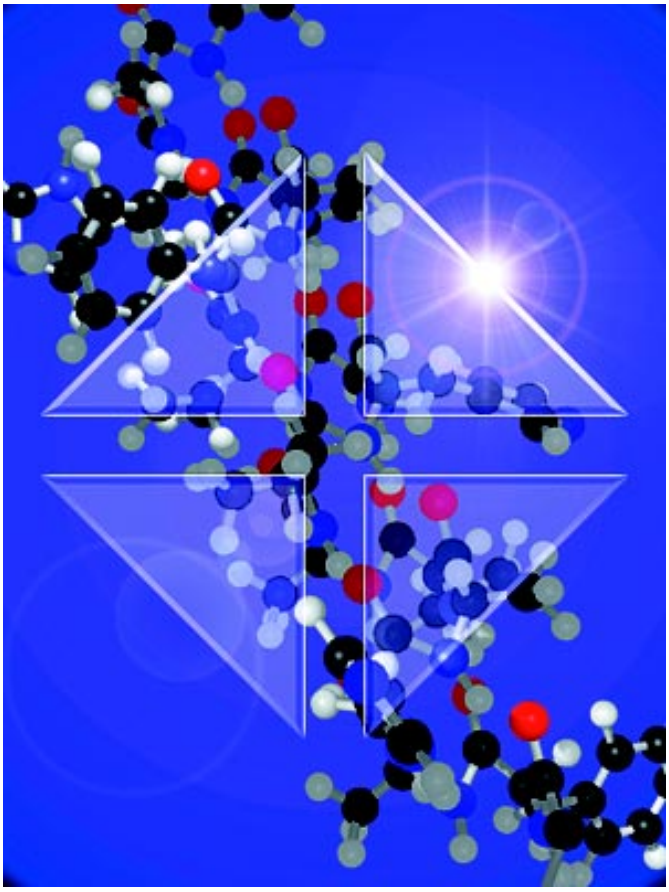
UNICORN

version 3.10

for oligonucleotide synthesis



User Manual

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Edition AA

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Preface

About this manual

This manual provides a full reference to UNICORN™ version 3.10 from Amersham Biosciences AB.
UNICORN is a complete package for control and supervision of oligosynthesis systems, suitable for use with Amersham Biosciences' OligoPilot II™ and OligoProcess™ systems. UNICORN consists of software which runs on an IBM-compatible PC under Microsoft Windows NT 4.00*, and hardware for interfacing the controlling PC to the synthesis module.

The manual is organised in 14 chapters and 6 appendices:

Introductory material	1. Introduction 2. UNICORN concepts 3. Logon and file handling
Methods and runs	4. Creating methods from method templates 5. Creating and editing methods 6. Performing a run 7. MethodQueues
Evaluation	8. Presenting results 9. Evaluating results
System management	10. Security features 11. Network setup 12. Installation 13. Administration 14. System settings
Appendices	A. Technical specifications B. General strategy for oligosynthesis C. Evaluation functions and instructions D. Feedback tuning E. File organisation F. Troubleshooting

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Assumptions

Two broad assumptions are made in this manual:

1. You should be familiar with the oligosynthesis systems in your installation. Refer to the appropriate System Manuals for details.

2. You should be familiar with the general principles of using Microsoft Windows NT version 4.0 on your PC. Although UNICORN is a self-contained program package and does not require any direct interaction by the user with Windows NT, the user interface principles follow the conventions set by Windows NT programs.

   Many of the menu commands in UNICORN can be activated using the toolbar buttons, keyboard shortcuts and the right mouse button menu. The availability of these command options is dependent on the active field or window in which you are currently working. The function of a toolbar button is displayed when you place the mouse pointer over a button. Right mouse button menu commands are quickly found through use of the program.

Typographical conventions

Menu commands, the names of dialogue boxes and windows, the contents of dialogue boxes windows, and option buttons are written with a **bold helvetica** typeface. Menu commands are written in the order of the menu name and then the command, separated by a colon. For example:

"Select **File:Save As** to display the **Save As** dialogue. Locate the destination drive and folder and enter a file name. Click on **Save**."

This directs you to click on the **File** menu and select the command, **Save As**. A dialogue called **Save As** is displayed in which you must locate the destination folder for the saved file and give the file a name. You then click on the button called **Save** in the dialogue to execute the save command.

A `typewriter-like` typeface is used for instructions as they appear in the text editor for methods and evaluation procedures. These are normally entered automatically by UNICORN.

Some menu commands also have shortcut keys on the keyboard, which are written within < > marks.

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1 Introduction

UNICORN is a control system developed and marketed by Amersham Biosciences AB for real-time control of oligosynthesis systems from a personal computer. The package operates together with OligoPilot II and OligoProcess from Amersham Biosciences. UNICORN runs under the operating system Microsoft Windows NT version 4.0.

Functional features of UNICORN 3.10 include:

- One PC may control up to 4 oligosynthesis systems directly.
- Network support allows up to 90 systems to be run from one PC.
- Method templates, providing method frameworks for most common applications, eliminating the need to program methods from scratch.
- Modular method definition in the method templates, reflecting the separate steps in a process.
- Dynamic graphical overview of active runs.
- User-definable alarm and warning limits for monitor signals.
- Programmed sequential operation.
- Batch operation and process documentation in accordance with the requirements of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP).
- Comprehensive data evaluation software.

In addition, UNICORN offers a comprehensive security system:

- Password control for all users, with access authorisation for other users' method and result files.
- Customised definition of access control levels.
- Audit trail for system operation.

Note: UNICORN must be correctly installed for stand-alone or network operation before the software can be used. Network considerations, software installation and administration of system and user definitions are described in Chapters 11, 12 and 13.

2 UNICORN concepts

This chapter introduces the basic concepts that are specific to UNICORN. For a description of how to work with the Windows NT operating system, see your Windows NT system documentation.

Material in this chapter is divided into 8 sections, dealing with:

- UNICORN control software
- UNICORN user interface
- Files and folders
- Methods and method structure
- System control
- Evaluation
- Network considerations
- Security and administration

2.1 UNICORN control software

UNICORN runs under the Windows NT operating system, and provides facilities for method-controlled operation of oligosynthesis systems as well as real-time monitoring and subsequent evaluation of the synthesis process.

2.1.1 Strategies

Part of UNICORN software (referred to as the strategy) is system specific. The strategy defines what is available in method and manual instructions, system settings, run data, curves and method templates. Most of this manual describes the user interface in UNICORN independent of the strategy. Strategy-dependent instructions are listed in Appendix B.

2.2 UNICORN user interface

2.2.1 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and logon reminding you about the Main menu toolbar buttons.

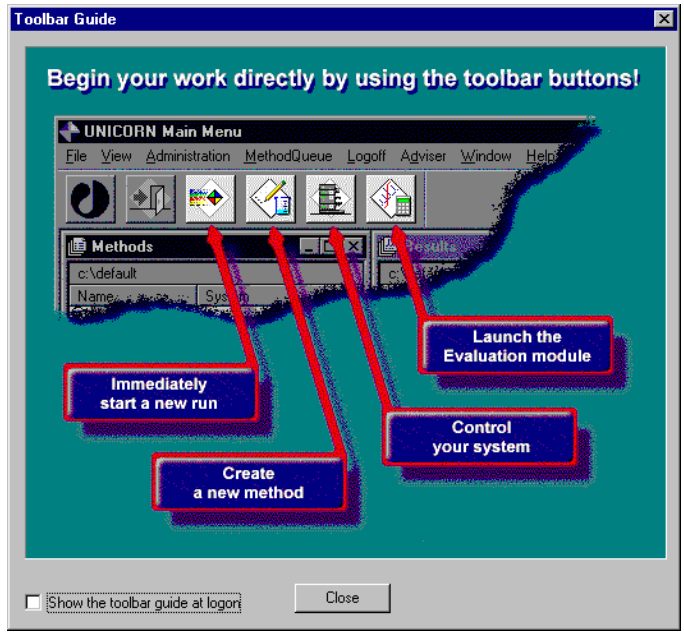


Figure 2-1. UNICORN Toolbar Guide dialogue.

The Main menu toolbar buttons allow you to begin using UNICORN quickly, for example, to create a new method in the Method editor, start an instant run, open a result file for evaluation, or execute manual instructions in System control (see Section 3.2).

2.2.2 Software modules

UNICORN control software consists of four integrated modules:

- The **Main menu**, with functions for file handling and administrative routines such as definition of available oligosynthesis systems and maintenance of user profiles.
- The **Method editor**, where methods for pre-programmed control of oligosynthesis systems are created and edited.

- The **System control** module, which permits manual or method-based control of oligosynthesis systems and on-line monitoring of synthesis processes. There may be up to four independent system control modules on one computer, for controlling up to four separate systems.
- The **Evaluation module**, with extensive facilities for presenting and evaluating stored results from synthesis processes.

These modules are present on the Windows NT taskbar.

To minimize a module to the taskbar, click on the Minimize button at the right-hand end of the window title bar. To minimize the whole of UNICORN click on the <Windows + M> keys on the keyboard.

Note: Minimizing a module window to the taskbar does not close the module. Once opened, UNICORN modules remain active until you quit the program. A minimised System control module may thus be actively in control of a running process.

2.2.3 On-line help

A comprehensive on-line help utility is included in UNICORN software. Entry to the general help utility can be accessed from the **Help** menu. Dialogue- or window-specific help topics can be obtained by clicking on the **Help** button in the dialogue or by pressing <F1> on the keyboard. In the dialogues for method instructions, procedure instructions and system settings, pressing <F1> when an instruction is highlighted will display an information box with short help on the function and use of the selected instruction.

2.3 Files and folders

UNICORN Main menu interface divides user files into two categories, for methods and results (see Figure 2-1). Only folders to which the current user has access are shown in the Main menu windows, **Method** window and **Results** window. Files may be displayed in several viewing options (see Chapter 3 for more details).

2.3.1 Method files

Method files contain instructions for controlling a run and are shown in the **Methods** window of the Main menu.

The **Methods** window also displays icons for MethodQueues, which allow several methods to be run in an automatic pre-programmed sequence on the same or different systems.

2.3.2 Result files

Result files are created by UNICORN when a method is run and contain:

- A copy of the method used in the run.
- Run data from the monitors in the oligosynthesis system (e.g. UV absorbance, flow rate, conductivity etc.).
- Saved results from evaluation of the run data (see Chapter 10).
- Run documentation including information on, for example, the run log, calibration settings, scouting parameters, text method etc.

2.4 Methods

Oligosynthesis runs are programmed as *methods* in UNICORN. This section gives a brief overview of the concepts and principles of methods. See Chapters 4 and 5 for a description of how to program methods, and Chapter 6 for how methods are used to control oligosynthesis systems.

2.4.1 Method structure

Blocks

Methods in UNICORN are divided into blocks. Blocks typically contain the subroutines that control the complete synthesis procedure. A synthesis cycle is generally based on the following order of subroutines:

- Detritylation
- Detrit wash
- Coupling
- Oxidation/thiolation
- Capping

Method templates supplied with UNICORN contain all the blocks that are likely to be used in a specific method. When the desired sequence is created, the blocks needed to build up the method to synthesize the sequence are automatically copied in from the method template. The methods derived from the method templates can be directly used to process the run.

Additionally, the methods are convenient starting points for developing customized methods. Fully adequate customised methods for many applications can be created simply by adjusting the values of

method variables (see below). New blocks can also be created in the Text instructions or in the cross reference list of the Sequence page in Run setup.

Method base

Method blocks are written in one of three method bases, which defines the unit for the breakpoints in the block:

- time (min)
- volume (ml or l according to the strategy)
- column volume (set by the user)

Different blocks in the same method may be written with different method bases: for example a column wash block might be written in terms of column volumes while a purge block might be best expressed in absolute volume.

Note: The term method base should not be confused with bases in a sequence or an oligonucleotide.

Instructions

The method is a call to blocks, with each block containing a series of instructions or sub-routines (see Figure 2-2). Each instruction is a request for specific operations in the system. A block may also contain other blocks which in turn contain their own series of instructions.

Double click on a block to expand/collapse the view of the instructions.

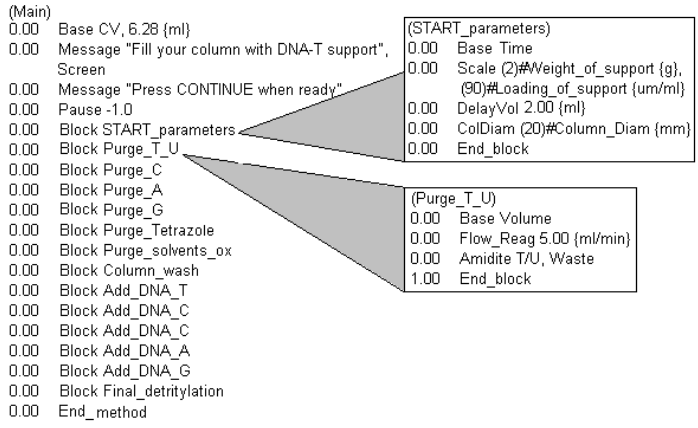


Figure 2-2. Relationship between blocks and instructions. The method (left) is written as a series of calls to blocks, each of which consists of instructions for performing one or more specified tasks (right).

Breakpoints

Each instruction in a method block is issued at a specified breakpoint according to the method base. The first instruction in a block is always at breakpoint 0, and all other breakpoints are counted from this point. For example, in the following instructions from a block:

```
0.00    Base Time
0.00    Flow_Reag 5.00 {ml/min}
9.00    Flow_Reag 0.00 {ml/min}
```

At breakpoint 0.00, the reagent flow rate is set to 5.00 ml/min. After nine minutes have elapsed, at the next breakpoint, the flow rate of the reagent will be set at 0.00 ml/min, i.e. no flow at all.

Method variables

Breakpoint values and instruction parameters may be defined as variables. This is a powerful facility for constructing a method which contains default parameter values. These default values may then easily be changed either to create variants of the same method or to adjust the parameter values at the start of a run (see Section 4.4).

Using variables makes it easy to adapt a method to a particular oligonucleotide synthesis run. For example, in the block below, the values of the start parameters variables can be seen:

```
(START parameters)
0.00  Base SameAsMain
0.00  Scale (2)#Weight_of_support {g},
      (90)#Loading_of_support {umol/g}
0.00  DelayVol 2.00 {ml}
0.00  ColDiam (20)#Col_Diam {mm}
4.00  End_block
```

The variables are expressed:

```
(variable value)#Variable_type {variable units}
```

In the block above, it is possible to see that the variable values have been set at 2 g weight of support, 90 µmol/g loading of support and a 20 mm column diameter.

By using variables, a method may be displayed either in detail as text instructions or in a condensed form as variable values in Run setup mode. This is illustrated in Figure 2-3. The Run setup mode is displayed when the method is run, allowing variable values to be set at the beginning of the run.

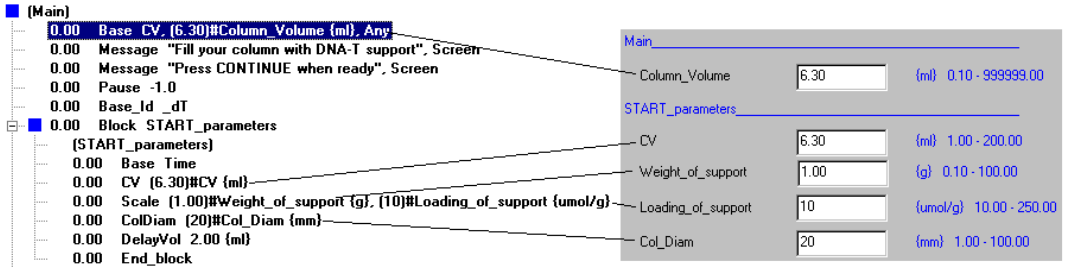


Figure 2-3. Relationship between variables in text instructions and in the Variables page of run set-up.

2.4.2 Method templates

Method templates are basic methods which provide convenient starting points for developing customised methods (see Chapter 4 for more details).

Method templates for most synthesis techniques are supplied with UNICORN installations for OligoPilot and OligoProcess. New methods are created by selecting a suitable system, technique and template and column. The method can then, if necessary, be modified on the Variables page or in the Text instructions. Fully adequate customised methods for many applications can be created simply by adjusting the values of method variables in a suitable template.

2.5 System control

2.5.1 Control facilities

The system control module allows independent control of up to four oligosynthesis systems from one computer, with continuous real-time monitoring of the synthesis process. The run status can be displayed as:

- numerical display of run data from selected monitors
- graphical display of curves from monitors
- a flow scheme showing the current open flow path in the system
- a logbook recording the control events in the run.

Systems can be controlled either manually with interactive commands or through pre-programmed methods.

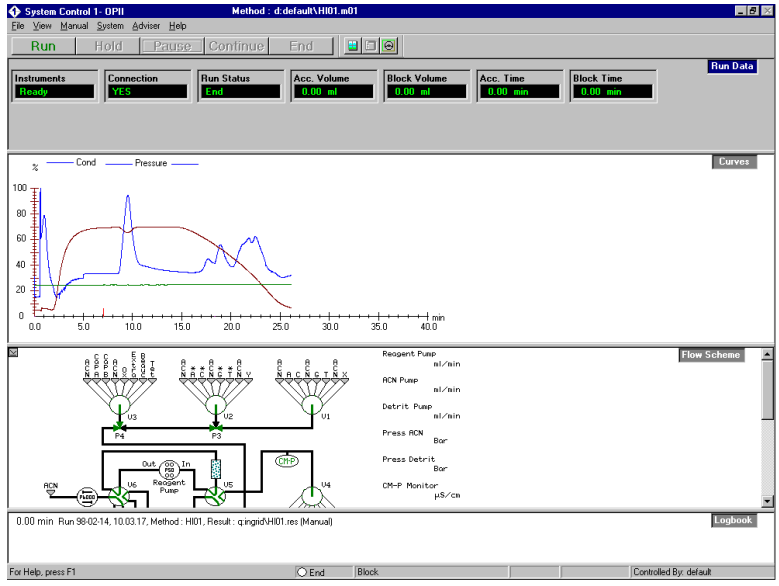


Figure 2-4. The system control screen.

By using MethodQueue facilities, several methods may be run in a pre-defined automatic sequence involving one or more oligosynthesis systems. With suitable oligosynthesis system equipment, this allows unattended operation of quite complex multi-step synthesis processes.

2.5.2 System connections

For controlling a synthesis process, the operator establishes a *connection* between the computer and the oligosynthesis system in one of the system control windows in UNICORN. Two kind of connections may be established:

- *Control mode connections* which permit full control of the connected system.
- *View mode connections* from which the progress of the synthesis can be monitored but the system cannot be controlled.

A system can be started from a computer in, for example, the laboratory. Control of the system can be released without affecting the run and the control of the system can be later taken from another computer station, for example, in the office.

Each oligosynthesis system can have only *one* control mode connection at any one time, but it can have *several* view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time.

2.6 Evaluation

The evaluation module (chapter 9 and 10) provides extensive facilities for presentation and evaluation of synthesis results. Essential features of evaluation include:

- *Trityl data.* This is stored in the result file and can be printed in a report as a table
- *Curve manipulation*. A wide range of operations can be performed on curves, such as addition and subtraction of two curves, differentiation, integration, normalization and scaling. The original raw data curves are always kept unmodified in the result file.
- *Curve comparisons*. Curves from different result files can easily be compared in the evaluation module.
- *Evaluation procedures.* Operations performed in the evaluation module can be recorded as an evaluation procedure and repeated for other result files with a single menu command. Evaluation procedures may be executed either automatically on completion of a method run or interactively from within the evaluation module.
- *Reports*. Comprehensive reports of the evaluation results can be generated for hard-copy documentation of the synthesis process. Generation and printing of reports may be included as an operation in an evaluation procedure to automate process evaluation and documentation.

2.7 Network considerations

UNICORN can be installed on a stand-alone PC workstation and/or PC workstations in a network.

2.7.1 Stand-alone installation

In a stand-alone installation, up to four oligosynthesis systems may be physically connected to and controlled from the workstation where UNICORN is installed.

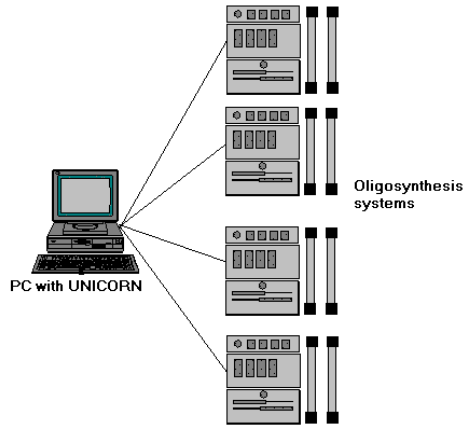


Figure 2-5. Stand-alone installation of UNICORN on a workstation, which can control up to four separate oligosynthesis systems.

2.7.2 Network control from a remote workstation

In a network installation, each oligosynthesis system is physically connected to a workstation, but may be controlled from any workstation in the network on which the UNICORN software is installed. A workstation to which a system is physically connected is referred to as a local station. Other workstations in a network installation are called remote stations.

During installation of UNICORN for the first time on a workstation in a network configuration, certain files are copied to the network server. These files include UNICORN user files and strategy files, and are the global settings for all UNICORN users in the network (see Chapter 13). However, UNICORN program files and templates are NOT copied or located on the network server and as such the server cannot be used to control a run. UNICORN program files and templates are instead locally installed on each workstation in the network, and the network is used as the medium of communication to establish control with the oligosynthesis systems.

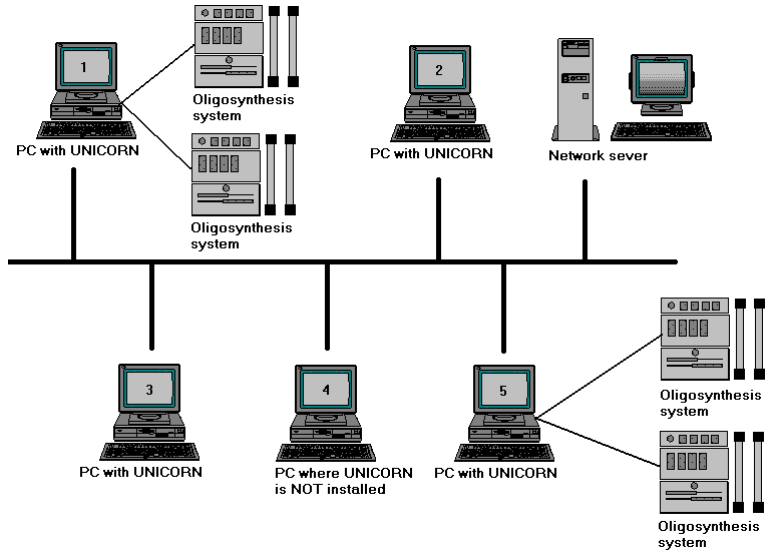


Figure 2-6. A network installation with 4 oligosynthesis systems and 5 workstations (PCs). The oligosynthesis systems physically connected to PCs 1 and 5 can be controlled locally. Alternatively, any of the PCs with UNICORN installed can be used to remotely control any of the oligosynthesis systems via the network. In this example, PC 4 is connected to the network but it cannot be used to control any oligosynthesis systems since it does not have UNICORN installed. Note also that the server does not have UNICORN program files installed and is not involved in the control process per se.

2.8 Security

Security features in UNICORN include:

- *Access security.* Use of UNICORN is restricted to authorised users. Each user is assigned an access level which defines the functions that the user is permitted to use.
- *Connection security.* Running systems may only be controlled from one connection. Systems may be locked with a password to prevent other users from changing run parameters.
- *Data security.* Result files can be saved automatically at pre-set intervals during a run to minimise data loss in the event of system failure. In a network installation, results are saved on the local station if network communication fails.

Security features are discussed in more detail in Chapter 10. Network and administrative aspects are discussed in Chapters 13 and 14 respectively.

3 Logon and file handling

3.1 Logging on

When you start the computer you must log on to Windows NT before you can log on to UNICORN and begin working. Logging on to Windows NT will automatically connect you up to the network if NT has been so configured. Network connection is not essential for local control of a system.

1. To start UNICORN, locate the program in the Windows NT Start button under **Programs:Unicorn:Unicorn 3.10**. Alternatively, double click on the UNICORN icon on the desktop if this option was selected during installation.

If UNICORN is already started and the previous user has logged off, click on the **Logon** menu command or click on **Logon/Logoff** button the in the Main menu module.

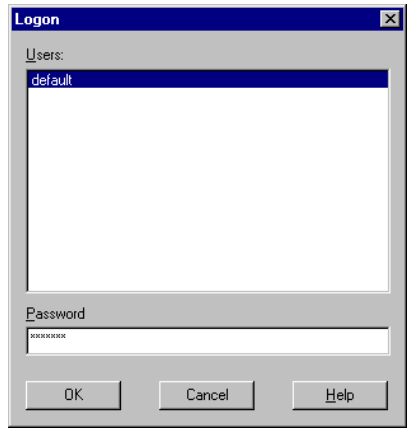


Figure 3-1. The Logon dialogue.

Click on your username in the list and type your password. Click on the **OK** button to log on. If you cannot remember your password, you cannot log on to UNICORN. Ask your system administrator or other user with sufficient authorisation to give you a new password.

Note: If UNICORN has been installed so that no password is required for logon, you need only select you username and click on **OK** to proceed.

Press the **Cancel** button to abandon the logon attempt.

Network installations

In a network installation, you must be logged on to the network before starting UNICORN. Any computer station in the network with UNICORN software installed can be used to log on to UNICORN. You can log on with the same username and password on multiple computers simultaneously.

Each oligosynthesis system can have only *one* control mode connection at any one time, but it can have *several* view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time. Multiple logons with the same username are treated internally as separate users for the purpose of System control.

Note: Do not confuse Windows NT/network logon with UNICORN logon. You log on to the network to gain access to network resources (shared drives, printers and other networked equipment). You log on to UNICORN to gain access to the oligosynthesis systems that are installed in the network. The username and password for logging on to the network are entirely independent of the those for logging on to UNICORN.

3.2 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and logon reminding you about the Main menu toolbar buttons.

The Main menu toolbar buttons allow you to begin using UNICORN quickly, for example, to create a new method in the Method editor, start an instant run, open a result file for evaluation, or execute manual instructions in System control.

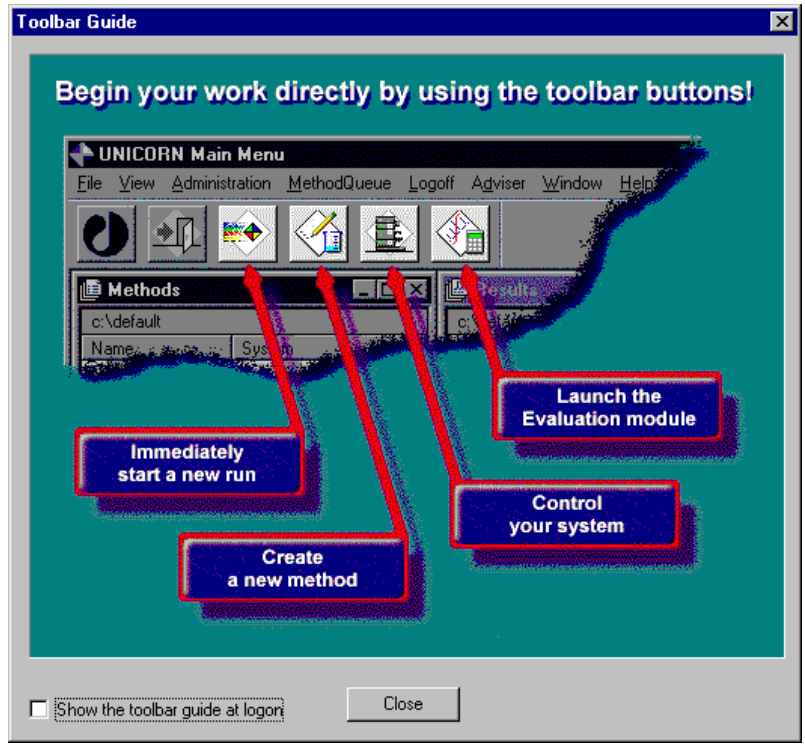


Figure 3-2. UNICORN Toolbar Guide dialogue.

The toolbar buttons are:

About UNICORN

This gives you information about the UNICORN version installed, copyright and web address for obtaining more information.


Logon/Logoff

This allows you to log on or off UNICORN as appropriate.

Instant Run

This opens the **Instant run** dialogue (Fig. 3-3) in which you can select the system to run, technique and template. Press on the **Run** button to view the Start protocol and to start the run (see Chapter 6).

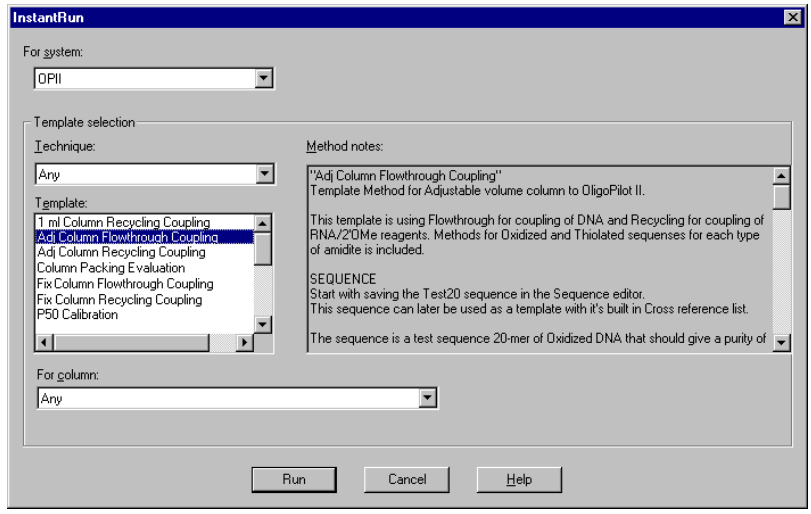


Figure 3-3. Instant Run dialogue.

Note: Use of this function requires that templates are defined. Standard systems are supplied with templates, but custom systems require that the user makes templates.

New Method This immediately starts the Method editor module and displays the **New Method** dialogue (see Section 4.1).

System Control This activates the first connected System control and displays the **Manual instruction** dialogue (see Section 6.3.2).

Evaluation This displays the **Open result** dialogue. Select a result file and click on **OK** to launch the Evaluation module (see Chapter 9).

3.3 UNICORN Main menu windows

The two Main menu windows display the folders to which you have access within UNICORN and the method and result files within the currently open folder respectively. You can only see method files written for systems to which you have access.

3.3.1 Creating a new folder

To create a new user-specific folder:

1. Select the appropriate window, **Methods** or **Results**, in which you want to create a new folder.

2. Select **File:New:Folder** or **New Folder** from the right mouse button menu. The **Create New Folder** dialogue is displayed.

3. Enter the name of the new folder and click on **OK**. The new folder is displayed in the appropriate window. Any user that has access set to the main folder in which the new folder was created also has access to the folders and files contained therein.

3.3.2 Opening and running method files

To open and edit a method file in the Method editor click on a file in the **Methods** window and select **File:Open**, or click on the file with the right mouse button and select **Open** from the menu. Alternatively, double click on a file to open it.

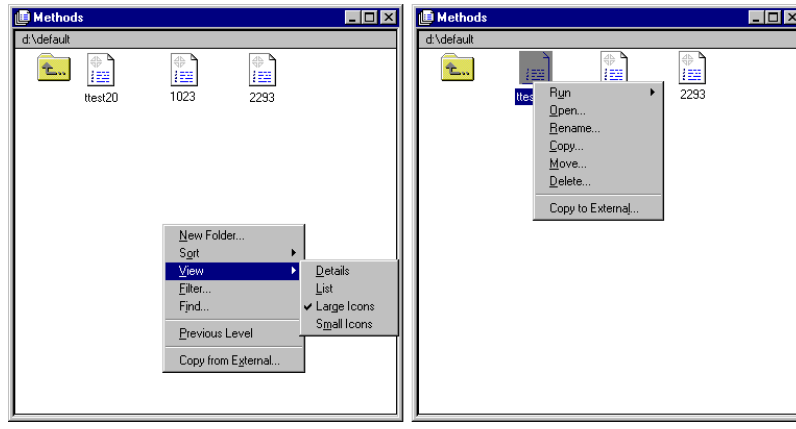
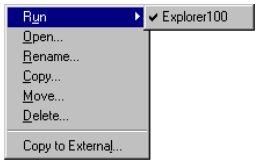


Figure 3-4. The Method window with right mouse button selected in the window (left) and the right mouse button menu for a selected icon within the window.



Method files can be run directly in the System control module. Alternatively, click on a file in the Main menu **Methods** window and select **File:Run**, or click on the file with the right mouse button and select **Run** from the menu.

3.3.3 Presenting files

The way files are presented in the windows can be set from the **File** menu or from the mouse right button menu. Presentation options are:

- view mode
- sorting order
- filter (for displaying only a chosen set of objects, e.g. methods for one system)

View mode

You can select to display the contents of the windows in several Windows NT views from the **View** menu or **View** options in the right mouse button menus. View the files either as a details list (**View:Details**), a simple list (**View:List**), large icons (**View:Large Icons**) or small icons (**View:Small Icons**).

The details list includes a small icon identifying the type of object, file name, file type, and the last modified date and time.

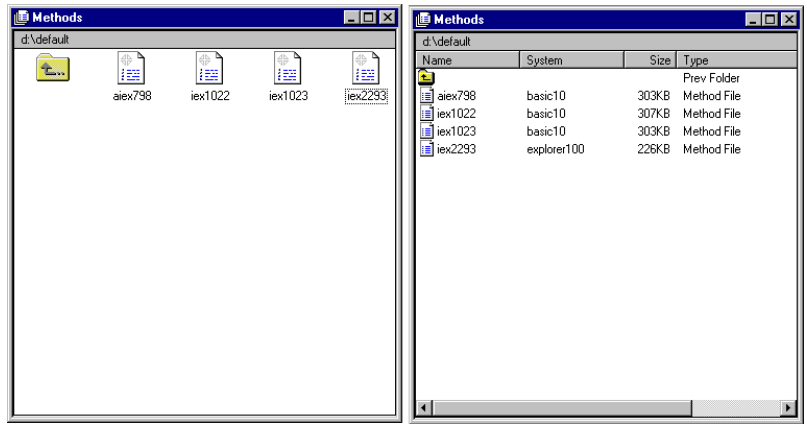


Figure 3-5. Icon and detail display modes illustrated for the Methods window.

Sorting order

In the details list viewing mode files can be sorted in the window according to one of:

Name	alphabetical order or reverse alphabetical order
System	alphabetical order or reverse alphabetical order (**Method** window only)
Size	smallest or largest files first
Type	alphabetical order of file extension type
Modified	last recently modified files first

To change the sorting order, choose **Sort** from the right mouse button menu or **File:Sort**, and choose the appropriate sorting order from the menu cascade. Alternatively, click on the column headers in the window for **Name**, **System**, **Size, Type** and **Modified** to change the file sorting accordingly. Click a second time on the same sorting option and the files are sorted in reverse alphabetical order, increasing file size etc. as appropriate to the selection. Changing the sorting order affects only the currently active window.

Filter

To restrict the files displayed according to file name or the system with which they are associated, choose **Filter** from the right mouse button menu or select **File:Filter**. Mark the system(s) for which you want to display files, and enter a file name specification if required. Click on **OK** to activate the filter. The filter affects the display in *both* windows.

You can use standard Windows wildcard characters in the file name specification (* stands for any number of characters, ? for any single character). For example:

test	will display only files named test
test*	will display all files with names beginning with test
*test	will display all files with names ending with test
?test	will display only 5-character names ending with test

If a filter is active, this is indicated in the title bar of the panel (e.g. Results : filtered igf*). To display all files, choose **Filter** and click on **View All**.

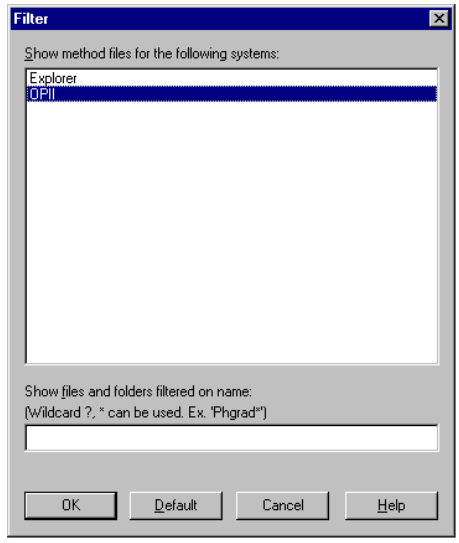


Figure 3-6. The Filter dialogue.

3.3.4 Finding files

To find a file:

1. Choose **Find** from the right mouse button menu or select **File:Find**. In the displayed **Find file** dialogue, enter a file name specification in the **Search for files filtered on name** field. You can use standard Windows wildcard characters in the file name specification (see above under Filter).

2. You can restrict the search further if required:

   - Choose file type from the pull-down menu for **Type** (**All**, **Folders**, **Method files** or **MethodQueue files** for the **Methods** window; **All**, **Folders** or **Result files** for the **Results** window).
   - Click on **Date range** and use the slide bar to set the date limits for the search. Click on **OK**.
   - Check **Search all folders** to search through all the folders to which you have access. If **Search all folders** is not checked, the search will be restricted to the current folder and sub-folders below.

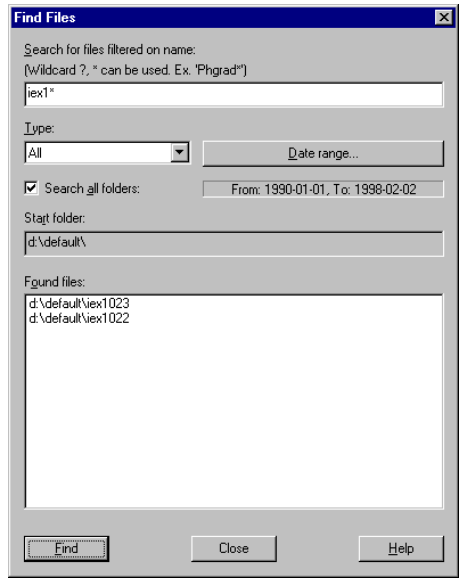


Figure 3-7. The Find file dialogue.

3. Click on **Find** when you have entered all parameters. The result of the search is shown in the **Found files** box.

4. Double-click on a file in this list to return to the Main menu with the selected file highlighted in the appropriate window. If you click on **Close** (with or without selecting a file), you will return to the Main menu with the window display unchanged.

3.3.5 Copying and moving files and folders

You can copy and move files and folders to another folder that is specific to your user logon name. You can also copy or move files to and from an external drive and folders available on the network. If you copy or move a folder, all files within the folder will also be copied or moved.

Copying or moving files and folders

1. Select one or more files or folders in either the **Methods** or **Results** window of the Main menu. To select multiple files or folders, use the standard Windows function keys <Ctrl> or >Shift>.

2. Click with the right mouse button on any file/folder icon and choose the **Copy** or **Move** command or select **File:Copy** or **File:Move**. The **Copy** or **Move** dialogue is displayed respectively.

3. Select an available folder or the diskette drive to which you want to copy or move the file/folder and click on **OK**. Copied files and folders are user-specific.

Note 1: You cannot copy or move files between the **Methods** and **Results** windows of the Main menu.

Note 2: Explicit authorisation is required to copy or move files (see Section 14.2).

Note 3: To copy a file within the same folder, open the file in the relevant UNICORN module, e.g. a method file in Method editor or a result file in the Evaluation module, and use the **File:Save as** command in the module to save the file with a different name from the original.

Note 4: When copying to a diskette (a:) use **Copy to external** so that the files are automatically compressed.

Note 5: If you are moving a method to another system, you must always use the **Copy to external**/**Copy from external** functions. this will give you the possibility of connecting the method to the appropriate system. The extension for the method file name is used to identify the system for which the method has been created. An incorrect extension may result in syntax errors in the method or the method not being visible in the **Methods** window of main menu.

Copying files to external

Copying files to external may be useful when you want to store all results, documentation etc. in a common project folder on the network, or want to back up the files in a special place.

To copy a method or result file to external:

1. Select the file to be copied in either the **Methods** window or **Results** window.

2. Select **Copy to external** from the right mouse button menu or select **File:Copy to external**. The **Copy to external** dialogue is displayed.

3. Select the destination drive and folder and click on the **Save** button.

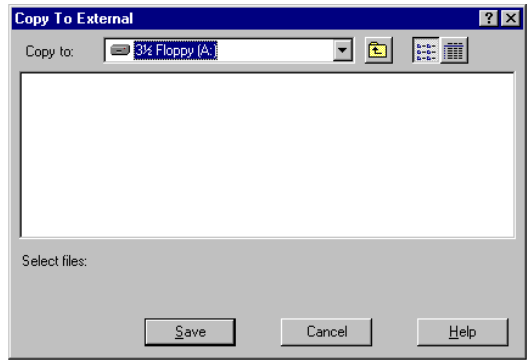


Figure 3-8. Copy to external dialogue.

Note: If you select the 3½" Floppy Drive (a:) as the destination drive, the files will be automatically compressed into a .zip file thus allowing approximately 5-10 times the storage capacity. Moreover, if the zipped file is greater than the storage capacity of the disk, the file saving is automatically spanned across several disks. Files are automatically decompressed when using the **Copy from external** operation (see below). The zip function does not work if you select the **Copy** function.

Copy files from external

Method and result files can be copied from external. If the selected files have been compressed using the **Copy to external** function, then these will be automatically decompressed. To copy a method or result file from external:

1. Select the destination folder in the **Methods** window or **Results** window.

2. Without selecting a file icon, bring up the right menu button menu and select **Copy from external** or select **File:Copy from external**. The **Copy from external** dialogue is displayed.

4. Select the wanted file(s) from the relevant source drive and folder. Click on the **Save** button.

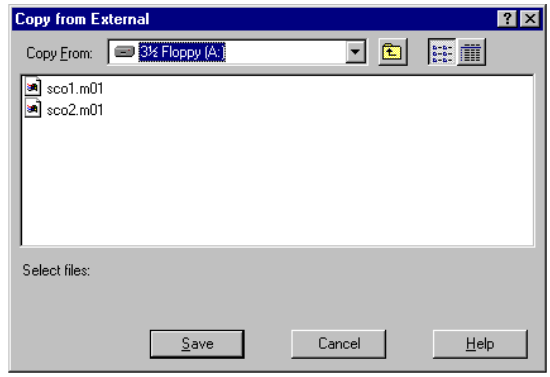


Figure 3-9. Copy from external dialogue, in this example, used to copy method files.

5. If result file(s) were selected, these will be copied into the previously designated folder in the **Results** window.

6. If method file(s) were selected, the **Method-System connection** dialogue is displayed.

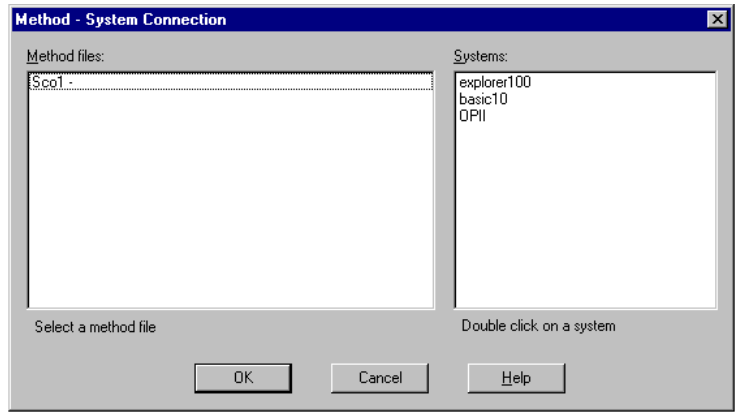


Figure 3-10. Method-System connection dialogue.

Each copied method listed in the **Method files** field must in turn be connected to the same type of system (same strategy) for which the method was originally created, listed in the **Systems** field. Highlight a method and double click on a system. Click on the **OK** button.

The **Method-System connection** dialogue is displayed again listing the remaining methods to be connected. Repeat the process until all methods have been connected.

Note: Method syntax errors may arise if a method created on one system is connected to a different type of system using the copy from external facility.

If at any time you press on the **Cancel** button, the **Method - System connection** dialogue is closed. However, it will reappear each time you perform other copy to/from external procedures for method files.

Method files that have been copied in and connected are displayed in the previously designated folder in the **Methods** window.

3.3.6 Deleting files

To delete a file or folder:

1. Select the item(s) to be deleted in the **Methods** or **Results** window of the Main menu. To select multiple files, hold down the <Ctrl> key while you click on the file names or icons.

2. Click with the right mouse button on any file icon and choose **Delete** from the menu, or choose **File:Delete**.

3. Confirm the deletion in the dialogue.

Note 1: Home folders cannot be deleted by this method (see Section 13.3).

Note 2: Explicit authorisation is required to delete files (see Section 13.2).

Note 3: A file that has been deleted cannot be recovered except by restoring a back-up copy.

3.3.7 Renaming files

To rename a file or folder:

1. Select a file or folder to be renamed in the **Methods** or **Results** window of the Main menu.

2. Click with the right mouse button on any file icon and choose **Rename** from the menu, or choose **File:Rename**. The **Rename** dialogue is displayed

3. Enter the new name for the file and click on **OK**.

3.3.8 Backup security

To protect important data against accidental deletion or loss in the event of hard disk failure, backup copies should be taken at regular intervals.

This can be best achieved by having the UNICORN folders on the server (if available) and working directly from these folders. Alternatively, you can use the **File:Copy to external** function to save files onto the network server. It is standard practice for backups to be made of the server folders. The responsibility for making backup copies rests entirely with the user. Amersham Biosciences cannot undertake to replace method programs lost as a result of computer failure or other incident.

3.4 Printer setup

UNICORN 3.1 uses the default printer and printer settings installed on your computer. To change the choice of printer, either change the default settings in Windows NT or set up your choice of destination printer for the current working session by selecting **File:Printer setup** in the Main menu module and selecting the desired printer.

3.4.1 Setting the margins

The default margins for the printers can be changed:

1. Locate the file UNICORN.INI found under C:\UNICORN\BIN, for example by using Windows NT Explorer.

2. Double click on the file to open it and locate the following lines:

```
EVAL     PrintMarginLeft  10
EVAL     PrintMarginRight  5
Eval     PrintMarginTop    5
Eval     PrintMarginBottom 5
```

   The values in the lines set the margins based as a percentage of the full width and height of the paper.

3. Change the values as appropriate and save the file.

Caution: Do *not* make any other changes in the UNICORN.INI file since this may severely affect the function of UNICORN.

3.5 Logging off

To log off from UNICORN click on the **Logoff** button or select the **Logoff** menu command.

Processes that are running when you log off will continue to run, and may be left locked with a locking password or unlocked (see Section 6.5 for more details). If the Method editor module was active at the time of logoff, it will be re-opened when the same user logs on again.

UNICORN will still be open after a user has logged off, and another user may log on. We recommend that you always log off when you leave the computer to prevent other users from accidentally changing or deleting your files or disturbing your runs.

3.6 Quitting UNICORN

To quit UNICORN and close the program, select **File:Quit program** in the Main menu. You will be prompted to save any unsaved data in the Method editor or Evaluation module. If a run is proceeding when you quit do not shut down Windows NT or turn off the computer while the run is in progress.

Note: You can not quit the program if you are performing a MethodQueue run.

Introductory material

Methods and runs

Evaluation

System management

Appendices

4 Creating methods from method templates

UNICORN is supplied with a set of method templates that can serve as the starting point for creating customised methods. These method templates are defined with variables for critical parameters in the synthesis, so that customised methods can be created for most purposes simply by setting appropriate values for the method variables. Different templates are provided for different system strategies. This chapter describes how to create and edit methods at this level. See Chapter 5 about advanced method editing facilities.

Briefly, the steps in creating a method by editing method variables are as follows:

1. Click on the **New Method** toolbar button in the Main menu, or select **File:New:Method** in the Main menu or **File:New** in the Method editor module. Select a system, technique, template and column.

2. Choose **View:Run setup** or press the **Run setup** button.

3. Adjust the values for the method variables.

4. Read the method notes.

5. Save the method.

Note: The fastest and easiest way to run a method is to click on the **Instant Run** toolbar button in the Main menu. This function runs a method template and the method is not saved in the Main menu **Methods** window. The method may, however, be recovered from the result file.

4.1 Creating a new method

To create a new method, do one of the following:

- click on the **New Method** toolbar button in the Main menu
- select **File:New:Method** in the Main menu

- click on the **New Method** toolbar button in Method editor
- select **File:New** in the Method editor

These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically.

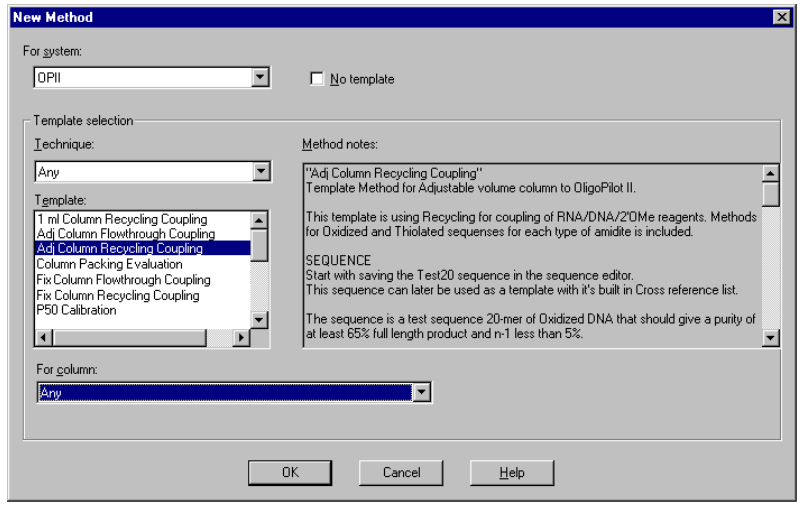


Figure 4-1. The New method dialogue.

1. Choose the system for which the method is intended.

   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

2. Select **Any** for the technique. The templates available for the selected technique will be displayed.

3. A list of ready-to-run method templates is displayed for the selected technique. Available templates are determined by the system strategy. Select one of these templates to create customised methods either by adjusting variable values or changing method instructions. For your first run you are recommended to select the method template, **Fix Column Recycling Coupling**.

   Click on a template to display information about the particular template in the **Method notes** field.

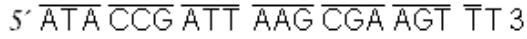
4. Choose a specific column to be used. Only columns for the selected technique are displayed. If you do not find your specific column it can be added to the list (see Section 5.9). Relevant column data are automatically copied into the method thus reducing the need to edit the method.

If **Any** is selected, you can use any column but must enter the column volume in the method on the Variables page. It is recommended that a specific column is selected.

5. Click on **OK** once you have made your selections. The method template will now be opened in Run setup view as an untitled method.

4.2 Saving and running a test sequence method

Several of the newly created templates already contain a partially built method for a pre-defined 20 base sequence, which is:



Note1: To view what the various symbols mean for the bases, please see the table in Section 4.3.

Note2: The synthesis reaction proceeds in the 3´- 5´ direction, so the 3´ base position is always the first base on the solid support before the start of the synthesis procedure.

This sequence can be viewed in the sequence editor in the Sequence page of Run setup, or the blocks for the sequence as blocks in the Text instruction panel. This partial method has two main uses:

- Used specifically with the **Fix Column Recycling Coupling** method template, you can save the method and directly perform a test run of UNICORN to synthesize the sequence (see below)
- Used with other templates containing the in-built sequence, you can replace the supplied sequence with a one of your own choice and then generate a ready-to-run method for that sequence (see Section 4.3).

To use the 20 base sequence for a test run of the instrument:

1. Create a new method according to Section 4.1.

2. Click on the Run set-up button on the toolbar or select **View:Run setup**.

3. Select the Sequence page to display the pre-defined sequence in the sequence editor field.

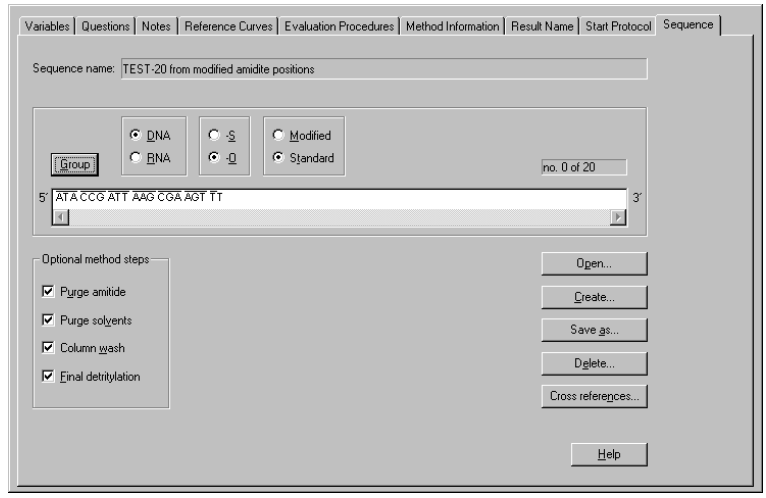


Figure 4-2. Sequence page in Run setup containing the 20 base sequence pre-defined in the method template.

4. Click on the **Create** button (see Section 4.3), which fully generates the method and inserts default variable values. The **Save As** dialogue is then displayed. Save the method with the name test20.

   The method must be saved before you can make a run. The test20 method will be displayed in the **Methods** window of the Main menu.

5. To run the test20 method, follow the instructions detailed in Section 4.7 and Chapter 6. You can change the method variables prior to the commencement of the run.

4.3 Creating a sequence and method

As described in Section 4.2, some of the method templates contain a partially built method for a pre-defined 20 base sequence. These templates are:

- **Fix Column Recycling Coupling**
- **Fix Column Flowthrough Coupling**
- **Adj Column Recycling Coupling**
- **Adj Column Flowthrough Coupling**

By replacing the pre-defined sequence in these method templates with a sequence of your choice, you can quickly and easily create a ready-to-run method.

1. Select a method template in the **New Method** dialogue box, as described in Section 4.1.

2. Click on the Run set-up button on the toolbar or select **View:Run setup**.

3. Select the Sequence page to display the pre-defined sequence. Select the pre-defined sequence in the sequence editor and delete it using the <Delete> key on the keyboard.

4. Enter a new sequence, up to a maximum of 200 bases, in the sequence editor in the 5´- 3´ direction. Remember that the synthesis reaction proceeds in the 3´- 5´ direction, so the 3´ base position is always the first base on the solid support. This base should be taken from the standard position and should be oxidated.

   Use the radio button combinations to select the base type to be added for each position. You are able to select DNA or RNA, whether the base is to be oxidated or thiolated, and whether the base is taken from the standard or modified reagent position. There are two extra physical reagent positions in OligoPilot II, labelled X and Y, both in the standard and modified positions. The extra characters Z and Q are also provided. The available combinations are as follows:

Radio button combination	**Bases as represented on the screen**
DNA, -O(xidated)**, Standard**	A, C, G, T, X, Y, Z, Q
DNA, -O(xidated)**, Modified**	$\overline{A}$, $\overline{C}$, $\overline{G}$, $\overline{T}$
DNA, -S (thiolated)**, Standard**	A, C, G, T, X, Y, Z, Q
DNA, -S (thiolated)**, Modified**	$\overline{A}$, $\overline{C}$, $\overline{G}$, $\overline{T}$
RNA, -O(xidated)**, Standard**	a, c, g, u, x, y, z, q
RNA, -O(xidated)**, Modified**	$\overline{a}$, $\overline{c}$, $\overline{g}$, $\overline{u}$
RNA, -S (thiolated)**, Standard**	a, c, g, u, x, y, z, q
RNA, -S (thiolated)**, Modified**	$\overline{a}$, $\overline{c}$, $\overline{g}$, $\overline{u}$

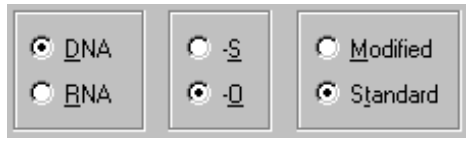


Figure 4-3. Radio buttons in the Sequence page used for choosing the base type to be included in the sequence.

5. Click on the **Group** button if you want the sequence to be displayed in groups of three bases, beginning from the 5´ end.

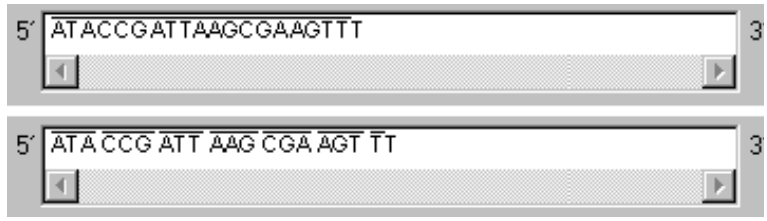


Figure 4-4. An ungrouped (top) and grouped (bottom) sequence in the sequence editor field of the Sequence page.

6. To save the sequence you have created, click on the **Save As** button and type in a name for your sequence. The name can be up to 256 characters in length. Click on **OK**. The name of the saved sequence will now be displayed in the Sequence page containing the specific sequence. Note that saved sequences are personal to the current user, i.e. users logged in under a specific username will not see the saved sequences of another user.

7. Place a check mark in those boxes beside the **Optional method steps** that you want to be included in your method.

8. Create the method for the sequence you have entered by clicking on the **Create** button. The **Create** button serves four main purposes:

   - to check the sequence for invalid combinations (ignoring the 3´ base), e.g. it is not possible to include both base 'A' (DNA) and base 'a' (RNA) in the same sequence since they both take up the same reagent bottle position on the instrument.
   - to generate a method based on the sequence and cross-reference list (see Section 5.1.1).

- update the method variables based on the generated method.
- display the **Save As** dialogue so that the method can be saved before performing a run.

Enter a name for the method, select the destination and click on **OK** (see Section 4.6 for more details). The method is saved with default values for the method variables. These can later be changed before you start a run (see Section 4.7 and Chapter 6) or you can change the variables and save the method under a new name.

Note: Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an already saved method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was saved with specially modified blocks (see Section 5.2) or cross-reference lists (see Section 5.1.1).

4.4 Editing method variables

The method templates are constructed from blocks representing the stages in a typical synthesis. Each block has a set of method variables, displayed on the Variables page in the run setup. You set default values for the variables in the Method editor, and can change these values for a particular run in the start protocol before the run is started.

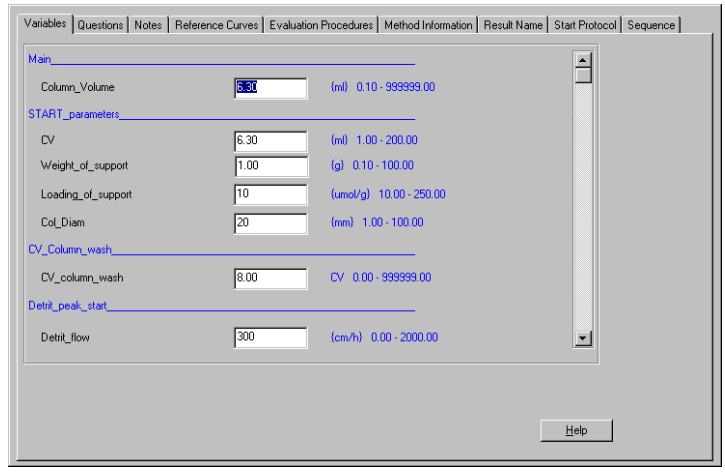


Figure 4-5. The Variables page in Run setup.

Work through the variable list, adjusting the values to suit your synthesis. To change a variable value, simply type the required value in the field. Remember that the values you enter here will be default values, suggested each time the method is run.

If the whole variable list does not fit on one screen, a scroll bar will be shown to the right of the list. Click on the arrows at the ends of the bar to scroll one variable at a time, or on the bar itself to scroll one screen at a time. You can also drag the slider button to scroll, but this is not recommended since you can easily miss variables by scrolling too far.

Typical blocks are illustrated with the list below, taken from a method created for OligoPilot II with the **Fix Column Recycling Coupling** template. The list is organised according to the blocks in the method, with mentioned variable parameters identified in italics. Other method templates have different structures and variables.

- Start_parameters

These variables together define the synthesis scale, i.e. Weight of the support, Loading of the support and Column diameter. CV (column volume) is also defined and is used for the calculation of special instructions such as Vol_Cap, Vol_amid and CT5_Cap.

- CV_column_wash

The number of column volumes (CV) to wash the column is set here. If zero is entered, no wash will take place.

- Detrit_peak_start

The flow rate of the detritylation solution is set here.

- Detrit_wash

The pressure of the detritylation wash and the number of column volumes of detritylation solution to be used are set here.

- DNA_Parameters

These variables together define the coupling of a base to the oligonucleotide sequence, i.e. how many equivalents of amidite should be added to the column with respect to the scale, the percentage volume of tetrazole to be used with respect to column volumes and the concentration of the amidite.

- DNA_Recycle

The amidite recycling flow rate and time used are set here.

- Oxidation_DNA

Oxidation stabilizes the phosphite group of the coupled amidite. The variables determine how many equivalents of iodine are used in the oxidation solution and the contact time between the oxidation solution and the support.

- Capping

The unreacted 5´-hydroxyl groups on the oligonucleotide are capped to prevent further participation in the synthesis reaction. The column volumes of capping solution and the contact time are set here.

Click on the x-axis button in the graphical display to change a base for the graphical display. Changing the display base will not affect the base in the method.

4.5 Method notes

Click on the **Notes** thumb-tab in the Run setup to show the **Notes** page, and read through the method notes. You can maximise each section in the notes page to fit more of the text on one screen. Click on the printer icon or choose **File:Print** to print the method notes.

The method notes provided with each template describe the important information about the template and, if relevant, how the system should be connected for the method to work correctly. If your system does not correspond to the description, either rearrange the valves and tubing connections in accordance with the method notes description or edit the method instructions (see Chapter 5) in accordance with your system setup.

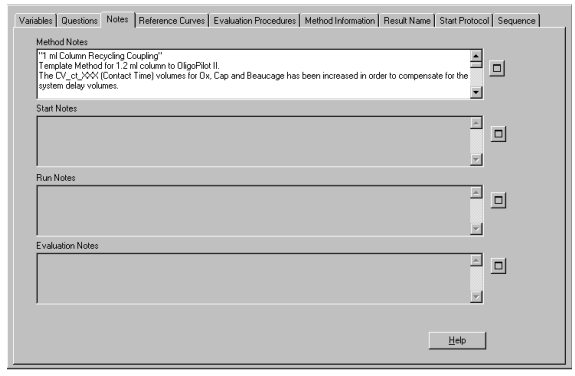


Figure 4-6. The Notes page in Run setup with the method notes maximised.

4.6 Saving the method

A new method created from a method template is untitled, and must be saved under a method name before it can be run. Click on the **Save Method** toolbar button or choose **File:Save** to save the method.

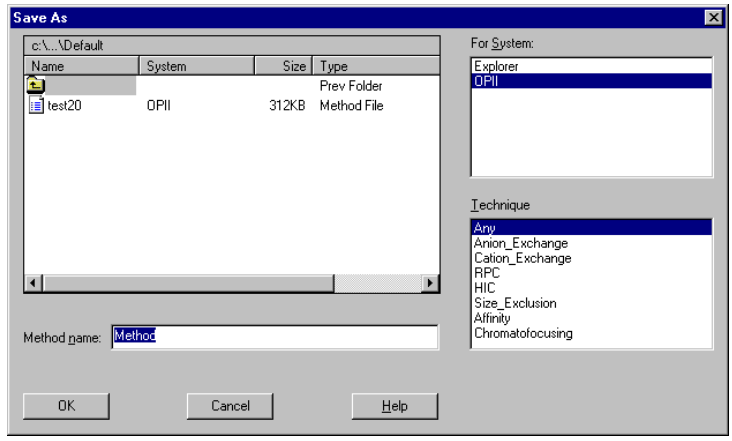


Figure 4-7. Save as dialogue for saving a method.

1. If required, select another folder than the default home folder in which to save the method.

2. Enter a **Method name** for the method. Method names may be up to 256 characters long. The method name must be unique for the chosen system within the folder (see steps 2 and 3).

3. If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

4. Choose the **Technique** for which the method was written.

5. Click on **OK**.

Note: The method templates are written for standard strategies. If you receive a syntax error message when the method is saved, one or more instructions in the method are invalid. These may be calls to blocks which are not defined, or instructions which are invalid in your customised strategy (this can also arise if a method is written for one system and saved for another). Invalid instructions are marked in red in text instruction mode in the Method editor (see Section 5.4.1), and must be deleted or replaced before the method can be run.

The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. Once the method has been saved, choosing **File:Save** saves the current state of the method under the same name. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described above.

4.7 Starting a run

This section briefly summarises how to start a run with a method. The method must be named and saved before it can be started. See Chapter 6 for more details of how to run a method.

Note: If you are editing the method in the Method editor and have made changes that you have not yet saved, these changes will not apply during the run. Similarly, if you edit the method while it is running, the run will not be affected. It is the version of the method that is saved on disk at the time when the method is started that controls the run.

1. Establish a control mode connection to the system where the method is to be run. See Section 6.5 for details. You cannot start a method without a control mode connection to the appropriate system.

2. Choose **File:Run** from System control for the required connection and select the method to run. Alternatively, click on the method in the **Methods** window of the Main menu and select **Run** from the right mouse button menu. Do *not* double-click on the method icon in the Main menu as this will open the Method editor.

3. Change the method variable values if required. The suggested values are those saved in the method. Any changes you make will apply only for the current run, and will be recorded in the run documentation.

4. Go through the rest of the Start protocol, entering information where appropriate. Use the **Next** and **Back** buttons to move through the Start protocol. If you click on **Cancel** on any page in the start protocol, the method will remain loaded in the System control module but will not start. Start protocol pages for most method templates are:

Variables	Adjust variable values as required for the run.
Questions	Fill in answers to the questions.
Notes	Read the method notes and enter start notes if required.
Evaluation Procedures	Select the **print_chromatogram** procedure if you want the results to be printed automatically.
Oligosynthesis Sequence	The sequence of the oligonucleotide to be synthesized.
Result	Set the result file name and path (folder) as required. The default result file name includes a 2-digit serial number.

5. The last page of the start protocol has a **Start** button. Click on this button to start the run.

4.8 Editing text instructions

Methods for most purposes can be created by adjusting the method variable values as described above. The method is actually programmed as a series of instructions that use these variables as parameters. To see and/or change the instructions, click on the **Text Mode** button on the Method editor toolbar or select **View:Text instructions** from the menu.

With the Text instruction editor, you have complete facilities for designing and editing your own customised methods. You will also use the Text instruction editor for refining and modifying methods based on the standard templates, e.g.

- Changing the method base (column volume, volume or time).
- Changing valve specifications for inlet and outlet (if the templates do not suit your system configuration).

- Adding or removing variables.
- Adding or removing instructions to change the method functionality.
- Adding or removing blocks to change the method structure.

To gain an understanding of how method templates are built up and can be modified, work through Chapter 5 which gives a full description of method editing facilities.

5 Creating and editing methods

This chapter describes the complete facilities for creating and editing methods in UNICORN. Refer to Section 2.3 for an overview of method concepts. For many applications, suitable methods can be created by changing the default variable values in one of the templates supplied with UNICORN (see Chapter 4). Use the more advanced editing facilities described here for:

- changing selected instructions in the method templates, e.g. changing the outlet valve position
- adding blocks and instructions, e.g. Watch instructions
- changing method instructions to adapt to non-standard system configurations
- creating new methods for applications which are not covered by the templates supplied

Advanced editing facilities can be used at three different levels. The chosen level is dependent on the type and extent of changes to be made:

- modifications at the sequence editor level, i.e. creating custom methods by assigning a specific base in the sequence editor to a freely selected block (see Section 5.1).
- adding new blocks to an existing method and/or modifying instructions in existing blocks (see Section 5.2).
- writing a new method “from scratch”, i.e. selection of **No Template** in the **New Method** dialogue.

5.1 The sequence editor

The sequence editor in the Sequence page of Run setup is a user interface which allows methods to be easily created. The bases in a sequence are each cross referenced to a specific block in the method template, with each block representing a series of instructions to be performed. Thus, in entering a sequence into the sequence editor and clicking on the **Create** button, the specific blocks that are cross-referenced to the bases are copied from the method template into the method. The method must be saved before it can be then used to run an instrument.

For example, in a sequence containing the bases 5´-...AC...3´, the DNA base C is assigned to a specific block in the method template called **Add_DNA_C**. Consequently, by clicking on the **Create** button in the Sequence page, the C base in the sequence causes the block **Add_DNA_C** to be copied into the method. The next base in the sequence, DNA base A, is assigned to the block name **Add_DNA_A**. Thus, the block cross referenced to base A will next be added to the method (see Figure 5-1). In the standard method templates, each block that is cross-referenced to a base is a self-contained set of instructions to perform a complete coupling cycle of one base, i.e. detritylation, detrit wash, coupling, oxidation or thiolation and capping (see Figure 5-1).

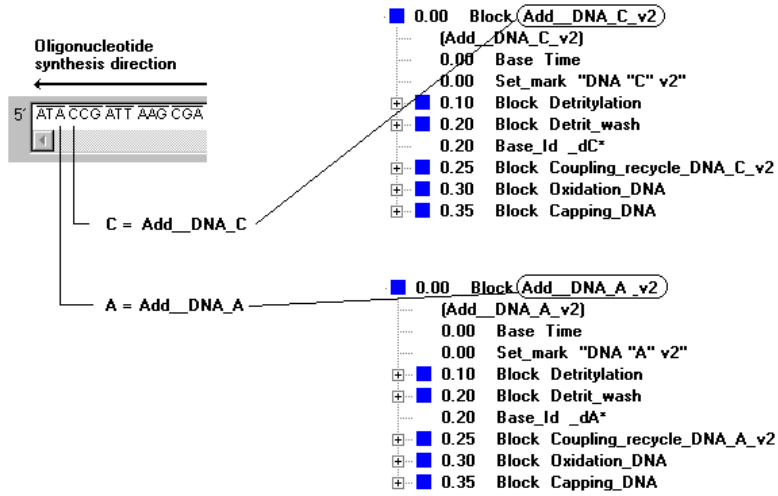


Figure 5-1. The relationship between the bases entered into the sequence editor and the blocks copied into the method. In the example, the sequence base C is cross referenced to the block name Add_DNA_C, which is then copied into the method. Similarly for the following base A, this is cross referenced to the block named Add_DNA_A which is also copied into the method. Note that each block contains a self-contained set of instructions for the complete coupling cycle of the specific base.

5.1.1 Modifying cross references in the sequence editor

Cross referencing a base to a method template block lends much flexibility to modifying the method creation process. UNICORN OS allows the user to change the block that is cross-referenced to any specific base type.

For example, you may decide that the DNA sequence 5´-ACTGGT-3´ should have a column wash step after the addition of each base in the sequence. By cross referencing a base not used in the current sequence, e.g. A, to the column wash block in the method template, this can be directly incorporated into the sequence to signal a column wash. Thus, in the sequence 5´-AAACATAGAGAT-3´, the appearance of A does not mean that a thiolated DNA-A base will be added to the oligonucleotide, but rather a column wash procedure is performed after the addition of the preceding base.

Alternatively, you may modify the instructions in an existing block or create a new block. It is thus a straightforward task to assign the new block to the base in question.

To change the assignment, do the following:

1. Create a new method by clicking on the **New Method** toolbar button or choose the **File:New:Method** menu command in the Method editor, or by choosing **File:New:Method** or **New:Method** in the Main menu. These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically. The **New Method** dialogue box will be displayed.

2. Choose the system for which the method is intended, e.g. OligoPilot or OligoProcess. From the **Technique** pull-down list, select **Any**. Next, select one of the available method templates in the **Template** pull-down list.

3. Click on **OK** once you have made your selections. The method template will now be opened as an untitled method.

4. Click on the Run setup button or select **View:Run** setup. Select the Sequence page.

5. Click on the **Cross references** button to display the **Cross reference list** dialogue.

   Select the **Coupling list** tag from the available **List** field. In the **Block** field you will see the all the bases and the block name that each is cross referenced to in the method template. For example:

   ```
   A = Add_DNA_A
   ```

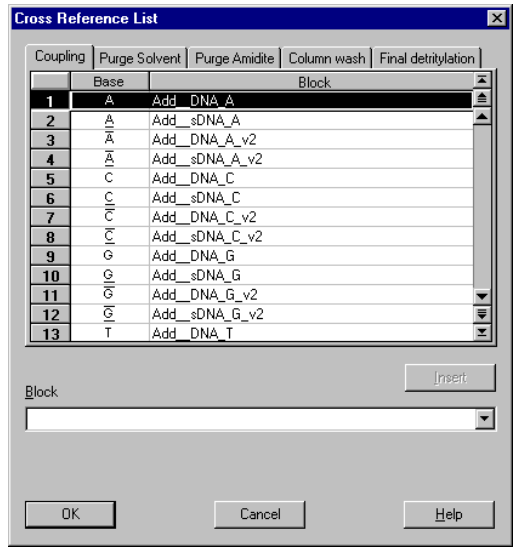


Figure 5-2. Cross reference list dialogue box.

6. To change the assignment of a base to a different block in the method template, first select the base in the **Block** field. Next, click on the **Block** pull-down list and select the method template block that you want assigned to the selected base. The base in the **Block** field will now be associated with the block name that you chose.

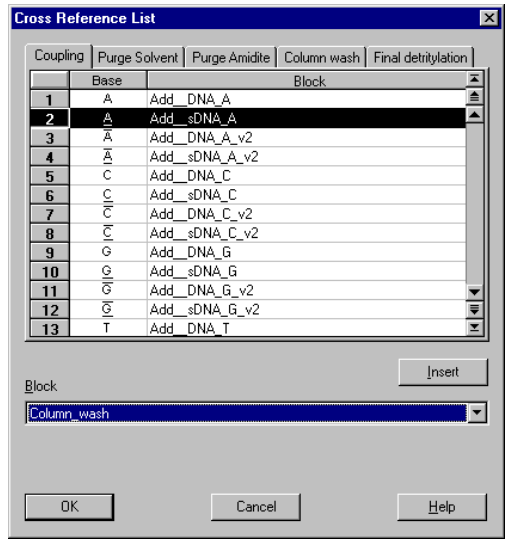


Figure 5-3. In the above example, the base A has been cross referenced to the block Column_wash.

You can select any block contained in the method template, even those that you have modified or created (see Section 5.3). Repeat this process for other bases as appropriate.

7. You can also reassign the cross references for the optional method steps selected in the Sequence page. The blocks for **Column wash**, **Final detritylation**, **Purge Amidite**, and **Purge Solvent** can be assigned by clicking on the appropriate tab and then using the **Block** pull down list.

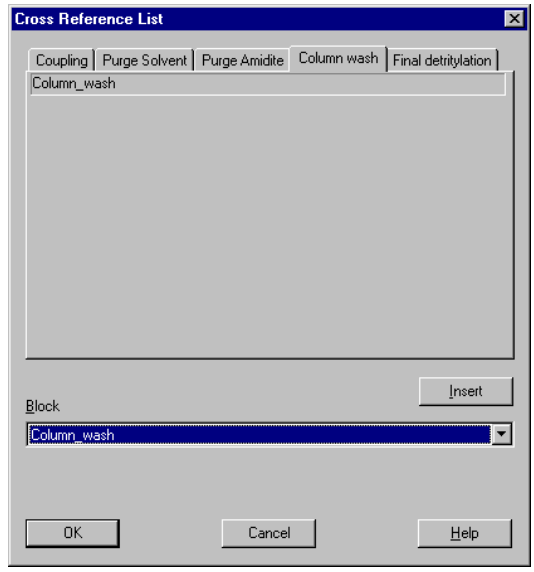


Figure 5-4. Fields for cross referencing the column wash and final detritylation blocks in a method.

7. Click on **Exit** to implement the new cross references.

8. Enter the appropriate sequence in the sequence editor field of the Sequence page. Create a method as described in Section 4.2.

9. Save the method (see Section 4.6).

   Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an already saved method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was previously saved with a specially modified cross-reference list. In such circumstances, open the appropriate method containing modified cross-reference list and access the Sequence page in Run setup. Delete the existing sequence in the sequence editor and enter the new sequence. Click on the **Create** button.

Note: Do *not* open a saved sequence from your personal sequence list in the Sequence page, otherwise the cross-reference list corresponding to the saved sequence will replace the modified cross-reference list present in the current method. Always manually enter your sequence over the original method sequence.

5.2 Text instruction editor

The next level of advanced editing to create new methods uses Text instructions. This involves modifying the instructions within the blocks of an existing method and/or adding new blocks to methods.

Note: Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an existing method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was previously saved with specially modified blocks.

This section introduces you to the Text instruction editor and the following sections present the components of a method and how to edit and create blocks.

The text instruction editor is used for entering and editing method instructions. Click on the **Text instructions** toolbar button or choose **View:Text Instructions** to display the text instruction editor.

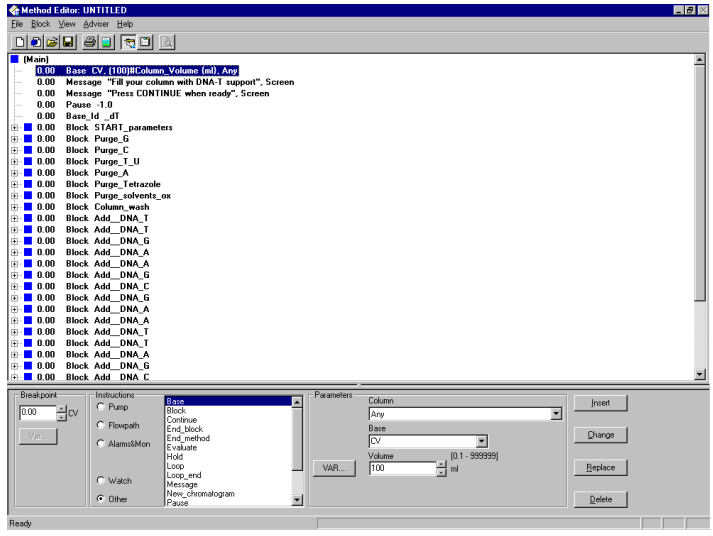


Figure 5-5. The Method editor in text instruction mode, showing the Block window (top), text instruction window (centre) and instruction box (bottom).

Up to four windows can be displayed together with the instruction box. Click on the **View Windows** toolbar button or choose the **View:Windows** menu command to open a dialogue for choosing which windows to display.

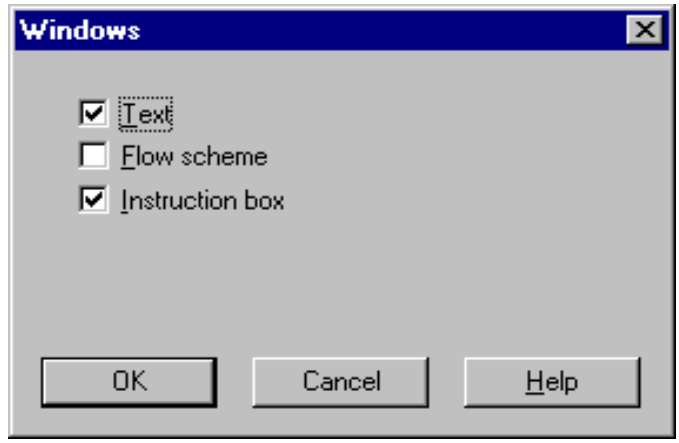


Figure 5-6. The Windows dialogue for selecting which windows to display in the Method editor.

Method editing operations which can be performed in the various windows are summarised in the table below.

Window	*Operations*	*See Section*
Text window	Display and hide block instructions. Select current instruction. Move instructions within a breakpoint.	5.4
Flow scheme window	For information only. The flow scheme picture is static and is therefore not updated according to system status or changes in the method.	5.4.5
Instruction box	Specify breakpoints, instructions, parameters and variables. Insert, change and delete instructions.	5.4.2 - 5.4.4

5.2.1 Run setup

The Run setup is a series of pages for defining the method properties. To access the Run setup pages, press the **Run setup** toolbar button or select **View:Run Setup** from the menu.

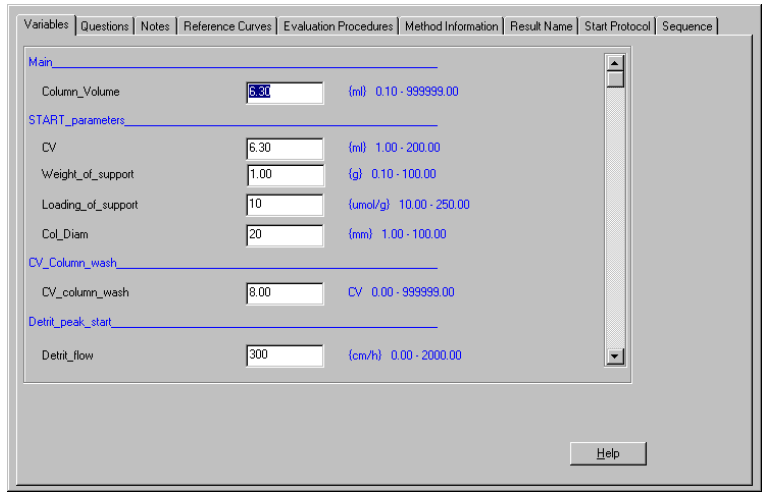


Figure 5-7. Run setup with the Variables page displayed.

To access a given page in the Run setup, click on the relevant tab. Pages in the Run setup editor are described in Section 5.6.

5.3 Method blocks

Viewing a method as a long list of individual text instructions would be confusing and inconvenient. Text instructions are therefore conveniently grouped into blocks of instructions that define a specific functional use. For example, one block might contain the instructions necessary for equilibrating a column, and another block contain instructions for adding a single defined nucleotide in the sequence, etc. By using such blocks it is easier to build up a total method for a run.

5.3.1 Viewing blocks

It is possible for one block to contain one or several other blocks. This is most evident, for example, in the Text instruction window.

In the text instruction window

In the text instruction window, the method is shown as a list of blocks, denoted by the blue square symbols. Beside each block is also a '+' symbol, which you can click on to expand the view of the instructions within the block. Note that a block can also contain other blocks as denoted by the blue square symbols.

Unexpanded blocks in a method

Expanded block in a method containing text instructions and blocks.

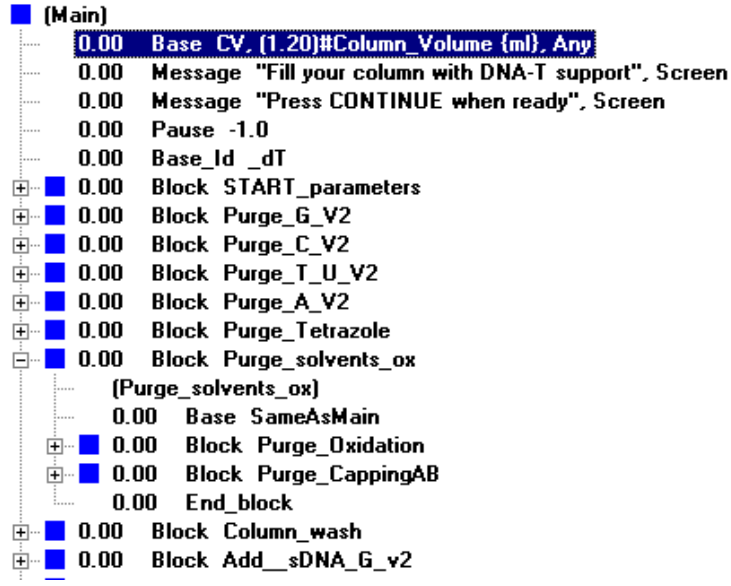


Figure 5-8. Text instructions showing blocks in a method.

To collapse the view, click instead on the '-' symbol for that block.

Alternatively you can double click on the block name to view or hide the instructions (see Section 5.4.1).

In the block window

The organisation of blocks in the method is shown graphically in the block window in text instruction mode.

Each block is represented by a grey bar with the block name and the length of the block. The line is shifted down to indicate calls to other blocks. In the example below, the blocks are called in sequence from the Main block at breakpoint 0. Blocks to which there is no valid call are not shown in this window.

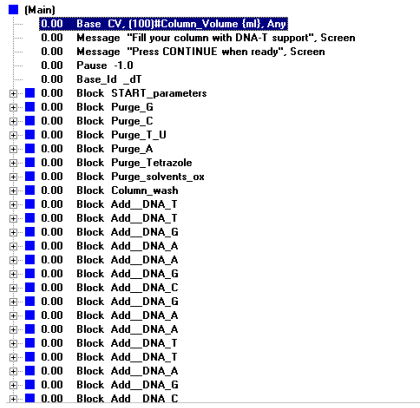


Figure 5-9. The Method editor block window.

Conditional (Watch) instructions are indicated by a green line showing the start and duration of the watch. The example above has a Watch instruction to start the fraction collector which is active throughout the gradient elution block. Loop instructions (to repeat a group of instructions) are also indicated in the block window.

If you click on the line representing a block in the block window, the first instruction in the block will be highlighted in the text window.

5.3.2 Calling blocks

To execute the instructions contained within a block in a method, the block must be *called* by the program. When a block is called the instructions in the block are executed in the order that they are written 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.

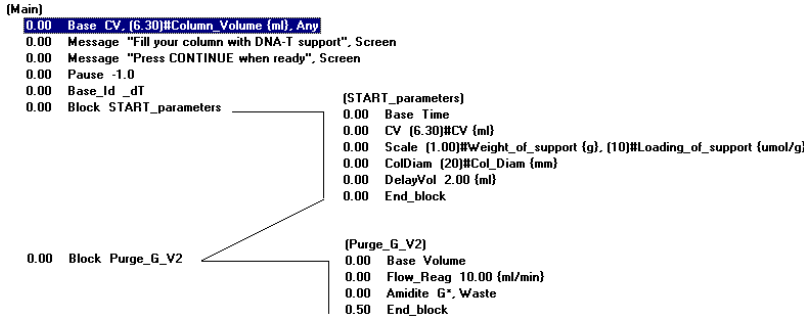


Figure 5-10. Illustration of the flow of process control through method blocks.

Calls may be of two types:

- *Unconditional calls* are made with the Block instruction.
- *Conditional calls* are made with a Watch instruction, which makes it possible to call a specified block or an instruction when a particular monitor signal meets a given condition. As long as the condition is not met, the block is not activated. There are different Watch instructions for each process monitor signal, and each Watch instruction can use various conditions to respond to absolute signal values or to rate of signal change.

  Note that the breakpoint when the Watch instruction is issued determines when the watch begins, not when the block is activated. The block will in fact never be activated if the watch condition is not met during the run.

  Once set, a watch remains active until the condition is met or a new Watch instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the Watch_off instruction.

  See Section 5.8.7 for more details of Watch instructions.

5.3.3 Adding blocks

To add a new block use the Text instruction editor and click on the **New block** toolbar button or select **Block:New**.

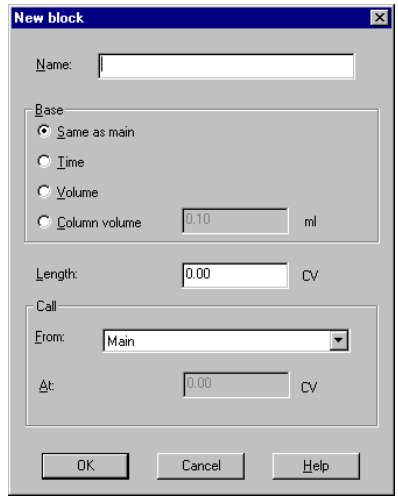


Figure 5-11. New block dialogue.

Alternatively you can enter the Block instruction in the Instruction box, enter a name for the block in the parameter field and click on **Insert**.

Block name

Enter a name for the block in the **Name** field. Block names may be up to 30 characters long, and may contain letters (A-Z), digits (0-9) and underscore characters.

Block names must be unique within the method. The case of letters is retained but is not significant (the names Add_DNA_W and add_dna_w are treated as identical).

Base

Choose a base for the block. If you choose **SameAsMain**, the new block will inherit the base from the Main block in the method. The corresponding Base instruction will be inserted in the block at breakpoint 0. If you choose **CV**, enter a value for the column volume. If you chose a specific column, a column volume is entered automatically.

Length

You can enter a length for the block if required. An End_Block instruction will automatically be inserted in the block at the corresponding breakpoint. This field may not be left blank.

Call

You can call the new block from an existing block (e.g. the Main block). The block is called by an instruction named Block. Choose the block from which the newly created block should be called in the **From** field and enter the breakpoint at which the call is to be made in the **At** field. If you do not want to call the block (e.g. when the block being created is to be activated by a Watch instruction), choose an empty line in the **From** field. These blocks are placed last in the method in the Unused category.

Note 1: If the Block instruction is placed at the same breakpoint as the End_Block instruction, the Block instruction will be placed immediately before End_Block.

Note 2: Do not call a block from within itself. You will generate a potentially infinite loop, which exceeds the maximum number of calls allowed in a method. A loop symbol is displayed at the beginning of the line if this occurs.

Press **OK** to add the new block.

Strategy for creating blocks

For blocks which are to be called unconditionally, you have the option of creating the Block instruction at the same time as you create the block, by selecting where the block will be inserted in the **From** field of the **New block** dialogue.

For blocks which are to be called conditionally with a Watch instruction, first create the block and save it under unused by selecting an empty line in the **From** field. Insert the Watch instruction into the text method and then make a call to the block. The block will move from unused to beneath the Watch instruction.

Note: If you call the block before inserting the Watch instruction, a copy of the block will be created, i.e. there will be two instances of the block in the text method.

5.3.4 Deleting blocks

To delete a block from the method using the right mouse button menu:

1. Click on the desired block in the method with the right mouse button to display the menu.

2. Select **Delete**. A warning dialogue is displayed requesting if you want to totally delete the block instruction from the method. Answer as appropriate:

   Yes The block is totally removed from the method. Blocks deleted in this fashion can not be called again in the method.

   No The block is deleted from the method and transferred the Unused line. Blocks deleted in this fashion can be called again in the method.

To delete a block from the method using the **Block:Delete** command:

1. Select the menu command **Block:Delete**. The **Delete block** dialogue is displayed with all blocks listed in alphabetical order.

2. Select the block to be deleted and click on **Delete**.

3. A warning dialogue is displayed requesting if you want to totally delete the block instruction from the method. Answer **Yes** or **No** as described for this function using the right mouse button menu command (above)

5.3.5 Renaming blocks

To rename blocks, choose **Block:Rename Block** or select a block in the method using the right mouse button and select **Rename** from the menu. In both instances the **Rename blocks** dialogue is displayed.

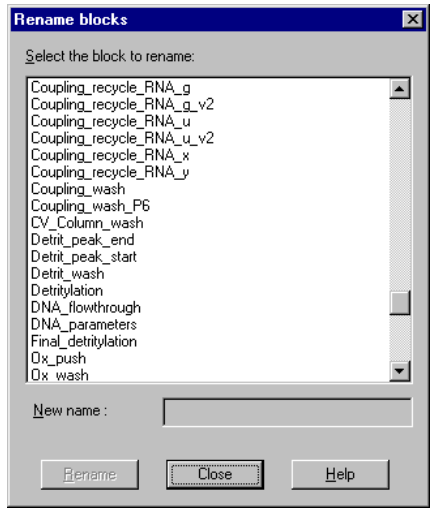


Figure 5-12. The Rename Blocks dialogue

By default, the block that is currently highlighted in the method text instructions is automatically selected in the dialogue. Enter the new name in the **New Name** field and press **Rename**. The dialogue remains open until you press **Close**, so that you can rename more than one block without closing the dialogue.

If the block you renamed is used in a Block or Watch instruction, the block name in these instructions will be changed automatically.

5.3.6 Copying, moving and importing blocks

You can copy, cut and paste blocks within a method or import blocks from another method.

Copying blocks

To copy blocks within a method:

1. Select the block to be copied using the right mouse button. Select **Copy** from the menu.

2. Select the instruction line *before* which you want the copied block to be pasted.

3. Click on the right mouse button and select **Paste** from the menu. A dialogue requests if you wish to rename the pasted block. Click on **Yes** to rename the block before insertion or **No** to directly insert the copied block.

   The pasted block is inserted with the same breakpoint value as the block selected for point of insertion.

Cutting and pasting blocks

To cut and paste blocks within a method:

1. Select the block to be copied using the right mouse button. Select **Cut** from the menu. The block is removed from the text instruction window.

Note: A cut block does not mean that it has been deleted (see Section (5.3.4) and can still be called from elsewhere in the method.

2. Select the instruction line before which you want the cut block to be pasted.

3. Click on the right mouse button and select **Paste** from the menu.

   The pasted block is inserted with the same breakpoint value as the block selected for point of insertion.

Importing blocks

Blocks may be imported from other method files to which you have access including the current method file in which you are working.

1. Select **Block:Import Block As**.

2. Choose the method from which you wish to import and mark the block to import. The name of the selected block is suggested in the **Block name** field.

3. Select a block from the **Call** drop-down list into which the imported block will be placed and enter a breakpoint value.

4. Click on **Import** to import the block. The dialogue remains open until you click on **Close**, so that you can import more than one block without closing the dialogue.

Note: If you use the import function to copy blocks within a method, the blocks are copied from the saved version of the method on disk. Any changes you have made in the method but not yet saved will not be copied.

The imported block may not have the same name as an existing block in the method. If the default name is not allowed for this reason, the **Import** button will be grey and locked. Change the name of the imported block so that the **Import** button becomes available.

The block is imported exactly as it appears in the source method. If the base of the imported block is defined as SameAsMain, the block will inherit the main base in the new method, regardless of the base in the source method. Also, the pasted block is inserted with the same breakpoint value as the block selected for point of insertion.

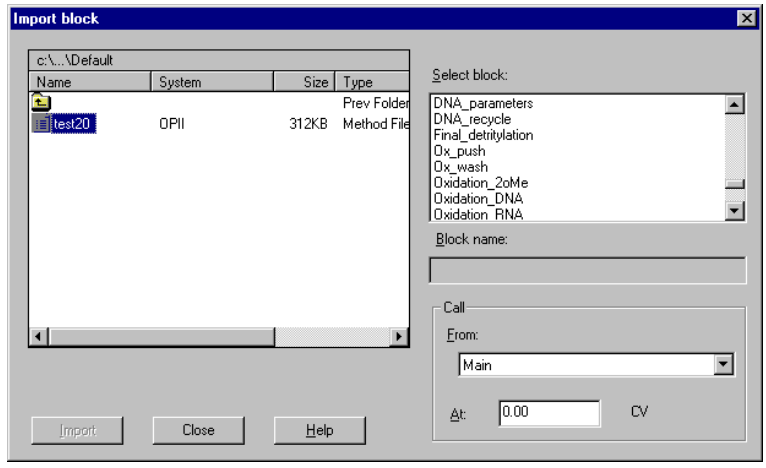


Figure 5-13. The Import Block dialogue.

5.4 Method instructions

Use the Instruction box in text instruction mode to enter, edit and delete instructions.

5.4.1 Viewing instructions

Instructions are displayed in the text Instruction box.

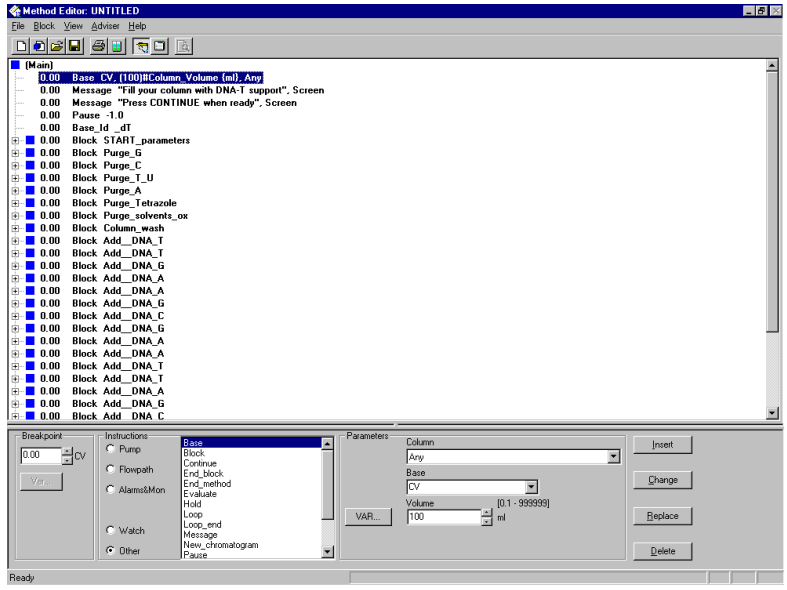


Figure 5-14. The text instruction window (top) with the instruction box (bottom).

Instructions are displayed in the text instruction window as follows:

Blue square beside text	Valid call instructions (i.e. Block and Watch instructions to other blocks in the method).
Bold text	Valid instructions.

Red bullet beside text	Instructions with invalid syntax. These may be: (a) calls to blocks which are not defined in the method, or (b) instructions which apply to a different system strategy (these can arise if a method is written for one system and saved for another). All such instructions must be deleted or changed before a method can be run (see Sections 5.4.3 and 5.4.4).
Normal text	Instructions which will not be executed because they are either after the end of a block or method or constitute a block to which there is no call.
 Text with a loop symbol	When a block is called from within itself this will generate a potentially infinite loop, which might exceed the maximum number of calls allowed in a method.

Double-click on a Block or Watch instruction to display or hide the instructions in the called block or click on the '+'/'-' symbol for the block respectively. Double-clicking on the **Main** keyword at the beginning of the method will show or hide instructions in all blocks in the method.

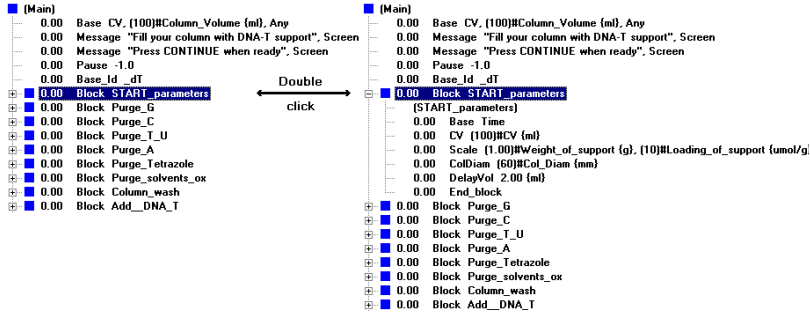


Figure 5-15. Displaying and hiding block instructions.

General oligosynthesis instructions are listed in Appendix B.

5.4.2 Adding instructions

To add a new instruction:

1. In the text instruction window, select an appropriate block and display the instructions within the block.

2. Select an instruction line in the block.

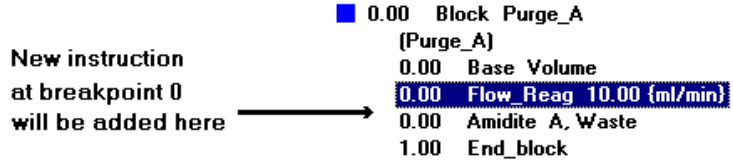


Figure 5-16. Instructions added at the same breakpoint as existing instructions are inserted after the highlight.

Note: Make sure that the selected instruction line is within the block, not the call to the block.

3. New instructions are added from the Instruction box. Open the Instruction box if not already displayed (**View:Windows**). For the new instruction:

   (a) Set the desired breakpoint in the **Breakpoint** field.

   (b) Choose the instruction type from the five or six options, depending on the strategy, and select the desired instruction from the displayed list. For short help on the purpose of each instruction, click on the instruction and press <F1>.

   (c) Enter values for instruction parameters in the **Parameters** field. If a scroll bar appears on the right-hand side of the **Parameters** field, additional parameters are required.

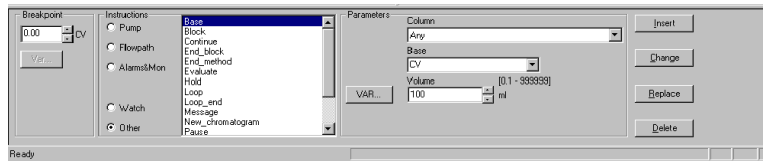


Figure 5-17. The Method editor Instruction box.

4. Click on **Insert**. The new instruction will be inserted in the block either

   (a) at the position of the breakpoint of the new instruction if there are no other instructions at that breakpoint

   (b) immediately after the currently highlighted instruction if the highlight is at the same breakpoint as the new instruction

   (c) as the last instruction at the breakpoint if there are several instructions at the same breakpoint as the new instruction and none of these is highlighted.

Note: Instructions that are placed at the same breakpoint are executed simultaneously, with the exception of Block instructions which are executed in the sequence in which they are written.

5.4.3 Deleting instructions

To remove an instruction:

1. Select the instruction in the text instruction window.

2. Press **Delete** in the Instruction Box, or press on the <Delete> key, or click on the right mouse button and select **Delete**.

An instruction that has been deleted can only be recovered by re-inserting the instruction. If you want to suspend execution of an instruction temporarily (e.g. during development work), you can replace the breakpoint with a value after the End_block or End_method instruction. Any instructions after the end of a block or method will not be executed.

Note: You cannot delete the Base instruction at the beginning of a block.

Caution: If you delete the End_block instruction, the block will end at the last instruction in the block.

5.4.4 Changing instructions

There are three possibilities for changing an instruction:

- change the breakpoint
- change parameters (including variables, see Section 5.5)
- select another instruction

To change an instruction:

1. Select the instruction in the text instruction window. The instruction with its current parameters will appear in the Instruction Box.

2. In the Instruction Box, make the required changes to the breakpoint or parameters or choose a new instruction.

3. Press **Change** or **Replace**. These buttons are equivalent unless changes are made to the breakpoint or the length of a gradient

Changing breakpoints

Change and **Replace** have different functions if the breakpoint is changed:

- **Change** 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 block.

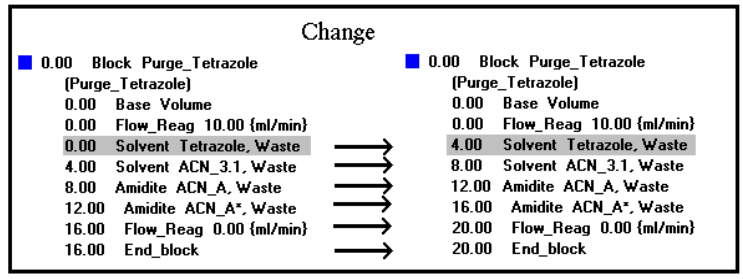


Figure 5-18. Change moves the selected instruction and all subsequent instructions.

- **Replace** 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:

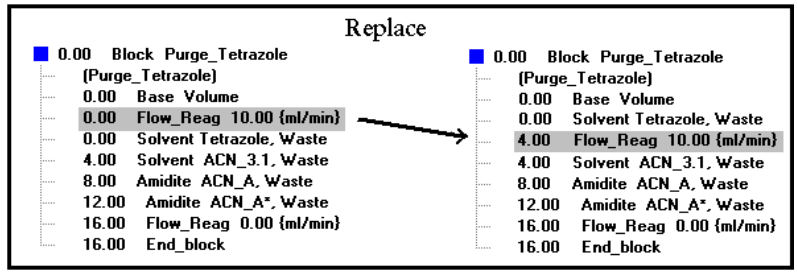


Figure 5-19. Replace moves only the selected instruction.

Moving instructions within a breakpoint

To change the order of instructions within the same breakpoint in a block, mark the instruction to move with the left mouse button and drag the instruction to its new location, holding the left mouse button down. You can only move instructions in this way within a group of instructions at the same breakpoint.

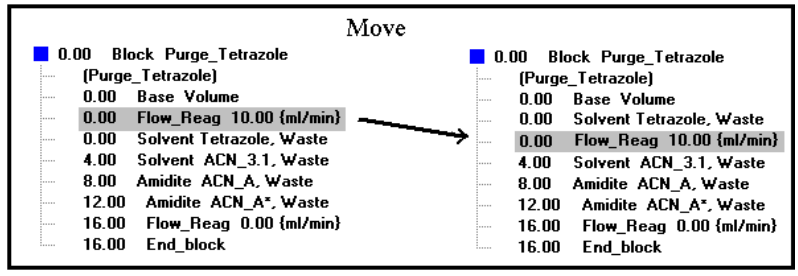


Figure 5-20. Instructions can be moved within the same breakpoint by dragging with the right mouse button.

Moving instructions between breakpoints

To move an instruction to another breakpoint:

1. Select the block to be moved using the right mouse button. Select **Cut** from the menu. The block is removed from the text instruction window.

Note: A cut block does not mean that it has been deleted (see Section (5.3.4) and can still be called from elsewhere in the method.

2. Select the instruction line before which you want the cut block to be pasted.

3. Click on the right mouse button and select **Paste** from the menu.

   The pasted block is inserted with the same breakpoint value as the block selected for point of insertion.

5.4.5 The flow scheme window

The flow scheme window displays the configuration of system components. This window is static and for information only, useful for example in identifying valves for flow path instructions.

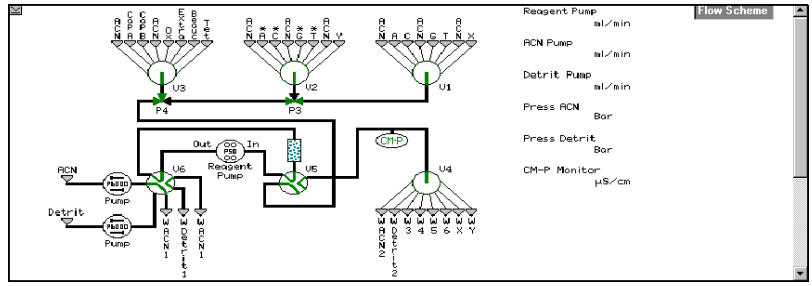


Figure 5-21. The Method editor flow scheme window.

5.5 Method variables

Variables can be assigned to many instruction parameters even including breakpoints. Variable values can be changed immediately before the start of a run without using the Method editor, allowing one method to be used for runs under a variety of conditions (see Chapter 4).

Variables are defined with names which can be explicit descriptions of the variable function, e.g. Sample_volume, Load_of_support. Suitable choice 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 run.

Each parameter defined as a variable is also assigned a default value, which is used if no changes are made to variable values at the start of a run. Up to 64 variables can be defined in a single method.

5.5.1 Identifying variables

Parameters defined as variables are identified in two ways:

- in the text instruction window, the parameter is given as the default value in parentheses followed by the variable name, e.g. (3.00)#Recycle_Time_DNA Recyle 0.00{cm/h}, OFF.

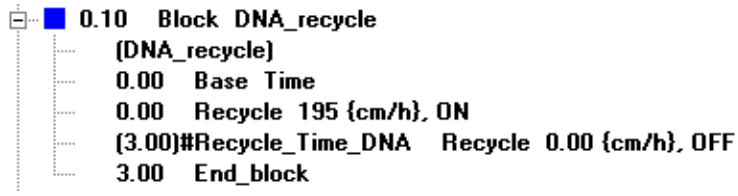


Figure 5-22. Default values for variables appear in parentheses in Text instructions

- when the instruction is shown in the Instruction box, the **VAR** button beside the parameter field is active in capital letters, i.e. **VAR** not **Var**.

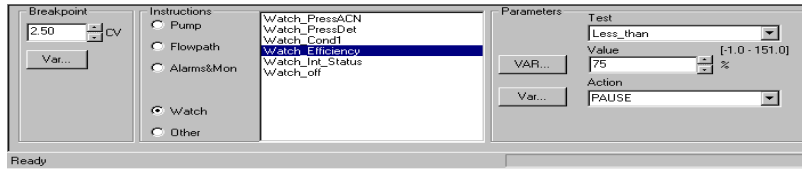


Figure 5-23. Parameters with variable definition are identified by an active (not greyed out) **VAR** button. In this example the Watch Efficiency **Value** is defined as variable and the **Action** position is fixed.

All variables are also listed on the Variables page of the Run setup (see Section 5.6.1), grouped according to the block in which they appear.

5.5.2 Defining variables

To define a new variable (i.e. convert an existing fixed value to a variable):

1. In the Text instructions window, select the instruction where you want to define the variable. The parameters for the instruction are shown in the instruction box.

2. Locate the breakpoint or the required parameter in the instruction box. Click on the Var button.

3. Enter a name for the variable in the dialogue and click on **OK**.

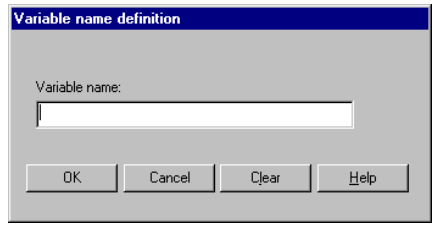


Figure 5-24. The Variable name definition dialogue.

Variable names may be up to 20 characters long, and may contain letters (A-Z), digits (0-9) and the underscore character. Use underscore characters instead of spaces if you want to separate words in a name (e.g. Flow_rate). Names must be unique within the method.

The case of letters is retained but is not significant. The names Flow_Rate and FLOW_RATE are treated as identical.

When you define a variable, the value in the parameter field applies as the default value for the variable.

Note: Only one variable which affects block length (breakpoint or gradient length) may be defined within each block. Any number of other parameters may however be defined as variables within a block.

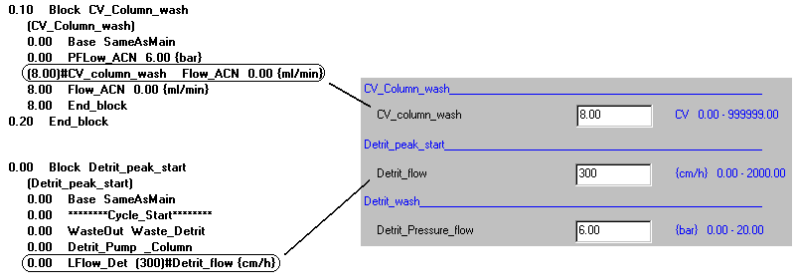


Figure 5-25. Relationship between variables in text instructions and in the Variables page of Run setup.

Default variable values can be changed either by editing the instruction in the Instruction box or by changing the value in the Variables page of Run setup. Changes made in the text instruction are automatically updated in the Variables page and vice versa (Figure 5.26).

Caution: If a breakpoint is defined as a variable, changing the variable value when the method is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using **Change** to alter a breakpoint or gradient length.

5.5.3 Removing a variable

To convert a variable to a fixed value:

1. In the text instruction window, select the instruction where you want to remove the variable. The parameters for the instruction will be shown in the instruction box.

2. Locate the required parameter in the Instruction box. Press the **VAR** button.

3. Click on **Clear** to delete the variable name and click on **OK**.

5.5.4 Renaming a variable

To change the name of an existing variable:

1. In the Text instruction window, select the instruction where you want to rename the variable. The parameters for the instruction will be shown in the instruction box.

2. Locate the required parameter in the instruction box. Press the **VAR** button.

3. Enter a new variable name in the dialogue and click on **OK**.

5.6 Run setup

The Run setup is a series of pages for defining the method properties. To access the Run setup pages, press the **Run setup** icon at the top of the Method editor toolbar. To access a given page in the Run setup, click on the respective tab.

5.6.1 Variables

The Variables page lists all variables used in the method with their default values, organised by method block. You can change the default values to create a variant of the method (see Chapter 4).

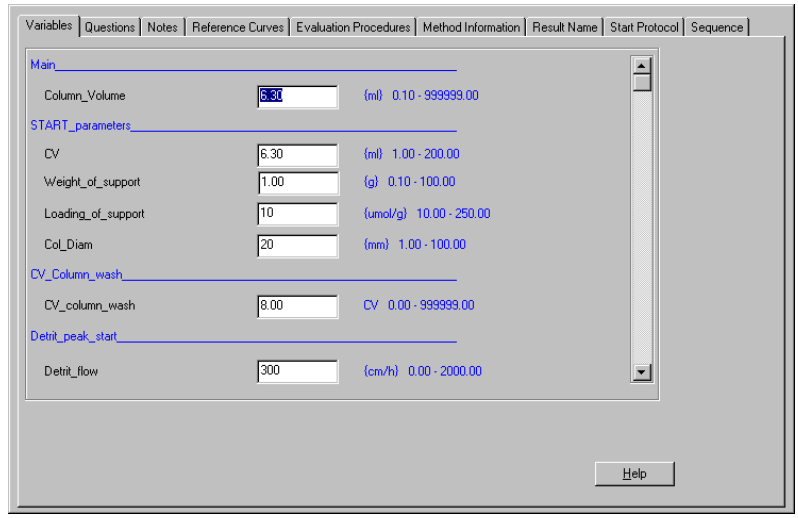


Figure 5-26. The Variables page in Run setup.

To change the default values, simply enter new values in the appropriate fields. Use the scroll bar to display additional variables if the variables occupy more than one screen. Click in the scroll bar to move one screen at a time, or on the arrows to move one variable at a time.

The changed values will be displayed for the corresponding instructions in the text instruction window. Remember to save the method with the changed variables.

Note: The **Variables** box must be checked in the Start protocol if you want to be able to change variable values at the start of a method.

5.6.2 Questions

Questions provide a means for entering structured run-specific information at the start of a run. Method templates supplied with UNICORN are defined with a set of questions for sample, column and eluent identification. To define questions which will be shown when the method is started, open the Questions page in Run setup.

Note: For questions to be shown in the start protocol, the **Questions** option must be checked in the Run set up Start Protocol page.

Questions may have the following status:

- **Mandatory**: these questions must be answered before a method is started.
- **Authorised**: answers to these questions must be acknowledged by a user with **Confirm/Unlock** authorisation (see Section 14.2). The user's password must be given to acknowledge the answers.
- **Chromatogram**: these questions will be printed with the answers on the same page as the chromatogram if Diagram header is chosen in an evaluation report (see Section 9.5).

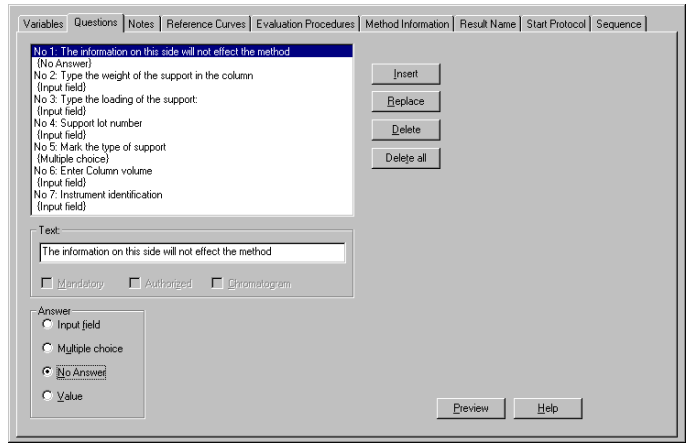


Figure 5-27. The Questions page in Run setup

Questions may be defined to accept four types of answers:

- **Input field** accepts any alphanumerical input as the answer. Input field questions may have a default answer. Example

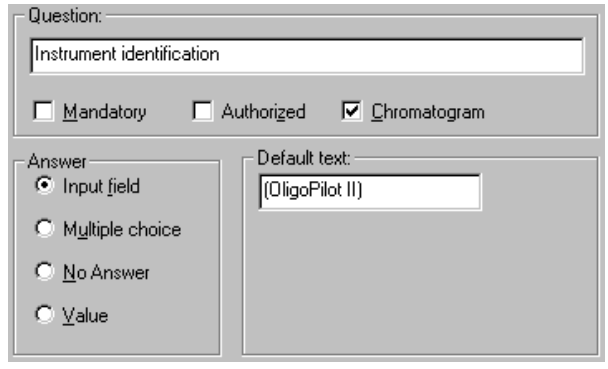


Figure 5-28. Options for input field questions.

- **Multiple choice** allows the user to choose one of a defined set of answers. To allow a blank answer, enter a space in one of the pre-defined answers. Example:

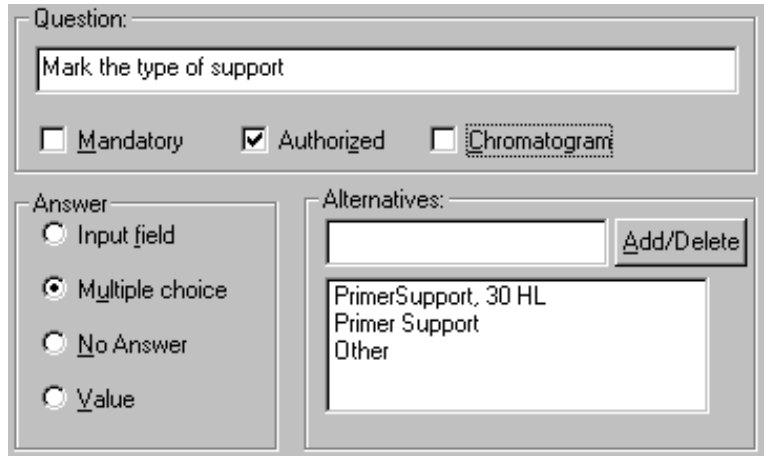


Figure 5-29. Options for multiple choice questions.

- **No answer** does not require an answer. This kind of "question" may be to display important information or to split a question over more than one line (by setting all but the last line to **No answer**). Example:

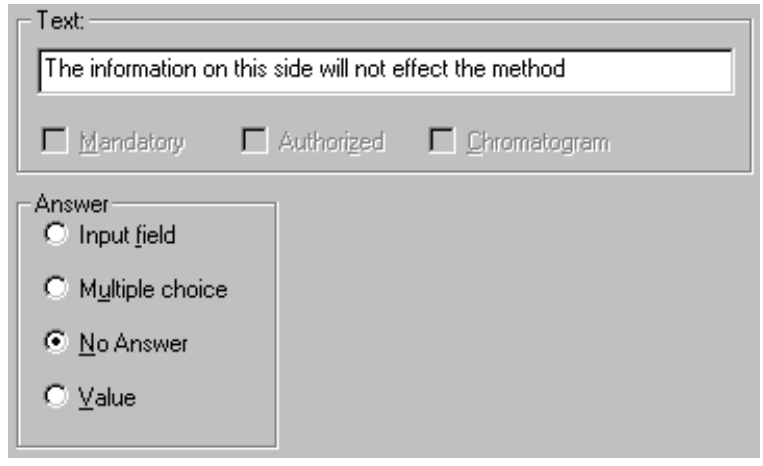


Figure 5-30. Options for no answer questions.

- **Value** accepts only numerical answers. Value questions may have specified maximum and minimum limits, and may be defined to accept only integer values.

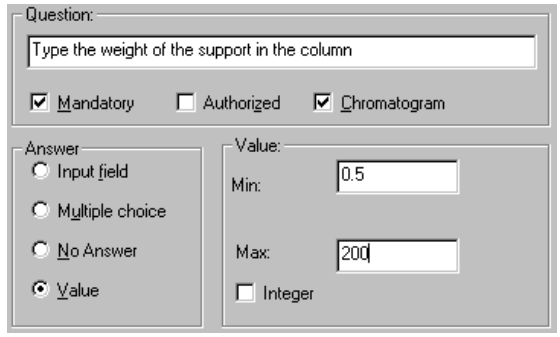


Figure 5-31. Options for value questions.

Press the **Preview** button to display the questions as they will appear when the method is run. (Alternative answers to multiple choice questions are not shown in this mode). From preview mode, press **Edit** to return to question editing mode.

Inserting a question

To insert a new question after an existing question:

1. Click on the existing question in the questions list.

2. Enter the text, status, type and answer for the new question as required. The **Answer** panel depends on the type of question:

   - Input field questions: Enter a default answer if required.
   - Multiple choice questions: Click on the input field under **Alternatives**, enter the answer and click on **Add/Delete**. Repeat this procedure to add other alternatives. New alternatives are always added at the end of the list. To remove an alternative, mark the alternative in the scroll list and click on **Add/Delete**.
   - Value questions: Enter maximum and minimum limits. Check the **Integer** box to if the question is to accept only whole numbers as answers.

3. Click on **Insert** to add the new question to the list.

   If the list is empty, the **Insert** operation creates the first question in the list.

Editing an existing question

To change the definition of an existing question, select the question to be changed. Change the text, status, type and answer as required and click on **Replace**.

Deleting a question

To remove a question, select the question and click on **Delete**. To remove all questions, click on **Delete All**.

5.6.3 Notes

Notes are descriptive comments that form part of the method documentation. There are four separate notes fields for method editing, start-up, run and evaluation respectively. Only the method notes can be edited from the Method editor: the other notes are accessible at the respective stages in a run.

To view the method notes, open the Notes page in the Run setup. Method templates are supplied with notes describing the system requirements for running the method. Read through these notes carefully before using a method. Click on the maximize notes button to expand a notes field to fill the notes page. Click on the same button again to restore the default display with all four notes fields visible.

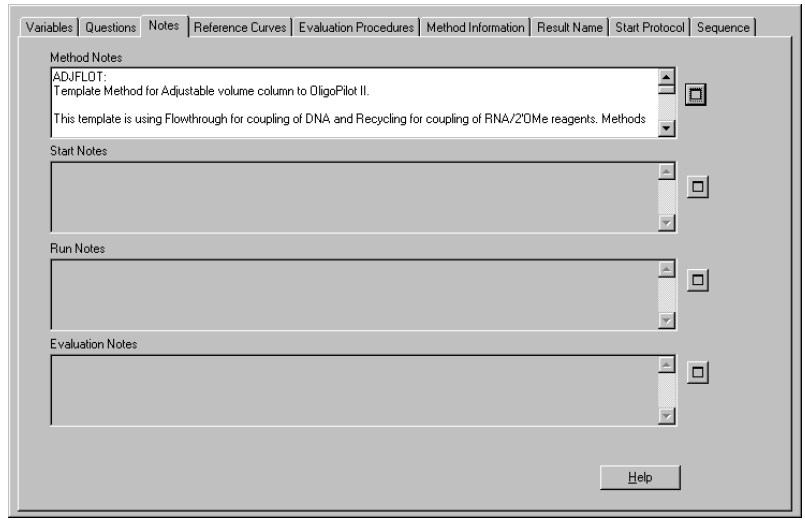


Figure 5-32. The Notes page in Run setup.

The notes are entered as free text and may be edited using standard Window editing functions to edit the notes. Words wrap automatically at the end of the field.

<Ctrl+X>	Cuts the marked text, saving it to the clipboard.
<Ctrl+C>	Copies the marked text to the clipboard.
<Ctrl+V>	Pastes the contents of the clipboard at the cursor.
<Delete>	Clears the marked text without saving it in the clipboard, or deletes the character to the right of the cursor if no text is marked.
<Backspace>	Clears the marked text without saving it in the clipboard, or deletes the character to the left of the cursor if no text is marked.

In the default method templates supplied with UNICORN, the method notes describe the system setup required by the method (e.g. eluent and sample inlets, outlets, column connections and so on). We recommend that you use method notes for this purpose in your own methods, to provide documentation of the method requirements. Bear in mind that method notes are saved with the method and apply to all runs made with the method. Use the start or run notes for run-specific information. The date and time when the method was created and last edited are saved automatically in the method information, and need not be entered in the method notes.

5.6.4 Evaluation procedures

Evaluation procedures can be called automatically at the end of a method to evaluate and/or print the results. Method templates supplied with UNICORN include procedures named:

Integrate_and_Print which integrates the first UV curve in the chromatogram and prints out the results

Print_Chromatogram which prints the chromatogram from the run with the scouting variables printed at the top.

User-defined procedures are created in the evaluation module and may be saved in method files (see Section 10.3). Procedures saved with one method file can be imported to another.

Note 1: A procedure in a method will not be updated when a procedure with the same name is changed in Evaluation. The same applies to report formats saved in a procedure.

Note 2: If you use an evaluation procedure to print results automatically from a run controlled from a remote station in a network installation, the results will be printed on the printer currently set up on the local station, not on the remote station. If however you execute the procedure interactively from the evaluation module on the remote station, the results will be printed on the printer set up on the remote station where you are working.

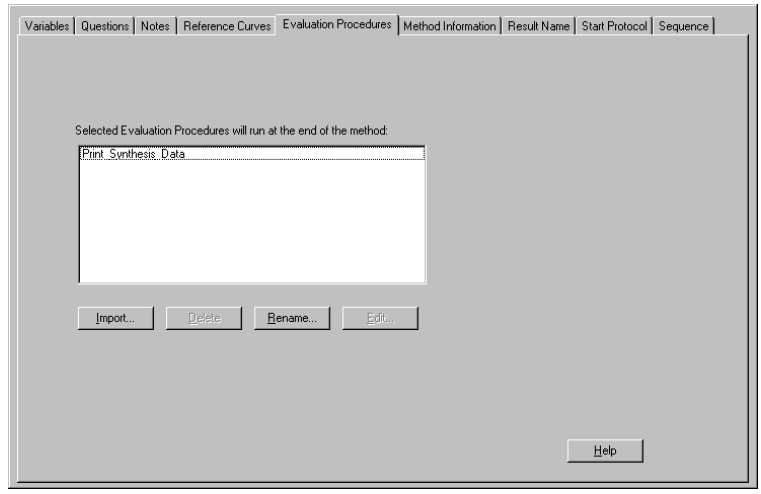


Figure 5-33. The Evaluation Procedures page in Run setup.

Defining and viewing procedures

Evaluation procedures are normally defined in the Evaluation module. Procedures imported to a method can also be viewed and edited in the Method editor; select the required procedure in the list and click on **Edit**. See Section 10.3.2 for a description of how to edit evaluation procedures.

Note: Evaluation procedures which process chromatogram data rely on consistent identification of curves in the result file for correct operation. If you include evaluation procedures with a method, make sure that references to curves in the procedure will be valid when the procedure is executed at the end of the run. See Section 10.3 for more details.

Selecting procedures to run

The Evaluation Procedures page lists all evaluation procedures associated with the method. Click on the procedure(s) which are to be executed at the end of the run. The procedures will be executed in the order they appear in the list.

Importing procedures

To import an evaluation procedure:

1. Select the Evaluation Procedures page and click on **Import.**

2. Choose a procedure from the **Select list**. You can also choose to import a procedure from another method. Select a method to show the procedures stored in the method. If you have chosen a method, click on **Evaluation Procedures** to return to the complete list.

3. If desired, change the procedure name in the **Import as** field.

4. Click on **Import**.

   The dialogue remains open until you click on **Close**, so that you can continue to add procedures from the same or different method files.

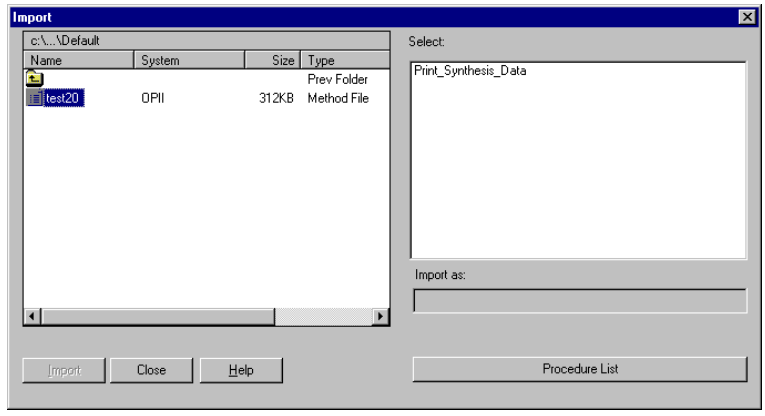


Figure 5-34. The Import procedures dialogue.

Deleting procedures

To remove one or more procedures from the method:

1. Select the Evaluation Procedures page and select the procedure(s) to be deleted.

2. Click on **Delete** and confirm the deletion.

Note: Procedures that you delete from the method are removed from the method file when you save the method.

Renaming procedures

To rename a procedure in a method:

1. Select the Evaluation Procedures page and click on **Rename**.

2. Select a procedure from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.

The dialogue remains open until you click on **Close**, so that you can rename more than one procedure without closing the dialogue.

Editing procedures

To edit a procedure in a method:

1. Select a procedure on the Evaluation Procedures page and click on **Edit**.

2. Edit the procedure as described in Section 10.3.

3. Choose **File:Exit** from the procedure editor menu. (**File:Save** is not available in the procedure editor window when you edit procedures in a method. Changes are saved automatically when you close the procedure editor).

Note: Report formats in procedures cannot be edited or viewed.

5.6.5 Method Information

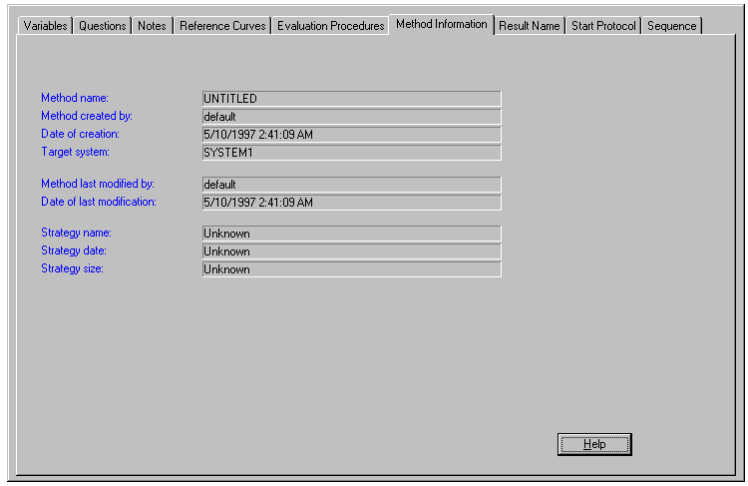


Figure 5-35. The Method Information page in Run setup.

The Method Information page displays information about the method, such as method name, target system for creation and date of last change, information about the strategy for which the method was created, estimated eluent consumption and duration in time of the method. These figures for the latter two are based on values for methods with variable length parameters, and will be changed if the values are changed.

The Method Information page is for information only and cannot be edited.

5.6.6 Sequence

The Sequence page contains the user interface between the desired sequence to be synthesized and the method created to run the synthesis procedure. The properties of this interface are described in Chapter 4 and Section 5.1.

5.6.7 Result Name

Use the Result Name page to specify how the result files will be named for the results of a run, and where the result file will be saved.

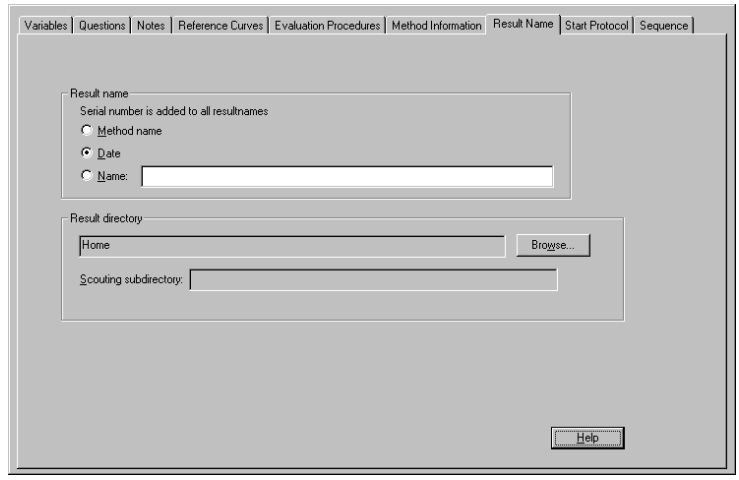


Figure 5-36. The Result Name page in Run setup.

The result file name is constructed by adding a 2-digit serial number to one of the base options listed below. The serial number is incremented automatically each time the method is run.

- the method name plus a 2-digit serial number
- the date of the run (in an 8-digit format determined by the country setting in Windows NT) plus a 2-digit serial number
- a freely specified name (within the file naming restrictions in the operating system) plus a 2-digit serial number

If the result file folder already contains files with the same file name base, the serial number is incremented automatically.

By default, result files are stored in the home folder of the user who starts the run. To change the folder where the result file will be stored, press the **Browse** button, double-click on the required folder icon and press **Close**.

Note: The result name may be specified as changeable in the Start protocol (see Section 5.6.10). In that case, the specification in the Result page serves to generate the suggested result name, which may be changed at the start of the run.

5.6.8 Start protocol

The Start protocol determines which items of the Run setup are displayed (and may in appropriate cases be altered) at the start of a run. Open the Start Protocol page and check the items that are to be displayed.

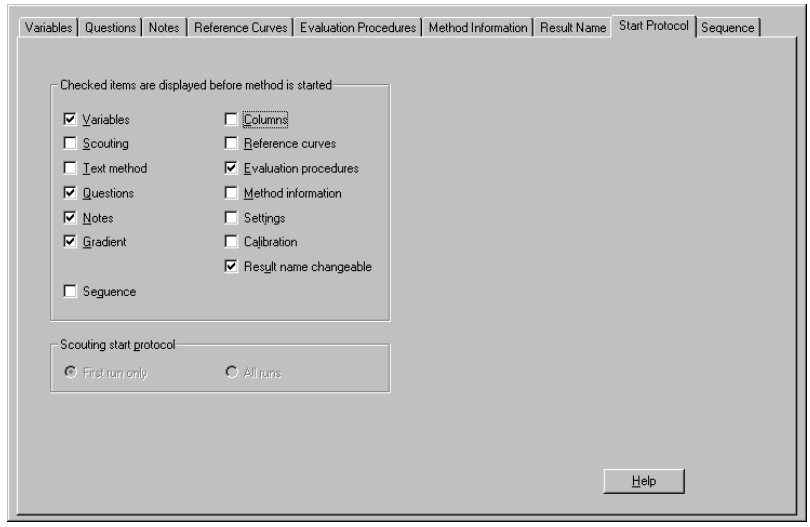


Figure 5-37. The Start Protocol page in Run setup.

Variables

If this box is checked, values for method variables will be displayed and can be changed at the start of the run. These values will override the default values for the particular run, and will be saved in the result file. The default values stored in the method are however not affected.

If the **Variables** box is not checked, the run will be executed with default values for all variables as defined in the method.

Text Method

Displays the method instructions. Double-click on a Block instruction, as denoted by the blue square and '+' mark, to display or hide the instructions in the called block (see Section 5.4.1). Method instructions cannot be changed from this display.

Questions

If this box is checked, questions defined in the method will be displayed at the start of the run.

Important!
If the **Questions** box is not checked, questions will not be displayed even if they are defined as mandatory. Since the answers to questions can form an important part of the run documentation, you are recommended always to check the **Questions** box.

Notes

If this box is checked, the notes page will be displayed at the start of the run. You can enter notes in the **Start notes** field but not in any of the other fields. You can use the scroll bar if necessary to read notes in the **Method notes** field.

The start of the run is the only occasion when you can enter start notes. If the **Notes** box is not checked, the notes will not be displayed and you cannot enter start notes for the run.

Columns

If this box is checked, you can view the available column definitions. The column definition used in the method run is selected with the Base instruction, and may be changed at the beginning of the run on the Variables page if the columns parameter is defined as a variable (see Section 5.6.1).

Note: Not used for Oligo systems.

Reference curves

If this box is checked, the reference curves associated with the method will be displayed at the start of the run. You can add, delete and rename curves at the start of the method. All curves in the list can be displayed in System control during the run.

If the **Reference curves** box is not checked, the curve settings saved in the method will apply.

Evaluation procedures

If this box is checked, the evaluation procedures set to execute at the end of the method will be displayed at the start of the run. You can change the choice of procedures to execute, but you cannot add or remove procedures. (Procedures are stored as part of the method file, which cannot be changed at the start of the run).

If the **Evaluation Procedures** box is not checked, the procedure settings saved in the method will apply at the end of the method.

Method information

If this box is checked, the method information (including creator, target system, strategy information and date and time of creation and latest change) will be displayed at the start of the run. You cannot edit the method information.

Settings

If this box is checked, the settings (including alarms, monitors and curve configuration) will be displayed for information at the start of the run.

To change settings, use the **System:Settings** command in System control before starting the run (see Chapter 15).

Calibration

If this box is checked, the monitor calibration settings will be displayed at the start of the run.

If the **Calibration** box is not checked, you can still calibrate the monitors before the run is started by using the **System:Calibrate** command in System control.

Result name changeable

If this box is checked, you can change the result name when the run is started. Click on the **Browse** button to change the result folder.

If the **Result name changeable** box is not checked, the result name will still be displayed, but neither the name nor the folder can be changed.

Sequence

This option should be selected to display the sequence that the method has been created for. This is for information only.

5.7 Saving the method

5.7.1 Saving a method

Click on the **Save** button on the toolbar or choose **File:Save** to save the method. The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. Once the method has been saved, choosing **File:Save** saves the current state of the method under the same name. If you want to save a copy of the

method under a new name, choose **File:Save As** and enter the details as described below.

1. Enter a name for the method. Method names may contain letters (A-Z) and digits (0-9). The case of letters is not significant. The method name must be unique for the chosen system within the folder (see steps 2 and 3 below).

2. By default, the method will be saved in your home folder. To change the folder, double-click on the appropriate folder icon in the Methods panel.

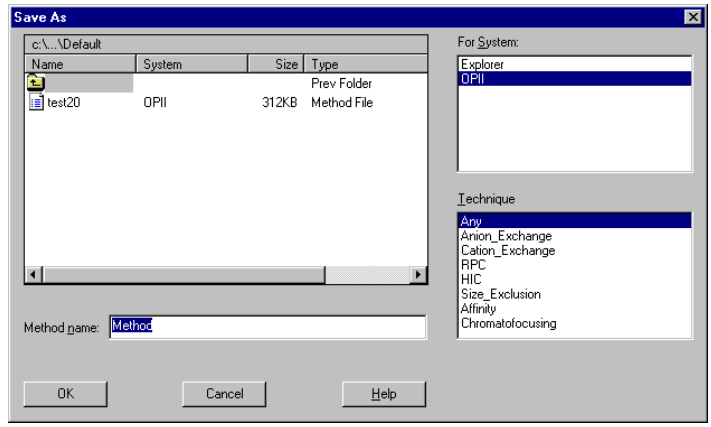


Figure 5-38. Save As dialogue for saving a method.

3. If you have more than one system available, choose the system for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

4. Click on **OK**.

5.7.2 Saving as a template

You can save the method as a template if you have **Edit global lists** authorisation.

1. Choose **File:Save as Template**. The **Save As Template** dialogue is displayed.

2. Enter a name for the template in the **Name** field, or choose an existing template name from the list. If you choose an existing name your will overwrite the existing template.

3. Choose the system for which the template is intended in the **For system** field.

4. Select the **Technique** from the lists as appropriate.

5. Click on **OK**.

The templates for each system are common for all users. Be restrictive in saving methods as templates. We recommend that only methods that are useful for all users are saved as templates.

5.7.3 Deleting a template

You can delete templates if you have **Edit global lists** authorisation.

1. Choose **File:Delete template**.

2. Select the system and the template to delete, and click on **Delete**.

3. Confirm the action.

Note: The templates for each system are common for all users. Be restrictive in deleting templates.

5.8 Printing the method

You can print a copy of the method including items from the method documentation (Run setup) and the Text instructions window.

1. In the Method editor select **File:Print** or click on the **Print** toolbar button.

   The **Print** dialogue is displayed. The dialogue contains print UNICORN modules although only those available from Method editor can be selected.

Note: It is recommended that you select the print command from the text instructions view so that you have access to the text method print options.

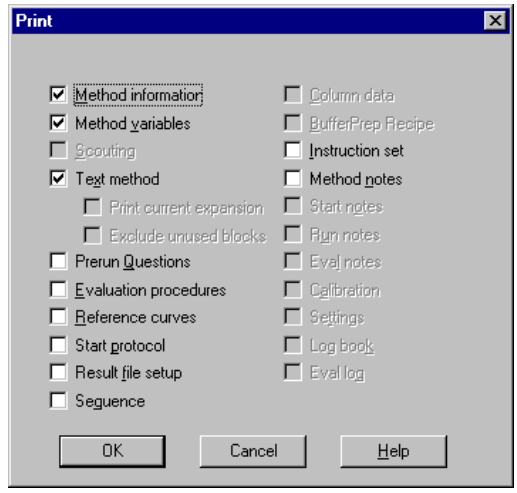


Figure 5-39. Print dialogue

2. Check the options that you want to print out.

3. Click on **OK** to print.

5.9 How to use selected unconditional method instructions

This section provides recommendations for how to use some common programming features in UNICORN methods.

5.9.1 Base instruction

Every method block must start with a Base instruction, defining the base for calculating breakpoints.

Note: Do not confuse the concept of a *method base* instruction with the bases in a sequence.

The base may be volume (ml or l depending on the scale defined in the system strategy), time (min), or column volume CV (defined as a numerical value or taken from the column definition). For all blocks other than the main block, the base may also be defined as SameAsMain, which means that the block will inherit the base defined in the main block. Different blocks may use different bases.

Use the base which most closely suits the purpose of the block. Column volume is recommended as the base for most steps in a run. In some

situations, it may be more suitable to use time or volume base for individual blocks.

Be careful when changing the base for an existing method. Changing between time and volume base can affect the relative duration of steps in the method if different steps use different flow rates.

Note: For method blocks which use a volume or column volume base, make sure that the flow rate is not zero. Volume breakpoints are calculated from the flow rate of the pump, and the method will not progress if the flow rate is zero.

The parameters for the Base instruction differ slightly according to whether a named column definition is used.

The Methodbase instruction

Volume or column volume base is calculated from the flow rate of the GradientPump (AB) or the sample pump, selected with the instructions Methodbase. If no Methodbase instruction is included in the method, the default setting GradientPump will be used.

5.9.2 Instructions at the same breakpoint

Instructions placed at the same breakpoint in a block are executed simultaneously, with the exception of successive CALL instructions which are executed in the sequence in which they are written. This can have important consequences in some situations. For example, the instruction sequence:

```
...
0.00  Block SameAsMain
0.00  WasteOut Waste_ACN
0.00  PFLow_ACN 2.00{bar}
(10.00)#CV_Column_wash End_block}
...
```

will set the waste valve to Waste ACN at the same time as 10 column volumes of ACN are pumped through at a pressure of 2.00 bar. Conversely, in the instruction fragment:

```
...
0.00  Call Normal, Detritylation
0.00  Call Normal, Detrit_wash
0.00  Call Normal, Coupling_recycle_DNA_T
      ...
```

the instructions contained in the first listed call to a block will be completed before proceeding onto the next instruction or call to block.

To ensure that instructions are executed in a defined sequence where this is important, separate the instruction breakpoints by 0.1 base units. The revised formulation for the first example above is:

```
...
0.00  Base SameAsMain
0.10  WasteOut Waste_ACN
0.20  PFlow_ACN 2.00{bar}
(10.00)#CV_Column_wash End_block
...
```

5.9.3 Block and method length

The length of a block is determined by the breakpoint of the last instruction in the block. A block in which all breakpoints are at 0 will take no time or volume during a run, e.g.

```
(START_parameters)
0.00    Base SameAsMain
0.00    Scale (1.75)#Weight_of_support{g}, (93)#Loading_of_support{umol/g}
0.00    ColDiam (10.00)#Col_Diam{mm}
0.00    DelayVol (1.3)#Delay_volume{ml}
0.00    End_block
```

To extend the length of a block without performing any other operation, set the breakpoint of the End_Block instruction appropriately, e.g.

```
(START_parameters)
0.00    Base SameAsMain
0.00    Scale (1.75)#Weight_of_support{g}, (93)#Loading_of_support{umol/g}
0.00    ColDiam (10.00)#Col_Diam{mm}
0.00    DelayVol (1.3)#Delay_volume{ml}
4.00    End_block
```

During a run, the overall time or volume is determined by the sum of the block lengths.

Note: The length of the main block does *not* indicate the overall length of the method (the main block often consists only of calls to other blocks and has zero length). The method length can be checked in the Gradient window of System control.

Depending on how conditional calls are used (see Section 5.8.8), the overall method time or volume may vary according to watch events during the run.

Log format

The difference between block time and accumulated time can be viewed in Method editor by viewing the **Log Format**.

To view the accumulated time for a method select **View:Log Format** or click on the **Log Format** toolbar button. The **Log Format** dialogue is displayed.

The **Log Format** dialogue displays the cumulative time or volume for the current method.

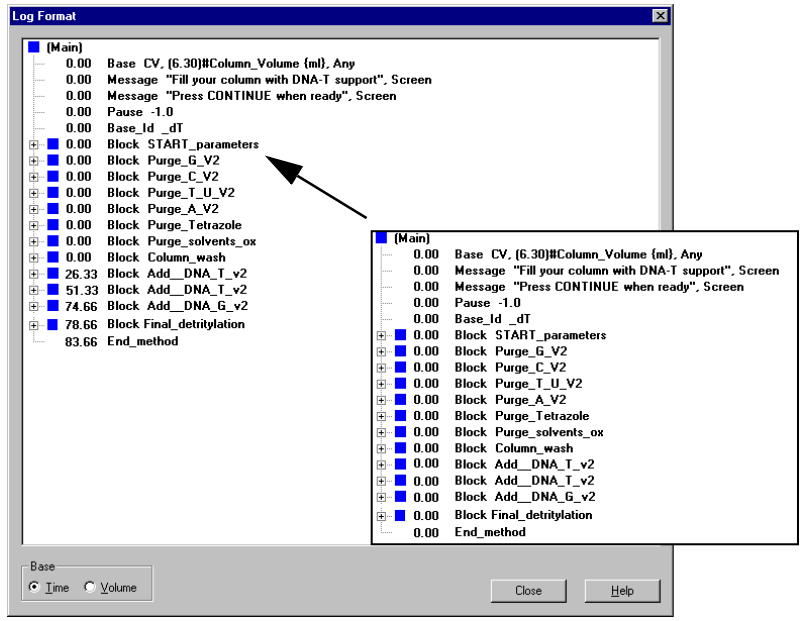


Figure 5-40. Log Format dialogue displaying the cumulative time for a method (in rectangle with arrow).

The concept of block time or volume versus accumulated time or volume is illustrated in the following table:

Accumulated time/vol	Block time/vol	Instruction
		(Main)
0	0	0.00 Block, Purge_G
0	0	0.00 Base Volume
0	0	. . .
1	1	1.00 End_block
1	0	0.00 Block, Purge_C
1	0	0.00 Base Volume
1	0	. . .
2	1	1.00 End_block
2	0	0.00 Block, Purge_A
2	0	0.00 Base Volume
2	0	. . .
3	1	1.00 End_block

Table illustrating the relationship between accumulated and block time/ volume for a simplified method fragment.

Depending on how conditional calls are used (see Section 5.8.8), the overall method time or volume may vary according to watch events during the run.

5.9.4 Messages and set mark

Use messages to inform the operator of the progress of the run. It is a good idea to issue messages at critical points in the method, e.g. when Watch instructions are used for conditional events or at the end of a gradient (see below). The example block below instructs the operator to fill the column with DNA-T support:

```
(Main)
0.00 Base CV, (6.30)#Column_volume{ml}, Any
0.00 Message "Fill your column with DNA-T
     support",Screen
0.00 Message "Press CONTINUE when ready", Screen
0.00 . . .
```

Messages which are set to Screen will be displayed on the screen during a run, and will remain there until acknowledged by the operator. Messages can also be set to Noscreen: these will be recorded in the run log but not displayed on the screen.

Set_mark

Other text messages can be inserted into the chromatogram at set points using the Set_mark instruction. The Set_mark instruction is also a convenient way of inserting a note into both the logbook and the chromatogram during a run. This contrasts from Message, which is displayed on the screen and entered into the logbook only.

Set_mark can be used to insert manual notes, for example when a problem occurs in the run. The instruction can also be incorporated into a method as shown in the following example where Set_mark is used to highlight the start of detritylation in a method.

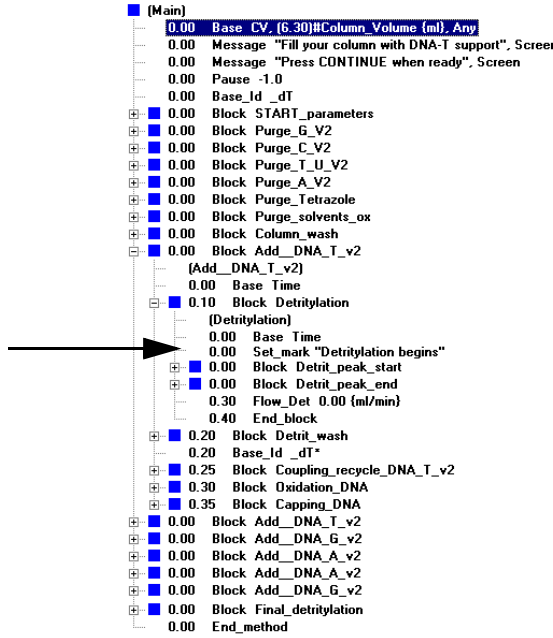


Figure 5-41. A method containing the Set_mark instruction (see arrows) to show the start of detritylation.

5.9.5 Pausing a method

A method can be programmed to pause at critical points. There are two instructions for this purpose:

Hold suspends execution of the method, but continues to pump eluent at the current flow rate and concentration settings.

Pause suspends execution of the method and stops the pumps so that the system comes to a standstill. In OligoPilot II, valves remain in the position they were in before the pause. The pause may be defined as indefinite or for a given number of minutes. This instruction is most useful for stopping the system in the event of an unexpected condition.

In both cases, the method may be resumed by pressing the **Continue** button in the System control toolbar (see Section 6.2).

Note: Never select Pause or Continue during a system Hold (Vol_amid, Vol_Ox, Vol_Thio, Vol_Cap), as this will interrupt the adding of reagent.

5.9.6 Linear flow rates

Linear flow rates (cm/h) for ACN, detrit solution and reagent can be specified by the instructions LFlow_ACN, LFlow_Det and LFlow_Reag respectively. To use these instructions, it is necessary for a column diameter to be defined in the Variables page of Run setup. The volume flow rate is calculated from the specified linear flow rate and the column diameter. The calculated volumetric flow rate is shown during runs.

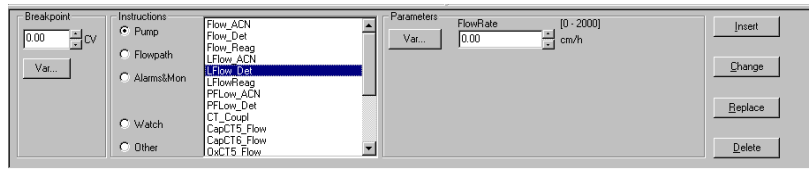


Figure 5-42. Setting linear flow rate in a method block. The linear flow rate option is only available if the column diameter is defined in the method.

Note: If a column diameter has not been defined in the method, linear flow will not be able to be used.

5.10 How to use selected conditional method instructions

Conditional instructions allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak elutes, or equilibrate the column until the eluent conductivity has reached a given value. This is facilitated by the Watch instructions.

5.10.1 Standard Watch conditions

The system strategy includes Watch instruction for each monitor defined in the system. A Watch is active from the point at which it is issued until either:

- the Watch condition is met
- a new watch is set for the same monitor
- a Watch_Off instruction is issued for the monitor

The conditions for which a Watch can be set are as follows for most monitors:

Greater_than	The signal exceeds a specified 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 (e.g. mAU/min).
Slope_less_than	The rate of change of the signal falls below a specified value, expressed in monitor units/minute (e.g. 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_maximum 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 below).
Peak_max	The signal falls to a specified fraction of the most recent peak maximum minus the Delta_Peak value (see below). Factor=1 detects peak maximum.
Stable_baseline	The signal is stable within the limits of the Delta_Base value (see below) for the period specified by the minutes parameter.
Int_Status	Equal to 0 or 1 to indicate if peak integration is switched off or on respectively.

To determine suitable values for watch conditions, it is often most convenient to examine data from a test run. For slope values, use the **Differentiate** function in the evaluation module to measure the slope of the test chromatogram (see section 10.2.2).

Note: The slope criteria operate on the arithmetic value of the slope, so that a value of -3 is less than a value of -2. The end of a peak is thus detected by Slope_greater_than with a negative value (the slope is negative but increasing).

Two conditions apply for air sensors (not available for OligoPilot II):

Equal 0 Air is not detected by the sensor.

Equal 1 Air is detected by the sensor.

Note: To use the Watch_AirSensor instruction for air sensors, the Alarm_AirSensor setting in **Alarms&Mon** must be disabled (use the Method editor to disable the alarm locally in a method, or the settings to disable the alarm for all methods, see Section 15.1). The Alarm_AirSensor setting overrides any Watch_AirSensor instruction, and if the alarm is enabled the method will pause when air is detected.

6 Performing a run

This chapter describes how to perform and monitor a run from the System control window. It is recommended that for your first run you use the 20 base pair sequence already supplied with the fixrec method template and which you should have saved as a method called test20 (See Section 4.2). This and other methods that you have created should be present in the Methods box of the Main Menu window.

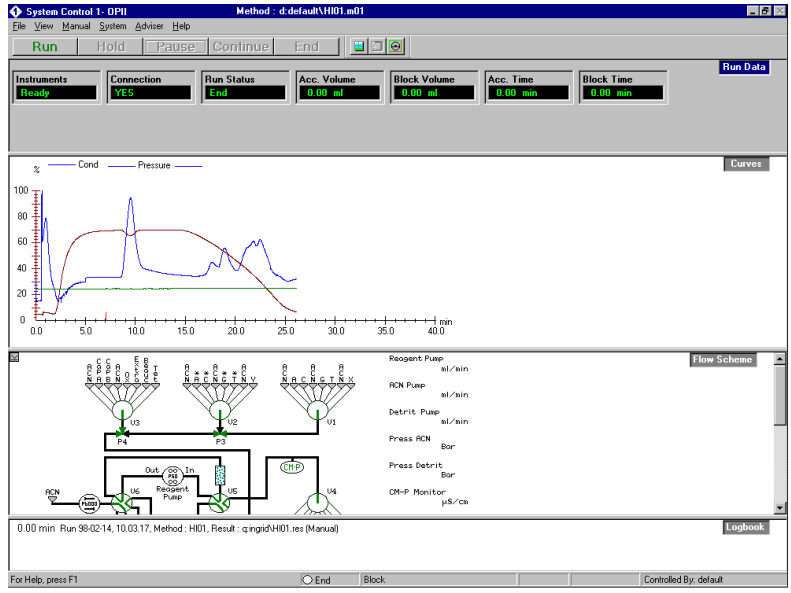


Figure 6-1. The System control workspace with run data, curves, flow scheme and logbook displayed.

6.1 Starting a method

You can only start a method if the system is connected and no method is currently running. You must have **Run methods** authorisation to start a method.

Before starting a method, make sure that:

- The correct system is connected in control mode (see Section 6.5).

  The name of the connected system is shown in the title bar of the System control workspace.

In the status bar, information is displayed about the following:
traffic light with status text,
current running block,
MethodQueue text,
connection status text.

If the correct system is not connected, you can connect up to the system if it is free by selecting **System:Connect**.

- The system monitors are correctly calibrated (see Section 6.6).

6.1.1 Starting from the Main menu

You start a method from the Main menu by selecting the method in the **Methods** window and selecting **File:Run**. Alternatively, you can click on a method file with the right mouse button and select **Run** followed by selecting one of the displayed available systems that have the same strategy as the system for which the method was created. By default, the system for which the currently selected method was created, is selected (checked). You can choose the default system or one of the other systems in this cascade list to run the method.

Note: Do not double-click on the method as this will open the Method editor with the method loaded.

6.1.2 Starting from System control

You can also start a method from the System control module. Click on the System control icon in the Windows NT taskbar. If you have more than one system installed, make sure you select the correct System control icon for the correct system.

From System control select **File:Run** and double-click on the method icon in the displayed dialogue.

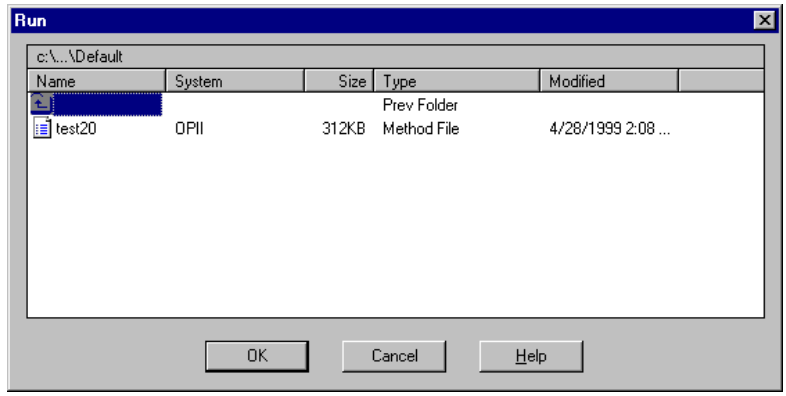


Figure 6-2. Starting a method from the System control menu.

For methods which are used frequently (e.g. column cleaning methods), it may be convenient to define the methods as commands in the **File** menu. To do this, choose **File:Menu** and select the required method. The method name will appear as a command in the **File** menu, and choosing the command will start the method.

6.1.3 Starting an Instant Run

You can Start a method template directly by clicking on the **Instant Run** button in the Main menu toolbar or by selecting **File:Instant Run** in System control. The method template will not, however, be saved as a method file, but it is possible to retrieve the method from the generated result file.

Note: Use of this function requires that templates are defined. Standard systems are supplied with templates but custom systems require that the user creates templates.

6.1.4 Start protocol

If the method is defined with a start protocol this will be displayed before the method actually starts. Work through the start protocol, answering questions as required. As each screen is completed, click on **Next** to move to the next screen or **Back** to return to the previous screen. The last screen has a **Start** button to start the run. At any stage, click on **Cancel** to abort the method start.

The following start protocol items may be displayed (see Section 5.4.4 for more details):

Variables	All the variables defined in the method instructions, organised by block. Values for variables can be changed here for the current run.
Text Method	Text instructions for the method. These are displayed for information only and cannot be changed at this stage.
Questions	Questions are data entry fields which are filled in by the operator when the run is started. Some questions may be mandatory and some may require authorised confirmation.
Notes	Method notes are shown and start notes can be entered.

Oligo Synthesis Sequence The sequence of oligonucleotide to be synthesized. This cannot be altered.

Reference Curve Reference curves which may be displayed in the System control workspace during the run can be selected here. See Section 5.6.6 for a description of reference curve selection.

Evaluation Procedures Evaluation procedures which will be executed automatically after completion of a run can be selected here. See Section 5.6.5 for a description of procedure definition and selection.

Method Information Information about the method being run.

System settings Displays the system settings for the run. If the settings are not suitable, cancel the method start, change the settings with the **System:Settings** command (see Chapter 15) and restart the method.

Calibration Displays calibration data for system monitors. If the calibration is not acceptable, cancel the method start, re-calibrate the monitor(s) with the **System:Calibrate** command (see Section 6.6) and restart the method.

Result Name The name of the result file is specified here. This page is displayed if there are any other pages in the start protocol. The names may be changed if this is permitted in the start protocol.

If any questions in the start protocol require authorised confirmation, you will be asked for a username and password when you attempt to leave the screen containing the questions. Only users with **Confirm** authorisation may authorise answers to such questions. Each question that requires an authorisation must have a separate authorisation.

Note: If the start protocol for a method in the queue is cancelled, the MethodQueue is paused. Select **MethodQueue:Display Running** in the Main menu and Restart or end the run in the displayed dialogue.

6.2 Monitoring a run

The System control workspace displays the status of the current system. On the Windows NT taskbar, there may be up to four System control modules available that can be connected to different systems. Separate systems may be controlled and displayed independently of each other.

Each System control workspace displays up to four windows for monitoring different aspects of the run. Click on the **View Windows** toolbar button or choose **View:Windows** from the menu to select which windows to display.

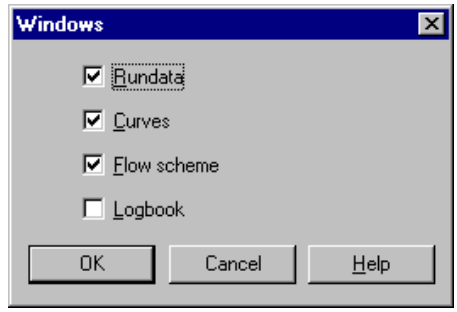


Figure 6-3. Dialogue for choosing windows to display in the System control workspace.

6.2.1 General window techniques

Windows in the System control are always displayed over the full width of the workspace. The boundaries between the displayed windows can be moved by selecting and dragging up or down to change the size of a specific window.

Any window can be maximized to the full view or restored to its original size by selecting the **Maximize** or **Restore** toggle command respectively in the associated right mouse button menu.

To hide a window from view, select **Hide** in the relevant right mouse button menu.

6.2.2 Run data

The run data window displays the current values for selected run parameters. Values are updated at least every 5 seconds (the actual interval is defined in the system strategy).

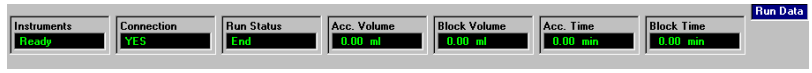


Figure 6-4. The run data window.

Run data layout

The general display for the run data window can be selected using layouts by doing the following:

1. Select **View:Properties** or select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed for all windows in System Control.

2. Select the **Run Data Groups** tab.

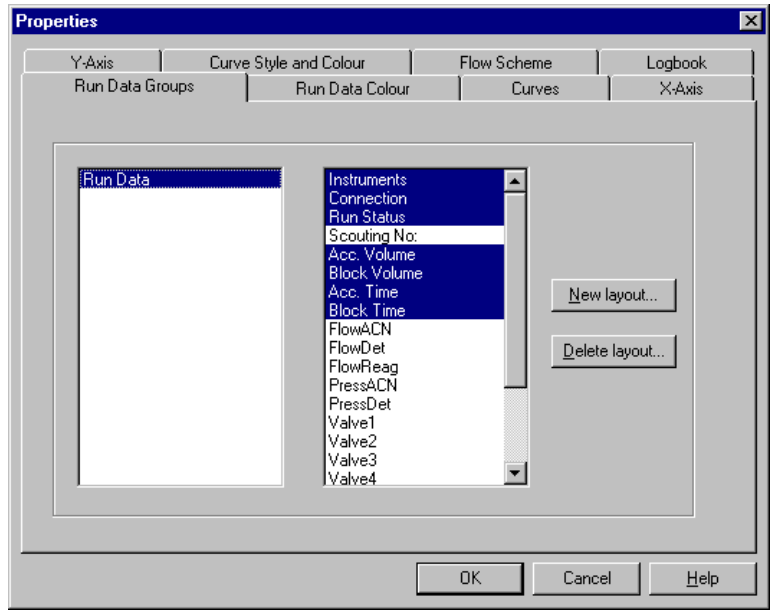


Figure 6-5. Properties dialogue, Run Data Groups tab.

3. Select individual run data parameters to view and click on **OK**.

Alternatively, select a layout, either:

an available layout	Select this from the list on the left
edit an available layout	Select this from the list on the left and modify the included readings in the list to the right
create a new layout	Click on the **New Layout** button and enter a name for the layout in the displayed dialogue. Finish by clicking on **OK**. Select the readings that you want to view from the list on the right.

Note: For systems with optional components, parameters are not shown for components that are not included in the system.

4. To delete a layout, select it and click on **Delete Layout**.

5. Click on **OK** to view the selection in the run data window. The name of the layout selected replaces the default layout name **Run Data**.

6. Toggle between the various layouts that you have created by selecting **Next Layout** in the right mouse button menu.

You can choose run data items to display without using named layouts, simply be selecting or deselecting items in the list. Note, however, that this will change the definition of the currently selected layout.

Run data style

You can change the colour of the text and background in the run data window.

To change the colour of the text or text background in the displayed readings boxes:

1. Select **Colour Settings:Text** or **Colour Settings:Background** from the right mouse button menu cascade.

2. Select a colour in the dialogue and click on **OK**.

Alternatively:

1. Select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed.

2. Select the **Run Data Colour** tab.

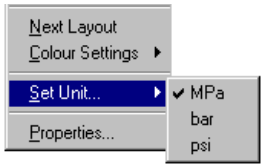
3. Click on the **Text** button or **Background** button and select a new colour. Click on **OK**. The result of the colour change is displayed in the tab.

4. Make further adjustments to the colours as appropriate.

5. Click on **OK** to close the dialogue and apply the changes.

Run data pressure units

If the Pressure data is displayed in the run data window you can set the displayed units.



1. Click on the **Pressure** data with the right mouse button to display the menu.

2. Select **Set Unit** and then the appropriate unit, either **MPa, bar** or **psi**, from the menu cascade.

   The selected unit is now displayed.

6.2.3 Curves

The curves window displays monitor signal values graphically.

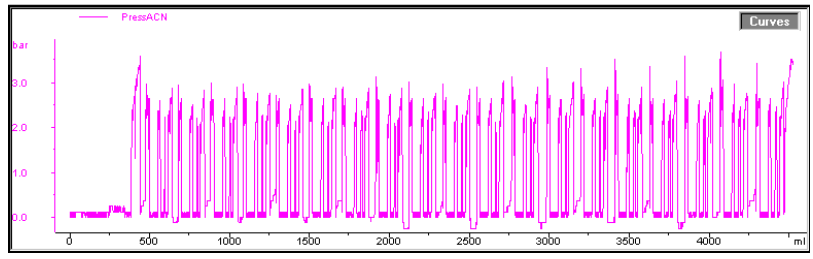


Figure 6-6. The Curves window.

To select the curves to be monitored on the screen:

1. Select **View:Properties** or select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed.

2. Select the **Curves** tab.

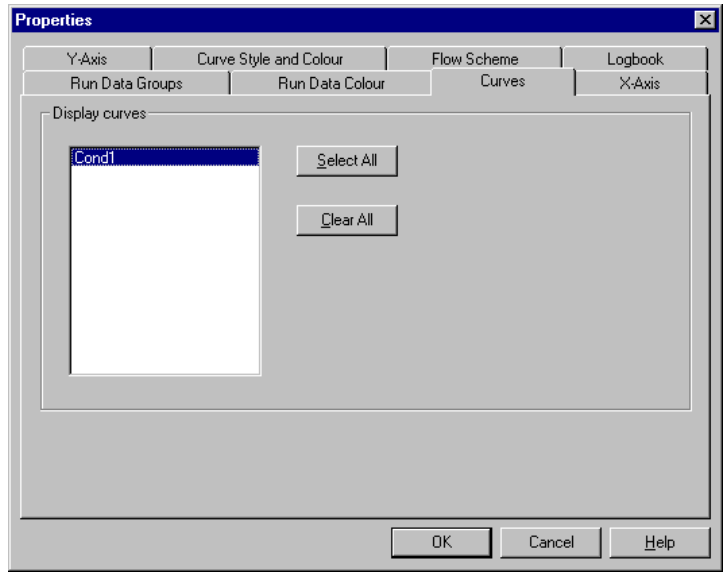


Figure 6-7. Properties dialogue, Curves tab.

3. Select the curves to be displayed from the list or click on **Select All** if you want to view all of the available curves. Note that curves will only be shown for components present in the oligosynthesis system. To clear the selection, click on **Clear All**. The curves in this list are those for which **Store** is set to **On** in the system settings (see Section 15.4) together with any reference curves defined in the method.

Note: Fraction marks, injection marks and set marks will always be shown and are not curves in the list.

4. Click on **OK**.

Vertical cursor line

To display a vertical cursor line select **Marker** from the right mouse button menu. Drag the cursor line with the mouse. Where the line bisects the curve, the X-axis and Y-axis values are displayed at the top of the window.

Changing the curve colours and styles

The curves window displays graphs for the selected curves in different colours, with any reference curves included with the method as dashed lines. The curve colours and styles can be changed:

1. Select **View:Properties** or select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed.

2. Select the **Curve Style and Colour** tab.

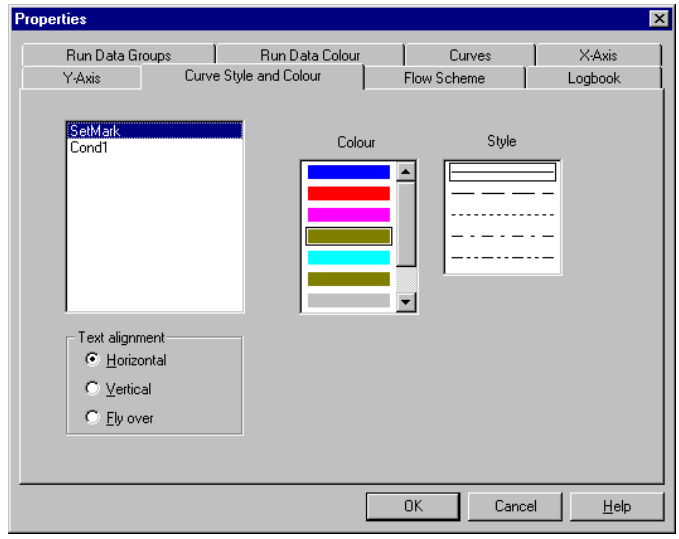


Figure 6-8. Properties dialogue, Curve Style and Colour tab.

3. Select a curve from the list and then select an appropriate colour and style.

4. Click on **OK**.

Changing the scale of y-axis

The y-axis is automatically scaled for each of the curves. To fix the scale of individual curves:

1. Select **View:Properties** or select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed.

2. Select the **Y-axis** tab.

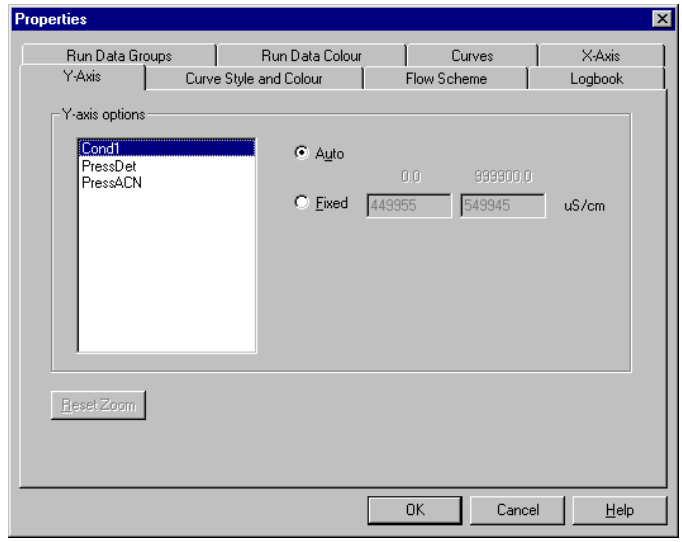


Figure 6-9. Properties dialogue, Y-Axis tab.

3. Select the appropriate curve and click on the **Fixed** button. Enter a minimum and maximum range in the fields within the specified limits.

4. Repeat steps 2 to 3 for other curves.

5. Click on **OK**.

Values on the y-axis apply to the curve with the same colour as the axis markings. Click on the legend to get the correct Y-axis.

Changing the scale of x-axis

Click on the x-axis to switch the display between time and volume units. (The run is controlled according to the time/volume base defined in the current block, regardless of the base in the curves display).

Alternatively, select the x-units in the **X-Axis** tab of the **Curve Properties** dialogue.

You can also set the viewed portion of the total run.

1. Select **View:Curve Properties** or select **Properties** from the right mouse button menu. The **Properties** dialogue is displayed.

2. Select the **X-Axis** tab.

3. Select the appropriate base, **Time** or **Volume**.

Note: Switching between bases may alter the resolution since the sampling frequency is adjusted according to the flow rate.

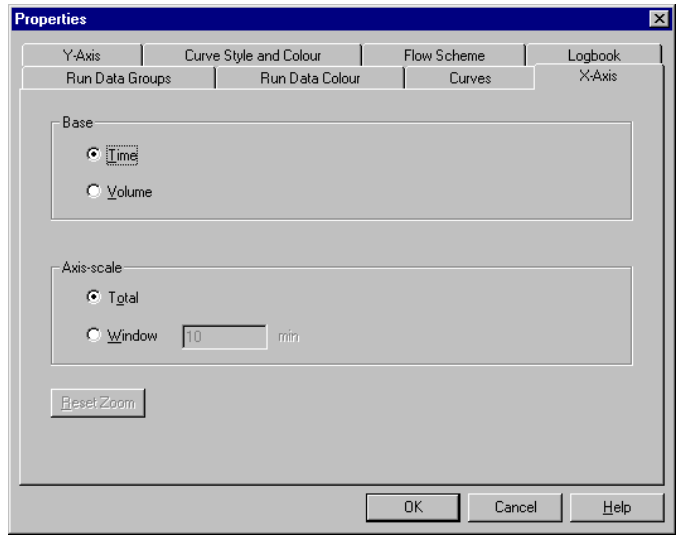


Figure 6-10. Properties dialogue, X-Axis tab.

4. Select the appropriate **Axis scale**, either **Total** or **Window**. The **Total** option will show the curves as far as they have come in the run. The **Window** option allows you to set the portion of the total window to be displayed, either in minutes or ml depending on the selected base.

5. Click on **OK**.

The zoom function

To zoom in on a selected region of the curve window:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

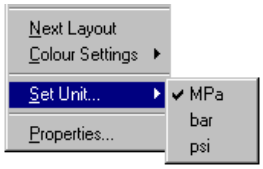
- reverse each zoom in action a step at a time by displaying the right mouse button menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the right mouse button menu and selecting **Reset Zoom**

Viewing hatch marks

You can display a grid in the curve window by selecting **Hatch** from the right mouse button menu.

Selecting curve pressure units

If the Pressure curve is displayed in the curves window you can set the displayed units.



1. Click on the pressure curve with the right mouse button to display the menu.

2. Select **Set Unit** and then the appropriate unit, either **MPa, bar** or **psi**, from the menu cascade.

   The selected unit is now displayed on the y-axis.

Alternatively:

1. Select **Properties** from the right mouse button menu.

2. In the displayed **Properties** dialogue select the **Y-Axis** tab.

3. Select the **Pressure** curve and then the appropriate pressure unit radio button. Click on **OK to implement** the change.

Selecting the text alignment

You can select the way that text is aligned for the set mark curves and fraction curve.

1. Select **Properties** from the right mouse button menu.

2. In the displayed **Properties** dialogue select the **Curve Style and Colour** tab.

3. Select the **SetMark** or **Fraction** curve as appropriate.

4. Select the appropriate **Text alignment** option from **Horizontal**, **Vertical** or **Flyover**. **Flyover** displays the text only if you place the mouse pointer over the generated mark. Click on **OK to implement** the change.

6.2.4 Flow scheme

The flow scheme is a graphical representation of the oligonucleotide synthesis system. During a run, the flow scheme displays open flow path(s) in colour and monitor signals with numerical displays. The flow scheme thus shows the current status of the run at a glance.

Stretching a flow scheme

The flow scheme can be stretched to fit the screen by selecting **Stretch** from the right mouse button menu. Alternatively, select the **Properties** option and the **Flow scheme** tab is shown in the **Properties** dialogue. Check the **Stretched** box to display a stretched view of the window.

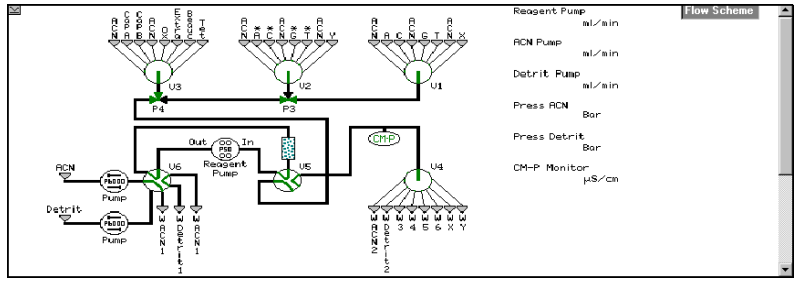


Figure 6-11. The flow scheme for a run.

Viewing multiple flow schemes

If there is more than one flow scheme picture for the system (there may be up to five pictures per system), then these can be selected and viewed in the right mouse button menu.

6.2.5 Logbook

All actions (including method start and end, base instruction, method instructions and manual interventions such as **Pause** or **Hold**) and unexpected conditions such as warnings and alarms are logged for every run, with date, time and current username where appropriate. (The date and time are taken from the system clock in the PC.) The logbook thus provides complete history of any given run. The log is saved in the result file.

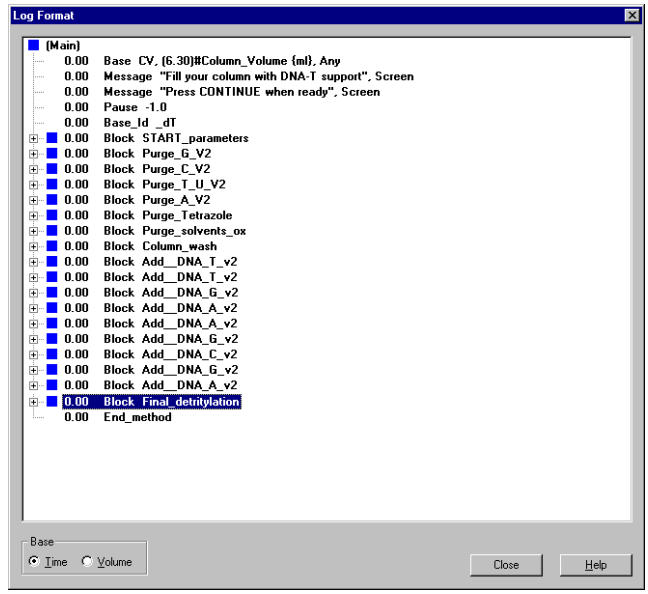
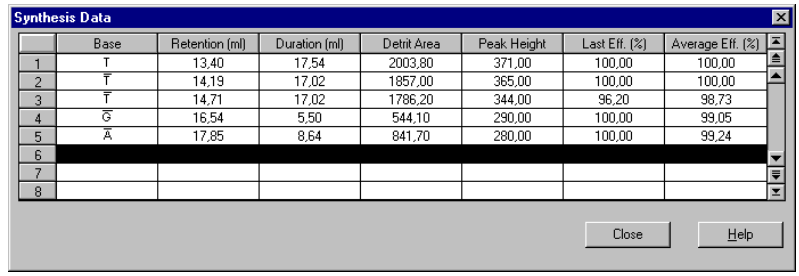


Figure 6-12. The logbook panel for a running method.

The logbook window can autoscroll to display the latest entries if you select the **Autoscroll** function from the right mouse button menu or else check the **Autoscroll** option in the **Properties** dialogue.

6.2.6 Synthesis Data

The **Synthesis Data** window can be accessed under **View:Synthesis Data** from within the System Control module (a summary **Synthesis Data** view is also available in the Evaluation module). For the current run, **Synthesis Data** displays a spreadsheet with summary information for each base in the chosen sequence. Units (time or volume) are identical to those in the chromatogram.



	Base	Retention (ml)	Duration (ml)	Detrit Area	Peak Height	Last Eff. (%)	Average Eff. (%)
1	T	13,40	17,54	2003,80	371,00	100,00	100,00
2	T	14,19	17,02	1857,00	365,00	100,00	100,00
3	T	14,71	17,02	1786,20	344,00	96,20	98,73
4	G	16,54	5,50	544,10	290,00	100,00	99,05
5	A	17,85	8,64	841,70	280,00	100,00	99,24
6							
7							
8							

Figure 6-13. The Synthesis Data window within System Control.

6.3 Manual control

6.3.1 The toolbar

The toolbar at the top of the System control workspace contains a set of buttons for starting and stopping the run, accessing documentation and locking the system.

Figure 6-14. The toolbar in the System control workspace.

Run — Starts a run when the system is in **End** state and a method is loaded.

Hold — Suspends execution of a method, but continues to pump liquid at the current flow rate and eluent concentration settings. Accumulated time and volume continue to be incremented.
Any method instructions which are set to the time/volume when **Hold** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Pause — Behaviour on Pause is strategy dependent. Pause suspends execution of a method and stops all pumps so that the system comes to a standstill. In OligoPilot and OligoProcess, valves remain in the position they were in before the pause. Accumulated time and volume is not incremented during **Pause**.

Any method instructions which are set to the time/volume when **Pause** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Continue — Resumes execution of a paused or held method.

End — Terminates method execution and puts the system into **End** state.

These commands can also be located under the System control **Manual** menu.

The available buttons in System control are dependent on the control status of the connection:

Status	*Available buttons*
End	**Run**
Running	**Hold**, **Pause**, **End**
Manual	**Run**, **Pause**, **End**
Hold	**Pause**, **Continue**, **End**
Method pause	**Hold**, **Continue**, **End**
Manual pause	**Run**, **Continue**, **End**

Other buttons on the toolbar are:

Opens a dialogue for choosing which window panels to display. Clicking on this button is equivalent to choosing the menu command **View:Window**.

Opens the documentation pages. Run notes may be entered in the Notes page. Other pages are displayed for information only.

The connection mode button has three states which indicate and change the connection mode (see table below)..

Button	*Connection mode*	*Click to change mode*
	System control workspace disconnected	Click to connect to a system
	Control mode connection	Click to leave control of system
	View mode connection	Click to establish a control mode connection (if possible)

The status bar also displays a text message indicating the connection status of the window:

Controlled by :<user> The indicated user has a control mode connection to the system. Other users may establish a view mode connection.

Locked by: <user> The indicated user has left the system in a locked state. Users who can supply the required password may unlock the system and establish a connection. Note that the password is case sensitive.

Note: It is possible to unlock with the "lock" password or with the UNICORN logon password. If using the UNICORN logon password, the user must have the **Unlock systems** access rights. The "lock" password is the password entered by the user who locked the system and is case sensitive.

System is available Any user may establish a connection.

System connections are described in more detail in Section 6.5.

6.3.2 Manual instructions

The oligonucleotide synthesis system can be controlled with manual instructions issued from the **Manual** menu. The available instruction options are dependent on the strategy, and only instructions for the components defined for the system are displayed. To save the results of a manual run, issue the instruction Record_on (in the **Other** instruction group) at the beginning of the run. UNICORN will prompt for a result file name at the beginning of the run.

The **Manual** menu opens a dialogue similar to the text instruction box in the Method editor (see Section 5.4):

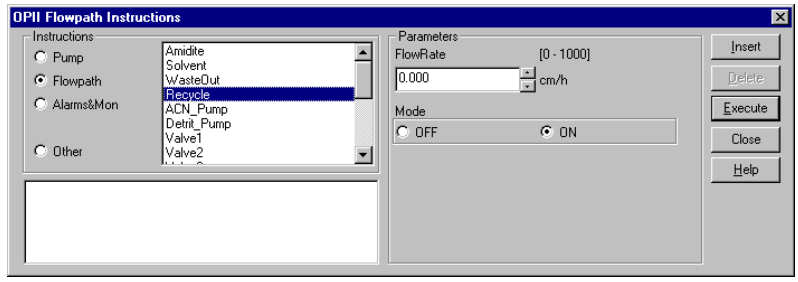


Figure 6-15. The Manual instruction dialogue.

The name of the connected system is displayed in the title bar of System control. Available instructions are determined by the strategy and selected optional components for the connected system. Instructions for the OligoPilot strategy is listed in Appendix B.

Manual instructions are entered in the same way as method instructions from the dialogue in the Method editor. The **Insert** button places the current instruction in the list at the bottom left of the dialogue. Clicking on **Execute** executes all instructions in the list at the same time, or executes the currently marked instruction if the list is empty. Note that although all instructions are executed simultaneously, some (for example gradient and fraction instructions) may take some time to complete in the liquid handling module.

The **Delete** button deletes selected instruction from the current list. Only one instruction can be deleted at a time.

If you close the dialogue by clicking on the **Close** button without choosing **Execute**, commands in the list will not be executed and will be deleted from the command list. Manual instructions can also be issued while a method is running. A manual setting applies until the next method instruction of the same type is executed (e.g. a manual Flow instruction will set the flow rate until the next Flow instruction in the method is executed). Manual instructions that you issue during a method are recorded in the logbook for the method run.

6.3.3 Alarms and warnings

The system settings (see Section 15.1) determine the acceptable limits of monitor signals during a run. The limits can also be set for the current run using an instruction in the method. Limits set with a method instruction override the limits set in system settings. If these limits are exceeded in a run, a warning (W) or alarm (A) dialogue with a message is displayed on the screen.

- The run continues if a warning is issued.
- An alarm pauses the system.

Warnings and alarms are displayed regardless of the activity currently in progress in UNICORN: you will be notified of an exceeded limit in a running system even if you are developing a method, evaluating data or monitoring a run on a different system. Warnings and alarms are also recorded in the logbook for the run.

In a network installation alarms and warnings are displayed on the controlling station and all stations viewing the system. An alarm can be acknowledged only from the computer connected in control mode: alarms are displayed but cannot be acknowledged on computers connected in view mode.

Note: For this reason, we discourage "passive" operation of a system, i.e. with no controlling connection.

6.4 If communication fails

This section summarises the consequences of system failure during a run. See Section 11.3 for more details.

If the results of a run were to be stored on a server or another location and there is network communication failure during a run that has been started from a remote station, the run will continue and the results will be saved in the Failed folder on the local station. A control mode connection can be established on the local station to control the running system.

6.5 Managing system connections

UNICORN installed on a given computer may have up to four System control windows (the actual number is determined when the software is installed, see Chapter 13), each of which may be connected to one oligonucleotide synthesis system at a time. Connections are managed using the **Connect** and **Disconnect** commands in the **System** menu. A network installation may have more than four systems in total, but each computer in the network can establish a maximum of four connections. Connection management is the same for stand-alone and network installations.

6.5.1 Establishing a connection

To connect a System control module to an oligonucleotide synthesis system, open a System control that is not currently in use (identified by the Disconnected icon in the System control tool bar) and choose **System:Connect**. The dialogue lists the systems to which you have access. Select the system to which you want to connect and click on **OK**.

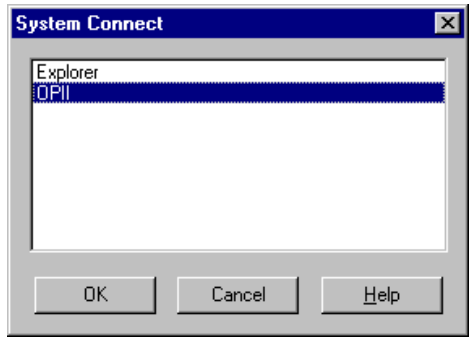


Figure 6-16. System connect dialogue.

To connect to a system from a remote station in a network installation, the local station (i.e. the computer physically connected to the oligonucleotide synthesis system) must be logged on to the network. The UNICORN drivers must be running on the local station although the UNICORN program does not need to be running.

A local station can be used to control the oligonucleotide synthesis systems directly connected to the PC without logging on to the network. Files stored on network drives will of course not be accessible. This mode of working places UNICORN into an “error” state and you should not ideally work in this state since global files such as the user settings file (musers30.mpm) etc. are stored on the network. Any changes made to these files while not logged on will apply locally and will be lost the next time you log on to the network to use UNICORN. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the Failed folder on the local station (see Section 11.3).

Several simultaneous connections can be established to one system, but only one may be in control mode, i.e. able to actively control the system. The other connections are in view mode, and can monitor the system activity but cannot issue commands.

6.5.2 Connection modes

The possible connection states of a System control module are indicated by the connection mode button and the status text on the status bar as summarised below:

Button	*Connection mode*	*Text*	*State/Action*
	Not connected	(none)	Click on the connection mode button or choose **System:Connect** to establish a connection.
	Control mode	**Controlled by: <user>**	Click on the connection mode button to leave the system but retain the connection with the System control module. You may leave the system locked or unlocked.

	View mode	**Controlled by: <other user>**	The indicated user has a control mode connection. Clicking on the connection mode button has no effect.
	View mode	**Locked by: <other user>**	The indicated user has left the system in a locked state. Click on the connection mode button to establish a control mode connection (you must supply the locking password, which is case sensitive, or your logon password if you have Unlock locked systems authorisation).
	View mode	**System is available**	A user has left the system in an unlocked state. Click on the connection mode button to establish a control mode connection.

In all modes you can choose **System:Disconnect** to disconnect the system from the control window.

6.5.3 Leaving and locking a system

A running or **End** state system with a control mode connection can be left and locked by clicking on the control mode button or selecting **System:Leave system**. When the system is left, the connection becomes a view mode connection. After leaving and locking scouting or MethodQueue runs, it is not possible to establish a control mode connection from another computer.

Figure 6-17. Leave Control dialogue.

You may leave the system unlocked or locked:

- **Unlocked** leaves the system unlocked. Any other user may establish a control mode connection to the system. Use this option if you do not intend to use the system in the near future.
- **Locked** locks the system with the password specified in the dialogue. A control mode connection can only be established by providing the correct password. Note that this password is independent of the user's logon password. A locked system can also be unlocked with the logon password for a user with **Unlock locked systems** authorisation. This authorisation should be restricted to a small number of users to prevent indiscriminate unlocking of locked systems.

6.5.4 Disconnecting a system

To disconnect an oligonucleotide synthesis system from a System control module, choose **System:Disconnect**. If you are disconnecting a control mode connection, you will be asked if you wish to leave control of the system under a password protected lock.

Logging off or quitting UNICORN automatically disconnects all connected systems, displaying the **Leave control** dialogue for each system. Systems which are disconnected in this way will be re-connected automatically when you log on to UNICORN again. (Note however that you may have disconnected from a control mode connection but establish a view mode connection on re-connect, if another user has taken control of the system in the meantime).

Note: You can disconnect a system during a run and the run will continue. It is not recommended to do this without locking the system, since this can leave a run on the system with no responsible user. You cannot, however, disconnect from MethodQueue runs.

6.5.5 Network considerations

In a network installation, an oligonucleotide synthesis system can be controlled from any computer in the network provided that the user has sufficient access rights in UNICORN. UNICORN software has to be installed but not necessarily running on the computer to which the system is physically connected. The computer has to be logged on to the network.

- A system which is controlled by another user through a control mode connection can be viewed through a view mode connection by any user with sufficient access on any number of computers in the network. This allows runs to be monitored from multiple display stations (although only the active connection can control the system).
- A system that is locked by a user can be unlocked on any computer in the network (not possible during MethodQueue runs) by any user with sufficient access rights (see above).
- *A UNICORN* user may connect to a system with up to eight different remote workstations in the network. Each successive multiple instance of the user automatically establishes the same System control connections as the first instance when the logon is performed. Multiple instances are however treated by UNICORN as separate users (although they are not distinguished in the System control workspace display) and only one of the instances may maintain a control mode connection to a system. Multiple instances may also disconnect and connect their System control modules independently of each other once the logon is performed. See Section 2.6.2.

6.6 Calibrating monitors

Certain system monitors need to be calibrated regularly for correct results. According to the routines established in the laboratory or process department, monitors may be calibrated at pre-set intervals by the system technician, or calibrated as required by the user before each run.

This section describes the calibration procedure for the ACN and Detrit solvent pumps in OligoPilot II. For other synthesis systems, users are recommended to read the relevant system manual for calibration procedures.

To calibrate the solvent pumps for OligoPilot II do the following:

1. Select System:Calibrate to display the Calibration box.

2. Select each of the pumps in turn and perform the procedures described below.

3. Click on Exit once the calibrations have been made.

Detrit solvent pump

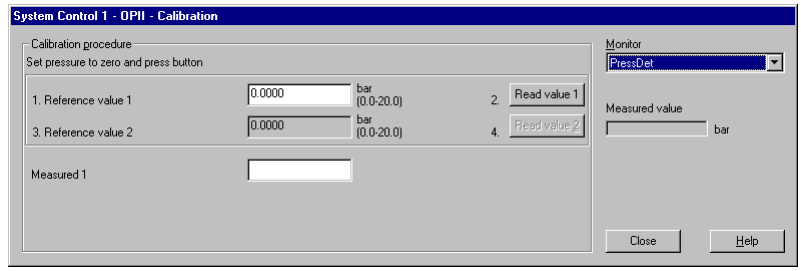


Figure 6-16. Dialogue box for pressure calibration of the detrit pump.

Select PressDet in the Monitor pull-down list to calibrate the pressure reading of the Detrit P-6000 solvent pump. The calibration is based on the maximum set pressure of the pump.

1. The value in the Reference value 1 box should be zero (0.0000 bar). If not, enter 0.0000. Click on the Read value 1 button.

2. Enter the desired maximum pressure value (<20.0 bar) in the Reference value 2 box. On the detrit pump, press and hold the SET button and manually set the pressure to the desired level. While holding the SET button on the pump, you can observe in the UNICORN Calibration dialogue that an internal Measured value is assigned. Click on the Read value 2 button and then release the SET button on the pump. Click on Save to save the calibration. Refer to the P-6000 Pump Instruction Manual for more detailed instructions.

Note: A value for the actual calibrated pump pressure can be obtained by multiplying the values obtained in the Measured value and Measured 1 fields.

ACN solvent pump

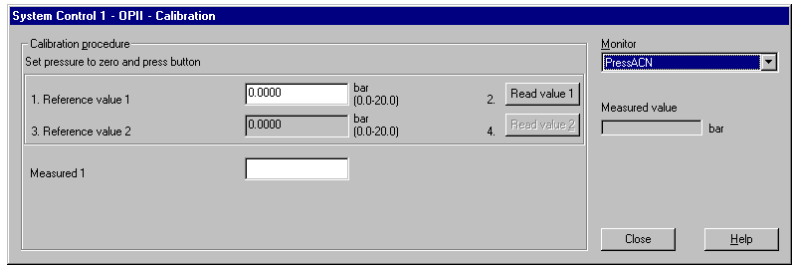


Figure 6-17. Dialogue box for pressure calibration of the ACN pump.

Select PressACN in the Monitor pull-down list to calibrate the pressure reading of the ACN P-6000 solvent pump. The calibration is based on the maximum set pressure of the pump.

1. The value in the Reference value 1 box should be zero (0.0000 bar). If not, enter 0.0000. Click on the Read value 1 button.

2. Enter the desired maximum pressure value (<20.0 bar) in the Reference value 2 box. On the ACN pump, press and hold the SET button and manually set the pressure to the desired level. While holding the SET button on the pump, you can observe in the UNICORN Calibration dialogue that an internal Measured value is assigned. Click on the Read value 2 button and then release the SET button on the pump. Click on Save to save the calibration. Refer to the P-6000 Pump Instruction Manual for more detailed instructions.

Note: A value for the actual calibrated pump pressure can be obtained by multiplying the values obtained in the Measured value and Measured 1 fields.

6.7 Maintenance

Some strategies support the possibility to view system information for the components in a synthesis unit and to define warnings on the components for maintenance purposes.

Note: OligoPilot II systems do not support these **Maintenance** functions.

6.7.1 Viewing system component information

To view the maintenance functions for a maintenance-supported oligosynthesis system:

1. Select **System:Maintenance**. The **Maintenance manager** dialogue is displayed. The **Info** tab is shown by default and UNICORN takes a few moments to scan the connected oligosynthesis system for the components present.

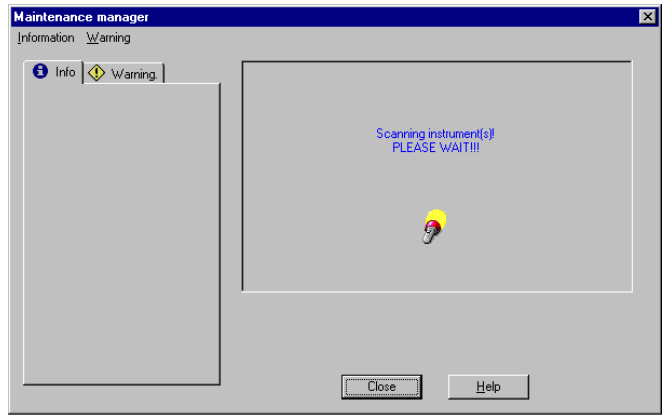


Figure 6-18. Maintenance manager dialogue, Info tab.

When UNICORN has completed its scan all system components are displayed.

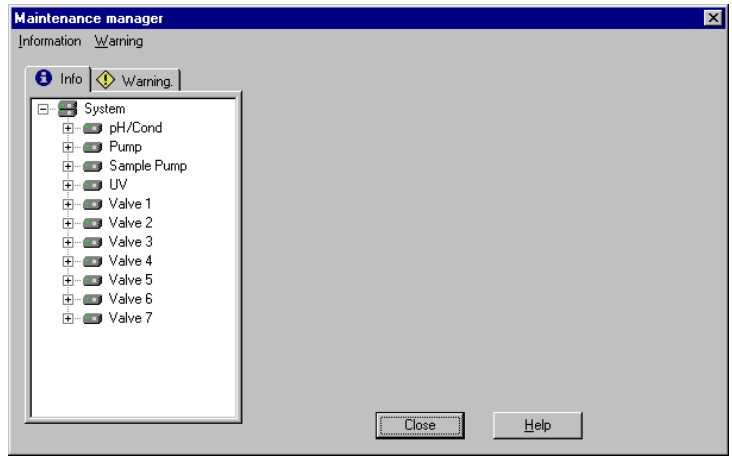


Figure 6-19. Maintenance manager dialogue, Info tab with system components displayed.

2. To view the information about a specific component, click on it. The information can be viewed on the right-hand side of the tab.

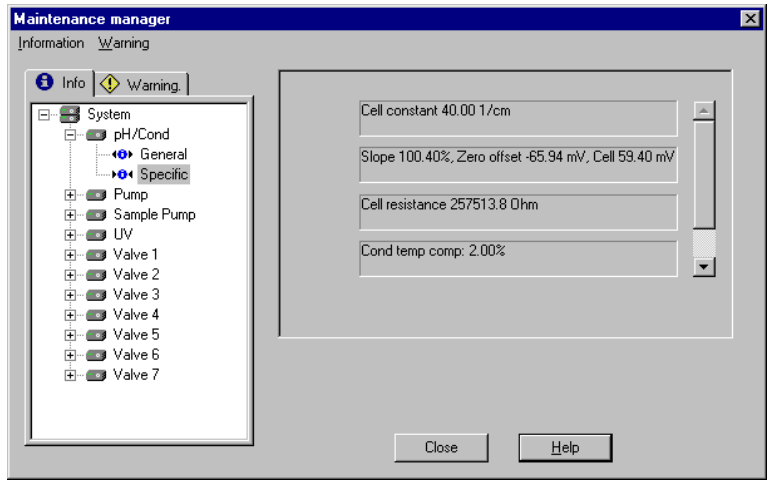


Figure 6-20. Maintenance manager dialogue, Info tab with information displayed for pH/Cond cells.

You can select to view **General** information, which contains information such as serial number, version number etc., or **Specific** information, which contains information such as how long the component has been used, how many hours for example a pump has run etc.

6.7.2 Setting up maintenance warnings

To set up a maintenance warning:

1. Click on the **Warning** tab. All of the system components are displayed.

2. Click on a specific component for which you want to set up a warning and select **Warning:New** from the menu. Alternatively, click with the right mouse button on the specific component and select **New** from the displayed menu.

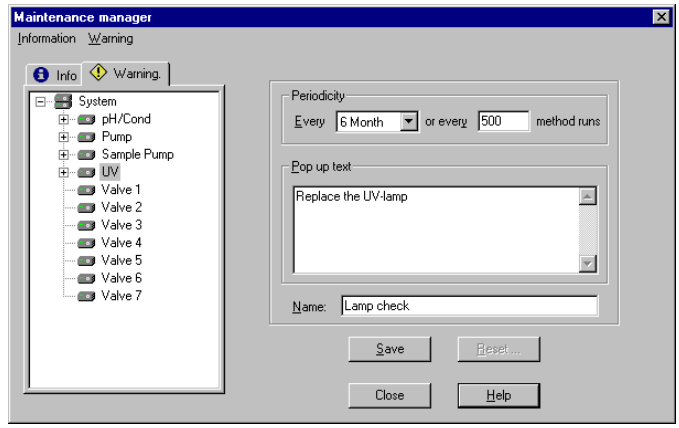


Figure 6-21. Maintenance manager dialogue, Warning tab.

3. Enter the appropriate values for **Periodicity**, enter the desired **Pop up text** and enter a **Name** for the warning type.

4. Click on **Save** to save the warning. The new warning is added to the specific component.

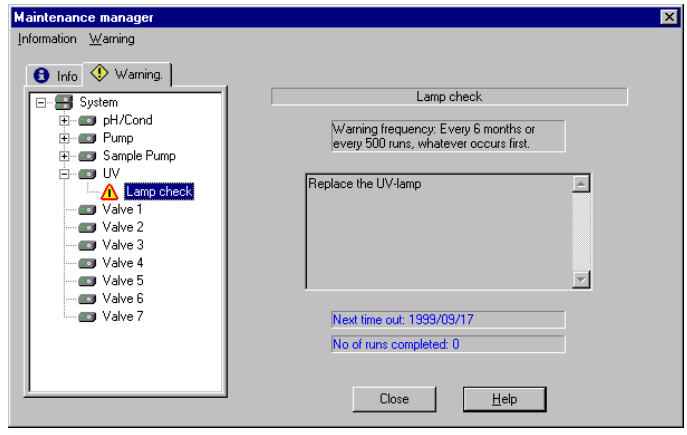


Figure 6-22. Maintenance manager dialogue, Warning tab with a defined warning set up for the UV lamp.

5. Repeat the process for setting up warnings for the same component or other components in the list.

6. Click on **Close** when you have made the appropriate selections.

6.7.3 Viewing and zeroing the warning parameters

A counter is set up linked to the **Periodicity** that you defined for a new warning message (see above). By entering the edit warning mode you are able to zero the warning parameters. You can do this by selecting the warning in the **Warning** tab followed by selecting **Warning:Edit** from the menu. Alternatively, click with the right mouse button on the warning and select **Edit** from the displayed menu. In the edit mode click on the **Reset** button, which displays the **Reset parameters** dialogue.

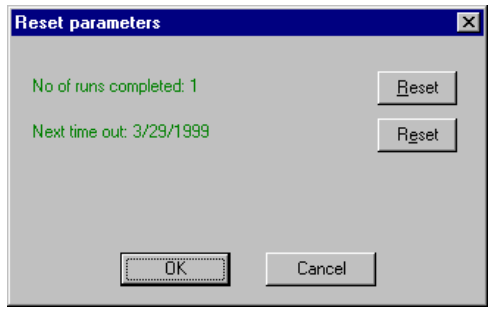


Figure 6-23. Reset parameters dialogue.

You can zero specific counters by clicking on the associated **Reset** button in the dialogue.

6.7.4 Getting a warning

When you end a method using an oligosynthesis system for which maintenance warnings have bee set up, a warning message will be displayed once the specific **Periodicity** parameter has been reached.

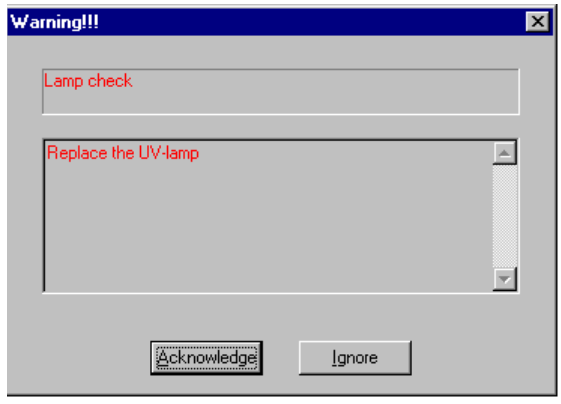


Figure 6-24. Warning dialogue for a lamp check.

7 MethodQueues

MethodQueues provide a means for linking several methods together, on the same or different systems. For example, if a system wash procedure is programmed in a separate method, it can be linked in a MethodQueue to a series of different process methods, ensuring that the same wash procedure is used before every process. Alternatively, the product of a separation on one system might form the starting material for a separation on the next, allowing fully automated multi-step processing.

Specific user authorisation is required to edit and run MethodQueues, separate from that required for editing and running methods.

7.1 Setting up a MethodQueue

7.1.1 Defining a MethodQueue

To create a new MethodQueue:

1. In Main menu select **File:New:MethodQueue** or **MethodQueue:New**. The **MethodQueue Editor** dialogue is displayed.

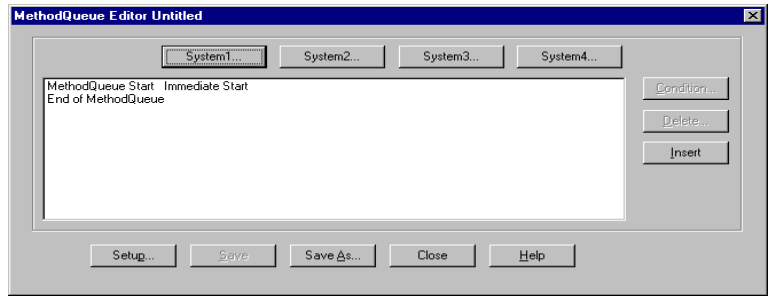


Figure 7-1. MethodQueue Editor dialogue

2. To add a method to the MethodQueue list, select the **End of MethodQueue** instruction in the list.

3. Click on the appropriate **System #** button. The **Load MethodQueue** dialogue is displayed.

Note: The number of available **System #** buttons is dependent on the number of systems that were selected during installation of UNICORN.

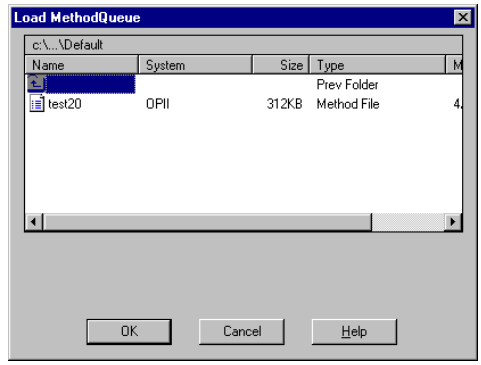


Figure 7-2. Load MethodQueue dialogue.

4. Use the dialogue to locate and select the required method. Click on **OK** or double click on the method item.

   The system type for the selected method, e.g. **Basic 10**, is assigned to the selected **System #** button and the **System #** name on the button is replaced with the name of the system.

   A line is also inserted into the MethodQueue list before the previously selected **End of MethodQueue** instruction. The new line contains the name of the selected method.

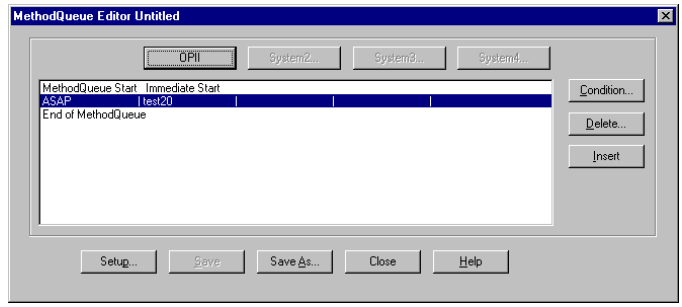


Figure 7-3. MethodQueue Editing dialogue with a method selected for a Basic 10XT system.

5. To add more method steps click on the **Insert** button and repeat steps 3-4. Note that you must use a new **System #** button to add methods written for a different system type.

   For example, if you have designated **System 1** to methods for the **Basic 10** system, then only methods written for Basic 10 can be added with this button. If you want to add to the list methods

written for, for example, Explorer 100 then you must use another free **System #** button such as **System 2**. In this case, **System 2** will be assigned and renamed **Explorer 100** and only methods written for this system can be added using this button.

6. By default each method step will start as soon as possible (**ASAP**) after the completion of the previous method step. To set the time interval for starting a selected step click on the **Condition** button. In the **Condition** dialogue set the time when the step is to start. Click on **OK**.

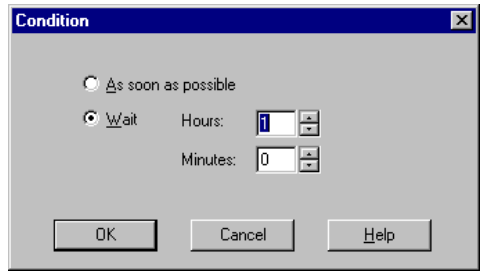


Figure 7-4. Condition dialogue.

The timing of MethodQueue steps performed on different systems can also be controlled by the Ready instruction (see Section 8.3.1) in the method.

7. Click on **Setup** to define the starting time for the MethodQueue. **Immediate start** sets the MethodQueue to start as soon as you request **Run** from the Main menu. **Start time** sets the MethodQueue to start at a pre-set time of day up to one week after **Run** is requested. If no day is specified, the MethodQueue will start as soon as the pre-set time is reached (i.e. within the next 24 hours).

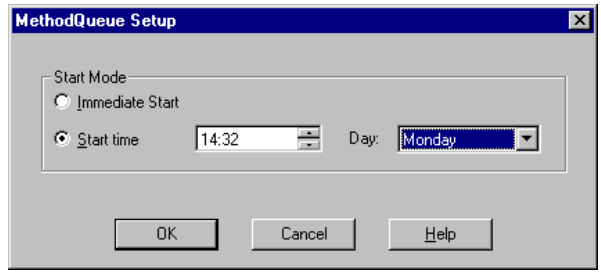


Figure 7-5. MethodQueue Setup dialogue.

Note: The MethodQueue setup time defines the starting time for the MethodQueue as a whole. Do not confuse this with **Condition**, which defines the relative starting time for a step within a MethodQueue.

8. Click on **Save** to save the MethodQueue. Enter a MethodQueue name in the dialogue.

Note: **Edit MethodQueue** authorisation is required to define a new MethodQueue.

7.1.2 MethodQueue folders and icons

MethodQueues are saved in a separate MethodQueue folder within the folder that you specified during the save. The MethodQueue folder is represented by a special icon in the **Methods** window of the main UNICORN menu.

MethodQueue folder.

Double-click on the MethodQueue folder icon to open it. A MethodQueue folder contains the MethodQueue definition and copies of all methods included in the MethodQueue.

MethodQueue definition (in MethodQueue folder).

It is important to realise that the MethodQueue works with copies of the original method files. If changes are made in the original method, these will *not* affect the method in the MethodQueue. To implement changes in a MethodQueue method, edit the method in the MethodQueue folder. Alternatively, edit the original method, then use the MethodQueue editor to update the MethodQueue, replacing the old method with the changed version. It is a good idea to make sure that MethodQueue definitions always contain updated methods, to avoid confusion between different versions of method files.

7.2 Editing MethodQueues

To edit an existing MethodQueue, open the MethodQueue icon with the right mouse button menu command, **Edit**. The **MethodQueue Editor** dialogue is displayed for the selected MethodQueue. **Edit MethodQueue** authorisation is required to edit a MethodQueue.

- To change the start condition or method name, select the line to be edited and click on the **Condition** or assigned system button respectively.

- To insert a new MethodQueue line after the currently selected line, click on **Insert**.
- To erase the currently selected line from the MethodQueue, click on **Delete**. In the dialogue box, check the system(s) for which the method is to be deleted.

7.3 Running a MethodQueue

Before starting a MethodQueue run, make sure that all systems used in the MethodQueue are connected with control mode connections (see Section 6.5) and are in **End** status. The system associated with the first **System** button in the MethodQueue definition must be connected to System control window 1, the second system to System control window 2 and so on. The MethodQueue will not start unless all required systems are connected in control mode.

To start a MethodQueue from the Main menu, select the MethodQueue icon and select **File:Run** or select **Run** from the right mouse button menu. The MethodQueue will start in accordance with the conditions defined in the MethodQueue setup. You cannot start or end a MethodQueue from the System control window.

Run MethodQueue authorisation is required to start a MethodQueue.

7.3.1 Method execution in MethodQueues

The start protocol for the first and each subsequent method step in the MethodQueue is displayed when the corresponding method is run. If you require unattended MethodQueue operation after the start of the first method step, make sure that subsequent method steps do not include a start protocol.

The **Condition** setting for each step in a MethodQueue determines the relative timing of the steps. If successive methods are run on the same system, the timing set in **Condition** applies from the completion of one method to the start of the next.

If successive methods are run on different systems, the Ready instruction in one method can be used to trigger the start of the next method, i.e with the Ready instruction you will be able to start the next method already before the current method has ended. The Condition setting then applies from the Ready instruction to the start of the triggered method. This is useful for example in situations where a method on one system prepares the starting material for the next, and then continues to wash the system:

System 1 System 2

Apply sample

Elute

READY ----------Apply sample

Wash Elute

....

7.4 Displaying MethodQueues

Choose **MethodQueue:Display running** to display pending and running MethodQueues. A pending MethodQueue is one for which **Run** has been requested, but which has not yet started either because the system is not available or because the Setup time has not been reached.

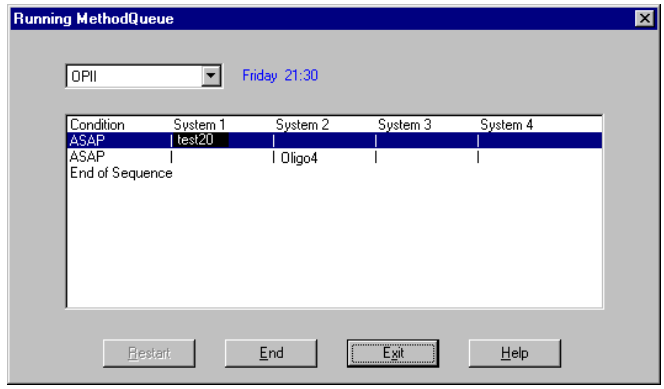


Figure 7-6. Displaying running and pending MethodQueues.

Use the list box at the top left corner of the dialogue to select a MethodQueue. The list shows pending and running MethodQueues.

For the selected MethodQueue, **Start** at shows the time when the MethodQueue is programmed to start. The actual time of start is shown for a currently running MethodQueue. The buttons in the MethodQueue display have the following functions:

Restart Restarts the currently running MethodQueue if a start protocol has been terminated by Cancel.

End	Terminates a running MethodQueue after the current step. Any methods currently in operation will continue to run, and must be terminated with End in the System control window if they are not to run to completion. Clicking on End for a pending MethodQueue deletes the MethodQueue from the pending list.
Exit	Closes the MethodQueue monitor panel.

Introductory material

Methods and runs

Evaluation

System management

Appendices

8 Presenting results

A result file is automatically generated at the end of a run and contains a complete record of the run, including method, system settings, curve data, run log and Trityl data. The Evaluation module offers extensive facilities for presenting synthesis run data.

This chapter describes how to:

- view the documentation from a run
- present the chromatograms and curves of your result file
- compare chromatograms and curves
- print reports

8.1 Opening a result file

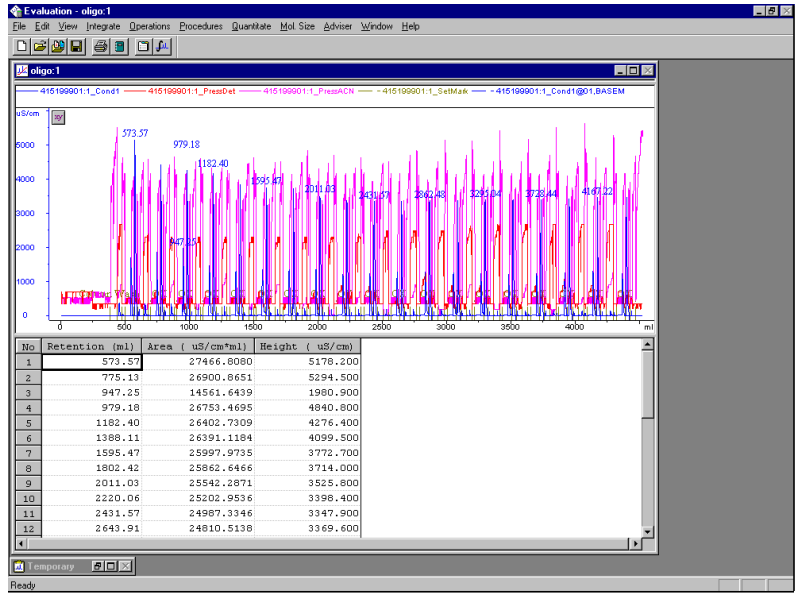


Figure 8-1. Evaluation module view.

To open a result file, either:

- double click on a result file icon in the **Results** window of the Main menu,
- select a result file icon in the **Results** window of the Main menu and select **File:Open**,

- click on the **Evaluation** icon in the Windows NT taskbar, select **File:Open** or click on the **Open** toolbar button, and select a result file from the **Open Result** dialogue,
- click on the **Evaluation** toolbar button in the Main menu and select a result file from the **Open Result** dialogue.

All contents of the opened result file are transferred to the Evaluation workspace. By default, the chromatograms in a run are shown as opened windows. The chromatogram window on top is the active window. There is also an minimized **Temporary** chromatogram window.

Note: The **Temporary** chromatogram window may be hidden from view - to view it, us the **Window:Cascade** or **Window:Tile** functions.

8.1.1 Chromatogram

A chromatogram includes a number of curves that have been created during a run, such as UV, conductivity, fraction marks, etc. The original raw data curves cannot be deleted or modified, although they can be used as the basis for evaluation procedures and subsequent creation of new curves. A chromatogram also contains the curves created and saved during an evaluation session. The default name for the first chromatogram in a result file is 1.

8.1.2 Temporary chromatogram

The **Temporary** chromatogram is essentially an empty chromatogram and is specific to the Evaluation module. Thus, curves can be copied into **Temporary** using **Edit:Copy:Curve** and comparisons and/or evaluations can be performed. This is particularly useful if you do not want to clutter up your original chromatograms with a large number of curves. It can also be used to keep blank run curves or curves to compare when opening different result files. Information contained within the **Temporary** chromatogram is automatically saved from one evaluation session to the next, but is not saved within the result files. Click on the window restore button or select **Windows:Temporary**. The contents of the temporary chromatogram can be removed by selecting **Edit:Clear temporary chromatogram**.

8.2 Basic presentation of chromatograms

This section gives directions on how to access result files and optimise the presentation of a chromatogram and its curves via the so called **Chromatogram Layout** dialogue. The last evaluation operation that is performed can be undone using **Edit:Undo**.

8.2.1 The chromatogram window

The chromatogram window is divided into three main views for header information, run curves and peak tables. The displayed areas for the views can be adjusted by dragging the borders with the mouse cursor between the views.

Viewing the curves

The first time a chromatogram window is opened and viewed, a default layout is applied to display all of the original curves. The default layout can be changed by the user (see Section 9.2.9).

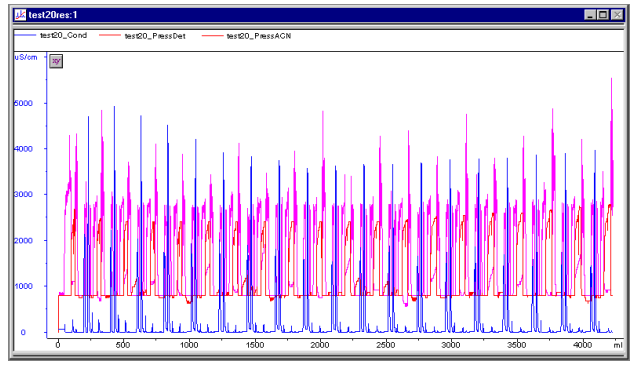


Figure 8-2. Displayed chromatogram in a newly opened result file.

Each curve is automatically assigned a default colour and style, with default information about each curve displayed in the key above the curves. This information includes the result file name, chromatogram name and curve name.

Each curve has a correspondingly coloured y-axis. To choose the appropriate y-axis scale, click on the y-axis until the desired scale is displayed, or simply click on the name of the curve of interest.

Optimising the workspace

Chromatograms can be minimised in the desktop by clicking on the minimize button in a chromatogram window. Icons can be neatly arranged in the workspace by selecting **Window:Arrange icons**.

To restore a window, click on the restore button for the iconised window or select the chromatogram name from the **Window** menu. You can also maximise a chromatogram window to fit in the whole Evaluation desktop by clicking on the maximize button.

To view several chromatogram windows side by side select **Window:Tile**. Alternatively, **Window:Cascade** will stack all of the open windows like a deck of cards.

8.2.2 Opening the Chromatogram Layout dialogue

Most of the changes that you are likely to make regarding chromatogram presentation, are made in the **Chromatogram Layout** dialogue. This is opened in one of two ways:

- Place the mouse cursor in the chromatogram window and select **Properties** from the right mouse button menu options. Note that the view from which you activate the **Properties** command determines the tab that is displayed in the **Chromatogram Layout** dialogue.
- Select **Edit:Chromatogram Layout**.

Note: You can apply any changes made in the **Chromatogram Layout** dialogue to all open chromatograms by checking the **Apply to all chromatograms** option.

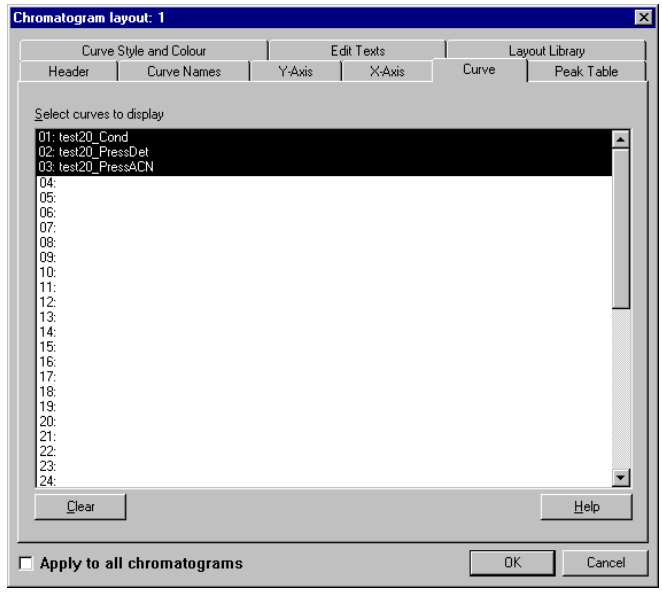


Figure 8-3. Chromatogram Layout dialogue, Curve tab.

The layout of the three views for header, curves and peak table can be modified in the various tabs that are displayed in the **Chromatogram Layout** dialogue. You can work freely in the **Chromatogram Layout** dialogue and all of the configurations are applied when you click on the **OK** button. If instead you want to close the dialogue without applying the changes you have made, click on the **Cancel** button. The main features of the **Chromatogram Layout** dialogue regarding chromatograms are described in the sections below. Features regarding peak tables are described in chapter 10.

8.2.3 Choosing the curve(s) you want to see

In the **Curve** tab of the **Chromatogram Layout** dialogue is a list of all curves contained within the chromatogram, numbered from 01 onwards. Select the curves you want to see in the chromatogram. Click on **OK** to return to the active chromatogram window.

8.2.4 Changing curve names

By default, names are sequentially built up from three components:

- result name,
- chromatogram name,
- curve name.

For example, a curve with the name 9139401:1_UV1_280, is derived from the result named 9139401. The chromatogram name is a number automatically given during a run, e.g. 1. The curve name corresponds to the curve type, e.g. UV1 for UV detection of an eluted component. If two or more curves of the same type were created within a result file, they will be numbered accordingly, e.g. UV1, UV2 etc. For systems using a variable wavelength detector, the wavelength for the UV curve is also given, e.g. 280.

If you do not want to display the entire names of the curves in both the dialogues and chromatogram windows:

1. Click on the **Curve Names** tab in the **Chromatogram Layout** dialogue.

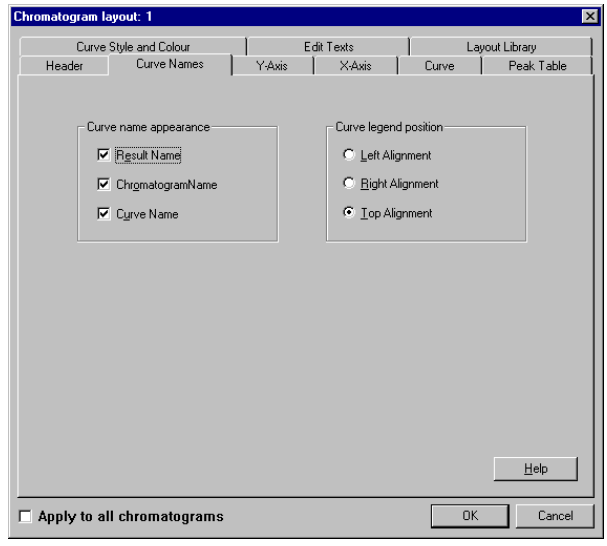


Figure 8-4. Curve Names dialogue.

2. Check the appropriate option boxes for the **Curve name appearance**.

3. Select the appropriate **Curve legend position** option.

It is usually sufficient to select the **Curve name** option if only one chromatogram is being evaluated. However, confusion may arise when more than one chromatogram is shown, so more complete names may be necessary.

8.2.5 Changing the colour and style of curves

All curves within a chromatogram are represented by a default colour and line style. Curves imported into the chromatogram or newly created curves are automatically assigned a colour and line style.

To reassign the colour and/or style of a specific curve, do the following:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. To change the colour and/or line style of a curve, select the curve of interest from the list.

3. Select the desired colour and/or style.

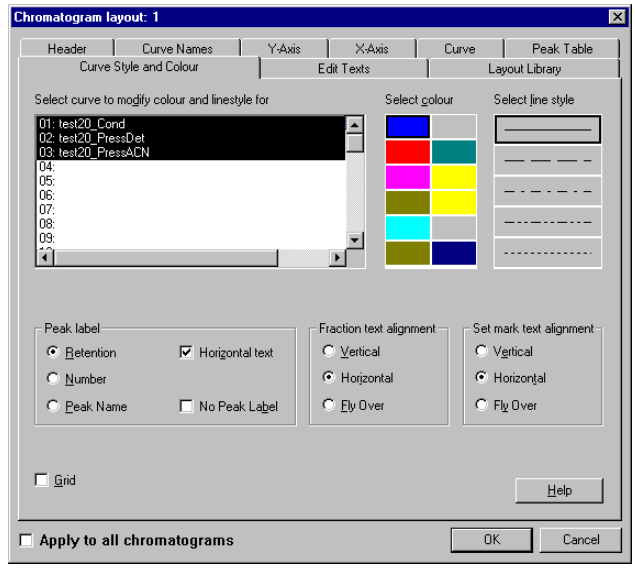


Figure 8-5. Curve Style and Colour dialogue.

8.2.6 Defining and positioning curve text

The **Curve Style and Colour** dialogue also allows for variable forms of peak labelling, as well as alignment of text within **Fraction and Set mark** curves.

Peaks may be labelled according to **Retention times** (the default label), by sequential **Number**, or by user-defined **Peak name** (see Section 10.1.6). The default **Peak label** text alignment is **Horizontal**, but can be changed to vertical by un-checking the **Horizontal** box. In addition, checking the **No Peak Label** box removes peak labels.

Both **Fraction** and **Set mark** text can be set to **Vertical**, **Horizontal** or **Fly Over** alignment by checking the appropriate box. **Fly Over** alignment sets text labels as hidden text, which appear only when the cursor is carefully positioned over a curve line.

8.2.7 Changing and fixing the axes

By default, the y-axes are automatically scaled for each curve to show the whole curve. The x-axis scale is automatically displayed to show the whole run (ml or minutes for OligoPilot and litres or minutes for OligoProcess).

It is possible to 'fix' the minimum and maximum values for the axes of any curve and thereby select a specific part of the curve to be displayed.

Y-Axis

1. Click on the **Y-Axis** tab in the **Chromatogram Layout** dialogue.

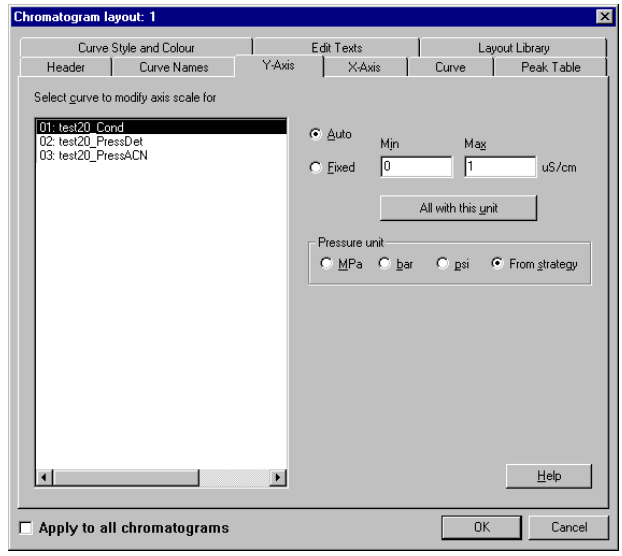


Figure 8-6. Chromatogram Layout dialogue, Y-axis tab.

2. Select the appropriate curve from the list for which you want to fix the scale. Click on the **Fixed** option.

3. Type in the desired minimum and maximum values for the y-axis. If you click on **All with this unit**, other curves that have the same y-axis units as the current scaled curve will be similarly scaled. Click on **OK**.

Note: On some systems, Y-Axis units for pressure curves may be changed by clicking on the appropriate **Pressure unit** (MPa, psi, bar). The default **Pressure unit** is **From strategy**, which is the unit defined in the original run strategy.

Note: **All with this unit** will only be applied to existing curves. It will not be applied to new curves created after this function was last used. New curves are automatically scaled.

X-Axis

1. Click on the **X-Axis** tab in the **Chromatogram Layout** dialogue.

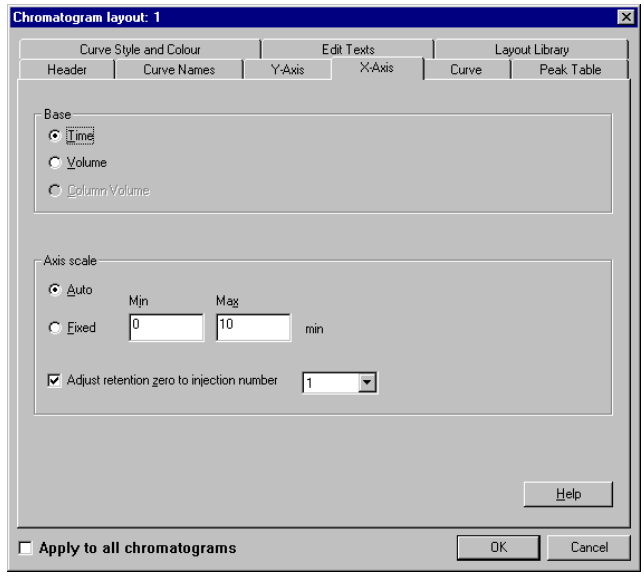


Figure 8-7. Chromatogram Layout dialogue, X-axis tab.

2. Select the appropriate choice from the **Base** field, either **Time** of retention, **Volume** or **Column volume**.

Note: Some calculated curves, e.g. baselines, exist in only one base and may seem to "disappear" when the base is changed. In addition, switching between Time and Volume base may alter the resolution, since the sampling frequency is not adjusted according to the flow rate.

3. Click on the **Axis Scale**, **Fixed** option. Type in the desired minimum and maximum values for the x-axis.

8.2.8 Viewing information about the run

You may wish to display header information at the top of a chromatogram detailing the variables, questions and/or notes. Header information cannot be displayed for imported chromatograms.

1. In the **Chromatogram Layout** dialogue, click on the **Header** tab.

2. Check the options to be included in the header of the chromatogram window.

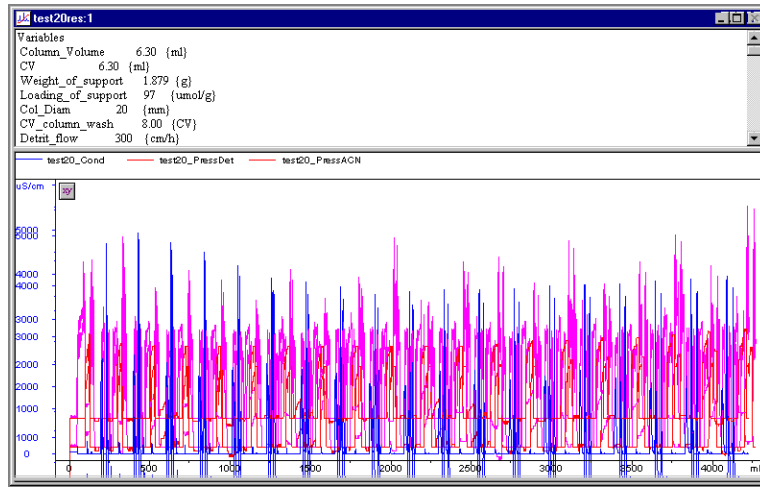


Figure 8-8. Chromatogram with header information displayed.

8.2.9 Saving and applying a layout

All configurations that you make in the **Chromatogram Layout** dialogue can be saved as a layout. It is possible to apply saved layouts to other chromatograms. All saved layouts are user specific.

To save a layout

1. Open the **Chromatogram Layout** dialogue and make the appropriate layout configuration within the various tabs. Note that you can return to the chromatogram window by clicking on **OK** to see the applied affects of a given configuration and return again to the **Chromatogram Layout** dialogue to perform further changes.

2. Select the ***Layout Library*** tab and click on **Save current layout as**.

3. Enter a name for the layout in the displayed dialogue. If you want the current layout to be the new default layout, check the **Save as default** option.

4. Click on **OK** to save the layout. The new name is added to the **Saved layouts** list.

To apply a layout

1. Select the **Layout Library** tab.

2. Select a layout from the **Saved layout** list and click on the **Apply selected layout** button. The layout is automatically applied to the active chromatogram window. If the same layout is to be applied to all chromatograms on the Evaluation workspace, select (check) the **Apply to all chromatograms** option.

8.2.10 Viewing a grid in the chromatogram window

You can display a grid in the chromatogram window:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. Check the **Grid** option.

To remove the grid uncheck the **Grid** option.

8.3 Other presentation possibilities

The Evaluation module allows you to perform operations on the curves to optimise the presentation.

8.3.1 Showing part of a curve

This section deals with the selection of just part of a curve for purposes of closer examination of details and for presentation. This can be done in three different ways:

- magnification using the zoom function
- fixing the axes
- cutting the curves

The zoom function

In the active chromatogram window, it is possible to zoom in on a designated area of the chromatogram. This is the easiest and quickest way to enlarge different parts of a curve.

1. Place the mouse pointer in any corner of the intended area to be magnified.

2. Press and hold the left mouse button. A magnifying-glass icon will replace the mouse pointer arrow on the screen.

3. Drag out a box from the point of origin to cover the area to be magnified. Release the mouse button.

   The selected region is now displayed in the entire chromatogram window, together with appropriate scales for the y- and x-axes.

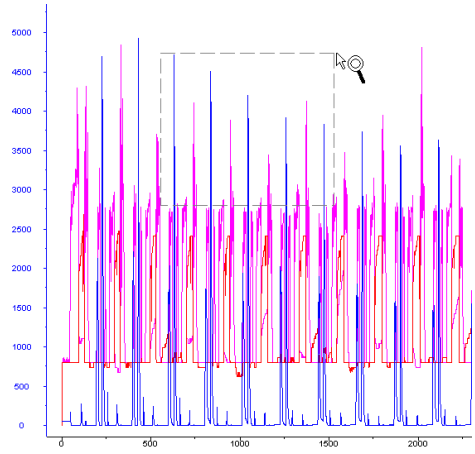


Figure 8-9. Illustration of the chromatogram Zoom function.

4. To move around in the chromatogram at the current zoom scale, use the cursor-arrow keys on the keyboard.

5. To undo the last zoom step, select **Undo zoom** from the right mouse button menu. To reset all zoom steps at once, i.e. no zoom applied, select **Reset zoom** from the right mouse button menu.

Alternatively:

Use the <Page Down> and <Page Up> keys to zoom in and zoom out respectively on the whole chromatogram.

Fixed scale axes

Another way to display only part of a curve is to choose, or 'fix', the minimum and maximum values of the y- and/or x- axes in the **Chromatogram Layout** dialogue (see Section 9.2.6).

Cutting curves

The cut curve function allows a region of the curve between two values on the x-axis to be cut and stored as a new curve. This is done in the following way:

1. Select **Operations:Cut curve**.

2. In the displayed dialogue select the curve(s) to be operated on. Click on **OK**.

3. The selected curve will now be shown in a new window which also contains two vertical cursor lines. To facilitate the cutting process, it is possible to use the zoom function within the window.

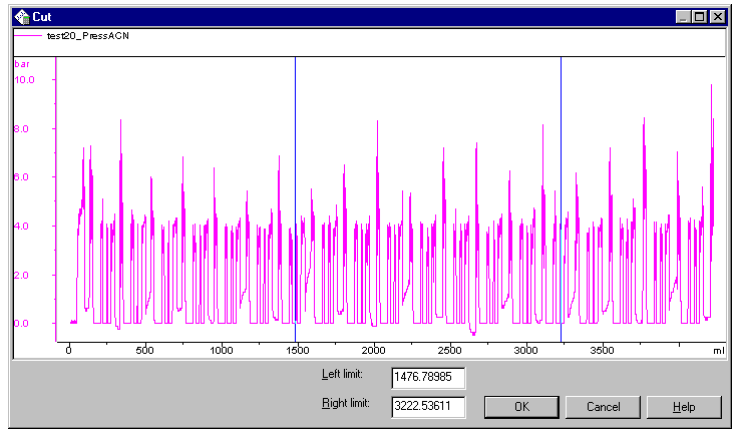


Figure 8-10. Cut window.

To select the region to be cut, either:

- drag the two cursor lines to define the left and right limits of the cut area, or,
- type the desired left and right limit values in the boxes marked **Left limit** and **Right limit**.

Note: The areas outside of the **Left limit** and **Right limit** will not be saved in the newly created cut curve. Thus, the x-axis of the new saved curve will not begin at zero unless designated as one of the limits. The original curve is not changed.

4. Click on **OK**. A new dialogue is displayed. Select whether to save the new cut curve in the **Source chromatogram**, i.e. the current active chromatogram, or in a **New chromatogram**. If you select the latter option, you can change the name of the chromatogram. Click on **OK**.

5. If the destination of the cut curve was the source chromatogram, the cut curve is automatically displayed in the source chromatogram. If the destination of the cut curve was a new chromatogram, this will be represented as a new, open chromatogram window.

8.3.2 Reducing noise and removing ghost peaks

Sometimes the chromatograms may contain curves with a noisy baseline. The noise can be caused by several things e.g. a dirty flow cell, air bubbles, electrical noise, dirty buffers etc. The amount of noise can usually be reduced by taking proper precautions, e.g. filtration of buffers and instrument maintenance.

Smoothing a curve

The smoothing function allows background noise to be reduced or removed from any selected curve. The type of smoothing function you should choose depends upon the type of noise encountered.

1. Select **Operations:Smooth**.

2. Select the source curve to be smoothed and its target destination. By default, smoothed curves are given the suffix, **SMTH**.

3. Select the **Filter type** to be applied in the smoothing operation. This selection can be based on the following criteria:

   - Choose **Moving average** if you have noise along most of the curve.
     Smoothing with this filter affects peak height but not retention. There is little effect on peak area.
   - Choose **Autoregressive** if you have periodic noise along the whole curve.
     Smoothing with this filter will affect peak height and retention, although has little affect on peak area.

- Choose **Median** if there is only one or a few noise spikes, e.g. caused by air bubbles, or if the noise is confined to only a small part of the curve.
  Smoothing with this filter may give flattened peaks and affect peak areas slightly but will not affect the retention.

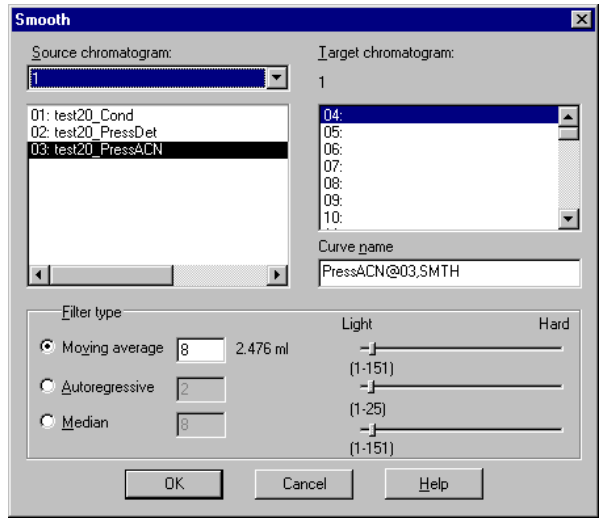


Figure 8-11. Smooth dialogue.

4. Select an appropriate smoothing parameter value from **Light** to **Hard** for the selected filter. The smoothing effect increases with increasing parameter values.

   Smoothing is always a compromise between noise removal and preservation of peak shape. The easiest way to find the optimum smoothing effect is to start with a low parameter value, e.g. the default value, and increase it until the best result is achieved. A useful strategy is to increment the parameter value by the default value for each try.

5. Click on **OK**.

The formulae for the filters are described in Appendix D.1.

8.3.3 Subtracting a blank run curve

This is a frequently used function in presentations, especially if the curves have a drifting baseline or "ghost" peaks.

Note: If the ghost peaks come from impurities in the eluents, all equilibration of the columns should be the same from run to run. If, for example, the equilibration volume with buffer A is larger before a blank run curve than before a synthesis, your ghost peaks might be higher in the blank run curve.

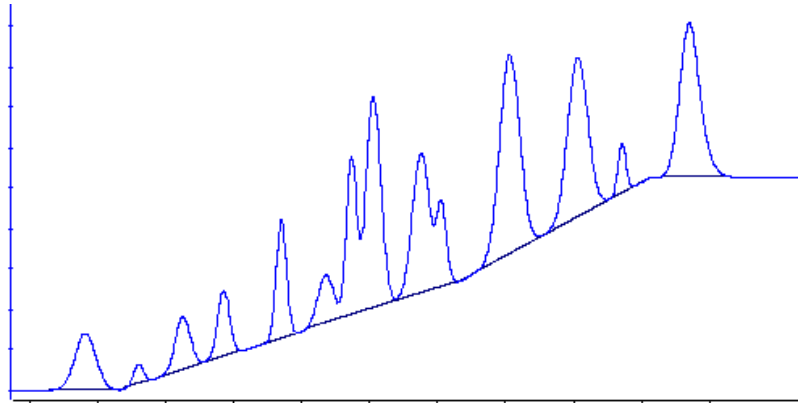


Figure 8-12. UV curve with baseline prior to subtraction of the baseline.

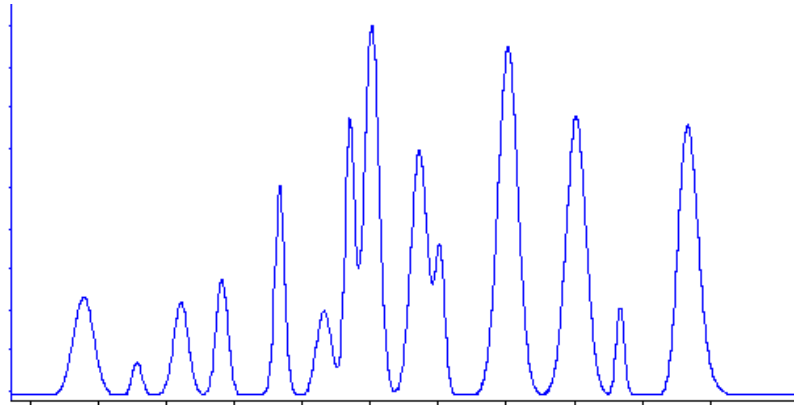


Figure 8-13. UV curve after subtraction of the baseline.

Alternative A: Importing a blank run curve

If a blank run curve was done, this may have been stored in another result file. To access the blank run curve:

1. Ensure that the destination chromatogram has been opened and is the active window on the workspace.

2. Select **File:Open** and then from the select **Curves** from the menu cascade. The **Open Curves** dialogue is displayed.

3. Locate and double click on the result file containing the blank run curve. The curves in the first chromatogram are displayed.

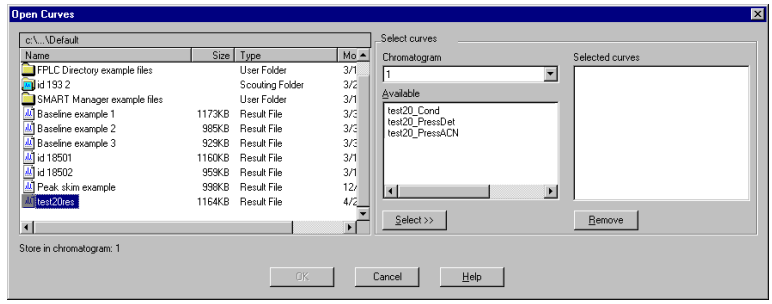


Figure 8-14. Open Curves dialogue with a result file selected.

4. Select the curve corresponding to the blank run curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list.

   To remove a curve from the list, select it and click on **Remove**.

   If there is more than one chromatogram in the result file and the blank run curve resides in another chromatogram, select the appropriate chromatogram from the drop-down list. The curves for that chromatogram are displayed from which you should make the appropriate selection.

5. To import the curve click on **OK**.

Note: For more detailed information about how to import curves, chromatograms and other results, see Section 9.4.

Alternative B: Calculating a baseline

If there is no blank run curve, you can instead create a baseline with **Integrate:Calculate baseline** (see Section 10.1.1).

Subtracting the blank run curve

Select **Operations:Subtract** to subtract the blank run curve or the baseline away from the sample curve. Click on **OK**. All resulting curves from the subtract operation receive the SUB suffix.

8.3.4 Adding curves

In some runs, several sequential chromatograms may have been created, for example, when the instruction **New chromatogram** has been used in the method, thus creating different chromatograms during the run. In order to view and evaluate the resultant curve of all the chromatogram parts, the curves must be added together. The common situation is when you have a number of chromatograms within the same result file and you want to add the curves. In some circumstances, curves may need to be imported from other result files.

To add curves:

1. Select and view the first chromatogram in the sequence.

2. Select **Operations:Add**. Add the first curve in the sequence to the second curve in the sequence from the appropriate chromatograms.

3. Add the result of the previous step to the next curve in the sequence.

4. Repeat this process until all curves have been added together. The final curve should be the cumulative curve for the whole run. All curves created using the **Add** operation receive the ADD suffix.

8.3.5 Entering text in the chromatogram

Basic annotations can be added to the chromatogram.

1. Place the mouse pointer in the curves view of the chromatogram window and select **Add text** from the right mouse button menu. Alternatively select the **Edit:Text:Add** command. The mouse pointer is replaced with an **ABC** pointer.

2. Position the pointer where you want to insert text in the chromatogram and click the left mouse button once.

3. In the dialogue that appears, type the desired text and then click on **OK**. Now the text can be viewed on the chromatogram. The text is saved at the position where it is placed in the chromatogram window and is not linked to any curve. The text cannot be moved within the window once it has been placed.

If you want to edit or delete an inserted text:

1. Open the **Chromatogram Layout** dialogue and select the **Edit Texts** tab. Alternatively, select the **Edit:Text:Edit** command and the **Edit Texts** tab is displayed automatically.

2. Select the specific text that you want to edit and make the appropriate changes in the **Selected text** field. Click on **Change text**.

3. Select the specific text that you want to delete and click on **Delete text**.

4. Click on **OK** to close the dialogue and apply the changes.

8.3.6 Renaming chromatograms, curves and peak tables

Sometimes, it may be desired to change the name of a chromatogram, curve or peak table. To do this, close the **Chromatogram Layout** dialogue and then:

1. Select **Edit:Rename** and the relevant menu cascade option **Chromatogram**, **Curve** or **Peak Table**.

2. Select the appropriate object in the displayed dialogue and type in the new name. Click on **OK**. The new name will replace the old one rather than creating a new curve or chromatogram.

Note: The original raw data curves cannot be renamed and are not therefore given as options.

8.4 Comparing different runs

The previous sections dealt with the manipulation of single curves within a chromatogram. The following sections describe how to make comparisons between two or more curves or chromatograms from different runs and detail how best to present them.

It is possible to:

- view several chromatograms at the same time
- overlay curves from different runs in one chromatogram
- stack curves from different runs in one chromatogram
- stretch curves to make comparisons easier
- create mirror images

8.4.1 Comparing chromatograms from different runs

To import chromatograms from other result files into an already opened result file, two functions can be used, namely **File:Open to compare** or **File:Open**. The former option is most useful in searching

for many chromatograms in a specific folder based on defined selection criteria. The latter option is best used to import any individual chromatograms from result files in different folders. The imported chromatograms are sequentially numbered (11, 12, 13, etc.) for identification purposes. Up to 10 chromatograms can be made available at the same time on the evaluation workspace.

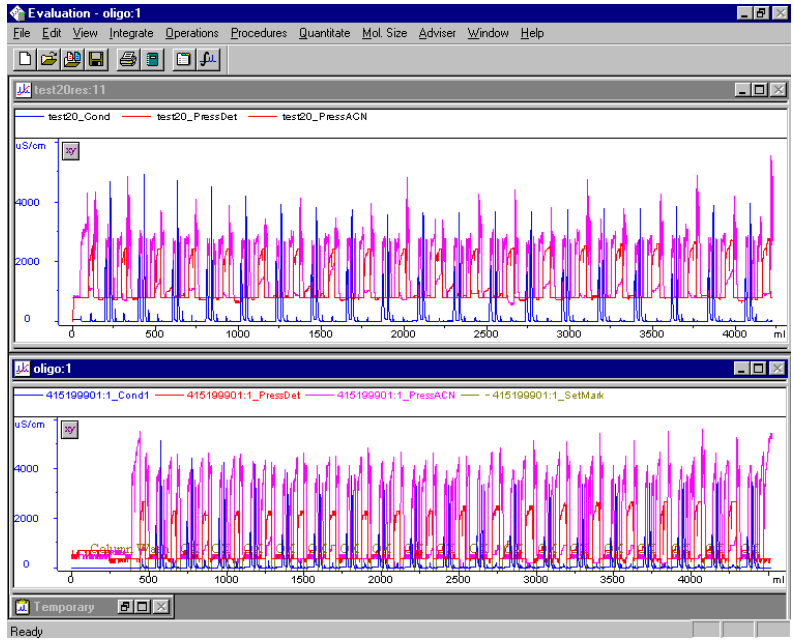


Figure 8-15. Windows:Tile function to display many chromatograms.

Alternative A: Import chromatograms using Open to compare

This method is useful, for example, when importing chromatograms from all files of a previous results folder.

1. Click on **File:Open to compare:Chromatograms**. The **Open Chromatograms to Compare** dialogue is displayed.

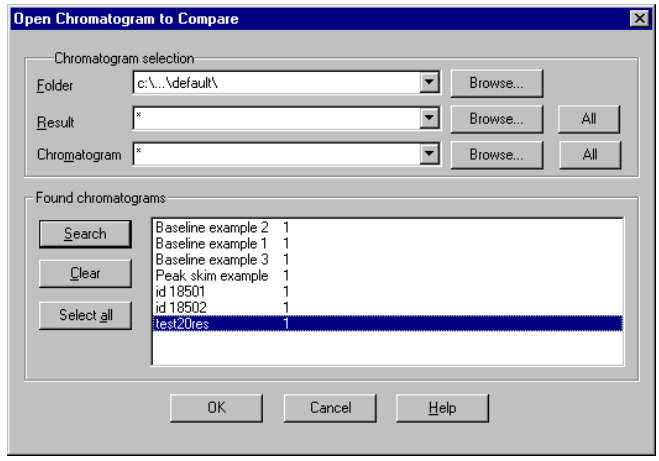


Figure 8-16. Open chromatograms to compare dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. The search for chromatograms will take place in all result files within the selected folder as denoted by the asterisk '*****'. You can instead select a specific result file using the **Browse** function. Moreover, you can use wildcard characters to search within result files with a specific name profile.

   You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

   User-entered search filters (to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used as a search delimiter (by inserting a ';' between strings) and search filters are automatically saved and stored within user profiles.

   To return to the default setting to search in all result files, click on **All**.

4. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the first found chromatograms from the list.

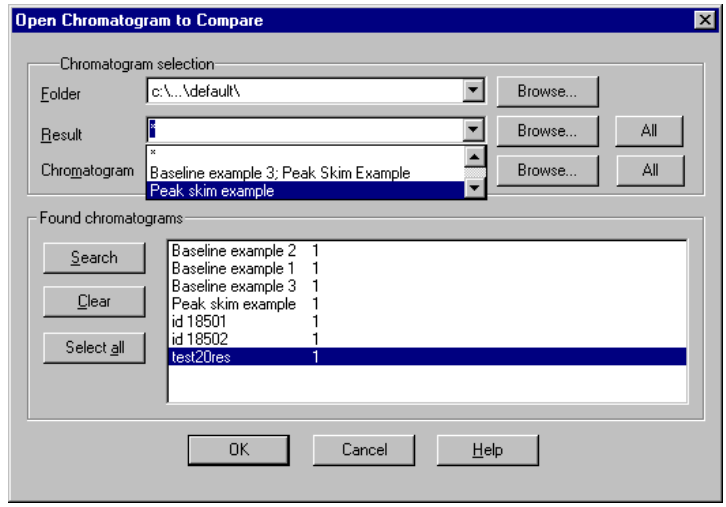


Figure 8-17. Open Chromatogram to Compare dialogue, showing search delimiters.

5. Select the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected for importing. If you want to clear the list of displayed chromatograms, click on **Clear**.

6. Click on **OK** and all selected chromatograms are shown on the Evaluation workspace.

Alternative B: Importing using Open

1. Select **File:Open:Chromatogram**.

2. Select the desired result file by double clicking on it, and all of the chromatograms contained within will be displayed. Normally it is only one chromatogram and is named "1".

3. Select the chromatogram(s) of interest and press the **Select** button. Selected chromatograms are added to the **Selected chromatograms** list. Chromatograms can be deselected by using the **Remove** command button.

4. Repeat steps 2-3 for chromatograms in other result files.

5. Click on **OK**.

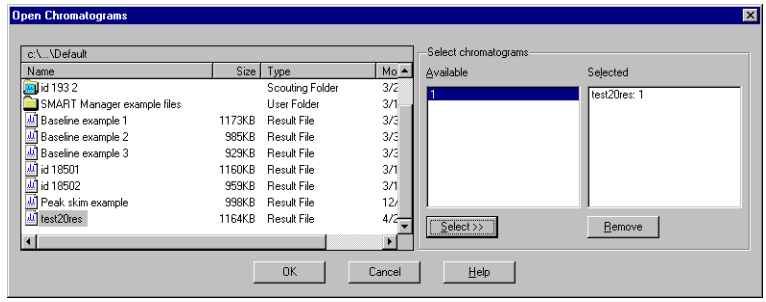


Figure 8-18. Open chromatogram dialogue.

Viewing all chromatograms

1. Simultaneously display the chromatograms by selecting **Window:Tile** or layer them by selecting **Window:Cascade**.

2. Chromatogram windows can be individually sized and the presentation of the curves changed.

3. If you want to have the same scale on all of the chromatograms, open the **Chromatogram Layout** dialogue for any chromatogram, make the changes and select (check) the **Apply to all chromatograms** option.

   Imported chromatograms cannot be shown with column volume as the x-axis base.

8.4.2 Comparing curves

Curves from different runs can be imported or copied into one chromatogram for comparison.

Alternative A: Importing curves using Open to compare

Result files contained in the same folder can be automatically searched to locate all curves of a specified type, for example, all UV curves. Moreover, the imported curves can be automatically overlaid, stacked or be presented as mirror images.

1. Select **File:Open to compare:Curves** or click on the **Open curves to compare** toolbar button. Select the search criteria for the folder, result, chromatogram and/or curve name using the respective **Browse** command buttons.

   Wildcard characters, * and ?, can also be used to further specify the search parameters (see Section 9.4.1 Alternative A).

User-entered search filters (to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used (by inserting a ';' between strings) as a search delimiter and search filters are automatically saved and stored within user profiles.

The UV curves are identified with number and sometimes wavelength. For example, UV1_280, UV2_280 and UV1_254 are all different curves. To search for all UV curves, enter UV* in the **Curve name** text field.

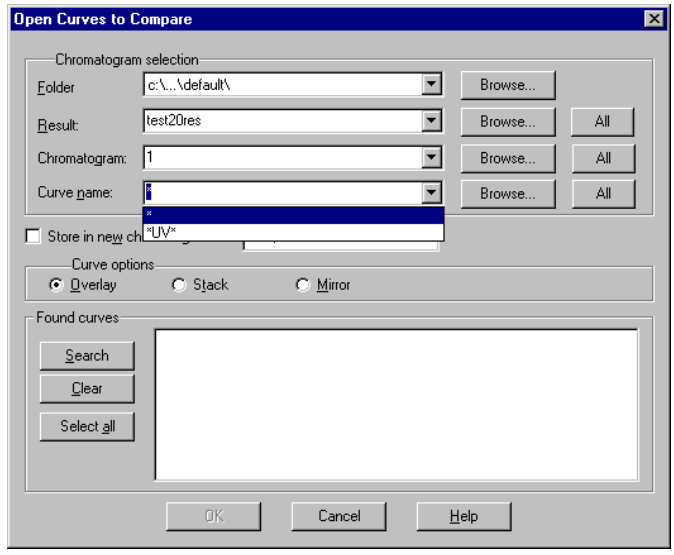


Figure 8-19. Open Curves to Compare dialogue.

2. Click on **Search** and a list of found curves will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing curves located in the previous search.

3. Select the curves that you want to be imported. Click on **Select All** if you want to import all of the curves.

4. You can import the curves into a new chromatogram by selecting (checking) the **Store in new chromatogram** option. This is recommended to keep the source chromatogram free of too many additional curves.

5. Select how the imported curves will be displayed by clicking on one of the buttons; **Overlay**, **Stack** or **Mirror**.

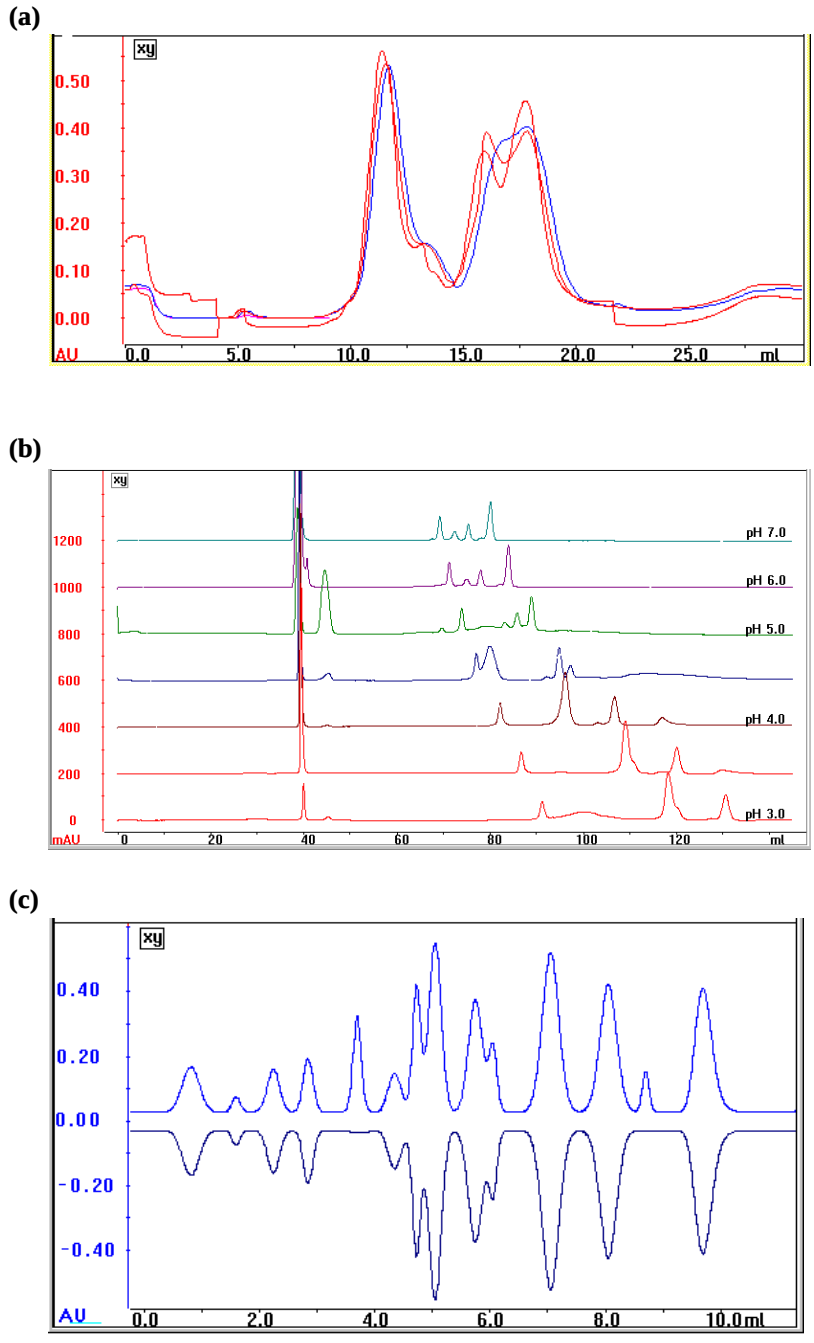


Figure 8-20. Different presentation options for comparison of imported curves; (a) overlaid curves, (b) stacked curves, (c) mirrored curves.

Overlay This presents the imported curves overlaid one on another.

Stack This presents the imported curves with a given offset y-axis value so that the curves are stacked and distinct from one another.

Mirror This should be ideally used to view two imported curves. One curve is inverted in the y-axis and thus appears to mirror the other curve.

6. When you have made you selection, click on **OK**.

   If you selected the **Stack** option (see step 5.), the **Stack Offset** dialogue is displayed. You can change the displayed value to increase or decrease the offset distance between the curves. If the selected curves have different y-axis units, the dialogue is displayed for each curve. Click on **OK**.

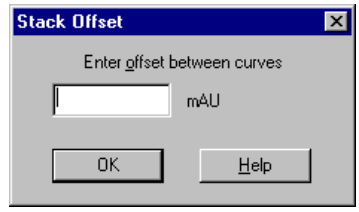


Figure 8-21. Stack Offset dialogue.

7. Imported curves are displayed in either the source chromatogram or in a new chromatogram that you created.

8. Select the curves that you want to view in the **Chromatogram Layout** dialogue. Curves can also be scaled individually or all with the same scale using the **All with this unit** function in the **Chromatogram Layout** dialogue (see Section 9.2.6).

   If you stacked the curves and you want to change the stack offset, the easiest way is to import the curves again with another offset value. The individual curves can also be moved (see Section 9.4.3).

9. If desired, select the **Store in new chromatogram** box, and give the chromatogram a new name.

Alternative B: Importing curves using Open

Using the **File:Open:Curves** function, individual curves may be imported into the active chromatogram.

1. Ensure that the destination chromatogram for the imported curve(s) is active on the screen.

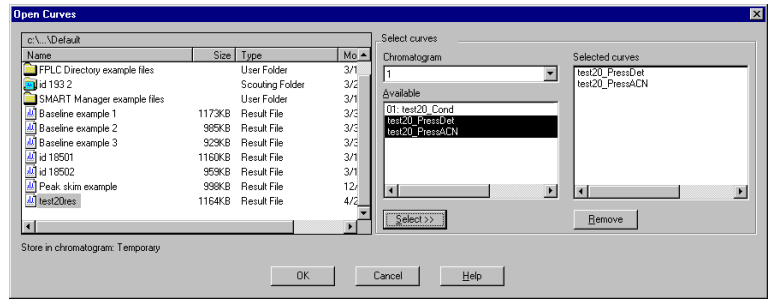


Figure 8-22. Open curves dialogue.

2. Select **File:Open**. The **Open Curves** dialogue is displayed.

3. Select the folder in which to search for curves.

4. Click on the result file of choice and, where appropriate, the specific chromatogram containing the desired curve. The chosen chromatogram and curves contained therein will be listed.

5. Select the desired curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list.

6. If you want to choose more curves from other chromatograms repeat steps 3-4. When all the desired curves have been selected, click on **OK**.

7. Restore the chromatogram window and open the **Chromatogram Layout** dialogue. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 9.2.6).

Alternative C: Copying curves into one chromatogram

Curves can be copied between chromatograms present in the Evaluation desktop. For effective comparison of curves, it is suitable to transfer all relevant curves to a single chromatogram. This is best achieved by:

- creating a new chromatogram using **File:New: Chromatogram**, and copying curves into it from other chromatograms, or,
- copying an existing chromatogram using **Edit:Copy:Chromatogram** and importing more curves into it, or,

- copying curves into the **Temporary** chromatogram (see Section 9.1.2). You can perform evaluations in the **Temporary** chromatogram and transfer the final curves to other destination chromatograms. The unwanted contents remaining in the **Temporary** chromatogram can then be removed using **Edit:Clear temporary** chromatogram.

To copy curves:

1. Select **Edit:Copy:Curve**.

2. Select the source chromatogram and the curve of interest. Select the target chromatogram. Click on the **Copy** button to effect the copy. Stay within the same dialogue to repeat this step for as many other curves you want, from the same or different chromatograms. When you have copied all desired curves, click on **Exit**.

3. Open the destination chromatogram and access the **Chromatogram Layout** dialogue. Select in the **Chromatogram Layout** dialogue the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 9.2.6).

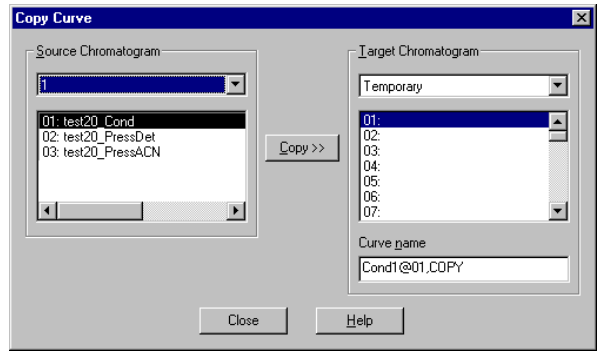


Figure 8-23. Copy curve dialogue.

8.4.3 Stacking and stretching curves

Several tools are available to stack and stretch curves from different runs to better visualise the differences. These tools are normalising curves, shifting curves and stretching curves. These allow you to manually reproduce the **Stack** and **Mirror** functions associated with the **Open to compare:Curves** operation (see Section 9.4.2 Alternative A), and more besides. If the curves have been stacked with the **Open to compare curves** operation and you want to change the stack offset, the

easiest way is to repeat the operation with another offset. The curves can also be stacked and stretched individually as described below. The operations presented below all require the curves to be present in one chromatogram (see Section 9.4.2).

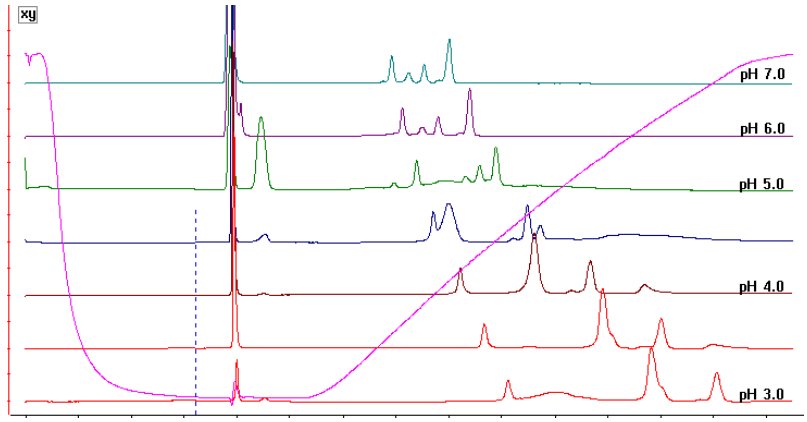


Figure 8-24. Stacking curves.

Alternative A: Stacking and stretching curves using the normalise function

The simplest method to align curves with respect to the x-axis or the y-axis for easier visualisation, is to use the normalise function.

To select the curve to be moved within a chromatogram:

1. Select **Operations:Normalise**. The **Normalise** dialogue is displayed.

2. Select the curve to be normalised and a reference curve to be normalised against. For example, if you want to stack curves, select the curve at the bottom of the stack to be normalised against and the curve to be moved as normalised. Click on **OK**.

3. The **Normalise** window is displayed. A box surrounds the curve selected to be normalised.

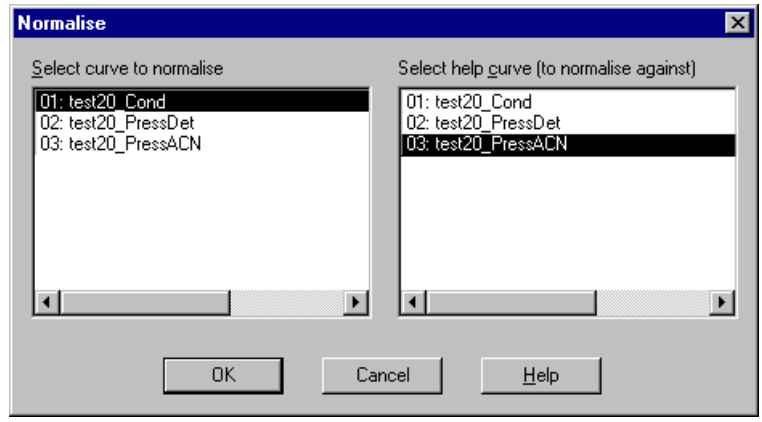


Figure 8-25. Normalise window.

You can now use the following functions:

Size Allows the selected curve to be stretched along its y-axis or x-axis. Click on **Size** and then drag the coloured box either along its y-axis or x-axis. This is useful for comparison of curves with, for example, different gradient lengths.

Move Allows the selected curve to be moved to any position on the chromatogram. Axes are automatically re-scaled to accommodate the new positioning. This function is useful for stacking curves. Click on **Move** and then move the curve with the mouse pointer. Click on the mouse button when the curve is in the correct position.

Normalise The curve to be normalised will be adjusted to the help curve. Thus, the height of the highest peak on both curves will be the same and will occur at the same retention point. The curve to be normalised is automatically moved along the x-axis and stretched along the y-axis.

4. When all operations have been performed, click on **OK** to save the new normalised curve. Open the **Chromatogram Layout** dialogue to select the normalised curve for viewing.

5. Repeat the procedure for all curves you want to stack or stretch.

Alternative B: Moving a curve using the Shift function

If more precise positioning of curves is required, then the shift function should be used. This function is similar to **Normalise:Move** except that each curve is repositioned by a precise value instead of by eye and the instruction logged in the evaluation log.

1. Select **Operations:Shift**. The **Shift** dialogue is displayed. Select the curve to be shifted.

2. Select the axis along which the shift is to be made, i.e. along the x-axis (**Shift retention**) or the y-axis (**Shift amplitude**). Enter the shift value and click on **OK**.

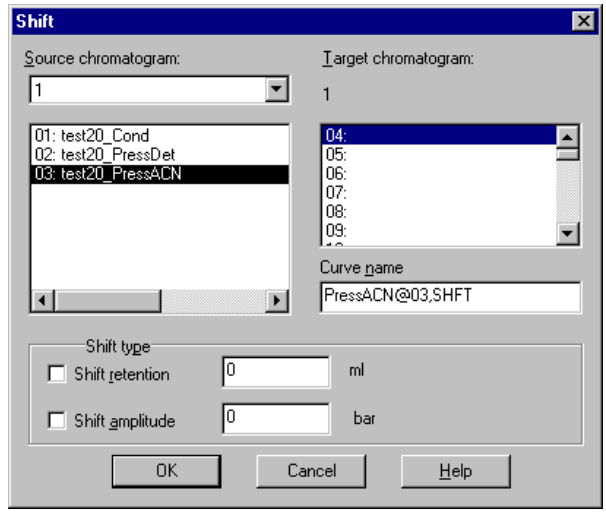


Figure 8-26. Shift dialogue.

Alternative C: Stretching and shrinking a curve using multiply

Curves can be stretched or shrunk in the x or y plane using the multiply function. This function is similar to **Normalise:Size** except that each curve is repositioned with precise numbers instead of by eye and the instruction logged in the evaluation log.

1. Click on **Operations:Multiply** and select the curve to be multiplied.

2. Select (check) the appropriate axis for multiplication, either **Multiply retention** and/or **Multiply amplitude**.

3. Insert the appropriate multiplication factor and click on **OK**.

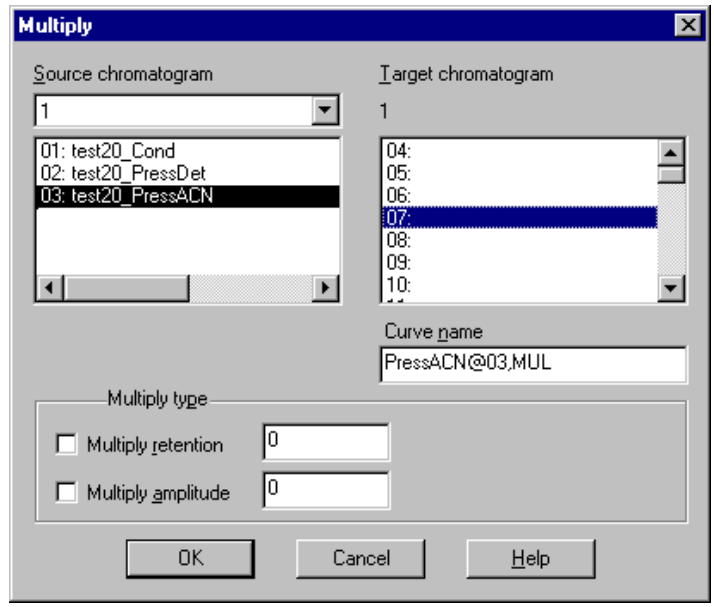


Figure 8-27. Multiply dialogue.

8.4.4 Mirror images of curves

A very useful way of comparing the features of two curves is to produce a mirror image of one curve. To achieve this:

1. Select **Operations:Multiply**.

2. Select the desired curve to be mirrored and select **Multiply amplitude** in the **Multiply type** field.

3. Type in a multiplication integer of -1 and click on OK.

4. Shift the mirror image curve downwards for an improved presentation (see Alternative B above).

Now the mirror image of the original curve will be displayed in the active window. Select/deselect for the other curves wanted in the active chromatogram window in the **Chromatogram Layout** dialogue.

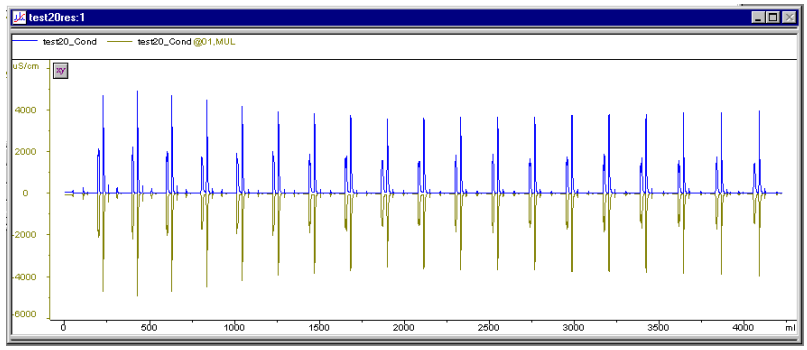


Figure 8-28. Two curves displayed in mirror image.

8.5 Saving results

Any changes to chromatograms, including all new created curves and all imported or created chromatograms, can be saved in either of two ways, using:

- **File:Save** or the **Save** toolbar button, which saves all changes in the original result file,
- **File:Save as**, which allows you to create a new result file in the specified target folder.

Note: All curves created during the manipulations will also be saved. This may not always be desirable. Before saving, remove unwanted curves from a chromatogram using **Edit:Delete:Curve**. The original curves can never be deleted.

8.6 Printing active chromatograms

To print out the open chromatogram(s) select **File:Print** or click on the **Print** toolbar button. If you want to print out several chromatograms ensure that these are open on the workspace before selecting **File:Print**.

A dialogue appears allowing you to select the print format of the chromatograms. Alternatively, you can click on the **Other** option and a dialogue appears that allows you to select the number of chromatograms in each column and row to be used in the printed document.

Click on **OK** to print out the chromatograms.

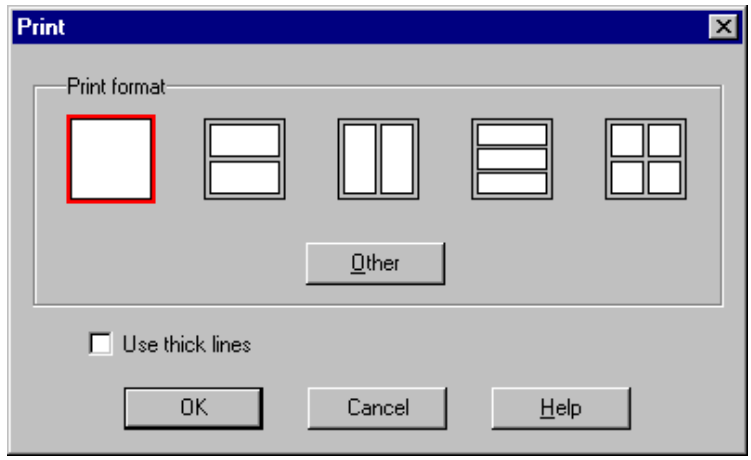


Figure 8-29. Print dialogue.

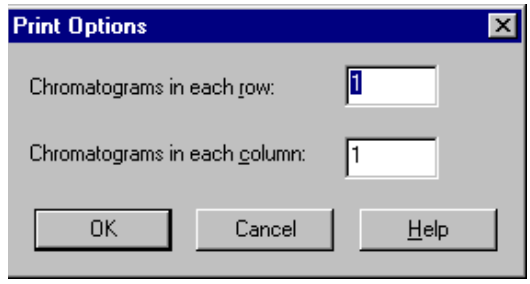


Figure 8-30. Print Options dialogue.

If the Documentation is open, click on the **Print** button to print any components in the documentation.

Chromatograms can also be printed from the **File:Report** dialogue (see 9.7 below).

8.7 Printing reports

The Evaluation module provides you with extensive tools with which to create detailed reports. You can create and save report formats based on either **Standard** layouts or on **Customised** layouts.

To open the report generator interface select **File:Report** or click on the **Report** toolbar button and the **Generate Report** dialogue is displayed.

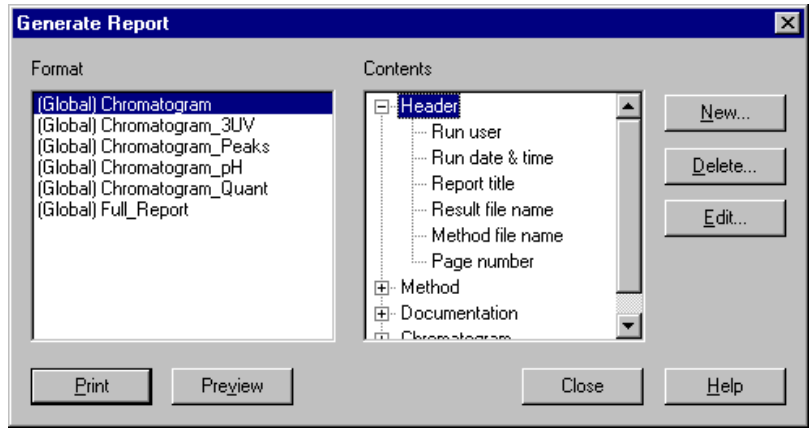


Figure 8-31. Generate Report dialogue.

In the dialogue you can see the available formats in the **Format** field. You can select a format from the list and directly apply it. Alternatively you can create a new report format or edit the existing formats.

Note: A report format saved with the **Current chromatogram** does not necessarily print the actual chromatogram as it appears on the screen in the Evaluation workspace.

Some global report formats are provided with the installation. Do not delete these formats, since you will then be unable to run the corresponding procedures selected in the method templates.

If you want to print a number of results with the same report format, create a procedure to print one result and then perform a batch run for the required results (see Section 10.3.6 for details).

8.7.1 Creating a new customised report format

You can create your own report format and save it for later use. The customised report interface allows you to choose from a variety of objects including chromatograms, methods, documentation, free text and more. Moreover, you can decide the placing, alignment and sizing of the objects according to the various options or else apply free placement and sizing.

To access the customised report interface, click on the **New** button in the **Generate Report** dialogue to display the **Create New Report Format** dialogue. Click on the **Customised format** radio button (the default setting), and then on **OK**. The **Customise Report** window is displayed.

‘

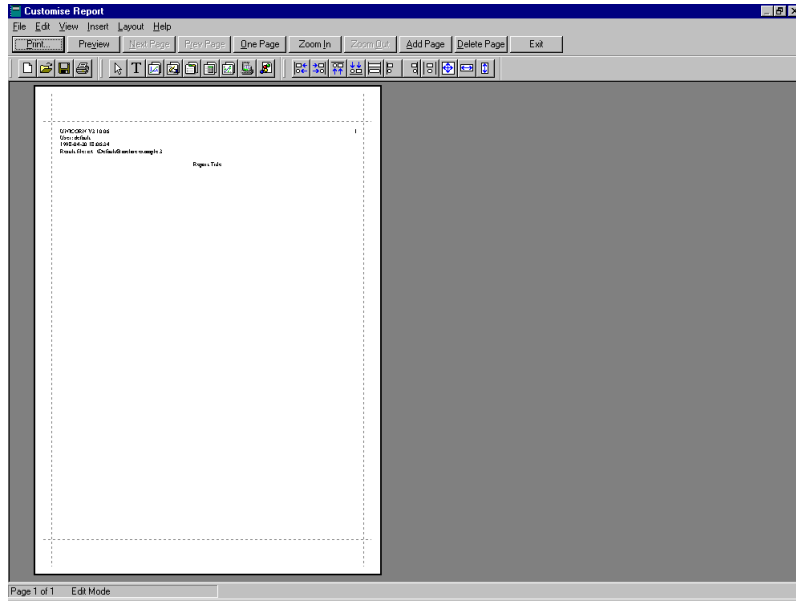


Figure 8-32. Customise Report window.

Adding and deleting pages

To add new pages to the report click on the **Add Page** button. A new page is added after the last page.

To delete a page while in single-page mode, click on the Delete Page button. Confirm the deletion. To delete a page in dual-page mode, click on an object on the page and then click on the **Delete Page** button. Confirm the deletion.

Adding objects

To add an object to the report:

1. Select an object from the **Insert** menu or click on the appropriate toolbar button.

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

3. 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 object inserted. Make the appropriate selections in the dialogue and then click on **OK** to view the inserted object.

The various object types and dialogues are as follows:

Free text

The **Setup Free Text** dialogue is used to define the desired text and the settings for the free text object.

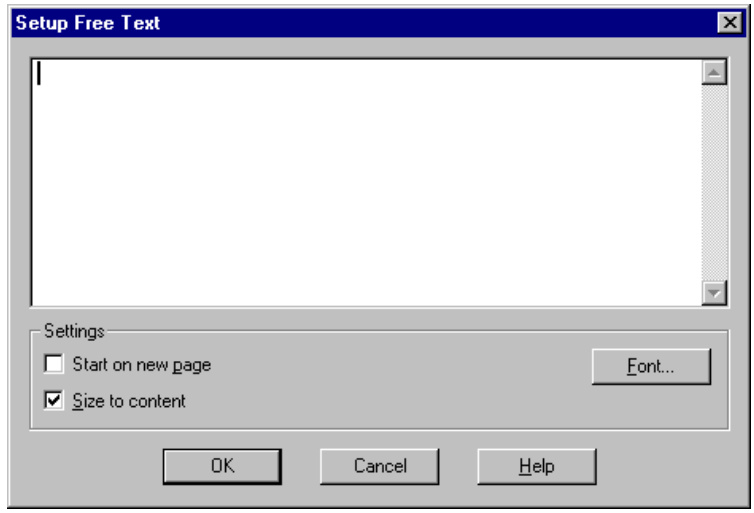


Figure 8-33. Setup Free Text dialogue

In the dialogue you can:

1. Enter text in the open field.
2. If appropriate insert the text box on a new page by checking **Start on new page**.
3. Automatically size the free text box by checking **Size to content**.
4. Select the font type, style, colour and size by clicking on the **Font** button.
5. Click on **OK** once you have made your selection. The free text object is inserted.

Two of the report options warrant more detailed description, namely **Synthesis data** and **Chromatogram**.

Synthesis Data

This option can be used to print out all of the relevant information concerning the actual synthesis, including the sequence, detritylation table and cross-reference list. The detritylation table contains information about the efficiency of the coupling reaction for the addition of each base to the oligonucleotide. Efficiency is automatically calculated by measuring the conductivity in the cell during the cleavage of DMTr at the detritylation step. The level of conductivity is determined by the amount of cleavage and is directly correlated to the area under the relevant peak in a coupling cycle. The area is determined by peak integration, which is automatically performed if one of the supplied method templates has been used to create a method. Click on the appropriate icon and select the options to be included.

The detritylation table alone can be viewed separately under **View: Synthesis Data**. This technique is useful if you would like to export **Synthesis Data** to an external spreadsheet such as Microsoft Excel. Use the cursor to highlight the desired sections of the table, and then Ctrl + C to copy the selection to the clipboard for export.

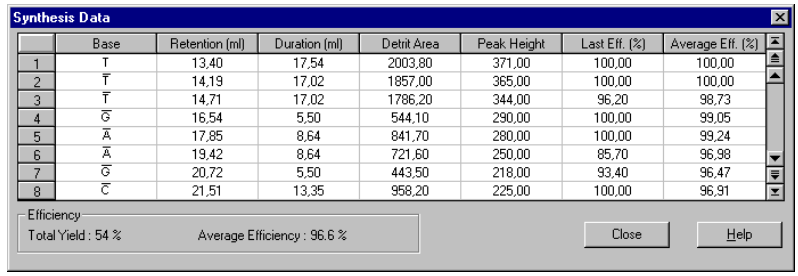


Figure 8-34. The Synthesis Data dialogue.

Chromatogram

The **Setup Chromatogram** dialogue is used to define the settings for the chromatogram object.

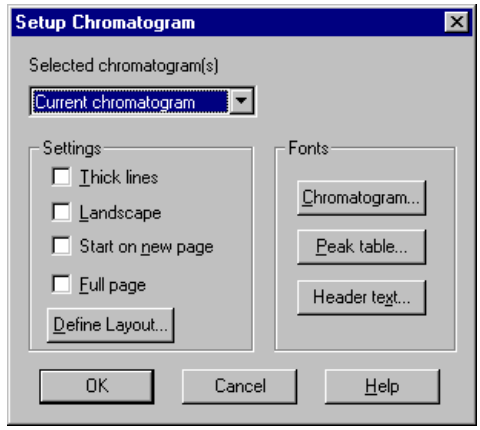


Figure 8-35. Setup Chromatogram dialogue

1. Select which chromatogram(s) to insert from the **Selected chromatogram(s)** drop-down list:

Current chromatogram	This inserts the chromatogram that is currently active in the Evaluation window.
All chromatograms	This inserts all chromatograms contained within the Evaluation window.
1, 2...etc.	This inserts the specific chromatogram corresponding to the selected number/name.

2. Check the appropriate **Settings** for the inserted chromatogram(s). You can select to view and print the chromatograms with **Thick lines**, view the chromatogram(s) in **Landscape** orientation, insert the chromatogram(s) so that they **Start on new page**, and/or show a chromatogram on a **Full page**.

3. To define the layout for the chromatogram(s) click on the **Define layout** button. The **Report Chromatogram Layout** dialogue is displayed. Make the appropriate selections in the various tabs for the report.

Note1: Selections made in this layout only affect the report and not the view of the chromatograms in the Evaluation window.

Note2: Appropriate variables can be selected for the chromatogram header, for example, if you wish to mark the chromatogram with the sample ID when using the autosampler.

Click on **OK** when you have made your selections to return to the **Setup Chromatogram** dialogue.

4. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Chromatogram**, **Peak table** and/or **Header text**.

5. When you have made your selections for the chromatogram(s) click on **OK** in the **Setup Chromatogram** dialogue. The chromatogram(s) are now inserted into the report.

Method

The **Setup Method** dialogue is used to define the settings for the method object.

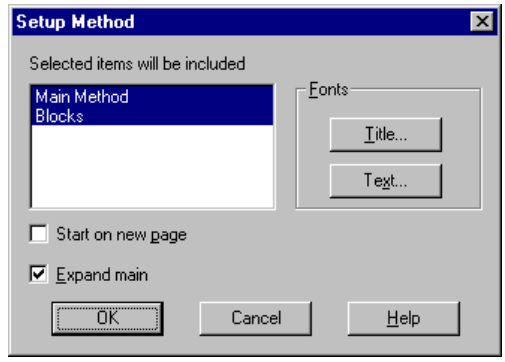


Figure 8-36. Setup Method dialogue.

1. Select the items to be included:

Main Method	This is the method on which the run was based.
Blocks	These are the blocks that were used in the method.

2. Select as appropriate for the inserted method to **Start on new page** and/or to **Expand main** to show the expanded view of the method.

3. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

4. When you have made your selections click on **OK** in the **Setup Method** dialogue. The method is now inserted into the report.

Documentation

The **Setup Documentation** dialogue is used to define the settings for the documentation object.

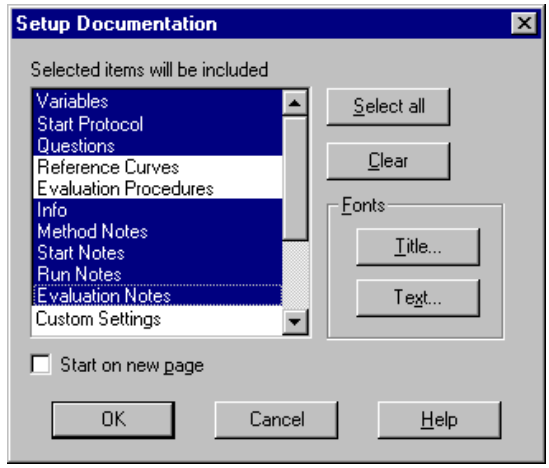


Figure 8-37. Setup Documentation dialogue.

1. Select the items to be included from the Evaluation Documentation menu. To clear the current selection click on **Clear**. To select everything click on **Select all**.

2. If required select **Start on new page**.

3. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

4. When you have made your selections click on **OK** in the **Setup Documentation** dialogue. Details from the selected documentation menu are now inserted into the report in the documentation object.

Evaluation log

The **Setup Evaluation Log** dialogue is used to define the settings for the evaluation log object.

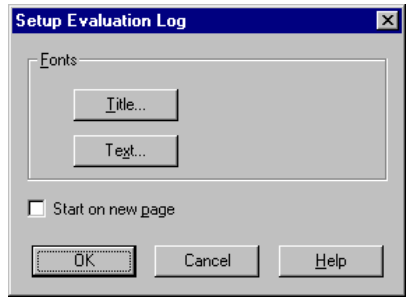


Figure 8-38. Setup Evaluation Log dialogue.

1. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

2. If required select **Start on new page**.

3. When you have made your selections click on **OK**. The evaluation log is now inserted into the report.

Moving and resizing objects

Objects can be freely moved, or moved and sized according to the various commands in the **Layout** menu or toolbar.

Note: Resizing the width of the objects with **Make same size** or **Make same width** can only be performed on chromatograms and free text.

1. To select an object for moving or sizing, click on the **Select** toolbar button and click on the object of interest. To select several objects hold down the <Ctrl> key while clicking on the objects.

2. For free object placement of an object hold down the left mouse button and drag the object to its new position. Similarly, to free size the object, click on one of the object border anchors either at the corners or in the middle of a border and drag the box to re-size it.

3. For defined placement and/or sizing of object(s), select from the following options:

Align left

For multiple selected objects on the same page, this function aligns the objects by their left borders.

Align right

For multiple selected objects on the same page, this function aligns the objects by their right borders.

Align top

For multiple selected objects on different pages, this function aligns the objects by their top borders.

Align bottom

For multiple selected objects on different pages, this function aligns the objects by their bottom borders.

Adjust to margins

For single or multiple selected object(s), this function resizes the width of the object(s) to span from the left to the right margin.

Adjust to left margin

For single or multiple selected object(s), this function moves the object(s) so that the left border(s) are aligned with the left margin.

Adjust to right margin

For single or multiple selected object(s), this function moves the object(s) so that the right border(s) are aligned with the right margin.

Adjust to centre

For single or multiple selected object(s), this function centres the object(s).

Make same size 	For multiple selected objects, this function resizes the objects to the same size as the currently active selection in the group of selected objects.
Make same width 	For multiple selected objects, this function resizes the objects to the same width as the currently active selection in the group of selected objects.
Make same height 	For multiple selected objects, this function resizes the objects to the same height as the currently active selection in the group of selected objects.

Viewing options

You have several viewing options available in the **View** menu or on the toolbar.

Preview/Edit	This toggles between looking at the print preview mode and edit mode
One Page/Two Pages	This toggles between viewing single pages or pairs of pages where there is more than one page
Next Page	This displays the next page or pair of pages where there is more than one page
Previous Page	This displays the previous page or pair of pages where there is more than one page
Zoom In	This increases the magnification of the view of the currently selected object or most recently selected object in a group of selected objects.

Zoom Out This decreases the view magnification of the currently selected object or most recently selected object in a group of selected objects.

Changing the page layout

To change the page layout double click on the header region of the page to display the **Page Setup** dialogue.

There are three tabs for changing different aspects of the page layout:

Page Layout, which allows you to set the page **Margins** and the **Units**, **cm** or **inch**. You can check the option to have the **Same header on all pages** and also you can check the option to **Draw a frame around the pages**.

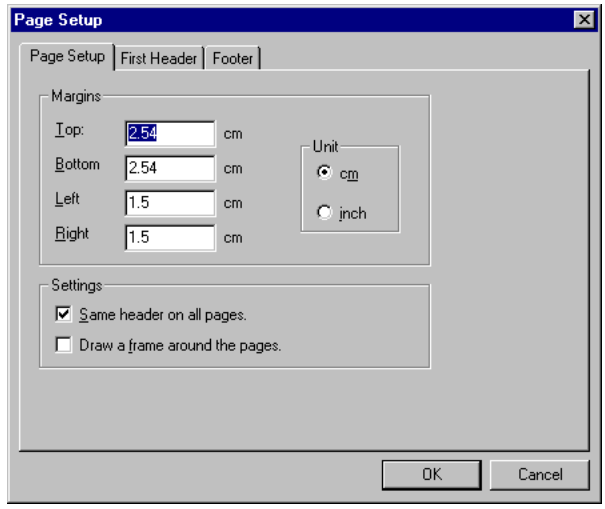


Figure 8-39. Page Setup dialogue, Page Setup tab.

First Header, which allows you to select the components to be included in the header for the first page.

Note: If you have not selected the setting **Same header** on all pages on the **Page Setup** tab, then a fourth tab option, **Header**, is visible in the **Page Setup** dialogue. The **Header** tab allows you to select items to include in the headers of the individual pages, except for the first page.

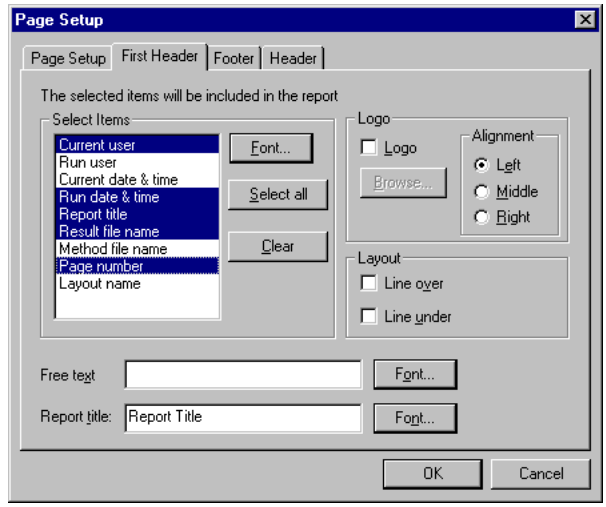


Figure 8-40. Page Setup dialogue, First Header tab.

1. Select the items to be included in the header. Click on **Select All** or **Clear** to facilitate your selection as appropriate.

2. If appropriate click on the **Font** button to alter the font characteristics.

3. Add **Free Text** and a **Report Title** as appropriate. You can also change the font style for these options.

4. If you have a logo in bitmap format this can be added to the header. Check the **Logo** option and then use the **Browse** function to locate your .bmp logo file.

5. Select the alignment of the logo, either **Left**, **Middle** or **Right**.

6. Select if you want a **Line under** or **Line over** the header.

7. Click on **OK** to implement the selection.

Footer, which allows you to select the components to be included in the footer.

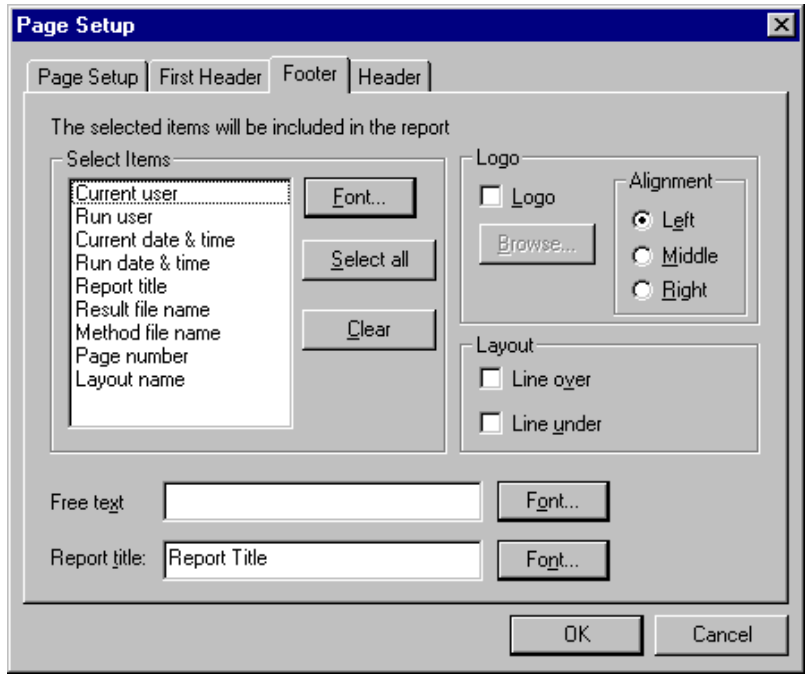


Figure 8-41. Page Setup dialogue, Footer tab.

Footer options are similar to those for **First Header** so you can have all information in either the header or footer or split the information between them as required.

Printing the report

To print the report select **File:Print** or click on the **Print** button. Select the page range in the displayed dialogue and then on **OK**.

Saving the report format

To save the report format:

1. Select **File:Save** or click on the **Save** toolbar button. If saving for the first time the **Save Report Format** dialogue is displayed.

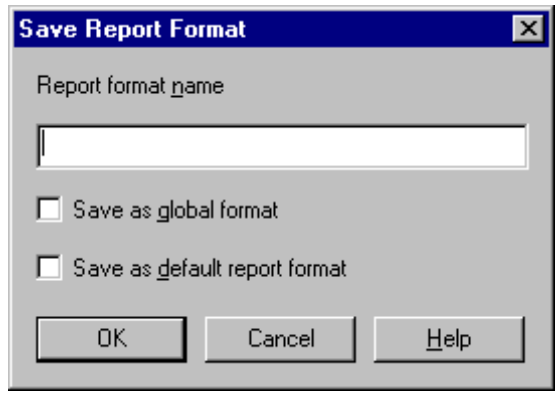


Figure 8-42. Save Report Format dialogue.

2. Enter a name for the format.

3. If you want the report format to be saved globally check the **Save as global format** option (if you have **Edit global lists** authorisation).

4. If you want the format to be used as the default format check the **Save as default report format** option.

5. Click on **OK**.

Note: If you selected the **Save as default report format** option, the format name is changed to DEFAULT.

To save a copy of the format under another name, select **File:Save As** and enter a new name in the **Save Report Format** dialogue.

Exiting the Customise Report window

To exit the **Customise Report** window select **File:Exit** or click on the **Exit** button. You will be prompted to save unsaved formats.

When you have exited the window the newly created format is displayed in the **Generate Format** dialogue.

8.7.2 Creating a new standard report format

It you do not want to create a new report layout using a customised format you can instead use the fixed layout in the **Standard** report formats. With **Standard** formats you can still select the objects that are included in the report and save the format for later use.

Selecting standard report options

1. Select the **New** button on the **Generate Report** dialogue. This prompts the **Create New Report Format** dialogue.

2. Choose **Standard format** and **OK**, and then use the tabs to select the report components within the displayed **Create Standard Report Format** dialogue. Note that the tabs for the standard report format are similar to the individual dialogues listed for objects in **Customised formats** (see Section 9.7.1).

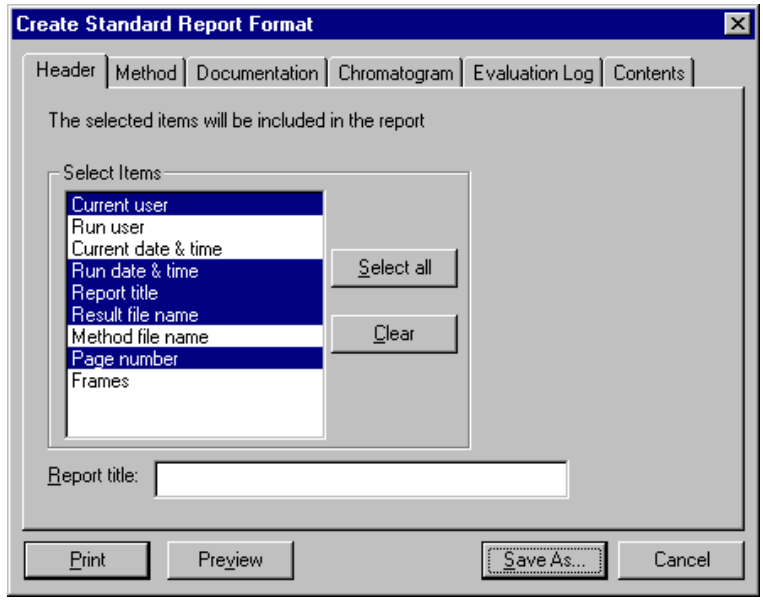


Figure 8-43. The Create Standard Report Format dialogue.

3. You can preview the report contents by clicking on the **Contents** tab. Clicking on the + symbols next to the content headings reveals the contents of each section of the report.

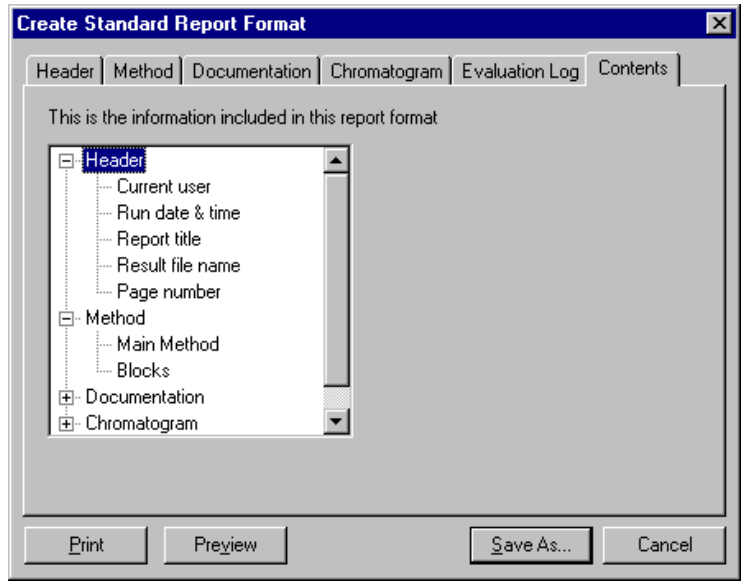


Figure 8-44. Create Standard Report Format dialogue, Contents tab.

Previewing and printing the report

Within any of the tabs on the **Create Standard Report Format** dialogue, you can preview the entire report printout by clicking **Preview**. Similarly, once you have made all of the desired adjustments to the report format, you can click on the **Print** button from within any of the tabs.

Saving the report format

1. Click on the **Save As** button.

2. In the displayed dialogue enter a name for the format.

3. If you want the report format to be saved globally check the **Save as global format** option (if you have **Edit global lists** authorisation).

4. If you want the format to be used as the default format check the **Save as default report format** option.

5. Click on **OK**.

Note: If you selected the **Save as default report format** option, the format name is changed to DEFAULT.

8.7.3 Modifying an existing report format

Another way of creating a new report format is to edit an existing format.

1. Select **File:Report** or click on the **Report** toolbar button and the **Generate Report** dialogue is displayed.

2. Select the report format of interest and click on the **Edit** button. Select either **Standard format** or **Customised format** and click **OK**.

Customised format

Choosing the **Customised format** button opens the **Customised Report** window. As these options are dealt with for creating a new customised report (see Section 9.7.1), they will be treated here only briefly. To add formatting to the page layout:

1. Add new objects as described in Section 9.7.1. Alternatively you can **Cut**, **Copy**, **Paste**, or **Delete** objects using the right mouse button menu.

2. Edit existing and new objects by moving them to new positions, resizing them or changing their properties. Change properties by clicking on an object with the right mouse button. This generates a popup menu that allows you to the highlighted section.

3. You can also modify the contents of the highlighted report section by choosing the **Properties** command. From the popup menu, click on **Properties**, make the desired choices on the resulting dialogue and press **OK**.

4. Once the desired formatting choices have been made, clicking on the **Preview** button will display the page layout. Press the **Print** button to print out the report. Also you can save the modified format under a new name by selecting **File:Save As**.

Standard format

Choosing the **Standard format** button generates a second **Edit Standard Report Format** dialogue with tabs representing format options for the major sections of the report. The options within the various tabs are identical to those available when creating a new standard format (see Section 9.7.2).

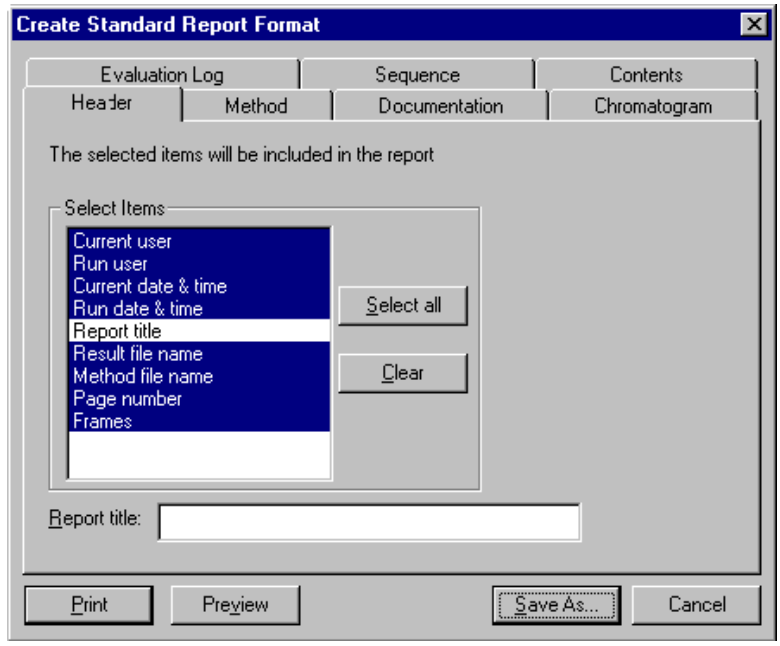


Figure 8-45. Standard format dialogue.

Once the desired formatting choices have been made, clicking on the **Preview** button will display the page layout. Press the **Print** button to print out the report. Also you can save the modified format under a new name by clicking on the **Save As** button.

8.8 Run documentation

The full documentation of a run is stored within the result file. A few of these are described below. To open the **Documentation**, either select **View:Documentation** or click on the **View Documentation** toolbar button. To print documentation contents, click on the **Print** button in the **Documentation** dialogue (see Section 9.6.3). The contents can be saved as a new method by selecting **Method** page and clicking on **Save as**.

Variables

These are the parameters that were used during the run.

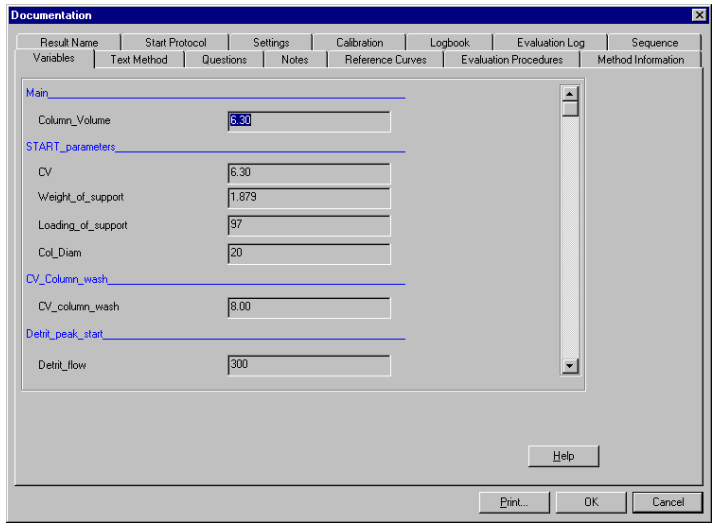


Figure 8-46. Documentation, Variables page.

Notes

This displays notes that you have made at various points during the run. You are also able to enter new comments in the **Evaluation Notes** field.

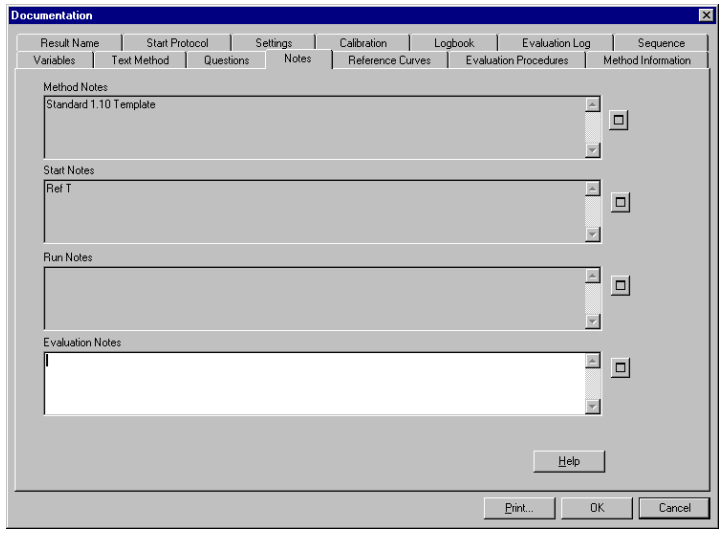


Figure 8-47. Documentation, Notes page.

Calibration

This displays what system calibrations were made, when and by whom.

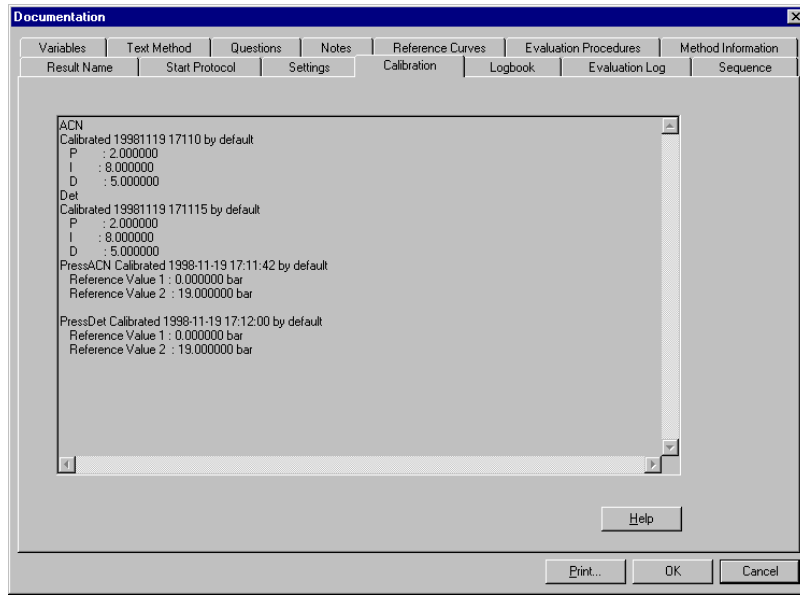


Figure 8-48. Documentation, Calibration page.

Log book

This displays exactly what happened during a run, including information concerning alarms, the method, manual changes, errors, the system and the oligonucleotide sequence. Selecting (checking) the Synthesis Data option provides information about the coupling efficiency of each base addition to the oligonucleotide. Trityl-ON synthesis will not display the last 5´ base in the Trityl table since the last base is not detriylated. Thus, in a synthesis of a 25-mer trityl-ON there will only be 24 Detrit values in the synthesis data. Synthesis data can also be obtained by printing a report (see Section 7.6).

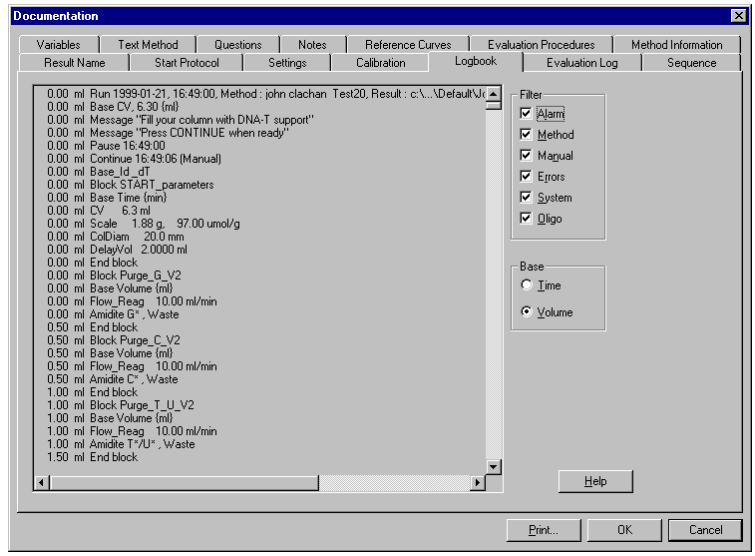


Figure 8-49. Documentation, Logbook page.

Evaluation Log

This lists all of the evaluation operations that you have performed for the current result file for all sessions, including procedures executed at the end of the method.

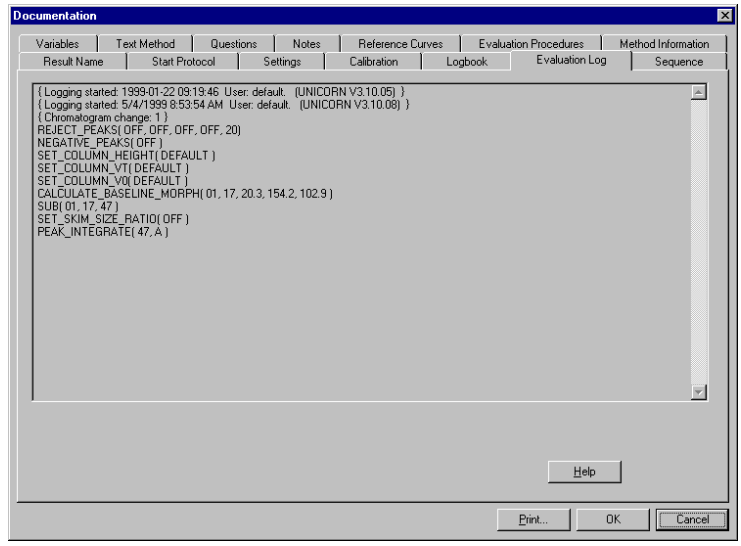


Figure 8-50. Documentation, Evaluation Log page.

8.9 Exiting Evaluation

If you want to quit from the Evaluation module of UNICORN, select **File:Exit**. You will then be asked if you want to save the results of the evaluation session that you have performed. If you answer **Yes**, the previous version of that result file will be unconditionally over-written. This may be undesirable if you have included the current result file within an evaluation procedure batch run (see Section 10.3.5).

9 Evaluating results

This chapter will mainly describe how to:

- integrate peaks
- automate evaluation operations
- export data and curves

9.1 Integrating peaks

Using peak integration, UNICORN allows you to identify and measure a number of curve characteristics including peak areas, retention times, and peak widths.

9.1.1 Baseline calculation for integration

Integrating peaks is divided into two steps: calculating the baseline and calculating peak areas. As a correct baseline is crucial for accurate calculation of peak areas, several ways of calculating the baseline are available in UNICORN:

- Using the **Calculate baseline** instruction for automatic calculation of the baseline, which gives, in most cases, a very accurate measurement. Baseline calculation can be performed using the Morphological algorithm or classical algorithm (see Sections 10.1.3 to 10.1.5). **Calculate baseline** is the most common alternative and it is strongly recommended that you read the information contained within Appendix D.2 which describes the principles of baseline creation.
- A blank run curve with the same chromatographic conditions as the corresponding sample can be used as the baseline for peak integration. Another approach that may improve the peak integration (if a blank run is available) is to first subtract the blank run from the source curve (see Section 9.3.3) and then perform peak integration on the resulting curve using the **Calculate baseline** option.

  In addition to blank runs, it is possible to select any curve present in the current chromatogram, e.g. an edited baseline (see Section 10.1.5), as baseline.

- Using a **Zero baseline**, i.e. no baseline subtraction at all.
- Reusing an already existing baseline for the selected curve by selecting the **Correlated baseline** option. This is the default alternative whenever possible.

9.1.2 Performing a basic integration

To perform a basic integration:

1. Select **Integrate:Peak integrate** or click on the **Peak Integrate** toolbar button. The **Integrate** dialogue is displayed.

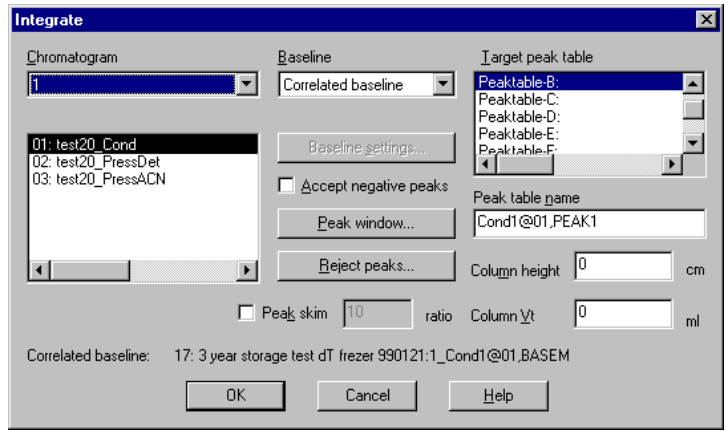


Figure 9-1. Integrate dialogue.

2. Choose the source curve to be analysed, usually the first UV curve, and a peak table destination from the peak table list. Any chromatogram can contain up to eight peak tables, designated A-H.

3. Make the appropriate **Baseline** selection from the above-mentioned possibilities. The **Calculate baseline** option with the default settings for the parameters is the most common choice.

Note: There are two choices of algorithm for baseline calculation, **Morphological** and **Classic**. The default setting is **Morphological**. See Section 10.1.3 for more details.

4. Click on **OK** to perform the peak integration when you are satisfied with your selections.

Following integration, the peaks in the chromatogram will be automatically labelled with their respective retention times. The start and end point of each peak will be marked by drop-lines.

The peak table will be displayed underneath the active chromatogram.

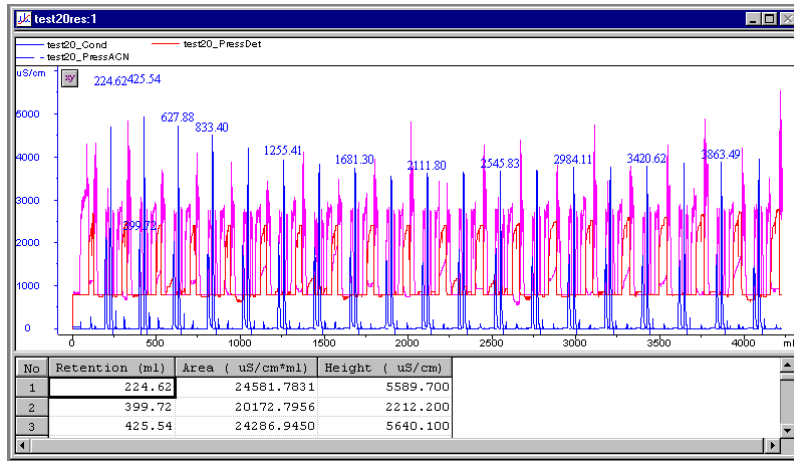


Figure 9-2. The results after peak integration.

In addition to peak areas, several other peak characteristics such as retention time and peak width are automatically calculated. The characteristics displayed in the peak table may be selected in the **Chromatogram Layout** dialogue, **Peak Tables** tab (see Appendix D.3).

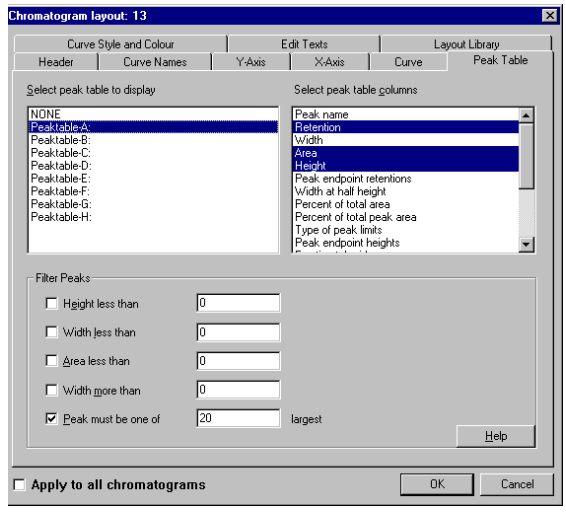


Figure 9-3. Chromatogram Layout dialogue, Peak Table tab.

Select the options that you want to be displayed from the **Select peak table columns list**. Most characteristics are automatically calculated

for each integrated peak when the peak integrate function is used, although only the selected items will be displayed in the peak table.

Changing peak labels

As alternatives to using retention times as peak labels, the peaks can be sequentially numbered or be marked with specific identification tags. The choice of label type is made in the **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue (see Section 9.2.5). Note that the labels may be displayed vertically for each peak by deselecting the **Horizontal text** option. If you do not want to view the peak labels, e.g. for presentation purposes, select the **No peak label** option.

Filtering peaks from view

It is possible to temporarily remove peaks from display in a peak table based upon the criteria you determine.

1. In the **Chromatogram Layout** dialogue, click on the **Peak Table** tab.

2. Select (check) the filter criteria in the peak table and specify the values used to filter the peaks, i.e.the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. Click on **OK**.

If you later want to include the peaks again you have to deselect the options. The difference between **Filter peaks** and **Reject peaks** is that the latter function permanently excludes peaks from the integration and affects the calculation of total peak area etc.

9.1.3 Optimising peak integration

If the results from the peak integration are unsatisfactory, there are several possibilities to improve the results.

Morphological and Classic baseline calculation

You can use one of two baseline calculation algorithms depending on the type of peaks to be integrated.

- The **Morphological** algorithm is set as the default and gives the best results in curves with drifting baseline and peak clusters. Optimising baseline calculation using the **Morphological** algorithm is also relatively easy since there are only three baseline parameters, namely structure width, noise window and minimum distance between points (see Section 10.1.4).

- The **Classic** algorithm has long been used as the standard for calculating the baseline (see Section 10.1.5). The **Classic** algorithm is particularly useful in integrating curves containing negative peaks. Thus, the **Classic** algorithm should be selected if the **Morphological** algorithm gives poor results from the presence of negative peaks or where quantitative data from negative peaks are important in the run.

To select the appropriate algorithm and change baseline settings:

1. Display the **Settings** or **Baseline Settings** dialogue, respectively, by (i) clicking on the **Baseline settings** button in the resulting **Integrate** dialogue, or (ii) selecting **Integrate:Calculate baseline**. These dialogues function identically. However, in the former case, the baseline is immediately used in a peak integration.

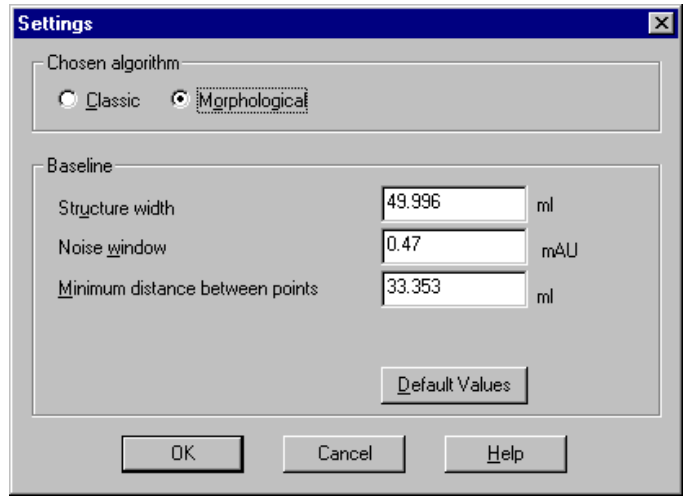


Figure 9-4. Settings dialogue.

2. Select the appropriate algorithm, **Classic** or **Morphological**.

3. Change the **Baseline** parameter values as appropriate.

Excluding peaks

It is possible to exclude peaks from integration based upon criteria you determine. Click on the **Reject peaks** command button in the **Integrate** dialogue. In the dialogue that is displayed, select (check) the criteria and parameters by which peaks will be excluded from the integration. You are able to define the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks.

The default criterion is to include only the 20 largest peaks.

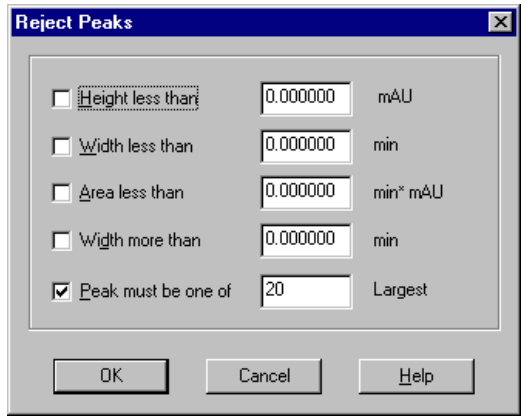


Figure 9-5. Reject Peaks dialogue.

Selecting part of a curve for integration

To select only a part of a curve for integration, click on the **Peak window** button in the **Integrate** dialogue. A chromatogram window will open containing the curve and two vertical cursor lines. These lines can be dragged to define a region between them that will be analysed. Alternatively, x-axis values for the **Left limit** and **Right limit** may be typed in. Click on **OK** to return to the main dialogue. The baseline will be calculated from the whole curve, but calculation of the areas beneath the peaks is only performed on the selected section of the curve. The default peak window includes the entire curve.

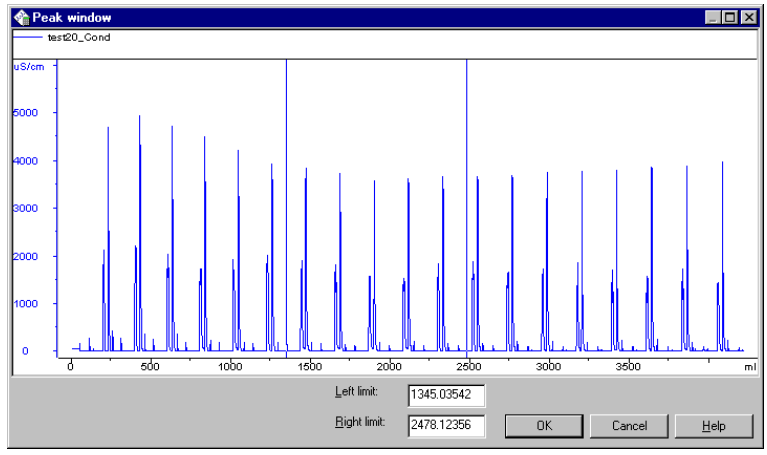


Figure 9-6. Peak Window chromatogram window.

Peak skimming

The area under a peak can be calculated either using drop-lines or peak skimming. Drop-lines are vertical marks that split two peaks at the valley. This is most commonly used for peaks of relatively similar size. In some circumstances, for example when a peak has a shoulder, use of a drop-line will cause too much area of the first peak to be lost to the peak that forms the shoulder. Thus, the skim peak function can be used when the smaller peak is skimmed off with a straight line starting at the valley between the peaks, and ending at the point on the other side of the smaller peak where the slope of the skim line is equal to the slope of the curve. In doing so, this skims the area under the second peak.

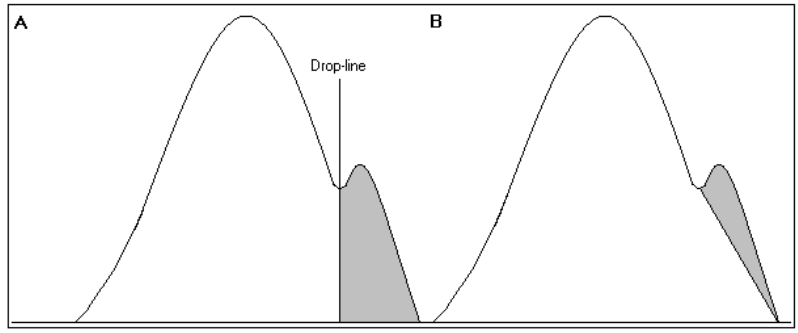


Figure 9-7. Illustration of how a drop-line (A) and a skimmed peak (B) affects the area under the main peak and the peak shoulder.

The **Skim peak** option can be checked in the **Integrate** dialogue. You can also set the **Ratio** value to determine when peak skimming should be applied to a peak instead of drop-lines (the default value for this ratio is 10). The ratio is based on the relationship:

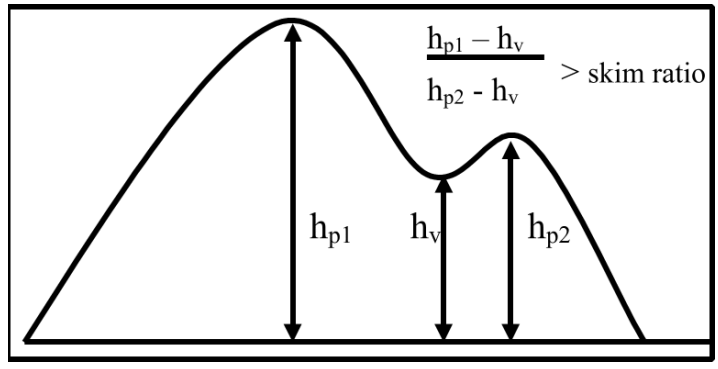


Figure 9-8. Skim peak ratio calculation.

Including negative peaks

If you want to include negative peaks in the integration, select (check) **Accept negative peaks** in the **Integrate** dialogue (or in the **Calculate baseline** dialogue). The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

Manually editing the baseline

In the event that the automatic baseline calculation does not produce a satisfactory baseline, it is possible to edit the baseline manually by inserting and deleting baseline points. This is done with the **Integrate:Edit baseline** function (see Section 10.1.6).

Manually editing the peak table

The **Integrate:Edit peak table** function (see Section 10.1.7) allows you to manually adjust the peak start and end points, split and join peaks.

9.1.4 Optimising the baseline parameters using a morphological algorithm

The first choice when trying to optimise the peak integration is to change the baseline parameters. A brief description of the parameters and a suggested way of estimating settings for the **Morphological** baseline parameters from the source curve, is given in Appendix D.2.

The **Morphological** algorithm can be described in terms of a straight line that "strolls" along the chromatogram parallel to the x-axis. Data points for the baseline are created wherever the straight line touches the curve and the points are joined at the end to create a baseline.

Structure width

When optimising the baseline parameters using a **Morphological** algorithm, changes to **Structure width** will in most cases give the best improvement in results. **Structure width** determines the length of the straight line (see above), which is set to a default value of the widest peak on the chromatogram multiplied by 1.5. In situations with drifting baseline for a curve, the morphological baseline follows the curve faithfully. Subsequent subtraction of the baseline thus creates a curve with the baseline at a more even level.

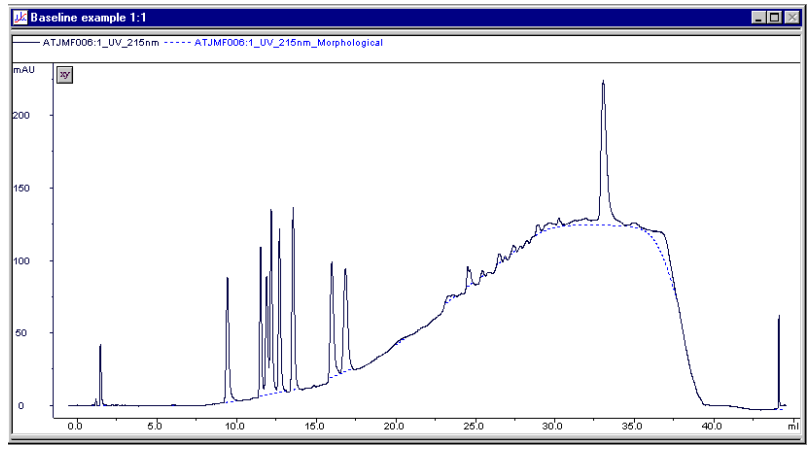


Figure 9-9. Fluctuating curve with a morphological baseline that follows the bases of the peaks at the different levels in the curve.

In some circumstances the default setting for the **Structure width** should be increased since a too low setting may result in a baseline that reaches too high up in the peaks of the curve (see figure below). This situation may arise when, for example, a wider peak is not recognised due to it containing a cluster of smaller peaks. The default **Structure width** is therefore set to a default value according to the largest width of the identified narrower peaks.

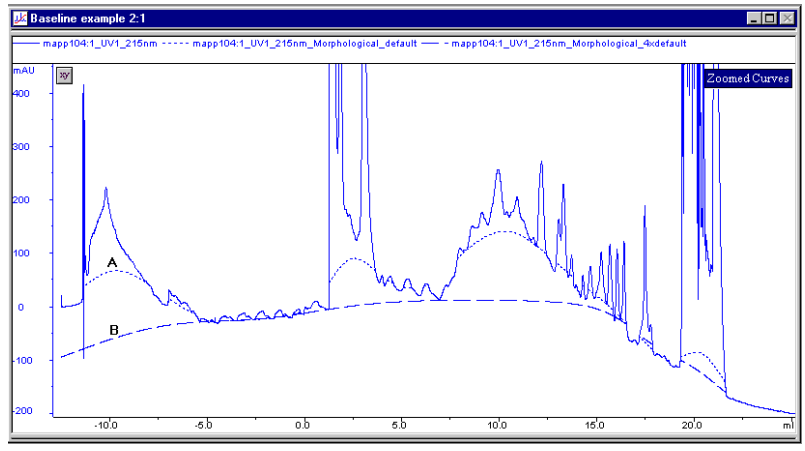


Figure 9-10. Chromatogram displaying a curve with two baselines; (A) default morphological algorithm settings and (B) morphological algorithm with increased structure width value.

Conversely, a too large **Structure width** value means that narrower peaks, especially in fluctuating curves, may not be properly followed. This may arise when an artifact in a curve is identified by the morphological algorithm as the widest peak and hence used to set the default **Structure width** value.

Minimum distance between points

The **Minimum distance between points** is a measure of the distance between the data points used to generate a baseline. The largest number of data points is produced at the slopes of the curves, and so by increasing the **Minimum distance between points** value fewer points will be collected on the slopes.

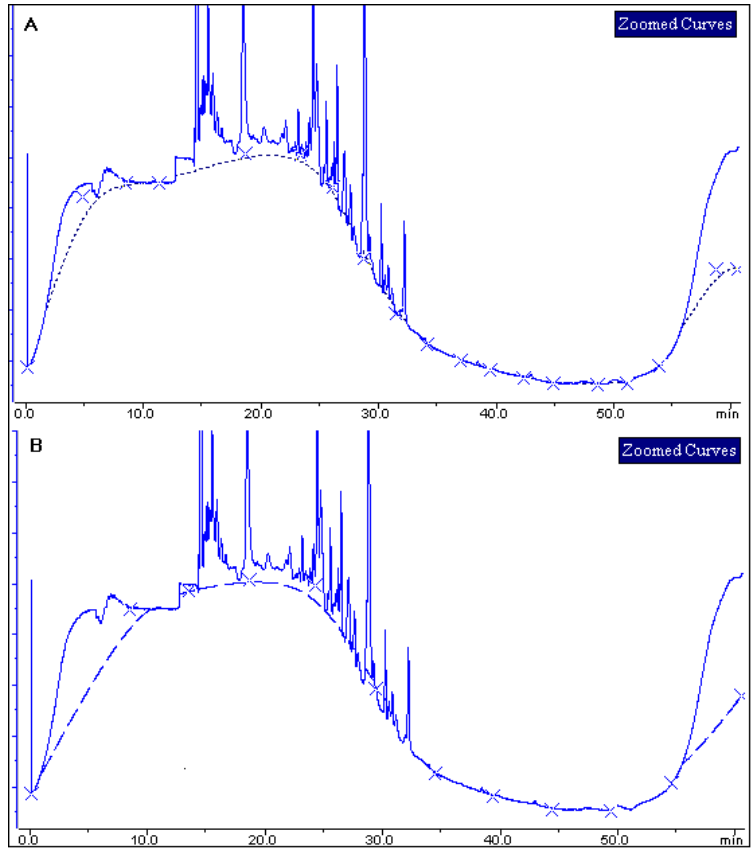


Figure 9-11. A curve in a chromatogram integrated using the morphological algorithm contains many data points when the Minimum distance between points parameter is set to a low value (A). The number of data points is reduced when the Minimum distance between points parameter is set to a higher value (B).

Noise window

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

The cause of this problem is that the **Noise window** parameter is too low. Increase the **Noise window** parameter in the baseline calculation. This may in some cases result in peak limits too high up on the peak slopes (see Section 10.1.5 for a description of **Noise window** with the classic algorithm).

Another possibility is to use the **Reject peaks** function in the **Integrate** dialogue to reduce the number of peaks based on an appropriate criterion, e.g. the number of peaks or the minimum peak height.

9.1.5 Optimising the baseline parameters using a classic algorithm

The first choice when trying to optimise the peak integration is to change the baseline parameters. A brief description of the parameters and a suggested way of estimating settings for the baseline parameters from the source curve, is given in Appendix D.2. The **Integrate:Edit baseline** function may provide additional information about the cause of the unsatisfactory peak integration by revealing where the baseline points have been placed by the automatic baseline calculation algorithm.

When optimising the baseline calculation, a change in the **Slope limit** will very often give the expected result. An example of the effect of a too high **Slope limit** is that the up-slopes of the peaks may be recognised as baseline segments. Baselines that deviate substantially from the source curve may arise from a combination of a too long **Shortest baseline segment** value with a too high **Slope limit**.

A recommended strategy is to change the baseline parameters step by step and to check the resulting baseline after each change. The size of the initial change depends on the cause of the peak integration problem, but some general guidelines can be given. When the desired effect is achieved it is advisable to go back and check a parameter value in between the two last settings to avoid an unnecessarily low or high value. The default baseline parameters can be restored by clicking on the **Default** command button.

Baseline parameter	*Recommended initial change*
Shortest baseline segment	20-50%
Noise window	10-30%
Slope limit	25-50%
Max baseline level	Usually not necessary to adjust

Below are some examples of common problems with peak integration and baseline calculation, as well as suggestions for improvements. The following problems will be discussed:

- Baseline does not follow the source curve
- Peak limits too high up on the peaks
- Noise detected as peaks
- Peaks missing
- Baseline on top of peaks

Baseline slope does not follow the source curve

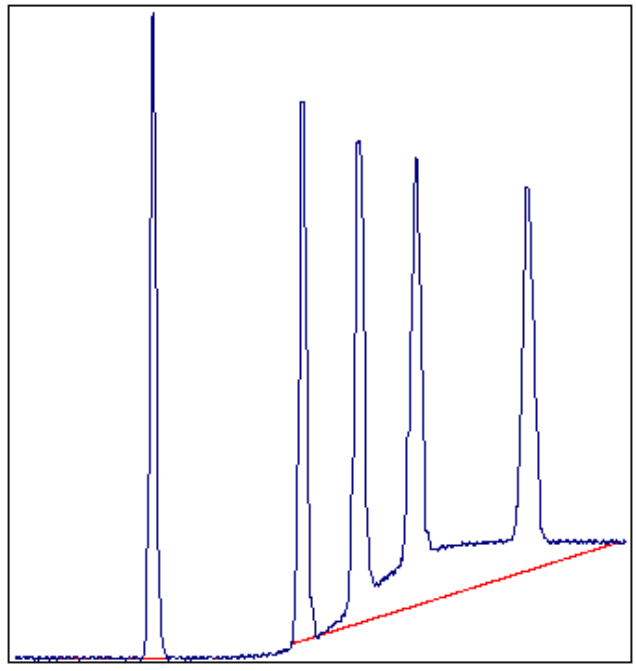


Figure 9-12. Baseline calculation with default parameters.

The calculated baseline does not follow the source curve, because short curve segments between peaks in the middle of the chromatogram are not identified as baseline segments. If you decrease the **Shortest baseline segment** by 50% the following baseline is calculated.

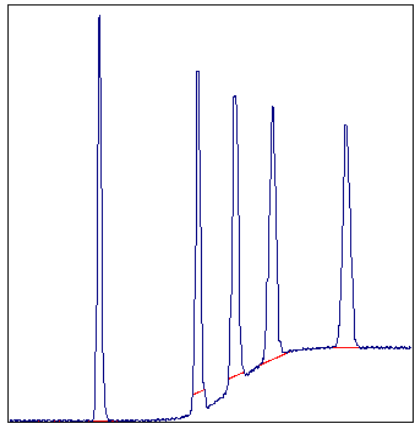


Figure 9-13. Baseline calculation with 50% lower Shortest baseline segment.

The baseline is, however, still unsatisfactory, due to the high slope of the short segments in the region between the second and fourth peak which are still not identified as baseline segments. If you increase the **Slope limit** by a factor 2.5, a correct baseline is calculated.

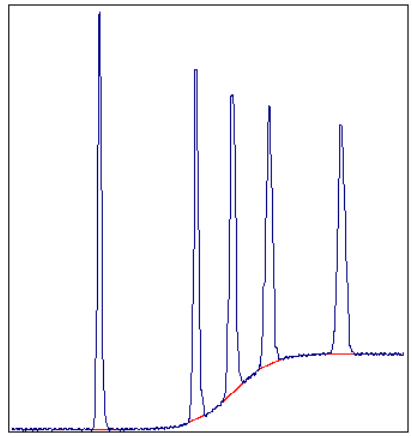


Figure 9-14. Correct baseline parameters.

Peak limits too high up on the peaks

This peak integration problem is in most cases caused by a too high value for the **Slope limit** and/or a too high value for the **Noise window**. This can be encountered when the chromatogram includes a very large flow-through or solvent peak. The large peak affects the calculation of the default baseline parameters, leading to too high values for **Slope limit** and **Noise window**.

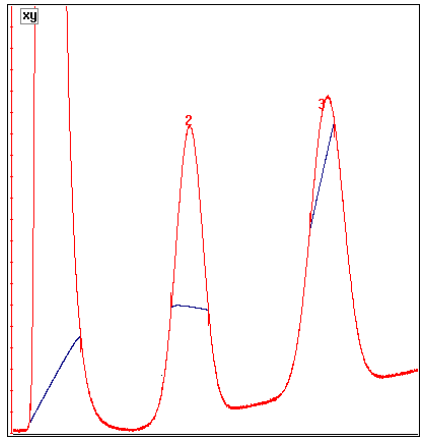


Figure 9-15. Peak start and end points on peaks caused by an excessively high Slope limit.

Note the difference between the situations in Figures 9-12and 9-15. In Figure 9-12, no baseline segments were detected between the second and fourth peaks (there are no blue crosses is this region when **Edit baseline** is selected). The baseline follows the curve as a best fit, and cannot be drawn above the curve unless **Accept negative peaks** is selected. In Figure 9-15, baseline segments are detected on the up and down slopes of the peaks (marked by blue crosses in these regions when **Edit baseline** is selected). By considerably decreasing the **Slope limit** in Figure 9-16, a better baseline can be constructed, leading to an improved peak integration.

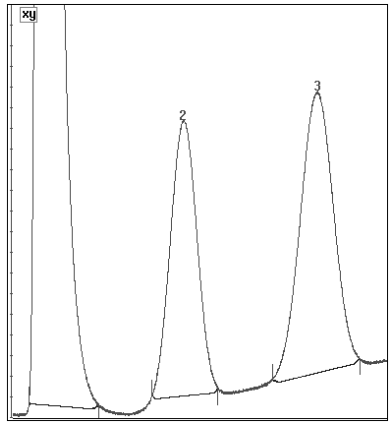


Figure 9-16. Peak integration with decreased Slope limit.

You may also have to decrease the **Noise window** to get a perfect peak integration.

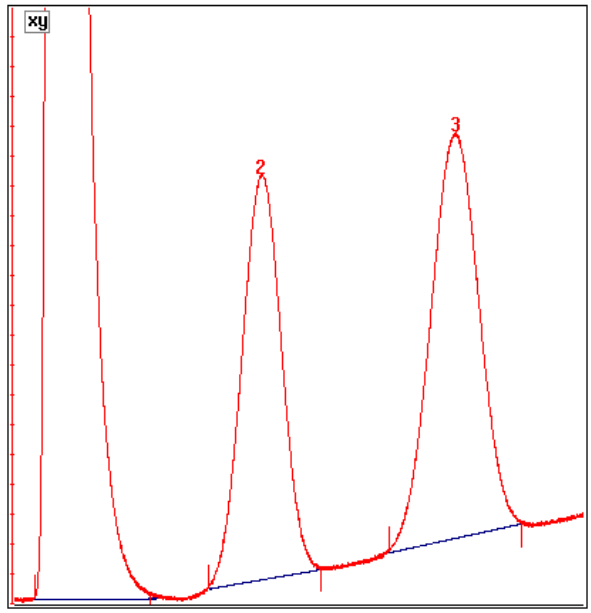


Figure 9-17. Correct baseline after decreasing both Slope limit and Noise window.

An alternative approach is to exclude the large peak from the peak integration, as its presence affects the default baseline parameters and the retention and area of the large peak is in most cases not interesting. Using the **Operations:Cut curve** function, the appropriate region of the chromatogram can be selected. The peak integration can then in most cases be performed with default baseline parameters on the cut curve. You can not use the **Peak window** function to remove the large peak as the baseline is calculated for the entire curve.

Noise detected as peaks

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

The cause of this problem is that the **Noise window** parameter is too low. Increase the **Noise window** parameter in the baseline calculation. This may in some cases result in peak limits too high up on the peak slopes (see example below).

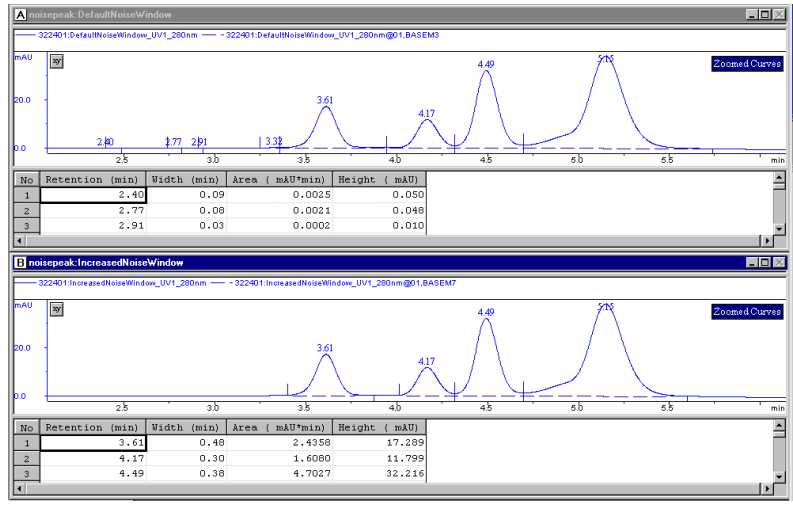


Figure 9-18. (A) Noise detected as peaks; (B) Peak integration after increase of Noise Window.

Another possibility is to use the **Reject peaks** function in the **Integrate** dialogue to reduce the number of peaks based on an appropriate criterion, e.g. the number of peaks or the minimum peak height.

Peaks missing

In cases where obvious peaks are not detected in the peak integration, a probable cause is that the **Noise window** parameter is too high.

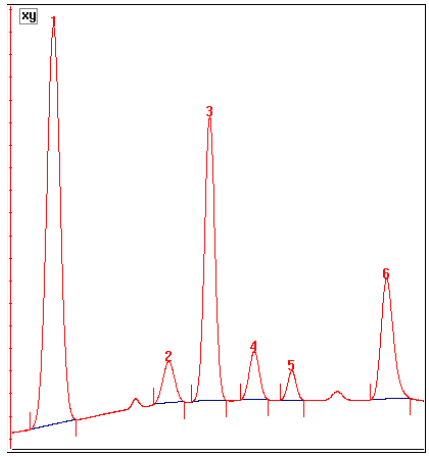


Figure 9-19. Peak integration with too high Noise window.

Decrease the **Noise window** parameter until the peaks are detected.

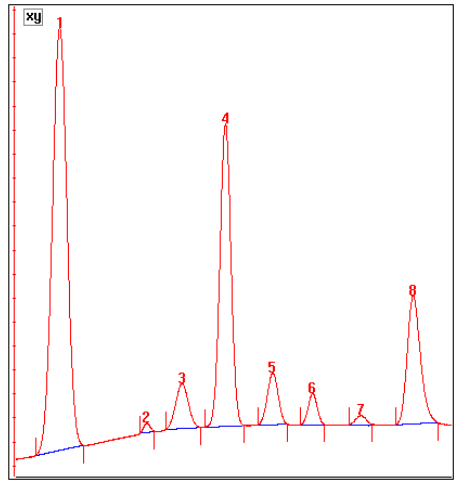


Figure 9-20. Correct peak integration after decreasing Noise window.

Another possible cause of missing peaks is that an improper reject criterion has been used. Check the criteria used for **Reject peaks** in the **Integrate** dialogue as well as Filter peaks in the **Chromatogram layout** function.

Baseline on top of peaks

In rare cases the top of a broad flat peak will be incorporated as a baseline segment.

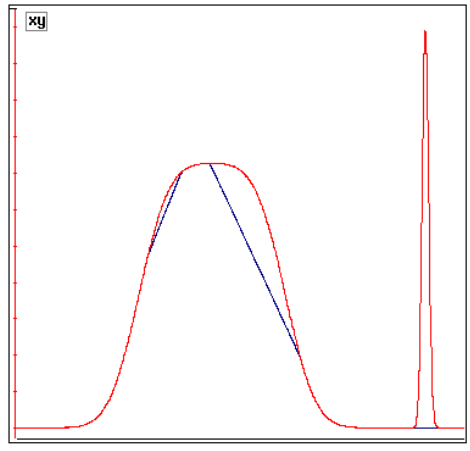


Figure 9-21. Baseline segment on top of peak.

This is one of the very few situations where it is useful to change the **Max baseline level**. Measure the height of the flat plateau of the peak using the **XY** icon on the chromatogram (see Section 10.1.8). Insert a value somewhat lower than the plateau height as the **Max baseline level** in the baseline calculation.

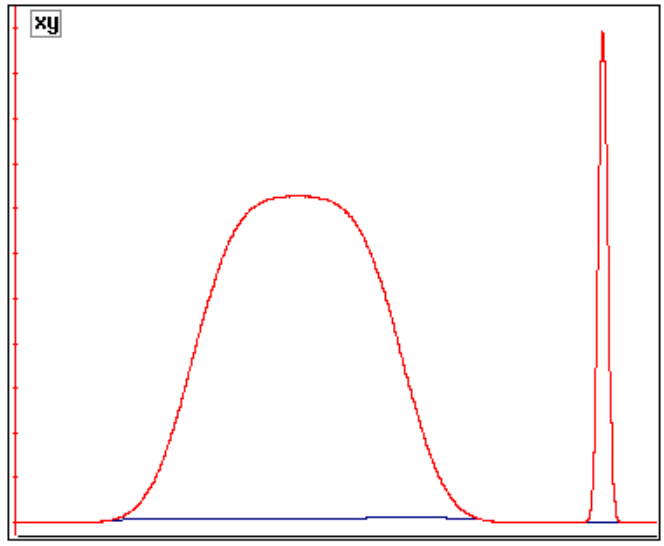


Figure 9-22. Correct baseline after decreasing Max baseline level.

If there are several rather short segments that erroneously have been incorporated in the baseline, an alternative remedy is to increase the **Shortest baseline segment** setting.

9.1.6 Manually editing a baseline

Once a baseline has been calculated, it is possible to add or remove baseline points on it and then draw a new baseline from the new set of data points. The edited baseline curve can then be used in a new peak integration.

1. Select **Integrate: Edit baseline**.

2. Choose the desired baseline from the displayed dialogue and click on **OK**. A window will appear displaying the baseline and the curve from which it was calculated. Additionally, blue crosses are displayed (the baseline points) and their co-ordinates in the **Point list**.

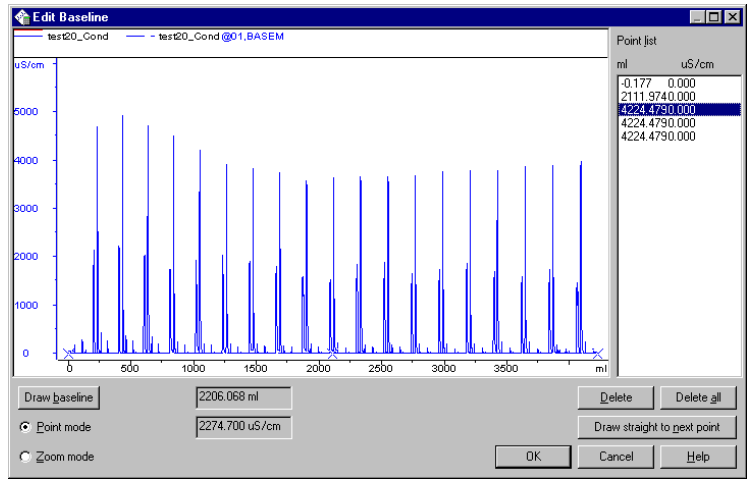


Figure 9-23. Edit Baseline chromatogram window.

Insertion of baseline data points

Select the **Point mode** button. Click on the left mouse button to place a new baseline point on the chromatogram. Each new point is represented by a cross and its co-ordinates are automatically entered into the **Point list**. This is useful when, for example, you want the baseline to go up to a high valley between two peaks.

To make your task easier you can click on **Zoom mode** and zoom in on specific regions of the chromatogram and then insert baseline data points. The right mouse button menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**.

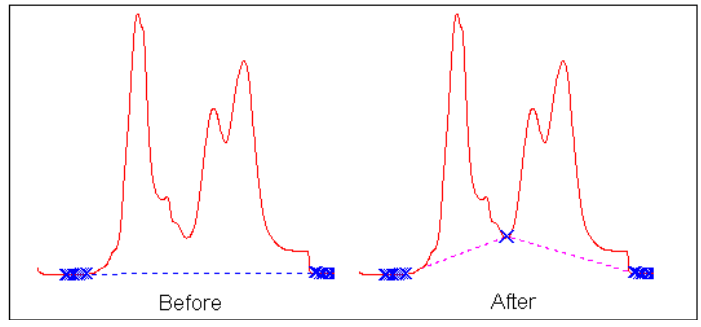


Figure 9-24. Baseline before and after editing.

Deleting baseline data points

If you want to delete a data point from the **Point list**, select the appropriate point in the list and press the **Delete** button. Alternatively, double click on the unwanted data point entry in the list to delete it. **Delete all** removes all baseline data points.

Drawing the new baseline

When you are satisfied with your baseline point selection, click on the **Draw baseline** button. The new baseline curve will be drawn as a spline function based on the previous and the new points. The spline function is guided by the points, but does not necessarily pass through them. You may also force a straight baseline between two points by selecting the first of the two points in the point list and then clicking on the **Draw straight to next point** button.

Click on **OK**, and the new baseline will be saved with the default name **Edited Baseline**. This may now be used as the baseline in a new peak integration.

9.1.7 Adjusting the peak limits

Once a peak table has been generated using the appropriate baseline, it is then possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak areas will be recalculated.

1. It is recommended that you first access **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and select either **Number** or **Retention** for labelling the peaks. The former option will sequentially number each of the peaks in the chromatogram which is opened during the edit mode of a peak table. The latter option will display the retention volume or time for each peak.

2. Select **Integrate:Edit peak table**. Select the desired peak table from the displayed dialogue. Note that name of the baseline on which the selected peak table was based, is displayed at the bottom of the panel.

3. Double click on the desired peak table in the list or click on **OK**. A chromatogram window is displayed containing the selected peak table with corresponding curve and baseline. The various editing features are described below. As an aid, it is possible to use the zoom function.

4. Once you have completed your changes, click on **OK** and verify the destination of the new (edited) peak table.

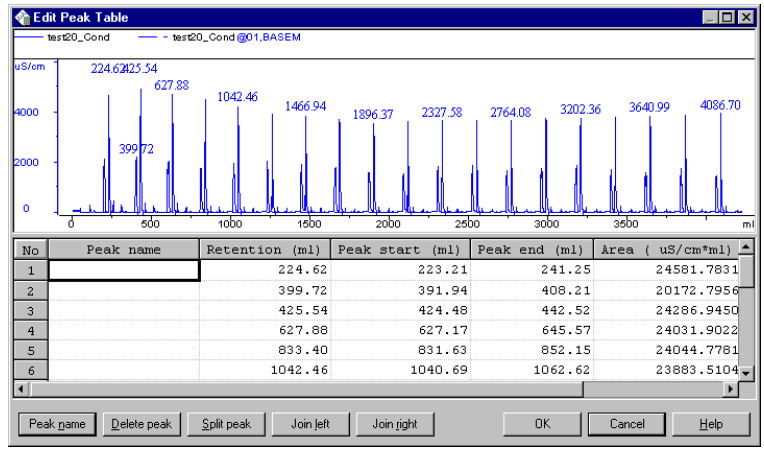


Figure 9-25. Edit Peak Table chromatogram window.

Deleting a peak in the peak table

To delete a peak from the table, click on the peak in the chromatogram or in the peak table and click on the **Delete peak** command button. Note that the remaining peaks will be renumbered after the deletion.

Splitting a peak

A peak is defined within delimiting drop-lines to the left and right of the peak. It is possible to split the peak into two new peaks by inserting a drop-line. The drop-line is always inserted at the middle point between two existing drop-lines. The area under each new peak will not be the same if the symmetry of the original peak was not perfect.

To make a split, select the desired peak in the list or mark in the curve and press the **Split Peak** button.

Note: The peaks will be renumbered according to the split. Refer to the description below about adjustment of the drop-lines.

Joining a peak

It is possible to join the areas of adjacent peaks if separated by a drop-line.

1. Select a peak either on the chromatogram or in the peak table.

2. Click on **Join left** or **Join right** if you want the peak to be joined with the peak to its left or right respectively. The original intervening drop-line is removed and all peaks are renumbered.

Adjusting peak start and end points

The beginning of each peak is marked with a drop-line above the curve, and the end of each peak is marked with a drop-line below the curve.

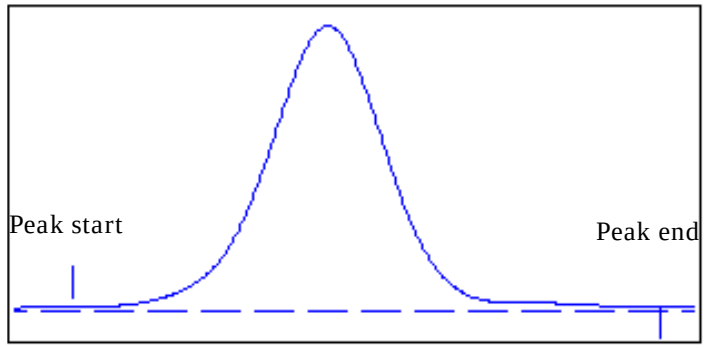


Figure 9-26. A drop-line at the start and end of a peak.

Where there are two peaks beside one another, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a drop-line below and above the line at the same point.

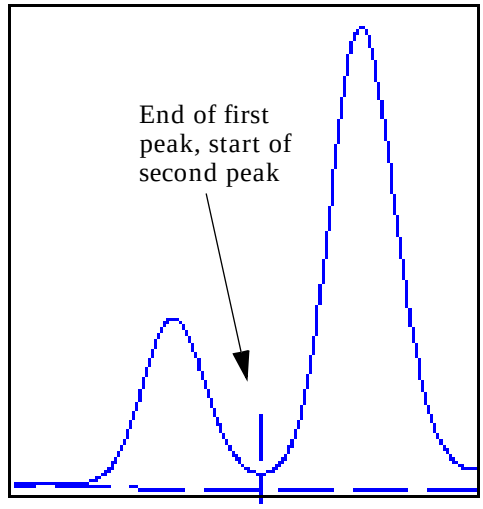


Figure 9-27. A drop-line between two peaks.

It is possible to move the drop-lines for a selected peak and thus affect the area beneath the peak.

1. Click on the peak of interest on the curve or in the peak table and two vertical cursor bars become superimposed on the left and right drop-lines that delimit the selected peak.

2. Drag the left and right drop-line bars to define the new left and right limits respectively for the selected peak. The drop-lines can never be moved beyond any other drop-line. The new left and right limits are now represented by a drop-line above and below the curve respectively, and the peak areas are automatically recalculated.

3. Drop-lines on preceding or following peaks can be similarly adjusted. Movement of these drop-lines can be up to, but never beyond, any other drop-line. A drop-line may also not be moved beyond a point where the peak meets the baseline.

Identification names for peaks

Double click on the peak of interest in the peak table spreadsheet cell and edit the name directly in the cell. You will see the peak names in the chromatogram (and the spreadsheet cell) only if you have earlier accessed **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and selected **Peak name** for labelling the peaks. Note also that in order to have **Peak name** listed within the spreadsheet cell, you must choose the **Peak name** option under the **Peak Table** tab within the **Chromatogram Layout** dialogue.

9.1.8 Measuring retention time and peak heights

It is possible to determine the co-ordinates of any point on a curve and thus obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations. Co-ordinates can be obtained in two ways:

- Direct measurement
- Viewing peak table data

Direct measurement

1. Double click on the small **XY** icon within the chromatogram area at the top of the y-axis. The active **XY** box contains coloured text which shows the x-axis co-ordinate and y-axis co-ordinate, both with their respective units. The colour and units of the y-axis information corresponds to a specific curve of the same colour.

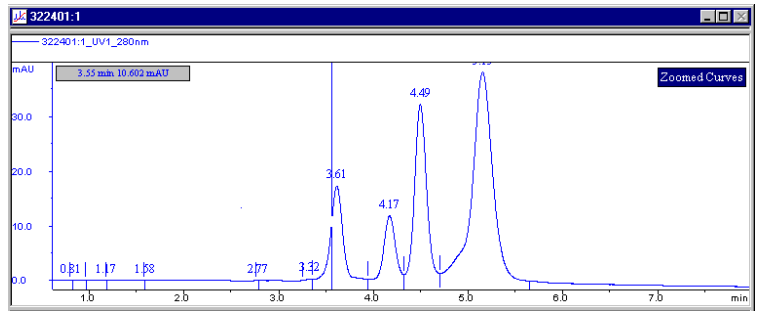


Figure 9-28. Active bar linked to XY box for determining curve point co-ordinates.

2. Click on the desired curve legend and the correct y-axis is displayed.

3. A vertical cursor line can also be seen on the chromatogram which is the same colour as the selected y-axis scale. Use the mouse pointer to drag the line back and forth along the chromatogram. Co-ordinates, corresponding to retention time and peak height, will change accordingly in the active **XY** box.

4. Double click on the active **XY** box to close this function.

Viewing peak table data

The retention time and amplitude of any peak can be directly viewed in a peak table after an integration, if selected for in the **Chromatogram Layout** dialogue.

9.1.9 Measuring HETP

HETP (height equivalent to a theoretical plate) calculations allow you to check how well the column has been packed.

1. Perform a run with injection of a non-interactive substance, for example, a small volume of acetone. Note that the injection must be at zero time.

2. In the **Integrate** dialogue, type in the column height (cm) in the appropriate field area. Perform the peak integration according to your other selected parameters.

3. To view the results of the integration, select **Plate height (HETP)** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue.

Every peak will have a HETP value. A narrow peak gives a low value corresponding to a well packed column. A broad peak gives a high value, indicating a column that is not optimally packed.

HETP is calculated as follows:

HETP = L/N

$N = 5.54 \times (V_R/w_h)^2$ assuming a Gaussian peak

where

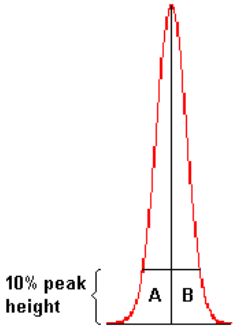
N = no. of theoretical plates

L = bed height in cm

V_R = peak retention (elution) volume or time

w_h = peak width at half height expressed in the same units as VR

9.1.10 Measuring peak asymmetry



This function can be used in combination with HETP to help assess column performance. A perfect peak will have no asymmetry and, after peak integration, give a value of 1.0. Any value less than 1.0 means that there is a left skew, i.e. the asymmetry falls on the leading side (left) of the peak. The reverse is true for values greater than 1.0 where the asymmetry comes on the tailing side (right) of the peak.

To view the asymmetry data select **Asymmetry** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window.

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.

9.1.11 Measuring resolution

Resolution is a measure of the relative separation between two peaks and can be used to determine if further optimisation of the chromatographic procedure is necessary. If the resolution value is 1.0, then 98% purity has been achieved at 98% of peak recovery, provided the peaks are Gaussian and approximately equal in size. Baseline resolution requires that the resolution value is greater than or equal to 1.5. At this resolution, purity of the peak is 100%.

To view the resolution data, select **Resolution** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window. The resolution value for each peak shows the resolution with respect to the previous peak.

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

$V_{R2} > V_{R1}$

V_{R1} = retention (elution) volume for peak 1

V_{R2} = retention (elution) volume for peak 2

w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)

w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

See Appendix Section D-3 for alternative calculations of the resolution.

9.2 Other evaluations

9.2.1 Peak purity and peak identification

Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity. The **Operations:Divide** function can be used when you have a result file with run detected at more than one wavelength.

1. Before dividing the curves, you must make sure that both curves have a baseline close to zero AU. This can be achieved with baseline subtraction.

2. Create a baseline for each UV curve according to the procedures detailed in Sections 10.1.1 and 10.1.2. Subtract the baselines from their respective UV curves using **Operations:Subtract**.

   Alternatively, you can subtract the corresponding blank runs from the UV curves if such exist.

3. When you have achieved two curves with a zero baseline, select **Operations:Divide** and select the two curves for division. You have the option to set **Threshold** values by checking the option and setting the threshold value for each curve. This sets the quotient to 1.0 if either of the sample values is closer to zero than the threshold value. This is to prevent very high quotient values being created if division is performed with values close to zero. Very low quotient values are also prevented. **Threshold** values are suggested by UNICORN although these can be changed.

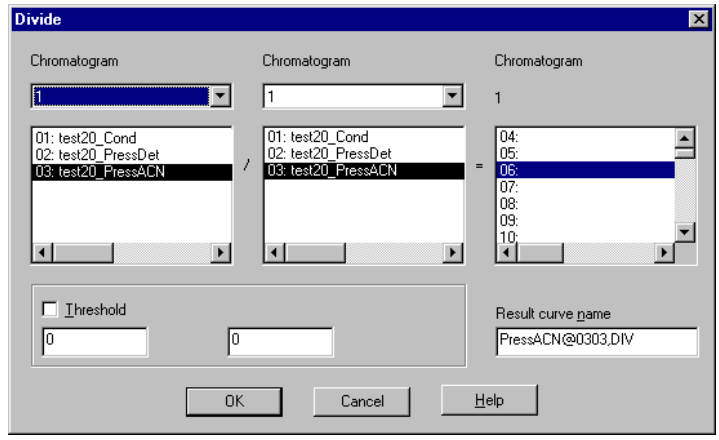


Figure 9-29. Divide dialogue.

4. The resulting curve can then be filtered by **Operations:Smooth** (see Section 9.3.2). It is suggested that you smooth using the median filter to remove noise that appears as spikes or occurs in a small area of the curve.

The ratio can be used to check peak purity. If the peak is pure, the absorbance spectra are the same over the whole peak and therefore the ratios should remain constant. If the absorbance ratio is not the same over the whole peak, then the peak is probably not pure.

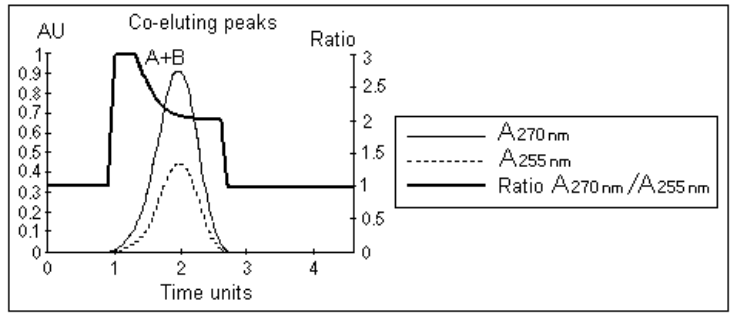


Figure 9-30. Simulated chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time.

The resulting ratio can also be used for peak identification as different compounds have a specific ratio between absorbencies at different wavelengths.

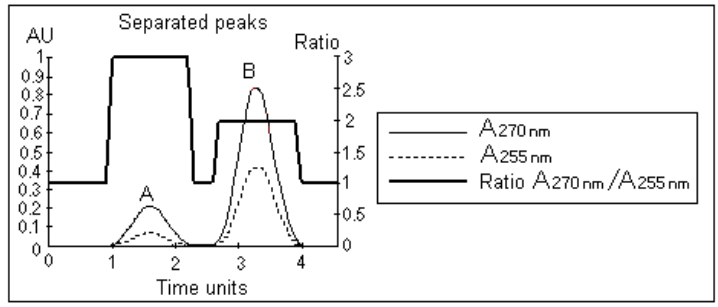


Figure 9-31. Simulated chromatogram of two components with differences in their absorbance spectra.

9.2.2 Finding the slope values for Peak Fractionation or Watch instructions

Peak fractionation parameters are set in the Method editor with the instruction Peak_FracParameters. StartSlope and EndSlope values are set. The procedures for finding suitable slope values for a particular run are described below.

It is also possible to set up conditional (Watch) instructions which allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak emerges. The slope of the curve may be set as a condition used to satisfy a Watch condition in the method during the run. It is therefore important to use accurate slope values for the specific Watch instruction parameter.

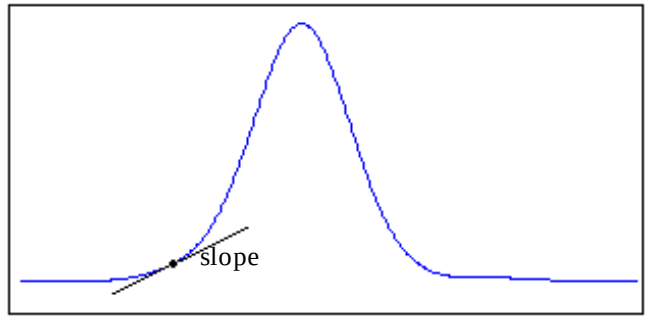


Figure 9-32. The slope of a curve.

To determine slope values, you must first make a run with the sample you intend to purify. Then use this result to find slope values in the

Evaluation module:

1. Ensure that you have selected **Time** as the x-axis scale for retention in the **Chromatogram Layout** dialogue, **X-Axis** tab.

2. Select **Operations:Differentiate**. Select the desired (UV) curve, check that a **First order** calculation is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

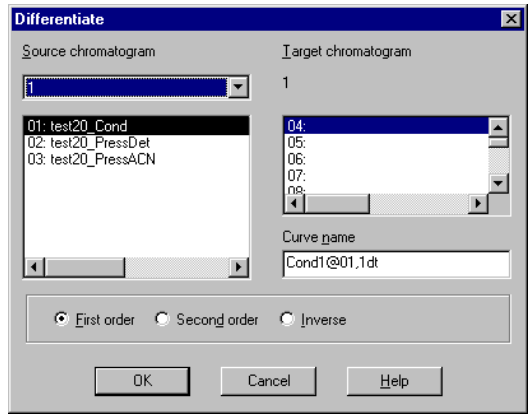


Figure 9-33. Differentiate dialogue.

3. Measure the y-axis values on the differentiated curve by clicking on the **XY** icon, choosing the y-axis differentiate scale and reading the curve co-ordinates in the active **XY** box. It may be necessary to smooth the differentiated curve. The units for the differentiated curve is mAU/min or AU/min. Co-ordinates are based upon the position of the vertical line on the chromatogram in relation to where it bisects the curve. Any y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

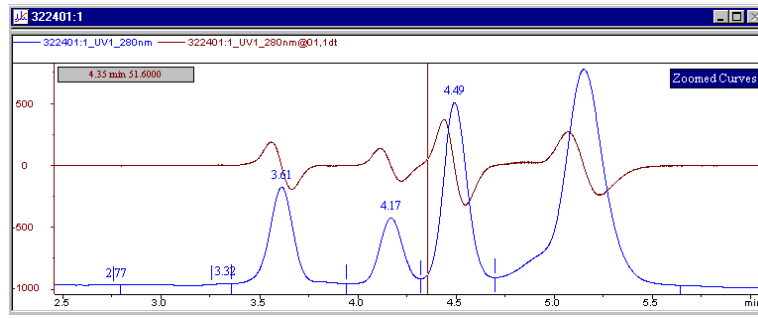


Figure 9-34. Measurement of the Slope limit after differentiation.

4. Use the zoom function to magnify the curve over an appropriate area. Place the vertical cursor bar at the beginning of a peak where you want the Watch conditions to be fulfilled, i.e. where the slope becomes higher. Read the actual slope value in the active **XY** box.

5. In the Method editor, enter the slope value as a parameter for the Watch instruction or enter the StartSlope and EndSlope values in the Peak_FracParameters instruction.

9.2.3 Creating a curve

It is possible to create a curve based on any external measurements.

1. Select **Operations:Create Curve**. In the dialogue that is displayed, select one or several help curve(s).

2. Select the minimum and maximum values of the y-axis. Also choose the appropriate units from the list that is displayed when you click on the drop-down arrow. The help curve determines the min and max values for the x-axis.

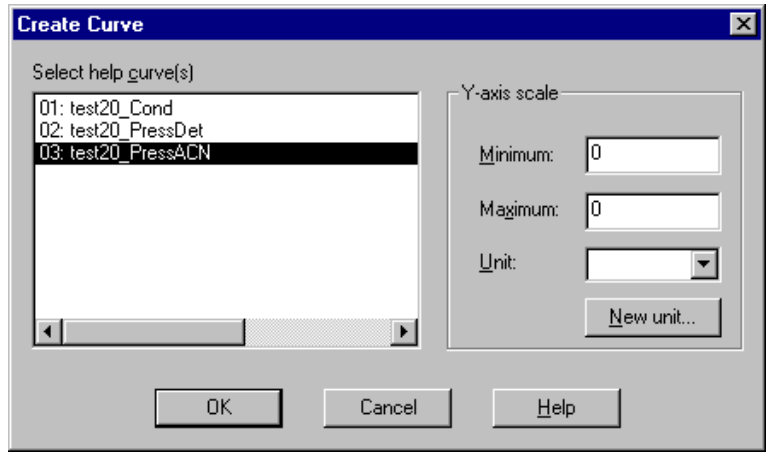


Figure 9-35. Create Curve dialogue.

3. If you want to create new unit, click on the **New unit** button and enter the new unit name and number of decimal places the values will receive.

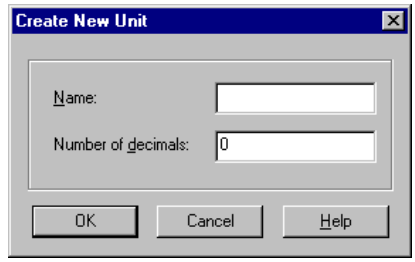


Figure 9-36. Create new unit dialogue.

Click on **OK** to return to the **Create curve** dialogue and again on **OK** when you have made your selections there.

4. With **Point Mode** selected, you can use the left button to insert new curve points on the chromatogram. The co-ordinates of each new point are automatically entered into the **Point list**.

   The co-ordinates of the mouse cursor are displayed beneath the curves thus allowing you to precisely position a new data point. The co-ordinates for the new curve are always displayed. Selecting **Zoom mode** allows you to use the mouse to select an area of the view and zoom in. The right mouse button menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**. In **Zoom mode** you can also drag the vertical cursor line and the co-ordinates presented reflect where the line bisects the curve of the same colour.

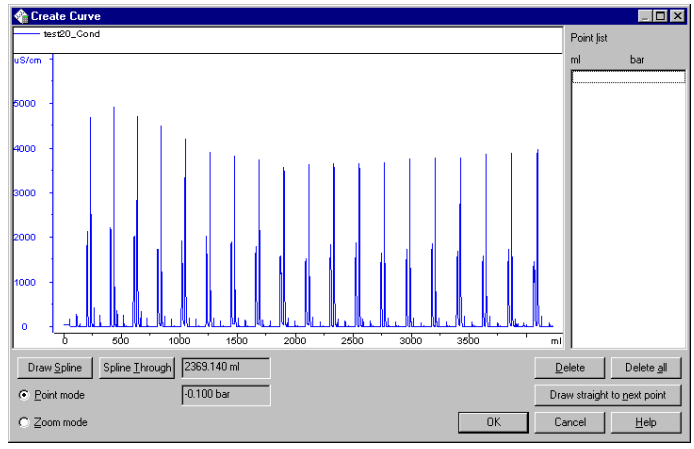


Figure 9-37. Create Curve chromatogram window.

5. To delete a point from the **Point list**, double click on the appropriate choice in the list. Alternatively, select the co-ordinates in the list and click on the **Delete** button. To delete all of the points in the list, click on the **Delete All** button.

6. To draw the curve, click on either **Draw spline** or **Spline through**. **Draw spline** creates a smooth curve from the data but does not necessarily pass through every point that you have entered. By contrast, **Spline through** creates a curve that passes through all of the data points.

7. In cases where you have created a curve using **Draw Spline**, you may want the curve to pass through a selection of those points currently lying away from the curve. You may force a straight line between two points by selecting the first of the two points in the **Point list** and then clicking on the **Draw straight to next point** button. This may have to be repeated for several consecutive points to achieve the desired curve.

8. Click on **OK** and save the curve. You can change the curve name from the default, **CreatedCurve**, and also the curve destination.

9.2.4 Measuring salt concentrations in the fractions

If you need to know the approximate conductivity or concentration of salt in your fractions, it is possible to calculate these from the conductivity curve.

Note: The conductivity signal is not linear above 0.3 M, but you will still gain a relatively good idea of the salt concentrations above 0.3 M.

A conductivity curve, usually given the name **Cond**, is stored in a chromatogram within a result file. This curve represents the real conductivity data in mS/cm and should be used for calculations. Another curve, **Cond%**, is also present and is the same as **Cond** but re-scaled to display percentage values.

To make the calculations:

1. Select **Operations:Fraction histogram**. Select the **Cond** curve in the left list and the fractions curve should already be selected in the middle list. If you have earlier pooled fractions, it is possible to select the desired fraction curve. Click on **OK**.

2. Select the appropriate fraction curve in the **Chromatogram Layout** dialogue. In the active chromatogram you will see the fraction

marks, the fraction histogram of the conductivity curve and any other selected curves. Double click on the **XY** box to display the vertical cursor line. Click on the desired curve legend and the corresponding y-axis is displayed.

3. Use the mouse to drag the vertical bar back and forth along the x-axis. For a given fraction, its conductivity is displayed in the active **XY** icon box.

9.3 Automated evaluation procedures

An evaluation procedure is a recorded sequence of interactive operations in the Evaluation module, which can be executed for automated data evaluation and report generation. It can be used for single chromatograms and for a number of chromatograms in different result files. The concept is analogous to the "macro" facility provided in many word processing and other programs. Evaluation procedures can also be called from methods, making run execution, evaluation and documentation fully automatic. Automation is achieved using the **Procedures** menu.

A procedure can be recorded and run using the **Procedures** menu commands or from the commands available in the **Procedure Editor** dialogue. The **Procedure Editor** dialogue also allows you to view and edit the instructions within a procedure. The evaluation module is locked during a batch run.

9.3.1 Recording a procedure

1. Open the appropriate results file in the Evaluation module.

2. To begin recording a procedure select **Procedures:Record on**. The **Procedure Editor** dialogue is displayed in Record mode.

3. Minimize the **Procedure Editor** dialogue.

4. Perform the evaluation steps that the procedure is to contain. These steps are recorded as you perform them.

5. To stop the recording, either:

   - select **Procedures:Record off**, or
   - restore the iconised **Procedure Editor** dialogue and click on the '**stop**' button or select the dialogue **Control:End Record** menu command.

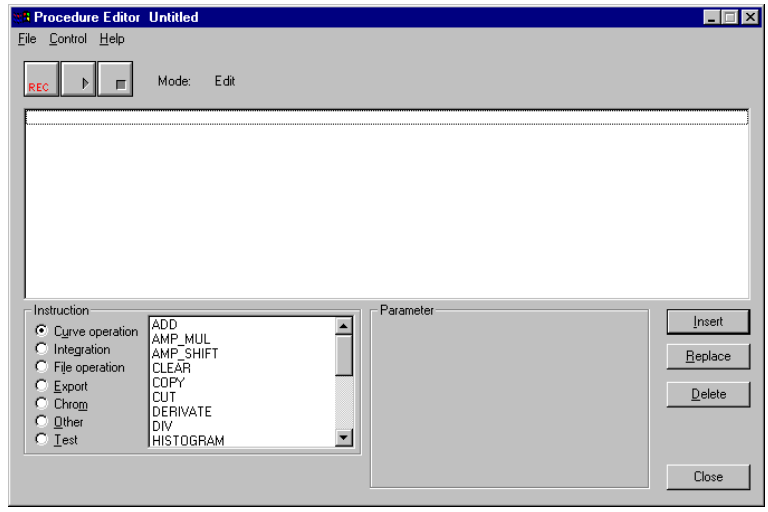


Figure 9-38. Procedure editor dialogue.

6. The recorded procedure can be viewed in the **Procedure Editor** dialogue. Restore the iconised dialogue if necessary.

7. More steps can be added to the evaluation procedure by repeating steps 2-6. The new steps are added to the previous procedure.

Note: New lines will be inserted into the procedure after the selected line in the currently listed procedure. This can be used to insert new instructions between existing instructions.

8. If required edit the evaluation procedure (see Section 10.3.2).

9. Select **File:Save** or **File:Save as** from the dialogue menu and enter a name for the procedure. The evaluation procedure is saved within UNICORN and is specific to your user name. If you have **Edit global list(s)** access you may also check the **Global procedure** option to make the procedure available to all users. Global procedures are marked with **[Global]** before the name. Even if the results of an evaluation session are not saved, the created evaluation procedure(s) are saved.

10. Choose dialogue **File:Exit** menu command.

Note: If you already have an existing procedure open for editing in the **Procedure Editor** dialogue (see Section 10.3.2) and you follow the above procedure, new instructions will be added to the currently open procedure, i.e. you will not be creating a new procedure.

To create a new procedure, select **File:New:Procedure** or **Procedure:Edit:New** to display the **Procedure Editor** dialogue. To begin recording, click on the **Rec** button or select the dialogue **Control:Record** menu command.

9.3.2 Editing an existing procedure

Evaluation operations are represented by instructions (see Appendix D.4) in the **Procedure Editor** dialogue. These may be modified to suit specific evaluation needs and be saved for later use.

Note: You are recommended to be restrictive in editing existing global procedures. It is recommended to open an existing procedure and save a copy under a new name and use this copy to perform any editing procedures.

1. Click on **Procedures:Edit:Open** and select the desired evaluation procedure from the list. The **Procedure Editor** dialogue is displayed for the selected procedure.

2. To view the parameters contained within a specific instruction in the procedure, select it and view the information contained within the **Instructions** and **Parameters** fields of the dialogue.

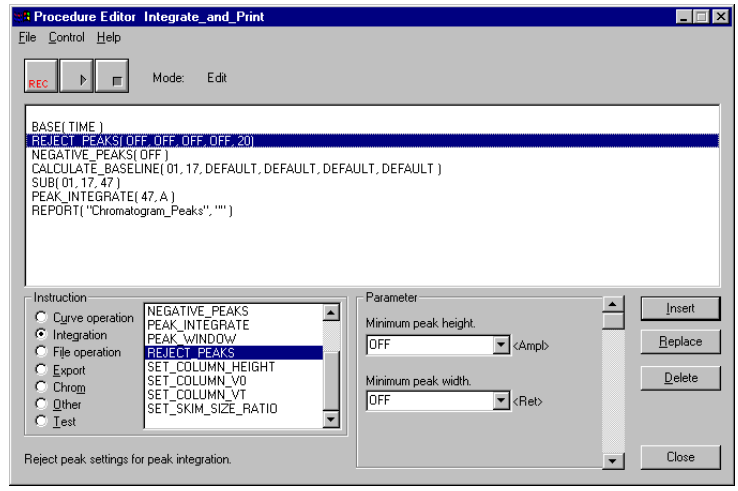


Figure 9-39. Editing procedures in the Procedure editor dialogue.

There are several types of instructions, as denoted by the options buttons, e.g. **Curve operation**, **Integration**, **File operation** etc., in the **Instructions** field. The appropriate option button and instruction therein will be automatically selected when you select an instruction in the procedure.

The specific parameters contained within the selected procedure instruction are displayed in the **Parameters** field.

A simple definition of the selected instruction is displayed at the bottom left-hand corner of the dialogue. You can also select an instruction and then press F1, or select **Help:Index:Instruction**. A list of procedure instructions with fuller descriptions and parameters can be found in Appendix D.3.

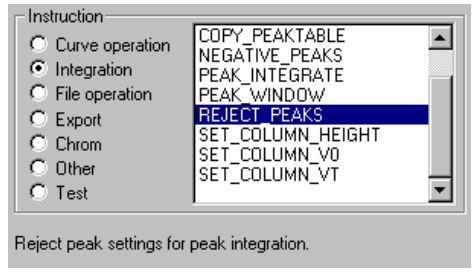


Figure 9-40. Instructions field in the Procedure editor dialogue.

3. To edit the parameters of a selected instruction, enter the new value in the appropriate place in the **Parameters** field. When you have made the desired changes, click on the **Replace** command button. You will now see that the selected instruction in the evaluation procedure is updated in accordance with the new parameters assigned to it.

4. You can insert new instructions *after* the currently selected procedure instruction. Select an instruction type and instruction in the **Instructions** field and enter the appropriate parameter values in the **Parameters** field. Click on the **Insert** command button. The new instruction will now be added to the evaluation procedure.

5. To remove an instruction from the evaluation procedure, select it in the procedure listing and click on the **Delete** button.

6. To save the edited procedure select **File:Save** or **File:Save as** from the dialogue menu commands. Name the procedure.

7. Choose **File:Exit** from the menu bar in the dialogue.

9.3.3 Renaming and removing procedures

Procedures that you have created can be renamed or deleted from the list of available procedures.

Note: It is recommended that you exercise caution in renaming and removing existing global procedures.

Renaming a procedure

1. Select **Procedures:Edit:Rename**.
2. Select a procedure in the **Rename Procedure** dialogue.
3. Click on **OK**.

Removing a procedure

1. Select **Procedures:Edit:Delete**.
2. Select a procedure in the **Delete Procedure** dialogue.
3. Click on **OK**.

9.3.4 Points to watch

In recording and editing evaluation procedures for automatic evaluation, beware of the following potential pitfalls:

- Make sure that the procedure addresses the right curves. Curves are identified by storage position alone: thus the instruction ADD (01,02,03) will try to add curve 01 to curve 02 and store the result in 03, regardless of the contents of 01 and 02. If 03 contains a curve which is not a raw data curve, the existing curve in 03 will be overwritten. If 03 contains a raw data curve, the procedure will stop with an error message. The raw data curves will always occupy the same positions for a given strategy, e.g. UV in position 01. If the operation is not valid when the procedure is run, the procedure will stop at the instruction with an error message. Any subsequent instructions in the procedure will not be executed.
- In calculating a baseline using the “classic” algorithm, UNICORN suggests default values for the four control parameters (see Section 10.1.2) based on the appearance of the curve. To instruct UNICORN to use default values appropriate for the curve every time the procedure is run, choose the Default setting in the appropriate fields for the parameters. For example;

CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)

can be changed to:

CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)

Similarly for baselines calculated using the **Morphological** algorithm:

CALCULATE_BASELINE_MORPH(01, 06, XXX, XXX, XXX, XXX)

can be changed to:

CALCULATE_BASELINE_MORPH (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)

9.3.5 Running evaluation procedures

To run a procedure for a specific chromatogram, first make sure that the desired chromatogram is active. Click on **Procedures:Run** and choose the desired evaluation procedure. Click on **OK** and the procedure runs at once. You can also open the **Procedure Editor** dialogue and select the dialogue **Control:Run** menu command or click on the 'Play' button.

9.3.6 Batch runs

It is possible to apply an evaluation procedure to a designated batch of result files even if they are not open on the Evaluation workspace. It is especially useful, for example, to perform integration with the same parameter settings on many results, or to print a number of results with the same settings. The batch run is done in the background of the Evaluation module and thus the results of the run are not seen. You will, of course, receive any print-outs or report documentation if this was one of the steps in the run procedure (see Section 9.5.3).

1. Select **Procedures:Batch run** and the **Open Procedure** dialogue is displayed. Select the evaluation procedure and click on **OK**. The **Batch Run** dialogue, which allows you to search for the result files and/or chromatograms on which you wish to perform the batch run.

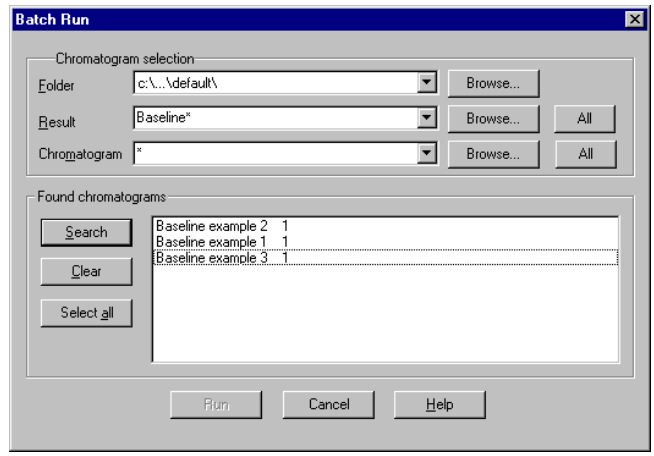


Figure 9-41. Batch run dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. By default, all chromatograms are searched for within the designated folder and result files, as denoted by the asterisk '*****'. As for result files you can select a specific result file using the **Browse** function or you can use wildcard characters to search for chromatograms with a specific name profile.

   You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

   User-entered search filters (up to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used as a search delimiter (by inserting a ';' between strings). Search filters are automatically saved and stored within user profiles.

To return to the default setting to search for all chromatograms, click on **All**.

4. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria.

5. Select the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected. If you want to clear the list of displayed chromatograms, click on **Clear**.

6. Click on **Run** to perform the batch run. Any created curves and peak tables will be saved in each result file automatically.

7. To view the results of a batch run on a specific result/ chromatogram, open this in the Evaluation workspace.

Note: If you include the currently opened result file in the batch run, it will stop temporarily and you may exclude this result file from the batch run. If you keep the current result file in the batch run, the result file will be saved and then re-opened to ensure that the contents of the Evaluation workspace reflects the results of the evaluation procedure. The subsequent result files in the batch run will always be processed automatically.

9.3.7 Evaluation procedures and reports

The creation of evaluation procedures, combined with batch runs, is a very useful tool to produce printed documentation simultaneously for many result files. This removes the necessity to open/import result files onto the Evaluation workspace.

1. Begin recording a new procedure by selecting **Procedures:Record on**.

2. Select **File:Report** and choose a report format (see Section 9.5.3).

3. Select **Print** in the **Generate Report** dialogue as the final instruction.

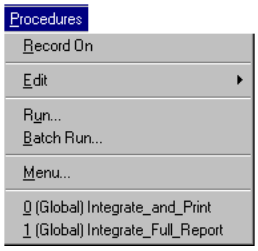
4. Stop the record function by selecting **Procedures:Record Off**.

5. Save the procedure.

6. Now do a batch run (see Section 10.3.5) on all the desired result files to get the printed reports. The procedure can also be saved with a method to get automatic printouts at the end of a run (Section 5.6.5).

Note: If the selected report format is changed in **File:Report** the new format will be applied when the procedure is run (except in cases where the procedure has been imported to a method - in these cases, the procedure is saved in the method file and cannot by changed). If the format is subsequently deleted, the procedure cannot be run.

9.3.8 Placing a procedure on the menu and running



It is possible to choose a maximum of 15 created evaluation procedures to be placed onto the **Procedures** menu.

Select **Procedures:Menu** and select the evaluation procedure to be added to the menu. Click on **OK**. Activate a chromatogram and select the **Procedures** menu. You will see the procedure that you added to the menu. Select this procedure and it is automatically run for the active chromatogram. The menu addition is remembered in UNICORN even if the results of the current evaluation session are not saved. To take away a procedure from the menu, deselect it from the **Procedures:Menu** list.

9.3.9 Exporting data or curves

Data and curves can be exported to other file formats. Select **File:Export** and then the appropriate cascade menu choice. Alternatively, the **Edit:Copy to clipboard** function can be used, which is the quickest and easiest way to copy a chromatogram into, for example, a spreadsheet application software.

9.3.10 Exporting results

Data can be exported to ASCII (text), and Lotus 1-2-3 (.wks or .xls) spreadsheet formats. Select the format that best matches your other application software. Peak tables are exported as text strings in the ASCII format, but as numerical values in the Lotus 1-2-3 (.wks or .xls) format. When exporting peak tables, all possible columns in the table are exported.

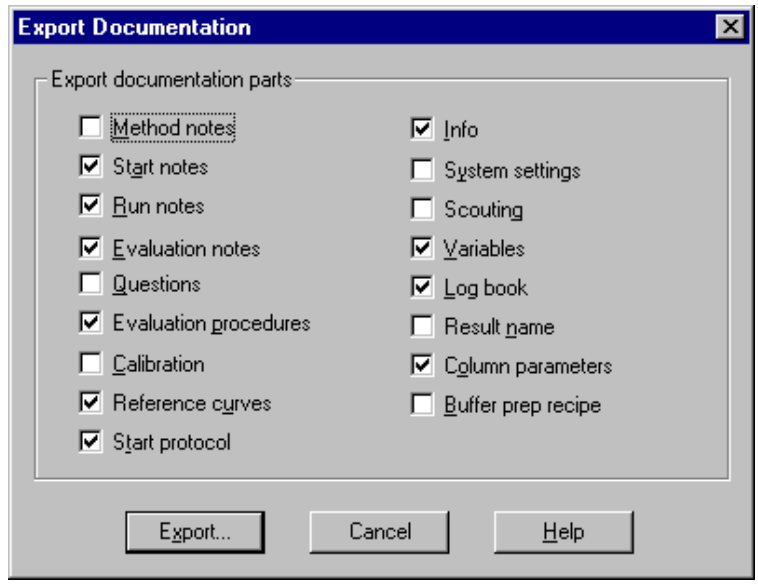


Figure 9-42. Export Documentation dialogue.

Curves are exported as two series of numerical co-ordinates referring to time/volume and signal respectively. Multiple curves can be exported in each file. When exporting a curve, you may choose to export only a portion of the curve by inserting the limiting retention values directly into the boxes in the **Cut curve** field or by visually selecting the part of the curve using the **Cut graphically** option. To optimise the size of exported files, you may want to reduce the number of data points that are exported.You can do this by clicking on the **Max no. samples** box, and then adjusting the number of data samples up or down. In addition, the **Reduce by factor** function decreases the frequency with which data points are sampled for export. For example, reducing the number of data points by a factor of five will export every fifth point to the file.

Pressing the **Export** button will then prompt a dialogue where you can choose the new file name and destination. The default name is the same as the name of the current file. The extension of the exported file will be .asc, .wks, or .xls, depending on the file format chosen.

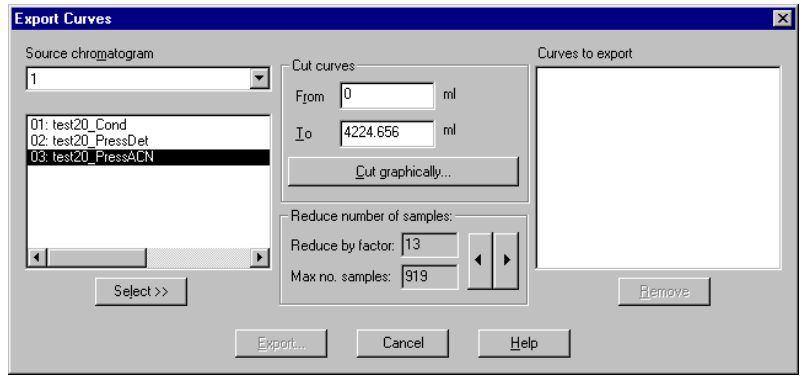


Figure 9-43. Export Curves dialogue.

9.3.11 Copying results to the clipboard

A related activity is **Edit:Copy to Clipboard** where the contents of the active window are copied to the Windows clipboard and can be later easily pasted into other programs, e.g. Microsoft® Word™. Curves and Documentation are copied as Windows enhanced metafiles (.emf), while Peak tables are copied as text. In the latter case, only the columns selected in the **Chromatogram Layout** dialogue will be copied.

9.3.12 Importing results and curves

You may import curves in ASCII (text) and Lotus 1-2-3 (.wks or .xls) spreadsheet formats, and results in SMART Manager and FPLCdirector formats. Select **File:Import** and then the appropriate menu choice. A new dialogue will then prompt you to choose the source folder and file. The imported curve or result file is automatically opened to the screen.

Introductory material

Methods and runs

Evaluation

System management

Appendices

10 Security features

This chapter considers security features in UNICORN, under the topics

- access security
- system connection security
- data security
- security recommendations for control stations

10.1 Access security

Operation of UNICORN is restricted to authorised users, defined in the system by the system administrator (see Section 14.3). User access may be protected by a password. The minimum password length for users is defined when UNICORN is installed (see Section 13.4). In installations where access security is important, passwords should be enforced and should be changed regularly (see Section 14.3.2).

Each user is assigned an access level and a folder access profile, defining the operations that the user can perform and the folders the user is allowed to access (see Section 14.3). For system security, administrative routines such as user and system definition should be permitted only to the system administrator.

Installation of UNICORN establishes a default user with full access rights in the system. It is important for system security that this user is deleted when the site-specific users have been created.

10.2 Connection security

To prevent conflicts in system control requests, each system in an installation may have only one control mode connection at any one time.

A running system may be left in a view mode connection and locked with a password (independent of the user's logon password) to prevent other users from establishing a control mode connection and interfering with controlling the synthesis process. If the system is left unlocked in a view mode connection, any user may establish a control mode connection to the system.

We recommend that systems are always locked when a user leaves the system. For controlled and locked systems, the responsible user is identified in the system control window for view mode connections. A system which is left unlocked with no control mode connection has no identified responsible user.

Systems may be locked even when they are idle, to allow users to reserve a system for later use.

10.3 Data security

10.3.1 Network communication failure

If the network communication fails while a method is running, any control mode connection on a remote station will lose control of the system and results destined for network drives cannot be saved in their correct folders. In this event, the run continues under control from the local station. Results are saved on the local station with the original result file name in the folder DRIVE:\Unicorn\Local \Fil\Failed, from which they can be retrieved after the run is complete. To retrieve results from the Failed folder:

1. Start UNICORN (if it is not already started) on the local station where the synthesis process was run.

2. Log on as a user with access to the Failed folder.

3. You can now open the result file in the evaluation module and move it to a suitable location on the network server when network communication is re-established so that it is accessible from remote stations.

Note: Result files are saved directly under the Failed folder, and are identified by result file name. Files with the same result file name base are distinguished by an incremental serial number, in the same way as result files in any other folder.

Access to the Failed folder

The system administrator may choose one of two policies concerning access to the Failed folder:

- Grant access to the Failed folder to all users, so that each user can retrieve his or her own result files. This places the responsibility for retrieving result files and deleting old files from the Failed folder on the individual users. Note that with this policy any user will be able to examine, copy, move and delete the other users'

results in the Failed folder. This policy has the advantage that the Failed folder can conveniently be used to temporarily store methods and results from runs performed from the local station when the network is not running.

- Grant access to the Failed folder only to one or a few users, who share the responsibility for retrieving results files and deleting old files from the Failed folder. This policy should be used if the installation requires restricted access to users' result files. Note that the user(s) with access to the Failed folder should also have access to other users' home folders, to be able to copy or move result files to suitable destinations.

In granting access to the Failed folder, it is sufficient to grant access to DRIVE:\Unicorn\Local\Fil\Failed, since this is the path to the Failed folder on each local station.

10.3.2 Local station failure

If the local station fails during a run, the run may continue but the results generated after the failure cannot be saved. An autosave feature saves a temporary result file on the local station during runs at a pre-set interval defined in the system definition (see Section 14.1). When the local station is restarted, the temporary result file will be transferred to the original result file destination (or to the Failed folder if the original destination was on a network drive which is not currently available). The result file will contain the results of the run up to and including the last autosave time before the failure. Results after this time will be lost.

10.4 Security recommendations for control stations

Oligosynthesis systems may be controlled without running the user interface modules. In a network installation, this situation can arise if a system is controlled from a remote station without starting UNICORN on the local station. In a stand-alone installation, the situation can arise if a user quits UNICORN after starting a run. In both cases, it is not apparent from the desktop that UNICORN control software is actually running, and there is therefore a risk that Windows NT may be shut down and the computer turned off in the belief that it is not in use. To prevent this:

- Do not quit UNICORN if you are controlling a system.
- Do not turn off local station computers in a network installation.
- If possible, start UNICORN application program on all local stations in a network installation and establish a view mode connection, as an indication that a connected system might be running.

11 Network setup

UNICORN 3.10 can be installed in a network environment, allowing synthesis systems to be controlled from any PC in the network where UNICORN is installed. This chapter describes how to set up the network environment before installing UNICORN. This chapter is only relevant if you will be running UNICORN to control synthesis systems remotely.

The Network setup should be performed by someone with experience in Windows NT and network installations.

11.1 Introduction

It is important to understand the basics of how UNICORN operates in a network setup to ease the installation. A good start is to read the following explanations of some of the aspects of UNICORN in a network setup.

Explanation of some of the aspects of UNICORN in a network:

Storage of data	Methods and log files are stored in a folder shared between the local and the remote UNICORN.
Communication	The remote and the local UNICORN use named pipes to send commands and data between them.
Log files in a network setup	While running, the local UNICORN system writes logs on the local harddisk. When the run is over it copies them to the network drive where log files are stored.
Named pipes communication	From the remote UNICORN commands are sent, such as "run method foo.met". From the local UNICORN messages and trend data is sent to the remote UNICORN.
Network failure in the middle of a run	The local UNICORN will continue the run and the log file will be on the local harddrive when the run is over.
Access to the network drive while running	When a method is begun the method is copied from the network drive to a local directory and it is read from there during the run.

Server in a UNICORN network setup:	UNICORN requires only a directory for log files and method templates accessible by both the local and the remote UNICORN. It is generally a good idea to use a directory on an NT server for easy backup.

The different network setups that UNICORN has been tested in are the following:

- NT domain with TCP/IP
- NT workstations connected to a Novell server

Note: The same version of UNICORN must be installed on both the computers with the synthesis system and the remote control computers. Mixing different versions will not work.

Establishing and maintaining a networked UNICORN installation requires some understanding of the working of the NT operating system and the concept of NT domains if used. We recommend that a competent network administrator that will also be involved in the installation of UNICORN software, should maintain the network.

The network administrator is not necessarily the same person as the system administrator for UNICORN. Once the network is set up, network functions are entirely transparent to UNICORN users, and the network administrator does not need to understand the use of UNICORN for controlling synthesis systems.

Security is very important in a networked UNICORN installation. Three aspects of security can be distinguished, and the responsibility for maintaining security is shared by the network administrator and the system administrator for UNICORN:

	Network administrator	*UNICORN administrator*
Data storage security (back-up routines)	Back-up routines for server and local disks.	Controlled user access to home and shared folders, e.g. placing all home folders on a shared disk to prevent data from being scattered throughout the network.
Network access security	Maintenance of user passwords and access rights to shared resources.	-
UNICORN access security	-	Maintenance of user profiles

PC's with UNICORN software can be categorised as follows:

- A local station is a PC to which synthesis systems are physically connected. UNICORN software must be installed on all local stations in the network.

- A remote station is a PC to which often no systems are physically connected, but which can control systems over a network link. UNICORN software is installed with the **Remote Only** option on remote stations.

Synthesis systems in the network can be controlled from either remote or local stations. A local station in a network can also be used as a remote station to control other systems.

Note: If a run is being controlled from a remote station and a network communication error occurs, the run will continue under the control of the local station. Results will be saved in the Failed folder on the local station. A control mode connection can be established on the local station to control the run.

For a synthesis system to be accessible in the network, the local station must be switched on and logged on to the network. The user interface for UNICORN does not however need to be started on the local station. System control from a remote station is managed through network level routines, which are started at log on time on the local station.

A local station can be used to control the synthesis system directly connected to the PC without logging on to the network. (The station must however have been logged on to the network once previously so that the necessary files are copied from the network server to the local computer.) This copying is performed automatically. Method and result files stored on the network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the Failed folder on the local station.

Note: It is still required that the user log on to the local Windows NT station using a valid user ID.

11.2 Requirements

The following are minimum network requirements for running UNICORN in a network installation:

- Windows NT workstation version 4.0.
- 3Com Etherlink III PCI network card or a Compaq NetFlex-3 network card.
- A valid network connection.
- The user right “Access this computer from network”.
- Protocols and services such that named pipes are usable over the network.
- Protocols and services such that folders can be connected to a drive unit.

The two last points are satisfied if the services; Server and Workstation are installed and one of the protocols TCP/IP or NWLink IPX/SPX.

User rights

The requirement in the above list regarding user right is satisfied if a default installation of the network is performed. The default is for everyone to have the user right “Access this computer from network”. However, if this is nor the case then the user rights should be set by a person with administrative privileges on the NT workstation using the following steps:

1. Select **Start:Programs:Administrative Tools (Common):User Manager**.
2. Select **Policies:User rights** from the dialogue menu.
3. Select **Access this computer from network** from the drop-down list.
4. Click on the **Add** button.
5. Select the group or person(s) who will get this right. It should be a group within which the person logged on to the local UNICORN station is a member, or that person is specifically selected.
6. Click on the **OK** button.

11.3 Installation guide

11.3.1 TCP/IP - NT domain

This is an example of how to setup the network so that UNICORN may run across the network when the network protocol is TCP/IP and there is an NT domain controller. The setup of the NT domain controller is not described here; a competent network administrator is generally needed to setup an NT domain server.

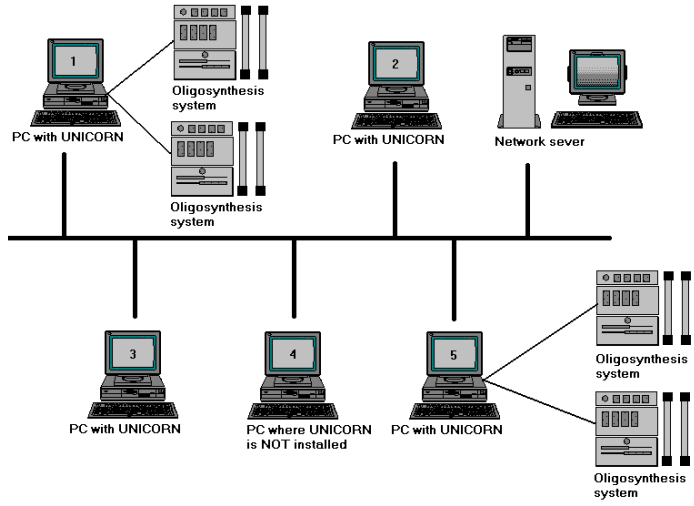


Figure 11-1. A network installation with 4 synthesis systems and 5 workstations (PCs). The synthesis systems physically connected to PCs 1 and 5 can be controlled locally. Alternatively, any of the PCs with UNICORN installed can be used to remotely control any of the synthesis systems via the network. In this example, PC 4 is connected to the network but it cannot be used to control any synthesis systems since it does not have UNICORN installed. Note also that the server does not have UNICORN installed and is not involved in the control process *per se*.

Creating user groups and users of UNICORN

This is performed by the network administrator:

1. Log on to the NT domain controller with administrative rights.

2. Enter the **User Manager** for the domain by selecting Start:Programs:Administrative Tools (Common).

3. Create a group that all UNICORN users will be part of. Select the **User** menu and the **New Local or Global Group** item.

4. In the dialogue that is displayed, enter a suitable name for the group and click on **OK**.

5. Create the users that will run UNICORN. Select the **User** menu and the **New User** item.

6. In the dialogue that is displayed, enter user name (maximum 20 characters and must be unique within the domain), description and password. Click on the **Groups** button.

7. In the dialogue that is displayed, select the newly created group of UNICORN users and click on the **<- Add** button. Finally click on the **OK** button in the **Group** membership dialogue.

8. Click on the **OK** button in the **New User** dialogue and the user is created.

9. When all users have been created, click on **Close** in the **New User** dialogue.

Sharing a folder

This is performed by the network administrator:

1. Log on to the NT computer designated to be the UNICORN file server with administrative rights.

2. Start Windows NT Explorer with Start:Programs:Windows NT Explorer.

3. Open the right mouse button menu for the folder that will serve as the UNICORN server disk. Select **Sharing**.

4. In the dialogue that is displayed, click on the **Shared As** radio button and enter a suitable share name, e.g. UNICORN. This name will be used when connecting to this folder.

5. Set up the permissions for the shared folder by clicking the **Permissions** button. Select **Everyone** in the list box and click on the **Remove** button. Click on the **Add** button and in the dialogue that is displayed make sure that the domain is selected in the top drop-down list. Select the newly created user group in the list and click on the **Add** button and finally select **Full Control** in the bottom drop-down list and click on the **OK** button.

6. Select **OK** to accept Share in the **Type of Access** dialogue.

Installing TCP/IP on the computer

This is performed by the network administrator:

1. Open the control panel with Start:Settings:Control Panel.

2. Double click on the **Network** icon.

3. A question will be displayed asking if you want to install Windows NT Networking. Click on **Yes**.

4. In the next dialogue select **Wired to the network** and click on **Next**.

5. Specify the network adapter to use by clicking on **Select from list**.

   If your adapter is shown in the displayed list, select it and click on **OK**.

   Alternatively click on **Have disk** and insert the disk with the latest version of your driver. Enter the path to it in the dialogue and click on **OK**.

6. Click on **Next**.

7. Make sure your selected adapter is shown in the list and click on **Next**.

8. Select **TCP/IP** as the protocol to use.

9. Click on **Next**.

10. Select all default the services and click on **Next**.

11. Configure the TCP/IP protocol. Information specific to the network will have to be entered. This information should be supplied by the network administrator. Click on **Next** when ready.

12. Click on **Next** again to install the selected components.

13. Insert the Windows NT CD-ROM disk and enter the path to it in the dialogue (Example: **D:**). Click on **Continue**.

14. A dialogue is displayed, displaying where the files will be read. Click on **Continue**.

15. The bindings are displayed. Click on **Next** to continue.

16. Click on **Next** to start the network.

17. On the primary Domain Controller, run Server Manager by selecting Start:Programs:Administrative Tools:Server Manager.

18. From the menu select **Computer** and then the **Add to Domain** item.

19. Select the **Windows NT** or **Server** radio button and enter the NT workstation name in the **Computer Name** field. Click on **Add**. The computer is added to the domain.

20. On the Windows NT workstation select the **Domain** radio button and enter the name for the domain. The network administrator should supply this name. Click on **Next** to continue. You will be welcomed to the domain.

21. Acknowledge and click on **Finish**.

22. Select the option to restart your computer now. The computer is restarted.

23. Log on to the Domain with administrator rights.

Installing TCP/IP on the computer

This is relevant when network has been installed earlier.

1. Log on to the computer with administrator rights.

2. Open the control panel with Start:Settings:Control Panel.

3. Double click on the **Network** icon.

4. In the **Network** dialogue, enter the **Adapters** tab and click on the **Add** button.

5. In the dialogue that is displayed select the correct driver for your network adapter and click on the **OK** button. Click on the **Have disk** button and insert the disk with the latest version of your driver and enter the path to it in the dialogue.

6. In the **Network** dialogue, enter the **Services** tab and click on the **Add** button.

7. In the dialogue that is displayed, select **Server** and click on the **OK** button.

8. Click on the **Add** button again.

9. In the dialogue that is displayed, select **Workstation** and click on the **OK** button.

10. In the **Network** dialogue, enter the **Protocols** tab and click on the **Add** button.

11. In the dialogue that is displayed, select **TCP/IP Protocol** and click on the **OK** button.

12. In the **Network** dialogue click on the **OK** button. When the window is writing this new data to the hard disk the configuration dialogues for the TCP/IP protocol will be displayed. Information specific to the network must be entered. This information should be supplied by the network administrator.

Installing UNICORN

1. Start Windows NT Explorer with Start/Programs:Windows NT Explorer.

2. Connect the shared folder on the UNICORN file server with the designated drive letter. You can do this by selecting Tools:Map Network Drive).

3. In the dialogue that is displayed select the drive letter in the upper drop-down list.

4. If the current user ID does not have permissions to the shared UNICORN folder on the server, enter a valid domain user ID in the **Connect As** field.

5. In the **Shared folders** list at the bottom of the dialogue the network can be seen, double click on the server with the shared folder.

6. Available shared folders on the selected server are displayed. Double click on the shared folder UNICORN will use.

7. A dialogue appears with a request to give a valid user name and password. The user name will be the ID previously entered in the **Connect As** field. Give the password and click on **OK**.

8. Install UNICORN (see Chapter 13), select **Network** version.

9. Re-boot the PC and log on as one of the domain users that will run UNICORN.

10. Connect the shared folder again, this is necessary since each user has his own connected network drives. Repeat steps 1, 2, 3,5 and 6 above and make sure the **Reconnect at Logon** option is checked.

11. Start UNICORN and set up system definitions and user profiles (see Chapter 13).

11.3.2 IPX/SPX - Novell server

This is an example of how to set up the network so that UNICORN can run across the network when the network protocol is IPX/SPX and uses a Novell server.

The general network organisation is described in the following picture.

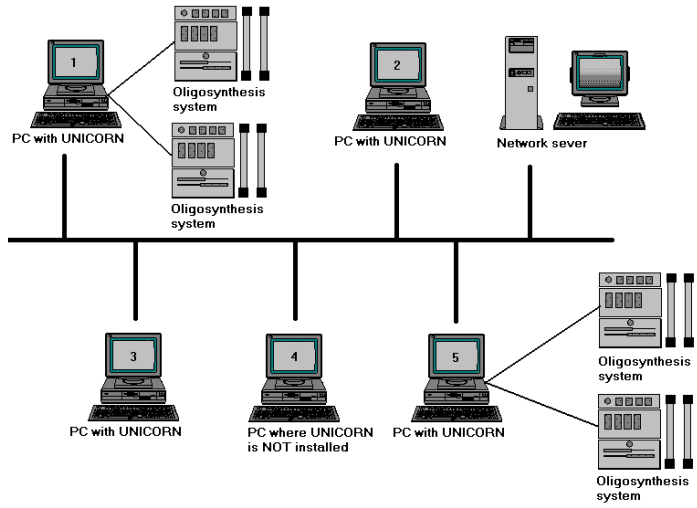


Figure 11-2. General network organisation based on a Novell network controller.

The setup of the Novell server is not described here; a competent network administrator is normally needed to setup a Novell server.

Creating user group and users of UNICORN on the Novell server

This is performed by the network administrator.

The network administrator creates the users that will be running UNICORN on the Novell server. The procedure for this is not described here.

Sharing a folder

This is performed by the network administrator.

The network administrator creates shared folder on the Novell server which UNICORN will use for shared data. The procedure for this is not described here.

Creating user group and users of UNICORN on the local computers

1. Log on to the client computer with administrator rights.

2. Enter the **User Manager** with Start:Programs:Administrative Tools (Common):User Manager.

3. Create the users that will run UNICORN. Select the **User** menu and the **New User** item.

4. In the dialogue that is displayed, enter the user name (the UNICORN users created by the network administrator), description and password (enter the password the user has in the Novell network). Make sure that the **Password Never Expires** option is checked and that the **User Must Change Password at Next Logon** option is not checked.

5. Click on **OK** in the **New User** dialogue and the user is created.

6. When completed, close **User Manager**.

Installing IPX/SPX on the computer

1. Open the control panel with Start:Settings:Control Panel.

2. Double click on the **Network** icon.

3. A question will be displayed asking if you want to install Windows NT Networking. Click on **Yes**.

4. In the next dialogue select **Wired to the network** and click on **Next**.

5. Specify the network adapter to use by clicking on **Select from list**.

   If your adapter is shown in the displayed list, select it and click on **OK**.

   Alternatively click on **Have disk** and insert the disk with the latest version of your driver. Enter the path to it in the dialogue and click on **OK**.

6. Click on **Next**.

7. Make sure your selected adapter is shown in the list and click on **Next**.

8. Select **NWLink IPX/SPX Compatible Transport** as the protocol to use. Deselect **TCP/IP**.

9. Click on **Next**.

10. Select all default the services and click on **Next**.

12. Select **Client Service for NetWare** and click on **OK**.

13. Click on **Next**.

14. Click on **Next** again to install the selected components.

15. Insert the Windows NT CD-ROM disk and enter the path to it in the dialogue (Example: **D:**). Click on **Continue**.

16. A dialogue is displayed, displaying where the files will be read. Click on **Continue**.

17. The bindings are displayed. Click on **Next** to continue.

18. Click on **Next** to start the network.

19. Select the **Workgroup** radio button and enter a name for the workgroup. The network administrator should supply this name, click on **Next** to continue.

20. Click on **Finish**.

21. Remove the CD-ROM disk.

22. Select the option to restart your computer now. The computer is restarted.

23. Log on with administrator rights.

24. The **Select NetWare Logon** dialogue is displayed. The network administrator must supply the **Default Tree and Context** to use. Select the **Default Tree and Context** radio button, enter the information from the network administrator and click on **OK**.

25. Since the name and password of the user you logged on as are not a valid account on the Novell server, a message will be displayed after a 30 second timeout, **NetWare Authentication Failure**. Click on **Yes** to keep the default tree and context.

Installing UNICORN

1. Start Windows NT Explorer with Start/Programs:Windows NT Explorer.

2. Connect the shared folder on the UNICORN file server with the designated drive letter. You can do this by selecting Tools:Map Network Drive).

3. In the dialogue that is displayed select the drive letter in the upper drop-down list.

4. Enter a valid domain user ID in the **Connect As** field.

5. In the **Shared folders** list at the bottom of the dialogue the network can be seen, double click on the Novell server with the shared folder.

6. Available shared folders on the selected server are displayed. Double click on the shared folder UNICORN will use.

7. A dialogue appears with a request to give a valid user name and password. The user name will be the ID previously entered in the **Connect As** field. Give the password and click on **OK**.

8. Install UNICORN (see Chapter 13), select **Network** version.

9. Re-boot the PC and log on as one of the users that will run UNICORN.

10. The **Select NetWare Logon** dialogue will be displayed. The network administrator must supply the **Default Tree and Context** to use. Select the **Default Tree and Context** radio button, enter the information from the network administrator and click on **OK**.

11. Connect the shared folder again, this is necessary since each user has his own connected network drives. Repeat steps 1, 2, 3,5 and 6 above and make sure the **Reconnect at Logon** option is checked.

12. Start UNICORN and set up system definitions and user profiles (see Chapter 13).

12 Installation

This chapter describes how to install UNICORN software.

12.1 Installation summary

The following installation procedures are required before UNICORN systems can be used:

1. Back up files if migrating from UNICORN OS 1.10to UNICORN 3.10.
2. Set up the network environment (for network installations only).
3. Install UNICORN hardware and software.
4. Define access levels for the installation.
5. Define users with home folders and access profiles.
6. Check the system settings for the attached systems.

12.2 Migrating from UNICORN OS 1.10 to UNICORN 3.10

12.2.1 Before migration

Caution: Before commencing with the migration from UNICORN OS 1.10 to UNICORN 3.10, ensure that all method files, MethodQueue files and result files are backed up on separate drive or medium and are separate to the UNICORN folders used on the network. When you access these files for the first time following installation of UNICORN 3.10, these will be reformatted so that they can be used by the new system.

- Document your user setup, access rights and folder structure so that they can be redone in the new version.
- Print out the system settings and calibration constants.

12.2.2 Migration and post-installation setup

1. Install UNICORN 3.10.

2. Install strategies either from diskette supplied with the original system using the **Have Disk** option, or by selecting from the standard systems supplied in the installation CD-ROM.

3. Set up the users, folders and access rights

4. Recalibrate the system.

5. Enter system settings.

6. Copy the methods and results you want using **Copy from External**.

7. If you are using multiple systems you might need to associate your copied files with a system in this process.

12.3 System requirements

For the hardware, software and network requirements, please refer to Appendix A.

12.4 Hardware installation

In most cases, an installation of your system will be performed by Amersham Biosciences authorised personnel. If your system is not pre-installed, then follow the steps below to install the expansion card in your PC. This procedure is only required in stand-alone installations, i.e. systems not connected to a network, and on local computers in network installations.

1. Turn off the power to your PC and remove the power cable from the mains socket.

2. Open the PC cover. Refer to your PC documentation if you are not sure how to do this.

3. Locate an empty full length expansion slot.

4. Take the expansion card out of the anti-static bag. Handle the card by its edges, and avoid touching the electronic components as far as possible, because discharges of static electricity can permanently damage electronic components on the card. If you are working in a room where static electricity tends to build up, discharge any

electricity from your body by touching an earthed metal surface (e.g. a water tap or radiator) before handling the card.

5. Two settings that are made on the expansion cards are for interrupt (IRQ) and address. These settings must be entered in the UNICORN software installation so that UNICORN can find the systems when started. To see which settings the card has, look at the card and compare it with the tables below. The IRQ is set by a jumper and the address is set with a dip-switch. Write down the settings on a paper for future use.

   When you are installing the UNICORN software and the setup program asks for the card settings, enter the correct values for IRQ and address. If for some reason the default settings can not be used on a specific computer, for example there is a conflict with another device that it already installed, the settings have to be changed.

   CU 900 (Oligosystems)

   Alternative IRQs for this card are 3, 4, 5, 7, 10, 11, 12 and 15. The manufacturing default setting is IRQ10.

   The address is made up of two parts, the base address and the node address. Valid base addresses for this card are any hex address between 0 and FF0, the node address can be altered between 1-4. The manufacturing default setting should be updated to reflect the current default for the address 100 (base address 100 and node address 1). Note that this is computer dependent. To change the settings for the address, refer to the tables

Node address	*Offset*	*S1:7*	*S1:8*
1	0	ON	ON
2	400h	OFF	ON
3	800h	ON	OFF
4	C00h	OFF	OFF

Base address	*S1:1*	*S1:2*	*S1:3*	*S1:4*	*S1:5*	*S1:6*
100 + offset	*ON*	*ON*	*ON*	*ON*	*OFF*	*ON*
110 + offset	*OFF*	*ON*	*ON*	*ON*	*OFF*	*ON*
120 + offset	*ON*	*OFF*	*ON*	*ON*	*OFF*	*ON*
130 + offset	*OFF*	*OFF*	*ON*	*ON*	*OFF*	*ON*
140 + offset	*ON*	*ON*	*OFF*	*ON*	*OFF*	*ON*
150 + offset	*OFF*	*ON*	*OFF*	*ON*	*OFF*	*ON*
160 + offset	*ON*	*OFF*	*OFF*	*ON*	*OFF*	*ON*
170 + offset	*OFF*	*OFF*	*OFF*	*ON*	*OFF*	*ON*
180 + offset	*ON*	*ON*	*ON*	*OFF*	*OFF*	*ON*
190 + offset	*OFF*	*ON*	*ON*	*OFF*	*OFF*	*ON*
1A0 + offset	*ON*	*OFF*	*ON*	*OFF*	*OFF*	*ON*
1B0 + offset	*OFF*	*OFF*	*ON*	*OFF*	*OFF*	*ON*
1C0 + offset	*ON*	*ON*	*OFF*	*OFF*	*OFF*	*ON*
1D0 + offset	*OFF*	*ON*	*OFF*	*OFF*	*OFF*	*ON*
1E0 + offset	*ON*	*OFF*	*OFF*	*OFF*	*OFF*	*ON*
220 + offset	*ON*	*OFF*	*ON*	*ON*	*ON*	*OFF*
230 + offset	*OFF*	*OFF*	*ON*	*ON*	*ON*	*OFF*
240 + offset	*ON*	*ON*	*OFF*	*ON*	*ON*	*OFF*
250 + offset	*OFF*	*ON*	*OFF*	*ON*	*ON*	*OFF*
260 + offset	*ON*	*OFF*	*OFF*	*ON*	*ON*	*OFF*
280 + offset	*ON*	*ON*	*ON*	*OFF*	*ON*	*OFF*
290 + offset	*OFF*	*ON*	*ON*	*OFF*	*ON*	*OFF*
2A0 + offset	*ON*	*OFF*	*ON*	*OFF*	*ON*	*OFF*
300 + offset	*ON*	*ON*	*ON*	*ON*	*OFF*	*OFF*
310 + offset	*OFF*	*ON*	*ON*	*ON*	*OFF*	*OFF*
320 + offset	*ON*	*OFF*	*ON*	*ON*	*OFF*	*OFF*

330 + offset	*OFF*	*OFF*	*ON*	*ON*	*OFF*	*OFF*
340 + offset	*ON*	*ON*	*OFF*	*ON*	*OFF*	*OFF*
350 + offset	*OFF*	*ON*	*OFF*	*ON*	*OFF*	*OFF*
3E0 + offset	*ON*	*OFF*	*OFF*	*OFF*	*OFF*	*OFF*

UNICORN Control Board, AT (Oligosystems)

Alternative IRQs for this card are 3, 5, 10 and 11. The manufacturing default setting is IRQ10.

The jumpers of the multinet card correspond to IRQs as follows:

Jumper setting	IRQ
W2	3
W3	5
W4	10
W5	11

Alternative addresses for this card are shown in the table. The value that should be entered as an address in the UNICORN software installation is the number listed in the Address DualPort column. The manufacturing default setting for the address is no. 12 and for this setting D0000 should be entered as the address in the UNICORN software installation.

No.	*S2:1*	*S2:2*	*S2:3*	*S2:4*	*Address DualPort*
0	*ON*	*ON*	*ON*	*ON*	*A0000*
1	*OFF*	*ON*	*ON*	*ON*	*A4000*
2	*ON*	*OFF*	*ON*	*ON*	*A8000*
3	*OFF*	*OFF*	*ON*	*ON*	*AC000*
4	*ON*	*ON*	*OFF*	*ON*	*B0000*
5	*OFF*	*ON*	*OFF*	*ON*	*B4000*
6	*ON*	*OFF*	*OFF*	*ON*	*B8000*

7	OFF	OFF	OFF	ON	BC000
8	ON	ON	ON	OFF	C0000
9	OFF	ON	ON	OFF	C4000
10	ON	OFF	ON	OFF	C8000
11	OFF	OFF	ON	OFF	CC000
12	ON	ON	OFF	OFF	D0000
13	OFF	ON	OFF	OFF	D4000
14	ON	OFF	OFF	OFF	D8000
15	OFF	OFF	OFF	OFF	DC000

6. Close the cover on your PC.

7. Connect the card to the liquid handling module CU connector using the communication cable provided. Additional liquid handling modules up to a total of four may be connected.

12.5 Software installation

UNICORN will normally be installed by your Amersham Biosciences representative. Follow the instructions below if you need to install the program yourself. If the system is connected to the network and installed to support remote control, make sure that the same version of UNICORN is installed on all stations in the network.

12.5.1 Installing UNICORN for the first time

The installation procedure described below assumes that the operating system Windows NT is correctly installed on your computer. Refer to the operating system documentation for details. For network installations, the network must be correctly set up.

UNICORN is supplied on a CD-rom. Files on the CD-rom are compressed and cannot simply be copied onto the hard disk. The installation procedure described below creates the required folder structure on the hard disk and decompresses the files. Do not attempt to decompress the distribution files using any other file decompression utility.

Follow the procedure below to install UNICORN. For a network installation, follow this procedure on each computer in the network. You can quit the installation at any point by clicking on either the

Cancel button or the **Exit** button. If you do this, however, the installation will be incomplete and the software cannot be used.

Note: Before the installation is performed on a local station be sure that you know which interrupt and address that the system(s) will use. It is possible to install the UNICORN software without this information, but the system(s) will not work. Refer to chapter 13.3 for information about the system settings.

1. For network installations, log on to the network and check that you have access to the server disk and folder where UNICORN network components are to be installed.

2. Insert the CD-rom disk into the CD-rom drive.

3. Select Run from the taskbar Start button menu.

4. Write the command `d:setup`, where `d:` is the unit for your CD-rom drive. Click on **OK**.

5. The UNICORN setup program is launched and you will see the **Welcome** dialogue. Click on **Next** to continue.

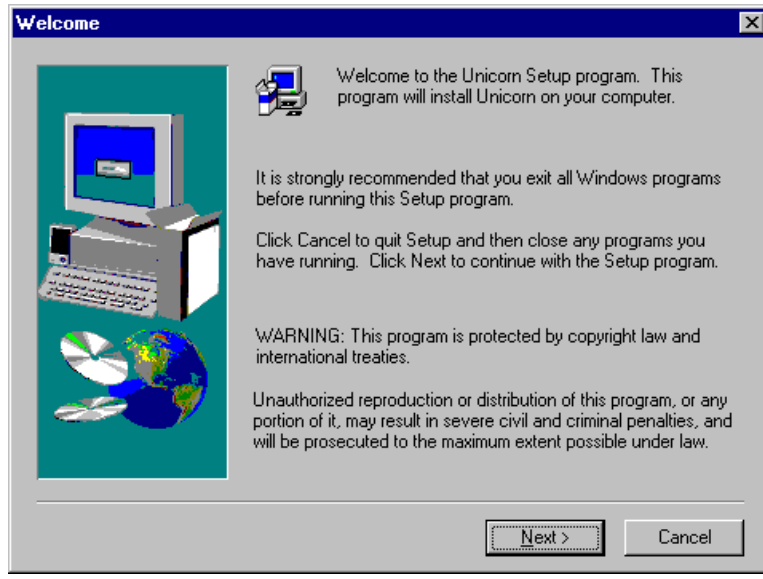


Figure 12-1. Welcome dialogue

6. The license agreement must be accepted to install UNICORN. Click on **Yes** to continue the setup program.

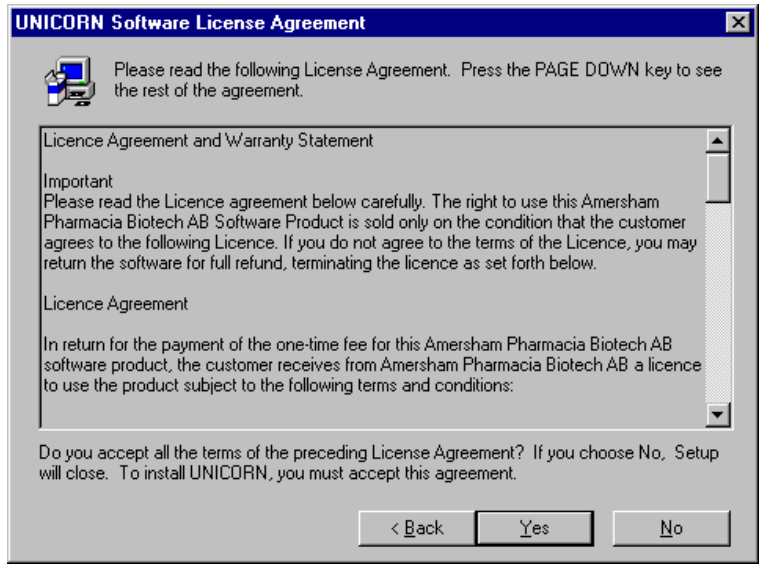


Figure 12-2. Unicorn Software License Agreement dialogue.

7. Enter your name, company, and product serial number for the software. Click on the **Next** button to continue.

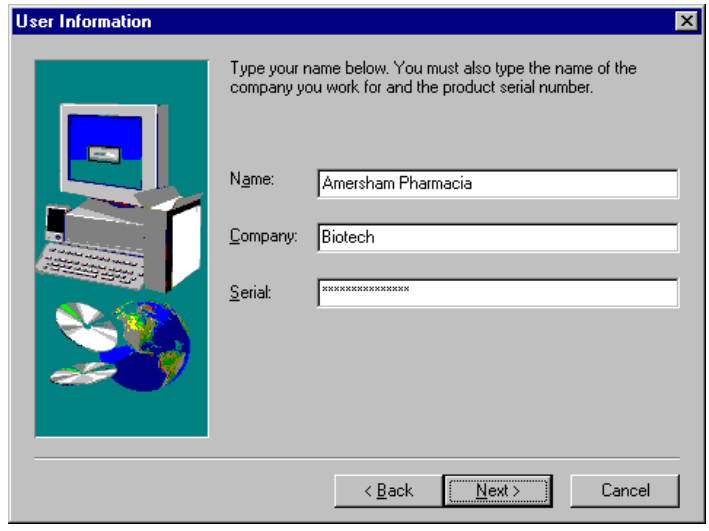


Figure 12-3. User information dialogue.

Note: The serial number can be found on the UNICORN License Agreement that is shipped with the software.

8. Select the components to install. The components **User information** and **Strategy and template files** contain sub-components. These sub-components can be accessed by clicking on the **Change** button. When all selections are made, click on the **Next** button to continue.

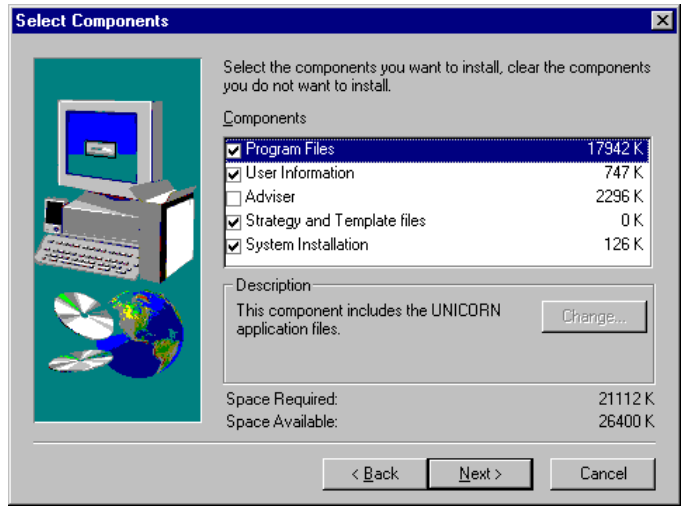


Figure 12-4. Select Components dialogue.

Stand-alone installation

- Check all components

Network installation

- **Program Files** must be checked on all stations, both local and remote.
- **User Information** contains global files for the UNICORN software, procedures, BufferPrep recipes, report formats, column files and the users file. Click on the **Change** button to select sub-components to install. These files need only be installed once in a network. For all following installations to the same network these files will be already be present on the server.

Note: The users file should be checked for the first installation only. If the users file is checked during later installation on other computers in the network, any users already defined will be deleted and only the default user will be available.

- **Adviser** should be checked on all stations, both local and remote.
- **Strategy and Template files** should be checked for each new strategy to be installed (usually when a new system is installed on a local computer). Each strategy needs only to be installed once since they are stored on the server.
- **System** should be checked on all stations where a system is connected, but not on demo or remote stations.

If global procedures, report formats, BufferPrep recipes, columns or a users file already exist, you will be asked if you want to replace the corresponding files. Normally you should answer **No**. If you answer **Yes**, all existing global procedures, report formats, BufferPrep recipes, columns and the users file will be deleted and replaced by the defaults.

9. Select the disk drive where the program is to be installed and click on the **Next** button to continue. This should be a physical disk drive (usually c:) on the computer where you are installing UNICORN, not a network disk drive.

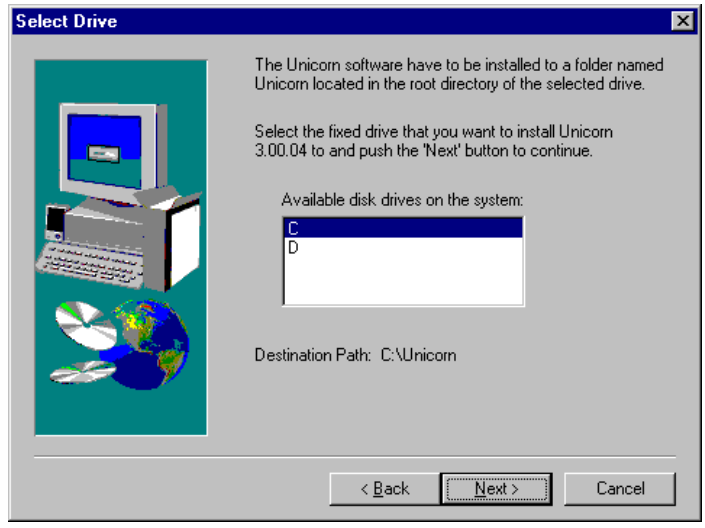


Figure 12-5. Select Drive dialogue.

10. Choose whether the setup type is stand-alone or network.

For a stand-alone installation the network options settings are ignored. A stand-alone installation can be either a local station or a demo station. If you want to install a demo station, make sure that **Demo system** is checked.

A network installation can be either a local station or a remote-only system. To get a remote-only system, i.e. a computer to which no systems are physically connected, check **Remote-only system**. For a network installation you will also have to select a server disk where the server files are to be located.

When you perform a network installation, the necessary parts of UNICORN software will be copied automatically to the network server disk.

Note: If you perform a stand-alone installation and later want to connect the system to a network, you must remove the current installation and install the software with the appropriate settings.

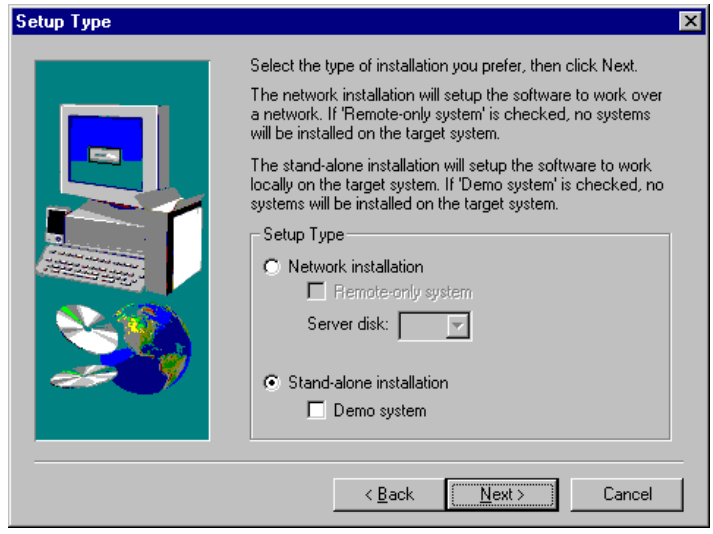


Figure 12-6. Setup Type dialogue.

11. Enter the minimum number of characters required for passwords (Valid numbers of password characters are 3 -15). Alternatively, if you do not require password protection, check **No password required**. With this setting, users can be defined with or without passwords.

Note: In a network installation, make sure that you enter the same password settings on each station in the network.

Choose the number of system control windows that should be available in the installation (maximum 4).

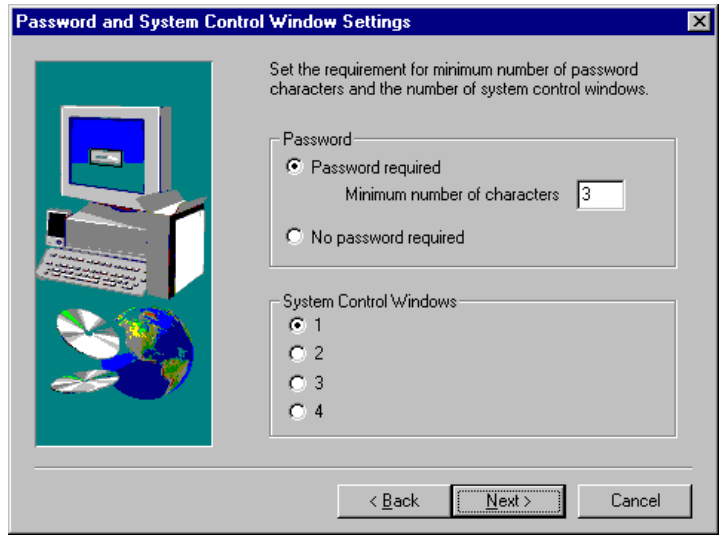


Figure 12-7. Password and System Control Window Settings dialogue.

12. Select the program folder in which you want the UNICORN icon to be placed. You can either create a new folder or select one that already exists. The folder will be placed in the programs menu under the Start button menu.

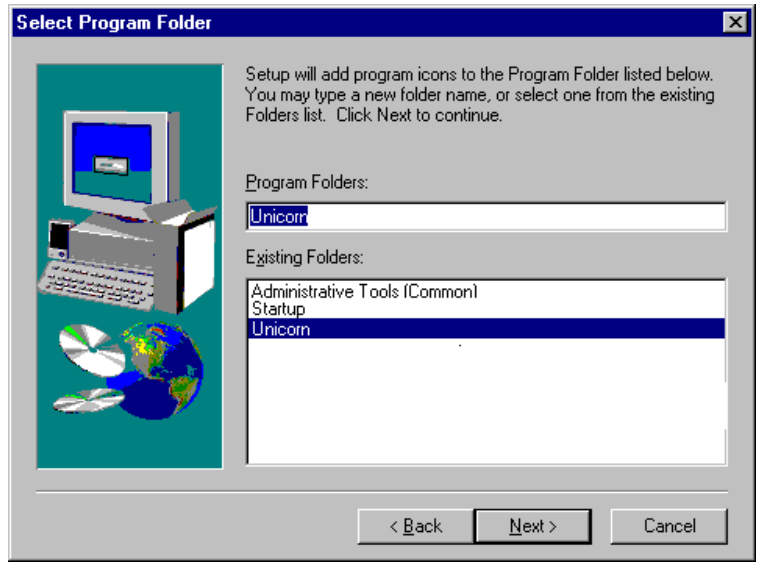


Figure 12-8. Select program Folder

13. At this point, the setup program is ready to start copy the files. A dialogue will display all the selections that have been made. If the settings are correct click on the **Next** button to start copying the files. If you want to make any changes you can click on the **Back** button.

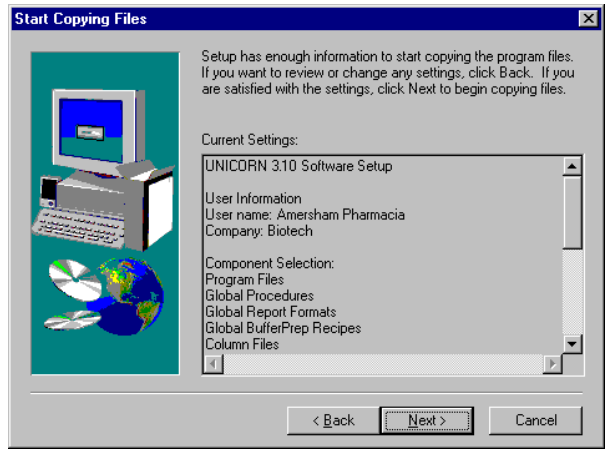


Figure 12-9. Start Copying Files dialogue.

14. If you have chosen to install systems, the system installation dialogue is displayed after the file copy process. Select a system (for OligoPilot II, select **Oligo**) and click on the **Change** button to set up the system. When all systems that are connected to the station have been defined click on the **Next** button to continue.

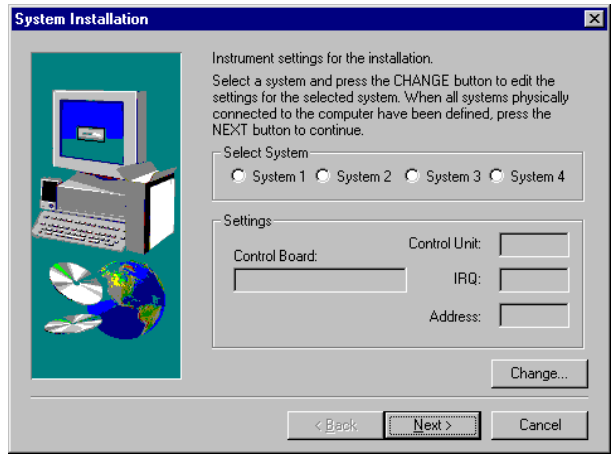


Figure 12-10. System Installation dialogue.

When you click on the **Change** button, the **System Setup** dialogue is displayed.

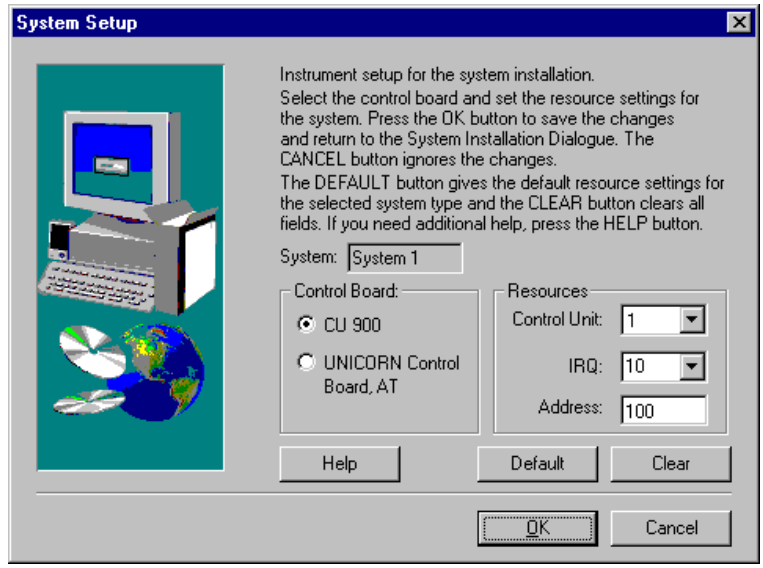


Figure 12-11. System Setup dialogue.

Select the type of control board that the system being installed is using, **CU 900** or **UNICORN control board, AT**. For each system you will have to set the correct settings for the control unit, interrupt and address. Refer to chapter 13.3 for the correct settings. The **Default** button gives the default resource settings for the selected system type. If you want to remove a system, click on the **Clear** button to clear all fields. All fields must contain a value before you can continue. Click on **OK** to save the changes or **Cancel** to abort the dialogue. In both cases you are returned to the **System Installation** dialogue. Install additional systems until you have installed all systems physically connected to the computer (the number of systems is not related to the number of system control windows installed in step 12). If you installed more than one system, make a note of which system is connected to which control unit. This information is useful to have when you set up the system table or if you must in the future reinstall UNICORN.

Note: If you want to define systems later or change the settings for an already defined system, run the setup program once again with only the **System Installation** option checked in the **Component Selection** dialogue.

15. If you have chosen to install strategy and/or template files, the **Strategy & Template Installation** dialogue is displayed. If the correct strategy is visible in the list, mark that strategy and click on the **Install** button. If you have the strategy/template files on a diskette or another source, click the **Have Disk** button to locate the strategy/template files.

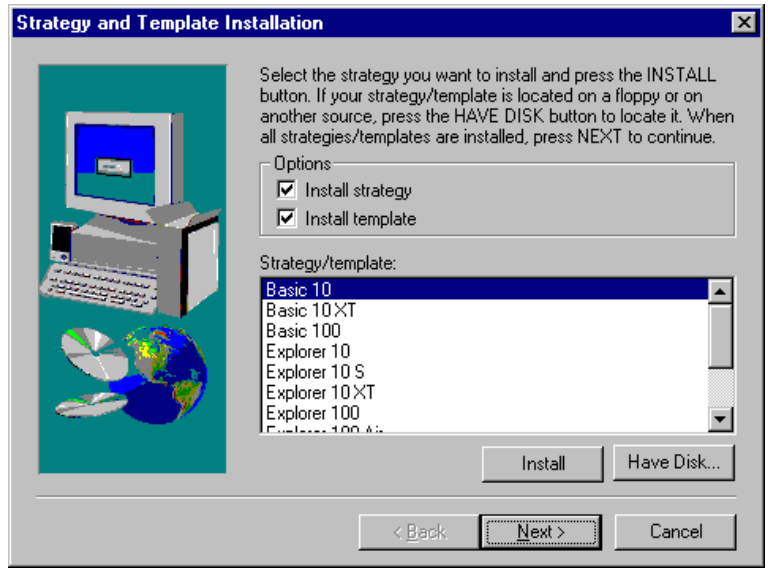


Figure 12-12. Strategy & Template Installation dialogue.

If the options for both strategy and template are checked the template files are installed automatically together with the strategy. If the strategy and template files are located on different diskettes, the setup program will ask for the template disk when it is needed.

When installing strategies, you will be prompted for a name for the strategy that is being installed. You can either keep the default name or enter another name. The name for a strategy can not be more than 8 characters.

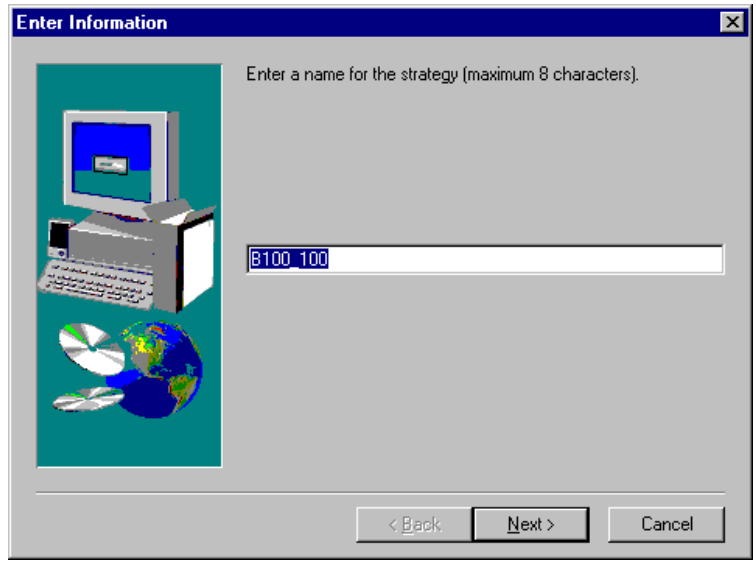


Figure 12-13. Enter Information dialogue.

If you are installing template files separately, you must enter the appropriate destination for the correct files. It is not possible to detect which template files correspond to which strategy. A list with all strategies installed on the station is displayed and you must select the strategy for which you want to install the template files.

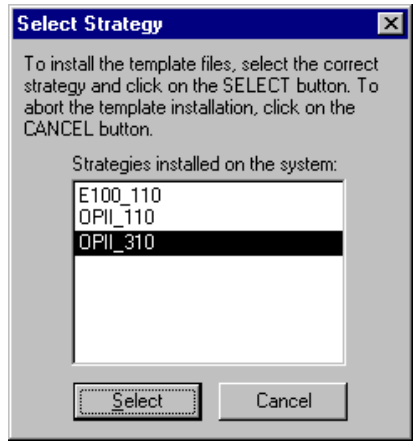


Figure 12-14. Select Strategy dialogue.

If you click on the **Select** button, the template files will be installed to the folder where the selected strategy is located. The **Cancel** button aborts the template installation. In both cases you are returned to the **Strategy & Template Installation** dialogue.

Choose to install additional strategies if you have more than one system configuration. Strategies are installed independently of systems. A strategy is assigned to a system when the system is defined.

16. The **System Table Settings** dialogue is only displayed when UNICORN is installed for the first time and only if a system table is not already present. The dialogue will be shown for each system defined in the **System Setup** dialogue (see step 14 above). Enter a name that you want to use for the system. If you want to connect a strategy to the system, select a strategy in the drop-down list box where all installed strategies are visible. When all selections have been made, click on the **OK** button to save the system in the system table. If you click the **Cancel** button, the system will not be written to the system table and you have to enter it yourself from within the UNICORN software (in the Main menu **Administration: System setup**).

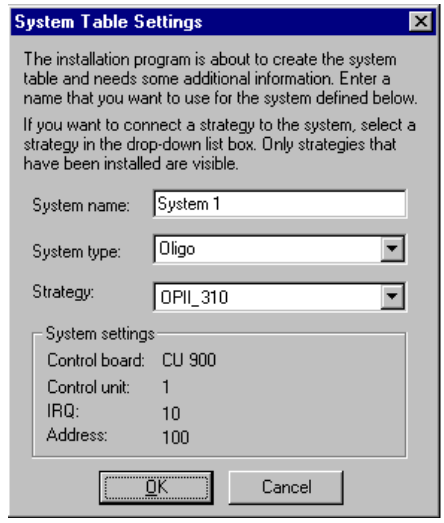


Figure 12-15. System Table Settings dialogue.

17. After the installation is complete the computer must be restarted for the installed software to be properly configured. Click on **Finish** to exit the **UNICORN Setup** dialogue and restart the computer.

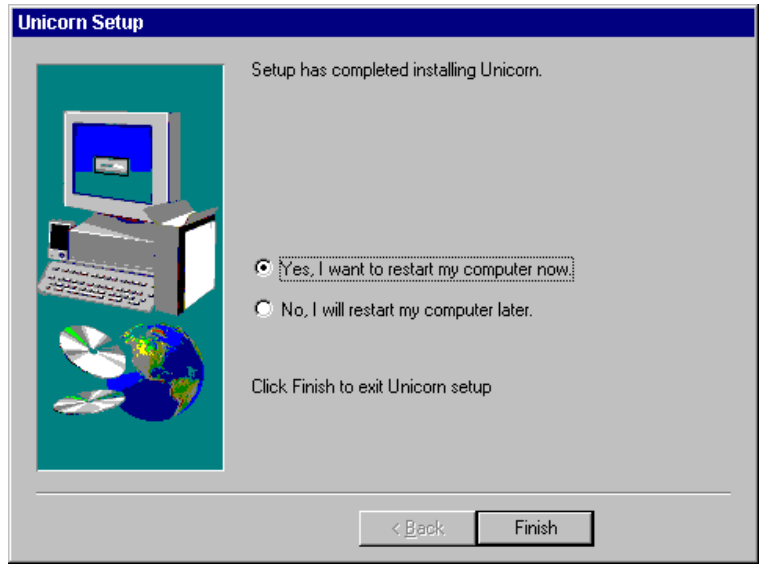


Figure 12-16. UNICORN Setup dialogue.

12.5.2 Installing selected software components after the initial installation

If your UNICORN installation should be damaged, for example due to accidental file deletion or hard disk failure, or if you want to install additional systems, strategies or templates, you can use the installation program to re-install selected parts of the software. The installation program detects the presence of existing UNICORN files, and suggests the components to install. Check some or all of **Program files**, **User information**, **Adviser**, **Strategy & template files** and **System installation** according to which part(s) you wish to install. The appropriate parts will be copied into the existing folder structure.

Note: For network installations, remember to log on to the network before installing any UNICORN software components.

Program files

Before re-installing the program files, be sure that the UNICORN software is not running. Check the **Program files** option in the **Components** list to re-install UNICORN. This will not affect any existing method or result files in the system. The program is always installed locally, even in network installations.

User information

User information contains several sub-components, global procedures, global report formats, global BufferPrep recipes, column files and the users file. To re-install these global files, check each sub-component.

Note: Any changes to the files will be lost since they will be replaced with their defaults.

If, for example, the existing user definitions are damaged you can check the **Users file** to re-install the default user. Any other users defined in the system including users installed from other stations in a network installation will be deleted. However, method and result files will not be erased. You can regain access to these files by re-defining users with appropriate folder access. Users are installed on the network server in a network installation.

System installation

Check the **System installation** option if you are installing a new or an additional system on a stand-alone computer or a local station in a network, or if you want to change the settings for an existing system. Systems are not installed on demo or remote-only systems.

Strategy files

Check the **Strategy files** option to re-install system strategies or to install additional strategies. Accept the suggested name or enter a new name for the strategy. This will not affect any existing method or result files in the system. Strategies are installed on the network server in a network installation.

Template files

Check the **Template files** option to re-install template files or to install template files for a new strategy. Normally, templates are installed together with strategies. If you are not installing a strategy at the same time, a dialogue will be shown displaying all strategies that are installed on the system. You must select the strategy to which the method template files correspond. Templates are installed on the network server in a network installation.

Adviser

Check the Adviser option to re-install the Adviser. This will not affect any existing method or result files in the system. The Adviser is always installed locally even in network installations.

13 Administration

There are three main aspects of administration of a UNICORN installation:

- System administration
- Access level and user administration
- Network administration where appropriate.

System administration concerns maintenance of software aspects of the installation, including definition of connected systems and monitoring of system usage (audit trails). These activities are described in the present chapter. System administration duties may also include routine monitor calibration (Section 6.6).

Access level and user administration concerns authorisation of access to the system, and should (at least in larger installations) be the responsibility of one person or a small group. These activities are described in the present chapter.

In a network installation, maintenance of the network functions will normally be carried out by the computer staff responsible for the company's network. Aspects of network administration relevant to UNICORN are considered in Chapter 12.

After installation, the following operations should be performed in UNICORN by the administrator before other users can use the program:

1. Set up system definitions for the synthesis systems (Section 14.1).
2. Define access levels for the installation (Section 14.2).
3. Define new users with home folders and access profiles (Section 14.3).

Note: These operations can be performed on any station in a network installation. It is however important that the administrator is logged on to the network on the station being used so that the changes will apply globally throughout the network.

13.1 System definitions

System definitions set up the synthesis systems which are connected directly to the local computer in UNICORN installation. This must be done for each new system installed. In a network installation, these definitions must be set up for each local computer in the network, but the actual set-up operations can be performed on any computer. Rights of access to system are controlled at the user administration level (Sections 14.2 and 14.3).

To manage system definitions, choose **Administration:System setup** in the Main menu. To use this menu command, you must have **Audit trail/ System setup** authorisation (see above).

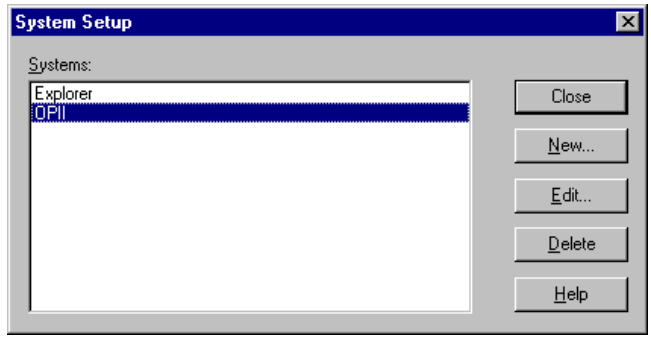


Figure 13-1. System setup dialogue.

13.1.1 Defining new systems

To define a new system, click on **New** in the **System setup** dialogue. The **New system** dialogue is displayed.

1. Enter the system name in the **System name** field. The system name can be set only when defining a new connection, and cannot later be edited since user access rights are linked directly to the system name. Names can be up to 30 characters long.

2. Select a **System Type**, either **Chromatography** or **Oligo**.

3. Select a strategy for the system from the pull-down list in the **Strategy** field. Available strategies are determined when UNICORN is installed (see Section 13.4). If you have several strategies installed, make sure that the selected strategy is appropriate for the synthesis system being defined. Click on the **Information** button to display information about the selected strategy.

4. The **Pipe server name** field is the same as the Windows NT computer name. Leave this value unchanged.

5. Select the control unit number (1-4) in **Control unit number**. This is the physical connection number for the synthesis system on the local computer (see Chapter 13).

6. Enter a value in the **Autosave interval** field if you want UNICORN to save a copy of the result file at pre-set intervals during a run. This minimises loss of data in the event of a computer failure. The recommended interval for most systems is 5 minutes. A shorter interval may slow down the user interface response. The control functions in UNICORN performance will however not be impaired.

Note: Normally, you should define systems before defining users. If you add system definitions after you have defined users, remember to grant access to the new systems to the appropriate users (see Section 14.3).

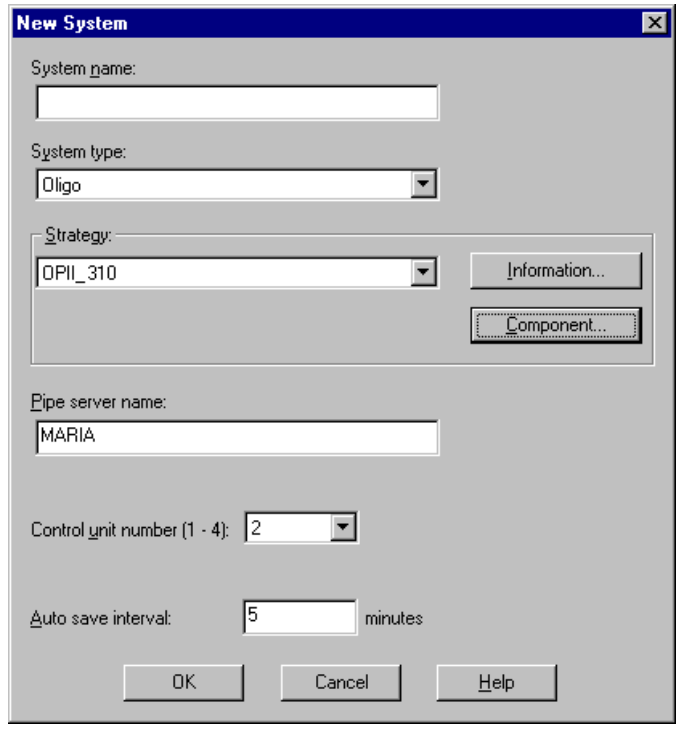


Figure 13-2. New system dialogue.

13.1.2 Editing system definitions

To edit an existing system, select the system in the **System setup** dialogue and click on **Edit**. Alter the parameters as appropriate (see Section 14.1.1 for a description of the dialogue contents).

Remember that if you change the system strategy the arrangement of tubing, pumps, columns etc. may need to be changed on the synthesis system. An attempt to control a system using the wrong strategy may cause malfunction and damage to the synthesis system.

13.1.3 Deleting system definitions

To delete a system definition, select the system(s) in the **System setup** dialogue and click on **Delete**. A system definition can only be deleted if the system is idle and no users are connected to the system.

13.2 Access levels

Access to UNICORN software is controlled by username and password authorisation (see Sections 14.3 and 3.1). Within the program, each authorised user is assigned an access level within the system which determines which functions the user can perform:

13.2.1 Defining access levels

Up to 10 different access levels can be defined. Initially, all levels are the same with access to all functions.

To edit an access level, select **Administration:Access levels** from the Main menu and select the level you wish to edit. To use this menu command, you must have **User setup/Levels** authorisation (see below). The levels are named **Level 1 - Level 10** by default: to change the name of a level, enter a new name click on the **Rename** button. Check the items to which users at this level are to have access, and click on **OK**. If you change the definition of a level to which users are already assigned, the changes will apply to all users at this level.

At least one access level must have **User setup/Levels** authorisation. UNICORN will not allow you to remove this authorisation from all levels. Click on the **Print** button in the dialogue to obtain a record of each authorisation level that you create.

The authorisation items are:

- **Method Editor**
  Required for using the Method editor for creating and editing methods (Chapters 4 and 5).

- **Evaluation**
  Required for using the evaluation module for processing result data (Chapters 9 and 10).

- **User setup/Levels**
  Required for defining and changing access levels and user.

Caution: We recommend that only one user in an installation or network is assigned this access.

- **Audit trail/System setup**
  Required for examining the audit trail and for defining connected systems (Section 14.4).

Caution: We recommend that only one user in an installation or network is assigned this access.

- **Delete, move - Home only**
  Required for deleting and moving files and folders within the user's home folder (Sections 3.2.5 and 3.2.6). Does not authorise these operations on other folders.

- **Delete, move**
  Required for deleting and moving files and folders outside the user's home folder (Sections 3.2.5 and 3.2.6). Also authorises these operations within the home folder.

- **Copy file(s)**
  Required for copying files (Section 3.2.5). The user must have access to both the source and target folders for moving or copying between folders.

- **Confirm**
  Required for authorised confirmation of answers to start protocol questions (Section 5.6.3).

- **Unlock locked system**
  Permits a user to unlock locked systems by providing the user's own logon passwords (locked systems can normally only be unlocked using the locking password, see Section 6.5.3). We recommend that this authorisation is restricted to a few users in an installation. The user who locks a system does not require this authorisation to unlock the same system.

- **Run methods**
  Required for starting methods (Section 6.1).

- **Manual interaction**
  Required for issuing manual commands in System control (Section 6.3).

- **Pause**
  Required for pausing a running process with the PAUSE button in System control (Section 6.3.1). The PAUSE instruction in methods does not require explicit authorisation.

- **Calibrate/Tune**
  Required for using the Calibrate and Tune commands in System control (Section 6.6 and Appendix D).

- **System settings**
  Required for changing system settings with the Settings command in System control (Chapter 15). Any user may view the system settings, but this authorisation is required to make changes to the settings.

- **Edit MethodQueue**
  Required for using the MethodQueue editor (Sections 8.1 and 8.2).

- **Run MethodQueue**
  Required for running MethodQueues (Section 8.3).

- **Edit global list(s)**
  Required for saving a method as a method template (Section 5.7.2), an evaluation procedure (Section 10.3), a report format, a column in the Column list or BufferPrep recipe (also Quantitation tables and Mol Size tables if the Analysis module is installed) as globally available. It is also required for deleting method templates, global procedures, global report formats, global columns or global BufferPrep recipes (also global Quantitation tables and global Mol Size tables). We recommend that this authorisation is restricted to only one user in an installation.

- **Maintenance**
  Required to gain access to the **System:Maintenance** command in System control.

- **Quit program**
  Required for ending a UNICORN session with the **File:Quit program** command in the Main menu.

13.2.2 Access level examples

Below are some examples illustrating the way access levels might be used in a multi-user installation.

System administrator

The administrator has special responsibility for maintaining user, system and audit information and for file management in the PC (folder structure, backup routines, etc.). The administrator may not edit or run methods or MethodQueues, issue manual instructions, calibrate monitors or change the system configuration.

Caution: We recommend that only one user in an installation or network is assigned system administrator rights. If several users can change user definitions and system connections, confusion can rapidly follow.

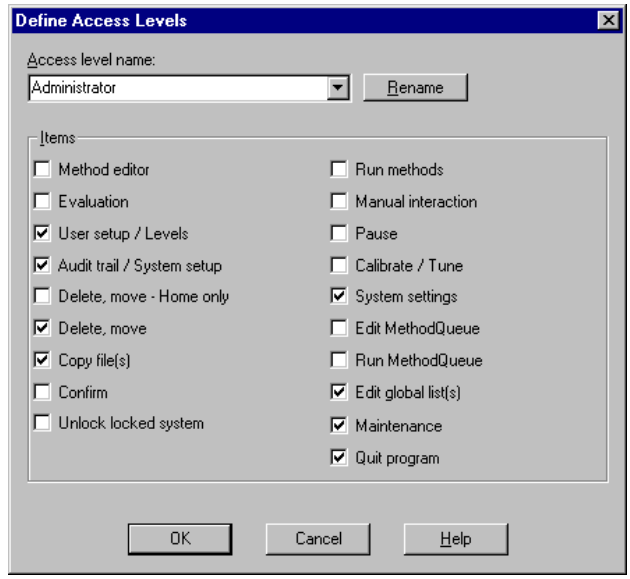


Figure 13-3. Suggested authorisation profile for the system administrator.

Development staff

Developers need to be able to edit and run methods and MethodQueues, issue manual instructions, configure system parameters, calibrate monitors and evaluate data. They may also copy, move or delete files.

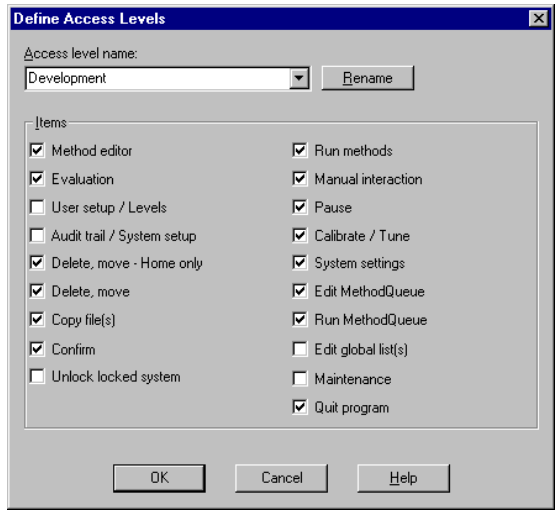


Figure 13-4. Suggested authorisation profile for development staff.

Process supervisors

Supervisors may pause a method and issue manual instructions as well as start methods and MethodQueues. Supervisors are also allowed to calibrate monitors, configure system parameters, unlock running processes, evaluate run data and quit UNICORN.

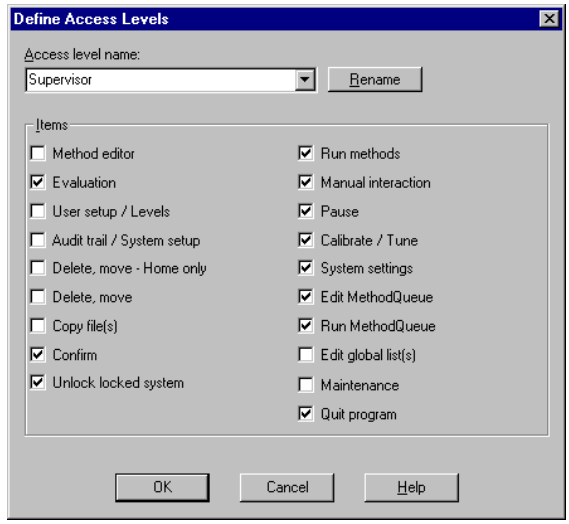


Figure 13-5. Suggested authorisation profile for process supervisors.

Process operators

Process operators are allowed to run and pause methods and MethodQueues but may not perform any other operations.

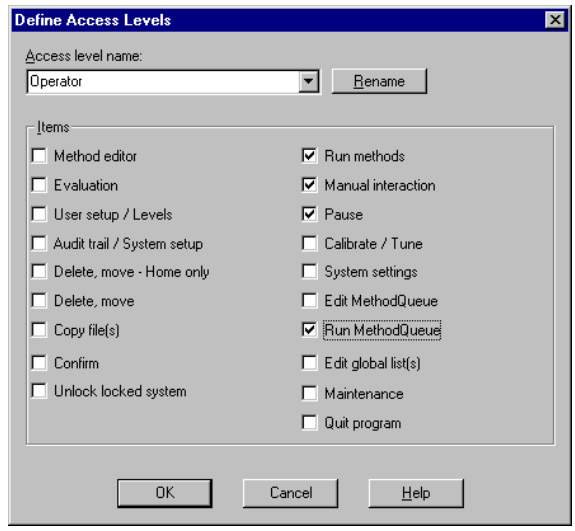


Figure 13-6. Suggested authorisation profile for process operators.

13.3 User administration

All UNICORN users are identified by a username and password. A new installation is provided with a default user (username:**default**, password:**default**). This user provides unrestricted access to all UNICORN functions.

Caution: As part of the installation procedure, new users should be created with passwords and restricted access rights as required. The default user should be deleted or redefined to prevent unauthorised access to the system.

Maintenance of user authorisation information is the responsibility of the system administrator. In a newly installed system, log on as user **default**.

To define, edit or delete users, choose **Administration:User setup** in the Main menu. To use this menu command, you must have authorisation (see above). All user administration is performed from this dialogue.

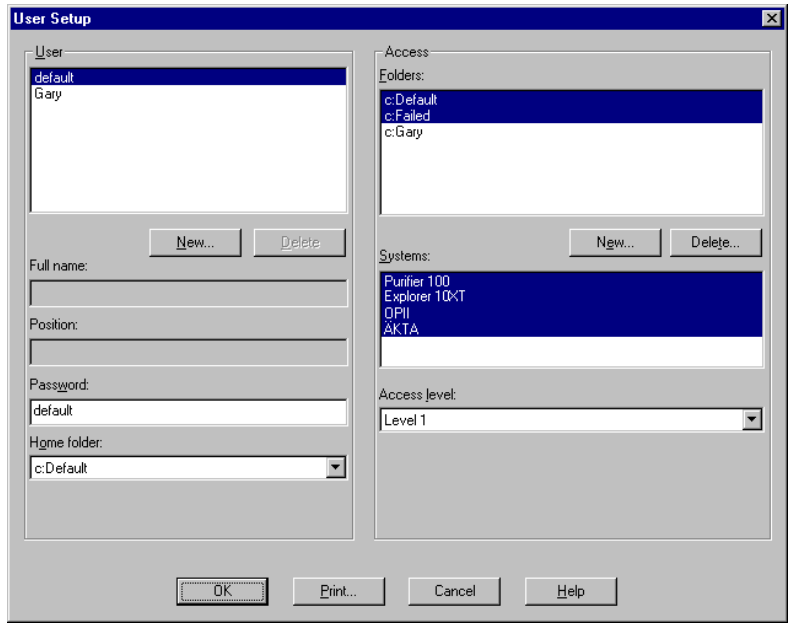


Figure 13-7. The User setup dialogue.

13.3.1 Defining new users

1. To define a new user, click on **New** in the **User** field and enter the username in the displayed dialogue.

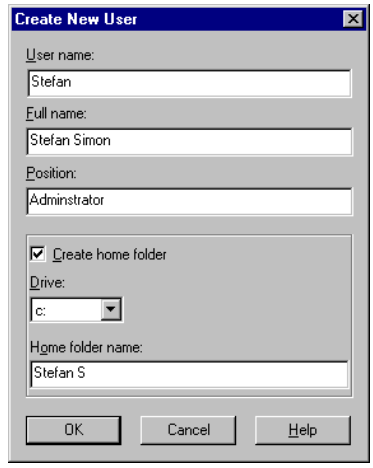


Figure 13-8. dialogue for defining a new user.

2. Enter the **Full name** of the user and **Position** as appropriate.

3. To create a new home folder for this user, check the **Create home folder** box and enter the folder name and drive in the appropriate fields. If you choose to create a home folder on a network drive, make sure that the drive is always accessible. Click on **OK** to create the user and return to the **User setup** dialogue.

Note: A home folder can always be created on a network drive even if UNICORN is not installed for network control. The computer only needs to be connected and logged on to the local network.

In general, each user should have a separate home folder.

Caution: In a network installation, always create home folders on a network drive which is accessible from all computers. If you create a home folder on the C: (local) drive, it will only be accessible from the computer on which it was created.

4. Enter the user's password in the **Password** field. The minimum number of characters in the password is defined when UNICORN is installed (see Section 13.3). The program may also be installed without password protection. The case of letters in passwords is significant.

Caution: Leaving the password as default can constitute a serious security risk.

All user passwords are visible in the **User setup** dialogue. For security reasons, make sure that access to this function is restricted.

5. Select a home folder from the **Home folder** drop-down list. You may choose any folder from this list, even if you created a home folder in the **New user** dialogue.

6. In the **Access** field, select other folders to which the user will have access from the folders list. Up to 20 folders can be set up to be shared. Selecting a folder here will give the user access to all files and folders therein. Folders that are not selected in this list will not be visible in the methods or results panel of the Main menu.

Note: All users should be given access to the Failed folder on each local station in a network installation. This will ensure that users can access results saved in the Failed folder in the event of a network communication error.

7. Select the system(s) to which the user will have access from the System(s) list and the access level of the user from the **Access level** drop-down list.

You can continue to define new users as long as the **User setup** dialogue is open. Click on **OK** to close the dialogue. If you close the dialogue by clicking on **Cancel**, all changes made since you opened the dialogue will be lost.

13.3.2 Changing user passwords

Every user can change his or her own password with the **Administration:Change Password** command in the Main menu. Enter the old password and the new password in the appropriate fields. Passwords are not displayed explicitly in this dialogue. The password will not be changed if either the old password is incorrect or the two copies of the new password differ from each other.

In addition, a user with **User setup/Levels** access can change the password for any user. To change a password for another user, open **Administration:User setup**, select the user and enter a new password in the **Password** field (see Section 14.3.1).

Change passwords regularly and avoid obvious passwords like "secret" and "open_sesame" for maximum security.

If you forget the password for the only user with **User setup/Levels access**, you must re-install the default user as described in Section 13.4.2.

13.3.3 Viewing and changing user definitions

To view the setup for any user, click on the user name in the **User setup** dialogue. To change the user definitions, make changes in the dialogue fields as appropriate and click on **OK**.

13.3.4 Deleting users

To delete a user, select the user from the username list and click on **Delete**. You may delete all users except the last user with **User setup/ Levels** access. This ensures that at least one user has the right to perform administration functions.

Deleting a user does not affect the user's home folder or method and result files.

13.3.5 Defining new home folders

To define an existing folder as a home folder for a user, select the folder in the **Home folder** field in the **User setup** dialogue. Any folder may be used as a home folder. As a recommendation in network installations, place the home folder on a drive which is addressed by the same drive letter from all computers in the network.

To create a new home folder at the same time as you create a user, check the **Create home folder** option in the **New user** dialogue and enter the folder name and drive in the appropriate fields.

To create new folders under a home folder, use the **New folder** command in the Main menu (see Section 3.2). This operation does not require special authorisation.

13.3.6 Deleting home folders

To delete home folders, click on **Delete** in the **Access** field of the **User setup** dialogue. Select a folder to delete in the **Delete Folder** dialogue that is displayed and click on **OK**.

Caution: It does not matter which folders are marked in the folders list in the **User setup** dialogue when you click on **Delete**. This list shows the folders to which the currently marked user has access, not the folders that will be deleted.

All methods, result files and folders within a selected folder will be deleted when the selected folder is deleted.

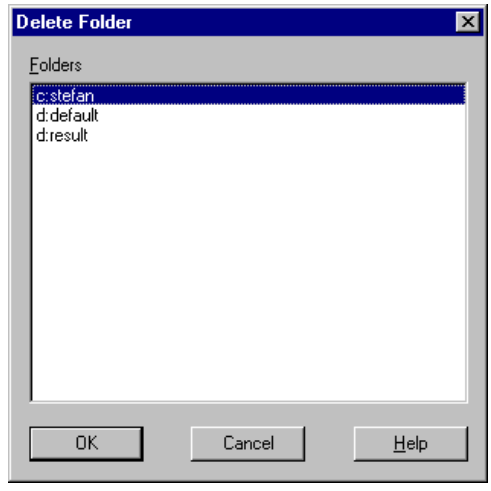


Figure 13-9. Delete Folder dialogue.

You cannot delete:

- a home folder to which a user is assigned. To delete such a folder, you must first either delete the user or change the home folder assignment for this user. Select the user in the **User setup** dialogue and assign a different home folder.
- a folder to which several users share access. To delete such a folder, remove the access rights from each user first.

13.3.7 Printing user setup information

You can print the settings for selected users.

1. In the **User Setup** dialogue click on the **Print** button. The Print dialogue is displayed.
2. Select individual users for which you want to print information or click on the **Select All** button.
3. Check the boxes for the **Print Item**s that you want to include.
4. Click on **OK** to print.

13.4 Audit trails

The audit trail, accessed under **Administration:Audit Trail** in the Main menu, provides a full record of UNICORN usage and system activity for the system administrator. The audit trail may be viewed in global mode (all systems in the installation) or system mode (one chosen system).

13.4.1 Examining audit trails

In the audit trail window, select whether you wish to view the **Global** or **System** trail.

Global audit trails

Check the items you want to display in the **Global** field. All items are recorded in the audit trail: the check boxes in the **Global** field only control which items are displayed. Global audit trail files are saved on the server disk in a network installation, and a network connection is required to examine global audit trails.

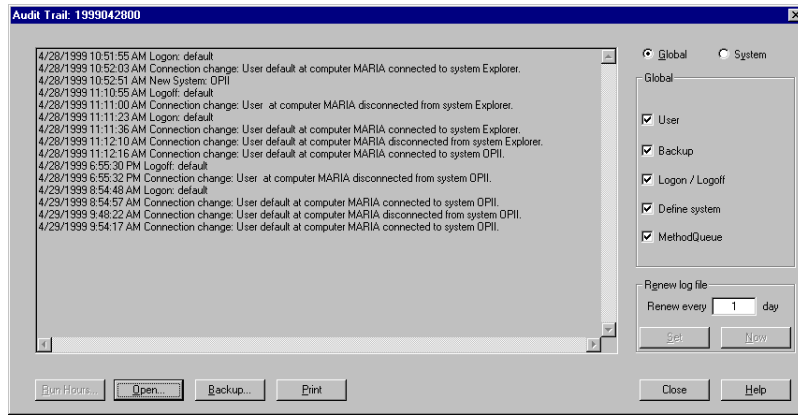


Figure 13-10. Global audit trails.

User Displays all user creation, deletion and re-definition operations.

Backup Displays backup operations for global audit trail files.

Log on/off Displays all logon and logoff attempts with the name of the user logging on or off, including failed logon attempts.

Define system Displays all system definition, deletion and redefinition events.

MethodQueue Displays MethodQueue start operations.

Sequence Displays sequence start operations.

System audit trails

Select the system for which the audit trail is to be displayed from the drop-down list and check the items you want to view. All items are recorded in the audit trail: the check boxes in the **System** field only control which items are displayed. System audit trail files are saved on the local station to which the system is physically connected, and may be examined from the local station without logging on to the network. System audit trail files can be viewed from any computer in a network installation.

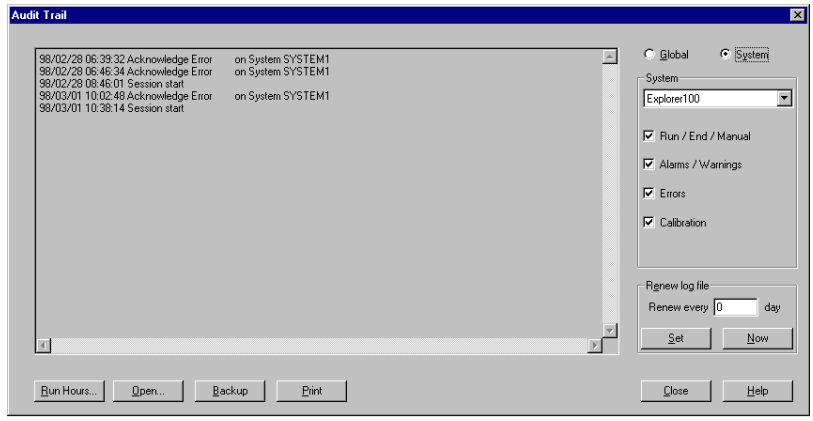


Figure 13-11. System audit trails.

Run/End/Manual Displays times for run start and completion and for manual operation.

Alarms/Warnings Displays alarms and warnings for the system.

Errors Displays system errors.

Calibration Displays monitor calibration operations.

Viewing older audit trails

When you choose **Administration:Audit trail**, the audit trail dialogue displays the current audit trail. To view an older audit trail, click on **Open** in the audit trail window and choose the file to open. The drive is automatically selected according to the type of audit trail file. If you have saved log files on diskette, these drives can be selected from the pull-down list. Files are named by date and serial number. Choose **Current log file** to return to the current audit trail after viewing older files.

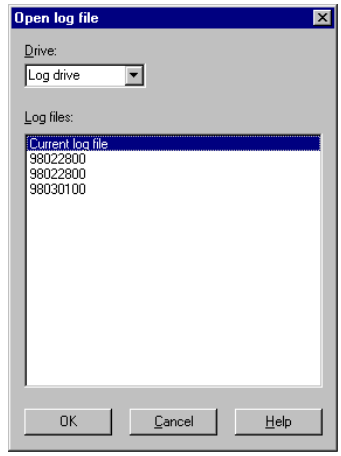


Figure 13-12. Open Log File dialogue.

Printing audit trails

Click on **Print** in the audit trail dialogue menu prints the audit trail file as currently displayed in the window.

13.4.2 Renewing audit trail files

The audit trail file is renewed at regular intervals between 1 and 30 days. To set the interval, choose **Administration:Audit Trail** from the Main menu. Enter the required interval in the **Renew every** field and click on **Set**. The new setting will take effect from the time the change is made, e.g. if the setting is changed to 7 days at 10 a.m. on a Monday, the file will be renewed at 10 a.m. every Monday.

You can also start a new audit trail file at any time by clicking on **Now**. This will not affect the automatic setting, e.g. if the audit trail is set to renew at 10 a.m. every Monday, and you click on **Now** on a Friday, a new file will be started immediately and another new file will be started on the following Monday morning.

13.4.3 Backing up audit trail files

Click **Backup** in the audit trail window to make copies of audit trail files on diskette. Choose whether to copy or move the files to diskette and click on **Backup**. The **Move** alternative is recommended to save disk space. Backup operations are recorded in the audit trail.

Note: The **Backup** command simply copies the audit trail file to diskette. It does not use any Windows NT backup commands.

System run hours

In the system audit trail, click on **Run hours** to display the accumulated run time for the system (i.e. the time the system has been in manual or run mode). Run hour values show the number of hours that the system has been used for manual or method-controlled runs. The Run hours record is useful in following up expected and actual lifetimes for liquid handling components. Click on **Reset** to reset the accumulated run hours to zero. The reset time is recorded in the audit trail.

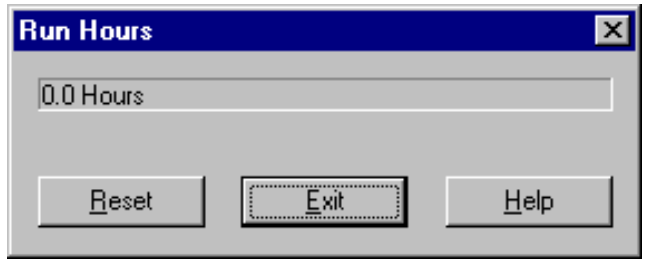


Figure 13-13. Run hours in the system audit trail.

13.5 Report Generator Wizard

UNICORN 3.10 contains a report generator Wizard for registration of errors or problems that you have detected or that occur during a run. The **Report Generator Wizard** takes you through the steps to generate your own report.

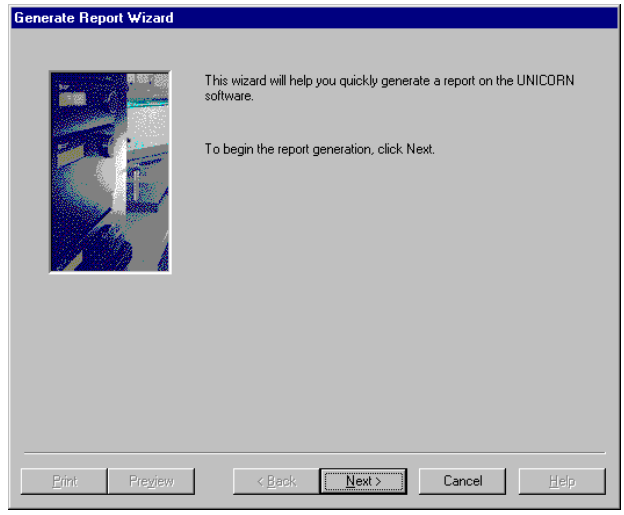
There are two ways of accessing the **Report Generator Wizard**:

- you can generate a report in the Main menu by selecting the menu command **Administartion:Create System Report**.
- when an error message appears in System control you can activate the report generator by clicking on the **Report** button in the message dialogue.

13.5.1 Generating a report from the main menu

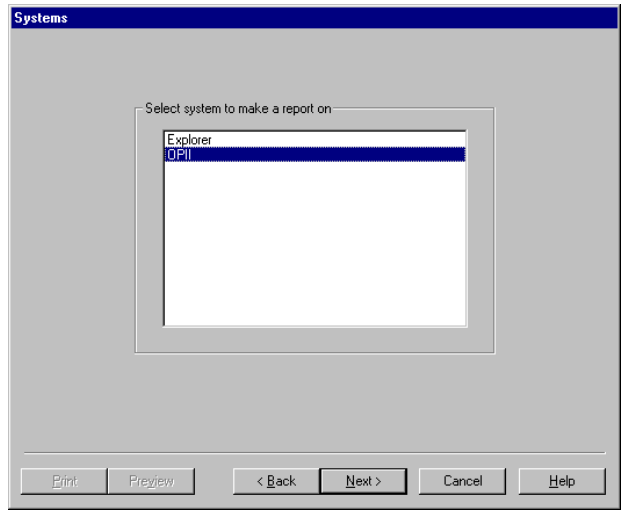
Generate Report Wizard

This first dialogue gives an introduction to the report generator.



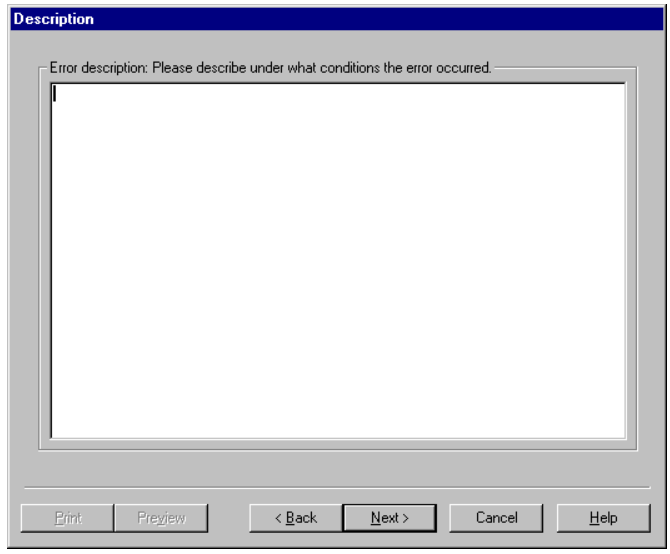
Systems

This dialogue is displayed only if you have accessed the wizard from the Main menu. This displays a list of the available systems for the logged on user. In order to proceed further, a system must be selected for which the report is to be generated.



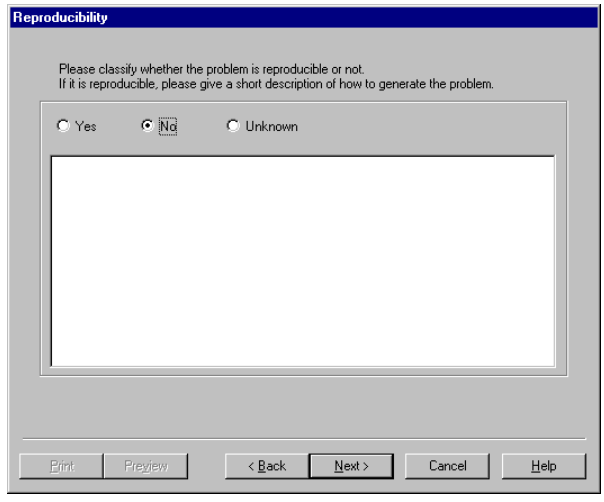
Description

You are required to enter a short description of the problem, the circumstances under which the problem occurs, and the consequences caused by the problem.



Reproducibility

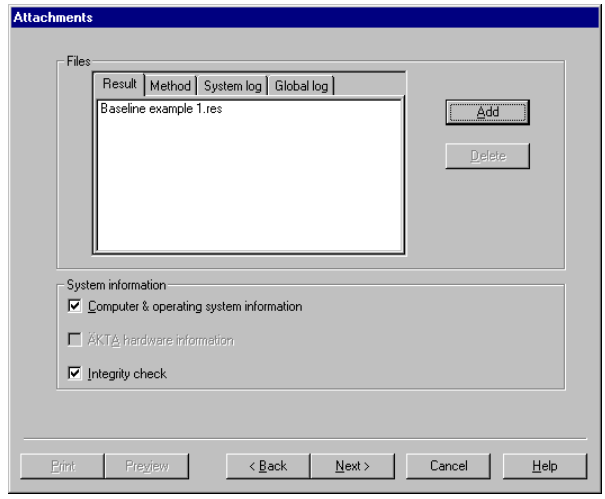
You are required to give information concerning the reproducibility of the problem. If the problem is reproducible then you can describe how to bring about the problem.



Attachments

You have the option of attaching result files, method files and/or log files to the report.

1. To make an attachment first select the appropriate tab, **Result**, **method**, **System log** or **Global log**.



2. Click on the **Add** button and browse in the displayed dialogue to find the file that you want to attach.

3. Select a file and click on **Open**. The selected file is added to the tab in the **Attachments** dialogue.

System information can also be included in the report by checking the appropriate option boxes. By default the following options are checked:

Computer & operating system information

This summarises information about the computer and operating system, e.g. type of processor, processor speed, RAM memory, hard disk capacity and printer.

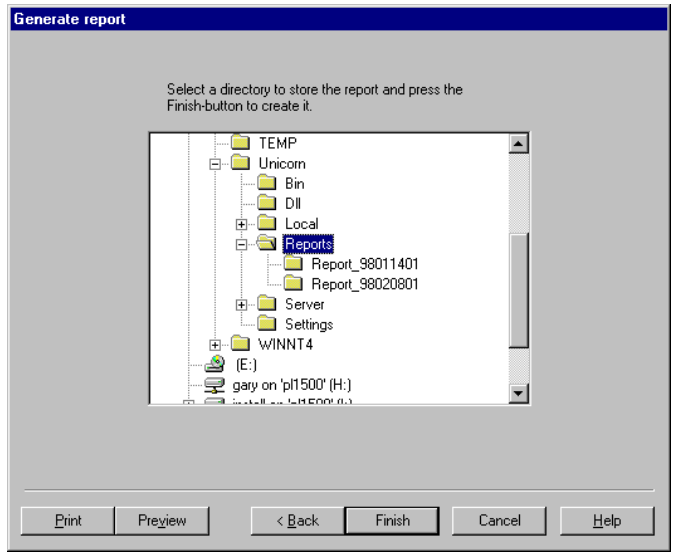
Integrity check

When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check involves a new checksum calculation being performed for the same files in their

folders and comparing the new calculated value with the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included.

Generate Report

The Generate Report dialogue displays a tree structure of all drives that are reached from the computer performing the report generation. By default the report is saved in the folder, Unicorn\Reports although you can change this folder to another one of your choice.



You will also notice that the **Print** and **Preview** buttons are enabled in this dialogue. **Preview** opens the report in NotePad, while **Print** prints out the report without any preview.

13.5.2 Generating a report from System Control

If you activate the **Generate Report Wizard** by clicking on the **Report** button in an error message dialogue in System control, the following dialogues are displayed:

Generate Report Wizard

See description above

Description

All of the problems/errors that are currently listed in the System control error message dialogue are recorded here. Help text is then shown specific to the selected error in the error message dialogue. By selecting a different error in the dialogue, the appropriate text is shown. All problems with the help texts are included in the report.

You also have the possibility to enter a short description of the problem, the circumstances under which the problem occurs, and the consequences caused by the problem.

Reproducibility

See description above.

Attachments

See description above. One exception is that log book files can be attached instead of result files.

Generate Report

See description above.

14 System settings

Each installed system has a set of default settings, for example, to define global system settings for alarms and warnings and to select which data will be stored in result files. The default system settings are valid for all runs unless you change the settings in one of two ways:

- you assign a new value to a parameter within a method. The specific change that you make in the method is valid only until End. After End the parameter returns to its default setting. Some parameter settings, however, can be set to override the global settings for the duration of the method. See Appendices B and C for settings defined in OligoPilot II system and other UNICORN System Controller strategies respectively.
- you assign a new value to the actual system setting. This new value is valid for all runs and remains until you again change the value or return the setting to its default value. Of course you can temporarily change the setting within a method as described in the previous point.

Changes to the default settings should be made when the system is installed. Also, certain settings may need to be adjusted if system components are changed (e.g. alarm and warning limits) or for specific run purposes (e.g. monitor and curve settings).

Note: For strategies where you can select the system components only the settings for the selected components will be displayed.

Assigning a new value to the actual system setting is performed in the System control module and **Configuration** access is required to make such changes (see Section 14.2).

1. To access the settings, click on the appropriate System control icon on the Windows NT taskbar and select **System:Settings**. The **Instructions** dialogue for the current system is displayed.

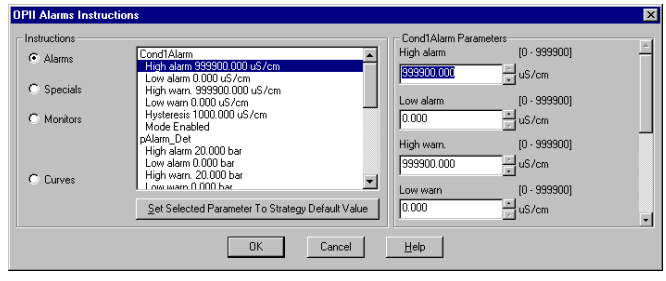


Figure 14-1. Instructions dialogue in System control. The illustration shows the Alarms group of settings.

2. The instructions are classified into **Instructions** groups including **Alarms**, **Special**, **Monitors** and **Curves**. To view the parameters and parameter settings for a particular group, click on the appropriate radio button.

3. The instructions for the selected instruction group are listed. Beneath each instruction is listed the parameters and the current setting.

4. To change the setting for a parameter, select a parameter from the list and change the associated parameter setting. The parameter is updated in the list.

5. To restore a setting to its default setting *as defined in the system strategy*, select the setting and click on **Set Selected Parameter to Strategy Default Value**.

6. When all required changes have been made, click on **OK**. Choosing **Cancel** will cancel all changes made since the dialogue was last opened.

Caution: Changes made to settings do not take effect until you click on **OK** to close the dialogue.

14.1 Alarms

Alarms define upper and lower alarm and warning limits for process monitor signals:

- If the signal exceeds the Alarm limits, a buzzer sounds and an alarm message is displayed, and the process is paused (i.e. method execution is suspended and all pumps are stopped). The situation must be acknowledged and corrected before the process can be restarted.
- If the signal exceeds the Warning limits, a warning message is issued without interrupting the process.

Alarm and warning messages are displayed on all stations with a connection to the system concerned, regardless of the activity currently being 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 control mode connection.

If allowed by the system strategy, limits for certain monitor signals may also be set locally in a method, overriding the global setting as long as the method is in operation. This feature allows for instance the

pH warning threshold to be set to one value during process operation and another during system cleaning.

Error messages from the monitors and pumps are reported if the respective **Error** settings are **Enabled**.

Caution: Alarms are not active unless the mode is set to **Enabled**. This can be viewed for each instruction in the list.

The hysteresis setting for a warning determines the extent to which the signal can oscillate around the warning limits without re-activating the warning (Figure 15-2).

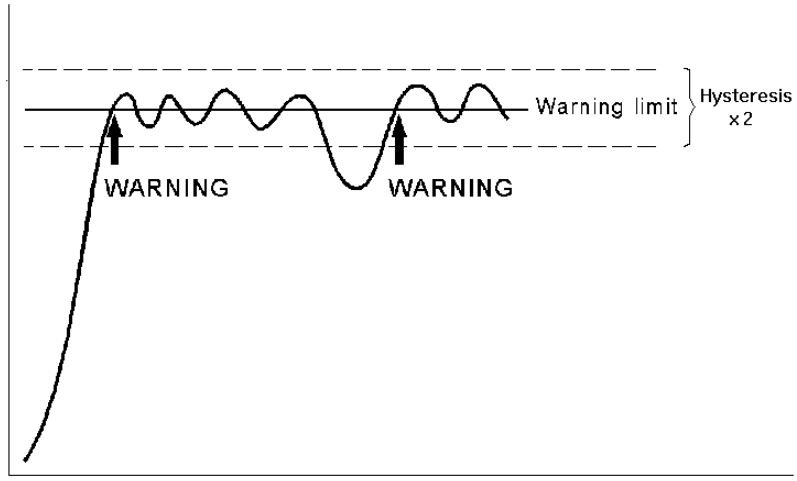


Figure 14-2. The hysteresis setting defines the limits within which the signal may oscillate up or down from the threshold without re-activating a warning. After the signal has activated a warning, the warning will not be repeated as long as the signal remains within a window defined by the hysteresis setting above and below the warning limit. This prevents repeated warnings from noisy or oscillating signals close to the warning boundary.

Hysteresis is only relevant for warnings, since an alarm puts the system in Pause at the first alarm.

Flow rate warning

If the software has calculated a flow rate that is more than the maximum capacity for a pump, a warning is displayed and the maximum flow rate will be used. This could mean a longer contact time than is set in the variables. this is mostly relevant for larger columns.

14.2 Specials

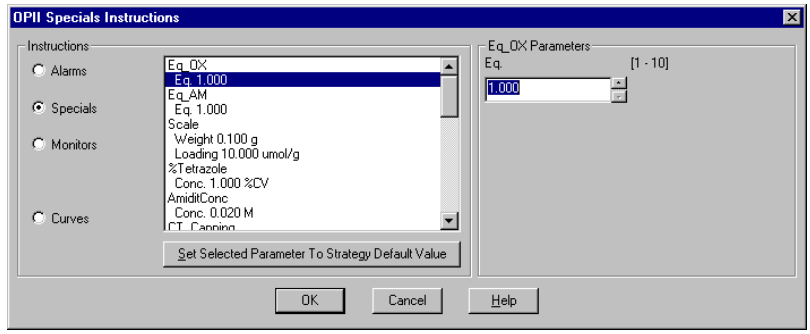


Figure 14-3. Special settings in System control.

Settings in the **Specials** group include:

Frac-Parameters — **DelayVol** determines the delay between peak detection on the UV monitor and the start of fraction collection. Fraction marks in the System control display and result file are adjusted with the delay volume. This value should be set to the volume of tubing between the UV monitor and the fraction collector outlet. **TubeChange** determines the flow handling during tube changes.

Peak_Frac-Parameters — Determines the parameters used for peak fractionation.

FracNum-bering Mode — Determines whether fraction numbers will be reset after each run (**Reset** or **Continue**).

Keyboard — Determines if the manual changes can be performed also from the instruments.

Methodbase — Determines which pump flow rate (GradientPump or SamplePump) will be used to calculate method volume.

PumpType Only for OligoPilot. Determines the types of pump used for pumps A, B and C.

PumpGain Only for OligoPilot. Sets the gain factor for the pump control signal. Normal value 1.0.

14.3 Curves

Curve settings determine which monitor signals will be stored as curves in the result file. Check that **Store** is set to **ON** for all signals that are to be stored.

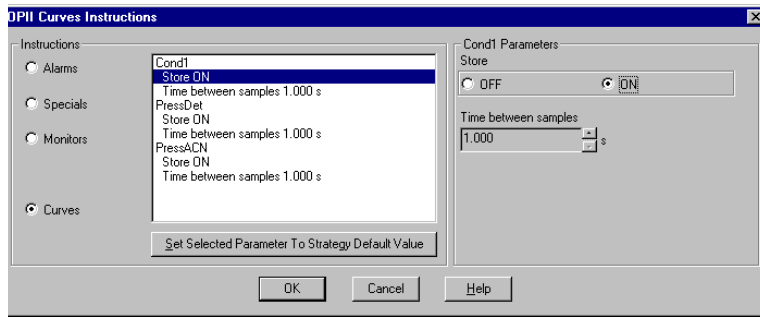


Figure 14-4. Curves settings in System control.

Settings in the **Curves** group include:

Store Determines whether the curve data is stored in UNICORN.

Time between samples The **Time between samples** determines the frequency with which curve data is recorded in UNICORN (this does not affect the reading frequency of the monitor itself). Default values are the shortest possible time between samples.

Caution: If a curve is set to **Store:OFF**, data from the monitor concerned cannot be displayed in the curves window during a process run, and will not be recorded in any way.

Introductory material

Methods and runs

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Appendices

A Technical specifications

A.1 System requirements

A.1.1 Hardware requirements

- Compaq PC, Pentium II/333 MHz or later (minimum Pentium/90 MHz)
- 64 Mb RAM (minimum 32 Mb) for one system
  128 Mb RAM (minimum 64 Mb) for two or more systems
- 1 Gb of available hard disk space, NTFS file system, (minimum 150 Mb)
- Colour monitor, 1024x768 pixels (minimum 800x600), small fonts, 64k colours
- 1 ISA slot per connected system
- CD-ROM drive
- 1.44 Mb (3.5") diskette drive
- Mouse
- Supported printers:
  HP DeskJet 660C
  HP DeskJet 690C
  HP DeskJet 870Cxi
  HP DeskJet 895 C
  HP DeskJet 2500 C
  HP LaserJet 4M
  HP LaserJet 5MP
  HP LaserJet 4000 N

A.1.2 Software requirements

Microsoft Windows NT Workstation 4.0 (with Service Pack 4 or later).

A.1.3 Network requirements

These are the recommended network requirements for running UNICORN in a network installation:

- Supported Network cards:
  3COM Etherlink III
  Compaq Netelligent 10/100 TX Embedded UTP Controller
  Compaq Integrated NetFlex-3 Controller
  AMD PCNET PCI Ethernet Adapter (Integrated)
- Novell NetWare version 4.50.189 or later or Microsoft Windows NT Server 4.0. The UNICORN software works on earlier versions as well even though some versions of the Novell Netware driver have known problems.
- A valid network connection

A.2 Control capacity

A.2.1 Stand-alone installations

Simultaneous control of up to four synthesis systems. Each module is separately configured in a system strategy supplied by Amersham Biosciences.

A.2.2 Network installations

A few basic facts about a network installation:

- Synthesis systems must be locally linked to a workstation and then the workstation is linked to the network, i.e. the synthesis systems are not directly linked up to the network.
- Each local workstation can be connected to four separate synthesis systems.
- A network can support up to 90synthesis systems, which are connected locally to the workstations in the network.
- A workstation can locally or remotely actively control up to four synthesis systems. This is achieved using the four possible System control windows in UNICORN available on each workstation.
- Each synthesis system may be controlled by only one active System Control window, and be viewed by seven different other System control windows in UNICORN.

A.3 Data sampling

Data from synthesis system monitors are stored temporarily in data buffers in the local system controller. Data are transferred from the buffers to disk storage by UNICORN whenever a chromatogram is closed (i.e. when the New_Chromatogram instruction is issued or the result file is closed). Data are also saved to disk at pre-set intervals during a run, minimising data loss in the event of power or communication failure.

The capacity of the data buffers is 16000 points for up to sixteen monitors (as listed in the **Curves** group of **System:Settings** in System control). If a buffer is filled during a run, the number of points is halved by deleting every second point, and the sampling frequency for subsequent points is halved. The initial sampling frequency for each monitor is set in the system strategy, and can be viewed and changed in the **Curves** group of **System:Settings**.

At an initial sampling frequency of 10 samples per second (10 Hz), the following resolutions apply for the curves:

Duration	Sampling frequency	No. of points	Resolution (s/point)
0-27 min	10 Hz	0-16000	0.1
27-53 min	5 Hz	8000-16000	0.2
53-107 min	2.5 Hz	8000-16000	0.4
107-203 min	1.25 Hz	8000-16000	0.8
...	...	...	...
...	...	...	...

To ensure maximum resolution for part of a run, issue a New_Chromatogram instruction at the beginning of the part. This empties the data buffers and resets the sampling frequency to that specified in the system settings.

B General strategy for Oligo Synthesis

This appendix alphabetically lists the instructions for methods, manual control, system settings and variables supported by the standard strategy for OligoPilot II. The user is referred to the appropriate synthesis system manual for specific details.

B.1 Method instructions

B.1.1 Pump

Instruction	*Description*
CT_Coupl	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by scale, Eq_Amidite, %Tet, CV, Delay volume
CapCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by CV and CV_Cap
OXCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by the Scale and Eq_Ox
ThioCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by CV and CV_Thio

CapCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by CV and CV_Cap
OXCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by the scale and Eq_Ox
ThioCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by CV and CV_Thio
Flow_ACN	Starts the flow with acetonitrile from Pump P6000 to the column or waste
Flow_Det	Starts the flow with detrit solution from Pump P6000 to the column or waste
Flow_Reag	Starts the flow with reagent, connected to valve x.x, to the column or waste
LFlow_ACN	Starts a linear flow of acetonitrile to the column or waste and is dependent on column diameter
LFlow_Det	Starts a linear flow of detrit solution to the column or waste and is dependent on column diameter
LFlow_Reag	Starts a linear flow of reagent to the column or waste and is dependent on column diameter
PFlow_ACN	Pressure-controlled flow of acetonitrile from Pump P6000 to the column or waste

PFlow_Det	Pressure-controlled flow of detrit solution from Pump P6000 to the column or waste
Vol_Amid	Set amidite and tetrazole with respect to the Scale (loading*weight), %Tetrazole, AmiditeConc, Eq_AM
Vol_Cap	Set capping with respect to CV and CV_Cap
Vol_Oxid	Set oxidation with respect to Scale (loading*weight), Eq_Ox, using the contact time flow rate
Vol_Thio	Set Thiolation with respect to CV and CV_Thio, using the contact time flow rate

B.1.2 Flowpath

Instruction	*Description*	*Comment*
Amidite:		Means:
ACN_A	to column 1.1: p1.0: p2.1 :5.2: 6.1	valve#pos#:
A	to column 1.2: p1.0: p2.1 :5.2: 6.1	port1pos#:
ACN_C	to column 1.4: p1.0: p2.1 :5.2: 6.1	port2pos1:
C	to column 1.3: p1.0: p2.1 :5.2: 6.1	valve5pos2:
ACN_G	to column 1.4: p1.0: p2.1 :5.2: 6.1	valve6pos1
G	to column 1.5: p1.0: p2.1 :5.2: 6.1	
ACN_T/U	to column 1.7: p1.0: p2.1 :5.2: 6.1	
T/U	to column 1.6: p1.0: p2.1 :5.2: 6.1	
ACN_A*	to column 2.1: p1.1: p2.1 :5.2: 6.1	
A*	to column 2.2: p1.1: p2.1 :5.2: 6.1	
ACN_C*	to column 2.4: p1.1: p2.1 :5.2: 6.1	
C*	to column 2.3: p1.1: p2.1 :5.2: 6.1	
ACN_G*	to column 2.4: p1.1: p2.1 :5.2: 6.1	
G*	to column 2.5: p1.1: p2.1 :5.2: 6.1	
ACN_T*/U*	to column 2.7: p1.1: p2.1 :5.2: 6.1	
T*/U*	to column 2.6: p1.1: p2.1 :5.2: 6.1	
ACN_X	to column 1.1: p1.0: p2.1 :5.2: 6.1	
X	to column 1.8: p1.0: p2.1 :5.2: 6.1	
ACN_Y	to column 2.1: p1.1: p2.1 :5.2: 6.1	
Y	to column 2.8: p1.1: p2.1 :5.2: 6.1	

Solvent:	
Beaucage	3.7: p1.0: 5.2: 6.1
ACN_3.1	to column 3.1: p1.0: 5.2: 6.1
Cap_A	to column 3.2: p1.0: 5.2: 6.1
Cap_B	to column 3.3: p1.0: 5.2: 6.1
ACN_3.4	to column 3.4: p1.0: 5.2: 6.1
OX	to column 3.5: p1.0: 5.2: 6.1
Extra_3.6	to column 3.6: p1.0: 5.2: 6.1
Tetrazole	to column 3.8: p1.0: 5.2: 6.1

Every amidite and solvent setting can also be set to waste. This means setting value 6 from position 1 to position 2.

WasteOut:	
Waste_ACN	valve 6.1
Waste_Detrit	valve 6.2
Waste_A	valve 6.3
Waste_C	valve 6.4
Waste_G	valve 6.5
Waste_T	valve 6.6
Waste_X	valve 6.7
Waste_Y	valve 6.8
ACN Pump	to waste/to column
Detrit Pump	to waste/to column
Valve:	
1	1-8
2	1-8
3	1-8
4	1-8
5	1-3
6	1-3
Port:	
1	0-1
2	0-1
Recycle:	
ON	flowrate LFlow 0-1000 cm/h
OFF	flowrate LFlow 0-1000 cm/h

B.1.3 Alarms&Monitors

Instruction	*Description*	*Comment*
%Tetrazole	Percent tetrazole of the column volume	1-100%
AmiditeConc		0.01-0.5 M
Base_Id	Identification of the base that gives the trityl peak - used because Last eff. only considers bases with the same ID.	
ColDiam	Sets the column diameter in mm - used in Lflow functions	1-100 mm
Cond1Alarm	Sets the alarm and warning limits for the signal from the monitor	
CV	Sets the column volume for calculation of CT flows and volume of Capping and Beaucage	1-200
CV_Cap	Column volume of capping, ½ CapA and ½ CapB	0.1-10 CV
CV_Thiolat	Sets the column volume for thiolation	0.1-1.0 CV
Cycle_Start	Marks the start point for an integration and thus where from retention is calculated	
DelayVol	The dead volume from port1 to the column inlet	0.1-10 ml
Eq_AM	Equivalents of amidites, dependent on the scale	1-10 eq
Eq_OX	Equivalents of oxidation, dependent on the scale	1-10 eq
pAlarm_Det		
pAlarm_ACN	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	

CycleStart	Indicates the synthesis cycle start in order to calculate retention, and used by Pause_at_Cycle_End to Pause a synthesis Also moves the marker in Synthesis Data in Run Control	
PeakCond1	Sets the minimum peak (MinPeak) Not to be regarded as a disturbance when using the instruction Watch, and the limits (±D_Baseline) used by the instruction Watch Stable_baseline	
PumpError	Enables\disables the alarms from the pumps	
Scale	Loading (µmol/g) * weight (g)	10-250 µmol/g and 0.1-100 g respectively
Integrate_Start	Indicates when the method can start looking for Int_Start_Level to begin integration of the trityl peak.	
CT_Thio	Sets the contact time of Beaucage	
CT_Oxidate	Sets the contact time of oxidation	
CT_Capping	Sets the contact time of capping	

B.1.4 Watch

Instruction	*Description*	*Comment*
Watch_PressDet	Watch on Pump P6000 for detritylation	0-20 bar
Watch_PressACN	Watch on Pump P6000 for detritylation	0-20 bar
Watch_Cond1	Watch on conductivity monitor	
Watch_IntStatus	Watch on integration	ON/OFF
Watch_Off	Cancels a watch on a specified monitor.	
Watch_Efficiency		-1.0-151%

B.1.5 Other

Instruction	*Description*	*Comment*
Base	Defines the base for a block for calculating breakpoints. Each block must have a defined base.	Volume Time Column volume
Block	Calls a block unconditionally.	Block name
Continue	Resumes execution of a paused or held method. This instruction has the same effect as clicking on the Cont. button in System control.	-
End_Method	Terminates method execution, equivalent to clicking on the End button in System control.	-
Evaluate	Calls an evaluation procedure. The procedure must be stored together with the current method.	Procedure name
Hold	Places the system in Hold state. This instruction has the same effect as clicking on the Hold button in System control.	-
Loop	Runs the instructions between a start and a number of loops	No of loops (1 - 9999)
Loop_end	Marks the end of a loop.	-
Message	Generates a user-defined message which is recorded in the log book and may be displayed on the screen.	"Message" Mode: Screen / Noscreen
New_ chromatogram	Opens a new chromatogram icon in the result file. All data collected after the instruction will be stored under the new icon until another New_chromatogram instruction is issued.	Chromatogram name
Pause	Places the system in the Pause state for the specified length of time.	Time (-1 (infinite) - 9999.9 in minutes)

Ready	Indicates that the next step in a process sequence may start.	-
Set_Mark	Inserts a note into both the logbook and the chromatogram during a run	Selected text
End_Block	Terminates a block and returns control to the point from which the block was called.	-
;	Inserts a comment in the method below the marked instruction.	Comment text

B.2 Manual control

B.2.1 Pump

This group contains the same instructions as the Pump group in method instructions (Section B.1.1).

B.2.2 Flowpath

This group contains the same instructions as the Flowpath group in method instructions (Section B.1.2).

B.2.3 Alarms&Monitors

This group contains the same instructions as the Alarms&Mon group in method instructions (Section B.1.3), in addition to the following:

Instruction	*Description*	*Parameters*
Efficiency_ Threshold	Can be used to set the watch on efficiency from off to on.	-
Pause_at_ Cycle_End	This sets the system to Pause at the next cycle start. Active only one time.	-

B.2.4 Other

Instruction	*Description*	*Parameters*
Block	Calls a block	Block name
Next_ breakpoint	Jump to the next breakpoint in the current method (only relevant when a method or block is running).	-
Record_on	Begins recording a run that has been started manually. A result file will be generated.	-
End_Timer	This is used to set the time out limit, in time or volume, for the End instruction.	Accumulated time or volume 0 - 9999 min or 0-9999 l or Disabled

B.3 System settings instructions

B.3.1 Alarms

Instruction	*Description*	*Comments*
Cond1Error	Enables/disables all alarms	Enabled/Disabled
Cond1Alarm	Sets the alarm and warning limits for the signal from the respective monitor	High alarm 0-99900 µS/cm Low alarm 0-99900 µS/cm High warn 0-99900 µS/cm Low warn 0-99900 µS/cm Hysteresis 0-99900 µS/cm Enabled/Disabled
Efficiency_ Threshold	Sets the threshold for an acceptable coupling efficiency. An efficiency below this threshold will pause the synthesis	Threshold 0-100% Enabled/Disabled
pAlarm_Det		

pAlarm_ACN	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	High alarm 0-20 bar Low alarm 0-20 bar High warn 0-20 bar Low warn 0-20 bar Hysteresis 0-5 bar Enabled/Disabled

B.3.2 Specials

Instruction	*Description*	*Parameters*
%Tetrazole	Percent tetrazole of the column volume	Conc 1-100% CV
AmiditeConc		Conc 0.01-0.5 M
ColDiam	Sets the column diameter in mm - used in Lflow functions	Diameter 10-100 mm
CV	Sets the column volume for calculation of CT flows and volume of Capping and Beaucage	Volume 1-200 ml
CV_Cap	Column volume of capping, ½ CapA and ½ CapB	0.1-10 CV
CV_Thiolat	Sets the column volume for thiolation	Volume 0.1-10 CV
DelayVol	The dead volume from port1 to the column inlet	Volume 0.1-10 ml
Eq_AM	Concentration of amidite	Eq 0.1-10
Eq_OX	Equivalents of oxidation, dependent on the scale	Eq 1-10

Int_Values	Sets the level where an integration starts and ends	StartLevel ø=2000 µS/cm StopLevel ø=2000 µS/cm
P50Gain	A scaling factor to calibrate the flow of the P50 pump	Gain 0.75-1.25
PumpError	Enables\disables the alarms from the pumps	ACN, Detrit and Reagent, each Enabled/ Disabled
Scale	Loading (mol/g) * weight (g)	Weight 0.1-1000 g Loading 10-250 µmol/g
CT_Thio	Sets the contact time for Beaucage	0.1-10 min
CT_Oxidate	Sets the contact time for Oxidate	0.1-10 min
CT_Capping	Sets the contact time for Capping	0.1-10 min

B.3.3 Monitors

Instruction	*Description*	*Parameters*
Cond1Keys		Keyboard Enabled/Displayed
Peak_Det Peak_ACN	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	MinPeak 0-20 bar D_Baseline 0-20 bar
PeakCond1	Sets the minimum peak (min/peak) Not to be regarded as a disturbance when using the instruction Watch, and the limits (±D_Baseline) used by the instruction Watch Stable_baseline	MinPeak 0-99900 µS/cm D_Baseline 0-99900 µS/cm

B.3.4 Curve

Instruction	*Description*	*Comment*
Cond1	Set the specific signal curve on/off	ON / OFF
PressDet	for storing in the Result file. The	
Press ACN	time between samples determines	Time between
	the frequency with which curve data	samples
	is recorded	0->1.000 s

B.3.5 Method variables

Variable	*Units*	*Description*
Col_Diam	mm	Column diameter for linear flow rates
Column_Volume	ml	Column volume for text method
Conc_of_DNA_		
amidite	M	Sets the DNA amidite concentration
Conc_of_RNA_		
amidite	M	Sets the RNA amidite concentration
CT_Oxidation_DNA	min	Sets the oxidation contact time
CT_Oxidation_RNA	min	Sets the oxidation contact time
CT_Thio	min	Sets the Beaucage reagent (thiolation) contact time
CT_Capping	min	Sets the capping reagent contact time
CV	ml	Column volume for calculation of functions in the strategy
CV_CT_Capping	CV	Sets the number of column volumes that the CT_Capping will last
CV_CT_Ox_DNA	CV	Sets the number of column volumes that the oxidation will last

CV_CT_Ox_RNA	CV	Sets the number of column volumes that the oxidation will last
CV_Capping	CV	Sets the number of column volumes of capping reagent
CV_Capping_wash	CV	Sets the number of column washes after Capping contact flow
CV_Column_wash	CV	Number of column volumes used in column wash block
CV_Coupling_wash	CV	Number of column volumes of wash after coupling
CV_detrit_wash	CV	Number of column volumes of detrit wash
CV_tetrazole_DNA	%	Sets the % of the column volume for tetrazole in DNA cycles
CV_tetrazole_RNA	%	Sets the % of the column volume for tetrazole in RNA cycles
CV_Thio	CV	Sets the number of column volume after of Beaucage reagent
Detrit_flow	cm/h	The linear flow used in detritylation block
Detrit_Pressure_Flow	bar	Maximum flow pressure of detrit
Efficiency_threshold	%	Minimum acceptable threshold efficiency
Eq_oxidation_DNA	eq	Number of equivalents of oxidation
Eq_oxidation_RNA	eq	Number of equivalents of oxidation
Eq_DNA_amidite	Eq	Number of equivalents of DNA amidite
Eq_RNA_amidite	Eq	Number of equivalents of RNA amidite
Loading_of_support	µmol/g	To calculate the scale
Recycle_Time	min	Sets the time of recycling of amidite and tetrazole
Weight_of_support	g	To calculate the Scale

C Evaluation functions and instructions

This appendix describes the functions implemented in the evaluation module. There are four sections in the appendix:

C.1 describes how the smoothing functions are calculated

C.2 gives an basic introduction into baseline calculation theory which is an essential part of peak integration

C.3 describes the peak table column components

C.4 the Procedure Editor instruction types are described which are used to build up an evaluation procedure

C.1 Smoothing algorithms

C.1.1 Moving Average

For each data point in the source curve, the processed curve is calculated as the average of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect.

The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

C.1.2 Autoregressive

The first data point in the source curve is copied to the processed curve. For each subsequent data point, the previous processed point is multiplied with the parameter value and added to the current source data point. The result is then divided by the parameter value plus 1 according to the following formulae:

$$t_1 = S_1$$

$$t_n = \frac{(p * t_{n-1} + S_n)}{(p + 1)}$$

where

t_n = current processed point

t_{n-1}= previous processed point

S_n = current source point

p = smoothing parameter value

Increasing the parameter value increases the smoothing effect. The filter algorithm accepts integer parameter values between 1 and 25.

C.1.3 Median

For each data point in the source curve, the processed curve is calculated as the median of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect. To completely remove a noise spike, the window width should in principle be slightly more than twice the width of the spike.

The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

C.2 Baseline calculation theory

The baseline calculation can schematically be described in three steps:

1. Defining baseline segments
2. Select baseline points
3. Draw the baseline.

C.2.1 Defining baseline segments

In the first step, baseline parameters are used to find the baseline segments. The parameters can be seen in the **Integrate:Calculate baseline** function or by pressing the **Baseline settings** button in the **Integrate:Peak integrate** function. The default values for the parameters are determined from the source curve.

The baseline segments are found by different parameters based on the type of algorithm selected.

Morphological algorithm

The **Morphological** algorithm searches for all parts of the source curve which:

1. come into contact at both extremes of the **Structure width**.

   This parameter is based on the widest detected peak in the curve.

2. fulfils the **Minimum distance between data points**.

   This parameter reduces the total number of data points created from a curve.

Classic algorithm

The **Classic** algorithm searches for all parts of the source curve which:

1. are longer than the **Shortest baseline segment**.

   This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.

2. have no point outside the **Noise window**.

   The noise window is defined as a rectangular corridor parallel to the slope of the curve and centred on the first and last points within the currently inspected segment.

3. slope less than the **Slope limit**.

   This limits the maximum slope of the baseline to differentiate baseline segments from peaks.

4. are lower than the **Max baseline level**.

Determines the highest acceptable signal level for the baseline. This parameter is by default set to have no influence on the baseline calculation and is seldom necessary to adjust.

The parameters can be illustrated as a rectangular box in which the source curve has to fit to be identified as a baseline segment, see Figure D-1. The length of the box corresponds to the **Shortest baseline segment** and the height of the box corresponds to the maximum level of noise on the baseline segments and is referred to as the **Noise window**.

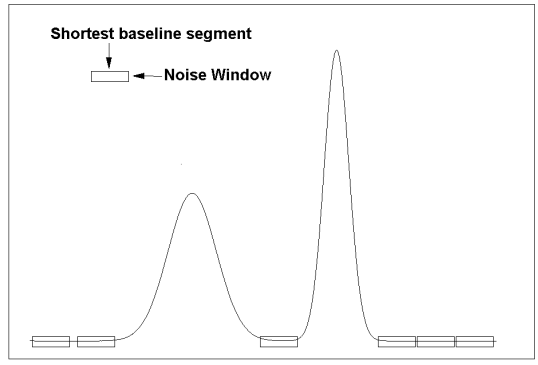


Figure C-1. Baseline box with Shortest baseline segment and Noise window.

The rectangular box is allowed to be tilted with a maximum slope corresponding to the **Slope limit**, see Figure D-2. The box is not allowed to move up above the **Max baseline level**.

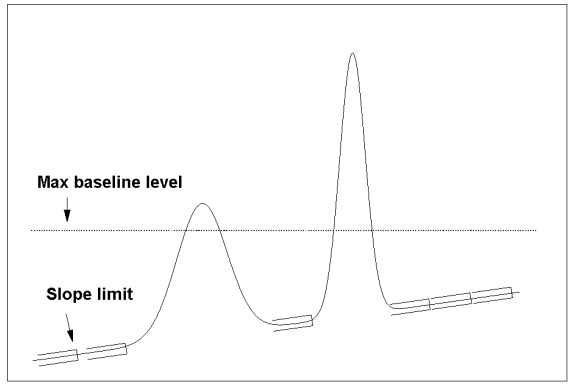


Figure C-2. Slope limit and Max baseline level.

When looking for baseline segments, the box is virtually moved along the source curve in steps of 1/3 of the **Shortest baseline segment**. A baseline segment is found whenever the currently examined part of the source curve fits completely within the box.

The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the **Slope limit**.

C.2.2 Selecting baseline points (for Classic algorithm)

In the second step, the baseline segments are replaced by a large number of baseline points. A baseline point is placed at the start and end of each segment. The line between these will also be filled with points. The baseline points are shown as pale blue crosses in the **Integrate:Edit baseline** function (see Section 10.1.5).

C.2.3 Drawing the baseline

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points, but the curve does not necessarily pass through them. The baseline will thus be a smoothly curved function passing close to or through the points. To reduce the effect of noise on the peak integration, the created baseline is adjusted by forcing it equal to the source curve in every position where the difference between the baseline and the source curve is small enough. If the **Accept negative peaks** option (see Section 10.1.4) is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

C.2.4 Estimating the baseline parameters from the source curve (for Classic algorithm)

The baseline parameters can sometimes be difficult to set. Rough estimates can be found by analysing the source curve.

Measuring the Shortest baseline segment using curve co-ordinates

If you are uncertain of the length of the **Shortest baseline segment**, you can try to measure it directly on your chromatogram. Locate the shortest segment of the curve that you consider as a part of the baseline and measure the length of the segment using the **XY** box on the chromatogram (see Section 10.1.7). Insert this value as the **Shortest baseline segment** value.

Measuring the noise level using curve co-ordinates

As for measuring the Shortest baseline segment, curve co-ordinates can be used in exactly the same manner to measure noise levels on the source curve. First use the Zoom function to select a part of the curve representative of the baseline noise. Measure the Y-axis co-ordinates, using the appropriately selected Y-axis scale, to calculate the noise range as the difference between the max. and min values. Add an extra 20% and insert this value as the **Noise window** value.

C.2.5 Measuring the Slope limit using Differentiate and curve co-ordinates (for Classic algorithm)

To measure the slope at any point on the curve:

1. Select **Operations:Differentiate**. A dialogue will appear.

2. Select the desired source curve, check that a **First order calculation** is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

3. Measure the Y-axis values on the differentiated curve using the **XY** curve co-ordinates function. Remember to select the appropriate Y-axis scale. Any Y-axis value is interpreted as the UV curve slope at the selected retention point.

   If the differentiated curve is very noisy, it can be filtered using a light Moving average filter in the **Operations:Smooth** function (see Section 9.3.2)

4. Determine the highest slope value of the baseline (non-peak) part of the curve. Add 10% and insert this value as the **Slope limit**.

C.3 Peak table column components

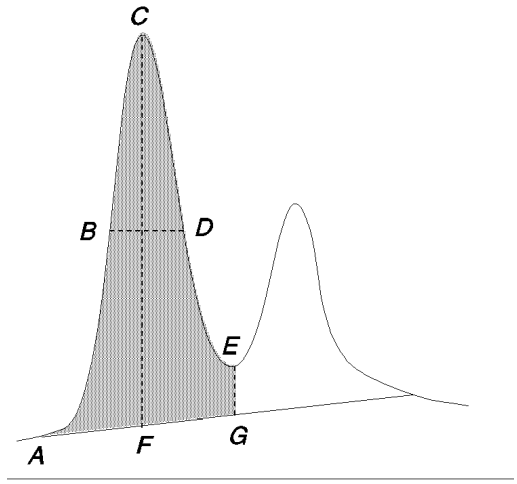


Figure C-3. Diagram illustrating peak parameters. See the parameter list below for explanations.

Peak name	Name of peak.
Retention	(time or volume base) Retention at the peak maximum (C in Figure C-3).
Width	(time or volume base) Difference in retention between the peak end and peak start (G-A in Figure C-3).
Area	(time or volume base) Calculated as the area between the curve and baseline, between the peak start and peak end (shaded in Figure C-3).
Height	Maximum amplitude above the baseline (C-F in Figure C-3).
Peak endpoint retention	(time or volume base) Retention value at peak start and peak end (A, G in Figure C-3).
Width at half height	(time or volume base) Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above baseline (D-B in Figure C-3, where BD bisects CF).

Percent of total area	(time or volume base) Peak area as a percent of the total area under the curve above the baseline. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Percent of total peak area	(time or volume base) Peak area as a percent of the sum of all integrated peaks. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or drop-line to the baseline.
Peak endpoint heights	Amplitude above the baseline at left (A in Figure D-3) and right peak limits (E-G in Figure C-3).
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in Figure C-3).
Sigma	Standard deviation for a Gaussian-shaped peak. For definition see below*
Resolution	Peak resolution. For definition, see Section 10.1.10 and below**.
Capacity factor	For a definition, see below ***. The Capacity factor will only be calculated when the chromatogram is in volume base. The total liquid volume, Vt, must be entered in the **Integrate** dialogue for this parameter to be calculated.
Kav	Gel phase distribution constant in gel filtration. For definition, see below ****. Kav will only be calculated when a gel filtration column was used and when the chromatogram is in volume base. The void volume, V0, must be entered in the **Integrate** dialogue for this parameter to be calculated.
Plate height (HETP)	Height equivalent to theoretical plate and plates/ metre. The column height must be entered in the Integrate dialogue for this parameter to be calculated. For definition, see Section 10.1.8 and below‡.
Asymmetry	Peak asymmetry (indicator of column packing). For definition, see Section 10.1.9 and below§.

Concentration	Values calculated by the Analysis module. For further details see the Analysis for UNICORN 3.10 User Manual.
Amount	As above
Molecular size	As above

***Sigma**

$$\text{Sigma} = \sqrt{\frac{(x_n - x_1)}{n - 1} \frac{\sum_{i=1}^{n} \left(y_i \, (x_i - x_{ymax})^2 \right)}{A_{peak}}}$$

where:

n is the number of data points

x is the volume or time value

y is the amplitude value

x_{ymax} is the volume or time value at the maximum amplitude value

A_{peak} is the area of the peak

The peak width for a Gaussian peak is (4 x Sigma).

****Resolution**

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

V_{R1} = retention volume for peak 1

V_{R2} = retention volume for peak 2

w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)

w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

The peak resolution is calculated with one of the following three algorithms:

1) $(V_{R2} - V_{R1})/((W_{b2} + W_{b1})/2)$

2) $(V_{R2} - V_{R1})/((Sigma_2 + Sigma_1) \times 2)$

3) $(V_{R2} - V_{R1})/(2 \times (W_{h2} + W_{h1})/2.354$

where V_{R1}, W_{b1}, $Sigma_1$ and W_{h1} are the retention, width, sigma and width at half height of the previous peak, and V_{R2}, W_{b2}, $Sigma_2$ and W_{h2} are the retention, width, sigma, and width at half height of the current peak, respectively. The UNICORN.INI variable (EVAL) ResolutionAlg determines which of the three algorithms is actually used. If this variable has the value 1, 2, or 3, then the algorithm used corresponds to the numbered list above. If the variable has the value 0, or if the varible is not defined or has a value other than 0, 1, 2, or 3, then the default (3) algorithm is used.

To change the peak resolution algorithm, edit the UNICORN.INI file by:

1. Minimize UNICORN and locate the file UNICORN.INI within C:\UNICORN\BIN.

2. Open the file and locate the following line:

   ```
   EVAL ResolutionAlg
   ```

   If the line does not exist then add it before the "`Begin`" line.

3. Choose the desired value for the algorithm.

4. Save the file.

Note: Do not make any changes in the UNICORN.INI file between the lines "`Begin`" and "`End`" as this may severely affect the functionality of UNICORN.

***** Capacity factor**

$$k' = \frac{V_R - V_t}{V_t}$$

where:

V_R = retention volume

V_t = total liquid volume

****** K_{av}**

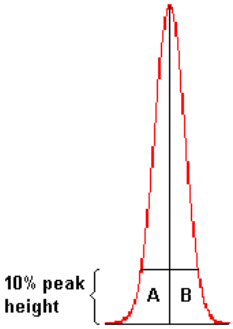
$$k_{av} = \frac{V_R - V_0}{V_C - V_0}$$

where:

V_R =retention volume

V_0 = void volume

V_C = column volume



§Asymmetry

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.

‡ HETP

HETP = L/N

$N = 5.54 \times (V_R/w_h)^2$

where

N = no. of theoretical plates

L = bed height in cm

V_R = peak retention volume or time

w_h = peak width at half height expressed in the same units as V_R

Plates/meter is the number of theoretical plates per meter, N x (100/L)

C.4 Evaluation procedure

C.4.1 Curve operations

Instruction	*Description*	*Parameters*
ADD	Adds two curves to gain a third curve which is the sum of the two curves. The two source curves must have the same y-axis unit and not be fraction or injection curves or else a run time error will occur.	First source curve Second source curve Target curve position
AMP_MUL	Multiplies the amplitude of the source curve by the multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor
AMP_SHIFT	Shifts the amplitude of the source curve by the shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor
CLEAR	Clears specified curve from the working memory of the computer.	Source curve
COPY	Copies the source curve to target curve position.	Source curve Target curve position
CUT	Cuts out the part of the source curve between Left and Right Limits and stores the result in the target curve position.	Source curve Target curve position Left limit Right-limit
DERIVATE	Differentiates the source curve (first or second order) and stores the result in target curve position. The y-axis of the target curve position will be a normalised scale without unit.	Source curve Target curve position First Order or Second Order

DIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any y-axis unit. The y-axis of the target curve position will be a normalised scale without unit.	First source curve Second source curve Target curve position
HISTOGRAM	Creates a histogram from any non-fraction curve (source curve 1) and a fraction curve (source curve 2_frac), and stores the result in the target curve position. If source curve 2 is not a fraction curve a run time error will occur. The y-axis of the target curve position will be the same as that of the first source curve.	First source curve Second source curve Target curve position
INTEGRATE	Performs a mathematical integration of the source curve and stores the result in Result curve. This instruction is not the same as Peak integrate which performs a real peak integration.	Source curve Target curve position
POOL_ FRACTIONS	Pools fractions from the source curve and stores the result in the target curve position. The fractions are pooled from the first selected fraction to the last selected fraction. If source curve is not a fraction curve, or First or Last is not an existing identification, a run time error will occur.	Source curve Target curve position First fraction to pool Last fraction to pool
RET_MUL	Multiplies the retention of the source curve by the Multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor
RET_SHIFT	Shifts the retention of the source curve by the Shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor

SMOOTH_AR	Smooths source curve with an autoregressive filter and stores the result in target curve position. The Filter parameter decides the strength of the filter.	Source curve Target curve position Filter
SMOOTH_MA	Smooths the source curve with a moving average filter and stores the result in Resulting Curve. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SMOOTH_MEDIAN	Smooths the source curve with a median filter and stores the result in target curve position. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SUB	Subtracts two curves to gain a third curve which is the difference of the two curves. The two source curves must have the same y-axis unit and not be fraction or injection curves.	First source curve Second source curve Target curve position
TDIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any y-axis unit. The threshold values are used to avoid division of numbers close to zero. At those points where source curve 1 has amplitude less than Threshold1, or source curve 2 has amplitude less than Threshold2, the result of the division is defined to be 1.0. The y-axis of the curve will be the same as that of the first source curve.	First source curve Second source curve Target curve position Threshold1 Threshold2

C.4.2 Integration

Instruction	*Description*	*Parameters*
CALCULATE_ BASELINE	Calculates a baseline from the source curve. The baseline is stored in the target curve position. DEFAULT can be selected in the Baseline parameters which will then calculate default baseline parameters for each new curve.	Source curve Target curve position Noise Window Shortest baseline segment Slope limit Max baseline level
CALCULATE_ BASELINE_ MORPH	Calculates a baseline from the curve crvSrc using a morphological method. The baseline is stored in curve crvDst.	Source curve Destination curve Noise Window Width Distance Between Points
CLEAR_ PEAKTABLE	Clears the peak table in Peak table source from the working memory of the computer.	Peak table source
COPY_ PEAKTABLE	Copies a peak table from Peak table source to Resulting peak table.	Peak table source Resulting peak table
NEGATIVE_ PEAKS	Controls the baseline behaviour in subsequent baseline calculations. If OnOff is ON then the baseline may be drawn above the curve and negative peaks may be detected by PEAK_INTEGRATE. If OnOff is OFF then the baseline is never drawn above the curve.	OnOff
PEAK_ INTEGRATE	Performs a peak integration on the source curve and stores the resulting peak table in Resulting peak table. It is assumed that the baseline is sub-tracted.	Source curve Resulting peak table

PEAK_ WINDOW	Specifies which part of the source curve that will be integrated. Peaks between retention Left limit and Right limit will be detected if the OnOff parameter is set to On. If OnOff is set to Off, the whole curve will be used for integration.	Source curve Left limit Right limit OnOff
REJECT_ PEAKS	Any combination of conditions is allowed. If all parameters are OFF then every detected peak are included in the peak table.	Height less than Width less than Width more than Area less than Peak must be one of xx largest
SET_ COLUMN_ HEIGHT	Sets the column height for the peak integration calculation of the HETP value. The Column height parameter is the height of the column in centimetres. If Column height is OFF then the HETP value is not calculated for the following integrations.	Column Height
SET_ COLUMN_V0	Sets the void volume for peak integration calculation of Kav.	Void volume
SET_ COLUMN_V T	Sets the total liquid volume for peak integration calculation of the capacity factor.	Total liquid volume
SET_ SKIM_SIZE_ RATIO	Sets the Skim size ratio to be used in the following peak integration(s)	Ratio

C.4.3 File Operations

CURVE_ OPEN	Opens the curve specified in the Result file defined in File name and stores it in target curve position. If "*" is entered as File name the current result file will be used. The File name parameter may include a path from the users root folder.	File name Curve name Target curve position

IMPORT_ CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name source curve Target curve position
IMPORT_ PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name Peak table source Resulting peak table
PEAKTABLE_ OPEN	Opens the specified Peak table in the Result file defined in File name and stores it in the Resulting peak table. If "*" is entered as File name the current Result file will be used. The File name parameter may include a path from the current users root folder.	File name Peak table name Resulting peak table

C.4.4 Export

Instruction	*Description*	*Parameters*
EXPORT_ CURVE_ ASCII	Exports the Source curve to the file defined in Export to File in ASCII format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ CURVE_W KS	Exports the source curve to the file defined in Export to File in WKS format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ EVAL_LOG _ ASCII	Exports an evaluation log in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ EVAL_LOG _ WKS	Exports an evaluation log in WKS format to the file defined in Export to file.	Export to file

EXPORT_ EVAL_LOG _ XLS	Exports an evaluation log as XLS format to the file defined in Export to file.	Export to file
EXPORT_ METHOD_ ASCII	Exports a method to the file defined in Export to file in ASCII format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ METHOD_ WKS	Exports a method to the file defined in Export to file in WKS format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ METHOD_ XLS	Exports a method to the file defined in Export to file in XLS format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ MULTI_ CURVES_ ASCII	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ MULTI_ CURVES_ WKS	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in WKS format to the file defined in Export to file.	Export to file
EXPORT_ MULTI_ CURVES_X LS	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in XLS format to the file defined in Export to file.	Export to file
EXPORT_ PEAKTABL E_ASCII	Exports the peak table in Peak table source to the file defined in Export to file in ASCII format.	Peak table source Export to file

EXPORT_ PEAKTABL E_WKS	Exports the peak table in Peak table source to the file defined in Export to file in WKS format.	Peak table source Export to file
EXPORT_ PEAKTABL E_XLS	Exports the peak table in Peak table source to the file defined in Export to file in XLS format.	Peak table source Export to file
EXPORT_S EL_ CURVES	Selects a curve for subsequent export (using the EXPORT_MULTI-CURVES_* instruction). The curve is cut according to the right and left cut limit and the number of points to be exported may be set by the Export every (for example, fifth point) parameter.	Source curve Left cut limit Right cut limit Export every
EXPORT_ DOC_WKS	Exports the documentation in the current result file in WKS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Proto-col ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibration ONOFF LogBook ONOFF ResultName ONOFF Column Parameters NAME Export to file

EXPORT_ DOC_XLS	Exports the documentation in the current result file in XLS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibra tion ONOFF LogBook ONOFF Result Name ONOFF Column Info ONOFF BufferPrep NAME Export to file
EXPORT_ DOC_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibra tion ONOFF LogBook ONOFF Result Name ONOFF Column Parameters NAME Export to file

C.4.5 Chromatogram functions

Instruction	*Description*	*Parameters*
COPY_ CHROM	Creates a copy of the specified chromatogram. If "*" is used as source then the current (default) chromatogram is used. If "*" is used as destination then a default name will be created for the copy.	From chromatogram name To chromatogram name
CREATE_ NEW_ CHROM	Creates a new chromatogram with the given name. If "*" is used for the chromatogram name a default name will be generated and used.	Name
DELETE_ CHROM	Deletes the named chromatogram. If trying to delete the current (default) chromatogram a run time error will be caused.	Chromatogram name
OPEN_ CHROM	Opens the specified chromatogram from the specified file.	File name Chromatogram name
RENAME_ CHROM	Renames the specified chromatogram. If "*" is used as From then the current (default) chromatogram is used.	From chromatogram name To chromatogram name
RESTORE_ DESTINATION _CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used in pair with the SET_DESTINATION_CHROM instruction.	
SET_ DESTINATION _CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used in pair with the RESTORE_DESTINATION _CHROM instruction.	Chromatogram name

C.4.6 Other

BASE	Sets the x-axis base in which the following calculations will be done in. If the value of x-axis base is DEFAULT then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure otherwise it will have no effect at all.	X-axis base
COMMENT	Inserts a comment below the marked instruction	Comment text
ENDLOOP	Marks the end of a LOOP statement.	
LOOP	The instructions between this statement and the ENDLOOP statement are repeated n times. It is possible to have loops within loops as long as the number of LOOP statements matches the number of ENDLOOP statements.	n Number of loops
REPORT	Prints a report with the specified named report layout and title. If Title is "*" then the title in the report layout is used. If ReportLayout is "*" then a default layout is used.	Report layout Report title
RUN_ PROGRAM	Starts a program as a separate process. The Program name string contains the program name and parameters to start it with.	Program name

D Feedback tuning

Some systems allow you to perform PID feedback tuning of the pump flow rate using the **System:Tune** command in System control. Other systems allow you to perform tuning with the insertion of a tune instruction in the method.

This appendix describes the principles of PID feedback tuning of pump flow rate to available systems. UNICORN process control software provides a graphical interface for feedback tuning.

Feedback control is aimed at eliminating discrepancies between the actual value and the requested value (in this case flow rate). OligoPilot II and OligoProcess use the measured flow rate to control overall pump speed and the measured conductivity to control the relative speeds of pumps A and B in gradient formation.

PID tuning uses three parameters to determine feedback control:

- The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable discrepancy between actual and requested flow rate (*a stationary error*).

- The I parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow. I can have values between 0 and infinity, where smaller values have greater effect and a value of infinity has no effect. (The value infinity is set as 9999 in UNICORN).

- In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have greater effect and a value of 0 has no effect. Most often, a simple PI-regulator is preferable for control of flow rate, and OligoProcess System is configured by default with the D parameter set to zero.

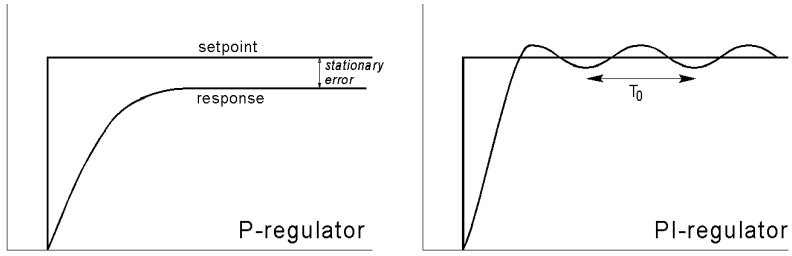


Figure D-1. A simple P-regulator (left) gives a stationary error. A PI-regulator (right) eliminates the stationary error but introduces stable oscillations in the response.

Tuning a feedback control system in practice is largely a matter of trial and error. The following recommendations summarize the Ziegler-Nichols method for finding suitable PID-values (small empirical adjustments in the suggested values may be required for optimal feedback control).

D.1 Flow rate tuning

1. Make sure that the flow path is open.

   Select **System:Tune** in System control. Select **Flow for Feedback Control**. Open the valves and start the pump with the column in-line.

2. Set P=0.05, I=9999 and D=0. Set the flow rate in **Setpoint**. Press **New parameters** and the new values will apply.

3. Note the response. Increase the value of P until the actual flow rate oscillates with a constant period and amplitude.

Note: When changing to new PID values, set the new values and flow rate and press **New parameters**. The flow rate must be changed in **Setpoint** every time the PID values are changed.

When the oscillation is satisfactory, note the P value (P0) and the oscillation period in seconds (T0).

4. Calculate suggested PID values for the required regulator type from the table below.

Regulator type	P	I	D
P	$0.5 * P_0$		
PI	$0.45 * P_0$	$0.83 * T_0$	
PID	$0.6 * P_0$	$0.5 * T_0$	$0.125 * T_0$

5. Adjust the PID parameters from these suggested starting values until the feedback behaviour is satisfactory.

6. When satisfied, press **Save** to save the PID parameter settings.

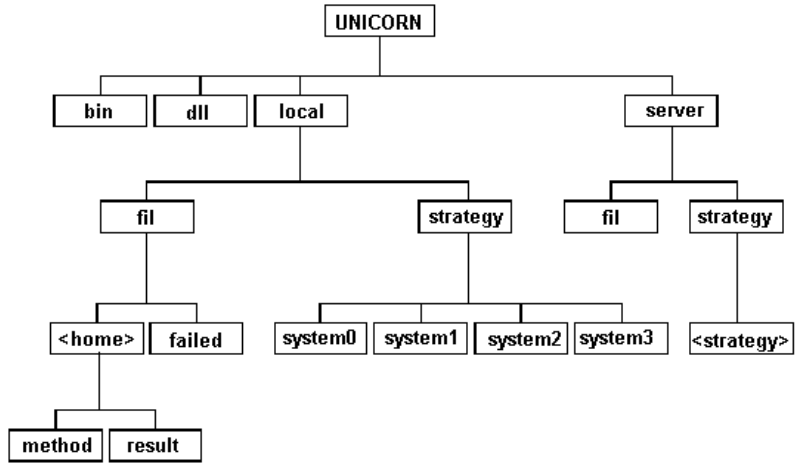
During tuning adjustments, UNICORN displays the effects of the current parameters graphically. The **Output** signal is the signal to the pump. The **Input** signal is the actual flow rate.

D.2 Gradient tuning

Tune gradient settings in the same way as flow rate settings, with the exception that the **Setpoint** parameter is %B. The **Input** signal is the actual eluent concentration (%B) as determined by the conductivity.

E File organisation

This appendix documents the file structure in a UNICORN installation.



E.1 Stand-alone installations

In a stand-alone installation, the entire folder structure resides on the local hard disk. System and user definition files and system strategies are duplicated in the local and server folders.

E.2 Network installations

E.2.1 Local and remote computers

The bin, dll and local folder structures are installed on each computer.

E.2.2 Network server

The server folder structure is installed on the network server. The local\fil folder structure is also installed if home folders are created on the server disk.

System and user definition files and system strategies are copied automatically from the network server to each station, so that local stations can be used as stand-alone systems in the event of a network communication failure.

folder	*Description*
UNICORN	The main UNICORN folder.
– bin	Executable modules.
– dll	Device drivers and dynamic linked library (dll) modules. the drivers are installed in the NT system folder for device drivers: \winnt\system32\drivers
– local	Holds a copy of the global system definitions (system.tab). Not available on remote computers.
– – fil	Holds a copy of the files containing user definitions (user30.mpm), Column list (columns.cmn) and BufferPrep recipes (global.rcp, comp.rcp). Not available on remote computers. Global audit trail files are also stored here.
– – – <home>	Home folders for users in the system. There may be any number of home folders in the system. In network installations, home folders may be created on the server disk as well as on local disks.
– – – – method	Holds method files, MethodQueue folders and user-created method folders.
– – – – result	Holds result files, scouting result folders and user-created result folders.
– – – failed	Holds result files in the event of network communication failure.
– – strategy	Holds sub-folders for each system physically connected to the local computer.
– – – system0 – – – system1 – – – system2 – – – system3	System information for systems physically connected to the local computer. Holds a copy of the strategy, flow scheme and templates, and files for the system settings and system audit trail. folder system0 corresponds to installed System 1. Folders for systems that are not installed are empty.
– server	Holds the original file for system definitions (system.tab). This file is copied to the local folder.

– – fil	Holds the original files for user definitions (users30.mpm), global procedures (globproc.gpl), global report formats (globproc.grf), BufferPrep recipes (global.rcp, comp.rcp) and the column list (columns.cmn). The user definition file, Column list and BufferPrep recipes are copied to the local\fil folder.
– – strategy	Holds the folder structure for installed strategies.
– – – <strategy>	Holds the original files for installed strategies. There is one <strategy> folder for each installed strategy. Each <strategy> folder also holds the corresponding template methods (if installed). Strategy files are copied to the local\strategy folder according to the systems installed on the local station.

F Troubleshooting

F.1 Logon problems

F.1.1 Unable to log on to UNICORN

Choose your username from the list and enter your password. If you have forgotten your password, ask the system administrator for a new one.

If you cannot log on using your correct username and password, the USERS30.MPM file in the \UNICORN\SERVER\FIL folder may be corrupt. Restore the file from the latest back-up copy or reinstall the default user (see Section 13.4.2).

If users are not available on a remote station in a network installation (the user list in the **Logon** dialogue box is empty), make sure that the computer is logged on to the network before starting UNICORN. A remote station accesses the user list directly from the network server.

If the user list on a local station in a network installation is not up to date, make sure that the computer is logged on to the network before starting UNICORN. The user list is stored locally on a local station, and is updated automatically from the network server if the computer is logged on to the network.

F.1.2 Error message "Strategy file error"

If you receive the error message "Strategy file error. Can't load strategy" in a stand-alone installation, the strategy file is probably corrupt. Reinstall the strategy as described in Section 13.4.2.

In a network installation, the error may appear if you try to create a method for a system not physically connected to the computer. Make sure that the computer is logged on to the network before UNICORN is started so that the strategy file on the server disk is accessible.

F.2 UNICORN access problems

F.2.1 Unable to access certain UNICORN functions

UNICORN functions to which you do not have access appear grey in the menu and cannot be used. Your user profile is determined by the system administrator from **Administration:User setup** in the main menu.

F.2.2 Connections are not available

Check the connection between the PC and the chromatography system. Check that the power to the chromatography system is turned on. If the connection appears to be correct and the power is turned on, switch off the chromatography system and quit UNICORN. Shut down and switch off the computer, then restart the entire system.

If a system is not available when you attempt to establish a connection, check that you have access rights to the system. Access rights are not automatically assigned for a newly defined system.

If you receive the error message "Cannot connect to system ..." in a network installation, check:

- that the local computer to which the system is connected is turned on and logged on to the network.
- that the computer from which you are trying to establish a connection is logged on to the network.
- that the limit of 8 connections to the system has not been exceeded.

If you can establish a connection but cannot control the system (the manual menu commands in system control are grey), check that no other user has a control mode connection, and that you have sufficient access rights to control the system manually.

F.2.3 Run data Connection in System control displays a "No x"

In System control, if the Run data option **Connection** says "No 1" or "No 2":

- Check that the UNICORN PC Control board is configured according to the settings made during the installation of the program, i.e. the same Control unit number, Address and IRQ must be set at the Control board (see Chapter 13.4).
- The communication may also fail if the UNICORN PC Control board configurations conflict with other boards in the PC. If this is the case select a free Address and a free IRQ during UNICORN installation and at the Control Board (see Chapter 13.4).

If the Run data option **Connection** says "No 3" there is no contact with the OCI:

- In Main menu select **Administration:System Setup**. In the dialogue select the system with problems and click on Edit. Check that the strategy, pipe server name and the control number are correct according to installation at the local station physically connected to the system (see Section 14.1)
- If connecting to a system remotely check that the local station (physically connected to the system) is turned on and that the network is functioning at both the remote and the local station.
- Check that the limit of eight connections to the system has not been exceeded.

F.3 Method and run problems

F.3.1 Cannot Quit or Logoff from UNICORN

If you are unable to Quit or Logoff from UNICORN for a connection, you may be running a scouting method or a MethodQueue. These functions require a control mode connection in order to start subsequent cycles correctly.

F.3.2 Monitor signals do not appear in the system control Curves panel

For monitor signals to be displayed in system control, they must be set to STORE ON in system settings.

Signals for which STORE ON is set can be chosen from the **View:Curve contents** dialogue box for display in the curves panel.

F.3.3 Error message "Couldn't create result file"

If you receive an error message "Couldn't create result file... Destination path could not be found" at the end of a method, the local computer was unable to access the folder specified in the result file path. This may arise if the specified folder is on the network server and network communication has been lost (see Section 11.3.1). The result file is saved in the FAILED folder on the local station.

F.3.4 The Method-System Connection dialogue keeps appearing

If the **Method-System Connection** dialogue keeps appearing you have some method(s) that you have not connected to a system, most likely from imported methods using the **Copy from external** function in the Main menu (see Section 3.3.5). Connect the method(s) to the appropriate system and the dialogue disappears.

F.3.5 The method editor window does not fit on the screen

If the Method editor window does not fit on the screen and has scroll bars, you may have the incorrect font size installed. For pre-installed Windows NT 4.0 the display screen resolution is set at "1024x768x65536" with "Large fonts". You need to install the "Small fonts", which requires that you have available the Windows NT Workstation 4.0 CD-ROM shipped with your Compaq computer. Insert the CD-ROM and follow the directions on the screen.

Note: Be sure to always install the latest NT4 service pack after installing something from the Windows NT 4.0 CD-ROM.

F.3.6 There are red instructions in a method

Red instructions, i.e. instructions with a red dot, in a method are syntax errors and may be due to the following:

- The method was connected to a wrong system, i.e. the strategy of the system is incompatible with the method.
- The method instructions do not correspond to the components you have chosen for your system. Check your system components under **Administration:System Setup:Components** in Main menu.
- The **Copy** function was used instead of **Copy from external** when importing a method from a diskette.
- The wrong system may have been selected in the **Save As** dialogue in Method editor.
- You may also have templates not intended for your system, which often happens for custom designed systems.

There are several actions that you can take:

- Check that the method has been connected to the correct system, either in the **System Method Connection** dialogue when using the **Copy from external** dialogue or in the **Save As** dialogue in Method editor.
- For custom designed systems go to the Method editor, 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.

F.3.7 I've logged out of Windows NT and then logged in again but I can not get system connection in UNICORN (only for local systems, not remote)

If you shut down Windows NT using the command **Start:Shutdown: Close all programs and log in as a different user**, you will not be able to obtain a system control connection in UNICORN the next time you or another user logs on. This is because the aforementioned shutdown procedure automatically shuts down a number of processes, including those needed for system connection, that are only started when the computer is booted up. In other words, you must restart the computer in order to obtain a system connection in UNICORN.

F.3.8 Print screen does not send a copy of the screen to the printer

Print screen only makes a copy of the screen to the clipboard and not to the default printer. If you wish to make a print out of the view on the screen, press the <Print Scrn> key and paste the image from the clipboard into an appropriate program, such as Microsoft®Paint, and then print out the image.

F.4 Evaluation problems

F.4.1 Incorrect date and time

The date and time recorded in the result file are taken from the PC system clock setting. If these are not correct, check the system clock setting.

F.4.2 Evaluation procedure aborts

Instructions in an evaluation procedure address curves by identification number irrespective of curve names. Make sure that the curves processed when the procedure is executed are compatible with those processed when it was recorded. An evaluation procedure aborts if you try to store resulting curves at the position of an original raw data curve.

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