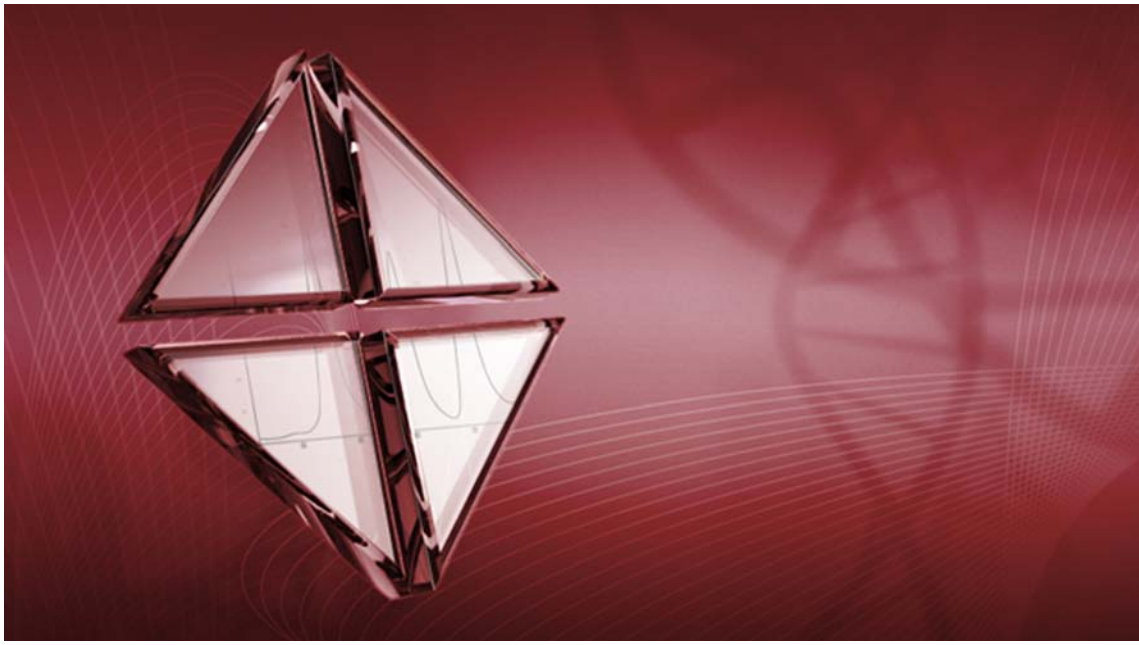
GE Healthcare
Life Sciences

UNICORN™ 6.1

Method Manual



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1 Introducing the UNICORN Method Editor

Introduction

This chapter contains:

- A general introduction to creating methods using the UNICORN software.
- Information about the user documentation for UNICORN, including an overview of related documents describing the use of the software.

Software declaration of conformity

UNICORN 6.1 is technically compatible with all relevant sections of FDA 21 CFR Part 11.

A part 11-system assessment checklist is available on request through the local GEHC representative.

In this chapter

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1.1 About the UNICORN Method Editor

Introduction

This section is a brief introduction to creating methods in UNICORN and a description of the scope of this manual.

What is UNICORN?

UNICORN is a complete software package for:

- control and supervision of chromatography systems.
- evaluation and analysis of the results from separation runs.

This manual describes UNICORN 6.1, which is designed for ÄKTA™ avant chromatography systems. This software version is not compatible with other ÄKTA systems.

Workflow

The workflow in UNICORN can be divided into four distinct stages. The flow chart below shows the work flow stages.

This manual describes step 1 of this workflow.

Tip: Step 2, how to perform method runs, is described in the *"ÄKTA avant and UNICORN 6.1 User Manual"*. Step 3, evaluate the results, and step 4, compile a report, are described in the *"UNICORN 6.1 Evaluation Manual"*.

Create a method

A method in UNICORN is a user-defined set of instructions that can be used to run an entire process on a system, for example a purification run or a column performance test. A method is comprised of one or several phases which are reusable sets of instructions. Examples of phases are equilibration steps and elution steps.

The UNICORN ***Method Editor*** module is a comprehensive tool for creating or editing phases and methods. UNICORN is delivered with templates for common procedures that can easily be edited for a specific process. Using the Method Editor it is possible to:

- build a method from a library of phases.
- create custom phases.
- create method queues to run multiple methods on up to three separate systems.
- keep track of column types or individual columns using the ***Column Handling*** tool.
- design and optimize purification schemes using the ***Design of Experiments*** and ***Scouting*** tools.
- automatically mix and titrate buffers using the ***BufferPro*** tool.

  etc.

1.2 About this manual

Introduction

This section describes the purpose of the manual, the general structure and conventions applied in the text, and some prerequisites that should be fulfilled before you start to apply any of the procedures described in the following chapters.

The purpose of the UNICORN Method Manual

The purpose of the UNICORN Method Manual is to provide a comprehensive guide to creating methods that can be run on an ÄKTA avant system. It covers the features and tools included in the Method Editor module of the UNICORN software with practical instructions.

The manual covers the following:

- how to create methods and phases.
- how to use ***BufferPro***.
- how to design and optimize experiments using ***Design of Experiments*** and ***Scouting***.
- how to use method queues.
- how to handle column types and individual columns.
- how to convert and scale methods created for ÄKTA avant 25 systems to be used with ÄKTA avant 150 systems (or conversely).

For advanced users, an overview of how to edit methods at the level of individual instructions is also given.

Note: The Method Manual does not describe the functions of every command in all panes and dialogs of the user interface. Refer to the online help for information about commands that are not described in this manual. The online help in the ***Method Editor*** module is accessed either by clicking Help buttons in software dialogs, by pressing the **F1** key, or selecting ***Help:Help for Method Editor***.

Document structure

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

A section is divided into blocks of information with separating lines. The blocks are identified by a label extending into the margin (such as the label Document Structure above). This makes it easier for you to quickly scan a page to find the exact topic you are looking for.

Typographical conventions

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

Example: ***Method Navigator***

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

Example: ***Edit:Import:Import Phase...*** i.e., the menu option ***Import Phase...*** in the sub-menu ***Import*** from the ***Edit***-menu.

Controls on the instrument, computer or keyboard keys are shown with a bold, regular typeface:

Example: Press the **Delete** key.

Text that the user must either type exactly as shown in the manual, or that UNICORN displays as a response (not a regular part of the graphic user interface), is represented by a monotype typeface within quotation marks:

Example: "Connection change"

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 and Windows™ work. In most cases universal computer functions will not be explained.
- UNICORN must be installed and configured correctly on your computer.
- Your user profile and access rights must be set up, and you must be able to log on to UNICORN and access a database.
- You need to understand the general concepts of liquid chromatography. Terminology and functionalities will be explained only when they differ from normal practice.

1.3 UNICORN 6.1 user documentation

Introduction

This section describes the user documentation that is delivered with an ÄKTA avant system.

User documentation

The user documentation listed in the table below is available from the ***Help*** menu in UNICORN or on the ÄKTA avant and UNICORN User Documentation CD.

Document	Main contents
ÄKTA avant and UNICORN 6.1 Installation Guide	Site preparation, stand-alone installation and test procedure.
Getting Started with ÄKTA avant and UNICORN 6.1	System overview and instructions to perform a basic run.
ÄKTA avant and UNICORN 6.1 User Manual	Instructions for safe handling of the system. Descriptions of components. Information about how to run and maintain the system.
UNICORN Help	Dialog descriptions for UNICORN (from the ***Help*** menu).
UNICORN 6.1 Method Manual	Overview and detailed descriptions of the method creation features in UNICORN. Instructions on how to use the software. Workflow descriptions for common operations.
UNICORN 6.1 Evaluation Manual	Overview and detailed descriptions of the evaluation features in UNICORN. Instructions on how to use the software. Workflow descriptions for common operations.
UNICORN 6.1 Administration and Technical Manual	Network setup and complete software installation. Administration of UNICORN and the UNICORN database.

2 The UNICORN Method Editor

About this chapter

This chapter gives an introduction to the ***Method Editor*** in UNICORN 6.1. It gives a brief description of the ***Method Editor*** interface and describes the concept of methods in UNICORN 6.1.

For information about how to create, open and edit methods as well as signing methods and importing/exporting methods, see *Chapter 3 Create and edit methods, on page 25*.

In this chapter

This chapter contains the following sections:

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2.1 The Method Editor

Introduction

The ***Method Editor*** provides complete facilities for:

- creating and editing methods
- copying, saving and deleting methods
- converting methods for use with different types of ÄKTA avant systems (e.g. from an ÄKTA avant 25 system to an ÄKTA avant 150 system)

The ***Method Editor*** also provides a number of tools to assist the user in optimizing runs and a tool for handling column types and individual columns (see below for more information). Functions like signing methods electronically and importing/exporting methods are also included.

Tools in the Method Editor

The table below describes the different tools included in the ***Method Editor***.

Tool	Description
Design of experiment (***DoE***)	***DoE*** is used to find out, in a systematic way, which run parameters affect a process to be run and how to find optimal values for these parameters to obtain the best possible result using a minimum number of runs. When creating a method and setting up an experimental design using ***DoE***, an optimized ***Scouting scheme*** will automatically be created. See *Chapter 5 Design of Experiments, on page 116* for more information.
Scouting	***Scouting*** is used to repeat a series of ***Method runs*** automatically, where the user can change the values of predetermined variables before starting the method. A ***Scouting scheme*** is defined as part of the method. See *Chapter 4 Scouting, on page 103* for more information.

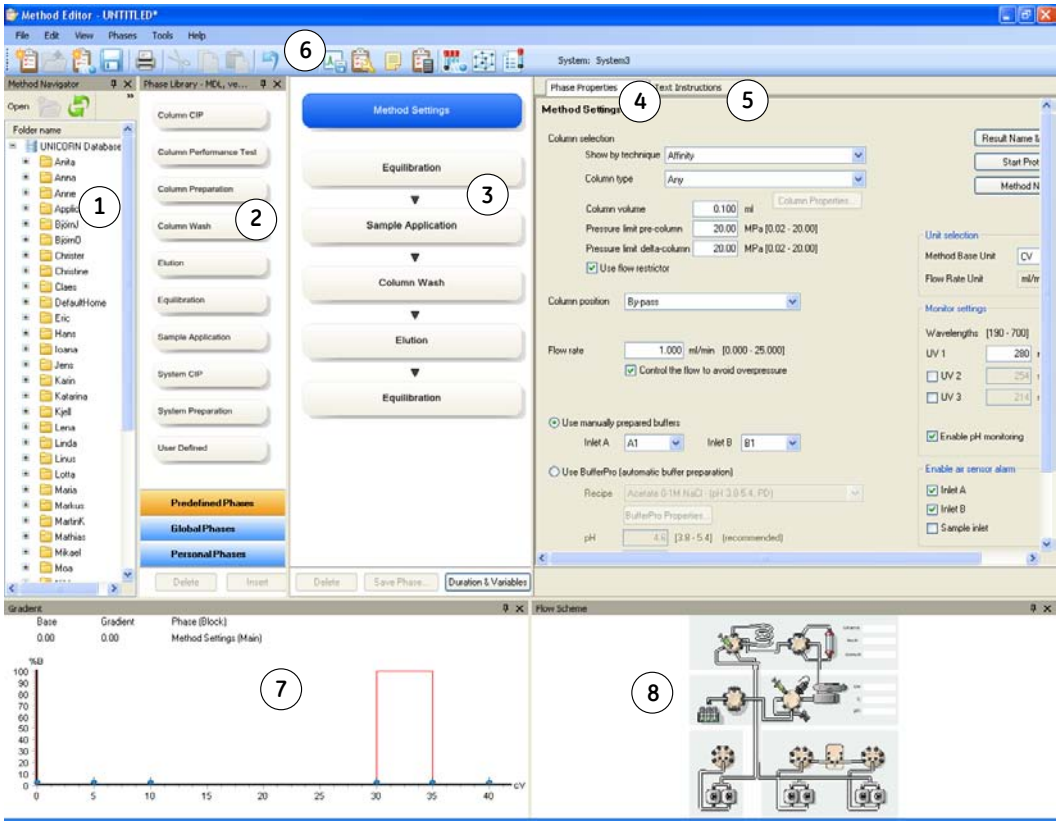
Tool	Description
BufferPro	***BufferPro*** allows a buffer of defined pH, and with defined salt concentrations to be prepared from four stock solutions (one Buffer stock solution, one Titrant, Water and a Salt stock solution). pH and salt concentration can be used as variable scouting parameters included in a ***Scouting scheme*** or in a ***Design of Experiments*** (***DoE***). ***BufferPro*** is optimized for cation and anion exchange chromatography, but can also be used when running other chromatographic techniques. See *Chapter 6 BufferPro, on page 203* for more information.
Column Handling	***Column Handling*** enables handling of column types and individual columns. See *Chapter 8 Column Handling, on page 241* for more information.

Illustration of the Method Editor

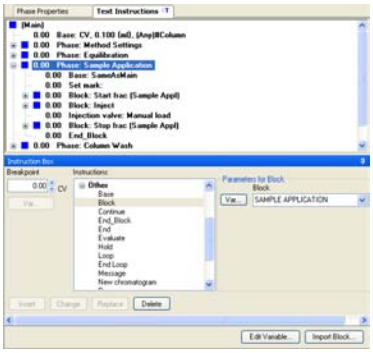
The basic ***Method Editor*** interface consists of two panes, the ***Method outline*** and the ***Phase Properties***/***Text Instructions*** pane.

By default, the ***Toolbar***, ***Phase Library*** pane and ***Gradient*** pane are also displayed in the ***Method Editor***. The display of these panes is however optional. Two more panes may be displayed in the ***Method Editor***, the ***Method Navigator*** and ***Flow Scheme***.

The illustration below shows the ***Method Editor*** with all the optional panes displayed.



Area	Description
1	***Method Navigator*** (optional pane): Shows all the user folders, methods and method queues that are available in the database.
2	***Phase Library*** (optional pane): Contains all available phases.
3	***Method Outline***: Shows the phases included in the opened method.
4	***Phase Properties*** tab: Select to display the ***Phase Properties***. ***Phase Properties*** shows the settings for the highlighted phase in the ***Method Outline***.

Area	Description
5	***Text Instructions*** tab: Select to display the ***Text Instructions***. ***Text Instructions*** shows the method in a text format. The illustration below shows the ***Text Instructions*** pane. 
6	***Toolbar*** (optional pane): Shows the toolbar icons.
7	***Gradient*** (optional pane): Shows the programmed gradient and break points for included phases and blocks.
8	***Flow Scheme*** (optional pane): Illustrates the flow path of the instrument graphically.

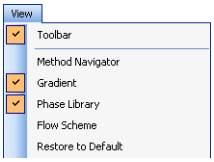
Note: For detailed information on the ***Toolbar*** and the different panes in the ***Method Editor***, see "Getting Help on the Toolbar and panes in the Method Editor" below.

Display optional panes

The optional panes in the ***Method Editor*** are displayed by selecting them in the ***View*** menu. To restore the appearance of the ***Method Editor*** to 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).

Note: Settings made by a user are automatically remembered by the software next time the same user opens the ***Method Editor***.

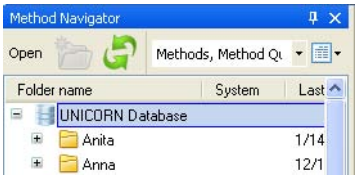
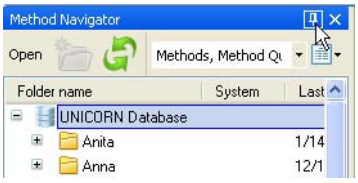
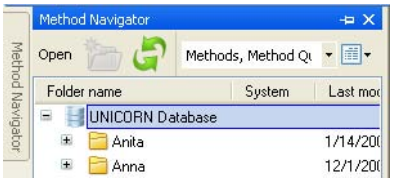
The illustration below shows the ***View*** menu with the default panes selected.

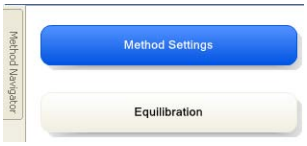


Auto Hide optional panes

The optional panes may either be displayed statically in the position where they open, or the ***Auto Hide*** function can be selected to automatically hide/display the pane when moving the mouse pointer over the pane.

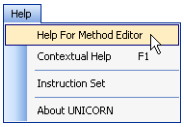
The table below describes how to turn on the ***Auto Hide*** function and how to hide/display, in this example, the ***Method Navigator*** pane.

Step	Action
1	If not already displayed, open the ***Method Navigator*** in the ***Method Editor*** by selecting ***View:Method Navigator***. *Result:* The ***Method Navigator*** pane is displayed. 
2	To turn on the ***Auto Hide*** function, click the vertical pin symbol in the top righthand corner.  *Result:* The pin symbol is rotated to horizontal position and a tab named ***Method Navigator*** is displayed to the left. 

Step	Action
3	Click outside the ***Method Navigator***. *Result:* The ***Method Navigator*** is hidden and only the ***Method Navigator*** tab is displayed. 
4	• To display the ***Method Navigator*** again, move the mouse pointer over the ***Method Navigator*** tab. • To turn off the ***Auto Hide*** function, click the horizontal pin symbol in the top righthand corner of the ***Method Navigator*** pane. *Result:* The ***Method Navigator*** pane is displayed statically.

Getting help on the Toolbar and panes in the Method Editor

The table below describes how to find detailed information about the ***Toolbar*** and the different panes in the ***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 displaying the ***Method Editor*** help start page.
2	To display help for a specific pane, click in the pane and press the **F1** keyboard key. *Result:* The online help page describing that pane is opened.

2.2 Methods in UNICORN 6.1

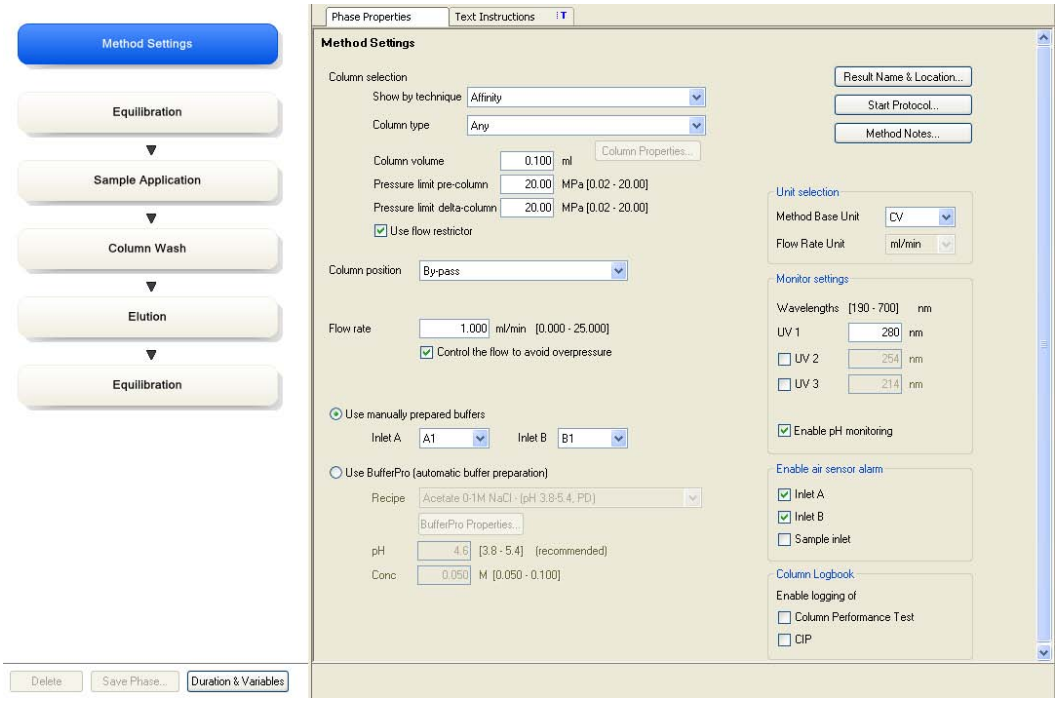
About methods

The program instructions for a chromatography run are defined in a ***Method***. The instructions are specific for each instrument configuration and component set up and follow certain syntactical and hierarchical rules.

Instructions are combined into blocks. Individual instructions and minor blocks are combined into the major method blocks, called ***Phases***. 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 *Chapter 3 Create and edit methods, on page 25* for information about creating and editing methods in the ***Method Editor***.

The illustration below shows the phases in a method in the ***Method Outline*** and the corresponding settings for the highlighted phase in the ***Phase Properties*** pane.



Method structure

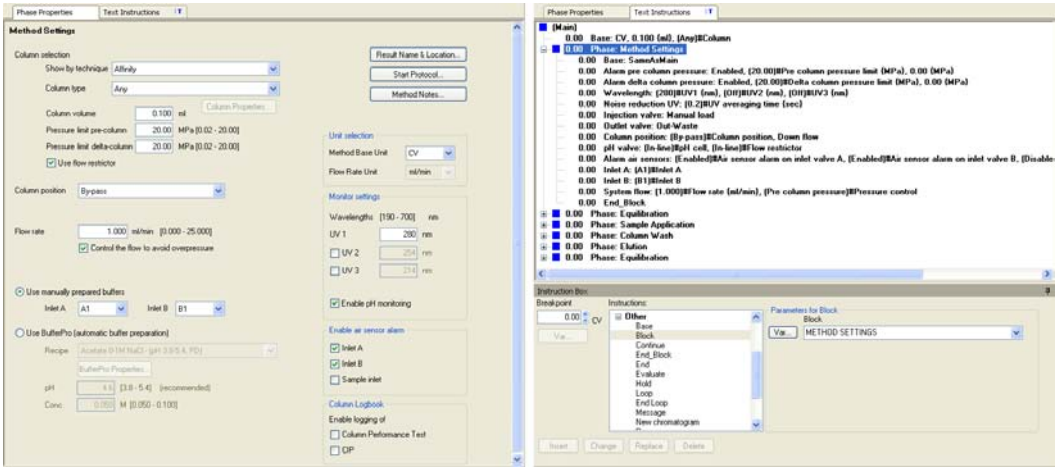
A method always starts with the ***Method Settings*** phase. This phase contains general settings that affect the rest of the method (e.g., ***Column type, Flow rate*** and ***Method Base Unit***). If changing ***Column type***, UNICORN will automatically calculate correct settings for volume, flow rate, and pressure limits. Subsequent phases reflect steps included in the chromatography run.

The figure below shows a method with the different phases in the ***Method Outline*** in the ***Method Editor***.

Working with methods

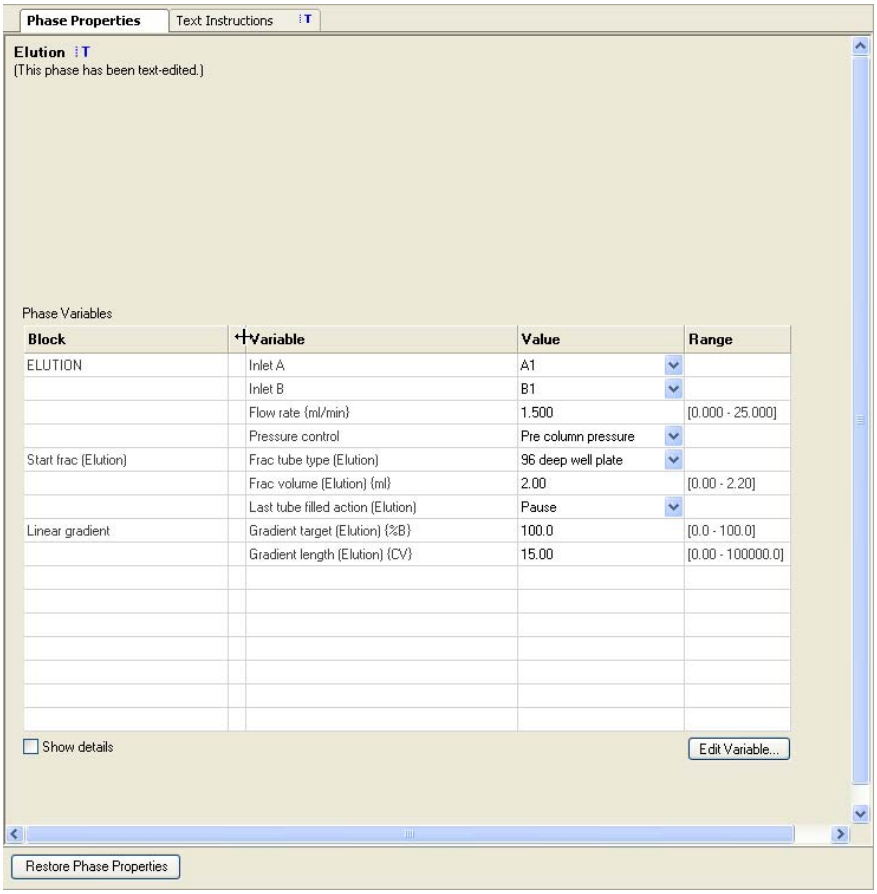
It is recommended to create and edit methods using ***Phase Properties***. Phases can easily be dragged-and-dropped into the ***Method Outline*** from the ***Phase Library*** and the phases are easily rearranged. Settings for each phase are set in the ***Phase Properties*** pane. When working like this, the text method is automatically built up in the ***Text Instructions*** pane and settings for blocks and instructions are updated accordingly.

The illustration below shows the phase properties settings and the text instructions for the ***Method Settings*** phase.



It is possible to use the text editor in ***Text Instructions*** to create a phase from scratch and to edit methods. Instructions are then created/edited one by one. This can be an option for fine-tuning or optimization of a method. If the text editor is used, ***Phase Properties*** will subsequently only show a list of variables for the phase, as shown in the following illustration. This can always be restored by clicking on the ***Restore Phase Properties*** button.

Phases that have been edited in the text editor are noted with a blue letter ***T*** as shown in the illustration below.



The phase ***User Defined*** is an empty phase designed for text editing methods. Such phases will only be displayed as a variable list in ***Phase Properties***, and may be saved in the personal or global phase library for reuse in other methods.

See *Chapter 9 Text edit methods, on page 286* for information about text editing methods.

Note: Do not mix text edited and non text edited phases unless you clearly understand the implications for the entire method of the instructions in the text edited phases.

Predefined and Empty methods

In UNICORN, a number of ***Predefined*** methods for different separation techniques and maintenance applications (e.g., preparation and cleaning of the system and columns) are supplied. When creating new methods, it is possible to use one of the ***Predefined*** methods or create a user defined method starting with an ***Empty method***. The phase ***Method Settings*** is mandatory in all methods.

See *Chapter 3 Create and edit methods, on page 25* for information about how to create new methods.

The table below gives a general description of ***Predefined*** and ***Empty*** methods.

Method	Description
Predefined	***Predefined*** methods include a number of relevant phases appropriate for the purification or maintenance to be performed. You may use the predefined methods as they are, or with adjusted settings as needed. See Section *Section 3.8 Predefined methods and phases, on page 81* for descriptions of the ***Predefined*** methods supplied with the software. **Note:** The ***Predefined*** methods are included in the instrument configuration files for each specific instrument.
Empty	***Empty*** methods include the mandatory phase ***Method Settings***. Other phases are then added by the user and settings adjusted as needed.

Predefined phases

UNICORN provides a number of ***Predefined Phases*** (e.g., ***Equilibration***, ***Column CIP*** and ***User Defined***) that can be used when building/editing methods in the ***Method Editor***. A predefined phase contains all necessary instructions to be run (except ***Method Settings*** which is mandatory in all methods, and ***User Defined*** that are special phases).

See *Section 3.8 Predefined methods and phases, on page 81* for descriptions of the pre-defined phases supplied with the software. See also *Chapter 3 Create and edit methods, on page 25*.

3 Create and edit methods

About this chapter

This chapter describes how to create, edit and handle chromatography and maintenance methods in UNICORN 6.1 using the ***Phase Properties*** pane. It also describes overall method options, how to sign methods electronically, how to print methods, how to convert and scale methods from one ÄKTA avant system type to another, and how to import/export methods. Descriptions of the predefined methods and phases supplied with the software are also included.

Note: It is recommended to work with phases using the ***Phase Properties*** pane. This chapter does not cover how to edit methods using the ***Text Instructions*** pane. For information about text editing methods, see *Chapter 9 Text edit methods, on page 286*.

In this chapter

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3.1 Working with methods - Overview

Introduction

In UNICORN 6.1 methods are built up using phases, where each phase corresponds to a step in a chromatography run with a number of properties associated to that phase. By building methods in this way, methods are easily created and edited. See *Section 2.2 Methods in UNICORN 6.1, on page 20* for more information about method structure, definitions and concepts of methods in UNICORN 6.1.

There are two different ways of creating and editing methods in UNICORN 6.1:

- Creating and editing methods using phases and the phase properties in the ***Phase-Properties*** pane (the recommended workflow described in this chapter).

  or

- Creating methods from scratch by text editing methods, creating and editing text instructions one-by-one.

Main steps when creating a new method

The main steps when creating a method are:

1 Create/open a method

   - Create a ***Predefined*** method (including a set of phases that may be edited)

     or

   - Create a new method from scratch (***Empty*** method) containing only the ***Method Settings*** phase

     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

   - ***Predefined*** methods: use as they are, or edit the ***Method Outline*** and/or ***Phase Properties***
   - ***Empty*** methods: add phases to the method (i.e., build the ***Method Outline***) and edit ***Phase Properties*** for the phases as appropriate
   - Opened methods: edit the ***Method Outline*** and/or ***Phase Properties***

3 Save the method

Main steps when editing a method

The main steps when editing a method are:

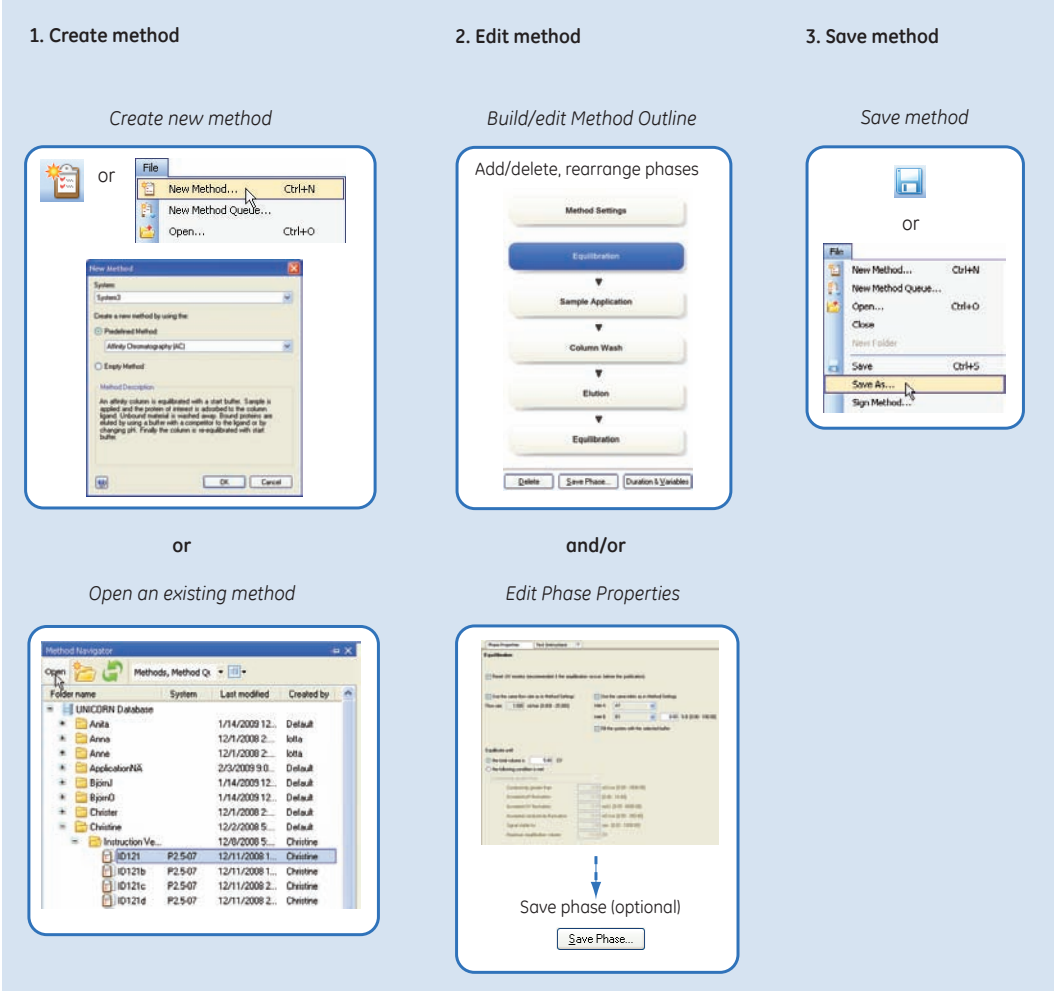
1 Open the method to be edited

2 Edit the ***Method Outline*** and/or edit the ***Phase Properties*** for the appropriate phases

3 Save the method

Illustration of workflow when creating or editing a new method

The illustration below shows the workflow in the ***Method Editor*** when creating or editing a method.



Overall method options

In addition to creating, editing and saving the method in the ***Method Editor***, a number of more general method options are available. These are settings for the method and are saved with the method.

Overall method settings can be divided into two groups. The table below shows the different groups.

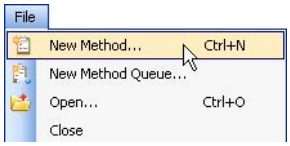
Method option	Description
General method options	• setting result name and the location of the results • setting up start protocols • adding/editing notes to the method • choosing to include evaluation procedures to be performed after the run • viewing and printing an estimate of the method duration time and the variables in the method See *Section 3.4 Set general method options for the method, on page 51* for more information.
Method options intended to assist the user in optimizing runs in UNICORN	• ***Scouting*** See *Chapter 4 Scouting, on page 103* for information. • ***Design Of Experiment*** (***DoE***) See *Chapter 5 Design of Experiments, on page 116* for information. • ***BufferPro*** See *Chapter 6 BufferPro, on page 203* for information.

3.2 Create and open methods

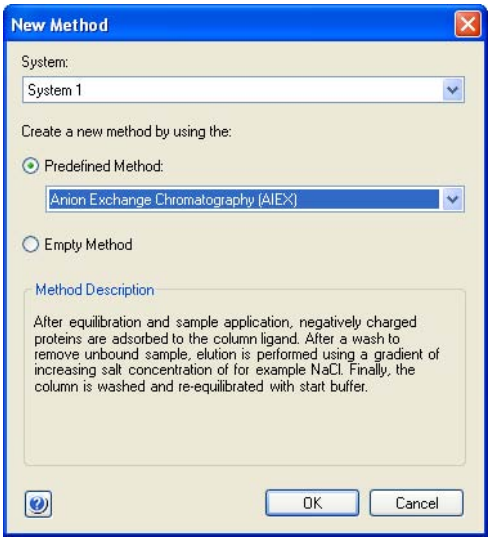
Create a new method

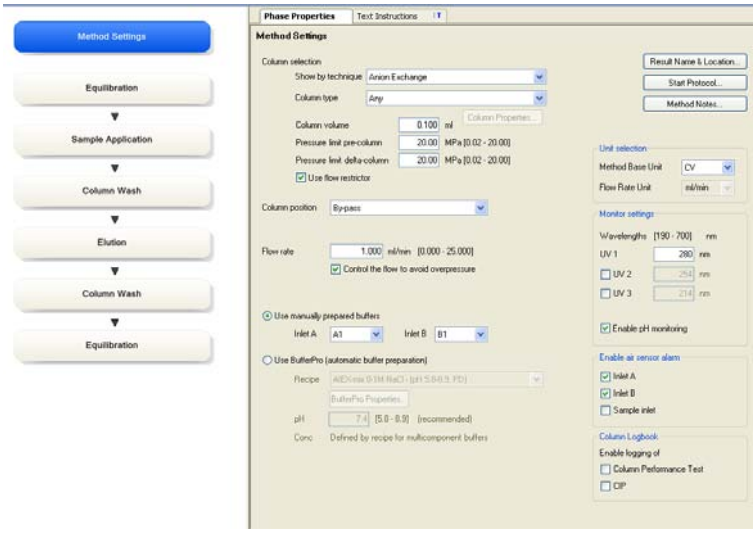
The table below describes how to create a new method:

Step	Action
1	In the ***Method Editor***: • click the ***Create a new method*** icon in the ***Toolbar***  *or* • select ***File:New Method...***



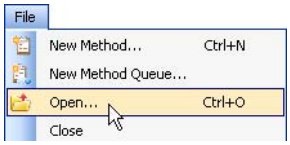
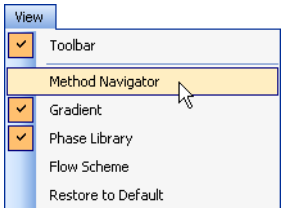
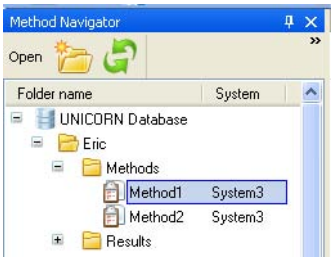
Result: The ***New Method*** dialog opens.



Step	Action
2	In the ***New Method*** dialog: • select a ***System*** • select a ***Predefined Method*** or select an ***Empty Method*** (to be created from scratch) • click ***OK*** *Result*: The ***Method Outline*** pane shows the included phases for the chosen method and the default settings for the phase highlighted in the ***Phase Properties*** pane. If an empty method was selected, only the ***Method Settings*** phase is shown (this phase is always included in all methods). 

Open a method

The table below describes how to open an existing method in the database:

Step	Action
1	In the ***Method Editor***: • Click the ***Open Method Navigator*** icon in the ***Toolbar***  *or* • select ***File:Open...***  *or* • select ***View:Method Navigator***  *Result:* The ***Method Navigator*** is displayed.
2	Select the method to be opened in the ***Folder name*** column. 

Step	Action
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 included phases. You can continue to edit the phases of the method using ***Phase Properties***. See *Section 3.3 Edit methods and phases, on page 34* for more information about how to edit a method. 

3.3 Edit methods and phases

About this section

This section describes how to edit the phase properties for a phase and how to edit the method outline of a method, that is, determine which phases that should be included in the method and determine the order of the phases in the method.

In this section

This section contains the following sub-sections:

Section	See page
3.3.1 Edit phase properties	35
3.3.2 Edit the method outline	43

3.3.1 Edit phase properties

Introduction

When editing ***Phase Properties*** for a phase, the changes affect either

- the whole method, when editing the ***Method Settings*** phase

  or

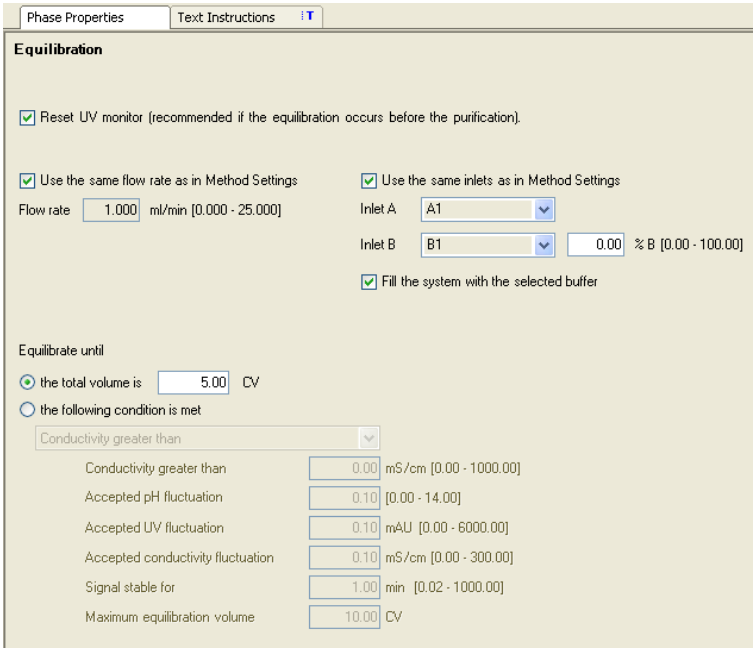
- only the phase that is being edited, when editing phases other than the ***Method Settings*** phase

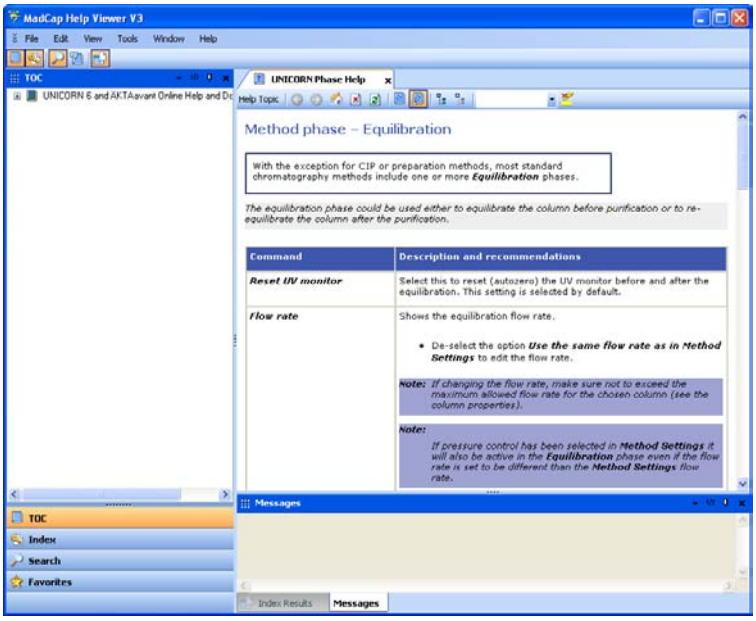
Getting help when editing Phase Properties

The table below describes how to get help information for the properties in a phase:

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

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



Step	Action
2	Click anywhere in the ***Phase Properties*** area to make it the active area in the software.
3	• 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

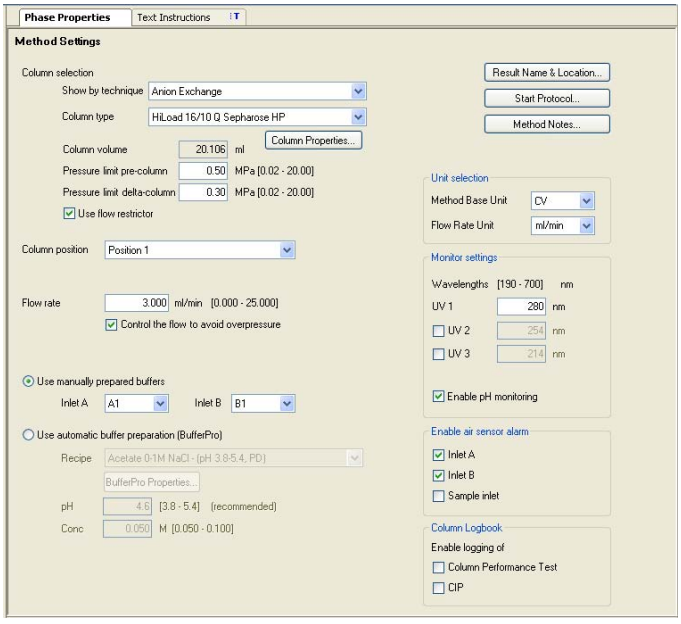
The following table describes how to edit a method phase in the ***Phase Properties*** tab:

Step	Action
1	Make sure the ***Phase Properties*** tab is selected.

Step	Action
2	• Select the ***Method Settings*** phase if you want to edit basic settings affecting the whole method (e.g., ***Column type, Flow rate*** and ***Method Base Unit***). Continue with steps 3-4. **Note:** You can also edit the ***Result name & Location***, the ***Start Protocol*** and ***Method Notes*** from the ***Method Settings*** phase. These are overall method options that also can be set using the corresponding ***Toolbar*** options and not described in this section. See *Section 3.4 Set general method options for the method, on page 51* for information on how to edit these settings. *or* • Select any other phase to edit the properties for that specific phase. Continue with step 5.

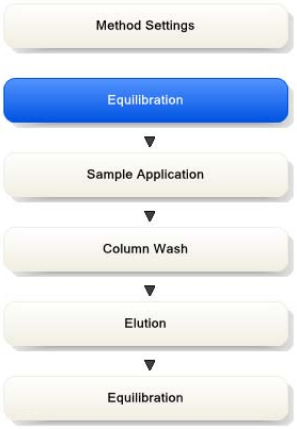
Step	Action
3	To edit the properties for the ***Method Settings*** phase, click ***Method Settings*** in the ***Method Outline***.

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

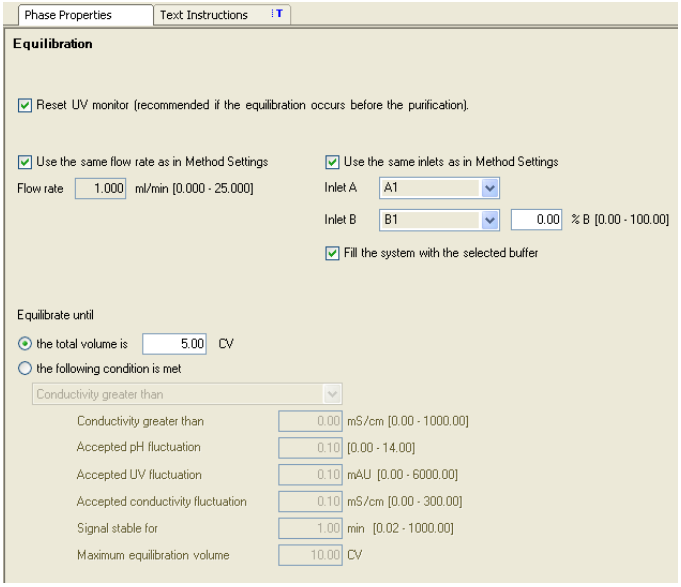


Step	Action
4	Edit the settings for the ***Method Settings*** phase in the ***Phase Properties*** pane as appropriate. If changing ***Column type***, UNICORN will automatically calculate correct settings for volume, flow rate, and pressure limits. **Note:** Settings in this phase will affect the whole method. **Note:** Allowed parameter ranges are shown in parenthesis beside the text boxes. *Result*: The method is updated with the new settings.

Step	Action
5	Select a phase in the method to be edited, for example, ***Equilibration***.



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



Step	Action
6	• Edit the settings as appropriate. **Note:** If there are, for example, two predefined ***Equilibration*** phases in your method, changing settings in one of them will not affect the other. To be able to see that they are different, it is recommended to rename one of them. See *Section 3.3.2 Edit the method outline, on page 43* for information about how to rename a phase. • Repeat steps 5-6 until the appropriate phases have been edited. *Result*: 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.
7	Save the method.

3.3.2 Edit the method outline

Introduction

The ***Method Outline*** shows the phases that are included in the method and the order of the phases in the method. Phases can be added, rearranged, renamed and deleted from the ***Method Outline***.

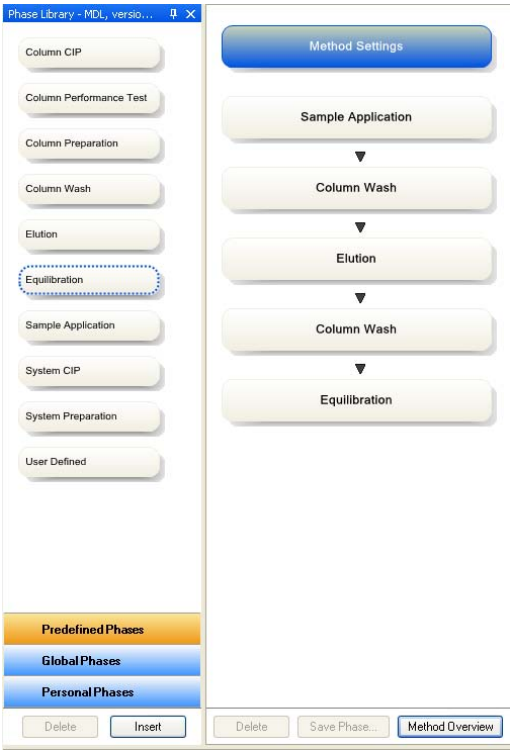
Add a phase to the method outline using drag-and-drop

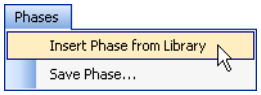
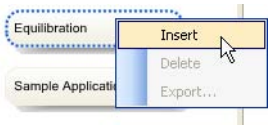
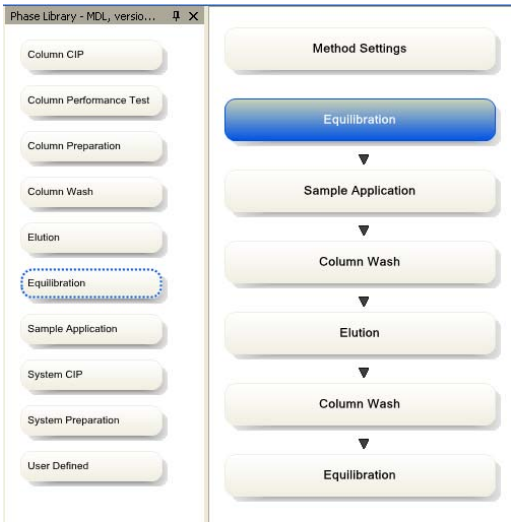
The table below describes how to add a phase to the method outline using drag-and-drop:

Step	Action
1	Select the appropriate phase in the ***Phase Library*** pane and drag-and-drop the phase to the requested position in the ***Method Outline*** pane. *Result:* The phase is included in the method at the requested position. If the ***User Defined*** phase was added, continue with step 2.
2	When the ***User Defined*** phase has been added to the ***Method Outline***, the phase name is enabled for editing.  Type a name for the phase and press the **Return** keyboard key. **Note:** The ***User Defined*** phase is marked with the letter ***T***, meaning that it is text edited. This phase contains only ***Base*** and ***End_Block*** instructions, so any functional instructions must be added by hand. To include instructions for the ***User Defined*** phase, select the ***Text Instructions*** tab. The ***Phase Properties*** tab will only show the variables used in this phase. See *Chapter 9 Text edit methods, on page 286* for information about how to work with instructions in the text Instructions pane.

Add a phase to the method outline using a button or menu command

The table below describes how to add a phase to the method outline using a button or menu command:

Step	Action
1	• Select the appropriate phase (e.g., ***Equilibration***) in the ***Phase Library*** • Select the appropriate phase (e.g., the ***Method Settings*** phase) in the ***Method Outline*** to determine where to place the new phase **Note:** When adding a phase to the ***Method Outline*** using a button or menu command, the new phase is always inserted below the currently selected phase in the ***Method Outline***. *Result:* The selected phase in the ***Phase Library*** is indicated by a blue dotted frame and the selected phase in the ***Method Outline*** is highlighted in blue. 

Step	Action
2	• Click the ***Insert*** button located below the ***Phase Library***  *or* • double-click the selected phase *or* • select ***Phases:Insert Phase from Library...***  *or* • right-click the phase in the ***Phase Library*** and select ***Insert...***  *Result:* The phase is included in the method and highlighted in blue. Continue with step 3 if adding the ***User Defined*** phase. 

Step	Action
3	When the ***User Defined*** phase has been added to the ***Method Outline***, the phase name is enabled for editing. Type a name for the phase and press the **Return** keyboard key. **Note:** The ***User Defined*** phase is marked with the letter ***T***, meaning that it is text edited. This phase contains only ***Base*** and ***End_Block*** instructions, so any functional instructions must be added by hand. To include instructions for the ***User Defined*** phase, select the ***Text Instructions*** tab. The ***Phase Properties*** tab will only show the variables used in this phase. See *Chapter 9 Text edit methods, on page 286* for information about how to work with instructions in the text Instructions pane.

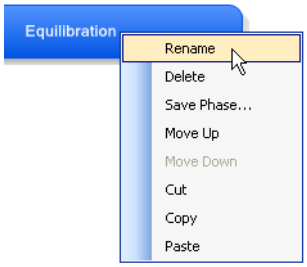
Rename phases

Note: It is only possible to rename phases in the ***Method Outline*** pane, not in the ***Phase Library***.

The table below describes how to rename a phase in the method:

Step	Action
1	Select the phase to be renamed in the ***Method Outline*** pane.

Step	Action
2	• right-click the phase and select ***Rename***

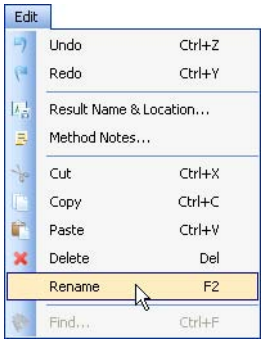


or

• press the **F2** keyboard key

or

• select ***Edit:Rename***

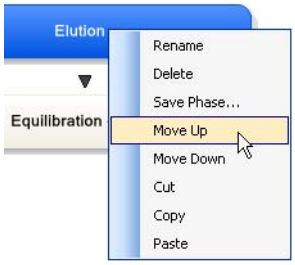


Result: The name in the phase becomes editable.

3	Type an appropriate name and click **Return** keyboard key. *Result:* The name of the phase is updated.

Rearrange phases within a method

The table below describes how to rearrange phases within a method:

Step	Action
1	Select the phase to be moved in the ***Method Outline*** pane.
2	• Drag-and-drop the phase to the requested position in the ***Method Outline*** pane. *Result:* The phase is moved to the requested position. *or* • Right-click the phase and select ***Move up*** or ***Move down***.  *Result:* The phase is moved one step up or down in the ***Method Outline***.

Delete a phase from the Method Outline

The table below describes how to delete a phase from the ***Method Outline***:

Step	Action
1	Select the phase to delete from the method in the ***Method Outline***.

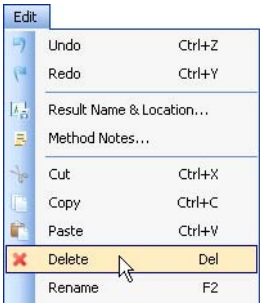
Step	Action
2	• Click the ***Delete*** button below the ***Method Outline*** pane.

or

- Press the **Delete** key on the keyboard.

or

- select ***Edit:Delete***



or

- Right-click on the phase and select ***Delete*** from the context menu.

Result: The phase is removed from the method.

Copy, cut and paste phases in a method

Note: It is only possible to copy, cut and paste phases in the ***Method Outline*** pane, not in the ***Phase Library***.

The features copy, cut and paste phases can be used to add/delete and rearrange phases in the ***Method Outline***. The table below describes the copy, cut and paste a phase features.

To...	then...
copy a phase	select the phase and: • right-click the phase and select ***Copy*** *or* • use the shortcut **Ctrl +C** *or* • select ***Edit:Copy***
cut a phase	select the phase and: • right-click the phase and select ***Cut*** *or* • use the shortcut **Ctrl +X** *or* • select ***Edit:Cut***
paste a phase	**Note:** The phase to be pasted will be pasted below the phase highlighted in the ***Method Outline***. Select the appropriate phase in the ***Method Outline***. Then: • right-click the highlighted phase in the ***Method Outline*** and select ***Paste*** *or* • use the shortcut **Ctrl +V** *or* • select ***Edit:Paste***

3.4 Set general method options for the method

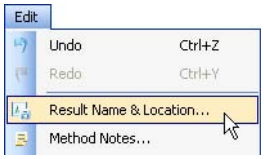
Introduction

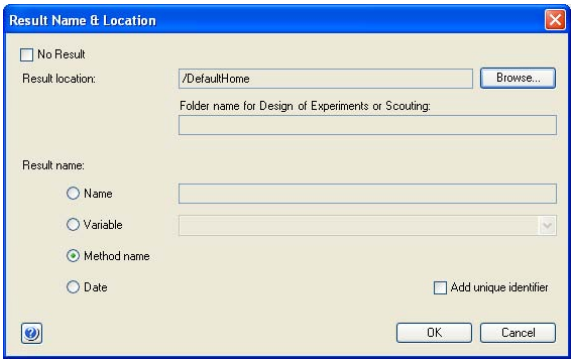
This section describes how to set and view options for an entire method. The following are covered in this section:

- Defining the name and location for the results.
- How to set up a ***Start Protocol*** that will be displayed before each method run.
- Adding or changing method notes.
- How to include evaulation procedures which can be executed during the run.
- Viewing the method duration time and volume.
- Viewing the variables used in the method.

Define Result Name & Location

The table below describes how to define the name of the result file created after the run and how to specify the folder in which to save the result file.

Step	Action
1	In the ***Method Editor***: • click the ***Result Name & Location*** icon  or • select ***Edit:Result Name & Location...***  or • click the ***Method Settings*** phase and click the ***Result Name & Location...*** button in the ***Phase Properties*** tab  *Result*: The ***Result Name & Location*** dialog opens.

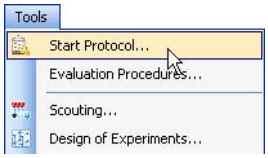
Step	Action
2	

In the ***Result Name & Location*** dialog:

- Set ***Result location*** by clicking the ***Browse*** button and select a folder in which to save the results. By default, the results will be saved in your home folder.
- Select ***Result name***.
  - ***Name***: The result name can be typed in manually
  - ***Variable***: The result name will be generated from the chosen variable (see *Section 9.2.4 Method variables, on page 315*)
  - ***Method name*** (default): The result name will be generated from the name of the method
  - ***Date***: The result name will be generated from the date of the run
- Check the ***Add unique identifier*** box if you want to include a unique identifier number to the file name. The number will be generated by UNICORN based on the run time of the method.
- Click ***OK*** to confirm and close the dialog.

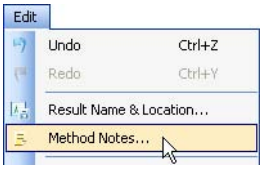
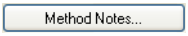
Set up a Start Protocol

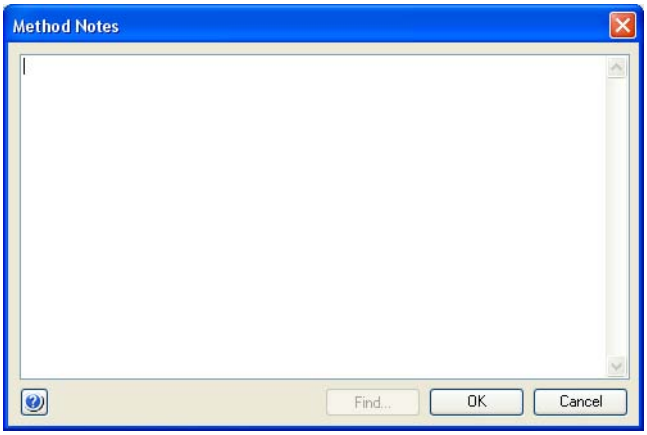
The table below describes how to set up a ***Start Protocol*** to be displayed before the run starts.

Step	Action
1	In the ***Method Editor***: • click the ***Start Protocol*** icon  or • select ***Tools:Start Protocol...***  or • click the ***Method Settings*** phase and click the ***Start Protocol...*** button in the ***Phase Properties*** tab  *Result*: The ***Start Protocol*** dialog opens.
2	 In the ***Start Protocol*** dialog: • Select items to display at method start. When selecting a method item, a description is shown to the right. ***Result Name and Location*** is selected by default. • Click ***OK*** to confirm and close the dialog.

Add/edit Method Notes

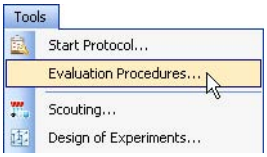
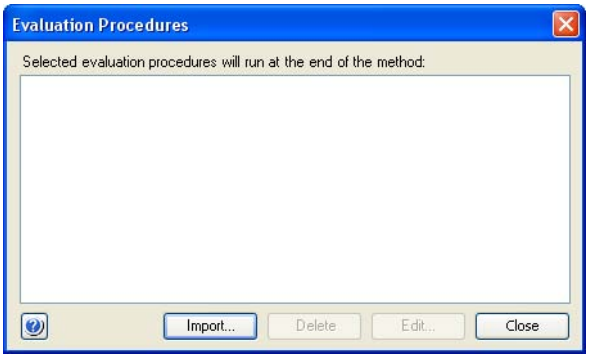
The table below describes how to add/edit notes to a method.

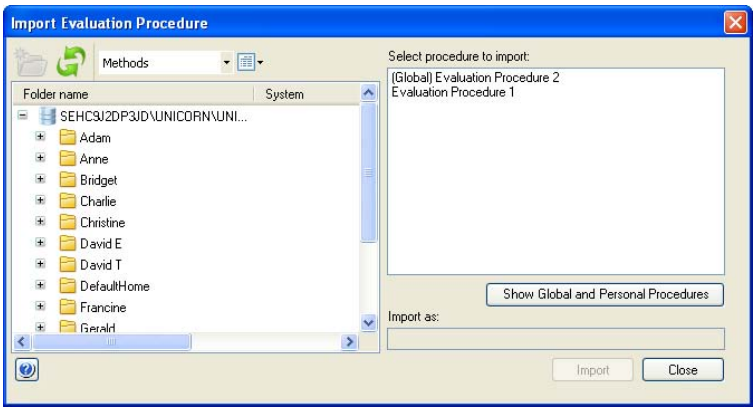
Step	Action
1	In the ***Method Editor***: • click the ***Method Notes*** icon  or • select ***Edit:Method Notes...***  or • click the ***Method Settings*** phase and click the ***Method Notes...*** button in the ***Phase Properties*** tab  *Result*: The ***Method Notes*** dialog opens.

Step	Action
2	 In the ***Method Notes*** dialog: • Enter/edit notes about the method. If notes already have been entered, it is possible to search for specific words using the ***Find...*** button. • Click ***OK*** to confirm and close the dialog. **Note:** Information will automatically be added to the ***Method Notes*** if the method has been converted for use with another ÄKTA avant system type than what it was originally created for, or scaled for another column type than what was originally selected.

Include Evaluation Procedures after the run

The table below describes how to include an evaluation procedure in the method. The evaluation procedure will be performed automatically after the run has finished. The evaluation procedures must have been defined in the ***Evaluation*** module, see the UNICORN 6.1 Evaluation Manual.

Step	Action
1	In the ***Method Editor***: • click the ***Evaluation Procedures*** icon  *or* • select ***Tools:Evaluation Procedures...***  *Result*: The ***Evaluation Procedures*** dialog opens.
2	 • If there are no evaluation procedures listed in the ***Evaluation Procedures*** dialog, click the ***Import...*** button to import an evaluation procedure. *Result:* The ***Import Evaluation Procedure*** dialog opens. Continue with step 3. • If an evaluation procedure that should be used in the run has been saved in the method earlier, it is shown in the ***Evaluation Procedures*** dialog. Continue with step 4.

Step	Action
3	

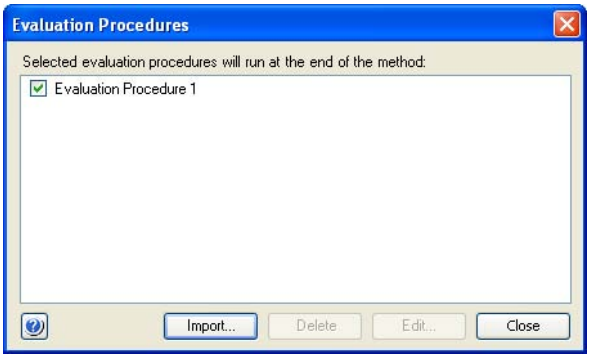
In the ***Import Evaluation Procedures*** dialog:

- Select the appropriate procedure to import in the ***Select procedure to import*** field.
- It is possible to change the name of the procedure to be displayed in your method by changing the name in the ***Import as*** field.
- Click ***Import*** to import the procedure

Note: Only ***Global*** procedures and your own ***Personal*** procedures are shown in the list.

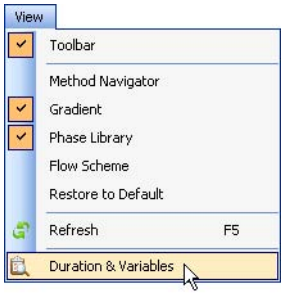
Note: It is also possible to import a procedure saved in another method by browsing to the appropriate folder and selecting the method containing the procedure. The procedure will be listed in the ***Select procedure to import*** field and can be imported as described above.

Result: The evaluation procedure is listed in the ***Evaluation Procedures*** dialog.

Step	Action
4	 Make sure the box in front of the evaluation procedure is checked to include it in the method. Click ***Close.*** *Result:* The evaluation procedure is included in the method. **Note:** It is possible to edit an existing evaluation procedure by selecting it and clicking ***Edit...***. The edits will only change the procedure that is included in the method. See the UNICORN 6.1 Evaluation Manual for information about how to edit an evaluation procedure.

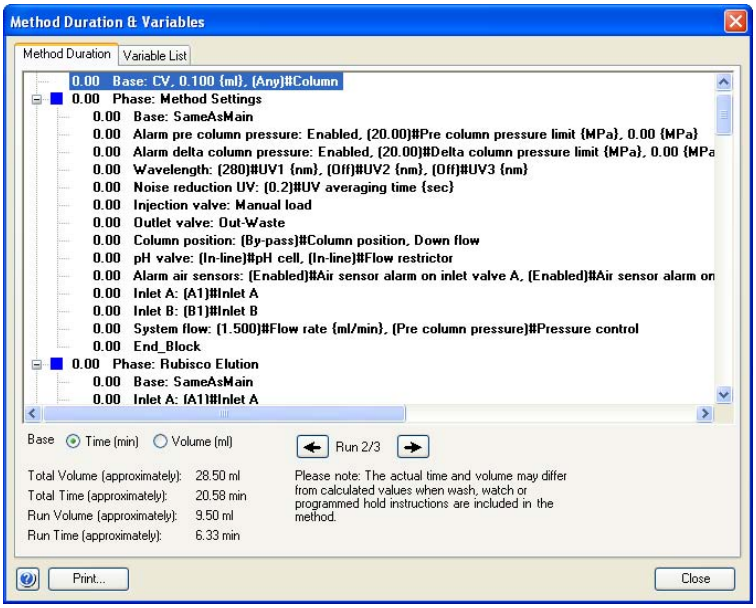
View and print the method duration time and variables

The table below describes how to view and print an estimation of the method duration time and the variables in the method:

Step	Action
1	In the ***Method Editor***: • click the ***Duration & Variables*** button below the ***Method Outline*** pane  *or* • select ***View:Duration & Variables***  *Result*: The ***Method Duration & Variables*** dialog opens displaying the ***Method Duration*** tab.

Step	Action

2



The ***Method Duration*** tab shows an estimation of the accumulated method time and volume for the current method below the text method.

If the method includes a ***Scouting*** series, an estimation of the accumulated method time and volume for the total series of runs is displayed below the text method.

Note: Click the arrow buttons to display the different scouting runs.

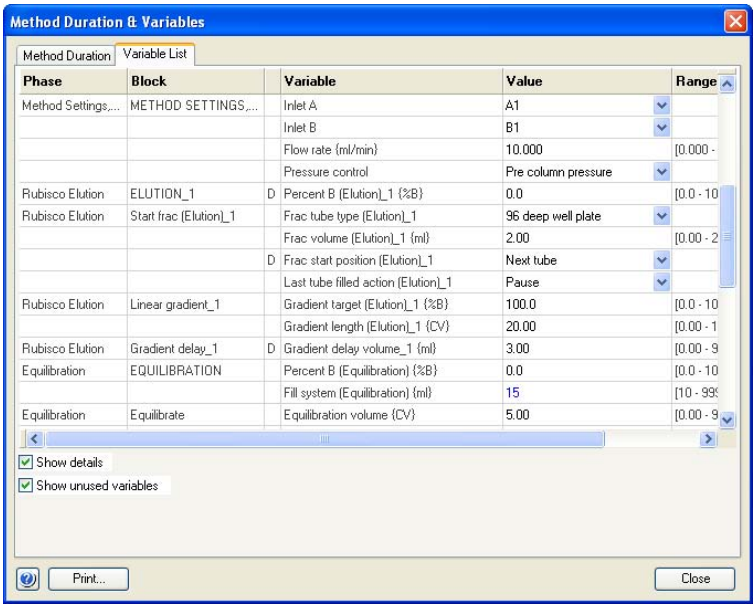
The accumulated time/volume is an approximation and does not take into account time or volume for ***Watch*** blocks, ***Wash*** commands or programmed ***Hold***.

3

- Select ***Time*** as ***Base*** to show the time in minutes in the text method.
- Select ***Volume*** as ***Base*** to show the volume in the text method.

4 To view the variables in the method, click the ***Variable List*** tab.

Result: The ***Variable List*** is displayed.

Step	Action
5	

The ***Variable List*** shows the variables in the method. It is also possible to see in which phases the variables are included and the different values. Variables with an ellipsis (**...**) after their name are used in multiple phases or blocks. It is not possible to change any values in this dialog.

- Check the ***Show details*** box to view variables classified as detailed. The letter ***D*** will be shown to the left of the detailed variables.
- Check the ***Show unused variables*** box to view unused variables in the method. The letter ***U*** will be shown to the left of unused variables.

6 To print the information in the ***Method Duration & Variables*** dialog, click the ***Print...*** button.

Result: The ***Print*** dialog opens.

7 Select a ***Printer*** from the drop-down list and click ***OK***.

Result: The information is printed.

3.5 Save methods and phases

Introduction

Methods and phases are saved in the UNICORN database.

Individual, edited phases may be saved to the ***Phase Library*** for later use in other methods on systems having the same instrument configuration and component configuration.

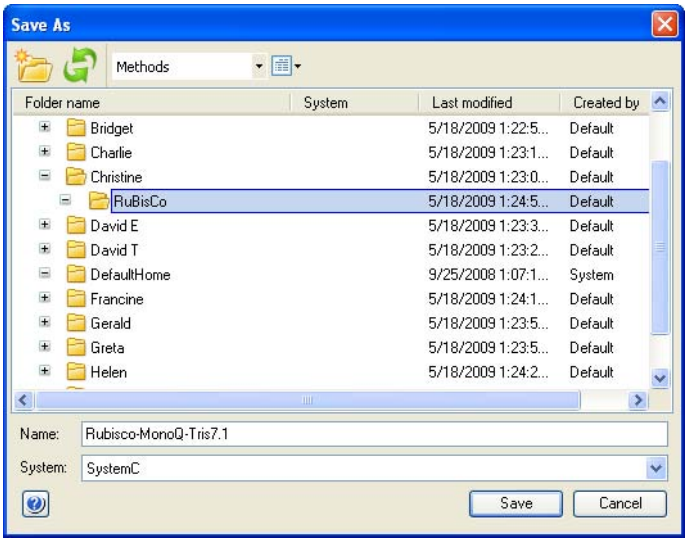
Note: You cannot save an edited method/phase to replace a predefined method or phase. If you want to save an edited variant of a predefined method or phase with specific settings, you must save it under another name. A predefined method or phase can not be overwritten.

Save a method

The table below describes how to save a method in UNICORN.

Step	Action
1	• Click the ***Save the Method*** icon  or • select ***File:Save*** or ***File:Save As***. *Result*: • If the method has been named and saved previously, the changes are saved immediately. *If not* • The ***Save As*** dialog opens. Proceed with steps 2-4 below.

Step	Action
2	Browse for an appropriate folder, or create a new one.



3

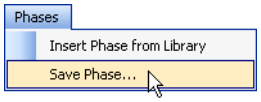
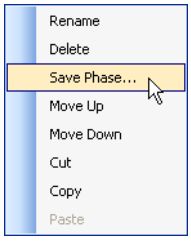
- Select the folder in which to save the results.
- Enter a method ***Name.***
- Select for which ***System*** to save the method

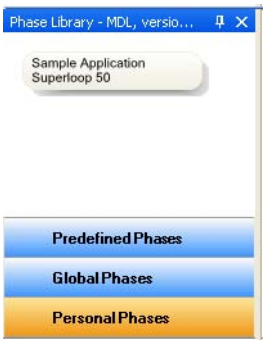
Step	Action
4	Click ***Save***. *Result:* The method is saved in the database. **Note:** An error message will appear if you are trying to save the method for: • a system using another instrument configuration and/or another component configuration than the method originally was created for and • the settings in the method depends on the component configuration (e.g., if an extra inlet A valve is used in the method, this setting cannot be used in a system lacking the extra inlet A valve.) It will still be possible to save the method but the phases in the method will be marked with an error symbol. In order to be able to subsequently run the method, either the method must be text-edited or the component configuration of the system changed in the ***Administration*** module.

Save a phase

The table below describes how to save a phase to the ***Phase Library***:

Step	Action
1	Select the phase to be saved in the method outline. **Note:** A ***Method Settings*** phase can not be saved as a separate phase with a new name. If editing properties for the ***Method Settings*** phase, the changes will be saved with the method.

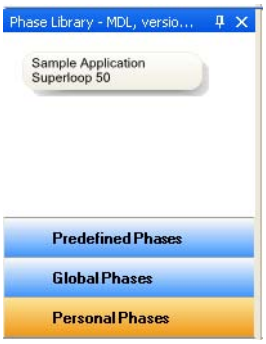
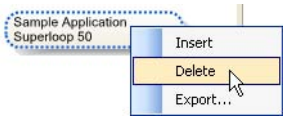
Step	Action
2	• click the ***Save Phase...*** button below the ***Method Outline*** pane  *or* • select ***Phases:Save Phase...***  *or* • right-click the phase and select ***Save Phase...***  *Result*: The ***Save Phase to Phase Library*** dialog opens. 
3	• Type a ***Phase name*** *or* • Choose a phase from the ***Phase name*** drop-down list. This phase will be replaced by the phase with the new settings.

Step	Action
4	In the ***For system*** field, the system that was selected when the current method was set up will be displayed by default. To save the phase for another system, choose the appropriate system from the ***For system*** drop-down list. **Note:** Only systems using the same instrument configuration and component configuration as the system that was selected when the current method was set up will be displayed in the ***For system*** field.
5	• Select if the phase shall be ***Global*** (available for all users) or ***Personal*** (for your own use only). • Click ***OK***. **Note:** It is not possible to replace a predefined phase by saving an existing phase. *Result*: The phase is saved and is available in the ***Global Phases*** or ***Personal Phases*** panel of the ***Phase Library***. 

Delete a phase from the Phase Library

It is possible to delete personal and global phases from the ***Phase Library***. Predefined phases cannot be deleted.

The table below describes how to delete a personal or global phase from the phase library:

Step	Action
1	Select the appropriate phase library: ***Personal Phases*** or ***Global Phases*** at the bottom of the ***Phase Library*** pane.  *Result*: The phases in that phase library are displayed. 
2	Select the phase to delete from the library.
3	• Click the ***Delete*** button at the bottom of the ***Phase Library*** pane.  *or* • Right-click the phase and select ***Delete***.  *Result*: The phase is removed from the ***Phase Library***.

3.6 Scale or convert methods

Introduction

UNICORN methods are always created specifically for a designated system and thus also for a specific system type (e.g. ÄKTA avant 25). However, it is often useful to convert a method that was originally created for a system of one type , for use with another a system of another type (e.g. ÄKTA avant 150). The converted method is created as a copy of the original method. The original method remains saved.

When a method is converted, it can also be scaled for use with a different column type than what it was originally created for. Both processes are described in this section.

Tip: If you wish to use a method for another system of the same ÄKTA avant system type that it was originally created for, you only need to choose ***File:Save As***, select the new system and save the method with another name.

To only change the selected column type, you should edit the method, change the column selection and save the edited method.

Prerequisites

The following items should be considered to ensure that the method conversion and scaling is successful:

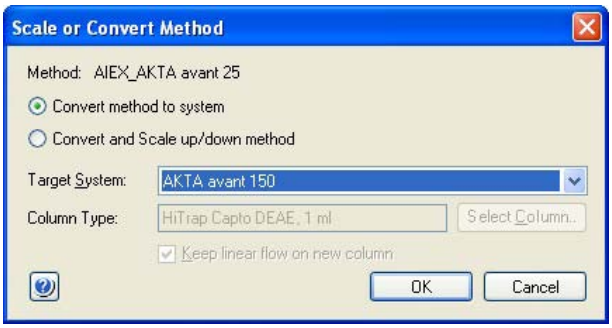
- The original method should use column volume (CV) as base
- All parameters that will require scaling should be defined as variables
- If linear flow is to be maintained in the scaled method, it must have been applied in the original method as well
- Scaling of the column type is not possible if the ***Any*** column type was selected in the original method
- Text edited phases will not be automatically updated during the conversion

Convert a method to another system type

The table below describes how to convert a method to be used with another system type.

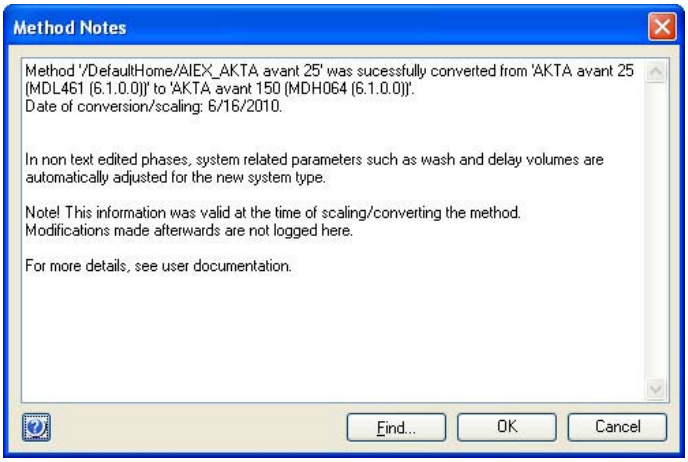
Step	Action
1	Open the method you want to convert in the ***Method Editor***.

Step	Action
2	Choose the menu command ***File:Scale or Convert Method*** *Result:* The ***Scale or Convert Method*** dialog opens.



3	Select the option ***Convert method to system***.
4	Choose the system to which the method should be converted in the ***Target System*** list. The list will show all available, active systems. Deactivated systems are not shown. **Tip:** You can convert methods that originally were created for systems that now are deactivated.

Step	Action
5	Click the ***OK*** button. *Result:* The method is converted as an untitled copy (***UNTITLED converted****). The ***Method Notes*** dialog opens, showing basic information about the conversion.



Note: The information shown in the ***Method Notes*** will not include details about scaled system related parameters (e.g. wash or delay volumes) or notes concerning method instructions that may have become invalid as a result of conversion between systems with different components and instrument configurations. You must verify that there are no phases with invalid instructions (i.e. phases marked with a red cross) in the new method before it can be used. See note below this instruction.

6

- Type any additional notes you wish to add in the text field and
- click the ***OK*** button to close the ***Method Notes*** dialog.

Step	Action
7	• Choose the ***File:Save As*** menu command to save the converted method or • click the ***Save*** icon  *Result:* The ***Save As*** dialog opens, with the folder where the original method is saved open by default.
8	• Select the desired target folder, • type a new method name in the ***Name*** field and • click the ***Save*** button.

Note: The original method remains after the converted method is saved. However, the converted method will replace the original if you choose to save the converted method with the same name in the same folder as the original.

Note: The flow rate and/or pressure settings in the method will automatically be adjusted if the maximum flow rate and/or pressure values for the target system is exceeded after the conversion. The maximum settings for the target system will be used by default.

Note: In case the original method contains instructions that are not supported by the new system, this will be indicated in the method outline of the converted method as a red cross on the phase containing these instructions.

To be able to run the method on the new system you need to replace or remove the invalid instructions in the ***Text Instructions*** pane. Invalid instructions are indicated with red square symbols in the text instructions.

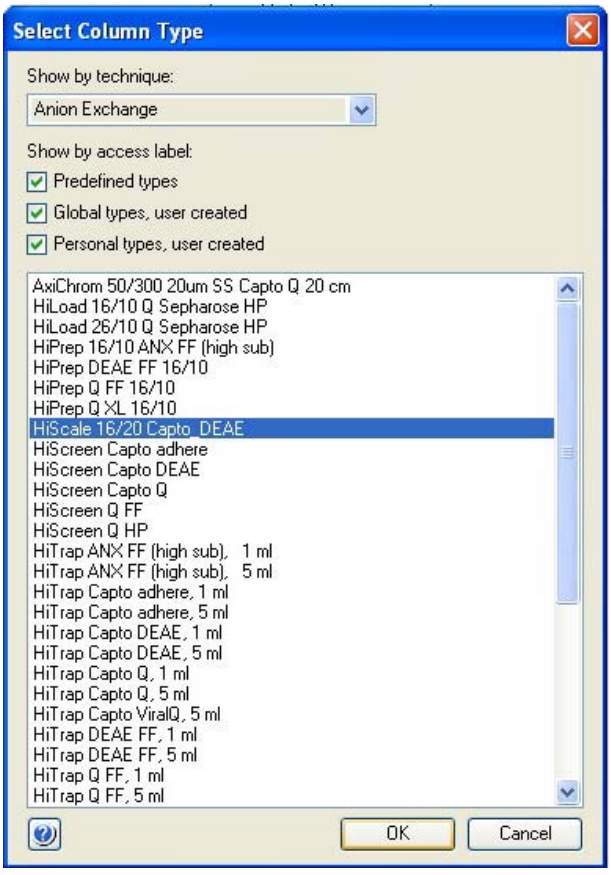
You can also replace the phase with a predefined phase from the phase library.

Convert and scale a method up or down

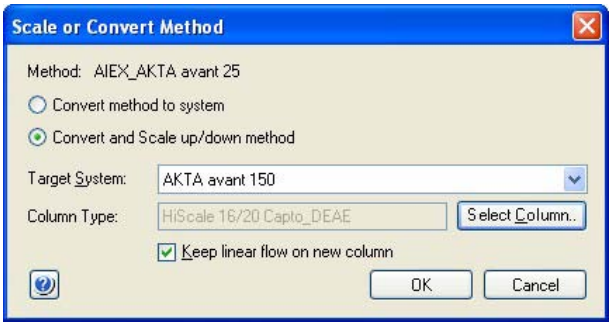
The table below describes how to convert a method to be used with another system type, and at the same time scale the method to be used with another column size.

Step	Action
1	Open the method you want to convert and scale in the ***Method Editor***.
2	Choose the menu command ***File:Scale or Convert Method*** *Result:* The ***Scale or Convert Method*** dialog opens.
3	Select the option ***Convert and Scale up/down method***.
4	Choose the system to which the method should be converted in the ***Target System*** list.

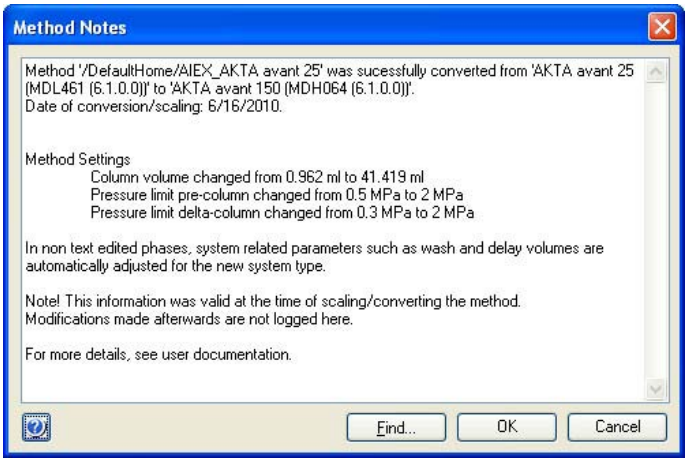
Step	Action
5	By default, the same column type that was selected in the original method is shown in the ***Column Type*** field. • Click the ***Select Column...*** button to select a new column type. *Result:* The ***Select Column Type*** dialog opens.



Step	Action
6	• Select the column type from the list of available column types and • click the ***OK*** button. *Result:* The ***Select Column Type*** dialog closes and the selected column is shown in the ***Column Type*** field of the ***Scale or Convert Method*** dialog.
7	If desired, select the ***Keep linear flow on new column*** option. **Note:** This option is applicable only if linear flow was selected in the original method. If linear flow is not selected, the default flow settings for the selected column type will be used.



Step	Action
8	Click the ***OK*** button. *Result:* The method is scaled as an untitled copy (***UNTITLED converted****). The ***Method Notes*** dialog opens, showing basic information about the conversion.



Note: The information shown in the ***Method Notes*** will not include details about scaled system related parameters (e.g. wash or delay volumes) or notes concerning method instructions that may have become invalid as a result of conversion between systems with different components and instrument configurations. You must verify that there are no phases with invalid instructions (i.e. phases marked with a red cross) in the new method before it can be used. See note below this instruction.

9

- Type any additional notes you wish to add in the text field and
- click the ***OK*** button to close the ***Method Notes*** dialog.

Step	Action
10	• Choose the ***File:Save As*** menu command *or* • click the ***Save*** icon  *Result:* The ***Save As*** dialog opens, with the folder where the original method is saved open by default.
11	• Select the desired target folder, • type a new method name in the ***Name*** field and • click the ***Save*** button.

Note: The original method remains after the converted method is saved. However, the converted method will replace the original if you choose to save the converted method with the same name in the same folder as the original.

Note: The flow rate and/or pressure settings in the method will automatically be adjusted if the maximum flow rate and/or pressure values for the target system is exceeded after the conversion. The maximum settings for the target system will be used by default.

Note: In case the original method contains instructions that are not supported by the new system, this will be indicated in the method outline of the converted method as a red cross on the phase containing these instructions.

To be able to run the method on the new system you need to replace or remove the invalid instructions in the ***Text Instructions*** pane. Invalid instructions are indicated with red square symbols in the text instructions.

You can also replace the phase with a predefined phase from the phase library.

The method after conversion

The conversion will adjust the following settings to the appropriate values for the selected new system:

- Gradient delay
- System wash volume (i.e. ***Fill system*** value)

- Volume for finalization of sample application (only applicable when ***Inject sample directly onto column*** and then ***Inject all sample using air sensor*** is selected)

The following settings may remain to be adjusted manually after the conversion:

- Sample volume
- Fractionation volumes
- User defined volumes in System CIP and System preparation
- System related settings in text edited phases

  (However, column related settings will be scaled also in text edited phases, provided they have been defined as variables)

Converting a method for use in a different database

The table below describes the necessary steps to be performed if you wish to convert a method for use with another ÄKTA avant system type, in another database than where the original method was created.

Tip: This procedure must be followed in order to convert methods from one stand-alone system to another stand-alone system.

Step	Action
1	Set up a new system in the target database. Use the same instrument configuration as the system for which the method was originally created. **Tip:** This system is created for the conversion only and can be set up inactivated.
2	Export the method from the original database.
3	Import the method into the target database, for use with the new, inactivated system.
4	Convert the method from the inactivated system to be used with the target system, as described in the applicable instruction above (i.e. with or without scaling of the column size).

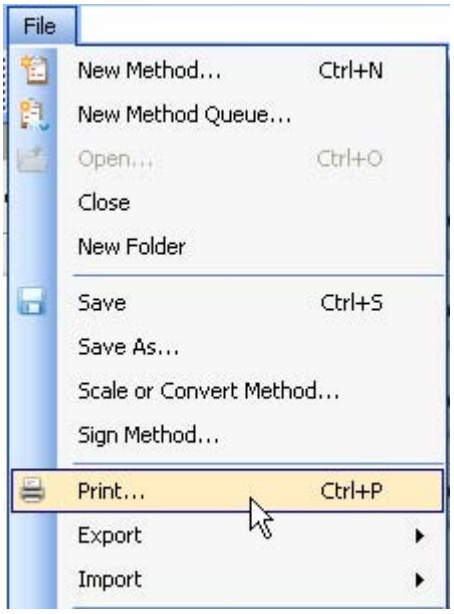
3.7 Print a method

Introduction

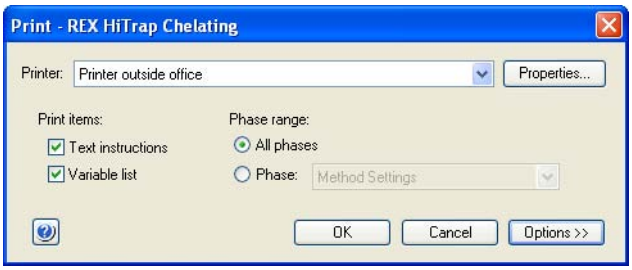
This section describes how to print a method's text instructions and variables. UNICORN uses the printers and printer settings that are installed on your computer.

Print a method

The table below describes how to print an opened method:

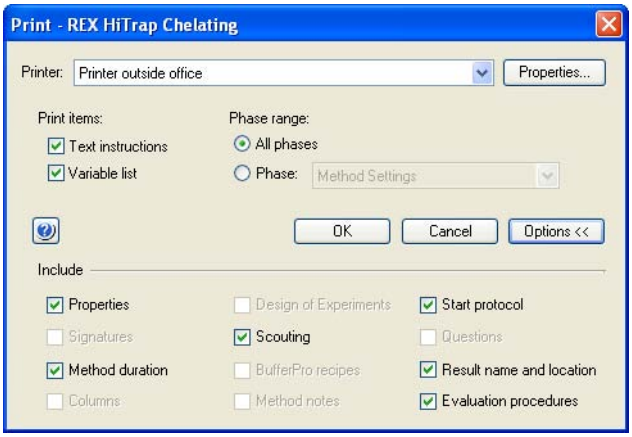
Step	Action
1	• Click the ***Print*** icon in the ***Toolbar***  or • select ***File:Print...***  *Result:* The ***Print*** dialog opens.

Step	Action
2	In the ***Print*** dialog:



- select ***Printer***
- select which ***Print items*** to be printed
- select to print all phases in the method or a specific phase in the ***Phase range*** field

3	By default, information about the overall method settings as well as any signatures and specific columns used in the method are printed. To exclude or add information, click ***Options>>*** and check/uncheck the appropriate boxes.



Note: Only options that are used in a method can be printed. Options that are not used are greyed out in the ***Print*** dialog.

4	Click ***OK***. *Result*: The method is printed.

3.8 Predefined methods and phases

Introduction

A predefined method contains a set of phases, each phase reflecting a specific stage of a chromatography or maintenance run. You can select additional phases from the phase libraries and add these to an existing method, or remove an undesired phase.

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 on the ***Phase Properties*** pane in the appropriate phases.

This section describes the predefined methods and phases.

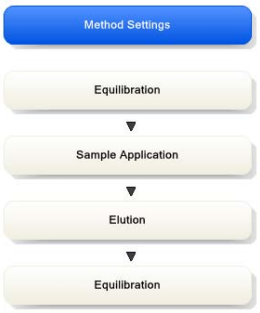
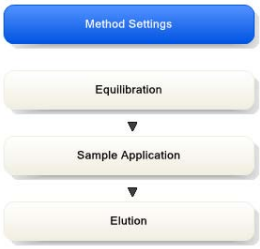
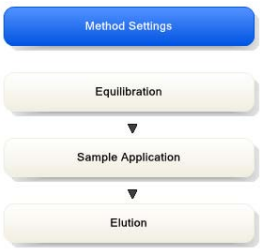
Predefined purification methods

The ***Method Editor*** has 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)	After equilibration and sample application, the protein of interest is adsorbed to the column ligand. After a wash to remove unbound sample, elution is performed either by using a buffer containing a competitor to displace the protein of interest, or by changing the pH or ionic strength. Finally, the column is re-equilibrated with start buffer.	

Predefined purification method	Principle	Included phases
Anion Exchange Chromatography (AIEX)	After equilibration and sample application, negatively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g. NaCl). Finally, the column is washed and re-equilibrated with start buffer.	
Cation Exchange Chromatography (CIEX)	After equilibration and sample application, positively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g. NaCl). Finally, the column is washed and re-equilibrated with start buffer.	

Predefined purification method	Principle	Included phases
Chromatofocusing (CF)	After equilibration and sample application, elution is performed using a pH gradient. The proteins separate and elute according to their isoelectric points. Finally, the column is re-equilibrated.	
Desalting	After equilibration and sample application, the proteins are eluted isocratically. This technique is commonly used for buffer exchange.	
Gel filtration (GF)	After equilibration and sample application, proteins separate and elute according to their size (largest first).	

Predefined purification method	Principle	Included phases
Hydrophobic Interaction Chromatography (HIC)	After equilibration and sample application (use a buffer containing a high salt concentration, for example 2 M Ammonium Sulphate) hydrophobic proteins are adsobed to the column ligand. After a wash to remove unbound sample, elution is performed using a gradient of decreasing salt concentration. Finally, the column is washed and re-equilibrated with start buffer.	
Reversed Phase Chromatography (RPC)	After equilibration and sample application, hydrophobic proteins adsorb to the column ligand. After a wash to remove unbound sample, elution is performed by generating a gradient of a non-polar, organic solvent such as Acetonitrile. Finally, the column is washed and re-equilibrated.	

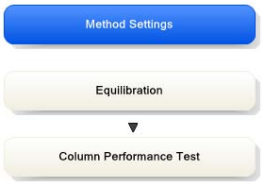
WARNING

Fraction collector. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using organic solvents, collect fractions through the Outlet valve.

Predefined maintenance methods

A number of predefined methods for preparation and cleaning are available. These maintenance methods are used to prepare the system, clean the system, and to fill the system with storage solution.

The table below describes the available predefined maintenance methods.

Predefined maintenance method	Principle	Included phases
Column CIP	The column is filled with a cleaning solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several cleaning solutions can be used. Suggestions for cleaning steps are available for a number of column types.	
Column Performance Test	After equilibration of the column, sample is injected via a capillary loop and eluted isocratically. A non-adsorbing sample like acetone or salt should be used. After the run, calculate column performance in the ***Evaluation*** module. The efficiency of the column is determined in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s). The result is logged in the column logbook.	
Column Preparation	The column is filled with buffer solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several preparation solutions can be used.	

Predefined maintenance method	Principle	Included phases
Intelligent Packing	Packs AxiChrom columns, with a predetermined column type, by a flow of hydraulic liquid that pushes the adaptor down. The user initiates the start of compression at the exact point when the adapter reaches the consolidated bed surface. The adapter compresses the bed according to the packing factor or target bed height as selected. Two Column Performance Test (upflow/downflow) phases are automatically performed after the AxiChrom column has been packed. Only available for ÄKTA avant 150.	
System CIP	The system is filled with cleaning solution. Select for example inlets, outlets and column positions to be cleaned. Three ***System CIP*** phases are included in the method to facilitate the use of three different cleaning solution. Additional ***System CIP*** phases can be added from the ***Phase Library*** if desired.	
System Preparation	The system is filled with preparation solution. Select for example inlets, outlets and column positions to be prepared. Two ***System Preparation*** phases are included in the method. Additional ***System Preparation*** phases can be added from the ***Phase Library*** if desired.	

Predefined phases

The table below describes the predefined phases.

Phase Name	Description
Method Settings	The first, and mandatory, phase in any method. Defines common parameters used in the subsequent phases. The ***Method Settings*** phase defines: • Column type • Pressure limits • Flow rate • Option to control the flow to avoid overpressure **Note:** Default values for pressure limits and flow rate are given for the selected column type • Column position • Flow restrictor use • Buffer preparation: - Manual, or - BufferPro (automatic buffer preparation) • Unit selection for Method base and Flow rate • Monitor settings: - UV monitor - pH monitor - Air sensor alarm settings • Settings for Column Logbook • Start Protocol • Result name and location **Note:** Some of these options may not be required by certain methods.
Equilibration	Equilibrates the column before purification, or re-equilibrates the column after purification.
Sample Application	Applies sample to the column. Defines the sample application technique, the sample volume, and the handling of flowthrough.

Phase Name	Description
Column Wash	Washes out unbound sample after sample application or removes strongly bound proteins after elution.
Elution	Elutes the sample from the column. Defines parameters for the elution and fractionation settings.
Column Preparation	Prepares the column before use by removing the storage solution and equilibrating the column. By adding steps, several preparation solutions can be used sequentially.
Column CIP	Cleans the column after purification runs by rinsing the column with a cleaning solution to remove unspecifically bound proteins. By adding steps, several cleaning solutions can be used sequentially.
System Preparation	Prepares the system before a run by removing storage solution and filling the system and inlets with buffer solution. One preparation solution is used per phase.
System CIP	Cleans the system after purification runs by rinsing the system with a cleaning solution. One cleaning solution is used per phase.
Column performance test	Tests the efficiency of a packed column in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s).
Intelligent Packing	A flow of hydraulic liquid pushes the adapter down. The user initiates the start of compression at the exact point when the adapter reaches the consolidated bed surface. The adapter compresses the bed according to the packing factor or target bed height as selected. Only available for ÄKTA avant 150.

3.9 Fraction collection

Introduction

For many purification schemes it is convenient to collect fractions of the eluent. Several of the predefined phases and methods include options for fraction collection in the ***Phase Properties*** pane.

This section describes briefly the various options available for fractionation in predefined methods and phases, and how to set up fraction collection when editing a method. More detailed information for individual settings can be found using the online help for the phase, see *Getting help when editing Phase Properties, on page 36*.

Fractionation overview

Fractionation is available in the ***Phase Properties*** pane in the predefined phases ***Sample Application*** , ***Column Wash*** and ***Elution***. These three phases are included in many of the predefined methods in UNICORN. This option will also be available in personal or global phases derived from these. See *Section 3.3.1 Edit phase properties, on page 35* for details on how to edit methods and phases.

For each phase, fractions can either be collected using the outlet valve or the fraction collector. If there is no risk of sample loss, the eluate may be sent to the waste and not collected. When fractionating to the outlet valve, a specific outlet valve position is selected. When collecting fractions in the fraction collector a tube or plate type is chosen and the fractions will be collected in the first available tube or plate of that type.

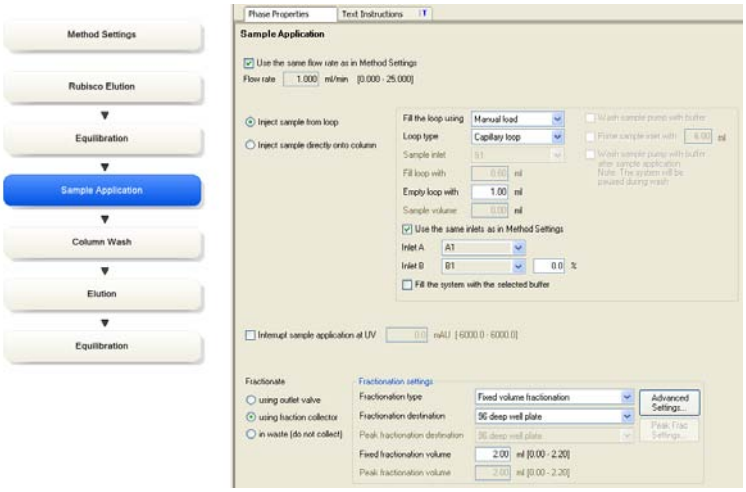
WARNING

Fraction collector. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using organic solvents, collect fractions through the Outlet valve.

Fractionation setup

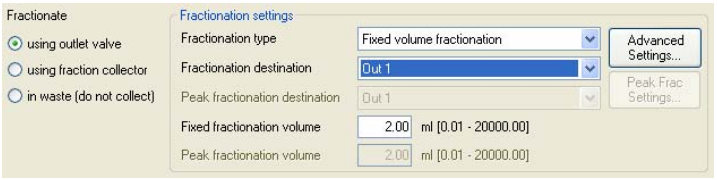
The following table describes the steps needed to set up fraction collection in the ***Phase Properties*** pane:

Step	Action
1	Select the phase for which fractionation is required in the method outline and select the ***Phase Properties*** pane.

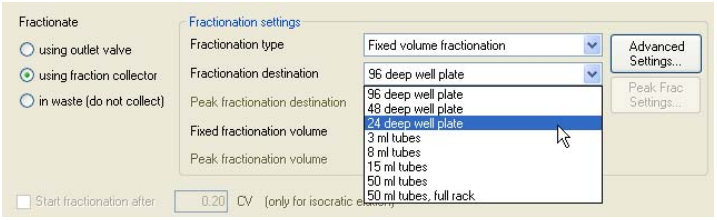


Note: Text edited phases will show the fractionation options as variables in the Phase Variables list, see *Chapter 9 Text edit methods, on page 286*.

Step	Action
2	Using the ***Fractionate*** radio buttons, select the fractionation type required for this phase: • ***using outlet valve*** enables fraction collection using the outlet valve. The Fractionation settings will change to reflect this choice, and the outlet valve position can be selected as the ***Fractionation destination***.



• ***using fraction collector*** enables fraction collection in the fraction collector. The Fractionation settings will change to reflect this choice, and the desired ***Fractionation destination*** can be chosen from the drop-down list.



• ***in waste (do not collect)*** will send the eluent to the waste.

3	Edit the ***Fractionation settings*** as appropriate. For detailed information on these settings see the online help for the phase, refer to *Getting help when editing Phase Properties, on page 36*.

3.10 Sign methods electronically

Introduction

Methods can be signed electronically to enhance data file security. Once a method has been signed, it is not possible to edit the method.

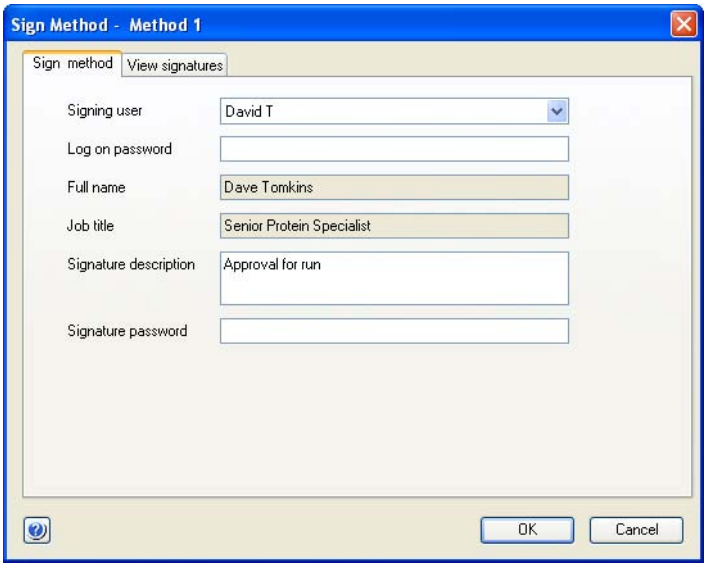
Tip: To edit a signed method create a new method using the settings in the signed method by selecting ***File:Save As...*** and save the method with a new name.

Sign a method electronically in the Method Editor

The table below describes how to sign a method electronically in the ***Method Editor***:

Step	Action
1	Select ***File:Sign Method...***.

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

Step	Action
2	 • The ***Signing user*** field shows the user currently logged on. If you are to sign the method but are not the one logged on to UNICORN, select your user name in the ***Signing user*** drop-down list. Your ***Full name*** and ***Job title*** are displayed. • Type your ***Log on password*** to UNICORN. • Type a ***Signature description*** if appropriate. • Type your ***Signature password*** and click ***OK***. *Result:* The method has been signed.

3.11 Import and export methods

Introduction

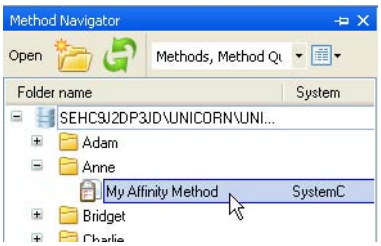
UNICORN methods are stored internally in the UNICORN database. It is however possible to export entire methods or individual phases to a zip file on the local computer so that they can be imported again later into the same database installation, or imported into another database installation.

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

Export a phase or method to UNICORN

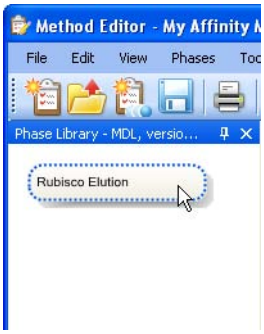
The following table outlines the steps needed to export a method or a phase for later import into UNICORN.

Step	Action
1	In the Method Navigator, select the method to be exported.



or

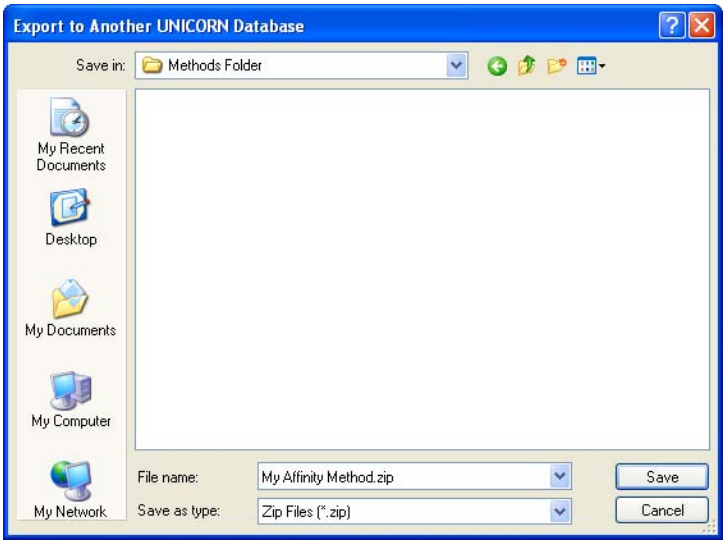
In the ***Phase Library*** pane, select the phase to be exported.



Note: Only ***Personal*** or ***Global*** phases may be exported.

Note: Only single methods or phases may be exported, it is not possible to write multiple methods or phases to a single zip file.

Step	Action
2	Choose ***File:Export:to UNICORN:Export Method to UNICORN...***. *Result*: The ***Export*** dialog opens.



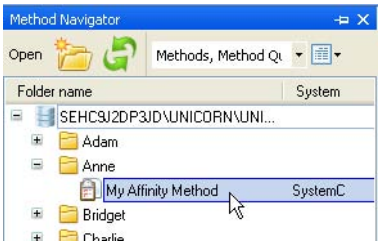
Note: In the case of phases, it is also possible to right-click on the phase name and select ***Export...*** from the context menu.

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

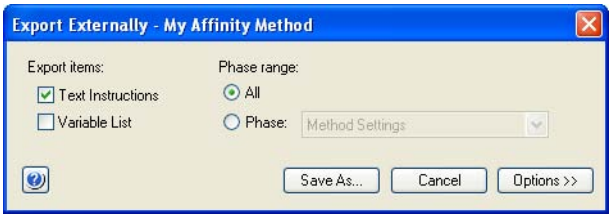
Export a method to a plain text file

The following table outlines the steps needed to export a method as a plain text file or to Excel.

Step	Action
1	In the Method Navigator, select the method to be exported.

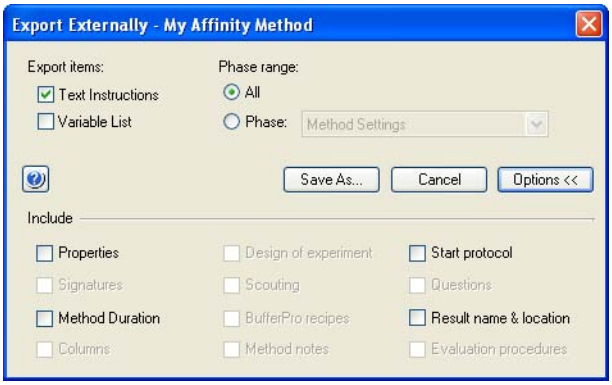


2	Choose ***File:Export:Export Method Externally...***. *Result*: The ***Export Externally*** dialog opens.

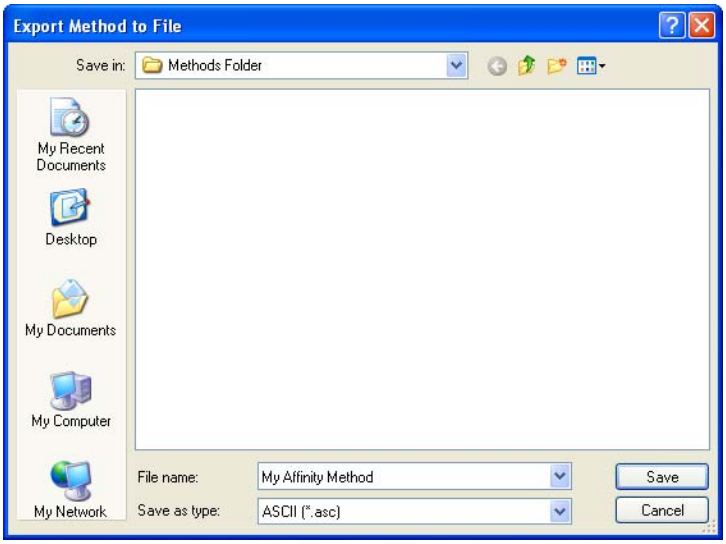


3	Choose which ***Export items*** to include by checking the appropriate boxes.
4	Choose whether to include ***All*** phases or only a specific ***Phase*** by selecting the appropriate ***Phase range*** option.

Step	Action
5	To add further information to export, click the ***Options >>*** button. *Result*: The ***Include*** options will be expanded.



6	Select information to add to the text file by checking the appropriate options. **Note:** Information that is not included in the method will appear greyed out and cannot be selected.
7	To save the text file with the selected information included click the ***Save As...*** button. *Result*: The ***Export*** dialog opens.

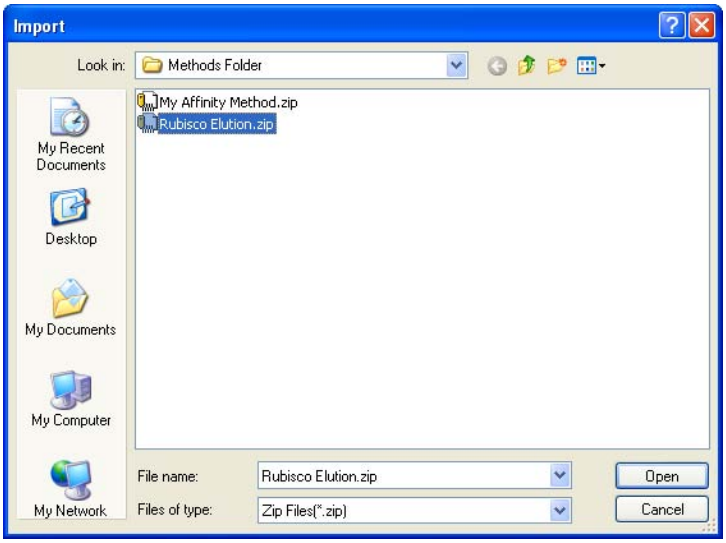


Step	Action
8	Choose whether to save to an ***ASCII*** file or to an ***Excel*** file from the ***Save as type*** drop-down menu.
9	Choose a file name and location and click the ***Save*** button to save the zip file.

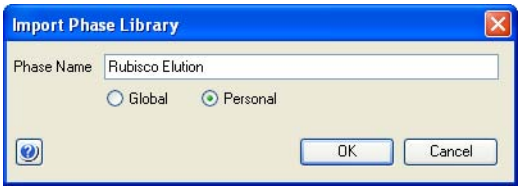
Import a phase into UNICORN

Phases that have previously been exported as zip files can be imported back into UNICORN. Plain text files or Excel files cannot be imported since there is no guarantee that they contain all the information UNICORN needs to recreate the phase. The following table outlines the steps needed to import a phase.

Step	Action
1	Select ***File:Import:Import Phase...***. *Result*: The ***Import*** dialog opens.



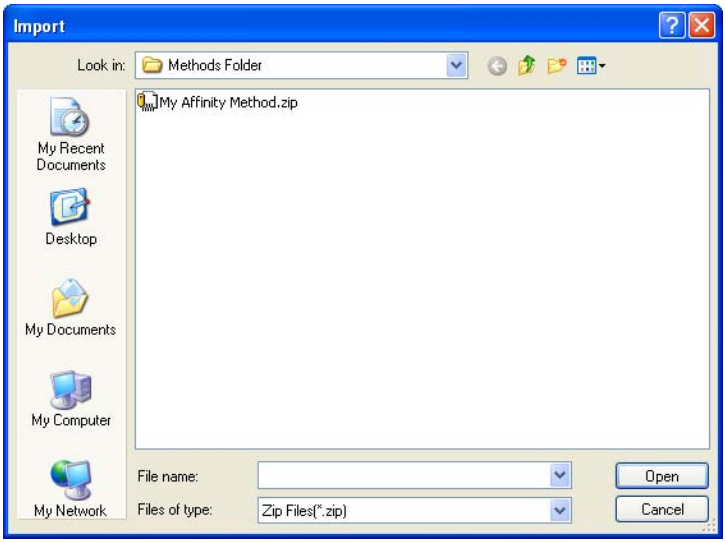
2	Browse to the required zip file in the ***Import*** dialog.

Step	Action
3	Open the file by selecting it and clicking the ***Open*** button, or by double-clicking on the file name. *Result*: The ***Import Phase Library*** dialog opens. 
4	Type a new ***Phase Name*** if required, and select whether the phase should be imported as ***Global*** or ***Personal***. ***Global*** phases are available to all users, ***Personal*** phases only to the currently logged-on user.
5	Click the ***OK*** button to import the phase.

Import a method into UNICORN

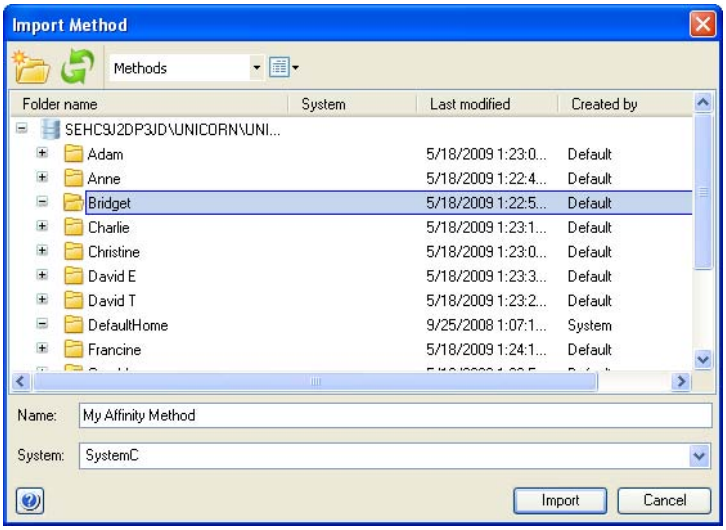
Methods that have previously been exported as zip files can be imported back into UNICORN. Plain text files or Excel files cannot be imported since there is no guarantee that they contain all the information UNICORN needs to recreate the method. The following table outlines the steps needed to import a method.

Step	Action
1	Select ***File:Import:Import Method...***. *Result*: The ***Import*** dialog opens.



2	Browse to the required zip file in the ***Import*** dialog.

Step	Action
3	Open the file by selecting it and clicking the ***Open*** button, or by double-clicking on the file name. Result: The ***Import Method*** dialog opens.



4	Browse to the required folder in the database and type in a new ***Name*** if necessary.
5	Select a ***System*** for the method from the drop-down menu.
6	Click the ***Import*** button to import the method. *Result*: The imported method will be opened in the ***Method Editor***. **Note:** If the imported method contains instructions that are not supported by the selected system, the phases containing these instructions will be marked with an error symbol.

4 Scouting

Introduction

Scouting is used to repeat a series of method runs automatically using different settings or with predetermined changes in the values for one or more ***Variables***. A ***Scouting scheme*** is defined as part of the method. This chapter gives an overview of scouting and the scouting workflow and describes how to set up and edit a ***Scouting scheme***.

Scouting is ideal for relatively simple variable combinations. When designing experiments to analyse several variables at the same time, it is advantageous to use the ***Design of Experiments*** (***DoE***) tool. This tool applies statistical methods for generating scouting runs that provide the most information with as few runs as possible, thus economising on time and sample amounts. For details on ***DoE***, see *Chapter 5 Design of Experiments, on page 116*.

In this chapter

This chapter contains the following sections:

Section	See page
4.1 Overview	104
4.2 Set up and edit a Scouting scheme	106

4.1 Overview

Introduction

Scouting can be used to generate a series of method runs where one or more ***Variable*** parameters are varied in the same method. The resulting ***Scouting scheme*** is defined and saved in the method.

This section gives an overview of scouting and the scouting workflow.

When to use scouting

Some typical situations where scouting is useful are for instance when the objective is to:

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

Scouting workflow overview

To perform a scouting experiment the following steps must be performed:

- **Create a method and decide appropriate run parameters (i.e.,variables) to be varied in the experiment**

  See *Chapter 3 Create and edit methods, on page 25* for information about how to create methods.

- **Set up a scouting scheme**

  This includes selecting variables, inserting runs/series of runs with different variable settings.

  To define new variables for a method, see *Section 9.2.4 Method variables, on page 315* for information.

- **Start and monitor the scouting run**

  This is performed in ***System Control***. See *ÄKTA avant and UNICORN 6.1 User Manual* for information.

Note: The ***Start protocol*** will only be displayed before the first run in the ***Scouting*** experiment.

- **Evaluate the results of the scouting run**

  This is performed in the ***Evaluation*** module. All results from ***Scouting*** runs performed at any one time are stored in the same folder. See *UNICORN 6.1 Evaluation Manual* for information.

4.2 Set up and edit a Scouting scheme

Introduction

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

This section describes how to set up and edit a ***Scouting scheme***.

Set up a scouting scheme

The table below describes how to set up a ***Scouting*** scheme where the flow rate is varied. In this example, the flow rate is varied between 0.5 and 3 ml/min.

Note: The ***Start protocol*** will only be displayed before the first run in the ***Scouting*** experiment.

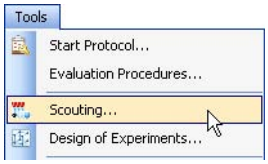
Step	Action
1	Create a method and decide appropriate run parameters to be varied in the experiment. The run parameters to be varied should be defined as ***Variables*** in your method. See *Chapter 3 Create and edit methods, on page 25* for information about how to create methods. See *Section 9.2.4 Method variables, on page 315* for information about how to define new variables. **Tip:** Many variables that can be used for scouting are already defined in either the Method Settings phase or the predefined phases. Note that some variables may be hidden or unused in the method. New variables often do not need to be defined.

Step	Action
2	In the ***Method Editor***:

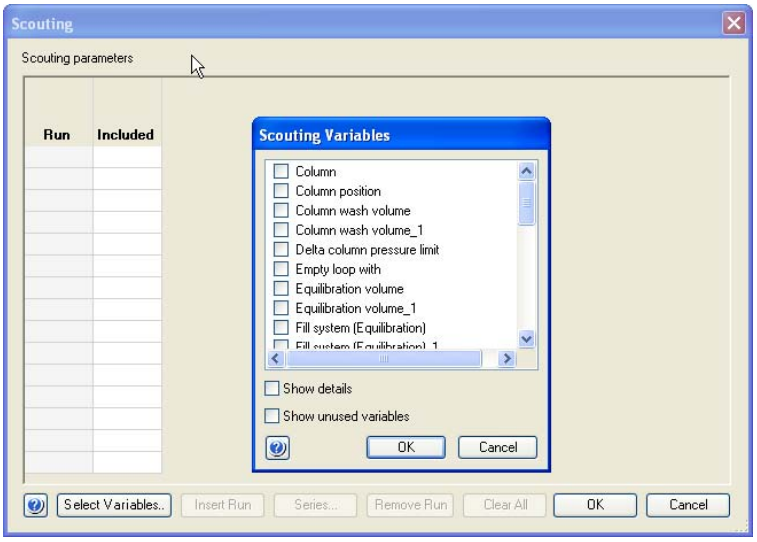
- Click the ***Scouting*** icon in the toolbar

or

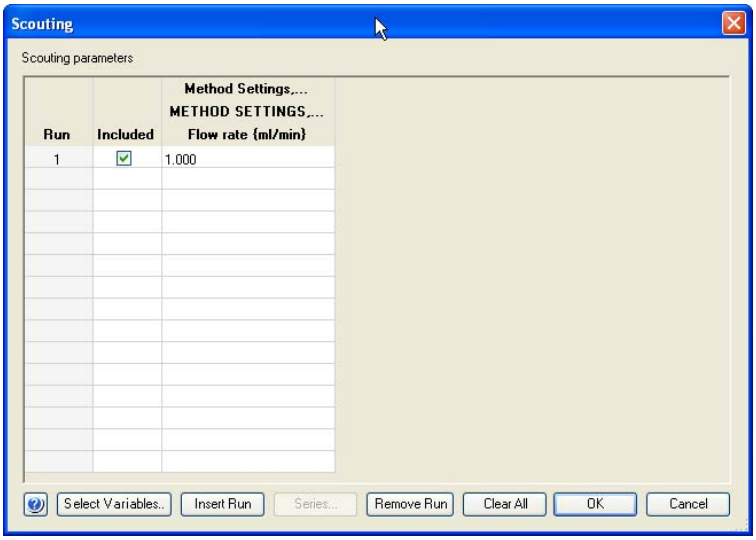
- Select ***Tools:Scouting***



Result: The ***Scouting*** dialog opens with the ***Scouting Variables*** dialog displayed on top.



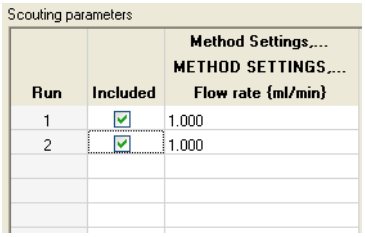
Note: When editing a scouting scheme, only the ***Scouting*** dialog is displayed.

Step	Action
3	• In the ***Scouting Variables*** dialog, select the appropriate variable(s) to be varied by checking the appropriate box(es). - Check the ***Show details*** box if you want to display variables defined as detailed variables in your method. These are rarely used as scouting variables. - Check the ***Show unused variables*** box if you want to display variables currently not used in the method. • Click ***OK***. *Result:* The ***Scouting*** dialog is updated with the selected variable(s) and their default value(s).  It is possible to insert runs one by one (see step 4) or insert series of runs (see step 5).

Step	Action
4	To insert runs one by one:

- In the ***Scouting*** dialog, select a row in the ***Scouting parameters*** table and click Insert Run.

  Result: A new row is added below the selected run. The variable value from the selected row is copied to the new run. All chosen variables are displayed in a separate column.



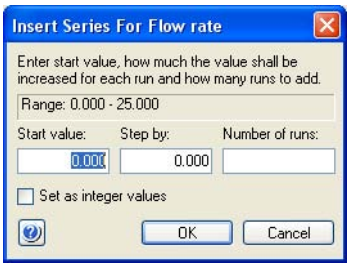
- In this example, click in the ***Flow rate {ml/min}*** column for the appropriate run and edit the flow rate value.

  Note: Changing variable values in the scouting scheme does *not* change the values in the ***Variable List*** in the ***Duration and Variables*** dialog or in the text instructions. The actual variable values used for each run in the scouting scheme are saved in the result file. To change the default values, the variable values must be edited in the ***Phase Properties*** pane.

- Repeat until all runs are included using the correct variable values.

  Note: The scouting scheme can also be edited just prior to starting the method run in the Start Protocol. Here variable values can be changed and individual runs included or excluded.

5	To insert a series of runs, click in the appropriate variable column in the ***Scouting parameters*** table and click Series.... This button is activated for variables with continuous values, such as flow rates or pressure limits. *Result:* The ***Insert Series*** dialog for the selected variable opens.

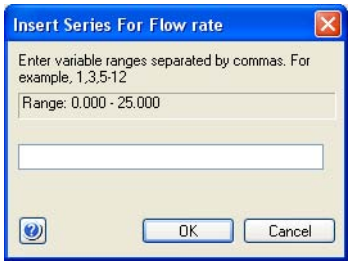


Step	Action
6	In the ***Insert Series*** dialog: • Enter ***Start value:***, ***Step by:*** and ***Number of runs:***. In this example, 0.5, 0.5 and 6. • Click ***OK***. *Result:* The ***Scouting parameters*** table is updated.

Scouting parameters

Run	Included	Method Settings,... METHOD SETTINGS,... Flow rate {ml/min}
1	☑	0.500
2	☑	1.000
3	☑	1.500
4	☑	2.000
5	☑	2.500
6	☑	3.000

Step	Action
7	Alternatively, to enter either consecutive or non-consecutive integer values: • Check the ***Set as integer values*** box in the ***Insert Series*** dialog. *Result:* The following alternative ***Insert Series*** dialog for the selected variable opens.



Step	Action
	• Enter the appropriate range, for example: 1-3,5-7 • Click ***OK***. *Result:* The ***Scouting parameters*** table is updated.

Scouting parameters

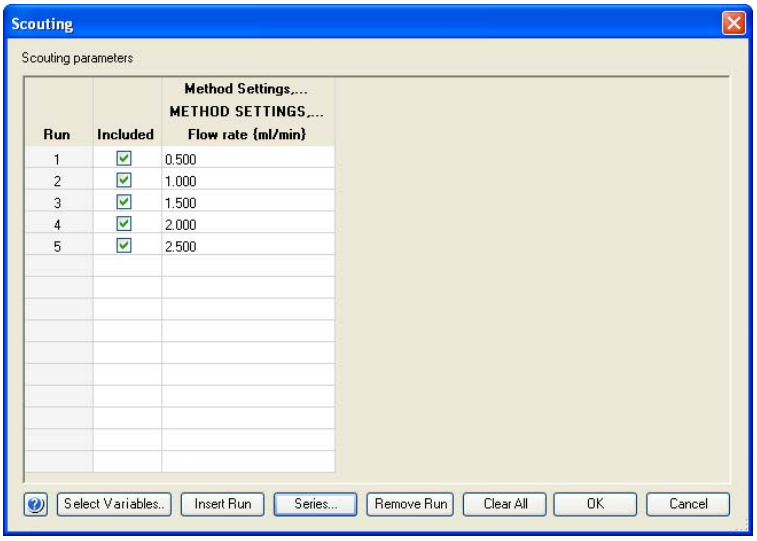
Run	Included	Method Settings,... METHOD SETTINGS,... Flow rate {ml/min}
1	☑	1.000
2	☑	2.000
3	☑	3.000
4	☑	5.000
5	☑	6.000
6	☑	7.000

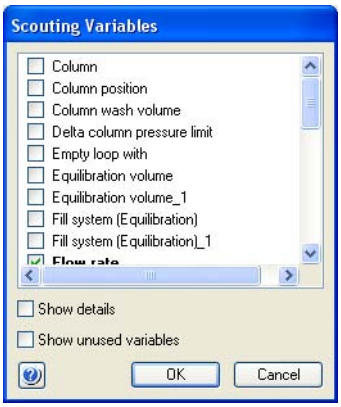
Step	Action
8	Click ***OK*** in the ***Scouting*** dialog to save the scouting scheme. Save the method.

Add, delete or edit variables in the Scouting scheme

The table below describes how to add, delete or edit variables in the ***Scouting scheme***.

Step	Action
1	Open the ***Scouting scheme*** (see block *Set up a scouting scheme, on page 106*).



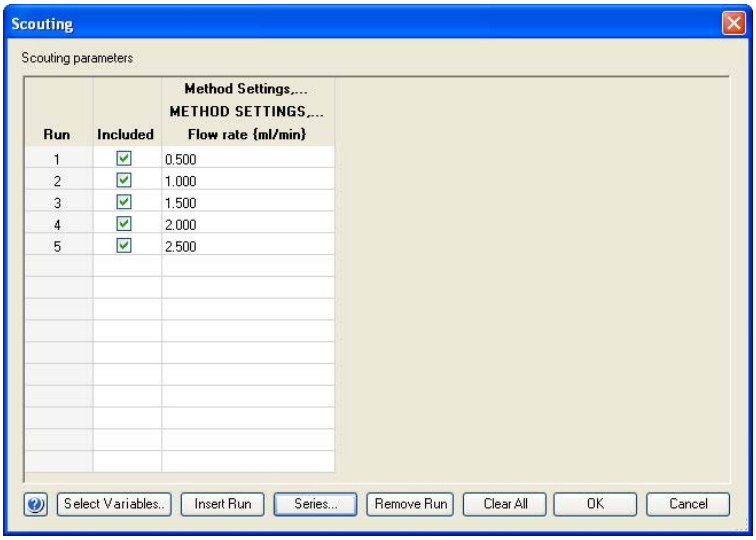
Step	Action
2	To add or delete variables in the ***Scouting scheme***, click Select Variables.. in the ***Scouting*** dialog. *Result:* The ***Scouting Variables*** dialog opens. 
3	• To add a variable to the ***Scouting scheme***, check the appropriate box in front of the variable. • To delete a variable from the ***Scouting scheme***, clear the box in front of the variable. If you can not find the appropriate variable: • Check the ***Show details*** box to display variables defined as detailed variables in your method. • Check the ***Show unused variables*** box to display variables currently not used in the method. To define a new variable, see *Section 9.2.4 Method variables, on page 315* for information. Click ***OK***. *Result:* The ***Scouting parameters*** table is updated with the changes.

Step	Action
4	To edit a variable value for a run: • Select the appropriate row and the variable value cell in the ***Scouting parameters*** table. • Type a new value for the variable. *Result:* The variable value is updated. **Note:** Changing variable values in the scouting scheme does *not* change the values in the ***Variable List*** in the ***Duration and Variables*** dialog or in the text instructions. The actual variable values used for each run in the scouting scheme are saved in the result file. To change the default values, the variable values must be edited in the ***Phase Properties*** pane.
5	Click ***OK***. *Result:* The ***Scouting parameters*** table is updated with the changes.
6	Add new scouting runs to the scouting scheme as required.
7	Click ***OK*** in the ***Scouting*** dialog to save the scouting scheme. Save the method.

Add/delete runs in the Scouting scheme

The table below describes how to add runs and series of runs to the ***Scouting scheme*** and how to delete runs.

Step	Action
1	Open the ***Scouting scheme*** (see *Set up a scouting scheme, on page 106*).



Step	Action
2	• *To insert a run after an existing run:* Select the appropriate row in the ***Scouting parameters*** table and click Insert Run. *Result:* A new row is added below the selected run to the ***Scouting parameters*** table. The variable value from the selected row is copied to the new run. Edit the variable value as appropriate. • *To insert a new series of runs:* - Click in the appropriate variable column in the ***Scouting parameters*** table and click Series.... - Set up a series in the ***Insert series*** dialog and click ***OK*** (see *Set up a scouting scheme, on page 106*). *Result:* The new set of runs are inserted in the ***Scouting scheme*** with the values provided. • *To delete runs from the Scouting scheme:* - Select the row(s) in the ***Scouting parameters*** table and click Remove Run. *Result:* The selected runs are removed from the ***Scouting scheme***. *or* - Click Clear All. *Result:* All runs are removed from the ***Scouting scheme***. No scouting will be performed when starting the run. • *To exclude a run from being used in the **Scouting** experiment but keep it in the **Scouting scheme**:* Clear the ***Included*** box in front of the appropriate run.
3	Click ***OK*** in the ***Scouting*** dialog to save the changes to the scouting scheme. Save the method.

5 Design of Experiments

About this chapter

This chapter describes how to set up an experimental design plan using the ***Design of Experiments*** tool in the ***Method Editor*** and how to evaluate the results of the runs in the ***Evaluation*** module. It also gives a brief overview of ***Design of Experiments*** and describes some basic terms and concepts used in the ***Design of Experiments*** tool in UNICORN.

In this chapter

This chapter contains the following sections:

Section	See page
5.1 Introduction to Design of Experiments	117
5.2 Create an experimental design	128
5.3 Run a scouting created with DoE	155
5.4 Evaluation of Design of Experiments	157

5.1 Introduction to Design of Experiments

Introduction

This section gives a brief introduction to the basic terms and concepts used in ***Design of Experiments*** (***DoE***).

What is Design of Experiments?

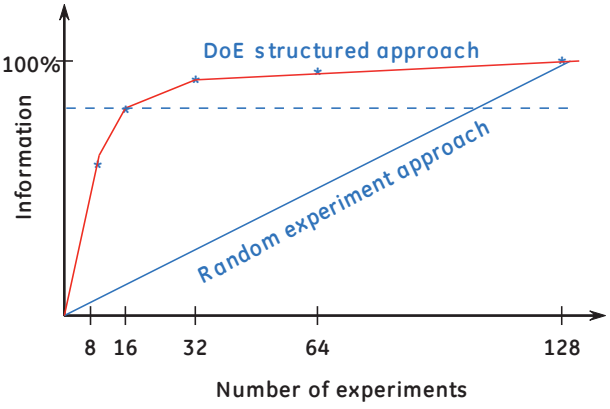
Design of Experiments (***DoE***) is a way to systematically vary several parameters simultaneously to obtain as much information about a process with as few experiments as possible.

Why use DoE?

Maximize the amount of information using a minimum number of runs

When trying to find optimal conditions for a process to obtain the best results, it is usually not possible to perform all experiments needed due to time or cost using a random experiment approach. The number of runs to be performed needs to be minimized at the same time as the information from the runs are maximized. ***DoE*** facilitates this by using a systematic approach for experimental set-up and statistical modelling for the results.

For example, it could be enough to obtain 80% information about a process. This level of information can be reached using a significantly lower number of experiments using ***DoE*** than using a random experiment approach as illustrated in the figure below.

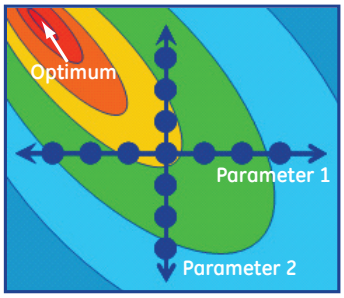


Estimate parameter interactions

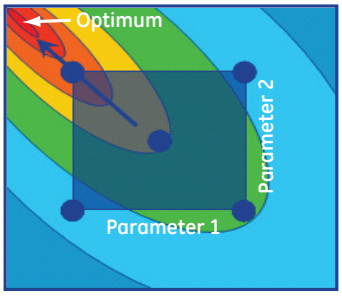
In the simplest traditional approach to optimization experiments, one parameter is varied while all others are fixed. In this way optimal values may be found for each parameter. Using this approach, interaction effects between parameters might be missed that could lead to better optimization of a process.

In the DoE approach, process parameters are allowed to vary simultaneously, thus allowing the effect of each parameter individually as well as the combined effect of parameters to be estimated. Each parameter may have an optimum, but when combining the parameters, values may be found that together give a new optimum, even better than the optima for the separate parameters. The illustration below shows the different approaches in a graphical way.

Traditional approach



DoE approach



Obtain reliable maps of the system

Experiments are performed to assess the conditions for best processes or to obtain the product characteristics required. In order to make the necessary decisions we need tools or ways to make this as intuitive and easy as possible. In the evaluation of ***DoE*** results, different plots are created from the model. Decision making becomes more reliable when using tools that benefit from the created model. This "map" of the process helps to decide on, for example, how to progress, or whether the process is already optimized. Is the process robust? What experiments can be performed to verify the process?

DoE in UNICORN

In UNICORN, ***DoE*** is used to systematically create an optimized set of experiments to be run. Depending on the objective and the number of parameters, a suitable design is suggested. An experimental plan is presented and a ***Scouting scheme*** is generated as a result from ***DoE*** containing the method runs to be performed. When the runs have been performed, the results can be analyzed in the ***Evaluation*** module. A model is created and a number of plots are generated to aid evaluation of the results. The model can be used to predict responses for new parameter settings and to optimize the parameter settings for a desired combination of responses (e.g., optimize the response combination "minimize the level of impurities and maximize the yield").

Factors and responses

The table below lists the definitions of the ***DoE*** terms factors and responses and how to use them in UNICORN.

Term	Definition	In UNICORN
Factor	The different parameters that may affect the process to be run. Factors may be either quantitative or qualitative. • Quantitative factors are characterized by being found on a continuous scale for example, pH, flow rate and conductivity. • Qualitative factors are characterized by being discrete (discontinuous), for example, column type, media type and buffer substance.	The factors are connected to a variable in the method. For example, the factor pH may be connected to the variable BufferPro pH. In pre-defined methods, most useful parameters are already defined as variables. **Note:** To be able to vary a value for a process parameter in the method it must be defined as a variable. Low and high values are entered for the quantitative factors. The factors will be varied within this range.
Response	The output parameter(s) from a process. For example, capacity, yield and purity.	When evaluating the ***DoE*** runs, the measured response values for each experiment are entered in UNICORN.

DoE design

The design is the setup of experiments with different combinations of factor settings resulting in a minimum number of experiments to be run to obtain as much information as possible.

UNICORN suggest a suitable design to be used in the experiment based on the:

- number of factors
- type of factors (quantitative or qualitative)
- experimental objective (screening, optimization or robustness testing)

There are different objectives and design types included in the ***DoE*** tool in UNICORN. See the following blocks for more information about design objectives and design types.

Design objectives

The table below describes the different design objectives:

Design objective	Used when you want to...
Screening	Determine which factors are important in your process and the appropriate ranges for these factors.
Optimization	Find the optimal factor settings for your process, that is, factor settings that give the desired responses.
Robustness Testing	Determine the process robustness by making minor adjustments of the factor settings and see if the responses are within the set specification limits. If the responses do not vary significantly due to the factor changes, the process is considered to be robust.

Example of how to use DoE for different objectives

To obtain maximum amount of protein after purification of a sample using a minimum number of runs, use ***DoE*** to find:

- important parameters (e.g., pH, conductivity) and the appropriate parameter ranges affecting this process
- the optimal parameter settings and any dependencies (interaction) between the parameters affecting the response of the product or process (e.g., yield or impurity level)

When the parameters affecting the process as well as their settings have been determined it is appropriate to test if the process is robust, that is, not affected by minor changes in the parameter settings. Neither the parameter settings selected or their interactions should affect the process if the process is to be considered robust.

A specific ***DoE*** setup is required for each step (i.e., screening for parameters or parameter settings, for optimization of parameter settings and for robustness testing). Each setup is a balance between the amount of information obtainable and the number of experiments that can be afforded. The process can be iterated and the initial screening results from one ***DoE*** can logically be used as input for the next ***DoE*** and so on.

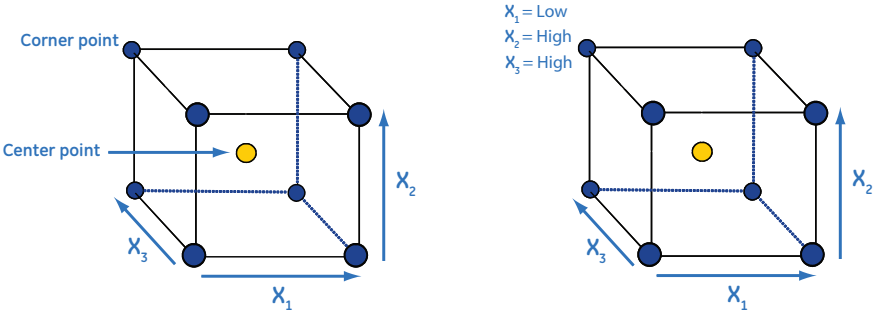
Design types

A design can be graphically illustrated by a box. The design box in the following examples illustrates designs where three different factors (X_1, X_2 and X_3) are varied simultaneously. Each corner point is the experiment for a specific combination of the settings of the three factors (e.g., low value for X_1 and X_2 and high value for X_3). The center point is the experiment where the different factors have the closest distance to all other factor settings, that is, the mean value.

The corner points are used to assess factor interaction effects. The center points are used to estimate the pure error and detect curvature. See *Model, on page 125* for detailed information about the terms interaction, curvature and pure error.

Illustration of the design box

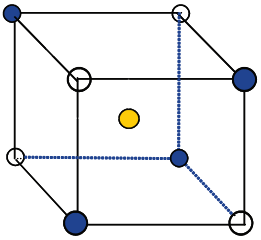
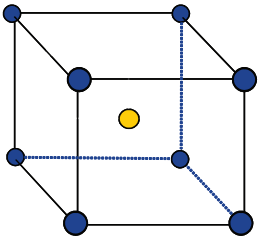
The illustration below (to the left) shows a design box with corner- and center points for the different factors X_1, X_2 and X_3. The illustration below (to the right) shows the factor values for one of the corner points. The arrow directions along the box edges denote the parameter change from low to high.

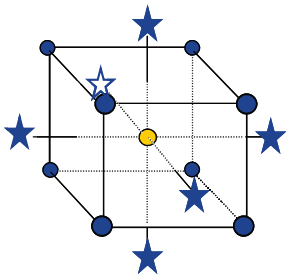


Different designs in UNICORN

There are several types of designs available in UNICORN.

The table below describes three design variants illustrated by the design box. Different designs are used based on the objective and the experiment setup. The center point experiments are always included in all designs.

Design type	Description
Fractional Factorial 	In the Fractional Factorial design, some of the corner point experiments are excluded as illustrated to the left (i.e., the white circles). This design will not give as much information as when using all corner point experiments but by excluding the corner points as shown in the illustration, the information loss is minimized. Information about which factors are important (main effects) and some information about factor interaction effects are obtained. This design type is good to use when you need to obtain information about the parameter settings and reduce the number of factors in your experiment before optimization. Fractional designs are suggested when performing: • ***Screening*** (because the information provided using this design is often enough to find the factors affecting the process) • ***Robustness Testing*** (because then optimal factor settings have already been found and only minor changes in the factor settings are studied) Less experiments are needed compared to using Full Factorial and Optimization designs.
Full Factorial 	The Full Factorial design uses all corner point experiments. This design is often suggested when performing ***Screening***. Information about which factors are important (main effects) and more information about factor interaction effects are obtained.

Design type	Description
Optimization designs 	For optimization studies and especially if curvatures are detected, the Full Factorial design can be extended with additional experiments outside the box, called star point experiments. The design box illustrates the experimental space (the low and high values of the different factors) and experiments outside the box, that is, star points enhancing the detection capability for curvatures. The default star point distance (CCC design, see below) can be edited in UNICORN. This design results in a higher number of experiments but more information can be obtained. It may be suggested when performing ***Optimization*** but often not as the first choice because a higher number of experiments must be performed. Information about which factors are important (main effects), information about factor interaction effects and curvature are obtained. See also *Model, on page 125* for information.

Designs supported by UNICORN

The table below briefly describes the design types that are supported by UNICORN.

Design type	Description
L-designs	L-designs are a type of Fractional Factorial design. Different variants are available in UNICORN. The table below gives a short description of the designs. **L-design** / **Description** ***L9*** — Fractional design at three levels for up to four factors. You can estimate square terms but not all interactions. ***L18*** — Fractional design with one factor at two levels and with up to 7 factors at three levels. ***L27*** — Fractional design at three levels for up to 13 factors. You can estimate square terms but not all interactions. ***L36*** — Fractional design at three levels for up to 13 factors. You can estimate square terms but not all interactions. L-designs are useful when performing ***Screening*** or ***Robustness Testing***.

Design type	Description
Plackett Burman	***Plackett Burman*** is a type of Fractional Factorial design with a lower resolution. This means that it is not possible to estimate any two-factor interactions using this design. ***Plackett Burman*** designs are useful when performing ***Screening*** or ***Robustness Testing***.
Rechtschaffner	***Rechtschaffner*** is a saturated fraction of the 2^n and 3^n factorial designs that supports all the first order interactions and quadratic terms. ***Rechtschaffner*** is useful when performing ***Optimization*** and you have at least three factors in your experimental plan.
Full Factorial 2 levels	***Full Factorial 2 levels*** is an orthogonal (balanced) design with all combinations of the factor levels. Main effects and all interactions are clear of each other (not confounded). Full Factorial 2 levels designs are useful when performing ***Screening*** or ***Robustness Testing***.
Full Factorial 3 levels	***Full Factorial 3 levels*** is a full factorial design with every factor varied at three levels. You can estimate the full quadratic model. ***Full Factorial 3 levels*** designs are useful when performing ***Screening***, ***Optimization*** or ***Robustness Testing***. They are however not the primary choice for ***Screening*** or ***Robustness Testing***.
CCC	The ***Central Composite Circumscribed*** (***CCC***) design is composed of a full or fractional factorial design and star points. ***CCC*** designs are useful when performing ***Optimization***.
CCF	The ***Central Composite Face*** (***CCF***) design is composed of a full or fractional factorial design and star points placed on the faces of the sides. ***CCF*** designs are useful when performing ***Optimization***.
Box Behnken	***Box Behnken*** is a three level Response Surface Modelling (RSM) design. All design points, except the center points, are located at the center of the edges of the hypercube, and are also on the surface of a sphere. You can estimate the full quadratic model. ***Box Behnken*** is useful when performing ***Optimization*** and you have at least three factors in your experimental plan.
Doehlert	***Doehlert*** is a RSM design constructed from regular simplexes. ***Doehlert*** designs are useful when performing ***Optimization***.

Model

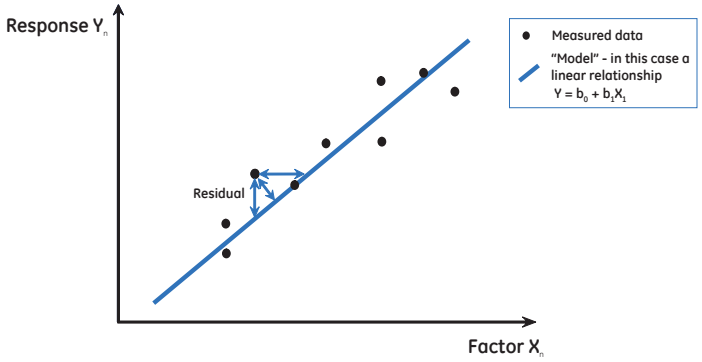
A model is created in the ***Evaluation*** module based on the response values measured or entered for each experiment in the ***DoE*** setup.

The model is a mathematical fit to all data (Multiple Linear Regression, MLR) and can be expressed as:

$$Y_n = f(X_1, X_2, ..., X_n)$$

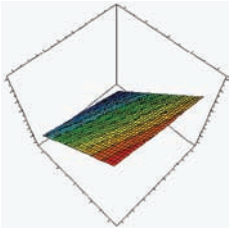
where **Y** is response and **X** is factor

The model can be explained in a graphical way as shown in the illustration below. In this case the "model" is a linear relationship. The residual (error) between the measured data and theoretical data according to the model is minimized.



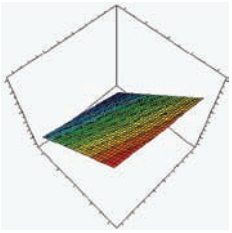
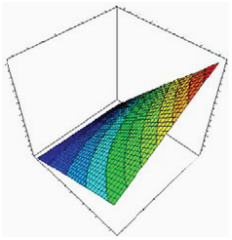
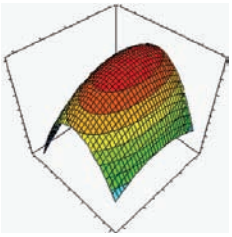
Model details

A more detailed description of the model is provided by the following formula as shown in the illustration below. The example is valid for three factors, X_1, X_2 and X_3 respectively.



As seen in the illustration above, the model can be divided into four parts, the Constant Term, the Linear Terms (main effects), the Two-way interaction terms and the Quadratic terms. The b-values are determined by the selected model. The Y-values are the response values that are entered in UNICORN.

The table below gives a brief description on how to interpret the different terms in the model.

Term	Graphical illustration	Description
Constant b_0	N/A	b_0 is the unknown constant term. It is the response of **Y** when the main effects are 0.
Linear (main effects) $b_1X_1 + b_2X_2 + b_3X_3$	Undistorted plane 	The main effects are described by the linear terms. In the graphical illustration, this part of the model can be viewed as an undistorted plane. It will give an overall idea of where the optimum for your process is but not details on how the sampling plane is twisted or which curvature the plane has. This part of the model usually gives sufficient information when the objective is screening or robustness testing. The fractional factorial designs will give enough input to create the linear part of the model.
Two-way interaction $b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$	Twisted plane 	The two-way interaction terms describe how the effect of one factor depends on the level of a second factor. In the graphical illustration, this part of the model can be viewed as twisted plane. This part is added to the model when the objective is screening. The fractional and full factorial designs will give input to the two-way interaction part of the model.
Quadratic $b_7X_1^2 + b_8X_2^2 + b_9X_3^2$	Curved plane 	The curvature of the sampling plane is described by the quadratic terms. This part is added to the model when the objective is optimization. The optimization designs will give enough information to create the quadratic part of the model.

DoE workflow in UNICORN

The main steps when performing a ***Design of Experiments*** in UNICORN are:

Step	Action
1	**Create a method for your process to be screened, optimized or tested for robustness** This includes defining the appropriate variables (if not already defined) that should be connected to the factors in ***DoE***. See *Chapter 3 Create and edit methods, on page 25* for information about how to create a method.
2	**Set up an experimental design** This is performed in the ***Method Editor*** in the ***Design of Experiments*** tool. See *Section 5.2 Create an experimental design, on page 128* for more information.
3	**Perform the runs in the Scouting scheme generated from DoE** See *ÄKTA avant and UNICORN 6.1 User Manual* for information about starting scouting runs. **Note:** The ***Scouting scheme*** generated from ***DoE*** does not normally need to be edited. If for some reason this is absolutely necessary, care must be taken so that the results can be used during evaluation of the ***DoE*** results.
4	**Perform statistical evaluation of a DoE scouting** This is performed in the ***Evaluation*** module. See *Section 5.4 Evaluation of Design of Experiments, on page 157* for more information.

5.2 Create an experimental design

Introduction

This section describes how to set up a ***DoE*** in the ***Method Editor***. A ***Scouting*** scheme is generated as a result containing the method runs to be run in ***System Control***.

In this section

This section contains the following sub-sections:

Section	See page
5.2.1 Set up an experimental design	129
5.2.2 Add responses and factors to an experimental design	138
5.2.3 Change design and design settings in a Design of Experiments setup	145
5.2.4 Divide the DoE runs into several scouting runs	149

5.2.1 Set up an experimental design

Introduction

This section describes how to set up a ***Design of Experiments*** in the ***Method Editor***.

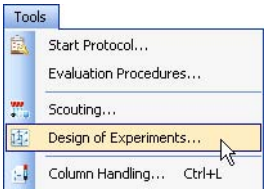
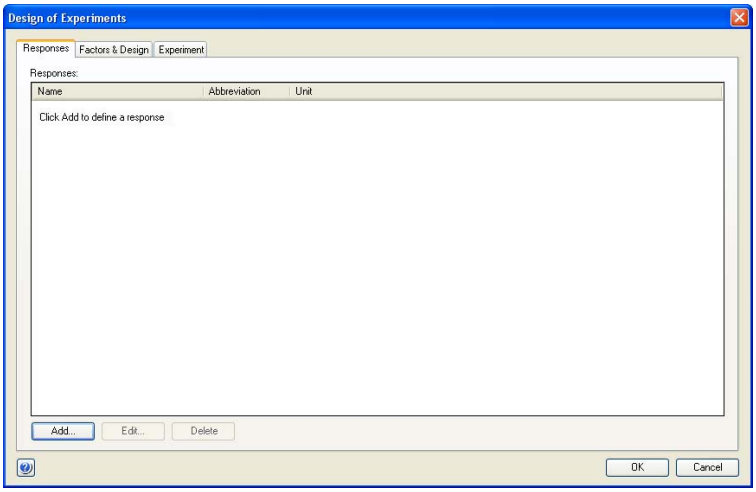
Create a method

Create a method for the process to be optimized. The table below briefly describes how to create a method.

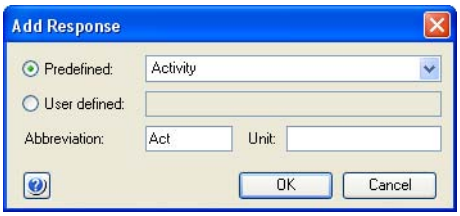
Step	Action
1	Create a method for the process to be optimized. See *Chapter 3 Create and edit methods, on page 25* for detailed information about how to create methods.
2	Decide which run parameters that should be screened for or optimized in the experiment. If the run parameters are not already defined as variables, define the parameters as variables to be able to vary the values i the ***DoE*** setup and to connect them to the appropriate factors. See *Section 9.2.4 Method variables, on page 315* for information about how to define new variables. **Note:** In the ***DoE*** setup factors are connected to the variables in the method.
3	Save the method.

Set up a new Design of Experiments

The table below describes how to set up a new ***Design of Experiments*** in the ***Method Editor***.

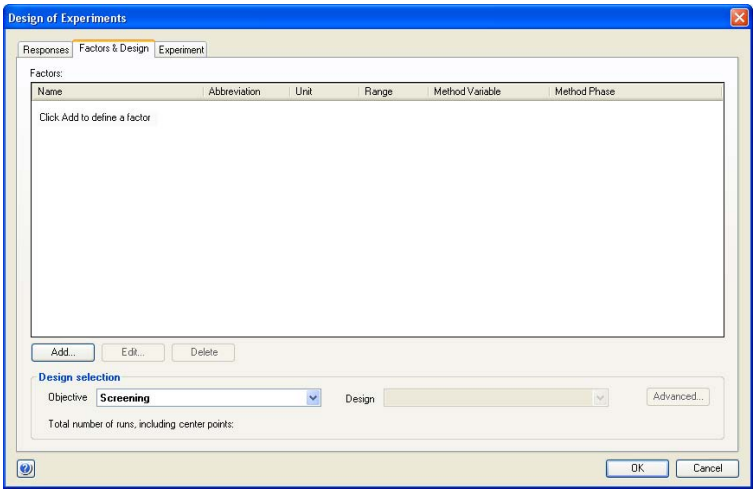
Step	Action
1	In the ***Method Editor***: • click the ***Design of Experiments*** icon in the ***Toolbar***  *or* • select ***Tools:Design of Experiments***  *Result:* The ***Design of Experiments*** dialog opens. 

Step	Action
2	To add responses to the ***Design of Experiments***, click ***Add...*** *Result:* The ***Add Response*** dialog opens. For detailed information about how to define and add a response, see *Section 5.2.2 Add responses and factors to an experimental design, on page 138*.

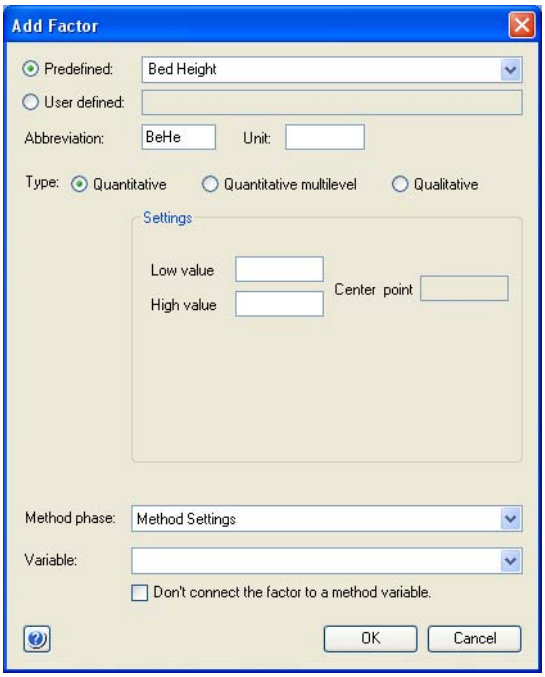


Note: It is possible to add new responses to the experimental design in ***Evaluation***. These new responses will not be added to the method file as opposed to responses added in the ***Method Editor***.

3	When all responses are defined, select the ***Factors & Design*** tab.

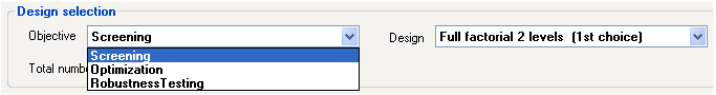


Step	Action
4	To add factors to the ***Design of Experiments***, click ***Add...*** *Result:* The ***Add Factor*** dialog opens. For detailed information about how to define and add a factor, see *Section 5.2.2 Add responses and factors to an experimental design, on page 138*.

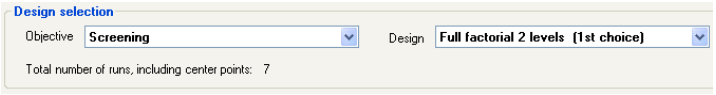


Step	Action
5	When all responses and factors have been defined, select the objective for the ***Design of Experiments***:

- In the ***Design selection*** area in the ***Factors & Design*** tab, select the appropriate objective from the ***Objective*** drop-down list.



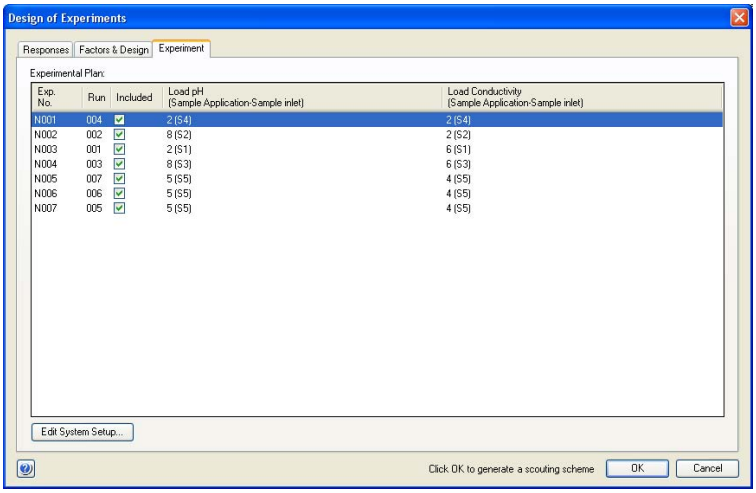
Result: Depending on the selected objective, UNICORN suggests a suitable design to obtain sufficient resolution with as few experiments as possible in the ***Design*** list. The total number of runs are displayed for the suggested design.



- It is possible to select the ***2nd choice*** design in the ***Design*** drop-down list if appropriate. The ***2nd choice*** design usually either requires a higher number of runs to be performed, or the resolution of the design is lower.

For information about how to view details for the selected design and/or to change to another design than the 1st or 2nd choice designs, see *Section 5.2.3 Change design and design settings in a Design of Experiments setup, on page 145*.

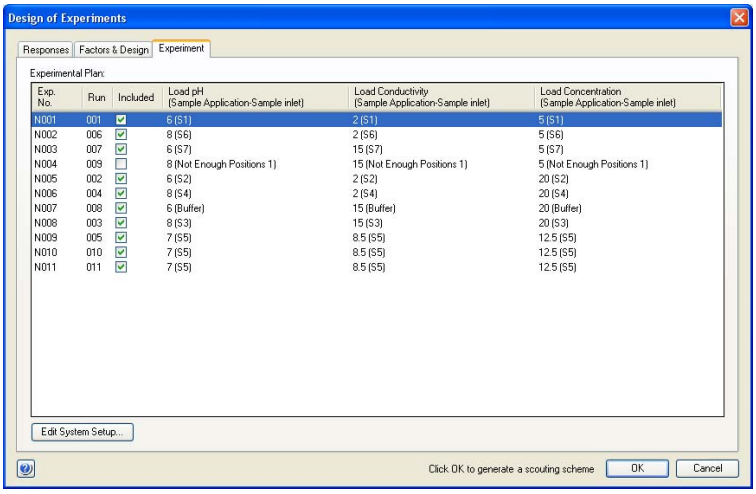
Step	Action
6	Click the ***Experiment*** tab. *Result:* The ***Experimental Plan*** is displayed.



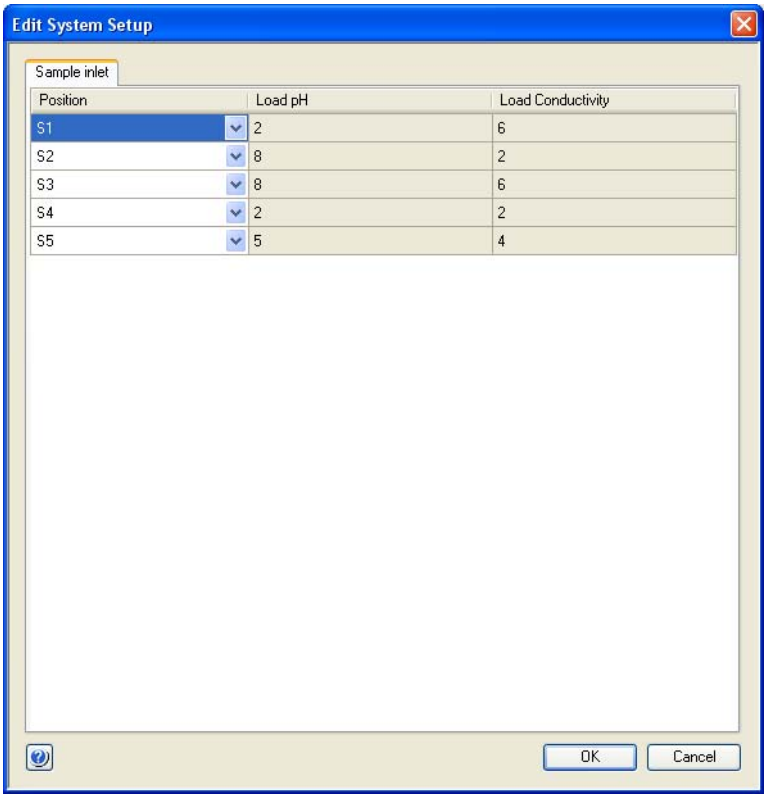
The ***Run*** column shows the run order for the optimized ***Scouting scheme*** that is generated from the ***DoE*** when clicking ***OK***.

Note: If excluding any of the runs in the ***Experimental Plan***, the results may not be reliable for use in the ***DoE*** evaluation.

Step	Action
7	In some cases it may be necessary to divide the ***DoE*** runs into two or more scouting runs, for example if there are too few sample inlet valves. Some of the runs can be excluded the first time and run during further rounds. If limitations in the hardware exist this will be indicated in the ***Experimental Plan*** on the ***Experiment*** tab by the text ***Not Enough Positions***. For information about how to proceed when, for example, the available sample inlet positions are not sufficient, see *Section 5.2.4 Divide the DoE runs into several scouting runs, on page 149*.



Step	Action
8	To view the system setup, click ***Edit System Setup...***. *Result:* The ***Edit System Setup*** dialog opens.



In this example the variable connected to both factors is the ***Sample inlet*** valve.

The ***Load pH*** and ***Load Conductivity*** values are set for each sample inlet.

To change the position for a certain combination of ***Load pH*** and ***Load Conductivity***:

- Select the appropriate position in the corresponding ***Position*** drop-down list.
  Note: It is not possible to change to a position already used.
- Click ***OK***.

Result: The changes are saved and you return to the ***Design of Experiments*** dialog.

Step	Action
9	• In the ***Design of Experiments*** dialog, click ***OK***. ***Result:*** The following dialog opens.  • Click ***OK***. *Result:* A ***Scouting scheme*** is generated with the runs to be performed. The method is displayed in the ***Method Editor***. **Note:** If you change the ***Scouting scheme***, the ***DoE*** experimental plan is changed and the results may not be reliable for use in the ***DoE*** evaluation.
10	Save the method including ***DoE***.

5.2.2 Add responses and factors to an experimental design

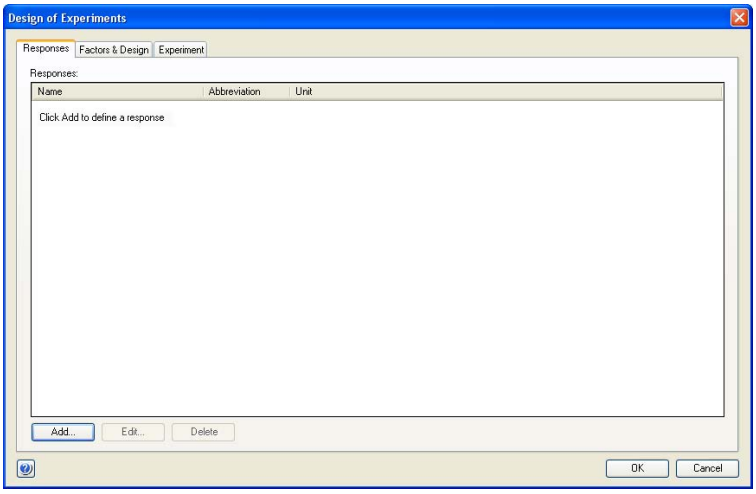
Introduction

This section describes how to add responses and factors to the ***Design of Experiments*** setup in the ***Method Editor***.

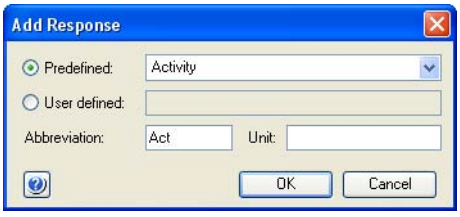
Add responses

The table below describes how to add responses to an experimental design:

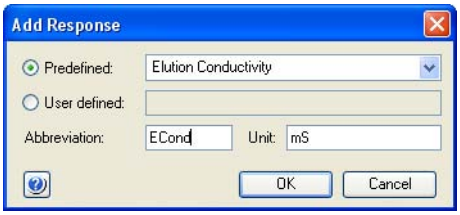
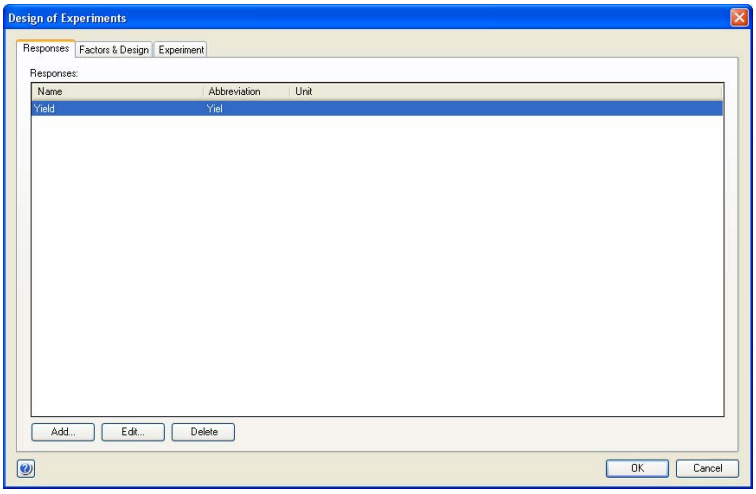
Step	Action
1	Select the ***Responses*** tab.



2	To add a response, click ***Add...*** *Result*: The ***Add Response*** dialog opens.



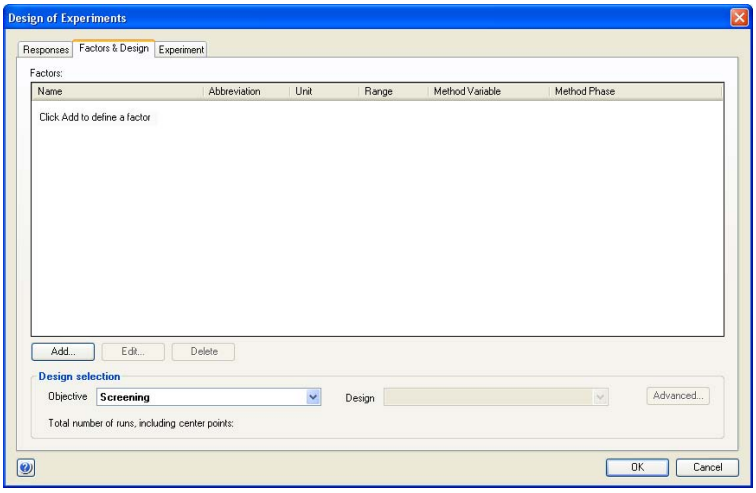
Note: It is possible to add new responses to the experimental design in ***Evaluation***. These new responses will not be added to the method file as opposed to responses added in the ***Method Editor***.

Step	Action
3	• To add a predefined response: Select the response to be added in the ***Predefined*** drop-down list. • To add a user defined response: Select ***User defined*** and type in your own response *Result:* ***Abbreviation*** is automatically filled in. • If applicable, enter unit for the response. 
4	Click ***OK***. *Result:* The selected response is added to the ***Responses*** list in the ***Design of Experiments*** dialog. 

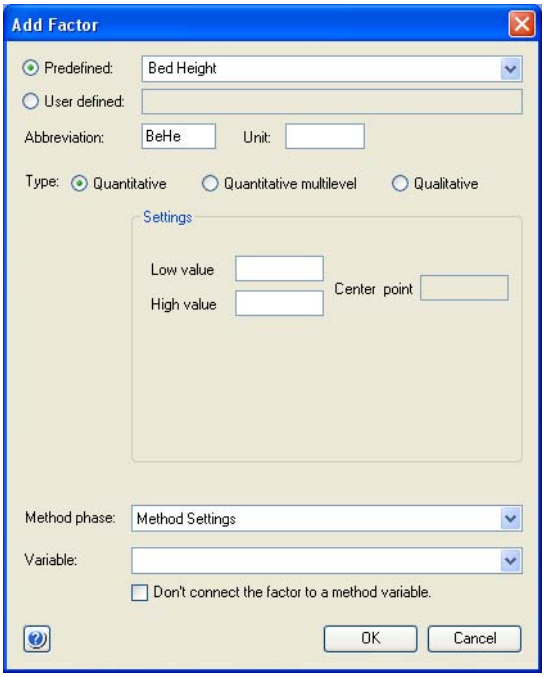
Add factors

The table below describes how to add factors to a ***Design of Experiments***:

Step	Action
1	Select the ***Factors & Design*** tab.



Step	Action
2	To define factors, click ***Add...***
	Result: The ***Add Factor*** dialog opens.



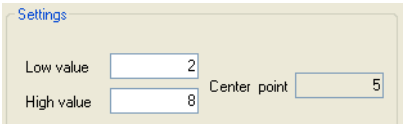
Step	Action
3	To add a predefined factor: • Select the factor to be added in the ***Predefined*** drop-down list. *Result:* ***Abbreviation*** and the correct ***Type*** radio button is selected. • If applicable, type in the ***Unit*** for the factor. To add a user defined factor: • Select ***User defined*** and type in your own factor. *Result:* ***Abbreviation*** is automatically filled in. • If applicable, type in the ***Unit*** for the factor. • Select what kind of factor it is by selecting the appropriate ***Type*** radio button. The table below describes the different types of factors:

Type of factor	Description
Quantitative	Quantitative factors are process parameters that can be measured and have values on a continuous scale (e.g., flow rate and pH values)
Quantitative multi-level	To specify more than two levels for a factor, select the Quantitative multilevel type. For example, if your are performing an experiment at three different temperatures, 4°C, 10°C and 25°C.
Qualitative	Qualitative factors are discrete discontinuous process parameters or categorical data (e.g., column type and type of salt used).

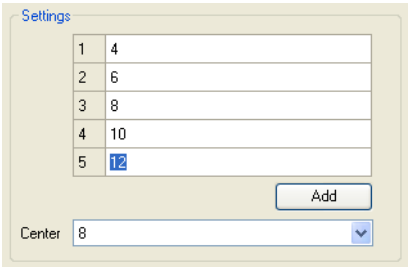
Step	Action
4	Enter settings for the selected factor:

- ***Quantitative*** factors:

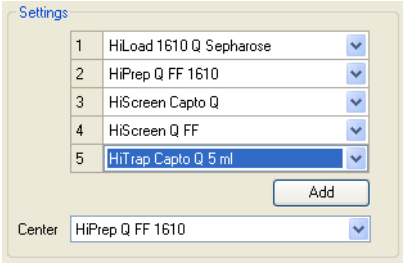
  Enter a ***Low value*** and a ***High value*** for the factor. The center point is automatically calculated.

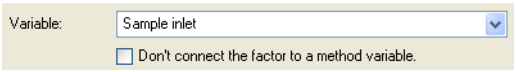
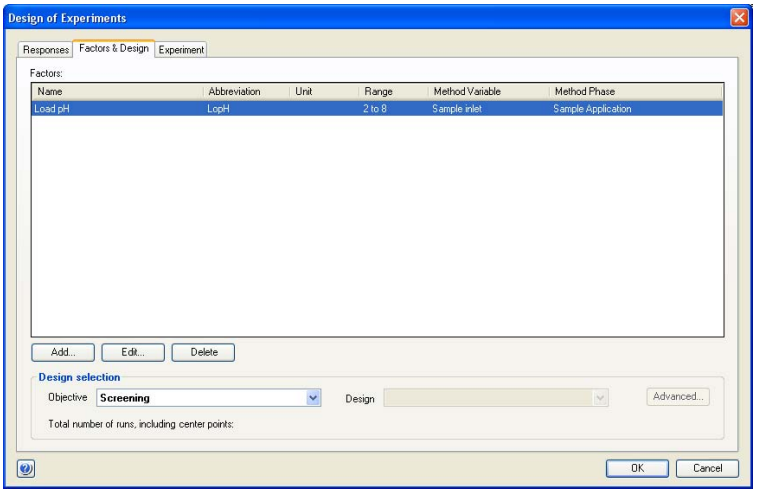


- ***Quantitative multilevel*** factors:
  - Enter the discrete values for the factor in the different rows.
  - To add more rows, click the ***Add*** button.
  - A center point is automatically selected. To select another center point, choose the appropriate one in the ***Center*** drop-down list.



- ***Qualitative*** factors:
  - Select or type in the parameters in the different rows.
  - To add more rows, click the ***Add*** button.
  - A center point is automatically selected. To select another center point, choose the appropriate one in the ***Center*** drop-down list.



Step	Action
5	Select to which phase the factor is connected in the ***Method phase*** drop-down list.  For example, if adding the predefined factor ***Load pH***, the pH at loading is controlled in the method phase ***Sample application***.
6	Select to which ***Variable*** the factor is connected in the ***Variable*** drop-down list.  For example, if adding the predefined factor ***Load pH***, the sample pH at loading is controlled by the ***Sample inlet*** valve position. **Note:** Variables connected to factors will be included in the ***Scouting*** scheme that is generated when completing the ***DoE*** setup.
7	If the factor is not connected to anything that can be controlled by UNICORN (e.g., if the experiment is performed in a cold room or in room temperature) check the box ***Don't connect the factor to a method variable***.
8	Click ***OK*** to add the factor to the ***Design of Experiments***. *Result:* The factor will be listed on the ***Factors & Design*** tab. 
9	To add more factors, repeat this procedure.

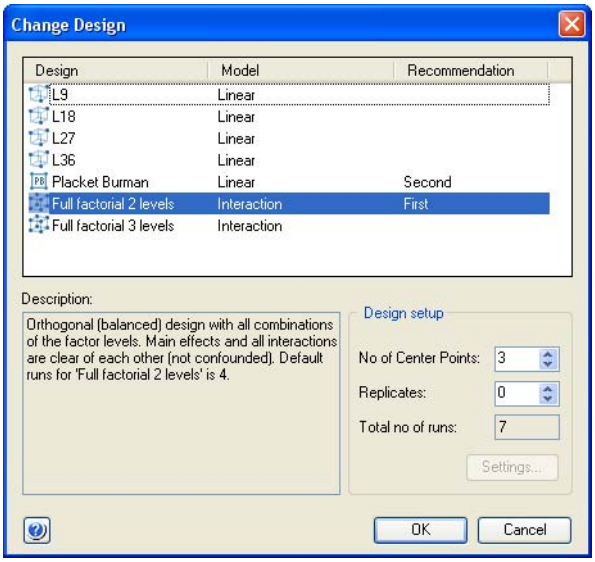
5.2.3 Change design and design settings in a Design of Experiments setup

Change design in a Design of Experiments setup

The design suggested by UNICORN can be changed to another design in the setup of the ***Design of Experiments***. The settings for a selected design can also be edited. The table below describes how to change the default design and design settings (i.e., the number of center points and replicates) in a ***Design of Experiments*** setup:

Step	Action
1	In the ***Factors & Design*** tab of the ***Design of Experiments*** dialog, the suggested design is displayed in ***Design*** drop-down list.

Step	Action
2	Click the ***Advanced...*** button to: • change to another design than the ***1st*** or ***2nd choice*** design available in the ***Design*** drop-down list (continue with step 3) *and/or* • edit the settings for the currently selected design in the ***Design*** table (continue with step 4) *Result:* The ***Change Design*** dialog opens displaying the designs that may be used for the current experimental setup and selected objective.



3	To change to another design, select the appropriate design in the ***Design*** table. *Result:* The ***Description*** field shows a short description of the selected design. For a description of which designs are supported by UNICORN and when they may be proposed, see *Designs supported by UNICORN, on page 123*.

Step	Action
4	The ***Design setup*** area shows the settings for the selected design.

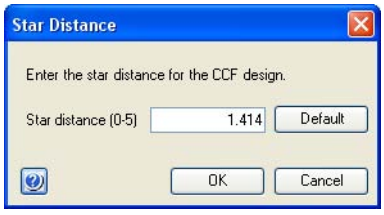
- Change the settings for ***No of Center Points*** and ***Replicates*** as appropriate.

  The table below describes the different settings:

Setting	Description
No of Center Points	The ***No of Center Points*** means that the center point experiment will be run the selected number of times. It is recommended to use at least three center points to be able to estimate the pure error, that is, the variation in the measurements.
Replicates	***Replicates*** means that the whole experiments series (corner and center points) will be replicated the selected number of times.
Total no of runs	This field lists the total number of runs to be performed based on the number of center points and replicates.

- The ***Settings...*** button is only active if a CCC design using star points is selected. To change the default star point distance in relation to the design box, click ***Settings...***.

  Result: The ***Star Distance*** dialog opens.



  Change the ***Star distance*** as appropriate and click ***OK***. To return to the default value, click ***Default***.

Step	Action
5	In the ***Change Design*** dialog, click ***OK***. *Result:* Changes in the ***Change Design*** dialog are saved and the settings in the ***Design setup*** area in the ***Design of Experiments*** dialog are updated. **Note:** If additional variables have been defined in the scouting scheme for a previously saved DoE method, these will be lost and need to be redefined.

5.2.4 Divide the DoE runs into several scouting runs

Introduction

If hardware limitations exist, for example too few sample inlet valve positions are available for the number of runs to be performed, the ***DoE*** runs can be divided into several scouting runs. This section describes how to divide a DoE run into several smaller runs.

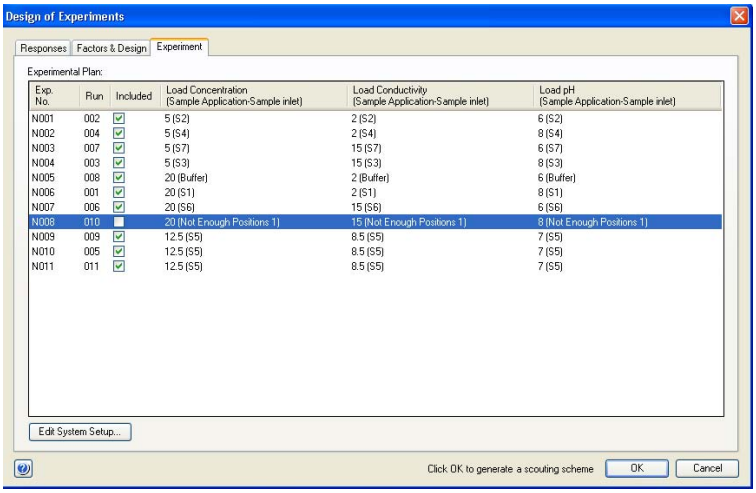
Divide DoE runs into several scouting runs directly in the Scouting scheme

It is possible to include/exclude runs directly in the generated ***Scouting*** scheme and edit, for example, the sample inlet positions. However, for complex experimental plans it is recommended to create multiple DoE methods, each using the same design but with different sub-sets of scouting runs (see below). As long as the designs are identical, the results can then be merged for analysis.

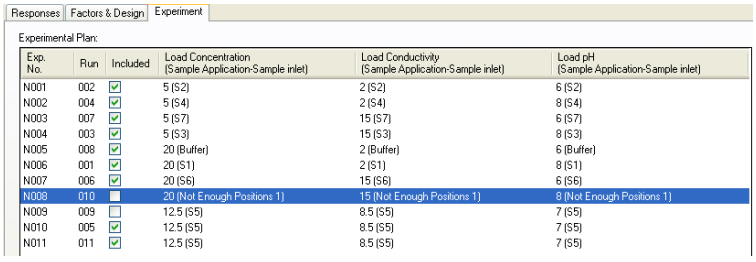
Divide DoE runs into several scouting runs in the DoE setup

The table below describes how to identify hardware limitations in a DoE run.

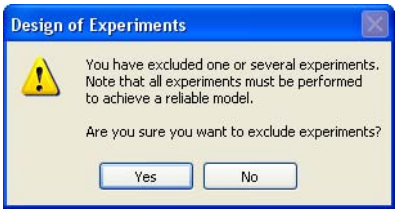
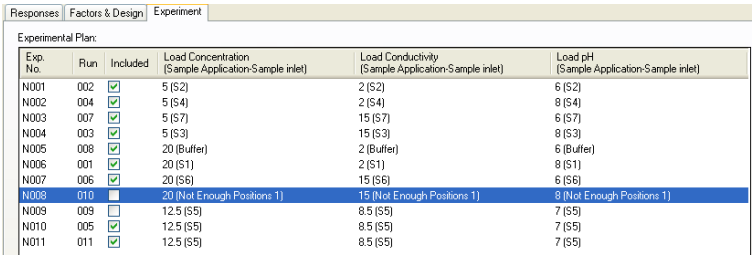
Step	Action
1	In the ***Design of experiments*** dialog, select the ***Experiment*** tab. *Result:* The ***Experimental Plan*** is displayed.



2	If limitations in the hardware exist this will be indicated in the ***Experimental Plan*** by the text ***Not Enough Positions*** for the run(s) in the ***Design of experiments*** dialog. These runs are also excluded from the ***Experimental Plan***.
3	Clear the ***Included*** box in front of the experiments to be excluded in the first set of runs. In the example below ***Run 009*** and ***Run 010*** are excluded from the first set of runs.



Note: Include at least one center point (runs 5, 9 and 11 in the above example) in each scouting run to have control of experimental variations.

Step	Action
4	Click ***OK***. *Result:* The following warning dialog opens. 
5	Click ***Yes*** in the warning dialog. *Result:* The following message is displayed. 
6	Click ***OK*** and save the method.
7	To define the second set of runs, open the ***Design of experiments*** dialog again and select the ***Experiment*** tab. *Result:* The ***Experimental Plan*** is displayed. 
8	Clear the ***Included*** boxes in front of **all** runs.

Exp. No.	Run	Included	Load Concentration (Sample Application-Sample inlet)	Load Conductivity (Sample Application-Sample inlet)	Load pH (Sample Application-Sample inlet)
N001	002	☑	5 (S2)	2 (S2)	6 (S2)
N002	004	☑	5 (S4)	2 (S4)	8 (S4)
N003	007	☑	5 (S7)	15 (S7)	6 (S7)
N004	003	☑	5 (S3)	15 (S3)	8 (S3)
N005	008	☑	20 (Buffer)	2 (Buffer)	6 (Buffer)
N006	001	☑	20 (S1)	2 (S1)	8 (S1)
N007	006	☑	20 (S6)	15 (S6)	6 (S6)
N008	010	☐	20 (Not Enough Positions 1)	15 (Not Enough Positions 1)	8 (Not Enough Positions 1)
N009	009	☐	12.5 (S5)	8.5 (S5)	7 (S5)
N010	005	☑	12.5 (S5)	8.5 (S5)	7 (S5)
N011	011	☑	12.5 (S5)	8.5 (S5)	7 (S5)

Step	Action
9	Click the ***Edit System Setup*** button. *Result:* The ***Edit System Setup*** dialog opens.

Sample inlet

Position	Load Concentration	Load Conductivity	Load pH
S1	20	2	8
S2	5	2	6
S3	5	15	8
S4	5	2	8
S5	12.5	8.5	7
S6	20	15	6
S7	5	15	6
Buffer	20	2	6
Not Enough Positions 1	20	15	8

Step	Action
10	Change the position for the inlet that did not have any position before to a valid position. The inlet that previously had the position must also be changed. **Example:** Change the position for, in this example, the sample inlet position indicated by ***Not Enough Positions 1*** to ***Buffer*** position.

Sample inlet

Position	Load Concentration	Load Conductivity	Load pH
S1	20	2	8
S2	5	2	6
S3	5	15	8
S4	5	2	8
S5	12.5	8.5	7
S6	20	15	6
S7	5	15	6
Buffer	20	2	6
Buffer	20	15	8

Then change the sample inlet position originally set to ***Buffer*** to ***Not Enough Positions 1***.

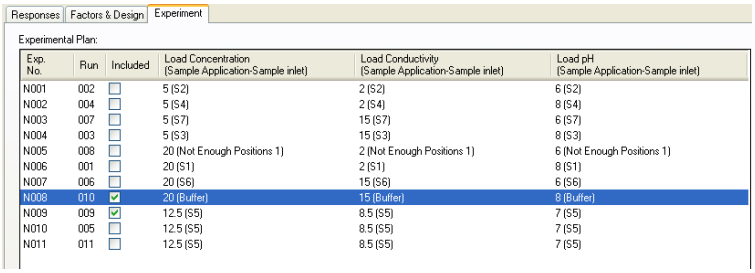
Sample inlet

Position	Load Concentration	Load Conductivity	Load pH
S1	20	2	8
S2	5	2	6
S3	5	15	8
S4	5	2	8
S5	12.5	8.5	7
S6	20	15	6
S7	5	15	6
Not Enough Positions 1	20	2	6
Buffer	20	15	8

The two inlet positions have been changed.

11	Click ***OK*** in the ***Edit System Setup*** dialog.

Step	Action
12	In the ***Experiment*** tab, check the boxes in front of the runs to be included in the second set of runs.



Exp. No.	Run	Included	Load Concentration (Sample Application-Sample inlet)	Load Conductivity (Sample Application-Sample inlet)	Load pH (Sample Application-Sample inlet)
N001	002	☐	5 (S2)	2 (S2)	6 (S2)
N002	004	☐	5 (S4)	2 (S4)	8 (S4)
N003	007	☐	5 (S7)	15 (S7)	6 (S7)
N004	003	☐	5 (S3)	15 (S3)	8 (S3)
N005	008	☐	20 (Not Enough Positions 1)	2 (Not Enough Positions 1)	6 (Not Enough Positions 1)
N006	001	☐	20 (S1)	2 (S1)	8 (S1)
N007	006	☐	20 (S6)	15 (S6)	6 (S6)
N008	010	☑	20 (Buffer)	15 (Buffer)	8 (Buffer)
N009	009	☑	12.5 (S5)	8.5 (S5)	7 (S5)
N010	005	☐	12.5 (S5)	8.5 (S5)	7 (S5)
N011	011	☐	12.5 (S5)	8.5 (S5)	7 (S5)

Note: In the example shown here, one of the center points (run 9) is also included.

13 Click ***OK*** in the ***Design of experiments*** dialog.

Result: A new ***Scouting scheme*** is generated. Click ***Yes*** and ***OK*** in any warning and messages dialog that appear.

14 Save the method with a **new** name.

Result: The two scouting runs are ready to be run in sequence.

Note: In this example, you must change samples in one of the sample inlets before starting the second scouting run. It is not possible to just create a method queue, start it and leave the system.

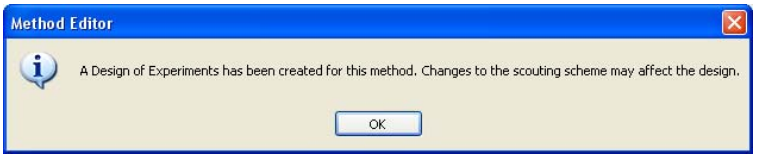
5.3 Run a scouting created with DoE

Introduction

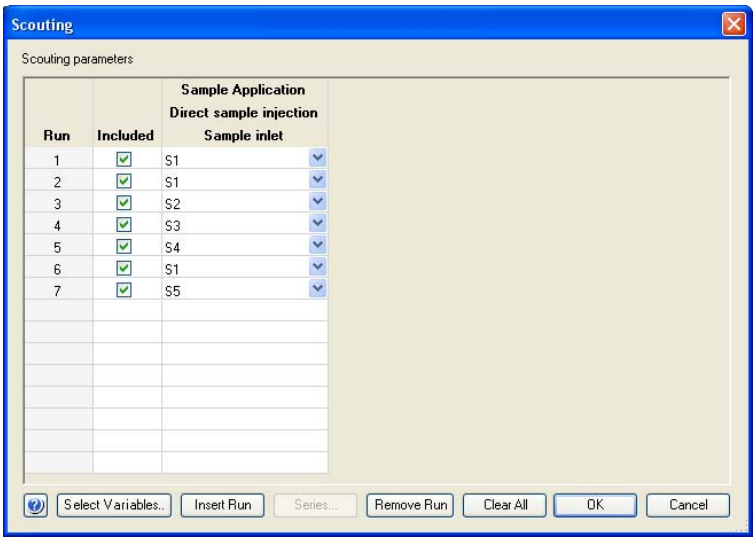
This section describes how to view the optimized ***Scouting*** scheme generated from ***DoE*** and how to print the method including ***DoE***. For information about how to start and monitor ***Scouting*** runs, see *ÄKTA avant and UNICORN 6.1 User Manual*.

View the Scouting scheme generated from DoE

When creating a ***Design of Experiments*** the final step is the generation of the optimized ***Scouting scheme***. The table below describes how to view the ***Scouting*** scheme generated from ***DoE***:

Step	Action
1	In the ***Method Editor***: • Click the ***Scouting*** icon  *or* • Select ***Tools:Scouting***. *Result:* The following dialog is displayed as a reminder of that a ***DoE*** has been created for the method. If you change the ***Scouting scheme***, the ***DoE*** experimental plan is changed and the results may not be reliable for use in the ***DoE*** evaluation. 

Step	Action
2	Click ***OK***. *Result:* The ***Scouting*** dialog opens displaying the ***Scouting scheme*** where it is possible to view the ***Scouting*** runs to be performed.
3	Click ***Cancel*** or ***OK*** to close the ***Scouting*** dialog.



Print method including DoE

Before starting the run, it is useful to print the method information to see, for example, which sample positions are used for the different runs. See *Section 3.7 Print a method, on page 79* for information about how to print the method.

5.4 Evaluation of Design of Experiments

Introduction

This section describes how to perform statistical evaluation of ***a DoE*** scouting.

In this section

This section contains the following sub-sections:

Section	See page
5.4.1 Workflow	158
5.4.2 Generate model	160
5.4.3 Analyze and evaluate the model - basic analysis	170
5.4.4 Analyze and evaluate the model - extended analysis	182
5.4.5 Edit the model	190
5.4.6 Use the model	193
5.4.7 Create and print reports	200

5.4.1 Workflow

Introduction

This section describes the workflow when evaluating a ***Design of Experiments*** scouting.

Workflow

The main steps when performing statistical evaluation of an experimental design are:

1 **Generate model**

This includes evaluating single ***DoE*** runs, opening the ***DoE*** result, and entering response data. The software will then generate a model.

2 **Analyze and edit the model**

This includes checking that the raw data is OK and performing a basic analysis of the model. The model may need refinement by removing insignificant terms, which should be done with care. Extended analysis can be performed for additional information.

3 **Use the model**

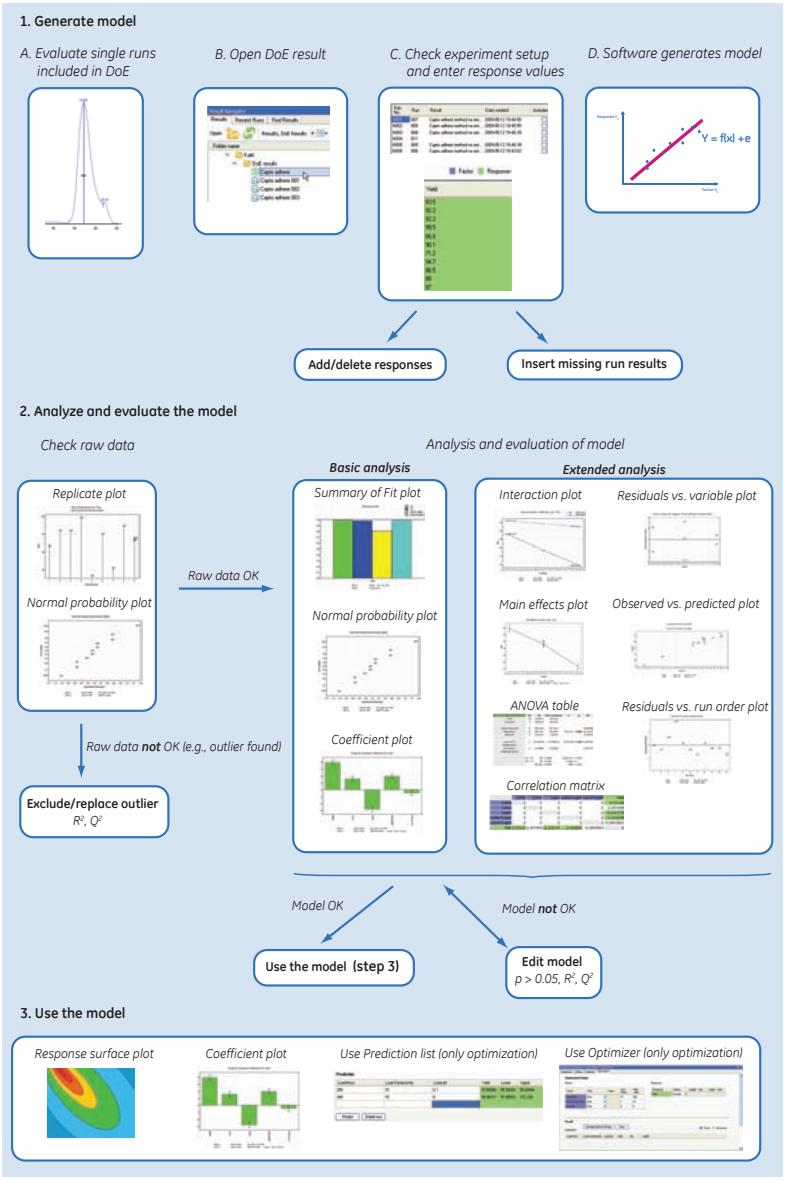
This includes generating a response surface plot as well as using the predictor and the optimizer.

- Generate response surface plot to get a map of the experimental area and information about how to proceed with new experiments.
- Use the predictor to predict response values based on entered factor settings (optimization experiments only).
- Use the optimizer to optimize responses based on entered criteria for factors and responses (optimization experiments only), for example maximizing response 1 and minimizing response 2 while keeping factor 1 constant and allowing the other factors to vary within a defined range.

Basic and extended reports can also be created for the experiment.

Illustration of workflow

The illustration below shows a possible workflow for evaluating a ***Design of Experiments*** scouting:



5.4.2 Generate model

Introduction

This section describes how to open single ***DoE*** runs for evaluation, how to open ***DoE*** results and how to generate a model.

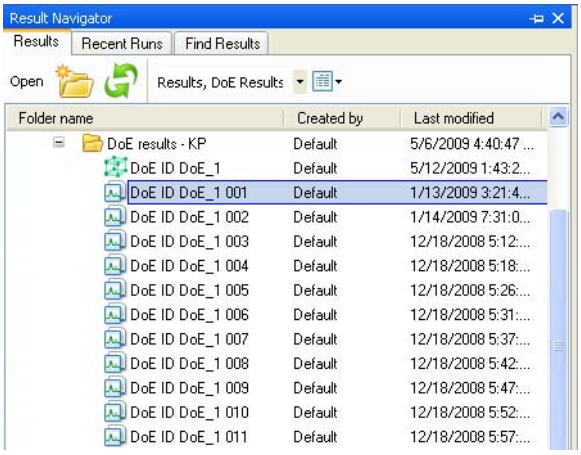
Evaluate the results of the single DoE runs

Before opening the ***DoE*** result in the ***Evaluation*** module, it is recommended to evaluate the single runs included in the ***Scouting*** run.

The table below describes how to open and evaluate single runs in the ***Evaluation*** module:

Step	Action
1	In the ***Evaluation*** module, click the ***Open Result Navigator*** icon in the ***Toolbar***.  *Result:* The ***Result Navigator*** is displayed.

Step	Action
2	Browse for the result and double-click the result name (single runs are indicated by the chromatogram icon).

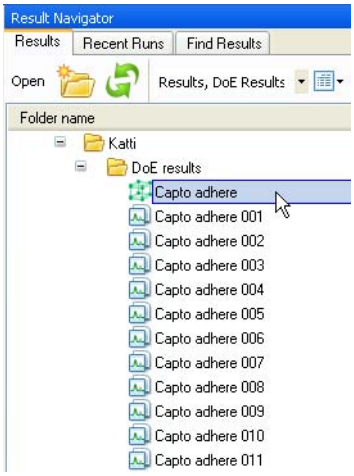
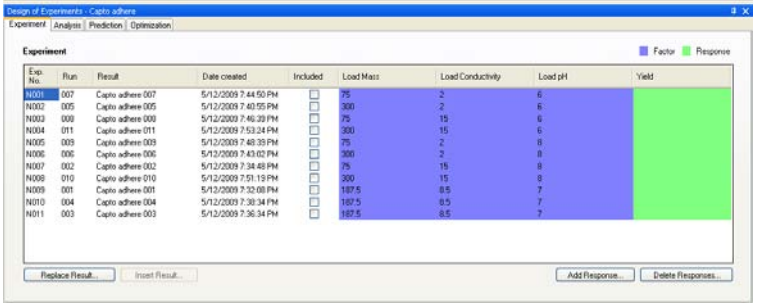


Result: The result of the run is opened and displayed in the ***Evaluation*** module.

3	Inspect the results visually and check that the runs have been performed as expected.
4	Evaluate the results for the run as appropriate. See *UNICORN 6.1 Evaluation Manual* for information about how to perform evaluation.
5	Save any changes. **Tip:** It is possible to have a ***Scouting*** run result open at the same time even if a ***DoE*** result is open.
6	Repeat this procedures for all the runs included in the ***DoE*** result.

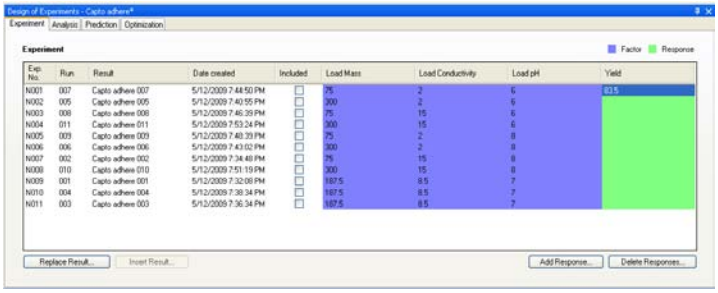
Open the DoE result

The table below describes how to open a ***DoE*** result:

Step	Action
1	In the ***Evaluation*** module, click the ***Open Result Navigator*** icon in the ***Toolbar***.  *Result:* The ***Result Navigator*** is displayed.
2	Browse for the ***DoE*** result and double-click the result name (***DoE*** results are indicated by the design box icon).  *Result:* The ***Design of Experiments*** box opens displaying the ***DoE*** scouting run. 

Generate model

The table below describes how to generate a model for the ***DoE*** result:

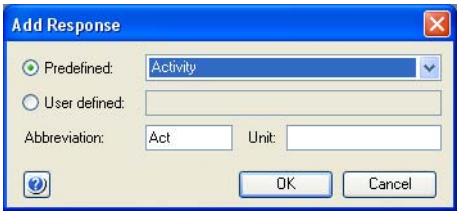
Step	Action
1	Responses defined in the method appear in the result in the ***Design of Experiments*** box. To add or delete responses to the experiment, use the ***Add Response...*** and ***Delete Response...*** buttons. See *Add responses, on page 164* and *Delete responses, on page 165* for more information.
2	To enter response data: • Click in a response cell for the appropriate response and experiment, and type in the data (the ***Yield*** column in the example shown here). *Result*: The data is entered in the cell.  **Tip:** Response data can be obtained from: - external measurements (e.g., biological activity) - peak data from UNICORN (e.g., HETP tests or resolution) • Repeat this procedure for all experiments and responses.
3	Select the runs to be included in the calculations for generating the model by checking the ***Included*** box for the appropriate runs (usually all runs). To insert a new run or to replace a failed run with a new run use the ***Replace Result...*** and ***Insert Result...*** buttons. See *Replace run results, on page 167* and *Insert new runs, on page 166* for more information about inserting and replacing runs. **Tip:** Instead of replacing a failed run with a new run, the run can be excluded from the model calculations by clearing the ***Included*** box in front of the appropriate run. This will however often result in some loss of information.

Step	Action
4	Click the ***Analysis*** tab. *Result:* A model is fitted to the entered data. For information about how to analyze the model, see *Section 5.4.3 Analyze and evaluate the model - basic analysis, on page 170*.

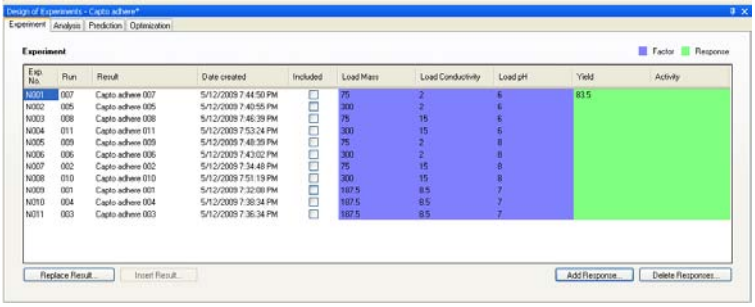
Add responses

Note: Responses added in the ***Evaluation*** module will not be included in the original method.

The table below describes how to add a new response to the experiment:

Step	Action
1	In the ***Design of Experiments*** tab in the ***Evaluation*** module, click the ***Add Response...*** button. *Result:* The ***Add Response*** dialog opens. 
2	Select the response to be added in the ***Predefined*** drop-down list or define your own response by selecting ***User defined*** and type in your own response. **Note:** ***Abbreviation*** is automatically filled in.
3	Enter the ***Unit*** for the response, if appropriate.

Step	Action
4	Click ***OK***. *Result:* The response is added to the ***DoE*** experiment.



Delete responses

The table below describes how to delete a response from the experiment:

Step	Action
1	In the ***Design of Experiments*** tab in the ***Evaluation*** module, click the ***Delete Response...*** button. *Result:* The ***Delete Responses*** dialog opens.

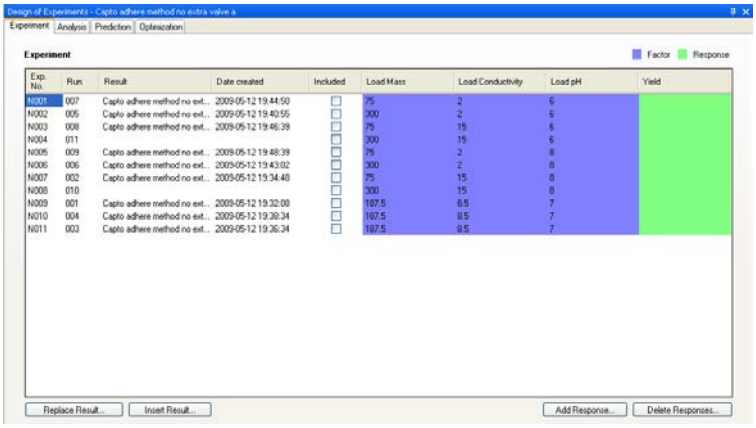
2	Check the box in front of the response to be deleted and click ***Delete***. *Result:* The response is deleted from the experiment.

Insert new runs

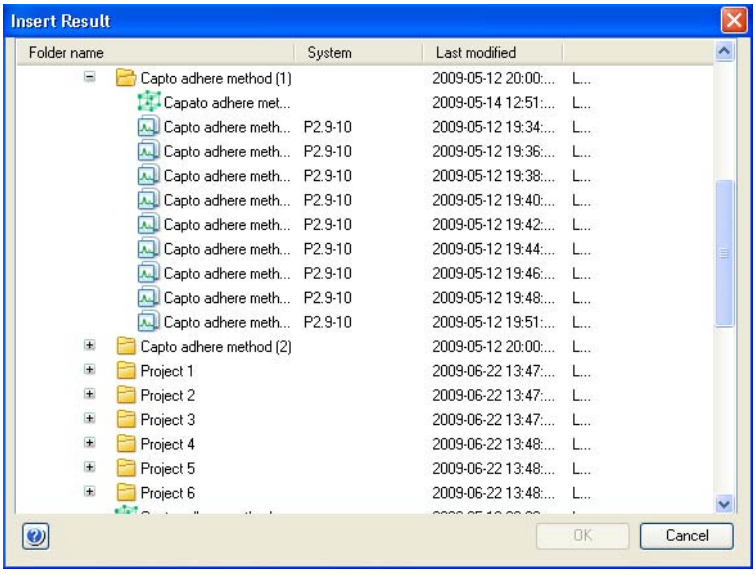
Runs can be inserted if there are runs missing in the experiment. This can be the case if the ***DoE*** run has been divided into two scouting runs. In that case there will be two ***DoE*** results.

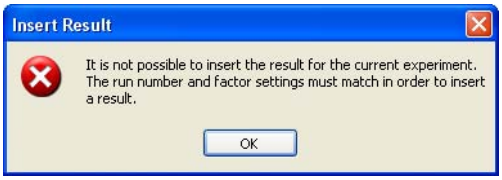
The table below describes how to add a missing run result to the experiment:

Step	Action
1	If runs are missing in the experiment, the rows for the missing runs are blank.



2	To insert the missing runs, click the ***Insert Result...*** button. *Result:* The ***Insert Result*** dialog opens.



Step	Action
3	Browse and select the run(s) that should be inserted. **Tip:** The run order number is found at the end of the result name. This number is the same as in the ***Run*** column. This makes it easier to locate the runs to be inserted.
4	Click ***OK***. *Result:* The runs are inserted in the experiment.
5	If a run does not match any of the missing runs in the experiment an error message will be displayed. Repeat from step 2 to insert the correct run.  **Note:** The run to be inserted must have the appropriate factor settings.

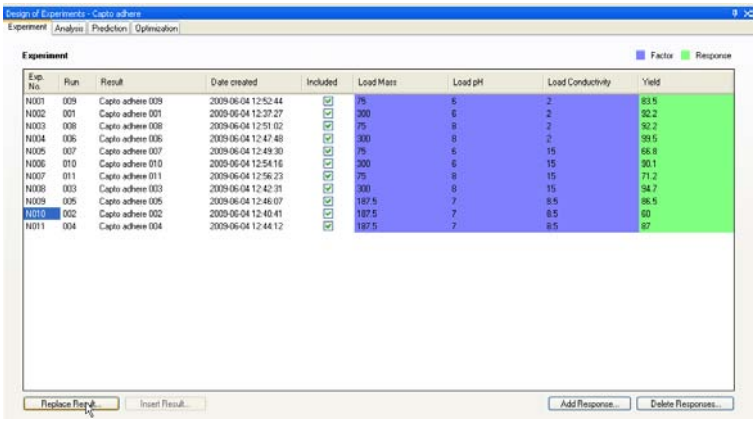
Replace run results

Runs can be replaced with a new run if, for example, the run has failed.

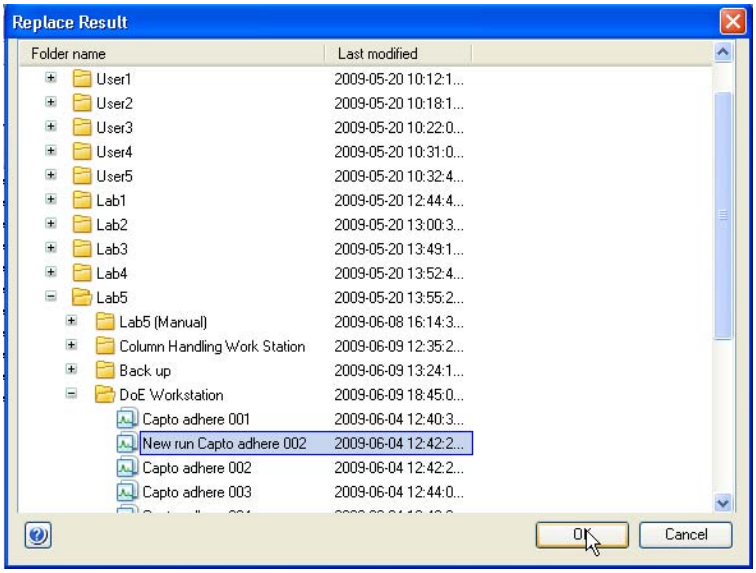
Note: If a run has failed, there is always a risk that experimental conditions that cannot be controlled may have affected the result (e.g., temperature in the lab, different batches of buffer preparation etc.) Therefore, it is not always a good idea to replace a failed run with a new one. Re-running a centre point experiment will help in keeping track of uncontrolled variations.

The table below describes how to replace a run result:

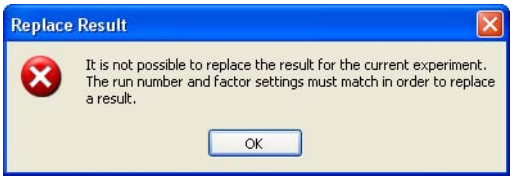
Step	Action
1	In the ***Experiment*** tab of the ***Design of Experiments*** box, select the result to be replaced in the ***Result*** column in the ***Experiment*** table.



2	Click the ***Replace Result...*** button. *Result*: The ***Replace Result*** dialog opens.



3	Browse and select the run that should replace the selected run.
4	Click ***OK***. *Result*: The new run is listed in the ***Experiment*** table.

Step	Action
5	If the run does not match the run to be replaced an error message will be displayed. Repeat from step 2 to insert the correct run.  **Note:** The run to be inserted must have the appropriate factor settings.

5.4.3 Analyze and evaluate the model - basic analysis

Introduction

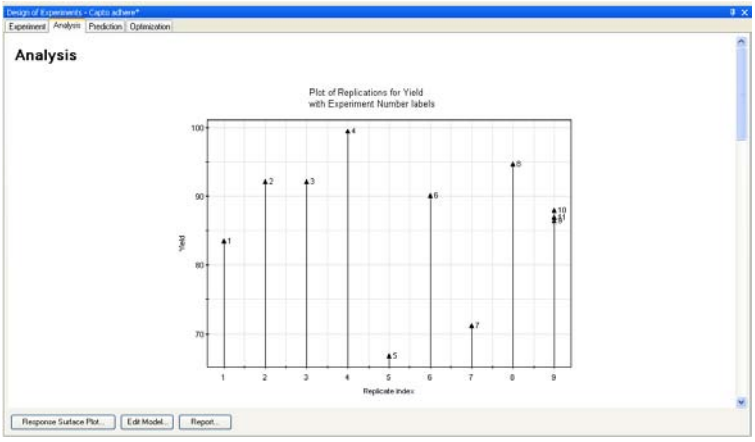
This section describes how to perform basic analysis of the model and how to evaluate the model.

Check the raw data

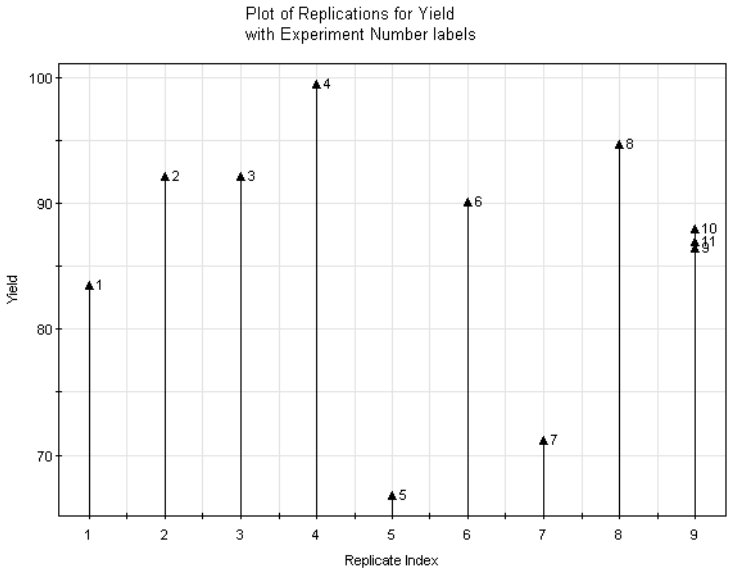
Before starting to analyze the model, the raw data must be checked to ensure that the correct conclusions can be drawn in the analysis and evaluation of the model.

The table below describes how to perform some initial checks that the raw data is OK:

Step	Action
1	Select the ***Analysis*** tab, if not already selected. *Result:* The ***Analysis*** tab opens showing 4 plots for each response: the Replicate plot, the Summary of fit plot, the Coefficient plot and the Normal probability plot of residuals. To be able to see all plots use the vertical scroll bar.



Step	Action
2	For each response, look at the replicate plot. This plot displays the variation in the response for replicated experiments and the variation among the replicates in relation to the variation across the entire design ("reproducibility").



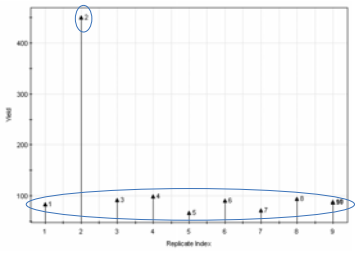
Each arrow in the plot represents an experiment.

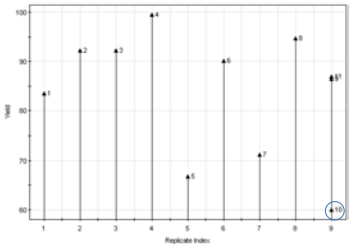
In a good replicate plot (as shown in the example above), the replicate runs should show as small a variation as possible (experiments ***9***, ***10*** and ***11***).

There should normally be some variation across the dataset of non-replicate experiments. However a single experiment should not deviate dramatically from the rest.

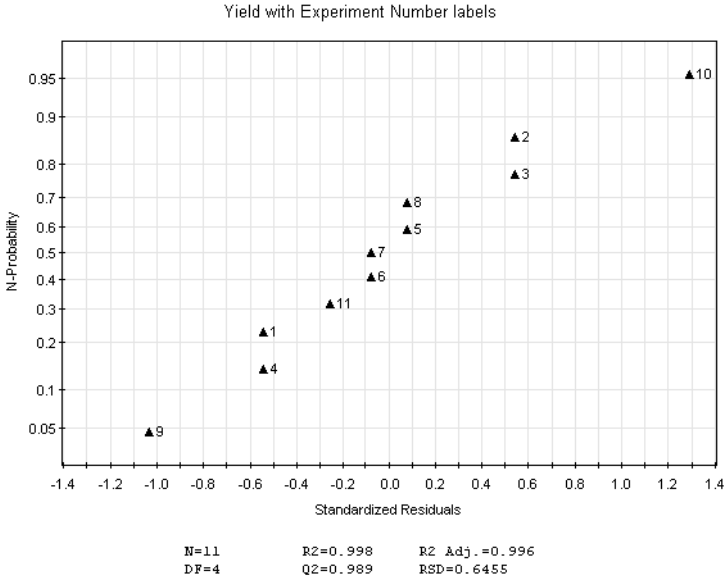
Note: When a robustness test has been performed, variations in the data should instead be as small as possible.

Step	Action
3	The replicate plots can also be used to identify outliers. If, for example, a single experiment deviates a lot from the rest of the experiments (see plot to the left below), or if a replicate deviates a lot from the rest of the replicates (see plot to the right below), this run could be an outlier.





Step	Action
4	Look at the normal probability plot of residuals. The residuals, or minimized errors between the measured data and the theoretical data calculated according to the model, should normally be distributed as shown in the diagram below.



In a normal distribution of residuals for a good model, the experiments should be distributed close to a straight line and also should lie within a ***Standardized Residuals*** range of -4 to +4 SD (standard deviations). Single experiments that deviate from this may be outliers.

A non-linear distribution of experiments may also indicate the presence of insignificant missing terms, for example curvature of the model. See *Analyze and interpret the model - basic analysis, on page 174* and *Section 5.4.4 Analyze and evaluate the model - extended analysis, on page 182*.

5	If the raw data is OK, continue with the basic analysis of the model described in *Analyze and interpret the model - basic analysis, on page 174*.

Step	Action
6	If outliers are detected, try to identify why. The table below gives a few examples of why outliers may be detected. You may also look at the plots in the extended analysis to get more information about the experiment.

Why outlier?	What to do	See...
Bad replicates	Check the individual result, and that correct response values have been entered. If the run has failed, consider performing new experiments and replace the run.	*Replace run results, on page 167* for information about how to replace a run result.
	The run may also be excluded from the experiment setup. Results that are true outliers should be excluded.	*Generate model, on page 163* for information about how to exclude a run from the experiment
Deviating experiments	Check that the correct response values have been entered. Check the individual result. Consider performing new experiments to verify the deviation. If the results are indeed valid, the model may be inappropriate for the area.	See *Section 5.4.2 Generate model, on page 160* for how to check entered response data.

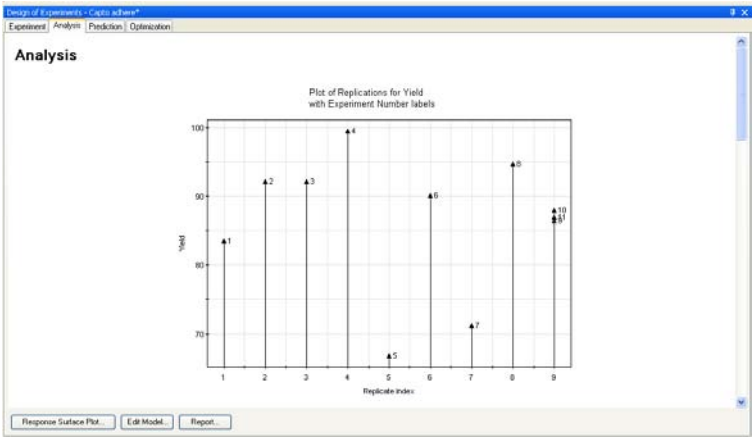
Analyze and interpret the model - basic analysis

Before you can use the model and draw conclusions from it, the model needs to be analyzed to investigate if the model gives a good reflection of the experiment data.

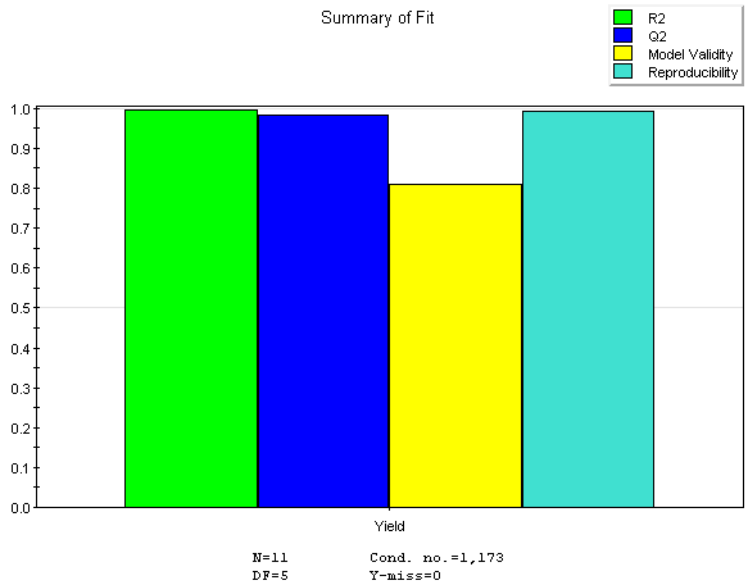
Note: The plots must be analyzed for each response. A model can be good for one response but not for another. In some cases a good model cannot be obtained when several responses are included in the same model. In this case, try fitting an individual model for each response separately.

The table below describes how to perform a basic analysis of the model:

Step	Action
1	Select the ***Analysis*** tab, if not already selected. *Result:* The ***Analysis*** tab opens showing four plots for each response: the Replicate plot, the Summary of Fit plot, the Coefficient plot and the Normal probability plot of residuals. To be able to see all plots use the vertical scroll bar.



Step	Action
2	Scroll down to display the ***Summary of Fit*** plot. If the experiment contains several responses, the plot will contain the four bars shown below for each response.



The bars in the plot describe different statistical calculations for each response, measuring how good the model is. It is the contribution of all values that together indicate if the model is good. A good model has high values for all parameters as seen in the plot above. The table below gives a description of the parameters:

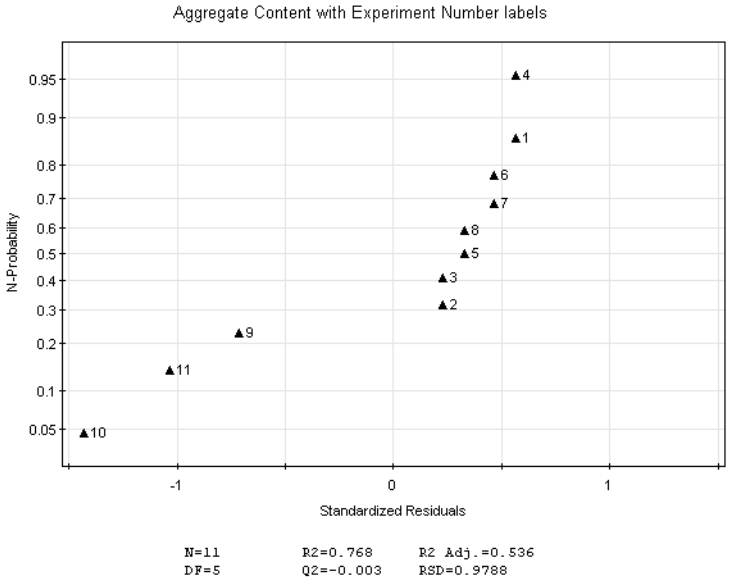
Step	Action

Coefficient value for...	Description
R2	R^2 describes how well the model fits the current data. It can vary between 0 and 1, where 1 equals a perfect model and 0 corresponds to no model at all. A high R^2-value is necessary for a good model but not sufficient on its own. A value of 0.75 indicates a rough but stable and useful model. **Note:** ***R2 Adj*** is the fraction of variations in the response data that is explained by the model, adjusted for degrees of freedom. R^2 does not take into account degrees of freedom.
Q2	Q^2 describes how well the model will predict new data. It can vary between -∞ and 1. The higher Q^2-value, the better indicator of how well the model will predict new data. Q^2>0.5 is good and Q^2>0.9 is excellent. Q^2 is a better indicator of the usefulness of the model than R^2. **Note:** R^2 should **not** exceed Q^2 by more than 0.2-0.3 for a good model.
Model Validity	Model validity is only available if replicated experiments have been performed. A model validity>0.25 indicates a good model. A model validity<0.25 indicates a significant "lack of fit", that is the model error is significantly larger than the pure error (reproducibility).
Reproducibility	A reproducibility<0.5 indicates that there is a large pure error and poor control of the experimental setup (high noise level).

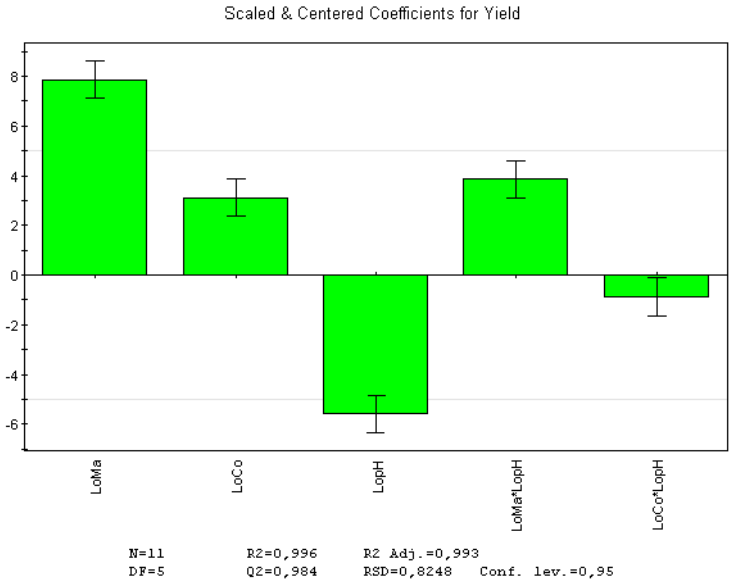
If the ***Summary of Fit*** plot does not look good, there may be several reasons for this. The table below lists a few.

Step **Action**

Summary of Fit plot value	Possible cause	What to do
Low Q^2 and model validity	Non-significant two-way interactions may be present?	Look at the coefficient plot (see step 3) and the Interaction plot to see if there are interaction effects.
	Curvature in the model. Is there a need of adding quadratic terms to the model?	Look at the ***Residual vs. variable*** plot (see *Residuals versus variables plot, on page 182*) and the ***ANOVA*** table (see *ANOVA table, on page 187*) to see if these also indicate curvature in the model. If you suspect curvature, try adding a quadratic term to the model.
Model with moderate R^2 (~ 0.6) and Q^2 (~0.4)	Important factors may be missing. Are there uncontrolled factors that may affect the experiment?	If needed, perform more experiments.
The model is good for one response but not the other	It might be difficult to fit the same model to all responses.	Consider dividing the experiment in two or more to be able to fit one model/response.

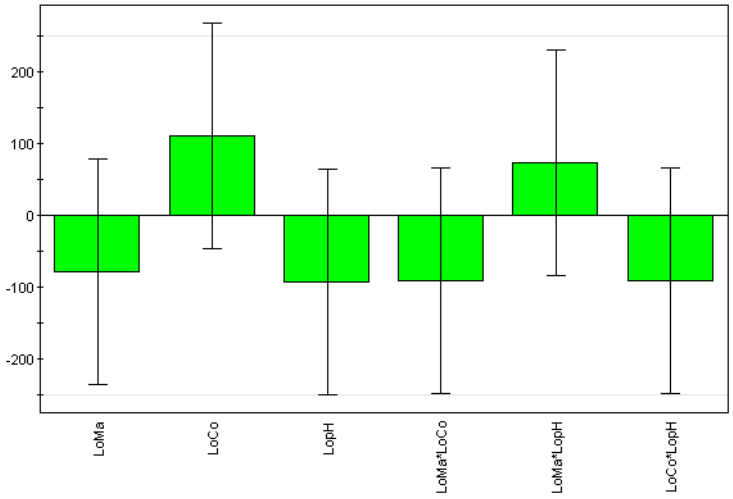
Step	Action
3	Look at the normal probability plot of residuals. If the model describes the experimental data well, the experiments should be distributed close to a straight line, and lie within a ***Standardized Residuals*** range of -4 to +4 SD (standard deviations). See *Check the raw data, on page 170*. If the centre points (points 9, 10 and 11 in the illustration below) are not linearly distributed, this may indicate curvature in the model rather than true outliers. A low Q^2, model validity and significant lack of fit may also indicate curvature.  If you suspect curvature, try adding a quadratic term to the model. See *Section 5.4.5 Edit the model, on page 190* for more information.

Step	Action
4	Look at the coefficient plot for each response. The coefficient plot can be used to see which factors that affect your response, in which way they affect the response(s) and if there are any non-significant terms in the model. In the example below the following terms have been included in the model: • the main effects, that is, the loading conditions for mass, pH and conductivity (LoMa, LoCo and LopH) • the two-way interaction effects for LoMa/LopH and LoCo/LopH **Note:** If an optimization design (CCC or CCF) has been used, quadratic terms for the model will also be included in the coefficient plot.



In the example above, the confidence limits (the black error bars shown on each green bar in the plot) do not cross zero. All of the terms are thus significant, with the ***LoCo*LopH*** two-way interaction term being least significant.

Positive bars have a positive influence on the response, in this example the ***Yield***, and negative bars a negative influence. From the above plot it is evident that increasing the ***LoMa*** (Load Mass) and ***LoCo*** (Load Conductivity) values, and decreasing the ***LopH*** (Load pH) value have a positive effect on the response.

Step	Action
5	Non-significant terms can be identified by the confidence limits for a coefficient (the black error barrs) crossing zero. The diagram below shows the extreme example where no terms are significant.  Insignificant terms should be removed from the model one at a time before reanalysing the model.
6	If the model does not look good or non-significant terms are present, edit the model or continue with the extended analysis before editing the model. See *Section 5.4.4 Analyze and evaluate the model - extended analysis, on page 182* and *Section 5.4.5 Edit the model, on page 190* for more information.
7	If the model looks good and all terms are significant, continue with *Section 5.4.6 Use the model, on page 193*.

5.4.4 Analyze and evaluate the model - extended analysis

Introduction

If you want to perform further analysis of the model in order to decide how to proceed, an extended report can be generated. The following plots and tables are displayed in the extended report in addition to the basic analysis:

- Residuals versus variables plot
- Residual versus run order plot
- Interaction plot
- Observed versus Predicted
- Main effects plot
- ANOVA table
- Correlation matrix

This section describes the plots in the extended report and gives information about how to evaluate the plots.

Open and view plots for extended analysis

To be able to view the plots for extended analysis create an extended report.

See *Create a report, on page 200* for information about how to create an extended report.

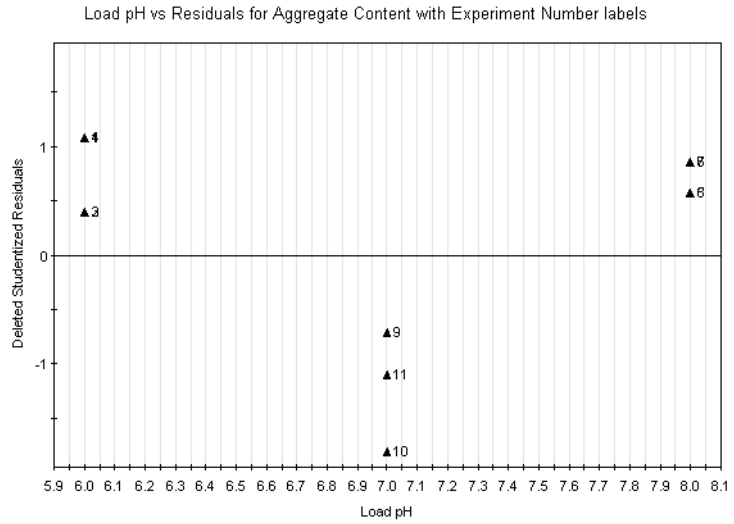
Residuals versus variables plot

The ***Residuals Plot vs. Variable*** shows the residuals (i.e., the minimized error between the measured and theoretical data according to the model) for one factor and one response.

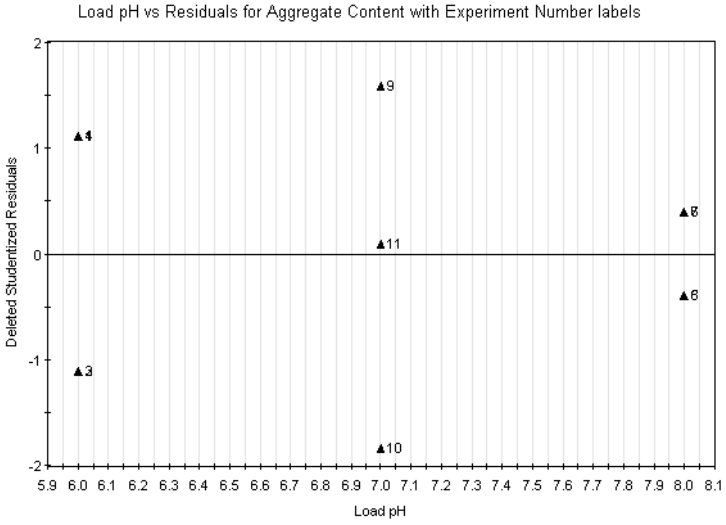
The residuals should be randomly distributed with no pattern. When a curved pattern can be seen in the plot this may indicate that a quadratic term is missing in the model. In this case try to add a quadratic term to the model and see if the model is improved.

See *Section 5.4.5 Edit the model, on page 190* for information about how to add a quadratic term to the model.

The illustration below shows an example of a plot indicating that a quadratic term is missing in the model.



The illustration below shows the plot for the same experiment when a quadratic term has been added to the model. Now the residuals are randomly distributed with no pattern.



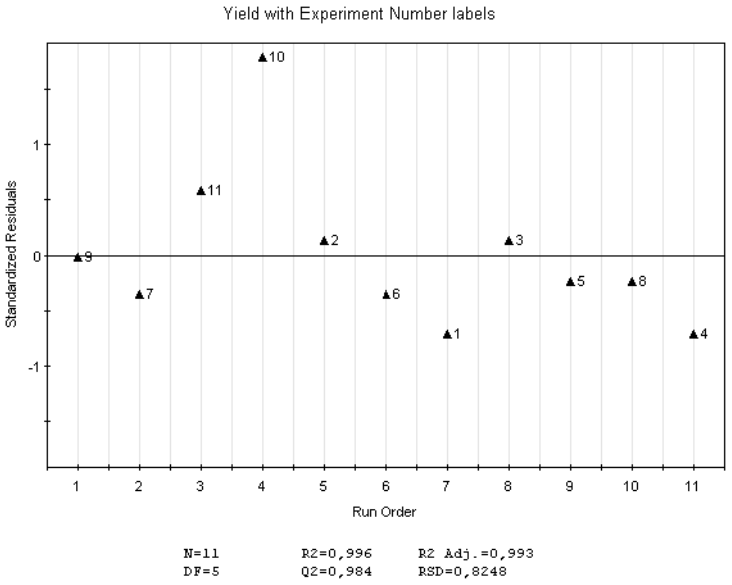
Note: When you find curvature in the model, the design for the experiment in the ***DoE*** setup should be changed to design allowing quadratic terms to be added to the model (Full factorial 3 levels, CCC, CCF, Box Behnken, Rechtschaffner or Doehlert). If the experimental setup is sufficiently stable the star point experiments alone can be run, otherwise it is recommended to rerun all experiments.

Residual versus run order plot

The ***Residuals Plot vs. Run order*** shows the residuals for the run order and one response.

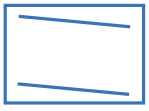
The residuals should be randomly distributed with no pattern. A pattern in the plot indicates a change in residuals over time. This could, for example, be the result when randomization errors exist in the experiment.

The illustration below shows a plot where the residuals are randomly distributed with no pattern.

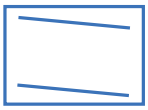
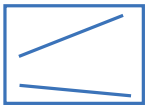
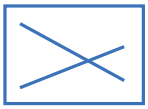


Interaction plot

The ***Interaction*** plot shows if there is any interaction (i.e., when the effect of one factor depends on another factor) between two factors. The illustration below shows an example of an interaction plot. In this example there is an interaction between load mass (***LoMa***) and load conductivity (***LoCo***).

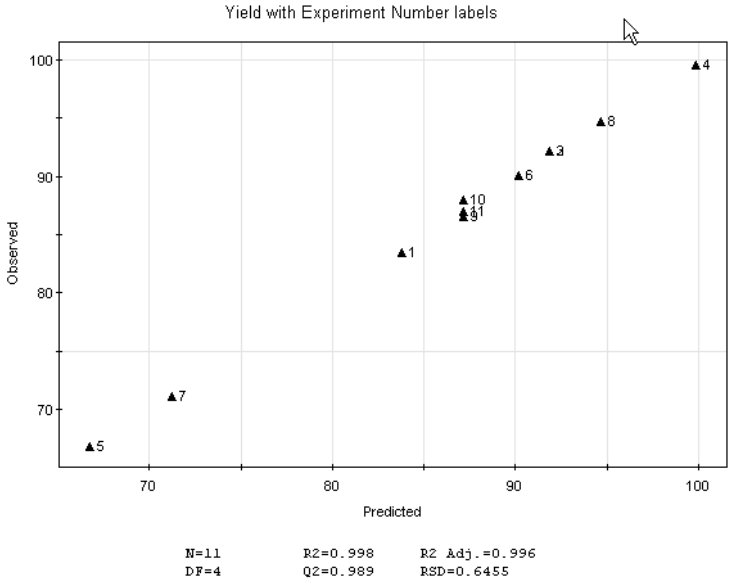


The table below describes how to interpret different interactions plot in a schematic way:

Plot	Description
	The two lines are parallel. This plot shows an example of no interaction between the two factors.
	The two lines are not parallel. This plot shows an example of interaction between the two factors.
	The two lines are crossing. This plot shows an example of strong interaction between the two factors.

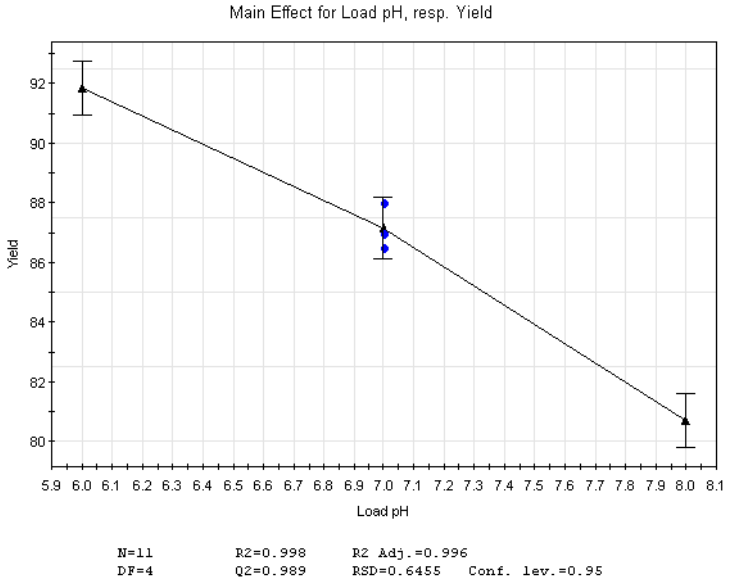
Observed versus Predicted for each Response plot

The ***Observed vs. Predicted for each Response (Y)*** plot can be used to judge the quality of the model. With a good model all the points will fall on the 45° line (illustrated in the plot below).



Main effects plot

The main effects plot displays the predicted response values when a factor varies from its low to its high level, all other factors in the design being set on their averages.



ANOVA table

The ANOVA (analysis of variance) table gives a numerical presentation of the variance analysis.

The illustration below shows an example of the ANOVA table.

Recovery MassOut/MassIn	DF	SS	MS (variance)	F	p	SD
Total	10	79468.2	7946.82			
Constant	1	78570.5	78570.5			
Total Corrected	9	897.672	99.7413			9.98706
Regression	6	893.347	148.891	103.271	**0.001**	12.2021
Residual	3	4.32527	1.44176			1.20073
Lack of Fit	1	0.0786042	0.0786042	0.0370193	**0.865**	0.280364
(Model Error)						
Pure Error	2	4.24666	2.12333			1.45717
(Replicate Error)						
	N = 10	Q2 = 0.988		Cond. no. = 3.635		
	DF = 3	R2 = 0.995		Y-miss = 0		
		R2 Adj. = 0.986		RSD = 1.201		

When looking at the ANOVA table, the p-values for regression, Lack of Fit (model error) and condition number give important information about the model. The table below describes these values in more detail.

Value	Description	Interpretation
Regression p-value	The regression p-value is a measure of the significance of the regression model.	p<0.05 indicates a significant regression model.
Lack of Fit p-value	The lack of fit p-value is a measure comparing the model error with the replicate error. This value is used in the calculation of Model Validity in the Summary of Fit plot.	p>0.05 indicates a good model. If p<0.05, this indicates that the model does not describe the relation between Y and X and that a quadratic term may be missing. See *Section 5.4.5 Edit the model, on page 190* for information about how to add a quadratic term to the model. A low p-value may also be due to other reasons, for example terms missing or that there is no correlation between X and Y that can be modelled.
Cond. no. (condition number)	The condition number can be used to investigate if the design is appropriate to use, especially if any of the default designs suggested in the ***Method Editor*** have been altered. Depending on the design, different condition numbers are expected for the model to be good.	• When the objective is screening and robustness testing - Good design when Cond. no.<3 - Questionable design when Cond .no.=3-6 - Poor design when Cond. no.>6 • When the objective is optimization - Good design when Cond. no.<8 - Questionable design when Cond .no.=8-12 - Poor design when Cond. no.>12

Correlation matrix

The correlation matrix gives a numerical presentation of the correlation between factors and responses and if the fit of the model is reasonable. The linear correlation coefficients R between all the terms in the model and all the responses are displayed in the correlation matrix.

Process factors are log-transformed, scaled, and centered and responses are log transformed. The value of the correlation coefficient R represents the extent of the linear association between two terms. The value of R ranges from -1 to 1. When R is near zero there is no linear relationship between the terms. Correlation coefficients above the threshold between a term in the model and the responses are colored green.

The illustration below shows an example of the correlation matrix.

	LoMa	LoCo	LopH	LoMa*LopH	LoCo*LopH	Yiel
LoMa	1	0	0	0	0	0,72118
LoCo	0	1	0	0	0	0,287094
LopH	0	0	1	0	0	-0,512175
LoMa*LopH	0	0	0	1	0	0,353699
LoCo*LopH	0	0	0	0	1	-0,0803863
Yiel	0,72118	0,287094	-0,512175	0,353699	-0,0803863	1

5.4.5 Edit the model

Introduction

Editing of the model may be necessary after analysis of the model, if the current model does not give a good fit. In the analysis you may for example:

- find insignificant terms that need to be removed
- find that the model may have curvature and that a quadratic term needs to be added

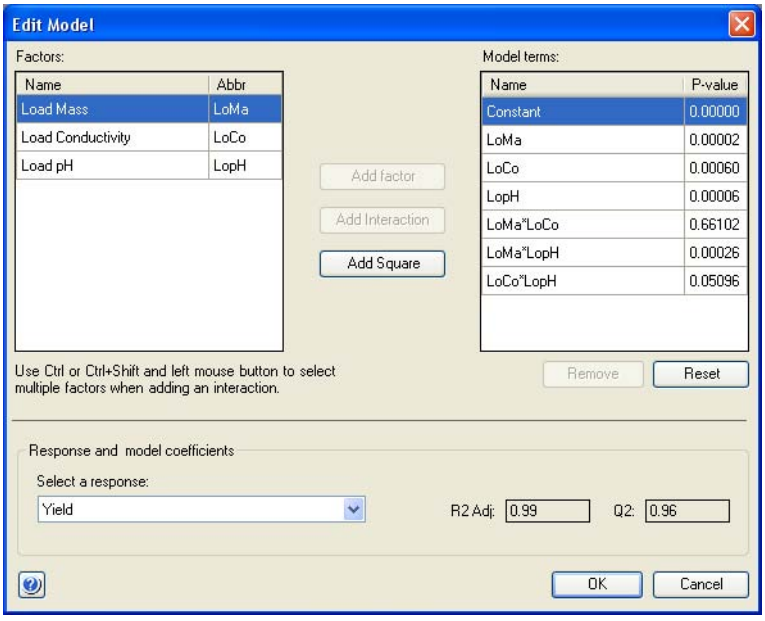
The refined model can be analysed to see if it better fits the data.

This section describes how to edit the model.

Edit the model

The table below describes how to edit a model:

Step	Action
1	In the ***Analysis*** tab, click Edit Model... . *Result:* The ***Edit Model*** dialog opens.



Step	Action
2	Note the ***R2 Adj*** and ***Q2*** values for the response(s) before starting to edit the model. Select different responses in the ***Select a response*** drop-down list. When editing the model, the ***R2 Adj*** and ***Q2*** values are updated. Higher values indicate a better model. See also *Analyze and interpret the model - basic analysis, on page 174* for a description of the values.
3	Non-significant terms may have been found in the analysis of the model (for example in the coefficient plot). To remove a non-significant term, select the term in the ***Model terms*** table and click ***Remove***. If the ***P-value***>0.05, the term is not significant. **Note:** Always remove non-significant terms from the model one by one, starting with the least significant interaction or quadratic term. When the first term has been removed, the significance of the other terms changes. The ***P-value*** can be used to determine which term to be removed next. **Note:** If you fit a model to two or more responses, a model term that is not significant for one response may be significant for another response. Then the term should not be removed. Before removing a term, always check that the term is not significant for any of the other responses by selecting the response in the ***Select a response*** drop-down list and checking the ***P-value*** for the term you want to remove. **Note:** If a main term is not significant but one of its interaction terms is significant the main term should not be removed. **Note:** If a main term is removed its interaction terms are also removed. *Result:* The term is removed from the model and the ***R2 Adj*** and ***Q2*** values are updated. If the model refinement gives a higher ***Q2*** value, the model refinement is justified. If one model is fitted to several responses, view the ***R2 Adj*** and ***Q2*** values for all responses.

Step	Action
4	Based on the previous analysis, add the appropriate terms to the model. • Add an interaction term by selecting the appropriate factors in the ***Factors*** table and clicking ***Add Interaction***. **Tip:** Use the **Ctrl** or **Shift** keyboard key when to select multiple factors. • Add a quadratic term to the model by selecting the appropriate factor in the ***Factors*** table and clicking ***Add Square***. **Note:** Quadratic terms can be added if any of the plots in the analyses indicates that a quadratic term is missing (in the ***Residuals vs. Variables*** plot, for example). **Note:** When you find curvature (i.e., a quadratic term needs to be added) in the model, the design for the experiment in the ***DoE*** setup should be changed to an extended Full Fractional (CCC or CCF) design. If the experimental setup is sufficiently stable the star point experiments alone can be added, otherwise it is recommended to rerun all experiments. *Result:* The terms are added to the model. If the model refinement gives higher ***R2 Adj*** and ***Q2*** values, the model refinement is justified. If one model is fitted to several responses, view the ***R2 Adj*** and ***Q2*** values for all responses.
5	To return to the original model settings, click ***Reset***.
6	When you are satisfied with the editing, click ***OK***. *Result:* The ***Edit Model*** dialog is closed and the ***Analysis*** tab displayed showing the new plots for the edited model.
7	Perform an analysis of the edited model to see if the new model is ***OK***. See *Analyze and interpret the model - basic analysis, on page 174* for information about how to analyze the model.

5.4.6 Use the model

Introduction

When you have found a good model, use the model to draw conclusions and to decide if more experiments are needed and what experiments to perform. The following plots and tools can be used in the evaluation:

- ***Response surface plot***

  Generate a response surface plot to get a graphical representation of the experimental region. From this, the most interesting area can be used to plan new experiments, verifying experiments and to better understand the impact of large interactions between factors.

- ***Prediction***

  Use the predictor to predict response values for entered factor settings.

- ***Optimization***

  Use the optimizer to enter response and factor settings criteria and obtain suitable factor setting combinations for the set response criteria.

Note: Information about significant terms and how they influence the response values have already been found in the analysis of the model by looking at the coefficient plot, interaction plot, main effects plot and correlation matrix. See *Analyze and interpret the model - basic analysis, on page 174* and *Section 5.4.4 Analyze and evaluate the model - extended analysis, on page 182* for information about how to evaluate these plots.

This section describes how to use the model.

Generate response surface plot and edit settings

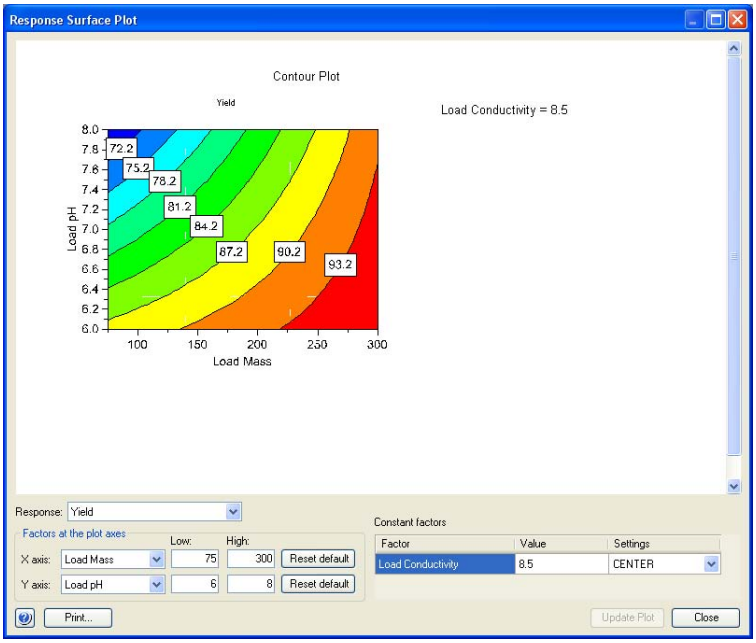
The response surface plot graphically displays the experimental region. It is helpful when you want to:

- get an overview of how different factor settings affect the response
- find the interesting experimental area
- get help in deciding where to start a new investigation
- get help in deciding where to make verifying experiments
- understand the impact of large interactions

Note: The underlying model must be good and have a high Q^2-value. See *Section 5.4.3 Analyze and evaluate the model - basic analysis, on page 170*.

The table below describes how to generate a response surface plot and how to evaluate the plot:

Step	Action
1	In the ***Analysis*** tab, click Response Surface Plot... . *Result:* The ***Response Surface Plot*** dialog opens.

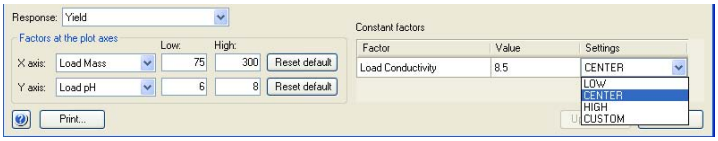


The ***Contour Plot*** shows a "map" of the model. The plot has a color scale from blue to red. For each color, the response value is displayed.

The factors selected are displayed on the ***X axis*** and ***Y axis*** in the ***Contour Plot*** (from ***Low*** to ***High*** as selected in the ***Factors at the plot axes*** area).

If you have more than two factors, the other factors will have constant values. The currently entered constant value(s) is displayed to the right of the contour plot. This means that this value is kept constant while the factors on the X- and Y-axes are varied.

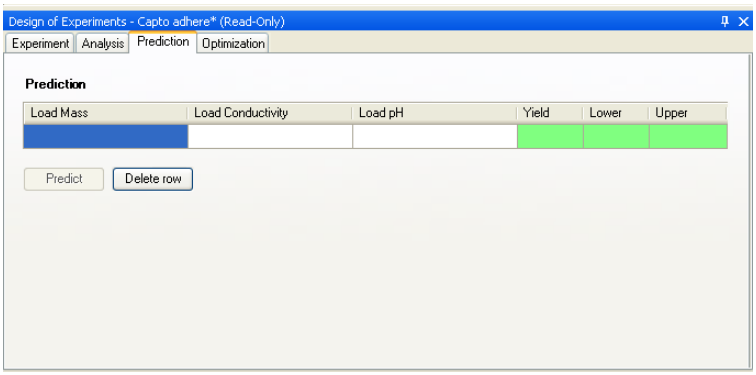
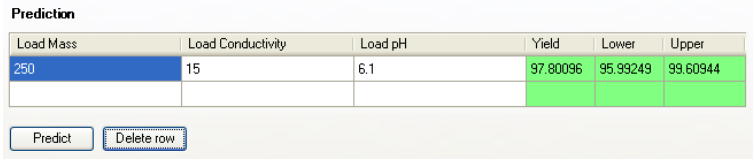
The red area indicates the area where the response is maximized using the factor settings within this area and the current constant value(s).

Step	Action
2	It is possible to change the factors and their corresponding settings for the response surface plot as well as the constant values for the other factor(s). This is done per response if you have several responses. In this way you can see what happens if constant values are changed and if other factors and/or factor settings are set on the contour plot axes. This will help you to decide if/which complementary experiments need to be performed. For example, you may want to investigate which factor settings to use in new ***DoE*** setup to narrow down the area of interest. The coefficient plot can be used to see which terms have the greatest positive or negative effect on the response. This information can be tested by changing the contour plot settings and updating the plot.
3	To change the ***Contour Plot*** settings: • Select the response for the contour plot in the ***Response*** drop-down list. • Select factors for the ***X axis*** and ***Y axis*** and their corresponding ranges. To return to the default values, click the ***Reset default*** button. • Select or enter values for the ***Constant*** factors by choosing in the ***Settings*** drop-down list as shown in the diagram below. If selecting ***CUSTOM***, click in the ***Value*** field and enter a value. 
4	Click the ***Update Plot*** button. *Result:* The ***Contour Plot*** is updated.
5	When you have obtained the appropriate information to help you in the decision on how to proceed it is possible to print the ***Contour Plot***. • Click the ***Print...*** button. *Result:* The ***Print Preview*** dialog opens. • Click ***Print...***. *Result:* The standard ***Print*** dialog opens. • Select the appropriate printer and click ***Print***.

Predict response values

It is possible to predict response values based on entered factor settings using the model. This is useful when you want to find out how detailed factor settings influence the response(s) in an optimization experiment. Factor settings are entered and response values are calculated when using the ***Prediction*** list.

The table below describes how to use the ***Prediction*** list:

Step	Action
1	Select the ***Prediction*** tab in the ***Design of Experiments*** box. *Result:* The ***Prediction*** list opens. 
2	Enter the appropriate settings for the different factors in their respective fields.
3	Click the ***Predict*** button. *Result:* The response value is calculated and displayed in the ***Yield*** field, together with the ***Lower*** and ***Upper*** confidence limits. The larger the confidence interval, the more uncertain the calculation is. 

Step	Action
4	To enter other factor settings, enter the settings in the empty row below and click ***Predict***. In this way it is possible to compare different response values for different factor settings.

Prediction

Load Mass	Load Conductivity	Load pH	Yield	Lower	Upper
250	15	6.1	97.80096	95.99249	99.60944
300	15	6	99.94317	97.65833	102.228

Predict | Delete row

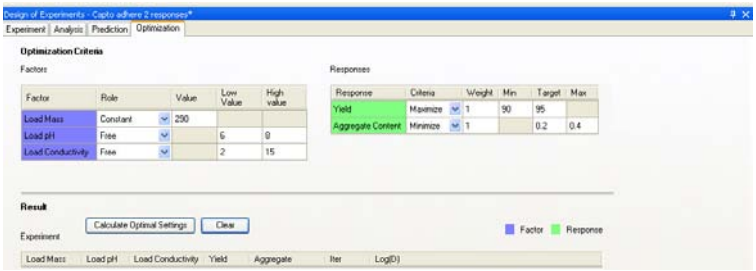
Repeat this procedure until you are satisfied.

Optimize response values and factor settings

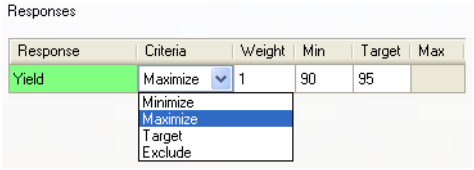
It is possible to optimize the response values using the optimizer. When using the optimizer, criteria for the response values and factor settings are entered (e.g., ***Yield***>90%) and factor settings are calculated. In this way, the experimental region can be moved to an optimum.

The table below describes how to use the optimizer:

Step	Action
1	Select the ***Optimization*** tab in the ***Design of Experiments*** box. *Result:* The ***Optimization Criteria*** and ***Result*** tables are displayed.



Step	Action
2	In the ***Responses*** area, select the ***Criteria*** for the response.



The following choices are available:

- ***Minimize***

  The response value should be minimized. Enter ***Target*** value and ***Max*** value for the response.

- ***Maximize***

  The response value should be maximized. Enter ***Target*** value and ***Min*** value for the response.

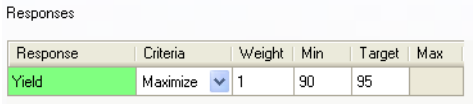
- ***Target***

  The response value should be optimized to reach the ***Target*** value. Enter ***Min***, ***Target*** and ***Max*** values for the response.

- ***Exclude***

  The response should not be included in the optimization (if you have several responses)

Result: The entered values are displayed.



Step	Action
3	In the ***Factors*** area, select ***Role*** and settings for each factor:

Factors

Factor	Role	Value	Low Value	High value
Load Mass	Free		100	300
Load Conductivity	Constant	15		
Load pH	Free		6	7.5

- If the role ***Free*** is selected, the factor settings to be calculated for the response can have values within the entered ***Low Value*** and ***High value*** range. Enter the ***Low Value*** and ***High value*** as appropriate (to get an idea of the new region of interest, use the response surface plot).
- If the role ***Constant*** is selected, the factor setting is constant. Enter the factor value in the ***Value*** field.

4 In the ***Result*** area, click the ***Calculate Optimal Settings*** button.

Result: The results are displayed in the ***Experiment*** table.

Result

Calculate Optimal Settings | Clear | Factor | Response

Experiment

Load Mass	Load Conductivity	Load pH	Yield	Iter	Log(D)
140	15	6	94.4676	0	-1.9455
300	15	6	99.9432	0	-10
299.975	15	6.0004	99.9412	80	-10
300	15	7.5	96.0432	0	-10
240	15	6.75	94.3998	0	-1.8414
200	15	6	96.521	0	-10
300	15	6	99.9432	0	-10
300	15	6	99.9432	0	-10

It is possible to see the combination factor settings that will give a certain response. The number of iterations for optimization is indicated in the ***Iter*** column. Lower (or more negative) ***Log(D)*** values (the logarithm of the distance to the target) indicate better results.

5.4.7 Create and print reports

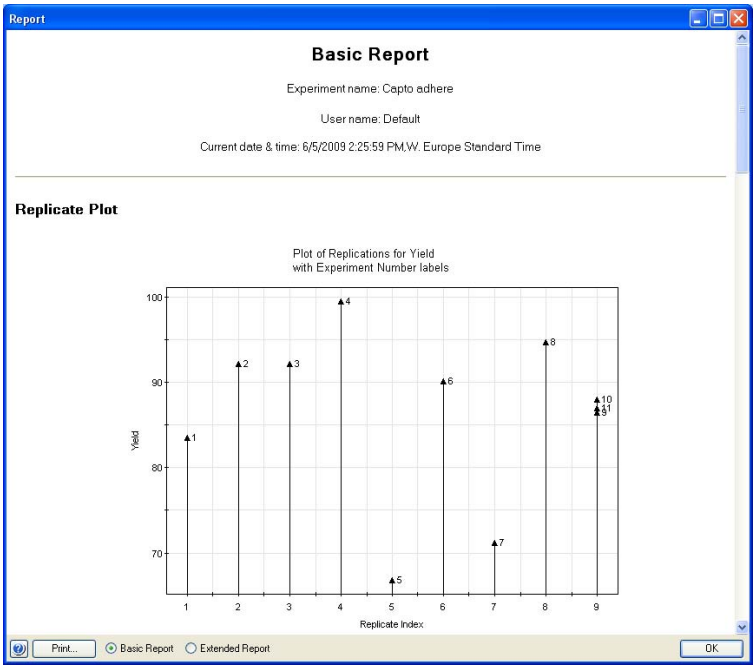
Introduction

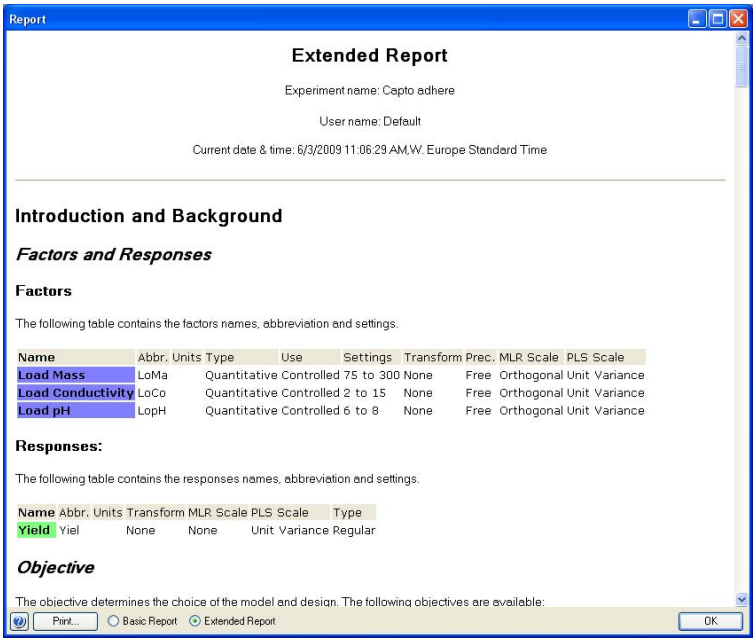
This section describes how to create basic and extended reports and how to print the reports.

Create a report

The table below describes how to create a report:

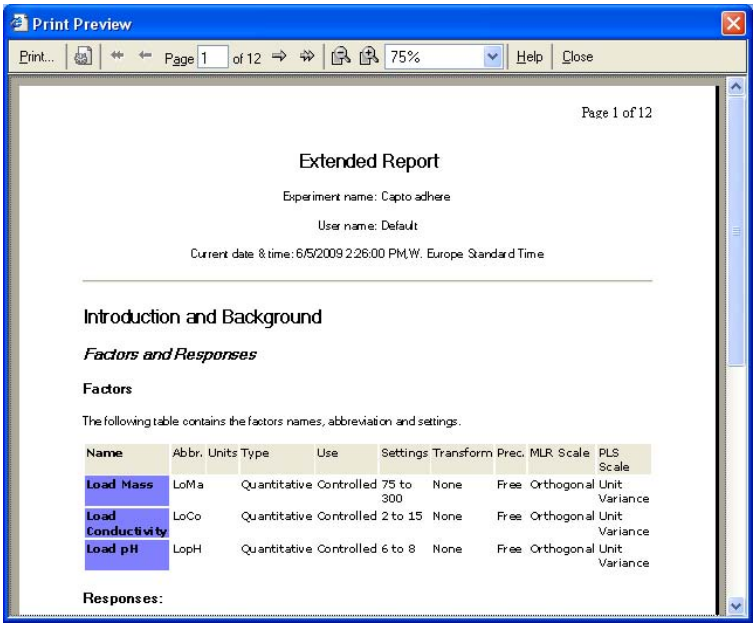
Step	Action
1	In the ***Analysis*** tab, click Report... . *Result:* The ***Report*** dialog opens displaying the ***Basic*** report by default. It displays the Replicate, Summary of Fit, Normal probability and Coefficient plots.



Step	Action
2	To display the extended report select the ***Extended Report*** radio button.  *Result:* The ***Extended Report*** opens in the ***Report*** dialog. This report includes all available plots as well as the experiment setup, objective and design used in the experiment. 
3	To view the information in the report use the vertical scrollbar.

Print a report

The table below describes how to print a report:

Step	Action
1	In the Report dialog, click the ***Print...*** button. *Result:* The ***Print Preview*** dialog opens. 
2	Click the ***Print...*** button. *Result:* The standard ***Print*** dialog opens.
3	Select the appropriate printer and click the ***Print*** button. *Result:* The report is printed.

6 BufferPro

About this chapter

This chapter describes how to create, edit and use buffer recipes created using the ***BufferPro*** tool in UNICORN. For information about how to prepare the system for using ***BufferPro***, see *ÄKTA avant and UNICORN User Manual*.

In this chapter

This chapter contains the following sections:

Section	See page
6.1 BufferPro - Overview	204
6.2 Create a method using BufferPro	206
6.3 Create and edit BufferPro recipes	207
6.4 Print a BufferPro recipe	216
6.5 Calculate buffer composition using BufferPro	219
6.6 Export and import BufferPro recipes	222
6.7 Predefined BufferPro recipes	226

6.1 BufferPro - Overview

Introduction

This section gives an introduction to the ***BufferPro*** tool in UNICORN, and includes a brief overview of the ***BufferPro*** recipes that are predefined.

What is BufferPro?

The ***BufferPro*** tool allows automatic mixing of buffers during a run. Four stock solutions are generally used in a recipe, the buffering agent, a titrant, a salt stock solution and water. ***BufferPro*** facilitates ***Scouting*** or ***Design of Experiments*** runs using pH as a variable.

BufferPro is optimized for use with anion or cation exchange chromatography, but can also be used with gel filtration where the salt concentration may also be used as a variable during ***Scouting*** or ***Design of Experiements***.

Commonly used buffer systems have predefined recipes in UNICORN from which new recipes can easily be created. New or edited recipes may be stored as ***personal*** or ***global*** recipes.

UNICORN uses a robust algorithm to calculate pH ranges for optimal buffering taking into account the buffer type, concentration, temperature and ionic strength. Once an optimal buffer has been found, it is possible using ***BufferPro*** to calculate the buffer composition for the production of bulk-scale buffer solutions if required.

For details on...	See...
Scouting	*Chapter 4 Scouting, on page 103*
Design of Experiments	*Chapter 5 Design of Experiments, on page 116*

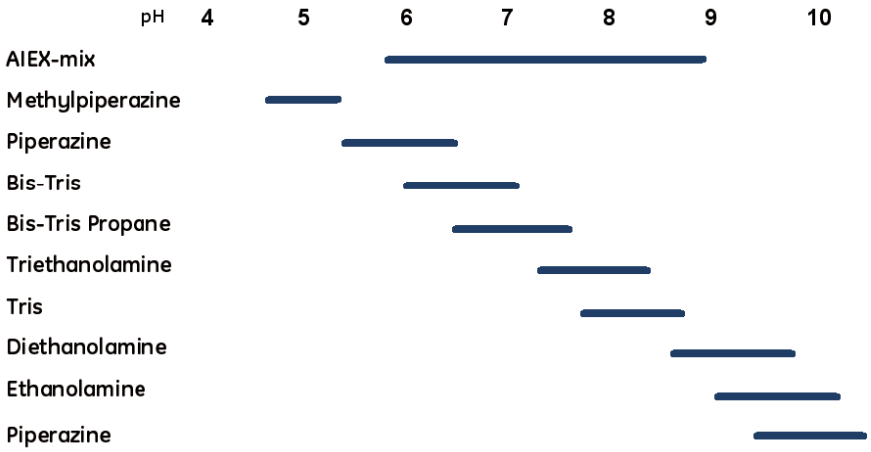
Workflow

- If required, create a new ***BufferPro*** recipe.
  Tip: Generally the predefined recipes will be sufficient.
- Create a method including ***BufferPro***.
- Save the method.

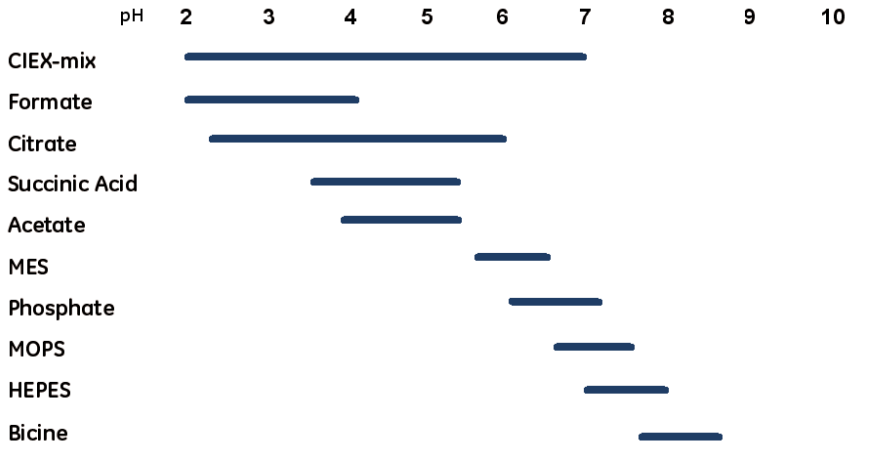
pH ranges for predefined buffers

The following diagrams show the optimal pH ranges for buffers commonly used in anion and cation exchange chromatography. Recipes for these buffers are predefined in UNICORN.

Anion exchange chromatography



Cation exchange chromatography



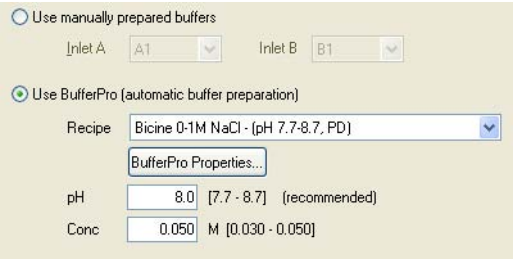
6.2 Create a method using BufferPro

Introduction

This section describes the how to use ***BufferPro*** recipes in a method. For details on how to edit methods see *Chapter 3 Create and edit methods, on page 25*.

Creating a BufferPro method

Step	Action
1	In the ***Method Settings*** phase of a method, select the ***Use BufferPro (automatic buffer preparation)*** option.



Note: It is not necessary to have the ***Enable pH monitoring*** option checked. The output from the pH monitor is not used by the BufferPro algorithm.

2 Select ***Recipe*** and enter ***pH*** and buffer concentration (***Conc***) within the specified range.

Note: For broad pH range multi-component buffers the concentration is fixed. For further information see *Section 6.7 Predefined BufferPro recipes, on page 226*.

Note: To obtain an even gradient, the gradient should run for at least 10 minutes, and the ***Flow rate*** should be not lower than 1 ml/min for ÄKTA avant 25 and 2 ml/min for ÄKTA avant 150.

3 ***Save*** the method.

6.3 Create and edit BufferPro recipes

Introduction

This section describes how to create, edit, rename and delete ***BufferPro*** recipes. Predefined recipes may not be overwritten, renamed or deleted. Edited recipes, including edited predefined recipes, can be saved as ***global*** or ***personal*** recipes. ***Global*** recipes are available for all users, ***personal*** recipes only for the current user.

Note: The predefined recipes can be used in the majority of cases. There is often no need to create a new recipe before creating a ***BufferPro*** method.

In this section

This section contains the following sections:

Section	See page
6.3.1 Create and edit a BufferPro recipe	208
6.3.2 Rename a BufferPro recipe	212
6.3.3 Delete a BufferPro recipe	214

6.3.1 Create and edit a BufferPro recipe

General considerations

The concentration of the buffer stock will affect the pH range and the settable concentration range in the method. The pH range will in general increase with increasing buffer concentration and decrease when lowered.

The titrant and buffer agent concentrations should be the same, since there may otherwise not be sufficient titrant to reliably obtain the entire pH range. For recipes titrated with strong acid/base, the concentration range that can be achieved is 15-25% of the buffer stock concentration. For conjugate acid/base titrants the corresponding range is 25-50% of the buffer stock concentration.

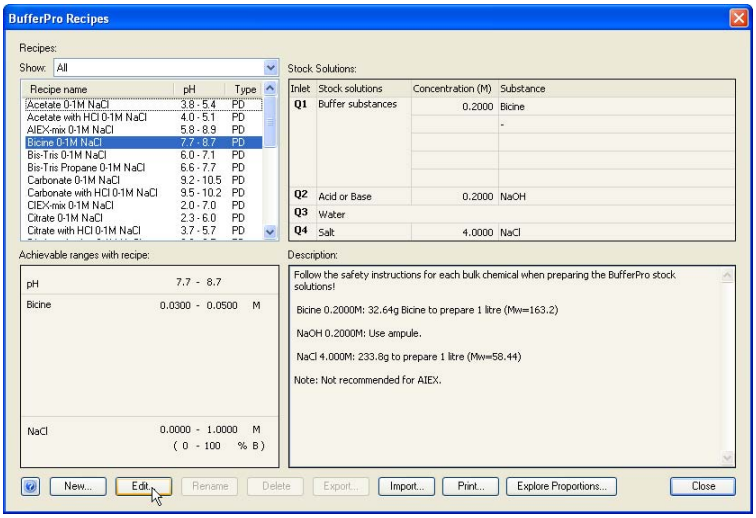
The following table describes how to create a new recipe and how to edit existing recipes.

Create/edit a recipe

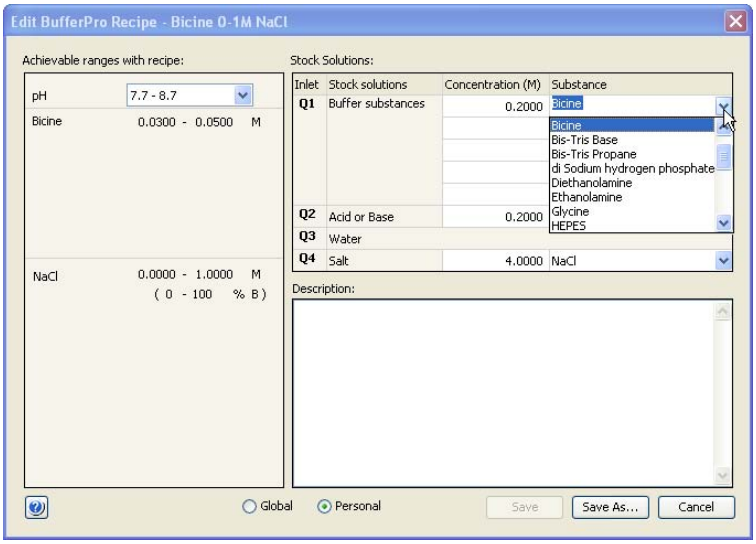
The following table describes how to create or edit a BufferPro recipe:

Step	Action
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.

Step	Action
2	To create a new recipe, click ***New...*** in the ***BufferPro Recipe*** dialog. To edit an existing recipe, select the recipe to be edited from the list and click ***Edit...***. **Note:** The available recipes may be filtered by type (***All***, ***Predefined***, ***Global*** or ***Personal***) by using the ***Show*** drop-down list.



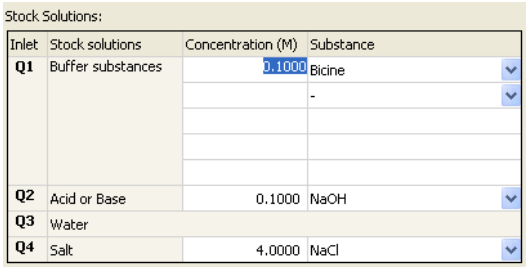
Step	Action
3	Select a ***Buffer substance*** from the drop-down list.

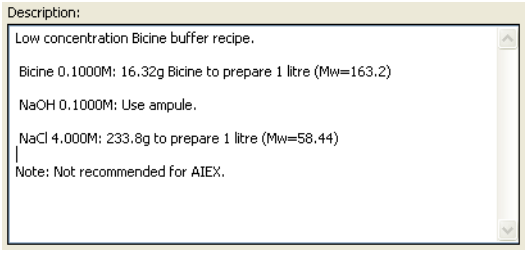
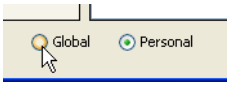
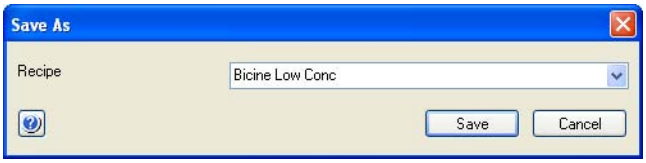


Note: Up to five buffer substances may be included in the recipe for the ***Q1*** inlet. If more than one substance is used, the concentration of the final buffer in ***BufferPro*** will be fixed, and is then dependent on the concentration of the stock solutions.

Note: To choose a conjugate acid-base pair as the buffer, select the base form as ***Buffer substance*** apart from phosphate where the acidic or basic form may be chosen. The conjugate acid or base will appear as an option in the ***Acid or Base*** drop-down list.

4	Select the concentration and edit the value.



Step	Action
5	Choose a titrant (***Acid or Base***) from the drop-down list and if required edit its ***Concentration***. **Note:** The titrant and the stock solution should generally have the same concentration. This is set as default for ***Acid or Base*** concentration.
6	Choose a ***Salt*** from the drop-down list and edit its ***Concentration*** if required. **Note:** The salt concentration of the stock solution should be four times larger than the desired maximum salt concentration for the gradient.
7	Enter a description of the buffer.  **Note:** Although the description is optional, it is highly recommended to add the recipe details for future reference.
8	Select to save the edited recipe as ***Global*** or ***Personal*** and click ***Save as...*** or ***Save***.  **Note:** Recipes can be changed from ***Personal*** to ***Global*** and vice versa by editing the recipe, changing the type then clicking on ***Save***. *Result*: The ***Save As*** dialog opens.
9	Enter a name and click ***Save***. 

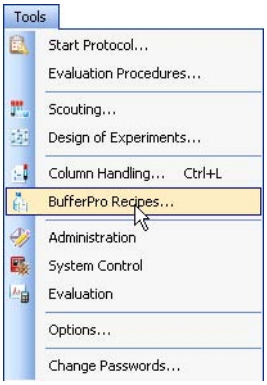
6.3.2 Rename a BufferPro recipe

Introduction

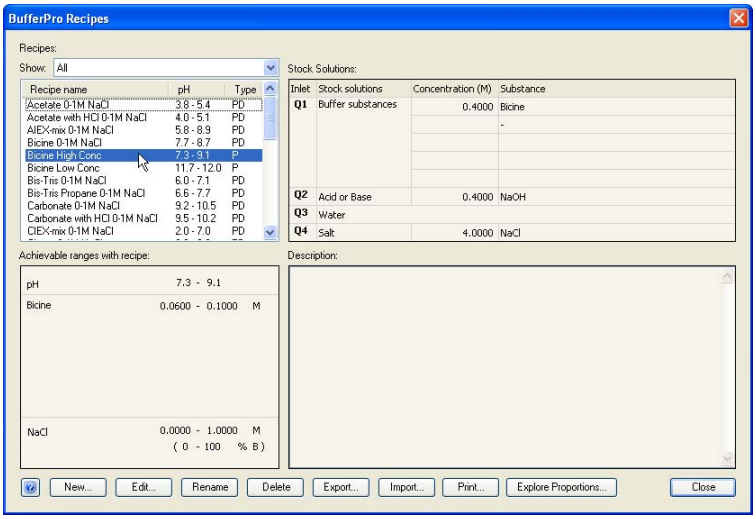
The following table describes the steps for renaming a ***BufferPro*** recipe.
Note: Predefined recipes (shown as ***PD*** in the ***Type*** column) can not be renamed.

Rename a recipe

Step	Action
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.

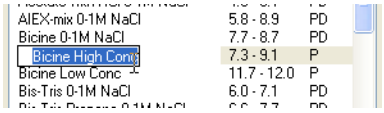


Step	Action
2	In the ***BufferPro Recipes*** dialog, select the recipe to be renamed.



Note: The available recipes may be filtered by type (***All***, ***Predefined***, ***Global*** or ***Personal***) by using the ***Show*** drop-down list.

3	Click ***Rename*** and enter the new name.



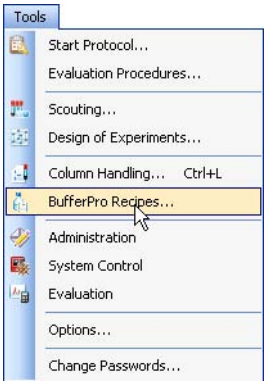
6.3.3 Delete a BufferPro recipe

Introduction

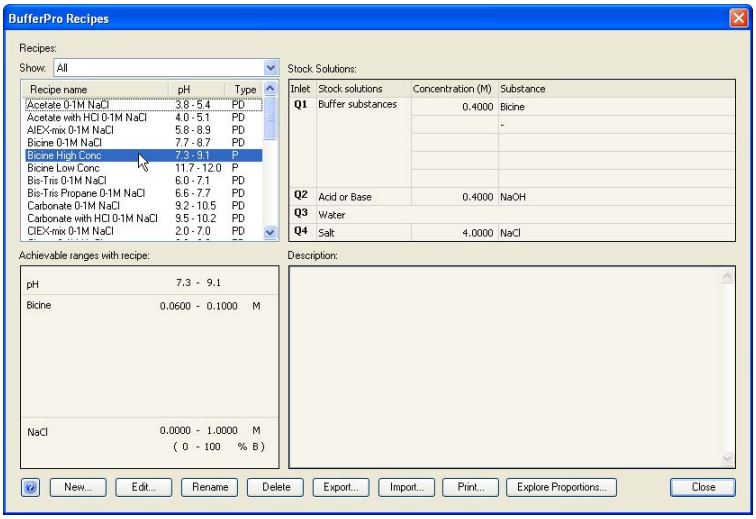
The following table describes the steps needed to delete a ***BufferPro*** recipe.
Note: Predefined recipes (shown as ***PD*** in the ***Type*** column) can not be deleted.

Delete a recipe

Step	Action
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.



Step	Action
2	In the ***BufferPro Recipes*** dialog, select the recipe to be deleted.



Note: The available recipes may be filtered by type (***All***, ***Predefined***, ***Global*** or ***Personal***) by using the ***Show*** drop-down list.

3	Click ***Delete***. A dialog will appear asking you to confirm the deletion.

6.4 Print a BufferPro recipe

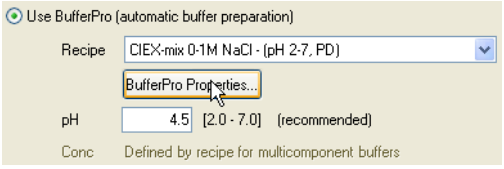
Introduction

This section describes how to print a BufferPro recipe from UNICORN. A recipe can be printed from the ***Phase Properties*** tab in ***Method Editor***, or from the ***BufferPro Recipes*** dialog.

It is also possible to include the ***BufferPro*** recipes when printing the whole method. See *Section 3.7 Print a method, on page 79*.

Printing from Method Editor

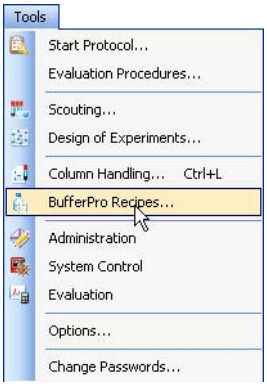
The following table describes how to print a recipe from the ***Phase Properties*** tab in ***Method Editor***.

Step	Action
1	In the ***Phase Properties*** tab in the ***Method Editor***, click the ***BufferPro Properties...*** button. 
2	In the ***BufferPro Properties*** dialog click the ***Print...*** button. *Result*: The ***Print*** dialog opens.
3	Choose a printer from the drop-down list in the ***Print*** dialog and click ***OK***.

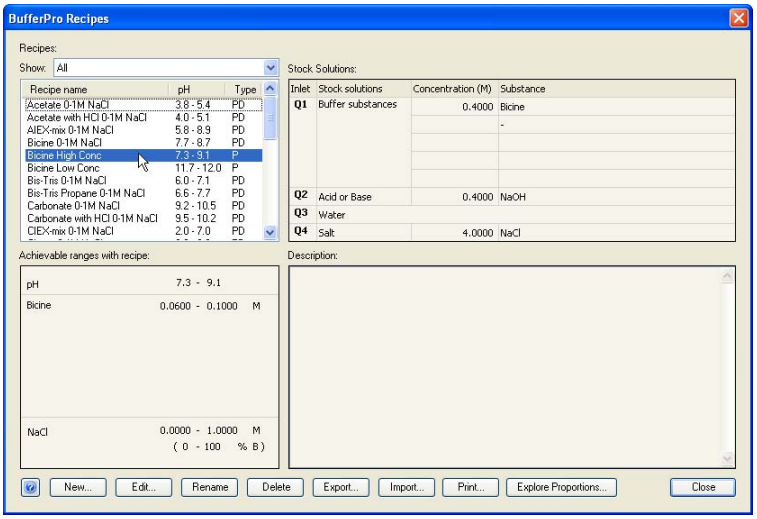
Printing from *BufferPro Recipes* dialog

The following table describes how to print a recipe from the ***BufferPro Recipes*** dialog.

Step	Action
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes***.



2	Choose the recipe to be printed from the list in the ***BufferPro Recipes*** dialog.



3	Click the ***Print...*** button. *Result*: The ***Print*** dialog opens.

Step	Action
4	Choose a printer from the drop-down list in the ***Print*** dialog and click ***OK***.

6.5 Calculate buffer composition using BufferPro

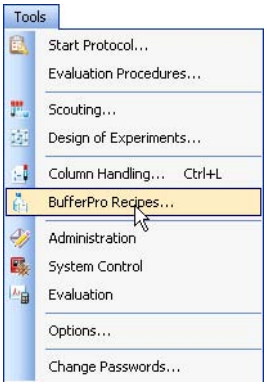
Introduction

This section describes how to calculate an exact buffer composition for a buffer previously optimized using ***BufferPro***. This is desirable when scaling up a purification procedure in order to prepare bulk-scale buffer solutions, for example ion exchange A and B buffers.

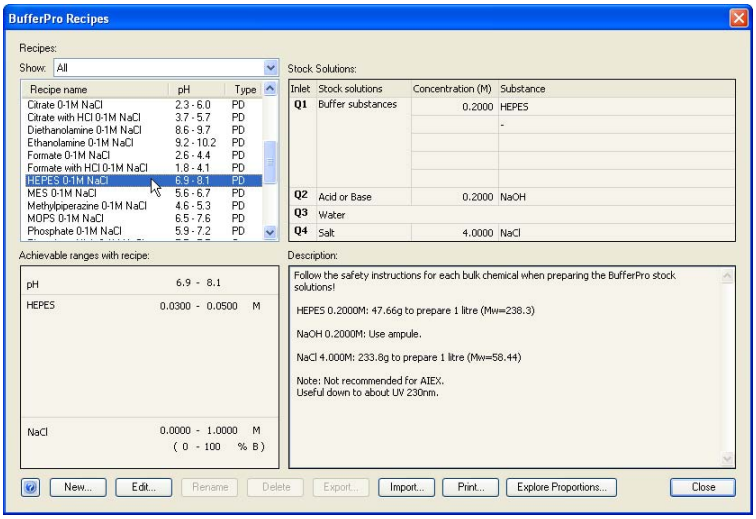
Calculating buffer composition

The following table describes the steps needed to calculate the buffer composition of a ***BufferPro*** recipe at a particular pH, buffer and gradient concentration, and temperature. In the examples shown in the table, a pH optimization scouting run has been performed. The buffer at which optimal separation was obtained was 50 mM HEPES, pH 7.8 at 25 °C, and the required peak eluted at 25% of the gradient.

Step	Action
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.

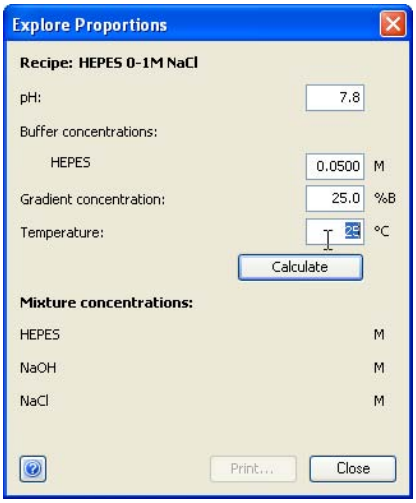


Step	Action
2	Choose the appropriate recipe from the list in the ***BufferPro Recipes*** dialog.



3	Click the ***Explore Proportions...*** button.

Step	Action
4	In the ***Explore Proportions*** dialog, enter the ***pH***, ***Buffer concentrations***, the desired ***Gradient concentration*** and ***Temperature***.

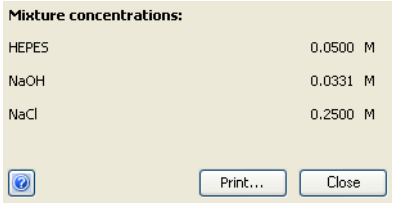


Note: The ***buffer concentrations*** may not exceed the limits of the recipe. If this is the case the ***Calculate*** button will be grayed out.

5 Click ***Calculate***.

Note: If the ***pH*** given is beyond the optimal buffering range of the buffer recipe, a warning will be displayed.

6 The actual concentrations of the components in the required buffer will be displayed.



Note: It is important that the molar amounts are as exact as possible when mixing the buffers. It has been found that four decimal places in molar concentration gives reproducible results.

7 The buffer composition can be printed by pressing the ***Print...*** button.

6.6 Export and import BufferPro recipes

Introduction

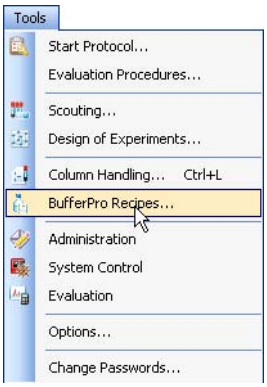
BufferPro recipes are stored internally in the UNICORN database. It is possible to export these recipes to a zip file on the local computer so that the recipe can be imported again later into the same database installation, or imported into another. This section describes how to export and import ***BufferPro*** recipes.

Exporting BufferPro recipes

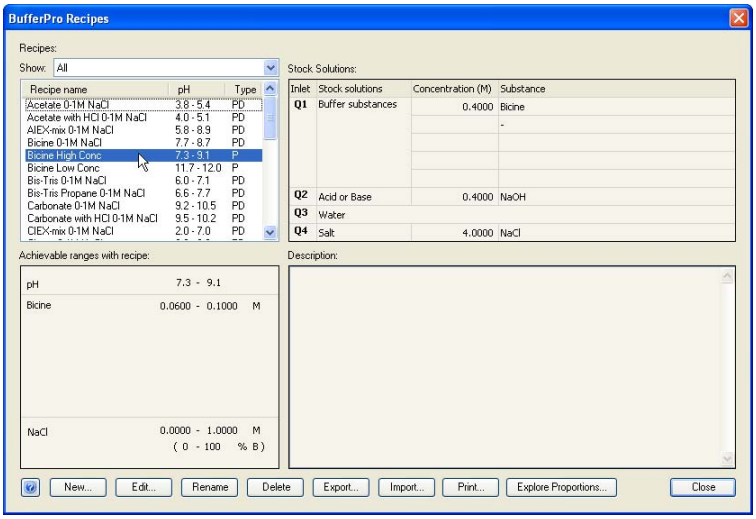
The following table illustrates the steps required to export one or several recipes.

Note: Predefined recipes can not be exported, since these recipes will always be found in a UNICORN installation.

Stage	Description
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.



Stage	Description
2	Choose the recipe to be exported from the list in the ***BufferPro Recipes*** dialog.



Note: Several recipes may be exported to the same zip file. To select a continuous range, click on the first recipe then Shift-click the last. To add single recipes to a selection, Ctrl-click them.

3 Click ***Export...***.

Result: The ***Export*** dialog opens.

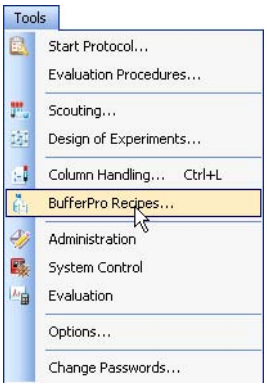
4 Choose a location on the computer disk and a filename for the zip file.

5 ***Save*** the file.

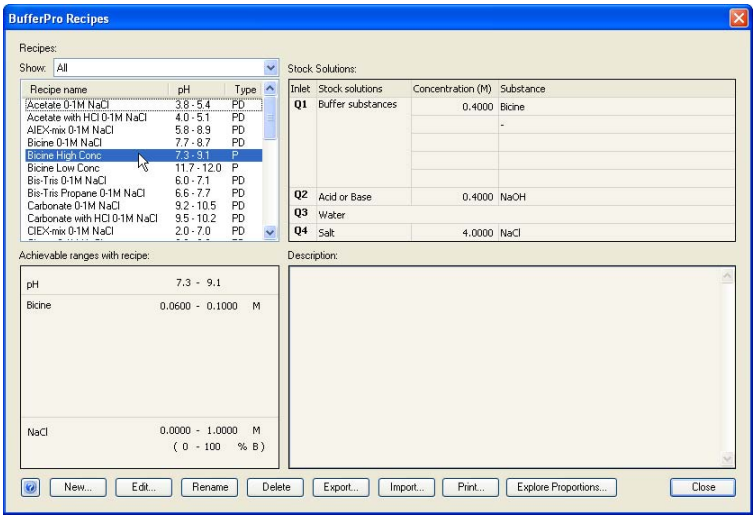
Importing BufferPro recipes

The following table illustrates the steps required to import one or several recipes.

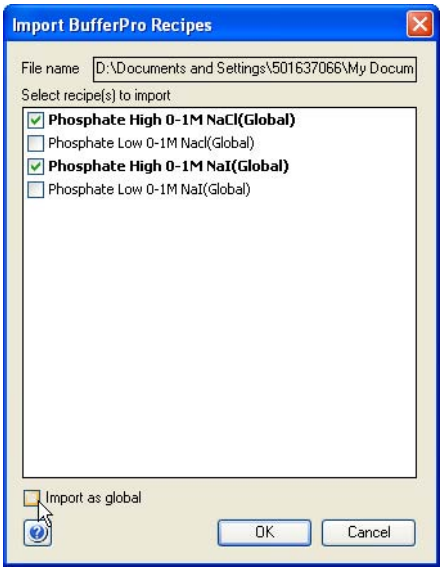
Stage	Description
1	In the ***Method Editor***, select ***Tools:BufferPro Recipes...***.



Result: The ***BufferPro Recipes*** dialog opens.



2	Click on the ***Import...*** button. *Result*: The Import dialog opens.

Stage	Description
3	Browse to the zip file containing the ***BufferPro*** recipe(s) on the computer disk and ***Open*** the file. *Result*: The ***Import BufferPro Recipes*** dialog opens. 
4	In the ***Import BufferPro Recipes*** dialog, uncheck any recipe(s) that you do not wish to import. Select whether the recipe(s) should be imported as ***global***, otherwise they will be imported as ***personal*** recipes.
5	Click ***OK*** to import the recipe(s).

6.7 Predefined BufferPro recipes

Introduction

This section describes in detail the predefined buffer systems in ***BufferPro*** that are delivered with UNICORN.

General points

The following points should be taken into consideration:

- The pKa of certain buffer substances can vary significantly with temperature. This means that the working pH range for optimal buffering will vary with temperature. It is possible to estimate the appropriate pH ranges using the ***Explore Proportions*** tool, see *Section 6.5 Calculate buffer composition using BufferPro, on page 219*.
- The two broad-range buffer systems, ***AIEX-mix 0-1M NaCl*** and ***CIEX-mix 0-1M NaCl*** may only be used at fixed concentration, since these are multi-component buffers.
- The working concentration for buffers that are mixed using conjugate acid-base pairs is 25-50% of the stock solution concentration. For buffers mixed using strong acid or base solutions the working concentration is 15-25% of the stock solution concentration. Although it may be possible to mix solutions outside this range, UNICORN will show a warning since the pH of the resulting buffer may not be reliable. If in doubt, check the pH of the eluent after running an experiment using a reliable lab pH meter.
- The pH range given is based on the narrowest range for effective buffering for the entire gradient (0-1M NaCl). The ionic strength of the mixed solution affects the apparent pKa of the buffering agent. For pH outside the recommended range the buffering capacity is unreliable and should be avoided. UNICORN will display a warning in case either the required concentrations or pH will not provide adequate buffering. If in doubt, check the pH of the eluent after running an experiment using a reliable lab pH meter.
- Certain buffer substances are not recommended for anion exchange and others not for cation exchange. For example, phosphate buffers are not suitable for anion exchange. Buffer suitability is noted in the predefined recipes in UNICORN.

pH and concentration ranges for predefined recipes

The following table gives the optimal pH and concentration ranges for buffer recipes that are predefined in ***BufferPro*** at 25 °C.

Buffer system	pH range at 25 °C	Concentration range (M)	Comment
AIEX-mix 0-1M NaCl	pH 5.8-8.9	Fixed at 25% of the concentration of the stock solution.	Broad range buffer system for Anion exchange chromatography.
CIEX-mix 0-1M NaCl	pH 2.0-7.0	Fixed at 25% of the concentration of the stock solution.	Broad range buffer system for Cation exchange chromatography.
Acetate 0-1M NaCl	pH 3.8-5.4	0.05 - 0.1	Titrated with conjugate acid
Acetate with HCl 0-1M NaCl	pH 4.0-5.1	0.03 - 0.05	Titrated with strong acid
Bicine 0-1M NaCl	pH 7.7-8.7	0.03 - 0.05	Titrated with strong base
Bis-Tris 0-1M NaCl	pH 6.0-7.1	0.03 - 0.05	Titrated with strong acid
Bis-Tris Propane 0-1M NaCl	pH 6.6-7.7	0.03 - 0.05	Titrated with strong acid
Carbonate 0-1M NaCl	pH 9.2-10.5	0.05 - 0.1	Titrated with conjugate acid
Carbonate with HCl 0-1M NaCl	pH 9.5-10.2	0.03 - 0.05	Titrated with strong acid
Citrate 0-1M NaCl	pH 2.3-6.0	0.05 - 0.1	Titrated with conjugate acid
Citrate with HCl 0-1M NaCl	pH 3.7-5.7	0.03 - 0.05	Titrated with strong acid
Diethanolamine 0-1M NaCl	pH 8.6-9.7	0.03 - 0.05	Titrated with strong acid
Ethanolamine 0-1M NaCl	pH 9.2-10.2	0.03 - 0.05	Titrated with strong acid

Buffer system	pH range at 25 °C	Concentration range (M)	Comment
Formate 0-1M NaCl	pH 2.6-4.4	0.05 - 0.1	Titrated with conjugate acid
Formate with HCl 0-1M NaCl	pH 1.8-4.1	0.03 - 0.05	Titrated with strong acid
HEPES 0-1M NaCl	pH 6.9-8.1	0.03 - 0.05	Titrated with strong base
MES 0-1M NaCl	pH 5.6-7.0	0.03 - 0.05	Titrated with strong base
Methylpiperazine 0-1M NaCl	pH 4.6-5.3	0.03 - 0.05	Titrated with strong acid
MOPS 0-1M NaCl	pH 6.5-7.6	0.03 - 0.05	Titrated with strong base
Phosphate 0-1M NaCl	pH 5.9-7.2	0.05 - 0.1	Titrated with conjugate acid
Phosphate with HCl 0-1M NaCl	pH 6.2-6.9	0.03 - 0.05	Titrated with strong acid
Piperazine 0-1M NaCl, low pH	pH 5.5-6.4	0.03 - 0.05	Titrated with strong acid
Piperazine 0-1M NaCl, high pH	pH 9.3-10.5	0.03 - 0.05	Titrated with strong base
Succinic Acid 0-1M NaCl	pH 3.4-5.6	0.03 - 0.05	Titrated with strong base
Triethanolamine 0-1M NaCl	pH 7.4-8.4	0.03 - 0.05	Titrated with strong acid
Tris 0-1M NaCl	pH 7.6-8.7	0.03 - 0.05	Titrated with strong acid

7 Method queues

Introduction

This chapter describes how to create and edit method queues in UNICORN. For information on how to create and edit individual methods, see *Chapter 3 Create and edit methods, on page 25*.

In this chapter

This chapter contains the following sections:

Section	See page
7.1 Method queues - overview	230
7.2 Create a method queue	231
7.3 Edit a method queue	235

7.1 Method queues - overview

Introduction

A method queue in UNICORN is a linked set of methods to be run. The method queue can contain methods to be run on up to three different systems. Each system may have up to ten methods queued.

For example, a method queue might be useful on a single system when a wash procedure is programmed in a separate method. This method can then be linked to a series of different process methods ensuring the same wash procedure is used in each process. On multiple systems, the product of a separation on the first system might be the starting material for a separation on the next, allowing fully automatic multi-step processing.

Note: When a method queue is started, an option is available to run the start protocol for the method queue only once. Notification limit warnings related to the number of times a column has been used, for example since the last CIP was performed, are only issued when the start protocol is performed. See *Set notification limits for an individual column, on page 269*. In a method queue this may therefore not always be shown exactly when the notification limit is reached. Each run will however be noted in the column history, which should be checked before critical runs in a method queue. See *View individual column history, on page 274*.

Main steps when creating a method queue

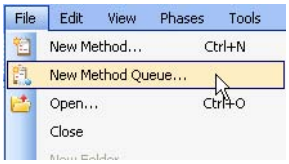
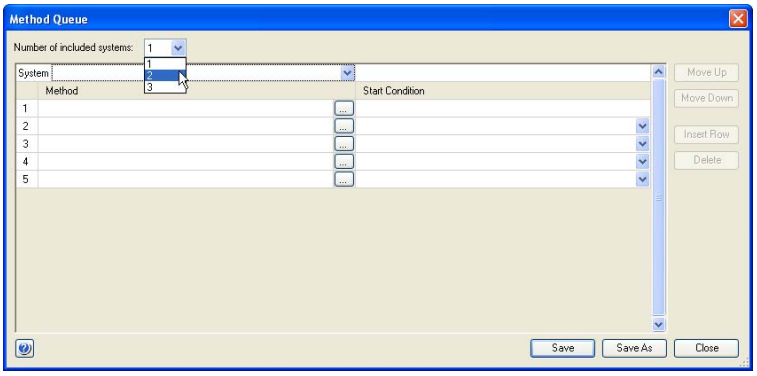
The main steps when creating a method queue are:

Step	Action
1	Create methods for the required system(s). See *Chapter 3 Create and edit methods, on page 25*.
2	Create/open a method queue • Create a new method queue *or* • Open an existing method queue that can be edited and saved with a new name
3	Save the method queue

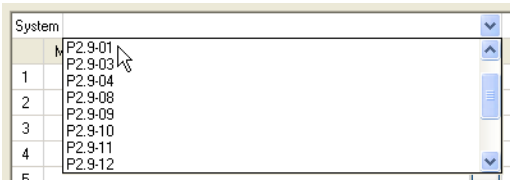
7.2 Create a method queue

Creating a method queue

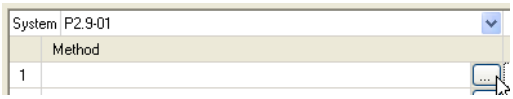
The following table describes how to create a method queue.

Step	Action
1	In the ***Method Editor***: • click the ***New Method Queue*** icon in the ***Toolbar***  *or* • Select ***File:New Method Queue...***  *Result*: The ***Method Queue*** dialog opens.
2	In the ***Method Queue*** dialog, choose the ***Number of included systems*** from the drop down list.  *Result*: A separate method queue block will be added to the dialog for each additional system if required.

Step	Action
3	Choose a system for each method queue block from the ***System*** drop down list.



4	Choose a ***Method*** to add to a method queue by pressing the browse button.



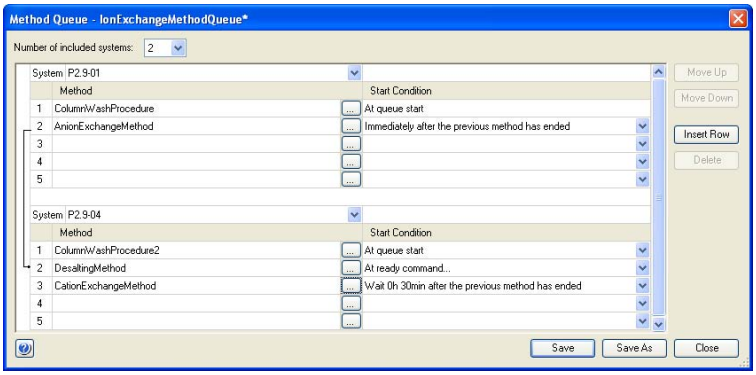
Result: The ***Select Method*** dialog opens.

5	In the ***Select Method*** dialog, browse to the required method and click ***OK***.

Result: The method is added to the method queue.

Note: For reasons of system compatability, the individual methods should be saved for the system on which they are queued.

Step	Action
6	Select a ***Start Condition*** for the method from the drop-down list. **Note:** The first ***Method*** for the first ***System*** will always have its ***Start Condition*** set to ***At queue start***. Available ***Start Conditions*** are: • ***At queue start*** The method will begin at the start of the method queue. Only available for the first method for each system. • ***Immediately after the previous method has ended*** The method will start when the previous has ended on the queue for that system. • ***Wait...*** The method will start after a specified ***Wait*** time has elapsed since the previous method in the queue for the system has ended. A separate dialog will open where the ***Wait*** time can be specified in ***Hours*** and ***Minutes***. The delay time will be shown in the ***Method Queue*** dialog once entered. • ***At ready command...*** The method will start when a ***Ready*** instruction in a method on another system has been executed. Using this start condition it is possible to connect methods running on different systems. A separate dialog will open where the ***System*** and ***Method*** can be chosen. An arrow to the left of the method queues will show the connected methods, as shown in the diagram.
7	Repeat steps 4 to 6 to add further methods to the ***Method*** list for each required system.



Step	Action
8	Click ***Save*** or ***Save As*** to save the completed method queue. **Note:** An error dialog will be displayed if any of the methods could be incompatible with the system on which they are queued.

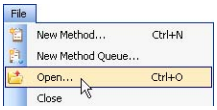
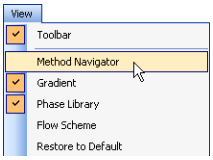
7.3 Edit a method queue

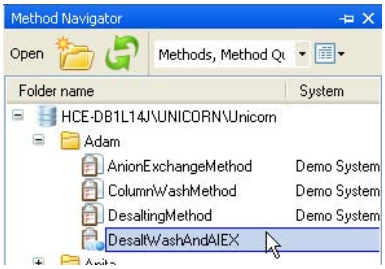
Introduction

This section describes how to open, delete and edit existing method queues. Methods can be inserted and deleted from a method queue, and their order in the queue can be changed.

Opening a method queue

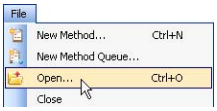
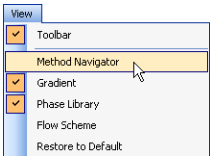
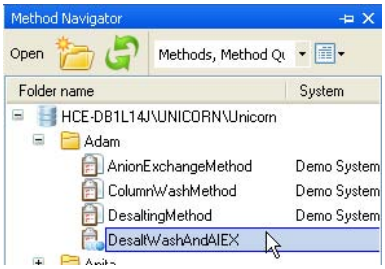
The table below describes how to open an existing method queue in the database:

Step	Action
1	In the ***Method Editor***: • Click the ***Open Method Navigator*** 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 queue to be opened in the ***Folder name*** column. 
3	To open the method queue, • Click the ***Open*** button located in the toolbar of the ***Method Navigator*** pane  *or* • double-click the selected method queue *Result:* The ***Method Queue*** dialog is opened with the details for the opened method queue. **Note:** If a method contained in the method queue has been altered since the last time it was saved, an information dialog will be displayed.

Delete a method queue

The table below describes how to delete a method queue from the database:

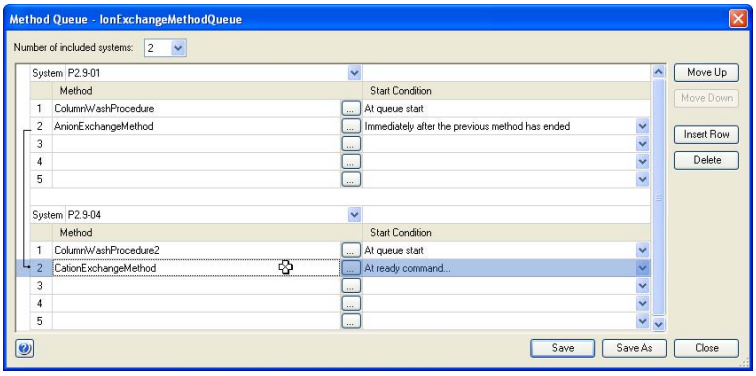
Step	Action
1	In the ***Method Editor***: • Click the ***Open Method Navigator*** icon in the ***Toolbar***  *or* • select ***File:Open...***  *or* • select ***View:Method Navigator***  *Result:* The ***Method Navigator*** is displayed.
2	Select the method queue to be deleted in the ***Folder name*** column. 

Step	Action
3	To delete the method queue, • select ***Edit:Delete*** *or* • press the **Delete** key *or* • right-click the selected method queue and select ***Delete*** from the context menu. *Result:* A dialog will appear asking to confirm the delete operation.

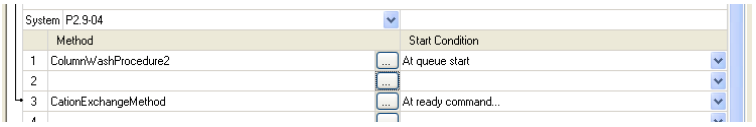
Insert a method into a method queue

The following table describes how to insert a method into the ***Method*** list for a system.

Step	Action
1	Open the method queue, see *Opening a method queue, on page 235*. *Result*: The ***Method Queue*** dialog opens with the details for the chosen method queue.
2	In the ***Method Queue*** dialog, select the position in the list at which a method will be inserted by clicking on the ***Method*** column.



Step	Action
3	Insert a new row by clicking on the ***Insert Row*** button. *Result*: An empty row will be inserted.

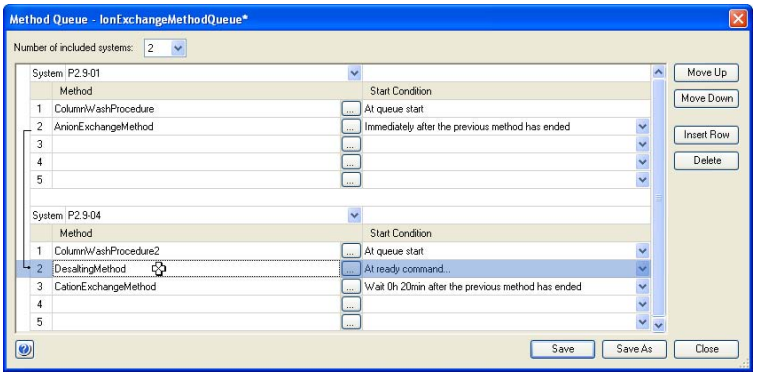


4	Add a ***Method*** and ***Start Condition*** to the ***Method*** list. See *Section 7.2 Create a method queue, on page 231*.
5	***Save*** the method queue.

Delete a method from a method queue

The following table describes how to delete a method from the method queue for a system.

Step	Action
1	Open the Method Queue, see *Opening a method queue, on page 235*. *Result*: The ***Method Queue*** dialog opens with the details for the chosen method queue.
2	In the ***Method Queue*** dialog, select the method to be removed by clicking on its name in the ***Method*** list.

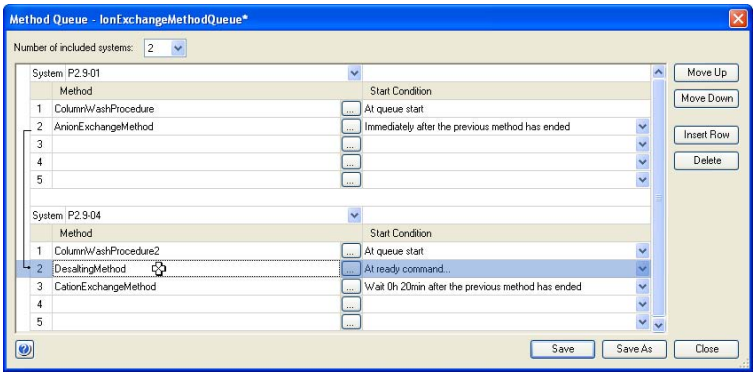


Step	Action
3	Delete the selected row by clicking on the ***Delete*** button. *Result*: The method will be deleted from the method queue.
4	***Save*** the method queue.

Change order of methods in a method queue

The following table describes how to change the order of methods in an existing method queue.

Step	Action
1	Open the method queue, see *Opening a method queue, on page 235*. *Result*: The ***Method Queue*** dialog opens with the details for the chosen method queue.
2	In the ***Method Queue*** dialog, select a method to be moved by clicking on its name in the ***Method*** list.



3	To move the selected method up in the ***Method*** list, click the ***Move Up*** button. *or* To move the selected method down in the ***Method*** list, click the ***Move Down*** button.
4	To change the order of further methods, repeat steps 2 and 3.
5	***Save*** the method queue.

8 Column Handling

Introduction

The ***Column Handling*** tool in UNICORN enables handling of column types and, if selected during installation, handling of individual columns using the ***Column Logbook***. The ***Column Handling*** tool can be opened from all available modules in UNICORN.

This chapter gives an overview of the ***Column Handling*** and ***Column Logbook*** tools.

In this chapter

This chapter contains the following sections:

Section	See page
8.1 Overview	242
8.2 Handling column types	247
8.3 Handling individual columns	260
8.4 Column performance	276
8.5 Intelligent Packing of AxiChrom™ columns	279

8.1 Overview

Introduction

This section gives an overview of the ***Column Handling*** tool and suggests a workflow when working with column types and individual columns.

Definitions

Term	Description
Column type	A type of column consisting of a particular hardware and media
Column *or* Individual column	An individual column of a column type

Example

A laboratory has two Mono Q™ HR 16/10 columns used in different projects. Both columns are of column type Mono Q HR 16/10. However each individual column may be treated and logged separately using ***Column Logbook*** in UNICORN, assuming this option has been enabled during installation.

Note: When creating methods and performing method runs, the bed height for the *column type* will be used. If you wish to use the actual bed height of a custom packed column (e.g. an AxiChrom column), you must create a specific column type that is equal to the individual column, using the packed bed height.

Open the Column Handling dialog

To open the ***Column Handling*** dialog:

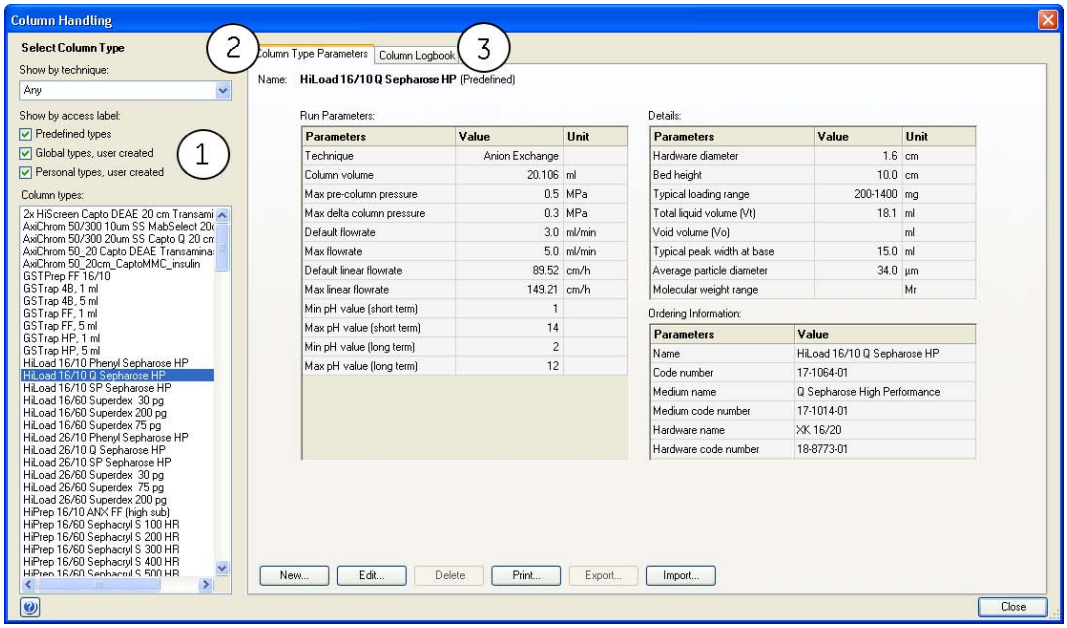
- select ***Tools:Column Handling...*** in any of the UNICORN modules

  or

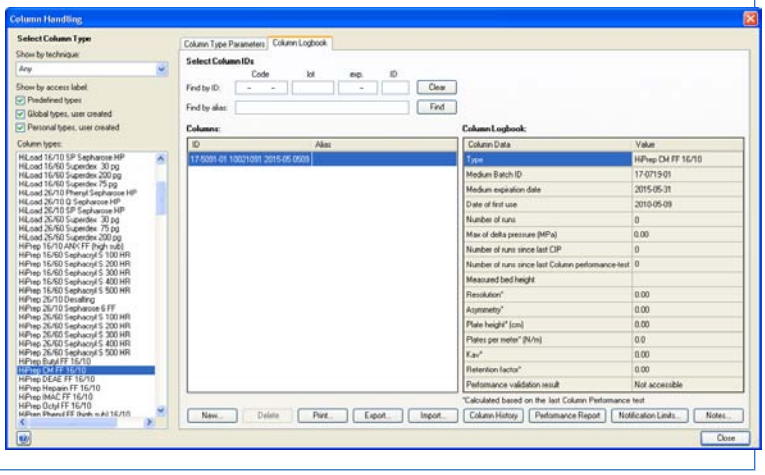
- click the ***Column Handling*** icon in the ***Toolbar*** where available

Illustration of the Column Handling dialog

The illustration below shows the ***Column Handling*** dialog displaying the ***Column Type Parameters*** tab.



Part	Function
1	***Select Column Type*** area: Shows the available column types in the ***Column Handling*** dialog. The list can be filtered to display column types for a specific technique and/or access label.
2	***Column Type Parameters*** tab: Shows the parameters for the selected column type in the ***Column types*** list. See *Section 8.2 Handling column types, on page 247* for more information.

Part	Function
3	***Column Logbook*** tab: Shows available individual columns for the selected column type in the ***Column types*** list. The parameters for the selected column in the ***Columns*** list are shown in the ***Column Logbook*** area to the right. See *Section 8.3 Handling individual columns, on page 260* for more information. 

Main Column Handling tasks

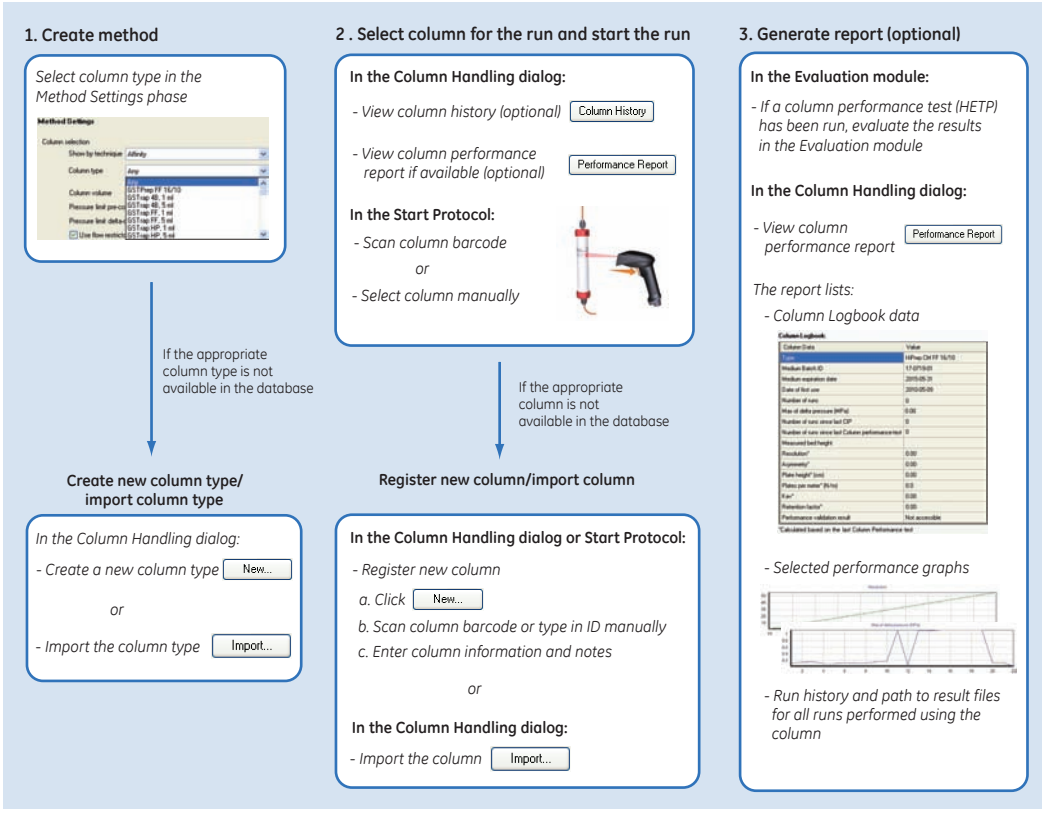
There are many possible workflows when working with column types and individual columns in UNICORN. The table below lists the main tasks that are performed in the ***Column Handling*** tool or the ***Select columns dialog*** in the ***Start Protocol*** (when starting the run in ***System Control***).

When working with...	the main tasks are...
Column types	• *Create new column types* • *Import/export column types* - Used to transfer column type data between different databases • *Edit column types* - Edit parameters and delete column types • *Print information about column types*

When working with...	the main tasks are...
Individual columns	• *Register new columns* - scan or manually type in the barcode - add notes (optional) **Tip:** New columns can be registered using the ***Column Handling*** dialog or before the run is started. • *Select columns to be used in the run* - view column history (optional) - view performance report (optional) - scan column barcode **Tip:** Individual columns to be used for a specific run can only be selected via the ***Start Protocol***. Before selecting a previously used column, it is possible to view the run history and a performance report (if available) in the ***Column Handling*** dialog. • *Edit columns* - add/edit notes - set notification limits - delete unused columns • *Print column information* • *Generate a performance report* • *Export and import columns from UNICORN*

Illustration of Column Handling workflow

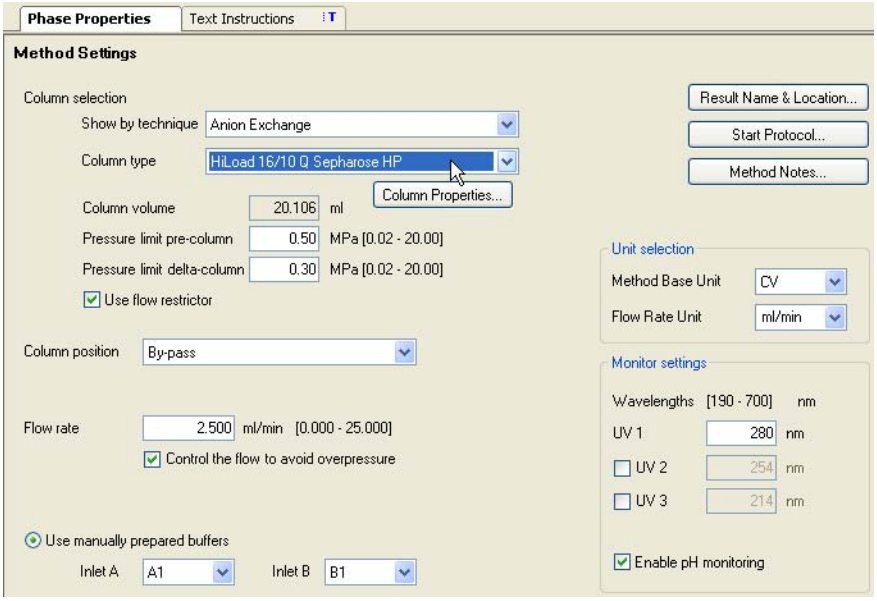
The illustration below shows a possible workflow when working with column types and individual columns:



8.2 Handling column types

Introduction

When you create a new method and select a column type in the ***Method Settings*** phase, the volume, flow rate, and pressure limits are automatically set. Most of the work regarding handling of column types is performed in the ***Method Editor***. The column type to be used in a method is selected when creating the method as shown in the illustration below.



Column types are either globally available to all users, or only personally available. A number of column types are predefined in UNICORN (see below for more information about predefined column types).

Note: When creating methods and performing method runs, the bed height for the column type will be used. The measured bed height of a custom packed individual column (e.g an AxiChrom column) may differ from the bed height of the parental column type. If you wish to use the correct bed height for method creation, you can edit the column type and enter the measured bed height of the individual column.

This section describes how to add, edit and delete column types. It also describes how to import and export column types and how to print information about selected column types.

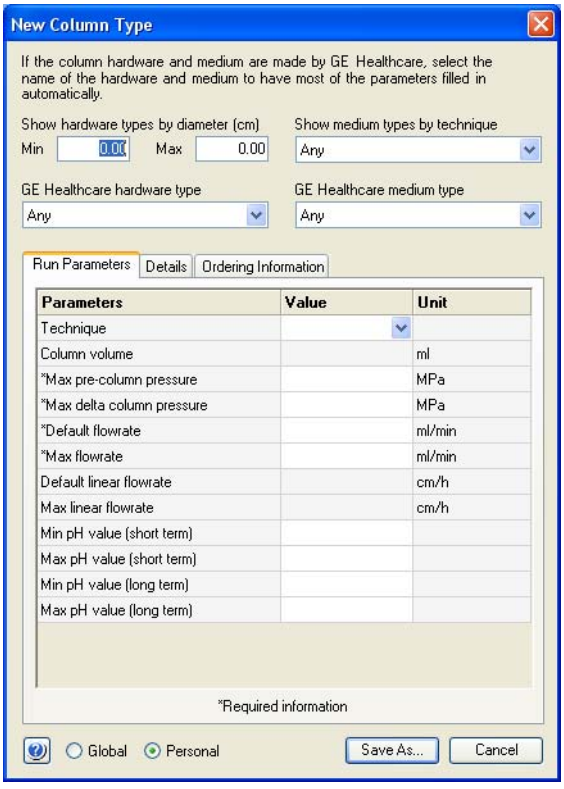
Predefined column types

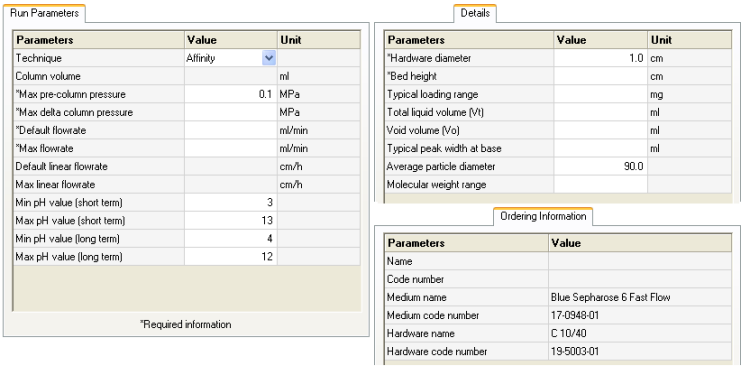
A number of GE Healthcare column types are predefined in UNICORN. For each column type, as many individual columns as needed can be registered. Parameters for the pre-defined column types can be edited by saving the column type with a new name and as a ***Personal*** or ***Global*** column type. The complete list of predefined column types can be found in the ***Column Handling*** dialog.

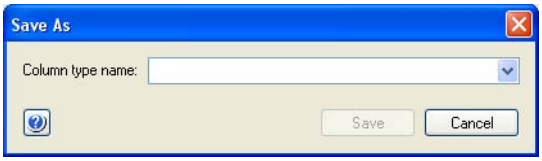
Create a new column type

The table below describes how to add a new column type with the ***Column Handling*** tool:

Step	Action
1	In the ***Column Type Parameters*** tab in the ***Column Handling*** dialog, click New... . *Result:* The ***New Column Type*** dialog opens.



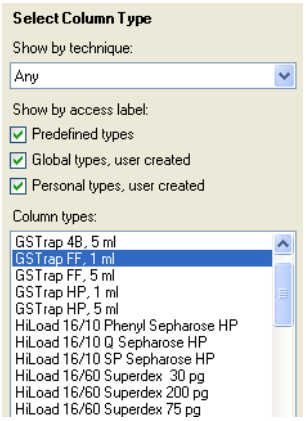
Step	Action
2	If adding a column type for which the column hardware and medium are not made by GE Healthcare, continue to step 4.
3	• Select the ***GE Healthcare hardware type*** for the new column type in the drop-down list. To filter the drop-down list to only show hardware types with certain diameters, enter the diameter range in cm in the ***Min*** and ***Max*** fields for ***Show hardware types by diameter (cm)*** above. • Select the ***GE Healthcare medium type*** for the new column type in the drop-down list. To filter the drop-down list to only show medium types for a specific separation technique, choose the appropriate technique in the ***Show medium types by technique*** drop-down list above. *Result:* The following parameters are automatically filled in (can be edited if appropriate):  Run Parameters Parameters / Value / Unit Technique: Affinity Column volume: ml *Max pre-column pressure: 0.1 MPa *Max delta column pressure: MPa *Default flowrate: ml/min *Max flowrate: ml/min Default linear flowrate: cm/h Max linear flowrate: cm/h Min pH value (short term): 3 Max pH value (short term): 13 Min pH value (long term): 4 Max pH value (long term): 12 *Required information Details Parameters / Value / Unit *Hardware diameter: 1.0 cm *Bed height: cm Typical loading range: mg Total liquid volume (Vt): ml Void volume (Vo): ml Typical peak width at base: ml Average particle diameter: 90.0 Molecular weight range Ordering Information Parameters / Value Name Code number Medium name: Blue Sepharose 6 Fast Flow Medium code number: 17-0948-01 Hardware name: C 10/40 Hardware code number: 19-5003-01
4	Enter the remaining parameter values for the new column type in the ***Run Parameters***, ***Details*** and ***Ordering Information*** tabs. Fields marked with ***** must be filled in. Values in the gray fields are calculated and automatically filled in based on entered values for the corresponding parameters.
5	Select whether the the new column type should be ***Global*** (available for all users) or ***Personal*** (only available for the current user).

Step	Action
6	Click ***Save As...*** to save the column type. *Result:* The ***Save As*** dialog opens. 
7	Type in a ***Column type name*** and click ***Save.*** *Result:* The column type is saved in the database and displayed in the ***Column types*** list.

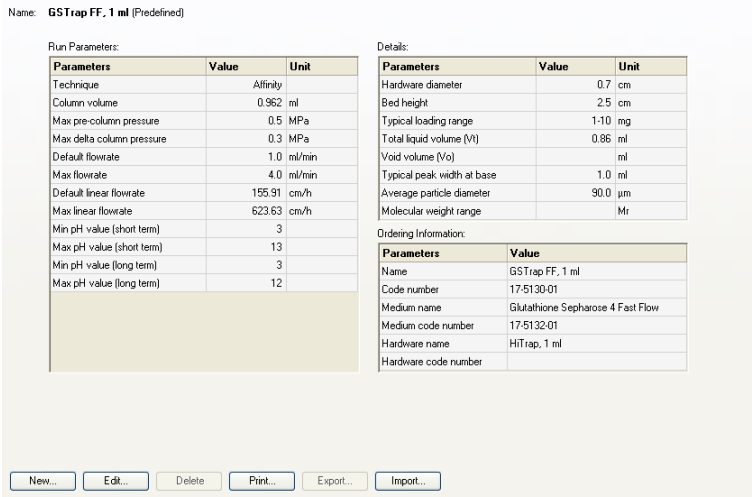
Edit parameters for a column type

The table below describes how to edit parameters for a column type:

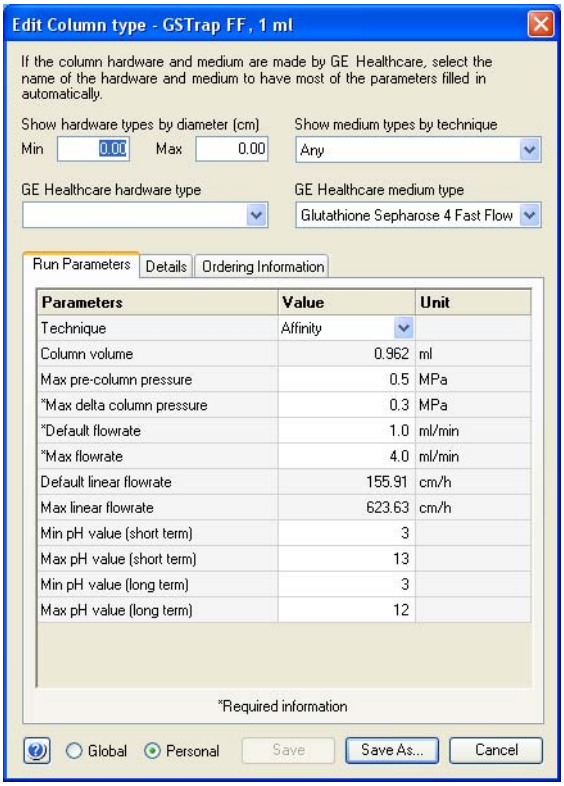
Step	Action
1	Select the appropriate column type for which to edit parameters in the ***Column types*** list.



Result: The parameters for the selected column type are displayed in the ***Column Type Parameters*** tab to the right.



Step	Action
2	In the ***Column Type Parameters*** tab, click Edit... *Result:* The ***Edit Column Type*** dialog opens.



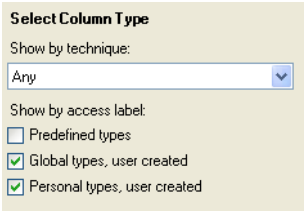
3	Edit the column type parameters as appropriate on the ***Run Parameters***, ***Details*** and ***Ordering Information*** tabs.
4	Select whether the edited column type should be ***Global*** (available for all users) or ***Personal*** (only available for the current user).

Step	Action
5	If editing parameters for a predefined column type, the column type must be saved with a new name. • Click ***Save As...*** to save the edited column type. *Result:* The ***Save As*** dialog opens.  • Edit the ***Column type name*** and click ***Save.*** *Result:* The column type is saved in the database and displayed in the ***Column types*** list.
6	If editing parameters for a ***Global*** or ***Personal*** column type, the column type can be saved with a new name (see step 5 above) or the changes can be applied to the current column type name. • Click ***Save***. *Result:* The changes for the column type are saved. **Note:** When editing parameters for ***Global*** column types, it is recommended to save the edited column type with a new name. Other users may otherwise not be aware that the parameters have been changed for that column type. **Note:** Methods that use the edited column type should be updated.

Delete column types

Note: It is not possible to delete predefined column types from the database. If a column type has any registered columns, it can not be deleted unless the individual columns are first deleted. See *Section 8.3 Handling individual columns, on page 260* for information about how to delete individual columns. If an individual column of a certain type has been used, it will not be possible to delete either the individual column or the column type.

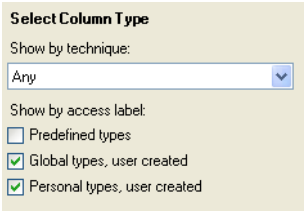
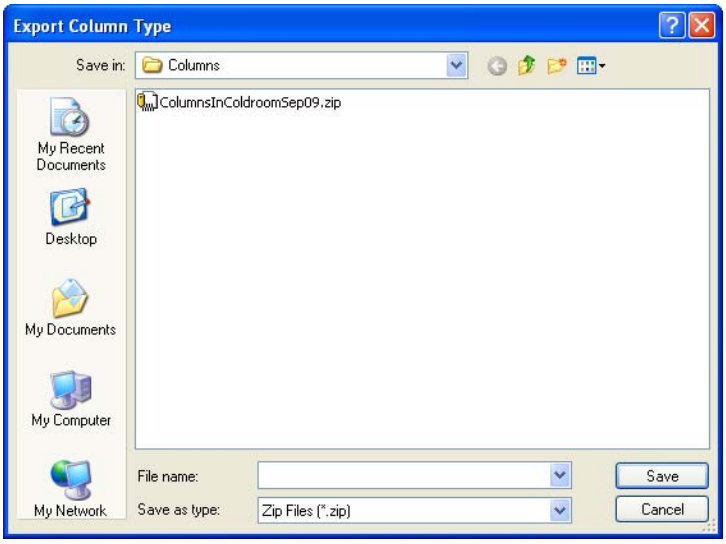
The table below describes how to delete ***Global*** and ***Personal*** column types from the database:

Step	Action
1	In the ***Select Column Type*** area, clear the ***Predefined types*** box.  *Result:* Only ***Global*** and ***Personal*** column types are displayed in the ***Column types*** list.
2	Select the column type(s) to be deleted in the ***Column types*** list. To select several column types use the **Ctrl** or **Shift** keyboard keys.
3	In the ***Column Type Parameters*** tab, click Delete. *Result:* The ***Confirm Column Type Delete*** dialog opens. 
4	Click ***Yes*** to delete the column type. *Result:* The column type is permanently deleted from the database.

Export column types

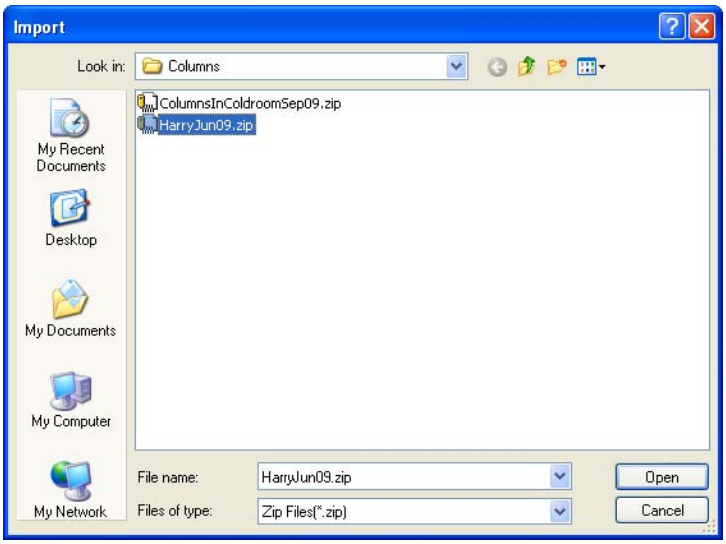
Note: It is not possible to export predefined column types from the database.

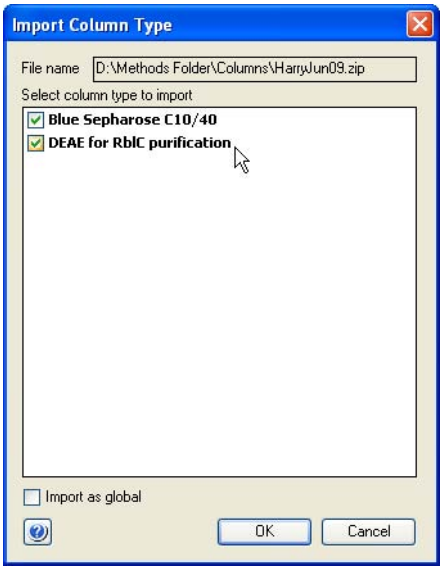
The table below describes how to export ***Global*** and ***Personal*** column types from the database:

Step	Action
1	In the ***Select Column Type*** area, clear the ***Predefined types*** box.  *Result:* Only ***Global*** and ***Personal*** column types are displayed in the ***Column types*** list.
2	Select the column type(s) to be exported in the ***Column types*** list. To select several column types use the **Ctrl** or **Shift** keyboard keys.
3	In the ***Column Type Parameters*** tab, click Export.... *Result:* The ***Export Column Type*** dialog opens. 
4	Select in which folder to save the information and type a name for the zip file to be exported. *Result:* The column type information is exported. This information can be imported into another database.

Import column types

The table below describes how to import column types into the database:

Step	Action
1	In the ***Column Type Parameters*** tab in the ***Column Handling*** dialog, click Import... . *Result:* The ***Import*** dialog opens. 

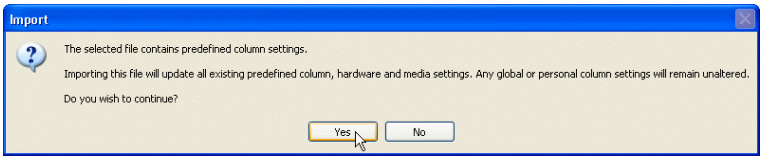
Step	Action
2	Locate the zip file with the column type information to be imported and click ***Open***. *Result:* The ***Import Column Type*** dialog opens displaying the names of the column types included in the zip file. 
3	Make sure that the check boxes in front of the column types to be imported are checked. If a column type should not be imported clear the corresponding check box.
4	Check the ***Import as Global*** box if the column types should be global (i.e., available for all users) when imported. Otherwise, the column types will be imported as personal column types.
5	Click ***OK***. *Result:* The column types are imported into the database. **Note:** If a column type to be imported has the same name as an existing column type in the database, you will be prompted to type a new name for that column type. Type in a name and click ***OK***.

Import new column list

Updated lists of predefined column types may be 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, both personal and global, will remain in the database.

Tip: A column list is available in the ***Misc*** folder of the UNICORN 6.1 installation DVD.

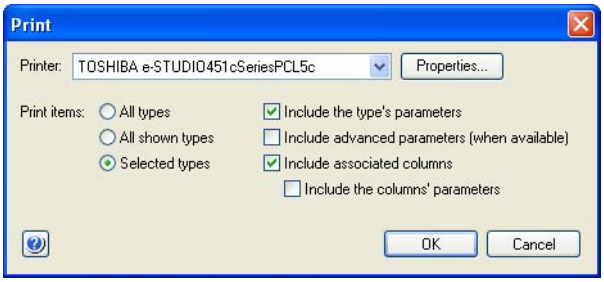
The table below describes how to import a new column list into the database:

Step	Action
1	In the ***Column Type Parameters*** tab in the ***Column Handling*** dialog, click Import... . *Result:* The ***Import*** dialog opens.
2	Locate the zip file with the column list to be imported and click ***Open***. *Result:* The ***Import*** confirmation dialog opens, explaining what will will happen when the zip file is imported. 
3	Click ***Yes***. *Result:* The new list of predefined column types is imported into the database.

Print information about column types

The table below describes how to print information about column types:

Step	Action
1	Select the column type(s) for which to print information in the ***Column types*** list. To select several column types use the **Ctrl** or **Shift** keyboard keys.

Step	Action
2	In the ***Column Type Parameters*** tab, click ***Print...*** *Result:* The ***Print*** dialog opens. 
3	Select ***Printer***.
4	Select for which column types to print information: • ***All types***: Prints information for all column types in the database • ***All shown types***: Prints information for all column types displayed in the ***Column types*** list • ***Selected types***: Prints information for the column type(s) selected in the ***Column types*** list
5	Select which type of information to include when printing the information: • Check the ***Include the type's parameters*** box to include the information from the ***Run parameters***, ***Details*** and ***Ordering Information*** fields in the ***Column Type Parameters*** tab. • Check the ***Include the associated columns*** box to include the Column ID and alias of the individual columns registered for the column type. Check the ***Include column's parameters*** to include the parameters for each individual column registered for the column type(s).
6	Click ***OK***. *Result:* The selected information for the column type(s) is printed.

8.3 Handling individual columns

Introduction

Individual columns are handled on the ***Column Logbook*** tab in the ***Column Handling*** dialog. The ***Column Logbook*** enables tracing of the run history for an individual column, for example, how many CIP runs have been performed using that column. Individual columns are always connected to a particular column type.

Note: The ***Column Logbook*** tab is only displayed if this option was selected when installing UNICORN.

Working with columns is primarily done in the ***Method Editor*** and ***System Control***, depending on the task to be performed.

In this section

This section covers the following:

Section	See page
8.3.1 Individual column identification	261
8.3.2 Register a new individual column	262
8.3.3 Find an individual column	266
8.3.4 Edit individual columns	268
8.3.5 Export and import individual columns	271
8.3.6 Print and view individual column information	274

8.3.1 Individual column identification

Matrix barcode

Most pre-packed GE Healthcare columns are marked with a matrix barcode on the column label. This barcode can be scanned using the 2D barcode scanner to register new individual columns or to find columns in the database.

Columns can also be labeled with UniTag labels. A UniTag label is a unique identifier for individual columns that are not pre-labelled with a matrix barcode, such as HiTrap™ columns, manually packed columns or columns from other sources. A number of UniTag labels are supplied with the system, and they can also be purchased separately.

The diagram below shows an example of a column label and a UniTag label with their matrix barcodes.

Column label UniTag label

8.3.2 Register a new individual column

Introduction

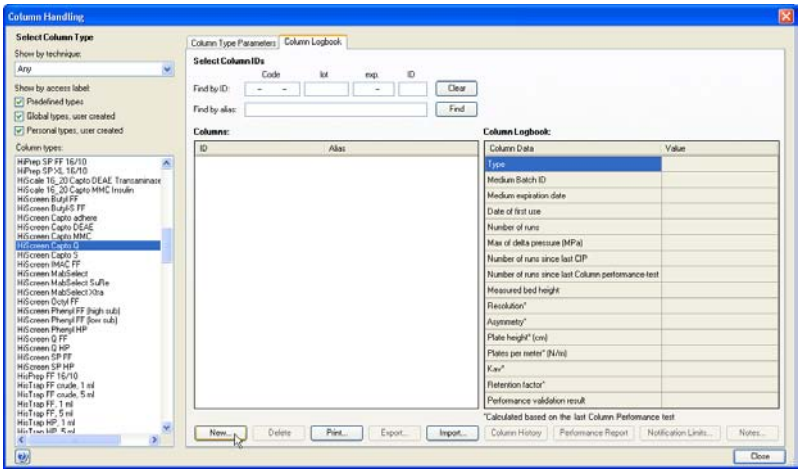
In order to take advantage of the column handling features of UNICORN, each individual column needs to be registered in the software.

Note: It is essential that the individual column is registered before a column performance test is performed. Otherwise the results will not be entered in the ***Column Logbook***. It is not possible to enter the performance test results afterwards.

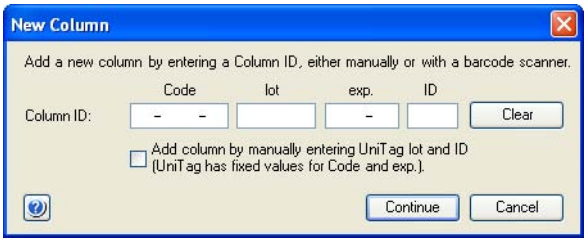
Register an individual column

The following table describes how to register an individual column in the ***Column Logbook***.

Step	Action
1	Select the ***Column Logbook*** tab and then click ***New.***

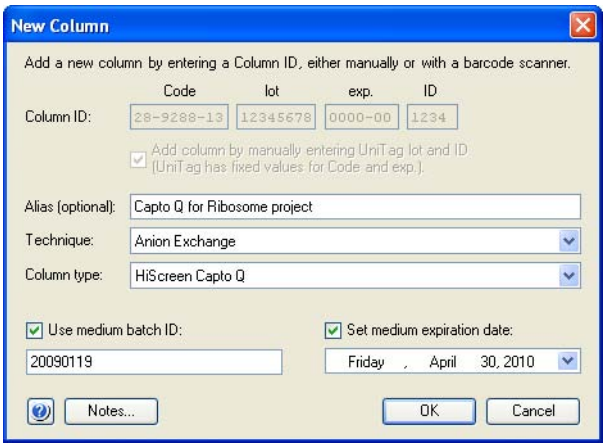


Result: The first ***New Column*** dialog opens.



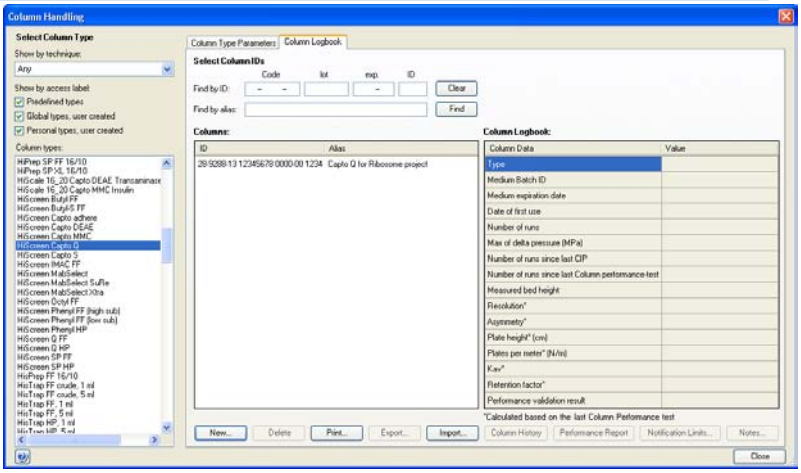
Step	Action
2	Register the column using the 2D barcode scanner as follows: • Make sure that the mouse pointer is placed in the first position of the ***Code*** field. • Point the 2D barcode scanner towards the data matrix tag on the column label or the UniTag label. • Press and hold the trigger.  • When the 2D barcode scanner beeps, the column ID is registered and the second ***New Column*** dialog opens.
3	If no 2D barcode scanner is available, enter the column ID manually: • If the column has a column label, enter the column ID shown in the ***Code*** field. • If the column has a UniTag label, check the box ***Add column by manually entering UniTag lot and ID*** and manually enter the number for the ***lot*** and ***ID*** fields. • Click ***Continue*** to open the second ***New Column*** dialog. **Note:** The ***lot*** field should contain eight digits, and the ***ID*** field should contain four digits. If the lot or ID numbers of the column contains fewer than eight or four digits respectively, insert leading zeros before the number. **Note:** If the column has no GE Healthcare label and you have run out of UniTag labels, check the box ***Add column by manually entering UniTag lot and ID***, then enter an arbitrary lot and ID. This procedure is possible, but not recommended.

Step	Action
4	In the second ***New Column*** dialog:



- Enter an ***Alias*** (optional).
  Tip: Alias can be used for easy identification of an individual column.
- Select ***Technique*** and ***Column type***.
  Note: For prepacked GE Healthcare columns with a matrix barcode, these are filled in automatically.
- Check the ***Use medium batch ID*** and type in the batch number of the medium.
- Check the ***Set medium expiration date*** and select expiration date for the medium to get a notification in UNICORN when this date is reached.
  Note: The expiration date cannot be set or changed after a column has been registered.
- Enter notes for the column by clicking the ***Notes...*** button and enter notes in the ***Notes*** dialog that opens.
- Click ***OK.***

Result: The entered information is saved and the registered column is displayed in the ***Column Handling*** dialog.

Step	Action
	

Column Handling
Select Column Type
Show by technique:
Any
Show by access label:
Predefined types
Global types, user created
Personal types, user created
Column types:
Column Type Parameters
Column Logbook
Select Column IDs
Code
lot
exp.
ID
Find by ID:
Clear
Find by alias:
Find
Columns:
ID
Alias
Column Logbook:
Column Data
Value
Type
Medium Batch ID
Medium expiration date
Date of first use
Number of runs
Max of delta pressure (MPa)
Number of runs since last CIP
Number of runs since last Column performance test
Measured bed height
Resolution*
Asymmetry*
Plate height* (cm)
Plates per meter* (N/m)
Kav*
Retention factor*
Performance validation result
*Calculated based on the last Column Performance test
New...
Delete
Print...
Export...
Import...
Column History
Performance Report
Notification Limits...
Notes...
Close

8.3.3 Find an individual column

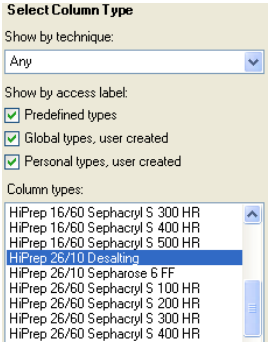
Introduction

Many features of the ***Column Handling*** tool require an individual column to be selected. This section describes how to find a column.

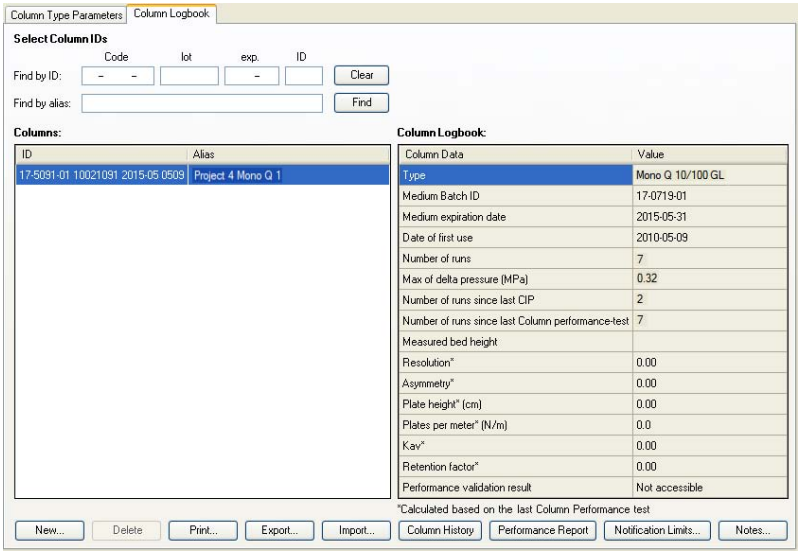
Find and select an individual column

The table below describes how to find/select a registered column in the ***Column Logbook***:

Step	Action
1	Select the ***Column Logbook*** tab.
2	Filter the list of column types by choosing the required technique in the pull-down menu ***Show by technique***, then select the column type to which the individual column belongs.



Step	Action
3	To select several column types, use the **Ctrl** and **Shift** keyboard keys. *Result:* The individual columns registered for the selected column type(s) are displayed in the ***Columns*** list. The ***Column Logbook*** area to the right shows parameters and information for the selected column.



Tip: To show all registered individual columns, select all the available column types by checking the boxes for ***Predefined***, ***Global*** and ***Personal*** types, then select all the ***Column Types*** in the list.

4

- If you have a short list of individual columns registered for the column type, just select the appropriate column in the ***Columns*** list. To select several columns, use the **Ctrl** and **Shift** keyboard keys.
- If you have many individual columns registered, find and select the appropriate column as described below:
  - position the cursor in the first position of the ***Code*** field, scan the column barcode or UniTag and click ***Find***

    or
  - type in the barcode in the ***Find by ID*** field and click ***Find***

    or
  - type in the alias in the ***Find by alias*** field and click ***Find***

    Result: The individual column is selected in the ***Columns*** list.

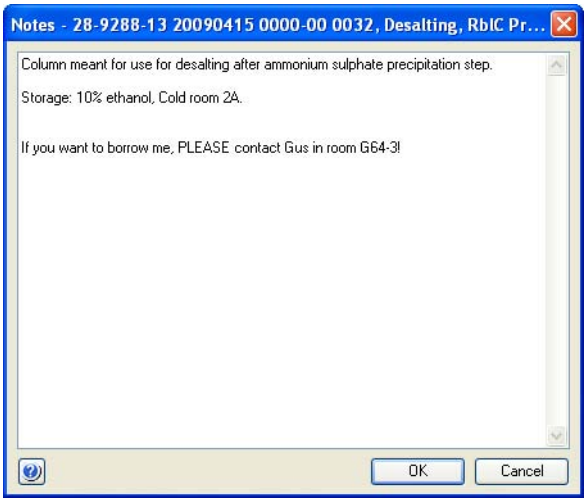
8.3.4 Edit individual columns

Introduction

This section describes the ways in which individual columns may be edited. This includes adding and editing notes, setting notification limits and deleting individual columns.

Add/edit notes for a column

The table below describes how to add/edit notes for an individual column:

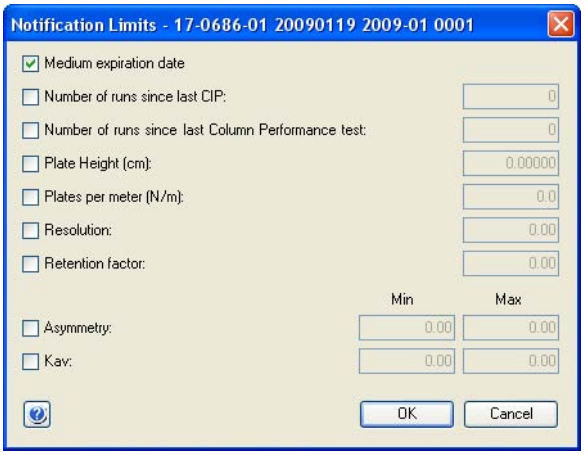
Step	Action
1	Select the individual column for which to add/edit notes in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column.
2	In the ***Column Logbook*** tab, click Notes... . *Result:* The ***Notes*** dialog for the selected column opens. 
3	Add/edit notes by typing in the dialog and click ***OK***. *Result:* The notes for the column are updated.

Set notification limits for an individual column

Notification limits can be set for individual columns. Once the limit is reached, the user receives a message stating what action should be taken before the column is used. Examples of such limits are the ***Medium expiration date***, that the column has been used a given number of times since the last CIP or column performance test were performed, or that the column performance parameters are poor so the medium should be repacked.

Note: Warnings related to the number of times a column has been used, for example since the last CIP was performed, are only issued when the start protocol is performed. In a method queue this may not always be shown for every run. Each run will however be noted in the column history.

The table below describes how to set notification limits for an individual column:

Step	Action
1	Select the individual column for which to set ***Notification Limits*** in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column.
2	In the ***Column Logbook*** tab, click Notification Limits... . *Result:* The ***Notification Limits*** dialog for the selected column opens. 
3	Check the appropriate boxes and enter notification values. When the values are reached or a value is outside the defined range, a warning will be displayed that action should be taken.
4	Click ***OK***. *Result:* The settings are saved and the dialog is closed.

Delete individual columns

The table below describes how to delete an individual column from the database:

Step	Action
1	Select the individual column to be deleted in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column. To select several columns in the ***Columns*** list, use the **Ctrl** and **Shift** keys.
2	In the ***Column Logbook*** tab, click Delete. *Result:* The ***Confirm Column Delete*** dialog opens. 
3	Click ***Yes*** in the ***Confirm Column Delete*** dialog. *Result:* The individual column is deleted. **Note:** Individual columns that have been used cannot be deleted.

8.3.5 Export and import individual columns

Introduction

The information for individual columns is stored in the UNICORN database. This information may be exported to a zip file in order to move the information to another UNICORN installation. This section describes how to export individual columns from UNICORN and how to import previously exported columns.

Export individual columns

Individual columns can be exported from the database to a zip file. The columns can then be imported to another database if appropriate.

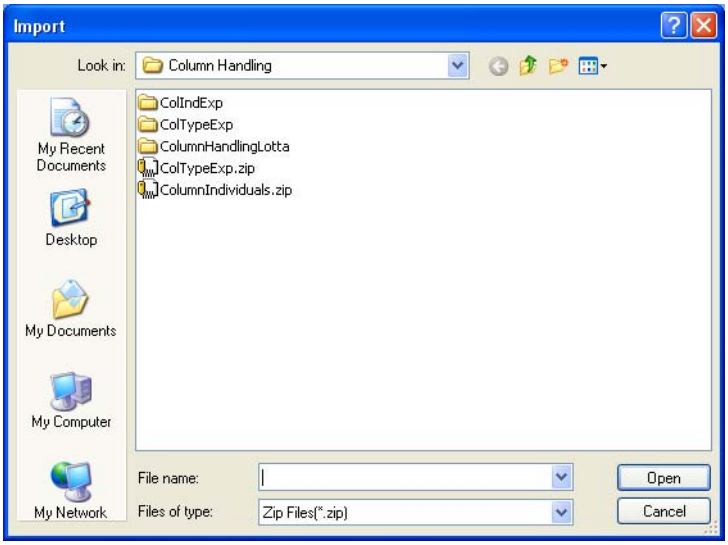
The table below describes how to export individual columns from the database:

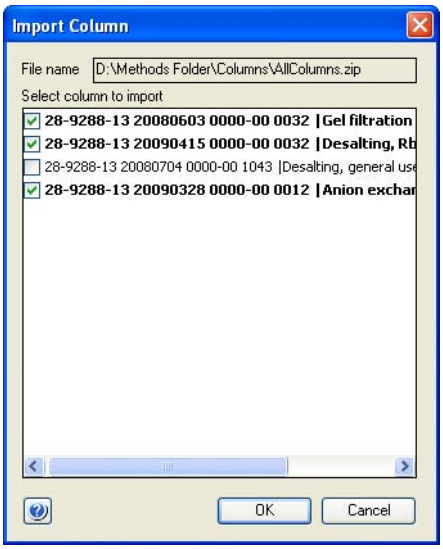
Step	Action
1	Select the individual column(s) to be exported in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column. To select several columns in the ***Columns*** list, use the **Ctrl** and **Shift** keys.
2	In the ***Column Logbook*** tab, click Export... . *Result:* The ***Export Column Type*** dialog opens.
3	Select in which folder to save the information and type a name for the zip file to be exported. *Result:* The individual column information is exported. The column information can be imported into another database.

Import individual columns

Individual columns that have been exported and saved locally can be imported into another database.

The table below describes how to import individual column information to a database:

Step	Action
1	In the ***Column Logbook*** tab, click Import... *Result:* The ***Import*** dialog opens. 

Step	Action
2	Locate the zip file with the column type information to be imported and click ***Open***. *Result:* The ***Import Column*** dialog opens displaying the barcodes and aliases of the individual columns included in the *.zip file. 
3	Make sure that the check boxes in front of the individual columns to be imported are checked. If a column should not be imported clear the corresponding check box.
4	Click ***OK***. *Result:* The columns are imported into the database. **Note:** If an individual column to be imported has the same barcode or alias name as an existing column in the database, a dialog will be displayed saying that the column already exists in the database and that it will not be imported.

8.3.6 Print and view individual column information

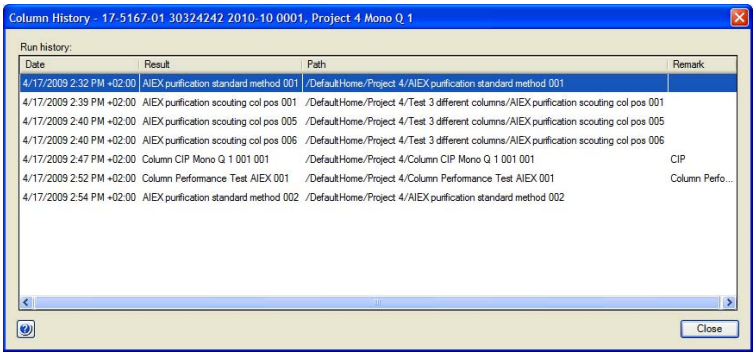
Introduction

This section describes how to view the run history for an individual column, and how to print column information.

View individual column history

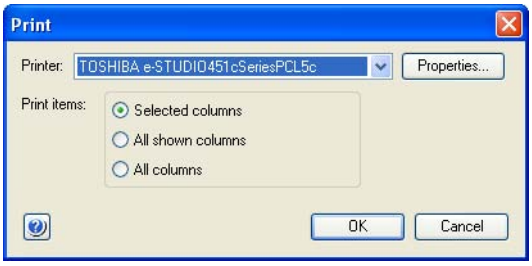
It is possible to view the run history for an individual column to see how many runs that have been performed using the column. The path to the result files for each run is also displayed. If the run was a column performance test or CIP run, this is shown as a remark.

The table below describes how to view the ***Column History*** for a column:

Step	Action
1	Select the individual column for which to view ***Column History*** in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column.
2	In the ***Column Logbook*** tab, click Column History. *Result:* The ***Column History*** dialog for the selected column opens.  The runs performed using the column are listed. The date, result name and location and any remarks for the run are displayed.
3	Click ***Close*** to close the dialog.

Print information about individual columns

The table below describes how to print information for individual columns:

Step	Action
1	Select the appropriate column(s) for which to print information in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column. To select several columns in the ***Columns*** list, use the **Ctrl** and **Shift** keys.
2	In the ***Column Logbook*** tab, click Print.... *Result:* The ***Print*** dialog opens. 
3	Select ***Printer***.
4	Select for which individual column(s) to print information: • ***Selected columns***: Prints information for the column(s) selected in the ***Columns*** list • ***All shown columns***: Prints information for all columns displayed in the ***Columns*** list • ***All columns***: Prints information for all columns in the database
5	Click ***OK***. *Result:* The Column ID, alias and parameters for the columns are printed.

8.4 Column performance

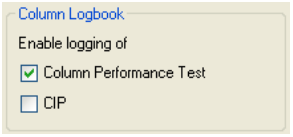
Introduction

Column performance can be tested by measuring the height equivalent to a theoretical plate (***HETP***) and asymmetry factor (***As***) values. Tests should be run directly after packing or obtaining a new column, regularly during the lifetime of the column and when separation performance is seen to deteriorate. By regularly monitoring the performance of an individual column, UNICORN can generate appropriate warnings when a cleaning procedure needs to be applied, or even when the column lifetime is approaching its end. For a description on how to set such notification limits see *Set notification limits for an individual column, on page 269*.

This section describes the workflow to run a ***Column Performance Test***, and how to generate a performance report for a specific column.

Column performance test

The following table describes the workflow for generating and analyzing a Column Performance Test result.

Step	Action
1	Create a ***Column Performance Test*** method, or a method containing a ***Column performance test*** phase. For details how to create methods see *Chapter 3 Create and edit methods, on page 25*. **Note:** The option ***Enable logging of Column Performance Test*** should be automatically selected in the Phase Properties for the Method Settings phase when this method is created. This can be deselected if logging of the performance test is not desired, but it should normally be kept selected. 

Step	Action
2	Run the method [1]. For details on running a method, see ÄKTA avant and UNICORN 6.1 User Manual. Suitable samples that can be used to monitor the column performance are for example 1% acetone (measuring the absorbance at 280 nm), or 2.0 M NaCl and eluting with 0.5 M NaCl. **Note:** A sample volume between 0.5% and 3% of the column volume and a flow rate between 15 and 30 cm/h is recommended. The calculated number of plates and the asymmetry factor will in part depend on the selected flow rate. To ensure that test results are comparable, always use the same flow rate for the tests.
3	Evaluate the Column Performance Test, see UNICORN 6.1 Evaluation Manual.

1 The individual column must be selected when the method run is started in order to register the results from the column performance test in the column logbook. The result cannot be logged for the individual column at a later time.

The individual AxiChrom column must also be selected when performing an Intelligent Packing method run, since this method includes column performance tests which should be logged.

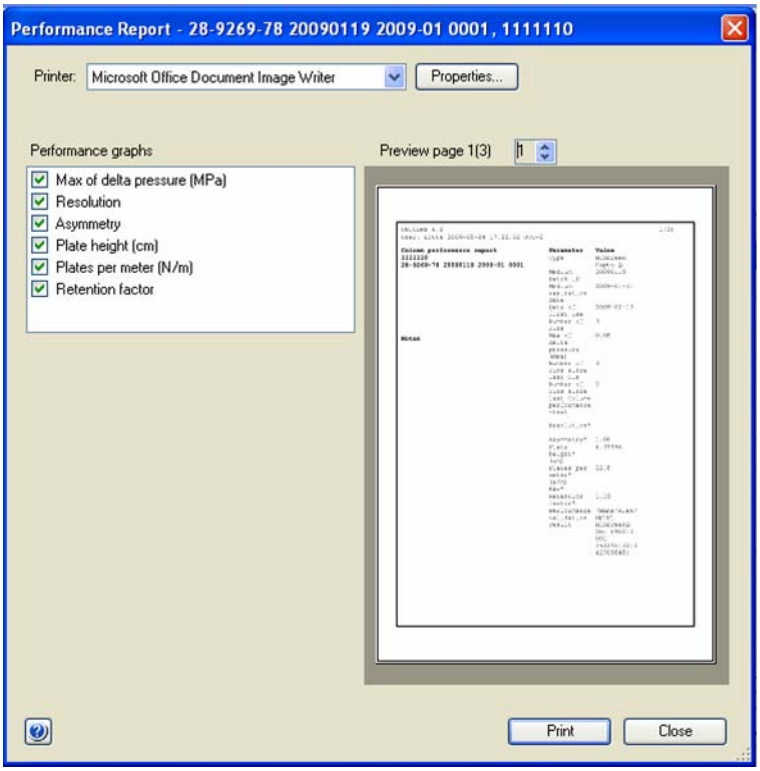
Create a performance report

A column performance report can be created before using an individual column to ensure that it is in good condition for use. The performance report contains the following information:

- Run and performance parameters
- Notes
- Performance graphs (optional)
- Run history

The table below describes how to generate a column performance report:

Step	Action
1	Select the individual column for which to generate a ***Performance Report*** in the ***Columns*** list in the ***Column Logbook*** tab. See *Section 8.3.3 Find an individual column, on page 266* for information about how to find and select a column.

Step	Action
2	In the ***Column Logbook*** tab, click Performance Report. *Result:* The ***Performance Report*** dialog for the selected column opens. 
3	Select ***Printer***.
4	Check the appropriate boxes in the ***Performance graphs*** area to include the corresponding graphs in the report. **Note:** The parameters and the corresponding values from the ***Column Logbook*** are always included on the first page in the report together with the latest performance test results. All runs are listed in the ***Run History*** at the end of the report, including ***Column Performance Test*** and ***CIP*** runs which are labelled.
5	A preview of the report is shown on the right side of the dialog. Use the buttons above the report to scroll the preview.
6	Click ***Print*** to print the information.
7	Click ***Close*** to close the dialog.

8.5 Intelligent Packing of AxiChrom™ columns

Introduction

UNICORN 6.1 features a solution for Intelligent Packing of AxiChrom columns. The AxiChrom column family feature hands-free packing using internal hydraulic axial compression. Used with ÄKTA avant 150 systems, Intelligent Packing of AxiChrom columns can be performed using either a predefined Intelligent Packing method, or by creating a user defined method including an Intelligent Packing phase.

The UNICORN 6.1 Method Manual provides an overview how to apply the Intelligent Packing method and how to create the individual AxiChrom column in UNICORN ***Column Handling***. More information about the use of the AxiChrom column is available in the operating instructions for the columns.

AxiChrom column types and individual AxiChrom columns

In order to obtain correct default parameter values for the Intelligent Packing method phase, a specific AxiChrom column type must be created, based on the AxiChrom hardware and the selected media. This is described below in *Create an AxiChrom column type*. The default values for the created AxiChrom column type will be applied (e.g. for the bed height) when this column type is used in a method.

When the column is to be packed, an individual AxiChrom column must be created. This is described below in *Create an individual AxiChrom column*. Registering this individual column for the column packing run will enable the results from the column performance tests to be registered in the column logbook for the column. Two performance tests are part of the Intelligent Packing method, one downflow and one upflow. When the evaluation of the tests are performed, the actual packed bed height should be used. This bed height will be registered in the column logbook. The evaluation procedure is described in the UNICORN 6.1 Evaluation Manual.

To ensure that the actual packed bed height is applied when creating or running methods using the AxiChrom column type, you must update the column type definition and replace the default value with the actual, registered value of the individual column. Until this is done, the default bed height will be applied.

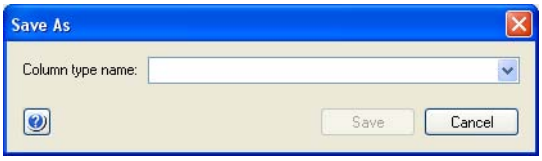
Tip: If it is essential that the exact packed bed height is used, you should create a column type for each individual AxiChrom column and update the bed height value after each time the column is repacked.

Create an AxiChrom column type

The table below describes how to create the AxiChrom column type:

Note: To ensure that the actual packed bed height is applied, you must

Step	Action
1	Choose the ***Tools:Column Handling*** menu command. *Result*: The ***Column Handling*** dialog opens.
2	In the ***Column Type Parameters*** tab in the ***Column Handling*** dialog, click New... . *Result*: The ***New Column Type*** dialog opens.

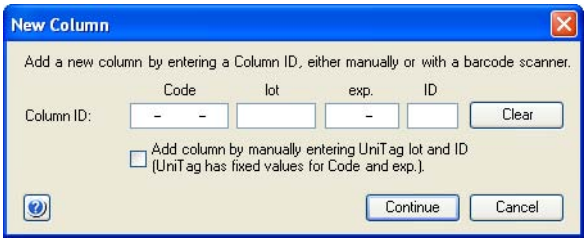
Step	Action
3	• Select the appropriate AxiChrom column hardware in the ***GE Healthcare hardware type*** drop-down list. • Select the ***GE Healthcare medium type*** for the new AxiChrom column type in the drop-down list. **Tip:** Only some of the available media are approved by GE Healthcare for use in the Intelligent Packing of AxiChrom columns. Click the ***GE approved media*** button in the Intelligent Packing phase to view a list of the approved media and bed heights. Other media can also be selected, but the packing procedure will then be performed with custom packing settings with a set of general default settings. *Result:* Based on the selections, some of the column type parameters are automatically filled in.
4	Enter the remaining parameter values for the new column type in the ***Run Parameters***, ***Details*** and ***Ordering Information*** tabs, for example • target bed height • max flow rate • max delta column pressure (based on chosen media) Fields marked with ***** must be filled in. Values in the gray fields are calculated and automatically filled in based on entered values for the corresponding parameters.
5	Select whether the the new column type should be ***Global*** (available for all users) or ***Personal*** (only available for the current user).
6	Click ***Save As...*** to save the column type. *Result:* The ***Save As*** dialog opens. 

Step	Action
7	Type in a ***Column type name*** and click ***Save.*** **Tip:** To simplify identification, it is recommended to choose a name for the column type comprised of hardware name, media name and bed height. However, the Method Editor will recognize the column from the selected hardware irrespective of the name. *Result*: The AxiChrom column type is saved in the database and displayed in the ***Column types*** list.

Create an individual AxiChrom column

Once a column type consisting of the AxiChrom hardware and selected media is created, you can proceed to register an individual column.

Note: It is essential to create an individual column before the column packing since it is impossible to afterwards manually register the results from the column performance tests in the ***Column Logbook***.

Step	Action
1	Select the ***Column Logbook*** tab and then click ***New.*** *Result*: The first ***New Column*** dialog opens. 
2	• Register the column either by scanning a UniTag or manually as described in *Section 8.3.2 Register a new individual column, on page 262*. and • click ***Continue***. *Result*: The second ***New Column*** dialog opens.

Step	Action
3	In the second ***New Column*** dialog: • Enter an ***Alias*** (optional). **Tip:** Alias can be used for easy identification of an individual column. • Select ***Technique*** and the AxiChrom ***Column type*** you created before. • Check the ***Use medium batch ID*** and type in the batch number of the medium in the column. • Check the ***Set medium expiration date*** and select expiration date for the medium to get a notification in UNICORN when this date is reached. **Note:** The expiration date cannot be set or changed after a column has been registered.
4	Enter notes for the column by clicking the ***Notes...*** button and typing information in the ***Notes*** dialog that opens.
5	Click ***OK***. *Result:* The entered information is saved and the registered column is displayed in the ***Column Handling*** dialog.

Note: Since the packing has not been performed at this point, the bed height for the column will be a target bed height based on the selected AxiChrom column type. This value is adjusted after the column performance tests are evaluated and the ***Column Logbook*** is updated.

Prepare an Intelligent Packing method

Use the predefined method ***Intelligent Packing*** to prepare a method for packing the AxiChrom column.

Step	Action
1	Open a new, predefined Intelligent Packing method.

Step	Action
2	In the ***Method Settings*** phase: • Select the AxiChrom column type you created previously **Note:** All the default settings for an Intelligent Packing method will be generated when the AxiChrom column is selected, including target bed height and media. The default column position will be selected. It is recommended not to change any settings in the ***Method Settings*** phase of the method.
3	In the ***Intelligent Packing*** phase: • Select ***GE approved packing settings*** (default) or • ***Custom packing settings*** **Tip:** The ***GE approved packing settings*** have been validated by GE Healthcare. If you wish to use other settings, for example other media or other bed height settings, you must select ***Custom packing settings***.
4	If you selected to enter your own ***Custom packing settings***, you can edit the following settings: • Select to - ***Pack by Packing Factor*** and choose a packing factor value *or* - ***Pack to the target bed height*** • Change the adapter velocity and • Select to use flow conditioning If you selected to use ***GE approved packing settings***, proceed with the step below. **Note:** It is not recommended to change the default position selections in the subsequent steps.
5	If necessary, select the Inlets for hydraulic chamber liquid and for the mobile phase.
6	If necessary, select the column position for the hydraulic chamber (only column position A is used) **Tip:** Click the ***Column Connection*** button to view information about the connections, including an illustration.

Step	Action
7	Enter the slurry start concentration to generate a slurry recipe, which is shown in a summary in the ***Start Notes*** at the start of the method run. You can view this recipe by clicking the ***Slurry Recipe*** button. **Note:** The accuracy of the slurry preparation will affect the packed bed height. **Note:** This function is not available when ***Custom packing settings*** is selected.
8	Verify the settings in the ***Equilibration*** phase.
9	Verify the settings in the downflow ***Column Performance Test*** phase.
10	Verify the settings in the upflow ***Column Performance Test*** phase.
11	Save the method.

Run the method and evaluate the packing

Once the ***Intelligent Packing*** method is ready, you can proceed to perform the actual packing of the individual column you have registered. Refer to the AxiChrom operating instruction for instructions how to prepare the column, connect it to the ÄKTA avant 150 system and perform the packing run.

The Intelligent Packing method includes two ***Column Performance Test*** phases, evaluating both the column upflow and downflow performance. Evaluate the results from these test as described in the UNICORN 6.1 Evaluation Manual and adjust the actual bed height according to the results if necessary.

9 Text edit methods

Introduction

Normally, methods are created and edited using the ***Method Outline***, ***Phase library*** and ***Phase Properties*** panes in the ***Method Editor*** (see *Chapter 3 Create and edit methods, on page 25* for information about how to create and edit methods using the ***Phase Properties*** pane). However, in some cases, you may want to edit a method or phase using the ***Text Instructions***. This can be an option for fine-tuning or optimization of a method.

This chapter gives an overview of the ***Text Instructions*** pane and describes how to use the ***Text Instructions*** pane to create and edit methods. It also describes some text instruction applications and how to access information about the text instructions.

In this chapter

This chapter contains these sections:

Section	See page
9.1 Overview	287
9.2 Working with methods in the Text Instructions pane	295
9.3 Specific instructions	326

9.1 Overview

Introduction

This section gives an overview of working with text instructions and a description of the ***Text Instruction*** pane.

In this section

This section contains the following sub-sections:

Section	See page
9.1.1 Working with text instructions	288
9.1.2 The Text Instructions pane	290

9.1.1 Working with text instructions

Introduction

Phases are normally edited in the ***Phase Properties*** pane. If you have selected a phase in the ***Method Editor*** and the ***Text Instructions*** tab is selected, the corresponding phase block is selected in the ***Text Instructions***.

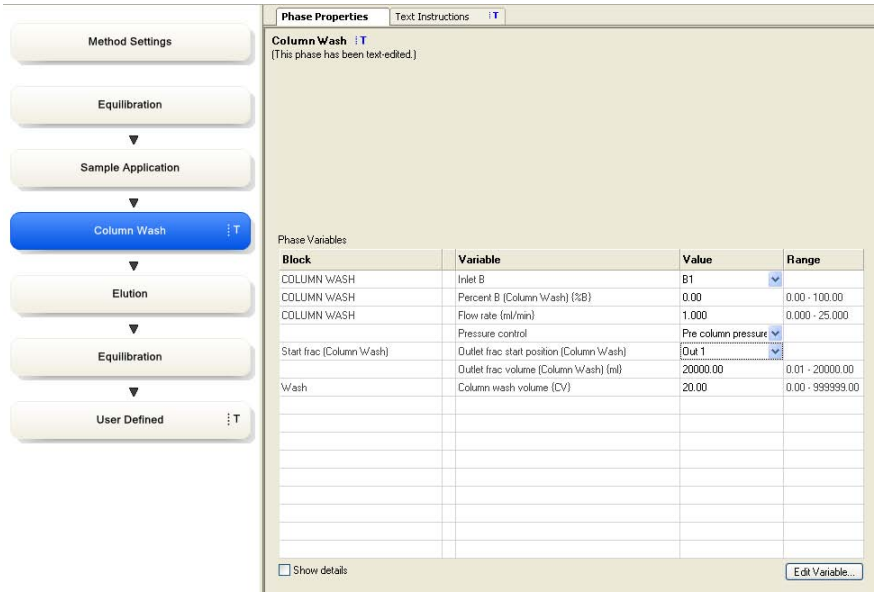
Changes made in the ***Phase Properties*** pane are automatically updated on the ***Text Instructions*** pane. However, if text editing the method in the ***Text Instructions*** pane, the settings in the ***Phase Properties*** pane will be replaced by a list of phase variables.

Text editing a method

Adding, editing or deleting any blocks or instructions in a phase in the ***Text Instructions*** area means text editing of the method. When a method has been text edited, one or several of the phases displayed in the ***Method Editor*** window are affected depending on the type of editing performed.

When text editing a method, the settings in the ***Phase Properties*** pane will be replaced with a list of phase variables that may be changed. The letter ***T*** next to the phase name in the ***Method Editor*** window indicates that the phase has been text edited.

The illustration below shows the ***Phase Properties*** pane when a method has been text edited and the indication (***T***) on the phase that has been text edited. The ***Phase Properties*** pane shows a list of phase variables.



Considerations when text editing a method

Before starting to text edit a method, consider the following:

- Editing instructions in the ***Text Instructions*** pane is only recommended for advanced users.
- If the text instructions for a method are edited manually, the phase properties will no longer show all optional settings but only the ***Phase Variables***. To restore the phase properties you have to undo the edited text instructions by clicking the ***Restore Phase Properties*** button which is displayed in the ***Phase Properties*** tab after text instructions have been edited.
- Several phases may be labelled as text edited when editing a single phase in the ***Text Instructions*** pane. This is the case when editing, for example, the phase ***Method Settings*** because several parameters are used in other phases.
- Do not mix text edited and non text edited phases unless you clearly understand the consequences.

9.1.2 The Text Instructions pane

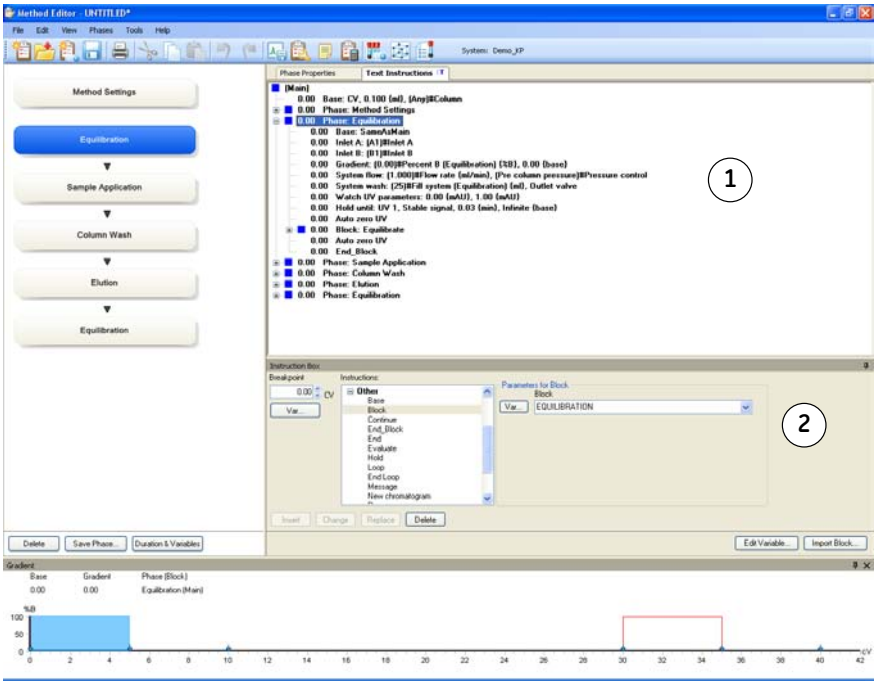
Introduction

This section gives an overview of the ***Text Instructions*** pane in the ***Method Editor*** and the structure of a text method.

Illustration of the Text Instructions pane

The ***Text Instructions*** pane consists of two areas, the ***Text Instructions*** area and the ***Instruction Box***.

The illustration below shows the ***Method Editor*** window with the ***Text Instructions*** tab selected. The phase ***Equilibration*** is selected in the ***Text Instructions*** area and the corresponding phase is highlighted in blue in the ***Method Outline*** and the ***Gradient*** panes.



The table below describes the different areas in the ***Text Instructions*** pane:

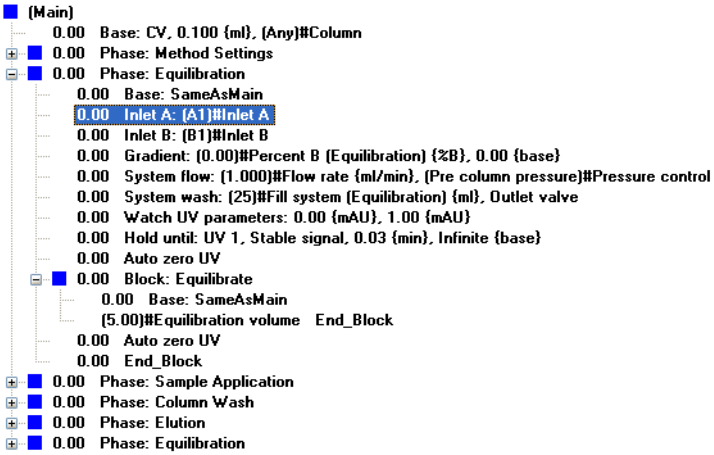
Area	Description
1	***Text Instructions*** area: Shows the method as a list of individual text instructions. The instructions are grouped into blocks (denoted by blue square symbols in the figure below) to obtain a logical overview of the method.
2	***Instruction Box***: Shows the available instructions. It can be displayed using the ***Auto Hide*** function (see *Auto Hide optional panes, on page 18* for more information). Use the ***Instruction Box*** to: • insert, change, replace and delete blocks and instructions in the method • delete phases • specify breakpoints, parameters and variables **Note:** It is not possible to add phases using the ***Instruction Box***. For information about how to add phases, see *Section 3.3.2 Edit the method outline, on page 43*.

Structure of the text method

A method in the ***Text Instructions*** area consists of a ***Main*** block that contains the ***Base*** instruction (mandatory) and the appropriate phases and blocks to be used in the method. Blocks containing valid instructions are denoted by blue square symbols (for a description of other icons that may appear, see *Description of icons and text formats in the text method, on page 293*).

Structuring the method into blocks enables reuse of instructions in the method. It also makes it possible to perform a sequence of instructions using watches (see *Section 9.3.3 Watch instructions, on page 333* for more information about watch instructions).

The illustration below shows an example of a method in the ***Text Instructions*** area:



The table below describes the different parts in the method:

Part	Description
Main	The main block contains the complete method. It contains the ***Base*** instruction (mandatory) and the appropriate phases with instructions to be executed in a method.
Phase	Blocks at the highest level in the method represents the major steps in the process flow and are called phases. Each phase can contain sub-blocks, that is, blocks at a lower level. **Note:** If the method has not been text edited, properties for the phase can be set in the ***Phase Properties*** pane. **Note:** New phases can only be added to the ***Method Outline*** using the ***Phase Library***. It is however possible to copy and paste an existing phase in the ***Text Instructions*** pane.
Block	Each block starts with a ***Base*** instruction, continues with the appropriate instructions and always ends with an ***End_Block*** instruction.
Sub-block	A sub-block is a block at a lower level than a phase that may contain conditional instructions or other instructions for specific events within a phase. Each sub-block starts with a ***Base*** instruction, continues with the appropriate instructions and always ends with an ***End_Block*** instruction.

Description of icons and text formats in the text method

The table below describes the icons and text formats that may appear in the the ***Text Instructions*** pane:

Icon/text format	Description
Blue square beside text 	A block containing instructions that can be run.
Blue square with a red cross 	A block containing one or more instructions that are not possible to run due to instrument configuration incompatibility (syntax errors).
Bold text	Instructions that can be run.
Red dot 	Instructions that are not possible to run. All such instructions must be deleted or changed before a method can be run. See *Section 9.2.3 Working with text instructions, on page 309*. The errors in the instructions may be of the following types: • Instructions that apply to a different instrument configuration (can occur if a method is written for one system and saved for another) • Instructions for deselected components in the ***System Setup***. • References to blocks that are not defined in the method (e.g., a ***Watch*** instruction but no instructions to be executed when the ***Watch*** is activated)
Normal text	Instructions that will not be run. Instructions with a red dot are formatted as normal text instead of bold text. Unused instructions are also formatted as normal text. Instead of deleting instructions they can be moved to unused instructions below the text method.

Icon/text format	Description
Text with a red loop symbol 	When a block is called from within itself this will generate a potentially infinite loop. It is not possible to run such a method.

9.2 Working with methods in the Text Instructions pane

Introduction

This section describes how to create or edit methods using specific text instructions. The general structure of the text method syntax is described, including the major hierarchy of the text method parts (phases and blocks).

In this section

This section contains these sub-sections:

Section	See page
9.2.1 Base instruction	296
9.2.2 Working with phases and blocks	300
9.2.3 Working with text instructions	309
9.2.4 Method variables	315

9.2.1 Base instruction

Introduction

Every method block must start with a ***Base*** instruction, defining the base for calculating breakpoints (see also *Structure of the text method, on page 291*). Different blocks can use different bases.

This section describes how to choose and edit settings for the ***Base*** instruction.

What base should I use?

Depending on the experiment, different bases should be used. Use the base that most closely suits the purpose of the block. Column volume (***CV***) is recommended as the base for most steps in a run. In some situations, however, it may be more suitable to use a time or volume base for individual blocks.

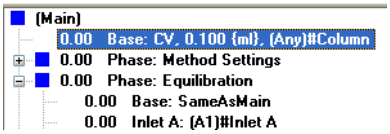
The table follows lists few examples when different bases should be used:

Use...	When...
CV	the method should be adjusted according to the selected column. In this way, you do not need to edit the method when changing column size.
Volume	the same volume should be used regardless of which column is used.
Time	a defined time is required and the volume used is not critical, or if the flow rate is zero.

Edit settings for a base instruction

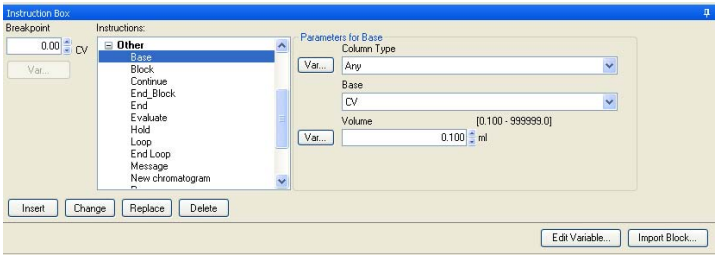
The following table describes how to edit settings for a base instruction:

Step	Action
1	Select the base instruction for which to edit the settings in the ***Text Instructions*** area.



Result:

- The settings for the selected ***Base*** instruction are displayed in the ***Parameters for Base*** area in the ***Instruction Box***.



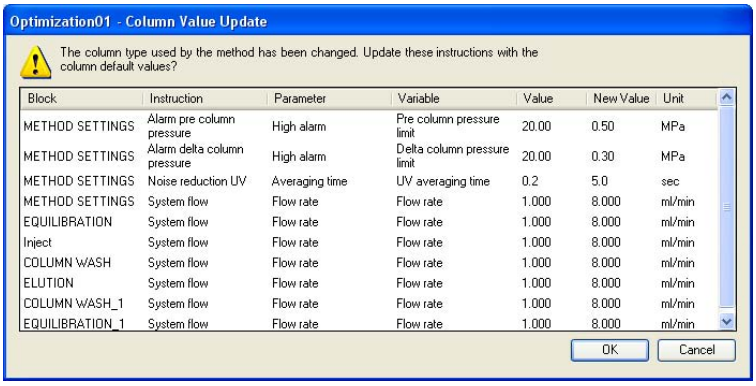
- If a ***Base*** instruction in a phase or block was selected using the same parameter settings as the ***Main*** block, this is displayed in the ***Instruction Box***.

2	• Select the appropriate ***Base*** from the ***Base*** drop-down list: - ***Volume*** (the unit depends on which ***Instrument Configuration*** used) - ***Time*** (minutes) - ***CV***, column volume (the corresponding volume in for example ml can be defined numerically or taken from the ***Column Type*** list) - ***SameAsMain*** (does not apply for the main block). The block will inherit the base defined in the main block. *Result:* The settings in the ***Parameters for Base*** area are updated.

Step	Action
3	Select the appropriate ***Column Type*** in the drop-down list. The table below gives a short descriptions of the available options:

Column parameter	Description
Any	Any column can be used in the block. If the ***Column Type*** is set to ***Any*** and the ***Base*** is set to ***CV***, enter the column volume in the ***Volume*** field.
ColumnSameAs-Main	The same column as in the main block will be used. When the ***Base*** is set to ***Volume*** but the flow still goes through the column, the ***Column Type*** can be set to ***SameAsMain*** to provide information on, for example, pressure limits for the column.
Named column type (e.g., HiTrap Q HP, 1 ml)	The named column type will be used in the block. The volume specified in the selected column definition will automatically be used for ***Volume*** parameter in the method block, and thus used to calculate column volumes (***CV***). The ***Volume*** parameter may then not be edited manually. The Column Type parameter can be defined as a variable. This may be useful if it is desirable to change column type when starting the method run in the ***Variable List*** during the ***Start Protocol*** (see *Set up a Start Protocol, on page 54*). See *Section 9.2.4 Method variables, on page 315* for information about how to define variables.

Step	Action
4	Click ***Change*** or ***Replace*** to save the settings for the selected ***Base*** instruction. *Result*: The parameters for the ***Base*** instruction are updated. **Note:** If the column type is changed, the ***Column Value Update*** warning dialog opens, displaying the changes that will be made in the method that will be made based on the column default values (see diagram below). If these changes are correct, click ***OK***, otherwise click ***Cancel***.



9.2.2 Working with phases and blocks

Introduction

This section describes how to add, delete and edit phases and blocks in the text method. It also describes how to import blocks from other methods.

Phases vs blocks

Because phases are blocks at the highest level in the text method, the same editing operations can be performed. In this section the name block will be used both for phase blocks and other blocks unless otherwise stated.

Exception

It is not possible to add a phase using the ***Instruction Box***. A new phase must be added from the ***Phase Library***. The ***User Defined*** phase is intended for this purpose, but any phase may be text edited.

See *Section 3.3.2 Edit the method outline, on page 43* for information about how to add phases to the ***Method Outline***.

Method blocks

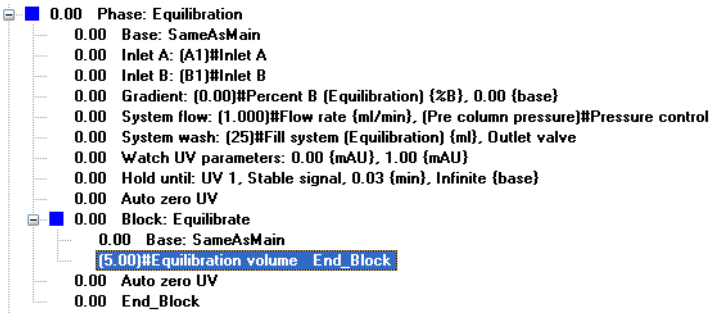
Instructions in each block are executed in the order they are written. The instructions within a block are executed until the block is finished or the ***End_Block*** instruction is executed. Any settings made in a block are valid throughout the method until the settings are changed.

However, if a conditional instruction, e.g., a ***Watch*** instruction controlling the start of a sub-block, is included in a phase the instructions in the sub-block are executed when the condition for that ***Watch*** is met (e.g., when a particular monitor signal meets a given condition).

Block length

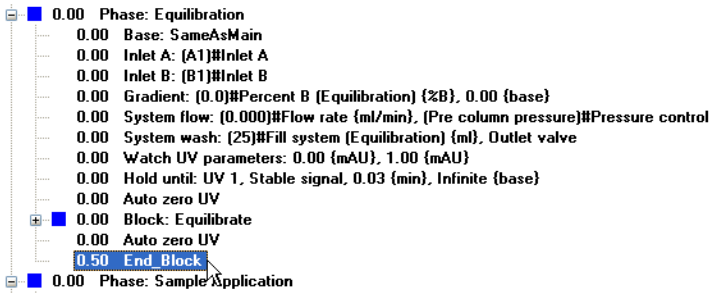
The length of a block is determined by the breakpoint of the last instruction in the block. Even if all breakpoints are set to 0, the instructions might take some time/volume because they are executed sequentially.

The illustration below shows an example of a method where ***Equilibration*** has a breakpoint set to 5:



In the example above, the value `5.00` will be 5 column volumes (***CV***) if the ***Base*** in the ***Main*** block is set to ***CV***, 5 minutes if ***Base*** is set to ***Time*** or 5 ml if ***Base*** is set to ***Volume***.

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



In this example, the block will end after 0.5 ml, since ***Base*** is set to ***Volume***.

An estimation of the time for running the method can be obtained in the ***Method Duration and Variables*** window. See *View and print the method duration time and variables, on page 60*.

View/hide instructions in blocks

The table below describes how to view or hide blocks and instructions in the ***Text Instructions*** pane:

If you want to...	then...
expand all blocks in the method	double-click ***Main***

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

Add phases

Phases can be added to a text method by:

- adding any phase to the method from the ***Phase library***. The phase ***User Defined*** is designed for use in creating text methods from scratch, and consists only of ***Base*** and ***End_block*** instructions. See *Section 3.3.2 Edit the method outline, on page 43* for information about how to add a phase to the ***Method Outline***.

  or

- by copying/pasting an existing phase in the text method and then edit it. See *Copy and paste blocks, on page 303* for information about how to copy and paste blocks.

Note: It is not possible to add a new phase using the ***Instruction Box***.

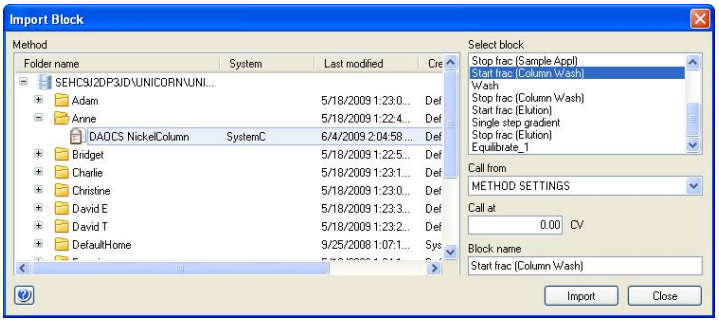
Add blocks in a phase

The table below describes how to add blocks in a phase:

Step	Action
1	Select the instruction or block after which you want to insert the new block.
2	Select ***Other:Block*** in the ***Instruction Box***.
3	• Enter a name for the block in the ***Block*** field. • Click the ***Insert*** button. *Result:* The block is inserted after the block that was selected in step 1.

Copy and paste blocks

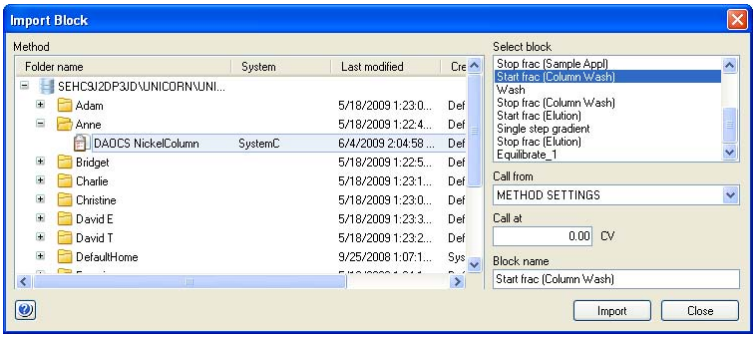
The table below describes how to copy and paste a block.

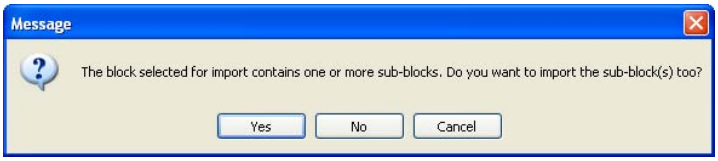
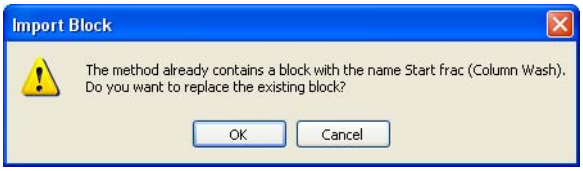
Step	Action
1	Select the block you want to copy. • click the ***Copy*** icon in the ***Toolbar***  *or* • right-click the block and choose ***Copy*** *or* • select ***Edit:Copy*** (**Ctrl+C**)
2	Select the instruction line just above the point where you want the block to be pasted. • click the ***Paste*** icon in the ***Toolbar***  *or* • right-click the instruction line and choose ***Paste*** *or* • select ***Edit:Paste*** (**Ctrl+V**) *Result*: A ***Rename*** dialog opens.

Step	Action
3	• Click ***Yes*** to rename the block after insertion A new block is created. The variables in the block will get new names so the variable values can be changed without affecting the original block. *or* • Click ***No*** to just insert the copied block with the same name. The block and variables names in the block are copied. If changing variable values in the pasted block, the values will be changed in the original block as well. *Result*: The block is inserted in position. **Note:** The pasted block is inserted with the same breakpoint value as the block or instruction selected for point of insertion. When a ***Phase*** is copied and pasted the ***Rename*** dialog is not opened.

Import blocks

The table below describes how to import blocks from other methods:

Step	Action
1	Click the ***Import Block...*** button. *Result*: The ***Import Block*** dialog opens.
2	Locate and select the method you wish to import the block from in the ***Method*** folder structure.  *Result*: All available blocks are listed in the ***Select block*** field.
3	Select a block to import from the method in the ***Select block*** list.

Step	Action
4	• Select the block where the imported block will be inserted in the ***Call from*** drop-down list. • Type the breakpoint that the imported block will be called at in the ***Call at*** text box. • If necessary, type a new name for the block in the ***Block name*** text box (optional).
5	• Click the ***Import*** button. • Confirm if you also want to import sub-blocks (if any)  *Result*: The block is imported into the method you are editing. Unless you have specified a breakpoint that is earlier, the block will be inserted at the end of the block that it is called from. **Note:** If you are importing a block with the same name as a block that already is included in the method you are editing, a warning will open and you will be asked to confirm that you wish to replace the original block with that name in the method. 

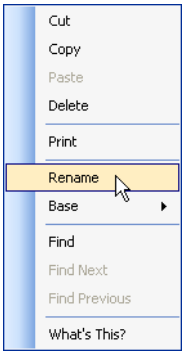
Move blocks

Blocks can be moved by drag and drop within the method. You can also use ***Cut*** and ***Paste*** as described below:

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

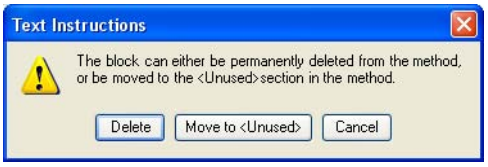
Rename blocks

The table below describes how to rename a block:

Step	Action
1	Right-click the block in the text pane and select ***Rename***.  *Result*: The block name is highlighted in a box.
2	Type in a new name. **Note:** If the block you renamed is called in a ***Block*** or ***Watch*** instruction, the block name in these instructions will be changed automatically.

Delete blocks

The table below describes how to delete a block:

Step	Action
1	• Right-click a block and choose ***Delete***. *or* • Select a block and click ***Delete*** in the ***Instruction box***. *or* • Select a block and press the **Delete** key on the keyboard. *Result*: A dialog will appear asking if the block should be deleted permanently or moved to unused blocks.  **Note:** If deleting a phase, the phase will be deleted right away.
2	• Click ***Delete*** to delete the block permanently. • Click ***Move to <Unused>*** to delete the block from the method and place it after the method.

9.2.3 Working with text instructions

Introduction

Instead of editing the method in the ***Phase Properties*** pane, instructions may be edited one at a time in the ***Text Instructions*** pane. The instructions in a block are always executed sequentially. This section describes the general principles for how to edit instructions.

Help texts for the instructions

It is possible to display help texts for the instructions that can be inserted in the ***Instruction Box***.

The table below describe how to display the help text for an instruction:

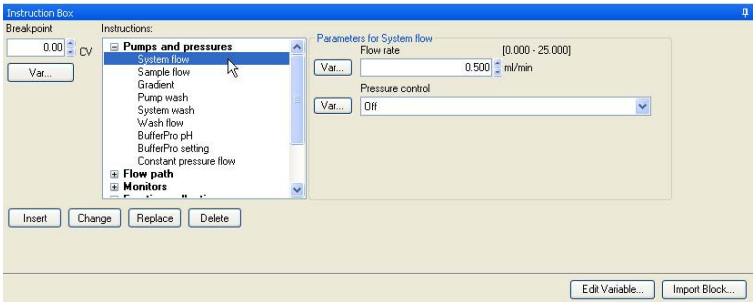
Step	Action
1	In the ***Instruction Box***, select the appropriate instruction for which to display help text.
2	Press **F1** on the keyboard. *Result:* A dialog with help text for the selected instruction will be displayed.

Insert a new instruction

The table below describes how to insert a new text instruction in the ***Text Instructions*** area:

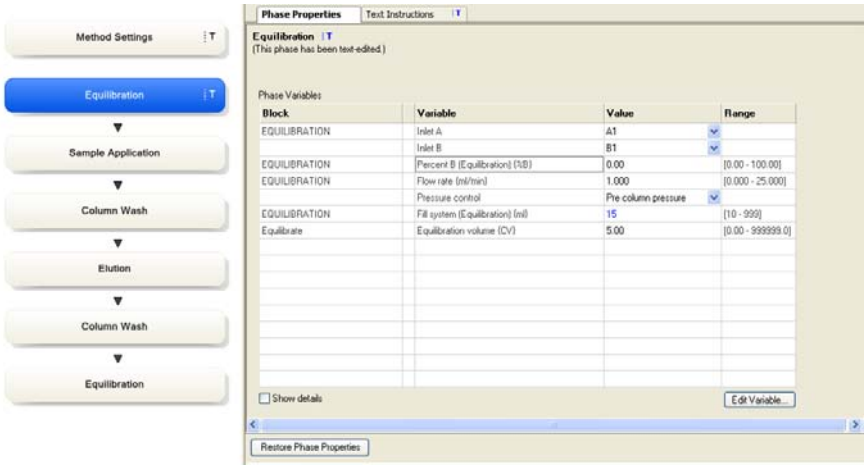
Step	Action
1	Select a block and display the instructions within the block.
2	Select the instruction in the block after which you want to add the new instruction.

Step	Action
3	Open the ***Instruction Box*** if it is hidden. Do the following: • Set the appropriate breakpoint in the ***Breakpoint*** box. • Choose the instruction type and the instruction in the ***Instructions*** field. For basic help on each instruction, select the instruction and press **F1**. • Type values for instruction parameters in the ***Parameters*** text boxes. The allowed range is shown in brackets beside the text box. If a scroll bar appears at the right side of the ***Parameters*** field, additional parameters are available.
4	Click the ***Insert*** button. *Result:* The instruction will be inserted in the block: • at the position of the breakpoint of the new instruction, if there are no other instructions at that breakpoint • immediately after the currently highlighted instruction, if the highlight is at the same breakpoint as the new instruction • as the last instruction at the breakpoint, if there are several instructions at the same breakpoint and none of these is highlighted.



Note: Once a method has been edited in text editing mode, the phases affected by the edited instruction are indicated with the letter "***T***", and the ***Phase Properties*** pane changes to show a variable list, as shown below. To restore the ***Phase Properties*** again you can click the ***Restore Phase Properties*** button to return the method to the state before the text edit. Any changes that were made in the ***Text Instructions*** pane will be removed.

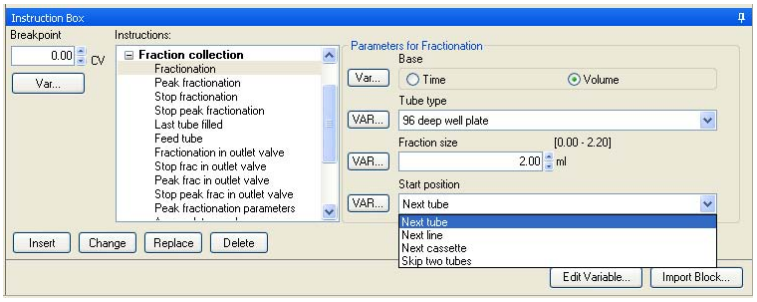
New phases from the ***Phase Library*** may be inserted in the method after text editing and the settings for these new phases can be edited in the ***Phase Properties*** pane or ***Text Instructions*** pane.



Change or replace an instruction

The table below describes how to edit instructions in the ***Text Instructions*** area:

Step	Action
1	Select an instruction in the text method. *Result*: The current ***Breakpoint*** and parameters for the selected instruction is displayed in the ***Instruction Box***.
2	Edit or select parameter values in the ***Instruction Box***:



Step	Action
3	To add the edited or a new instruction to the method, click one of the following buttons: • ***Insert*** • ***Change*** • ***Replace*** **Note:** The ***Insert*** button adds the edited instruction immediately below the instruction that was selected in the method. The ***Change*** and ***Replace*** buttons are equivalent unless changes are made to the breakpoint or gradient length. Both buttons replace the highlighted instruction with the newly edited instruction. The differences are explained below.

Effects of the Change button and the Replace button on breakpoints

The table below describes the difference in function between the ***Change*** and ***Replace*** buttons when changing breakpoints:

Button	Function
Change	This button shifts all subsequent instructions in the block according to the change in the breakpoint. ***Change*** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in a block.
Replace	This button moves the selected instruction but does not change the breakpoint of any other instruction. ***Replace*** can change the relative order of instructions in the method.

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

The ***Length*** parameter in the ***Gradient*** instruction affects the length of a gradient. The change will have different results depending on which button is used. The table below describes this:

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

Move instructions

A selected instruction may be dragged-and-dropped in a new location to change the order of instructions. The symbol shown in the illustration below will be displayed if the instruction cannot be dropped in a specific location.

Delete instructions

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

Step	Action
1	Select the instruction in the ***Text Instructions*** pane.
2	• Right-click the instruction and choose ***Delete***. *or* • Click the ***Delete*** button in the ***Instruction box***. *or* • Press the **Delete** key on your keyboard.

End_Block instruction

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

9.2.4 Method variables

Introduction

Variables are used when you want to vary parameter values in a method. Variables must be defined when you want to:

- perform scouting and ***Design of Experiments*** (***DoE***) where different parameters are varied to find, for example, optimal settings for a process.

  See *Chapter 4 Scouting, on page 103* and *Chapter 5 Design of Experiments, on page 116* for more information.

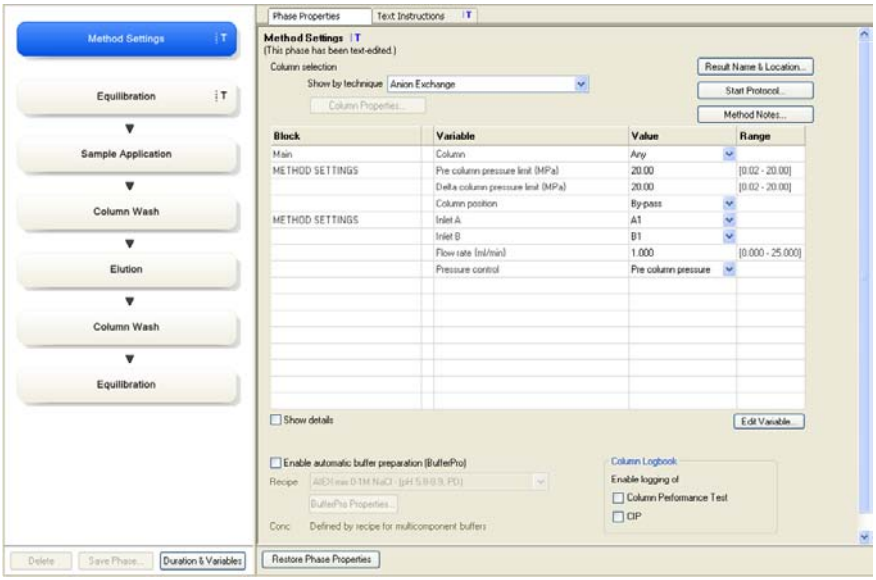
- change parameter values in the ***Start Protocol*** immediately before the start of a method run without using the ***Method Editor***, allowing one method to be used for runs under a variety of conditions. Each parameter defined as a variable is assigned a default value, which is used if no changes are made to variable values at the start of a run.

  For information about how to change variable values in the ***Start Protocol***, see *ÄKTA avant and UNICORN 6.1 User Manual*.

Viewing method variables

All variables in a method are listed on the ***Variable List*** tab in the ***Method and Duration*** dialog, grouped according to the phase and block in which they appear. For information about how to view the variables in a method, see *View and print the method duration time and variables, on page 60* for more information.

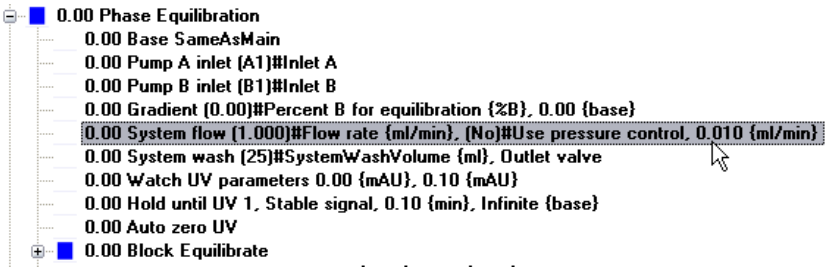
If the method has been text edited the phase variables for the selected phase will be displayed in the ***Phase Properties*** pane. If the ***Method Settings*** phase has been edited, some additional parameters (e.g., enabling of ***BufferPro*** and ***Column Logbook*** settings) will also be displayed. It is possible to edit variable names, values and the other settings displayed in the ***Phase Properties*** pane.



Identifying variables in the Text Instructions area

Parameters that are defined as variables in the text method are indicated in the ***Text Instructions*** area.

The parameter is given as the default value in parentheses followed by the variable name. The illustration below shows an example of this:



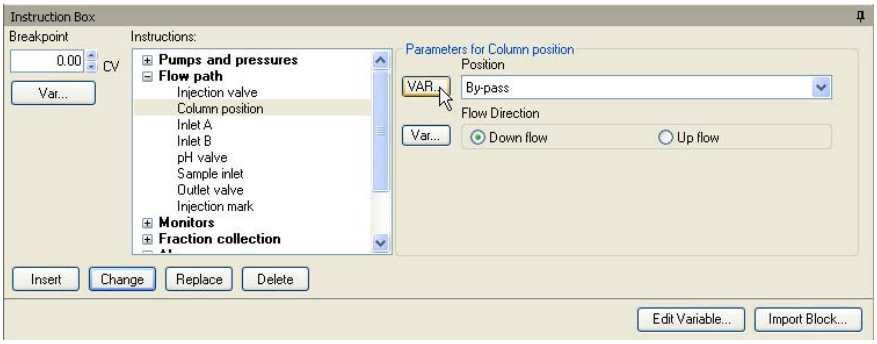
For example, in ***(1.000)#Flow rate (ml/min)***:

- `(1.000)` is the default value for the variable
- `(ml/min)` is the variable unit
- `Flow rate` is the variable name

Identifying variables in the Instruction Box

Parameters that are defined as variables in the text method are also indicated in the ***Instruction Box*** for the selected instruction in the ***Text Instructions*** area.

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



Variable name conventions

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

When defining and/or renaming variables, consider the following:

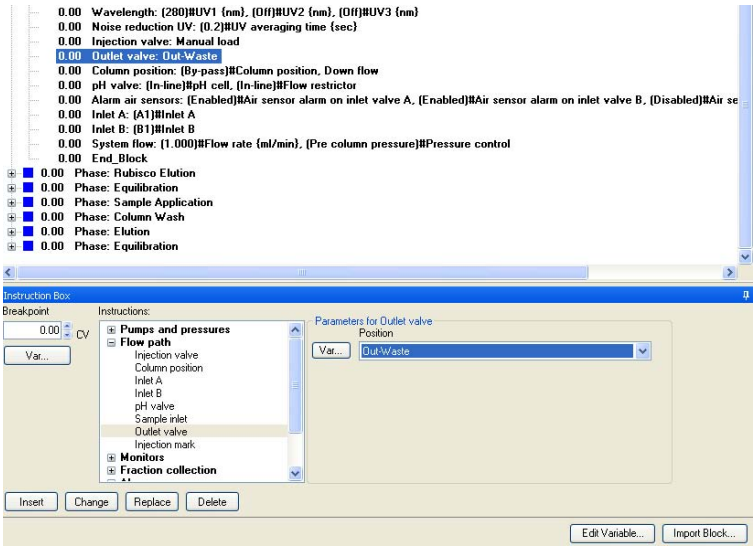
- The names can be up to 50 characters long and the following characters can be used:
  - Letters (`A-Z`)
  - Digits (`0-9`)
  - The underscore character (_)
  - The `Space` character
- The case of letters is retained, but not significant. The names ***Flow Rate*** and ***FLOW RATE*** are treated as identical.

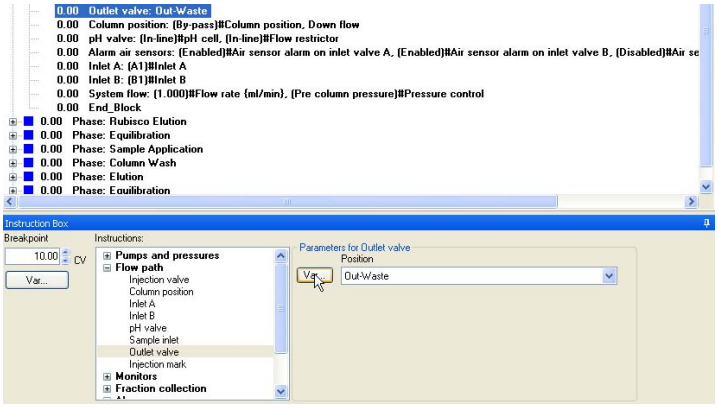
For information about defining and renaming variables, see *Define new variables, on page 318* and *Edit variables, on page 320*.

Define new variables

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

Step	Action
1	Select the instruction where you want to define the variable in the ***Text Instructions*** area. *Result:* The parameters for the instruction are shown in the ***Instruction box***.



Step	Action
2	• Locate the breakpoint or the required parameter in the ***Instruction box***. • Click the ***Var...*** button.  *Result:* The ***New Variable*** dialog opens. 
3	• Type a name for the variable (see *Variable name conventions, on page 317* for information about how to name variables). • Select the ***Visible in details only*** check box if you want to set the variable as a "details" variable. Detail variables become visible in the ***Variable List*** if the ***Show details*** check box is selected. This option can be used to simplify the workflow later. • Click ***OK***. *Result:* The ***Var...*** button changes to ***VAR...*** to confirm the new variable. **Note:** If a breakpoint or gradient length is defined as a variable, changing the variable value in the ***Variable List*** tab when the method run is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using the ***Change*** button to alter a breakpoint or gradient length (see *Section 9.2.3 Working with text instructions, on page 309* for how the ***Change*** button affects instructions within gradients).

Step	Action
4	Click ***Change***. *Result:* The variable is saved and displayed in the ***Text Instructions*** area.

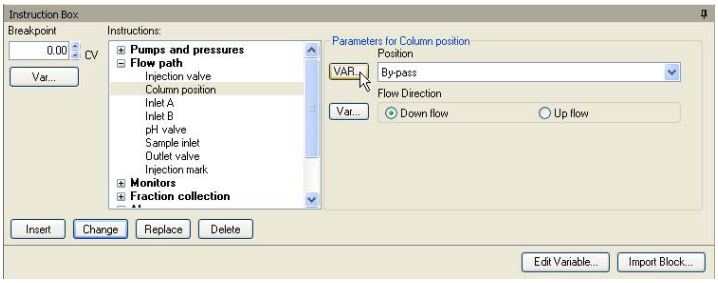
Edit variables

Editing a variable includes renaming and deleting the variable and choosing whether the variable should be a detailed variable or not. For information about how to edit the variable values, see *Edit variable values, on page 324*.

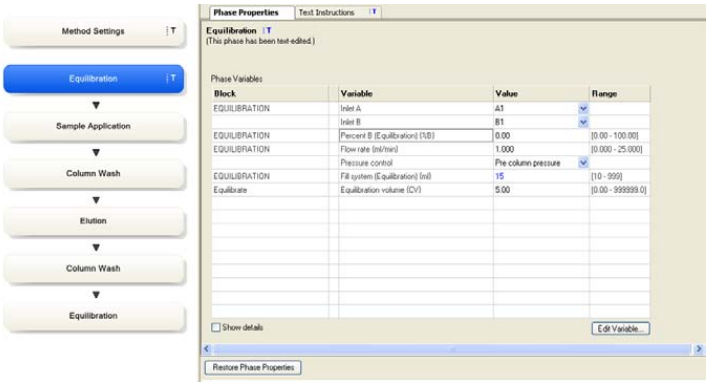
Edit a variable using the Edit variable button

The table below describes how to edit a variable using the ***Edit Variable*** button:

Step	Action
1	• In the ***Instruction Box***, click ***Edit Variable...***.

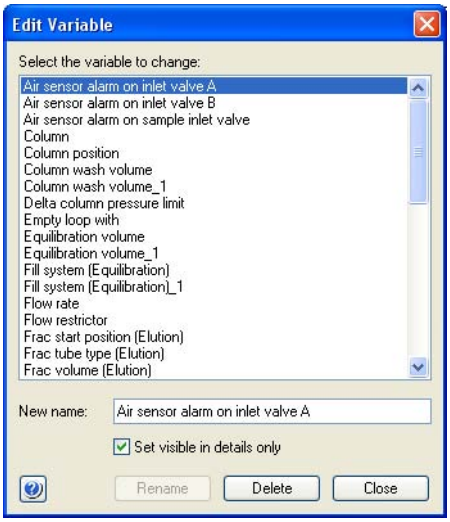


	• Alternatively, if the phase containing the variable has been text edited, select the ***Phase Properties*** tab to display the phase variables, select the variable and click ***Edit Variable...***.



Result: The ***Edit Variable*** dialog opens displaying all variables (if opened from the ***Text Instructions*** pane) or the phase variables (if opened from the ***Phase Properties*** tab).

Step	Action
2	Select the variable to be edited (if not already selected). Do one or several of the following as appropriate:



- Type in a new name in the ***New name*** field and click ***Rename***.
- Check the ***Set visible in details only*** if the variable should be a detailed variable. Uncheck the box to set it to a normal variable.
- Click ***Delete*** to delete the variable.

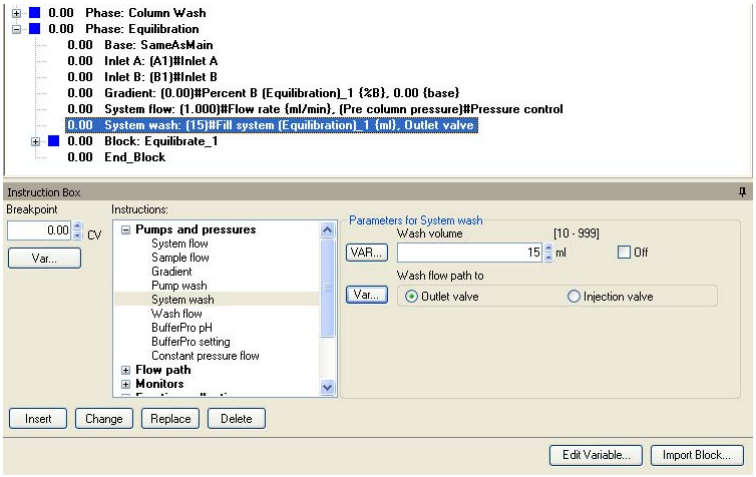
  Confirm that you want to delete the variable in the dialog that appears.

3	Click ***Close*** to close the dialog.

Edit a variable using the VAR.. button in the Instruction Box

The table below describes how to edit a variable using the ***Instruction Box***:

Step	Action
1	Select the instruction containing the variable to be edited in the ***Text Instructions*** area. *Result:* The parameters for the instruction are shown in the ***Instruction box***.



2	Click the ***VAR...*** button for the appropriate variable. *Result:* The ***Edit Variable*** dialog opens.

3	Do one or several of the following as appropriate: • Type in a new name in the ***Variable name*** field. • Check the ***Visible in details only*** if the variable should be a detailed variable. Uncheck the box to set it to a normal variable. • Click ***Clear*** to delete the variable.
4	Click ***OK***.
5	To save the changes, click ***Change*** in the ***Instruction Box***. *Result:* The text instruction is updated.

Edit variable values

To edit default variable values, you can either

- edit the value in the ***Phase Properties*** tab if the text method has been edited.

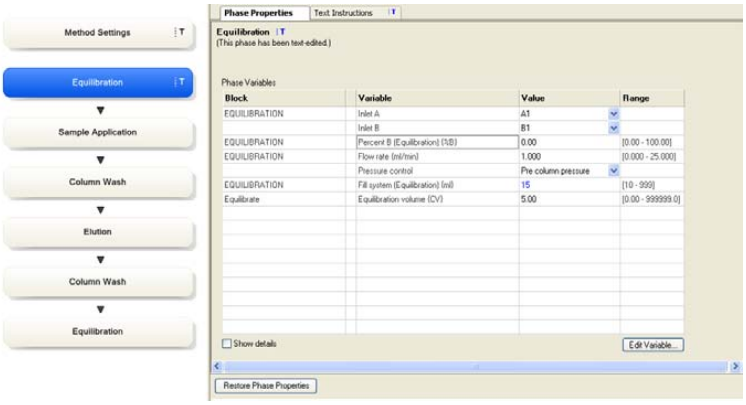
  or

- edit the instruction in the ***Instruction box*** of the ***Text Instructions***.

Changes made in the ***Phase Properties*** tab are automatically updated on the ***Text Instructions*** tab and vice versa.

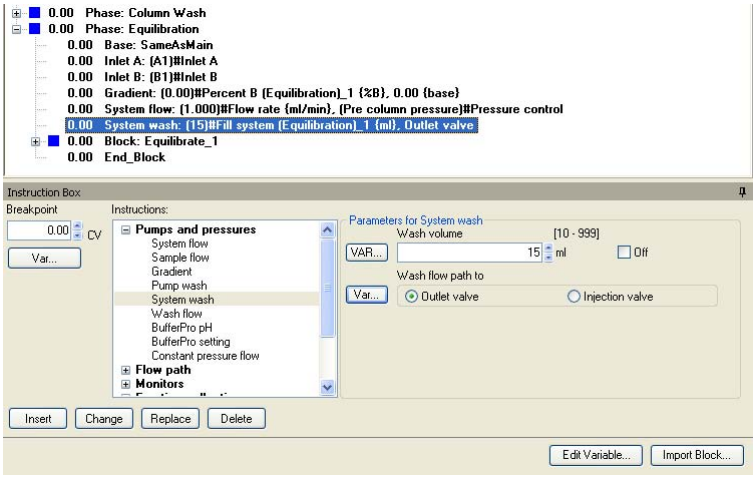
Edit variable values in the phase variables list

If the phase containing the variable value to be edited has been text edited, it is possible to edit the variable value on the ***Phase Properties*** tab. The table below describes how to edit variable values in the ***Phase Properties*** tab for a text edited phase:

Step	Action
1	Select the ***Phase Properties*** tab to display the ***Phase Variables*** list. 
2	Change the variable value for the appropriate variable in the ***Value*** field by choosing a new value in the drop-down list or typing in the field. **Tip:** To show detailed variables, check the ***Show details*** box. *Result:* The variable value is updated.
3	Repeat this procedure for the appropriate variables.

Edit variable values in the Instruction Box

The table below describes how to edit variable values in the ***Instruction Box***:

Step	Action
1	Select the instruction containing the variable value to be edited in the ***Text Instructions*** area. *Result:* The parameters for the instruction are shown in the ***Instruction box***. 
2	Change the value for the appropriate variable(s) (indicated by ***VAR...***).
3	Click ***Change***. *Result:* The settings are saved and the text instruction updated in the ***Text Instructions*** area.

9.3 Specific instructions

Introduction

This section describes some text instruction applications, for example:

- Gradient instructions
- Alarms
- Conditional instructions
- Messages, set marks, pause and hold instructions

In this section

This section contains these sub-sections:

Section	See page
9.3.1 Gradients and eluent concentrations	327
9.3.2 Alarm instructions	330
9.3.3 Watch instructions	333
9.3.4 Pause or hold a method	340
9.3.5 Messages and Set marks	343

9.3.1 Gradients and eluent concentrations

Introduction

Gradient instructions allow definition of an A- and B-buffer mix. The starting point for the ***Gradient*** is always the current eluent composition. The instruction can be read as follows: "form a ***Gradient*** to reach ***Target*** after ***Length***". Linear gradients and step gradients can be created using ***Gradient*** instructions.

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

Linear gradients

A gradient can be defined as a linear gradient. The eluent composition changes linearly over time.

Example of instruction

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

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

Step gradients

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

Example of instruction

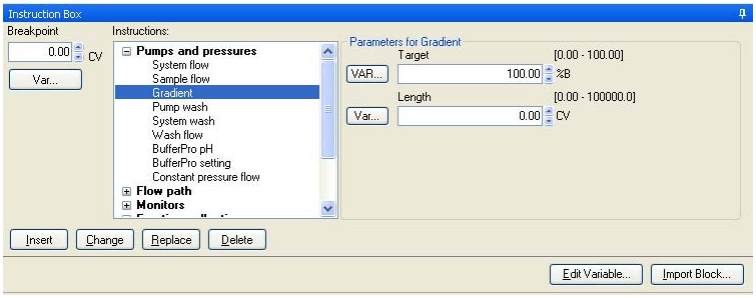
```
10.00 Gradient 50{%B}, 0{base}
```

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

Insert a Gradient text instruction

The table below describes how to insert a ***Gradient*** instruction:

Step	Action
1	At a suitable ***Breakpoint*** in the method, select the instruction line immediately before where you want to insert the gradient (this decides when the gradient begins).
2	• Expand the ***Pumps and pressures*** item in the ***Instructions*** field of the ***Instruction Box***. • Select ***Gradient***. • In the ***Parameters for Gradient*** field, select appropriate values for: - ***Target*** (final eluent composition expressed in % eluent B) - ***Length*** (duration of the gradient) **Tip:** To form a step gradient, set the ***Length*** parameter to zero. **Tip:** For many purposes, it can be useful to define the length of the gradient as a variable. When this is done, breakpoints for instructions issued during or after the gradient in the same block are automatically shifted in proportion to the length of the gradient when the variable value is changed. This is the same functionality as the ***Change*** button command in the ***Instruction Box***.



Step	Action
3	Edit the ***Breakpoint*** for the gradient, if appropriate. **Note:** The breakpoint for a ***Gradient*** instruction defines the time or volume (according to method base) for the start of the gradient. A gradient with a non-zero duration occupies time and volume in the method, and breakpoints for other instructions may be set to occur before the gradient is completed. The instruction is simply carried out at the requested breakpoint, while the gradient is forming.
4	Click the ***Insert*** button. *Result:* The new ***Gradient*** instruction is inserted in the method in the ***Text Instructions*** area.

Instruction after a gradient

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

Instructions that affect gradients

The table below describes the instructions that affect the gradient:

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

9.3.2 Alarm instructions

Introduction

This section is a description of how alarms work in UNICORN and of the ***Alarms*** text instructions. It also describes the differences between ***Alarms*** and ***Warnings***.

Alarms and Warnings

The ***Alarms*** parameter settings define the high and low ***Alarm*** limits for process monitor signals. You can define these limits either in the system settings or as part of a method. Settings in the method will override the system settings.

The limits that will generate a ***Warning*** from the system are defined in the instrument configuration files and you cannot edit these settings.

Conditions can also be applied to process monitor signals such that a block of instructions will execute when a particular condition is satisfied (for example, when the absorbance of the eluent exceeds a certain limit). This is done using ***Watch*** instructions which are described in *Section 9.3.3 Watch instructions, on page 333*.

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

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

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

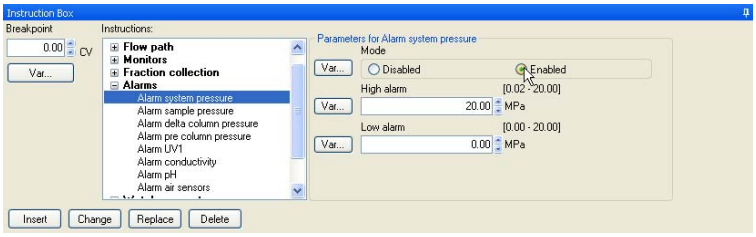
Alarms in a network

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

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

Insert an Alarm text instruction

The table below describes how to insert an alarm instruction into the method.

Step	Action
1	Select the instruction line immediately before where you want to insert the ***Alarm***, at a suitable ***Breakpoint*** in the method. (This will decide when the alarm conditions begin.)
2	• Select ***Alarms*** in the ***Instructions*** field of the ***Instruction Box***. • Select the desired alarm from the list.
3	Select appropriate values for ***High alarm*** and for ***Low alarm*** in the ***Parameters*** field.  **Note:** There are no high and low settings for ***Air sensors***, only enabled or disabled.
4	Click the ***Enabled*** radio button.
5	Click the ***Insert*** button. *Result:* The new ***Alarm*** instruction is inserted in the method.

Available alarms

The alarms available depend on the instrument configuration. Alarms for the following monitor readings may be set:

- System pressure
- Sample pressure
- Delta column pressure
- Pre-column pressure
- UV1
- Conductivity
- pH
- Air sensors

9.3.3 Watch instructions

Introduction

Watch instructions allow the progress of a method run to be determined by events during the method run. For example, start collecting fractions when the first peak elutes.

The ***Instrument Configuration*** files include ***Watch*** instructions for each monitor defined in the system. These instructions are used to monitor method runs, and instruct the system to call a specified block or an instruction when a particular signal meets a given condition. As long as the condition is not met, the block is not activated.

Note: ***Watch*** instructions available for the instrument configuration are listed in the ***Instruction box***.

When is a Watch active?

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

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

- the ***Watch*** condition is met.
- a new watch is set for the same monitor.
- a ***Watch off*** instruction is issued for the monitor.

  or

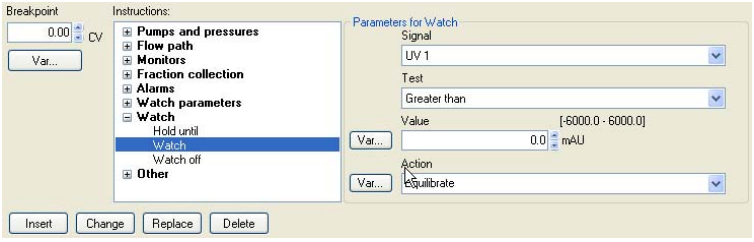
- the method ends.

Insert a Watch text instruction

The table below describes how to insert a watch instruction in the text method. Setting up additional Watch parameters is described afterwards, see *Insert a Watch parameters instruction, on page 337*.

Step	Action
1	At a suitable ***Breakpoint*** in the method, select the instruction line immediately before where you want to insert the watch (this decides when the watch begins).

Step	Action
2	• expand ***Watch*** in the ***Instructions*** field. • select the desired ***Watch*** type: - ***Hold until*** Subsequent instructions in the block will execute when the conditions have been met - ***Watch*** A specified action will be performed when the conditions have been met - ***Watch off*** Cancels the watch on the specified signal



3	Select a signal for the watch from the Signal drop-down menu. See *Monitor signals to watch, on page 335* for available signals that can be selected.
4	For watch types ***Hold until*** or ***Watch***, select the appropriate ***Parameters for Watch***: • ***Test*** See *Test options in the Parameters field, on page 335* for a description of the different ***Test*** options. • ***Value/Slope/Minutes/Factor*** depending on the selected test • select an appropriate ***Action***. See *Actions when a Watch condition is met, on page 336* for a description of the different ***Watch Action*** options.

Step	Action
5	Click the ***Insert*** button. *Result:* The new ***Watch*** instruction is inserted in the ***Text Instructions*** area. **Note:** A ***Watch off*** instruction can be added to the method at a breakpoint where the watch no longer is needed.

Note: Watch parameters may be set as variables so that the method easily can be adjusted for different run conditions.

Monitor signals to watch

The monitor signals that can be watched differ depending on the ***Instrument Configuration*** but may include the following:

- pH
- Cond
- UV (1,2 and 3)
- Pressure (System, Sample, Pre-column and Delta-column)
- Flow (System and sample)
- Air sensor (System pump A and B, sample pump)

The buffer concentration may also be set as a watch parameter.

Test options in the Parameters field

The table below describes the ***Test*** options that are available for the ***Watch*** instruction in the ***Parameters for Watch*** field:

Option	Explanation
Greater than	The signal exceeds a certain value.
Less than	The signal falls below a specified value.
Slope greater than	The rate of change of the signal exceeds a specified value, expressed in monitor units/minute (for example, mAU/min).
Slope less than	The rate of change of the signal falls below a specified value, expressed in, for example, mAU/min.

Option	Explanation
Less than or valley	The signal falls below a specified value or a valley is detected. A valley is detected only after a ***Peak max*** has been detected, and the valley is defined by a local minimum followed by an increase to 102% of the local minimum value plus the ***Delta peak*** value (see *The Delta peak setting, on page 338*).
Peak max	The signal falls to a specified fraction of the most recent peak maximum minus the ***Delta peak*** value.
Stable signal	The signal is stable, within the accepted fluctuation given by the relevant ***Watch parameters*** instruction (see *Insert a Watch parameters instruction, on page 337*), for the period specified by the minutes parameter.
Equals	Air sensor test parameter, explained below.

Note: In order to set a valid slope value, use the ***Differentiate*** function in the ***Evaluation*** module to measure the slope of the test chromatogram.

Actions when a Watch condition is met

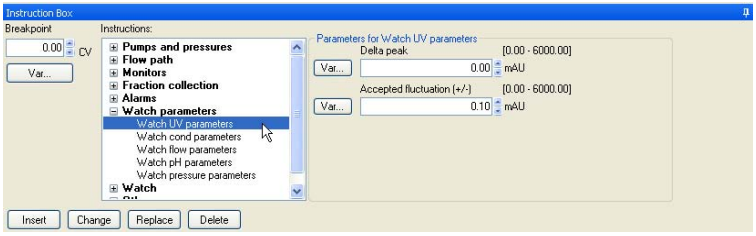
The selection in the ***Action*** drop-down list will determine what happens when the condition of a Watch instruction is met. The table below describes the possible actions:

Instruction	Effect
Block name	Calls the named block. **Note:** All available method blocks are listed in alphabetical order in the drop-down list, before the general actions which are listed below.
Continue	Continues the method if paused or held.
End_block	Ends the current block and return to the point from which the block was called.
Hold	Holds the method, the flow continues. See *Hold instruction, on page 340*.
End_method	Ends the method.
Next_breakpoint	Indicates that the run may execute the next breakpoint.

Instruction	Effect
Pause	Pauses the method, the flow is stopped. See *Pause instruction, on page 340*.

Insert a Watch parameters instruction

Watch parameters instructions are used to define accepted limits and fluctuations for a signal in a ***Watch*** instruction. ***Watch parameters*** instructions should therefore be inserted just before the ***Watch*** instruction on which the limits are required.

Step	Action
1	Select the instruction line immediately before the ***Watch*** instruction to which the parameters will apply.
2	• Expand ***Watch parameters*** in the ***Instructions*** field of the ***Instruction Box***. • Select the desired watch parameters from the list. • Select appropriate values for the ***Accepted fluctuation*** and ***Delta peak*** (for the ***Watch UV parameters*** and ***Watch cond parameters*** instructions) in the ***Parameters*** field. For information about the ***Delta peak*** setting and how to use it, see *The Delta peak setting, on page 338*. 
3	Click the ***Insert*** button. *Result:* The new ***Watch parameters*** instruction is inserted in the method in the text area.

The Delta peak setting

The ***Delta peak*** setting in the ***Watch parameters*** helps the software to detect valleys, peaks and peak maxima, and to filter noise in the chromatogram.

The ***Delta peak*** value should be set

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

  and

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

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

Use of the Delta peak setting

The ***Delta peak*** setting in the ***Watch parameters***

- sets the threshold for signal increase after a local minimum that will be interpreted as a valley for the ***Less than or valley*** condition. A valley and a new peak are detected when the signal increases to 102% of the local minimum plus the ***Delta peak*** value.
  Note: A valley is detected only after a ***Peak max*** has been detected.

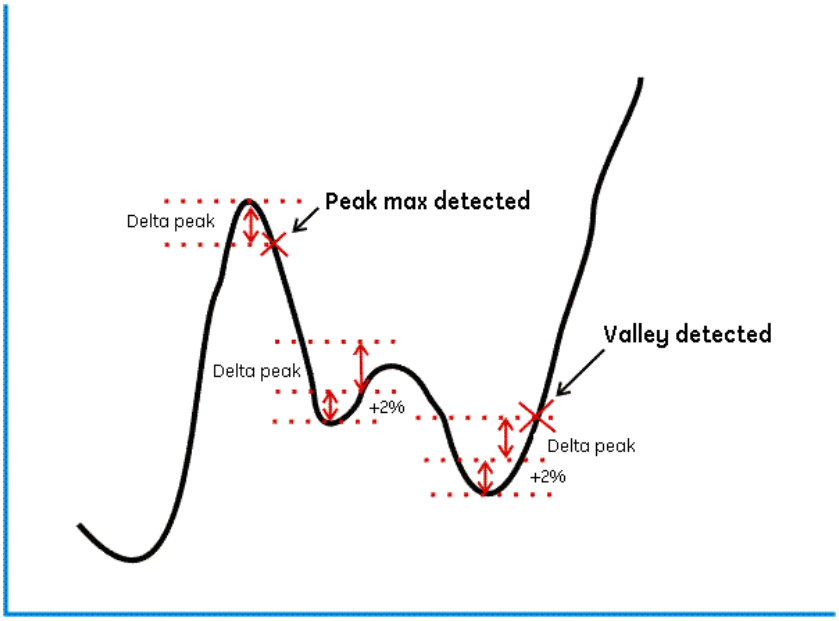
  Example:

  If there is a local minimum at 50 mAU and a ***Delta peak*** of 10 mAU, a valley will be detected at:

  ```
  (1.02 x 50) + 10 = 61 mAU
  ```

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

  The schematic figure below illustrates the ***Delta peak*** setting where ***Peak max*** is detected when the signal falls by ***Delta peak*** from a local maximum if the ***Peak max Factor*** is set to ***1*** in ***Watch:Watch:Parameters for Watch***:



Watch test parameter for air sensors

Two ***Watch*** conditions are available for air sensors. The table below describes the conditions and their explanations:

Equals	Explanation
No air	No air detected.
Air	Air detected.

Note: To use the ***Watch*** parameters for an air sensor, the corresponding ***Alarm air sensors*** setting must be disabled.

9.3.4 Pause or hold a method

Introduction

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

Pause instruction

The ***Pause*** instruction suspends execution of the method and stops the pumps so that the system comes to a standstill. The valves remain in the position they were in before the pause.

The pause may be defined as ***Infinite*** or for a specified number of minutes.

Resume the method

It is possible to define the pause time for the method in the ***Pause*** instruction. The method will continue when the set time has elapsed.

The method may also be resumed if you click the ***Continue*** icon on the ***System Control*** toolbar:

Note: If the pause is set to Infinite, the method must be resumed manually by clicking the ***Continue*** icon.

Hold instruction

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

Resume the method

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

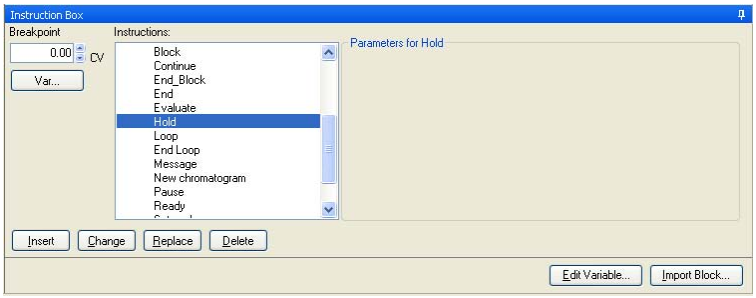
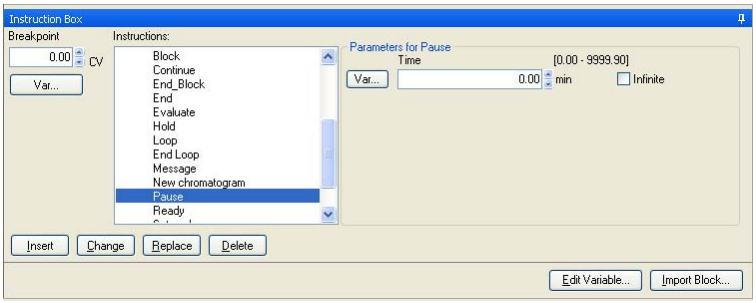
Note: With the ***Hold*** instruction, the method must always be resumed manually by clicking the ***Continue*** icon.

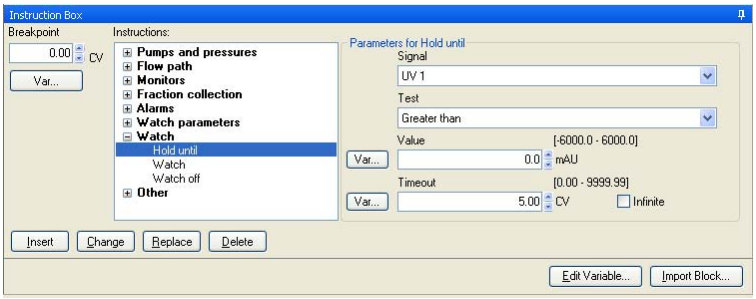
Hold until instruction

The ***Hold until*** instruction is a special kind of ***Watch*** instruction. The method is put on hold until a specific condition is met (***Signal***, ***Test*** or ***Value***) or the ***Timeout*** is reached. Thereafter the remaining instructions in the method are executed. See *Section 9.3.3 Watch instructions, on page 333* for a description of ***Watch*** instructions.

Insert a Pause, Hold or Hold until instruction

The table below describes how to insert a ***Pause***, ***Hold*** or ***Hold until*** instruction:

Step	Action
1	At a suitable ***Breakpoint*** in the method, select the instruction line immediately before where you want to insert the ***Pause***, ***Hold*** or ***Hold until*** instruction (this decides when the instruction begins).
2	To insert a ***Hold*** instruction, select ***Other:Hold*** in the ***Instructions*** field of the ***Instruction Box***. 
3	To insert a ***Pause*** instruction, select ***Other:Pause*** and enter the ***Time*** for the method to be paused in the ***Time*** field. To pause the method for infinite time, check the ***Infinite*** box. 

Step	Action
4	To insert a ***Hold until*** instruction: • select ***Watch:Hold until*** in the ***Instructions*** field of the ***Instruction Box*** • select the appropriate parameters for the ***Hold until*** instruction in the ***Parameters for Hold until*** area. See *Section 9.3.3 Watch instructions, on page 333* for descriptions of the available settings. 
5	Click the ***Insert*** button. *Result:* The new instruction is inserted in the ***Text Instructions*** area. **Note:** Instructions that share the same breakpoint as the ***Hold until*** instruction, but are placed after it in the method, will be executed after the ***Hold until*** conditions have been met.

9.3.5 Messages and Set marks

When to use a message

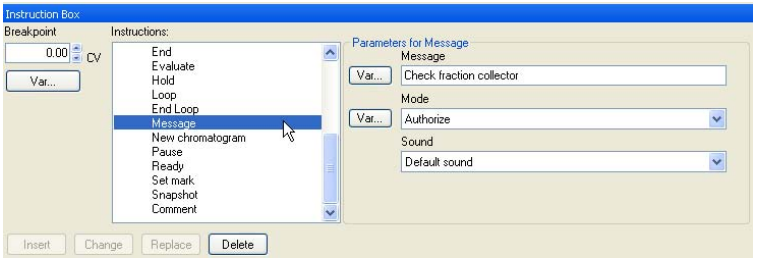
Messages are used to inform the operator of the progress of the run or to prompt the user for an action. It is a good idea to issue messages at critical points in the method, for example, in combination with a ***Pause*** instruction to inform the operator that the inlet tube needs to be moved to another inlet.

Insert a Message instruction

The ***Message*** instruction can be used to set up a message that will be displayed for the user during the execution of the method run. The message can be for information in a screen only, or it can require a signature before the user can control the system. The messages are all added to the logbook text.

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

Step	Action
1	• Select ***Other*** in the ***Instructions*** field of the ***Instructions box***. • Select ***Message*** in the instructions list.
2	Type a message in the ***Message*** text box in the ***Parameters*** field.
3	Select one of the display options on the ***Mode*** menu: • ***Screen***, that is, only a text message is displayed. • ***Noscreen***, that is, the message will not be displayed but only inserted into the logbook. • ***Authorize***, that is, the message will require a signature from the user before the user can interact with the system again.



Step	Action
4	• Select a sound on the ***Sound*** menu if desired. • Click the ***Insert*** button.

Note: If the ***Message*** instruction is inserted in a conditional block it will only be displayed if the conditions of the block (for example a ***Watch***) is fulfilled.

When to use a Set mark

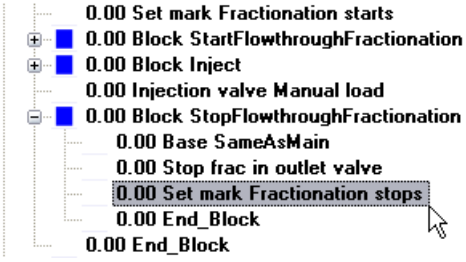
Set mark instructions are useful text messages. They can be used

- to highlight certain stages in a method
- to insert manual notes, for example, when a specific event occurs in a run (only in ***System Control***)

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

Example of a Set mark

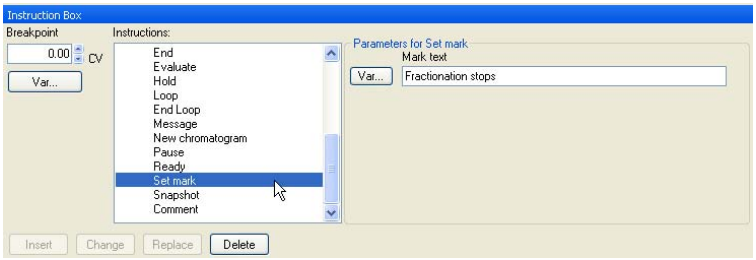
The illustration below shows an example where ***Set marks*** are used to highlight the start and end of fractionation in a method:



Insert a Set mark

Set marks are inserted from the ***Instructions box***. The table below describes how to do this:

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



10 Troubleshooting

Introduction

This chapter describes different problems which may arise when creating methods in UNICORN, and how to solve the problems. It also describes how to generate a system error report describing performance problems.

In this chapter

This chapter contains these sections:

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10.1 Troubleshooting methods	347
10.2 System Error Reports	353

10.1 Troubleshooting methods

Introduction

This section describes how to solve the following method problems:

- The ***Phase Properties*** tab only shows a variables table
- You cannot find settings that you need in the ***Phase Properties***
- There are red instructions in a method
- Breakpoints are not calculated correctly
- Volumes are smaller or larger than expected after a method is converted
- A column cannot be selected for scaling
- The **Print Screen** command does not send a copy of the screen to the printer
- Undefined inlets are used briefly for CIP or preparation phases

The Phase Properties tab only shows a Phase Variables table

The table below describes how to restore the options and settings to the ***Phase Properties*** tab:

Problem description	Solution
The ***Phase Properties*** tab shows only a variables table and not the regular options and settings for the selected phase. The phase is marked with the letter "T" in the method outline.	The phase has been edited in the ***Text Instructions*** tab. Click the ***Restore Phase Properties*** button to return to the default settings and restore the ***Phase Properties*** options and settings. Note that if the text edited settings also involve subsequent phases and the general ***Method Settings***, all these phases are changed as well and you must restore them all individually.

Options are not available in the phase properties

The table below describes what to do if the standard settings available in the ***Phase Properties*** for a phase are not suitable for your specific application needs:

Problem description	Solution
Options that you need are not available for selection or editing in the ***Phase Properties***.	• Add a ***User Defined*** phase to the method and edit the properties in the ***Text Instructions*** tab or • Text edit the phase where the option is required.

There are red instructions in a method

Red instructions (instructions with a red dot) in a method are syntax errors and may have several causes. A phase containing syntax errors is marked in the method outline with a red cross. The table below describes some solutions to syntax error problems:

Problem description	Solution
The method instructions do not correspond to the components you have chosen for your system.	Check your system components under ***System Properties*** in the ***Administration*** module and that the correct instrument configuration is selected.
Syntax errors are not corrected by changing the component configuration.	Close and reopen the method.

Problem description	Solution
Syntax errors appear because the method was connected to the wrong system. That is, the instrument configuration of the system is incompatible with the method.	• Edit the method so it can be run on the currently chosen system. Red instructions must be removed. • Save the method for a system that has all components installed. **Note:** The red instructions must be replaced. • Reselect the required component under ***System Properties*** in the ***Administration*** module (if the component is actually present on the system). Reopen the method and replace the red instructions with the corresponding instruction for the added component.
Syntax errors appear because the system's instrument configuration has been updated with a new instrument configuration that differs in the instruction set.	Select the red instruction and either delete it or replace it with a corresponding instruction (if available) from the ***Instruction box***. Repeat this for all red instructions before saving the method.
Syntax errors appear because the method was converted for use with a system with a component set up differ from the component set up of the system for which the method was originlly created.	Select the red instruction and either delete it or replace it with a corresponding instruction (if available) from the ***Instruction box***. Repeat this for all red instructions before saving the method.
A phase is marked as incorrect (with a red cross). This may appear if • the instrument configuration has been changed • components have been removed or • the method was converted from a system with a different component set up	Replace the phase with a compatible phase from the ***Phase Library***. This phase will automatically be adapted to the current instrument configuration and component settings.

Breakpoints are not correctly calculated

The table below describes how to solve problems with calculation of breakpoints in the method, for example in the ***Method Duration and Variables*** dialog.

Problem description	Solution
Method breakpoints are not calculated. All values are shown as zero.	If the method block uses volume or column volume base, the breakpoints are calculated from the pump flow rate. Check that the flow rate is not zero.

A converted method generates unexpected results

The table below describes how to solve problems when a converted method generates unexpected results.

Problem description	Solution
When running the method, volumes are generally smaller or larger than expected	• Ensure that the method uses Column Volume (CV) as base unit • Verify that all parameter settings that need manual adjustments after the conversion are updated and • Review all text edited phases to locate system parameters that must be edited. For more information, see *Section 3.6 Scale or convert methods, on page 69*.

A column cannot be selected when converting and scaling a method

The table below describes how to solve problems when a column cannot be selected for conversion and scaling of a method.

Problem description	Solution
When converting the method including scaling of the column, the field for column scaling is inactive	The reason for this may be that either the option ***Scale*** was not selected, or that the ***Any*** column was selected in the original method. If ***Scale*** was selected, either • select a column in the original method and repeat the conversion or • convert the method to the new system first and select a column in the converted method afterwards.

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

The table below describes how to solve a printing problem:

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

Inappropriate inlet settings for CIP or preparation

The table below describes how to ensure that the inlet settings are correct for a CIP or preparation phase:

Problem description	Solution
When a CIP or preparation phase (CIP column, CIP system, Prepare column or Prepare system) is started, inlets that are not defined in the phase are briefly used. **Note:** This happens for a very short time and it will normally not cause any problems.	Check which inlets are chosen in ***Method Settings***. Choose the same inlets as required for the CIP or preparation phase.

10.2 System Error Reports

Introduction

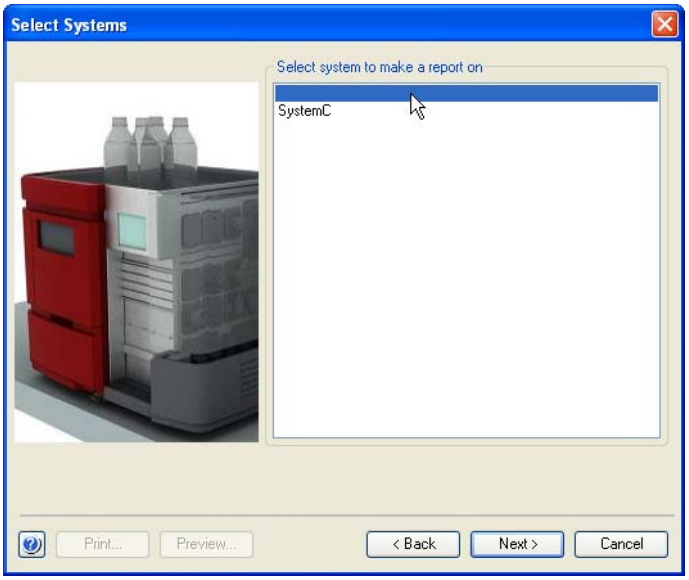
The ***Generate System Error Report Wizard*** is used to generate problem reports. The report can provide useful background information for the support staff when trying to provide solutions to problems or suggestions for improved system performance.

Step 1: Create an error report

This table below describes how to activate the ***Generate System Error Report Wizard*** and create a report:

Step	Action
1	• Choose the ***Reports:Create System Error Report*** menu command in the ***Administration*** module [1]. *Result*: The ***Generate System Error Report Wizard*** opens. **Note:** If an error occurs during a method run you can also start the wizard by clicking the ***Report*** button in the error message dialog. • Click the ***Next*** button.

1 You can also create an error report from ***System Control*** by choosing ***System:Create System Error Report***. A system must be connected. The report will be created for the connected system and step 2 in the instruction above will be omitted.

Step	Action
2	The ***Select Systems*** dialog is displayed, showing all accessible systems. **Tip:** In this and all subsequent dialogs you can always click the ***Back*** button to return to previous dialogs and change the entries. • Select the system that the error is connected to. • Click the ***Next*** button. **Note:** If the problem is general, related to the UNICORN software and cannot be connected to a specific system you do not have to select a system in this step. Ensure that the empty space above the first system is selected (as illustrated below) and click ***Next*** to proceed. However, it is an advantage in subsequent troubleshooting if at least one system can be referenced. 
3	The ***Error Description*** dialog opens. Add the following information: • A short description of the problem. • The circumstances under which the problem occurs. • The consequences of the problem. Click the ***Next*** button.

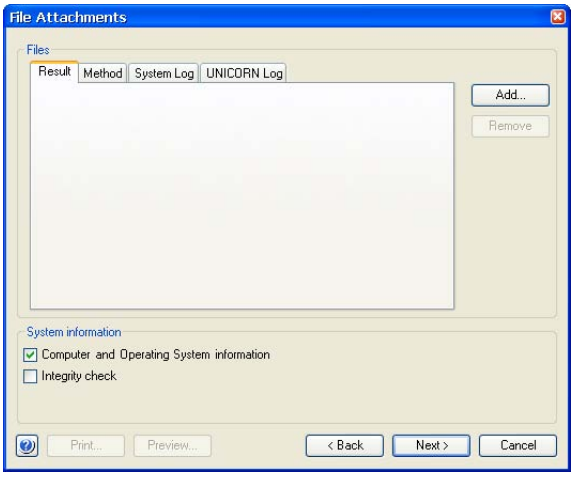
Step	Action
4	The ***Error Reproducibility*** dialog opens. • Specify whether the problem is reproducible or not. Select one of these alternatives: • - ***Yes*** (Provide a short description in the text box of how the problem can be reproduced.) - ***No*** - ***Unknown***. • Click the ***Next*** button. *Result*: The ***File Attachment*** dialog opens.
5	Go to step 2 below.

Step 2: Attach example files

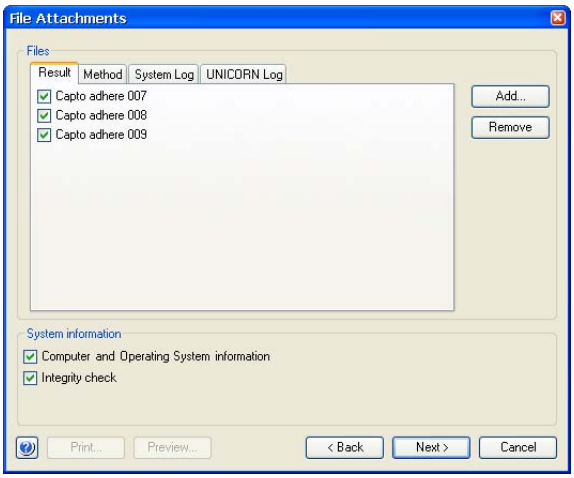
You can attach results, methods and/or UNICORN log files to the problem report.

The table below describes how to attach a file:

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



Step	Action
2	• Depending on the character of the file to be attached, select the appropriate tab: ***Result***, ***Method***, ***System Log*** or ***UNICORN Log***. • Attach a file: - Click the ***Add*** button. - Select a file in the dialog and click the ***Attach*** button. *Result*: The selected file is added to the tab in the ***File Attachments*** dialog.



Tip: To remove a file from the tab, select the checkbox and click the ***Remove*** button.

When attaching the ***UNICORN Log***, a separate dialog will open. Choose the time period for the attached log.

Step	Action
3	To include more information in the report, select the appropriate check boxes in the ***System information*** field. **Computer and Operating System information** A summary of the computer and operating system information, for example type of processor, processor speed, RAM, hard disk capacity and printer. **Integrity check** When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check means that a new checksum calculation is performed for the same files in their folders. This new calculated value is compared to the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included. • Click the ***Next*** button. *Result*: The ***Generate System Error Report*** dialog is displayed.
4	Go to step 3 below.

Step 3: Generate and save the report

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

Step	Action
1	By default, the report is saved as a zip file in the UNICORN folder on your local computer. If you want to save the report in another location, click the ***Browse*** button and select a destination folder.
2	You also have these options: • Click the ***Preview*** button to open the report in Notepad. • Click the ***Print*** button to print the report without any preview.
3	• Click the ***Finish*** button. *Result*: The report is generated and saved and the wizard dialog closes.

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