ÄKTA design

Making your first runs

Begin here with ÄKTA FPLC

18-1140-80

Amersham Biosciences

Important user information

All users must read this entire manual to fully understand the safe use of ÄKTA*FPLC*.

WARNING!

The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Caution!

The Caution sign is used to call attention to instructions or conditions that must be followed to avoid damage to the product or other equipment in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certification

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences except for alterations described in this manual.

WARNING!

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Contents

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1 About this guide

This guide is written for users who are not familiar with UNICORN™ software and ÄKTA*FPLC*™. Here you will learn the basics of UNICORN and how to operate ÄKTA*FPLC* from UNICORN.

UNICORN is a software package for control and supervision of the ÄKTA™*FPLC* chromatography system. It runs on an IBM-compatible PC under Windows™, and includes hardware for interfacing the controlling PC to the separation equipment of ÄKTA*FPLC*.

In this guide you will learn how to:

- create methods
- prepare the system for runs
- perform runs
- make simple evaluations
- make reports
- perform automatic method optimization (Scouting)

Follow the guide from page to page in front of the computer. The time will be well spent.

Note: *To follow the instructions it is not necessary to read the comments (written with smaller font) containing additional information.*

1.1 Pre-requisites

Before using the system, see the separate Installation guide:

- the system and the software must be installed and functioning, and
- the monitor and the pump must be calibrated

as described in the guide.

IMPORTANT! Before using ÄKTA*FPLC*, read all the safety information in ÄKTA*FPLC* System Manual.

1.2 Typographical conventions

Menu commands and dialog box prompts are identified in the text by bold text. A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu.

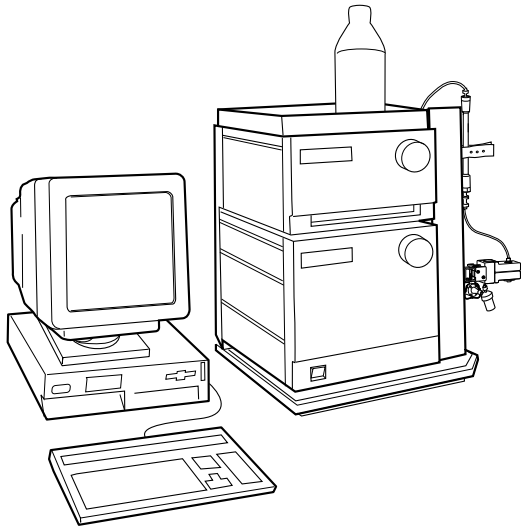
2 The system and the software

2.1 General

ÄKTA*FPLC* is a fully automated liquid chromatography system designed for method development and research applications. The system has two main instrument components stacked on the base platform.They are:

- Pump P-920, a high performance laboratory pump for constant flow delivery.
- Monitor UV-900, a high precision on-line combined monitor for measuring UV absorption, conductivity and pH (optional).

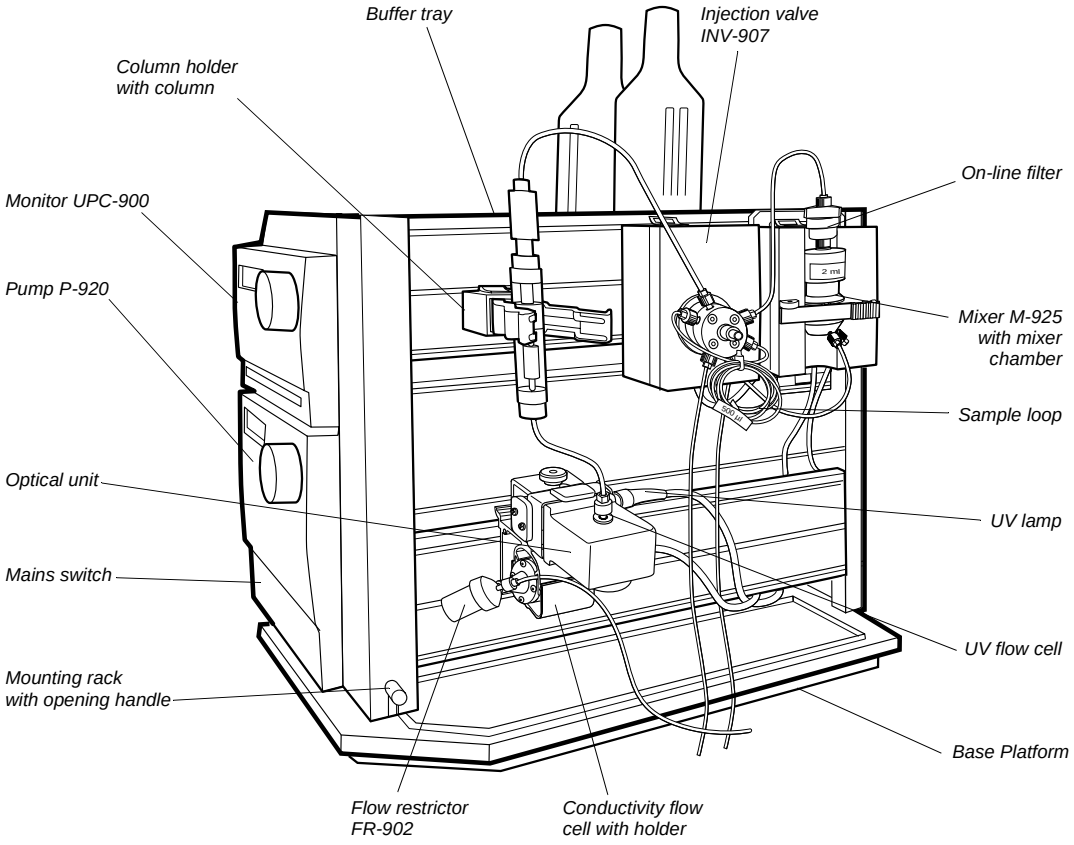
If installing a fraction collector, it should be placed on the right-hand side of the system.



Components, such as the mixer, column and different valves, are mounted on a system rack on the right side of the separation unit.

Pump P-900, Monitor UV-900 and Monitor pH/C-900 can also be controlled individually from the modules, without UNICORN software. In this guide, however, you will only learn how to operate the chromatography system from UNICORN.

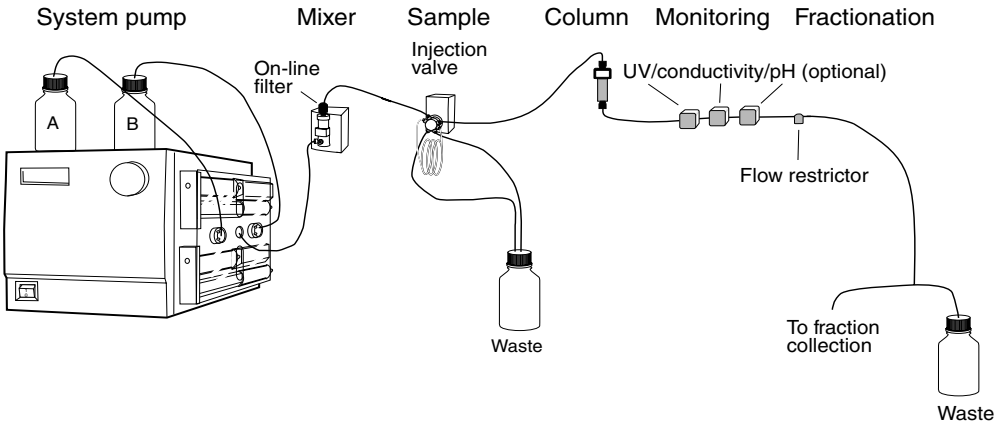
Switch on the chromatography system with the ON/OFF button located on the front of the base platform to the bottom left.



The system is controlled from UNICORN software.

Comment:

The flow path between the different modules and components in the separation unit is shown and described below. It is not necessary to go through this in detail to make your first runs. Look at the right-hand side of the system if you want to follow the description.



1 The pump has 4 pump cylinders, two for pump A and two for pump B. Pump A is the upper pair of cylinders and the pump valve closest to the front.

2 Pump inlets A and B are placed in buffer A and B respectively and the buffer solutions are pumped to a mixer.

3 The flow path continues from the mixer via an on-line filter to the injection valve.

4 A sample loop is connected to the injection valve. The sample loop is filled manually using a syringe. To perform this, connect a fill port to the injection valve.

5 From the injection valve, the flow is directed to the column, and then to the UV flow cell in the optical unit and the conductivity flow cell located below the optical unit.

Note: *In the standard configuration, the pH flow cell is not included. In optional configurations, when the pH flow cell is mounted in the flow path, it is connected between the conductivity flow cell and the flow restrictor.*

6 The flow path continues to the fraction collection or waste.

2.2 UNICORN overview

1 Switch on the computer. Log on to Windows by first pressing **Ctrl-Alt-Del** and then clicking **OK**. After a while the Windows desktop appears.

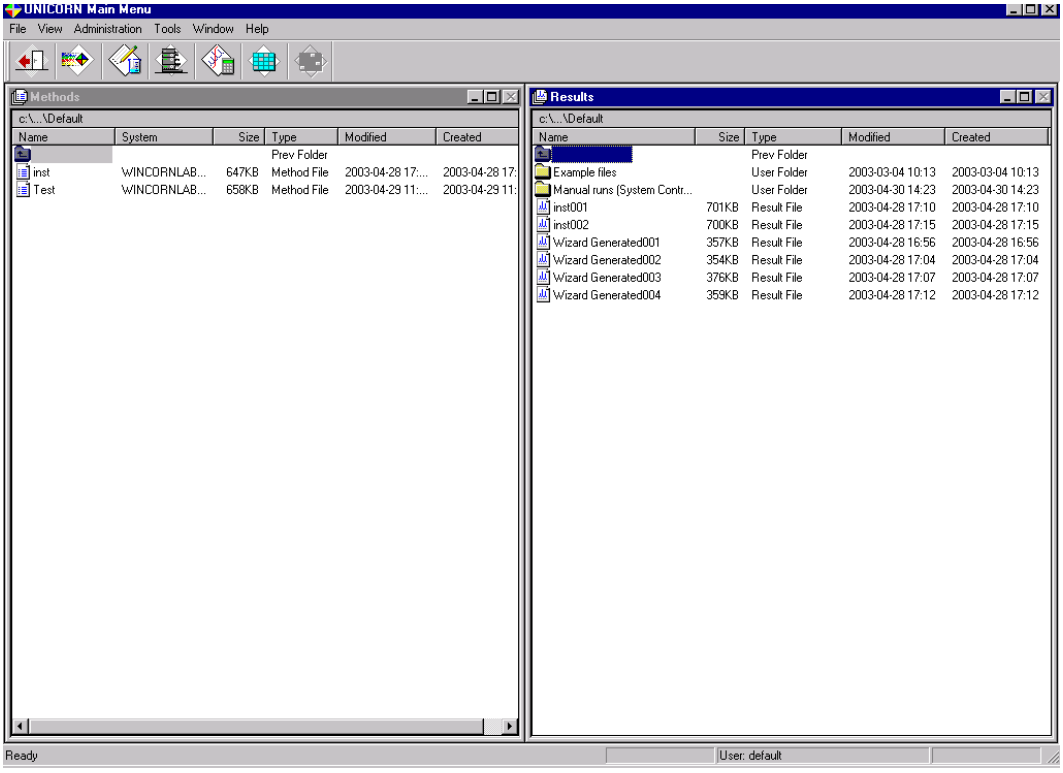
2 Start UNICORN by double-clicking on the UNICORN icon.

3 An information window appears during start-up.

4 In the **Logon** dialog, select a user from the **Users** list and enter the password. If you log in for the very first time, select user **default** and enter the password **default**. Click **OK**.

Note: *You should enter users and individual passwords before starting using ÄKTA*FPLC *on a regular basis.*

5 The four UNICORN modules open, and their respective short-cut button is placed in the task bar at the bottom of the screen. The UNICORN **Main Menu** window appears on the screen.



6 The **Main Menu** window is the central part of the UNICORN displays. It is mainly used for file handling. From this window you can navigate through the control system.
In the **Methods** pane to the left in **Main Menu**, all method files that you create are displayed. A method file contains a series of instructions for controlling a run.
In the **Results** pane to the right, all result files are displayed. A result file is the result from a run, including all documentation (e.g. the method used) and the generated chromatogram.

In general, UNICORN consists of 4 different modules of which the **Main Menu** is one. The other modules are represented by icons in the toolbar. These modules are:

- Method Editor — opens the Method editor with a dialog window for creating new methods.

- System control — opens a dialog window for controlling the system and running your methods.

- Evaluation — opens a dialog window for evaluating your results.

To swap between the module windows, click their respective button in the task bar at the bottom of the screen.

Additional buttons are provided in the toolbar. These are:

- Instant run — opens a dialog where you directly can choose a method to run. This is handy for starting routine runs instantly.

- Logon/Logoff — opens a dialog to control the log-on/ log-off process.

- Method Queue* — opens a dialog window for defining a new Method Queue.

- Existing Method Queue — opens a dialog window for showing the Method Queue that is running.

* Method Queues are used to link several methods together.

2.3 Help

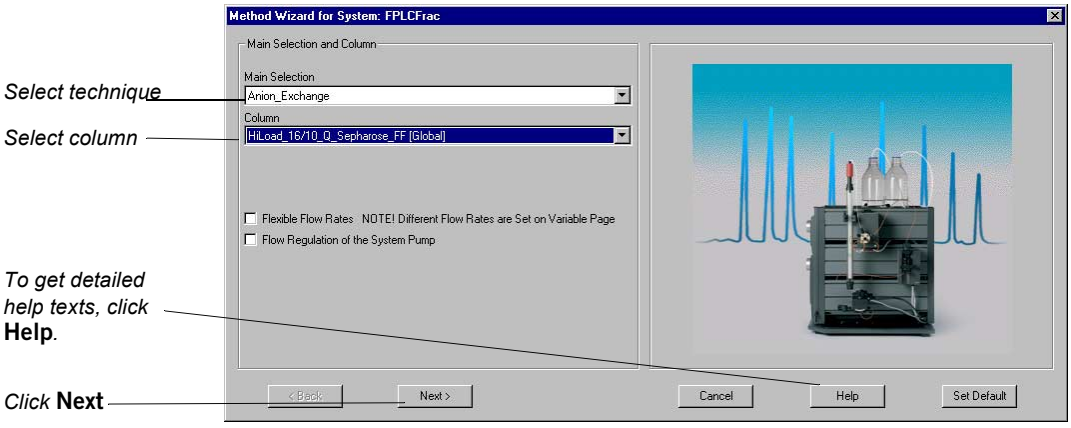
Comprehensive on-line help is available. To get help about an instruction or module, place the cursor on the instruction/module and press the **F1** key. Alternatively, click on the **Help** menu in the upper right corner of each module and select **Help for.....** to get general help about the current instruction or module and find new help topics, or **Index** for a specific topic. In any dialog, click on the **Help** button to get help on how to use the current active dialog.

3 Creating a method

The UNICORN software is supplied with a *Method Wizard* used for creating new methods. The wizard is a number of dialog windows with questions and instructions that help you creating the method.

To create a method:

1 Click the **Method Wizard** icon  in the **Method Editor** module. If required, choose which system you want to use and click **OK**. The **Method Wizard** window appears.



Note: *You can restore all settings to default values by clicking* **Set Default** *(only possible in this dialog).*

2 Select a chromatographic technique, for example **Anion_Exchange.**

3 Select the column you intend to use. The correct column volume, the recommended flow rate, and the correct pressure limit for that column will then be automatically implemented in the method.

Comment:
If you manually alter the default values, and thereby exceed the recommended values for the selected column, you will get a warning when you save your method.

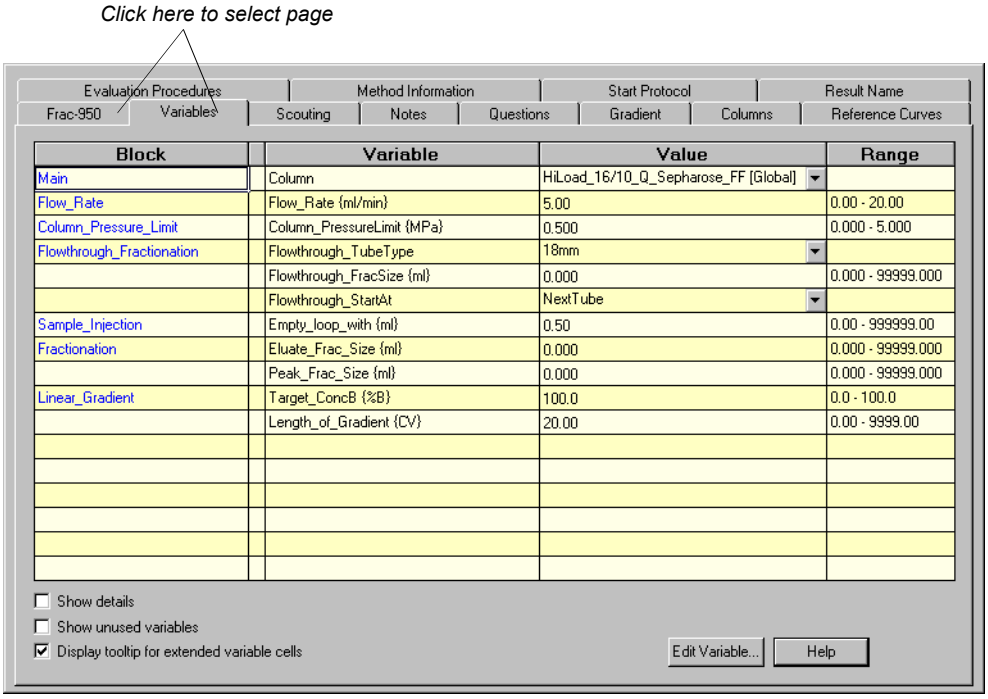
If you want to perform a test run without a column, you should still select a column (a small one is recommended) to get suitable default parameters in the method. Then, when running the method, use a piece of tubing to replace the column.

Comment:
If you do not find your column in the list, you can add one. Refer to the UNICORN User Manual.

4 If required, select **Flexible Flow rates** and/or **Flow Regulation of the System Pump**.

5 Click **Next** to go through the subsequent windows. In each window, select the appropriate parameter values.

6 Click **Finish** in the last window. The **Run Setup** window appears.



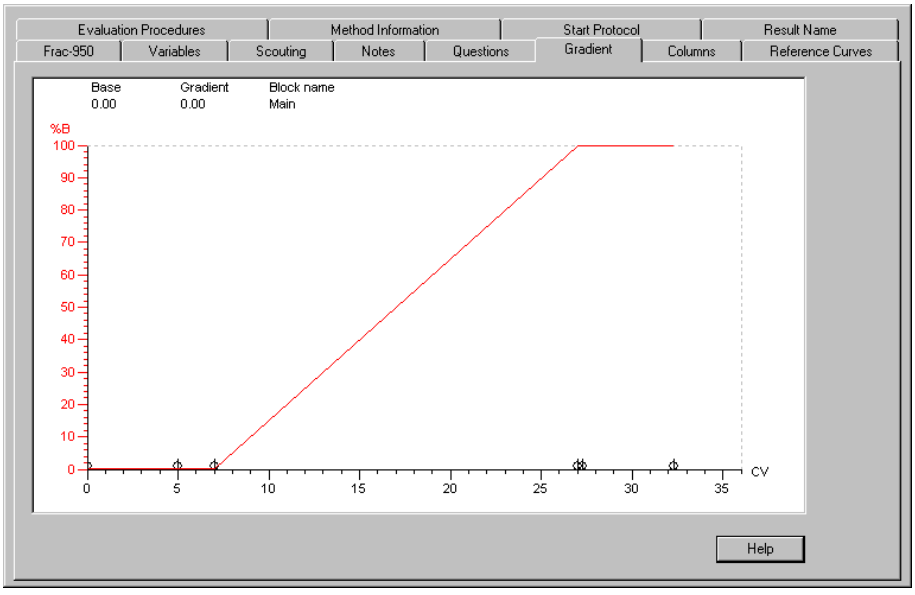
Run setup consists of a number of pages. You will only look at a few now. Select a page by clicking the respective tab at the top of the window.

7 On the **Variables** page, the method is presented by a number of blocks. The blocks represent typical steps in a chromatographic run, such as:

- Start instructions
- Column equilibration
- Sample injection
- Wash out unbound sample
- Fractionation
- Gradient
- Clean after elution
- Re-equilibration

Each block contains a number of **Variables** with suitable default values. The values can be changed to suit your application. Some of the variables are normally hidden but can be shown by checking the **Show details** box.

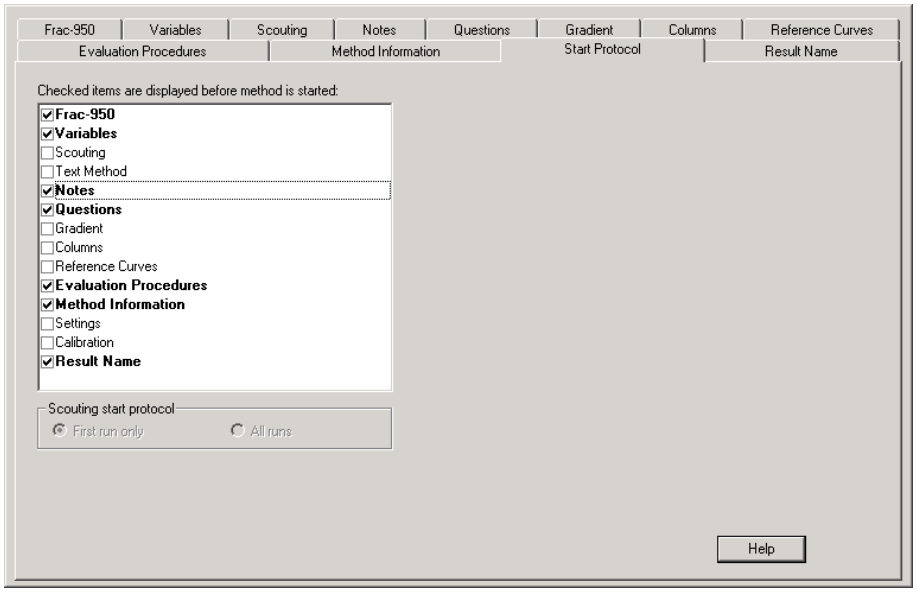
8 Click the **Gradient** tab to view the method graphically.



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

Click the x-axis to view the method in time, volume or column volumes.

9 Click the **Start Protocol** tab to decide which of the **Run Setup** pages to be displayed at the start of a method run.



10 To save the method, select **File:Save.** In the **Save** dialog, enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click **OK**. In the **UNICORN Main Menu** module, the method appears in the **Methods** window.

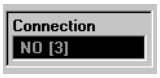
Comment:
The method name, followed by three consecutive numbers starting with 001 will then be used as default name for the result file of your method after runs.

Now you are ready to start a run. Go to chapters 4 and 5.

You can also go to chapter 8 to learn how to alter variables systematically and automatically in repeated runs. This is know as scouting and is a convenient, easy-to-use function.

4 Preparing the system for a run

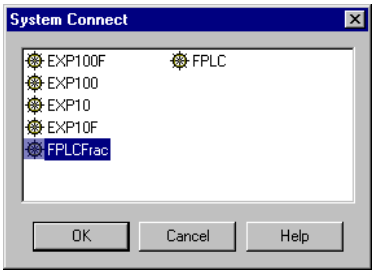
4.1 System connection



Before you can start a run, you must connect to the system. Connecting means that the **System Control** module is set up for a particular system. If you are not connected, the text **NO** is shown in the **Connection** panel in the **Run Data** window. Once you are connected, the text changes to **YES**.

1 Click on the **1.System Control** button in the task bar at the bottom of the monitor

2 To connect to a system: Select **System:Connect...**. The **System connect** dialog appears.



3 Select a system symbol. If you are not connected to a network, only one system will be shown. Click **OK**.

When connected, the text **YES** is shown in the **Connection** panel in the **Run Data** pane. You only have to connect once. If you do not select **System:Disconnect,** you will be automatically connected to the system the next time you log on to UNICORN.

4.2 General system preparation

1 Immerse inlet tubing A in buffer A and inlet tubing B in buffer B.

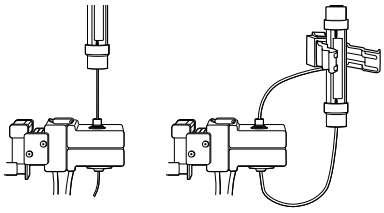
2 Check that the tubing marked G5 from the FR-902 Flow restrictor outlet is connected correctly. Check that the three waste tubings are put into waste bottles.

3 If there is air in the inlet tubing, or if you suspect air in the pump, purge the pump as described in Pump P-920 User Manual.

4 Calibrate the pH monitor (optional) if required. Refer to the UNICORN User Manual or the Monitor UPC-900 User Manual.

5 Connect the column between port 1 of the injection valve and the UV flow cell. Use a suitable length of 0.50 mm PEEK tubing (orange) supplied with your system.

6 Insert a sufficient number of tubes into the fraction collector.



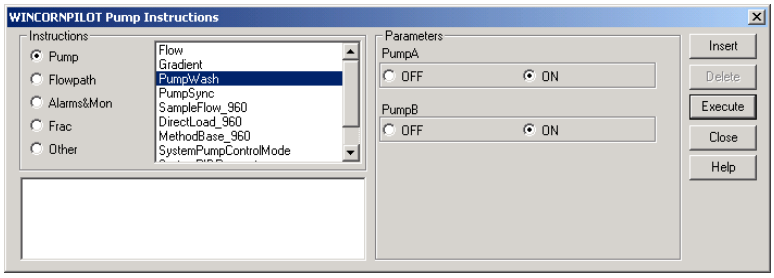
5 mm flow cell Connect from above

2 mm flow cell Connect from below

4.2.1 Filling the inlet tubing

1 Select **Manual:Pump** in the **System Control** module.

2 Select the instruction **PumpWash**. The **Pump Instructions** dialog opens.



3 Select **ON** for PumpA.

4 Select **ON** for PumpB.

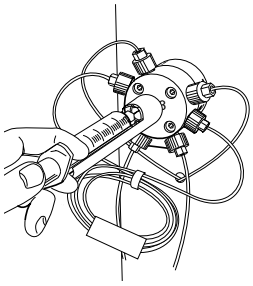
5 Click **Execute** to start filling the tubing. The injection valve will automatically switch to waste during the pump wash.

6 When finished, click **End** in the **System Control** toolbar.

7 In the **Pump Instructions** dialog, click **Close** to close the dialog.

4.2.2 Filling the Sample loop

1 Check that the correct loop is mounted between port 2 and 6 on the injection valve.



2 Connect an injection fill port or a union luer female/1/16” male to port 3 on the injection valve and apply the sample manually with a syringe.

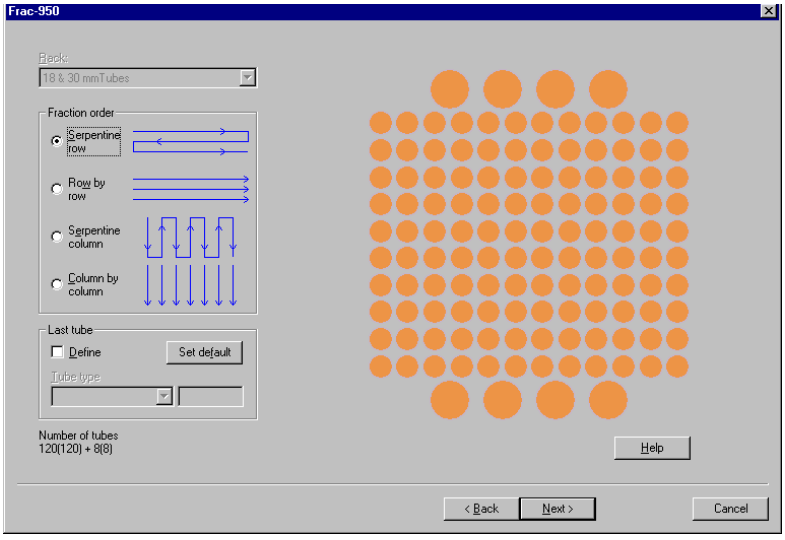
5 Starting a run

1 Open the **System Control** module.

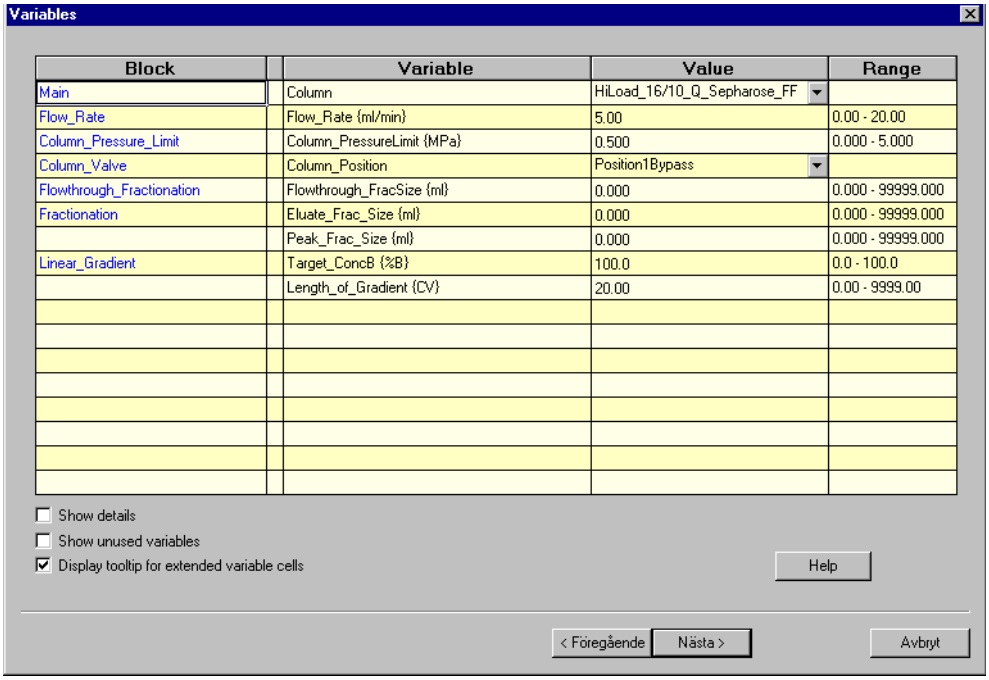
2 Select **File:Run...** Select the method to start. Click **OK** (the method will not start yet).

A Start protocol appears consisting of a number of **Run Setup** pages. The pages that are displayed depending on your selections in the Method Editor.

3 If using a Frac-950, the **Frac-950** page appears. In the **Frac-950** page you set up the Frac-950 fraction collector. Define the order of fractionation and if desired, set up the last tube used in the fractionation. The system will be paused when the last tube is reached and the fractionation will stop.



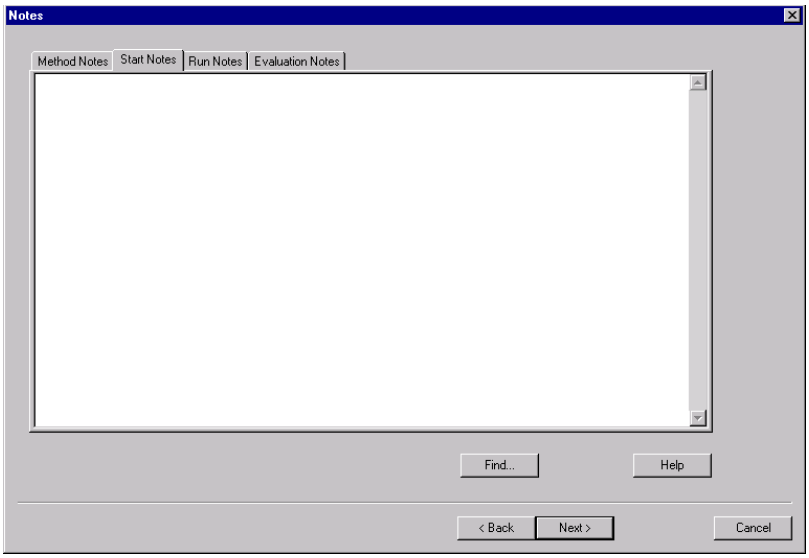
4 Click **Next.** For example, the next page can be **Variables.** This is the same page you were working on in the Method Editor. Here you can verify and fine tune the method before proceeding. This is very convenient when repeating runs with minor adjustments.



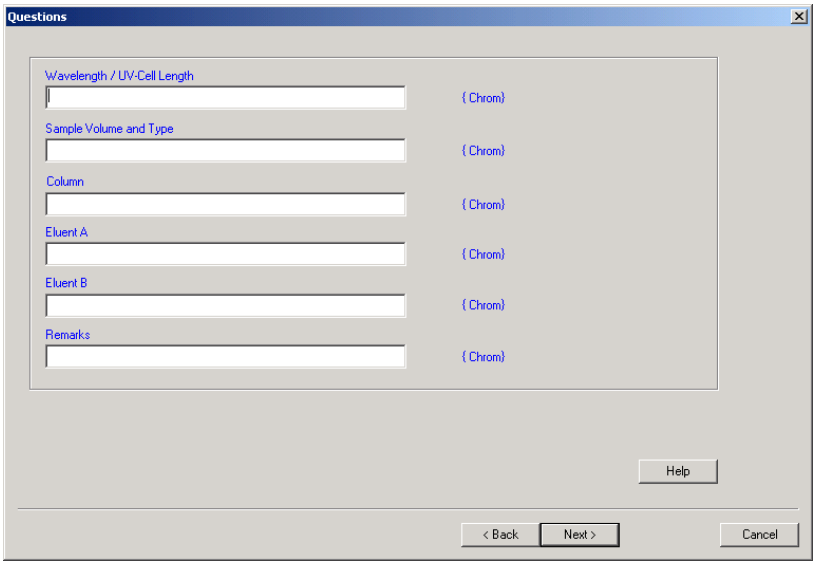
Note: *When starting run no. 2 immediately after run no. 1 with the same method but, for example, a different flow rate, you simply:*
Click the Run button in System Control.
Change the flow rate on the Variables page.
Continue through the start protocol by clicking Next and then start the run.
You do not need to change the method in the Method Editor.

Go through the **Variables** page to check that the method is OK (this is not necessary if this was done in the Method Editor).

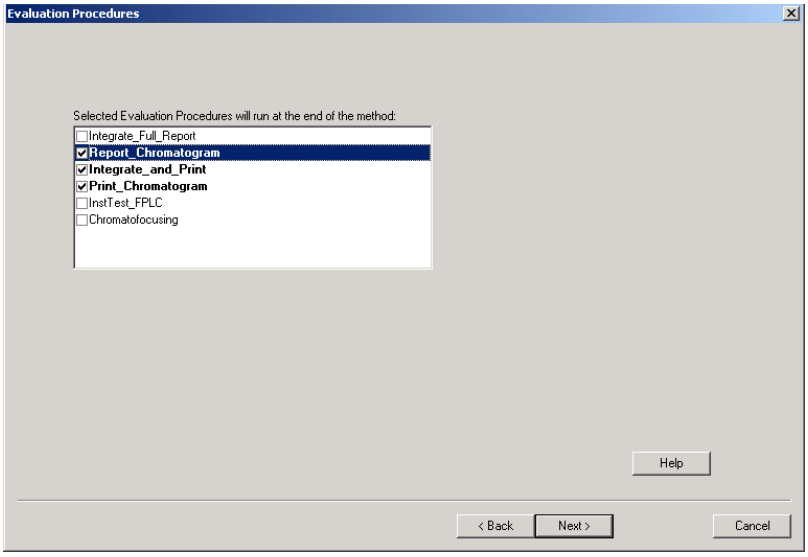
5 Click **Next.** For example, the **Notes** page appears. You can write your own comments in the **Starts Note** tab.



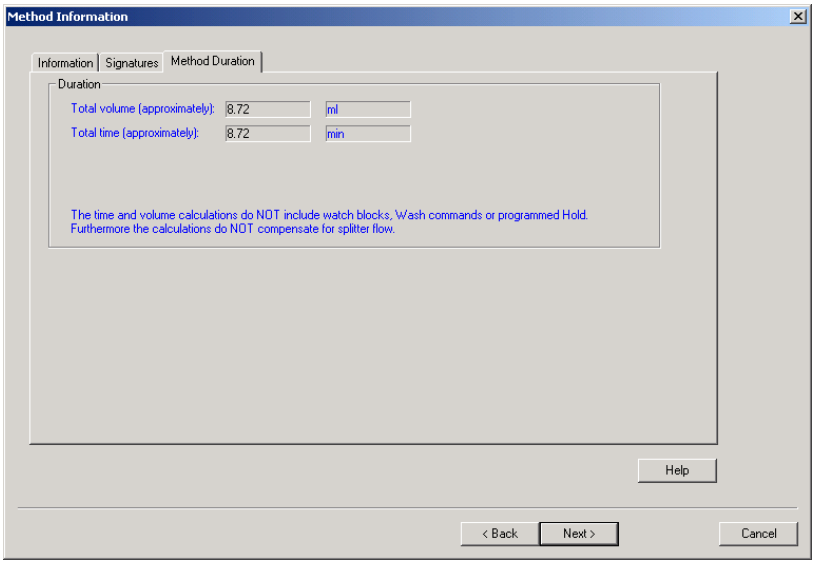
6 Click **Next.** For example, the **Questions** page appears. Type the answers on the questions. The answers will be saved in the result file.



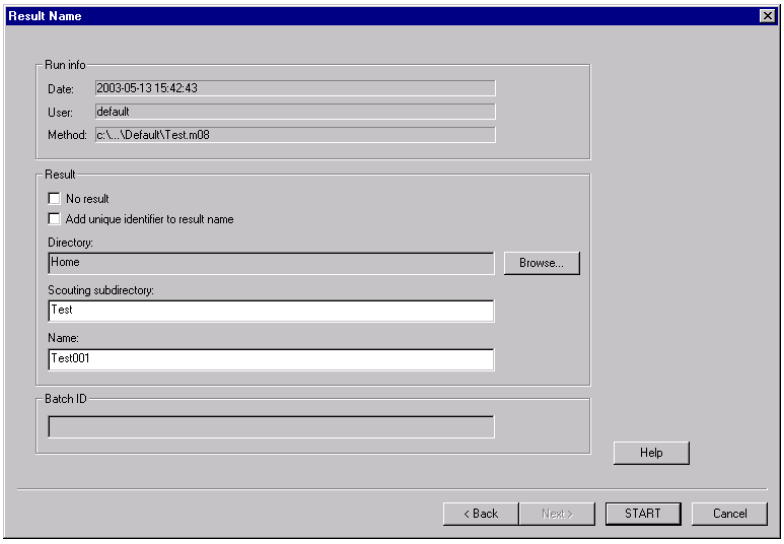
7 Click **Next.** For example, the **Evaluation Procedures** page appears. Evaluation procedures are automated evaluation operations that are performed after the run. For instance, select **Print_Chromatogram** and the chromatogram will automatically be printed after the run.



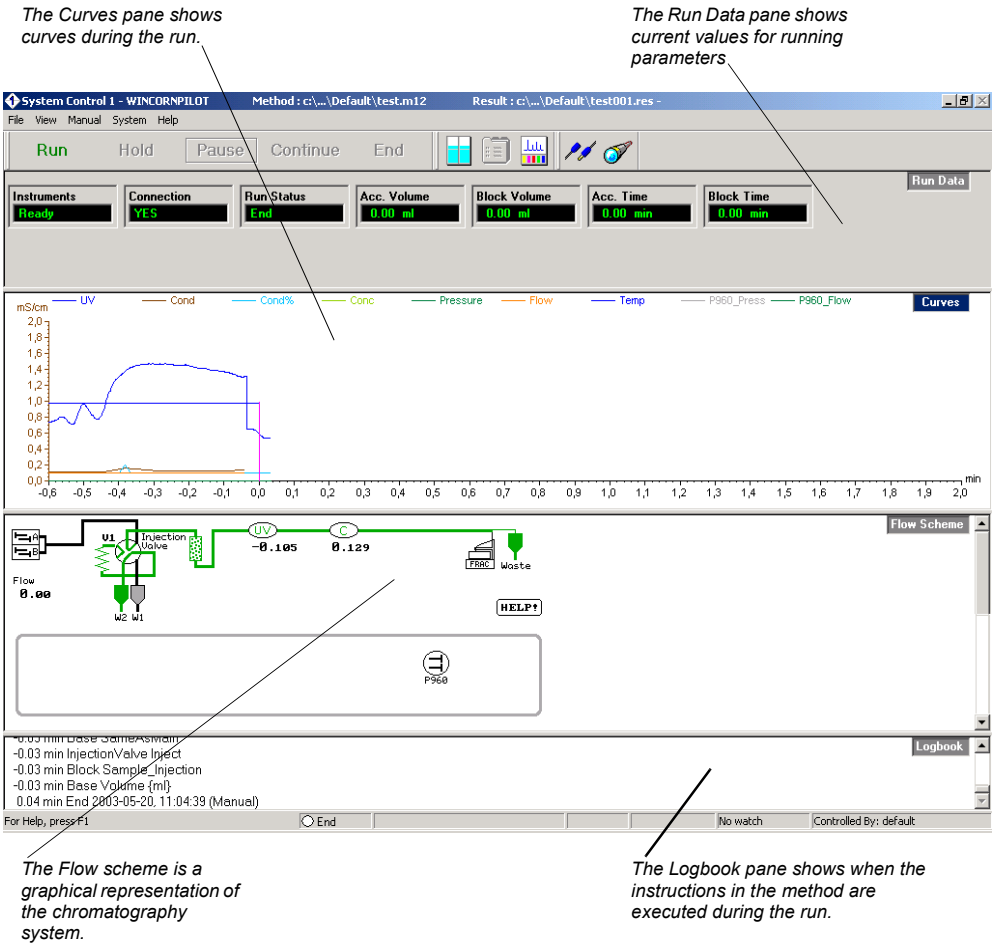
8 Click **Next.** For example, the **Method Information** page appears. Here you see information about the run. Under the **Method Duration** tab the approximate volume of buffer used (A+B) is shown as well as how long time the method will take.



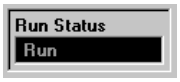
9 Click **Next**. The **Result Name** page appears. Name the result file and define in which directory the result should be stored. A default name (the method name followed by 001) and a directory are suggested.To change the result name and directory, click **Browse**.



10 Click **START.** The run starts.You will view the run in the **System Control** module.



6 Viewing a run

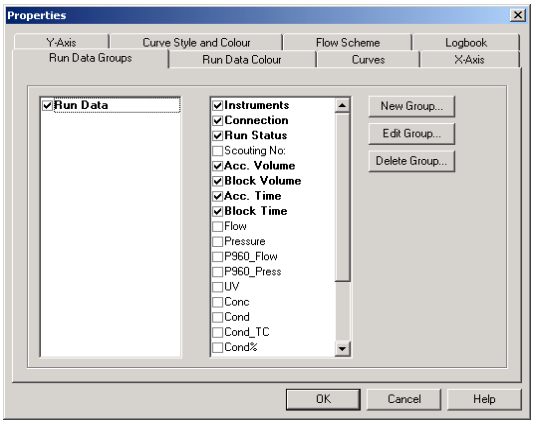


When the system pump is running, the text **Run** is shown in the **Run Status** panel in the **Run Data** pane.

1 To choose which panes to display, select **View:Windows**. In the **Customise panes** dialog, select, for example, **Rundata**, **Curves** and **Logbook**. Click **OK**.

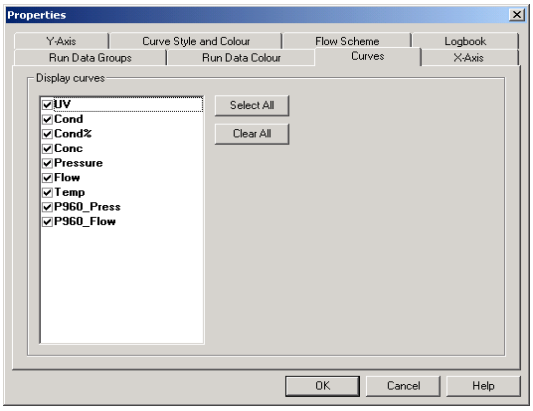
2 To customize the pane's display after your own needs, you can choose parameters in the **Properties** dialog. In the respective pane, select the right-click command **Properties** and click the requested tab.

3 The **Run Data** pane at the top shows current values for running parameters. Under the **Run Data Groups** tab, select the parameters you want to display and click **OK**.



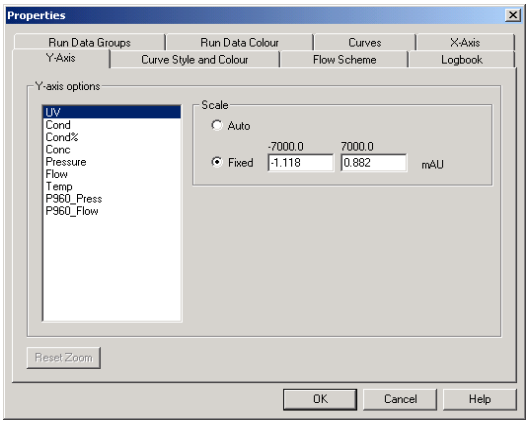
4 The **Curves** window shows the curves during the run. All curves are stored in the result file.

Under the **Curves** tab, select which curves to show during the run. Click **OK**.



5 Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.

For example, to fix the Y-axis scale for a curve, click the **Y-axis** tab. Mark the curve, click **Fixed**, and enter the max. and min. values. You can repeat this for other curves. Click **OK**.

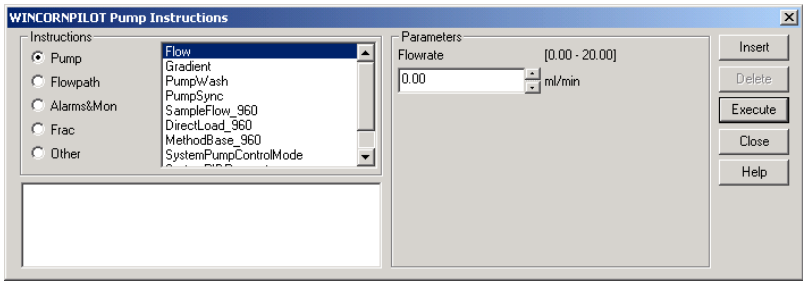


6 To maximize the **Curves** pane, right-click in the **Curve Data** pane and select **Maximise**. Go back to normal size by clicking **Restore**.

7 To shift to a scale for another curve, click on the Y-axis scale, or click on the curve name at the top of the **Curves** pane. The color of a curve, its Y-scale, and its name are always the same. Click the **X-axis** to shift between time and volume.

8 The **Logbook** is shown at the bottom. The **Logbook** shows exactly when the instructions in the method are executed during the run. The **Logbook** is stored in the result file.

9 You can make manual changes during the run. Select **Manual:Pump**. The **Pump Instructions** dialog opens.



If, for example, you want to change the flow rate, select **Pump** and then **Flow**. Enter a new flow rate under **Parameters** and click **Execute**. The new flow rate will be used until the end of the run or until a new flow rate instruction is reached in the method.

Close the box by clicking **Close**. All manual interactions are recorded in the **Logbook**.

10 If you want to stop the run before it is finished, click the **End** button at the top.

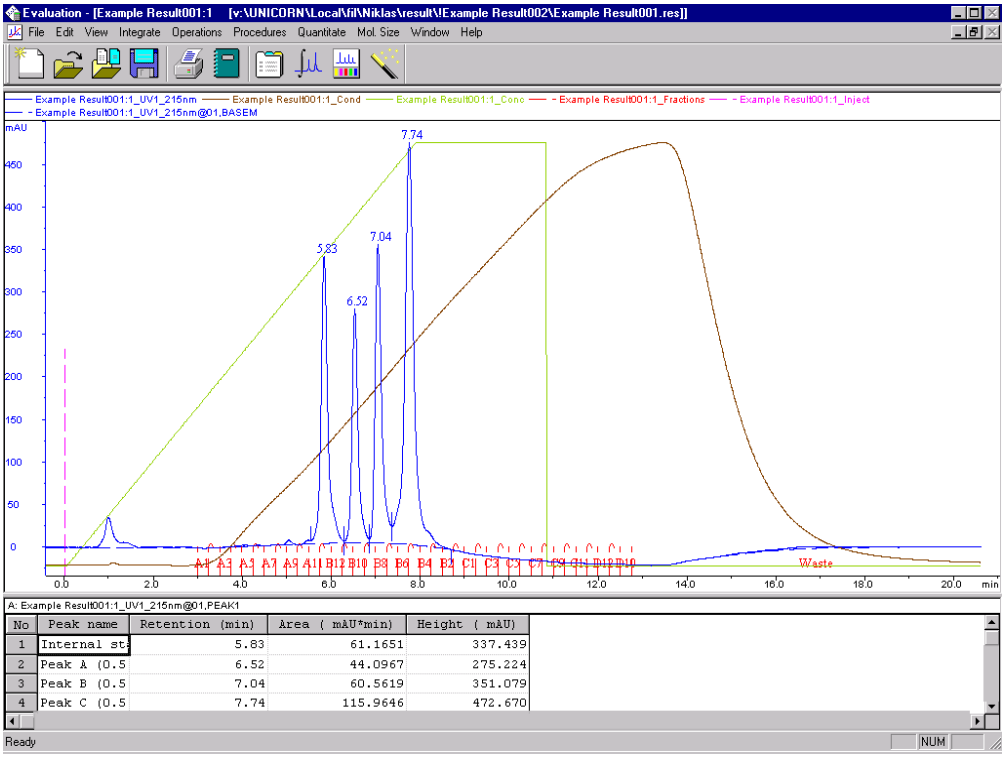
7 Viewing and printing the result

If you are satisfied with the automated print-out obtained after the run (if selected), you do not need to alter anything described in this section. However, if you want to alter the chromatogram layout, this section will teach you the basics of the evaluation module.

7.1 Viewing

1 After a run you can view the result. Open the UNICORN **Main Menu**. Double-click on a result file icon in the list to the right.

2 The **Chromatogram** window is opened automatically in the **Evaluation** module when you open a result file. The **Chromatogram** window contains all the curves. Note that the term chromatogram is used here when talking about the whole window containing all the different curves.



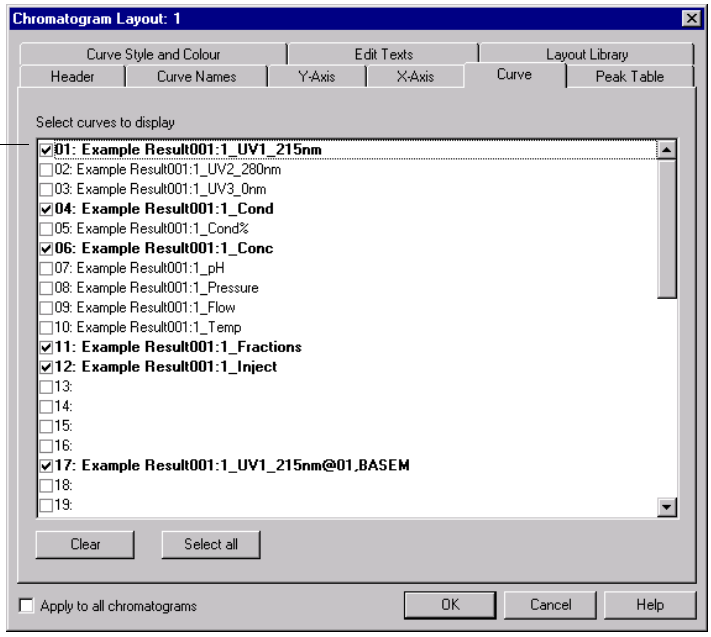
The result file from a run contains a complete record of the run, including method, system settings, curve data and run log.

Note: *Original raw data curves can never be modified, renamed, or deleted from a result file.*

3 Maximize the **Chromatogram** window by clicking on the larger square in the upper right corner.

4 All changes regarding the presentation of the curves are done in the **Chromatogram Layout** dialog. Right-click in the **Chromatogram** window and select **Properties...**, or select **Edit: Chromatogram layout...** to activate this dialog.

Highlight curves to view



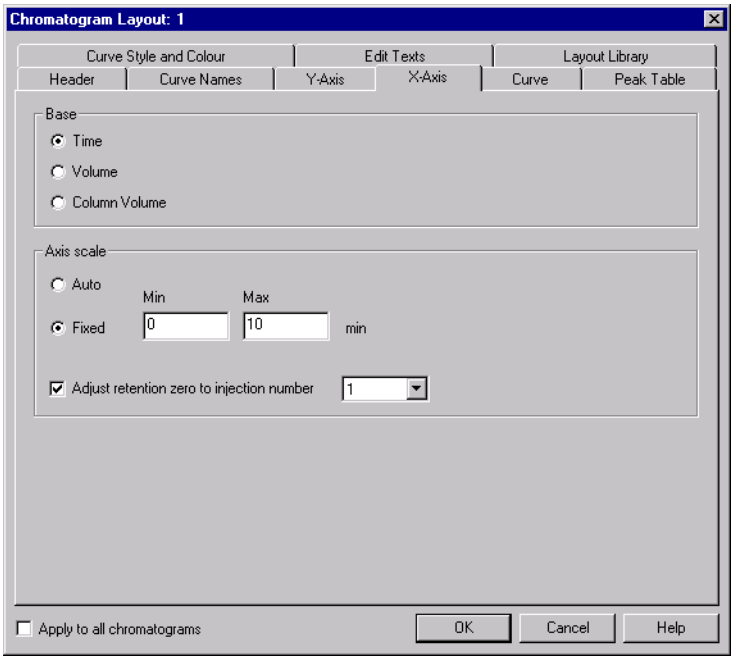
5 Highlight the curves to view under **Curve**. Curves are named as **Resultfile001:1_"curve"** where a curve can be, for example, UV_wavelength, Cond, pressure...etc. Clear all curves except, for example, the UV, Cond and Conc curves. Click **OK** at the bottom of the **Chromatogram Layout** dialog.

6 To zoom in a peak of interest, left click-and-drag to create a rectangle. When you release the mouse button, the part within the rectangle will be enlarged. You can zoom further on the enlarged part. Click on the right mouse button and select **Undo** or **Reset zoom** to return to the complete chromatogram.

7 Click on the Y-axis scale to change to a scale for another curve. The style and colour of a curve, its Y-scale and its X-scale can all be changed.

8 Open the **Chromatogram Layout** dialog again. Click the **Y-axis** and **X-axis** tabs to set the scale for the different curves. Normally, the curves are scaled with auto scaling, i.e. the highest and lowest values for each curve set the scale.

- To fix the Y-axis scale, mark a curve, click **Fixed**, and enter the **Min** and **Max** values for that curve. You can repeat this for other curves.
- To fix the X-axis scale, click **Fixed** in the X-axis field, and enter the **Min** and **Max** values for the X-axis.

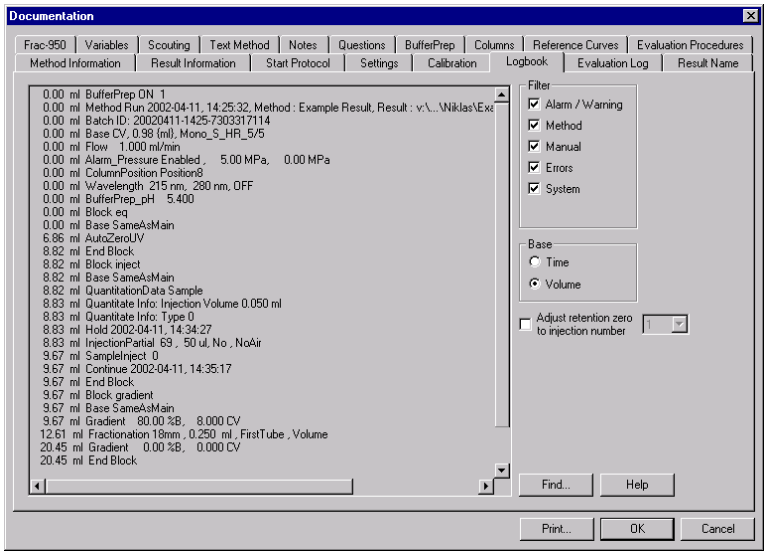


9 To save changes in the chromatogram layout, click the **Layout Library** tab. Click **Save Current layout as...**. In the **Save layout** dialog, enter a name of the layout and click **OK**.

Note: *The saved layout settings can be applied to any result file.*

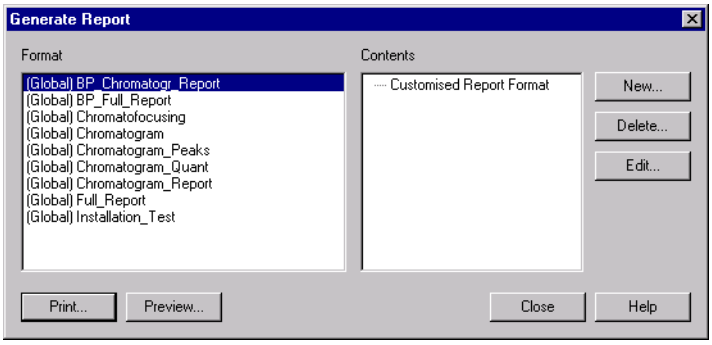
10 Click **OK** at the bottom of the **Chromatogram Layout** dialog to execute all the changes.

11 Click the **View Documentation** button. A number of pages appear as in the Run Setup in the Method Editor. All documentation about the run is stored here, e.g. the method, answers to questions, variables, logbook...etc. For example, click the **Notes** and **Logbook** tabs to check the contents. Close the **Documentation** window by clicking on the **X** in the upper right corner.



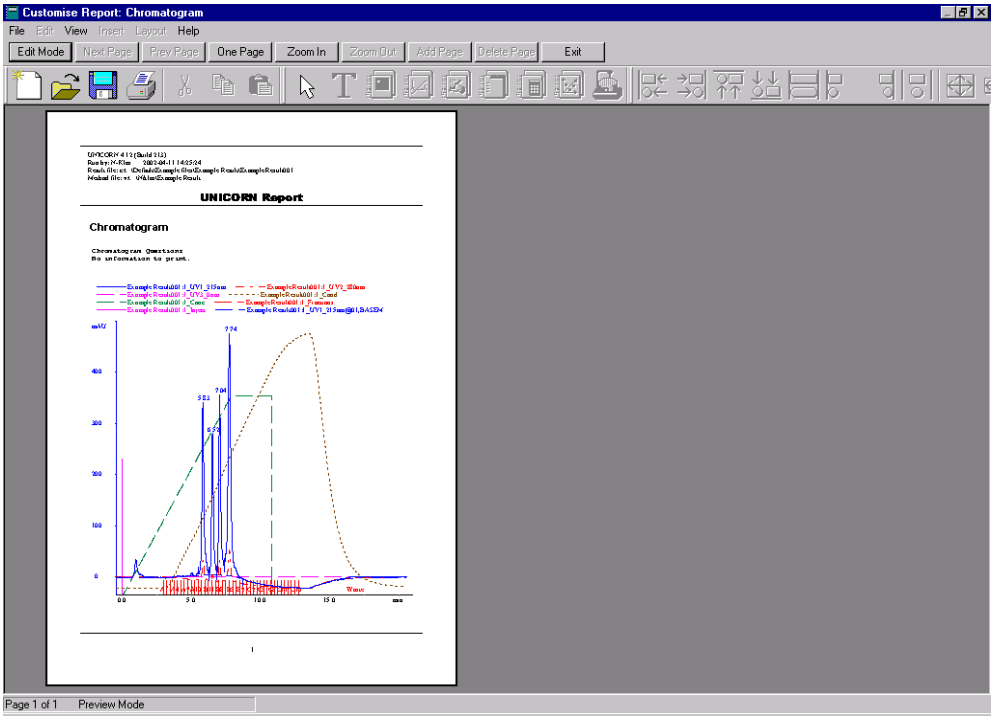
7.2 Printing and making a report

1 To print the chromatogram, select **File:Report**. The **Generate Report** dialog opens.



2 Select, for example, format **(Global) BP Chromatogram.** This will create a report containing the chromatogram and the questions on one page.

3 Click **Preview** to view the report on the screen.



Add information to the report

1 Click **Edit Mode** to enable changes in the report.

2 To add an empty page to the report, click **Add Page**.

3 Select from the **Insert** menu, the item to include. Items available are:

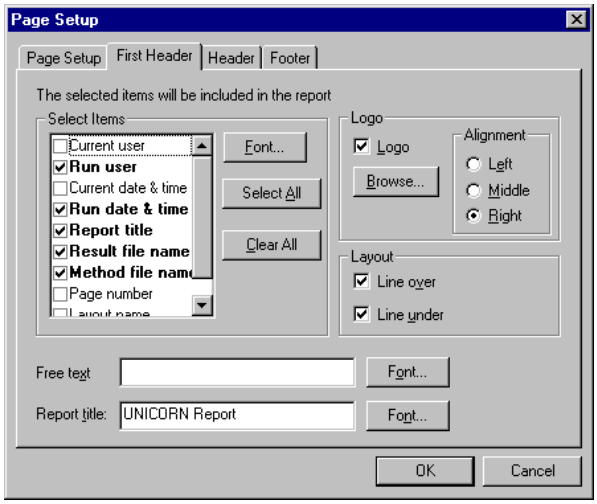
- Free text
- Picture
- Text method
- Chromatogram
- Documentation
- Evaluation log

4 Move the mouse pointer into the page area of the window. You will notice that the mouse pointer has an additional symbol according to the item type you selected to insert.

5 Click-and-drag to create a box of the desired size. Release the mouse button. A dialog is displayed specific to the type of item inserted. Make the appropriate selections in the dialog and then click **OK** to view the inserted item.

Change page layout

1 If you want to change the page layout, select **Edit:Page Setup**. The **Page Setup** dialog opens and you can e. g. select page size and items to be included in the header and in the footer. The information selected here will be printed in the report. Click **OK**.



2 To print the report, click **Print**.

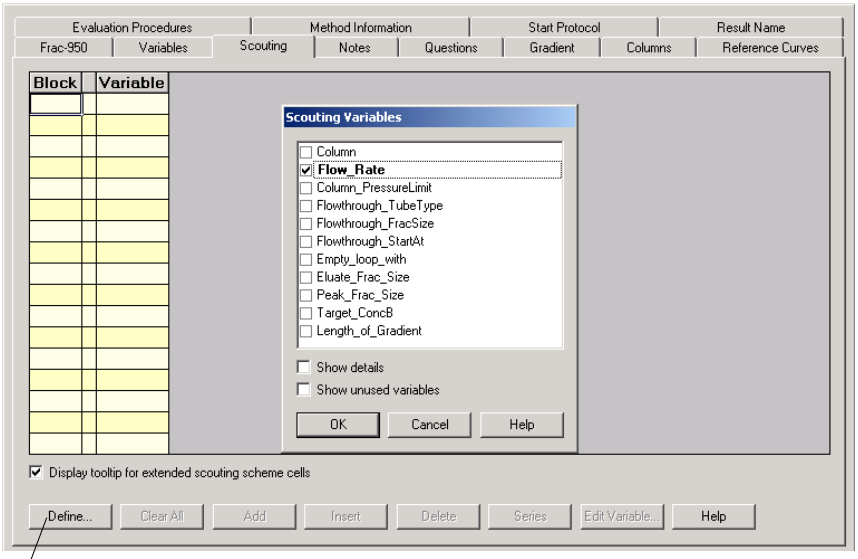
8 Scouting

Scouting allows any run parameters, e.g. flow rate, to be systematically varied automatically, in repeated runs.

Below is a description of how to perform a flow rate scouting.

1 Create a new method as described in chapter *3 Creating a method*.

2 When the **Run Setup** window appears, click the **Scouting** tab.



Define other Scouting variables

3 A list of all the variables will appear. Select the variable **Flow_Rate** and any other variable you wish to alter, e.g. **Peak_Frac_Size**.

4 Click **OK**. The selected scouting variables will appear to the left with their default values inserted.

Note: *Values for variables selected for scouting are greyed on the Variables page and cannot be changed there.*

5 To change a variable value, position the cursor in the Run value field and double-click with the left mouse button. Type the new value.

Block	Variable	Run1
Flow_Rate	Flow_Rate (ml/min)	1.00

6 To add a table column for the next run, click **Add**. A second column appears with the values from the previous run copied. Change the values as required.

If you want to insert a new run column after a specific column in the scouting scheme, position the cursor in the column and click **Insert**. A new column with identical values appears directly after the selected column.

7 Repeat step 6 until you have defined all the runs you require. If necessary, use the horizontal scroll bar to see more runs.

8 Click **Run1**, **Run2**, etc. at the top of the scheme with the right mouse button to toggle between **Run** and **Excluded** for the different runs. Those marked **Excluded** will not be run. A scouting scheme is now defined.

9 To save the scouting method, select **File:Save**.

10 Prepare the system, and start the run as described in chapters 4 and 5.

When the method is started all the runs in the scheme will be performed automatically and the set flow for each run will be prepared automatically. Each run in the scouting scheme will generate a separate result file which are all stored in a special scouting directory.

9 Going further

Once you are used to the system and software you may want to learn more about it and its capabilities. Below is a list of operations and descriptions that you may find of interest, they are cross-referenced to other manuals in the ÄKTA*FPLC* manual package.

To learn about	**Read manual/section**
Protein purification strategies	The Method Handbook
Different sample applications options	ÄKTA*FPLC* System Manual
Different fraction collection options	ÄKTA*FPLC* Optional Configurations User Manual
Columns	ÄKTA*FPLC* System Manual
Calibrating monitors and pumps	UNICORN 4.12 User Manuals
Comparing chromatograms	UNICORN 4.12 User Manuals
Intergrating curves	UNICORN 4.12 User Manuals
Measuring HETP and resolution	UNICORN 4.12 User Manuals
Exporting curves and data to other programs	UNICORN 4.12 User Manuals
Finding information about a certain menu instruction in UNICORN	Click on Help button in the dialogue box that appears, or look in the index in the UNICORN 4.12 User Manuals
Controlling Pump P-900 and Monitor UPC-900 from the dials on the instruments themselves	ÄKTA*FPLC* System Manual to unlock the dials. Chapter 3 in the User Manual for each instrument, found in the binder ÄKTAdesign Components
Details about each component	See each individual manual in the binder ÄKTAdesign Components

Security features	UNICORN 4.12 User Manuals
Controlling the system from a remote computer	UNICORN 4.12 User Manuals

Index

Short instructions

The following short instructions are intended as a guide for users who are fully familiar with the safety precautions and operating instructions described in this manual. The instructions assume that the unit is installed according to the installation instructions.

1 Select **File:Method Wizard** in the **Method Editor** module or click  .

2 If necessary, select a system and click **OK**.

3 Go through the selections on the Method Wizard pages (click **Next** to go to next page).

4 Click **Finish** on the last page.

5 Select **File:Save** in the **Method Editor** module and give the method a name. Click **OK**.

6 Click the **System Control** button in the task bar 

.

7 Select **File:Run**. Select the method and click **Run**.

8 The start protocol will appear. Check the method on the **Variables** page and change values as you require. Click **Next** a few times.

9 On the **Evaluations procedures** page, select **Print_Chromatogram** to get a print-out automatically after the run.

10 Click the **Start** button on the last page, the run starts.

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