GE Healthcare
Life Sciences

UNICORN™ 5.31

User Reference Manual

Chapter 12 to 15, Appendices

Table of Contents

12 Evaluation

Introduction

This chapter describes:

- How to evaluate results with the focus on how to integrate peaks.
- How to automate evaluation operations.
- How to export data and curves.

In this chapter

This chapter contains these sections.

12.1 Peak integration

Introduction

Peak integration is used to identify and measure a number of curve characteristics including peak areas, retention time and peak widths. This section describes:

- How to perform peak integrations.
- How to optimize peak integrations.

In this section

This section contains these sub-sections:

12.1.1 Baseline calculation

Introduction

The first step when you integrate peaks is to calculate a baseline. A correct baseline is crucial for accurate calculation of the peak areas. This section describes the options for how to calculate baselines in the ***Integrate*** dialog box.

Baseline options

UNICORN™ offers several options for how to create an accurate baseline:

- To use the automatic ***Calculate baseline*** function.
- To create a baseline based on a blank curve.
- To use a ***Zero baseline***.
- To reuse an existing baseline.

The Calculate baseline function

The ***Calculate baseline*** instruction provides automatic calculation of the baseline. In most cases the measurement is very accurate. The calculation can be performed using the ***Morphological*** algorithm or the ***Classical*** algorithm.

Baselines based on a blank curve

A blank curve can be used as the baseline for peak integration.

- You can use a blank curve with the same chromatographic conditions as the corresponding sample.

or

- You can subtract the blank run from the source curve and then perform peak integration on the resulting curve with the ***Calculate baseline*** instruction.

Note: In addition to blank run curves, it is also possible to select any curve from the current chromatogram as the baseline, e.g. an edited baseline.

Zero baseline

To use a ***Zero baseline*** means that there is no baseline subtraction at all.

Reuse an existing baseline

To reuse an existing baseline for the selected curve is the default alternative whenever there is an existing baseline available. The option ***Correlated baseline*** is selected if this is the case.

12.1.2 How to perform a peak integration

How to perform a peak integration

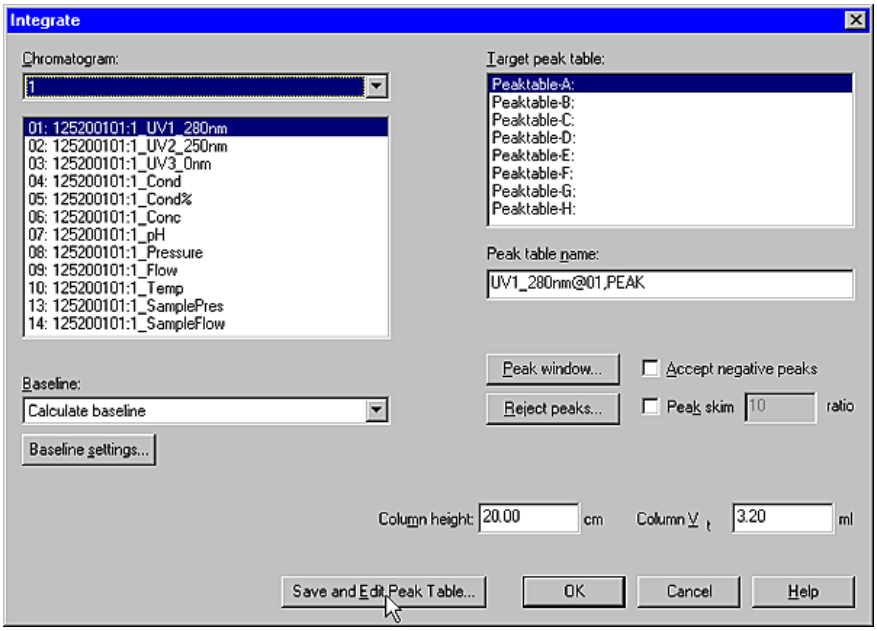
The table below describes how to perform a basic peak integration.

Step	Action
1	Open a result file in the ***Evaluation*** module.
2	• Choose ***Integrate:Peak Integrate***. *or* • Click the ***Peak Integrate*** toolbar icon.  *Result*: The ***Integrate*** dialog box opens.
3	• Select a source curve. • Select a baseline or a calculation method from the ***Baseline*** list. • Click ***OK*** to integrate with the default selections. *or* • Proceed with steps 4 to 6 to change the default selections. *Note*: See also *Section 12.1.3 How to optimize the baseline with a morphological algorithm, on page 410* and *Section 12.1.4 How to optimize the baseline with a classic algorithm, on page 414*.
4	• Click the ***Baseline settings*** button to change the calculation algorithm in the ***Settings*** dialog box. The default algorithm is ***Morphological***. • Change the selections or values. • Click ***OK***
5	• Click the ***Peak window*** button to edit the peak window limits if necessary. • Click the ***Reject peaks*** button to set the parameters for peak rejection if necessary. • Edit the ***Column height*** or ***Column V*** values if necessary.

Step	Action
6	• Click ***OK*** to integrate and close the dialog box. *or* • Click ***Save and Edit Peak Table*** to save the integration and open the integrated curve for editing. - See *Section 12.1.5 How to edit the baseline manually, on page 423* - See *Section 12.1.6 How to edit the peaks, on page 427* - See *Section 12.1.7 How to integrate part of a curve and how to exclude or skim peaks, on page 437*

Illustration

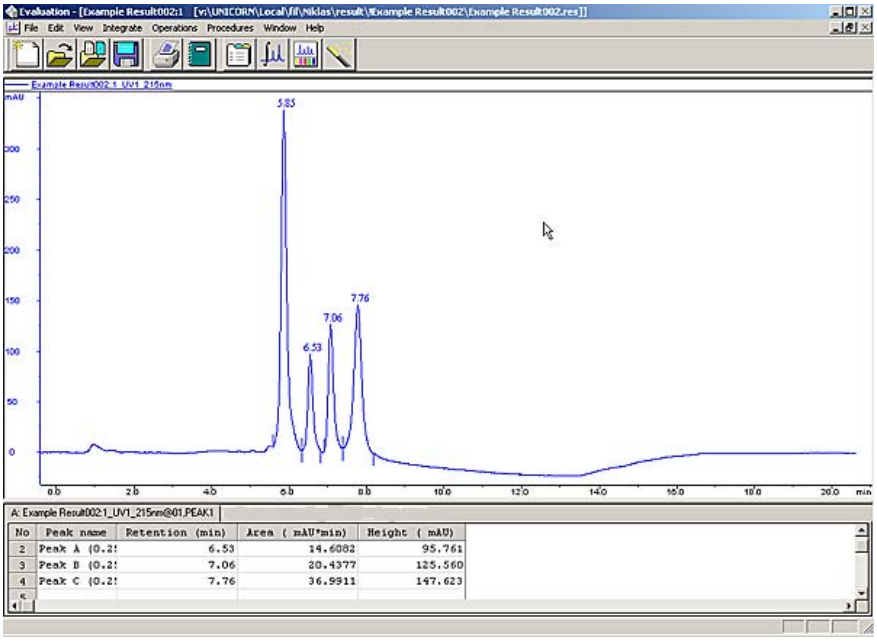
This is an illustration of the ***Integrate*** dialog box:



Peak integration results

The peak table is displayed underneath the active chromatogram. The start point and end point of each peak are marked by vertical marks, ***drop-lines***, in the chromatogram. The peaks are automatically labelled according to what is selected in the ***Curve Style and Color*** tab of the ***Chromatogram Layout*** dialog box.

This is an illustration of the results after a peak integration:



Note: Peak tables can be copied from one chromatogram to another with the ***Edit:Copy*** command. However, to display the table you must right-click in the chromatogram, choose ***Properties*** and then select the new peak table on the ***Peak Table*** tab of the ***Chromatogram Layout*** dialog box.

How to display peak characteristics

The peak retention times and several other peak characteristics are calculated automatically. The table below describes how to display other peak characteristics.

Step	Action
1	• Right-click in the active chromatogram. • Select ***Properties*** from the shortcut menu. *Result*: The ***Chromatogram Layout*** dialog box opens.
2	Click the ***Peak Table*** tab.
3	• Select options from the ***Select peak table columns*** list. • Click ***OK***. *Result*: The selected items will be displayed in the peak table.

How to filter peaks from view

Peaks can be removed from display in a peak table. The table below describes how to filter the peaks:

Step	Action
1	• Right-click in the active chromatogram or peak table. • Select ***Properties*** from the shortcut menu. *Result*: The ***Chromatogram Layout*** dialog box opens.
2	Click the ***Peak Table*** tab.
3	• Click the check boxes in the ***Filter Peaks*** field to select the filter criteria. • Specify filter values. • Click ***OK***.

To filter peaks vs. to reject peaks

The table below describes the major differences in the effect of filtering peaks compared to excluding the peaks by rejection.

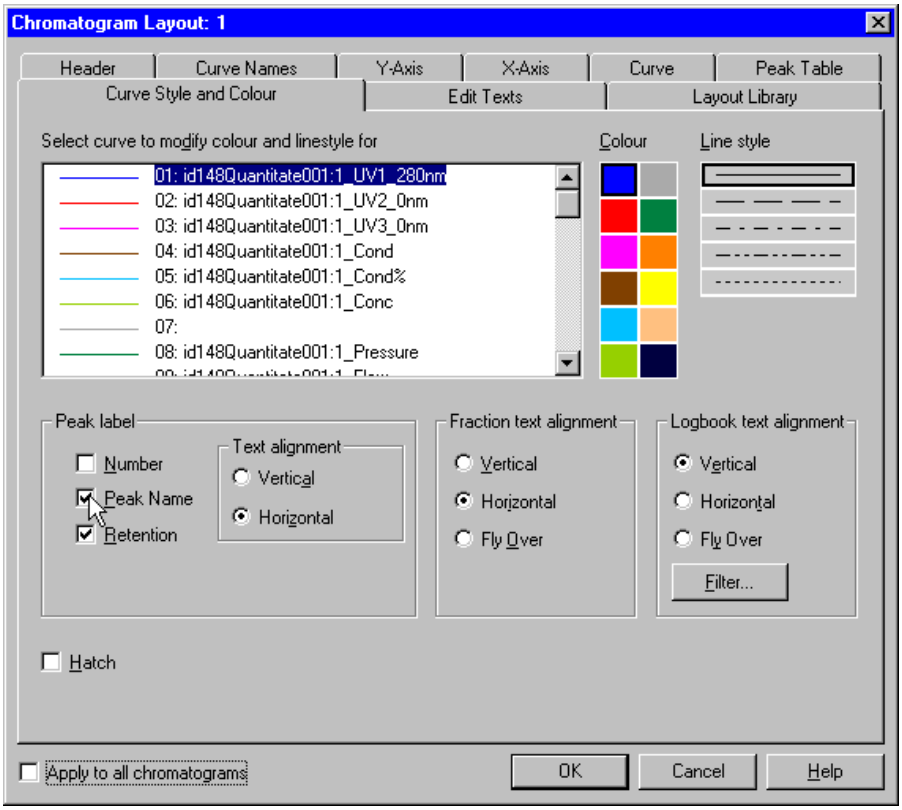
Filter peaks...	Reject peaks...
excludes the peaks from display,	permanently excludes peaks from the integration,
does not exclude the peaks from the calculation of the total peak area,	excludes the peaks from the calculation of the total peak area,
can be reversed.	cannot be reversed.

Peak labels

Peaks can be labelled with their retention, sequentially numbered, or be marked with specific identification names. See table below for an instruction on how to display peak labels.

The label type can be selected on the ***Curve Style and Colour*** tab in the ***Chromatogram Layout*** dialog box. De-select all label options to hide the labels, e.g. for presentations.

The illustration below shows the ***Chromatogram Layout*** dialog box with the ***Curve Style and Colour*** tab opened:



How to display peak labels

The table below describes how to display peak labels:

Step	Action
1	• Choose ***Edit:Chromatogram Layout***. *or* • Click the ***Chromatogram Layout*** icon.  *Result*: The ***Chromatogram Layout*** dialog box opens.

Step	Action
2	Click the ***Curve Style and Colour*** tab.
3	Select one or more of the following labelling options in the Peak label field: • ***Number*** *Result*: The peaks will be numbered sequentially. • ***Peak Name*** *Result*: Peak names will be displayed. See *Section 12.1.6 How to edit the peaks, on page 427* for information about how to name the peaks. • ***Retention*** *Result*: The retention volume or time will be displayed. • Click ***OK***.

12.1.3 How to optimize the baseline with a morphological algorithm

Introduction

The first choice when you want to optimize the peak integration is to change the baseline parameters. This section describes how to optimize the baseline with a morphological algorithm.

The Morphological algorithm

The ***Morphological*** algorithm can be described as a line that follows the chromatogram parallel to the X-axis. Data points for the baseline are created whenever the line touches the curve, and the points are joined at the end to create a baseline.

The ***Morphological algorithm*** gives the best result in curves with drifting baseline and peak clusters. The morphological baseline follows the curve faithfully, and a curve with a baseline at a more even level can be created by subtracting the morphological baseline.

The ***Morphological algorithm*** does *not* work well if there are negative peaks or if quantitative data from negative peaks are important in the run.

Note: The ***Morphological algorithm*** is the default baseline setting.

How to set a Morphological baseline

The table below describes how to choose a ***Morphological algorithm*** and define baseline settings.

Step	Action
1	Select ***Integrate:Peak Integrate***. *Result*: The ***Integrate*** dialog box opens.
2	Click the ***Baseline settings*** button in the ***Integrate*** dialog box. *Result*: The ***Settings*** dialog box opens.
3	• Select the ***Morphological*** algorithm. • Change the ***Baseline*** parameters if necessary. See more information about the parameters below this table. • Click ***OK***.

Note: The same settings can be edited in the ***Calculate Baseline*** dialog box when a new baseline is created. Choose ***Integrate:Calculate Baseline*** to open the dialog box.

Morphological algorithm parameters

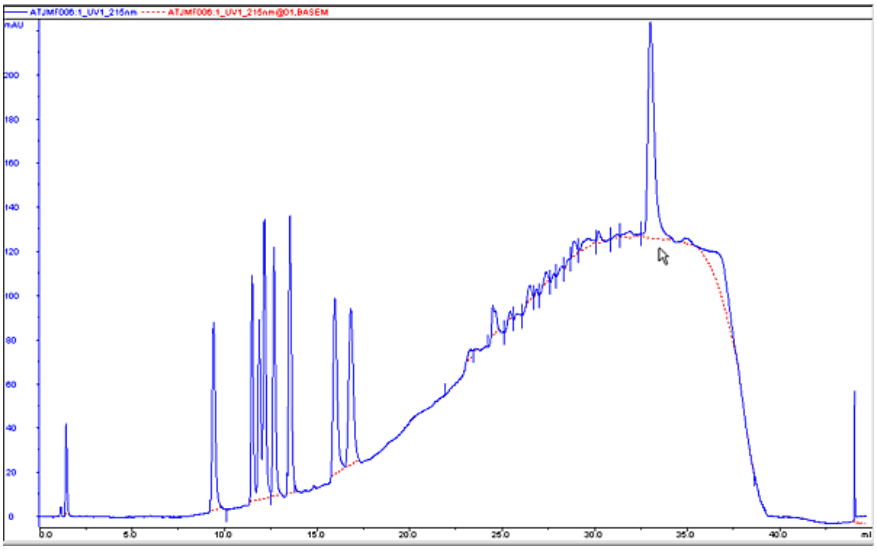
The parameters for the ***Morphological algorithm*** are:

- ***Structure width***
- ***Noise window***
- ***Minimum distance between points***

Structure width

Structure width determines the length of the straight line that follows the chromatogram. The default value is set at the widest peak in the chromatogram multiplied by 1.5.

The illustration below is an example of how a morphological baseline follows the peaks at the different levels in the curve:



The correct structure width settings

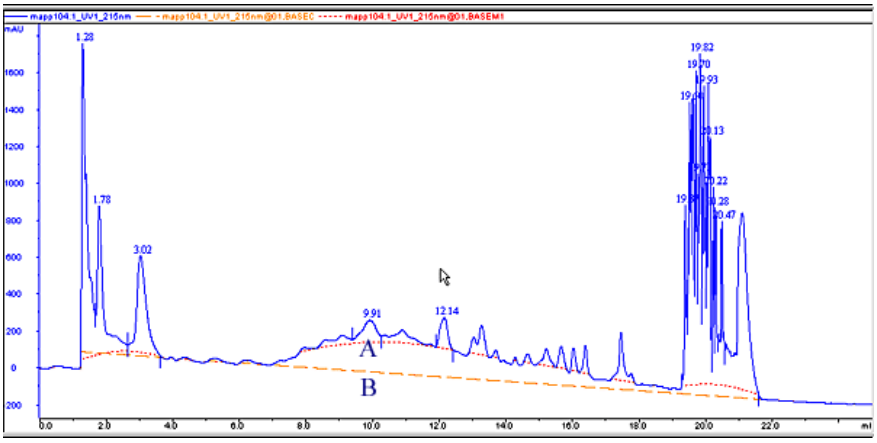
Too low settings

Too low ***Structure width*** settings can result in a baseline that reaches too high up in the peaks of the curve. Sometime a wider peak is not recognized because it contains a cluster of smaller peaks. The ***Structure width*** is then set to a value according to the largest width of the identified narrower peaks, and must be increased.

Too high settings

Too high ***Structure width*** settings mean that narrower peaks, especially in fluctuating curves, are not properly followed. This happens when an artifact in a curve is identified as the widest peak by the morphological algorithm, and then is used to set the default ***Structure width*** value.

The illustration below is an example of baselines using the default morphological algorithm settings (A) and a morphological algorithm with an increased ***Structure width*** value (B).



Noise window

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

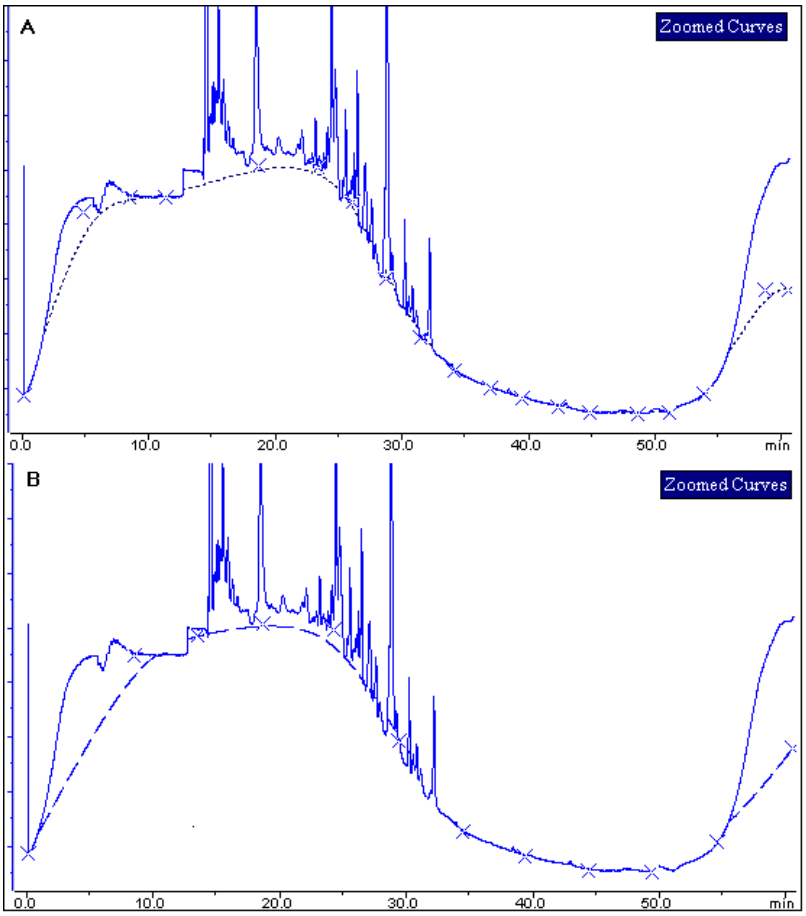
The solution to this is to increase the ***Noise window*** parameter. However, this can result in peak limits too high up on the peak slopes.

Note: You can also use the ***Reject peaks*** function in the ***Integrate*** dialog box to reduce the number of peaks based on the total number of accepted peaks or the minimum peak height.

Minimum distance between points

The ***Minimum distance between points*** is a measure of the distance between the data points used to generate a baseline. The largest number of data points is produced at the slopes of the curves. If you increase the ***Minimum distance between points*** value, fewer points will be collected on the slopes.

The illustration below is an example of a baseline (A) that is created with the ***Minimum distance between points*** parameter set at a low value. The number of data points is reduced when the ***Minimum distance between points*** parameter is set to a higher value (B).



12.1.4 How to optimize the baseline with a classic algorithm

Introduction

The first choice when you want to optimize the peak integration is to change the baseline parameters. This section describes how to optimize the baseline with a classical algorithm.

What is the Classic algorithm?

The ***Classic algorithm*** searches for all parts of the source curve that are longer than a defined minimum baseline segment and fall within limiting parameters. Together, the parameter values define the limits for a rectangular box. A part of the source curve must fit entirely inside this rectangular box to be identified as a baseline segment.

The ***Classic algorithm*** is particularly useful when you need to integrate curves with negative peaks and when quantitative data from negative peaks are important.

Classic algorithm parameters

The parameters for the ***Classic algorithm*** are:

- ***Shortest baseline segment***
- ***Noise window***
- ***Max baseline level***
- ***Slope limit***

See more information about the parameters below.

How to set a Classic baseline

The table below describes how to set a ***Classic algorithm*** and define a baseline.

Step	Action
1	Click the ***Baseline settings*** button in the ***Integrate*** dialog box. *Result*: The ***Settings*** dialog box opens.

Step	Action
2	• Select the ***Classic*** algorithm. • Change the ***Baseline*** parameters. See more information about the parameters below this table. • Click ***OK***.

Note: The same settings can be edited in the ***Calculate Baseline*** dialog box when a new baseline is created. Choose ***Integrate:Calculate Baseline*** to open the dialog box.

Test your parameter changes

The best way to optimize the baseline is to change the baseline parameters step by step and then check the resulting baseline after each change. When the desired effect is accomplished it is best to go back and try a parameter value in between the two last settings to avoid an unnecessarily low or high value.

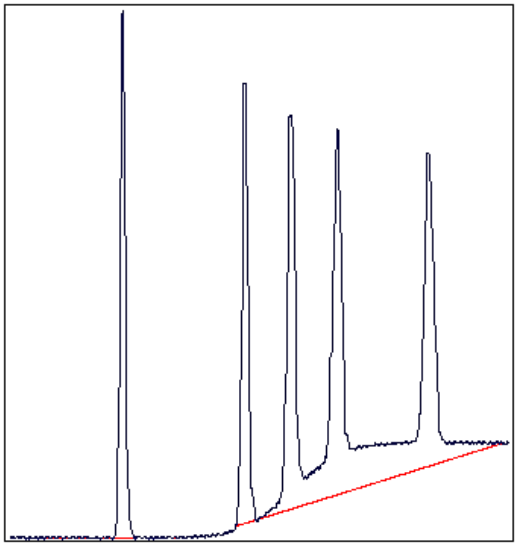
How much the values should be changed depends on the cause of the peak integration problem. The table below is a general guideline.

Baseline parameter	Recommended initial change
Shortest baseline segment	20-50%
Noise window	10-30%
Max baseline level	Usually not necessary to adjust
Slope limit	25-50%

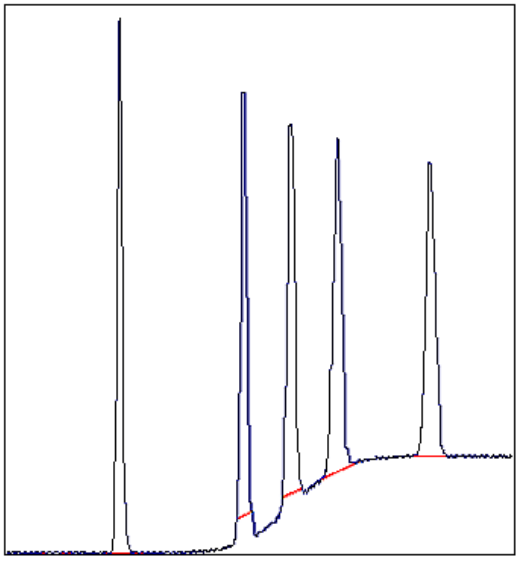
Note: If necessary, click the ***Default*** button to restore the default values.

Shortest baseline segment

If a too high ***Shortest baseline segment*** value is set, short curve segments between peaks in the middle of the chromatogram are not identified as baseline segments. The calculated baseline does not follow the source curve, see below:



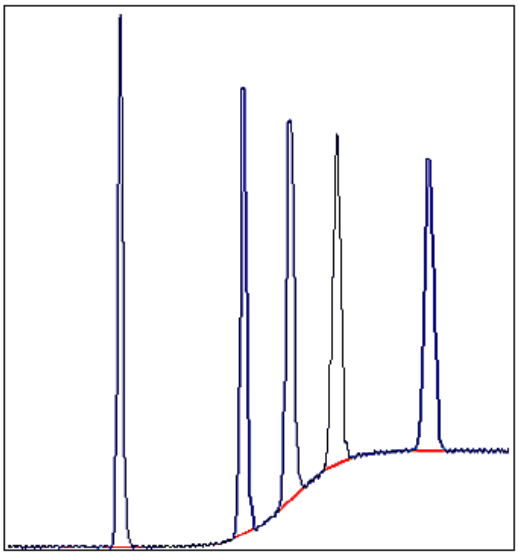
The ***Shortest baseline segment*** value is decreased by 50% in this example:



Slope limit

A changed ***Slope limit*** will often improve the baseline calculation. The ***Slope limit*** sets the maximum slope of the curve to define when a peak is recognized. A too high ***Slope limit*** will cause the up-slopes of the peaks to be recognized as baseline segments.

The example above was improved by the shorter baseline segments but the high slope of the short segments in the region between the second and the fourth peak still makes the baseline unacceptable. In the example below the ***Slope limit*** is increased by a factor of 2.5, which produces a correct baseline:

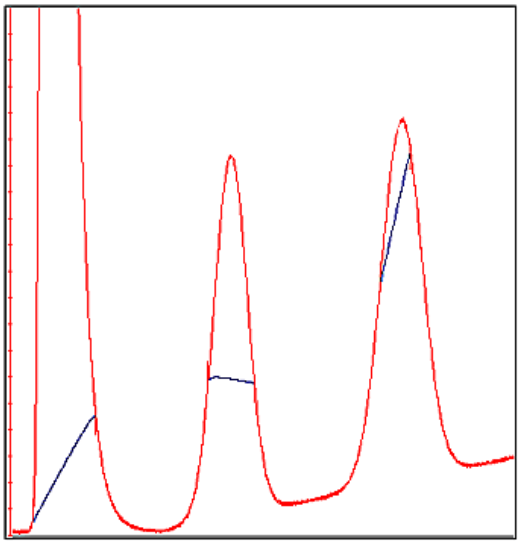


Too high slope limit

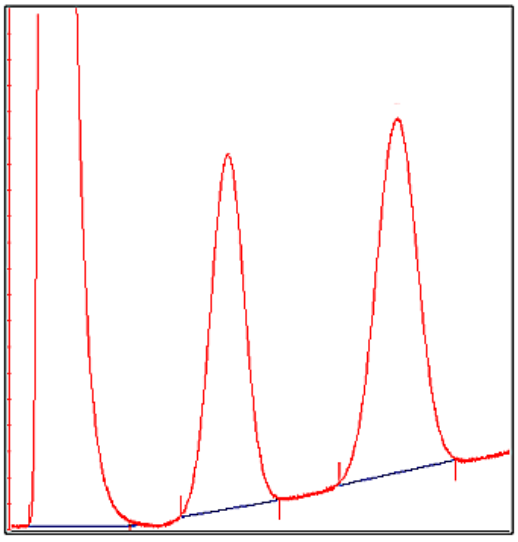
A too high ***Slope limit*** value can cause peak limits too high up on the peaks. This can be the case when the chromatogram includes a very large flow-through or solvent peak. The large peak affects the calculation of the default parameters and leads to too high values for the ***Slope limit***.

Note: A too high value for the ***Noise window*** can have the same effect and be caused by the same situation, often also in combination with a high ***Slope limit***.

Peak limits are defined on peaks in the example below due to the high ***Slope limit***:



The example below has a much lower ***Slope limit***, and a lower ***Noise window***:

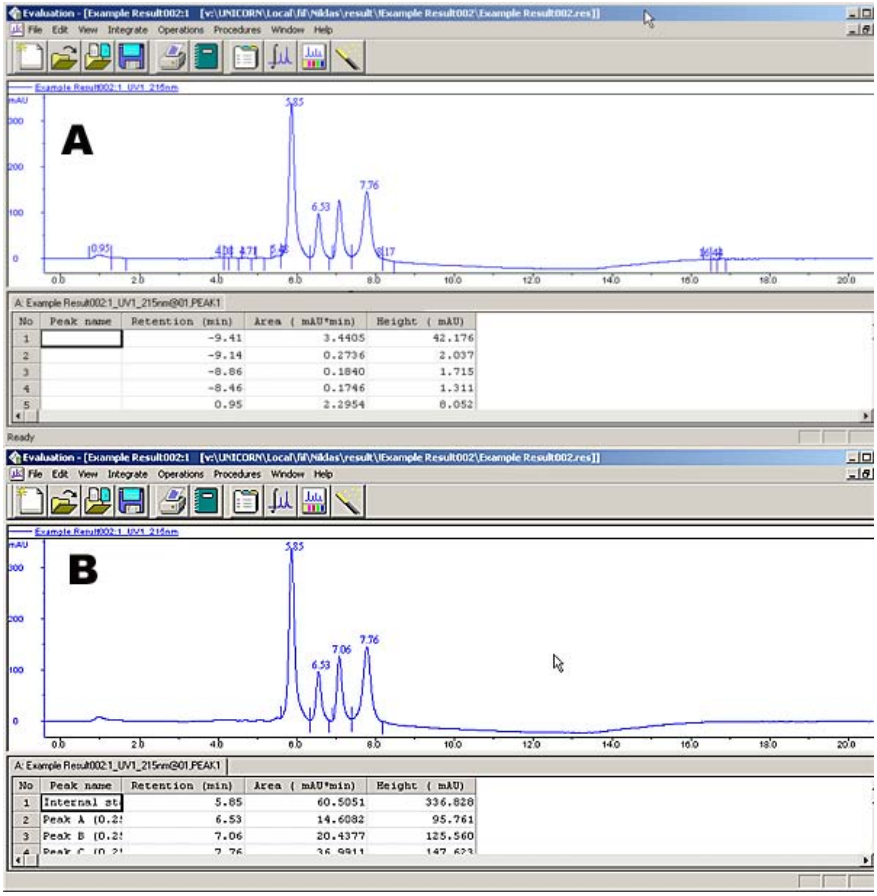


Noise window

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

The solution to this is to increase the ***Noise window*** parameter. However, this can result in peak limits too high up on the peak slopes.

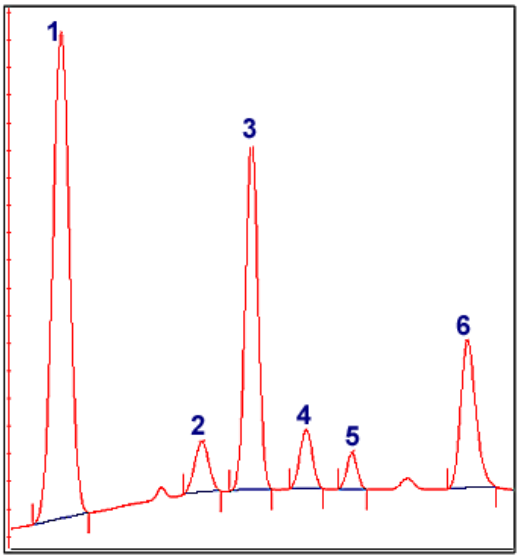
The illustration below is an example of noise detected as peaks (A) and the result of a second peak integration with an increased ***Noise window*** (B).



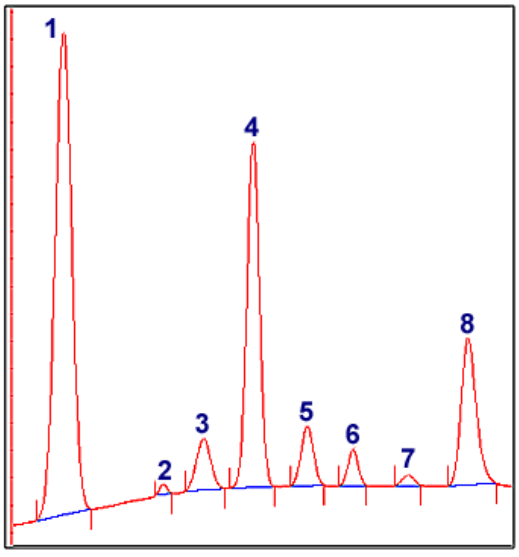
Note: You can also use the ***Reject peaks*** function in the ***Integrate*** dialog box to reduce the number of peaks based on the total number of accepted peaks or the minimum peak height.

Missing peaks

Sometimes obvious peaks are not detected in the peak integration. The probable cause is that the ***Noise window*** is set too high. See the illustration below:



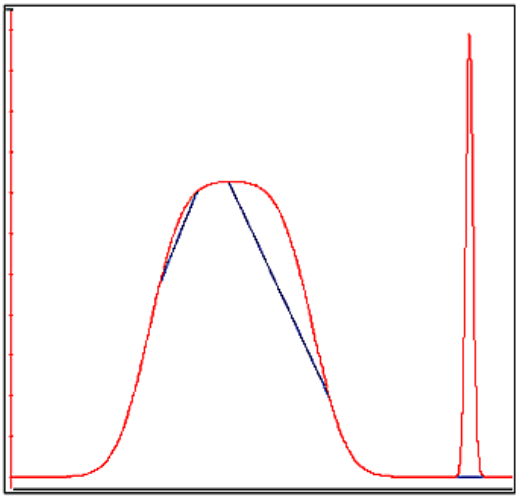
All peaks are detected if the ***Noise window*** is decreased, see example below:



Note: Missing peaks can also be caused by improper settings for ***Reject peaks*** in the ***Integrate*** dialog box, or ***Filter peaks*** in the ***Chromatogram layout*** dialog box.

When to change the Max baseline level

In rare cases the top of a broad, flat peak can be incorporated as a baseline segment. This is one of the very few situations where it is useful to change the ***Max baseline level***. The illustration below is an example:



How to set the Max baseline level

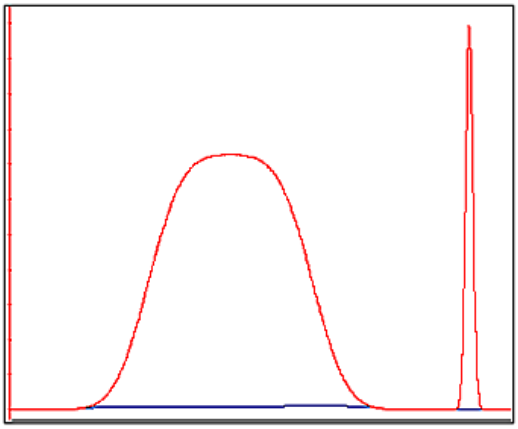
The table below describes how to set the ***Max baseline level***.

Step	Action
1	Right-click in the chromatogram and select ***Marker***. *Result*: A vertical line is set in the chromatogram. A text box in the top left corner of the chromatogram displays the X-axis and Y-axis values of the curve at the point where the vertical ***Marker*** line crosses the curve.
2	• Move the ***Marker*** with your mouse. • Measure the height of the peak you want to exclude from the baseline.
3	Choose ***Integrate:Calculate baseline***.

Step	Action
4	• Select the ***Classic*** checkbox as the ***Chosen algorithm***. • Type a new value for ***Max baseline level***. Set the level slightly lower than the value that you measured in step 2. • Click ***OK***.

Example of a correct baseline

The illustration below is an example of a correct baseline after the ***Max baseline level*** has been changed:



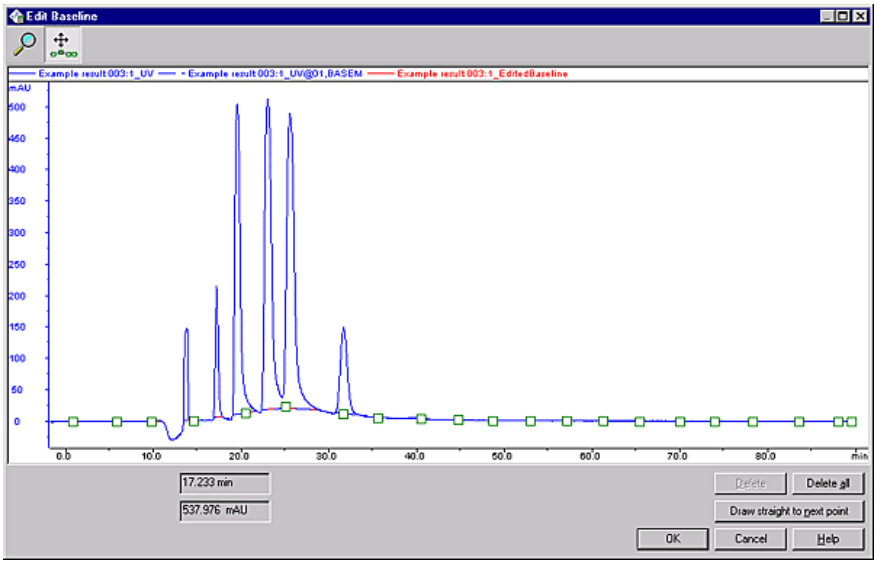
12.1.5 How to edit the baseline manually

The Edit Baseline dialog box

You can edit the baseline manually in the ***Edit Baseline*** dialog box in the ***Evaluation*** module:

- Select ***Integrate:Edit Baseline*** to display the dialog box.

The ***Edit Baseline*** dialog box displays the baseline and the curve it was calculated from. The baseline points are marked with green squares. Hold the cursor above the baseline point to display its coordinates. See the illustration below:



How to use the zoom function

The table below describes how to use the zoom function in the ***Edit Baseline*** dialog box.

Step	Action
1	• Click the ***Zoom*** icon. *Result*: The cursor is changed into a magnifying glass.

Step	Action
2	• Press and hold the left mouse button. • Drag the cursor over the area you want to zoom in on. • Release the mouse button. *Result*: The area is enlarged. Right-click and select ***Reset zoom*** to restore the full view.

How to edit and insert data points

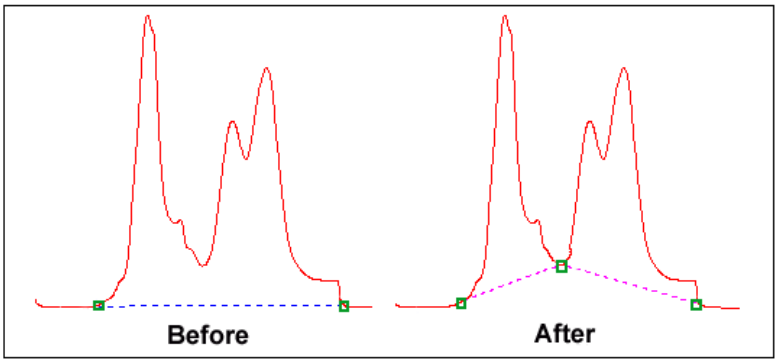
The table below describes how to edit and insert baseline data points:

Step	Action
1	Select ***Integrate:Edit Baseline***. *Result*: If there are more than one baseline available, the ***Select Baseline to Edit*** dialog box opens. If not, proceed to step 2. • Select the baseline you want to edit from the list. • Click ***OK***. *Result*: The ***Edit Baseline*** dialog box opens
2	• Click the ***Set Curve Points*** icon.  *Result*: The cursor is changed into a cross.
3	**Add a data point** • Click the left mouse button to place a new baseline point in the chromatogram. *Result*: A new point is created, marked by a green square. The baseline curve is redrawn as a spline function based on the old and the new points. The baseline is guided by the points, but does not necessarily pass through them.

Step	Action
4	**Delete a data point** • Double-click the data point. *or* • Click the data point to select it and click the ***Delete*** button. *or* • Right-click the data point and select ***Delete Point*** from the shortcut menu. *Result*: The data point is deleted and the curve is redrawn.
5	**Move a data point** • Select the data point and drag it to a new position. *Result*: The baseline curve is redrawn.
6	Click ***OK***. *Result*: The ***Save Edited Baseline*** dialog box opens.
7	• Confirm the location and type a new name if necessary. • Click ***OK***. *Result*: The new baseline is saved.

Edited baseline

The illustration below is an example of a baseline before and after editing:



How to draw a straight line

The table below describes how to force a straight baseline between two points.

Step	Action
1	Select the first of the two points in the point list.
2	Click the ***Draw straight to next point*** button. *Result*: The baseline is drawn through the points as a straight line.

12.1.6 How to edit the peaks

Introduction

Once a peak table has been generated based on an appropriate baseline, it is possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak values will all be recalculated.

How to open the peak table for editing

The table below describes how open the peak table for editing. The editing options are described below this table:

Step	Action
1	• Select ***Integrate:Edit Peak Table***. *Result*: If there are more than one peak table available, the ***Select Peak Table to Edit*** dialog box opens. The name of the baseline on which the peak table was based is displayed at the bottom of the panel.
2	• Select the peak table from the list and click ***OK***. • Select one or more ***Help Curves*** to be displayed for reference if necessary. *Result*: The ***Edit Peak Table*** dialog box opens. *Note*: The ***Edit Peak Table*** dialog box will be opened immediately if you select ***Save and Edit Peak Table*** as the last step of the peak integration.
3	Perform the changes (described in the instructions below).
4	Click ***OK***. *Result*: The ***Save Edited Peak Table*** dialog box opens. The dialog box displays a suggested name and location for the peak table.
5	Confirm the name and location and click ***OK***.

How to adjust the baseline

The baseline can be adjusted graphically (see also *Section 12.1.5 How to edit the baseline manually, on page 423*) in the ***Edit Peak Table*** dialog box. The table below describes this:

Step	Action
1	• Click the ***Set Curve Points*** icon. *Result*: The cursor is changed into a cross.
2	Perform the operations below as desired: • Click to insert a new data point. • Double-click on a data point or right-click the point and select ***Delete Point*** from the short-cut menu to delete the point. • Click a data point and drag the point to a new position to move the baseline. *Note*: ***Accept negative peaks*** must be selected before the peak integration if you want to be able to drag a data point to move the baseline above the curve.

How to calculate a new baseline

The baseline can be recalculated in the ***Edit Peak Table*** dialog box. The table below describes how to do this:

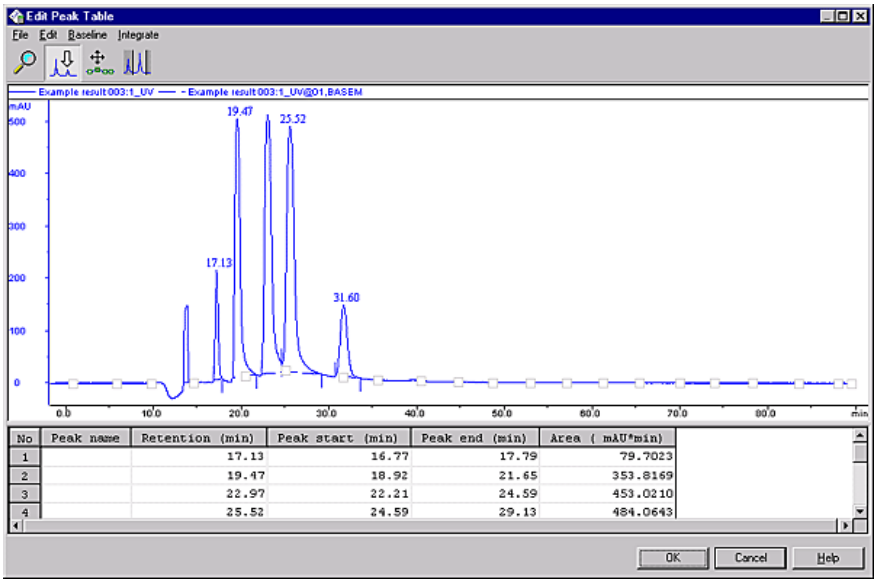
Step	Action
1	• Select ***Baseline:New:Calculate***. *or* • Right-click and select ***New Calculate*** from the shortcut menu. *Result*: The ***Settings*** dialog box opens.
2	• Select an algorithm (***Morphological*** is default).
3	• Adjust the ***Baseline*** parameters as desired. *or* • Click the ***Default Values*** button for the default values.

Step	Action
4	• Click ***OK***. *Result*: The baseline is recalculated.

Note: Select ***Baseline:New:Zero Baseline*** to replace the calculated baseline with a zero baseline.

The Edit Peak Table dialog box

The illustration below shows the ***Edit Peak Table*** dialog box.



How to delete a peak

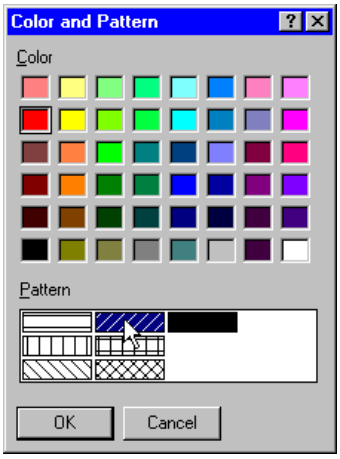
The table below describes how to delete a peak in the ***Edit Peak Table*** dialog box:

Step	Action
1	• Click the ***Edit peaks*** icon.  • Click the peak in the curve or in the peak table to select the peak.
2	• Right-click and select ***Delete Peaks*** from the shortcut menu. *or* • Select ***Edit:Delete Peaks***. *Result*: The peak is deleted and the remaining peaks are renumbered.

How to add color to a peak

The table below describes how to add a fill color and a pattern to a peak in the ***Edit Peak Table*** dialog box:

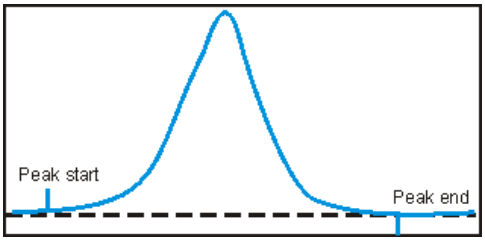
Step	Action
1	• Click the ***Edit peaks*** icon.  • Move the cursor over the peak you want to edit. *Result*: The cursor is changed into a larger arrow. • Click to select the peak.

Step	Action
2	• Right-click and select ***Fill Peak*** from the shortcut menu. *or* • Select ***Edit:Fill Peak***. *Result*: The ***Color and Pattern*** dialog box opens.  • Select a color and a pattern. • Click ***OK***. *Result*: The peak is filled according to the selections.

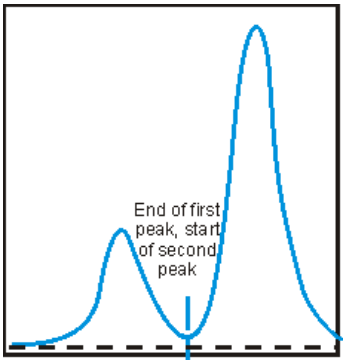
Note: The color and pattern selections will override the general ***Fill settings*** that can be selected for all peaks on the ***Peak Table*** tab in the ***Chromatogram Layout*** dialog box.

Peak start and end points

The beginning of each peak is marked with a drop-line above the curve, and the end of each peak is marked with a drop-line below the curve. The illustration below shows an example of start and end point drop-lines:



Where there are two peaks beside one another, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a drop-line below and above the curve at the same point. See the illustration below:



How to split a peak

It is possible to split the peak into two new peaks by inserting a drop-line. The table below describes how to split a peak in the ***Edit Peak Table*** dialog box:

Step	Action
1	• Click the ***Edit peaks*** icon.  • Click the peak in the curve or in the peak table to select the peak.
2	• Right-click and select ***Split Peak*** from the shortcut menu. *or* • Select ***Edit:Split Peaks***. *Result*: A new drop-line is inserted at the middle point between the two existing drop-lines and the peak is split.

Note: The area under each new peak will not be the same if the symmetry of the original peak was not perfect.

How to join peaks

It is possible to join the areas of adjacent peaks if they are separated by a drop-line. The table below describes how to join adjacent peaks in the ***Edit Peak Table*** dialog box:

Step	Action
1	• Click the ***Edit peaks*** icon.  • Click the peak in the curve or in the peak table to select the peak.

Step	Action
2	• Right-click and select ***Join Left*** or ***Join Right*** from the shortcut menu. *or* • Select ***Edit:Join Left*** or ***Edit:Join Right***. *Result*: The original intervening drop-line is removed and all peaks are renumbered.

How to add peak names

The table below describes how to add names in the ***Edit Peak Table*** dialog box to identify the peaks:

Step	Action
1	• Click the ***Edit peaks*** icon.  • Click the peak in the curve or in the peak table to select the peak.
2	• Right-click and select ***Peak Name*** from the shortcut menu. *or* • Choose ***Edit:Peak name***. *or* • Double-click the peak in the peak table or the curve. *Result*: The ***Edit Peak Name*** dialog box opens. The number and retention of the selected peak is displayed.
3	Type a name in the ***Peak name*** textbox and click ***OK***.

How to adjust peak areas with drop-lines

The table below describes how to move the drop-lines to adjust the peak area in the ***Edit Peak Table*** dialog box.

Step	Action
1	• Click the ***Edit peaks*** icon.  • Click the peak in the curve or in the peak table to select the peak. *Result*: Two vertical bars become superimposed over the drop-lines that delimit the selected peak. The area between the bars is filled with a yellow fill pattern.
2	Drag the bars to define the new limits for the selected peak. *Result*: The drop-lines are moved and the peak areas are automatically re-calculated.

Note: A drop-line can never be moved beyond another drop-line or beyond a point where the peak meets the baseline.

How to use the zoom function

The table below describes how to use the zoom function in the ***Edit Peak Table*** dialog box.

Step	Action
1	• Click the ***Zoom*** icon.  *Result*: The cursor is changed into a magnifying glass.

Step	Action
2	• Press and hold the left mouse button. • Drag the cursor over the area you want to zoom in on. • Release the mouse button. *Result*: The area is enlarged. Right-click and select ***Reset zoom*** to restore the full view.

The Integrate menu

If needed you can use the selections on the ***Integrate*** menu to perform a peak integration in the ***Edit Peak Table*** dialog box. This is useful for example if you want to re-integrate the curve using different settings or integrate only part of a curve with different settings.

See *Section 12.1.7 How to integrate part of a curve and how to exclude or skim peaks, on page 437* for more information.

12.1.7 How to integrate part of a curve and how to exclude or skim peaks

Introduction

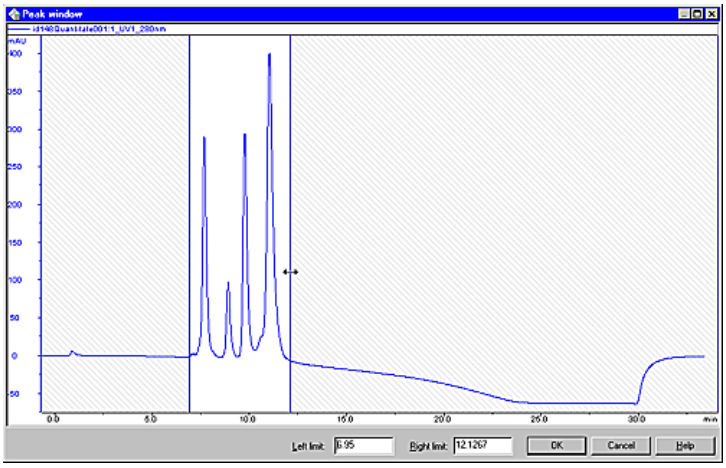
There are several possibilities to improve the results if the peak integration is unsatisfactory. This section describes:

- How to select only part of a curve for integration.
- How to exclude peaks.
- How to skim peaks.

These operations can be performed both in the ***Integrate*** dialog box in preparation for the peak integration, or in the ***Edit Peak Table*** dialog box to adjust an unsatisfactory peak integration. This section describes both alternatives.

How to select part of a curve

The table below describes how to select only a part of a curve for peak integration in the ***Integrate*** dialog box:

Step	Action
1	• Choose ***Integrate:Peak Integrate***. *Result*: The ***Integrate*** dialog box opens. • Click the ***Peak Window*** button. *Result*: The ***Peak window*** dialog box opens. 
2	• Type new X-axis values for the ***Left limit*** and the ***Right limit***. *or* • Drag the vertical cursor lines to define the limits.
3	Click ***OK***. *Result*: The baseline will be calculated from the whole curve, but the calculation of the peak areas is only performed on the selected section.

How to exclude peaks

You can define criteria to exclude peaks from integration. The table below describes how to define peaks to be excluded in the ***Integrate*** dialog box.

Step	Action
1	Click the ***Reject peaks*** button. *Result*: The ***Reject Peaks*** dialog box opens.
2	• Select the appropriate checkboxes and type values for height, width and area. • Define how many of the largest peaks you want to include. • Click ***OK***.

How to include negative peaks

Select the ***Accept negative peaks*** checkbox of the ***Integrate*** dialog box to include negative peaks in the integration.

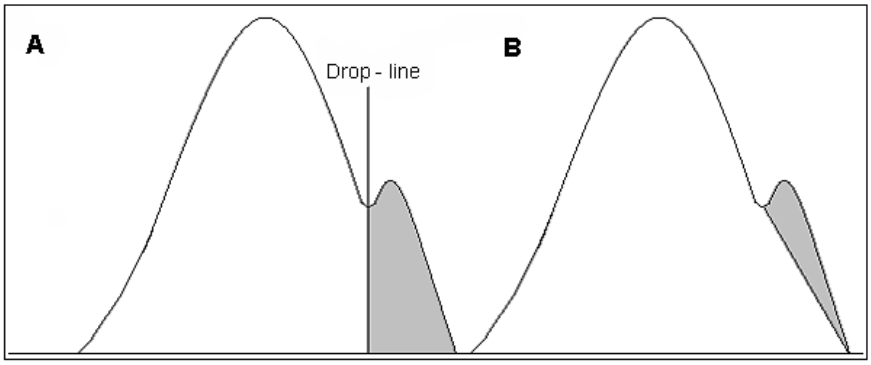
Result: The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

Peak skimming vs. drop-lines

The area under a peak can be calculated either using separating drop-lines or peak skimming:

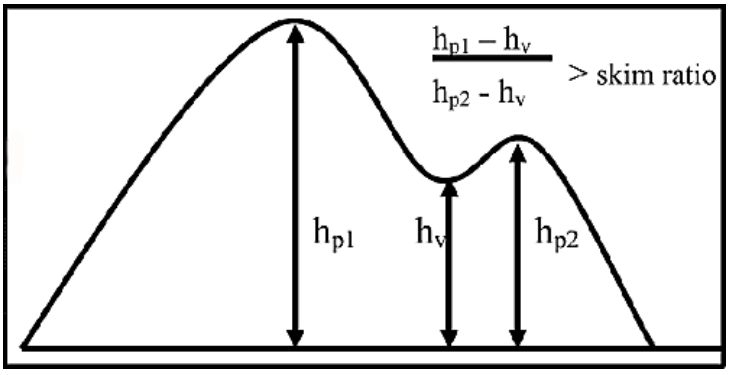
- ***Drop-lines*** are vertical marks that split two peaks at the valley. Drop-lines are used mostly for peaks of relatively similar size. When a peak has a shoulder, splitting with drop-lines will cause the first peak to lose too much of its area to the peak that forms its shoulder.
- The ***Peak skim*** option can be used to skim off the smaller peak with a straight line that starts in the valley between the peaks and ends at the other side of the smaller peak, at the point where the skim line and the curve slope are equal.

The illustration below is an example of how a drop-line (A) and a skimmed peak (B) affects the area under the main peak and the peak shoulder. The peak shoulder area is marked in gray:



How to skim peaks

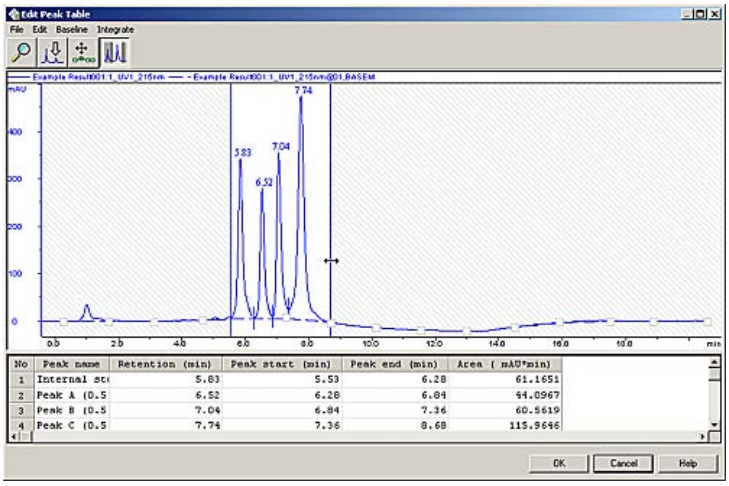
The table below describes how to select a ratio to skim peaks in the ***Integrate*** dialog box:

Step	Action
1	Select the ***Peak skim*** checkbox.
2	Determine the ratio when peak skimming should be applied based on the relationship in the illustration below:  *Note*: The default ratio value is 10.
3	Type the ratio value in the text box.

How to integrate part of a curve

Part of a curve can be selected in the ***Edit Peak Table*** dialog box and integrated with settings that differ from the rest of the curve. The table below describes how to do this.

Step	Action
1	• Choose ***Integrate:Edit Peak Table***. *Result*: The ***Select Peak Table to Edit*** dialog box opens. • Select the peak table to edit and click ***OK***. *Result*: The ***Edit Peak Table*** dialog box opens.
2	• Click the ***Peak Window*** icon. *Result*: Two vertical cursor lines are displayed. • Drag the cursor lines to the beginning and the end of the selected part of the curve.



Note: All operations described below will only affect the selected part of the curve.

Step	Action
3	If desired, change the integration parameters: **Reject peaks** • Choose ***Integrate:Settings***. *Result*: The ***Reject Peaks*** dialog box opens. • Change the settings as desired and click ***OK***. **Skim peaks** • Choose ***Integrate:Peak Skim***. *Result*: The ***Peak Skim*** dialog box opens. • Select the ***Skim Peaks*** checkbox and type a ratio. • Click ***OK***.
4	• Choose ***Integrate:Peak Integrate***. *Result*: The selected part of the curve is peak integrated based on the changed parameters.

12.1.8 Measurements

Introduction

It is possible to determine the coordinates of any point on a curve and to obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations.

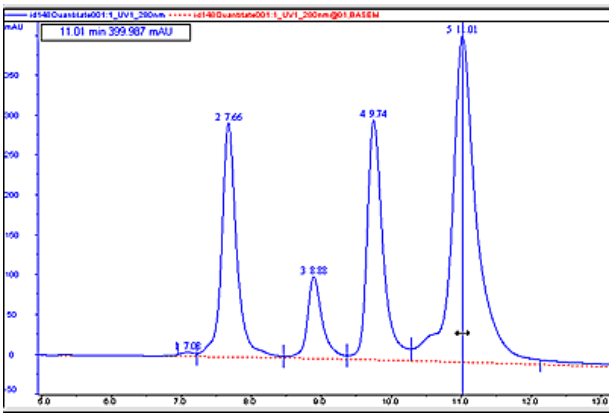
Measurement options

Coordinates can be obtained in two ways:

- Through direct measurement.
- From peak table data.

How to make direct measurements

The table below describes how to make direct measurements in a chromatogram:

Step	Action
1	Right-click in the chromatogram and select ***Marker***. *Result*: A vertical line is set in the chromatogram. A text box in the top left corner of the chromatogram displays the X-axis and Y-axis values of the curve at the point where the vertical ***Marker*** line crosses the curve. See the illustration below:  *Note*: The color of the ***Marker*** is the same as the selected curve.
2	Move the ***Marker*** with your mouse to display the peak data.
3	Click the curve name legend above the chromatogram to change to another curve. *Result*: The Y-axis is changed to the one corresponding to the new curve.
4	Right-click and select ***Marker*** again to de-select the function.

How to set a reference point

The table describes how to set a reference point:

Step	Action
1	Right-click in the chromatogram and select ***Set Marker Ref. Point*** to define a reference point for the marker position.
2	When the marker is moved from the reference point, the X-axis and Y-axis values for the new position are displayed together with: • the new position in relation to the position of the reference point, • the minimum, maximum and average values for the curve interval between the reference point and the new position.

How to record a Snapshot

The table below describes how to record a ***Snapshot*** of the current curve values:

Step	Action
1	• Right-click in the chromatogram and select ***Snapshot*** from the shortcut menu. *Result*: The ***Snapshot*** dialog box opens.
2	The dialog box displays all the curve data that was current at the moment the snapshot was taken. • Click the ***Save to file*** button to save the snapshot as an Excel file. • Click the ***Print*** button to print the snapshot.

How to select peak table data

The retention time and amplitude of any peak can be viewed directly in a peak table after an integration. This data and more is selected in the ***Chromatogram Layout*** dialog box. The table below describes how to select peak table data.

Step	Action
1	Click the ***Chromatogram Layout*** icon.  *Result*: The ***Chromatogram Layout*** dialog box opens.
2	Click the ***Peak Table*** tab.
3	• Select the checkboxes on the ***Select peak table columns*** list for all items that you want to display in the table. • Click ***OK***.

12.2 Other evaluations

Introduction

This section describes how the results can be used for other types of evaluations.

In this section

This section contains these sub-sections.

12.2.1 Peak purity and peak identification

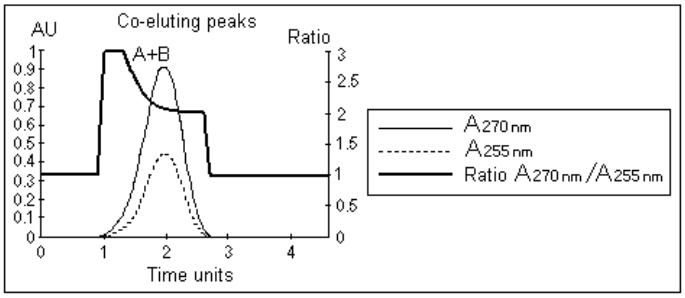
Introduction

Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity.

Peak purity

The absorbance ratio can be used to check peak purity. If the peak is pure, the absorbance spectra are the same over the whole peak and the ratios should therefore remain constant. The peak is probably not pure if the absorbance ratio is not the same over the whole peak.

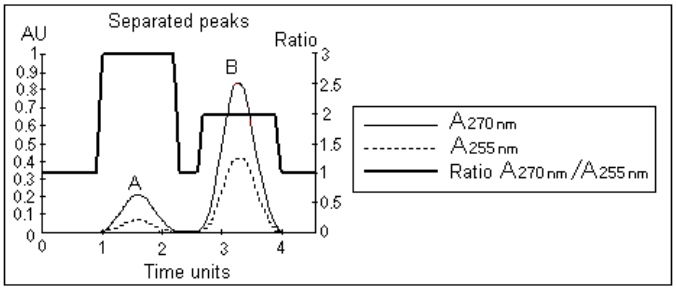
The illustration below shows a simulated chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time:



Peak identification

The absorbance ratio can be used for peak identification. Different compounds have a specific ratio between absorbancies at different wavelengths.

The illustration below shows a simulated chromatogram of two components with differences in their absorbance spectra:



How to divide the curves

Both curves must have a baseline close to zero AU before they can be divided. This is achieved with baseline subtraction. The table below describes how to subtract the baseline from an earlier integration and divide the curves:

Step	Action
1	Create a baseline for each UV curve.
2	Select ***Operations:Subtract***. *Result*: The ***Subtract*** dialog box opens.
3	• Select the UV curve in the first list of curves. • Select its baseline in the second list of curves. • Click ***OK***. *Note*: You can also subtract corresponding blank runs if there are blank runs available.
4	Repeat steps 2 and 3 for the second UV curve.
5	Select ***Operations:Divide***. *Result*: The ***Divide*** dialog box opens.
6	• Select the first result curve from the subtractions in the first list of curves. • Select the second result curve from the subtractions in the second list of curves.

Step	Action
7	Click the checkbox for ***Threshold*** and type values for each curve. This results in the following: • The quotient is set to 1.0 if either of the sample values is closer to zero than the threshold value. Very high quotient values are prevented if division is performed with values close to zero. Very low quotient values are also prevented. *Note*: Default ***Threshold*** values are entered by UNICORN. The values can be changed.
8	Click ***OK***.

How to filter the result curve

The resulting curve can be filtered to reduce noise and to remove ghost peaks. The table below describes how to filter the curve.

Step	Action
1	Select ***Operations:Smooth***. Result: The ***Smooth*** dialog box opens.
2	• Select the ***Source Chromatogram***. • Select a ***Filter Type***. *Note*: The ***Median*** filter is recommended to remove noise that appears as spikes or occurs in a small area of the curve. • Click ***OK***.

12.2.2 How to find slope values

Introduction

With ÄKTAdesign systems it is possible to only collect peaks during fractionation. The way to find suitable slope values for a particular run is described in this section.

Where to use slope values

The slope values can be used in the ***Method Editor***

- as ***StartSlope*** and ***EndSlope*** values in the ***Peak_FracParameters*** instruction.
- as parameters for the ***Watch*** instruction.

Using slope values for Watch instructions

The slope values can be used in the ***Method Editor*** as parameters for the ***Watch*** instruction.

Conditional ***Watch*** instructions can be set up to let the progress of a run be determined by the events during the run, e.g. start to collect fractions when the first peak emerges.

The slope of the curve can be set as a condition to satisfy a ***Watch*** condition in the method during the run. It is important to use accurate slope values for the specific ***Watch*** instruction parameter.

A sample run

You must first make a separation run with the sample you intend to purify. The result from this separation run is then used to find the slope values.

Retention scale

Time should be used as the X-axis scale for retention.

Step	Action
1	Click the ***Chromatogram Layout*** icon.

Step	Action
2	• Click the ***X-axis*** tab. • Select ***Time***. • Click ***OK***.

How to differentiate the curve

The slope values are measured on a differentiated curve. The table below describes how to create a differentiated curve.

Step	Action
1	Select ***Operations:Differentiate***. *Result*: The ***Differentiate*** dialog box opens.
2	• Select the UV curve you want in the ***Source chromatogram*** list. • Click the ***First order*** radio button. • Click ***OK***. *Result*: The differentiated curve opens in the chromatogram.

How to measure the slope values

Sometimes the differentiated curve must be filtered to reduce noise and ghost peaks before the measurements. See section *Section 12.2.1 Peak purity and peak identification, on page 448*.

The table below describes how to measure the slope values on the differentiated curve.

Step	Action
1	Click the name of the differentiated curve (above the chromatogram window) to select the curve.
2	Use the zoom function to magnify the curve over an appropriate area.
3	Right-click and select ***Marker*** from the short-cut menu. *Result*: A vertical cursor bar opens in the chromatogram.

Step	Action
4	Place the ***Marker*** at the beginning of a peak where you want the ***Watch*** conditions to be fulfilled, i.e. where the slope becomes higher.
5	Read the actual slope value in the active ***Marker*** text box in the top left corner of the chromatogram window.

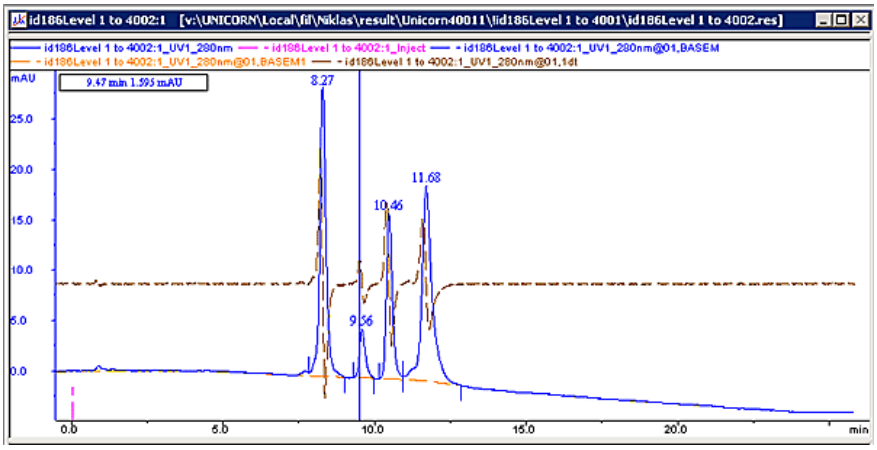
Note: The unit for the differentiated curve is mAU/min or AU/min. Any Y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

Peak fractionation for ÄKTAdesign

If your system is an ÄKTA™ design system, measure the slope at the beginning and the end of the smallest, flattest peak of all the peaks of interest, and use these values.

Illustration: Slope value measurement

The illustration below shows a measurement of the slope limit after differentiation:



12.2.3 How to simulate a peak fractionation

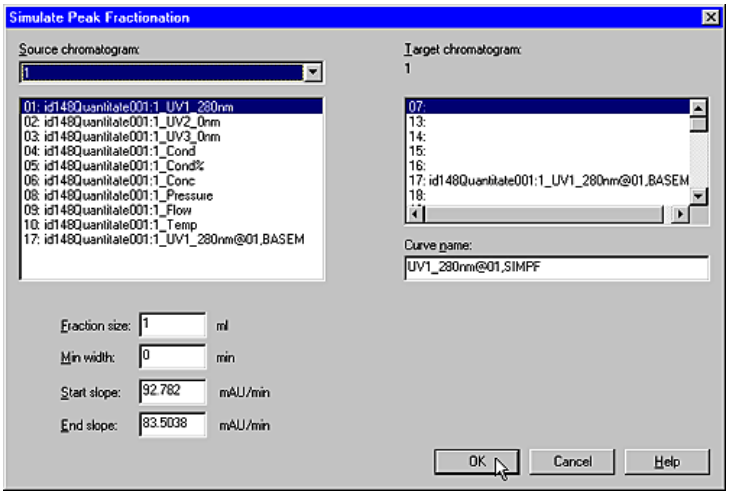
Introduction

You can create a curve that simulates a peak fractionation to test the outcome before the actual peak fractionation is run. This section describes how this is done.

How to simulate a peak fractionation

The table below describes how to simulate a peak fractionation in the ***Evaluation*** module.

Step	Action
1	Choose ***Operations:Simulate Peak Fractionation***. *Result*: The ***Simulate Peak Fractionation*** dialog box opens.
2	Select the ***Source Chromatogram*** and the curve the simulated peak fractionation is to be generated for.
3	If necessary, select a ***Destination Curve*** and type a new ***Curve name***.
4	Type new values in the ***Parameters*** text boxes.
5	Click ***OK***. *Result*: The simulated peak fraction curve is displayed on the chromatogram.



12.2.4 How to create curves

Introduction

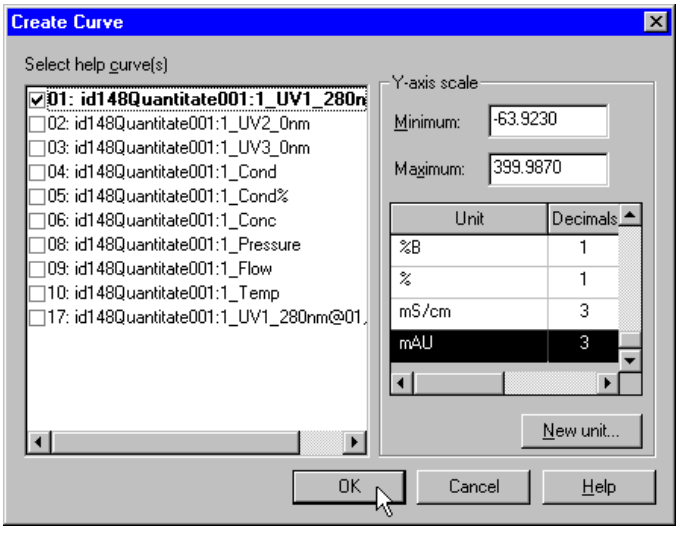
You can draw a curve of your own in the ***Evaluation*** module. This section describes how this is done.

Note: The right to create and rename curves is defined in the user access rights and may be restricted.

How to create curves - step 1

The table below describes how to set up a chromatogram window to create a curve in the ***Evaluation*** module.

Step	Action
1	Open a result file.
2	Select ***Operations:Create Curve***. *Result*: The ***Create Curve*** dialog box opens.
3	Select one or more ***Help curves***.



Step	Action
4	Select • minimum and maximum values for the Y-axis. • appropriate units from the ***Unit*** list. *Note*: The help curve determines the minimum and maximum values for the X-axis.
5	Click ***OK***. *Result*: The ***Create Curve*** chromatogram window opens.

How to create new units

In the ***Create Curve*** dialog box you can also create new units for the curve. The table below describes how this is done.

Step	Action
1	Click the ***New unit*** button. *Result*: The ***Create New Unit*** dialog box opens.
2	Type a new unit name and a number of decimal places.
3	Click ***OK***. *Result*: The ***Create New Unit*** dialog box is closed. The new unit is now available in the ***Create Curve*** dialog box.

How to create curves - step 2

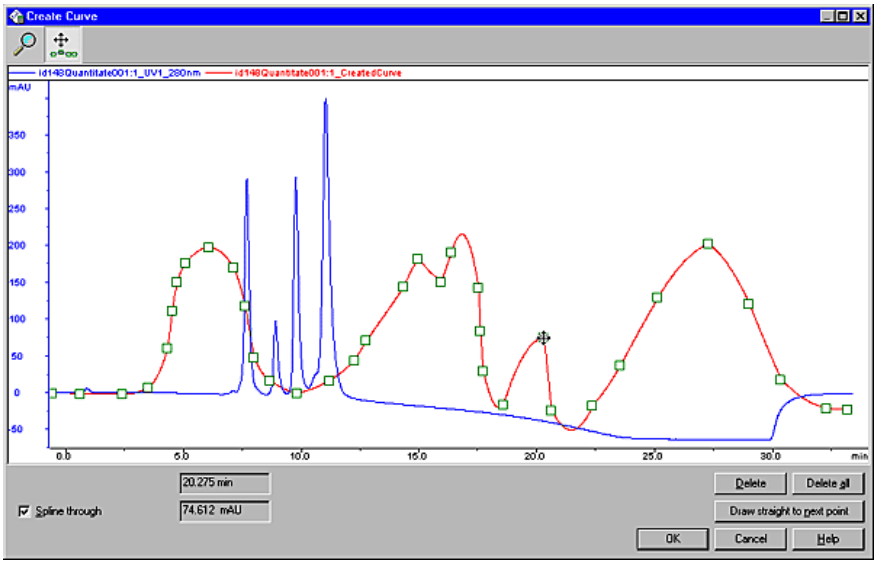
The new curve is created in the ***Create Curve*** window. The table below describes how to work in this window.

Step	Action
1	Click the ***Set Curve Points*** icon. 

Step	Action
2	• Click to insert curve points in the chromatogram. • Add more points to draw the curve. *Result*: A green square marks the new curve point. The curve is drawn from the previous point. Hold the cursor over the inserted point to see the coordinates displayed. **Curve mode** • The regular spline mode draws the curve as a smooth line near but not through every point. • Click the ***Spline through*** checkbox to draw the curve through all of the curve points.
3	**Move a point** • Select the point and drag it to the new position. *Result*: The curve is redrawn.
4	**Delete a curve point** • Double-click the curve point. *or* • Select the point and click the ***Delete*** button. *or* • Select the point, right-click and choose ***Delete Point*** from the shortcut menu.
5	• Click the ***Zoom*** icon to focus on details in the curve. *Note*: Right-click and select ***Reset zoom*** to return to the full view. • Right-click in the chromatogram window and select ***Marker***. • Position the ***Marker*** bar over peaks in the help curve to measure the coordinates. *Result*: The coordinates are displayed in the ***Marker*** text box in the top left corner of the chromatogram. *Note*: Click the ***Marker*** text box to display the coordinates for the created curve. Click again to return to the help curve coordinates.
6	Click ***OK***. *Result*: The ***Save Curve*** dialog box opens.
7	Type a new name if desired and click ***OK***.

Curve example

The illustration below is an example of a curve created by using the ***Draw Spline*** command in the ***Create Curve*** chromatogram window.



How to force the curve through points

In cases where you have created a curve and not selected the ***Spline through*** option, you may want the curve to pass through some of the points that are outside the created curve. The table below describes how to force the curve through these points:

Step	Action
1	• Select the curve point immediately before the curve point you want to connect to.
2	• Click the ***Draw straight to next point*** button. *Result*: The curve is adjusted so that it is drawn as a straight line between the two points.

12.2.5 How to use the Fraction Histogram

Introduction

The ***Fraction Histogram*** dialog box in the ***Evaluation*** module can be used to create a curve for the average fraction absorbance.

How to create a Fraction Histogram

The table below describes how to create a ***Fraction Histogram*** curve.

Step	Action
1	Select ***Operations:Fraction histogram***. *Result*: The ***Fraction histogram*** dialog box opens.
2	• Select the desired UV curve. *Note*: The fractions curve should already be selected on the middle list. If you have previous pooled fractions and created a pooled fraction curve, select the desired fraction curve.
3	• Click ***OK***. *Result*: The average fraction absorbance values are displayed as a new curve in the chromatogram.

12.3 Automated evaluation procedures

Introduction

An evaluation procedure is a recorded sequence of interactive operations in the ***Evaluation*** module, which can be executed for automated data evaluation and report generation. The concept is similar to the "macro" facilities in other programs. This section describes how to work with automated evaluation procedures.

In this section

This section contains these sub-sections.

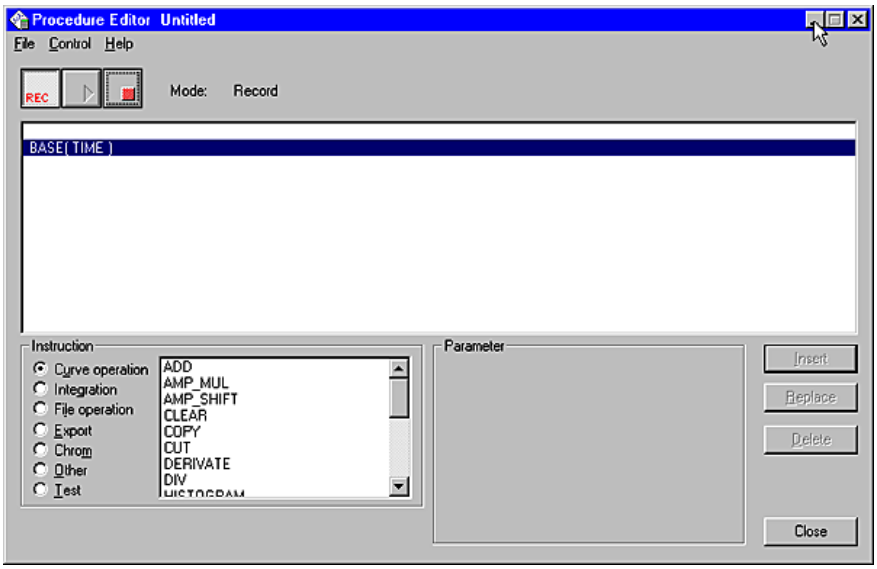
12.3.1 How to create a new procedure

Introduction

You can use the ***Procedure Editor*** to record or create a new procedure. The ***Procedure Editor*** can also be used to view and edit the instructions within a procedure. This section describes how to use the ***Procedure Editor*** to record new procedures.

The Procedure Editor dialog box

The illustration below shows the ***Procedure Editor*** in ***Record mode***.



How to record a procedure

The table below describes how to record a new procedure.

Step	Action
1	Open the result file in the ***Evaluation*** module.
2	Choose ***Procedures:Record On***. *Result*: The ***Procedure Editor*** dialog box opens in record mode.

Step	Action
3	Minimize the ***Procedure Editor*** dialog box.
4	Perform the evaluation steps that the procedure is to contain. *Result*: The steps are recorded in the order that they are performed.
5	**Stop the recording** • Choose ***Procedures:Record Off***. *or* • Restore the minimized ***Procedure Editor*** dialog box and click the ***Stop*** button. *or* • Restore the minimized ***Procedure Editor*** dialog box and select ***Control:End Record***.
6	Choose ***File:Save*** or ***File:Save As*** in the dialog box. *Result*: The ***Save As*** dialog box opens.
7	• Type a name for the new procedure in the ***Procedure name*** text box. • Select the ***Global procedure*** checkbox if desired (see further information below). • Click ***OK***. *Result*: The procedure is saved and available for future use.
8	Click the ***Close*** button to close the dialog box.

How to create a Global procedure

You can choose to save the new procedure as a ***Global procedure***. This makes the procedure available to all users. The procedure will have ***(Global)*** before the name to designate that it is available to all users.

You must have ***Edit global list(s)*** authorization to be able to save ***Global procedures***.

How to build a procedure with instructions

You can select instructions in the ***Procedure Editor*** dialog box to build a complete procedure step by step. The procedure instructions are described in *Section B.4 Procedure instructions, on page 607*. The table below describes how to create a new procedure with instructions.

Step	Action
1	Choose ***Procedures:Edit:New***. *Result*: The ***Procedure Editor*** opens in ***Edit mode***.
2	• Select an instruction from the ***Instruction*** list. • Type the appropriate parameters in the ***Parameter*** field. • Click ***Insert***.
3	Repeat step 2 until the procedure is complete.
4	Choose ***File:Save***.
5	Type a procedure name and click ***OK***.
6	Click the ***Close*** button in the ***Procedure Editor***.

12.3.2 How to edit a procedure

Introduction

Evaluation operations are represented by instructions in the ***Procedure Editor*** dialog box. The instructions can be modified to suit other specific evaluation needs and be saved for later use. This section describes how to use the ***Procedure Editor*** to edit a procedure.

How to edit a procedure

The table below describes how to edit an existing procedure:

Step	Action
1	Select ***Procedures:Edit:Open***. *Result*: The ***Open Procedure*** dialog box opens.
2	Select the procedure from the list and click ***OK***. *Result*: The ***Procedure Editor*** opens in ***Edit Mode***.
3	Select an instruction in the procedure window. *Result*: The instruction parameters are displayed in the ***Instruction*** and ***Parameter*** fields. A short definition of the selected instruction is displayed at the bottom left corner.
4	Type new values in the ***Parameter*** text boxes and click the ***Replace*** button. *Result*: The old parameters are replaced by the new parameters.
5	**Add a new instruction** • Select the instruction in the procedure immediately before where you want the new instruction. • Select a type and an instruction in the ***Instruction*** field. • Type parameter values in the ***Parameter*** field. • Click the ***Insert*** button. *Result*: The new instruction is inserted after the selected instruction.
6	**Remove an instruction** Select an instruction in the procedure and click the ***Delete*** button to remove the instruction from the procedure.
7	Choose ***File:Save*** and click the ***Close*** button to close the dialog box.

Descriptions of the procedure instructions

Appendix *Section B.4 Procedure instructions, on page 607* contains a list of procedure instructions with descriptions.

How to add instructions to a procedure when recording

If you start recording again you can add more instructions to a procedure that is already open in the ***Procedure Editor***:

- The new instructions will be added to the end of the present procedure.

or

- The new instructions will be inserted after the selected instruction if an instruction has been selected.

Invalid instructions

The procedure will stop and display an error message if an instruction calls for an invalid operation when the procedure is run. Any subsequent instructions in the procedure will not be executed.

Address the right curves

Curves are identified only by their storage position. An instruction can become invalid if it addresses the wrong curve:

Example

- The instruction ***ADD (01,02,03)*** will try to add curve 01 to curve 02 and store the result in position 03.
- A curve in position 03 that is not a raw data curve will be overwritten.
- A raw data curve in position 03 cannot be overwritten and the procedure will be stopped at that point.

Default values for classic baseline instructions

When a classic or morphological algorithm is used to calculate a baseline, UNICORN will suggest default values for the four control parameters based on the appearance of the curve. To instruct UNICORN to use default values appropriate for the curve every time the procedure is run, choose the default setting in the appropriate fields for the parameters.

Example

- `CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)`

Can be changed to:

- `CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)`

Global procedures

It is not advisable to edit existing global procedures. Open the global procedure instead and save a copy under a new name. Use this copy for editing purposes.

12.3.3 How to run a procedure

Introduction

You can run the saved procedures either for a specific chromatogram or as batch runs.

How to run a single procedure

The table below describes how to run a procedure for a specific chromatogram.

Step	Action
1	Open a result file.
2	Select ***Procedures:Run***. *Result*: The ***Run Procedure*** dialog box opens.
3	Select the procedure from the list and click ***OK***. *Result*: The procedure is executed.

Note: You can also open the procedure in the ***Procedure Editor*** dialog box and choose ***Control:Run*** or click the ***Play*** button.

Batch runs

It is possible to apply an evaluation procedure to a designated batch of result files if they are not open in the ***Evaluation*** module. An open file will not run and an error message will be displayed.

The batch run is performed in the background of the ***Evaluation*** module and the results of the run are not seen, with the exception of prints and documentation that are defined as steps in the procedure. For example, batch runs are useful

- to perform integration with the same parameter settings on many results,
- to print a number of results with the same settings.

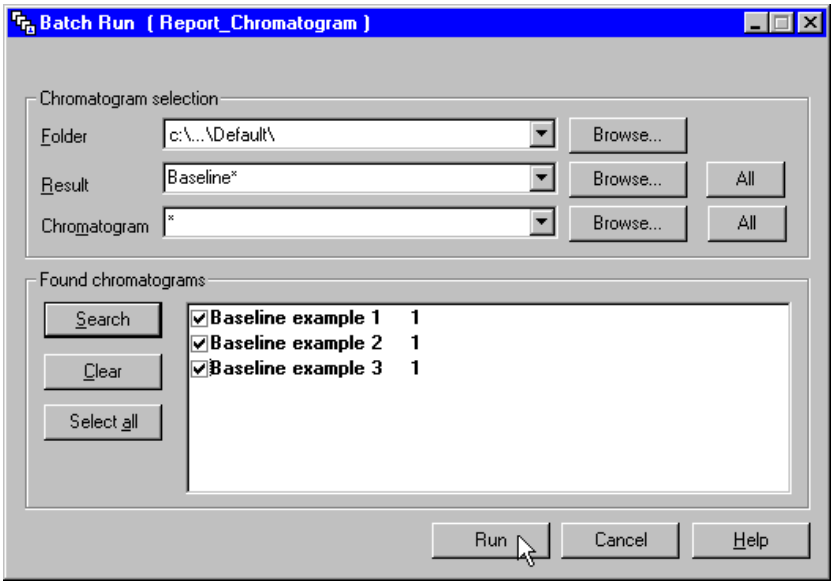
How to perform a batch run

The table below describes how to perform a batch run:

Step	Action
1	Choose ***Procedures: Batch run***. *Result*: The ***Open Procedure*** dialog box opens.
2	Select the procedure from the list and click ***OK***. *Result*: The ***Batch Run*** dialog box opens.
3	Use the ***Browse*** button to find and select the folder to search for result files and chromatograms. *Note*: The search will only be performed in the selected folder. You can use standard wildcard characters and define restricting search criteria for the ***Result*** and ***Chromatogram*** fields. Up to 10 user-defined search filters can be saved in the drop-menus.
4	Click the ***Search*** button. *Result*: A list of found chromatograms is displayed.
5	Select the chromatograms you want to perform the run on. • The ***Select All*** button selects all chromatograms. • The ***Clear*** button removes all chromatograms from the list.
6	Click the ***Run*** button. *Result*: The batch run is performed and any created curve or peak table will automatically be saved in each result file.

The Batch Run dialog box

The illustration below is an example of search results in the ***Batch Run*** dialog box:



How to batch-run reports

Evaluation procedures combined with batch runs can be a useful tool to produce printed documentation simultaneously for many result files, e.g. for a number of scouting runs. The table below describes how to create a procedure to batch-run reports.

Step	Action
1	Choose ***Procedures:Record On*** to record a procedure.
2	Choose ***File:Report***. *Result*: The ***Generate Report*** dialog box opens.
3	Choose a report format.
4	Click the ***Print*** button as the final instruction.
5	Choose ***Procedures:Record Off***.

Step	Action
6	Save the procedure. *Note*: A printing procedure can also be saved with a method to produce automatic prints at the end of a run.

Note: When for example a batch run is performed, the latest version of the procedure will be used. However, procedures that are saved with a method are not affected if the original procedure is edited at a later time.

How to add procedures to the menu

You can add up to 15 created evaluation procedures to the ***Procedures*** menu in the ***Evaluation*** module. The table below describes how to add procedures to the menu:

Step	Action
1	Select ***Procedures:Menu***. *Result*: The ***Edit Procedures Menu*** dialog box opens.
2	• Select the checkboxes of the procedures you want to display on the menu. • Click ***OK***. *Result*: The selected procedures are included on the ***Procedures*** menu.

Remove a procedure

Open the ***Edit Procedures Menu*** dialog box and select the checkbox again to de-select and remove a procedure from the menu.

12.3.4 How to rename and remove procedures

Introduction

The procedures that you have created can be renamed or removed from the list of available procedures. This section describes how this is done.

How to rename a procedure

The table below describes how to rename a procedure.

Step	Action
1	Choose ***Procedures:Edit:Rename***. *Result*: The ***Rename Procedure*** dialog box opens.
2	Select a procedure. *Result*: The procedure name is displayed in the ***New name*** text box.
3	Type the new name.
4	Click OK. *Result*: The procedure name is changed.

How to delete a procedure

The table below describes how to delete a procedure.

Step	Action
1	Choose ***Procedures:Edit:Delete***. *Result*: The ***Delete Procedure(s)*** dialog box opens.
2	Select a procedure.
3	• Click ***OK***. • Click the ***Yes*** button to confirm. *Result*: The procedure is deleted.

Global procedures

It is not advisable to edit existing global procedures. Open the global procedure instead and save a copy under a new name. Use this copy for editing purposes.

13 The Analysis module

Introduction

This chapter describes how to use the ***Analysis*** module. This module is an optional feature that must be ordered separately and installed after the regular UNICORN installation.

The ***Analysis*** module is accessed in the ***Evaluation*** module. The ***Analysis*** module uses functions in the ***Evaluation*** module that are presented in the previous chapters. It is recommended that you are familiar with the contents of those chapters before you begin with this chapter.

In this chapter

This chapter contains these sections.

13.1 General information about the module

Introduction

This section is an overview of the ***Analysis*** module including:

- Definitions of terminology that will be used in this chapter.
- A description of how to install the ***Analysis*** module.
- A description of the new procedure instructions that become available when the ***Analysis*** module is installed.

Module functions

The ***Analysis*** module is an optional extra module that adds functionality to the regular UNICORN ***Evaluation*** module. Basically the ***Analysis*** module is used:

- to determine the absolute quantity or concentration of a component.
- to determine the molecular size of a component.

Module menus

The ***Analysis*** module is accessed in the ***Evaluation*** module. After the installation, two new ***Evaluation*** module menus are added:

- ***Quantitate***.
- ***Mol.Size***.

Note: The menus are only available when a result file is open in the ***Evaluation*** module.

Quantitate

The ***Quantitate*** function provides a wide range of techniques for quantitative analysis:

- External standard quantitation
- Internal standard quantitation
- Standard addition
- Recovery calculations

Quantitate uses peak data from standard runs to produce calibration curves which can then be used to evaluate the amount and concentration of components in a sample.

Molecular Size

The ***Molecular Size*** (***Mol.Size***) function determines the molecular size of components in a sample. The function uses a molecular size curve prepared from one or more standards.

Term definitions

The table below lists definitions for some terminology that is used in this chapter.

Term	Definition
Amount	This specifically refers to the injected amount. In most cases, the word "amount" is used as an abbreviation for "concentration or amount". Both concentration and injected amount can be used to produce the calibration curve. When analyzing the sample, both amount and concentration are calculated.
Calibration curve	The relationship between amount and peak size of a component. The relationship can be shown as a curve and as a mathematical expression.
Level	A known amount or concentration of a standard. The levels are numbered 1-20 in decreasing or increasing order of concentration.
Molecular size curve	The relationship between molecular size and retention volume for a number of components. The relationship can be shown as a curve and as a mathematical expression.
Molecular size table	All necessary data required to determine the molecular size of one or several components in a sample. The molecular size table contains the molecular size curve.
Peak size	Used generally as a common term for "peak area or peak height".
Peak table	The result of a peak integration presented in tabular form. The peak table can include, for example, height, area and retention volume. After the analysis, the peak table contains the values for concentration, amount (and molecular size).
Quantitation table	All necessary data required to quantitate one or several components in a sample. The quantitation table contains calibration curve(s) and peak identification settings.
Sample	A sample with an unknown concentration of the component(s) of interest. The concentration is determined by ***Quantitation***. For molecular size calculations, the sample contains a component or several components of unknown molecular size.

Term	Definition
Sample run	A chromatographic sample run of a sample to be analyzed.
Spiking	The addition of a known quantity of the component of interest to the sample prior to the sample preparation for the run.
Standard	A defined concentration of one or several components. The concentration does not have to be the same for all components in the standard. One or several standards are used to produce a calibration curve. For molecular size calculations, the standard contains components of known molecular size.
Standard run	A chromatographic standard run of a specific concentration level of a standard.

How to install the Analysis module

The table below describes how to install the ***Analysis*** module.

Step	Action
1	Close all other applications.
2	Insert the installation CD in the CD drive.
3	Open ***My Computer***.
4	Double-click the CD drive icon. *Result*: The file window opens.
5	Double-click ***Setup.exe***.
6	Follow the instructions on the screen.
7	Remove the CD after the installation is complete.

Note: See the license agreement for information on the legal aspects of the installation.

The Analysis module in a network

One or several computers in a network may have the Analysis module installed. The module does not need to be installed on all network computers that run UNICORN. All installations must be made in accordance with the license agreement.

13.2 Quantitation overview

Introduction

Quantitation is used to determine the amount or concentration of components in a sample. This section is an overview over quantitation in general and the four quantitation techniques that the ***Analysis*** module provides. The section also contains information about the reliability of quantitation.

In this section

This section contains these sub-sections.

13.2.1 General information about quantitation

Introduction

This section is a brief presentation of the quantitation techniques that the ***Analysis*** module provides. The section also contains an outline of the steps in a quantitation and the procedure instructions for quantitation that are added when the ***Analysis*** module is installed.

About quantitation

Most quantitation techniques use peak integration data from standards to produce calibration curves. These curves show the relationship between the amount of the components of interest and the peak sizes at different concentration levels of the standard. The relationship can be linear, quadratic or point-to-point. Quantitation is usually based on a number of test runs using a standard at several concentration levels.

The amount and concentration of the component(s) of interest in the sample are then determined from the peak size of the component using the calibration curve.

Note: Quantitation should only be performed on chromatograms that have been integrated and saved. Time is the recommended base unit for quantitation and it must be used for all integrations.

Quantitation steps

The table below describes the general steps in quantitation. The steps are described in detail in the sections about the different quantitation techniques.

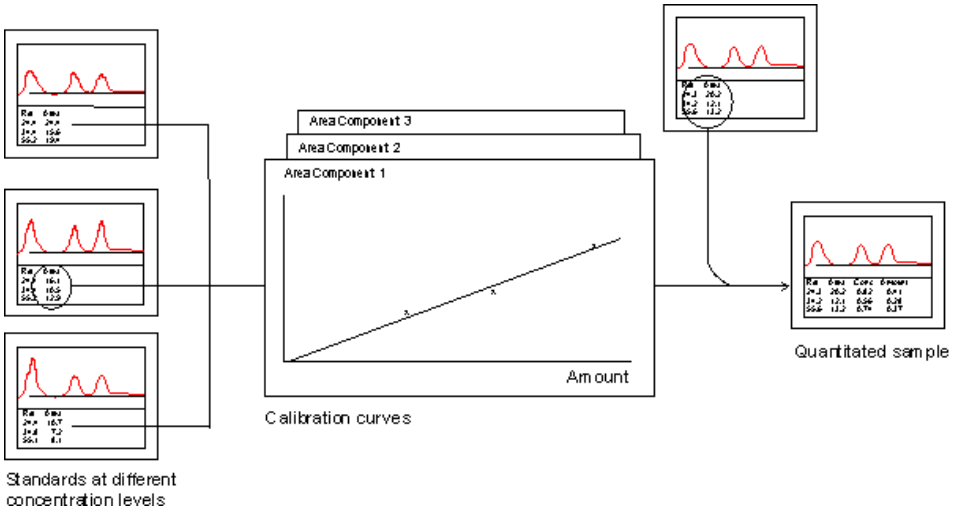
Step	Action
1	Run the different concentration levels of the standard.
2	• Integrate the curves to produce peak tables. • Check the integration.
3	Identify the components for which calibration curves will be produced.
4	Enter the known concentrations for the different standards to produce a calibration curve for each selected component.
5	Run the sample and integrate the curve.

Step	Action
6	Let the program calculate the concentration and amount of the components of interest in the sample.

Note: The steps above do not apply to ***Standard addition***. See "Standard addition quantitation" below.

Illustration of the work flow

The quantitation work flow is illustrated below.



The four quantitation techniques

The Analysis module provides four different quantitation techniques:

- External standard quantitation
- Internal standard quantitation
- Standard addition quantitation
- Recovery calculation

Each technique is described below.

External standard quantitation

One or several component(s) of interest are run to produce a calibration curve. The amount and concentration of the component in the sample is then determined from the calibration curve. This technique is fairly simple and usually produces accurate results.

Internal standard quantitation

Peak areas of the components of interest are related to the peak area of an internal standard added in a fixed amount to each concentration level of the standard and to the sample. This technique reduces errors that are caused by changes occurring between the separation runs and is therefore the technique that can produce the highest precision if a suitable internal standard can be selected.

Standard addition quantitation

The sample is spiked with a known amount of the component of interest. The areas of the spiked and unspiked sample are then compared and the amount in the unspiked sample is determined. No calibration curves from standards are used. Only one component can be quantitated. Compared to other techniques, results can be obtained more quickly when you are performing a small number of sample runs with standard addition. However, the precision is limited.

Recovery calculation

Recovery is used to determine the losses that can occur during the sample preparation process. The sample is spiked with a known amount of the component of interest. The amount in the spiked sample is then determined from a calibration curve and is compared with the amount in an unspiked sample. The recovery can only be determined for one component each time.

Analysis procedure instructions

The table below describes the new procedure instructions for quantitation that become available when the ***Analysis*** module is installed.

Instruction	Description
`QUANTITATE`	The instruction calculates the concentration and amounts in the sample from a quantitation table. Amount and concentration columns will be added to the peak table.

Instruction	Description
UPDATE	The instruction updates a quantitation table with new data from one standard concentration level. The default ***Limit (+/-)*** value of 12.5% will be used. The quantitation table will not be updated if the peak area or peak height of the new and the previous results differ more than the ***Limit*** value. *Note*: Either peak area or height is selected for the ***Limit*** value.

Default values

The DEFAULT value for the injection value will be taken from the injection volume reported by the ***Autosampler A-900*** from the method. DEFAULT can only be used when the injection is performed by the autosampler.

The DEFAULT value for the concentration level for the standard will be taken from the level entered in the ***QuantitationData*** instruction in the method.

13.2.2 External standard quantitation

General information

External standard quantitation is based on the use of a standard prepared in a number of concentration levels. A run is performed for each concentration level and calibration curves are produced to show the relationship between amount and peak size for each component. The calibration curves are used to quantitate the components in the sample.

Note: The standard should contain known amounts of all the components that are to be quantitated in the sample.

How to improve quantitation

External standard quantitation can be based on the use of a single standard concentration level, but the calibration curve is then limited to a linear through-the-origin relationship. The use of a number of different concentration levels of the standard broadens the range of the calibration curve. It also allows the development of non-linear calibration curves and improves precision. Multiple runs at each level improve precision further.

The description in this section is based on the use of a standard

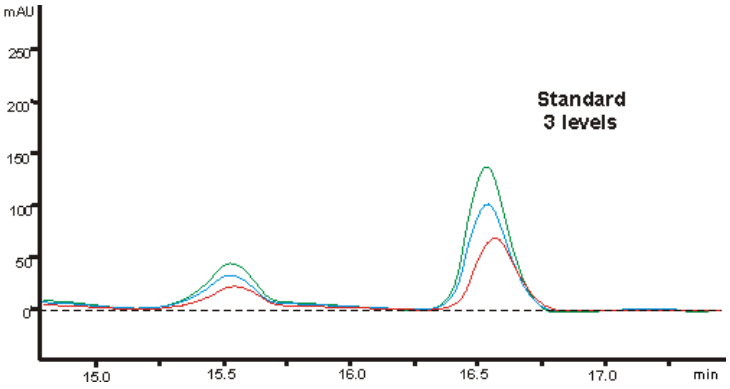
- that contains two components,
- which is run at three different concentration levels.

How to perform External standard quantitation

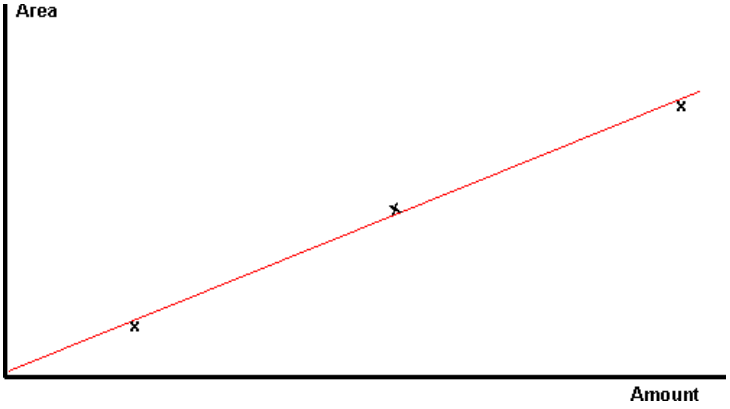
The table below describes briefly how ***External standard quantitation*** is performed (based on the use of a standard which contains two components and which is run at three different concentration levels).

Step	Action
1	Perform a run for each standard level.

Step	Action
2	Integrate the curves to produce a peak table for each run.



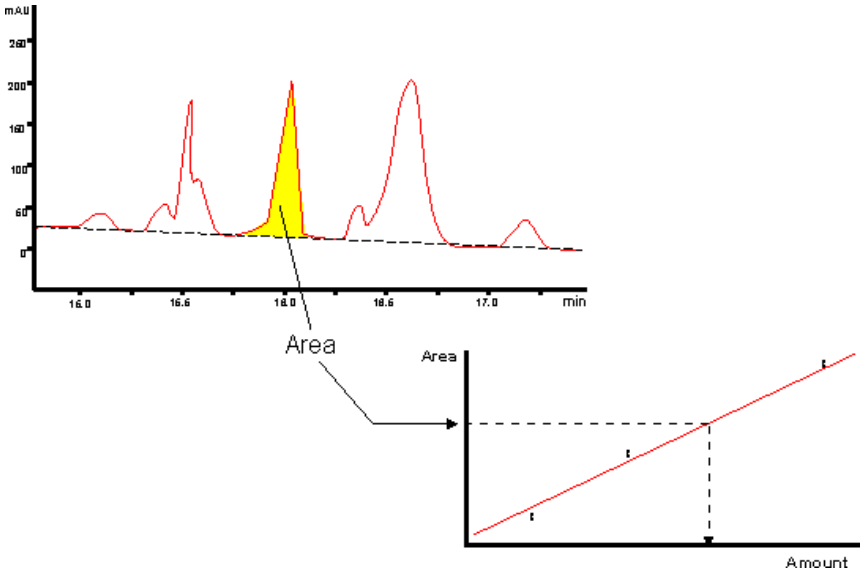
3	Use the peak tables from the standard runs to produce a calibration curve for each component. This curve shows the relationship between amount and peak size. Below is a calibration curve for one of the components:



4	Perform a run with the sample and peak integrate the curve.
5	Identify the components of interest by the peak identification settings from the sample peak table. Use the peak size(s) to calculate the concentration and amount from the calibration curve(s).

Illustration - how to use the calibration curve

The illustration below describes how the calibration curve is used to determine the amount based on the sample peak area.



Reliability

External standard quantitation normally produces accurate results and is fairly simple. The following reliability factors are specific to the technique.

- Precision is limited by changes that may take place between the runs, for example column degradation and mobile phase variations.
- There is no compensation for losses of sample during the sample preparation process prior to analysis.

13.2.3 Internal standard quantitation

General information

Internal standard quantitation uses peak tables prepared from the standard, similar to the ***External standard quantitation***. However, a fixed quantity of an additional component is added to every separation run, including the sample. The peak sizes of the standards and the sample are then related to the peak size of the internal standards to compensate for any changes that may have occurred between the runs.

General assumption

The internal standard technique relies on the assumption that any changes in the injected amount of the component(s) of interest, e.g. due to sample preparation losses, correspond to equal changes in the injected amount of the internal standard component.

Advantages

Internal standard quantitation reduces errors that are caused by changes in the system between successive runs with the sample and the standard concentration levels. For example, there may be unpredictable losses during the sample preparation procedure or unintentional changes in the amounts that are injected.

What is a suitable internal standard?

A suitable internal standard must meet the following conditions:

- It must be well separated from the components in the sample (not just from the components of interest).
- It must *not* be present naturally in the sample(s).
- It must have similar chemical properties to the component(s) of interest.

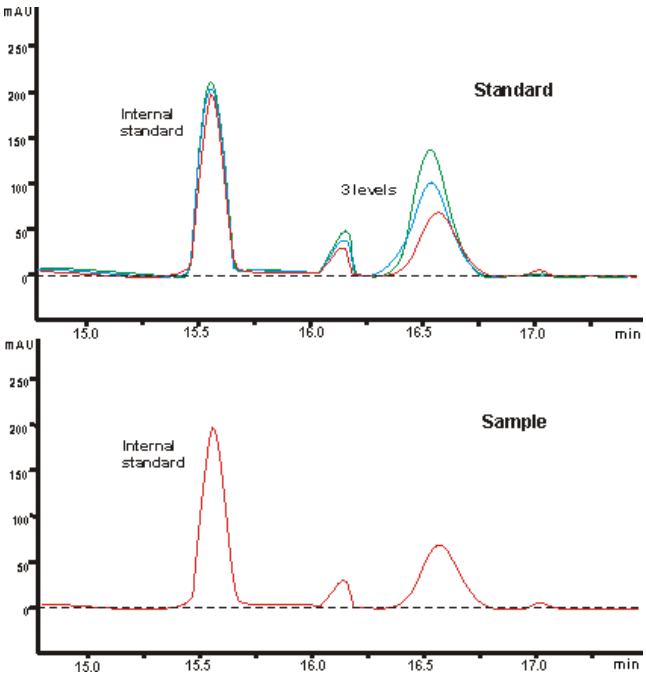
To be able to compensate for losses during the sample preparation, all the standard concentration levels must be subjected to the same sample preparation procedure as the samples.

Note: If there are several components of interest, they must all be chemically similar.

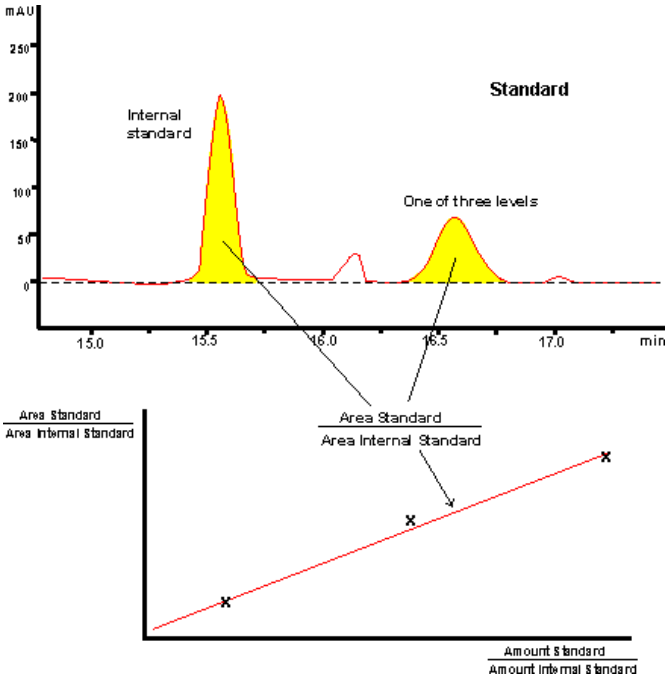
How to perform Internal standard quantitation

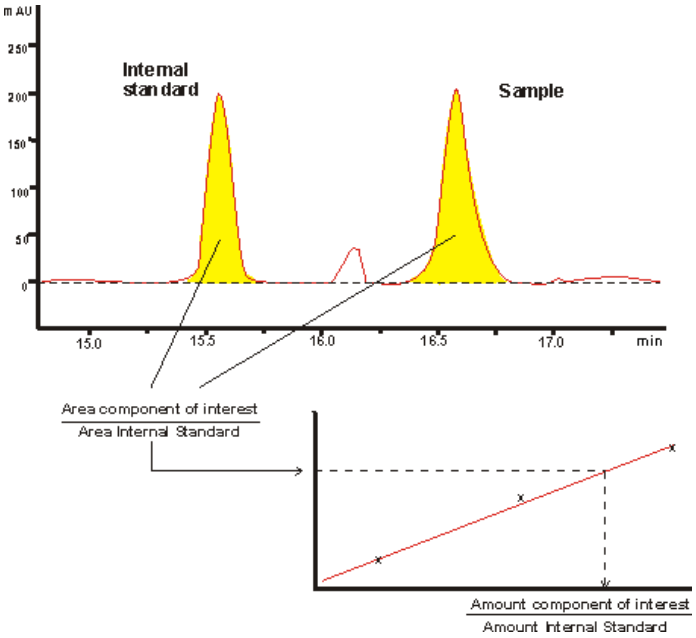
The table below describes briefly how ***Internal standard quantitation*** is performed.

Step	Action
1	Prepare a series of concentration levels from the standard.
2	Add an additional component, the internal standard, in the same concentration to all the standards and to the sample.
3	Perform a run for each standard and the sample.
4	Integrate the curves to produce a peak table for all standard runs and for the sample. *Result*: Each curve contains a peak from the internal standard. Changes in the size of the internal standard peak indicate changes in the system. See illustration below:



Step	Action
5	• Plot all peak sizes relative to the size of the internal standard peak to produce a calibration curve for each component. • The standard peak area, relative to the internal standard peak area, is used to produce a point on the calibration curve. See illustration below:



Step	Action
6	• Prepare data from the sample in the same way as the data from the standard runs to produce peak sizes relative to the internal standard peak size. • The resulting relative value is applied to the calibration curve to determine the amount and concentration of the component of interest. See illustration below: 

Reliability

Internal standard quantitation is potentially the most reliable of the quantitation techniques. However, if the internal standard component is not selected carefully, the reliability will probably be worse than with the external standard technique. There are some specific factors that can affect the reliability:

- There is an increased risk of overlap when the extra component (the internal standard) is added if the sample contains many peaks.
- The addition of the internal standard must be accurate in both the standards and samples, otherwise, the precision of the quantitation will be reduced dramatically.

13.2.4 Standard addition quantitation

General information

Standard addition quantitation is a simple way to obtain measurements of amount in your sample (concentration is not calculated). It requires only a first sample run and a second sample run which has been spiked with a known amount of the component of interest. The technique is straight-forward and relatively fast when you are running only a few samples. Standard addition can be useful when you want to use the internal standard technique but do not have a suitable internal standard.

Disadvantages

The disadvantages of ***Standard addition quantitation*** are

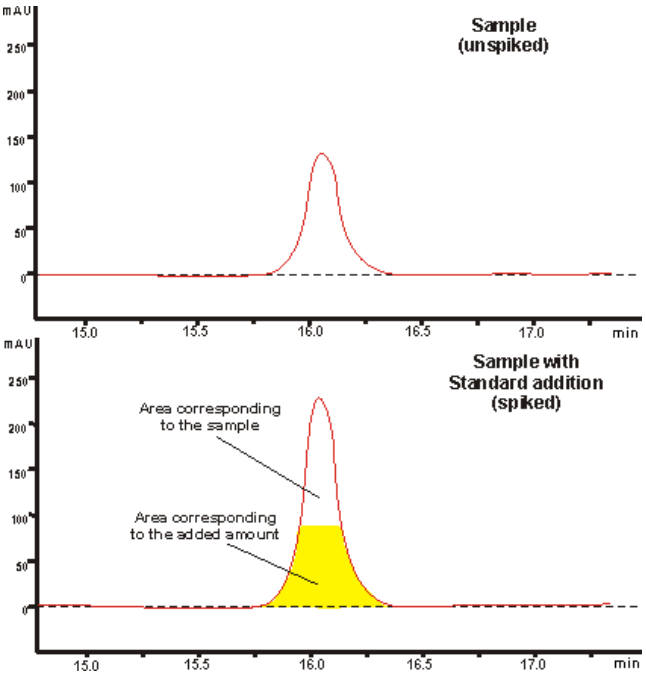
- its limited precision compared to the external and internal standard techniques
- its lack of ability to measure more than one component.

How to perform Standard addition quantitation

The table below describes briefly how ***Standard addition quantitation*** is performed.

Step	Action
1	Perform a sample run.

Step	Action
2	Perform a second run with a sample that has been spiked with a known quantity of the component of interest prior to the sample preparation. See illustration below:



3	Perform peak integration on both curves in the ***Evaluation*** module to produce a peak table for both the spiked and the unspiked sample. *Result*: The difference in peak area between the spiked and the unspiked sample represents the peak area from the added amount.
4	With the assumption of a linear proportionality between the peak area and amount, and with the added amount known, the software calculates the amount of the component of interest in the sample: $\text{Unspiked sample amount} = \text{Amount added} \times \frac{\text{Peak area from unspiked sample}}{(\text{Peak area spiked sample} - \text{Peak area unspiked sample})}$

Reliability

Standard addition is the least precise of the quantitation techniques since it is restricted to a single concentration level and the amount in the sample is calculated by extrapolation. Below are factors that determine if standard addition can be used with reliable results:

- The component of interest must be completely resolved from all other components in the chromatogram. Overlapping peaks will produce unreliable results.
- The peak integration parameters (baseline settings) must be correctly selected. The default settings will be satisfactory in many cases, but the integration results have to be checked for all chromatograms.
- The standard addition technique assumes a linear through-the-origin relationship between the amount of component and peak size. This is a good approximation for small quantities under normal conditions.
- Standard addition has no way of compensating for changes that are made between the runs. However, if losses during sample preparation are constant between the two runs, they may be accounted for by spiking the sample prior to the sample preparation.
- A spike amount which is of the same order of magnitude as the sample must be used to maximize precision.
- All the runs must be performed consecutively to reduce systematic errors and thereby maximize precision.

13.2.5 Recovery calculation

General information

Recovery calculation is used to determine losses that can occur during the sample preparation process. Recovery can also be used to determine the recovery factor of a preparative purification or a chromatographic process. The recovery factor can only be determined for a single component.

A calibration curve is produced using a concentration series of an external standard. The calibration range must cover the amount in both the sample and the spiked sample. Two runs are performed, one with the sample and a second with the sample that was spiked prior to the sample preparation with a known amount of the component of interest. Quantitation of the data from the two sample runs allows the recovery factor of the sample preparation to be calculated.

Note: The recovery is measured as the recovery for the sample preparation, not for the separation during the chromatographic analysis.

The recovery factor

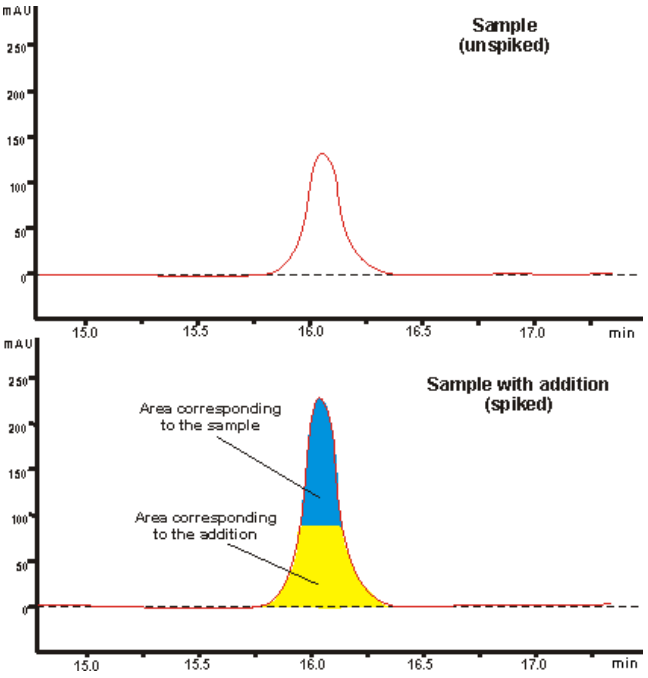
The recovery factor can be used to manually compensate for losses during sample preparation. The apparent amount in a sample is divided by the recovery factor to obtain the corrected amount.

How to perform Recovery calculation

The table below describes briefly how ***Recovery calculation*** is performed.

Step	Action
1	Perform a run with each level of the standard.
2	Peak integrate the curves to produce a peak table for each level.
3	Use the data from the peak tables to produce a calibration curve. *Note*: This is the same process that is used in the ***External standard quantitation***.
4	Spike a portion of the sample with a known amount of the component of interest prior to the sample preparation.
5	Run both the spiked and an unspiked sample.

Step	Action
6	Peak integrate both samples to produce peak tables for the unspiked sample and the spiked sample.
7	The amounts for unspiked and spiked sample are calculated from the calibration curve. The difference between these amounts provides the apparent amount of the addition. See illustration below:



8	The ratio of this apparent amount compared to the amount actually added to the sample determines the recovery of the system. $\text{Recovery factor} = \frac{\text{Apparent amount added*}}{\text{Actual amount added}}$ *Apparent amount added = Amount of spiked sample - Amount of unspiked sample *Example*: If 2 mg of the component of interest had been added to the sample and quantitation indicated an apparent amount added of 1.6 mg, the recovery factor would then be 0.8.

Reliability

Below are some specific factors that determine if the recovery factor result is reliable:

- A spike amount that is of the same order of magnitude as the sample must be used to maximize the precision.
- It is assumed that the recovery is the same for both the sample and the spiked sample. However, if the recovery varies according to the amount of the component of interest, the results are unreliable.

13.2.6 General reliability factors for the quantitation techniques

Reliability factors

The following factors are valid for all quantitation techniques, except for ***Standard addition***:

- Quantitation requires that the components of interest are completely resolved from all other components in the chromatogram. Overlapping peaks will produce unreliable results.
- The peak integration parameters (baseline settings) must be correctly selected. The default settings will be satisfactory in many cases, but the integration results have to be checked for all chromatograms.
- All integrations must be performed using the same X-axis base unit. For highest reliability, time is the recommended unit.
- The concentration levels of the standard have to be accurately prepared. Errors in the amount or concentration values will lead to unpredictable results.
- Self-imposed limitations, such as the use of a small number of concentration levels of the standard, also limits precision.
- Precision is improved by the appropriate choice of the concentration range of the standard. The range should extend across the presumed amount in the sample.
- Use of the most appropriate curve model will maximize precision.
- Accuracy is improved if several runs are performed at each level.
- All the runs should be performed consecutively to reduce systematic errors and thereby maximize precision.

Further information

Refer to statistical reference books for more detailed information about quantitative analysis. An example is "Statistics for Analytical Chemistry", 3rd Edition 1993, J.C. Miller and J.N. MIller, Ellis Horwood PTR Prentice Hall.

13.3 How to prepare for quantitation

Introduction

This section describes how to use peak data from standards to prepare quantitation tables and calibration curves for use with ***External standard***, ***Internal standard*** and ***Recovery*** quantitation.

In this section

This section contains these sub-sections.

13.3.1 Preparations before quantitation

Description

The table below describes the preparations before the quantitation.

Step	Action
1	Create a method to be used for all the standard runs. The method and the injection volume must be the same for all the runs.
2	Perform at least one run for each concentration level of the standard.
3	Peak integrate the curves to produce a peak table for each of the standard curves. *Note*: When integrated, all standards must use the same X-axis base unit. Time is the recommended unit for the highest reliability.
4	Check that each integration is correct and consistent.
5	Select ***File:Save*** to save all the peak tables.

Concentration levels

The standard series should include standard concentrations that extend beyond the lower and upper limits of the sample amount. If an internal standard is used, the internal standard must be added in the same concentration in all standards.

Methods created from a wizard

If the method is created from a wizard for ÄKTAdesign systems, you may select the correct standard concentration level in the variable ***Quantitation_Type***. You can also set the level after the run has been performed. Each level is an alias for a specific concentration of the standard.

The list below describes how the levels are applied:

- Level 1 should be selected for the standard with the highest or lowest concentration.
- The levels must be set in consecutive order of changing concentration of the standard.
- All runs with the same concentration must be given the same level.

Reject irrelevant peaks

If many small irrelevant peaks are detected, it may be an advantage to re-integrate after adjusting the ***Reject peaks*** criteria. The number of largest peaks to detect has a default value of 20 and it may be helpful if this is set to a smaller value.

13.3.2 How to create a quantitation table

Introduction

The quantitation table contains all the necessary data, such as the calibration curves, that are needed to quantitate one or several components in a sample. This section describes how the quantitation table is created.

How quantitation tables are created

Quantitation tables are created in the same way for both external standard quantitation and for recovery calculations. They both use absolute values of standard peak data.

For quantitation with internal standard, the peak sizes relative to the size of the internal standard peak are used to create a calibration curve.

Four process steps

The creation of the quantitation table can be divided into four steps:

1 Standard data input

2 Component selection and definition

3 Peak identification

4 Calibration curve and quantitation table creation

Step 1 - How to input the standard data

The table below describes how to input the standard data in the ***Evaluation*** module.

Step	Action
1	Select ***Quantitate:Edit Quantitation Table:New*** on the menu bar. *Result*: The ***New Quantitation Table*** dialog box opens with the name of the active chromatogram displayed in the ***Source chromatogram*** field.

Step	Action
2	• Double-click a result file in the ***Select peak table*** list if you want to select a source chromatogram from another result file. If desired, the standard can be expressed in ***Concentration*** instead of in ***Amount***. • Click the ***Concentration*** checkbox and edit the injection volume in the ***Inj. volume*** field. *Note*: The software will always calculate both amount and concentration for the sample. • Highlight the standard peak table of level 1 on the ***Peak table(s)*** list and click the ***Select*** button. *Note*: This should be the table for the highest or lowest concentration of the standard. *Result*: The peak table is added to the ***Level/Peak table(s)*** list.
3	• The level is automatically copied onto the list if it already was set in the method. If so, continue with step 4. • If a level has *not* been set, the ***Select Level*** dialog box opens. Select 1 on the ***Level*** menu and click ***OK***.
4	• Click another result file in the ***Results*** field and select the new source chromatogram. *Result*: The peak tables associated with this chromatogram are displayed on the ***Peak table(s)*** list.
5	• Repeat steps 3 and 4 until all the standard peak tables have been selected. *Note*: Increase the level number for each new standard concentration in consecutive order of decreasing or increasing concentration. • Click the ***Current*** button at any time to return to the chromatogram that was active before you activated ***Quantitate***. • Highlight unwanted tables on the list and click ***Remove***. • Click ***OK*** to finish the selection. *Result*: The ***Define Component(s)*** dialog box opens. Continue to "Step 2, How to select and define components" below this table.

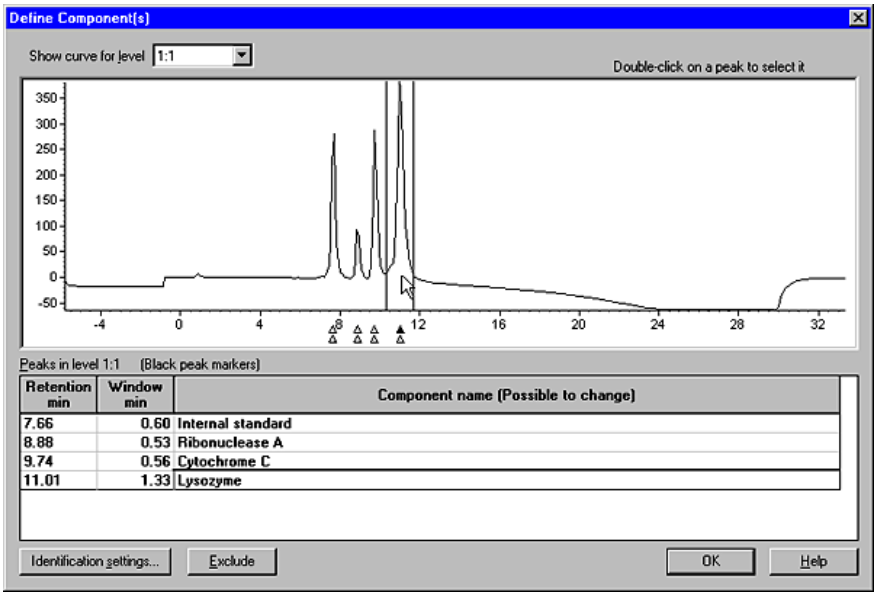
Standard concentration levels

It is useful to think of each level as an alias for a specific concentration of the standard. You can incorporate up to 10 peak tables at each level, prepared from runs repeated at the same concentration. ***Quantitate*** will later allocate each with an incrementing suffix, e.g. 1:1, 1:2 etc.

The Define Component(s) dialog box

The components that will be used to produce the calibration curves are selected in the ***Define Component(s)*** dialog box. ***Quantitate*** must be able to identify these components on all levels. This dialog box is used to set the criteria by which peaks are identified.

The illustration below shows the ***Define Component(s)*** dialog box.



Examine the components

The ***Define Component(s)*** dialog box initially displays the components from level 1:1, that is the peak table from the highest or lowest concentration of the standard. The ***Show curve for level*** list is used to examine the curve for each standard run. The size of the components are reduced or increased progressively as you select levels further down on the list, which reflects the decreasing or increasing concentration of the standard.

If an internal standard has been incorporated, its peak remains about the same size on each level.

Peaks detected during the peak integration

Each component peak that was detected during the peak integration, i.e. that is present in the peak table, is identified by a lower triangle (black in level 1:1, green in other levels). There may be different peaks detected for different levels. Upper triangles will later identify the peaks that are selected for quantitation.

Step 2 - How to select and define components

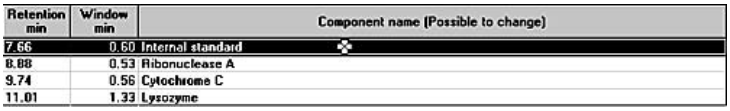
The table below describes how to select and define the components.

Step	Action
1	Select level 1:1 in the ***Show curve for level*** list and click a peak. *Result*: The peak is highlighted in the table.
2	• Double-click the peak. *or* • Click the ***Include*** button. *Result*: The peak is selected for quantitation, marked with an upper triangle and "***component no.***" is listed as the ***Component name***. The selected peak is affected on all levels. *Note*: More than one peak can be selected to produce calibration curves for several components.
3	Highlight the component name and type a new name.
4	Double-click the internal standard peak (if applicable) and type a new name.
5	Continue to "Step 3, How to identify the peaks" below this table.

The Define component(s) peak table columns

The peak table within the ***Define Component(s)*** dialog box has three columns:

- The (absolute) ***Retention*** value of the component in level 1:1.
- The width of each component's window. If you change the width of the window by adjusting the cursor lines, this is reflected in the ***Window*** column.
- The ***Component name***, with the currently selected component highlighted.



Retention min	Window min	Component name (Possible to change)
7.66	0.60	Internal standard
8.88	0.53	Ribonuclease A
9.74	0.56	Cytochrome C
11.01	1.33	Lysozyme

Step 3 - How to identify the peaks

Description

When a component is selected, vertical cursor lines show the current identification window. The software uses this window to search for peaks on other levels and in the sample runs. A peak found in the window is assumed to be the component of interest. You can change the limits by dragging a limit cursor line. Both cursor lines move symmetrically so that the limits center on the component peak.

The window should be set wide enough to include peaks on the other levels despite minor variations in retention volumes. However, the window should also be narrow enough to exclude unwanted peaks that will interfere with the quantitation.

Instruction

The table below describes how to adjust the window width for the best results.

Step	Action
1	Drag the cursor lines to set the window to a suitable width.
2	• Use the ***Show curve for level*** menu to display all levels and check that the width is suitable (the window width is the same on all levels). • Click the lower green or black triangle to display the actual retention for a peak.
3	Repeat steps 1 and 2 for all selected peaks. *Note*: Overlapping windows are not allowed.

Step	Action
4	If necessary, click the ***Identification settings*** button to edit the settings. See "How to adjust the identification settings" below this table.
5	• Click the ***OK*** button to accept the default identification settings. *Result*: The ***Quantitation table*** dialog box opens.
6	Continue to "Step 4, How to create a calibration curve and a quantitation table" below this table.

Identification settings

The criteria by which peaks are identified are set in the ***Identification Settings*** dialog box. The criteria are valid for all the selected peaks in the ***Define Component(s)*** dialog box. These settings also affect the information provided in the peak table in the dialog box.

How to adjust the identification settings

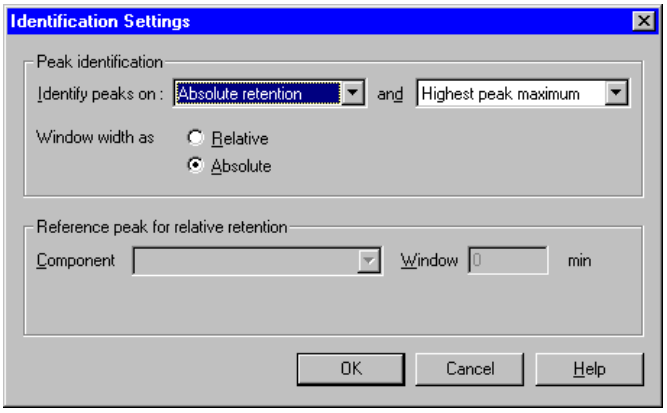
Description

By default, peaks are identified by their absolute retention values and the highest peak maximum within the window. In most cases, it is not necessary to change these default settings. Peak identification by absolute retention works well when there has been little or no drift in retention between successive runs of the standard. Quantitate will find corresponding peaks in these successive runs providing any drift in retention does not move a peak outside the peak window.

Instruction

If you have drifting retention that makes peak identification difficult you can choose to identify peaks according to their position relative to a reference peak. The table below describes how to adjust the identification settings in the ***Define Component(s)*** dialog box.

Step	Action
1	Identify a component peak that can be used as the reference. *Note*: Choose a peak that is well separated from any other peaks. This enables the window to be set relatively wide and the system can accommodate a larger drift in retention value.

Step	Action
2	Click ***Identification Settings***. *Result*: The ***Identification Settings*** dialog box opens.  See "How to identify peaks within a window" below.
3	Select ***Relative retention*** on the ***Identify peaks on*** droplist. (See "Absolute and Relative window width" below)
4	Scroll down the ***Component*** menu and select the component to be used as the reference peak.
5	• Type the window width for the reference peak (an absolute value). *Note*: Set the width fairly wide to accommodate a larger drift in the retention value. Make sure that there are no other large peaks within the window. • Click ***OK***. *Result*: A column for the relative retention is added in the peak table, Ret/Ref. The column displays the value of each component relative to the retention value of the reference component. This reference component is marked ***Ref.*** in the ***Window%*** column. The ***Window%*** column shows the window width for each peak expressed as a percentage of its relative retention value.

How to identify peaks within a window

Quantitate must be advised of how the peaks are to be identified if any of the windows includes more than one peak. The second droplist in the ***Peak identification*** field of the ***Identification Settings*** dialog box offers the following options:

- ***Highest peak maximum*** (default).
- ***Closest to retention***, i.e. closest to the center of the window (see the retention column in the peak table.)
- ***Maximum peak area***.

Examine the nature of the peaks enclosed by the window and select the option that differs between the wanted and the unwanted peaks. Use ***Closest to retention*** if there are large peaks from components that are not going to be quantitated.

Note: The selection applies to all peaks, even the internal standard and reference if used.

Absolute and Relative window width

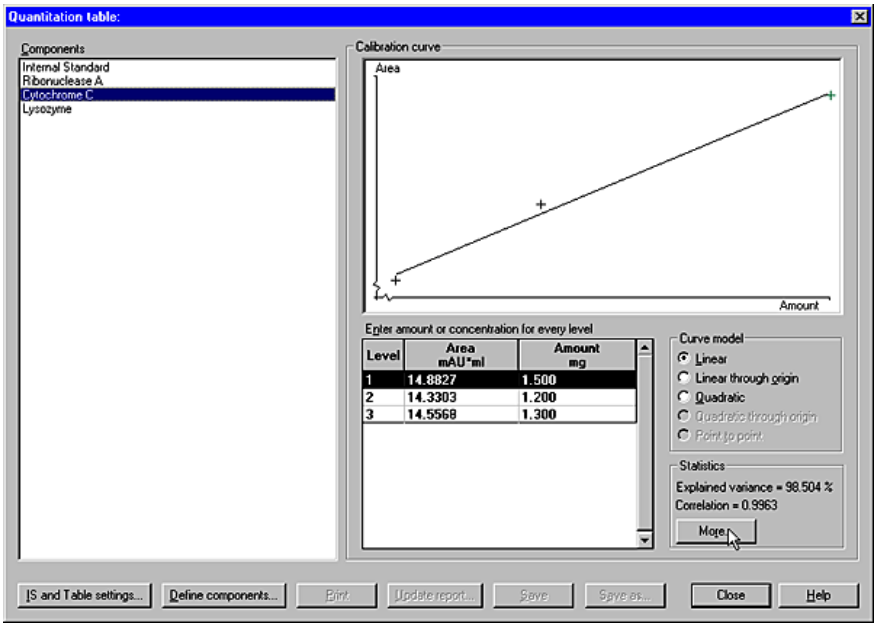
When the ***Peak identification*** is set to ***Absolute retention***, the peak window width can be displayed as ***Absolute*** or ***Relative***. Select the appropriate button in the ***Identification Settings*** dialog box.

- Select ***Absolute*** to show the window width of each peak in minutes (or the base volume unit).
- Select ***Relative*** to display the width of each component as a percentage of its retention.

If ***Peak identification*** is set to ***Relative*** retention, ***Window*** is set automatically to ***Relative*** except for the reference peak.

Step 4 - How to create a calibration curve and a quantitation table

When the component selection and identification settings are completed (see Step 3), the ***Quantitation table*** dialog box is opened:



The table below describes how to enter data for the standards and create a quantitation table and a calibration curve.

Step	Action
1	• Click the ***IS and Table settings*** button if you want to use an internal standard or base the calibration curve on peak height (see "How to select an Internal Standard" below this table).
2	• Verify that the selected components in the ***Components*** list are correct. - If an internal standard is used, the related component is labelled ***(IS)***. - If relative retention has been used, the reference component is labelled ***(Ref)***. • Click the ***Define components*** button to change the components.

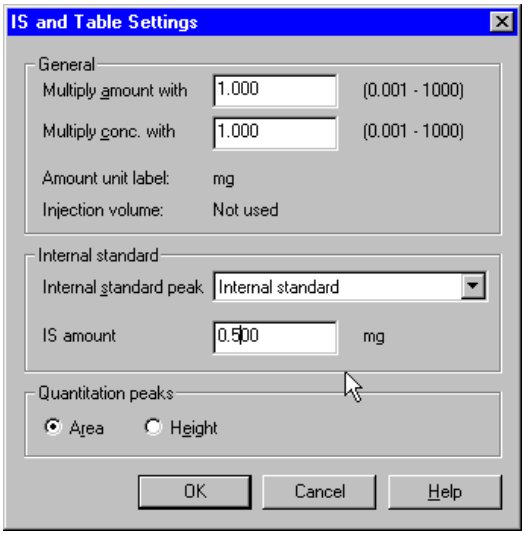
Step	Action
3	• Select the first component at the top of the ***Components*** list. *Note*: Do not select an internal standard component (if available) as the amount for this has already been entered and does not change between the levels. • Highlight the ***Amount/Concentration*** for ***Level 1***. • Type the amount or concentration of the component in the standard at this level. *Note*: This is the amount corresponding to the injected volume, not the total amount used when the standard level was prepared. • Repeat this for the other levels for this component.
4	Click the ***Curve model*** radio button for the best curve model: • ***Linear*** (recommended). • ***Linear through origin***. • ***Quadratic***. • ***Quadratic through origin***. • ***Point to point***. *Result*: The curve is displayed in the ***Calibration curve*** window. Each component level is labelled with crosses. If more than one run has been performed for any level, all points in that level will be shown. The average of these points is calculated and this value is used to produce the calibration curve.
5	Repeat steps 3 and 4 for all the remaining components. *Result*: The quantitation table is complete with a calibration curve for each component.
6	• Save the quantitation table and click ***Close***. *or* • Click the ***Save as*** button. *Result*: The ***Save quantitation table*** dialog box opens. *Note*: The ***Save*** button is used to save updates in an existing quantitation table. However, this will overwrite the original table. You might prefer to use ***Save as*** and create a new name for the edited table to preserve the original.

Step	Action
7	• Specify if the table is to be globally accessible to any user or restricted to your personal user ID. The default is global. • Type a name in the ***Quantitation table name*** field. • Click the ***OK*** button.

How to select an Internal Standard

The table below describes how to select an internal standard in the ***Quantitation table*** dialog box.

Step	Action
1	Click the ***IS and Table settings*** button. *Result*: The ***IS and Table Settings*** dialog box opens. The illustration below shows the ***IS and Table Settings*** dialog box with an ***Internal standard*** selected.



2	Type the amount and concentration multipliers in the ***General*** field. *Note*: These values are normally set to 1. See remarks below.

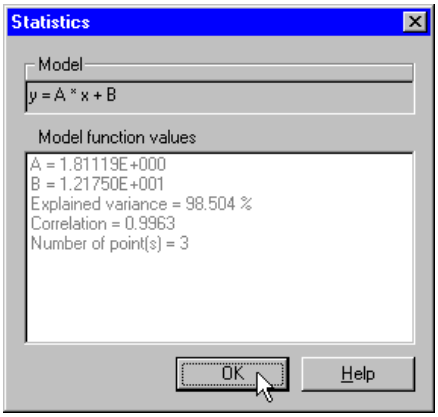
Step	Action
3	Select the internal standard component on the ***Internal standard peak*** droplist. *Note*: The default option is ***Not selected***, which is used for external standard quantitation and measurements of the recovery factor.
4	Type the injected internal standard amount for the standard and sample runs in the ***IS amount*** text box.
5	Select if the quantitation will be based on ***Area*** (default) or ***Height*** in the ***Quantitation peaks*** field. *Note*: Select ***Height*** if the peaks are not completely separated from those of other components.
6	Click ***OK***.

Note: The amount and concentration of the sample are multiplied by the multiplier values when the calibration curve is applied to a sample. Change the default values if you want to determine the amount or concentration in the starting volume of the sample instead of in the injected volume of the sample.

Quantitation statistics

The ***Statistics*** field in the ***Quantitation table*** dialog box displays the ***Correlation*** and ***Explained variance*** values when available.

Click the ***More*** button to open the ***Statistics*** dialog box for a complete display of available data.



Statistical reference values

- The correlation (only available for linear models) should be as close as possible to 1.00.
- The explained variance value should be as close as possible to 100%.

Note that the value is usually rather high even for poor models. A value of 90% indicates a very poor model.

The explained variance is not shown for curve models that are drawn through the origin.

Note: If the point-to-point curve model is selected, no statistics are available.

13.3.3 How to edit and update a quantitation table

How to open an existing table

The table below describes how to open an existing quantitation table for editing in the ***Evaluation*** module.

Step	Action
1	Select ***Quantitate:Edit Quantitation Table:Open***. *Result*: The ***Open quantitation table*** dialog box opens.
2	Select a quantitation table from the ***Quantitation table(s)*** list. *Note*: By default the list will show the quantitation tables that are globally available. Click the ***Personal*** radio button to display the tables that are restricted to your own user ID.
3	Click ***OK***. *Result*: The ***Quantitation table*** dialog box opens.

Note: ***Quantitate*** includes an update function that can be used to add new peak size data to an existing quantitation table in a simplified way. This function does *not* allow you to redefine components in the ***Define Component(s)*** dialog box.

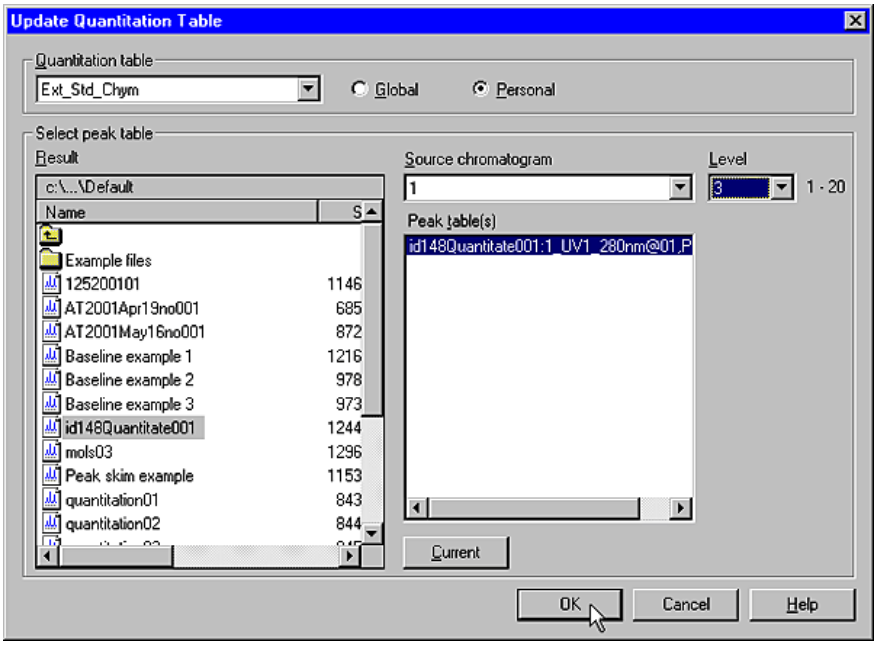
The update function

The update function can be used to add new peak size data to an existing quantitation table. This enables precision to be improved through the use of data from a number of standard runs. It also simplifies the process of renewing the calibration curves before each analysis.

Note: The injection volume must always be the same for the new run as it was for the previous standard runs.

The Update Quantitation Table dialog box

The illustration below shows the ***Update Quantitation Table*** dialog box.



How to prepare the calibration curve for updating

The table below describes how to open the function and prepare the calibration curve for updating.

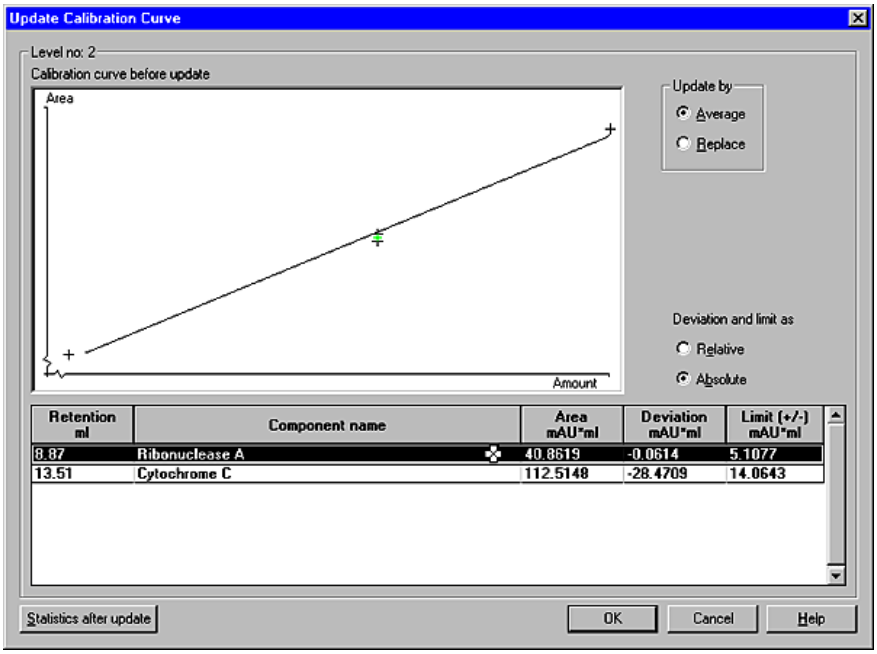
Step	Action
1	Perform a peak integration for the new run and save the result.
2	Select ***Quantitate:Edit Quantitation Table:Update***. *Result*: The ***Update Quantitation Table*** dialog box opens.
3	• Select the ***Personal*** radio button if the table is located in your personal folder. • Select the quantitation table that is to be updated in the ***Quantitation table*** field.

Step	Action
4	• Double-click the result file in the ***Select peak table*** list to access the new data. • Click the ***Current*** button if you want to use the result file that is open in the ***Evaluation*** module.
5	• Select the chromatogram on the ***Source chromatogram*** list. • Select the peak table that contains the new data in the ***Peak table(s)*** list.
6	• Select the level you wish to update on the ***Level*** list. • If the selected quantitation table is based on concentration, verify or edit the ***Inj. Volume*** field. • Click ***OK***. *Result*: The ***Update Calibration Curve*** dialog box opens. See "How to update a calibration curve" below.

The Update Calibration Curve dialog box

Data on the selected components for the curve to be updated are shown in the ***Component name*** table. When a component is highlighted, its calibration curve is displayed above in the ***Calibration curve before update*** field.

The calibration curve to be updated is shown without taking the new point into consideration. A new point is shown either in green or red. If it is green, the area falls within the set ***Limit (+/-)*** value and this point will be used for calculation of the new calibration curve, instead of the old point. If it is red, it falls outside this range.



How to update a calibration curve

Peak size deviation

The ***Deviation*** column of the ***Update Calibration Curve*** dialog box shows how much the peak size for the proposed new point differs from the existing size. The ***Limit (+/-)*** column displays the set limit for the deviation. The default value is +/- 12.5% of the existing peak size. You can edit the ***Limit (+/-)*** value. Use the ***Deviation and limit as*** radio buttons to specify if both of these columns are expressed in ***Absolute*** or ***Relative*** (%) units.

Instruction

The table below describes how to use the ***Update Calibration Curve*** dialog box for calibration curve updates.

Step	Action
1	Choose to update by ***Average*** or by ***Replace***. The same selection applies to all components. See explanations for the options below this table.

Step	Action
2	Select each component table rows in turn and check that the new point falls within acceptable limits.
3	Click the ***Statistics after update*** button. *Result*: The ***Statistics after update*** dialog box opens.
4	• Use the statistical data to check the curve model. *Note*: The old non-updated calibration curve is still shown, but the statistics apply to the data after the update. If the new point is red, the statistics shown will be those for the old curve. • Click ***OK*** to close the ***Statistics after update*** dialog box.
5	Repeat steps 2-4 for each component.
6	Click ***OK***. *Result*: The ***Update report*** dialog box opens. This report provides a summary of the proposed update so that you can assess its viability. See illustration below.
7	• Click the ***Print*** button for a print-out of the ***Update report***. *and/or* • Click ***Save*** or ***Save as*** to save the updated table.

Update by Average

The ***Average*** option means that the average area value is calculated from the old point (representing the average of the old points at this level) together with the new point. The green point represents the new average value and not the position of the point from the new peak table.

Update by ***Average*** may be used if you want to increase the precision of the calibration curve by performing several runs at each level.

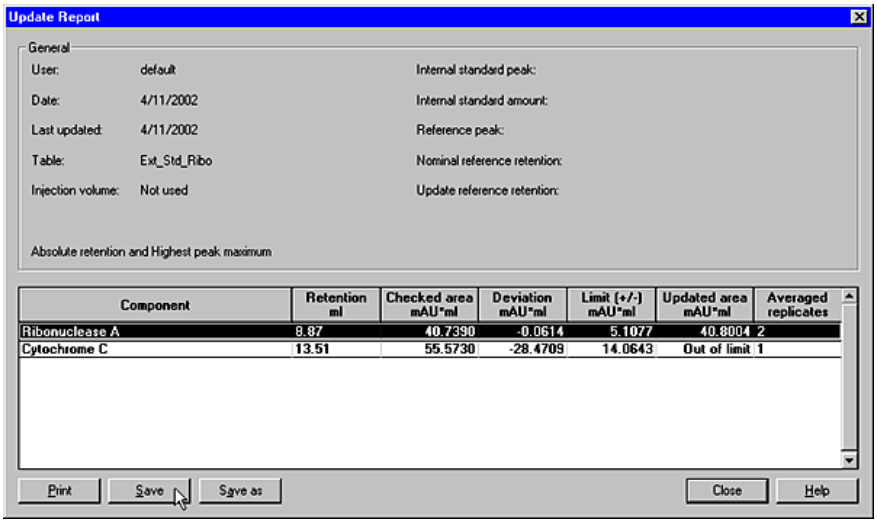
Update by Replace

The ***Replace*** option means that the old point (representing the average of the old points at this level) will be replaced with the new point shown in green. The data for the old point can then not be recovered.

Update by ***Replace*** may be used to simplify the process of renewing the calibration curve before each analysis. Instead of manually producing a new quantitation table, you may renew an existing table by running all standard levels again and updating the table with ***Replace***. The old data will then be deleted.

The Update Report dialog box

The illustration below shows the ***Update Report*** dialog box.



Features

The list below describes some features of the dialog box.

- Components that will not be updated are shown in the column ***Updated area*** (or ***Updated ratio*** if an internal standard is used) with the text `Out of limit`.
- The column ***Averaged replicates*** shows the number of points used to calculate the average area value. After each update by ***Average***, the number is increased by one. After an update by ***Replace***, the number will be one.
- ***Nominal reference retention*** shows the retention for the reference peak in level 1:1.
- ***Update reference retention*** shows the retention for the reference peak in the new peak table.

How to rename a quantitation table

The table below describes how to rename an existing quantitation table.

Step	Action
1	Select ***Quantitate:Edit Quantitation Table:Rename***. *Result*: The ***Rename quantitation table*** dialog box opens.
2	• Select ***Personal*** to display the quantitation tables that are restricted to your own user ID, if needed. • Select the quantitation table you wish to rename on the ***Quantitation table(s)*** list. • Click in the ***Quantitation table name*** text box and type a new name. • Click the ***Rename*** button. • Click the ***Close*** button.

Note: You must have ***Edit global list(s)*** rights to be able to rename a global quantitation table.

How to delete a quantitation table

The table below describes how to delete an existing quantitation table.

Step	Action
1	Select ***Quantitate:Edit Quantitation Table:Delete***. *Result*: The ***Delete quantitation table*** dialog box opens.
2	• Select ***Personal*** to display the quantitation tables that are restricted to your own user ID, if needed. • Select the quantitation table you wish to delete on the ***Quantitation table(s)*** list. • Click the ***Delete*** button. • Click the ***Yes*** button to confirm. • Click the ***Close*** button.

Note: You must have ***Edit global list(s)*** rights to be able to delete a global quantitation table.

13.4 How to quantitate the sample

Introduction

This section describes how to use calibration curves to quantitate samples.

Calibration curves are applicable to external and internal standard quantitation and to recovery factor measurement. Standard addition measurements are also described.

In this section

This section contains these sub-sections.

13.4.1 External and internal standard quantitation

Introduction

This section describes how to perform quantitation in the ***Evaluation*** module using either an external standard or an internal standard.

The processes involved in both external standard and internal standard quantitation of a sample are very similar. The procedural differences mainly concern the creation of the quantitation tables. A quantitation table is specific to either external standard or internal standard quantitation.

Method for the sample runs

The method that is used for the sample runs must be the same as for the standard runs. If the method is created from a wizard or a template for ÄKTAdesign systems, select ***Sample*** in the variable ***Quantitation_Type*** on the ***Variables*** tab in the ***Run Setup***.

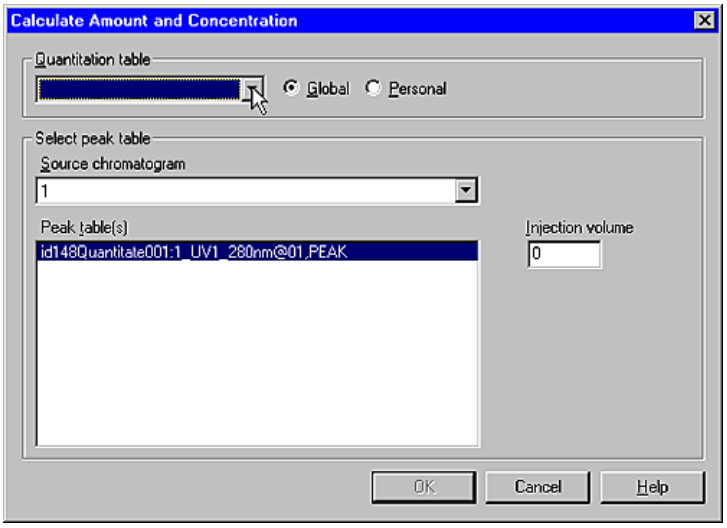
How to prepare for the quantitation

The table below describes briefly how to prepare for the quantitation.

Step	Action
1	Prepare a quantitation table for the components of interest. See *Section 13.3.2 How to create a quantitation table, on page 499* for further information.
2	Perform a sample run. *Note*: If internal standard quantitation is used, the internal standard must have been added to the sample prior to the sample preparation procedure. The injected amount must be the same as on the standard levels.
3	Open the sample result file and peak integrate the sample curve to produce a peak table. *Note*: The sample curve must use the same X-axis base unit as the standards during the integration. Time is the recommended unit for highest reliability.
4	Select ***File:Save*** to save the peak table.

How to calculate the amount and concentration

The table below describes how to calculate the amount and concentration in the sample.

Step	Action
1	Select ***Quantitate:Calculate amount and conc.*** *Result*: The ***Calculate Amount and Concentration*** dialog box opens. 
2	• Select a quantitation table on the ***Quantitation table*** droplist. • Select the chromatogram that contains the sample curve on the ***Source chromatogram*** droplist. • Select the sample peak table from the ***Peak table(s)*** list. • Check the ***Injection volume*** value and type a new value if necessary. *Note*: For internal standard quantitation, the injection volume must be the same as used for the standard runs. • Click the ***OK*** button. *Result*: The peak table is updated.

How to view the quantitation results

The results of the quantitation are shown in the ***Concentration*** and ***Amount*** peak table columns of the ***Evaluation*** module.The ***Peak Names*** are shown in the table and the type of quantitation is also listed. See illustration below:

A: ID 26500210_UV2_215nm@02,PEAK1

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)	Conc (mg/ml)	Amount (mg)
1	Component 1	8.49	106.7160	584.031		
2	Component 2	9.24	75.4414	466.665	7.957	0.398
3	Component 3	9.77	98.1309	593.788	7.939	0.397
4	Component 4	10.45	175.9518	694.142	7.900	0.395
5						
6	Total number of detected peaks			60		
7	Total area (mAU*min)			886.0878		
8	Area in evaluated peaks (mAU*min)			456.2401		
9	Ratio peak area / total area			0.514893		
10	Total peak width (min)			2.49		
11	Column height (cm)			5.00		
12	Calculated from			ID 265002:10_UV2_215nm		
13	Baseline			ID 265002:10_UV2_215nm@02,BASEM		
14	Peak rejection on					
15	Maximum number of peaks ()			20		
16	Int standard applied.					
17						

The quantitation table used for the quantitation

When the result file is saved, it includes the quantitation table that was used for the quantitation. You can view the table that was used by selecting ***Quantitate:Edit Quantitation Table:View Current***.

If the amount cannot be calculated

If the amount cannot be calculated, one of the following signs is shown in the peak table ***Amount*** column:

Sign	Function
>	This means that the value is higher than the highest value in the calibration curve, i.e. outside the valid range of the calibration curve.
<	This means that the value is lower than the lowest value in the calibration curve, i.e. outside the valid range of the calibration curve.
-	This means that the value cannot be calculated. For example, this sign might indicate that the peak could not be identified.

13.4.2 Standard addition quantitation

Stages in standard addition

Standard addition is performed in five stages:

Stage	Description
1	Perform two runs.
2	Copy the curves into one result file.
3	Integrate the curves to produce the peak tables.
4	Select the component to be used.
5	Evaluate the amount of a component in the sample.

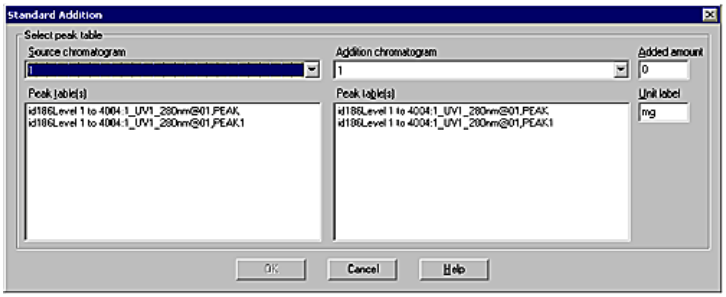
How to prepare for the Standard addition quantitation

The table below briefly describes how to prepare for the quantitation.

Step	Action
1	Perform a sample run with the unspiked sample and a run with the spiked sample.
2	Open one of the two result files. Use ***File:Open:Curves*** to copy the second curve to the opened result file.
3	Peak integrate the sample curves to produce the peak tables for the unspiked and the spiked samples. *Note*: The sample curves must use the same X-axis base unit. Time is the recommended unit for highest reliability.
4	• Check that the integrations are correct. • Optimize the peak integration if necessary.
5	Select ***File:Save*** to save the peak table.

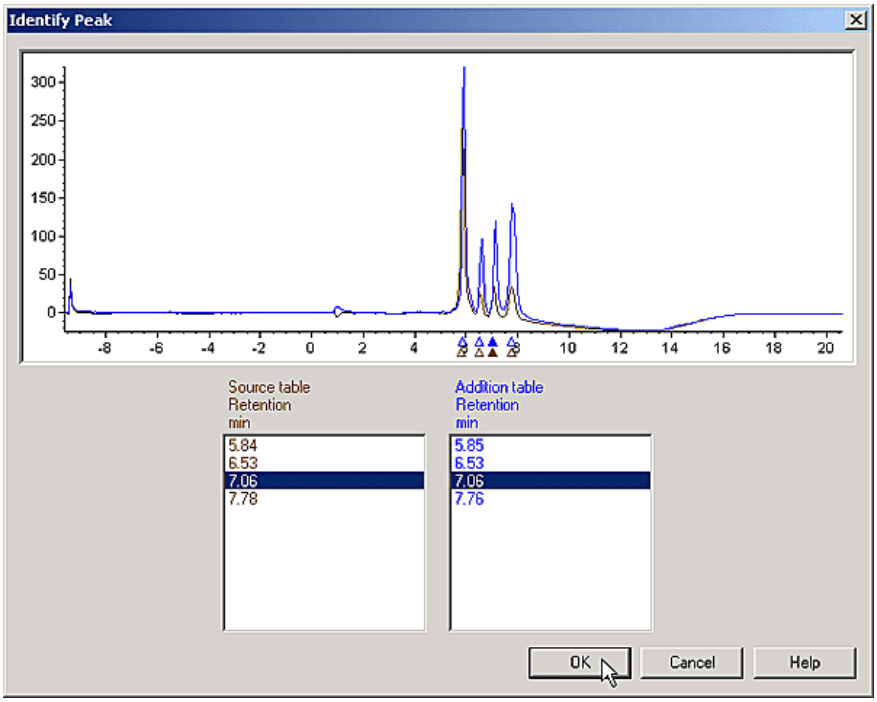
How to select the component and identify the sample peaks

The table below describes how to select the component to be used for the standard addition and how to identify the sample peaks.

Step	Action
1	Select ***Quantitate:Standard addition*** *Result*: The ***Standard Addition*** dialog box opens. 
2	• Select the chromatogram that contains the peak table for the unspiked sample in the ***Source chromatogram*** droplist. • Select the unspiked sample peak table from the ***Peak table(s)*** list to the left.
3	Repeat step 2 in the ***Addition chromatogram*** section to the right to select the addition peak table for the spiked sample.
4	• Edit the default unit ***mg*** in the ***Unit label*** field if necessary. • Type the amount of the component that was added as the spike in the ***Added amount*** field. • Click ***OK***. *Result*: The ***Identify Peak*** dialog box opens.
5	To locate and select the peak of the unspiked sample, do the following: • Click its triangle marker (black) or select its reference in the ***Source table***.
6	• Repeat step 5 to select the spiked sample. The triangle color is blue. Use the ***Addition*** table. • Click the ***OK*** button.

The Identify Peak dialog box

The illustration shows the ***Identify Peak*** dialog box, described in the table above.



How to view the quantitation results

The amount of the component of interest is displayed in the peak table ***Amount*** columns of the ***Evaluation*** module.

No	Retention (min)	Area (mAU*min)	Height (mAU)	Amount (mg)
1	5.84	52.9263	282.754	
2	6.53	4.8881	30.365	
3	7.06	6.4036	38.687	0.913
4	7.78	11.3658	42.744	
5				
6	Total number of detected peaks			220
7	Total area (mAU*min)			92.9166
8	Area in evaluated peaks (mAU*min)			75.5839
9	Ratio peak area / total area			0.813459

13.4.3 How to calculate the recovery factor

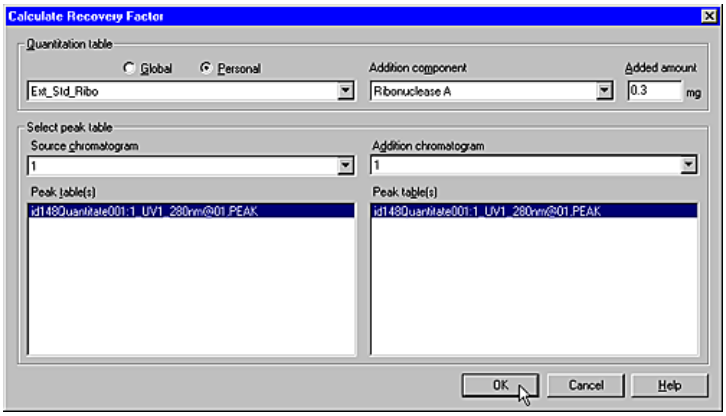
How to prepare for the quantitation

The table below briefly describes how to prepare for the quantitation.

Step	Action
1	Prepare a quantitation table for the components of interest. *Note*: An external standard quantitation must be used. Internal standard quantitation tables cannot be used.
2	Perform a sample run with the unspiked sample and a run with the spiked sample.
3	Peak integrate the sample curves to produce the peak tables for the unspiked and the spiked samples. *Note*: The sample curves must use the same X-axis base unit as the standards during the integration. Time is the recommended unit for highest reliability.
4	• Check that the integration is correct. • Optimize the integration if necessary.
5	• Open one of the sample result files. • Use ***File:Open:Peak Tables*** to copy the other peak table to that result file.
6	Select ***File:Save*** to save the result.

How to calculate the recovery

The table below describes how to calculate the recovery factor.

Step	Action
1	Select ***Quantitate:Calculate Recovery***. *Result*: The ***Calculate Recovery Factor*** dialog box opens. 
2	• Select ***Global*** or ***Personal*** quantitation tables. • Select a quantitation table on the ***Quantitation table*** droplist. *Note*: Only external standard quantitation tables will be shown. • Select the chromatogram that contains the unspiked sample peak table on the ***Source chromatogram*** droplist. • Select the unspiked sample peak table from the ***Peak table(s)*** list to the left.
3	• Repeat step 2 to select the peak table for the spiked sample on the ***Addition chromatogram*** fields. • Select the component that was added prior to the sample preparation on the ***Addition component*** droplist. • Type the injected amount of this component in the ***Added amount*** field. • Click the ***OK*** button.

How to view the recovery factor calculation results

The recovery factor calculated by the software is placed at the bottom of the peak table in the ***Evaluation*** module. You need to scroll to the end of the table to see it.

No	Retention (min)	Area (mAU*min)	Area/Peak area ((time) %)	Height (mAU)
	Recovery Factor ()			0.989
	Component name			Chymotrypsinogen A
	Calculated from			id 18501:1_UV1_280nm
	Baseline			id 18501:1_UV1_280nm@01,BASEC
	Peak rejection on			
	Maximum number of peaks ()			20
	Recovery applied,			
	Current peak filter settings			
	Maximum number of peaks ()			20

Note: The checkbox ***Do not show global peak table data*** must be de-selected in the ***Peak Table*** tab of the ***Chromatogram Layout*** dialog box.

If the recovery cannot be calculated

If the recovery cannot be calculated, one of the following signs is shown in the peak table ***Amount*** column:

Sign	Function
>	This means that one of the amounts/concentrations is higher than the highest value in the calibration curve, i.e. outside the valid range of the calibration curve.
<	This means that one of the amounts/concentrations is lower than the lowest value in the calibration curve, i.e. outside the valid range of the calibration curve.
-	This means that the recovery factor cannot be calculated. For example, this sign might indicate that the peak could not be identified in both runs.

13.5 Automated quantitation

Introduction

Some method wizards designed for quantitation are available for ÄKTAdesign systems supplied with ***Autosampler A-900*** or ***A-905***. These can be used to quantitate a sample automatically or to update a quantitation table.

The procedures described in this chapter are designed for use with the systems mentioned above.

In this section

This section contains these sub-sections.

13.5.1 How to set up for automated quantitation

Introduction

This section describes how to create a quantitation table for automated quantitation.

Basic conditions for the quantitation table

A quantitation table must be produced from standards before samples can be quantitated. The list below describes the basic conditions for the quantitation table:

- The same method must be used for all standard and sample runs.
- Each level is an alias for a specific concentration of the standard.
- All runs with the same concentration must be assigned the same level.
- Level 1 must be selected for the standard with the highest or lowest concentration.
- The levels must be set in order of decreasing or increasing concentration of the standard.

How to prepare the quantitation table

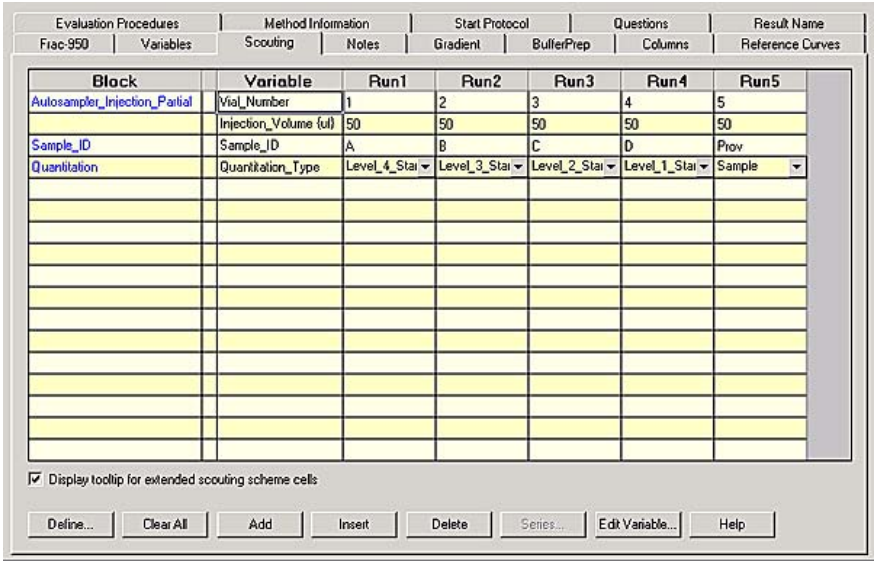
The table below describes how to prepare the quantitation table for automated quantitation.

Step	Action
1	• Use the ***Method Wizard*** to create a method. • Select ***Autosampler*** from the ***Injection Technique*** droplist in the ***Sample Injection*** dialog box.
2	Proceed with the following dialog boxes in the ***Method Wizard*** and click the ***Finish*** button on the last dialog box. *Result*: The ***Run Setup*** opens.

Step	Action
3	Click the ***Scouting*** tab. (See illustration below) • Select the ***Quantitation_Type*** variable from the ***Scouting Variables*** dialog box. • Select other scouting variables of interest, e.g. ***Sample_ID***, ***Vial_Number*** etc. • Click ***OK***.
4	• Double-click the ***Quantitation_Type*** variable table cell. • Select the correct standard concentration level. *Note*: This corresponds to the text instruction ***QuantitationData***. You can also set this level after the run has been completed. For more information about scouting see *Section 7.1 How to set up a Scouting Scheme, on page 214*.
5	Click the ***Evaluation Procedures*** tab. • Select the ***Integrate_and_Print*** procedure. *Result*: This procedure will automatically use default baseline settings and integrate the first UV curve.
6	Save the method.
7	Perform all the standard runs.
8	In the ***Evaluation*** module, select ***Quantitate:Edit Quantitation Table:New***.
9	Create a quantitation table manually from the standard runs. See *Section 13.3.2 How to create a quantitation table, on page 499*.

The Scouting tab

The illustration below shows the ***Scouting*** tab in the ***Run Setup***, used to enter standard data, before the standard concentration level is defined.



13.5.2 How to perform automated quantitation

Instruction

The table below describes how to set up sample runs to perform automated quantitation.

Step	Action
1	• Select ***File:Open*** in the ***Method Editor*** module. • Select a method that has been used for standard runs in the ***Open*** dialog box. (See *Section 13.5.1 How to set up for automated quantitation, on page 531*) • Click ***OK***.
2	• Click the ***Scouting*** tab in the ***Run Setup***. • Click the ***Clear All*** button to clear the scouting scheme. • Double-click each ***Quantitation_Type*** table cell and select ***Sample*** for all sample runs.
3	Click the ***Evaluation Procedures*** tab. • Select only the ***Quantitate_Sample*** procedure. (Click the ***Import*** button to import the ***Quantitate_Sample*** procedure if it isn't displayed on the list) *Result*: This procedure automatically integrates the first UV curve with default baseline settings and uses the selected quantitation table to quantitate the sample. The amounts and concentrations are then printed.
4	Click the ***Quantitate*** button. *Result*: The ***Quantitation table*** dialog box opens.
5	Select the quantitation table from the ***Global*** or ***Personal*** folder and click ***OK***. *Result*: The quantitation table is copied into the ***Quantitate_Sample*** procedure. *Note*: The procedure cannot be executed if a quantitation table has not been selected. Time must have been selected as the X-axis base unit.
6	Save the method with a new name.
7	Perform the run(s). *Result*: The amount and concentration of the components in the samples will be printed automatically after each run.

13.5.3 How to perform automated update

Introduction

This section describes how to update quantitation tables automatically, also in scouting runs. See also *Section 13.3.3 How to edit and update a quantitation table, on page 512*.

How to perform automated update with the Replace option

The table below describes how to automatically update a quantitation table with the ***Replace*** option (default).

Step	Action
1	Open a method in the ***Method Editor***.
2	• Click the ***Scouting*** tab in the ***Run Setup***. • Click the ***Clear All*** button to clear the scouting scheme. • Double-click each ***Quantitation_Type*** table cell and select the correct concentration level for the standards.
3	Click the ***Evaluation Procedures*** tab. • Select the ***Update_Quantitation*** procedure. (Click the ***Import*** button to import the ***Update_Quantitation*** procedure if it isn't displayed on the list) *Result*: This procedure automatically integrates the first UV curve with default baseline settings and updates the selected quantitation table with the new standard. An update report is then printed.
4	Click the ***Quantitate*** button. *Result*: The ***Quantitation table*** dialog box opens.
5	Select the quantitation table from the ***Global*** or ***Personal*** folder. Time must be selected as the X-axis base unit. *Result*: The quantitation table is copied into the ***Update_Quantitation*** procedure. *Note*: You can only perform one run at each level since the old points in the quantitation table will be replaced after each run.
6	Save the method with a new name.

Step	Action
7	Perform the run(s). *Result*: The quantitation table will be updated automatically after each run.

Note: The quantitation table will not be updated if the peak area or peak height of the new and the previous results differ more than the ***Limit*** value. The ***Limit*** value is defined either for peak area or height.

How to perform automated update with the Average option

The table below describes how to automatically update a quantitation table with the ***Average*** option.

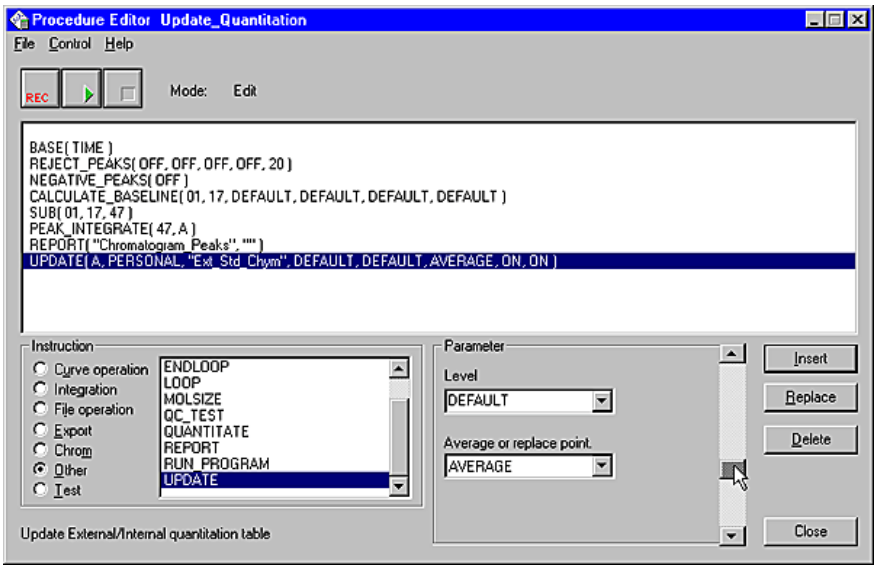
Step	Action
1	Open a method in the ***Method Editor***.
2	• Click the ***Scouting*** tab in the ***Run Setup*** dialog box. • Click the ***Clear All*** button to clear the scouting scheme. • Double-click each ***Quantitation_Type*** table cell and select the correct concentration level for the standards.
3	• Click the ***Evaluation Procedures*** tab. • Select the ***Update_Quantitation*** procedure. • Click the ***Quantitate*** button. *Result*: The ***Quantitation table*** dialog box opens.
4	Select the quantitation table from the ***Global*** or ***Personal*** folder and click ***OK***. *Result*: The quantitation table is copied into the ***Update_Quantitation*** procedure.
5	Click the ***Edit*** button on the ***Evaluation Procedures*** tab. *Result*: The ***Procedure Editor*** dialog box opens. See illustration below.
6	Select the existing ***UPDATE*** instruction.

Step	Action
7	Use the scroll bar in the ***Parameter*** field to locate the ***Average or replace point*** droplist. • Select the ***AVERAGE*** option. • Click the ***Replace*** button to the right of the scroll bar.
8	Select ***File:Close*** in the ***Procedure Editor*** dialog box to return to the ***Run Setup***.
9	Save the method and perform the runs. *Result*: The quantitation table will be updated automatically after each run. New average values will be calculated from the old points together with the new points.

Note: The quantitation table will not be updated if the peak area or peak height of the new and the previous results differ more than the ***Limit*** value. The ***Limit*** value is defined either for peak area or height.

The Procedure Editor dialog box

The ***Procedure Editor*** dialog box is illustrated below:



How to perform automated update in scouting runs - step 1

It is possible to run both standards and samples in the same scouting run and continuously update a previously created quantitation table with new values. The table below describes how to set up the evaluation procedures for the updates.

Step	Action
1	Open the same method that was used to create the quantitation table from the standard runs and open the ***Run Setup***.
2	Click the ***Evaluation Procedures*** tab.
3	Select ***Update_Quantitation*** and click ***Quantitate***. *Result*: The ***Quantitation table*** dialog box opens.
4	Select the quantitation table and click ***OK***.
5	Deselect the ***Update_Quantitation*** procedure on the ***Evaluation Procedures*** tab.
6	Repeat steps 3 to 5 for the ***Quantitate_Sample*** procedure. *Note*: Make sure that both procedures are deselected after this is completed. Otherwise they will be run twice.
7	Proceed with the instructions how to edit the instructions (see table below).

How to perform automated update in scouting runs - step 2

The table below describes how to edit the text instructions.

Step	Action
1	Click the ***Text Instructions*** icon.
2	Select the last instruction in the method in the ***Text*** pane.
3	• Click the ***Other*** radio button in the ***Instructions*** field of the ***Instruction box***. • Select ***Evaluate*** on the ***Instructions*** list.

Step	Action
4	• Select ***Update_Quantitation*** in the ***Procedure*** droplist of the ***Parameters*** field.  • Click the ***Var...*** button in the ***Parameters*** field. *Result*: The ***Variable Name Definition*** dialog box opens.
5	Type a variable name, for example `Procedure` and click ***OK***. *Result*: The ***Evaluate*** instruction is inserted in the method. By defining the evaluation procedure as a variable, different procedures can be selected on the ***Scouting*** tab for different scouting runs.
6	Proceed with the instructions on how to set up the scouting runs for the standards (see table below).

How to perform automated update in scouting runs - step 3

The table below describes how to set up the scouting runs for the standards.

Step	Action
1	Select ***View:Run Setup*** and click the ***Scouting*** tab.
2	Click the ***Define*** button. *Result*: The ***Scouting Variables*** dialog box opens.

Step	Action
3	Edit the scouting variables list to include: • ***Procedure*** • ***Vial_Number*** • ***Injection_volume*** • ***Sample_ID*** • ***Quantitation_Type*** *Note*: The ***Procedure*** variable will appear at the beginning of the list of variables, even though the ***Evaluate*** instruction is inserted at the end of the method.
4	Set up all the standard runs in the scouting scheme: • Select the ***Update_Quantitation*** procedure. • Ensure that ***Quantitation_Type*** is set to the correct standard level for each run. *Result*: The quantitation table will now be updated with new values after each run. Since the runs will be performed with the ***Replace*** (the default selection) option, you can only perform one run at each level.
5	Proceed with the instructions on how to set up the scouting runs for the samples in step 4 (see table below).

Note: The quantitation table will not be updated if the peak area or peak height of the new and the previous results differ more than the ***Limit*** value. The ***Limit*** value is defined either for peak area or height.

How to perform automated update in scouting runs - step 4

The table below describes how to set up the scouting runs for the samples.

Step	Action
1	Select the ***Quantitate_Sample*** procedure.

Step	Action
2	Select ***Sample*** in the variable ***Quantitation_Type*** for all the sample runs. *Result*: All samples will be run automatically and the amount and concentration of the components of interest will be printed after each run. *Note*: The result files will include an additional chromatogram (labelled 12) that contains a small part of the curves collected during the execution of the evaluation procedure.

How to change the scouting runs to be updated by average

The table below describes how to change the scouting runs so that the quantitation table is updated by average after each standard run.

Step	Action
1	• Click the ***Evaluation Procedures*** tab in the ***Run Setup Editor***. • Click the ***Import*** button. *Result*: The ***Import*** dialog box opens.
2	• Select the current method from the ***Method*** file menu. • Highlight ***Update_Quantitation*** in the ***Select*** field. • Type a new name, e.g. `Update_Average` in the ***Import as*** text box. • Click the ***Import*** button. • Click the ***Close*** button. *Result*: The new procedure is added to the list of ***Evaluation Procedures***.
3	Select the new procedure and click the ***Edit*** button. *Result*: The ***Procedure Editor*** dialog box opens.
4	Highlight the existing ***Update*** instruction.
5	Use the scroll bar in the ***Parameter*** field to locate the ***Average or replace point*** droplist. • Select the ***AVERAGE*** option. • Click the ***Replace*** button.

Step	Action
6	Select ***File:Close*** in the ***Procedure Editor*** dialog box to return to the ***Run Setup***.
7	Select the ***Update_Average*** procedure and click the ***Quantitate*** button. *Result*: The ***Quantitation table*** dialog box opens.
8	Select the quantitation table and click ***OK***.
9	Deselect all the procedures on the ***Evaluation Procedures*** tab, otherwise they will be run twice.
10	Click the ***Scouting*** tab. • Select the ***Update_Average*** procedure for the second and all following runs at each standard level concentration. *Note*: The ***Update_Quantitation*** procedure (***Update by Replace***) should still be used for the first run at each level.

13.6 How to measure molecular size

Introduction

The molecular size of components in a sample can be determined by size exclusion chromatography. A molecular size calibration curve must first be created with components of known molecular size. The retention is inversely related to the molecular size.

This section describes how to measure the molecular size.

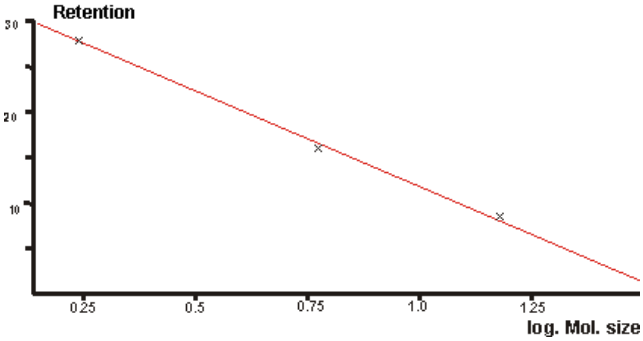
In this section

This section contains these sub-sections.

13.6.1 Overview of molecular size determination

How to create a molecular size curve

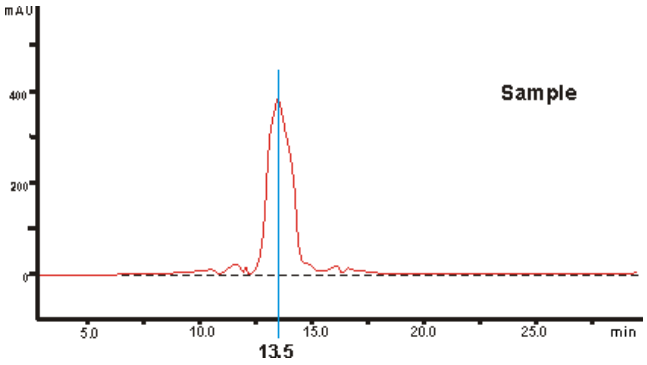
The table below is a brief description of how to create a molecular size curve.

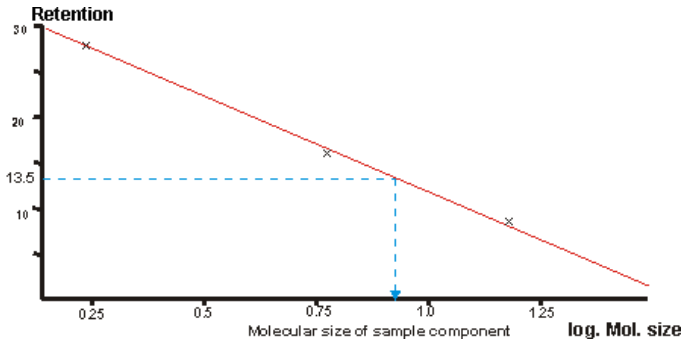
Step	Action
1	Perform a run with one or more standards to create a standard curve. *Note*: The standards should contain a number of components of known molecular size and these should extend beyond the size limits that are expected in the test sample.
2	Peak integrate the standard curve to produce a peak table.
3	Use the peak table from the standard to produce a molecular size table. Each peak is represented by a retention value.
4	Select the relevant peaks and input data for the corresponding molecular sizes. *Result*: The software plots these values as a molecular size curve. This curve has an inverse relationship between the logarithm of the molecular size and retention. 

How to calculate the molecular size in the sample

The table below is a brief description of how to use the molecular size table to calculate the molecular size of the components in the sample.

Step	Action
1	Use the sample peak table to obtain retention values for each of the components of interest.
2	Use the molecular size curve to obtain the molecular sizes of the components in the sample. The molecular sizes are presented in the peak table.





13.6.2 How to determine the molecular size

Introduction

This section describes the technique for measuring molecular size in detail.

Before you start

Before you create the molecular size curve, you need to do the following:

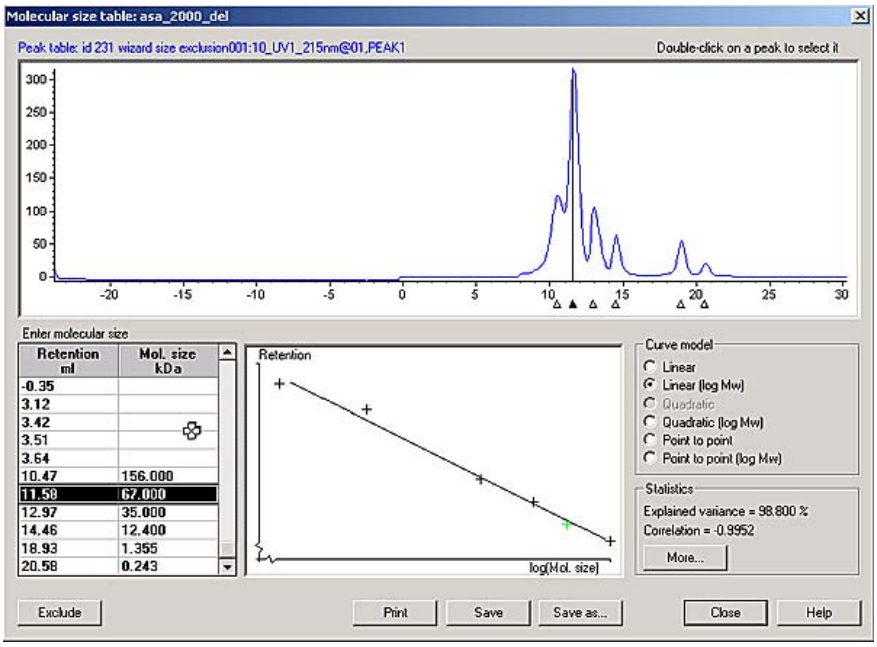
- Perform chromatographic runs with an appropriate standard with components of known molecular size. The standard should contain components of sizes that extend over the range that is expected in the sample. If you are using many components, it may be better to split them into two or more standard runs.
- Peak integrate the curves to produce peak tables. The standard curves must all use the same X-axis base unit during the integration. Volume is the recommended unit for molecular size determination.
- Save the results.

The Molecular size table dialog box

This dialog box is used to select the peaks that will be used to produce the molecular size curve. Each curve and its peak table name is color coded. All the available peaks for all the curves are listed together in the ***Retention/Mol.size*** table.

Triangles show that a peak has been selected. The name of its source peak table is shown above the curve window. This is useful when you wish to know which peak has been selected of two closely spaced peaks from different peak tables.

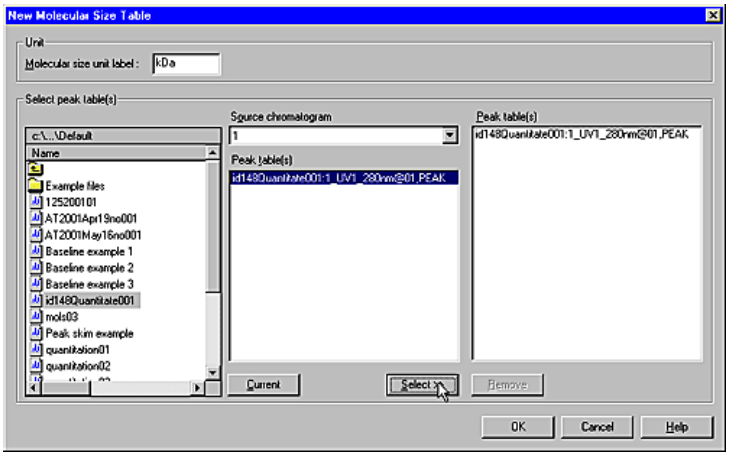
The illustration below shows the ***Molecular size table*** dialog box.


Molecular size table: asa_2000_del
Peak table: id 231 wizard size exclusion001:10_UV1_215nm@01,PEAK1
Double-click on a peak to select it
Enter molecular size
Retention ml
Mol. size kDa
-0.35
3.12
3.42
3.51
3.64
10.47 156.000
11.58 67.000
12.97 35.000
14.46 12.400
18.93 1.355
20.58 0.243
Retention
log(Mol. size)
Curve model
Linear
Linear (log Mw)
Quadratic
Quadratic (log Mw)
Point to point
Point to point (log Mw)
Statistics
Explained variance = 98.800 %
Correlation = -0.9952
More...
Exclude
Print
Save
Save as...
Close
Help

How to create and save a molecular size table

The table below describes how to create and save a molecular size table in the ***Evaluation*** module.

Step	Action
1	Open a result file and select ***Mol. Size:Edit Mol. Size Table:New***. *Result*: The ***New Molecular Size Table*** dialog box opens.



2	• Double-click the result file in the ***Select peak table(s)*** list. • Select the source chromatogram on the ***Source chromatogram*** droplist.
3	Highlight a peak table that was prepared from the standard in the source ***Peak table(s)*** list and click the ***Select*** button.
4	Repeat step 3 to select more peak tables. *Note*: The runs must all have been made under the same conditions.
5	To deselect a table, highlight the table in the ***Peak table(s)*** list to the right and click the ***Remove*** button.
6	Repeat steps 2 to 4 to select peak tables from other result files.

Step	Action
7	• Type the appropriate size measurement unit in the ***Molecular size unit label*** field (default kDa). • Click ***OK*** when the ***Peak table(s)*** list to the right contains all the required peak tables. *Result*: The ***Molecular size table*** dialog box opens.
8	Use one of the following ways to select a peak: • Click the peak in the curve. • Click the peak entry in the ***Retention/Mol. size*** table.
9	Double-click in the ***Mol. size*** column cell and type the known molecular size from the standard.
10	Repeat step 8 and 9 for all components of known molecular size.
11	To remove unwanted entries, click the peak entry in the table and click the ***Exclude*** button.
12	Select the appropriate curve model in the ***Curve model*** field (see "The molecular size curve" below).
13	Click the ***Save as*** button. *Result*: The ***Save molecular size table*** dialog box opens.
14	• Choose if the table is to be globally accessible to any user or restricted to your personal user ID. The default is global. • Type a name in the ***Molecular size table name*** field. • Click ***OK***.

The molecular size curve

The molecular size curve shows the relationship between molecular size and the corresponding retention. The curve is plotted from the ***Retention/Mol. size*** data that you have typed in the table as described above. Before this can be done, a curve model is needed, which describes the relationship between molecular size and retention. Each of the peaks selected is represented by a point in this curve, which is drawn according to the best fit that can be achieved using the selected model. Select one of the available models in the ***Curve model*** field:

- ***Linear***

- ***Linear (logMw)*** (Theoretically, this is the best choice.)
- ***Quadratic***
- ***Quadratic (logMw)***
- ***Point to point***
- ***Point to point (logMw)***

Molecular size Statistics

With the exception for the two point-to-point models, the molecular size curves can be expressed as mathematical expressions. The expressions and related items can be viewed in the ***Statistics*** dialog box.

- Click the ***More*** button in the ***Statistics*** field of the ***Molecular size table*** to open the dialog box.

The expression is shown at the top of the window, followed by the values for the constant that it contains.

Statistical reference values

- The correlation value (only for linear models) should be as close to -1.00 as possible.
- The explained variance value should be as close to 100% as possible.

Note: Explained variance values are usually high. A value of 90% indicates a very poor model.

How to open an existing table

The table below describes how to open an already existing molecular size table for editing in the ***Evaluation*** module.

Step	Action
1	Select ***Mol. Size:Edit Mol. Size Table:Open***. *Result*: The ***Open molecular size table*** dialog box opens.
2	• Select a molecular size table from the ***Molecular size table(s)*** list. *Note*: By default the list will show the molecular size tables that are globally available. Click the ***Personal*** radio button to display the tables that are restricted to your own user ID. • Click ***OK***. *Result*: The ***Molecular size table*** dialog box opens.

How to rename a molecular size table

The table below describes how to rename an existing molecular size table.

Step	Action
1	Select ***Mol. Size:Edit Mol. Size Table:Rename***. *Result*: The ***Rename molecular size table*** dialog box opens.
2	• Select ***Personal*** to display the tables that are restricted to your own user ID, if needed. • Select the molecular size table you wish to rename in the ***Molecular size table(s)*** list. • Click in the ***Molecular size table name*** text box and type a new name. • Click the ***Rename*** button.

Note: You must have ***Edit global list(s)*** rights to be able to rename global tables.

How to delete a molecular size table

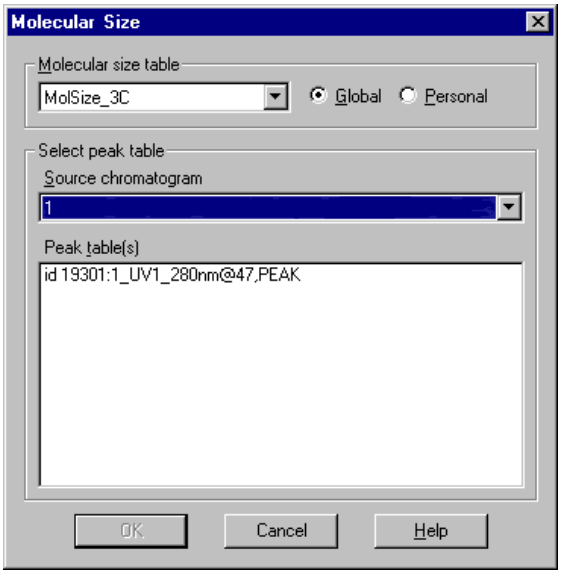
The table below describes how to delete an existing molecular size table.

Step	Action
1	Select ***Mol. Size:Edit Mol. Size Table:Delete***. *Result*: The ***Delete molecular size table*** dialog box opens.
2	• Select ***Personal*** to display the tables that are restricted to your own user ID, if needed. • Select the molecular size table you wish to delete in the ***Molecular size table(s)*** list. • Click the ***Delete*** button. • Click the ***Yes*** button to confirm.

Note: You must have ***Edit global list(s)*** rights to be able to delete global tables.

How to calculate the molecular size

The table below describes how the molecular size curve is used to calculate the molecular sizes of the components in the sample.

Step	Action
1	Perform a sample run and peak integrate the curve to produce a peak table. *Note*: The sample curve must use the same X-axis base unit as the standards. Use volume for molecular size calculations.
2	Select ***Mol. Size:Calculate Mol. Size*** in the ***Evaluation*** module. *Result*: The ***Molecular Size*** dialog box opens. 
3	• Select ***Global*** or ***Personal*** according to the location of the molecular size table. • Select the molecular size table on the ***Molecular size table*** droplist. • Select a chromatogram on the ***Source chromatogram*** droplist. • Select a peak table on the ***Peak table(s)*** list and click ***OK***. *Result*: The results of the molecular size calculation are shown in the ***Mol. size*** peak table column. (See illustration below)

The Mol. size peak table column

The illustration below shows the ***Mol. size*** peak table column.

No	Retention (min)	Mol. size (kDa)
8	6.42	>
9	7.90	38.22
10	8.42	31.15
11	9.59	19.57
12	10.43	14.05
13	11.71	<

When the result file is saved, it includes the molecular size table that was used for the molecular size determination. You can view the table that was used by selecting ***Mol. Size:Edit Mol. Size Table:View Current***.

If the molecular size cannot be calculated

If the molecular size cannot be calculated, one of the following signs is shown in the peak table ***Mol. size*** column:

Sign	Function
>	This means that the molecular size is larger than the largest size in the molecular size curve, i.e. outside the valid range of the curve.
<	This means that the molecular size is smaller than the smallest size in the molecular size curve, i.e. outside the valid range of the curve.
-	This means that the retention value is negative.

Molecular size procedure instruction

The table below describes the new procedure instruction for molecular size measurement that becomes available when the ***Analysis*** module is installed.

Instruction	Description
`MOLSIZE`	The instruction calculates the molecular sizes from a molecular size curve. A ***Mol.size*** column will be added to the peak table.

14 System settings

Introduction

This chapter describes some of the general UNICORN system settings.

In this chapter

This chapter contains these sections:

14.1 General information about system settings

System settings

The system settings

- define settings for alarms and warnings
- select the data that will be stored in result files

When to change the system settings

Each system has a set of default settings.

- Changes to the default settings should be made when the system is installed.

Certain system settings may need to be adjusted in the following cases:

- If system components are changed: e.g. the alarm and warning limits
- For specific separation runs: e.g. the monitor and curve settings.

Note: Only the settings for the selected components will be shown for strategies where you select the system components.

How to change the default settings

The table below describes the two different ways to change the default system settings.

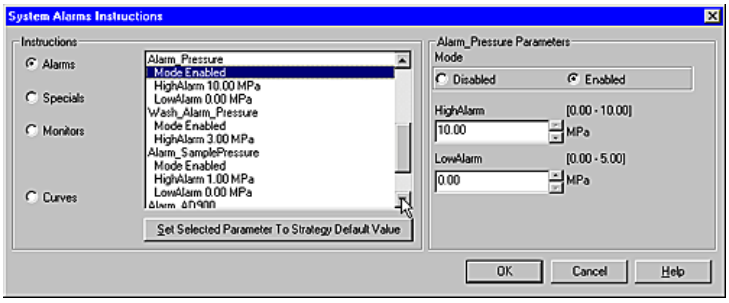
Change	Effect
To assign a new value to a parameter within a method.	The specific change is valid only until ***End*** in the method. After ***End*** the parameter returns to its default setting. **Note:** Only some parameters can be changed in the method.
To assign a new value to the system setting.	The new value is valid for all runs and remains until you change the value again or return the setting to its default value. See *"How to assign a new value to a system setting"* below. Like the default values, the new value can be changed temporarily in a method.

Note: You must have ***System settings*** authorization to assign a new value to an actual system setting.

How to assign a new value to a system setting

The table below describes how to assign a new value to a system setting in the ***System Control*** module.

Step	Action
1	Select ***System:Settings***. *Result*: The ***Instructions*** dialog box for the connected system opens. The illustration below shows the dialog box opened with the ***Alarms*** group of settings selected.
2	Click the radio button to select one of the following instruction groups: • ***Alarms*** • ***Specials*** • ***Monitors*** • ***Curves*** *Result*: The instructions for the group are displayed. The parameters are listed below each instruction. The title bar of the dialog box shows the selected instruction group.
3	• Select a parameter from the list. • Change the setting value in the ***Parameters*** field. *Result*: The parameter is updated with the new value in the list.
4	Click the ***Set Selected Parameter To Strategy Default Value*** button to return to the default value (if necessary). *Result*: The default setting that was defined in the system strategy is restored. Only the selected parameters will be restored.



Step	Action
5	Click ***OK***.

Limits for monitor signals in methods

If the system strategy allows, limits for certain monitor signals can be set in the method. These limits will only work locally in the method and override the global settings as long as the method is in operation. This feature can be used to set the pH warning threshold to one value during the process operation and another during the system cleaning.

14.2 Alarms

Introduction

This section is a description of the ***Alarms*** system settings.

Alarms and Warnings

The ***Alarms*** settings define the upper and lower ***Alarm*** and ***Warning*** limits for process monitor signals.

The table below describes the difference between ***Alarms*** and ***Warnings***.

If the signal exceeds...	then...
the ***Alarm*** limits	• an alarm sounds • an alarm message is displayed • the process is paused (i.e. the method execution is suspended and all pumps are stopped) • the alarm is noted in the logbook. The situation must be acknowledged and corrected before the process can be continued.
the ***Warning*** limits	• a warning message is displayed • the process continues • the warning is noted in the logbook.

Note: The message text in an ***Alarm*** dialog box and the corresponding text in the logbook are both color-coded in red. ***Warning*** texts are color-coded in orange both in the dialog box and in the logbook. The text in the logbook is changed into black when the ***Alarm*** or ***Warning*** is acknowledged.

Note: The ***Alarms*** are not active unless the mode is set to ***Enabled***.

Alarms in a network

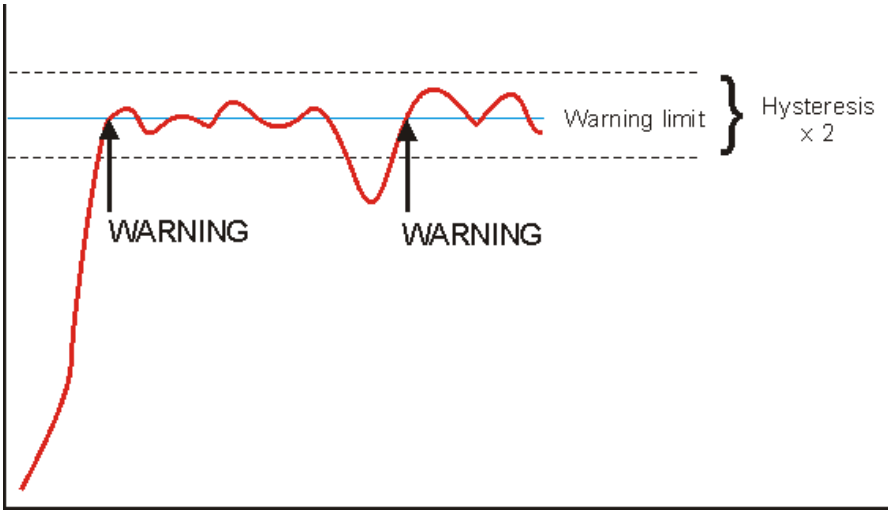
Alarms and warning messages are displayed on all stations with a connection to the concerned system. This is regardless of the activity that is currently performed in UNICORN and regardless of the identity and access rights of the current user.

Alarms and warnings can only be acknowledged from the station that is connected in control mode.

The hysteresis setting

The hysteresis setting (not available for ÄKTAdesign systems) for a warning determines to which extent the signal can oscillate up or down from the warning limit threshold without re-activating a warning.

After the signal has activated a warning, the warning will not be repeated as long as the signal remains within a window defined by the hysteresis setting above and below the warning limit. This prevents repeated warnings from noisy or oscillating signals close to the warning boundary.



Note: Hysteresis is only relevant for warnings, since an alarm puts the system into ***Pause*** mode at the first alarm.

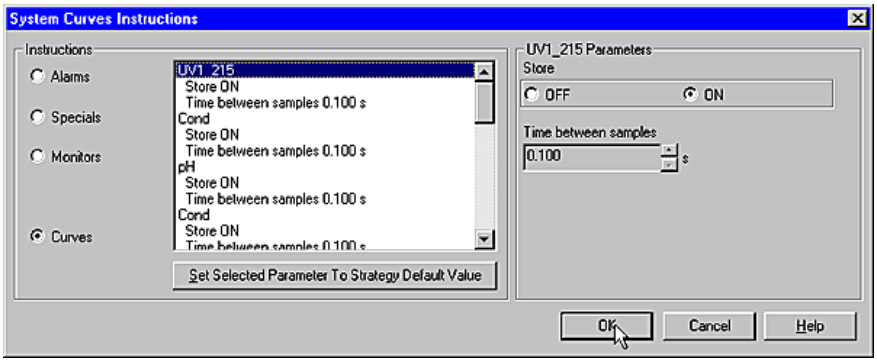
14.3 Curves

Introduction

This section is a short description of the ***Curves*** system settings.

The Instructions dialog box

The illustration below shows the ***Instructions*** dialog box with the ***Curves*** instructions selected.



Curve settings

The curve settings determine which monitor signals that will be stored as curves in the result file. Verify that ***Store:ON*** is set in the ***Instructions*** dialog box for all signals that are to be stored.

Note: If a curve is set to ***Store:OFF***, data from the specific monitor cannot be displayed in the curves window during a process run. The data will not be recorded in any way.

Store and Time between samples

The table below describes the function of the two curve settings.

Setting	Function
Store (OFF/ON)	This setting determines whether the curve data is stored or not.

Setting	Function
Time between samples	This setting determines with which frequency curve data is recorded. It does not affect the reading frequency of the actual monitor. Default value is the shortest possible time between samples.

15 System maintenance and error reporting

Introduction

This chapter describes the system maintenance and error reporting functions.

In this chapter

This chapter contains these sections:

15.1 System maintenance functions

Introduction

Some strategies support the capacity to view system information for the components in a chromatography unit. The system information can be used to issue maintenance warnings for the components.

This section describes the system maintenance functions.

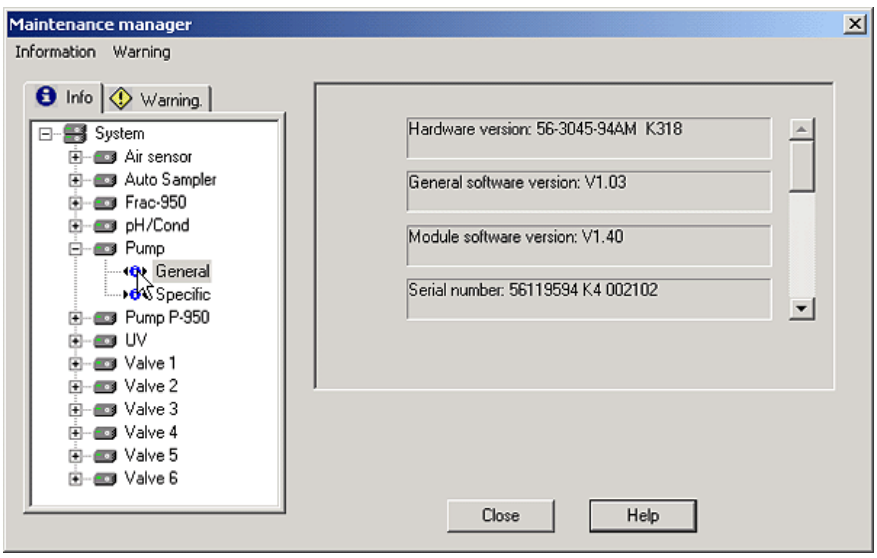
How to open the Maintenance manager

The system maintenance functions are controlled in the ***Maintenance manager*** dialog box in the ***System Control*** module.

- Select ***System:Maintenance***.

Result: The ***Maintenance manager*** dialog box opens with the ***Info*** tab selected. The connected chromatography system is scanned for its components. After a while the components are displayed.

The illustration below shows the ***Maintenance manager*** dialog box with the ***Info*** tab selected and general information about the pump displayed:



How to display component information

Click a component in the list to display the component information.

You can choose two different views:

- ***General***, e.g. serial number, version number etc.
- ***Specific***, e.g. how many hours a pump has been run etc.

How to set up a maintenance warning

The table below describes how to set up a maintenance warning.

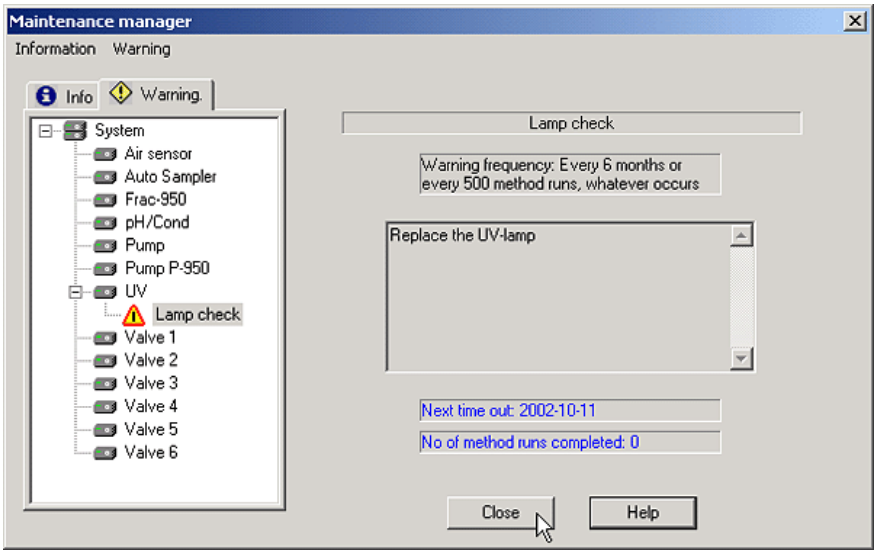
Step	Action
1	Click the ***Warning*** tab.
2	Select a component. • Choose ***Warning:New***. *or* • Right-click the component and select the ***New*** option on the shortcut menu.
3	• Type the appropriate value in the ***Periodicity*** field. • Type a warning text in the ***Pop up text*** box. • Type a name for the warning type in the ***Name*** text box. • Click the ***Save*** button.
4	Repeat steps 2 and 3 to set up more warnings.
5	Click the ***Close*** button.

How to view the warning parameters and counters

The component that has been set up for a maintenance warning is marked by an icon and the name of the warning.

- Select the warning to display the parameters.

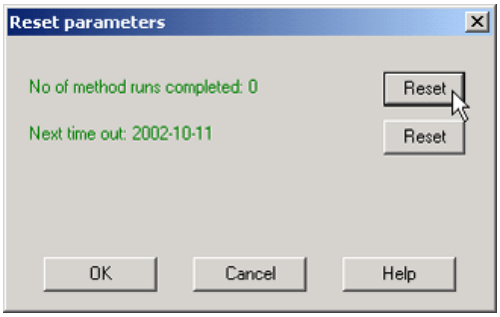
Counters show the remaining time or number of operations before the next maintenance warning. See the illustration below:



How to reset the counters

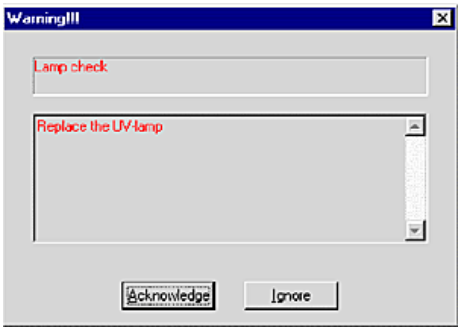
The table below describes how to reset the maintenance warning counters.

Step	Action
1	• Select ***System:Maintenance*** in the ***System Control*** module to open the ***Maintenance manager*** dialog box. • Click the ***Warning*** tab.
2	Select the warning you want to reset on the component list. • Choose ***Warning:Edit***. *or* • Right-click and select ***Edit*** on the shortcut menu. *Result*: The ***Maintenance manager*** dialog box changes into edit mode and the text boxes are activated.

Step	Action
3	• Type new text if necessary. • Click the ***Reset*** button. *Result*: The ***Reset parameters*** dialog box opens. 
4	• Click one or both of the ***Reset*** buttons to reset the counters. • Click ***OK***.

How to acknowledge a warning

Once a specific ***Periodicity*** parameter has been reached, a warning message will be displayed. The table below describes how to acknowledge the warning.

Step	Action
1	The ***Warning*** dialog box opens. 

Step	Action
2	• Click the ***Acknowledge*** button if you have corrected the problem. • Click the ***Ignore*** button if you haven't corrected the problem. **Note:** You will be reminded later about the unsolved problem if you click the ***Ignore*** button.

15.2 How to generate problem reports

Introduction

UNICORN contains a ***Generate Report Wizard*** for registration of errors or problems that you have detected or that occur during a run. The ***Generate Report Wizard*** takes you through the steps to generate your report.

There are two ways of accessing the ***Generate Report Wizard***:

- From the ***UNICORN Manager***
- From the ***System Control***.

In this section

This section contains these sub-sections:

15.2.1 How to generate a problem report from the UNICORN Manager

Introduction

The ***Generate Report Wizard*** is used to generate problem reports. This section describes how to generate a problem report from the ***UNICORN Manager***.

Step 1: How to create the report

The table below describes how to create a report with the ***Generate Report Wizard***.

Step	Action
1	Select ***Administration:Create System Report*** in the ***UNICORN Manager*** module.
2	The first step is a ***Welcome*** screen. • Click the ***Next*** button. *Result*: The ***Systems*** dialog box opens with a list of the available systems for the logged-on user.
3	Select a system for which the report is to be generated and click the ***Next*** button. *Result*: The ***Description*** dialog box opens.
4	Add the following information in the dialog box: • a short description of the problem • the circumstances under which the problem occurs • the consequences of the problem. Click the ***Next*** button. *Result*: The ***Reproducibility*** dialog box opens.

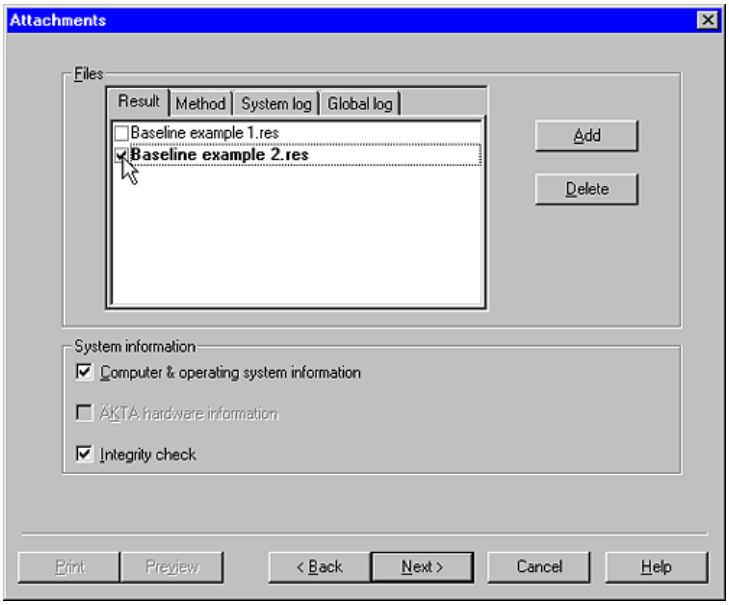
Step	Action
5	Specify whether the problem is reproducible or not. Select one of these alternatives: • ***Yes*** (Provide a short description in the text box of how the problem can be reproduced.) • ***No*** • ***Unknown***. Click the ***Next*** button to proceed to attach example files (see table below).

Step 2: How to attach a file

You can attach result files, method files and/or log files to the problem report.

The table below describes how to attach a file:

Step	Action
1	The ***Attachments*** dialog box is displayed:



Step	Action
2	• Depending on the character of the file to be attached, select the appropriate tab: ***Result***, ***Method***, ***System log*** or ***Global log***. • Attach the file: - Click the ***Add*** button. - Select a file in the dialog box and click the ***Attach*** or ***OK*** button. *Result*: The selected file is added to the tab in the ***Attachments*** dialog box. **Note:** To remove a file, select the check box and click the ***Delete*** button.
3	To include more information in the report, select the appropriate check boxes in the ***System information*** field. By default, all options are checked. **Computer & operating system information** A summary of the computer and operating system information, for example type of processor, processor speed, RAM, hard disk capacity and printer. **ÄKTA hardware information** A summary of the specific ÄKTAdesign hardware, for example the instrument and PROM version for every instrument that is connected. **Integrity check** When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check means that a new checksum calculation is performed for the same files in their folders. This new calculated value is compared with the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included. Click the ***Next*** button. *Result*: The ***Generate report*** dialog box is displayed.
4	Proceed to *Step 3: How to generate and save the report* below.

Step 3: How to generate and save the report

The table below describes how to generate and save the report:

Step	Action
1	By default, the report is saved in the folder `Unicorn\Reports`. If you want to save the report at another location, select a folder in the tree structure.
2	You also have these options: • Click the ***Preview*** button to open the report in Notepad. • Click the ***Print*** button to print the report without any preview.
3	Click the ***Finish*** button to generate and save the report.

15.2.2 How to generate a problem report from the System Control

Introduction

The ***Generate Report Wizard*** is used to generate problem reports. When an error message appears in ***System Control***, you can activate the report wizard from the error message dialog box. The ***Generate Report Wizard*** can also be activated anytime if you choose ***System:Report***.

Step 1: How to create the report

When an error message appears in ***System Control***, follow the instructions in this table to activate the ***Generate Report Wizard*** and create a report:

Step	Action
1	• Click the ***Report*** button in the error message dialog box. *or* • Choose ***System:Report***.
2	The first step is a ***Welcome*** screen. Click the ***Next*** button. *Result*: The ***Description*** dialog box is displayed and shows a list of the problems/errors that have occurred. All the problems/errors that have occurred, together with help texts, are automatically recorded and included in the report. **Tip:** If you select a specific error in the ***Description*** dialog box, the appropriate help text is shown in the error message box.
3	Add the following information in the ***Description*** dialog box: • A short description of the problem. • The circumstances under which the problem occurs. • The consequences of the problem. Click the ***Next*** button. *Result*: The ***Reproducibility*** dialog box opens.

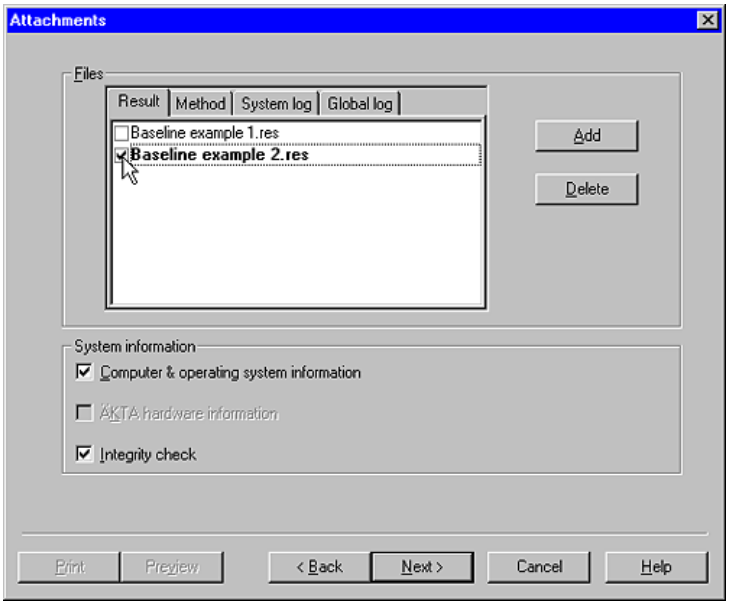
Step	Action
4	Specify whether the problem is reproducible or not. Select one of these alternatives: • ***Yes*** (Provide a short description in the text box of how the problem can be reproduced.) • ***No*** • ***Unknown***. Click the ***Next*** button to proceed to attach example files (see table below).

Step 2: How to attach a file

You can attach method files and/or log files to the problem report.

The table below describes how to attach a file:

Step	Action
1	The ***Attachments*** dialog box is displayed:



Step	Action
2	• Depending on the character of the file to be attached, select the appropriate tab: ***Result***, ***Method***, ***System log*** or ***Global log***. • Attach a file: - Click the ***Add*** button. - Select a file in the dialog box and click the ***Attach*** or ***OK*** button. *Result*: The selected file is added to the tab in the ***Attachments*** dialog box. **Note:** To remove a file, select the checkbox and click the ***Delete*** button.
3	To include more information in the report, select the appropriate check boxes in the ***System information*** field. By default, all options are checked. **Computer & operating system information** A summary of the computer and operating system information, for example type of processor, processor speed, RAM, hard disk capacity and printer. **ÄKTA hardware information** A summary of the specific ÄKTAdesign hardware, for example the instrument and PROM version for every instrument that is connected. **Integrity check** When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check means that a new checksum calculation is performed for the same files in their folders. This new calculated value is compared with the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included. Click the ***Next*** button. *Result*: The ***Generate*** report dialog box is displayed.
4	Go to step 3 below.

Step 3: How to generate and save the report

The table below describes how to generate and save the report.

Step	Action
1	By default, the report is saved in the folder: `Unicorn\Reports`. If you want to save the report in another location, select a folder in the tree structure.
2	You also have these options: • Click the ***Preview*** button to open the report in Notepad. • Click the ***Print*** button to print the report without any preview.
3	Click the ***Finish*** button to generate and save the report.

Appendix A Troubleshooting

Introduction

This appendix describes different problems which may arise in UNICORN and how to solve the problems.

In this appendix

This appendix contains these sections:

A.1 Logon

In this section

This section describes how to solve the following log on problems:

- Unable to log on to UNICORN
- Error message "Strategy file error".

Unable to log on to UNICORN

The table below describes some log on problems and their solutions:

Problem description	Solution
You have forgotten your password.	Ask the system administrator to supply a new password.
Username and password not accepted You cannot log on although you use your correct username and password. *Reason*: The file `USERS30.MPM` in the folder `\UNICORN\SERVER\FIL` could be corrupt.	• Restore the file USERS30.MPM from the latest back-up copy *or* • reinstall the default user.
No user names: Remote station Both these conditions must apply: • The ***User name*** drop-down box in the ***Logon*** dialog box is empty. • You are trying to log on from a remote station in a network installation.	Make sure that the computer is logged on to the network before you start UNICORN. *Note*: A remote station accesses the user list directly from the network server.
No user names: Local station The user list on a local station in a network installation is not up to date.	Make sure that the computer is logged on to the network before starting UNICORN. *Note*: The user list is stored locally on a local station, and is updated automatically from the network server if the computer is logged on to the network.

Error message "Strategy file error"

The table below describes some problems and their solutions:

Problem description	Solution
Stand-alone installation If you receive the error message "Strategy file error" in a *stand-alone* installation, the strategy file is probably corrupt.	Reinstall the strategy as described in the Administration and technical manual "Install selected software components after the initial installation".
Network installation In a *network* installation, the error message "Strategy file error" may appear if you try to create a method for a system not physically connected to the computer.	Make sure that the computer is logged on to the network before UNICORN is started, so that the strategy file on the server disk is accessible.

A.2 UNICORN access

In this section

This section describes how to solve the following UNICORN access problems:

- Unable to access certain UNICORN functions
- Connection problems
  - Connections are not available
  - System is not available
  - Error message in a network installation
  - You cannot control the system
- ***Run data Connection*** in ***System Control*** displays a "NO [1]", "NO [2]" or "NO [3]".

Unable to access certain UNICORN functions

The table below describes an access problem and its solution:

Problem description	Solution
UNICORN functions to which you do not have access appear grey in the menu and cannot be used.	Choose ***Administration:User Setup*** in the ***UNICORN Manager*** to change the user profile. *Note*: Contact the system administrator if you are not authorized to change your user profile.

Connection problems

The table below describes some connection problems and their solutions:

Problem description	Solution
The connections are not available.	• Check the connection between the PC and the chromatography system. • Check that the power to the chromatography system is turned on.

Problem description	Solution
The connections are not available even though • the connection between PC and chromatography system appears to be correct • the power is turned on.	• Quit UNICORN. • Shut down and switch off the computer. • Switch off the chromatography system. • Restart the entire system.
A system is not available when you attempt to establish a connection.	Check that you have access rights to the system. Access rights are not automatically assigned for a newly defined system.
You receive the error message "Cannot connect to system..." in a network installation.	• Check that the local computer to which the system is connected is turned on and logged on to the network. • Check that the computer where you try to establish a connection is logged on to the network. • Check that the limit of 8 connections to the system has not been exceeded.
You can establish a connection but cannot control the system, that is the ***Manual*** menu commands in the ***System Control*** are grey.	• Check that no other user has a control mode connection. • Check that you have sufficient access rights to control the system manually.

Note: The ***Method Wizard*** can be used on a local system even if the network connection is not established.

The Connection field in System Control displays a "NO [X]"

The table below describes some connection problems and their solutions:

Problem Description	Solution
The ***Connection*** field in the ***Run data*** pane in ***System Control*** says "NO [1]" or "NO [2]".	• Check that the UNICORN PC Control board is configured according to the settings made during the installation of the program. The same Control unit number, Address and IRQ must be set at the Control board, see the Administration and technical manual "Hardware installation". • The communication may also fail if there is a conflict between the UNICORN PC Control board configurations and other boards in the PC. If so, select a free Address and a free IRQ during UNICORN installation and at the Control Board, see the Administration and technical manual "Hardware installation".
The ***Connection*** field in the ***Run data*** pane in ***System Control*** says "NO [3]".	• Choose ***Administration:System Setup*** in the ***UNICORN Manager***. - Select the system with problems in the dialog box and click the ***Edit*** button. - Check that the strategy, computer name and the control number are correct according to the installation at the local station which is physically connected to the system. See the Administration and technical manual "System definitions". • If you connect remotely to a system - check that the local station which is physically connected to the system is turned on - check that the network is functioning at both the remote and the local station. • Check that the limit of eight connections to the system has not been exceeded.

A.3 Methods and method runs

In this section

This section describes how to solve the following method and method run problems:

- Cannot perform ***Quit*** or ***Logoff***
- Monitor signals do not appear in the Curves pane in ***System Control***
- Error message "Couldn't create result file... Destination path could not be found"
- The ***Method-System Connection*** dialog box keeps appearing
- The ***Method Editor*** window does not fit on the screen
- There are red instructions in a method
- After Windows™ logout and login you cannot get a system connection
- The ***Print screen*** command does not send a copy of the screen to the printer

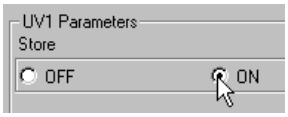
Cannot perform Quit or Logoff

The table below describes a problems and its solutions:

Problem description	Solution
You are unable to perform ***Quit*** or ***Logoff*** from UNICORN for a connection.	You might be running a ***Scouting*** method or a ***MethodQueue***. These functions require a control mode connection in order to start subsequent cycles correctly. *Action*: Stop the ***Scouting*** method or ***MethodQueue*** before you quit or log off.

Monitor signals do not appear in the Curves panel in System Control

The table below describes a problem and its solution:

Problem description	Solution
Monitor signals do not appear in the ***Curves*** pane in ***System Control***.	• Choose ***System:Settings*** in ***System Control*** *Result*: The ***System Instructions*** dialog box opens. • Choose the ***Curves*** group in the ***Instructions*** field. • Set the ***Store*** option to ***ON***.  Signals for which ***Store*** is set to ***ON*** can be selected from the ***View:Properties:Curves*** dialog box in ***System Control***.

Error message "Couldn't create result file... Destination path could not be found"

The table below describes a problem and its solution:

Problem description	Solution
If you receive the error message "Couldn't create result file... Destination path could not be found" at the end of a method, the local computer was unable to access the folder specified in the result file path.	This may happen if the specified folder is on the network server and network communication has been lost. The result file is saved in the ***Failed*** folder on the local station.

The Method-System Connection dialog box keeps appearing

The table below describes a problem and its solution:

Problem description	Solution
If the ***Method-System Connection*** dialog box keeps appearing you have some method(s) which is not connected to a system. *Reason*: Most likely you have imported some method(s) with the command ***File:Copy from External*** in the ***UNICORN Manager***.	Connect the method(s) to the appropriate system.

The Method Editor window does not fit on the screen

The table below describes a problem and its solution:

Problem description	Solution
The ***Method Editor*** window does not fit the screen and has scroll bars. *Reason*: The incorrect font size might be installed.	• The display screen resolution may be set to "1024x768x65536" with "Large fonts". You need to install the "Small fonts". This requires that you have the Windows XP or Windows 7 DVD-ROM that was shipped with your computer. • Insert the CD-ROM and follow the directions on the screen.

Note: Always install the latest service pack after you have installed something from the Windows XP/Windows 7 DVD-ROM.

There are red instructions in a method

The table below describes some solutions to syntax error problems:

Problem description	Solution
Red instructions (instructions with a red dot) in a method are syntax errors and may be due to the following: • The method was connected to the wrong system, that is the strategy of the system is incompatible with the method. • The method instructions do not correspond to the components you have chosen for your system. Check your system components under ***Administration:System Setup*** in the ***UNICORN Manager***. • The ***Copy*** function was used instead of ***Copy from external*** when a method was imported from a diskette. • The wrong system may have been selected in the ***Save As*** dialog box in the ***Method Editor***. • You may also have templates not intended for your system, which might be the case for custom designed systems. • The systems strategy has been updated with a new strategy that differs in the instruction set.	There are several actions that you can take: • Check that the method has been connected to the correct system in either of these ways: - in the ***System Method Connection*** dialog box when you use the ***Copy from external*** dialog box - in the ***Save As*** dialog box in ***Method Editor***. • If the system is custom designed, open the ***Method Editor***, select the red instruction and either delete it or replace it with a corresponding instruction (if available) from the ***Instruction box***. Repeat this for all red instructions before saving the method.

Print screen does not send a copy of the screen to the printer

The table below describes how to solve a printing problem:

Problem description	Solution
The ***Print screen*** command only makes a copy of the screen to the clipboard and not to the default printer.	If you want to print the view on the screen, press the ***<Print Scrn>*** key and paste the image from the clipboard into an appropriate program, such as Microsoft™ Paint, and then print out the image.

A.4 Evaluation

In this section

This section describes how to solve the following evaluation problems:

- Incorrect date and time in the result file
- Evaluation procedure aborts

Incorrect date and time in the result file

The table below describes a problem and its solution:

Problem description	Solution
The result file shows incorrect date and time.	Check the system clock setting. The date and time recorded in the result file are taken from the PC system clock setting.

Evaluation procedure aborts

The table below describes a problem and its solution:

Problem description	Solution
The evaluation procedure aborts.	Instructions in an evaluation procedure refer to curves by identification number irrespective of the curve names. Make sure that the curves processed when the procedure is executed are compatible with those processed when it was recorded. An evaluation procedure aborts if you try to store resulting curves at the position of an original raw data curve.

A.5 ÄKTAdesign system specific problems

In this section

This section describes how to solve the following problems:

- Connected to a system but no system contact
- Flow scheme not displayed properly

Connected to a system but no system contact

The table below describes a problem and its solution:

Problem description	Solution
You are connected to a system but have no system contact. *Indications*: In the ***System Control***, • the option ***Connection*** in the ***Run data*** pane says "Yes", • the option ***Instruments*** says "Scanning", • there is no contact with the system after a period of waiting.	• Check that the system is turned on. • Check that all the cable connections are intact. • If the above actions do not help, try to restart both the computer and the system.

Flow scheme not displayed properly

The table below describes a problem and its solution:

Problem description	Solution
The flow scheme is not displayed properly.	Choose ***Settings:Control Panel: Display:Settings*** in the Windows Start menu to check that you have selected 65536 colors.

Appendix B Evaluation functions and instructions

Introduction

This appendix describes the functions that are implemented in the ***Evaluation*** module.

In this appendix

This appendix contains these sections:

B.1 Smoothing algorithms

Introduction

This section describes how the smoothing functions are calculated. Choose ***Operations:Smooth*** in the ***Evaluation*** module to view and edit the options.

Moving Average

The table below describes the process when the ***Moving Average*** smoothing algorithm is used.

Stage	Description
1	For each data point in the source curve, the processed curve is calculated as the average of the data points within a window centered on the source data point. • The width of the window is determined by the parameter value, expressed as number of data points.
2	When the source point is less than half the window size from the beginning of the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible. • If you increase the window width, the smoothing effect is also increased.

Note: The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given, it is incremented by one (1).

Autoregressive

The table below describes the process when the ***Autoregressive*** smoothing algorithm is used:

Stage	Description
1	The first data point in the source curve is copied to the processed curve.
2	For each subsequent data point, the previous processed point is multiplied with the parameter value and added to the current source data point.

Stage	Description
3	The result is then divided by the parameter value plus 1 according to the following formulae: $t_1 = S_1$ $t_n = \frac{(p * t_{n-1} + S_n)}{(p+1)}$ Where: t_n = current processed point. t_{n-1} = previous processed point. S_n = current source point. p = smoothing parameter value. *Note*: If you increase the parameter value, the smoothing effect is also increased.

Note: The filter algorithm only accepts integer parameter values between 1 and 25.

Median

The table below describes the process when the ***Median*** smoothing algorithm is used.

Stage	Description
1	For each data point in the source curve, the processed curve is calculated as the median of the data points within a window centered on the source data point. • The width of the window is determined by the parameter value, expressed as number of data points.
2	When the source point is less than half the window size from the beginning of the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible. • If you increase the window width, the smoothing effect is also increased. • To completely remove a noise spike, the window width should in effect be slightly more than twice the width of the spike.

Note: The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given, it is incremented by one.

Savitzky-Golay

The table below describes the process when the ***Savitzky-Golay*** smoothing algorithm is used.

Stage	Description
1	The algorithm is based on performing a least squares linear regression fit of a polynominal of degree k over at least k+1 data points around each point in the curve to smoothen the data. The derivate is the derivate of the fitted polynominal at each point. The calculation uses a convolution formalism to calculate 1st through 9th derivatives.
2	The calculation is performed with the data in low X to high X order. If the input trace goes from low to high, it is reversed for the calculation and is re-reversed afterwards.

Note: See Gorry, Peter A, General Least-Squares Smoothing and Differentation by the Convolution (Savitsky-Golay) Method (Analytical Chemistry 1990, Volume 62, 570-573) for more information on the Savitzky-Golay algorithm.

B.2 Baseline calculation theory

Overall process

The table below describes the overall process of a baseline calculation.

Stage	Description
1	The baseline segments are defined.
2	The baseline points are selected.
3	The baseline is drawn.

Baseline segment definition

Baseline parameters are used to find the baseline segments. The default values for the parameters are determined from the source curve. The baseline segments are found by different parameters that are based on the type of algorithm that is selected.

Note: The parameters can be displayed in the ***Evaluation*** module if you choose ***Integrate:Calculate baseline*** function. You can also click the ***Baseline settings*** button in the ***Integrate:Peak integrate*** dialog box.

Morphological algorithm

The ***Morphological*** algorithm searches for all parts of the source curve where:

- The curve parts come into contact at both ends of a horizontal line of the length defined in the ***Structure width*** parameter. The default value of this parameter is based on the widest detected peak in the curve. The horizontal line is moved along the curve up the peak until it reaches the contact points. The curve parts below the horizontal line and the line will now form a "curve" with a plateau. The center point in the plateau formed by the horizontal line will be the data point for the baseline.
- The data points fulfil the ***Minimum distance between data points***. This parameter reduces the total number of data points that are created from a curve.

Classic algorithm

The ***Classic*** algorithm searches for all parts of the source curve where:

- The curve parts are longer than the ***Shortest baseline segment***. This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.
- The curve has no point outside the ***Noise window***. The noise window is defined as a rectangular corridor parallel to the slope of the curve and centered on the first and last points within the currently inspected segment.
- The slope is less than the ***Slope limit***. This limits the maximum slope of the baseline to differentiate baseline segments from peaks.
- The curve parts are lower than the ***Max baseline level***. This parameter determines the highest acceptable signal level for the baseline.

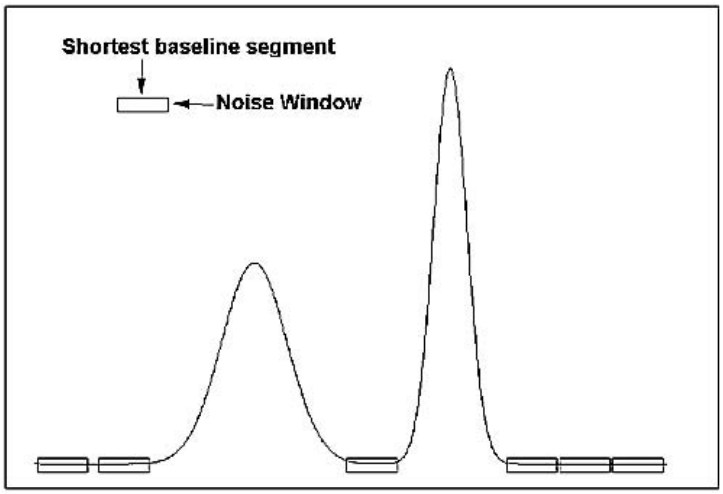
Baseline parameters

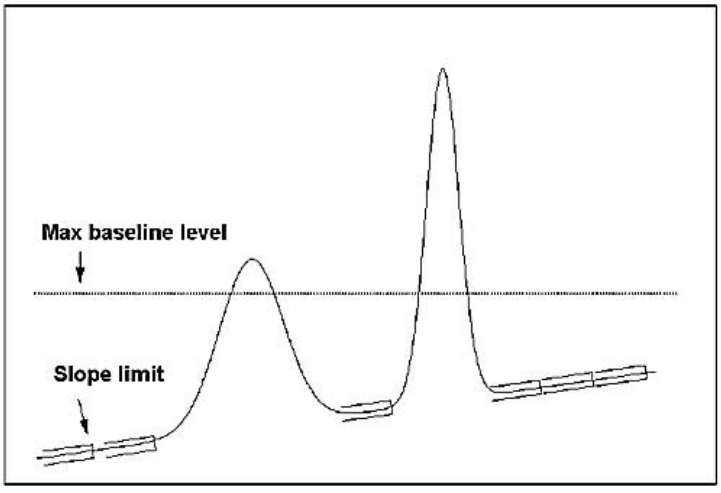
The baseline parameters can be illustrated as a rectangular box that the source curve has to fit into in order to be identified as a baseline segment, where:

- The length of the box corresponds to the ***Shortest baseline segment***.
- The height of the box corresponds to the maximum level of noise on the baseline segments. This is referred to as the ***Noise window***.
- The box is allowed to be tilted with a maximum slope corresponding to the ***Slope limit***.
- The box is not allowed to move up above the ***Max baseline level***.

Baseline parameters - illustration

The illustrations below shows the baseline parameters graphically.





Baseline segment identification

The table below describes the baseline segment identification process:

Stage	Description
1	The box is virtually moved along the source curve in steps of one third of the ***Shortest baseline segment*** length to look for baseline segments.
2	A baseline segment is found whenever the currently examined part of the source curve fits completely within the box.
3	The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the ***Slope limit***.

Baseline points (Classic algorithm)

When the baseline segments have been defined and joined, they are replaced by baseline points at the start and end of each segment. The line between these is also filled with points.

Note: The baseline points are shown as green squares in the ***Integrate:Edit baseline*** function of the ***Evaluation*** module.

Baseline drawing

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points. However, the curve does not necessarily pass through the baseline points. The baseline will be a smoothly curved function passing close to or through the points.

To reduce the effect of noise at the peak integration, the created baseline is forced equal to the source curve in every position where the difference between the baseline and the source curve is small enough. Choose ***Integrate:Calculate Baseline***. If the ***Accept negative peaks*** option is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

How to measure the baseline segment (Classic algorithm)

You can try to measure the ***Shortest baseline segment*** length directly on your chromatogram. The table below describes how to do this:

Step	Action
1	Locate the shortest segment of the curve that you consider a part of the baseline.
2	Use the marker box on the chromatogram to measure the length of the segment.
3	Choose ***Integrate:Calculate Baseline*** and insert this value as the ***Shortest baseline segment*** value.

How to measure noise level (Classic algorithm)

Curve coordinates can also be used to measure noise levels on the source curve. The table below describes how to do this:

Step	Action
1	Use the ***Zoom*** function to focus on a part of the curve that is representative for the baseline noise.
2	Select an appropriate Y-axis scale.
3	Measure the Y-axis coordinates.
4	• Calculate the noise range as the difference between the max. and min. values. • Add an extra 20%. • Choose ***Integrate:Calculate Baseline*** and insert this value as the ***Noise window*** value.

How to measure the slope limit (Classic algorithm)

The table below describes how to measure the slope at any part of the curve.

Stage	Description
1	Select ***Operations:Differentiate*** in the ***Evaluation*** module. *Result*: The ***Differentiate*** dialog box opens.
2	• Select the desired source curve. • Select the ***First order*** calculation option. • Click ***OK***. *Result*: The differentiated curve will appear in the active chromatogram.
3	Select an appropriate Y-axis scale, right-click and select ***Marker*** to measure the Y-axis values for the differentiated curve with the curve coordinates function. *Result*: The Y-axis value is interpreted as the UV curve slope at the selected retention point.
4	• Determine the highest slope value of the baseline (non-peak) part of the curve. • Add 10%. • Select ***Integrate:Calculate Baseline*** and use this value as the ***Slope limit***.

Note: If the differentiated curve is very noisy, it can be filtered with a light ***Moving average*** filter in the ***Operations:Smooth*** function.

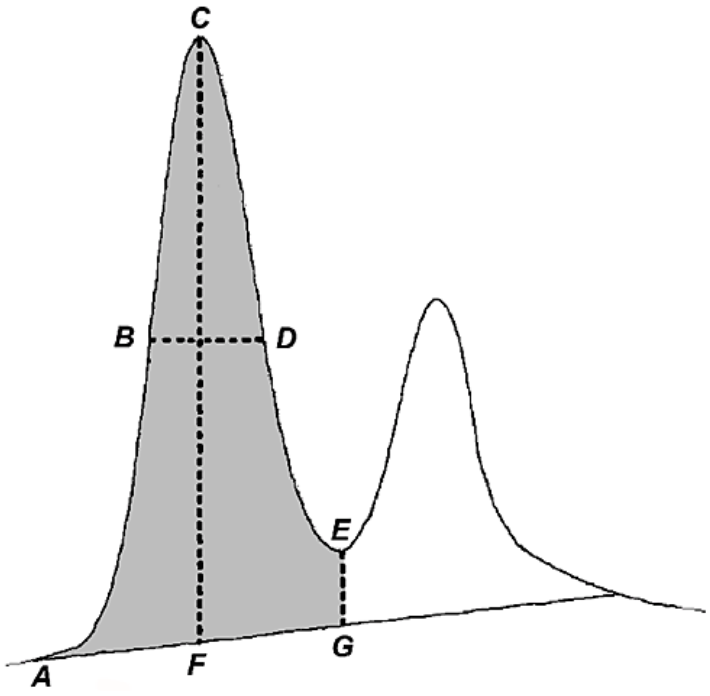
B.3 Peak table column components

Introduction

This section contains a list of peak parameters with explanations and calculation formulae when applicable.

Peak parameters - illustration

The diagram below illustrates the peak parameters. See the parameter list below for explanations.



Peak parameter descriptions

The list below contains descriptions of the peak parameters.

Parameter	Description
Amount	Values calculated by the ***Analysis*** module. (Only available if the ***Quantitation*** module is installed.)
Area	Calculated as the area between the curve and baseline, between the peak start and peak end, time or volume base. (Gray area in the diagram above.)
Asymmetry	Peak asymmetry (indicator of column packing). See definition below this table.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end. (A, F and G in the diagram above.)
Capacity factor	The capacity factor will only be calculated when the chromatogram is in volume base. The total liquid volume, Vt, must be entered in the ***Integrate*** dialog box for this parameter to be calculated. See definition below this table.
Concentration	Values calculated by the ***Analysis*** module. (Only available if the ***Quantitation*** module is installed.)
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Height	Maximum amplitude above the baseline. (C-F in the diagram above)
Kav	Gel phase distribution constant in gel filtration. Kav will only be calculated when a gel filtration column was used and when the chromatogram is in volume base. The void volume, V0, must be entered in the ***Integrate*** dialog box for this parameter to be calculated. See definition below this table.
Molecular size	Values calculated by the ***Analysis*** module. (Only available if the ***Quantitation*** module is installed.)
Plate height (HETP)	Height equivalent to theoretical plate and plates/meter. The column height must be entered in the ***Integrate*** dialog box for this parameter to be calculated. See definition below this table.
Peak endpoint heights	Amplitude above the baseline at left (A in the diagram above) and right peak limits (E-G in the diagram above).
Peak endpoint retention	Retention value at peak start and peak end, time or volume base. (A and G in the diagram above.)

Parameter	Description
Peak name	Name of the peak.
Percent of total area	Peak area as a percent of the total area under the curve above the baseline. Time or volume base. *Note*: This value can differ in time and volume base if the flow rate is not constant throughout the method.
Percent of total peak area	Peak area as a percent of the sum of all integrated peaks. *Note*: This value can differ in time and volume base if the flow rate is not constant throughout the method.
Resolution	Peak resolution. See definition below this table.
Retention	Retention at the peak maximum, time or volume base. (C in the diagram above.)
Sigma	Standard deviation for a Gaussian-shaped peak. See definition below this table.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or dropline to the baseline or skim line.
Width	Difference in retention between the peak end and peak start, time or volume base. (G-A in the diagram above.)
Width at half height	Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above the baseline. Time or volume base. (B-D in the diagram above, where BD bisects CF.)

Note: In the ***Options*** dialog box in the ***UNICORN Manager*** you can select if negative retentions should be displayed or not. The default selection is that negative retention is not displayed.

Sigma formula

The formula below is used to calculate ***Sigma***.

$$\text{Sigma} = \sqrt{\frac{\sum_{i=1}^{n} \left(y_i \left(x_i - x_{ymax} \right)^2 \right)}{A_{peak}}}$$

Where:

- *n* is the number of data points.
- *x* is the volume or time value.
- x_{ymax} is the volume or time value at the maximum amplitude value.
- A_{peak} is the area of the peak.

Note: The peak width for a Gaussian peak is (4 x Sigma).

Peak resolution algorithms

The peak resolution is calculated with one of the following three algorithms:

1 $(V_{R2} - V_{R1}) / ((W_{b2} + W_{b1}) / 2)$

2 $(V_{R2} - V_{R1}) / ((Sigma_2 + Sigma_1) \times 2)$

3 $((V_{R2} - V_{R1}) / (2 \times (W_{h2} + W_{h1}))) / 2.354$

Where:

- V_{R1}, W_{b1}, $Sigma_1$ and W_{h1} are the retention, width, Sigma and width at half height of the previous peak.
- V_{R2}, W_{b2}, $Sigma_2$ and W_{h2} are the retention, width, Sigma and width at half height of the current peak.

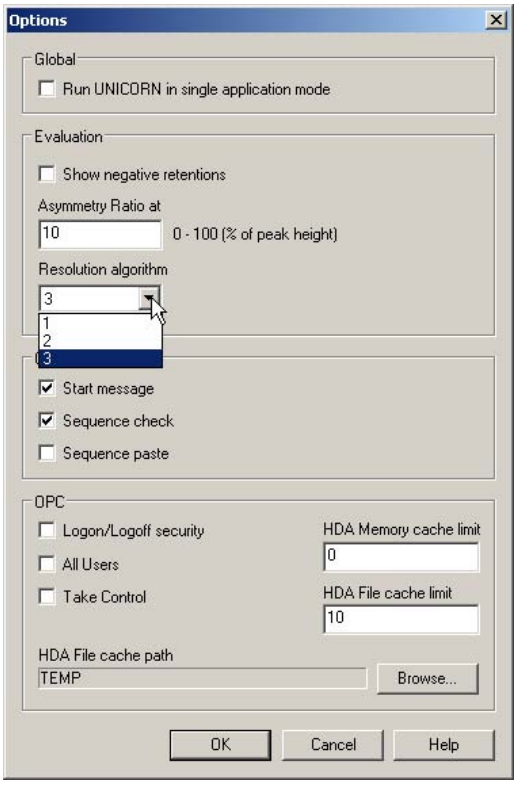
Note: The ***Resolution algorithm*** variable in the ***Options*** dialog box in the ***UNICORN Manager*** determines which of the three algorithms is used. If this variable has the value 1, 2 or 3, then the algorithm with the corresponding number in the list above is used. The default value is 3.

How to change the peak resolution algorithm

The table below describes how to change the peak resolution algorithm in the ***UNICORN Manager***.

Step	Action
1	• Choose the ***Administration:Options...*** menu item. *Result*: The ***Options*** dialog box opens.

Step	Action
2	• Select the desired algorithm number described as described in ***Peak resolution algorithms*** above, in the ***Resolution algorithm*** droplist.



	• Click ***OK***.
	Result: The dialog box closes and the peak resolution algorithm is changed.

Note: You must repeat the peak integrations after the change to update the values based on the new algorithm.

Capacity factor formula

The formula below is used to calculate the ***Capacity factor***.

$$k' = \frac{V_R - V_t}{V_t}$$

Where:

- V_R = retention volume.
- V_t = total liquid volume.

Kav formula

The formula below is used to calculate ***Kav***.

$$k_{av} = \frac{V_R - V_0}{V_C - V_0}$$

Where:

- V_R = retention volume.
- V_0 = void volume.
- V_C = column volume.

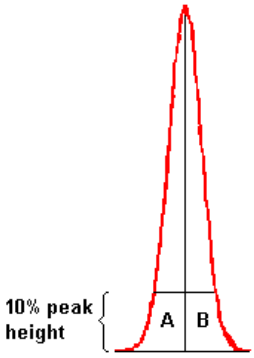
Asymmetry formula

The formula below is used to calculate the ***Asymmetry***.

Asymmetry = B / A

Where:

- A is a partial peak width, measured at a percentage of the peak height, for the leading part of the peak.
- B is a partial peak width, measured at a percentage of the peak height, for the tailing part of the peak.



How to change the Asymmetry Ratio

The ***Asymmetry Ratio*** is selected in the ***Options*** dialog box in the ***UNICORN Manager***. The table below describes how to select a value:

Step	Action
1	• Choose the ***Administration:Options...*** menu item. *Result*: The ***Options*** dialog box opens.
2	• Type a ratio value in the ***Asymmetry Ratio at*** text box. • Click ***OK***. *Result*: The ratio value is changed and the dialog box closes.

Note: You must repeat the peak integrations after the change to update the values based on the new asymmetry ratio. The default ratio is 10%.

HETP formula

The formula below is used to calculate the ***HETP*** value.

HETP = L/N

$N = 5.54 \times (V_R/w_h)^2$ assuming a Gaussian peak.

Where:

- N = no. of theoretical plates.
- L = bed height in cm.
- V_R = peak retention (elution) volume or time.
- w_h = peak width at half height expressed in the same units as V_R.

B.4 Procedure instructions

Introduction

This section contains lists of procedure instructions with descriptions. These instructions are used in the ***Procedure Editor***. Choose ***Procedures:Edit:New*** in the ***Evaluation*** module to view the ***Instruction*** list.

Curve operation

The table below contains a list of instructions for curve operations.

Instruction	Description
ADD	Adds two curves to produce a third curve, which is the sum of the two curves. The two source curves must have the same Y-axis unit and not be fraction or injection curves, or else a run time error will occur.
AMP_MUL	Multiplies the amplitude of the source curve by the multiplication factor and stores the result in the target curve position.
AMP_SHIFT	Shifts the amplitude of the source curve by the shift factor and stores the result in the target curve position.
CLEAR	Clears the specified curve from the working memory of the computer.
COPY	Copies the source curve to the target curve position.
CUT	Cuts out the part of the source curve between the ***Left*** and ***Right*** limits and stores the result in the target curve position.
DERIVATE	Differentiates the source curve (first or second order) and stores the result in the target curve position. The Y-axis of the target curve position will be a normalized scale without unit.
DIV	Divides two curves to produce a third curve, which is the quotient of the two curves. The two source curves can have any Y-axis unit. The Y-axis of the target curve position will be a normalized scale without unit.

Instruction	Description
HISTOGRAM	Creates a histogram from any non-fraction curve (source curve 1) and a fraction curve (source curve 2_frac), and stores the result in the target curve position. If source curve 2 is not a fraction curve a run time error will occur. The Y-axis of the target curve position will be the same as that of the first source curve.
INTEGRATE	Performs a mathematical integration of the source curve and stores the result in a ***Result*** curve. This instruction is not the same as ***Peak integrate***, which performs a real peak integration.
POOL_FRACTIONS	Pools fractions from the source curve and stores the result in the target curve position. The fractions are pooled from the first selected fraction to the last selected fraction. If the source curve is not a fraction curve, or ***First*** or ***Last*** is not an existing identification, a run time error will occur.
RET_MUL	Multiplies the retention of the source curve by the ***Multiplication*** factor and stores the result in the target curve position.
RET_SHIFT	Shifts the retention of the source curve by the ***Shift*** factor and stores the result in the target curve position.
SIMULATE_PEAK_FRAC	Simulates ***Peak Fractionation***.
SMOOTH_AR	Smooths the source curve with an autoregressive filter and stores the result in the target curve position. The ***Filter*** parameter decides the strength of the filter.
SMOOTH_MA	Smooths the source curve with a moving average filter and stores the result in the ***Resulting Curve***. The ***Filter width*** parameter decides how many samples wide the filter is.
SMOOTH_MEDIAN	Smooths the source curve with a median filter and stores the result in target curve position. The ***Filter width*** parameter decides how many samples wide the filter is.
SMOOTH_SG	Smooths the curve with the Savitzky-Golay algorithm.
SUB	Subtracts two curves to produce a third curve, which is the difference of the two curves. The two source curves must have the same Y-axis unit and not be fraction or injection curves.

Instruction	Description
TDIV	Divides two curves to produce a third curve, which is the quotient of the two curves. The two source curves can have any Y-axis unit. The threshold values are used to avoid division of numbers close to zero. At those points where source curve 1 has an amplitude less than ***Threshold1***, or the source curve 2 has an amplitude less than ***Threshold2***, the result of the division is defined to be 1.0.

Integration

The table below contains a list of instructions for integration.

Instruction	Description
CALCULATE_BASELINE	Calculates a baseline from the source curve. The baseline is stored in the target curve position. ***DEFAULT*** can be selected in the ***Baseline*** parameters, which will then calculate default baseline parameters for each new curve.
CALCULATE_BASELINE_MORPH	Calculates a baseline from the curve crvSrc using a morphological method. ***DEFAULT*** can be selected in the ***Baseline*** parameters, which will then calculate default baseline parameters for each new curve. The baseline is stored in curve crvDst.
CLEAR_PEAKTABLE	Clears the peak table in ***Peak table source*** from the computer memory.
COPY_PEAKTABLE	Copies a peak table from ***Peak table source*** to ***Resulting peak table***.
NEGATIVE_PEAKS	Controls the baseline behavior in subsequent baseline calculations. If ***ONOFF*** is ***ON*** then the baseline can be drawn above the curve and negative peaks can be detected by ***PEAK_INTEGRATE***. If ***ONOFF*** is ***OFF*** then the baseline is never drawn above the curve.
PEAK_INTEGRATE	Performs a peak integration on the source curve and stores the resulting peak table in ***Resulting peak table***. It is assumed that the baseline is subtracted.
PEAK_WINDOW	Specifies which part of the source curve that will be integrated. Peaks between retention ***Left*** limit and ***Right*** limit will be detected if the ***ONOFF*** parameter is set to ***ON***. If ***ONOFF*** is set to ***OFF***, the whole curve will be used for integration.

Instruction	Description
REJECT_PEAKS	Any combination of conditions is allowed. If all parameters are ***OFF*** then every detected peak is included in the peak table.
SET_COLUMN_HEIGHT	Sets the column height for the peak integration calculation of the ***HETP*** value. The ***Column height*** parameter is the height of the column in centimetres. If ***Column height*** is ***OFF*** then the ***HETP*** value is not calculated for the following integrations.
SET_COLUMN_V0	Sets void volume for ***Kav*** peak integration calculation.
SET_COLUMN_VT	Sets the total liquid volume for peak integration calculation of the capacity factor.
SET_SKIM_SIZE_RATIO	Sets the ***Skim size ratio*** to be used in the following peak integration(s).
WINDOW_PEAK_INTEGRATE	Integrates the curve within the peak window. All curve parts outside the peak window remain unchanged.

File operation

The table below contains a list of instructions for file operations.

Instruction	Description
CURVE_OPEN	Opens the curve specified in the ***Result*** file defined in ***File name*** and stores it in target curve position. If "*" is entered as ***File name*** the current result file will be used. The ***File name*** parameter may include a path from the users root folder.
IMPORT_CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.
IMPORT_PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.
PEAKTABLE_OPEN	Opens the specified ***Peak table*** in the ***Result*** file defined in ***File name*** and stores it in the ***Resulting peak table***. If "*" is entered as ***File name*** the current ***Result*** file will be used. The ***File name*** parameter may include a path from the current users root folder.

Export

The table below contains a list of instructions for export operations.

Instruction	Description
EXPORT_CURVE_AIA	Exports the curve in AIA format.
EXPORT_CURVE_ASCII	Exports the ***Source curve*** to the file defined in ***Export to File in ASCII format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. In the part of the source curve limited by the ***Left*** limit and ***Right*** limit every <n> sample is exported.
EXPORT_CURVE_WKS	Exports the source curve to the file defined in ***Export to File in WKS format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. In the part of the source curve limited by ***Left*** limit and ***Right*** limit every <n> sample is exported
EXPORT_EVAL_LOG_ASCII	Exports an evaluation log in ASCII format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_EVAL_LOG_WKS	Exports an evaluation log in WKS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_EVAL_LOG_XLS	Exports an evaluation log in XLS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_METHOD_ASCII	Exports a method to the file defined in ***Export to file in ASCII format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If all parameters are ***OFF*** then no method is exported. If ***Main*** is ***ON*** then the main method is included and if ***Blocks*** is ***ON*** then all blocks are included in the exported file.

Instruction	Description
EXPORT_METHOD_WKS	Exports a method to the file defined in ***Export to file in WKS format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters are ***OFF*** then no method is exported. If ***Main*** is ***ON*** then the main method is included and if ***Blocks*** is ***ON*** then all blocks are included in the exported file.
EXPORT_METHOD_XLS	Exports a method to the file defined in ***Export to file in XLS format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters are ***OFF*** then no method is exported. If ***Main*** is ***ON*** then the main method is included and if ***Blocks*** is ***ON*** then all blocks are included in the exported file.
EXPORT_MULTI_CURVES_ASCII	Exports multiple curves (previously defined with ***EXPORT_SEL_CURVES*** instructions) in ASCII format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_MULTI_CURVES_WKS	Exports multiple curves (previously defined with ***EXPORT_SEL_ CURVES*** instructions) in WKS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_MULTI_CURVES_XLS	Exports multiple curves (previously defined with ***EXPORT_SEL_ CURVES*** instructions) in XLS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_NORMALISE_RETENTION	Normalizes retention when exporting multiple curves.

Instruction	Description
EXPORT_PEAK-TABLE_ASCII	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file in ASCII format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_PEAK-TABLE_WKS	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file in WKS format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_PEAK-TABLE_XLS	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file in XLS format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_PEAK-TABLE_XML	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file in XML format***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question.
EXPORT_SEL_CURVES	Selects a curve for subsequent export (using the ***EXPORT_MULTI-CURVES_**** instruction). The curve is cut according to the right and left cut limit and the number of points to be exported may be set by the ***Export*** parameter (for example, every fifth point).
EXPORT_DOC_400_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.

Instruction	Description
EXPORT_DOC_400_WKS	Exports the documentation in the current result file in WKS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.
EXPORT_DOC_400_XLS	Exports the documentation in the current result file in MS Excel XLS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.
EXPORT_DOC_WKS	Exports the documentation in the current result file in WKS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.
EXPORT_DOC_XLS	Exports the documentation in the current result file in XLS format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file

Instruction	Description
EXPORT_DOC_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in ***Export to file***. If "*" is entered as ***File name*** the current ***Result*** file will be used. If "?" is entered followed by text, e.g. "Enter a file name", as ***File name***, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.

Chromatogram functions

The table below contains a list of instructions for chromatogram functions.

Instruction	Description
COPY_CHROM	Creates a copy of the specified chromatogram. If "*" is used as source then the current (default) chromatogram is used. If "*" is used as destination then a default name will be created for the copy.
CREATE_NEW_CHROM	Creates a new chromatogram with the given name. If "*" is used for the chromatogram name a default name will be generated and used. *Note*: It is a recommendation not to use only numbers as names for new chromatograms.
DELETE_CHROM	Deletes the named chromatogram. If trying to delete the current (default) chromatogram a run time error will be caused.
OPEN_CHROM	Opens the specified chromatogram from the specified file.
RENAME_CHROM	Renames the specified chromatogram. If "*" is used as ***From*** then the current (default) chromatogram is used.
RESTORE_DESTINATION_ CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used in pair with the ***SET_DESTINATION_CHROM*** instruction.
SET_DESTINATION_CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used in pair with the ***RESTORE_ DESTINATION_CHROM*** instruction.

Other instructions

The table below contains a list of instructions for other operations.

Instruction	Description
BASE	Sets the X-axis base that the following calculations will be made in. If the value of the X-axis base is ***DEFAULT***, then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure, otherwise it will have no effect at all.
Comment	Inserts a comment below the marked instruction.
ENDLOOP	Marks the end of a ***LOOP*** statement.
LOOP	The instructions between this statement and the ***ENDLOOP*** statement are repeated n times. It is possible to have loops within loops as long as the number of ***LOOP*** statements matches the number of ***ENDLOOP*** statements.
MOLSIZE	Calculates the molecular sizes from a molecular size curve. A ***Mol. size*** column will be added to the ***Peak table***.
QC_TEST	Performs a QC test.
QUANTITATE	Calculates the concentration and amounts in the sample from a quantitation table. ***Amount*** and ***Concentration*** columns will be added to the ***Peak table***.
REPORT	Prints a report with the specified named report layout and title. If ***Title*** is "*" then the title in the report layout is used. If ***Report Layout*** is "*" then a default layout is used.
RUN_PROGRAM	Starts a program as a separate process. The ***Program name*** string contains the program name and parameters to start it with.
UPDATE	Updates a ***Quantitation table*** with new data from one standard concentration level. The default ***Limit(+/-)*** value of 12.5% will be used.

Test instructions

The ***Instruction*** field also contains a group of test instructions. These instructions are only available for the UNICORN software development team.

Instruction	Description
AUTOSAMPLER_PEAK_INTERVALS	Sets the area intervals for the ***AUTOSAMPLER_PEAK_TEST***.
AUTOSAMPLER_PEAK_TEST	Locates the first peak in the peak table. Compares the area of the peak in the peak table with the specified maximum and minimum areas.
EXPORT_TEST_RESULT_TO_FILE	Finishes the current result and saves the output file as an ASCII file in a destination and with a file name specified in the variable DestFilename (.txt). A complete search path may be incluede in the file name.
GRADIENT_TEST_INTERVALS	Sets the level intervals for the ***GRADIENT_TEST***.
GRADIENT_TEST	The theoretical straight line between the 0% and 100% levels are calculated. The deviation between the curve and the ideal straight line is compared in both directions from the center position (50%) until the deviation exceeds the defined maximum deviation. The calculated deviation points are checked against the defined limits.
STEP_RESPONSE_INTERVALS	Sets the level intervals for the ***STEP_RESPONSE_TEST***.
STEP_RESPONSE_TEST	The relative amplitude is calculated at the specified retentions (The 0% and 100% amplitudes are used for reference). The calculated relative amplitudes are checked against the specified error margins. The 0% level amplitude is verified to be within the specified interval from the absolute 0 level.
TEST_CURVE_AMPLITUDE_CHANGE	Verifies that the curve amplitude has changed more than or equal to the value of the Delta parameter between the defined to and from retention points. A print parameter may be set to On to generate printed results.
TEST_CURVE_AMPLITUDE_STABLE	Verifies that the curve amplitude is stable between the defined to and from retention points. The actual curve value is compared to a set amplitude parameter. If the difference exceeds a set Delta value, the test is failed. A print parameter may be set to On to generate printed results.

Instruction	Description
TEST_INFO	Adds selected information items to the output file, e.g. system name, UNICORN version etc. Also, a specified free text can be added. A print parameter may be set to On to generate printed results.
TEST_LOG-BOOK_EVENT	Verifies if a specified text is present in the logbook curve between the defined to and from retention points. The test can be defined to be passed either if the text is present or not. A failed or passed text will be added to the output file. A print parameter may be set to On to generate printed results.
UV_RESPONSE_IN-TERVALS	Sets the level intervals for the ***UV_RESPONSE_TEST***.
UV_RESPONSE_TEST	The amplitudes for the 0% and 100% levels are calculated and the difference between the values are calculated. The results of (1) Curve2_Difference / Curve1_Difference and (2) Curve2_Difference / Curve3_Difference are calculated. The calculated points are checked if they are outside the defined limits from the 50% level.

Appendix C Curve fit models and statistics

Introduction

The ***Analysis*** module (optional) is used to produce calibration curves and molecular size curves for analytical purposes. The quality of the curve fit model determines the accuracy of the curves. This appendix describes

- The available curve fit models.
- The statistical measurements in the ***Analysis*** module.

In this appendix

This appendix contains these sections:

C.1 Curve fit models

Calibration curve models

The ***Analysis*** module provides a comprehensive range of curve fit models. The following models are available for calibration curves:

- ***Linear***.
- ***Linear through origin***.
- ***Quadratic***.
- ***Quadratic through origin***.
- ***Point to point***.

Note: The average peak size for all points at a specific level is used to calculate the calibration curve.

Molecular size curve models

The following curve fit models are available for molecular size curves:

- ***Linear***.
- ***Linear (log Mw)***.
- ***Quadratic***.
- ***Quadratic (log Mw)***.
- ***Point to point***.
- ***Point to point (log Mw)***.

Statistics

The ***Analysis*** module provides values for the appropriate constants that are used in each curve equation for all models, except for the point to point models. It also provides statistical data that you can use to assess the quality of fit of the curve to the data.

- Click the ***More...*** button in the ***Statistics*** field of the ***Quantitation table*** or ***Mol. size table*** dialog boxes to view the applied model statistics.

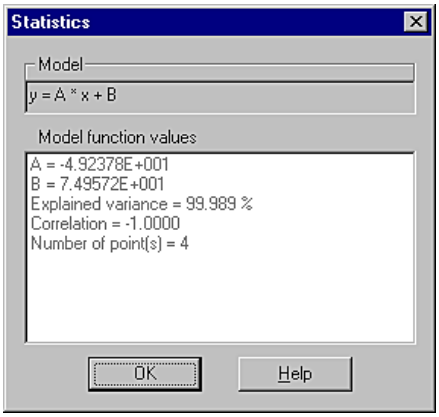
The Linear model

The table below describes the features of the ***Linear*** curve fit model.

Feature	Description
Equation.	y = Ax + B
Mathematical model.	The constants A and B are determined by linear least squares regression.
Minimum number of required points.	2 (at least 4 points recommended)
Measuring range for the calibration curve.	Within the highest and lowest values for the points.

Note: A variant of this model is available for the production of a molecular size curve. This uses the logarithm of the molecular size as the x value in the expression above.

The illustration below is an example of the statistical information for an applied ***Linear*** curve model:



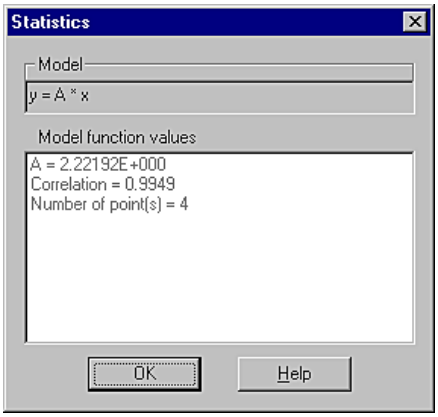
The Linear through origin model

The table below describes the features of the ***Linear through origin*** curve fit model:

Feature	Description
Equation.	y = Ax
Mathematical model.	The constant A is determined by linear least squares regression.
Minimum number of required points.	1 (at least 2 points recommended)

Feature	Description
Measuring range for the calibration curve.	From the point with the highest value down to the origin.

The illustration below is an example of the statistical information for an applied ***Linear through origin*** curve model:



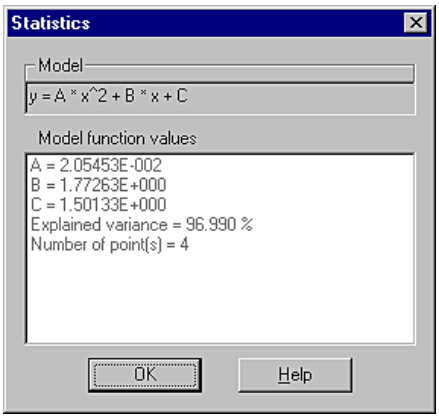
The Quadratic model

The table below describes the features of the ***Quadratic*** curve fit model:

Feature	Description
Equation.	$y = Ax^2 + Bx + C$
Mathematical model.	The constants A, B and C are determined by linear least squares regression.
Minimum number of required points.	3 (at least 6 points recommended)
Measuring range for the calibration curve.	Within the highest and lowest values for the points.

Note: A variant of this model is available for the production of a molecular size curve. This uses the logarithm of the molecular size as the x value in the expression above.

The illustration below is an example of the statistical information for an applied ***Quadratic*** curve model:

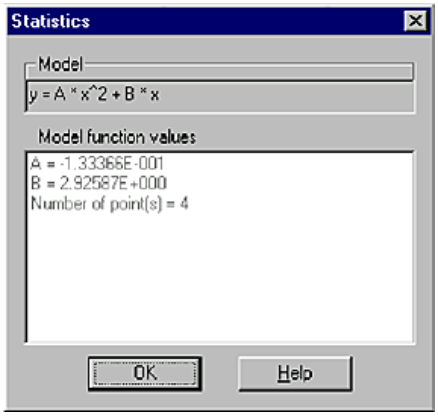


The Quadratic through origin model

The table below describes the features of the ***Quadratic through origin*** curve fit model:

Feature	Description
Equation.	$y = Ax^2 + Bx$
Mathematical model.	The constants A and B are determined by linear least squares regression.
Minimum number of required points.	2 (at least 4 points recommended)
Measuring range for the calibration curve.	From the point with the highest value down to the origin.

The illustration below is an example of the statistical information for an applied ***Quadratic through origin*** curve model:

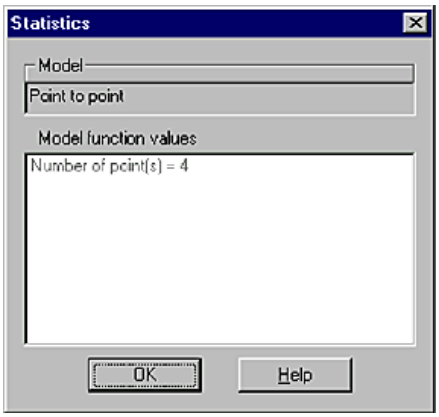


The Point to point model

The table below describes the features of the ***Point to point*** curve model.

Feature	Description
Equation.	No single equation.
Mathematical model.	As these curves are not based on a single equation, no statistical data is available. The statistics table only contains information on the number of points in the curve.
Minimum number of required points.	2
Measuring range for the calibration curve.	Within the highest and lowest values for the points.

The illustration below is an example of the statistical information for an applied ***Point to point*** curve model:


Statistics
Model
Point to point
Model function values
Number of point(s) = 4
OK
Help

C.2 Statistics

Introduction

This section explains the correlation and explained variance calculations that are used by the ***Analysis*** module.

Correlation

The ***Analysis*** module calculates the correlation coefficient for linear models. This shows how well the data are linearly related. The correlation is displayed in the ***Statistics*** table.

If you are producing a calibration curve that relates peak area or height to amount or concentration, you aim to achieve a high positive correlation coefficient. A value of +1 indicates a perfect fit of all the data to the straight line. A molecular size curve has a negative slope, so the aim is towards a correlation coefficient of -1.

Too few data points

If you only have two data points for a ***Linear*** model, or only one point for a ***Linear through origin*** model, the fitted straight line will inevitably pass exactly through the points. By definition, this leads to a correlation of exactly +1, but this does not indicate a good fit, but instead indicates too few data points. In these cases the ***Statistics*** table will display a "**---**" symbol instead of the correlation value.

Correlation calculation

The correlation is derived as follows:

$$Correlation = \frac{\sum_i \left[(x_i - \bar{x})(y_i - \bar{y})\right]}{\sqrt{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]}}$$

Where:

- $\bar{x}$ is the average of the x value.
- $\bar{y}$ is the average of the y value.

For the molecular size model "Linear log(Mw)":

- $\bar{x}$ is the average of the logarithms of the molecular sizes.

Explained variance

Explained variance provides a measurement of how much of the variation in the data points (xy pairs) is due to the model. The remaining variation can be attributed to noise, i.e. random errors, or to the fact that an inappropriate model has been selected. This makes it possible to use the explained variance value for model selection, e.g. to decide if a quadratic model fits the data better than a linear model. This would be confirmed by a higher explained variance value.

Note: The explained variance is not calculated for curve models drawn through the origin.

Explained variance calculation

The explained variance is equal to R^2 adjusted for degrees of freedom. The illustration below shows the mathematical model:

$$\text{Explained variance (\%)} = 100 \text{ x } \left[1 - \frac{SS_{residuals}/(n - k - 1)}{SS_{total}/(n - 1)} \right]$$

Where:

$SS_{residuals} = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$ (Residual Sum of Squares)

$SS_{total} = \sum_{i=1}^{n} (y_i - \bar{y})^2$ (Total Sum of Squares)

- $\bar{y}$ is the average of all y values.
- $\hat{y}_i$ is a function value using the fitted model.

For example: $\hat{y}_i = Ax_i^2 + Bx_i + C$

- n is the number of points (xy pairs).
- k is the number of x terms in the model.

For example, 1 for "**Linear**" and 2 for "**Quadratic**".

Undefined value for explained variance

You can only obtain a value for explained variance if you have sufficient data points on the curve. For instance, if you only have two points for a ***Linear*** model, or only three points for a ***Quadratic*** model, the fitted curve will pass exactly through the points. By definition, this leads to an undefined value for explained variance. In these cases the ***Statistics*** table will show a "**---**" symbol instead of an explained variance value.

Appendix D The Column list

Introduction

The ***Column List*** includes all available columns and their specific parameters. This appendix describes how to edit the ***Column List***.

In this appendix

This appendix contains this section:

D.1 How to edit the Column List

Introduction

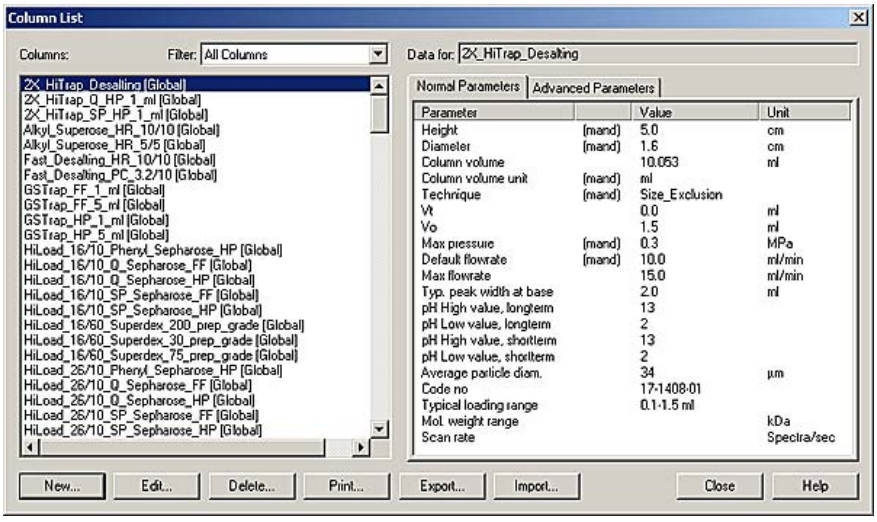
This section describes how to edit the list of available columns.

Available columns

When you create a new method and select a column, certain column-specific parameters are automatically copied into the method. The list of available columns is found in the ***For column*** field of the ***New Method*** dialog box. The ***Column List*** is not linked to a particular method, although the columns are edited within the ***Method Editor***.

Columns are either globally available to all users, or only personally available. It is best not to edit the globally available columns, unless you save the changes under a new column name, since other users may not appreciate the changes.

Note: It is recommended that only a limited number of users are given access to the right to edit global columns. This is essential to avoid unintentional changes.



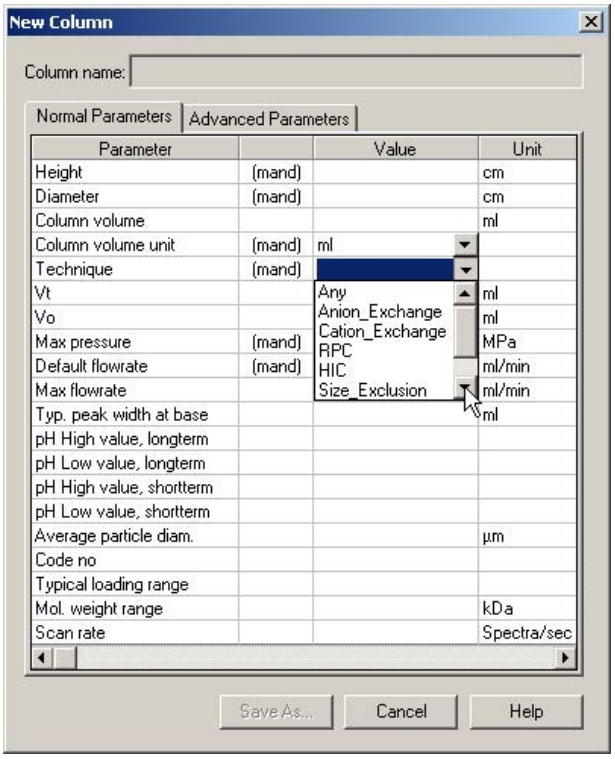
How to print the column list

The table below describes how to print the column list data.

Step	Action
1	• Click the print button. *Result*: The ***Print Column List*** dialog box opens.
2	• Select to print a global or your personal column list. • Click ***OK***. *Result*: The column list is printed on the default Windows printer.

The New Column dialog box

The illustration below shows the ***New Column*** dialog box:



How to add a new column

The table below describes how to add a new column to the ***Column List***.

Step	Action
1	• Choose ***Edit:Column List*** in the ***Method Editor***. *Result*: The ***Column List*** dialog box opens. *Note*: Select a column from the list to display the parameters in the field to the right. Most column parameters are displayed in the ***Normal Parameters*** tab. Additional parameters for special columns may be displayed in the ***Advanced Parameters*** tab.
2	• Click the ***New*** button. *Result*: The ***New Column*** dialog box opens.
3	• Select the appropriate parameter tab. • Type the desired parameter values. • Click the ***Save as*** button. *Note*: Mandatory parameters are labelled ***mand***. The column cannot be saved unless all mandatory parameters are filled in. *Result*: The ***Save as*** dialog box opens.
4	• Type the name of the new column. • Click the ***Save as global*** checkbox if the column should be available to other users. *Note*: You must have ***Edit global lists*** authorization to save a column for global use. A global column cannot have the same name as a personal column. • Click ***OK***. *Result*: The new column is added to the ***Column List***.

Note: See column instruction to determine the back pressure over the system and the column.

The normal column parameters

The table below is a list of all the available normal column parameters:

Parameter	Unit	Comment
Height	cm	• Mandatory. • Calculation of N/m.
Diameter	cm	• Mandatory.
Column volume	nl, µl, ml or liter	• Mandatory. • Automatically calculated from ***Height*** and ***Diameter***. • User cannot set this parameter directly.
Column volume unit	nl, µl, ml or liter	• Not mandatory. • The column volume is calculated in the set unit.
Technique		• Mandatory. • Decides which technique the column should be available for.
Vt	nl, µl, ml or liter	• Not mandatory. • Total liquid volume. • Used to calculate the capacity factor after an integration.
Vo	nl, µl, ml or liter	• Not mandatory. • Void volume. • Used to calculate K_{av} after integration.
Max pressure	MPa	• Mandatory. • Used for setting pressure limit in a method automatically.
Default flowrate	nl/min, µl/min, ml/min or liter/min	• Mandatory. • Used to set the flowrate in a method automatically.

Parameter	Unit	Comment
Max flowrate	nl/min, µl/min, ml/min or liter/min	• Not mandatory. • Used to give a warning if a higher flowrate is chosen when saving or starting a method.
Typ. peak width at base	nl, µl, ml or liter	• Not mandatory. • Used to set averaging time for UV detector. • used to set peak fractionation parameters.
pH high value, longterm		• Not mandatory. • Used to give a warning when saving or starting the method if the ***BufferPrep_pH*** value is higher than the set value.
pH low value, longterm		• Not mandatory. • Used to give a warning when saving or starting the method if the ***BufferPrep_pH*** value is lower than the set value.
pH high value, shortterm		• Not mandatory. • Used to give a warning when saving or starting the method if the ***BufferPrep_pH*** value is higher than the set value.
pH low value, shortterm		• Not mandatory. • Used to give a warning when saving or starting the method if the ***BufferPrep_pH*** value is lower than the set value.
Average particle diameter	µm	• Not mandatory. • Information only.
Code no		• Not mandatory. • Information only.

Parameter	Unit	Comment
Typical loading range	mg	• Not mandatory. • Information only.
Mol. weight range	kDa	• Not mandatory • Information only
Scan rate	spectra/sec	• Not mandatory. • Information only.

Note: The values for the parameters ***Max pressure***, ***Default flowrate*** and ***Typical peak width at base*** (used to set average time and peak fractionation parameter ***MinWidth***) are only copied into the method if the corresponding instructions are available as variables.

How to edit column parameters

The table below describes how to edit column parameters in the ***Method Editor***:

Step	Action
1	Choose ***Edit:Column List***. *Result*: The ***Column List*** dialog box opens.
2	Select a column and click the ***Edit*** button. *Result*: The ***Edit Column*** dialog box opens.
3	Select the desired parameters and change the value settings.
4	• Click the ***Save*** button. *or* • Click the ***Save as*** button to save the column under a new name.

Note: If a column has been selected and saved in a method, and the parameters for the column are changed later, the column in the method will not be updated automatically. When you open the method you will be asked if you want to update the parameters. The recommendation is that you answer ***Yes***.

How to delete a column

The table below describes how to delete a column:

Step	Action
1	Choose ***Edit:Column List***. *Result*: The ***Column List*** dialog box opens.
2	Select a column and click the ***Delete*** button. *Result*: The ***Delete Column*** dialog box opens.
3	• Click the checkbox for each column you want to delete. • Click ***OK***. *Result*: The selected columns are deleted.

How to export a column

The column information for a system can be transferred to another by using the export and import functions in the column list. The table below describes how to export a column:

Step	Action
1	Choose ***Edit:Column List***. *Result*: The ***Column List*** dialog box opens.
2	Click the ***Export*** button. *Result*: The ***Export Column*** dialog box opens.
3	• Click the checkbox for each column you want to export. • Click ***Export***. *Result*: The ***Export Column to file*** dialog box opens.
4	• Select the desired folder in the navigation window. • Type a new file name if neccessary. • Choose the type of file to export (column file or Excel file) • Click the ***Save*** button. *Result*: The column file is saved and the dialog box closes.

Note: If a column is selected in the ***Column List*** when the ***Export Column*** dialog box is opened, this column will automatically be selected in the ***Export Column*** dialog box.

How to import a column

The table below describes how to import a column:

Step	Action
1	Choose ***Edit:Column List***. *Result*: The ***Column List*** dialog box opens.
2	Click the ***Import*** button. *Result*: The ***Import Column*** dialog box opens.
3	• Click the ***Browse*** button to locate the column file. *Result*: The ***Import Column from file*** dialog box opens.
4	• Select a column file. • Click ***Open***. *Result*: The ***Import Columns*** dialog box opens.
5	• Select the columns to import from the list. • Select ***Import as global*** to add the columns to the global column list if desired. • Click ***Import***. *Result*: The selected columns are imported and available in the column list.

Note: Select ***Import as global*** to import the columns to the global column list.

Appendix E How to create and edit BufferPrep recipes

Introduction

The ***BufferPrep*** function is available for some ÄKTAdesign systems. This appendix describes how to create and how to edit the recipes for ***BufferPrep***.

In this appendix

This appendix contains these sections:

E.1 How to create a BufferPrep recipe

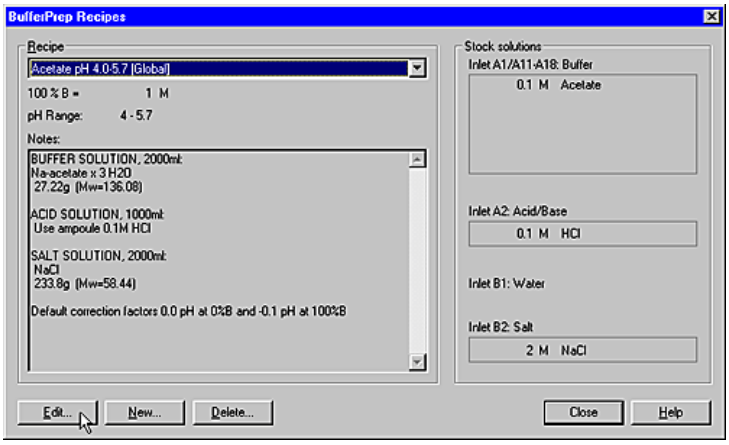
About BufferPrep recipes

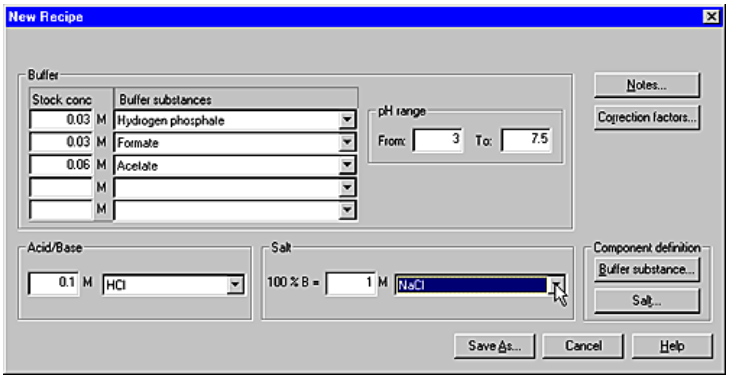
New ***BufferPrep*** recipes are created in the ***Method Editor***. The list of recipes is not linked to a specific method. Which recipe to use in a certain method is selected on the ***BufferPrep*** tab in the ***Run Setup***.

How to create a recipe

The table below describes how to create a new ***BufferPrep*** recipe in the ***Method Editor***:

Step	Action
1	Choose ***Edit:BufferPrep Recipes***. *Result*: The ***BufferPrep Recipes*** dialog box opens. The illustration below shows the ***BufferPrep Recipes*** dialog box with a recipe selected:



Step	Action
2	Click the ***New*** button. *Result*: The ***New Recipe*** dialog box opens. The illustration below shows a complete example of a ***BufferPrep*** recipe in the ***New Recipe*** dialog box. 
3	Select buffers from the ***Buffer substances*** droplists and type stock concentrations in the corresponding ***Stock conc*** box. See “How to define a new buffer substance” below if the desired substance is not available.
4	Select either ***HCl*** (acid) or ***NaOH*** (base) from the ***Acid/Base*** droplist and type the required stock concentration (typically 0.1 M)
5	Select a salt from the ***Salt*** droplist and type the maximum outlet concentration of the salt for ***100%B*** (typically 1.0 M). See “How to define a new salt” below if the desired salt is not available.
6	Type the desired pH range minimum and maximum values in the ***From*** and ***To*** boxes. See “How to select the pH range” below this table.
7	• Click the ***Notes*** button (optional). • Type your notes about the recipe in the displayed dialog box. • Click ***OK*** to return to the ***New Recipe*** dialog box.
8	Click ***Save as*** to save the recipe under a new name. *Note*: A warning message will appear if any of the recipe values are unfeasible.

Step	Action
9	• Type a name in the dialog box. • Click ***OK***. *Result*: The new recipe is added to the recipe list.

Note: It is recommended that restricted access be given to the right to edit global recipes.

The recipes are either globally available to all users, or only personally available. It is best not to edit the globally available recipes, unless you save the changes under a new recipe name, since other users may not appreciate the changes.

Buffer concentration

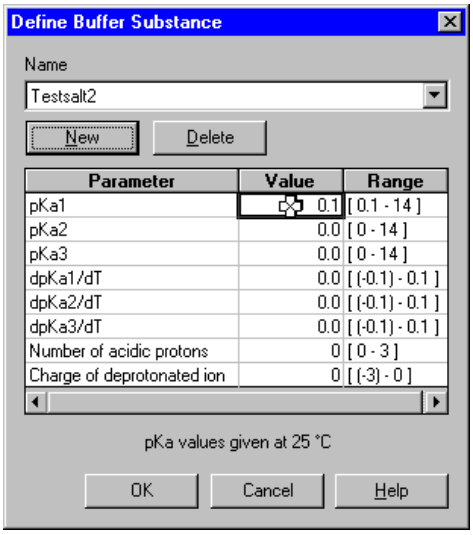
Use buffer concentrations that are 2-4 times higher than the concentration that is used in the normal preparation. When ***BufferPrep*** is used, the buffer will be diluted 2-10 times depending on the amount of acid/base that has to be used to reach the desired pH value.

Up to five different buffering components can be selected. To prevent a too high ionic strength, the sum of the concentrations for all selected buffers should be between 0.03 M and 0.2 M (typically 0.1 M).

How to select the pH range

The useful pH range depends on the pKa value. The table below describes how to determine a pH range based on the pKa value:

Step	Action
1	Choose ***Edit:BufferPrep Recipes*** and click the ***New*** button.

Step	Action
2	Click the ***Buffer substance*** button in the ***New Recipe*** dialog box. *Result*: The ***Define buffer substance*** dialog box opens. 
3	Select the buffer component from the ***Name*** droplist and note the displayed pKa value.
4	Click ***Cancel*** to return to the ***New Recipe*** dialog box.
5	Use the pKa value to determine the pH range. Typically a range of +/- 0.5 units around the pKa value is useful. *Note*: Check buffer tables for the exact ranges.

How to define a new buffer substance

Note: Before you can define a new buffer substance you must ensure that all pKa values are available for the substance. The pKa values should be true (i.e. the pKa value at in-definite dilution) and not apparent pKa values (i.e. measured at a non-zero concentration). The pKa values should be given at 25° C.

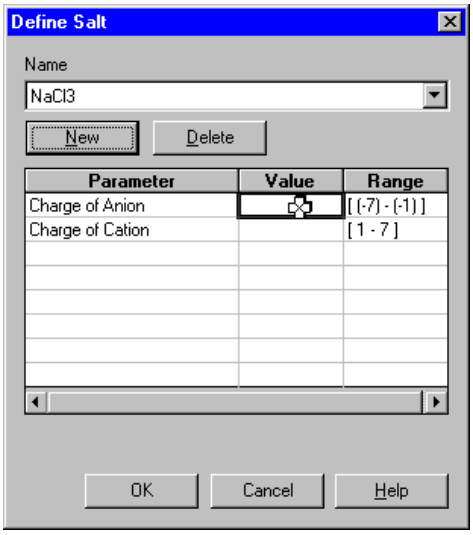
The table below describes how to define a new buffer substance:

Step	Action
1	Choose ***Edit:BufferPrep Recipes*** and click the ***New*** button.
2	Click the ***Buffer substance*** button in the ***New Recipe*** dialog box. *Result*: The ***Define buffer substance*** dialog box opens.
3	Click the ***New*** button. *Result*: The ***New component*** dialog box opens.
4	Type a name for the new component and click ***OK*** to return to the ***Define buffer substance*** dialog box.
5	Type appropriate values in the ***Value*** cells for each pKa and dpKa/dT parameter. *Note*: All values must fall within the stated ***Range*** limits. Up to three values can be entered for each buffering component. When the component has less than three pKa values, the other values should be set to zero. A dpKa/dT value of zero means that the pKa does not change with temperature.
6	• Type the number of acidic protons for the buffer substance in the form that it is actually weighed in. *Example*: The number is 2 for NaH_2PO_4, 1 for Na_2HPO_4 and 0 for Tris. • Type the charge of the completely de-protonated ion. This will be a negative value for an acid and zero for a base. *Example*: The value is -3 for NaH_2PO_4 and 0 for Tris. • Click ***OK***. *Result*: The new buffer substance is added to the list of available substances.

How to define a new salt

Before you can define a new salt you must ensure that the new salt is inert, i.e. a salt with no buffering properties. The table below describes how to define a new salt:

Step	Action
1	Choose ***Edit:BufferPrep Recipes*** and click the ***New*** button.

Step	Action
2	Click the ***Salt*** button in the ***New Recipe*** dialog box. *Result*: The ***Define salt*** dialog box opens. 
3	Click the ***New*** button. *Result*: The ***New component*** dialog box opens.
4	Type a name for the new salt and click ***OK*** to return to the ***Define salt*** dialog box.
5	• Type the appropriate charge of anion value in the corresponding ***Value*** cell. *Example*: The value for Cl^- is - 1. The value for SO_4^{2-} is -2. • Type the appropriate charge of the cation value in the corresponding ***Value*** cell. *Example*: The value for Na^+ is 1. The value for Mg^{2+} is 2. • Click ***OK***. *Result*: The new salt is added to the list of available salts.

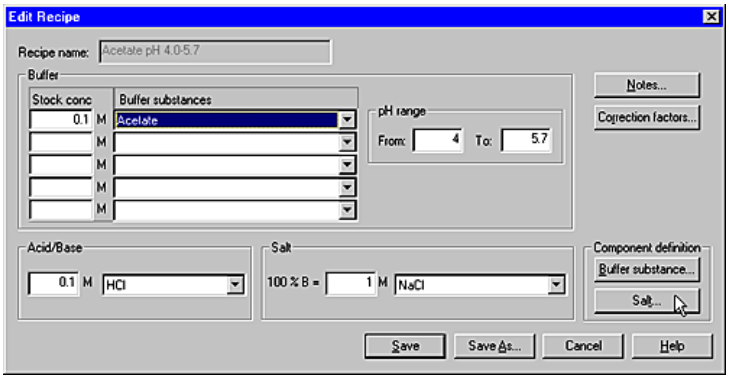
E.2 How to edit a BufferPrep recipe

Introduction

This section describes how to edit a ***BufferPrep*** recipe in the ***Method Editor***.

How to edit a recipe

The table below describes how to edit a ***BufferPrep*** recipe:

Step	Action
1	Choose ***Edit:BufferPrep Recipes***. *Result*: The ***BufferPrep Recipes*** dialog box opens.
2	Select a recipe and click the ***Edit*** button. *Result*: The ***Edit Recipe*** dialog box opens. 
3	Change the substances and parameters as desired and click the ***Save*** button or the ***Save as*** button to save the new recipe.

Changes to recipes in methods

If a recipe has been selected and saved in a method, and the recipe is later changed, the corresponding recipe in the method will not be updated automatically. When you open the method you will be asked if you want to update the parameters in the method recipe. The recommendation is that you answer ***Yes***.

Note: The question will not appear if you only change the ***Correction factors***. The ***Correction factors*** in the method recipe will not be updated.

How to determine if the Correction factors need to be changed

Correction factors can be set to fine-tune a recipe around a specific pH, to obtain high pH accuracy. The table below describes how to run the ***BufferPrep*** manually at 0% and 100% in the ***System Control*** module, to determine if the ***Correction factors*** need to be changed:

Step	Action
1	Choose ***Manual:Other***. *Result*: The ***System Other instructions*** dialog box opens.
2	• Select the recipe from the ***Recipe Name*** droplist and click the ***Execute*** button. *Result*: The recipe instruction is added.
3	• Click the ***Pump*** radio button and select ***BufferPrep_pH***. • Set the pH value in the ***pH*** parameter box and click the ***Execute*** button. *Result*: The ***BufferPrep*** pH value is added and the run starts.
4	• Select ***Flow***. • Set the flow rate in the ***FlowRate*** parameter box and click ***Execute***. *Result*: The new flow rate is added.
5	Check the pH reading when it is stable in the ***BuffPre_pH*** meter in the ***Run Data*** pane. *Note*: At least 30 ml of eluent must pass through before the reading stabilizes. To display the ***BuffPre_pH*** meter, right-click and select ***Properties***. Select ***BuffPre_pH*** on the ***Run Data Groups*** panel and click the ***OK*** button.

Step	Action
6	• Select ***Gradient*** in the ***Instructions*** list. • Type 100% in the ***Target*** parameter box, 0 in the ***Length*** parameter box and click ***Execute***. *Result*: The gradient instruction is added.
7	Check the pH reading when it is stable at 100%. See "How to change the ***Correction factors***" below.

How to change the Correction factors

If the readings described in the instruction above are acceptable at both 0% and 100%, the ***Correction factors*** do not need to be changed. If the ***Correction factors*** do not produce an acceptable result, they must be adjusted in the ***Method Editor*** module. The table below describes how to change the ***Correction factors***:

Step	Action
1	Choose ***Edit:BufferPrep Recipes***. *Result*: The ***BufferPrep Recipes*** dialog box opens.
2	Select the recipe from the ***Recipe*** droplist and click the ***Edit*** button. *Result*: The ***Edit Recipe*** dialog box opens.
3	Click the ***Correction factors*** button. *Result*: The ***Correction Factors*** dialog box opens.
4	• Type the deviation at 0% and 100%. *Example*: If the pH is set to 7.0 and the actual pH is 7.1, the ***Correction factor*** is 0.1. If the actual pH is 6.9, the ***Correction factor*** is -0.1. • Click ***OK***. • Click the ***Save*** button or the ***Save as*** button to save the recipe.

Note: If there already are ***Correction factors***, the measured pH deviation should be added to the old factors.

Appendix F Method examples

Introduction

This appendix contains practical method examples that can be applied in typical situations. The examples cover three different topic groups:

- ***Watch*** instructions
- ***Messages***
- ***Quality control***

Watch instructions allow the progress of a method run to be determined by the events during the method run, for example start collecting fractions when the first peak elutes, or equilibrate the column until the eluent conductivity has reached a given value. This is facilitated by the ***Watch*** instructions.

The system strategy includes ***Watch*** instructions for each monitor defined in the system. These instructions are used to survey method runs, and instruct the system to call a specified block or an instruction when a particular monitor signal meets a given condition. As long as the condition is not met, the block is not activated.

Messages can be used in a method to provide information to the operator but also for interaction between the system and the operator.

A ***Quality control*** procedure in a method can be used to ensure that the quality of the results remain consistent in a series of runs.

In this appendix

This appendix contains the following sections:

F.1 Simple equilibration

Introduction

This section contains an example of how a ***Watch*** instruction for simple equilibration can be inserted into a method.

Example instruction

This is an example instruction as it would be presented in the ***Text*** pane.

```
0.00 Block EQUILIBRATE
(Equilibrate)
0.00 Base SameAsMain
0.00 Watch_Cond Less_than, 5 {mS/cm}, CONTINUE
0.00 Hold
0.10 Watch_UV1 Less_than, 100 {mAU}, CONTINUE
0.10 Hold
0.10 End_Block
```

If you are not using ÄKTA instruments

If you are not using ÄKTA™ instruments, a delay should be added after the ***Hold/Pause*** instruction so that the following instruction will not be executed simultaneously with the ***Hold/Pause*** instruction.

This is what happens

The table below describes what happens in the above example:

Stage	Description
1	The ***Watch*** is started on the conductivity signal and the method is then put on ***Hold***.
2	***Continue*** is issued and ***Watch_cond*** is turned off automatically when the ***Watch_cond*** condition is fulfilled.
3	Method execution continues issuing a ***Watch_UV*** command. Again the method is put on ***Hold*** until the ***Watch*** condition is fulfilled.

Note: Even though the line

```
Watch_Cond Less_than, 5 {mS/cm}, Continue
```

is in the method placed before ***Hold***, the method is put on hold first and then continued only after the conductivity has reached a level less than 5 mS/cm. This is because ***Hold*** is an instruction that will be executed at its breakpoint, while ***Continue*** is not an instruction but rather an action for the ***Watch*** instruction.

Evaluation of the method

This method works satisfactorily although one drawback is that it might never end, and thus consume all of the buffer if the conditions for some reason are unfulfilled. See appendices *Section F.2 Equilibration with simple safeguard, on page 652* and *Section F.3 Equilibration with extra safeguard, on page 653*.

F.2 Equilibration with simple safeguard

Introduction

This section contains an example of how a ***Watch*** instruction for simple safeguard can be inserted into a method.

Example instruction

This is an example instruction as it would be presented in the ***Text*** pane:

```
0.00 Block EQUILIBRATE
(Equilibrate)
0.00 Base SameAsMain
0.00 Watch_UV1 Less_than, 100 {mAU} END_BLOCK
5.00 Watch_Off UV1
5.00 Message “The Condition was never reached”, Screen, “No sound”
5.00 End_Block
```

This is what happens

This is what happens in the above example:

The column is equilibrated until the UV has reached a level below 100 mAU or until the column has been equilibrated with five column volumes of buffer, whichever condition is met first. In this way, it is possible to equilibrate the column without the risk of running out of buffers.

F.3 Equilibration with extra safeguard

Introduction

This section contains an example of how a ***Watch*** instruction for extra safeguard can be inserted into a method.

Example instruction

This is an example instruction as it would be presented in the ***Text*** pane:

```
0.00 Block EQUILIBRATE
(Equilibrate)
0.00 Base SameAsMain
0.00 Block COND_LESS_THAN
(Cond_less_than)
0.00 Base SameAsMain
0.00 Watch_Cond Less_than, 5 {mS/cm} END_BLOCK
6.00 Message “Low conductivity not reached”, Screen, “No sound”
6.00 Pause INFINITE {Minutes}
6.00 End_Block
0.00 Block COND_STABLE
(Cond_stable)
0.00 Base SameAsMain
0.00 WatchPar_Cond 0.500 {mS/cm}, 2 {mS/cm}
0.00 Watch_Cond Stable_Baseline, 5 {Minutes}, END_BLOCK
10.00 Message “Conductivity not stable”, Screen, “No sound”
10.00 Pause INFINITE {Minutes}
10.00 End_Block
0.00 End_Block
```

Note: If you are not using ÄKTA instruments, a delay should be added after the ***Hold/Pause*** instruction so that the following instruction will not be executed simultaneously with the ***Hold/Pause*** instruction.

This is what happens

The table below describes what happens in the above example:

Stage	Description
1	The column is equilibrated until the conductivity is below 5 mS/cm.
2	If this value is not reached within 6 column volumes, the method is paused and a message is displayed.
3	Equilibration of the column is continued until the conductivity value is "stable" (allowed to vary by max. ±2 mS/cm) over a period of at least 5 minutes.
4	If this condition is not met within 10 column volumes, the method is again paused.

Note: At each pause, the operator can decide whether to continue or abort the run.

F.4 Collection of absorbance peaks

Introduction

This section contains an example of how to collect absorbance peaks through outlets F3 and F4.

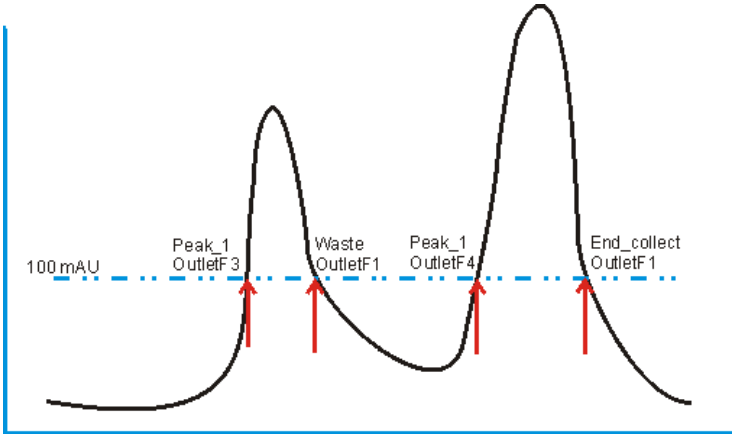
Example instruction

This is an example instruction as it would be presented in the ***Text*** window:

```
0.00 Block ELUTION
(Elution)
0.00 Base SameAsMain
0.00 Gradient 100.0 {%}, 20.00 {base}
0.00 Watch_UV1 Greater_Than, 100 {mAU}, Peak_1
(Peak_1)
0.00 Base SameAsMain
0.00 OutletValve F3
0.00 Watch_UV1 Less_Than_Or_Valley, 100 {mAU}, Waste
(Waste)
0.00 Base SameAsMain
0.00 OutletValve WasteF1
0.00 Watch_UV1 Greater_Than, 100 {mAU}, Peak_2
(Peak_2)
0.00 Base SameAsMain
0.00 OutletValve F4
0.00 Watch_UV1 Less_Than, 100 {mAU}, End_collect
(End_collect)
0.00 Base SameAsMain
0.00 OutletValve WasteF1
0.00 End_Block
0.00 End_Block
0.00 End_block
0.00 End_Block
20.00 End_Block
```

Illustration

The illustration below shows peaks collected by the method in the example above.



This is what happens

In this example, one or two absorbance peaks are collected through outlets F3 and F4 respectively with waste fractions collected through outlet valve F1 (waste). Each called block (except ***End_collect***) resets the ***Watch*** condition so that the method reacts correctly to subsequent changes in the UV absorbance.

Invalid Watch instructions

The design of a method of this kind (with several ***Watch*** instructions for the same monitor) is important. The construction in the following three lines appears simpler but is incorrect:

0.00 Watch_UV Greater_than, 100 {mAU}, Peak_2

0.00 Watch_UV Less_than, 100 {mAU}, End_collect

0.00 End_block

Here, the second ***Watch*** instruction will annul the first, since a signal can only be watched for one condition at a time.

F.5 Collection of three absorbance peaks

Introduction

This section contains an example of how to collect three absorbance peaks through outlets F3, F5 and F7 with waste fractions through outlets F4, F6 and F8.

The maximum number of peaks collected in this example is three due to the limited number of positions on the outlet valve.

Recommendations

Waste container needed

The waste fractions between the peaks are collected through the outlet valve positions F4, F6 and F8, so ensure that the tubing from these positions is lead to a suitably large container.

Condition for UV threshold

The UV threshold for collecting the waste fraction must be below the threshold for collecting the peak fraction so that the "waste" condition will not be fulfilled simultaneously or immediately after peak collection.

Example instruction

This is an example instruction as it would be presented in the ***Text*** window:

```
0.00 Block Eluate_Fractionation
(Eluate_Fractionation)
0.00 Base SameAsMain
0.00 Watch_UV1 Greater_Than, 5 {mAU}, Peak
(Peak)
0.00 Base SameAsMain
0.00 OutletValve Feed
0.00 Watch_UV1 Less_Than_Or_Valley, 4.75 {mAU}, Waste
(Waste)
0.00 Base SameAsMain
0.00 OutletValve Feed
0.00 Watch_UV1 Greater_Than, 5 {mAU}, Peak1
(Peak1)
```

```
0.00 Base SameAsMain
0.00 OutletValve Feed
0.00 Watch_UV1 Less_Than_Or_Valley, 4.75 {mAU}, waste1
(Waste1)
0.00 Base SameAsMain
0.00 OutletValve Feed
0.00 Watch_UV1 Greater_Than, 5 {mAU}, Peak2
(Peak2)
0.00 Base SameAsMain
0.00 OutletValve Feed
0.00 Watch_UV1 Less_Than_Or_Valley, 4.75 {mAU}, Waste2
(Waste2)
0.00 Base SameAsMain
0.00 OutletValve WasteF1
0.00 End_block
0.00 End_block
0.00 End_block
0.00 End_block
0.00 End_block
0.00 End_block
0.00 End_block
```

This is what happens

The table below describes what happens in the above example:

Stage	Description
1	When the UV reaches 5 mAU or more, the outlet valve is switched to the position for collecting the first peak.
2	When the UV reading goes down to 4.75 mAU, the outlet valve switches to the next position to separate the waste fraction from the collected peak fraction.

Stage	Description
3	This process is repeated twice for the next two peaks so that when the UV reading rises above 5 mAU, the position switches to collect the peak fraction and the position switches again to collect the waste fraction when the UV reading falls again.

F.6 Messages

When to use a message

Messages are used to inform the operator of the progress of the run. Messages can also be used for interaction between the operator and the system when necessary. A message can be for information in a screen only, or it can require a signature before the user can control the system. The messages are all added to the logbook text. This appendix describes how to add a message to a method. The appendix also gives two examples of how a message can be used.

How to add a Message instruction

The table below describes how to add a ***Message*** instruction to the method.

Step	Action
1	• Select ***Other*** in the ***Instructions*** field of the ***Instruction box***. • Select ***Message*** in the instructions list.
2	Type a message in the ***Message*** text box in the ***Parameters*** field.
3	Select one of the display options on the ***Mode*** menu: • ***Screen***, i.e. only a text message is displayed. • Noscreen, i.e. the message will not be displayed but only inserted into the logbook. • ***Authorize***, i.e. the message will require a signature from the user before the user can interact with the system again.
4	• Select a sound on the ***Sound*** menu if desired. • Click the ***Insert*** button.

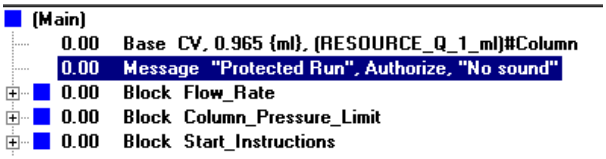
Note: If the ***Message*** instruction is inserted in a conditional block it will only be displayed if the conditions of the block (for example a ***Watch***) is fulfilled.

All messages are erased when the system reaches the ***End*** status. This also includes ***Authorize*** messages.

Protecting a method run with a message

A message can be set up in the beginning of a method to protect the method run from unauthorized interference. Once the message is issued, the system is locked from interaction by any user unless the user provides an authorization signature. The only command that is available without authorization is ***Pause***.

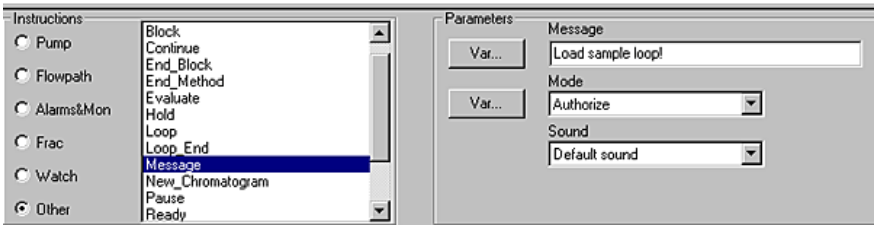
The illustration below shows the text instruction for the message described above:



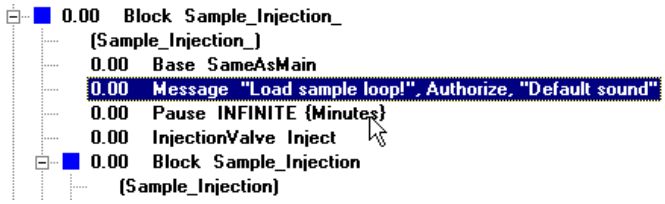
Pausing a method run for a manual sample injection

A message can be set up to pause the method until a sample has been injected manually. If a message requiring an authorization is followed by a ***Pause*** instruction the system will be paused until the message is acknowledged and signed. No other interaction with the system is available to the user. The operator will see a screen with a reminder to inject the sample before the method run proceeds.

The illustration below shows the selected message instruction in the ***Instruction box*** and the parameters for the message described above:



The illustration below shows the text instruction for the message described above:



Note: The message instruction must be followed immediately by the ***Pause*** instruction as shown above.

F.7 Quality control procedure

Introduction

When a series of runs is performed, irregularities in samples or in system or column performance can produce errors that will make the results inaccurate.

A quality control procedure can be added to a method to be used for a test run during the series of runs. The control procedure can ensure that the results remain within acceptable limits. If the result from the test run is unacceptable, the system can be paused so that the error is not repeated in subsequent runs.

How to create the quality control procedure

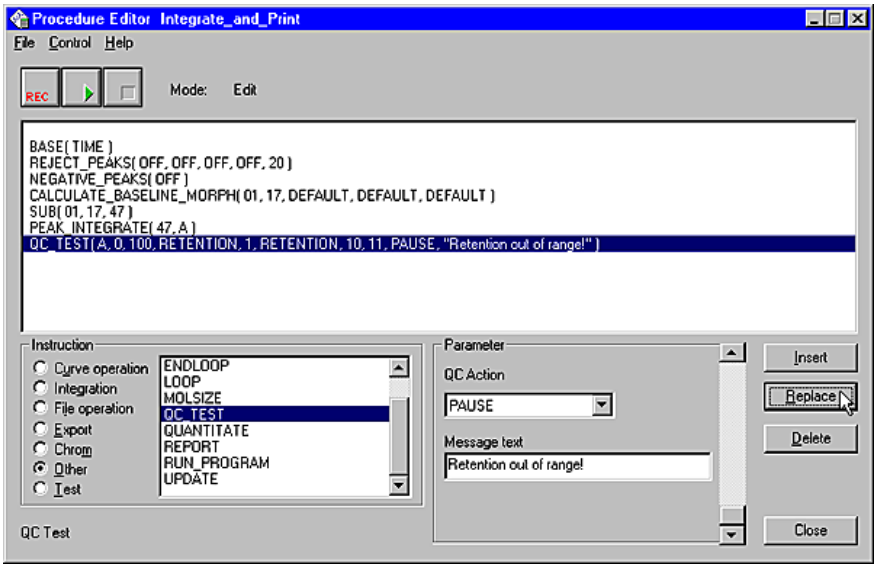
The easiest way to create the quality control procedure is to edit an existing procedure that includes a peak integration. The table below describes how to do this.

Step	Action
1	• Choose ***Procedures:Edit:Open*** in the ***Evaluation*** module. *Result*: The ***Open Procedure*** dialog box opens.
2	• Select the procedure ***(Global) Integrate_and_Print***. • Click the ***OK*** button. *Result*: The ***Procedure Editor*** opens with the procedure displayed.
3	• Select the ***REPORT*** instruction in the procedure. • Choose ***Other*** and ***QC_TEST*** in the ***Instruction*** field.
4	• Type appropriate values in the ***Parameter*** field. See "QC_TEST Parameter descriptions" below. • Click the ***Replace*** button. *Result*: The ***REPORT*** instruction is replaced by the ***QC_TEST*** instruction.

Step	Action
5	• Choose ***File:Save As***. *Result*: The ***Save As*** dialog box opens. • Type a name for the procedure (for example ***QC_test***). • Select the ***Global procedure*** check box if the procedure is to be available to all users. *Note*: If you select ***File:Save*** to save the procedure it will replace the ***(Global) Integrate_and_Print*** procedure.

Illustration: The Procedure Editor

The illustration below shows the ***Procedure Editor*** with the ***QC_TEST*** instruction displayed:



QC_TEST parameter descriptions

The table below describes the parameters for the ***QC_TEST*** instruction.

The example values are used in the illustration above.

Parameter	Description
Peak table source	The peak table indicated in the ***PEAK_INTEGRATE*** instruction (Example: ***A***).
Left limit	The retention value where the control instruction will begin (Example: ***0***).
Right limit	The retention value where the control instruction will end (Example: ***100***). *Note*: The control instruction will be applied to the run up to the sequence in the method where the control instruction is inserted: • The controlled part of the run will end at the ***Right limit*** if this retention value is reached before the control instruction is reached in the method. • If not, the controlled part of the run will end when the control instruction is reached in the method.
Peak selection on	The criteria for peak identification (Example: ***RETENTION***).
Order number	The sequential order number of the peak (Example: 1).
Peak table parameter	The peak table parameter that will be tested by the control instruction (Example: ***RETENTION***).
Less than	Values less than the parameter value will be out of the acceptable range (Example 10).
Greater than	Values greater than the parameter value will be out of the acceptable range (Example 11).
QC Action	The action the system will take when the controlled value is out of the acceptable range (Example: ***PAUSE***).
Message text	Free text message that is displayed when the controlled value is out of the acceptable range (Example: ***Retention out of range!***)

Note: All values must be included before the instruction can be inserted.

How to add the quality control procedure to a method

The table below describes how to add the quality control procedure to a method.

Step	Action
1	• Open the method in the ***Method Editor***. • Click the ***Run Setup*** icon.  *Result*: The ***Run Setup*** for the method opens.
2	• Select the ***Evaluation Procedures*** tab. • Click the ***Import*** button. *Result*: The ***Import*** dialog box opens.
3	• Select the quality control procedure you created and saved (Example: ***QC_test***) in the ***Select*** field. • Click the ***Import*** button. *Result*: The quality control procedure is added to the available evaluation procedures. • Click the ***Close*** button.
4	• Click the check box to de-select the quality control procedure. *Note*: If the quality control procedure is selected it will initiate a new manual run at the end of the method run.
5	• Click the ***Text Instructions*** icon.  • Select the last instruction in the method. • Select ***Other:Evaluate*** in the ***Instructions*** field. • Select the quality control procedure in the ***Procedure*** list. • Click the ***Insert*** button.

Step	Action
6	• Choose ***File:Save*** *or* • Click the ***Save*** icon. *Result*: When the method run is performed the quality control procedure will create a second chromatogram. If the controlled value is outside the acceptable range, the system will be paused.

Index

D

E

F

G

I

L

M

P

Q

R

S

T

U

V

W

Y

Z

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