GE Healthcare

UNICORN 5.1

User Reference Manual

UNICORN™

Table of Contents

Part 1

Part 2

1 Introducing UNICORN

Introduction

This chapter contains:

- A general overview of the UNICORN™ system.
- Information about the user documentation for UNICORN and how to use it.

In this chapter

This chapter contains the following sections

Topic	See
About UNICORN	1.1
About this manual	1.2
About the UNICORN user documentation	1.3

1.1 About UNICORN

Introduction

This section is a general overview of the UNICORN system.

What is UNICORN?

UNICORN is a complete package for control and supervision of chromatography systems. It consists of control software and a controller card for interfacing the controlling PC to the chromatography liquid handling module.

Liquid chromatography is used in separation processes, for analytical purposes or in the biochemical process industry.

UNICORN is a trademark of GE Healthcare.

Operating environment

UNICORN runs on a PC under Microsoft® Windows® 2000 or Microsoft Windows XP. It is designed to run under English keyboard settings.

Windows functions

Most Windows functions are also available in UNICORN, including

- cut and paste
- right-click short-cut menus

Note: Drag and drop is not available. File and folder handling in UNICORN also differs from the general Windows file manager standard.

Bar code reader

You can connect a bar code reader to the PC and use the reader to enter information instead of using the keyboard. This can be useful for example when entering information like batch IDs.

Compatible chromatography systems

UNICORN can be used with a number of systems including

- ÄKTA™ design systems
- BioProcess™ systems

Note: All examples in this guide are based on an ÄKTAexplorer™ 100 system that operates with the E100F400 strategy.If you use another system you may find that the descriptions and instructions do not match your system on every point. In that case you also need to refer to the user documentation for your specific chromatography system.

System networks UNICORN can be installed on a stand-alone computer to control only a single, locally attached system. However, a stand-alone computer can control up to four separate systems. In a network installation each computer workstation can operate many systems regardless if they are locally connected or not. Each system can only be operated by one workstation at a time, but several may view the output data.

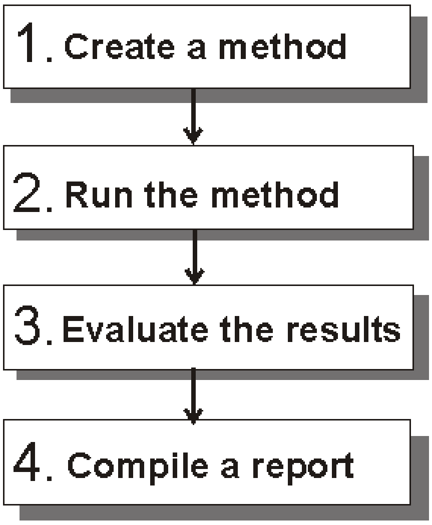
Software modules The UNICORN control software consists of four integrated modules:

Module	Function
UNICORN Manager	File handling and administration, e.g. definition of systems and user profile etc.
Method Editor	To create and edit methods for pre-programmed control of chromatography systems.
System Control	To control and monitor the separation processes online, through method-based or manual control.
Evaluation	To evaluate and present stored results from separation processes.

Note: All modules are active when the program is operating, and are not closed when they are minimized. A minimized System Control unit may control a process. All modules will normally open when the program is started. However, a user profile may be set up so that not all modules are available. Only the available modules will be displayed.

Work flow

The work flow in UNICORN can be divided into four distinct stages. Each stage is described in separate chapters in this manual. The flow chart below shows the work flow stages.



Help functions

An online help utility is included in the UNICORN software. The table below describes how to access the help utility.

If you want to access...	Then...
the general help utility.	open the **Help** menu in any of the software modules.
context-specific help topics.	• click the **Help** button in the dialog box *or* • press the **F1** key on your keyboard.
the online manuals.	open the **Help** menu in any of the software modules and select **Manuals**.

Security

The table below describes the main security functions in UNICORN:

Feature	Function
Access Security	Only authorized users can access UNICORN. Each user is assigned an access level, which defines the functions that the user is permitted to use.

Feature	**Function**
Connection Security	A running system can only be controlled from one connection. Systems may be locked with a password to prevent other, un-authorized users from changing parameters.
Data Security	Result files from an ongoing separation run can be saved automatically at preset intervals to minimize data loss if the system fails. The results are saved locally if the network communication fails.
Electronic Signatures	Method and result files can be signed electronically for enhanced security and accountability.

1.2 About this manual

Introduction

This section is a general description of the manual, the contents and the pre-requisites for the examples and instructions that are presented in the User Reference Manual.

The purpose of the User Reference Manual

The purpose of the User Reference Manual is to present a comprehensive guide to the UNICORN system for a user either with previous experience of this system or from other, similar chromatography systems. The system is presented in detail, along with practical instructions of how to operate a model system.

Systems covered by this manual

This manual and the corresponding version of Getting Started with UNICORN covers the following systems:

- ÄKTAexplorer
- ÄKTApurifier™
- ÄKTAFPLC™
- ÄKTAbasic™
- ÄKTApilot™
- Ettan™ LC

Note: Adapted versions of this manual are available for ÄKTAxpress™, ÄKTA oligopilot™ and BioProcess systems.

The model system

For practical reasons the user documentation is based on a model system that consists of:

- **ÄKTAexplorer 100**
- Strategy **E100F400**
- **Frac-950**

Note: If you use another system you may find that the descriptions and instructions do not match your system on every point. In that case you also need to refer to the user documentation for your specific chromatography system.

Refer to other manuals

The User Reference Manual does not contain information about the installation procedure or network configuration. You will find this information in the Administration and Technical Manual.

Sometimes you may find it more convenient to refer to the Getting Started with UNICORN guide for a linear, step-by-step instruction how to perform a task.

Note: When you install the UNICORN software you choose which manuals you wish to install. You can also install the manuals after the program installation.

Document structure

The manual is divided into chapters. Each chapter starts with a brief overview that presents the contents and the headings for the sections that the chapter contains. Most sections begin with an introduction that summarizes the content. Some sections are divided into sub-sections.

A section is divided into blocks of information with separating lines. The blocks are identified by a label in the margin. This makes it easier for you to quickly scan a page to find the exact topic you are looking for.

Typographical representations

Menu commands, field names and other text items from the software are quoted exactly as they appear on the screen, in a bold typeface:

Example: **Run Setup**

Search paths are shown in a bold typeface with a separating colon between each level:

Example: **View:Panes:Customize** (i.e. the menu command **Customize** in the sub-menu **Panes** from the **View**-menu).

Text entries that UNICORN generates or that the user must type is represented by a monotype typeface:

Example: `Connection change`

Pre-requisites

The following pre-requisites must be fulfilled before you can use this manual the way it was intended:

- You need to have a general understanding of how your PC and Windows works. In most cases universal computer functions will not be explained.
- UNICORN must be installed and configured correctly on your computer.
- You need to understand the concepts of liquid chromatography. Terminology and functionalities will be explained only when they differ from normal practise.
- Before you try to operate a chromatography system based on the instructions in this manual you need to study and understand the safety information that is part of the system documentation.

1.3 About the UNICORN user documentation

Introduction

The user documentation for UNICORN is divided into three separate manuals. This section is an overview of the contents and the relationship between the manuals.

The manuals

The three manuals are:

- Getting Started with UNICORN
- UNICORN User Reference Manual (See **1.2 About this manual** on page 14).
- UNICORN Administration and Technical Manual

User info about Getting Started

The questions and answers in the table below describe the features of the Getting Started manual.

Question	Answer
Who should read Getting Started?	Users that are new to the UNICORN system and with limited experience from other chromatography systems.
What do I need before I start?	A basic knowledge of PC and Windows functions and an understanding of the concepts and terminology of liquid chromatography.
What are the contents of Getting Started?	Basic descriptions of UNICORN and its use, based on a model system.
How should I use Getting Started?	Read in front of your computer and test the instructions at the same time.

User info about the User Reference Manual

The questions and answers in the table below describes the features of the User Reference Manual.

Question	Answer
Who should read the User Reference Manual?	• Users that are experienced with previous UNICORN system versions. • Users with vast experience from other chromatography systems.

Question	Answer
What do I need before I start?	Knowledge of PC and Windows functions and an understanding of the concepts and terminology of liquid chromatography. Preferably previous experience with UNICORN.
What are the contents of the User Reference Manual?	• Detailed descriptions of UNICORN. • Instructions on how to use the system, with suggested alternatives. Most instructions are based on a model system.
How should I use the User Reference Manual?	Depending on your previous experience you can either read whole chapters from the beginning to the end, or only selected sections for reference.

User info about The Administration and Technical Manual

The questions and answers in the table below describes the features of the Administration and Technical Manual.

Question	Answer
Who should read the Administration and Technical Manual?	System administrators.
What do I need before I start?	• General knowledge of UNICORN. • Knowledge of PC, Windows and general network administration functions. • An understanding of the concepts and terminology of liquid chromatography.
What are the contents of the Administration and Technical Manual?	Detailed instructions of: • How to install and maintain UNICORN in a network environment. • How to create and administrate user profiles. Most instructions are based on a model system.

Question	Answer
How should I use the Administration and Technical Manual?	• If you are an experienced administrator of previous UNICORN versions you can read selected sections for reference. • If this is your first experience of UNICORN we recommend that you study the manual in detail.

2 UNICORN concepts

Introduction

This chapter contains:

- Definitions and descriptions of some of the specific concepts that are presented in this manual and in other UNICORN manuals.
- An overview of the UNICORN user interface.
- A Quick Start Guide that can be used as a shortcut for experienced users that want to start right away.

Note: General concepts and common chromatography terminology are not explained here.

In this chapter

This chapter contains the following sections

Topic	See
Concept definitions	2.1
The UNICORN user interface	2.2
Quick Start Guide	2.3

2.1 Concept definitions

Introduction	This chapter contains explanations and definitions of a number of UNICORN concepts that are used in this manual. The concepts are organized in alphabetical order.
Alarms	Systems settings or method instructions specify acceptable limits for monitor signals during a separation run. An **Alarm** dialog box will be displayed on the screen if the monitored values exceed or fall below specified limits. The system will be paused.
Batch run	You can perform a **Batch run** of a number of result files in the **Evaluation** module. The files do not have to be open and the run operates in the background. The procedure is useful if you want to print a number of results with the same settings, or if you want to perform integration with the same parameter settings on many results.
BufferPrep	**BufferPrep** is a function to prepare a buffer of different pH and salt concentrations online from four stock solutions. This eliminates the need to manually prepare new buffers every time the pH needs to be changed. *Note*: **BufferPrep** is only available for some ÄKTAdesign systems.
Chromatogram	A chromatogram is a collection of data represented by a number of curves that have been created during a separation run, including UV, conductivity, pH, fraction marks etc. The original raw data curves cannot be deleted or modified. They can be used as a basis for evaluation procedures and subsequent creation of new curves. A chromatogram can also contain curves that have been created and saved during an evaluation session.
Curves	The monitor signals from the chromatography run are displayed graphically as curves.
Method	The program instructions for a chromatography run are defined in a **Method**. A Method can be divided into blocks that represent steps in the separation process. Each block consists of a series of instructions that request specific operations in the system.
MethodQueue	**MethodQueues** are used to link several methods together, on the same or on different systems.

Example: A **MethodQueue** can be set up to conduct a CIP study of a number of columns, through a controlled series of scouting runs.

Method Wizard

The **Method Wizard** is a user-friendly tool to create new methods. The **Wizard** takes the user step-by-step through the creation process.

Method Wizards are supplied with UNICORN installations for ÄKTAdesign systems.

Result files

UNICORN creates **Result files** when a method is run. The **Result files** contain:

- Run data from the monitors in the chromatography system.

Example: UV absorbance, flow rate, conductivity etc.

- Documentation from the run.

Example: Logbook entries, calibration settings, scouting parameters, text method etc.

- Saved results from evaluations of the run data.

Example: Peak integrations, simulated peak fractionations etc.

Scouting

Scouting is used to repeat a series of **Method runs** automatically with predetermined changes in the values for one or more **Variables**. A **Scouting Scheme** is defined as part of the method.

Scouting is used for optimizing chromatographic processes.

Strategy

Part of the UNICORN software is specific for the system that it is set up to operate. The system specific part is usually referred to as the **Strategy**. The **Strategy** defines available method and manual instructions, system settings, run data, curves and Method Wizards.

Note: The examples in this guide are generally based on the **E100F400** strategy.

Template

Templates are basic methods that can be used as a starting point for developing customized methods. The method variables in a suitable **Template** is adjusted to create a method for another application.

Variable

Instruction parameters and values at breakpoints in the **Method**may be defined as **Variables**. **Variables** makes it easy to adapt a method to a particular chromatography run.

- A framework **Method** with default parameters can be changed to create variants.
- A **Method** can be used in automatic **Method Scouting**, where one or more parameter **Variables** are changed systematically.

Warnings

Systems settings or method instructions specify acceptable limits for monitor signals during a separation run. A **Warning** dialog box may be displayed on the screen if a specified limit is exceeded. The system will still continue to run after a **Warning**.

2.2 The UNICORN user interface

Introduction

This section is an overview of the four UNICORN modules with descriptions of some of the elements of the user interface. The section also contains a description of the search functions in UNICORN.

Note: A user profile can be set up so that the user only has limited access to the modules described in this chapter. Only the available modules will open when the program is started.

In this section

This section contains the following sub-sections

Topic	See
UNICORN Manager	2.2.1
The Method Editor module	2.2.2
The System Control module	2.2.3
The Evaluation module	2.2.4
Search functions	2.2.5
Help functions and manuals	2.2.6
Snapshots	2.2.7

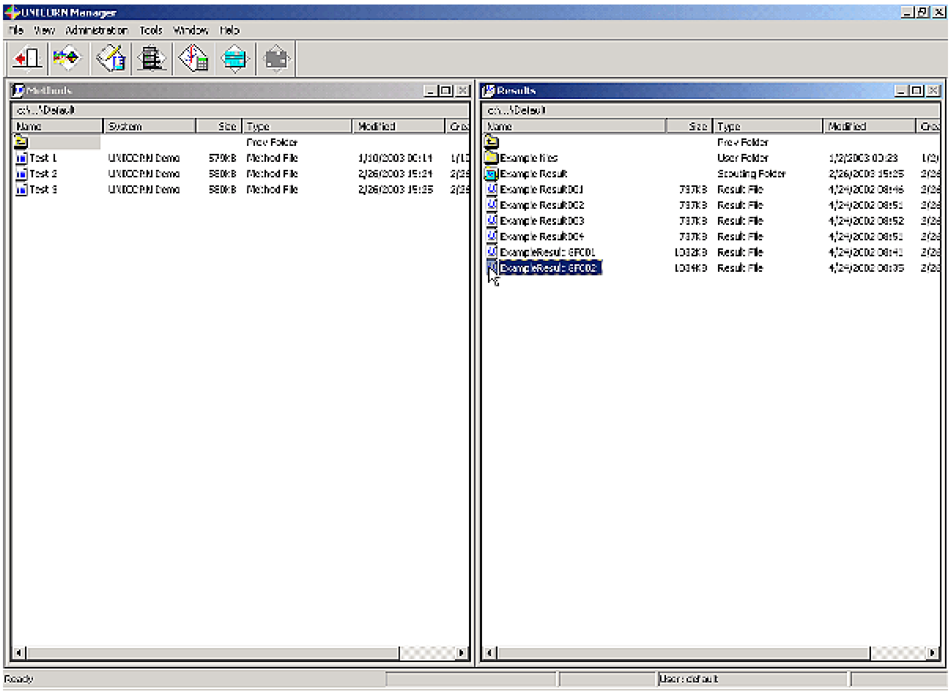
2.2.1 UNICORN Manager

Introduction

The **UNICORN Manager** is mainly used for file and folder administration.

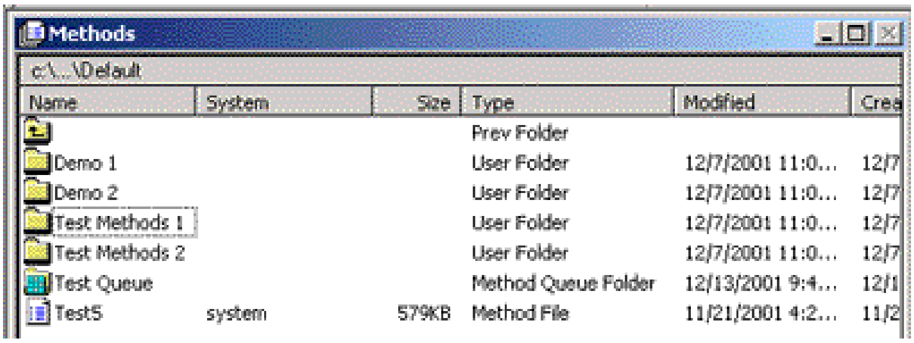
The UNICORN Manager windows

The module is divided into two windows, the **Methods** window and the **Results** window. See the illustration below:



The Methods window

The **Methods** window contains all the saved methods, **MethodQueues** and all the folders containing methods that are available to the user. See the illustration below:



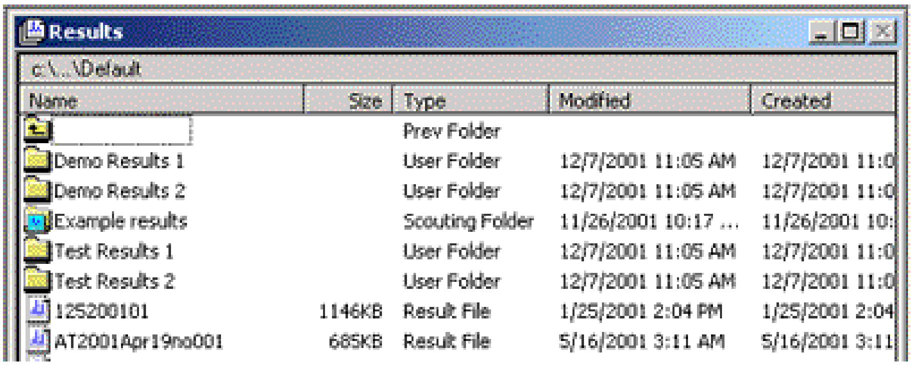
c:\...\Default

Name	System	Size	Type	Modified	Crea
			Prev Folder		
Demo 1			User Folder	12/7/2001 11:0...	12/7
Demo 2			User Folder	12/7/2001 11:0...	12/7
Test Methods 1			User Folder	12/7/2001 11:0...	12/7
Test Methods 2			User Folder	12/7/2001 11:0...	12/7
Test Queue			Method Queue Folder	12/13/2001 9:4...	12/1
Test5	system	579KB	Method File	11/21/2001 4:2...	11/2

Note: The icons for **MethodQueue** folders are different from the regular folder icon.

The Results window

The **Results** window contains all the saved results and all the result folders.



c:\..\Default

Name	Size	Type	Modified	Created
		Prev Folder		
Demo Results 1		User Folder	12/7/2001 11:05 AM	12/7/2001 11:0
Demo Results 2		User Folder	12/7/2001 11:05 AM	12/7/2001 11:0
Example results		Scouting Folder	11/26/2001 10:17 ...	11/26/2001 10:
Test Results 1		User Folder	12/7/2001 11:05 AM	12/7/2001 11:0
Test Results 2		User Folder	12/7/2001 11:05 AM	12/7/2001 11:0
125200101	1146KB	Result File	1/25/2001 2:04 PM	1/25/2001 2:04
AT2001Apr19no001	685KB	Result File	5/16/2001 3:11 AM	5/16/2001 3:11

Note: The icons for **Scouting** folders are different from the regular folder icon.

Toolbar icons in the UNICORN Manager

The table below describes the toolbar icons in the module.

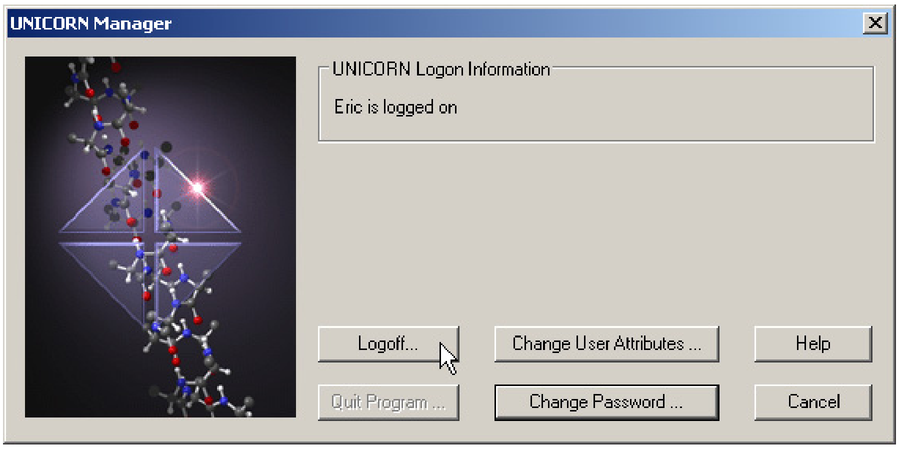
Icon	Function
	The **Logon/Logoff** icon is used to log on or log off the system. *Note*: The arrow in the **Logoff** icon points away from the door.
	The **Instant Run** icon immediately starts a run from a selected template or from a wizard.
	The **New Method** icon opens the **Method Editor** module and displays the **New Method** dialog box.
	The **System Control** icon activates the first connected **System Control** module and displays the **Manual instruction** dialog box.
	The **Evaluation** icon opens the **Open Result** dialog box. Select a result file and click **OK** to start the **Evaluation** module.
	The **MethodQueue** icon opens the **MethodQueue Editor**.
	The **Existing MethodQueue** icon opens the **Running MethodQueue** dialog box to display **MethodQueues** in progress.

Limited access to the UNICORN Manager

Some user groups may be defined to have only a limited access to the **UNICORN Manager** functions. The available functions in the limited version are:

- **Log off**
- **Change User Attributes**
- **Change Password**
- **Quit Program**
- **Help**

There is also a **Cancel** button which minimizes the dialog box. The illustration below shows the limited access version of the **UNICORN Manager**.



UNICORN Logon Information

Eric is logged on

Note: For more information about how to change passwords and user attributes please refer to **3.4 How to change your passwords and user attributes** on page 59. For more information about how to log off and quit the program, please refer to **3.1 Log on routines and log off routines** on page 47.

2.2.2 *The Method Editor module*

Introduction

The **Method Editor** module provides complete facilities for advanced editing of the methods.

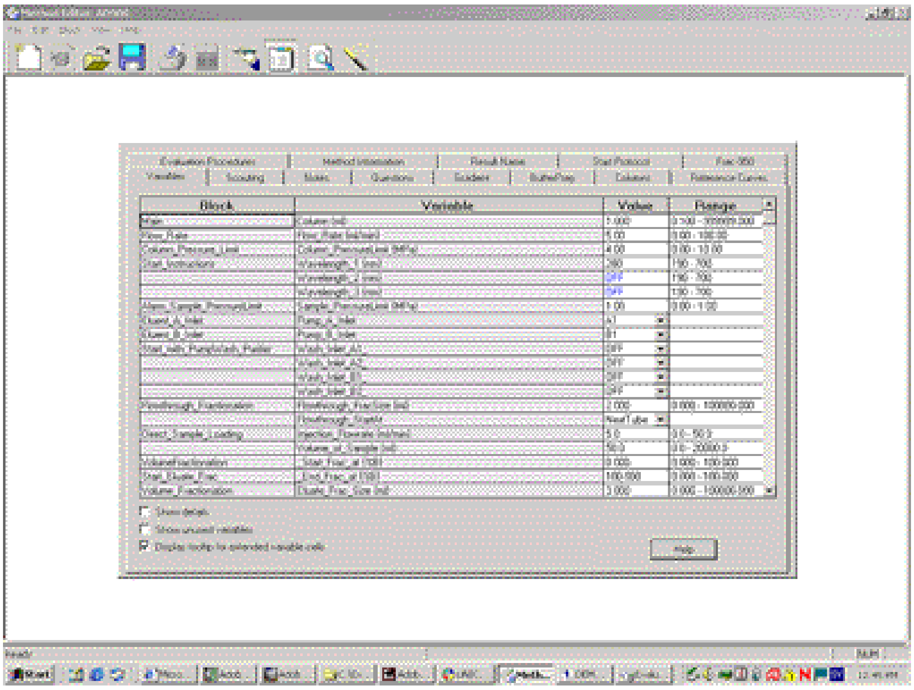
Two modes

The **Method Editor** interface operates in two modes:

- **Run Setup**
- **Text Instructions**

Run Setup

Run Setup is a dialog box with a number of tabs that define the method properties. See the illustration below:

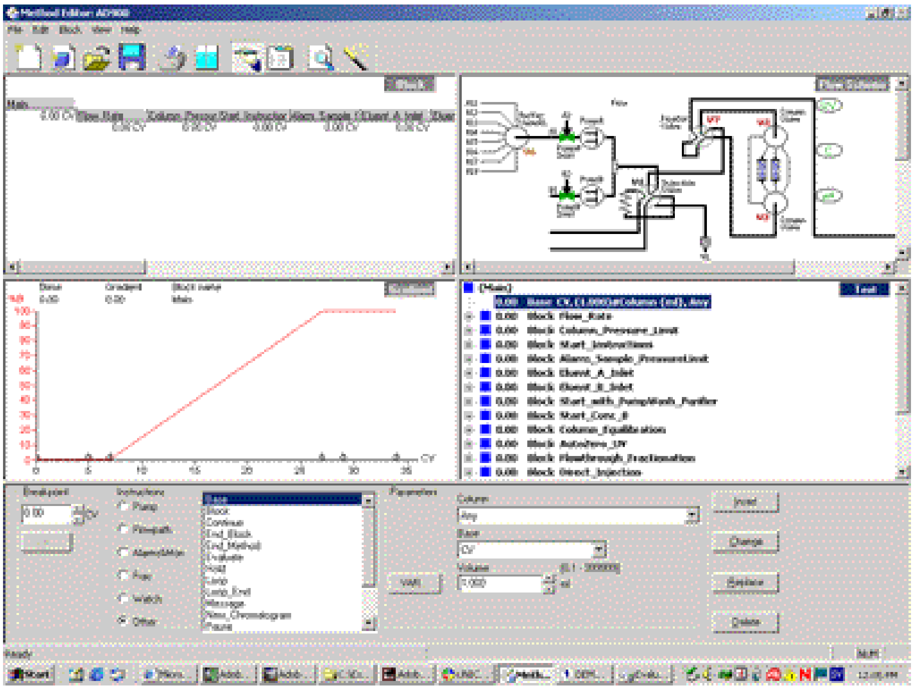


Text Instructions

Text Instructions are used for advanced editing. Up to five different display panes can be open at the same time:

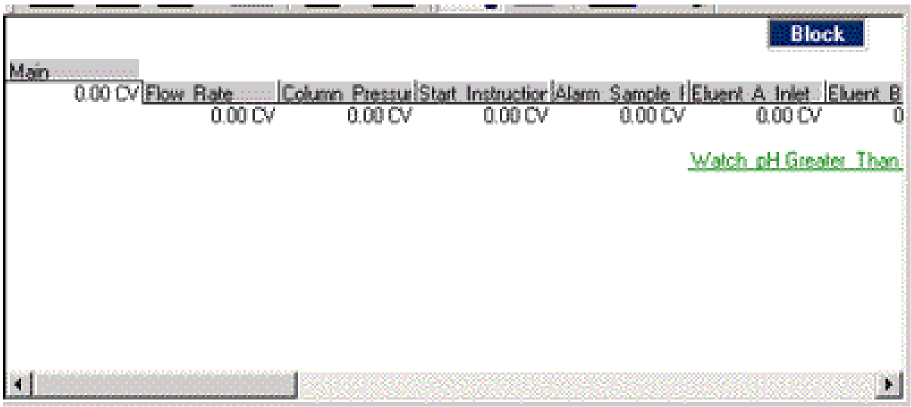
- The **Block** pane.
- The **Flow Scheme** pane.
- The **Gradient** pane.
- The **Text** pane.
- The **Instruction box** pane.

See the illustration below:



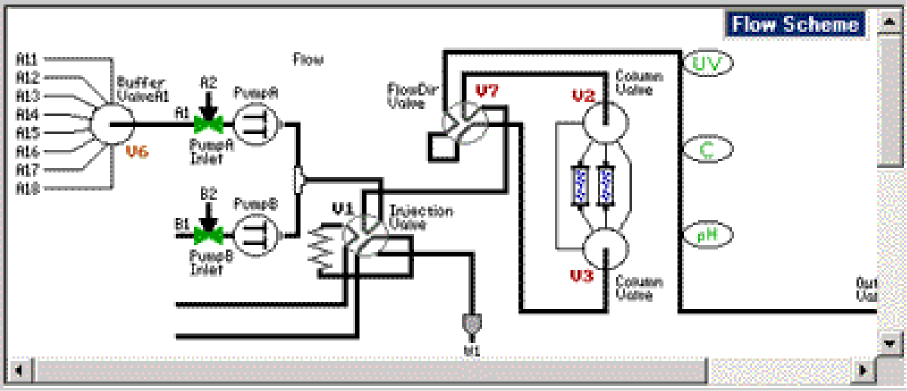
The Block pane

The **Block** pane contains a graphical representation of the method organized in blocks. See the illustration below:



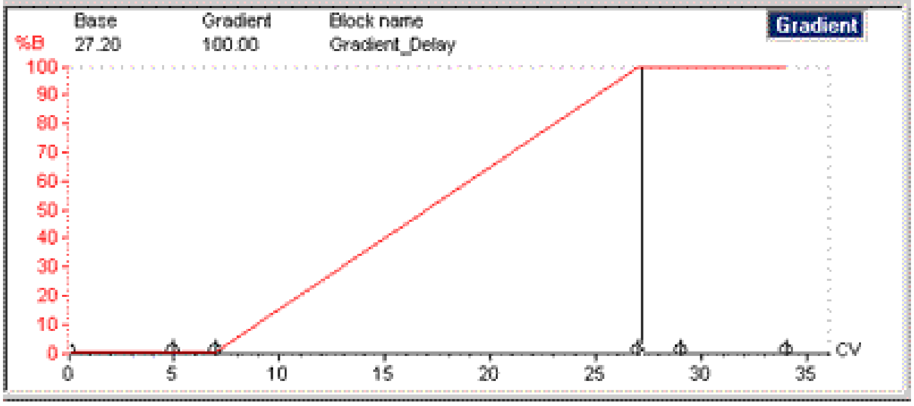
The Flow Scheme pane

The **Flow Scheme** pane displays the configuration of the system components. The pane is static and for information only. See the illustration below:



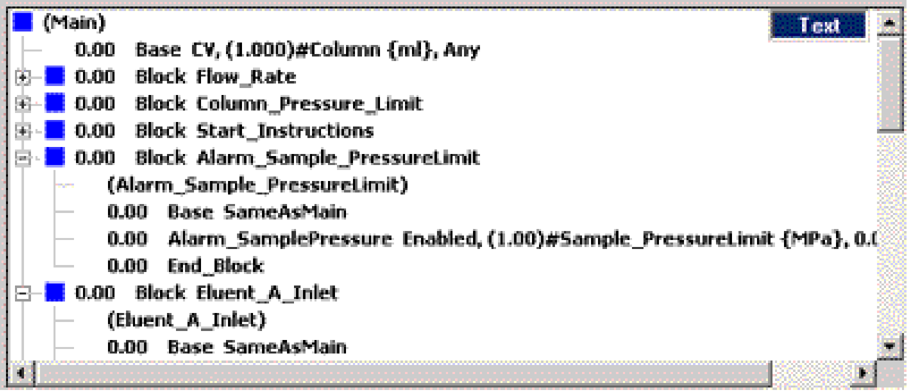
The Gradient pane

The **Gradient** pane provides a graphical overview of the block structure and eluent gradient in the current method. See the illustration below:



The Text pane

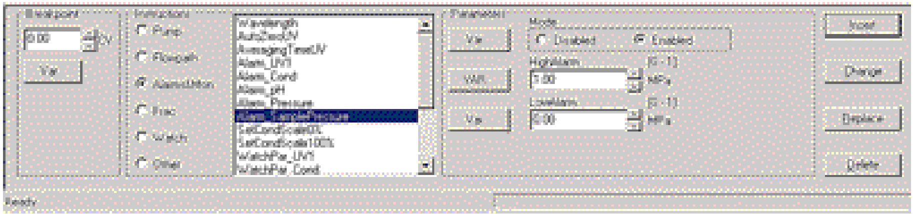
The **Text** pane displays the method as a list of text instructions. The instructions can be organized in blocks, denoted by blue square symbols. The blocks can be expanded to show the instructions within the block. See the illustration below:



```
■ (Main)
    0.00  Base  CV, (1.000)#Column {ml}, Any
⊞ ■ 0.00  Block  Flow_Rate
⊞ ■ 0.00  Block  Column_Pressure_Limit
⊞ ■ 0.00  Block  Start_Instructions
⊟ ■ 0.00  Block  Alarm_Sample_PressureLimit
        (Alarm_Sample_PressureLimit)
        0.00  Base  SameAsMain
        0.00  Alarm_SamplePressure  Enabled, (1.00)#Sample_PressureLimit {MPa}, 0.(
        0.00  End_Block
⊟ ■ 0.00  Block  Eluent_A_Inlet
        (Eluent_A_Inlet)
        0.00  Base  SameAsMain
```

The Instruction box pane

The **Instruction box** pane is used to enter, edit or delete instructions. See the illustration below:



Toolbar icons in the Method Editor

The table below describes the toolbar icons in the module.

Icon	Function
	The **New** icon opens the **New Method** dialog box. The dialog box is used to create a new method.
	The **New Block** icon opens the **New Block** dialog box, which is used to add blocks to a method.
	The **Open** icon displays all available method files and method folders in the **Open** dialog box.
	The **Save Method** icon saves the edited method.
	The **Print** icon opens the **Print** dialog box. Select the method elements that you want to print.
	The **Customise Panes** icon opens the **Customise Panes** dialog box, which is used to select the panes that are open in **Text Instructions** mode.
	The **Text Instructions** icon opens the **Method Editor** in **Text Instructions** mode.
	The **Run Setup** icon opens the **Method Editor** in **Run Setup** mode.
	The **Log Format** icon opens the **Log Format** dialog box, which is used to display the accumulated time or volume for a method.
	The **Method Wizard** icon opens the **Method Wizard**, which is used to create new methods.

2.2.3 *The System Control module*

Introduction

The **System Control** module is used to perform and monitor separation runs.

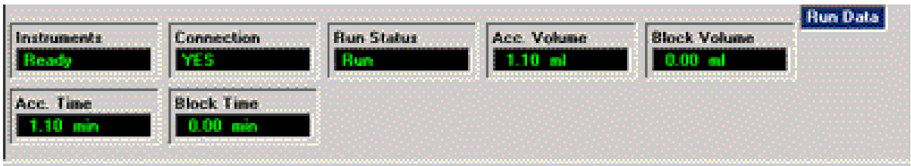
The System Control panes

The **System Control** module contains four different display panes that can be opened all at once or in any combination:

- The **Run Data** pane.
- The **Curves** pane.
- The **Flow Scheme** pane.
- The **Logbook** pane.

The Run Data pane

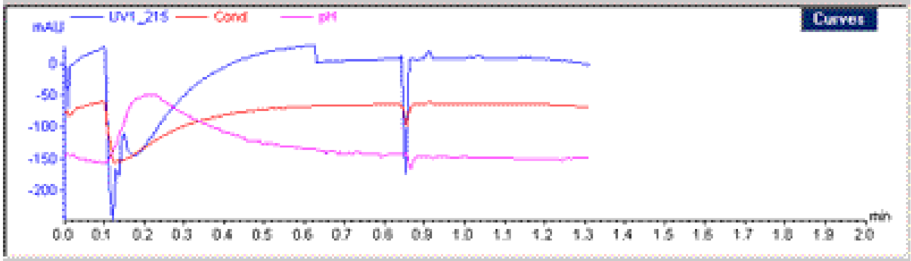
The **Run Data** pane displays the current values for the selected run parameters. The values are updated at regular intervals, which are defined in the system strategy. See the illustration below:



Instruments: Ready
Connection: YES
Run Status: Run
Acc. Volume: 1.10 ml
Block Volume: 0.00 ml
Acc. Time: 1.10 min
Block Time: 0.00 min

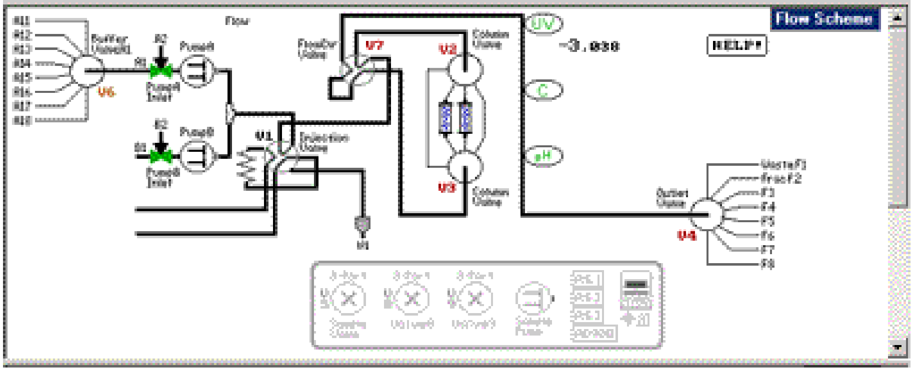
The Curves pane

The **Curves** pane displays monitor signal values graphically. See the illustration below:



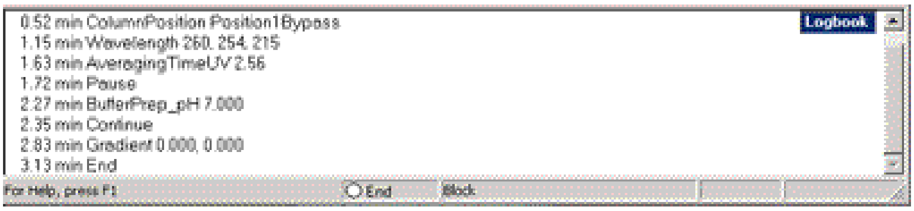
The Flow Scheme pane

The **Flow Scheme** is a graphical representation of the chromatography system. During a run, the **Flow Scheme** displays open flow paths in color. Monitor signals can be displayed numerically. See the illustration below:



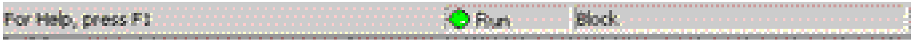
The Logbook pane

The **Logbook** pane displays all actions during a separation run, e.g. method start and end, base instruction, method instructions and manual instructions such as **Pause** or **Hold**. See the illustration below:


```
0.52 min ColumnPosition Position1Bypass
1.15 min Wavelength 260, 254, 215
1.63 min AveragingTimeUV 2.56
1.72 min Pause
2.27 min BufferPrep_pH 7.000
2.35 min Continue
2.83 min Gradient 0.000, 0.000
3.13 min End
```

The Status bar

The **Status bar** in the bottom of the **System Control** module displays the current status of the separation run. See the illustration below:



The current system status is represented by the colored dot:

- A green dot represents a running system.
- A red dot represents a system in **Pause** state.
- A yellow dot represents a system in a **Hold** state.
- A white dot represents a system in an **End** state.

Toolbar icons in the System Control

The table below describes the toolbar icons in the module:

Icon	Function
Run	The **Run** icon opens the **Run** dialog box, which shows all available methods. If a method is loaded, **Run Setup** opens.

Icon	Function
Hold	The **Hold** icon suspends execution of the method, while liquid is still pumped at the current flow rate and eluent concentration.
Pause	The function of the **Pause** icon depends on the strategy. The **Pause** icon suspends execution of the method and stops all pumps so that the system comes to a standstill.
Continue	The **Continue** icon resumes the execution of a paused or held method.
End	The **End** icon terminates the method execution and puts the system into an **End** state.
▥	The **Customise Panes** icon opens the **Customise Panes** dialog box, which is used to select the display panes that are open.
📋	The **View Documentation** icon opens the documentation pages. Run notes can be entered in the **Notes** page and settings can be changed.
📊	The **View Properties** icon opens the **Properties** dialog box, which is used to control the data display in the **System Control** panes.
🔌	The **Connect System** icon is used to connect a system.
🔌	The **Disconnect System** icon is used to disconnect the system.
☸	The **Take Control of the System** icon is used to leave the view mode for the system and change into a control mode.
🔭	The **Leave Control of the System** icon is used to leave the control mode for the system and change into a view mode.

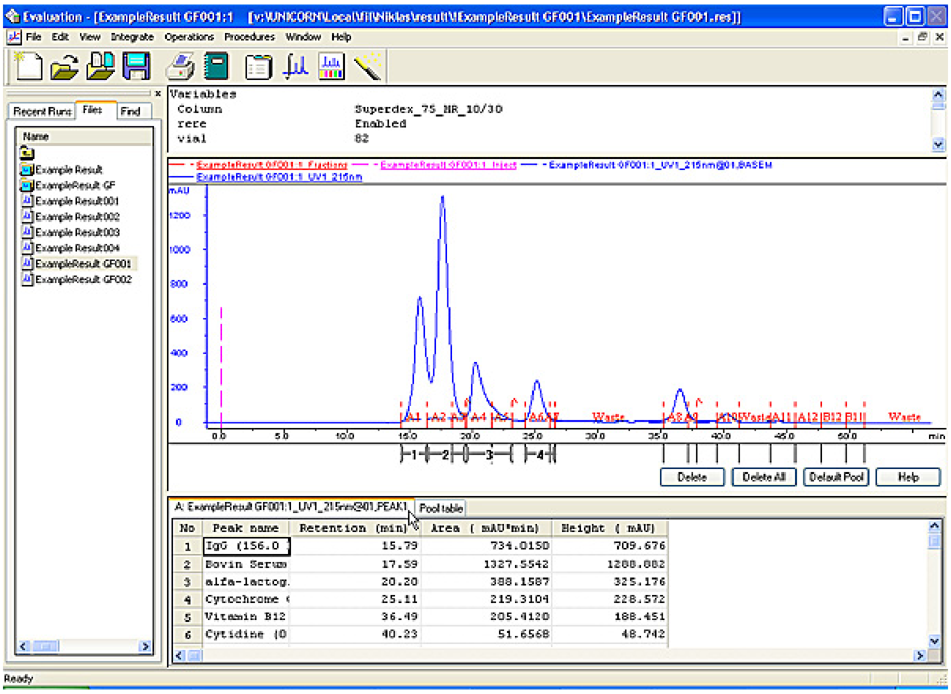
2.2.4 *The Evaluation module*

Introduction

The **Evaluation** module provides extensive facilities to present and to evaluate curve data.

The module window

Opened result files are displayed in the **Evaluation** module window. See the illustration below:



```
Variables
 Column                 Superdex_75_HR_10/30
 rere                   Enabled
 vial                   82
```

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)
1	IgG (156.0	15.79	734.0150	709.676
2	Bovin Serum	17.59	1327.5542	1288.882
3	alfa-lactog.	20.20	388.1587	325.176
4	Cytochrome (	25.11	219.3104	228.572
5	Vitamin B12	36.49	205.4120	188.451
6	Cytidine (0	40.23	51.6568	48.742

Toolbar icons in the Evaluation module

The table below describes the toolbar icons in the module:

Icon	Function
🗋	The **New** icon opens an empty chromatogram.
📂	The **Open** icon displays all available result files and result folders in the **Open Result** dialog box.
📂	The **Open Curves to Compare** icon opens the **Open Curves to Compare** dialog box, which is used to select and open curves for comparison.
💾	The **Save** icon saves the edited result file.

Icon	Function
🖨	The **Print** icon opens the **Print Chromatograms** dialog box.
📓	The **Report** icon opens the **Generate Report** dialog box, which is used to select a report format.
📋	The **View Documentation** icon opens the **Documentation** dialog box, which is used to view and edit the result documentation.
∫	The **Peak Integrate** icon opens the **Integrate** dialog box, which is used to select peaks to integrate in a modified peak table.
📊	The **Chromatogram Layout** icon opens the **Chromatogram Layout** dialog box, which is used to select and format curves and display items in the chromatogram.
🪄	The **Multifile Peak Compare** icon opens the **Multifile Peak Compare Wizard**, which is used to compare peak data from different result files.

2.2.5 Search functions

Introduction

This section describes the general search functions that can be used to locate for example chromatograms, curves and text strings in UNICORN. These functions can be used in several program modules, dialog boxes and wizards.

Search the Folder list

The search will take place in the displayed folder only. To select another folder, click the **Browse** button and open the desired folder.

Search the Result list

- The search will take place in *all* result files within the selected folder as denoted by the asterisk (*). To select specific result file(s), click the **Browse** button and select the result file(s).
- You can use wildcard characters to search for chromatograms within result files with a specific name profile.
    - * represents any number of characters
    - ? represents any single character

Wildcard character examples:

`iex` will search files named “iex”

`iex*` will search all files with names that begin with “iex”

`*iex` will search all files with names that end with “iex”

`?iex` will search only 4-character names that end with iex

Search the Chromatogram list

The asterisk (*) indicates that all chromatograms within a result file will be selected. Click **Browse** to select one or several specific chromatograms.

Search the Curve name list

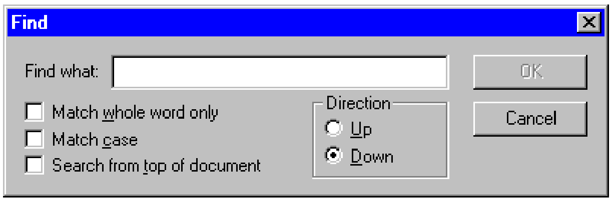
The UV curves are identified by number and sometimes wavelength. For example, UV1_280, UV2_280 and UV1_254 are all different curves. To search for all UV curves, select `*UV*` in the **Curve name** text field.

Searches for Sample ID

A **Sample ID** can be used as a search criteria if it has been defined as a variable. The **Sample ID** can be entered in searches for result files both in the **UNICORN Manager** and in the **Evaluation** module.

Find a text string

The **Find** command is used to search for text strings:



Field	Description
Find what	Type the text string you want to find.
Match whole word only	Select the check-box if you only want complete string matches, not partial matches.
Match case	Select the check-box if you only want matches which correspond according to upper-case and lower-case letters.
Search from top of document	Select the check-box to start the search from the top of the document, otherwise the search will start from the cursor position.
Direction	Choose whether to search upwards or downwards in the document.

Commands

Use the commands below to find more occurrences of a text string after you have found the first one:

- Press **F3** to search for the next occurrence of the string *or* right-click and choose **Find next**.
- Right-click and choose **Find previous** to search for a previous occurrence.

General information about searches

- The default setting is to search in all result files or chromatograms.
- User-entered search filters (to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used as a search delimiter (insert ";" between strings), and search filters are automatically saved and stored within user profiles.
- Click **All** to return to the default setting to search in all result files or chromatograms.

2.2.6 Help functions and manuals

Introduction

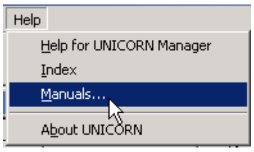
There are different ways to get help and instructions in the UNICORN application:

- From the **Help** menu in each module
- From the context-sensitive help in each dialog box
- By selecting the **Online Manual** from the **Help** menu
- By pressing the <F1> key
- By right-clicking an instruction in the **Method Editor** and selecting the **What's This?** menu item

The Help menu

- From the **Help** menu in each module you can access the **Help** file.
- From the **Help** menu of the **UNICORN Manager** module you can also access the installed manuals.

The illustration below shows the **Help** menu of the **UNICORN Manager** module:



The Help file

The table below describes how to open and use the Help file:

Step	Action
1	Choose **Help:Index**. *Result*: The Help file is displayed
2	• Type a word you want help on in the text box in the left pane. *Result*: The closest matches are displayed in the list. • Select a match and click the **Display** button. *Result*: The associated help text is displayed in the right pane.
3	• You can also click the **Contents** tab to view the contents of the Help file divided into sections. • Click the plus signs to expand the tree structure. • Click a topic to read the associated help text.

Manuals

When UNICORN was installed, the administrator selected which manuals to install. Therefore the available manuals may be different on your system than in the illustration below.

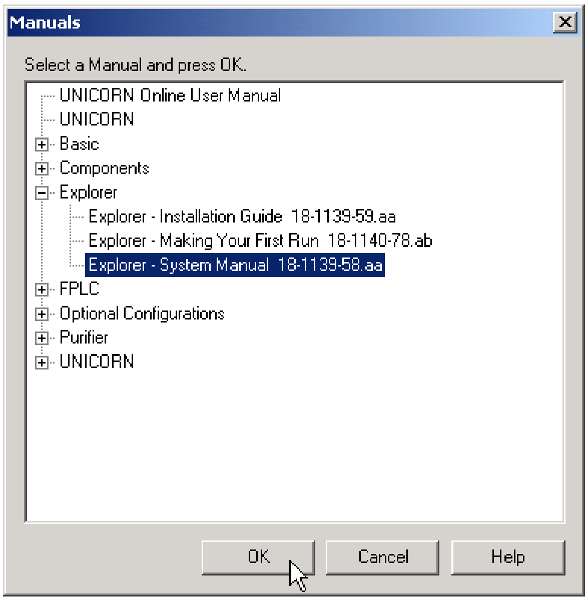
Note: Manuals can be added after the UNICORN installation. See the Administration and Technical manual for more information.

How to open a manual

To open a manual

- choose **Help:Manuals** in the **UNICORN Manager** module.

Result: The **Manuals** dialog box is opened.



Select a Manual and press OK.

- UNICORN Online User Manual
- UNICORN
- Basic
- Components
- Explorer
  - Explorer - Installation Guide 18-1139-59.aa
  - Explorer - Making Your First Run 18-1140-78.ab
  - Explorer - System Manual 18-1139-58.aa
- FPLC
- Optional Configurations
- Purifier
- UNICORN

- Select the manual and click the **OK** button.

Note: Some manuals are only available in PDF format.

Context-sensitive help

In each dialog box there is a **Help** button. If you press that button, either of the following will be displayed:

- A message box with relevant information, for example the dialog box options.
- The Help file, with relevant information displayed in the right pane.

2.2.7 Snapshots

Introduction

A **Snapshot** provides information about a method run at a certain point in time. It contains information about the values of all the variables at the selected point.

Snapshot functionality is available in

- the **Method Editor**, where Snapshot instructions can be inserted in a method to be recorded during the method run.
- the **Evaluation** module, where you can take Snapshots from a result file using the Marker.
- the **System Control** module, where you can take Snapshots during a run using the Marker.

How to view recorded Snapshots

The table below describes how to view Snapshots which have been recorded during a method run using the **Snapshot** text instruction.

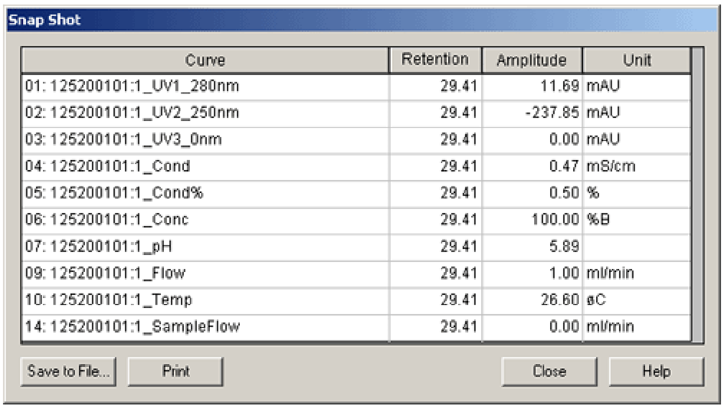
Note: How to insert the **Snapshot** text instruction in a method is described in **5.3 How to use Text instructions** on page 95.

Step	Action
1	In the **Evaluation** module, • choose **View:Documentation** *or* • click the **View Documentation** icon. *Result*: the **Documentation** dialog box is displayed.
2	• Select the **Result Information** tab. • Select the **Snapshots** sub-tab. *Result*: The recorded Snapshot information for a chromatogram is displayed in a list.
3	You can • select other chromatograms in the **Select chromatogram** drop-down box. • select the **Rows** or **Columns** radio button to display each Snapshot as a row or a column. • select the **Time** or **Volume** radio button depending on which quantity you want as a base.

Step	Action
4	To print the Snapshot information • click the **Print** button • select the **Snapshot** check box in the **Print** dialog box. • click **OK**.
5	Click **OK** (or the **Cancel** button) to exit the **Documentation** dialog box.

How to take Snapshots in the Evaluation module

The table below describes how to take Snapshots in the **Evaluation** module:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>• Open a result file in the Evaluation module.
• Right-click and select Marker in the menu.
<i>Result</i>: A vertical line indicating a certain point is displayed.</td></tr>
<tr><td>2</td><td>Click the marker line and drag it to the desired point where you want to take a Snapshot.</td></tr>
<tr><td>3</td><td>Right-click and select Snapshot in the menu.
<i>Result</i>: The Snapshot is displayed in the Snap Shot dialog box.


<table>
<tr><th>Curve</th><th>Retention</th><th>Amplitude</th><th>Unit</th></tr>
<tr><td>01: 125200101:1_UV1_280nm</td><td>29.41</td><td>11.69</td><td>mAU</td></tr>
<tr><td>02: 125200101:1_UV2_250nm</td><td>29.41</td><td>-237.85</td><td>mAU</td></tr>
<tr><td>03: 125200101:1_UV3_0nm</td><td>29.41</td><td>0.00</td><td>mAU</td></tr>
<tr><td>04: 125200101:1_Cond</td><td>29.41</td><td>0.47</td><td>mS/cm</td></tr>
<tr><td>05: 125200101:1_Cond%</td><td>29.41</td><td>0.50</td><td>%</td></tr>
<tr><td>06: 125200101:1_Conc</td><td>29.41</td><td>100.00</td><td>%B</td></tr>
<tr><td>07: 125200101:1_pH</td><td>29.41</td><td>5.89</td><td></td></tr>
<tr><td>09: 125200101:1_Flow</td><td>29.41</td><td>1.00</td><td>ml/min</td></tr>
<tr><td>10: 125200101:1_Temp</td><td>29.41</td><td>26.60</td><td>øC</td></tr>
<tr><td>14: 125200101:1_SampleFlow</td><td>29.41</td><td>0.00</td><td>ml/min</td></tr>
</table>
</td></tr>
</table>

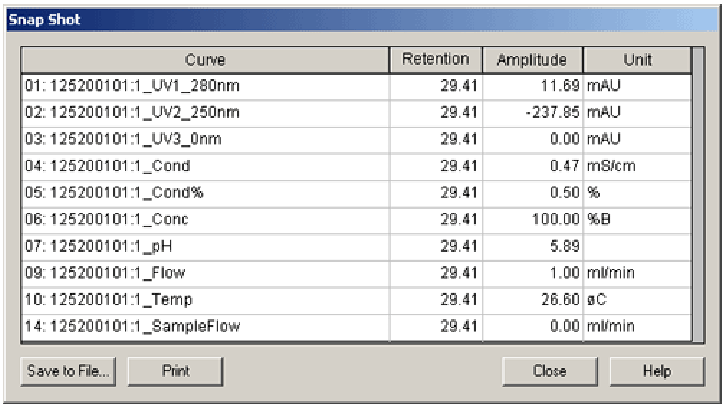
Step	Action

4	• Click the **Save to File** button if you want to save the information as an Excel file (**.xls**) or a tabbed text file (**.txt**).
	• You can also copy the information to the clipboard:
	  - Click and drag the mouse in the table to select the information you want to copy.
	  - Press **CTRL**+**C**.
	  The information can now be pasted in a text editor.
	• Click the **Print** button if you want to print the information.
	• Click the **Close** button.

5	Repeat steps 2 to 4 if you want to view more Snapshots.

How to view Snapshots during a method run

The table below describes how to view Snapshots in the **System Control** module during a method run:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>A method is running and the System Control is displayed:
• Right-click in the Curves pane and select Marker in the menu.
<i>Result</i>: A vertical line is displayed.</td></tr>
<tr><td>2</td><td>Click the marker line and drag it to the desired point where you want to take a Snapshot.</td></tr>
<tr><td>3</td><td>Right-click in the Curves pane and select Snapshot in the menu.
<i>Result</i>: The Snapshot is displayed in the Snap Shot dialog box.


<table>
<tr><th>Curve</th><th>Retention</th><th>Amplitude</th><th>Unit</th></tr>
<tr><td>01: 125200101:1_UV1_280nm</td><td>29.41</td><td>11.69</td><td>mAU</td></tr>
<tr><td>02: 125200101:1_UV2_250nm</td><td>29.41</td><td>-237.85</td><td>mAU</td></tr>
<tr><td>03: 125200101:1_UV3_0nm</td><td>29.41</td><td>0.00</td><td>mAU</td></tr>
<tr><td>04: 125200101:1_Cond</td><td>29.41</td><td>0.47</td><td>mS/cm</td></tr>
<tr><td>05: 125200101:1_Cond%</td><td>29.41</td><td>0.50</td><td>%</td></tr>
<tr><td>06: 125200101:1_Conc</td><td>29.41</td><td>100.00</td><td>%B</td></tr>
<tr><td>07: 125200101:1_pH</td><td>29.41</td><td>5.89</td><td></td></tr>
<tr><td>09: 125200101:1_Flow</td><td>29.41</td><td>1.00</td><td>ml/min</td></tr>
<tr><td>10: 125200101:1_Temp</td><td>29.41</td><td>26.60</td><td>øC</td></tr>
<tr><td>14: 125200101:1_SampleFlow</td><td>29.41</td><td>0.00</td><td>ml/min</td></tr>
</table>
</td></tr>
</table>

Step	Action
4	• Click the **Save to File** button if you want to save the information as an Excel file (**.xls**) or a tabbed text file (**.txt**). • You can also copy the information to the clipboard: - Click and drag the mouse in the table to select the information you want to copy. - Press **CTRL**+**C**. The information can now be pasted in a text editor. • Click the **Print** button if you want to print the information. • Click the **Close** button.
5	Repeat steps 2 to 4 if you want to view more Snapshots.

2.3 *Quick Start Guide*

Introduction

This guide is intended for users who are fully familiar with the safety precautions and operating instructions that are described in all manuals, i.e. experienced users of previous versions of UNICORN. The instructions assume that all installations were made according to the instructions, that the model system is used and is connected.

Quick Start instructions

The table below describes the easiest way to create a method, run the system and generate a printed chromatogram. The procedure is based on an **Instant run**.

Step	Action
1	Click the **Instant run** icon in the **UNICORN Manager** module. *Result*: The **Instant run** dialog box opens.
2	• Select **Wizard**. • Select a system (if necessary). • Click the **Run** button. Result: The **Method Wizard** opens in the **System Control** module.
3	Go through all selections on the **Method Wizard** pages. Click the **Next** button to proceed from page to page.
4	Click the **Run** button on the last page. *Result*: The start protocol opens.
5	Verify the method on the **Variables** page and change values as required. Click the **Next** button to proceed through several pages.
6	Select **Print_Chromatogram** in the **Evaluation procedures** page. *Result*: A printout will automatically be generated after the run.
7	Click the **Start** button on the last page. *Result*: The run starts.

3 General system operations

Introduction

This chapter describes how to start the program, assign user properties and set up the system.

Refer to the Administration and Technical Manual for installation and network configuration instructions.

In this chapter

This chapter contains the following sections

Topic	See
Log on routines and log off routines	3.1
How to create a new user	3.2
How to assign user properties	3.3
How to change your passwords and user attributes	3.4
How to connect to the chromatography system	3.5
How to back up and restore system data	3.6
How to set up a printer	3.7

3.1 Log on routines and log off routines

Introduction

This section describes how to start and quit the UNICORN program, and how to log on and log off.

Username and password

Normally the system administrator defines the users and creates your first password. The program can also be set up so you can log on without a password.

Note: The first time after UNICORN has been installed, you may need to log on as a default user and create a user profile. This process is described in **3.2 How to create a new user** on page 52.

How to start the program

Note: if UNICORN is already started by a previous user, proceed to How to log on.

There are two ways to start the program:

If you start with...	**Then...**
a UNICORN icon on your desktop	double-click the icon UNICORN
the Windows Start menu in Windows 2000	locate the program under **Programs:Unicorn** and click the **UNICORN** logo
the Windows Start menu in Windows XP	locate the program under **All programs:Unicorn** and click the **UNICORN** logo

How to log on

The table below describes how to log on to UNICORN.

Step	Action
1	• Select **Tools:Logon** in the **UNICORN Manager** module *or* • Click the **Logon/Logoff** icon in the **UNICORN Manager** module *Result*: the **Logon** dialog box is displayed. *Note*: You do not have to perform this step if you start up UNICORN. When you start UNICORN the **Logon** dialog box is automatically displayed.
2	Select your username from the list.
3	Type your password (optional).
4	Click **OK**.

The four program modules

The program has four modules. When you start the program and log on you work in the **UNICORN Manager** module. UNICORN also automatically opens the **Method Editor**, the **System Control** and the **Evaluation** modules. These modules are minimized until you activate them. Up to four System Control module windows may open if UNICORN was set up to control more than one system at the installation.

Note: If the access rights are limited to only some modules, the other modules will not open.

Log off after you are finished

Always log off when you leave the computer to prevent others from accidentally changing or deleting your files, or disturbing your UNICORN runs. There are two ways to log off in the **UNICORN Manager**:

- Select **Tools:Logoff**

or

- Click the **Logon/Logoff** icon.

Note: In case your access to the **UNICORN Manager** is restricted you will still be able to log off.

Processes can run after log off

The process will continue even if you log off while a separation run is in progress. You can leave the process locked and set a password to protect it from interference. The table below describes how to log off and set a password for a running process.

Step	Action
1	• Select **Tools:Logoff** in the **UNICORN Manager** module. *or* • Click the **Logoff** icon. *Result*: A confirmation box opens.
2	Click **Yes** to confirm that you want to log off. *Result*: The **Leave Control of system** dialog box opens.
3	Click the **Locked** radio button.
4	Type a password in the **Password** text box.
5	Click **OK**.

Unlocked Log off

It is not recommended that you log off and leave a running system unlocked. This means that the run is in progress without a user that is responsible for the process.

Automated workstation lock or logout

The system administrator may set an automatic workstation lock or log off after a specified time for a user. If there are no keyboard entries or mouse movements within the time limit, the workstation will be locked or logged off.

Note: A locked workstation can be activated again only by the previous user if the regular log in password is entered. If another user wants to log on and use the workstation the previous user can be logged off without entering the correct password. The previous user's files will be closed and the new user will only have access to his own files. Automated logout will not happen while a **MethodQueue** or a **Scouting** scheme is operating.

How to log on and unlock the system

When you log on again after leaving the system locked with a process running or after an automated workstation lock, you will be asked to unlock the system.

Step	Action
1	Log on to the system. *Result*: The **System Unlock Confirmation** dialog box opens.

Step	Action
2	Type your login password or the password that the system was locked with in the **Password** text box.
3	Click **OK**

Note: You can connect in view mode only without providing the password.

Systems locked by other users

You can unlock a system that has been locked by another user if you have the correct password.

You may still be able to unlock a system even if you do not have the password. Any user with **Unlock locked systems** authorization can override another user's lock by entering his or her own logon password. However, it is recommended that this authorization is limited to only a few users.

How to quit UNICORN

UNICORN will still be open after you have logged off. To close the program you must log in again and quit UNICORN (you cannot quit the program if you are not logged in). The table below describes how to do this.

Step	Action
1	• Select the **File:Quit Program** menu command in the **UNICORN Manager** module. *or* • Click the close icon in the top right-hand corner of the program window. *Result*: A confirmation box opens.
2	Click **Yes** to confirm that you want to quit.
3	A **Warning** opens if you have any unsaved data in the Method Editor or Evaluation module. • Click **Yes** to continue to close the program. Your unsaved data will be lost when the program is closed. • Click **No** to return to the program and save your data.
4	The **Leave Control of system** dialog box opens. Select the locked or unlocked option as in the logoff procedure. *Note*: This step only happens when a system is connected.
5	Click **OK**.

Note: Do not shut down Windows 2000/XP or turn off the computer if you quit UNICORN with a separation run in progress. If you are performing a **Scouting run** or a **MethodQueue run** you cannot quit the program at all.

In case your access to the **UNICORN Manager** is restricted you will still be able to quit the program.

3.2 How to create a new user

Introduction

This section describes how to create a new user and assign a home folder for the user's methods and results.

Default user

A default user is created when the system is installed. The default user has unrestricted access to all UNICORN functions. You log on with this profile when you access a newly installed system for the first time.

The table below describes how to log on as the default user.

Step	Action
1	Select user **default** from the user drop-list.
2	Type password `default` if necessary. *Note*: The **default** user is the only user that is allowed to use the user name as password.
3	Click **OK** or press the **Enter** key.

User name: default

Password: *******

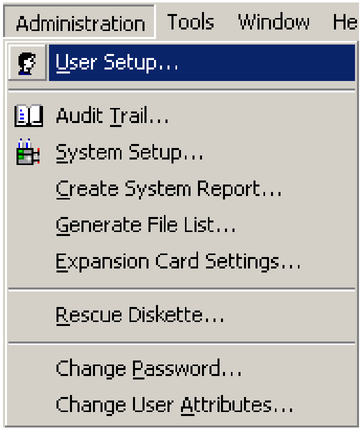
Note: We recommend that the default user is deleted when regular user profiles are created.

How to open User Setup

All user administration is performed in the **User Setup** dialog box in the **Main Menu** module. It is accessible only to authorized users (and the default user).

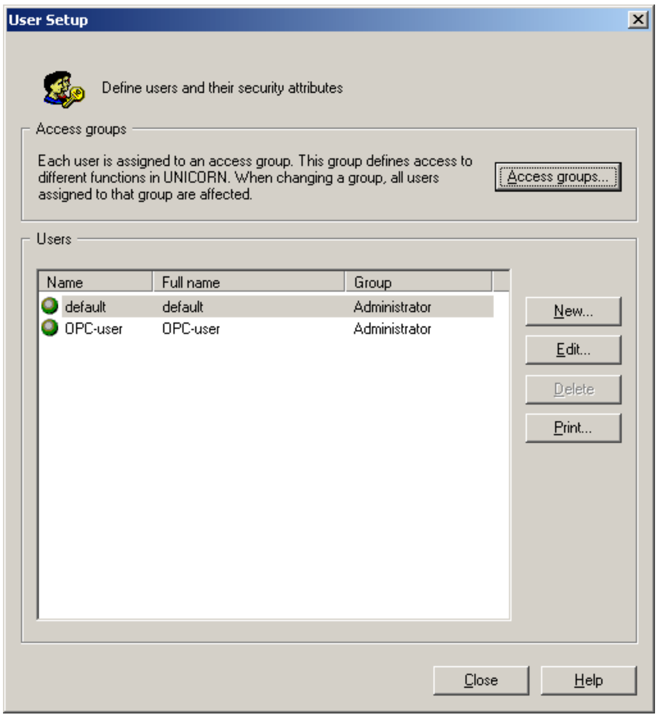
User Setup is found on the **Administration** menu.

- Choose **Administration:User Setup**.



The User Setup dialog box

The illustration below shows the **User Setup** dialog box.



Define users and their security attributes

Each user is assigned to an access group. This group defines access to different functions in UNICORN. When changing a group, all users assigned to that group are affected.

Name	Full name	Group
default	default	Administrator
OPC-user	OPC-user	Administrator

How to create a new user

The table below describes how to create a new user.

Step	Action
1	Click the **New** button in the **User Setup** dialog box. *Result*: The **Create New User** dialog box opens.
2	Enter a user name in the **User name** text box.
3	Enter the full name of the user in the **Full name** text box.
4	Enter the position of the user in the **Position** text box.
5	Select or create a **Home folder**: • Select a **Drive** and a folder from the **Name** drop-list and proceed to step 9. *or* • If you need to create a new home folder, proceed with step 6.
6	Click **New**. *Result*: the **Create New Folder** dialog box opens.
7	Select a **Drive** and type a folder name.
8	Click **OK** to create the folder and return to the **Create New User** dialog box.
9	Click **OK**. *Result*: The new user is created and added to the **User Setup** list.
10	• Click **Close**. *or* • Click the **New** button and repeat steps 1 - 8 to create more users.

Home folders

Each user must be assigned to a home folder. The **Default** folder can be used if you do not want to assign an individual home folder.

Note: If you create a home folder on the C: (local) drive it will not be accessible from other computers. If you select a network, make sure that is addressed by the same drive letter from all computers in the network.

3.3 How to assign user properties

Introduction

A user is assigned properties that define password rules, and the folders and chromatography systems that the user can access. This section describes how to assign properties.

How to open User properties

The user properties are defined in the **User Setup** dialog box in the **UNICORN Manager** module. The table below describes how to open **User Setup**.

Step	Action
1	Select **Administration:User Setup**.
2	Select a user in the **Users** list.
3	Click the **Edit** button. *Result*: The **User properties** dialog box opens.

The **User properties** dialog box is used to edit the user definition and assign properties for passwords, folder and system access, and available manual instructions.

How to edit the user definition

The table below describes how to edit the user definition in the **User properties** dialog box.

Step	Action
1	Select the **User** item.
2	Select an access group from the **Group** drop-down box. *Note*: A pre-defined access group is assigned a certain level of access to UNICORN.
3	Select a folder from the **Home folder** drop-down box.
4	Click the check boxes to select **Administrator Attributes**.
5	Click **OK** to finalize or select another definition to edit.

How to edit the user attributes

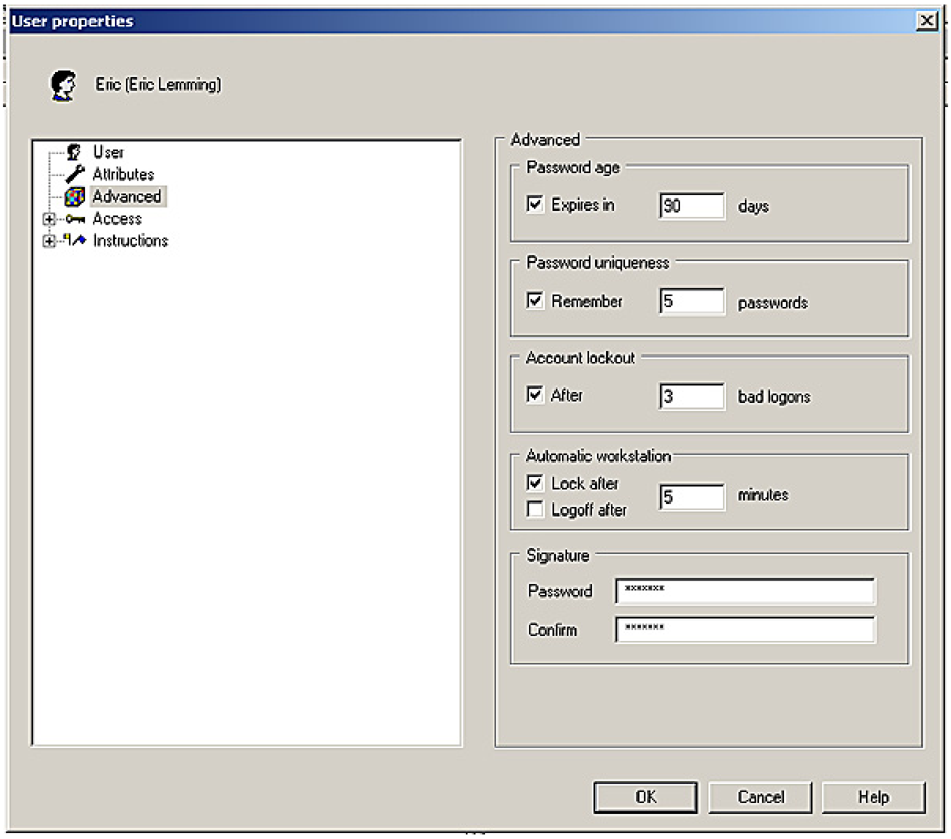
The table below describes how to edit the attributes in the **User Attributes** window pane.

Step	Action
1	Select the **Attributes** item.

Step	Action
2	Select applicable attribute items in the User Attributes pane: • Use large toolbar icons • Show unused variables • Show variable details • Default overwrite of baselines and peak tables • Prompt for column before manual runs
3	Type which curve to display in the **Quick view** dialog box.
4	• Select a size definition and type a value for the **Fraction mark** height. • Select a size definition and type a value for the **Injection mark** height. • Select a size definition and type a value for the **Logbook mark** height.
5	Click **OK** to finalize or select another definition to edit.

The Advanced dialog page

The **Advanced** window pane is used to define password policies for the user. Normally this is only used by the system administrator.



Advanced

Password age

☑ Expires in 90 days

Password uniqueness

☑ Remember 5 passwords

Account lockout

☑ After 3 bad logons

Automatic workstation

☑ Lock after
☐ Logoff after
5 minutes

Signature

Password *******
Confirm *******

Note: This dialog page is only available if a required password was selected when the software was installed.

How to define access to folders and systems

The **Access** dialog page is used to define the folders and systems that the user has access to. Click the check box for each selected folder and system.

Up to 20 folders can be set up to be shared. The user has access to all files and sub-folders in the selected folders. Only selected folders will be visible in the methods or results panels of the **UNICORN Manager** module.

Note: All users should have access to the **Failed** folder on each local station in a network installation. This will ensure that users can access results that were saved in the **Failed** folder in case of a network communication error.

How to define available manual instructions

The **Instructions** dialog page is used to define the manual instructions and system sounds that are available to the user as well as which monitors the user is allowed to calibrate. Click the check box for each selected instruction, sound or monitor.

Access groups

The level of access to UNICORN functions for each user is determined by the **Access group** that the user is assigned to. The access authorizations can be edited for each group, normally by the systems administrator. Refer to the Technical and Administration Manual if you need to edit an **Access group**.

Note: User access can be limited to only some UNICORN modules. If that is the case the unavailable modules will not be displayed. E.g. if the **UNICORN Manager** is unavailable you will only have access to a dialog box with the basic functions to change limited user attributes, passwords and to log out and quit the program.

3.4 How to change your passwords and user attributes

Introduction

Every user can change his or her passwords and some user attributes even if user administration is handled exclusively by the system administrator. The changes are made in the **UNICORN Manager**.

How to change passwords

The table below describes how to change your logon and signature passwords.

Step	Action
1	Select **Administration:Change Password**. *Result*: The **Change Password** dialog box opens.
2	Type your old logon password in the **Old** text box. *Note*: Your passwords will only be shown as asterisks.
3	Type a new password in the **New** text box.
4	Repeat the new password exactly in the **Confirm** text box.
5	Repeat steps 2 to 4 in the **Signature password** section if necessary.
6	Click **OK**.

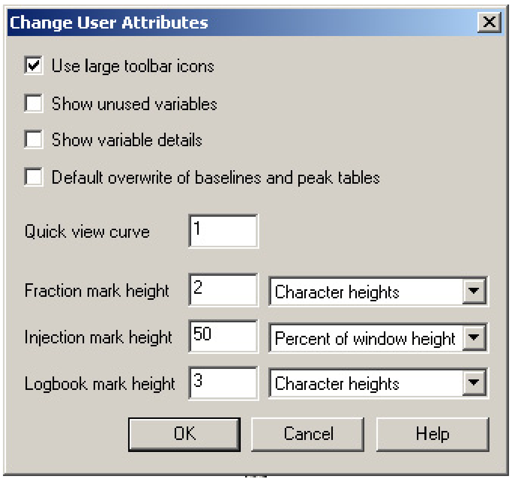
About passwords

The list below is a summary of facts and advice about UNICORN passwords:

- The system can be set up to operate without required passwords.
- The minimum number of password characters is set up at installation.
- Passwords can be any combination of letters and numbers.
- Passwords are case sensitive.
- Avoid using obvious passwords, e.g. your username, your telephone number, etc.
- The settings in the **User properties** determine the expiration for a password. Change passwords regularly even if your user profile is set up without password expiration.

How to change user attributes

The table below describes how to change your user attributes.

Step	Action
1	Select **Administration:Change User Attributes**. *Result*: The **Change user attributes** dialog box opens.  Change User Attributes ☑ Use large toolbar icons ☐ Show unused variables ☐ Show variable details ☐ Default overwrite of baselines and peak tables Quick view curve: 1 Fraction mark height: 2 Character heights Injection mark height: 50 Percent of window height Logbook mark height: 3 Character heights
2	**Dialog check box options** The dialog check box options are described below: • **Use large toolbar icons** Display large toolbar icons in all modules. • **Show unused variables** Show variables that are not used in the method on the **Variable** page of the **Start Protocol**. • **Show variable details** Show detailed method variables on the **Variable** page of the **Start Protocol**. • **Default overwrite of baselines and peak tables** When new baselines and peak tables are created, the old ones are overwritten.
3	**Mark heights** Select a size definition and type the height for the following marks: • Fraction mark • Injection mark • Logbook mark
4	Click **OK**.

3.5 *How to connect to the chromatography system*

Introduction

A computer can have up to four chromatography systems connected at a time. This section describes how to connect to the systems, and different connection modes.

How to establish a connection

The table below describes how to connect a chromatography system that is locally connected to your computer.

Step	Action
1	Open a **System Control** module. *Note*: Each UNICORN installation may have up to four System Control modules. The number of modules are selected when the software is installed.
2	• Select the **System:Connect** menu command. *or* • Click the **Connect to system** toolbar icon. *Result*: The **System Connect** dialog box opens.
3	Select the system you want to connect.
4	Click **OK**.

Remote connections

Each computer workstation may have up to four chromatography systems connected locally. In a network installation you may connect a system that is physically connected to another computer, the local station. Your system is then a remote station.

The local station that is connected to the chromatography system must be logged on to the network and the UNICORN drivers must be running. However, the connection will work even if the UNICORN program is not running on the local station.

Network log on

Ensure that your workstation is logged on to the network before you start a chromatography system that is directly connected to the station. You can operate a local system without logging on to the network, but there are several disadvantages to this:

- Files stored on network drives are not accessible.
- Changes made to global files, e.g. user settings files, will apply only locally and will be lost the next time you log on to the network.
- Result files that are directed to a network drive will be stored in the **Failed** folder on the local station.

Connection modes

Several workstations can connect to a single chromatography system at the same time but only one workstation can be in control mode. The other connections are in view mode and the connected workstations can only monitor the system activity, but not issue any commands.

The system status is indicated on the status bar at the bottom of the **System Control** window. The table below describes the different connection modes, the corresponding status texts and some of the various actions you can take to change the connection mode.

Connection mode	Status Text	Possible action to change connection mode
Not connected	 Not Connected	Connect to a system.
Control mode.	 Controlled By: default	Disconnect from or leave control of the system. (The system is controlled by you.)
View mode	 Controlled By: Eric	No connection possible. (The system is controlled by another user.)
View mode	 Locked By: Eric	Click the **Connect to system** icon and supply a password. (The system is locked by another user.)

Connection mode	Status Text	Possible action to change connection mode
View mode	 System is available	Connect to the system. (The system has been left unlocked.)

How to leave control of a system

The table below describes how to leave control of a system so that it is available to be controlled by other users.

Step	Action
1	• Select **System:Leave Control**. *or* • Click the **Leave control of system** icon. *Result*: **The Leave Control of system** dialog box opens.
2	Click the radio buttons to select to leave the system unlocked or locked.
3	Enter a password (if the system is to be locked).
4	Click **OK**.

How to disconnect a system

The table below describes how to disconnect from a system.

Step	Action
1	• Select **System:Disconnect**. *or* • Click the **Disconnect from system** icon.

Step	Action
2	*Result*: If the system is in view mode • the system is disconnected. If the system is in control mode • the **Leave Control of System** dialog box opens.
3	Select to leave the system locked or unlocked.
4	Click **OK**. *Result*: The system is disconnected.

How to disconnect when quitting

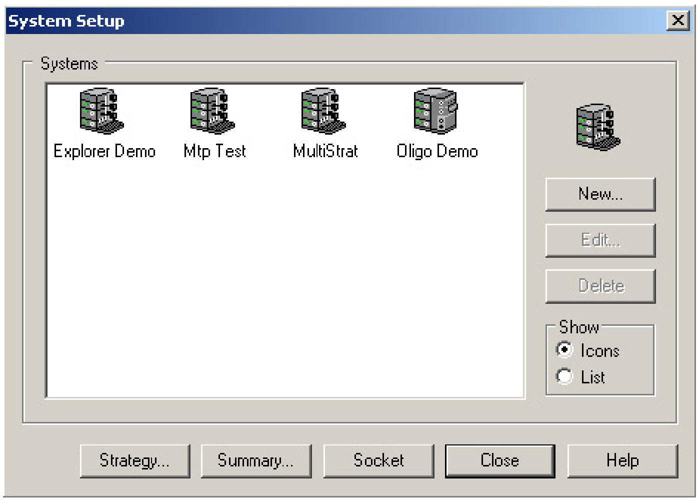
When you log off or quit from UNICORN you automatically disconnect all connected systems. A **Leave Control of System** dialog box will be opened for each system that was connected.

Note: If you disconnect from a system in control mode and re-connect to it, you may be connected in view mode. Another user may have taken control in the meantime.

How to view or print a system summary

You can view and print a total summary of a selected system from the **System Table Summary** dialog box.

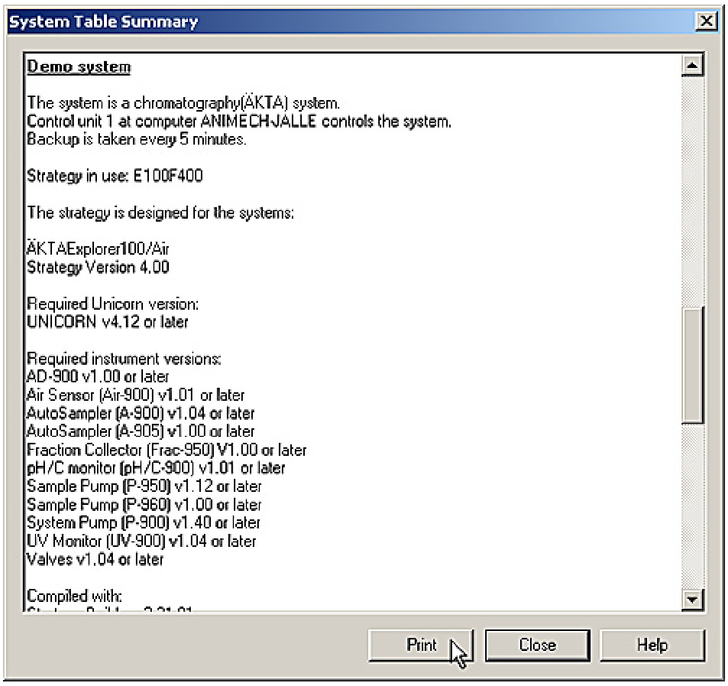
The table below describes how to view and print an information summary of a selected systemthe systems:

Step	Action
1	Choose **Administration:System Setup** in the **UNICORN Manager**. *Result*: The **System Setup** dialog box is displayed: 

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>

- Select the system you want a summary of.
- Click the **Summary** button.

Result: The **System Table Summary** dialog box is displayed:



<pre>
Demo system

The system is a chromatography(ÄKTA) system.
Control unit 1 at computer ANIMECHJALLE controls the system.
Backup is taken every 5 minutes.

Strategy in use: E100F400

The strategy is designed for the systems:

ÄKTAExplorer100/Air
Strategy Version 4.00

Required Unicorn version:
UNICORN v4.12 or later

Required instrument versions:
AD-900 v1.00 or later
Air Sensor (Air-900) v1.01 or later
AutoSampler (A-900) v1.04 or later
AutoSampler (A-905) v1.00 or later
Fraction Collector (Frac-950) V1.00 or later
pH/C monitor (pH/C-900) v1.01 or later
Sample Pump (P-950) v1.12 or later
Sample Pump (P-960) v1.00 or later
System Pump (P-900) v1.40 or later
UV Monitor (UV-900) v1.04 or later
Valves v1.04 or later

Compiled with:
</pre>

</td></tr>
<tr><td>3</td><td>

- Click the **Print** button to print the information.
- Click the **Close** button to exit the dialog box.

</td></tr>
</table>

3.6 *How to back up and restore system data*

Introduction

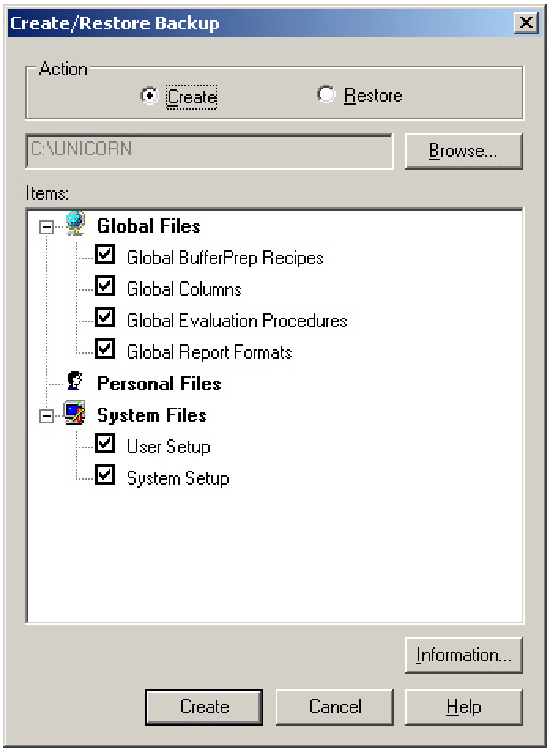
You can create a backup file with system information and store it on a diskette or another drive. The backup file will contain information about

- Global Files
- Personal Files
- System Files

Afterwards you can use the backup file to restore the system definitions in case they are corrupted.

How to create a backup file

The table below describes how to create a backup file and store it for example on a rescue diskette:

Step	Action
1	Insert a diskette into the computer if you want to store the backup file on a diskette.
2	Choose **Administration:Create/Restore Backup** in the **UNICORN Manager** to display the **Create/Restore Backup** dialog box: 

Create/Restore Backup

Action: ● Create ○ Restore

C:\UNICORN

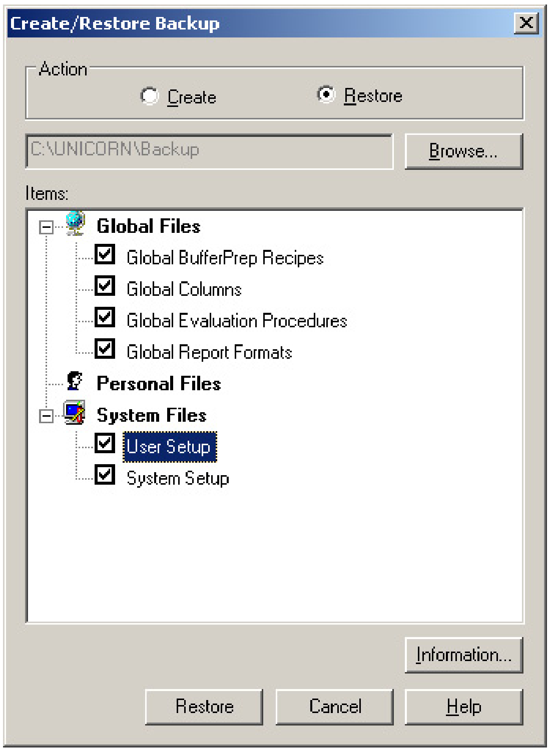
Items:

- **Global Files**
  - ☑ Global BufferPrep Recipes
  - ☑ Global Columns
  - ☑ Global Evaluation Procedures
  - ☑ Global Report Formats
- **Personal Files**
- **System Files**
  - ☑ User Setup
  - ☑ System Setup

Step	Action
3	- In the **Action** field, make sure that the **Create** option is selected. - Click the **Browse** button to select where to store the backup file. .*Note*: Select `A:\` to store the file on the diskette. - In the **Items** field, select which information to include on the backup file. - Click the **Create** button to create the backup file and store it in the selected location. *Note*: You can click the **Information** button to see which information files will be included in the backup file.

How to restore the system data

The table below describes how to restore the system data from a backup file, located for example on a rescue diskette:

Step	Action
1	If the backup file is located on a diskette, insert the diskette into the computer.
2	Choose **Administration:Create/Restore Backup** in the **UNICORN Manager** to display the **Create/Restore Backup** dialog box:  Action: ○ Create ● Restore C:\UNICORN\Backup Items: **Global Files** ☑ Global BufferPrep Recipes ☑ Global Columns ☑ Global Evaluation Procedures ☑ Global Report Formats **Personal Files** **System Files** ☑ User Setup ☑ System Setup

Step	Action
3	• In the **Action** field, select the **Restore** option. • Click the **Browse** button to select the folder where the backup file is located.*Note*: Select `A:\` if the file is located on the diskette. • In the **Items** field, select which information to include from the backup file. • Click the **Restore** button to restore the system definitions. *Note*: You can click the **Information** button to see which information files are included in the backup file.

3.7 How to set up a printer

Introduction

UNICORN uses the default printer and printer settings that are installed on your computer. You can change your printer by changing the default Windows settings, but you can also set up a printer in UNICORN for the current working session.

How to set up a printer

The table below describes how to set up a printer in UNICORN.

Step	Action
1	Select the **File:Printer Setup** menu command in the **UNICORN Manager** module. *Result*: The **Print Setup** dialog box opens.
2	Select a printer from the **Name** drop-down box.
3	Change all printer properties as necessary.
4	Click **OK**.

Note: To save created reports electronically you can select to print the files in PDF-format. To be able to do this you must have a full version of Adobe™ Acrobat™ installed and select PDF Writer or Distiller™ in the **Printer Setup**.

4 Files and folders in UNICORN

Introduction

All UNICORN data is organized in files and folders. Files and folders are handled like in any other Windows application, with some exceptions. This chapter describes how to work with UNICORN files and folders, with the focus on the topics that are specific for UNICORN.

In this chapter

This chapter contains the following sections

Topic	See
How to create folders	4.1
How to open and preview files	4.2
How to arrange and locate your files	4.3
How to copy, delete, rename and backup files and folders	4.4

4.1 *How to create folders*

Introduction

This section describes how folders are organized in UNICORN and how to create a new user-specific folder for the user's methods and results.

UNICORN folders

The files and folders are displayed in the two **UNICORN Manager** module windows.

- All method files and corresponding folders are listed in the **Methods** window.
- The result files and folders are listed in the **Results** window.
- You can only see folders that you have access to.
- You can only see method files that are written for systems that you have access to.

How to create a user-specific folder

The table below describes how to create a user-specific folder.

Step	**Action**
1	Select the window you want to create the folder in: **Methods** or **Results**. (*Result*: The window title bar is highlighted.)
2	• Select **File:New:Folder**. *or* • Right-click and select the **New Folder** shortcut. *Result*: The **Create New Folder** dialog box opens.
3	Type a name for the new folder.
4	Click **OK**.

4.2 How to open and preview files

Introduction

This section describes how to open your saved method files and result files. You can also preview your result files to identify the correct file before you open it.

How to open a method file

You open a method file in the **UNICORN Manager** module. Click the file in the **Methods** window to select it and

- choose **File:Open**.

or

- right-click the file and choose **Open** from the short-cut menu.

or

- double-click the file.

Result: The file is opened for editing in the **Method Editor** module.

Note: A method file cannot be opened on two workstations simultaneously.

How to open a result file in UNICORN Manager

You can open a result file in the **UNICORN Manager** module. Click the file in the **Results** window to select it and

- choose **File:Open**.

or

- right-click the file and choose **Open** from the short-cut menu.

or

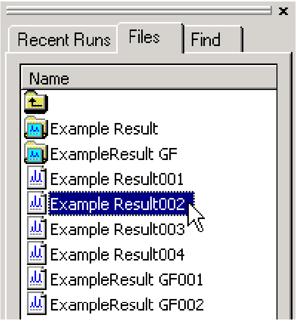
- double-click the file.

Result: The file is opened for editing in the **Evaluation** module.

How to open a result file in the Evaluation module

The table below describes how to open a result file from the **File Navigator** in the **Evaluation** module.

Step	Action
1	• Click the **Files** tab.

Step	Action
2	• Locate and double-click the result file  *Result*: The result file opens.

Note: The **File Navigator** is opens by default in the **Evaluation** module. If it has been closed, select **View:File Navigator** in the **Evaluation** module.

Quick View

Quick View is a preview function for result files to make it easier to select the correct result file.

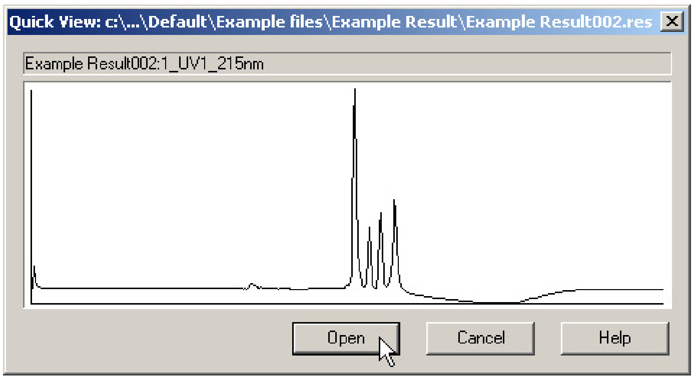
You can preview the first curve in the first chromatogram. You can also select to view another curve as default by selecting another curve number in your **User Attributes** settings, see **3.4 How to change user attributes** on page 60.

Several files can be opened for comparison.

How to use Quick View

The table below describes how to preview result files in Quick View.

Step	Action
1	Select one or more result files in the **Result** window of the **UNICORN Manager**.

Step	Action
2	- Choose **File:Quick View**. *or* - Right-click and choose **Quick View** from the short-cut menu. *Result*: The **Quick View** dialog box opens. 
3	- Click the **Next** and **Previous** buttons to move between the result files (if more than one is selected). - Click the **Open** button when the right file is displayed. *Result*: The result file that is displayed in the dialog box opens in the **Evaluation** module.

4.3 *How to arrange and locate your files*

Introduction

This section describes how to arrange the way the files are displayed in your UNICORN workspace and how to locate files through a search.

Different view modes

You can choose how the files and folders are displayed in the **UNICORN Manager** windows. The options are the standard Windows alternatives:

- Details
- List
- Large icons
- Small icons.

How to change the view mode

If you want to change the view you either:

- Select **View** and the option that you want,

or

- Right-click and select **View** and the option that you want from the shortcut menu.

Sort order in detailed view

The files can be sorted in a different order when a window is displayed in detailed view. The table below shows the options.

Sorted by:	**Order**
Name	Alphabetical order or reverse alphabetical order.
System	Alphabetical order or reverse alphabetical order (**Method** window only).
Size	Smallest or largest files first.
Type	Alphabetical order of file extension type.
Modified	Most recently modified files first.
Created	Most recent creation dates first.

How to change the sorting order

Select one of the methods below to change the sorting order:

- Select **View:Sort** and the option that you want,

or

- Right-click and select **Sort** and the option that you want from the short-cut menu.

or

- Click the column header for the option that you want to sort by (a second click on the same header will reverse the order).

Note: Only the currently active window is affected.

How to filter Method files

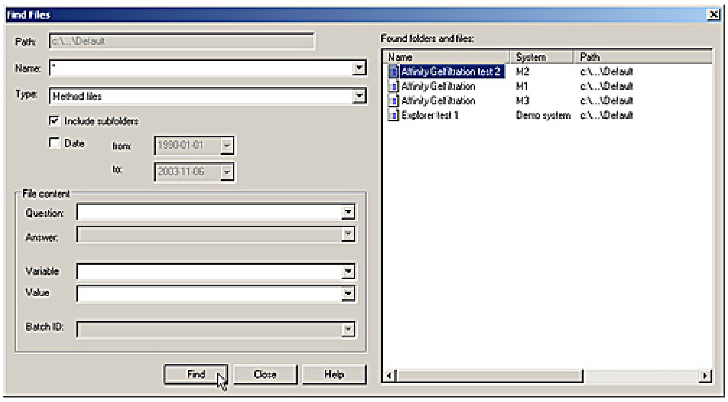
The files in the **Method** window can be filtered to show only methods for selected systems. You can also limit the displayed files by using standard Windows wildcard characters. The title bar of the **Method** window indicates if a filter has been activated.

The table below describes how to activate a filter.

Step	Action
1	• Select **View:Filter**. *or* • Right-click and select **Filter** from the shortcut menu. *Result*: The **Filter** dialog box opens.
2	Click the check-boxes for the systems for which you want to show files.
3	Enter a file name specification (if necessary).
4	Click **OK**.

How to find files

The table below describes how to perform a search for files.

Step	Action
1	Click either the **Methods** or **Results** window and: • Select the **File:Find** menu command. *or* • Right-click and select **Find** from the shortcut menu. *Result*: The **Find files** dialog box opens.  Find Files Path: c:\...\Default Name: * Type: Method files ☑ Include subfolders ☐ Date from: 1990-01-01 to: 2003-11-06 File content Question: Answer: Variable Value Batch ID: Found folders and files: Name / System / Path Affinity Gelfiltration test 2 / M2 / c:\...\Default Affinity Gelfiltration / M1 / c:\...\Default Affinity Gelfiltration / M3 / c:\...\Default Explorer test 1 / Demo system / c:\...\Default
2	Add search criteria to the dialog box, for example: • Type a name in the **Name** field. • Select a file type from the **Type** drop-down box. • Select if the search should include subfolders. • Select date limits in the **Date** drop-down boxes. • Type text strings to match **Question** or **Answer** texts. • Type a variable name and, if desired, a value. • Type a **Batch ID**. *Note*: You can search for a sample ID provided the sample ID is defined as a variable.
3	Click **Find**. *Result*: The search results are listed in the **Found folders and files** field. The search is limites to either methods or results and to the folder (including its subfolders) that is currently displayed.

Step	Action
4	Double-click a file in this list. *Result*: The dialog box is closed and the selected file is highlighted in the **UNICORN Manager** window. *Note*: If you click **Close** you will return to the **UNICORN Manager** window with no file highlighted regardless if you have selected one in the dialog box or not.

4.4 How to copy, delete, rename and backup files and folders

Introduction

UNICORN has some file and folder handling functions that are slightly different from the general Windows functions. This section focuses on the differences.

Note: You need explicit authorization in your user profile to copy, move and delete files.

How to copy or move files and folders

There are some restrictions to how you can copy or move files and folders:

- Files and folders can only be copied or moved to folders that are specific to your user name.
- You can also copy files to and from the folders that you have access to on the network.
- Method files or folders cannot be copied to the **Results** window.
- Result files and folders cannot be copied to the **Methods** window.

If you copy a folder you will also at the same time copy all files and folders that it contains. The table below describes how to copy files and folders.

Note: Follow the same steps but select **Move** to move files and folders.

Step	Action
1	Select one or more files and folders in either the **Methods** or **Results** window of the **UNICORN Manager**.
2	• Select **File:Copy**. *or* • Right-click and select **Copy** from the short-cut menu. *Result*: The **Copy** dialog box is opened.
3	Select a target folder or floppy disk drive.
4	Click **OK**.

The function Copy to External

Use the function **Copy to External** when you need to copy files and folders outside of your own user folders. **Copy to External** should be used specifically when you need:

- to copy a method to another system (the method can then be connected to the appropriate system),
- to copy to a floppy disk drive. (The files are automatically compressed into a zip-file. The file will also automatically be spanned across several disks if necessary.)

How to Copy to External

The table below describes how to use the function **Copy to External**.

Step	Action
1	Select the file you want to copy.
2	• Select **File:Copy to External**. *or* • Right-click and select **Copy to External** from the shortcut menu. *Result*: the **Copy to External** dialog box opens.
3	Select the destination drive and folder.
4	Click the **Save** button.

The function Copy from External

The function **Copy from External** can be used to import files and folders:

- If the files were saved using the function **Copy to External** they will automatically be decompressed.
- Copied method files must be connected to the same type of system they originally were created for. This is part of the **Copy from External** procedure.
- Method files that have been copied in and connected are displayed in the designated folder in the **Methods** window.

How to use Copy from External

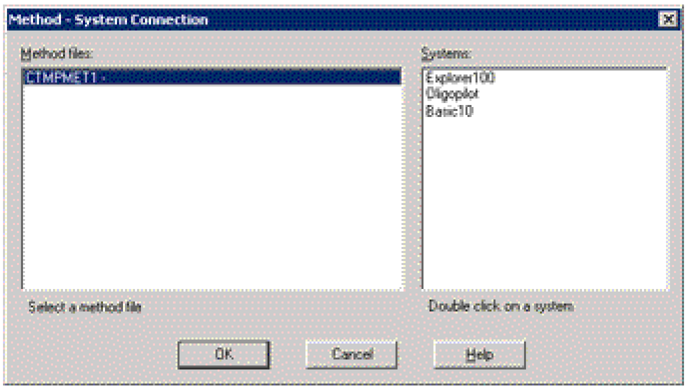
The table below describes how to use the function **Copy from External**.

Step	Action
1	Select a destination folder in the **Methods** or the **Results** window.
2	• Select **File:Copy from External**. *or* • Right-click and select **Copy from External**. *Note*: Do not select a file icon. *Result*: The **Copy from External** dialog box opens.
3	Select the files you want to copy.

Step	Action
4	Click **Save**. *Result*: - Result files are copied into the designated folder in the **Results** window. - If method files were selected, the **Method-System Connection** dialog box opens.

How to connect a method to a system

The table below describes how to connect a method to a system.

Step	Action
1	Select a method and double-click a system.  *Result*: The method is connected and the system name is added after the method name in the **Method files** list.
2	Repeat step 1 until all methods are connected to a system.
3	Click **OK**.

How to rename files and folders

The table below describes how to rename files and folders in the **Methods** or **Results** windows in the **UNICORN Manager** module.

Step	Action
1	Select the item that you want to rename.

Step	Action
2	- Select **File:Rename**. *or* - Right-click and select **Rename** from the shortcut menu. *Result*: The **Rename** dialog box opens.
3	Type a new name.
4	Click **OK**.

How to delete files and folders

The table below describes how to delete files and folders in the **Methods** or **Results** windows in the **UNICORN Manager** module.

Note: Home folders cannot be deleted this way.

Step	Action
1	Select the item that you want to delete.
2	- Select **File:Delete**. *or* - Right-click and select **Delete** from the shortcut menu. *or* - Press the **Delete** key.
3	Confirm the delete action in the confirmation dialog box

Backup security

Backup copies should be taken regularly to avoid data loss in the event of hard disk failure or accidental deletion. You can use the function **Copy to External** to save your files on the network server.

Note: GE Healthcare cannot accept responsibility for the replacement of method programs that were lost as a result of computer failure or other incidents.

5 How to create a method

Introduction

Chromatography runs are programmed as **Methods** in UNICORN. Before you can proceed with a chromatography run you need either to use an existing method or create a new method. This chapter describes how to create new methods. It also contains instructions for signing a method.

In this chapter

This chapter contains the following sections

Topic	**See**
How to use the Method Wizard	5.1
How to use the Method templates	5.2
How to use Text instructions	5.3
How to sign the method	5.4

5.1 How to use the Method Wizard

Introduction

This section describes how to use a **Method Wizard** to create a new method. For most purposes customized methods can be created simply by setting appropriate values for the method variables.

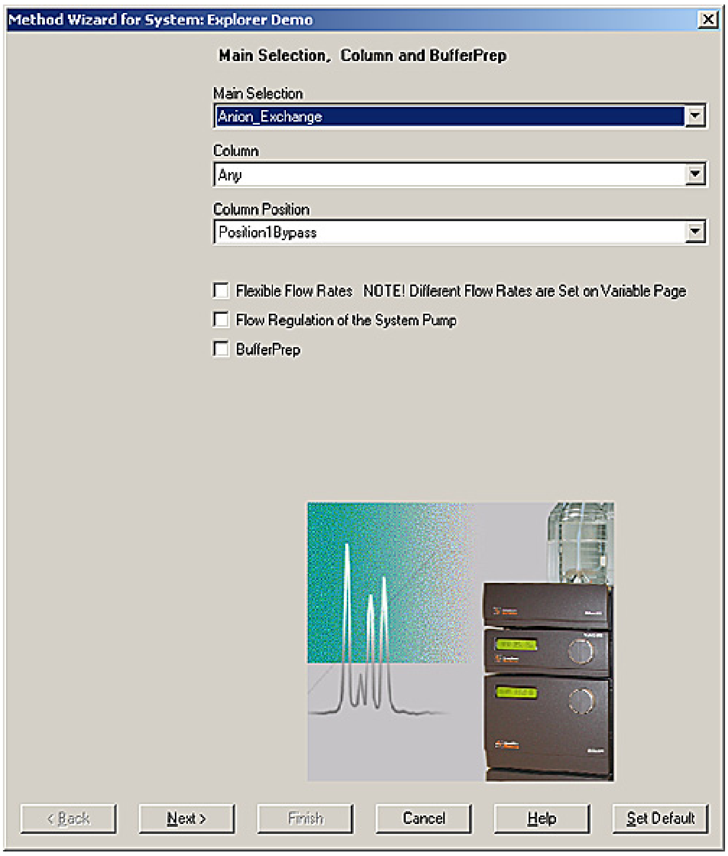
Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

Are wizards always available?

Method Wizards are available for some **ÄKTAdesign** systems delivered with standard strategies. **Method Wizards** are not available for process systems.

How to create a new method

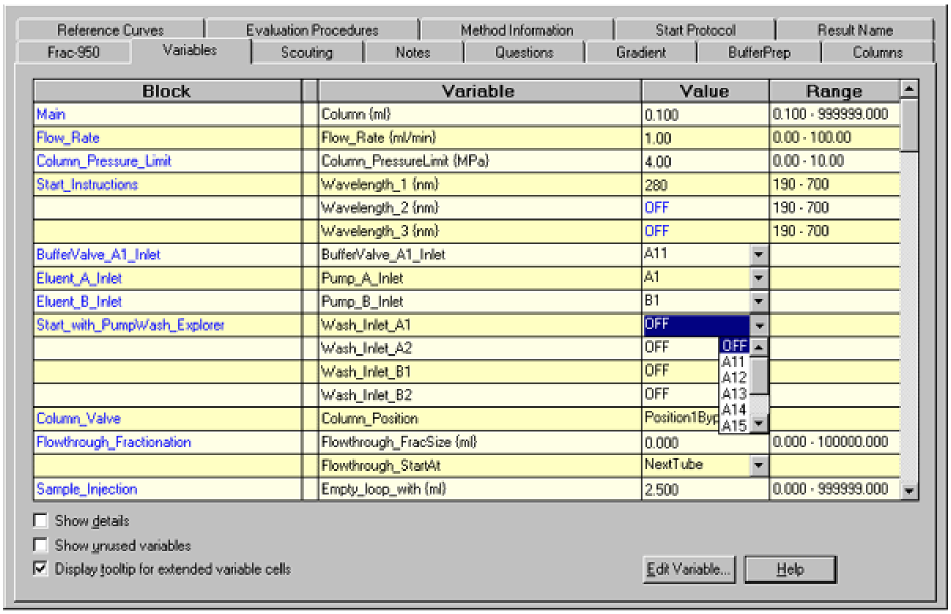
The table below describes how to create a method with the **Method Wizard**.

Step	Action
1	Click the **Method Wizard** icon on the **Method Editor** module or choose **File:Method Wizard**. *Result:* The **Method Wizard** dialog box appears.  Method Wizard for System: Explorer Demo Main Selection, Column and BufferPrep Main Selection: Anion_Exchange Column: Any Column Position: Position1Bypass ☐ Flexible Flow Rates NOTE! Different Flow Rates are Set on Variable Page ☐ Flow Regulation of the System Pump ☐ BufferPrep *Note:* If several systems are available you must first select which system you want to use.
2	Select the appropriate parameter values and click the **Next** button. *Note:* Click **Set Default** on the first wizard page to restore all settings to the default values.
3	In each new dialog box, select the appropriate parameter values and click the **Next** button to continue. *Note:* Select a column even if you want to perform a test run without a column. Use a small column. Replace the column with a piece of tubing when you run the method.

Step	Action
4	Click the **Finish** button in the last dialog box. *Result*: The **Run Setup** opens.

The Run Setup

The **Run Setup** consists of a number of tabs. Click on the appropriate tab at the top to select it.



Block	Variable	Value	Range
Main	Column (ml)	0.100	0.100 - 999999.000
Flow_Rate	Flow_Rate (ml/min)	1.00	0.00 - 100.00
Column_Pressure_Limit	Column_PressureLimit (MPa)	4.00	0.00 - 10.00
Start_Instructions	Wavelength_1 (nm)	280	190 - 700
	Wavelength_2 (nm)	OFF	190 - 700
	Wavelength_3 (nm)	OFF	190 - 700
BufferValve_A1_Inlet	BufferValve_A1_Inlet	A11	
Eluent_A_Inlet	Pump_A_Inlet	A1	
Eluent_B_Inlet	Pump_B_Inlet	B1	
Start_with_PumpWash_Explorer	Wash_Inlet_A1	OFF	
	Wash_Inlet_A2	OFF	
	Wash_Inlet_B1	OFF	
	Wash_Inlet_B2	OFF	
Column_Valve	Column_Position	Position1Byp	
Flowthrough_Fractionation	Flowthrough_FracSize (ml)	0.000	0.000 - 100000.000
	Flowthrough_StartAt	NextTube	
Sample_Injection	Empty_loop_with (ml)	2.500	0.000 - 999999.000

☐ Show details
☐ Show unused variables
☑ Display tooltip for extended variable cells

The Variables tab

The method is represented by a number of blocks on the **Variables** tab. The blocks are typical steps in a chromatographic run.

Each block contains a number of **Method Variables** with suitable default values that can be changed to suit your application. Only the most commonly used variables are initially shown on the page. Click the **Show details** check box to display all variables in the method.

Default values for columns

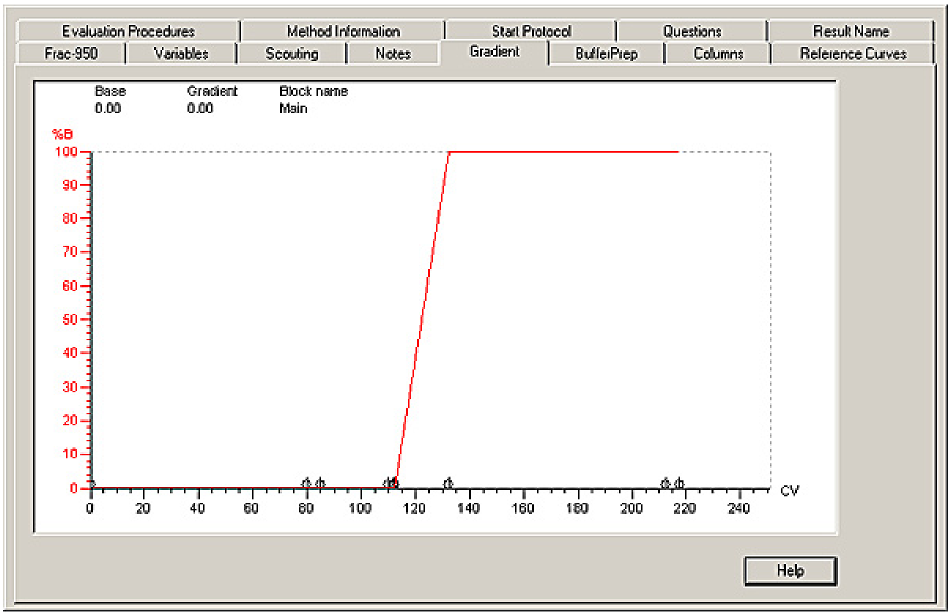
When you select a column, default values will be set for several parameters including the following:

- the correct column volume
- the recommended flow rate
- the correct pressure limit.

Note: If you exceed the recommended values for the selected column you will receive a warning when you save your method.

The Gradient tab

The **Gradient** tab shows the method graphically:



Base 0.00
Gradient 0.00
Block name Main

The length of each block is marked at the bottom of the graph.

- Click the X-axis to view the method in time, volume or column volumes.
- Drag the Y-axis to display a marker in the gradient. The **Base** value, **Gradient** value and **Block name** at the current marker position will be displayed in the upper part of the graph.

How to save the new method

A new method created from a **Wizard** is untitled, and must be saved under a method name before it can be used. The table below describes how to save a new method.

Step	**Action**
1	Click the **Save Method** toolbar or choose **File:Save**.
2	• If required, save the method in a folder other than the default home folder. • Enter a **Method name** for the method. The total path can be up to 256 characters long. The method name must be unique for the chosen system within the folder.
3	• If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can be run on any system that uses the same strategy. Remember that different systems may have different configurations and control capabilities. • Choose the **Technique** for which the method was written.

Step	Action
4	Click **OK**. *Result:* The method is saved, but remains open in the **Method Editor**, so that you can continue editing if you wish.

Note: You might want to sign your method. If you do so, you can choose to lock the method so that nobody will be able to change the method. See **5.4 How to sign the method** on page 96 for further instructions.

5.2 How to use the Method templates

Introduction

This section describes how to create methods based on an existing template.

Note: A custom system, for example a process system, requires that the users create their own templates by saving methods as templates. Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

How to create a new method

The table below describes how to create a method from the **UNICORN Manager** module.

Note: The **New Method** dialog box is also accessible from the **Method Editor** module using the same commands.

Step	Action
1	• Choose the **File:New:Method** menu command *or* • click the **New Method** icon. *or* • right-click in the **Methods** window and select **New:Method** from the shortcut menu. *Result*: The **New Method** dialog box opens in the **Method Editor** module.
2	• Select the system for which you want to create the method in the **For system** drop-down list. • Select **Template** in the **Use** field. • Select a chromatographic technique from the **Technique** drop-down list. • Select a method template from the **Template** list. • Select a column from the **For column** list and click **OK**. *Result:* The method template will be opened as an untitled method in the **Run Setup** in the **Method Editor**. *Note*: If **Any** is selected in the **For column** list, you can use any column but must enter the column volume in the method on the **Variables** tab. It is recommended that a specific column is selected.

Note: Only columns for the selected technique are displayed. If Any is selected as technique, all columns are displayed. Right-click in the textbox to open a list of the column categories to limit the number of displayed columns. If you type the beginning of a column name in the textbox UNICORN will automatically complete the column name.

If you do not find your specific column it can be added to the list. The column value, recommended flow rate, pressure limit and averaging time for the selected column will be automatically copied into the method, thus reducing the need to edit the method.

Method notes

Click the **Notes** and then the **Method Notes** tabs in the **Run Setup**. The notes describe important information about the template and how the system should be connected so that the method will work correctly.

Note: If your system does not correspond to the description on the **Method Notes** tab, either:

- rearrange the valves and tubing connections in accordance with the method notes description

or

- edit the method instructions in accordance with your system setup.

How to save the new method

A new method created from a method template is untitled, and must be saved under a method name before it can be used.

The table below describes how to save a new method.

Step	Action
1	Click the **Save Method** toolbar icon or choose **File:Save**.
2	• If required, save the method in a folder other than the default home folder. • Enter a **Method name** for the method. The total path can be up to 256 characters long. The method name must be unique for the chosen system within the folder.

Step	Action
3	• If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can be run on any system that uses the same strategy. Remember that different systems may have different configurations and control capabilities. • Choose the **Technique** for which the method was written. • Click **OK**. *Result:* The method is saved, but remains open in the **Method Editor**, so that you can continue editing if you wish.

Note: You might want to sign your method. If you do so, you can choose to lock the method so that nobody else will be able to change the method. See **5.4 How to sign the method** on page 96 for further instructions.

5.3 How to use Text instructions

Introduction

You can use the **Text Instructions** editor in the **Method Editor** to build your method step by step. You can also use the editor to modify instructions in methods created by wizards or based on templates.

Advanced editing facilities are available when you work directly in the **Text Instructions** editor. This section is a very brief description of this process. See **6 How to edit methods** on page 97 for detailed instructions.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

When do I use Text Instructions?

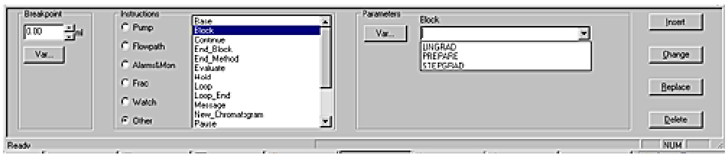
Use **Text Instructions** when you want:

- to change selected instructions in the method, for example the outlet valve position
- to add blocks or instructions, for example **Watch** instructions
- to change method instructions to adapt to non-standard system configurations
- to create new methods for applications not covered by the supplied templates or wizards.

How to edit Text Instructions

Open the **Text Instructions** editor by following the steps in the table below.

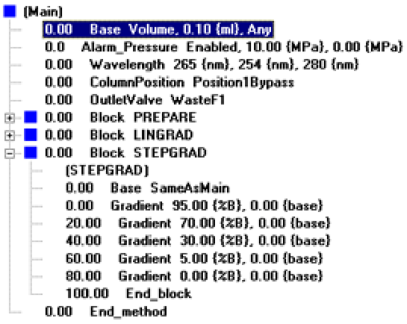
Step	Action
1	Select the **Method Editor** module and click the **Text Instructions** icon.
2	• Click the **Customise Panes** icon and select **Text** and **Instruction Box**. • Click **OK**.

Step	Action
3	Select instructions in the **Instruction box** in the lower part of the **Method Editor**, and use the **Insert**, **Change**, **Replace** or **Delete** buttons. All text entries are shown in the **Text** pane. Applicable variables can be edited for each selection. The illustration below shows the **Instruction box**: 

Instructions can be organized in blocks

Individual text instructions can be grouped in blocks of instructions (marked by blue square symbols) for a specific functional use, e.g. to load a sample, to equilibrate a column etc. A block may contain other blocks or individual instructions.

This is an example of text instructions in the **Text** pane:



```
(Main)
  0.00   Base  Volume, 0.10 {ml}, Any
  0.0   Alarm_Pressure  Enabled, 10.00 {MPa}, 0.00 {MPa}
  0.00   Wavelength  265 {nm}, 254 {nm}, 280 {nm}
  0.00   ColumnPosition  Position1Bypass
  0.00   OutletValve  WasteF1
+ 0.00   Block  PREPARE
+ 0.00   Block  LINGRAD
- 0.00   Block  STEPGRAD
      (STEPGRAD)
      0.00   Base  SameAsMain
      0.00   Gradient  95.00 {%B}, 0.00 {base}
      20.00   Gradient  70.00 {%B}, 0.00 {base}
      40.00   Gradient  30.00 {%B}, 0.00 {base}
      60.00   Gradient  5.00 {%B}, 0.00 {base}
      80.00   Gradient  0.00 {%B}, 0.00 {base}
      100.00   End_block
  0.00   End_method
```

How to save the new method

A new method is untitled, and must be saved under a method name before it can be used.

The table below describes how to save a new method.

Step	Action
1	Click the **Save Method** toolbar or choose **File:Save**.
2	• If required, save the method in a folder other than the default home folder. • Enter a **Method name** for the method. The total path can be up to 256 characters long. The method name must be unique for the chosen system within the folder.

Step	Action
3	• If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can be run on any system that uses the same strategy. Remember that different systems may have different configurations and control capabilities. • Choose the **Technique** for which the method was written. • Click **OK**. *Result:* The method is saved, but remains open in the **Method Editor**, so that you can continue editing if you wish.

Note: You might want to sign your method. If you do so, you can choose to lock the method so that nobody will be able to change the method. See **5.4 How to sign the method** on page 96 for further instructions.

How to display descriptions of instructions

A dedicated strategy is available for each system in the **ÄKTAdesign** platform. Although the majority of the instructions are general, some of them differ slightly between the individual strategies.

The list below describes two ways to display descriptions of the instructions in your particular strategy:

- Select the instruction in the **Instruction Box** of the **Method Editor** and press **<F1>**

or

- Right-click the instruction in the **Text** pane and choose the menu option **What's This?**

How to print descriptions of instructions

The table below describes how to print descriptions of the instructions in your particular strategy:

Step	Action
1	Select **File:Print** in the **Method Editor**.
2	• Select the **Instruction set** option to print the full set of instructions. • Click **OK**.

How to add a Snapshot

The **Snapshot** instruction can be used to record the curve values at a specific point in the method run. For example, a snapshot can be inserted to record the curve values immediately before an injection. The values are recorded in the result file and can be viewed in the **Snapshots** tab of the **Documentation** dialog box (See **10.7 Run documentation** on page 280). Up to 500 snapshots can be recorded in each result file. The table below describes how to add a snapshot instruction to a method:

Step	Action
1	• In the **Text** pane, select the instruction immediately before the position where you want to insert the **Snapshot** instruction.
2	• Select **Other** in the **Instructions** field of the **Instructions box**. • Select **Snapshot** in the instructions list.
3	Type a name in the **Name** text box in the **Parameters** field. • Click the **Insert** button.

Note: Snapshots can also be taken in the **System Control** and **Evaluation** modules. However, these snapshots will only record the data for a specific moment. For more information about the **Snapshot** function see **2.2.7 Snapshots** on page 41.

5.4 How to sign the method

Instruction

If you sign the method, you can choose to lock it so that nobody will be able to change it.

The table below describes how to sign the method.

Step	Action
1	Choose **File:Sign Method** in the **Method Editor**. *Result:* The **Sign the Method** dialog box is displayed.
2	Click the **Signing** tab and do the following: • Select a user in the **User** drop-down list box. In most instances, you will want to use the current user shown on the list. • In the **Meaning** field, provide a short text description explaining the meaning behind the signature (for example "Method now fully tested and approved"). • Type your signature password in the **Password** field. If desired, select the **Lock** box to lock the method permanently from further changes by other users. • If needed, view a list of all signatures associated with the current method on the **View Signatures** tab. • Click **OK** on either the **Signing** or **View Signatures** tab.

6 How to edit methods

Introduction

This chapter describes the complete facilities for editing methods in UNICORN. For many applications, suitable methods can be created by changing the default values in one of the wizard-generated methods supplied with UNICORN.

Use the more advanced editing facilities described here when you want

- to change selected instructions in the method, for example, change the outlet valve position
- to add blocks and instructions
- to change method instructions to adapt to non-standard system configurations.

In this chapter

This chapter contains the following sections

Topic	See
The Method Editor interface	6.1
Method blocks	6.2
Method instructions	6.3
How to use method variables	6.4
Run Setup	6.5
How to use selected method instructions	6.6
Standard Watch conditions	6.7
How to save or delete a method template	6.8
How to print a method	6.9
How to export a method	6.10

6.1 The Method Editor interface

Introduction

This section contains a general description of the **Method Editor** user interface and the editing operations that can be performed in the different parts of the module.

In this section

This section contains the following sub-sections

Topic	See
Method Editor module	6.1.1
Text Instructions editor	6.1.2

6.1.1 *Method Editor module*

Two modes

The **Method Editor** interface operates in two modes:

- **Text Instructions** editor for entering and editing method instructions (see **6.1.2 Text Instructions editor** on page 100)
- **Run Setup** for defining method properties (see **6.5 Run Setup** on page 128).

How to open the Method Editor dialog boxes

The table below describes how to open the dialog boxes in the **Method Editor**:

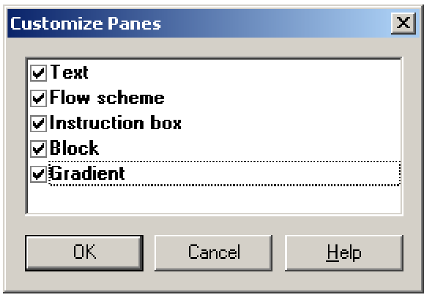
If you want to open...	**then...**
the **Text Instructions** editor	click the **Text Instructions** icon. *or* choose **View:Text Instructions**.
the **Run Setup**	click the **Run Setup** icon. *or* choose **View:Run Setup**.
the **Log Format**	click the **Log Format** icon. *or* choose **View:Log Format**.
the **Method Wizard**	click the **Method Wizard** icon. *or* choose **File:Method Wizard**.

6.1.2 *Text Instructions editor*

How to select panes to be displayed

You have a choice of four panes that can be open together with the **Instruction box** in the **Text Instructions** editor, all at once or one at a time.

Follow the steps in this table to select the panes to be displayed:

Step	Action
1	• In the **Method Editor**, choose **View:Text Instructions** *or* • click the **Text Instructions** icon.
2	• Choose **View:Panes:Customize** (or select additional panes here) *or* • click the **Customize Panes** icon.
3	**Select panes** • Select panes in the dialog box and click the **OK** button.  ☑ Text ☑ Flow scheme ☑ Instruction box ☑ Block ☑ Gradient **Deselect panes** • Deselect panes in the **Customize Panes** dialog box and click the **OK** button. *or* • right-click a window and select **Hide**.

Method editing operations performed in the different panes

This table shows the method editing operations that can be performed in the different panes:

The pane...	Is used...	See section
Text	• to display instructions • to display and hide block instructions. • to select current instruction. • to edit instructions • to cut, copy and paste instructions. • to move instructions within a breakpoint.	**6.2.1 How to view method blocks** on page 103 **6.3 Method instructions** on page 116
Flow scheme	• for information only. This window is not updated according to system status and changes in the method.	**9.2.4 The Flow Scheme pane** on page 212
Instruction box	• to specify breakpoints, instructions, parameters and variables. • to insert, change and delete instructions.	**6.3.2 How to add method instructions** on page 118
Block	to select or display blocks.	**6.2 Method blocks** on page 102
Gradient	to display block duration and eluent gradient throughout the method.	**6.5.5 The Gradient tab** on page 139

6.2 Method blocks

Introduction

This section contains a description of how to organize a method in blocks of instructions in order to make it more structured, and of how to work with method blocks.

In this section

This section contains the following sub-sections

Topic	See
How to view method blocks	6.2.1
How to call method blocks	6.2.2
How to add method blocks	6.2.3
How to delete method blocks	6.2.4
How to rename method blocks	6.2.5
How to find, copy and move method blocks	6.2.6
How to import method blocks	6.2.7

6.2.1 *How to view method blocks*

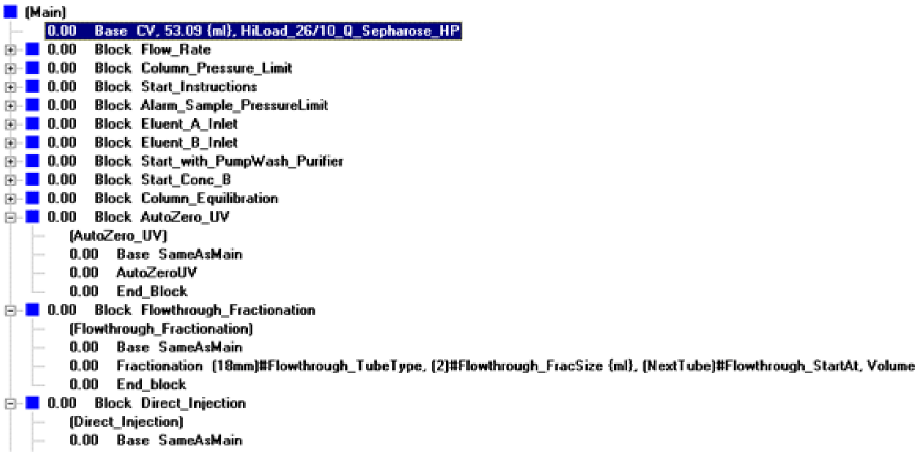
Instructions can be grouped into blocks

To view a method as a long list of individual text instructions can be confusing and inconvenient. Text instructions can therefore be grouped into blocks of instructions that define a specific functional use. For example, one block might contain the instructions necessary to equilibrate a column, and another block contains instructions to load a sample, etc.

The Text pane

In the **Text** pane of the **Method Editor**, the method is shown as a list of blocks, denoted by the blue square symbols. Note that a block can also contain sub-blocks.

The figure below shows the text instructions in blocks:



```
[Main]
  0.00  Base  CV, 53.09 {ml}, HiLoad_26/10_Q_Sepharose_HP
  0.00  Block  Flow_Rate
  0.00  Block  Column_Pressure_Limit
  0.00  Block  Start_Instructions
  0.00  Block  Alarm_Sample_PressureLimit
  0.00  Block  Eluent_A_Inlet
  0.00  Block  Eluent_B_Inlet
  0.00  Block  Start_with_PumpWash_Purifier
  0.00  Block  Start_Conc_B
  0.00  Block  Column_Equilibration
  0.00  Block  AutoZero_UV
    [AutoZero_UV]
    0.00  Base  SameAsMain
    0.00  AutoZeroUV
    0.00  End_Block
  0.00  Block  Flowthrough_Fractionation
    [Flowthrough_Fractionation]
    0.00  Base  SameAsMain
    0.00  Fractionation  [18mm]#Flowthrough_TubeType, [2]#Flowthrough_FracSize {ml}, [NextTube]#Flowthrough_StartAt, Volume
    0.00  End_block
  0.00  Block  Direct_Injection
    [Direct_Injection]
    0.00  Base  SameAsMain
```

The table below describes how to view or hide the instructions:

If you want...	then...
to view the instructions	click the "+" symbol *or* double-click the block name.
to hide the instructions	click the "-" symbol *or* double-click the block name.

The Block pane

The organization of blocks in the method is shown graphically in the **Block** pane of the **Method Editor**.

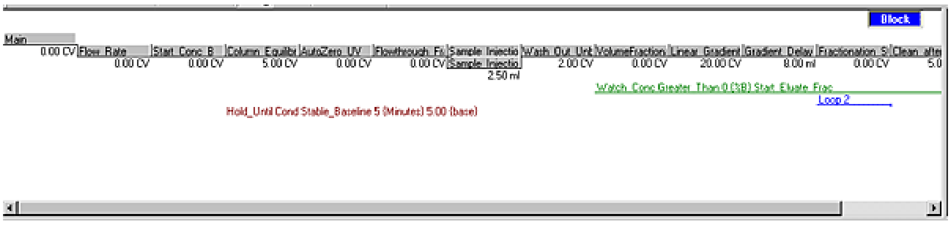
Description

Each block is represented by a gray bar with the block name and the length of the block. The line is shifted down to indicate calls to other blocks.

Click on the line that represents a block in the **Block** window to expand the block in the **Text** pane and select the first instruction in the block.

Figure

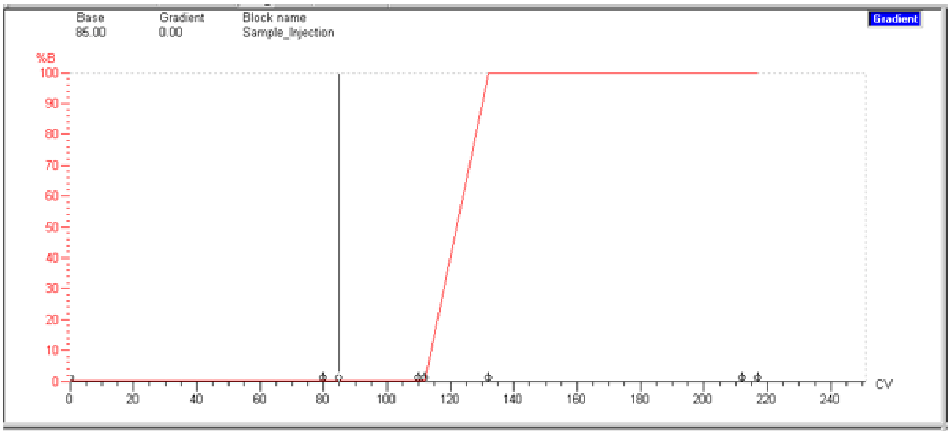
The figure below is an example with a **Watch** instruction to start the fraction collector which is active throughout the gradient elution block. **Loop** (to repeat a group of instructions) and **Hold_until** instructions are also indicated in the **Block pane**.



The Gradient pane

Blocks are represented in the **Gradient** pane of the **Method Editor** by marks on the X-axis. The marks show the length of each block. The name of the block in which the cursor line is currently placed is shown at the top of the pane.

The figure below describes the **Gradient** pane:


Base 85.00
Gradient 0.00
Block name Sample_Injection

6.2.2 How to call method blocks

General description

To execute the instructions contained within a block in a method, the block must be called by the program. When a block is called, the instructions in the block are executed in the order that they are written until the block is finished or the **End_Block** instruction is executed. Any settings made in a block are valid throughout the method until the settings are changed.

Types of calls

There are two types of calls:

- Unconditional calls, which are made with a **Block** instruction.
- Conditional calls, which are made with a **Watch** instruction. This makes it possible 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.

Watch instructions

Watch instructions are indicated by a green line that show the start and duration of the watch. These instructions can use various conditions to respond to absolute signal values or to rate of signal changes.

The breakpoint when the **Watch** instruction is issued determines when the watch begins, not when the block is activated. Once set, a watch remains active until the condition is met or a new **Watch** instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the **Watch_off** instruction.

See **F Method examples** on page 569 for more details on **Watch** instructions.

6.2.3 How to add method blocks

Two ways to add method blocks

You can add method blocks to a method in two ways, using either

- the **Instruction box** of the **Text Instructions** editor,

or

- the **New Block** dialog box reached via the **New Block** icon.

Both these alternatives are described below.

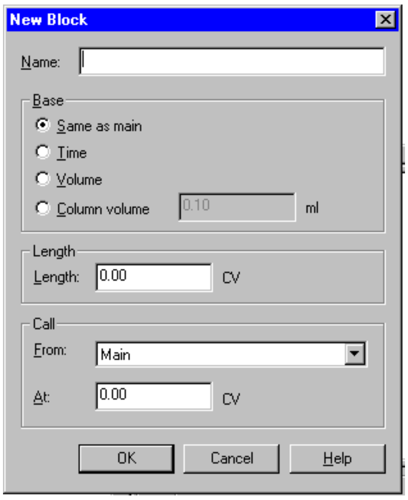
How to add blocks with the Instruction box

The table below describes how to add blocks with the **Instruction box**:

Step	Action
1	In the **Text pane** of the **Text Instructions** editor, select the instruction or block that you want to precede the new block.
2	Select **Other:Block** in the **Instruction box**.
3	• Enter a name for the block in the **Block** field. • Click the **Insert** button. *Result:* The block is inserted after the block that was selected in step 1.

The New Block dialog box

The illustration below shows the **New Block** dialog box that can be used when adding new method blocks:



Name:

Base: ● Same as main

Column volume: 0.10 ml

Length: 0.00 CV

From: Main

At: 0.00 CV

How to add blocks with the New Block dialog box

The table below describes how to add blocks with the menu options of the **New Block** dialog box:

Step	Action
1	Choose **Block:New** in the **Method Editor** *or* click the **New Block** icon. *Result:* The **New Block** dialog box is displayed.
2	Enter the relevant information in the **New Block** dialog box, and click **OK**. *Result:* The new block is added to the method, and placed last of all blocks. *Note*: The block can be placed in other positions by selecting something other than **Main** in the **From** droplist.

The fields of the New Block dialog box

The table below describes the fields of the **New Block** dialog box:

Field	Description
Name	Block names can be up to 30 characters long, and can contain letters (A-Z), digits (0-9) and the underscore character. Block names must be unique within the method. The case of letters is retained but not significant (the names **Start_Frac** and **START_FRAC** are treated as identical).
Base	One of the following options can be selected: • **SameAsMain**: the new block will inherit the base from the **Main** block in the method. The corresponding **Base** instruction will be inserted in the block at breakpoint 0. • **Time**: The block will be based on time. • **Volume**: The block will be based on volume. • **Column volume**: The block will be based on column volume.
Length	A block continues until the breakpoint for the **End_Block** instruction has been reached. An **End_Block** instruction will automatically be inserted in the block at the defined breakpoint. This field must not be left blank.

Field	Description
Call	You can call the new block from an existing block (for example the **Main** block). Select values in the two fields: • **From** The block from which the newly created block should be called. • **At** The breakpoint at which the call is to be made. If you do not want to call the block (for example when the block being created is to be activated by a **Watch** instruction), choose the **<Unused>** line from the **From** drop-down list. Blocks using this line are placed last in the method in the **Unused** category. *Note*: You should not call a block from within itself. If you do, you will generate a potentially infinite loop that exceeds the maximum number of calls allowed in a method. A loop symbol is displayed at the beginning of the line if this occurs.

6.2.4 *How to delete method blocks*

Four ways to delete blocks

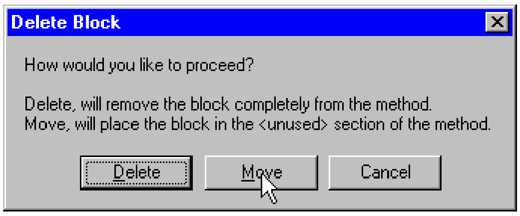
There are four ways to delete blocks:

- To right-click a block and choose **Delete** from the shortcut menu
- To select a block and click **Delete** in the **Instruction box**
- To select a block and press the <Delete> key on the keyboard
- To select a block and use the **Block:Delete Block** command

Note: When you use any of the first three ways, the **Method Editor** dialog box will give you the option to transfer the block to the `Unused` section.

Delete options

The **Delete Block** dialog box is displayed when you delete a block with one of the first three options mentioned above.



How would you like to proceed?

Delete, will remove the block completely from the method.
Move, will place the block in the <unused> section of the method.

Options

Choose from the following options:

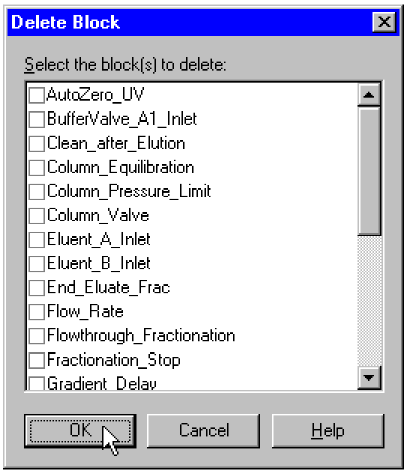
- **Delete**: The block is totally removed from the method. If the block is called several times in the method, all the blocks will be deleted. Blocks deleted in this fashion cannot be called again in the method.

  Note: If the block contains sub-blocks, another dialog box is displayed, asking you if you want to delete the sub-blocks as well.

- **Move**: The block is deleted from the method and transferred to the `Unused` section. If the block is called several times in the method, however, only the row with the block currently marked in the **Text** pane will be deleted. In this case, the block will not be placed in the `Unused` section (since the block is still used in the method). Blocks deleted in this fashion can be called again in the method.

How to use the Block:Delete Block command

The table below describes how to delete a block using the **Block:Delete Block** command:

Step	Action
1	Select the menu command **Block:Delete Block** in the **Method Editor**. *Result:* The **Delete Block** dialog box is displayed with all blocks listed in alphabetical order. 
2	Select the blocks you want to delete and click **OK**.
3	Click **Yes** to confirm.

How to delete unused blocks

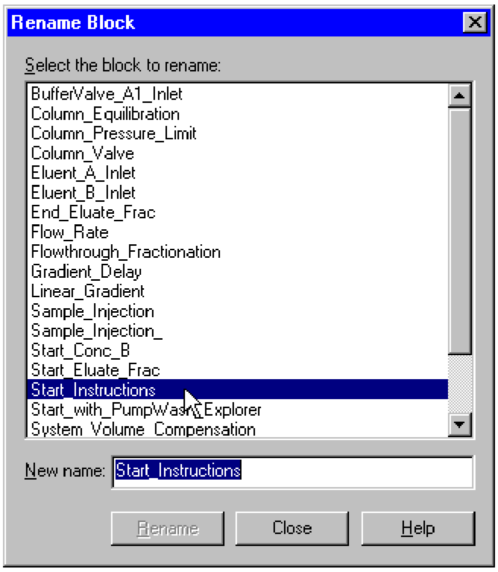
The table below describes how to delete an unused method block.

Step	Action
1	Highlight the method block. • Press the <delete> key *or* • Right-click and choose **Delete** on the shortcut menu. *Result*: The **Delete Block** dialog box opens. Note that the **Move** button is not available.
2	Click the **Delete** button. *Result*: The unused block is deleted and cannot be called upon again in the method.

6.2.5 *How to rename method blocks*

Instruction

The table below describes how to rename blocks:

Step	Action
1	Right-click the block you want to rename in the Text pane and select **Rename**. *Result:* The **Rename Block** dialog box is displayed.  New name: Start_Instructions *Note:* By default, the block that is currently selected in the **Text** window is automatically selected in the dialog box.
2	Enter the new name in the **New name** field and click **Rename**.
3	• If needed, repeat step 3 for other blocks. • Click **Close**. *Note:* If the block you renamed is called in a **Block** or **Watch** instruction, the block name in these instructions will be changed automatically.

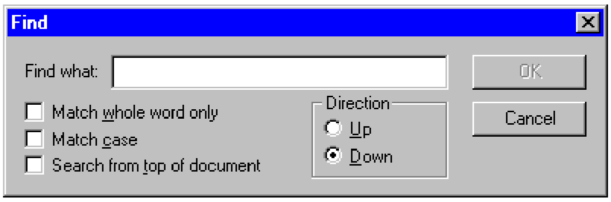
6.2.6 *How to find, copy and move method blocks*

Introduction

By using the **Edit** options in the **Method Editor**, you can find, copy and paste and move blocks within a method.

How to find text strings in the method text

The table describes how to find text strings in the method text.

Step	Action
1	Choose **Edit:Find** in the **Method Editor**, *or* right-click an instruction or a block in the **Text** window and select **Find**. *Result:* The **Find** dialog box is displayed. 
2	• Enter the text you want to search for, search direction and case matching criteria. • Click **OK**.

How to copy and paste a block

The table describes how to copy a block.

Step	Action
1	• Right-click the block you want to copy. • Choose **Copy**.
2	• Right-click the instruction line just above the point where you want the block to be pasted. • Choose **Paste**. *Result:* A dialog box asks if you wish to rename the pasted block.
3	Click **Yes** to rename the block before insertion, or **No** to insert the copied block directly. *Result:* The pasted block is inserted with the same breakpoint value as the block or instruction selected for point of insertion.

How to move a block

The table describes how to move a block.

Step	Action
1	• Right-click the block you want to move. • Choose **Cut**.
2	• Right-click the instruction line just above the point where you want the block to be pasted. • Choose **Paste**. *Result:* The block is now removed from its original breakpoint and pasted at the new breakpoint. The pasted block is inserted with the same breakpoint value as the block or instruction selected for point of insertion.

6.2.7 How to import method blocks

Introduction

You can import method blocks from other method files. You can also use this function to copy blocks within a method. In the latter case, it is important to note that it is the *saved* version of the method that will be copied, not changes that have been made after you last saved the method.

The block is imported exactly as it appears in the source method. If the base of the imported block is defined as **SameAsMain**, the block will inherit the main base in the new method, regardless of the base in the source method. Also, the imported block is inserted with the same breakpoint value as the block selected for point of insertion.

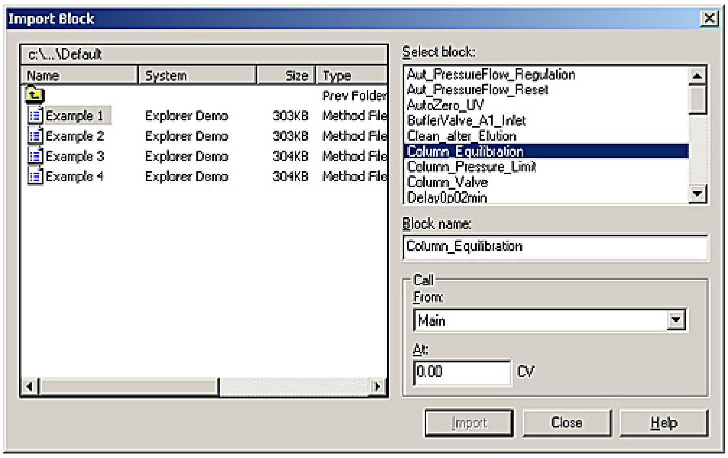
Instruction

The table below describes how to import method blocks:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>

Choose **Block:Import Block As** in the **Method Editor**.

Result: The **Import Block** dialog box is displayed.



Import Block

c:\...\Default

Name	System	Size	Type
			Prev Folder
Example 1	Explorer Demo	303KB	Method File
Example 2	Explorer Demo	303KB	Method File
Example 3	Explorer Demo	304KB	Method File
Example 4	Explorer Demo	304KB	Method File

Select block:
Aut_PressureFlow_Regulation
Aut_PressureFlow_Reset
AutoZero_UV
BufferValve_A1_Inlet
Clean_after_Elution
Column_Equilibration
Column_Pressure_Limit
Column_Valve
Delay0p02min

Block name: Column_Equilibration

Call
From: Main
At: 0.00 CV

</td></tr>
<tr><td>2</td><td>

- Select the method from which you want to import a block.
- Select the block.

Result: The name of the selected block is displayed in the **Block name** field.

</td></tr>
</table>

Step	Action
3	In the **Call** field, do the following: • On the **From** drop-down list, select a block into which the block will be imported. • In the **At** field, select the breakpoint value for the block to be imported. Click the **Import** button. *Note:* The imported block cannot have the same name as an existing block in the method. If the default name is not allowed for this reason, the **Import** button will be gray and locked. If this occurs, change the name of the imported block so that the **Import** button becomes available.
4	• Repeat steps 2 and 3 if needed. • Click the **Close** button.

6.3 Method instructions

Introduction

This section describes how to work with the individual method instructions, in order to edit method blocks and methods.

In this section

This section contains the following sub-sections

Topic	See
How to read method instructions	6.3.1
How to add method instructions	6.3.2
How to delete method instructions	6.3.3
How to change or move method instructions	6.3.4

6.3.1 *How to read method instructions*

Description of instruction markings

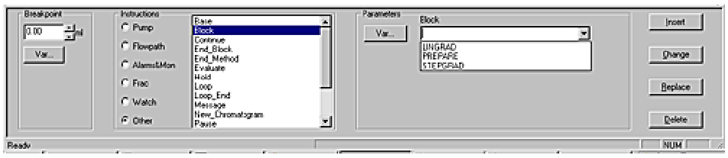
Method instructions are displayed in the **Text** pane of the **Text Instructions Editor**. The table below explains the meaning of the markings:

Marking	Explanation
Blue square beside text 🟦	Valid call instructions, that is, **Block** and **Watch** instructions to other blocks in the method.
Blue square with a red cross ☒	Call instruction that contains one or more invalid instructions.
Bold text	Valid instructions.
Red dot ●	Instructions with invalid syntax. All such instructions must be deleted or changed before a method can be run. See **6.3.4 How to change or move method instructions** on page 121. The instructions may be of the following types: • Calls to blocks which are not defined in the method • Instructions that apply to a different system strategy (can occur if a method is written for one system and saved for another) • Instructions for components that have not been selected in the **System Setup**.
Normal text	Instructions that will not be executed because • they are positioned after the end of a block or method *or* • they constitute a block to which there is no call.
Text with a loop symbol 🔄	When a block is called from within itself this will generate a potentially infinite loop, which might exceed the maximum number of calls allowed in a method.

6.3.2 *How to add method instructions*

Instruction

The table below describes how to add a method instruction in the **Text Instructions Editor**:

Step	Action
1	Select a block in the **Text** pane, and display the instructions within the block.
2	Select an instruction line in the block. Make sure that the selected instruction line is in the block, not the call to the block.
3	Open the **Instruction box** if it is not already displayed (**View: Panes**). Do the following: • Set the desired breakpoint in the **Breakpoint** field. • Choose the instruction type and the instruction in the **Instructions** field. For basic help on each instruction, click the instruction and press <F1>. • Type values for instruction parameters in the **Parameters** fields. If a scroll bar appears at the right side of the **Parameters** field, additional parameters are required. 
4	Click the **Insert** button. *Result:* The instruction will be inserted in the block • at the position of the breakpoint of the new instruction, if there are no other instructions at that breakpoint • immediately after the currently highlighted instruction, if the highlight is at the same breakpoint as the new instruction • as the last instruction at the breakpoint, if there are several instructions at the same breakpoint and none of these is highlighted. *Note:* Instructions that are placed at the same breakpoint are executed simultaneously, with the exception of **Block** instructions which are executed in the sequence in which they are written.

Pause, Hold and Hold_until instructions

If you use ÄKTA systems, the **Pause**, **Hold**, and **Hold_until** instructions will stop execution at this breakpoint, that is, instructions following after **Pause**, **Hold** and **Hold_until** at the same breakpoint will not be executed until a **Continue** instruction is issued.

6.3.3 How to delete method instructions

Instruction

The table below describes how to delete method instructions in the **Text Instructions Editor**:

Step	Action
1	Select the instruction in the **Text** pane.
2	Use one of the following alternatives: • Right-click the instruction and choose **Delete** in the displayed menu, *or* • press the **Delete** button in the **Instruction box**, *or* • press the **Delete** key on your keyboard.

End_Block instruction

If you delete the **End_Block** instruction, the block will end at the last instruction in the block. If a gradient is currently being formed, the gradient will continue into the next block.

How to suspend execution temporarily

An instruction that has been deleted can only be recovered by re-inserting the instruction. If you want to suspend execution of an instruction temporarily (for example during development work), you can replace the breakpoint with a value after the **End_Block** or **End_Method** instruction.

6.3.4 How to change or move method instructions

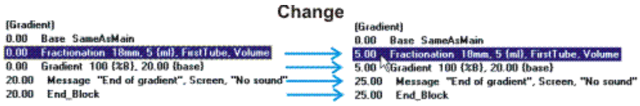
How to change an instruction

The table below describes how to change an instruction in the **Text** pane of the **Text Instructions Editor**:

Step	Action
1	Select the instruction. *Result:* The instruction with its current parameters is displayed in the **Instruction box**.
2	Make the required changes to the breakpoint or parameters *or* select a new instruction in the **Instruction Box**.
3	Click the **Change** button *or* the **Replace** button. *Note*: These buttons are equivalent unless changes are made to the breakpoint or the length of a gradient. See below.

Effects of the Change button and the Replace button on breakpoints

The table below describes the difference in function between the **Change** button and the **Replace** button when you change breakpoints:

Button	Function
Change	This button shifts all subsequent instructions in the block according to the change in the breakpoint. **Change** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in a block. The illustration shows an example where **Fractionation** is changed from breakpoint 0 to 5:  (Gradient) 0.00 Base SameAsMain 0.00 Fractionation 18mm, 5 (ml), FirstTube, Volume 0.00 Gradient 100 {%B}, 20.00 {base} 20.00 Message "End of gradient", Screen, "No sound" 20.00 End_Block (Gradient) 0.00 Base SameAsMain 5.00 Fractionation 18mm, 5 (ml), FirstTube, Volume 5.00 Gradient 100 {%B}, 20.00 {base} 25.00 Message "End of gradient", Screen, "No sound" 25.00 End_Block

Button	Function
Replace	This button moves the selected instruction but does not change the breakpoint of any other instruction. **Replace** can change the relative order of instructions in the method. The illustration shows an example where **Fractionation** is changed from breakpoint 0 to 5:  {Gradient} 0.00 Base SameAsMain 0.00 Fractionation 18mm, 5 {ml}, FirstTube, Volume 0.00 Gradient 100 {%B}, 20.00 {base} 20.00 Message "End of gradient", Screen, "No sound" 20.00 End_Block {Gradient} 0.00 Base SameAsMain 0.00 Gradient 100 {%B}, 20.00 {base} 5.00 Fractionation 18mm, 5 {ml}, FirstTube, Volume 20.00 Message "End of gradient", Screen, "No sound" 20.00 End_Block

---

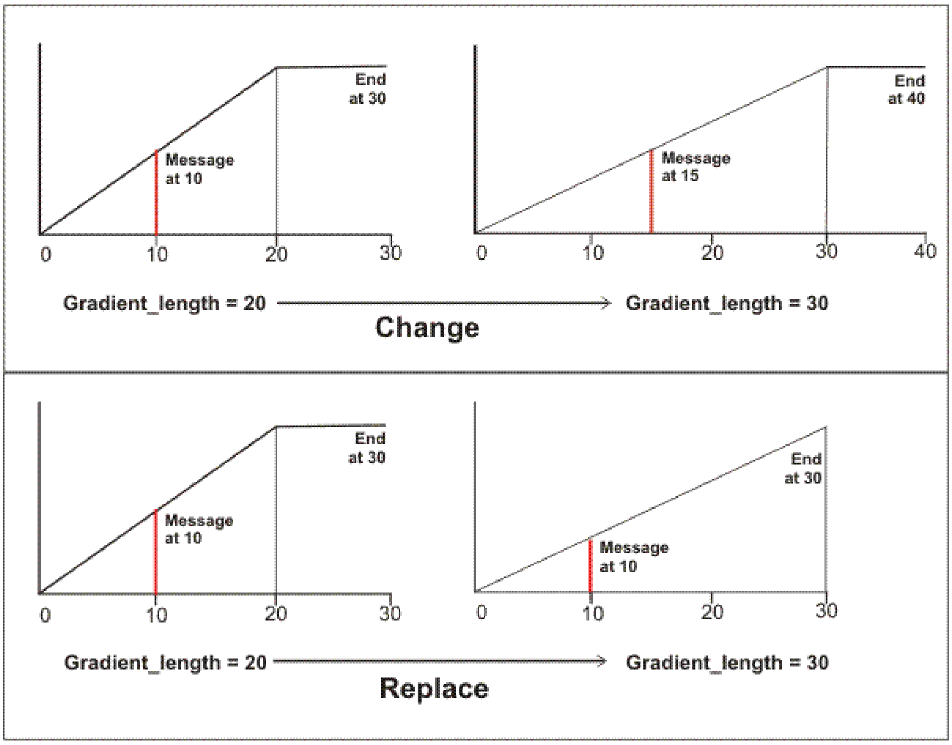
Effects of the Change button and the Replace button on gradient length

The **Length** parameter in the **Gradient** instruction affects the length of a gradient. Depending on which button you use, the change will have different results. The table below describes this:

Command	Function
Change	If this button is used to change the length of a gradient, the breakpoints for any instructions issued during the progress of the gradient will be adjusted proportionately so that they are always placed at the same relative position within the gradient. Instructions issued after the end of the gradient will be shifted by the amount of the change. Since the gradient works over time, any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length. *Note:* Moving the **End_block** instruction in a gradient block with the **Change** button does not affect the length of the gradient.
Replace	If this button is used to change the length of a gradient, other instructions are not affected.

Illustration of the effects of the Change button vs. the Replace button on gradients

The illustration shows the different effects of the **Change** button and the **Replace** button on instructions within and after gradients:



How to move an instruction

Move an instruction within the same breakpoint

Select the instruction in the **Text** pane of the **Text Instructions Editor** and drag it to its new location to change the order of instructions within the same breakpoint in a block.

Move an instruction to another breakpoint

The table below describes how to move an instruction to another breakpoint:

Step	Action
1	• Select the instruction in the **Text** pane of the **Text Instructions Editor**. • Choose **Edit:Cut**.
2	Select the instruction line just *above* the point where you want the cut instruction to be pasted. Choose **Edit:Paste**. *Result:* The instruction is now removed from its original breakpoint and pasted at the new breakpoint. The pasted instruction is inserted with the same breakpoint value as the instruction selected for point of insertion.

6.4 *How to use method variables*

Introduction

Method variables can be used to edit suitable methods. Variables can be assigned to most instruction parameters including breakpoints. Variables also form the foundation for automatic method scouting.

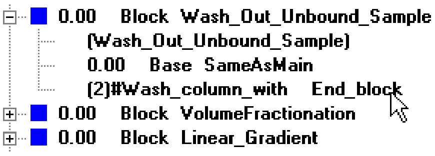
Each parameter defined as a variable is also assigned a default value, which is used if no changes are made to variable values at the start of a run. Up to 500 variables can be defined in a single method.

All variables are listed on the **Variables** tab of the **Run Setup**, grouped according to the block in which they appear. See **6.5.2 The Variables tab** on page 131.

Identifying variables

Parameters defined as variables can be identified in two ways:

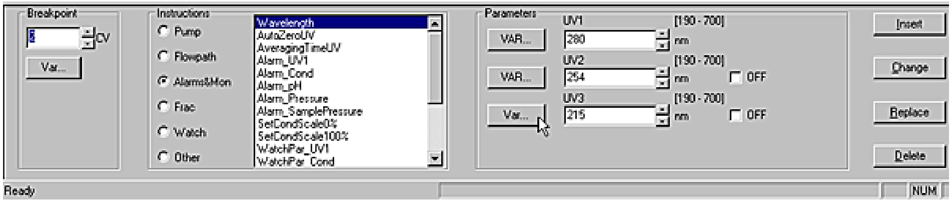
- In the **Text** pane in **Text instructions**, the parameter is given as the default value in parentheses followed by the variable name. The illustration below shows an example of this:



```
0.00   Block  Wash_Out_Unbound_Sample
   (Wash_Out_Unbound_Sample)
   0.00   Base  SameAsMain
   (2)#Wash_column_with   End_block
0.00   Block  VolumeFractionation
0.00   Block  Linear_Gradient
```

- When the instruction is shown in the **Instructions** field of the **Instruction box**, the **VAR** button beside the parameter field is displayed in capital letters, that is **VAR** not **Var**.

  The illustration below shows an example of the **Instruction box** where **UV1** and **UV2** are defined as variables and the **UV3** position is fixed.



UV1 280 nm
UV2 254 nm
UV3 215 nm

When to change variable values

Variable values can be changed immediately before the start of a method run without using the **Method Editor**, allowing one method to be used for runs under a variety of conditions.

How to change variable values

To change default variable values, you can either

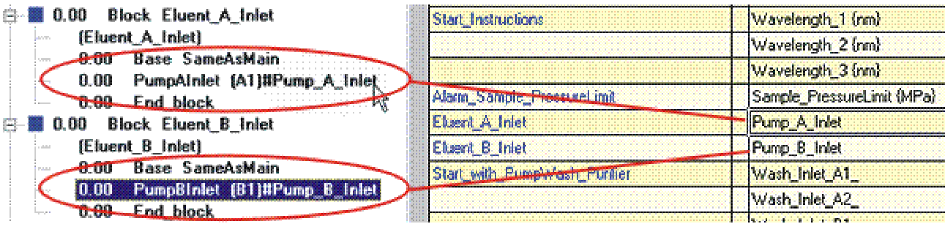
- edit the instruction in the **Instruction box**

or

- change the value in the **Variables** tab of **Run Setup**.

Changes made in the **Text** pane are automatically updated on the **Variables** tab and vice versa.

The figure below illustrates the relationship between variables in the **Text** pane and on the **Variables** tab of **Run Setup**:



```
0.00   Block  Eluent_A_Inlet
       (Eluent_A_Inlet)
       0.00   Base  SameAsMain
       0.00   PumpAInlet  (A1)#Pump_A_Inlet
       0.00   End_block
0.00   Block  Eluent_B_Inlet
       (Eluent_B_Inlet)
       0.00   Base  SameAsMain
       0.00   PumpBInlet  (B1)#Pump_B_Inlet
       0.00   End_block
```

Start_Instructions	Wavelength_1 (nm)
	Wavelength_2 (nm)
	Wavelength_3 (nm)
Alarm_Sample_PressureLimit	Sample_PressureLimit (MPa)
Eluent_A_Inlet	Pump_A_Inlet
Eluent_B_Inlet	Pump_B_Inlet
Start_with_PumpWash_Purifier	Wash_Inlet_A1_
	Wash_Inlet_A2_

Breakpoints or gradient lengths

If a breakpoint or gradient length is defined as a variable, changing the variable value in the **Variables** tab when the method run is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using the **Change** button to alter a breakpoint or gradient length (see **6.3.4 How to change or move method instructions** on page 122 for how the **Change** button affects instructions within gradients).

How to define new variables

Only one variable that affects block length (breakpoint or gradient length) may be defined within each block. However, any number of parameters may be defined as variables within a block. The table below describes how to define a new variable.

Step	Action
1	Select the instruction where you want to define the variable in the **Text** pane of **Text instructions**. *Result:* The parameters for the instruction are shown in the **Instruction box**.
2	• Locate the breakpoint or the required parameter in the **Instruction box**. • Click the **Var** button. *Result:* The **Variable Name Definition** dialog box opens.

Step	**Action**
3	• Enter a name for the variable. • Select the **Visible in details only** check box if you want to set the variable as a "details" variable. Detail variables only become visible on the **Variables** tab if the **Show details** check box is selected. This option is useful for hiding less important variables. • Click **OK**. *Result:* The **Var** button changes to **VAR** to confirm the new variable. The variable is displayed in the **Text** pane.

Variable names

Variables are defined with names that can be explicit descriptions of the variable function, for example **Sample_volume** and **Gradient_length**. Suitable choices of variable names can make the method easier to read and understand, and also help the operator in setting variable values at the start of a method run.

The names can be up to 32 characters long and the following characters can be used:

- Letters (A-Z)
- Digits (0-9)
- The underscore character (_)

The case of letters is retained, but not significant. The names **Flow_Rate** and **FLOW_RATE** are treated as identical.

How to rename a variable

The table below describes how to rename a variable:

Step	**Action**
1	Select the instruction that includes the variable you wish to rename in the **Text** pane of **Text instructions**. *Result:* The parameters for the instruction are shown in the **Instruction box**.
2	• Locate the required parameter in the **Parameters** field. • Click the **VAR** button.
3	Enter a new variable name in the dialog box and click **OK**.

Note: Variables can also be renamed in the **Edit Variables** dialog box in the **Method Editor**. See **6.5.2 The Variables tab** on page 132 for more information.

How to remove a variable

The table below describes how to remove a variable by converting it into a fixed value:

Step	Action
1	In the **Text** pane of **Text instructions**, select the instruction with the variable you want to remove. *Result:* The parameters for the instruction are shown in the **Instruction box**.
2	• Locate the required parameter in the **Parameters** field. • Click the **VAR** button.
3	• Click the **Clear** button to delete the variable. • Click **OK**. *Result:* The **VAR** button changes to **Var** to confirm that the variable is removed.

Note: Variables can also be deleted in the **Edit Variables** dialog box in the **Method Editor**. See **6.5.2 The Variables tab** on page 132 for more information.

6.5 Run Setup

Introduction

Run Setup is a part of the **Method Editor**. It has several tabs for defining method properties. This section describes how to use the tabs and the information displayed on the tabs.

In this section

This section contains the following sub-sections

Topic	See
Overview of Run Setup	6.5.1
The Variables tab	6.5.2
The Scouting tab	6.5.3
The Questions tab	6.5.4
The Gradient tab	6.5.5
The Notes tab	6.5.6
The Evaluation Procedures tab	6.5.7
The Reference Curves tab	6.5.8
The Columns tab	6.5.9
The BufferPrep tab	6.5.10
The Method Information tab	6.5.11
The Result Name tab	6.5.12
The Frac-950 tab	6.5.13
The Start Protocol tab	6.5.14
How to export the values in the Run Setup	6.5.15

6.5.1 *Overview of Run Setup*

Introduction

To access **Run Setup**, either

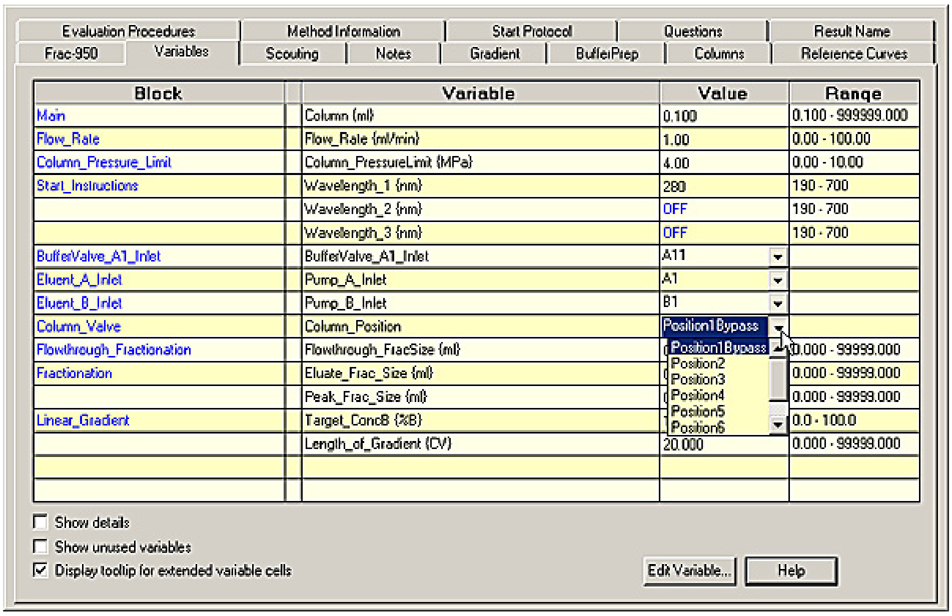
- Click the **Run Setup** icon on the **Method Editor** toolbar,

or

- Select **View: Run Setup**.

Illustration of Run Setup

The illustration below shows an example of the **Run Setup** with the **Variables** tab selected:



Block	Variable	Value	Range
Main	Column (ml)	0.100	0.100 - 999999.000
Flow_Rate	Flow_Rate (ml/min)	1.00	0.00 - 100.00
Column_Pressure_Limit	Column_PressureLimit (MPa)	4.00	0.00 - 10.00
Start_Instructions	Wavelength_1 (nm)	280	190 - 700
	Wavelength_2 (nm)	OFF	190 - 700
	Wavelength_3 (nm)	OFF	190 - 700
BufferValve_A1_Inlet	BufferValve_A1_Inlet	A11	
Eluent_A_Inlet	Pump_A_Inlet	A1	
Eluent_B_Inlet	Pump_B_Inlet	B1	
Column_Valve	Column_Position	Position1Bypass Position1Bypass Position2 Position3 Position4 Position5 Position6	
Flowthrough_Fractionation	Flowthrough_FracSize (ml)		0.000 - 99999.000
Fractionation	Eluate_Frac_Size (ml)		0.000 - 99999.000
	Peak_Frac_Size (ml)		0.000 - 99999.000
Linear_Gradient	Target_ConcB (%B)		0.0 - 100.0
	Length_of_Gradient (CV)	20.000	0.000 - 99999.000

☐ Show details
☐ Show unused variables
☑ Display tooltip for extended variable cells

The tabs

The table below contains brief descriptions of the tabs of **Run Setup**. If you want more detailed descriptions, see sections on the respective tabs:

Tab	**This tab...**
Frac-950	allows the user to choose rack type and the fractionation order for the Frac-950 fraction collector.
Variables	lists all variables used in the method with their default values, organized by method block.
Scouting	shows the scouting scheme used for the method. The scouting scheme can also be set up from this tab.

• p 129

Tab	This tab...
Notes	shows the descriptive comments that form a part of the method documentation.
Gradient	provides a graphical overview of the block structure and eluent gradient tab in the current method.
BufferPrep	displays information about the selected buffer preparation recipe for the current method.
Columns	displays the columns used in the current method.
Reference curves	displays the reference curves that will appear in the **System Control curve** dialog box during the run of the current method.
Evaluation Procedures	shows the evaluation procedures that will run at the end of the current method.
Method Information	displays information about the method, such as method name, target system, and last date of change.
Start Protocol	determines which items of the **Run Setup** that are displayed at the start of the run.
Questions	displays the questions used in the method. Questions provide a means for entering run-specific information at the start of a run. Use this tab when you want to define questions.
Result name	specifies how the result files will be named for the results of a run, and where the result file will be saved.

6.5.2 *The Variables tab*

Introduction

The **Variables** tab lists all variables used in the method with their default values, organized by method block. You can change the default values to create a variant of the method.

Note: The variables of a block are only displayed once on the **Variables** tab, even if the block is called several times in a method. **Variables** are displayed only if the method contains variables.

Check boxes

There are three check boxes on the **Variables** tab. The table below describes these boxes:

Check box	Select this box if you want...
Show details	detail variables to be shown. Detail variables are indicated by a **D** in the column immediately to the left of the **Variable** column.
Show unused variables	unused variables to be shown. Unused variables are indicated by a **U** in the column immediately to the left of the **Variable** column.
Display tooltip for extended variable cells	to display useful tips when you move the cursor to fields that can have several functions.

Note: The options to show detail and unused variables can be set up as default options in the **Administration:Change User Attributes** settings in the **UNICORN Manager**.

How to change the default values

Enter new values in the appropriate fields to change the default variable values. For some variables, pre-set values are available on drop-down menus. Save the method when you have made your changes.

Note: The **Variables** box must be selected on the **Start Protocol** tab if you want to be able to change variable values at the start of a method.

Blue values

For variables with values shown in blue, the value input can be toggled between **OFF**, **INFINITE** or other single position values, and a variable range. To change the value, right-click the value cell.

Variables can also be changed in the Text Instructions Editor

Variables can be changed in the **Text Instructions Editor** as well as on the **Variables** tab of the **Method Editor**. Changed values will be displayed for the corresponding instructions in both windows.

How to delete or rename variables

The table below describes how to delete or rename a variable in the **Run Setup**.

Step	Action
1	• Click the **Edit Variable...** button on the **Run Setup Variables** tab. *or* • Choose the **Edit:Variable...Method Editor** menu option. *Result*: The **Edit Variables** dialog box opens. The variables are listed alphabetically.
2	Select the variable to edit.
3	**Rename** • Type a new variable name in the **New name** text box. • Click the **Rename** button. *Result*: The variable is renamed. **Delete** • Click the **Delete** button. • Confirm that you want to delete the variable. *Result*: The variable is deleted.

How to change a variable into a detail variable

Detail variables are only shown if the **Show details** checkbox is selected on the **Variables** tab. The table below describes how to set up a detail variable.

Step	Action
1	• Click the **Edit Variable...** button on the **Run Setup Variables** tab. *or* • Choose the **Edit:Variable...Method Editor** menu option. *Result*: The **Edit Variables** dialog box opens. The variables are listed alphabetically.
2	Select the variable to be changed.
3	• Select the **Set visible in details only** checkbox. • Click the **Close** button. *Result*: The variable is marked by the detail indicator **D**.

How to change a detail variable into a regular variable

The table below describes how to change a detail variable into a regular variable.

Step	Action
1	• Click the **Edit Variable...** button on the **Run Setup Variables** tab. *or* • Choose the **Edit:Variable...Method Editor** menu option. *Result*: The **Edit Variables** dialog box opens. The variables are listed alphabetically.
2	Select the variable to be changed.
3	• De-select the **Set visible in details only** checkbox. • Click the **Close** button. *Result*: The detail variable indicator **D** is removed.

6.5.3 The Scouting tab

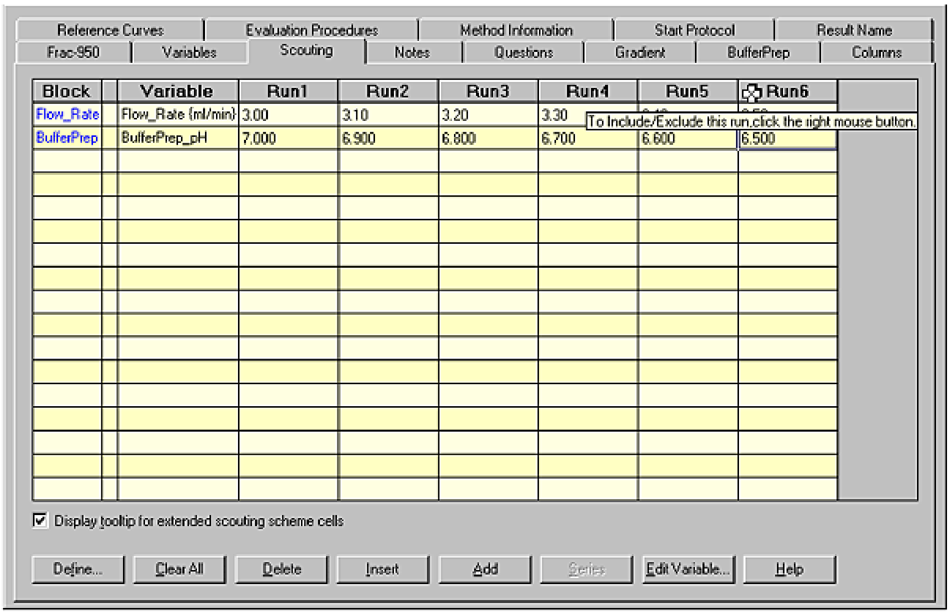
Introduction

A scouting scheme is a series of runs where chosen variable values are varied. You can define up to 99 runs in a scouting scheme. When a method is run with scouting, the method is automatically repeated for each selected run in the scouting scheme. Typically, scouting will vary one or more variables in a series of runs, for example, flow rate or elution gradient. See **7 Scouting** on page 182 for instructions on how to set up a scouting scheme, and **9.4 How to perform a scouting run.** on page 224

Note: The **Scouting** tab is available only if the method contains variables.

Example of a scouting scheme

The illustration below shows a scouting scheme for six flow rates and different pH values:



Block		Variable	Run1	Run2	Run3	Run4	Run5	Run6
Flow_Rate		Flow_Rate {ml/min}	3.00	3.10	3.20	3.30		
BufferPrep		BufferPrep_pH	7.000	6.900	6.800	6.700	6.600	6.500

To Include/Exclude this run,click the right mouse button.

☑ Display tooltip for extended scouting scheme cells

Note: The **Edit Variable...** button on the **Scouting** tab opens the same **Edit Variables** dialog box that can be accessed from the **Variables** tab.

6.5.4 *The Questions tab*

Introduction

The **Questions** tab of **Run Setup** is used for viewing and adding questions that the system asks a user at the start of a run. These questions provide a means for entering structured run-specific information. Method wizards and templates supplied with UNICORN are defined with a set of questions for sample, column and eluent identification.

Note: For questions to be shown in the start protocol, the **Questions** option must be checked on the **Start Protocol** tab of **Run Setup**.

Question status

Different types of questions have different status. The illustration below shows the **Question** field, an example of a question and the status alternatives that can be used:

Question: Sample Volume and Type

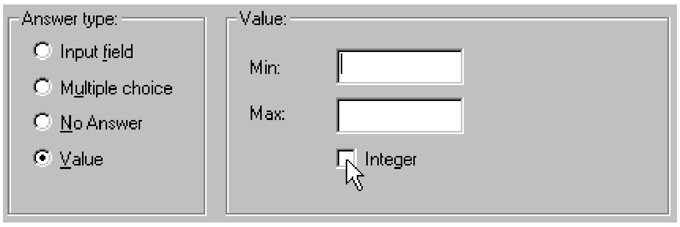
☐ Mandatory ☐ Authorized ☑ Chromatogram

The table below explains the different alternatives:

Question status	Explanation
Mandatory	These questions must be answered before a method is started.
Authorized	These questions must be signed with the users signature password to unlock and continue the method.
Chromatogram	These questions will be printed with the answers on the same page as the chromatogram, if a question is chosen in an evaluation report.

Answer type

A question has to be defined to accept one of four types of answers. The illustration below shows an example where the **Value** option has been selected. The appearance of the box to the right of the **Answer type** field depends on the answer type option selected:



Answer type:
○ Input field
○ Multiple choice
○ No Answer
● Value

Value:
Min:
Max:
☐ Integer

The table below describes the different answer types:

Answer type	**This option...**
Input field	accepts any alphanumerical input as the answer. Input field questions may have a default answer.
Multiple choice	allows the user to choose one of a defined set of answers. To allow a blank answer, enter a space in one of the predefined answers.
NoAnswer	is used to • display important information *or* • to split a question over more than one line by setting all but the last line in a question to **No answer**. (Normally, each question consists of one line only.) It is impossible to give an answer to questions with this option selected.
Value	accepts only numerical answers. **Value** questions must have specified maximum and minimum limits, and may be defined to accept only integer values.

How to insert a question

The table below describes how to insert a question:

Step	**Action**
1	If there are questions on the list, select the question that should be followed by the new question.
2	Enter the question text, status, answer type and answer option as required.

Step	Action
3	The **Answer type** determines what is displayed in the question definition field to the right of the **Answer type** field. For each answer type, do as follows: **Input field** Enter a default answer if required. **Multiple choice** • Click in the text field under **Alternatives**. • Enter the answer. • Click the **Add/Delete** button. *Result:* The new alternative is added at the end of the list. • Repeat this procedure to add new alternatives. To remove an alternative, mark the alternative in the scroll list and click the **Add/Delete** button. **No answer** No action taken. **Value** Enter maximum and minimum limits. Select the **Integer** box if the question is to accept integers only as answers.
4	Click the **Insert** button. Result: The new question is added to the list.

How to preview questions

The table below describes how to preview the questions as they will appear in the **Start Protocol**.

Step	Action
1	• Select a question. • Click the **Preview** button. *Result*: The question is displayed.
2	Click the **Edit** button to return to the question editing mode.

How to edit a question

The table below describes how to edit a question:

Step	Action
1	Select the question you want to edit.
2	Change the text, status, type and answer as required
3	Click the **Replace** button.

How to delete a question

Do one of the following to delete a question:

- Select a question and click the **Delete** button to remove the selected question.
- Click the **Delete all** button to delete all questions.

6.5.5 *The Gradient tab*

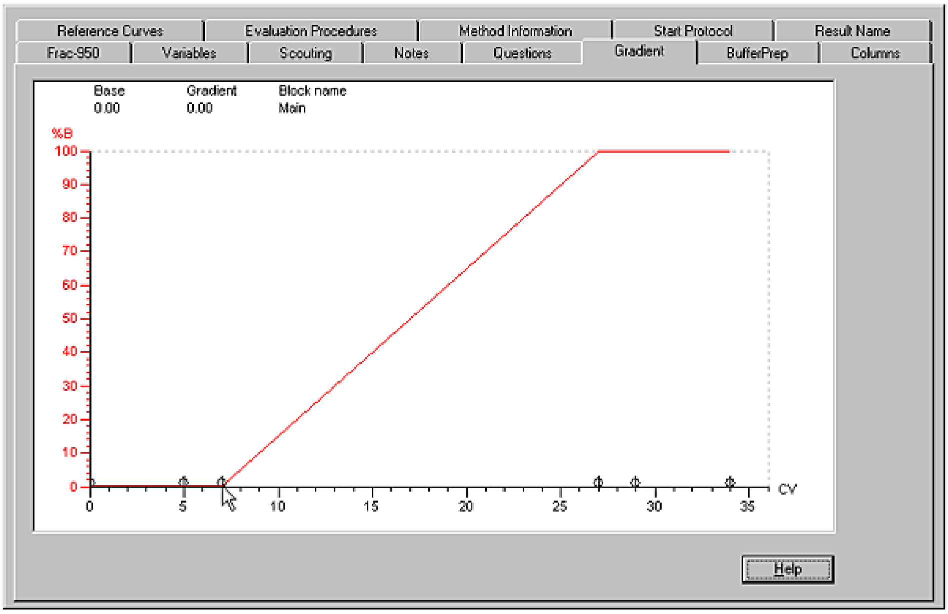
Introduction

The **Gradient** tab provides a graphical overview of the block structure and eluent gradient in the current method. The description of this tab can also serve as a description of the **Gradient** pane of the **Text Instructions**.

Note: For scouting runs, click **Run X** to see the gradient for each run.

Illustration

The illustration below shows an example of a **Gradient** tab:



Base 0.00

Gradient 0.00

Block name Main

How to zoom in on a selected region

The table below describes how to zoom in on a selected area of the **Gradient** tab:

Step	Action
1	• Press and hold the left mouse button and drag a rectangle on the screen to select the area you want to zoom in on. • Release the mouse button. *Result:* The display is now zoomed in on the selected area.
2	Repeat the process for further magnification of selected areas.

How to reduce the scale of the zoom function

To reduce the scale of the zoom function, right-click the tab and choose either:

- **Undo Zoom** to reverse each zoom-in action a step at a time, or
- **Reset Zoom** to reverse all of the zoom-in actions to the default scale setting.

How to use the vertical marker line

A vertical marker line can be dragged from the Y-axis with the mouse. As you drag the marker line, the current position is identified at the top of the tab in terms of the block name, X-position in the currently displayed base and eluent concentration in per cent of eluent B.

How to change the base shown on the X-axis

You can change the base shown on the **Gradient** X-axis. The alternatives are time, volume and column volumes. Changing the base for the display does not affect the base in the method instructions, which means that you can check how long a method will take simply by setting the axis scale to time, even if the method blocks are written in volume or column volume base.

The list below describes two ways to change the base shown on the X-axis:

- Click the X-axis to toggle between the base types.

or

- Right-click anywhere on the **Gradient** tab.

  Result: A sub-menu is displayed.
- Select **Base** and make the appropriate choice: **Time**, **Volume** or **CV**.

How to view hatch marks

You can display a hatched background on the **Gradient** tab. The table below describes how to do this:

Step	Action
1	Right-click anywhere on the **Gradient** tab. *Result:* A sub-menu is displayed.
2	Select **Hatch**. *Result:* The **Gradient** background is hatched.
3	To hide the hatch marks, repeat steps 1 and 2.

6.5.6 The Notes tab

Introduction

Notes are descriptive comments that form part of the method documentation. Method templates are supplied with notes describing the system requirements for running the method. Read through these notes carefully before using a method.

Sub-tabs

There are four sub-tabs:

- **Method Notes**
- **Start Notes**
- **Run Notes**
- **Evaluation Notes**

Only the **Method Notes** can be edited from the **Method Editor**; the other notes are accessible at the respective stages in a run.

Recommended usage

We recommend that you use **Method Notes** to describe the system setup required by the method (for example eluent and sample inlets, outlets and column connections).

Use the **Start Notes** or **Run Notes** for run-specific information.

Note: **Method Notes** are saved with the method and apply to all runs made with the method.

How to write method notes

To write method notes in your own methods, place the cursor in the white area of the **Notes** tab and type the relevant text. Use standard Windows editing functions to edit the notes.

How to search for text strings

You can search for text strings in the method notes. The table below describes how to perform a search.

Step	Action
1	Click the **Find** button. *Result*: The **Find** dialog box opens.
2	• Type the text string in the **Find what** text box. • Select search criteria and click **OK**. *Result*: The located text string is highlighted in the text area.

6.5.7 The Evaluation Procedures tab

Introduction

The **Evaluation Procedures** tab lists all evaluation procedures associated with the method. Evaluation procedures can be called automatically at the end of a method to evaluate and/or print the results.

Many UNICORN strategies are supplied with method templates or wizards that include a number of evaluation procedures. User defined procedures are created in the evaluation module and can be saved in method files (see **12.3 Automated evaluations procedures** on page 394).

Changes in the Evaluation module

A procedure in a method will not be updated when a procedure with the same name is changed in the **Evaluation** module. The same applies to report formats saved in a procedure.

References to curves

Evaluation procedures that process chromatogram data rely on consistent identification of curves in the result file for correct operation. If you include evaluation procedures with a method, make sure that references to curves in the procedure will be valid when the procedure is executed at the end of the run (see **12.3 Automated evaluation procedures** on page 394 for more details).

How to print evaluation results

If you use an evaluation procedure to print results automatically from a run controlled from a remote station in a network installation, the results will be printed on the printer currently set up on the local station, not on the remote station.

If you execute the procedure interactively from the **Evaluation** module on the remote station, the results will be printed on the printer set up on the remote station where you are working.

How to define and view evaluation procedures

Evaluation procedures are defined in the **Evaluation** module.

Procedures imported to a method can also be viewed and edited in the **Method Editor**. To do this, select the required procedure on the list and click the **Edit** button.

How to select procedures to run

To select procedures to run, select the procedure(s) that are to be executed at the end of the run. The procedures will be executed in the order they appear on the list.

How to import evaluation procedures

The table describes how to import global evaluation procedures:

Note: Procedures saved with one method file can be imported to another.

Step	Action
1	Select the **Evaluation Procedures** tab and click the **Import** button. *Result:* The **Import** dialog box is displayed.
2	Choose either option 1 or 2 below. **Option 1: Select a global UNICORN procedure** 1. Select a procedure on the **Select** list. *Result:* The evaluation procedure name is displayed in the **Import as** field. **Option 2: Select a procedure from another method** 1. Select a method, that contains a procedure, in the left part of the dialog box. *Result:* The procedures of the selected method will be displayed on the **Select** list. 2. Select the desired procedure on the **Select** list. *Result:* The method name is displayed in the **Import as** field. *Note:* Click **Procedure List** to return to the list of UNICORN's global evaluation procedures.
3	If desired, change the procedure name in the **Import as** field. *Note:* The imported evaluation procedure cannot have the same name as an existing evaluation procedure in the method. If the default name is not allowed for this reason, the **Import** button will be gray and disabled. When you change the name in the **Import as** field, the button will become available again.
4	Click the **Import** button. *Result:* The evaluation procedure is imported into the method.
5	Repeat steps 2 - 4 until you have imported all procedures.
6	Click the **Close** button.

How to delete evaluation procedures

The table describes how to delete evaluation procedures from the method:

Step	Action
1	Select the **Evaluation Procedures** tab.
2	Select the procedure(s) that you want to delete.
3	Click the **Delete** button and confirm the deletion when prompted. *Result:* The deleted procedures are immediately removed from the method file.

How to rename evaluation procedures

The table describes how to rename evaluation procedures in a method.

Step	Action
1	Select the **Evaluation Procedures** tab and click the **Rename** button. *Result:* The **Rename** dialog box is displayed.
2	• Select a procedure from the list and change the name in the **Rename item to** field. • Click **Rename**.
3	Repeat step 2 until you have renamed all procedures required.
4	Click the **Close** button.

How to edit an evaluation procedure

The table describes how to edit evaluation procedures in a specific method:

Step	Action
1	Select a procedure on the **Evaluation Procedures** tab and click the **Edit** button. *Result:* The **Procedure Editor** dialog box is displayed, with information about the selected procedure.
2	Enter the new parameter values in the appropriate place of the **Parameters** field, and click the **Replace** button. *Result:* The selected instruction in the evaluation procedure is updated in accordance with the new parameters assigned to it.

Step	Action
3	If needed, insert new instructions after the currently selected procedure instruction. Do the following: 1. Select an instruction type and instruction in the **Instructions** field. 2. Enter the appropriate parameter values in the **Parameters** field. 3. Click the **Insert** button. *Result:* The new instruction is added to the evaluation procedure.
4	To remove an instruction from the evaluation procedure, select it and click the **Delete** button.
5	Select **File:Save as** to save the edited procedure with a new name. Click the **Close** button.
6	Select **File:Close** from the menu in the **Procedure Editor** dialog box. *Result:* The **Procedure Editor** dialog box is closed and the procedure is saved automatically.

6.5.8 The Reference Curves tab

Introduction

Reference curves are curves from existing result files that you can display in the **Curves** pane of **System Control** during a run.

How to choose and display reference curves

You can include up to five reference curves in a method. You choose which curves to display during the run with the **View:Properties:Curves** command in **System Control** (see **9.2.3 The Curves pane** on page 206). Reference curves are only displayed during the run. Reference curves are not saved in the result file.

How to add reference curves

The table below describes how to add a reference curve from a result file:

Step	Action
1	Select the **Reference Curves** tab and click the **Import** button. *Result:* The **Import Reference Curve** dialog is displayed.
2	• In the left field, select the result file containing the curve to be added. *Result:* The **Select** list displays the available curves for the result file. • Select the curve you want to add from the **Select** list.
3	• If desired, change the curve name in the **Import as** field. *Note:* The curve name has to be changed if a reference curve with that name already exists. • Click **Import**.
4	Repeat steps 2 and 3 if you want to add more curves.
5	Click the **Close** button to close the **Import Reference Curve** dialog box.

How to delete reference curves

The table describes how to delete reference curves.

Step	Action
1	Select the curves you want to delete.
2	Click the **Delete** button and confirm the action when prompted.

Note: Deleting curves from the method does not affect the curves in the result file from which they were imported.

How to rename reference curves

The table below describes how to rename a reference curve in a method:

Step	Action
1	Click the **Rename** button.
2	• Select a curve from the list. • Change the name in the **Rename item to** field. • Click the **Rename** button.
3	Repeat steps 2 and 3 if you want to rename more reference curves.
4	Click the **Close** button. *Result:* The reference curve name is changed.

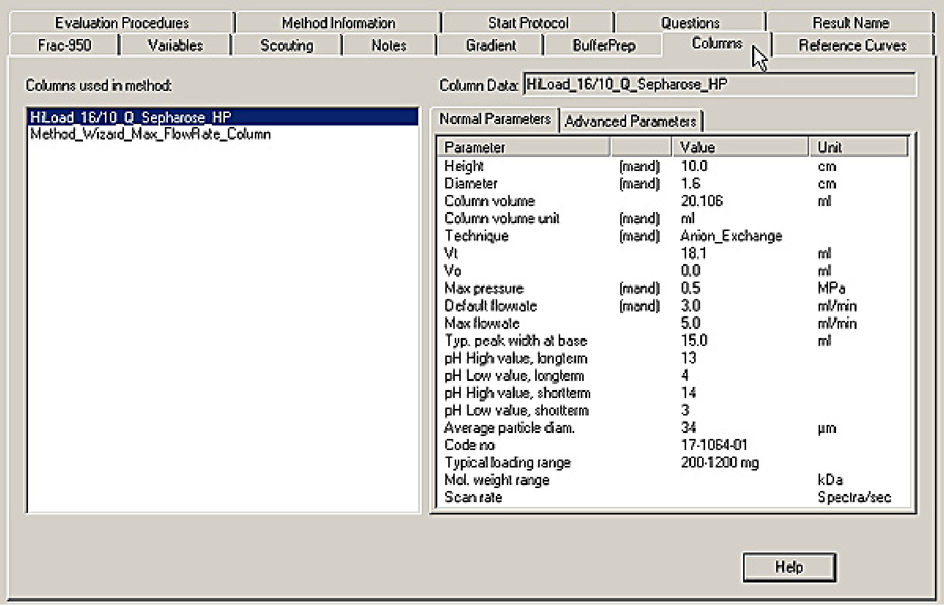
6.5.9 The Columns tab

Display of the column parameters

The **Columns** tab shows the parameters of the column selected for your method. The column parameters are displayed in the **Column Data** field. If you perform scouting runs with different columns, all of these will be listed. Select the appropriate column to display the parameters.

Illustration

The illustration shows an example of the **Columns** tab:



Columns used in method:
HiLoad_16/10_Q_Sepharose_HP
Method_Wizard_Max_FlowRate_Column

Column Data: HiLoad_16/10_Q_Sepharose_HP

Parameter		Value	Unit
Height	(mand)	10.0	cm
Diameter	(mand)	1.6	cm
Column volume		20.106	ml
Column volume unit	(mand)	ml	
Technique	(mand)	Anion_Exchange	
Vt		18.1	ml
Vo		0.0	ml
Max pressure	(mand)	0.5	MPa
Default flowrate	(mand)	3.0	ml/min
Max flowrate		5.0	ml/min
Typ. peak width at base		15.0	ml
pH High value, longterm		13	
pH Low value, longterm		4	
pH High value, shortterm		14	
pH Low value, shortterm		3	
Average particle diam.		34	µm
Code no		17-1064-01	
Typical loading range		200-1200 mg	
Mol. weight range			kDa
Scan rate			Spectra/sec

6.5.10 The BufferPrep tab

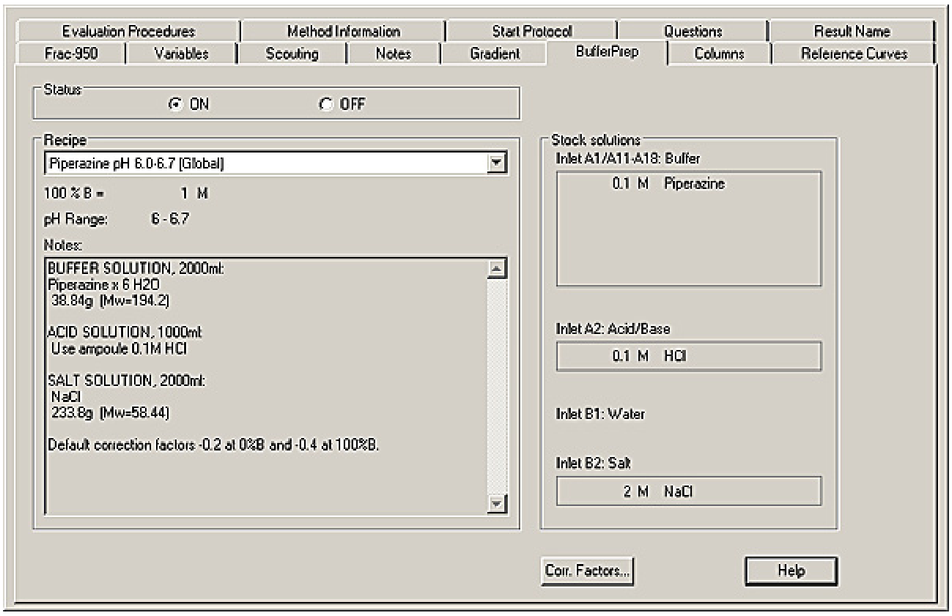
BufferPrep usage

BufferPrep allows a buffer of different pH and salt concentrations to be prepared on-line from four stock solutions. This removes the need to manually prepare new buffers every time the pH needs to be changed. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter. **BufferPrep** is optimized for cation and anion exchange chromatography. For a complete description of **BufferPrep**, see the user manual for ÄKTAdesign systems.

Note: **BufferPrep** is only available for some ÄKTAdesign systems.

Illustration

The illustration below shows an example of the **BufferPrep** tab:



Status
● ON ○ OFF

Recipe
Piperazine pH 6.0-6.7 [Global]

100 % B = 1 M

pH Range: 6 - 6.7

Notes:

BUFFER SOLUTION, 2000ml:
Piperazine x 6 H2O
38.84g (Mw=194.2)

ACID SOLUTION, 1000ml
Use ampoule 0.1M HCl

SALT SOLUTION, 2000ml:
NaCl
233.8g (Mw=58.44)

Default correction factors -0.2 at 0%B and -0.4 at 100%B.

Stock solutions

Inlet A1/A11-A18: Buffer
0.1 M Piperazine

Inlet A2: Acid/Base
0.1 M HCl

Inlet B1: Water

Inlet B2: Salt
2 M NaCl

Stock solutions

The solutions and the inlets to which they should be connected are displayed to the right of the dialog box. Accuracy of preparation is essential. The four stock solutions consist of:

- a mix of buffering components (there can be up to five different buffering components enabling a broad pH range to be covered),
- an acid (HCl) or base (NaOH) for pH on-line titration,
- distilled water,
- an inert salt (for example NaCl) for salt gradient formation.

How to create a BufferPrep method

If a suitable template or wizard is not available, you can create a **BufferPrep** method yourself. The instruction **BufferPrep_pH** must be available at breakpoint zero at the beginning of the method. The method must not contain the instructions **PumpAInlet** or **PumpBInlet**.

The table shows one way to create a **BufferPrep** method.

Step	Action
1	In the **Text Instruction** editor: • Insert a **BufferPrep-pH** block at breakpoint zero at the beginning of the method. • Define **BufferPrep-pH** as a variable.
2	Change to the **Run Setup**. • Select the **BufferPrep** tab. • Click the **ON** radio button in the **Status** field. • Select a **Recipe** from the drop-down list box. There are two main alternatives: - AIEX or CIEX, which are recipes covering a broad pH range, - single buffer recipes for more narrow pH ranges. *Result:* All information relevant to the selected recipe will be displayed on the tab.
3	Prepare the required stock solutions.
4	Do one of the following: • Select the **Variables** tab. Set the required pH for the method run in the variable **BufferPrep_pH**, *or* • If you want to perform pH scouting, click the **Scouting** tab and select **BufferPrep_pH** as a scouting variable. Enter the pH values for the different runs.

BufferPrep recipes

The recipe saved in the method (the one selected on the **BufferPrep** tab) cannot be edited, although fine tuning is possible. However, the recipes on the list of all **BufferPrep** recipes can be edited. New recipes can also be created (see **E How to create and edit BufferPrep recipes** on page 560).

How to fine tune the BufferPrep recipe with correction factors

In order to obtain high pH accuracy, the recipe can be fine tuned around a specific pH by setting correction factors. The table below describes how to fine tune the recipe with correction factors:

Step	Action
1	In **System Control**, select **Manual: Other**.
2	Select **BufferPrep Recipe** and **Recipe Name**. Click the **Execute** button.
3	• Set the pH in the instruction **BufferPrep_pH** in group **Pump**. • Click the **Execute** button.
4	• Set the flow rate to be used during the run in the **Flow** instruction. • Click the **Execute** button.
5	Check the pH reading when stable. Allow at least 30 ml of eluent to pass through before expecting a steady pH reading.
6	• Change to 100% B by setting the Gradient instruction in **Manual:Pump** to 100% for **Target** and 0 for **Length**. • Click the **Execute** button.
7	Check the pH reading when stable at 100% B.
8	• If the readings are acceptable at both 0% and 100%, the correction factors do not need to be changed. • If the readings are not acceptable, click the **Corr. Factors** button in the **BufferPrep** tab in the method.
9	Enter the deviation at 0% and 100%. (e.g., if the pH is set to 7.0 and the actual pH is 7.1 enter 0.1. Enter -0.1 if the pH is 6.9). *Note:* If correction factors already exist, the measured pH deviation should be added to the old factors.
10	Save the method.

Note: When changing the correction factors for the recipe selected in the method, the recipe with the same name on the list of all **BufferPrep** recipes is not affected. The changes will only apply in the specific method.

6.5.11 *The Method Information tab*

Introduction

The **Method Information** tab displays information about the method. This tab is for information only and cannot be edited.

There are three sub-tabs on this tab: **Information**, **Signatures**, and **Method duration**.

The Information sub-tab

The **Information** sub-tab displays

- method information such as method name, creation date, creator and date of last change,
- target system,
- strategy information such as strategy name, date and size.

The **Strategy Notes** button displays what systems, programs and file versions the strategy is designed for.

The Signatures sub-tab

The **Signatures** sub-tab has five information fields for all signatures. The table below describes the content of each field:

Field	Description
Date	Date of the signature.
Meaning	Short description explaining the meaning behind the signature.
User Name	User name of the user who signed the method.
Full Name	Full name of the user who signed the method.
Position	Position of the user.

The Method Duration sub-tab

The **Method Duration** sub-tab presents

- the estimated total time
- the estimated buffer volume required for the method.

If the method includes a scouting scheme, click the **Run 'x'** button to see values for the different scouting runs.

6.5.12 The Result Name tab

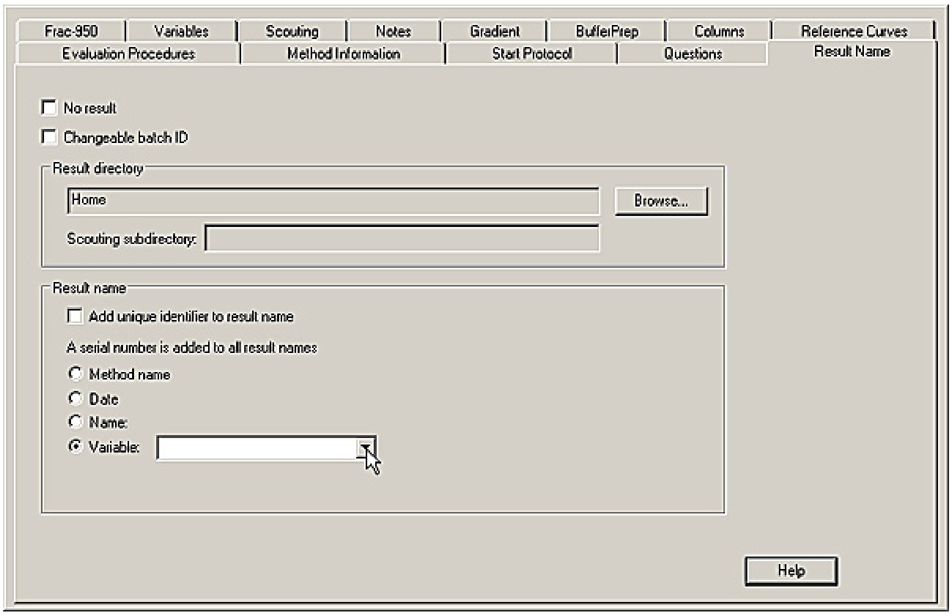
Introduction

The **Result Name** tab is used to specify:

- how the result files will be named for the results of a run
- where the result file will be saved
- the name of the special scouting folder where results from scouting runs will be stored.

Illustration

The illustration below shows an example of the **Result Name** tab:



☐ No result

☐ Changeable batch ID

Result directory

Home

Scouting subdirectory:

Result name

☐ Add unique identifier to result name

A serial number is added to all result names

○ Method name

○ Date

○ Name:

● Variable:

Construction of the result file name

The result file name is constructed by one of the base options listed below. The serial number is changed automatically each time the method is run.

Base options of the result file name are:

- The **Method name** plus a 3-digit serial number,
- The **Date** of the run (in an 8-digit format determined by the country setting in Windows 2000 or XP) plus a 3-digit serial number,
- A freely specified **Name** (within the file naming restrictions of the operating system) plus a 3-digit serial number.
- A selected **Variable** (from the droplist) plus a 3-digit serial number.

Note: If a result names includes decimal points (e.g. numeric variables) or underscore characters, these characters will automatically be replaced by spaces. Points and underscores are not allowed in the result names.

Serial numbers and unique identifiers

If the result file folder already contains files with the same file name base, the serial number is changed automatically. For scouting runs, the 3-digit serial number will be the number of the executed run column in the scouting scheme.

A unique identifier can also be generated automatically, in addition to the serial number. The identifier is a string of numbers inserted between the result file name and the three-digit serial number.

- Select **Add unique identifier to result name** in the **Result** name field.

Batch ID for each test run

UNICORN will automatically issue a **Batch ID** to each method run. This ID is displayed before the **Base** in the logbook and can be used to identify individual runs. See illustration in **9.2.5 The Logbook pane** on page 213. If **Changeable batch ID** is selected, another ID string can be typed in the **Start Protocol**.

Specify result name as changeable

The result name can be specified as changeable in the **Start Protocol** (see **6.5.14 The Start Protocol tab** on page 158). In that case, the information you supply on the **Result Name** tab will be the suggested result name, but you can change this at the start of the run.

How to save the result files in a different folder

By default, result files are stored in the home folder of the user who starts the run. The table below describes how to change the folder where the result file will be stored:

Step	Action
1	If the run contains information that is not important, you can save disk space by selecting the **No result** check box, thereby storing the result in the **Temporary** folder (named **Manual Runs**, where only the latest 10 result files are saved). If not, go to step 2.
2	Click the **Browse** button.
3	• Double-click the required folder icon. • Click the **OK** button.

How to save scouting results

Scouting results will be saved in a special folder as specified by the result file path. To select a folder, type a name for the folder in the **Scouting** subdirectory field. Each time the scouting method is run, a new folder will be created with the name and a serial number (entering IEXSC will create folders IEXSC001, IEXSC002, etc.).

6.5.13 *The Frac-950 tab*

Introduction

The **Frac-950** tab is used for defining options for **Frac-950**. The user can choose rack type and fractionation order.

How to set up fractionation

The table describes how to set up the fractionation.

Step	Action
1	Select rack type from the **Rack** drop-down list.
2	Select the order for fractionation by using the **Fraction order** radio buttons.

Manual runs

In **System Control**, for manual runs, the **Frac-950** tab cannot be used. Instead, use the manual fractionation instructions, starting with **Man_**.

- Choose **Manual:Frac** to open the **Frac Instructions** dialog box.

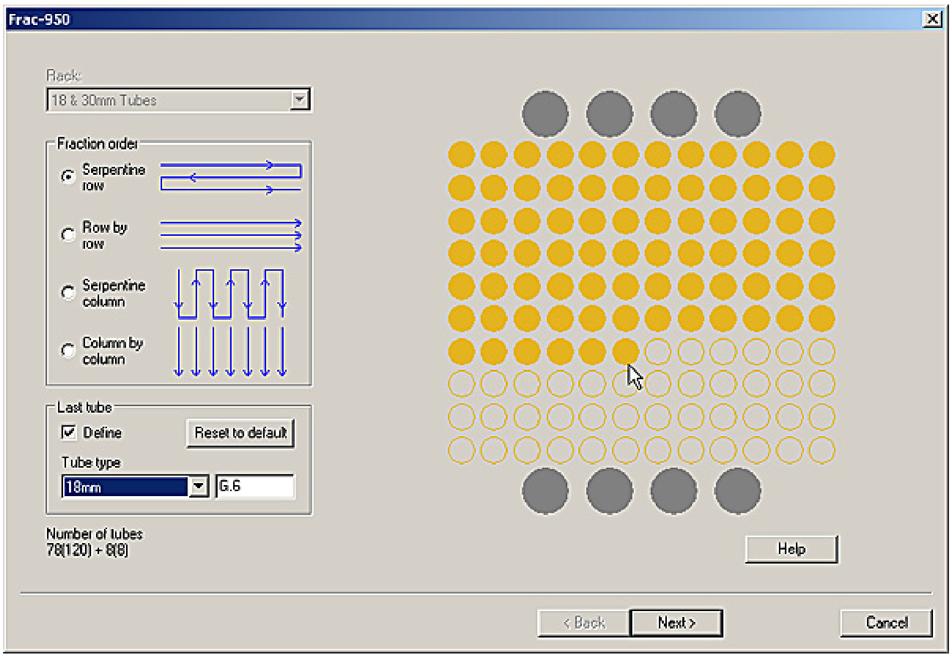
Total number of tubes

The total number of tubes sampled may differ if a last tube has been chosen. The **Number of tubes** equation in the bottom left corner of the **Frac-950** dialog box of the **Start Protocol** shows the current number of available tubes chosen for fractionation, followed by the total possible number in parentheses.

How to select the last tube

You can select a position for the last tube to be used in the fractionation process. If the process attempts to go further than the selected last tube during a method run, an alarm will be executed.

The last tube position can only be selected on the **Frac-950** dialog box in the **Start Protocol** when you start a method run, or when you do an instant run. The illustration below shows an example of this dialog box:



Rack: 18 & 30mm Tubes

Fraction order: ● Serpentine row

Last tube: ☑ Define

Tube type: 18mm 6.6

Number of tubes 78(120) + 8(8)

The lower right box within the **Last tube** field shows the currently selected last tube. The table below describes how to re-define the last tube:

Step	Action
1	In the **Frac-950** dialog box, select the **Define** box in the **Last tube** field to select the last tube position.
2	Place the cursor over the appropriate tube (circle) within the tube matrix and click again.

Note: When using different sized tubes in the same rack, the last tube can be set for both tube sizes. Use the **Tube type** drop-down list to choose the desired tube size, and then follow the procedures outlined above to select the last tube.

How to set the last tube to default setting

If you want to return to the default last tube position, click the **Reset to default** button of the **Frac-950** dialog box in the **Start Protocol** when you start a method run.

6.5.14 *The Start Protocol tab*

Introduction

The **Start Protocol** tab determines which items of the **Run Setup** are displayed at the start of a method run. Click the **Start Protocol** tab and select the items that you want to be displayed.

Checkboxes

The table below describes the check boxes of the **Start Protocol** tab:

Checkbox	Displays...
Frac-950	the **Frac-950** setup parameters, which can be changed.
Variables	values for method variables that can be changed at the start of the run. These values will override the default values for the particular run and be saved in the result file. The default values stored in the method are not affected.
Scouting	the scouting scheme which can be changed at the start of the run. Changes will override the default settings and values for the particular run and be saved in the result file.
Text Method	method instructions. They cannot be changed from this display.
Notes	the **Notes** tab.
Gradient	the gradient.
BufferPrep	the recipe selected in the method. The recipe cannot be changed during the start of a run.
Columns	the available column definitions.
Reference curves	the reference curves associated with the method.
Evaluation procedures	the evaluation procedures set to be executed at the end of the method.
Method information	the method information.
Settings	the settings.
Calibration	the monitor calibration settings.

Checkbox	Displays...
Questions	questions defined in the method.You are recommended to always use this option, since the answers to questions can form an important part of the UNICORN run documentation.
Result name	the result name, which is changeable if this option has been selected. Click the **Browse** button to change the result folder. If the box is not selected, the result name will still be displayed, but you will not be able to change the name or folder.

Scouting start protocol field

The table below describes the options in the **Scouting start protocol** field.

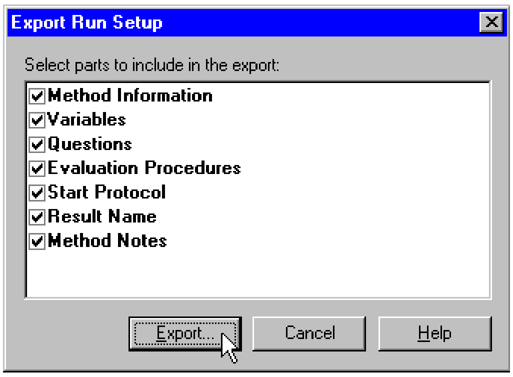
Option	If you check this option...
First run only	parameters for the scouting runs can be adjusted at the beginning of the first run only. After that, the runs will be performed automatically without operator intervention.
All runs	the **Scouting start protocol** will be displayed at the beginning of each run in the scouting scheme.

6.5.15 *How to export the values in the Run Setup*

Instruction

You can easily export the values in the **Run Setup** to a file, and save it in ASCII format. This is useful when you want to enable others to read the methods without having access to UNICORN on their computers.

The table below describes how to export the values in the **Run Setup** and save them to a file.

Step	Action
1	In the **Text instructions Editor** or the **Run Setup**, select **File:Export:Run Setup**. *Result:* The **Export Run Setup** dialog box is displayed.  Select parts to include in the export: ☑ Method Information ☑ Variables ☑ Questions ☑ Evaluation Procedures ☑ Start Protocol ☑ Result Name ☑ Method Notes
2	• Select the boxes to select the parts of **Run Setup** that you want to export. • Click the **Export** button. *Result:* The **Export** dialog box is displayed.
3	• Type a file name and select the target drive and folder. • Click the **Save** button.

6.6 How to use selected method instructions

Introduction

This section provides recommendations for how to use some common programming features in UNICORN methods. They are available from the **Instruction box** in the **Method Editor**.

In this section

This section contains the following sub-sections

Topic	See
Base instruction	6.6.1
Instructions at the same breakpoint	6.6.2
Block and method length	6.6.3
Messages and Set_Marks	6.6.4
How to delay a method	6.6.5
Linear flow rates	6.6.6
Gradients and eluent concentrations	6.6.7

6.6.1 Base instruction

Bases

Every method block must start with a **Base** instruction, defining the base for calculating breakpoints.

Different blocks can use different bases. The base can be one of the following:

- **volume** (the unit depends on the scale defined in the system strategy)
- **time** (minutes)
- **column volume**, CV (defined as a numerical value or taken from the column definition)
- **SameAsMain** (all blocks apart from the main block), which means that the block will inherit the base defined in the main block.

Method blocks that use a volume or column volume base

Make sure that the flow rate is not zero. Volume breakpoints are calculated from the flow rate of the pump, and the method will not progress if the flow rate is zero.

What base should I use?

Use the base that most closely suits the purpose of the block. Column volume is recommended as the base for most steps in a run. In some situations, however, it may be more suitable to use a time or volume base for individual blocks.

To change the base for an existing method

Be careful when changing the base for an existing method. Changing between time and volume bases can affect the relative duration of steps in the method if different steps use different flow rates.

Column parameter: named column

If a named column is selected for the **Column** parameter in the **Other:Base** instruction, the volume specified in the selected column definition will automatically be used for column volume in the method block. The column volume for base **CV** cannot then be changed in the instruction or defined as a variable. However, the **Column** parameter should be defined as a variable. Choosing a column definition also enables linear flow rate and column performance calculations.

Column parameter: Any

If the **Column** parameter in the **Other:Base** instruction is set to **Any** and the **Base** parameter is set to **CV**, the column volume is set numerically by the **Volume** parameter. The column volume may be defined as a variable, allowing the scale of the run to be decided when the method is actually run.

How to select columns for a template or wizard

In cases where a template or wizard-generated method and column are chosen, it is easy to select other columns for that method on the **Variables** tab in **Run Setup**.

Note: This might not be possible for methods that you have created yourself.

How to select columns for a method not selected from a template

The table below describes how to select columns for a method, not selected from a template.

Step	Action
1	In the **Instruction box** of the **Text instruction** dialog box, mark the **Other:Base** instruction.
2	• Select the required column from the drop-down list for the **Column** parameter. • Click the **Var...** button to define the **Column** parameter as a variable. This is an optional but recommended step that will make it easy to change the column selection for different runs.
3	• Enter a variable name and click **OK**. • Click **Yes** to confirm.

Column definition

A column definition can be chosen and defined as a variable even if the base for the block is set to volume or time. Parameters in the column definition will then be used for linear flow rate and column performance calculations.

Recommendation

A selected column definition applies locally within the block for which it is selected, and is not transferred to other blocks. We strongly recommend that the column definition be selected for the main block.

Update parameters

If you want parameters (for example, flow, pressure and averaging time) to be updated when you change the column, you must define these as variables.

Pump:Methodbase instruction

Volume or column volume base is calculated from the flow rate of the **SystemPump** or the **SamplePump**, selected with the instruction **Pump:Methodbase**. If no **Pump:Methodbase** instruction is included in the method, the default setting **SystemPump** will be used.

6.6.2 Instructions at the same breakpoint

Description

Instructions placed at the same breakpoint in a block are executed simultaneously.

Exceptions

Exceptions are successive **Block** instructions, which are executed in the sequence in which they are written. This can have important consequences in some situations.

The instruction sequence below shows an example of instructions with the same breakpoint, where the **AutoZero_UV** will start *after* the **Wash** block is completed.

Breakpoint	Instruction
`0.00`	`Block WASH`
`0.00`	`AutoZero_UV`
`0.00`	`Block ELUATE`

6.6.3 Block and method length

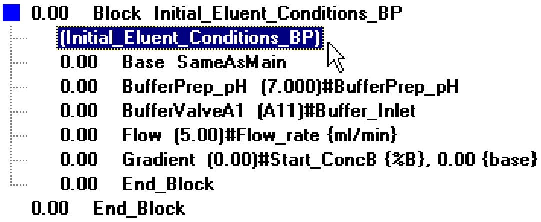
General description

The time or volume of a method run is determined by the sum of the block lengths. In turn, the length of a block is determined by the breakpoint of the last instruction in the block.

Note: Depending on how conditional calls are used (see **6.7 Standard Watch conditions** on page 172), the overall method time or volume may vary according to watch events during the run.

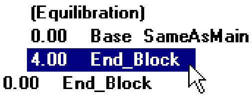
Block length

A block in which all breakpoints are set to 0 will take no time or volume during a method run. The illustration below shows an example of this:



```
0.00   Block  Initial_Eluent_Conditions_BP
          (Initial_Eluent_Conditions_BP)
          0.00   Base  SameAsMain
          0.00   BufferPrep_pH  (7.000)#BufferPrep_pH
          0.00   BufferValveA1  (A11)#Buffer_Inlet
          0.00   Flow  (5.00)#Flow_rate {ml/min}
          0.00   Gradient  (0.00)#Start_ConcB {%B}, 0.00 {base}
          0.00   End_Block
0.00   End_Block
```

To extend the length of a block without performing any other operation, set the breakpoint of the **End_block** instruction appropriately, for example, as in the illustration below:



```
    (Equilibration)
    0.00   Base  SameAsMain
    4.00   End_Block
0.00   End_Block
```

How to view the accumulated method time or volume

The Log Format window in the **Method Editor** shows the accumulated method time or volume for the current method. The accumulated time/volume is an approximation and does not take into account time or volume for **Watch** blocks, **Wash** commands or programmed **Hold**. Also it does not compensate for splitter flow.

The table below describes how to view the accumulated method time or volume:

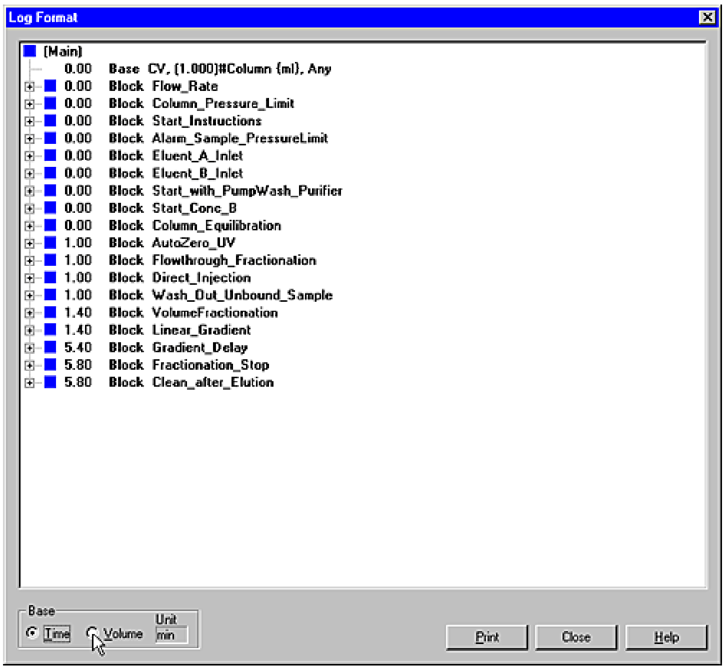
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>

Select **View:Log Format**

or

click the **Log Format** icon.

Result: The **Log Format** dialog box is displayed.



```
(Main)
   0.00   Base  CV, (1.000)#Column (ml), Any
   0.00   Block  Flow_Rate
   0.00   Block  Column_Pressure_Limit
   0.00   Block  Start_Instructions
   0.00   Block  Alarm_Sample_PressureLimit
   0.00   Block  Eluent_A_Inlet
   0.00   Block  Eluent_B_Inlet
   0.00   Block  Start_with_PumpWash_Purifier
   0.00   Block  Start_Conc_B
   0.00   Block  Column_Equilibration
   1.00   Block  AutoZero_UV
   1.00   Block  Flowthrough_Fractionation
   1.00   Block  Direct_Injection
   1.00   Block  Wash_Out_Unbound_Sample
   1.40   Block  VolumeFractionation
   1.40   Block  Linear_Gradient
   5.40   Block  Gradient_Delay
   5.80   Block  Fractionation_Stop
   5.80   Block  Clean_after_Elution
```

Base: ● Time ○ Volume Unit: min

</td></tr>
<tr><td>2</td><td>If the method is a scouting run, click **Run X** to move between runs.</td></tr>
</table>

6.6.4 *Messages and Set_Marks*

When to use a message

Messages are used to inform the operator of the progress of the run. It is a good idea to issue messages at critical points in the method, for example, when **Watch** instructions are used for conditional events.

How to add a Message instruction

The **Message** instruction can be used to set up a message that will be displayed for the user during the execution of the method run. The 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. See **F.6 Appendix Messages** on page 579 for examples.

The table below describes how to add a **Message** instruction to the method.

Step	Action
1	• Select **Other** in the **Instructions** field of the **Instructions 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.

Note: All messages are erased when the system reaches the **End** status. This also includes **Authorize** messages.

When to use a Set_Mark

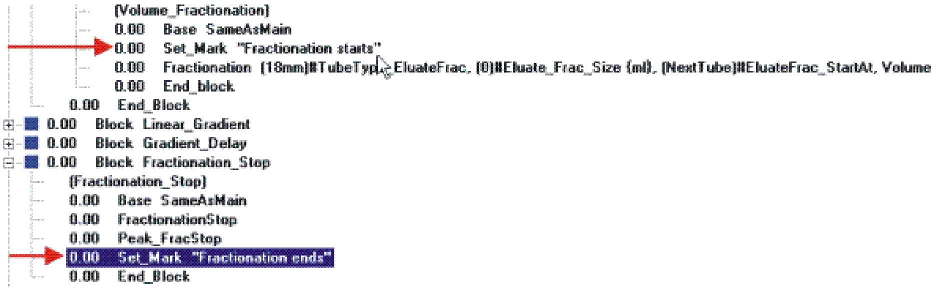
Set_Mark instructions are useful text messages. They can be used

- to insert manual notes, for example, when a problem occurs in a run
- to highlight certain stages in a method.

Set_Marks differ from **Messages** in that they are inserted into the chromatogram at set points as well as into the logbook during a method run.

Example of a Set_Mark

The illustration below shows an example where **Set_Marks** are used to highlight the start and end of fractionation in a method:



```
    (Volume_Fractionation)
    0.00   Base  SameAsMain
    0.00   Set_Mark  "Fractionation starts"
    0.00   Fractionation  (18mm)#TubeTyp EluateFrac, (0)#Eluate_Frac_Size (ml), (NextTube)#EluateFrac_StartAt, Volume
    0.00   End_block
  0.00   End_Block
0.00   Block  Linear_Gradient
0.00   Block  Gradient_Delay
0.00   Block  Fractionation_Stop
  (Fractionation_Stop)
  0.00   Base  SameAsMain
  0.00   FractionationStop
  0.00   Peak_FracStop
  0.00   Set_Mark  "Fractionation ends"
  0.00   End_Block
```

How to issue a Set_Mark

Set_Marks are issued from the **Instructions box** of the **Text Instructions** editor. The table below describes how to do this:

Step	Action
1	Select **Other:Set_Mark** in the **Instructions box**.
2	Type the message in the **Mark text** field.
3	Click the **Insert** button. *Result:* A new line with the **Set_Mark** is added to the text instruction.

6.6.5 How to delay a method

Introduction

A method can be programmed to be delayed at critical points. There are three instructions for this purpose: **Hold**, **Pause** and **Hold_Until**. These instructions are described below.

Hold

The **Hold** instruction suspends the execution of the method, but continues to pump eluent at the current flow rate and concentration settings. For example, this instruction is useful for giving the operator time to load a sample loop.

Resume the method

The method may be resumed if you click **Continue** on the **System Control** toolbar.

Pause

The **Pause** instruction suspends execution of the method and stops the pumps so that the system comes to a standstill. In ÄKTAdesign systems valves remain in the position they were in before the pause. The pause may be defined as indefinite or for a given number of minutes. This instruction is most useful for stopping the system in the event of an unexpected condition.

Resume the method

The method may be resumed if you click **Continue** on the **System Control** toolbar.

Hold_Until

The **Hold_Until** instruction is a special kind of **Watch** instruction. The method is put on hold until a specific condition is met (signal, test or value) or the time-out is reached. Thereafter the remaining instructions in the method are executed.

Instructions that share the same breakpoint as the **Hold_Until** instruction, but are placed after it in the method, will be executed after the **Hold_Until** conditions have been met.

6.6.6 Linear flow rates

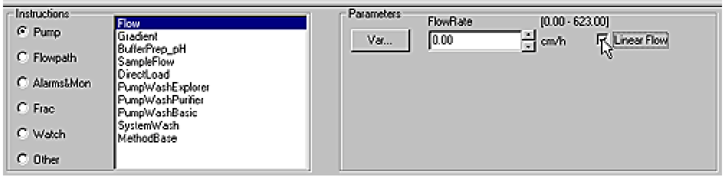
Introduction

Linear flow rates (cm/h) can be specified for **Flow** instructions. The volume flow rate is calculated from a specified linear flow rate and the column diameter as given in the column definition.

How to use linear flow rates

The table describes how to use linear flow rates.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select a specific column on the Variables tab of the Run Setup,
<i>or</i>
Insert a column for the Base instruction of the block in the Text Instructions Editor.</td></tr>
<tr><td>2</td><td>In the Instruction box of the Text Instructions editor, select Flow and select the Linear Flow option as shown in the illustration below:


</td></tr>
</table>

Note: If the column is changed, you will be asked if the linear flow rate or the default flow rate should be used. If the linear flow rate cannot be used due to the max flow rate of the system or new column, you will be advised that the max flow rate will be used instead.

6.6.7 *Gradients and eluent concentrations*

Introduction

Gradient instructions are given in the **Text Instructions** editor of the **Method Editor**. This type of instruction defines gradients and immediate changes in eluent concentration.

Parameters of the Gradient

The table below shows the two parameters of the **Gradient** instruction:

Parameter	Description
Target	Final eluent composition expressed in % eluent B.
Length	Duration of the gradient.

Example of a Gradient instruction

The starting point for the **Gradient** is always the current eluent composition. The instruction can be read as follows: “form a **Gradient** to reach **Target** after **Length**”.

Example of instruction

```
10.00 Gradient 50{%B}, 20{base}
```

The example instruction above forms a gradient to 50%B (**Target**) starting at breakpoint 10 with duration 20 method base units (**Length**). The example instruction will finish at breakpoint 30. If the current eluent concentration is greater than 50%, the gradient will be negative.

How to form a step gradient instruction

A step gradient is an immediate change in eluent composition. To form a step gradient, set the **Length** parameter to 0 in the **Gradient** instruction.

Example of instruction

```
10.00 Gradient 50{%B}, 0{base}
```

The example instruction above forms a step from the current eluent composition to 50%B at breakpoint 10. The method continues with 50%B.

Breakpoints for gradients

The breakpoint for a **Gradient** instruction defines the time or volume (according to method base) for the start of the gradient. A gradient with a non-zero duration occupies time and volume in the method, and breakpoints for other instructions may be set to occur before the gradient is completed. For most instructions, the instruction is simply carried out at the requested breakpoint, while the gradient is forming.

Instructions that affect gradients

The table below describes the instructions that affect the gradient:

Instruction	Effect
Gradient	A new gradient will start at the requested breakpoint. Any remaining duration of the previous gradient is ignored.
Flow	The eluent flow rate will change at the requested breakpoint. If the current base is volume or column volume, the duration of the gradient will be changed. If the method base is time, the volume of the gradient will be changed.
End_Method	The whole method will stop, interrupting the gradient.
End_Block	The gradient formation will continue uninterrupted unless a new **Gradient** instruction is issued in the next block. For example, this means that a block can be called conditionally during gradient formation without interrupting the gradient.

Gradients with variable length

For many purposes, it can be useful to define the length of the gradient as a variable. When this is done, breakpoints for instructions issued during or after the gradient in the same block are automatically shifted in proportion to the length of the gradient, with the same functionality as **Change** in the **Text Instructions** editor.

Instruction after a gradient

Any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length, since gradients function over time.

6.7 Standard Watch conditions

Introduction

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 eluates, 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.

Note: **Watch** instructions are shown in the **Instruction box** of the **Text Instructions** editor, indicated in the **Block** pane by a green line that shows the start and duration of the watch.

When is a Watch active?

The breakpoint when the **Watch** instruction is issued determines when the watch begins, not when the block is activated.

A watch is active from the point at which it is issued until

- the **Watch** condition is met

  or
- a new watch is set for the same monitor

  or
- a **Watch_Off** instruction is issued for the monitor.

How to insert a Watch instruction

Watch instructions are inserted in the **Instruction box** of the **Text Instructions Editor**. The table below describes how to do this.

Step	Action
1	In the **Breakpoint** field, select the appropriate breakpoint. This decides when the watch begins.
2	• Select **Watch** in the **Instructions** field. • Select a **Watch instruction** from the list. • Select appropriate values under **Test**, **Value** and **Action** in the **Parameters** field.
3	Click the **Insert** button. *Result:* The new **Watch** instruction is inserted on the list of actions in the **Text** window.

Test options in the Parameters field

The table below describes the **Watch** options that are available on the **Test** drop-down list of the **Parameters** field:

Option	Explanation
Greater_Than	The signal exceeds a certain value.
Less_Than	The signal falls below a specified value.
Slope_Greater_Than	The rate of change of the signal exceeds a specified value, expressed in monitor units/minute (for example, mAU/min).
Slope_Less_Than	The rate of change of the signal falls below a specified value, expressed in monitor units/minute (for example, mAU/min).
Less_Than_Or_Valley	The signal falls below a specified value or a valley is detected. A valley is detected only after a **Peak_Max** has been detected, and the valley is defined by a local minimum followed by an increase to 102% of the local minimum value plus the **Delta_Peak** value (see below).
Peak_Max	The signal falls to a specified fraction of the most recent peak maximum minus the **Delta_Peak** value. Factor=1 detects peak maximum.
Stable_Baseline	The signal is stable within the limits of the **Delta_Base** value for the period specified by the minutes parameter.

Note: For slope values, use the **Differentiate** function in the **Evaluation** module to measure the slope of the test chromatogram. The **Simulate Peak Fractionation** technique can also be used to find the slope values.

Watch conditions for air sensors and AuxIn

Two **Watch** conditions are available for systems with air sensors, although they may be handled differently depending on the system. The table below describes the conditions and their explanations:

Condition	Explanation
Equal 0	No air detected.
Equal 1	Air detected.

Note: To use the **Watch_AirSensor** instruction for air sensors, the **Alarm_AirSensor** setting must be disabled.

Actions when a Watch condition is met

The table below describes possible actions when a watch condition is met:

Instruction	Effect
Block name	Calls the named block.
Pause, **Hold**	Pauses or holds the method.
Continue	Continues the method if paused or held.
End_block	Ends the current block and return to the point from which the block was called.
End_method	Ends the method.
Ready	Indicates that the next step in a **MethodQueue** may start.

How to enter settings for Delta_Peak and Delta_Base

Permanent settings

Permanent settings for **Delta_Peak** and **Delta_Base** are entered with the **WatchPar** instruction (for example **WatchPar_UV**, **WatchPar_Cond**) under **System:Settings** in the **System Control** module (see the Administration and Technical Manual).

Temporary settings

Temporary settings that apply only for the duration of a given run can be entered in the Instructions field of the **Instruction box** in the **Text Instructions** editor. Select **Alarms&Mon** and then **WatchPar**.

The Delta_Peak setting

The **Delta_Peak** setting helps the software to detect valleys, peaks and peak maximum, and to ignore noise in the chromatogram.

The **Delta_Peak** value should be set

- large enough so that signal noise does not activate the conditions

  and

- small enough so that the condition is activated close to the valley or peak.

As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. If you set a too high value you can prevent a new peak from being detected after a local minimum.

Use of the Delta_Peak setting

The **Delta_Peak** setting

- sets the threshold for signal increase after a local minimum that will be interpreted as a valley for the **Less_Than_Or_Valley** condition. A valley and a new peak are detected when the signal increases to 102% of the local minimum plus the **Delta_Peak** value.

  Note: A valley is detected only after a **Peak_Max** has been detected.

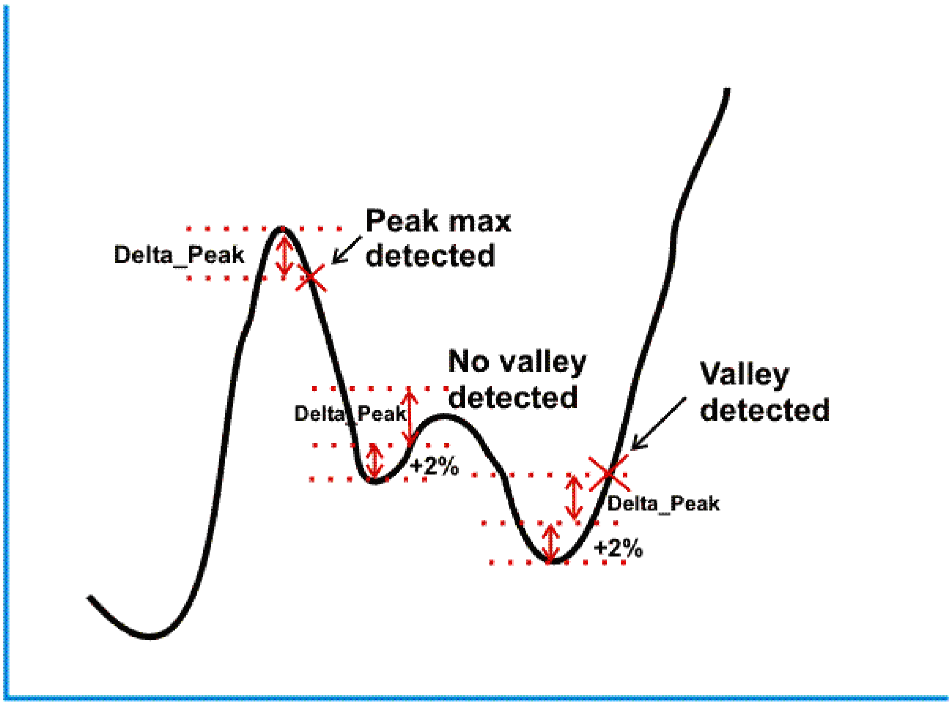
  Example:

  If there is a local minimum at 0.05 AU and a **Delta_Peak** of 0.01 AU, a valley will be detected at:

  `(1.02 x 0.05)+0.01=0.111 AU`

- sets the threshold for signal decrease after a local maximum that will activate the **Peak_Max** condition. **Peak_Max** is detected when the signal falls to the specified fraction of the most recent peak maximum minus the **Delta_Peak** value.

  The figure below illustrates the **Delta_Peak** setting where **Peak_Max** is detected when the signal falls by **Delta_Peak** from a local maximum if the **Peak_Max factor** is set to 1:



The Delta_Base setting

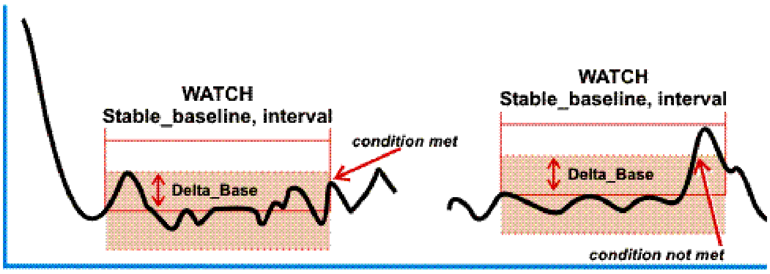
The **Delta_Base** setting helps the software to determine when the baseline is considered to be stable. In other words, it defines the permitted variation for the **Stable_Baseline** condition. For this condition to be activated, the signal may not vary by more than the **Delta_Base** value up or down over the time interval specified in the **Stable_Baseline** condition in the **Watch** instruction.

Note: The **Delta_Base** setting affects the **Stable_Baseline** condition only.

The condition Watch Stable_Baseline

The condition **Watch Stable_Baseline** is met if the signal does not deviate by more than **±Delta_Base** from the baseline during the time interval specified for the watch. The baseline value is determined by the signal at the start of the watch. If the condition is not met, a new interval is started with a new baseline value defined by the signal level at the start of the new interval.

The illustration below shows an example of this:



6.8 How to save or delete a method template

How to save a method as a template

You can save a method that you have created yourself as a template if you have **Edit global lists** authorization (see the Administration and Technical Manual).

Recommendation

The templates for each system are common for all users. Be restrictive in saving methods as templates. We recommend that only methods that are useful for all users be saved as templates.

The table below describes how to save a method as a template:

Step	Action
1	Choose **File:Save as Template** in the **Method Editor**. *Result:* The **Save as Template** dialog box is displayed.
2	Enter a name for the template in the **Name** field, *or* choose an existing template name from the **Templates** list that shows the available templates within the chosen system.
3	• Select the system for which the template is intended in the **For system** field. • Select the appropriate technique on the **Technique** list. • Click **OK**. *Result:* The method is saved as a template.

How to delete a template

The table below describes how to delete a template:

Step	Action
1	Choose **Edit:Delete template** in the **Method Editor**.
2	• Select the system and the template that you want to delete. • Click the **OK** button and the **Yes** button to confirm.

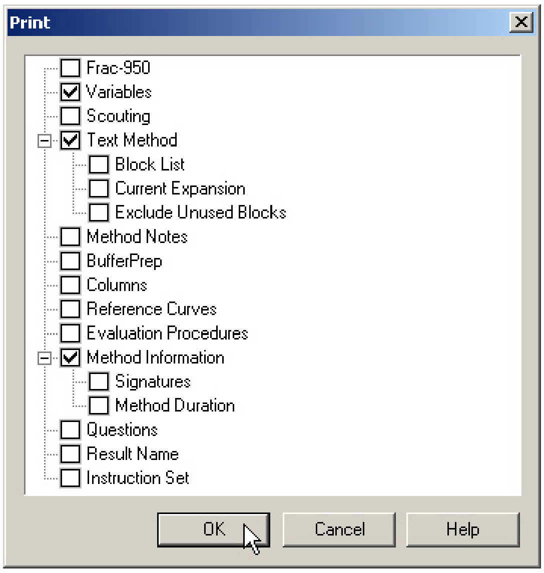
6.9 How to print a method

Instruction

You can print a copy of the method, including items from the method documentation, in **Run Setup** and the **Text Instructions** editor.

The table below describes how to print a method:

Step	Action

1	In the **Method Editor**, select **File:Print**
	or
	click the print icon.
	Result: The **Print** dialog box is displayed, showing the available items from the **Method Editor**.
	
	Print
	☐ Frac-950
	☑ Variables
	☐ Scouting
	☑ Text Method
	  ☐ Block List
	  ☐ Current Expansion
	  ☐ Exclude Unused Blocks
	☐ Method Notes
	☐ BufferPrep
	☐ Columns
	☐ Reference Curves
	☐ Evaluation Procedures
	☑ Method Information
	  ☐ Signatures
	  ☐ Method Duration
	☐ Questions
	☐ Result Name
	☐ Instruction Set

2	• Select the options you want to print. • Click **OK**. *Note:* For comments on the different alternatives, see "The Print dialog box" below.

The Print dialog box

The table below describes some of the check box options in the **Print** dialog box:

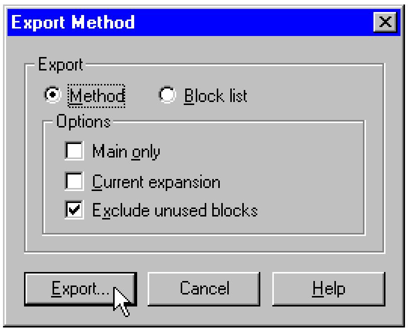
Check box	If you select this box...
Text Method	all instructions will be printed, including those in unused blocks.
Text Method:Current Expansion	the method will be printed according to the current expansion in the **Text** pane. (Only available from the **Text Instructions** editor.)
Exclude Unused Blocks	only blocks that are used in the method will be printed.
Text Method: Block List	only the main method and a list of the blocks that are used in the method will be printed.

6.10 How to export a method

Instruction

You can easily export a method to another file, and save it in another format, for instance .rtf. This is useful when you want to enable others to read the methods without having access to UNICORN on their computers.

The table below describes how to export a method and save it to another file:

Step	Action
1	In the **Text Instructions** editor or the **Run Setup**, select **File:Export:Method**. *Result:* The **Export Method** dialog box is displayed.  Export Method Export: ● Method ○ Block list Options: ☐ Main only ☐ Current expansion ☑ Exclude unused blocks
2	Do the following: • Select whether the current method should be exported as a **Method** or as a **Block list**. • Select the appropriate boxes in the **Options** field to define the level of detail in the information. • Click the **Export** button. *Result:* The **Export Method to file** dialog box is displayed.
3	• Enter a file name and select the target drive and folder. • Click the **Save** button.

7 Scouting

Introduction

Scouting is used to repeat a series of **Method runs** automatically with predetermined changes in the values for one or more **Variables**. A **Scouting Scheme** is defined as part of the method.

This chapter describes how to set up a **Scouting Scheme** and define columns. The chapter also provides some usage examples.

In this chapter

This chapter contains the following sections

Topic	See
How to set up a Scouting Scheme	7.1
How to define different columns for scouting	7.2

7.1 How to set up a Scouting Scheme

Introduction

This section describes how to set up a method for scouting.

Any parameter can be scouted, provided that it can be defined as a variable in the method.

When to use scouting

Scouting is a facility for automatically repeating a run with systematic variation of one or more parameters. Some typical situations where scouting is useful are when you want:

- to screen for the best column
- to find the optimal pH
- to test column capacity (sample volume)
- to find the optimal flow rate for binding and elution
- to optimize gradient length and slope
- to optimize step gradients.

Variable values

The variables that appear in the scouting scheme are usually a subset of those on the **Variables** tab of the **Run Setup**. The values in the scouting scheme can only be set on the **Scouting** tab, while the default values in the method can be set either on the **Variables** tab or in the **Text instruction** pane.

Changing variable values in the scouting scheme does *not* change the values on the **Variables** tab or in the text instructions. Values for variables selected for scouting are grey on the **Variables** tab and cannot be changed there.

Any changes that you make to variable values when a scouting scheme is run are saved in the result file. Results from a scouting run are saved in a scouting folder.

Scouting tab buttons

There are seven buttons on the **Scouting** tab of the **Run Setup** plus the **Help** button. The table describes the functions of these buttons:

Click the button...	if you want...
Define...	to define new scouting variables. The **Scouting Variables** dialog box is displayed, and you can select variables to be used in the scouting series. *Note:* The variables that have been selected for scouting *cannot* be changed on the **Variables** tab.

Click the button...	if you want...
Clear All	to clear all runs. This converts the scouting run to a non-scouting run so that it contains only the original method and values.
Delete	to remove a run from the **Scouting** tab. Click on any variable in the run you want to remove, and then click the **Delete** button.
Insert	to insert a new scouting run before an existing run. Click on a run column and then click the **Insert** button. The new run will inherit the variable values from • the preceding run, *or* • from the default values in the method if the run is inserted at the beginning of the scouting series
Add	• to add a scouting run if there are no runs previously in the scheme. Default values will be used for the first run. *or* • to add a scouting run after all other runs in the series. The new run inherits the values from the run that precedes the new run.
Series...	to set up a series of runs with differing inputs.
Edit Variable...	to rename or delete a variable, or change a variable into a detail variable.

How to set up or edit a Scouting Scheme

The table below describes how to set up or edit a scouting scheme.

Step	Action
1	Create a method. If you do not use a template or wizard, define appropriate variables in the method.
2	In the **Run Setup**, click the **Scouting** tab. *Result:* If no scouting variables have been previously defined, the **Scouting Variables** dialog box is displayed. If not, click the **Define** button.

Step	Action
3	Select the variables you want to scout. If you cannot find the variable you want, use the following options: • **Show details** to display variables created with the **Visible in details only** option. • **Show unused variables** to display all variables, including those that are not used in the method. Click **OK**. *Result:* The selected scouting variables will appear in a column, with default valued inserted.
4	Make any required changes in the scouting variable values.
5	To add a new **Run** column, click the **Add** button to copy the values from the last run column, and then change variable values as required.
6	Repeat steps 3 to 5 as required until you have defined all the scouting runs you need.
7	To exclude scouting runs from the default scouting scheme, right-click the heading of the run. To include the scouting run again, right-click it again.
8	Click the **Start Protocol** tab in **Run Setup**. **Select from the following options:** • The **Scouting** box: Select this to display the **Scouting** page at the start of a run. This allows the operator to adjust the values for scouting variables before the method run starts. • The **First run only** button: Select this to display the start protocol before the first run only. The settings entered in the **Start Protocol** for the first run will apply throughout the run, and the scouting series will be performed automatically without user intervention. • The **All runs** button: Select this to display the **Start Protocol** before each run in the scheme. This gives the operator an opportunity to change variable values or fill the sample loop before each run. *Note:* The operator must then click the **Start** button before each run.

How to set up series

The table below describes how to set up series.

Step	Action
1	Select a cell on the **Scouting** tab, and click the **Series** button. *Result:* The **Insert Series** dialog box is displayed.
2	In the **Insert Series** dialog box, type the selected series values (within the specified range limits), separated by commas, and click OK. *Result:* A new set of runs is inserted on the **Scouting** tab with the values provided.

How to delete or rename scouting variables

Scouting variables can be deleted or renamed in the scouting scheme in the same way as in the **Variables** tab. The table below describes how to delete or rename a variable in the **Scouting** tab.

Step	Action
1	Click the **Edit Variable...** button on the **Scouting** tab. *Result*: The **Edit Variables** dialog box opens. The variables are listed alphabetically.
2	Select the variable to edit.
3	**Rename** • Type a new variable name in the **New name** text box. • Click the **Rename** button. *Result*: The variable is renamed. **Delete** • Click the **Delete** button. • Confirm that you want to delete the variable. *Result*: The variable is deleted.

How to change a scouting variable into a detail variable

Detail variables are indicated with a **D** to the left of the **Variable** column on the **Scouting** tab. The table below describes how to set up a detail variable.

Step	Action
1	Click the **Edit Variable...** button on the **Scouting** tab. *Result*: The **Edit Variables** dialog box opens. The variables are listed alphabetically.
2	Choose the variable to be changed.
3	• Select the **Set visible in details only** checkbox. • Click the **Close** button. *Result*: The variable is indicated by a **D**. *Note*: De-select the checkbox to make the variable fully visible again.

How to copy contents to factorial design programs

The contents of the **Scouting** tab can be copied and pasted into a third-party factorial design program. Processed values can then be pasted back into the **Scouting** tab. The table below describes how to do this:

Step	Action
1	Select the text, etc., that you want to copy.
2	Press **Ctrl+C**.
3	Place the cursor where you want to insert the copied text.
4	Press Ctrl+V.

7.2 How to define different columns for scouting

Instruction

You can define different columns for use in the various scouting runs. However, in selecting a different column, other variables may also be changed between runs. The table describes how to scout columns.

Step	Action
1	Choose a method with a column (not **Any**). Alternatively, you can have a method with CV as the main base and a column (not **Any**) selected as a variable called "column".
2	Click the **Define** button on the **Scouting** tab. *Result:* The **Scouting Variables** dialog box is displayed.
3	Select **Column** and click **OK**.
4	Click the **Column** drop-down menu item within the desired run. *Result:* A menu is displayed.
5	Select a column. *Result:* The **Column Value Update** dialog box is displayed.
6	The dialog box asks you whether you want to update the instructions with column default values. Select one of the following: **Yes** The method for the scouting run is updated with variable parameter values for the selected column, consisting of UV average time, pressure limit, flow rate, etc. These parameter values are added to the scouting variables on the **Scouting** tab. Note that the updated parameter values may differ from the values for the same variables in other scouting runs. **No** No changes are made. The method retains the parameter values corresponding to the column that were either originally selected during creation of the method, or included in an earlier version of the method on the **Scouting** tab for which the default values for that column were accepted. *Note*: If the method contains a linear flow rate instruction, the user can keep the linear flow rate by selecting a check box in the dialog box.

8 MethodQueues

Introduction

MethodQueues provide a means for linking several methods together, on the same or different systems. For example, if a system wash procedure is programmed in a separate method, it can be linked in a **MethodQueue** to a series of different process methods, ensuring that the same wash procedure is used before every process. Alternatively, the product of a separation on one system might form the starting material for a separation on the next, allowing fully automated multi-step processing.

In this chapter

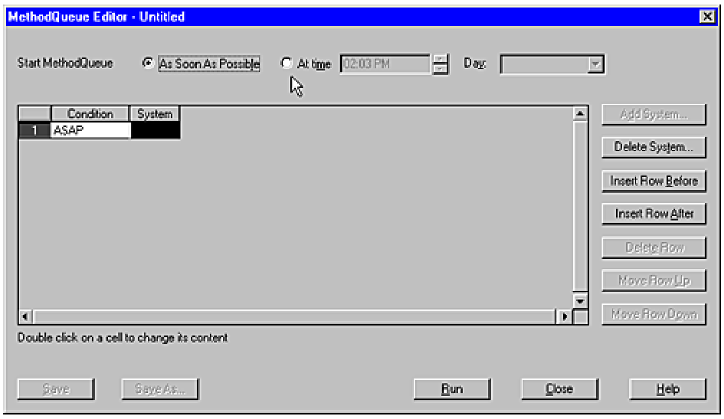
This chapter contains the following sections

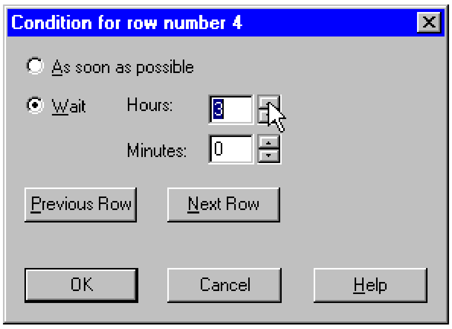
Topic	**See**
How to create a new MethodQueue	8.1
How to edit a MethodQueue	8.2

8.1 How to create a new MethodQueue

Instruction

The table below describes how to create a **MethodQueue** in the **UNICORN Manager** module.

Step	Action
1	- Select **File:New:MethodQueue**. *or* - Right-click in the **Methods** window and select **New:MethodQueue** on the shortcut menu. *or* - Click the **MethodQueue** icon. *Result:* The **MethodQueue Editor** dialog box is displayed.  MethodQueue Editor - Untitled Start MethodQueue ● As Soon As Possible ○ At time 02:03 PM Day: Condition: 1 ASAP Double click on a cell to change its content
2	The default selection for **Start MethodQueue** is **As soon as possible**. - Click the **At time** radio button and select a time and weekday for the start of the **MethodQueue**, if desired.
3	- Double-click the cell in the first row of the **System** column. *Result:* The **Method for row number 1 System** dialog box opens. *Note*: See "How to set up MethodQueues on several systems" below if you have more than one system available.
4	- Select a method and click **OK**. *Result:* The method is displayed in the **System** column.

Step	**Action**
5	• Click the **Insert Row After** button and repeat steps 3 and 4 to add more methods to the **MethodQueue**. *Note:* The timing of **MethodQueue** steps performed on different systems can also be controlled by the **Ready** instruction in the method (see "Relative timing of steps" below). By default, each method step will start as soon as possible (**ASAP**) after the completion of the previous method step. Use the **Condition** cell of the chosen method to set another time interval for starting a selected step. • In the **Conditions** column, double-click the cell for the method to be delayed. *Result*: The **Condition for row number X** dialog box opens.  Condition for row number 4 ○ As soon as possible ● Wait Hours: 3 Minutes: 0 *Note*: Use the **Previous Row** and **Next Row** buttons to select other methods for editing. • Click the **Wait** radio button, select the number of hours and minutes that the method is to be delayed and click **OK**. *Result*: The execution of the **MethodQueue** will be held for the selected number of hours and minutes and then resume. • Click the **Save** button to save the method. Result: The **Save MethodQueue** dialog box opens. • Type a file name and click the **Save** button.

How to set up MethodQueues on several systems

If you have more than one system available, the **System** column will not be displayed at first in the **MethodQueue Editor**. The table below describes how to set up a **MethodQueue** for several systems.

Step	Action
1	- Click the **MethodQueue** icon. - Click the **Add System** button and select a system for the first **MethodQueue** step from the **Add System** dialog box.
2	- Repeat this for each system when you want to use a different system in the **MethodQueue**. *Result*: Another system column will be added for each additional system.

Relative timing of steps

The setting of the **Condition** dialog box (reached by double-clicking a **Condition** cell in the **MethodQueue Editor** dialog box), determines the relative timing of the steps of a **MethodQueue**. If successive methods are run on the same system, the timing set in **Condition** applies from the completion of one method to the start of the next.

If successive methods are run on different systems, you can use the **Ready** instruction in one method to trigger the start of the next method. In this way, you will be able to start the next method before the current method has ended. The **Condition** setting then applies from the **Ready** instruction to the start of the triggered method. This is useful in situations where a method on one system prepares the starting material for the next, and then continues to wash the system. See the example below:

Instruction to System 1	Instruction to System 2
Apply sample	
Eluate	
READY	**Apply sample**
Wash	**Eluate**

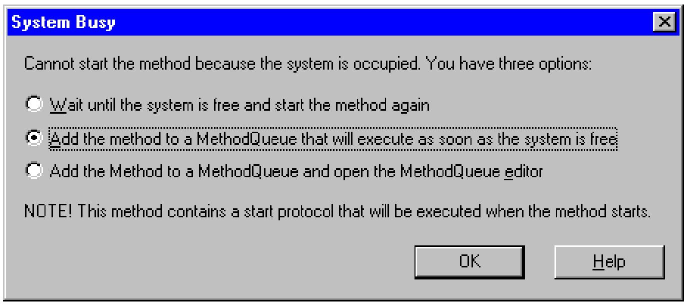
Unattended operation of the MethodQueue

The **Start Protocol** for each method step in the **MethodQueue** is displayed when the corresponding method is run. If you want the **MethodQueue** to operate unattended you must ensure that the methods do not include a **Start Protocol**.

See **5 How to create a method** on page 83 for more information.

How to hold a method in queue while the system is busy

The table below describes how you can create a **MethodQueue** if you try to start a new method run while the system is still busy with another method run.

Step	Action
1	Right-click on the method in the **UNICORN Manager** module and select **Run:***system name* on the shortcut menu. *Result*: The **System Busy** dialog box opens.  System Busy Cannot start the method because the system is occupied. You have three options: ○ Wait until the system is free and start the method again ● Add the method to a MethodQueue that will execute as soon as the system is free ○ Add the Method to a MethodQueue and open the MethodQueue editor NOTE! This method contains a start protocol that will be executed when the method starts.
2	- Select the **Add the method to a MethodQueue that will execute as soon as the system is free** option. - Click **OK**. *Result*: A **MethodQueue** will automatically be created in the default queue folder. The name of the **MethodQueue** will be the same as the method name, followed by a five-digit sequence number.
3	The method will be executed as soon as the system is free. *Note*: A warning note is displayed in the **System Busy** dialog box if the method includes a **Start Protocol**. The **Start Protocol** must be completed at the start of the method run before it can be executed.

8.2 How to edit a MethodQueue

Method Queues are saved in a separate folder

MethodQueues are saved in a separate folder within the folder that you specified when you saved the **MethodQueue**. The **MethodQueue** folder is represented by a special icon in the **Methods** window of the **UNICORN Manager**.

A **MethodQueue** folder contains the **MethodQueue** definition and copies of all included methods.

How to edit a MethodQueue file

The **MethodQueue** files are *copies* of the original method files. If changes are made in the original method, these will not affect the method in the **MethodQueue**.

To avoid confusion between different versions of method files, make sure that **MethodQueue** definitions always contain updated methods. To implement changes in a **MethodQueue** method, do one of the following:

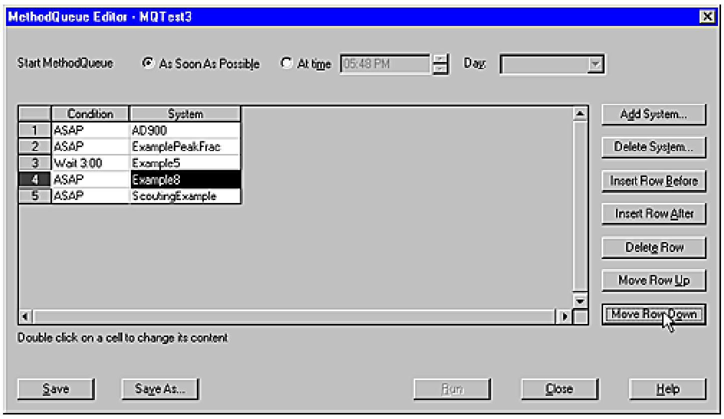
- Edit the method in the **MethodQueue** folder,

  or

- Edit the original method, then use the **MethodQueue** editor to update the **MethodQueue**, and replace the old method with the changed version.

Instruction

The table below describes how to edit an existing **MethodQueue**.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Right-click the selected MethodQueue folder icon in the UNICORN Manager, and select Edit from the displayed menu.
<i>Result:</i> The MethodQueue Editor dialog box is displayed.

MethodQueue Editor - MQTest3
Start MethodQueue ● As Soon As Possible ○ At time 05:48 PM Day:
<table>
<tr><th></th><th>Condition</th><th>System</th></tr>
<tr><td>1</td><td>ASAP</td><td>AD900</td></tr>
<tr><td>2</td><td>ASAP</td><td>ExamplePeakFrac</td></tr>
<tr><td>3</td><td>Wait 3:00</td><td>Example5</td></tr>
<tr><td>4</td><td>ASAP</td><td>Example8</td></tr>
<tr><td>5</td><td>ASAP</td><td>ScoutingExample</td></tr>
</table>
Double click on a cell to change its content
</td></tr>
</table>

Step	Action
2	Select a table row to edit and do the following as required: • Double-click the **System** cell and select a new method from the **Method for row...** dialog box. • Double-click the **Condition** cell and edit the delay time for the method. • Click the **Add System** button to add a new system to the queue and use it for a **MethodQueue** step. • Click the **Delete System** button to remove a system and all associated methods from the **MethodQueue**. • Click the **Insert Row Before** or **Insert Row After** buttons to add new rows before or after the selected row. • Click the **Delete Row** button to remove the selected row. • Click the **Move Row Up** or **Move Row Down** to move the selected row one step up or down in the queue.
3	• Click the **Save** button. • Click the **Run** button to execute the **MethodQueue** immediately or the **Close** button to close the dialog box.

9 How to perform method runs

Introduction

This chapter describes how to perform and monitor different kinds of method runs from the **System Control** module. It also describes how to control the system with manual commands and instructions.

In this chapter

This chapter contains the following sections

9.1 How to start a method run

Before you start

Before you start a method, make sure that

- the correct system is connected in control mode

Note: If the system is connected via a **CU-950 Advanced** unit, the Ethernet connection must not be broken during the start-up phase of the method run.

How to start from the UNICORN Manager

You can start a method from the **UNICORN Manager** in two ways:

- Select a method in the **Methods** window and select **File:Run**.
- Select a method, right-click and select **Run** from the displayed menu.

How to start from System Control

The table below describes how to start a method run from **System Control**:

Step	Action
1	Select **File:Run** *or* click the **Run** button. *Result:* The **Run** dialog box is displayed. *Note*: The **Run** button will open the method that was used for the previous run, if a run has been performed since you logged on.
2	Select a method and double-click the method icon. *Result*: The method run starts. If the method includes a **Start Protocol** this must be completed before the actual method run starts. Se further instructions below.

How to add methods to the File menu

For methods that are used frequently (for example column cleaning methods or routine separations), it may be convenient to define the methods as commands in the **File** menu.

The table below describes how to define a method as a command:

Step	Action
1	Choose **File:Menu** in **System Control** and select the required method.
2	Click the **Add** button and click **OK**. *Result:* The method name will appear as a command in the **File** menu. If you choose the command, the method will start.

How to start an instant run

You can start a method template or wizard directly if your system has defined templates or wizards.

To do this, either

- click the **Instant Run** icon in the **UNICORN Manager** toolbar

or

- select **File:Instant Run** in **System Control**.

How to use the Start Protocol

If the method is defined with a **Start Protocol**, this will be displayed before the method actually starts.

The table below describes how to use the **Start Protocol**:

Step	Action
1	• Start the method run. • Work through the start protocol, answering questions as required. The start protocol items that can be displayed are described in **6.5.14 The start protocol tab** on page 157. • As each screen is completed, click the **Next** button to move to the next screen or the **Back** button to return to the previous screen.
2	Click the **Start** button in the last window to start the run.

Confirm/Sign authorization for the Start Protocol

If there are any questions in the **Start Protocol** that require authorized confirmation, you will be asked for a user name and password when you attempt to leave the screen containing the questions. Only users with **Confirm/Sign** authorization may authorize answers to such questions. Each question that requires an authorization must have a separate authorization.

How to start a method when the system is busy

If the system is busy with a method run in progress, you can still start a new method. You will have the option to place the method in a **MethodQueue**, which can be executed as soon as the system becomes available again. The table below describes how to do this.

Step	Action
1	• While a method run is in progress, right-click on the next method you want to run and select **Run:System**. *Result*: The **System Busy** dialog box opens.
2	• Select the **Add the method to a MethodQueue that will execute as soon as the system is free** option. • Click **OK**. *Result*: A **MethodQueue** will automatically be created in the default queue folder. The name of the **MethodQueue** will be the same as the method name, followed by a five-digit sequence number.
3	The method will be executed as soon as the system is free. *Note*: A warning note is displayed in the **System Busy** dialog box if the method includes a **Start Protocol**. The **Start Protocol** must be completed at the start of the method run before it can be executed.

Note: See **8.2 How to edit a MethodQueue** on page 194 for more information.

9.2 How to monitor a method run

Introduction

This section describes how to monitor a method run by using the **System Control** module and how to customize the different panes.

In this section

This section contains the following sub-sections

Topic	See
How to customize System Control panes	9.2.1
The Run Data pane	9.2.2
The Curves pane	9.2.3
The Flow Scheme pane	9.2.4
The Logbook pane	9.2.5

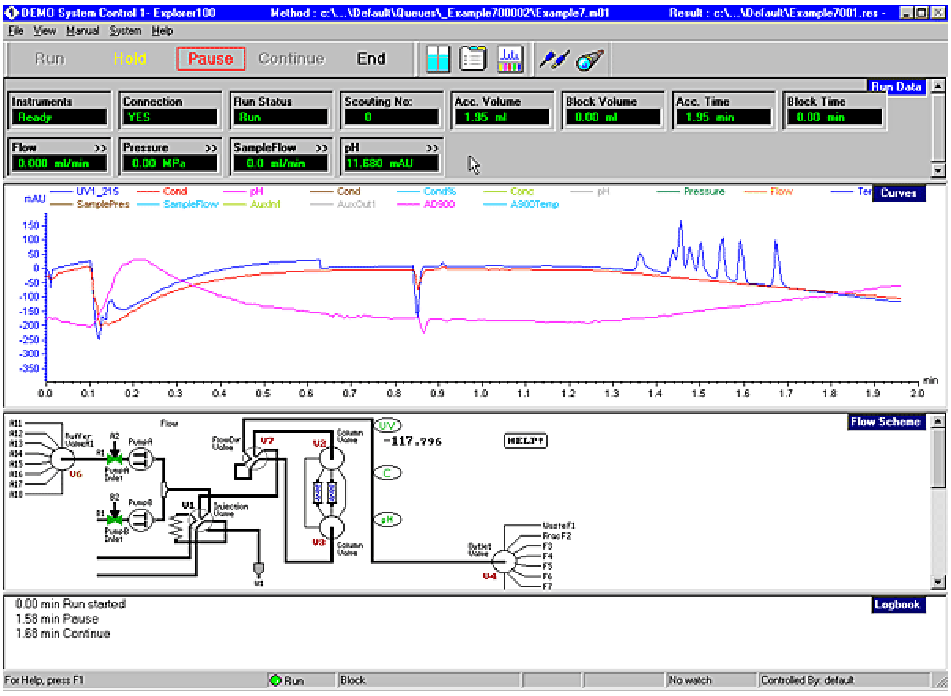
9.2.1 How to customize System Control panes

Introduction

The **System Control** module displays the status of the current system. On the Windows taskbar, there may be up to four **System Control** modules available that can be connected to different systems. Separate systems may be controlled and displayed independently of each other.

Illustration

The illustration shows the **System Control** module with the **Run Data**, **Curves**, **Flow scheme** and **Logbook** panes displayed.


DEMO System Control 1 - Explorer100 Method : c:\...\Default\Queues_Example700002\Example7.m01 Result : c:\...\Default\Example7001.res -

Instruments: Ready
Connection: YES
Run Status: Run
Scouting No: 0
Acc. Volume: 1.95 ml
Block Volume: 0.00 ml
Acc. Time: 1.95 min
Block Time: 0.00 min
Flow: 0.000 ml/min
Pressure: 0.00 MPa
SampleFlow: 0.0 ml/min
pH: 11.680 mAU

0.00 min Run started
1.58 min Pause
1.68 min Continue

How to select what panes to display

Each **System Control** module displays up to four panes for monitoring different aspects of the run. To select what panes to display, either

- click the **Customize Panes** icon,

or

- choose **View:Panes**.

How to customize System Control panes

Change the size

Select a split-bar and drag up and down to change the size of a specific pane.

Maximize, restore or hide

Right-click a pane and select the appropriate option to:

- maximize,
- restore

  or
- hide the pane.

9.2.2 The Run Data pane

Description

The **Run Data** pane displays the current values for selected run parameters. The update interval is defined in the system strategy.

The figure below displays an example of the **Run Data** pane:

Instruments	Connection	Run Status	Acc. Volume	Acc. Time	Pressure	pH
Ready	YES	Run	0.98 ml	0.99 min	0.00 MPa	11.270 mAU

How to change the appearance of the pane

The appearance of the pane can be changed so that it includes more or fewer data displays. The table below describes how this is done:

Step	Action
1	In **System Control**, select **View:Properties** *or* right-click on the pane and select **Properties** on the menu. *Result:* The **Properties** dialog box is displayed.
2	Select the **Run Data Groups** tab and, if desirable, do one or more of the following: • *Select* an available group to be displayed in the list to the left. • *Edit* an available group: Select the group from the list on the left, and click the **Edit Group** button. Modify the included readings in the list to the right, and click **OK**. • *Create* a new group: Click the **New group** button and select the readings that you want to view from the list. Enter a name for the group, and click **OK**. • *Delete* a group: Click the **Delete Group** button and select a group in the **Delete Layout** dialog box, click **OK** and confirm the deletion.
3	Select the run data parameters that you want to display in the list to the right.
4	Click **OK** to view the selected items in the **Run Data** pane. The name of the selected layout replaces the default layout name **Run Data**.

How to change text color or text background

The table describes how to change the text color or background in the displayed reading boxes.

Step	Action
1	Right-click on the pane and select Properties. *Result:* The **Properties** dialog box is displayed.
2	Select the **Run Data Color** tab.
3	Click the **Text** or **Background buttons.** Select a new color, and click **OK**. *Result:* The color change is displayed in the test field.
4	Make further adjustments to the colors as appropriate.
5	Click **OK** to apply the changes.

How to set the pressure units

If the **Pressure** reading box is displayed in the **Run Data** pane, you can set the displayed units. The table below describes how this is done:

Step	Action
1	Right-click on the **Pressure** reading box to display the menu.
2	Select **Set Unit** and the appropriate unit (**MPa**, **bar** or **psi**). *Result:* The selected unit is displayed.

How to view and select manual instructions

Some strategies directly link specific manual instructions to the reading boxes in the **Run Data** pane. This is indicated by a double arrow (**>>**). A particular reading box can have one or more instructions attached to it. In cases where there is more than one instruction, one of the instructions is the main instruction.

There are two ways to view the manual instructions:

Option 1:

- Double-click the reading box.

  Result: The dialog box for manual instructions is displayed, showing the instruction, or main instruction if there is more than one.

Option 2:

- Right-click the reading box. Select **Instructions** in the displayed menu. Another menu shows the specific manual instruction(s).
- Click an instruction to select it.

Result: The dialog box for manual instructions is displayed in which you can execute the appropriate command.

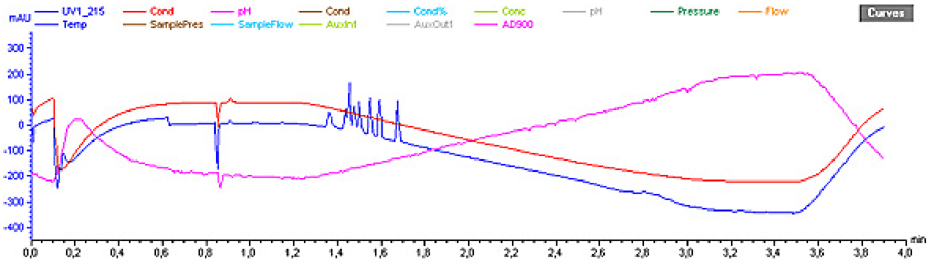
For more details on how to use manual instructions, please see **9.3.2 Manual instructions** on page 220.

9.2.3 *The Curves pane*

Introduction

The **Curves** pane of the **System Control** module displays monitor signal values graphically.

The figure below shows an example of the **Curves** pane:



How to select curves to be displayed

You can decide which curves you want to display in the **Curves** pane. Curves will only be shown for components present in the chromatography system.

The table describes how to select the curves to be displayed on the screen.

Step	Action
1	In **System Control**, select **View:Properties**. *Result:* The **Properties** dialog box is displayed.
2	Select the **Curves** tab. *Note:* The curves in the list are those for which **Store** is set to **On** in the system settings, together with any reference curves defined in the method.
3	In the **Display curves** list, select the curves you want to display. If you want all curves to be displayed, click the **Select All** button. If you do not want any curves to be displayed, click the **Clear All** button. Click **OK**.

How to display a vertical marker line

The table below describes how to display a vertical marker line:

Step	Action
1	Right-click the **Curves** pane and select **Marker**.
2	Drag the marker line with the mouse. *Result:* Where the line bisects the curve, the X-axis and Y-axis values are displayed at the top right corner of the pane.

Note: Right-click and select **Snapshot** to record the marker position values. See **2.2.7 Snapshots** on page 41 for more information about the **Snapshot** function.

How to set a reference point

When the vertical marker is displayed, you can set a reference point to display curve data. The table describes how to set a reference point:

Step	Action
1	• Display a **Marker** in the **Curves** pane. • Right-click 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 change the curve colors and styles

The **Curves** pane displays graphs for the selected curves in different colors, with any reference curves included with the method as dashed lines.

The table below describes how to change the curve colors and styles:

Step	Action
1	Select **View:Properties**. *Result:* The **Properties** dialog box is displayed.
2	Select the **Curve Style and Color** tab.
3	• Select a curve from the **Curve** list. • Select an appropriate color and style.

How to change the scale of the Y-axis

In most cases, the Y-axis is automatically scaled for each of the curves. Values on the Y-axis apply to the curve with the same color as the axis markings. To get the correct Y-axis, click the legend. The table below describes how to fix the scale of individual curves.

• p 207

Step	Action
1	• Select **View:Properties**. *Result:* The **Properties** dialog box is displayed. • Select the **Y-axis** tab.
2	• Select the appropriate curve. • Select **Fixed** and type a minimum and maximum range in the fields within the specified limits.
3	Repeat step 2 for other curves if needed.
4	Click **OK**.

How to change the scale of the X-axis

The table below describes how to change the scale of the X-axis:

Step	Action
1	• Select **View:Properties**. *Result:* The **Properties** dialog box is displayed. • Select the **X-axis** tab.
2	Select the appropriate base, **Time** or **Volume**. *Note:* Curves are collected in time and recalculated for display in volume. Thus, the resolution of the two bases may appear slightly different.
3	Select the appropriate **Axis scale**: • **Total** will show the curves as far as they have come in the run. • **Window** allows you to set the portion of the total pane to be displayed, either in minutes or ml depending on the selected base. • **Adjust retention zero to injection** sets the retention value to zero at the point of the first injection. • Click **OK**.

How to switch between time and volume units

- Click the legend of the X-axis

or

- right-click and select **Base Type**

to switch the display between time and volume units. The run is controlled according to the time/volume base defined in the current block, regardless of the base in the curves display.

How to zoom in the Curves pane

The table below describes how to zoom in on a selected region of the curve pane:

Step	Action
1	• Press and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed. • Release the mouse button. *Result:* The display is now zoomed in on the selected area.
2	Repeat the process for further magnification of selected areas.

How to zoom out

To reduce the scale of the zoom, right-click in the **Curves** pane, and select one of the following options:

- **Undo Zoom**: reverses each zoom-in action a step at a time.
- **Reset Zoom**: reverses all zoom-in actions to the default scale.

How to select curve pressure units

If the **Pressure** curve is displayed in the **Curves** pane, you can set the displayed units. The table below describes how to do this:

Step	Action
1	Right-click in the **Curves** pane, and select **Properties** in the displayed menu. *Result:* The **Properties** dialog box is displayed.
2	Select the **Y-Axis** tab.
3	Select the **Pressure** curve and select the appropriate **Pressure unit** button. Click **OK**.

How to edit text in the Curves pane

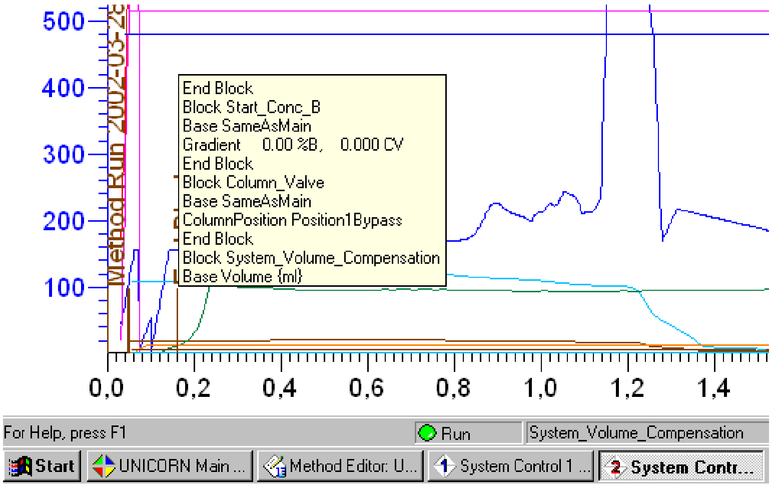
You can select the way that text is aligned for the **Logbook** and **Fraction** curves. You can also select to show only part of the **Logbook** information. The table below describes how to do this:

Step	Action
1	Right-click in the **Curves** pane, and select **Properties** in the displayed menu. *Result:* The **Properties** dialog box is displayed.
2	Select the **Curve Style and Color** tab.
3	Select the following: • **Logbook** or **Fraction** curve in the **Curve** list as appropriate. • Select the appropriate **Logbook text alignment** or **Fraction text alignment** option: - Horizontal - Vertical - Fly over (displays the text if you place the mouse pointer over the generated mark).
4	To filter the type of **Logbook** information overlaid on the **Curves** pane, do the following: • Click the **Filter** button. *Result:* The **Filter Logbook** dialog box is displayed. • Select the appropriate check boxes and set the maximum block depth. • Click **OK** to return to the **Curve style and Color** tab.
5	Click **OK**.

How to view the complete logbook information

At some breakpoints there can be more logbook information than what is possible to conveniently display in the **Curves** pane. The additional information that is not displayed is indicated by an arrow point symbol by the break point.

- Hold the mouse cursor over the break point to display the complete information in a flyover text box, as shown in the illustration below.



End Block
Block Start_Conc_B
Base SameAsMain
Gradient 0.00 %B, 0.000 CV
End Block
Block Column_Valve
Base SameAsMain
ColumnPosition Position1Bypass
End Block
Block System_Volume_Compensation
Base Volume {ml}

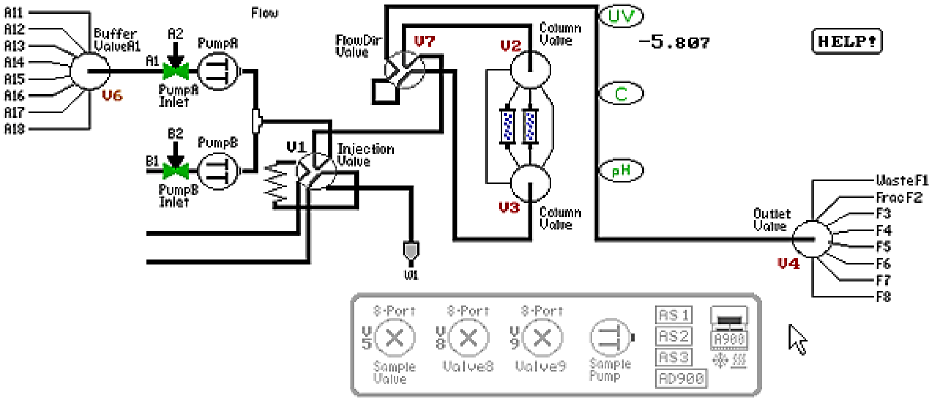
9.2.4 *The Flow Scheme pane*

Introduction

The flow scheme is a graphical representation of the chromatography system that shows the current status of the run. During a run, the flow scheme displays open flow path(s) in color and monitor signals with numerical displays.

Illustration

The illustration below shows an example of a flow scheme for a run:



How to stretch a flow scheme

The flow scheme can be stretched to fit the screen. To do this, right-click in the pane and select **Stretch** in the shortcut menu.

How to view and select flow scheme manual instructions

Some strategies link specific manual instructions directly to the components in the flow scheme pane. The components in the flow scheme that are associated with instructions are indicated with double arrows (**>>**). A particular component can have one or more instructions attached to it. In cases where there is more than one instruction, one of the instructions is the main instruction.

To display and select instructions:

- double-click a component

or

- right-click a component, select **Instructions** and an instruction in the shortcut menu.

Result: The manual instructions dialog box for the selected instruction type opens.

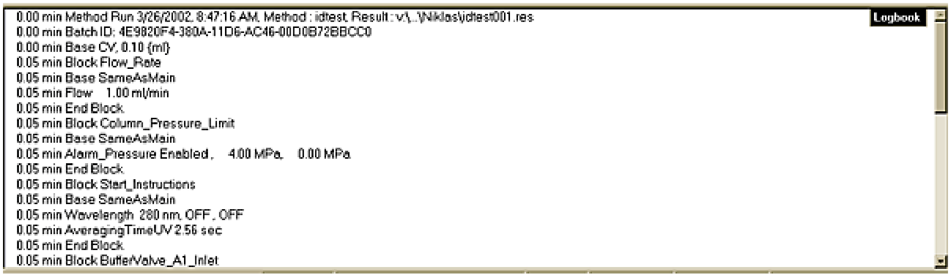
9.2.5 *The Logbook pane*

Introduction

All actions (including method start and end, base instruction, method instructions and manual interventions such as **Pause** or **Hold**) and unexpected conditions such as warnings and alarms are logged for every run, with date, time and current user name where appropriate. The logbook thus provides a complete history of any given run. The log is saved in the result file.

Illustration

The illustration below shows an example of the **Logbook** pane:



```
0.00 min Method Run 3/26/2002, 8:47:16 AM, Method : idtest, Result : v:\...\Niklas\idtest001.res
0.00 min Batch ID: 4E9820F4-380A-11D6-AC46-00D0B72BBCC0
0.00 min Base CV, 0.10 {ml}
0.05 min Block Flow_Rate
0.05 min Base SameAsMain
0.05 min Flow    1.00 ml/min
0.05 min End Block
0.05 min Block Column_Pressure_Limit
0.05 min Base SameAsMain
0.05 min Alarm_Pressure Enabled ,    4.00 MPa,    0.00 MPa
0.05 min End Block
0.05 min Block Start_Instructions
0.05 min Base SameAsMain
0.05 min Wavelength  280 nm, OFF , OFF
0.05 min AveragingTimeUV 2.56 sec
0.05 min End Block
0.05 min Block BufferValve_A1_Inlet
```

Note: The second logbook line is the **BatchID** that is automatically generated.

Autoscroll

The **Logbook** pane can autoscroll to display the latest entries. Right-click in the pane, and select **Autoscroll**. You can also select the **Autoscroll** option in the **Properties** dialog box (**View: Properties** and select the **Logbook** tab).

How to filter the logbook contents

You can choose to display only selected items in the logbook. The table below describes how to activate the filter.

Step	Action
1	• Right-click in the **Logbook** pane and choose **Properties**. *Result*: The **Properties** dialog box opens.
2	• Choose the **Logbook** tab. • Select the items you want to display in the logbook (all items are selected by default). • Click the **OK** button. *Result*: Only the selected items will be displayed in the logbook. The **Logbook** title in the upper right corner will show the text **(Filter on)** to indicate that not all items are visible. All items will still be logged in the result file.

How to find logbook text entries

The logbook can be searched for specific text entries. The table below describes the function:

Step	Action
1	Right-click in the **Logbook** pane and choose **Find**. *Result*: The **Find** dialog box opens.
2	• Type the text you want to locate. • Select search criteria if necessary. • Click **OK**. *Result*: The located logbook entry is highlighted.

9.3 Manual system control

Introduction

This section describes how to control the system with manual commands and instructions.

In this section

This section contains the following sub-sections

Topic	**See**
The toolbar and status bar	9.3.1
Manual instructions	9.3.2
Alarms and warnings	9.3.3

9.3.1 *The toolbar and status bar*

Toolbar buttons

The toolbar at the top of the **System Control** module contains three sets of buttons:

- **Manual Direct Commands** buttons for starting and stopping the run
- **Windows** buttons to access dialog boxes for pane selection, documentation and layout properties
- **System Access** buttons to control the system connection.

Show and hide

The toolbars can be shown and hidden by choosing **View:Toolbars** and selecting the relevant boxes.

The figure below shows the toolbar:

Manual Direct Commands

The available **Manual Direct commands** buttons in **System Control** are dependent on the control status of the connection. The table below shows when each button is available:

Control Status	Available buttons
End	**Run**
Running	**Hold**, **Pause**, **End**
Manual	**Run**, **Pause**, **End**
Hold	**Pause**, **Continue**, **End**
Method pause	**Hold**, **Continue**, **End**
Manual pause	**Run**, **Continue**, **End**

Direct command button functions

The table below describes the functions of the **Manual direct command** buttons.

Button	Function
Run	Opens the **Run** dialog box, which shows all available methods, as the first step in a method run. If a method is loaded, **Run Setup** opens. The run will start immediately if a start protocol isn't part of the method.

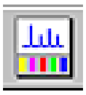
Button	**Function**
Hold	Suspends execution of a method, but continues • to pump liquid at the current flow rate and eluent concentration settings. All settings remain unchanged. • to increase accumulated time and volume. Method instructions are not executed until the **Continue** button is pressed.
Pause	Behavior of the **Pause** button is strategy-dependent. The **Pause** button suspends execution of a method and stops all pumps so that the system comes to a standstill. For ÄKTAdesign systems, valves remain in the position they were in before the pause. Accumulated time and volume is not increased during a **Pause**. Method instructions are not executed until **Continue** is pressed.
Continue	Resumes execution of a paused or held method.
End	• Terminates method execution • Puts the system into an **End** state. *Note*: You can choose to save the partial result or discard it.

Note: The commands can also be found on the **Manual** menu.

Windows buttons

The table below describes the functions of the **Windows** buttons:

Button	**Function**
◫	Opens a dialog box where you can choose which window panes to display. This button is equivalent to the menu command **View:Panes**.

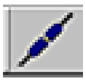
Button	Function
	Opens the documentation pages. Run notes can be entered on the **Notes** tab and settings can be changed. Other tabs are displayed for information only. This button is equivalent to the menu command **View:Documentation**.
	Opens the properties pages. This button is equivalent to the menu command **View:Properties**.

System Access buttons

There are two functions of the **System Access** buttons:

Disconnect/Connect system

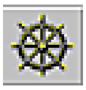
The **Disconnect** button is used to disconnect the system and leave it in a locked or unlocked state.



The **Connect** button connects the system.

Leave/Take control of the system

The **Leave control** button leaves the system in a locked or unlocked state.



The **Take control** button takes control of the system.

Status bar, connection status

The status bar displays a message indicating the connection status of the window. The table below describes the different messages:

Message	Connection status
Controlled by: <user>	The indicated user has a control mode connection to the system. Other users can establish a view mode connection.

Message	Connection status
Locked by:<user>	The indicated user has left the system in a locked state. Users who can supply the required password can unlock the system and establish a connection. The password is case sensitive. *Note:* It is possible to unlock with the "lock" password or with the UNICORN logon password. Anyone who uses the UNICORN logon password must have **Unlock systems** access rights. The "lock" password is the password entered by the user who locked the system.
System is available	Any user can establish a connection.

Status bar, Watch status

The status bar displays a message indicating if a **Watch** is active in the method.

- Click the **Active watch** status message to open the **Watch** dialog box with information about the active **Watch** instruction.

9.3.2 *Manual instructions*

Introduction

The chromatography system can be controlled with manual instructions issued from the **Manual** menu in the **System Control** module. The available instruction options are dependent on the strategy.

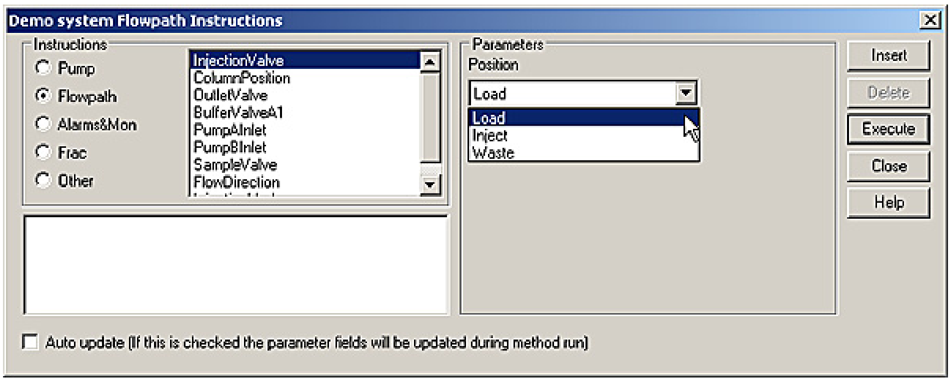
Manual instructions during a method run

Manual instructions can be issued while a method is running. A manual setting applies until the next method instruction of the same type is executed

Example: A manual **Flow** instruction will set the flow rate until the next **Flow** instruction in the method is executed. Manual instructions that you issue during a method are recorded in the logbook for the method run.

The manual instructions dialog box

The **Manual** menu in **System Control** opens a dialog box similar to the **Instruction box** in the **Method Editor**. The name of the connected system is displayed on the title bar of the dialog box. See an example in the illustration below:



☐ Auto update (If this is checked the parameter fields will be updated during method run)

Note: The parameter values will be updated continually during the run if the **Auto update** checkbox is selected.

Column protection

Your user attributes may include a requirement to always set pressure alarms.

Step	Action
1	When you try to execute a pump instruction the **Column protect mode** dialog box opens.
2	• Click the **Yes** button in the dialog box to select a column and retrieve the correct maximum pressure value. • Click **OK** to close the column list. • Click the **Insert** button to add the **Alarm_Pressure** instruction.

Step	Action
3	• If necessary, repeat step 2 to add an **Alarm_SamplePressure** instruction.

How to use manual instructions

Manual instructions are entered in the same way as method instructions from the dialog box in the **Method Editor**. The table below describes how to add a manual instruction:

Step	Action
1	• Select an instruction group and a component in the **Instructions** field. • Select instruction parameters in the **Parameters** field.
2	• Click the **Insert** or **Execute** buttons as needed. (See the descriptions of the different functions below)

The buttons of the manual instructions dialog box

The table below describes the functions of the manual instructions buttons:

Button	Function
Insert	This button places the current instruction in the list at the bottom left of the dialog box.
Delete	This button deletes the selected instruction from the current list only. One instruction can be deleted at a time.
Execute	This button • executes all instructions in the list at the same time *or* • executes the currently marked instruction if the list is empty. *Note:* Although all instructions are executed simultaneously, some (for example gradient and fraction instructions) may take some time to complete in the liquid handling module.

Button	Function
Close	If you click the **Close** button without first clicking the **Execute** button, commands in the list • will not be executed • will be deleted from the command list.

How to save manual results

When you choose to run the system manually - as opposed to a **Method run** - the results are automatically stored in a folder called **Manual Runs**. The **Manual Runs** folder stores the ten most recent results from your manual runs. To save a result file from the **Manual Runs** folder more permanently, you need to move or copy it to another location.

An alternative way to save the results from a manual run is to record the results manually in a result file. The table below shows how to do this:

Step	Action
1	• Choose **Manual:Other**. • Select the instruction **Record On** at the beginning of the run.
2	• Click the **Execute** button. *Result*: UNICORN will prompt for a result file name.

9.3.3 Alarms and warnings

Introduction

Alarms and warnings are displayed regardless of the activity currently in progress in UNICORN. You will be notified of an exceeded limit in a running system even if you are developing a method, evaluating data or monitoring a method run on a different system. Warnings and alarms are also recorded in the logbook for the run.

Limits for monitor signals

The system settings determine the acceptable limits of monitor signals during a run. The limits can also be set for the current run by an instruction in the method. Limits set with a method instruction override the limits set in system settings. If these limits are exceeded in a run, a warning or alarm dialog box is displayed on the screen.

Effects of alarms and warnings

Alarms and warnings have different effects on the system:

- *Warning*: The run continues.
- *Alarm*: The system is paused.

In a network system

In a network installation, alarms and warnings are displayed on the controlling station and all stations viewing the system. An alarm can be acknowledged only from the computer connected in control mode. Alarms are displayed but cannot be acknowledged on computers connected in view mode.

9.4 How to perform a scouting run

More information on scouting runs

See **7.1 How to set up a scouting scheme** on page 183 for information on how to set up scouting runs.

Instruction

The table below describes how to perform a scouting run:

Step	Action
1	Start the method (see **9.1 How to start a method run** on page 197). *Result:* The **Start Protocol** will display the scouting scheme as defined in the method (assuming that the **Scouting** box is selected on the **Start Protocol** tab of the **Run Setup**).
2	Check through the settings for the scouting scheme in the **Scouting** tab, and if required, do the following: • Change the scouting variable values. • Right-click the top of the **Run** column to toggle the run status between **Run** and **Excluded**.
3	Work through the rest of the **Start Protocol**. Click the **Start** button.

Results of a scouting run

The results of a scouting run are saved in a special scouting folder as defined in the **Results** tab of the **Start Protocol**. Within the folder, each run is saved in a separate result file named according to the usual naming rules (see **6.5.12 The result name tab** on page 153).

If the **Start Protocol** is displayed for each run in a scouting scheme, you are able to change the result file name during scheme execution.

How to change scouting variables during a run

- At any time during a run, you can click the **View Documentation** icon in **System Control** and change the scouting variables on the **Scouting** tab for runs which have not yet been started.

- Settings for the run that is currently in progress cannot be changed.
- You can add more scouting runs to the scheme as long as the last run has not been started.
- You can use this feature to adjust variables for scouting even if the start protocol is not displayed at the beginning of each run.

9.5 *How to perform a MethodQueue run*

Instruction

The table below describes how to run a **MethodQueue**:

Step	Action
1	• Make sure that all systems used in the **MethodQueue** are connected with control mode connections.
2	Select a **MethodQueue** in the **Methods** pane in the **UNICORN Manager** and • choose **File:Run** *or* • right-click the **MethodQueue** icon in the **Methods** pane and select **Run** from the shortcut menu. *or* • double-click the **MethodQueue** icon in the **Methods** pane and click the **Run** button in the **MethodQueue Editor** dialog box. *Result:* The **MethodQueue** will start in accordance with the conditions defined in the **MethodQueue** setup.

See **8.1 How to create a new MethodQueue** on page 190 for information about how to create a **MethodQueue**.

Unattended MethodQueue operation

The **Start Protocol** for the first and each subsequent method step in the **MethodQueue** is displayed when the corresponding method is run. If you require unattended **MethodQueue** operation after the start of the first method step, make sure that subsequent method steps do not include a **Start Protocol**.

Note: If the **Start Protocol** for a method in the queue is cancelled, the **MethodQueue** is paused. Select **MethodQueue:Display Running** in the **UNICORN Manager** and **Restart** or **End** the run in the displayed dialog box.

MethodQueues when the system is busy

You can choose to place a method in a **MethodQueue** if the system is already busy with another method run (See **8.1 How to create a new MethodQueue** on page 190). In a similar manner you can also start a new **MethodQueue** while another **MethodQueue** is in progress. It will be placed in queue and executed when the first queue is completed.

How to display and edit pending and running MethodQueues

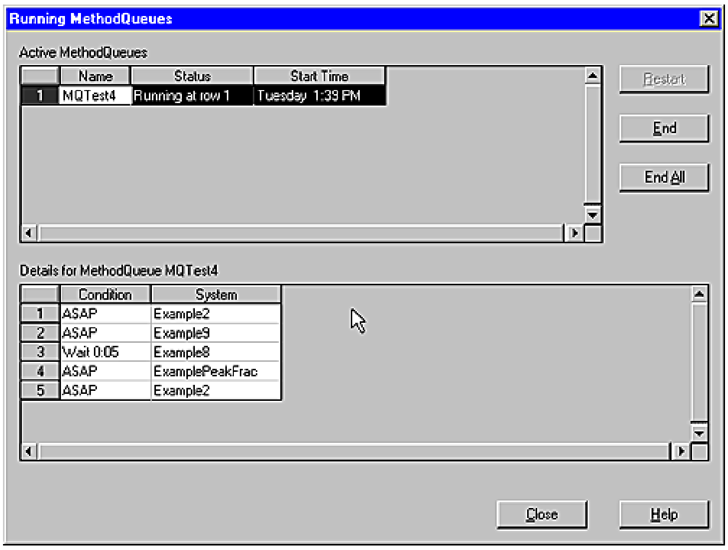
Definition: A pending **MethodQueue** is one for which **Run** has been requested but which has not yet started, either because the system is not available or because the setup time has not been reached.

The table describes how to display running and pending **MethodQueues**.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>

Click the **Running MethodQueue** icon.

Result: The **Running MethodQueue** dialog box is displayed.



Active MethodQueues

<table>
<tr><th></th><th>Name</th><th>Status</th><th>Start Time</th></tr>
<tr><td>1</td><td>MQTest4</td><td>Running at row 1</td><td>Tuesday 1:39 PM</td></tr>
</table>

Details for MethodQueue MQTest4

<table>
<tr><th></th><th>Condition</th><th>System</th></tr>
<tr><td>1</td><td>ASAP</td><td>Example2</td></tr>
<tr><td>2</td><td>ASAP</td><td>Example9</td></tr>
<tr><td>3</td><td>Wait 0:05</td><td>Example8</td></tr>
<tr><td>4</td><td>ASAP</td><td>ExamplePeakFrac</td></tr>
<tr><td>5</td><td>ASAP</td><td>Example2</td></tr>
</table>

</td></tr>
<tr><td>2</td><td>

Select a **MethodQueue** in the **Active MethodQueues** list box.

Result: Information on the selected **MethodQueue** is displayed in the **Details for...** field of the dialog box.

Choose from the following:

- The **Restart** button restarts the currently running **MethodQueue** if a **Start Protocol** has been terminated by **Cancel**.
- The **End** button terminates a running **MethodQueue** after the current step. Any methods currently in operation will continue to run and must be terminated with the **End** button in the **System Control** window if they are not to run to completion. If you click the **End** button for a pending **MethodQueue**, it is deleted from the pending list.
- The **End All** button terminates the running **MethodQueue** and deletes all **MethodQueues** from the pending list.
- The **Close** button closes the dialog box.

</td></tr>
</table>

• p 227

9.6 *If the network connection fails*

Results will be saved in the Failed folder

If the results of a method run are stored on a server or other location, and there is a network communication failure during a method run that has been started from a remote station, the method run will continue and the results will be saved in the **Failed** folder on the local station. A control mode connection can be established on the local station to control the running system. See the Administration and Technical Manual for more details.

---

10 How to view results

Introduction

A result file is automatically generated at the end of a method run and contains a complete record of the method run, including method, system settings, curve data and method run log. The **Evaluation** module offers extensive facilities for presentation and evaluation of curve data.

This chapter describes how to present the chromatograms and curves of your result file and how to create and print reports.

In this chapter

This chapter contains the following sections

Topic	See
How to open a result file	10.1
How to use the File Navigator	10.2
Basic presentation of chromatograms	10.3
How to optimize the presentation of a chromatogram	10.4
How to print active chromatograms	10.5
How to create and print reports	10.6
Run documentation	10.7

10.1 How to open a result file

Introduction

All contents of the result files are opened in the **Evaluation** module. By default, the chromatograms in a run are shown as opened windows. The chromatogram window on top is the active window. There is also a minimized **Temporary** chromatogram window. See **10.3 Basic presentation of chromatograms** on page 235 for further information about chromatograms.

Note: It is not possible to open the same result file from two different locations simultaneously.

How to open a result from the UNICORN Manager

To open a result file from the **UNICORN Manager**, do one of the following:

- Double-click a result file in the **Results** window of the **UNICORN Manager**,

or

- Select a result file icon in the **Results** window of the **UNICORN Manager** and select **File:Open**,

or

- Click the **Evaluation** icon in the **UNICORN Manager**, open the **Evaluation** module and select a result file from the **Open Result** dialog box.

How to open a result in the Evaluation module

To open a result file in the **Evaluation** module:

- Do the following:
  - Select **File:Open**
  - Select a result file from the **Open Result** dialog box.

or

- Do the following:
  - Select **View:File Navigator**
  - Locate and select a result file from the **File Navigator**.

Note: See **10.2 How to use the File Navigator** on page 231 for detailed instructions on how to locate files and set up File Navigator preferences.

10.2 How to use the File Navigator

Introduction

The **File Navigator** can be used to locate and open result files in the **Evaluation** module. Recent runs are also listed based on the user preferences.

How to open the File Navigator

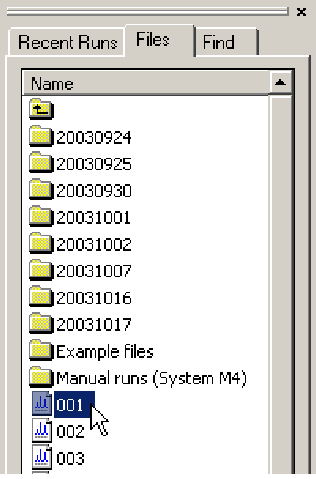
To open the **File Navigator**:

- Click the **Evaluation** module icon in the Windows task bar.
- Select **View:File Navigator**

Result: The **File Navigator** opens in the **Evaluation** module. The **File Navigator** can be resized and dragged to other positions in the module.

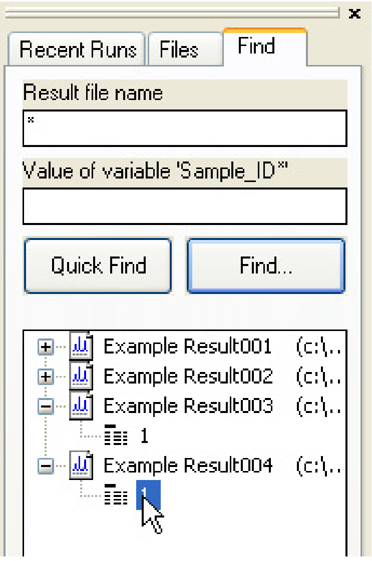
How to open files from the Files list

The table below describes how to use the **Files** list to locate and open a result file.

Step	Action
1	• Click the **Files** tab *Result*: The **Files** list opens in the **File Navigator**. The list is identical to the **Results** window in the **UNICORN Manager** and shows all user available folders and files. 
2	• Navigate to the desired folder • Double-click the desired result file *Result*: The result file opens in the **Evaluation** module.

How to use Find to search for files

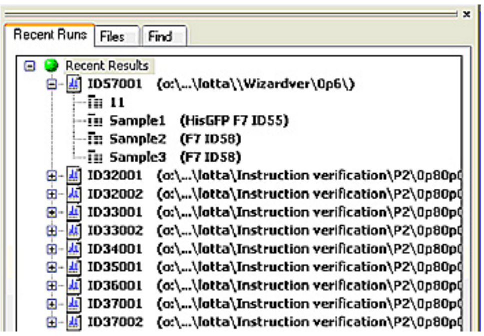
The **Find** function in the File Navigator is used to locate result files in the available folders. The table below describes how to use the **Find** function to locate and open a result file.

Step	Action
1	• Click the **Find** tab *Result*: The **Find** list opens in the **File Navigator**. 
2	• Type a file name or part of a file name in the **Result file name** text box. Standard wildcard characters can be used. *or* • Type a Sample ID value in the **Value of variable Sample_ID** text box. *Note*: The defined variable name must begin with **Sample_ ID**.
3	• Click the **Quick Find** button *Result*: The located result files are listed in the **File Navigator**.
4	• Double-click the desired result file or chromatogram *Result*: The file or chromatogram opens in the **Evaluation** module.

Note: Click the **Find** button to open the **Find Files** dialog box where more search functions are available. See **4.3 How to arrange and locate your files** on page 78 for more information.

How to open a Recent Run

The **Recent Runs** list shows all the available recorded recent runs based on the selected user preferences. The table below describes how to use the **Recent Runs** list to locate and open a result file.

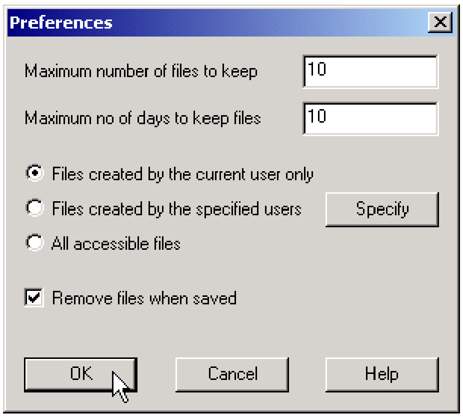
Step	Action
1	- Click the **Recent Runs** tab *Result*: The **Recent Runs** list opens in the **File Navigator**.  Recent Results ID57001 (o:\...\lotta\\Wizardver\0p6\) 11 Sample1 (HisGFP F7 ID55) Sample2 (F7 ID58) Sample3 (F7 ID58) ID32001 (o:\...\lotta\Instruction verification\P2\0p80p ID32002 (o:\...\lotta\Instruction verification\P2\0p80p ID33001 (o:\...\lotta\Instruction verification\P2\0p80p ID33002 (o:\...\lotta\Instruction verification\P2\0p80p ID34001 (o:\...\lotta\Instruction verification\P2\0p80p ID35001 (o:\...\lotta\Instruction verification\P2\0p80p ID36001 (o:\...\lotta\Instruction verification\P2\0p80p ID37001 (o:\...\lotta\Instruction verification\P2\0p80p ID37002 (o:\...\lotta\Instruction verification\P2\0p80p *Note*: Until the files and chromatograms in the list have been opened and saved they are noted in bold text. When they are opened and saved the text is changed to plain text.
2	- If needed, click the **Refresh** button in the bottom of the **File Navigator** *Result*: The **Recent Runs** list is updated with all runs that were performed since the **File Navigator** was opened the last time.
3	- Locate the desired run - Double-click the file *Result*: The result file opens in the **Evaluation** module.

Note: Click the **+** signs to view or select individual chromatograms from the result files. Individual result files can be selected and removed from the list by clicking the **Remove** button. The **Remove all** button clears the whole list.

Note: **Remove** only clears the list, the files are not deleted.

How to set preferences for Recent Runs

The **File Navigator** will display **Recent Runs** based on the individual user preference settings. The table below describes how to adjust the preference settings:

Step	Action
1	• Click the **Preferences** button *Result*: The **Preferences** dialog box opens.  Preferences Maximum number of files to keep 10 Maximum no of days to keep files 10 ● Files created by the current user only ○ Files created by the specified users ○ All accessible files ☑ Remove files when saved
2	• Type the maximum number of files to keep on the list • Type the maximum number of days to keep the files on the list
3	Select which files to display on the list: • Only files created by the current user • All files created by specified users *Note*: Click the **Specify** button to open a dialog box and select from a list with all accessible users. • All accessible files regardless of the creator
4	• Choose **Remove files when saved** if the files are to be removed from the list when they have been saved. • Click the **OK** button. *Result*: All new results will be displayed on the **Recent Runs** list based on the changed preferences.

How to close the File Navigator

To close the **File Navigator**:

- Click the small cross in the top right-hand corner of the File Navigator.

Result: The **File Navigator** closes.

10.3 Basic presentation of chromatograms

Introduction

This section describes how to access result files and optimize the presentation of a chromatogram and its curves via the **Chromatogram Layout** dialog box.

In this section

This section contains the following sub-sections

Topic	See
Introduction and temporary chromatograms	10.3.1
The chromatogram window	10.3.2

10.3.1 Introduction and temporary chromatograms

Contents of a chromatogram

Chromatograms can be viewed in the **Evaluation** module.

A chromatogram includes a number of curves that have been created during a method run, such as UV, conductivity, pH, fraction marks, etc. A chromatogram also contains the curves created and saved during an evaluation session. The original raw data curves cannot be deleted or modified, but they can be used as the basis for evaluation procedures and subsequent creation of new curves.

Temporary chromatograms

A **Temporary** chromatogram is essentially an empty chromatogram that is specific to the **Evaluation** module. It is also user-specific, so that all users have their own.

Information contained within a **Temporary** chromatogram is automatically saved from one evaluation session to the next, but is not saved within the result files.

How to copy curves into Temporary

Curves can be copied into **Temporary** and comparisons or evaluations can be performed. This is particularly useful if you do not want to clutter up your original chromatograms with a large number of curves. It can also be used to keep blank run curves or curves to compare when you open different result files.

The table below describes how to copy curves into **Temporary**:

Step	Action
1	Open a result file.
2	Select **Edit:Copy:Curves.** *Result:* The **Copy Curve** dialog box is displayed.
3	Select a source chromatogram and a curve to be copied in the **Source Chromatogram** fields.
4	Select **Temporary** as the target chromatogram and a position for the new curve in the **Target Chromatogram** fields.
5	Click the **Copy** button. *Result:* The curve is copied into the **Temporary** chromatogram. Click the **Close** button.

How to clear a temporary chromatogram

The table below describes how to clear the contents of a temporary chromatogram:

Step	Action
1	Open the relevant result file.
2	• Select **Edit:Clear Temporary Chromatogram**. • Click the **Yes** button to confirm.

10.3.2 The chromatogram window

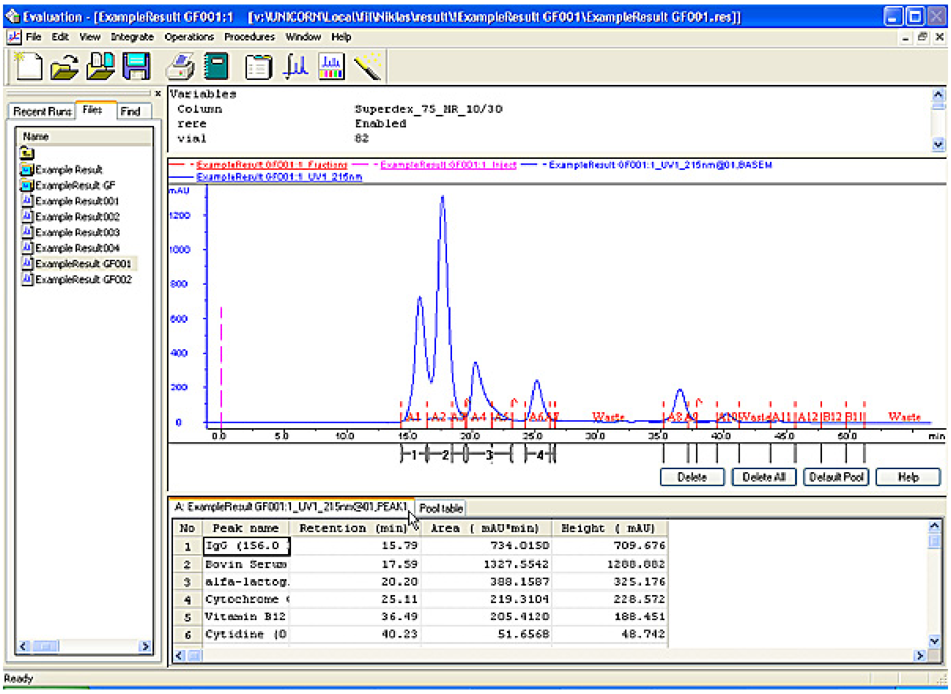
Main views

The chromatogram window is divided into four main views:

- File Navigator
- header information
- curves
- peak and pool tables

The displayed areas for the views can be adjusted by dragging the borders with the mouse cursor between the views.

The picture below shows an example of the window with all views present:



```
Variables
 Column                     Superdex_75_HR_10/30
 rere                       Enabled
 vial                       82
```

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)
1	IgG (156.0	15.79	734.0150	709.676
2	Bovin Serum	17.59	1327.5542	1288.882
3	alfa-lactog.	20.20	388.1587	325.176
4	Cytochrome (	25.11	219.3104	228.572
5	Vitamin B12	36.49	205.4120	188.451
6	Cytidine (0	40.23	51.6568	48.742

How to view header information

You can display header information at the top of a chromatogram, with details on variables, scouting variables, questions and/or notes. Header information cannot be displayed for imported chromatograms.

The table below describes how to display header information:

Step	Action
1	Open a result file.
2	In the **Evaluation** module, select **Edit:Chromatogram Layout...** *Result:* The **Chromatogram Layout** dialog box is displayed.

Step	Action
3	• Click the **Header** tab. • Select the options you want in the header. • Click **OK**.
4	• In the chromatogram window, place the cursor at the top of the curve window (just below the toolbar) until the window sizing tool appears. • Drag the cursor down to display the header window.

How to view peak table information

The table below describes how to display peak table information if the result has been integrated:

Step	Action
1	Open a result file.
2	• Choose **Edit: Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box opens.
3	• Click the **Peak Table** tab. • Select a peak table in the **Select peak table to display** list. • Select what peak table columns to display. • Check if global peak table data should be displayed or not. • Click **OK**.

How to view the Pool table

If fractions are pooled, the **Pool Table** is displayed in the same pane as the **Peak Table**.

- Click the **Pool Table** tab to display the **Pool Table** information.

See **11.5 How to pool fractions** on page 291 for more information on how to create the **Pool Table**.

Run curves, default appearance and information

The first time a result file is opened and viewed, a default layout is applied to display all the original curves. The default layout can be changed by the user (see **10.4.5 How to save and apply a layout** on page 251).

Information for each curve

Each curve is automatically assigned a default color and style, with default information about each curve displayed in the key above the curves. This information includes

- result file name
- chromatogram name
- curve name.

Choose the Y-axis scale

Each curve has a correspondingly colored Y-axis. To choose the appropriate Y-axis scale

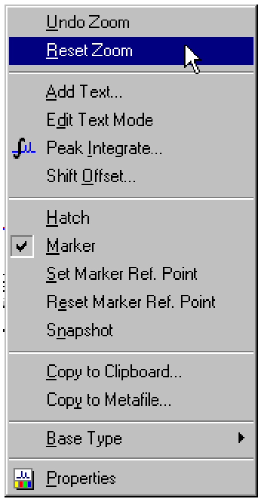
- click on the Y-axis until the desired scale is displayed

or

- click on the name of the curve.

Run curves, shortcut menu

When viewing curves in the **Evaluation** module, you can access a menu that provides a quick alternative to menu commands. Right-click the run curves view to display the menu shown in the picture below:



Optimizing the workspace

The chromatogram window can be minimized and maximized using ordinary Windows commands. The table below describes extra features to optimize the workspace:

Use the command	if you want...
Window:Arrange icons	to arrange icons of minimized windows.

Use the command	if you want...
Window:Tile	to view several chromatogram windows side by side.
Window:Cascade	to stack the open windows like a deck of cards.

How to display a vertical marker line

The table below describes how to display a vertical marker line:

Step	Action
1	Right-click the **Curves** pane and select **Marker**.
2	Drag the marker line with the mouse. *Result:* Where the line bisects the curve, the X-axis and Y-axis values are displayed at the top right corner of the pane.

Note: Right-click and select **Snapshot** to record the marker position values. See **2.2.7 Snapshots** on page 41 for more information about the **Snapshot** function.

How to set a reference point

The table describes how to set a reference point:

Step	Action
1	• Display a **Marker** in the **Curves** pane. • Right-click 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 reference point, • the minimum, maximum and average values for the curve interval between the reference point and the new position.

How to display the logbook overlay

The table below describes how to display the logbook entries as an overlay in the chromatogram.

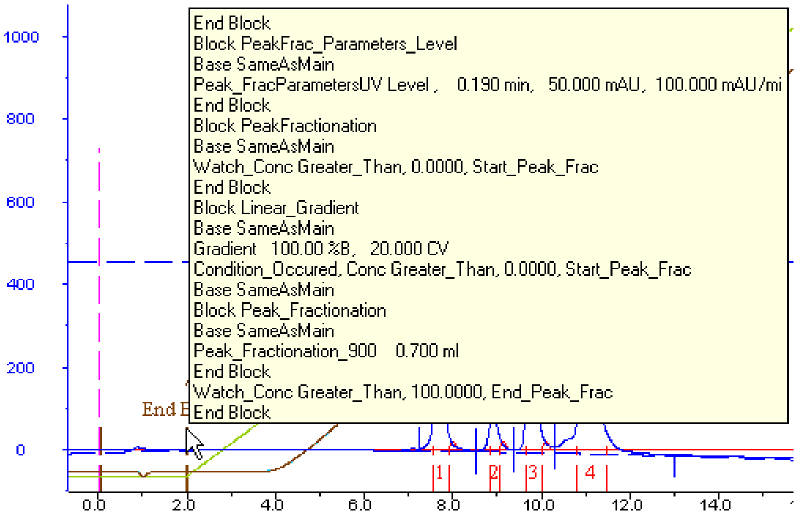
Step	Action
1	• Right-click in the chromatogram window and choose **Properties** on the shortcut menu. *Result*: The **Chromatogram Layout** dialog box opens.

Step	Action
2	• Choose the **Curve** tab. • Select the **Logbook** curve.
3	• Choose the **Curve Style and Color** tab. • Click the **Filter** button in the **Logbook text alignment** field. *Result*: The **Filter Logbook** dialog box opens.
4	• Select all the logbook items you want to display and click **OK**. • Click **OK** in the **Chromatogram Layout** dialog box. *Result*: The selected logbook items are displayed in the chromatogram window.

How to view the complete logbook information

At some breakpoints there can be more logbook information than what is possible to conveniently display in the chromatogram window. The additional information that is not displayed is indicated by an arrow point symbol by the break point.

- Hold the mouse cursor over the break point to display the complete information in a flyover text box, as shown in the illustration below:



```
End Block
Block PeakFrac_Parameters_Level
Base SameAsMain
Peak_FracParametersUV Level ,   0.190 min,   50.000 mAU,  100.000 mAU/mi
End Block
Block PeakFractionation
Base SameAsMain
Watch_Conc Greater_Than, 0.0000, Start_Peak_Frac
End Block
Block Linear_Gradient
Base SameAsMain
Gradient   100.00 %B,   20.000 CV
Condition_Occured, Conc Greater_Than, 0.0000, Start_Peak_Frac
Base SameAsMain
Block Peak_Fractionation
Base SameAsMain
Peak_Fractionation_900    0.700 ml
End Block
Watch_Conc Greater_Than, 100.0000, End_Peak_Frac
End Block
```

10.4 How to optimize the presentation of a chromatogram

Introduction

This section describes some of the ways you can optimize the presentation of a chromatogram.

In this section

This section contains the following sub-sections

Topic	See
How to make changes in the Chromatogram Layout dialog box	10.4.1
The Curve tab and Curve Names tab	10.4.2
The Curve Style and Color tab	10.4.3
How to change and fix the axes	10.4.4
How to save and apply a layout	10.4.5
How to show part of a curve	10.4.6
How to change the size of Fraction, Injection and Logbook marks	10.4.7

10.4.1 *How to make changes in the Chromatogram Layout dialog box*

Instruction

The **Chromatogram Layout** dialog box is used to make changes regarding chromatogram presentation. The main features of the **Chromatogram Layout** dialog box regarding chromatograms are described in the subsequent sections in this chapter. Features regarding peak tables are described in **12.1.2 How to perform a peak integration** on page 344.

The table below describes how to make changes in the **Chromatogram Layout** dialog box:

Step	Action
1	Open a result file.
2	• Right-click the chromatogram window and select **Properties** *or* • Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed. The view from which you activate the **Properties** command determines the tab that is displayed in the **Chromatogram Layout** dialog box.
3	Carry out the changes on the different tabs to get the desired layout for header, curves and peak table. Select **Apply to all chromatograms** if you want to apply changes made in the **Chromatogram Layout** dialog box to all open chromatograms. Click **OK**.

10.4.2 The Curve tab and Curve Names tab

The Curve tab

The **Curve** tab of the **Chromatogram Layout** dialog box contains a list of all the curves included in the chromatogram. Select the curves you want to display in the chromatogram, and click **OK**.

Curve name appearance

You select options for the curve name appearance on the **Curve Names** tab. This is an example of a default curve name:

Result:11_UV1_280

The table below describes the three components that make up the default curve name:

Component	Description	Example
Result name	Name of the result.	Result
Chromatogram name	Number given automatically during a run or a name defined by the **New_Chromatogram** instruction.	11
Curve name	Curve type, for example detection of an eluted component. In this example, the system uses a variable wavelength detector, so the wavelength (280) for the UV curve is also given.	UV1_280

How to choose curve name appearance

You can choose to view only part of the curve name. The table below describes how to do this:

Step	Action
1	Open a result file.
2	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	Click the **Curve Names** tab.

Step	Action
4	• Select the appropriate boxes for **Curve name appearance**. • Select the appropriate **Curve legend position**. • Click **OK**.

Note: It is usually sufficient to select the **Curve Name** option if only one chromatogram is being evaluated. However, confusion can arise when more than one chromatogram is shown, so more complete names might be necessary.

10.4.3 The Curve Style and Color tab

Introduction

All curves within a chromatogram are represented by a default color and line style. Curves imported into the chromatogram or newly created curves are automatically assigned a color and line style.

Peak label settings

Peaks can be labeled on the **Curve Style and Color** tab of the **Chromatogram Layout** dialog box. Use a combination of the following labels:

- **Retention** (the default label)
- sequential **Number**
- user-defined **Peak name**.

Fraction text and Logbook text alignment settings

Both **Fraction text** and **Logbook text** can be set to the following alignment options:

- **Vertical**
- **Horizontal**
- **Fly Over**, which sets text labels as hidden text that appears only when the cursor is carefully positioned over a fraction mark.

How to change the color and style of a curve

The table below describes how to change the color and style of a curve:

Step	Action
1	Open a result file.
2	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	Click the **Curve Style and Color** tab.
4	• Select the curve you want to change from the list. • Select the desired color and style. • Click **OK**.

How to display and filter logbook information

The table below describes how to display and filter logbook curve information:

Step	Action
1	Open a result file.

Step	Action
2	• Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	• Click the **Curve** tab. • Select the logbook curve.
4	• Click the **Curve Style and Color** tab. • Click the **Filter...** button in the **Logbook text alignment** field. *Result:* The **Filter Logbook** dialog box is displayed.
5	• Select the type of logbook information you want to show. • Set the maximum block depth to show. • Click **OK**.

How to display a hatched background

The table below describes how to display a hatched background in the chromatogram window:

Step	Action
1	Open a result file.
2	• Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	• Click the **Curve Style and Color** tab. • Select the **Hatch** box. • If desired, select the **Apply to all chromatograms** box and click **OK**. *Result:* Hatch marks are displayed as a background.

Note: You can also right-click in the **Chromatogram** window and select **Hatch**.

10.4.4 How to change and fix the axes

How to change and fix the Y-axis

The table below describes how to change and fix the Y-axis:

Step	Action
1	Open a result file.
2	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	Click the **Y-Axis** tab.
4	• Select the appropriate curve from the list. • Click the **Fixed** option.
5	• Type the desired minimum and maximum values. • Click the **All with this unit** button if you want other curves with the same Y-axis units as the current scaled curve to be similarly scaled. *Note:* The values will only be applied to existing curves. They will not be applied to new curves created after this function was last used. • Click the appropriate **Pressure unit** (**MPa**, **psi**, **bar**) option to change Y-axis units for pressure curves. *Note:* Default **Pressure unit** is **From strategy**, which is the unit defined in the original run strategy. • Click **OK**.

How to add a second Y-axis

The table below describes how to add a second Y-axis to the chromatogram.

Step	Action
1	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
2	Click the **Y-Axis** tab.
3	• Select the appropriate curve from the **Right Axis** droplist. • Click the **OK** button.

How to change and fix the X-axis

The table below describes how to change and fix the X-axis:

Step	Action
1	Open a result file.
2	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	Click the **X-Axis** tab.
4	Select the appropriate option in the **Base** field: • **Time** of retention • **Volume** • **Column Volume** *Note:* Some calculated curves, for example baselines, exist in only one base and might seem to disappear when the base is changed. Curves are collected in time and recalculated for display in volume. Thus, switching the base between **Time** and **Volume** can slightly alter the resolution.
5	• Click the **Fixed** option in the **Axis scale** field to set the axis limits manually. • Type the desired minimum and maximum values. • If desired, de-select the **Adjust retention zero to injection number** checkbox. This checkbox is selected by default. The function sets the time/volume to zero at the injection mark, that is when the sample was injected. The time and volume before injection will become negative values. • Click **OK**.

10.4.5 *How to save and apply a layout*

Introduction

All configurations that you make in the **Chromatogram Layout** dialog box can be saved as a layout. It is possible to apply saved layouts to other chromatograms. All saved layouts are user-specific.

How to save a layout

The table below describes how to save a layout:

Step	**Action**
1	Open a result file.
2	Choose **Edit:Chromatogram Layout**. *Result:* The **Chromatogram Layout** dialog box is displayed.
3	Make the appropriate layout configuration within the various tabs. **View your changes** Click **OK** if you want to return to the chromatogram window to see the applied affects of a given configuration. Return to the **Chromatogram Layout** dialog box to perform further changes.
4	• Select the **Layout Library** tab. • Click the **Save current layout as** button. *Result:* The **Save Layout** dialog box is displayed.
5	• Type a name for the layout. • If you want the current layout to be the new default layout, select the **Save as default** option. • Click **OK**. *Result:* The new name is added to the **Saved layouts** list. • Click **OK**.

How to apply a layout

The table below describes how to apply a layout:

Step	**Action**
1	Select the **Layout Library** tab on the **Chromatogram Layout** dialog box.

Step	Action
2	• Select a layout from the **Saved layouts** list. • Click the **Apply selected layout** button. *Result:* The layout is automatically applied to the active chromatogram window. • If the same layout is to be applied to all chromatograms on the **Evaluation** workspace, select the **Apply to all chromatograms** checkbox. • Click **OK**.

10.4.6 How to show part of a curve

Introduction

You can select a part of a curve in order to examine details more closely.

You can

- use the zoom to magnify

or

- cut the axes.

It is also possible fix the axes, see **10.4.4 How to change and fix the axes** on page 249.

How to use the zoom function

In the active chromatogram window, you can zoom in on a designated area of the chromatogram. This is the easiest and quickest way to enlarge different parts of a curve. The table below describes how to do this:

Step	Action
1	Open a result file.
2	• Place the mouse pointer in any corner of the area you want to magnify. • Press and hold the left mouse button. A magnifying glass icon will be added to the mouse pointer arrow on the screen. • Drag a box to cover the area to be magnified, and release the mouse button. *Result:* The selected region is now displayed in the entire chromatogram window, together with appropriate scales for the Y and X axes.
3	Use the arrow keys on the keyboard to move around in the chromatogram at the current zoom scale.
4	**Undo zoom** Right-click in the window and select **Undo zoom** to undo the last zoom step. **Reset zoom** Right-click in the window and select **Reset zoom** to reset all zoom steps at once.

How to cut a curve and store as a new curve

The table below describes how to cut the curve between two values on the X-axis and store this part of the curve as a new curve:

Step	Action
1	Open a result file.
2	Choose **Operations:Cut curve**. *Result*: The **Select Curve(s) to Operate On** dialog box opens.
3	• Select the curves to be operated on. • Click **OK**. *Result:* The selected curves are shown in the **Cut** dialog box which contains two vertical cursor lines.
4	To select the region to be cut, either • drag the two cursor lines to define the left and right limits of the cut area *or* • type the desired left and right limit values in the **Left limit** and **Right limit** boxes. *Note:* The areas outside of the **Left limit** and **Right limit** will not be saved in the newly created cut curve. Thus, the X-axis of the new curve will not begin at zero unless this is designated as one of the limits. The original curve is not changed.
5	Click **OK**. *Result:* The Save Cut Curves dialog box opens.

Step	Action
6	• Select whether to save the new cut curve in - the **Source chromatogram**, that is the current active chromatogram, *or* - a **New chromatogram** (if you select this option, you can change the name of the chromatogram. Note that it is a recommendation not to use only numbers as names for chromatograms.). • Click **OK**. Result: - If the destination of the cut curve was the source chromatogram, the cut curve is automatically displayed in the source chromatogram. - If the destination of the cut curve was a new chromatogram, this will be represented as a new, open chromatogram window.

10.4.7 How to change the size of Fraction, Injection and Logbook marks

Introduction

The sizes of **Fraction**, **Injection** and **Logbook** marks are all determined by your user settings. The settings are applied for all your chromatograms.

Instruction

The table below describes how to change the size of the **Fraction**, **Injection** and **Logbook** marks:

Step	Action
1	• Choose **Administration:Change User Attributes** in the **UNICORN Manager** module. *Result*: The **Change user attributes** dialog box opens.
2	Select the unit for the **Fraction mark height**: • **Percent of window height** • **Character Heights** • **Pixels** Type a new size value in the **Fraction mark height**box.
3	• Repeat step 2 for the **Injection** and **Logbook** marks if necessary. • Click **OK**.

10.5 How to print active chromatograms

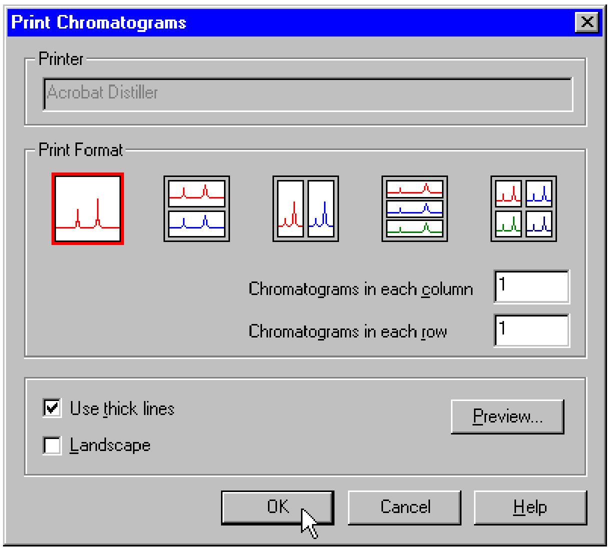
Introduction

This section describes how to print the chromatograms that are open in the **Evaluation** module.

The Print Chromatograms dialog box

This is an illustration of the **Print Chromatograms** dialog box.

Note: The selected print format is outlined in red.



Printer: Acrobat Distiller

Chromatograms in each column: 1

Chromatograms in each row: 1

☑ Use thick lines

☐ Landscape

Instruction

The table below describes how to print active chromatograms.

Step	Action
1	Open all chromatograms that you want to print in the **Evaluation** module.
2	• Select **File:Print**. *or* • Click the **Print** toolbar icon. *Result*: The **Print Chromatograms** dialog box opens.
3	Select print format and layout options.

Step	Action
4	• Click **OK** to print. *or* • Proceed with step 5 to preview and edit the layout.
5	Click the **Preview** button. Result: The **Customise Report** window opens.
6	• Click the **Edit Mode** button to make changes, e.g. change the order of the chromatograms (see **10.6.1 How to create and print a customized report** on page 260 for more information about how to edit). • Click the **Preview** button to return to preview mode.
7	• Select **File:Print**. *or* • Click the **Print** toolbar icon. *Result*: The **Print** dialog box opens.
8	• Select the print range and number of copies. • Click **OK**.

10.6 How to create and print reports

Introduction

The **Evaluation** module provides extensive tools to create detailed reports. This section describes how to create and print reports that are based either on a standard or a customized layout.

In this section

This section contains the following sub-sections

Topic	See
How to create and print a customized report	10.6.1
How to create and print a standard report	10.6.2
How to edit an existing report format	10.6.3

10.6.1 How to create and print a customized report

Introduction

You can choose from a variety of objects to include in a report, including chromatograms, methods, documentation, free text and more in the customized report interface. You can also place, align and size the objects as you please. This section describes how to create a customized report format.

Should you need to store store your reports in an electronic format you can save them as PDF files. This section also describes how to do this..

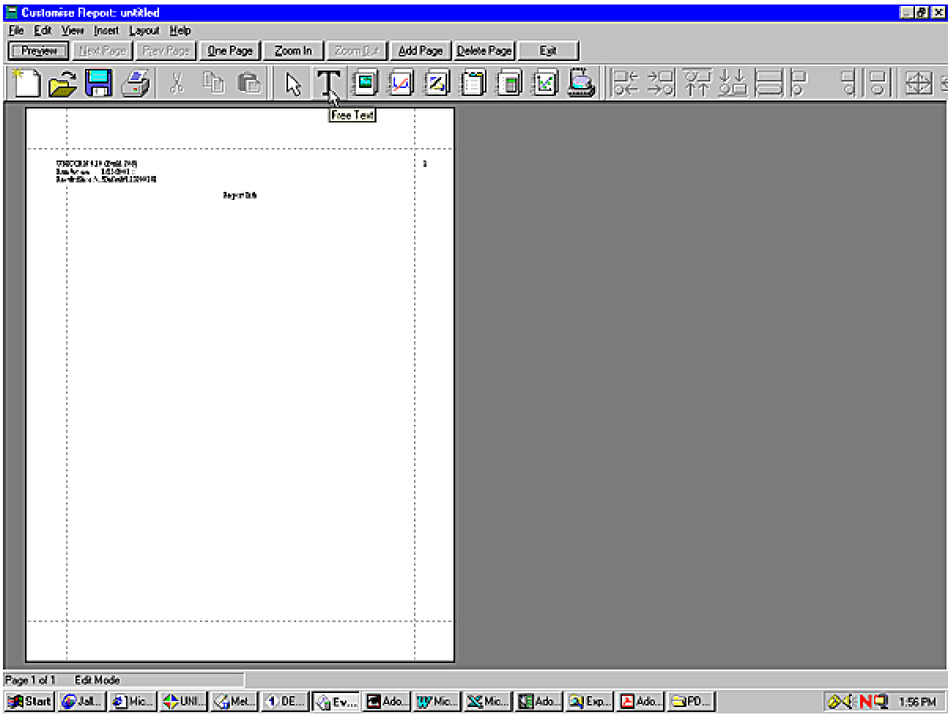
How to open the Report Editor in edit mode

The table below describes how to open the **Report Editor** in **Edit mode** to create a customized report format.

Step	Action
1	Open a result file in the **Evaluation** module.
2	• Select **File:Report**. *or* • Click the **Report** icon. *Result*: The **Generate Report** dialog box opens.
3	• Click the **New** button. *Result*: The **Create New Report Format** dialog box opens.
4	• Select the **Customised format** and click **OK**. *Result*: The **Report Editor** opens in **Edit mode**.

The Edit mode window

The illustration below shows the **Report Editor** window in **Edit mode** with a blank report open:



Toolbar button functions in the Report Editor

The table below describes the different functions of the Edit mode toolbar buttons in the **Report Editor**:

Toolbar button	Function
Preview/Edit	This button toggles between a print preview of the report and the **Edit mode**.
Next Page	This button displays the next page or pair of pages (where there are more than one page).
Prev Page	This button displays the previous page or pair of pages (where there are more than one page).
One Page/Two Pages	This button toggles between single page view and pairs of pages view, when there is more than one page.
Zoom In	This button increases the magnification of the view.
Zoom Out	This button decreases the magnification of the view.
Add Page	This button adds a blank page to the report.
Delete Page	This button deletes the current page from the report.

Toolbar button	Function
Exit	This button closes the **Customize Report** window.

How to add and delete report pages

The table below describes how to add or delete report pages in the **Report Editor**:

If you want...	then...
to add new pages,	• click the **Add Page** toolbar button. *Result*: A new page is added after the last page.
to delete a page while in **One Page** mode,	• select the page with **Next Page** or **Prev Page**, • click the **Delete Page** toolbar button and confirm the deletion.
to delete a page in **Two Page** mode,	• select the page with **Next Page** or **Prev Page**, • click an object on the page, • click the **Delete Page** toolbar button and confirm the deletion.

How to change the page layout

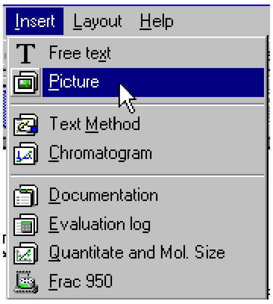
The page layout is changed in the **Page Setup** dialog box. The table below describes how to set up the page layout:

Step	Action
1	Double-click anywhere on the report page in the **Report Editor** (not on an object). *Result*: The **Page Setup** dialog box opens.
2	• Type new values for the **Margins** if necessary. • Select the appropriate **Settings** and **Unit**. *Note*: An extra **Header** tab will appear if you de-select the option to have the same header on all pages. The **First Header** tab is used for the first page header only, and the **Header** tab is used for all subsequent pages. • Click the **First Header** tab.
3	• Select all the items you want to include in the header from the **Select Items** list. • Click the **Font** button to change the font for all items if necessary.

Step	Action
4	• Type header text in the **Free text** box and click the **Font** button to alter the default font if necessary. • Type the report title in the **Report title** box and click the **Font** button to alter the default font if necessary.
5	• Select the **Logo** check box and click the **Browse** button if you want to locate and select a logo image file. • Select the **Alignment** for the logo, if necessary. *Note*: The logo file must be in bitmap format (**.bmp**) and smaller than 64 kB. Larger logo files or files in other formats must be inserted as **Picture** objects.
6	If you want to have a line under or over the header, select the appropriate option in the **Layout** field.
7	• Repeat steps 3 to 6 on the **Footer** tab and the subsequent pages **Header** tab. *Note*: All **Header** and **Footer** tabs contain the same options. You can have all information in either the header or footer or split information between the header and footer as required. • Click **OK**.

How to add objects to the report

The table below describes how to add objects to the report. The various objects are described below this table.

Step	Action
1	• Click the appropriate icon in the **Report items** toolbar. *or* • Choose an object from the **Insert** menu. 

Step	Action
2	• Press and hold the left mouse button on the report page, and drag out a box to the size of the item you want to insert. *Note*: The mouse pointer shows a symbol for the type of item you have selected. • Release the mouse button. *Result*: A **Setup** dialog box opens. The dialog is specific to the type of item that you want to insert.
3	• Select the desired options and click **OK**. *Result*: The object is inserted onto the page.

Note: If you want to edit an object later, double-click the object box.

How to add free text

The table below describes how to add free text to the report:

Step	Action
1	• Click the **Free Text** icon. T • Press and hold the left mouse button on the report page and drag out a box to the size of the text. Release the button. *Result*: The **Setup Free Text** dialog box opens.
2	• Type text in the edit field. • Select if the text is to start on a new page. • Select if the text box should be automatically sized. • Select if the text should appear in the same position on all pages, for example as header and footer text.
3	• Click the **Font** button to change the default font. *Result*: The **Font** dialog box opens. • Make the necessary changes and click **OK** to return. • Click **OK**. *Result*: The text object is inserted onto the page.

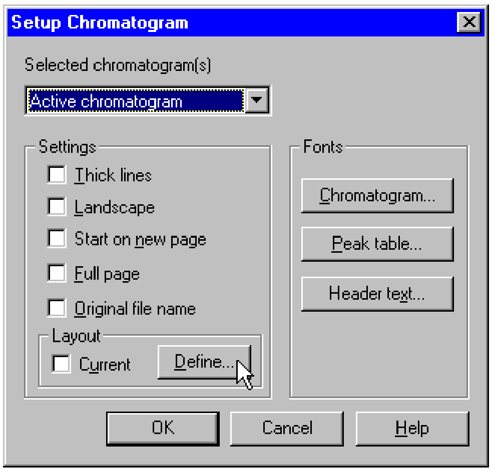
How to add a picture

The **Picture** dialog box is useful to insert logos, pictures or other figures in the report. The table below describes how to add a picture object to the report:

Step	Action
1	• Press and hold the left mouse button on the report page and drag out a box to the size of the picture item. Release the mouse button. • Click the **Picture** icon. *Result*: The **Picture** dialog box opens.
2	• Click the **Browse** button to locate the desired picture file. • Select the picture file and click the **Open** button. *Note*: The file formats **.bmp**, **.emf**, **.jpg** and **.tif** can be used. *Result*: A preview of the selected picture is displayed.
3	• Select the desired **Settings** and click **OK**. *Result*: The picture is inserted onto the page.

How to add a chromatogram or peak table

The table below describes how to add a chromatogram to the report. The layout can also be defined to include a peak or pool table if desired.

Step	Action
1	• Click the **Chromatogram** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the chromatogram. Release the mouse button. *Result*: The **Setup Chromatogram** dialog box opens.  Setup Chromatogram Selected chromatogram(s): Active chromatogram Settings ☐ Thick lines ☐ Landscape ☐ Start on new page ☐ Full page ☐ Original file name Layout ☐ Current
2	Select which chromatogram(s) to insert from the **Selected chromatogram(s)** droplist. • **Active chromatogram** inserts the chromatogram that currently is active in the **Evaluation** module. • **All chromatograms** inserts all chromatograms that are open in the **Evaluation** module. • **1, 2...etc.** inserts the corresponding chromatogram.
3	• Select the desired **Settings**. • If desired, change the **Fonts**. *Note*: Separate fonts can be selected for the **Chromatogram**, the **Peak table** and the **Header text**.

Step	Action
4	• Click the **Define** button in the **Layout** field if you want to re-define the layout of the chromatogram. *Result*: The **Report Chromatogram Layout** dialog box opens. • Make the appropriate changes and click **OK** to return to the **Setup Chromatogram** dialog box. *Note*: The changes that you make will only affect the report and not the view of the chromatograms in the **Evaluation** module.
5	Click **OK**. *Result*: The chromatogram is inserted onto the page.

Note: All curves can be de-selected in the **Report Chromatogram Layout** dialog box leaving only the selected peak table(s) in the report.

How to include a method

The table below describes how to include a method in the report:

Step	Action
1	• Click the **Method** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the button. *Result*: The **Setup Method** dialog box opens.
2	Select the items to be included in the report: • **Main Method** is the method on which the run was based. • **Blocks** are the blocks that were used in the method.
3	• Select the appropriate **Settings**. *Note*: **Expand main** displays the expanded method view. • If desired, change the **Fonts**. • Click **OK**. *Result*: The method object is inserted onto the page.

How to add documentation

The table below describes how to add documentation to the report:

Step	Action
1	• Click the **Documentation** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the button. *Result*: The **Setup Documentation** dialog box opens.
2	Select the items to be included in the report: • **Select All** includes all items in the report. • **Clear All** removes all selections.
3	• If desired, change the **Fonts**. • Select if the documentation should start on a new page. • If **Select All**, **Logbook** or **Run summarySelect All** or **Logbook** was selected, make the necessary changes to the **Base** and **Logbook filter** settings. • Click **OK**. *Result*: The selected documentation items are inserted into the report.

How to add the Evaluation Log

The table below describes how to add the **Evaluation Log** to the report:

Step	Action
1	• Click the **Evaluation Log** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the mouse button. *Result*: The **Setup Evaluation Log** dialog box opens.
2	• If desired, change the **Fonts**. • Select if the **Evaluation Log** should start on a new page. • Click **OK**. *Result*: The **Evaluation Log** is inserted into the report.

How to include Quantitate and Molecular Size data

The table below describes how to include **Quantitate** and **Molecular Size** data in the report.

Note: This option is only available if the **Analysis** module has been installed.

Step	Action
1	• Click the **Quantitate and Mol Size** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the mouse button. *Result*: The **Setup Quantitate** dialog box opens.
2	• If desired, change the **Fonts**. • The default option is that the **Quantitate and Molecular Size** data will start on a new page. • Click **OK**. *Result*: The **Quantitate and Molecular Size** data is inserted into the report.

How to include Frac-950 data

The table below describes how to include **Frac-950** data in the report.

Note: This option is available only if a **Frac-950** has been installed and if the result file contains data from the **Frac-950**.

Step	Action
1	• Click the **Frac-950** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the mouse button. *Result*: The **Setup Frac-950** dialog box opens.

Step	Action
2	• If desired, change the **Fonts**. • Select if the **Frac-950** data should start on a new page. • The **Include rack layout** option is selected by default. This will display the rack layout that was used in the run. • Click **OK**. *Result*: The **Frac-950** data is inserted into the report.

How to move and resize objects freely

The table below describes how to select, move and resize objects freely:

If you want...	then...
to select a single object,	• click the **Select** icon, • click the object of interest.
to select several objects,	• click the **Select** icon, • press and hold the <Ctrl> key while you click the objects.
to move the selected object(s),	click on the objects, hold down the left mouse button and drag the object(s) to the new position.
to resize the selected object(s),	click one of the object border anchors, either in the corners or in the middle of a border, and drag the box to the new size. *Note*: Some **Text** objects cannot be resized.

Alignment toolbar icon functions

Objects can be placed in exact positions and sized in relation to other objects. The table below describes the function of the **Alignment** toolbar icons in the **Report Editor**:

Toolbar icon	Function
	Align left Matches the left alignment of all selected objects to that of the highlighted object.

Toolbar icon	**Function**
	Align right Matches the right alignment of all selected objects to that of the highlighted object.
	Align top Matches the top alignment of all selected objects to that of the highlighted object.
	Align bottom Matches the bottom alignment of all selected objects to that of the highlighted object.
	Adjust to margins Stretches the selected object(s) to the left and right margins.
	Adjust to left margin Adjusts the selected object(s) to the left margin.
	Adjust to right margin Adjusts the selected object(s) to the right margin.
	Adjust to centre Adjusts the selected object(s) to the center of the page.
	Make same size Adjusts the selected objects to the same size as the highlighted reference object.
	Make same width Adjusts the selected objects to the same width as the highlighted reference object.
	Make same height Adjusts the selected objects to the same height as the highlighted reference object.

Note: The **Make same size** and **Make same width** functions can only be used to resize the width of chromatograms, free text and picture objects.

How to print the report

The table below describes how to print the report:

Step	Action
1	• Choose **File:Print**. *or* • Click the **Print** icon. *Result*: The **Print** dialog box opens. *Note*: Printers are set up in the **File** menu of the **UNICORN Manager**.
2	• Select the printing range. • Select the number of copies. • Click **OK**.

Note: You can also print the report from the **Generate Report** dialog box.

How to save the report in PDF format

The table below describes how to save the finished report as a PDF file:

Step	Action
1	• Click the **UNICORN Manager** icon on the Windows taskbar. *Result*: The **UNICORN Manager** opens. • Choose **File:Printer Setup**. *Result*: The **Print Setup** dialog box opens.
2	• Select an Adobe Acrobat printer from the **Printer Name** list (e.g. Acrobat Distiller). • Click the **Properties** button and edit the document properties if needed. • Select the appropriate paper size and orientation. • Click **OK**.
3	• Click the **Evaluation** icon on the Windows taskbar. *Result*: The **Evaluation** module opens
4	• Print the report as described in "How to print the report". *Result*: The report is created as a PDF file and saved in the location specified in your Acrobat settings.

Note: You must have a full installation of Adobe Acrobat or a suitable printer driver to be able to do this.

How to save the report format

The table below describes how to save the finished report format:

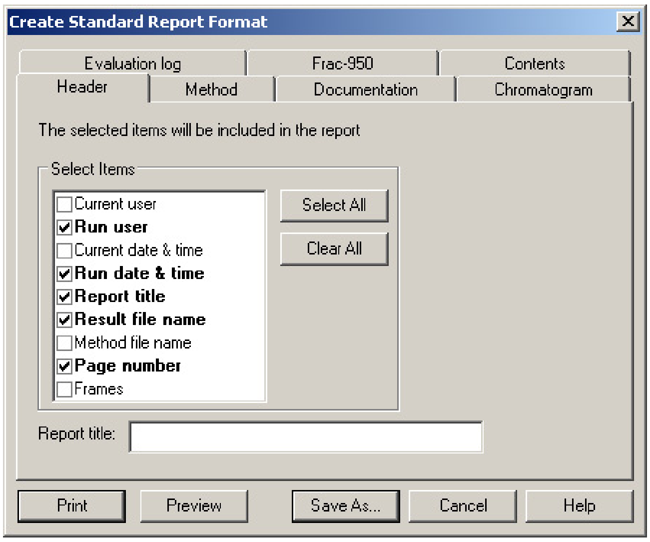
Step	Action
1	• Choose **File:Save**. *or* • Click the **Save** icon. 💾 *Result*: The **Save Report Format** dialog box opens.
2	• Type a name for the format. • Select if you want to save the format for global use. • Select if you want to save the format as default. *Note*: The name for the default format will automatically be changed to `DEFAULT`. • Click **OK**.

10.6.2 *How to create and print a standard report*

How to create a Standard report

You can only select a number of pre-formatted items when you create a **Standard** report format. If you want to edit the layout in detail you must create a **Customized** report format. See **10.6.1 How to create and print a customized report** on page 260.

The table below describes how to create and save a **Standard** report format:

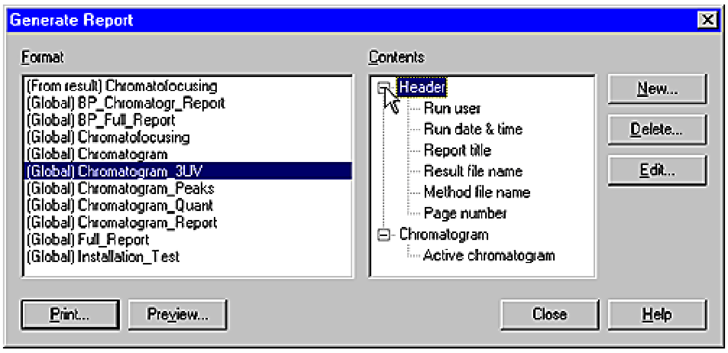
Step	Action
1	Open a result file.
2	• Select **File:Report**. *or* • Click the **Report** icon. *Result*: The **Generate Report** dialog box opens.
3	Click the **New** button. *Result*: The **Create New Report Format** dialog box opens.
4	• Select **Standard format** and click **OK**. *Result*: The **Create Standard Report Format** dialog box opens. The illustration below shows the **Create Standard Report Format** dialog box with the **Header** tab selected:  The selected items will be included in the report ☐ Current user ☑ **Run user** ☐ Current date & time ☑ **Run date & time** ☑ **Report title** ☑ **Result file name** ☐ Method file name ☑ **Page number** ☐ Frames Report title:
5	Click the appropriate tabs and select the check boxes for each item that you want to include in the report.

Step	Action
6	Click the **Chromatogram** tab and select the chromatogram(s) you want to include. • Select the **Current** option in the **Layout** field to apply the current layout in the **Evaluation** module. *or* • Click the **Define** button in the **Layout** field to open the **Curve** tab in the **Report Chromatogram Layout**. - Select the curves that you want to include in the report and click **OK**.
7	• Click the **Contents** tab to see a list of all the selected items. • Click the **Preview** button to see the entire report layout. • Click the **Close** button to return. • Click the **Print** button to print a test report.
8	• Click the **Save As** button. *Result*: The **Save Report Format** dialog box opens. • Type a name in the **Report format name** text box. - Select the **Save as global format** check box to make the format available to other users. - Select the **Save as default report format** check box if desired (The format is saved as DEFAULT). • Click **OK**. *Result*: The **Generate Report** dialog box opens again. The new report is saved and available in the **Format** list.
9	• Click the **Close** button *or* • Click the **New** button to create another **Standard** report.

How to print a standard report

The table below describes how to print a **Standard** report format in the **Evaluation** module.

Step	Action
1	Open a result file.

Step	Action
2	• Select **File:Report**. *or* • Click the **Report** icon. *Result*: The **Generate Report** dialog box opens. 
3	• Select a **Standard** report format. *Note*: The contents of a **Standard** report format is displayed in the **Contents** field. • Verify in the **Contents** field that the report format contains all the elements that you want to include. Click the **Edit** button to modify the report format if needed.
4	• Click the **Print** button. *Result*: The **Print** dialog box opens. • Choose what pages and how many copies to print. • Click **OK**. *Note*: Printers are set up in the **File** menu of the **UNICORN Manager**.

10.6.3 *How to edit an existing report format*

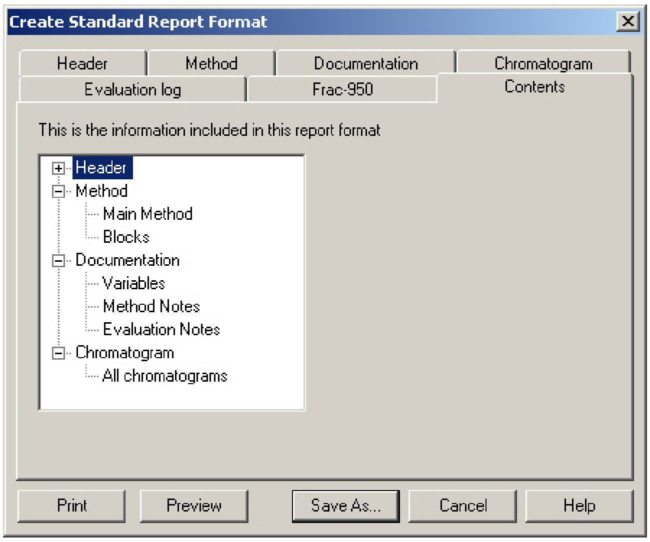
Introduction

This section describes how to edit an existing report format.

How to edit a standard report

The table below describes how to edit a standard report format in the **Evaluation** module.

Step	**Action**
1	Open a result file.
2	• Select **File:Report**. *or* • Click the **Report** icon. *Result*: The **Generate Report** dialog box opens.
3	• Select a **Standard** report format to edit. • Click the **Edit** button. *Result*: The **Edit Report Format** dialog box opens. • Select **Standard format** and click **OK**. *Result*: The **Edit Standard Report Format** dialog box opens.
4	Click the appropriate tabs and select the check boxes for each item that you want to include in the report format. *Note*: See **10.6.2 How to create and print a standard report** on page 274 for more information.

Step	Action
5	Click the **Contents** tab to see a list of all the selected items.  This is the information included in this report format
6	• Click the **Save As** button. *Result*: The **Save Report Format** dialog box opens. • Type a name in the **Report format name** text box. - Select the **Save as global format** check box to make the format available to other users. - Select the **Save as default report format** check box if desired (The format is saved as DEFAULT). • Click **OK**. *Result*: The **Generate Report** dialog box opens again. The new report format is saved and available in the **Format** list.
7	• Click the **Close** button. *or* • Click the **Edit** button to edit another report format.

How to edit a customized report

The table below describes how to edit a customized report format in the **Evaluation** module.

Step	Action
1	Open a result file.

Step	Action
2	• Select **File:Report**. *or* • Click the **Report** icon. *Result*: The **Generate Report** dialog box opens.
3	• Select a **Customized Report Format** to edit. • Click the **Edit** button. *Result*: The report format opens in the **Report Editor**.
4	• Double-click the report item you want to edit. • Make the desired changes in the dialog box. • Continue to edit all items until the format is complete. *Note*: See **10.6.1 How to create and print a customized report** on page 260 for more information.
5	• Select **File:Save As**. *Result*: The **Save Report Format** dialog box opens. • Type a name in the **Report format name** text box. - Select the **Save as global format** check box to make the format available to other users. - Select the **Save as default report format** check box if desired (The format is saved as DEFAULT). • Click **OK**. *Result*: The new report format is saved and available in the **Format** list.

10.7 Run documentation

Introduction

The full documentation for a method run is stored in the result file. This section describes:

- some of the contents of the run documentation,
- how to view and print the run documentation,
- how to save the method from the run as a new method.

How to view and print the run documentation

The table below describes how to view and print the run documentation.

Step	Action
1	Open a result file.
2	• Choose **View: Documentation** in the **Evaluation** module. *or* • Click the view **Documentation** icon. *Result*: The **Documentation** dialog box opens. See further information about some of the tabs below.
3	• Click the **Print** button. *Result*: The **Print** dialog box opens. • Select the documentation items you want to print and click **OK**.

The tabs of the Documentation dialog box

The table below describes the contents of some of the **Run Documentation** tabs.

Documentation tab	Contents
Variables	The **Variables** tab lists the parameters that were used during the method run.
Scouting	The **Scouting** tab displays the whole scouting scheme, with the values for the current result file displayed in yellow cells.

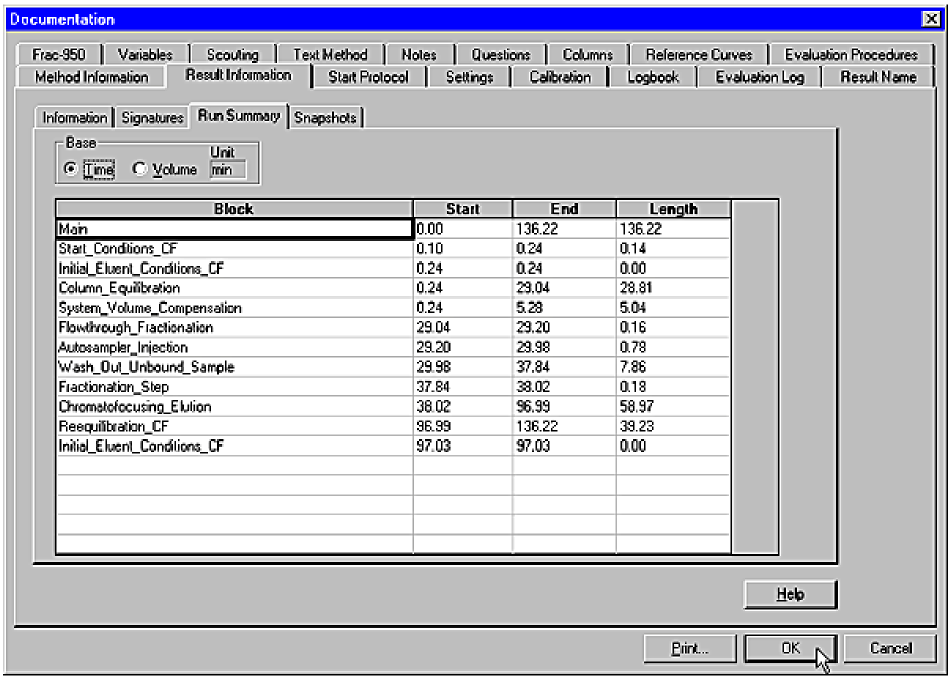
Documentation tab	Contents
Notes	The **Notes** tab displays the notes that you have made at various times during the method run. You are also able to type new comments on the **Evaluation Notes** sub-tab. *Note*: Click the **Find** button to search for a specific text string in the **Notes**.
Calibration	The **Calibration** tab displays the system calibrations and when and by whom they were made.
Logbook	The **Logbook** tab displays what happened during a method run. You can view information concerning alarms, the method, manual changes during the run, errors, etc. *Note*: Click the **Find** button to search for a specific text string in the **Logbook**.
Evaluation Log	The **Evaluation Log** lists all of the evaluation operations that you have performed for the result file during all sessions, including at the end of the method.
Method Information	The **Method Information** tab displays information about the method, such as the method name, the target system and the date of the last change. Information about the strategy includes name, date and size. There is also a sub-tab for **Signatures**.
Frac-950	The **Frac-950** tab displays the setup parameters for the fraction collector provided it is included in the strategy.
Result Information	See "The Result Information tab" in this section.

The Result Information tab

The **Result Information** tab displays information about the result file, such as

- the result file name
- the system that was used
- the last date it was changed.

Information about the strategy includes name, date and size. The **Run Summary** sub-tab is a summary of the run expressed in volume or time per block. There is also a sub-tab for **Signatures** and a sub-tab where all **Snapshots** that have been taken during the run are displayed.



Base: ● Time ○ Volume Unit: min

Block	Start	End	Length
Main	0.00	136.22	136.22
Start_Conditions_CF	0.10	0.24	0.14
Initial_Eluent_Conditions_CF	0.24	0.24	0.00
Column_Equilibration	0.24	29.04	28.81
System_Volume_Compensation	0.24	5.28	5.04
Flowthrough_Fractionation	29.04	29.20	0.16
Autosampler_Injection	29.20	29.98	0.78
Wash_Out_Unbound_Sample	29.98	37.84	7.86
Fractionation_Step	37.84	38.02	0.18
Chromatofocusing_Elution	38.02	96.99	58.97
Reequilibration_CF	96.99	136.22	39.23
Initial_Eluent_Conditions_CF	97.03	97.03	0.00

Save the method used for the run as a new method

You can save the method and the variables that were used for the run as a new method:

Step	Action
1	• Select the **Text Method** tab in the **Documentation** dialog box. • Click the **Save as** button. *Result*: The **Save As** dialog box opens.

Step	Action
2	• Select the appropriate destination folder. • Type a name in the **Method name** text box. • Select a system in the **For System** field. • Select a technique in the **Technique** field. • Click **OK**. *Result*: The method is saved.

11 How to edit results

Introduction

This chapter describes

- how to edit the results that are presented in the **Evaluation** module
- how to import and compare runs
- how to import and export results.

For more information about how to view results, see chapter **10 How to view results** on page 229.

In this chapter

This chapter contains the following sections

Topic	See
How to reduce noise and remove ghost peaks	11.1
How to subtract a blank run curve	11.2
How to add curves	11.3
How to enter and edit text in the chromatogram	11.4
How to pool fractions	11.5
How to match protein activity to a curve	11.6
How to rename chromatograms, curves and peak tables	11.7
How to import and compare different runs	11.8
How to import and export results	11.9
How to sign results electronically	11.10
How to save results and exit the Evaluation module	11.11

11.1 How to reduce noise and remove ghost peaks

Introduction

Sometimes the chromatograms contain curves with a noisy baseline. The noise can be caused by several factors, for example a dirty flow cell, air bubbles, electrical noise, dirty buffers, etc. The amount of noise can usually be reduced by taking proper precautions, for example filtration of buffers and instrument maintenance.

You can also use the smoothing function to reduce or remove background noise from a selected curve. Smoothing is always a compromise between noise removal and preservation of peak shape.

How to smooth a curve

The table below describes how to select a smoothing function and smooth a curve:

Step	Action
1	Select **Operations:Smooth**. *Result:* The **Smooth** dialog box is displayed.
2	Select the curve to be smoothed and its target destination.
3	Select the **Filter type** to be applied. The options are: • **Moving average**. Use this if you have noise along most of the curve. It affects peak height but not retention. There is little effect on the peak area. • **Autoregressive**. Use this if you have periodic noise along the whole curve. It affects peak height and retention, although this has little effect on the peak area. • **Median**. Use this if there is only one or a few noise spikes, for example caused by air bubbles, or if the noise is confined to only a small part of the curve. It can flatten peaks and affect peaks areas slightly, but does not affect retention. • **Savitzky-Golay**. Use this to calculate the smoothing and differentiation of data by a least squares technique.
4	Select an appropriate smoothing parameter value from **Light** to **Hard** for the selected filter in the **Filter Parameters** field. Use the slider, or insert a value manually in the text field. The smoothing effect increases with increasing parameter values. • Click **OK**.

Tip: Start with a low parameter value, for example the default value, and increase it until the best result is achieved. A useful strategy is to increase the parameter value by the default value for each try.

Note: By default, smoothed curves are given the suffix **SMTH**. The default curve name can be changed as needed.

11.2 How to subtract a blank run curve

Introduction

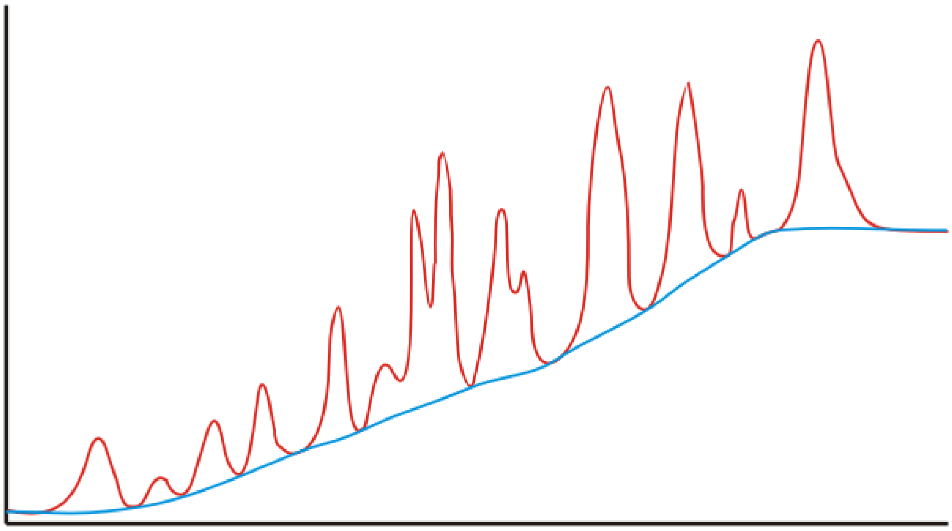
Subtracting a blank run curve is a frequently used function in presentations, especially if the curves have a drifting baseline or ghost peaks.

Ghost peaks

If the ghost peaks come from impurities in the eluents, all equilibration of the columns should be the same from method run to method run. If, for example, the equilibration volume with buffer A is larger before a blank run curve than before a separation, your ghost peaks might be higher in the blank run curve.

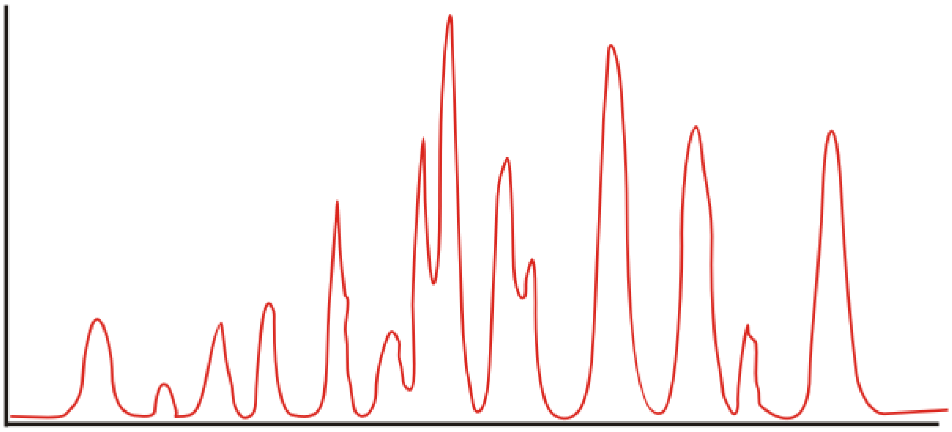
Example of a UV curve with baseline

The illustration below shows the UV curve with baseline prior to subtraction of the baseline:



Example of a UV curve after subtraction of the baseline

The illustration below shows the UV curve after subtraction of the baseline:



How to import a blank run curve

If a blank run curve was made, this might have been stored in another result file. If there is no blank run curve, you can create one with **Integrate:Calculate baseline**. The table below describes how to import the blank run curve:

Step	Action
1	Ensure that the destination chromatogram has been opened and is the active window on the screen.
2	Choose **File:Open:Curves**. *Result:* The **Open Curves** dialog box is displayed.
3	Double-click the result file that contains the blank run curve. *Result:* The curves in the first chromatogram are displayed.
4	• Select the appropriate chromatogram in the **Chromatogram list**. *Result:* The curves for that chromatogram are displayed on the **Available** list. • Select the curves that correspond to the blank run curve and click the **Select** button. *Result:* The selected curve is displayed on the **Selected curves** list.
5	• If you want to remove a curve from the list, select it and click the **Remove** button. • Click **OK** to import the curve.

Note: For more detailed information on how to import curves, chromatograms and other results see **11.8 How to import and compare different runs** on page 299.

How to subtract a blank run curve

You can subtract the blank run curve or the baseline from the sample curve. The table below describes how to do this:

Step	Action
1	Select **Operations:Subtract.** *Result:* The **Subtract** dialog box is displayed.
2	Select the sample chromatogram and curve in the left field and the baseline or blank run curve to be subtracted in the middle field. Click **OK**.

Note: All resulting curves from the subtract operation receive the **SUB** suffix by default. The default curve name can be changed as needed.

11.3 How to add curves

Introduction

In some method runs, several sequential chromatograms might have been created. This can occur, for example, when the instruction **New chromatogram** has been used in the method, thus creating different chromatograms during the run.

In order to view and evaluate the resultant curve of all the chromatogram parts, the curves must be added together. Usually, you have a number of chromatograms within the same result file and you want to add the curves. In some circumstances, curves might need to be imported from other result files.

Instruction

The table below describes how to add curves:

Step	Action
1	Select and view the first chromatogram in the sequence.
2	Choose **Operations:Add**. *Result:* The **Add** dialog box is displayed.
3	• Select the first curve in the desired sequence in the left field. • Select the second curve in the sequence in the middle field. • Click the **OK** button to add the two curves together in a new result curve.
4	• Open the **Add** dialog box again. • Select the result curve (**.ADD**) from the previous addition in the left field. • Select the next curve in the sequence in the middle field. • Click **OK** to add the two curves together in a new result curve.
5	Repeat steps 3 and 4 until all curves have been added together. The final curve should be the cumulative curve for the whole run.

Note: All curves created using the **Add** operation receive the **ADD** suffix by default. The default curve name can be changed as needed. The original curves are distinguished in the chromatogram by underlined curve names.

11.4 How to enter and edit text in the chromatogram

How to enter text

Text can be added to the chromatogram. The table below describes how to do this:

Step	Action
1	• Right-click the curves view of the chromatogram window and select **Add text** from the menu. *or* • Choose **Edit:Text:Add**.
2	• Click where you want to insert text in the chromatogram. *Result*: A text box opens. • Type the text. • Click outside the text box to set the text.

How to edit the text

The table below describes how to edit inserted text:

Step	Action
1	Choose **Edit:Text:Edit**. *Result:* The **Edit Texts** tab of the **Chromatogram Layout** dialog box is displayed.
2	• Select the text that you want to edit and make the appropriate changes in the **Selected text** field. • Click the **Change text** button or the **Delete text** button. • Use the **Font** and **Set Orientation** buttons if needed, and make the desired changes in the resulting dialog boxes. • Click **OK** to apply the changes.

Shortcut option

You can also right-click outside the text box and select **Edit Text Mode** from the shortcut menu. This activates all the text boxes in the chromatogram. The list below describes how to edit the text:

- Click the text and type the new text.
- Click outside the text box to set the text.

11.5 How to pool fractions

Introduction

Fractions are collected sequentially during a separation. Each fraction contains a set volume of sample. This section describes how to pool the information on several fractions into a new curve.

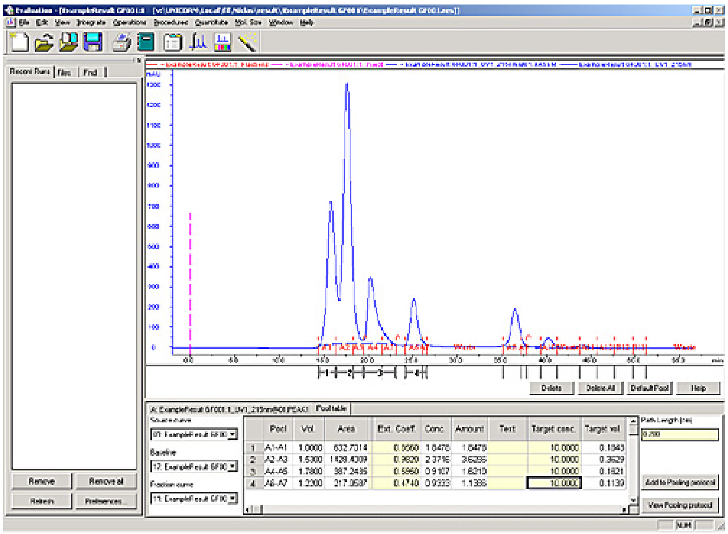
How to view the contents of a fraction

Each fraction is numbered according to its order in the sequence. The information is saved as a curve under the name **Fractions**.

- Select this curve on the **Curve** tab in the **Chromatogram Layout** dialog box to display the contents of each fraction in relation to the information displayed on the UV detection curve.

How to pool fractions

The table below describes how to pool fractions.

Step	Action
1	• Choose **Operations:Pool**. *Result*: UNICORN™ will automatically pool suitable fractions. The pooled fractions are listed in a table below the chromatogram and the pooled peaks are numbered sequentially in the chromatogram.  *Note*: Only adjacent fractions will be pooled. The fraction numbers for each pool are listed in the table as a range in retention order, e.g. A6-A7 etc.

Source curve: 01: ExampleResult GF00

Baseline: 17: ExampleResult GF00

Fraction curve: 11: ExampleResult GF00

Path Length (cm): 0.200

	Pool	Vol	Area	Ext. Coeff	Conc	Amount	Text	Target conc.	Target vol
1	A1-A1	1.0000	632.7014	0.5560	1.0470	1.0470		10.0000	0.1047
2	A2-A3	1.5300	1428.4309	0.3820	2.3716	3.6285		10.0000	0.3629
3	A4-A5	1.7800	387.2436	0.5960	0.9107	1.6210		10.0000	0.1621
4	A6-A7	1.2300	217.3587	0.4740	0.9233	1.1386		10.0000	0.1139

Step	Action
2	The pooled fractions can be adjusted manually: **To include or exclude adjacent fractions in a pool** • Click the numbered marker under the pool and drag the sideline. **To add more pools** • Click between the droplines under a fraction to create a new pool, and drag the sidelines to include more adjacent fractions. **To delete pools** • Click the numbered marker to select the pool and click the **Delete** button. Click the **Delete All** button to clear all pools. **To restore the pools created by UNICORN** • Click the **Default Pool** button.
3	Other curves can be selected for the operation: • Select another source curve from the **Source curve** droplist and click the **Default Pool** button. *or* • Select another baseline curve from the **Baseline** droplist and click the **Default Pool** button. *or* • Select another fraction curve from the **Fraction curve** droplist and click the **Default Pool** button. *Result*: The pooled fractions in the list are replaced by the pooled fractions for the selected curve.

How to create a pool fraction curve

The pooled fractions can be stored as a new curve.

Note: You must store the pooled fractions as a new curve in order to be able to proceed with other operations using the pooled fractions.

Step	Action
1	• Choose **Operations:Create Pool Fraction** curve. *Result*: The **Create Pool Fraction Curve** dialog box opens.

Step	Action
2	• Select a position where the curve will be stored from the **Save curve in** list. • If needed, type a new name in the **Curve name** text box. *Note*: The suggested curve name will have the default suffix **POOL**. • Click the **OK** button. *Result*: The **Pool Fraction** curve is displayed in the chromatogram.

How to show only the pooled fractions

The active chromatogram will now show both the original and the pooled fraction curves. The table below describes how to show only the pooled fractions.

Step	Action
1	• Choose **Edit:Chromatogram Layout**. *or* • Right-click in the chromatogram and choose **Properties** from the shortcut menu. *Result*: The **Chromatogram Layout** dialog box opens.
2	• Select the **Curve** tab. • De-select the check box for the original fraction curve (remove the check mark). *Result*: The original fraction curve is de-selected and is not displayed.

How to calculate concentration and amount in the pools

Protein concentrations

The protein concentration in the fractions are calculated using the following formula:

Concentration [mg/ml] = A / (d * 1000 *Ext.Coeff.)

A = Average fraction absorbance = **Area / Volume [mAu]**.

d = UV-cell path length [cm]

Ext.Coeff. = Protein coefficient at used wavelength. [l g^{-1} cm^{-1}]

Protein amounts

The total amount of protein found in the pool fraction is calculated using the following formula:

Amount [mg] = Concentration [mg/ml] * pooled fraction volume [ml]

How to calculate the concentrations and amounts:

- Type the UV path length expressed in centimeters in the **Path Length** text box.
- Type the extiction coefficient in the **Ext.Coeff.** table cell for each pool.

Result: The sample concentration and amount for each pool is calculated in the corresponding table cell.

How to determine a pool target volume

The **Target conc.** and **Target vol.** cells are used to calculate the pool volume at a specific concentration level. The result can then be used to determine if the pool needs to be concentrated further or diluted.

- Type the desired concentration level (mg/ml) in the **Target conc.** table cell.

Result: The corresponding target volume is calculated in the **Target vol**. table cell using the following formula:

Target vol. = Conc. * (Vol./Target conc.)

How to use the Pooling Protocol

A protocol of the pooled fractions can be printed for use when handling the samples. The table below describes how to add pools to the **Pooling Protocol** and send the list to a printer or export the list to a file.

Step	Action
1	• Open a result file in the **Evaluation** module. • Pool fractions as described in How to pool fractions above. • Click the **Add to Pooling Protocol** button. *Result*: The pooled fractions from the active result file is added to the **Pooling Protocol**.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>

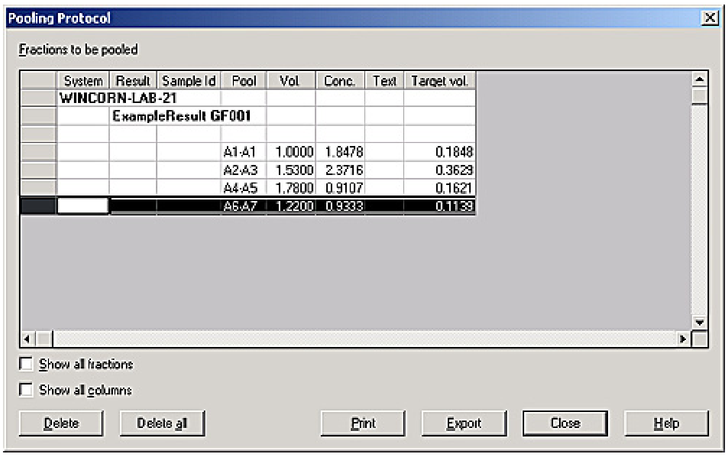
- Repeat step 1 to add pooled fractions from other result files.

Note: You will be asked to save the current file when you open the next. The pool table will not be saved.

</td></tr>
<tr><td>3</td><td>

- Click the **View Pooling Protocol** button.

Result: The **Pooling Protocol** dialog box opens.



<table>
<tr><th>System</th><th>Result</th><th>Sample Id</th><th>Pool</th><th>Vol.</th><th>Conc.</th><th>Text</th><th>Target vol.</th></tr>
<tr><td>WINCORN-LAB-21</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>ExampleResult GF001</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td>A1-A1</td><td>1.0000</td><td>1.8478</td><td></td><td>0.1848</td></tr>
<tr><td></td><td></td><td></td><td>A2-A3</td><td>1.5300</td><td>2.3716</td><td></td><td>0.3629</td></tr>
<tr><td></td><td></td><td></td><td>A4-A5</td><td>1.7800</td><td>0.9107</td><td></td><td>0.1621</td></tr>
<tr><td></td><td></td><td></td><td>A6-A7</td><td>1.2200</td><td>0.9333</td><td></td><td>0.1139</td></tr>
</table>

☐ Show all fractions

☐ Show all columns

</td></tr>
</table>

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>

- Click the **Show all fractions** checkbox to display the individual fractions instead of fraction ranges for the pools.
- Click the **Show all columns** checkbox to display all the information columns from the **Pool table**.

Possible actions in the Pooling Protocol

To delete a single pool...

- select a pool and click the **Delete** button

To clear the whole protocol...

- click the **Delete all** button.

To print the protocol on the default Windows™ printer...

- click the **Print** button to print the protocol on the default Windows printer.

To export the protocol...

- click the **Export** button to save the protocol in one of the following formats:
  - text (.txt)
  - Excel (.xls)
  - HTML (.htm)
  - XML (.xml)

Note: The protocol is automatically saved for the user. The pooling protocol will be available again when the user starts UNICORN the next time.

</td></tr>
<tr><td>5</td><td>

- Click the **Close** button to close the **Pooling Protocol** dialog box.

Result: If the protocol was exported or only edited, the dialog box will close. If the protocol was printed, a dialog box will open asking if you want to delete the list and start a new.

</td></tr>
</table>

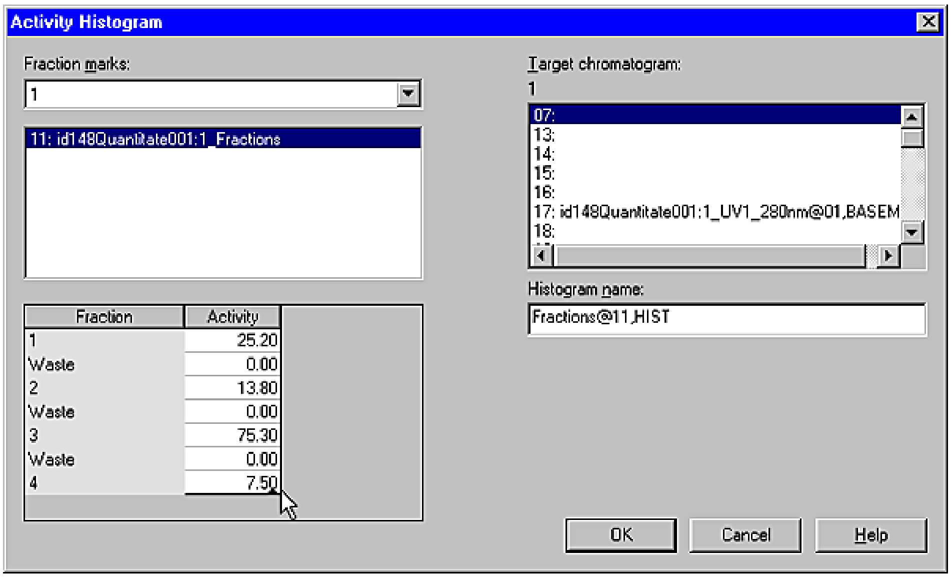
11.6 How to match protein activity to a curve

Introduction

You can compare data from the results of protein activity assays, such as ELISA, with the data contained in the UV curve. The activity curve and the UV curve can be compared in a combined presentation.

The Activity Histogram dialog box

The illustration below shows the **Activity Histogram** dialog box:



Fraction marks: 1

11: id148Quantitate001:1_Fractions

Target chromatogram: 1

07:
13:
14:
15:
16:
17: id148Quantitate001:1_UV1_280nm@01,BASEM
18:

Histogram name: Fractions@11,HIST

Fraction	Activity
1	25.20
Waste	0.00
2	13.80
Waste	0.00
3	75.30
Waste	0.00
4	7.50

How to enter protein activity values for comparison

The table below describes how to enter the values from a protein activity assay in a comparison histogram:

Step	Action
1	Choose **Operations:Activity Histogram**. *Result*: The **Activity Histogram** dialog box opens.
2	By default, the fraction curve for the specific chromatogram is selected. • If necessary, change the source and target chromatograms. All the component fractions of the fraction curve are listed in the **Fraction/Activity** field. • Type an activity value for each fraction in the **Activity** column. • Click **OK**.

11.7 How to rename chromatograms, curves and peak tables

Instruction

The table below describes how to rename chromatograms, curves or peak tables in the **Evaluation** module:

Step	Action
1	Choose **Edit:Rename** and the relevant option **Chromatogram**, **Curve** or **Peak Table**. *Result*: The **Rename** dialog box opens.
2	• Select the appropriate object. • Type a new name in the **Name** field. • Click **OK**.

Note: The original raw data curves cannot be renamed. They will not be listed as options in the dialog box.

11.8 How to import and compare different runs

Introduction

This section describes

- how to make comparisons between curves or chromatograms from different runs
- how to present curves or chromatograms from different runs.
- how to compare curve parameters among curves from different runs
- how to view several chromatograms at the same time
- how to overlay curves from different runs in one chromatogram
- how to stack curves from different runs in one chromatogram
- how to stretch curves to make comparisons easier
- how to create mirror images

In this section

This section contains the following sub-sections

Topic	See
How to use the Multifile Peak Compare wizard	11.8.1
How to import and compare chromatograms	11.8.2
How to import and compare curves	11.8.3
How to stack and stretch curves	11.8.4
How to produce a mirror image	11.8.5

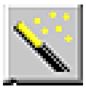
11.8.1 How to use the Multifile Peak Compare wizard

Introduction

This section describes how to use the **Multifile Peak Compare** wizard to make comparisons between different results, for example, by comparing area, retention etc. The difference can be presented graphically or in a spreadsheet.

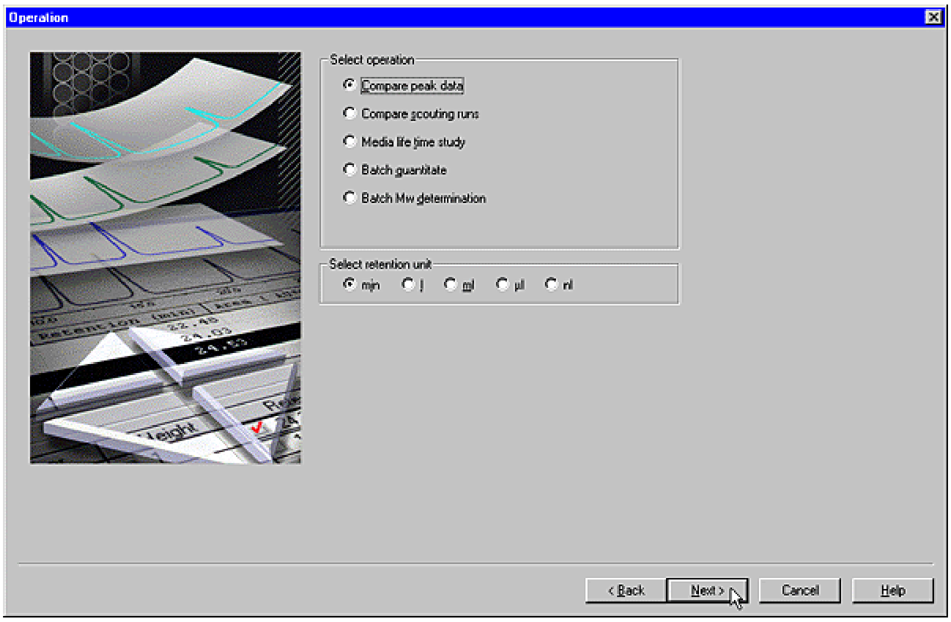
Step 1: How to select the Operation

The table below describes how to select the operation:

Step	Action
1	In the **Evaluation** module, • choose **File:Multifile Peak Compare:Start Wizard** *or* • click the **Multifile Peak Compare** toolbar icon:  *Result*: The **Multifile Peak Compare** wizard entry dialog box is displayed.
2	Click the **Next** button to display the **Operation** dialog box.
3	Select • one of the available operations (see descriptions of the operations below this table) • a retention unit. If you select **Batch quantitate**: • Select a quantitation table in the **Select quantitation table** field. If you select **Batch Mw determination**: • Select a molecular size table in the **Select mol. size table** field. Click the **Next** button to proceed to the **Data Selection** dialog box.

The Operation dialog box

The illustration below displays the **Operation** dialog box:



Operation

Select operation

● Compare peak data
○ Compare scouting runs
○ Media life time study
○ Batch quantitate
○ Batch Mw determination

Select retention unit

● min ○ l ○ ml ○ µl ○ nl

The operation options

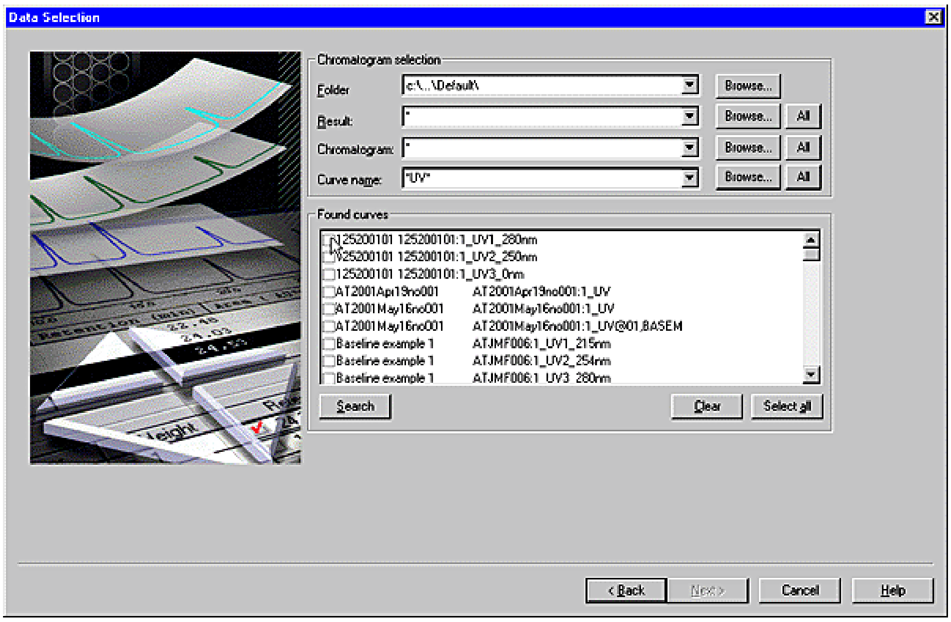
The table below is a brief description of the operation options:

Operation	Description
Compare peak data	This option is used to compare different results.
Compare scouting runs	This option is used to compare the results from scouting runs. The scouting variables can be displayed.
Media life time study	This option features different default values than the **Compare peak data** option, specially selected to measure changes in the column media.
Batch quantitate	This option is used to run several quantitations. This is an alternative to **Quantitate:Calculate Amount and Conc.** which is used to quantitate single results. A quantitation table must be created before this option can be used. This option is available only if the **Analysis** module has been installed.

Operation	Description
Batch Mw determination	This option is used to batch run molecular size calculations. This is an alternative to **Mol. Size:Calculate Mol.Size**, which is used for single calculations. A molecular size table must be created before this option can be used. This option is available only if the **Analysis** module has been installed.

The Data Selection dialog box

The illustration below shows the **Data Selection** dialog box.



Folder: c:\...\Default\

Result: *

Chromatogram: *

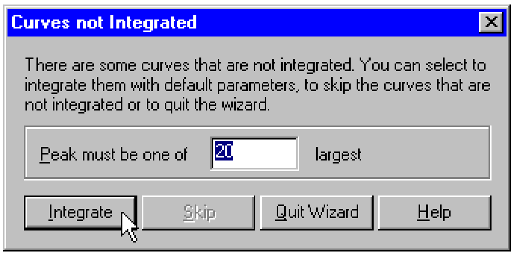
Curve name: "UV"

- ☐ 125200101 125200101:1_UV1_280nm
- ☐ 125200101 125200101:1_UV2_250nm
- ☐ 125200101 125200101:1_UV3_0nm
- ☐ AT2001Apr19no001 AT2001Apr19no001:1_UV
- ☐ AT2001May16no001 AT2001May16no001:1_UV
- ☐ AT2001May16no001 AT2001May16no001:1_UV@01,BASEM
- ☐ Baseline example 1 ATJMF006:1_UV1_215nm
- ☐ Baseline example 1 ATJMF006:1_UV2_254nm
- ☐ Baseline example 1 ATJMF006:1_UV3_280nm

Step 2: How to select data to compare

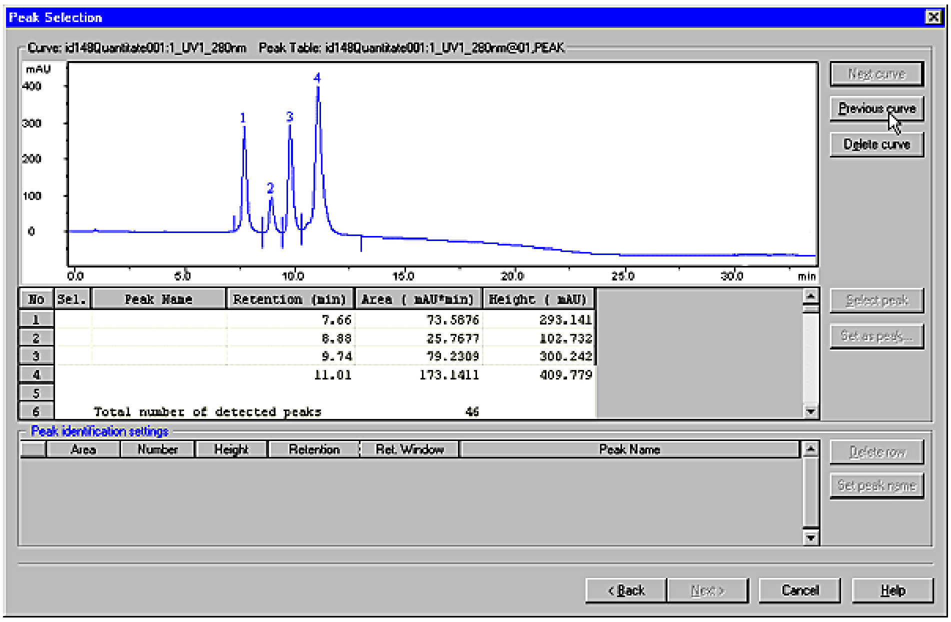
The table below describes how to select data to compare:

Step	Action
1	• Use the drop-down lists and **Browse** buttons in the **Chromatogram selection** field to specify the result files, chromatograms and curves for comparison. • Click the **All** button if you want to select all available results, chromatograms or curves.

Step	Action
2	- Click the **Search** button in the **Found curves** field. *Result*: A list of all curves that matched the search criteria is displayed in the **Found curves** field.
3	- Select the check boxes (or click the **Select All** button) of the desired curves within the **Found curves** field. - Click the **Next** button to proceed to the **Peak Data Selection** dialog box.
4	- If all the chosen curves have been integrated, go to "Step 3: How to select the peaks" in this section. - If any of the chosen curves have *not* been integrated, the **Curves not Integrated** dialog box is first displayed:  Curves not Integrated There are some curves that are not integrated. You can select to integrate them with default parameters, to skip the curves that are not integrated or to quit the wizard. Peak must be one of 20 largest - If desired, change the default value for the peak number selection filter. - Click the **Integrate** button. *Result*: The **Peak Selection** dialog box is displayed. *Note*: If the results from the automatic peak integration is not satisfactory you must cancel the wizard and perform the integration manually. See **12.1.2** on page 344.

The Peak Selection dialog box

The illustration below displays the **Peak Selection** dialog box:



Curve: id148Quantitate001:1_UV1_280nm Peak Table: id148Quantitate001:1_UV1_280nm@01,PEAK

No	Sel.	Peak Name	Retention (min)	Area (mAU*min)	Height (mAU)
1			7.66	73.5876	293.141
2			8.88	25.7677	102.732
3			9.74	79.2309	300.242
4			11.01	173.1411	409.779
5					
6		Total number of detected peaks		46	

Dialog box description

The dialog box displays the following properties for the first of the chosen curves:

- The integrated peak and the associated peak table
- The **Peak identification settings** table. Its purpose is to identify the peak parameter to be used in the comparison.

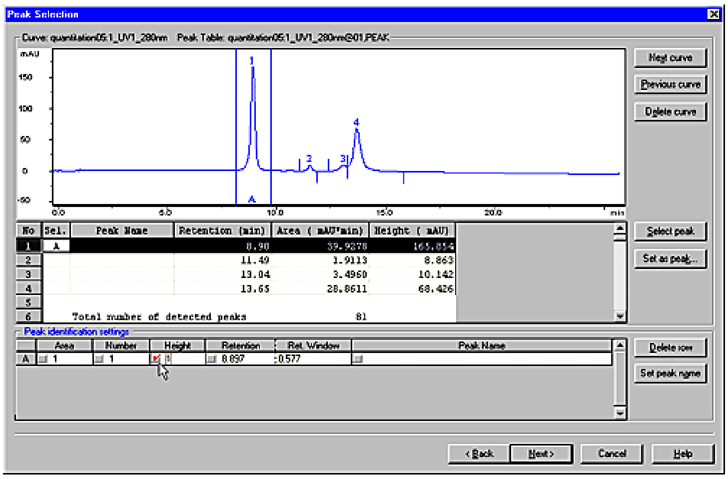
How to adjust improper peak integrations

The table below describes what to do if the peaks in the curve window do not appear to be integrated properly (for example if ghost peaks are labelled).

Step	Action
1	Click the **Cancel** button to quit the wizard.
2	Perform a peak integration (see **12.1.2 How to perform a peak integration** on page 344) and verify that the resulting curve is properly integrated.
3	Repeat the **Multifile Peak Compare** wizard operation.

Step 3: How to select the peaks

The table below describes how select peaks in the **Peak Selection** dialog box:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Choose a curve in the curve window:
Double-click the peak, or click the peak once and then click the Select peak button.
<i>Result</i>: The peak is assigned a letter (A, B, C....) and the peak parameters are displayed in the Peak identification settings table.</td></tr>
<tr><td>2</td><td>Set the desired peak identification criterion:
Click the desired parameter value in the Peak identification settings table.
<i>Example</i>: If you have selected the highest peak in the curve and want to compare the highest peak among all curves, select the Height check box.
In the illustration below, the initial (A) peak and the Height check box have been selected:


Curve: quantitation05:1_UV1_280nm Peak Table: quantitation05:1_UV1_280nm@01.PEAK
<table>
<tr><th>No</th><th>Sel.</th><th>Peak Name</th><th>Retention (min)</th><th>Area (mAU*min)</th><th>Height (mAU)</th></tr>
<tr><td>1</td><td>A</td><td></td><td>8.90</td><td>39.9278</td><td>165.854</td></tr>
<tr><td>2</td><td></td><td></td><td>11.49</td><td>1.9113</td><td>8.863</td></tr>
<tr><td>3</td><td></td><td></td><td>13.04</td><td>3.4960</td><td>10.142</td></tr>
<tr><td>4</td><td></td><td></td><td>13.65</td><td>28.8611</td><td>68.426</td></tr>
<tr><td>5</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>6</td><td></td><td>Total number of detected peaks</td><td></td><td>81</td><td></td></tr>
</table>
<table>
<tr><th></th><th>Area</th><th>Number</th><th>Height</th><th>Retention</th><th>Ret. Window</th><th>Peak Name</th></tr>
<tr><td>A</td><td>☐ 1</td><td>☐ 1</td><td>☒</td><td>☐ 8.897</td><td>±0.577</td><td>☐</td></tr>
</table></td></tr>
<tr><td>3</td><td>If desired, you can assign a name to a chosen peak:
Click the name of the row, for example A.
Click the Set peak name button.
Type a new name and click OK.
<i>Note</i>: This can be useful when you compare multiple peak parameters and you wish to have peak names other than “Peak A”, “Peak B”, etc. to simplify peak identification and clarity f.ex. when comparing peak data between batch quantitated results.</td></tr>
<tr><td>4</td><td>Repeat steps 1-3 for other desired peaks in the current curve.</td></tr>
</table>

Step	Action
5	Use the **Next curve** and **Previous curve** buttons to navigate forward and backward among your selected curves and manually check the selections made by the software if necessary.
6	**Other possible actions you can perform** • If the current curve does not prove useful for your comparison, click the **Delete curve** button to delete it from the comparison. • Click the **Back** button to navigate back to the **Data Selection** dialog box and add new curves to your comparison. See also How to change the peak identification below.
7	When all peak selections and identification settings are complete, click the **Next** button to proceed to the **Peak Data Selection** dialog box.

Note: Click and drag in the curve window to zoom into selected peaks to simplify accurate peak identification. Right-click and click the **Reset Zoom** button to reset the zoom to the full view.

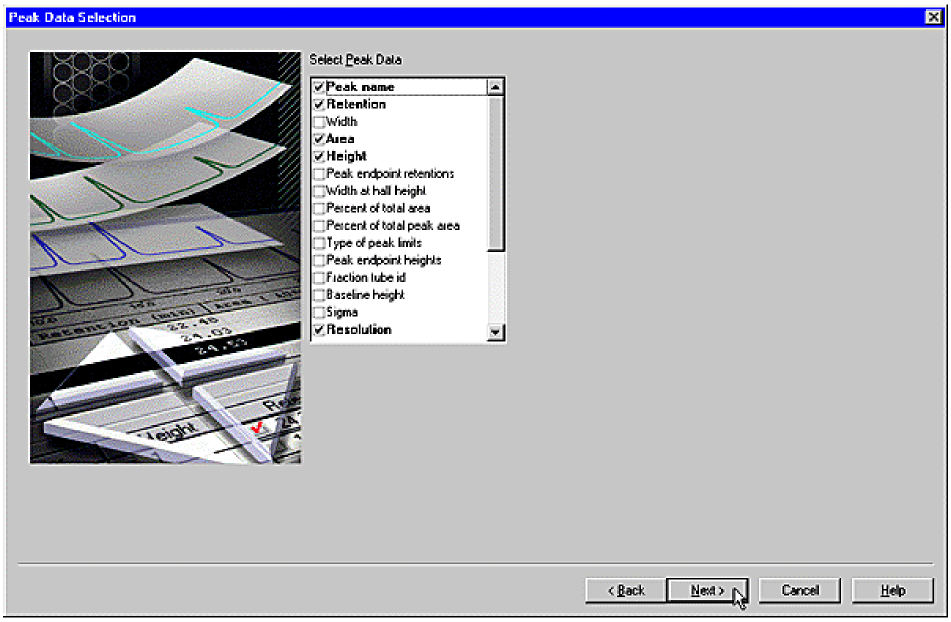
How to change the peak identification

In the **Peak identification settings table**, each column identifies a peak parameter to be compared among all peaks. If UNICORN has identified other peaks than the intended ones, you can change the peak identification manually. The table below describes how to change the identification:

If you want to...	then...
remove a peak identification	• click the desired peak in the curves window • click the **Set as peak** button • choose **None** in the **Set As Peak** dialog box • click **OK**.
replace or add a peak identification	• click a peak in the curves window • click the **Set as peak** button • choose a letter in the **Set As Peak** dialog box • click **OK**.
remove a row from the table	• select the row • click the **Delete row** button. *Note*: If you click **Delete row** without first selecting a row, the first row (A) is deleted by default.

Step 4: How to select the Peak Data

The illustration below displays the **Peak Data Selection** dialog box:



Select Peak Data

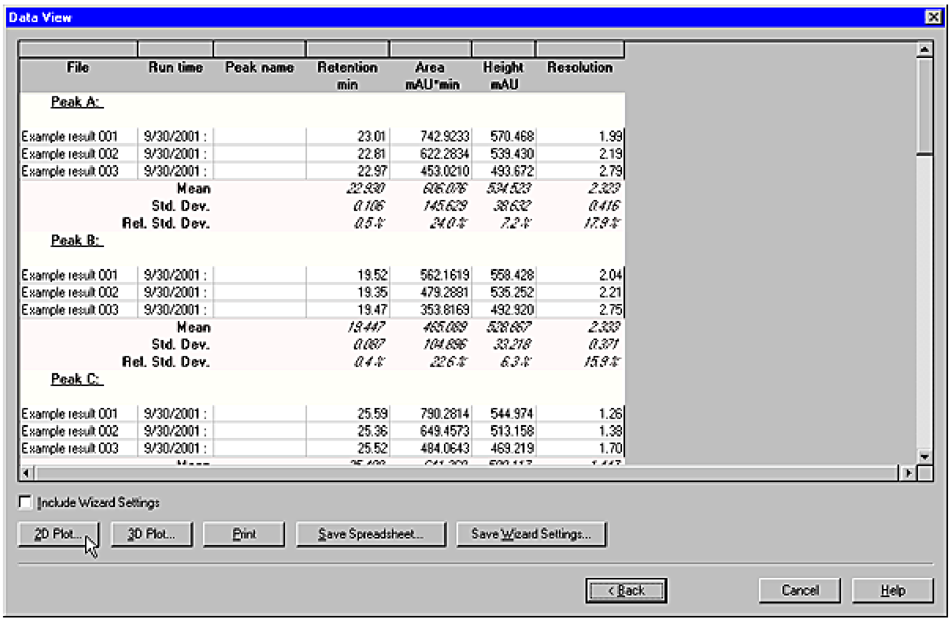
- ☑ Peak name
- ☑ Retention
- ☐ Width
- ☑ Area
- ☑ Height
- ☐ Peak endpoint retentions
- ☐ Width at half height
- ☐ Percent of total area
- ☐ Percent of total peak area
- ☐ Type of peak limits
- ☐ Peak endpoint heights
- ☐ Fraction tube id
- ☐ Baseline height
- ☐ Sigma
- ☑ Resolution

The table below describes how to select the peak data:

Step	Action
1	• In the **Select Peak Data** list, select the peak characteristics on the list that you want to include in your comparisons. • If available, select the appropriate **Scouting** variables.
2	• Click the **Next** button and proceed to step 5, How to use the **Data View** dialog box below. *Note*: If **Media life time study** was chosen in the **Operation** dialog box when the wizard was started, **2D Plot** is selected in the **Data View** dialog box.

Step 5: How to use the Data View dialog box

The **Data View** dialog box presents a comparison of the chosen data for the designated peak comparisons. The illustration below shows the dialog box:



File	Run time	Peak name	Retention min	Area mAU*min	Height mAU	Resolution
Peak A:						
Example result 001	9/30/2001 :		23.01	742.9233	570.468	1.99
Example result 002	9/30/2001 :		22.81	622.2834	539.430	2.19
Example result 003	9/30/2001 :		22.97	453.0210	493.672	2.79
		Mean	22.930	606.076	534.523	2.323
		Std. Dev.	0.106	145.629	38.632	0.416
		Rel. Std. Dev.	0.5 %	24.0 %	7.2 %	17.9 %
Peak B:						
Example result 001	9/30/2001 :		19.52	562.1619	558.428	2.04
Example result 002	9/30/2001 :		19.35	479.2881	535.252	2.21
Example result 003	9/30/2001 :		19.47	353.8169	492.920	2.75
		Mean	19.447	465.089	528.867	2.333
		Std. Dev.	0.087	104.896	33.218	0.371
		Rel. Std. Dev.	0.4 %	22.6 %	6.3 %	15.9 %
Peak C:						
Example result 001	9/30/2001 :		25.59	790.2814	544.974	1.26
Example result 002	9/30/2001 :		25.36	649.4573	513.158	1.38
Example result 003	9/30/2001 :		25.52	484.0643	469.219	1.70

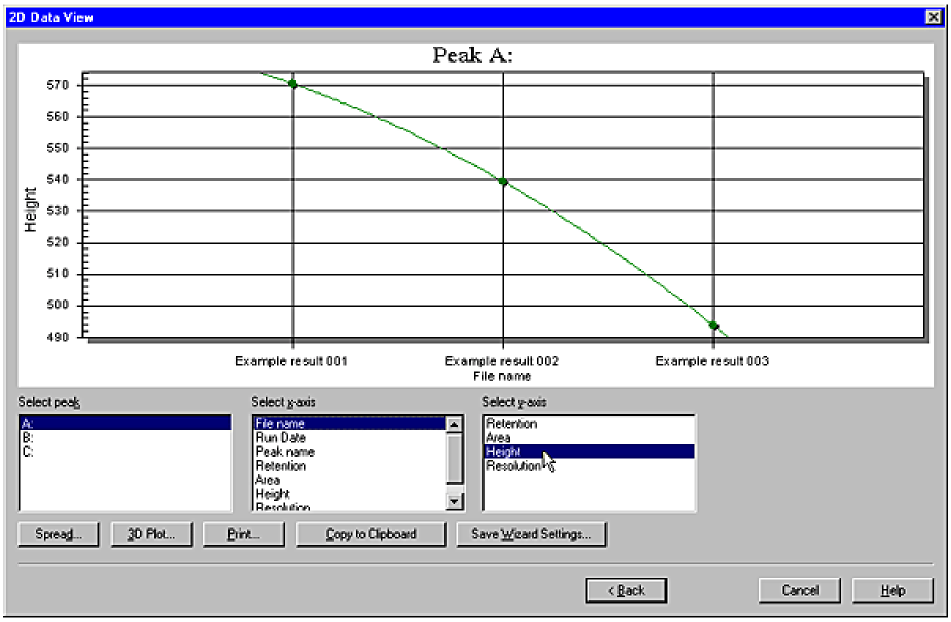
☐ Include Wizard Settings

The table below describes how to use the command buttons of the dialog box:

Command button	Function
2D Plot	Displays the data in 2-dimensional plot. See "How to use the 2D Data View dialog box" below.
3D Plot	Displays the data in 3-dimensional plot. See "How to use the 3D Data View dialog box" below..
Print	Prints the spreadsheet.
Save Spreadsheet	Allows you to save the data in different formats: • Excel (.xls) • Tabbed text (.txt) • FarPoint spread (.ss3)
Save Wizard Settings	See "How to save the Wizard Settings" below.
Cancel	Ends the **Multifile Peak Compare** wizard.

How to use the 2D Data View

The **2D Data View** dialog box presents a two-dimensional plot of a selected peak. See also "How to use the 2D Data View shortcut menu" below. The illustration below shows the dialog box:



Select peak: A:

Select x-axis: File name

Select y-axis: Height

The list boxes

Use the list boxes to select which peak to plot and the units of the x- and y-axes.

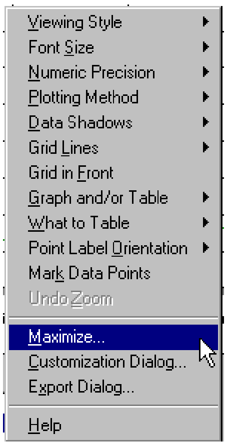
The command buttons

The table below describes how to use the command buttons of the dialog box:

Command button	Description
Spread	Returns to the **Data View** dialog box.
3D Plot	Displays the data in 3-dimensional plot. See "How to use the 3D Data View dialog box" below.
Print	Prints the spreadsheet.
Copy to Clipboard	Stores a figure for transfer to an external program.
Save Wizard Settings	See "How to save the Wizard Settings" below.
Cancel	Ends the **Multifile Peak Compare** wizard.

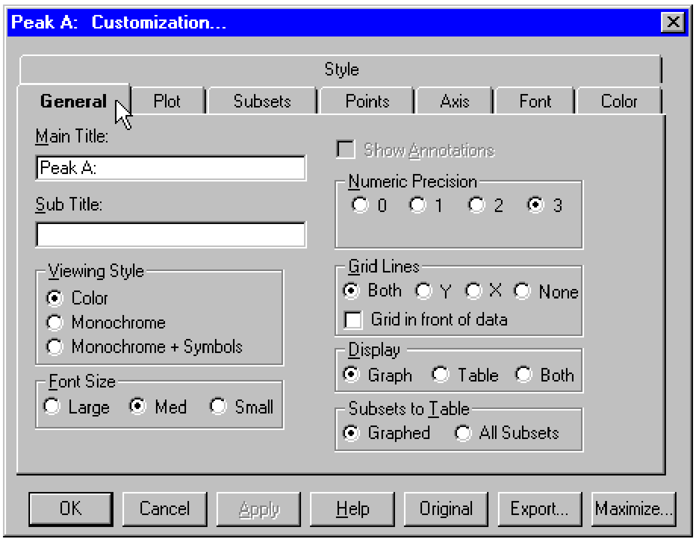
How to use the 2D Data View shortcut menu

Click the right mouse button in the plot area of the **2D Data View** dialog box to open the shortcut menu. See illustration below:



A wide array of plot presentation options can be found on the shortcut menu. Two of them are described below:

- Select **Customization Dialog** to open a dialog box which allows further customization of the graph:


Peak A: Customization...

Main Title: Peak A:

Sub Title:

Viewing Style: ● Color ○ Monochrome ○ Monochrome + Symbols

Font Size: ○ Large ● Med ○ Small

☐ Show Annotations

Numeric Precision: ○ 0 ○ 1 ○ 2 ● 3

Grid Lines: ● Both ○ Y ○ X ○ None

☐ Grid in front of data

Display: ● Graph ○ Table ○ Both

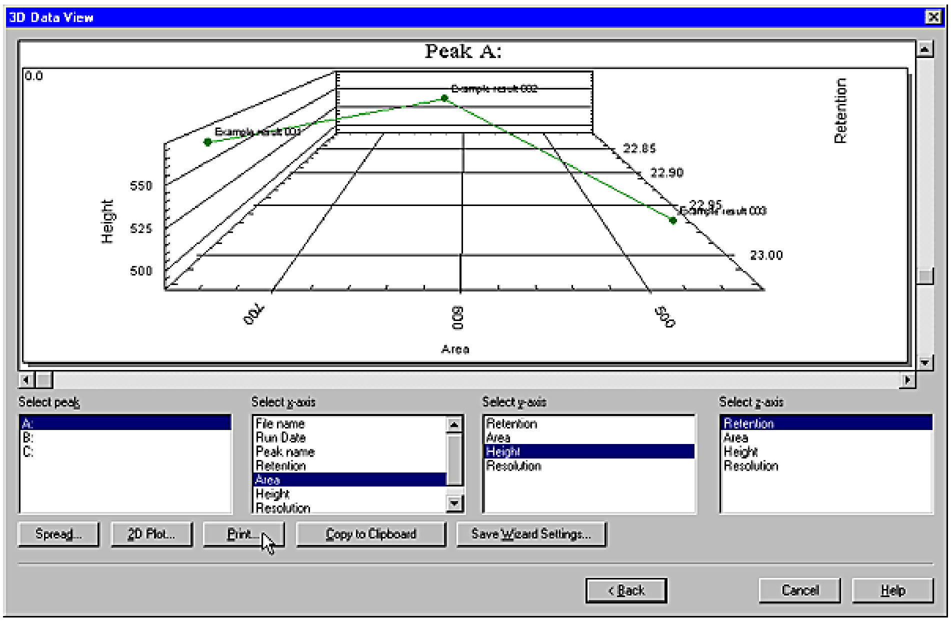
Subsets to Table: ● Graphed ○ All Subsets

- Select **Export Dialog** to export the view.

  Note: You can also click the **Export** button from the **Customization** dialog box.

How to use the 3D Data View dialog box

The **3D Data View** dialog box presents a three-dimensional plot of a selected peak. See also "How to use the 3D Data View shortcut menu" below. The illustration below shows the dialog box:



Select peak: A:, B:, C:

Select x-axis: File name, Run Date, Peak name, Retention, Area, Height, Resolution

Select y-axis: Retention, Area, Height, Resolution

Select z-axis: Retention, Area, Height, Resolution

The list boxes

Use the list boxes to select which peak to plot and the units of the x-, y- and z-axes.

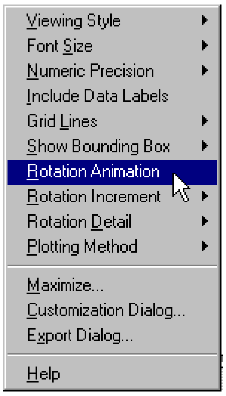
The command buttons

The table below describes how to use the command buttons of the dialog box:

Command button	Function
Spread	Returns to the **Data View** dialog box.
2D Plot	Displays the data in 2-dimensional plot. See "How to use the 2D Data View dialog box" above.
Print	Prints the spreadsheet.
Copy to Clipboard	Stores a figure for transfer to an external program.
Save Wizard Settings	See "How to save the Wizard Settings" below.
Cancel	Ends the **Multifile Peak Compare** wizard.

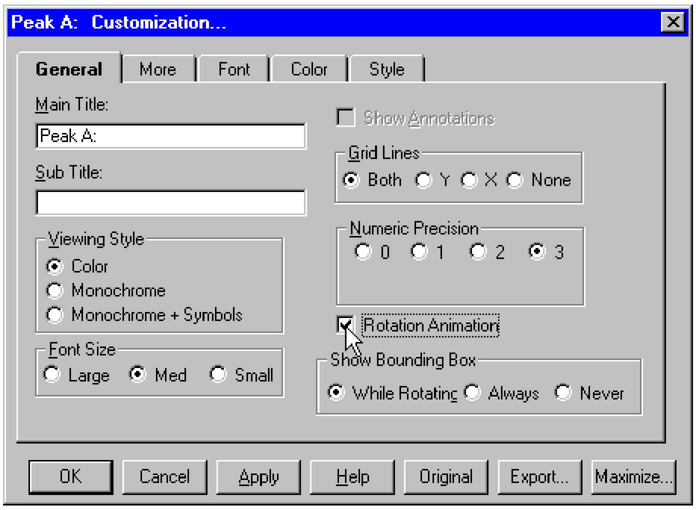
How to use the 3D Data View shortcut menu

Click the right mouse button in the plot area of the **3D Data View** dialog box to open the shortcut menu. See illustration below:



The **3D Data View** shortcut menu differs some from the **2D Data View** shortcut menu and allows the figure to be viewed by animated rotation. The shortcut menu displays different plot presentation options.

- Select **Customization Dialog** to open a dialog box that allows further customization of the graph:



Main Title: Peak A:

Sub Title:

Viewing Style: ● Color ○ Monochrome ○ Monochrome + Symbols

Font Size: ○ Large ● Med ○ Small

☐ Show Annotations

Grid Lines: ● Both ○ Y ○ X ○ None

Numeric Precision: ○ 0 ○ 1 ○ 2 ● 3

☑ Rotation Animation

Show Bounding Box: ● While Rotating ○ Always ○ Never

- Select **Export Dialog** to export the view.

Note: You can also click the **Export** button from the **Customization** dialog box.

How to save the Wizard Settings

The wizard settings can be saved from either of these dialog boxes:

- The **Data View** dialog box
- The **2D Data View** dialog box
- The **3D Data View** dialog box

The table below describes how to save the wizard settings:

Step	Action
1	• Click the **Save Wizard Settings** button. *Result*: The **Save Wizard Settings** dialog box opens.
2	Type a name in the **Wizard settings name** field.
3	• If the settings are to be used by all users on the system, select the **Global wizard settings** check box. • Click **OK**. • Click **Cancel** to close the wizard. *Note*: The **Global wizard settings** check box can also be used to toggle between lists of stored global and stored user settings.

How to open the saved wizard settings

The table below describes how to open the saved wizard settings:

Step	Action
1	• Choose the **File:Multifile Peak Compare:Start Wizard With Settings** menu item. *Result*: The **Select Wizard Settings** dialog box opens.
2	• Select the desired saved settings from the list. • Click **OK**. *Result*: The **Multifile Peak Compare** wizard opens with the saved settings. *Note*: The **Global wizard settings** check box is used to toggle between lists of stored global and stored user settings.

11.8.2 *How to import and compare chromatograms*

Introduction

This section describes

- how to import chromatograms from other result files,
- how to compare with chromatograms in an already opened result file.

Commands to use

Two commands in the **Evaluation** module can be used to import chromatograms from result files into an already opened result file:

- **File:Open to compare**

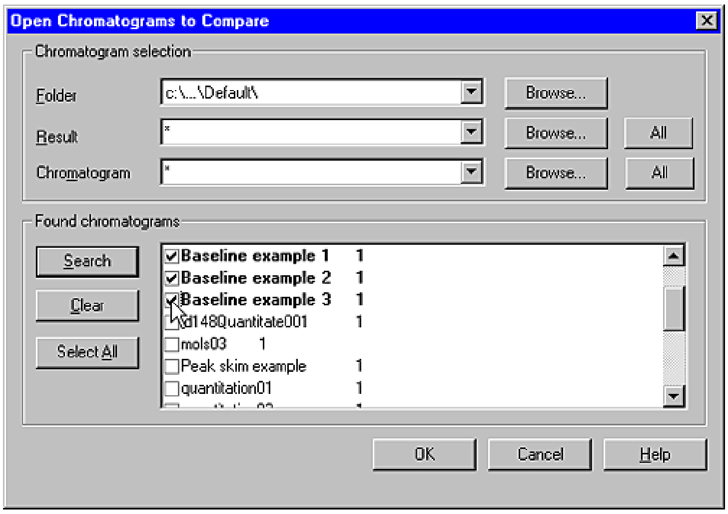
  This is the preferred option when you search for many chromatograms in a *specific* folder based on defined selection criteria. See "How to import chromatograms with the command **File:Open to compare**" below.

- **File:Open**

  This is the preferred option to import any individual chromatograms from result files in *different* folders. See "How to import chromatograms with the command **File:Open**" below.

How to import chromatograms with the command File:Open to compare

The table below describes how to import chromatograms with the **File:Open to compare** command. The search is performed at specific locations or with specific search criteria. This method is useful if you, for example, want to import chromatograms from all files of a scouting folder.

Step	Action
1	Choose **File:Open to compare:Chromatograms** in the **Evaluation** module. *Result*: The **Open Chromatogram to Compare** dialog box is displayed.  Open Chromatograms to Compare Chromatogram selection Folder c:\...\Default\ Result * Chromatogram * Found chromatograms ☑ **Baseline example 1** 1 ☑ **Baseline example 2** 1 ☑ **Baseline example 3** 1 ☐ d148Quantitate001 1 ☐ mols03 1 ☐ Peak skim example 1 ☐ quantitation01 1 Browse... Browse... All Browse... All Search Clear Select All OK Cancel Help

Step	Action
2	• Click the **Search** button in the **Found chromatograms** field and a list of chromatograms will be displayed based on the designated search criteria. • A new search can be performed with new search criteria without erasing the first found chromatograms from the list. • Select the chromatograms that you want to import. If you click the **Select All** button, all the displayed chromatograms will be imported. • If you want to clear the list of displayed chromatograms, click the **Clear** button. • Click **OK**. *Result*: All the selected chromatograms are shown in the **Evaluation** workspace. *Note*: If the names of the imported chromatograms already are used they will be sequentially numbered for identification purposes. Up to 10 chromatograms can be made available at the same time in the **Evaluation** workspace.

How to import chromatograms with the command File:Open

The table below describes how to import chromatograms one by one, using the command **File:Open**:

Step	Action
1	Choose **File:Open:Chromatogram** in the **Evaluation** module. *Result*: The **Open Chromatograms** dialog box is displayed.
2	Double-click a result file to select it. *Result*: All the chromatograms contained in the result file will be displayed in the **Available** field.
3	• Select the chromatogram(s) of interest and click the **Select** button. *Result*: Selected chromatograms are added to the **Selected chromatograms** list. *Note*: Chromatograms can be deselected with the **Remove** button.

Step	Action
4	• Repeat steps 2-3 if you want to import chromatograms from other result files. • Click **OK**. *Note*: If the names of the imported chromatograms already are used they will be sequentially numbered for identification purposes. Up to 10 chromatograms can be made available at the same time in the **Evaluation** workspace.

How to display and compare the imported chromatograms

The table below describes how to simultaneously display and compare the imported chromatograms:

Step	Action
1	In the **Evaluation** module, select • **Window:Tile** to display the chromatograms side by side. *or* • **Window:Cascade** to display the chromatograms in layers. *Note*: Chromatogram windows can be individually sized and the presentation of the curves changed.
2	**Display all chromatograms on the same scale** • Open the **Chromatogram Layout** dialog box for any chromatogram • Make the changes to the chromatogram axes. • Select the **Apply to all chromatograms** option.

Note: Imported chromatograms cannot be shown with column volume as the X-axis base.

11.8.3 How to import and compare curves

Introduction

This section describes how to import or copy curves from different runs into one chromatogram for comparison.

Commands to use

Two commands can be used to import curves from result files into one chromatogram:

- **File:Open to compare**

  This is the preferred option if you want to automatically search result files that are stored in the same folder to locate all curves of a specified type, for example, all UV curves. This is especially useful for comparison of curves from scouting runs. Moreover, the imported curves can be automatically overlaid, stacked or presented as mirror images. See "How to use File:Open to compare" below.

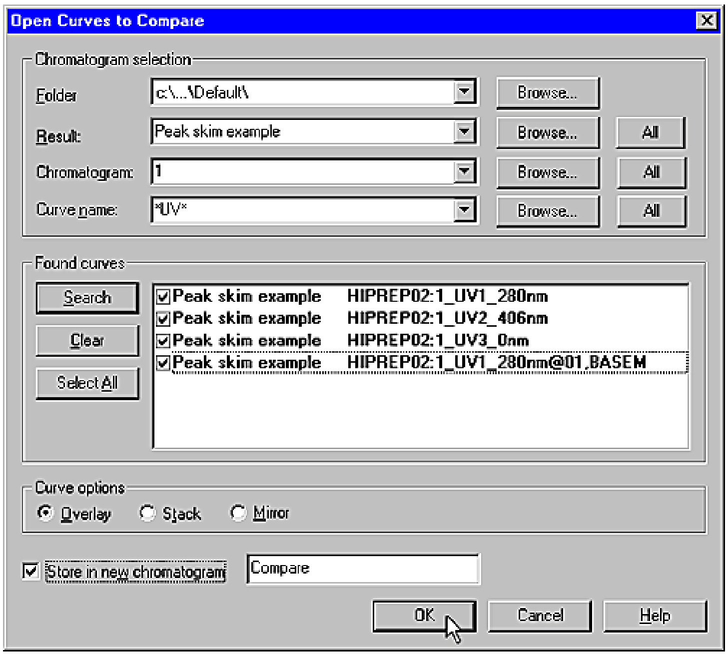
- **File:Open:Curves**

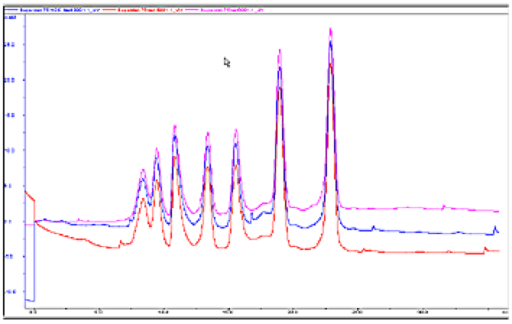
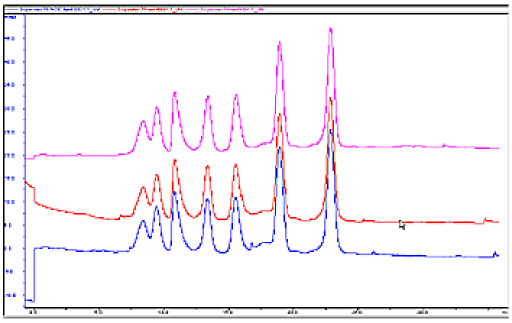
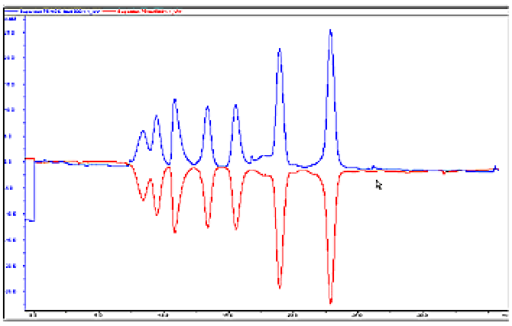
  This is the preferred option to import individual curves. See " How to use File:Open:Curves" below.

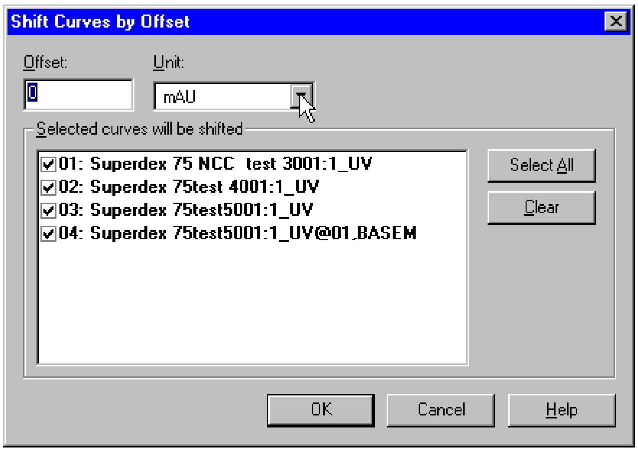
Note: Original curves are underlined in the chromatogram, imported and created curves are not underlined.

How to use File:Open to compare

The table below describes how to import curves to a chromatogram with the command **File:Open to compare**:

Step	Action
1	In the **Evaluation** module, - **choose File:Open to compare:Curves** *or* - click the **Open curves to compare** toolbar button. *Result*: The **Open Curves to Compare** dialog box opens.  Folder: c:\...\Default\ Result: Peak skim example Chromatogram: 1 Curve name: *UV* ☑ Peak skim example HIPREP02:1_UV1_280nm ☑ Peak skim example HIPREP02:1_UV2_406nm ☑ Peak skim example HIPREP02:1_UV3_0nm ☑ Peak skim example HIPREP02:1_UV1_280nm@01,BASEM ● Overlay ○ Stack ○ Mirror ☑ Store in new chromatogram: Compare
2	- Select the desired search criteria in the **Folder**, **Result**, **Chromatogram** and **Curve name** droplists of the **Chromatogram selection** section. - Click **Search** and a list of found curves will be displayed based on the selected search criteria. *Note*: A new search can be performed with new search criteria without erasing curves located in the previous search. - Select the check boxes for the curves that you want to import. Click the **Select All** button if you want to import all the curves. - If you select the **Store in new chromatogram** option, the curves will be imported into a new chromatogram. This is recommended to keep the source chromatogram free of too many additional curves.

Step	Action
3	Select how to display the imported curves in the **Curve options** field and click **OK**. See the options below: **Overlay**  The curves are presented overlaid on one another. **Stack**  The curves are presented with a given offset Y-axis value so that the curves are stacked and distinct from one another. **Mirror**  For comparison of *two* imported curves. One curve is inverted in the Y-axis and thus appears to mirror the other curve.

Step	Action
4	If you selected the **Stack** option in step 3, the **Shift Curves by Offset** dialog box is displayed:  Offset: 0 Unit: mAU Selected curves will be shifted ☑ 01: Superdex 75 NCC test 3001:1_UV ☑ 02: Superdex 75test 4001:1_UV ☑ 03: Superdex 75test5001:1_UV ☑ 04: Superdex 75test5001:1_UV@01,BASEM • You can set the **Offset** value to increase or decrease the offset distance between the curves. • Click **OK**. *Result*: Depending on your previous choices, the imported curves are now displayed in the source chromatogram or in a newly created chromatogram. *Note*: If curves with several different units have been selected, the curves with each different unit will be grouped together with separate offset from the other groups.
5	**Change some comparison settings** • Choose **Edit:Chromatogram Layout** to open the **Chromatogram Layout** dialog box. • Select or de-select the check boxes on the **Curve** tab to compare a different set of curves. • On the **Y-Axis** tab, the curves can be scaled - individually - all with the same scale (click the **All with this unit** button). • Click **OK** to display the curves.
6	If you stacked the curves and want to change the stack offset • choose **Operations:Shift offset** • type a new **Offset** value and click **OK**. *Note*: The individual curves can also be moved (see **11.8.4 How to stack and stretch curves** on page 324).

How to use File:Open:Curves

The table below describes how to import individual curves into an active chromatogram with the **File:Open:Curves** command:

Step	Action
1	Make sure that the destination chromatogram for the imported curve(s) is active on the screen. • Select **File:Open:Curves** in the **Evaluation** module. *Result*: The **Open Curves** dialog box is displayed.
2	**Select curves in the Open curves dialog box** • Select the folder and the result file in the upper part of the dialog box. • Select a chromatogram on the **Chromatogram** drop-down list. Usually there is just one chromatogram. *Result*: The available curves are listed on the **Available** list. • Click the check boxes on the **Available** list for the curves that you want to import and click the **Select** button. *Result*: The selected curve(s) is displayed in the **Selected curves** list. To remove a curve from the **Selected curves** list, click the check box and then click the **Remove** button.
3	• Repeat step 2 if you want to import curves from other chromatograms. • Click **OK** when you have selected the curves you want.
4	**Change some comparison settings** • Choose **Edit:Chromatogram Layout** to open the **Chromatogram Layout** dialog box. • Select or de-select the check boxes on the **Curve** tab to compare a different set of curves. • On the **Y-Axis** tab, the curves can be scaled - individually - all with the same scale (click the **All with this unit** button). • Click **OK** to display the curves.

How to copy curves into one chromatogram

A practical way to compare curves is to create a chromatogram and copy curves from different chromatograms into the new chromatogram. The comparisons are then performed in the new chromatogram.

The table below describes how to copy curves into a chromatogram:

Step	Action
1	Perform *either* A or B below: **A. Create a new chromatogram** • Choose **File:New:Chromatogram** to create a new chromatogram. **B. Use the Temporary chromatogram** • Choose **Window:Temporary**.
2	**Open the source chromatogram(s)** Choose **File:Open:Chromatogram** to open the chromatogram(s) that contains the curves you want to copy. *Result*: The **Open Chromatogram** dialog box opens.
3	• Select the result file. • Click the check box for the source chromatogram in the **Available** list. • Click the **Select** button. • Click **OK**. *Result*: The source chromatogram opens.
4	**Copy the curves** • Choose **Edit:Copy:Curves**. *Result*: The **Copy Curve** dialog box is displayed.
5	• Select the source chromatogram and a curve of interest in the **Source Chromatogram** field. • Select the target chromatogram (the one you created, or **Temporary**) in the **Target Chromatogram** field. • Click the **Copy** button. • Repeat this step for as many curves as you want, from the same or other chromatograms. *Note*: You can open more source chromatograms with the **File:Open:Chromatogram** command. • Click the **Close** button when you have copied all curves.

Step	Action
6	**Change some comparison settings** • Make sure the target chromatogram is open and that its window is active. • Choose **Edit:Chromatogram Layout** to display the **Chromatogram Layout** dialog box. • Select the curves that you want to view on the **Curve** tab and click **OK**. • The curves can be scaled individually or all with the same Y-axis scale. Use the **All with this unit** button on the **Y-Axis** tab to scale all curves with the same scale.
7	**If you used the Temporary chromatogram** • If you used the **Temporary** chromatogram you can perform evaluations in the **Temporary** chromatogram and transfer the final curves to other destination chromatograms. • All of the contents in the **Temporary** chromatogram can be removed with **Edit:Clear Temporary Chromatogram**.

Alternative way to copy curves

An alternative way to copy curves into one chromatogram is to

- create a new chromatogram by copying an existing chromatogram and saving it under a new name
- import more curves into the new chromatogram according to the instructions described above in this section.

11.8.4 How to stack and stretch curves

Functions

You can stack and stretch curves from different runs to better visualize the differences. To achieve this you can use the following functions:

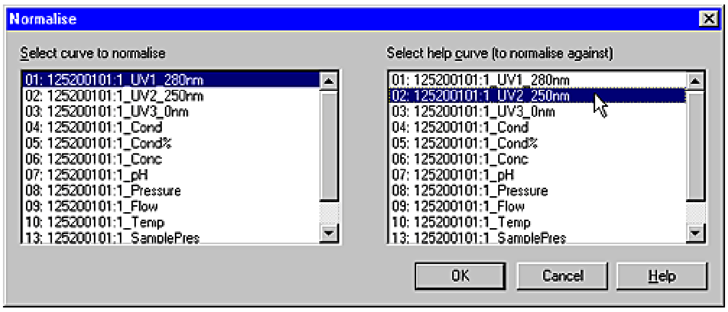
- **Normalise**
- **Shift**
- **Multiply**.

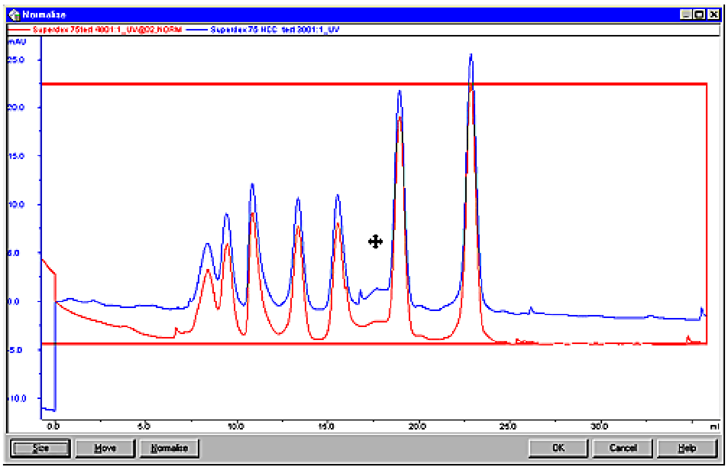
Note: All the functions require the curves to be present in one chromatogram.

How to use the Normalise function

The **Normalise** function provides the simplest method to align curves with respect to the X-axis or the Y-axis for easier visualization.

The table below describes how to use the **Normalise** function:

Step	Action
1	• Make sure that a chromatogram with the relevant curves is open in the **Evaluation** module. • Choose **Operations:Normalise**. *Result*: The **Normalise** dialog box is displayed.  Normalise Select curve to normalise 01: 125200101:1_UV1_280nm 02: 125200101:1_UV2_250nm 03: 125200101:1_UV3_0nm 04: 125200101:1_Cond 05: 125200101:1_Cond% 06: 125200101:1_Conc 07: 125200101:1_pH 08: 125200101:1_Pressure 09: 125200101:1_Flow 10: 125200101:1_Temp 13: 125200101:1_SamplePres Select help curve (to normalise against) 01: 125200101:1_UV1_280nm 02: 125200101:1_UV2_250nm 03: 125200101:1_UV3_0nm 04: 125200101:1_Cond 05: 125200101:1_Cond% 06: 125200101:1_Conc 07: 125200101:1_pH 08: 125200101:1_Pressure 09: 125200101:1_Flow 10: 125200101:1_Temp 13: 125200101:1_SamplePres OK Cancel Help

Step	Action
2	- Select the curve you want to normalise in the left (**Select curve to normalise**) field. - Select the reference curve you want to normalise *against* in the right (**Select help curve**) field.*Example*: If you want to *stack* the curves, select the curve at the bottom of the stack as the reference curve. - Click **OK**. *Result*: The **Normalise** window is displayed, where a box surrounds the curve selected to be normalised. 

Step	Action
3	In the **Normalise** window, you can use the following command buttons: **Size** Allows the arrow keys to be used to stretch the selected curve along its Y-axis or X-axis. This is useful for comparison of curves with, for example, different gradient lengths. • Click the **Size** button and use the arrow keys to stretch the the curve either along its Y-axis or X-axis. **Move** Allows the arrow keys to be used to move the selected curve to any position on the chromatogram. Axes are automatically re-scaled to accommodate the new positioning. This function is useful for stacking curves. • Click the **Move** button and use the arrow keys to move the curve into position. The curve can also be moved with the mouse pointer. Click the mouse button when the curve is in the correct position. *Note*: The curve can also be moved and sized with the mouse pointer. **Normalise** The curve to be normalised will be adjusted to the help curve. Thus, the height of the highest peak on both curves will be the same and will occur at the same retention point. • Click the **Normalise** button. The curve to be normalised is automatically moved along the X-axis and stretched along the Y-axis. • Click **OK** to save the new normalised curve. *Result*: The **Save Curve** dialog box opens.
4	Choose a curve position to save the curve in and click **OK**.
5	• Choose **Edit:Chromatogram Layout** to open the **Chromatogram Layout** dialog box. • Select the normalised curve for viewing on the **Curve** tab. • Click **OK**.
6	Repeat steps 1-5 for all curves you want to stack or stretch.

How to move a curve with the Shift function

If you want to position a curve more precisely, the **Shift** function should be used. The function is similar to **Normalise:Move** but each curve is repositioned by a precise value instead of by eye and the instruction is logged in the evaluation log. The table below describes how to use the **Shift** function:

Step	Action
1	• Make sure that a chromatogram with the relevant curves is open in the **Evaluation** module. • Choose **Operations:Shift**. *Result*: The **Shift** dialog box is displayed.
2	• Select the curve to be shifted in the **Source chromatogram** list. • Select a curve position in the **Target chromatogram** list. • Type a new **Curve name** or accept the default. • Select the axis/axes along which the shift is to be made: - along the X-axis (**Shift retention**) - along the Y-axis (**Shift amplitude**). • Type the shift value(s). • Click **OK**.

How to stretch and shrink a curve with the Multiply function

Curves can be stretched or shrunk on the x or y plane with the **Multiply** function. This function is similar to **Normalise:Size**, but each curve is repositioned with precise numbers instead of by eye and the instruction logged in the evaluation log. The table below describes how to use the **Multiply** function:

Step	Action
1	• Make sure that a chromatogram with the relevant curves is open in the **Evaluation** module. • Choose **Operations:Multiply**. *Result*: The **Multiply** dialog box is displayed.

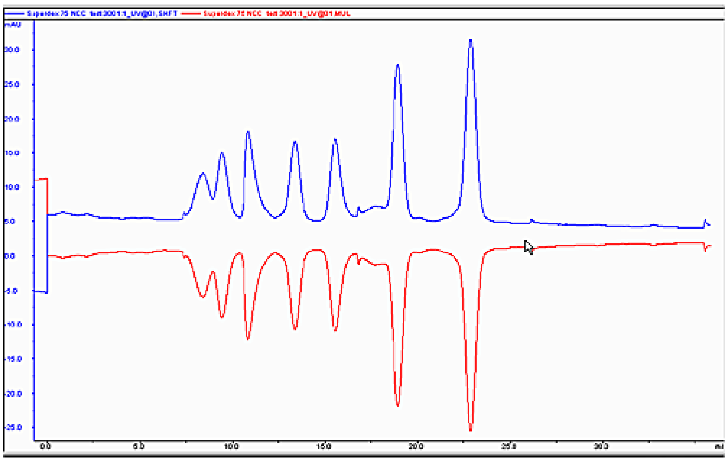
Step	Action
2	• Select the curve to be multiplied in the **Source chromatogram** list. • Select a curve position in the **Target chromatogram** list. • Type a new **Curve name** or accept the default. • Select the axis/axes along which the multiplication is to be made: - along the X-axis (**Multiply retention**) - along the Y-axis (**Multiply amplitude**). • Type the multiply value(s). • Click **OK**.

11.8.5 How to produce a mirror image

Instruction

A very useful way to compare the features of two curves is to produce a mirror image of one curve. The table below describes how to do this:

Step	Action
1	• Make sure that a chromatogram with the relevant curves is open in the **Evaluation** module. • Choose **Operations:Multiply**. *Result*: The **Multiply** dialog box is displayed.
2	• Select the curve to be multiplied in the **Source chromatogram** list. • Select a curve position in the **Target chromatogram** list. • Type a new **Curve name** or accept the default. • Select the **Multiply amplitude** check box. • Type the multiply value `-1`. • Click **OK**. *Result*: The mirror image of the original curve is displayed in the active chromatogram window.

Step	Action
3	**Shift the mirror image curve downwards** Shift the mirror image curve downwards for an improved presentation: - Choose **Operations:Shift**. *Result*: The **Shift** dialog box is displayed. - Select the curve to be shifted in the **Source chromatogram** list. - Select the same curve number in the **Target chromatogram** list box as in step 2. - Select the Shift amplitude check box since the shift is to be made along the Y-axis. - Type a shift value. - Click **OK**. The illustration below shows the original curve and the mirror image displayed. 
4	If you want to display other curves in the active chromatogram window, - choose **Edit:Chromatogram Layout** to open the **Chromatogram Layout** dialog box - select the curves that you want to display - click **OK**.

11.9 How to import and export results

Introduction

Curves and data can be imported and exported in different formats. This section describes how to import and export results.

In this section

This section contains the following sub-sections

11.9.1 How to import results

Introduction

This section describes how to import curves in different formats and how to import result data from **SMART Manager** or **FPLCdirector™**.

Curve formats

You can import curve files in the following formats:

- AIA (.cdf)
- ASCII (text)
- Lotus 1-2-3 spreadsheet (.wks)

How to import curves

The table below describes how to import curves.

Step	Action
1	Choose **File:Import:Curve**. *Result*: A menu with the available curve formats opens.
2	Choose the correct curve format. *Result*: The **Choose File to Import From** dialog box opens.
3	Locate the file that contains the curve and double-click the file. *Result*: The **Import Curves** dialog box opens.
4	• Select the curve(s) to import and click the **OK** button. *Result*: The curves are opened in the **Evaluation** module.

How to import data from SMART Manager and FPLCdirector

The table below describes how to import data from **SMART Manager** and **FPLCdirector**:

Step	Action
1	Choose **File:Import:Result**. *Result*: A menu box with the available data sources opens. This box opens immediately after **Import** if no result file is open in the **Evaluation** module.
2	Choose **FPLCdirector** or **SMART**. *Result*: The **Import FPLCdirector Result** dialog box or the **Import SMART Result** dialog box opens.

Step	Action
3	Locate and double-click the result file. *Result*: The result file is opened in the **Evaluation** module.

Copy from a floppy disk

When you import **SMART** or **FPLCdirector** files from a floppy disk it is best to first copy the files to the hard disk and then import the files.

11.9.2 How to export results

Introduction

This section describes how to export curves in different formats and how to copy data and curves to the clipboard.

Data formats

You can export data in the following formats:

- AIA (.cdf)
- ASCII (.asc)
- Lotus 1-2-3 (.wks)
- Excel (.xls)
- XML (.xml)

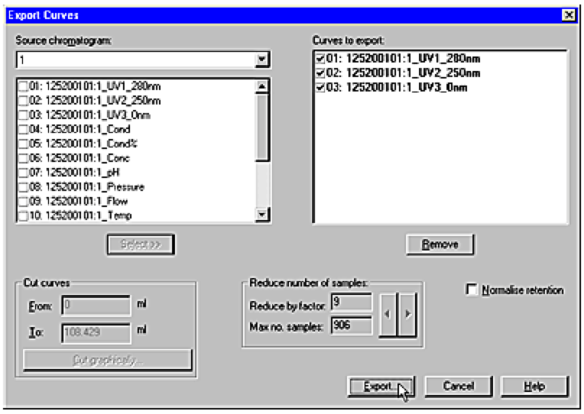
Export options

Select **File:Export** in the **Evaluation** module to export data from an open result file. The following export options are available:

- **Curves**
- **Export curve to AIA**
- **Peak table**
- **Method**
- **Documentation**
- **Evaluation log**

How to export curves

The table below describes how to export curves in the **Evaluation** module.

Step	Action
1	Choose **File:Export:Curves**. *Result*: The **Export Curves** dialog box opens.  Export Curves Source chromatogram: 1 ☐01: 125200101:1_UV1_280nm ☐02: 125200101:1_UV2_250nm ☐03: 125200101:1_UV3_0nm ☐04: 125200101:1_Cond ☐05: 125200101:1_Cond% ☐06: 125200101:1_Conc ☐07: 125200101:1_pH ☐08: 125200101:1_Pressure ☐09: 125200101:1_Flow ☐10: 125200101:1_Temp Curves to export: ☑01: 125200101:1_UV1_280nm ☑02: 125200101:1_UV2_250nm ☑03: 125200101:1_UV3_0nm Cut curves From: 0 ml To: 108.429 ml Reduce number of samples: Reduce by factor: 9 Max no. samples: 906 ☐ Normalise retention

Step	Action
2	• Select the curve(s) you want to export. • Enter parameters to limit the curve(s) if necessary. • Click the **Select** button. • Repeat Step 2 to select more curves.
3	Click the **Export** button. *Result*: The **Export Curves to File** dialog box opens.
4	Select the export file format from the **Save as type** droplist. • **ASCII files (*.asc)** • **Lotus 1-2-3 files (*.wks)** • **Excel files (*.xls)** • **AIA files (*.cdf)**
5	• Select a destination folder. • Type a file name and click **OK**.

Note: Curves are exported as series of numerical coordinates that refers to the time/volume and signal respectively.

How to limit the exported curves

You can optimize the exported curves to only the parts that you want to focus on, in the **Export Curves** dialog box. The table below describes how to use these editing options.

Dialog box option	Instruction
Cut curves	Enter retention values in the text boxes to limit the curve to only a portion of the original curve.
Cut graphically	This button opens the **Export Cut** dialog box. Move the vertical markers to the correct cutoff points.
Reduce number of samples	Adjust the factor value or the maximum number of samples. To reduce the number of samples by a factor of five means that only every fifth point will be sampled for export.

Dialog box option	Instruction
Normalise retention	Select the **Normalise retention** check-box to have all exported curves normalized to a common X-axis.

How to export curves in AIA format

The table below describes how to export curves in AIA format.

Step	Action
1	Select **File:Export:Export curve to AIA**. *Result*: The **Export curve in AIA format** dialog box opens.
2	• Select the source chromatogram and the curve you want to export. • Click the **Export** button. *Result*: The **Export Curves to File** dialog box opens.
3	• Select a destination folder. • Type a file name. • Click **OK**.

How to export peak tables

The table below describes how to export peak tables.

Step	Action
1	Choose **File:Export:Peak Table**. *Result*: The **Export Peak Table** dialog box opens.
2	• Select the source chromatogram and the peak table you want to export. • Click the **Export** button. *Result*: The **Export Peak Table to File** dialog box opens.
3	Select the export file format from the **Save as type** drop-list. • **ASCII files (*.asc)** • **Lotus 1-2-3 files (*.wks)** • **Excel files (*.xls)** • **XML files (*.xml)**

Step	Action
4	• Select a destination folder. • Type a file name. • Click **OK**.

Note: Peak tables are exported as text strings in ASCII format and numerical values in the Lotus 1-2-3 formats. All possible columns in the peak table are exported.

How to export methods, documentation and evaluation logs

The table below shows how to export methods, documentation and evaluation logs:

Step	Action
1	Select the data you want to export.
2	• Select options in the dialog box. • Click the **Export** button.
3	• Select a destination folder and type a file name. • Click **OK**.

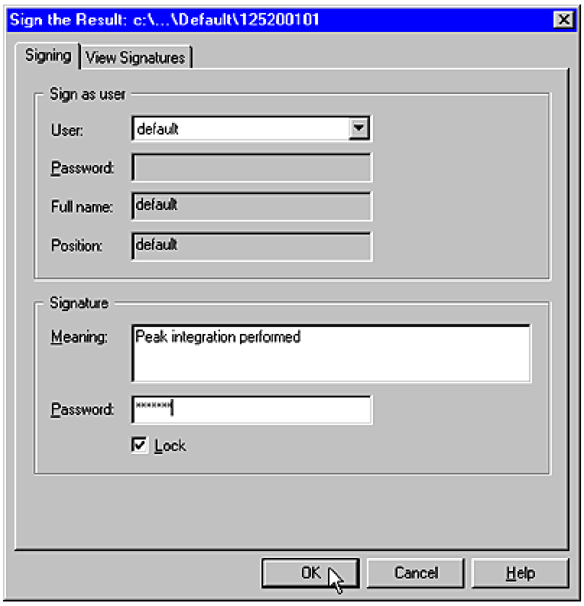
Copy to the clipboard

You can also use the **Windows** clipboard to copy the contents of the active window and paste it into other programs, e.g. **Microsoft Word**. Curves and documentation are copied as Windows enhanced metafiles (.emf) and peak tables are copied as text. Only the peak table columns that are selected in the spreadsheet will be copied.

11.10 *How to sign results electronically*

Instruction

Result files can be signed electronically to enhance data file security. The table below describes how to sign a result file electronically in the **Evaluation** module:

Step	Action
1	Choose **File:Sign Result**. *Result*: The **Sign the Result** dialog box opens.  Sign the Result: c:\...\Default\125200101 Sign as user User: default Password: Full name: default Position: default Signature Meaning: Peak integration performed Password: ******* ☑ Lock
2	• The **Sign as user** field shows the properties for the current user. You can also choose another user from the droplist. If you choose a new user, the corresponding password must be typed in the **Password** text box. • Type a short text description for the signed operation in the **Meaning** field (e.g. Peak integration performed). • The **Lock** check box is selected as default, to lock the result file from further changes. • Type your signature password in the **Password** field and click **OK**. *Note*: You should only lock the result when you are sure that the result file will not be modified anymore.

Signatures associated with the result

The **View Signatures** tab of the **Sign the Result** dialog box provides a list of all signatures associated with the current result. The information on this tab is for viewing purposes only and cannot be changed.

11.11 How to save results and exit the Evaluation module

Introduction

After you have finished the evaluation process, you can save all the changes you have made to the chromatograms, including newly created curves and chromatograms that you have imported and created.

How to delete unwanted curves

All the curves that you created during your manipulations will be saved in the chromatogram. If some of these curves are not be needed anymore, select **Edit:Delete:Curves** in the **Evaluation** module to remove the curves.

Note: The original curves that were created during the run can never be deleted.

How to save the results

You can either save your edited results in the original file or in a new result file. The table below describes how to save the results in the **Evaluation** module.

If you want to save the edited results...	then...
in the original result file	• select **File:Save**. *or* • click the **Save** toolbar icon. 💾
in a new result file	• select **File:Save as**.

Note: The previous version of the result file will be overwritten if you save the changes. This cannot be reversed. However, the raw data curves remain unchanged.

How to exit the Evaluation module

The table below describes how to exit the **Evaluation** module:

Step	Action
1	Choose **File:Exit**. *Result*: If there are unsaved changes, a dialog box opens with an option to save the changes before exit.
2	Select **Yes** if you want to save the changes. *Result*: The result file is closed in the **Evaluation** module and the **UNICORN Manager** module is displayed.

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 the following sections

Topic	See
Peak integration	12.1
Other evaluations	12.2
Automated evaluation procedures	12.3

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 the following sub-sections

Topic	See
Baseline calculation	12.1.1
How to perform a peak integration	12.1.2
How to optimize the baseline with a morphological algorithm	12.1.3
How to optimize the baseline with a classic algorithm	12.1.4
How to edit the baseline manually	12.1.5
How to edit the peaks	12.1.6
How to integrate part of a curve and how to exclude or skim peaks	12.1.7
Measurements	12.1.8

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

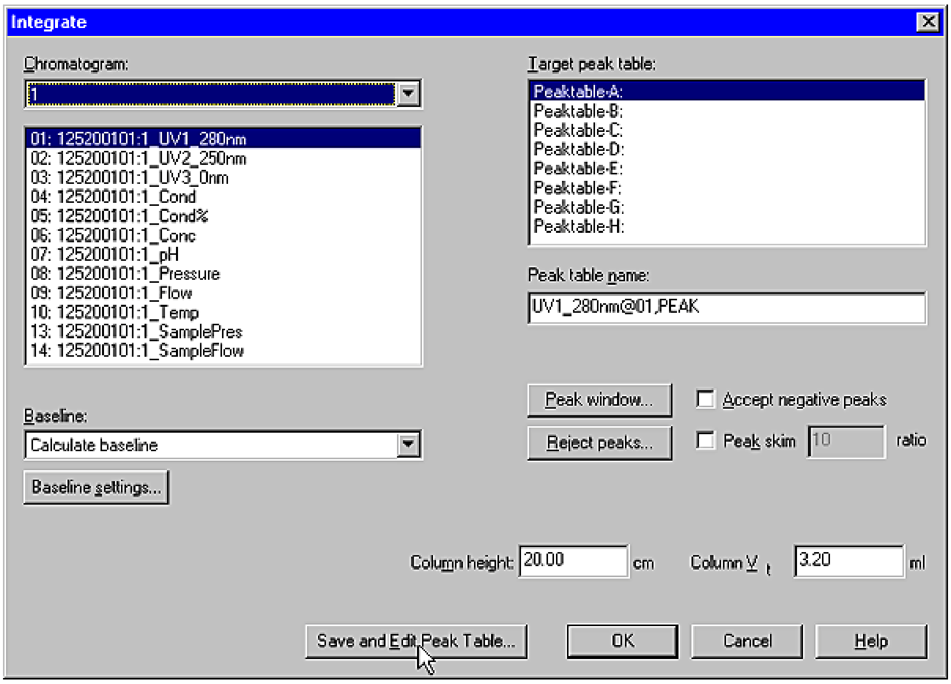
The table below describes how to perform a basic peak integration.

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 **12.1.3 How to optimize the baseline with a morphological algorithm** on page 350 and **12.1.4 How to optimize the baseline with a classic algorithm** on page 354.
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 **12.1.5 How to edit the baseline manually** on page 362 - See **12.1.6 How to edit the peaks** on page 365 - See **12.1.7 How to integrate part of a curve and how to exclude or skim peaks** on page 373

Illustration

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



Chromatogram: 1

01: 125200101:1_UV1_280nm
02: 125200101:1_UV2_250nm
03: 125200101:1_UV3_0nm
04: 125200101:1_Cond
05: 125200101:1_Cond%
06: 125200101:1_Conc
07: 125200101:1_pH
08: 125200101:1_Pressure
09: 125200101:1_Flow
10: 125200101:1_Temp
13: 125200101:1_SamplePres
14: 125200101:1_SampleFlow

Target peak table:
Peaktable-A:
Peaktable-B:
Peaktable-C:
Peaktable-D:
Peaktable-E:
Peaktable-F:
Peaktable-G:
Peaktable-H:

Peak table name: UV1_280nm@01,PEAK

Baseline: Calculate baseline

☐ Peak skim 10 ratio

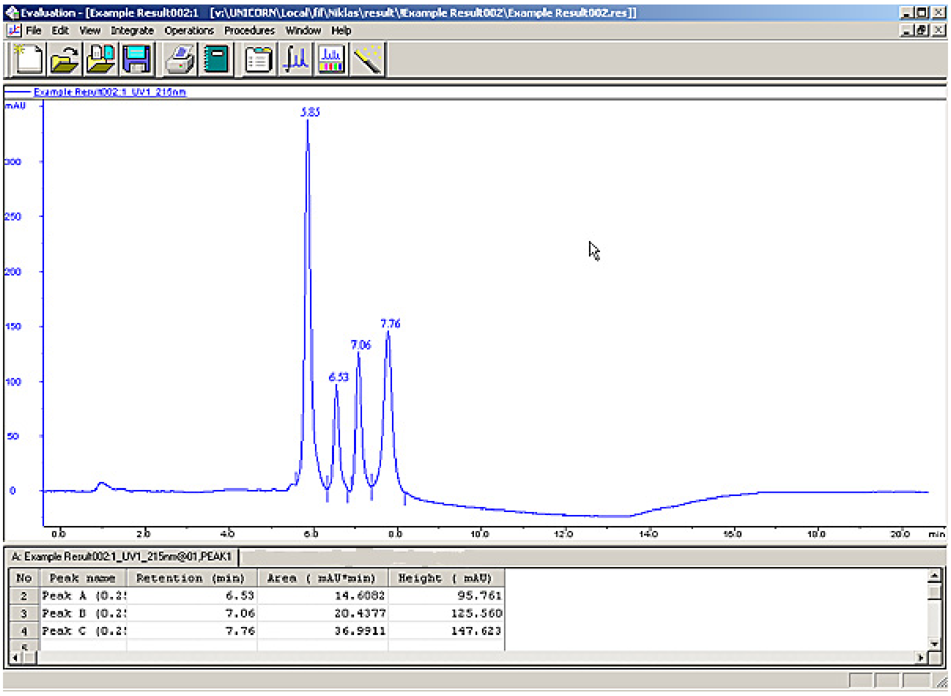
Column height 20.00 cm

Column V t 3.20 ml

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:



No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)
2	Peak A (0.2:	6.53	14.6082	95.761
3	Peak B (0.2:	7.06	20.4377	125.560
4	Peak C (0.2:	7.76	36.9911	147.623

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.

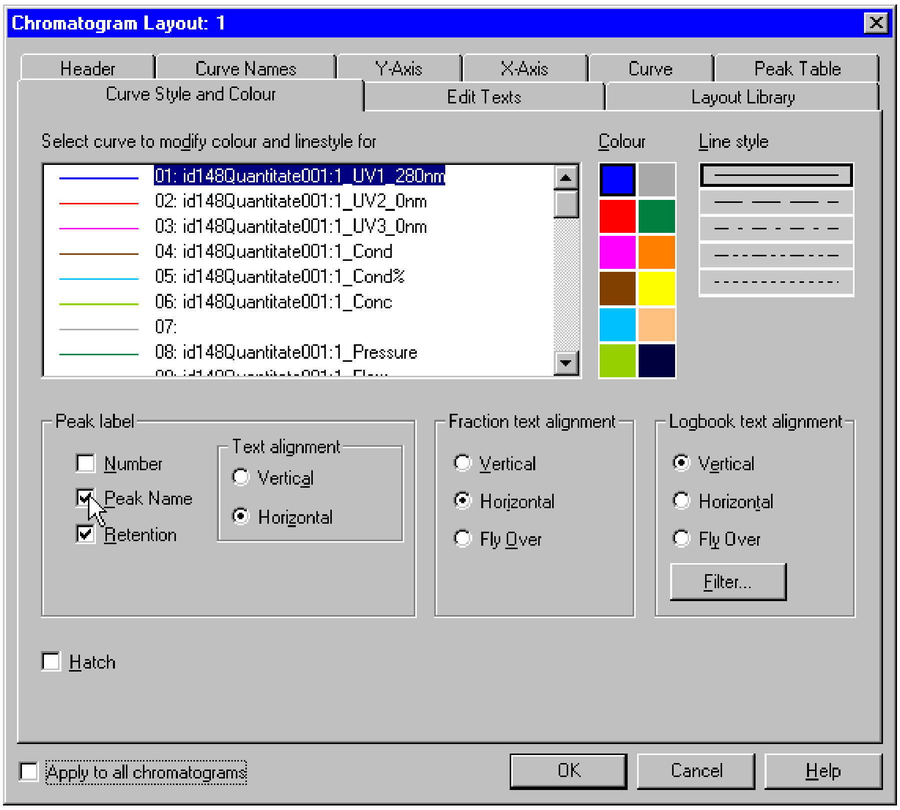
Filter peaks...	Reject peaks...
excludes the peaks from display,	permanently excludes peaks from the integration,
does not exclude the peaks from the calculation of the total peak area,	excludes the peaks from the calculation of the total peak area,
can be reversed.	cannot be reversed.

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:



Select curve to modify colour and linestyle for

01: id148Quantitate001:1_UV1_280nm
02: id148Quantitate001:1_UV2_0nm
03: id148Quantitate001:1_UV3_0nm
04: id148Quantitate001:1_Cond
05: id148Quantitate001:1_Cond%
06: id148Quantitate001:1_Conc
07:
08: id148Quantitate001:1_Pressure

Peak label
☐ Number
☑ Peak Name
☑ Retention

Text alignment
○ Vertical
● Horizontal

Fraction text alignment
○ Vertical
● Horizontal
○ Fly Over

Logbook text alignment
● Vertical
○ Horizontal
○ Fly Over

☐ Hatch

☐ Apply to all chromatograms

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.
2	Click the **Curve Style and Colour** tab.

Step	Action
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 **12.1.6 How to edit the peaks** on page 365 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

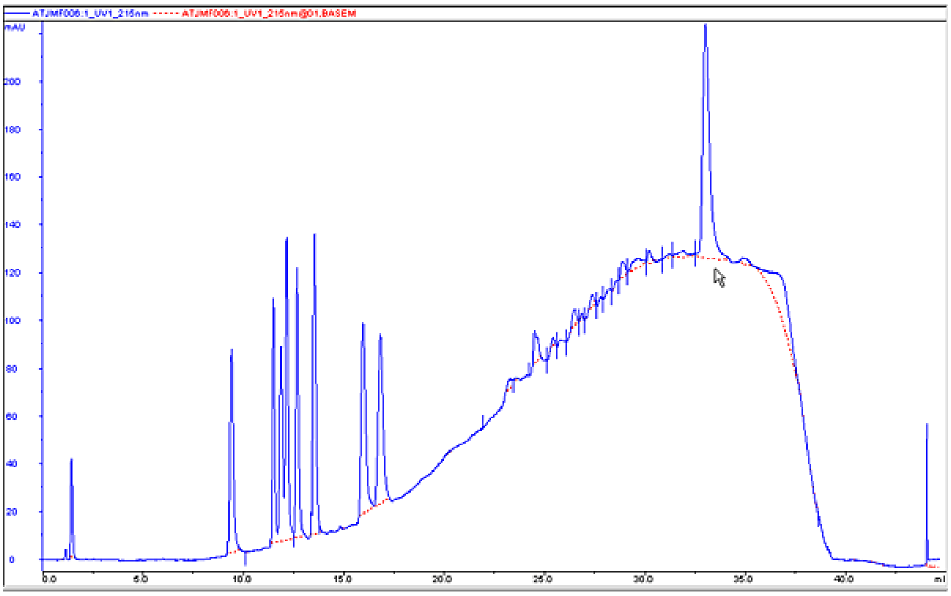
The parameters for the **Morphological algorithm** are:

- **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

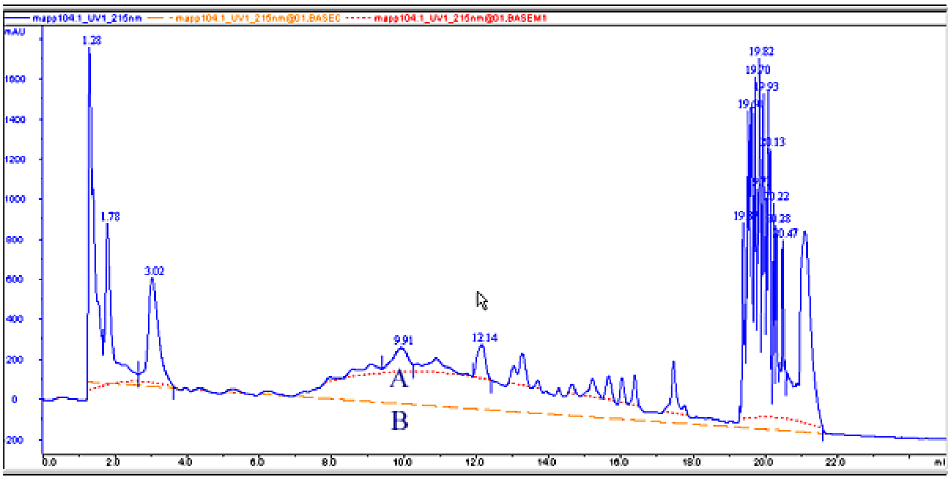
Too low settings

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.

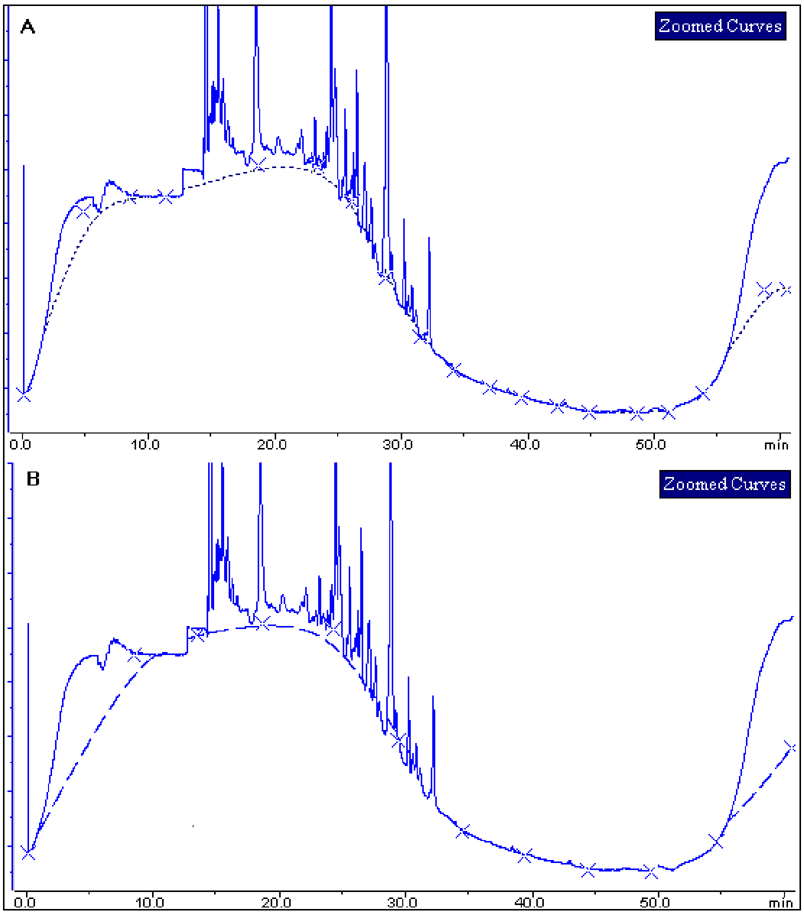
The solution to this is to increase the **Noise window** parameter. However, this can result in peak limits too high up on the peak slopes.

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.
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.

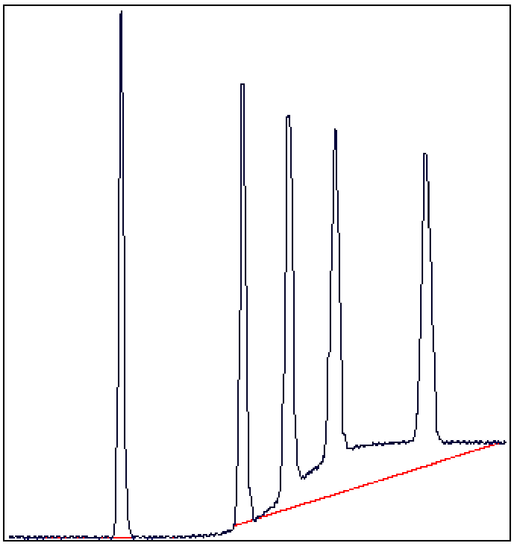
How much the values should be changed depends on the cause of the peak integration problem. The table below is a general guideline.

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%

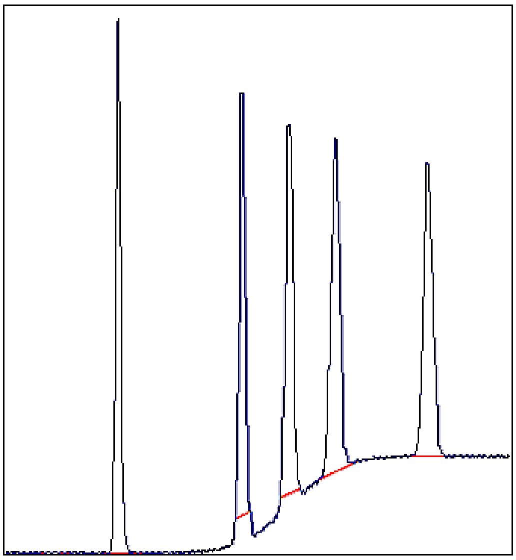
Note: If necessary, click the **Default** button to restore the default values.

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:



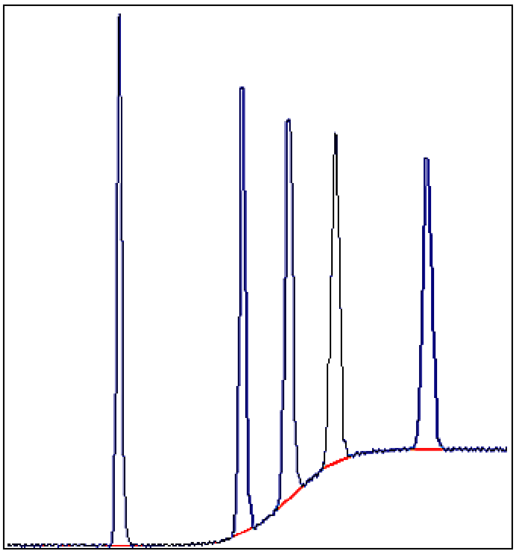
The **Shortest baseline segment** value is decreased by 50% in this example:



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:

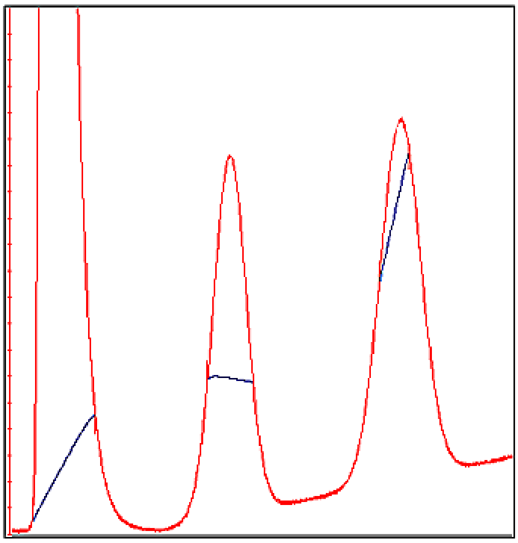


Too high slope limit

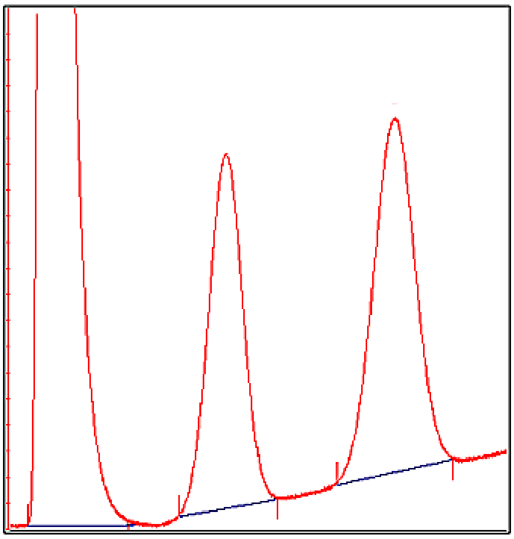
A too high **Slope limit** value can cause peak limits too high up on the peaks. This can be the case when the chromatogram includes a very large flow-through or solvent peak. The large peak affects the calculation of the default parameters and leads to too high values for the **Slope limit**.

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**:

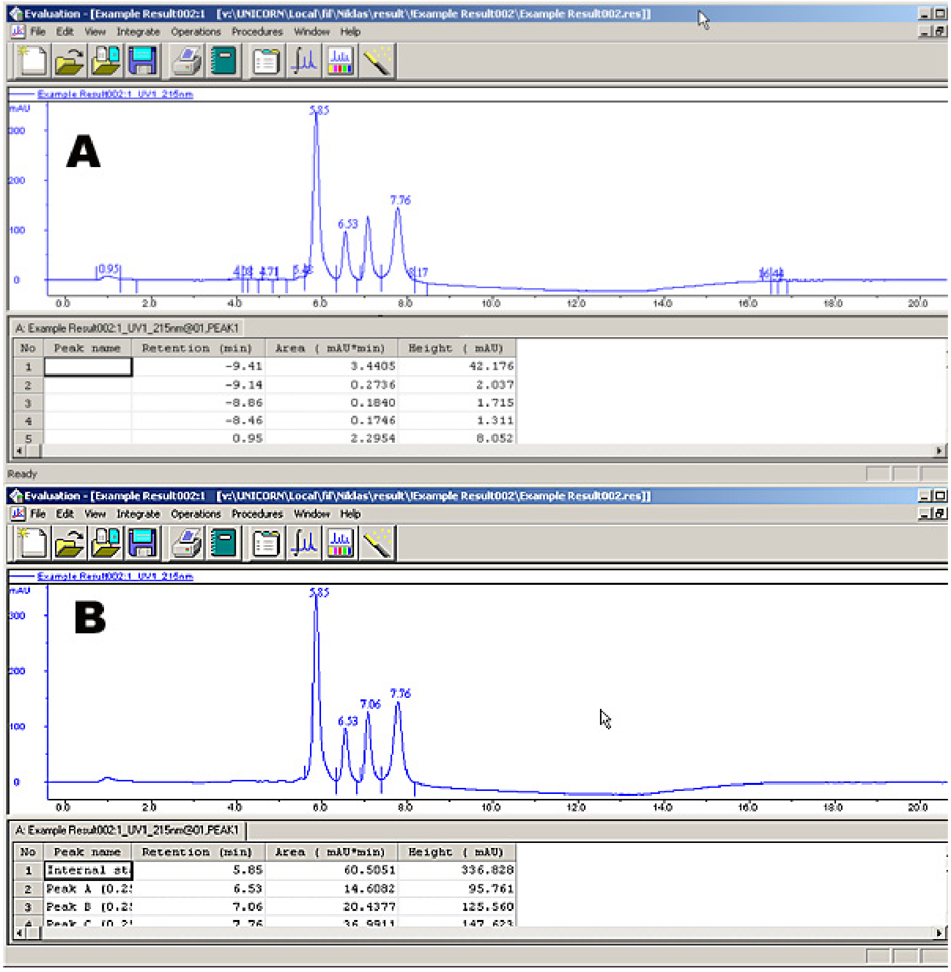


Noise window

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).



A: Example Result002:1_UV1_215nm@01_PEAK1

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)
1		-9.41	3.4405	42.176
2		-9.14	0.2736	2.037
3		-8.86	0.1840	1.715
4		-8.46	0.1746	1.311
5		0.95	2.2954	8.052

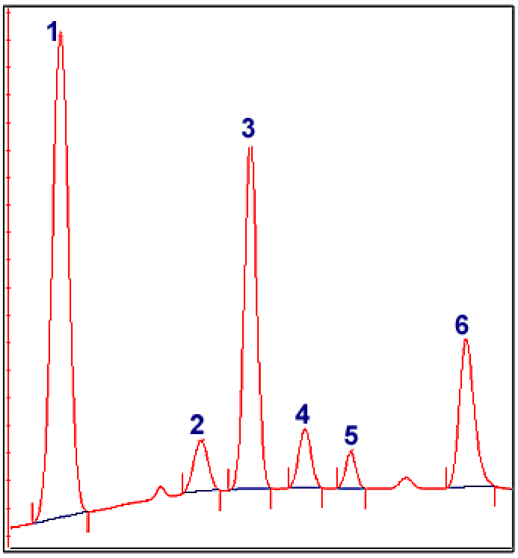
A: Example Result002:1_UV1_215nm@01_PEAK1

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)
1	Internal st	5.85	60.5051	336.828
2	Peak A [0.2	6.53	14.6082	95.761
3	Peak B [0.2	7.06	20.4377	125.560
4	Peak C [0.2	7.76	36.9911	147.623

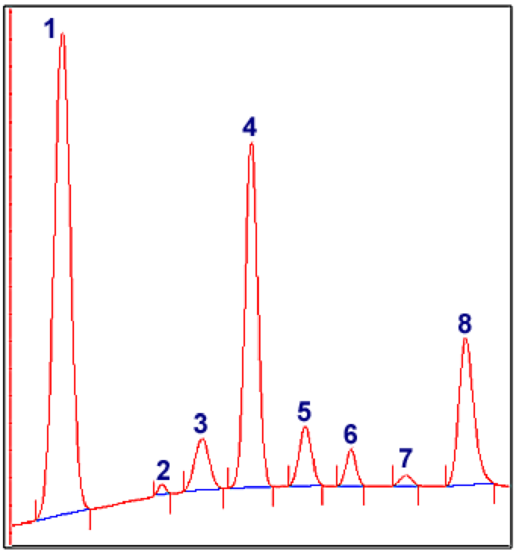
Note: You can also use the **Reject peaks** function in the **Integrate** dialog box to reduce the number of peaks based on the total number of accepted peaks or the minimum peak height.

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:



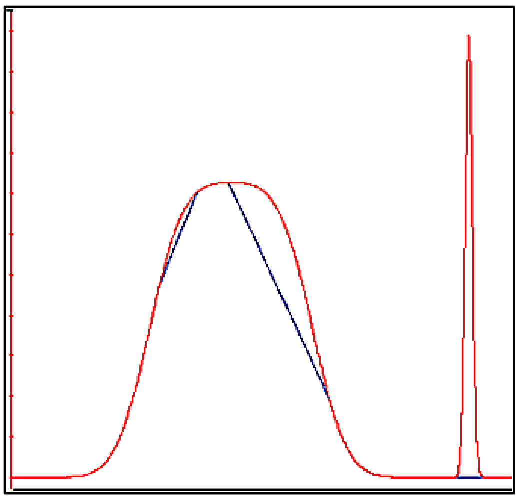
All peaks are detected if the **Noise window** is decreased, see example below:



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:



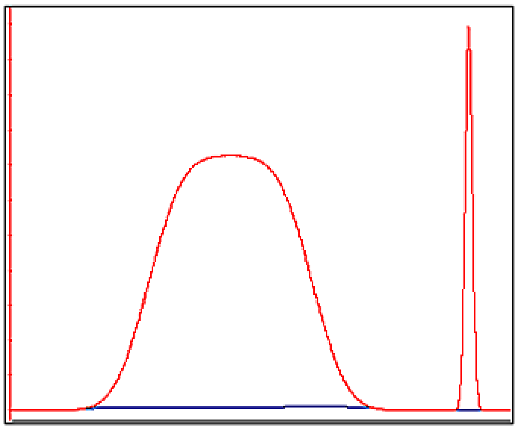
How to set the Max baseline level

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**.
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:



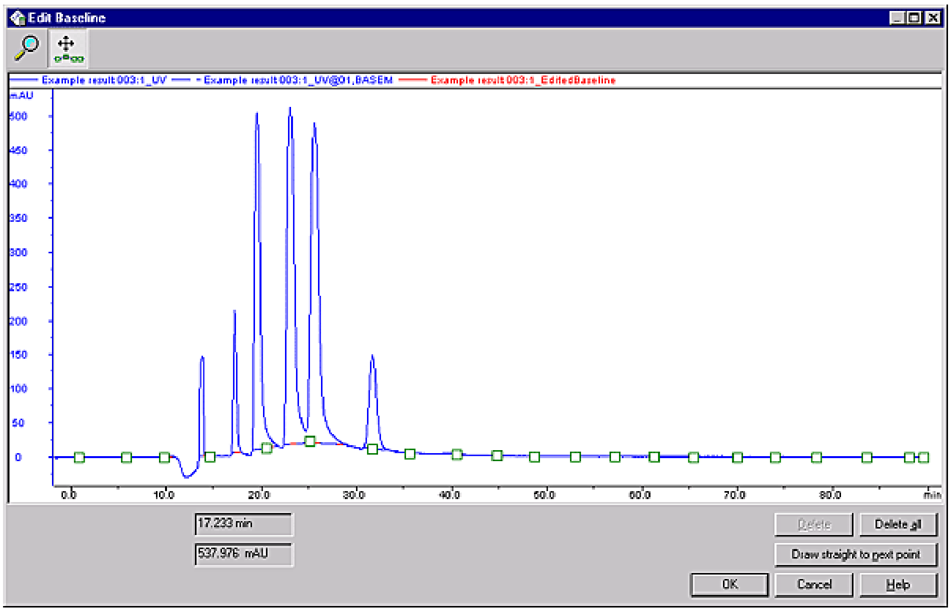
12.1.5 How to edit the baseline manually

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:



17.233 min

537.976 mAU

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.
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

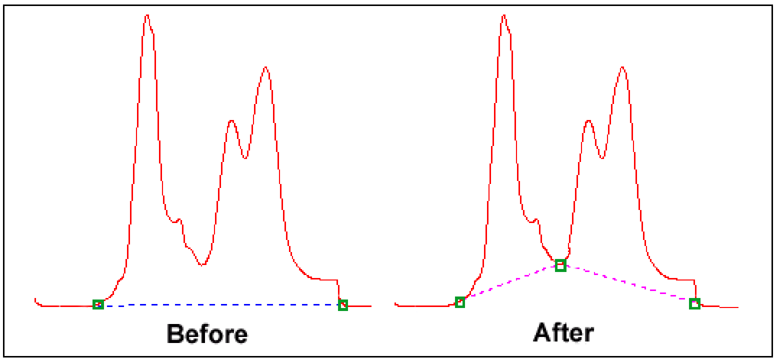
The table below describes how to edit and insert baseline data points:

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.
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.

Step	Action
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 **12.1.5 How to edit the baseline manually** on page 362) 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.

Step	Action
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

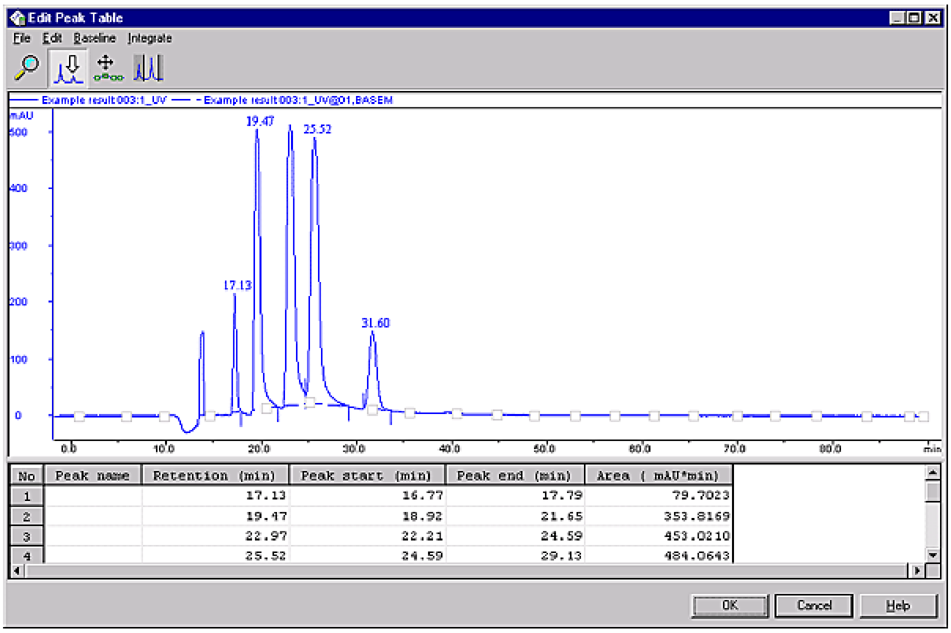
The baseline can be recalculated in the **Edit Peak Table** dialog box. The table below describes how to do this:

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.
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.



No	Peak name	Retention (min)	Peak start (min)	Peak end (min)	Area (mAU*min)
1		17.13	16.77	17.79	79.7023
2		19.47	18.92	21.65	353.8169
3		22.97	22.21	24.59	453.0210
4		25.52	24.59	29.13	484.0643

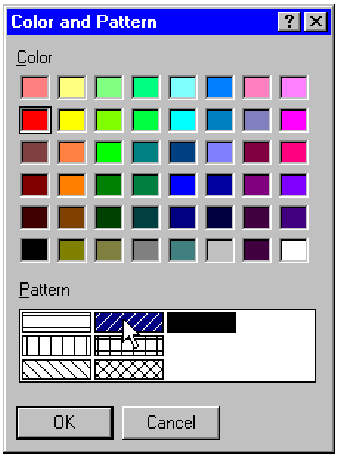
How to delete a peak

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

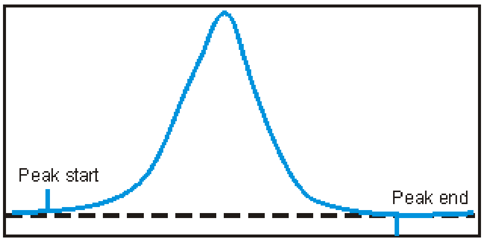
The table below describes how to add a fill color and a pattern to a peak in the **Edit Peak Table** dialog box:

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.
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.

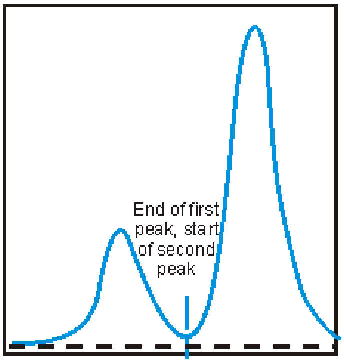
Note: The color and pattern selections will override the general **Fill settings** that can be selected for all peaks on the **Peak Table** tab in the **Chromatogram Layout** dialog box.

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.
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 recalculated.

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.
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 **12.1.7 How to integrate part of a curve and how to exclude or skim peaks** on page 373 for more information.

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

Introduction

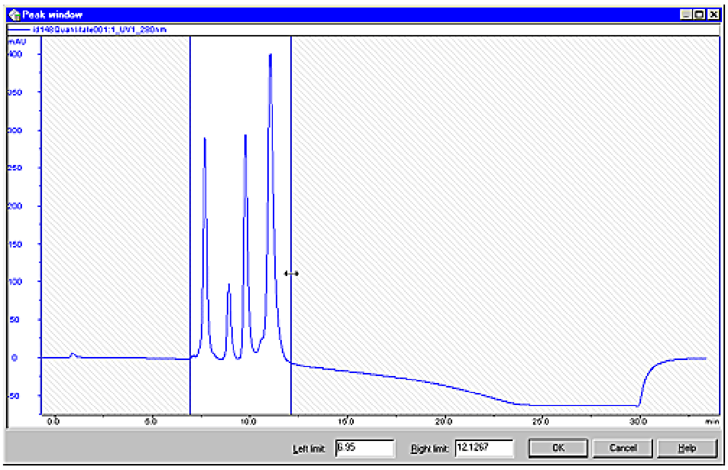
There are several possibilities to improve the results if the peak integration is unsatisfactory. This section describes:

- 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.  Left limit: 6.95 Right limit: 12.1267
2	• Type new X-axis values for the **Left limit** and the **Right limit**. *or* • Drag the vertical cursor lines to define the limits.

Step	Action
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.

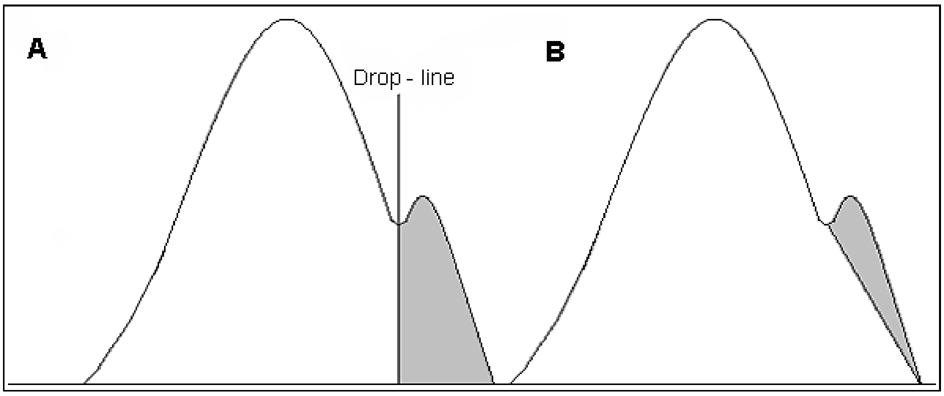
Result: The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

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.

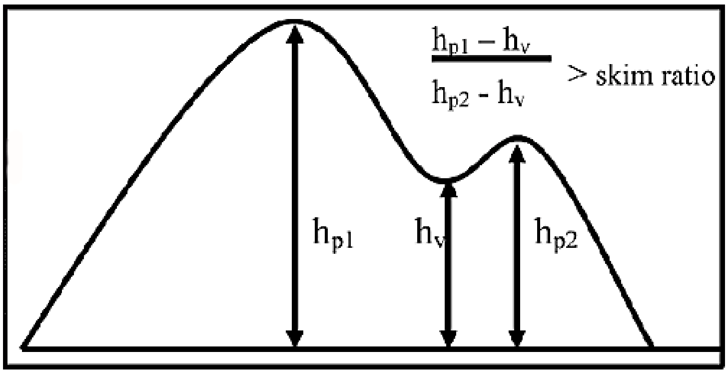
The illustration below is an example of how a drop-line (A) and a skimmed peak (B) affects the area under the main peak and the peak shoulder. The peak shoulder area is marked in gray:



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.

Step	Action
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.

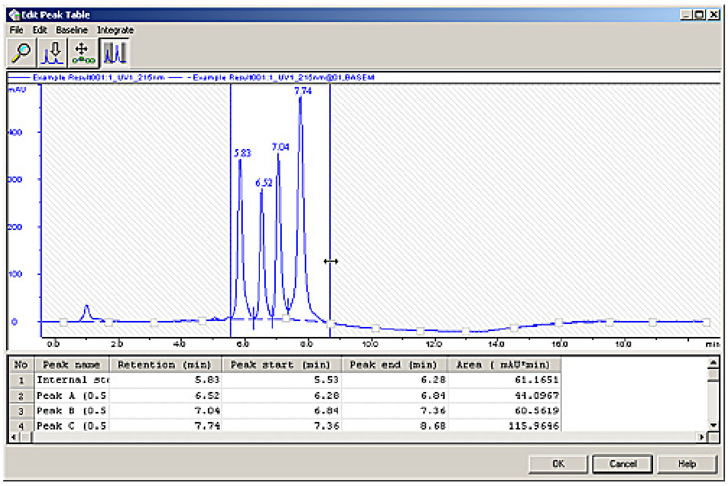
Step	Action
1	• Choose **Integrate:Edit Peak Table**. *Result*: The **Select Peak Table to Edit** dialog box opens. • Select the peak table to edit and click **OK**. *Result*: The **Edit Peak Table** dialog box opens.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>

- 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.



<table>
<tr><th>No</th><th>Peak name</th><th>Retention (min)</th><th>Peak start (min)</th><th>Peak end (min)</th><th>Area (mAU*min)</th></tr>
<tr><td>1</td><td>Internal st</td><td>5.83</td><td>5.53</td><td>6.28</td><td>61.1651</td></tr>
<tr><td>2</td><td>Peak A (0.5</td><td>6.52</td><td>6.28</td><td>6.84</td><td>44.0967</td></tr>
<tr><td>3</td><td>Peak B (0.5</td><td>7.04</td><td>6.84</td><td>7.36</td><td>60.5619</td></tr>
<tr><td>4</td><td>Peak C (0.5</td><td>7.74</td><td>7.36</td><td>8.68</td><td>115.9646</td></tr>
</table>

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

</td></tr>
<tr><td>3</td><td>

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**.

</td></tr>
<tr><td>4</td><td>

- Choose **Integrate:Peak Integrate**.

Result: The selected part of the curve is peak integrated based on the changed parameters.

</td></tr>
</table>

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.

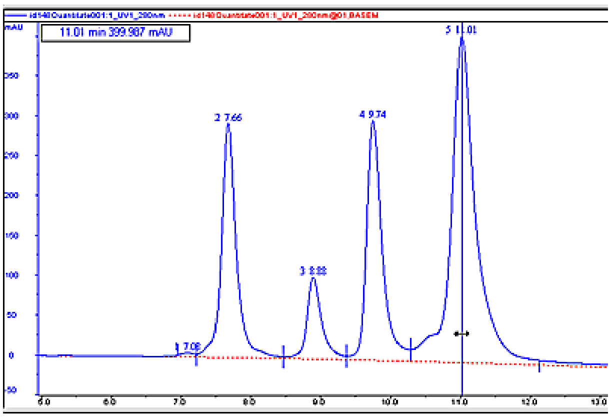
Measurement options

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.

Step	Action
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 the following sub-sections

Topic	See
Peak purity and peak identification	12.2.1
How to find slope values	12.2.2
How to simulate a peak fractionation	12.2.3
How to create curves	12.2.4
How to use the Fraction Histogram	12.2.5

12.2.1 Peak purity and peak identification

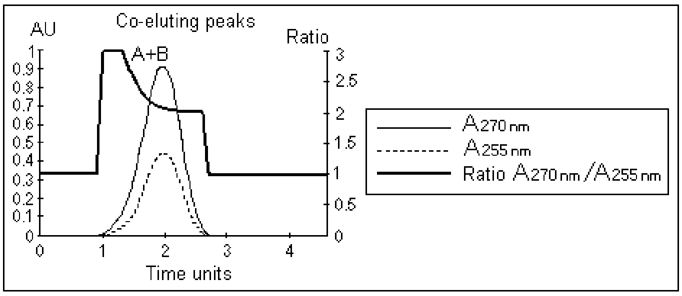
Introduction

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.

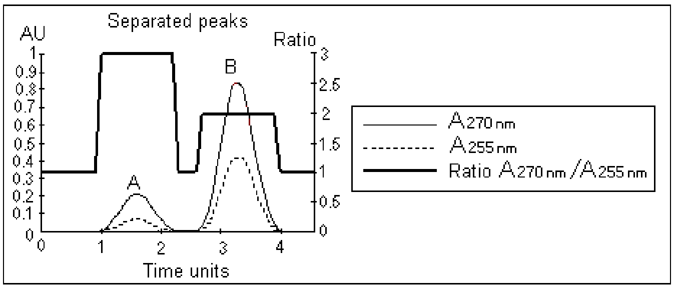
The illustration below shows a simulated chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time:



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.

Step	Action
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.
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.

Step	Action
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.

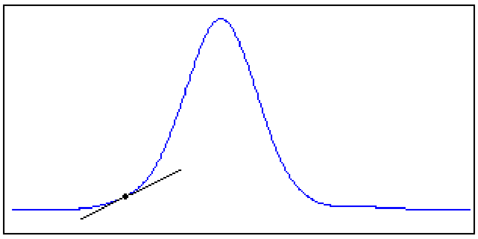
Using slope values for Watch instructions

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.
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 **12.2.1 Peak purity and peak identification** on page 382.

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.
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.

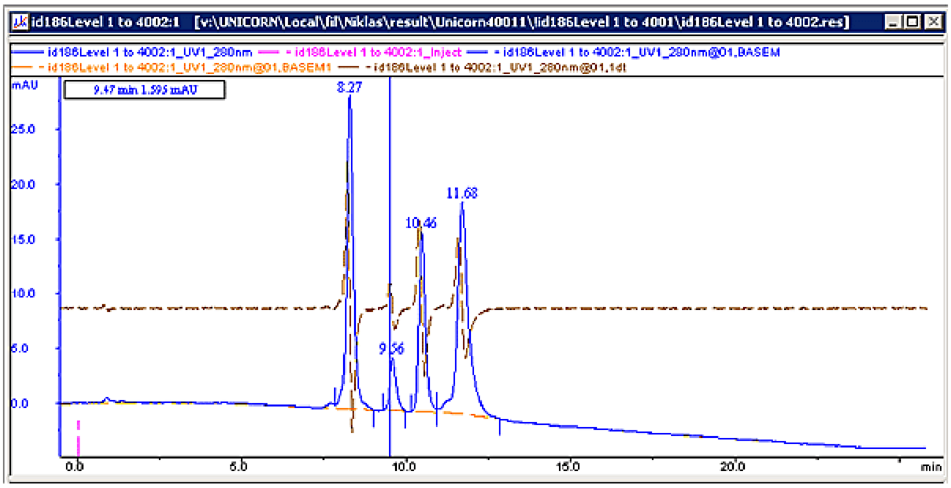
Note: The unit for the differentiated curve is mAU/min or AU/min. Any Y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

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:



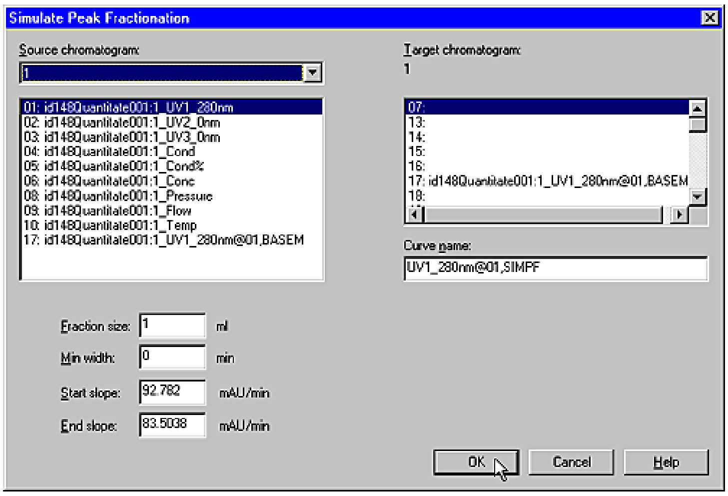
12.2.3 How to simulate a peak fractionation

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.  Source chromatogram: 1 01: id148Quantitate001:1_UV1_280nm 02: id148Quantitate001:1_UV2_0nm 03: id148Quantitate001:1_UV3_0nm 04: id148Quantitate001:1_Cond 05: id148Quantitate001:1_Cond% 06: id148Quantitate001:1_Conc 08: id148Quantitate001:1_Pressure 09: id148Quantitate001:1_Flow 10: id148Quantitate001:1_Temp 17: id148Quantitate001:1_UV1_280nm@01,BASEM Target chromatogram: 1 07: 13: 14: 15: 16: 17: id148Quantitate001:1_UV1_280nm@01,BASEM 18: Curve name: UV1_280nm@01,SIMPF Fraction size: 1 ml Min width: 0 min Start slope: 92.782 mAU/min End slope: 83.5038 mAU/min
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*

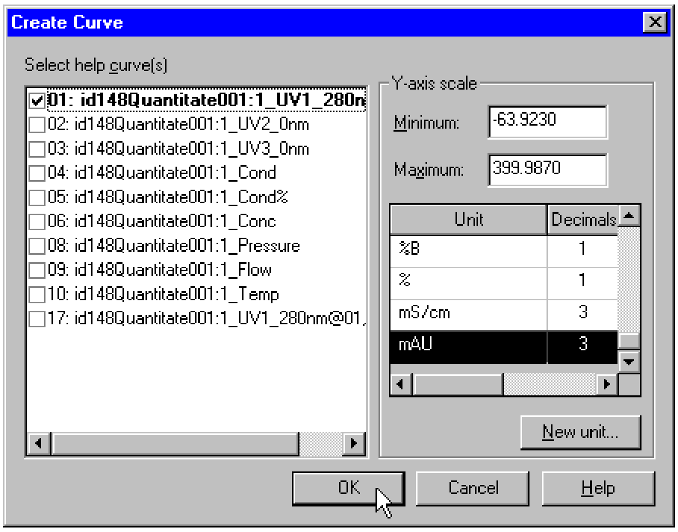
Introduction

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.  Create Curve Select help curve(s) ☑ **01: id148Quantitate001:1_UV1_280n** ☐ 02: id148Quantitate001:1_UV2_0nm ☐ 03: id148Quantitate001:1_UV3_0nm ☐ 04: id148Quantitate001:1_Cond ☐ 05: id148Quantitate001:1_Cond% ☐ 06: id148Quantitate001:1_Conc ☐ 08: id148Quantitate001:1_Pressure ☐ 09: id148Quantitate001:1_Flow ☐ 10: id148Quantitate001:1_Temp ☐ 17: id148Quantitate001:1_UV1_280nm@01, Y-axis scale Minimum: -63.9230 Maximum: 399.9870 Unit: %B, Decimals: 1 Unit: %, Decimals: 1 Unit: mS/cm, Decimals: 3 Unit: mAU, Decimals: 3
3	Select one or more **Help curves**.
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

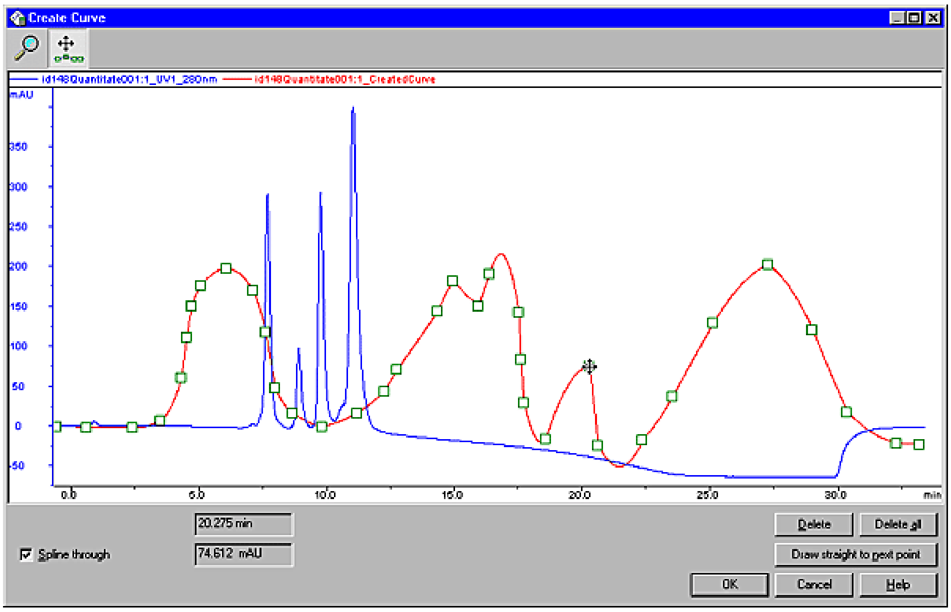
The new curve is created in the **Create Curve** window. The table below describes how to work in this window.

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

Step	Action
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.



☑ Spline through

20.275 min

74.612 mAU

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 the following sub-sections

Topic	See
How to create a new procedure	12.3.1
How to edit a procedure	12.3.2
How to run a procedure	12.3.3
How to rename and remove procedures	12.3.4

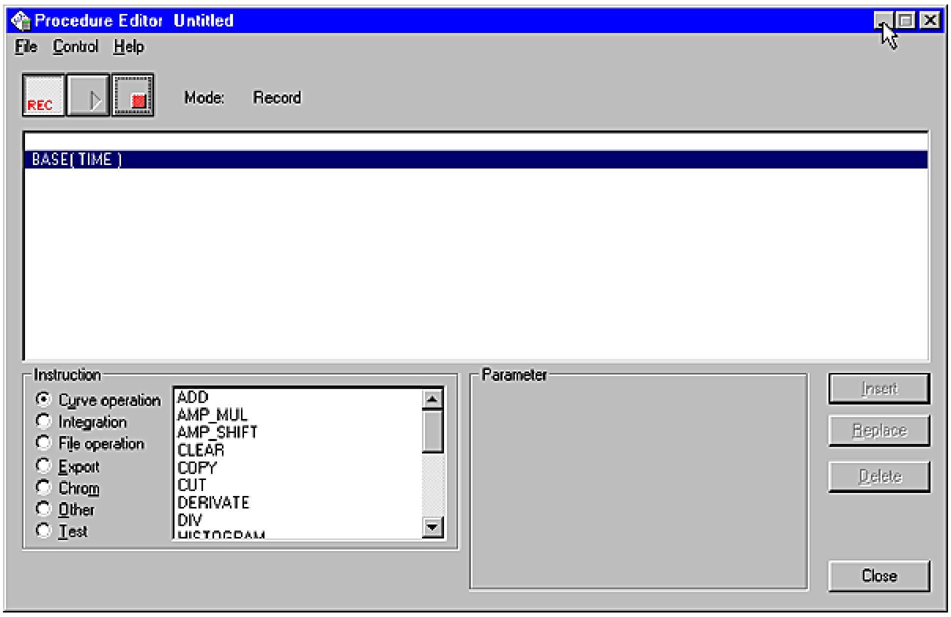
12.3.1 How to create a new procedure

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**.



Mode: Record

BASE(TIME)

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.
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.

Step	Action
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 **B.4 Procedure instructions** on page 526. 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**.

Step	Action
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 **B.4 Procedure instructions** on page 526 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

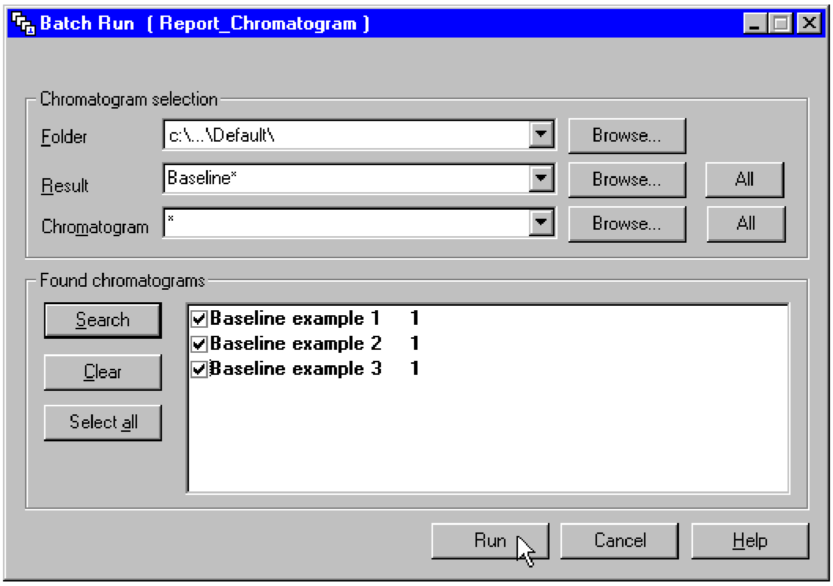
The table below describes how to perform a batch run:

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.

Step	Action
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:



Batch Run (Report_Chromatogram)

Chromatogram selection

Folder: c:\...\Default\

Result: Baseline*

Chromatogram: *

Found chromatograms

☑ Baseline example 1 1
☑ Baseline example 2 1
☑ Baseline example 3 1

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**.
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 the following sections

Topic	See
General information about the module	13.1
Quantitation overview	13.2
How to prepare for quantitation	13.3
How to quantitate the sample	13.4
Automated quantitation	13.5
How to measure molecular size	13.6

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).

Term	Definition
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.
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.

• p 407

Step	Action
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 the following sub-sections

Topic	See
General information about quantitation	13.2.1
External standard quantitation	13.2.2
Internal standard quantitation	13.2.3
Standard addition quantitation	13.2.4
Recovery calculation	13.2.5
General reliability factors for the quantitation techniques	13.2.6

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

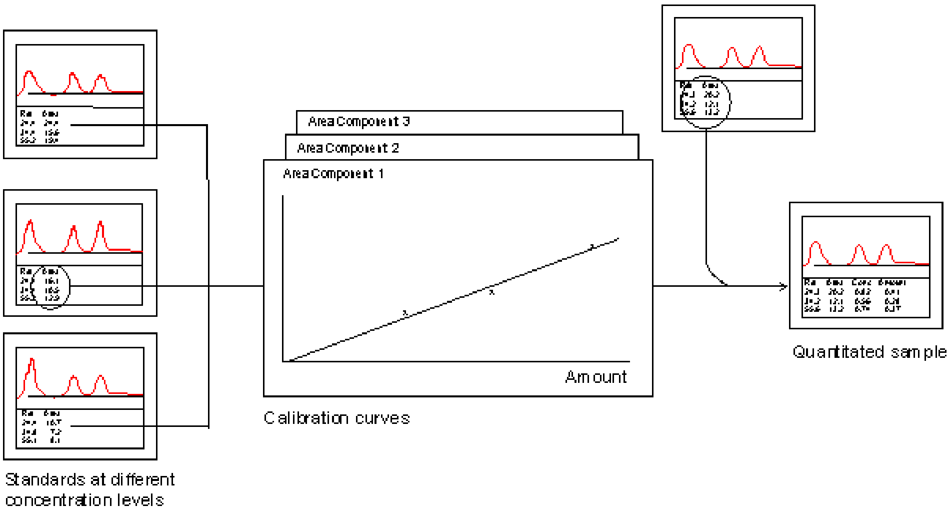
The table below describes the general steps in quantitation. The steps are described in detail in the sections about the different quantitation techniques.

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.
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.
`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

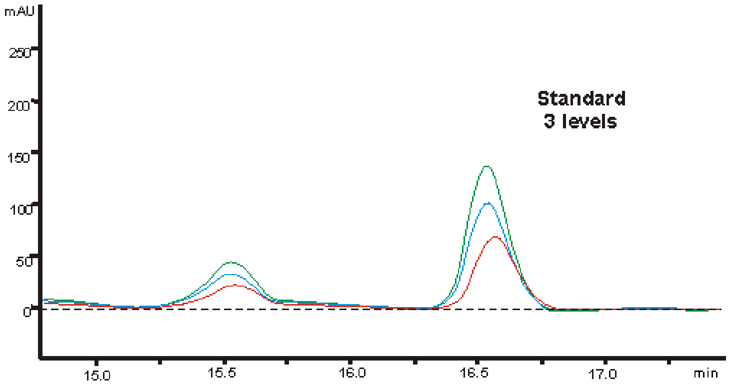
External standard quantitation can be based on the use of a single standard concentration level, but the calibration curve is then limited to a linear through-the-origin relationship. The use of a number of different concentration levels of the standard broadens the range of the calibration curve. It 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.
2	Integrate the curves to produce a peak table for each run. 

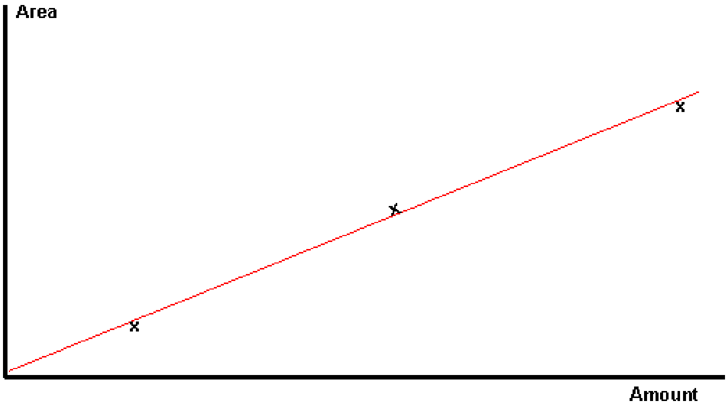
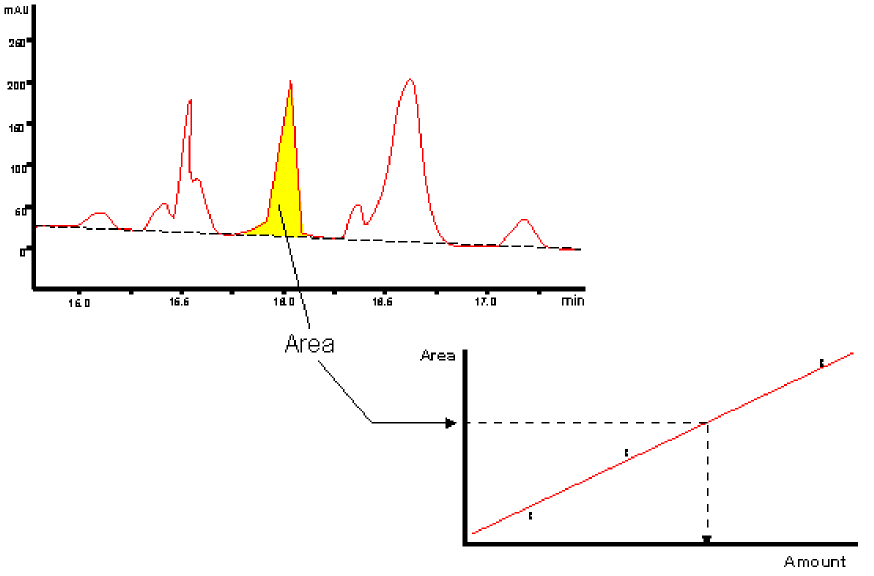
Step	Action
3	Use the peak tables from the standard runs to produce a calibration curve for each component. This curve shows the relationship between amount and peak size. Below is a calibration curve for one of the components: 
4	Perform a run with the sample and peak integrate the curve.
5	Identify 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.

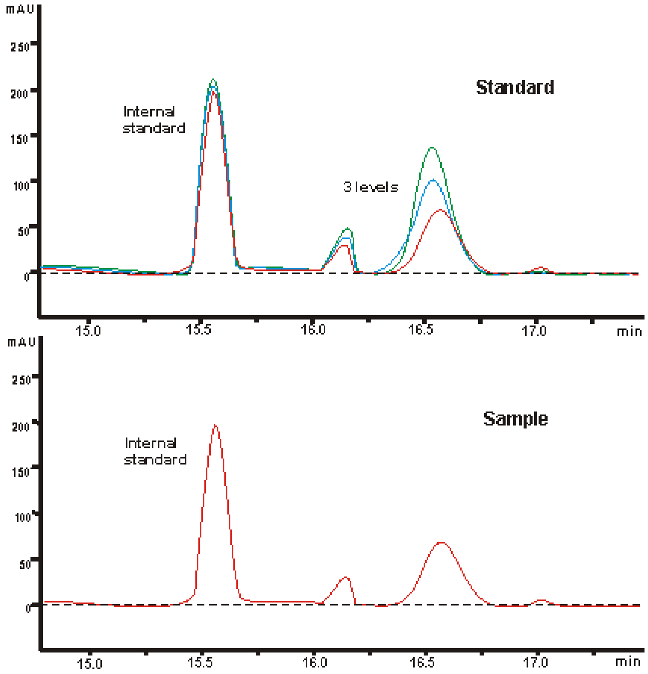
To be able to compensate for losses during the sample preparation, all the standard concentration levels must be subjected to the same sample preparation procedure as the samples.

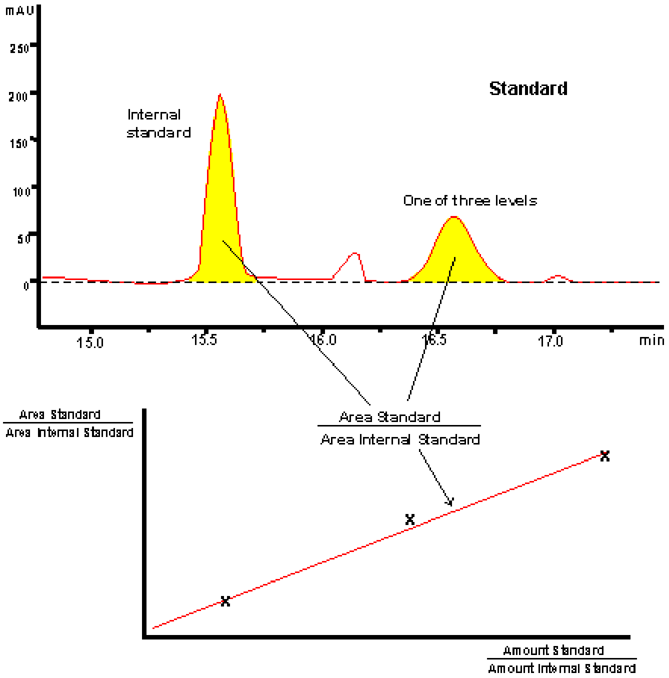
Note: If there are several components of interest, they must all be chemically similar.

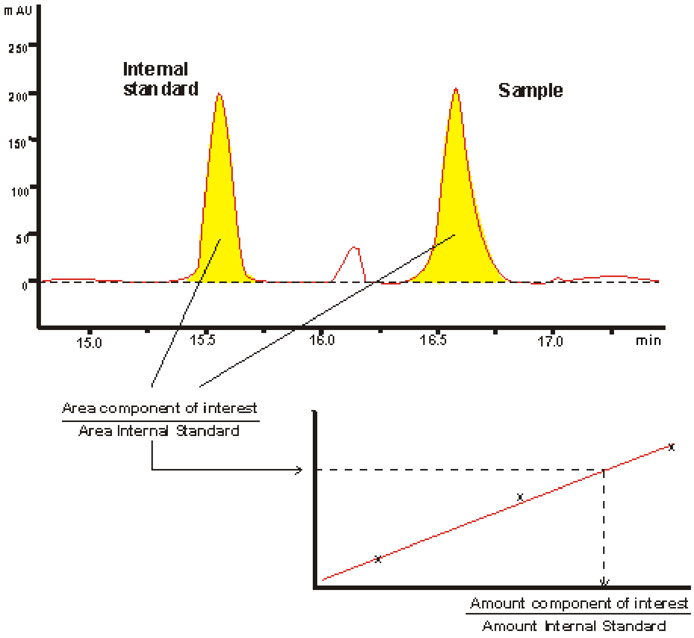
How to perform Internal standard quantitation

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.

Step	Action
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.

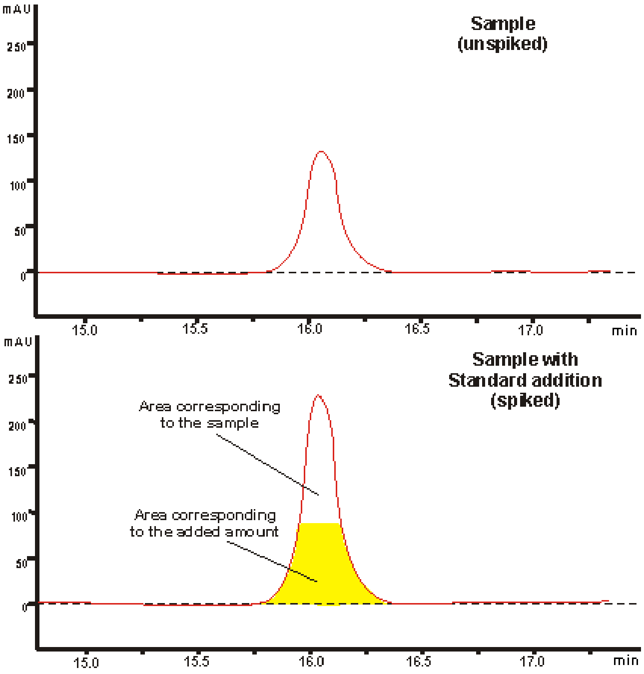
Disadvantages

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.
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: 

Step	Action
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: $\text{Unspiked sample amount} = \text{Amount added} \times \frac{\text{Peak area from unspiked sample}}{(\text{Peak area spiked sample - Peak area unspiked sample})}$

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.

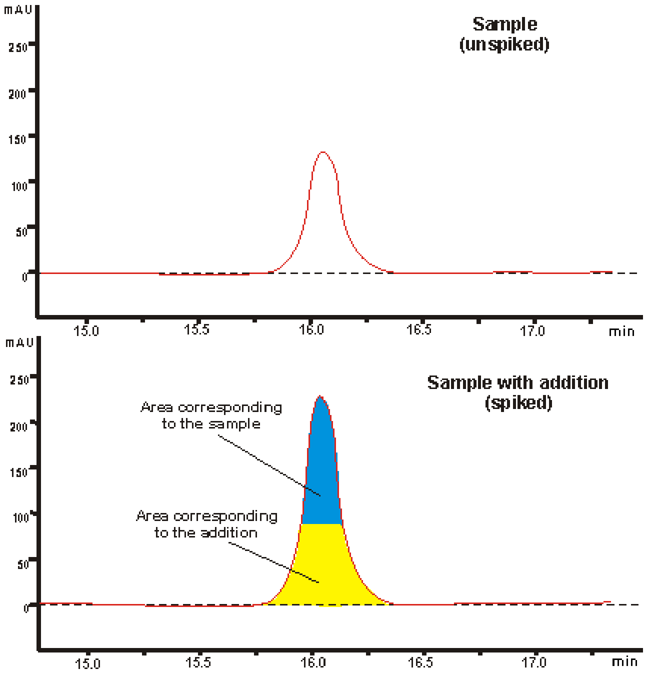
The recovery factor

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.
6	Peak integrate both samples to produce peak tables for the unspiked sample and the spiked sample.

Step	Action
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 the following sub-sections

Topic	**See**
Preparations before quantitation	13.3.1
How to create a quantitation table	13.3.2
How to edit and update a quantitation table	13.3.3

• p 425

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

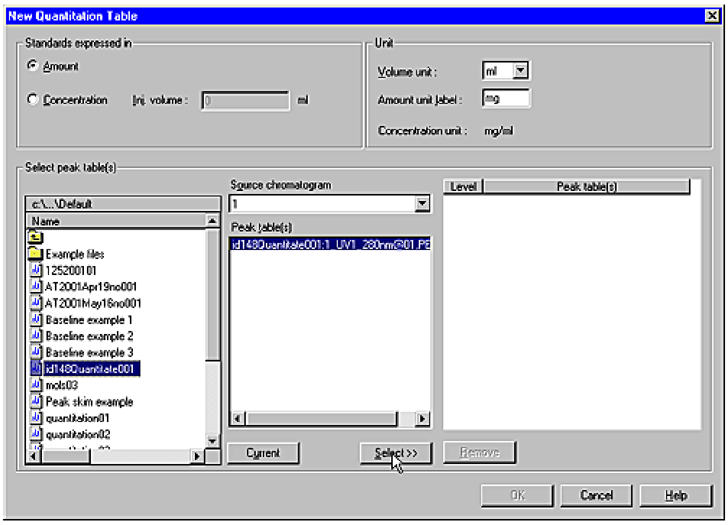
Step 1 - How to input the standard data

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.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>

- Double-click a result file in the **Select peak table** list if you want to select a source chromatogram from another result file.



● Amount
○ Concentration Inj. volume: 0 ml
Volume unit: ml
Amount unit label: mg
Concentration unit: mg/ml
c:\...\Default
Source chromatogram: 1
Peak table(s): id148Quantitate001:1_UV1_280nm@01,PE

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.

</td></tr>
<tr><td>3</td><td>

- 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**.

</td></tr>
<tr><td>4</td><td>

- 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.

</td></tr>
</table>

Step	Action
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.

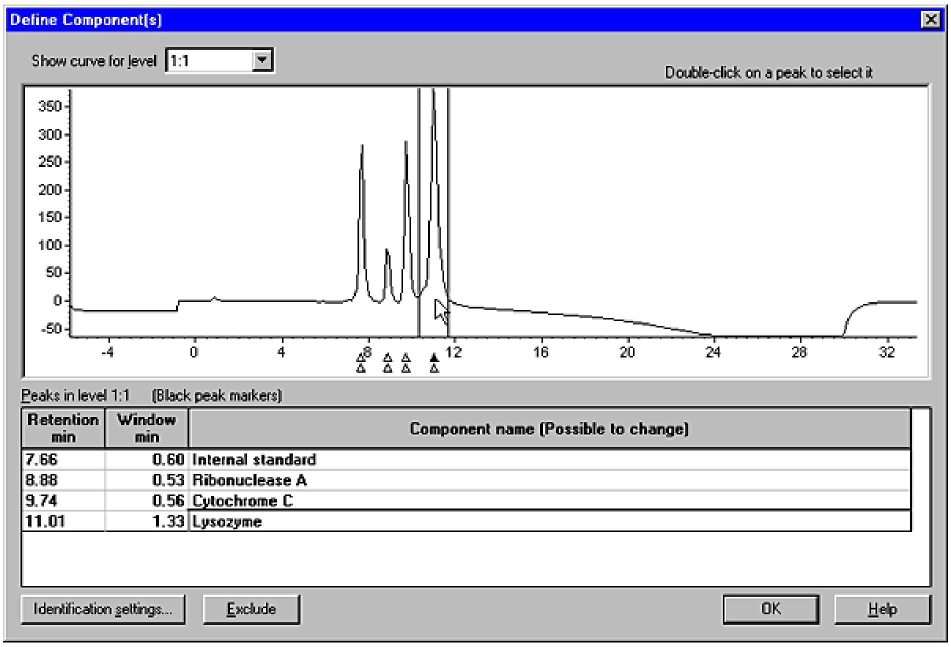
Standard concentration levels

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.



Peaks in level 1:1 (Black peak markers)

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

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.
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.

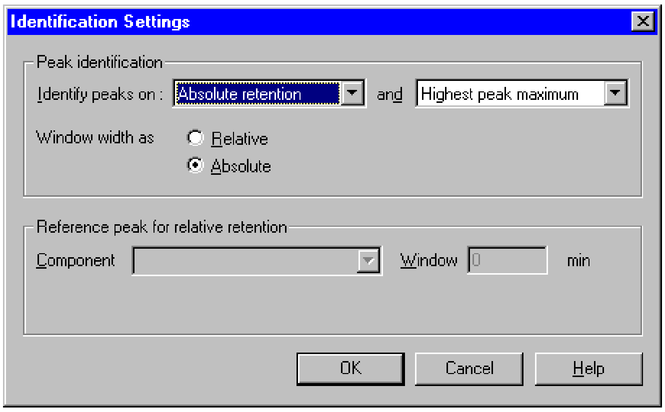
How to adjust the identification settings

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.
2	Click **Identification Settings**. *Result*: The **Identification Settings** dialog box opens.  Identification Settings Peak identification Identify peaks on : Absolute retention and Highest peak maximum Window width as ○ Relative ● Absolute Reference peak for relative retention Component: (empty) Window 0 min 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.

Step	Action
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

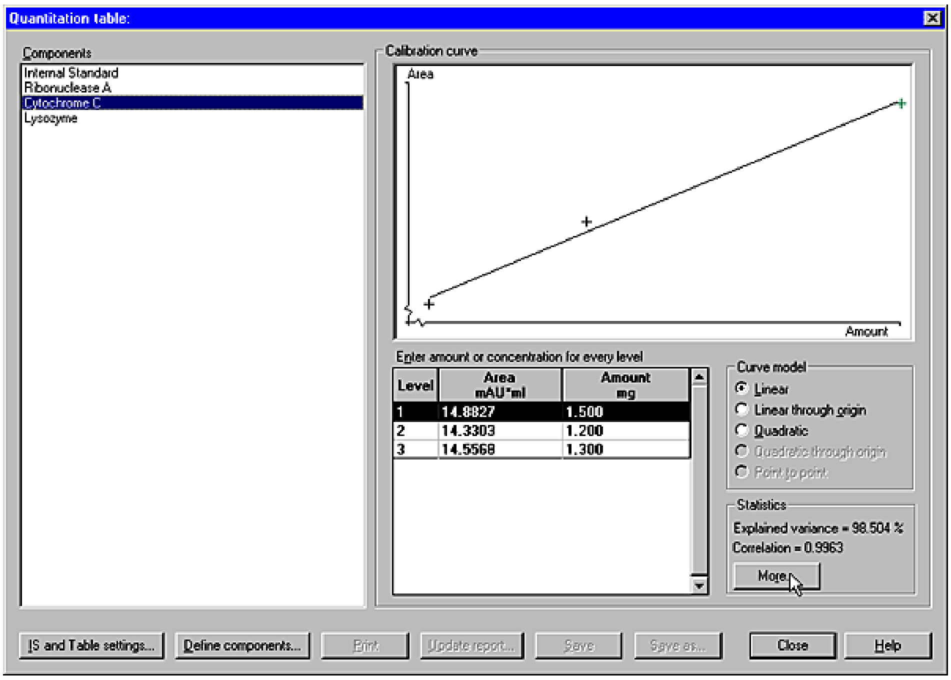
When the **Peak identification** is set to **Absolute retention**, the peak window width can be displayed as **Absolute** or **Relative**. Select the appropriate button in the **Identification Settings** dialog box.

- 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:



Enter amount or concentration for every level

Level	Area mAU*ml	Amount mg
1	14.8827	1.500
2	14.3303	1.200
3	14.5568	1.300

● Linear
○ Linear through origin
○ Quadratic
○ Quadratic through origin
○ Point to point

Explained variance = 98.504 %
Correlation = 0.9963

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

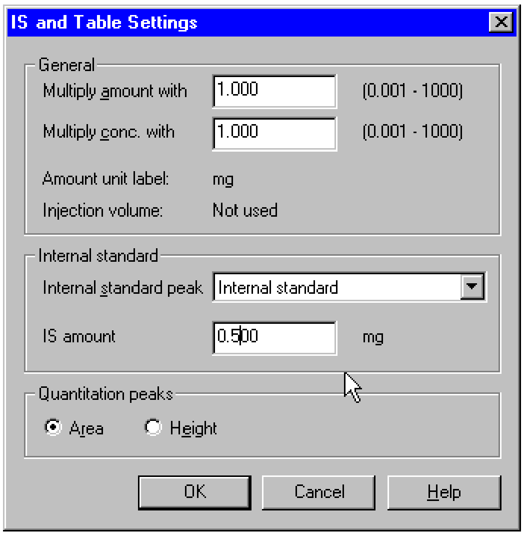
Step	Action
1	• Click the **IS and Table settings** button if you want to use an internal standard or base the calibration curve on peak height (see "How to select an Internal Standard" below this table).
2	• Verify that the selected components in the **Components** list are correct. - If an internal 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.  IS and Table Settings General Multiply amount with: 1.000 (0.001 - 1000) Multiply conc. with: 1.000 (0.001 - 1000) Amount unit label: mg Injection volume: Not used Internal standard Internal standard peak: Internal standard IS amount: 0.500 mg Quantitation peaks ● Area ○ Height
2	Type the amount and concentration multipliers in the **General** field. *Note*: These values are normally set to 1. See remarks below.
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.

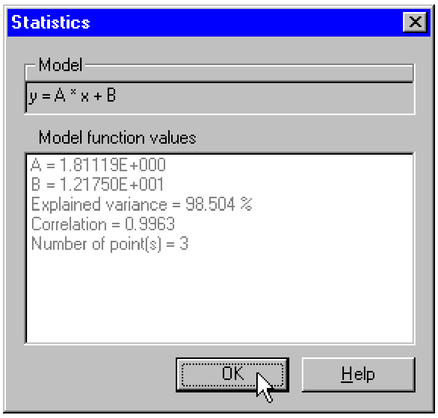
Step	Action
5	Select if the quantitation will be based on **Area** (default) or **Height** in the **Quantitation peaks** field. *Note*: Select **Height** if the peaks are not completely separated from those of other components.
6	Click **OK**.

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.



y = A * x + B

A = 1.81119E+000
B = 1.21750E+001
Explained variance = 98.504 %
Correlation = 0.9963
Number of point(s) = 3

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.

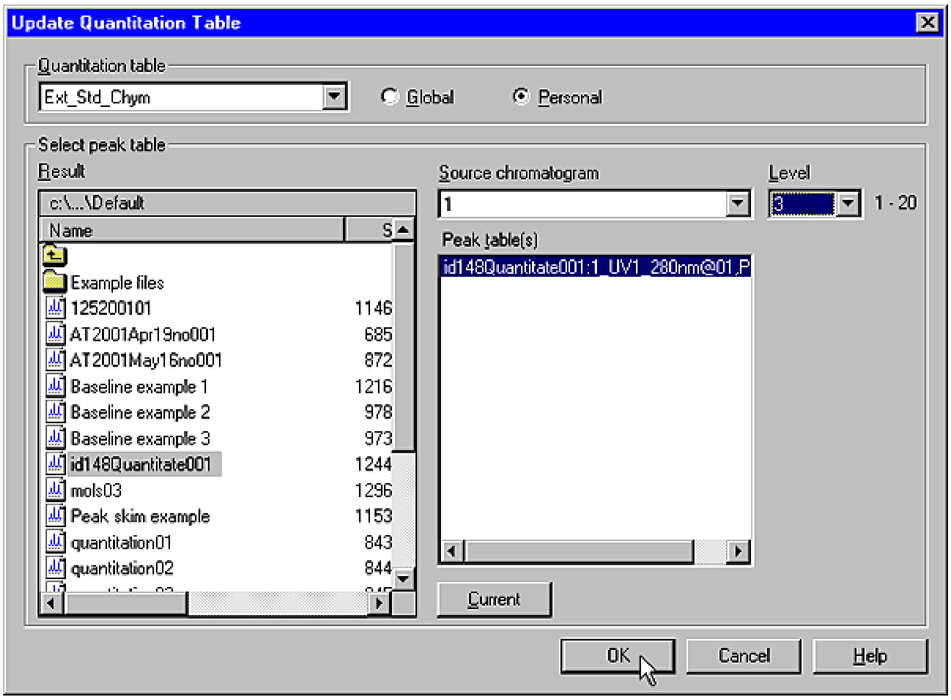
The update function

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.



Quantitation table: Ext_Std_Chym ○ Global ● Personal

c:\...\Default

Name	S
Example files	
125200101	1146
AT2001Apr19no001	685
AT2001May16no001	872
Baseline example 1	1216
Baseline example 2	978
Baseline example 3	973
id148Quantitate001	1244
mols03	1296
Peak skim example	1153
quantitation01	843
quantitation02	844

Source chromatogram: 1

Level: 3 1 - 20

Peak table(s): id148Quantitate001:1_UV1_280nm@01_P

How to prepare the calibration curve for updating

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

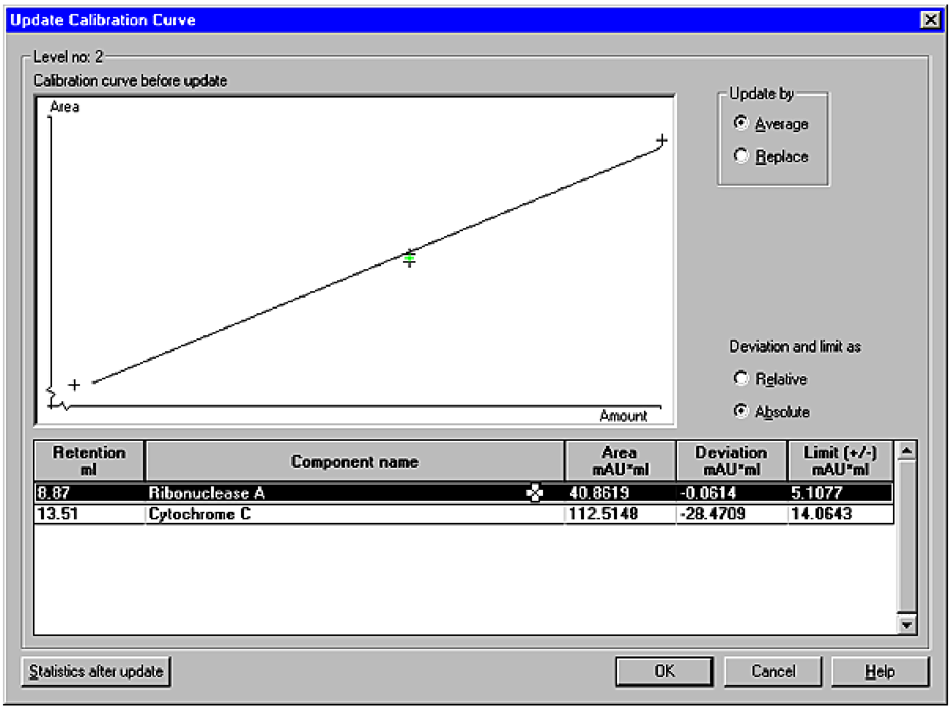
Step	Action
1	Perform a peak integration for the new run and save the result.
2	Select **Quantitate:Edit Quantitation Table:Update**. *Result*: The **Update Quantitation Table** dialog box opens.
3	• Select the **Personal** radio button if the table is located in your personal folder. • Select the quantitation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

Step	**Action**
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.



Level no: 2

Update by: ● Average ○ Replace

Deviation and limit as: ○ Relative ● Absolute

Retention ml	Component name	Area mAU*ml	Deviation mAU*ml	Limit (+/-) mAU*ml
8.87	Ribonuclease A	40.8619	-0.0614	5.1077
13.51	Cytochrome C	112.5148	-28.4709	14.0643

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.
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.

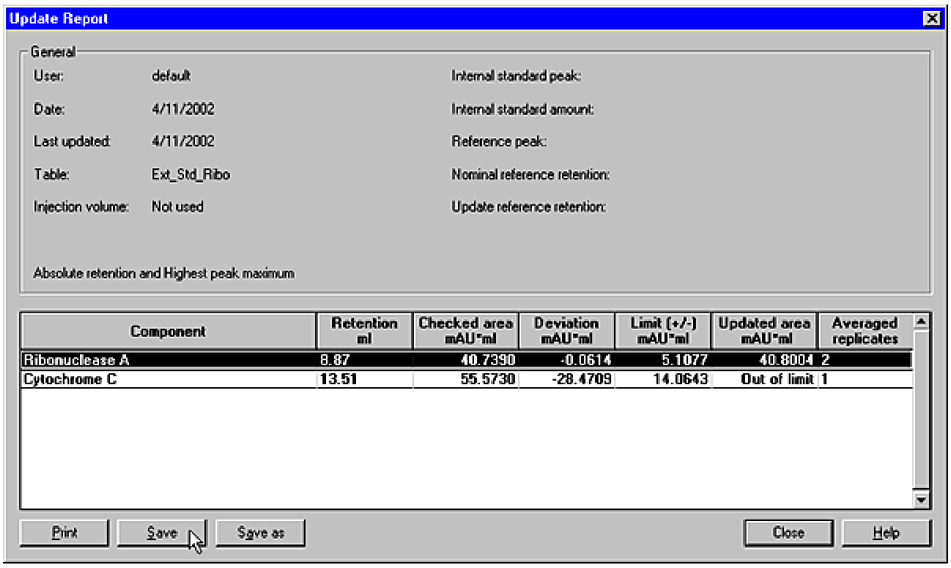
Update by Replace

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.



User: default
Date: 4/11/2002
Last updated: 4/11/2002
Table: Ext_Std_Ribo
Injection volume: Not used
Internal standard peak:
Internal standard amount:
Reference peak:
Nominal reference retention:
Update reference retention:

Absolute retention and Highest peak maximum

Component	Retention ml	Checked area mAU*ml	Deviation mAU*ml	Limit (+/-) mAU*ml	Updated area mAU*ml	Averaged replicates
Ribonuclease A	8.87	40.7390	-0.0614	5.1077	40.8004	2
Cytochrome C	13.51	55.5730	-28.4709	14.0643	Out of limit	1

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.

Step	Action
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 the following sub-sections

Topic	See
External and internal standard quantitation	13.4.1
Standard addition quantitation	13.4.2
How to calculate the recovery factor	13.4.3

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**.

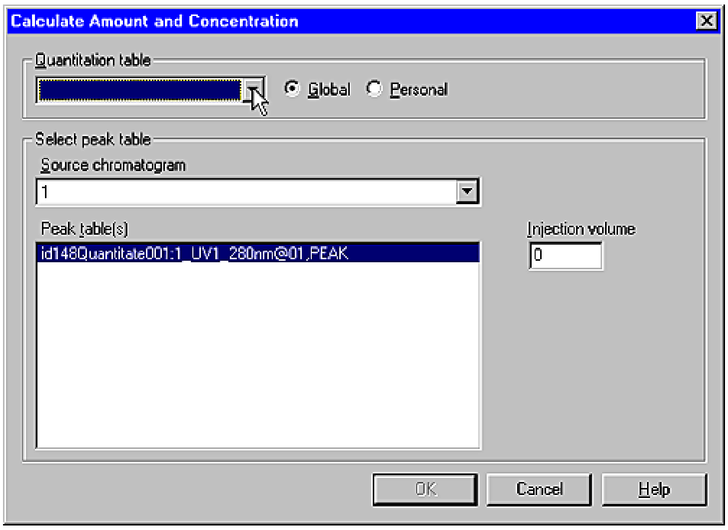
How to prepare for the quantitation

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

Step	**Action**
1	Prepare a quantitation table for the components of interest. See **13.3.2 How to create a quantitation table** on page 427 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.

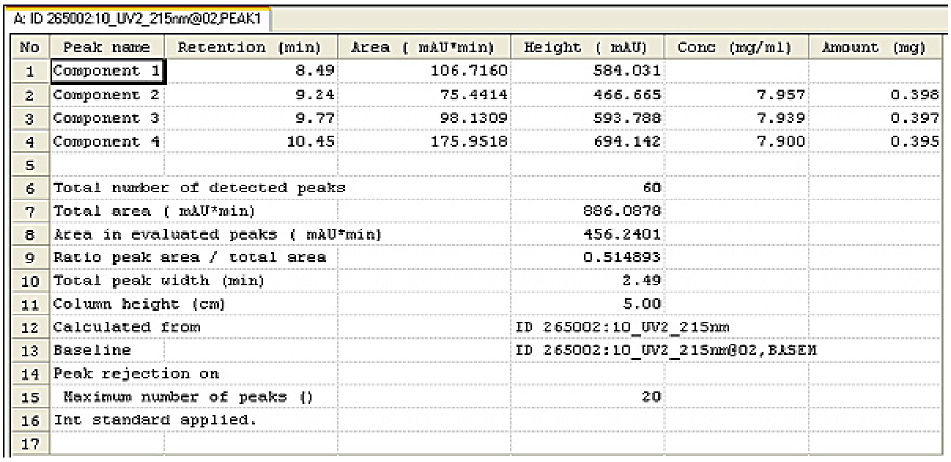
How to calculate the amount and concentration

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.  Calculate Amount and Concentration Quantitation table: ● Global ○ Personal Select peak table Source chromatogram: 1 Peak table(s): id148Quantitate001:1_UV1_280nm@01,PEAK Injection volume: 0
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 265002:10_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.

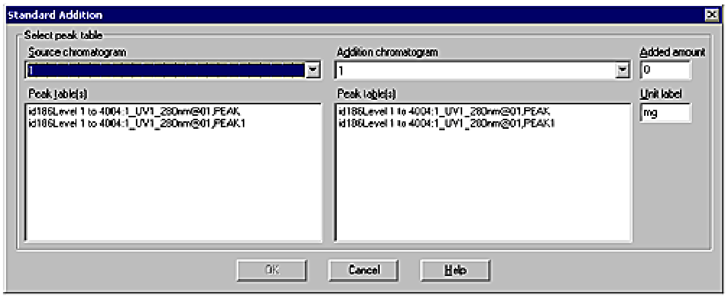
How to prepare for the Standard addition quantitation

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.

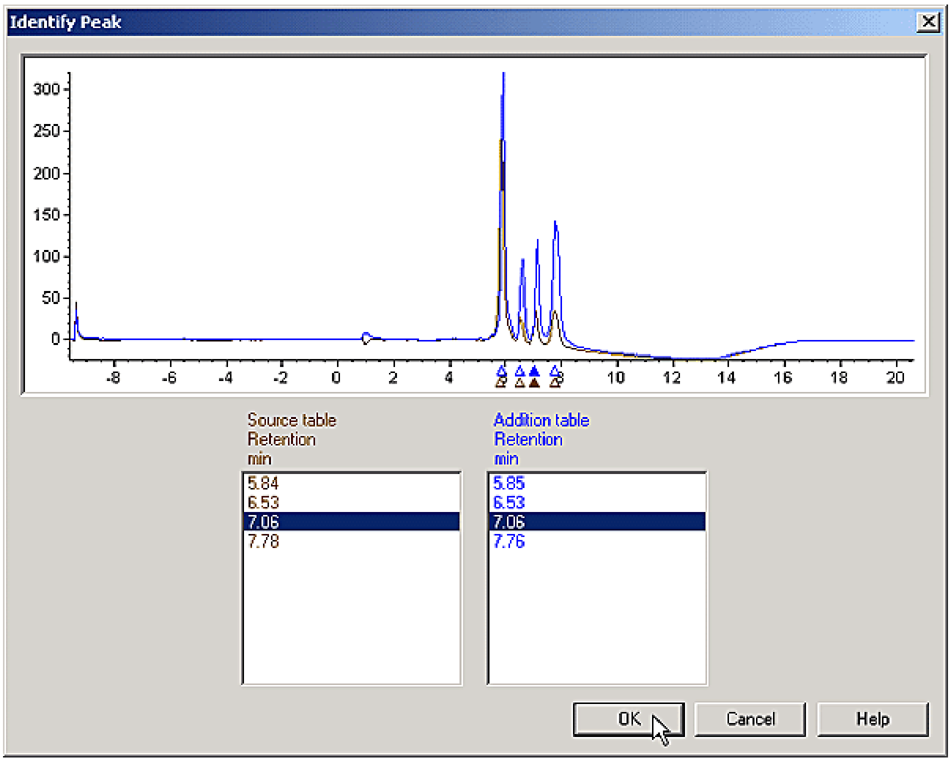
How to select the component and identify the sample peaks

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.  Source chromatogram: 1 Peak table(s): id186Level 1 to 4004:1_UV1_280nm@01,PEAK id186Level 1 to 4004:1_UV1_280nm@01,PEAK1 Addition chromatogram: 1 Peak table(s): id186Level 1 to 4004:1_UV1_280nm@01,PEAK id186Level 1 to 4004:1_UV1_280nm@01,PEAK1 Added amount: 0 Unit label: mg
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

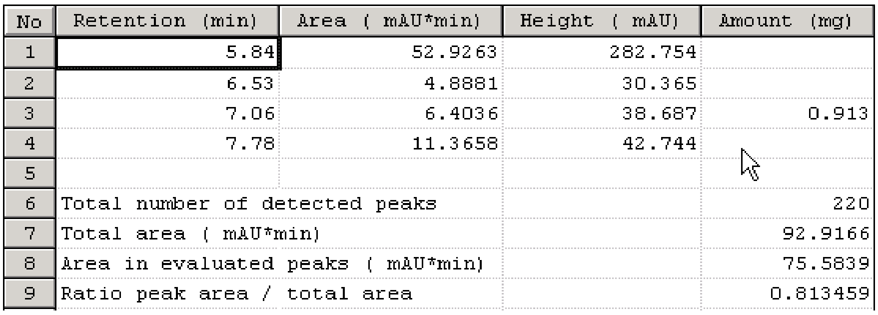
The illustration shows the **Identify Peak** dialog box, described in the table above.



Source table Retention min	Addition table Retention min
5.84	5.85
6.53	6.53
7.06	7.06
7.78	7.76

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*

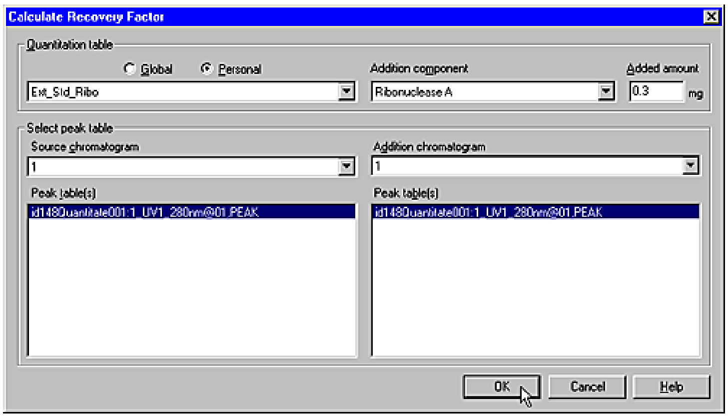
How to prepare for the quantitation

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.

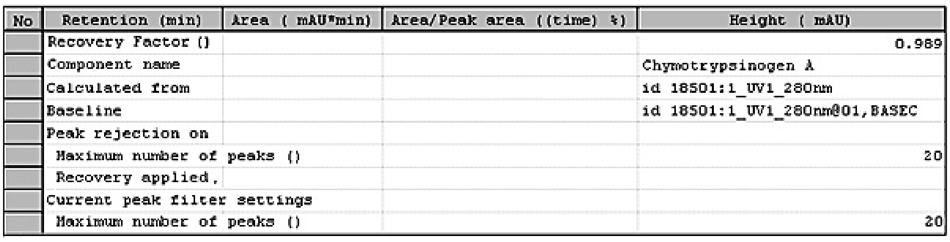
How to calculate the recovery

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.  Calculate Recovery Factor Quantitation table: ○ Global ● Personal Ext_Std_Ribo Addition component: Ribonuclease A Added amount: 0.3 mg Select peak table Source chromatogram: 1 Peak table(s): id148Quantitate001:1_UV1_280nm@01.PEAK Addition chromatogram: 1 Peak table(s): id148Quantitate001:1_UV1_280nm@01.PEAK
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 the following sub-sections

Topic	See
How to set up for automated quantitation	13.5.1
How to perform automated quantitation	13.5.2
How to perform automated update	13.5.3

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

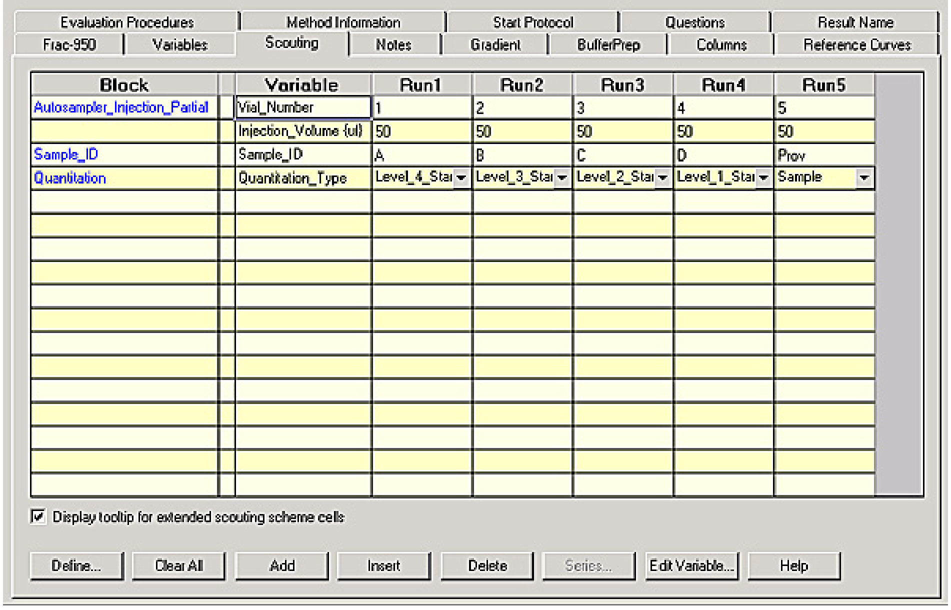
The table below describes how to prepare the quantitation table for automated quantitation.

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.
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 **7.1 How to set up a scouting scheme** on page 183.

Step	Action
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 **13.3.2 How to create a quantitation table** on page 427.

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.



Block	Variable	Run1	Run2	Run3	Run4	Run5
Autosampler_Injection_Partial	Vial_Number	1	2	3	4	5
	Injection_Volume (ul)	50	50	50	50	50
Sample_ID	Sample_ID	A	B	C	D	Prov
Quantitation	Quantitation_Type	Level_4_Sta	Level_3_Sta	Level_2_Sta	Level_1_Sta	Sample

☑ Display tooltip for extended scouting scheme cells

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 **13.5.1 How to set up for automated quantitation** on page 457) • 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 **13.3.3 How to edit and update a quantitation table** on page 439.

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.
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

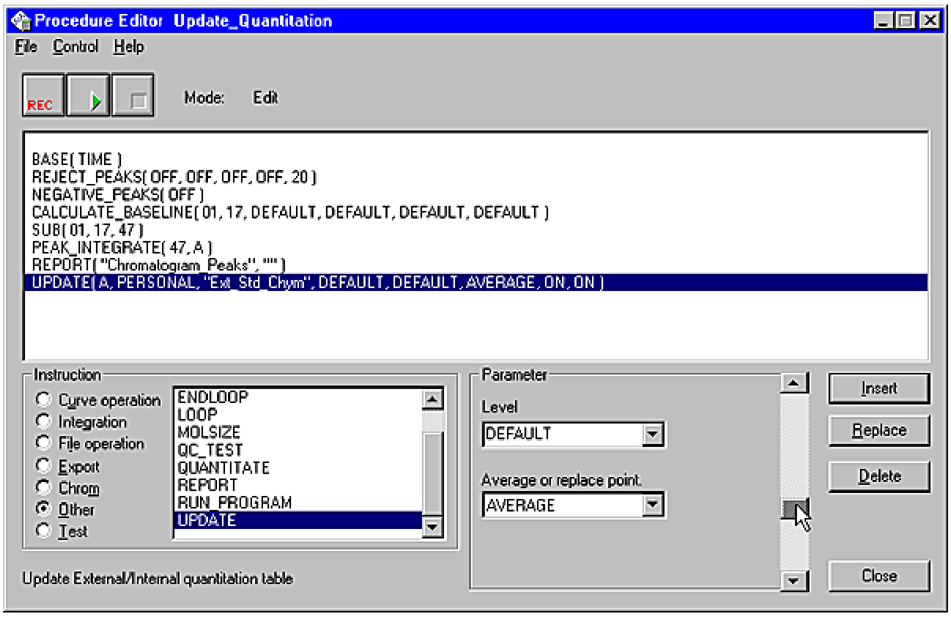
The table below describes how to automatically update a quantitation table with the **Average** option.

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.
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:



```
BASE( TIME )
REJECT_PEAKS( OFF, OFF, OFF, OFF, 20 )
NEGATIVE_PEAKS( OFF )
CALCULATE_BASELINE( 01, 17, DEFAULT, DEFAULT, DEFAULT, DEFAULT )
SUB( 01, 17, 47 )
PEAK_INTEGRATE( 47, A )
REPORT( "Chromatogram_Peaks", "" )
UPDATE( A, PERSONAL, "Ext_Std_Chym", DEFAULT, DEFAULT, AVERAGE, ON, ON )
```

Level: DEFAULT

Average or replace point: AVERAGE

Update External/Internal quantitation table

How to perform automated update in scouting runs - step 1

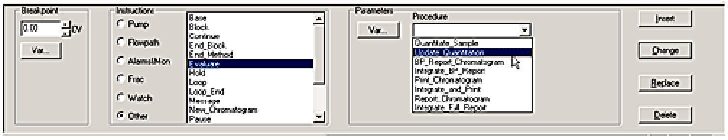
It is possible to run both standards and samples in the same scouting run and continuously update a previously created quantitation table with new values. The table below describes how to set up the evaluation procedures for the updates.

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.

Step	Action
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.
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.
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.
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.

Step	Action
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

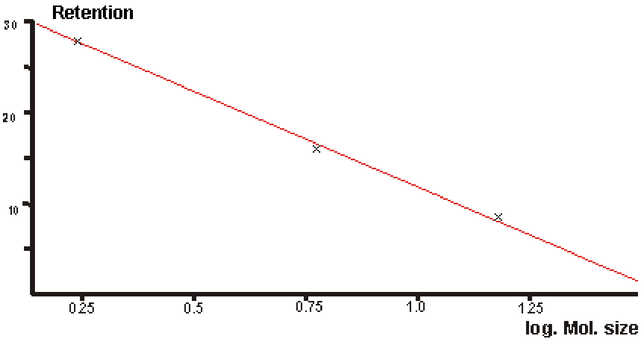
This section contains the following sub-sections

Topic	See
Overview of molecular size determination	13.6.1
How to determine the molecular size	13.6.2

13.6.1 Overview of molecular size determination

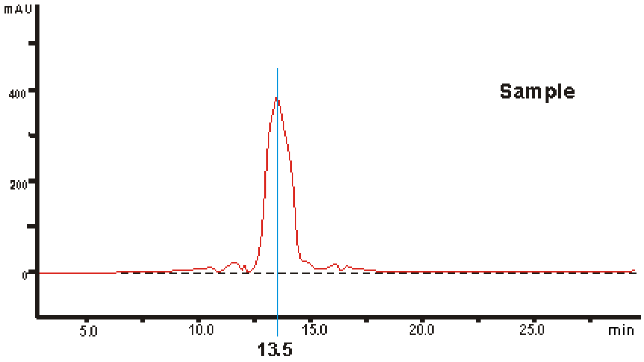
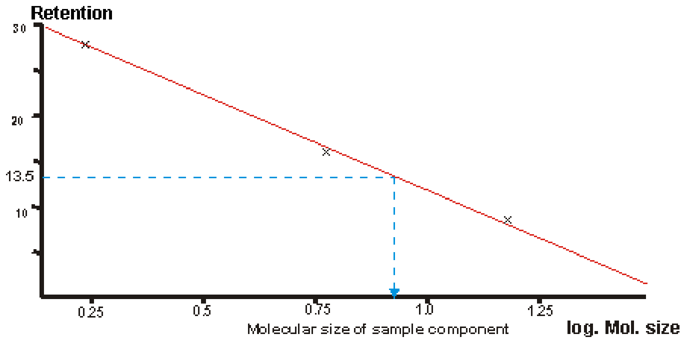
How to create a molecular size curve

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:

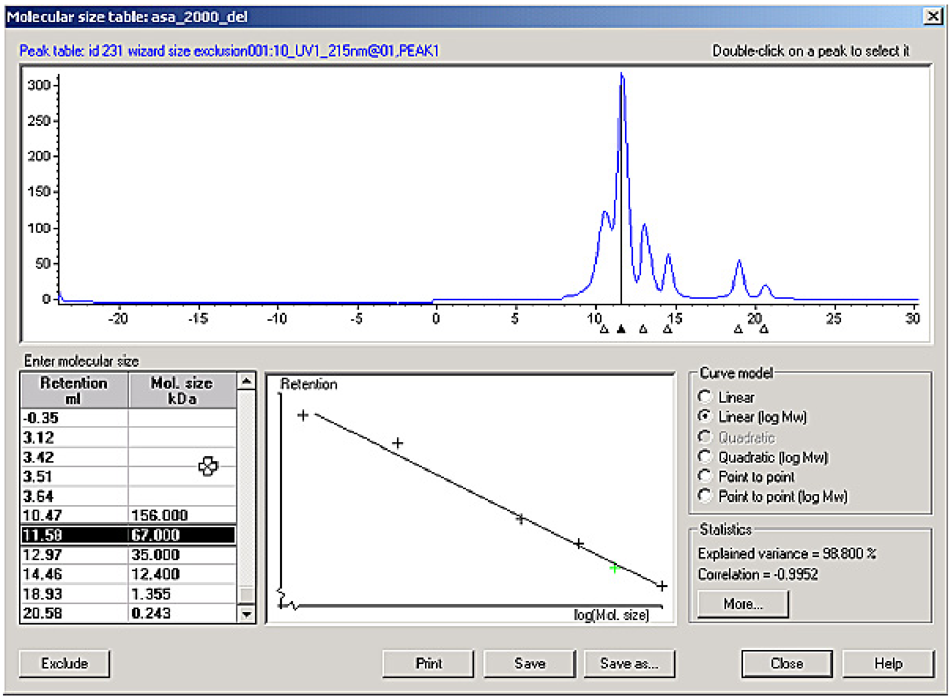
- Perform chromatographic runs with an appropriate standard with components of known molecular size. The standard should contain components of sizes that extend over the range that is expected in the sample. If you are using many components, it may be better to split them into two or more standard runs.
- 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

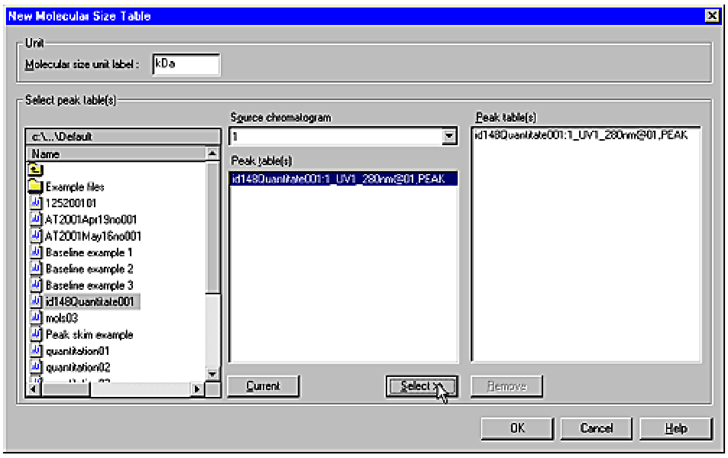
Retention ml	Mol. size kDa
-0.35	
3.12	
3.42	
3.51	
3.64	
10.47	156.000
11.58	67.000
12.97	35.000
14.46	12.400
18.93	1.355
20.58	0.243

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

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.  Molecular size unit label : kDa Source chromatogram: 1 Peak table(s): id148Quantitate001:1_UV1_280nm@01,PEAK Peak table(s): id148Quantitate001:1_UV1_280nm@01,PEAK
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.
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.

Step	Action
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.

Step	Action
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.

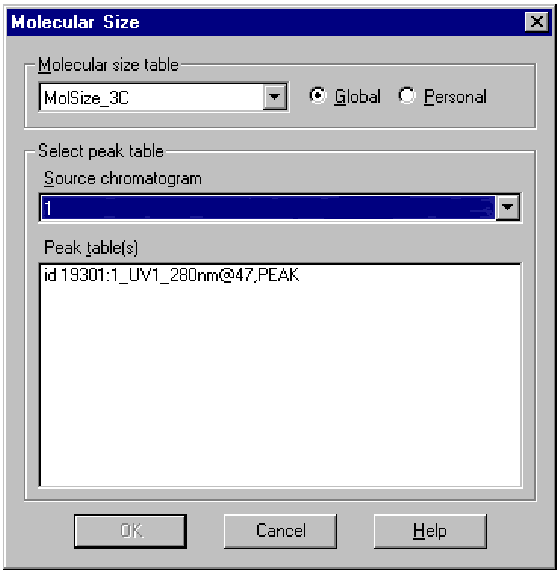
Step	Action
1	Select **Mol. Size:Edit Mol. Size Table:Delete**. *Result*: The **Delete molecular size table** dialog box opens.
2	• Select **Personal** to display the tables that are restricted to your own user ID, if needed. • Select the molecular size table you wish to delete in the **Molecular size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

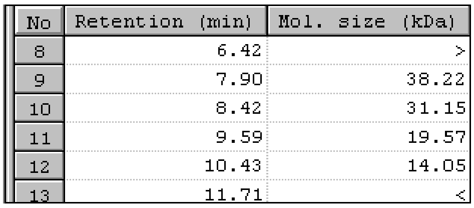
The table below describes how the molecular size curve is used to calculate the molecular sizes of the components in the sample.

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.

Step	Action
2	Select **Mol. Size:Calculate Mol. Size** in the **Evaluation** module. *Result*: The **Molecular Size** dialog box opens.  Molecular size table: MolSize_3C ● Global ○ Personal Select peak table Source chromatogram: 1 Peak table(s): id 19301:1_UV1_280nm@47,PEAK
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 system settings.

In this chapter

This chapter contains the following 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

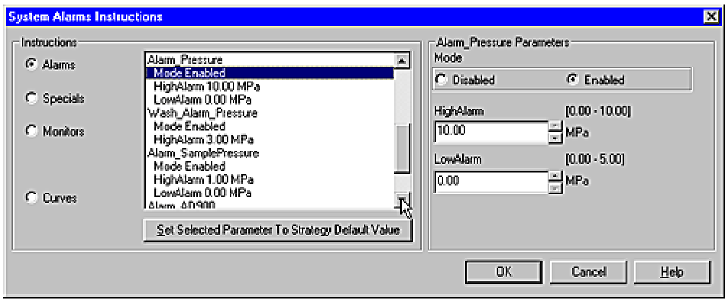
The table below describes the two different ways to change the default system settings.

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.  System Alarms Instructions Instructions ● Alarms ○ Specials ○ Monitors ○ Curves Alarm_Pressure Mode Enabled HighAlarm 10.00 MPa LowAlarm 0.00 MPa Wash_Alarm_Pressure Mode Enabled HighAlarm 3.00 MPa Alarm_SamplePressure Mode Enabled HighAlarm 1.00 MPa LowAlarm 0.00 MPa Alarm_Pressure Parameters Mode ○ Disabled ● Enabled HighAlarm [0.00 - 10.00] 10.00 MPa LowAlarm [0.00 - 5.00] 0.00 MPa
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.
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

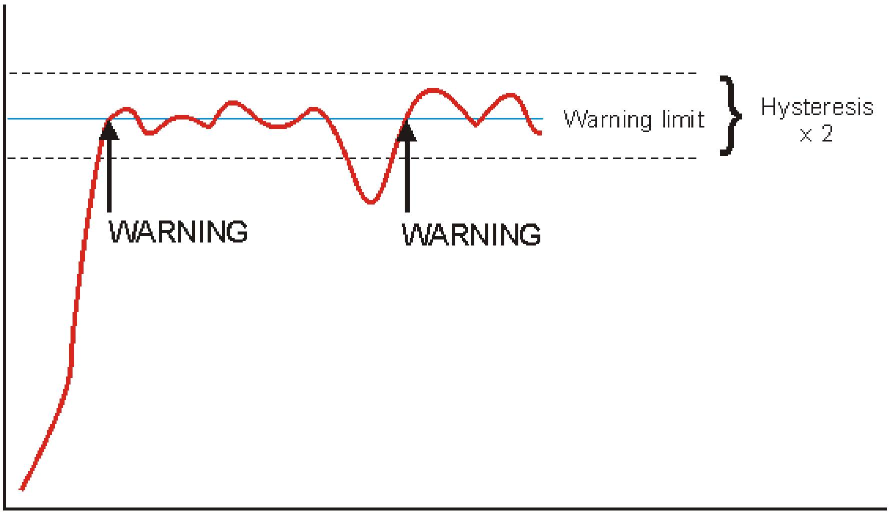
Alarms and warning messages are displayed on all stations with a connection to the concerned system. This is regardless of the activity that is currently performed in UNICORN and regardless of the identity and access rights of the current user.

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.

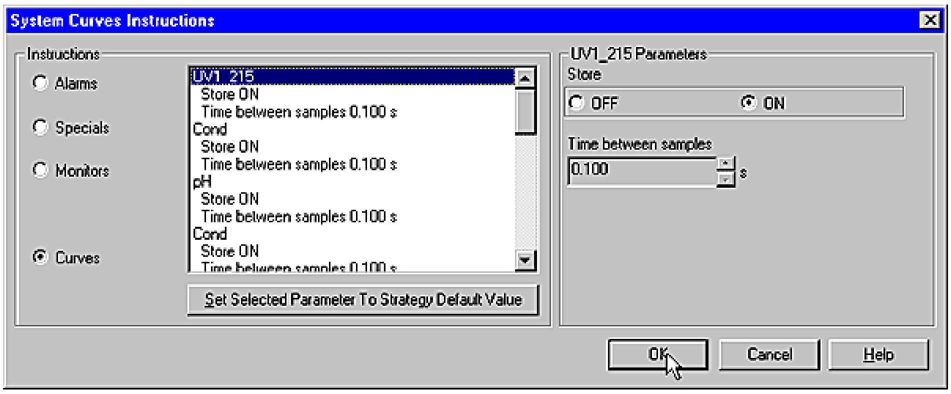
14.3 Curves

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.



UV1_215
Store ON
Time between samples 0.100 s
Cond
Store ON
Time between samples 0.100 s
pH
Store ON
Time between samples 0.100 s
Cond
Store ON
Time between samples 0.100 s

Store ○ OFF ● ON

Time between samples 0.100 s

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.

Warning: 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.
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 the following sections

Topic	See
System maintenance functions	15.1
How to generate problem reports	15.2

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.

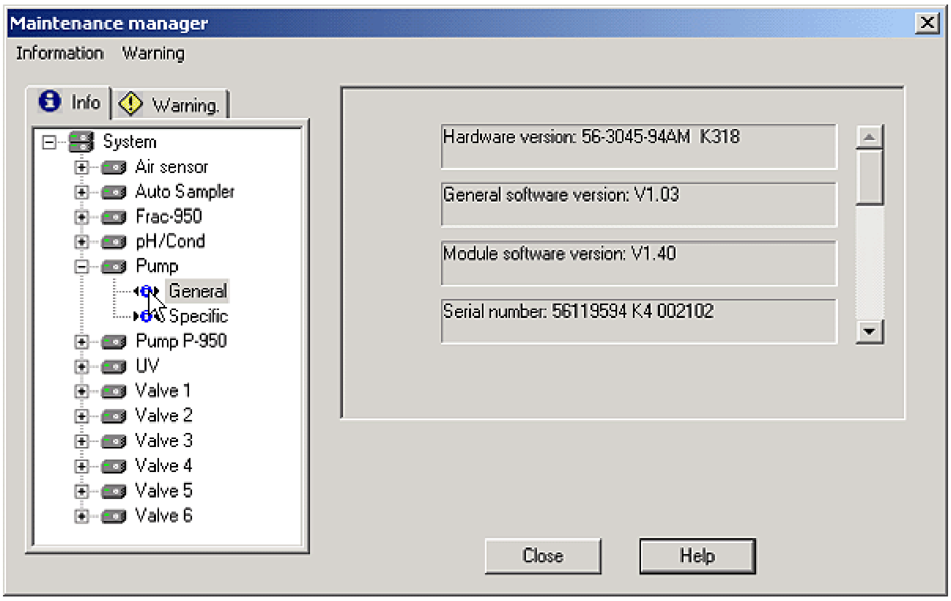
How to open the Maintenance manager

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:



Hardware version: 56-3045-94AM K318

General software version: V1.03

Module software version: V1.40

Serial number: 56119594 K4 002102

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.

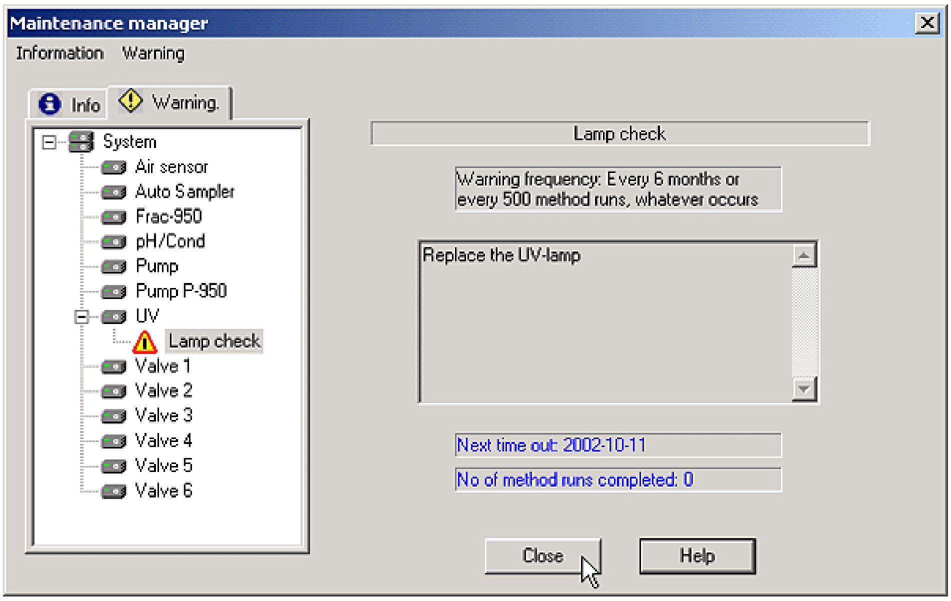
Step	Action
1	Click the **Warning** tab.
2	Select a component. - Choose **Warning:New**. *or* - Right-click the component and select the **New** option on the shortcut menu.
3	- Type the appropriate value in the **Periodicity** field. - Type a warning text in the **Pop up text** box. - Type a name for 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:



Lamp check

Warning frequency: Every 6 months or every 500 method runs, whatever occurs

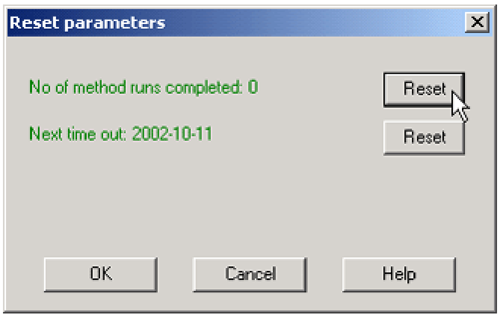
Replace the UV-lamp

Next time out: 2002-10-11

No of method runs completed: 0

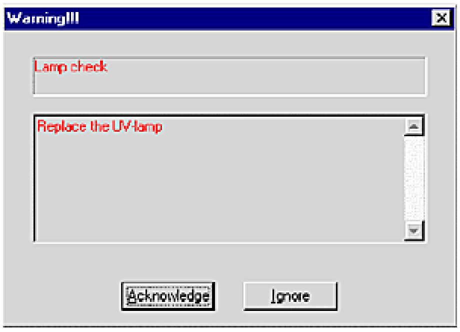
How to reset the counters

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.
3	• Type new text if necessary. • Click the **Reset** button. *Result*: The **Reset parameters** dialog box opens.  Reset parameters No of method runs completed: 0 Next time out: 2002-10-11
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.  Warning!!! Lamp check Replace the UV-lamp
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 the following sub-sections

Topic	See
How to generate a report from the UNICORN Manager	15.2.1
How to generate a report from the System Control	15.2.2

15.2.1 How to generate a 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

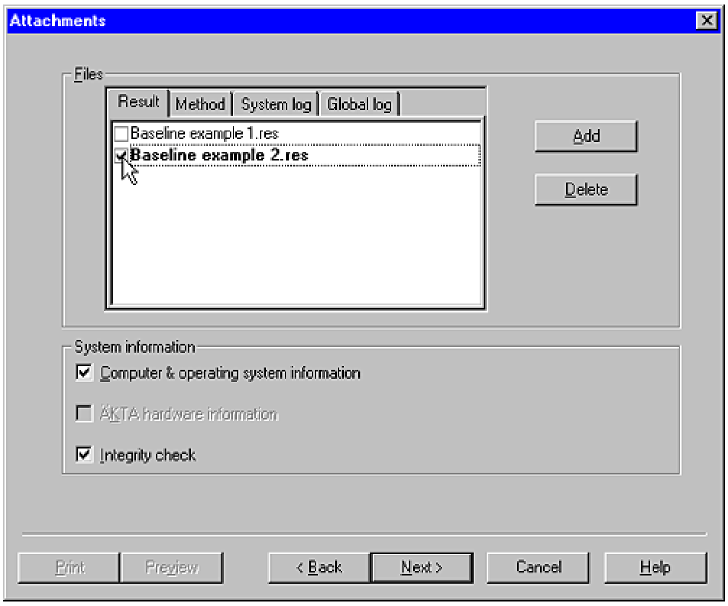
The table below describes how to create a report with the **Generate Report Wizard**.

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. • Select a system for which the report is to be generated and click the **Next** button. *Result*: The **Description** dialog box opens.
3	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.
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 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:  ☐ Baseline example 1.res ☑ Baseline example 2.res ☑ Computer & operating system information ☐ ÄKTA hardware information ☑ Integrity check

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.

Step	Action
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 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:

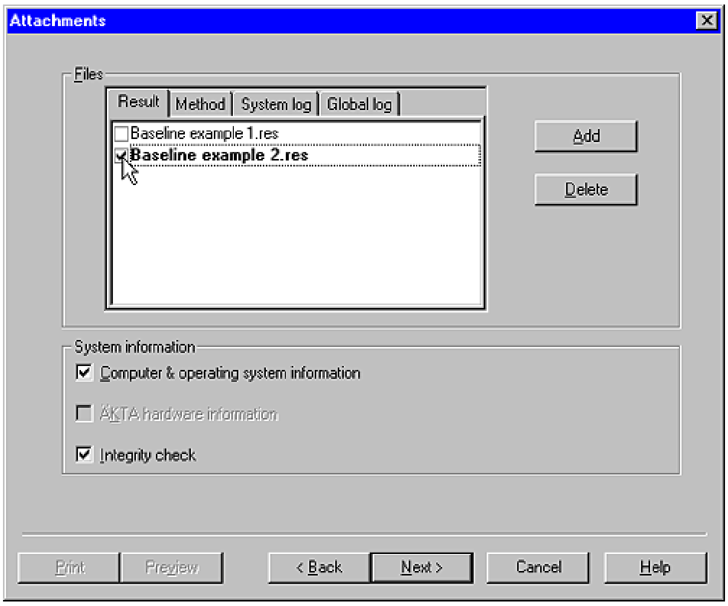
Step	**Action**
1	• Click the **Report** button in the error message dialog box. *or* • Choose **System:Report**.
2	The first step is a **Welcome** screen. Click the **Next** button. *Result*: The **Description** dialog box is displayed and shows a list of the problems/errors that have occurred. All the problems/errors that have occurred, together with help texts, are automatically recorded and included in the report. • 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:  ☐ Baseline example 1.res ☑ Baseline example 2.res ☑ Computer & operating system information ☐ ÄKTA hardware information ☑ Integrity check

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.

A Troubleshooting

Introduction

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

In this chapter

This chapter contains the following sections

Topic	See
Logon	A.1
UNICORN access	A.2
Methods and method runs	A.3
Evaluation	A.4
ÄKTAdesign system specific problems	A.5

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.
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.

Problem description	Solution
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”.

Problem Description	Solution
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

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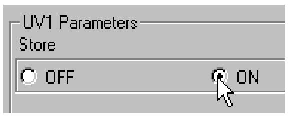
Cannot perform Quit or Logoff

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**.  UV1 Parameters Store ○ OFF ● 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 2000 or Windows XP CD-ROM that was shipped with your Compaq 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 2000/XP CD-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.

After Windows logout and login you cannot get a system connection

The table below describes a system connection problem. This applies only to local systems, not remote systems:

Problem description	Solution
You have logged out of Windows 2000 and then logged in again, but you cannot get a system connection in UNICORN. *Reason*: If you shut down Windows 2000 with the command **Start:Shutdown:Close all programs and log in as a different user**, you will not be able to obtain a **System Control** connection in UNICORN the next time you or another user logs on. This is because the described shutdown procedure automatically shuts down a number of processes, including those needed for system connection. The services are only started when the computer is booted up.	Restart the computer in order to obtain a system connection in UNICORN.

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.

B Evaluation functions and instructions

Introduction

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

In this chapter

This chapter contains the following sections

Topic	See
Smoothing algorithms	B.1
Baseline calculation theory	B.2
Peak table column components	B.3
Procedure instructions	B.4

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.

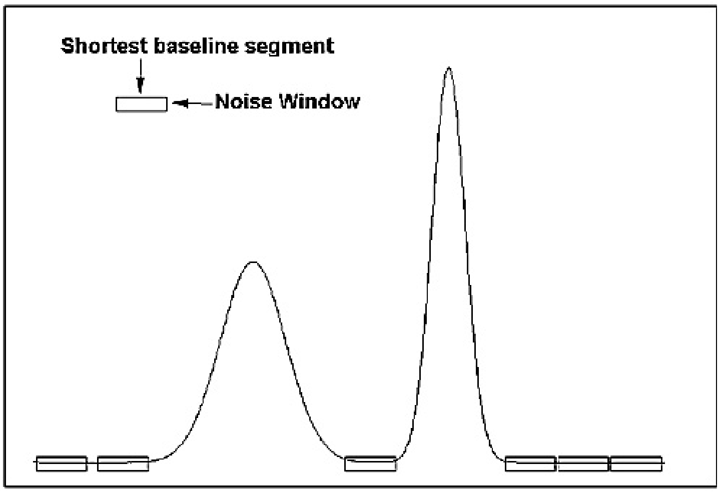
Baseline parameters

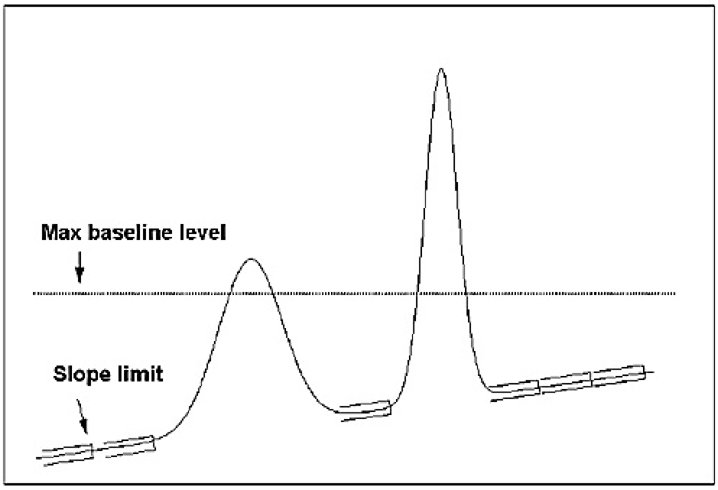
The baseline parameters can be illustrated as a rectangular box that the source curve has to fit into in order to be identified as a baseline segment, where:

- 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.

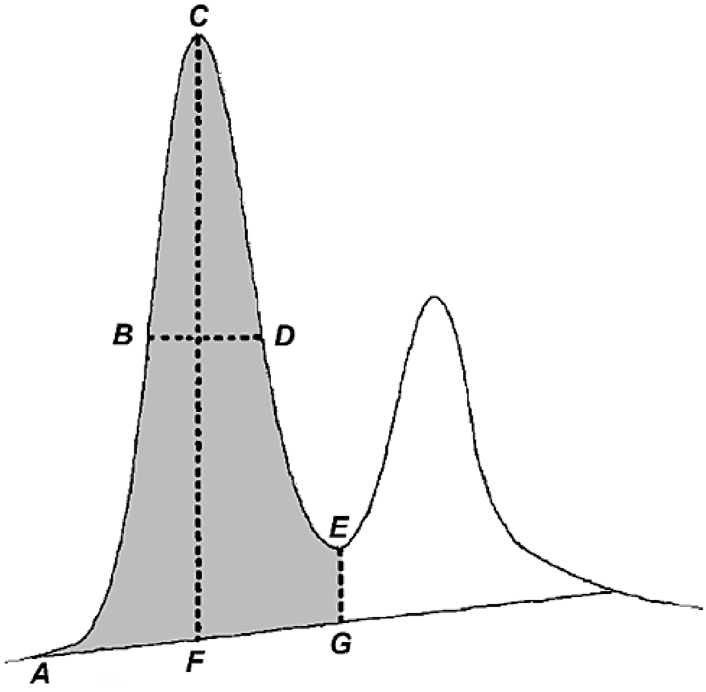
B.3 Peak table column components

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.

Parameter	**Description**
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).

Parameter	Description
Peak endpoint retention	Retention value at peak start and peak end, time or volume base. (A and G in the diagram above.)
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.)

Parameter	Description
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. $(V_{R2} - V_{R1}) / ((\text{Sigma}_2 + \text{Sigma}_1) \times 2)$
3. $((V_{R2} - V_{R1}) / (2 \times (W_{h2} + W_{h1}))) / 2.354$

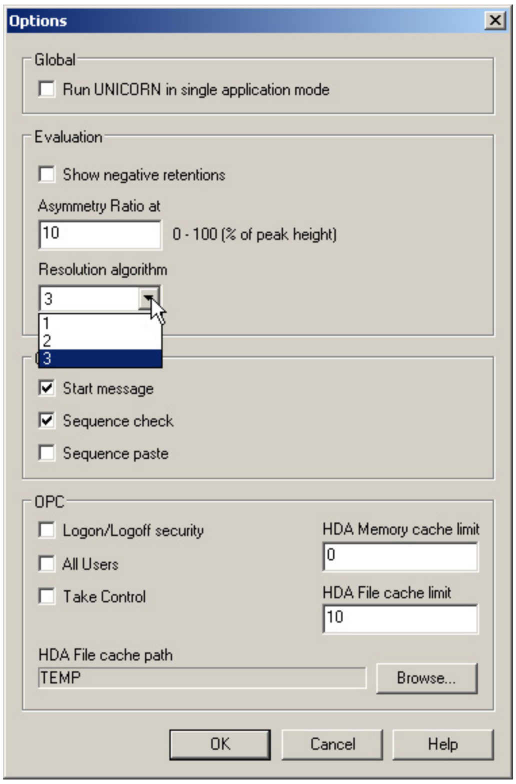
Where:

- 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.
2	- Select the desired algorithm number described as described in **Peak resolution algorithms** above, in the **Resolution algorithm** droplist.  Options Global ☐ Run UNICORN in single application mode Evaluation ☐ Show negative retentions Asymmetry Ratio at: 10 0 - 100 (% of peak height) Resolution algorithm: 3 ☑ Start message ☑ Sequence check ☐ Sequence paste OPC ☐ Logon/Logoff security ☐ All Users ☐ Take Control HDA Memory cache limit: 0 HDA File cache limit: 10 HDA File cache path: TEMP - 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**.

$$k^1 = \frac{V_R - V_t}{V_t}$$

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.

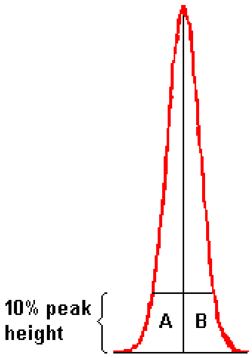
Asymmetry formula

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.

Instruction	Description
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.
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**.

Instruction	Description
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.
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.

Instruction	Description
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

Instruction	Description
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.

Instruction	Description
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.
EXPORT_PEAKTABLE_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_PEAKTABLE_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.

Instruction	Description
EXPORT_PEAKTABLE_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_PEAKTABLE_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.

Instruction	Description
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.

Instruction	Description
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.

Instruction	Description
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.

Instruction	**Description**
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.

Instruction	Description
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.
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_LOGBOOK_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_INTERVALS	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.

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 chapter

This chapter contains the following 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

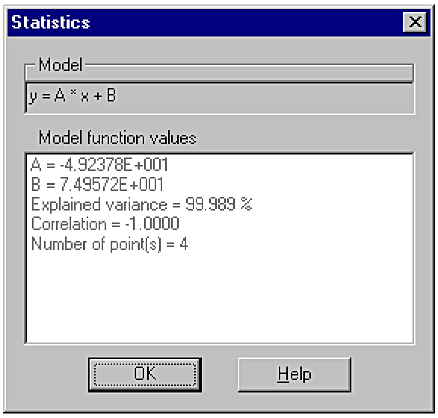
The table below describes the features of the **Linear** curve fit model.

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)

Feature	Description
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:



```
y = A * x + B
```

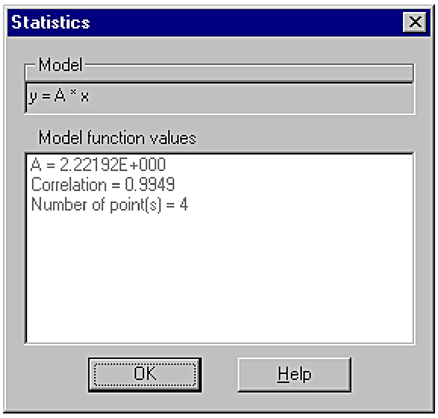
```
A = -4.92378E+001
B = 7.49572E+001
Explained variance = 99.989 %
Correlation = -1.0000
Number of point(s) = 4
```

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)
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:



```
y = A * x
```

```
A = 2.22192E+000
Correlation = 0.9949
Number of point(s) = 4
```

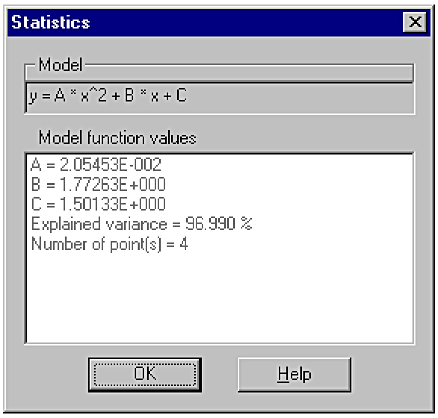
The Quadratic model

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:



```
y = A * x^2 + B * x + C
```

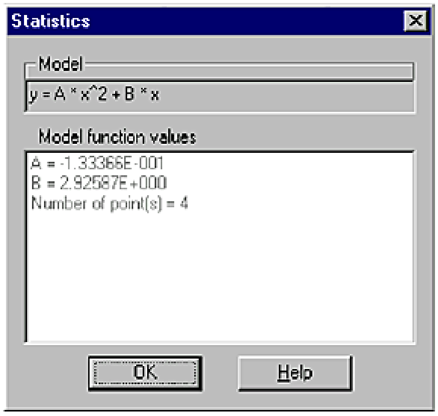
```
A = 2.05453E-002
B = 1.77263E+000
C = 1.50133E+000
Explained variance = 96.990 %
Number of point(s) = 4
```

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:



```
y = A * x^2 + B * x
```

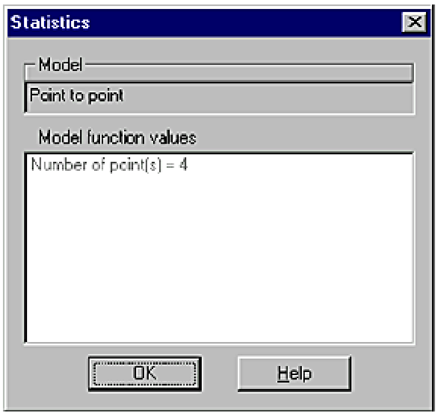
```
A = -1.33366E-001
B = 2.92587E+000
Number of point(s) = 4
```

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:



Model: Point to point

Number of point(s) = 4

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:

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

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

- $\bar{y}$ 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.

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 chapter

This chapter contains the following section

D.1 How to edit the Column List

Introduction

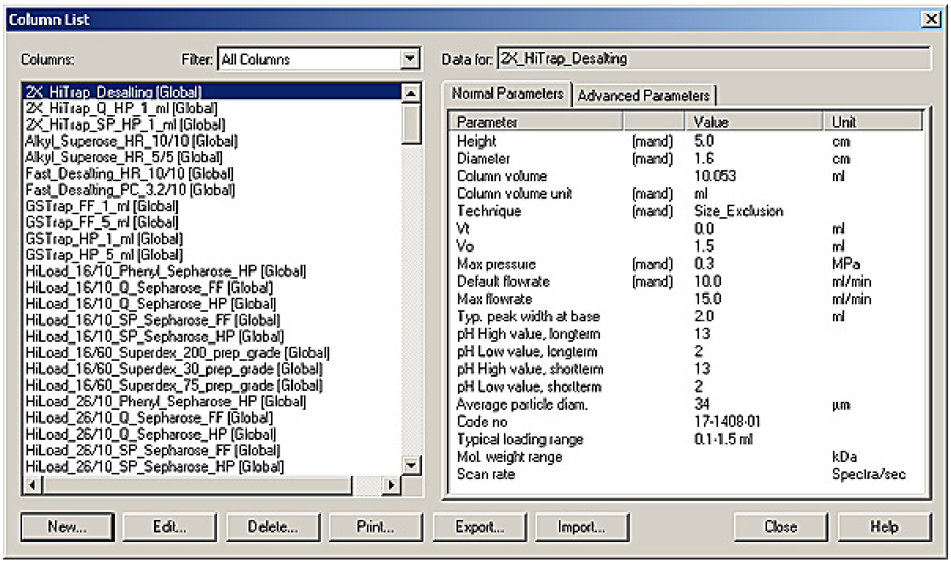
This section describes how to edit the list of available columns.

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.



Filter: All Columns

Data for: 2X_HiTrap_Desalting

2X_HiTrap_Desalting [Global]
2X_HiTrap_Q_HP_1_ml [Global]
2X_HiTrap_SP_HP_1_ml [Global]
Alkyl_Superose_HR_10/10 [Global]
Alkyl_Superose_HR_5/5 [Global]
Fast_Desalting_HR_10/10 [Global]
Fast_Desalting_PC_3.2/10 [Global]
GSTrap_FF_1_ml [Global]
GSTrap_FF_5_ml [Global]
GSTrap_HP_1_ml [Global]
GSTrap_HP_5_ml [Global]
HiLoad_16/10_Phenyl_Sepharose_HP [Global]
HiLoad_16/10_Q_Sepharose_FF [Global]
HiLoad_16/10_Q_Sepharose_HP [Global]
HiLoad_16/10_SP_Sepharose_FF [Global]
HiLoad_16/10_SP_Sepharose_HP [Global]
HiLoad_16/60_Superdex_200_prep_grade [Global]
HiLoad_16/60_Superdex_30_prep_grade [Global]
HiLoad_16/60_Superdex_75_prep_grade [Global]
HiLoad_26/10_Phenyl_Sepharose_HP [Global]
HiLoad_26/10_Q_Sepharose_FF [Global]
HiLoad_26/10_Q_Sepharose_HP [Global]
HiLoad_26/10_SP_Sepharose_FF [Global]
HiLoad_26/10_SP_Sepharose_HP [Global]

Parameter		Value	Unit
Height	(mand)	5.0	cm
Diameter	(mand)	1.6	cm
Column volume		10.053	ml
Column volume unit	(mand)	ml	
Technique	(mand)	Size_Exclusion	
Vt		0.0	ml
Vo		1.5	ml
Max pressure	(mand)	0.3	MPa
Default flowrate	(mand)	10.0	ml/min
Max flowrate		15.0	ml/min
Typ. peak width at base		2.0	ml
pH High value, longterm		13	
pH Low value, longterm		2	
pH High value, shortterm		13	
pH Low value, shortterm		2	
Average particle diam.		34	µm
Code no		17-1408-01	
Typical loading range		0.1-1.5 ml	
Mol. weight range			kDa
Scan rate			Spectra/sec

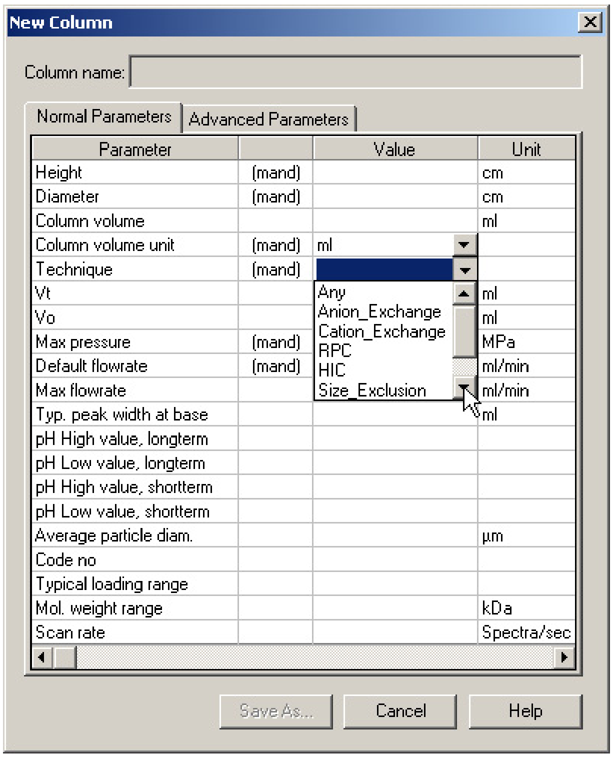
How to print the column list

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:



Parameter		Value	Unit
Height	(mand)		cm
Diameter	(mand)		cm
Column volume			ml
Column volume unit	(mand)	ml	
Technique	(mand)		
Vt			ml
Vo			ml
Max pressure	(mand)		MPa
Default flowrate	(mand)		ml/min
Max flowrate			ml/min
Typ. peak width at base			ml
pH High value, longterm			
pH Low value, longterm			
pH High value, shortterm			
pH Low value, shortterm			
Average particle diam.			µm
Code no			
Typical loading range			
Mol. weight range			kDa
Scan rate			Spectra/sec

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.

Step	Action
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.

Parameter	Unit	Comment
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.
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.

Parameter	Unit	Comment
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.
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.

Step	Action
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.

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 chapter

This chapter contains the following sections

Topic	See
How to create a BufferPrep recipe	E.1
How to edit a BufferPrep recipe	E.2

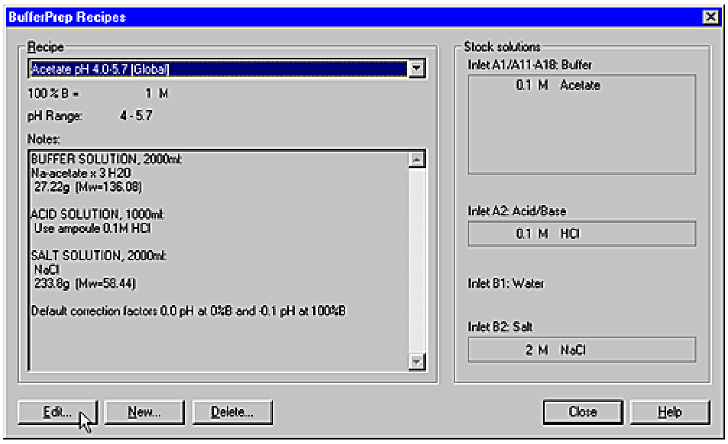
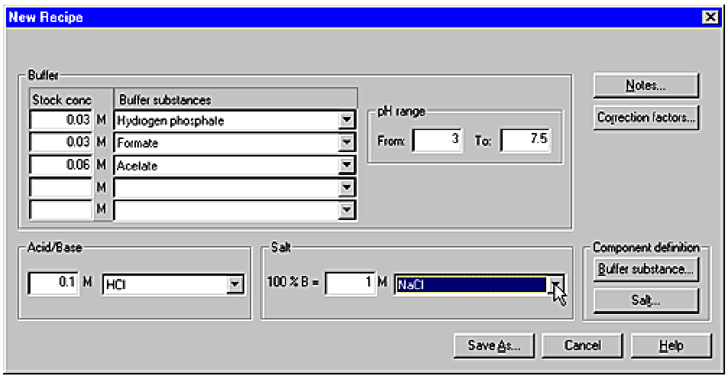
E.1 *How to create a BufferPrep recipe*

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:  BufferPrep Recipes Recipe: Acetate pH 4.0-5.7 [Global] 100 % B = 1 M pH Range: 4 - 5.7 Notes: BUFFER SOLUTION, 2000ml: Na-acetate x 3 H20 27.22g (Mw=136.08) ACID SOLUTION, 1000ml: Use ampoule 0.1M HCl SALT SOLUTION, 2000ml: NaCl 233.8g (Mw=58.44) Default correction factors 0.0 pH at 0%B and -0.1 pH at 100%B Stock solutions Inlet A1/A11-A18: Buffer 0.1 M Acetate Inlet A2: Acid/Base 0.1 M HCl Inlet B1: Water Inlet B2: Salt 2 M NaCl
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.  New Recipe Buffer Stock conc / Buffer substances 0.03 M Hydrogen phosphate 0.03 M Formate 0.06 M Acetate pH range From: 3 To: 7.5 Acid/Base 0.1 M HCl Salt 100 % B = 1 M NaCl

Step	Action
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.
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.

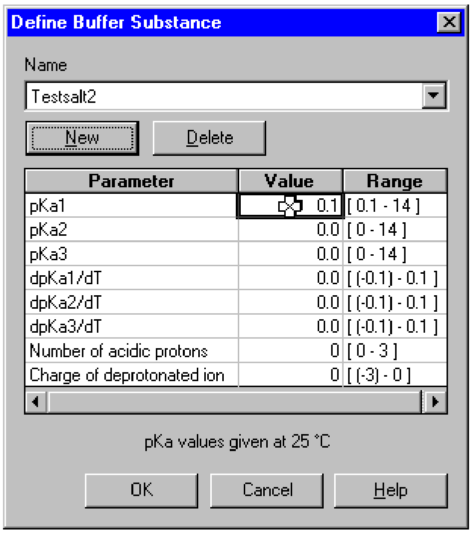
Buffer concentration

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:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Choose Edit:BufferPrep Recipes and click the New button.</td></tr>
<tr><td>2</td><td>Click the Buffer substance button in the New Recipe dialog box.
<i>Result</i>: The Define buffer substance dialog box opens.


Name: Testsalt2
<table>
<tr><th>Parameter</th><th>Value</th><th>Range</th></tr>
<tr><td>pKa1</td><td>0.1</td><td>[0.1 - 14]</td></tr>
<tr><td>pKa2</td><td>0.0</td><td>[0 - 14]</td></tr>
<tr><td>pKa3</td><td>0.0</td><td>[0 - 14]</td></tr>
<tr><td>dpKa1/dT</td><td>0.0</td><td>[(-0.1) - 0.1]</td></tr>
<tr><td>dpKa2/dT</td><td>0.0</td><td>[(-0.1) - 0.1]</td></tr>
<tr><td>dpKa3/dT</td><td>0.0</td><td>[(-0.1) - 0.1]</td></tr>
<tr><td>Number of acidic protons</td><td>0</td><td>[0 - 3]</td></tr>
<tr><td>Charge of deprotonated ion</td><td>0</td><td>[(-3) - 0]</td></tr>
</table>
pKa values given at 25 °C</td></tr>
<tr><td>3</td><td>Select the buffer component from the Name droplist and note the displayed pKa value.</td></tr>
<tr><td>4</td><td>Click Cancel to return to the New Recipe dialog box.</td></tr>
<tr><td>5</td><td>Use the pKa value to determine the pH range. Typically a range of +/- 0.5 units around the pKa value is useful.
<i>Note</i>: Check buffer tables for the exact ranges.</td></tr>
</table>

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 indefinite 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:

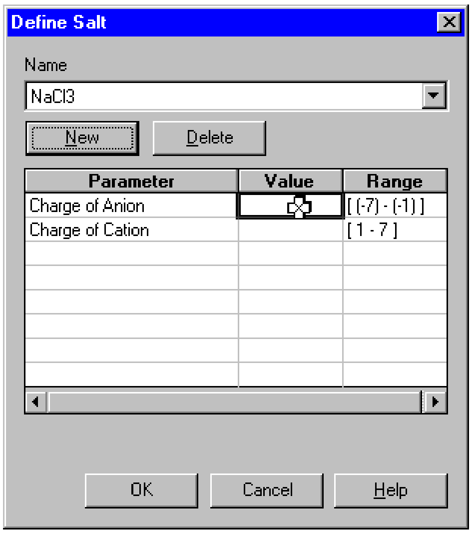
Step	Action
1	Choose **Edit:BufferPrep Recipes** and click the **New** button.

Step	Action
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.  Name: NaCl3 Parameter / Value / Range Charge of Anion / / [(-7) - (-1)] Charge of Cation / / [1 - 7]
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.

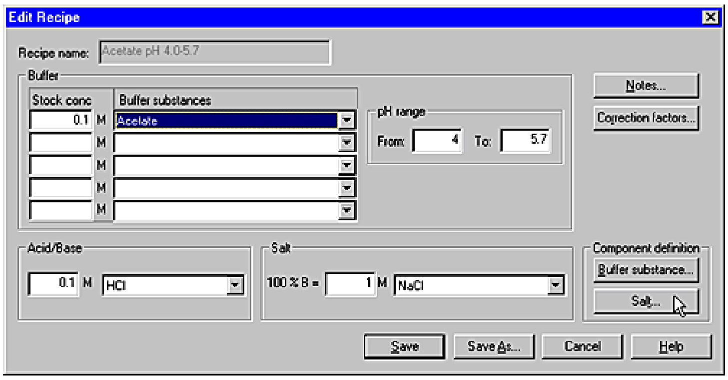
E.2 How to edit a BufferPrep recipe

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.  Edit Recipe Recipe name: Acetate pH 4.0-5.7 Buffer Stock conc 0.1 M Buffer substances Acetate pH range From: 4 To: 5.7 Acid/Base 0.1 M HCl Salt 100 % B = 1 M NaCl
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.
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.

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 chapter

This chapter contains the following sections

Topic	See
Simple equilibration	F.1
Equilibration with simple safeguard	F.2
Equilibration with extra safeguard	F.3
Collection of absorbance peaks	F.4
Collection of three absorbance peaks	F.5
Messages	F.6
Quality control procedure	F.7

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 **F.2 Equilibration with simple safeguard** on page 572 and **F.3 Equilibration with extra safeguard** on page 573.

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.

Stage	Description
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.

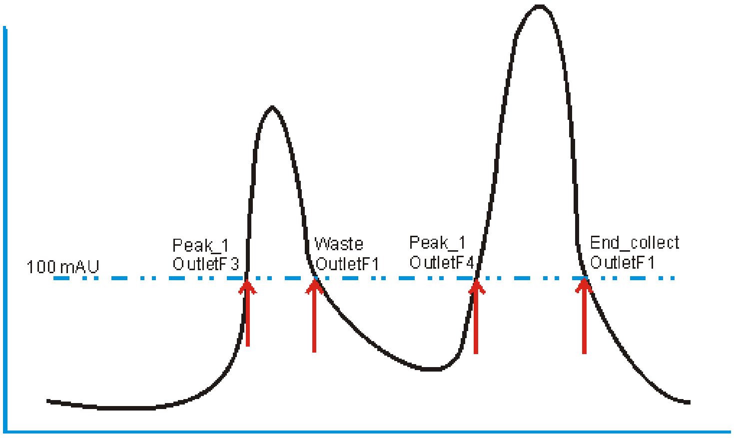
Example instruction

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
                      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.
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.

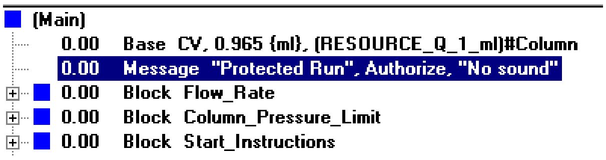
Note: If the **Message** instruction is inserted in a conditional block it will only be displayed if the conditions of the block (for example a **Watch**) is fulfilled.

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:

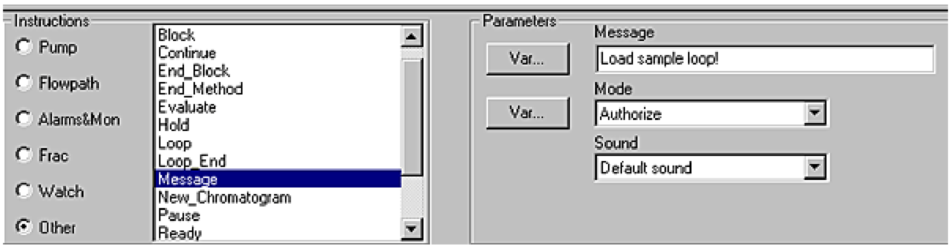


```
(Main)
   0.00  Base  CV, 0.965 {ml}, (RESOURCE_Q_1_ml)#Column
   0.00  Message  "Protected Run", Authorize, "No sound"
   0.00  Block  Flow_Rate
   0.00  Block  Column_Pressure_Limit
   0.00  Block  Start_Instructions
```

Pausing a method run for a manual sample injection

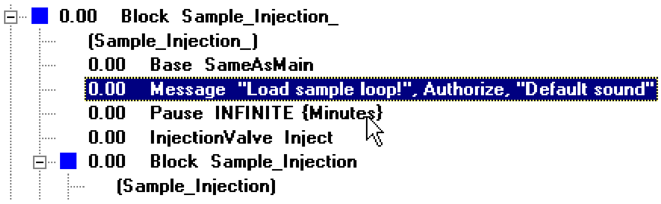
A message can be set up to pause the method until a sample has been injected manually. If a message requiring an authorization is followed by a **Pause** instruction the system will be paused until the message is acknowledged and signed. No other interaction with the system is available to the user. 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:



Message: Load sample loop!
Mode: Authorize
Sound: Default sound

The illustration below shows the text instruction for the message described above:



```
0.00  Block  Sample_Injection_
   (Sample_Injection_)
   0.00  Base  SameAsMain
   0.00  Message  "Load sample loop!", Authorize, "Default sound"
   0.00  Pause  INFINITE {Minutes}
   0.00  InjectionValve  Inject
   0.00  Block  Sample_Injection
      (Sample_Injection)
```

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.

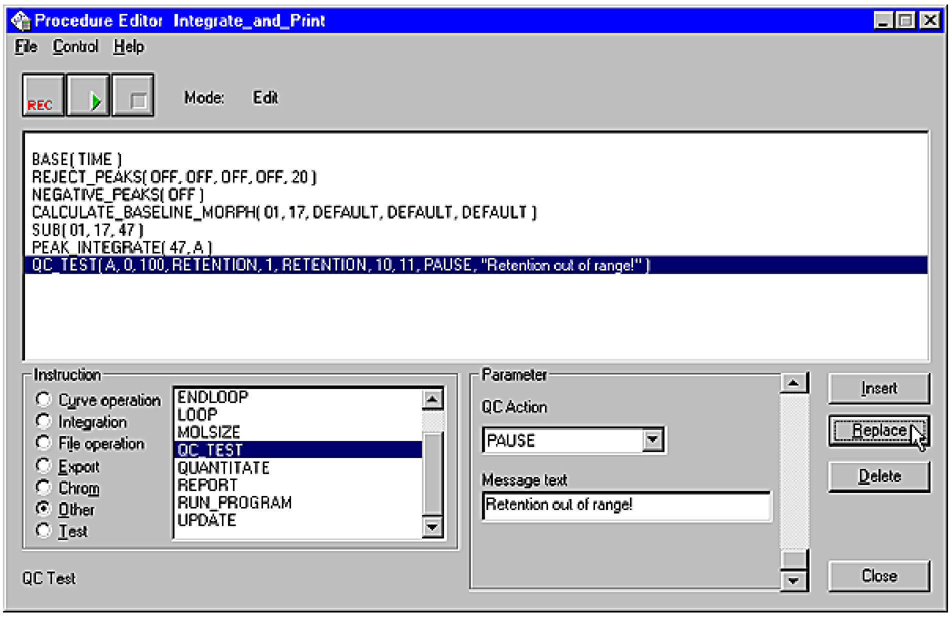
How to create the quality control procedure

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.
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:



Mode: Edit

```
BASE( TIME )
REJECT_PEAKS( OFF, OFF, OFF, OFF, 20 )
NEGATIVE_PEAKS( OFF )
CALCULATE_BASELINE_MORPH( 01, 17, DEFAULT, DEFAULT, DEFAULT )
SUB( 01, 17, 47 )
PEAK_INTEGRATE( 47, A )
QC_TEST( A, 0, 100, RETENTION, 1, RETENTION, 10, 11, PAUSE, "Retention out of range!" )
```

Instruction

○ Curve operation
○ Integration
○ File operation
○ Export
○ Chrom
● Other
○ Test

ENDLOOP
LOOP
MOLSIZE
QC_TEST
QUANTITATE
REPORT
RUN_PROGRAM
UPDATE

Parameter

QC Action: PAUSE

Message text: Retention out of range!

QC Test

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**).

Parameter	Description
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.

Step	Action
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.
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.

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