

ÄKTA *design*

Making your first run

Begin here with
ÄKTA*FPLC*

18-1140-80



amersham pharmacia biotech

Important user information



Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!

The Warning sign is used to call attention to the necessity to follow an instruction in detail 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 shall be followed to avoid damage to the product or other equipment. 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 or optimal use of the product.

Should you have any comments on this instruction, we will be pleased to receive them at:

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Trademarks

ÄKTA, FPLC and UNICORN are trademarks of Amersham Pharmacia Biotech Limited or its subsidiaries.

Amersham is a trademark of Nycomed Amersham plc. Pharmacia and Drop Design are trademarks of Pharmacia & Upjohn Inc.

Windows is a trademark of Microsoft Corp.

Terms and Conditions of Sale

All goods and services are subject to the terms and conditions of sale of the company within the Amersham Pharmacia Biotech group which supplies them. A copy of these terms and conditions of sale is available on request.

Addresses

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Amersham Pharmacia Biotech UK Limited
Amersham Place
Little Chalfont
Bucks, HP7 9NA
England

Amersham Pharmacia Biotech Inc.
800 Centennial Avenue
PO Box 1327
Piscataway, NJ 08855
USA

Amersham Pharmacia Biotech Europe GmbH
Postfach 5480
D-79021 Freiburg
Germany

© Amersham Pharmacia Biotech AB 1999
– All rights reserved

Contents

1	About this guide	1
2	The system and the software	2
3	Creating a method	9
4	Preparing the system for a run	16
5	Starting a run	18
6	Viewing a run	22
7	Viewing and printing the result	24
8	Scouting	29
9	Going further	31

Short instructions on back page

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 Microsoft Windows™ NT 4, and includes hardware for interfacing the controlling PC to the separation equipment of ÄKTA FPLC.

This manual describes UNICORN for a system with Frac-950 installed.

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

The system and the software must be installed and functioning and the monitor and pump calibrated as described in the separate Installation guide.

IMPORTANT! Before using ÄKTA FPLC, read all the safety information in section 1.2 in ÄKTA FPLC System Manual.

1.2 Typographical conventions

Menu commands and dialogue 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.

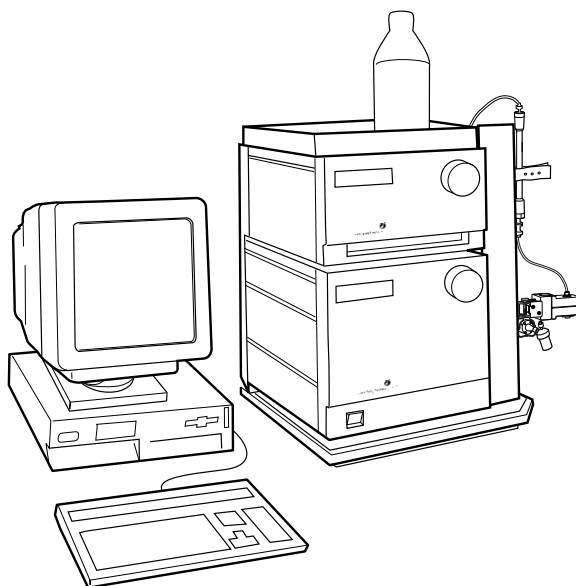
2 The system and the software

2.1 General

ÄKTAFPLC 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 UPC-900, a high precision on-line combined monitor for measuring UV absorption, conductivity and pH (optional).

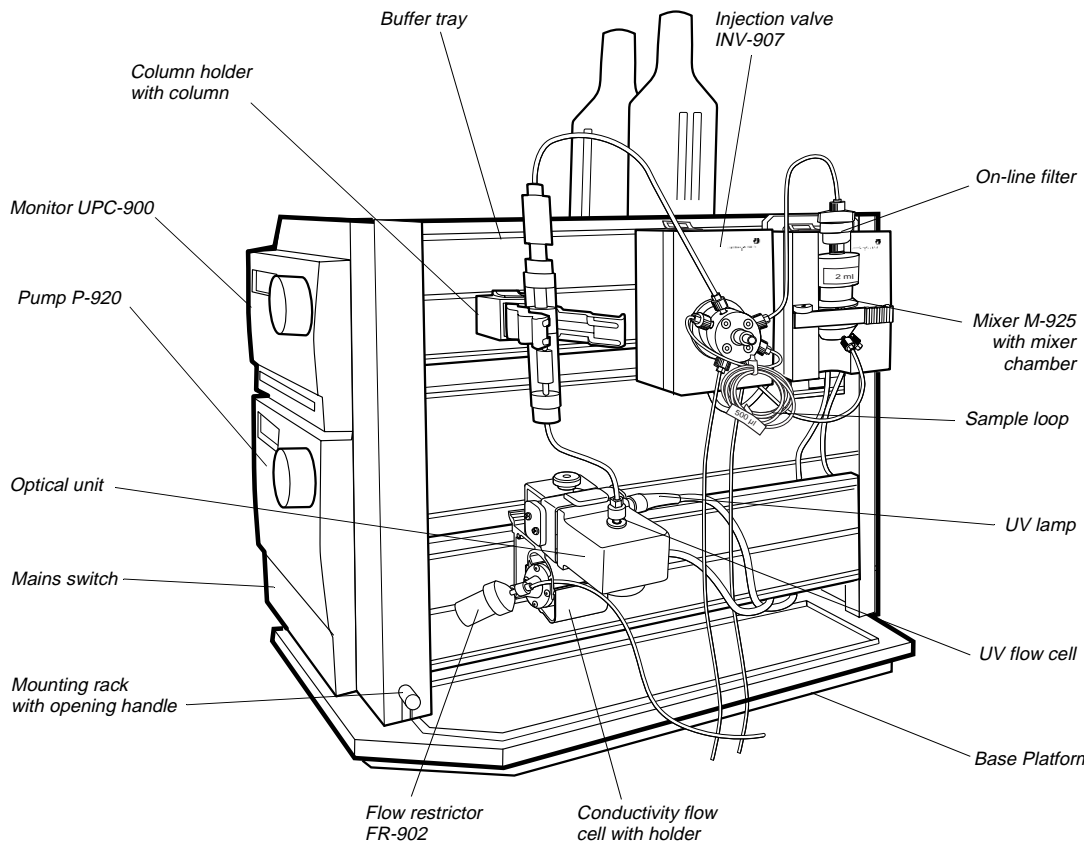
If a fraction collector (optional) should be installed, it should be placed to the right of the system. This manual describes UNICORN with Frac-950 installed.



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-920 and Monitor UPC-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 system to the bottom left of P-920.

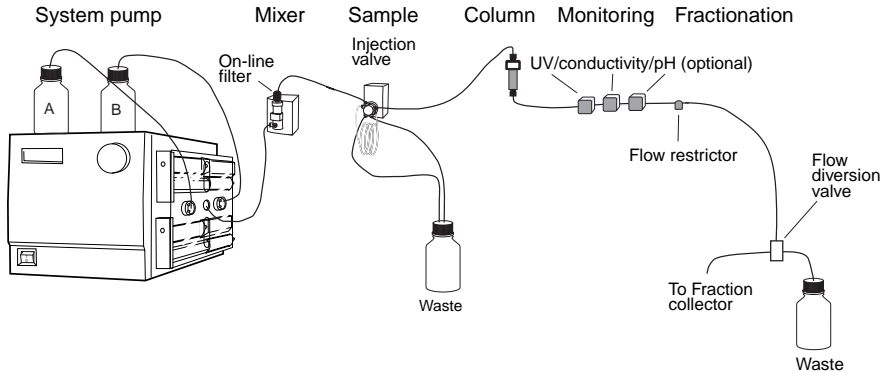


The system is controlled from UNICORN software.

2 The system and the software

Comment:

The flow path between the different modules and components in the system 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.

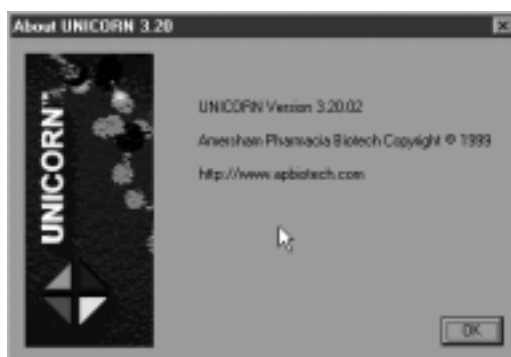
Note: In standard configuration, the pH flow cell is not included. In optional configurations, when the pH flow cell is mounted in the flow path, it is connected between the conductivity flow cell and the Flow restrictor.

6. The flow path continues to the Flow diversion valve. This valve is used to switch the outlet flow between waste and fraction collection.

2.2 UNICORN overview



- 1 Switch on the computer. Log in to Windows NT 4 by first pressing **Ctrl-Alt-Del**, and then clicking on **OK**. After a while the Windows NT 4 desktop appears.
- 2 Start UNICORN by double-clicking on the UNICORN icon.
- 3 An information window appears during start-up.



- 4 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 on **OK**.

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



2 The system and the software

- Eventually, the UNICORN Main menu window appears on the screen. At delivery, a Toolbar guide is displayed providing a quick guide on how to use the toolbar items. The Toolbar guide is inhibited by unchecking the square at the bottom left. Click on **Close**.



- The Main menu window is the central part of UNICORN displays from which you navigate through the control system. It is mainly used for file handling. In the Methods window 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 window 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:



- New method opens a dialogue window for creating new methods.



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



- Evaluation opens a dialogue window for evaluating your results.

A module is opened by clicking on its icon.

Additional buttons are provided in the Toolbar. These are:



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



- Logon/Logoff opens a dialogue window to control the logon/logoff process.



- about UNICORN opens an information window about the UNICORN version and how to contact Amersham Pharmacia Biotech via the world wide web (internet).

2.3 Help and Adviser

Comprehensive on-line help is available. Click on the **Help** menu in the upper right corner of each module and select **H**elp for..... to get general help about the current module and find new help topics, or **H**elp **I**ndex for a specific topic. Double-click on green text to get more help. In any box, click on the **Help** menu to get help on how to use the current active box.


Click on **A**dviser in the menu bar, and choose the appropriate system to get detailed information about chromatographic columns (Media Adviser), and information about ÄKTA_{FPLC} (System Adviser).

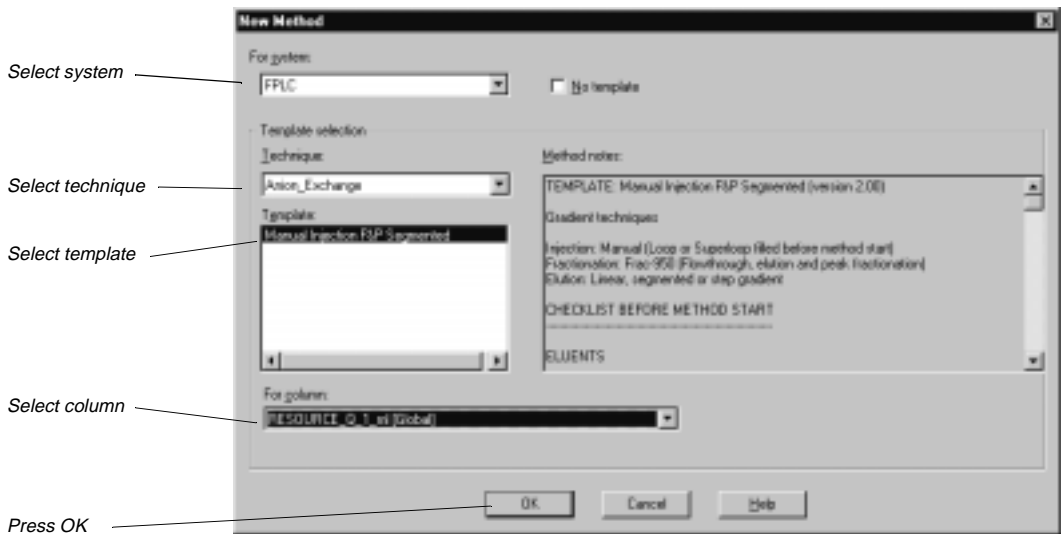


3 Creating a method

UNICORN is supplied with a number of almost ready-made methods called *method templates*. Different method templates are available for different chromatographic techniques. The method templates can be run as they are or you can easily modify them to design your own method in a very short time. Let's start!



- 1 Click on  in the Main menu Toolbar. The New Method window will appear.



- 2 Select a system. Then select a chromatographic technique, for example **Anion_Exchange**.
- 3 A list of available templates will appear. By clicking on a template, an explanation for the template appears to the right in the Method Notes field. Select the template **Manual Injection FP Segmented**, which is suitable for the first run.

Comment:

The other templates are briefly described at the end of this chapter.

- 4 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.

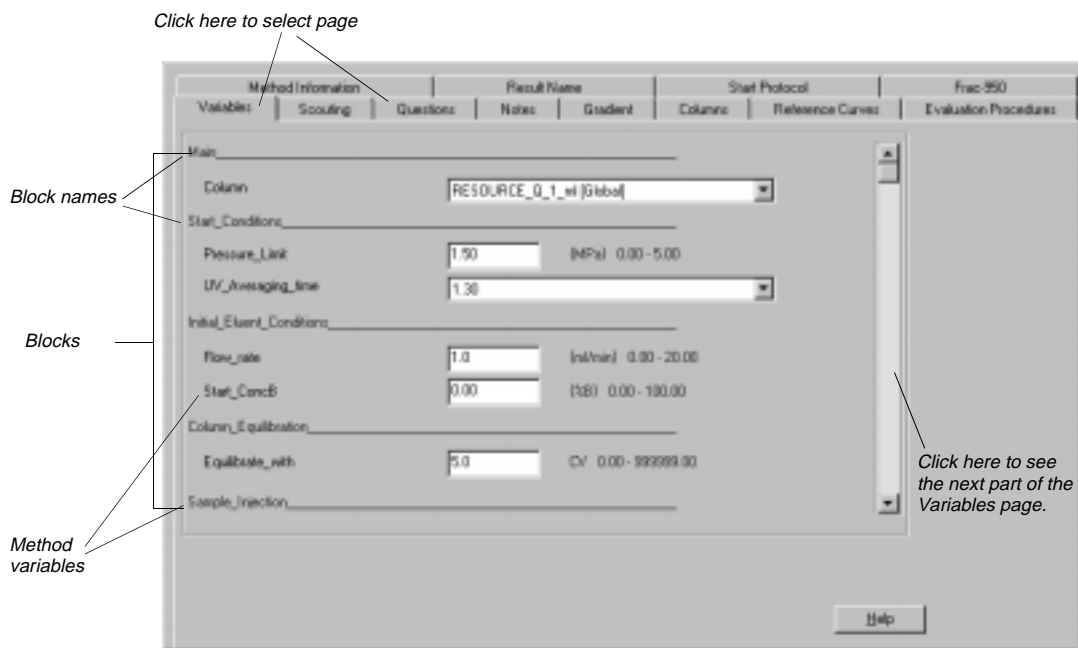
3 Creating a method

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. See section 5.9 in UNICORN User Manual.

- 5 Click on **OK**. The method template will now be opened as an untitled method.
- 6 Select **View:Run setup** (may already be checked).



- 7 **Run setup** consists of a number of pages. You will only look at **Variables** and **Notes** now. You select a page by clicking on the respective tab at the top of the run setup screen.

8 On the **Variables** page, the method is presented by a number of **Blocks** (name in blue). The **Blocks** represent the typical steps in a chromatographic run:

- Start conditions.
- Column equilibration.
- Sample injection.
- Wash out unbound sample.
- Fractionation.
- Gradient.
- Clean after gradient.
- Re-equilibration.

Each block contains a number of **Method variables** (name in black) with suitable default values. The values are easily changed to suit your requirements. Click on the scroll bar to see the next part of the **Variables** page.

The only values you must change in the **Manual Injection FP Segmented** template are for:

- **Flowthrough_FracSize**, in the block **Flowthrough_Fractionation**.

Enter a suitable fraction size. The fraction collection will start at sample injection and continues to the start of the gradient. Zero means that no fractions will be collected.

If using Frac-950, select the tube position for where to start flowthrough fractionation.

Flowthrough_Fractionation

Flowthrough_FracSize 0.00 - 100000.00

Start_Flowthrough_at

- **Empty_loop_with**, in the block **Sample_Injection**

Enter a value of 5 x the volume of the sample loop to apply all the sample onto the column.

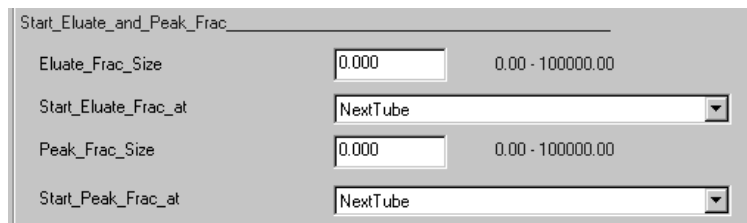
Sample_Injection

Empty_loop_with ml 0.00 - 999999.00

- **Eluate_Frac_Size**, in the block Start_Eluate_and_Peak_Frac

Enter a suitable fraction size. The fraction collection will start at the beginning of the gradient. Zero means no fraction collection.

If using Frac-950, select the tube position for where to start the elution fractionation.



Start_Eluate_and_Peak_Frac		
Eluate_Frac_Size	<input type="text" value="0.000"/>	0.00 - 100000.00
Start_Eluate_Frac_at	<input type="text" value="NextTube"/>	
Peak_Frac_Size	<input type="text" value="0.000"/>	0.00 - 100000.00
Start_Peak_Frac_at	<input type="text" value="NextTube"/>	

- **Peak_Frac_Size**, in the block Start_Eluate_and_Peak_Frac

Enter a suitable peak fraction size. The collection of peak fractions will start at the beginning of the peak. Zero means that peak fractions will not be collected.

If using Frac-950, select the tube position for where to start peak fractionation.

Comment:

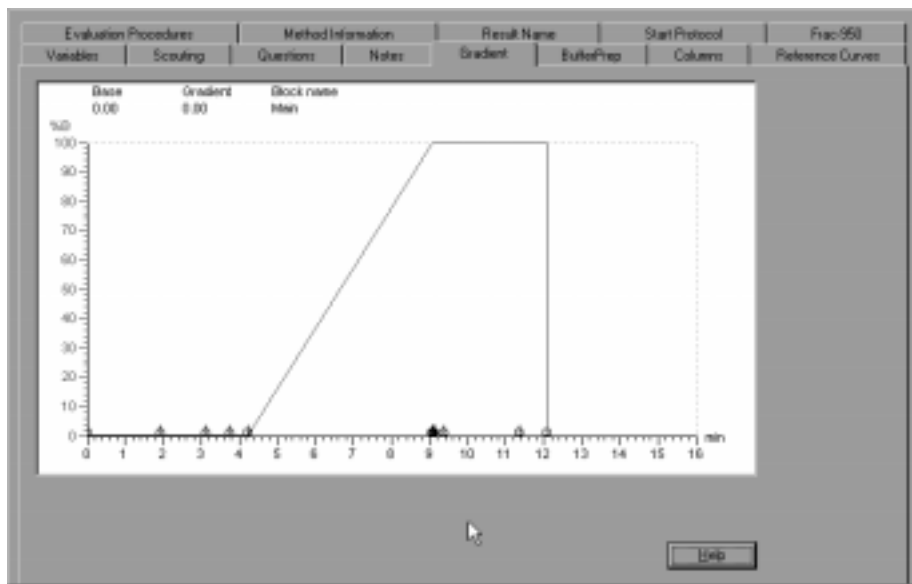
Each method template is unique, but they are all built up with the same principle. Below is a description of all the blocks in the Manual Injection FP Segmented template.

- **Main**
The column selected is shown here.
- **Start conditions**
Defines the general settings during a run.
In the column list, typical peak width and max pressure are pre-defined.
UV averaging time and column pressure limit are automatically set to a default value for the column selected.
If Superloop is used, the pressure limit should be reduced to match the max pressure of the superloop.
- **Initial eluent conditions**
Set the start concentration of eluent B (default 0%B).
The flow rate is set equal to the default value of the selected column.
- **Column equilibration**
The number of column volumes (CV) required to equilibrate the column is set here.
An autozero of the UV monitor is done at the end of equilibration.
- **Flowthrough fractionation**
The flowthrough collection starts at sample injection and continues to the start of the gradient. If the value is set to zero, fractions will not be collected.
- **Sample injection**
To apply all the sample onto the column, empty the sample loop with a volume 5 times the volume of the sample loop.
For a Superloop, set the value equal to the sample volume.
- **Wash out unbound sample**
To wash out unbound sample with the start eluent, set the length of the wash here.

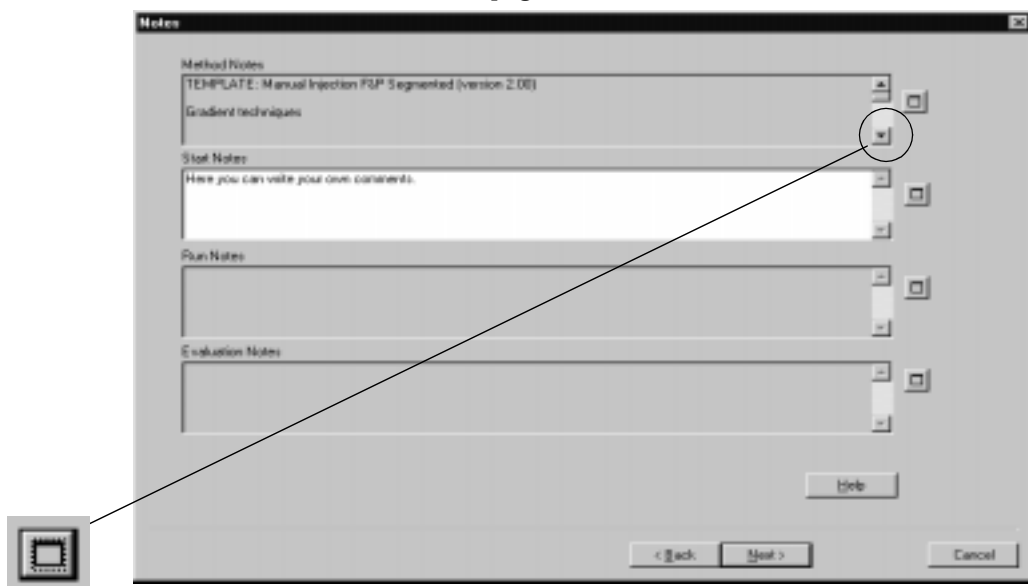
- **Eluate and peak fractionation**
Set when to start the collection of eluate and peak fractions (default: gradient start).
- **Start eluate and peak frac**
For collecting fractions with Frac-950 during elution, set eluate fraction volume and start tube position here.
Select volume and starting position for peak fractionation.
The default value for the minimum peak width is calculated from the typical peak width of the selected column, but may have to be adjusted, for example if the flowrate is changed
The eluate fractions are collected throughout the entire elution, by default. To end eluate fractionation before the gradient end, set the concentration value here.
When the Eluate fracsize is set to zero, the entire eluate is diverted to waste.
- **Gradient**
- **Gradient segment 1, 2 and 3**
The start concentration of eluent B, the end concentration and the length of the gradient is set here. A linear gradient is created. Three separate gradients can be defined.
A step gradient is defined by setting the concentration of eluent B and zero length in one segment.
- **Clean after gradient**
Set the concentration of eluent B for column cleaning and the duration (in column volume units) here.
- **Re-equilibration**
To reequilibrate the column with the initial eluent conditions, set the appropriate length of reequilibration (in column volume units).

3 Creating a method

- 9 Click on the **Gradient** page to view the method graphically. The length of each block is marked at the bottom of the graph. Click in the graph. The name of the block at that position is shown in the upper part of the chromatogram. Click on the x axis to view the method in time, volume or column volumes.



- 10 Click on the **Notes** page.



Maximise the method notes field with the button to the right of the field. The method notes contain comments and information about the method, e. g. how sample injection, fractionation and elution are performed.

- 11 Select **File: Save**. Enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click on **OK**.

Comment:

The method name, followed by two consecutive numbers starting with 01 will then be used as default name for the result file of your method after runs.

- 12 You have now created a method.

- 13 Click on the UNICORN menu icon at the bottom of the screen. Your saved method appears in the window to the left.

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 vary any variables systematically and automatically in repeated runs. This is known as scouting and is a convenient, easy to use function.

Comment:

All templates are named according to the following abbreviations (**Type of injection Y Type of gradient**).

Type of injection identify the type of sample application used:

Manual injection = the sample loop or Superloop is filled manually with a syringe

Y identifies the type of fractionation used:

FP = straight, volume based fractionation and additional peak fractionation

Type of gradient identify the technique for which the template is written:

Step = affinity chromatography

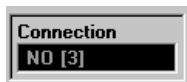
Isocratic = size exclusion techniques (gel filtration)

Segmented = gradient based techniques, referring to RPC, HIC and ion exchange chromatography.

4 Preparing the system for a run

4.1 System connection

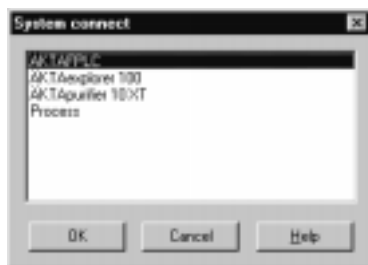
- 1 Click on the **1. System Control** button at the Task bar at the bottom of the monitor.
- 2 If the text **NO** is written in the **Connection** panel in the **Run Data** window, follow step 3 below. If it says **YES**, go directly to *General system preparation*.



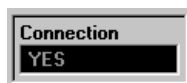
Comment:

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

- 3 Select **System:Connect...** The System connect dialogue window appears:



- 4 Select a system from the list. If you are not connected to a network, only one system will be shown. Click on **OK**.
- 5 When connected, the text **YES** is written in the **Connect** panel in the **Run Data** window. 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 login to UNICORN.

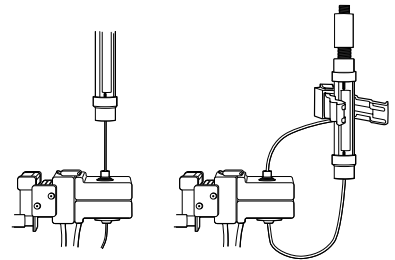


4.2 General system preparation

- 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 waste tubings are put into a waste bottle.
- 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 chapter 6.6 in UNICORN 3.1 User Manual or Section 3.6 in 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.



5 mm flow cell
Connect from above

2 mm flow cell
Connect from below

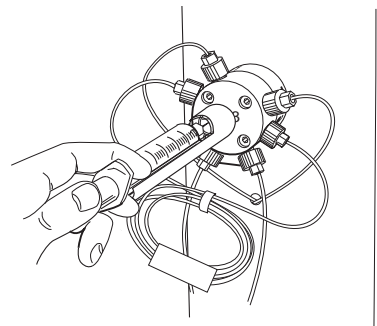
- 6 Insert a sufficient number of tubes into the fraction collector.

- 7 Click on the System Control icon. Fill the inlet tubings with the correct solutions by selecting **Manual:Pump**. Then select the instruction **PumpWash**. Click on **Execute** to fill the inlet tubings.



- 8 The Injection valve will switch to waste during the pump wash. Then click on **Exit**.

- 9 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 Click on the System Control icon if it is not open.
- 2 Select **File:Run...** Select the method to start. Click on **OK** (the method will not start yet).
- 3 A Start protocol appears consisting of a number of pages.
- 4 The first page you see is **Variables**. This is the same page you were working on in the method editor. Here you can fine tune the method before starting it. This is very convenient when repeating runs with minor adjustments.

Comment:

When starting run no. 2 immediately after run no. 1 with the same method but, for example, a different flow rate, you simply:

- 1 Click on the Run button in System Control.
- 2 Change the flow rate on the Variables page.
- 3 Continue through the start protocol by clicking on Next and then start the run.

You do not need to change the method in the Method editor.

Variables

Main

Column: RESOURCE_0_1 ml

Start_Conditions

Pressure_Limit: 1.50 (MPa) 0.00 - 5.00

UV_Averaging_time: 1.30

Initial_Eluent_Conditions

Flow_rate: 1.0 (ml/min) 0.00 - 20.00

Start_CoxB: 0.00 (dB) 0.00 - 100.00

Column_Equilibration

Equilibrate_with: 5.0 CV 0.00 - 99999.00

Sample_injection

Help

< Back Next > Cancel

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

- Click on the **Next** button at the bottom of the window; this takes you to **Questions**. Enter the answers to the questions. The answers will be saved in the result file. Some questions may have been defined as mandatory (mand). These must be answered before the run can be started.

The 'Questions' dialog box contains the following fields:

- Wavelength/UV cell pathlength (Mand)
- Sample volume and type (Mand)
- Column (Mand)
- Eluent A (Mand)
- Eluent B (Mand)
- Remarks (Mand)

- Click on **Next**. This takes you to **Notes**. You can write your own comments in the start notes.

The 'Notes' dialog box is divided into four sections:

- Method Notes:** Contains the text 'TEMPLATE: Manual Injection FSP Segmented (version 2.80)' and 'Gradient techniques'.
- Start Notes:** A large text area with the prompt 'Here you can write your own comments.'
- Run Notes:** An empty text area.
- Evaluation Notes:** An empty text area.

- Click on **Next**. This takes you to **Evaluation Procedures**. Evaluation Procedures are automated evaluation operations that are performed after the run. Mark **Print Chromatogram**. The chromatogram will then automatically be printed after the run.

The 'Evaluation Procedures' dialog box displays the following text:

Selected Evaluation Procedures will run at the end of the method

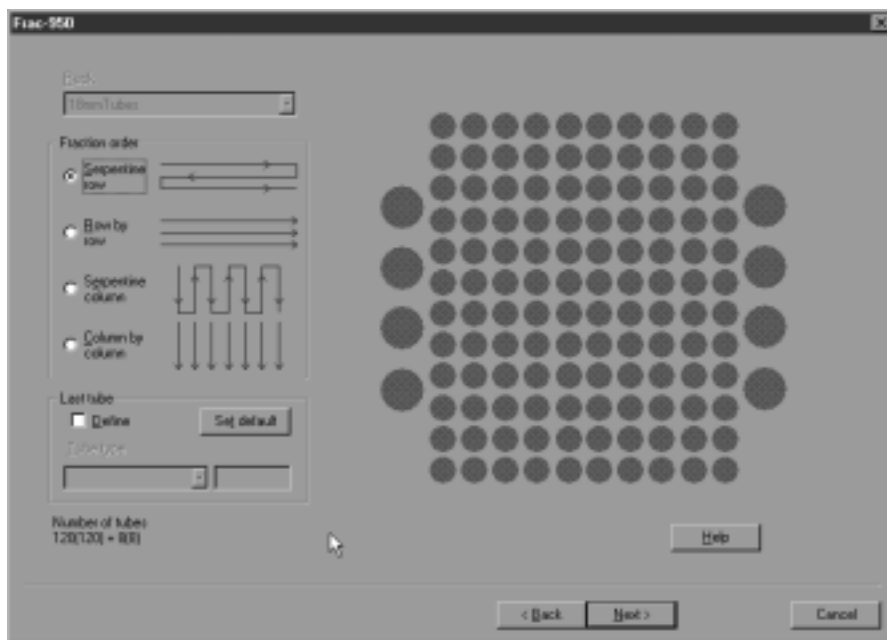
- Print_Chromatogram
- Integrate_and_Print
- Report_Chromatograms
- Integrate_Full_Report

- 9 Click on **Next**. This takes you to **Method Information**.

Here you see information about the run. The approximate volume of buffer used (A+B) is shown as well as how long the method will take.

- 10 Click on **Next**. If using a Frac-950, this takes you to **Frac-950**. Otherwise, it takes you directly to **Result Name** (next step).

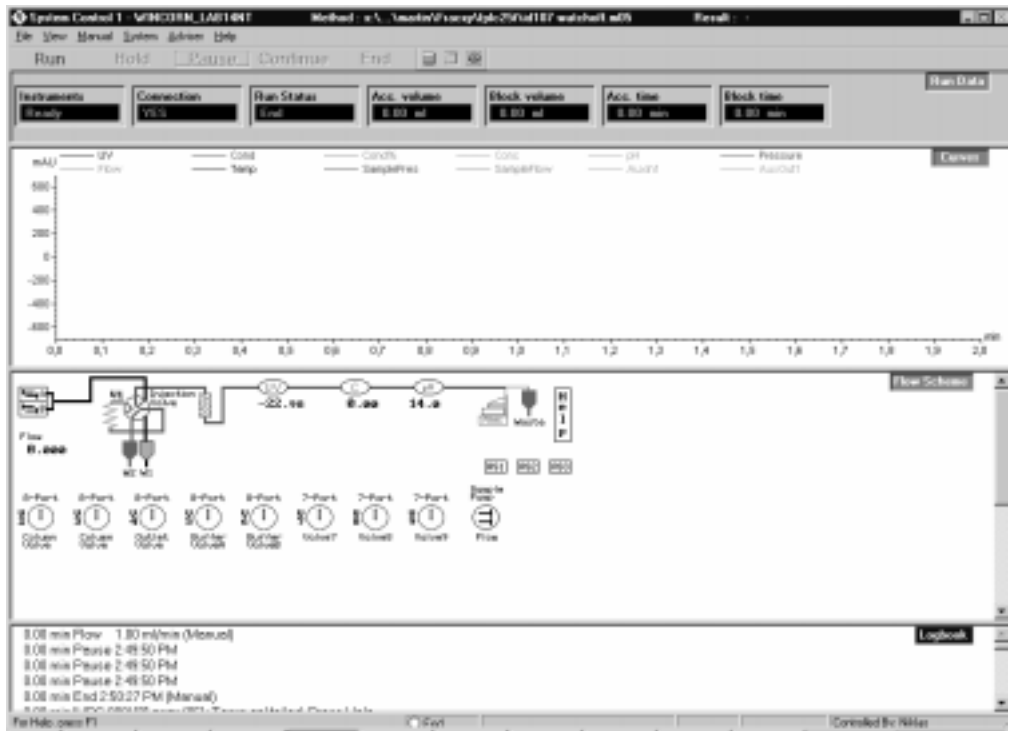
Here you setup the Frac-950 fraction collector. Define the rack type to be used and the order of fractionation. Set up the last tube used in the fractionation. The fractionation will stop when the last tube is reached.



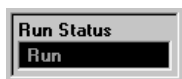
- 11 Click on **Next**. This takes you to **Result Name**.

Here you name the result file and define in which directory the result should be stored. A default name (the method name followed by 01) and a directory are suggested. But you can change the result name and directory (click on **Browse...**) if you so wish.

12 Click on **START**. The run will start.

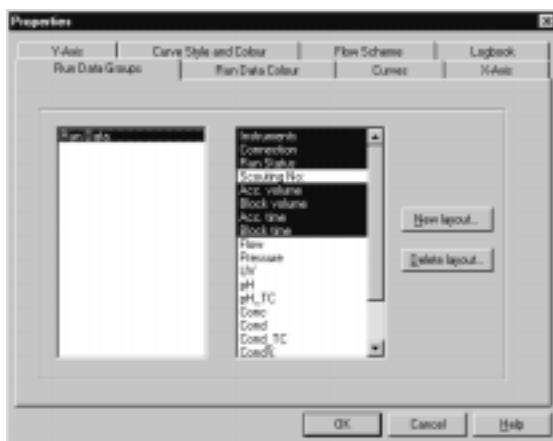


6 Viewing a run

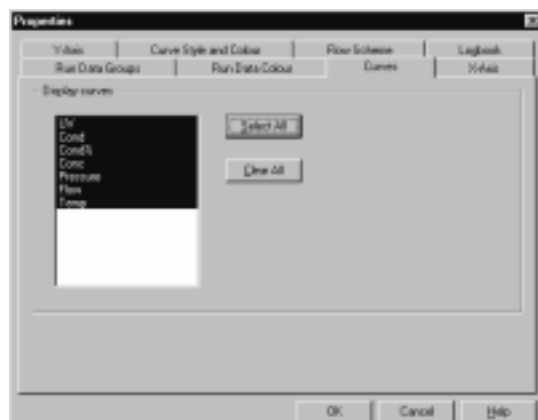


When the system pump is running, the text **Run** is shown in the **Run Status** panel in the **Run Data** window.

- 1 Select **View: Windows**. Check **Rundata**, **Curves** and **Logbook**. Click on **OK**.
- 2 The **Run Data** window at the top shows current values for running parameters.
- 3 Position the cursor in the **Run Data** window. Click on the right mouse button and select **Properties....** Now you can select which parameters you want to see in the **Run Data** window. For example, select **Acc. time**, **Block time**, **flow** and **pressure**. Click on **OK**.



- 4 The **Curves** window shows the curves during the run.
- 5 Position the cursor in the **Curves** window. Click on the right mouse button and select **Properties....** Here you can select which curves to show during the run. All curves are stored in the result file.



- 6 By clicking the different tabs in the **Curve Properties** window you can set the properties for the different curves. Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.
- 7 To fix the Y-axis scale for a curve, mark the curve, click on **Y-axis**, click on **Fixed**, and enter the max. and min. values. You can repeat this for other curves. Click on **OK**.
- 8 To maximise the **Curve Data** window, position the cursor in the **Curve Data** window. Click on the right mouse button and select **Maximize**. Go back to normal size by clicking on **Restore**.
- 9 Click on the Y-axis scale, or click on the curve name at the top of the **Curve Data** window to shift to a scale for another curve. The colour of a curve, its Y-scale, and its name are always the same. Click on the **X-axis** to shift between time and volume.
- 10 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.
- 11 You can make manual changes during the run. Select **Manual:Pump**. The Instructions box is opened.



- 12 If, for example, you want to change the flow rate, select **Pump** and then **Flow**. Enter a new flow rate under Parameters and then click on **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 on **Close**. All manual interactions are recorded in the **Logbook**.
- 13 If you want to stop the run before it is finished, click on the **End** button at the top.

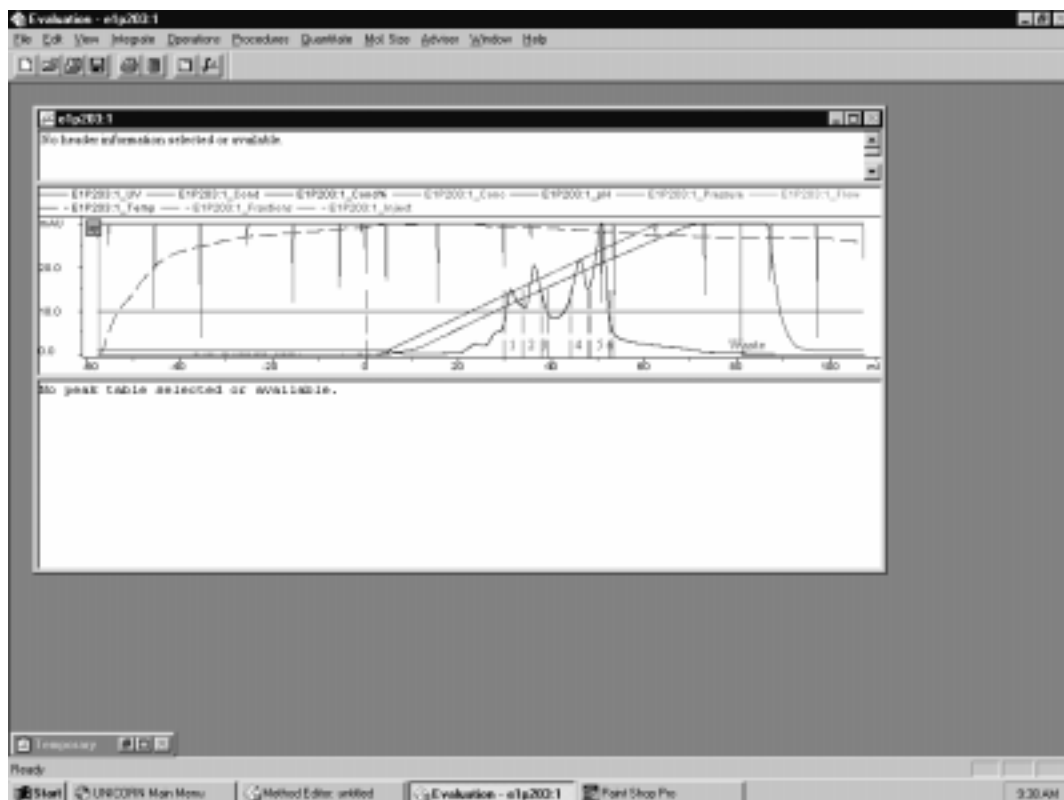


7 Viewing and printing the result

If you are satisfied with the automated printout obtained after the run (if selected), you need not 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. Click on the UNICORN menu icon. Double-click on a result file icon in the list to the right.
- 2 The **Chromatogram** window is opened automatically in the Evaluation workspace 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.

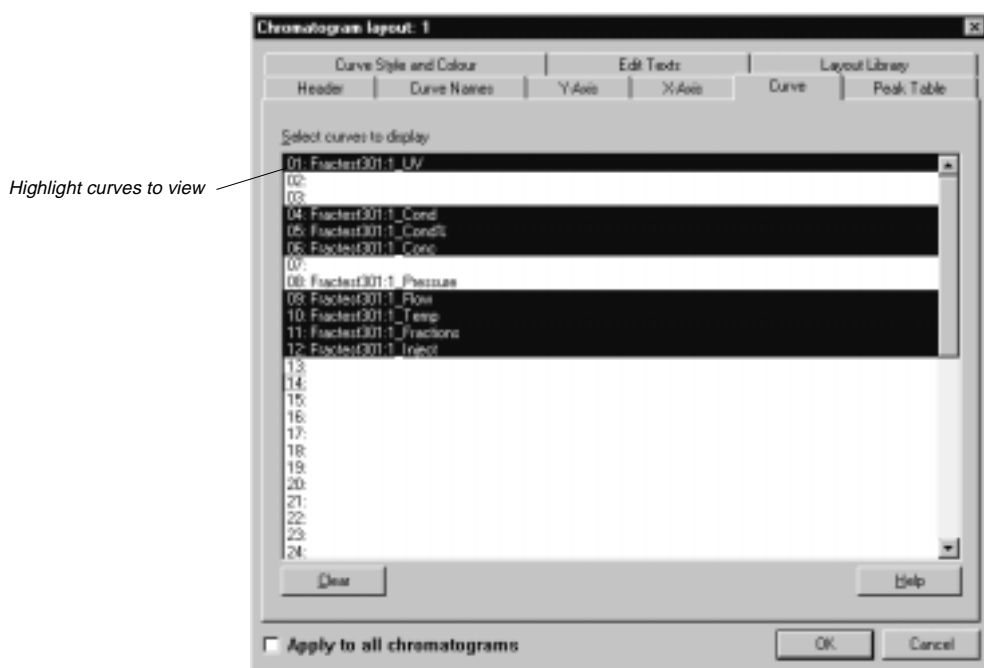


The result file from a run holds a complete record of the run, including method, system settings, curve data and run log.

Comment:

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

- 3 Maximise 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** window. Position the cursor in the **Chromatogram** window. Click on the right mouse button and select **Properties.....**, or select **Edit: Chromatogram layout...** to activate this window.



- 5 Highlight the curves to view under Curves. Curves are named as **Resultfile:1 "curve"** where a curve can be, for example, UV_wavelength, Cond, pressure...etc. De-select all curves except, for example, the UV, Cond and Conc. curves. Click on **OK** at the bottom of the **Chromatogram Layout** window.
- 6 You can easily zoom in on the curves. Place the cursor in the chromatogram, click on the mouse button and holding it pressed down, move the mouse. A rectangle appears on the screen. 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.

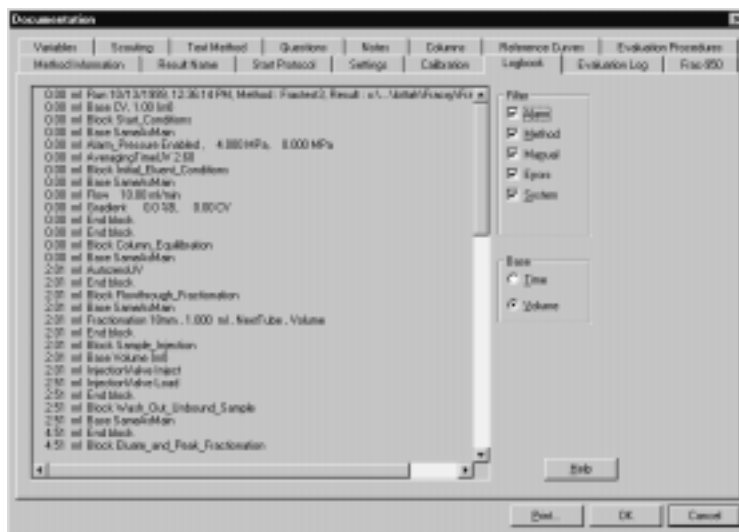
7 Viewing and printing the result

- 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.
- Open the **Chromatogram Layout** window again. Click on 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 on **Fixed**, and enter the max. and min. values for that curve. You can repeat this for other curves.
- To fix the X-axis scale, click on **Fixed** in the X-axis field, and enter the min. and max. values for the X-axis. Click on **OK**.
- Click on **OK** at the bottom of the **Chromatogram Layout** window to execute all the changes.

Comment:

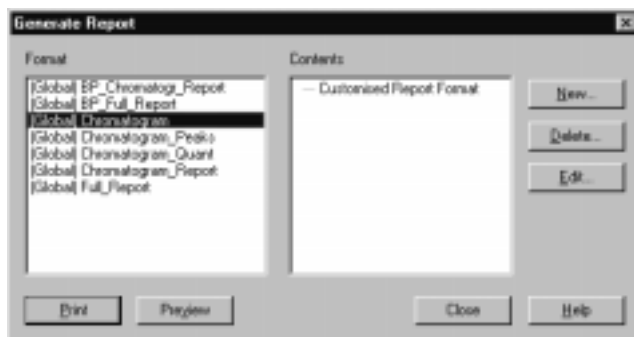
When you have made the necessary changes in the Chromatogram layout box, they can be saved as a Layout. Click on the Save as button at the top of the Chromatogram layout box to save the layout. Give the layout a name and click on OK. Layouts can be selected in Apply layout at the top of the box and all your saved selections will apply. Saved layouts can be applied to any result file.

- Minimise the chromatogram window by clicking on the smaller squares in the upper right corner.
- Click on 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 on the **Notes** and **Logbook** pages to check the contents. Close the **Documentation** window by clicking on the **X** in the upper right corner.

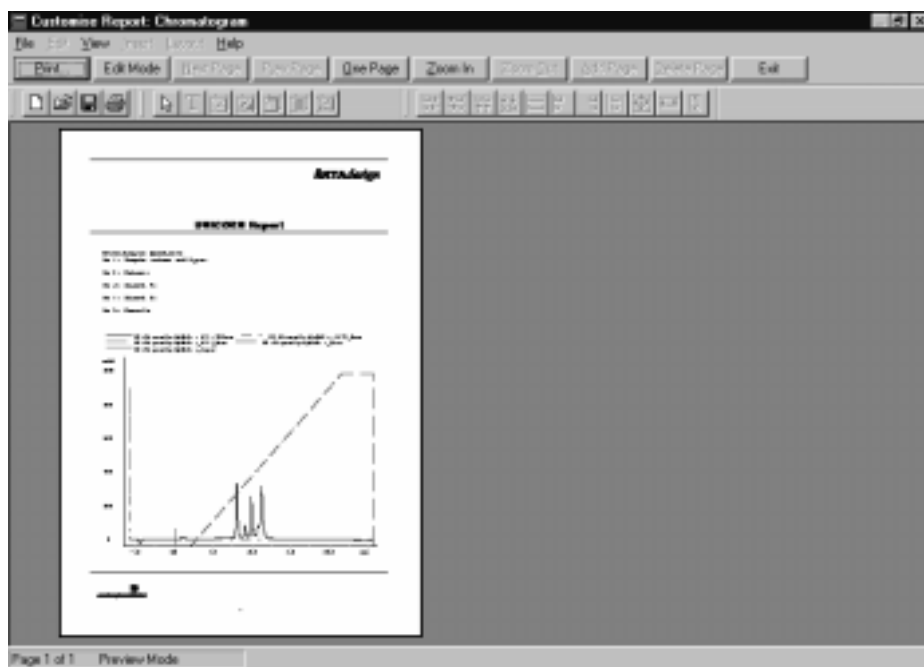


7.2 Printing and making a report

- 1 To print out the chromatogram select **File:Report**.
- 2 Select standard format **(Global) Chromatogram**. This will create a report containing the chromatogram and the questions on one page.



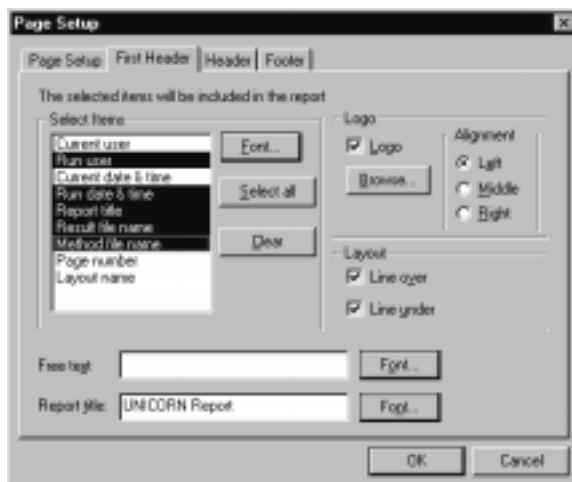
- 3 Click on **Preview** to view the report on the screen.



- 4 Click on **Edit Mode** to enable changes in the report.
- 5 To include more information in the report, click on **Add Page**. A new page is added to the report.

7 Viewing and printing the result

- 6 Under **Insert**, click on the item to include. Items available are:
 - Free text
 - Chromathogram
 - Text method
 - Documentation
 - Evaluation log
- 7 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.
- 8 Press down and hold the left mouse button and drag out a box to the desired size. Release the mouse button. A dialogue is displayed specific to the type of item inserted. Make the appropriate selections in the dialogue and then click **OK** to view the inserted item.
- 9 If you want to change the page layout, select **Edit: Page Setup**. The **Page Setup** window is opened 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 on **OK** at the bottom of the **Page Setup** window.



- 10 Click on **Print** to print out the report.

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.

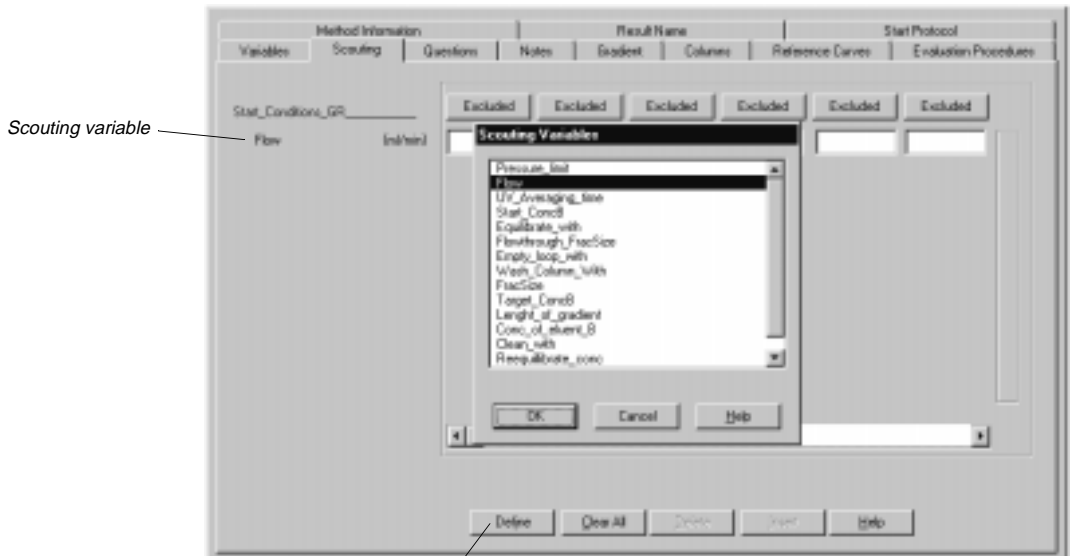
- 1 Select **File:New:Method** in Main menu. Select system. Then select **Anion_exchange** as technique.
- 2 Select template **Manual injection FP Segmented**. Select a column and click on **OK**.

Comment:

A special template is not required for scouting. scouting can be performed with any template for any technique, or without any template at all.

- 3 Select **View:Run setup** in the Method editor (may already be checked).
- 4 Click on the **Scouting** page.

Each Run (excluded) column represents one run



Define other Scouting variables

- 5 A list of all the variables will appear. Mark the variable **Flow** and any other variable you wish to alter, e.g. **Start_ConcB**. Click on **OK**.

Comment:

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

- 6 Click on any cell in the column under **Excluded**. This will change the text to Run 1, Run 2, Run 3 and so on, in the scouting scheme and insert the default values for the scouting variables.
- 7 Make any changes you require to the variable values.

Comment:

For variables with text values (e.g column position) double-click on the variable field and select the required value from the list that appears.

- 8 Click on next run column in the next run, and click on any cell in that column to copy the values from preceding run. Then change the valves as required.
- 9 Repeat step 8 until you have defined all the runs you require. Use the horizontal scroll bar to see more runs.
- 10 Click on the buttons at the top of the scheme to toggle between **Run** and **Excluded** for the different runs. Those marked **Excluded** will not be run. A scouting scheme is now defined.
- 11 Click on the **Variables** page. Change and check the values for the same variables as in the basic template (see chapter 3).
- 12 Select **File:Save**.
- 13 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.
- 14 Prepare the system and start the run as described in sections 4 and 5.

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 chapter/section
Protein purification strategies	The Method Handbook
Different sample applications options	2.2–2.6 in ÄKTA [®] FPLC System Manual
Different fraction collection options	ÄKTA [®] design Optional Configurations User Manual
Columns	2.1 in ÄKTA [®] FPLC System Manual
Calibrating monitors and pumps	6.6 in UNICORN 3.1 User Manual
Comparing chromatograms	9.4 in UNICORN 3.1 User Manual
Integrating curves	10.1 in UNICORN 3.1 User Manual
Measuring HETP and resolution	10.1.9 and 10.1.11 in UNICORN 3.1 User Manual
Exporting curves and data to other programs	10.3 in UNICORN 3.1 User Manual
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 UNICORN 3.1 User Manual

Controlling Pump P-900 and Monitor UPC-900 from the dials on the instruments themselves	2.11 in ÄKTAFPLC System Manual to unlock the dials. 3 in the User Manual for each instrument, found in the binder ÄKTAdesignComponents
Details about each component	See each individual manual in the binder ÄKTAdesignComponents
Security features	11 in UNICORN 3.1 User Manual
Controlling the system from a remote computer	6.5 in UNICORN 3.1 User Manual

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:New:Method** in the main menu or click on
- 2 Select **System, Technique, Template** and **Column**. Click on **OK**.
- 3 Select **File:Save** in the **Method** editor and give the method a name. Click on **OK**.



- 4 Click on
- 5 Select **File:Run**. Select the method and click on **Run**.
- 6 The start protocol will appear. Check the method on the **Variables** page and change values as you require.
- 7 Click on the **Next** button and go through all the other pages.
- 8 On the **Evaluations procedures** page, select **Print_Chromatogram** to get a print-out automatically after the run.
- 9 Click on the **Start** button on the last page and the run will start.