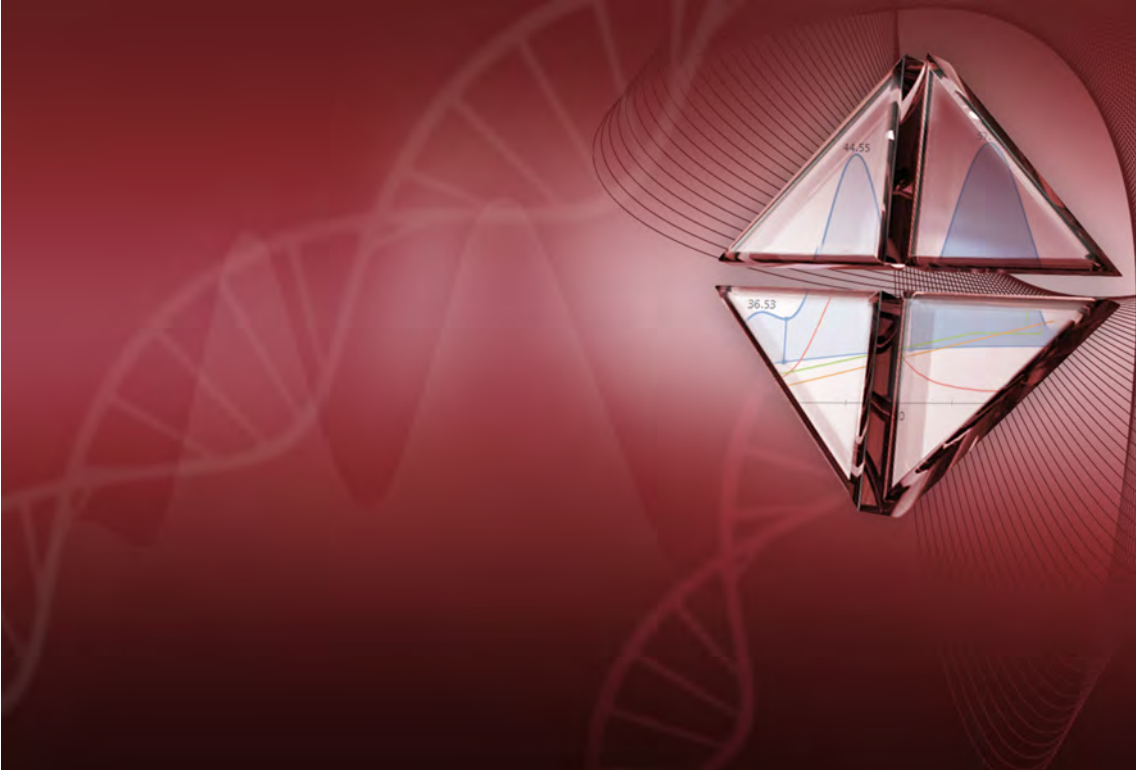


UNICORN™ 7.0

Method Manual



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Table of Contents

1	Introducing the UNICORN Method Editor	7
1.1	About the UNICORN Method Editor	8
1.2	About this manual	10
1.3	Associated documentation	13
2	The UNICORN Method Editor	14
2.1	The Method Editor	15
2.2	Methods in UNICORN 7.0	21
3	Create and edit methods	27
3.1	Working with methods - Overview	28
3.2	Open a method	33
3.3	Working with predefined methods	35
3.3.1	<i>Predefined methods and phases</i>	36
3.3.2	<i>Create a predefined method</i>	48
3.3.3	<i>Edit phase properties</i>	50
3.3.4	<i>Fraction collection</i>	58
3.4	Working with wizard generated methods	61
3.4.1	<i>Create a wizard generated method</i>	62
3.4.2	<i>Wizard generated methods</i>	64
3.4.3	<i>Frac-950</i>	67
3.5	Working with empty methods	69
3.5.1	<i>Create an empty method</i>	70
3.5.2	<i>Edit an empty method</i>	72
3.6	Working with methods in general	74
3.6.1	<i>Edit the method outline</i>	75
3.6.2	<i>Set general method options for the method</i>	83
3.6.3	<i>Print a method</i>	94
3.6.4	<i>Sign methods electronically</i>	96
3.7	Save methods and phases	98
3.8	Scale or convert methods	104
3.9	Import and export methods	113
4	Scouting	126
4.1	Overview	127
4.2	Set up and edit a Scouting scheme	129
5	Design of Experiments	139
5.1	Introduction to Design of Experiments	140
5.2	Create an experimental design	152
5.2.1	<i>Set up an experimental design</i>	153
5.2.2	<i>Add responses and factors to an experimental design</i>	162
5.2.3	<i>Change design and design settings in a Design of Experiments setup</i>	169
5.2.4	<i>Divide the DoE runs into several scouting runs</i>	173
5.3	Run a scouting created with DoE	178

Table of Contents

5.4	Evaluation of Design of Experiments	180
5.4.1	Workflow	181
5.4.2	Generate model	183
5.4.3	Analyze and evaluate the model - basic analysis	192
5.4.4	Analyze and evaluate the model - extended analysis	204
5.4.5	Edit the model	211
5.4.6	Use the model	214
5.4.7	Create and print reports	224
6	BufferPro	227
6.1	BufferPro - Overview	228
6.2	Create a method using BufferPro	230
6.3	Create and edit BufferPro recipes	231
6.3.1	Create and edit a BufferPro recipe	232
6.3.2	Rename a BufferPro recipe	237
6.3.3	Delete a BufferPro recipe	239
6.4	Print a BufferPro recipe	241
6.5	Calculate buffer composition using BufferPro	243
6.6	Export and import BufferPro recipes	247
6.7	Predefined BufferPro recipes	251
7	Method queues	254
7.1	Method queues - overview	255
7.2	Create a method queue	256
7.3	Edit a method queue	260
7.4	Import and export method queues	266
8	Import and export folders	270
9	Column Handling	273
9.1	Overview	274
9.2	Handling column types	280
9.3	Handling individual columns	293
9.3.1	Individual column identification	294
9.3.2	Register a new individual column	295
9.3.3	Find an individual column	298
9.3.4	Edit individual columns	300
9.3.5	Export and import individual columns	303
9.3.6	Print and view individual column information	306
9.4	Column performance	308
9.5	Intelligent Packing of AxiChrom columns	312
9.5.1	AxiChrom column types and individual AxiChrom columns	314
9.5.2	Predefined Intelligent Packing method	318
9.5.3	Wizard generated Intelligent Packing method	320
10	Text edit methods	325
10.1	Overview	326
10.1.1	Working with text instructions	327

10.1.2	<i>The Text Instructions pane</i>	329
10.2	Working with methods in the Text Instructions pane	334
10.2.1	<i>Base instruction</i>	335
10.2.2	<i>Working with phases and blocks</i>	339
10.2.3	<i>Working with text instructions</i>	347
10.2.4	<i>Method variables</i>	353
10.3	Specific instructions	364
10.3.1	<i>Gradients and eluent concentrations</i>	365
10.3.2	<i>Alarm instructions</i>	368
10.3.3	<i>Watch instructions</i>	371
10.3.4	<i>Pause or hold a method</i>	378
10.3.5	<i>Messages and Set marks</i>	381
11	Troubleshooting	384
	Index	390

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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 7.0 is technically compatible with all relevant sections of FDA 21 CFR Part 11. A part 11-system assessment checklist is available on request from your local GE representative.

In this chapter

This chapter contains these sections:

Section	See page
1.1 About the UNICORN Method Editor	8
1.2 About this manual	10
1.3 Associated documentation	13

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.
-

Workflow

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



This manual describes step 1 of this workflow.

Tip: *Step 2, how to perform method runs, is described in the UNICORN System Control Manual. Step 3, evaluate the results, and step 4, compile a report, are described in the UNICORN 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 composed of one or several predefined or user defined phases which are reusable sets of instructions. Examples of predefined phases are equilibration and elution phases. An empty user defined phase is also available.

The UNICORN **Method Editor** module is a comprehensive tool for creating or editing methods either by using predefined methods and phases, wizard generated methods or user defined text edited methods. Depending on the system, the Method Editor can for example be used to:

- build a method from a library of phases.
 - build a method with guidance from a method wizard.
 - 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.
-

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™ system. It covers the features and tools included in the Method Editor module of the UNICORN software with practical instructions. Some functionality is only available for some systems.

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.

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 function 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***

Menu 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 monospaced typeface within quotation marks:

Example: "Connection change"

File system paths are represented by a monospaced typeface:

Example: C:\Program Files\GE Healthcare\UNICORN

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.

1 Introducing the UNICORN Method Editor

1.2 About this manual

- Your user profile and access group 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 Associated documentation

Introduction

This section describes the user documentation that is delivered with UNICORN.

User documentation

The user documentation listed in the table below is available from the **Help** menu in UNICORN and as printed books.

Document	Main contents
UNICORN 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 Evaluation Manual	Overview and detailed descriptions of the Evaluation Classic module. Workflow descriptions for common operations. Description of the evaluation algorithms used in UNICORN.
UNICORN Administration and Technical Manual	Overview and detailed description of network setup and complete software installation. Administration of UNICORN and the UNICORN database.
UNICORN System Control Manual	Overview and detailed description of the system control features in UNICORN. Includes general operation, system settings and instructions on how to perform a run.
UNICORN Online Help	Dialog descriptions for UNICORN (from the Help menu).

2 The UNICORN Method Editor

About this chapter

This chapter gives an introduction to the **Method Editor** in UNICORN 7.0. It gives a brief description of the **Method Editor** interface and describes the concept of methods in UNICORN 7.0.

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 27*.

In this chapter

This chapter contains the following sections:

Section	See page
2.1 The Method Editor	15
2.2 Methods in UNICORN 7.0	21

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 system types.

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 Experiments (DoE) (system specific)	<p>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.</p> <p>When creating a method and setting up an experimental design using DoE, an optimized Scouting scheme will automatically be created.</p> <p>See <i>Chapter 5 Design of Experiments, on page 139</i> for more information.</p> <p>Note: <i>DoE requires an e-license.</i></p>
Scouting	<p>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.</p> <p>See <i>Chapter 4 Scouting, on page 126</i> for more information.</p>

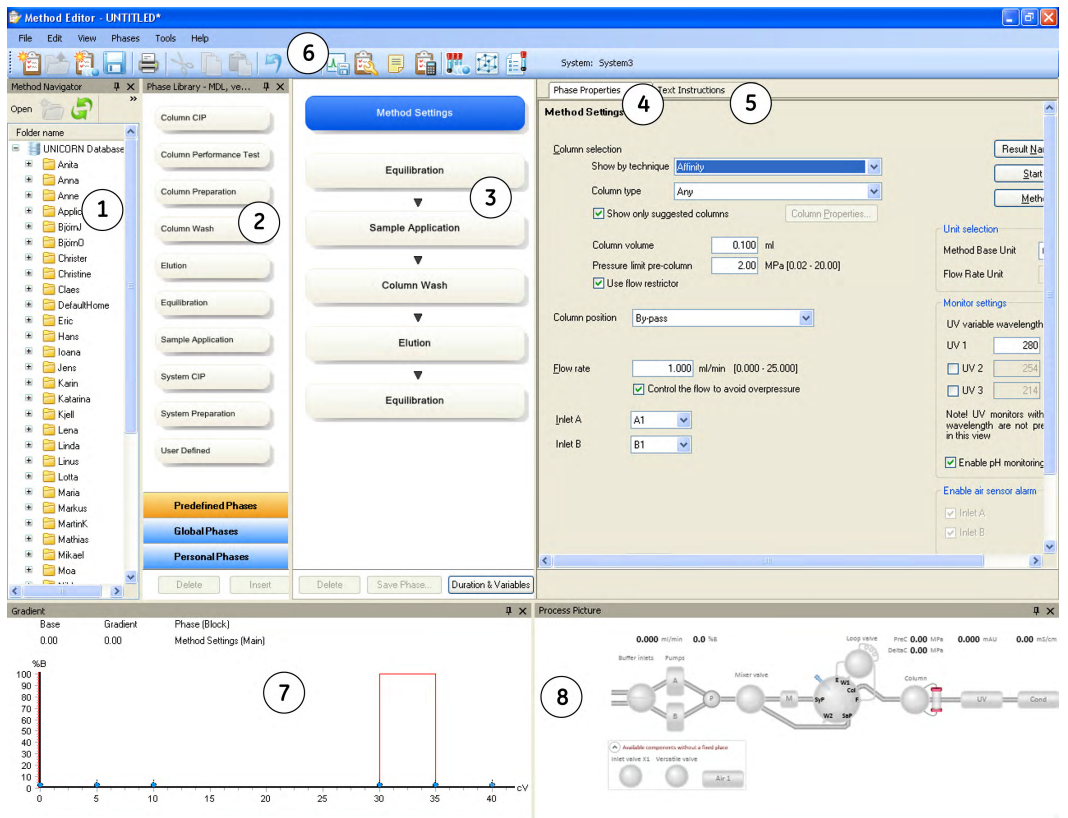
Tool	Description
BufferPro (system specific)	<p>BufferPro allows a buffer of defined pH, and with defined salt concentrations to be prepared from four stock solutions (one Buffer stock solution, one Titrant, Water and a Salt stock solution). pH and salt concentration can be used as variable scouting 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.</p> <p>See <i>Chapter 6 BufferPro</i>, on page 227 for more information.</p>
Column Handling	<p>Column Handling enables handling of column types and individual columns.</p> <p>See <i>Chapter 9 Column Handling</i>, on page 273 for more information.</p> <p>Note: <i>Parts of Column Handling requires an e-license.</i></p>

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 **Process Picture**.

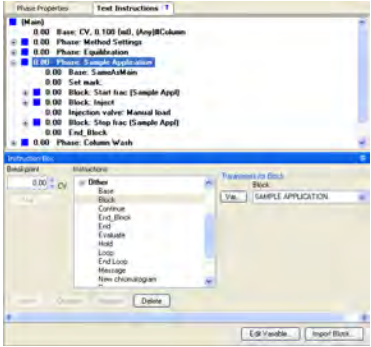
The illustration below shows the **Method Editor** with all the optional panes displayed.



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 . For wizard generated and text edited methods, the Phase Properties shows a list of variables.

2 The UNICORN Method Editor

2.1 The Method Editor

Area	Description
5	<p>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.</p> 
6	<p>Toolbar (optional pane): Shows the toolbar icons.</p>
7	<p>Gradient (optional pane): Shows the programmed gradient and break points for included phases and blocks.</p>
8	<p>Process Picture (optional pane): Illustrates the flow path of the instrument graphically.</p>

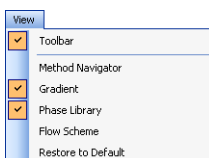
Note: For detailed information on the **Toolbar** and the different panes in the **Method Editor**, see "Getting Help on the Toolbar and panes in the Method Editor" below.

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 and are applied 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

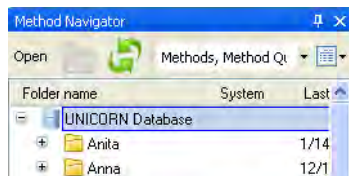
The optional panes may either be displayed statically in the position where they open, or the **Auto Hide** function can be selected to automatically hide/display the pane when moving the mouse pointer over the position of the pane.

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

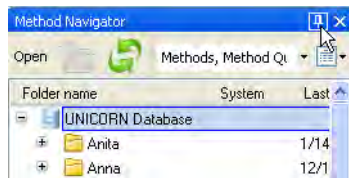
Step	Action
------	--------

- | | |
|---|--|
| 1 | If not already displayed, open the Method Navigator in the Method Editor by selecting View:Method Navigator . |
|---|--|

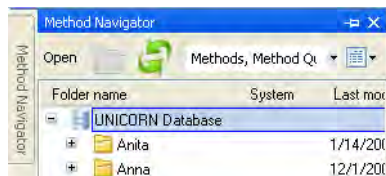
Result: The **Method Navigator** pane is displayed.



- | | |
|---|---|
| 2 | To turn on the Auto Hide function, click the vertical pin symbol in the top right hand corner. |
|---|---|

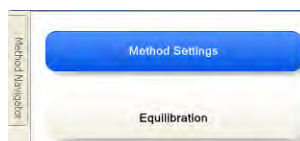


Result: The pin symbol is rotated to horizontal position and a tab named **Method Navigator** is displayed to the left.



- | | |
|---|---|
| 3 | Click outside the Method Navigator . |
|---|---|

Result: The **Method Navigator** is hidden and only the **Method Navigator** tab is displayed.



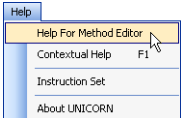
2 The UNICORN Method Editor

2.1 The Method Editor

Step	Action
4	<ul style="list-style-type: none">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 right hand corner of the Method Navigator pane. <p><i>Result:</i> The Method Navigator pane is displayed statically.</p>

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	<p>To display detailed information about the Toolbar and different panes in the Method Editor interface, select Help:Help For Method Editor.</p>  <p><i>Result:</i> The online help opens displaying the Method Editor help start page.</p>
2	<p>To display help for a specific pane, click in the pane and press the F1 keyboard key.</p> <p><i>Result:</i> The online help page describing that pane is opened.</p>

2.2 Methods in UNICORN 7.0

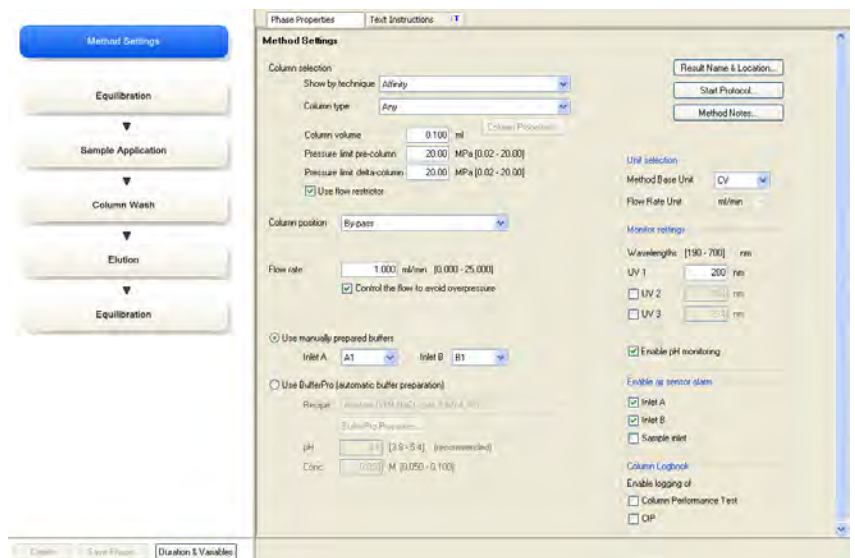
About methods

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**. In a predefined method (only available for some systems) 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 27* for information about creating and editing methods in the **Method Editor**.

The illustration below shows the phases in a predefined method in the **Method Outline** and the corresponding settings for the highlighted phase in the **Phase Properties** pane. The image is specific for systems that can use predefined methods.



Method structure

A method always starts with the **Method Settings** phase. This phase contains general settings that affect the rest of the method. For example, all systems have the settings **Column type** and **Column volume** and some systems have **Flow rate** and **Method Base Unit**. If **Column type** is changed for a predefined method, UNICORN will automatically calculate correct settings for volume, flow rate, and pressure limits. For wizard generated methods, UNICORN will only check that the pressure and flow limits are not exceeded when saving a method. Subsequent phases reflect steps included in the chromatography run.

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

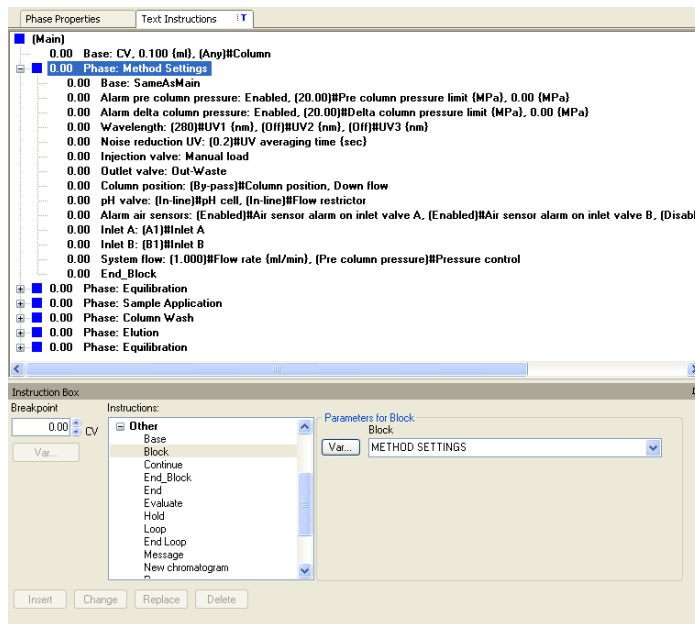


Working with methods

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 illustrations below show the text instructions and the phase properties settings for the **Method Settings** phase in a predefined method.

Note: A wizard generated method consists of a basic method settings phase and a user defined phase that contains the variable list.



2 The UNICORN Method Editor

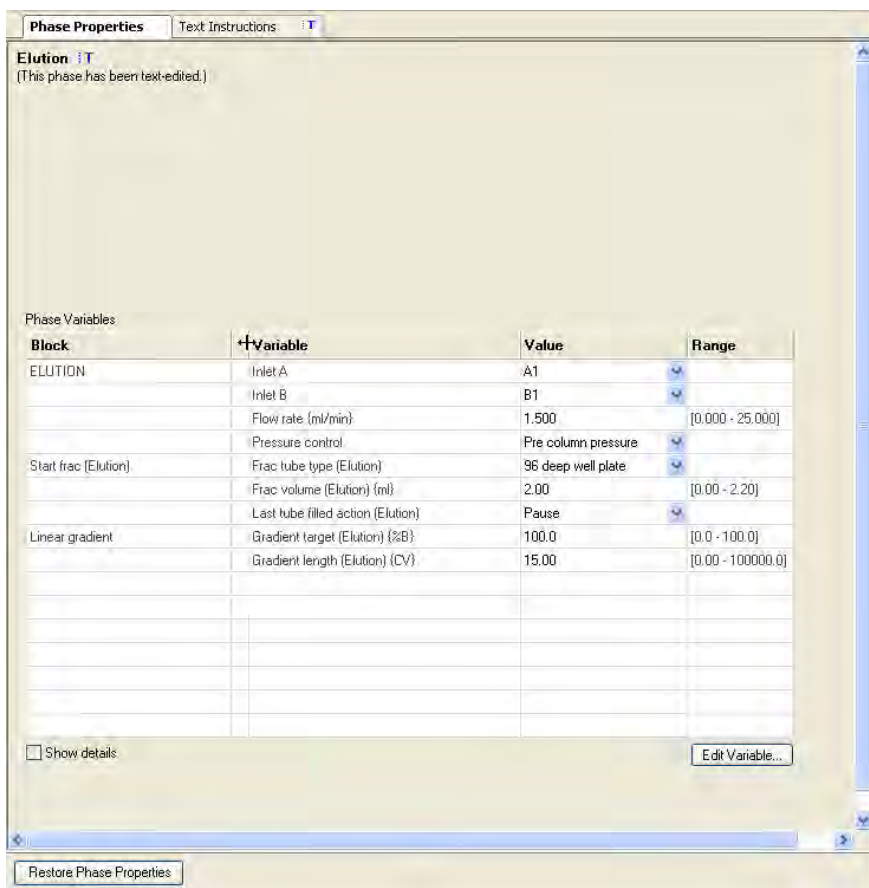
2.2 Methods in UNICORN 7.0

The screenshot displays the 'Method Settings' window in UNICORN 7.0. The window has two tabs: 'Phase Properties' and 'Text Instructions', with 'Text Instructions' being the active tab. The 'Method Settings' section is divided into several sub-sections:

- Column selection:** 'Show by technique' is set to 'Affinity', 'Column type' is 'Any', and 'Column volume' is '0.100 ml'. There are buttons for 'Column Properties...' and 'Result Name & Location...'. 'Start Protocol...' and 'Method Notes...' buttons are also present.
- Pressure limits:** 'Pressure limit pre-column' and 'Pressure limit delta-column' are both set to '20.00 MPa [0.02 - 20.00]'. A 'Use flow restrictor' checkbox is checked.
- Flow rate:** 'Flow rate' is '1.000 ml/min [0.000 - 25.000]'. A 'Control the flow to avoid overpressure' checkbox is checked.
- Column position:** Set to 'By-pass'.
- Buffer preparation:** 'Use manually prepared buffers' is selected. 'Inlet A' is 'A1' and 'Inlet B' is 'B1'. The 'Recipe' is 'Acetate 0.1M NaCl - (pH 3.8-5.4, PD)'. 'pH' is '4.6 [3.8 - 5.4] (recommended)' and 'Conc' is '0.050 M [0.050 - 0.100]'. There is a 'BufferPro Properties...' button.
- Unit selection:** 'Method Base Unit' is 'CV' and 'Flow Rate Unit' is 'ml/min'.
- Monitor settings:** 'Wavelengths [190 - 700] nm' are listed: 'UV 1' is '280 nm', 'UV 2' is '254 nm', and 'UV 3' is '214 nm'. 'Enable pH monitoring' is checked.
- Enable air sensor alarm:** 'Inlet A' and 'Inlet B' are checked, while 'Sample inlet' is unchecked.
- Column Logbook:** 'Enable logging of' includes 'Column Performance Test' (unchecked) and 'CIP' (unchecked).

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 or edited one by one. This can be an option for fine-tuning or optimization of a method. If the text editor is used for a predefined phase, **Phase Properties** will subsequently only show a list of variables for the phase, as shown in the following illustration. For a predefined phase 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 10 Text edit methods*, on page 325 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.

Method types

UNICORN supplies a number of **Predefined** methods for different separation techniques and maintenance applications (e.g., preparation and cleaning of the system and columns). The phase **Method Settings** is mandatory in all methods.

See *Chapter 3 Create and edit methods*, on page 27 for information about how to create new methods.

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

Method	Description
Predefined	<p>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.</p> <p>See Section <i>Section 3.3.1 Predefined methods and phases, on page 36</i> for descriptions of the Predefined methods supplied with the software.</p> <p>Note: The Predefined methods are included in the instrument configuration files for each specific instrument.</p>
Wizard generated	Wizard generated methods contain a Method Settings phase and a User Defined phase with a variable list.
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 **User Defined** phase which is available for all systems, and a number of other **Predefined Phases** which are available for some systems.

Predefined phases (for example **Equilibration** and **Column CIP**) can be used when building or 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. The **User Defined** phase is an empty phase that can be built by adding text instructions in the text editing field.

See *Section 3.3.1 Predefined methods and phases, on page 36* for descriptions of the predefined phases supplied with the software. See also *Chapter 3 Create and edit methods, on page 27*.

3 Create and edit methods

About this chapter

This chapter describes how to create, edit and handle chromatography and maintenance methods in UNICORN 7.0 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 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 10 Text edit methods, on page 325.*

In this chapter

This chapter contains the following sections:

Section	See page
3.1 Working with methods - Overview	28
3.2 Open a method	33
3.3 Working with predefined methods	35
3.4 Working with wizard generated methods	61
3.5 Working with empty methods	69
3.6 Working with methods in general	74
3.7 Save methods and phases	98
3.8 Scale or convert methods	104
3.9 Import and export methods	113

3.1 Working with methods - Overview

Introduction

In UNICORN 7.0 the predefined methods are built up using phases, where each phase corresponds to a step in a chromatography run with a number of properties associated with that phase. The wizard generated methods consist of a method settings phase and a user defined phase containing all instructions for the method. See *Section 2.2 Methods in UNICORN 7.0, on page 21* for more information about method structure, definitions and concepts of methods in UNICORN 7.0.

There are three different ways of creating and editing methods in UNICORN 7.0:

- Creating and editing methods using phases and the phase properties in the **Phase-Properties** pane.
 - Creating methods using the wizard.
 - Creating methods by text editing, creating and editing text instructions one-by-one.
-

Main steps when defining a new method using phases

The main steps when defining a method are:

- 1 Create/open a method
 - Create a **Predefined** method (including a set of phases that may be edited) or
 - Open an existing method that can be edited and saved with a new name or overwritten
 - 2 Build/edit the **Method Outline** and/or edit the **Phase Properties** for the appropriate phases
 - **Predefined** methods: use as they are, or edit the **Method Outline** and/or **Phase Properties**
 - Opened methods: edit the **Method Outline** and/or **Phase Properties**
 - 3 Save the method
-

Main steps when defining a new method using the wizard

The main steps when defining a method are:

- 1 Create/open a method
 - Create a method using the method wizard
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
 - Wizard generated methods: use as they are, or edit the **Phase variable list** in the user defined phase
 - Opened methods: edit the **Method Outline** and/or **Phase variable list**
 - 3 Save the method
-

Main steps when defining an empty method

The main steps when defining an empty method are:

- 1 Create/open a method
 - Create a new **Empty** method containing the **Method Settings** phase.
 - 2 Build/edit the **Method Outline** and edit the **Text instructions** for the phases
 - Add user defined or predefined phases to the method (i.e., build the **Method Outline**) and edit the phases as appropriate.
 - 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

3 Create and edit methods

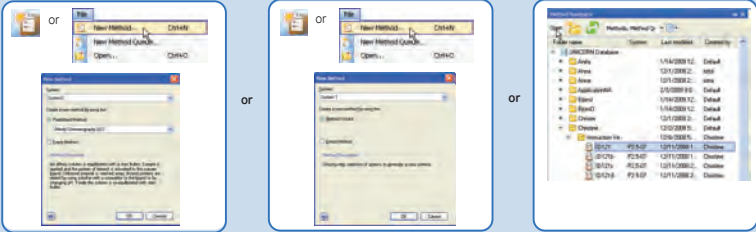
3.1 Working with methods - Overview

- Edit the **Phase Properties** for the appropriate phases or the phase variables for a wizard generated or text created method.
and/or
 - Text edit user defined phases
- 3 Save the method
-

Illustration of workflow when creating or editing a new method

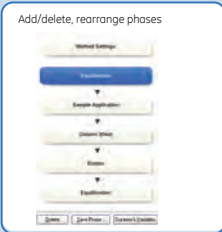
The illustration below shows the workflow in the **Method Editor** when creating or editing a method. The available options depend on the instrument configuration.

1. Create method
Create new method using phases or *Create new method using the wizard* or *Open an existing method*

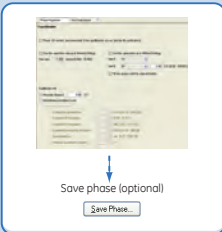


2a. Edit methods using phases
Build/edit Method Outline and/or *Edit Phase Properties* and/or *Text edit instructions*

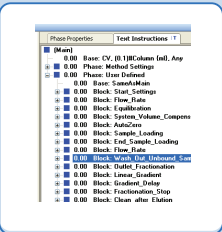
Add/delete, rearrange phases



Edit Phase Properties




Text edit instructions

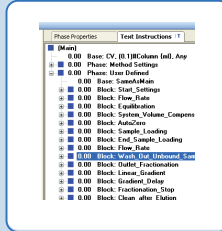


2b. Edit wizard created methods
Edit Phase Variables and/or *Text edit instructions*

Edit Phase Variables

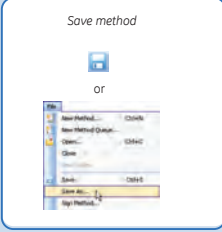


Text edit instructions



3. Save method

Save 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	<ul style="list-style-type: none"> • 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 <p>Note: <i>This information only applies to Evaluation Classic.</i></p> <ul style="list-style-type: none"> • viewing and printing an estimate of the method duration time and the variables in the method <p>See Section 3.6.2 <i>Set general method options for the method</i>, on page 83 for more information.</p>
Method options intended to assist the user in optimizing runs in UNICORN	<ul style="list-style-type: none"> • Scouting See Chapter 4 <i>Scouting</i>, on page 126 for information. • Design of Experiments (DoE) See Chapter 5 <i>Design of Experiments</i>, on page 139 for information. • BufferPro See Chapter 6 <i>BufferPro</i>, on page 227 for information.

3.2 Open a method

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

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

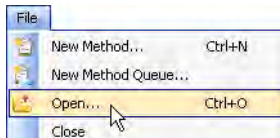
1 In the **Method Editor**:

- Click the **Open Method Navigator** icon in the **Toolbar**



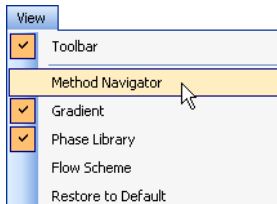
or

- select **File:Open...**



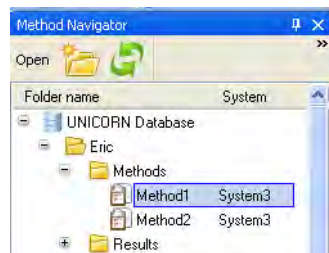
or

- select **View:Method Navigator**



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

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



3 Create and edit methods

3.2 Open a method

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**.



3.3 Working with predefined methods

About this section

This section describes how to work with methods and phases in systems that have access to the full phase library and predefined methods.

In this section

This section contains these subsections:

Section	See page
3.3.1 Predefined methods and phases	36
3.3.2 Create a predefined method	48
3.3.3 Edit phase properties	50
3.3.4 Fraction collection	58

3.3.1 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 phases that are not required.


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.	

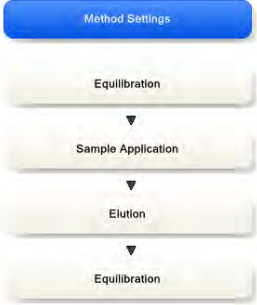
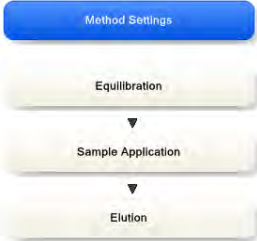
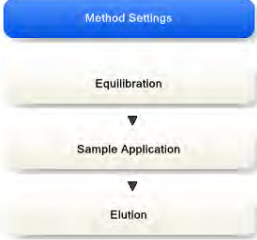
Predefined purification method	Principle	Included phases
<p>Affinity Chromatography (AC) with Tag Removal</p>	<p>After equilibration, the sample of tagged protein is applied. After washing to remove unbound sample the column is equilibrated with cleavage buffer, a protease is applied and the flow is stopped. The cleaved protein and the protease are eluted by starting the flow. The next step regenerates the column, eluting the tag, uncleaved protein and protease (if tagged). Finally, the column is re-equilibrated.</p> <p>Required components</p> <p>The required components for this phase are Inlet valve (2-ports or 7-ports) and Mixer valve.</p> <p>Required solutions</p> <p>The method phases are pre-configured to use the following solutions in the following inlets and sample loop:</p> <p>Inlet A1: Equilibration buffer Inlet A2: Sample Inlet B1: Cleavage buffer Inlet B2: Affinity Regeneration buffer Sample loop: Protease</p> <p>Note:</p> <p><i>The protease application phase is pre-configured to empty the loop with the protease with a volume of 0.7 ml. The recommended protease volume is 0.7 CV (column volume). If a column volume different than 1 ml is used, this value needs to be changed to correspond to 0.7 CV.</i></p>	 <p>The flowchart displays the following phases in order from top to bottom:</p> <ul style="list-style-type: none"> Method Settings Equilibration Sample Application Wash Out Unbound Sample Optional Wash Condition Column with Cleavage Buffer Protease Application Finalize Protease Application Incubation Elution Cleaved Protein Affinity Regeneration Equilibration

3 Create and edit methods

3.3 Working with predefined methods

3.3.1 Predefined methods and phases


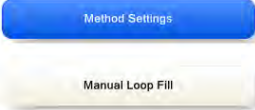
Predefined purification method	Principle	Included phases
Anion Exchange Chromatography (AIEX)	After equilibration and sample application, negatively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (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
<i>Chromatofocusing (CF)</i>	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.	 <p>The diagram shows a vertical sequence of five steps: 'Method Settings' (highlighted in blue), 'Equilibration', 'Sample Application', 'Elution', and 'Equilibration'. Each step is connected to the next by a downward-pointing triangle.</p>
<i>Desalting</i>	After equilibration and sample application, the proteins are eluted isocratically. This technique is commonly used for buffer exchange.	 <p>The diagram shows a vertical sequence of four steps: 'Method Settings' (highlighted in blue), 'Equilibration', 'Sample Application', and 'Elution'. Each step is connected to the next by a downward-pointing triangle.</p>
<i>Gel filtration (GF)</i>	After equilibration and sample application, proteins separate and elute according to their size (largest first).	 <p>The diagram shows a vertical sequence of four steps: 'Method Settings' (highlighted in blue), 'Equilibration', 'Sample Application', and 'Elution'. Each step is connected to the next by a downward-pointing triangle.</p>

3 Create and edit methods

3.3 Working with predefined methods

3.3.1 Predefined methods and phases


Predefined purification method	Principle	Included phases
<i>Hydrophobic Interaction Chromatography (HIC)</i>	After equilibration and sample application (use a buffer containing a high salt concentration, for example 2 M ammonium sulfate) hydrophobic proteins are adsorbed 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.	
<i>Manual Loop Fill</i>	The sample application loops of the loop valve are manually filled with samples. The method will guide the user through the process by pausing and displaying on-screen instructions. Up to five loops can be filled with different samples. Partial or complete loop fill can be chosen. Automatic washing of loops and flow path are integrated in the method. The required components for this phase are the loop valve.	

Predefined purification method	Principle	Included phases
<p>NHS-coupling</p>	<p>A column packed with NHS-activated Sepharose™ is washed with 1 mM HCl, followed by immediate application of the protein for covalent coupling onto the column. After incubation the nonspecifically bound protein is washed out and the remaining active groups are deactivated with ethanolamine buffer, followed by further washes.</p> <p>Required components</p> <p>The required component for this phase is an Inlet valve (2-ports or 7-ports).</p> <p>Required solutions</p> <p>The method phases are preconfigured to use the following solutions in the following inlets and sample loop:</p> <p>Inlet A1: Coupling buffer, for example 0.2 M NaHCO₃ + 0.5 M NaCl pH 8.3.</p> <p>Inlet A2: Activation solution, for example 1 mM HCl</p> <p>Inlet B1: High pH buffer, for example 0.5 M ethanolamine, 0.5 M NaCl pH 8.3</p> <p>Inlet B2: Low pH buffer, for example 0.1 M Sodium acetate, 0.5 M NaCl pH 4.0</p> <p>Sample loop: Ligand in coupling buffer</p> <p>Note:</p> <p><i>The NHS coupling phase is preconfigured to empty the loop with the ligand with a volume of 1 ml. The recommended ligand volume is 1 CV (column volume). If a column volume different than 1 ml is used, this value needs to be changed to correspond to 1 CV.</i></p>	

3 Create and edit methods

3.3 Working with predefined methods

3.3.1 Predefined methods and phases

Predefined purification method	Principle	Included phases
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.	








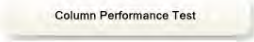

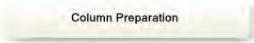
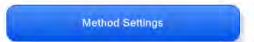


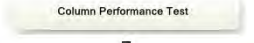

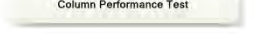
WARNING

Built-in fraction collector. Do not fractionate flammable liquids on instruments with built-in fraction collector. Flammable gas might be formed inside the closed cabinet. 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	<p>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.</p>	 
Column Performance Test	<p>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.</p>	   
Column Preparation	<p>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.</p>	 
Intelligent Packing	<p>Packs AxiChrom™ columns, with a predetermined column type, by motorized power or 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 phases (upflow and/or downflow) can be performed after the AxiChrom column has been packed.</p> <p>Only available for some types of AxiChrom columns.</p> <p>Note: <i>Intelligent packing is also available for systems that use wizard generated methods.</i></p>	     

3 Create and edit methods

3.3 Working with predefined methods

3.3.1 Predefined methods and phases

Predefined maintenance method	Principle	Included phases
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	<p>The first, and mandatory, phase in any method. Defines common parameters used in the subsequent phases.</p> <p>The Method Settings phase defines:</p> <ul style="list-style-type: none"> • Column type • Pressure limits • Flow rate • Option to control the flow to avoid overpressure <p>Note: <i>Default values for pressure limits and flow rate are given for the selected column type.</i></p> <ul style="list-style-type: none"> • Column position • Flow restrictor use • Buffer preparation: <ul style="list-style-type: none"> - Manual, or - BufferPro (automatic buffer preparation) • Unit selection for Method base and Flow rate • Monitor settings: <ul style="list-style-type: none"> - pH monitor - Air sensor alarm settings - UV monitor <p>Note: <i>The first wavelength of U9-M and the fixed wavelength for U9-L or U9-L 2nd is always turned on. The second and third wavelengths for UV monitor U9-M can be set on or off.</i></p> <ul style="list-style-type: none"> • Settings for Column Logbook • Start Protocol • Result name and location <p>Note:</p>

3 Create and edit methods

3.3 Working with predefined methods

3.3.1 Predefined methods and phases

Phase Name	Description
	<p><i>Some of these options may not be required by certain methods.</i></p> <p>Note: <i>The Method Settings phase differs for systems that have the Method settings phase and the User defined phase as the only available predefined phases.</i></p>
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.
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 nonspecifically 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).
Manual Loop Fill	Is used to manually fill the additional sample loops mounted on the loop valve. The filling options are: <ul style="list-style-type: none">• Partial loop fill• Complete loop fill

Phase Name	Description
<i>Miscellaneous</i>	Can be added to any method at suitable places. The instructions can help the user to better organize the graphical output of the results or introduce a controlled delay in the method run.
<i>Intelligent Packing</i>	<p>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.</p> <p>Only available for some systems and some types of AxiChrom columns.</p>

3.3.2 Create a predefined method

The table below describes how to create a new method using phases:

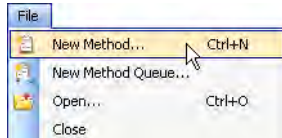
Step	Action
------	--------

- | | |
|---|---|
| 1 | In the Method Editor : <ul style="list-style-type: none">click the Create a new method icon in the Toolbar |
|---|---|

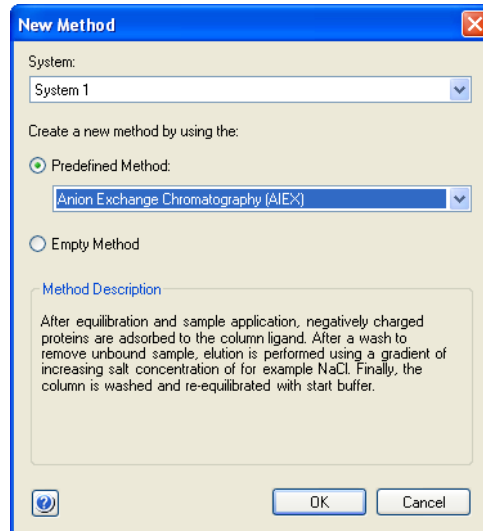


or

- select **File:New Method...**



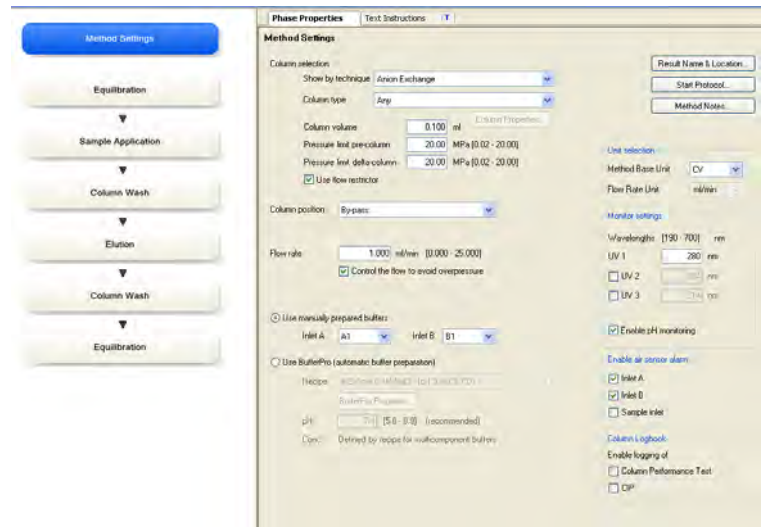
Result: The **New Method** dialog opens.



Step **Action**

- 2 In the **New Method** dialog:
- select a **System**
 - select a **Predefined Method**
 - click **OK**

Result: The **Method Outline** pane shows the included phases for the chosen method and the **Phase Properties** pane shows the default settings for the currently highlighted phase.



3.3.3 Edit phase properties

Introduction

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

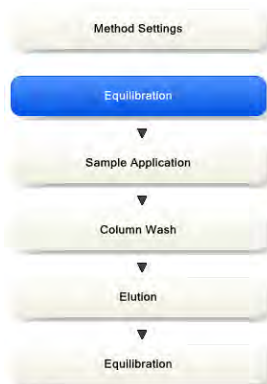
- the whole method, when editing the **Method Settings** phase
or
 - only the phase that is being edited, when editing phases other than the **Method Settings** phase
-

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.

Phase Properties

Text Instructions

Equilibration

Reset UV monitor (recommended if the equilibration occurs before the purification).

Use the same flow rate as in Method Settings

Flow rate ml/min [0.000 - 25.000]

Use the same inlets as in Method Settings

Inlet A

Inlet B % B [0.00 - 100.00]

Fill the system with the selected buffer

Equilibrate until

the total volume is CV

the following condition is met

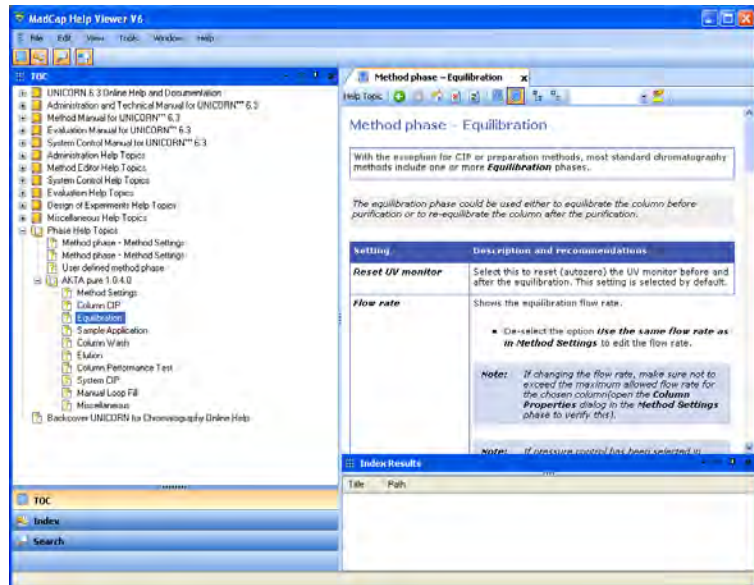
Conductivity greater than mS/cm [0.00 - 1000.00]

Conductivity greater than	0.00	mS/cm [0.00 - 1000.00]
Accepted pH fluctuation	0.10	[0.00 - 14.00]
Accepted UV fluctuation	0.10	mAU [0.00 - 6000.00]
Accepted conductivity fluctuation	0.10	mS/cm [0.00 - 300.00]
Signal stable for	1.00	min [0.02 - 1000.00]
Maximum equilibration volume	10.00	CV

3 Create and edit methods
 3.3 Working with predefined methods
 3.3.3 Edit phase properties

- | Step | Action |
|------|---|
| 2 | Click anywhere in the Phase Properties area to make it the active area in the software. |
| 3 | <ul style="list-style-type: none"> Press the F1 keyboard key. or Select Help:Contextual Help |

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



View and edit phases using Phase Properties

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	<ul style="list-style-type: none"><li data-bbox="425 274 1213 373">• 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. <p data-bbox="473 391 534 422">Note:</p> <p data-bbox="473 433 1213 591"><i>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 3.6.2 Set general method options for the method, on page 83 for information on how to edit these settings.</i></p> <p data-bbox="473 609 495 640">or</p> <ul style="list-style-type: none"><li data-bbox="425 651 1213 709">• Select any other phase to edit the properties for that specific phase. Continue with step 5.

3 Create and edit methods
3.3 Working with predefined methods
3.3.3 Edit phase properties

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.

The 'Phase Properties' dialog box for the 'Method Settings' phase. It is divided into several sections:

- Column selection:** Includes dropdowns for 'Show by technique' (Anion Exchange), 'Column type' (HiLoad 16/10 Q Sepharose HP), and 'Column volume' (20.106 ml). It also has input fields for 'Pressure limit pre-column' (0.50 MPa) and 'Pressure limit delta-column' (0.30 MPa), and a checked 'Use flow restrictor' checkbox.
- Column position:** A dropdown menu set to 'Position 1'.
- Flow rate:** An input field for '3.000 ml/min' and a checked 'Control the flow to avoid overpressure' checkbox.
- Buffer preparation:** Two radio buttons: 'Use manually prepared buffers' (selected) and 'Use automatic buffer preparation (BufferPro)'. Under manual, 'Inlet A' is 'A1' and 'Inlet B' is 'B1'. Under automatic, a 'Recipe' dropdown is set to 'Acetate 0.1M NaCl - (pH 3.8-5.4, PD)'. Below this are input fields for 'pH' (4.6) and 'Conc.' (0.050 M).
- Buttons:** 'Result Name & Location...', 'Start Protocol...', and 'Method Notes...'.
- Unit selection:** 'Method Base Unit' (CV) and 'Flow Rate Unit' (ml/min).
- Monitor settings:** 'Wavelengths' (190-700 nm) and three UV detectors (UV 1: 280 nm, UV 2: 254 nm, UV 3: 214 nm) with checkboxes.
- Enable or sensor alarm:** Checkboxes for 'Inlet A', 'Inlet B', and 'Sample inlet'.
- Column Logbook:** Checkboxes for 'Enable logging of' 'Column Performance Test' and 'CIP'.

Step	Action
4	<p>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.</p> <p>Note: <i>Settings in this phase will affect the whole method.</i></p> <p>Note: <i>Allowed parameter ranges are shown in parentheses beside the text boxes.</i></p> <p>Result: The method is updated with the new settings.</p>

3 Create and edit methods
3.3 Working with predefined methods
3.3.3 Edit phase properties

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.

Phase Properties Text Instructions IT

Equilibration

Reset UV monitor (recommended if the equilibration occurs before the purification).

Use the same flow rate as in Method Settings Use the same inlets as in Method Settings

Flow rate 1.000 ml/min [0.000 - 25.000] Inlet A A1 [v]

Inlet B B1 [v] 0.00 % B [0.00 - 100.00]

Fill the system with the selected buffer

Equilibrate until

the total volume is 5.00 CV

the following condition is met

Conductivity greater than [v]

Conductivity greater than	0.00	mS/cm [0.00 - 1000.00]
Accepted pH fluctuation	0.10	[0.00 - 14.00]
Accepted UV fluctuation	0.10	mAU [0.00 - 6000.00]
Accepted conductivity fluctuation	0.10	mS/cm [0.00 - 300.00]
Signal stable for	1.00	min [0.02 - 1000.00]
Maximum equilibration volume	10.00	CV

Step	Action
6	<ul style="list-style-type: none"><li data-bbox="435 274 795 305">• Edit the settings as appropriate. <p data-bbox="473 329 531 360">Note:</p> <p data-bbox="473 369 1188 524"><i>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 3.6.1 Edit the method outline, on page 75 for information about how to rename a phase.</i></p> <ul style="list-style-type: none"><li data-bbox="435 542 1124 573">• Repeat steps 5-6 until the appropriate phases have been edited. <p data-bbox="435 587 1188 675"><i>Result:</i> The method is updated with the new settings. The edited settings remain in place while subsequent phases are edited. If the method is closed and not saved, the settings will revert back to the earlier values.</p>
7	Save the method.

3.3.4 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 51*.

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 3.3.3 Edit phase properties, on page 50* 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

Built-in fraction collector. Do not fractionate flammable liquids on instruments with built-in fraction collector. Flammable gas might be formed inside the closed cabinet. When running RPC methods, or other procedures using organic solvents, collect fractions through the Outlet valve.

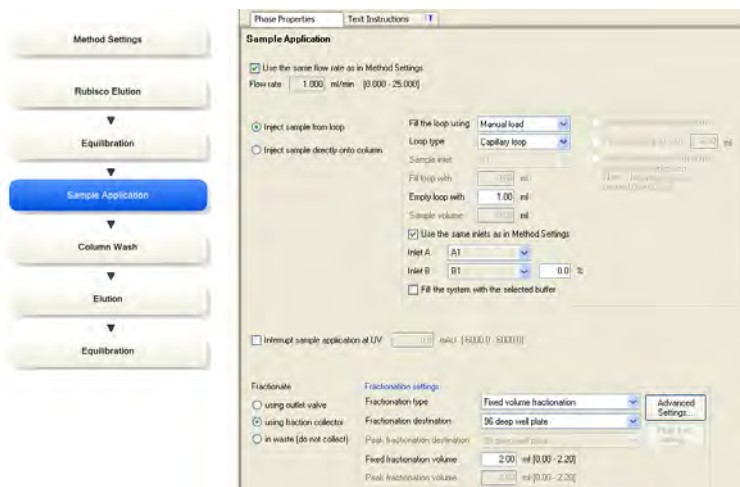
Fractionation setup

The following table is an example of how to set up fraction collection in the **Phase Properties** pane:

Note: *The setup can vary between different fraction collectors.*

Step **Action**

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



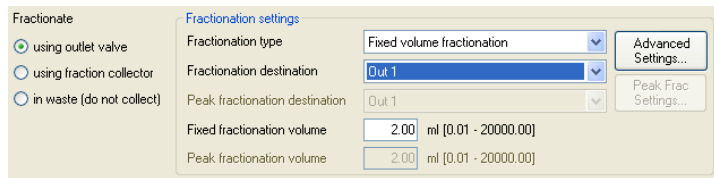
Note:

Text edited phases will show the fractionation options as variables in the **Phase Variables** list, see Chapter 10 Text edit methods, on page 325.

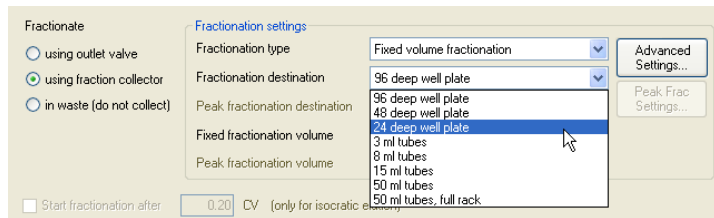
Step **Action**

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 direct 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 51.

3.4 Working with wizard generated methods

About this section

For instruments with an instrument configuration that includes a method wizard, it is possible to create wizard generated methods. This section describes how to create and edit wizard generated methods. The wizard generated methods only contain a method settings phase and a user defined phase. The sections also describes **Frac-950** fractionation.

In this section

This section contains these subsections:

Section	See page
3.4.1 Create a wizard generated method	62
3.4.2 Wizard generated methods	64
3.4.3 Frac-950	67

3 Create and edit methods

3.4 Working with wizard generated methods

3.4.1 Create a wizard generated method

3.4.1 Create a wizard generated method

The table below describes how to create a new method using the wizard:

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

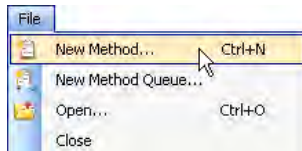
1	In the Method Editor :
---	-------------------------------

- click the **Create a new method** icon in the **Toolbar**

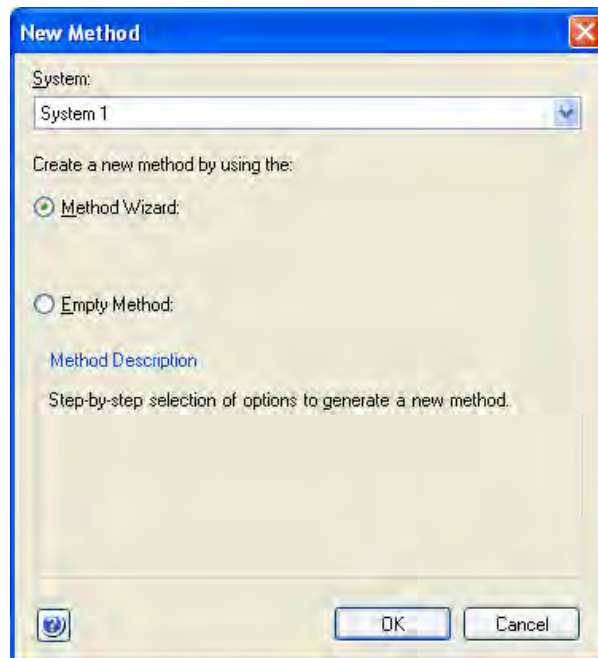


or

- select **File:New Method...**



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



Step	Action
2	<p>In the New Method dialog:</p> <ul style="list-style-type: none">• select a System <p>Note:</p> <p><i>The dialog changes depending on the type of the selected system. For systems that use predefined methods, refer to Section 3.3 Working with predefined methods, on page 35.</i></p> <ul style="list-style-type: none">• select the Method Wizard radio button• click OK <p><i>Result:</i> The Method Wizard opens.</p>
3	<ul style="list-style-type: none">• Perform the Method Wizard sequence until the final dialog is reached. <p>Note:</p> <p><i>Performance tests, CIP and other special methods are also created in the Method Wizard.</i></p> <p>Refer to Wizard Help for more information.</p> <ul style="list-style-type: none">• Click Finish.

3 Create and edit methods

3.4 Working with wizard generated methods

3.4.2 Wizard generated methods

3.4.2 Wizard generated methods

The methods generated by the wizard will consist of two phases.

- The **Method Settings** phase, which contains column type, result name and location, start protocol and method notes. Logging of Column performance test and Column CIP is selected in this phase.
- The **User Defined** phase, which contains a list of **Phase variables** that can be text edited.

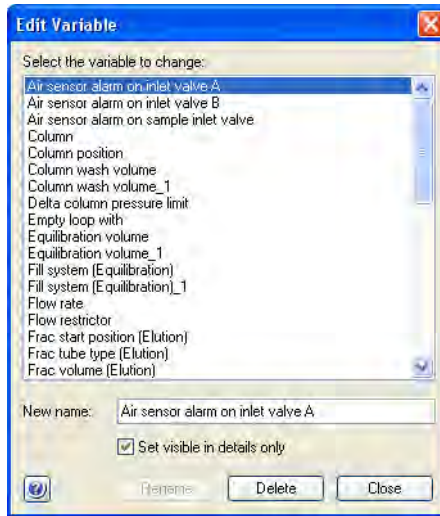
Edit phase variables

Editing a variable includes renaming and deleting the variable and choosing whether the variable should be a detailed variable or not.

The table below describes how to edit a variable for a selected phase:

Step	Action
1	Select the Text Instructions tab to display the phase variables, select the variable Value and click Edit Variable... <i>Result:</i> The Edit Variable dialog opens displaying all the phase variables.

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 variable values

The table below describes how to edit default variable values in the **Phase Variables** list. For information on how to edit variables from **Text Instructions**, see *Section 10.2 Working with methods in the Text Instructions pane, on page 334*.

Step	Action
1	Select the Phase Properties tab to display the Phase Variables list.

3 Create and edit methods

3.4 Working with wizard generated methods

3.4.2 Wizard generated methods

Step	Action
2	<p>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.</p> <p>Tip: <i>To show detailed variables, check the Show details box.</i></p> <p><i>Result:</i> The variable value is updated.</p>
3	<p>Repeat this procedure for the appropriate variables.</p>

Changes made in the **Phase Properties** tab are automatically updated on the **Text Instructions** tab and vice versa.

3.4.3 Frac-950

Introduction

For many purification schemes it is convenient to collect fractions of the eluent. The wizard generated methods include options for fraction collection in the **Phase Properties** pane using **Frac-950** if Frac-950 has been chosen as a component for the system.

This section describes how to edit a **Frac-950** fractionation. More detailed information for individual settings can be found in the online help.

Frac-950 is a system specific option.

Fractionation

For many purification schemes it is convenient to collect fractions of the eluent.

The fractionation settings are available in the **Phase Properties** tab in the **Method Settings** phase. Wizard generated methods support fractionation if **Frac 950** has been chosen as a component for the system.

Note: *Scouting is not available for Frac 950 variables.*

The following table describes how to edit a **Frac-950** fractionation for a Wizard generated method.

3 Create and edit methods

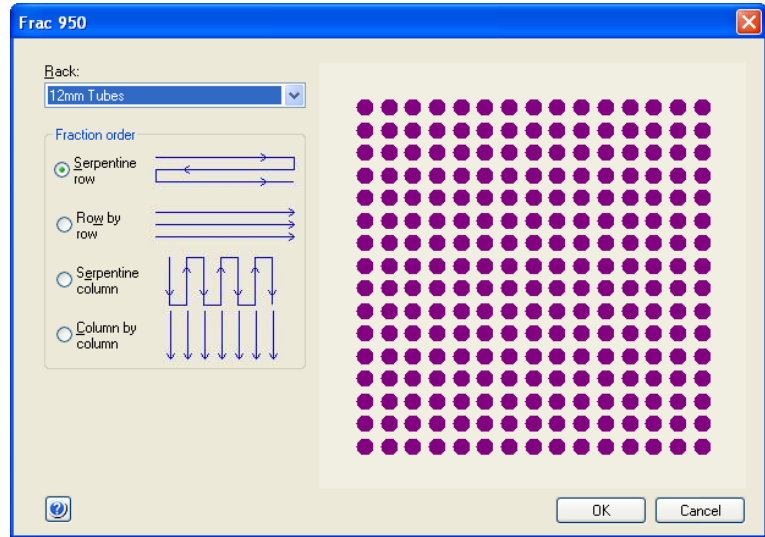
3.4 Working with wizard generated methods

3.4.3 Frac-950

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

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">Click the Frac-950... button in the Phase Properties tab in the Method settings phase.orSelect Edit:Frac-950... |
|---|---|

Result: The **Frac-950** dialog opens.



Note:

The button is only active if Frac-950 has been chosen as a component for the system.

- | | |
|---|---|
| 2 | <ul style="list-style-type: none">Select Rack for the fractionation in the drop down list.Select what kind of Fraction order to use in the fractionation.Click OK. |
|---|---|

3.5 Working with empty methods

About this section

This section describes how to work with methods and phases in systems that create methods using text instructions. From the start, an empty method only contains a method settings phase.

In this section

This section contains these subsections:

Section	See page
3.5.1 Create an empty method	70
3.5.2 Edit an empty method	72

3.5.1 Create an empty method

The table below describes how to create a new empty method:

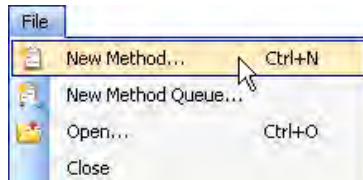
Step	Action
------	--------

- | | |
|---|---|
| 1 | In the Method Editor : <ul style="list-style-type: none">click the Create a new method icon in the Toolbar |
|---|---|



or

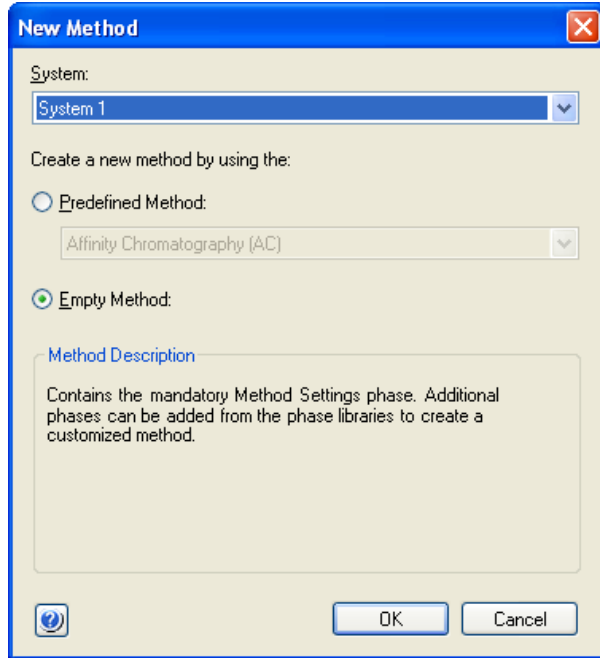
- select **File:New Method...**



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

Step **Action**

2 The **New Method** dialog looks different depending on the system.



In the **New Method** dialog:

- select a **System**
- select the **Empty Method** radio button
- click **OK**

Result: An empty method that consists of the mandatory **Method Settings** phase is created.

3.5.2 Edit an empty method

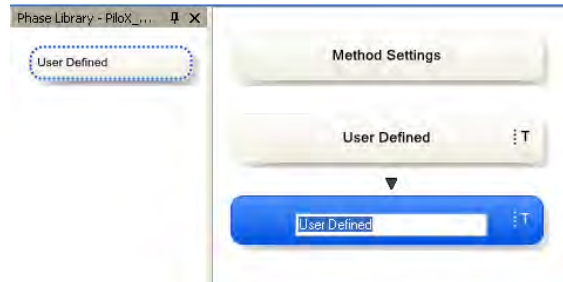
Introduction

A new empty method only has the **Method Settings** phase in the **Method Outline**. **User Defined** and **Predefined** phases can be added, rearranged, renamed and deleted from the **Method Outline**.

Add a phase to the method outline

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

Step	Action
1	Select the phase in the Phase Library pane and drag-and-drop the phase to the requested position in the Method Outline pane. In the example, a User Defined phase is used. <i>Result:</i> The phase is included in the method at the requested position.
2	When the User Defined phase has been added to the Method Outline , the phase name is enabled for editing.



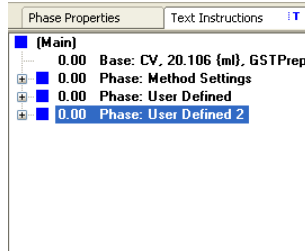
Type a name for the phase and press the **Return** keyboard key.

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 manually.

Step **Action**

- 3 To include instructions for the **User Defined** phase, select the **Text Instructions** tab and text edit the method.



For detailed instructions on how to text edit methods, refer to *Chapter 10 Text edit methods, on page 325*

Note:

The **Phase Properties** tab will then show a list of variables used in the phase.

3.6 Working with methods in general

About this section

This section includes descriptions of how to work with methods and phases in general.

In this section

This section contains these subsections:

Section	See page
3.6.1 Edit the method outline	75
3.6.2 Set general method options for the method	83
3.6.3 Print a method	94
3.6.4 Sign methods electronically	96

3.6.1 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. All systems have a user defined phase available and some system has several predefined phases available in the phase library. <i>Result:</i> 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 10 Text edit methods, on page 325 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 a menu command. It is possible to define several phases and store in the library to use in new methods.

Step	Action
1	<ul style="list-style-type: none">• Select the appropriate phase (e.g., Equilibration or User Defined) 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**

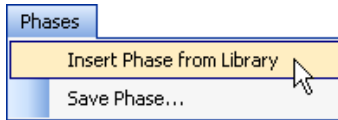


or

- 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.



3 Create and edit methods
3.6 Working with methods in general
3.6.1 Edit the method outline

Step	Action
3	<p>When the User Defined phase has been added to the Method Outline, the phase name is enabled for editing.</p> <p>Type a name for the phase and press the Return keyboard key.</p> <p>Note:</p> <p>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 show a list of variables used in this phase. See Chapter 10 Text edit methods, on page 325 for information about how to work with instructions in the text instructions pane.</p>

Rename phases

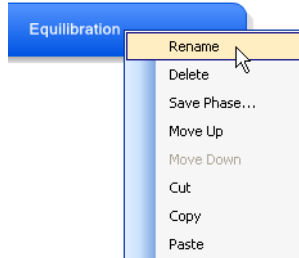
Note: *It is only possible to rename phases in the **Method Outline** pane, not in the **Phase Library**.*

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**

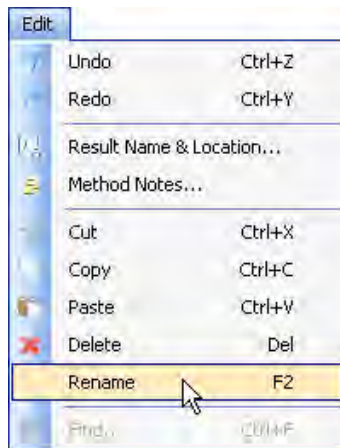


or

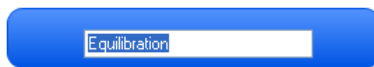
- press the **F2** keyboard key

or

- select **Edit:Rename**



Result: The name in the phase becomes editable.



- 3
- Type an appropriate name and click **Return** keyboard key.

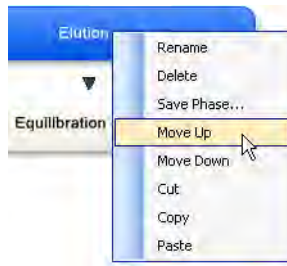
Result: The name of the phase is updated.



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	<ul style="list-style-type: none">• Drag-and-drop the phase to the requested position in the Method Outline pane. <i>Result:</i> The phase is moved to the requested position. <i>or</i>• 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**.

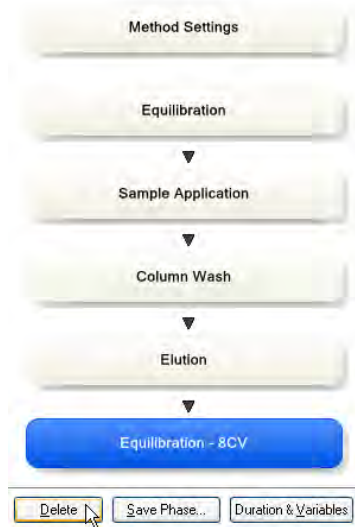
Delete a phase from the Method Outline

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.

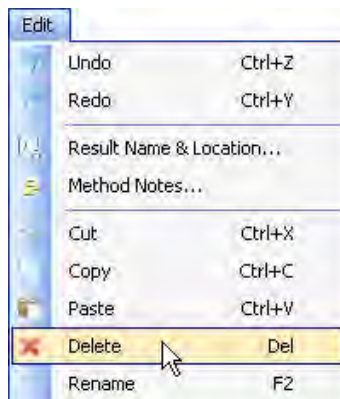


or

- 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: <ul style="list-style-type: none"> right-click the phase and select Copy or use the shortcut Ctrl +C or select Edit:Copy
cut a phase	select the phase and: <ul style="list-style-type: none"> right-click the phase and select Cut or use the shortcut Ctrl +X or select Edit:Cut
paste a phase	<p>Note: <i>The phase to be pasted will be pasted below the phase highlighted in the Method Outline.</i></p> <p>Select the appropriate phase in the Method Outline. Then:</p> <ul style="list-style-type: none"> right-click the highlighted phase in the Method Outline and select Paste or use the shortcut Ctrl +V or select Edit:Paste

3.6.2 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 evaluation 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
------	--------

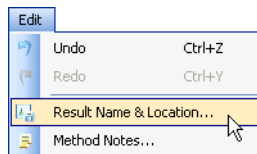
1	In the Method Editor :
---	-------------------------------

- click the **Result Name & Location** icon



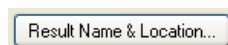
or

- select **Edit:Result Name & Location...**



or

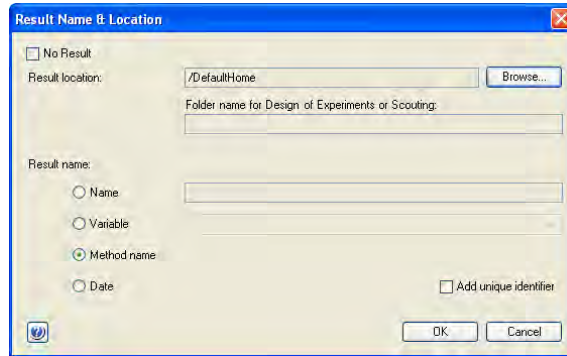
- click the **Method Settings** phase and click the **Result Name & Location...** button in the **Phase Properties** tab



Result: The **Result Name & Location** dialog opens.

Step **Action**

2


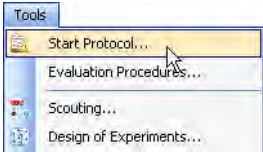



In the **Result Name & Location** dialog:

- 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 10.2.4 Method variables, on page 353)
 - **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	<p>In the Method Editor:</p> <ul style="list-style-type: none">click the Start Protocol icon  <p>or</p> <ul style="list-style-type: none">select Tools:Start Protocol...  <p>or</p> <ul style="list-style-type: none">click the Method Settings phase and click the Start Protocol... button in the Phase Properties tab  <p><i>Result:</i> The Start Protocol dialog opens.</p>
2	<p>In the Start Protocol dialog:</p> <ul style="list-style-type: none">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
------	--------

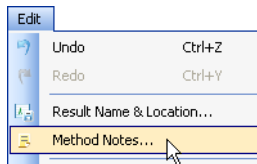
1	In the Method Editor :
---	-------------------------------

- click the **Method Notes** icon



or

- select **Edit:Method Notes...**



or

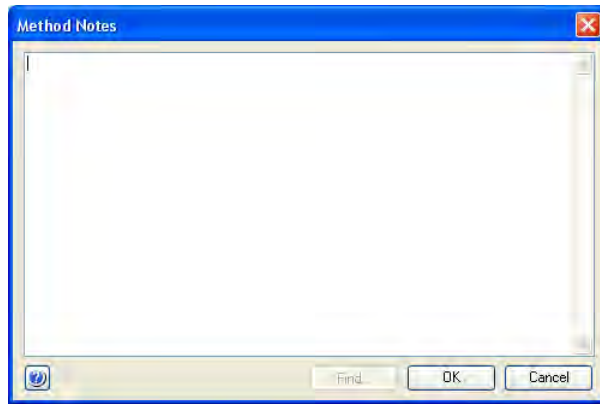
- click the **Method Settings** phase and click the **Method Notes...** button in the **Phase Properties** tab



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:

*For some systems information will automatically be added to the **Method Notes** if the method has been converted for use with another system type than it was originally created for, or scaled for another column type than was originally selected, or if it is a wizard generated intelligent packing method.*

Include Evaluation Procedures after the run

Note: *This information only applies to Evaluation Classic.*

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 Evaluation Manual.

3 Create and edit methods

3.6 Working with methods in general

3.6.2 Set general method options for the method

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

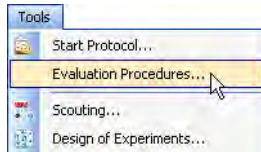
1 In the **Method Editor**:

- click the **Evaluation Procedures** icon



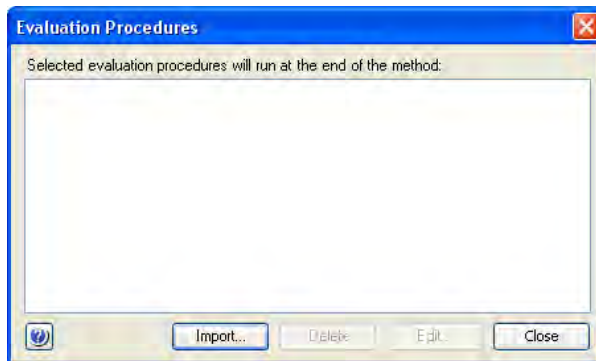
or

- select **Tools:Evaluation Procedures...**



Result: The **Evaluation Procedures** dialog opens.

2



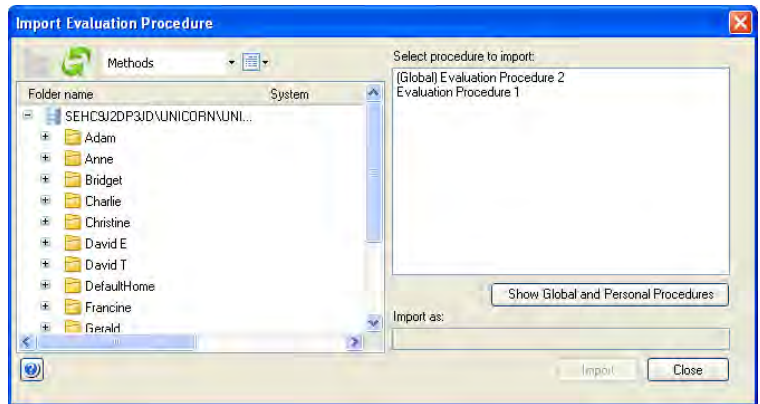
- If there are no evaluation procedures listed in the **Evaluation Procedures** dialog, click the **Import...** button to import an evaluation procedure.

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.

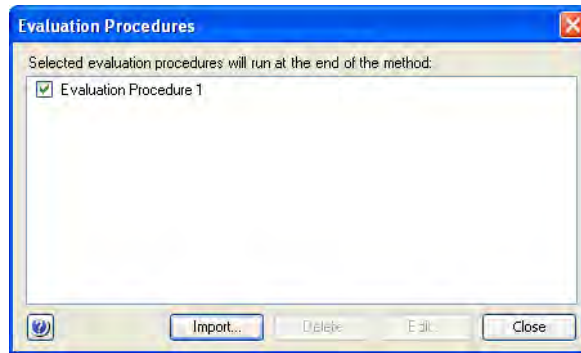
3 Create and edit methods

3.6 Working with methods in general

3.6.2 Set general method options for the method

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:

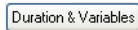
*It is possible to edit an existing evaluation procedure by selecting it and clicking **Edit**.... The edits will only change the procedure that is included in the method. See the UNICORN Evaluation Manual for information about how to edit an evaluation procedure.*

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:

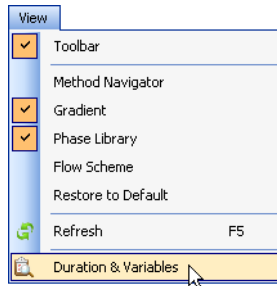
Step	Action
------	--------

- 1 In the **Method Editor**:
 - click the **Duration & Variables** button below the **Method Outline** pane



or

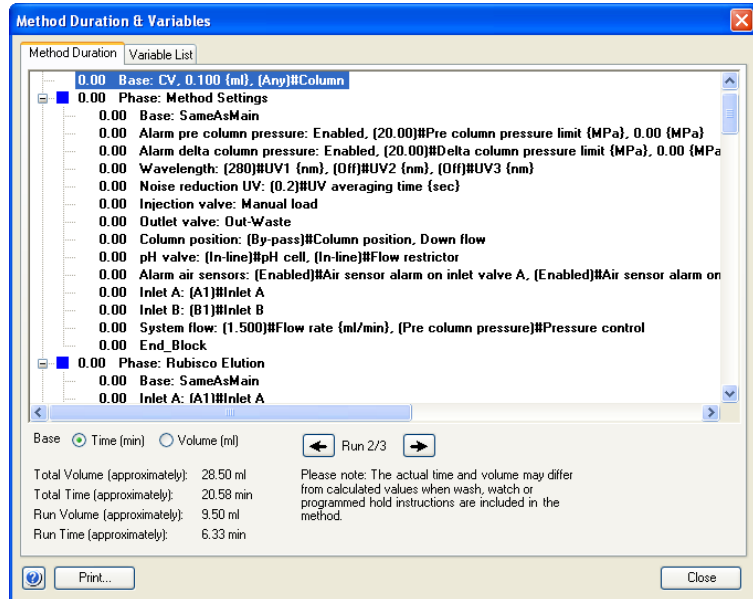
- select **View:Duration & Variables**



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.

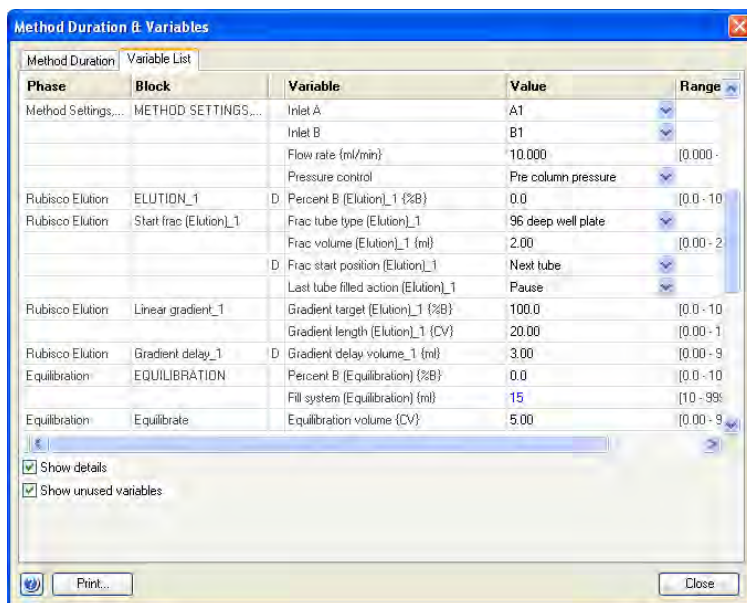
4

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.

3.6.3 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:

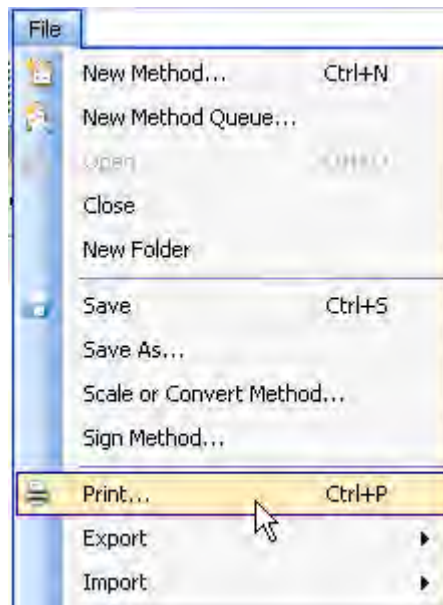
Step	Action
------	--------

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">Click the Print icon in the Toolbar |
|---|---|



or

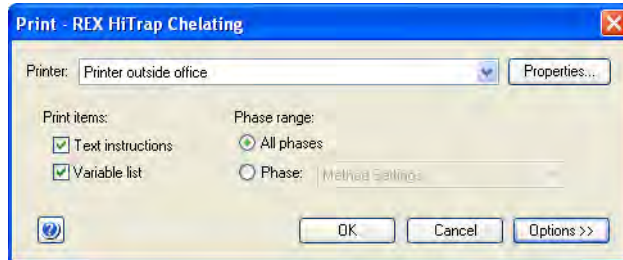
- select **File:Print...**



Result: The **Print** dialog opens.

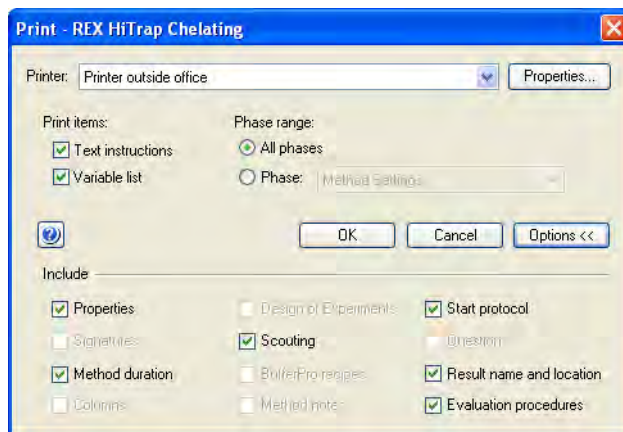
Step **Action**

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 grayed out in the **Print** dialog.

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

3.6.4 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**:

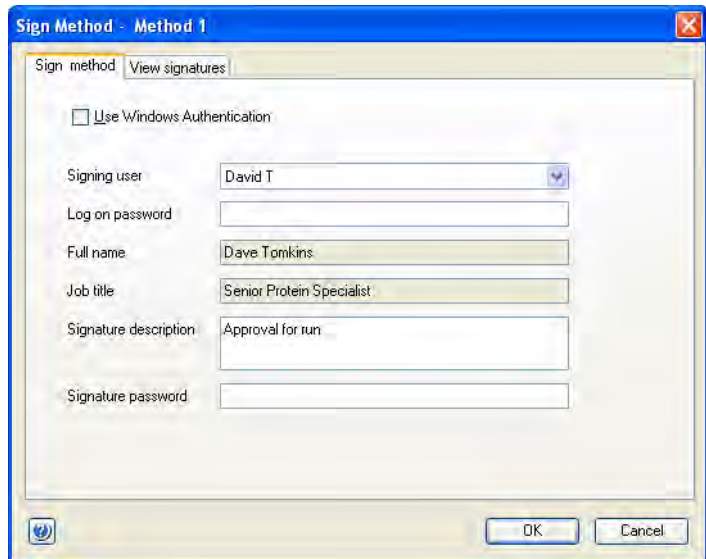
Step	Action
1	Select File:Sign Method...



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

Step **Action**

2



- The **Signing user** field shows the user currently logged on.

Note:

*If you want to **Use Windows Authentication**, select the check box, and log in as a network user.*

If you want to sign the method but are not 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.

3.7 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 cannot be overwritten.*

Save a method

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

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

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">Click the Save the Method icon |
|---|---|



or

- select **File:Save** or **File:Save As**.

Result:

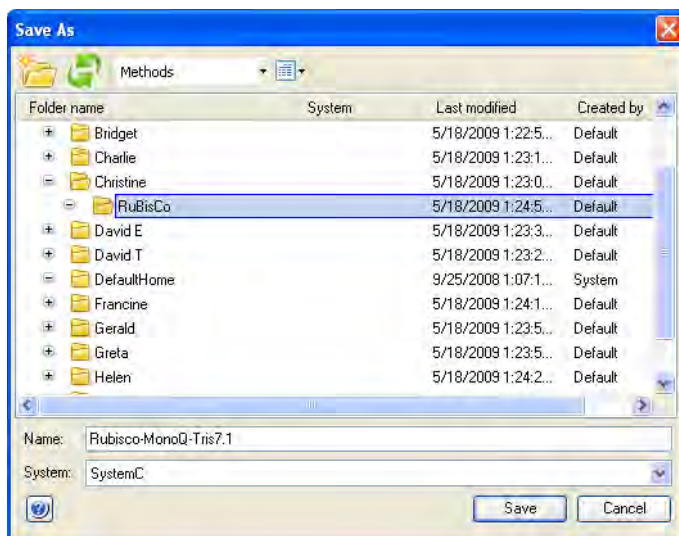
- If the method has been named and saved previously, the changes are saved immediately.

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

- 4 Click **Save**.

Result: The method is saved in the database.

Note:

For some systems 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 depend 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.*

3 Create and edit methods

3.7 Save methods and phases

Adapt a method

For ÄKTA pure it is possible to open and save methods created with systems with another set of components than the currently used system. When a method is opened for a system that has been changed or if a method is saved for a different system, a dialog is opened where the user can choose to either adapt the method or to keep the text method unchanged.

If you select..	Then..
Adapt method	<p>the method will be adapted to a new set of components. All functions and positions that are still available will be unchanged. For example, valve positions present on both the original and the new system will be unchanged in the adapted method. Non compatible settings will be adjusted in order to properly adapt the method for new components. Settings that have been changed are described and saved in Method Notes, which are displayed after the method has been adapted.</p> <p>Change the settings in Phase Properties if required.</p>
Keep text method	<p>the text method will be kept and nothing in the text method will be changed. Non-compatible settings will still be present in the method but they will not be functional. All Phase Properties of the method will be replaced by tables of Phase Variables.</p> <p>Note: <i>Some predefined methods require certain components to be functional. Adapting these methods to systems that do not include the required components is not possible.</i></p>

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 cannot be saved as a separate phase with a new name. If properties for the **Method Settings** phase are changed, the changes will be saved with the method.

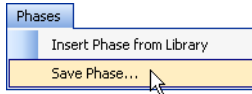
Step **Action**

- 2
- click the **Save Phase...** button below the **Method Outline** pane



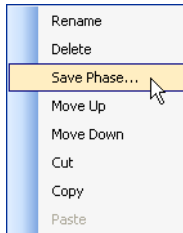
or

- 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.

3 Create and edit methods

3.7 Save methods and phases

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 | <ul style="list-style-type: none">• 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.

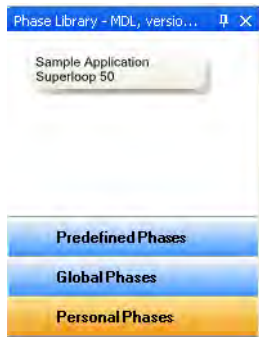
The table below describes how to delete a personal or global phase from the phase library:

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**.

3.8 Scale or convert methods

Introduction

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

The possibility to scale or convert methods is only available for some systems, and only between systems that use the same instructions.

A method can also be scaled for use with a different column type than it was originally created for, when the method is converted. Conversion and scaling is described in this section.

Tip: *If you wish to use a method for another system of the same 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. This is possible for all systems.*

Instructions marked with a red cross must be edited.

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

Prerequisites

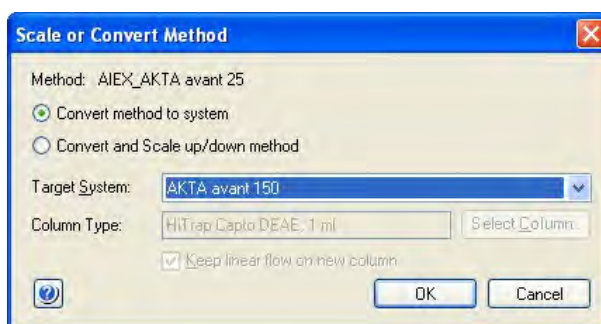
The following items should be considered to ensure that the method conversion and scaling is successful:

- 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 . |
| 2 | Choose the menu command File:Scale or Convert Method
<i>Result:</i> 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.

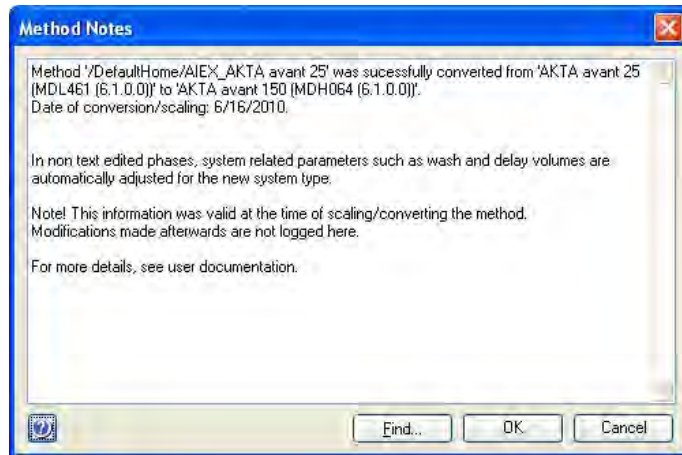
3 Create and edit methods

3.8 Scale or convert methods

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 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 | <ul style="list-style-type: none">• Type any additional notes you wish to add in the text field and• click the OK button to close the Method Notes dialog. |
|---|---|

- | | |
|---|--|
| 7 | <ul style="list-style-type: none">• 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.

Step	Action
8	<ul style="list-style-type: none"> • Select the desired target folder, • type a new method name in the Name field and • click the Save button.
Note:	<i>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.</i>
Note:	<i>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.</i>
Note:	<p><i>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.</i></p> <p><i>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.</i></p> <p><i>You can also replace the phase with a predefined phase from the phase library.</i></p>

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 <i>Result:</i> 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.

3 Create and edit methods

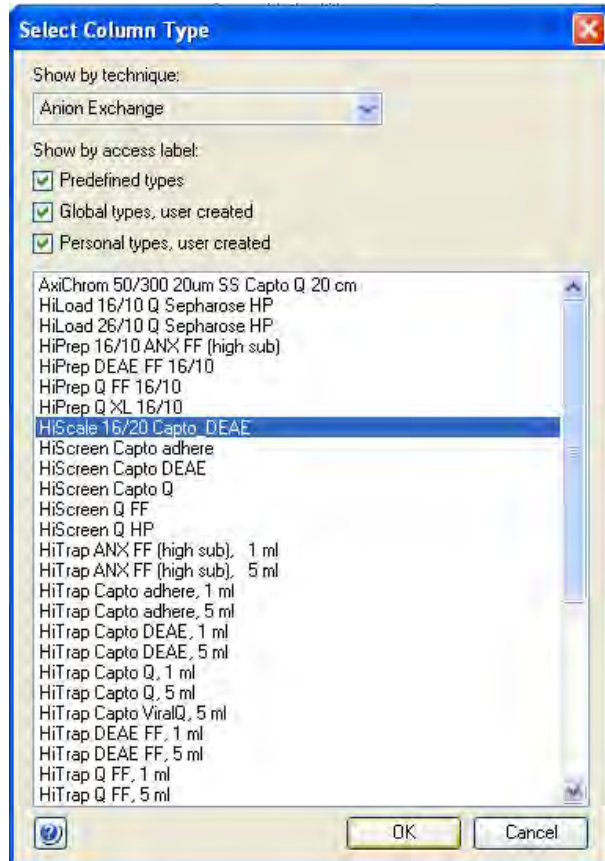
3.8 Scale or convert methods

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.

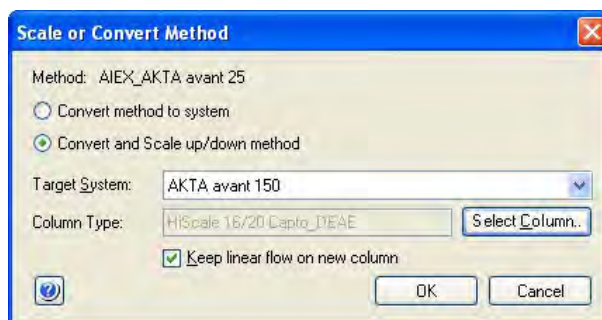
Result: The **Select Column Type** dialog opens.



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

- | | |
|---|--|
| 6 | <ul style="list-style-type: none">• 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. |
|---|--|

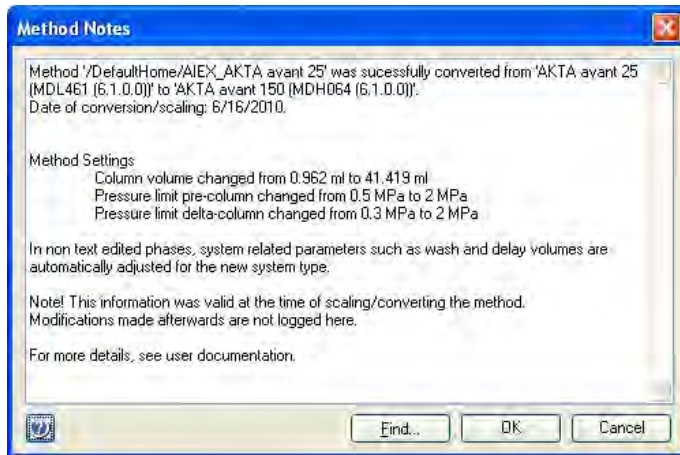
Note:

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 | <ul style="list-style-type: none">Choose the File:Save As menu command
orclick the Save icon |
|----|---|



Result: The **Save As** dialog opens, with the folder where the original method is saved open by default.

- | | |
|----|---|
| 11 | <ul style="list-style-type: none">Select the desired target folder,type a new method name in the Name field
andclick 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)

3 Create and edit methods

3.8 Scale or convert methods

The following settings may require manual adjustment 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 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 should be set up inactivated.

Since the new system will not be used for anything other than the method conversion, the system name, IP address and serial number can be selected at random. For example:

- **System name:** `Method conversion system`
- **Instrument serial number:** `123456789`
- **Fixed IP address:** `10.1.1.1`

Refer to UNICORN Administration and Technical Manual, *Define a new system* for more information on how to set up a new system.

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).
---	---

3.9 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

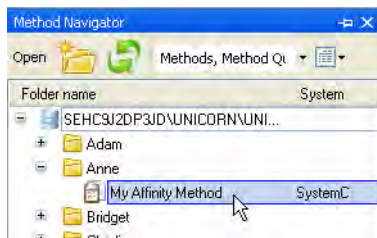
The following table outlines the steps needed to export a method or a phase for later import into UNICORN.

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

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

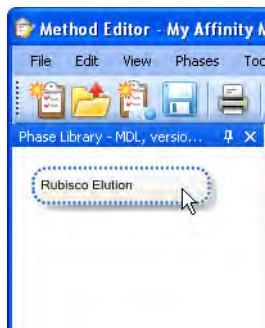
Note:

Several methods in the same folder can be selected and exported at the same time. You can select several results at once by using the **Shift** or **Ctrl** key when selecting.



or

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



Note:

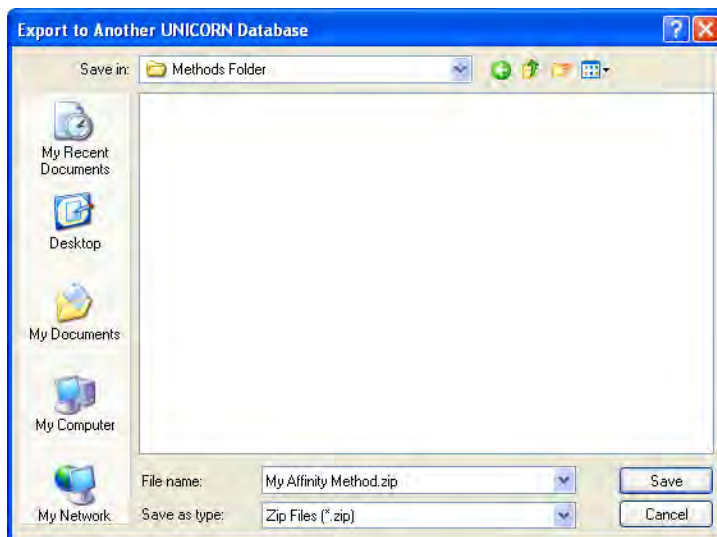
Only **Personal** or **Global** phases may be exported.

Note:

Only single phases may be exported.

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

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



Note:

To export phases, right-click on the phase name and select **Export...** from the context menu.

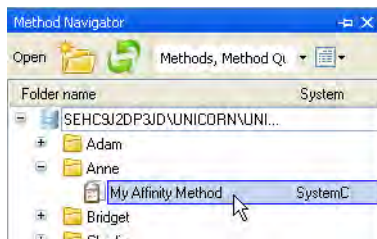
- | | |
|---|--|
| 3 | Choose a file name and location and click the Save button to save the zip file. |
|---|--|

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.

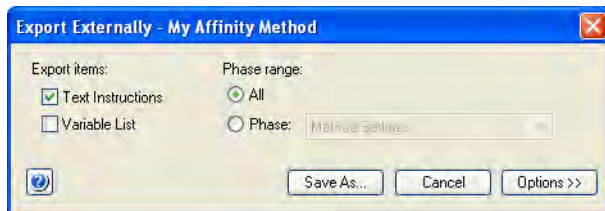
Step	Action
------	--------

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



- | | |
|---|---|
| 2 | Choose File:Export:Export Method Externally... |
|---|---|

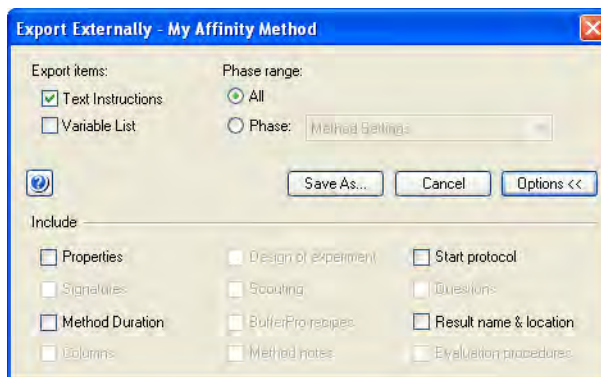
Result: The **Export Externally** dialog opens.



- | | |
|---|---|
| 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.



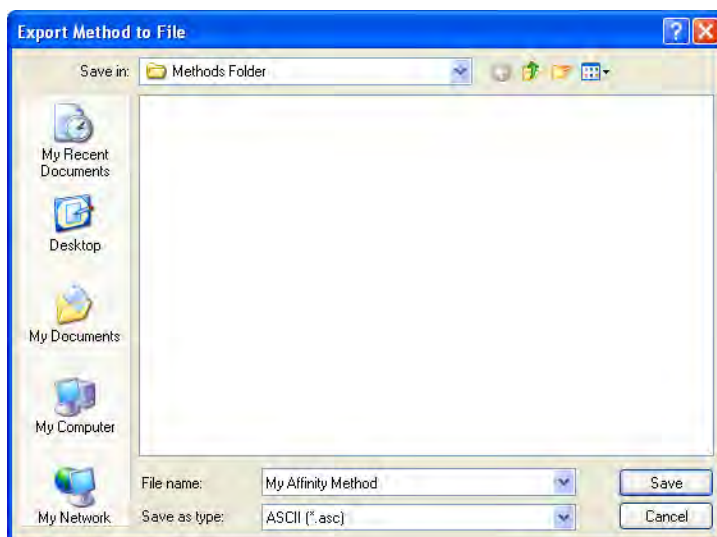
- 6 Select information to add to the text file by checking the appropriate options.

Note:

Information that is not included in the method will appear grayed 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.



3 Create and edit methods

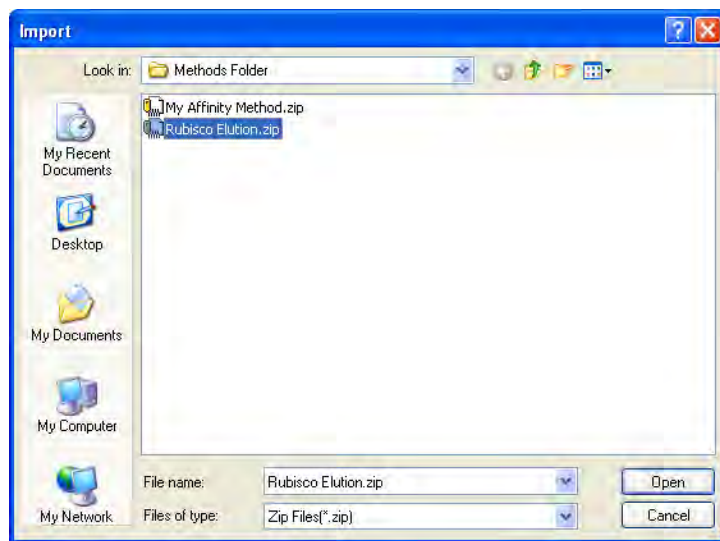
3.9 Import and export methods

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. The following table outlines the steps needed to import a phase.

Step	Action
1	Select File:Import:Import Phase... <i>Result:</i> The Import dialog opens.

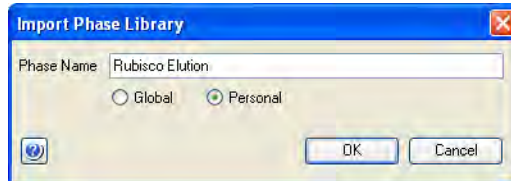


2	Browse to the required zip file in the Import dialog.
---	--

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.
-

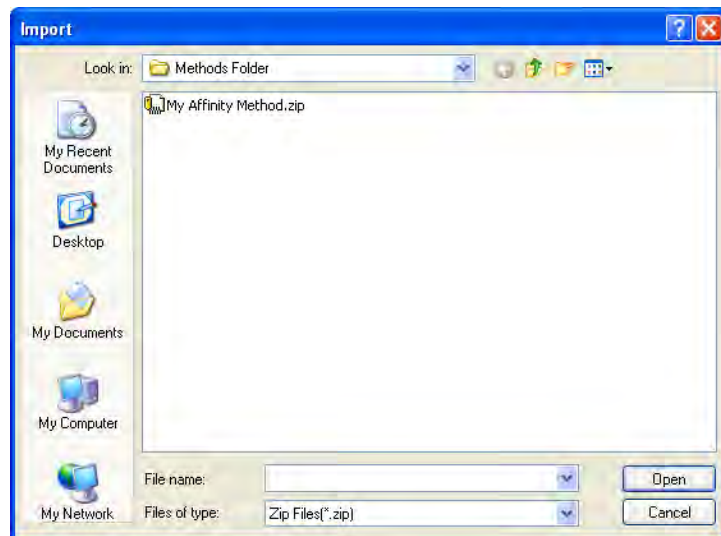
Import a method into UNICORN

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.



Note:

When importing methods from UNICORN 5, choose format **UNICORN 5 Method Files** in the **Files of type** field.

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

Note:

For methods imported from UNICORN 5, browse to the required method file.

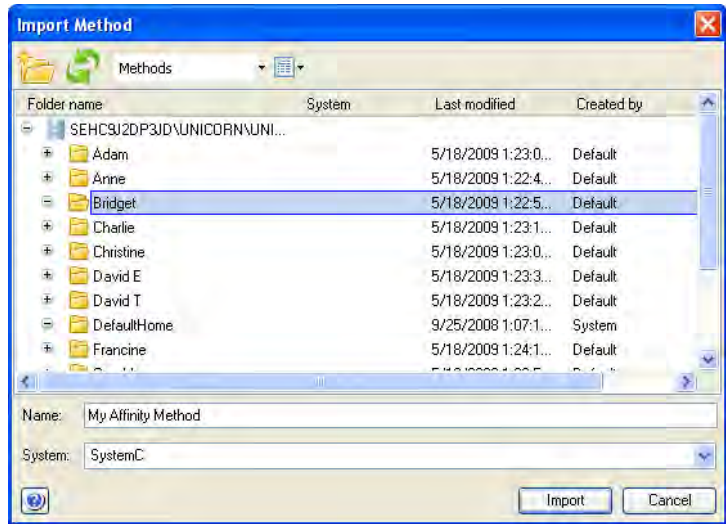
Step **Action**

- 3 Open the file by selecting it and clicking the **Open** button, or by double-clicking on the file name.

Note:

*Several methods in the same folder can be selected and imported at the same time. You can select several results at once by using the **Shift** or **Ctrl** key when selecting.*

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**.

If several methods were chosen in the first step, repeat the procedure in the **Import Method(s)** dialog for each method.

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.

Import a method from UNICORN 5

Methods that have been created in UNICORN 5.11 and subsequent versions can be imported into UNICORN 7.0 in the same way as described above. However, some information will not be imported.

Note: *The Data collection utility can only be used if UNICORN 5 is installed on Windows XP.*

Changes in data after import

When methods are imported from UNICORN 5, there is some information that will not be included and some information that will be added. It is important to review the method after import, to verify that all essential information is there. The method can be manually edited, after the import. All functionality in UNICORN 7.0 is not available for imported methods, since necessary data might be missing.

Affected information	Description
Installation test methods	Installation test methods that were generated by the method wizard in UNICORN 5 do not work in UNICORN 7.0. These methods must be regenerated by the method wizard in UNICORN 7.0.
Scouting data	Scouting on any of the Frac-950 instructions (Fractionation and Peak_Fractionation) parameters will not be migrated.
Reference curves	Reference curves are not migrated since this functionality is not available in UNICORN 7.0.

Affected information	Description
Column data	<p data-bbox="671 280 844 311">Delta pressure</p> <p data-bbox="671 323 1190 569">A UNICORN 5 method with an included column can be migrated, but where UNICORN 5 uses Max pressure in the method, UNICORN 7.0 uses the terms Max delta column pressure and Max pre column pressure. UNICORN 7.0 will set both Max delta column pressure and Max pre column pressure to be the Max pressure value from UNICORN 5 when a method without these parameters is imported.</p> <p data-bbox="671 575 832 606">Max flow rate</p> <p data-bbox="671 618 1190 797">A UNICORN 5 method with an included column can be migrated, but Max flow rate is not mandatory in UNICORN 5 and may not have been set. When a method without Max flow rate is migrated UNICORN 7.0 will set the Max flow rate to be the same as the default flow rate for the column.</p> <p data-bbox="671 802 821 833">Column type</p> <p data-bbox="671 846 1190 1093">The UNICORN 7.0 Column list differs from the UNICORN 5 Column list. Any column type included in a method is migrated and the values are not changed, but the column type will not be imported to the UNICORN 7.0 Column list. The column type of pre-packed columns from GE from the UNICORN 5 Column list should be exchanged for the corresponding column type in the UNICORN 7.0 Column list.</p> <p data-bbox="671 1099 1190 1228">Custom packed columns from the UNICORN 5 Column list have to be redefined in Column handling in UNICORN 7.0, in order to get the full use of the Column handling tool.</p>

Affected information	Description
Evaluation procedures	<p>If an evaluation procedure instruction is invalid in UNICORN 5 the instruction is removed from the method during migration.</p> <p>Non-imported instructions</p> <p>Some evaluation procedure instructions are not imported. A removed instruction is replaced in UNICORN 7.0 by a comment that informs the user that an instruction is missing.</p> <p>The following evaluation procedure instructions will not be imported:</p> <ul style="list-style-type: none"> • <i>CURVE_OPEN</i> • <i>PEAKTABLE_OPEN</i> • <i>EXPORT_DOC_ASCII</i> • <i>EXPORT_DOC_WKS</i> • <i>EXPORT_DOC_XLS</i> • <i>COPY_CHROM</i> • <i>CREATE_NEW_CHROM</i> • <i>OPEN_CHROM</i> • <i>DELETE_CHROM</i> • <i>RENAME_CHROM</i> • <i>SIMULATE_PEAK_FRAC</i> • <i>POOL_FRACTIONS</i> • <i>RET_MUL</i> • <i>EXPORT_DOC_400_xxx</i> • <i>EXPORT_NORMALISE_RETENTION</i> • Export of curves in AIA • Quantitate functions • All instructions for exporting to WKS format

Affected information	Description
	<p data-bbox="671 280 911 311">Changed instruction</p> <p data-bbox="671 323 1190 384">The following evaluation procedure instructions are changed when imported:</p> <ul data-bbox="671 402 1190 633" style="list-style-type: none"><li data-bbox="671 402 1190 524">• The evaluation procedure instruction EXPORT_MULTI_CURVES_XLS is migrated from UNICORN 5 to the equivalent instruction EXPORT_MULTI_CURVES_CSV in UNICORN 7.0.<li data-bbox="671 542 1190 633">• The instruction RUN_PROGRAM is implemented in UNICORN 7.0 but programs that have a user interface cannot be launched.
Report layouts	<p data-bbox="671 651 1002 682">Report layouts are not migrated.</p> <p data-bbox="671 693 1177 784">If a non-imported report layout is referred to in an evaluation procedure, the layout will be replaced with the default layout.</p>

4 Scouting

About this chapter

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 economizing on time and sample amounts. For details on **DoE**, see *Chapter 5 Design of Experiments, on page 139*.

In this chapter

This chapter contains the following sections:

Section	See page
4.1 Overview	127
4.2 Set up and edit a Scouting scheme	129

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 27* 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 10.2.4 Method variables, on page 353* for information.

- **Start and monitor the scouting run**

This is performed in **System Control**. See *UNICORN System Control 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 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.

Note: *Fractionation using **Frac-950** is an exception and cannot be scouted.*

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	<p>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.</p> <p>See <i>Chapter 3 Create and edit methods, on page 27</i> for information about how to create methods.</p> <p>See <i>Section 10.2.4 Method variables, on page 353</i> for information about how to define new variables.</p> <p>Tip: <i>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.</i></p>

4 Scouting

4.2 Set up and edit a Scouting scheme

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

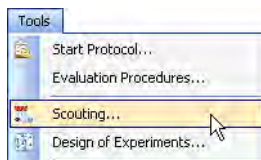
2	In the Method Editor :
---	-------------------------------

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

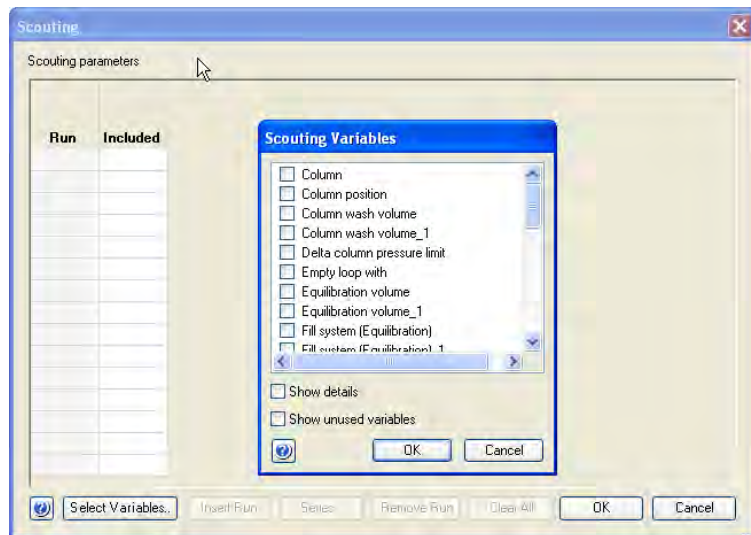


or

- Select **Tools:Scouting**



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



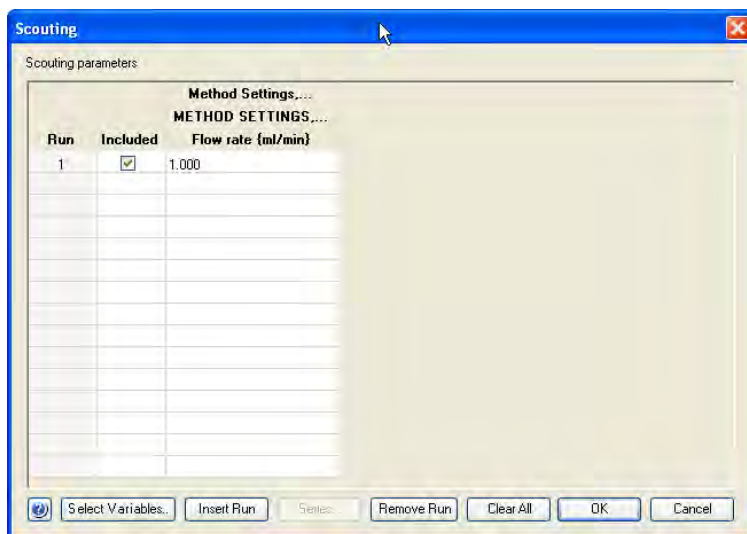
Note:

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).

4 Scouting

4.2 Set up and edit a Scouting scheme

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

4 To insert runs one by one:

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

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

Run	Included	Method Settings....
		METHOD SETTINGS.... Flow rate (ml/min)
1	<input checked="" type="checkbox"/>	1,000
2	<input checked="" type="checkbox"/>	1,000

- 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.

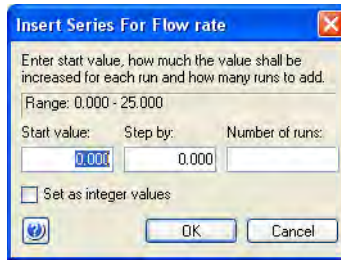
Note:

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.

Step **Action**

5 To insert a series of runs, click in the appropriate variable column in the **Scouting parameters** table and click . 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.



6 In the **Insert Series** dialog:

- Enter **Start value:**, **Step by:** and **Number of runs:**. In this example, 2, 2 and 6.
- Click **OK**.

Result: The **Scouting parameters** table is updated.

Scouting parameters		Method Settings,....
Run	Included	METHOD SETTINGS,.... Flow rate (ml/min)
1	<input checked="" type="checkbox"/>	2.0
2	<input checked="" type="checkbox"/>	4.0
3	<input checked="" type="checkbox"/>	6.0
4	<input checked="" type="checkbox"/>	8.0
5	<input checked="" type="checkbox"/>	10.0
6	<input checked="" type="checkbox"/>	12.0

4 Scouting

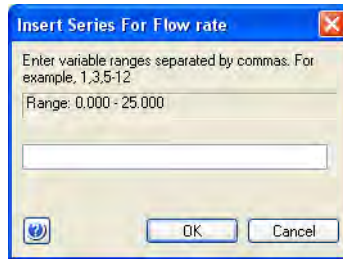
4.2 Set up and edit a Scouting scheme

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.



- Enter the appropriate range, for example: 1-3,5-7
- Click **OK**.

Result: The **Scouting parameters** table is updated.

Run	Included	Method Settings... METHOD SETTINGS... Flow rate (ml/min)
1	<input checked="" type="checkbox"/>	1.000
2	<input checked="" type="checkbox"/>	2.000
3	<input checked="" type="checkbox"/>	3.000
4	<input checked="" type="checkbox"/>	5.000
5	<input checked="" type="checkbox"/>	6.000
6	<input checked="" type="checkbox"/>	7.000

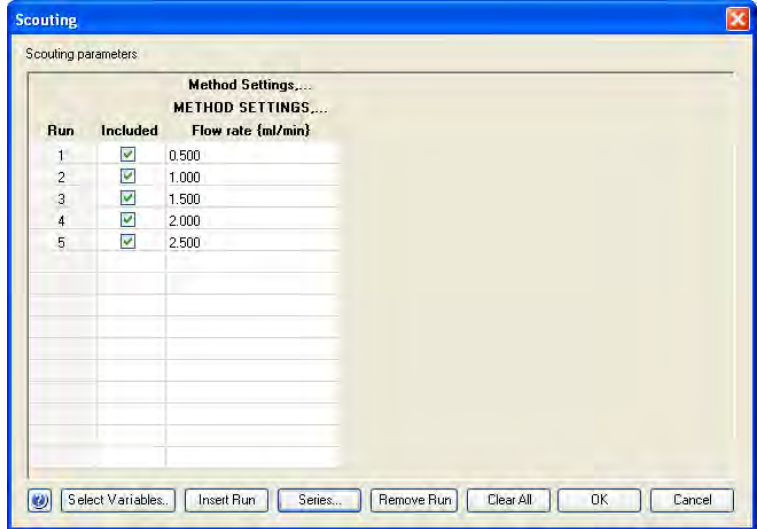
8 Click **OK** in the **Scouting** dialog to save the scouting scheme.
Save the method.

Add, delete or edit variables in the Scouting scheme

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

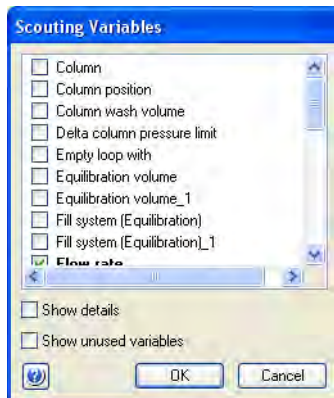
Step	Action
------	--------

- | | |
|---|--|
| 1 | Open the Scouting scheme (see <i>Set up a scouting scheme, on page 129</i>). |
|---|--|



- 2 To add or delete variables in the **Scouting scheme**, click **Select Variables...** in the **Scouting** dialog.

Result: The **Scouting Variables** dialog opens.



4 Scouting

4.2 Set up and edit a Scouting scheme

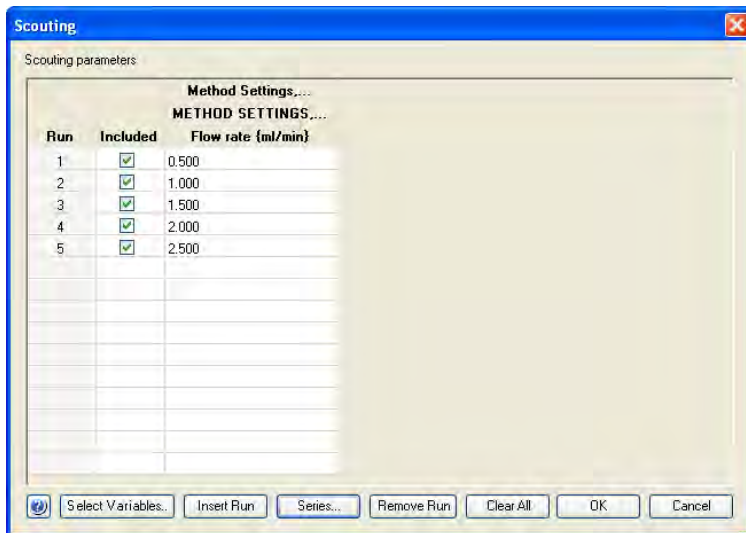
Step	Action
3	<ul style="list-style-type: none">To add a variable to the Scouting scheme, check the appropriate box in front of the variable.To delete a variable from the Scouting scheme, clear the box in front of the variable. <p>If you cannot find the appropriate variable:</p> <ul style="list-style-type: none">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. <p>To define a new variable, see <i>Section 10.2.4 Method variables, on page 353</i> for information.</p> <p>Click OK.</p> <p><i>Result:</i> The Scouting parameters table is updated with the changes.</p>
4	<p>To edit a variable value for a run:</p> <ul style="list-style-type: none">Select the appropriate row and the variable value cell in the Scouting parameters table.Type a new value for the variable. <p><i>Result:</i> The variable value is updated.</p> <p>Note:</p> <p><i>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.</i></p>
5	<p>Click OK.</p> <p><i>Result:</i> The Scouting parameters table is updated with the changes.</p>
6	<p>Add new scouting runs to the scouting scheme as required.</p>
7	<p>Click OK in the Scouting dialog to save the scouting scheme.</p> <p>Save the method.</p>

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 <i>Set up a scouting scheme, on page 129</i>). |
|---|--|



4 Scouting

4.2 Set up and edit a Scouting scheme

Step	Action
2	<ul style="list-style-type: none">• <i>To insert a run after an existing run:</i> Select the appropriate row in the Scouting parameters table and click <input type="button" value="Insert Run"/>. <i>Result:</i> 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.• <i>To insert a new series of runs:</i><ul style="list-style-type: none">- Click in the appropriate variable column in the Scouting parameters table and click <input type="button" value="Series..."/>.- Set up a series in the Insert series dialog and click OK (see <i>Set up a scouting scheme, on page 129</i>). <i>Result:</i> The new set of runs are inserted in the Scouting scheme with the values provided.• <i>To delete runs from the Scouting scheme:</i><ul style="list-style-type: none">- Select the row(s) in the Scouting parameters table and click <input type="button" value="Remove Run"/>. <i>Result:</i> The selected runs are removed from the Scouting scheme. or- Click <input type="button" value="Clear All"/>.<i>Result:</i> All runs are removed from the Scouting scheme. No scouting will be performed when starting the run.• <i>To exclude a run from being used in the Scouting experiment but keep it in the Scouting scheme:</i> 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 gives a brief overview of **Design of Experiments** and describes some basic terms and concepts used in the **Design of Experiments** tool in UNICORN. It also describes how to set up an experimental design plan using the **Design of Experiments (DoE)** tool in the **Method Editor** and how to evaluate the results of the runs in the **Evaluation** module.

Note: *This information only applies to Evaluation Classic.*

Design of Experiments is only available for some systems.

In this chapter

This chapter contains the following sections:

Section	See page
5.1 Introduction to Design of Experiments	140
5.2 Create an experimental design	152
5.3 Run a scouting created with DoE	178
5.4 Evaluation of Design of Experiments	180

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?

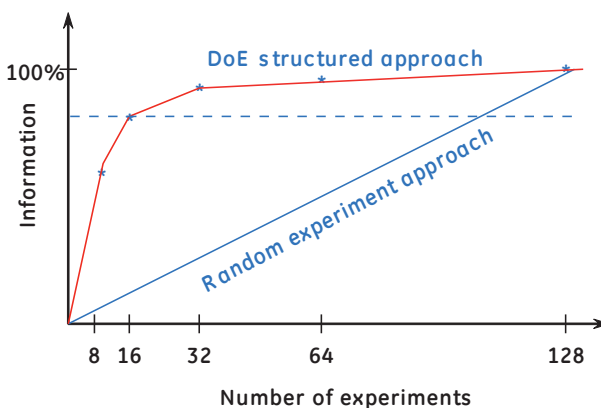
Design of Experiments is a way to systematically vary several parameters simultaneously to obtain as much information about a process with as few experiments as possible.

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.

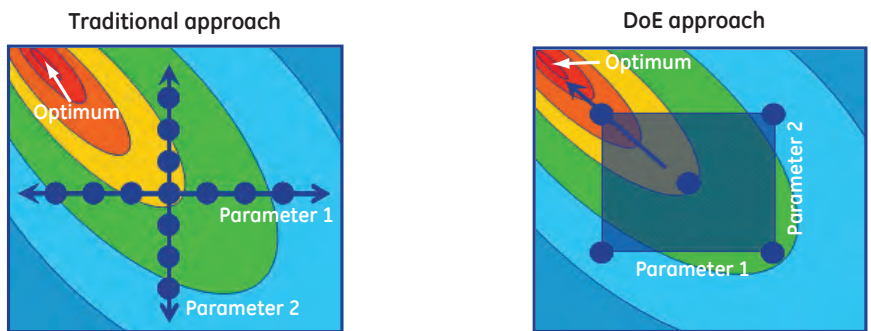
For example, it could be enough to obtain 80% information about a process. This level of information can be reached using a significantly lower number of experiments using **DoE** than using a random experiment approach as illustrated in the figure below.



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.



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	<p>The different parameters that may affect the process to be run.</p> <p>Factors may be either quantitative or qualitative.</p> <ul style="list-style-type: none"> 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. 	<p>The factors are connected to a variable in the method. For example, the factor pH may be connected to the variable BufferPro pH.</p> <p>In predefined methods, most useful parameters are already defined as variables.</p> <p>Note: <i>To be able to vary a value for a process parameter in the method it must be defined as a variable.</i></p> <p>Low and high values are entered for the quantitative factors. The factors will be varied within this range.</p>
Response	<p>The output parameter(s) from a process. For example, capacity, yield and purity.</p>	<p>When evaluating the DoE runs, the measured response values for each experiment are entered in UNICORN.</p>

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.

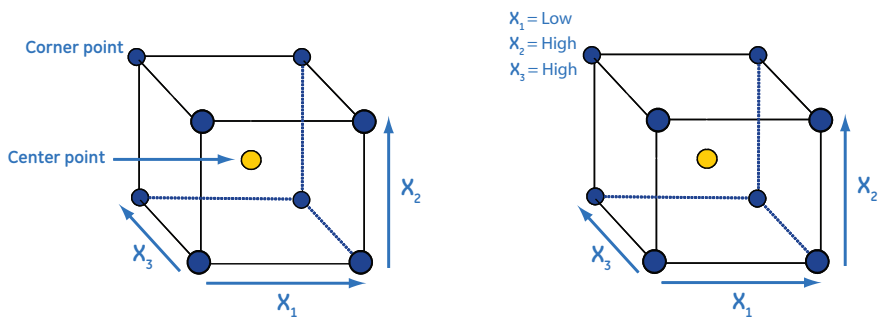
Design types

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 148 for detailed information about the terms interaction, curvature and pure error.

Illustration of the design box

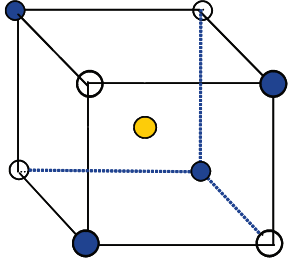
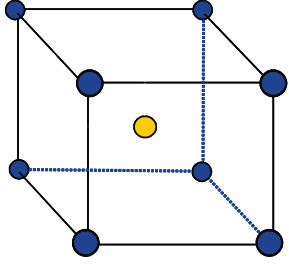
The illustration below (to the left) shows a design box with corner- and center points for the different factors X_1 , X_2 and X_3 . The illustration below (to the right) shows the factor values for one of the corner points. The arrow directions along the box edges denote the parameter change from low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



Different designs in UNICORN

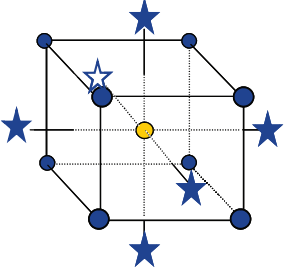
There are several types of design available in UNICORN.

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
<p data-bbox="164 283 361 311">Fractional Factorial</p> 	<p data-bbox="477 283 1188 438">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.</p> <p data-bbox="477 451 1188 606">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.</p> <p data-bbox="477 618 1002 646">Fractional designs are suggested when performing:</p> <ul data-bbox="477 664 1188 828" style="list-style-type: none"> • 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) <p data-bbox="477 842 1154 906">Less experiments are needed compared to using Full Factorial and Optimization designs.</p>
<p data-bbox="164 920 297 948">Full Factorial</p> 	<p data-bbox="477 920 1188 984">The Full Factorial design uses all corner point experiments. This design is often suggested when performing Screening.</p> <p data-bbox="477 997 1188 1061">Information about which factors are important (main effects) and more information about factor interaction effects are obtained.</p>

5 Design of Experiments

5.1 Introduction to Design of Experiments

Design type	Description
<p>Optimization designs</p> 	<p>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.</p> <p>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.</p> <p>The default star point distance (CCC design, see below) can be edited in UNICORN.</p> <p>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.</p> <p>Information about which factors are important (main effects), information about factor interaction effects and curvature are obtained. See also <i>Model</i>, on page 148 for information.</p>

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 quadratic 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	<p>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.</p> <p>Plackett Burman designs are useful when performing Screening or Robustness Testing.</p>
Rechtschaffner	<p>Rechtschaffner is a saturated fraction of the 2^n and 3^n factorial designs that supports all the first order interactions and quadratic terms.</p> <p>Rechtschaffner is useful when performing Optimization and you have at least three factors in your experimental plan.</p>
Full Factorial 2 levels	<p>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).</p> <p>Full Factorial 2 levels designs are useful when performing Screening or Robustness Testing.</p>
Full Factorial 3 levels	<p>Full Factorial 3 levels is a full factorial design with every factor varied at three levels. You can estimate the full quadratic model.</p> <p>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.</p>
CCC	<p>The Central Composite Circumscribed (CCC) design is composed of a full or fractional factorial design and star points.</p> <p>CCC designs are useful when performing Optimization.</p>
CCF	<p>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.</p> <p>CCF designs are useful when performing Optimization.</p>
Box Behnken	<p>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.</p> <p>Box Behnken is useful when performing Optimization and you have at least three factors in your experimental plan.</p>
Doehlert	<p>Doehlert is a RSM design constructed from regular simplexes.</p> <p>Doehlert designs are useful when performing Optimization.</p>

Model

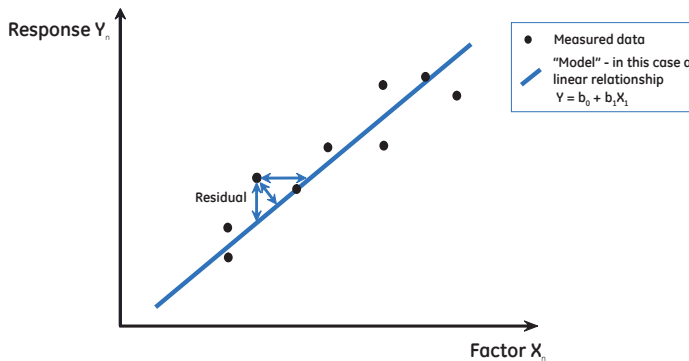
A model is created in the **Evaluation** module based on the response values measured or entered for each experiment in the **DoE** setup.

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

$$Y_n = f(X_1, X_2, \dots, X_n)$$

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.

Model

$$Y = \underbrace{b_0}_{\text{Constant Term}} + \underbrace{b_1X_1 + b_2X_2 + b_3X_3}_{\text{Linear terms (main effects)}} + \underbrace{b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3}_{\text{Two-way Interaction terms}} + \underbrace{b_7X_1^2 + b_8X_2^2 + b_9X_3^2}_{\text{Quadratic terms}}$$

Description of the variables and constants in the model

Known variables (entered in UNICORN)

X_1, X_2, \dots, X_M are the input variables to the equation

They correspond to the low and high factor values entered in the Method Editor.

Y_1, Y_2, \dots, Y_M are the output variables to the equation

They correspond to the measured response values entered in the Evaluation module.

Unknown constants determined by the model selected in UNICORN

b_0 is the unknown Constant Term. It is the response of Y when the main effects are 0.

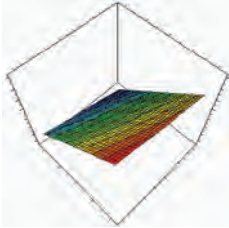
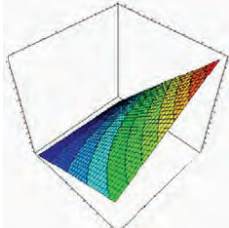
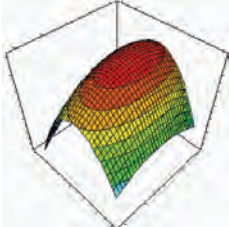
b_1, b_2, \dots, b_M are unknown constants determined by the model in UNICORN.

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.

5 Design of Experiments

5.1 Introduction to Design of Experiments

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 	<p>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.</p> <p>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.</p> <p>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.</p>
Two-way interaction $b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$	Twisted plane 	<p>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.</p> <p>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.</p>
Quadratic $b_7X_1^2 + b_8X_2^2 + b_9X_3^2$	Curved plane 	<p>The curvature of the sampling plane is described by the quadratic terms.</p> <p>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.</p>

DoE workflow in UNICORN

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

Step	Action
1	<p>Create a method for your process to be screened, optimized or tested for robustness</p> <p>This includes defining the appropriate variables (if not already defined) that should be connected to the factors in <i>DoE</i>.</p> <p>See <i>Chapter 3 Create and edit methods, on page 27</i> for information about how to create a method.</p>
2	<p>Set up an experimental design</p> <p>This is performed in the <i>Method Editor</i> in the <i>Design of Experiments</i> tool.</p> <p>See <i>Section 5.2 Create an experimental design, on page 152</i> for more information.</p>
3	<p>Perform the runs in the Scouting scheme generated from DoE</p> <p>See UNICORN System Control Manual for information about starting scouting runs.</p> <p>Note:</p> <p><i>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.</i></p>
4	<p>Perform statistical evaluation of a DoE scouting</p> <p>This is performed in the <i>Evaluation</i> module.</p> <p>See <i>Section 5.4 Evaluation of Design of Experiments, on page 180</i> for more information.</p>

5.2 Create an experimental design

About this section

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 these subsections:

Section	See page
5.2.1 Set up an experimental design	153
5.2.2 Add responses and factors to an experimental design	162
5.2.3 Change design and design settings in a Design of Experiments setup	169
5.2.4 Divide the DoE runs into several scouting runs	173

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 <i>Chapter 3 Create and edit methods, on page 27</i> 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 <i>Section 10.2.4 Method variables, on page 353</i> for information about how to define new variables. Note: <i>In the DoE setup factors are connected to the variables in the method.</i>
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*.

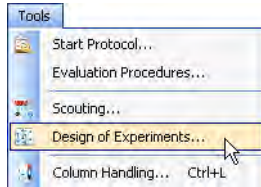
Step	Action
------	--------

- | | |
|---|---|
| 1 | In the <i>Method Editor</i> : <ul style="list-style-type: none">click the <i>Design of Experiments</i> icon in the <i>Toolbar</i> |
|---|---|

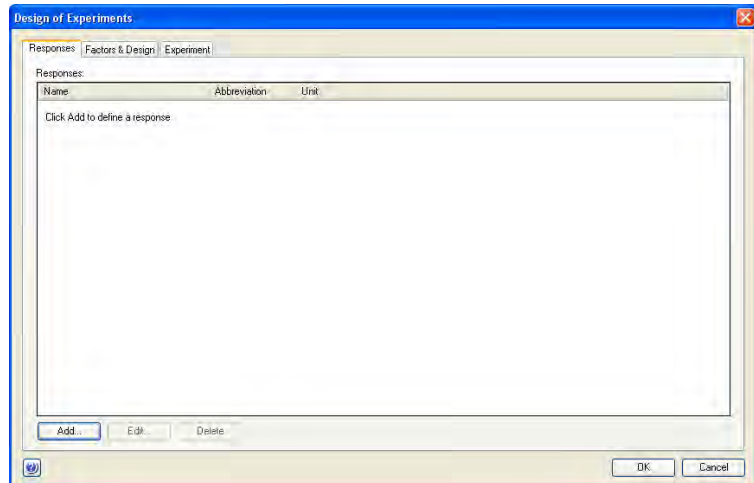


or

- select *Tools:Design of Experiments*



Result: The *Design of Experiments* dialog opens.

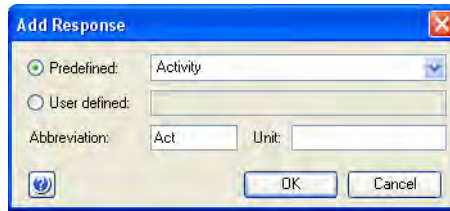


Step **Action**

2 To add responses to the **Design of Experiments**, click **Add...**

Result: The **Add Response** dialog opens.

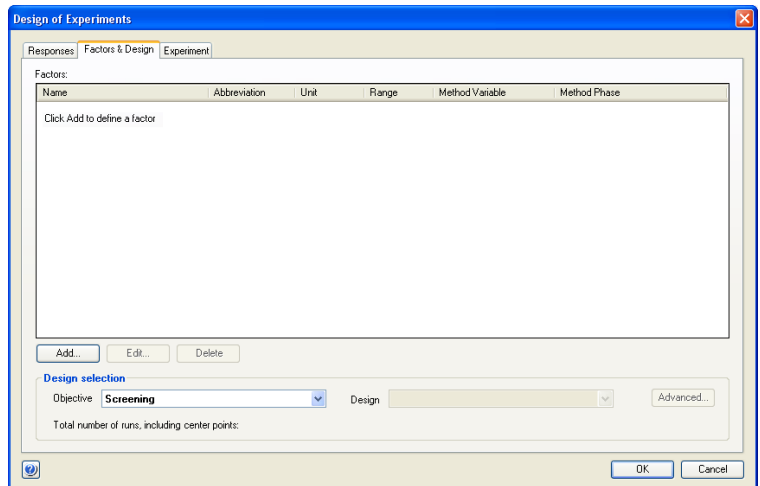
For detailed information about how to define and add a response, see Section 5.2.2 *Add responses and factors to an experimental design, on page 162.*



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.



5 Design of Experiments

5.2 Create an experimental design

5.2.1 Set up an experimental design

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 162.

Add Factor

Predefined: Bed Height

User defined:

Abbreviation: BeHe Unit:

Type: Quantitative Quantitative multilevel Qualitative

Settings

Low value: High value: Center point:

Method phase: Method Settings

Variable:

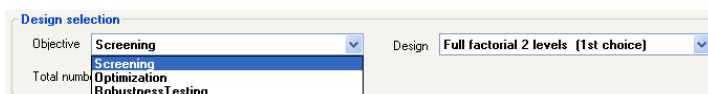
Don't connect the factor to a method variable.

OK Cancel

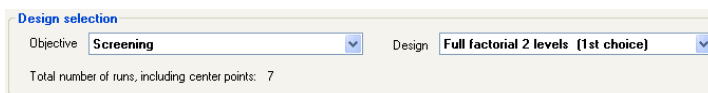
Step **Action**

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.

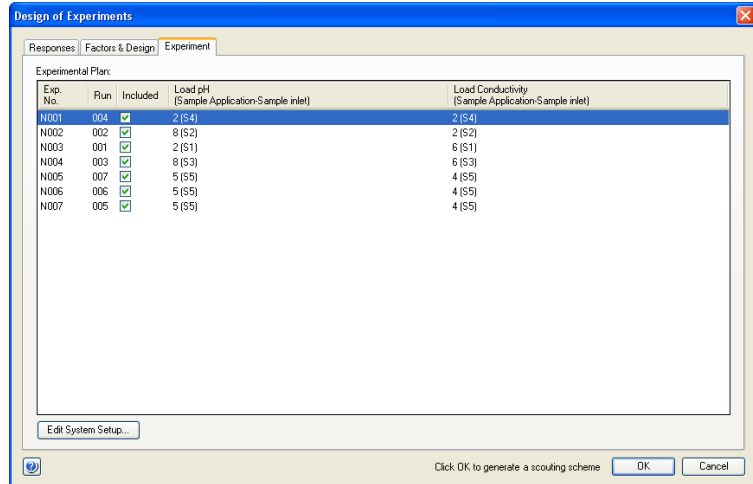
For information about how to view details for the selected design and/or to change to another design than the 1st or 2nd choice designs, see *Section 5.2.3 Change design and design settings in a Design of Experiments setup, on page 169.*

5 Design of Experiments
5.2 Create an experimental design
5.2.1 Set up an experimental design

Step **Action**

6 Click the **Experiment** tab.

Result: The **Experimental Plan** is displayed.



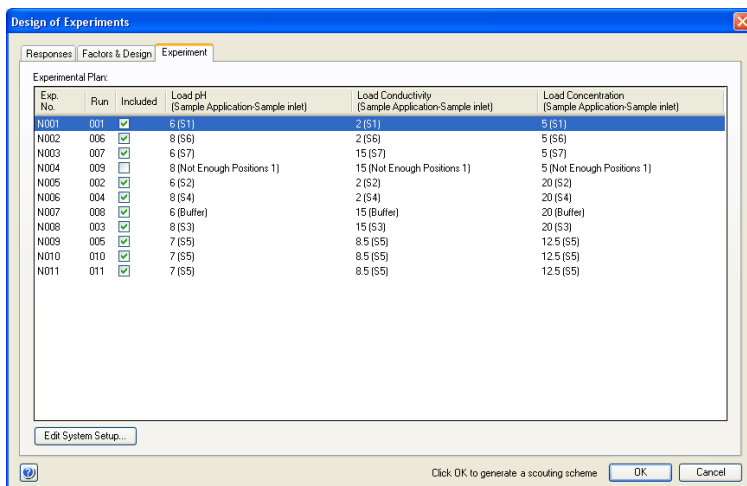
The **Run** column shows the run order for the optimized **Scouting scheme** that is generated from the **DoE** when clicking **OK**.

Note:

*If any of the runs in the **Experimental Plan** are excluded, 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 173*.



5 Design of Experiments

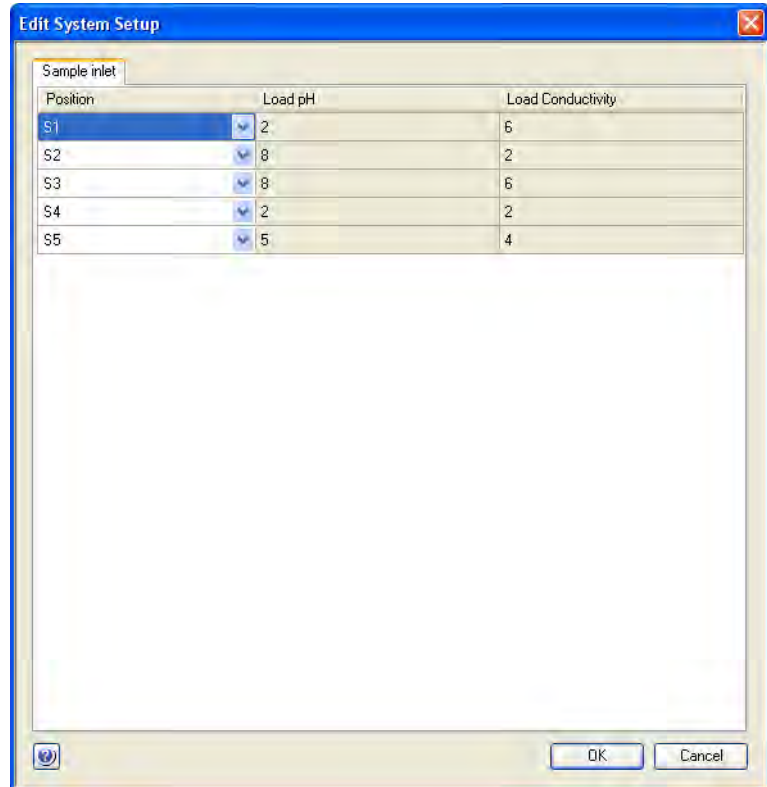
5.2 Create an experimental design

5.2.1 Set up an experimental design

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 | <ul style="list-style-type: none">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

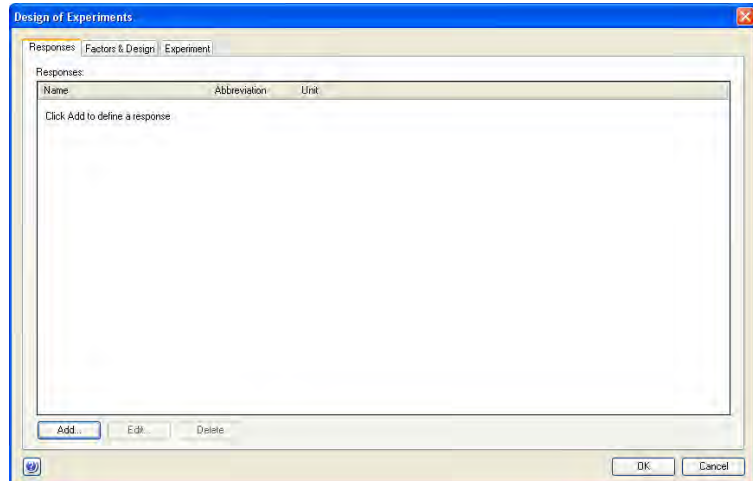
Introduction

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:

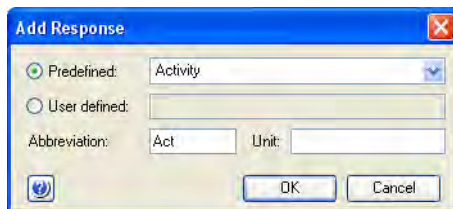
Step	Action
1	Select the Responses tab.



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

2	To add a response, click Add...
---	--

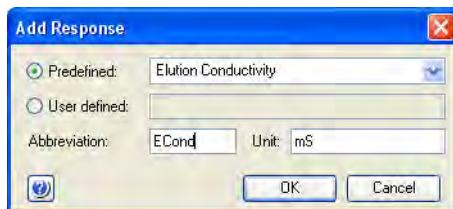
Result: The **Add Response** dialog opens.



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**.*

- | | |
|---|--|
| 3 | <ul style="list-style-type: none">• 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
<i>Result: Abbreviation is automatically filled in.</i>• If applicable, enter unit for the response. |
|---|--|



5 Design of Experiments

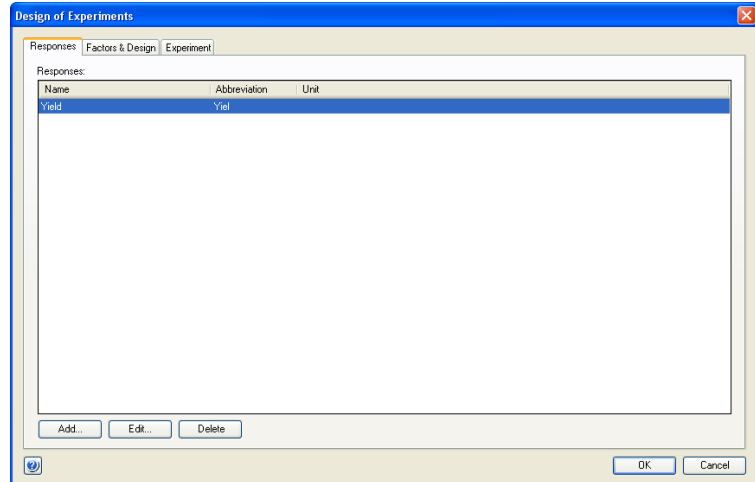
5.2 Create an experimental design

5.2.2 Add responses and factors to an experimental design

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

4	Click OK .
---	-------------------

Result: The selected response is added to the **Responses** list in the **Design of Experiments** dialog.

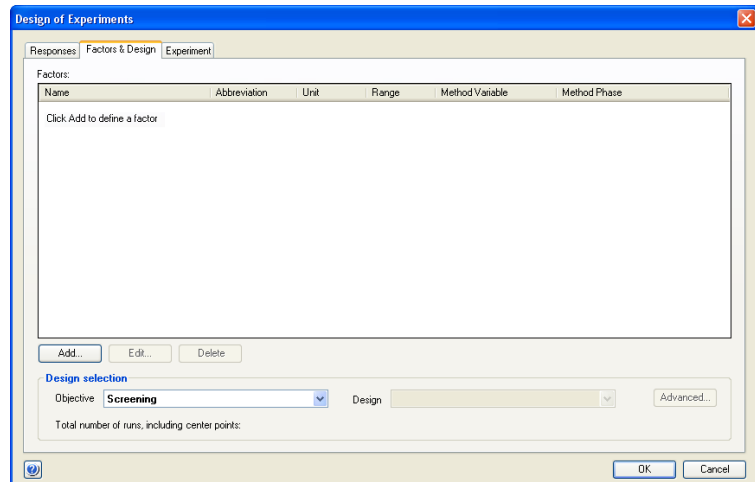


Add factors

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

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

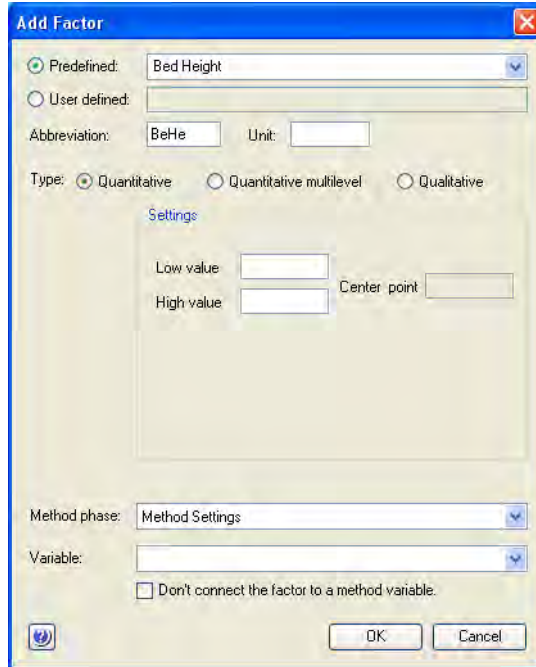
1	Select the Factors & Design tab.
---	---



Step **Action**

2 To define factors, click **Add...**

Result: The **Add Factor** dialog opens.



5 Design of Experiments

5.2 Create an experimental design

5.2.2 Add responses and factors to an experimental design

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

- | | |
|---|--|
| 3 | <p>To add a predefined factor:</p> <ul style="list-style-type: none">• Select the factor to be added in the Predefined drop-down list.
<i>Result: Abbreviation</i> 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:

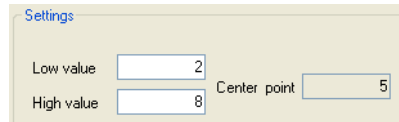
Type of factor	Description
Quantitative	Quantitative factors are process parameters that can be measured and have values on a continuous scale (e.g., flow rate and pH values)
Quantitative multi-level	To specify more than two levels for a factor, select the Quantitative multilevel type. For example, if you 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.



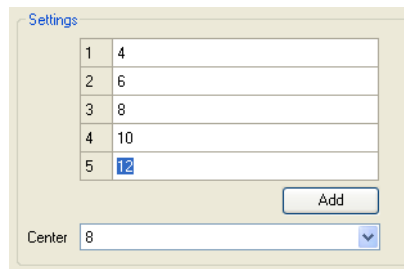
Settings

Low value Center point

High value

- **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.



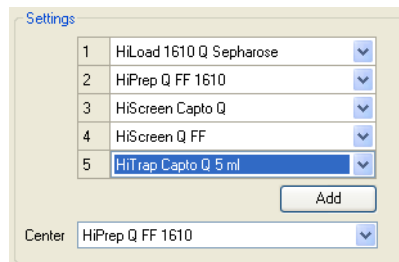
Settings

1	4
2	6
3	8
4	10
5	12

Center ▼

- **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.



Settings

1	HiLoad 1610 Q Sepharose ▼
2	HiPrep Q FF 1610 ▼
3	HiScreen Capto Q ▼
4	HiScreen Q FF ▼
5	HiTrap Capto Q 5 ml ▼

Center ▼

5 Design of Experiments

5.2 Create an experimental design

5.2.2 Add responses and factors to an experimental design

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

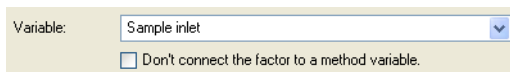
- 5 Select the phase to which the factor is connected in the **Method phase** drop-down list.



Method phase:

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.



Variable:
 Don't connect the factor to a method variable.

For example, if adding the predefined factor **Load pH**, the sample pH at loading is controlled by the **Sample inlet** valve position.

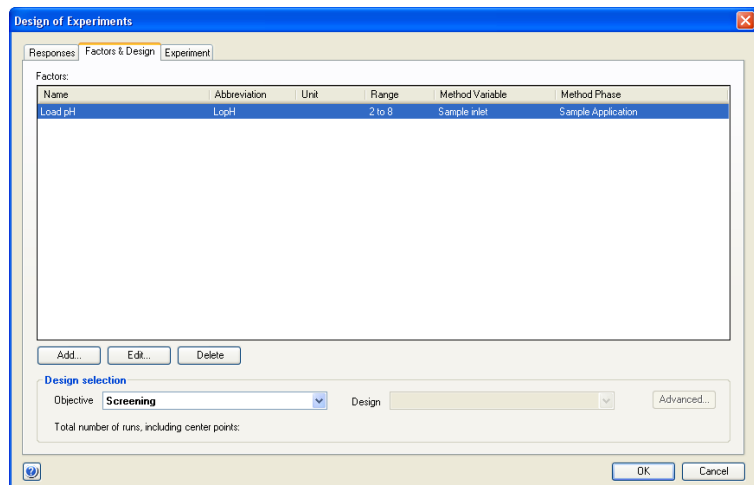
Note:

*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.



5 Design of Experiments

5.2 Create an experimental design

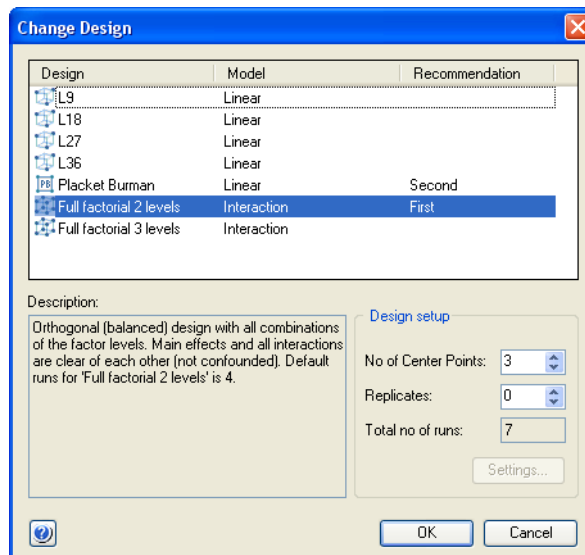
5.2.3 Change design and design settings in a Design of Experiments setup

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 146*.

Step **Action**

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

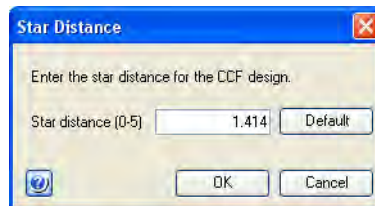
- Change the settings for **No of Center Points** and **Replicates** as appropriate.

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**.

5 Design of Experiments

5.2 Create an experimental design

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

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

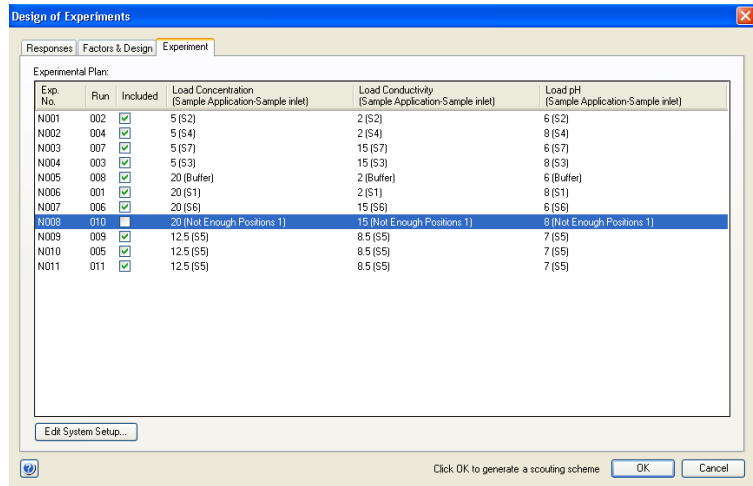
It is possible to include/exclude runs directly in the generated **Scouting** scheme and edit, for example, the sample inlet positions. However, for complex experimental plans it is recommended to create multiple **DoE** methods, each using the same design but with different sub-sets of scouting runs (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

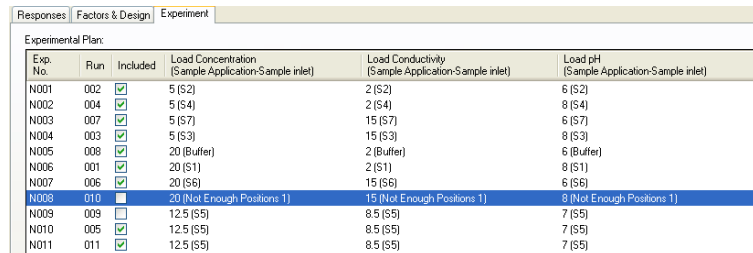
The table below describes how to identify hardware limitations in a DoE run.

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.



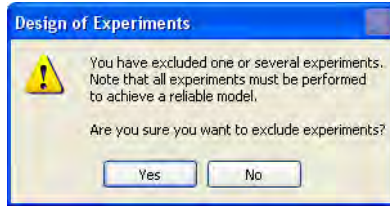
Note:

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.

Experimental Plan:						
Exp. No.	Run	Included	Load Concentration (Sample Application-Sample inlet)	Load Conductivity (Sample Application-Sample inlet)	Load pH (Sample Application-Sample inlet)	
N001	002	<input checked="" type="checkbox"/>	5 (S2)	2 (S2)	6 (S2)	
N002	004	<input checked="" type="checkbox"/>	5 (S4)	2 (S4)	8 (S4)	
N003	007	<input checked="" type="checkbox"/>	5 (S7)	15 (S7)	6 (S7)	
N004	003	<input checked="" type="checkbox"/>	5 (S3)	15 (S3)	8 (S3)	
N005	008	<input checked="" type="checkbox"/>	20 (Buffer)	2 (Buffer)	6 (Buffer)	
N006	001	<input checked="" type="checkbox"/>	20 (S1)	2 (S1)	8 (S1)	
N007	006	<input checked="" type="checkbox"/>	20 (S6)	15 (S6)	6 (S6)	
N009	010	<input type="checkbox"/>	20 (Not Enough Positions 1)	15 (Not Enough Positions 1)	9 (Not Enough Positions 1)	
N009	009	<input type="checkbox"/>	12.5 (S5)	8.5 (S5)	7 (S5)	
N010	005	<input checked="" type="checkbox"/>	12.5 (S5)	8.5 (S5)	7 (S5)	
N011	011	<input checked="" type="checkbox"/>	12.5 (S5)	8.5 (S5)	7 (S5)	

8 Clear the **Included** boxes in front of **all** runs.

5 Design of Experiments

5.2 Create an experimental design

5.2.4 Divide the DoE runs into several scouting runs

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

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.

Step Action

- 11 Click **OK** in the **Edit System Setup** dialog.
- 12 In the **Experiment** tab, check the boxes in front of the runs to be included in the second set of runs.

Experimental Plan:						
Exp. No.	Run	Included	Load Concentration (Sample Application-Sample inlet)	Load Conductivity (Sample Application-Sample inlet)	Load pH (Sample Application-Sample inlet)	
N001	002	<input type="checkbox"/>	5 (S2)	2 (S2)	6 (S2)	
N002	004	<input type="checkbox"/>	5 (S4)	2 (S4)	8 (S4)	
N003	007	<input type="checkbox"/>	5 (S7)	15 (S7)	6 (S7)	
N004	003	<input type="checkbox"/>	5 (S3)	15 (S3)	8 (S3)	
N005	008	<input type="checkbox"/>	20 (Not Enough Positions 1)	2 (Not Enough Positions 1)	6 (Not Enough Positions 1)	
N006	001	<input type="checkbox"/>	20 (S1)	2 (S1)	8 (S1)	
N007	006	<input type="checkbox"/>	20 (S6)	15 (S6)	6 (S6)	
N008	010	<input checked="" type="checkbox"/>	20 (Buffer)	15 (Buffer)	8 (Buffer)	
N009	009	<input checked="" type="checkbox"/>	12.5 (S5)	8.5 (S5)	7 (S5)	
N010	005	<input type="checkbox"/>	12.5 (S5)	8.5 (S5)	7 (S5)	
N011	011	<input type="checkbox"/>	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 UNICORN System Control 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
------	--------

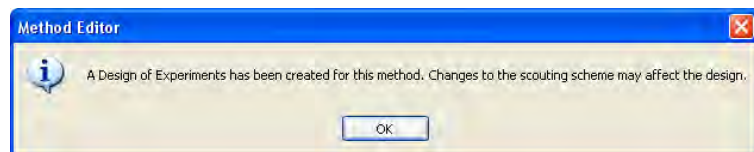
- | | |
|---|--|
| 1 | In the Method Editor : <ul style="list-style-type: none">Click the Scouting icon |
|---|--|



or

- Select **Tools:Scouting**.

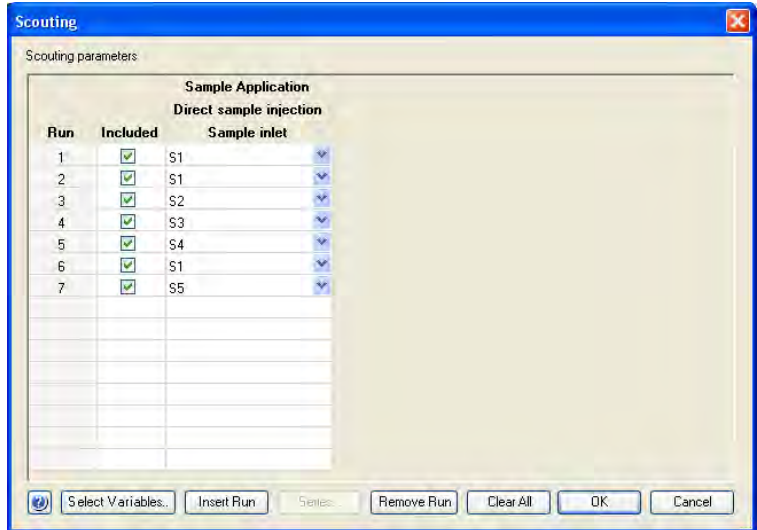
Result: The following dialog is displayed as a reminder of that a **DoE** has been created for the method. If you change the **Scouting scheme**, the **DoE** experimental plan is changed and the results may not be reliable for use in the **DoE** evaluation.



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 3.6.3 Print a method, on page 94* for information about how to print the method.

5.4 Evaluation of Design of Experiments

About this section

Note: *This information only applies to Evaluation Classic.*

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

In this section

This section contains these subsections:

Section	See page
5.4.1 Workflow	181
5.4.2 Generate model	183
5.4.3 Analyze and evaluate the model - basic analysis	192
5.4.4 Analyze and evaluate the model - extended analysis	204
5.4.5 Edit the model	211
5.4.6 Use the model	214
5.4.7 Create and print reports	224

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**

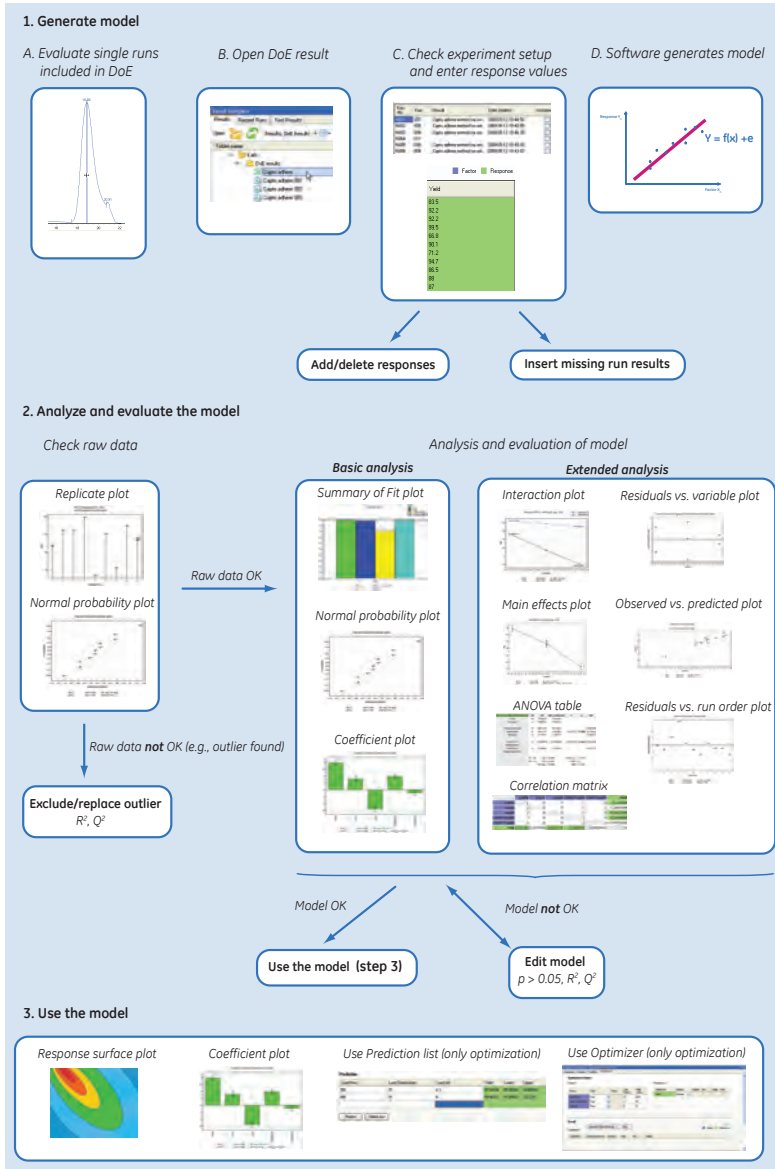
This includes generating a response surface plot (including a sweep spot plot) as well as using the predictor and the optimizer.

- 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:

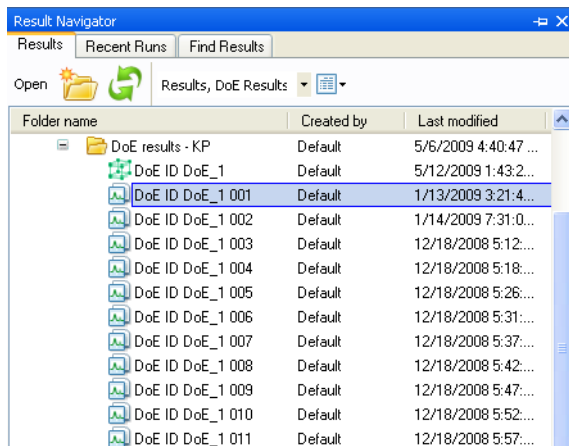
Step	Action
------	--------

- 1 In the **Evaluation** module, click the **Open Result Navigator** icon in the **Toolbar**.



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

- 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.

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.2 Generate model

Step	Action
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 <i>UNICORN Evaluation Manual</i> for information about how to perform evaluation.
5	Save any changes. Tip: <i>It is possible to have a Scouting run result open at the same time even if a DoE result is open.</i>
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:

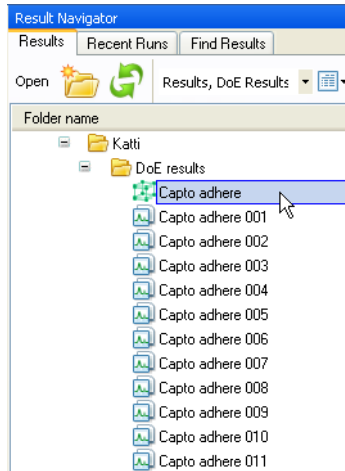
Step	Action
1	In the Evaluation module, click the Open Result Navigator icon in the Toolbar .



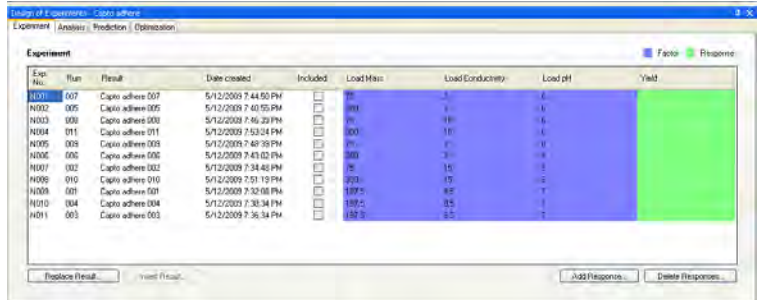
Result: The **Result Navigator** is displayed.

Step **Action**

- 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.

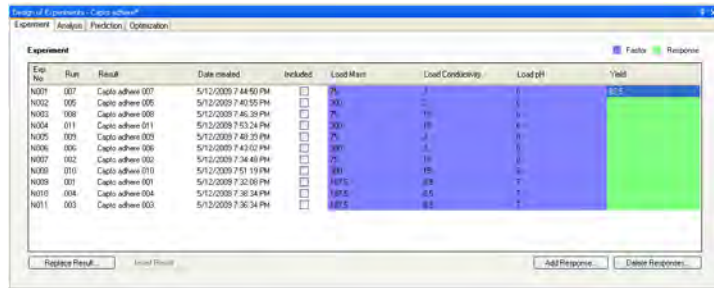
To add or delete responses to the experiment, use the **Add Response...** and **Delete Response...** buttons. See *Add responses, on page 187* and *Delete responses, on page 188* for more information.

Step Action

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 190* and *Insert new runs, on page 188* 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.*

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 192*.

Add responses

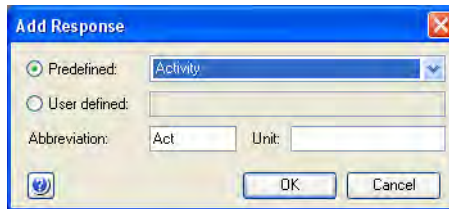
Note: Responses added in the **Evaluation** module will not be included in the original method.

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. |
| 4 | Click OK . |

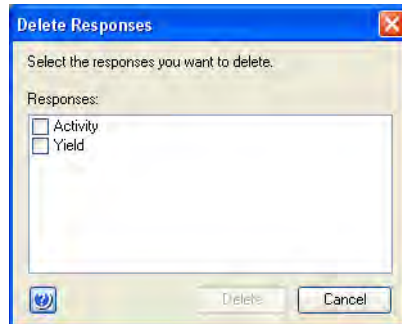
Result: The response is added to the **DoE** experiment.

Exp. No.	Plan	Result	Date created	Included	Load Mass	Load Conductivity	Load pH	Yield	Activity
N001	007	Capto adhere 007	5/12/2009 7:44:50 PM	<input type="checkbox"/>	25	10	6	83.5	
N002	005	Capto adhere 005	5/12/2009 7:40:55 PM	<input type="checkbox"/>	100	1	6		
N003	008	Capto adhere 008	5/12/2009 7:46:39 PM	<input type="checkbox"/>	75	15	6		
N004	011	Capto adhere 011	5/12/2009 7:53:24 PM	<input type="checkbox"/>	100	10	6		
N005	000	Capto adhere 000	5/12/2009 7:48:30 PM	<input type="checkbox"/>	75	21	6		
N006	006	Capto adhere 006	5/12/2009 7:43:02 PM	<input type="checkbox"/>	100	1	6		
N007	002	Capto adhere 002	5/12/2009 7:34:48 PM	<input type="checkbox"/>	75	15	6		
N008	010	Capto adhere 010	5/12/2009 7:51:19 PM	<input type="checkbox"/>	100	15	6		
N009	001	Capto adhere 001	5/12/2009 7:32:09 PM	<input type="checkbox"/>	100.0	10.0	7		
N010	004	Capto adhere 004	5/12/2009 7:38:34 PM	<input type="checkbox"/>	100.0	10.0	7		
N011	003	Capto adhere 003	5/12/2009 7:36:34 PM	<input type="checkbox"/>	100.0	10.0	7		

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.
<i>Result:</i> The Delete Responses dialog opens. |



- | | |
|---|---|
| 2 | Check the box in front of the response to be deleted and click Delete .
<i>Result:</i> The response is deleted from the experiment. |
|---|---|

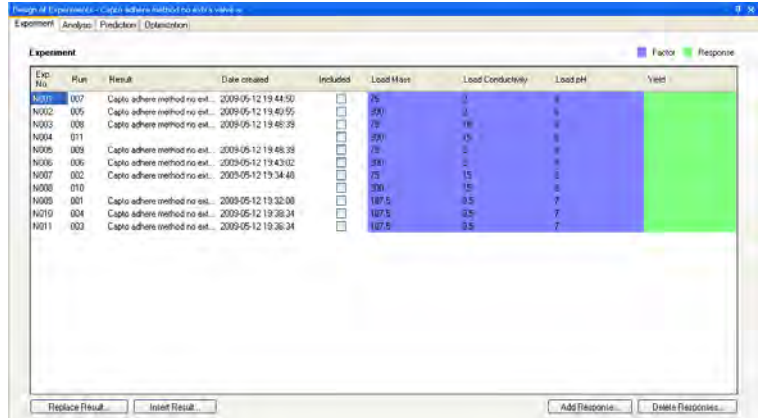
Insert new runs

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:

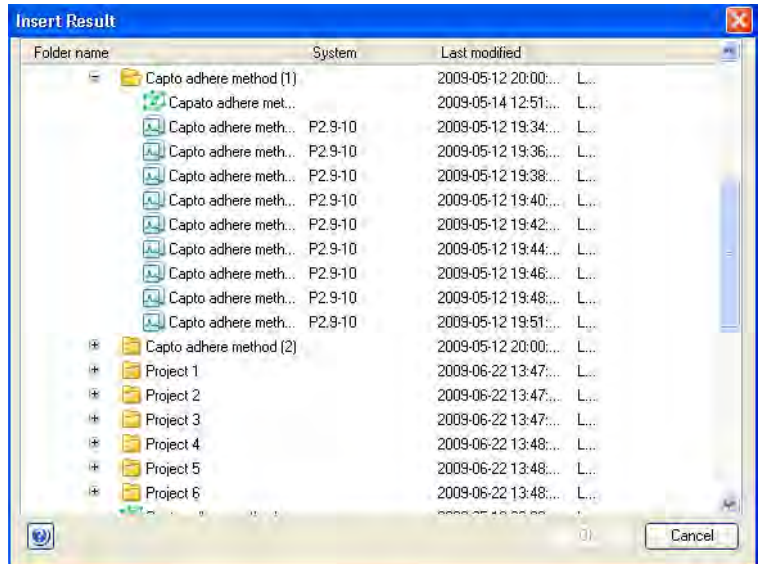
Step Action

- 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.



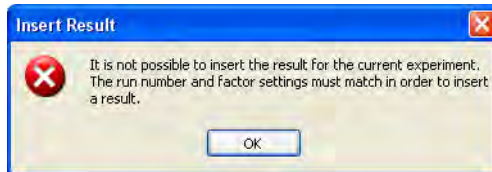
- 3 Browse and select the run(s) that should be inserted.

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.*

Step Action

- 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

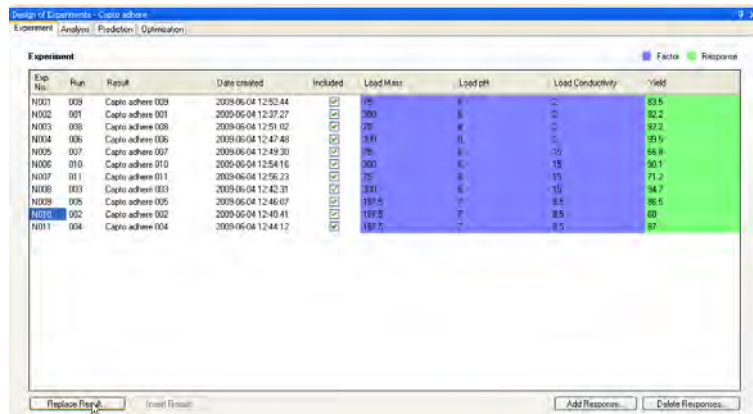
Runs can be replaced with a new run if, for example, the run has failed.

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. Rerunning a center point experiment will help in keeping track of uncontrolled variations.*

The table below describes how to replace a run result:

Step Action

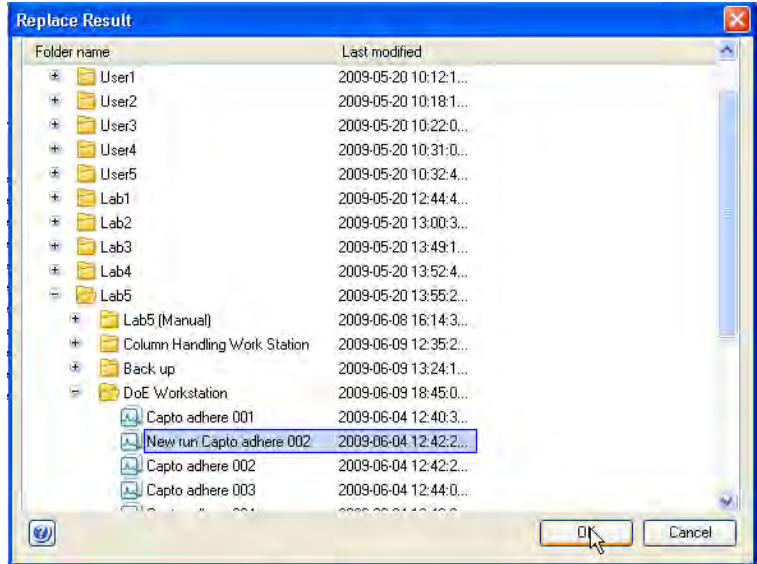
- 1 In the **Experiment** tab of the **Design of Experiments** box, select the result to be replaced in the **Result** column in the **Experiment** table.



Step **Action**

2 Click the **Replace Result...** button.

Result: The **Replace Result** dialog opens.

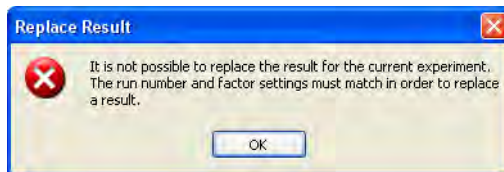


3 Browse and select the run that should replace the selected run.

4 Click **OK**.

Result: The new run is listed in the **Experiment** table.

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

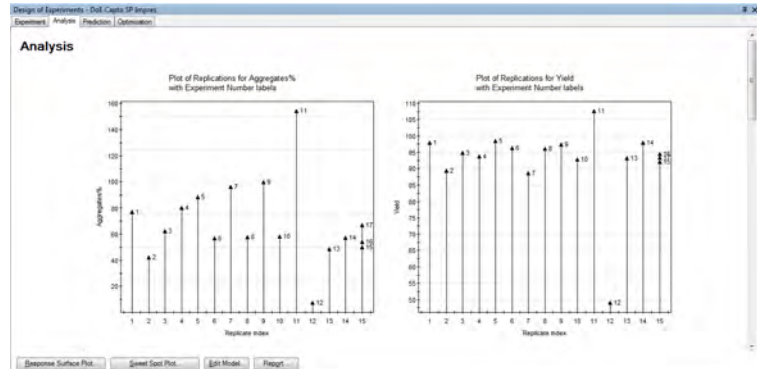
Before starting to analyze the model, the raw data must be checked to ensure that the correct conclusions can be drawn in the analysis and evaluation of the model.

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

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

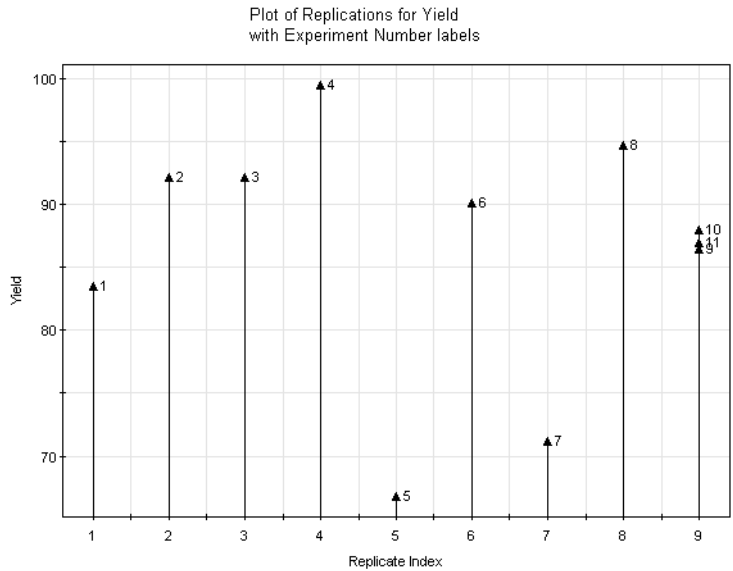
- | | |
|---|--|
| 1 | Select the Analysis tab, if not already selected. |
|---|--|

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.

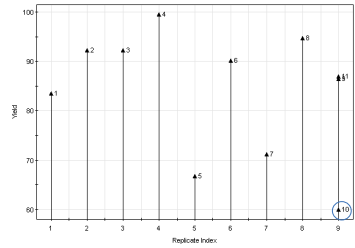
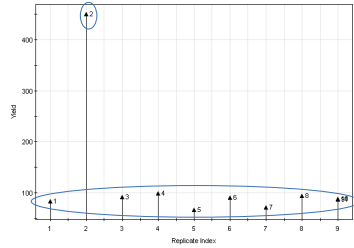
5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.3 Analyze and evaluate the model - basic analysis

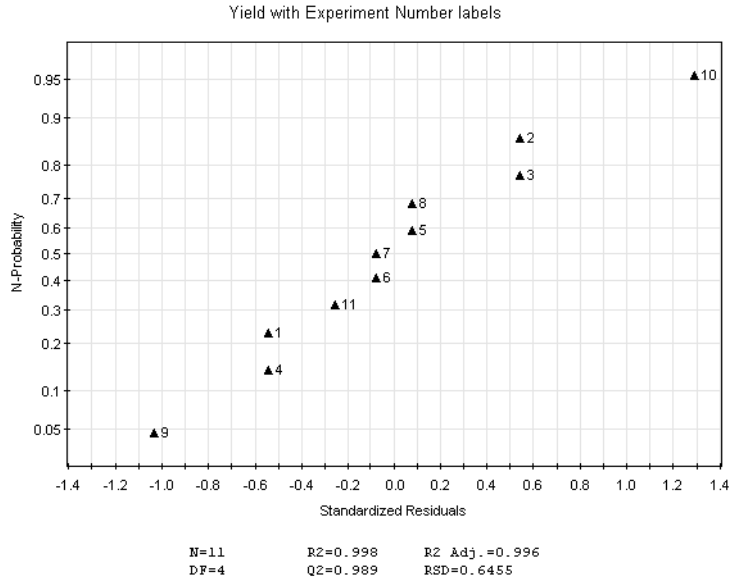
Step Action

- 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 196* and *Section 5.4.4 Analyze and evaluate the model - extended analysis, on page 204*.

- 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 196*.

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.3 Analyze and evaluate the model - basic analysis

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.	<i>Replace run results, on page 190</i> 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.	<i>Generate model, on page 185</i> 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 <i>Section 5.4.2 Generate model, on page 183</i> 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.

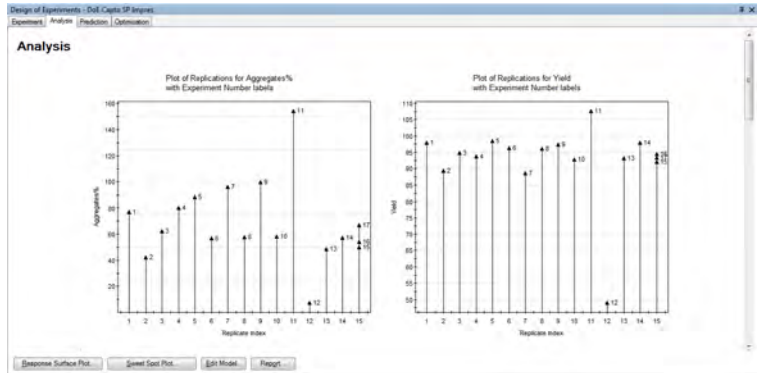
Note: *The plots must be analyzed for each response. A model can be good for one response but not for another. In some cases a good model cannot be obtained when several responses are included in the same model. In this case, try fitting an individual model for each response separately.*

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.



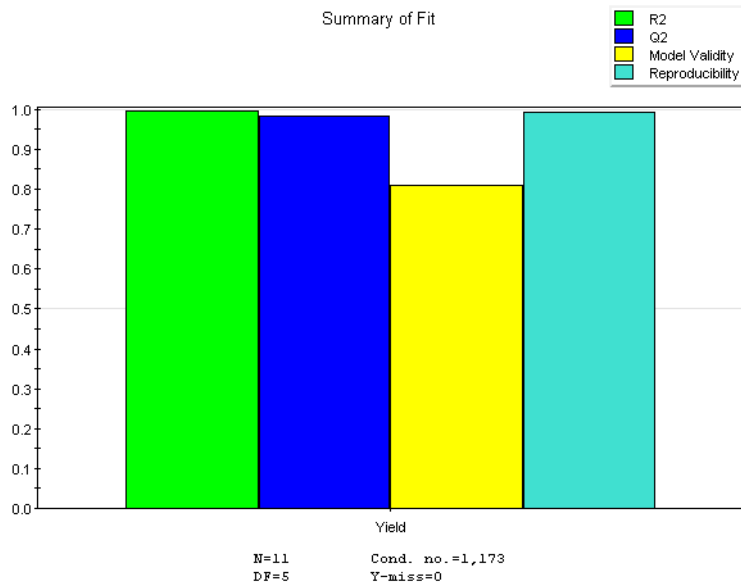
5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.3 Analyze and evaluate the model - basic analysis

Step	Action
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- | | |
|---|---|
| 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	<p>R² 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 R²-value is necessary for a good model but not sufficient on its own.</p> <p>A value of 0.75 indicates a rough but stable and useful model.</p> <p>Note: R² Adj is the fraction of variations in the response data that is explained by the model, adjusted for degrees of freedom. R² does not take into account degrees of freedom.</p>
Q²	<p>Q² describes how well the model will predict new data. It can vary between -∞ and 1. The higher Q²-value, the better indicator of how well the model will predict new data.</p> <p>Q²>0.5 is good and Q²>0.9 is excellent.</p> <p>Q² is a better indicator of the usefulness of the model than R².</p> <p>Note: R² should not exceed Q² by more than 0.2-0.3 for a good model.</p>
Model Validity	<p>Model validity is only available if replicated experiments have been performed.</p> <p>A model validity>0.25 indicates a good model.</p> <p>A model validity<0.25 indicates a significant "lack of fit", that is the model error is significantly larger than the pure error (reproducibility).</p>
Reproducibility	<p>A reproducibility<0.5 indicates that there is a large pure error and poor control of the experimental setup (high noise level).</p>

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

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.3 Analyze and evaluate the model - basic analysis

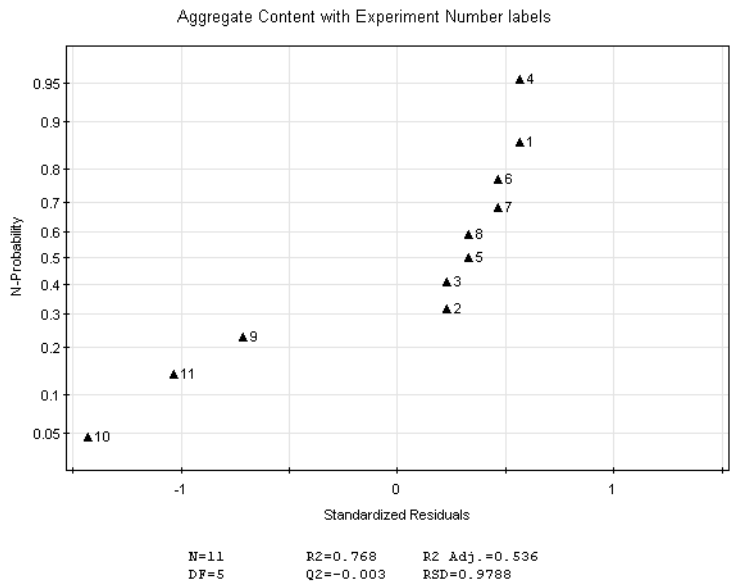
Step Action

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 <i>Residuals versus variables plot</i> , on page 204) and the ANOVA table (see <i>ANOVA table</i> , on page 208) 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 192*.

If the center 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 211* for more information.

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.3 Analyze and evaluate the model - basic analysis

Step	Action
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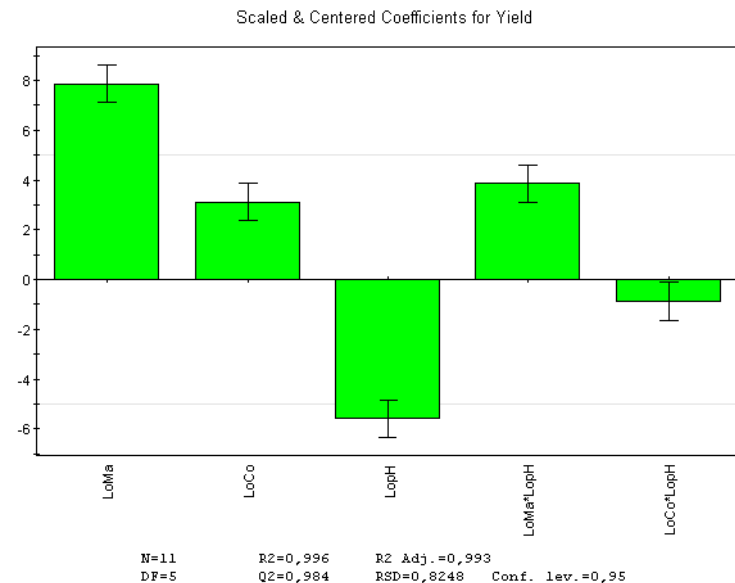
4	Look at the coefficient plot for each response. The coefficient plot can be used to see which factors that affect your response, in which way they affect the response(s) and if there are any non-significant terms in the model.
---	--

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.

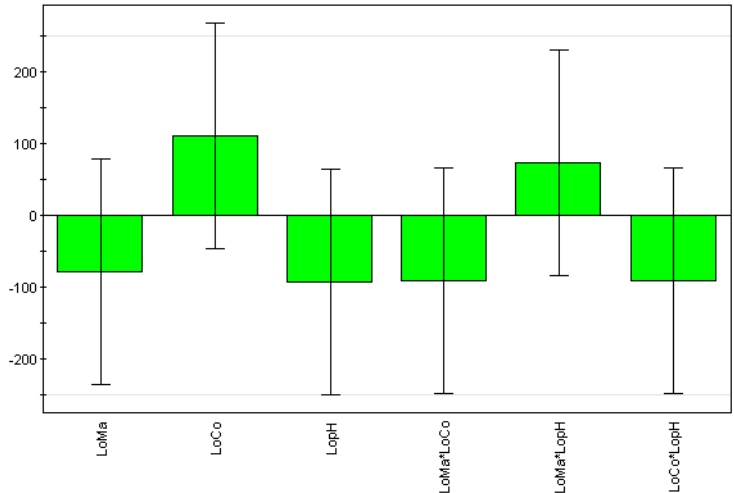


In the example above, the confidence limits (the black error bars shown on each green bar in the plot) do not cross zero. All of the terms are thus significant, with the **LoCo*LopH** two-way interaction term being least significant.

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 bars) 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 204* and *Section 5.4.5 Edit the model, on page 211* 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 214*.

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 224 for information about how to create an extended report.

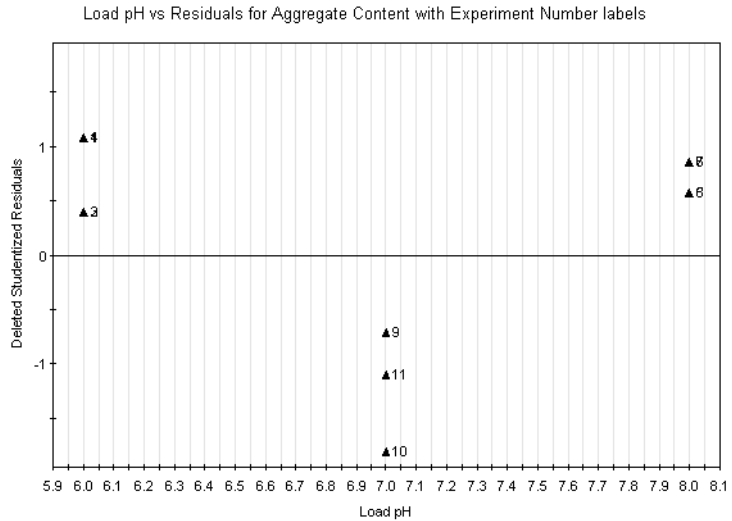
Residuals versus variables plot

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.

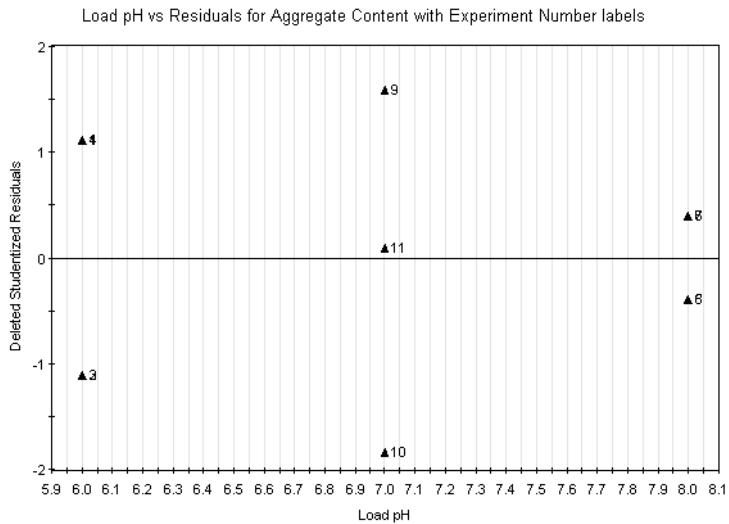
The residuals should be randomly distributed with no pattern. When a curved pattern can be seen in the plot this may indicate that a quadratic term is missing in the model. In this case try adding a quadratic term to the model and see if the model is improved.

See *Section 5.4.5 Edit the model*, on page 211 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.

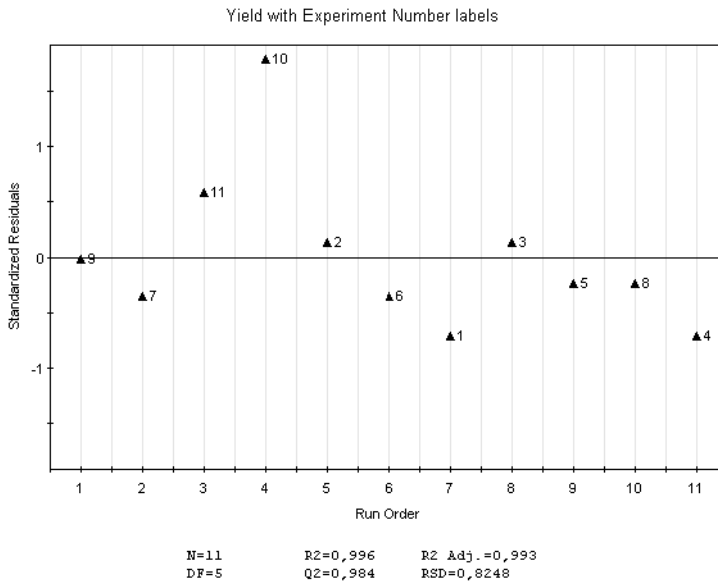


Note: When you find curvature in the model, the design for the experiment in the DoE setup should be changed to design allowing quadratic terms to be added to the model (Full factorial 3 levels, CCC, CCF, Box Behnken, Rechtschaffner or Doehlert). If the experimental setup is sufficiently stable the star 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.


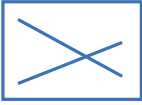


Interaction plot

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**).

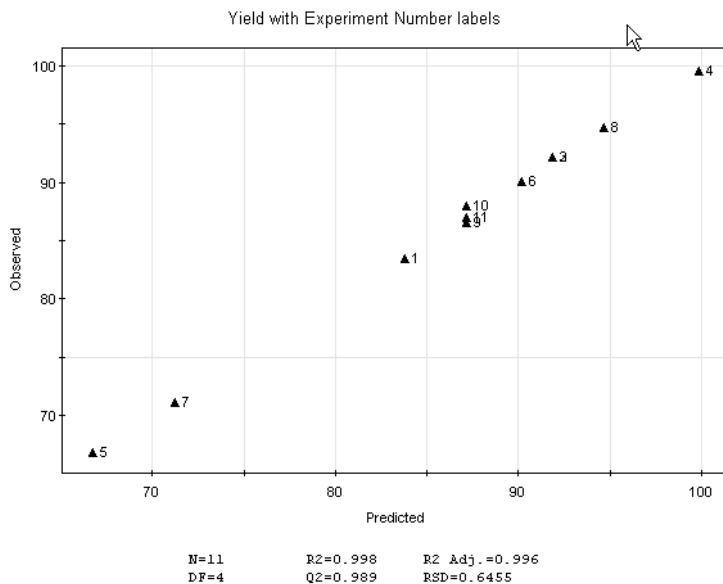
The table below describes how to interpret different interactions plot in a schematic way:

Plot	Description
	The two lines are parallel. This plot shows an example of no interaction between the two factors.

Plot	Description
	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.

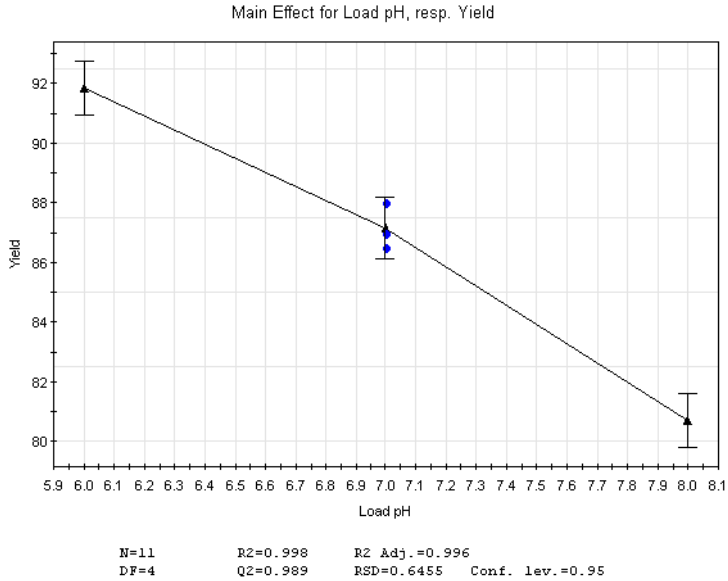
Observed versus Predicted for each Response plot

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 (Model Error)	1	0.0786042	0.0786042	0.0370193	0.865	0.280364
Pure Error (Replicate Error)	2	4.24666	2.12333			1.45717
	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>$p > 0.05$ indicates a good model.</p> <p>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.</p> <p>See Section 5.4.5 <i>Edit the model</i>, on page 211 for information about how to add a quadratic term to the model.</p> <p>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 modeled.</p>
Cond. no. (condition number)	<p>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.</p> <p>Depending on the design, different condition numbers are expected for the model to be good.</p>	<ul style="list-style-type: none"> • When the objective is screening and robustness testing <ul style="list-style-type: none"> - Good design when Cond. no. < 3 - Questionable design when Cond. no. = 3-6 - Poor design when Cond. no. > 6 • When the objective is optimization <ul style="list-style-type: none"> - Good design when Cond. no. < 8 - Questionable design when Cond. no. = 8-12 - Poor design when Cond. no. > 12

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.4 Analyze and evaluate the model - extended analysis

Correlation matrix

The correlation matrix gives a numerical presentation of the correlation between factors and responses and shows 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.

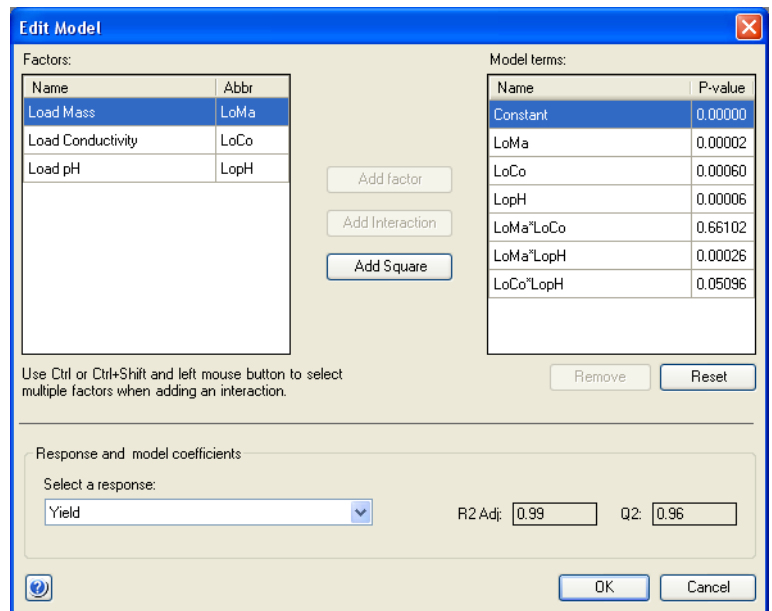
Edit the model

The table below describes how to edit a model:

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

1	In the Analysis tab, click  .
---	---

Result: The **Edit Model** dialog opens.



Factors:

Name	Abbr
Load Mass	LoMa
Load Conductivity	LoCo
Load pH	LopH

Model terms:

Name	P-value
Constant	0.00000
LoMa	0.00002
LoCo	0.00060
LopH	0.00006
LoMa*LoCo	0.66102
LoMa*LopH	0.00026
LoCo*LopH	0.05096

Response and model coefficients

Select a response:

R2 Adj: Q2:

5 Design of Experiments

5.4 Evaluation of Design of Experiments

5.4.5 Edit the model

Step	Action
2	<p>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.</p> <p>When editing the model, the R2 Adj and Q2 values are updated. Higher values indicate a better model. See also <i>Analyze and interpret the model - basic analysis, on page 196</i> for a description of the values.</p>
3	<p>Non-significant terms may have been found in the analysis of the model (for example in the coefficient plot).</p> <p>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.</p> <p>Note:</p> <p><i>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.</i></p> <p>Note:</p> <p><i>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.</i></p> <p>Note:</p> <p><i>If a main term is not significant but one of its interaction terms is significant the main term should not be removed.</i></p> <p>Note:</p> <p><i>If a main term is removed its interaction terms are also removed.</i></p> <p>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.</p>

Step	Action
4	<p>Based on the previous analysis, add the appropriate terms to the model.</p> <ul style="list-style-type: none">• Add an interaction term by selecting the appropriate factors in the Factors table and clicking Add Interaction. <p>Tip: <i>Use the Ctrl or Shift keyboard key to select multiple factors.</i></p> <ul style="list-style-type: none">• Add a quadratic term to the model by selecting the appropriate factor in the Factors table and clicking Add Square. <p>Note: <i>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).</i></p> <p>Note: <i>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.</i></p> <p><i>Result:</i> 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.</p>
5	To return to the original model settings, click Reset .
6	<p>When you are satisfied with the editing, click OK.</p> <p><i>Result:</i> The Edit Model dialog is closed and the Analysis tab displayed showing the new plots for the edited model.</p>
7	Perform an analysis of the edited model to see if the new model is OK . See <i>Analyze and interpret the model - basic analysis</i> , on page 196 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.
- **Sweet Spot Plot**
Generate a sweet spot plot to get a graphical representation of the experimental region where two response criteria are satisfied.
- **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 has 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 196 and Section 5.4.4 Analyze and evaluate the model - extended analysis, on page 204 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

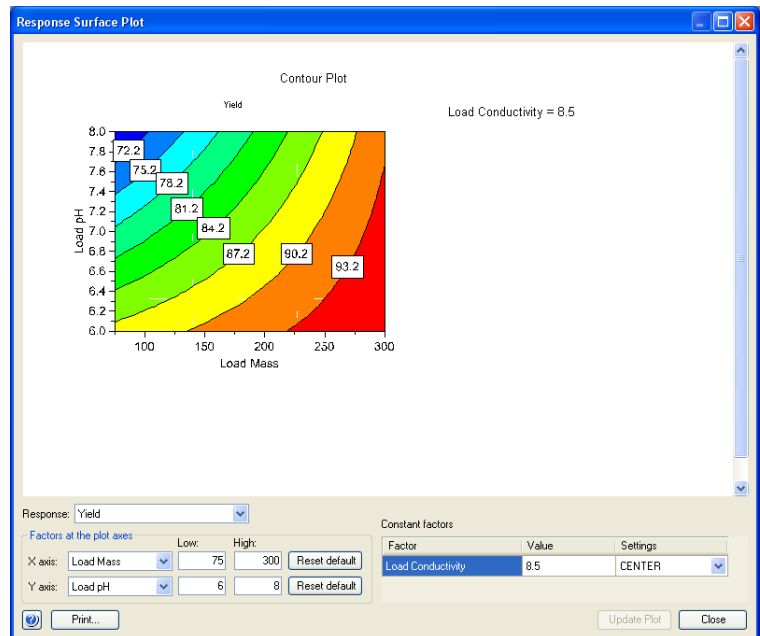
Note: The underlying model must be good and have a high Q^2 -value. See Section 5.4.3 Analyze and evaluate the model - basic analysis, on page 192.

The following table 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**

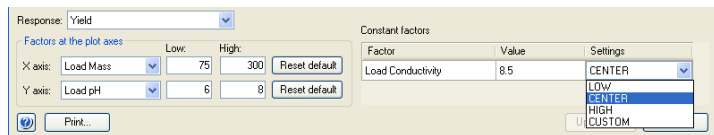
2 It is possible to change the factors and their corresponding settings for the response surface plot as well as the constant values for the other factor(s). This is done per response if you have several responses.

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** 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** list as shown below. If selecting **CUSTOM**, click in the **Value** field and enter a value.



4 Click **Update Plot**.

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 **Print**.
Result: The **Print Preview** dialog opens.
 - Click **Print**.
Result: The standard **Print** dialog opens.
 - Select the appropriate printer and click **Print**.
-

Generate sweet spot plot and edit settings

The sweet spot plot graphically displays the range where two or more selected response criteria are satisfied. It is helpful when you want to:

- get an overview of what factor ranges will give a desired 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

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 192.*

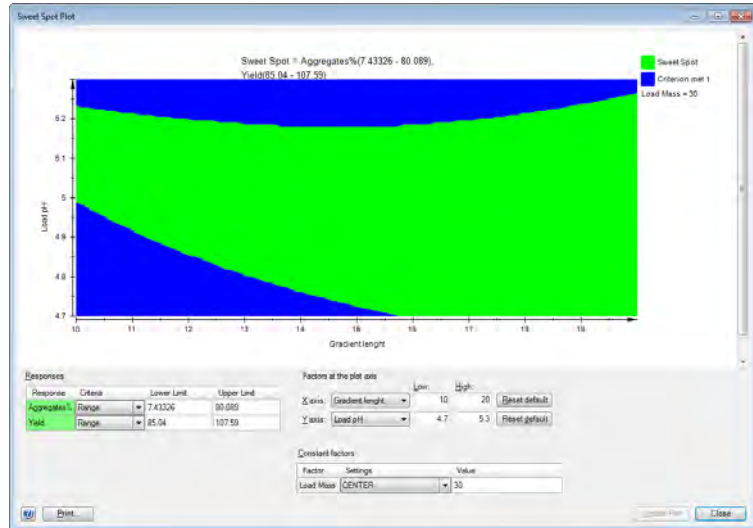
Note: *The sweet spot plot will only provide useful information of two or more factors are set as responses.*

The following instruction describes how to generate a sweet spot plot and how to evaluate the plot:

Step **Action**

1 In the **Analysis** tab, click **Sweet Spot Plot**.

Result: The **Sweet Spot Plot** dialog opens.



The plot shows a map of the model, visualizing factor ranges where all response criteria are satisfied. Areas where all criteria are not satisfied are colored different shades of blue. Areas of the plot where all response criteria are satisfied are colored green. The green areas are called "sweet spots". The current sweet spot criteria is listed above the plot.

The sweet spot is defined as a prediction interval. That is, the likelihood that a future observation will fall within the sweet spot criterion is larger further away from the blue border.

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

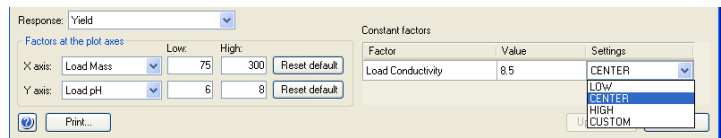
Step Action

2 It is possible to change the response criteria for the selected responses. Response criteria can be ranges, upper and lower limits and excluded ranges. Criteria for the responses are set individually. You can also change the value(s) of the constant factor(s).

In this way you can see what happens if you redefine the response criteria and if constant factors are changed.

To change the **Sweet Spot Plot** settings:

- Select the response criteria for the sweet spot plot in the **Criteria** lists. There is one criterion for each of the responses.
- 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** list. If selecting **CUSTOM**, click in the **Value** field and enter a value.



3 Click **Update Plot**.

Result: The **Sweet Spot Plot** is updated.

4 When you have obtained the appropriate information to help you in the decision on how to proceed it is possible to print the **Sweet Spot Plot**.

- Click **Print**.
Result: The **Print Preview** dialog opens.
- Click **Print**.
Result: The standard **Print** dialog opens.
- Select the appropriate printer and click **Print**.

Predict response values

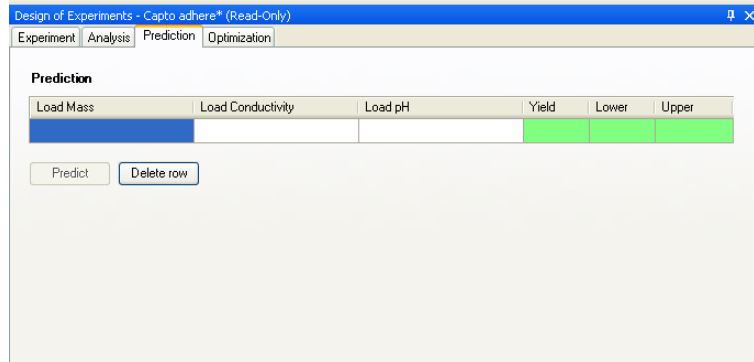
It is possible to predict response values based on entered factor settings using the model. This is useful when you want to find out how detailed factor settings influence the response(s) in an optimization experiment. Factor settings are entered and response values are calculated when using the **Prediction**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

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

Step Action

- 1 Select the **Prediction** tab in the **Design of Experiments** box.

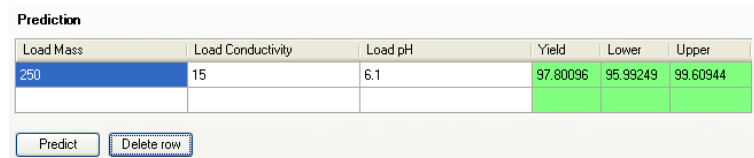
Result: The **Prediction** list opens.



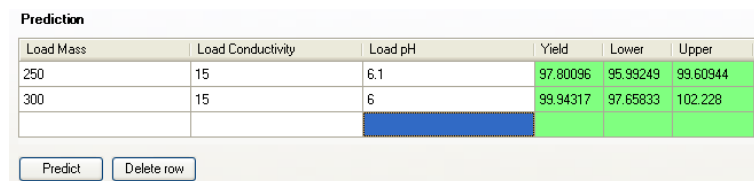
- 2 Enter the appropriate settings for the different factors in their respective fields.

- 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.



- 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.



Repeat this procedure until you are satisfied.

Optimize response values and factor settings

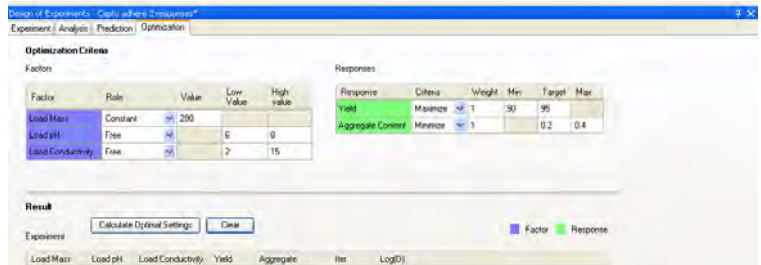
It is possible to optimize the response values using the optimizer. When using the optimizer, criteria for the response values and factor settings are entered (e.g., **Yield**>90%) and factor settings are calculated. In this way, the experimental region can be moved to an optimum.

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.

Responses

Response	Criteria	Weight	Min	Target	Max
Yield	Maximize	1	90	95	

Dropdown menu for Criteria:

- Minimize
- Maximize
- Target
- Exclude

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.

Responses

Response	Criteria	Weight	Min	Target	Max
Yield	Maximize	1	90	95	

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.

Step **Action**

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

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

Result

Experiment

Calculate Optimal Settings Clear

Factor Response

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 of 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.

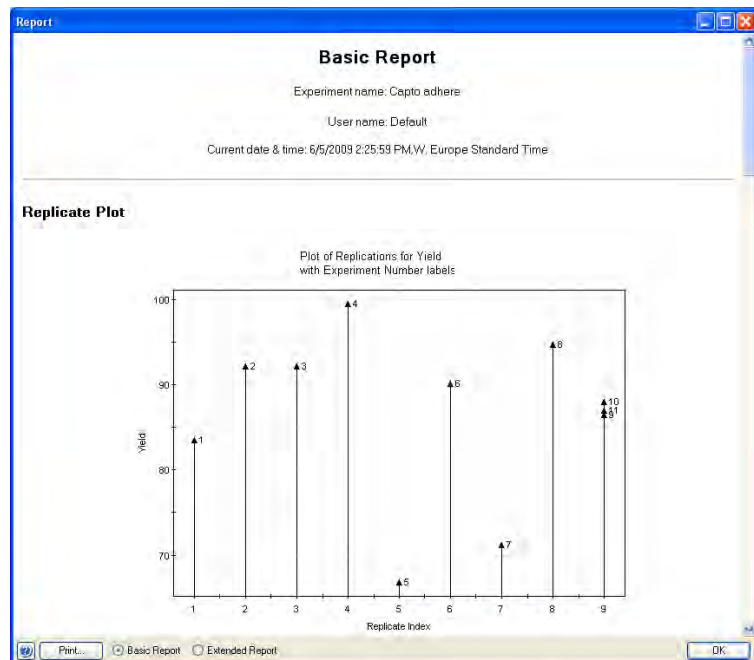
Create a report

The table below describes how to create a report:

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

1	In the Analysis tab, click  .
---	---

Result: The **Report** dialog opens displaying the **Basic** report by default. It displays the Replicate, Summary of Fit, Normal probability and Coefficient plots.

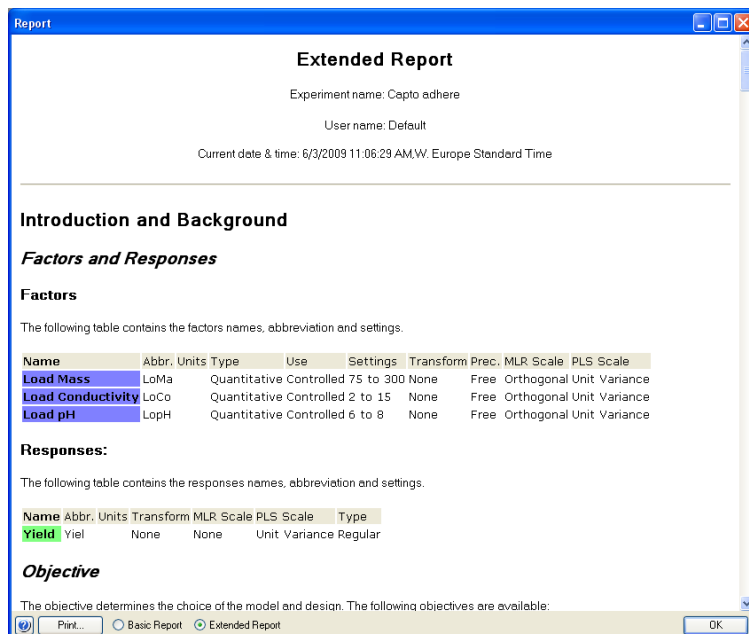


Step Action

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.



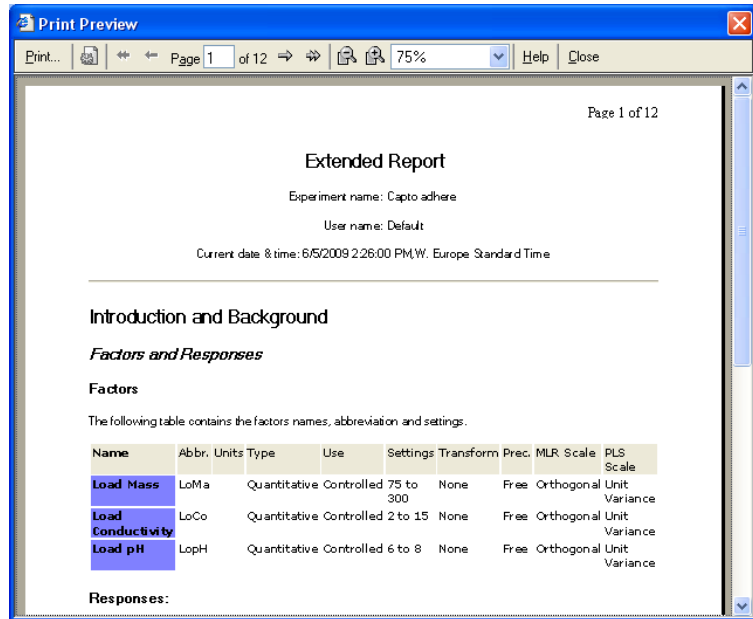
3 To view the information in the report use the vertical scrollbar.

Print a report

The table below describes how to print a report:

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

- | | |
|---|--|
| 1 | In the Report dialog, click the Print... button.
<i>Result:</i> The Print Preview dialog opens. |
|---|--|



- | | |
|---|--|
| 2 | Click the Print... button.
<i>Result:</i> The standard Print dialog opens. |
| 3 | Select the appropriate printer and click the Print button.
<i>Result:</i> 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.

BufferPro is only available for some systems.

In this chapter

This chapter contains the following sections:

Section	See page
6.1 BufferPro - Overview	228
6.2 Create a method using BufferPro	230
6.3 Create and edit BufferPro recipes	231
6.4 Print a BufferPro recipe	241
6.5 Calculate buffer composition using BufferPro	243
6.6 Export and import BufferPro recipes	247
6.7 Predefined BufferPro recipes	251

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 full use of **BufferPro** is only available for some systems but all systems have the possibility to use the **BufferPro** recipes and calculate buffer composition as described in *Section 6.5 Calculate buffer composition using BufferPro, on page 243*.

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 Experiments**.

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	<i>Chapter 4 Scouting, on page 126</i>
Design of Experiments	<i>Chapter 5 Design of Experiments, on page 139</i>

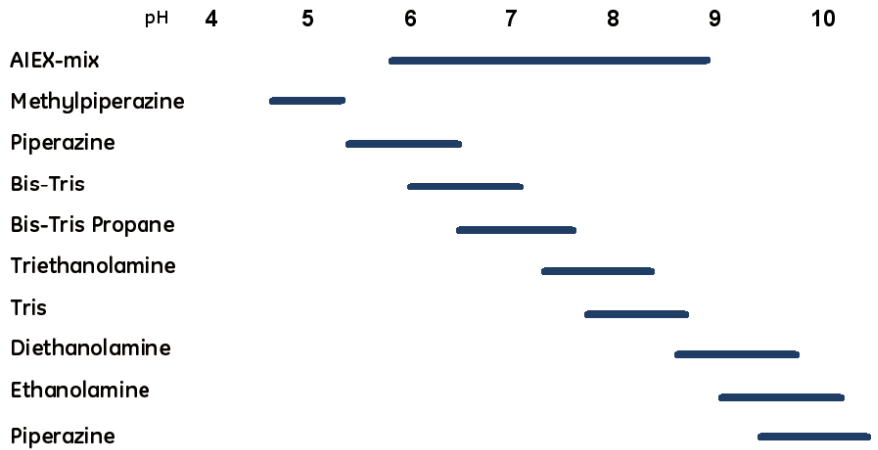
Workflow

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

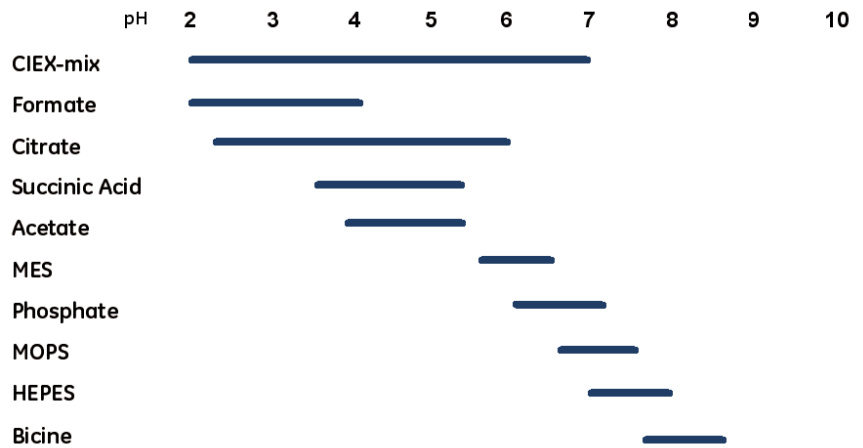
pH ranges for predefined buffers

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 27.

Creating a BufferPro method

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

The screenshot shows the BufferPro configuration interface. At the top, there are two radio buttons: 'Use manually prepared buffers' (unselected) and 'Use BufferPro (automatic buffer preparation)' (selected). Below the radio buttons are two dropdown menus for 'Inlet A' (set to 'A1') and 'Inlet B' (set to 'B1'). Under the selected radio button, there is a 'Recipe' dropdown menu showing 'Bicine 0-1M NaCl - (pH 7.7-8.7, PD)'. Below the recipe is a 'BufferPro Properties...' button. At the bottom, there are three input fields: 'pH' with the value '8.0' and a range '[7.7 - 8.7] (recommended)'; 'Conc' with the value '0.050' and a range 'M [0.030 - 0.050]'; and a 'M' label.

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 251.

- | | |
|---|-------------------------|
| 3 | Save the method. |
|---|-------------------------|

6.3 Create and edit BufferPro recipes

About this section

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.*

Note: *For systems that do not have the **BufferPro** tool, it is still possible to edit **BufferPro** recipes in imported methods.*

In this section

This section contains these subsections:

Section	See page
6.3.1 Create and edit a BufferPro recipe	232
6.3.2 Rename a BufferPro recipe	237
6.3.3 Delete a BufferPro recipe	239

6 BufferPro

6.3 Create and edit BufferPro recipes

6.3.1 Create and edit a BufferPro recipe

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.

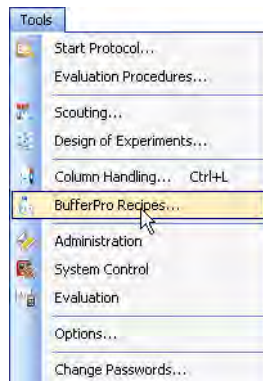
The titrant and buffer agent concentrations should be the same, since there may otherwise not be sufficient titrant to reliably obtain the entire pH range. For recipes titrated with strong acid/base, the concentration range that can be achieved is 15-25% of the buffer stock concentration. For conjugate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

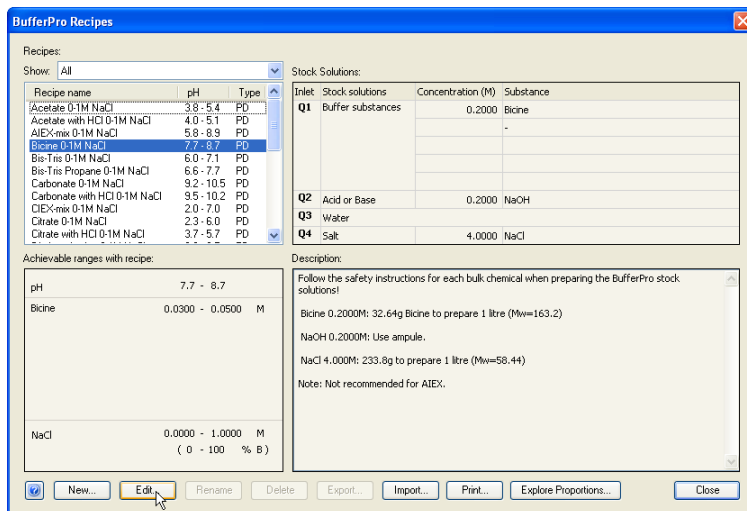


Step Action

- 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.



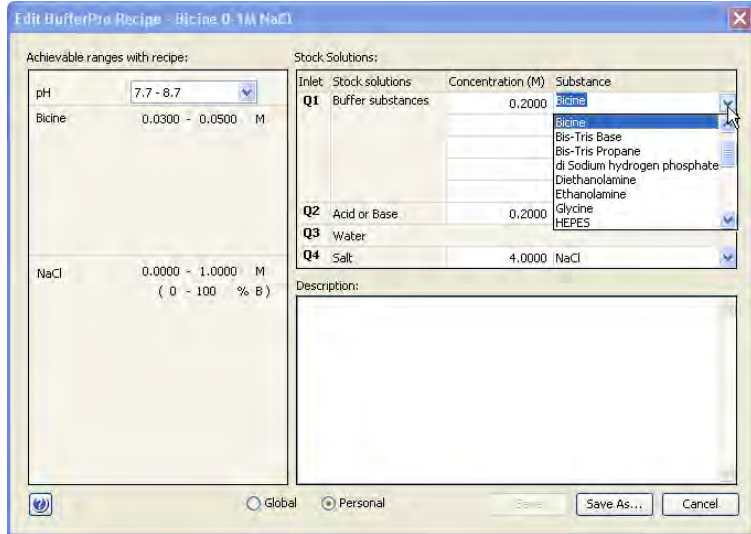
6 BufferPro

6.3 Create and edit BufferPro recipes

6.3.1 Create and edit a BufferPro recipe

Step Action

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



Note:

For ÄKTA avant, 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.

Stock Solutions:			
Inlet	Stock solutions	Concentration (M)	Substance
Q1	Buffer substances	0.1000	Bicine
			-
Q2	Acid or Base	0.1000	NaOH
Q3	Water		
Q4	Salt	4.0000	NaCl

- | Step | Action |
|------|--|
| 5 | Choose a titrant (Acid or Base) from the drop-down list and if required edit its Concentration . |

Note:

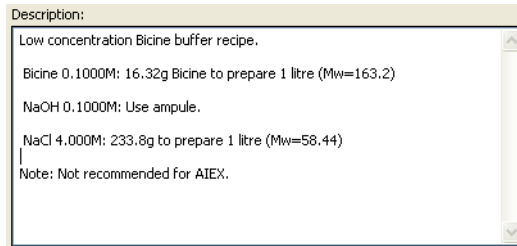
*The titrant and the stock solution should generally have the same concentration. This is set as default for **Acid or Base** concentration.*

- | | |
|---|---|
| 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.

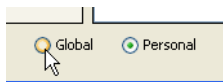
- | | |
|---|------------------------------------|
| 7 | Enter a description of the buffer. |
|---|------------------------------------|



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.*

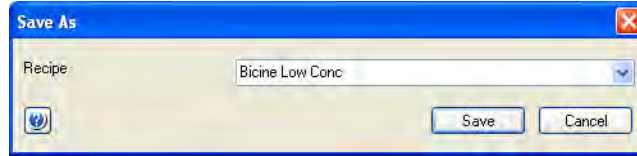
6 BufferPro

6.3 Create and edit BufferPro recipes

6.3.1 Create and edit a BufferPro recipe

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

9	Enter a name and click Save .
---	--------------------------------------



6.3.2 Rename a BufferPro recipe

Introduction

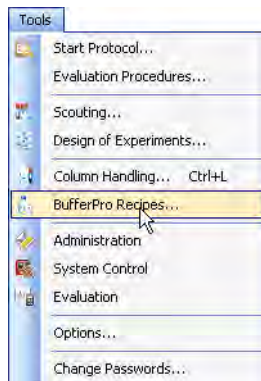
The following table describes the steps for renaming a **BufferPro** recipe.

Note: *Predefined recipes (shown as **PD** in the **Type** column) cannot be renamed.*

Rename a recipe

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

1	In the <i>Method Editor</i> , select <i>Tools:BufferPro Recipes...</i>
---	--



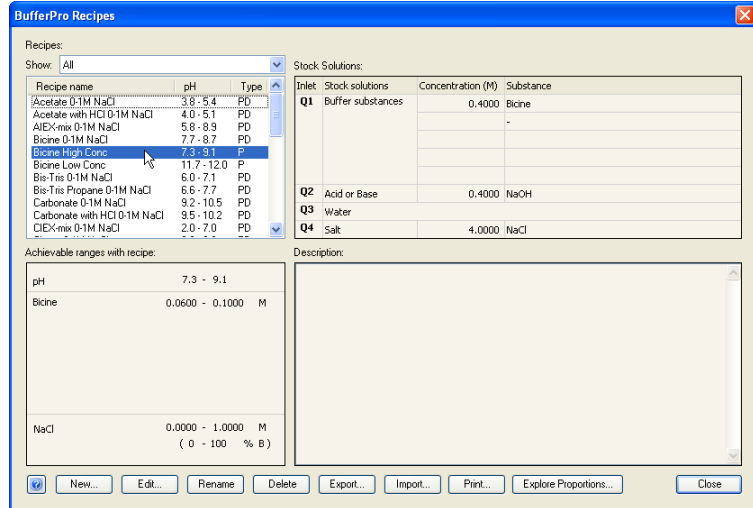
6 BufferPro

6.3 Create and edit BufferPro recipes

6.3.2 Rename a BufferPro recipe

Step Action

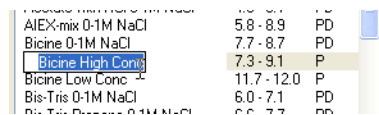
2 In the **BufferPro Recipes** dialog, select the recipe to be renamed.



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

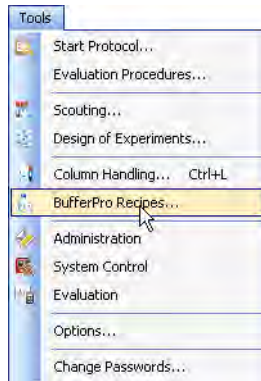
The following table describes the steps needed to delete a **BufferPro** recipe.

Note: *Predefined recipes (shown as **PD** in the **Type** column) cannot be deleted.*

Delete a recipe

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

1	In the Method Editor , select Tools:BufferPro Recipes...
---	--



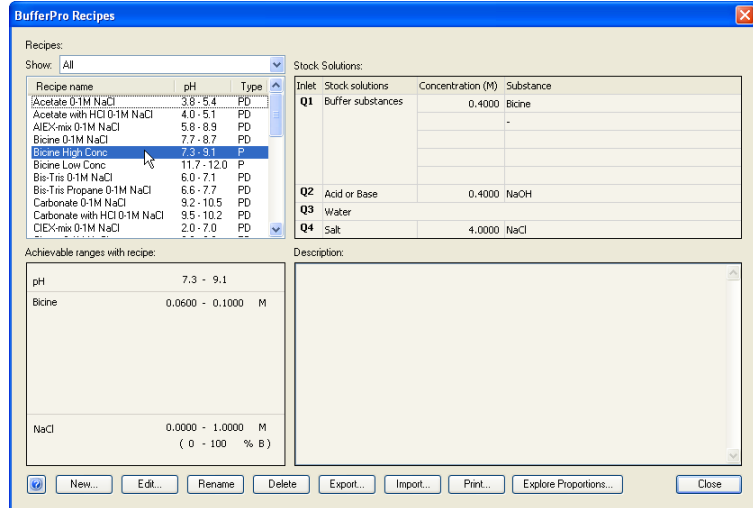
6 BufferPro

6.3 Create and edit BufferPro recipes

6.3.3 Delete a BufferPro recipe

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

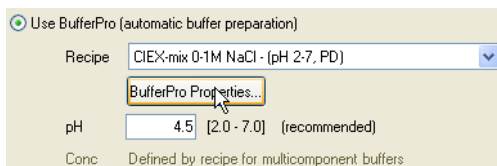
This section describes how to print a BufferPro recipe from UNICORN. A recipe can be printed from the **Phase Properties** tab in **Method Editor**, or from the **BufferPro Recipes** dialog.

It is also possible to include the **BufferPro** recipes when printing the whole method. See [Section 3.6.3 Print a method, on page 94](#).

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.



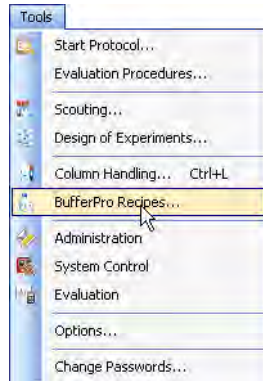
2	In the BufferPro Properties dialog click the Print... button. <i>Result:</i> The Print dialog opens.
3	Choose a printer from the drop-down list in the Print dialog and click OK .

Printing from *BufferPro Recipes* dialog

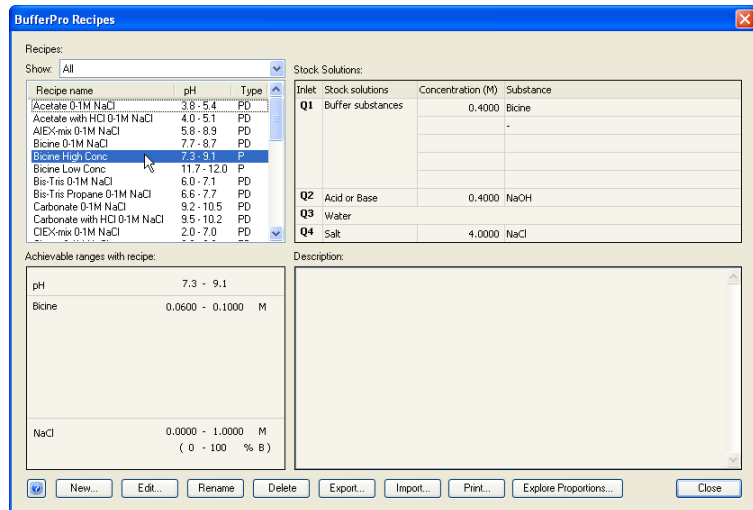
The following table describes how to print a recipe from the *BufferPro Recipes* dialog.

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

6.5 Calculate buffer composition using BufferPro

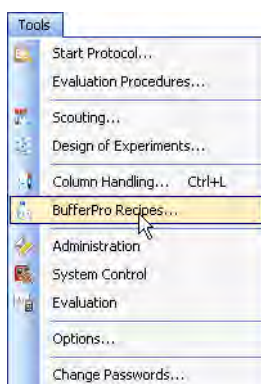
Introduction

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 <i>Method Editor</i> , select <i>Tools:BufferPro Recipes...</i>

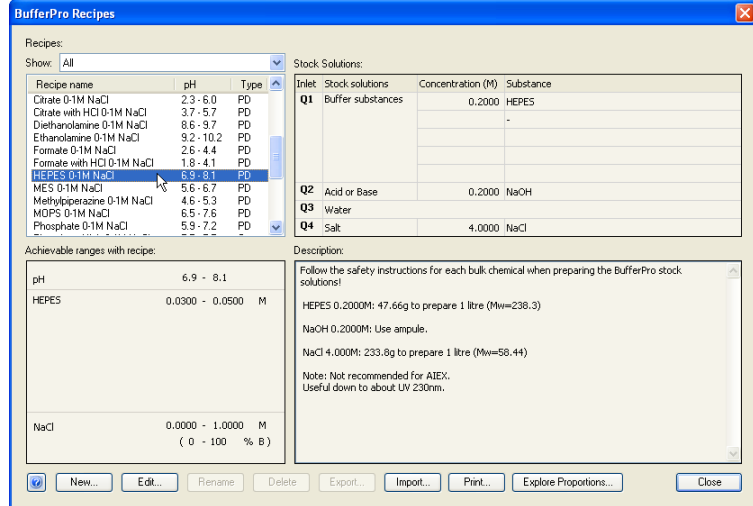


6 BufferPro

6.5 Calculate buffer composition using BufferPro

Step Action

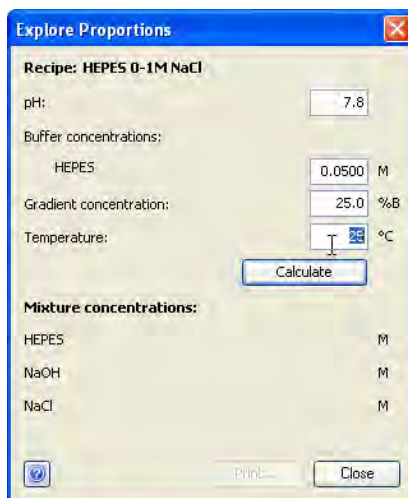
2 Choose the appropriate recipe from the list in the **BufferPro Recipes** dialog.



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:

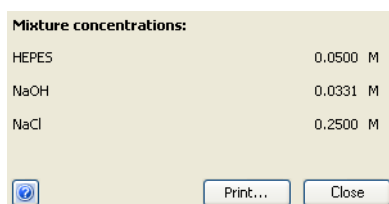
The **buffer concentrations** may not exceed the limits of the recipe. If this is the case the **Calculate** button will be grayed out.

- 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.

6 BufferPro

6.5 Calculate buffer composition using BufferPro

Step	Action
7	The buffer composition can be printed by pressing the <i>Print...</i> 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.

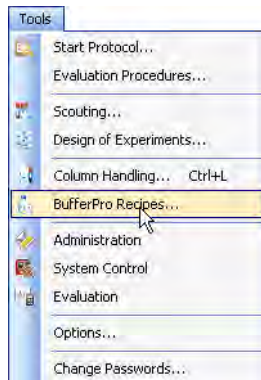
Exporting BufferPro recipes

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

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

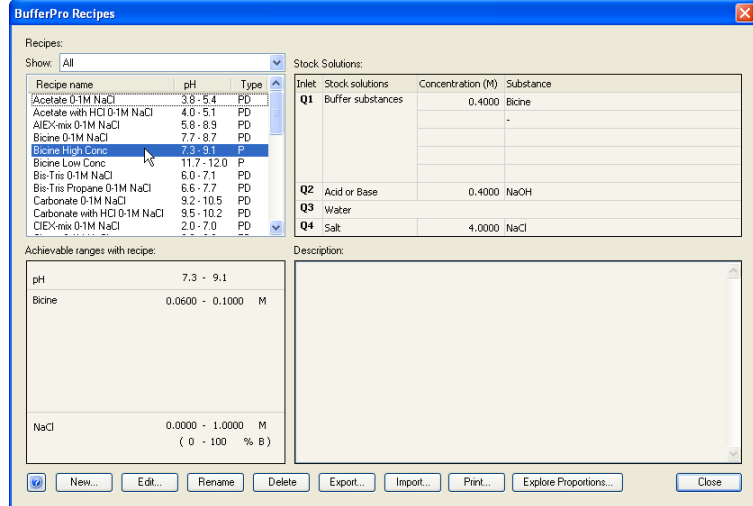
Stage	Description
-------	-------------

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.

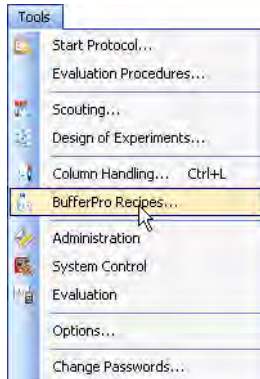
- 3 Click **Export....**
- Result: The **Export** dialog opens.
- 4 Choose a location on the computer disk and a filename for the zip file.
- 5 **Save** the file.

Importing BufferPro recipes

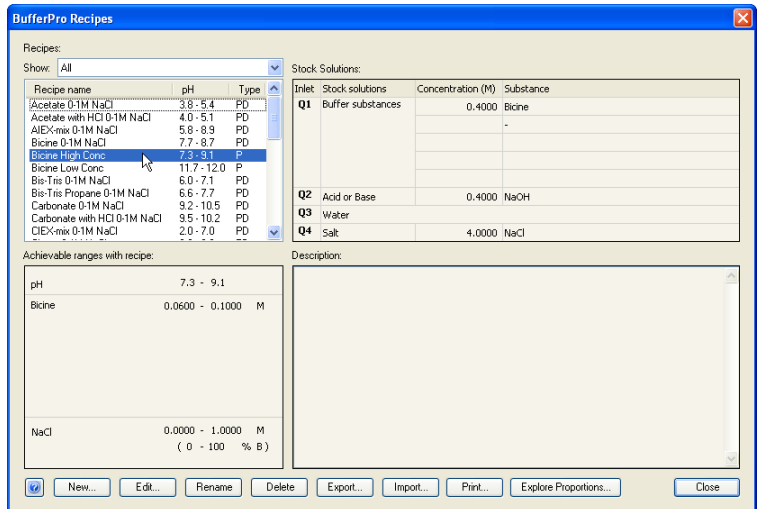
The following table illustrates the steps required to import one or several recipes.

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.

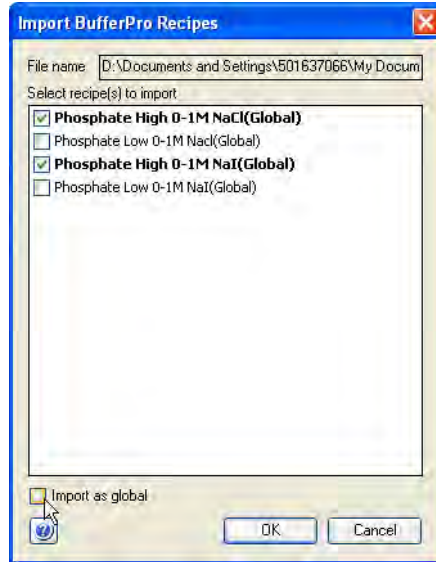
6 BufferPro

6.6 Export and import BufferPro recipes

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 pK_a 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 243*.
 - The two broad-range buffer systems, **ALEX-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 pK_a 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
ALEX-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. It also describes how to import and export method queues into and from UNICORN, respectively. For information on how to create and edit individual methods, see *Chapter 3 Create and edit methods*, on page 27.

In this chapter

This chapter contains the following sections:

Section	See page
7.1 Method queues - overview	255
7.2 Create a method queue	256
7.3 Edit a method queue	260
7.4 Import and export method queues	266

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 301. 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 306.*

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 <i>Chapter 3 Create and edit methods, on page 27</i> .
2	Create/open a method queue <ul style="list-style-type: none">• Create a new method queueor• 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.

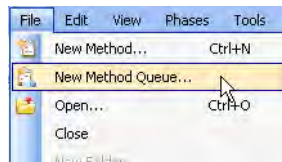
Step	Action
------	--------

- | | |
|---|--|
| 1 | In the Method Editor : <ul style="list-style-type: none">click the New Method Queue icon in the Toolbar |
|---|--|



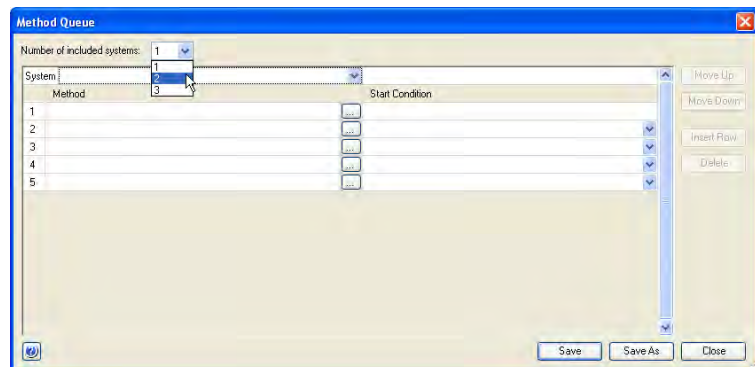
or

- Select **File:New Method Queue...**



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

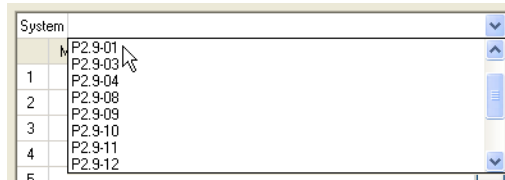
- | | |
|---|--|
| 2 | In the Method Queue dialog, choose the Number of included systems from the drop down list. |
|---|--|



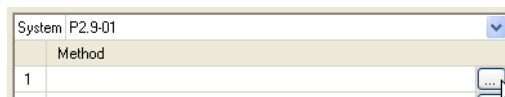
Result: A separate method queue block will be added to the dialog for each additional system if required.

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 compatibility, the individual methods should be saved for the system on which they are queued.

Step **Action**

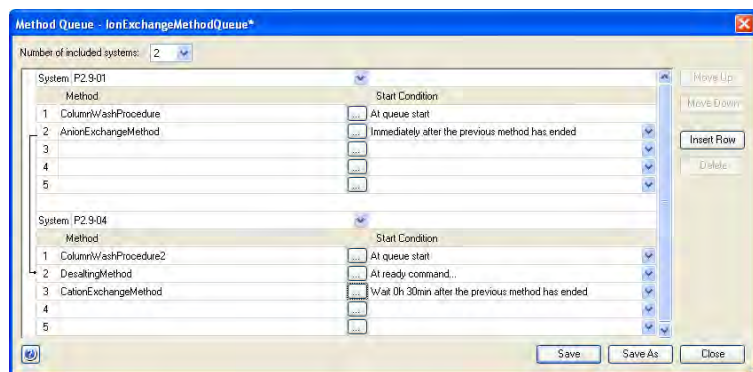
6 Select a **Start Condition** for the method from the drop-down list.

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.



Step	Action
7	Repeat steps 4 to 6 to add further methods to the Method list for each required system.
8	Click Save or Save As to save the completed method queue.
Note: <i>An error dialog will be displayed if any of the methods are incompatible with the system on which they are queued.</i>	

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
------	--------

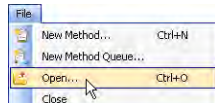
1 In the **Method Editor**:

- Click the **Open Method Navigator** icon in the **Toolbar**



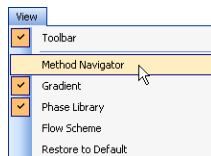
or

- select **File:Open...**



or

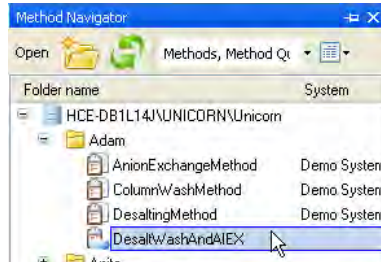
- select **View:Method Navigator**



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**

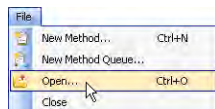
1 In the **Method Editor**:

- Click the **Open Method Navigator** icon in the **Toolbar**



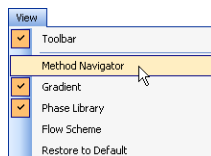
or

- select **File:Open...**



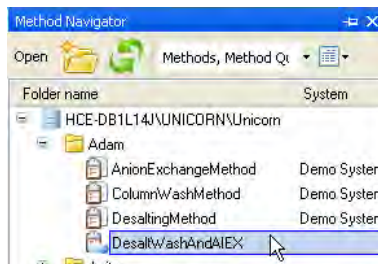
or

- select **View:Method Navigator**



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

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



3 To delete the method queue,

- select **Edit>Delete**

or

- press the **Delete** key

or

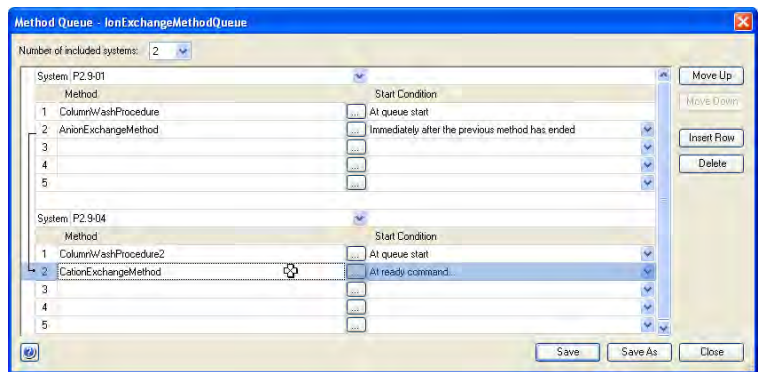
- right-click the selected method queue and select **Delete** from the context menu.

Result: A dialog will appear asking to confirm the delete operation.

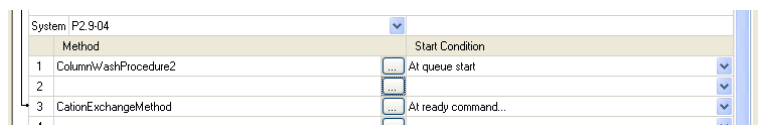
Insert a method into a method queue

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

- | Step | Action |
|------|--|
| 1 | Open the method queue, see <i>Opening a method queue, on page 260</i> .
<i>Result:</i> 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. |



- | | |
|---|--|
| 3 | Insert a new row by clicking on the Insert Row button.
<i>Result:</i> An empty row will be inserted. |
|---|--|



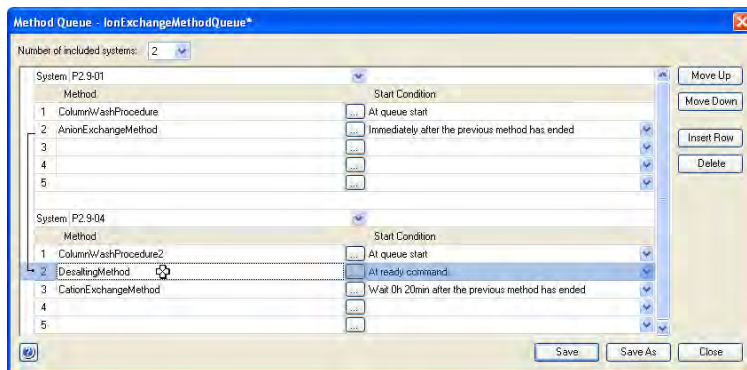
- | | |
|---|---|
| 4 | Add a Method and Start Condition to the Method list. See <i>Section 7.2 Create a method queue, on page 256</i> . |
| 5 | Save the method queue. |

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 <i>Opening a method queue, on page 260</i> .
<i>Result:</i> 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. |



- | | |
|---|--|
| 3 | Delete the selected row by clicking on the Delete button.
<i>Result:</i> The method will be deleted from the method queue. |
| 4 | Save the method queue. |

Change order of methods in a method queue

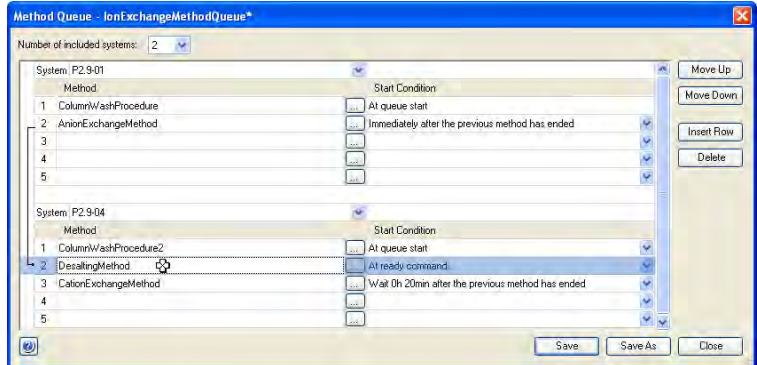
The following table describes how to change the order of methods in an existing method queue.

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

- | | |
|---|--|
| 1 | Open the method queue, see <i>Opening a method queue, on page 260</i> .
<i>Result:</i> The Method Queue dialog opens with the details for the chosen method queue. |
|---|--|

Step **Action**

- 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.

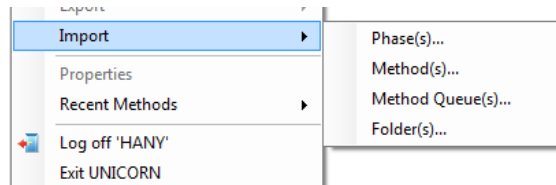
7.4 Import and export method queues

Import a method queue

Method queues can be imported into UNICORN. The following table describes how to do this.

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

1	Select File:Import:Method Queue(s)...
---	--



Result: The **Import** dialog opens.

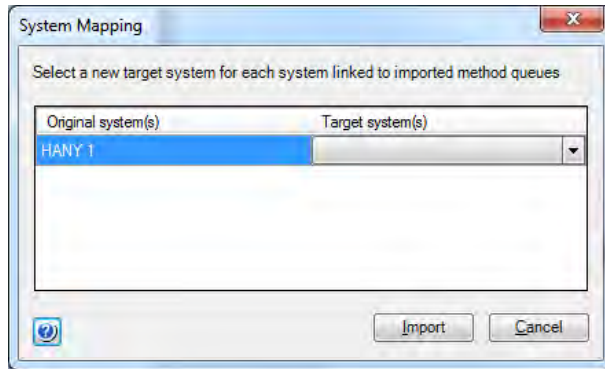
2	Browse to the method queue file (*.UMQ) in the Import dialog.
---	--

- | | |
|---|--|
| 3 | <ul style="list-style-type: none"> • Select the file. • Click Open. • Select the Import location of choice. |
|---|--|

Result: The **System Mapping** dialog opens.

Step **Action**

4



Select a **Target system** for each **Original system** listed.

Note:

To proceed with the import, there must be at least the same number of systems active in UNICORN as the number of original systems.

Note:

The **System Mapping** dialog appears once for every method queue selected.

5

Click the **Import** button to import the method queue. If several method queues are selected, the import of all the method queues starts.

Note: *Method queues may also be imported by importing an entire folder, see [Import folders](#), on page 270.*

Note: *During the method queue import an **Import Report** is generated at the target location.*

Export a method queue to UNICORN

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

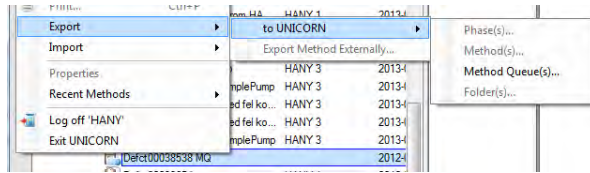
Step	Action
------	--------

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

Note:

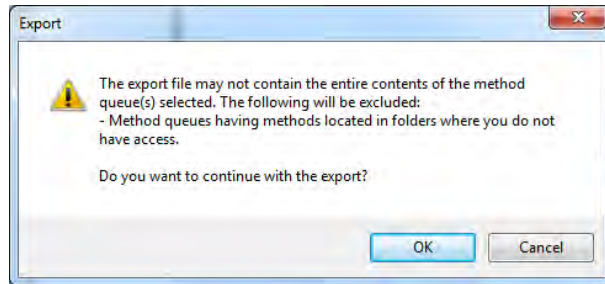
Several method queues in the same folder can be selected and exported at the same time. You can select several method queues at once by using the **Shift** or **Ctrl** key when selecting.

2	Choose File:Export to UNICORN:Method Queue(s)...
---	---



Result: An **Export** dialog opens

3	
---	--



- Click **OK** to continue the export.

Result: The **Export to Another UNICORN Database** opens.

4	Choose a file name and location and click the Save button to save the .UMQ file.
---	--

Note:

Export of multiple method queues results in the generation of multiple export files.

Note: Method queues may also be exported by exporting an entire folder, see *Export folders*, on page 271.

Note: *During the method queue export an **Export Report** is generated at the target location.*

8 Import and export folders

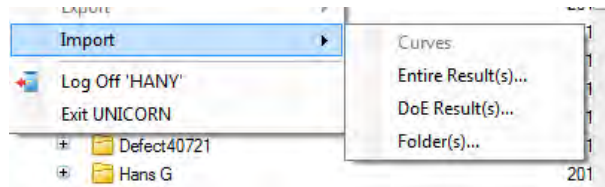
Introduction

This chapter describes how to import and export folders containing for example methods, method queues and different types of result files.

Import folders

Folders containing for example methods, method queues and/or DoE Results can be imported into UNICORN. The table below describes how to do this.

Step	Action
1	Select File:Import:Folder(s)...

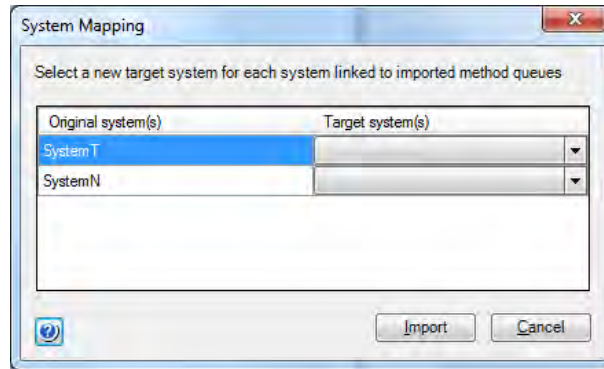


Result: The **Import** dialog opens.

- | | |
|---|---|
| 2 | Browse to the folder of interest (*.UFol) in the Import dialog. |
| 3 | Open the folder by selecting it and clicking the Open button. |

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

- 4 If the folder contains method queues a **System Mapping** dialog opens.



Select a **Target system** for each **Original system** listed.

Note:

To proceed with the import, there must be at least the same number of systems active in UNICORN as the number of original systems.

- 5 Click the **Import** button to import the folder with all its content.

Note:

For a folder containing several method queues, the **System Mapping** dialog is only shown once.

Note: During the import of a folder an **Import Report** is generated at the target location.

Export folders

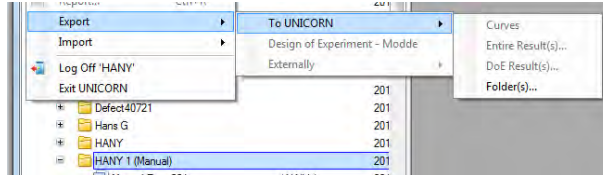
Folder export is recommended for bulk export of all items residing in a folder. During folder export everything in the folder is exported regardless of application or object navigator filter settings. If the folder contains compound objects, such as method queues or DoE results, all individual items located outside the exported folder will be placed together with the exported folder.

The following table outlines the steps needed to export a folder containing for example methods, method queues and/or DoE Results for later import into UNICORN.

8 Import and export folders

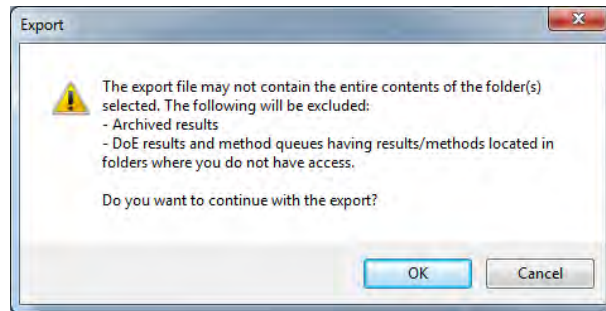
Step	Action
------	--------

- | | |
|---|---|
| 1 | <ul style="list-style-type: none">• Select a Folder in the Object Navigator. To select several Folders, press Shift while you click the folders.• Choose File:Export:to UNICORN:Folder(s)... |
|---|---|



Result: An **Export** dialog opens.

2



Click **OK** to continue the export.

Result: The **Export to Another UNICORN database** dialog opens.

- | | |
|---|--|
| 3 | Choose a location and click the Save button to save the folder. |
|---|--|

Result:

- The folder is exported as a **.UFol** file.
- An **Export Report** is generated at the target location.

Note:

Export of multiple folders results in the generation of a single export file.

9 Column Handling

About this chapter

The **Column Handling** tool in UNICORN enables handling of column types and, if enabled, 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
9.1 Overview	274
9.2 Handling column types	280
9.3 Handling individual columns	293
9.4 Column performance	308
9.5 Intelligent Packing of AxiChrom columns	312

9.1 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 with a defined bed height.
Column <i>or</i> Individual column	An individual column of a column type.

Example

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

Note: *When creating methods and performing method runs, the bed height for the column type will be used. If you wish to use a custom packed column (e.g. an AxiChrom column), you must create a specific column type with the defined hardware and media, and the obtained bed height.*

Open the Column Handling dialog

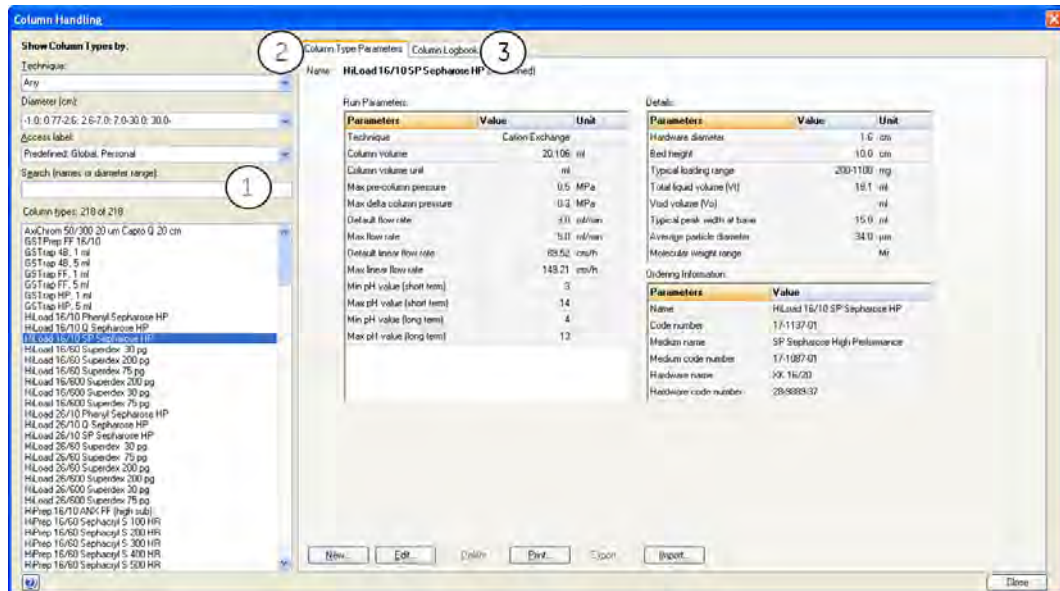
To open the **Column Handling** dialog:

- select **Tools:Column Handling...** in any of the UNICORN modules
or
- click the **Column Handling** icon in the **Toolbar** where available



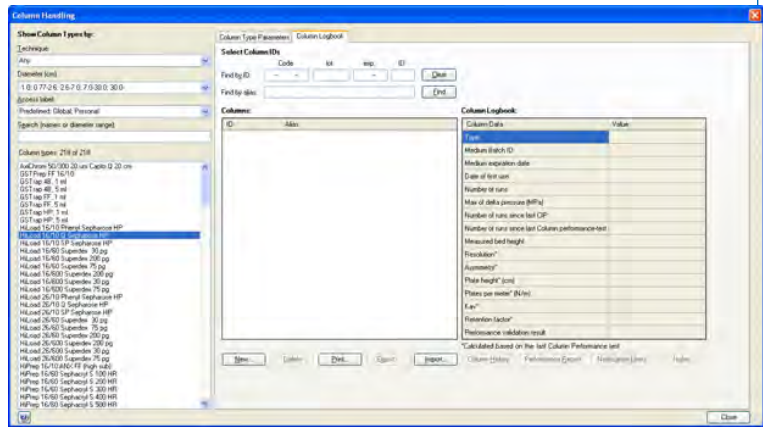
The Column Handling dialog

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



Part	Function
1	<p>Select Column Types by area:</p> <p>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.</p>
2	<p>Column Type Parameters tab:</p> <p>Shows the parameters for the selected column type in the Column types list. See <i>Section 9.2 Handling column types, on page 280</i> for more information.</p>

Part	Function
3	<p>Column Logbook tab:</p> <p>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 9.3 <i>Handling individual columns, on page 293</i> for more information.</p> <p>Note:</p> <p>A Column Logbook e-license is needed for the Column Logbook functionality.</p>



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**).

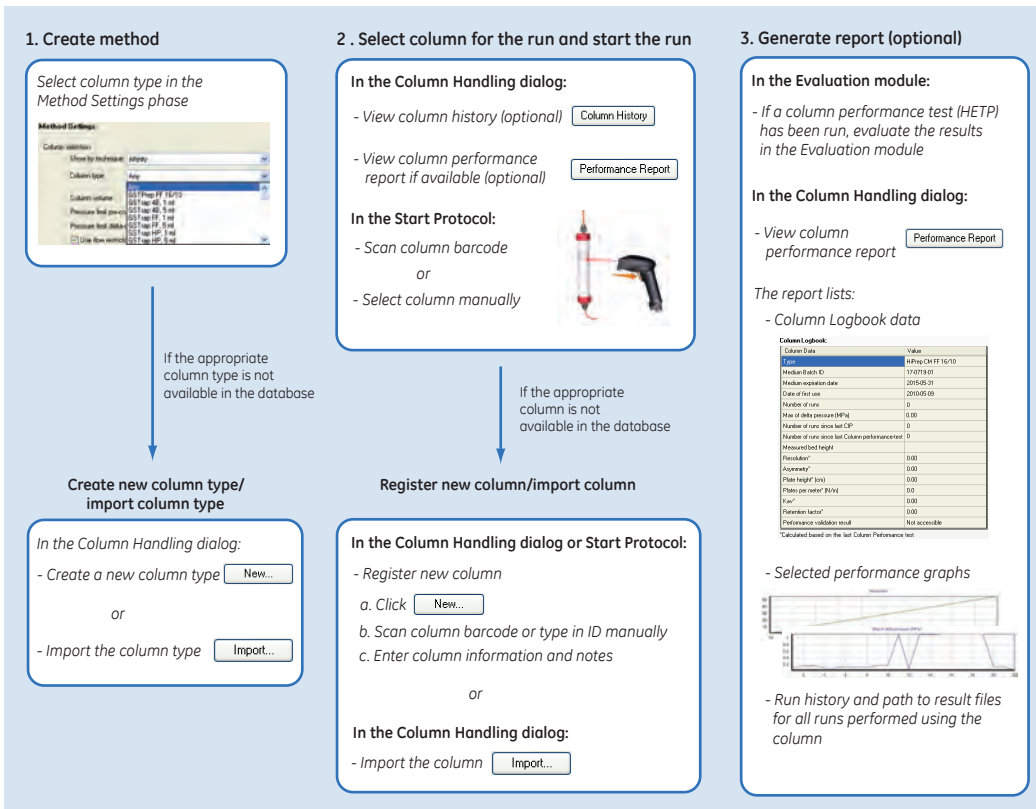
When working with...	the main tasks are...
Column types	<ul style="list-style-type: none">• <i>Create new column types</i>• <i>Import/export column types</i><ul style="list-style-type: none">- Used to transfer column type data between different databases• <i>Edit column types</i><ul style="list-style-type: none">- Edit parameters and delete column types• <i>Print information about column types</i>

When working with...	the main tasks are...
<p>Individual columns</p>	<ul style="list-style-type: none"> • Register new columns <ul style="list-style-type: none"> - scan or manually type in the barcode - add notes (optional) <p>Tip: New columns can be registered using the Column Handling dialog or before the run is started.</p> • Select columns to be used in the run <ul style="list-style-type: none"> - view column history (optional) - view performance report (optional) - scan column barcode <p>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.</p> • Edit columns <ul style="list-style-type: none"> - 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:

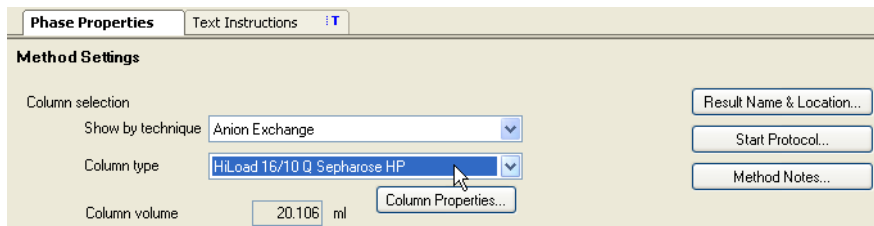
Note: *Generate report is a part of the illustrated workflow. This information only applies to Evaluation Classic.*



9.2 Handling column types

Introduction

When you create a new method and select a column type in the **Method Settings** phase or in the wizard window, the column volume and column pressure limits are automatically set. For systems using predefined methods, the default flow rate and the pressure for the column are automatically set as well. 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 the column type was used in the packing method. 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 column types from GE are predefined in UNICORN. For each column type, as many individual columns as needed can be registered. Parameters for the predefined 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 |
|---|--|



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

If the column hardware and medium are made by GE Healthcare, select the name of the hardware and medium to have most of the parameters filled in automatically.

Show hardware types by diameter (cm) Show medium types by technique
 Min Max Any

GE Healthcare hardware type GE Healthcare medium type
 Any Any

Run Parameters Details Ordering Information

Parameters	Value	Unit
*Technique		
Column volume		ml
*Column volume unit	ml	
*Max pre-column pressure		MPa
*Max delta column pressure		MPa
*Default flow rate		ml/min
*Max flow rate		ml/min
Default linear flow rate		cm/h
Max linear flow rate		cm/h
Min pH value (short term)		
Max pH value (short term)		
Min pH value (long term)		
Max pH value (long term)		

*Required information

Global Personal Save As... Cancel

- | | |
|---|--|
| 2 | If adding a column type for which the column hardware and medium are not made by GE, continue to step 4. |
|---|--|

Step Action

- 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 as required):

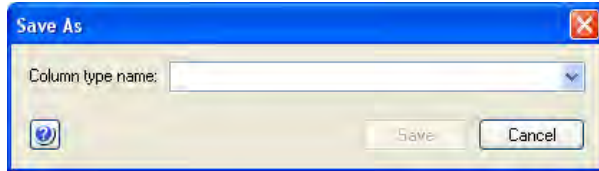
Run Parameters			Details		
Parameters	Value	Unit	Parameters	Value	Unit
*Technique	Affinity	▼	*Hardware diameter	5.0	cm
Column volume		ml	*Bed height		cm
*Column volume unit	ml	▼	Typical loading range		mg
*Max pre-column pressure		MPa	Total liquid volume (V _l)		ml
*Max delta column pressure		MPa	Void volume (V ₀)		ml
*Default flow rate		ml/min	Typical peak width at base		ml
*Max flow rate		ml/min	Average particle diameter	90.0	
Default linear flow rate		cm/h	Molecular weight range		
Max linear flow rate		cm/h			
Min pH value (short term)	4		Ordering Information		
Max pH value (short term)	10		Parameters	Value	
Min pH value (long term)	4		Name		
Max pH value (long term)	10		Code number		
*Required information			Medium name	2'5' ADP Sepharose 4B	
			Medium code number	17-0700-01	
			Hardware name	AxiChrom 50/300 glass, 10 um steel	
			Hardware code number	See user manual for AxiChrom	

- 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.
For systems that cannot utilize the delta column pressure signal, it is recommended to use the same pressure as the pre-column pressure.
- 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 | Choose filtering options from the drop-down lists to show the column types by Technique , Diameter or Access label . 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.

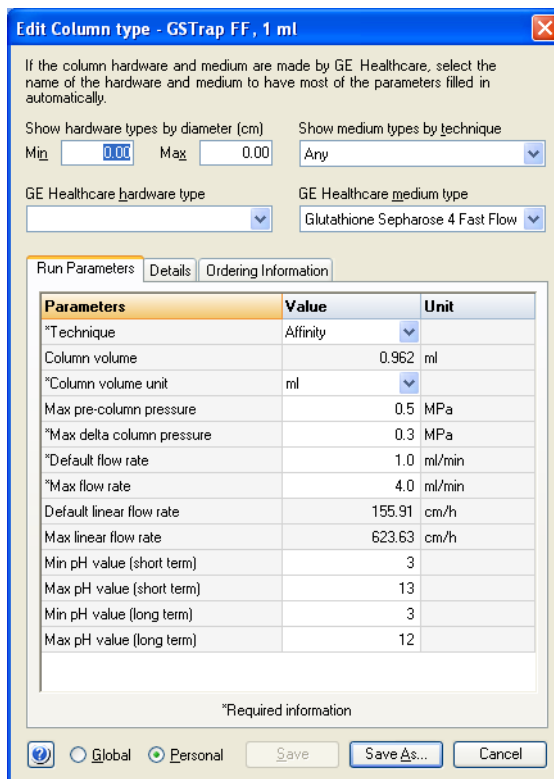
Name: **GS Trap FF, 1 ml** (Predefined)

Run Parameters:			Details:		
Parameters	Value	Unit	Parameters	Value	Unit
Technique	Affinity		Hardware diameter	0.7	cm
Column volume	0.962	ml	Bed height	2.5	cm
Column volume unit		ml	Typical loading range	1-10	mg
Max pre-column pressure	0.5	MPa	Total liquid volume (Vl)	0.86	ml
Max delta column pressure	0.3	MPa	Void volume (Vo)		ml
Default flow rate	1.0	ml/min	Typical peak width at base	1.0	ml
Max flow rate	4.0	ml/min	Average particle diameter	90.0	µm
Default linear flow rate	155.91	cm/h	Molecular weight range		Mr
Max linear flow rate	623.63	cm/h			
Min pH value (short term)	3		Ordering Information:		
Max pH value (short term)	13		Parameters	Value	
Min pH value (long term)	3		Name	GS Trap FF, 1 ml	
Max pH value (long term)	12		Code number	17-5130-01	
			Medium name	Glutathione Sepharose 4 Fast Flow	
			Medium code number	17-5132-01	
			Hardware name	HITrap, 1 ml	
			Hardware code number		

Buttons:

Step Action

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



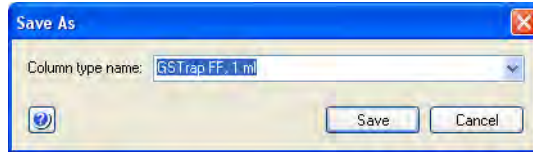
- Edit the column type parameters as appropriate on the **Run Parameters**, **Details** and **Ordering Information** tabs.
- Select whether the edited column type should be **Global** (available for all users) or **Personal** (only available for the current user).

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

5	When parameters for a predefined column type are edited, 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	When parameters for a Global or Personal column type are edited, 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.

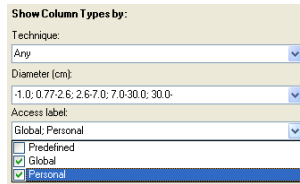
Delete column types

Note: *It is not possible to delete predefined column types from the database. If a column type has any registered columns, it cannot be deleted unless the individual columns are first deleted. See Section 9.3 Handling individual columns, on page 293 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 in the **Access label** drop-down list.

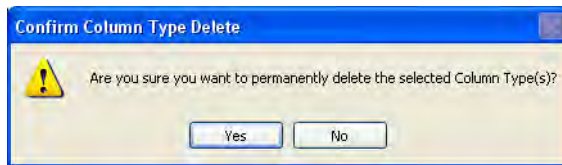


Result: Only **Global** and **Personal** column types are displayed in the **Column types** list.

- 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 .

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.

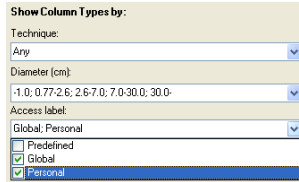
Export column types

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 in the **Access label** drop-down list.

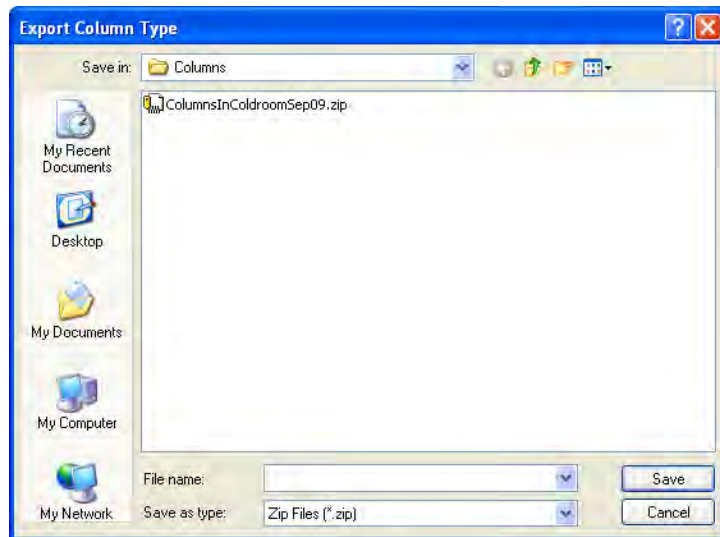


Result: Only **Global** and **Personal** column types are displayed in the **Column types** list.

- 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 .

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

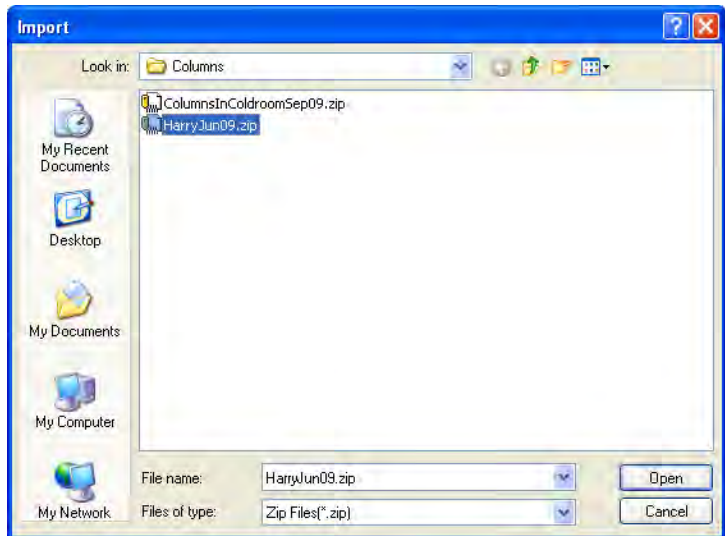
The table below describes how to import column types into the database:

Step	Action
1	In the Column Type Parameters tab in the Column Handling dialog, click <input type="button" value="Import..."/>

Result: The **Import** dialog opens.

Note:

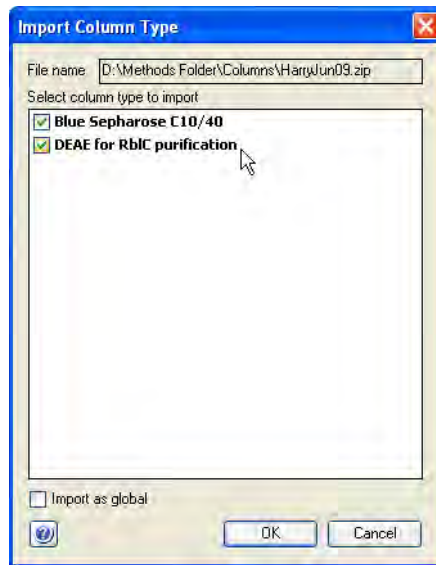
It is not possible to import column types from UNICORN 5. They have to be re-created in UNICORN 7.0.



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:

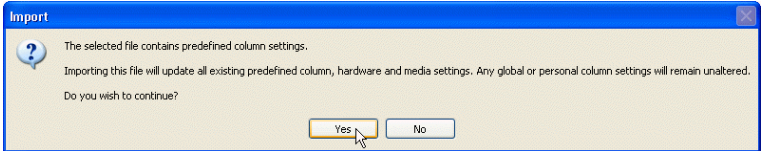
*If a column type to be imported has the same name as an existing column type in the database, you will be prompted to type a new name for that column type. Type in a name and click **OK**.*

Import new column list

Updated lists of predefined column types may be provided by GE. 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. The import of a new column list will also update the column hardware list and the approved media list.

Tip: A column list is available in the **Misc** folder of the UNICORN 7.0 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 . <i>Result:</i> The Import dialog opens.
2	Locate the zip file with the column list to be imported and click Open . <i>Result:</i> The Import confirmation dialog opens, explaining what will happen when the zip file is imported.
	
3	Click Yes . <i>Result:</i> The new list of predefined column types is imported into the database.

Print information about column types

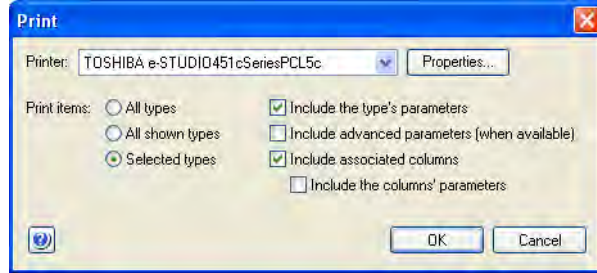
The table below describes how to print information about column types:

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 the column types for which information is to be printed:

- **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.

9.3 Handling individual columns

About this section

Individual columns are handled on the **Column Logbook** tab in the **Column Handling** dialog. The **Column Logbook** enables the run history for an individual column to be traced, 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 has been selected and a **Column Logbook** e-license exists.*

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 contains these subsections:

Section	See page
9.3.1 Individual column identification	294
9.3.2 Register a new individual column	295
9.3.3 Find an individual column	298
9.3.4 Edit individual columns	300
9.3.5 Export and import individual columns	303
9.3.6 Print and view individual column information	306

9.3.1 Individual column identification

Matrix barcode

Most prepacked GE 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 prelabelled with a matrix barcode, such as HiTrap™ columns, manually packed columns or columns from other sources. A number of UniTag labels may be 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.



9.3.2 Register a new individual column

Introduction

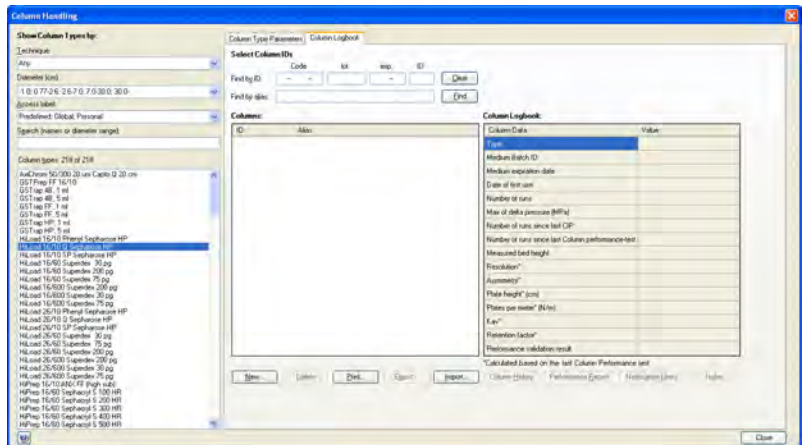
In order to take advantage of the column handling features of UNICORN, each individual column needs to be registered in the software.

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.*

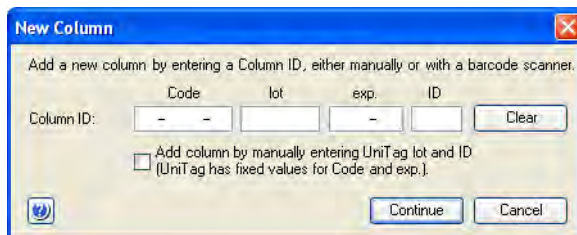
Register an individual column

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.



9 Column Handling

9.3 Handling individual columns

9.3.2 Register a new individual column

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**, expiration date (**exp**) and **ID** fields.
- Click **Continue** to open the second **New Column** dialog.

Note:

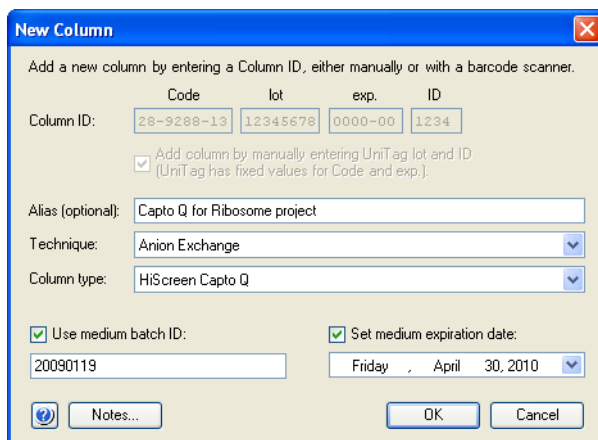
*The **lot** field should contain eight digits, and the **ID** field should contain four digits. If the lot or ID numbers of the column contains fewer than eight or four digits respectively, insert leading zeros before the number.*

Note:

*If the column has no GE 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 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. If an expiration date was set in the column ID field, this date will be suggested in the medium expiration date. The expiration date of the medium overrules the expiration date of the column.

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.

9.3.3 Find an individual column

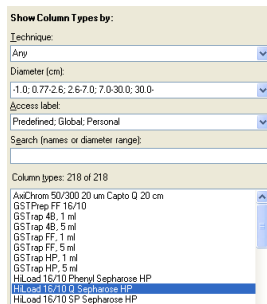
Introduction

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

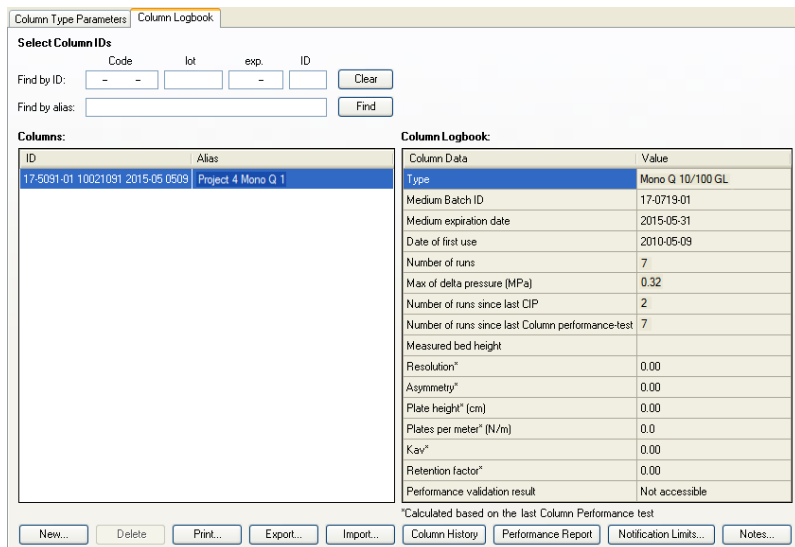
The table below describes how to find/select a registered column in the **Column Logbook**:

Step	Action
1	Select the Column Logbook tab.
2	Filter the list of column types by: <ul style="list-style-type: none">• choosing the required technique in the drop-down menu Technique and/or• choosing the diameter in the drop-down menu Diameter and/or• choosing the access labels in the drop-down menu Access label and/or• enter a search criteria in the Search field. 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.

9.3.4 Edit individual columns

Introduction

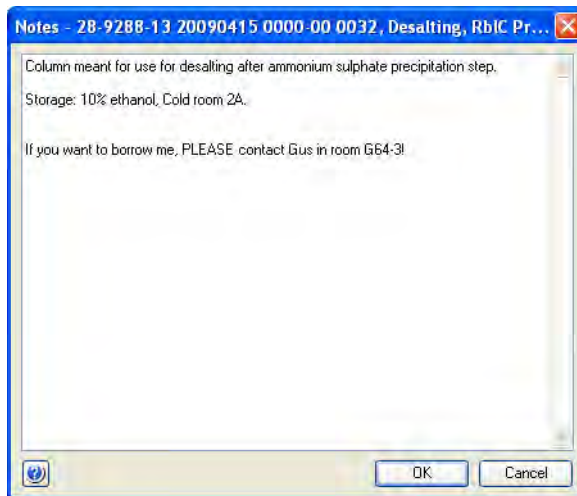
This section describes the ways in which individual columns may be edited. This includes adding and editing notes, setting notification limits and deleting individual columns.

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 <i>Section 9.3.3 Find an individual column, on page 298</i> for information about how to find and select a column.

- 2 In the **Column Logbook** tab, click .
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.
-

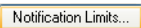
Set notification limits for an individual column

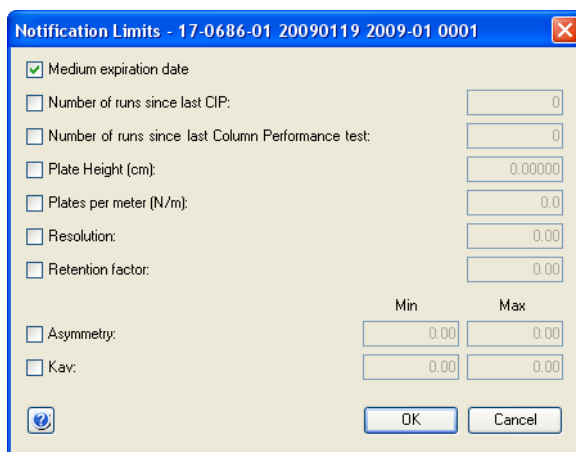
Notification limits can be set for individual columns. Once the limit is reached, the user receives a message stating what action should be taken before the column is used. Examples of such limits are the **Medium expiration date**, that the column has been used a given number of times since the last 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 9.3.3 Find an individual column, on page 298 for information about how to find and select a column.

2 In the **Column Logbook** tab, click .
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 <i>Section 9.3.3 Find an individual column, on page 298</i> 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 .
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.

9.3.5 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 <i>Section 9.3.3 Find an individual column, on page 298</i> 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 <input type="button" value="Export..."/> . <i>Result:</i> 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. <i>Result:</i> The individual column information is exported. The column information can be imported into another database.

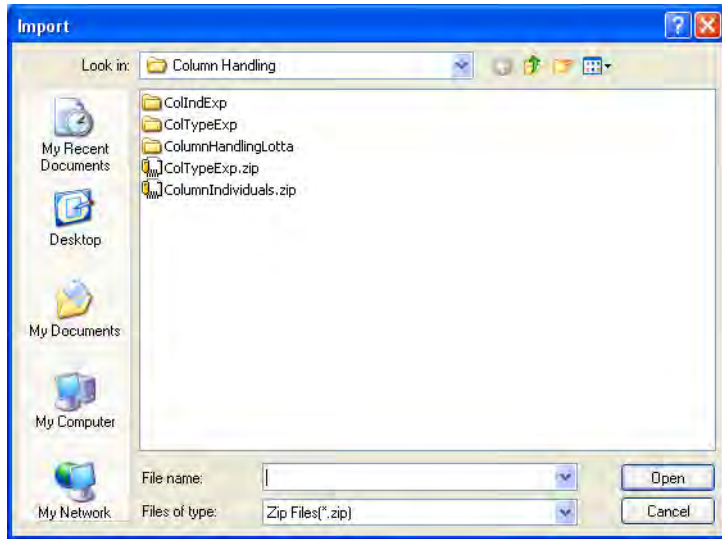
Import individual columns

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

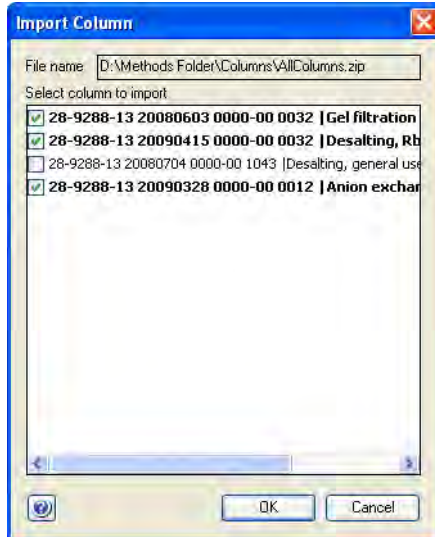
The table below describes how to import individual column information to a database:

Step **Action**

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



- 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.



Step	Action
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 . <i>Result:</i> The columns are imported into the database. Note: <i>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.</i>

9.3.6 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.

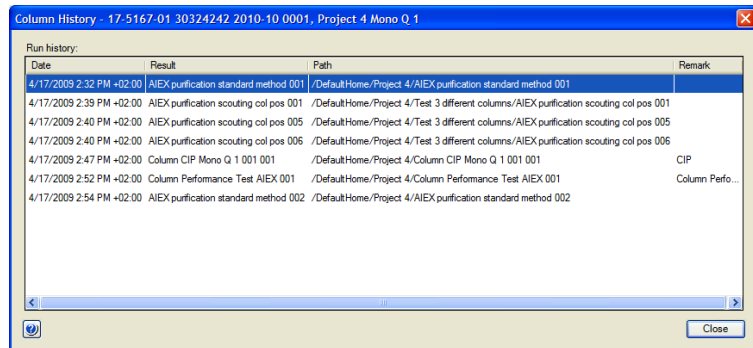
The table below describes how to view the **Column History** for a column:

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

1	Select the individual column for which to view Column History in the Columns list in the Column Logbook tab. See <i>Section 9.3.3 Find an individual column, on page 298</i> for information about how to find and select a column.
---	--

2	In the Column Logbook tab, click <input type="button" value="Column History"/> .
---	---

Result: The **Column History** dialog for the selected column opens.




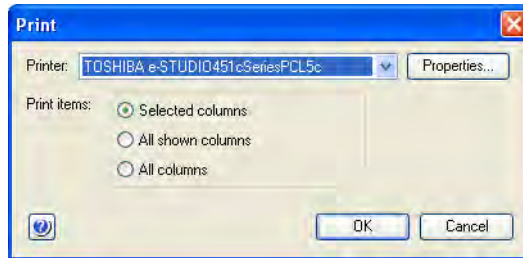
The runs performed using the column are listed. The date, result name and location and any remarks for the run are displayed.

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 <i>Section 9.3.3 Find an individual column, on page 298</i> 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  .
<i>Result:</i> The Print dialog opens. |



- | | |
|---|--|
| 3 | Select Printer . |
| 4 | Select for which individual column(s) to print information: <ul style="list-style-type: none">• 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 .
<i>Result:</i> The Column ID, alias and parameters for the columns are printed. |

9.4 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 of how to set such notification limits see *Set notification limits for an individual column*, on page 301.

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

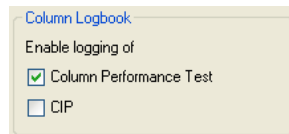
Column performance test

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. For details how to create methods see <i>Chapter 3 Create and edit methods</i> , on page 27.

Note:

*For methods created using phases, 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.*



*For wizard created methods and text created methods the **Enable logging of Column Performance Test** is not automatically selected, and must be selected in the method settings phase for a column performance test to be logged.*

Step	Action
2	<p>Run the method. For details on running a method, see UNICORN System Control Manual.</p> <p>Note:</p> <p><i>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.</i></p> <p><i>When using a predefined intelligent packing method the column type is requested to be selected and an individual column will also be requested at method start. When creating a wizard generated intelligent packing method it is an option to create separate methods for the packing and for the column performance test and therefore the column type and the individual column may only be needed for the column performance test method.</i></p> <p><i>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.</i></p> <p>Note:</p> <p><i>A sample volume between 0.5% and 3% of the column volume and a flow velocity between 15 and 30 cm/h is recommended.</i></p> <p><i>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.</i></p>
3	<p>Evaluate the Column Performance Test, see UNICORN Evaluation Manual.</p>


Create a performance report

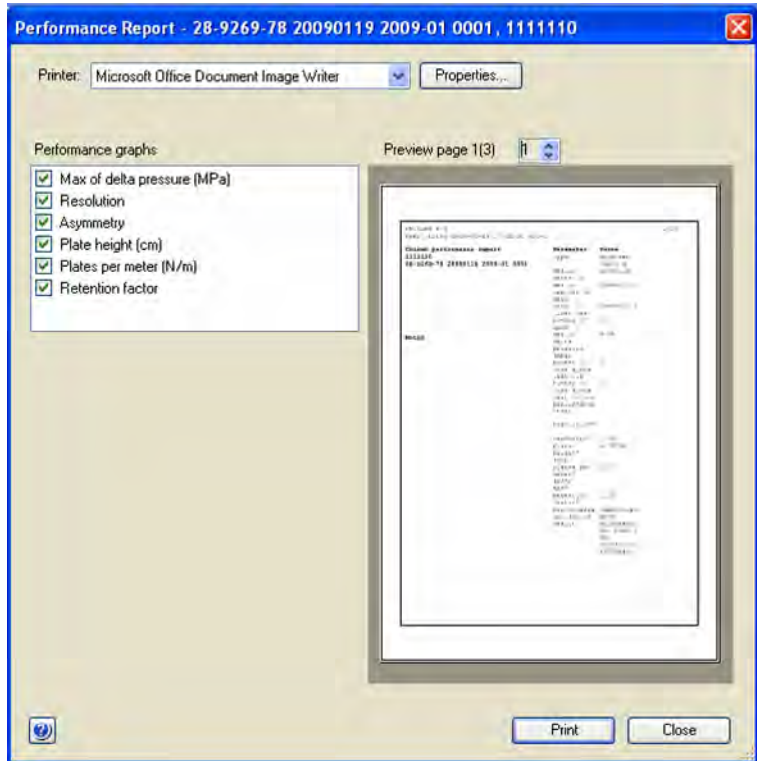
A column performance report can be created before using an individual column. The report shows if the column is in good condition for use. The performance report contains the following information:

- 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 9.3.3 Find an individual column, on page 298* for information about how to find and select a column.
- 2 In the **Column Logbook** tab, click .
Result: The **Performance Report** dialog for the selected column opens.



- 3 Select **Printer**.

Step	Action
4	<p>Check the appropriate boxes in the Performance graphs area to include the corresponding graphs in the report.</p> <p>Note: <i>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 labeled.</i></p> <p>Note: <i>Not all systems have feedback on the max delta pressure.</i></p>
5	<p>A preview of the report is shown on the right side of the dialog. Use the buttons above the report to scroll the preview.</p>
6	<p>Click Print to print the information.</p>
7	<p>Click Close to close the dialog.</p>

9.5 Intelligent Packing of AxiChrom columns

Introduction

UNICORN 7.0 features a solution for Intelligent Packing of AxiChrom columns. The AxiChrom column family features hands-free packing using internal hydraulic axial compression. Intelligent packing of AxiChrom columns can be performed in several ways.

For systems that create methods using phases, the Intelligent Packing method can be performed using either a predefined Intelligent Packing method, or by creating a user defined method including an Intelligent Packing phase.

For systems that have the Method Wizard, the Intelligent Packing method is created using the Intelligent Packing Wizard.

The workflows for the systems can differ. For a predefined Intelligent Packing method the Column Performance tests are included, but for a wizard created Intelligent Packing method, it is possible to separate the packing and the Column performance test in two different methods.

If Column Handling is used, and the method contains a Column Performance test, it is essential to use the correct column type, previously created in column handling, in the method settings phase.



In this section

This section contains these subsections:

Section	See page
9.5.1 AxiChrom column types and individual AxiChrom columns	314

Section	See page
9.5.2 Predefined Intelligent Packing method	318
9.5.3 Wizard generated Intelligent Packing method	320

9.5.1 AxiChrom column types and individual AxiChrom columns

It is essential to create an individual column before a Column Performance test is done since it is impossible to afterwards manually register the results from the Column Performance test in the **Column Logbook**. This is described later in *Create an AxiChrom column type, on page 314*.


When the evaluation of the tests is 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 Evaluation Manual.

Tip: *If it is essential that the exact packed bed height is used when running methods, a column type for each individual AxiChrom column should be created with the exact bed height value each time the column is repacked.*

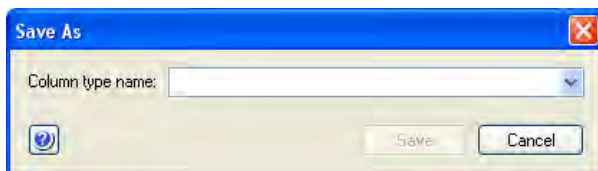
Create an AxiChrom column type

The table below describes how to create the AxiChrom column type:

Note: *If a column type was used when running a column packing method, the column type bed height might need to be updated with the achieved bed height to ensure that the actual packed bed height is applied when creating or running methods using the column type.*

Step	Action
1	Choose the Tools:Column Handling menu command. <i>Result:</i> The Column Handling dialog opens.
2	In the Column Type Parameters tab in the Column Handling dialog, click  . <i>Result:</i> The New Column Type dialog opens.

Step	Action
3	<ul style="list-style-type: none"> • 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. <p>Tip: <i>Only some of the available media are approved by GE for use in the Intelligent Packing of AxiChrom columns. For systems equipped with predefined methods and phases it is possible to click the GE approved media button in the Intelligent Packing phase to view a list of the approved media and bed heights. Systems that use the intelligent packing wizard will be guided to the approved media in the wizard.</i></p> <p><i>Other media can also be selected, but the packing procedure will then be performed with a set of general default settings.</i></p> <p><i>Result:</i> Based on the selections, some of the column type parameters are automatically filled in.</p>
4	<p>Enter the remaining parameter values for the new column type in the Run Parameters, Details and Ordering Information tabs, for example</p> <ul style="list-style-type: none"> • target bed height • max flow rate • max delta column pressure (the max delta column pressure value is used as the max pressure value for systems that cannot utilize the delta column pressure) <p>Fields marked with * must be filled in.</p> <p>Values in the gray fields are calculated and automatically filled in based on entered values for the corresponding parameters.</p>
5	<p>Select whether the new column type should be Global (available for all users) or Personal (only available for the current user).</p>
6	<p>Click Save As... to save the column type.</p> <p><i>Result:</i> The Save As dialog opens.</p>



9 Column Handling

9.5 Intelligent Packing of AxiChrom columns

9.5.1 AxiChrom column types and individual AxiChrom columns

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 composed 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

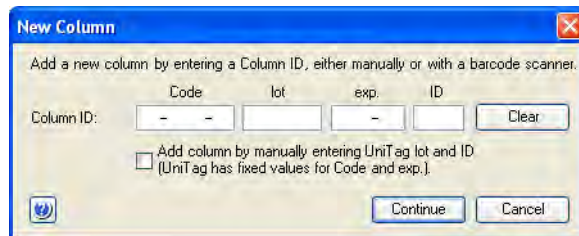
Once a column type consisting of the AxiChrom hardware and selected media is created, you can proceed to register an individual column.

Note: It is essential to create an individual column before a Column Performance test since it is impossible to afterwards manually register the results from the Column Performance test in the **Column Logbook**.

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

1	Select the Column Logbook tab and then click New .
---	--

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



- | | |
|---|--|
| 2 | <ul style="list-style-type: none">Register the column either by scanning a UniTag or manually as described in Section 9.3.2 Register a new individual column, on page 295 andclick Continue. |
|---|--|

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

Step	Action
3	<p>In the second New Column dialog:</p> <ul style="list-style-type: none">• Enter an Alias (optional). <p>Tip: <i>Alias can be used for easy identification of an individual column.</i></p> <ul style="list-style-type: none">• Select Technique and the AxiChrom Column type you previously created.• 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. <p>Note: <i>The expiration date cannot be set or changed after a column has been registered.</i></p>
4	<p>Enter notes for the column by clicking the Notes... button and typing information in the Notes dialog that opens.</p>
5	<p>Click OK.</p> <p><i>Result:</i> The entered information is saved and the registered column is displayed in the Column Handling dialog.</p>
Note:	<p><i>For a predefined intelligent packing method, the packing has not been performed at this point and the bed height for the column will be a target bed height based on the selected AxiChrom column type. This value can be adjusted after the Column Performance tests are evaluated and the Column Logbook is updated. For systems using the method wizard it is possible to pack the column first and then run the Column Performance. In this case the column type and individual column can be created after the packing and selected only for the column performance test method.</i></p>

9.5.2 Predefined Intelligent Packing method

For systems that support predefined methods, use the **Intelligent Packing** method to prepare a method for packing the AxiChrom column.

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

1	Open a new, predefined Intelligent Packing method.
---	--

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. 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.

Step	Action
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: <i>Click the Column Connection button to view information about the connections, including an illustration.</i>
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: <i>The accuracy of the slurry preparation will affect the packed bed height.</i> Note: <i>This function is not available when Custom packing settings is selected.</i>
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 chromatography 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 Evaluation Manual and adjust the actual bed height according to the results if necessary.

9.5.3 Wizard generated Intelligent Packing method

For systems that support the method wizard, use the **Intelligent Packing wizard** to prepare a method for packing the AxiChrom column.

Note: *If column handling is used, it is essential to select hardware, media and bed height in the wizard as well as creating the corresponding column type and individual column in column handling.*

The table below describes how to create an **Intelligent Packing** method using the Wizard.

Step	Action
1	Create a new method in the Method Editor.
2	In the New Method window: <ul style="list-style-type: none">• Choose the correct system in the drop-down menu and• choose Method Wizard and click OK.
3	Follow the wizard to create a new method. Intelligent packing is found in the Special method droplist. Press F1 to use the Wizard help if needed. Note: <i>The Intelligent packing method is only active if Intelligent packing has been chosen as a component for the system.</i>

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

4	The created method opens in the Method Editor .
---	--

Note:

If column handling is used, replace the column type **Any** in the **Method Settings** phase with the corresponding column type created in column handling. It is important to use a column type with the same hardware, media and bed height as defined in the method. Information about the column type for which the method was created can be found in **Method Notes**.

When the column type has been created in column handling it is found in the column type list. See *Create a new column type*, on page 281, for information on how to create column types.

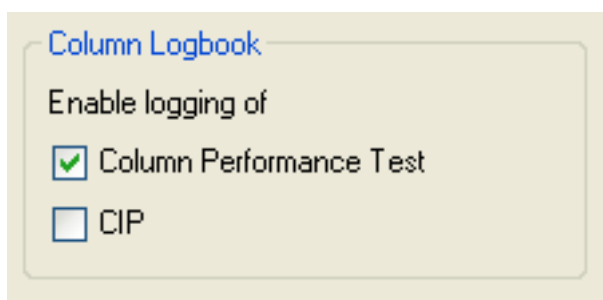
Note:

Columns that do not use the AxiChrom Master for packing will get a warning when saving the method, if the column type was replaced with the column type corresponding to the defined type in the intelligent packing method. This is because the packing pressure is, and shall be, higher than the maximum pressure for the column. Save the method with correct packing pressure by pressing **Save** anyway. This warning will appear every time the method is used.

Tip:

To avoid the pressure warning it is advised to generate two separate methods, one for packing and one for column performance test, and only replace the column type for the column performance test method.

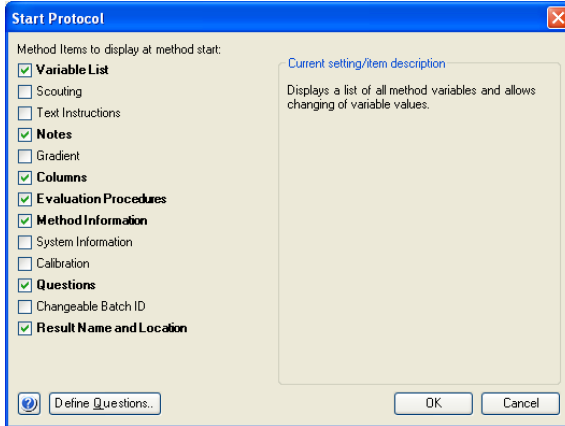
5	Select the Phase properties tab in the Method Settings phase and select the Enable logging of Column Performance Test check box.
---	---



Step **Action**

- 6
- Click the **Start Protocol...** button.

Result: The **Start Protocol** dialog opens.



- Select the check boxes of the items you want to display at the start of the method.

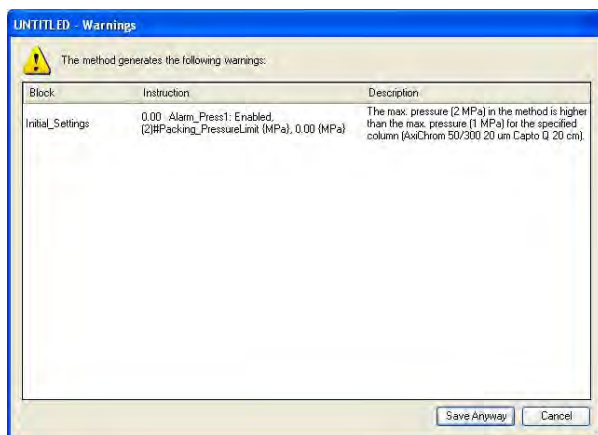
Tip:

*It is recommended to select the **Notes** checkbox. This will allow the method summary page in **Method Notes** to be available at method start.*

Step **Action**

Warning

When saving a created packing method for AxiChrom columns that do not use the AxiChrom master for packing, a warning appears if the column type has been replaced in the method with the corresponding column type created in column handling.



This warning is issued because UNICORN compares the column type max pressure and the method max pressure at saving and method start, and the packing pressure for the AxiChrom column has to be higher than the maximum pressure for the column during run. Press **Save Anyway** to save the method and keep the correct packing pressure. This warning will appear every time the packing method is used.

Tip:

To avoid the message it is advised to generate two separate methods, one for packing and one for column performance test (packing test) and replace the column type for the column performance test method but not for the packing method. This will also allow to generate the column type after packing and thereby apply the measured bed height rather than the target bed height for the column type.

7 Save the method.

Note: It is recommended not to change any variables in a wizard generated intelligent packing method.

9 Column Handling

9.5 Intelligent Packing of AxiChrom columns

9.5.3 Wizard generated Intelligent Packing 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 column. If a column type is used, it is also necessary to create and select a column individual prior to start.

Refer to the AxiChrom operating instruction for instructions on how to prepare the column, connect it to the chromatography system and perform the packing run.

10 Text edit methods

Introduction

For some instruments it is necessary to create and edit methods or phases using **Text Instructions**. But it can also be an option for fine-tuning or optimizing 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.

Note: *Text instruction may differ depending on instrument configuration. The instructions shown in this chapter are examples.*

In this chapter

This chapter contains these sections:

Section	See page
10.1 Overview	326
10.2 Working with methods in the Text Instructions pane	334
10.3 Specific instructions	364

10.1 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
10.1.1 Working with text instructions	327
10.1.2 The Text Instructions pane	329

10.1.1 Working with text instructions

Introduction

If a predefined phase in the **Method Editor** is selected, the corresponding phase block is selected in the **Text Instructions** when changing to the **Text Instructions** tab.

Changes made in the **Phase Properties** pane are automatically updated on the **Text Instructions** pane. If a predefined method or a method based on predefined phases is edited in the **Text Instructions** pane, the settings in the **Phase Properties** pane will be replaced by a list of the phase variables. A wizard generated method or an empty method consists of a method settings phase and a user defined phase. The user defined phase will always have a list of the 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.

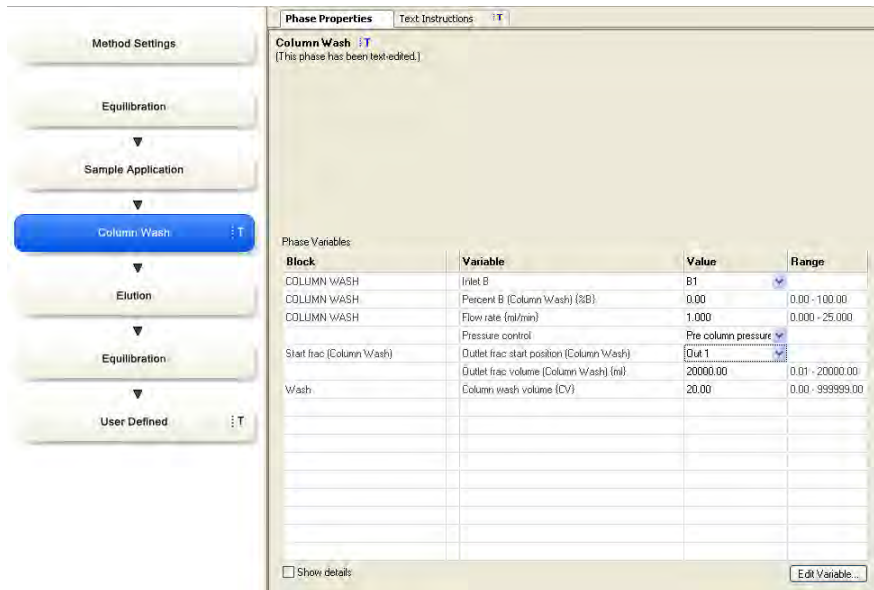
The letter **T** next to the phase name in the **Method Editor** window indicates that the phase has been text edited.

The illustration below shows the **Phase Properties** pane when a phase generated 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.

10 Text edit methods

10.1 Overview

10.1.1 Working with text instructions



The screenshot displays the 'Phase Properties' window with the 'Text Instructions' tab selected. The main area shows the text '[This phase has been text-edited.]'. Below this, a table titled 'Phase Variables' lists various parameters for the 'Column Wash' phase.

Block	Variable	Value	Range
COLUMN WASH	Inlet B	B1	
COLUMN WASH	Percent B (Column Wash) (%B)	0.00	0.00 - 100.00
COLUMN WASH	Flow rate (ml/min)	1.000	0.000 - 25.000
	Pressure control	Pre column pressure	
Start frac (Column Wash)	Outlet frac start position (Column Wash)	Out 1	
	Outlet frac volume (Column Wash) (ml)	20000.00	0.01 - 20000.00
Wash:	Column wash volume (CV)	20.00	0.00 - 999999.00

At the bottom of the window, there is a checkbox labeled 'Show details' and a button labeled 'Edit Variable...'.

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 phase generated method are edited manually, the phase properties will no longer show all optional settings but only the **Phase Variables**. To restore the phase properties in a phase generated method you have to undo the edited text instructions by clicking the **Undo text editing** button which is displayed in the **Phase Properties** tab after text instructions have been edited.
- Several phases may be labeled 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.

10.1.2 The Text Instructions pane

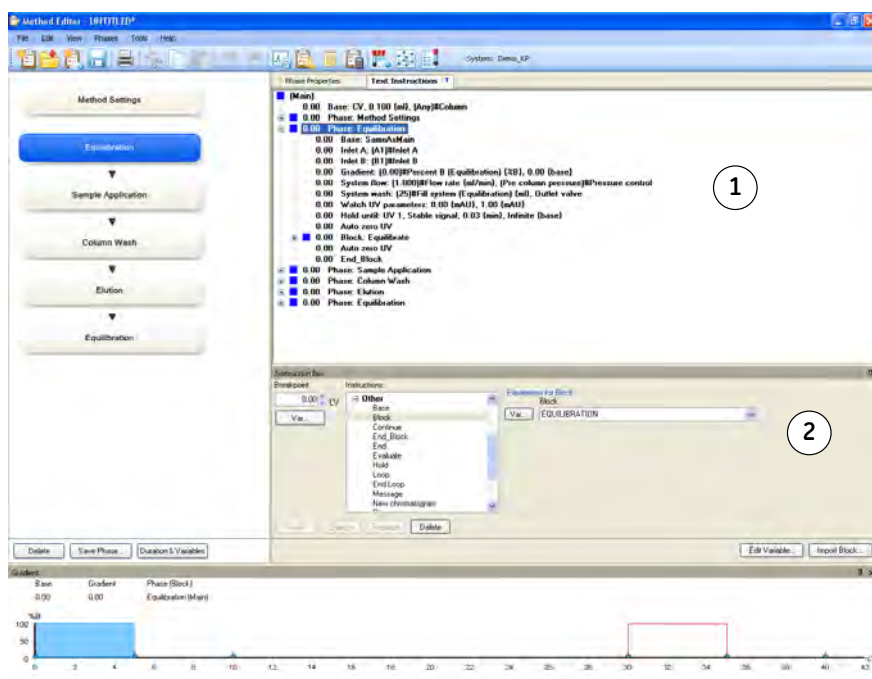
Introduction

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 when creating a method for a system that allows phase generated methods. The **Text Instructions** tab is 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:

10 Text edit methods

10.1 Overview

10.1.2 The Text Instructions pane

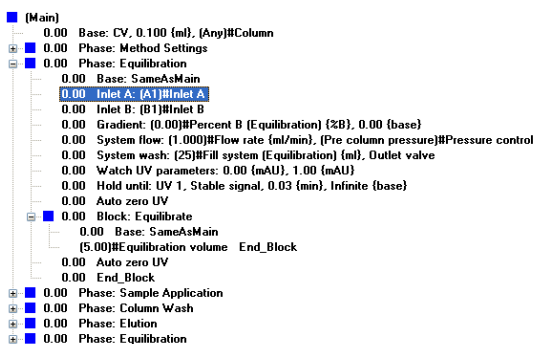
Area	Description
1	Text Instructions area: Shows the method as a list of individual text instructions. The instructions are grouped into blocks (denoted by blue square symbols in the figure below) to obtain a logical overview of the method.
2	Instruction Box : Shows the available instructions. It can be displayed using the Auto Hide function (see <i>Auto Hide optional panes, on page 19</i> for more information). Use the Instruction Box to: <ul style="list-style-type: none">• insert, change, replace and delete blocks and instructions in the method• delete phases• specify breakpoints, parameters and variables Note: <i>It is not possible to add phases using the Instruction Box. For information about how to add phases, see Section 3.6.1 Edit the method outline, on page 75.</i>

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 332*).

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 10.3.3 Watch instructions, on page 371* for more information about watch instructions).

The illustration below shows an example of a phase generated 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	<p>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.</p> <p>Note: <i>If a phase generated method has not been text edited, properties for the phase can be set in the Phase Properties pane.</i></p> <p>Note: <i>A wizard generated method only contains the method settings phase and the user defined phase containing all steps in the process flow.</i></p> <p>Note: <i>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.</i></p>
Block	Each block starts with a Base instruction, continues with the appropriate instructions and always ends with an End_Block instruction.

10 Text edit methods




10.1 Overview


10.1.2 The Text Instructions pane

Part	Description
Sub-block	<p>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.</p> <p>Each sub-block starts with a Base instruction, continues with the appropriate instructions and always ends with an End_Block instruction.</p>

Description of icons and text formats in the text method

The table below describes the icons and text formats that may appear in 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 	<p>Instructions that are not possible to run. All such instructions must be deleted or changed before a method can be run. See <i>Section 10.2.3 Working with text instructions, on page 347</i>.</p> <p>The errors in the instructions may be of the following types:</p> <ul style="list-style-type: none">• 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).

Icon/text format	Description
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.
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.

10.2 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
10.2.1 Base instruction	335
10.2.2 Working with phases and blocks	339
10.2.3 Working with text instructions	347
10.2.4 Method variables	353

10.2.1 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 330). 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.

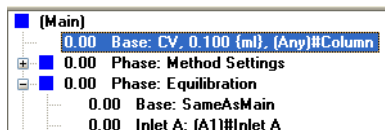
The table follows lists few examples when different bases should be used:

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

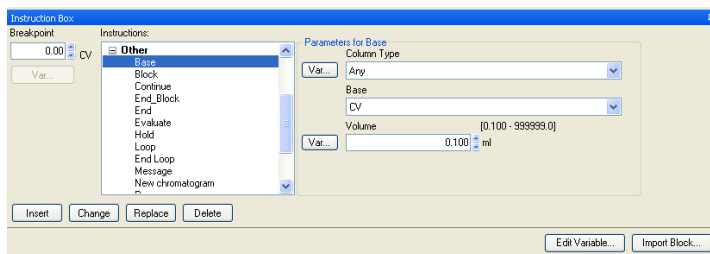
The following table describes how to edit settings for a base instruction:

Step	Action
1	Select the base instruction for which to edit the settings in the <i>Text Instructions</i> area.

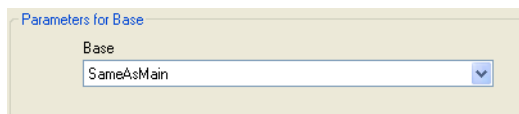


Result:

- 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**.



- 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 <i>Set up a Start Protocol</i> , on page 85). See Section 10.2.4 <i>Method variables</i> , on page 353 for information about how to define variables.

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.1 Base instruction

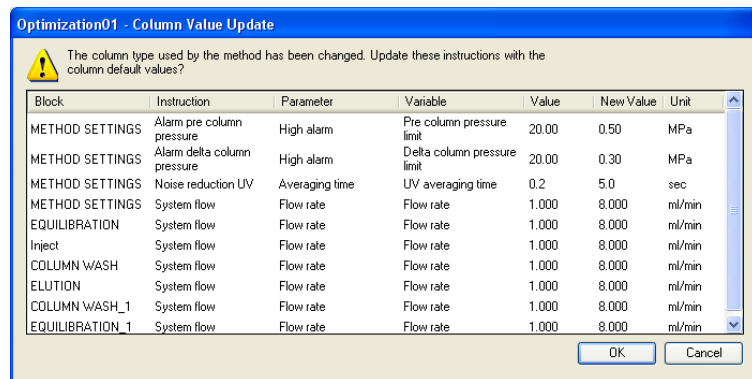
Step	Action
------	--------

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 methods, based on the column default values (see diagram below). If these changes are correct, click **OK**, otherwise click **Cancel**.*



10.2.2 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 3.6.1 Edit the method outline, on page 75* 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:

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.2 Working with phases and blocks

```
0.00 Phase: Equilibration
0.00 Base: SameAsMain
0.00 Inlet A: (A1)#Inlet A
0.00 Inlet B: (B1)#Inlet B
0.00 Gradient: (0.00)#Percent B (Equilibration) {%B}, 0.00 {base}
0.00 System flow: (1.000)#Flow rate (ml/min), (Pre column pressure)#Pressure control
0.00 System wash: (25)#Fill system (Equilibration) {ml}, Outlet valve
0.00 Watch UV parameters: 0.00 {mAU}, 1.00 {mAU}
0.00 Hold until: UV 1, Stable signal, 0.03 {min}, Infinite {base}
0.00 Auto zero UV
0.00 Block: Equilibrate
0.00 Base: SameAsMain
[5.00]#Equilibration volume End_Block
0.00 Auto zero UV
0.00 End_Block
```

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:

```
0.00 Phase: Equilibration
0.00 Base: SameAsMain
0.00 Inlet A: (A1)#Inlet A
0.00 Inlet B: (B1)#Inlet B
0.00 Gradient: (0.0)#Percent B (Equilibration) {%B}, 0.00 {base}
0.00 System flow: (0.000)#Flow rate (ml/min), (Pre column pressure)#Pressure control
0.00 System wash: (25)#Fill system (Equilibration) {ml}, Outlet valve
0.00 Watch UV parameters: 0.00 {mAU}, 1.00 {mAU}
0.00 Hold until: UV 1, Stable signal, 0.03 {min}, Infinite {base}
0.00 Auto zero UV
0.00 Block: Equilibrate
0.00 Auto zero UV
0.50 End_Block
0.00 Phase: Sample Application
```

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 91*.

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
view the instructions in a block	<ul style="list-style-type: none">click the "+" symbolordouble-click the block name.

If you want to...	then...
hide the instructions in a block	<ul style="list-style-type: none"> 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 3.6.1 Edit the method outline, on page 75* 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 342* 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	<ul style="list-style-type: none"> Enter a name for the block in the Block field. Click the Insert button.
<i>Result:</i> The block is inserted after the block that was selected in step 1.	


10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.2 Working with phases and blocks

Copy and paste blocks

The table below describes how to copy and paste a block.

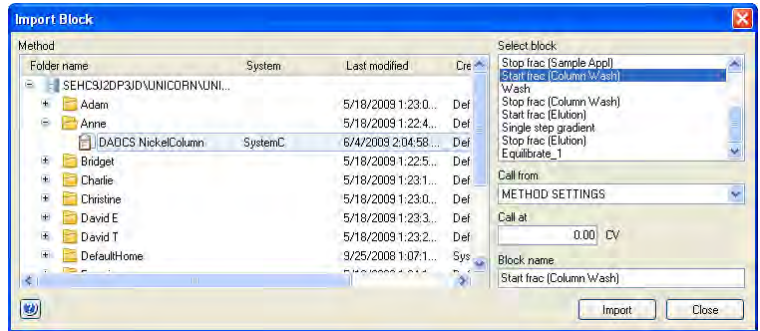
Step	Action
1	<p>Select the block you want to copy.</p> <ul style="list-style-type: none">click the Copy icon in the Toolbar  <p><i>or</i></p> <ul style="list-style-type: none">right-click the block and choose Copy <p><i>or</i></p> <ul style="list-style-type: none">select Edit:Copy (Ctrl+C)
2	<p>Select the instruction line just above the point where you want the block to be pasted.</p> <ul style="list-style-type: none">click the Paste icon in the Toolbar <p><i>or</i></p> <ul style="list-style-type: none">right-click the instruction line and choose Paste <p><i>or</i></p> <ul style="list-style-type: none">select Edit:Paste (Ctrl+V) <p><i>Result:</i> A Rename dialog opens.</p>
3	<ul style="list-style-type: none">Click Yes to rename the block after insertion <p>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.</p> <p><i>or</i></p> <ul style="list-style-type: none">Click No to just insert the copied block with the same name. <p>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.</p> <p><i>Result:</i> The block is inserted in position.</p> <p>Note:</p> <p><i>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.</i></p>

Import blocks

The table below describes how to import blocks from other methods:

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

- | | |
|---|---|
| 1 | Click the Import Block... button.
<i>Result:</i> 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. |
| 4 | <ul style="list-style-type: none">• 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). |

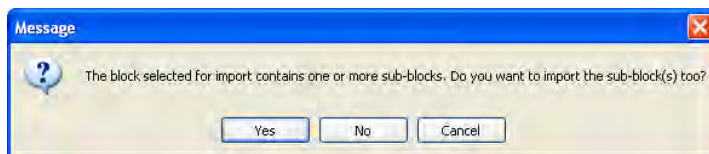
10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.2 Working with phases and blocks

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

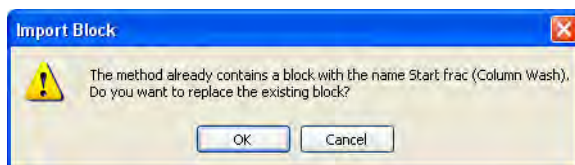
- | | |
|---|--|
| 5 | <ul style="list-style-type: none">Click the Import button.Confirm if you also want to import sub-blocks (if any) |
|---|--|



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. <ul style="list-style-type: none">click the Cut icon in the Toolbar |
|---|--|



or

- right-click the block and choose **Cut**
- or*
- select **Edit:Cut**

Step	Action
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.

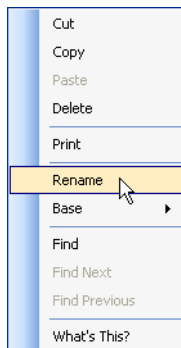
Note:

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.

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

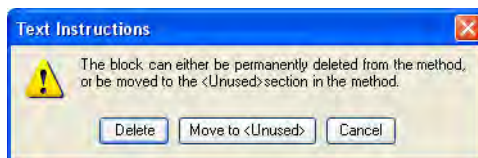
10.2.2 Working with phases and blocks

Step	Action
2	Type in a new name. Note: <i>If the block you renamed is called in a Block or Watch instruction, the block name in these instructions will be changed automatically.</i>

Delete blocks

The table below describes how to delete a block:

Step	Action
1	<ul style="list-style-type: none">Right-click a block and choose Delete.<i>or</i>Select a block and click Delete in the Instruction box.<i>or</i>Select a block and press the Delete key on the keyboard. <p><i>Result:</i> A dialog will appear asking if the block should be deleted permanently or moved to unused blocks.</p>



Note:

If deleting a phase, the phase will be deleted right away.

2	<ul style="list-style-type: none">Click Delete to delete the block permanently.Click Move to <Unused> to delete the block from the method and place it after the method.
---	---

10.2.3 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. <i>Result:</i> 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.

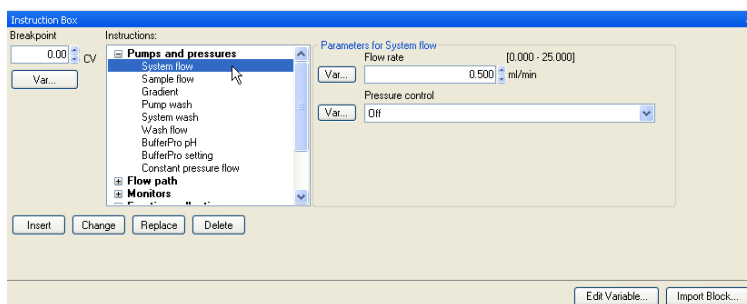
10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.3 Working with text instructions

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

- | | |
|---|---|
| 3 | <p>Open the Instruction Box if it is hidden. Do the following:</p> <ul style="list-style-type: none">• 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. |
|---|---|

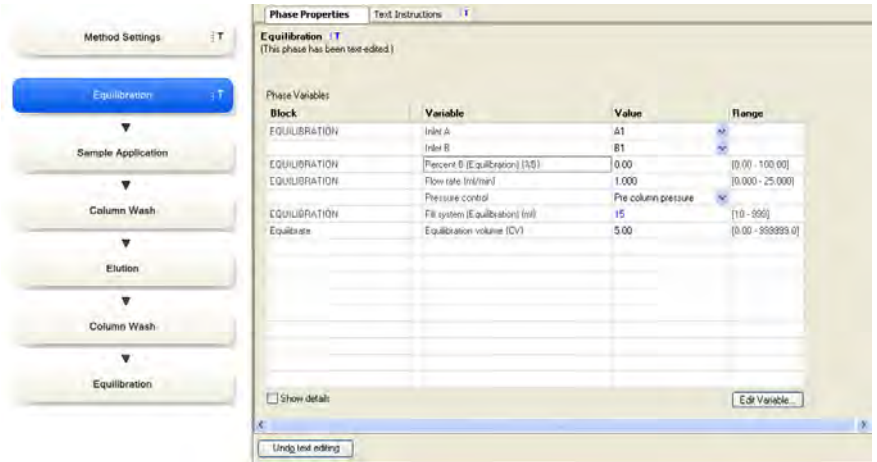


- | | |
|---|--|
| 4 | <p>Click the Insert button.</p> |
|---|--|

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.

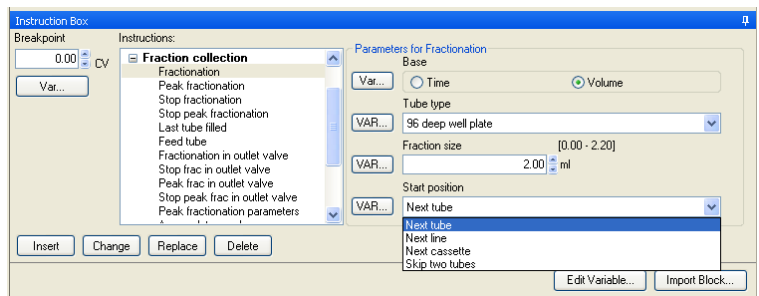
Note: *Once a phase generated method has been edited in text editing mode, the phases affected by the edited instruction are indicated with the letter "T", and the **Phase Properties** pane changes to show a variable list, as shown below. For a phase generated method you can click the **Restore Phase 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.
<i>Result:</i> 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 : |



10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.3 Working with text instructions

Step	Action
3	<p>To add the edited or a new instruction to the method, click one of the following buttons:</p> <ul style="list-style-type: none">• Insert• Change• Replace <p>Note:</p> <p><i>The Insert button adds the edited instruction immediately below the instruction that was selected in the method.</i></p> <p><i>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.</i></p>

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	<p>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.</p> <p>Note: <i>Moving the End_block instruction in a gradient block with the Change button does not affect the length of the gradient.</i></p>
Replace	<p>If this button is used to change the length of a gradient, other instructions are not affected.</p>

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.



10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.3 Working with text instructions

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	<ul style="list-style-type: none">• Right-click the instruction and choose Delete.• <i>or</i>• Click the Delete button in the Instruction box.• <i>or</i>• 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.

10.2.4 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 126* and *Chapter 5 Design of Experiments, on page 139* 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.
-

Viewing method variables

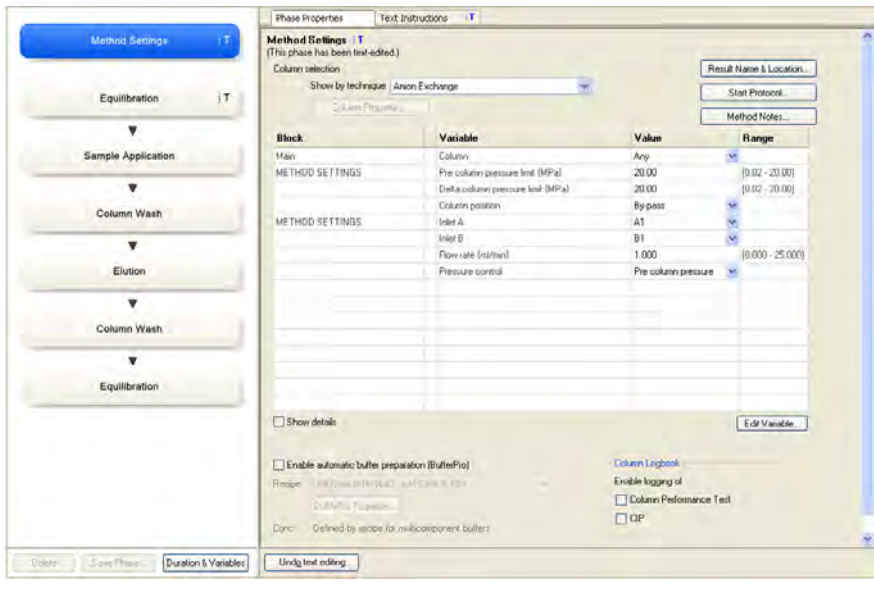
All variables in a method are listed on the **Variable List** tab in the **Method and Duration** dialog, grouped according to the phase and block in which they appear. For information about how to view the variables in a method, see *View and print the method duration time and variables, on page 91* for more information.

If a method has been text edited or created using the wizard 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 will also be displayed. It is possible to edit variable names, values and the other settings displayed in the **Phase Properties** pane.

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.4 Method variables



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:

```
0.00 Phase Equilibration
  0.00 Base SameAsMain
  0.00 Pump A inlet (A1)#Inlet A
  0.00 Pump B inlet (B1)#Inlet B
  0.00 Gradient (0.00)#Percent B for equilibration {%B}, 0.00 {base}
  0.00 System flow (1.000)#Flow rate {ml/min}, (No)#Use pressure control, 0.010 {ml/min}
  0.00 System wash (25)#SystemWashVolume {ml}, Outlet valve
  0.00 Watch UV parameters 0.00 {mAU}, 0.10 {mAU}
  0.00 Hold until UV 1, Stable signal, 0.10 {min}, Infinite {base}
  0.00 Auto zero UV
  0.00 Block Equilibrate
```

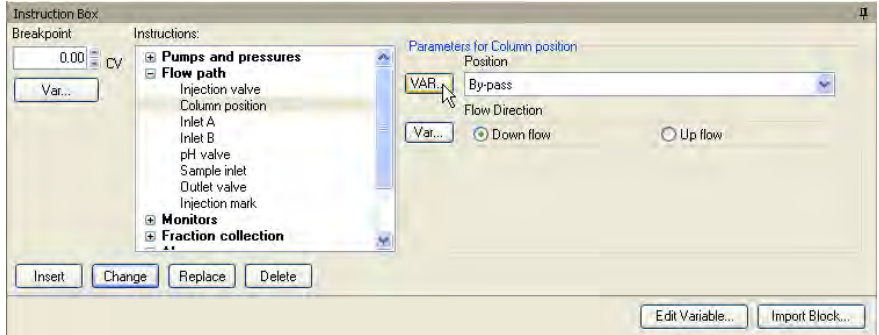
For example, in **(1.000)#Flow rate {ml/min}**:

- (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 356 and *Edit variables*, on page 358.

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.4 Method variables

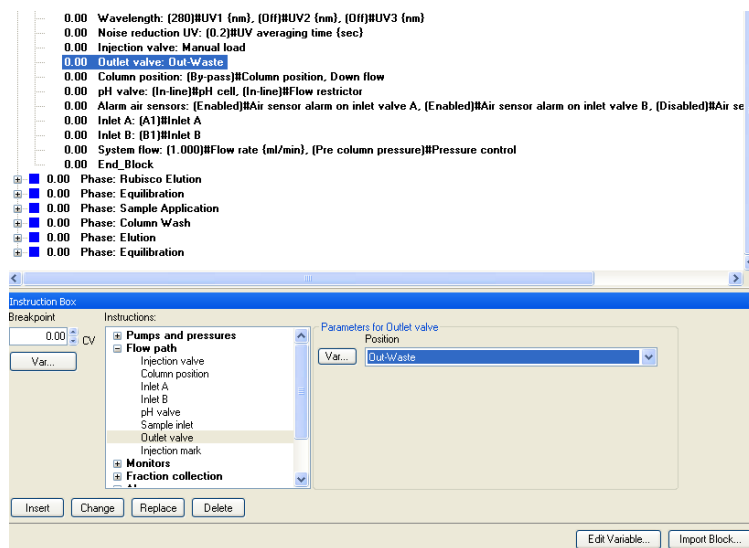
Define new variables

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
------	--------

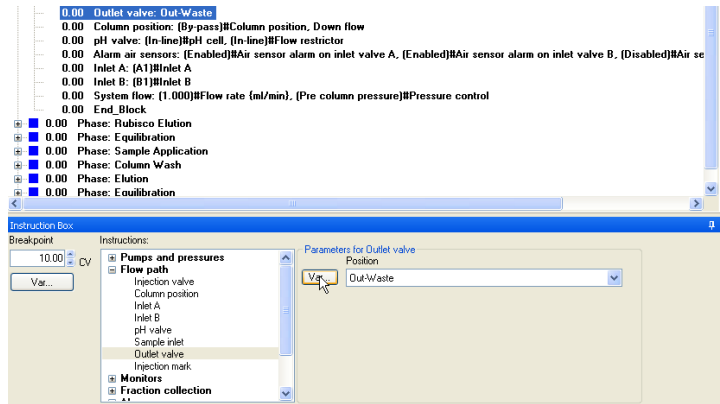
- | | |
|---|--|
| 1 | Select the instruction where you want to define the variable in the Text Instructions area. |
|---|--|

Result: The parameters for the instruction are shown in the **Instruction box**.

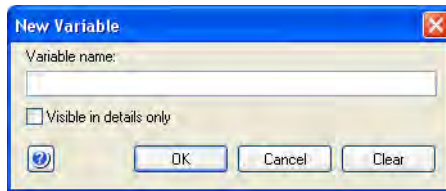


Step **Action**

- 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 355 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 10.2.3 Working with text instructions, on page 347 for how the **Change** button affects instructions within gradients).

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.4 Method variables

Step	Action
4	Click Change . <i>Result:</i> The variable is saved and displayed in the Text Instructions area.

Edit variables

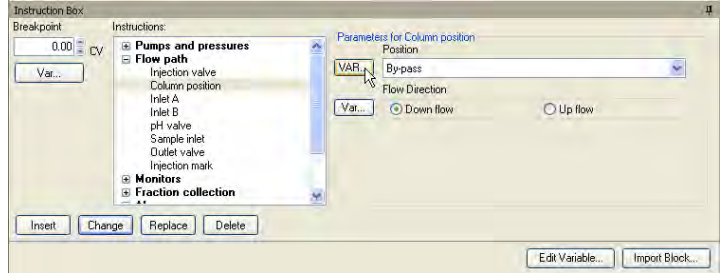
Editing a variable includes renaming and deleting the variable and choosing whether the variable should be a detailed variable or not. For information about how to edit the variable values, see *Edit variable values*, on page 362.

Edit a variable using the Edit variable button

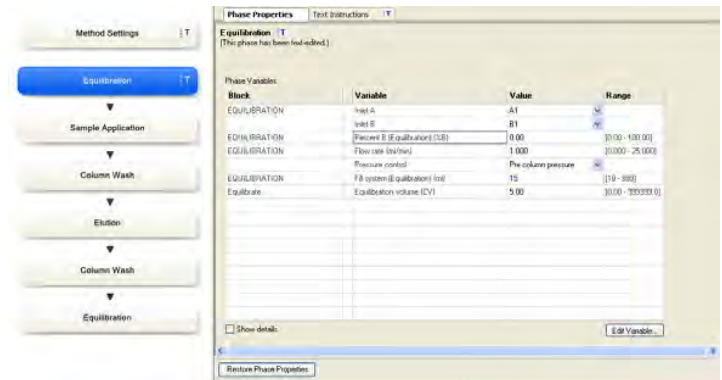
The table below describes how to edit a variable using the **Edit Variable** button:

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).

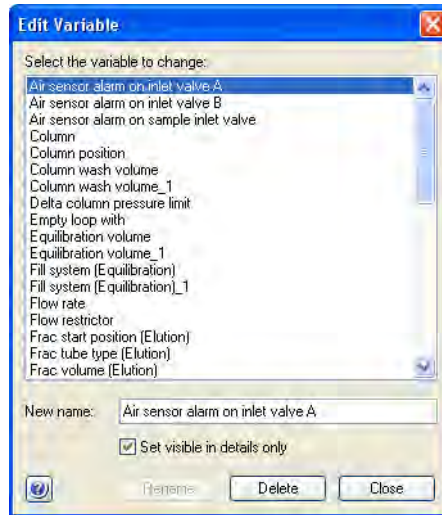
10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.4 Method variables

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. |
|---|---|

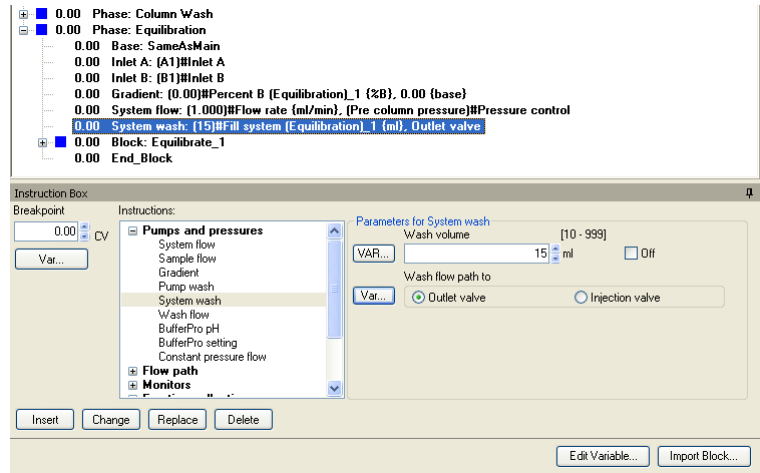
Edit a variable using the VAR.. button in the Instruction Box

The table below describes how to edit a variable using the **Instruction Box**:

Step **Action**

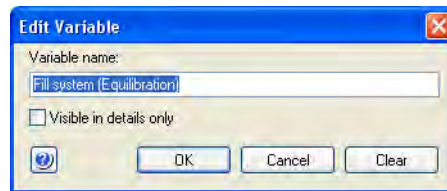
- 1 Select the instruction containing the variable to be edited in the **Text Instructions** area.

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.

10 Text edit methods

10.2 Working with methods in the Text Instructions pane

10.2.4 Method variables

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**.

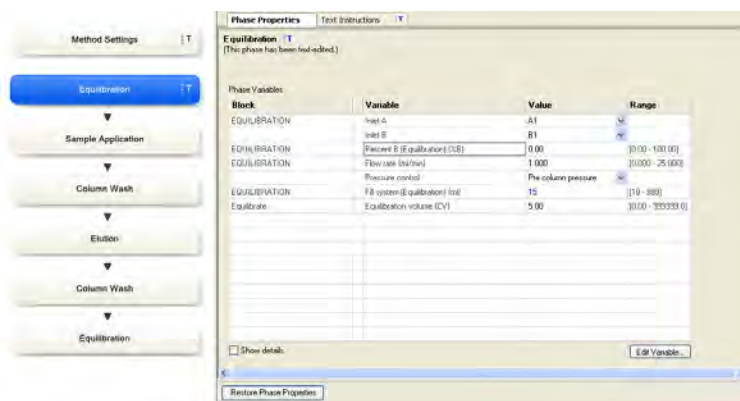
Changes made in the **Phase Properties** tab are automatically updated on the **Text Instructions** tab and vice versa.

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.

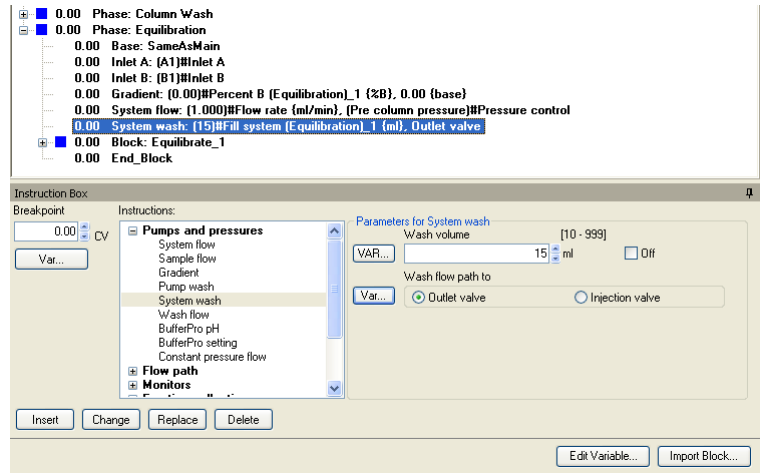
Edit variable values in the Instruction Box

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.

10.3 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
10.3.1 Gradients and eluent concentrations	365
10.3.2 Alarm instructions	368
10.3.3 Watch instructions	371
10.3.4 Pause or hold a method	378
10.3.5 Messages and Set marks	381

10.3.1 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:

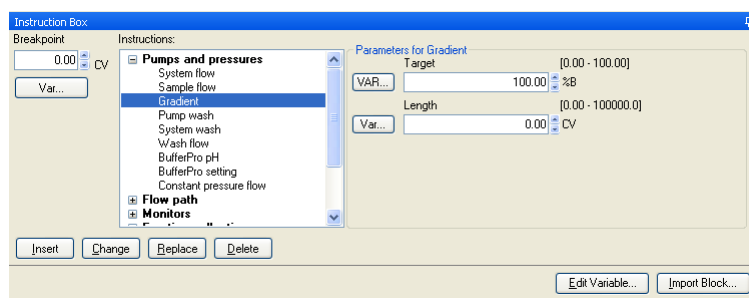
Step	Action
1	At a suitable Breakpoint in the method, select the instruction line immediately before where you want to insert the gradient (this decides when the gradient begins).
2	<ul style="list-style-type: none"> Expand the Pumps and pressures item in the Instructions field of the Instruction Box. Select Gradient. In the Parameters for Gradient field, select appropriate values for: <ul style="list-style-type: none"> - 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	<p>Edit the Breakpoint for the gradient, if appropriate.</p> <p>Note: <i>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.</i></p>
4	<p>Click the Insert button.</p> <p>Result: The new Gradient instruction is inserted in the method in the Text Instructions area.</p>

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	<p>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.</p> <p>Note: <i>If the flow is changed, the slope of the gradient will also change.</i></p>
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.

10.3.2 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 10.3.3 Watch instructions, on page 371*.

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

If the signal exceeds...	then...
Alarm limits	<ul style="list-style-type: none">• an alarm sounds <p>Note: <i>The alarm can be disconnected in System Settings.</i></p> <ul style="list-style-type: none">• 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. <p>The situation must be acknowledged and corrected before the process can be continued.</p>
Warning limits	<ul style="list-style-type: none">• 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

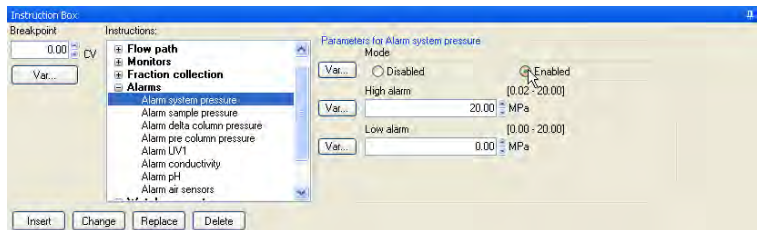
Alarms and warning messages are displayed on all stations with a connection to the concerned system. This is regardless of the activity that is currently performed in UNICORN and regardless of the identity and access rights of the current user.

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 | <ul style="list-style-type: none">• 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.

10 Text edit methods

10.3 Specific instructions

10.3.2 Alarm instructions

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
-

10.3.3 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.
-

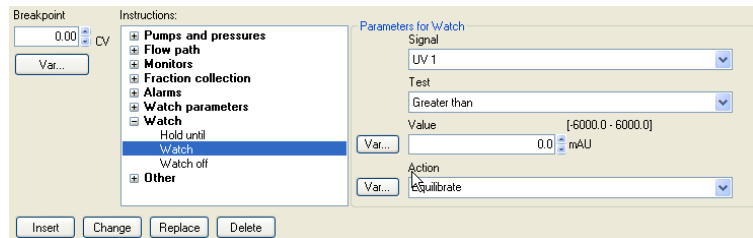
Insert a Watch text instruction

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 375.

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 373 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 373 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 374 for a description of the different **Watch Action** options.

Step	Action
5	<p>Click the Insert button.</p> <p><i>Result:</i> The new Watch instruction is inserted in the Text Instructions area.</p> <p>Note: A Watch off instruction can be added to the method at a breakpoint where the watch no longer is needed.</p>
Note:	<p>Watch parameters may be set as variables so that the method easily can be adjusted for different run conditions.</p>

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).

Option	Explanation
Slope less than	The rate of change of the signal falls below a specified value, expressed in, for example, mAU/min.
Less than or valley	The signal falls below a specified value or a valley is detected. A valley is detected only after a Peak max has been detected, and the valley is defined by a local minimum followed by an increase to 102% of the local minimum value plus the Delta peak value (see <i>The Delta peak setting, on page 376</i>).
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 <i>Insert a Watch parameters instruction, on page 375</i>), 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. This information only applies to Evaluation Classic.*

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	<p>Calls the named block.</p> <p>Note: <i>All available method blocks are listed in alphabetical order in the drop-down list, before the general actions which are listed below.</i></p>
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 <i>Hold instruction, on page 378</i> .

Instruction	Effect
End_method	Ends the method.
Next_breakpoint	Indicates that the run may execute the next breakpoint.
Pause	Pauses the method, the flow is stopped. See <i>Pause instruction, on page 378</i> .

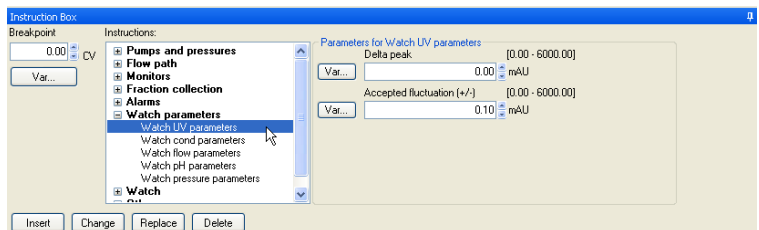
Insert a Watch parameters instruction

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 376*.



- 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.

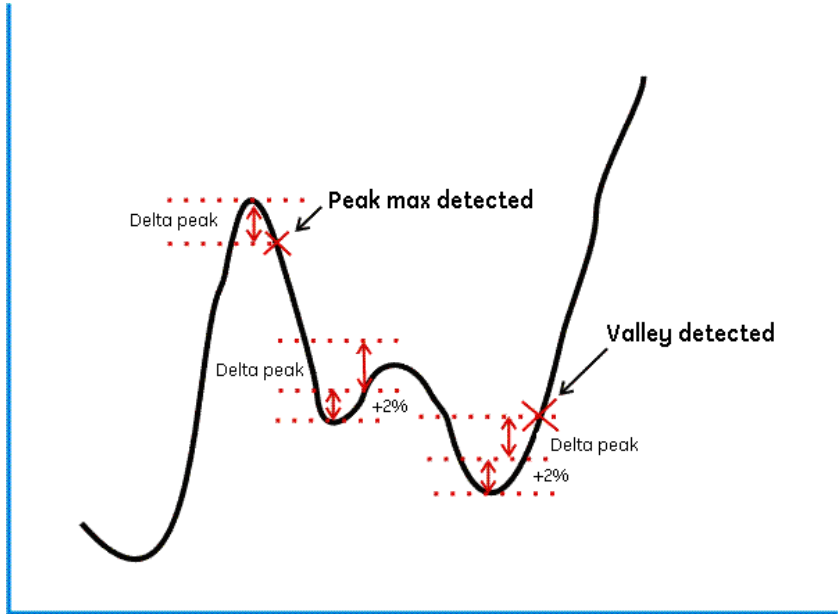
Example:

If there is a local minimum at 50 mAU and a **Delta peak** of 10 mAU, a valley will be detected at:

$$(1.02 \times 50) + 10 = 61 \text{ mAU}$$

- 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.

10.3.4 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.*

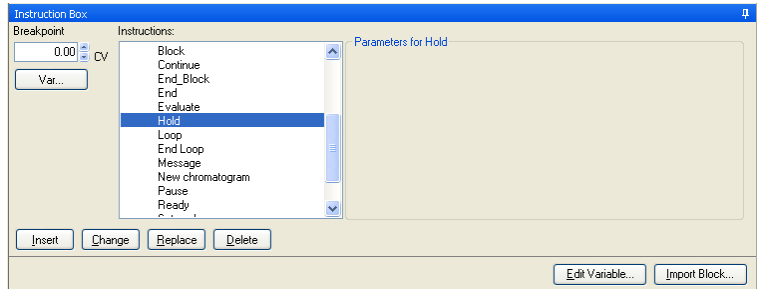
Hold until instruction

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 10.3.3 Watch instructions, on page 371* for a description of **Watch** instructions.

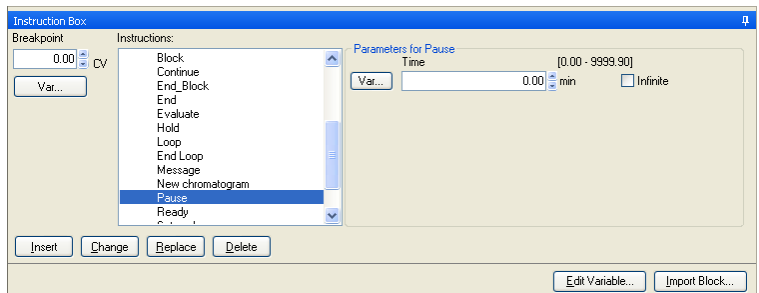
Insert a Pause, Hold or Hold until instruction

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 .

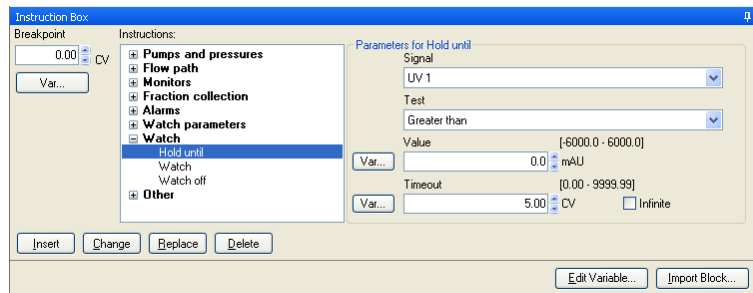


3	To insert a Pause instruction, select Other:Pause and enter the Time for the method to be paused in the Time field. To pause the method for infinite time, check the Infinite box.
---	---



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 10.3.3 *Watch instructions*, on page 371 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.*

10.3.5 Messages and Set marks

When to use a message

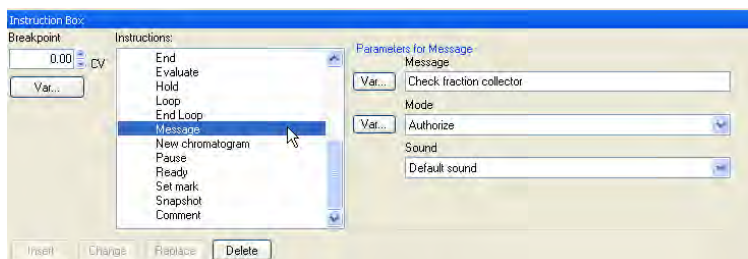
Messages are used to inform the operator of the progress of the run or to prompt the user for an action. It is a good idea to issue messages at critical points in the method, for example, in combination with a **Pause** instruction to inform the operator that the inlet tube needs to be moved to another 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 on 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	<ul style="list-style-type: none">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 | <ul style="list-style-type: none">• 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

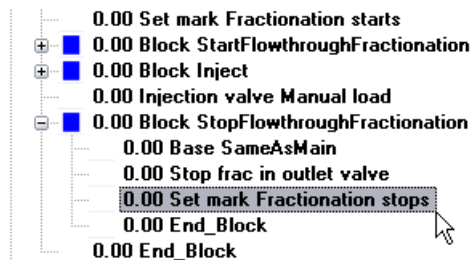
Set mark instructions are useful text messages. They can be used

- 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:

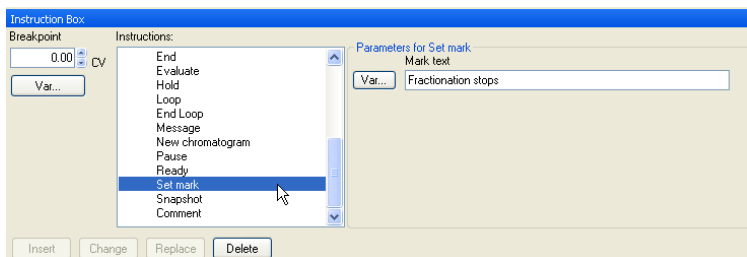


Insert a Set mark

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.

11 Troubleshooting

Introduction

This chapter describes different problems which may arise when creating methods in UNICORN, and how to solve the problems.

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
<p>The Phase Properties tab shows only a list of variables and not the regular options and settings for the selected phase. The phase is marked with the letter "T" in the method outline.</p> <p>Note: <i>User defined phases only have a list of variables.</i></p>	<p>The phase has been edited in the Text Instructions tab. Click the Undo text editing button to return to the default settings and restore the Phase Properties options and settings.</p> <p>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.</p>

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 predefined phase are not suitable for your specific application needs:

Problem description	Solution
<p>Options that you need are not available for selection or editing in the Phase Properties.</p>	<ul style="list-style-type: none"> • 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.
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.	<ul style="list-style-type: none"> • Edit the method so it can be run on the currently chosen system. • Save the method for a system that has all components installed. <p>Note: <i>The red instructions must be replaced or removed.</i></p> <ul style="list-style-type: none"> • 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 originally 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.

Problem description	Solution
<p>A phase is marked as incorrect (with a red cross).</p> <p>This may appear if</p> <ul style="list-style-type: none"> • the instrument configuration has been changed • components have been removed or • the method was converted from a system with a different component set up 	<p>Replace the phase with a compatible predefined phase from the Phase Library. This phase will automatically be adapted to the current instrument configuration and component settings.</p> <p>Note: <i>If predefined phases are not available for the system, the red instructions in the phase have to be deleted or replaced.</i></p>

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
<p>Method breakpoints are not calculated. All values are shown as zero.</p>	<p>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.</p>

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	<ul style="list-style-type: none"> • 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. <p>For more information, see <i>Section 3.8 Scale or convert methods, on page 104</i>.</p>

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	<p>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</p> <ul style="list-style-type: none"> • 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 predefined 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: <i>This happens for a very short time and it will normally not cause any problems.</i>	Check which inlets are chosen in Method Settings . Choose the same inlets as required for the CIP or preparation phase.

Export of a method to a network drive fails

The table below describes how to solve a method export problem:

Problem description	Solution
Export of a method to a network drive fails.	Ensure that the destination network drive is mapped and that you have the appropriate access rights.

Predefined method cannot be created after new Instrument Configuration installation

The table below describes how to solve a predefined method creation problem:

Problem description	Solution
A new instrument configuration is installed. After this, it is still impossible to create a new, predefined method.	The Method Editor must be restarted after importing the new instrument configuration.

Incorrect Bar code reader usage closes Column Handling dialog

The table below describes how to solve a bar code reader usage problem:

Problem description	Solution
Using the bar code reader to read column bar codes without first selecting a proper input field will cause the Column Handling dialog to close.	Use the bar code reader only as described in the user documentation.

Index

A

- Add runs
 - DoE, 188
 - Scouting, 137
- Affinity chromatography
 - predefined method, 36–37
- Alarms
 - Available monitors, 370
 - General description, 368
 - Insert alarm in method, 369
- Anion exchange
 - predefined method, 38
- ANOVA (analysis of variance) table
 - Design of Experiments, 208
- Auto Hide
 - Optional Method Editor panes, 19

B

- Barcodes
 - Column identification, 294
- Base instruction
 - CV (Column volume), 335
 - Edit settings, 336
 - Text instructions, 335
 - Time, 335
 - Volume, 335
- Blocks
 - Adding, 341
 - Copy/paste, 342
 - Deleting, 346
 - Import from other methods, 343
 - Moving, 344
 - Rename, 345
 - Text editing, 339
- Breakpoints
 - In methods, 386
- BufferPro
 - Considerations when preparing buffers, 251
 - Create method using, 230
 - Create recipe, 232
 - Delete recipe, 239

- Edit recipe, 232
- Explore proportions, 243
- Export recipe, 247
- Import recipe, 249
- Overview, 228
- pH ranges, 252
- Predefined buffers, anion exchange, 229
- Predefined buffers, cation exchange, 229
- Predefined recipes, 252
- Print recipe, 241–242
- Rename recipe, 237
- Workflow, 228

Buffers

- Anion exchange, 229
- Calculate composition of, 243
- Cation exchange, 229
- Considerations when preparing, 251
- pH ranges, 252
- Predefined, 229

C

- Cation exchange
 - predefined method, 38
- Chromatofocusing
 - predefined method, 39
- CIP
 - column maintenance method, 43
 - column predefined phase, 46
 - System, predefined phase, 100
 - system maintenance method, 44
 - system predefined phase, 46
- Column CIP
 - maintenance method, 43
 - predefined phase, 46
- Column Handling
 - Barcodes, 294
 - Column types, 280

- Individual columns, 293
 - Overview, 274
 - Registering new individual columns, 295
 - Workflow, 278
 - Column list
 - Importing predefined column types, 291
 - Column performance test
 - maintenance method, 43
 - predefined phase, 46
 - Predefined phase, 100
 - Column preparation
 - maintenance method, 43
 - predefined phase, 46
 - Columns
 - Adding or editing notes, 300
 - Barcodes, 294
 - Column Handling
 - Overview, 274
 - Column Handling Workflow, 278
 - Column performance test, 308
 - Column types, 280
 - Create an AxiChrom column type, 314
 - Create new column type, 281
 - Deleting column types, 286
 - Deleting individual columns, 302
 - Editing column type, 284
 - Editing individual columns, 300
 - Exporting column types, 287
 - Exporting individual columns, 303
 - Find an individual column, 298
 - Handling individual columns, 293
 - Importing column types, 289
 - Importing individual columns, 303
 - Importing new column lists, 291
 - Notification limits, 301
 - Performance report, 309
 - Predefined column types, 280
 - Predefined Intelligent Packing method, 318
 - Printing column type information, 291
 - Printing individual column information, 307
 - Registering new individual columns, 295
 - Scale a method for use with another column type, 107
 - Unable to select for method scaling, 387
 - View column history, 306
 - Wizard generated Intelligent Packing method, 320
 - Column types
 - Create new column type, 281
 - Deleting, 286
 - Editing, 284
 - Exporting, 287
 - Importing, 289
 - Predefined column types, 280
 - Printing information, 291
 - Column volume (CV)
 - Base instruction, 335
 - Column wash
 - predefined phase, 46
 - Correlation matrix
 - Design of Experiments, 210
 - Create method
 - Workflow, 31
 - CV (Column volume)
 - Base instruction, 335
- ## D
- Desalting
 - predefined method, 39
 - Design of Experiments
 - Add factors in experimental design, 164
 - Add responses, 162, 187
 - Add runs, 188
 - Analysis, advanced, 204
 - Analysis, basic, 196
 - ANOVA (analysis of variance) table, 208

- Change experimental design, 169
 - Check data, 192
 - Coefficient plot, 202
 - Correlation matrix, 210
 - Create report, 224
 - Delete responses from model, 188
 - Edit model, 211
 - Evaluation of results, overview, 181
 - Evaluation of single runs, 183
 - Experimental design, set-up, 153
 - Experimental designs, 146
 - Fractional Factorial design, 145
 - Full Factorial design, 145
 - Generate model, 185
 - Interaction plot, 206
 - Main effects plot, 208
 - Model curvature, 195, 205
 - Models, overview, 148
 - Multiple scouting runs, 174
 - Normal probability plot of residuals, 195, 201
 - Observed vs predicted plot, 207
 - Open result, 184
 - Optimization designs, 146
 - Optimize response values, 221
 - Outliers, 192
 - Overview, 140
 - Predict response values, 219
 - Print method, 179
 - Print report, 226
 - Replace run results, 190
 - Replicate plot, 193
 - Report, creating, 224
 - Report, printing, 226
 - Residual vs run order plot, 206
 - Residual vs variable plot, 204
 - Response surface plot, 215
 - Summary of Fit plot, 198
 - sweet spot plot, 217
 - View scouting scheme, 178
 - Workflow, 151
 - Documentation, 13
 - Downscale
 - Scale a method for a smaller column type, 107
- E**
- Edit
 - BufferPro recipe, 232
 - Column, individual, 300
 - Column type, 284
 - DoE model, 211
 - Method notes, 86
 - Method outline, 75
 - Method phases, 52
 - Method queue, 260
 - Scouting scheme, 129
 - Text instructions, 349
 - Variables, 358
 - Electronic signature
 - Signing a method, 96
 - Elution
 - predefined phase, 46
 - End_Block
 - Text instruction, 339
 - Equilibration
 - predefined phase, 46
 - Evaluation
 - DoE results, overview, 181
 - Single DoE runs, 183
 - Evaluation procedures
 - Include after run, 87
 - Experimental design
 - Add factors in DoE, 164
 - Add responses in DoE, 162
 - Change design in DoE, 169
 - Design types in UNICORN, 146
 - Setup in DoE, 153
 - Exporting
 - BufferPro recipes, 247
 - Column types, 287
 - Individual columns, 303
 - Method queues, 268
 - Methods, 114
 - Phases, 114
- F**
- Fractional Factorial design
 - Description, 145

- Fraction collection
 - Frac 950, 67
 - Overview, 58
 - Setup, 58
- Full Factorial design
 - Description, 145
- G**
- Gel filtration
 - predefined method, 39
- Gradients
 - Gradient breakpoints, 367
 - Length as variable, 366
 - Linear, 365
 - Step, 365
 - Text instructions, 367
- H**
- Help
 - Text instructions, 347
 - Viewing, 20
- Hold
 - Insert instruction, 379
 - Text instruction, 378
- Hold until
 - Insert instruction, 379
 - Text instruction, 379
- Hydrophobic interaction chromatography (HIC)
 - predefined method, 40
- I**
- Importing
 - Blocks from other methods, 343
 - BufferPro recipe, 249
 - Column types, 289
 - Individual columns, 303
 - Method queues, 266
 - Methods, 120
 - Import from UNICORN 5, 122
 - Phases, 118
 - Predefined column types, 291
- Individual columns
 - Adding or editing notes, 300
 - Column Logbook, 293
 - Column performance test, 308
 - Deleting, 302
 - Editing, 300
 - Exporting, 303
 - Finding, 298
 - Importing, 303
 - Notification limits, 301
 - Performance report, 309
 - Printing information, 307
 - View column history, 306
- Instrument
 - Components, 385
 - Instrument configuration, 385
- Intelligent Packing
 - Predefined method, 43, 318
 - Predefined phase, 47
 - Wizard generated method, 320
- Ion exchange
 - predefined method, 38
- M**
- Maintenance methods
 - column CIP, 43
 - column performance test, 43
 - column preparation, 43
 - system CIP, 44
 - system preparation, 44
- Manual loop fill
 - predefined phase, 46
- Manual Loop Fill
 - predefined method, 40
- Message
 - Text instruction, 381
- Method
 - Options, 32
- Method blocks
 - Adding, 341
 - Block length, 339
 - Copying, 342
 - Deleting, 346
 - Hide instructions, 340
 - Import from other methods, 343
 - Moving, 344
 - Rename, 345
 - View instructions, 340
- Method editor, 15
- Method Editor

- General interface description, 16
- Optional panes, 18
- Saving a method, 98
- Saving a phase, 100
- Tools available in, 15
- Viewing help topics, 20
- Method notes
 - Add to method, 86
 - Edit, 86
- Method outline
 - Description, 22
 - Duration time and volume, 91
 - Edit method outline, 75
- Method queues, 254
 - Change method order, 264
 - Create, 256
 - Delete method from, 264
 - Deleting, 261
 - Editing, 260
 - Export a method queue, 268
 - Insert method into, 263
 - Multiple systems, 256
 - Opening, 260
- Methods
 - Add notes, 86
 - affinity chromatography, 36–37
 - After conversion, 111
 - anion exchange, 38
 - Breakpoints in, 386
 - BufferPro, 230
 - BufferPro, using, 230
 - cation exchange, 38
 - chromatofocusing, 39
 - CIP methods, 388
 - Convert a method to another system type, 105
 - Convert and scale a method for another system type and column type, 107
 - Convert method for use in a different database, 112
 - Create empty method, 70
 - Create new method, 48, 62
 - Definition, 21
 - desalting, 39
 - Duration time and volume, 91
 - Edit empty method, 72
 - Edit method outline, 75
 - Edit methods, 52
 - Edit notes, 86
 - Edit phase variables, 64
 - Electronic signature, 96
 - Empty method
 - Edit method outline, 72
 - Empty methods, 25
 - Evaluation procedures, include, 87
 - Export a method, 114
 - Export as text, 116
 - gel filtration, 39
 - hydrophobic interaction chromatography (HIC), 40
 - Import a method, 120
 - Import a method from UNICORN 5, 122
 - Import method queues, 266
 - Intelligent Packing, 43
 - manual loop fill, 40
 - Method outline, 75
 - NHS-coupling, 41
 - Open a method, 33
 - predefined maintenance methods, 42
 - predefined methods, 36
 - Predefined methods, 25
 - Prepare methods, 388
 - Print, 94
 - Print duration time and volume, 91
 - Print variables, 91
 - Result name and location, 83
 - reverse phase chromatography (RPC), 42
 - Saving, 98
 - Saving a phase, 100
 - Start protocol, setup, 85
 - Structure of, 22
 - Syntax errors in, 385
 - Text editing, 325
 - Troubleshooting, 384
 - unable to create predefined method, 389
 - unable to export method to network drive, 388

- Unexpected results from converted methods, 387
- Variables, 353
- View variables, 91
- Wizard generated method
 - Phases, 64
 - Working with, 23
- Method settings
 - predefined phase, 45
- Method variables
 - Viewing, 353
- Miscellaneous
 - predefined phase, 47
- Model curvature
 - Design of Experiments, 195, 201, 205

N

- NHS-coupling
 - predefined method, 41
- Notes
 - Column notes, 300
 - Method notes, 86

P

- Pause
 - Insert instruction, 379
 - Text instruction, 378
- Phase properties
 - Edit phase, 52
 - Help, 51
- Phases
 - CIP phases, 388
 - column CIP, 46
 - column performance test, 46
 - Column performance test, 100
 - column preparation, 46
 - column wash, 46
 - Deleting phases from library, 102
 - Edit phases, 52
 - elution, 46
 - equilibration, 46
 - Export a phase, 114
 - Help, 51
 - Import a phase, 118
 - Intelligent Packing, 47
 - manual loop fill, 46

- method settings, 45
- miscellaneous, 47
- Phase library, 386
- Phase properties, 52, 384
- predefined phases, 45
- Predefined phases, 26
- Prepare phases, 388
- sample application, 46
- Syntax errors in, 385
- system CIP, 46
- System CIP, 100
- system preparation, 46
- Text editing, 339, 384
- Phase variables
 - Edit, 64
 - Edit values, 65
- pH range
 - BufferPro predefined buffers, 252
 - Common buffers, 229
- Plots
 - Coefficient plot, DoE, 202
 - Interaction plot, DoE, 206
 - Main effects plot, DoE, 208
 - Normal probability plot of residuals, DoE, 195, 201
 - Observed vs predicted plot, DoE, 207
 - Replicate plot, DoE, 193
 - Residual vs run order plot, DoE, 206
 - Residual vs variable plot, DoE, 204
 - Response surface plot, DoE, 215
 - Summary of Fit, DoE, 198
 - sweet spot plot, DoE, 217
- Print
 - Methods, 94
- Printing
 - BufferPro recipe, 241–242
 - Column performance report, 309
 - Column type information, 291
 - DoE methods, 179
 - DoE report, 226
 - Individual column information, 307

- Method duration time and variables, 91
- Print screen, 388
- Response surface plot, DoE, 215
- Sweet spot plot, DoE, 217

R

- Response surface plot
 - Generating in DoE, 215
- Response values
 - Optimize in DoE, 221
 - Predicting in DoE, 219
- Result
 - Name and location, 83
 - Open DoE result, 184
- Reverse phase chromatography (RPC)
 - predefined method, 42

S

- Sample application
 - predefined phase, 46
- Scouting
 - Add runs, 137
 - Add variable, 135
 - Change variable values, 132, 136
 - Delete runs, 137
 - Delete variable, 135
 - Edit variable, 135
 - Overview, 127
 - Scouting scheme, 129
 - Usage, 127
 - Variables in, 135
 - Workflow, 127
- Scouting scheme
 - Add runs, 137
 - Delete runs, 137
 - Editing, 129
 - How to set up, 129
 - View in DoE, 178
- Set mark
 - Insert instruction, 383
 - Usage, 382
- Start protocol
 - Set up start protocol, 85
- Sweet spot plot
 - Generating in DoE, 217
- Syntax errors

- Icons in text instructions, 332
- In methods, 385
- In phases, 385
- In text instructions, 385

System

- Components, 385
- Instrument configuration, 385
- System CIP
 - maintenance method, 44
 - predefined phase, 46
 - Predefined phase, 100
- System preparation
 - maintenance method, 44
 - predefined phase, 46

T

- Text editing
 - Blocks, 339
 - Help texts, 347
 - Methods, 325
 - Phases, 339
 - Text instructions, 327
- Text instructions
 - Add a new instruction, 347
 - Alarm instructions, 369
 - Base instruction, 335
 - Change, 350
 - Change and Replace, differences, 350
 - Delete instructions, 352
 - Delta peak settings, 376
 - Edit an instruction, 349
 - End_Block, 339
 - Gradient breakpoints, 367
 - Gradient instructions, 367
 - Help, 347
 - Hold instruction, 378
 - Hold until, 379
 - Icons and text formats, 332
 - Linear gradient, 365
 - Message, 381
 - Message instructions, 381
 - Move an instruction, 351
 - Pause instruction, 378
 - Replace, 350
 - Set mark, 382
 - Step gradient, 365
 - Syntax errors in, 385

- Text editing a method, 327
- Text instructions pane, 329
- Text method symbols, 332
- Watch instructions, 371
- Watch parameters, 375
- Watch parameter test options, 373
- Time
 - Base instruction, 335
- Tools
 - In Method Editor, 15
- Troubleshooting
 - Methods, 384
- Typographical conventions, 11
- U**
- UNICORN workflow, 8
- Upscale
 - Scale a method for a larger column type, 107
- V**
- Variables
 - Breakpoints or gradient lengths, 357
 - Defining, 356
 - Deleting, 358
 - Edit values, 362
 - Identification in text instructions, 354–355
 - Method variables, 353
 - Renaming, 358
 - Scouting, use in, 135
 - Variable names, 355
 - Viewing, 353
- Volume
 - Base instruction, 335
- W**
- Warnings
 - Compared to Alarms, 368
- Watch instructions
 - Actions, 374
 - Air sensors, 377
 - Delta peak settings, 376
 - Description, 371
 - Insert instruction, 371
 - Parameter test options, 373
 - Signals available, 373
- Watch parameters
 - Delta peak settings, 376
- Watch parameters instructions
 - Insert text instruction, 375

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First published Nov. 2014

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