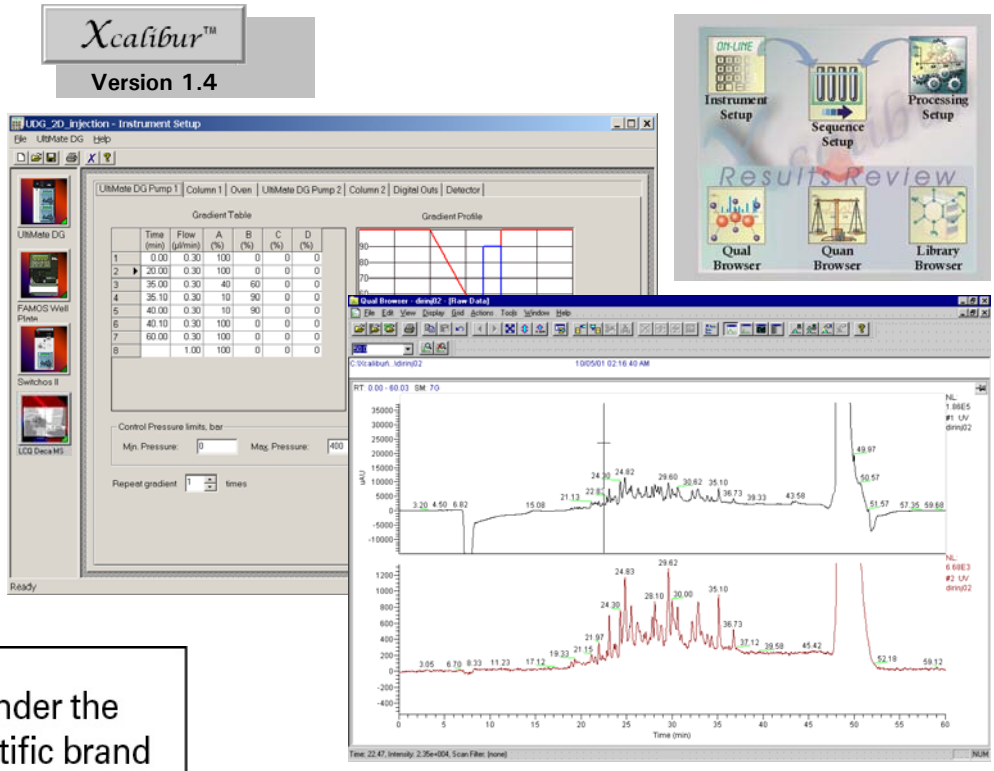
VI Plug-ins for Xcalibur®

User's Manual

P/N 161106



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CHAPTER 1

Introduction

1.1 Features of the Virtual Instrument Plug-ins for Xcalibur™

The LC Packings Virtual Instrument (VI) Plug-ins allows full control of all modules of the LC Packings UltiMate™ System via Thermo Electron's Xcalibur single-platform mass spectrometer control software. The following modules are supported by the VI Plug-ins:

- **UltiMate or UltiMate Plus** Capillary- and Nano HPLC System.
- **UltiMate or UltiMate Plus** Dual Gradient Capillary- and Nano HPLC System.
- **FAMOS**™ Well Plate Microautosampler.
- **FAMOS**™ Carousel Microautosampler.
- **Switchos II**™ Advanced Microcolumn Switching Unit.
- **Peak Parking Kit**.

These specialized software modules have been designed to work with the Windows® NT-based Xcalibur software package to provide a unique combination of functionality, power and ease-of-use. They provide the following features:

- Single-point control of all instruments.
- Fully automated.
- Full control of all instrument functions, including flow sensor support.
- Acquisition and display of high resolution UV data.
- Up to 4 different user-selectable wavelengths can be recorded simultaneously.
- Monitoring separation column and trap column pressure.

1.2 System Requirements

The plug-ins provided with the CD 'Virtual Instrument Plug-ins for Xcalibur V1.3 / V1.4, Version 2.0, May 2004' support all LC Packings UltiMate system configurations. The instrument firmware versions and Xcalibur VI plug-in version listed in TABLE 1-1 need to be installed to fully control the LC Packings UltiMate HPLC System, the FAMOS Microautosampler and the Switchos II Advanced Microcolumn Switching Unit from the Xcalibur V1.4 or V1.3 software package.

TABLE 1-1 Required Instrument Firmware and Xcalibur VI Plug-in Requirements

Instrument	Xcalibur VI Plug-in Revision / Date	Firmware Requirements
UltiMate™ / UltiMate™ Plus Capillary- and Nano HPLC System	V 2.0 May 2004	Micropump V6.00, UV Detector V2.31, Solvent Organizer: V1.00, or higher
UltiMate™ Dual Gradient / UltiMate™ Plus Dual Gradient Capillary- and Nano HPLC System	V 2.0 May 2004	Micropump V6.00, UV Detector V2.31, Solvent Organizer: V1.00, or higher
FAMOS™ Well Plate **FAMOS™** Carousel Microautosampler	V 2.0 May 2004	Well Plate V2.02, Carousel V1.14, or higher
Switchos II™ Advanced Microcolumn Switching Unit	V 2.0 May 2004	Loading Pump V6.00, Switchos II V1.00, or higher

Note: The plug-in required for the UltiMate or UltiMate Plus Capillary- and Nano HPLC system is different to the plug-in required for the UltiMate Dual Gradient or UltiMate Plus Dual Gradient system. Please make sure to install the plug-in that is appropriate for your UltiMate instrument configuration.

Note: The LC Packings Virtual Instrument Plug-ins will also function correctly under Xcalibur version 1.2.

The LC Packings VI Plug-Ins for the Xcalibur software is designed for use with the LC Packings UltiMate and UltiMate Plus Capillary- and Nano HPLC Systems. When the LC Packings UltiMate system is mentioned, the reader should assume that the material applies to both systems.

1.3 About this Manual

Note: The LC Packings VI Plug-Ins for the Xcalibur software is designed for use with the LC Packings UltiMate and UltiMate Plus Capillary- and Nano HPLC Systems. When the LC Packings UltiMate system is mentioned, the reader should assume that the material applies to both systems.

The manual assumes that the user is familiar with the Xcalibur software package and provides information about controlling the LC Packings UltiMate system, the FAMOS Microautosampler and the Switchos II from the Thermo Electron Xcalibur software. For more information about how to use the Xcalibur software package refer to the user's manual provided with Xcalibur.

The manual is divided in 3 chapters:

- CHAPTER 1 - Introduction
  - System Requirements (Section 1.2).
- CHAPTER 2 - Installation and Getting Started
  - Electrical Connections (Section 2.2).
  - Software Installation (Section 2.3).
  - Instrument Configuration (Section 2.4).
  - Instrument Setup (Section 2.5).
  - Sequence Setup (Section 2.6).
  - Displaying the UV Data and the Column Pressure Data (Section 2.7).
  - Identifying the Plug-In Version (Section 2.8).
- CHAPTER 3 Programming Examples for the UltiMate Dual Gradient
  - Comprehensive 2-D Nano LC Setup (IEX/RP) (Section 3.2)
  - Parallel Nano–LC (Section 3.3)

All information provided in this user's manual refers to the plug-ins version 2.0 from May 2004 in conjunction with Xcalibur 1.4. Refer to Section 2.8 to identify your plug-ins versions.

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CHAPTER 2

Installation and Getting Started

2.1 Overview

The instructions provided below are provided for installation of the LC Packings VI-Plug-in for Xcalibur in conjunction with Thermo Electron's Xcalibur™ V1.4 or V1.3 software packages and the LC Packings UltiMate™ Capillary- and Nano HPLC System, the UltiMate™ Dual Gradient Capillary- and Nano HPLC System, the FAMOS Microautosampler and the Switchos II Advanced Microcolumn Switching Unit. In addition, instructions are provided to install the LC Packings Peak Parking Kit as part of the UltiMate System in this environment.

Please refer to the User's Manuals of the LC Packings instruments as well as the documentation provided with Thermo Electron's Xcalibur software package for additional information.

Chapter 2 provides the following installation information:

- Electrical Connections (Section 2.2).
- Software Installation (Section 2.3).
- Instrument Configuration (Section 2.4).
- Instrument Setup (Section 2.5).
- Sequence Setup (Section 2.6).
- Displaying the UV Data and the Column Pressure (Section 2.7).

Note: The plug-in required for the UltiMate or UltiMate Plus Capillary- and Nano HPLC system is different to the plug-in required for the UltiMate Dual Gradient or UltiMate Plus Dual Gradient system. Please make sure to install the plug-in that is appropriate for your UltiMate instrument configuration.

For additional information about how to install the LC Packings VI-Plug-in for Xcalibur in conjunction with Xcalibur™ V1.2, please refer also to Section 2.3.4

2.2 Electrical Connections

2.2.1 RS-232 Communication Cables

2.2.1 A UltiMate and Switchos

To control the modules of the UltiMate system and the Switchos Advanced Microcolumn Switching Unit, RS-232 communication cables need to be installed. Make certain that the RS-232 connectors of the system modules are connected as described in the user's manual provided with the instruments. Typical configurations are presented in FIGURE 2-1 and FIGURE 2-2. The connections to be made depend on the configuration of your system (e.g. if the flow sensor is not installed, the corresponding connection is missing).

FIGURE 2-1 shows the RS-232 connections of an UltiMate system and a Switchos II (UV Detector installed, no flow sensor installed).

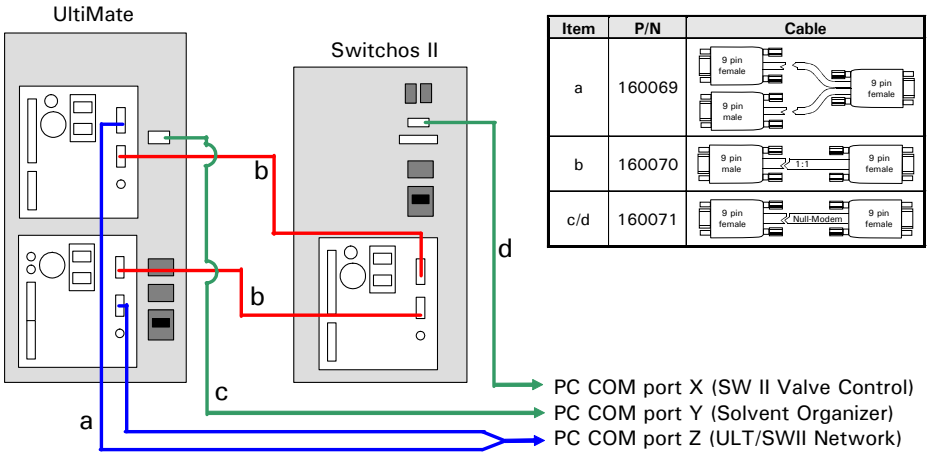


FIGURE 2-1 PC Connections of the UltiMate System (without Flow Sensor) and Switchos

FIGURE 2-2 shows the RS-232 connections of an UltiMate Dual Gradient system in conjunction with a Switchos II (2 flow sensors installed).

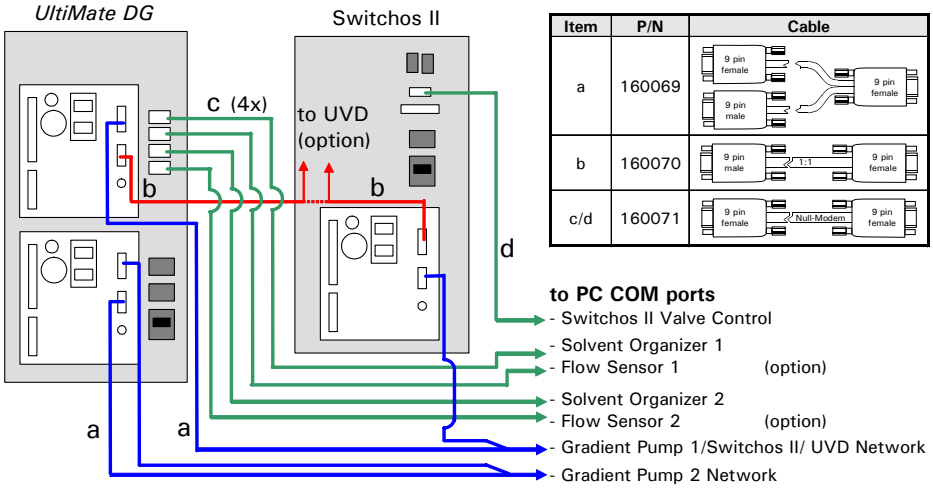


FIGURE 2-2 PC Connections of the UltiMate Dual Gradient System (with Flow Sensors) and Switchos

2.2.1 B FAMOS

Connect the FAMOS (if available in your configuration) to a free COM port of the PC using the FAMOS communication cable supplied with the instrument.

2.2.2 Connecting the START Signal

To start a run sequence, UltiMate Micropump(s), the UltiMate UV Detector (if included) and the Switchos II Loading Pump (if included) needs a start pulse. This is normally generated by the FAMOS Microautosampler ('Start Instrument'). Depending on the system configuration either the MARKERS output or the AUXILIARIES output is used.

When installing the instruments in conjunction with the LC Packings Peak Parking Kit, the start signal of the FAMOS must be decoupled during the run sequence from the START input of the Micropump to assure proper control by the Peak Parking 'Freeze-and-Resume' signal. In most configurations, the START signal is also used to start the MS data acquisition.

CAUTION **Caution: Make certain to connect the GROUND pins of all instruments together (e.g. the ground pin of the UltiMate UV Detector to the ground pin of the Micropump and to the ground pin of the Switchos II Loading Pump).**

The following sections show the setup of typical instrument configurations.

2.2.2 A UltiMate and FAMOS

The run sequence is started via the Inject Marker contact closure of the MARKERS output of the FAMOS Microautosampler.

To start a run sequence, connect the START inputs of the UltiMate Micropump, the UltiMate UV Detector to the P4 MARKERS output of the FAMOS Microautosampler as presented in FIGURE 2-3.

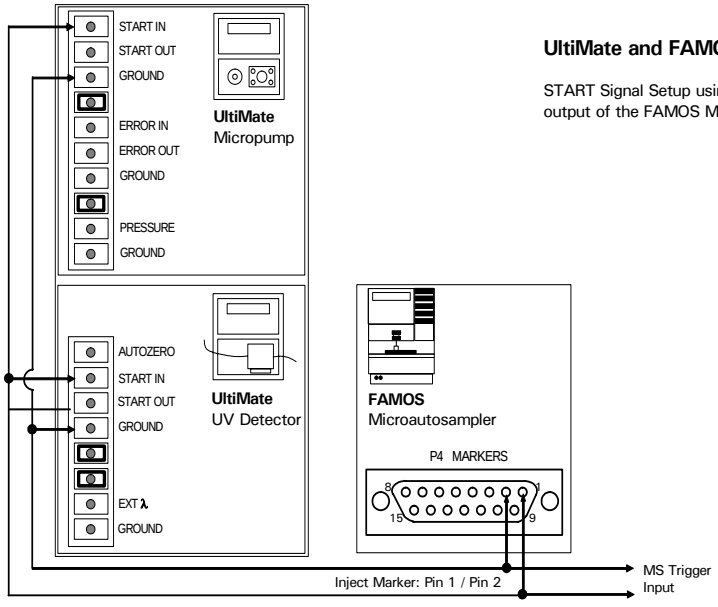


FIGURE 2-3 START Signal Setup – UltiMate and FAMOS

2.2.2 B UltiMate, FAMOS and Switchos II

When using the Switchos II for sample preparation the run sequence is started from the AUX 4 contact closure of the AUXILIARIES output of the FAMOS Microautosampler. This allows for a delayed start (e.g. after a pre-concentration step)

To start a run sequence, connect the START inputs of the UltiMate Micropump and the UltiMate UV Detector, the Switchos II to the P5 AUXILIARIES output of the FAMOS Microautosampler as presented in FIGURE 2-4.

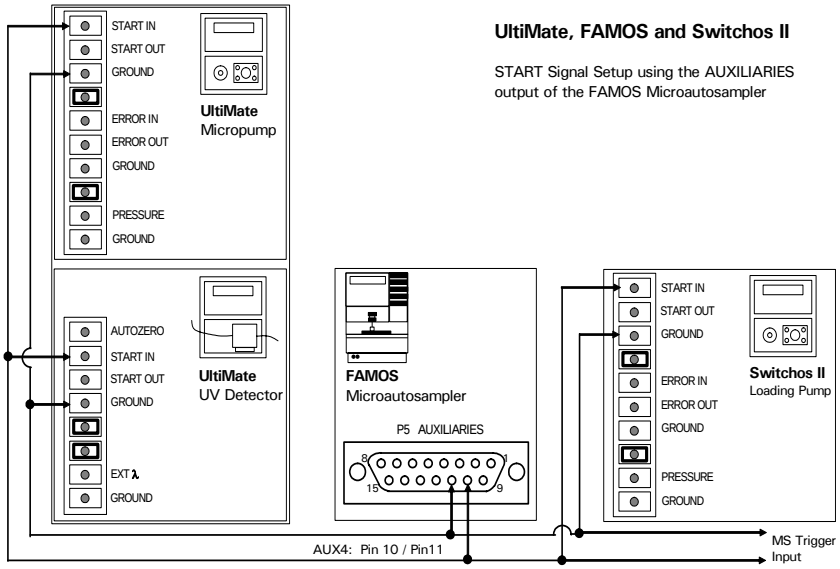


FIGURE 2-4 START Signal Setup – UltiMate, FAMOS and Switchos II

2.2.2 C UltiMate Dual Gradient, FAMOS and Switchos II

To start a run sequence, connect the START inputs of the two UltiMate Gradient Pumps, the UltiMate UV Detector (if present) and the Switchos II to the P5 AUXILIARIES output of the FAMOS Microautosampler as presented in FIGURE 2-5.

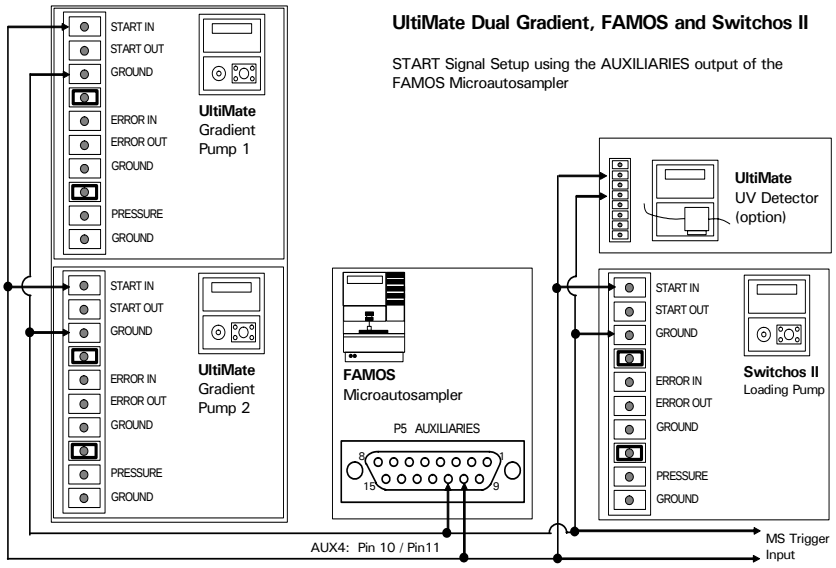


FIGURE 2-5 START Signal Setup – UltiMate Dual Gradient, FAMOS and Switchos II

2.2.2 D UltiMate, FAMOS, Switchos II and Peak Parking Kit

When the UltiMate System is installed in conjunction with the Peak Parking Kit, the START input of the UltiMate Micropump needs to be decoupled from the start signal of the FAMOS after the gradient start.

Note: The Peak Parking Kit can not be configured in conjunction with the UltiMate Dual Gradient version.

To decouple the START signal, use the EVENT8 relay output of the UltiMate Micropump as presented in FIGURE 2-6.

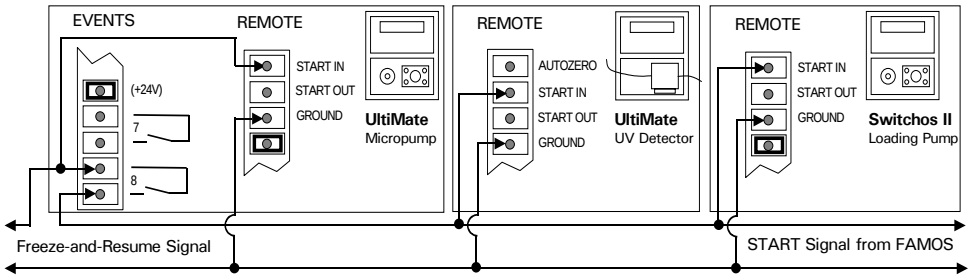


FIGURE 2-6 START Input Setup – Installation including the Peak Parking Kit

The relay contact of EVENT8 needs to be closed before the gradient is started. Immediately after the start, EVENT8 should open. The control module of the Peak Parking Kit can then control the START input of the Micropump (freeze and resume the gradient) without interfering with the other instruments.

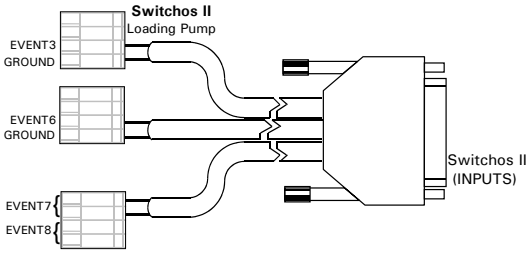
Note: Do not connect the 'Freeze-and-Resume Signal' of the Peak Parking Kit directly to the UltiMate UV Detector. This may lead to an incorrect display of the current run time.

Note: Instead of using the EVENT8 output of the UltiMate Micropump, any other available relay output can be used (e.g. EVENT1 of the UltiMate UV Detector).

2.2.3 Controlling the Switchos II Valves

The switching valves (Valve A and B) and the Solvent Selection Valve of the Switchos II Advanced Microcolumn Switching Unit are controlled by the EVENT outputs of the Switchos II Loading Pump.

Connect the INPUTS connector of the Switchos II to the EVENTS outputs of the Loading Pump using the cable provided with the instrument (FIGURE 2-7).



Pump	Switchos II
EVENT3	Valve A
EVENT6	Valve B
EVENT7	SSV
EVENT8	*n/a*

FIGURE 2-7 Switchos II Control Cable

Note: The Valves of the Switchos II Advanced Microcolumn Switching Unit (e.g. SSV, Valve A and Valve B) are controlled by the Switchos II Loading Pump.

2.3 Software Installation

Since there are two different plug-ins to control different instrument configurations, the first step of the software installation procedure is to identify your instrument configuration.

An install wizard will guide through the installation procedure. Carefully read the instructions in the various windows that appear during the installation process and take the appropriate actions. In most cases this will be to choose the **Next** button.

- Follow the instructions provided in Section 2.3.1, if your system configuration includes a single gradient UltiMate or UltiMate Plus system.
- Follow the instructions provided in Section 2.3.2, if your configuration system includes an UltiMate Dual Gradient or UltiMate Plus Dual Gradient system.

2.3.1 Single Gradient UltiMate based System

To install the VI Plug-in for Xcalibur for UltiMate control, proceed as follows:

a) Insert the CD ROM that is provided in the CD drive of your PC.

b) Identify the folder 'UltiMate Setup Files' and double click the **Setup.exe** file.

c) Click **Next** to open the 'Choose Destination Location' box (FIGURE 2-8).

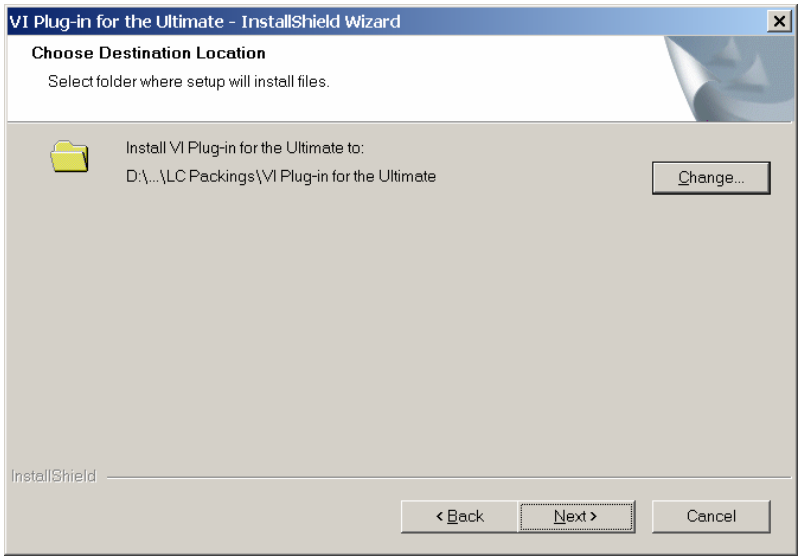


FIGURE 2-8 The Choose Destination Location Box

d) The setup program suggests 'X:\program files\LC Packings\...' as the default installation subdirectory. 'X' represents the letter of the drive where Windows® is installed. The user may change this to any valid path.

e) Click **Next** to open the 'Select Component' box. Check the instruments that are present in your instrument configuration (FIGURE 2-9).

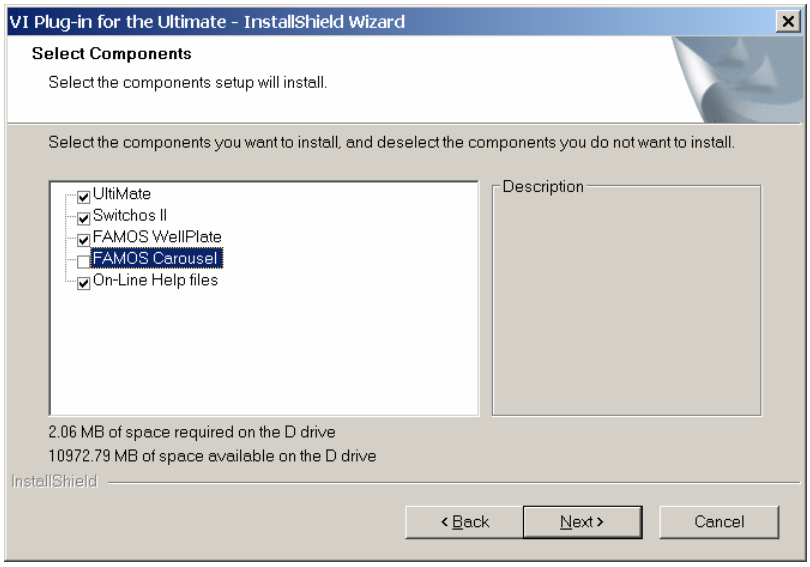


FIGURE 2-9 The Select Components Box for the UltiMate Components

f) Click **Next** to open the 'InstallShield Wizard Complete' window and then choose **Install** to complete the installation. Click **Back** if you want to change a setting.

2.3.2 UltiMate Dual Gradient based System

To install the VI Plug-in for Xcalibur for UltiMate Dual Gradient control, proceed as follows:

a) Insert the CD ROM that is provided in the CD drive of your PC.

b) Identify the folder 'UltiMate DUAL GRADIENT Setup Files' and double click the **Setup.exe** file.

c) Follow the steps c) – e) as described in Section 2.3.1 to present the 'Select Component' box (FIGURE 2-10)

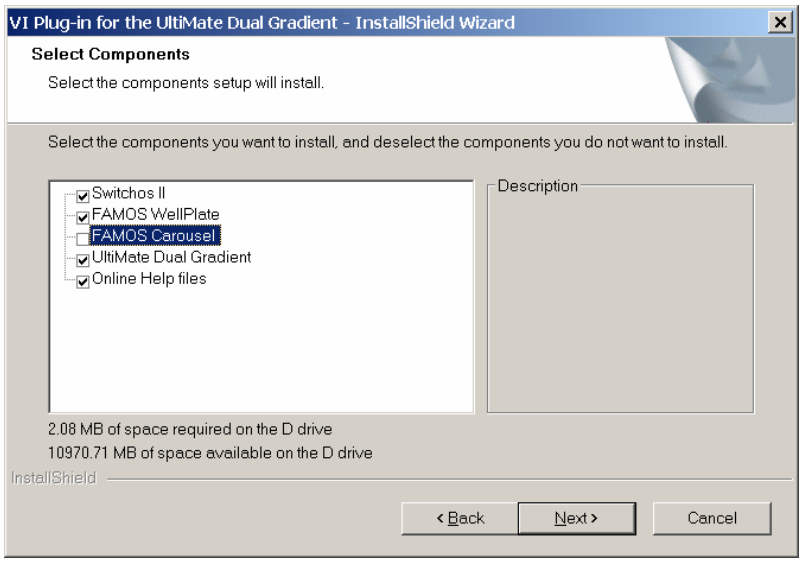


FIGURE 2-10 The Select Components Box for the UltiMate Dual Gradient Components

d) Check the instruments that are present in your instrument configuration (FIGURE 2-10).

e) Click **Next** to open the 'InstallShield Wizard Complete' window and then choose **Install** to complete the installation. Click **Back** if you want to change a setting.

2.3.3 Modify, Reinstall or Uninstall the VI Plug-Ins

During the installation procedure, the installation program checks if already a copy of the LC Packings Plug-Ins are installed on your computer. Then setup procedure will open the **Setup Maintenance Program** screen automatically.

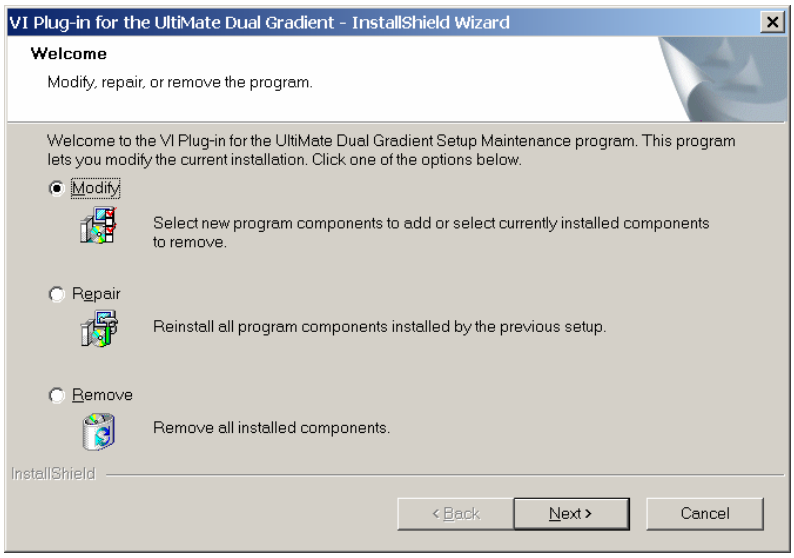


FIGURE 2-11 The Setup Maintenance Program Window

a) Check the appropriate box (e.g. if you want to install the plug-in for the UltiMate Dual Gradient system, you need to 'Remove' the plug-in for the single gradient version first).

b) To continue installation, check the 'Modify' option and choose the **Next** button.

c) The installation window will appear.

- Follow the instructions provided in Section 2.3.1, if your system configuration includes a single gradient UltiMate or UltiMate Plus system.

- Follow the instructions provided in Section 2.3.2, if your configuration system includes an UltiMate Dual Gradient or UltiMate Plus Dual Gradient system.

2.3.4 Installation in Conjunction with Xcalibur V1.2

When installing the plug-in in conjunction with Xcalibur V1.2, it is necessary to add a few important files manually to the original Xcalibur V1.2 installation. These files are located in the folder **Virtual Instruments** on the LC Packings VI Plug-Ins CDROM that is provided.

To add the Virtual Instrument files to the original Xcalibur path:

a) Identify the files 'VIRawfile.dll', 'VIRawfileX.dll' and 'VIRawfileReg.bat' in the **Virtual Instruments** folder on the CDROM that is provided.

b) Copy all three files to the to the folder **X:\Xcalibur\System\Programs** (X represents the letter of the drive where the Xcalibur software is installed).

c) Double click on the 'VIRawfileReg.bat' file. A dialog box with the registration information appears (FIGURE 2-12).

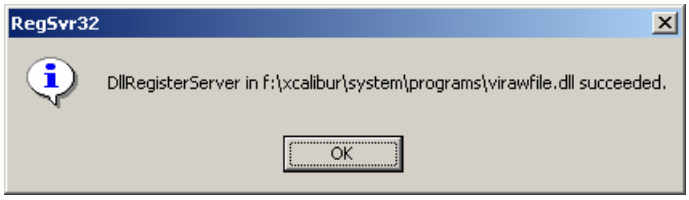


FIGURE 2-12 The Registration Information Dialog Box

d) Confirm the message with **OK**. The dialog box disappears and the installation is completed.

Note: If the registration fails, it may be necessary to edit the batch file (VIRawfileReg.bat) and change the drive letter from the default 'C:' to the drive that you are using on your particular system.

IMPORTANT NOTE: Do not use the 'VIRawfile.dll' and 'VIRawfileX.dll' file of Xcalibur V1.2 together with Xcalibur V1.4 or V1.3 or vice versa (e.g. copying the files from the CD ROM into the Xcalibur V1.4 or V1.3 original installation path)! Although the names are identical the files are different in the two versions!

2.4 Instrument Configuration

The next step of the installation procedure is to select and configure the instruments to be controlled. As with the installation, the configuration procedure depends on the current system configuration. After the selection of the instruments to be controlled (Section 2.4.1):

- Follow the instructions provided in Section 2.4.2 if your system configuration includes a single gradient UltiMate or UltiMate Plus system.
- Follow the instructions provided in Section 2.4.3 if your system configuration includes an UltiMate or UltiMate Plus Dual Gradient system.

2.4.1 Selecting the Instruments to be controlled

To select the instruments to be controlled:

a) Click the desktop icon *Instrument Configuration* to present the **Instrument Configuration** window (FIGURE 2-13). Additional instruments may appear in this window, depending on your specific installation.

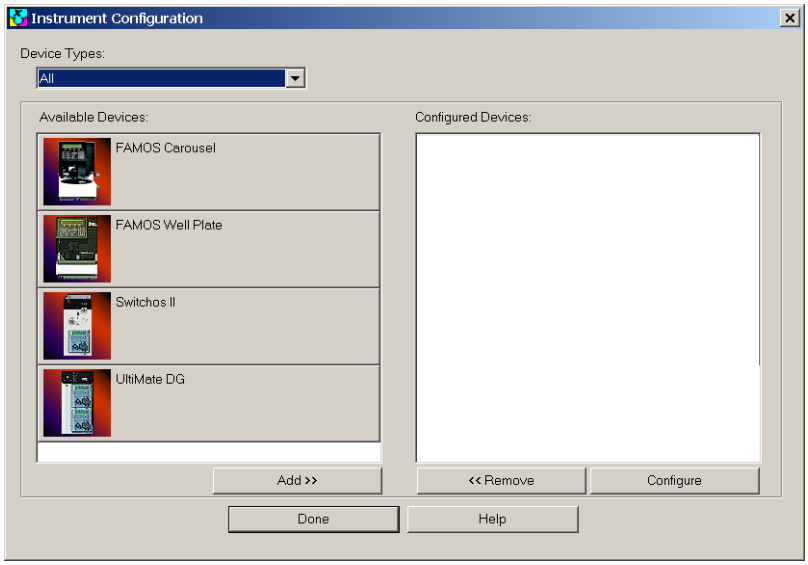


FIGURE 2-13 The Instrument Configuration Window

b) Choose an instrument in the left panel and press the **Add>>** button. Repeat this process for each instrument you want to be controlled by Xcalibur.

c) Once you have finished the selection of the required instruments, the window will look similar to FIGURE 2-14.

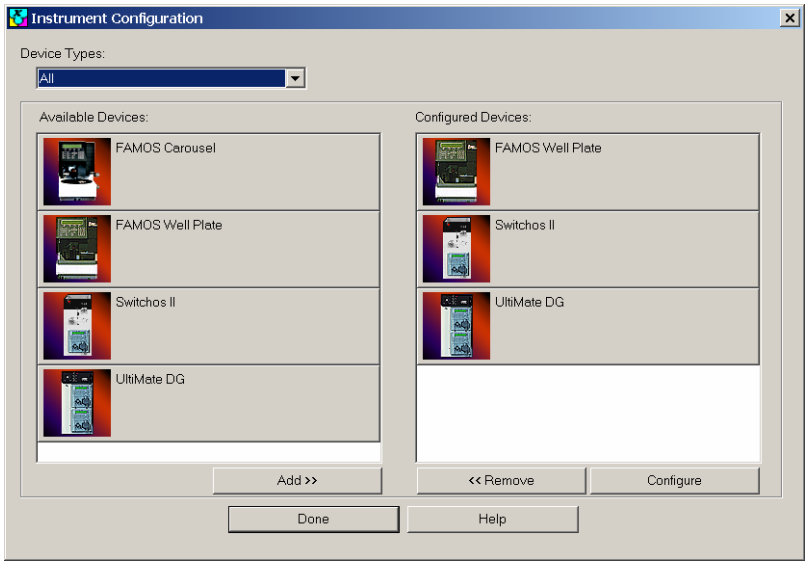


FIGURE 2-14 The Instrument Configuration Window – 3 Instruments selected

Note: Use either the Well Plate or the Carousel version of the FAMOS Microautosampler, but not both.

d) The next step is the configuration of the instruments as described in the following sections. To configure the components, either double click on an instrument in the right panel or just select the instrument by a single click and then choose the **Configure** button in the Instrument Configuration Window (FIGURE 2-14).

e) After all instruments have been configured, choose the **Done** button.

2.4.2 Configuration of the UltiMate System

2.4.2 A The UltiMate Micropump

To configure the UltiMate Micropump, choose the **Pump** tab (FIGURE 2-15).

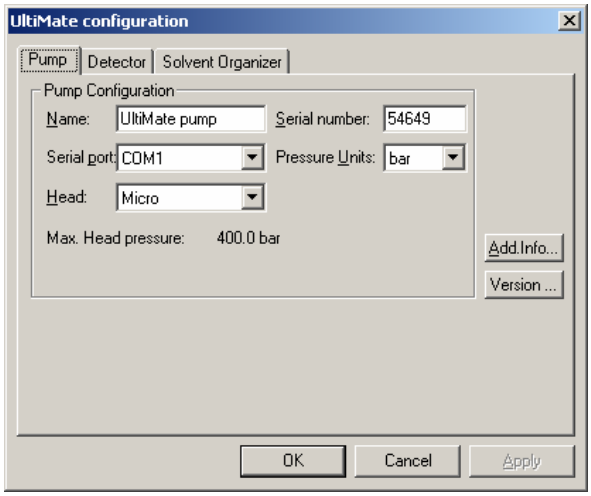


FIGURE 2-15 The UltiMate Micropump Configuration Window

a) Select the 'Serial port' by which the UltiMate System is controlled.

b) Enter the 'Serial Number' of the Micropump (which can be found by selecting the appropriate screen of the **GLP** menu of the pump).

c) Choose the 'Pressure Units' and select the Micro option from the 'Head' menu.

2.4.2 B The UltiMate UV Detector

To configure the UltiMate UV Detector (if included in the configuration), choose the **Detector** tab (FIGURE 2-16).

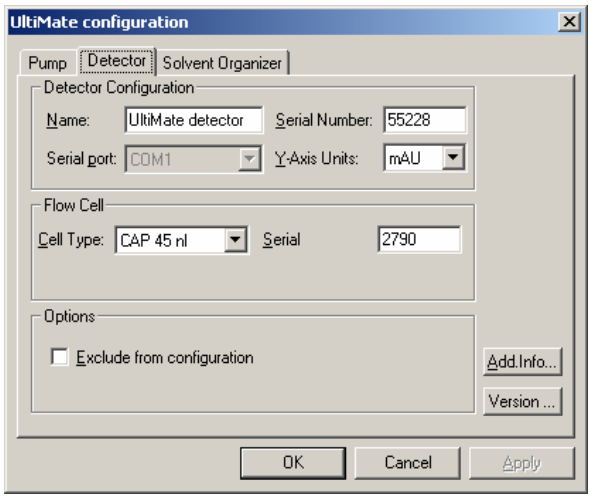


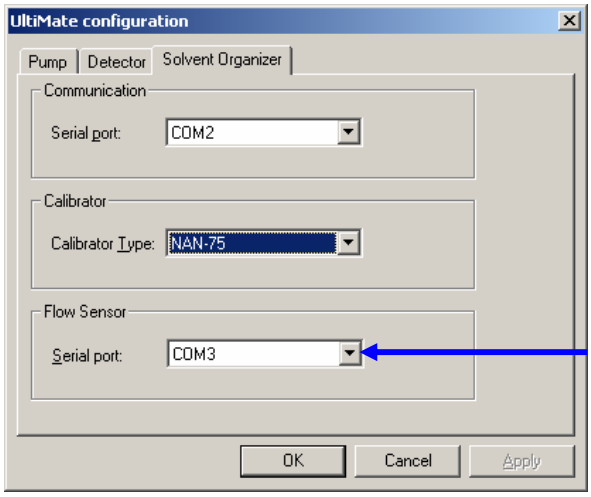
FIGURE 2-16 The UltiMate UV Detector Configuration Window

d) Enter the 'Serial Number' of the UV Detector (which can be found by selecting the appropriate screen of the **GLP** menu of the detector).

e) Select the 'Y-Axis Units', 'Cell type' and enter the serial number of the flow cell.

2.4.2 C The UltiMate Solvent Organizer and Flow Sensor

To configure the UltiMate Solvent Organizer and the flow sensor (if included), choose the **Solvent Organizer** tab (FIGURE 2-17).



Select 'None' if no flow sensor is installed.

FIGURE 2-17 The UltiMate Solvent Organizer Configuration Window

f) Select the 'Serial port' by which the UltiMate Solvent Organizer is controlled.

g) Select the 'Calibrator Type'.

h) Select the 'Serial port' to which the flow sensor is connected (if included).

Note: The 'Calibrator Type' will determine the 'Inner Diameter' of the column in the column parameter setup window (Section 2.5.1 C). If you want to use columns of different diameters, make sure that the calibrator setting is changed as appropriate.

2.4.3 Configuration of the UltiMate Dual Gradient System

2.4.3 A The UltiMate Gradient Pumps

To configure the UltiMate Gradient Pump 1 (upper pump) and the Gradient Pump 2 (lower pump), choose the **Pump** tab (FIGURE 2-18).

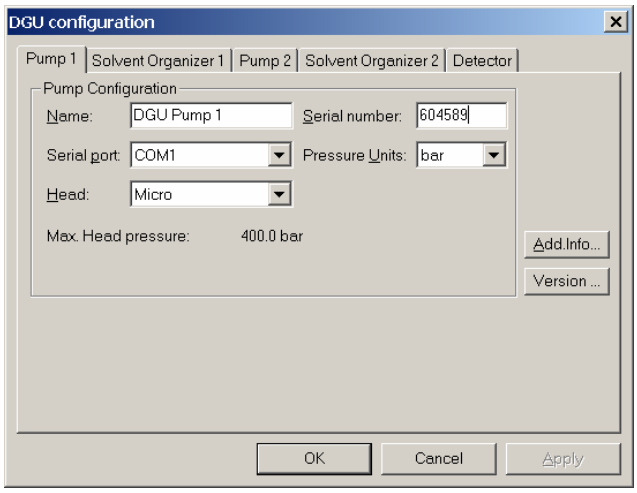


FIGURE 2-18 The UltiMate Pump1 Configuration Window

a) Select the 'Serial port' by which the UltiMate Gradient Pump 1 is controlled. The same communication port controls Switchos II Loading Pump (if included) and the UV Detector (if included).

b) Enter the 'Serial Number' of the Gradient Pump 1 (which can be found by selecting the appropriate screen of the **GLP** menu of the pump).

c) Choose the 'Pressure Units' and select the Micro option from the 'Head' menu.

d) Repeat steps a) to c) for Gradient Pump 2 (FIGURE 2-19).

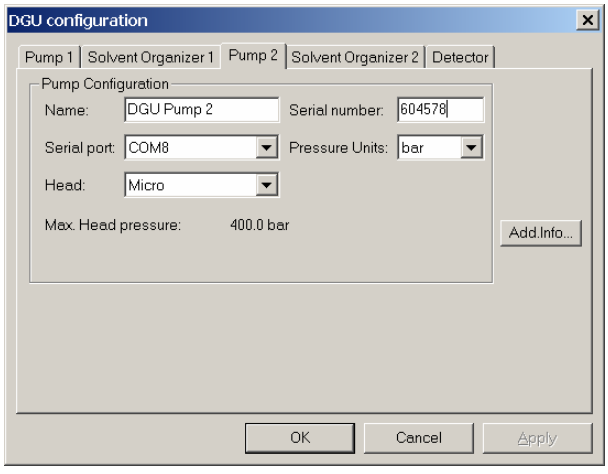


FIGURE 2-19 The UltiMate Pump 2 Configuration Window

2.4.3 B The UltiMate Solvent Organizers and Flow Sensors

To configure the UltiMate Solvent Organizer 1 and the flow sensor 1 (if included), choose the **Solvent Organizer1** tabs (FIGURE 2-20).

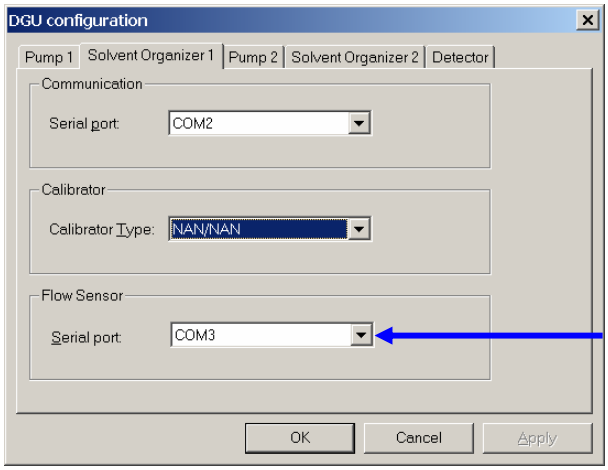


FIGURE 2-20 The UltiMate Solvent Organizer Configuration Window

a) Select the 'Serial port' by which the UltiMate Solvent Organizer 1 is controlled.

b) Select the 'Calibrator Type' that is installed in your system.

c) Select the 'Serial port' to which the flow sensor is connected (if included).

d) Repeat steps a) – c) for Solvent Organizer 2 and flow sensor 2 (if included). The calibrator type must be the same type than selected in step b).

Note: The 'Calibrator Type' will determine the 'Inner Diameter' of the column in the column parameter setup window (Section 2.5.1 C). If you want to use columns of different diameters, make sure that the calibrator setting is changed as appropriate.

2.4.3 C The UltiMate UV Detector

The UV Detector is not part of the UltiMate Dual Gradient system. However, a standalone instrument can be added to the configuration.

To configure the UltiMate UV Detector (if included in the configuration), choose the **Detector** tab (FIGURE 2-21).

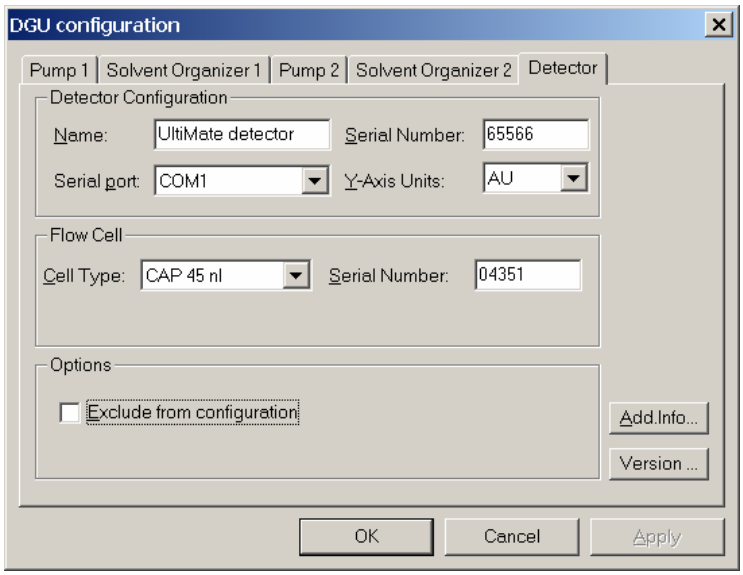


FIGURE 2-21 The UltiMate UV Detector Configuration Window

a) Enter the 'Serial Number' of the UV Detector (which can be found by selecting the appropriate screen of the **GLP** menu of the detector).

b) Select the 'Y-Axis Units', 'Cell type' and enter the serial number of the flow cell.

If you do not intend to use the UV Detector in a particular method but if you don't want to remove the instrument permanently from the instrument configuration (e.g. unplug the communication cables), check the 'Exclude from configuration' box in the 'Options' area (FIGURE 2-21). If desired, you can also switch off the Deuterium lamp in the **Instrument Setup** (2.5.1 B).

2.4.4 Configuration of the FAMOS Well Plate Microautosampler

To configure the FAMOS Well Plate Microautosampler, select the instrument as discussed in Section 2.4. The FAMOS Well Plate configuration window will appear (FIGURE 2-22).

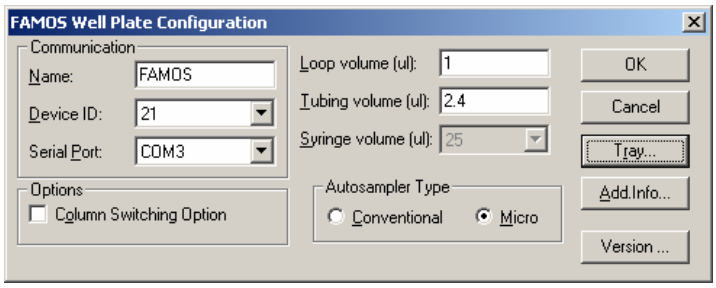


FIGURE 2-22 The FAMOS Well Plate Configuration Window

a) Select the 'Serial port' by which the FAMOS is controlled. The 'Device ID' of the FAMOS Well Plate Microautosampler is '21' and the 'Device ID of the FAMOS Carousel it is '20'.

b) Select the 'Loop volume', the 'Tubing volume' and the 'Autosampler type' according to your specific setup. If you wish to inject sample volumes from 0.01 to 10 µL choose the Micro option. The range of the Conventional option is 0.1 to 1000 µL.

Note: Select the 'Column switching' option only if a column switching valve is installed in your FAMOS (right side of the FAMOS Microautosampler).

c) Choose the **Tray...** button to open the tray configuration window (FIGURE 2-23).

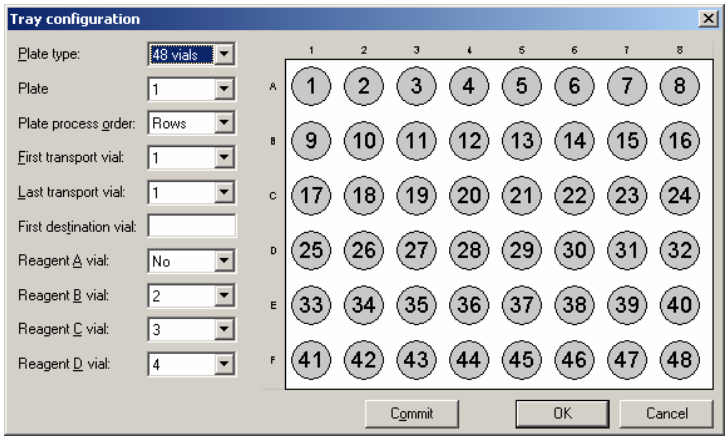


FIGURE 2-23 The Tray Configuration Window

d) Configure the tray parameter according to your specific needs and choose the **Commit** button to store your tray configuration.

CAUTION

Caution: The 4 extra vial positions in the FAMOS Well Plate can only be used once (e.g. if the 'First transport vial' is set to '1' then 'Reagent A vial' must be set to 'no', etc). Failure to do this may lead to unpredictable behavior of the FAMOS Microautosampler and may damage the needle!

2.4.5 Configuration of the Switchos II

To configure the Switchos II, select the instrument as discussed in Section 2.4. The Switchos II configuration window will appear (FIGURE 2-24).

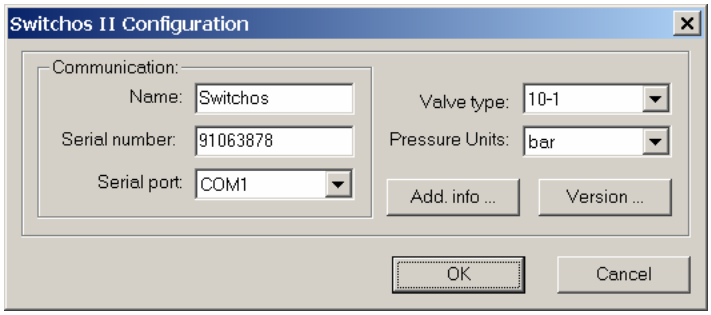


FIGURE 2-24 The Switchos II Configuration Window

a) Enter the 'Serial Number' of the Loading Pump (which can be found by selecting the appropriate screen of the **GLP** menu of the pump).

Note: The 'Serial' number of the Switchos II must always have the format '910nnnnn', where 'nnnnn' represents the original serial number, which can be found on the back panel of the instrument. The 'Serial port' is defined in the UltiMate System configuration.

b) Select the 'Valve type' which is installed on the Switchos II (e.g. select '10-1' in case 10-port valves are installed and '6-1' in case of 6-port valves).

c) Select the 'Pressure Units' used to display the trap column pressure.

2.5 Instrument Setup

The working conditions of the instruments (e.g. gradient time table, injection method, defining the wavelength) will be defined in the **Instrument Setup** window.

To access the **Instrument Setup** window:

a) Activate the Xcalibur software by double clicking the *Xcalibur* icon. The Xcalibur **Home Page** will be presented (FIGURE 2-25).

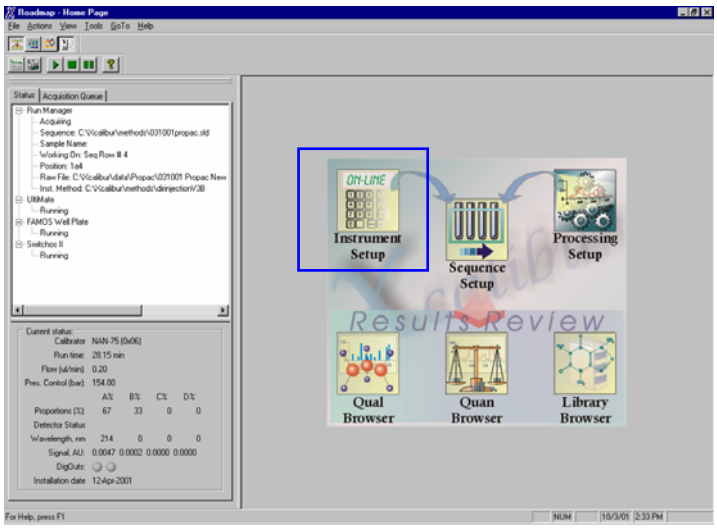


FIGURE 2-25 The Xcalibur Home Page

b) Click the *Instrument Setup* icon. If your system includes a single gradient UltiMate system, the **Instrument Setup** window presented in FIGURE 2-26 will appear. FIGURE 2-27 presents the Dual Gradient setup.

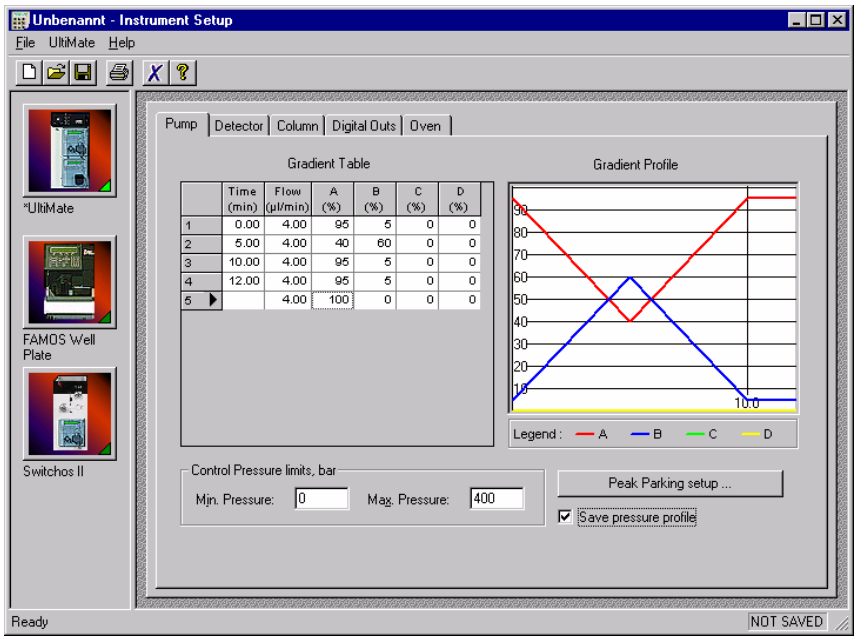


FIGURE 2-26 The Instrument Setup Window of the UltiMate System

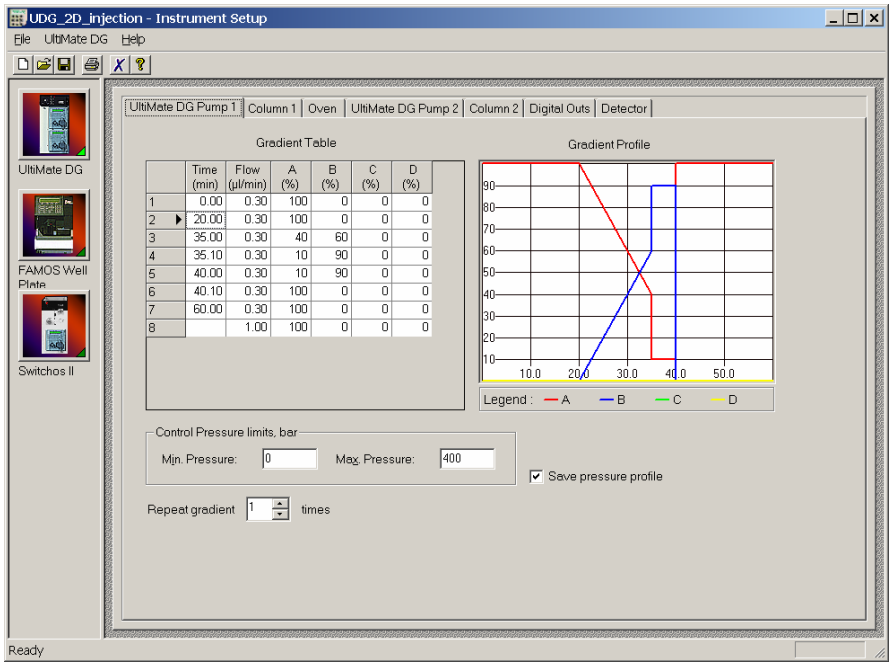


FIGURE 2-27 The Instrument Setup Window of the UltiMate Dual Gradient

c) Continue with the next setup step or quit the setup by saving your method using the **SaveAs** command from the **File** menu.

Note: When using a mass spectrometer, this instrument will also be displayed in the left bar of the *Instrument Setup* window.

d) The setup of the single gradient version is different from the dual gradient version:

- Follow the instructions provided in Section 2.5.1 if your system configuration includes a single gradient UltiMate or UltiMate Plus system.
- Follow the instructions provided in Section 2.5.2 if your system configuration includes an UltiMate Dual Gradient or an UltiMate Plus Dual Gradient system.

2.5.1 Setting up the UltiMate System

Open the **Instrument Setup** window as described in Section 2.5. Follow the instructions provided in following sections, once you have setup all instruments, save your method under any suitable name.

2.5.1 A Setting up the UltiMate Micropump

To set up the UltiMate Micropump:

a) Choose the **Pump** tab in the **Instrument Setup** window to display the pump setup window (FIGURE 2-26).

b) Create your gradient by filling out the timetable.

c) To enable storage of the column pressure, check the 'Save pressure profile' box.

Note: The 'Save pressure profile' option is a useful diagnostic tool. If this option stays unchecked, the pressure profile cannot be recorded!

d) Continue with the next setup step or quit the setup by saving your method.

2.5.1 B Setting up the UltiMate UV Detector

To set up the UltiMate UV Detector:

a) Choose the **Detector** tab in the **Instrument Setup** window to display the detector setup window (FIGURE 2-28).

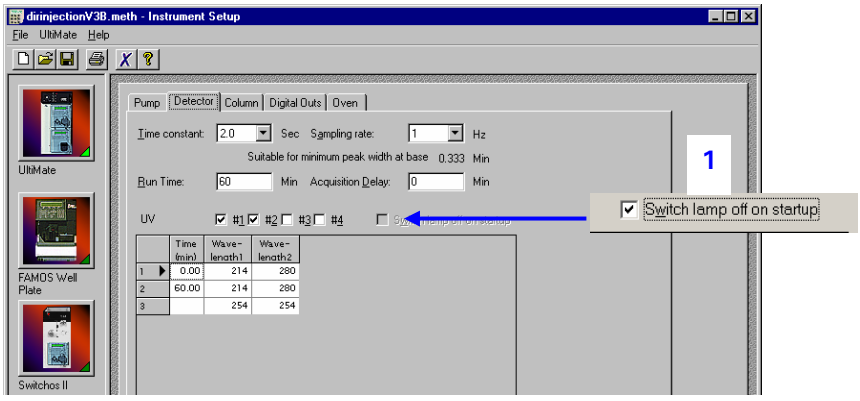


FIGURE 2-28 The UV Detector Setup Window

b) Fill out the detector parameters (e.g. Time constant, Sampling rate, Run Time, etc.). The checkboxes 'UV #1, #2, #3, #4' determine how many channels are used to collect data, the appropriate number of 'Wavelength #n' columns are represented in the detector timetable. If the detector is not used (temporarily), uncheck all 'UV #n' boxes and check the 'Switch off the on startup) box (item 1, FIGURE 2-28).

c) Right clicking on a column causes a popup menu to appear. Use this menu to insert lines, delete lines or to fill out entire columns with the same value.

d) Continue with the next setup step or quit the setup by saving your method.

2.5.1 C Setting up the Column Parameters

To set up the column parameters:

a) Choose the **Column** tab in the **Instrument Setup** window to display the column setup window (FIGURE 2-29).

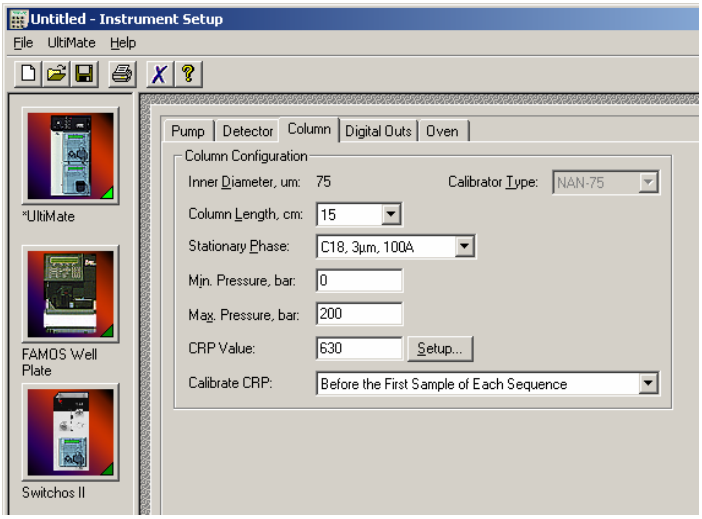


FIGURE 2-29 The Column Parameters Setup Window

b) Enter the column parameters (e.g. length, stationary phase).

Note: The column diameter is automatically set in conjunction with the calibrator setting during the *Instrument Configuration* step (Section 2.4). When columns of different diameters are used, they must be defined via the calibrator setting in the *Instrument Configuration* step (Section 2.4).

c) Enter the values for 'Min. Pressure' and 'Max. Pressure'. A minimum pressure greater than 0 bar assures that the system will shut down if either a solvent bottle is empty or if a considerable leak occurs.

The Column Resistance Parameter (CRP) is used to ascertain the correct flow rate through the column, based on the flow rate of the gradient pump and the split ratio defined by the calibrator and the column.

d) For manual flow correction, press the **Setup...** button and enter the measured flow in the relevant box, then press the button **Correct Flow**.

If your system is equipped with a flow sensor (e.g. the UltiMate Plus offers this option), the flow rate can automatically be corrected when the 'Calibrate CRP' option is enabled.

To enable the automatic CRP correction:

e) Select either the 'Before the First Sample of Each Sequence' or the 'Before Each Sample' option. If 'Never' is selected, no automatic calibration is performed. Proceed as described in step d) for manual correction.

f) Continue with the next setup step or quit the setup by saving your method.

2.5.1 D Setting up the Digital Outputs

To use and to set up the various event outputs available on the UltiMate Micropump and the UltiMate UV Detector:

a) Choose the **Digital Outs** tab in the **Instrument Setup** window to display the digital outputs setup window (FIGURE 2-30).

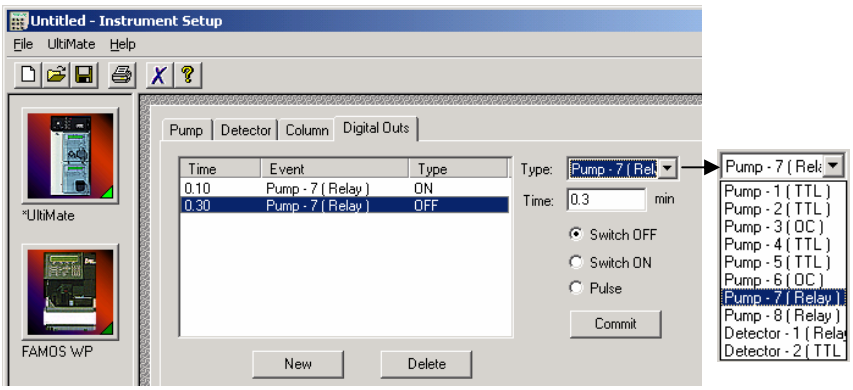


FIGURE 2-30 The Digital Outs Setup Window

b) Click the **New** button, enter the time when the event should start, choose which event you need from the dropdown list and then indicate whether this output should be switched on, switched off or if it should generate a pulse.

c) Click the **Commit** button to store the new input.

d) Repeat steps a) – c) for each event at the appropriate time.

e) Continue with the next setup step or quit the setup by saving your method.

2.5.1 E Setting up the Oven

a) Choose the **Oven** tab in the **Instrument Setup** window to display the oven setup window (FIGURE 2-31).

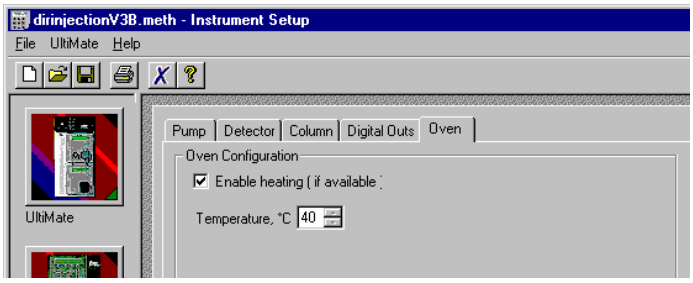


FIGURE 2-31 The Oven Setup Window

b) Enable the heating and set the oven temperature according to your specific needs (if the oven option is installed in your UltiMate system).

c) Continue with the next setup step or quit the setup by saving your method.

The setup of the UltiMate is finished at this point. To setup the FAMOS Microautosampler or the Switchos Advanced Microcolumn Switching Unit, continue with Section 2.5.3 or Section 2.5.4, respectively

2.5.2 Setting up the UltiMate Dual Gradient

Open the **Instrument Setup** window as described in Section 2.5. Follow the instructions provided in following sections, once you have setup all instruments, save your method under any suitable name.

2.5.2 A Setting up the UltiMate Gradient Pumps

To set up the UltiMate Gradient Pumps:

a) Choose the **UltiMate DG Pump 1** tab in the **Instrument Setup** window to display the pump setup window (FIGURE 2-32).

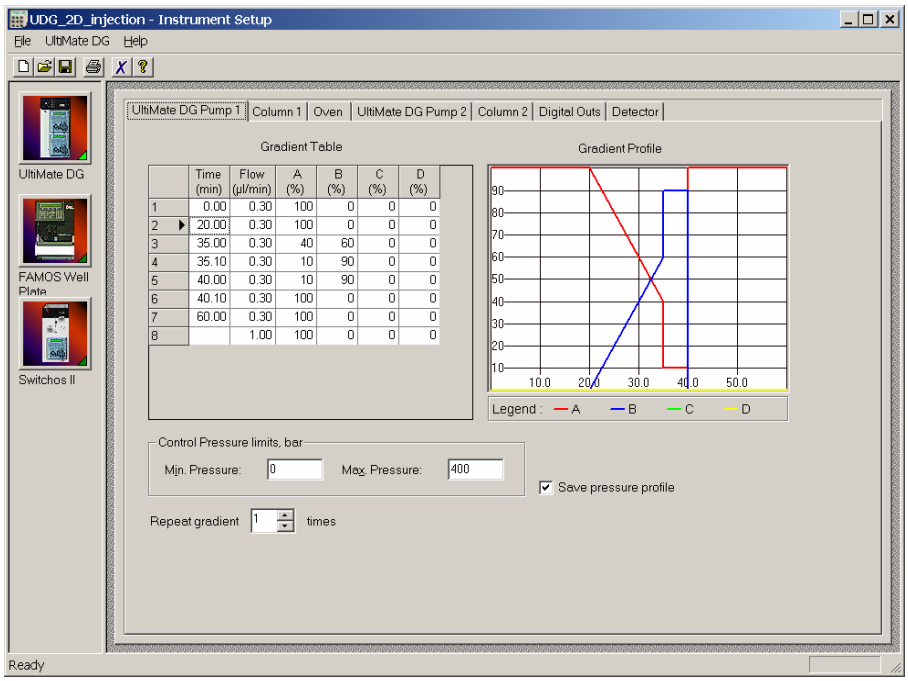


FIGURE 2-32 The Gradient Pump 1 Setup Window

b) Create your gradient by filling out the timetable.

c) To repeat the gradient multiple times, adjust the 'Repeat gradient' field.

d) To enable storage of the column pressure, check the 'Save pressure profile' box.

Note: The 'Save pressure profile' option is a useful diagnostic tool. If this option stays unchecked, the pressure profile cannot be recorded!

e) To program the Gradient Pump 2, choose the **UltiMate DG Pump 2** tab and repeat steps a) – d).

f) Continue with the next setup step or quit the setup by saving your method.

2.5.2 B Setting up the Column Parameters and Flow Sensors

To set up the column parameters and the flow sensors:

a) Choose the **Column 1** tab in the **Instrument Setup** window to display the column 1 setup window (FIGURE 2-33).

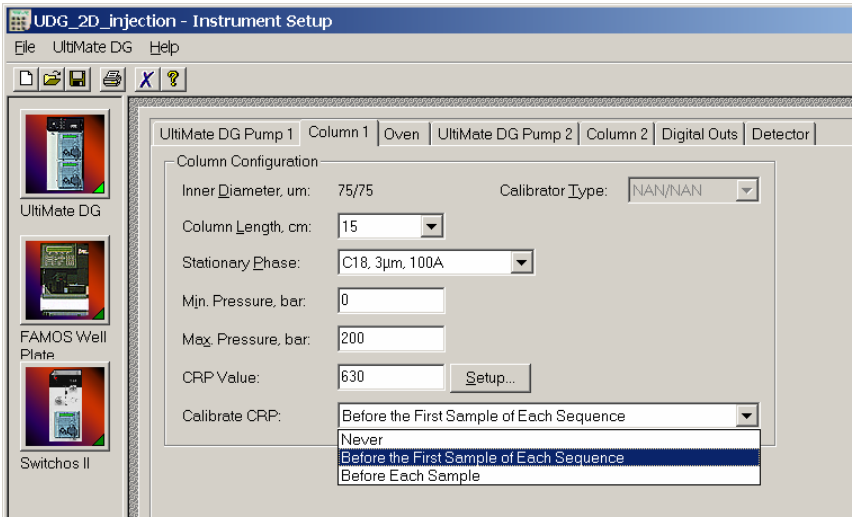


FIGURE 2-33 The Column 1 Parameters Setup Window

b) Enter the column parameters (e.g. length, stationary phase).

Note: The column diameter is automatically set in conjunction with the calibrator setting during the *Instrument Configuration* step (Section 2.4). When columns of different diameters are used, they must be defined via the calibrator setting in the *Instrument Configuration* step (Section 2.4).

c) Enter the values for 'Min. Pressure' and 'Max. Pressure'. A minimum pressure greater than 0 bar assures that the system will shut down if either a solvent bottle is empty or if a considerable leak occurs.

The Column Resistance Parameter (CRP) is used to ascertain the correct flow rate through the column, based on the flow rate of the gradient pump and the split ratio defined by the calibrator and the column.

d) For manual flow correction, press the **Setup...** button and enter the measured flow in the relevant box, then press the button **Correct Flow**.

If your system is equipped with a flow sensor (e.g. the UltiMate Plus offers this option), the flow rate can automatically be corrected when the 'Calibrate CRP' option is enabled.

To enable the automatic CRP correction:

e) Select either the 'Before the First Sample of Each Sequence' or the 'Before Each Sample' option. If 'Never' is selected, no automatic calibration is performed. Proceed as described in step d) for manual correction.

f) Choose the **Column 2** tab for the column 2 and the flow sensor 2 setup (FIGURE 2-33) and repeat steps a) - f).

g) Continue with the next setup step or quit the setup by saving your method.

2.5.2 C Setting up the Oven, Digital Outputs, UltiMate UV Detector

To program the oven temperature, the digital outputs of the Gradient Pump 1 and 2 and to setup the UV Detector (if included) for data acquisition, follow the instructions provided in

- Setting up the Oven – Section 2.5.1 E
- Setting up the Digital Outputs – Section 2.5.1 D
- Setting up the UltiMate UV Detector – Section 2.5.1 B

2.5.3 Setting up the FAMOS Microautosampler

2.5.3 A Standard Injection Mode

To set up the FAMOS Microautosampler:

a) Choose the *FAMOS* icon in the **Instrument Setup** window to display the FAMOS setup window (FIGURE 2-34).

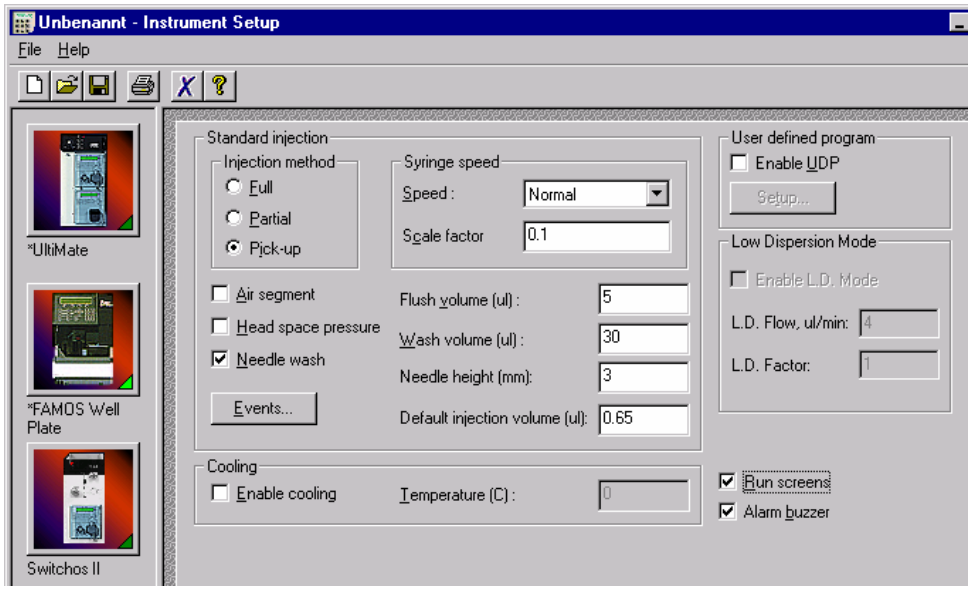


FIGURE 2-34 The FAMOS Setup Window

b) Make your choices by selecting the various radio buttons and check boxes. The sample injection volume can be entered in the run sequence table for each specific sample. The 'Default injection volume' is the value used to fill out the run sequence table. Subsequently each line or block of lines may be edited.

Note: For small sample amounts, *Head space pressure* and *Air segment* should not be used. The *Flush volume* is normally 2 times the tubing volume. For the FAMOS Well Plate Microautosampler, the minimum *Wash volume* in the micro mode is 1 µL, in conventional mode it is 300 µL. For the FAMOS Carousel Microautosampler, the minimum wash volume is 300 µl in either mode. Entering an invalid value will lead to an error message during the run time!

2.5.3 B User Defined Program (UDP) Mode

The **U**ser **D**efined **P**rogram mode offers full access to all functions of the FAMOS Microautosampler (e.g. this mode allows two or more injections per run – refer to the user's manual of the FAMOS Microautosampler for more details).

To program your UDP:

a) Choose the *FAMOS* icon in the **Instrument Setup** window to display the FAMOS setup window (FIGURE 2-35).

b) Select the 'Enable UDP' option and choose the **Setup** button to present the **User Defined Program** window (FIGURE 2-35, item 1).

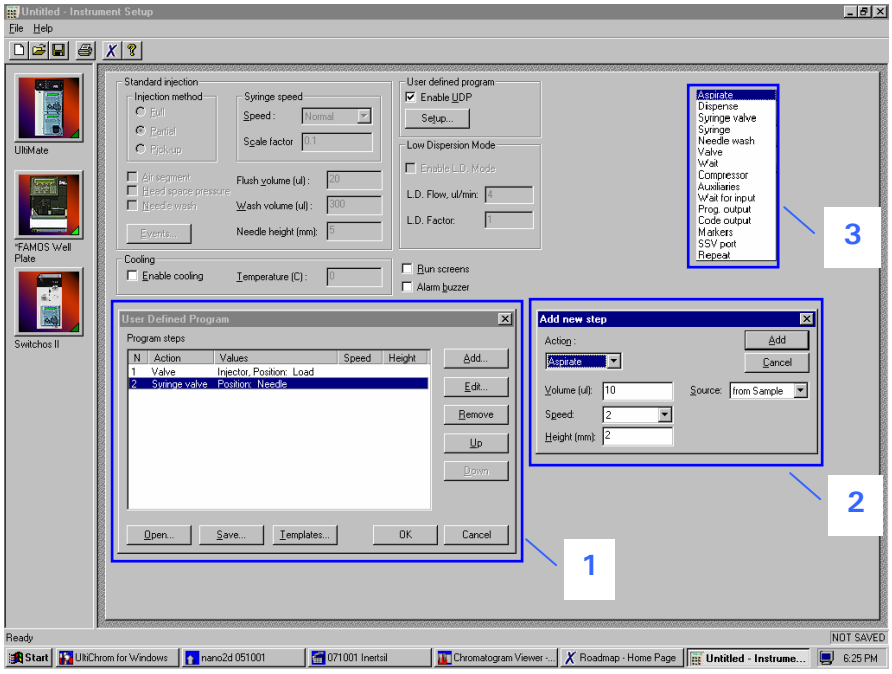


FIGURE 2-35 The User Defined Program Window

c) Choose the **Add** button to open the **Add new step** window and select the required program step from the 'Action' list (FIGURE 2-35, items 2 and 3).

d) To add another program step (e.g. to add an 'Aspirate' step as presented in FIGURE 2-35), choose the **Add** button in this window.

e) Once you have defined all program steps, choose **Save...** to save your UDP.

Note: Some UDPs have been pre-defined for easier start. Choose the 'Template' button to open one of the pre-defined UDPs (e.g. Macro1 – µL Pickup with a 100 µL loop, Macro2 – single bead, Macro3 – gel extraction).

2.5.4 Setting up the Switchos II Advanced Microcolumn Switching Unit

To set up the Switchos II Advanced Microcolumn Switching Unit:

a) Choose the Switchos II icon in the **Instrument Setup** window to display the Switchos II setup window (FIGURE 2-36).

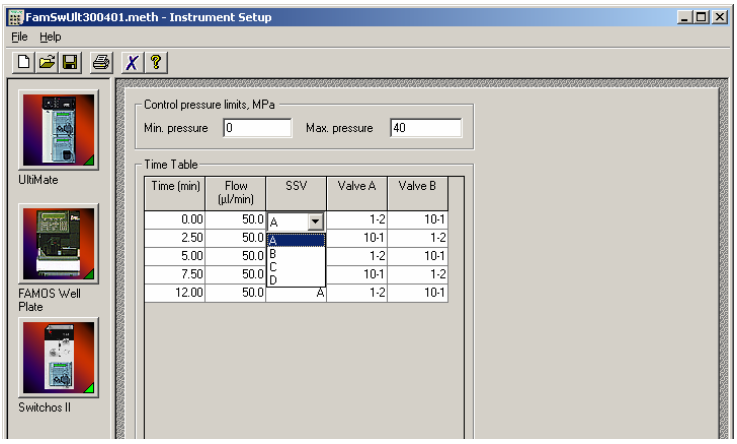


FIGURE 2-36 The Switchos II Setup Window

b) Double click on a cell in the columns *SSV*, *ValveA* or *ValveB* to open a pull down menu. Select the position of the switching valve A, B or the solvent selection valve according to the needs of your application.

c) Enter the flow rate.

d) Insert an additional line by right clicking on a cell and choosing *Insert, a*nd then enter the new time.

e) Repeat steps b) - d) until all steps required for your application have been programmed.

f) Continue with the next setup step or quit the setup by saving your method.

Note: On the start of each run, the Solvent Selection Valve of the Switchos II Advanced Microcolumn Switching Unit will be initialized to solvent A. During this procedure, the Loading pump may be connected to another solvent channel rather than channel A for a short moment (e.g. the valve moves first to channel B). To avoid the possibility that air bubbles can enter the pump head, make sure that all four solvent bottles are filled.

2.5.5 Setting up the Peak Parking Kit

Note: The Peak Parking Kit not can be configured in conjunction with the UltiMate and UltiMate Plus Dual Gradient version.

To set up the Peak Parking Kit:

a) Choose the **Peak Parking setup ...** button on the **Pump** tab in the **Instrument Setup** window (FIGURE 2-37).

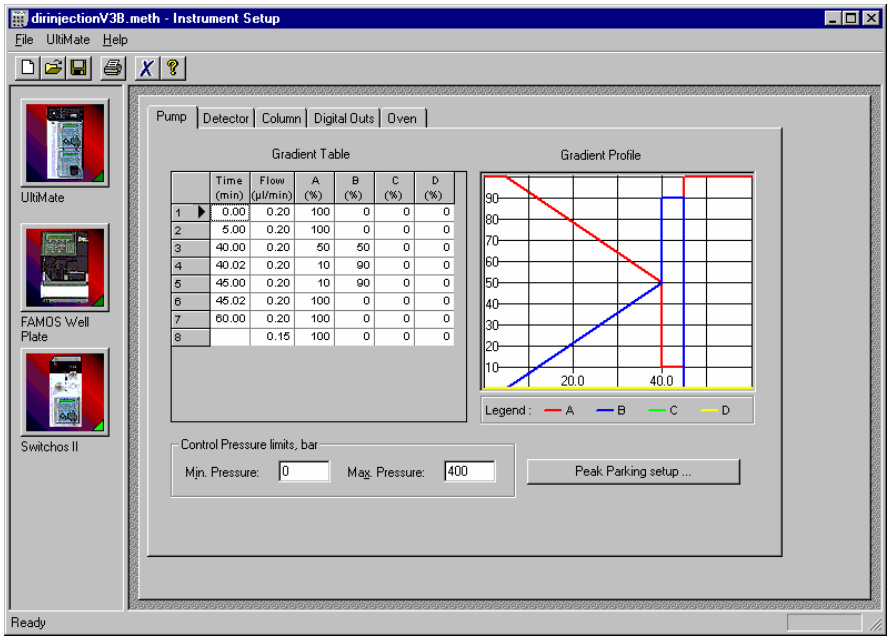


FIGURE 2-37 The Pump Setup Tab in the Instrument Setup Window

b) Clicking this button opens the **Peak Parking Setup** window (FIGURE 2-38).

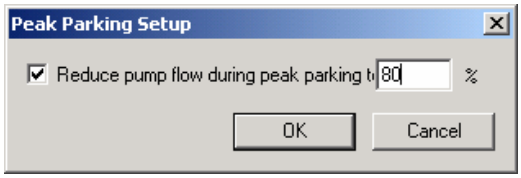


FIGURE 2-38 The Peak Parking Setup Window

c) To enable the peak parking option, check the *Reduce pump flow during peak parking to* box and enter an appropriate flow rate reduction.

d) Confirm your settings by choosing the **OK** button.

e) Save your method.

Note: For more detailed information about the peak parking technique and how to determine the reduced flow rate in the parking mode, refer to the user's manual provided with the kit.

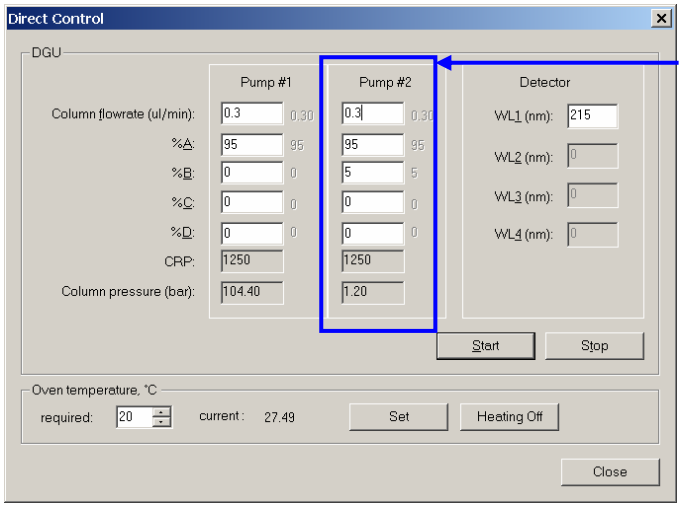
2.5.6 The 'Direct Control' Option

To equilibrate and to prepare a run the sequence, the 'Direct Control' option of the *Instrument Setup* window lets you start the flow delivery of the UltiMate Micropump(s), set the UltiMate oven temperature and set the detector wavelengths.

To setup the 'Direct Control' parameters:

a) Choose the *UltiMate* or *UltiMate DG* icon in the **Instrument Setup** window (FIGURE 2-26).

b) Choose the **UltiMate** menu from the menu bar and then choose **Direct Control**. The Direct Control window appears (FIGURE 2-39).



UltiMate [Plus] Dual Gradient only.

FIGURE 2-39 The Direct Control Window

c) Enter the appropriate parameters that meet the needs of your application (e.g. the same parameters as used in the first program line of the run sequence you want to start).

d) Choose the **Start** button to initialize the system and to start the flow delivery.

e) Start your run sequence when the system is prepared.

Note: It is recommended that you close the 'Direct Control' window once the run sequence is started. If you press the 'Stop' button in the 'Direct Control' window, the run sequence will be interrupt.

Note: The CRP (Column Resistance Parameter) value is read-only and is a check that you are working with the correct column. The Column pressure is a means to verify that the UltiMate Micropump(s) is (are) really delivering solvent and that the column pressure is within reasonable limits.

2.6 Sequence Setup

After creating a method that meets the needs of your application, a sequence must be created.

2.6.1 Standard Sequence Setup

To create a new sequence:

a) Click the *Sequence Setup* icon on the Xcalibur **Home Page** (FIGURE 2-25) to present the **Sequence Setup** window (FIGURE 2-40).

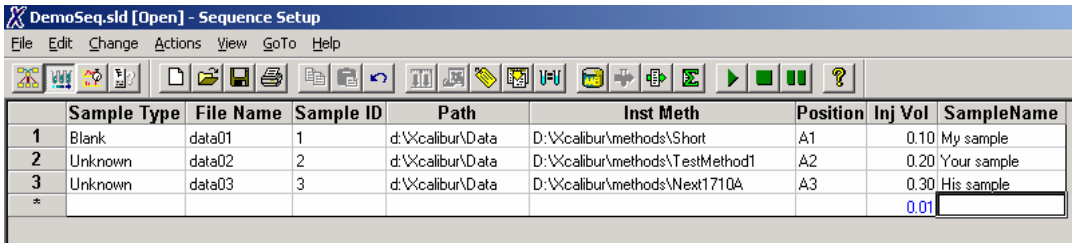


FIGURE 2-40 The Sequence Setup Window

b) Fill out the table to meet the needs of your application. Start by double clicking on the first **Inst Meth** entry. Choose a method from the dialog box that is presented.

c) Enter the sample **Position** and the desired injection volume (**Inj. Vol.**).

d) The **File Name** is set to 'data01, data02, etc.' by default. You may change this according to your needs.

Note: If you intend to re-use the table, save it using any valid name (the sequence will run, even if you have not saved it).

Note: At this point, we will equilibrate the system and then run the sequence. This can be done by either using the 'Direct Control' option from the *Instrument Setup* window or by performing a blank run. A detailed discussion of the 'Direct Control' option is presented in Section 2.5.6.

2.6.2 Starting the Sequence

To start the sequence:

a) Start the sequence by choosing the **Actions** menu and then **Run Sequence** or by choosing the button in the toolbar. The **Run Sequence** window will appear (FIGURE 2-41).

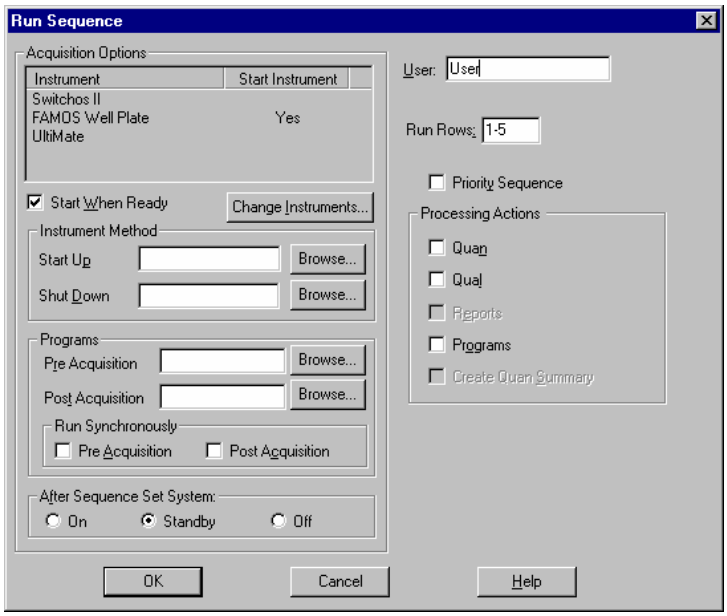


FIGURE 2-41 The Run Sequence Window

b) Define the *Run Rows* and fill in the various parameters according to the needs of your application. Click **OK** to start the sequence.

Note: FIGURE 2-41 shows the FAMOS Well Plate Microautosampler as 'Start Instrument', which means that the FAMOS is started by Xcalibur. After the FAMOS has completed its activities, a pulse from the FAMOS will start the gradient program of the UltiMate system and the data acquisition of the Mass Spectrometer.

Note: When the system is under Xcalibur control, the START IN input of the Micropump(s), the UV Detector and the Switchos II Loading Pump (if installed) must be connected in parallel (Section 2.2.1).

2.6.3 Sequence Setup with a large Number of Samples

If you need to program a large number of samples (e.g. when using microtitre plates), follow the description below:

a) Choose **File** menu and then **New** to open the **New Sequence Template** window (FIGURE 2-42).

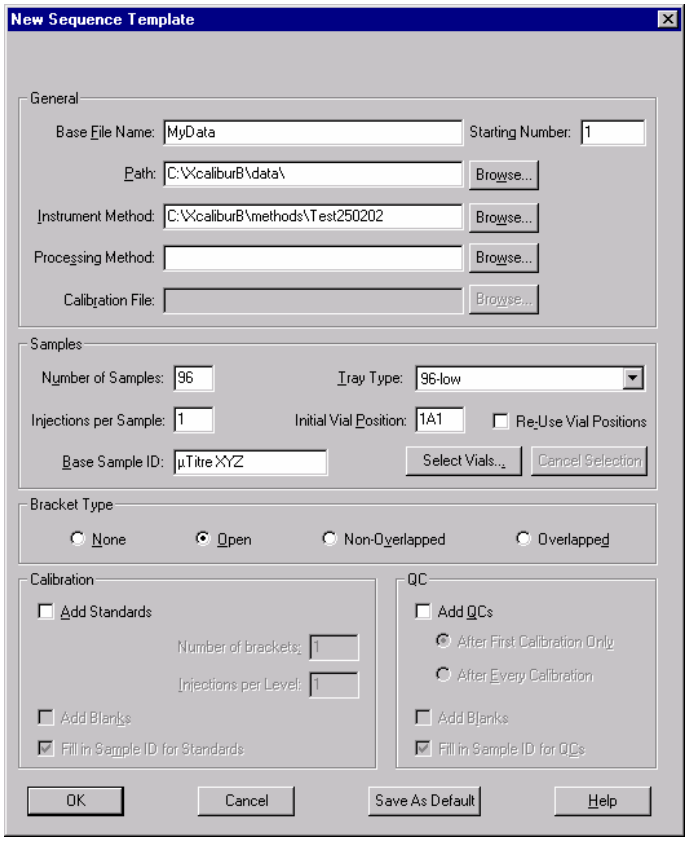
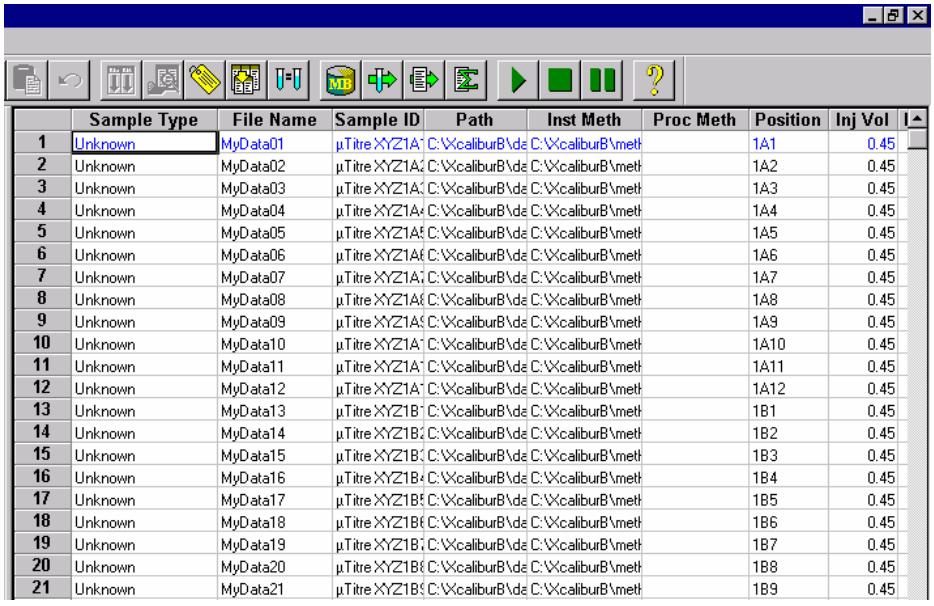


FIGURE 2-42 The New Sequence Template Window

b) Fill in the various parameters and choose **OK**. A run table as presented in FIGURE 2-43 will be automatically generated.



	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol
1	Unknown	MyData01	µTitre XYZ1A1	C:\XcaliburB\da	C:\XcaliburB\meth		1A1	0.45
2	Unknown	MyData02	µTitre XYZ1A2	C:\XcaliburB\da	C:\XcaliburB\meth		1A2	0.45
3	Unknown	MyData03	µTitre XYZ1A3	C:\XcaliburB\da	C:\XcaliburB\meth		1A3	0.45
4	Unknown	MyData04	µTitre XYZ1A4	C:\XcaliburB\da	C:\XcaliburB\meth		1A4	0.45
5	Unknown	MyData05	µTitre XYZ1A5	C:\XcaliburB\da	C:\XcaliburB\meth		1A5	0.45
6	Unknown	MyData06	µTitre XYZ1A6	C:\XcaliburB\da	C:\XcaliburB\meth		1A6	0.45
7	Unknown	MyData07	µTitre XYZ1A7	C:\XcaliburB\da	C:\XcaliburB\meth		1A7	0.45
8	Unknown	MyData08	µTitre XYZ1A8	C:\XcaliburB\da	C:\XcaliburB\meth		1A8	0.45
9	Unknown	MyData09	µTitre XYZ1A9	C:\XcaliburB\da	C:\XcaliburB\meth		1A9	0.45
10	Unknown	MyData10	µTitre XYZ1A1	C:\XcaliburB\da	C:\XcaliburB\meth		1A10	0.45
11	Unknown	MyData11	µTitre XYZ1A1	C:\XcaliburB\da	C:\XcaliburB\meth		1A11	0.45
12	Unknown	MyData12	µTitre XYZ1A1	C:\XcaliburB\da	C:\XcaliburB\meth		1A12	0.45
13	Unknown	MyData13	µTitre XYZ1B1	C:\XcaliburB\da	C:\XcaliburB\meth		1B1	0.45
14	Unknown	MyData14	µTitre XYZ1B2	C:\XcaliburB\da	C:\XcaliburB\meth		1B2	0.45
15	Unknown	MyData15	µTitre XYZ1B3	C:\XcaliburB\da	C:\XcaliburB\meth		1B3	0.45
16	Unknown	MyData16	µTitre XYZ1B4	C:\XcaliburB\da	C:\XcaliburB\meth		1B4	0.45
17	Unknown	MyData17	µTitre XYZ1B5	C:\XcaliburB\da	C:\XcaliburB\meth		1B5	0.45
18	Unknown	MyData18	µTitre XYZ1B6	C:\XcaliburB\da	C:\XcaliburB\meth		1B6	0.45
19	Unknown	MyData19	µTitre XYZ1B7	C:\XcaliburB\da	C:\XcaliburB\meth		1B7	0.45
20	Unknown	MyData20	µTitre XYZ1B8	C:\XcaliburB\da	C:\XcaliburB\meth		1B8	0.45
21	Unknown	MyData21	µTitre XYZ1B9	C:\XcaliburB\da	C:\XcaliburB\meth		1B9	0.45

FIGURE 2-43The Run Table

c) Adjust the rows according your needs.

2.7 Displaying the UV Data and the Column Pressure Data

The following section provides detailed information about the most important options related to the Ultimate System when displaying UV and column pressure data. For general information refer to the Xcalibur user's manual.

To display the chromatogram:

d) Choose *Real Time Plot View* from the **View** menu on the Xcalibur **Home Page** (FIGURE 2-44) or the toolbar button  to display the **Real Time Plot** of the chromatogram. All standard Xcalibur plot options may be used to adapt this view to your requirements.

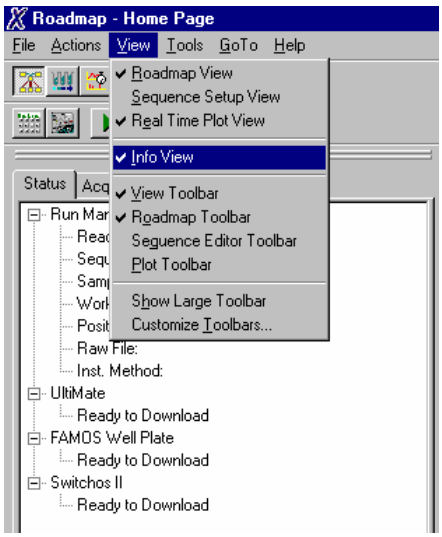


FIGURE 2-44 The View Menu (Home Page)

e) Use the items *Ranges*... and *Display options*... of the ***View*** menu in the **Real Time Plot** window to adapt the chromatogram plots to your individual needs. When choosing *Ranges*... the **Chromatogram Ranges** window appears (FIGURE 2-45).

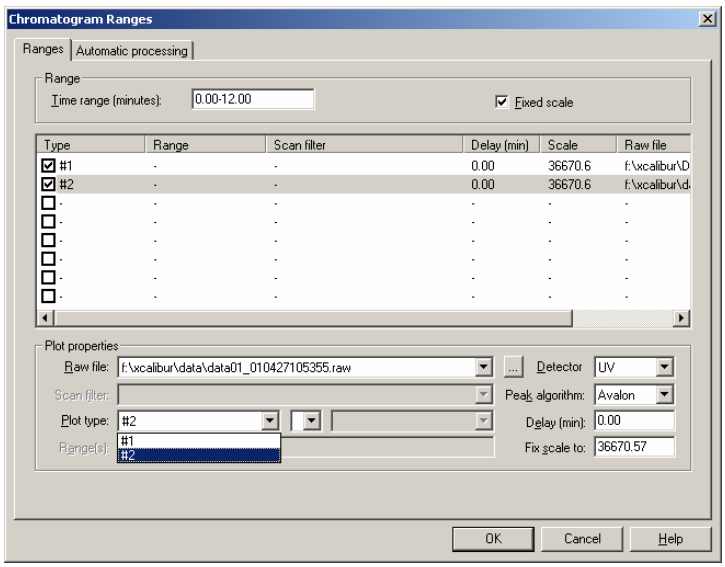


FIGURE 2-45 The Chromatogram Ranges Window

f) To display UV data from more than one channel (wavelength), check the next empty check box and define its contents with the list box *Plot type*. By default only '#1' is checked.

g) Finish by choosing the **OK** button.

The separation column pressure is displayed using the free UV channel 'UV2' of Xcalibur while the trap column pressure uses the free UV channel 'UV3'.

To display the separation column data:

a) Choose *Ranges*... of the ***View*** menu in the **Real Time Plot** window and select 'UV2' as **Detector** (FIGURE 2-46). The **Plot type** is automatically set to 'Pressure (UltiMate)'.

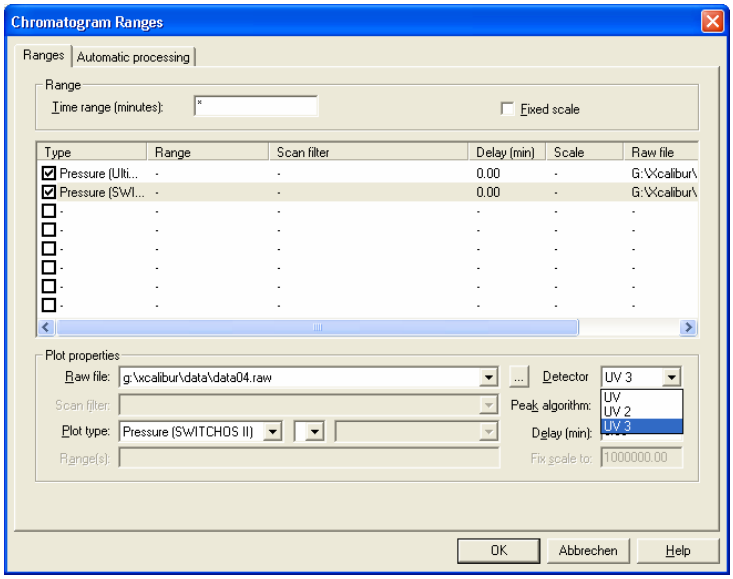


FIGURE 2-46 The Chromatogram Ranges Window

b) Use *Display options*... of the ***View*** menu in the **Real Time Plot** window to adapt the chromatogram plots to your individual needs.

Note: The option to display UV data from more than one channel is available only if the UV Detector setup for collecting UV data indicates that more than one channel has been selected (Section 2.5.1 B). The same is valid for the pressure data: if no data are collected, no data can be displayed!

To display the trap column data:

c) Repeat steps a) and b). Select 'UV3' as **Detector** (FIGURE 2-46). The **Plot type** is automatically set to 'Pressure (Switchos)'.

To adapt the display options:

a) Choose *Real Time Plot View* from the **View** menu on the Xcalibur **Home Page** (FIGURE 2-44) or the toolbar button to display the **Real Time Plot** of the chromatogram.

b) Select the UV Data plot and choose the *Display Options* item from **View** menu in the **Real Time Plot** window, the **Display Options** window will appear (FIGURE 2-47).

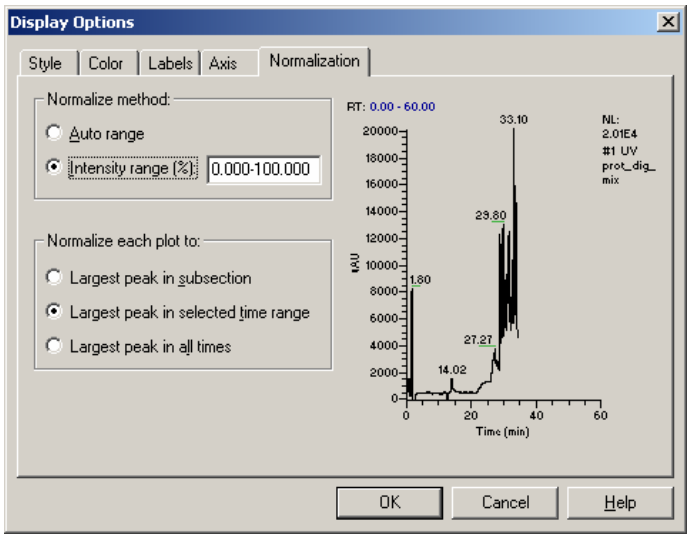


FIGURE 2-47 The Display Options Window (UV Data)

c) Set the 'Intensity range' or the 'Auto range' option. In this case negative values can be displayed.

d) In addition, it might be useful to add some offset value to the axis. Do this by choosing the **Axis** tab and checking the *offset* checkboxes.

e) Select the column pressure plot and adapt the display options of the column pressure according to steps a) to d).

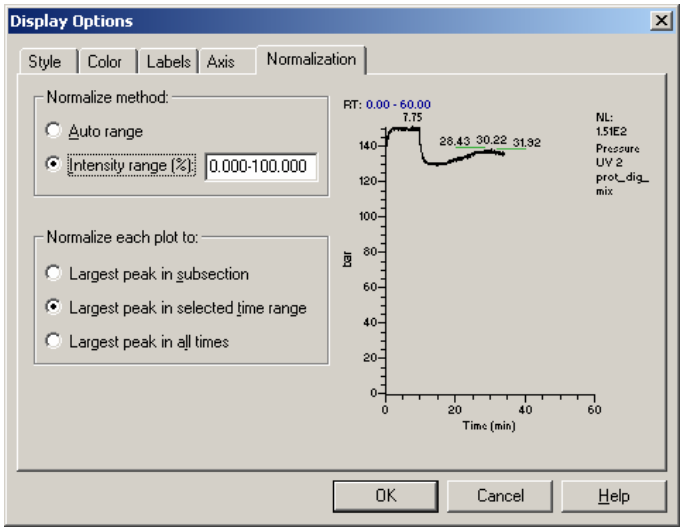


FIGURE 2-48 The Display Options Window (Column Pressure)

FIGURE 2-49 gives a good impression of how the chromatogram and the column pressure display looks with the options presented in FIGURE 2-47 and FIGURE 2-48:

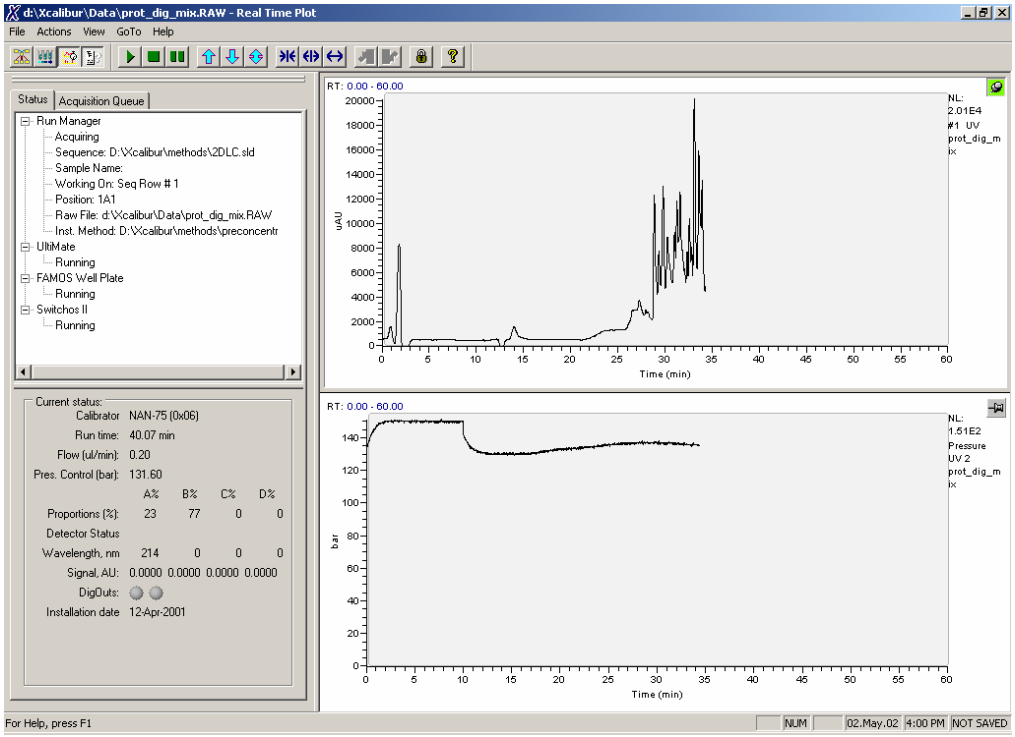


FIGURE 2-49 Presenting the UV Data and the Column Pressure

2.8 Identifying the Plug-In Version

To verify which plug-in version of the individual instruments currently is installed on your PC:

a) Open the **Instrument Configuration** window and choose one of the instruments that are presented in the 'Configured Devices' field, e.g. click on the FAMOS Well Plate icon (item1, FIGURE 2-50).

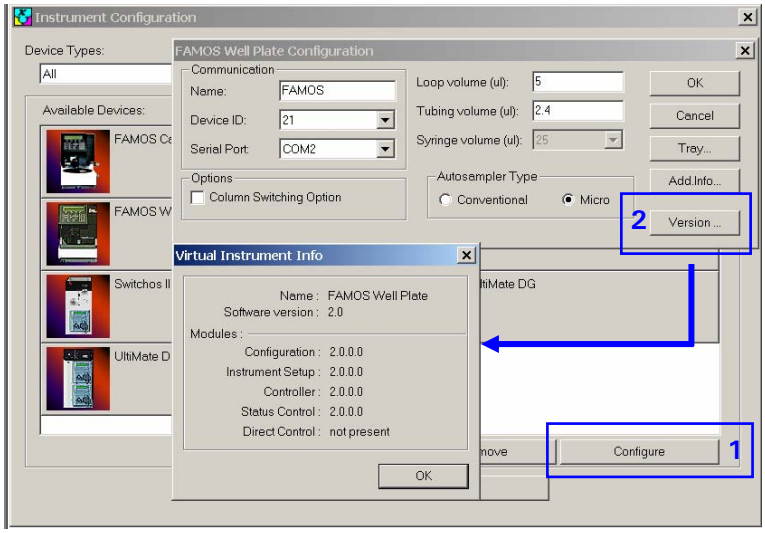


FIGURE 2-50 Reading the FAMOS Well Plate Plug-In Version

b) Choose the **Configure** button (item 1, FIGURE 2-50) to open the configuration box.

c) Choose the **Version** button (item 2, FIGURE 2-50) to open the **Virtual Instrument Info** window.

FIGURE 2-51 presents the **Virtual Instrument Info** window of the UltiMate Dual Gradient Micropump 1.

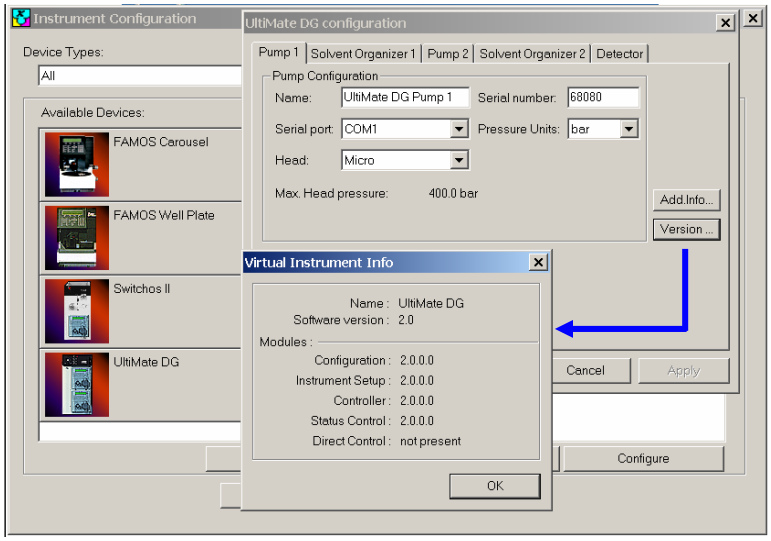


FIGURE 2-51 Reading the UltiMate Micropump Plug-In Version

TABLE 2-1 lists the software version and detailed information about the plug-ins installed with the LC Packings CD ROM 'Virtual Instrument Plug-ins for Xcalibur V1.3 / V1.4, Version 2.0, May 2004'.

TABLE 2-1 Plug-In Version Information

Instrument	Software Version	Detailed Information	
UltiMate Micropump	V2.0	Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A
UltiMate UV Detector		Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A
UltiMate Dual Gradient	V2.0	Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A
FAMOS Carousel	V2.0	Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A
FAMOS Well Plate	V2.0	Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A
Switchos	V2.0	Configuration: Instrument Setup: Controller: Status: Direct Control:	2.0.0.0 2.0.0.0 2.0.0.0 2.0.0.0 N/A

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CHAPTER 3

Programming Examples for the UltiMate Dual Gradient

3.1 Overview

This chapter provides additional information that helps you to setup programs and sequences for the UltiMate Dual Gradient used in one of the following applications:

- Comprehensive 2-D Nano LC Setup (IEX/RP) – Section 3.2
- Parallel Nano–LC – Section 3.3

It is assumed that the UltiMate Dual Gradient System is used in combination with the FAMOS™ Microautosampler, the Switchos™ Advanced Microcolumn Switching Unit and the 6-port Nano Switching Valve (used with the Parallel Nano-LC application only).

For basic information about the electrical connections, the system configuration and the sequence setup, please refer to sections 2.2, 2.4 - 2.6.

Note: These application examples should be considered as starting points. In order to run your particular sample, you may need to optimize the separation parameters (e.g. loading time, valve switching procedure, gradients, etc).

3.2 Comprehensive 2-D Nano LC Setup (IEX/RP)

This section discusses the principle of a comprehensive two-dimensional HPLC application and shows how to setup and control the UltiMate Dual Gradient system in conjunction with the LC Packings VI plug-ins for Xcalibur.

3.2.1 Principle of a Comprehensive 2D-LC Application

At the beginning of the sequence the FAMOS Microautosampler injects the sample onto the Ion Exchange (IEX) column (e.g. a SCX column packed with Poros 10 S material). The gradient for this IEX column is performed by gradient pump 2 (e.g. for a total run time of 865 minutes in this example).

Two reversed phase trap columns (TC1 and TC 2) trap the fractions eluted from the SCX column alternately. The UltiMate gradient pump 1 performs the Nano flow gradients for both trap columns and the Nano separation column. The Switchos loading pump is used to wash the buffer salt from the two trap columns after the loading step and to equilibrate these columns after the elution step.

Fractionating and switching of the trap columns is performed by valve B of the Switchos device. Valve A controls the 'injection' of the trapped fractions onto the Nano separation column and the washing of the trap columns by the Switchos loading pump.

The fluidic setup that is used for this example is presented in FIGURE 3-1. The details of the required system setup and fluidic connections on the two Switchos valves are presented in Sections 3.2.1 A and 3.2.1 B.

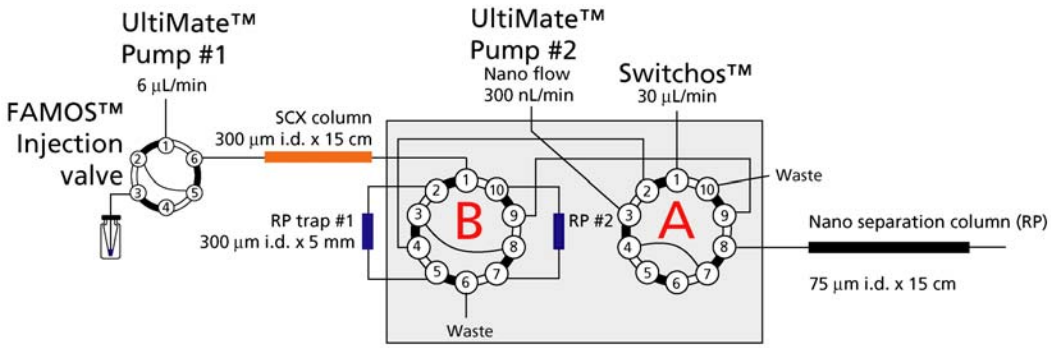


FIGURE 3-1 Fluidic Setup for the Comprehensive 2-D LC Setup

FIGURE 3-2 presents an overview of the gradients to be performed. FIGURE 3-3 presents the timing of the first 4 steps (programs) for the 2-D setup which is discussed in this example.

Section 3.2.2 provides programming information. All programs used with this example can be found on the LC Packings VI Plug-Ins for Xcalibur CD ROM in the folder 'DUAL GRADIENT Programming Examples'.

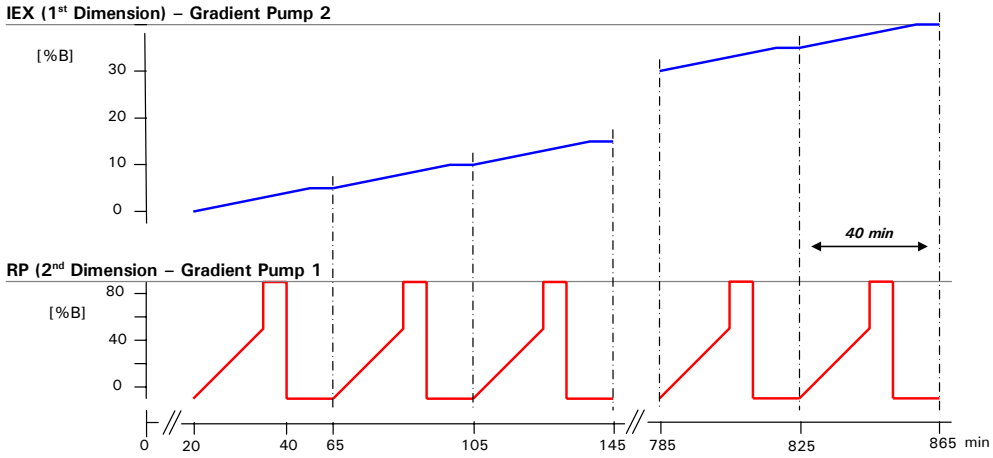
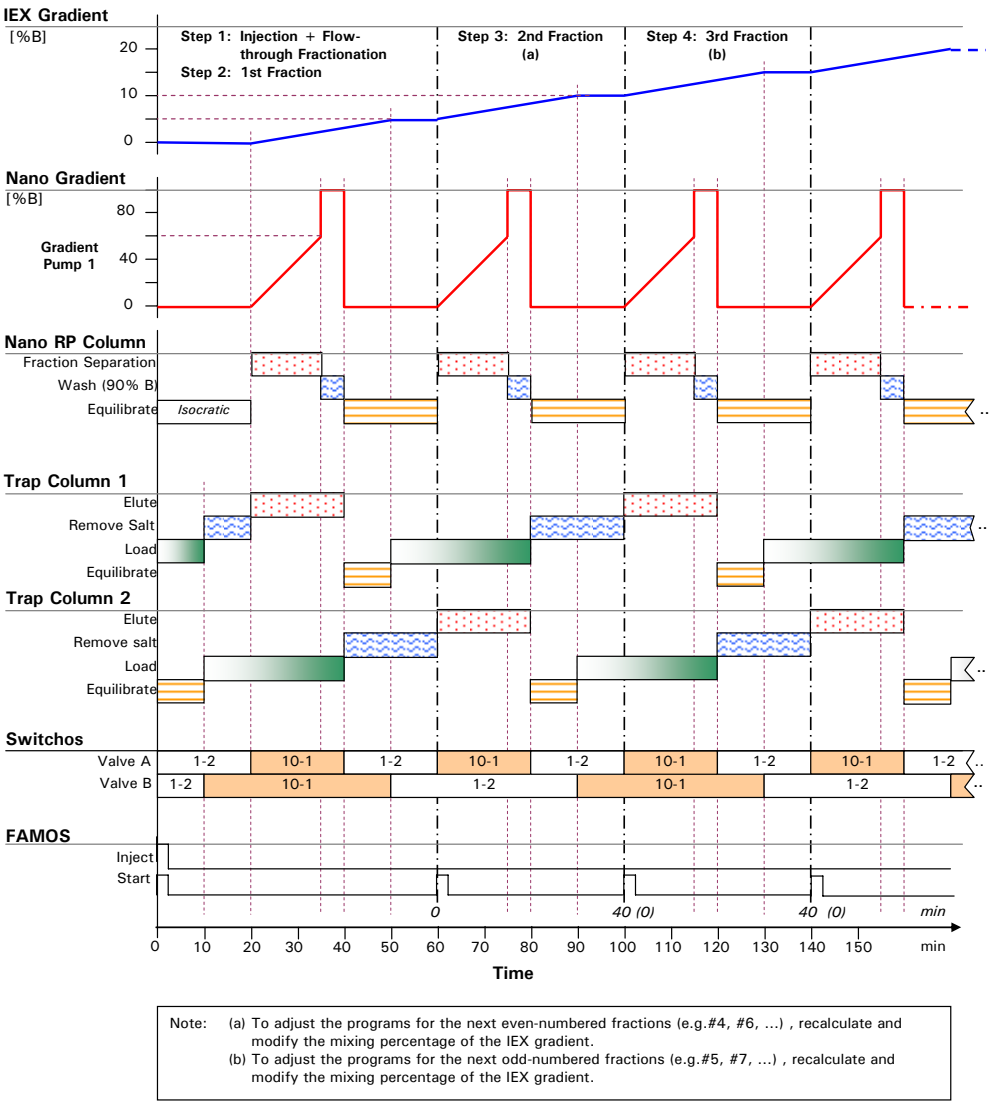


FIGURE 3-2 Gradients used for the Comprehensive 2-D Setup



Note: (a) To adjust the programs for the next even-numbered fractions (e.g.#4, #6, ...) , recalculate and modify the mixing percentage of the IEX gradient.
(b) To adjust the programs for the next odd-numbered fractions (e.g.#5, #7, ...) , recalculate and modify the mixing percentage of the IEX gradient.

FIGURE 3-3 The First 4 Steps of the Comprehensive 2-D Application

The salt content (in this example: 0.5 mol/L KCl in B of SCX mobile phase) in the SCX mobile phase is increased from 0% to 40%. When the first trap column is loaded, the second trap column is eluted and equilibrated, so all three processes are maintained in one program. Only in the first program the FAMOS Microautosampler performs a sample injection onto the SCX column, sample is defined as "Unknown". For all the following runs where the fractions are processed, the FAMOS will not perform any sample injection, but only start the gradient programs using an **U**ser **D**efined **P**rogram (UDP) to generate the start pulse.

3.2.1 A System Setup

TABLE 3-1 provides information of the system setup (e.g. solvents and concentrations).

TABLE 3-1 System Setup

System Component		Description	P/N
UltiMate Dual Gradient System Gradient pump #2	*SCX gradient*		
	IEX column	300 µm I.D. x 15 cm, packed with 10 µm, POROS 10S	162122
	Mobile phase A	5 mmol/L KH_2PO_4 buffer (pH = 3, adjusted with H_3PO_4) + 5% acetonitrile	
	Mobile phase B	5 mmol/L KH_2PO_4 buffer (pH = 3, adjusted with H_3PO_4) + 5% acetonitrile, 0.5 mol/L KCl	
	Flow rate	6 µL/min	
	CRP Value	50	
UltiMate Dual Gradient System Gradient pump #1	*Reversed phase (RP) gradient*		
	Nano separation column	75 µm I.D. x 15 cm, packed with 3 µm C18 100Å PepMap™	160321
	Mobile phase A	98% water, 2% acetonitrile, 0.08% formic acid	
	Mobile phase B	20% water, 80% acetonitrile, 0.1% formic acid	
	Flow rate	300 nL/min	
	CRP Value	625	
UV Detector (if available)	Flow Cell	UZ-View™ flow cell, 10 mm path length, volume 3 nL, for Nano LC	160015
	Wavelength	214 nm	
	Time Constant	2 s	
FAMOS Microautosampler	Sample	Protein mixture digest, 100 pmol, lyophilized Note: sample preparation according to the enclosed instruction sheet, diluted 1:50	161088
	Loop	5 µL	
	Injection volume	5 µL (full loop injection)	
Switchos II	Trap columns	300 µm I.D. x 5 mm, packed with 5 µm C18 100Å PepMap™	160292
	Wash solvent	A: 0.05% trifluoroacetic acid in water	
	Wash flow rate	30 µL/min	
	Wash time	20 min	

3.2.1 B Fluidic Connections - Switchos

TABLE 3-2 Description of the Fluidic Connections for the 2-D LC Setup

Switchos [Valve. Port #]	**Connected to**
A.1	Switchos loading flow, 130 µm ID x 50 cm connection tubing
A.2	25 µm ID x 25 cm length FS transfer tubing from Valve B Port #4
A.3	UltiMate Nano flow, 30 µm ID x 50 cm PEEKSil connection tubing
A.4	Loop to Port #7, 30 µm ID x 10 cm length
A.5	Not used
A.6	Not used
A.7	Loop from Port #4 (see A.4)
A.8	Nano separation column, 75µm ID x 15 cm length
A.9	25 µm ID x 25 cm length FS transfer tubing from Valve B Port #9
A.10	Waste
B.1	IEX Column outlet tubing
B.2	RP Trap column 1 outlet tubing, 30 µm ID x 10 cm length connection tubing
B.3	Loop to Port #8 , 30 µm ID x 10 cm length connection tubing
B.4	25 µm ID x 25 cm length FS transfer tubing to Valve A Port #2
B.5	RP Trap column 1 (see B.2)
B.6	Waste
B.7	RP Trap column 2 outlet tubing, 30 µm ID x 10 cm length connection tubing
B.8	Loop from Port #3 (see B.3)
B.9	25 µm ID x 25 cm length FS transfer tubing to Valve A Port #9
B.10	RP Trap column 2 (see B.7)

3.2.2 Programming the 2D-C Application

This section provides information about how you program the injection step, the flow-through step and the 1st fraction step. In addition, it provides information how to generate the programs for the following fractions. All programs are provided on the LC Packings VI Plug-Ins for Xcalibur CD ROM in the folder 'DUAL GRADIENT Programming Examples'.

- 'UDG_2D_Injection' is used for sample injection, the 'flow-through fraction' (non-binded peptides) step and the first fraction step.

- 'UDG_2D_Fraction1', 'UDG_2D_Fraction2', etc. are different programs that each describes a part of the IEX gradient and one full reversed phase gradient.

It is recommended to perform a blank run in the beginning of the sequence for conditioning of the IEX column.

To adjust the programs for new fractions, recalculate and modify the mixing percentage of the IEX gradient. Use the program 'UDG_2D_Fraction1' for all even-numbered fractions and the program 'UDG_2D_Fraction2' for all odd-numbered fractions.

To program the injection and flow through step and first fraction step:

a) Open the **Instrument Setup** window (Section 2.5).

b) Choose the **UltiMate DG Pump 1** and **UltiMate DG Pump 2** tab to display the pump setup windows and program the required gradients as discussed in Section 2.5.2 A. The following pictures present typical gradients for the gradient pump 1 (FIGURE 3-4) and gradient pump 2 (FIGURE 3-5).

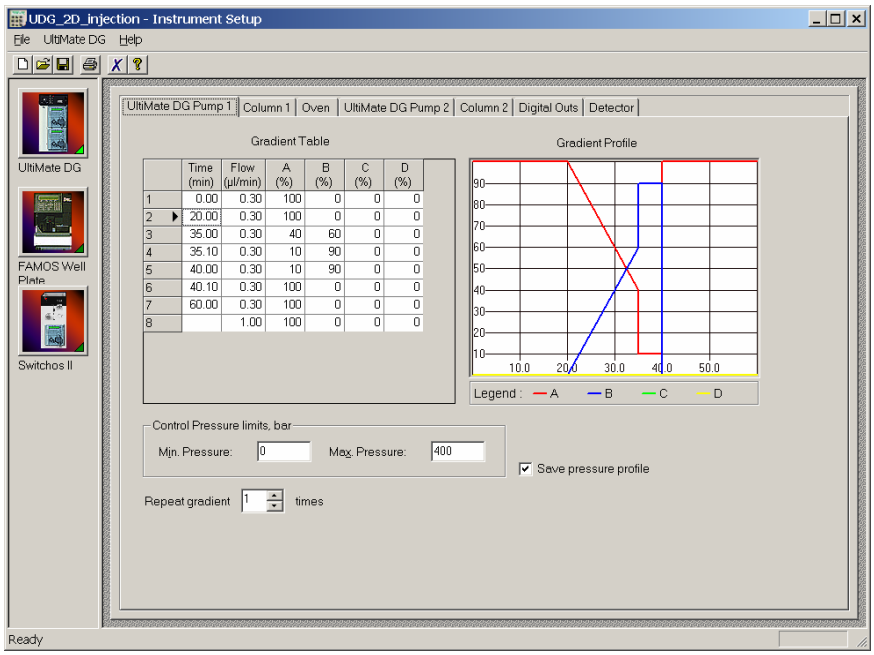


FIGURE 3-4 Gradient Program for the Gradient Pump 1

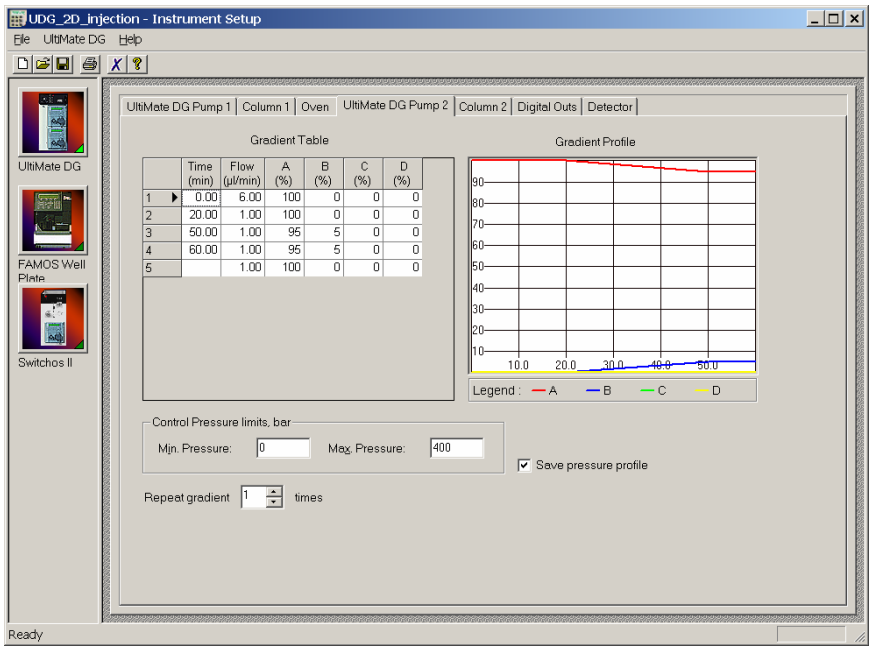


FIGURE 3-5 Gradient Program for the Gradient Pump 1

c) If the UV Detector is part of the configuration, refer to Section 2.5.1 B about how to set up the instrument.

d) Choose the **Column 1** and **Column 2** tabs to display and to setup the column parameters and the CRP correction mode as discussed in Section 2.5.2 B. The following pictures present a typical setup for the column 1 (Nano column) (FIGURE 3-6) and column 2 (IEX column) (FIGURE 3-7).

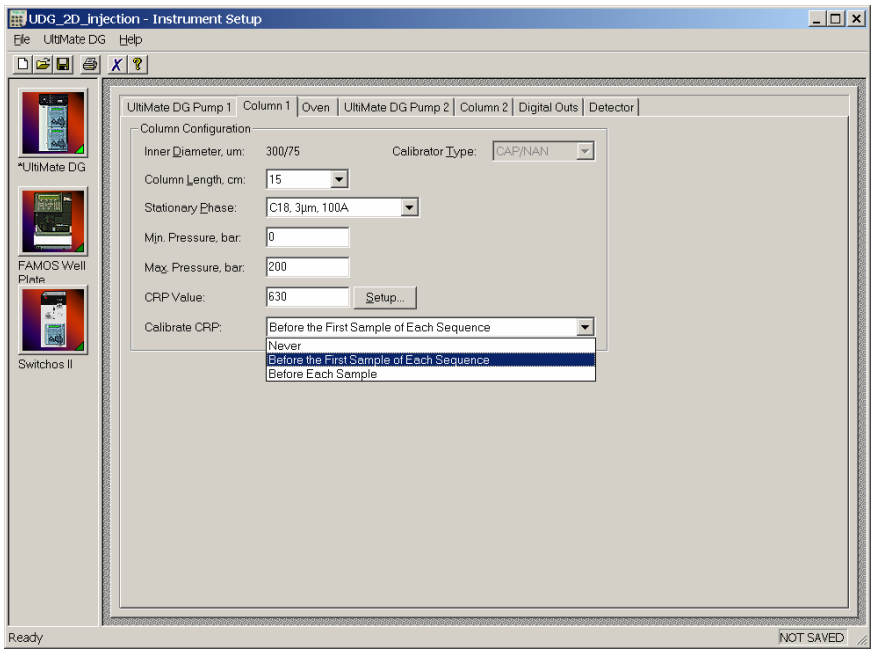


FIGURE 3-6 The Column 1 (Nano Column) Parameters Setup Window

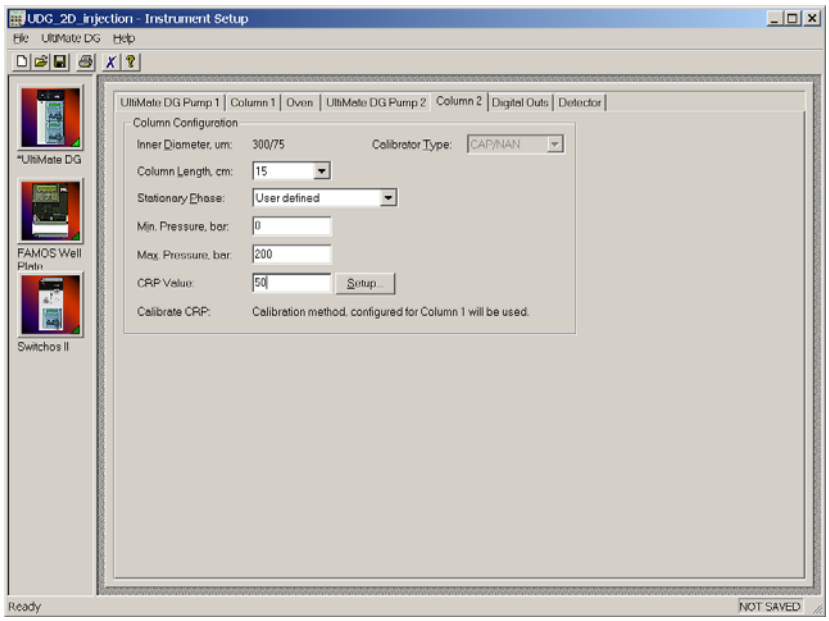


FIGURE 3-7 The Column 2 (IEX column) Parameters Setup Window

e) Choose the *Switchos II* icon in the **Instrument Setup** window to display the Switchos II setup window and to program the valve positions. For the 2D-LC application, the position for valve A and B are opposite in consecutive RP runs as presented in FIGURE 3-8.

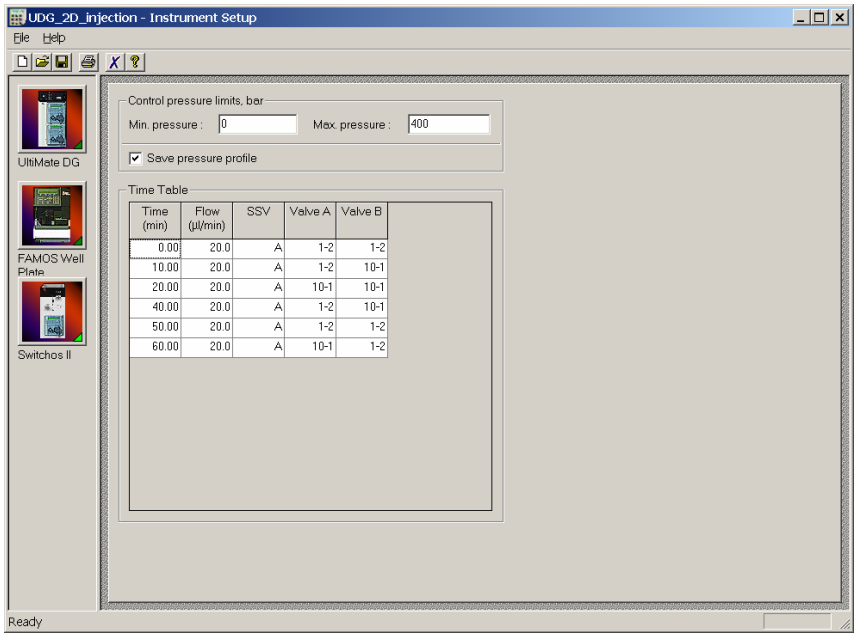


FIGURE 3-8 The Switchos Setup Window

f) Choose the *FAMOS* icon in the **Instrument Setup** window to display the FAMOS setup window (FIGURE 3-9) to program the injection parameters as discussed in Section 2.5.3 A. In the 2D-LC application, sample is injected only in the first line of the sequence.

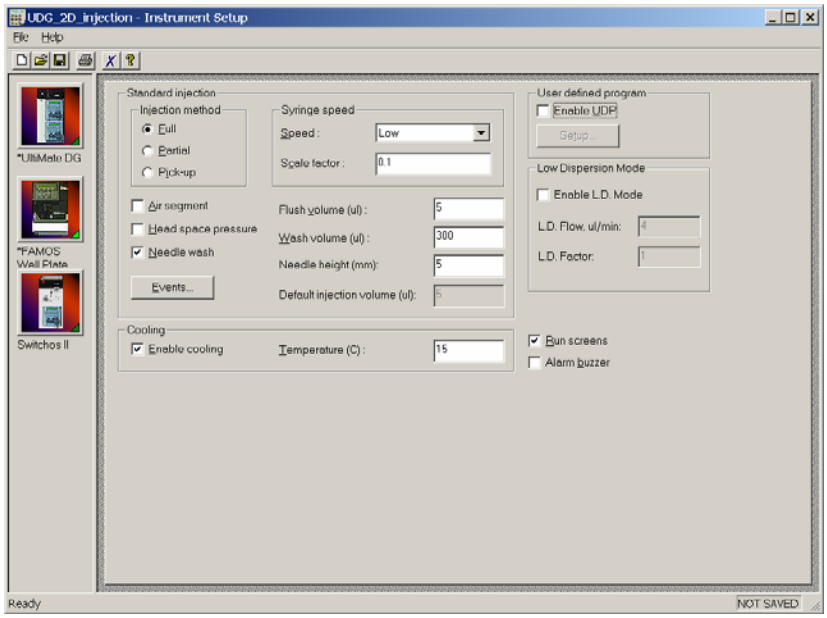


FIGURE 3-9 The FAMOS Setup Window

For the following SCX fractions, a **U**ser **D**efined **P**rogram (UDP) is used on the FAMOS to generate the start signal.

To generate the start signal for the following fractions:

a) Check the 'Enable UDP' option and choose the **Setup** button (FIGURE 3-10).

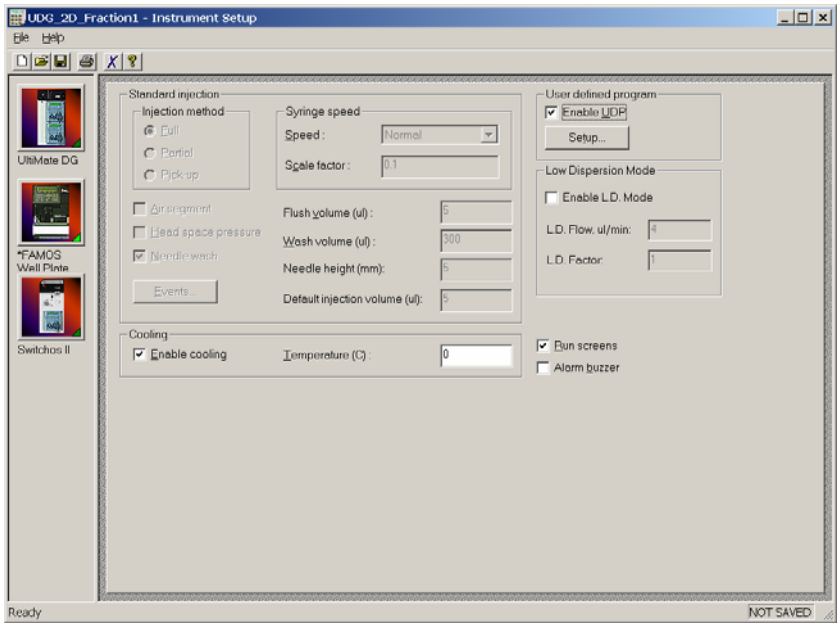


FIGURE 3-10 The User Defined Program Window

b) Program the 'Markers' output (P4) as presented in FIGURE 3-11.

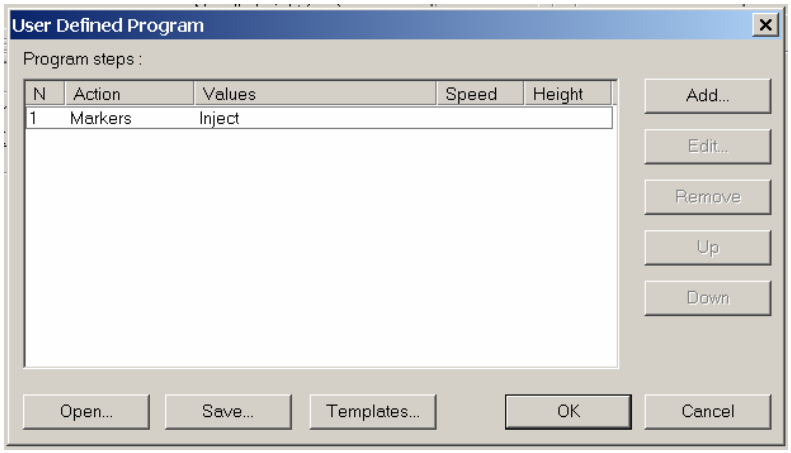


FIGURE 3-11 Inject Marker to start the RP Micropump

3.2.3 Sequence Setup

To setup the sequence for a 2D-LC application, click on the *Sequence Setup* icon on the Xcalibur Home Page to present the **Sequence Setup** window (FIGURE 3-12).

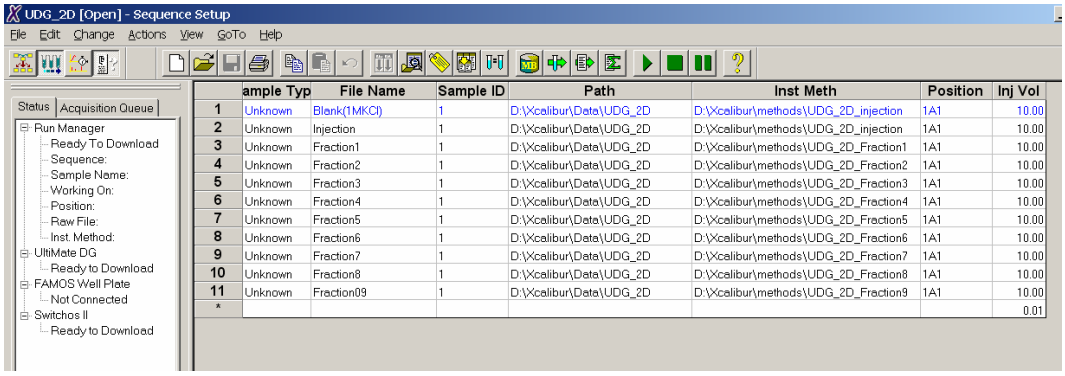


FIGURE 3-12 The Sequence Setup Window

FIGURE 3-12 shows the sequence table for comprehensive 2-D LC as provided on the CD ROM. The first line shows a blank injection which is optional but strongly recommended. The second line shows the sample injection onto the SCX column and the gradient of flow through fraction. All following lines are RP gradients to elute the trapped peptides from the trap column.

Fill out the table to meet the needs of your application. Start by double clicking on the first **Inst Meth** entry. Choose a method from the dialog box that is presented. Enter the sample Position and the desired injection volume (Inj. Vol.). The File Name is set to ‘data01, data02, etc.’ by default. You may change this according to your needs.

Note: If you intend to re-use the table, you should save it under any valid name. However, the sequence will run, even if you have not saved it.

3.3 Parallel Nano–LC

This section discusses the principle of a Parallel Nano-LC application and shows how to setup and control the UltiMate Dual Gradient system in conjunction with the LC Packings VI plug-ins for Xcalibur.

3.3.1 Principle of a Parallel Nano-LC Application

In the Parallel Nano-LC application, two almost identical programs are used in an alternating sequence. In the first program the gradient pump 1 runs a solvent gradient while gradient pump 2 performs a wash and reconditioning step (of the trap column as well as of the separation column). In the second program the gradient pump1 performs a wash and reconditioning step while the gradient pump 2 runs a solvent gradient. The LC Packings ultra low dispersion Nano Switching Valve connects the column on which the gradient is performed and from which the peaks are eluted to the mass spectrometer

The fluidic setup that is used for the Parallel Nano-LC application is presented in FIGURE 3-13.The figure shows the flow path and the positions of the three valves during the elution of the first trap column (performing the first solvent gradient) and the loading of the second trap column.

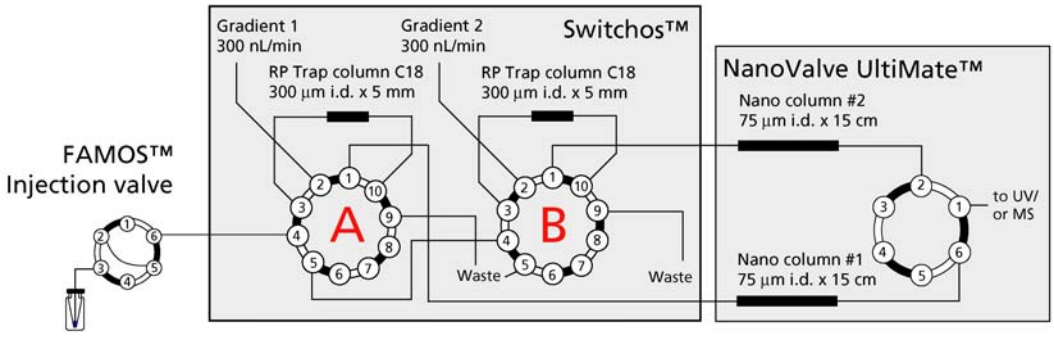


FIGURE 3-13 Fluidic Connections for the Parallel Nano-LC Application

The details of the required system setup, the fluidic connections on the two Switchos valves and on the Nano Switching Valve are presented in Sections 3.3.1 A, 3.3.1 B and 3.3.1 C, respectively.

FIGURE 3-14 presents the timing of the first 4 steps (programs) for the Parallel Nano-LC setup which is discussed in this example.

Section 3.3.2 provides programming information. All programs used with this example can be found on the LC Packings VI Plug-Ins for Xcalibur CD ROM in the folder 'DUAL GRADIENT Programming Examples'.

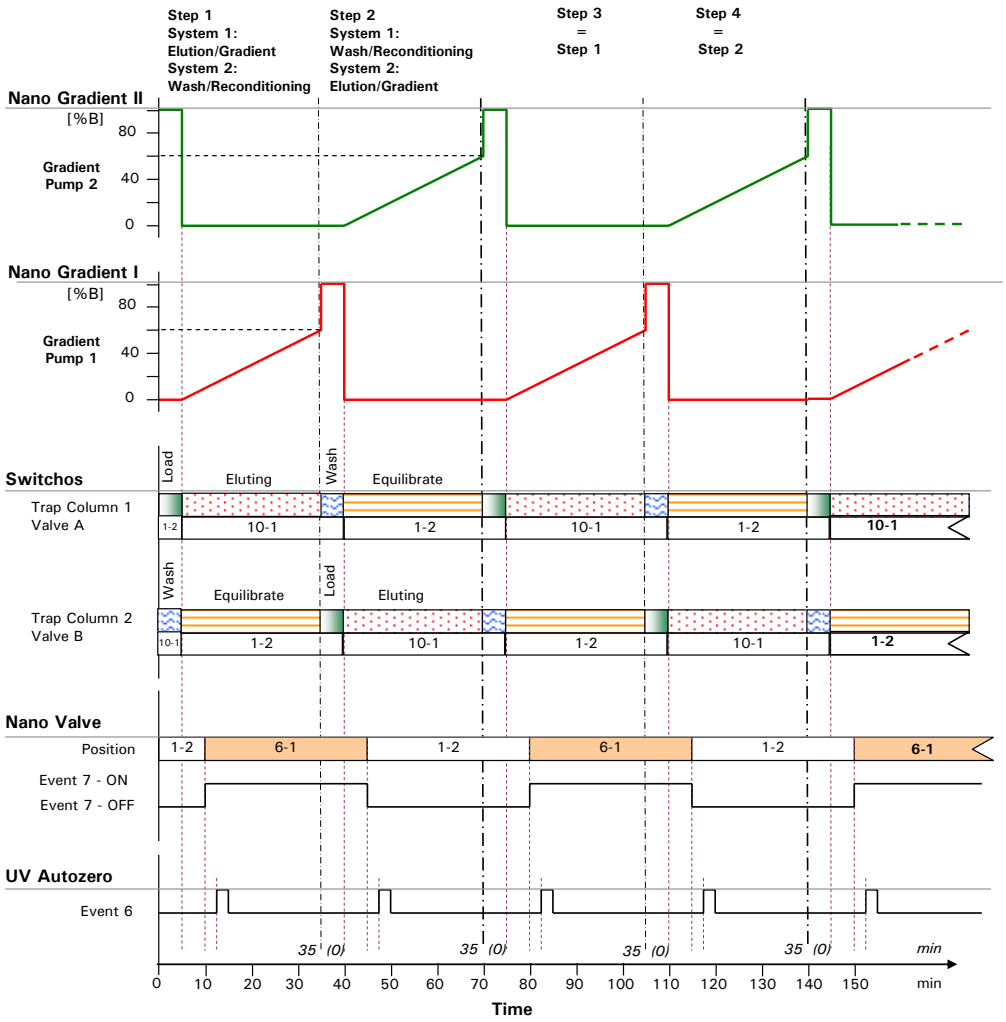


FIGURE 3-14 The First 4 Steps of the Parallel Nano-LC Application

3.3.1 A System Setup

TABLE 3-3 provides information of the system setup (e.g. solvents and concentrations).

TABLE 3-3 System Setup

	System Component	Description
Columns	RP column	2x PepMap C18 (15 cm x 75 μm ID, 3 μm, P/N 160321)
	Trap column	2x PepMap C18 (1 mm x 300 μm ID, 3 μm, P/N 160458)
UltiMate Dual Gradient System	Mobile phase A	0.1% formic acid in water
	Mobile phase B	0.08% formic acid in water/acetonitrile (20:80, v/v)
	Flow rate	300 nL/min
	CRP Value	630
	Gradient program 1	0 - 50%B in 30 min (Gradient Pump 1)
	Gradient program 1	90%B hold 5 min, 0%B 5 - 30 min
	Gradient program 2	90%B hold 5 min, 0%B 5 - 30 min
	Gradient program 2	0 - 50%B in 30 min (Gradient Pump 2)
UV Detector (if available)	Wavelength	214 nm
FAMOS Microauto sampler	Sample	Cytochrome C digest (P/N 161089), diluted to 100 fmol/μL
	Loop	10 μL
	Injection volume	5 μL, partial loop fill
Switchos II	Loading solvent	0.1% TFA in water
	Loading flow rate	30 μL/min
	Loading time	3 minutes
6-Port Nano Switching Valve	Column switching	0 – 10 min Step 1 Pos 1 – 2 Step 2 Pos 6 – 1 10—35 min Pos 6 – 1 Pos 1 – 2

Section 3.3.2 provides programming information. All programs used with this example can be found on the LC Packings VI Plug-ins for Xcalibur CD ROM in the folder 'DUAL GRADIENT Programming Examples'.

3.3.1 B Fluidic Connections – Switchos

TABLE 3-4 lists the required port connections and tubing dimensions which are to be connected to the Switchos valves.

TABLE 3-4 Switchos Fluidic Connections for the Parallel Nano-LC Application

Switchos [Valve. Port #]	Connected to
A.1	PepMap Nano column 1
A.2	Ultimate gradient 1, 20 μm I.D. x 65 cm, P/N 160035
A.3	Trap column 1, 30 μm I.D. PEEK shielded fused silica tubing, P/N 160182
A.4	FAMOS injection valve ; 130µm x 40cm, P/N 160181 (a)
A.5	To valve B, port 4; 130µm x 20cm, , P/N 160181 (a)
A.6	Not in use
A.7	Not in use
A.8	Not in use
A.9	Waste
A.10	Trap column 1, connecting tubing see above
B.1	PepMap Nano column 2
B.2	Ultimate gradient 2
B.3	Trap column 2, 30 μm I.D. PEEK shielded fused silica tubing, P/N 160182
B.4	To valve A, port 5 ; 130 µm x 20 cm, P/N 160181 (a)
B.5	Waste, 200 µm I.D. Tefzel tubing
B.6	Not in use
B.7	Not in use
B.8	Not in use
B.9	Waste, 200 µm I.D. Tefzel tubing
B.10	Trap column 2, connecting tubing see above
Note:	a) Tubing set, consisting of 130 μm I.D. x 100 cm PEEK tubing

3.3.1 C Fluidic Connections – 6-port Nano Valve

TABLE 3-5 lists the required port connections and tubing dimensions which are to be connected to the Nano Switching Valve.

TABLE 3-5 Fluidic Connections of the Nano Valve

Port #	Connected to
1	Mass spectrometer , I.D. 20 µm, P/N160475 (a)
2	Nano column, I.D. 20 µm, P/N160475 (a)
3	Waste
4	Not used
5	Waste
6	Nano column 1, I.D. 20 µm, P/N160475 (a)
Note:	a) Fused silica tubing I.D. 20μm / O.D. 280μm, 5 meter

Note: The position of the Nano Switching Valve is controlled by the event output 7 of the UltiMate gradient pump 2. For more details about the electrical connection and control of this module, refer to the user's manual provided with this instrument.

3.3.2 Programming the Parallel LC Application

This section discusses the programs used to perform consecutive separations of a cytochrome c digest to test and understand the working principle of a Parallel Nano-LC application.

- 'Parallel_Column1' performs a gradient run on gradient pump 1 and a wash and reconditioning on gradient pump 2.
- 'Parallel_Column2' performs a gradient run on gradient pump 2 and a wash and reconditioning on gradient pump 1.

It is recommended to perform a two blank runs in the beginning of the sequence.

Use the program 'Parallel_Column1' for all odd-numbered injections and the program 'Parallel_Column2' for all even-numbered injections.

To program the first step (the first 35 minutes run) of the Parallel Nano-LC example:

a) Open the **Instrument Setup** window (Section 2.5).

b) Choose the **UltiMate DG Pump 1** and **Pump 2** tab to display the pump setup windows and program the required gradients as discussed in Section 2.5.2 A. The following pictures present the gradients for the gradient pump 1 (FIGURE 3-15) and gradient pump 2 (FIGURE 3-16).

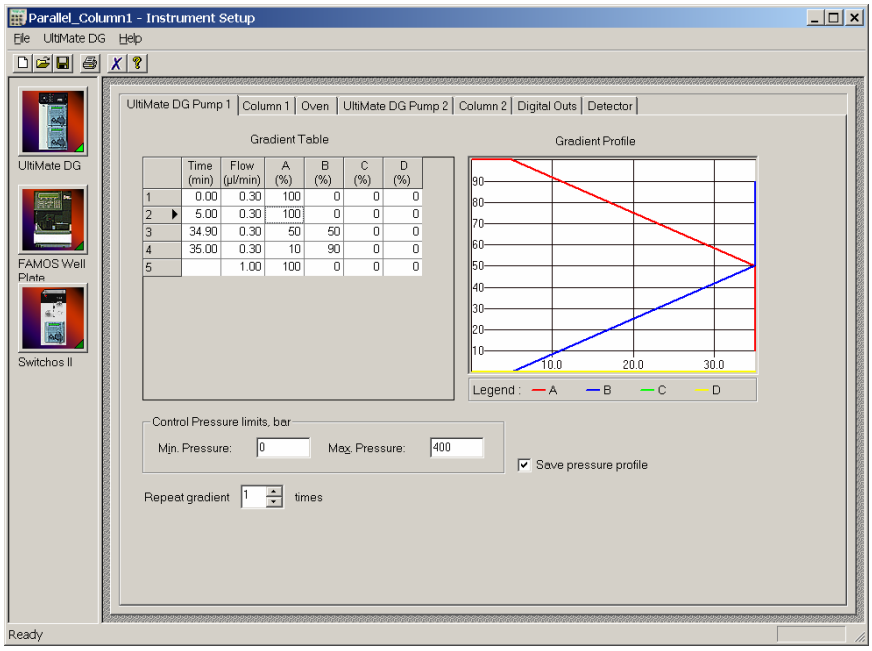


FIGURE 3-15 Gradient Program for the Gradient Pump 1 – Gradient Elution

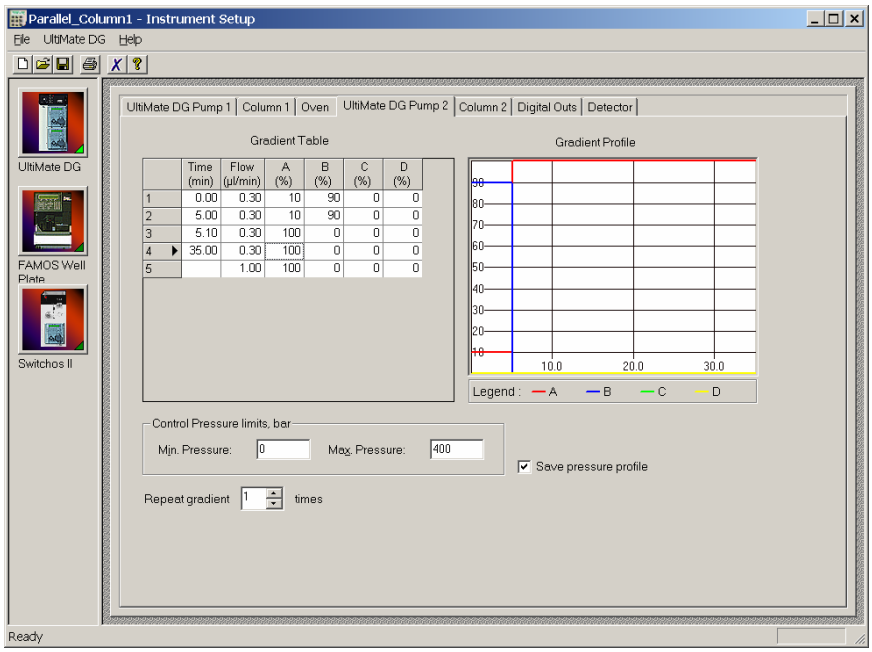


FIGURE 3-16 Gradient Program for the Gradient Pump 2 - Wash and Reconditioning

c) Choose the **Column 1** and **Column 2** tabs to display and to setup the column parameters and the CRP correction mode as discussed in Section 2.5.2 B. The following pictures present a typical setup for the two identical Nano separation columns (FIGURE 3-17).

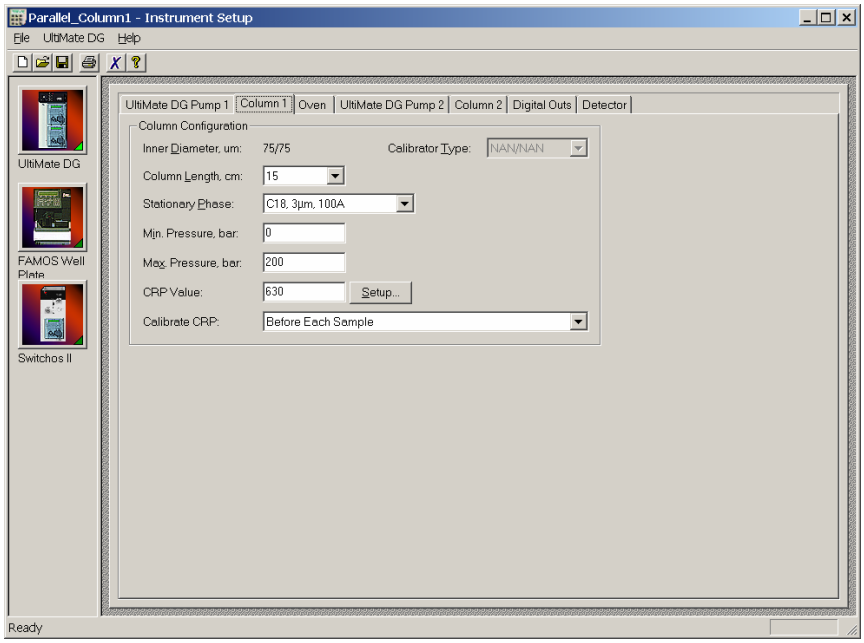


FIGURE 3-17 The Column 1 (Nano Column 1) Parameters Setup Window

d) To control the position of the Nano Switching Valve and the autozero signal of the UV Detector (if installed) by the event output 7 and 6 of the UltiMate gradient pump 2, select the **Digital Outs** tab and program the outputs as presented in FIGURE 3-18.

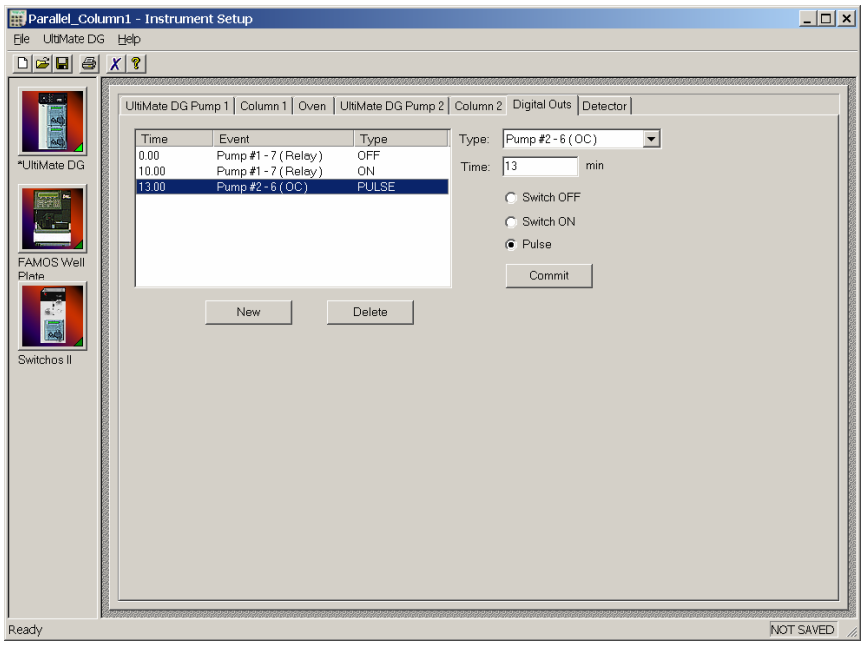


FIGURE 3-18 Controlling the Nano Switching Valve and the UV Autozero

e) If the UltiMate UV Detector is part of the configuration, select the **Detector** tab and setup the wavelength parameter as presented in FIGURE 3-19. For more programming details refer to Section 2.5.1 B.

Note: The exact timing of the UV Detector autozero signal depends on the delay volume of the connecting tubing between the columns and the detector flow cell. To get best performance, you may need to change this setting.

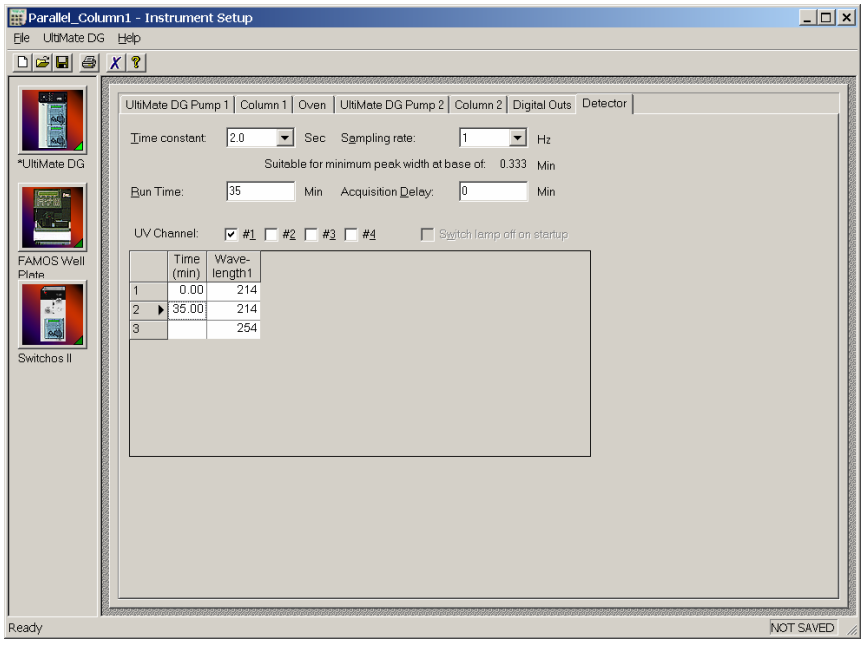


FIGURE 3-19 The UV Detector Setup Window

f) Choose the *Switchos II* icon in the **Instrument Setup** window to display the Switchos II setup window and to program the valve positions. For the Parallel Nano-LC application, the position for valve A and B are opposite in consecutive RP runs as presented in FIGURE 3-20.

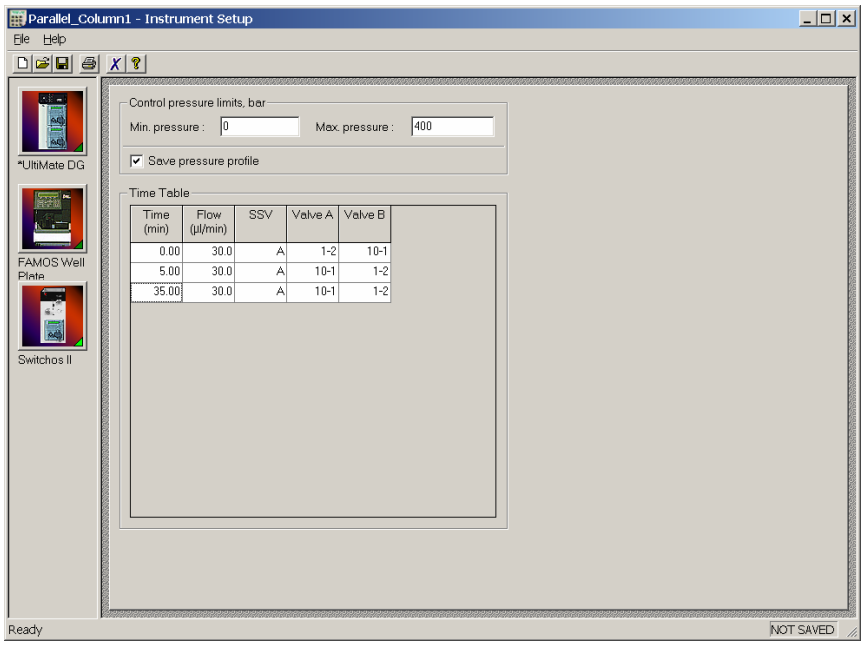


FIGURE 3-20 The Switchos Setup Window

g) Choose the *FAMOS* icon in the **Instrument Setup** window to display the FAMOS setup window (FIGURE 3-21) to program the injection parameters as presented in TABLE 3-3.

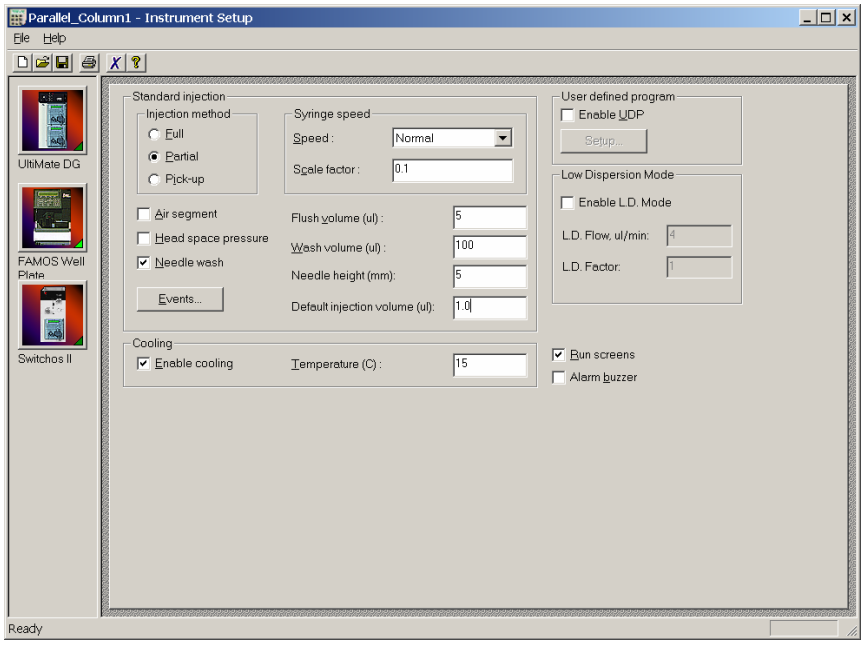


FIGURE 3-21 The FAMOS Setup Window

To program the second step (the next 35 minutes run):

a) Program the gradient pump 1 (which performs the wash and reconditioning step now) as presented in FIGURE 3-22 and the gradient pump 2 as presented in FIGURE 3-23.

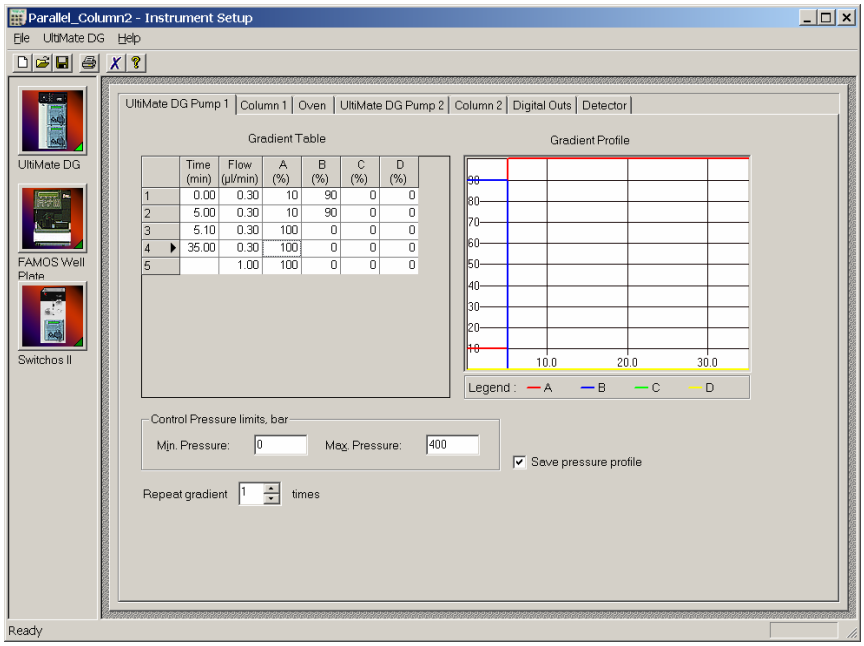


FIGURE 3-22 Gradient Program for the Gradient Pump 1 - Wash and Reconditioning

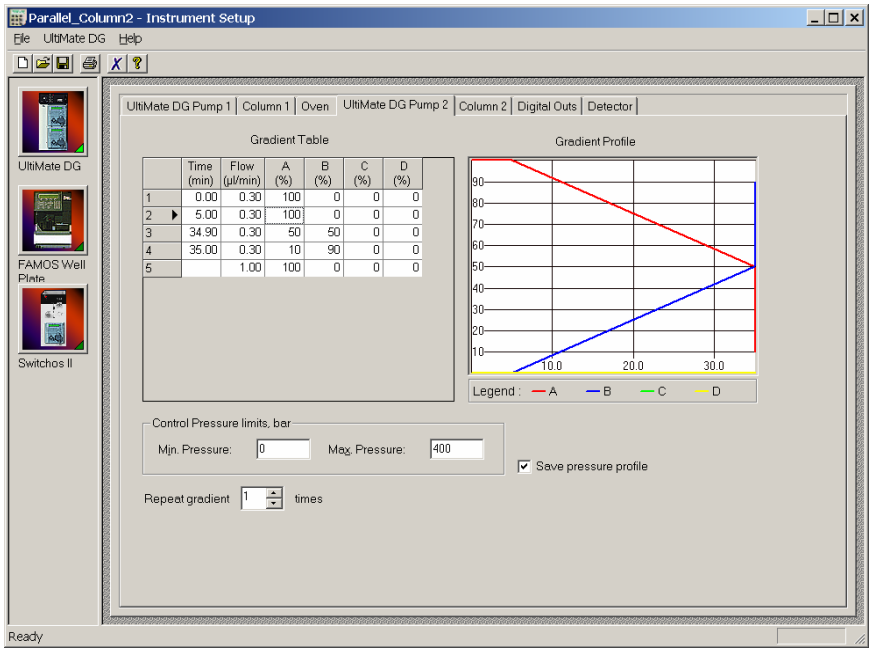


FIGURE 3-23 Gradient Program for the Gradient Pump 2 – Gradient Elution

b) To switch the Nano Switching Valve back after 10 minutes and to generate the autozero signal, program the event outputs of gradient pump 2 as presented in FIGURE 3-24.

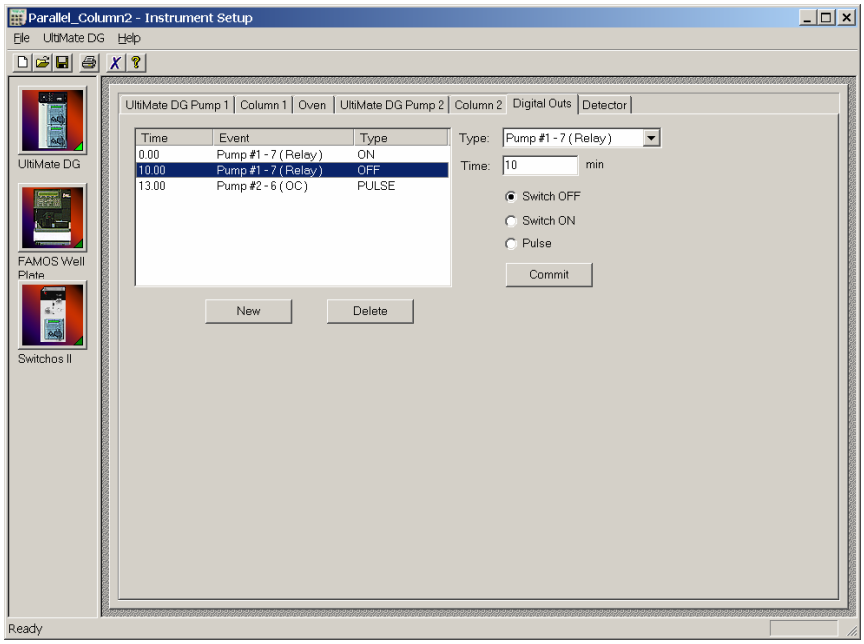


FIGURE 3-24 Controlling the Nano Switching Valve and the UV Autozero

c) Setup the UV Detector (if installed), the Switchos and the FAMOS as discussed in step e) to step h) above.

To program the following programs, use the program 'Parallel_Column1' for all odd-numbered injections and the program 'Parallel_Column2' for all even-numbered injections.

3.3.3 Sequence Setup

To setup the sequence for a 2D-LC application, click on the *Sequence Setup* icon on the Xcalibur Home Page to present the **Sequence Setup** window (FIGURE 3-25).

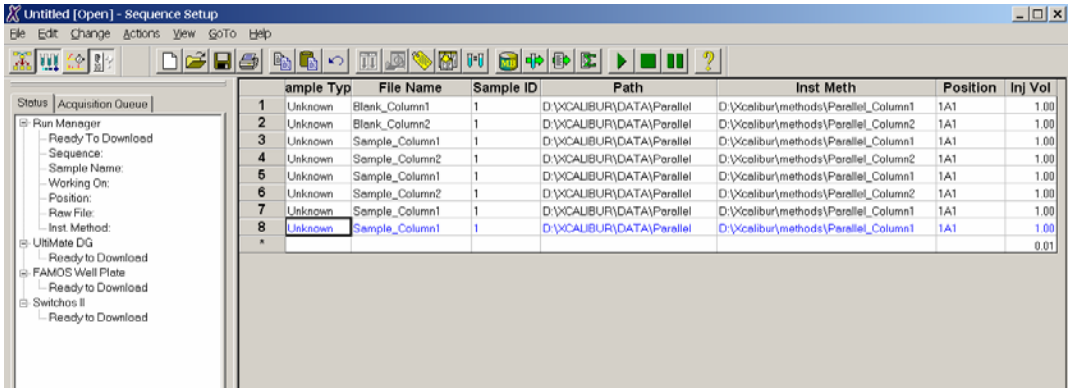


FIGURE 3-25 The Sequence Setup Window

FIGURE 3-25 shows the sequence table for Parallel Nano-LC as provided on the CD ROM. The first two lines show blank injections which are optional but strongly recommend. The third line shows the first sample injection (step 1), etc.

Fill out the table to meet the needs of your application. Start by double clicking on the first **Inst Meth** entry. Choose a method from the dialog box that is presented. Enter the sample Position and the desired injection volume (Inj. Vol.). The File Name is set to 'data01, data02, etc.' by default. You may change this according to your needs.

Note: If you intend to re-use the table, you should save it under any valid name. However, the sequence will run, even if you have not saved it.

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