

GE Healthcare
Life Sciences

UNICORN™ start 1.0

User Manual



Table of Contents

1	Introduction	7
1.1	Important user information	8
1.2	User documentation	10
1.3	UNICORN start overview	11
2	Installation of UNICORN start	13
2.1	Computer requirements	14
2.2	Installation overview	16
2.3	Additional software installed by UNICORN start	17
2.4	Installation procedure	19
2.4.1	<i>Install UNICORN start</i>	20
2.4.2	<i>Configure e-license</i>	31
2.5	Uninstall UNICORN start	39
2.6	Removing additional software components	44
3	Getting started	45
3.1	Launch UNICORN start	46
3.2	Help functions	54
4	System control	55
4.1	Overview	56
4.2	Connect to system	60
4.3	Start a run	61
4.4	Monitor a run	66
5	Create a method	75
5.1	Overview	76
5.1.1	<i>Method Editor</i>	77
5.1.2	<i>Methods in UNICORN start</i>	80
5.2	Create and edit methods	83
5.2.1	<i>Working with methods</i>	84
5.2.2	<i>Open a method</i>	86
5.3	Predefined methods and phases	88
5.3.1	<i>Definitions</i>	89
5.3.2	<i>Create a predefined method</i>	93
5.3.3	<i>Edit phase properties</i>	95
5.4	Fraction collection	99
5.4.1	<i>Introduction</i>	100
5.4.2	<i>Fractionation setup</i>	101
5.4.3	<i>Fractionation without Frac30</i>	102
5.4.4	<i>Fractionation with Frac30</i>	104
5.5	Import and export methods	109
5.6	Column handling	115

6	View and present the results	117
6.1	Introduction	118
6.2	Evaluation module interface	119
6.2.1	<i>User interface</i>	120
6.2.2	<i>Result navigator</i>	123
6.2.3	<i>Chromatogram</i>	125
6.3	View and present the results	126
6.3.1	<i>Open and view results</i>	127
6.3.2	<i>Locate results</i>	133
6.4	Optimize the presentation of chromatograms	138
6.4.1	<i>Customize the chromatogram layout</i>	139
6.4.2	<i>Edit curve presentation</i>	143
6.4.3	<i>Change the axes</i>	148
6.4.4	<i>Print chromatograms and peak data</i>	152
6.4.5	<i>Save report as PDF</i>	154
7	Evaluate and compile the results	155
7.1	Peak integration	156
7.1.1	<i>Perform a peak integration</i>	157
7.1.2	<i>Baseline calculation</i>	160
7.1.3	<i>Display peak data</i>	161
7.1.4	<i>Edit the integration parameters</i>	164
7.1.5	<i>Integrate part of a curve</i>	177
7.1.6	<i>Exclude peaks</i>	178
7.2	Baseline operations	180
7.2.1	<i>Edit the baseline manually</i>	181
7.2.2	<i>Optimize the baseline with a morphological algorithm</i>	184
7.2.3	<i>Optimize the baseline with a classic algorithm</i>	188
7.3	Fraction and peak operations	197
7.3.1	<i>The Fraction Histogram</i>	198
7.3.2	<i>Match protein activity to a curve</i>	201
7.4	Compare different runs	203
7.4.1	<i>Open and compare chromatograms</i>	204
7.4.2	<i>Open and compare curves</i>	207
7.4.3	<i>Shift curves</i>	213
7.4.4	<i>Create a mirror image</i>	214
7.5	Rename folders, results, chromatograms, curves and peak tables	216
7.6	Save results	217
7.7	Import and export results	219
7.7.1	<i>Import results</i>	220
7.7.2	<i>Export results</i>	222
8	USB Export and Import Operations with ÄKTA start	229
8.1	Export a method to a USB memory stick	230
8.2	Import results from a USB memory stick	231

9	Administration	232
9.1	System administration	233
9.2	Database administration	239
9.2.1	<i>Database overview</i>	240
9.2.2	<i>Database maintenance</i>	241
10	Troubleshooting	253
10.1	Installation and uninstallation	254
10.2	System connections	255
10.3	System access errors	257
10.4	Database functions	258
10.5	Evaluation errors	259
A	Evaluation functions and instructions	261
A.1	Smoothing algorithms	262
A.2	Baseline calculation theory	265
A.3	Peak table column components	271
	Index	278

1 Introduction

About this user manual

This user manual provides the instructions needed to install and operate UNICORN start, and analyze chromatography run data.

In this chapter

This chapter contains the following sections:

Section	See page
1.1 Important user information	8
1.2 User documentation	10
1.3 UNICORN start overview	11

1.1 Important user information

Purpose of UNICORN start User Manual

The purpose of this user manual is to provide a guide describing system control, method editor and result evaluation functions of UNICORN start.

The manual covers the following topics:

- System control
- Create and edit methods
- View, evaluate and present results
- Administration and database management
- Troubleshooting

Refer to the ÄKTA™ *start Operating Instructions* for further information on how to perform a chromatography run.

Note: *The UNICORN start User Manual does not describe all functions or every command in all panes and dialogs of the user interface. Refer to the UNICORN start Online Help for more information about commands that are not described in this manual.*

Notes and tips

Note: *A Note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software items are identified in the text by **bold italic** text. A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu.

Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

Text entries that UNICORN start generates or that the user must type are represented by a monotype typeface (e.g., C:\Program Files\GE Healthcare\UNICORN\UNICORN start 1.0\Database).

Prerequisites

The following prerequisites must be fulfilled before you can use this manual the way it is intended:

- You need to have a general understanding of how your PC/laptop and Microsoft™ Windows™ work.
 - You need to understand the general concepts of liquid chromatography.
-

1.2 User documentation

User documentation

From the **Help** menu in UNICORN start or on the UNICORN start DVD, the following user documentation is available.

Document	Main contents
<i>UNICORN start User Manual</i>	Overview and detailed descriptions of the different features in UNICORN start. Instructions on how to use the software. Workflow descriptions for common operations. Dialog descriptions and user instructions, context-linked from all software features.
<i>UNICORN start Online Help</i>	Dialog descriptions for UNICORN start (accessible from the Help menu only).

The user documentation listed in the table below is available on the ÄKTA start User Documentation CD. A display help is available from the Instrument Display.

Documentation	Main contents
<i>ÄKTA start Operating Instructions</i>	Instructions needed to install, operate and maintain ÄKTA start in a safe way.
<i>ÄKTA start Display Help</i>	Dialog descriptions of the functionality menu for ÄKTA start (only accessible from the Instrument Display).
<i>ÄKTA start Maintenance Manual</i>	Detailed instrument and module descriptions, including instructions needed to maintain and troubleshoot ÄKTA start.
<i>ÄKTA start System Cue Card</i>	A condensed guide to prepare and run chromatographic techniques on ÄKTA start.
<i>ÄKTA start Maintenance Cue Card</i>	A condensed guide to handling routine maintenance operations and troubleshooting ÄKTA start.

1.3 UNICORN start overview

UNICORN start is a complete software package for:

- Control, edit methods and monitor chromatography run on ÄKTA start.
- Evaluation and analysis of the results generated from ÄKTA start.

Illustration of the system

The illustration below shows the ÄKTA start System with UNICORN start installed on a computer.



Part	Description
1	ÄKTA start (instrument).
2	Frac30 (Fraction collector).
3	UNICORN start (software installed on a computer).

UNICORN start modules

The four modules of UNICORN start and their main features are listed below.

Module	Features
System Control	<ul style="list-style-type: none"> • Provides an intuitive and easy-to-use interface to control ÄKTA start. • Performs and monitors quick start or user defined chromatography method runs. • Performs and monitors chromatography runs from pre-defined or user-defined methods. • Performs system performance method runs.
Method Editor	<ul style="list-style-type: none"> • Provides the flexibility to automate the chromatography runs. • Allows creation of methods from predefined chromatography templates like Affinity, Gel Filtration, Ion Exchange and Desalting. • Gives flexibility to create a customized method by dragging and dropping the chromatography phases such as Prime and Equilibration, Sample Application, Elution and Fractionation, etc. • Allows exporting methods to a USB memory stick for importing them into ÄKTA start.
Evaluation	<ul style="list-style-type: none"> • Allows viewing and presentation of results, including creation of PDF reports. • Allows various evaluation operations on curves and chromatograms including comparison, peak integration etc. • Allows importing results from ÄKTA start and exporting results to other formats.
Administration	<ul style="list-style-type: none"> • Allows administration of the UNICORN start database for backup, restore, archive and retrieve operations. • Allows reviewing of UNICORN start and system logs.

2 Installation of UNICORN start

In this chapter

This chapter describes how to install UNICORN start. It contains the following sections:

Section	See page
2.1 Computer requirements	14
2.2 Installation overview	16
2.3 Additional software installed by UNICORN start	17
2.4 Installation procedure	19
2.5 Uninstall UNICORN start	39
2.6 Removing additional software components	44

2.1 Computer requirements

Installation requirements

Before starting the installation procedure, make sure that the following requirements are met for the computer (PC or laptop):



NOTICE

UNICORN start is tested using US-English operating system version. Using other language versions of the operating system may cause errors.



NOTICE

Changing the default font and font size on windows may cause problems in the UNICORN start user interface.



NOTICE

A screen resolution of 1280x1024 or higher is recommended. Parts of the UNICORN start user interface may not be displayed properly using a lower specification.



NOTICE

Windows power save features should be turned off to avoid conflicts with system operations.

Component	Specification
Operating System	<ul style="list-style-type: none">Recommended: Windows 7 Professional x86 or x64 with SP1 (English US).Also compatible with Windows XP professional with SP3 (English US).
RAM	4 GB (minimum)

Component	Specification
Minimum free disk space	12 GB (minimum)
Display	<ul style="list-style-type: none"> Laptop: 14" (minimum) Desktop: 17" (minimum)
Processor	<ul style="list-style-type: none"> Laptop: Dual Core with 3 GHz clock speed (minimum) Desktop: Dual Core with 2.5 GHz clock speed (minimum)
USB port	2.0
Other	<ul style="list-style-type: none"> DVD drive Network interface card

- Note:**
- *UNICORN start is not tested for operation in a mixed Windows XP/Windows 7 environment.*
 - *UNICORN start is tested using US-English operating system version. Using other language versions of the operating system may cause errors.*
 - *A screen resolution of 1280x1024 or higher is recommended. Parts of the UNICORN start user interface may not be displayed properly using a lower specification.*
 - *Changing the default font and font size on windows may cause problems in the UNICORN start user interface.*
 - *The Windows basic color scheme is recommended.*
 - *Using Windows 7 Aero color scheme is not recommended.*
 - *Windows power save features should be turned off to avoid conflicts with system operations.*
 - *UNICORN start is not compatible with the Windows 7 feature High DPI Awareness which allows the graphical user interface to be scaled. The interface scale must remain at 100% scale to avoid issues with clipping and misalignment of parts of the UNICORN user interface. Normally, the scale is set at 100% by default.*

2.2 Installation overview

Installation summary

The table below provides an overview of the UNICORN start installation procedure for setting up a UNICORN start workstation.

Stage	Description
1	Installation of additional software necessary to run UNICORN start. <i>See Section 2.3 Additional software installed by UNICORN start, on page 17.</i>
2	Installation of UNICORN start. <i>See Section 2.4 Installation procedure, on page 19.</i>

Do not copy the DVD-ROM or decompress the files



NOTICE

Do not run the installation from a network drive.



NOTICE

Do not attempt to decompress the files using any other file decompression utility.

UNICORN start is supplied on a DVD-ROM. Files on the DVD-ROM are compressed and the installation cannot be performed by simply copying the files onto the local hard drive. However, the installation files can be copied to a hard drive and the installation may be run from the hard drive instead. Do not run the installation from a network drive.

During the installation procedure, the required folder structure is created on the hard drive and the files are decompressed. Do not attempt to decompress the files using any other file decompression utility.

2.3 Additional software installed by UNICORN start

Required software

The following additional software will be installed, if not found, before the installation of UNICORN start begins:

- MadCap™ HelpViewer
- MS Windows Installer 4.5 (x86)
- Microsoft .Net Framework 4.0
- Windows PowerShell™ 1.0
- Microsoft Visual C++™ 2008 SP1 Redistributable Package (x86)
- Microsoft Core XML Services (MSXML) 6.0
- Microsoft SQL Server™ Express Edition 2008 R2 (x86)

The installation will be initiated automatically by the UNICORN start installation program. Any of the listed software that are already installed will not be included in this installation step. You may need to restart the computer several times to proceed from one application installation to the next.

All software listed above are already installed on computers delivered from GE Healthcare.

Note: *By default, the software listed above is installed on the computer C:\ drive. However, you can manually change to another computer drive e.g., D:\ drive. If the available space on the drive is insufficient, the installation may fail and the installation program will attempt to repeat the installation after each restart. Make sure that enough space is available on the drive before starting the installation. The actual required space will vary depending on what is previously installed on the computer, and subsequent selections in the UNICORN start installation. However, the installation program needs a total of 6 GB of free space to initiate the installation.*

Required disc space



NOTICE

Make sure that enough space is available on the drive before starting the installation.

2 Installation of UNICORN start

2.3 Additional software installed by UNICORN start

The installation program needs 6 GB of free space to initiate the installation. If the available space on the drive is insufficient, the installation may fail and the installation program will attempt to repeat the installation after each restart.

Make sure that enough space is available on the drive before starting the installation. The actual required space will vary depending on what is previously installed on the computer, and subsequent selections in the UNICORN start installation.

The help viewer application

As part of the UNICORN start installation, a special viewer for the *UNICORN start Online Help* is also installed. This application, the **MadCap HelpViewer**, is accessed from the UNICORN start user interface either by clicking the help buttons in the dialogs, by pressing the **F1** key or by selecting **Help** from the menu bar.

2.4 Installation procedure

In this section

This section contains the following subsections:

Section	See page
2.4.1 Install UNICORN start	20
2.4.2 Configure e-license	31

2 Installation of UNICORN start

2.4 Installation procedure

2.4.1 Install UNICORN start

2.4.1 Install UNICORN start

Introduction



NOTICE

Make sure that enough space is available on the drive before starting the installation.

The installation of UNICORN start includes all software components necessary to operate UNICORN start and a connected ÄKTA start instrument.

The components that are installed are described in the table below.

Software component	Characteristics
UNICORN start client	UNICORN client software for the workstation, including the four UNICORN start modules: Administration , Method Editor , System Control and Evaluation .
UNICORN start database	An instance of Microsoft SQL Server Express Edition that stores the results and methods created by the UNICORN start modules.
Instrument server	A Windows service necessary to control and manage an instrument that is connected to the workstation.

Instructions



NOTICE

Make sure that enough space is available on the drive before starting the installation.

To install UNICORN start, follow the steps below:

Step	Action
------	--------

- | | |
|---|--|
| 1 | Insert the installation DVD in the DVD drive.
The UNICORN start 1.0 installation starts. |
|---|--|



Tip:

If the DVD drive is not set up to allow automatic start, browse the DVD contents and double click the file `Setup.exe` to initiate the installation.



NOTICE

Do not click on the `.msi` file to start the installation. The `.msi` file does not check the pre-required software for the installation to succeed.

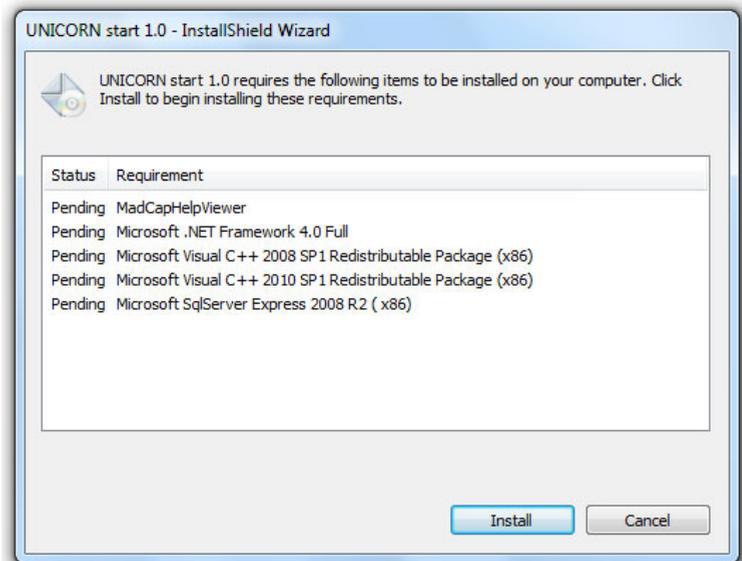
2 Installation of UNICORN start

2.4 Installation procedure

2.4.1 Install UNICORN start

Step	Action
------	--------

- | | |
|---|---|
| 2 | If there are any required software components to be installed (as mentioned in <i>Section 2.3 Additional software installed by UNICORN start, on page 17</i>), the following dialog appears: |
|---|---|



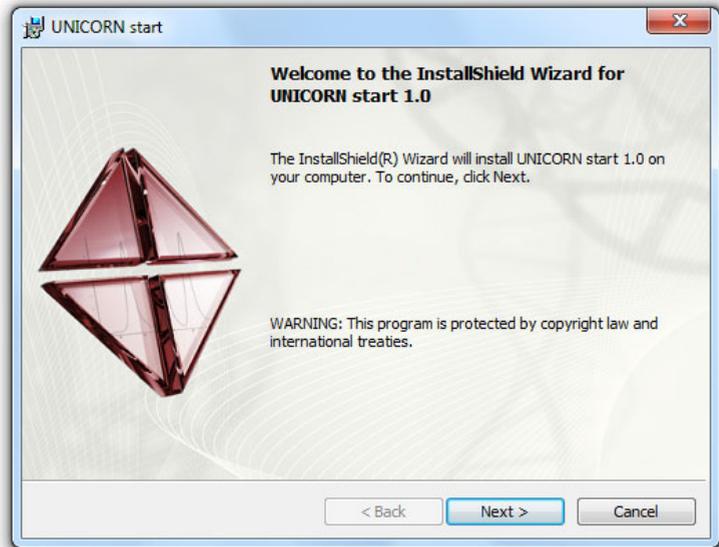
Note:

Multiple restarts of the computer may occur during the installation process of these software components.

If the installation does not start automatically after a restart, please manually restart the installation as described in step 1.

Step **Action**

- 3 The **UNICORN start 1.0 InstallShield Wizard** window opens. Click **Next** to continue with the installation.



2 Installation of UNICORN start

2.4 Installation procedure

2.4.1 Install UNICORN start

Step	Action
------	--------

- | | |
|---|---|
| 4 | <p>Read the license agreement carefully.</p> <ul style="list-style-type: none">• Select <i>I accept the terms in the license agreement.</i>• Click <i>Next</i> to proceed. |
|---|---|

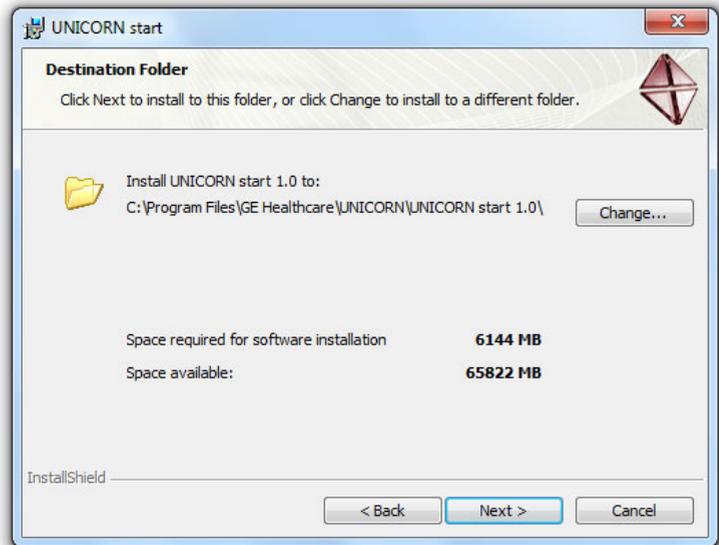


Step **Action**

- 5 Click **Next** to install UNICORN start in the default folder, or click **Change** to install in a different folder.

Note:

By default, UNICORN start suggests C:\Program Files\GE Healthcare\UNICORN\.



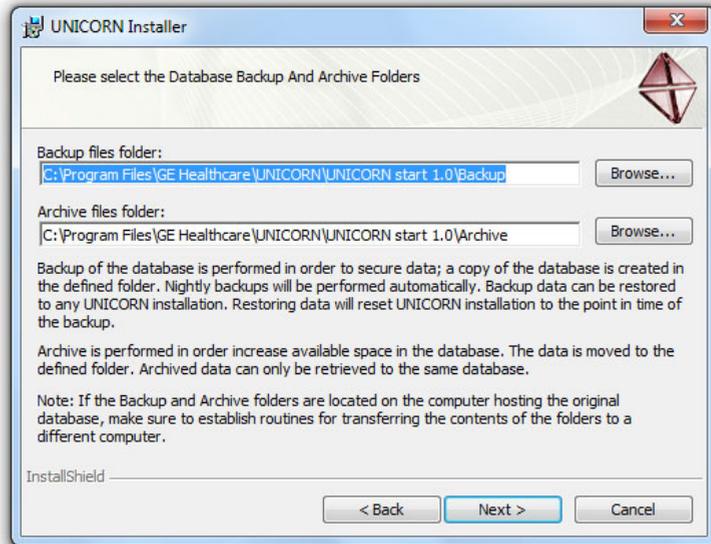
2 Installation of UNICORN start

2.4 Installation procedure

2.4.1 Install UNICORN start

Step	Action
------	--------

- | | |
|---|---|
| 6 | <ul style="list-style-type: none">• Enter locations for Backup and Archive folders.• Click Next to proceed. |
|---|---|



Note:

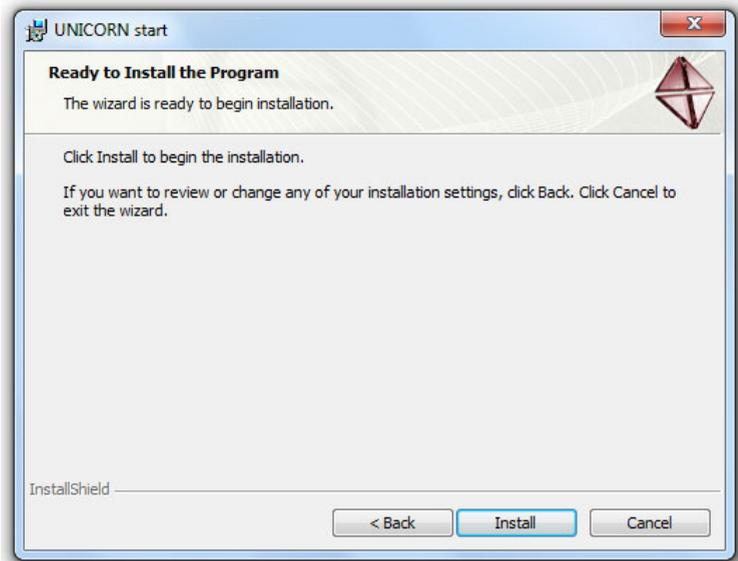
To ensure data safety, it is recommended that the backup and archive folders are copied or moved at regular intervals to another server computer or some other storage media.

Tip:

The **UNICORN Configuration Manager** is located at **Start:Programs: GE Healthcare**. This dialog can be used to edit the backup and archive information after the installation.

Step **Action**

- 7 Click **Install** to begin installation of UNICORN start, ÄKTA start driver and UNICORN services.



Note:

The SQL Server software is installed on the C:\ drive by default. If the available space for this is insufficient, the installation may fail. The required space varies depending on what is previously installed on the computer but at least 2 GB should be available to ensure that the installation is completed correctly.

2 Installation of UNICORN start

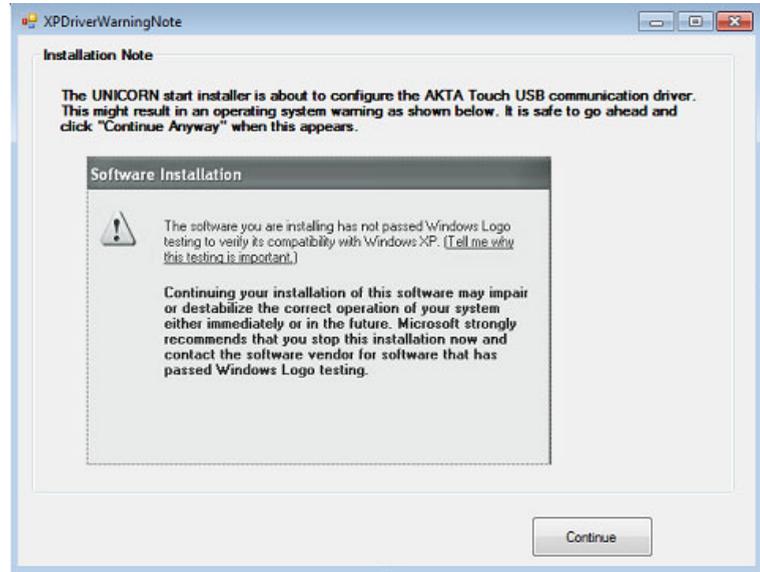
2.4 Installation procedure

2.4.1 Install UNICORN start

Step	Action
------	--------

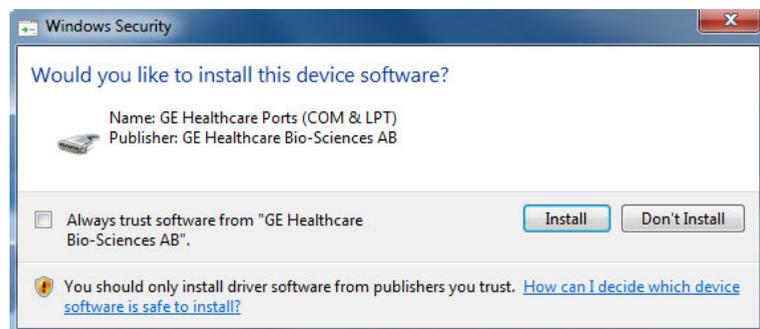
Note:

If a warning is displayed when the ÄKTA start USB communication driver is installed on a Windows XP system, click the **Continue** button to proceed.



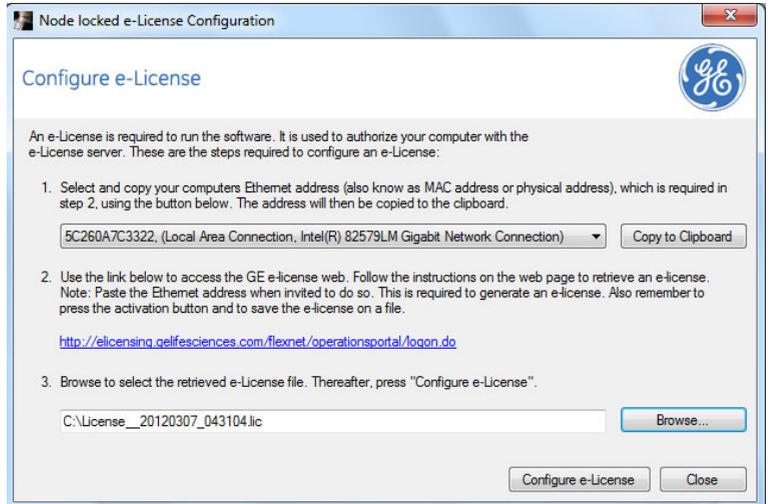
Note:

If a windows security warning is displayed when the ÄKTA startUSB communication driver is installed, click **Install** button to proceed.



Step **Action**

8 Proceed by configuring the e-license, see Section 2.4.2 Configure e-license.

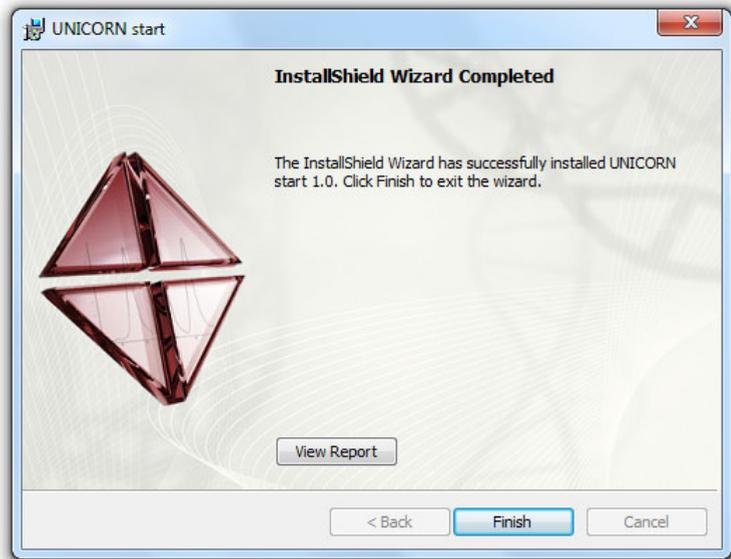


2 Installation of UNICORN start

2.4 Installation procedure

2.4.1 Install UNICORN start

Step	Action
9	Click the Finish button in the UNICORN start InstallShield Wizard Completed window.



Note:

The installation report can be viewed by clicking the **View Report** button.

2.4.2 Configure e-license

Introduction



NOTICE

Make sure that your PC/laptop is connected to the internet before initiating e-license configuration.

After completing a UNICORN start installation, the software prepares to configure an e-license for UNICORN start.

Note: *Make sure that your PC/laptop is connected to the internet before initiating e-license configuration. An internet connection needs to be established in order to get access to the GE Healthcare e-licensing web site. For details about retrieving an e-license, see Section 2.4.2 Access the e-licensing web site*

2 Installation of UNICORN start

2.4 Installation procedure

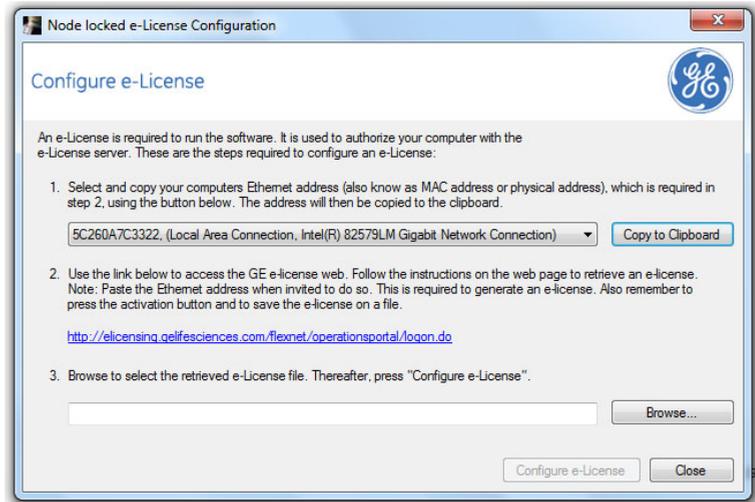
2.4.2 Configure e-license

Initiate license configuration

Follow the instructions below to perform the final installation step and initiate the e-license configuration.

Step	Action
------	--------

1	In the Configure e-License dialog:
---	---



Click the **Copy to Clipboard** button to copy the **Ethernet address**.

Note:

UNICORN start attempts to select the right Ethernet address from the available set of network interface cards. If you anticipate the selected network configuration to change in this computer please choose a more permanent Ethernet address from the list.

2	Click the hyperlink in the dialog to proceed to the e-licensing web site.
---	---

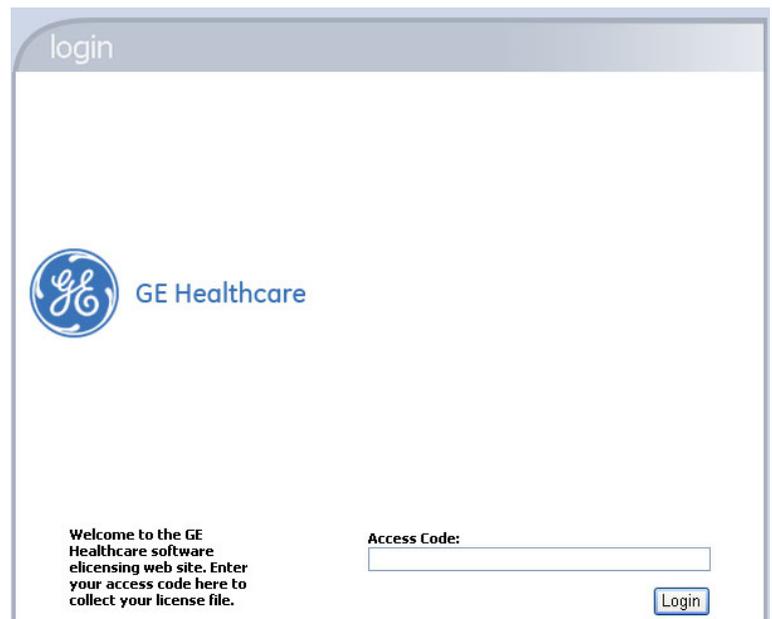
Access the e-licensing web site

You must retrieve the e-license from the GE Healthcare e-licensing web site. Follow the instructions below to access this site from the configuration dialog.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the hyperlink in the Configure e-License dialog. |
|---|---|

Result: The **GE Healthcare software elicensing web site** opens in your web browser.



The screenshot shows a web browser window with a grey header containing the word "login". The main content area features the GE Healthcare logo on the left and the text "GE Healthcare" to its right. Below the logo, there is a message: "Welcome to the GE Healthcare software elicensing web site. Enter your access code here to collect your license file." To the right of this message is a text input field labeled "Access Code:" and a blue "Login" button.

- | | |
|---|--|
| 2 | Enter the Access Code presented on the DVD label, and then click the Login button. |
|---|--|

Result: The **operations portal** opens.

2 Installation of UNICORN start

2.4 Installation procedure

2.4.2 Configure e-license

Retrieve the e-license

Follow the instructions below to retrieve the e-license.

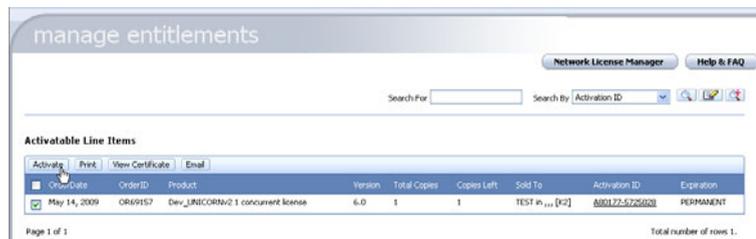
Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the Collect License hyperlink. |
|---|---|



Result: The **manage entitlements** page opens.

- | | |
|---|--|
| 2 | Select the checkbox for your license and click the Activate button. |
|---|--|



Result: A **manage entitlements** data input page opens.

Step	Action
------	--------

- | | |
|---|--|
| 3 | <p>Enter the requested data:</p> <ul style="list-style-type: none"> • Start date, if requested by the GE Healthcare software e-licensing web site. • Customer data: <ul style="list-style-type: none"> - Name - Department - Organization - Order Email |
|---|--|

Note:

You must also note if you want software updates and information about related products or not. All items marked with red dots are mandatory.

- Click the **Next** button to proceed to the next page.

Note:

*The options **Ship to Email** or **Ship to Address** can be selected if, for some reason, the e-license cannot be saved to a file during the configuration. Normally, this will not be necessary.*

- | | |
|---|--|
| 4 | <p>Under the heading Nodelocked Hosts, click the Add New Host button.</p> <p><i>Result:</i> The create license host dialog opens.</p> |
|---|--|

Generate Licenses

Nodelocked Hosts

Add New Host		Remove
<input type="checkbox"/>	Host	
No license hosts selected		

Licenses will be generated for the hosts listed above. Create a new one by clicking on Add New Host.

2 Installation of UNICORN start

2.4 Installation procedure

2.4.2 Configure e-license

Step	Action
------	--------

- | | |
|---|---|
| 5 | Click in the empty Nodelocked Hosts field and press the Ctrl and V keys simultaneously to paste your Ethernet address from the clipboard into this field. |
|---|---|

create license host

Nodelocked Hosts: ETHERNET = 5C260A7C3322

This is the identifier for the machine where FLEXenabled application will run. Select the identifier type from the drop down and enter the appropriate value in the text box.

OK Cancel

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Note:

If you use another computer to retrieve the e-license, you must write down the Ethernet address from the computer you are installing UNICORN start, and type this address in the **Nodelocked Hosts** field.

Click the **OK** button to proceed.

Result: Your computer address is added to the **Nodelocked Hosts** list.

Generate Licenses

Nodelocked Hosts

Add New Host Remove

Host

No license hosts selected

licenses will be generated for the hosts listed above. Create a new one by clicking on Add New Host.

Please enter values for the hosts and click 'Verify' to validate the license. Or click 'Generate' to generate the license.

Cancel Back Verify Generate

Selected Line Items

Activation Id	Product	Version	Expiration Date
W22622-249201	Unicom start, 1 node-locked license, internal FOC	3.0	PERMANENT

- | | |
|---|--|
| 6 | If the displayed information is correct, click the Generate button. |
|---|--|

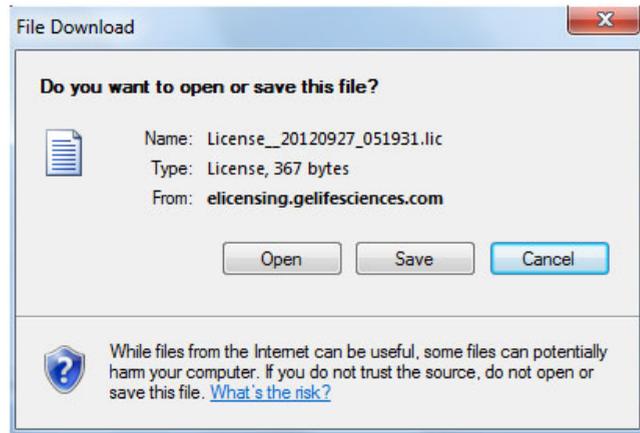
Result: The **License summary** page opens.

Activate Print View Certificate Email

OrderDate	OrderID	Product	Version	Total Copies	Copies Left	Expiration
Oct 26, 2012	sw best	Unicom start, 1 node-locked license, internal FOC	3.0	1	1	PERMANENT

Page 1 of 1 Total number of rows 1.

- | Step | Action |
|------|--|
| 7 | Select the Product checkbox for your license and then click the Save to File button.
<i>Result:</i> A File download dialog opens. Save the license file in a temporary folder or on your Windows desktop. |



- | | |
|---|--|
| 8 | Click the Complete button and Logout to close the GE Healthcare software e-licensing web site. |
|---|--|

2 Installation of UNICORN start

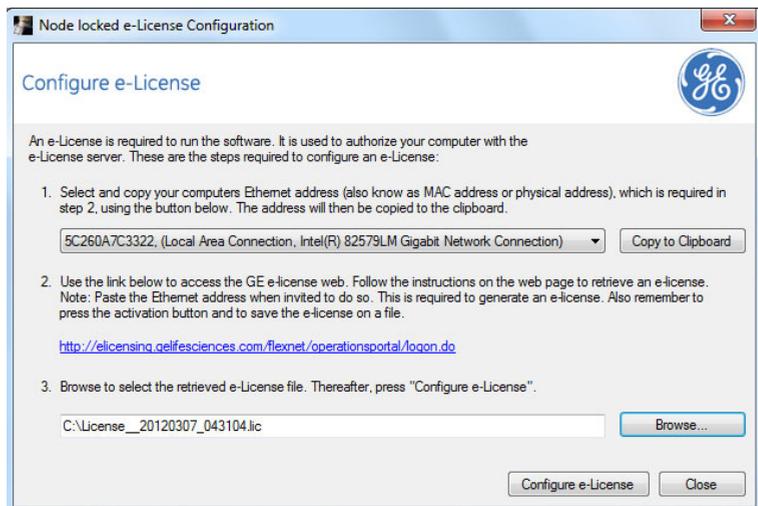
2.4 Installation procedure

2.4.2 Configure e-license

Locate and connect the e-license

The table below describes how to connect and confirm the e-license in the UNICORN start *e-License Configuration* dialog.

Step	Action
1	<ul style="list-style-type: none">Click the Browse button to locate the license file andClick the Open button to add the search path to the file in the <i>e-License Configuration</i> dialog.



2	Click the Configure e-License button. <i>Result:</i> A confirmation dialog opens, showing that the configuration has been successful.
3	Click OK in the confirmation dialog, and Close in the <i>e-License Configuration</i> dialog to complete the process.

2.5 Uninstall UNICORN start

Introduction

This section describes how to remove UNICORN start using the installation program. UNICORN start can also be removed using the Windows **Add or Remove Programs** dialog.

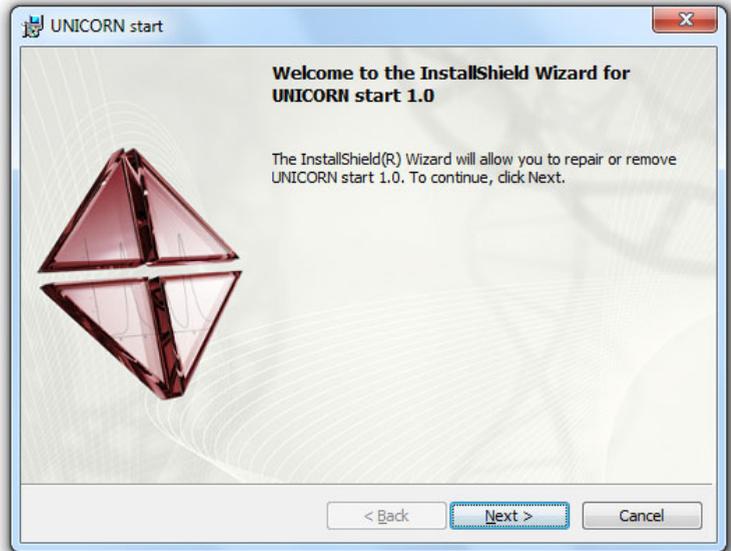
Remove the installation

To uninstall UNICORN start, follow the steps described below:

Note: *Before removing UNICORN start, a backup must be made, otherwise all data will be lost if proceeding. For instructions about how to back up data, see Section 9.2.2 Database maintenance, on page 241.*

Step	Action
------	--------

- | | |
|---|---|
| 1 | Open the UNICORN start installation program Setup.exe . This will open the UNICORN start InstallShield Wizard . |
|---|---|



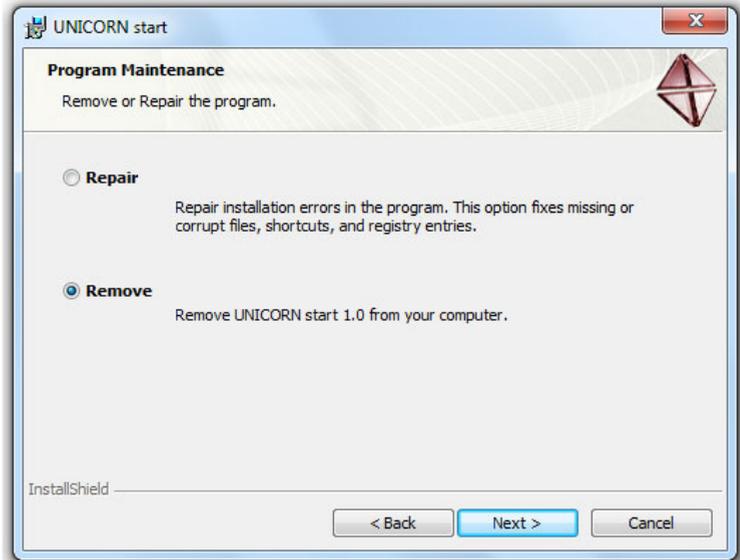
2 Installation of UNICORN start

2.5 Uninstall UNICORN start

Step	Action
------	--------

2	Click the Next button.
---	-------------------------------

UNICORN start Program Maintenance dialog opens.



Step **Action**

- 3 Select **Remove** and click the **Next** button.

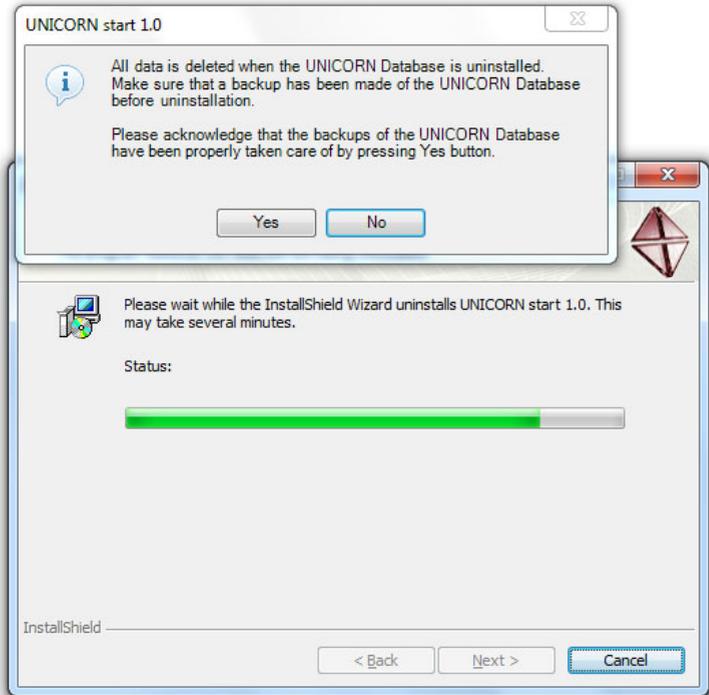


2 Installation of UNICORN start

2.5 Uninstall UNICORN start

Step	Action
------	--------

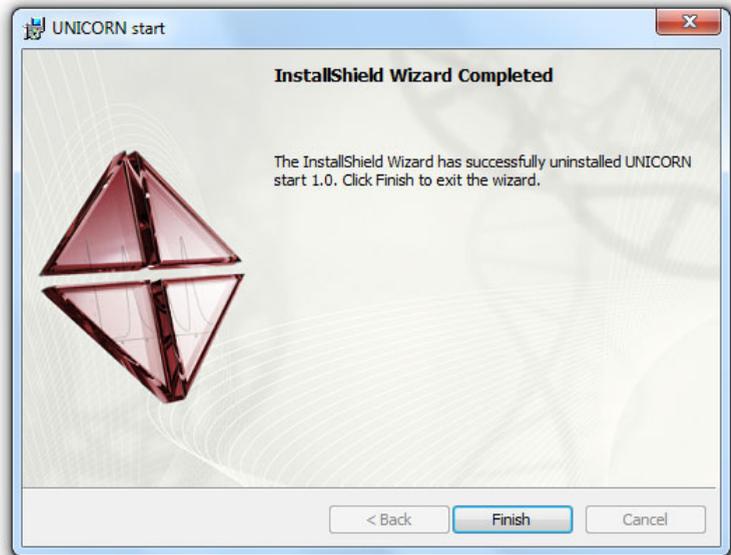
- | | |
|---|--|
| 4 | Make sure that a backup of the database has been made. If no backup has been made, all data will be lost if proceeding. For instructions about how to back up data, see <i>Section 9.2.2 Database maintenance, on page 241</i> . |
|---|--|



Step	Action
------	--------

- | | |
|---|---|
| 5 | Click the Yes button to proceed. |
|---|---|

The *UNICORN start InstallShield Wizard Completed* window appears.



- | | |
|---|--|
| 6 | Click the Finish button to complete the uninstallation. |
|---|--|

Note:

After removing UNICORN start, some parts will still remain in the installation folder, for example logs and database backups. If desired, these objects must be removed manually.

2.6 Removing additional software components

Some software components required to operate UNICORN cannot be removed automatically by the installation wizard. This is because the same components may be used for other purposes as well as for UNICORN start. The components may also have been updated after the original installation, and UNICORN start can no longer determine if they were part of the installation package. If any of the components listed below are not in use by other applications, they need to be removed manually:

- Microsoft .Net Framework 4.0 (including all language versions).
- Microsoft Visual C++ 2005 Redistributable Package
- Microsoft SQL Server 2008 Express Edition

Note: *The UNICORN start instance of the Microsoft SQL Server needs to be uninstalled manually. Follow the instructions described in the table below to uninstall the Microsoft SQL Server.*

The table below describes how to remove the components manually.

Step	Action
1	Open the Windows Control Panel .
2	Double-click the Add or Remove Programs icon. Result: The Add or Remove Programs dialog opens. All programs installed on the client computer are listed.
3	Select the software component from the list and click the Change/Remove button.
4	Follow the instructions in the dialogs that opens, until the software is removed.

3 Getting started

In this chapter

This chapter provides a general description of the operation of UNICORN start, including log on and log off functions, instructions on how to structure files and folders, and how to use the *UNICORN start Online Help*. The chapter contains the following sections:

Section	See page
3.1 Launch UNICORN start	46
3.2 Help functions	54

3.1 Launch UNICORN start

Start and log on

Follow the instructions below to start and log on to UNICORN start.

Step	Action
------	--------

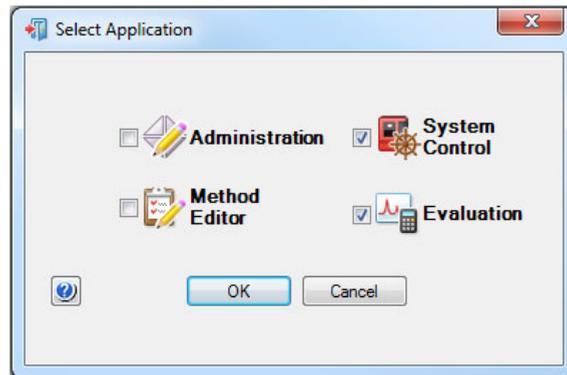
- | | |
|---|---|
| 1 | <ul style="list-style-type: none">• Double-click the UNICORN start icon on the desktop, |
|---|---|



or

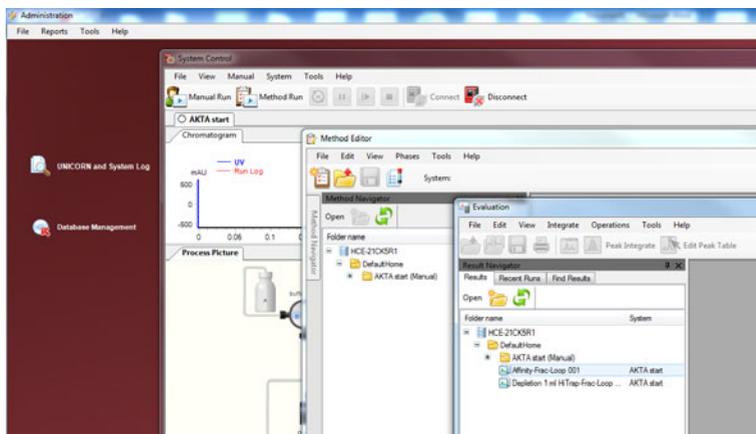
- Select **Start:All Programs:GE Healthcare:UNICORN start 1.0:UNICORN start 1.0**.

Result: The **Select Application** dialog opens.



Step **Action**

- 2 In the **Select Application** dialog, select the module(s) to be opened.
Result: The selected UNICORN start modules open.

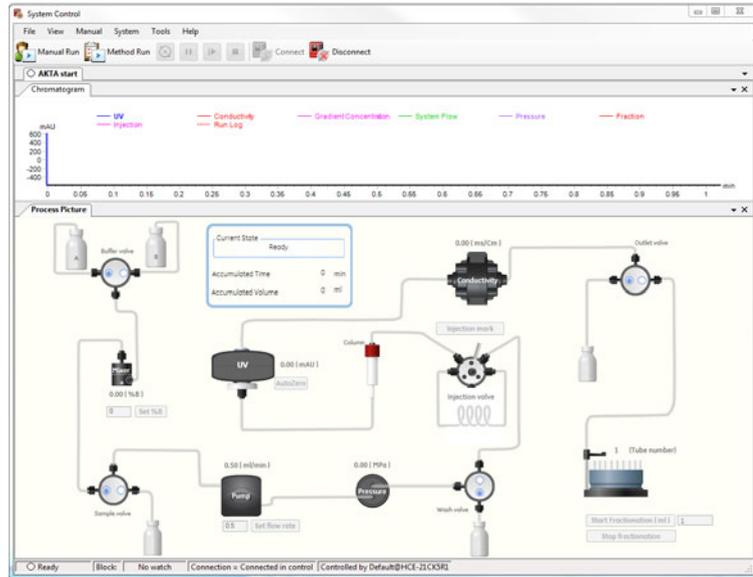


- 3 Select **System Control**.

Step Action

4

Click  **Connect** to connect to the ÄKTA start instrument.
Result: UNICORN start connects to the ÄKTA start.



NOTICE

Make sure that the computer which is used for running UNICORN start is connected to the ÄKTA start instrument, using the USB cable.

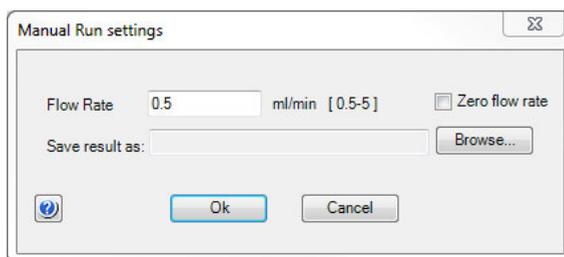
Start and evaluate a Manual run

Follow the instructions below to start a Manual run using UNICORN start.

Step	Action
------	--------

1

Click  **Manual Run** in the toolbar to start a manual run. The **Manual Run settings** dialog opens.



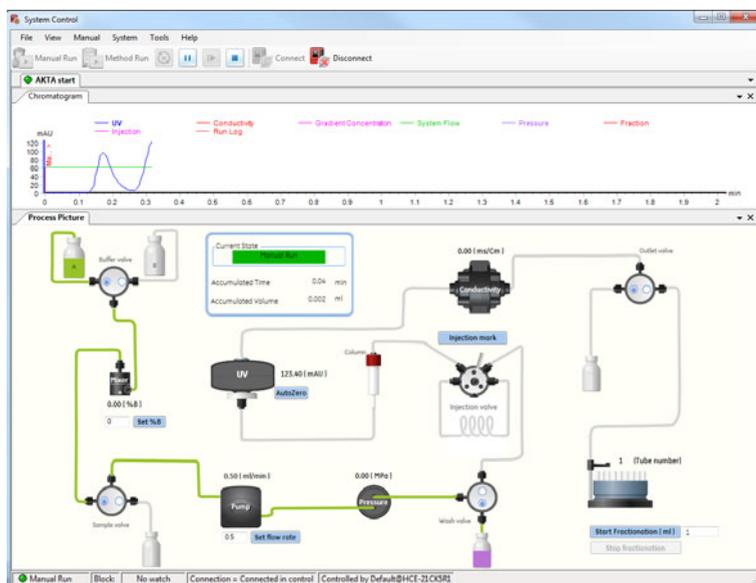
2

Enter the desired **Flow Rate** as ml/min.

3

Click the **OK** button to start the manual run.

The run starts and **System Control** shows the current flow path as well as the real time chromatogram.



3 Getting started

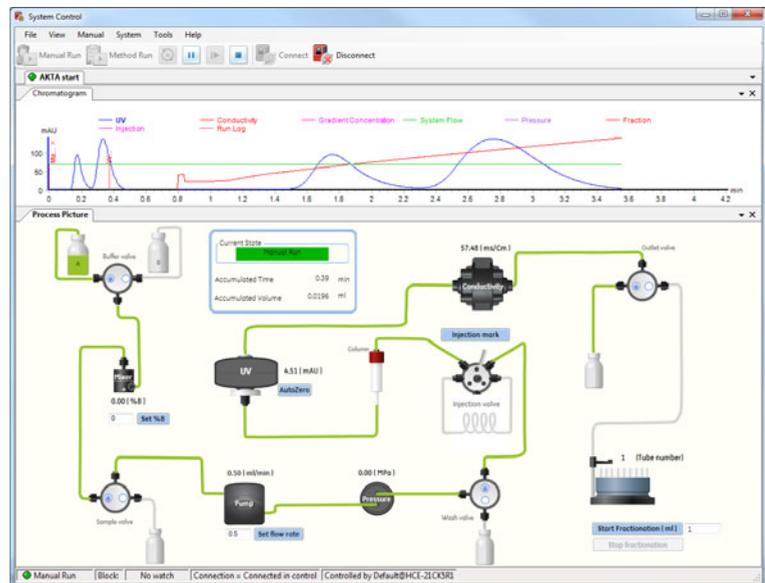
3.1 Launch UNICORN start

Step	Action
------	--------

- | | |
|---|---|
| 4 | Select the upper radio button to change the position of the wash valve. |
|---|---|



Result: The change in flow is reflected in the process picture.



- | | |
|---|---|
| 5 | End the run by clicking the  button. |
|---|---|

Result: The run stops.

- | | |
|---|---|
| 6 | Open the Evaluation module by selecting it on the Windows task bar or by selecting Tools:Evaluation . |
|---|---|

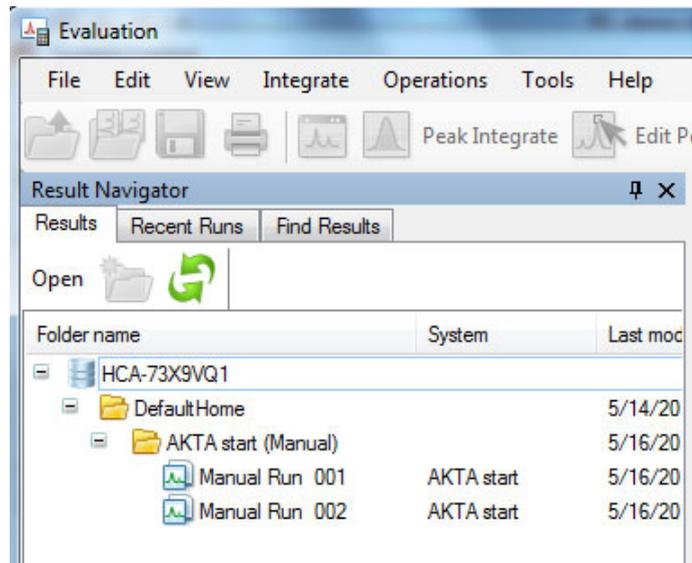
Result: The **Evaluation** module opens.

Step **Action**

7

Open the **Result Navigator** by clicking the  icon or the **Result Navigator** icon.

Result: The **Result Navigator** opens.

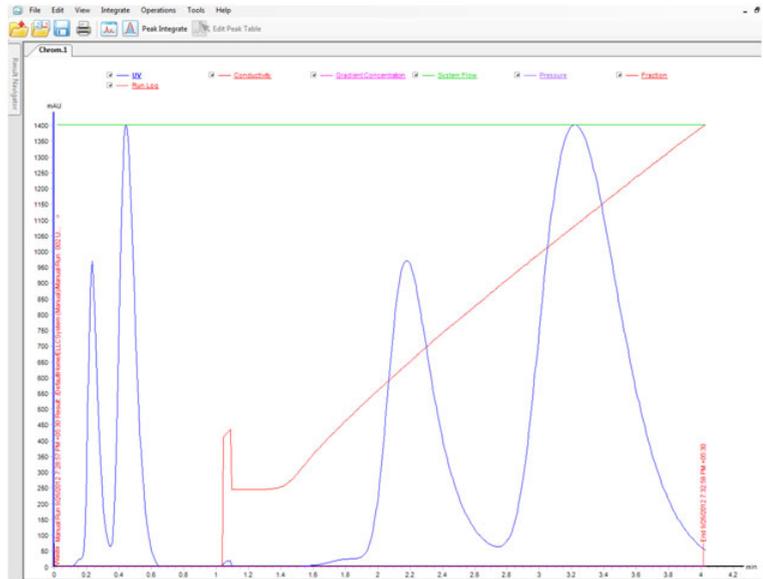


3 Getting started

3.1 Launch UNICORN start

Step Action

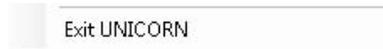
- 8 Double-click the most recent run to open the chromatogram.
The chromatogram result opens.



- 9  Click the  icon to save a PDF result and select a location to save the file.
The PDF result is generated and saved in the location selected above.

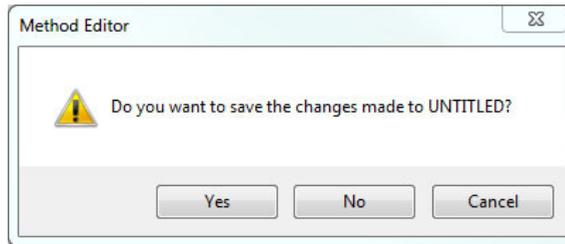
Exit UNICORN start

To exit UNICORN start select **File:Exit UNICORN**. This can be performed from any of the UNICORN start modules.



Result: All open UNICORN start modules close.

Note: *If an updated method or result is open and not saved during exit or log off from UNICORN start, a warning is displayed. Click **Yes** to save, **No** to exit without saving, or **Cancel** to stay logged on.*



3.2 Help functions

The help viewer application

As part of the UNICORN start installation, a special viewer for the online help is installed. This application, the **MadCap HelpViewer**, can be accessed from the UNICORN start user interface either by clicking help buttons in dialogs, by pressing the **F1** key or by selecting **Help** from the menu bar.

By default, this application places a shortcut icon on the desktop, which can be used to open the online help without logging on to UNICORN start. However, if the desktop icon is not present, this application can also be accessed from **Start:All Programs:MadCap Software:MadCap HelpViewer V6**.

To start the UNICORN start online help:

Step	Action
1	Double-click the shortcut icon.
	
2	Choose File:Open .
3	Browse to the folder UNICORN\Documentation\Help.
4	Select the file Manual.mchelp.
5	Click the Open button.
	<i>Result:</i> The online help portal page opens.

Note: Sometimes Windows may need to register **MadCap HelpViewer** as the application for files of the type `.mchelp` before it recognizes calls from help buttons in UNICORN start. If that happens, open the viewer manually as described above.

4 System control

In this chapter

This chapter gives an overview of the **System Control** module and describes how to start method and manual runs. It contains the following sections:

Section	See page
4.1 Overview	56
4.2 Connect to system	60
4.3 Start a run	61
4.4 Monitor a run	66

4.1 Overview

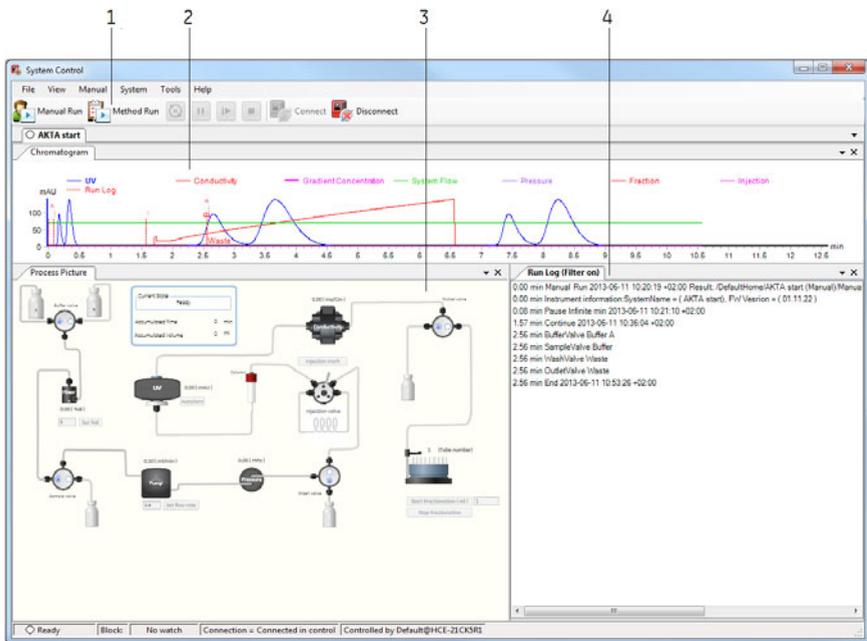
Introduction

The **System Control** module is used to start, monitor and control a chromatography run. Runs can be performed in two different ways, as listed below:

- Using a pre-defined method template.
- Performing the chromatography run by providing manual instructions.

System Control user interface

The illustration below shows the different panes and areas of the **System Control** module.



Note: The status bar in the bottom of the window shows the current connection and control status for the displayed system.

Pane	Function
1	Toolbar: Includes buttons and icons for system control.

Pane	Function
2	Chromatogram: Illustrates data as curves such as UV absorbance (mAU), Conductivity (mS/cm), Flow rate (ml/min), Pressure (mPa), Gradient (%B), Run log and Fraction.
3	Process Picture: Illustrates the current flow path.
4	Run Log: Displays the current state of the system.

Toolbar icons in the System Control module

The table below describes the toolbar icons in the **System Control** module.

Icon	Function
 Start a Method Run	Starts a method run. Note: <i>It is mandatory to select a method from the Method Navigator dialog to start the run.</i>
 Start a Manual Run	Starts a manual run.
Hold 	Temporarily holds the run, with currently set flow rate, valve positions, and B concentration.
Pause 	Temporarily pauses the run by stopping the pump.

4 System control

4.1 Overview

Icon	Function
Continue 	Continues the run that has been paused
End 	Terminates the ongoing run.
Connect 	Connects the software to the instrument.
Disconnect 	Disconnects the software from the instrument.

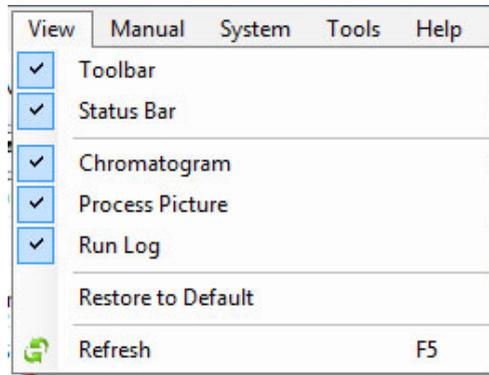
Select panes to display

The table below describes how to display panes:

Step	Action
------	--------

1	In the System Control menu bar, select View .
---	---

Step	Action
2	Select the panes to display.



Selected panes are displayed on the screen.

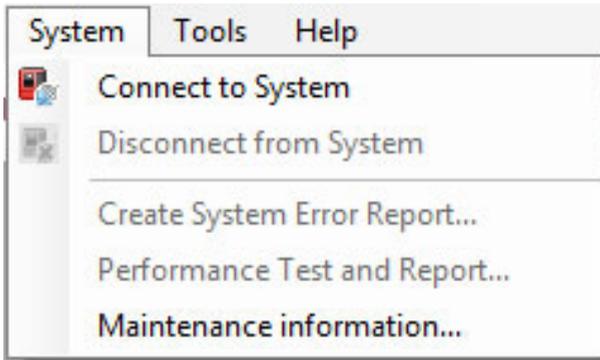
Instrument states

Information about the status of instrument connections and chromatography runs is displayed as **Current State** in the chromatogram.

4.2 Connect to system

Connect the ÄKTA start system to UNICORN start

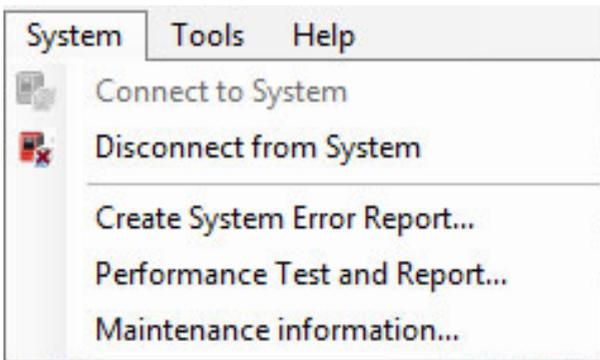
Click on the  **Connect** icon to connect UNICORN start to the ÄKTA start instrument. Alternatively, navigate to **System:Connect to System** in the **System Control** module.



Disconnect from system

Click on the  **Disconnect** icon to disconnect UNICORN start from the ÄKTA start instrument.

Alternatively, navigate to **System:Disconnect from System** in the **System Control** module.



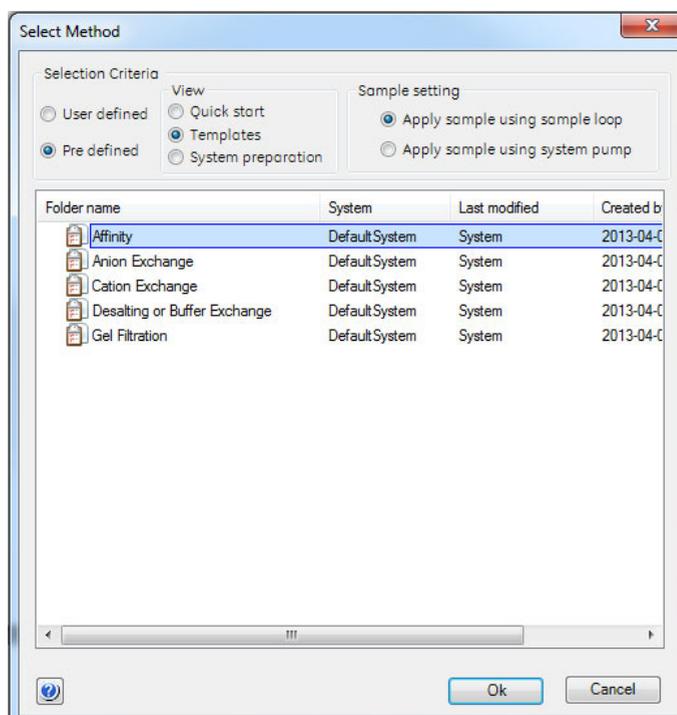
4.3 Start a run

Start a method run

Follow these steps to start a method run:

Step	Action
------	--------

1	<p>Click the  icon in the toolbar.</p> <p>Or,</p> <p>select Method Run from File menu in the System Control toolbar.</p> <p>This opens the Select Method dialog.</p>
---	---



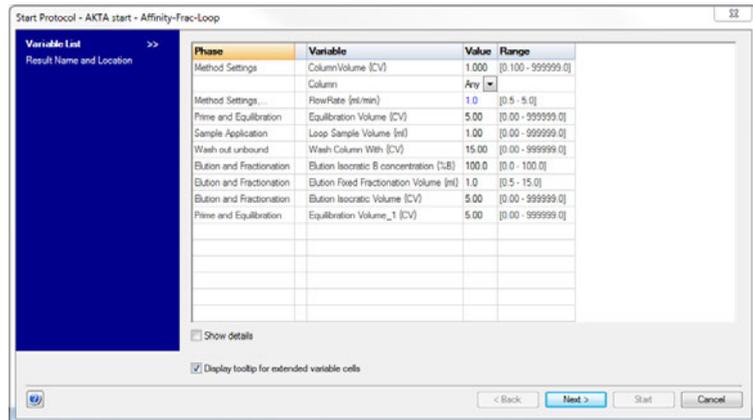
2	Select the desired Selection criteria : User defined or Pre defined .
---	--

4 System control

4.3 Start a run

Step Action

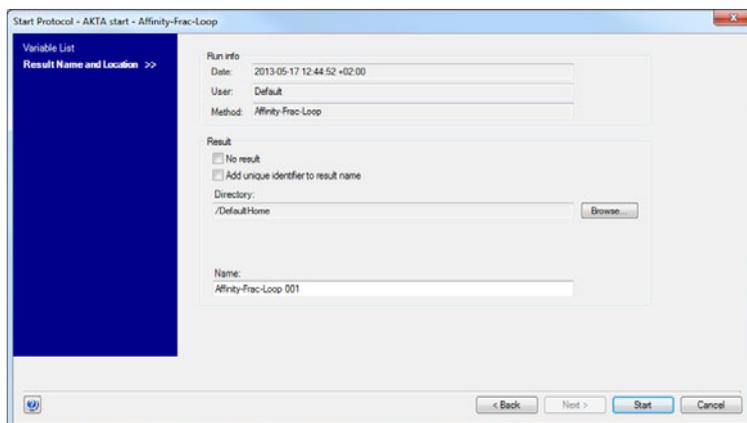
- 3 For **User defined**, find and select a suitable method in the folder pane.
For **Pre defined**, select **View** and find a suitable method in the folder pane, and then select **Sample setting**:
 - View:
 - **Quick start**
 - **Templates**
 - **System preparation**
 - Sample setting:
 - **Apply sample using sample loop**
 - **Apply sample using system pump**
- 4 Click the **OK** button to start the selected method run.
This opens the **Start protocol** dialog.



- 5 Review the **Variable List** and change method parameters if required, and then click **Next** to proceed to the next page.

Step	Action
------	--------

- | | |
|---|---|
| 6 | Specify a result name and then click Start to start the run. |
|---|---|

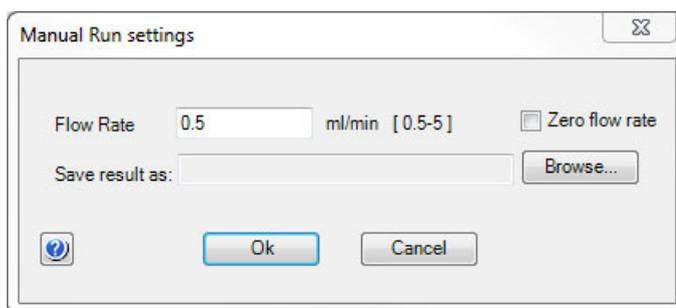


Start a manual run

Follow these steps to start a manual run:

Step	Action
------	--------

- | | |
|---|---|
| 1 |  Click the Manual Run icon in the toolbar.
This opens the Manual Run settings dialog. |
|---|---|



- | | |
|---|--|
| 2 | Enter the desired flow rate. |
| 3 | Click the Browse button and select the location where the result should be saved. |

4 System control

4.3 Start a run

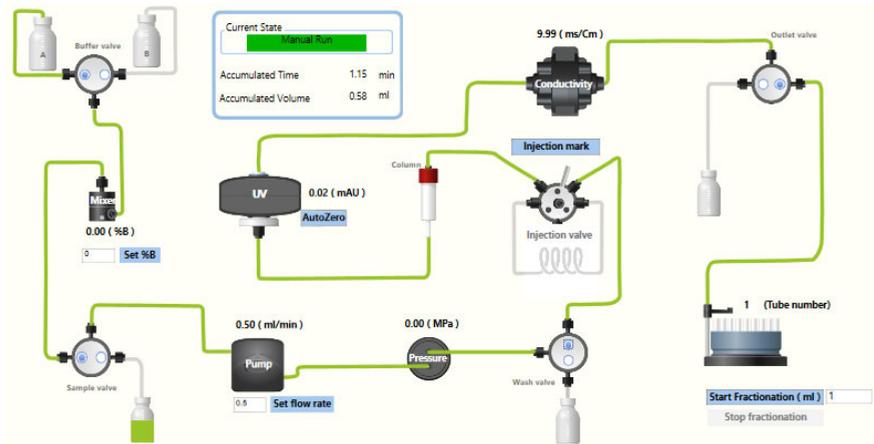
Step	Action
------	--------

4	Click the OK button to start the manual run.
---	---

Manual Instructions

To open the **Manual Instructions** dialog, select **Manual:Execute Manual Instructions**. The following manual instructions can be performed from the process picture (see the illustration below):

- All valve changes can be executed with a single click.
- % B concentrations can be set.
- Flow rate can be changed.
- Fractionation can be started or stopped.
- Autozero can be performed; an injection mark can be set.



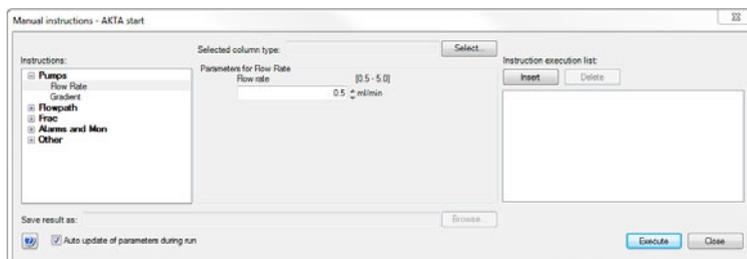
Perform manual instructions during a method run

The instruction below is an example of how to manually interact with an ongoing method run. The example shows how to increase the system flow.

Step	Action
------	--------

1	Open the Manual Instruction dialog according to the above instruction.
---	---

- | Step | Action |
|------|---|
| 2 | In the Instructions list, select the instruction group Pumps and instruction Flow rate . |
| 3 | Enter a new value for Flow rate . |



- | | |
|---|---|
| 4 | To execute several instructions at the same breakpoint, select and edit an instruction and click Insert . Repeat for several instructions. |
| 5 | To refresh the parameter fields during a method run, check the Auto update of parameters during run checkbox. |
| 6 | To execute the instructions, click the Execute button. |

4.4 Monitor a run

Introduction

This section describes the data shown in **System Control** during a run, and the procedure to customize the view of the different panes. It also describes how to enable alarm and error notifications.

You can follow the ongoing method run in the **System Control** module.

- Selected curves are shown in the **Chromatogram** pane.
- The current flow path is shown in the **Process Picture** pane.

To find an overview of the **System Control** user interface, see *Section 4.1 Overview, on page 56*.

Open the Customize dialog

To customize displayed information and data in the different panes:

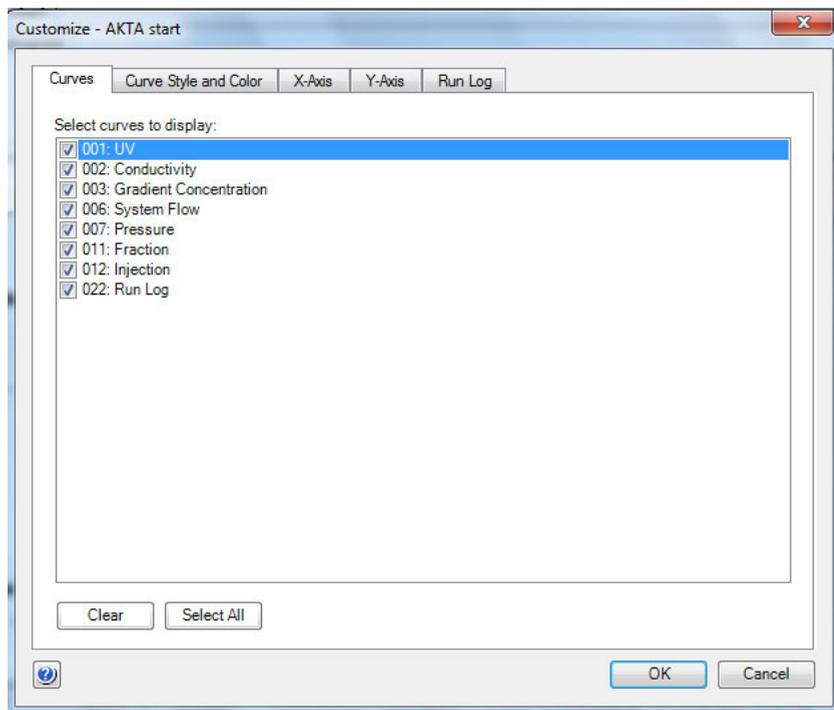
- Select **Customize** from the **Tools** menu in the **System Control** toolbar.



or

- Right-click in the different panes (except **Process Picture**) and select **Customize**.

Result: The **Customize** dialog is opened.



Note: Further information about the settings in the **Customize** dialog can be found in the Online Help.

Customize Chromatogram

The **Chromatogram** pane displays registered curves during the run.

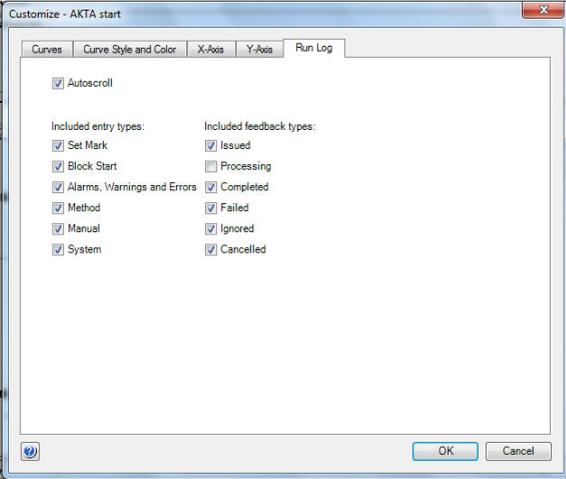
Tab in <i>Customize</i> dialog	Customization
Curves	Choose curves to be displayed
Curve style and color	Customize the appearance of the displayed curves
X-axis	Choose base unit (time or volume) and set axis scale
Y-axis	Set scale for the different curves and select which axes to display

4 System control

4.4 Monitor a run

Customize Run Log

The **Run Log** pane displays registered actions during the run.

Tab in <i>Customize</i> dialog	Customization
Run Log	<p>Selects what to display in the log.</p> <p>Note: <i>All information in the run log is saved. The selections define what is displayed.</i></p> 

Select and zoom a curve in the chromatogram

The table below shows how to select a curve and to zoom in the chromatogram.

Command	Instruction
Select a curve	<ul style="list-style-type: none">• Position the mouse marker over a curve name: The curve line becomes bold to facilitate the identification of desired curve.• Click the curve: The corresponding legend text is shown in bold type and the Y-axis scale changes to the unit applicable for the curve.

Command	Instruction
Zoom	<ul style="list-style-type: none">• Click and hold the left mouse button with the pointer positioned in the top left corner of the area you want to zoom in on.• Drag the pointer downwards and to the right. A dotted rectangle marks the selected area. Select the area you want to zoom in on, and release the left mouse button. (The text Zoomed mode is shown in the chromatogram)• To reset zoom, right-click and select Reset zoom.• To reset the last zoom action, right-click and select Undo zoom.
Right-click menu options	<ul style="list-style-type: none">• Right-click and select options and actions. See <i>Online Help</i> for information.

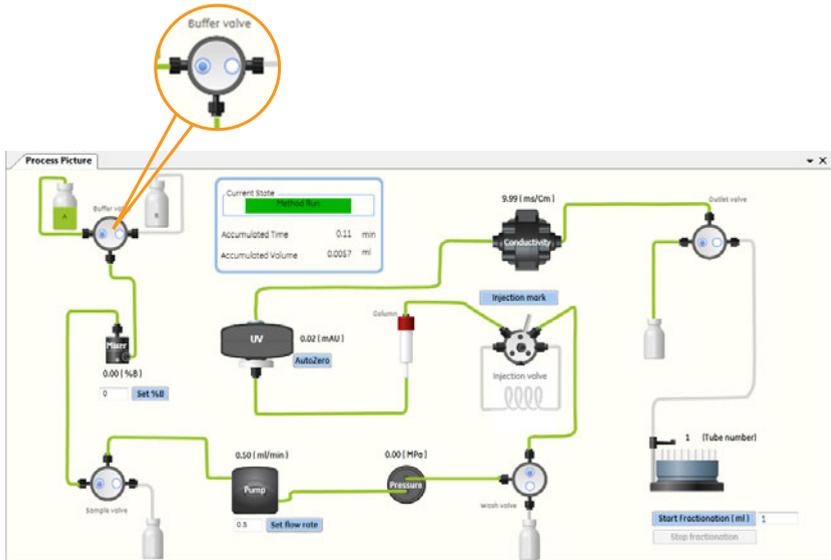
4 System control

4.4 Monitor a run

Illustration of the Process Picture pane

The **Process Picture** pane shows the current flow path during the run. Color indication is applied and real-time data from monitors are shown.

The following illustration shows an example of a process picture. In this example, the orange marking indicates where you can shift between options by clicking the radio button for e.g., Buffer A or B.



The table describes how different colors indicate the current state of the flow paths; *open* or *closed*.

Color	Indication
Green	Open flow path
Gray	Closed flow path

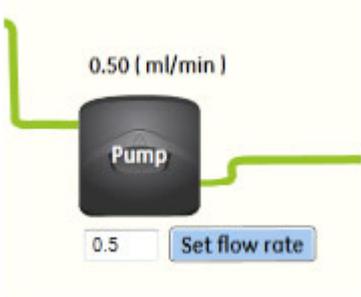
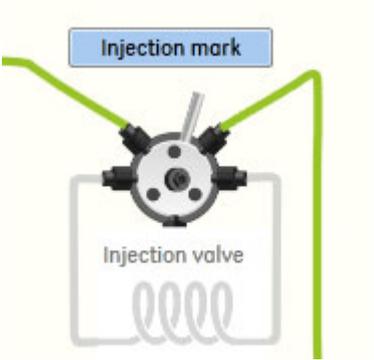
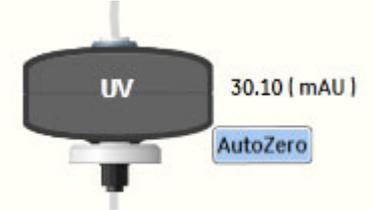
Options in the Process Picture pane

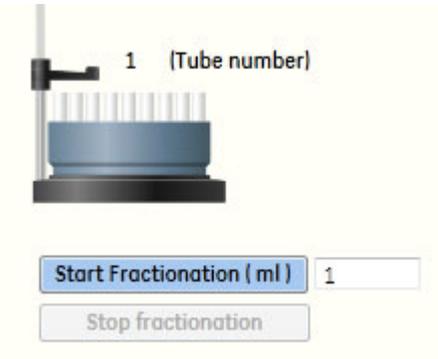
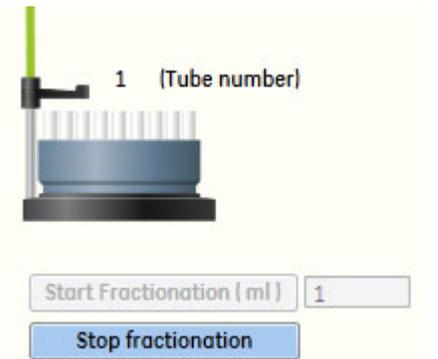
The following table describes how to change valve positions, set values, start/stop fractionation and start/stop peak collection in the **Process Picture** pane.

Option	Description
Change valve positions	<p>Click the radio buttons to change between the valve positions.</p> 
Set % B concentration	<p>Enter a value for setting the B concentration and then click Set %B.</p> 

4 System control

4.4 Monitor a run

Option	Description
Set flow rate	<p>Enter a value for flow rate and then click Set flow rate to set the flow rate.</p>  <p>The screenshot shows a black cylindrical component labeled 'Pump' with a green tube connected to its top. Above the pump, the text '0.50 (ml/min)' is displayed. Below the pump, there is a small input field containing the number '0.5' and a blue button labeled 'Set flow rate'.</p>
Introduce Injection mark	<p>Click the Injection mark button to introduce an injection mark in the chromatogram.</p>  <p>The screenshot shows a central injection valve with a grey coil below it. A blue button labeled 'Injection mark' is positioned above the valve. Green tubes are connected to the top of the valve.</p>
Preform UV baselining	<p>Click the AutoZero button to perform UV baselining.</p>  <p>The screenshot shows a black cylindrical component labeled 'UV' with a grey tube extending from its bottom. To the right of the component, the text '30.10 (mAU)' is displayed. Below this text is a blue button labeled 'AutoZero'.</p>

Option	Description
Start/Stop fractionation	<ul style="list-style-type: none"><li data-bbox="641 287 1190 347">Enter a fraction volume per tube (ml) and then click Start Fractionation to start the fractionation.  <ul style="list-style-type: none"><li data-bbox="641 771 1182 798">Click Stop Fractionation to stop the fractionation.  <p data-bbox="641 1239 1190 1361">Note: <i>This option is visible only if a fraction collector has been enabled. To enable the fraction collector, refer to the ÅKTA start Operating Instructions.</i></p>

4 System control

4.4 Monitor a run

Option	Description
Peak collection	<ul style="list-style-type: none">Click Start peak collection to start the peak based collection.  <ul style="list-style-type: none">Click Stop peak collection to stop the peak based collection.  <p>Note: <i>This option is visible only if the fraction collector has been disabled.</i></p>

5 Create a method

In this chapter

This chapter contains a general introduction to creating and editing methods using UNICORN start. It also describes overall method options, how to print methods, and how to import/export methods. Descriptions of the pre-defined methods and phases supplied with the software are included as well. The chapter contains the following sections:

Section	See page
5.1 Overview	76
5.2 Create and edit methods	83
5.3 Predefined methods and phases	88
5.4 Fraction collection	99
5.5 Import and export methods	109
5.6 Column handling	115

5 Create a method

5.1 Overview

5.1 Overview

About this section

This section gives an introduction to the **Method Editor** module of UNICORN start. It also gives a brief description of the **Method Editor** interface and describes the concept of methods in UNICORN start.

For information on how to create, open and edit methods and import/export methods, see *Section 5.2 Create and edit methods, on page 83*.

In this section

This section contains the following topics:

Section	See page
5.1.1 Method Editor	77
5.1.2 Methods in UNICORN start	80

5.1.1 Method Editor

Introduction

The **Method Editor** module is used to:

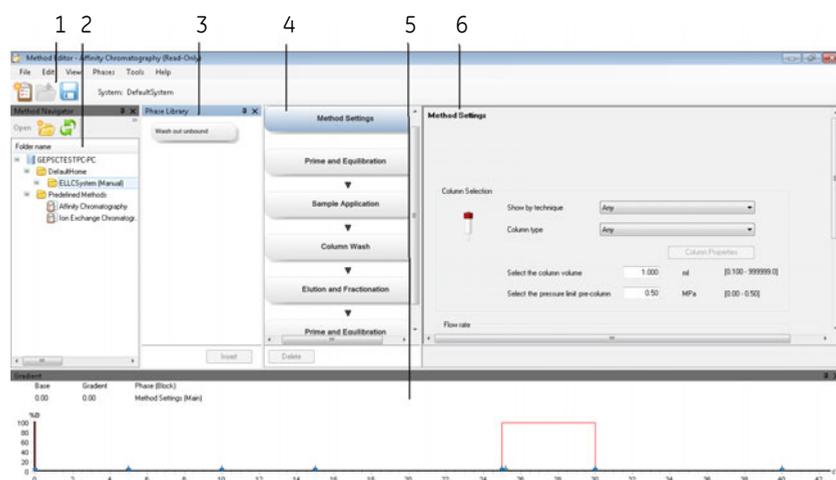
- Create and edit methods.
- Copy, save and delete methods.

Method Editor also assists the user in optimizing runs and allows handling of column types and user defined columns. For more information, see the following sections.

Method Editor interface

The **Method Editor** interface consists of different panes that allows the user to get an overview of the method, a deep dive into each phase of a method and what methods and phases the database contains.

The **Method Editor** interface with all the optional panes is illustrated next.



Pane	Description
1	Toolbar (optional pane): Shows the toolbar icons.
2	Method Navigator (optional pane): Shows all the user folders and methods that are available in the database.

5 Create a method

5.1 Overview

5.1.1 Method Editor

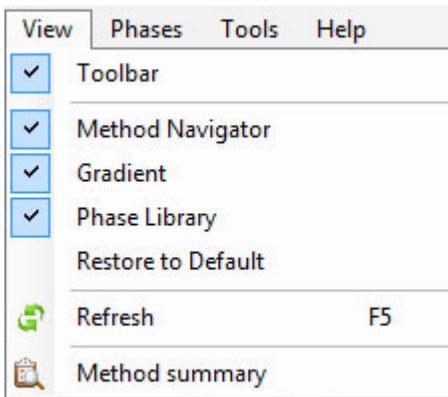
Pane	Description
3	Phase Library (optional pane): Contains all available phases.
4	Method outline : Shows the phases included in the open method.
5	Gradient (optional pane): Shows the programmed gradient and break points for included phases.
6	Phase properties : Shows the settings for the highlighted phase in the method outline.

Display optional panes

The optional panes in **Method Editor** are displayed by selecting them in the **View** menu.

To restore the appearance of **Method Editor** and display the default panes, select **Restore to Default** in the **View** menu. Then, the **Toolbar**, **Gradient** and **Phase Library** are displayed. The appearance of the optional panes can also be controlled using the auto hide function (see below for more information).

The illustration below shows the **View** menu with the default panes selected.



Method Navigator auto hide

The **Method Navigator** may be displayed in the left portion of the **Method Editor** module, or the **Auto Hide** function can be selected by clicking the pin symbol in the top right-hand corner of the pane.

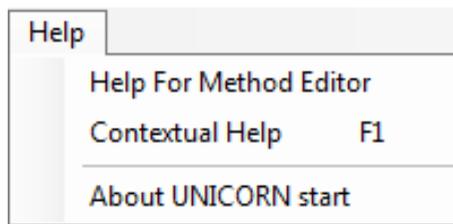
Pin direction	Function
	Auto Hide is off. Click the pin symbol to turn the function on.
	Auto Hide is on. Click the pin symbol to turn the function off.

If **Auto Hide** is selected, the **Method Navigator** opens automatically when the mouse pointer is placed over its tab. It remains open as long as the mouse pointer remains over the pane. The pane closes automatically when the pointer is moved outside the pane.

Getting help on the toolbar and panes in Method Editor

The table below describes how to find detailed information about the toolbar and the different panes in **Method Editor** by opening the online help.

Step	Action
1	To display detailed information about the toolbar and different panes in the Method Editor interface, select Help:Help For Method Editor .



Result: The online help opens and displays the **Method Editor** help start page.

2	To display help for a specific pane, click in the pane and press the F1 key. <i>Result:</i> The online help page describing that pane is opened.
---	--

5 Create a method

5.1 Overview

5.1.2 Methods in UNICORN start

5.1.2 Methods in UNICORN start

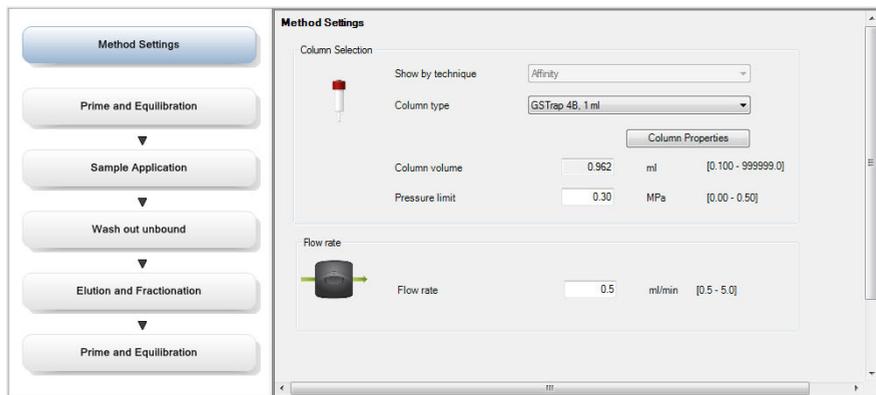
About methods

The program instructions for a chromatography run are defined in a method. These instructions are specific for each instrument configuration and follow certain syntactical and hierarchical rules.

In a predefined method, each phase reflects a step in the chromatography run, for example, equilibration or sample application. A number of settings are available for each type of phase. By building methods in this way, methods are easily created and edited.

See *Section 5.2 Create and edit methods, on page 83* for information about creating and editing methods in **Method Editor**.

The illustration below shows the phases in a predefined method in the method outline pane (left) and the corresponding settings for the highlighted phase in the phase properties pane (right).



Method structure

A method always starts with the **Method Settings** phase. This phase contains the general settings that affect the rest of the method. If **Column type** is changed for a predefined method, UNICORN start automatically calculates the correct settings for column volume, flow rate, and pressure limits that will be used throughout the method. Subsequent phases reflect steps included in the chromatography run.

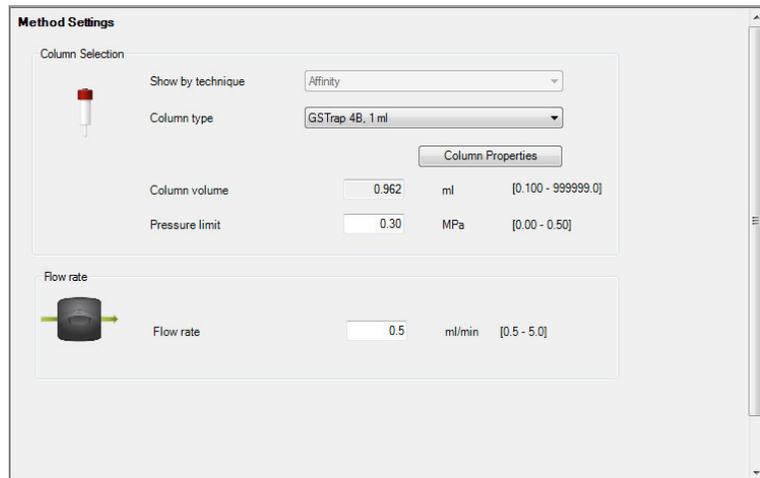
A predefined method with the different phases in the method outline pane is illustrated next.



Working with methods

It is recommended to create and edit methods using phase properties. Phases can easily be dragged-and-dropped into the method outline from the **Phase Library** and the phases are easily rearranged. Settings for each phase are set in the phase properties pane.

The phase properties settings for the **Method Settings** phase in a predefined method are illustrated next.



5 Create a method

5.1 Overview

5.1.2 Methods in UNICORN start

Method types

UNICORN start provides a number of predefined methods for different chromatography techniques, and for preparation and maintenance of the instrument (e.g., preparation and cleaning of the system and columns). Customized methods may be created, by dragging and dropping the phases from the phase library followed by setting the parameters. The phase **Method Settings** is mandatory to all methods.

See Section 5.2 *Create and edit methods*, on page 83 for information about how to create new methods.

The table below gives a general description of the different method types.

Method	Description
Predefined Method	Predefined methods include a number of relevant phases appropriate for the purification or maintenance to be performed. These predefined methods can be used as they are, or with adjusted settings as needed. See Section 5.3 <i>Predefined methods and phases</i> , on page 88 for descriptions of the predefined methods provided with the software.
Customized Method	Customized methods include the mandatory phase Method Settings . Other phases are then added by the user from the Phase Library pane and settings adjusted as needed.

Predefined phases

UNICORN start provides a number of predefined phases.

Predefined phases (for example, **Prime and Equilibration**) can be used when building or editing methods in **Method Editor**. A predefined phase contains all the necessary instructions needed for a particular phase. All predefined phases can easily be viewed in the **Phase Library** pane.

See Section 5.3.1 *Definitions*, on page 89 for descriptions of the predefined phases supplied with the software.

5.2 Create and edit methods

About this section

This section describes how to create and edit methods in UNICORN start using the phase properties pane.

In this section

This section contains the following topics:

Section	See page
5.2.1 Working with methods	84
5.2.2 Open a method	86

5.2.1 Working with methods

Introduction

In UNICORN start, the predefined methods for ÄKTA start are built up of phases, where each phase corresponds to a step in a chromatography run, with a number of properties associated with that phase. See *About methods, on page 80* for more information about method structure, definitions and concepts of methods in UNICORN start.

Methods can be created and edited by using phases and phase properties.

Main steps when defining a new method using predefined phases

The main steps when defining a method are:

Step	Description
1	Create/open a method <ul style="list-style-type: none">• Create a predefined method (including a set of phases that may be edited) or• Open an existing method that can be edited and saved with a new name or overwritten.
2	Build/edit the method outline and/or edit the phase properties for the appropriate phases <ul style="list-style-type: none">• Predefined Methods: use as they are, or edit the method outline and/or phase properties.• Opened methods: edit the method outline and/or phase properties.
3	Save the method.

Main steps when defining a customized method

The main steps when defining an empty method are:

Step	Description
1	Create/open a method <ul style="list-style-type: none">• Create a new empty method containing the Method Settings phase.

Step	Description
2	Build/edit the method outline and edit the phase properties for the phases <ul style="list-style-type: none"><li data-bbox="435 316 1209 378">• Add predefined phases to the method (i.e., build the method outline) and edit the phases as appropriate.<li data-bbox="435 396 680 425">• Rename the phases.
3	Save the method.

5.2.2 Open a method

Follow the instructions to open an existing method in the database:

Step	Action
------	--------

1 In **Method Editor**:

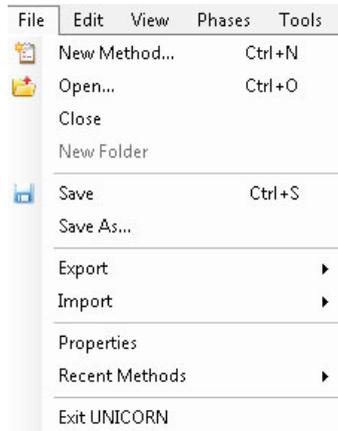
- Click the



icon in the toolbar

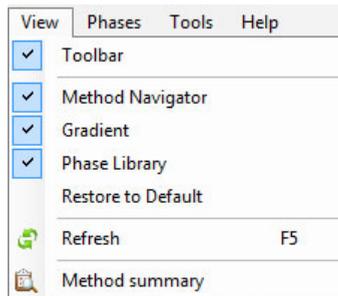
or

- Select **File:Open**



or

- Select **View:Method Navigator**



Result: The **Method Navigator** is displayed.

Step	Action
------	--------

2 Select the method to be opened in the **Folder name** column.



3 To open the method,

- Click the **Open** button located in the toolbar of the **Method Navigator** pane



or

- Double-click the selected method

or

- Right-click on the method name and select **Open** from the context menu

Result: The method is opened and displayed in the method outline pane with the included phases. The phases of the method are still editable using phase properties.

5.3 Predefined methods and phases

In this section

This section describes how to work with methods and phases. It contains the following subsections:

Section	See page
5.3.1 Definitions	89
5.3.2 Create a predefined method	93
5.3.3 Edit phase properties	95

5.3.1 Definitions

Introduction

A predefined method contains a set of phases, each phase reflecting a specific stage of a chromatography or maintenance run. Additional phases can be selected from the phase library and added to an existing method and phases that are not required can be removed.

The predefined purification methods have default values with suitable running conditions for the chosen column type such as flow and pressure limits. Other settings (e.g., sample application technique, sample volume, elution profile and fractionation) are set in the phase properties pane of the appropriate phases.

This section describes the predefined methods and phases.

Predefined purification methods

Method Editor has five predefined methods for different separation techniques. The methods include a number of relevant phases.

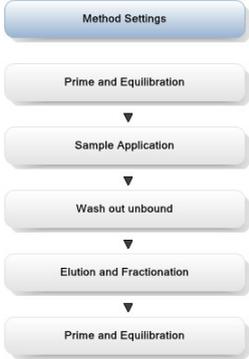
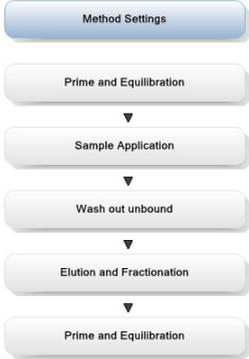
The table below describes the available predefined purification methods and which phases that are included.

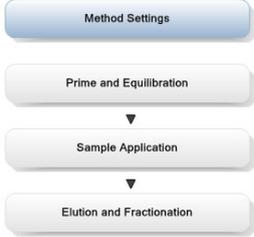
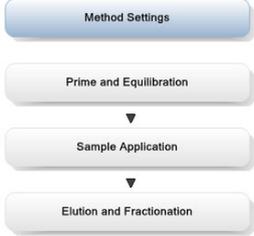
Predefined purification method	Principle	Included phases
Affinity Chromatography (AC)	Separates molecules based on the reversible interaction between the target protein and the specific ligand attached to a chromatography matrix. The steps include equilibration, sample application, wash unbound step to remove unbound non-specific proteins, elution with with fractionation followed by re-equilibrating the column.	

5 Create a method

5.3 Predefined methods and phases

5.3.1 Definitions

Predefined purification method	Principle	Included phases
Anion Exchange Chromatography (AIEC)	Based on the reversible interaction between negatively charged protein and positively charged chromatography media. The steps include, equilibration with binding buffer, sample application, elution with change in salt concentration (e.g. NaCl) or change in pH of elution buffer and re-equilibration with binding buffer.	 <pre>graph TD; A[Method Settings] --> B[Prime and Equilibration]; B --> C[Sample Application]; C --> D[Wash out unbound]; D --> E[Elution and Fractionation]; E --> F[Prime and Equilibration];</pre>
Cation Exchange Chromatography (CIEC)	Based on the reversible interaction between positively charged protein and negatively charged chromatography media. The steps include, equilibration with binding buffer, sample application, elution with change in salt concentration (e.g. NaCl) or pH of elution buffer and re-equilibration with binding buffer.	 <pre>graph TD; A[Method Settings] --> B[Prime and Equilibration]; B --> C[Sample Application]; C --> D[Wash out unbound]; D --> E[Elution and Fractionation]; E --> F[Prime and Equilibration];</pre>

Predefined purification method	Principle	Included phases
Desalting	<p>A gel filtration technique that allows rapid group separation of high molecular weight substances from low molecular weight substances. Small molecules like salt, free labels and other impurities are efficiently separated from the high molecular weight substances of interest. The method involves equilibration followed by sample application, elution and fractionation using single buffer.</p>	 <pre> graph TD A[Method Settings] --> B[Prime and Equilibration] B --> C[Sample Application] C --> D[Elution and Fractionation] </pre>
Gel filtration (GF)	<p>Also known as size-exclusion chromatography, is a chromatography technique that separates molecules based on differences in the molecular size. The method involves equilibration, sample application and elution and fractionation.</p>	 <pre> graph TD A[Method Settings] --> B[Prime and Equilibration] B --> C[Sample Application] C --> D[Elution and Fractionation] </pre>

Predefined phases

The table below describes the predefined phases.

Phase Name	Description
Method Settings	<p>The first, and mandatory, phase in any method. Defines common parameters used in the subsequent phases.</p> <p>The Method Settings phase defines:</p> <ul style="list-style-type: none"> • Column selection • Flow rate • Pressure limit <p>Note: <i>Default values for pressure limits and flow rate are given for the GE Healthcare selected column type.</i></p> <p>Note: <i>Some of these options may not be required by certain methods.</i></p>
Prime and Equilibration	Primes the flow path by filling it with buffers of interest, and equilibrates the column before a purification, or re-equilibrates the column after a purification.
Sample Application	Applies sample to the column. Defines the sample application mode technique, sample volume, and handling of the flow through.
Washout unbound	Washes out unbound sample after sample application.
Elution and Fractionation	Elutes the sample from the column. Defines parameters for the elution and fractionation settings.

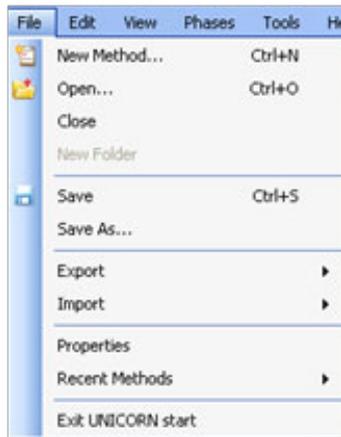
5.3.2 Create a predefined method

Follow the instructions to create a new method using phases:

Step	Action
------	--------

1 In **Method Editor**:

-  Click the  icon in the toolbar
or
- Select **File:New Method**



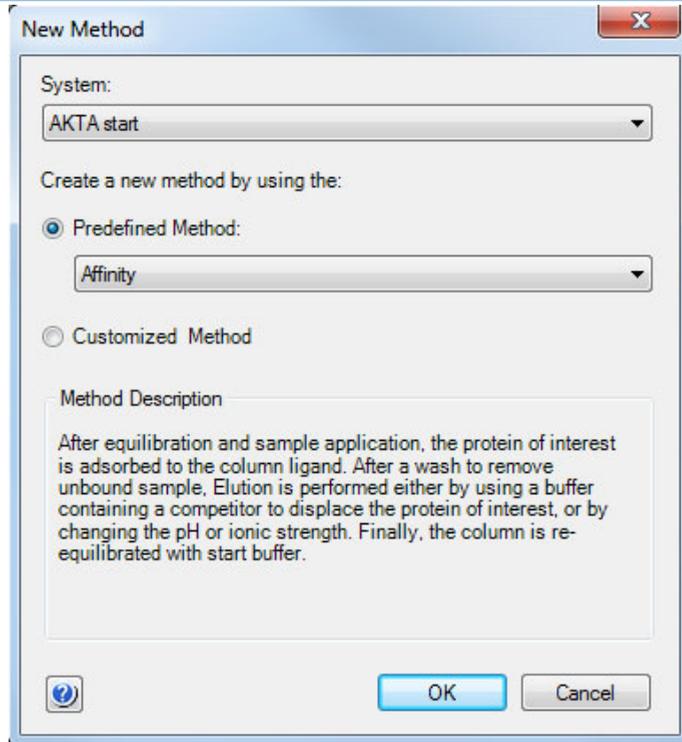
Result: The **New Method** dialog opens.

5 Create a method

5.3 Predefined methods and phases

5.3.2 Create a predefined method

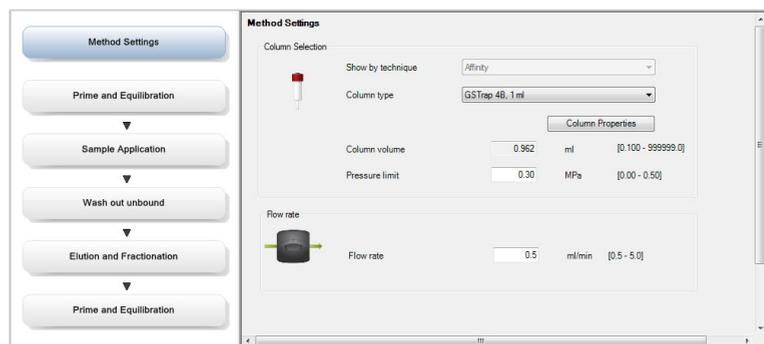
Step	Action
------	--------



2 In the **New Method** dialog:

- Select a **Predefined Method**.
- Click the **OK** button.

Result: The method outline pane shows the included phases for the chosen method and the phase properties pane shows the default settings for the currently highlighted phase.



5.3.3 Edit phase properties

Introduction

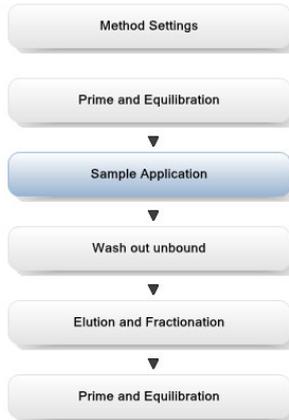
When editing phase properties, the changes affect either:

- The whole method, when editing the **Method Settings** phase.
 - Only the phase that is being edited, when editing phases other than the **Method Settings** phase.
-

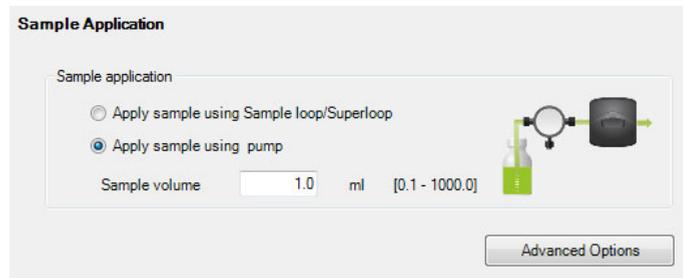
Getting help when editing phase properties

Follow the instructions to get help for the properties in a phase:

Step	Action
1	Select a phase in the method to be edited, for example, Sample Application .



Result: The properties for the selected phase are displayed in the phase properties pane.



Note:

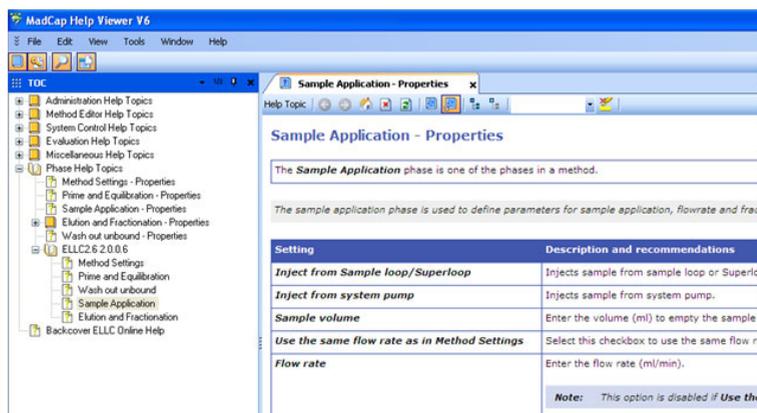
Click the **Advanced Options** button to display more phase properties settings.

2	Click anywhere in the phase properties area to make it the active area in the software.
---	---

Step	Action
------	--------

- | | |
|---|---|
| 3 | <ul style="list-style-type: none"> Press the F1 keyboard key. or Select Help:Contextual Help |
|---|---|

Result: The Online help for the selected phase is displayed.



View and edit phases using phase properties

Follow the instructions to edit a method phase in the **Phase properties** pane:

Step	Action
------	--------

- | | |
|---|---|
| 1 | Make sure the phase properties pane is selected. |
| 2 | <ul style="list-style-type: none"> Select the Method Settings phase if the basic settings affecting the whole method need to be edited (e.g., Column type, and Flow rate). Continue with steps 3 and 4. or Select any other phase to edit the properties for that specific phase. Continue with step 5. |
| 3 | To edit the properties for the Method Settings phase, click Method Settings in the method outline pane. |

Result: The phase properties of the **Method Settings** phase is displayed.

5 Create a method

5.3 Predefined methods and phases

5.3.3 Edit phase properties

Step	Action
4	<p>Edit the settings for the Method Settings phase in the phase properties pane as appropriate. If changing Column type, UNICORN start automatically calculates the correct settings for volume, flow rate, and pressure limits.</p> <p>Note: <i>Settings in this phase affect the whole method.</i></p> <p>Note: <i>Allowed parameter ranges are shown in parentheses beside the text boxes.</i></p> <p><i>Result:</i> The method is updated with the new settings.</p>
5	<p>Select a phase in the method to be edited, for example, Sample Application.</p> <p><i>Result:</i> The properties for the selected phase are displayed in the phase properties pane.</p>
6	<ul style="list-style-type: none">• Edit the settings as appropriate.• Repeat steps 5 and 6 until the appropriate phases have been edited. <p><i>Result:</i> The method is updated with the new settings. The edited settings remain in place while subsequent phases are edited. If the method is closed and not saved, the settings will revert back to the earlier values.</p>
7	<p>Save the method.</p>

5.4 Fraction collection

In this section

This section describes how to set up different options for fraction collection, with or without Frac30. It contains the following subsections:

Section	See page
5.4.1 Introduction	100
5.4.2 Fractionation setup	101
5.4.3 Fractionation without Frac30	102
5.4.4 Fractionation with Frac30	104

5 Create a method

5.4 Fraction collection

5.4.1 Introduction

5.4.1 Introduction

Collecting the protein fractions during either sample application, wash unbound or elution step, is an important activity during a chromatography run. Some of the predefined phases and methods include options for fraction collection in the phases.

Note: *The Fraction Collector needs to be turned ON from the ÄKTA start Instrument Display for it to be available as an option within UNICORN start.*

Fractionation

Fractionation settings are available in the phase properties pane in the predefined phases **Sample Application**, **Washout Unbound** and **Elution and Fractionation**. These three phases are included in many of the predefined methods of UNICORN start. See [Section 5.3.3 Edit phase properties, on page 95](#) for details on how to edit methods and phases.

For each phase, fractions can either be collected with or without fraction collector (using **Outlet Valve**). If there is no risk of sample loss, the eluate may be sent to the waste and not collected.

5.4.2 Fractionation setup

Options

The following fractionation options are available based on the instrument configuration in use.

Instrument Configuration	Fractionation options
ÄKTA start, UNICORN start and Frac30	<ul style="list-style-type: none"> • Fixed volume fractionation • Peak fractionation <ul style="list-style-type: none"> - Level based - Slope based
ÄKTA start and UNICORN start	<ul style="list-style-type: none"> • Single Peak collection <ul style="list-style-type: none"> - Level based - Slope based
ÄKTA start and Frac30	<ul style="list-style-type: none"> • Fixed volume fractionation
ÄKTA start	<ul style="list-style-type: none"> • Collection of elution volume

Delay volume handling

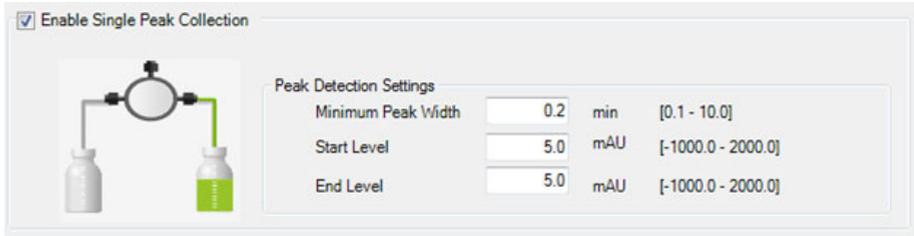
Option	Description
Fractionation with Frac30	Delay volume is collected in the first tube (T1) followed by the rest of the fractions in subsequent tubes.
Fractionation without Frac30	Delay volume is collected in the collection container along with fraction (total collected volume = Delay volume + fraction volume).

For setting the delay volume refer to section *Prepare the fraction collector* in the *ÄKTA start Operating Instructions*.

For detailed instructions on fractionation operations using ÄKTA start, see *ÄKTA start Operating Instructions*.

5.4.3 Fractionation without Frac30

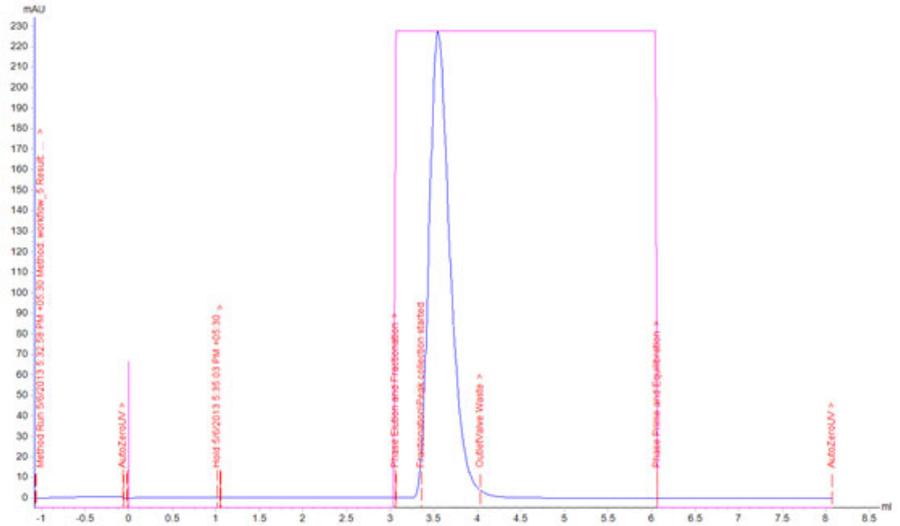
Single peak collection



Single Peak Collection option is available when Frac30 has been disabled from the instrument. Single Peak Collection can be chosen and configured in the **Elution and Fractionation** phase as depicted above.

Setting	Description
Start level	UV absorbance (mAU) that triggers the start of peak collection.
End level	UV absorbance (mAU) that triggers the end of peak collection.

A representative chromatogram depicting fractionation by setting level [Abs(mAU)] values for collecting fractions from UNICORN start using **Outlet Valve** (without Frac30) is shown below.



Note: Make sure to standardize and set the optimized level values and fraction volume in order to collect the peak of interest in a single collection tube. For collecting multiple peaks, make sure to change the collection tube manually after collecting each peak.

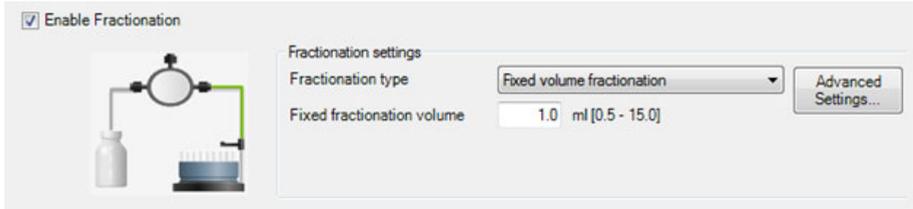
- Note:**
- The UV monitor to **Outlet Valve** volume (0.27 ml) is constant for all instruments.
 - Make sure to use recommended length and inner diameter of polyether ether ketone tubing from **UV** monitor to **Outlet Valve** to avoid incorrect calculation of delay volume.
 - Make sure to update the length and inner diameter of the polyether ether ketone tubing in case the tubing is not of the recommended length and inner diameter.

5.4.4 Fractionation with Frac30

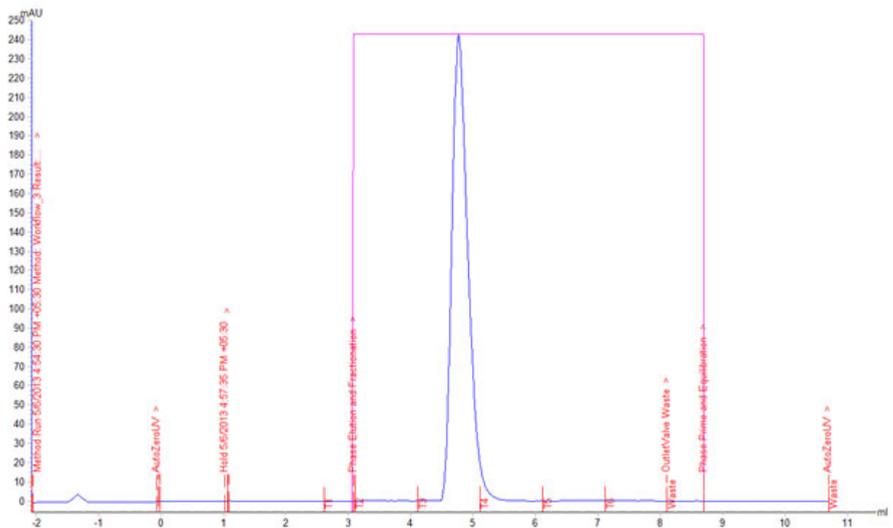
Fixed volume fractionation

Fixed volume fractionation can be chosen and configured in the **Sample Application**, **Washout unbound** and the **Elution and Fractionation** phases as depicted.

Fixed fractionation volume is the volume that will be collected in every fraction per tube.



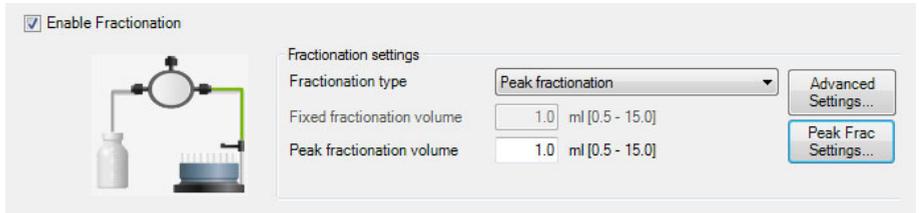
A representative chromatogram depicting fractionation using fixed volume fraction collection from UNICORN start with Frac30 is shown below.



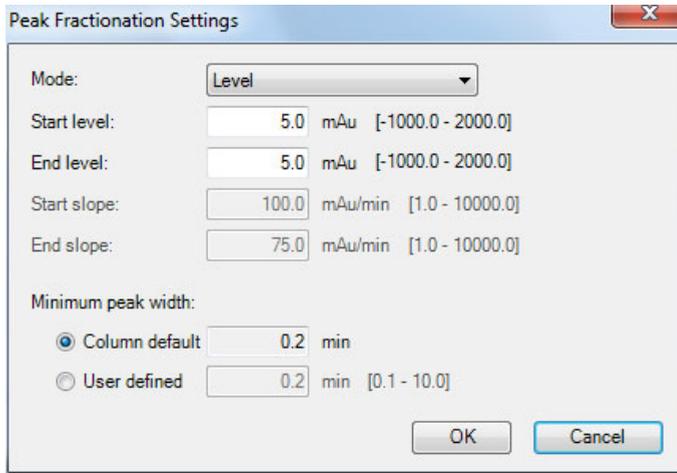
Note: Make sure to set fraction volumes that suit the column being used, and to fill adequate number of collection tubes.

Peak fractionation: Level based

Peak fractionation can be chosen and configured in the **Elution and Fractionation** phase as depicted.



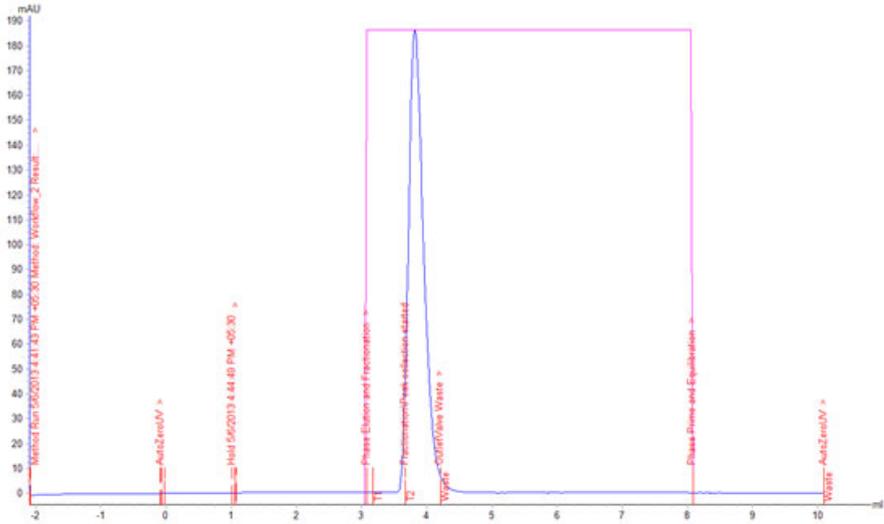
Level based peak fractionation can be configured by editing the **Peak Frac Settings**.



Setting	Description
Start level	UV absorbance (mAU) that triggers the start of peak fractionation.
End level	UV absorbance (mAU) that triggers the end of peak fractionation.
Column default	Selecting this option displays the default minimum peak width based on the column type selected in Method Settings.
User defined	To choose a custom value for the minimum peak width.

A representative chromatogram depicting fractionation by setting level [Absorbance (mAU)] values for collecting fractions from UNICORN start with Frac30 is shown below.

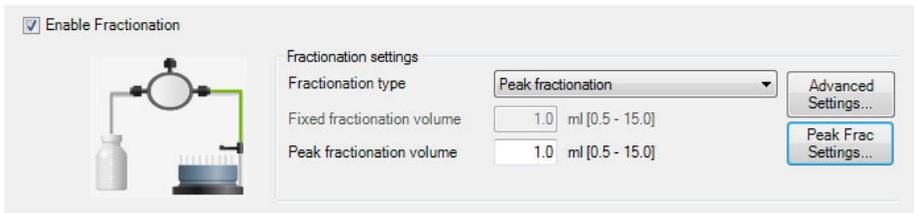
5 Create a method
5.4 Fraction collection
5.4.4 Fractionation with Frac30



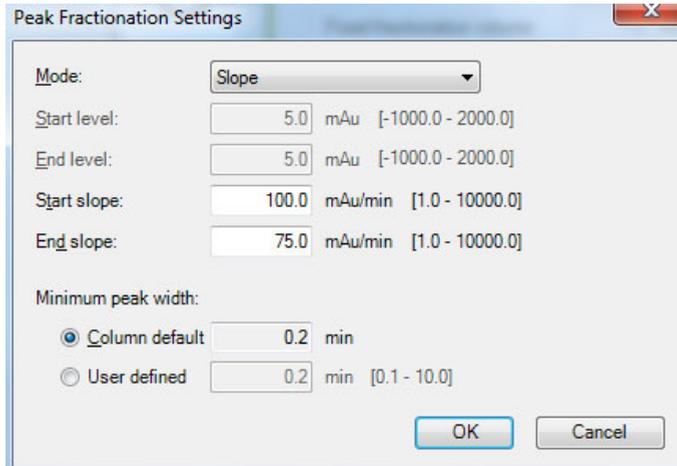
Note: Make sure to standardize and set the optimized level values and fraction volume in order to collect the peak of interest in a single collection tube.

Peak fractionation: Slope based

Peak fractionation can be chosen and configured in the **Elution and Fractionation** phase as depicted.



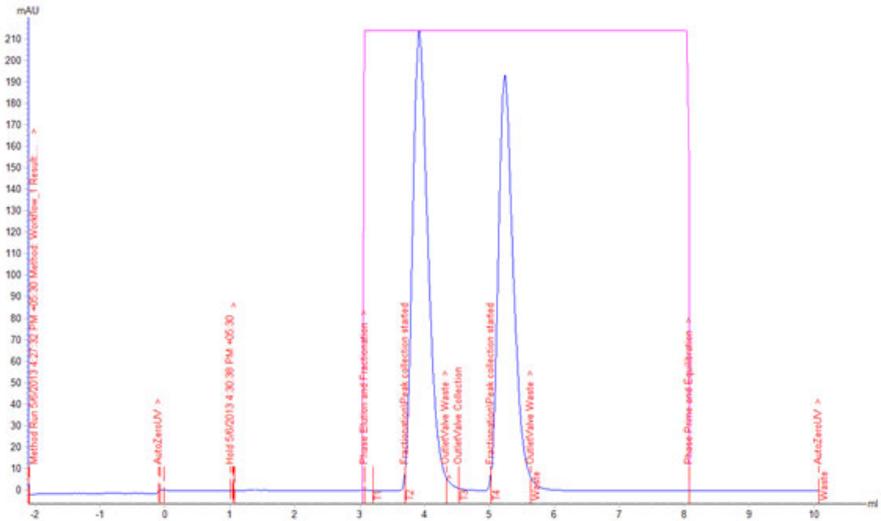
Slope based peak fractionation can be configured by editing **Peak Frac Settings**.



Setting	Description
Start slope	Slope value (mAU/min) that triggers the start of peak fractionation.
End slope	Slope value (mAU/min) that triggers the end of peak fractionation.
Column default	Selecting this option displays the default minimum peak width based on the column type selected in Method Settings.
User defined	To choose a custom value for the minimum peak width.

A representative chromatogram depicting fractionation by setting slope [Abs(mAU)/Time (min)] values for collecting fractions from UNICORN start with Frac30 is shown below.

5 Create a method
5.4 Fraction collection
5.4.4 Fractionation with Frac30



- Note:**
- Make sure to standardize and set the optimized slope values and fraction volume in order to collect the peak of interest in single collection tube.
 - It is recommended to set lower slope values for collection smaller peaks and larger values for collecting higher peaks so that the peak of interest is collected in a single collection tube. The suitable slope values could be derived from UV curve with the UNICORN start differentiate operation available in the **Evaluation** module.

5.5 Import and export methods

Introduction

UNICORN start methods are stored internally in the UNICORN start database. It is however possible to export entire methods or individual phases to a zip file on the local computer so that they can be imported again later into the same database installation, or imported into another database installation.

Alternatively, methods or phases can be exported as plain text files or Excel files, which may be useful for documentation purposes.

Export a method to another UNICORN start database

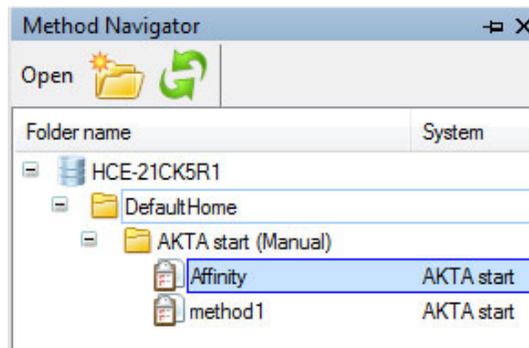
Follow these steps to export a method into a UNICORN start database.

Step	Action
------	--------

- | | |
|---|--|
| 1 | In the Method Navigator pane, select the method to be exported. |
|---|--|

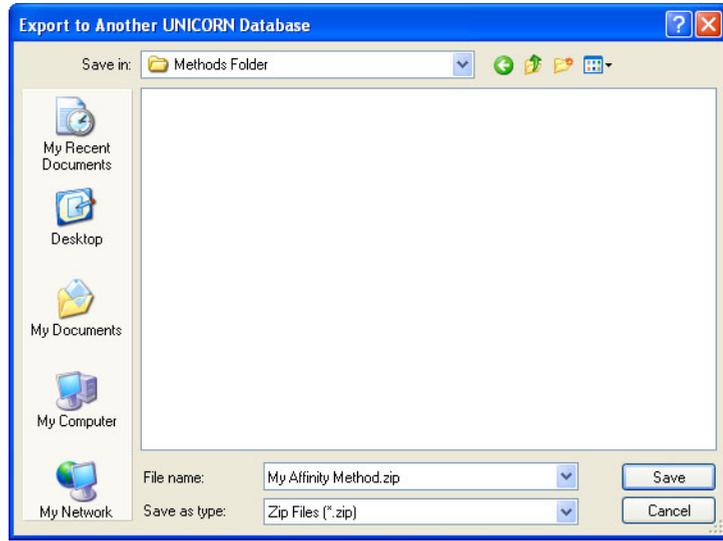
Note:

*Several methods in the same folder can be selected and exported at the same time. Also, several results can be selected simultaneously by using the **Shift** or **Ctrl** key.*



Step	Action
------	--------

- | | |
|---|---|
| 2 | Choose File:Export:to UNICORN:Export Method to UNICORN .
<i>Result:</i> The Export to Another UNICORN Database dialog opens. |
|---|---|



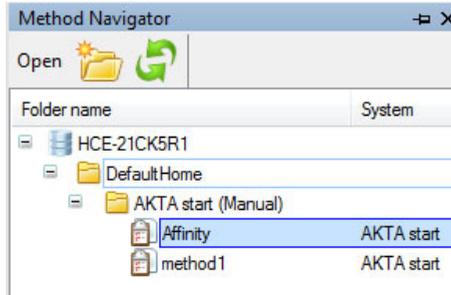
- | | |
|---|--|
| 3 | Choose a file name and location and click the Save button to save the zip file. |
|---|--|

Export a method to a plain text file or to Excel

Follow these steps to export a method as a plain text file, .asc or to Excel.

Step	Action
------	--------

- 1 In the **Method Navigator** pane, select the method to be exported. It is only possible to export one method at a time to an external file.



- 2 Choose **File:Export:Export Method Externally**.
Result: The **Export Externally** dialog opens.



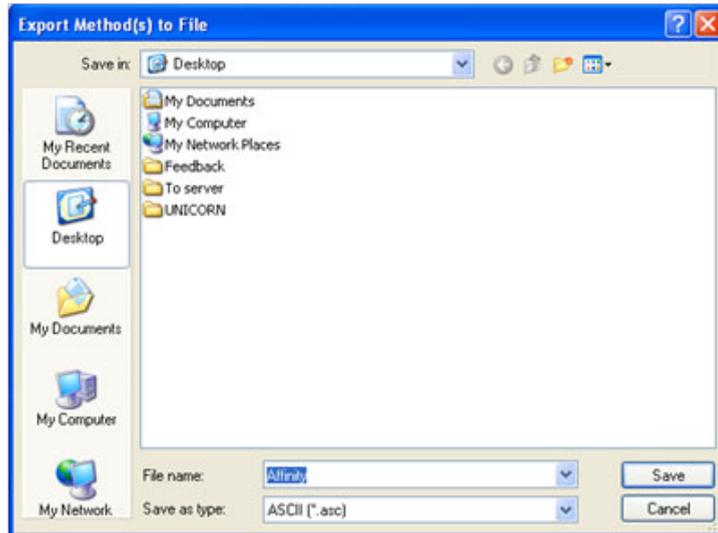
Only **Variable list** can be exported externally.

- 3 Click the **Save As** button to save the exported variable list.

Step	Action
------	--------

- | | |
|---|---|
| 4 | To save the text file with the selected information included click the Save As button. |
|---|---|

Result: The **Export Method(s) to File** dialog opens.



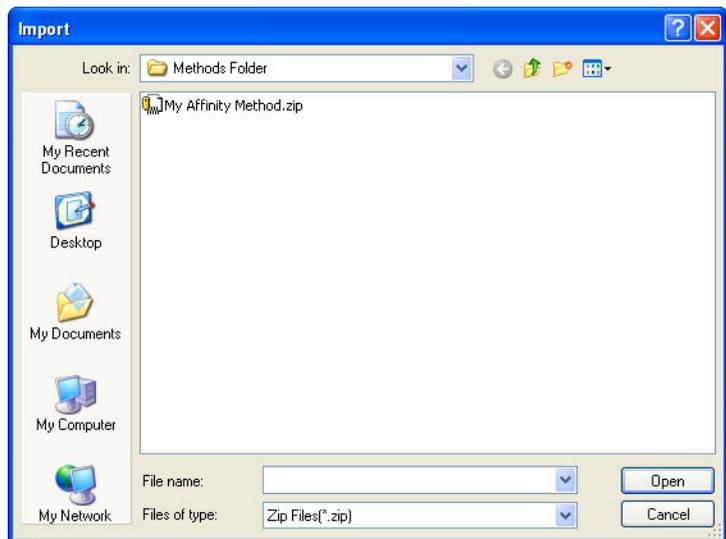
- | | |
|---|--|
| 5 | Choose whether to save to an ASCII file or to an Excel file from the Save as type drop-down list. |
| 6 | Choose a file name and location and click the Save button to save the zip file. |

Import a method into UNICORN start

Methods that have previously been exported as zip files can be imported back into UNICORN start. Plain text files or Excel files can be imported if they contain all the information that UNICORN start needs to recreate the method. Follow these steps to import a method.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Select File:Import:Import Method .
<i>Result:</i> The Import dialog opens. |
|---|---|



- | | |
|---|--|
| 2 | Browse to the required zip file in the Import dialog. |
|---|--|

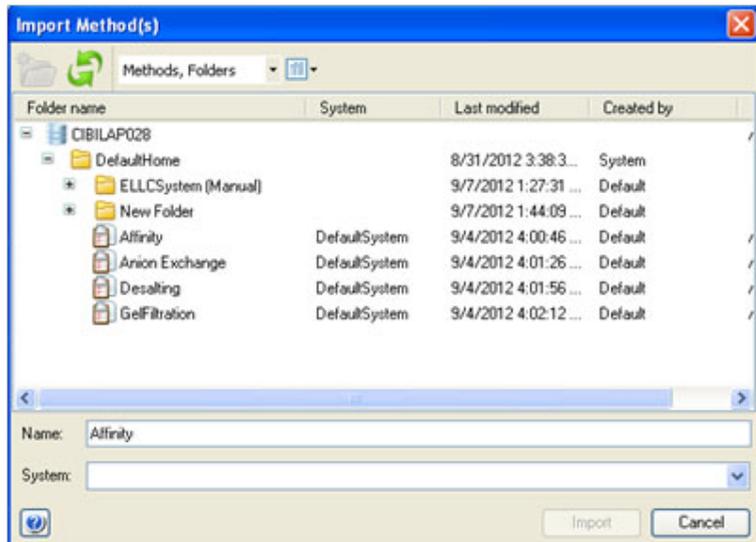
Step **Action**

- 3 Select the file and click the **Open** button, or double click on the file name to open it.

Note:

*Several methods in the same folder can be selected and imported at the same time. Several results can be selected simultaneously by using the **Shift** or **Ctrl** key.*

Result: The **Import Method(s)** dialog opens.



- 4 Browse to the required folder in the database and type in a new **Name** if necessary.

- 5 Click the **Import** button to import the method.

Result: The imported method is opened in **Method Editor**.

To import several methods, repeat the procedure in the **Import Method** dialog for each method.

5.6 Column handling

Introduction

This section describes how to update predefined column types provided by GE Healthcare. For details on how to add, edit, delete, import, export and print specific column types refer to *UNICORN start Online Help*.

Updated lists of predefined column types are provided by GE Healthcare. When a new list is imported, it will replace all the predefined column types in the database with the updated column types. Only predefined column types will be replaced, user defined column types will remain in the database.

Tip: For column list updates please visit www.gelifesciences.com/unicornstart.

Import column list into the database

Follow these steps to import a new column list into the database.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Click the column handling icon in the Toolbar. |
|---|--|



- | | |
|---|---|
| 2 | In the Column Type Parameters tab, in the Column Handling dialog, click the Import button. |
|---|---|

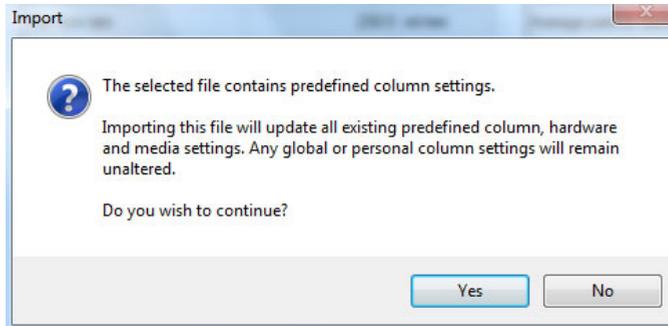


Result: The **Import** dialog opens.

- | | |
|---|---|
| 3 | Locate the zip file with the column list to be imported and click Open . |
|---|---|

Step **Action**

Result: The **Import** confirmation dialog opens, explaining what happens when the zip file is imported.



4 Click **Yes**.

Result: The new list of predefined column types is imported into the database.

6 View and present the results

In this chapter

This chapter describes how to view results, edit and optimize the representation of the results. It contains the following sections:

Section	See page
6.1 Introduction	118
6.2 Evaluation module interface	119
6.3 View and present the results	126
6.4 Optimize the presentation of chromatograms	138

6.1 Introduction

A result is automatically generated at the end of a run and contains a complete record of the chromatography run, including system settings, monitored data and run log. The complete result is saved in the UNICORN start database.

UNICORN start **Evaluation** module is used to view and analyze the result data and optimize the result presentation, for example, by:

- Integrating peaks.
- Measuring peak properties such as area and height.
- Comparing curves and chromatograms from different runs.

When a satisfactory evaluation is obtained, a report may be compiled. The report can be anything from a simple print of a chromatogram, showing selected curves, to a comprehensive report containing chromatograms from several results.

6.2 Evaluation module interface

In this section

This section gives an overview of the **Evaluation** module. It contains the following sub-sections:

Section	See page
6.2.1 User interface	120
6.2.2 Result navigator	123
6.2.3 Chromatogram	125

6 View and present the results

6.2 Evaluation module interface

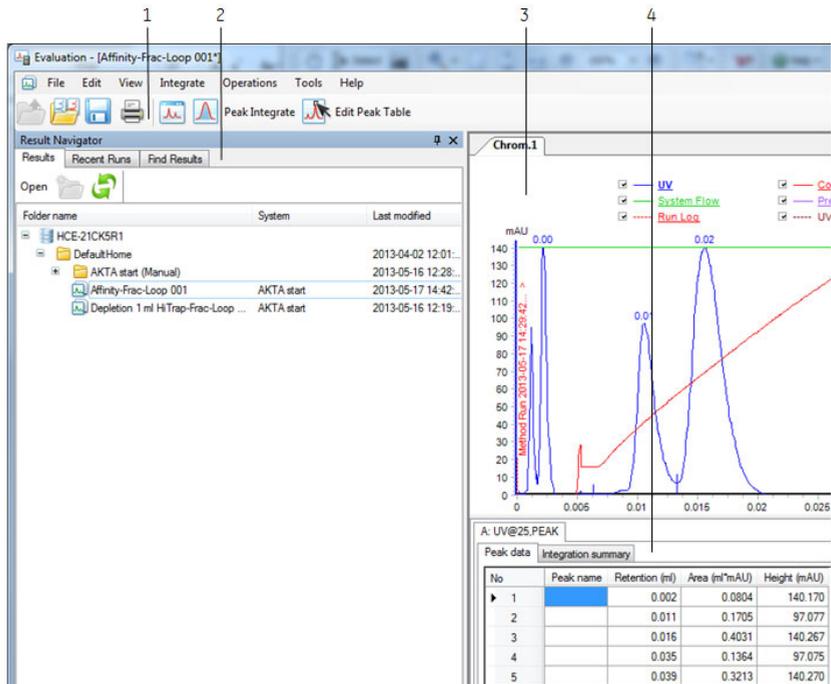
6.2.1 User interface

6.2.1 User interface

The interface consists of a pane where the current result is displayed and, the **Result Navigator**. Both panes are described below.

Evaluation module interface

Different parts of the **Evaluation** module are illustrated below:



Pane	Function
1	Toolbar
2	Result navigator
3	Chromatogram
4	Peak summary table

Toolbar icons in the Evaluation module

The table below describes the toolbar icons in the **Evaluation** module.

Icon	Function
	<p>Opens the Result Navigator.</p> <p>The Result Navigator is described below.</p>
	<p>Opens the Add To Compare dialog.</p> <p>You may use the dialog to search for curves to compare in specified folders, results or chromatograms. This dialog is described in <i>Section 7.4 Compare different runs, on page 203</i>.</p> <p>Note: <i>This dialog can also be opened from the File menu.</i></p>
	<p>Saves the current result.</p> <p>This icon is used to save the current result that has been edited and not saved yet.</p> <p>Note: <i>This function can also be selected in the File menu.</i></p> <p>Note: <i>To save the result with a different name, select File: Save As. The Save As dialog opens, and the result can be named and saved in the selected folder.</i></p>
	<p>Opens the Print Chromatograms dialog.</p> <p>This dialog is used for printing the chromatograms on a selected printer. The chromatogram can also be previewed before printing. This dialog is described in <i>Section 6.4.4 Print chromatograms and peak data, on page 152</i>.</p> <p>Note: <i>This dialog can also be opened from the File menu.</i></p>
	<p>Saves the report as PDF.</p> <p>This opens the Save As dialog where the result can be named and saved in the selected folder.</p> <p>Note: <i>This dialog can also be opened from the File menu.</i></p>

6 View and present the results

6.2 Evaluation module interface

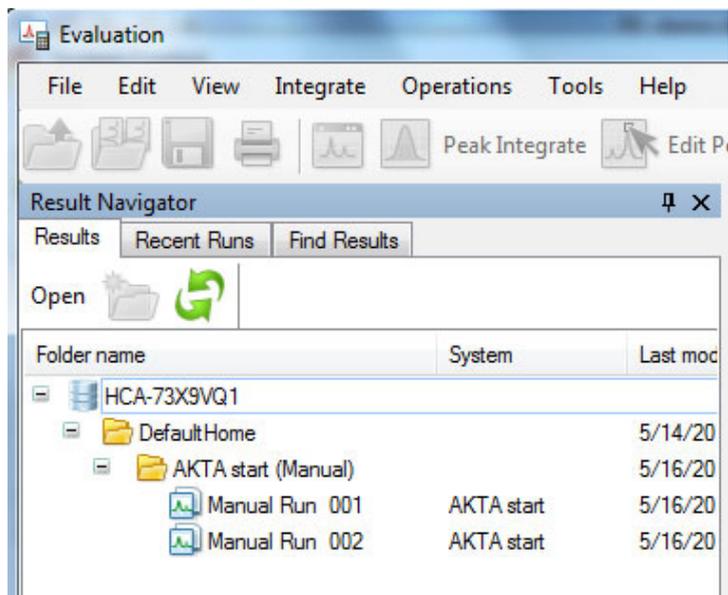
6.2.1 User interface

Icon	Function
	<p>Performs peak integration of the selected UV curve. This dialog is described in detail in <i>Section 7.1 Peak integration, on page 156</i>.</p> <p>Note: <i>This dialog can also be opened from the Integrate menu.</i></p>
	<p>Opens the Edit Peak Table dialog.</p> <p>This wizard can be used to edit peak tables.</p> <p>Note: <i>This dialog can also be opened from the File menu.</i></p>

6.2.2 Result navigator

Illustration of *Result Navigator* pane

The **Result Navigator** shows all the folders and individual results.



Result Navigator auto hide

Result Navigator may be displayed in the left portion of the **Evaluation** module, or the auto hide function can be selected by clicking the pin symbol in the top right-hand corner of the pane.

Pin di- rection	Function
	Auto Hide is off. Click the pin symbol to turn the function on.

6 View and present the results

6.2 Evaluation module interface

6.2.2 Result navigator

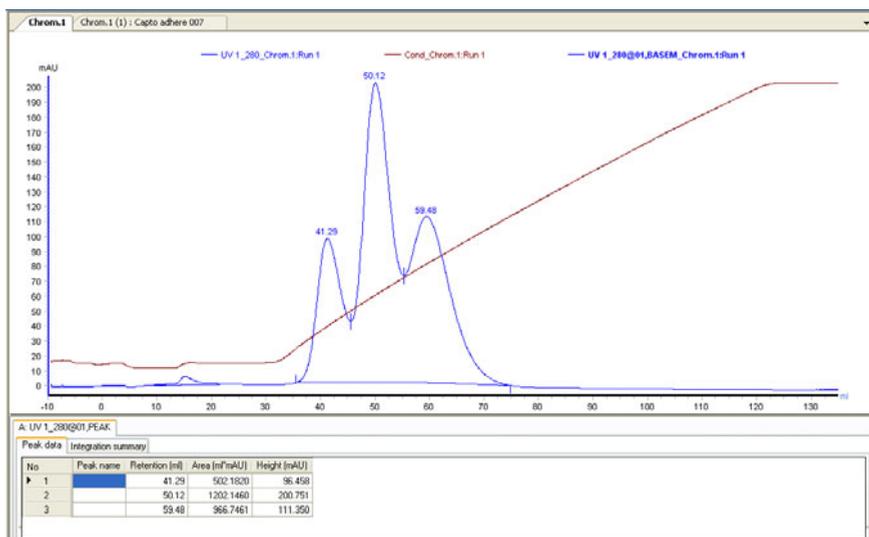
Pin direction	Function
	Auto Hide is on. Click the pin symbol to turn the function off.

If auto hide is on, the **Result Navigator** opens automatically when the mouse pointer is placed over its tab. It remains open as long as the mouse pointer remains over the pane. The pane closes automatically when the pointer is moved outside the pane.

6.2.3 Chromatogram

The Chromatogram pane

The **Chromatogram** pane is illustrated below.



The current result contains two chromatograms. The chromatograms are displayed by clicking the corresponding tab. A **Peak Table** is displayed below the chromatogram. **Peak Table** are described in detail in *Section 7.1.3 Display peak data, on page 161*.

Docking Chromatogram panes

If a result contains several chromatograms, each chromatogram is displayed in its own pane. The chromatograms can be stacked on top of each other and selected by clicking the corresponding tab. They can also be arranged in various positions using a docking function. The arrangement of chromatograms is described in *Section 7.4.1 Open and compare chromatograms, on page 204*.

6 View and present the results

6.3 View and present the results

6.3 View and present the results

In this section

This section describes how to present the chromatograms and curves of the result file, and how to create and print reports. It contains the following subsections:

Section	See page
6.3.1 Open and view results	127
6.3.2 Locate results	133

6.3.1 Open and view results

Introduction

A result file is opened in the **Evaluation** module where all of its content can be analyzed and reports created. The **Evaluation** module user interface and toolbar icons are described in *Section 6.2.1 User interface, on page 120*.

The result files and their folder locations are shown in the **Result Navigator**. There are different ways to locate a specific result, *Section 6.2.2 Result navigator, on page 123* describes this. In this section a preview function to identify a certain result and locate specific curves is described, it is called **Quick View**.

This section also describes how to highlight curves in a chromatogram, read curve values using a marker and save curve data as a **Snapshot**.

How to open specific chromatograms and curves is described in *Section 7.4.1 Open and compare chromatograms, on page 204* and *Section 7.4.2 Open and compare curves, on page 207*.

Open a result in the **Evaluation** module

There are four ways to open a result from the **Result Navigator**:

- Select a result and press the **Enter** key.
- Double-click a result.
- Right-click a result and select **Open** from the shortcut menu.
- Select a result and click the **Open** toolbar icon in the **Result Navigator**.



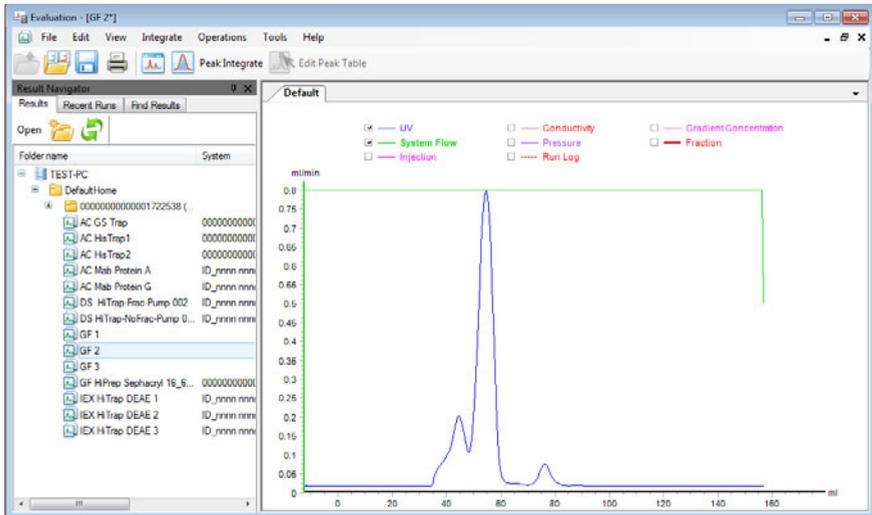
Only one result at a time may be opened this way. If a new result is opened, the previous result closes automatically. However, several chromatograms from different results can be opened simultaneously. This is described in *Section 7.4.1 Open and compare chromatograms, on page 204*.

6 View and present the results

6.3 View and present the results

6.3.1 Open and view results

Illustration of a result opened in the *Evaluation* module



A result may contain several chromatograms and corresponding peak tables. Each chromatogram can be shown by clicking the corresponding tab. The chromatograms can also be displayed simultaneously by selecting **View:Tile All Chromatograms**.

Highlight or select a curve

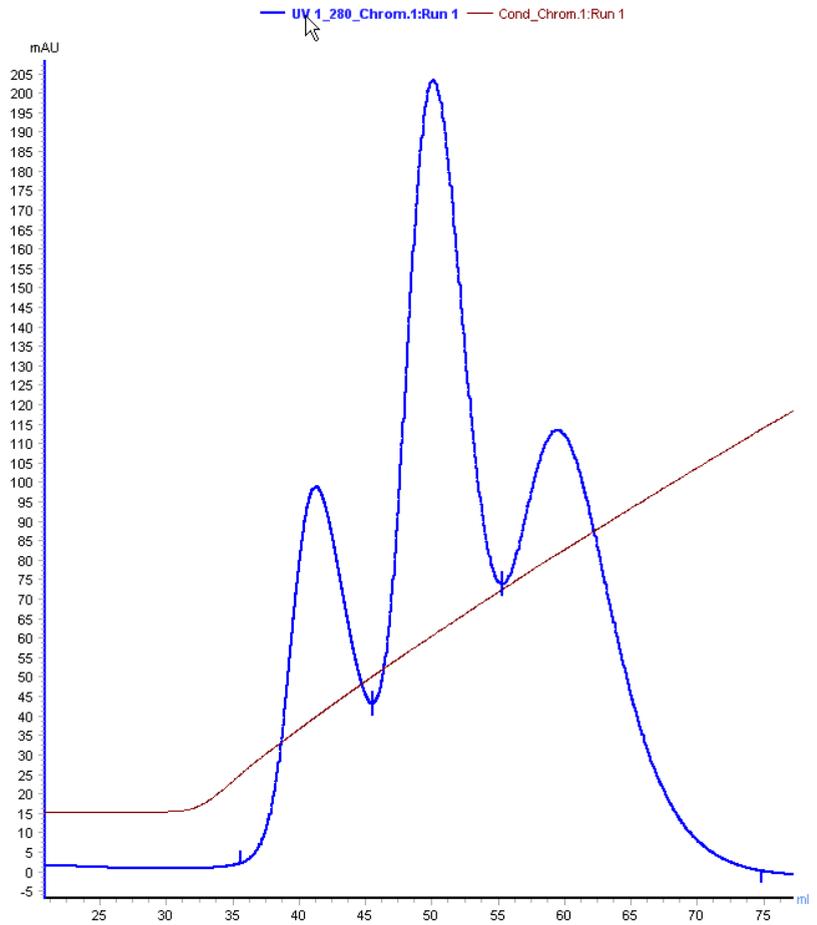
An individual curve in the chromatogram can be selected or highlighted. The table below describes the differences:

If you...	Then...
hold the mouse pointer over a curve segment	a pop-up box displays the curve name.
hold the mouse pointer over a curve name	the curve and the short line segment in front of the curve name become bold.
click a curve segment or a curve name	the Y-axis shows the values for this specific curve.

6 View and present the results

6.3 View and present the results

6.3.1 Open and view results



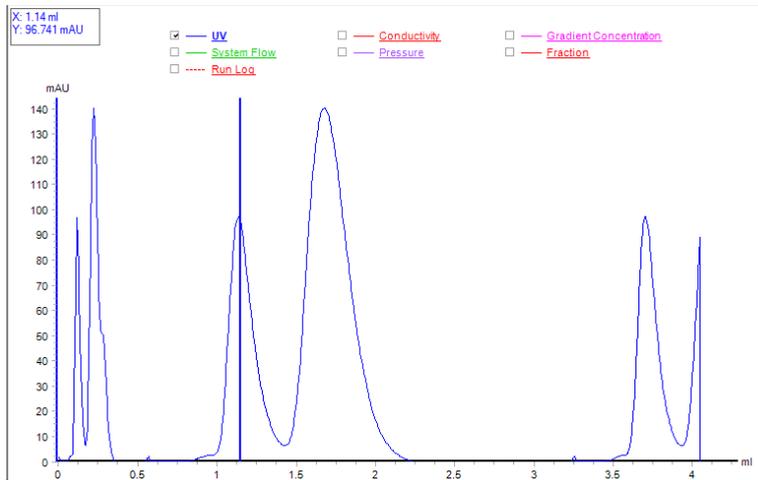
6 View and present the results

6.3 View and present the results

6.3.1 Open and view results

Insert a vertical marker

The vertical marker is used to measure the values for a specific curve position. Right-click in the chromatogram and choose **Vertical marker**. Move the marker along the X-axis and read the X-axis and Y-axis values of the selected curve in the box in the top left corner of the chromatogram.



Note: The marker measures the curve that currently is selected if several curves are displayed in the chromatogram. The marker has the same color as the selected curve.

Set marker reference

The vertical marker can be used for more measurements than just the readings from a specific curve position. Follow these steps to use the marker to determine **Delta Peak** and **Mean** Y-axis values.

Step	Action
------	--------

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">Position the marker to where the measurement should begin.Right-click and select Set vertical marker reference point. |
|---|---|

Result: The reference point is set to the position shown in the box in the top left corner of the chromatogram.

6 View and present the results

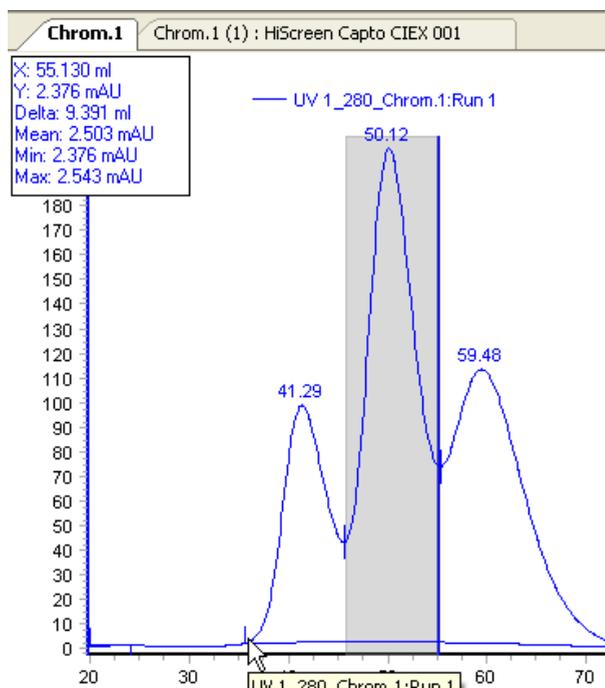
6.3 View and present the results

6.3.1 Open and view results

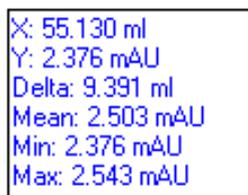
Step	Action
------	--------

2	Drag the marker to the point where the measurement should end.
---	--

Result: The measured area is colored as illustrated below:



3	Read e.g. the Volume , Delta and Mean values from the box:
---	---



Note: If the X-axis has been set to volume base, X will hear equal to the volume for the selected area.

6 View and present the results

6.3 View and present the results

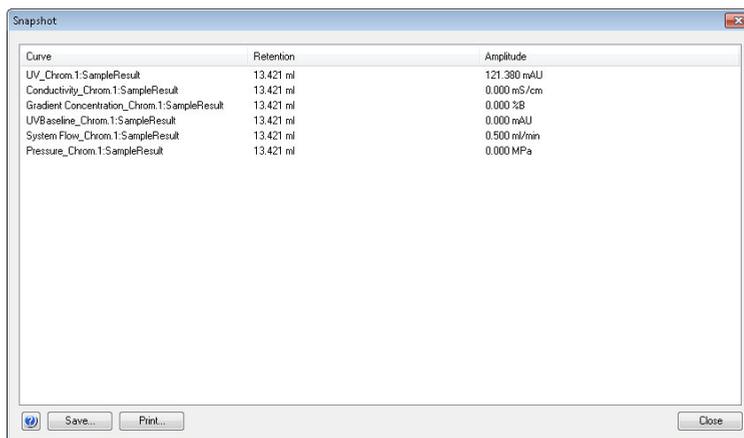
6.3.1 Open and view results

Snapshots

It is possible to take a snapshot of all the curve values at the marker position. Follow these steps to take a snapshot:

Step	Action
------	--------

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">• Position the marker to where the snapshot should be taken.• Right-click and select Snapshot from the shortcut menu. |
|---|---|



- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Click the Save button to save the Snapshot.• Alternatively, click the Print button to print the Snapshot. The Print dialog opens and the Snapshot can be printed. |
| 3 | Click the Close button to close the Snapshot dialog. |

Note: *The snapshot only records the values of the curves that are displayed in the current view.*

6.3.2 Locate results

Introduction

In the **Method Editor** and **Evaluation** modules of UNICORN start there are navigator panes that can be used to locate and open methods and result files, and to arrange files in folders.

The **Evaluation** module contains a **Result Navigator** pane that is of the same type as the navigator pane in the **Method Editor** module, but it also contains a pane for **Recent Runs** and a specific search pane, **Find Results**. If desired, the **Result Navigator** can also display methods.

Show the navigator pane

To open and display the navigator panes:

- Choose the **Result Navigator** menu command in the **View** menu.

Or,

-



Click the (open result navigator) toolbar icon.

Note: As described in the general overview of the **Evaluation** module in Section 6.2 Evaluation module interface, on page 119, Auto Hide can be selected for the navigator panes.

Navigator toolbar

The table below shows the navigator toolbar icons:

Icon	Function
	<p>Opens the selected result.</p> <p>Note: Selected items can also be opened by selecting the File:Open menu command or by double-clicking the file in the navigator pane. The Open command from the right-click shortcut menu can also be used to open selected chromatograms, curves or peak tables.</p>

6 View and present the results

6.3 View and present the results

6.3.2 Locate results

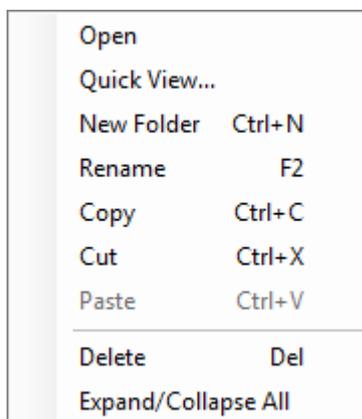
Icon	Function
	<p>Creates a new folder in the folder that is currently selected.</p> <p>Note: <i>This function can also be accessed by selecting the File:New Folder menu command or selecting the New Folder command from the right-click shortcut menu.</i></p>
	<p>Performs a Refresh in the navigator pane to update all items to the current status.</p> <p>Note: <i>This function can also be accessed from the View menu. However, note that the Refresh menu command updates both the items in the navigator pane and items open in the module. The navigator toolbar icon updates only the navigator pane.</i></p>
	<p>Opens the View Details drop-down list where the following optional information columns may be selected for display in the navigator pane:</p> <ul style="list-style-type: none">• System• Created by• Last modified

Expand and collapse folders

When a folder is selected in the **Result Navigator** pane, the subfolders and results are displayed.

If the folder structure is expanded to lower levels, collapse all folders below a selected top folder, to simplify browsing.

- Right-click a folder and choose the **Expand/Collapse All** shortcut.



Result: The folder structure is collapsed to the selected folder level.

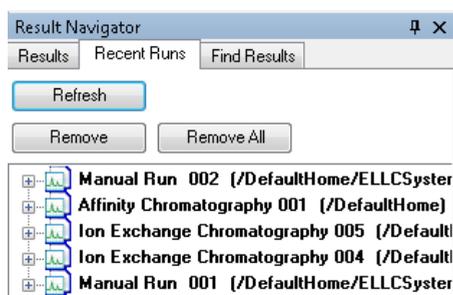
The Recent Runs pane

The **Recent Runs** pane shows all the available recorded recent runs based on the selected preferences. Follow the instructions below to use **Recent Runs** to locate and open a result.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the Recent Runs tab in the Result Navigator . |
|---|---|

Result: The **Recent Runs** pane opens.



- | | |
|---|---|
| 2 | If needed, click the Refresh button. |
|---|---|

Result: The **Recent Runs** list is updated with all runs that were performed since the **Result Navigator** was opened the last time.

6 View and present the results

6.3 View and present the results

6.3.2 Locate results

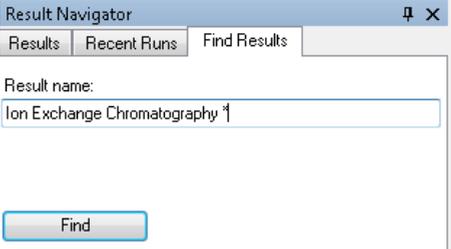
Step	Action
3	Locate and double-click the desired result. <i>Result:</i> The result opens in the Evaluation module.
Note:	<i>Click the + signs to view or select individual chromatograms from the results. Individual results can be selected and removed from the list by clicking the Remove button. The Remove all button clears the whole list. The Remove and Remove all buttons only clear the list, the results are not deleted.</i>

Search for results using Find Results

The **Find Results** function in the **Result Navigator** is used to locate results in the available folders.

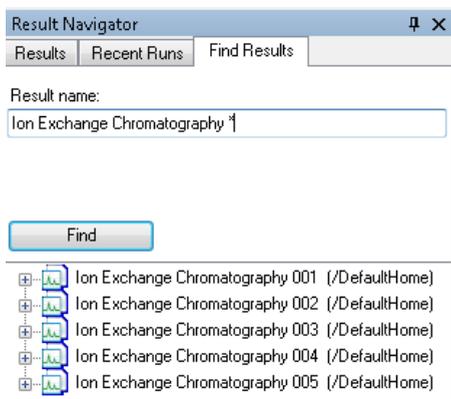
Note: *There is also a common **Find** function which is available in the **Edit** menu of all modules except the **Administration** module.*

The table below describes how to use the **Find Results** function to locate and open a result.

Step	Action
1	Click the Find Results tab. <i>Result:</i> The Find Results pane opens.
	
2	Type a result name or part of a result name in the Result name text box. Note: <i>The standard wildcard character * can be used to represent a number of characters before or after the partial result name.</i>

Step	Action
------	--------

3	<i>Result:</i> The located results are listed.
---	--



4	Double-click the desired result or chromatogram.
---	--

Result: The file or chromatogram opens in the **Evaluation** module.

Close the navigator pane

To close the navigator pane:

- Click the small cross in the top right-hand corner of the navigator.

Result: The navigator closes.

6.4 Optimize the presentation of chromatograms

In this section

This section describes some of the ways to optimize the presentation of a chromatogram. It contains the following subsections:

Section	See page
6.4.1 Customize the chromatogram layout	139
6.4.2 Edit curve presentation	143
6.4.3 Change the axes	148
6.4.4 Print chromatograms and peak data	152
6.4.5 Save report as PDF	154

6.4.1 Customize the chromatogram layout

Introduction

The **Customize** dialog box is used to change the chromatogram presentation. The main features of the **Customize** dialog box regarding chromatograms are described in the subsequent sections. Features regarding peak tables are described in *Section 7.1 Peak integration, on page 156*.

Instruction

Follow the instructions below to make changes in the **Customize** dialog box:

Step	Action
------	--------

- | | |
|---|---|
| 1 | Open a result. |
| 2 | <ul style="list-style-type: none">• Right-click the chromatogram and select Customize.or• Choose Tools:Customize. |

Result: The **Customize** dialog box is displayed.

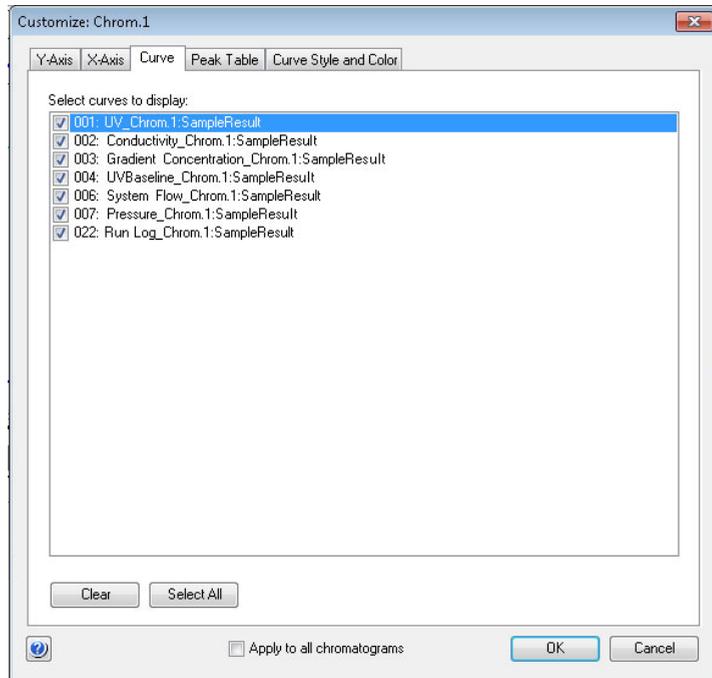
6 View and present the results

6.4 Optimize the presentation of chromatograms

6.4.1 Customize the chromatogram layout

Step	Action
------	--------

- | | |
|---|--|
| 3 | <ul style="list-style-type: none">Carry out the changes on the different tabs to get the desired layout for header, curves and peak table. For more info, see below. |
|---|--|



- Select **Apply to all chromatograms** if you want to apply the changes made in the **Customize:** dialog to all open chromatograms.

- | | |
|---|---------------------------------------|
| 4 | Click OK to apply the changes. |
|---|---------------------------------------|

Tabs in the Customize dialog

The table below lists the tabs of the **Customize** dialog and outlines the editing functions of each tab.

Tab	Use this tab to...
Y-Axis	Determine how the Y-axis is displayed. This is described in detail in Section 6.4.3 Change the axes, on page 148 .

Tab	Use this tab to...
X-Axis	Determine how the X-axis is displayed. This is described in detail in Section 6.4.3 Change the axes, on page 148 .
Curve	Select the curves to display in the chromatogram.
Peak Table	Select the Peak Table settings. This is described in detail in Section 7.1.3 Display peak data, on page 161 .
Curve Style and Color	Determine how the curves are displayed. This is described in detail in Section 6.4.2 Edit curve presentation, on page 143 .

Note: The selected settings in all tabs can be applied to all open chromatograms.

Layout options in the right-click menu

You can also change the chromatogram layout by choosing some menu items from the right-click shortcut menu. The table below describes the layout options available on this menu:

Choose...	when you want to...
Grid	Add a grid to the background of the chromatogram.
Filter Curves	<p>Add a list of all available curves to the chromatogram, with checkboxes to select the curves to display:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> UV 1 280 Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> UV 2 0 Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> UV 3 0 Chrom.1:HiScreen Capto CIEX 001 <input checked="" type="checkbox"/> Cond Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> % Cond Chrom.1:HiScreen Capto CIEX 001 <input checked="" type="checkbox"/> Conc B Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> pH Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> System flow Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> System pressure Chrom.1:HiScreen Capto CIEX 001 <input type="checkbox"/> Cond temp Chrom.1:HiScreen Capto CIEX 001 <p>Note: Click the arrow buttons to show additional curves that do not fit in the available space.</p>
Select Active Curve Only	Show only the active curve in the chromatogram. (this option is available only when Filter Curves is selected)

6 View and present the results

6.4 Optimize the presentation of chromatograms

6.4.1 Customize the chromatogram layout

Choose...	when you want to...
Select All Curves	Show all curves in the chromatogram. (this option is available only when Filter Curves is selected)
Legend	Show the list of all displayed curve names over the curve area of the chromatogram.
<ul style="list-style-type: none">• Time or• Volume or• Column volume	Select the unit of the X-axis.

6.4.2 Edit curve presentation

Select curves to display

The **Curve** tab of the **Customize** dialog contains a list of all the curves included in the chromatogram. Select the curves to be displayed in the chromatogram, and click the **OK** button.

Curve style and color settings

All curves within a chromatogram are represented by a default color and line style. Curves that are imported into the chromatogram or newly created curves are automatically assigned a color and line style. The settings are available in the **Curve Style and Color** tab. The tab also contains settings for peak labels, fraction marks and run log texts.

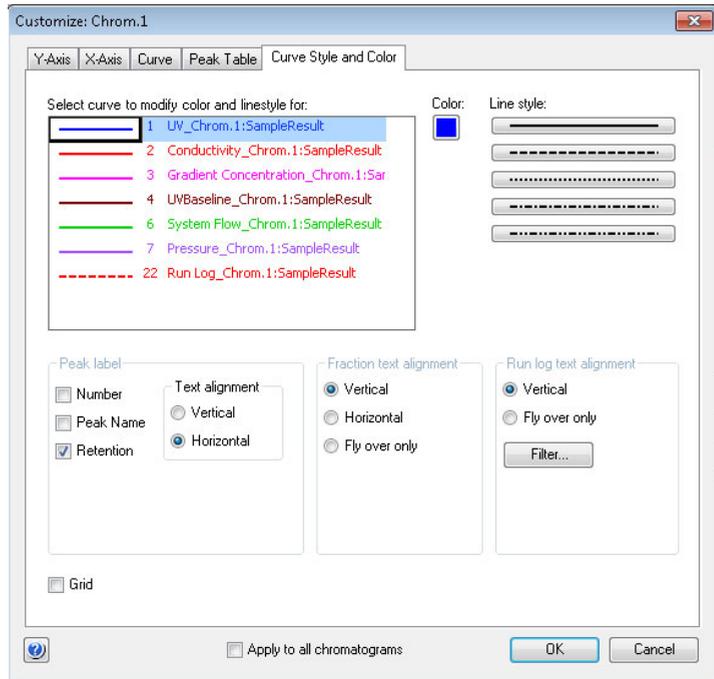
Change the color and style of a curve

The table below describes how to change the color and style of a curve:

Step	Action
1	Open the Customize dialog.
2	Click the Curve Style and Color tab.

Step **Action**

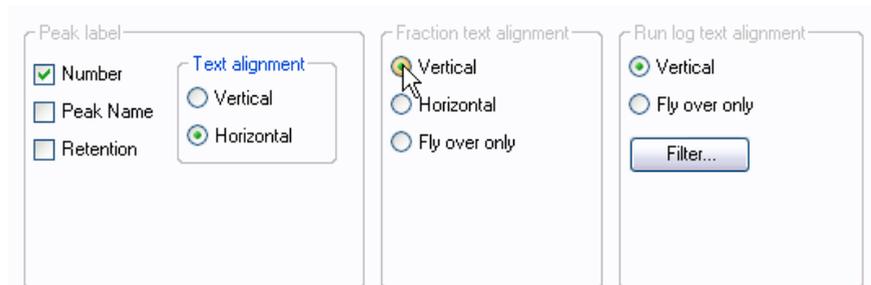
- 3
- Select the curve to change, from the list.
 - Click the **Color** button to open the **Color** dialog and choose a color from the palette.
 - Click a button to choose a **Line style**.



- Repeat step 3 to modify other curves.
- 4 Click the **OK** button to apply the changes.

Align Fraction and Run log texts

Both **Fraction** and **Run log** text can be set to be displayed either when the mouse pointer is positioned over a fraction mark (**Fly over only**) or at all times. The **Fraction** text can be aligned both vertically and horizontally. Default setting for both text types is **Vertical**. The **Vertical** selection for **Run log** text is the setting to show the text at all times.



Filter Run log information

Follow the instructions below to select the run log entry and feedback types to show in the chromatogram:

- | Step | Action |
|------|--|
| 1 | Open the Customize dialog. |
| 2 | Click the Curve Style and Color tab. |
| 3 | Click the Filter button in the Run log text alignment field. |



Result: The **Filter Run log** dialog opens.

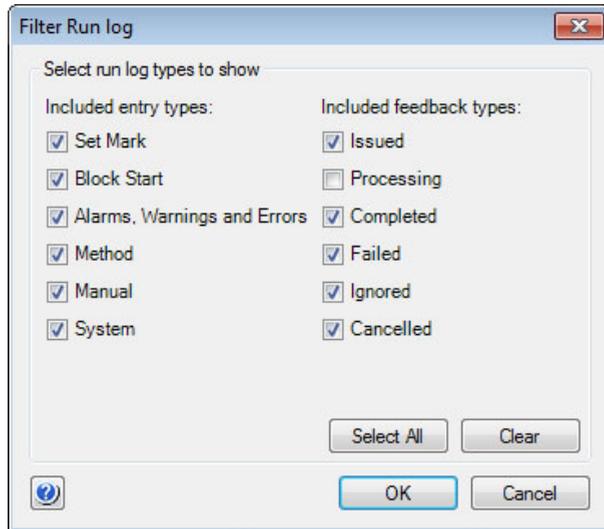
6 View and present the results

6.4 Optimize the presentation of chromatograms

6.4.2 Edit curve presentation

Step	Action
------	--------

- | | |
|---|--|
| 4 | <ul style="list-style-type: none">• Select the entry types to show in the chromatogram.• Select the feedback types to include in the run log entries. |
|---|--|



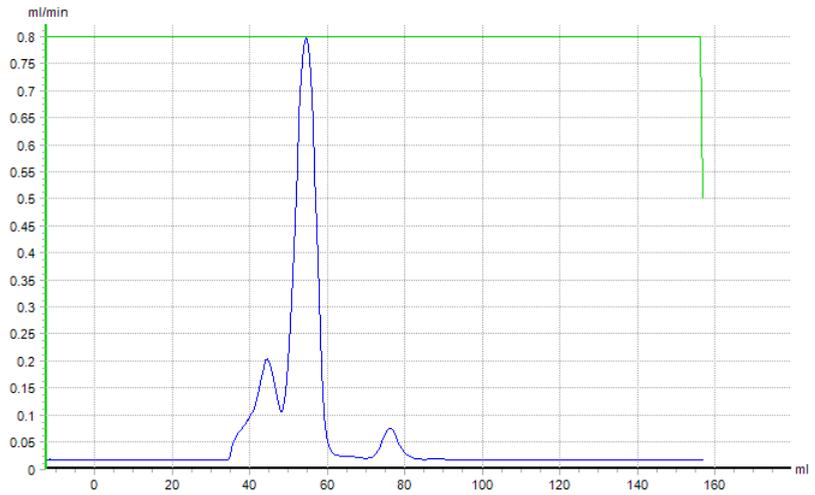
- Click the **OK** button.

Result: The **Filter Run log** dialog closes.

- | | |
|---|---|
| 5 | Click the OK button in the Customize dialog to apply the changes. |
|---|---|

Add a grid

It is possible to add a grid to the background of the chromatogram by selecting the **Grid** checkbox.



Note: This can also be accessed by right-clicking in the chromatogram and selecting **Grid** from the shortcut menu.

6.4.3 Change the axes

Introduction

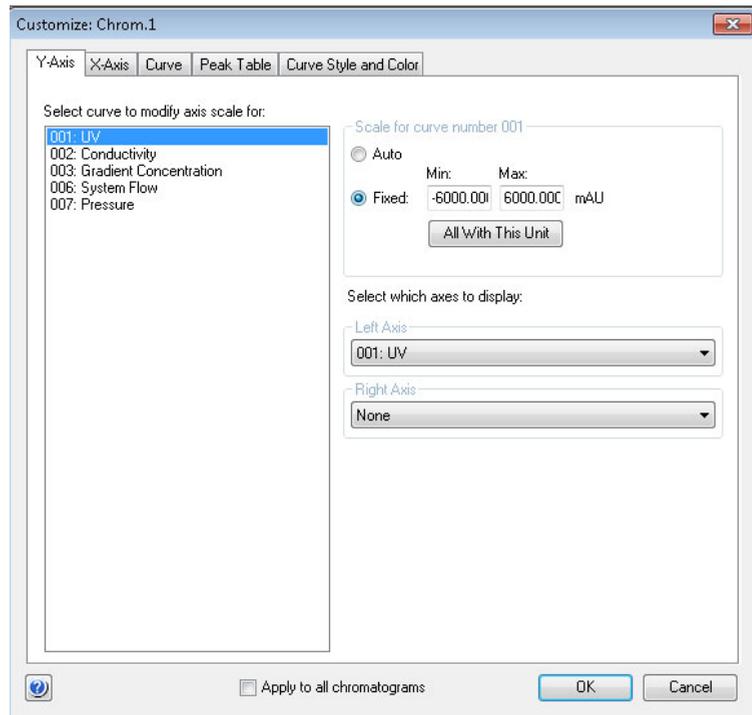
This section describes how to change the chromatogram axes. These changes are made in the **Y-Axis** and **X-Axis** tabs of the **Customize** dialog.

Change and fix the Y-axis

Follow the instructions below to change and fix the scale of the curves in the chromatogram Y-axis:

Step	Action
------	--------

- | | |
|---|--|
| 1 | Click the Y-Axis tab. |
| 2 | <ul style="list-style-type: none">• Select the appropriate curve from the list.• Select the Fixed option in the Scale for curve number field. |



Step	Action
3	<ul style="list-style-type: none">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. <p>Note: <i>The values will only be applied to existing curves. They will not be applied to new curves created after this function was last used.</i></p>
4	Click the OK button to apply the changes.

Select Y-axes to display

Follow the instructions below to select the Y-axes to display in the chromatogram.

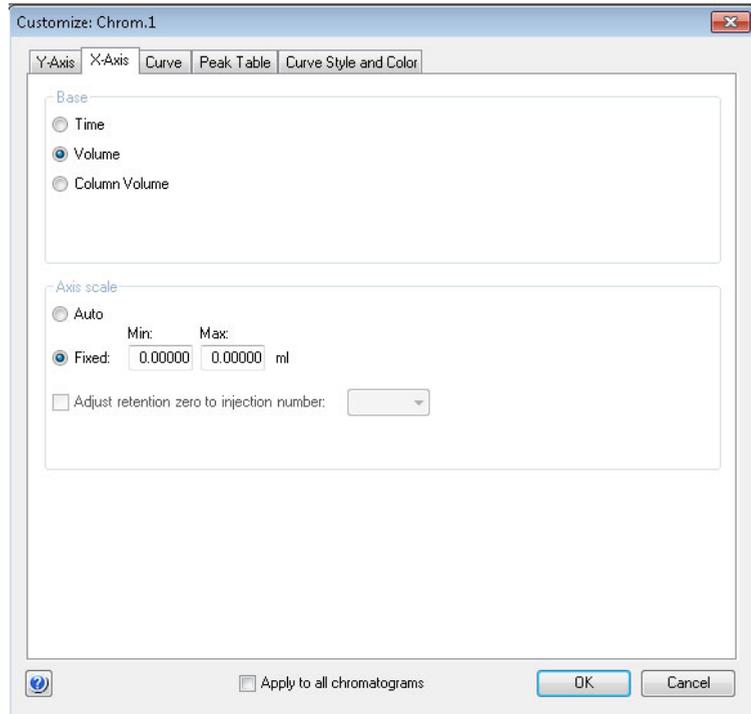
Step	Action
1	Click the Y-Axis tab.
2	<ul style="list-style-type: none">Select a curve for the left Y-axis in the Left Axis drop-down list.To add a second Y-axis, proceed with step 3. If not, jump to step 4.
3	Select a second curve from the Right Axis drop-down list.
4	Click the OK button to apply the changes.

Change and fix the X-axis

Follow the instructions below to change and fix the scale of the X-axis in the **Customize** dialog:

Step	Action
------	--------

- | | |
|---|------------------------------|
| 1 | Click the X-Axis tab. |
|---|------------------------------|



- | | |
|---|---|
| 2 | Select the appropriate option in the Base field: |
|---|---|

- **Time**
- **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 of the displayed curves.*

Step	Action
3	<ul style="list-style-type: none">• Click the Fixed option in the Axis scale field to set the axis limits manually.• Type the desired minimum and maximum values.• If desired, select the Adjust retention zero to injection number checkbox and choose an injection number from the drop-down list. <p>Note: <i>This sets the time/volume to zero at the selected injection mark. The time and volume before selected injection will become negative values.</i></p>
4	Click the OK button to apply the changes.

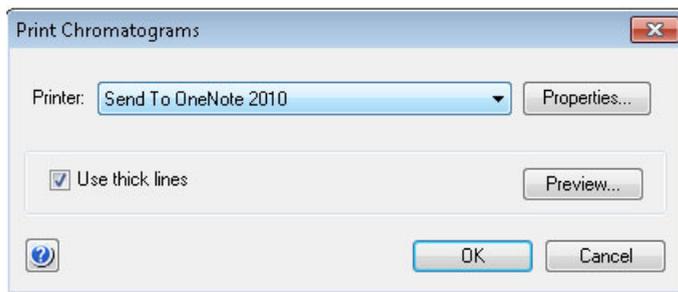
6.4.4 Print chromatograms and peak data

Introduction

This section describes how to print the chromatograms that are open in the *Evaluation* module.

The Print Chromatograms dialog box

The *Print Chromatograms* dialog is illustrated below.



Instructions

Follow the instructions below to print active chromatograms.

Step	Action
------	--------

1	Open all chromatograms to be printed.
---	---------------------------------------

- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Select <i>File:Print</i>.or• Click the |
|---|--|



toolbar icon.

Result: The *Print Chromatograms* dialog opens.

- | Step | Action |
|------|--|
| 3 | <ul style="list-style-type: none">Click the OK button to open the Print dialog and proceed with step 7.
orProceed with step 4 to preview and edit the layout. |

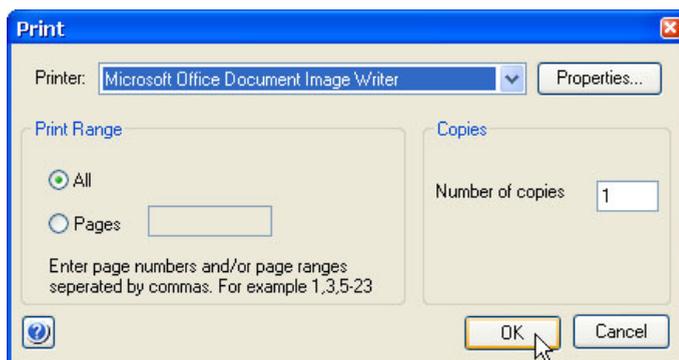
Note:

Click and drag the chromatogram tabs to change the order of the chromatograms on print.

- | | |
|---|--|
| 4 | Click the Preview button.
<i>Result:</i> The Customize Report window opens. |
|---|--|

- | | |
|---|--|
| 5 | <ul style="list-style-type: none">Select File:Print.or Click the  toolbar icon. |
|---|--|

Result: The **Print** dialog opens.



Note:

It is possible to print directly from the **Customize Report** window or click the **Exit** button to return to the **Print Chromatogram** dialog.

- | | |
|---|---|
| 6 | <ul style="list-style-type: none">Select the print range and number of copies.If necessary, click the Properties button to open the printer properties dialog where the settings for the printer can be changed.Click the OK button to print the chromatograms. |
|---|---|

- 6 View and present the results
- 6.4 Optimize the presentation of chromatograms
- 6.4.5 Save report as PDF

6.4.5 Save report as PDF

To save the report as PDF, select **File:Report As PDF**.

Result: The report is created as a PDF file and saved in the location specified in the dialog.

Note:

A PDF report can also be generated by clicking the  (icon) in the toolbar of the Evaluation module.

7 Evaluate and compile the results

About this chapter

This chapter describes how to perform a number of editing and evaluation operations on the results, for example:

- Peak integrations
- Baseline operations (editing and optimization)
- Fraction and peak operations
- Comparison of different runs

This chapter also describes how to import and export results.

For information about how to view results, see *Chapter 6 View and present the results, on page 117*.

In this chapter

This chapter contains the following sections:

Section	See page
7.1 Peak integration	156
7.2 Baseline operations	180
7.3 Fraction and peak operations	197
7.4 Compare different runs	203
7.5 Rename folders, results, chromatograms, curves and peak tables	216
7.6 Save results	217
7.7 Import and export results	219

7 Evaluate and compile the results

7.1 Peak integration

7.1 Peak integration

About this section

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

In this section

This section contains the following subsections:

Section	See page
7.1.1 Perform a peak integration	157
7.1.2 Baseline calculation	160
7.1.3 Display peak data	161
7.1.4 Edit the integration parameters	164
7.1.5 Integrate part of a curve	177
7.1.6 Exclude peaks	178

7.1.1 Perform a peak integration

Follow the instructions below to perform a basic peak integration.

Step	Action
------	--------

1	Open a result file in the Evaluation module.
---	---

- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Choose Integrate:Peak Integrate.or• Click the |
|---|--|



toolbar icon.

Result:

Peak integration of the UV curve is performed.

Peak integration results

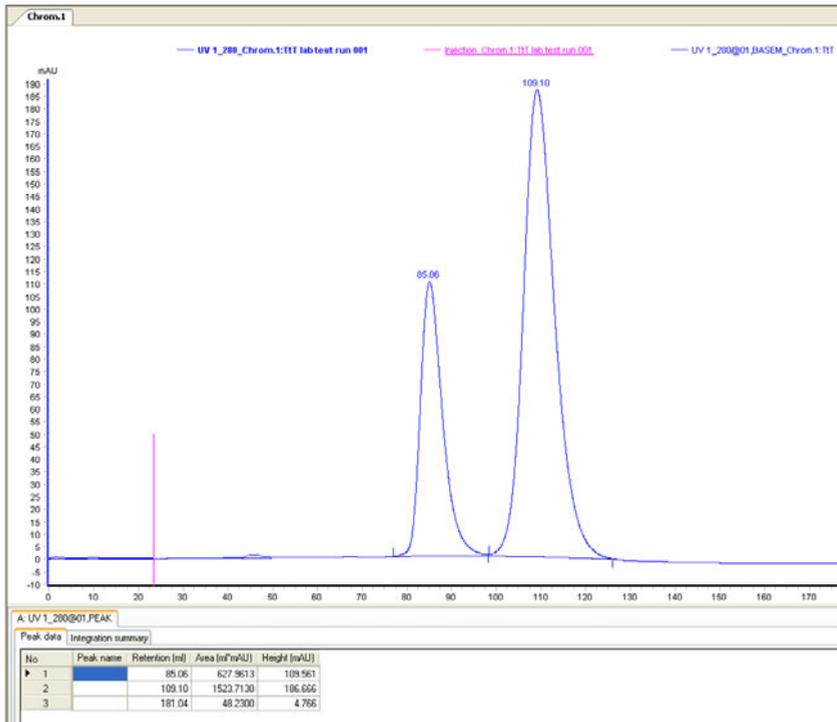
The peak table is displayed below the active chromatogram. The start point and end point of each peak are marked by vertical marks, **drop-lines**, in the chromatogram. The peaks are automatically labelled according to what is selected in the **Curve Style and Color** tab of the **Chromatogram Layout** dialog box. A summary of the integration and the settings used can be viewed by clicking the **Integration summary** tab.

The result after a peak integration is illustrated below:

7 Evaluate and compile the results

7.1 Peak integration

7.1.1 Perform a peak integration



Note: Peak tables can be copied from one chromatogram to another by selecting **Edit:Copy between chromatograms**. The copied peak table name has the default suffix **COPY**.

Edit peak integration data

Step Action

1



Click the  (icon) or select **File:Integrate>Edit Peak Table**.

Result:

The **Edit Peak Table** dialog opens.

Step	Action
2	<p>Edit different parameters of the curve from the Edit Peak Table dialog:</p> <ul style="list-style-type: none"><li data-bbox="439 316 937 347">• <i>Section 7.1.2 Baseline calculation, on page 160</i><li data-bbox="439 365 1053 396">• <i>Section 7.1.4 Edit the integration parameters, on page 164</i><li data-bbox="439 414 883 445">• <i>Section 7.1.6 Exclude peaks, on page 178</i><li data-bbox="439 464 919 495">• <i>Section 7.2 Baseline operations, on page 180</i> <p><i>Result:</i></p> <p>The peaks are integrated and updated automatically.</p>

7 Evaluate and compile the results

7.1 Peak integration

7.1.2 Baseline calculation

7.1.2 Baseline calculation

Introduction

The first step when integrating peaks is to calculate a baseline. A correct baseline is important for accurate calculation of the peak areas. The baseline is per default calculated using the morphological algorithm, when a one-click peak integration is performed. This section describes the options available to calculate baselines manually.

Baseline options

UNICORN start offers several options to create an accurate baseline:

- **Classical** algorithm
 - **Morphological** algorithm
 - **Zero baseline**
-

The Calculate baseline function

Automatic calculation of the baseline can be performed using the **Classical** or **Morphological** algorithm, the latter being the default one. In most cases, the measurement is very accurate.

Zero baseline

The baseline will appear as a straight line at zero absorbance.

7.1.3 Display peak data

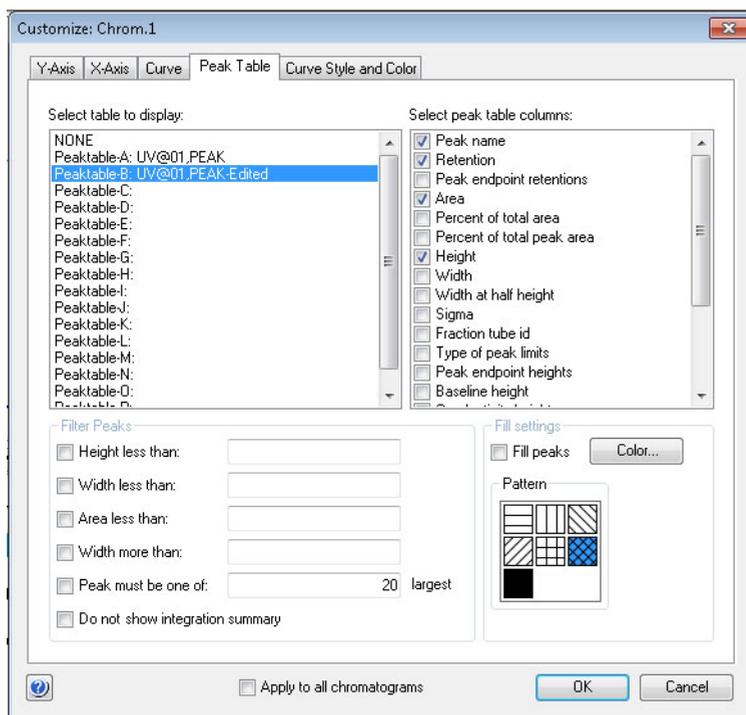
Introduction

There are a number of options available to display and edit peak data and peak table contents. This section describes some of these options. Other options are described in *Section 7.1.4 Edit the integration parameters, on page 164*.

Peak characteristics

The peak retention times and several other peak characteristics are calculated automatically. Follow the instructions below to display other peak characteristics.

Step	Action
1	<ul style="list-style-type: none"> Right-click in the active chromatogram. Select Customize from the shortcut menu. <p><i>Result:</i> The Customize dialog opens.</p>
2	Click the Peak Table tab.



7 Evaluate and compile the results

7.1 Peak integration

7.1.3 Display peak data

Step	Action
------	--------

- | | |
|---|--|
| 3 | <ul style="list-style-type: none">• Select options from the Select peak table columns list.• Click the OK button. |
|---|--|

Result: The selected items are displayed in the peak table.

How to filter peaks

Peaks can be excluded from display in a peak table. Follow the instructions below to filter the peaks:

Step	Action
------	--------

- | | |
|---|--|
| 1 | <ul style="list-style-type: none">• Right-click in the active chromatogram or peak table.• Select Customize from the shortcut menu. <p><i>Result:</i> The Customize dialog opens.</p> |
| 2 | Click the Peak Table tab. |
| 3 | <ul style="list-style-type: none">• Click the check boxes in the Filter Peaks field to select the filter criteria.• Specify filter values.• Click the OK button. |

To filter peaks vs. to reject peaks

The table below describes the major differences in the effects 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.

Measurement options

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.

Coordinates can be obtained in two ways:

- Through direct measurement using the **Vertical marker**. This is described in *Section 6.3.1 Open and view results, on page 127*.
 - From peak table data.
-

7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

7.1.4 Edit the integration parameters

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 are then automatically renumbered and the peak values recalculated.

These changes can be made in the **Edit Peak Table** dialog. In this dialog, peak names that may be used as labels in the chromatogram can also be added.

Open the peak table for editing

Follow the instructions below to open the peak table for editing. The editing options are described below the instructions.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Select Integrate>Edit Peak Table .
<i>Result:</i> The Edit Peak Table dialog opens. |
|---|---|

Note:

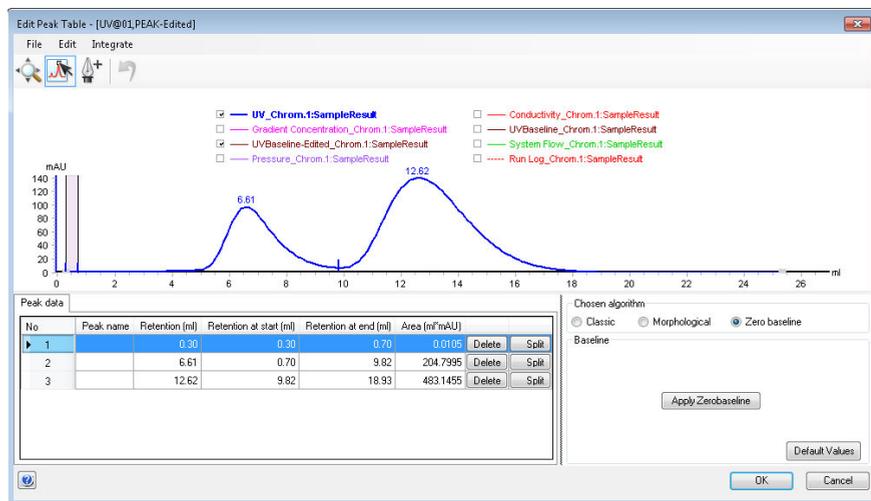
If there are several peak tables in the result, the dialog opens for the peak table tab that is selected. The name of the baseline on which the peak table is based is displayed in the dialog.

- | | |
|---|---|
| 2 | Perform the changes (described in the instructions below). |
| 3 | Click the OK button.
<i>Result:</i> The Save Edited Peak Table dialog opens. The dialog displays a suggested name and location for the peak table. |
| 4 | Confirm the name and location and click the OK button. |
-

Note: *The **Edit Peak Table** dialog is opened immediately if **Save and Edit Peak Table** is selected.*

The Edit Peak Table dialog

The illustration below shows the **Edit Peak Table** dialog:



Toolbar icons in the Edit Peak Table dialog

The table below describes the toolbar icons in the **Edit Peak Table** dialog.

Icon	Function
	Activates the Zoom mode. When it is activated, you can use the mouse pointer to drag out an area to be zoomed in. This is described in <i>The zoom function</i> below.
	Activates the Edit Peaks mode. In this mode, you can select a peak and edit the start and end points. This is described in <i>Edit the peaks manually</i> below.
	Activates the Set Curve Points mode. In this mode, you can select baseline curve points for editing and add new points to re-draw the baseline. This is described in <i>Adjust the baseline</i> below.

7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Icon	Function
	Returns the result to the state it was in before the last change (Undo). Note: <i>This function can also be selected in the Edit menu.</i>

The zoom function

Follow the instructions below to use the zoom function in the **Edit Peak Table** dialog.

Step	Action
------	--------

1	Click the Zoom icon.
---	-----------------------------

Result: The mouse pointer is changed into .

- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Position the mouse pointer over the left topmost position of the area you want to zoom in.• Press and hold the left mouse button. |
|---|--|

3	Drag the cursor over the area you want to zoom in.
---	--

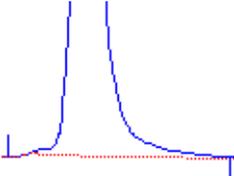
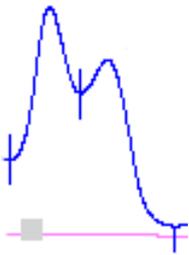
4	Release the mouse button.
---	---------------------------

Result: The area is enlarged.

Note: *A **Zoomed mode** note will be displayed in the top right-hand corner of the chromatogram when the zoom function has been used. Right-click and select **Reset Zoom** from the shortcut menu to return to the full display.*

Peak start and end points

The beginning of each peak is marked with a dropline above the curve, and the end of each peak is marked with a dropline below the curve. The table below shows examples of start and end point droplines:

Stage	Description
	Single peak.
	Double peaks: the end of the first peak will be at the same point as the beginning of the next peak.

Edit the peaks manually

The start and end points of a peak can be adjusted graphically by moving the droplines in the **Edit Peak Table** dialog.

Step	Action
1	Click the Edit Peaks icon. <i>Result:</i> The mouse pointer is changed into  .
2	Click the peak you want to edit. <i>Result:</i> The peak is highlighted and marker lines show the start and end points.

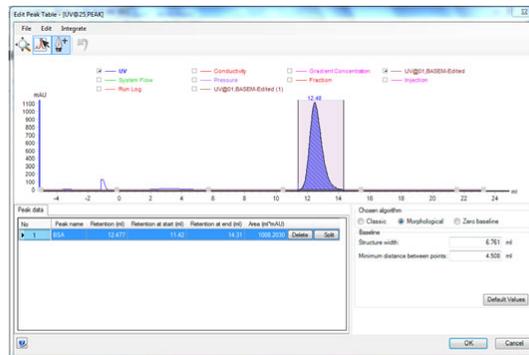
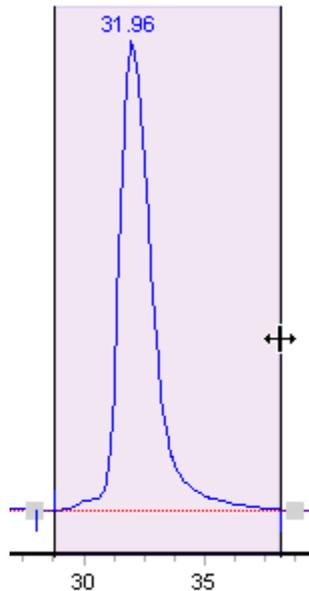
7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Step	Action
------	--------

- | | |
|---|---|
| 3 | Position the mouse pointer over the marker line that you want to change.
<i>Result:</i> The pointer is changed into a double-arrow symbol. |
|---|---|



- | | |
|---|--|
| 4 | Drag the marker to a new position with the mouse pointer.
<i>Result:</i> The start or end point droplines of the peak are changed and the corresponding values are updated in the peak table. |
|---|--|

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

Adjust the baseline

The baseline can be adjusted graphically in the **Edit Peak Table** dialog.

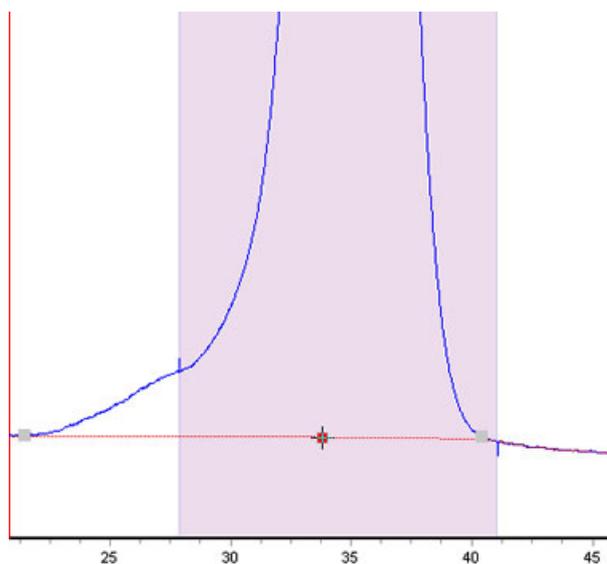
Step	Action
------	--------

1	Click the Set Curve Points icon.
---	---

Result: The mouse pointer is changed into .

2	Perform one or more of 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 shortcut menu to delete the point.
- Click a data point and drag the point to a new position to move the baseline.

Result: The baseline is edited and the peak table values are recalculated accordingly.

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.

7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Calculate a new baseline

The baseline can be recalculated in the **Edit Peak Table** dialog. To alter the baseline settings:

Step	Action
1	Select an algorithm (Morphological is default).
2	<ul style="list-style-type: none">Adjust the Baseline parameters as desired.<ul style="list-style-type: none">Structure widthMinimum distance between pointsorClick the Default Values button for the default values.
3	Click OK . <i>Result:</i> The baseline is recalculated.

Note: Select **Zero Baseline** and click the **Apply Zerobaseline** button to replace the calculated baseline with a zero baseline.

Add color to a peak

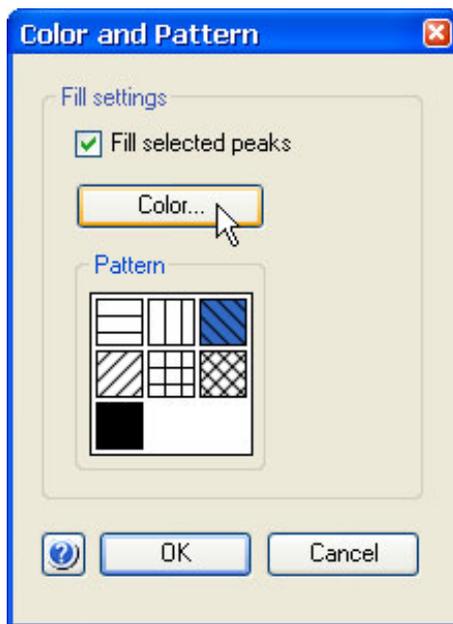
The table below describes how to add a fill color and a pattern to an individual peak in the **Edit Peak Table** dialog:

Step	Action
1	Click the Edit Peaks icon. <i>Result:</i> The mouse pointer is changed into  .
2	Click to select the peak.

Step	Action
------	--------

- | | |
|---|--|
| 3 | <ul style="list-style-type: none">• Right-click and select Fill Peaks from the shortcut menu or• Select Edit:Fill Peak. |
|---|--|

Result: The **Color and Pattern** dialog opens.



- | | |
|---|--|
| 4 | Select the Fill selected peaks check box. |
|---|--|

Result: The **Color** button and **Pattern** field are activated.

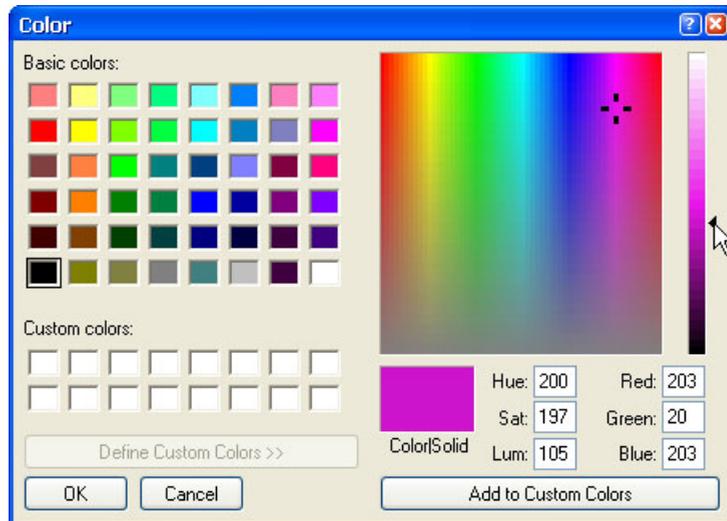
7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Step	Action
------	--------

- | | |
|---|---|
| 5 | Click the Color button.
<i>Result:</i> The Color dialog opens. |
|---|---|



- | | |
|---|---|
| 6 | <ul style="list-style-type: none">• Select one of the Basic colors• or• Click the Define Custom Colors button to open the settings field and define new colors (illustrated above).• After you have selected a color, click OK to close the dialog. |
| 7 | <ul style="list-style-type: none">• Select a fill pattern in the Pattern field.• 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 **Customize** dialog for the chromatogram.*

Split a peak

It is possible to split a peak into two new peaks by inserting a dropline. Follow the instructions below to split a peak in the **Edit Peak Table** dialog:

Step	Action
1	Click the Edit peaks icon. <i>Result:</i> The mouse pointer is changed into  .
2	Click the peak in the curve or in the peak table to select the peak.
3	<ul style="list-style-type: none">Right-click and select Split Peak from the shortcut menu orSelect Edit:Split Peaks. <i>Result:</i> A new dropline is inserted at the middle point between the two existing droplines and the peak is split. The peak numbering and the peak table are updated accordingly.

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

Join peaks

It is possible to join the areas of adjacent peaks if they are separated by a dropline. Follow the instructions below to join adjacent peaks in the **Edit Peak Table** dialog:

Step	Action
1	Click the Edit peaks icon. <i>Result:</i> The mouse pointer is changed into  .
2	Click the peak in the curve or in the peak table to select the peak.
3	<ul style="list-style-type: none">Right-click and select Join Left or Join Right from the shortcut menu orSelect Edit:Join Left or Edit:Join Right. <i>Result:</i> The original intervening dropline is removed, all peaks are renumbered and the peak table is updated.

7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Add peak names

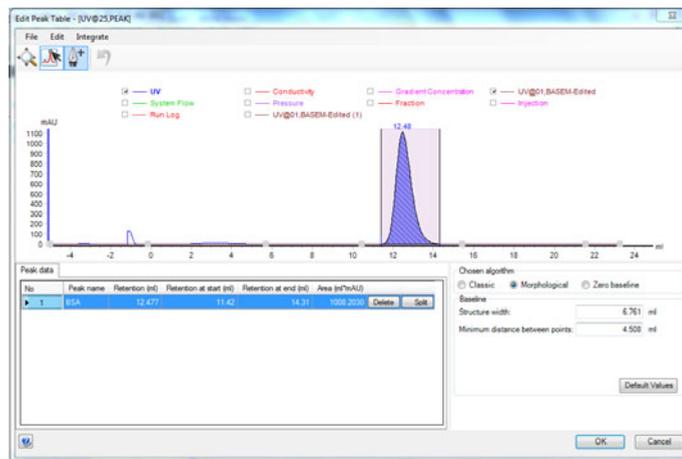
Follow the instructions below to add names in the **Edit Peak Table** dialog, to identify the peaks:

Step	Action
------	--------

1	Click the Edit peaks icon.
---	-----------------------------------

Result: The mouse pointer is changed into .

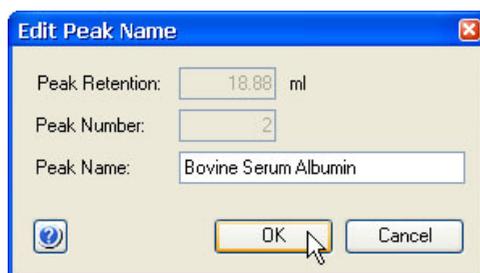
2	Click the peak in the curve or in the peak table to select the peak.
---	--



Step	Action
------	--------

- | | |
|---|--|
| 3 | <ul style="list-style-type: none"> Right-click and select Peak Name from the shortcut menu or Choose Edit:Peak name or Double-click the peak. |
|---|--|

Result: The **Edit Peak Name** dialog opens. The number and retention of the selected peak is displayed.



- | | |
|---|--|
| 4 | Type a name in the Peak name textbox. |
|---|--|

- | | |
|---|-------------------|
| 5 | Click OK . |
|---|-------------------|

Result: The new name is added in the corresponding **Peak name** cell in the peak table. It is also shown below the peak in the chromatogram. If you have selected to show peak names in the chromatogram, it will also be displayed over the peak.

Note: You can also add a peak name by selecting the **Peak name** cell in the peak table, click again in the cell and then type the peak name. A pencil symbol is displayed by the peak number to indicate that you are able to enter the text.

Delete peaks

Follow the instructions below to delete a peak in the **Edit Peak Table** dialog:

Step	Action
------	--------

- | | |
|---|-----------------------------------|
| 1 | Click the Edit Peaks icon. |
|---|-----------------------------------|

Result: The mouse pointer is changed into .

- | | |
|---|--|
| 2 | Click the peak in the curve or in the peak table to select the peak. |
|---|--|

7 Evaluate and compile the results

7.1 Peak integration

7.1.4 Edit the integration parameters

Step	Action
3	<ul style="list-style-type: none">• Right-click and select Delete Peaks from the shortcut menu or• Select Edit>Delete Peaks or• Press the Delete key. <p><i>Result:</i> The peak is deleted, the remaining peaks are renumbered and the peak table is updated.</p>

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.

7.1.5 Integrate part of a curve

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.

This partial integration can be performed both in the **Integrate** dialog in preparation for the peak integration, or in the **Edit Peak Table** dialog to adjust an unsatisfactory peak integration. Both alternatives are described here.

Select part of a curve for integration

Follow the instructions below to select only part of a curve for peak integration in the **Integrate** dialog box:

Step	Action
------	--------

- | | |
|---|---|
| 1 | <ul style="list-style-type: none"> Choose Integrate:Peak Integrate or Click the |
|---|---|



icon.

No	Peak name	Retention (ml)	Retention at start (ml)	Retention at end (ml)	Area (ml ² mAU)		
1		0.40	0.30	0.80	0.0120	Delete	Split
2		6.48	0.80	9.78	204.2648	Delete	Split
3		12.57	9.78	18.85	481.4632	Delete	Split
4		31.22	29.83	32.92	2.5213	Delete	Split
5		37.01	32.92	37.71	0.1025	Delete	Split
6		41.10	37.71	41.10	0.2023	Delete	Split

- | | |
|---|-------------------|
| 4 | 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.

7 Evaluate and compile the results

7.1 Peak integration

7.1.6 Exclude peaks

7.1.6 Exclude peaks

Introduction

The results of the peak integration can sometimes be improved if some peaks are excluded. Also, more accurate results may be obtained if smaller peaks are skimmed off larger peaks with shoulders, as described in this section.

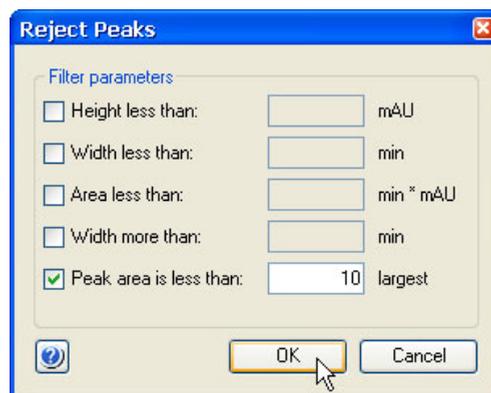
Exclude peaks

Follow the instructions below to define peaks to be excluded in the **Peak Integrate** dialog during a peak integration.

Step	Action
------	--------

1	Click the Reject peaks button.
---	---------------------------------------

Result: The **Reject Peaks** dialog opens.



- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Select the appropriate checkboxes and type values for peak height, width and area.• Define how many of the largest peaks you want to include. |
|---|--|

3	Click OK .
---	-------------------

4	Proceed with the peak integration.
---	------------------------------------

Note: You can also exclude peaks from the peak integration in the **Edit Peak Table** dialog. Select **Integrate:Settings** to open the **Reject Peaks** dialog.

Include negative peaks

Select the **Accept negative peaks** checkbox of the **Peak Integrate** dialog to include negative peaks in the integration. The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

7.2 Baseline operations

About this section

In order to achieve the best possible peak integration result, the baseline may have to be edited or optimized. The edit baseline operations can be accessed through the edit peak dialog.

In this section

This section contains the following subsections:

Section	See page
7.2.1 Edit the baseline manually	181
7.2.2 Optimize the baseline with a morphological algorithm	184
7.2.3 Optimize the baseline with a classic algorithm	188

7.2.1 Edit the baseline manually

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 manually.

How to use the zoom function

Follow the instructions below to use the zoom function in the **Edit Baseline** dialog.

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.
 - 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

Follow the instructions below to edit and insert baseline data points:

Step	Action
------	--------

1 Select **Integrate:Edit Baseline**.

Result: If there is more than one baseline available, the **Select Baseline to Edit** dialog opens. If not, proceed to step 2.

- Select the baseline you want to edit from the list.
- Click **OK**.

Result: The **Edit Baseline** dialog opens

7 Evaluate and compile the results

7.2 Baseline operations

7.2.1 Edit the baseline manually

Step	Action
------	--------

2	Click the Set Curve Points icon.
---	---



Result: The mouse pointer is changed into a pen symbol and a cross.

3	Add, delete and/or move data points according to the table below.
---	---

6	Click OK .
---	-------------------

Result: The **Save Edited Baseline** dialog opens.

- | | |
|---|---|
| 7 | <ul style="list-style-type: none">• Confirm the location and type a new name if necessary.• Click OK. |
|---|---|

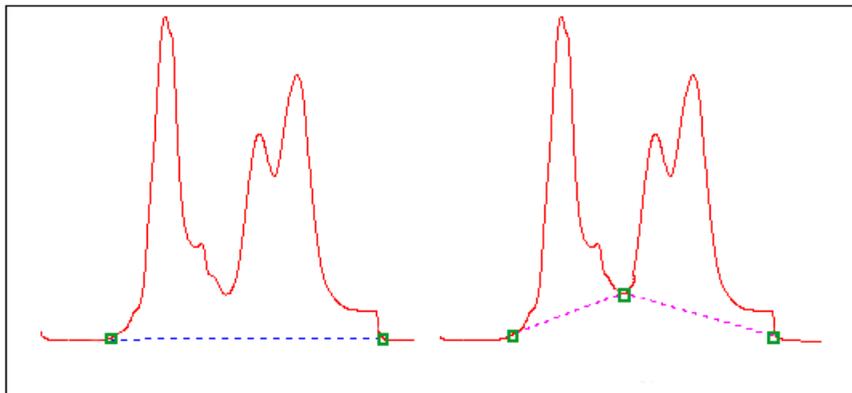
Result: The new baseline is saved.

If you want to...	Then...
Add a data point	<p>Click the left mouse button to place a new baseline point in the chromatogram.</p> <p><i>Result:</i> A new point is created, marked by a red square. The baseline curve is redrawn as a smooth spline function based on the old and the new points. The baseline is guided by the points, but does not necessarily pass through them.</p> <p>Tip: <i>If you want to create straight baselines, read the instruction later in this section.</i></p>
Delete a data point	<p>Click the data point to select it and click the Delete button.</p> <p><i>Result:</i> The data point is deleted and the curve is redrawn.</p> <p>Note: <i>Click the Delete All button to delete all the data points at once.</i></p>
Move a data point	<p>Select the data point and drag it to a new position.</p> <p><i>Result:</i> The baseline curve is redrawn.</p>

Tip: Click the **Undo** button to cancel the last edit if the result is not satisfactory.

Edited baseline

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



How to draw a straight line

Follow the instructions below 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. <i>Result:</i> The baseline is drawn through the points as a straight line.

7 Evaluate and compile the results

7.2 Baseline operations

7.2.2 Optimize the baseline with a morphological algorithm

7.2.2 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.

Morphological algorithm description

A 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.

A 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.

A morphological algorithm does not work well if there are negative peaks or if quantitative data from negative peaks are important in the run.

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

How to set a morphological baseline

Follow the instructions below to choose a **Morphological** algorithm and define baseline settings.

Step	Action
------	--------

1



Click the  icon or select **Integrate>Edit Peak Table** to perform peak integration.

2

- Choose **Calculate baseline** in the **Baseline** drop-down menu.
- Click the **Baseline settings** button.

Result: The **Baseline Settings** dialog opens.

Step	Action
------	--------

- | | |
|---|---|
| 3 | <ul style="list-style-type: none">• Select the Morphological algorithm.• Change the Baseline parameters if necessary. See more information about the parameters below this table. <ul style="list-style-type: none">• Click OK. |
|---|---|

Morphological algorithm parameters

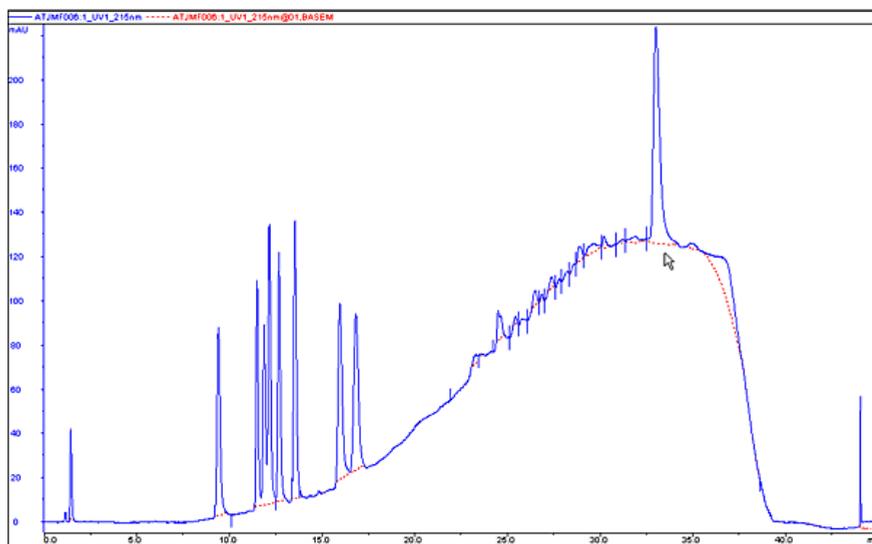
The parameters for the **Morphological** algorithm are:

- **Structure width**
- **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:



7 Evaluate and compile the results

7.2 Baseline operations

7.2.2 Optimize the baseline with a morphological algorithm

The correct structure width settings

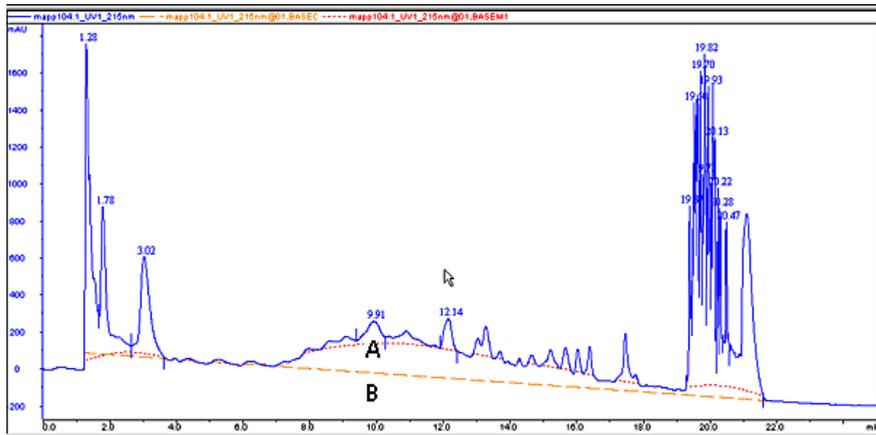
Settings too low

Structure width settings that are too low can result in a baseline that reaches too high up in the peaks of the curve. Sometimes 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.

Settings too high

Structure width settings that are too high 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.

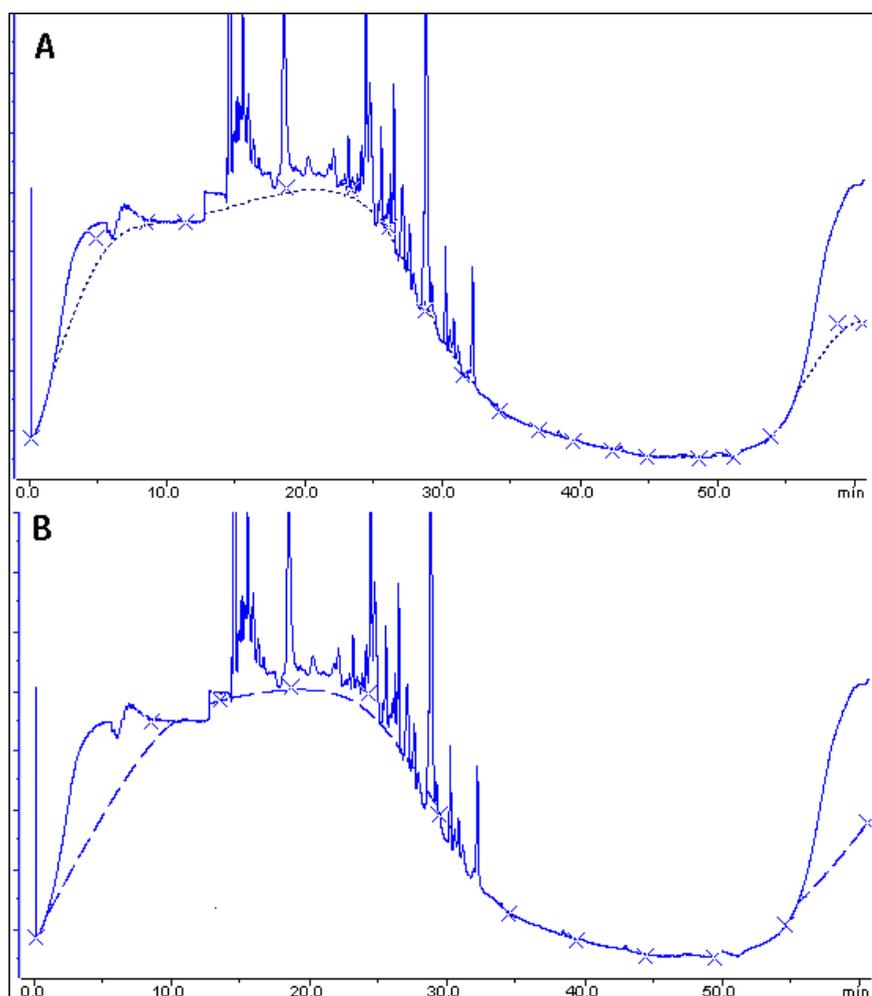
The illustration below is an example of baselines using the default morphological algorithm settings (A) and a morphological algorithm with an increased **Structure width** value (B).



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).



7 Evaluate and compile the results

7.2 Baseline operations

7.2.3 Optimize the baseline with a classic algorithm

7.2.3 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 classic algorithm.

Classic algorithm description

A 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.

A 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

Follow the instructions below to set a **Classic** algorithm and define a baseline.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the Baseline settings button in the Peak Integrate dialog.
<i>Result:</i> The Baseline Settings dialog opens. |
|---|---|

Step	Action
------	--------

- | | |
|---|---|
| 2 | <ul style="list-style-type: none">• Select the Classic algorithm.• Change the Baseline parameters. <p>See more information about the parameters below this table.</p> <ul style="list-style-type: none">• Click OK. |
|---|---|

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

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%

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

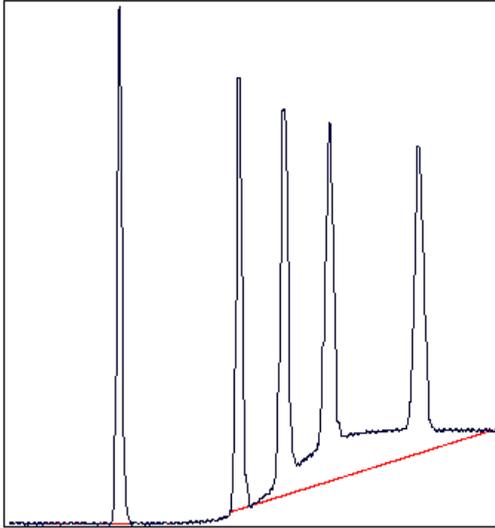
7 Evaluate and compile the results

7.2 Baseline operations

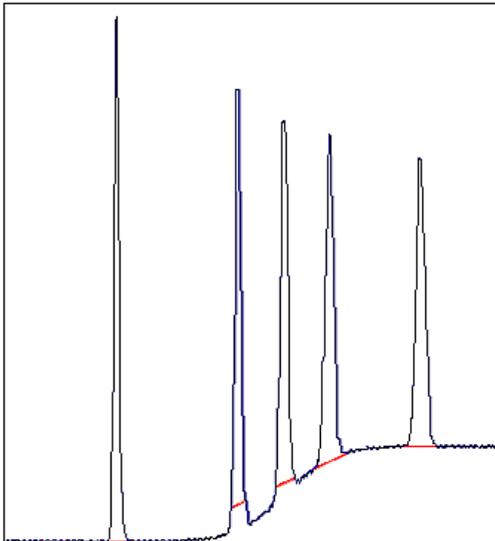
7.2.3 Optimize the baseline with a classic algorithm

Shortest baseline segment

If an excessively 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:



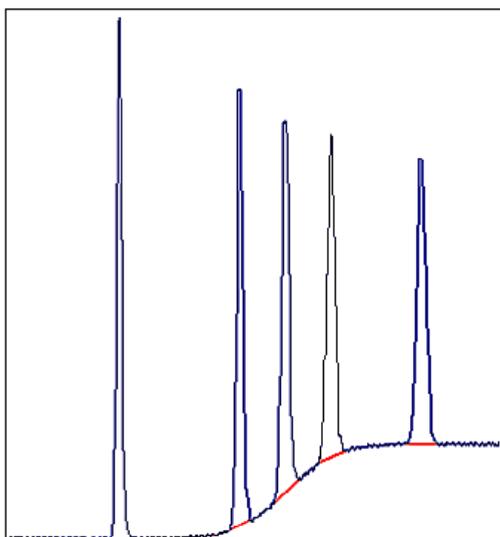
The **Shortest baseline segment** value is decreased by 50% in this example:



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. An excessively 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:



Slope limit too high

An excessively 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 flowthrough or solvent peak. The large peak affects the calculation of the default parameters and leads to excessively high values for the **Slope limit**.

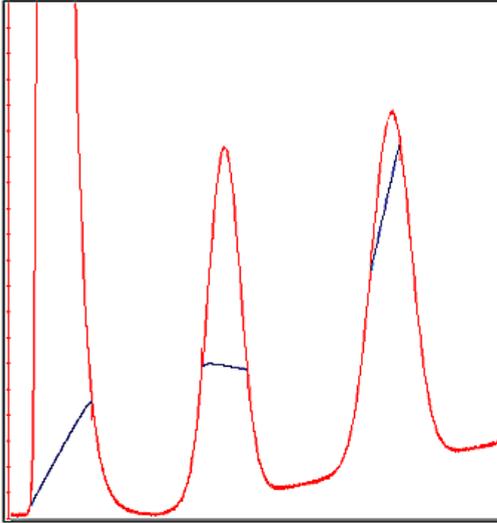
Note: *An excessive value for the **Noise window** can have the same effect and be caused by the same situation, often also in combination with a high **Slope limit**.*

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

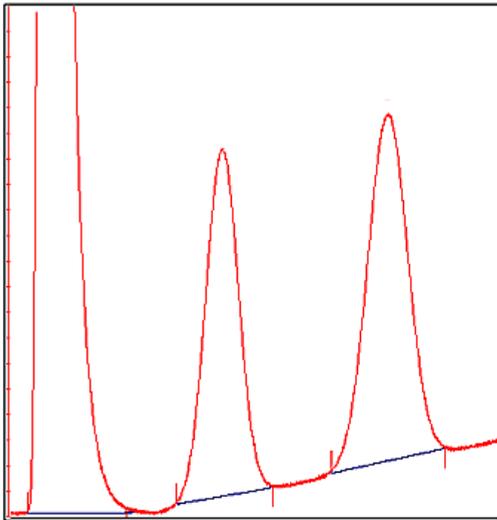
7 Evaluate and compile the results

7.2 Baseline operations

7.2.3 Optimize the baseline with a classic algorithm

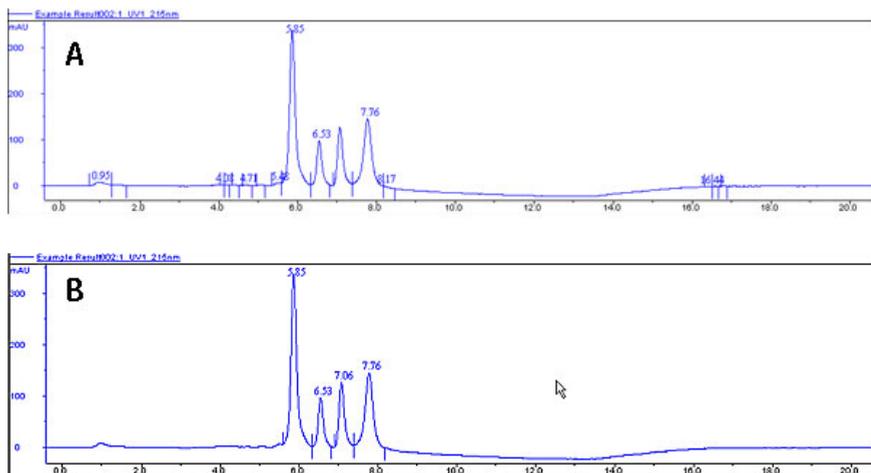


The example below has a much lower **Slope limit**, and a lower **Noise window**:



Noise window

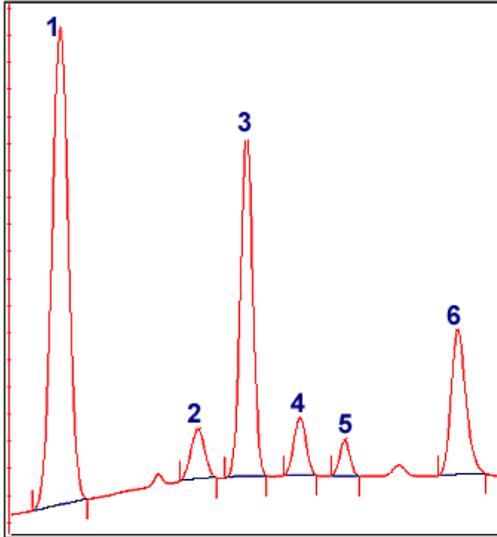
Occasionally too many peaks occur after the peak integration, usually because noise on the baseline is erroneously detected as peaks. The solution to this is to increase the **Noise window** parameter. However, this can result in peak limits too high up on the peak slopes. The illustration below is an example of noise detected as peaks (A) and the result of a second peak integration with an increased **Noise window** (B).



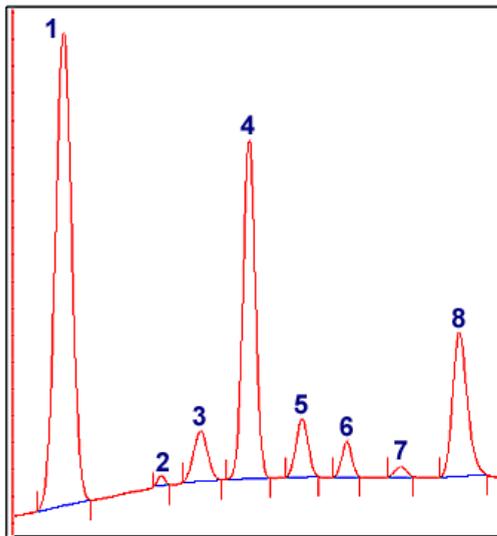
Tip: You can also use the **Reject peaks** function in the **Peak Integrate** dialog to reduce the number of peaks based on the total number of accepted peaks or the minimum peak height.

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:



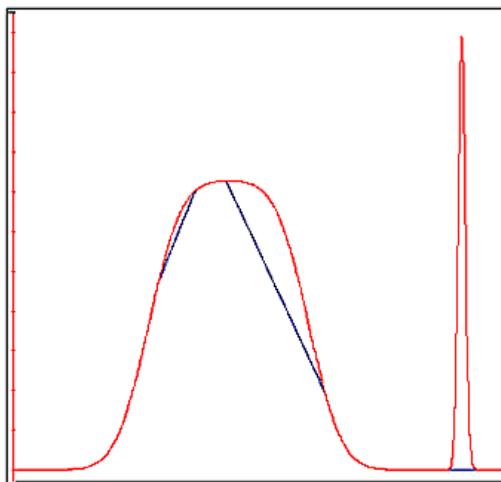
All peaks are detected if the **Noise window** is decreased- see example below:



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

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

Follow the instructions below to set the **Max baseline level**.

Step	Action
1	Right-click in the chromatogram and select Marker . <i>Result:</i> 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	<ul style="list-style-type: none">• Move the Marker with your mouse.• Measure the height of the peak you want to exclude from the baseline.
3	Choose Integrate:Calculate baseline .

7 Evaluate and compile the results

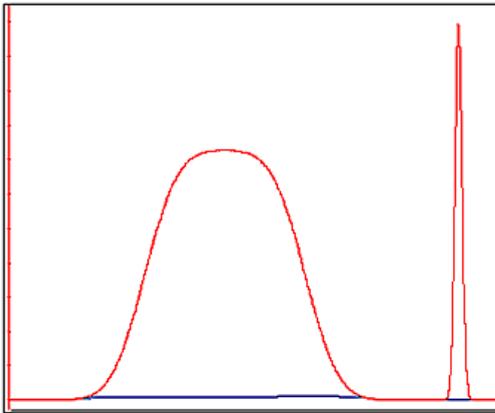
7.2 Baseline operations

7.2.3 Optimize the baseline with a classic algorithm

Step	Action
4	<ul style="list-style-type: none">• Select Classic as 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:



7.3 Fraction and peak operations

About this section

This section describes additional operations that you can perform on your results:

- Create an average fraction absorbance curve, the ***Fraction Histogram***.
 - Match protein activity to a curve using the ***Activity Histogram***.
 - Use the absorbance ratio to verify peak purity or identify peaks.
-

In this section

This section contains the following subsections:

Section	See page
7.3.1 The Fraction Histogram	198
7.3.2 Match protein activity to a curve	201

7 Evaluate and compile the results

7.3 Fraction and peak operations

7.3.1 The Fraction Histogram

7.3.1 The Fraction Histogram

Introduction

The **Fraction Histogram** dialog in the **Evaluation** module can be used to create a curve for the average fraction absorbance. It can also be used to create a table showing the amount of protein and the concentration in each fraction.

Create a Fraction Histogram curve

Follow the instructions below to create a **Fraction Histogram** curve.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Select Operations:Fraction Histogram .
<i>Result:</i> The Fraction Histogram dialog opens. |
| 2 | Select the desired UV curve in the left Source chromatogram and curve field.
Tip:
<i>The fractions curve should already be selected on the middle field.</i> |
| 3 | Click OK .
<i>Result:</i> The average fraction absorbance values are displayed as a new curve in the chromatogram. |

Tip: *By default, the **Fraction Histogram** curve will be assigned to the first available curve position, which is shown in the **Target chromatogram and curve** list. A default curve name with the ending **HIST** is suggested in the **Curve name** field. You can choose another curve position and type another curve name in the **Curve name** field if desired.*

Protein concentrations in the fractions

The protein concentration in the fractions are calculated using the following formula:

$$\text{Concentration [mg/ml]} = A / (d * 1000 * \text{Ext. coef.})$$

$$A = \text{Average fraction absorbance} = \text{Area} / \text{Volume [mAU]}$$

$$d = \text{UV cell path length [cm]}$$

$$\text{Ext. coef.} = \text{Protein coefficient at used wavelength. [l g}^{-1} \text{ cm}^{-1}\text{]}$$

Protein amounts in the fractions

The total amount of protein found in the fraction is calculated using the following formula:

$$\text{Amount [mg]} = \text{Concentration [mg/ml]} * \text{fraction volume [ml]}$$

Calculate the protein concentration and amount

Follow the instructions below to calculate the protein concentration and amount in the fractions using the **Fraction** table in the **Fraction Histogram** dialog:

Step	Action
1	Select Operations:Fraction Histogram . <i>Result:</i> The Fraction Histogram dialog opens. Note: <i>If the path length is not shown, it can be entered manually.</i>
2	Type the extinction coefficient for each fraction in the corresponding Ext. coef table cell. <i>Result:</i> The fraction protein concentration and amount are calculated and displayed in the corresponding Conc. and Amount table cells. Note: <i>The values are calculated using a zero baseline (i.e., no baseline subtraction is applied).</i>

Export the Fraction table

The complete **Fraction** table can be exported as an Excel file:

Step	Action
1	Click the Export Table button in the Fraction Histogram dialog. <i>Result:</i> The Export Fraction Table to Excel file dialog opens.
2	Browse to the folder where you want to save the file.
3	Type a name in the File name field.
4	Click the Save button. <i>Result:</i> The table is saved in Excel format.

7 Evaluate and compile the results

7.3 Fraction and peak operations

7.3.1 The Fraction Histogram

Print the Fraction table

The complete **Fraction** table can be printed directly from the **Fraction Histogram** dialog:

Step	Action
1	Click the Print Table button. <i>Result:</i> The Print dialog opens.
2	If necessary, select a printer and the number of copies to print. You may also click the Properties button in this dialog, to change the general printer settings.
3	Click OK . <i>Result:</i> The table is printed on the selected printer.

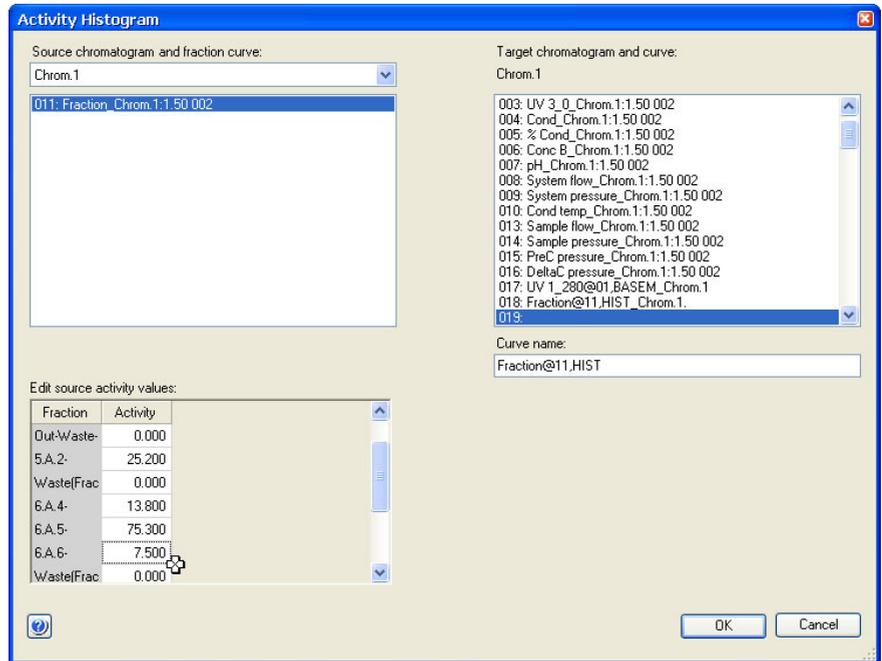
7.3.2 Match protein activity to a curve

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

The illustration below shows the **Activity Histogram** dialog:



7 Evaluate and compile the results

7.3 Fraction and peak operations

7.3.2 Match protein activity to a curve

Enter protein activity values for comparison

Follow the instructions below to enter the values from a protein activity assay in a comparison histogram:

Step	Action
------	--------

-
- | | |
|---|--|
| 1 | <p>Choose Operations:Activity Histogram.</p> <p><i>Result:</i> The Activity Histogram dialog opens.</p> |
| 2 | <p>By default, the fraction curve for the current chromatogram is selected in the Source chromatogram and fraction curve list.</p> <ul style="list-style-type: none">• If necessary, change the source and target chromatograms. <p>All the component fractions of the fraction curve are listed in the Fraction column of the Edit source activity values field.</p> |
| 3 | <ul style="list-style-type: none">• Type an activity value for each fraction in the Activity column.• Click OK. <p><i>Result:</i> A histogram curve showing the activity values is added to the chromatogram.</p> |

Tip: *By default, the **Activity Histogram** curve will be assigned to the first available curve position, which is shown in the **Target chromatogram and curve** list. A default curve name with the ending **HIST** is suggested in the **Curve name** field. You can choose another curve position and type another curve name in the **Curve name** field if you so wish.*

7.4 Compare different runs

About this section

This section describes how to:

- Make comparisons between curves or chromatograms from different runs.
 - Present curves or chromatograms from different runs.
 - Compare curve parameters among curves from different runs.
 - View several chromatograms at the same time.
 - Overlay curves from different runs in one chromatogram.
 - Stack curves from different runs in one chromatogram.
 - Create mirror images
-

In this section

This section contains the following subsections:

Section	See page
7.4.1 Open and compare chromatograms	204
7.4.2 Open and compare curves	207
7.4.3 Shift curves	213
7.4.4 Create a mirror image	214

7.4.1 Open and compare chromatograms

Introduction

This section describes how to:

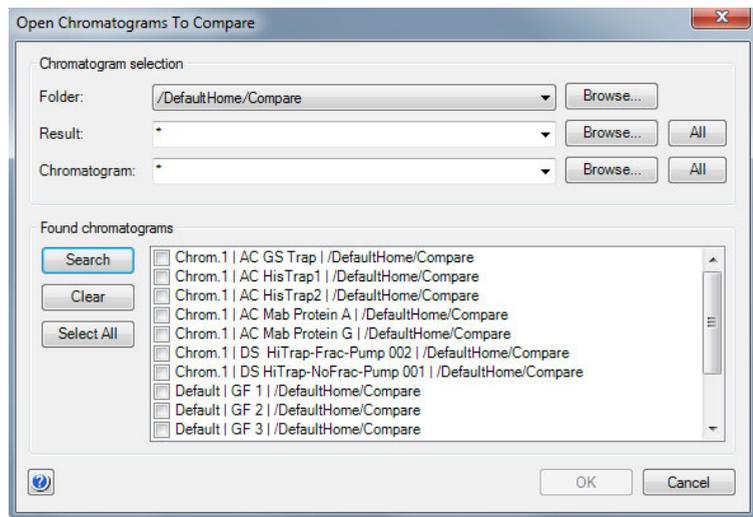
- Import chromatograms from other result files to an open result.
- Compare chromatograms in a result.

Import chromatograms with the command *File:Add to compare*

Follow the instructions below to import chromatograms with the **File:Add to compare** command. The search is performed at specific locations or with specific search criterias. This method is useful if, for example, you want to import chromatograms from all results of a specific folder.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Choose File:Add to compare:Chromatograms in the Evaluation module.
<i>Result:</i> The Open Chromatogram to Compare dialog box is displayed. |
|---|---|



Step	Action
2	<ul style="list-style-type: none">Click the Browse... button beside the Folder drop-down list This opens a browse dialog where you can select the result folder where you want to search for chromatograms. <p>Note: <i>The search will not include the contents of subfolders. You must select the subfolders individually for the search.</i></p> <ul style="list-style-type: none">Repeat this step to define specific results or chromatograms if desired. <p>Tip: <i>Click the drop-down list arrow buttons to view searchpaths that you have used previously. You can click selections in these lists to search the same locations again.</i></p>
3	<ul style="list-style-type: none">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 initial 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.
4	<ul style="list-style-type: none">Click the OK button. <p>Result: All the selected chromatograms are opened in the Evaluation module.</p> <p>Note: <i>If the names of the imported chromatograms are already used they will be sequentially numbered for identification purposes. Each chromatogram will be opened in its own tab. However, depending on the number of chromatograms and the length of the names, not all tabs may be visible at once. Click the arrow symbol on the top right corner of the chromatogram pane to show a list of all open chromatograms. You may select the chromatogram you want to view from this list.</i></p>

7 Evaluate and compile the results

7.4 Compare different runs

7.4.1 Open and compare chromatograms

Display all chromatograms on the same scale

Step	Action
1	Click the Customize icon or select Tools:Customize to open the Customize dialog for any chromatogram.
2	Select the X-Axis tab and make the changes to the Base and the Axis scale .
3	Select the Apply to all chromatograms option and click OK .

7.4.2 Open and compare curves

Introduction

This section describes how to import or copy curves from different runs into one chromatogram for comparison.

7 Evaluate and compile the results

7.4 Compare different runs

7.4.2 Open and compare curves

File:Add to compare

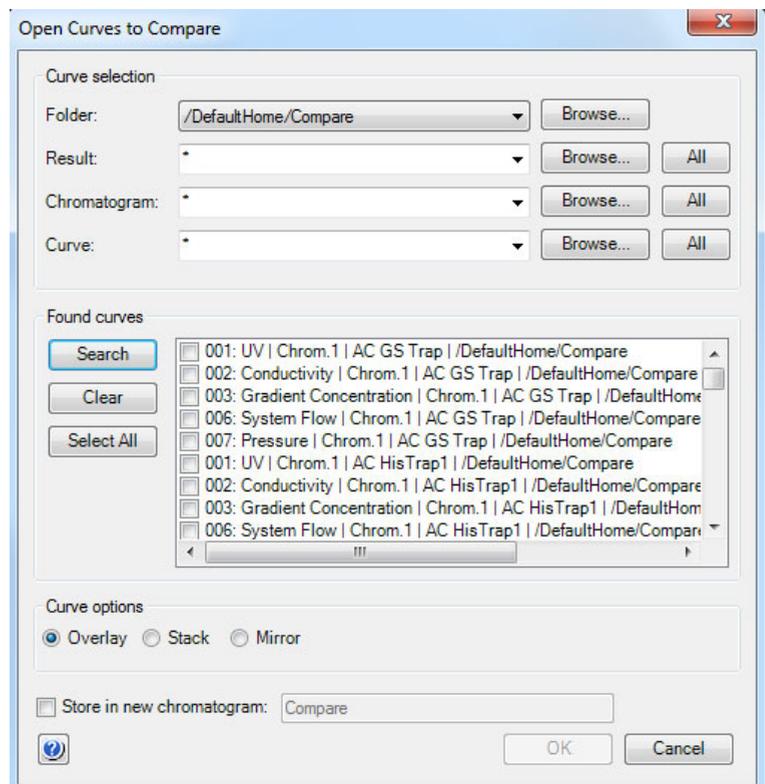
Follow the instructions below to import curves to a chromatogram with the command **File:Add to compare**:

Step	Action
------	--------

- | | |
|---|--|
| 1 | In the Evaluation module, <ul style="list-style-type: none">• Choose File:Add to compare:Curves
or• Click the Open curves to compare toolbar button. |
|---|--|



Result: The **Open Curves to Compare** dialog opens.



Step	Action
2	<ul style="list-style-type: none"><li data-bbox="435 269 1192 334">• Select the desired search criteria in the Folder, Result, Chromatogram and Curve drop-down list of the Curve selection section.<li data-bbox="435 347 1192 413">• Click Search and a list of curves will be displayed based on the selected search criteria. <p data-bbox="435 425 477 455">Tip:</p> <p data-bbox="435 462 1163 527"><i>A new search can be performed with new search criteria without erasing curves located in the previous search.</i></p> <ul style="list-style-type: none"><li data-bbox="435 540 1192 606">• 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.<li data-bbox="435 618 1192 717">• 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.
3	<ul style="list-style-type: none"><li data-bbox="435 729 1192 806">• Select how to display the imported curves in the Curve options field. The options are described in "Curve options" below.<li data-bbox="435 811 564 846">• Click OK.

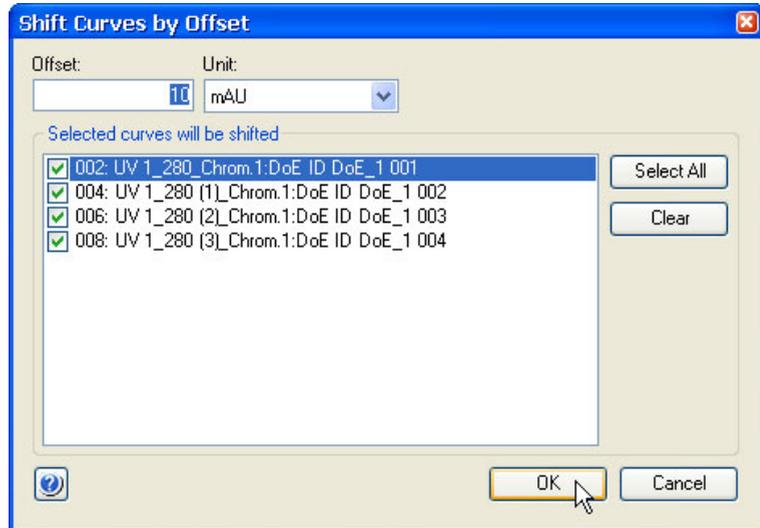
7 Evaluate and compile the results

7.4 Compare different runs

7.4.2 Open and compare curves

Step	Action
------	--------

- | | |
|---|---|
| 4 | If you selected the Stack option in step 3, the Shift Curves by Offset dialog is displayed: |
|---|---|



- 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 a separate offset from the other groups.

7 Evaluate and compile the results

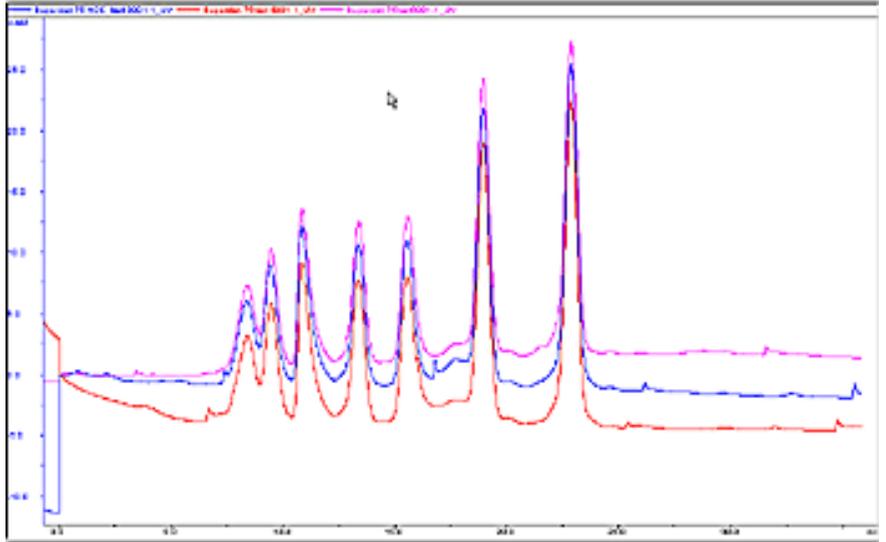
7.4 Compare different runs

7.4.2 Open and compare curves

Curve options

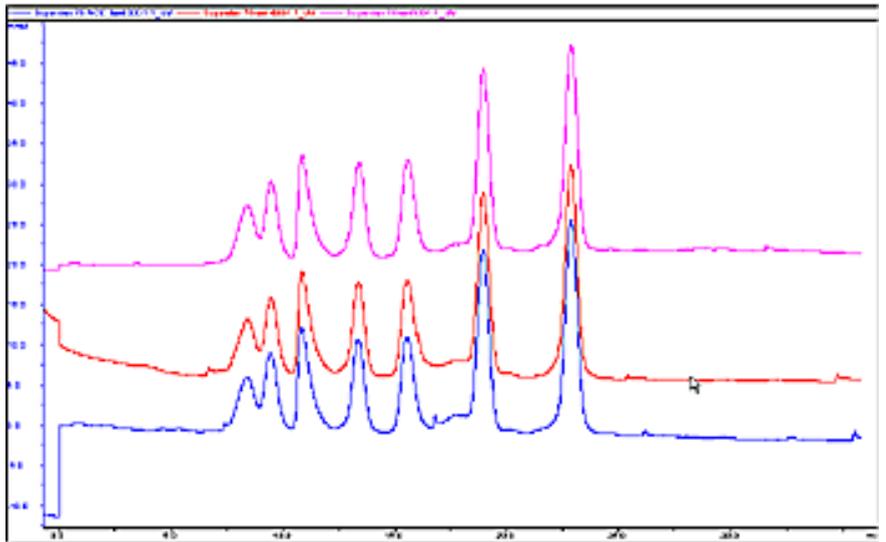
The illustrations below show the different curve presentation options:

Overlay



The curves are presented overlaid on one another.

Stack



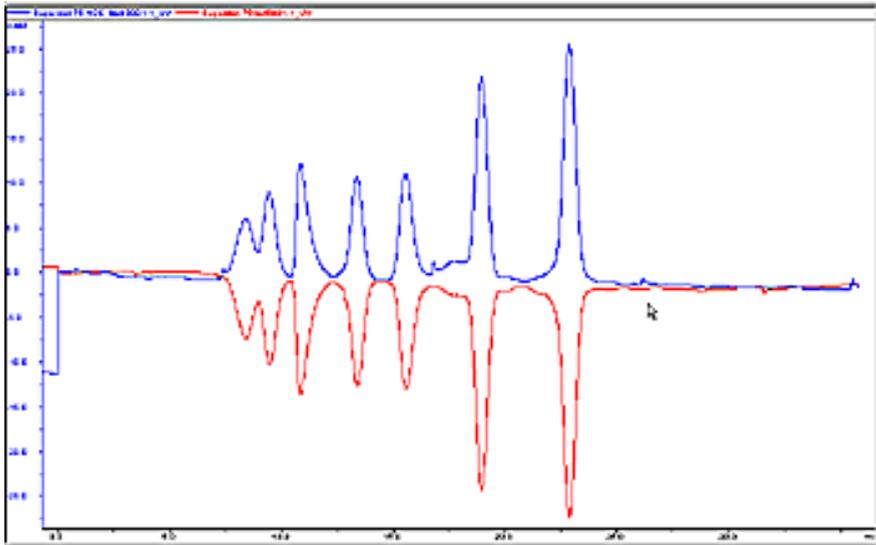
7 Evaluate and compile the results

7.4 Compare different runs

7.4.2 Open and compare curves

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.

Change comparison settings

Follow the instructions below to change other comparison settings:

Step	Action
1	Click the Customize icon to open the Customize dialog.
2	Select or de-select the check boxes on the Curve tab to compare a different set of curves.
3	<ul style="list-style-type: none">• Select the Y-Axis tab to change the curve scales either<ul style="list-style-type: none">- individuallyor- all with the same scale (click the All With This Unit button).• Click OK to update the curves.

7.4.3 Shift curves

Introduction

You can use the **Shift** function to stack and move curves from different runs to better visualize the differences. Each curve is repositioned along the x- or y-axis by a precise value and the instruction is logged in the evaluation log.

Note: The **Shift** function requires that the curves are present in one chromatogram.

Move a curve with the Shift function

Follow the instructions below to use the **Shift** function:

Step	Action
1	<ul style="list-style-type: none">• Make sure that a chromatogram with the relevant curves is open in the Evaluation module.• Choose Operations:Shift. <p><i>Result:</i> The Shift dialog is displayed.</p>
2	<ul style="list-style-type: none">• Select the curve to be shifted in the Source chromatogram and curve list.• Select a curve position in the Target chromatogram and curve list.• Type a new Curve name or accept the default. The default curve name ending is SHFT.
3	<ul style="list-style-type: none">• Select the axis/axes along which the shift is to be made:<ul style="list-style-type: none">- along the X-axis (Shift retention)- along the Y-axis (Shift amplitude).• Type the shift value(s).• Click OK.
4	Repeat steps 1 to 3 to shift other curves in the chromatogram.

7 Evaluate and compile the results

7.4 Compare different runs

7.4.4 Create a mirror image

7.4.4 Create a mirror image

Instruction

A very useful way to compare the features of two curves is to produce a mirror image of one curve. To create a mirror image of a chromatogram:

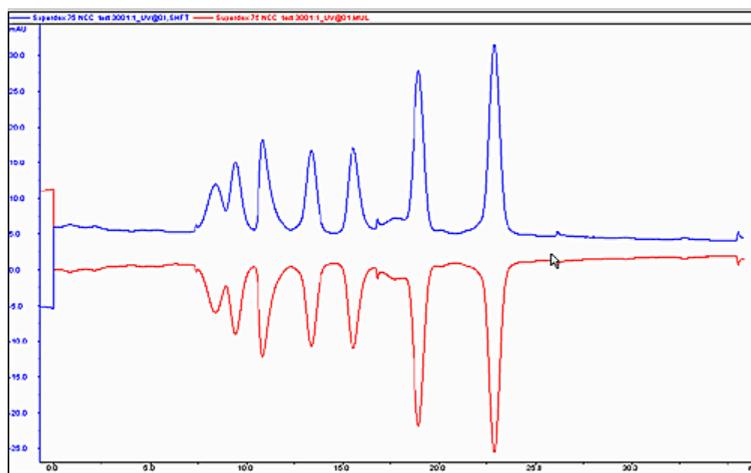
Step	Action
1	<ul style="list-style-type: none">• Open a chromatogram to insert the relevant curves into.• Choose File:Open to Compare:Curves. <p><i>Result:</i> The Open Curves to Compare dialog is displayed.</p>
2	<ul style="list-style-type: none">• Select the desired search criteria in the Folder, Result, Chromatogram and Curve drop-down list of the Curve selection section.• Click Search and a list of found curves will be displayed based on the selected search criteria. <p>Tip: <i>A new search can be performed with new search criteria without erasing curves located in the previous search.</i></p>
3	<ul style="list-style-type: none">• Select the checkboxes for the curves that you want to import.• Select Mirror in the Curve options field.• Click OK. <p><i>Result:</i> The curves are displayed with one of the curves as a mirror image in the active chromatogram window.</p>

Step Action**4 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 and curve** list.
- Select the same curve number in the **Target chromatogram and curve** list box as in step 2.
- Select the **Shift amplitude** checkbox since the shift is to be made along the Y-axis.
- Type a shift value.
- Click **OK**.

The illustration below shows a curve and a mirror image curve displayed.



7.5 Rename folders, results, chromatograms, curves and peak tables

Rename folders and files

Folders and files may be renamed in the **Result Navigator** either by

- Right-clicking the object and choosing **Rename** from the shortcut menu or
- Choosing **Edit:Rename:Navigator Object**

This will highlight the name of the selected object and you can type a new name instead.

Note: *You cannot rename assigned home folders.*

Rename chromatograms, curves and peak tables

Follow the instructions below 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 . <i>Result:</i> The Rename dialog opens.
2	<ul style="list-style-type: none">• Select the appropriate object.• Type a new name in the Name field.• Click OK.

Note: *The original chromatogram or original raw data curves cannot be renamed. They will not be available in the **Rename** dialog.*

7.6 Save results

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.

Delete unwanted curves

All the curves that you created during your manipulations will be saved in the chromatogram. If some of these curves are no longer needed, 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.*

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	<ul style="list-style-type: none"> Select File:Save. or Click the Save toolbar icon. 
In a new result file	<ul style="list-style-type: none"> 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.*

Exit the Evaluation module

The table below describes how to exit the **Evaluation** module:

If you want to...	then...
Close only the Evaluation module	Click the red cross icon in the top right corner of the module window.
Exit UNICORN start	Choose File:Exit UNICORN .

Note: *If there are unsaved changes, a dialog box opens with an option to save the changes before exit.*

7.7 Import and export results

About this section

Curves and data can be imported and exported in different formats. This chapter describes how to import and export results.

In this section

This chapter contains the following subsections:

Section	See page
7.7.1 Import results	220
7.7.2 Export results	222

7 Evaluate and compile the results

7.7 Import and export results

7.7.1 Import results

7.7.1 Import results

Import curves

Individual curves saved in ASCII format (as ***.asc** or ***.txt** files) may be imported into the active chromatogram. Follow the instructions below to import curves:

Step	Action
1	Choose File:Import:Curves . <i>Result:</i> The Import dialog box opens.
2	<ul style="list-style-type: none">Locate the file that contains the curve and click the file.Click the Open button. <i>Result:</i> The Import Curves dialog opens, showing the available curves in the file.
3	<ul style="list-style-type: none">Select the curve(s) to import.Click the OK button. <i>Result:</i> The curves are opened in the active chromatogram.

Note: A curve can only be imported in the base unit that it was exported in. For volume base, the curve is shown in chromatogram with ml and CV as x-axis. For time base, the curve is shown in in chromatogram with min as x-axis.

Import results

Results that have been exported from a UNICORN start database may be imported into the UNICORN start database again. Follow the instructions below to import results:

Step	Action
1	<ul style="list-style-type: none">Choose File:Import:Entire Result <i>Result:</i> The Import dialog opens.
2	<ul style="list-style-type: none">Locate and click the zip-file that contains the result. To select several files, press Shift while you click the files.Click the Open button. <i>Result:</i> The Import Result dialog opens.

Step	Action
3	<ul style="list-style-type: none"><li data-bbox="435 276 899 309">• Select the destination folder for the result.<li data-bbox="435 327 714 360">• Click the Import button. <p data-bbox="435 373 1191 433"><i>Result:</i> The result files are individually unzipped and imported into the selected folder.</p>
Note:	<p data-bbox="435 464 1191 525"><i>A result file cannot be imported into a folder if the folder already contains a result with the same name. In that case you must rename the file.</i></p>

7 Evaluate and compile the results

7.7 Import and export results

7.7.2 Export results

7.7.2 Export results

Introduction

This section describes how to export entire results and result items, including

- Curves
- Documentation
- Peak tables

The section also describes how to copy items using the Windows clipboard.

Export options

Select **File:Export** in the **Evaluation** module to export data from an open result file. The following export options are available:

If you choose...	Then the options are...
to UNICORN	<ul style="list-style-type: none">• Curves or <ul style="list-style-type: none">• Entire Result
Externally	<ul style="list-style-type: none">• Curves• Peak Table• Documentation or <ul style="list-style-type: none">• Copy to Metafile

These options are explained further below.

Export results

One or the several of the complete results may be exported for use in another UNICORN database. To export entire results:

Step	Action
1	<ul style="list-style-type: none"> • Select a result in the Result Navigator. To select several results, press Shift while you click the results. • Select File:Export:To UNICORN:Entire Result. <p><i>Result:</i> The Export to Another UNICORN Database dialog opens if one result was chosen. The Browse For Folder dialog opens if several results were chosen.</p>
2	<p>Result files are exported as compressed .zip files only.</p> <ul style="list-style-type: none"> • Select an export location. Make a new folder if desired. • Type a name in the File name field in the Export to Another UNICORN Database dialog if one result was chosen. <p>Note:</p> <p><i>As the results are exported individually, it is not applicable to enter a destination file name if several results are selected for export.</i></p>
3	<ul style="list-style-type: none"> • Click Save in the Export to Another UNICORN Database dialog or OK in the Browse For Folder dialog. <p><i>Result:</i> The result is saved as a zip file. If several results were exported, each result is saved as an individual zip file.</p>

7 Evaluate and compile the results

7.7 Import and export results

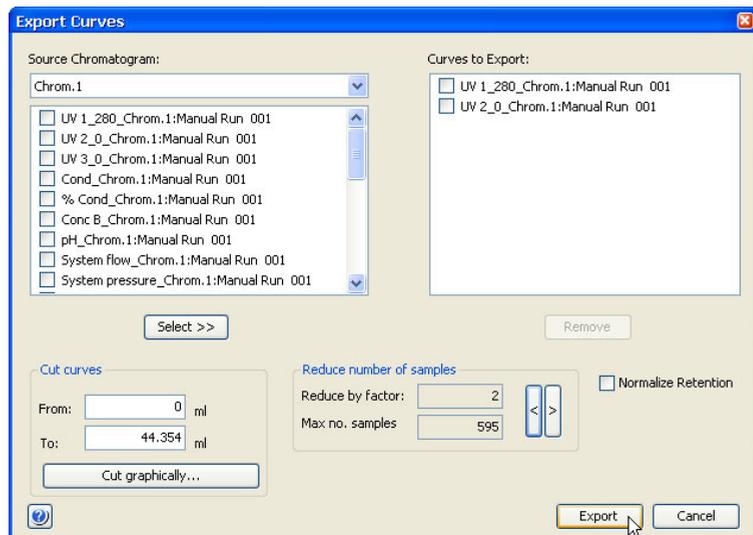
7.7.2 Export results

Export curves

Follow the instructions below to export curves in the **Evaluation** module.

Step	Action
------	--------

- | | |
|---|--|
| 1 | <ul style="list-style-type: none">• Choose File:Export:To UNICORN:Curves.or• Choose File:Export:Externally:Curves <p><i>Result:</i> The Export Curves dialog opens.</p> |
|---|--|



- | | |
|---|--|
| 2 | <ul style="list-style-type: none">• Select the curve(s) you want to export.• Enter parameters to limit the curve(s) if necessary (described in "Limit the exported curves" below).• Click the Select >> button.• Repeat this step to select more curves. |
| 3 | <p>Click the Export button.</p> <p><i>Result:</i> The Export Curves to File dialog opens.</p> |

Step	Action
4	Select the export file format from the Save as type drop-down list. <ul style="list-style-type: none"> • ASCII files (*.asc) or • Comma Separated Values (*.csv)
5	<ul style="list-style-type: none"> • Select a destination folder. • Type a file name and click Save.

Note: *Curves are exported as series of numerical coordinates that refers to the time/volume and signal respectively.*

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. The table below describes how to use these editing options.

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 a graphical Export Curves dialog. Move the vertical markers to the correct cut-off points. <div data-bbox="602 1099 1187 1485" data-label="Figure"> </div>
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.

7 Evaluate and compile the results

7.7 Import and export results

7.7.2 Export results

Option	Instruction
Normalize retention	Select the Normalize retention check box to have all exported curves normalized to a common X-axis.

Export peak tables

Follow the instructions below to export peak tables.

Step	Action
------	--------

- | | |
|---|---|
| 1 | Choose File:Export:Externally:Peak Table .
<i>Result:</i> The Export Peak Table dialog opens. |
| 2 | <ul style="list-style-type: none">• Select the source chromatogram and the peak table you want to export.• Click the Export button. <i>Result:</i> The Export Peak Table To File dialog opens. |
| 3 | Select the export file format from the Save as type drop-down list: <ul style="list-style-type: none">• ASCII files (*.asc)• Excel files (*.xls)• XML files (*.xml) |
| 4 | <ul style="list-style-type: none">• Select a destination folder.• Type a file name.• Click Save. |

Note: *Peak tables are exported as text strings in ASCII or XML format and numerical values in the Excel format.*

Export the chromatogram to a Windows meta file

You can export an image of the open chromatogram as a Windows enhanced metafile. This image format can be used in other applications, for example *Microsoft™ Word* or *PowerPoint™*. To export a chromatogram to a Windows meta file:

Step	Action
1	<ul style="list-style-type: none">• Choose File:Export:Externally:Copy to Metafileor• Right-click in the chromatogram and choose Copy to Metafile <p><i>Result:</i> The Export Chromatogram to Windows Enhanced Metafile dialog opens. The only available file type is Enhanced Meta File (*.emf)</p>
2	<ul style="list-style-type: none">• Select a destination folder.• Type a file name.• Click Save.

Copy to the clipboard

You can use the Windows clipboard to copy the contents of the active window and paste it into other programs. To copy to the clipboard:

Step	Action
1	<ul style="list-style-type: none">• Choose Edit:Copyor• Right-click and choose Copy to Clipboardor• Click the Copy icon



Result:

- If the active window only contains a chromatogram, it will immediately be copied to the clipboard as a Windows metafile.
- If the active window also contains a peak table, the **Copy to Clipboard** dialog opens.

7 Evaluate and compile the results

7.7 Import and export results

7.7.2 Export results

Step	Action
2	Select one of the options: <ul style="list-style-type: none">• <i>Copy curve window as enhanced meta file</i>• <i>Copy selected peak table data as tabbed text</i>
3	Click the OK button.

8 USB Export and Import Operations with ÄKTA start

About this chapter

This chapter describes how to export a method from ÄKTA start and how to import results generated with ÄKTA start (without UNICORN start).

In this chapter

This chapter contains the following sections:

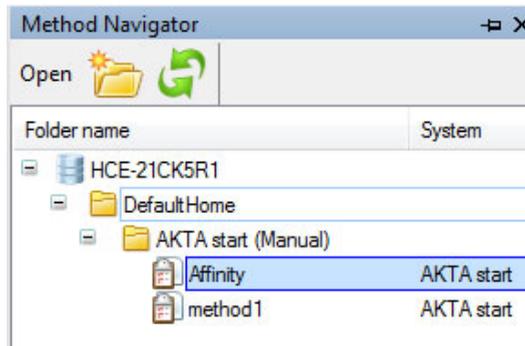
Section	See page
8.1 Export a method to a USB memory stick	230
8.2 Import results from a USB memory stick	231

8.1 Export a method to a USB memory stick

Instructions

Follow the instructions below to export a method to a USB memory stick:

- | Step | Action |
|------|--|
| 1 | In the Method Navigator pane, select the method to be exported. It is only possible to export one method at a time to an external file. |



- | | |
|---|---|
| 2 | Choose File:Export:Export Method To USB .
<i>Result:</i> The File Explorer dialog opens. |
| 3 | Locate the USB flash drive and enter a file name. |
| 4 | Click the Save button. |

8.2 Import results from a USB memory stick

Instructions

Results can also be imported from a ÄKTA start instrument into the UNICORN start database using a USB stick. To import results from a USB stick:

Step	Action
1	Select File: Import: Import Result from USB . <i>Result:</i> The Import dialog opens.
5	<ul style="list-style-type: none">• Locate the USB drive.• Locate and click the zip-file that contains the result. To select several files, press Shift while you click the files.• Click the Open button. <i>Result:</i> The Import Result dialog opens.
6	<ul style="list-style-type: none">• Select the destination folder for the result.• Click the Import button. <i>Result:</i> The result files are individually unzipped and imported into the selected folder.

9 Administration

In this chapter

This chapter describes the system and database administration aspects of UNICORN start. It contains the following sections:

Section	See page
9.1 System administration	233
9.2 Database administration	239

9.1 System administration

Purpose

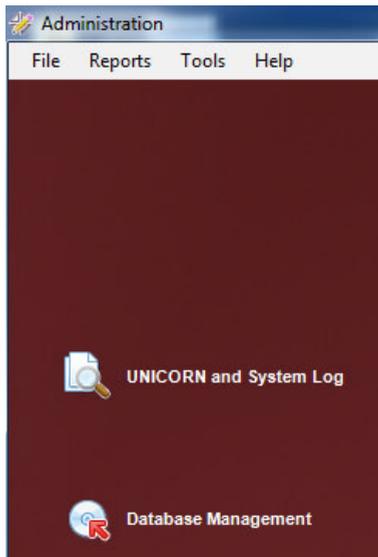
UNICORN start, system, run and result related logs provide the system administrator with a full record of UNICORN start usage and system activity.

Different types of logs

There are two types of logs:

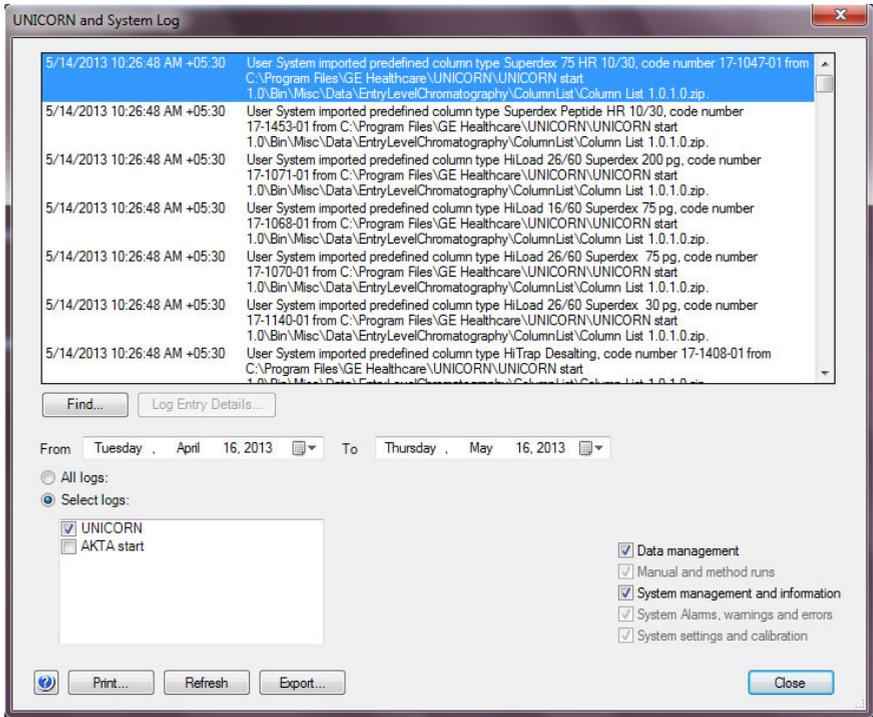
- UNICORN start log
which records all system independent events,
- System log
which records events related to the ÄKTA start.

UNICORN start and system logs are available in the UNICORN start **Administration** module, by clicking the **UNICORN and System Log** icon.



UNICORN and System Log dialog

The **UNICORN and System Log** dialog is illustrated next.



Tip: Click the **Refresh** button to update the displayed events to the latest records.

View selected log entries

By default, the **UNICORN and System Log** dialog shows all the log entries for UNICORN start, ÄKTA start up to a month before the current date. Specific log entries can be selected so that only the items of interest are displayed. It is also possible to combine selections to narrow down the displayed items, for example, to the log entries for a specific user at a specific system on a specific date.

The table below describes how select specific log entries for display:

To view log entries...	Then...
From a specific time period	<ul style="list-style-type: none"> • Click the down arrow by the From field and select the starting date for the time period in the calendar and • Click the down arrow by the To field and select the ending date.
For specific systems	<ul style="list-style-type: none"> • Click the Select logs option button and • Select UNICORN from the list below.
From the UNICORN log	<ul style="list-style-type: none"> • Click the Select logs option button and • Select UNICORN from the list below.
For specific events	<p>Select one or several of the items below:</p> <ul style="list-style-type: none"> • Data management (folder, method and result activities, for example, create, copy, changes, etc.) • Manual and method runs (run start and end, created results, etc.) • System management and information (connections, session start and end, create new, activate or deactivate systems, etc.) • System alarms, warnings and errors (alarm and error descriptions, acknowledgement and action records, etc.) • System settings and calibration (changed settings and calibration actions)

Long log entries

Long entries in the logs may sometimes be shown only in part, ending the entry with four period characters (i.e., "...."). The full entry can be viewed by double-clicking the **Log Entry Details** button.

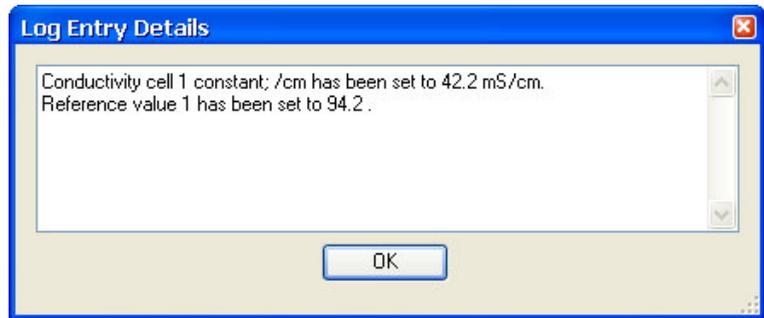
Step	Action
------	--------

1	Select the entry and double-click the Log Entry Details button.
---	--

Tip:

This button is enabled when a log entry is selected.

2	The full log entry is shown in a separate dialog.
---	---

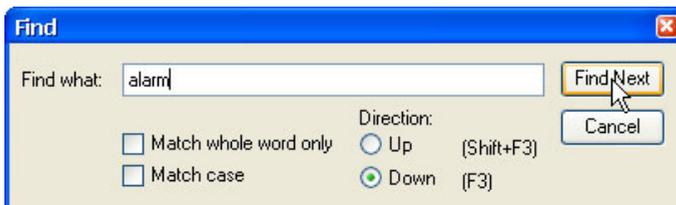


Tip:

The complete entries are included in printed logs.

Find specific log entries

You can search for specific log entry text by clicking the **Find** button and entering text to search for in the **Find what** field of the **Find** dialog:



Export logs

You can export the selected log entries to an XML file by clicking the **Export** button. Using the XML file format, the log entries can be imported into XML-based reporting systems.

Archive logs

To reduce the size of the logs, older records can be archived. The archiving procedure is part of the **Database Management** routines, and it is described in *Archive data*, on page 246. The archived logs can be retrieved by using the **Database Management** retrieval procedure.

Note: *It is possible to retrieve logs to the same database that they were originally archived from. That is, the retrieval procedure cannot be used to import logs from one database to another.*

Note: *It is recommended that all archived logs be saved on a different hard drive than where the active database is stored.*

Print the logs

Follow the instructions below to print selected log entries:

Step	Action
1	Select the log entries to print.
2	Click the Print button. <i>Result:</i> The Print dialog opens.

Step **Action**

- 3
- Select a printer from the drop-down list.
 - If desired, click the **Properties** button and select printer settings and
 - Click the **OK** button.

Result: The selected log entries are printed.

```
UNICORN start 1.0                                     1 (49)
User: Default 9/20/2012 8:51:21 PM +05:30
                                UNICORN and System logs

UNICORN and System logs
Selected date interval: 9/20/2012 12:00:00 AM +05:30 to 9/20/2012 8:49:48 PM
+05:30

Selected log(s):
UNICORN

No selection on users.
Selected log entry type(s):
Data management
System management and information

8/31/2012 3:45:50 PM +05:30:
User System imported predefined column type Superdex 75 HR 10/30, code number
17-1047-01 from C:\Program Files\GE Healthcare\UNICORN\UNICORN start 1.0
\Bin\Misc\Data\EntryLevelChromatography\ColumnList\Column List 1.0.1.0.zip.
8/31/2012 3:45:50 PM +05:30:
User System imported predefined column type Superdex Peptide HR 10/30, code
number 17-1453-01 from C:\Program Files\GE Healthcare\UNICORN\UNICORN start
1.0\Bin\Misc\Data\EntryLevelChromatography\ColumnList\Column List
1.0.1.0.zip.
8/31/2012 3:45:50 PM +05:30:
User System imported predefined column type HiLoad 26/60 Superdex 200 pg,
code number 17-1071-01 from C:\Program Files\GE Healthcare\UNICORN\UNICORN
start 1.0\Bin\Misc\Data\EntryLevelChromatography\ColumnList\Column List
1.0.1.0.zip.
8/31/2012 3:45:50 PM +05:30:
User System imported predefined column type HiLoad 16/60 Superdex 75 pg, code
number 17-1068-01 from C:\Program Files\GE Healthcare\UNICORN\UNICORN start
1.0\Bin\Misc\Data\EntryLevelChromatography\ColumnList\Column List
1.0.1.0.zip.
8/31/2012 3:45:50 PM +05:30:
```

9.2 Database administration

About this section

This section describes the standard database solution used for data storage and the maintenance procedures required to operate the database.

This section also contains a general instructions on how to upgrade the database server software to editions with greater data handling capacity.

In this section

This chapter contains these sections.

Section	See page
9.2.1 Database overview	240
9.2.2 Database maintenance	241

9.2.1 Database overview

About the UNICORN database

Data storage in UNICORN start is handled by a database. The database is a relational database. SQL (Structured Query Language) is the querying language used for the retrieval, insertion, updating, deleting and general management of the data.

The UNICORN start database server installation for a stand-alone workstation includes a distributable version of Microsoft SQL Server Express. It is described in *SQL Server Express facts* below.

The installation and basic set up of the database is an integrated part of the installation sequence for the options including the database server functions. It will not require any user settings other than the initial selection of the option and selection of destination folders for backup and archives.

Normally, the database maintenance functions offered by UNICORN start are sufficient for the UNICORN start administrator. Using management tools such as SQL Server Management Studio should not be necessary and it is not recommended since improper changes may corrupt the database.

SQL Server Express usage with UNICORN start

The list below includes some properties of Microsoft SQL Server Express distributed with UNICORN start:

- Primarily suited for small-scale applications.
 - Up to 1 GB of server RAM can be utilized.
 - Utilizes one single physical CPU.
 - Operates databases up to 10 GB, excluding log files.
-

9.2.2 Database maintenance

Once the database is installed and a system is running and producing data, it is essential that routines and schedules for backup and archiving of data are established. This section describes how to perform these routines.

Database backup

During the database server installation, a target folder for the regular, scheduled backup was set up. By default, backups were also scheduled at the installation. The default backup settings are:

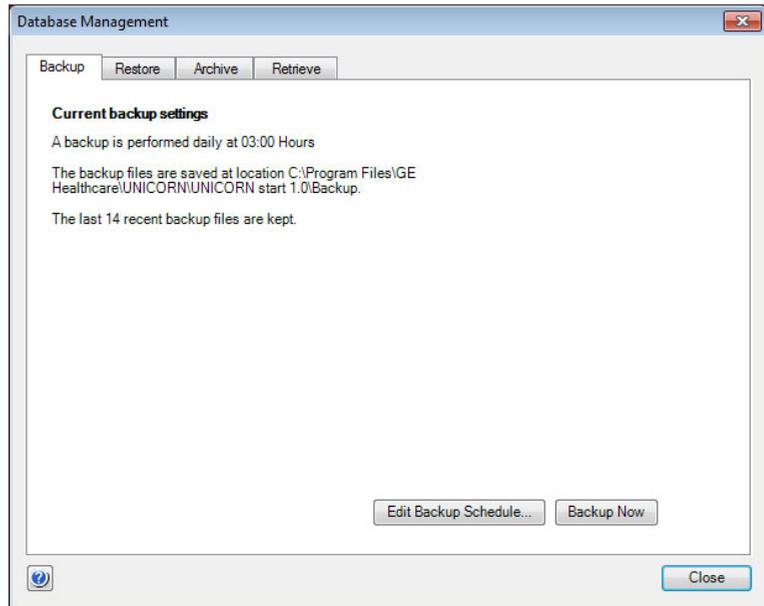
- Daily backup
 - At 03:00 local time
 - In the selected target folder
 - With the last 14 recent backup files kept.
(when additional files are saved, the oldest backup is deleted)
-

Verify or edit the database backup schedule

Follow the instructions below to schedule the database backup:

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the Database Management icon in the Administration module.
<i>Result:</i> The Database Management dialog opens. |
|---|---|



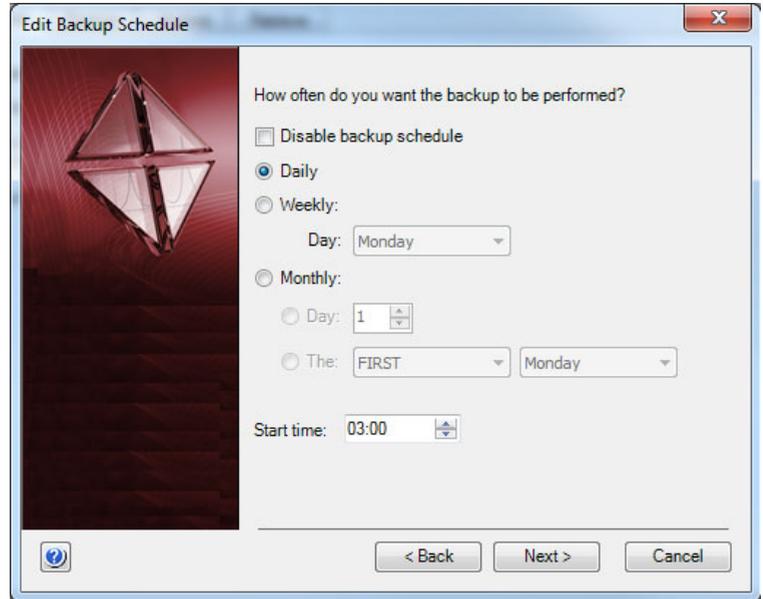
Tip:

The **Backup** tab shows the current schedule. If the settings are suitable, click the **Close** button at this point.

- | | |
|---|---|
| 2 | Click the Edit Backup Schedule button.
<i>Result:</i> The Edit Backup Schedule wizard opens. |
|---|---|

Step **Action**

- 3 Click the **Next** button to open the next wizard dialog:



- 4 Select the frequency and the start time for the scheduled backups.

Note:

It is possible to disable the scheduled backups at this point.

- 5 Click the **Next** button to proceed.

- 6 Choose the number of backup files that are to be kept.

Note:

It is possible to keep all files. However, depending on the number of results that are created, this may cause disk space problems.

- 7 Click the **Next** button to proceed.

The final wizard page shows a summary of the selected settings.

- 8 Click the **Finish** button to accept and apply the schedule.

Note:

The backup files will be named UNICORN_SCHEDULED_BACK-UP_<Date>_<Time>.BAK by default.

Note: *It is recommended that another physical drive than where the active database is stored is selected for the scheduled backups at the installation of UNICORN start. If this is not possible, it is recommended that the backup files are copied to another physical drive or storage media at regular intervals.*

Note: *Make sure that the database server computer is turned on at the time when the scheduled backup will occur. If it is not turned on and operational, the backup will fail, it will not be postponed.*

Manual backup

Extra backups can be made manually between the scheduled backups. Follow the instructions below to make extra backups:

Step	Action
1	Click the Database Management icon in the Administration module. <i>Result:</i> The Database Management dialog opens.
2	Click the Backup Now button in the Backup tab. <i>Result:</i> A Backup Now confirmation dialog opens.
3	Click the OK button to start the backup. <ul style="list-style-type: none">• This process may take several minutes. A progress dialog is displayed while the backup is performed. <i>Result:</i> A Backup confirmation dialog opens when the backup is completed. The backup file will be named UNICORN_MANUAL_BACK-UP_<Date>_<Time>.BAK by default.
4	Verify that the backup has been performed in the default folder and then either <ul style="list-style-type: none">• Click the Close buttonor• Click Go To Backup File to open the backup folder and access the files.

Tip: *It is recommended to copy or move the backup files to an external storage device to minimize the risk for loss of data. The backup also includes the database content and log reports of UNICORN start.*

Restore backup data

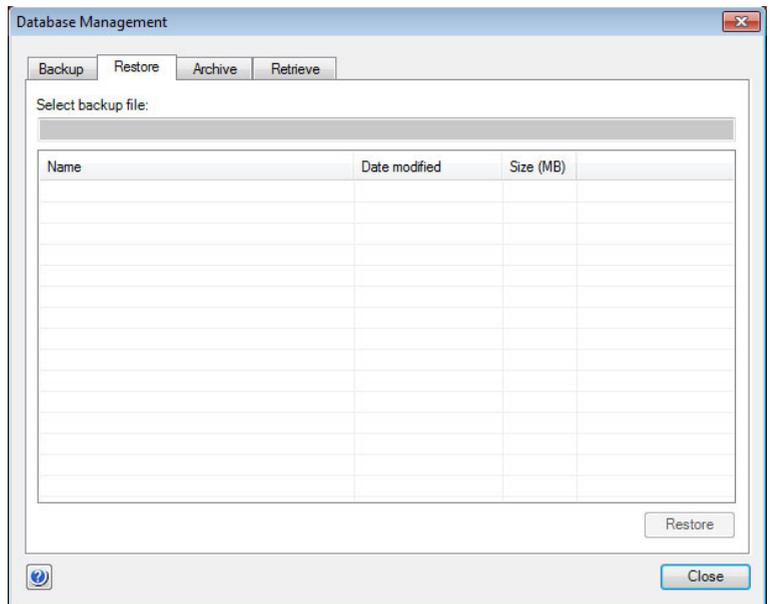
It is possible to restore data from a previous backup, for example, if data has been deleted by mistake. The restored data replaces all the current data in the database with the data generated up to the point of time when the backup was made. All changes performed after the time of the backup will be lost.

This procedure can be used to move data from one database instance to another but all data in the database instance where the backup is restored will be overwritten.

Follow the instructions below to restore data from a previous backup:

Step	Action
------	--------

- | | |
|---|---|
| 1 | Click the Database Management icon in the Administration module.
<i>Result:</i> The Database Management dialog opens. |
| 2 | Click the Restore tab. All the available backup files are shown in the displayed list. |



- | | |
|---|--|
| 3 | Select the backup file to restore and click the Restore button.
<i>Result:</i> A warning dialog opens. This dialog suggests that a backup of the database be made before restoring the previous backup. This ensures that all the essential data is saved before it is replaced with the data from the backup. |
|---|--|

Step	Action
4	<p>Either</p> <ul style="list-style-type: none">• Click the Yes button to backup before restoringor• Click the No button to proceed without making a new backup. <p><i>Result:</i> A second warning dialog opens. This dialog shows the backup file that is restored and explains the implications for the current data. All other UNICORN start modules are closed and no other actions may be performed during the restoration.</p>
5	<p>Click the OK button to proceed.</p> <p><i>Result:</i> A progress dialog is displayed while the restoration is performed. The process is complete when the dialog closes.</p>

Archive data

When the database size reaches a level where performance is affected and the remaining space is not enough for the immediate storage needs, selected parts of the result data can be archived. This can either be the entire result contents from before a specified date, or selected results. It is also possible to archive logs. It is normally a good idea to archive data that is not immediately needed at regular intervals.

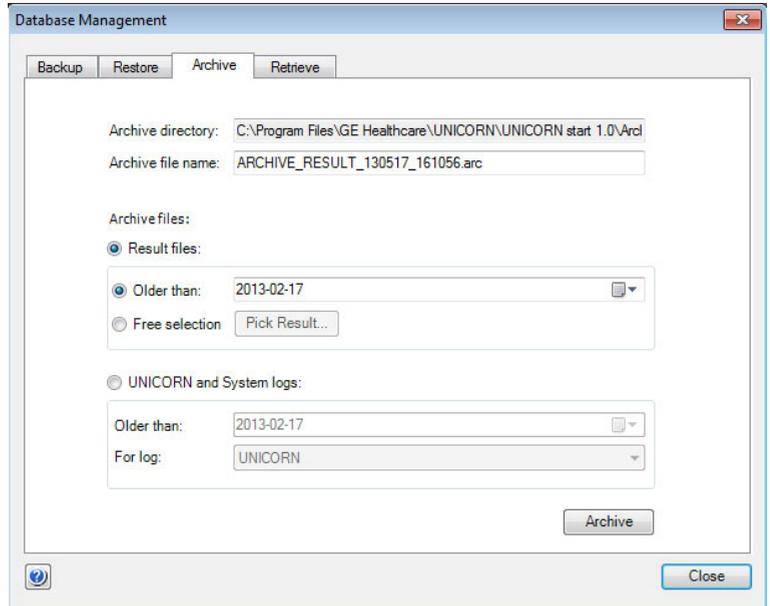
Archiving will reduce the size of the database. It is not the same as exporting result data, which creates copies of the results and does not reduce the size of the database at all.

Follow the instructions below to archive complete result data or logs:

Step	Action
1	<p>Click the Database Management icon in the Administration module.</p> <p><i>Result:</i> The Database Management dialog opens.</p>

Step **Action**

2 Click the **Archive** tab.



The archive directory and a suggested archive file name are shown in the dialog. The directory was selected at the installation and cannot be edited here. However, the file name can be changed. The default name suggested by UNICORN start includes the date of archiving, and reflects if the selected content is results or logs.

3 Choose the data to be archived:

- If **Result files** is selected
 - then choose a latest date from the drop-down list calendar
 - or
 - select **Free selection** and click the **Pick Result** button.
(this option is described in *Archive selected results* below)

If **UNICORN and System log** is selected

- Then choose a latest date from the drop-down list calendar and
- Select the log to archive - UNICORN or a system log - from the **For log** list.

Step	Action
4	<p>Click the Archive button.</p> <p><i>Result:</i> A warning dialog opens. This dialog suggests that a backup of the database be made before archiving the selected data.</p> <p>Note: <i>It is recommended to always perform a backup before archiving.</i></p>
5	<p>Either</p> <ul style="list-style-type: none">• Click the Yes button to backup before restoring or• Click the No button to proceed without making a new backup. <p><i>Result:</i> A progress dialog opens while the data is archived. Depending on the selections above, this may take several minutes. When archiving is completed, a confirmation dialog opens.</p>
6	<p>Click the Close button to close the confirmation dialog.</p>

Note: *It is recommended to save the archived data on another storage media than where the active database is stored.*

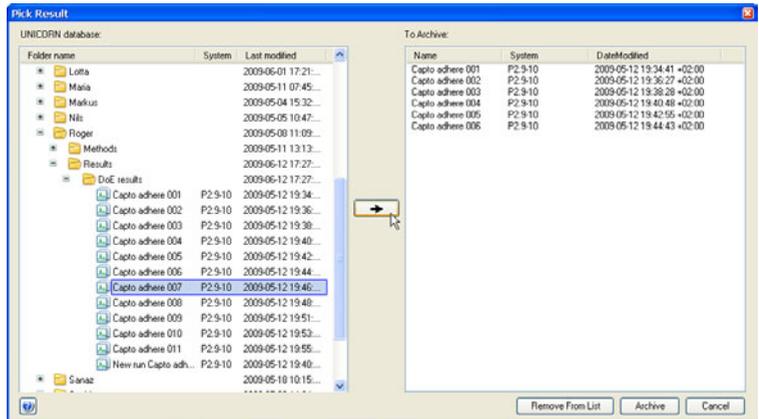
Archive selected results

Follow the instructions below to select individual results for archiving:

Step	Action
1	<p>In the Database Management dialog, select the Archive tab.</p> <ul style="list-style-type: none">• Select Result files• Select Free selection and• Click the Pick Result button <p><i>Result:</i> The Pick Result dialog opens.</p>

Step **Action**

- 2 In the **Pick Result** dialog:
- Locate and select the result to archive
and
 - Click the arrow button to add it to the **To Archive** list.



- 3 Repeat step 2 until all the results have been added to the archive list.
- It is possible to remove results from the list by selecting them and clicking the **Remove From List** button.
- 4 Click the **Archive** button.
- Result:* A warning dialog opens. This dialog suggests that a backup of the database be made before archiving the selected data.
- 5 Either
- Click the **Yes** button to backup before restoring
or
 - Click the **No** button to proceed without making a new backup.
- Result:* A progress dialog opens while the data is archived. Depending on the selections above, this may take several minutes. When the archiving is completed, a confirmation dialog opens.
- 6 Click the **Close** button to close the confirmation dialog.

Retrieve archived data

Results and logs that have been archived can be retrieved from the archive and included in the active database again. Individual results are selected from the archive directory, not the entire archive. However, when logs are retrieved, the entire log is restored.

Note: *Only archived results and logs can be retrieved back into the same database that they were originally archived from. This procedure cannot be used to migrate data from one database to another.*

Tip: *Archived results can be shown in the **Evaluation** module **Result Navigator** by selecting the **Show All** option. This can help in locating the archived results to be retrieved. Archived results are represented by a special icon:*

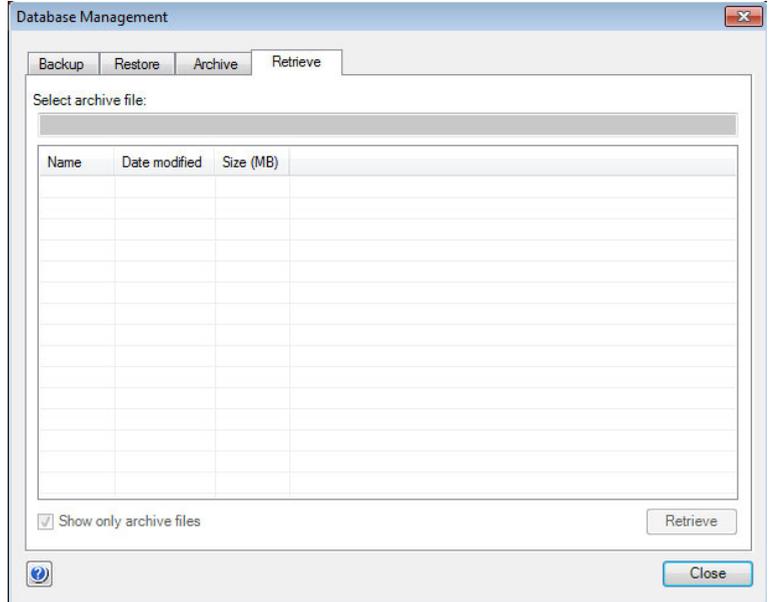


Follow the instructions below to

Step	Action
1	Click the Database Management icon in the Administration module. <i>Result:</i> The Database Management dialog opens.

Step **Action**

- 2 Click the **Retrieve** tab.

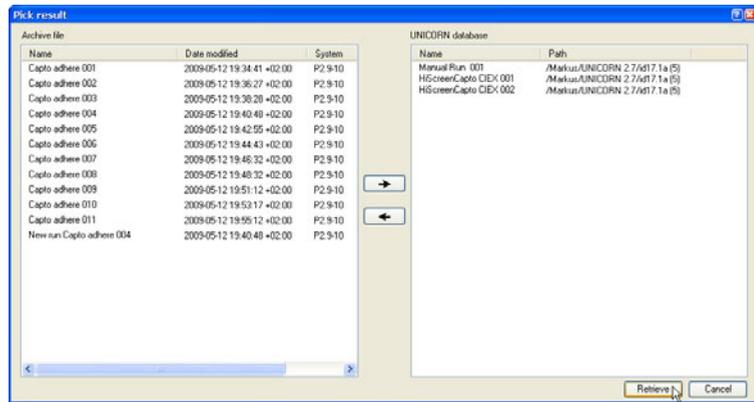


Note:

Archived results or logs can be retrieved. All archived files have the extension `.arc`. The **Show only archived files** option displays only these files. By deselecting this option additional files that have the extension `.log` are also displayed. These log files record the archiving operations and are not connected to the actual UNICORN start results or logs.

Step **Action**

- 3 Select the archive file to be retrieved and click the **Retrieve** button.
- If a log has been selected to be retrieved:
- The log is restored to the active database and a confirmation dialog is shown.
(This completes the retrieving process.)
- If archived results has been selected to be retrieved:
- The **Pick Result** dialog opens.
(Proceed with the steps below.)



- 4 Select an archived result in the **Archive file** list and click the right arrow button.
- Result:* The result is moved to the **UNICORN database** list. The destination folder search path is shown in the list. It is possible to remove selected results from the **UNICORN database** list by clicking the left arrow button.
- 5 Click the **Retrieve** button in the **Pick Result** dialog.
- Result:* The results are restored to the active database and a confirmation dialog is displayed.
-

Note: *The retrieved results are restored to the folder where they were originally archived from. All archived material is, however, still saved in the archive.*

10 Troubleshooting

About this chapter

This chapter describes different operational scenarios which may arise in UNICORN start and their solutions or consequences.

In this chapter

This chapter contains the following sections:

Section	See page
10.1 Installation and uninstallation	254
10.2 System connections	255
10.3 System access errors	257
10.4 Database functions	258
10.5 Evaluation errors	259

10.1 Installation and uninstallation

Problem description	Solution
SQL Server Express or .net framework installation fails.	<ul style="list-style-type: none"> • Make sure that any other installation or an operating system upgrade is not in progress. Retry the installation after the existing installation is complete. Alternatively, try restarting the computer and install again. • If the above fails in addition check the following locations for additional clues on SQL Server failure: C:\Program Files\Microsoft SQL Server\100\Setup Bootstrap\Log\Summary.txt or for 64 bit operating system C:\Program Files (x86)\Microsoft SQL Server\100\Setup Bootstrap\Log\Summary.txt • SQL Server installation can fail due to an incompatible Operating System, a pending reboot, incompatible pre-existing SQL Server version among other reasons.
Unable to configure a license.	<ol style="list-style-type: none"> 1 Open the .lic license file and note the address mentioned as the value for HOSTID. 2 Compare this value to the address generated by the configure e-license tool found at Start:Programs:GE Healthcare menu. 3 If none of the addresses match the HOSTID value you may have an incorrect file or the file was generated for another machine. Contact your GE Healthcare representative to generate a new license file.
Prerequisite software fail to install.	Make sure that there is enough disk space on the C:\ drive.
An error message stating that your access rights are insufficient appears when you try to uninstall UNICORN start from the Windows 7 Add or Remove Programs dialog.	This is caused by Windows 7 UAC. Turning off UAC may solve this problem. If not, use the software removal procedure from the UNICORN start installation DVD.

10.2 System connections

Problem description	Solution
<p>The connections are not available even though:</p> <ul style="list-style-type: none"> • The connection between the PC and the ÄKTA start instrument appears to be correct. • The power is turned on. 	<ul style="list-style-type: none"> • Unplug and then replug the USB cable, wait for a minute and try to reconnect from UNICORN start. • If the above solution fails: <ol style="list-style-type: none"> 1 Switch off the ÄKTA startinstrument. 2 Exit UNICORN start. 3 Restart the computer. 4 Restart the ÄKTA startinstrument. 5 Log on to UNICORN start and try to connect again. • If the connection still does not work, run the UNICORN Diagnostics Manager from the folder All Programs:GE Health-care:UNICORN start 1.0, for description see section Running the Diagnostics manager.
<p>You are connected to a UNICORN start system but have no contact with the ÄKTA start instrument. The system hangs at initialization.</p>	<ul style="list-style-type: none"> • Check that the instrument is turned on. • Check that all cable connections are intact.
<p>The UNICORN start system connection is lost when the PC\Laptop goes to sleep or the laptop lid is closed.</p>	<p>Turn of Windows power save options to prevent this from happening.</p>

Running the *Diagnostics manager*

Start the UNICORN start **Diagnostics Manager** from the folder **All Programs:GE Healthcare:UNICORN start 1.0.** The following statuses will be displayed:

Status	Description
Port	<p>If the port status is not displayed in green, it may indicate that some other application is blocking the ports reserved for UNICORN start communication.</p> <p>The process name which is blocking the port will be displayed.</p>
Service	<p>If the service status is not displayed in green, it may indicate that the service used for UNICORN start communication and data base operations is not running.</p> <p>Try to restart the service from the application.</p>
USB	<p>Examples of status messages:</p> <ul style="list-style-type: none">• USB device is working properly.• USB device cannot work properly until the computer is restarted.• USB device is disabled. <p>Note:</p> <p><i>There are often multiple USB devices connected to the computer and a failure indication here might not indicate a failure in connecting to ÄKTA start.</i></p> <p><i>Try the following:</i></p> <ul style="list-style-type: none">• <i>Unplug and replug the USB cable.</i>• <i>Restart the computer.</i>

10.3 System access errors

Problem description	Solution
<p>UNICORN start modules are not available for selection in the Log On dialog.</p>	<p>Check that the modules in question are not already open.</p>
<p>The help viewer cannot be opened using help buttons or the F1 key.</p>	<ol style="list-style-type: none"> <li data-bbox="783 438 1186 493">1 Open the MadCap HelpViewer from the Windows desktop icon.  <ol style="list-style-type: none"> <li data-bbox="783 657 1186 684">2 Try the Help button or F1 key again.
<p>The Microsoft Office Document Image Writer causes UNICORN start to terminate.</p>	<p>This writer application will not work. Set another printer driver as the default printer.</p> <ol style="list-style-type: none"> <li data-bbox="783 820 1186 875">1 Open the Windows Control Panel (in Category View). <li data-bbox="783 902 1186 930">2 Choose Hardware and Sound. <li data-bbox="783 957 1186 984">3 Choose Devices and Printers. <li data-bbox="783 1011 1186 1084">4 Right-click on an alternate printer and choose Set as default printer from the menu.

10.4 Database functions

Problem description	Solution
The database is not available at log on	<ul style="list-style-type: none">• Wait a couple of minutes to allow the SQL Server services to start and try to log on again. (This may be necessary when logging on after a computer re-start)• Verify in the Windows Services dialog that the SQL Server and SQL Server Browser services are running properly.
The scheduled database backups are not performed	Re-set the scheduled backup in the Database backup wizard.
Unable to connect to the database server.	Verify in the Windows Services dialog that the SQL Server service MSSQL\$UNICORNSTART and the SQL Server Browser service are running. <ul style="list-style-type: none">• If the services are <i>not</i> running, try to start them.• If the services are running, try to restart the same.

10.5 Evaluation errors

Maximum number of curves exceeded

Problem description	Solution
The maximum number of curves (100) are exceeded when importing curves	The injection curve is also included for each curve that is imported. Delete the unnecessary curves before importing more curves.

Distorted peaks

Problem description	Solution
Peaks appear distorted	A high UV averaging time value will distort and delay the peaks.

Retention time differences

Problem description	Solution
The retention time in the peak table differs from that in the chromatogram.	Verify that the integration was performed on the currently displayed chromatogram. Note: <i>Only applicable if there are more than one chromatogram in the result.</i>
The retention value in the peak label differs from that in the x-axis.	Verify that the x-axis base has not changed after the peak integration was performed, but is the same as in the peak table. This is applicable for ml and CV bases.

Problem description	Solution
The retention value is not displayed in the peak label.	<ul style="list-style-type: none"> • Verify that the x-axis base has not changed after the peak integration was performed, but is the same as in the peak table. This is applicable for volume and time bases. • Verify that the chromatogram has not been changed, for example by selecting or deselecting Adjust retention zero to injection number in the Customize dialog, X-Axis tab.

General evaluation errors

Problem description	Solution
Computer restarts abruptly.	If it is a 64 bit computer\laptop there are known incompatibilities between Intels graphic driver (igdkmd64.sys) and Windows 7. Please ask your administrator to update to the latest graphics driver .

Note: *In case you are unable to resolve your problem, please contact your local GE Healthcare representative.*

Note: *For UNICORN start product updates please visit:
www.gelifesciences.com/unicornstart.*

Appendix A Evaluation functions and instructions

Introduction

This appendix contains background information about the algorithms and calculation theories applied in UNICORN.

It also describes the peak table column components and the instructions that are available for automated evaluation procedures.

In this appendix

This appendix contains these sections:

Section	See page
A.1 Smoothing algorithms	262
A.2 Baseline calculation theory	265
A.3 Peak table column components	271

A.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. <ul style="list-style-type: none">The width of the window is determined by the Filter Parameter value, expressed as number of data points.
2	When the source point is less than half the window size from the beginning or the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible. <ul style="list-style-type: none">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 adding the number 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 filter parameter value and added to the current source data point.

Stage	Description
3	<p>The result is then divided by the parameter value plus 1 according to the following formulae:</p> $t_1 = S_1$ $t_n = \frac{(p \cdot t_{n-1} + S_n)}{(p+1)}$ <p>Where:</p> <p>t_n = current processed point</p> <p>t_{n-1} = previous processed point</p> <p>S_n = current source point</p> <p>p = smoothing parameter value</p> <p>Note: <i>If you increase the parameter value, the smoothing effect is also increased.</i></p>
Note:	<i>The filter algorithm only accepts integer parameter values between 1 and 25.</i>

Median

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

Stage	Description
1	<p>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.</p> <ul style="list-style-type: none"> The width of the window is determined by the parameter value, expressed as number of data points.
2	<p>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.</p> <ul style="list-style-type: none"> 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.

A Evaluation functions and instructions

A.1 Smoothing algorithms

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 adding the number one (1).*

Savitzky-Golay

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

Stage	Description
1	<p>The algorithm is based on performing a least squares linear regression fit of a polynomial of degree k over at least $k+1$ data points around each point in the curve to smooth the data.</p> <p>The derivative is the derivative of the fitted polynomial at each point.</p> <p>The calculation uses a convolution formalism to calculate 1st through 9th derivatives.</p>
2	<p>The calculation is performed with the data in low X to high X order.</p> <p>If the input trace goes from low to high, it is reversed for the calculation and is re-reversed afterwards.</p>
Tip:	<p><i>See Gorry, Peter A, General Least-Squares Smoothing and Differentiation by the Convolution (Savitsky-Golay) Method (Analytical Chemistry 1990, Volume 62, 570-573) for information about the Savitzky-Golay algorithm.</i></p>

A.2 Baseline calculation theory

Introduction

There are two methods to calculate the baseline, based on a morphological and classic algorithm.

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.

Tip: *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.*

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:

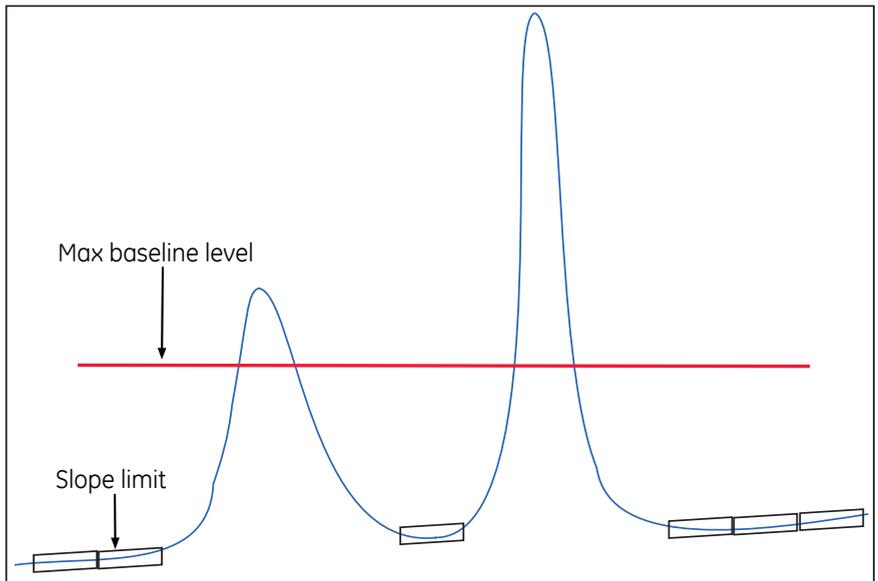
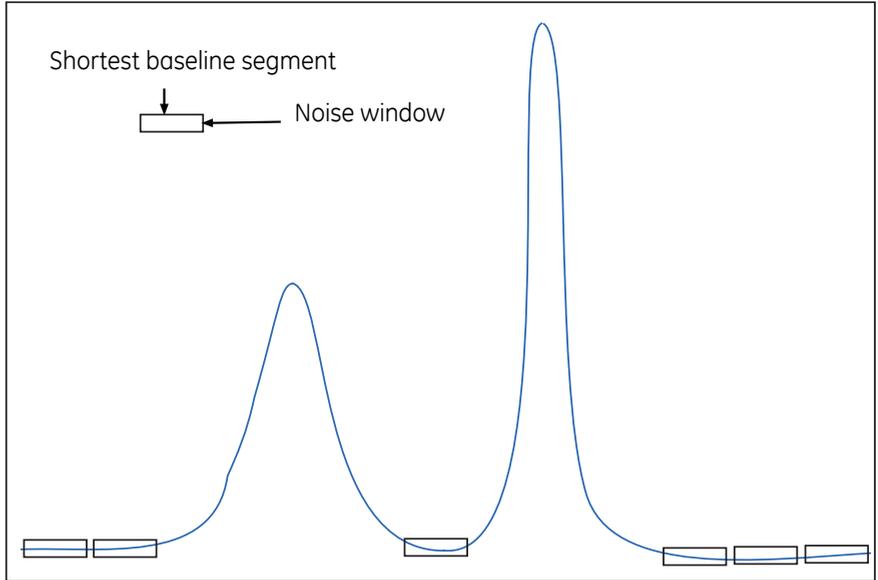
- The curve parts are longer than the **Shortest baseline segment**. This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.
- The curve has no point outside the **Noise window**. The noise window is defined as a rectangular corridor parallel 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.

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 (Classic algorithm)

The illustrations below show the baseline parameters graphically.



Baseline segment identification (Classic algorithm)

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.

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

Baseline drawing (Classic algorithm)

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.

Measure a Classic algorithm baseline segment

You can try to measure the **Shortest baseline segment** length directly on your chromatogram. To measure the shortest baseline segment:

Step	Action
1	Locate the shortest segment of the curve that you consider a part of the baseline.
2	Right-click in the chromatogram and choose Vertical marker .
3	Use the Vertical marker to measure the length of the segment by reading the start and end point values.
4	Choose Integrate:Calculate Baseline and insert this value as the Shortest baseline segment value.

Measure noise level (Classic algorithm)

Curve coordinates can also be used to measure noise levels on the source curve. To measure noise levels:

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	<ul style="list-style-type: none">• 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.

Measure the slope limit (Classic algorithm)

Follow the instructions below to measure the slope at any part of the curve.

Stage	Description
1	Select Operations:Differentiate in the Evaluation module. <i>Result:</i> The Differentiate dialog opens.
2	<ul style="list-style-type: none">• Select the desired source curve.• Select the First order calculation option.• Click OK. <i>Result:</i> The differentiated curve will appear in the active chromatogram.
3	Select an appropriate Y-axis scale, right-click and select Vertical marker to measure the Y-axis values for the differentiated curve with the curve coordinates function. <i>Result:</i> The Y-axis value is interpreted as the UV curve slope at the selected retention point.
4	<ul style="list-style-type: none">• 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.
Tip:	<i>If the differentiated curve is very noisy, it can be filtered with a light Moving average filter in the Operations:Smooth function.</i>

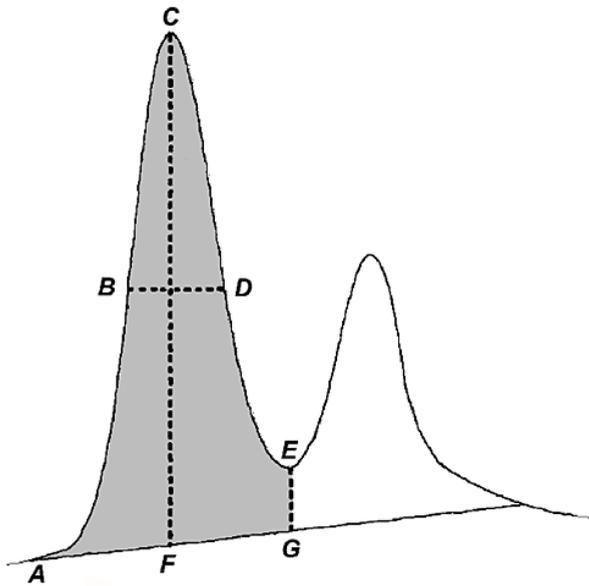
A.3 Peak table column components

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
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).

A Evaluation functions and instructions

A.3 Peak table column components

Parameter	Description
Asymmetry	Peak asymmetry (indicator of column packing). See definition below this table.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in the diagram above).
Retention factor	The retention factor will only be calculated when the chromatogram is in volume base. The total liquid volume, V_t , must be entered in the Peak Integrate dialog for this parameter to be calculated. See definition below this table.
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, V_0 , must be entered in the Peak Integrate dialog for this parameter to be calculated. See definition below this table.
Plate height (HETP)	Height equivalent to theoretical plate and plates/meter. The column height must be entered in the Peak Integrate dialog for this parameter to be calculated. See definition below this table.
Peak endpoint heights	Amplitude above the baseline at left (A in the diagram above) and right peak limits (E-G in the diagram above).
Peak endpoint retention	Retention value at peak start and peak end, time or volume base (A and G in the diagram above).
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: <i>This value can differ in time and volume base if the flow rate is not constant throughout the method.</i>
Percent of total peak area	Peak area as a percent of the sum of all integrated peaks. Note: <i>This value can differ in time and volume base if the flow rate is not constant throughout the method.</i>

Parameter	Description
Resolution	Peak resolution. See definition below this table.
Retention	Retention at the peak maximum, time or volume base (C in the diagram above).
Sigma	Standard deviation for a Gaussian-shaped peak. See definition below this table.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or dropline to the baseline or skim line.
Width	Difference in retention between the peak end and peak start, time or volume base (G-A in the diagram above).
Width at half height	Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above the baseline. Time or volume base. (B-D in the diagram above, where BD bisects CF)

Note: *In the **Options** dialog (which is available in all UNICORN modules from the **Tools** menu) you can select if negative retentions should be displayed or not. The default selection is that negative retention is displayed.*

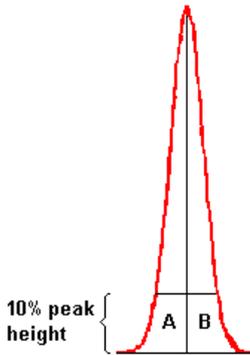
Asymmetry formula

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

$$\text{Asymmetry} = B / A$$

Where:

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



Change the Asymmetry Ratio

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

Step	Action
1	<ul style="list-style-type: none">Choose the Tools:Options menu item. <p><i>Result:</i> The Options dialog opens.</p>
2	<ul style="list-style-type: none">Type a ratio value in the Asymmetry Ratio at text box.Click OK. <p><i>Result:</i> The ratio value is changed and the dialog closes.</p>
Note:	<p><i>You must repeat the peak integrations after the change to update the values based on the new asymmetry ratio. The default ratio is 10%.</i></p>

Retention factor formula

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

$$k^1 = \frac{V_R - V_t}{V_t}$$

Where:

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

K_{av} 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.
-

HETP formula

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

$$\text{HETP} = L/N$$

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

Where:

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

Peak resolution algorithms

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

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

$$2 \quad (V_{R2} - V_{R1}) / ((\text{Sigma}_2 + \text{Sigma}_1) \times 2)$$

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

Where:

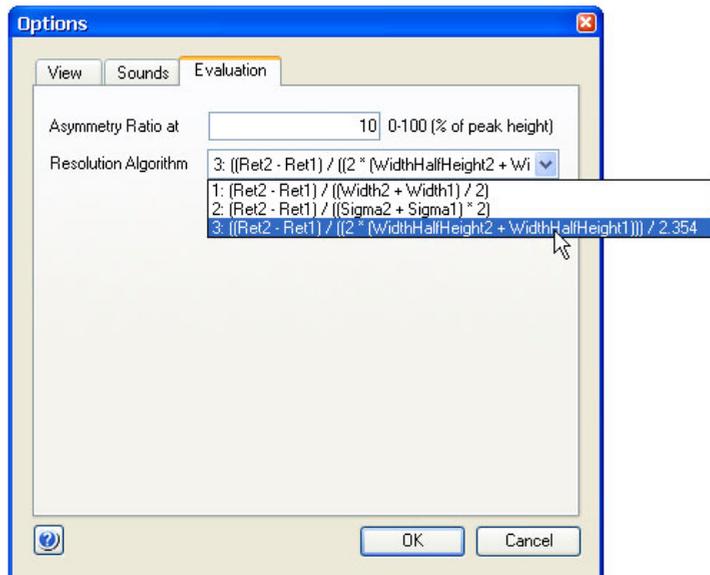
- V_{R1} , W_{b1} , Sigma_1 and W_{h1} are the retention, width, Sigma and width at half height of the previous peak.
- V_{R2} , W_{b2} , Sigma_2 and W_{h2} are the retention, width, Sigma and width at half height of the current peak.

Note: The **Resolution Algorithm** variable in the **Options** dialog determines which of the three algorithms is used. The default setting is algorithm 3.

Change the peak resolution algorithm

Follow the instructions below to change the peak resolution algorithm in the **Options** dialog.

- | Step | Action |
|------|---|
| 1 | <ul style="list-style-type: none"> Choose the Tools:Options menu item in any of the UNICORN modules. <p><i>Result:</i> The Options dialog opens.</p> |
| 2 | Click the Evaluation tab. |
| 3 | <ul style="list-style-type: none"> Select the desired algorithm as described in <i>Peak resolution algorithm</i> above, in the Resolution Algorithm droplist. |



- Click **OK**.
- Result:* The dialog 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.

Sigma formula

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

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

Where:

- n is the number of data points.
- x is the volume or time value.
- $x_{y_{max}}$ 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).

Index

A

- Affinity chromatography
 - Predefined method, 89
- ÄKTA start
 - system description, 11
- Anion exchange
 - Predefined method, 90
- Auto Hide
 - Method Navigator in the Evaluation module, 79, 123

B

- Baseline
 - Adjust the baseline graphically, 169
 - Calculate a new, 170
 - Definition of a segment, 265
 - How to edit manually, 181
 - Parameters, 266
 - The Calculate function, 160

C

- Cation exchange
 - Predefined method, 90
- Chromatogram
 - Select and zoom, 68
- Chromatograms
 - Background grid, 147
 - Change and fix the X-axis, 150
 - Change and fix the Y-axis, 148
 - Change curve color and style, 143
 - Customize - select curves to display, 143
 - Export as Windows metafile image, 227
 - Fraction text alignment, 145
 - How to import curves with File:Open to compare, 208
 - How to make layout changes, general, 139
 - How to rename, 216

- Insert a marker for measurements, 130
- Measure using marker references, 130
- Print active chromatograms, 152
- Run log text display, 145
- Select Y-axes to display, 149
- The command File:Open to compare, 204
- Classic algorithm
 - Definition, 188, 266
 - How to set, 188
 - How to set Max baseline level, 195
 - Measure baseline segments, 269
 - Measure noise level, 269
 - Measure the slope limit, 270
 - Missing peaks, 194
 - Noise window, 193
 - Parameters, 188
 - Shortest baseline segment, 190
 - Slope limits, 191
 - When to change the Max baseline level, 195
- Column handling
 - Import column list, 115
- Column wash
 - Predefined phase, 92
- Curves
 - Change and fix the X-axis, 150
 - Change and fix the Y-axis, 148
 - Change curve color and style, 143
 - Export options, 222
 - Fraction text alignment, 145
 - Highlight in a chromatogram, 128
 - How to create a fraction histogram, 198
 - How to delete unwanted curves, 217

- How to export, 224
 - How to move using the Shift function, 213
 - How to produce a mirror image, 214
 - How to rename, 216
 - How to shift a mirror image, 215
 - How to use the Open to compare command, 208
 - Import, 220
 - Measure using a marker reference, 130
 - Run log text display, 145
 - Select Y-axes to display, 149
- D**
- Database maintenance
 - archive results or logs, 246
 - archive selected results, 248
 - disable scheduled backup, 243
 - manual database backup, 244
 - restore backup data, 245
 - retrieve archived results or logs, 250
 - schedule backup, 242
 - Desalting
 - Predefined method, 91
 - Docking panes
 - In the Evaluation module, 125
- E**
- Edit
 - Method phases, 97
 - Elution
 - Predefined phase, 92
 - Equilibration
 - Predefined phase, 92
 - Evaluation
 - How to exit the module, 218
 - How to make chromatogram layout changes, general, 139
 - Evaluation module
 - The Chromatogram pane, 125
 - Toolbar icons, 121
 - Toolbar icons in the Edit Peak Table dialog, 165
- Exporting
 - Methods, 109
 - Phases, 109
- F**
- File Navigator
 - How to close, 137
 - Files
 - How to rename, 216
 - Folders
 - How to rename, 216
 - Fraction collection
 - Overview, 100
 - Fraction Histogram
 - How to create a curve, 198
 - Protein amount calculation, 199
 - Protein concentration calculation, 198
- G**
- Gel filtration
 - Predefined method, 91
- H**
- Help
 - Viewing, 79
- I**
- Importing
 - Column list, 115
 - Methods, 113
 - Installation
 - overview of workstation installation and configuration, 16
 - Ion exchange
 - Predefined method, 90
- L**
- Log off
 - UNICORN, 53
 - Log on
 - UNICORN, 46, 49
 - Logs
 - archive, 237
 - export, 237

- print, 237
 - search for specific text, 236
 - types of logs, 233
 - UNICORN and System Log dialog, 234
 - view selected UNICORN and system log entries, 234
- M**
- Method Editor
 - Optional panes, 78
 - Viewing help topics, 79
 - Method Navigator
 - Expand or collapse folders, 134
 - Toolbar icons, 133
 - Method outline
 - Description, 80
 - Methods
 - Affinity chromatography, 89
 - Anion exchange, 90
 - Create new method, 93
 - Definition, 80
 - Desalting, 91
 - Edit methods, 97
 - Empty methods, 82
 - Export a method, 109
 - Export as text, 111
 - Gel filtration, 91
 - Import a method, 113
 - Open a method, 86
 - Predefined methods, 82, 89
 - Structure of, 80
 - Working with, 81
 - Method settings
 - Predefined phase, 92
 - Morphological algorithm
 - Definition, 265
 - Description, 184
 - How to set, 184
 - Incorrect structure width, 186
 - Minimum distance between points, 187
 - Structure width, 185
- P**
- Panes
 - Display panes in System Control, 58
 - Peak integration
 - Adjust the peak start and end points manually, 167
 - Differences between to filter peaks and to reject peaks, 162
 - How to perform, 157
 - Select characteristics to display, 161
 - Select part of a curve for peak integration, 177
 - Peaks
 - Add a fill color and pattern, 170
 - Add peak names, 174
 - Adjust the start and end points manually, 167
 - Change the Asymmetry Ratio value, 274
 - Change the peak resolution algorithm, 276
 - Delete peaks, 175
 - Drop-lines, description, 167
 - How to filter from view, 162
 - Include negative peaks in integration, 179
 - Join peaks, 173
 - Open the peak table for editing, 164
 - Peak parameters, 271
 - Split a peak, 173
 - Peak table
 - How to export, 226
 - How to rename, 216
 - Phase properties
 - Edit phase, 97
 - Help, 96
 - Phases
 - Column wash, 92
 - Edit phases, 97
 - Elution, 92
 - Equilibration, 92
 - Export a phase, 109
 - Help, 96
 - Method settings, 92
 - Phase properties, 97
 - Predefined phases, 82, 92
 - Sample application, 92
 - Process Picture

- actions in the process picture, 70
- Protein activity
 - Match to UV curve, 202
- Protein amount in fractions
 - Calculate in Fraction Histogram dialog, 199
- Protein concentration
 - Calculate in Fraction Histogram dialog, 198

R

- Recent runs
 - Listed in the Result Navigator, 135
- Result files
 - Export options, 222
 - How to export for use in another UNICORN database, 223
 - How to rename, 216
 - How to save, 217
 - Import, 220
 - Open a result, 127
- Result Navigator
 - Expand or collapse folders, 134
 - Recent Runs list, 135
 - Toolbar icons, 133
 - Use Find Results to locate a file, 136
- Run
 - monitor a run, 66
 - Select a method, 61

S

- Sample application
 - Predefined phase, 92
- Smoothing algorithms
 - Autoregressive, 262
 - Median, 263
 - Moving average, 262
 - Savitzky-Golay, 264
- Snapshots

- Take a Snapshot of curve values in a result, 132
- Software installation
 - configure e-license, 31
 - removing prerequisite software, 44
- States
 - Display in System Control, 59
- System Control
 - Customize, 66
 - Display panes, 58
 - Instrument status, 59
- System Log
 - dialog illustration, 234
 - general description, 233

T

- Troubleshooting
 - access to UNICORN start functions, 257
 - database functions, 258
 - Diagnostics manager, 256
 - distorted peaks, 259
 - maximum number of curves exceeded, 259
 - system or computer connections, 255

U

- UNICORN
 - Exit, 53
 - Log off, 53
 - Log on, 46, 49
 - Start, 46, 49
- UNICORN start Log
 - dialog illustration, 234
 - general description, 233

Z

- Zero baseline
 - Definition, 160
- Zoom
 - Chromatogram, 68

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