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UltiMate™

Fully Integrated Micro-, Capillary- and Nano HPLC System

User's Manual

P/N 160534

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Notice: The UltiMate system is covered by a limited warranty. A copy of this warranty is included with this manual. The customer is required to perform routine maintenance as described in the User's Manual on a periodic basis to keep the warranty in effect.

All information in this manual is subject to change without notice and does not represent a commitment on the part of LC Packings, BV.

The material included in this manual is provided to assist users in the operation, maintenance and repair of UltiMate HPLC systems. It is assumed that the individual using this manual has sufficient training in the use of analytical instrumentation and is aware of the potential hazards including (but not limited to) electrical hazards, chemical solvent hazards, exposure to UV radiation and the exposure to pressurized solvents.

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Warranty

LC Packings (Netherlands) BV, warrants that the products manufactured and sold by it to be free from defects in material and workmanship for normal use and service from the date of delivery to original purchaser for a period of one (1) year from the date of shipment. This limited warranty does not cover, and no warranty is provided, for parts that by their nature are required to be replaced periodically as a function of use of the normal operation of the system. These items include, without limitation: HPLC columns, fuses, tubing, detector sources, pump piston seals, injector rotors, check valves, filters, any software, etc. In addition, damage due to corrosion, misuse, negligence, accident, alteration of the system or repair by an unauthorized individual is not covered by the warranty. It is understood that the performance characteristics of the instrument require that the mobile phase be degassed with He as described in the User's Manual.

This warranty covers products sold under the LC Products trademark. If a different warranty than the above is indicated in the sales literature, the warranty indicated in the sales literature will prevail. If the system includes equipment supplied by LC Packings but manufactured by a third party, LC Packings makes no warranty of any kind, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose. LC Packings will make available to you, to the extent permitted, the warranties of the manufacturer of the relevant equipment following your timely written request.

If any product covered by this warranty becomes defective during the warranty period, it will be repaired or replaced by LC Packings at no charge to the customer (the repair/replace decision is solely at the option of LC Packings). All warranty requests must be received by LC Packings during the warranty period.

LC Packings will pay for surface transportation to the applicable LC Packings Office (North America – Sunnyvale CA, Europe and Asia - Amsterdam, the Netherlands), if the instrument proves defective within thirty (30) days from the date of shipment (this does not include air freight, drayage, labor, crating charges, customs clearance charges, etc.). The user should carefully follow the directions indicated on the Return Goods Instruction Sheet in the User's Manual. After thirty days, all transportation costs will be at the expense of the customer.

Software Warranty

If, at any time during the period ending ninety (90) days after delivery of any product to you, you report and document any error in any software provided with such product and developed by LC Packings or any failure of any such software substantially to conform to LC Packings software description that limits or prevents use of the software by you, we will use reasonable efforts to correct any such error or failure, will replace such software or will terminate your license to use the software and refund the price of the related product. In connection with any such termination and refund, you will return the related product to LC Packings upon request.

The warranty will apply only to those portions of the software that were developed by LC Packings and that incorporated all program corrections and modifications, if any, delivered to you. It will not apply to any error or failure due to machine error or to the misuse by or negligence of any person or entity other than LC Packings or to any software, which is modified by any person, or entity other than LC Packings.

Liability

Under no circumstances shall LC Packings be liable for damage to persons or property. This warranty is the only warranty given by LC Packings with respect to products and software provided with the products and is given in lieu of all other warranties, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose.

Your exclusive remedies and LC Packings's sole liability for any non-conformity or defect in the products and such software will be those expressed herein. Under no circumstances will LC Packings's liability arising from the performance or failure to perform of any product or software, in contract, in tort (including negligence), or otherwise, exceed the purchase price of the product and software. In no event will LC Packings be liable, in contract, in tort (including negligence), or otherwise for special, incidental, consequential or analogous damages, including, without limitation, damages resulting from loss of use, loss of profits, loss of business or loss of goodwill, even if LC Packings has been advised of the possibility of such damages.

This warranty comprises the entire warranty between LC Packings and the customer. It overrides any warranty related language that may appear in the customer purchase order or other documentation provided by the customer.

This warranty shall be governed by, and construed and enforced in accordance with, the laws of the Netherlands. It is non-transferable and shall run to the benefit of the original purchaser only. Any change, alteration or amendment to this warranty is not valid unless it has been approved in writing by an officer of LC Packings.

North America
LC Packings / Dionex

500 Mercury Drive
Sunnyvale, CA 94088-3603
USA

Technical Call Center
USA/CA: (800) 346-6390

Europe and Asia
LC Packings (Netherlands) BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
Fax: + 31 20 685 3452

Instructions for Returning Instruments

Before you return any item for repair, please contact the nearest LC Packings office or its local distributor for instructions and obtain a return authorization number.

Pack the equipment carefully, preferably in its original shipping container and ship it to the LC Packings Service Department, using the appropriate address.

North America
LC Packings / Dionex

500 Mercury Drive
Sunnyvale, CA 94088-3603
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Technical Call Center
USA/CA: (800) 346-6390

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LC Packings (Netherlands) BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
Fax: + 31 20 685 3452

IMPORTANT:

1) Make certain that the return authorization number together with the HEALTH AND SAFETY form (if applicable) is attached outside of the package so that we can properly track and account for your system.

2) Please include the following

   a) Company letterhead with the following information.

      - Your Name
      - Complete Mailing Address
      - Telephone Number, fax number and e-mail address
      - Return Authorization Number
      - A detailed description of the problem.
      - The name of the LC Packings personnel to whom you have spoken to regarding the problem
      - Return Shipping Information (if appropriate)

   b) Relevant chromatograms

   c) A purchase order (if the system is not in warranty)



Note: The completed and signed HEALTH AND SAFETY form must be returned to LC Packings service department (fax or mail) prior to the return of any component, or attached outside the shipping package. In addition, the provided RMA number must be clearly marked on the outside of the shipping package. Failure to complete and return this form will result in the package returned without the parts being inspected or credit issued.

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Warnings

The Danger sign, Warning sign and the Caution sign shown below are included in various locations in this manual. These signs provide the following information:



Danger: The information in a danger statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to personal injury or loss of life.



Warning: The information in a warning statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to severe injury and/or damage or destruction to parts or all of the equipment.



Caution: The information in a caution statement relates to a condition that could lead to damage to equipment and/or lead to invalid analytical results.



Note: The information in a note statement relates to important information that should be read and understood before continuing.

Safety Precautions



Note: The following precautions should be followed to minimize the possibility of personal injury and/or damage to property.



Note: Make certain that you are familiar with the contents of this manual before working on the system.

1) The system should be installed in a well-ventilated laboratory. If the mobile phase includes volatile or flammable solvents, make certain that they are not allowed to enter the workspace.

2) If the mobile phase includes volatile or flammable solvents, avoid open flames and sparks.

3) If a leak occurs, turn off power to the instrument and remedy the situation immediately.

4) All components of the system should be plugged into a common power line that is directly connected to a true ground.

5) When the panels are removed, dangerous electrical connections will be exposed. Disconnect the instrument from all power sources before removing the panels.

6) The D_2 lamp in the UV Detector emits radiation from 190 nm to 700 nm. Radiation below 400 nm is hazardous to the eyes and the user should refrain from viewing an illuminated lamp.

7) Always replace blown fuses with fuses of the same size and rating indicated on the fuse holder and panel. Refer to Section 3.5.16 of this manual for more information on fuses.

8) Repair or replace faulty power cords and all communication cables.

9) Many organic solvents and buffers are toxic. Make certain that you know the toxicological properties of all mobile phases that you are using.

10) The toxicological properties of many samples may not be well known. If you have any doubt about a sample, treat it as if it contained a potentially harmful substance.

11) Wear protective eye goggles when handling mobile phases or operating the instrument. An eye wash facility and a sink should be close to the unit. If any mobile phase splash on the eyes or skin, wash the affected area and seek medical attention.

12) Dispose of all waste mobile phase in an environmentally safe manner that is consistent with all local regulations. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose flammable and/or toxic solvents through the municipal sewage system.

13) PEEK tubing is used in a variety of locations. While this polymer has superb chemical resistance to most organic solvents, it tends to swell when it is contact with $CHCl_3$, DMSO and THF. In addition, it is attacked by concentrated acids such as Sulfuric Acid and Nitric Acid (swelling or attack by acid is not a problem with short flushing procedures).

    Do not use PEEK tubing that is stressed, bent or has a kink.

14) Wear protective eye goggles when handling fused silica tubing (i.e. installation, cutting etc.)

15) If a buffer is used as a part of the mobile phase, flush the system with several volumes of a methanol/water (50/50) before it is shut down. This will prevent salt buildup inside the unit.

16) Do not use the instrument in ways other than those indicated in the instructions given in this manual.

CE

DECLARATION OF CONFORMITY

We

LC Packings Nederland B.V.
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

declare that our product

UltiMate™ Fully Integrated Micro-, Capillary- and Nano HPLC System

is in confirmation with the following documents:

EEC directives 89/392, incl. 91/368 and 93/44 (machine safety) and EEC directives 73/23 and 93/68 (low voltage safety), applied with the following standard:

EN61010-1	Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree II)



WARNING

LC Packings will not accept any liability for damages direct or indirect caused by connecting this instrument to devices which do not meet relevant safety standards.

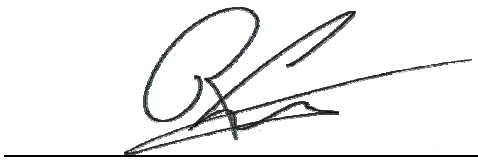
EEC directives 89/336 and 92/31 (EMC requirements), applied with the following standards:

EN 50081-1	Generic emission standard
EN 50082-1	Generic immunity standard
EN 61000-3-2	Harmonic current emissions



Use shielded cables and connectors for all remote connections.

Amsterdam, December 8, 2000



Robert van Ling, QA manager

D962R1

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CHAPTER 1

Introduction

1.1 Overview

The LC Packings UltiMate™ is a fully integrated micro-scale high performance liquid chromatograph. The system is designed to use micro-HPLC columns, nL/min to µL/min flow rates and a specially designed flow cell to obtain the most sensitive UV detection. It provides superb sensitivity, reproducibility and separation efficiency and is designed to be easy to use. The UltiMate system is fully controlled by the CHROMELEON™ Chromatography Management System. Typical applications include the analysis of proteins and peptides, pharmaceuticals and their metabolites and amino acids.

This manual is designed to assist the chromatographer who uses the system to separate and quantize complex samples and should be used in conjunction with the user's manual for the CHROMELEON Software for Windows™.

Note: Individuals using this manual should note the safety issues discussed on pages xiii-xiv.

1.2 Fluidics Pathway

The fluidics pathway of the UltiMate system is shown in FIGURE 1- 1.

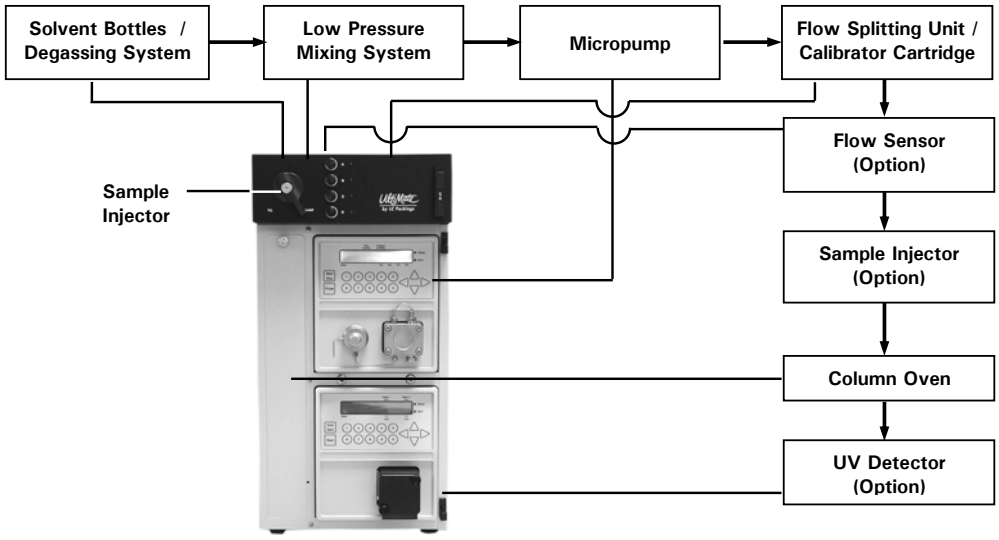


FIGURE 1- 1 Schematic Design - UltiMate System

- **Solvent Bottles and Degassing System** - provides mobile phase to the system. The Helium degassing system is provided to improve check valve reliability and diminish baseline noise (the solvent bottles and degassing system are located in the Solvent Organizer module).
- **Low Pressure Mixing System** - rapid response solenoid valves that are controlled by the Micropump are used to generate the desired mobile phase composition. Either isocratic or gradient mobile phases can be readily generated via the CHROMELEON software. The low pressure mixing system, which includes a set of proportioning valves is located in the Solvent Organizer module.
- **Micropump** - delivers the mobile phase that is generated by the low pressure mixing system to the flowsplitting unit. The Micropump is controlled by the CHROMELEON software via a token-ring network. In most applications, the flow rate is set to 200 μL/min.
- **Flowsplitting Unit** - consists of the calibrator cartridge and waste restrictor (located in the Solvent Organizer). Flow rates from 50 nL/min to 200 μL/min can be provided. The flow rate through the microcolumn is determined by the flow rate of the Micropump and the type of the calibrator cartridge.
- **Calibrator Cartridge** - is located in the Solvent Organizer. A variety of calibrator cartridges are available and are readily interchanged as described in Chapter 3.
- **Flow Sensor (Option)** - is located in the Solvent Organizer. Two types of flow sensor are available (Nanoflow / Capflow) and are readily interchanged as described in Appendix A.
- **Column Oven Compartment** - The temperature stabilized column oven compartment can accommodate microcolumns up to 30 cm long. The oven temperature can be controlled from a few degrees above ambient temperature up to 70 °C.
- **Sample Injector (Option)** - A manual 6 port low dispersion sample injector is optionally available. As an alternative, the FAMOS™ Microautosampler, which is controlled by the CHROMELEON software can be used to deliver samples to the column. This autosampler can be used for automated sample preparation (e.g. for high throughput screening) as well as with 96 or 384 well plates (Well Plate version).
- **Detector (Option)** - The UV detector (if included with the UltiMate) is the most sensitive and versatile detector for micro-separations. The detector is fitted with the U-Z View™ flow cell, which is a patented longitudinal Z shaped flow cell that provides exceptional sensitivity. A variety of cells are available with volumes from 3 nL to 10 μL. The detector is controlled by the CHROEMLEON software via a token-ring network.

1.3 Software Control of the *UltiMate* System

- The CHROMELEON Chromatography Management System, which is a Windows based program is used to control instrument settings for the low pressure mixing system (which generates the mobile phase), the flow sensor (option), the Micropump, the UV-Detector (option), the microautosampler (when installed) and the column oven. In addition, the application software is used to analyze the chromatogram and provide quantitative information about the compounds in the sample. The Micropump and the UV-Detector can be controlled on a local basis for various diagnostic and testing purposes.

1.4 How is this Manual Structured



Note: This manual covers the standard (stainless steel based) version of the UltiMate Capillary HPLC System as well as the inert version. All installation procedures and system tests shown are using the standard version. If you are using an inert version, please refer to the appropriate section for specific information that relates to this configuration and the appropriate part numbers for replacement parts.

This manual includes the following information:

Chapter 2: *Installation and Getting Started* describes how to install the UltiMate System and the CHROMELEON Software. It includes the various steps that should be performed to setup the system, define a server configuration, set up a program with the CHROMELEON software and prepare the system for operation.

Chapter 3: *The Solvent Organizer* describes the design and use of the degassing system, the low pressure mixing system, and the flow splitting unit with the calibrator cartridge. It includes a standard system test protocol to monitor the performance of the system. In addition, it includes information about the maintenance/troubleshooting of these components and describes a series of tests to verify the performance of several subsystems.

Chapter 4: *The UltiMate Micropump* describes the Micropump that is used with the UltiMate system. This chapter discusses the user interface, presents an overview of the design and operation of the pump, and provides information about the maintenance and troubleshooting of the components of this unit.

Chapter 5: *The UltiMate UV Det*ector describes the UV/VIS detector that is used with the UltiMate system. This chapter discusses the user interface, presents an overview of the design and operation of the detector, and provides information about the maintenance and troubleshooting of the components of this unit.

In addition, a series of appendices are provided to supply ancillary technical information, system specifications, etc.

Appendix A: *Optional Configuration* provides information about the various available system configurations (e.g. the flow sensor, the manual injection valve, the system without UV Detector).

Appendix B: *UltiMate Dual Gradient System* describes the UltiMate Dual Gradient System and installation of the instrument in conjunction with the CHROMELEON® Chromatographic Management System.

Appendix C: *Column Installation Instructions* provides guidelines for the installation of columns.

Appendix D: *Maintenance of the Manual Injector* describes the manual injection valve that is included in the UltiMate Capillary HPLC System (optional) and presents information about the disassembly and reassembly of the valve.

Appendix E: *CHROMELEON® - Additional Program Examples* provides a number of programs that the user can prepare for activities that are commonly performed with the UltiMate™ Capillary HPLC System.

1.5 Conventions used in this Manual

The following conventions will be used in this manual:



Danger: The information in a danger statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to personal injury or loss of life.



Warning: The information in a warning statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to severe injury and/or damage or destruction to parts or all of the equipment.



Caution: The information in a caution statement relates to a condition that could lead to damage to equipment and/or lead to invalid analytical results.



Note: The information in a note is provided to assist the reader, highlight specific material or clarify a procedure. If this information is ignored, it may be more difficult to successfully perform the indicated procedure (but there is no risk of personal injury or damage to the system).

When the user is to press one or more keys on a component or on the personal computer, select a command on the menu bar, select a check box, select a radio button, etc., the key(s), checkbox or radio button will be indicated in bold face:

> Enter the desired flow rate and press **Enter**.

If two (or more) keys are to be pressed at the same time to perform a specific function, this will be indicated as follows:

> Press **Alt** + **V** to access ...

When the location of the command is not obvious, its location will be also be indicated in bold face:

> Click on **Add Device** on the **Edit** menu to open ...

When a window or dialog box is indicated, the title of the window will be indicated in Italics:

> Click on **Add Device** on the **Edit** menu to open the *Add device to Timebase* box

In many instances, CHROMELEON provides a variety of ways to perform a given task or access a given command. This may include the use of function keys, icons on the Tool bar, the use of the Alt key + a letter (e.g. **Alt** + **V** to open the **View** menu), etc. For the sake of brevity, we will describe only one approach to selecting the desired response, but the reader should recognize that many pathways might exist for a given task.

1.6 Options

A variety of system configurations and options are available for the *UltiMate* including:

- **UltiMate Dual Gradient:** UltiMate Capillary HPLC System equipped with two gradient micropumps and two 3-channel low pressure mixing systems (see Appendix D).
- **Manual Injection Valve:** A manual 6 port low dispersion sample injector (see Appendix A).
- **Flow Sensor:** High precision and fast flow measuring device (see Appendix A).
- **UV Detector:** Most sensitive detector for micro-separations fitted with the U-Z View™ flow cell (see Appendix A and Chapter 5).
- **FAMOS™ Well Plate Microautosampler:** Microautosampler (Well Plate format) for 96 and 384 well plates (for high throughput screening).
- **FAMOS™ Carousel Microautosampler**: Microautosampler (Sample Tray format) for 96 vials (1.5 mL) or 160 microvials (0.5 mL)
- **Switchos™ II Advanced Microcolumn Switching Device:** Valve switching device including a loading pump and a 4 channel solvent selection valve.
- **Probot™ Micro Fraction Collector**: High precision x/y/z robot for fraction collection of the column eluate onto MALDI targets, sequencing membranes and well plates (96, 384, 1536 well plates).

Please contact LC Packings or your nearest representative for additional information.

1.7 For Additional Information

For additional information, refer to the following documentation:

- User's Manual for CHROMELEON Software.
- User's Manual for the FAMOS Microautosampler (if applicable).
- User's Manual of the Switchos II Advanced Microcolumn Switching Unit (if applicable).
- User's Manual of the Probot Micro Fraction Collector (if applicable).
- Documentation for Microsoft Windows.
- A series of application notes and a bibliography of papers and reviews on micro-, capillary- and nano-LC are available at the LC Packings website (www.lcpackings.com).

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CHAPTER 2

Installation and Getting Started

2.1 Overview

The UltiMate™ Capillary HPLC system is shipped as an integrated unit that includes the components discussed in Chapter 1. A variety of accessories are included that are indicated on the packing list. The following components need to be installed during installation:

- the calibrator cartridge (if not already installed)
- the Z-shaped capillary flow cell
- the microcolumn
- the CHROMELEON® Software

When the UltiMate system is part of an instrument setup with a FAMOS™ Microautosampler and/or a Switchos™ II Advanced Microcolumn Switching Unit, these instruments must be interfaced to the Capillary HPLC system. Sections 2.5 and 2.6 provide basic information how to install these instruments in conjunction with the UltiMate system.

If the system is equipped with optional components (e.g. a flow sensor or a manual injection valve is installed), please refer to Appendix A for installation details.

The CHROMELEON Software must be installed on the personal computer that will be used to control the system and the personal computer must be interfaced with the UltiMate system.

This chapter describes how the UltiMate system is installed in the user's facility in conjunction with the CHROMELEON software package. If the UltiMate is to be installed together with a different control software package (e.g. the mass spectrometer software), refer to the documents provided with this software package.

A standard system test is provided in Section 3.3.3 that could be performed after the system is installed to ensure that the system is operating properly. In addition, Section 3.6 provides a series of checkout procedures for the Micropump and the UV Detector.

2.2 Location of the UltiMate System in the Laboratory

The UltiMate system should be installed in a facility with the following environmental conditions:

- The temperature range should be maintained between 10 and 40°C. The system should be installed in an area in which the temperature is fairly constant (do not place the system near a window, an air conditioning duct or a heating duct). The humidity should be maintained between 20 and 80 % relative humidity.
- If flammable or toxic solvents are to be used, a suitable ventilation system should be provided.
- The use of open flames in the laboratory should be prohibited.
- Corrosive vapors or dust should not be present as these materials can adversely affect the long-term performance of the system.

The UltiMate system requires approximately 250 mm (10") of linear bench space. In addition, 600 mm (24") of bench space should be provided for the personal computer, 300 mm (12") of bench space should be provided for the autosampler (if appropriate), 190 mm (7.5") of linear bench space should be provided for the Switchos II (if appropriate) and 400 cm (16") should be provided for the printer (if appropriate). The lab bench should be capable of supporting the entire system (for the UltiMate system, FAMOS and Switchos II we recommend that the lab bench be capable of supporting at least 100kg (225 lb.)).

The power consumption of the UltiMate is 250 VA (the power consumption of the FAMOS Microautosampler it is 250 VA and of the Switchos II it is 100 VA).



Danger: The UltiMate System must be connected to a power source that is connected to a true ground. In addition, all other components of the system (e.g. the FAMOS Microautosampler) should be connected to the same ground.

2.3 Unpacking the System

When the system is received, carefully unpack the unit and verify receipt of all components according to the packing list (some components include sub-packing lists). It is recommended that all packing materials be saved in the event that it is necessary to return any item to the factory.

If there is external damage to the shipping boxes, the damage should be reported to the shipping agent and LC Packings upon receipt of the goods. If internal damage is observed or if any items are missing, this should be reported to the shipping agent and to LC Packings as soon as it is observed.



Note: If there is any apparent damage to the system, the user should investigate the nature of the damage before plugging the unit into the mains to ensure that powering up of the system will not create a hazardous condition or damage internal components. If the damage appears significant, call LC Packings or its local representative before connecting the unit to the mains.

2.4 Installing the UltiMate in Conjunction with CHROMELEON

Section 2.4 provides information how to install the UltiMate Capillary HPLC System in conjunction with the CHROMELEON Chromatography Management System software. In addition, Sections 2.5 and 2.6 provide installation details for other system components such as the FAMOS Microautosampler and the Switchos II Advanced Microcolumn Switching Unit in same system.

Installation information is provided in the following sections:

- **Section 2.4** – Installing the UltiMate Capillary HPLC System (consisting of a Micropump, an UV Detector, but no manual injection valve installed).
- **Sections 2.5** - Installing the FAMOS Well Plate Microautosampler (with cooling option installed, 'Micro' configuration).
- **Sections 2.6** - Installing the Switchos II Advanced Microcolumn Switching Unit.

In addition, refer to the user manuals provided with these components for additional information. Please refer to Appendix A for details how to install system components not listed above or systems that with different options (e.g. a flow sensor or a manual injection valve is installed).

2.4.1 Electrical Connections

All electrical connections are made via the rear panel of the UltiMate system (FIGURE 2-1). The necessary cables are included with the instrument.

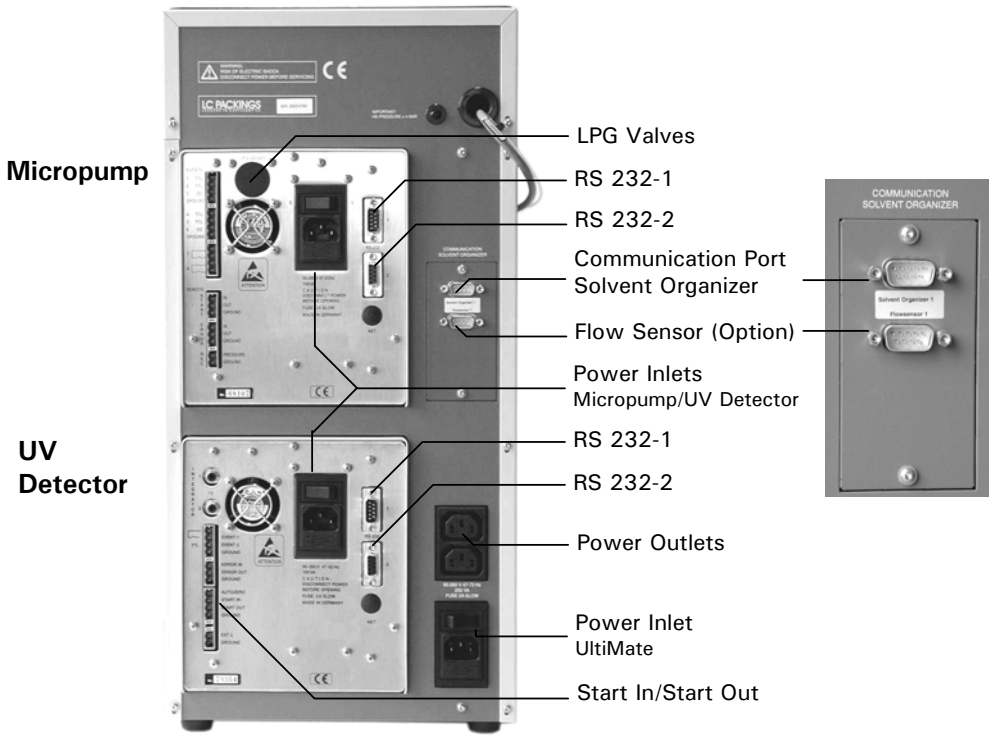


FIGURE 2-1 Rear View of UltiMate with Communication Ports/Power Connections

2.4.1.A Communication Ports

The following connections should be made:

a) Connect the Y-Cable between a free COM port on the PC, the RS 232-1 port on the Micropump and the RS 232-2 port on the UV-Detector (item a, FIGURE 2-2).

b) Connect the RS 232-2 port on the Micropump to the RS 232-1 port on the UV-Detector with the Serial Cable (item b, FIGURE 2-2).

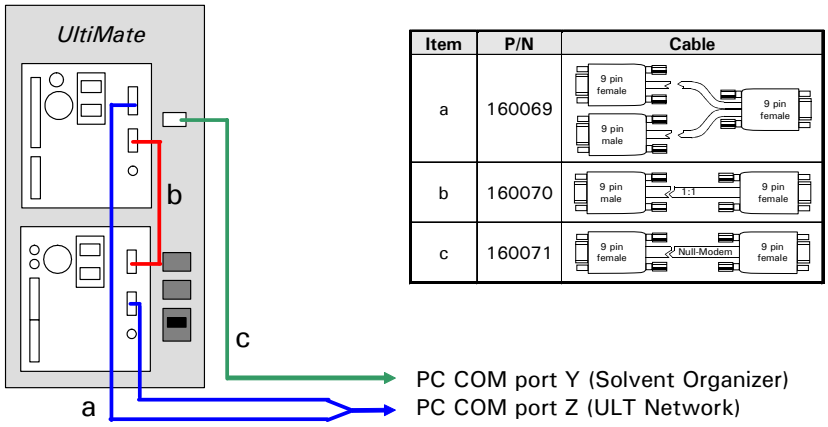


FIGURE 2-2 Setting up the RS-232 Connections – UltiMate

c) Connect the Communication Solvent Organizer port to a free COM port on the computer, using the Solvent Organizer Communication cable (item c, FIGURE 2-2).

2.4.1.B Solvent Organizer Cable

Carefully insert the black Solvent Organizer Cable in the LPG VALVES connector on the rear panel of the Micropump (FIGURE 2-1 and FIGURE 2-3).

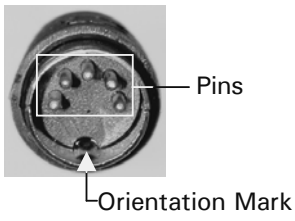


FIGURE 2-3 Connector Plug to Solvent Organizer Module

Note: Make certain that the pins are positioned properly and the orientation mark on the plug is at the bottom before inserting it into the Micropump (FIGURE 2-3).

2.4.1.C Power Cables

A built-in relay will supply power to the two power outlets on the rear side of UltiMate if the system is powered on. In 'standby' mode (e.g. the red LED of the ON/Stand by button is illuminated) the Micropump and UV Detector are not powered.

a) Use the extension cords to connect the power inlets of Micropump and UV-Detector to the power outlets on the rear side of UltiMate (FIGURE 2-1).

b) Connect the power inlet of UltiMate to the power line.



Danger: The UltiMate must be connected to a power source that is connected to a true ground. In addition, all other components of the system (e.g. the FAMOS Microautosampler) should be connected to the same ground.

2.4.2 He Connection

Connect the Helium line (¼" O.D.) that is supplied to the Helium inlet on the back of the UltiMate (directly above the Micropump). The Helium pressure should be set to approximately 1 bar.



Caution: Do not operate the He lines at a pressure greater than 4 bar (60 PSI).

2.4.3 Waste Bottle

Insert the waste lines into a waste reservoir of sufficient volume. The reservoir should be able to hold at least 1 L of mobile phase.

2.4.4 Calibrator Installation

The UltiMate is shipped with the calibrator cartridge installed. Follow the instructions below if a different calibrator cartridge needs to be installed or replaced.

The system is provided in different configurations and a variety of calibrators are available (TABLE 2-1). The calibrator(s) that is(are) provided for a given system is dependent on the column size and flow rate that are to be used (see the shipping list for details).

TABLE 2-1 Calibrator Cartridge Selection Guide

Configuration	Column Size	Calibrator Type	Part Number
Micro LC	1 mm I.D. 0.8 mm I.D.	MIC-1000 MIC- 800	160057 160058
Capillary LC	300 µm I.D. 180 µm I.D.	CAP-300 CAP-180	160059 160060
Nano LC	100 µm I.D. 75 µm I.D. 50 µm I.D.	NAN-100 NAN-75 NAN-50	162052 160061 162051
Monolithic Column Setup	200 µm I.D.	MON-200	161406

The appropriate calibrator should be installed by placing it in the slot on the upper front of the UltiMate and carefully tightening the nuts (do not overtighten) as shown in FIGURE 2-4.



FIGURE 2-4 Mounting the Calibrator Cartridge

2.4.5 Flow Cell Installation

The flow cell is installed by inserting the cell from the top into the cell compartment (FIGURE 2-5). When you are installing the flow cell, make certain that the side with the three holes faces the detector housing and align the pin on the detector housing with the hole on the flow cell. Additional information is provided on the instruction sheet that comes with the flow cell and in Chapter 5.

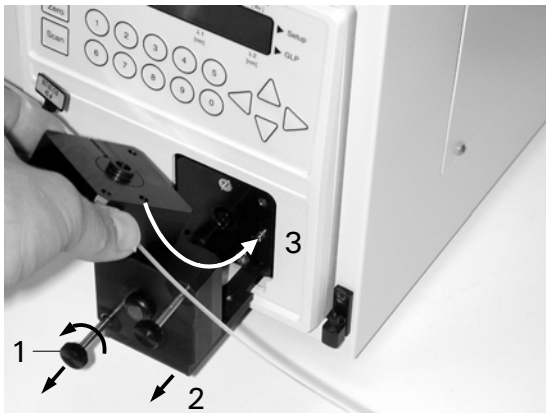


FIGURE 2-5 Installing the Flow Cell

The system is provided in different configurations and a variety of flow cells are available (TABLE 2-2). The flow cell that is provided for a given system is dependent on the column size and flow rate that are to be used (see the shipping list for details).

TABLE 2-2 Flow Cell Selection Guide

Configuration	Column Size	Flow Cell Type	P/N
Micro LC	0.8-1 mm I.D.	UZ-M10	160011
Capillary LC	180-300 µm I.D.	UZ-C10	160013
Nano LC	50-100 µm I.D.	UZ-N10	160015
Monolithic Column Setup	200 µm I.D.	UZ-MON	161719

2.4.6 Fluidic Connections

To connect the Nano/Micro flow outlet of the UltiMate system:

a) Remove the fluidics access plate from the solvent organizer (Section 3.7.5). The top view of the fluidics compartment is shown in FIGURE 2-6.

Note: If your system is equipped with a manual injection valve follow the instruction provided in Appendix A.3.3 to bypass this valve.

Note: If your system is equipped with a flow sensor follow the instructions provided in Appendix A.4 to connect this system.

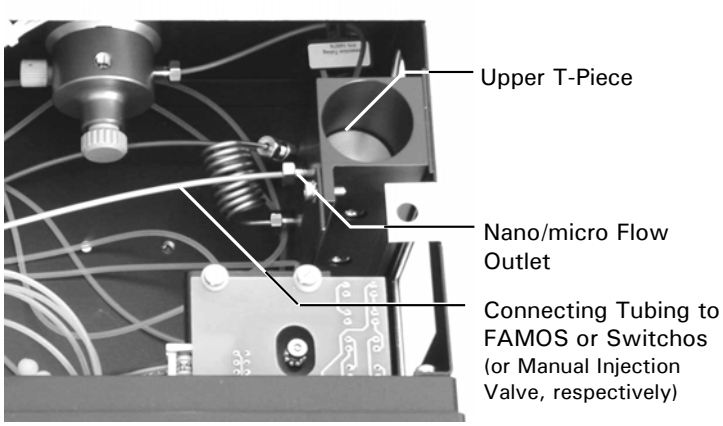


FIGURE 2-6 The Nano/Micro Flow outlet of Solvent Organizer

b) Remove the black cap from the hole that is located in the left side panel of UltiMate.

c) Connect the appropriate tubing to the Nano/Micro flow outlet.

Note: Section 2.5 provides details to connect the UltiMate to a FAMOS Microautosampler and Section 2.6 shows how to connect the system to Switchos II. In addition, Appendix C provides instructions how to install the different Nano and Micro columns.

d) Guide the capillary through the hole in the side panel of the UltiMate and connect it to the instrument that is to be connected to the UltiMate system.

e) Replace the fluidics access plate.

2.5 Installing the UltiMate with the FAMOS Autosampler

If the system includes a FAMOS Microautosampler, it should be installed via the following (basic) steps. Refer to the user's manual provided with this instrument for more details or if the instrument is to be installed in conjunction with a different control software package.

2.5.1 Electrical Connections

a) Connect the serial cable from the COMMUNICATION port on the FAMOS to a free RS232 serial COM port on the computer (FIGURE 2-7).

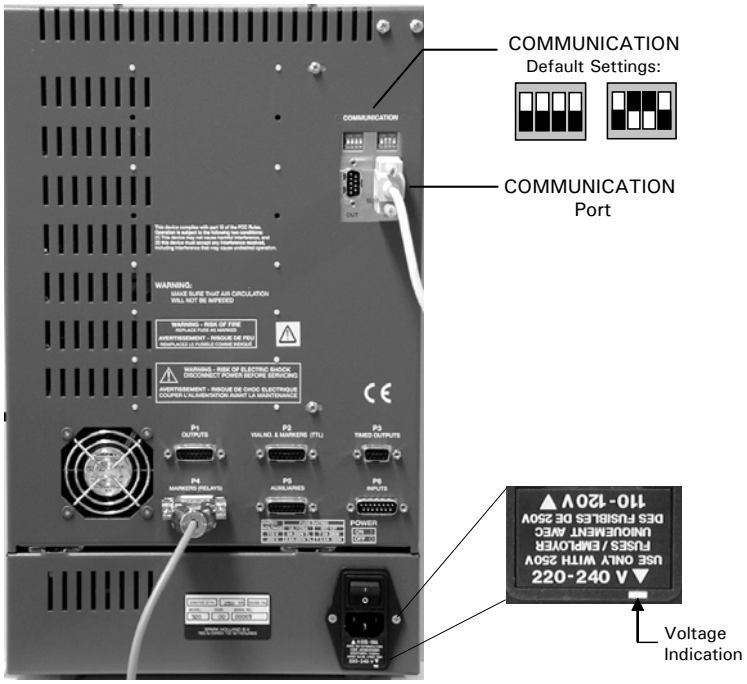


FIGURE 2-7 Rear Panel – FAMOS Autosampler

Note: CHROMELEON uses a 'virtual' start signal. The Inject Marker output of the FAMOS Microautosampler is not connected to the START IN of the UltiMate UV Detector (or the Micropump).

b) Check the voltage setting of the FAMOS and if it matches with the mains voltage, connect the power inlet to the power line.

DANGER

Danger: The FAMOS must be connected to a power source that is connected to a true ground.

2.5.2 Fluidic Connections

The outlet of the UltiMate system (Section 2.4.6) is connected to the FAMOS injection valve and the separation column by the following steps:

a) Remove the black cap from the hole that is located in the left side panel of UltiMate and remove the oven cover plate.

b) Connect the appropriate tubing to the upper T piece (TABLE 2-3). Guide the capillary through the hole in the side panel of the UltiMate and connect this capillary to port 1 ("pump") of the FAMOS injection valve.

TABLE 2-3 Connecting Tubing for Installation of the FAMOS Autosampler.

Column I.D. [µm]	Calibrator Type	Connecting Tubing I.D. [µm]	Capillary Upper T piece – FAMOS injection valve	Capillary FAMOS injection valve-column oven
Connecting Tubing for the UltiMate - Standard Version				
1000	MIC-1000	150	P/N 160031	P/N 160032
800	MIC-800	150		
300	CAP-300	50	P/N 160033	P/N 160034
180	CAP-180	50		
75	NAN-75	20	P/N 160035	P/N 160036
200	MON-200	50	P/N 160033	N/A
Connecting Tubing for the UltiMate - Inert Version				
1000	MIC-1000	150	P/N 161013	P/N 161014
800	MIC-800	150		
300	CAP-300	50	P/N 161013	P/N 161014
180	CAP-180	50		
75	NAN-75	20	P/N 161011	P/N 161012

Note: The tubing supplied with the UltiMate depends on the configuration of the UltiMate (e.g. MICro, NANo, CAPillary configuration or MONlithic column are used).

c) Connect the appropriate tubing to port 6 ("column") of the FAMOS injection valve (TABLE 2-3).

d) Guide the capillary end with the union through the hole in the side panel of the UltiMate and guide the union through the hole on top of the oven.

e) Connect the column to the union and insert the union into the clip. Guide the capillary end through one of the holes of the oven cover plate.

Note: Alternatively, the column can be directly connected to port 6 ("column") of the FAMOS Autosampler instead of using the connecting tubing "injection valve - column oven" (e.g. when using a Nano-LC column).

2.6 Installing the UltiMate with the Switchos II

If the system includes a Switchos II Advanced Microcolumn Switching Unit, it should be installed via the following (basic) steps. Refer to the user's manual provided with this instrument for more details if the instrument is to be installed in conjunction with a different control software package.

2.6.1 Electrical Connections

2.6.1.A Communication Ports

The two RS-232 serial interfaces enable digital data transfer between the loading pump, the UltiMate Micropump, the UltiMate UV Detector and the PC. These devices communicate with each other to form an integrated network.

To set up the RS-232 network connections:

a) Connect the RS-232 1 port of the UltiMate Micropump, the RS-232 2 port of the UltiMate UV Detector and the COM port of the PC using the Y-shape cable (item a, FIGURE 2-8).

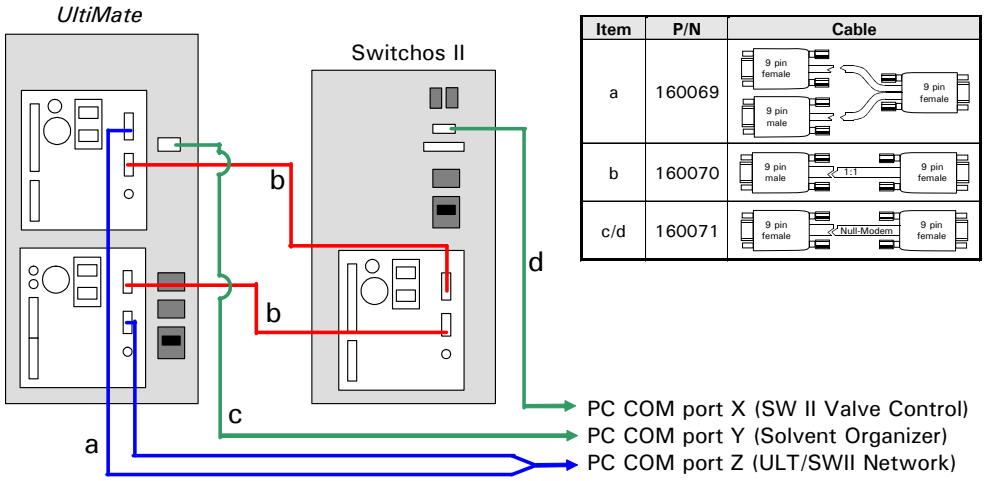


FIGURE 2-8 Setting up the RS-232 Connections - UltiMate and Switchos

b) Connect the RS-232 1 port of the Switchos II loading pump to the RS-232 2 port of the UltiMate Micropump using the Serial Communication Cable (item b, FIGURE 2-8). Connect the RS-232 2 port of the Switchos II loading pump to the RS-232 1 connector of the UltiMate UV Detector with the same type of cable.

c) Connect the UltiMate Solvent Organizer COMMUNICATION port to a free COM port on the computer using the Solvent Organizer Com cable (item c, FIGURE 2-8).

d) Connect the Switchos II COMMUNICATION port to a free COM port on the computer using the Solvent Organizer Com cable (item d, FIGURE 2-8).

2.6.1.B INPUTS Connector

CHROMELEON controls the valve positions via the COMMUNICATION port (Section 2.6.1.A). Refer to the Switchos II user's manual if the instrument is to be installed in conjunction with a different control software package.

2.6.1.C Power Connector

Since the Switchos II is fitted with a universal power supply for input voltages from 90 to 260 V, manual setting of the supply voltage is not required. The power cord should be inserted in the socket directly below the Main Power switch on the right side of the rear panel. In addition, the loading pump should be plugged into the socket above the Main Power switch.



Danger: The Switchos II must be connected to a power source that is connected to a true ground.

2.6.2 He Connection

Connect the Helium line (1/4 " O.D.) that is supplied to the Helium inlet on the rear panel of the Switchos II. To connect the Helium line of the Switchos II to the Helium line of the UltiMate Capillary HPLC System, use the T-piece (P/N 161470) that is supplied with the Switchos II instrument. The Helium pressure should be set to approximately 1 bar.



Caution: Do not operate the He lines at a pressure greater than 4 bar (60 PSI).

2.6.3 Fluidic Connections

The Switchos II is used in a broad range of applications and the user can configure the unit to meet the specific needs of the laboratory. FIGURE 2-9 shows a typical pre-concentration setup using the Switchos II in conjunction with the FAMOS Microautosampler and the UltiMate Capillary HPLC System. Additional examples are presented in the user's manual of the Switchos II.

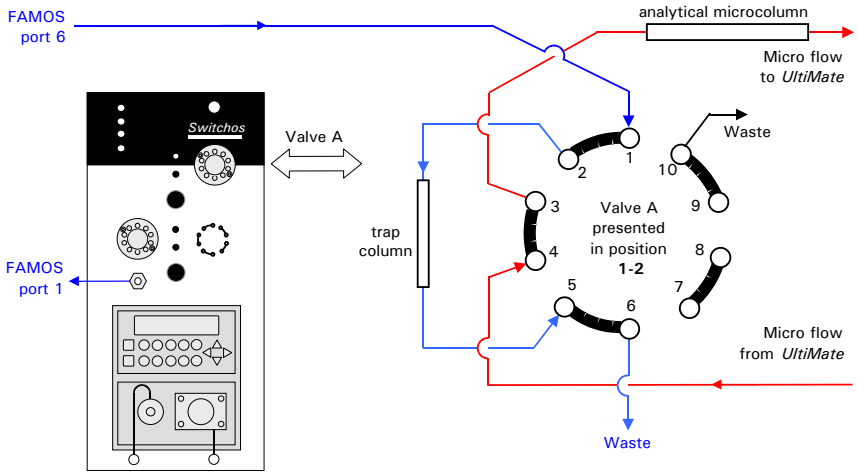


FIGURE 2-9 Typical Pre-Concentration Setup

To setup the Switchos II for the pre-concentration application (FIGURE 2-9):

a) Connect port 1 of the injection valve of the FAMOS Microautosampler to the Nano/Micro flow outlet of the Switchos II loading pump using the 130 µm I.D. PEEK tubing provided with the instrument:

Type of Connection	Standard Version	Inert Version
short (50cm)	P/N 160180	P/N 160180
long (100 cm)	P/N 160181	P/N 160181

b) Connect port 6 of the injection valve of the FAMOS Microautosampler to the port 1 of valve A of the Switchos II as described in item a).

c) Connect the outlet flow of the UltiMate System (e.g. from the upper T-Piece of the flow splitter) to port 4 of valve A of the Switchos II. Use the appropriate tubing for your application.

Type of Application	Standard Version	Inert Version
Capillary LC	P/N 161479	P/N 161261 (1)
Nano LC	P/N 160178	P/N 161259 (1)
(1) use PEEK fingertight fittings only		

d) Connect port 3 of valve A of the Switchos II to the UltiMate column bulkhead or directly to the micro column located in the UltiMate column compartment. Use the appropriate tubing for your application.

Type of Application	Standard Version	Inert Version
Capillary LC	P/N 161480	P/N 161262 (1)
Nano LC	P/N 160179	P/N 161260 (1)
(1) use PEEK fingertight fittings only		

e) Connect ports 6 and 10 of valve A of Switchos II to waste (e.g. using 200 - 500 µm I.D. PTFE tubing).

f) Connect the trap column between ports 2 and 5 of valve A of the Switchos II, using the appropriate tubing supplied with the trap column.



Caution: Do not use a stainless steel nut and/or ferrule with inert (PAEK) injection/switching valves. The use of stainless steel nuts or ferrules may damage the valve. Use only the supplied fittings (PEEK).

2.7 Installing the CHROMELEON® Software

The UltiMate system is fully controlled by the Dionex™ CHROMELEON® Chromatographic Management System, which is a Windows® application.

The following sections describe how to install a copy of CHROMELEON 6.50 SP1 on your PC to control a single UltiMate system (Timebase). For more detailed information or different instrument setups refer to the CHROMELEON manual and Appendix A and Appendix B.

2.7.1 System Requirements

The computer should have the following minimum requirements as indicated in TABLE 2-4:

TABLE 2-4 Minimum PC Requirements for Chromeleon 6.5

Item	Specification
Operating System	Windows® NT® 4 (≥SP6a) Windows® 2000 (SP1/SP2/SP3) Windows® XP (SP1)
Processor	Minimum Pentium® II (266 MHz) Pentium III (400 MHz) recommended
RAM	Minimum 128 MB 256 MB or more recommended
Hard disk space	Minimum 10 GB 20 GB or more recommended
Video display	1024 × 768 pixels, 16 colors 1024 × 768 pixels, 65536 colors recommended
Communication ports	Minimum 2 free 9-pin male RS-232 8 free 9-pin male RS-232 ports recommended Usage of external ports rather than internal recommended
Peripherals	Windows-compatible keyboard, mouse, printer, and CD ROM.

2.7.2 Loading the CHROMELEON Software

To load the CHROMELEON software on the Personal Computer:

a) Close all user programs that may be running on the computer.

b) Insert the CD-ROM that contains the program into the CD Drive of your computer.

   If the computer is configured for Autorun, the first installation screen will be presented (FIGURE 2-10).

   If the computer is not configured for Autorun, click on **Run** on the **Start** menu and use the **Browse** dialog box to access and run **Setup.exe** file. This file is located in the X:\CM folder (where X is the letter for the CD-ROM drive) and presents the first installation screen.



FIGURE 2-10 The CHROMELEON Setup Window

c) Click on the **Install MDAC 2.5 RTM** to install the Microsoft® Distributed Access Components. If these components are already installed on your PC the setup program will terminate.

d) Restart the Computer.

e) Double click on the **Launch CHROMELEON setup** to initialize the installation program (FIGURE 2-10).

f) A Wizard guides you through the installation. This procedure will install the CHROMELEON core application components: CHROMELEON Server and CHROMELEON Client. The default settings provided by the setup program should be used unless a conflict exists.

Note: Do not install the 'License Server'. It is not used in single client installations, and a special server license is required.

g) After CHROMELEON has been installed, the CHROMELEON icon will appear on the desktop (item 1, FIGURE 2-11). The icons for the Server Monitor and the Server Configuration (item 2 and 3, FIGURE 2-11) can be found in the standard CHROMELEON directory (default: C:/Chromel/Bin folder). The icons can be added to the desktop if desired



FIGURE 2-11 The CHROMELEON Icons

2.7.3 Entering the Software License Number

Each CHROMELEON station (PC), regardless of whether it is a server and/or a client, receives its license from a copy protection device, e.g. a dongle or a hard-protect plug-in card (PAL). PALs and dongles store the licensed serial number for one CHROMELEON station. For large installations, a CHROMELEON License Server is provided for the license management.

The following section describes how to enter the software license number (key code) in conjunction with a dongle. This procedure requires that you first launch the Server Monitor and then start the CHROMELEON server. The Server Configuration must be launched to enter the serial number.

To enter the software license number:

a) Shut down your PC, and then install the dongle that is provided with your CHROMELEON packages in either the USB port ort the printer port of your PC (depending on which dongle version was ordered). Restart the PC.

b) Click either on the Server Monitor icon (if copied to your desktop) or start the Server Monitor via the **Start** menu, the *Server Monitor* box will appear (FIGURE 2-12).

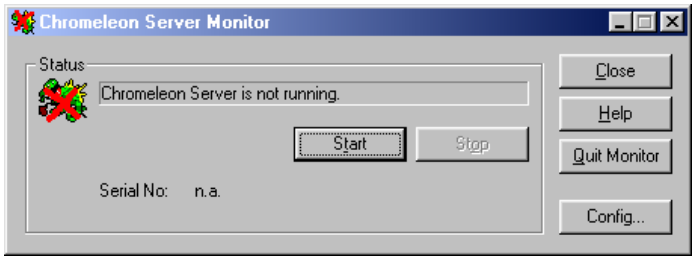


FIGURE 2-12 The Server Monitor - not running, no S/N entered

c) Click on the Start button to start the CHROMELEON Server. During the software installation the server will run in Evaluation Mode (FIGURE 2-13).

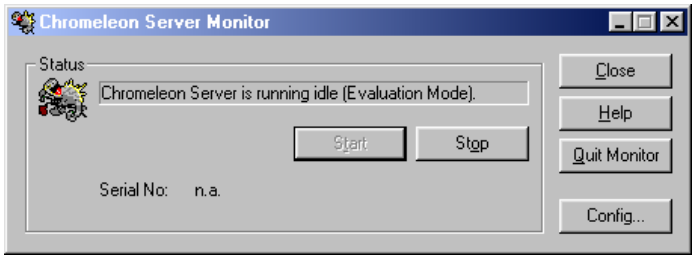


FIGURE 2-13 The Server Monitor - Evaluation Mode

d) Click either on the Server Configuration icon (if copied to your desktop) or start the Server Configuration via the **Start** menu, to present the *Server Configuration* box (FIGURE 2-14).

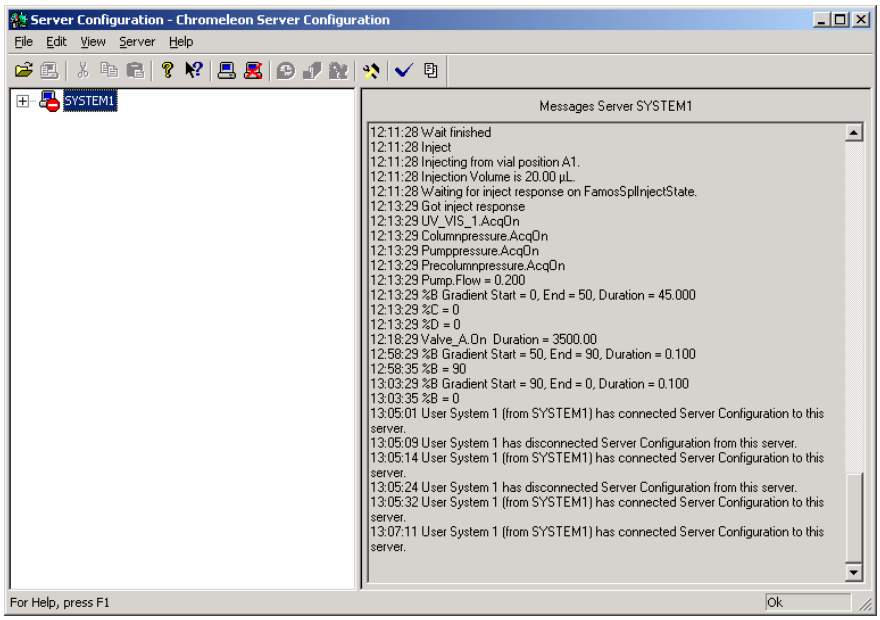


FIGURE 2-14 The Server Configuration Box

e) Click on **Edit** and then on **Properties to** open the configuration box (FIGURE 2-15). Select **Dongle** and enter the 12- or 24-digit key code as stated on the 'Certificate of Compliance' provided with your CHROMELEON package.

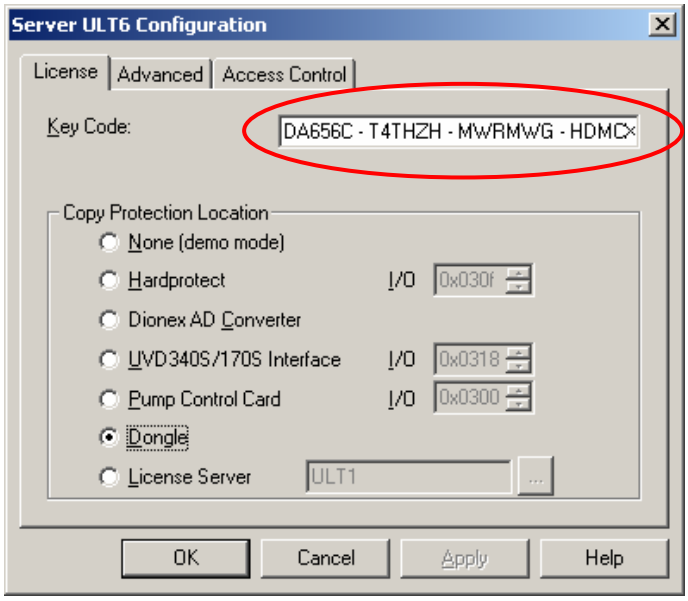


FIGURE 2-15 Server Configuration – Entering the Key Code (using a Dongle)

f) After entering the key code, the CHROMELEON server must be restarted to run in normal mode.

Note: The serial number of the dongle and the key code entered here must match. If they do not match, CHROMELEON cannot operate correctly.

Note: If you do not have a key code yet, select *None* to enable the evaluation mode. The evaluation mode allows testing the CHROMELEON Chromatography Management System in your working environment for one hour.

2.8 Configuring the UltiMate System

The following sections describe how to add a Timebase, how to add the UltiMate instrument to this Timebase and how to configure the system. In addition, basic information is provided how to install other components of the LC Packings UltiMate System. For more detailed and latest information refer to the manuals provided with these instruments.

2.8.1 Adding a new Timebase

A Timebase describes the instrument configuration as it is used for your experiment. Normally, it consists of different devices (e.g. the LC Packings UltiMate/Switchos device, which controls the network communication of these devices).

To add a Timebase:



a) Start the CHROMELEON server, and then start the CHROMELEON Server Configuration (FIGURE 2-16).

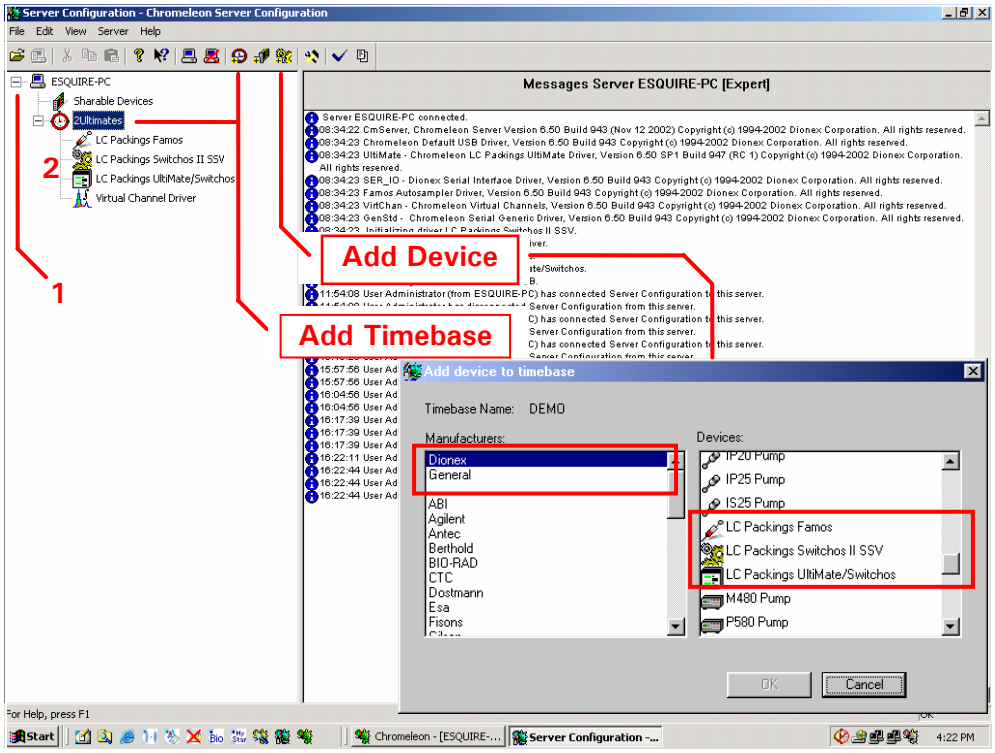


FIGURE 2-16 The Server Configuration Box

b) Select the name for the server (if more than one is configured) of which you want to modify the configuration. Click the '+' character in front of the server name to view its current configuration (item 1, FIGURE 2-16).

c) Click on **Add Timebase** on the **Edit** menu (or the corresponding button) to add a new Timebase to the selected server.

2.8.2 Adding and Configuring the UltiMate System

To add and configure the UltiMate System:

d) Click on **Add Device** on the **Edit** menu to open the *Add device to Timebase* box and add the 'LC Packings UltiMate / Switchos' device to your Timebase (item 2, FIGURE 2-16). The *Components* box will appear (FIGURE 2-17).

Note: The 'LC Packings UltiMate/Switchos' device controls the network communication between the UltiMate Micropump, the UV Detector (if installed) and the Switchos II Loading Pump (if installed).

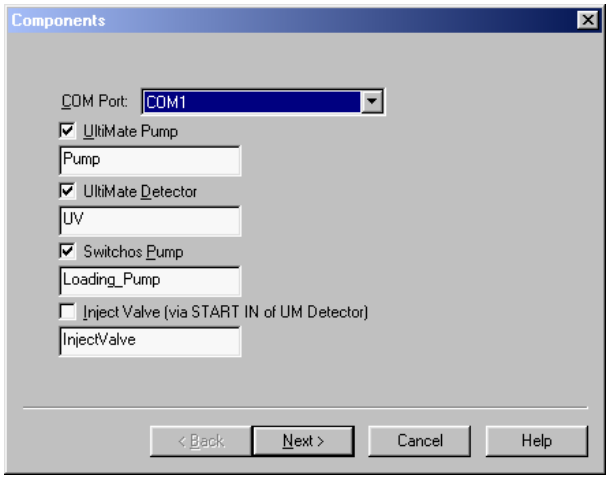


FIGURE 2-17 The UltiMate/Switchos Component Box

e) Select the **COM Port** and check the boxes corresponding to the instruments connected to this port (e.g. if a Micropump, a UV Detector and a Switchos II is present, the result looks as presented in FIGURE 2-17).

Note: If you want to start the program by the contact closure of the (optional) manual injection valve (or any other external event) via the START IN input of the UltiMate UV Detector, check the 'Inject Valve' box.

f) Click on **Next** to open the *Flow Options* dialog box (FIGURE 2-18).

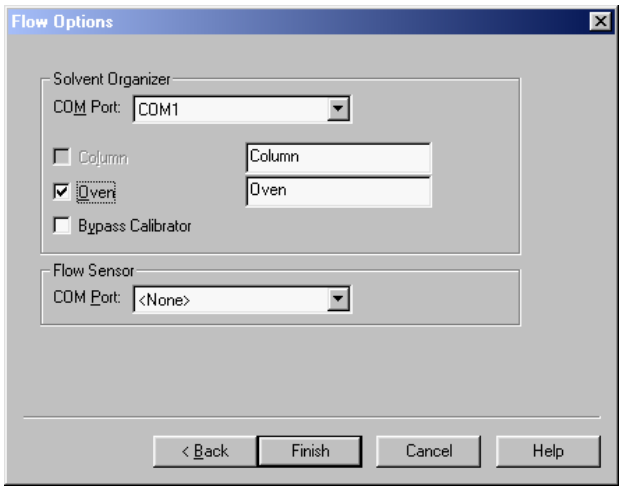


FIGURE 2-18 The Flow Options Box

g) Select the **COM Port** to control the Solvent Organizer and check the **Oven** box if an oven is installed in your UltiMate System (FIGURE 2-18). For details how to setup the **Flow Sensor** option (if installed), refer to Appendix A.

h) Click **Finish** to close the box and to confirm the settings.

To verify or change the configuration, double-click on the 'LC Packings UltiMate/Switchos' device (item 2, FIGURE 2-16).

2.8.3 Adding and Configuring the FAMOS Well Plate Microautosampler

To add and configure the FAMOS Well Plate Microautosampler:

a) Click on **Add Device** on the **Edit** menu to add the 'LC Packings FAMOS' device to this Timebase (item 2, FIGURE 2-16). The FAMOS *General* box will appear (FIGURE 2-19).

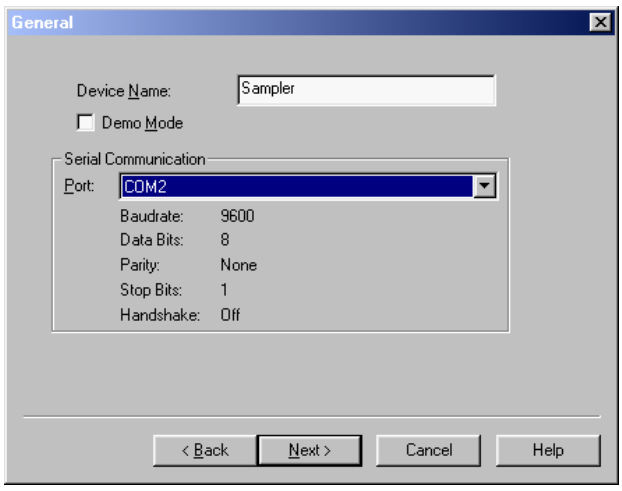


FIGURE 2-19 FAMOS General Box

b) Select the **Port** and click on **Next,** the FAMOS *Rack* box (FIGURE 2-20) appears.

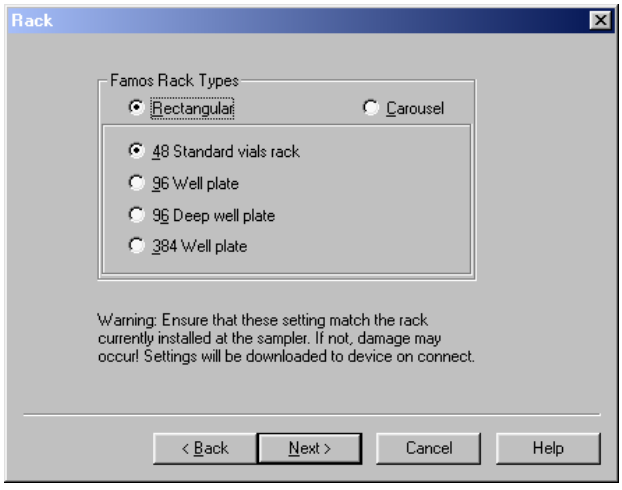


FIGURE 2-20 FAMOS Rack Box

c) Check **Carousel** if you install the Carousel version or check **Rectangular** (and then the **Rack Type)** if you are going to install the FAMOS Well Plate version.

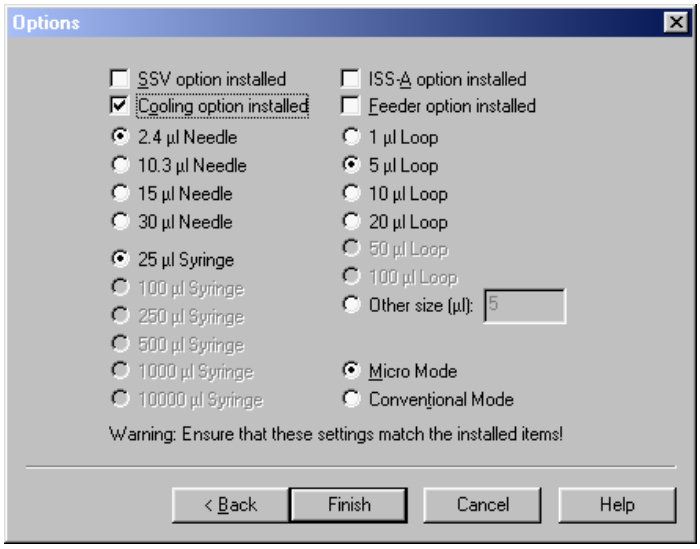


FIGURE 2-21 FAMOS Options Box

d) Setup the *Options* box according to the current configuration of the FAMOS to be installed (FIGURE 2-21). Select **Micro Mode** if a 2.4 µL (fused silica) needle is installed and **Conventional Mode** if a 15 µL needle is installed.

e) Click **Finish** to close the box and to confirm the settings.

To verify or change (e.g. to configure additional relay outputs) the configuration, double-click on the 'LC Packings FAMOS' device (item 2, FIGURE 2-16).

2.8.4 Adding and Configuring the Switchos II Unit

To communicate with the Loading Pump of the Switchos II Advanced Microcolumn Switching Unit:

a) Double-click on the 'LC Packings UltiMate/Switchos' device (item 2, FIGURE 2-16). The *LC Packings UltiMate/Switchos* box appears (FIGURE 2-22).

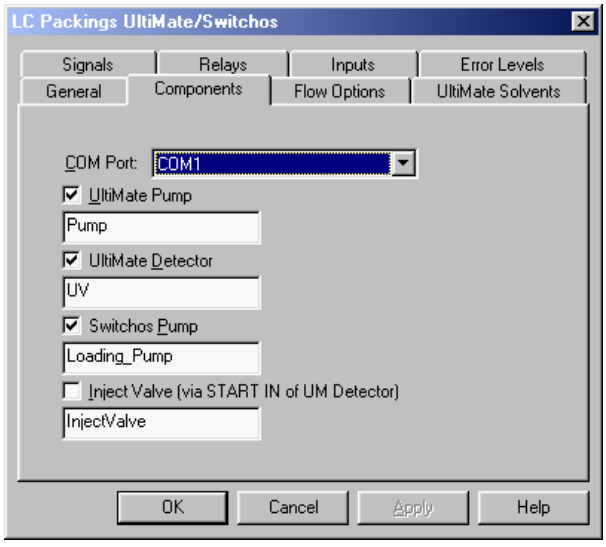


FIGURE 2-22 The UltiMate Configuration Box – Components Tab

b) Make sure that the **SwitchosPump** box is checked on the **Compenents** tab.

The valves of the Switchos II can either be controlled by the event outputs of the Switchos II Loading Pump via the INPUTS connector or directly via the COMMUNICATION connector and via a free COM port of the PC.

2.8.4.A Switchos II controlled by Events

To control the unit by the event outputs of the Loading Pump, select the **Relays** tab of the *LC Packings UltiMate/Switchos* box and then check the outputs SSV, Valve_A, and Valve_B (FIGURE 2-23).

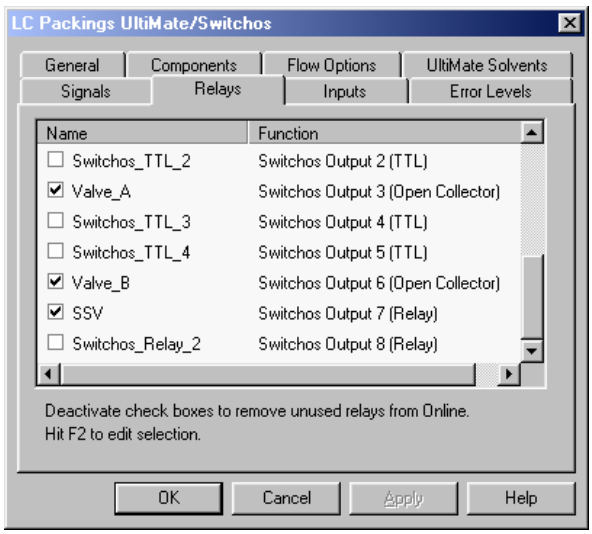


FIGURE 2-23 The UltiMate Configuration Box – Relays Tab

2.8.4.B Switchos II controlled by a RS-232 COM port

To control the unit by serial communication:

a) Add the 'LC Packings Switchos II SSV' device to the Timebase (FIGURE 2-16). This will present the *LC Packings Switchos II SSV* configuration box (FIGURE 2-24).

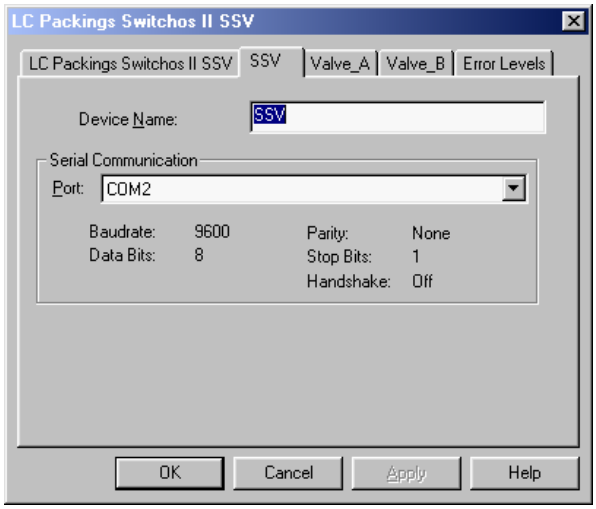


FIGURE 2-24 The Switchos II Configuration Box

b) Select the COM port which controls the unit, and click **OK** to confirm the setting.

To verify or change the configuration, double-click on the 'LC Packings Switchos II SSV' device (item 2, FIGURE 2-16).

Note: If there are not enough COM ports available, the valves of the Switchos II can be controlled by the event outputs of the Loading Pump (Section 2.8.4.A).

2.8.5 Adding and Configuring the Virtual Channel Driver

To monitor and record various parameters of the UltiMate (e.g. the column pressure or the oven temperature) a **Virtual Channel Driver** is needed.

To add a Virtual Channel Driver:

a) Click on **Add Device** in the *Server Configuration Box* and select **Virtual Channel Driver** from the **General**, to present the *Virtual Channel Driver* box (FIGURE 2-25).

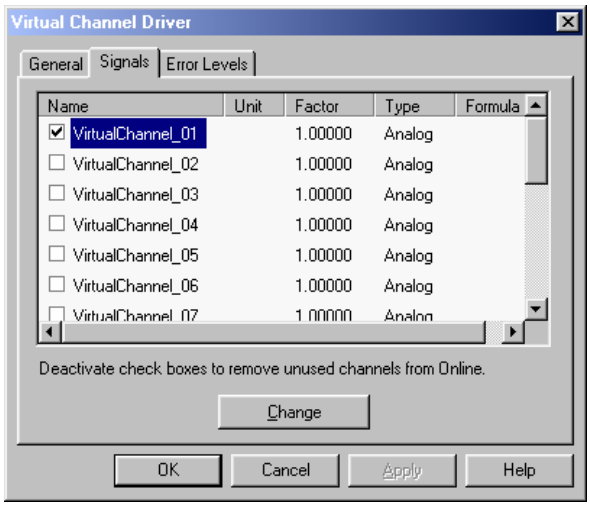


FIGURE 2-25 The Virtual Channel Driver Box

b) Check the first channel and click on **Change** to configure it. To readout the column pressure, configure the *Signal Configuration Box* as indicated in FIGURE 2-26.

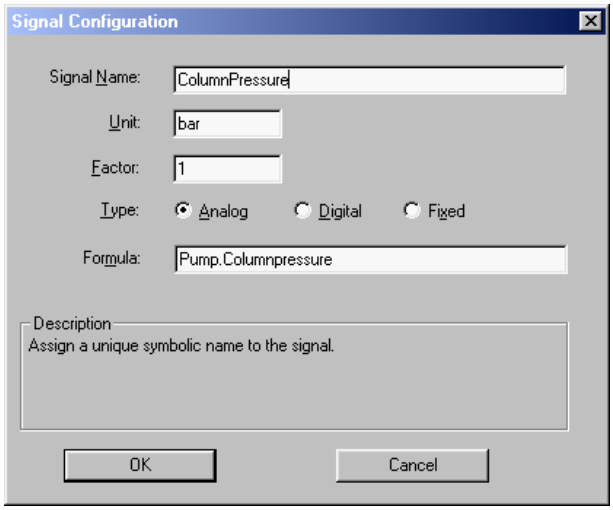


FIGURE 2-26 Virtual Channel Setup for Column Pressure Readout

To monitor other parameters (e.g. the UltiMate Micropump pressure), check a new channel (FIGURE 2-25) and configure the signal as presented in Table 2-5.

Table 2-5 Signal Name and Formula Definitions

Device (a)	Signal Name	Unit	Factor	Formula
ULT	PumpPressure	bar	1.0	pump.masterpressure
ULT	ColumnPressure	MPa	0.1	pump.columnpressure
SW II	TrapColumnPressure	psi	14.5	loading_pump.trapcolumnpressure
ULT	OvenTemperature	°C	1.00	Oven.Temperature
FMS	TrayTemperature			Sampler.Temperature

Note: a) ULT = UltiMate , FMS = FAMOS , SW II – Switchos II

A typical setup is presented FIGURE 2-27.

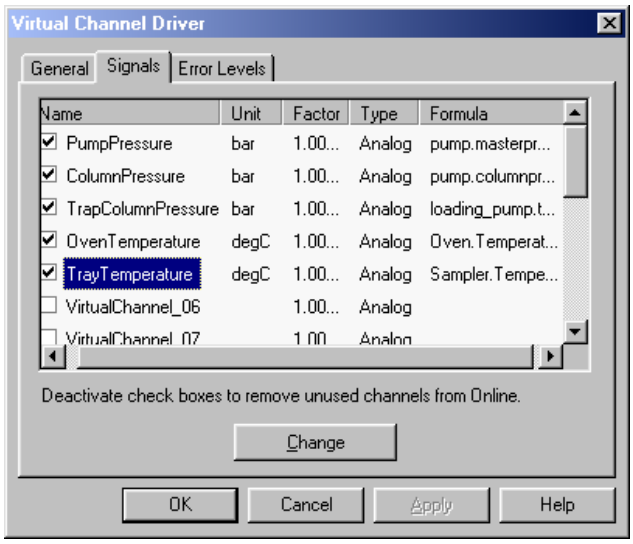


FIGURE 2-27 Example for a typical Virtual Channel Setup

Note: Make sure to use the same signal names and the same formulas as indicated in Table 2-5. If you modify the signal names or the formulas, they may not be recognized by the (predefined) panels and some functions may not work properly.

2.9 Using CHROMELEON®

2.9.1 Starting CHROMELEON

Click on the CHROMELEON Server icon to start the CHROMELEON server (which is the interface between the CHROMELEON user interface and the instruments). Click on the CHROMELEON icon to start the software.

2.9.2 The Control Panel

The Control Panel (abbreviated: the 'panel') controls and monitors the instruments of one Timebase. With regard to appearance and function, it is a special type of window. You can determine the number of available controls and their functionality via the design tools, depending on your individual requirements (refer to the CHROMELEON user's manual for more detailed information).

For the most common used UltiMate configurations a number of standard panels are available. Refer to Table 2-6 and choose the panel which corresponds to your instrument configuration, and then load this panel from the 'Dionex Templates/ Panels/Dionex LC' directory.

Table 2-6 Panel Name vs. Instrument Configuration

Control Panel Name	Instrument Configuration
Ultimate.pan	UltiMate
Ultimate_FAMOS.pan	UltiMate and FAMOS,
Ultimate_FAMOS_Switchos.pan	UltiMate, FAMOS, Switchos II (Switchos II valves are controlled by the Loading Pump – Section 2.8.4.A)
Ultimate_FAMOS_SwitchosII.pan	UltiMate, FAMOS, Switchos II (Switchos Valves are controlled by serial communication – Section 2.8.4.B)

Depending on your authorization, you can create a completely new control panel. In order to create or change properties of a control panel, change to the **Layout Mode** on the **Edit** menu. A new control panel is saved as a PAN file (*.pan) and is then available to the user (refer to the CHROMELEON user's manual for more detailed information).

2.9.2.A Connecting the Control Panel to a Timebase

A panel needs to be connected to a certain Timebase to allow the control of the instruments of this Timebase (Section 2.8).

To connect the control panel to a Timebase:

a) Click on the **Connect to Timebase...** command on the **Control** menu to select the Timebase you want to connect the panel to.

b) Use on the **Save as...** command on the **File** menu to save this new assignment.

2.9.2.B Starting the Flow Delivery and Baseline Monitoring

To gain control of the different Ultimate modules, each module of the configuration need to be connected to the control panel. Check the **Connect** boxes to connect the individual modules (item 1; FIGURE 2-28).

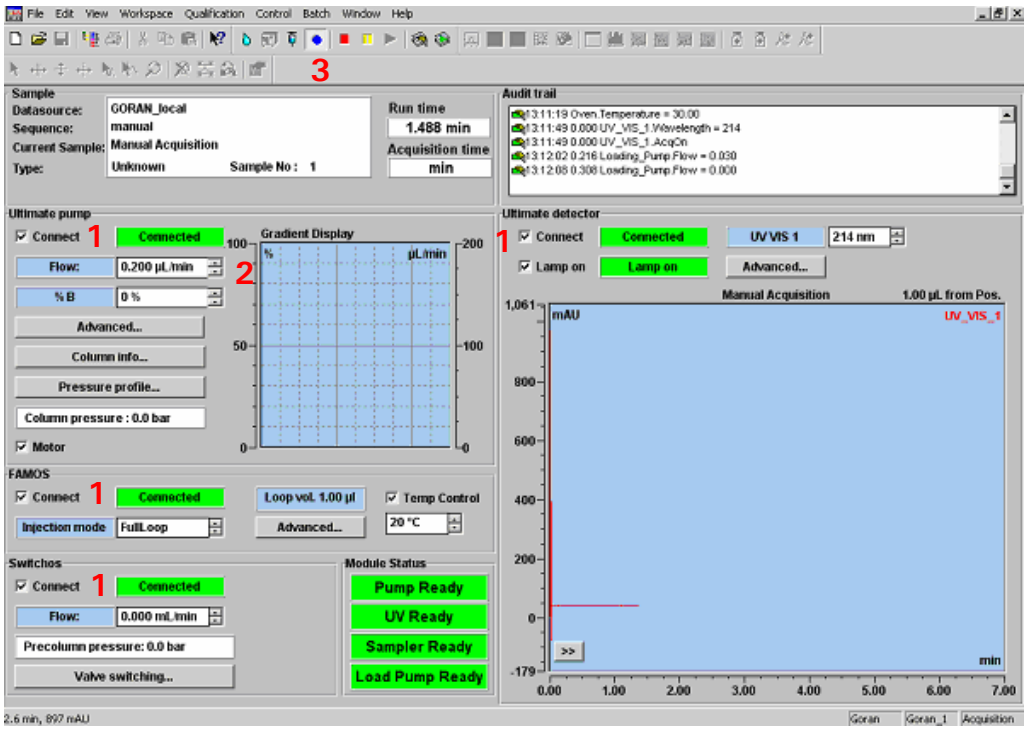


FIGURE 2-28 The Control Panel for UltiMate, FAMOS and Switchos

Flow delivery and baseline monitoring can now be started. Enter the column flow rate in the **Flow** field in the *UltiMate pump* box (item 2; FIGURE 2-28) and click on the blue circle in the toolbar to start baseline monitoring (item 3; FIGURE 2-28).

2.9.3 Creating a Program File – Using the Wizard

CHROMELEON programs are text files and modifying a program is normally done by editing in the text window. When creating a new program file, a Program Wizard starts automatically and guides you through the programming to simplify the procedure. Enter the required information; in most cases the pre-defined values can be used.

The wizard provides a ready-to-use program based on your entries, without the need to type a special programming syntax. Press F1 key for additional help and more detailed information. Section 2.9.4 and Appendix E provide programming examples.

2.9.4 Creating a Program – An Example

The following section shows an example for the creation of a program file. For a column switching experiment (a pre-concentration step) the UltiMate system

(Nano configuration), the Switchos II Advanced Microcolumn Switching Unit and the FAMOS Microautosampler are used and need to be programmed.

The experiment has the following features:

- NanoLC separation on a 75 µm I.D., 15 cm PepMap column.
- Injection of 10 µl of sample.
- Sample loading onto a trap column for 5 minutes at a flow rate of 30 µL/min (pre-concentration step).
- Solvent gradient from 0 - 50%B in 30 minutes.
- Analysis time of 50 minutes.
- Recording of the UV signal at 214 nm, the column pressure, the trap column pressure and the pump pressure.

CHROMELEON automatically provides a program wizard when a new program is to be created, which guides through creation of the program file.

To create a new program using the Program Wizard:

a) Select the **New** command on the **File** menu, and then select **Program File** from the *New* box. The Program Wizard starts automatically.

b) Select the Timebase from the *Select Timebase Options* box.

c) Setup the column oven temperature (if applicable) in the *Oven Options* box.

d) Setup the trap column pressure limits of the Switchos II Loading Pump (e.g. 0 bar and 200 bar) and fill in the time table in the *Loading_Pump Options* box.

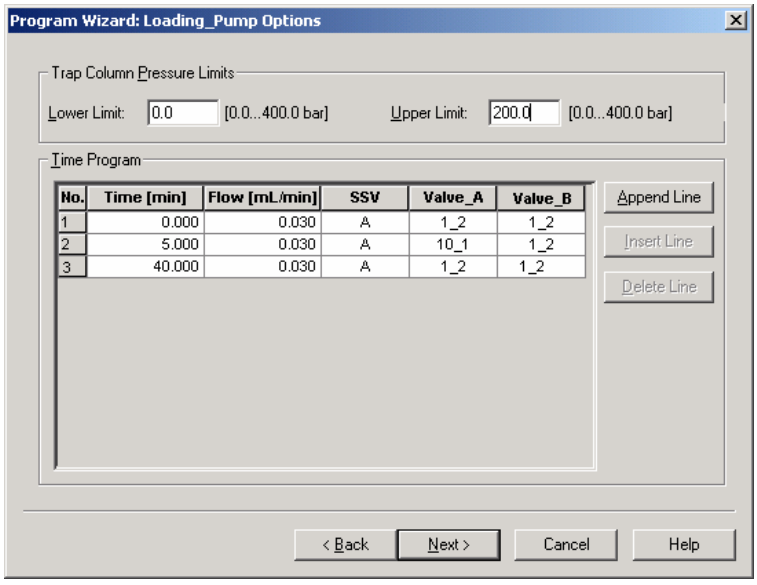


FIGURE 2-29 The Loading Pump Options Box

e) Setup the gradient conditions in the *Pump Options* box, use the 'Multi-step Gradient' option to run a gradient.

f) Define the gradient in the *Flow Gradient Options* box (FIGURE 2-30).

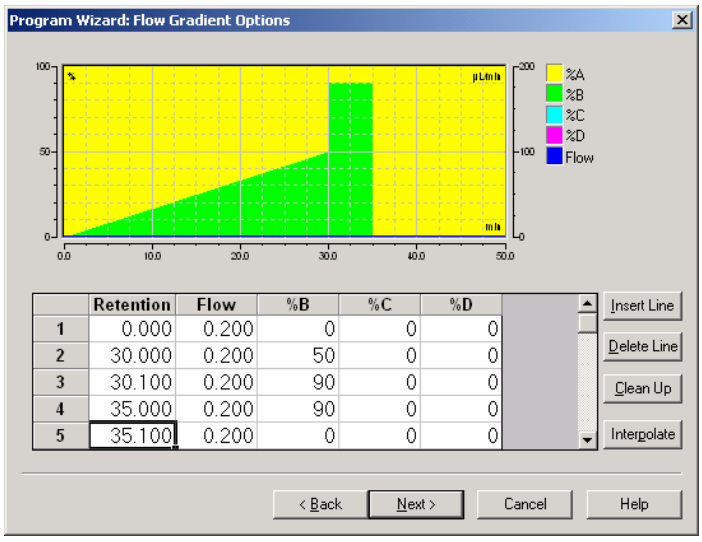


FIGURE 2-30 The Flow Gradient Options Box

Note: The *Flow Gradient* box is not present if the 'isocratic' option was selected in the *Pump Options* box in the previous step.

g) Setup the pressure limits for the separation column and the UltiMate pump, and define separation column parameters (e.g. I.D., length and stationary phase) in the *UltiMate Pump Options* box (FIGURE 2-31).

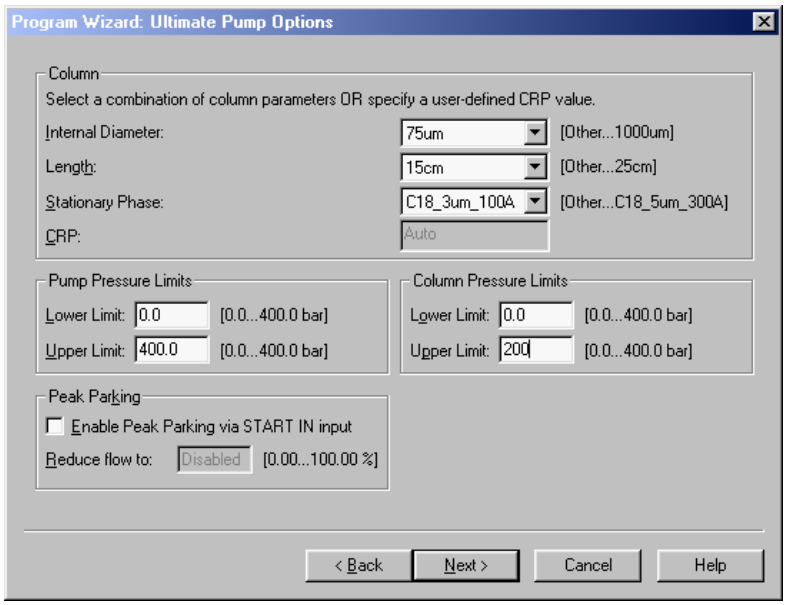


FIGURE 2-31 The UltiMate Pump Options Box

Note: If you do not specify the 'Column' parameters (e.g. the option 'other' is selected), you need to enter a CRP (Column Resistance Parameter) value. The CRP is required to calculate the (master) flow rate of the UltiMate Micropump.

h) Specify the detector settings (e.g. the data collection rate) in the *UV Options* box.

i) Define the type of injection you want to use and the corresponding injection parameters in the *Sampler Options* box.

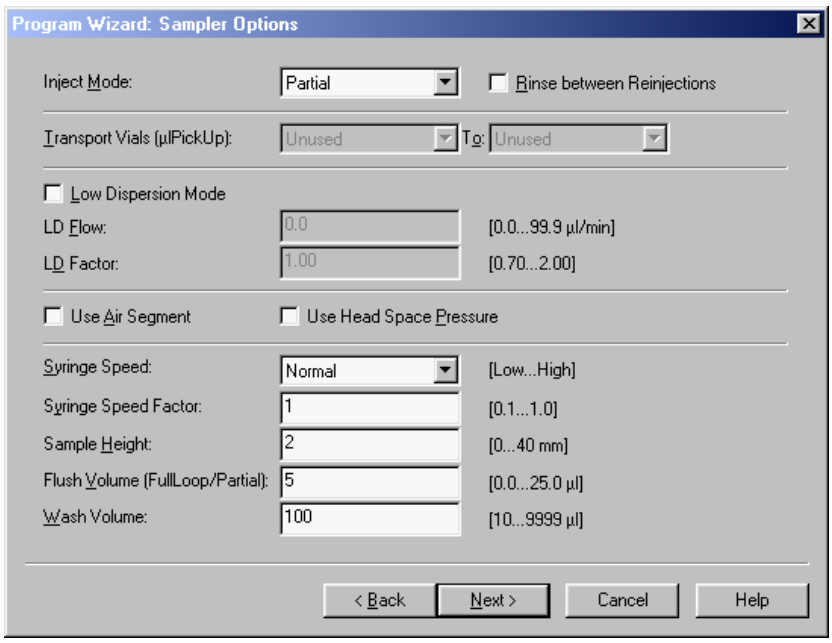


FIGURE 2-32 The Sampler Options Box

Note: If you want to create a 'User Defined Program', please refer to Section 2.10 for more details.

j) Select the number of UV channels used and the data acquisition time in the *Acquisition Options* box (FIGURE 2-33).

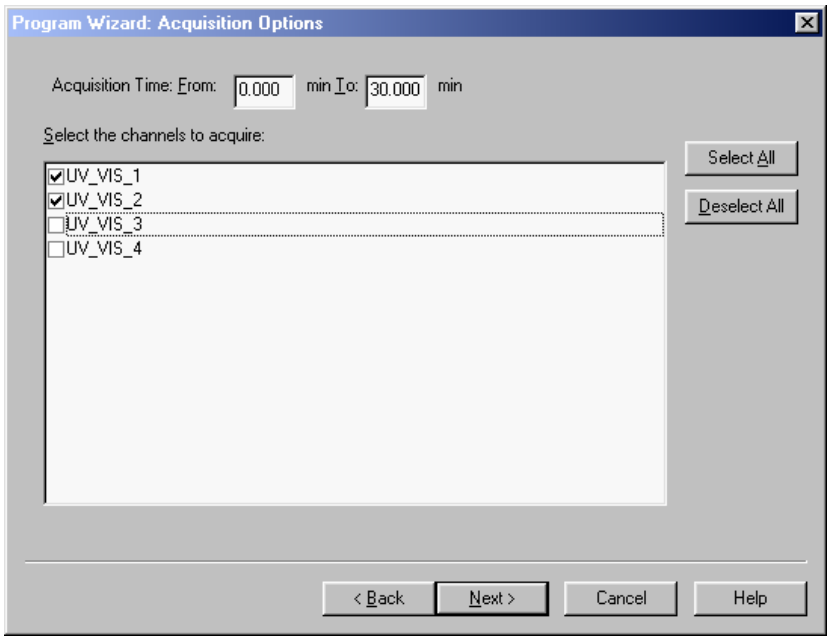


FIGURE 2-33 The Acquisition Option Box

k) Depending on how many channels you are using the corresponding number of *UV_VIS_n Options* boxes need to be filled in to program e.g. the different wavelengths (FIGURE 2-34).

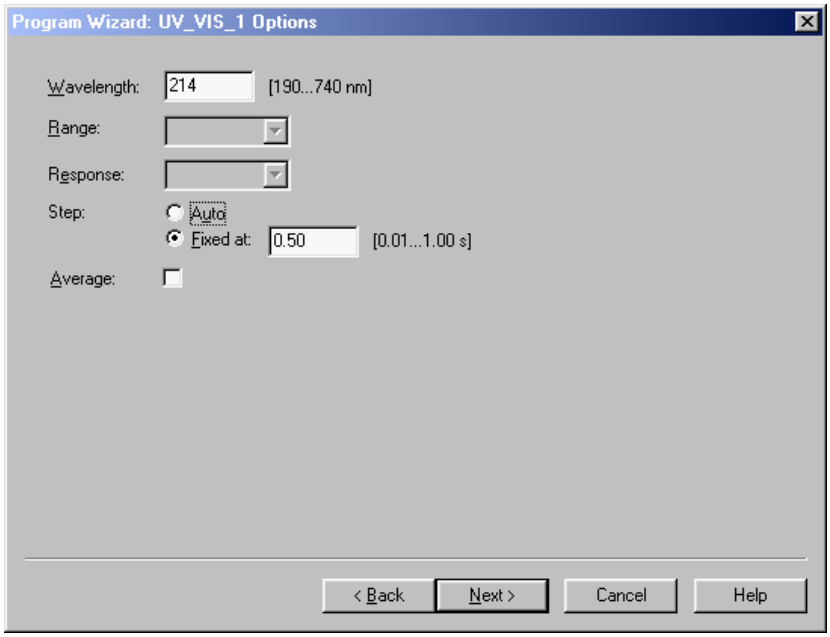


FIGURE 2-34 The UV_VIS_*n* Options Box

l) Finally the program name and path must be specified.

Four additional examples are presented in Appendix E. While these examples may not meet the specific needs of the analyst, it is likely that they can be used with minor modification.

- NanoLC separation with full loop injection.
- NanoLC separation with partial loop injection and with pre-concentration.
- NanoLC separation with UDP injection and with pre-concentration.
- NanoLC separation with manual Injection.

2.9.5 Changing a Program File

A CHROMELEON program can easily be changed by a double click on the program name in the CHROMELEON browser. The program appears in a text format. Program lines are inserted by describing the parameter or by using the F8 command.

2.9.6 Creating a Sequence File – Using the Wizard

The Sequence Wizard helps you to quickly create a basic sample list consisting of analysis and standard samples. The Sequence Wizard is opened via the **File/New** command in the Browser. Creating a Sequence is performed in five easy steps:

- Step 1: Selecting the Timebase
- Step 2: Generating the analysis (unknown) samples
- Step 3: Generating the standard samples
- Step 4: Determining the Program File and the analysis method
- Step 5: Saving the sequence and assigning a name

Refer to the CHROMELEON Online Help (**F1** key) for a detailed description of the mentioned steps.

Each step is performed in a separate box. Use the **<Back** and **Next>** buttons to browse through the input screens.

In the fifth step, click the **Finish** button to save the sequence and close the wizard.

FIGURE 2-35 presents a typical setup of the *Unknown Samples* box (step 2) and a typical CHROMELEON Sequence is presented in FIGURE 2-36.

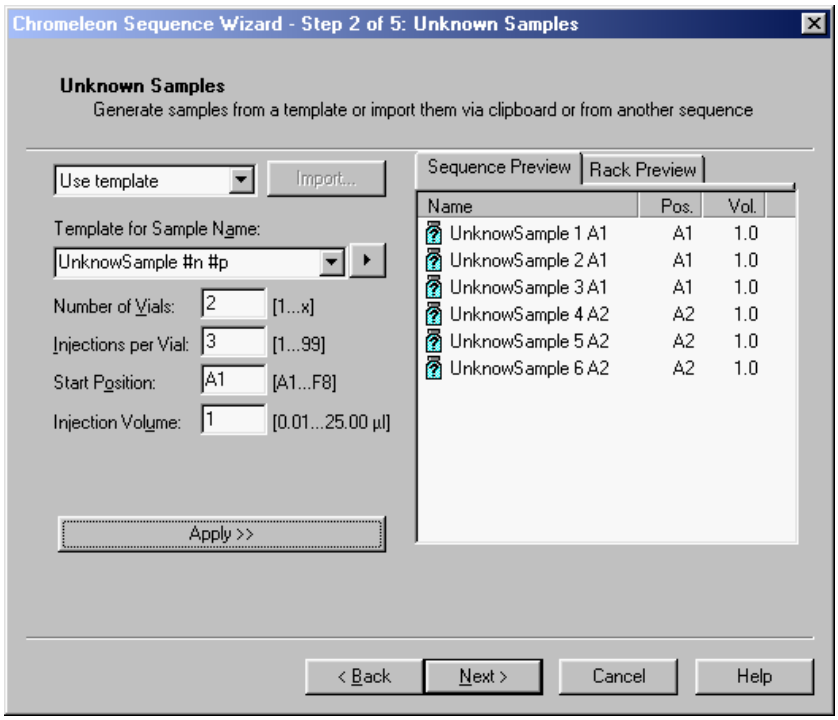


FIGURE 2-35 Typical 'Unknown Sample Setup (Step2)

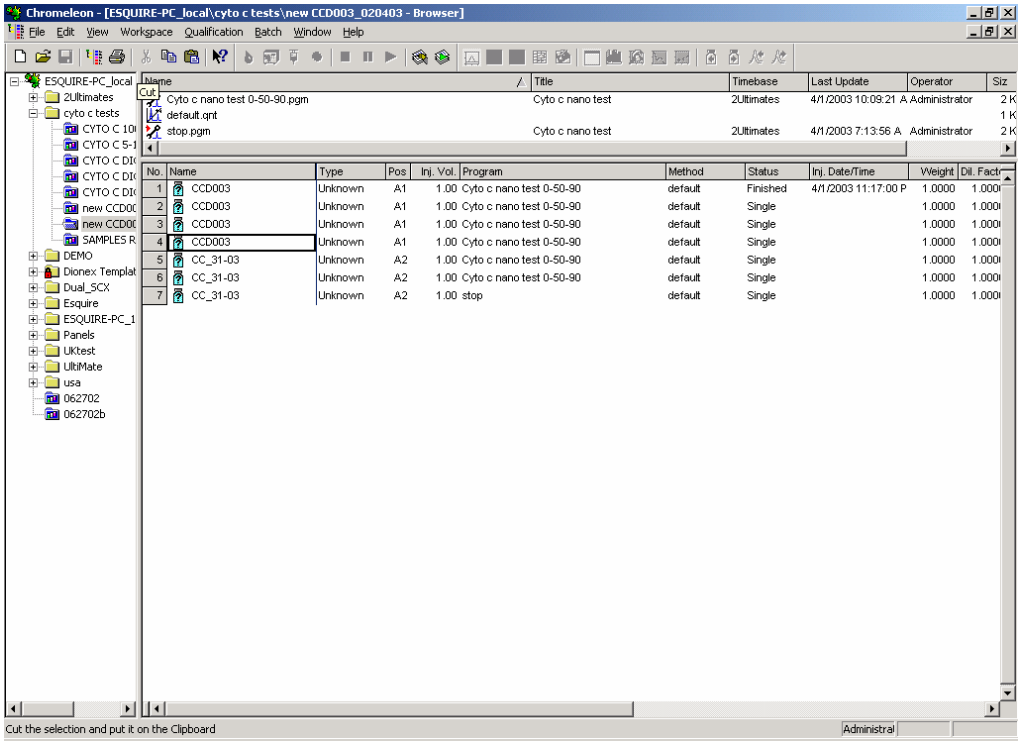


FIGURE 2-36 Typical Sequence for CHROMELEON

2.10 Creating a User Defined Program

In addition to the three available standard injection modes (full loop, partial loop and µl pick-up), so-called **U**ser **D**efined **P**rograms (UDP) can be created. This option allows programming of each single step of what the FAMOS Microautosampler should do.

To open the wizard, which then guides through the UDP programming, select the 'UserProg' option in the inject mode field in the *Sampler Options* box (FIGURE 2-32).

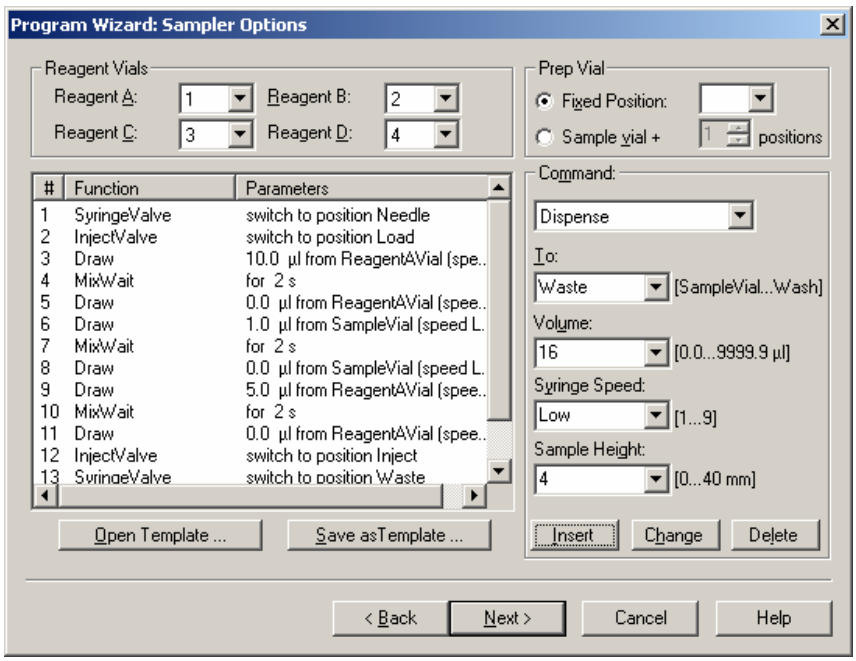


FIGURE 2-37 User Defined Program Wizard for the FAMOS

To insert a new program line:

a) Select a command from the Command list (item 1) and define the parameter (e.g. Dispense the volume of 16 uL to the Waste position at Low speed and at a needle height of 4 mm).

b) Click on **Insert** to add it to the program, click on **Change** to overwrite an existing program line.

c) Define the Reagent vials and/or Prep vials according to the needs of your application.

d) Click **Next** to continue.

Note: For getting easily started, use one of the templates provided and modify it according to the needs of your application.

2.11 Preparing the System for Operation

2.11.1 ON/Standby Switch

The ON/standby switch (FIGURE 2-38) is located on the top front panel. Once the system is powered on by the main power switch on the rear panel, it can be switched into standby mode (e.g. when not used during the night).

If you press the switch for more than 2 seconds, the Micropump and the UV Detector will be turned-off and the LED will be illuminated. In addition, the Solvent Organizer is set into standby mode at the same time. When the system is in standby mode, pressing the switch for a short time will power on the system again (and toggle the LED).



FIGURE 2-38 The ON/Standby Switch

Note: The ON/Standby switch must be pressed for at least 2 seconds to switch the UltiMate system into standby mode.

2.11.2 Installing a Backflushing System

Note: The pump head of the Micropump should be backflushed with propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the pistons may result; this will dramatically shorten the life of these components.

To fill the backflushing system:

a) Remove the silicon tubing connecting the two backflushing port from one port and attach the end to a container (e.g. small beaker) to collect the flushing liquid.

b) Fill the 5 mL priming syringe (accessories kit) with the backflushing liquid and connect it to the open backflushing port using 1/16”silicon tubing (accessories kit).

c) Force the liquid through the pump head until liquid appears in the container (FIGURE 2-39).

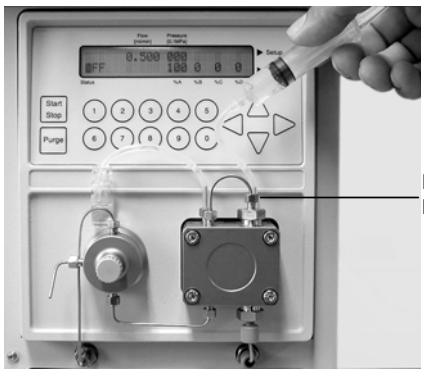


FIGURE 2-39 Filling the Backflushing System of Pump Head

d) To prevent vaporization of the flushing liquid, place the connecting tubing back onto the port.

The fluid should be changed/re-filled on a daily basis.

If the Micropump is to be used to deliver buffers with high salt concentration, it is necessary to use a continuous piston backflushing system.

2.11.3 Purging the Micropump and the Solvent Lines

To Purge the Micropump:

a) Fill solvent reservoirs A and B with the mobile phase to be used, e.g. the standard test procedure to be used in Section 3.3.3 employs the following mobile phases :

A: 0.05% TFA in water/acetonitrile (95/5, v/v)

B: 0.04% TFA in water/acetonitrile (20/80, v/v)

b) Fill solvent reservoirs C and D (which are not to be used in the test separation) with methanol/water (1:1).

Note: All four solvent lines must contain mobile phase and must be purged to assure proper functioning of the system.

c) Open the He regulating valves and verify that the He shut-off valve on top of each solvent reservoir (FIGURE 2-40) is open (i.e. the white line should be vertical). Allow vigorous sparging to continue for approximately 10 min before continuing. After the vigorous sparging is complete, reduce the flow to a maintenance level (see Section 3.3.1).

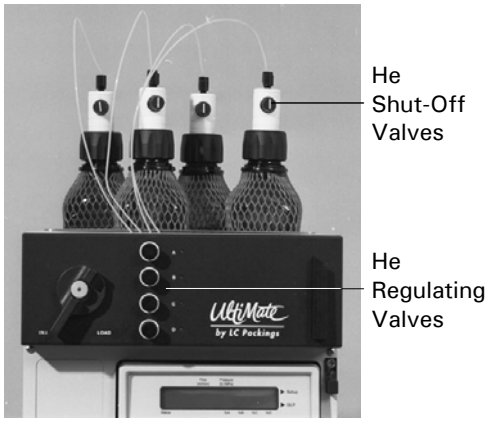


FIGURE 2-40 Solvent Organizer with He Shut-Off Valves and He Regulating Valves

d) Purge the solvent lines to the Micropump:

- Connect the 5 mL syringe to the outlet of the purge valve (FIGURE 2-41) using a piece of silicone tubing.

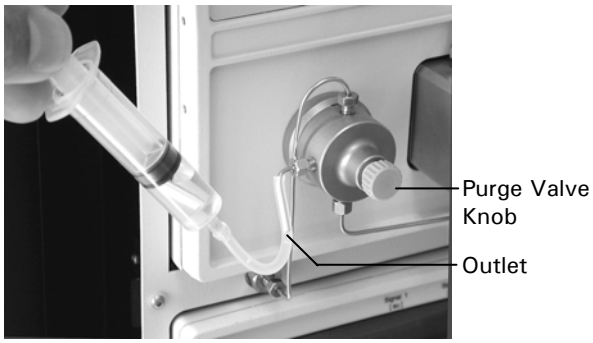


FIGURE 2-41 The Purge Valve

- Open the Purge Valve.
- Power up the UltiMate System via the switch on the back panel.
- Press the **PURGE** key on the Micropump and set the flow to 0.0 mL/min. Use the cursor keys to select the solvent line A and purge the line using the plastic 5 mL syringe. After line A is purged, continue with lines B, C and D.
- Set the purge flow to 1.0 mL/min (if the firmware version for the Micropump is lower than Version 1.3, the maximum purge flow rate is 0.5 mL/min). Allow the system to purge for at least 3 min. After line A has been purged, repeat the process for all other lines.

e) Close the Purge Valve.

f) Close the He regulating valves for the solvent reservoirs that will not be used (e.g. lines C and D).

g) Close the He shut-off valve on top of each solvent reservoir that will not be used (the white line should be horizontal).

2.11.4 Purging the Column Pressure Sensor

Note: When the UltiMate is installed, it may be necessary to purge the column pressure sensor as it may be partially filled with air. When the column pressure sensor is partially filled with air, pressurization of the column will take longer than usual.

a) Make certain that a Capillary LC calibrator or a bypass cartridge (P/N 160062) is placed in the UltiMate.

b) Disconnect the connecting tubing from the Nano/Micro flow outlet of the upper T-piece (FIGURE 2-42).

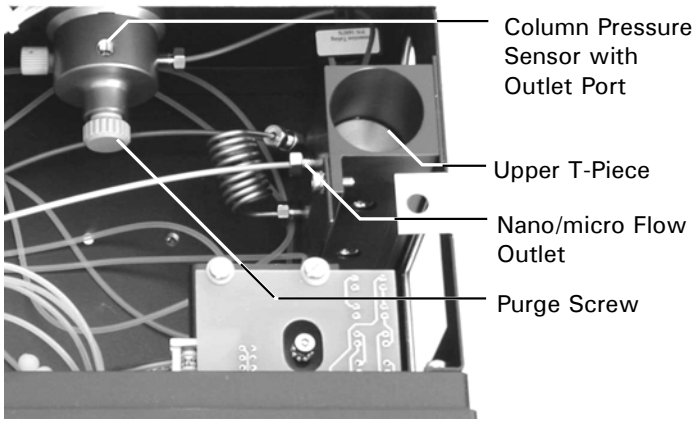


FIGURE 2-42 Purge Valve – Column Pressure Sensor

c) Open the purge valve of the column pressure sensor (FIGURE 2-42) a quarter turn counter clockwise.

d) Set the Flow to 200 µL/min and start the delivery of mobile phase for at least 5 min until the solvent level fills the entire outlet port.

e) Close the purge valve.

Note: Purging with a NANO calibrator in place will take a long period of time because the flow rate is exceedingly low (up to 1 h).

2.12 Installation of the Column

When installing the column in the oven compartment, guide the appropriate connecting capillary (the dimensions depend on your application, see Sections 2.5.2 and 3.10) through the foam material on top of the oven into the fluidic compartment. Then guide the capillary through the hole on the left side and connect it to the FAMOS injection valve or to the manual injection valve (if installed).

When installing a Nano column, use one of the slots around the oven cover plate to keep the connection as short as possible (e.g. item 1, FIGURE 2-43)

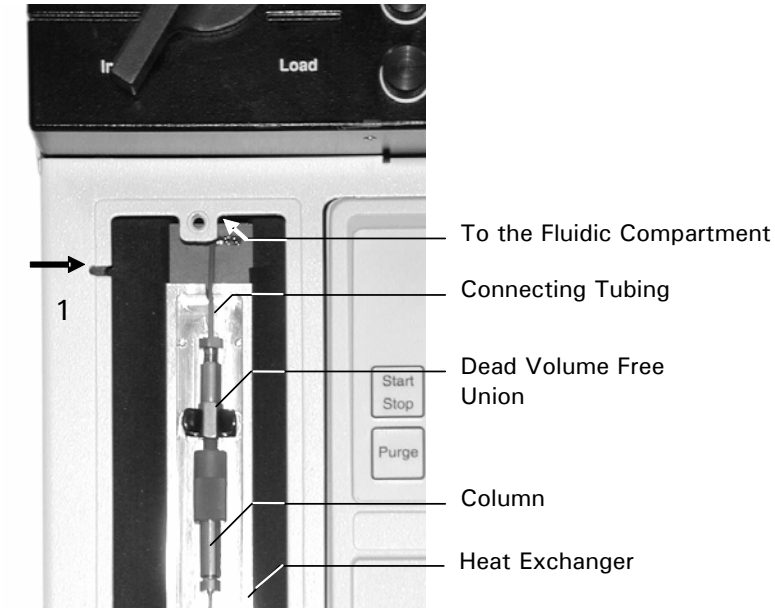


FIGURE 2-43 **Installing of a 180 µm – 800 µm Column in the UltiMate Oven**

2.12.1 UltiMate System

To install the column in the column compartment:

a) Install the column in the column compartment (FIGURE 2-43).

b) Guide the column outlet capillary through one of the slots in the column compartment cover plate.

c) Connect the column outlet to the flow cell inlet using a Teflon connector (Nano and Pico columns, FIGURE 2-44) or a fingertight fitting (Microbore columns).

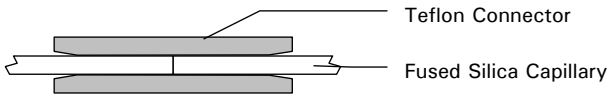


FIGURE 2-44 Connecting Fused Silica Capillaries

Note: Refer to the instruction sheet supplied with the column and Appendix C for more details. Make certain that the connection does not have any void ('dead') volume by making it as short as possible and ensure that there is no gap between the end of the column outlet capillary and the end of the detector inlet capillary.

2.12.2 UltiMate System with FAMOS Autosampler

a) Bypass the manual injection valve of the UltiMate as described in Appendix 3.3.

b) Install the column in the column compartment (FIGURE 2-43).

Note: Alternatively, the column can be directly connected to port 6 ("column") of the FAMOS Autosampler instead of using the bulkhead fitting (e.g. interfacing a Nano-LC system to the FAMOS Autosampler).

d) Connect the column outlet to the flow cell inlet using a Teflon connector (Nano and Pico columns, FIGURE 2-44) or a fingertight fitting (Microbore columns).

Note: Refer to the instruction sheet supplied with the column and Appendix C for more details. Make certain that the connection does not have any dead volume by making it as short as possible and ensure that there is no gap between the end of the column and the end of the detector capillary.

Note: The tubing supplied with the UltiMate depends on the configuration of the UltiMate System (Micro, Nano or Capillary).

2.13 The Standard System Test

2.13.1 Preparing the UltiMate System for the Standard System Test

A standard system test is provided in Chapter 3 to allow the installer to verify that the instrument is functioning properly.

The system should be configured as described in Chapter 3. When the FAMOS Microautosampler is installed, the injection system should be configured as described in Section 2.4.6.

2.13.2 Performing the Standard System Test

The standard system test involves the separation of a Cytochrom C digest. The test, which is described in detail (and the expected results) is presented in Section 3.5, is used to evaluate the following:

- Reproducibility of the gradient
- Dead volume of the system
- Sensitivity of the UV Detector

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CHAPTER 3

The UltiMate™ Solvent Organizer

3.1 Overview

The UltiMate™ Capillary HPLC System (FIGURE 3-1) is an integrated system that consists of the following modules:

- UltiMate Solvent Organizer with column oven and manual injection valve (option)
- UltiMate Micropump with μ-Pump Head
- UltiMate UV Detector (option) with U-Z View™ Capillary flow cell

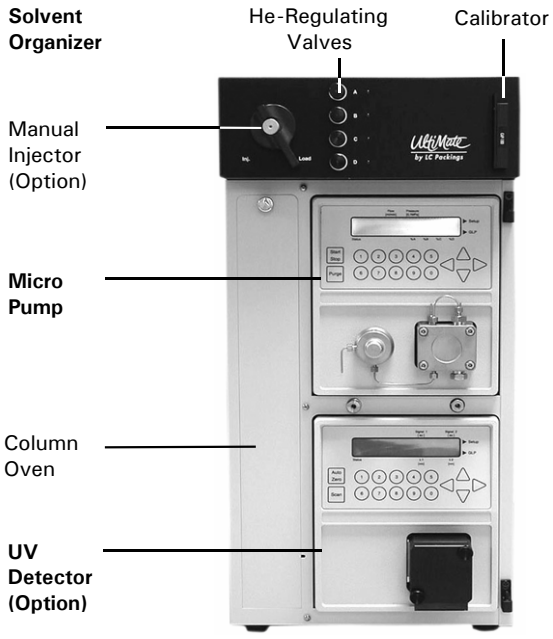


FIGURE 3-1. The UltiMate Capillary HPLC System

This chapter describes the Solvent Organizer and includes the following information:

- Design of the Fluidics System (Section 3.2)
- Operation of the UltiMate Capillary HPLC System (Section 3.3)
- Sample and Mobile Phase Considerations (Section 3.3.2)
- System Checkout (Section 3.3.3)
- Maintenance (Section 3.4)
- Disassembly and Replacement of User Replaceable Components (Section 3.5)
- Checking System Components (Section 3.6)
- Troubleshooting (Section 3.7)
- List of Spare Parts (Section3.8)
- Specifications (Section 3.9)

The Micropump is described in Chapter 4 and the UV Detector is described in Chapter 5.

3.2 Design of the Fluidics System

The fluidics system of the UltiMate Solvent Organizer consists of the following modules:

- Solvent Bottles and Degassing Unit which provides degassed mobile phase to the low pressure mixing system (Section 3.2.1).
- A four channel low-pressure mixing system to generate mobile phases consisting of up to quaternary gradients (Section 3.2.2).
- An integrated flow-splitting unit to deliver the low flow rates required for Micro-, Capillary- and Nano-HPLC (Section 3.2.3).
- A pressure sensor assembly for monitoring column pressure (Section 3.2.4).
- An external loop manual injection valve (option, Section 3.2.5).

All modules, except the Solvent Bottle assembly, are located in the fluidics compartment in the top of the UltiMate housing.

3.2.1 Solvent Bottles and Degassing Unit

Each of the four solvent channels can be degassed independently. The He inlet on the rear of the system provides He via a 5 port manifold to the four regulating valves located on the upper front panel. He lines connect the valves to the bottle cap assemblies (FIGURE 3-2).

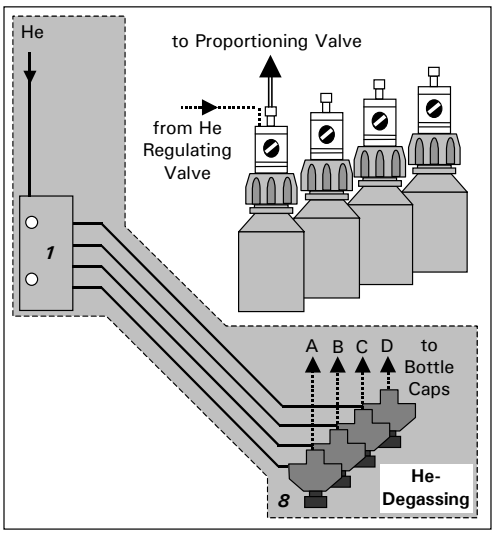


FIGURE 3-2. Solvent Bottle Assembly and He-Degassing Unit

The Solvent Bottle Caps on each bottle include a He shut-off valve and a combined sparging frit and solvent inlet filter. The He shut-off valve is shown in FIGURE 3-3 and the sparging/filter unit is shown in FIGURE 3-4.



FIGURE 3-3. He Shut-off Valve

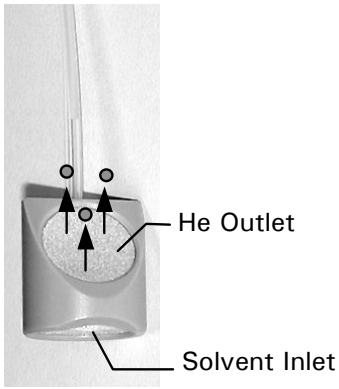


FIGURE 3-4. Sparging/Filter Unit

3.2.2 Four Channel Low Pressure Mixing System

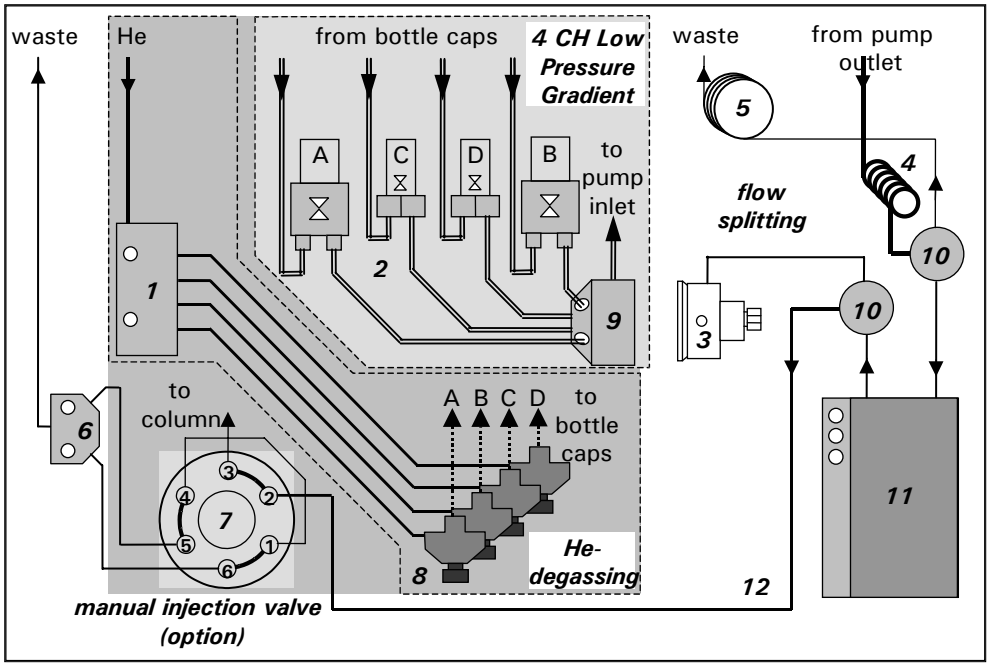
Four proportioning valves are used to generate the mobile phase defined by the user via the CHROMELEON software. The proportioning valves are controlled by the UltiMate Micropump; 0.5 mm Tefzel tubing is used to connect the proportioning valves to the sparging/filter unit. The solvent lines run through the bottle cap assemblies.

Note: If two solvents are used to generate the mobile phase, channels A and B should be employed. When three or four solvents are employed, channels A and B should be used for the organic components and channels C and D for the aqueous components.

3.2.3 Integrated Flowsplitting Unit

The flow rate delivered by the Micropump is typically 200 µL/min. The flow rate used for separation of the sample is determined by the flow resistance of the waste restrictor, the flow resistance of the calibrator and the resistance of the analytical microcolumn. The desired flow rate is entered by the user via the CHROMELEON software.

The overall design of the Fluidics Module is shown in FIGURE 3-5.



1 5 Port He Manifold
2 Proportioning Valves A, B
Proportioning Valves C, D
3 Column Pressure Sensor
4 Static Mixer
5 Waste Restrictor
6 3 Port Waste Manifold
7 Manual Injection Valve (Option)
8 He Regulating Valves
9 5 Port Mixing Manifold
10 Lower and upper T-Pieces of the Flow Splitter
11 Calibrator (Section 3.5.14)
12 Connection Capillary (Section 3.5.14)

FIGURE 3-5. Flow Diagram of Solvent Organizer

A variety of calibrators are available to define the flow rate (refer to Sections 3.5.14 and 3.8.2 for details).

Note: The I.D. of the capillaries between the upper T-Piece and the manual injection valve and the manual injection valve and the column depend on the application (Micro-, Capillary– or Nano LC, see Section 3.5.14 for details).

3.2.4 Column Pressure Sensor Assembly

A pressure transducer unit with purge valve is connected as a dead end to measure the column pressure.

3.2.5 External Loop Manual Injection Valve

If you purchased the UltiMate system with the manual injection valve option, an external loop manual injection valve (with 0.25 mm internal bore) is mounted in the upper front panel of the UltiMate housing. Sample loading is performed through the injection port in the handle of the valve. The two waste ports of the valve are connected in a T-piece and a waste line runs to the rear of the UltiMate.



Note: Maintenance of the Manual Injection Valve is described in Appendix D.

3.2.6 FAMOS Autosampler

The FAMOS Microautosampler, which replaces the manual injection valve offers the capability to automatically inject the sample in three different modes (Flushed Loop, Partial Loop and μL Pick-Up). For full details about this system, please refer to the manual provided with the autosampler.

3.3 Operation of the UltiMate Capillary HPLC System

3.3.1 Getting Started

Although the UltiMate system is controlled via the CHROMELEON® software, initial system preparation is done with the Micropump on an off-line basis (i.e. the Micropump is operated locally).

To prepare the system for a separation:

a) Power up the UltiMate system and make certain that power is provided to the pump and detector.

b) Check that the pump head backflushing system is operating. A solution of iso-propanol/water (1:1) is commonly used but other solvents can be used (if any of the buffer components are not soluble in this mixture, reduce the fraction of propanol). If desired, you can reduce evaporation of the backflushing solution by connecting the two backflush ports (Section 2.7) A 5 mL syringe is provided to backflush the head and fill the reservoir.

The pump head of the Micropump should be backflushed with iso-propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the piston may result; this will dramatically shorten the life of these components.

c) Inspect all fittings. If there is a salt deposit by a joint, it is probable that a leak has occurred and the fitting should be cleaned and tightened. When you tighten a fitting, do not overtighten. Check that the solvent filters are clean, if not they are not clean, they should be replaced.

d) Fill the solvent reservoirs with the mobile phases to be used for the separation.

CAUTION **Caution: Only use the shielded solvent reservoirs supplied with the UltiMate.**

CAUTION **Caution: Do not operate the He lines at a pressure greater than 4 bar (60 PSI).**

Note: All four solvent bottles must be filled and purged (even if the separation requires less than four mobile phases) to assure proper function of the system. If two solvents are used to generate the mobile phase, bottles A and B should be used. When three or four solvents are used, channels A and B should be used for the organic solvents, while channel C and D should be used for the aqueous phases. Fill solvent bottles that will not be used with methanol/water (1/1).

Note: The solvents must be degassed via the He degassing technique described below. If other techniques are used (e.g. vacuum degassing) the performance of the system will be seriously degraded and the performance specifications will not be obtained.

e) Open the He Shut-off valves by rotating the knob so that the line on the valve is vertical (see FIGURE 3-6) and open the He Regulating Valves for maximum sparging (FIGURE 3-7A). Allow sparging to continue for

approximately 10 minutes at a rapid rate, then lower the flow rate to maintenance mode (FIGURE 3-7B).

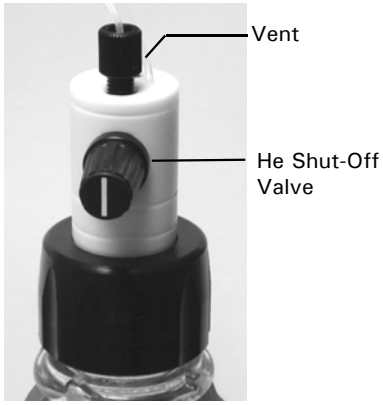


FIGURE 3-6. He Shut-Off Valve



(A) (B)

FIGURE 3-7. (A) Rapid Sparging (B) Sparging – Maintenance Mode (Shield removed to provide clarity)

f) Connect the 5 mL syringe to the purge outlet on the purge valve on the pump using 1/16" ID silicon tubing (FIGURE 3-8).

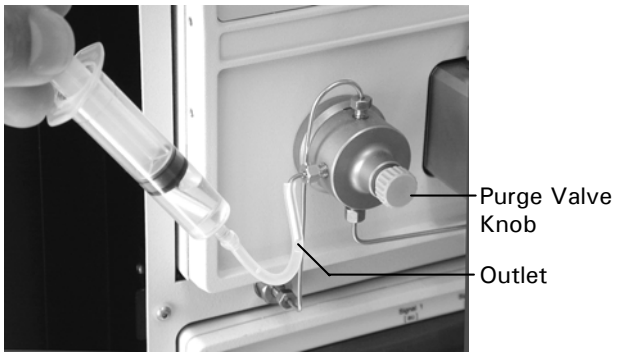


FIGURE 3-8. The Purge Valve

g) Open the purge valve on the pump by turning the purge valve knob (FIGURE 3-8) approximately 1 turn counterclockwise.

h) Press the PURGE key on the pump, set the flow rate to 0.0 mL and select A (Section 4.4). Withdraw solvent from bottle A using the syringe until no air is observed. Repeat this process for all four channels.

i) Set the purge flow to 1.0 mL/min and allow the system to purge for at least 3 min. After line A has been purged, repeat the process for all other lines.

j) Close the purge valve on the pump by turning the purge valve knob clockwise.

k) Close the He regulating valves for each of the solvent reservoirs that are not going to be used in the separation.

l) Close the He shut-off valve on top of the solvent reservoirs that will not be used (the white line should be horizontal).

m) Place the Micropump under computer control and deliver mobile phase through the entire HPLC system at the flow rate and composition that is used as the initial conditions for the analysis that you intend to perform. As the system is delivering mobile phase, check for leaks, monitor the baseline and check that the pressure is similar to what was observed when the system was last used.

n) If the system includes a FAMOS Autosampler, check that the temperature is correct (if the cooling option is installed), the syringe is bubble free and the wash syringe has sufficient wash solution for the day's work.

o) Check that the temperature of the column oven is correct (if the column is installed in the oven).

p) Run a blank run and then separate a standard to make sure that the system performance matches that from previous separations.



Caution: Close all He shut-off valves when not using the system. If they stay open, solvents may enter the He line and may cause contamination of the system.

3.3.2 Sample and Mobile Phase Considerations

To optimize performance of the system, we recommend that all samples and mobile phases are free of particulate matter. Samples and mobile phases should be filtered through a 0.22 μm membrane filter. The filter should be checked to ensure that extractable materials are not present.



Caution: It is strongly recommend that only bottled HPLC water and solvent be used. If water from water purification systems is used, polymeric contamination may seriously damage the flow cell. This is especially true if sample pre-concentration or 2D separations are performed. This polymeric contamination may also seriously damage the flow cell (e.g. coating of the capillary walls).

If a gradient is used, make certain that the sample and the buffer are soluble in all compositions of the mobile phase that will be used in the separation. This test should be run in a beaker or test tube so that particulate matter does not enter the system. If any cloudiness is observed in the test, the gradient should be adjusted and repeated.

After you have finished using the system, flush the system with a water/methanol or water/acetonitrile mobile phase before shutting it down.

The solvents must be degassed via the He degassing technique described before. If other techniques are used (e.g. vacuum degassing) the performance of the system will be seriously degraded and the performance specifications will not be obtained.



The pump head of the Micropump should be backflushed with iso-propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the piston may result; this will dramatically shorten the life of these components.

3.3.3 System Checkout

This section includes a standard test protocol that can be used to monitor the performance of the Ultimate system. This test uses a standard lyophilized cytochrome C digest (P/N 161089) and is identical to that used as part of the factory operation qualification at the LC Packings manufacturing facility. The standard system test is used to evaluate the following:

- Reproducibility of the Gradient
- Sensitivity of the Detector
- Dead Volumes

To perform the Standard System on a Nano LC system:

a) Configure the system as indicated in TABLE 3-1 ('Nano LC').

TABLE 3-1. System Configuration for Standard System Test

Type of Application	Column I.D. [µm]	Flow Rate [µL/min]	Calibrator Type	Flow Cell Type	Connecting capillary		Injection Loop Size [µL]
					Low flow outlet - FAMOS	FAMOS - Column	
Nano LC (P/N)	75 (160321)	± 0.3	NAN-75 (160061)	UZ-N10 (160015)	(160035)	(160036)	1 (160109)
Cap. LC (P/N)	300 (160295)	± 4	CAP-300 (160059)	UZ-C10 (160013)	(160033)	(160034)	5 (160110)
Note: The part numbers listed above are for the standard version only, refer to Section 3.8 for more information about inert systems.							

b) Prepare the following mobile phases:

Solvent A: 0.05 % TFA in water/acetonitrile (95/5, v/v)
Solvent B: 0.04 % TFA in water/acetonitrile (20/80, v/v)
Solvent C + D: methanol/water (1/1)

c) Prepare the lyophilized cytochrome C digest as described in the instruction sheet provided with the sample (P/N 161089). Dilute the reconstituted sample 8 times in 0.05% TFA to obtain a final concentration of 1 pmol/µL.

d) Load the Gradient Program shown in TABLE 3-2 (use a CRP value of 625).

TABLE 3-2. Gradient Program - Standard System Test

Time [min]	Flow rate (1) [µL/min]	Solvent A [%]	Solvent B [%]	Wavelength [nm]
0.0	0.3	100	0	214
30.0	0.3	50	50	214
31.0	0.3	10	90	214
36.0	0.3	10	90	214
37.0	0.3	100	0	214
55.0	0.3	100	0	214
(1) Use a CRP value of 625				

e) Set-up the software for saving the pressure profile.

f) Start a blank run (without injection) and then inject 1 µL of the diluted test sample. To measure the reproducibility, run at least 8 injections. The chromatograms should be similar to FIGURE 3-9.

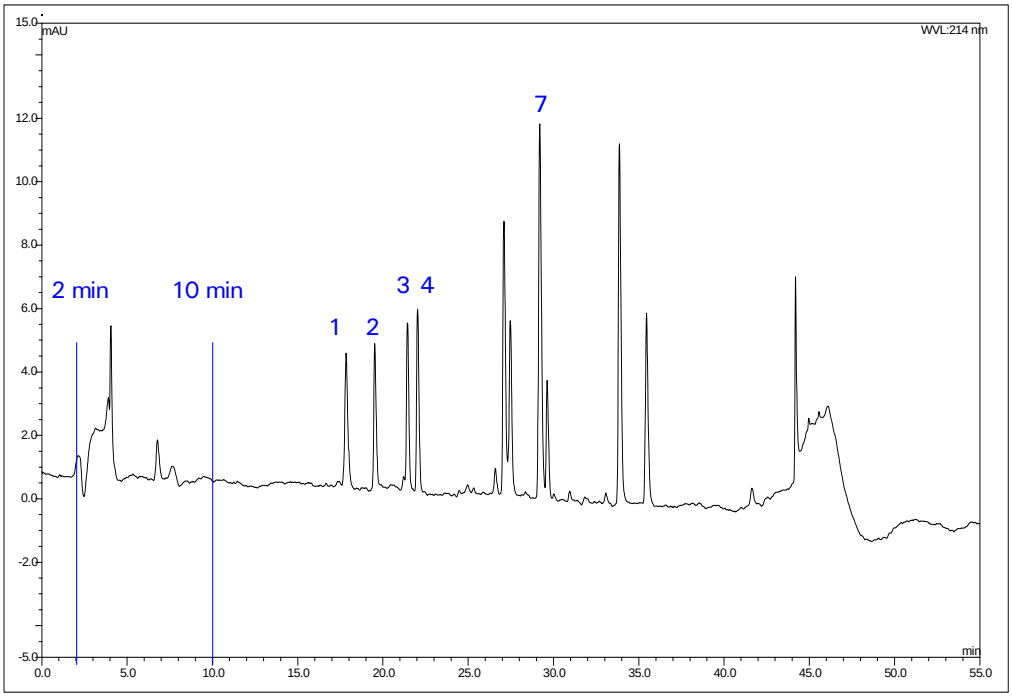


FIGURE 3-9. Standard System Test (Nano LC) – Chromatogram

The acceptance criteria are:

a) Injection profile should be between 2 and 10 min.

b) Components 1 through 4 should be baseline separated.

c) The peak width at half height for component 7 should be < 20 s.

d) The peak height for component 7 should be > 7 mAU.

e) The reproducibility of retention time should be better then 0.5 % relative standard deviation for component 7.

f) The column pressure profile (FIGURE 3-10) should not contain any spikes. The column pressure under initial conditions (100% Solvent A) should be between 8 to 12 MPa (110–150 bar, 1595-2175 PSI) and the maximum pulsation should not exceed 0.2 MPa (2 bar, 29 PSI).

g) The measured flow should be 0.30 µL with a CRP value of 625 (i.e. the flow rate displayed on the UltiMate Micropump is 187 µL/min).

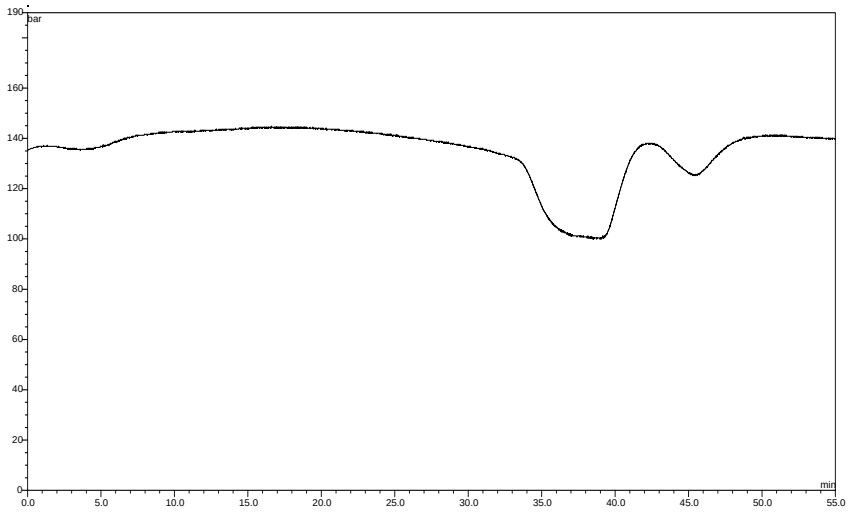


FIGURE 3-10. Standard System Test (Cap LC) – Pressure Profile

Note: If you performing the test with a Capillary LC system, check that it is configured according to TABLE 3-1 (page 3-10). The reconstituted cytochrome c digest sample (P/N 161089) is injected in a concentration of 8 pmol/µL. Use a CRP value of 50.

The acceptance criteria are:

a) Injection Profile: 2-7 min

b) Peak height for Peak 7: > 50 mAU

c) Reproducibility of Retention time: 0.5 %

d) Column Pressure: 70-100 Bar (1015-1450 PSI)

A typical chromatogram from a Cap LC system is shown in FIGURE 3-11.

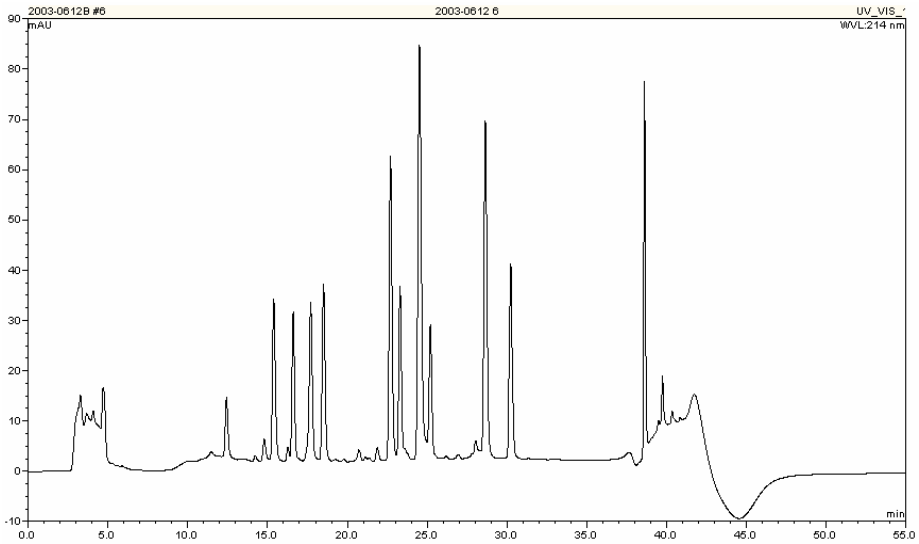


FIGURE 3-11. Standard System Test (Cap LC) – Chromatogram

Note: If you are performing the standard system test on a Micro LC system, please contact LC Packings for further information.

3.4 Maintenance

Maintenance refers to a variety of activities that should be performed on a routine basis to optimize the performance of the system.

In some cases (e.g. replacement of critical components), we recommend that a factory trained service engineer should be called to perform the operation. This will ensure optimal long term performance and maximum uptime. LC Packings provides a broad range of service support activities to ensure that the UltiMate Capillary HPLC System is functioning in a suitable manner. These activities can be customized to meet the specific needs of the customer. For further information, please contact your local LC Packings office or representative.



Note: The frequency of the various activities described below is a good starting point. As the user gains experience it will be found that some activities can be done less frequently and others done more frequently. The frequency depend on a variety of factors including the nature of the sample and the mobile phase and the number of samples.

TABLE 3-3 Recommended Maintenance Schedule

Frequency	Operation	Reference
Every day	Check that there are no leaks of the fluidics connections and the Micropump	
	Check/refill the pump head's backflushing liquid	Section 2.10.2
Every week	Perform a check of the column flow rate	Section 3.6.7
Every 3 months	Inspect the condition of all tubing (cracks, nicks, cuts, clogging)	
	Inspect the connections in the fluidics compartment for leakage (and tighten as necessary)	
Every 6 months (or 2000 h of usage)	Replace piston seals	Section 4.5.5
	Replace inline filter	Section 3.5.3
	Replace Solvent Filter/Sparging Unit	Section 3.5.2
Every year	Check injection valve (option)	Appendix D
	Replace check valves	Section 4.5.6
	Perform the standard system test	Section 3.3.3

3.5 Disassembly and Replacement of User Replaceable Components

3.5.1 General Information and Hints

This section provides information and procedures how to disassemble the UltiMate system and how to replace units or assemblies (steps or procedures which are obvious from the appearance of the unit are not described). All necessary calibration and adjustment procedures can be found in Section 3.9.

In most cases, re-assembly of a component is identical to its disassembly, except that the steps are performed in the reverse order. If no comment is made, it should be assumed that assembly of a component or installation of a component is identical to disassembly or removal, except that the actions are in the reverse order.

Note: When disassembling and reassembling the UltiMate, make certain that each component is clean and take care to ensure that the system is assembled in a dust free environment.

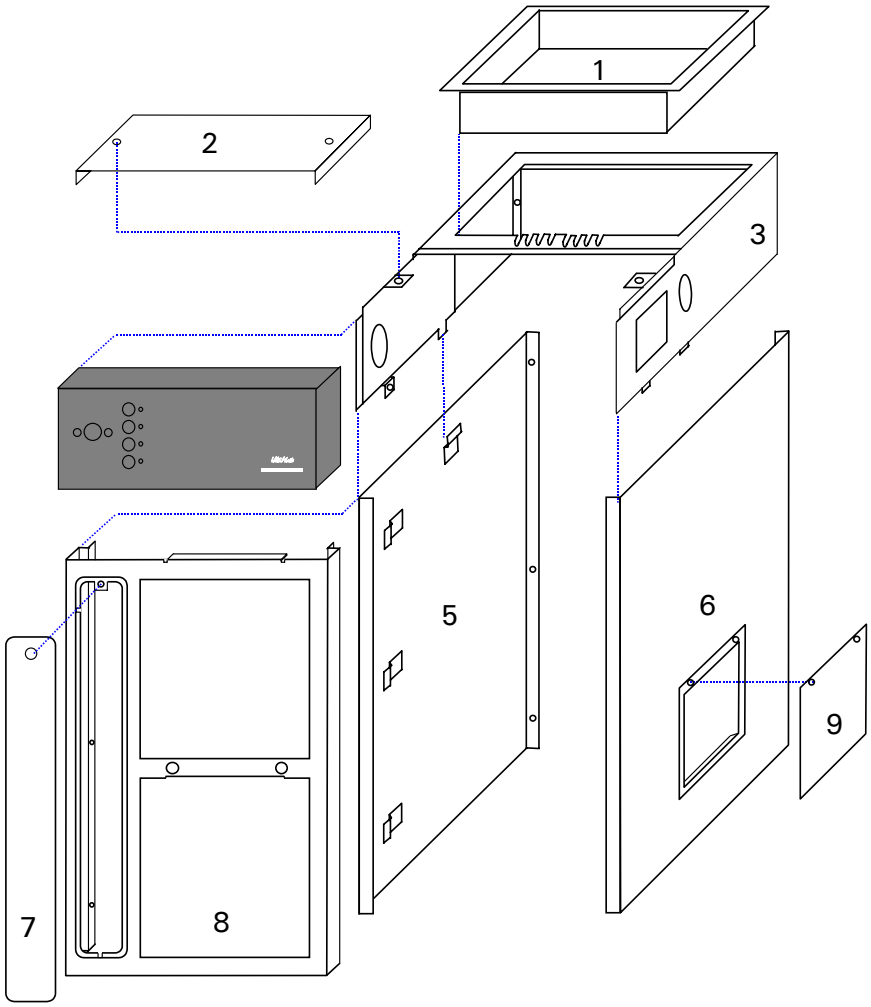
Note: If you have loosened a fitting, check to ensure that the connection is sufficiently tightened so that it does not leak.

When an electrical connection is removed, remove the cable(s) carefully and take care not to break connectors or cables.

DANGER **Danger: Hazardous voltages are present inside the instrument. Disconnect the instrument from the electrical supplies before removing the side panels.**

WARNING **Warning: Before disassembling the unit, make sure the system is flushed with methanol/water (50/50) at a flow rate of 100 µL/min to remove any hazardous solvents or samples. After the system has been flushed, make certain that the power has been removed from all components.**

A perspective drawing that presents the housing of the UltiMate is presented in FIGURE 3-12.



1 Solvent Bottle Tray
2 Fluidics Access Plate
3 Top Cover
4 Top Front Panel
5 Left Side
6 Right Side Panel
7 Cover Plate Column Compartment
8 Front Panel
9 Lamp Access Plate

FIGURE 3-12. Perspective Drawing–UltiMate Housing

3.5.2 Replacing the Sparging/Filter Frit

The Sparging/Filter Unit is connected via tubing which slides off the unit. Over time, the filter may become clogged by particulate matter and should be replaced. In some cases, the filter can be cleaned via an ultrasonic bath (depending on the nature of the particulate matter).

Note: A clogged solvent filter may lead to poor analytical performance (Section 3.7 - Troubleshooting).

To replace the Sparging/Filter Frit:

a) Unscrew the Solvent Bottle Caps from the solvent bottles and take the tubing with the frit out.

b) Remove the old frit by pulling the tubing (item 1, FIGURE 3-13) off the filter body (item 2, FIGURE 3-13).

c) Insert the He tubing and the solvent tubing into a new Sparging/Filter Frit (P/N 160044). The He tubing should be placed in the hole closer to the He outlet.

d) Gently pull at each line to make sure that it is secure.

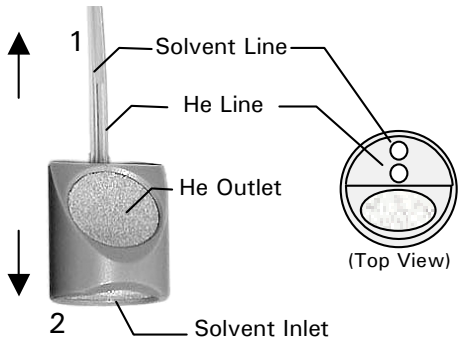


FIGURE 3-13. Sparging/Filter Frit

3.5.3 Replacing the High Pressure In-line Filter

The high pressure in-line filter serves to remove particulate matter from the mobile phase and is considered as a consumable item. If excessive pressure is required to deliver the mobile phase (indicated on the pump display), it is probable that the filter should be replaced.

Note: A clogged high pressure filter may lead to poor analytical performance and/or a maximum pressure shut-off of the UltiMate Micropump (Section 3.7 - Troubleshooting).

To replace the high pressure in-line filter:

a) Remove the capillary (item 1, FIGURE 3-14) between the purge valve and the filter unit.

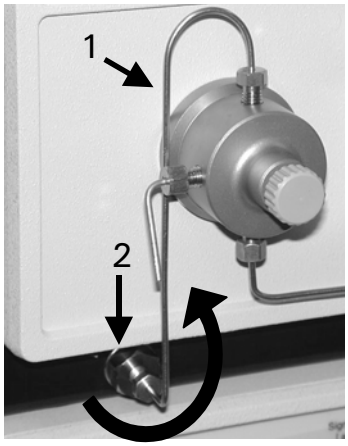
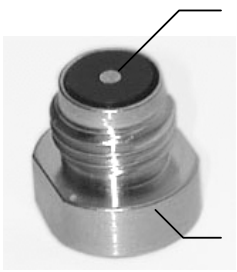


FIGURE 3-14. Tubing - High Pressure Inline Filter

b) Use the socket driver (P/N 160073) or a wrench (11 mm) to remove the filter holder (item 2, FIGURE 3-14)

c) Remove the filter and place a new filter into the holder (FIGURE 3-15). Refer to the table to select the proper replacement filter.



Description	P/N
Filter	
Standard (color: black)	160072
Inert (color: blue)	161107
Filter Frit Holder	
Standard (stainless steel)	163021
Inert (titanium)	162108

FIGURE 3-15. In-Line Filter in Filter Holder

d) Re-mount the filter holder.

CAUTION **Caution: Make certain that the filter remains in the filter holder when screwing the holder in and do not overtighten the filter holder. Overtightening could result in distortion of the filter which may lead to leakage and/or premature failure of the component.**

3.5.4 Accessing the Lamp in the Detector

From time to time, it may be necessary to access the Lamp in the detector (i.e. for replacement). The plate on the right side of the UltiMate housing must be removed to access the region of the lamp.

To access and replace the lamp:

a) Remove the two screws indicated in FIGURE 3-16 and remove the lamp access plate by pushing it towards the bottom of the instrument and pulling it out at the same time.

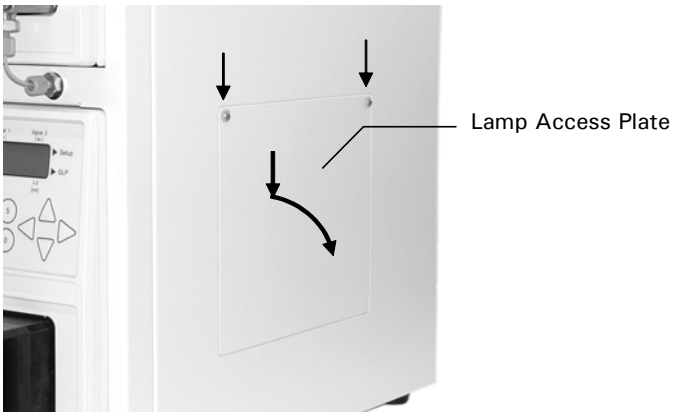


FIGURE 3-16. Removing the Lamp Access Plate

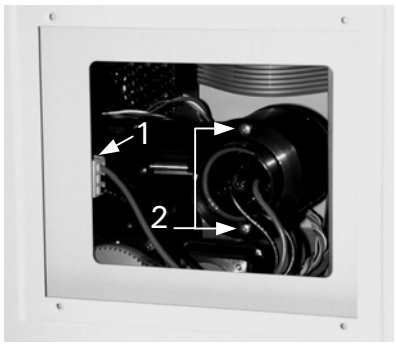


FIGURE 3-17. Accessing the D2 Lamp

b) Unplug the 3 pin connector of the lamp (item 1, FIGURE 3-17), remove the two screws (item 2, FIGURE 3-17) and pull the lamp out carefully.

c) When putting in a new lamp (P/N 160063) make sure that it is correctly seated.

d) Increment the lamp counter in the LAMP Screen (the working time counter will automatically be reset)

Section 5.5.3 presents a detailed discussion about the removal of the lamp.

WARNING **Warning: The lamp in the detector emits UV-radiation. Make certain that power is turned off to the detector before attempting to access the lamp.**

WARNING **Warning: Both the lamp and the optical bench become very hot during operation. Allow the components to cool down before accessing any part.**

3.5.5 Removing the Fluidics Access Plate

The fluidics access plate of the Solvent Organizer must be removed in order to get access to the fluidics compartment.

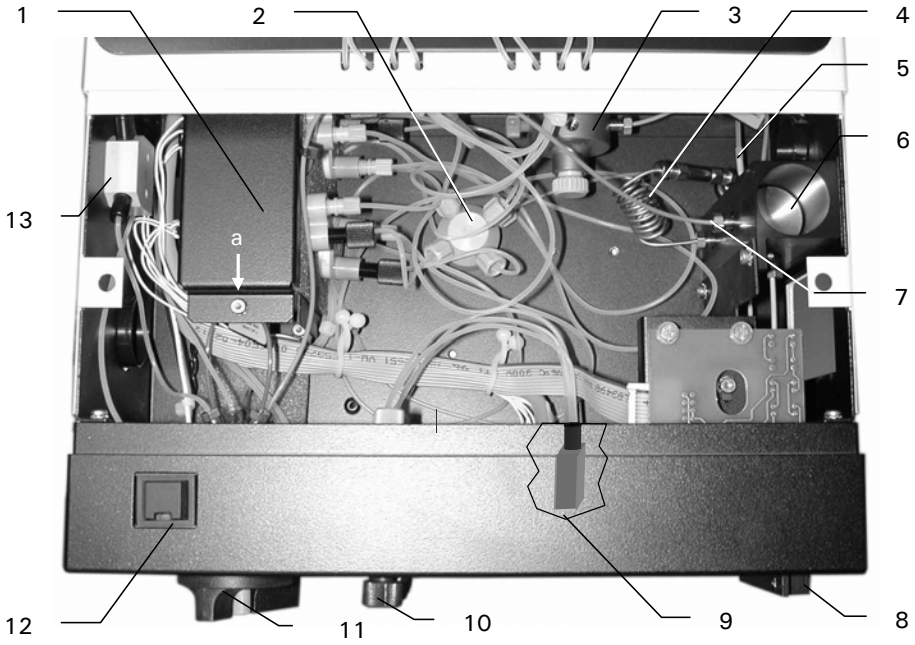
To Remove the Fluidics Access Plate:

a) Loosen the fluidics access plate by turning the two pop screws a quarter turn counter clockwise (FIGURE 3-18).



FIGURE 3-18. Removal of Fluidics Access Plate

b) Lift the fluidics access plate. The fluidics compartment is presented in FIGURE 3-19.



Item	Description	P/N Standard System	P/N Inert System
1	Proportioning Valves A, B Proportioning Valves C, D	160052 160051	160052 160051
2	5 Port Mixing Manifold	160089	160089
3	Column Pressure Sensor	-	-
4	Static Mixer	160684	161059
5	Waste Restrictor	160077	161043
6	T-Pieces, Flow Splitter, Upper Lower	 160694 160695	 162299 162298
7	Connection Capillary (to manual injection valve – if installed)	see Section 3.5.14	
8	Calibrator Cartridge	see Sections 3.5.14 and 3.8.2	
9	5 Port He Manifold	160087	160087
10	He Regulating Valves	160086	160086
11	Manual Injection Valve (option)	160068	161047
12	ON/Standby Switch	-	-
13	3 Port Waste Manifold	160088	160088

FIGURE 3-19. Fluidics Compartment

3.5.6 Removing the Side Panels

The side panels must be removed to gain access to the electronics or to remove the pump and/or detector.

DANGER

Danger: Disconnect the instrument from the electrical supply before removing the side panels.

To remove the side panels:

a) Remove the tubing to the pump inlet and outlet on the front panel.

b) Remove the six screws on the rear of each side panel (items 1-6; FIGURE 3-20).

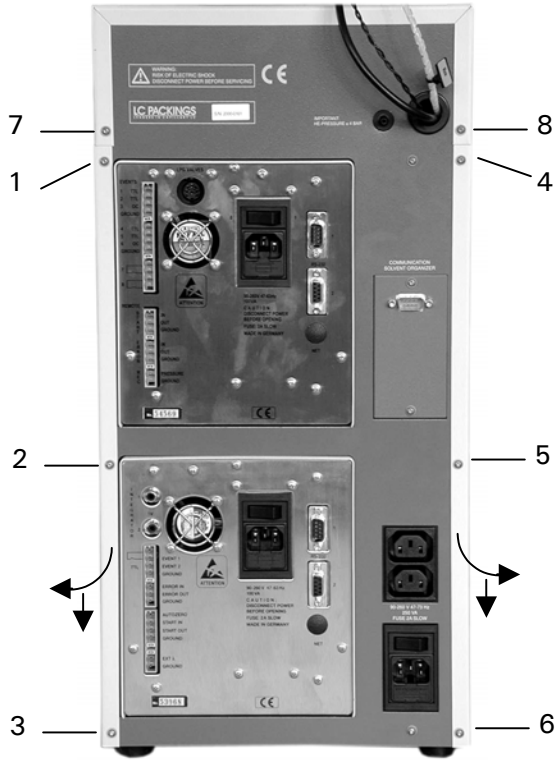


FIGURE 3-20. Rear View of UltiMate

c) Remove each the side panel by moving it towards the rear and towards the bottom of the instrument at the same time.

When left side panel is removed, the view is as shown in FIGURE 3-21 and when the right side panel is removed, the view is as shown in FIGURE 3-22.

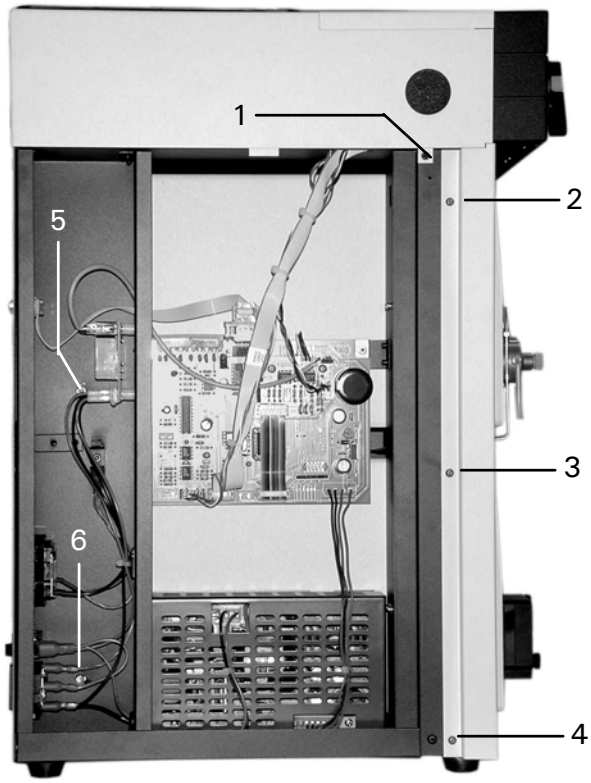


FIGURE 3-21. Left Side Panel Removed

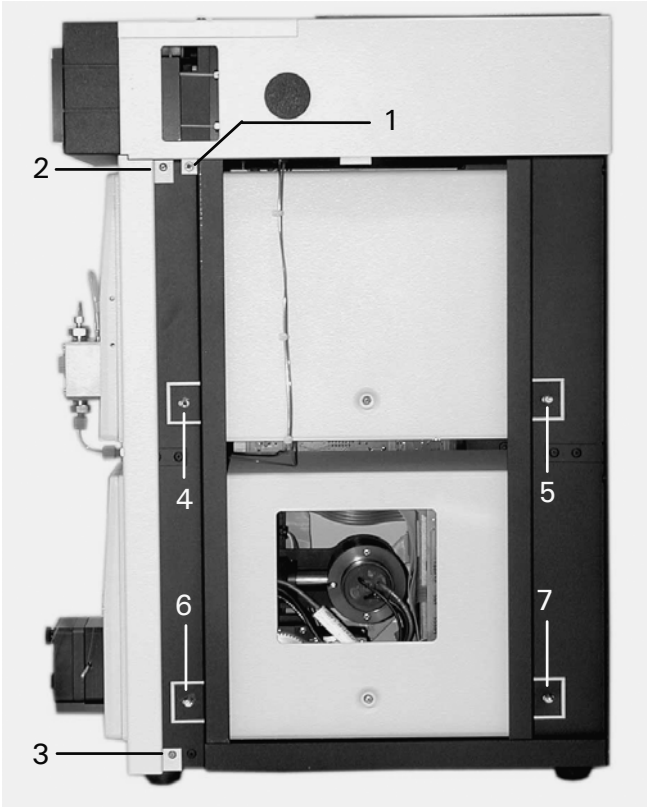


FIGURE 3-22. Right Side Panel Removed

3.5.7 Removing the Micropump and UV Detector

DANGER **Danger: Hazardous voltages are present inside the instrument. Disconnect the instrument from the electrical supply before removing the Micropump and the UV Detector.**

To remove the Micropump and UV Detector:

a) Disconnect all cables on the rear panel of Micropump and UV Detector.

b) Remove the capillary inlet and outlet connections from the Micropump.

c) Remove the flow cell from the UV Detector.

d) Remove the side panels of the UltiMate (Section 3.5.6).

e) Remove the screw on the left side and the two screws on the right of each device (items 5, 6; FIGURE 3-21 and items 1-4, FIGURE 3-22).

f) Remove the Micropump from the front of the UltiMate.

g) Remove the UV Detector from the front of the UltiMate.

CAUTION **Caution: When removing the Micropump or the UV-Detector from the UltiMate, carefully pull the devices out of the housing. To avoid any damage to the component, make certain that they are not allowed to drop as they are being removed from the housing.**

Note: When re-installing the Micropump and the UV-Detector, take care that the mobile phase tubing is not bent or overly flexed.

3.5.8 Removing the Top Cover

To remove the top cover:

a) Remove all solvent bottles and the solvent bottle tray.

b) Remove the fluidics access plate (Section 3.5.5) and the side panels (Section 3.5.6).

c) Remove the two screws of the top cover on the back side (items 7, 8; FIGURE 3-20), the screw on the right side (FIGURE 3-23) and the corresponding screw on the left side.

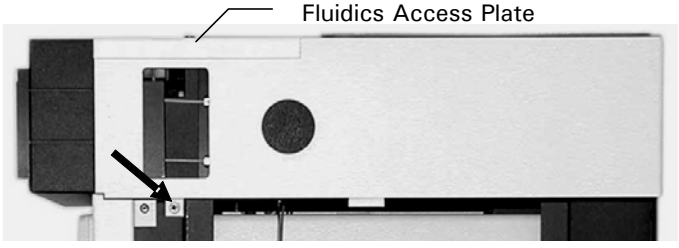


FIGURE 3-23. Removing the Top Cover (Side Panels removed)

d) Remove the Top Cover.

3.5.9 Removing the Top Front Panel

To remove the Top Front Panel:

a) Remove the top cover (Section 3.5.8).

b) Remove all fluidic components attached to the injection valve (if installed) and the He supply connection attached to the He manifold (items 11,9, FIGURE 3-19).

c) Disconnect all proportioning valves and the 5 pin connector from the Valve Connection PCB.

d) Disconnect the cable of the ON/Standby button from the interface PCB.

e) Remove the screws on the left and right side that attach the top front panel to the frame, and then remove the top front panel.

3.5.10 Replacing the Proportioning Valves and the Mixing Manifold

The Proportioning Valves (mounted in a piece of foam) and the Mixing Manifold are mounted in the Fluidics Compartment (FIGURE 3-19).

To remove the Manifold:

a) Remove the fluidic access plate of the UltiMate (Section 3.5.5).

b) Disconnect all fluidic and electrical connections from the 5-port mixing manifold and the proportioning valves (item 1 and 2, FIGURE 3-19).

c) Remove the screw of the (item a, FIGURE 3-19)

d) Remove the valve block out of the compartment. If a single valve is to be replaced, it is not necessary to remove the entire assembly; instead the valve can be carefully removed from the foam and the new valve inserted.

When re-installing the valves and/or manifold, take care to ensure that the solvent withdrawing lines are connected to the correct valve. The two ends of each valve are equivalent (i.e. the inlet and outlet of each valve is not specified). The electrical connections should be connected to the appropriate terminals.

3.5.11 Replacing the Manual Injection Valve

To remove the manual injection valve (option):

a) Remove the solvent bottles and solvent bottle tray.

b) Remove the fluidics access plate (Section 3.5.5).

c) Disconnect all fluidic connections from the injection valve (item 11, FIGURE 3-19) except for the injection loop.

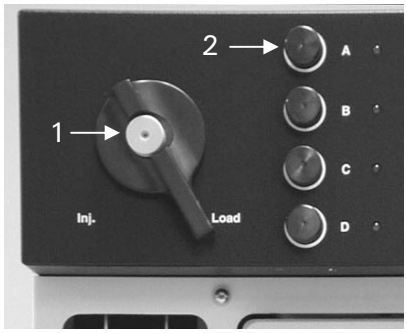


FIGURE 3-24. Manual Injection Valve

d) Disconnect the cable from the START IN connector on the UV Detector, remove the plug and remove the cable from the system. This cable crosses the fluidics compartment and the compartment into which the solvent tray is placed and exits the system in the rear.

e) Unscrew the injection port on the front and pull out the handle (item 1, FIGURE 3-24).

f) Remove the two Allen screws that attach the valve to the top front panel.

Note: For further information, refer to the Valco service instructions for the valve (see Appendix D).

When replacing the valve, ensure that the handle is re-installed in the correct position.

3.5.12 Replacing the T-Pieces of the Flow Splitter

To replace the T-Pieces of the Flow Splitter:

a) Remove the fluidics access plate (Section 3.5.5).

b) Remove the top cover (Section 3.5.8).

c) Remove the calibrator and all fluidic connections from the T-Piece assembly.

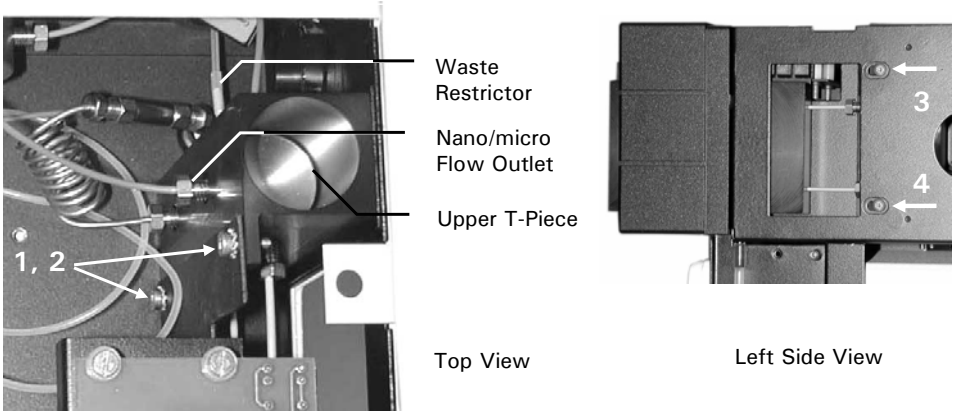


FIGURE 3-25 Removing the T-Piece Assembly

d) Remove the four screws for the T-piece assembly (items 1–4, FIGURE 3-25).

Remove the T-pieces from the T-Piece holder. Each T-piece is locked in the holder by one pin screw (items 1 and 2, FIGURE 3-26).

Note: The upper T-piece is different from the lower one.

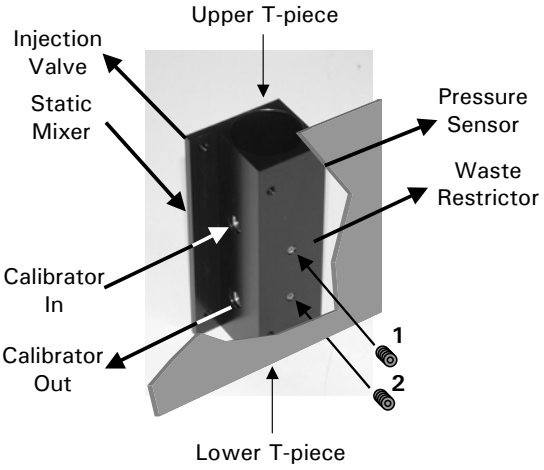


FIGURE 3-26. T-Piece Assembly

3.5.13 Replacing the Waste Restrictor

The waste restrictor determines the operating pressure of the Micropump and in combination with the calibrator cartridge and the column that is installed it determines the flow rate through the separation column.

a) Remove the solvent bottles and remove the solvent tray.

b) Loosen the waste restrictor connection from the lower T-piece (FIGURE 3-25) and from the union (FIGURE 3-27).

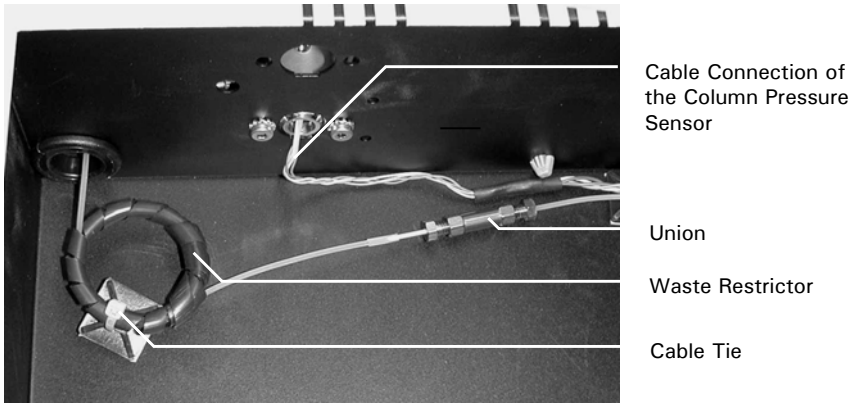


FIGURE 3-27. Waste Restrictor and Union (Solvent Tray removed)

c) Remove the cable tie (FIGURE 3-27) and remove the waste restrictor.

d) Replace the waste restrictor and the cable clip.

e) Replace the solvent tray.

3.5.14 Replacing the Connection Capillary between the Nano/Micro Flow outlet the Manual Injection Valve

The Connecting Capillary between the Calibrator (upper T-piece) and the Manual Injection Valve (option) is shown in FIGURE 3-27. TABLE 3-1 indicates the appropriate configuration for Micro-, Capillary- and Nano LC systems.

TABLE 3-4 System Configuration Information

Application	Column I.D. [µm]	Flow rate [µL/min]	Calibrator	Flow cell UZ-	P/N for Connecting capillary		Injection loop size
					Calibrator/ Inj. Valve	Inj. Valve/ Column	
Micro LC	1000	40	MIC-1000	M10 or M30	75 µm 160074 (161040)	75 µm 160076 (161042)	5 µL 160029 (161016)
	800	20	MIC-800				
Cap LC	300	4	CAP-300	C10 or C30	75 µm 160074 (161040)	75 µm 160076 (161042)	5 µL 160029 (161016)
	180	2	CAP-180				
Nano LC	75	0.2	NAN-75	N10 or N30	20 µm 160078 (161044)	20 µm 160079 (161045)	1 µL 160028 (161015)

3.5.15 Replacing the Main Fuse



Danger: Disconnect the instrument from the electrical supply before inspecting/changing the fuse.

To change the Fuse:

a) Pull out the fuse holder (item 1, FIGURE 3-28).

b) Replace the blown fuse by a fuse of identical rating. Close the fuse compartment.

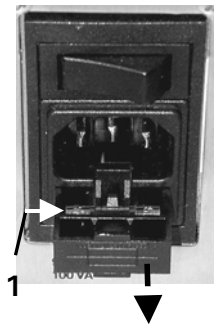


FIGURE 3-28 Fuse Compartment

3.6 Checking System Components

3.6.1 Overview

This section provides procedures and information for checking various units or assemblies of the UltiMate Solvent Organizer.

Note: Unless otherwise noted, pure methanol should be used as the solvent and these tests should be performed at 25°C.

Fill each bottle with methanol before performing the tests described below.

3.6.2 Solvent Bottles and Degassing Unit – Basic Test

The He lines, regulating valves and connections can be tested by the following procedure:

a) Close the Shut-Off Valves (FIGURE 3-29) and apply 4 bar (60 PSI) of He pressure.

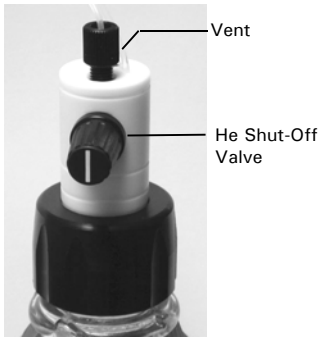


FIGURE 3-29. The He Shut-Off Valve (Opened Position)

b) Open the He Regulating Valve on the front panel for solvent bottle A. The regulating valve should be turned 90°. Check to see if there are any leaks (i.e. are He bubbles observed in the solvent bottle).

c) Open the shut-off valve on the bottle cap for solvent bottle A, close the He regulating valves and verify that the bubbles of He exit on the side frit of the sparging/filter unit. Check to make sure that it is possible to regulate the He flow by opening the He regulating valves until the maximum is reached (90°).

d) Repeat steps (b) and (c) for each solvent bottle.

3.6.3 Low Pressure Mixing System – Basic Test

To check the four channel mixing system:

a) Enter the PURGE screen of the Micropump and set the flow rate to 0.0 mL/min.

b) Select channel A. The LED corresponding to Valve A should be illuminated and you should hear a click indicating that the valve was opened.

c) Repeat step (b) for each valve.

3.6.4 Four Channel Low Pressure Mixing System – Fluid Path Test

To determine if the proportioning valves close and open properly and if the resistance of the flow path is within the specifications:

a) Fill each solvent bottle until the fluid level corresponds to the top of the UltiMate housing.

b) Open the He shut-off valves on the bottle cap assemblies.

c) The four channels should be well flushed using the Purge function of the Micropump. If is not possible to flush the lines, check for clogged or dirty solvent filters or bent solvent lines.

d) Disconnect the solvent inlet line from the pump head and connect a 500µl syringe (i.e. the plunger should be removed) using an adapter (P/N 160259) as shown in FIGURE 3-30.



FIGURE 3-30. Placing the Syringe on the Solvent Inlet Line

e) Enter the PURGE screen of the Micropump, set the flow rate to 0.00 mL/min and select channel A. The LED corresponding to Valve A should be illuminated.

f) Measure the flow rate from the inlet tubing for one minute. The flow rate should be greater than 0.15 mL/min for a proportioning valve that opens properly.

g) Repeat steps (e) and (f) for each valve.

Note: If the flow is less than 0.15 mL/min, repeat the test with the solvent filters removed. If the flow is within specifications after the filters have been removed, they are clogged, dirty or defective and must be replaced.

h) Switch off the pump. The flow should stop immediately.

Note: As an alternative to using a syringe, this test can be performed by allowing the solvent to drip from the solvent inlet line and counting the number of drops per minute. For a proportioning valve that opens properly, at least 15 drops per minute should be observed. If the flow rate is less than 15 drops/min, repeat the test with the solvent filters removed. If the flow is within specifications after the filters have been removed, they are clogged, dirty or defective and must be replaced.

3.6.5 Four Channel Low Pressure Mixing System – Performance Test

To check the performance of a Micro LC or Capillary LC gradient system:

a) Add methanol to bottles A and C, and a 0.5 % solution of acetone in to bottles B and D [approximately 250 mL of methanol (methanol with acetone)] should be placed in each bottle).

b) Connect the outlet of the column bulkhead directly to the flow cell (remove the column if one is present).

c) Degas the solvents.

d) The four channels should be well flushed using the Purge function of the Micropump.

e) Prepare the step gradient program indicated below. Check that the CRP is set to the default conditions (50).

TABLE 3-5 Step Gradient

Time (min)	Wavelength [nm]	Flow [µL/min]	% B
0:00	254	Cap: 4 Micro: 40	0
5:00	„	„	0
5:01	„	„	10
10:00	„	„	10
10:01	„	„	20
15:00	„	„	20
15:01	„	„	30
20:00	„	„	30
20:01	„	„	40
25:00 (*)	„	„	40

(*) Continue the step gradient until the concentration of B is 100 % and hold it for five minutes, then allow the gradient to return to 0% B.

f) Flush channel B well and monitor the baseline until it is stable. The signal should be close to the upper end of scale and the maximum signal noise should be less than 0.5 % full scale.



Note: If the maximum absorbance observed during this test is greater than 0.25 AU (i.e. the signal goes off scale), add methanol to bottle B (or D) so that the signal remains on scale when this test is run.

g) Flush channel A until the baseline is stable. The signal noise should be less than 0.5% full scale/min.

h) Initiate the gradient and collect the absorbance data. A typical trace is shown in FIGURE 3-31.

i) The maximum deviation from the theoretical composition is ± 2 % B (FIGURE 3-31 shows a typical step gradient trace).

Note: This test should be performed under standard capillary or micro conditions, without a column connected. If the flow rate indicated on the display of the Micropump (when any of the cursor keys are pressed) is not exactly 200 µL, check the CRP value.

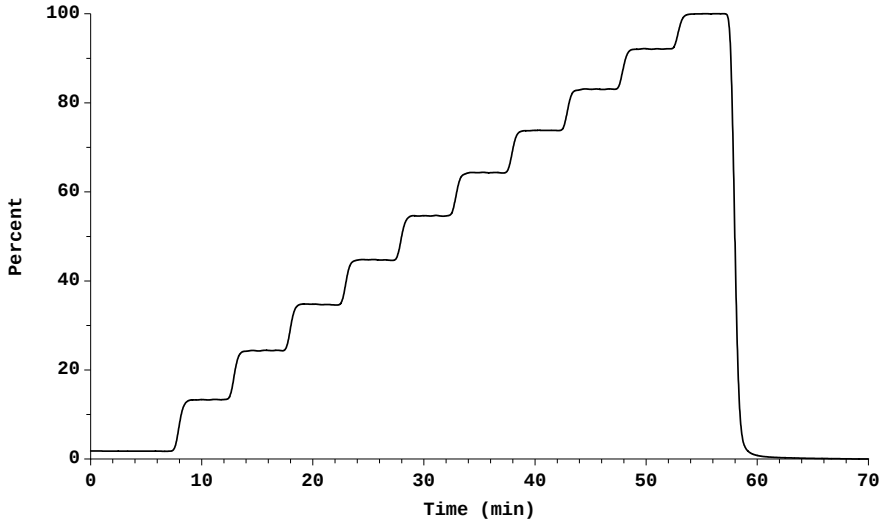


FIGURE 3-31. Test Report – Step Gradient

j) After you have completed this test for proportioning valves A and B, repeat for valves C and D.

The acceptance criteria for this test are:

a) Step length: 5 min (as programmed)

b) Response delay: 2 - 5 min (from performing the first step till the response of the detector)

c) Noise: less than 0.5 % of full scale (0.5 % B)

d) Step Change Time: less than or equal to 2 min/10% B step (1)

e) Signal at 100 % B: absolute signal less than or equal to 250 mAU

(1) The signal begins to change after the response delay (due to the dead volume of the system. Once the signal intensity changes, it should take less than 2 minutes for the slope of the signal to become zero again.

3.6.6 Integrated Flow Splitting Unit – Basic Pressure Test

Note: The operating pressure of the Micropump depends on the mobile phase used, the pump flow and the Calibrator that is installed.

To determine if the flow splitting unit functions within an appropriate pressure range:

a) Install a CAP-300 or NAN-75 calibrator cartridge.

b) Fill solvent channel A with methanol and purge the Micropump.

c) Set flow rate to 0.2 mL/min and start flow of the methanol. The backpressure must stabilize at 12 ± 2 MPa (120 ± 20 bar or 1750 PSI).

3.6.7 Integrated Flow Splitting Unit – Flow Rate Test

This test is provided to determine if the flow splitting unit is working properly by measuring the flow at the column bulkhead.

To determine the flow rate at the low flow outlet:

a) Disconnect the column.

b) Connect a 50 µl syringe to the connecting tubing using an appropriate union and an adapter (P/N 160259) as described in Section 3.6.4.

c) Set flow rate of the UltiMate Micropump to 0.2 mL/min and initiate delivery of the mobile phase.

d) Measure the flow rate via the calibrated syringe and a stopwatch. The split ratio is determined by the calibrator that is installed. The appropriate flow for the various calibrators is shown in TABLE 3-6.

TABLE 3-6 Calibrator/Flow Rate Data

Calibrator Type	Flow [µL/min]	
MIC-1000	70	± 5
MIC-800	65	± 5
CAP-300	7	± 2
CAP-180	2.5	± 0.5
NAN-75	0.9	± 0.1
Mon-200	16	± 4
Bypass Calibrator	200	± 10

3.7 Troubleshooting

The troubleshooting section will help to identify and diagnose operating problems and instrument failures.

Problem	Possible Cause	Solution
Back pressure on Micropump is too high.	• Contamination of the high pressure in-line filter • Clogged waste restrictor • Pressure sensor of the pump needs to be calibrated	- Check filter - Replace waste restrictor - Contact LC Packings
Back pressure on Micropump is too low/zero.	• Leaking/broken waste restrictor • Pump problems	- Check for leakage - Replace waste restrictor - Check pump (e.g perform droplet test – Sect. 3.6)
No flow through column - no column pressure	• Solvent Bottle(s) empty • Air in column pressure sensor • Leakage between Calibrator and column • Calibrator clogged	- Purge column pressure sensor - Check for leakage
No flow through column - high column pressure	• Column or connecting capillary clogged	- Replace calibrator - Replace capillaries or column
Flow through column too low - low column pressure	• CRP value too low • Calibrator partially clogged • Waste restrictor broken (pump pressure too low) • Leakage between Calibrator and column	- Check for leakage - Change CRP value - Replace Calibrator - Replace waste restrictor
Flow through column too low - column pressure high	• Column or connecting capillary partially clogged	- Replace capillaries or column - Replace column
Flow through column too high	• CRP value too high • waste restrictor partially clogged (pump pressure too high ?)	- Change CRP value - Replace waste restrictor
Reproducibility > 0.5 % RSD	• Column temperature not stable • Column pressure not stable	- Purge pump - Check degassing - Replace check valves - Use column oven - Check/replace valve
Sensitivity low	• Injected amount too low • Flow cell contaminated	- Check injection valve/autosampler - Clean flow cell
Peaks too wide/bad separation	• No peak focussing • Dead volume in system • Bad column	- Check sample solvent - Check connections - install new column

3.8 List of Spare Parts

Note: Please refer to Chapter 4 and Chapter 5 for the lists of spare parts for the UltiMate Micropump and UV Detector.

3.8.1 Solvent Bottle Cap Assembly and He-Degassing Unit

Part Number	Description
160027	Solvent Bottle 500 mL
160026	Solvent Bottle 250 mL
162140	1000 ml Bottle Cap ASSY with bottle (a)
160042	Bottle Cap Assembly
160043	Bottle Caps (6 pieces)
160086	He-Regulating Valve
160046	Replacement Needle for He-regulating Valve
160097	He Barbed Adapter
160087	5-Port He Manifold, including Finger Tight Fittings
160091	Union (T) for 6 mm O.D. gas tight tubing
160096	He -Tubing, 6mm O.D.
160092	Nut for 6 mm O.D. gas tight tubing
160093	Back/front ferrule for 6 mm gas tight tubing
Note:	**(a) For replacement of solvent bottle A and B, in combination with two 250 mL bottles (C and D) only.**

3.8.2 Integrated Flowsplitting Unit

Part Number	Description
	Common Parts
161406	Calibrator cartridge for Monolithic Capillary Columns, I.D. 200 μm
160057	Calibrator cartridge for Micro columns, 1.0 mm I.D. (40 µL/min)
160058	Calibrator cartridge for Micro columns, 800 µm I.D. (20 µL/min)
162001	Calibrator cartridge for Micro columns, 500 µm I.D. (10µL/min)
160059	Calibrator cartridge for Capillary columns, 300 µm I.D. (4.0 µL/min)
160060	Calibrator cartridge for Capillary columns, 180 µm I.D. (2.0 µL/min)
162052	Calibrator cartridge for Nano columns, 100 µm I.D. (300 nL/min)
160061	Calibrator cartridge for Nano columns, 75 µm I.D. (180 nL/min)
162051	Calibrator cartridge for Nano columns, 50 µm I.D. (80 nL/min)
160062	Bypass cartridge for flow rates from 0.2 - 1.0 ml/min
160522	Purge Screw
160527	O-Ring 6mm x 1.7 mm for purge screw
160088	3 Port Waste Manifold
163009	Housing for T-Pieces
	Standard Version (stainless steel)
160075	Connecting tubing, calibrator – pressure sensor
160694	T-Piece (upper)

160695	T-Piece (lower)
160684	Static Mixer, 300 µL a)
160077	Waste tubing (restrictor)
	Inert Version
161041	Connecting tubing, calibrator – pressure sensor, INERT
162299	T-Piece (upper), INERT
162298	T-Piece (lower), INERT
161059	Static Mixer, 300 µL, INERT a)
161043	Waste tubing (restrictor), INERT
Note:	a) the standard version has 9 windings , the inert version has 13 windings

3.8.3 Four Channel Low Pressure Mixing System

Part Number	Description
160052	Proportioning Valve, channel A, B (made of Kalrez®)
160051	Proportioning Valve, channel C, D (made of EPDM)
160089	5-Port mixing manifold, including finger tights

3.8.4 Replacement Filters

Part Number	Description
	Standard Version
160072	In-line replacement filter 0.5 µm
163021	Filter Frit Holder for UltiMate/Switchos, stainless steel
	Inert Version
161107	In-line replacement filter 0.5 µm – INERT
162108	Filter Frit Holder for UltiMate/Switchos, Titanium, INERT
	Common Parts
160044	Solvent Filter

3.8.5 Manual Injection Valve

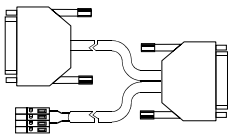
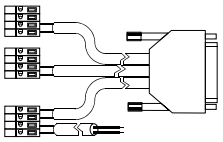
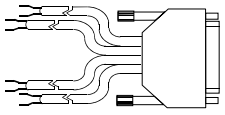
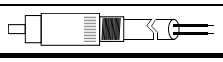
Part Number	Description
	Standard Version
160068	Low dispersion injection valve for UltiMate™
160066	Replacement rotor for low dispersion injection valve
160067	Replacement stator for low dispersion injection valve
160028	Injection Loop, 1 µl
160029	Injection Loop, 5 µl
160074	Connecting tubing, calibrator – injection valve, 75 µm I.D.
160078	Connecting tubing, calibrator – injection valve, 20 µm I.D.
160076	Connecting tubing, manual injection valve - column, 75 µm I.D.
160079	Connecting tubing, manual injection valve - column, 20 µm I.D.
	Inert Version
161047	Low dispersion injection valve for UltiMate™ INERT
161046	Replacement rotor for low dispersion injection valve, INERT
161004	Replacement stator for low dispersion injection valve
161015	Injection Loop, 1 µl, INERT
161016	Injection Loop, 5 µl, INERT

161040	Connecting tubing, calibrator – injection valve, 75 µm I.D., INERT
161044	Connecting tubing, calibrator – injection valve, 20 µm I.D., Nano, INERT
161042	Connecting tubing, manual injection valve - column, INERT
161045	Connecting tubing, manual injection valve - column, Nano, INERT
	Common Parts
160090	PEEK needle guide for manual injection valve
160088	3-Port waste manifold, including finger tights

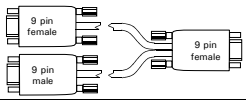
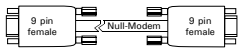
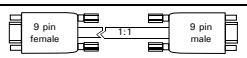
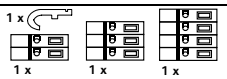
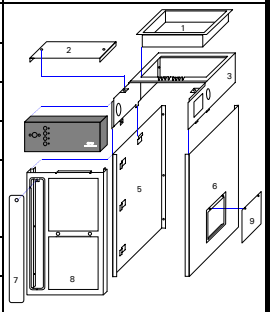
3.8.6 FAMOS Connecting Tubing

Column I.D. [µm]	Calibrator Type	Connecting Tubing I.D. [µm]	Capillary Upper T piece – FAMOS injection valve	Capillary FAMOS injection valve-column oven
Connecting Tubing for the UltiMate - Standard Version				
1000	MIC-1000	150	P/N 160031	P/N 160032
800	MIC-800	150		
300	CAP-300	50	P/N 160033	P/N 160034
180	CAP-180	50		
75	NAN-75	20	P/N 160035	P/N 160036
200	MON-200	50	P/N 160033	N/A
Connecting Tubing for the UltiMate – Inert Version				
1000	MIC-1000	150	P/N 161013	P/N 161014
800	MIC-800	150		
300	CAP-300	50	P/N 161013	P/N 161014
180	CAP-180	50		
75	NAN-75	20	P/N 161011	P/N 161012

3.8.7 Cables for Interfacing other Instruments

Part Number	Description	
160171	Cable for controlling Switchos by FAMOS P5 Auxiliaries	
160172	Cable for controlling Switchos by UltiMate Event Outputs	
160174	Contact Closure Cable	
160037	Integrator Cable	

3.8.8 Cables/Tubing/Housing

Part Number	Description	
160069	Y-Communication cable	
160070	Serial communication cable	
160071	Solvent organizer communication cable	
160081	Connector set	
160083	Silicon tubing, 2.0 mm I.D.	2 meter length
163016	Solvent bottle tray [1]	 (see FIGURE 3-12)
161037	Fluidics Access Plate [2]	
160686	Top Cover [3]	
162106	Top Front Panel (Man. Injector installed) [4]	
163018	Top Front Panel (no Man. Injector installed) [4]	
163015	Left side panel [5]	
163014	Right side panel [6]	
163012	Cover plate column compartment [7]	
163013	Lamp access plate [9]	

3.8.9 Special Tools

Part Number	Description
160073	Socket driver 11mm, for In-line filter replacement
160259	Syringe Adapter
162142	Tool for pre-assembling of INERT fittings

3.9 Specifications

3.9.1 Solvent Organizer

Reservoirs	Standard: 4 x 500 mL (safety) bottles with shut-off valve and 10 μm in-line filter (optional 2x 1000 ml, 2 x 250 mL).
Solvent Degassing	Built-in 4 channel He degasser.
GLP Features	Full traceability of solvents used (composition, purity, supplier, etc.) in the CHROMELEON® software.

3.9.2 Temperature Stabilized Column Compartment

Dimensions (WxDxH)	18 mm (0.7 in) x 12 mm (0.5 in) x 354 mm (13.9 in), for any microcolumn with a length up to 300 mm (12 in) temperature stabilized from ambient +5° C up to 70° C
Connection	Dead volume free union, preinstalled with manual injector (option).
GLP Features	Complete column specification and identification during system set-up (CHROMELEON® software).

3.9.3 Operating Software

CHROMELEON® Chromatography Management System	Full control of UltiMate instrument settings and run sequences, including data acquisition (maximum 4 channels), data evaluation and presentation. Recommended version: CM 6.50 SP2 or higher
Recommended Computer Configuration	IBM compatible 400 MHz Pentium III computer, 256 Mb RAM, 20 Gb hard drive, 17" Super VGA display (1024 x 768 resolution), CD-ROM drive, 8 free com ports.
Third Vendor software	Single-point control from the following MS platform software packages: Analyst®, (Applied Biosystems™/MDS Sciex™) HyStar™ (Bruker-Daltonics®), MassLynx™ (Waters® Corporation) Xcalibur® (ThermoFinnigan™)

3.9.4 Manual Injector (Option)

Valve	Manual, low dispersion 6 port valve with position feedback for automated injection mark (start data acquisition).
Injection Loop	Standard: 5 μL (optional: 1 μL).
Fill Port for Needle	2" x 22 gauge.

3.9.5 Flow Sensor (Option)

	Nanoflow Sensor	Capflow Sensor
Flow Rate Range (a)	20 – 1500 nL/min	0.2 – 7 µL/min
Flow Accuracy (a)	+/- 5 % of measured value	
Dwell Volume (b)	Dwell volume free	
Flow Path	Fused silica and PEEK	
Solvent Compatibility	All common HPLC solvents	
Software Control	CHROMELEON® 6.5/SP2 or higher	
Note:	a) Solvent: water b) Connecting tubing: NAN < 200 nL, CAP < 2 µL	

3.9.6 General

Power Requirements	90 - 260 V, 47-63 Hz, 250 VA
Fuse	Slow-Blow, 2 A
Dimensions (WxDxH)	250 mm (10 in) x 400 mm (16 in) x 530 mm (21 in).
Weight	24.5 kg (53.5 lb).
	18.5 kg (40.3 lb) (no UV Detector installed).
He Supply	1-2 bar (15-30 PSI), maximum 4 bar (60 PSI)
Production Quality	ISO 9001:2000 certified manufacturing. CE certified (LVD and EMC).
Operating Temperature	10-40 °C, 20-80% relative humidity.
Wetted Parts	**Standard Version (Stainless Steel)**
	PTFE, graphite fiber filled PTFE, PEEK, glass, sapphire, ceramic, fused silica, stainless steel Types 316 and V4A.
pH Range	2-10
	Inert Version
Wetted Parts	PTFE, graphite fiber filled PTFE, PEEK, glass, sapphire, fused silica, titanium.
pH-Range	2-10

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CHAPTER 4

The UltiMate™ MICROPUMP

4.1 System Overview

4.1.1 Description of the UltiMate Micropump

The UltiMate™ Micropump (FIGURE 4-1) is a modern, high pressure pump for high performance liquid chromatography. The standard configuration of the pump includes stainless steel pump head (with titanium inlays) with the capability of delivering from 0.001 to 0.500 mL/min. The Micropump delivers pressurized mobile phase with very low residual pulsation.

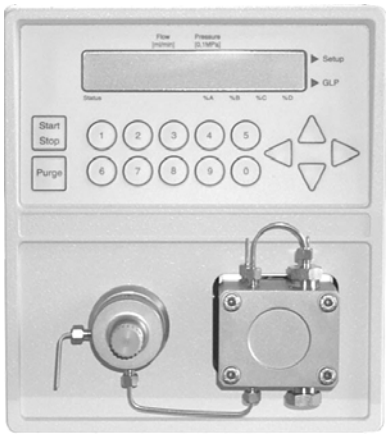


FIGURE 4-1. The UltiMate Micropump

The UltiMate Micropump is a component in the LC Packings UltiMate Capillary HPLC system. The pump delivers mobile phase to the UltiMate Solvent Organizer that provides the appropriate flow rate and mobile phase composition for Micro, Capillary and Nano HPLC. It generates a low pressure gradient by controlling the 4 gradient valves of the Solvent Organizer under control of the CHROMELEON® Chromatography Management software and delivers the pressurized mobile phase to the mixer (Section 3.2).

If desired, the UltiMate Micropump could be used as a stand-alone pump for HPLC. While this chapter primarily describes the use of the pump as a component in the UltiMate Capillary HPLC system, it also includes information which should be useful if the pump is used with other devices.

4.1.2 Features of the UltiMate Micropump

The UltiMate Micropump is designed to provide essentially pulse-free mobile phase delivery over a range from 0.001 to 0.500 mL/min. The overall mode of operation involves a "1½ piston" design, which uses a main piston and an auxiliary piston. To minimize pulsation, an electronic residual pulsation compensation program is included in the pump (in addition to the low pulsation 1½ piston design).

The Micropump includes a piston backflushing system to rinse the back of the plunger seal. This mechanism is provided to prevent seal failure due to the crystallization of buffer salts that are commonly used in HPLC.

A purge mechanism is provided to withdraw solvent from the bottles. This feature is used to remove air from the delivery lines and the pump head.

When the Micropump is installed in the UltiMate system, the pump is used to generate a low pressure gradient.

This chapter includes the following information:

- Installing the Micropump (Section 4.2).
- The User Interface (Section 4.3).
- Using the Micropump (Section 4.4).
- Maintenance (Section 4.5).
- Troubleshooting (Section 4.6).
- Error Messages (Section 4.7).
- Service Codes (Section 4.8).
- Spare Parts Lists (Section 4.9).
- Specifications (Section 4.10).

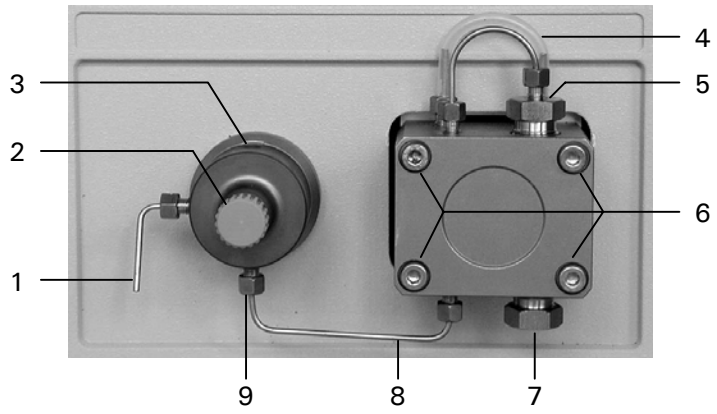
4.2 Installing the Micropump

4.2.1 Installation

When the Micropump is included as a component of an LC Packings UltiMate Capillary HPLC system, the Micropump is installed into the system as described in Chapter 2. The instructions provided below are provided for installation of the Micropump as a stand-alone component in an HPLC system.

4.2.2 Fluidics

The Pump Head and Purge Valve region of the Micropump is presented in FIGURE 4-2.



1 Purging Outlet
2 Purging Screw
3 Eluent Outlet
4 Backflushing System
5 Connection Tubing for Pump Head
6 Pump Head Screws
7 Eluent Inlet
8 Connecting Tubing for Pressure Sensor
9 Inlet to Pressure Sensor

FIGURE 4-2. The Pump Head/Purge Valve Region

To connect the fluidics (stand-alone instrument):

a) The Eluent Inlet (item 7, FIGURE 4-2) is connected to the solvent bottle using 1/16" O.D. 1 mm I.D. tubing (P/N 160045) and a 10 μm solvent filter (P/N 160044) should be used to ensure that particulate matter does not enter the micropump. The mobile phase should be degassed by sparging with He (recommended) or via vacuum.

b) The Eluent Outlet should be connected to the next component in the HPLC system (in most instances this will be an in-line filter such as P/N 160001).

c) Install the Piston Backflushing system.

   i) Remove the silicon tubing connecting the two backflushing ports from one port and attach the end to a container (e.g. a small beaker) to collect the flushing liquid.

ii) Fill the 5 mL priming syringe (accessories kit) with the backflushing liquid (a solution of iso-propanol:water (1:1) is commonly used) and connect it to the open backflushing port using 1/16"silicon tubing (accessories kit).

iii) Force the liquid through the pump head until liquid appears in the container.

iv) To prevent vaporization of the flushing liquid, place the connecting tubing back onto the port (see item 4, FIGURE 4-2).

The fluid should be changed on a daily basis.

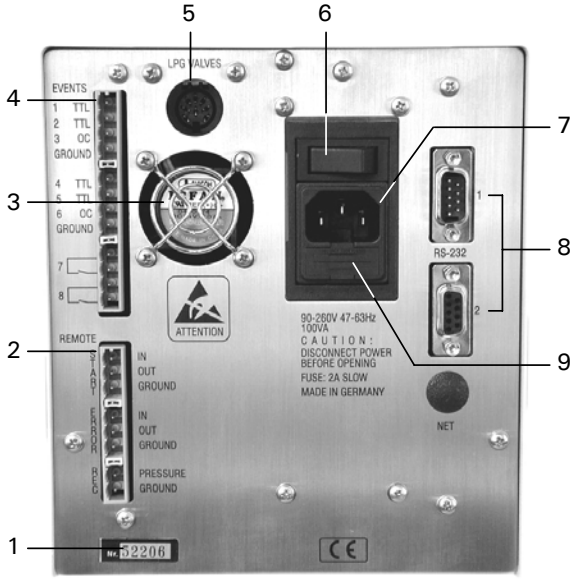
Note: If desired, a continuous backflush system can be used. In this approach, two containers of the backflushing liquid should be used; these should be attached to the two tubes connected to the ports on the pump and one container should be higher than the other to promote the flow of fluid by gravity.

If the Micropump is to be used to deliver buffers with high salt concentration, it is necessary to use a continuous piston backflushing system.

d) Prime the Pump (Section 4.4.2).

4.2.3 Electrical Connections

All electrical connections are made on the rear panel of the pump (FIGURE 4-3).



1 Serial Number
2 Terminal Sockets REMOTE
3 Cooling Fan
4 Terminal Sockets EVENTS
5 Plug for Solvent Organizer
6 Main Power Switch
7 Power Connector
8 RS-232 Interface
9 Fuse Compartment

FIGURE 4-3. Rear Panel of the Micropump

A. The EVENTS and REMOTE Terminal Sockets

The EVENTS terminal sockets and the REMOTE terminal sockets (which are located on the left side of the rear panel) are used to connect the pump to other devices.

Note: Communication using these sockets is performed in conjunction with CHROMELEON software.

CAUTION **Caution: Avoid touching the electrical contacts on the terminal strips. Electrostatic discharges could damage internal components of the pump.**

Connections to the terminal sockets should be made using the connectors provided with the accessory kit.

To make a connection:

a) Insert the rounded end of the lever latch into the square opening of the selected connector of the plug strip (item 1, FIGURE 4-4).

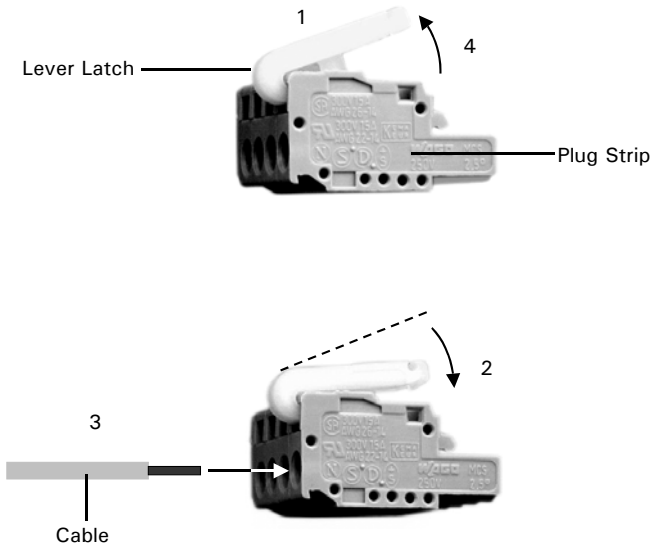


FIGURE 4-4. Connector for Terminal Strips

b) Press the lever latch down as indicated by the arrow so that it is flush with the top of the plug strip (item 2, FIGURE 4-4).

c) Insert the uninsulated end of the wire into the opening under the catch (item 3, FIGURE 4-4).

d) Release the catch and remove the lever latch from the plug (item 4, FIGURE 4-4).

The cable is now firmly anchored in the plug strip.

B. EVENTS Terminal Sockets

The different output configurations and the maximum current available are shown in FIGURE 4-5.

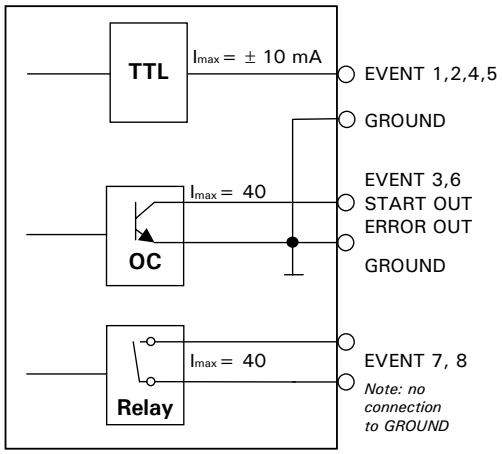


FIGURE 4-5. Output Circuits

Both relay outputs are potential-free contact closures, while OC and TTL outputs are related to GROUND. Connect the output to the input that is to be closed and connect the ground to the ground of the device to be closed.

C. REMOTE Terminal Sockets

To connect cables to a remote socket, connect one wire to the desired input and the other to ground. The remote sockets are used to start other components of the system, obtain a start message, to send/receive an error message or to monitor the system pressure on a recorder.

START Connections – a signal is produced or received at the start of a program

START IN is activated by a 0 Volt signal or short circuit

START OUT is an OC (open collector) output that is active for 500 ms.

ERROR Connections – a signal is transmitted or received when an error is occurring.

ERROR IN - when receiving a 0V (error) signal from an external device, the message "Error signal was detected" will be indicated on the display and the pump will stop.

ERROR OUT - an OC signal that remains active until the error is removed and any key is depressed.

Note: A minimum current of 7 mA is required to drive the inputs (take care about the voltage drop of the driving source, e.g. the saturation voltage of an open collector source).

REC (Recorder) Connection– transmits an analog signal corresponding to the pump pressure to a recorder (1 V corresponds to 40 MPa (5825 PSI); the offset is approximately 10 mV).

D. RS 232 Serial Interfaces

The two RS232 serial interfaces enable digital transfer between the pump and other devices. These devices communicate with each other to form an integrated network.

E. Solvent Organizer Cable

Carefully insert the black Solvent Organizer Cable in the LPG VALVES connector on the rear panel of the Micropump (item 5, FIGURE 4-3).

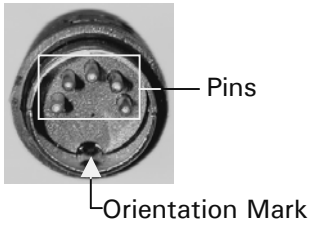


FIGURE 4-6. Connector Plug to Solvent Organizer Module

Note: Make certain that the pins are positioned properly and the orientation mark on the plug is at the bottom before inserting it into the Micropump (FIGURE 4-6).

F. Power Connector

The Micropump is fitted with a universal power supply for input voltages from 90 to 260 V. Manual setting of the supply voltage is not required. The power cord should be inserted in the socket directly below the Main Power switch.

CAUTION **Caution: Make certain that the system is properly grounded to a true earth ground. Connecting the instrument to a ungrounded power line can cause injuries and serious damage to the system.**

4.3 The User Interface

4.3.1 Overview

The User Interface is used to:

- Start (stop) the delivery of mobile phase
- Start (stop) the Purge operation
- Set system parameters
- Review GLP data

4.3.2 Powering up the Micropump

a) When the Micropump is powered up via the Main Power switch on the back panel, the pump will go through a initialization/self-test protocol. During this time, a number of messages indicating that various components are functioning properly. When the message TESTING MOTOR is presented, the pump drive wheel will be advanced and an audible noise should be heard. During the initialization, all four proportioning valves will be powered up (and the LED's lit) for a short period of time. At the end of the initialization process only one proportioning valve will be powered (and one LED lit).

b) During the initialization, the Kernel Version and the Firmware Version number will be presented on the display as shown in FIGURE 4-7 (the Kernel version will be presented if the firmware version is V5.00 or greater).

When the system has successfully passed all tests, the main screen will appear as shown in bottom of FIGURE 4-7 (a detailed discussion of the display is presented in Section 4.3.3).

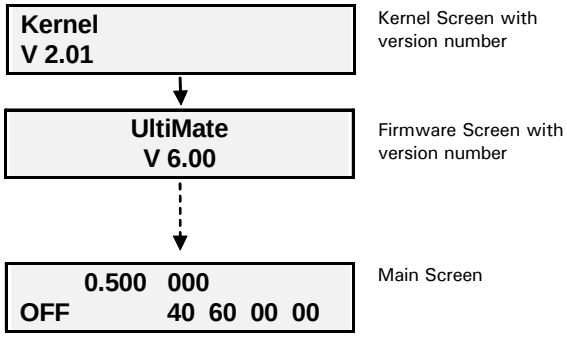
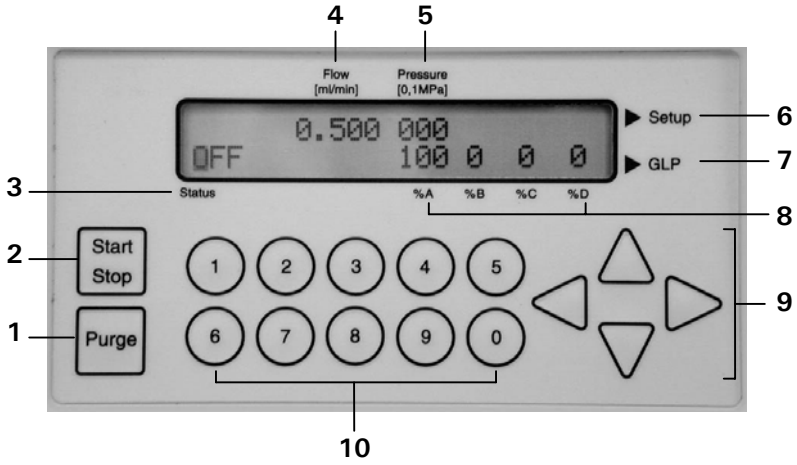


FIGURE 4-7. The Start-Up Sequence

Note: The Kernel version number and Firmware version number should be recorded, as they may be useful for troubleshooting purposes.

4.3.3 The Front Panel

All communication between the user and the system is provided by the front panel of the pump (FIGURE 4-8).



1 START/STOP Purge
2 START/STOP Pump
3 Status of Pump
4 Flow Rate
5 Pressure
6 Setup Menu Access
7 GLP Menu Access
8 % value for solvents
9 Arrow Keys for Cursor
10 Data Input Keys

FIGURE 4-8. The Front Panel

The upper front panel includes:

a) The **Display Screen**, which indicates a variety of system parameters. In addition, it provides access to the Setup menu (Section 4.3.5) which is used to set a variety of system parameters and the GLP menu (Section 4.3.6) which is used to monitor system activity, service and error codes.

b) The **Start/Stop** key, which initiates the delivery of mobile phase using the parameters indicated on the display screen.

CAUTION **Caution: Do not start the pump unless there is liquid in the pump head. Operating the pump without solvent will lead to damage of the pump head.**

c) The **Purge** key, which is used to start the purge mechanism (see Section 4.4).

d) The **Numerical** keypad**,** which is used to indicate the desired value for a given parameter.

e) The **Arrow** keys, which are provided to move the cursor to the desired character for editing.

4.3.4 The Main Screen

The Main Screen includes a series of parameters that are used to set the principal operating conditions for delivering the mobile phase such as the flow rate and mobile phase composition when the low pressure gradient mode is employed (Section 4.3.5). In automated mode these parameters are set via CHROMELEON.

To edit a parameter on the Main screen, use the ▲,▼,◀ and ▶ arrow keys to move the cursor to the appropriate field and press the desired number key. As an example, if you wanted to change the flow rate from 0.500 mL/min to 0.250 mL/min, move the cursor to the first character in the field, press 0, 2, 5, 0 and press any arrow key to confirm. The range for each of the main display parameters is indicated in TABLE 4-1.

TABLE 4-1. Main Screen Parameters

Parameter	Range
Flow Rate	0.001-0.500 mL/min
Pressure	0-40 MPa (5825 PSI) (1)
%A, %B, %C, %D	0-100 (sum will automatically be adjusted to 100 %)
System Status	ON/OFF

(1) The Maximum Pressure value can be set via the PRESSURE LIMITS screen accessed via the SETUP Screen (Section 4.3.5) or by this field. When this field is being edited, it is bracketed by a pair of vertical lines to indicate that this is a programmed value rather than the actual pressure. During operation of the pump, the actual pressure is indicated. However, if the cursor is moved to the Pressure field during operation, the Maximum Pressure value will be indicated and the brackets will be presented.

The SETUP Menu (Section 4.3.5), and the GLP Menu (Section 4.3.6) are accessed by moving the cursor to the right most position in either row and pressing the ▶ arrow key (with firmware 5.00 or greater, it is necessary to press the key for about a second).

4.3.5 The SETUP Menu

The SETUP Menu includes two screens and is provided to allow the user to set the pressure limits and the time/date. To access the SETUP Menu:

a) Move the cursor to the right most position of the upper line of the display.

b) Press the ▶ key (with firmware 5.00 or greater, it is necessary to press the key for about a second).

When you open the SETUP Menu, the display screen will present the **PRESSURE LIMITS** screen (Figure 4-8). The values indicated on this screen indicate the desired pressure range. The pump will automatically shut off if the pressure (in MPa) is above the maximum value. If the minimum pressure limit is reached, the pump will stop in 60 seconds unless the pressure rises above the minimum level. If the minimum pressure is set to 000, the minimum pressure check will not be performed.

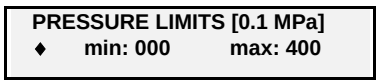


FIGURE 4-9. The PRESSURE LIMITS Screen

To set the minimum and maximum pressure for the pump:

a) Move the cursor to the appropriate field.

b) Enter the desired value via the keypad (e.g. if you want to change the maximum pressure to 125 enter 1, 2, 5).

c) Press any arrow key to confirm your entry.

Once you have accessed the PRESSURE LIMITS screen, you can access the DATE/TIME screen (FIGURE 4-10) by moving the cursor to the ♦ (diamond) in the lower left corner and pressing the ▲ or ▼ arrow. As an alternative, you can return to the main screen by moving the cursor to the ♦ (diamond) in the lower left corner and pressing the ◀ arrow.

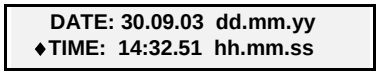


FIGURE 4-10. The DATE/TIME Screen

This screen is used to set the date and time for the system and is edited in the same way as the PRESSURE LIMITS screen.

Note: In this manual, the various display messages and menus shown correspond to a Micropump with firmware version V6.00 installed. If a different version of the firmware is used, there may be small differences in the screens and/or actions that occur when a given command is performed.

4.3.6 The GLP Menu

The GLP Menu describes a variety of reports on system usage and system status such as total operating information, service information and error reports.

To access the GLP Menu:

a) Move the cursor to the right most position of the bottom line of the display.

b) Press the ▶ key (with firmware 5.00 or greater, it is necessary to press the key for about a second).

The overview of the GLP Menu is presented in FIGURE 4-11. Selection of the screen to be accessed and returning to the Main screen is identical to that of the SETUP Menu (Section 4.3.5).

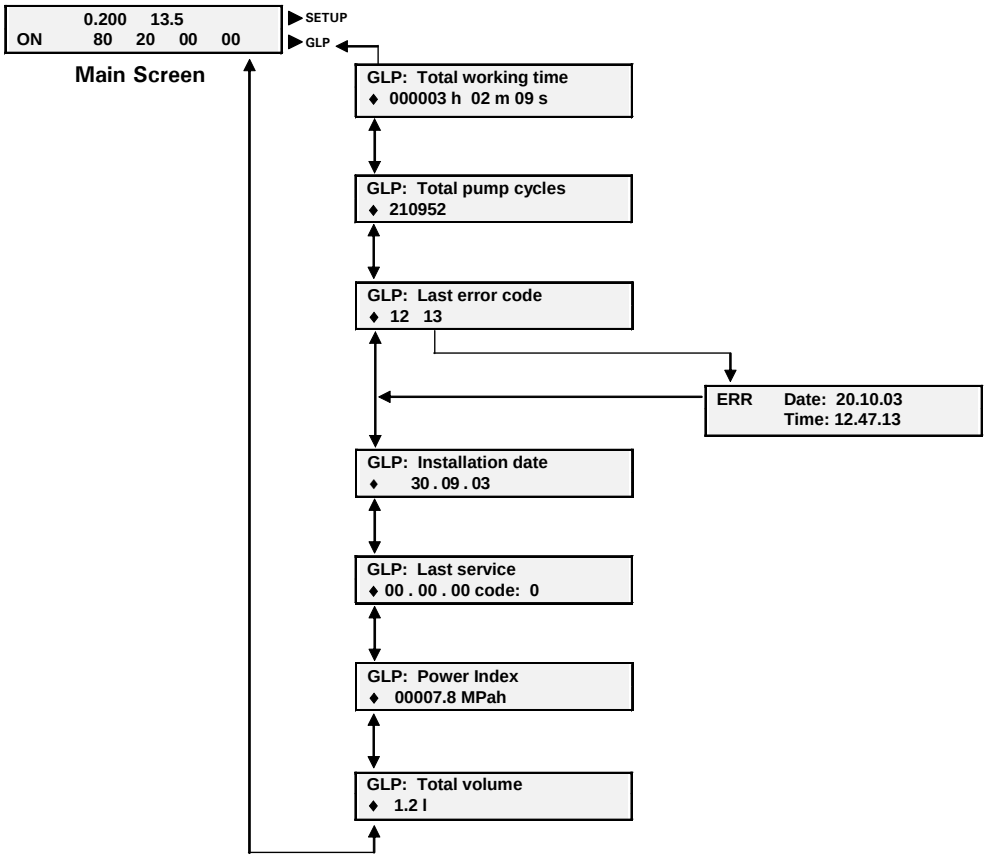
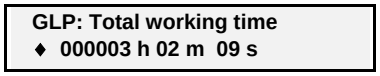


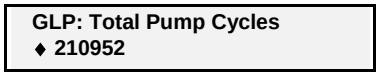
FIGURE 4-11. The GLP Menu Overview

To view the GLP Menu screens:

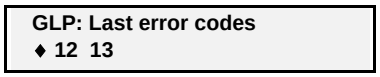
When you first access GLP Menu, the **GLP Total working time** screen is accessed. The selection of the screen to be accessed and method of returning to the Main screen is identical to that of the SETUP menu (Section 4.3.5). These screens are not editable by the operator.



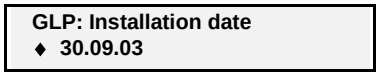
The **GLP: Total working time** screen indicates the overall time that the pump has been delivering mobile phase.



The **GLP: Total Pump Cycles** screen indicates the number of cycles that the pump has performed.



The **GLP: Last error codes** screen indicates the last 5 error codes reported by the system since the last. If you click on the ▲ or ▼ arrow, the time and date of the error is presented. Error codes are defined in Section 4.8.



The **GLP: Installation date** screen indicates when the system was installed.



The **GLP: Last service** screen indicates when the unit was last serviced and the three digit code describing the service (Section 4.8) and is set by the service engineer.



The **GLP: Power Index** screen provides the product of the pressure and the time that the pump has delivered solvent, and is a measure of the overall use of the pump.



The **GLP: Total Volume** screen indicates the volume of solvent delivered by the Micropump since it was installed.

4.4 Using the Micropump

4.4.1 Starting the Pump

When the Micropump is employed in the UltiMate, it is under the control of the CHROMELEON software. Certain preliminary activities such as priming and purging the pump are performed while the pump is operating in local mode. Before starting the pump, degas the mobile phases to be used.

4.4.2 Priming the Pump

To prime the pump:

a) Switch the pump on.

b) Cap the eluent outlet of the purge valve/pressure sensor.

c) Connect a syringe to the purging outlet (item 1, FIGURE 4-2), on the pump (use a 1/16" ID silicon tubing).

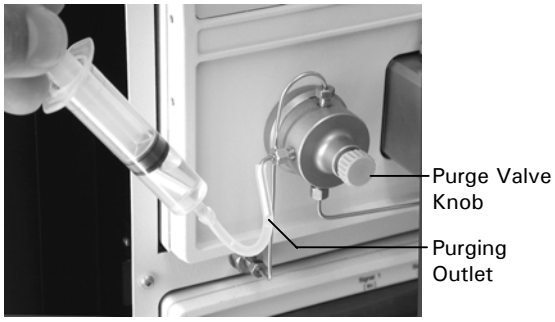


FIGURE 4-12. Purging the System

d) Open the purge valve on the pump by turning the purge valve knob (FIGURE 4-12) approximately 1 turn counterclockwise.

e) Press the PURGE key on the pump (item 1, FIGURE 4-8) to present the PURGE screen (FIGURE 4-13). Set the flow rate to 0.000 mL/min and select valve A by using the ◀ and ▶ arrow keys (items 9 and 10, FIGURE 4-8). Selecting the MIX field enables mixing of the solvents according to the composition setting in the main screen.

```
      2.000   125
PURGE:  MIX    A    B    C    D
```

FIGURE 4-13. The PURGE Screen

f) Withdraw solvent from bottle A using the syringe until no air is observed.

g) Repeat this process for solvent bottles B, C and D.

h) Press the PURGE key and close the purge valve.

4.4.3 Purging the Pump

To purge the pump:

a) Open the purge valve on the pump by turning the purge valve knob (FIGURE 4-12) approximately 1 turn counterclockwise.

b) Press the PURGE key on the pump (item 1, FIGURE 4-8) to present the PURGE screen (FIGURE 4-13). Set the flow rate to 2.000 mL/min and select valve A by using the ◀ and ▶ arrow keys (items 9 and 10, FIGURE 4-8).

c) Allow the system to operate for approximately 2 minutes, repeat step (b) for solvent bottles B, C and D. Ensure that no air bubbles are observed in the mobile phase.

d) Press the PURGE key and close the purge valve.

At this point, you can operate the system via the CHROMELEON software. If you are operating the pump on a local basis, simply edit the main screen parameters and press START.



Caution: Purging without opening the purge valve knob may cause damage to your column and/or system.

4.4.4 Hints for Successful Operation of the Micropump

The following points should be kept in mind when using the Micropump

- Always use a 10 μm solvent filter (e.g. P/N 160044) to ensure that particulate matter does not enter the pump.
- Protect the HPLC by using an in-line filter (e.g. P/N 160001 + P/N 160219).
- The piston backflushing system should be used at all times and the backflushing solution should be refreshed frequently.
- If buffers are delivered by the pump, let the pump deliver mobile phase at a low flow rate (e.g. 25 μL/min) when the system is not being used (e.g. overnight) to prevent any problems such as crystallization of buffer salts inside the pump. During this operation, it is acceptable to recycle the mobile phase (i.e. deliver the pumped mobile phase back into the solvent delivery bottle).
- If a low pressure gradient that includes a buffer is used, make certain that the buffer constituents are soluble in the composite mobile phase to be used for the separation.
- Install the unit in a location where ambient temperature locations are minimized. Avoid placing the unit in direct sunlight, near a heating duct or near an air conditioning duct.
- If PEEK tubing is installed, avoid the use of concentrated nitric acid or sulfuric acid and solvents such as $CHCl_3$, DMSO and THF.
- Always degas the solvents properly.

4.5 Maintenance

4.5.1 Overview

This section describes a series of activities that should be performed on a routine basis to ensure long term safe and trouble free operation of the system. TABLE 4-2 includes a typical maintenance schedule. The frequency of the maintenance activities in this table is somewhat dependent on the nature of the application (e.g. the solvents used, the quantity of the mobile phase delivered by the Micropump, the level of cleanliness of the facility). The user should monitor the system activity to determine the appropriate frequency of these items.

TABLE 4-2. Recommended Maintenance Schedule

Frequency	Operation	Reference
Every 2 months	Replacing Solvent Filters	Section 4.5.2
Every 2000 h (typical lifetime is 2500 h)	Replacing Piston Seals	Section 4.5.5
When pump head is disassembled	Cleaning the Piston Rod	Section 4.5.4
When pressure fluctuates significantly	Cleaning Check Valves	Section 4.5.6



Warning: Before starting to disassemble the Micropump, make sure the instrument is flushed properly using isopropanol/water (50/50) and a flow rate of 2 mL/min in PURGE mode for 10 minutes. After the pump has been purged, switch off the instrument and disconnect it from the electrical supply.

This section provides information and procedures about how to replace user replaceable parts. In most cases, re-assembly of a component is identical to its disassembly, except that the steps are performed in the reverse order. If no comment is made, it should be assumed that the assembly of a component or installation of a component is identical to disassembly or removal, except that the actions are in the reverse order.



Note: When disassembling or reassembling the Micropump, make sure that each component is clean and take care to ensure that the system is assembled in a clean environment.

4.5.2 Replacing the Solvent Filter

The Sparging/Filter unit is connected via tubing which slides off the unit. Over time, the filter may become clogged by particulate matter and should be replaced. In some cases, the filter can be cleaned via an ultrasonic bath (depending on the nature of the particulate matter).

4.5.3 Removing the Pump Head

To remove the Pump Head:

a) Remove the inlet and the connecting capillary (FIGURE 4-14).

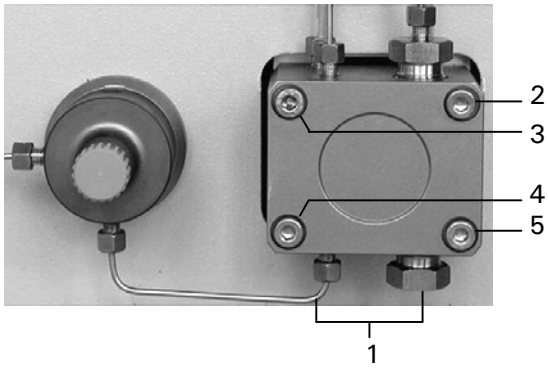


FIGURE 4-14. Disassembly of the Pump Head

b) Loosen two diagonally opposed screws (e.g. screws 2 and 4 in FIGURE 4-14 by a quarter turn at a time and remove the screws.

c) Carefully loosen the two remaining screws (e.g. screws 3 and 5 in FIGURE 4-14), alternating from one to the other, approximately half a turn at a time and remove the screws.

d) Remove the pump head.

Note: If the purpose of removing the pump head is to check the piston rods, there is no need to disassemble the pump head any further.

To replace the Pump Head:

a) Position the pump head onto the housing and carefully line up the screw holes.

b) Tighten all four set screws by hand.

c) Alternating from one to the next, tighten two diagonally opposed screws (e.g. screws 2 and 3) half a turn at a time until the pump head is correctly seated onto the housing.

d) Tighten the two other screws.

e) Check that the screws that were tightened in step c are well tightened.

4.5.4 Inspecting/Replacing Piston Rods

To remove the piston rods:

a) Remove the pump head as described in Section 4.6.2.

b) Pull out each piston rod (item 3, FIGURE 4-16) in a straight line using a pair of pliers. Pull the rods straight out rather than at an angle.

c) Check pistons for any damage or scratches. If the pistons are dirty (e.g. if buffer salts are deposited on them), the material can usually be removed by cleaning with toothpaste.

4.5.5 Disassembling the Pump Head/Replacing Seals

Two different pump heads are available on the UltiMate Micropump. Before starting to disassemble, identify the pump head version and refer to the appropriate section:

- The Standard Version (stainless steel) – Section 4.5.5.A.
- The Inert Version (titanium Inlays) – Section 4.5.5.B.

FIGURE 4-15 presents the two different versions. The inert pump head has four (4) bushings instead of two (2) bushings:

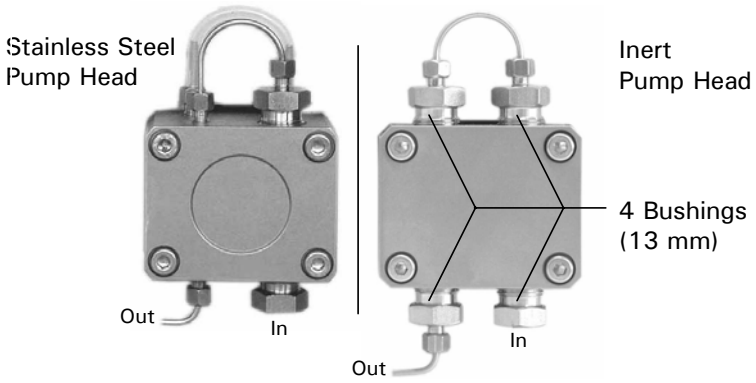


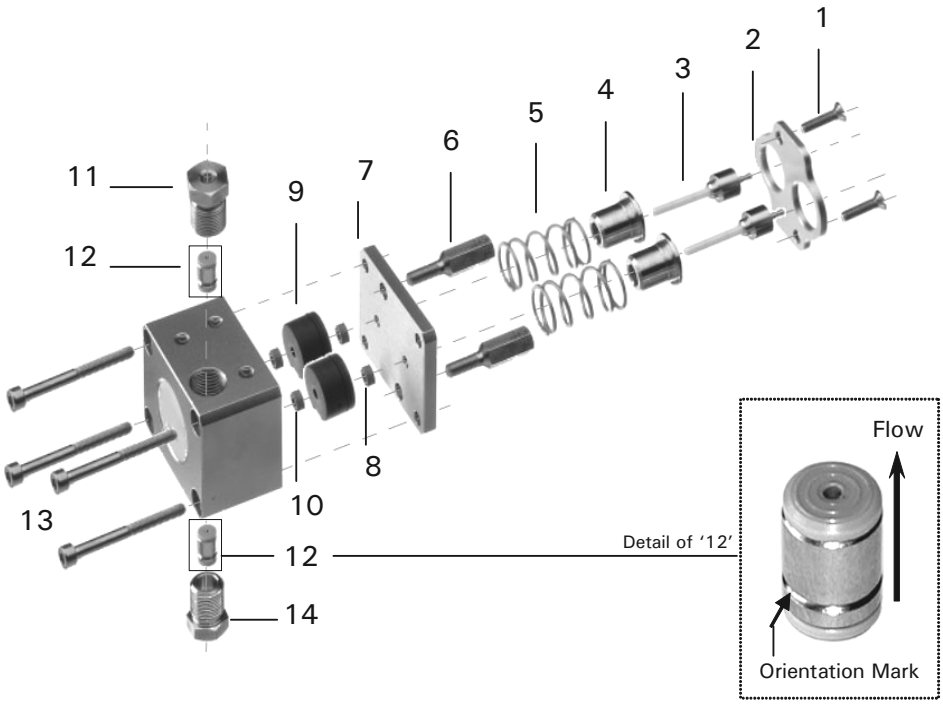
FIGURE 4-15 Identifying the Different Pump Head Versions

4.5.5.A The Stainless Steel Pump Head

To disassemble the stainless steel pump head:

a) Remove the pump head (see Section 4.5.3).

b) Remove the piston rods (see Section 4.5.4).



Item	Description	P/N
1	Recessed-head screw	-
2	Retaining plate	-
3	Piston rod	160047
4	Guide for spring	-
5	Springs	-
6	Spacing bolt	-
7	Pressure plate	-
8	Piston seal, backflushing	160048[1]
9	Seal holder	161090
10	Piston seal, high pressure	160048[1]
11	Check valve bushing, outlet	-
12	Check valve cartridge	160049
13	Set screw	-
14	Check valve bushing, inlet	-

Note: 1) Set of 4 piston seals (includes two high pressure seals and two backflushing seals)

FIGURE 4-16. The Stainless Steel Pump Head Assembly

c) Loosen the two screws (item 1, FIGURE 4-16) that attach the retaining plate (item 2, FIGURE 4-16) to the pump head. As you remove the screws, alternate from one to the other to avoid damaging the retaining plate.

Note: Because the screws that hold the retaining plate to the pump head are very tight, it may be helpful to either clamp the pump head or to press one of its side surfaces against a table with one hand while loosening the screws. Since the springs (item 5, FIGURE 4-16), exert a significant amount of force, care should be taken when the screws are loosened.

d) Loosen the spacing bolts (item 6, FIGURE 4-16). As you remove the screws, alternate from one to the other. These bolts are also seated very tightly, and it may be helpful to either clamp the pump head or to press one of its side surfaces against a table with one hand while loosening the screws.

e) Remove the spacing bolts and then remove the pressure plate (item 7, FIGURE 4-16).

f) Use an appropriate tool (e.g. a screwdriver) to remove the backflushing seal (item 8, FIGURE 4-16).

Note: The seals should only be replaced when the pump leaks. The useful lifetime depends on the mobile phase to be pumped and the back pressure of the system. The typical lifetime of the seals is in the order of 2500 h. It is recommended that both seals be replaced if one is defective.

g) Screw in a M3 screw into the seal holder and use a small screwdriver to remove the low pressure seal holder (item 1, FIGURE 4-17).

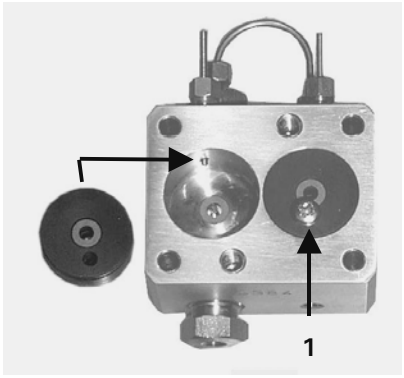


FIGURE 4-17. The Low Pressure Seal Holder

h) Use a small screwdriver to remove the high-pressure piston seal (item 10, FIGURE 4-16).

i) Place a new high pressure seal on the piston and use it to properly align the seal (FIGURE 4-17). The spring side should be placed as indicated in FIGURE 4-18.

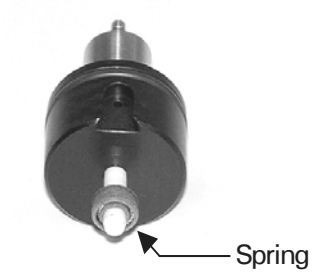


FIGURE 4-18. Aligning the High Pressure Seal

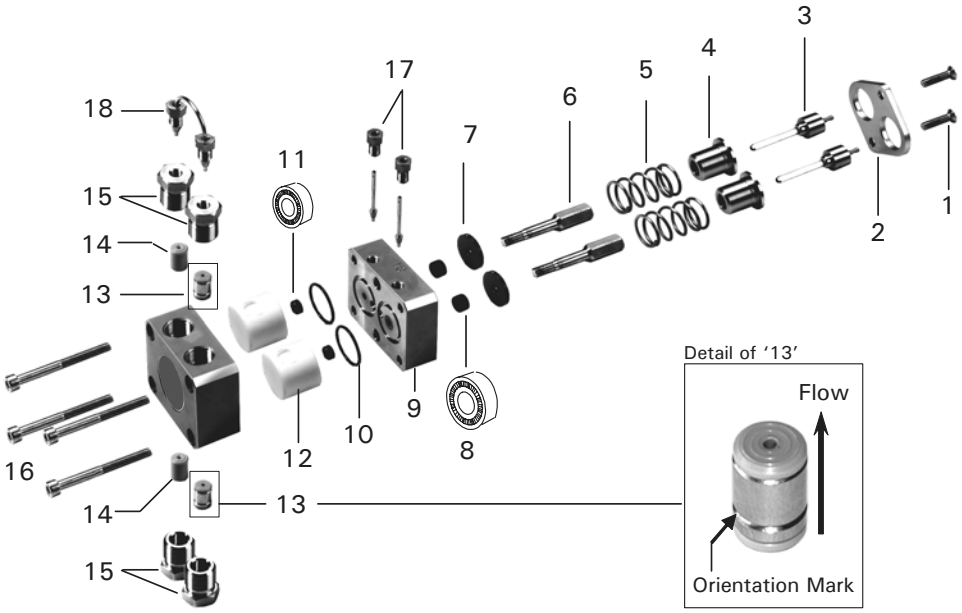
Note: When you are working on the pump head, take special care to avoid knocking or hitting the pistons.

j) Rebuild the pump head and make certain that all screws are securely tightened. As you insert the check valves, make certain that the orientation mark on each check valve is placed so that the mark is on the bottom.

4.5.5.B The Inert Pump Head (Titanium Inlays)

To disassemble the inert pump head (titanium inlays):

a) Remove the pump head (Section 4.5.3) and the piston rods (Section 4.5.4).



Item	Description	P/N
-	Inert Pump Head	161019
1	Recessed-Head Screw	-
2	Retaining Plate	-
3	Piston Rod	160047
4	Guide for Spring	-
5	Spring	-
6	Spacing Bolt	-
7	Support Washer	-
8	Piston Seal (backflushing)	161020 [1]
9	Pressure Plate	-
10	O-Ring	160084
11	Piston Seal (high pressure)	161020 [1]
12	Titanium Inlay	161400
13	Check Valve Cartridge	160049
14	PEEK Adapter	162300
15	Check Valve Bushing	-
16	Set Screw	-
17	Backflushing Connection	-
18	Connecting Tubing Pump Head	161023

Note: 1) Set of 4 piston seals (includes two high pressure seals and two backflushing seals)

FIGURE 4-19. The Inert Pump Head Assembly

b) Loosen the two screws that attach the retaining plate to the pump head (items 1, 2; FIGURE 4-19). As you remove the screws, alternate from one to the other as long as the springs are loaded to avoid damaging the retaining plate.

Note: Since the springs (item 5; FIGURE 4-19) exert a significant amount of force, care should be taken when the screws are loosened.

c) Remove the spring guides, the springs and the support washers (items 4, 5, 7; FIGURE 4-19).

d) You can remove/replace the piston seals (backflushing seals) now. Use an appropriate tool (e.g. a screwdriver) to remove the seals (item 8; FIGURE 4-19).

Note: The seals should only be replaced when the pump leaks. The useful lifetime depends on the mobile phase to be pumped and the back pressure of the system. The typical lifetime of the seals is in the order of 2500 h. It is recommended that both seals be replaced if one is defective.

e) Loosen the spacing bolts (item 6; FIGURE 4-19).

Note: Because the bolts that hold the pressure plate to the pump head are very tight, it may be helpful to either clamp the pump head or to press one of its side surfaces against a table with one hand while loosening the screws.

f) Remove the two spacing bolts and then remove the pressure plate (item 9; FIGURE 4-19).

g) You can remove/replace the piston seals (high pressure seals) now. Use an appropriate tool (e.g. a small screwdriver) to remove the seals (item 11; FIGURE 4-19).

Note: The seals should only be replaced when the pump leaks. The useful lifetime depends on the mobile phase to be pumped and the back pressure of the system. The typical lifetime of the seals is in the order of 2500 h. It is recommended that both seals be replaced if one is defective.

h) To remove the titanium inlays, remove the four bushings, the check valves and the PEEK adapters (items 13, 14, 15; FIGURE 4-19).

Note: The two titanium inlays (item 12; FIGURE 4-19) should not be removed unless any leakage is observed.

i) Carefully insert new seals. The spring side should be placed as indicated in FIGURE 4-19.

j) Rebuild the pump head and make certain that all screws are securely tightened. As you insert the check valves, make certain that they are inserted in the right position (item 13; FIGURE 4-19) and that the orientation mark on each check valve is placed so that the mark is on the bottom. Insert the two PEEK adapters (item 14; FIGURE 4-19). Tighten the check valve bushings by hand.

k) Alternating from one to the next, tighten the upper and the lower bushing half a turn at a time.

l) Tighten all 4 bushings.

CAUTION

Caution: Make certain that the orientation mark on each check valve is placed so that the mark is on the bottom. Installing the check valves in the wrong direction may lead to a damage of the pump head.

4.5.6 Check Valve Cartridge

4.5.6.A Replacing the Check Valve Cartridge – Stainless Steel Pump Head

To remove/clean the check valve cartridge(s):

a) Unscrew the two check valve bushings (items 11 and 14, FIGURE 4-16) and remove the check valve cartridges.

b) As you replace the check valves, make certain that the orientation mark on each check valve is placed so that the mark is on the bottom. Tighten the two check valve bushings by hand.

c) Tighten the two check valve bushings.

4.5.6.B Replacing the Check Valve Cartridge – Inert Pump Head

The inert pump head is equipped with two check valves (item 13; FIGURE 4-19), one on the inlet side of the main piston (lower right) and on the outlet of the main piston (upper right) while two PEEK adapters are used on the auxiliary piston (lower and upper left side).

To remove/clean the check valves cartridge(s):

a) Unscrew the two check valve bushings (items 15, FIGURE 4-19) and remove the check valve cartridges.

b) As you replace the check valves, make certain that they are inserted in the right position (item 13; FIGURE 4-19). Tighten the two check valve bushings by hand.

c) Alternating from one to the next, tighten the upper and the lower bushing half a turn at a time.

d) Tighten the two check valve bushings.



Caution: Make certain that the orientation mark on each check valve is placed so that the mark is on the bottom. Installing the check valves in the wrong direction may lead to a damage of the pump head.

4.5.6.C Cleaning the Check Valve Cartridge

a) Remove the check valve cartridge as described in Section 4.5.5.A (Standard Pump Head) or Section 4.5.5.B (Inert Pump Head), respectively.

b) To clean the whole check valve cartridge, place it in an ultrasonic bath with a suitable cleaning solution for about 5 minutes. Methanol works well in most cases.



Note: The components of the check valve should be cleaned only if the valve has stopped functioning. It is not necessary to do this on a routine basis.

c) To clean the individual components of the check valve cartridge, use a knife and carefully remove the valve seals from the housing (FIGURE 4-20).

d) Remove the individual components and clean them (e.g. ultrasonic bath).

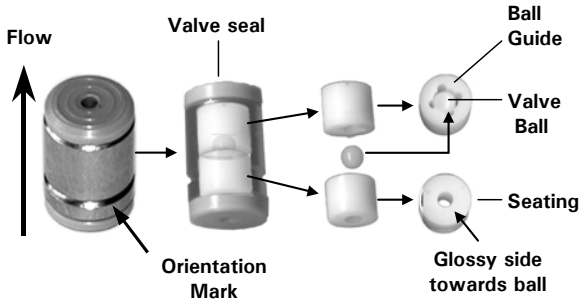


FIGURE 4-20. Check Valve Assembly

CAUTION **Caution: Be sure to identify the glossy side of the seating and assemble the check valve (see FIGURE 4-20). Incorrect assembly can lead to damage and leakage of the check valve.**

e) Replace the check valve cartridge as described in Section 4.5.6.A (Standard Pump Head) or Section 4.5.6.B (Inert Pump Head), respectively.

Note: Make certain to insert the check valves and the PEEK spacers in their proper positions.

4.5.7 Replacing the Main Fuse

WARNING **Warning: Disconnect the instrument from the electrical supply before inspecting/changing the fuse.**

To change the Fuse:

a) Pull out the fuse holder (item 1, FIGURE 4-21).

b) Replace the blown fuse by a fuse of identical rating (2 A, Slo-Blo fuse).

c) Close the fuse compartment.

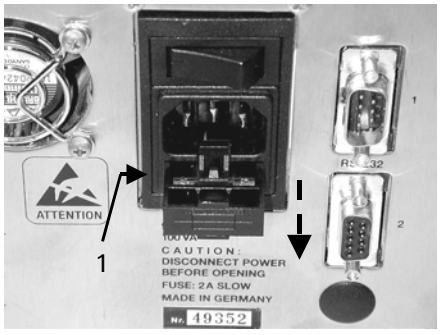


FIGURE 4-21. The Fuse Compartment

4.6 Troubleshooting

Problem	Probable Cause	Solution
Flow delivery stops after 1 minute during purging, error message **"minimum pressure ..."** is displayed	System pressure dropped below the minimum pressure (Pmin) set in SETUP menu PRESSURE LIMITS	Set "min" value to 0 MPa to disable checking Note: For firmware versions lower than V5.xx, check for minimum pressure condition during purging.
Flow delivery stops during purging, error message **„maximum pressure ...**" is displayed	System pressure exceeded the maximum pressure (Pmax) set in SETUP menu PRESSURE LIMITS **or** in monitor screen	Open the purge valve knob
	In firmware versions V5.xx, the pressure during purging is limited to 0.5 MPa	
No solvent delivery, pump motor is running	Air in solvent/pump head	Purge pump and check degassing
	Clogged solvent inlet filter	Replace solvent inlet filter
	Leakage in the pump head	Check for any leakage
Leaking pump head	Loose inlet/ outlet fitting	Tighten the fittings
	Loose check valve(s)	Tighten the check valves
	Defective piston seals	Replace seals
Flow fluctuation	Flow restriction in the solvent inlet fluid path	Check tubings, filters, proportioning valves, etc.
	Air in solvent inlet fluid path	Purge pump, check degassing
	Defective valves	Clean/replace check valves
Flow rate too high or too low (max. ± 10%)	Air in solvent inlet fluid path	Purge pump, check degassing
	Pump out of calibration	Contact LC Packings
Excessive pressure fluctuation	Sticking or dirty check valve(s)	Clean or replace check valves
	Air in solvent inlet fluid path	Purge pump, check degassing
	Defective piston seals	Replace piston seals
Piston seals have a short life span	Dirty solvent	Filter the solvent properly
	Scratches on pump pistons	Replace pistons and seals
Pump does not start, no power-up message, LCD backlight is not lit	Blown fuse(s)	Check fuses
	Faulty power supply or DC/DC converter	Contact LC Packings

4.7 Error Messages

The error messages in TABLE 4-3 may be displayed on the display:

TABLE 4-3. Error Messages

No.	Error Message	Note	Probable Cause	Solution
4	**No programs to run**	G,E	NOTE: reserved for future use	
5	**Motor failure**	G, E	Faulty cable connection	Contact LC Packings
			Faulty motor	Contact LC Packings
9	**ERROR signal was detected**	G, E	ERROR IN input is activated	Check device driving the input
10	**Max current error ! Switch instrument off**	G, E	Pump outlet is blocked	Check capillary connections
			Check valve wrong assembled	Check check valves
11	**Max temperature error**	G, E	Overheating of motor driver stage	Power the system down and allow to cool. Power up again, if message reappears, contact LC Packings.
			Faulty cable connection	Power system down and allow to cool. Power up again, if message reappears, contact LC Packings.
12	**Max. Pressure exceeded! **.* MPa. System halted**	G, E	Refer to Troubleshooting List NOTE: The actual pressure at the time when the error occurred will be indicated	Refer to Troubleshooting List
13	**Min. Pressure error! **.* MPa. System halted**	G, E		
14	**Net error**	G, E	Network is not established or is broken.	Power system down and power up again, if message reappears, contact LC Packings. Check connections, cables.
21	**Motor is blocked ! Switch instrument off**	G, E	Pump outlet is blocked	Check capillary connections
			Check valve assembled incorrectly	Check the check valves

No.	Error Message	Note	Probable Cause	Solution
27	**Calibration curve was destroyed**	G,E	RAM/RTC Battery discharged	Contact LC Packings
			Battery backed-up RAM/RTC corrupted	Contact LC Packings
NOTE: Errors 27 and 28 will be displayed during the power up sequence until it has been reset by LC Packings personnel.				
28	**Calibration values were destroyed**	G,E	RAM/RTC Battery discharged	Contact LC Packings
			Battery backed-up RAM/RTC corrupted	Contact LC Packings
35	**Current pressure exceeds * MPa**	E	Pump operates in a pressurized system during offset correction	Open purge screw
			Offset value of pressure sensor too high	Contact LC Packings
36	**Instrument operates in SLAVE mode**	E	An attempt to enter data was made during network operation.	Operate instrument in standalone mode.

Note: G: stored as GLP information, E: Error screen presented

4.8 Service Codes

The codes in TABLE 4-4 are entered by the service engineer when service is performed and appear in the screen on the GLP menu (other numbers from 1-199 can be used for reference if other service is performed and you should contact your service engineer for assistance).

TABLE 4-4. Service Codes

Code	Work Carried Out
100	Replacement/maintenance of pump head
101	Replacement of piston seals, high pressure
102	Replacement of piston seals, low pressure
103	Replacement of piston seals, high and low pressure
104	Replacement of pistons (including new seals)
105	Replacement/maintenance of check valve(s)
110	Replacement/maintenance of pump drive assembly
111	Replacement of motor assembly
112	Replacement of camshaft assembly
120	Replacement / adjustment of the pressure transducer
130	Replacement of any electronic assembly (e.g. CPU PCB, Main PCB, etc.)
140	Software update
99	non listed service
0	default, no code entered

4.9 Spare Parts Lists

4.9.1 Major Items

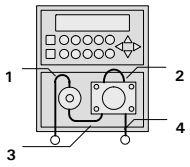
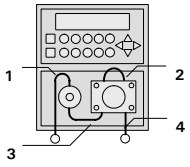
Part Number	Description
160007	UltiMate™ Micro/Loading pump

4.9.2 Filters

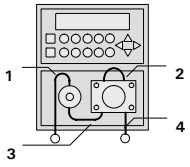
Part Number	Description
160044	Solvent filter including 10 µm frit
161001	Frit Holder for UltiMate™ (complete high-pressure filter assembly)
161102	Frit Holder for UltiMate™ INERT (complete high-pressure filter assembly)
160219	0.5 µm replacement in-line frit
161107	0.5 µm replacement in-line frit, INERT
	Note: The P/N's listed above apply for standalone instruments. Refer to Section 3.10 for filters used in the UltiMate system

4.9.3 Pump Head and Tubing

4.9.3.A Inert Pump Head (Titanium Inlays)

Part Number	Description	
161399	Inert Pump Head (Titanium Inlays)	
160049	Check Valve Cartridge (1 piece)	
160050	Check Valve Cartridges (set of 5)	
161020	Piston Seals [Graphite Fibre Filled PTFE], 1/4" O.D. and 0.315" O.D. (set of 4)	
160047	Sapphire Piston	
161400	Titanium Inlay for the Inert Pump Head	
162300	PEEK Adapter for inert Pump Head	
160084	O-Ring, 17 mm x 1.5 mm	
161026	Torque Wrench	
	Connecting Tubing for the UltiMate - Inert Version	
161024	Connecting Tubing Pressure Sensor to High Pressure Filter, Titanium [1]	
161023	Connecting Tubing for Pump Head, Titanium [2]	
161022	Connecting Tubing Pump Outlet to Pressure Sensor, Titanium [3]	
160053	PEEK Connecting Tubing Pump Inlet [4]	
	Connecting Tubing for the UltiMate - Standard Version	
160056	Connecting Tubing Pressure Sensor to High Pressure Filter, Stainless Steel [1]	
162010	Connecting Tubing for Pump Head, Stainless Steel [2]	
162009	Connecting Tubing Pump Outlet to Pressure Sensor, Stainless Steel [3]	
160053	PEEK Connecting Tubing Pump Inlet [4]	
	Note: The 'Inert' and the 'Standard' system are using the same type of pump head, but different connecting tubing.	

4.9.3.B Standard Pump Head (Stainless Steel)

Part Number	Description	
160082	Pump Head	
160049	Check Valve Cartridge (1 piece)	
160050	Check Valve Cartridges (set of 5)	
160047	Sapphire Piston	
160084	O-Ring, 17 mm x 1.5 mm	
161090	Seal holder for stainless steel pump head	
160048	Piston Seals [Graphite Fiber Filled PTFE, 1/4" O.D.] (set of 4)	
	Connecting Tubing	
160056	Connecting Tubing Pressure Sensor to High Pressure Filter [1]	
160055	Connecting Tubing for Pump Head [2]	
160054	Connecting Tubing Pump Head Outlet to Pressure Sensor [3]	
160053	PEEK Connecting Tubing Pump Inlet [4]	

4.9.4 Pressure Sensor Assembly

Part Number	Description
160527	O-Ring, 6 mm x 1.7 mm
160522	Purge Screw

4.10 Specifications

4.10.1 General

Power Requirements	90-260 V, 47-63 Hz, 100 VA Maximum
Fuse	2 Slo-Blo, 2A
Dimensions (WxDxH)	160 mm (6.4 in) x 340 mm (13.4 in) x 185 mm (7.3 in).
Weight	5.5 kg (12 lb.).
Production Quality	ISO 9001:2000 certified manufacturing. CE certified (LVD and EMC).
Operating Temperature	10-40°C, 20-80% relative humidity.

4.10.2 Flow Characteristics

Pump Type	Reciprocating double piston pump with electronic residual pulsation suppression
Flow Rate Range	0-500 µL/min
Flow Rate Accuracy	< 0.5 %
Flow Rate Selection	Predefined by Calibrator in Solvent Organizer (when used in conjunction with UltiMate)
Gradient Formation	Low pressure quaternary mixing with high speed micro proportioning valves
Gradient Accuracy	<0.5 % RSD (typically < 0.02 % RSD) (when used in conjunction with UltiMate)
Delay Volume	Calibrator Dependent Micro HPLC ~ 120 µL Capillary HPLC ~ 12 µL Nano HPLC ~ 0.6 µL (when used in conjunction with UltiMate)
Rapid Purge Valve	Yes
Pressure Range	0 - 40 MPa
GLP Features	Tracking of instrument usage, total pump cycles, total volume electronic records of installation, maintenance, error codes

4.10.3 Instrument Control

User Interface	Keypad, 16 Keys, Liquid Crystal Display 2 x 24 characters
Instrument Control	2 x RS-232C terminals for remote control operation via CHROMELEON® Software
GLP Features	Tracking of instrument usage, total pump cycles, total volume, electronic records of installation, maintenance, error codes

4.10.5 Inputs/Outputs

Solvent Generation	4 Low Pressure Gradient Proportioning Valves
Events	4 TTL (max 10 mA)* 2 Open Collector (max 40 mA)* 2 Relay (normally open) START-, ERROR IN: Optocoupler, max 10 mA* START-, ERROR OUT: Open Collector, max 40 mA* * Events controlled by CHROMELEON Software
Recorder Output	2.5 mV/MPa (1 V = 40 MPa)

CHAPTER 5

The UltiMate™ UV DETECTOR

5.1 System Overview

5.1.1 Description of the UltiMate UV Detector

The UltiMate™ UV Detector (FIGURE 5-1) is a rapid-scanning spectrophotometer that can monitor up to four different wavelengths at a time. This section provides an overview of the detector, which can be fitted with a D_2 or a Tungsten lamp and operated in the range from 190 to 740 nm. It can scan at the rate of 100 nm/sec and has an ordinate range of 0-4 AU. A variety of flow cells are available to meet the needs of the application ranging from 3 nL to 100 μL. When the unit is integrated into the UltiMate system, it is controlled by the CHROMELEON® Chromatography Management software, but it can be controlled on a local level via the keypad as a stand-alone detector.

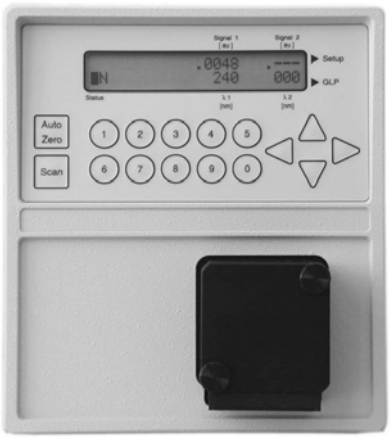


FIGURE 5-1. The UltiMate UV Detector

If desired, the UltiMate UV Detector could be used as a stand-alone detector for HPLC. While this chapter primarily describes the use of the detector as a component in the UltiMate Capillary HPLC system, it also includes information which should be useful if the detector is used with other devices.

This chapter describes the UV Detector and includes the following information:

- Installation of the UV Detector (Section 5.2)
- The User Interface (Section 5.3)
- Testing the Detector (Section 5.4)
- Cleaning and Replacement of Components (Section 5.5)
- Error Messages (Section 5.7)
- Troubleshooting (Section 5.6)
- Service Codes (Section 5.7)
- Spare Parts Lists (Section 5.9)
- Specifications (Section 5.10)

5.1.2 Features and Design of the UltiMate UV Detector

The major features of the detector include:

- **Fixed Wavelength and Scanning Capabilities:** The detector can simultaneously measure the absorbance of the eluent at two wavelengths or it can provide a scan (when controlled by CHROMELEON, it can simultaneously measure the absorbance of up to four wavelengths).
- **Low Noise and Baseline Drift:** The noise level of the detector is less than 1×10^{-5} AU and the baseline drift is approximately 1.5×10^{-4} AU/h.
- **Autotest and Autocalibration:** The detector goes through a self-test and the monochromator position is automatically calibrated when it is powered up.
- **A Variety of Flow Cells:** Flow cells for Microbore, Capillary and Nano LC can be used available. In addition, an on-column fiber optic cell holder is available.
- **Broad Wavelength Range:** A deuterium lamp (standard) and a tungsten lamp (optional) provide cover the wavelength range from 190 to 740 nm.
- **Rapid Scanning:** The monochromator is scanned at a high rate so that it is not necessary to stop the HPLC flow.
- **A Second Order Filter:** A wedge filter is used to suppress to secondary grating orders when a deuterium lamp is used above 380 nm.
- **Storage of Various Operating Parameters:** The detector stores a variety of parameters such as lamp usage, service information and error codes for GLP compliance.

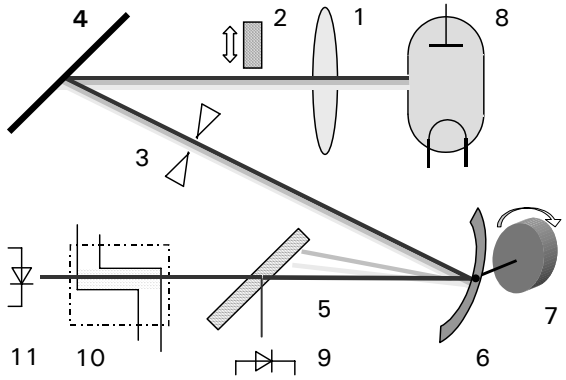
5.1.3 Design of the UltiMate UV Detector

A. Optical Bench

The Optical Bench includes the following components:

- Lamp (either D_2 or Tungsten lamp)
- Optics (lens, mirror and slit to focus the light beam)
- Motor driven monochromator grating to select the wavelength
- Solenoid driven cut-off filter
- Flow Cell
- Diodes for Signal and Reference Detection

The optical ray diagram is presented in FIGURE 5-2.



1 Lens
2 Cut-Off Filter
3 Slit
4 Mirror
5 Beam Splitter (a)
6 Grating
7 Grating Motor (b)
8 Lamp
9 Reference Detection Diode
10 Flow Cell
11 Signal Detection Diode

(a) The light beam is split before entering the flow cell to generate a reference signal and the measuring signal.
(b) A 5-phase stepper motor rotates the grating with a resolution of 2000 steps/revolution and is driven in half step mode.

FIGURE 5-2. Optical Bench (Optical Ray Diagram)

An autocalibration procedure is automatically performed every time the detector is powered up. After the power-up check, the D_2 lamp is allowed to reach the operating temperature and ignited; once the lamp is ignited, an automated wavelength calibration process is initiated. The automated wavelength calibration process first checks for 0-order radiation at 000 nm, then it checks for the H_α-line at 656 nm. During this process, the display indicates 'Calibrating... '. Each time the detector performs a wavelength change, it checks the 0-order light position again.

Note: When a Tungsten lamp is installed, only 0-order light is checked.

The detector can monitor the absorbance at two user-selected wavelengths. If desired, the absorbance ratio between the two wavelengths (λ_2/λ_1 or λ_1/λ_2) can be reported. In addition, the detector can scan over three predefined wavelength ranges and output the scan data to a chart recorder to gain additional information about the nature of the eluted compounds.

B. Lamps

Two sources can be used with the UV Detector, a D_2 lamp (standard) and a Tungsten lamp (optional). The wavelength range is from 190 to 740 nm. A secondary filter is included and is activated above 380 nm to suppress short wavelength radiation when the deuterium lamp is used above 380 nm.

C. Flow Cells

LC Packings offers a number of dedicated flow cells for Microbore-, Capillary and Nano-LC, which are listed in TABLE 5-1. Typical flow cells are shown in FIGURE 5-3.

TABLE 5-1. Flow Cell Selection – Type and Part Numbers

Technique	10 mm UZ-View™	30 mm UV-Booster™ (a)
Micro LC (10-100 µL/min)	UZ-M10 P/N 16011	UZ-M30 P/N 160012
Capillary LC (1-10 µL/min)	UZ-C10 P/N 160013	UZ-C30 P/N 160014
Nano LC (0.1-1.0 µL/min)	UZ-N10 P/N 160015	UZ-N30 P/N 160016
Nano LC Flow Cell for Monolithic Capillary Columns (0.1-3.0 µL/min)	UZ-MON P/N 161719	-

a) The High Sensitivity UV-Booster cells have a longer path length to increase sensitivity.



FIGURE 5-3. 10 mm UZ-View™ and 30 mm UV-Booster™ Flow Cells

The 10 mm UZ-View cells are designed for very fast separations with virtually no loss in chromatographic resolution. The UV booster cells have a longer path length and provide increased sensitivity with a minimum loss in resolution. These cells provide a linear range of up to 4 orders of magnitude and are ideally suited for quantitative applications. Detailed specifications are provided in Section 5.10.4.

5.2 Installation of the UV Detector

5.2.1 Installation

When the UV Detector is included as a component of a LC Packings UltiMate system, the UV Detector is installed into the system as described in Chapter 2. The instructions provided below are provided for installation of the UV Detector as a stand-alone component in an HPLC system.

5.2.2 Installing the Flow Cell

The UV Detector is delivered with a dummy flow cell. Before operating the detector, the dummy cell should be removed and the desired cell should be installed. LC Packings supplies a broad range of cells for the detector as presented in TABLE 5-1.

Note: Do not discard the dummy flow cell as it is used in troubleshooting activities.

To install a flow cell:

a) Loosen and remove the two knurled screws (item 1, FIGURE 5-4) which secure the front of the cell housing.

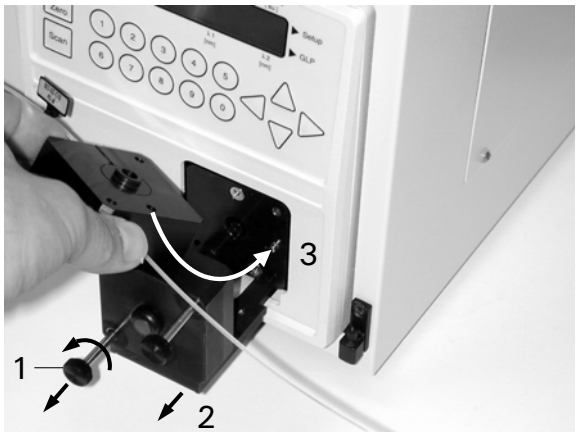


FIGURE 5-4. Cell Region of UV Detector

b) Pull the measuring cell housing (item 2, FIGURE 5-4) approximately 3 cm from the body of the detector.

c) Remove the dummy cell by lifting from the top.

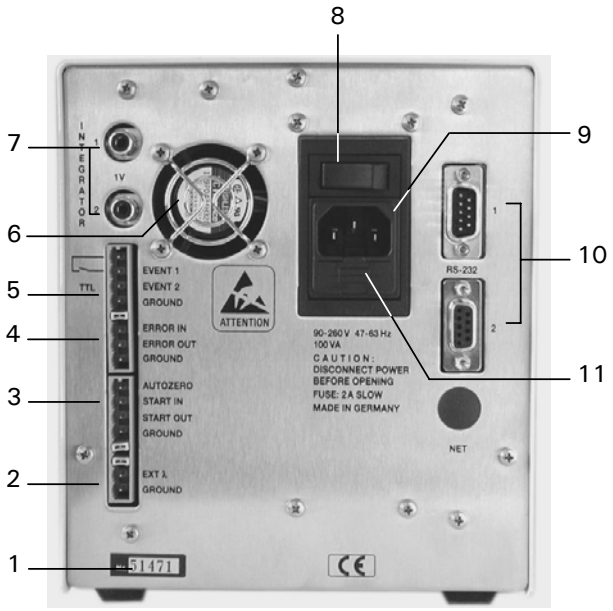
d) Place the new flow cell in the area. Make certain that the indentation on the back side of the cell corresponds with the metal pin of the UV detector housing (item 3, FIGURE 5-4).

e) Slide the measuring cell housing into the body of the unit and tighten the screws.

The cell inlet should be connected to the end of the column by a short piece of tubing. The length of the tube should be as short as possible to minimize post-column loss of chromatographic resolution.

5.2.3 Electrical Connections

All electrical connections are made on the rear panel of the detector (FIGURE 5-5).



1 Serial Number
2 Terminal Ext λ (external wavelength control)
3 Terminal Strip START, AUTOZERO
4 Terminal Strip ERROR
5 Terminal Strip EVENTS
6 Cooling Fan
7 Analog Outputs (to recorder or integrator)
8 Main Power Switch
9 Power Connector
10 RS-232 Interface
11 Fuse Compartment

FIGURE 5-5. Rear Panel of the Detector

A. The Terminal Sockets

The two banks of sockets, which are located on the left side of the rear panel, are used to connect the detector to other devices (FIGURE 5-5).

Note: Communication using these sockets is performed in conjunction with CHROMELEON software.

CAUTION **Caution: Avoid touching the electrical contacts on the terminal strips. Electrostatic discharges could damage internal components of the pump.**

Connections to the terminal sockets should be made using the connectors provided with the accessory kit. To make a connection:

a) Insert the rounded end of the lever latch into the square opening of the selected connector of the plug strip (item 1, FIGURE 5-6).

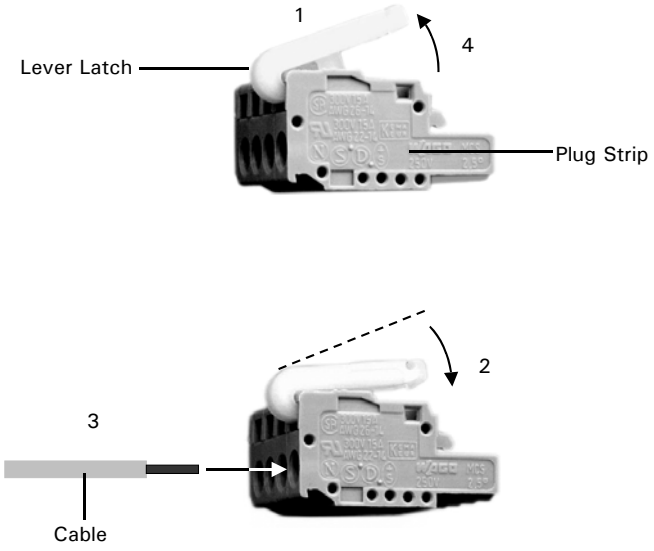


FIGURE 5-6. Connector for Terminal Strips

b) Press the lever latch down as indicated by the arrow so that it is flush with the top of the plug strip (item 2, FIGURE 5-6).

c) Insert the uninsulated end of the wire into the opening under the catch (item 3, FIGURE 5-6).

d) Release the catch and remove the lever latch from the plug (item 4, FIGURE 5-6).

The cable is now firmly anchored in the plug strip.

B. Output Terminal Sockets

The different output configurations and the maximum current available for the Output Terminal Sockets is shown in FIGURE 5-7.

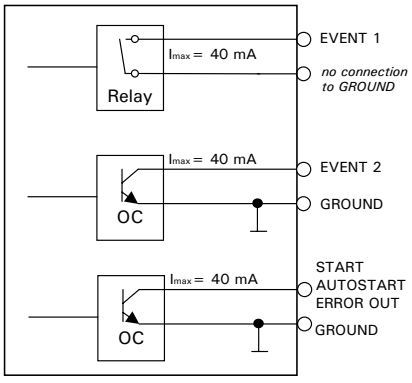


FIGURE 5-7. Output Circuits

The Relay output is a potential-free contact closure, while the Open Collector outputs must be grounded via the GROUND socket. Connect the output to the input that is to be closed and connect the ground to the ground of the device to be closed.

Note: Output Event 2 is an OC output. On some units it is described as a TTL output.

The Output sockets are used when the system is to send a signal to another device. An example of the use of an Event socket is the control of the collection of a fraction using switching valves or a fraction collection such as PROBOT™. The relay will close when the appropriate event is set to 1 in the method and the signal level chosen for fraction collection is exceeded, thus producing a short circuit that will switch the valve to the next position.

The ERROR OUT socket is used to inform some other component in the system that an error has occurred within the detector. It is an Open Collector that remains active as long as an error in the detector is observed (e.g. the lamp does not start).

C. Input Terminal Sockets

The input circuits are shown in FIGURE 5-8. The inputs START, ERROR IN and AUTOZERO are isolated via optocouplers to minimize the effects of electromagnetic interference.

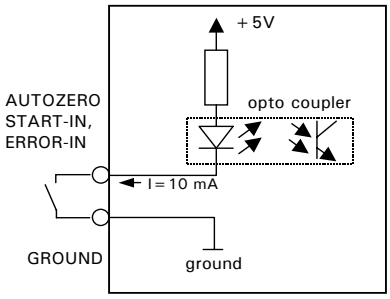


FIGURE 5-8. Input Circuits

A contact closure to GROUND will lead to a current of approximately 10 mA, and the LED will light. It is possible to use Open Collector output stages instead of relay contacts to drive the inputs.

Note: A minimum current of 7 mA is required to drive the inputs (take care about the voltage drop of the driving source, e.g. the saturation voltage of an open collector source).

The START IN socket is used if a CHROMELEON method or run sequence is to be initiated by a signal from an external device (e.g. the mass spectrometer) and is activated by a 0 V signal or short circuit. If the 'virtual' start signal feature is used, the START IN socket is not connected.

The ERROR IN socket is used to indicate that another component of the system is not operating properly. When a 0 V signal is received, the message '`Error signal was detected`' appears on the display, the program will be stopped and the lamp will be switched off.

The AUTOZERO socket is used to set an external autozero and is equivalent to the AUTOZERO button on the front panel.

The EXT λ socket is used to control the wavelength by an external analog voltage signal; a 1 V signal will set the wavelength to 100 nm, a 3 V signal sets it to 300 nm, etc. The maximum wavelength is 740 nm, which is set by a signal of 7.4 V.

D. RS 232 Serial Interfaces

The two RS232 serial interfaces enable digital transfer between the detector and other devices. These devices communicate with each other to form an integrated network.

E. Integrator Output

Two Integrator Outputs are available to readout the analog signals of channel 1 and 2 and to easily interface the UV Detector to devices of other manufacturers via an AD converter. Refer to Section 5.3.5 for more information about the setup of the output signals (e.g. maximum voltage swing).

Carefully insert the Integrator Cable (P/N 160037) into the Integrator Output on the rear panel of the detector (item 7, FIGURE 5-5) and connect to the appropriate device.

F. Power Connector

The UV Detector is fitted with a universal power supply for input voltages from 90 to 260 V. Manual setting of the supply voltage is not required. The power cord should be inserted in the socket directly below the Main Power switch.



Danger: Make certain that the system is properly grounded to a true earth ground. Connecting the instrument to an ungrounded power line can cause injuries and/or damage the instrument.

5.3 The User Interface

5.3.1 Overview

The User Interface is used to:

- Set general system parameters
- Monitor absorbance
- Store and retrieve GLP data
- Initiate scans

5.3.2 Powering up the UV Detector

a) When the UV Detector is powered up via the Main Power Switch on the back panel, it will go through an initialization/self-test protocol. During this time, a number of messages indicating that various components are functioning properly.

b) During the initialization, the Kernel Version and the Firmware Version number will be presented on the display as shown in FIGURE 5-9.

c) When the detector is powered up, the KERNEL version number is displayed for a few seconds. After that, the current firmware version will be displayed for a short time.

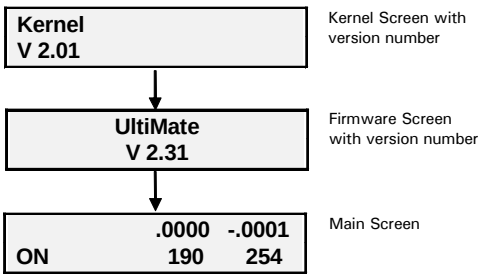


FIGURE 5-9. The Start-Up Sequence

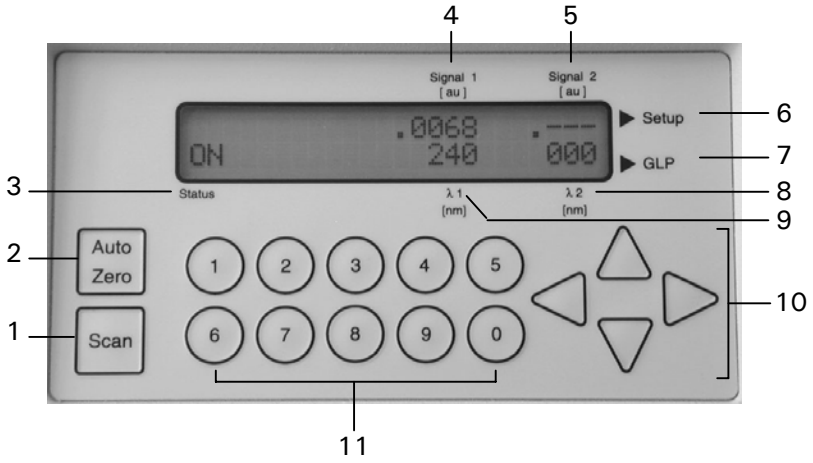
When the system has successfully passed all tests, the main screen will appear as shown in bottom of FIGURE 5-9 (a detailed discussion of the display is presented in Section 5.3.3).

Note: In this manual, the various display messages and menus shown correspond to a UV Detector with firmware version V2.31. If a different version of the firmware is used, there may be small differences in the screens and/or actions that occur when a given command is performed.

Note: The Kernel version number and Firmware version number should be recorded, as they may be useful for troubleshooting purposes.

5.3.3 The Front Panel

Communication between the user and the system is provided by the front panel of the detector (FIGURE 5-10) or via CHROMELEON. This section describes the display and the use of the keypad when the detector is used on a local basis.



1 Scan Button
2 Auto Zero Button
3 Status
4 Signal from Wavelength 1
5 Signal from Wavelength 2
6 Setup Menu Access
7 GLP Menu Access
8 Wavelength 2
9 Wavelength 1
10 Arrow Keys for Cursor
11 Data Input Keys

FIGURE 5-10. The Front Panel

The upper front panel includes:

a) The **Display Screen**, which indicates a variety of system parameters. In addition, it provides access to the Setup menu (Section 5.3.5) which is used to set a variety of system parameters and the GLP menu (Section 5.3.6) which is used to monitor system activity, service and error codes.

b) The **Scan** key, which initiates the scanning of grating in the monochromator.

c) The **AutoZero** key, which sets the current intensity for a given wavelength to zero.

d) The **Numerical Keypad,** which is used to indicate the desired value for a given parameter.

e) The **Arrow keys**, which are provided to move the cursor to the desired character for editing.

5.3.4 The Main Screen

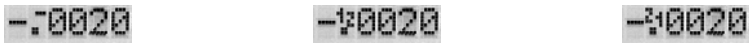
The Main Screen is used to set the wavelength(s) to be used to monitor the eluent, indicate the output of the signal, change the lamp status to ON(OFF), and access the SETUP and GLP menus. In automated mode, these parameters are set via CHROMELEON.

To edit the wavelength, use the ▲,▼,◀ and ▶ arrow keys to move the cursor to the appropriate field and press the desired number key. As an example, if you wanted to change the λ1 from 254 nm to 280 nm, move the cursor to the first character in the field, press 2, 8, 0 and press any arrow key to confirm (the range of the wavelength is 190-740 nm). If a single wavelength is desired, set the other wavelength to 0. If the wavelength is above 380 nm and the deuterium lamp is employed, the second order filter is placed in position. This is indicated by a + superscript by the wavelength.

The default signal output is the absorbance for each channel. In addition, several other data presentation formats are provided:

- Inversion of the signal (1/λ1)
- Ratio (λ1/λ2 and λ2/λ1) - Ratio output monitors the relative absorbance of the eluant at two different wavelengths. This mode provides information about the purity of the eluted compound since the absorbance ratio will likely change if two (or more) compounds co-elute.

To select the desired output format, move the cursor to either of the intensity fields and click any numerical key until the signal field indicates the appropriate indicator, as shown in FIGURE 5-11.



Signal Inversion λ_1/λ_2 Ratio λ_2/λ_1 Ratio

FIGURE 5-11. Output Option Indicator

If desired, you can change the output scale or monitor the output for the reference or signal channel via the SETUP menu (Section 5.3.5).

5.3.5 The SETUP Menu

The SETUP menu includes a variety of fields that are used to set instrumental parameters which are not changed on a routine basis. To access the SETUP Menu:

a) Move the cursor to the right most position of the upper line of the display.

b) Press the ▶ key the key for about a second.

When you open the SETUP menu, the display screen will present the TIME CONSTANT screen, which is one of the screens in the SETUP Menu (FIGURE 5-12). The various screens indicated in FIGURE 5-12 can be accessed by placing the cursor on the ♦ (diamond) key on the lower left corner of the screen and clicking the ▲ or ▼ arrow.

When the ▲ arrow is pressed, the FRACTION DELAY screen is presented and if the ▼ arrow is pressed, the LAMP screen is presented.

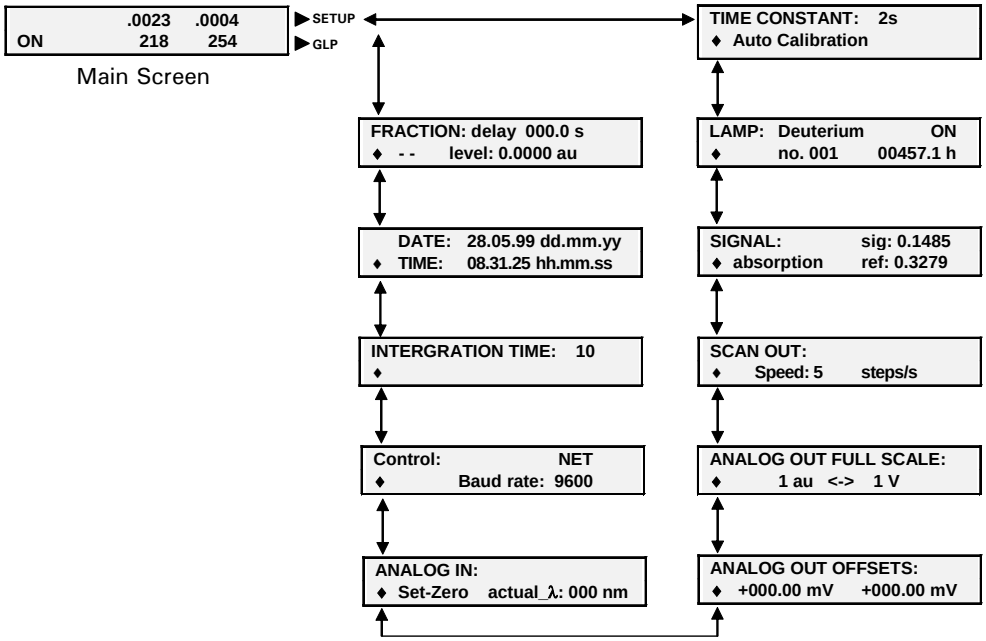


FIGURE 5-12. The Setup Menu

Some screens provide a limited number of allowable options. As an example, the TIME CONSTANT can be 0.1, 0.2, 0.5, 1, 2.5, and 10 sec and the desired option is set by moving the cursor to the data field via the ▶ arrow and selecting the value with the ▲ or ▼ arrow. In contrast, other fields (e.g. ANALOG OUT OFFSETS), require a specific numeric entry which is set by moving the cursor to the first character and editing in the same way as the wavelength setting.

In this section, we will describe each command and how to edit the individual screens. When the cursor is in a position other than the ♦ position, the ▲ or ▼ arrow or the numerical keypad can be used in editing the parameter. After you have completed the editing of a screen, move the cursor on the ♦ (diamond) key and click the ▼ or ▲ arrow to access the next (previous) screen. To return to the main screen, move the cursor to the ♦ position and click the ◀ key.

TIME CONSTANT: 1s
♦ AUTOCALIBRATION

Indicate the desired time constant (0.1, 0.2 0.5, 1, 2, 5,10 sec). A large value will smooth the data (a value of 1 or 2 sec is ideal for Microbore, Capillary and Nano applications). For very fast separations (a few seconds), 0.2 sec is recommended.

The AUTOCALIBRATION field is used to perform the wavelength calibration procedure without powering off the instrument.



Note: The Autocalibration procedure is automatically performed every time the detector is powered up.

LAMP: Deuterium ON
♦ no. 001 00457.1

Used to indicate the type of lamp to be used (Deuterium/Halogen) and the number of working hours for the Deuterium lamp. When a Deuterium

lamp is used, you can reset the working hours counter to (the screen will ask you to verify that the counter should be reset). The no. field is automatically incremented by unity when a lamp is changed (and cannot be edited by the user.

This function should be used only when replacing a deuterium lamp.

The ON/OFF field is used to switch the lamp off without turning the detector off.



Used to select the type of data to be presented on the main screen, via the analog output and transferred via the network to CHROMELEON (options are absorbance, signal, and reference). The **sig** and **ref** fields on this screen indicate the present intensity on the reference and the signal channel respectively. The values range between 0 and 1 and are dependent on the wavelength, the solvent the condition of the cell, the lamp output, etc.

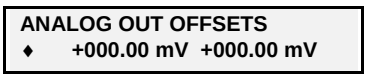
Note: Signal and reference intensities are only used for troubleshooting. If you select this mode, make certain that you return to the absorbance display before collecting analytical data.



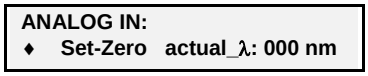
Used to set the rate for transferring the scan data. In a narrow wavelength range (e.g. 190-380 nm), the "steps/sec" setting closely approximates the scan speed "nm/s".



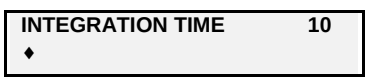
Used to select the full signal range and the maximum output voltage signal. There are 16 steps from 10^{-4} to 10 AU, The potential can be set to 0.1, 1 and 10 V. Both settings are made via the up/down arrow keys.



Used to set an offset for the integrator output. This typical use for this feature is when two analog channels are being monitored (e.g. when using a two pen recorder to monitor both channels). The output of the voltage is set to zero during the instrument self test.



Used to correct any offset value of the external wavelength control signal (Set-Zero) and to adjust/scale the external voltage signal.



Used to indicate and adjust the integration (sampling) time of the A/D converters (in 'ms'). See Section 5.4.4.

Note: This SETUP screen is available with firmware version 2.31 or greater only.

CONTROL: Net ♦ baud rate: 9600

Used to select the mode of operation (networking or external voltage control). The baud rate setting should not be changed.

DATE: 30.09.03 dd.mo.yy ♦TIME: 08.57.10 hh.mm.ss

Used to enter the time and date (these values are used for the GLP menu and service related activities, see Section 5.3.6).

FRACTION: delay 000.0 sec ♦-- E1 level : 0.0000 au

Used to program operation of a fraction collector via the event output of the detector. The desired delay between the observation of the event and the triggering of the event (due to the volume of the tubing connecting the detector and the fraction collector) is indicated on the top line. The event contact (e.g. E1) is indicated on the lower left of the screen and the trigger level is indicated on the lower right side of the screen. The event contact on the back of the detector should be connected to the desired item.

5.3.6 The GLP Menu

The GLP Menu describes a variety of reports on system usage and system status such as total operating information, service information and error reports.

To access the GLP Menu:

a) Move the cursor to the right most position of the bottom line of the display.

b) Press the ▶ key (with firmware 1.5 or greater, it is necessary to press the key for about a second).

The overview of the GLP Menu is presented in FIGURE 5-13. Selection of the screen to be accessed and returning to the Main screen is identical to that of the Setup Menu (Section 5.3.5).

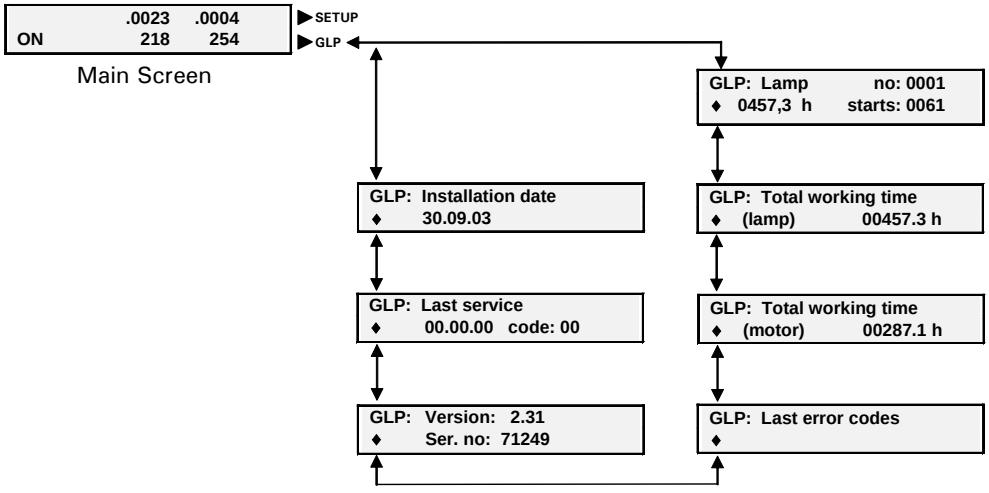


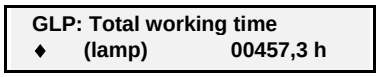
FIGURE 5-13 The GLP Menu Overview

To view the GLP Menu screens:

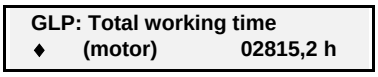
When you first access GLP Menu, the **GLP Lamp** screen is accessed. The selection of the screen to be accessed and method of returning to the Main screen is identical to that of the Setup menu (Section 5.3.5). These screens are not editable by the operator.



The **GLP: Lamp** screen indicates the lamp number (increments by one when a new lamp is installed), the time that the lamp has been used and the number of starts.



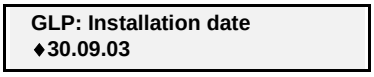
The **GLP: Total Working Time (lamp)** indicates the number of hours that the all lamps have been used. It is reset as described above.



The **GLP: Total Working Time (Motor)** indicates the number of hours that the motor has been used.



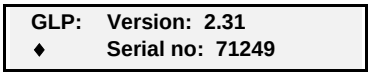
The **GLP: Last error codes** screen indicates the last 5 error codes reported by the system since the last. If you click on the ▲ or ▼ arrow, the time and date of the error is presented. Error codes are defined in Section 5.6



The **GLP: Installation date** screen indicates when the system was installed.



The **GLP: Last service** screen indicates when the unit was last serviced and the four-digit code describing the service (Section 5.7). It is set by the service engineer.



The **GLP: Version/Serial no.** indicates the currently installed firmware version and the serial number of the unit. The serial number also represents the network address.

5.3.7 Wavelength Scanning

The scanning capabilities of the UV detector are activated by pressing the **SCAN** button on the front panel, which presents the SCAN screen (FIGURE 5-14).

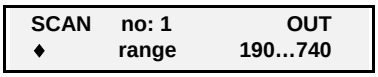


FIGURE 5-14. The Scan Screen

The SCAN screen can be used to perform up to four scans which can be stored in memory and recalled as desired. The number of the scan (1-4) can be selected via the ▲ or ▼ arrow on the **no.** field. To select the scan range, place the cursor on **range** and use the ▲ or ▼ arrow to select the desired range (190 - 740, 190 - 380 or 381 - 740 nm).

When you are ready to make a scan, a background scan should be taken. This scan is obtained with the mobile phase in the cell, and the data is stored in memory. A background scan is initiated by pressing on the AUTOZERO button for one second.

To initiate a scan, select the desired scan no. and press the SCAN button. The appropriate range will be scanned at the rate of approximately 100 nm/sec. At the conclusion of the scan, you can move the cursor to the OUT notation and press the ▲ or ▼ arrow. The data will be sent to the recorder at the rate indicated in the SCAN OUT screen (Section 5.3.5).



Note: The memory will store four scans (one for each scan number) which can be downloaded as desired. If you start a scan for a given scan number (e.g. no. 1), the data will be erased if you start another scan with the same scan number. When you change the wavelength range setting for a scan, all stored scan data will be erased. It is therefore recommended that you download each scan as soon as it has been collected.

5.4 Testing the Detector

5.4.1 Lamp Intensity

To test the lamp:

a) Set the wavelength to 240 nm.

b) Install the dummy cell and access the SIGNAL screen on the SETUP menu.

c) Monitor the signal value (SIG) and the reference value (REF). Both the signal and the reference values should be greater than 0.1000 and should not exceed 0.9000.



Note: If the values are not within the specifications, install a new lamp and perform the test again. If the values are still not within the specifications check/adjust the 'Integration Time' setting (Section 5.4.4).

5.4.2 Flow Cell

To test the flow cell:

a) Make certain that the flow cell to be tested is flushed properly and clean (Section 5.5.2).

b) Fill the flow cell with well degassed chromatography grade water.

c) Set the wavelength to 240 nm.

d) Access the SIGNAL screen on the SETUP menu (Section 5.3.5).

e) Monitor the Reference signal ("ref"). The value should be greater than 0.1000 (this step verifies that the lamp is providing sufficient energy).

f) Monitor the Sample signal ("sample") and compare the result with the signal intensity limits presented in TABLE 5-2.

TABLE 5-2 Signal Intensity Limits

Flow Cell	Limit of signal intensity	
UZ-C10	≥ 15	% of the intensity of the reference channel
UZ-M10	≥ 15	
UZ-N10	≥ 15	
UZ-C30	≥ 5	
UZ-M30	≥ 5	
UZ-N30	≥ 5	

5.4.3 Drift and Noise Test

To perform the Drift and Noise Test:

a) Prepare data acquisition and system setup using CHROMELEON Software.

b) Install the dummy cell and let the base line stabilize.

c) Set the system to the conditions indicated below:

- λ1 = 254 nm, λ2 = 0 nm, λ3 = 0 nm, λ4 = 0 nm
- Time Constant = 2 sec
- Data Acquisition Rate = 1 Hz
- Data Acquisition Length = 24 h (once the data is acceptable, data collection can be terminated).

Note: When a new lamp is installed, optimum performance is obtained after the lamp has approximately 24 hours of usage. If this test performed on a new lamp, the noise and drift levels will be larger than the specified values.

FIGURE 5-15 shows the signal/time plot from a typical new lamp. It can be seen that the lamp output stabilizes after a few hours and the noise and drift measurements can be made as soon as the output is fairly stable. For the lamp described by FIGURE 5-15, good data was obtained after approximately 6 hr. It is recommended that the expansion capabilities of CHROMELEON be used to view the plot (a typical expansion plot is shown in FIGURE 5-16).

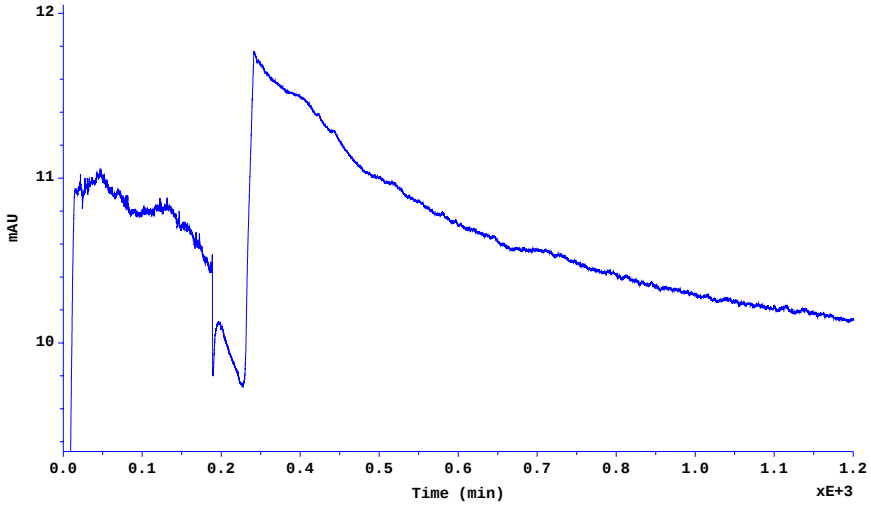


FIGURE 5-15. 24h Drift and Noise Test - Results

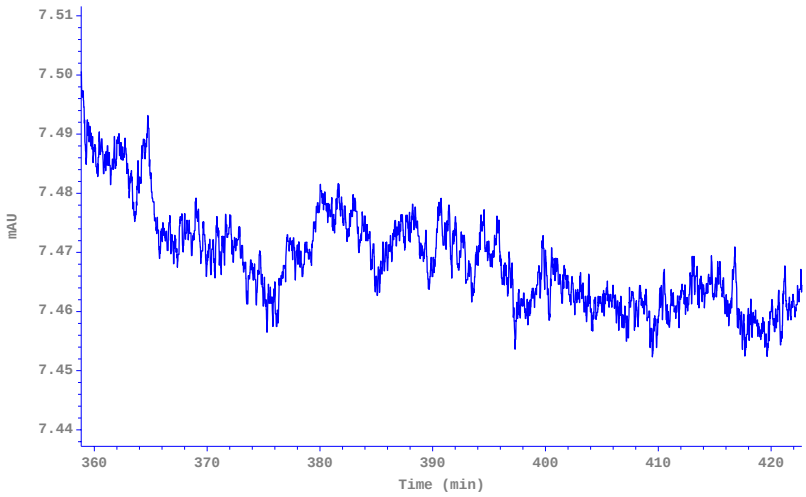


FIGURE 5-16. Noise Test - 1h Result Expanded

The drift specification is < 250 µAU/hr, the noise specification is <15 µAU (peak to peak) and no spikes or steps should be observed.

Note: If the specifications are not met after approximately 8 h of burn-in time, let the lamp burn in for a longer period of time. Once the data is acceptable, data collection can be terminated.

If the unit does not pass this test, check the lamp (Section 5.4.1).

The typical lifetime of a D_2 lamp is approximately 1500 h. It should be noted that the use of 30 mm booster cells may require a greater light intensity. This may lead to a somewhat shorter lamp lifetime, requiring that the lamp should be replaced on a more frequent basis.

5.4.4 Adjusting the Integration Time

The integration time determines the sampling time of the Analog to Digital converter (which converts the absolute intensity signals into a digital value). A large integration time leads to a higher value for the digital signal presentation and a larger intensity in the SIGNAL screen on the SETUP menu. However, a large Integration time may lead to an overflow condition and/or lead to distortion in the observed chromatogram.

Note: This SETUP screen is available with firmware version 2.31 or greater only.

To set the Integration Time:

Note: Before adjusting the integration time, make sure that the D_2 lamp has not been in use for more than 500 h.

a) Install the dummy cell.

b) Set wavelength λ1 to 240 nm.

c) Check the intensity value on the SIGNAL screen in the SETUP menu. The values for 'Sig' and 'Ref' should be in the range from 0.45 to 0.90 and the 'Sig' value must be greater than the 'Ref' value.

d) Adjust the values as required by changing the Integration time in the INTEGRATION TIME screen on the SETUP menu.

5.5 Cleaning and Replacement of Components

5.5.1 General Information and Hints



Warning: Before starting to disassemble the detector, make sure the flow cell is flushed properly, switch off the instrument and disconnect it from the electrical supply.

The user is expected to change the lamp. In addition, the user may change the flow cell (Section 5.2.2).



Note: When disassembling or reassembling the UV Detector, make sure that each component is clean and take care to ensure that the system is assembled in a clean environment.



Note: When replacing the lamp, take care that you do not touch any optical components and make certain that you do not scratch any components. If you get fingerprints on an optical component, they should be removed with a clean lint free cloth saturated with methanol.

5.5.2 Cleaning the Flow Cell

If the noise level for the detector is excessively high with a cell in position, it is possible that eluted compounds have been deposited on the cell walls. In this event, flush the cell with HPLC grade Methanol and measure the baseline. If this does not solve the problem, flush the cell with 0.1 M Nitric Acid.



Warning: Nitric Acid may damage the eyes and skin. Always wear protective clothing and eye goggles when using it

If extraneous peaks are observed, it is likely that strongly retained compounds are being eluted from the column. While this is not a “detector problem”, per se, we mention it because it is observed via the detector. When this occurs, it is suggested that the column is flushed with a strong solvent or replaced.

5.5.3 D_2 Lamp



Warning: The D_2 lamp emits short wavelength radiation. Viewing the lamp without suitable eye protection can cause serious eye damage.



Warning: Both the lamp and the optical bench become very hot during operation. Allow the components to cool down before accessing any part.



Caution: Switch the instrument off. Changing the D_2 Lamp with the detector powered on may damage the instrument.

The following section describes how to replace the D_2 lamp in the built-in detector as well as a stand-alone detector.

To replace the D_2 Lamp of the built-in UV Detector:

a) Remove the two screws of the Lamp Access Plate of the UltiMate system (FIGURE 5-17).

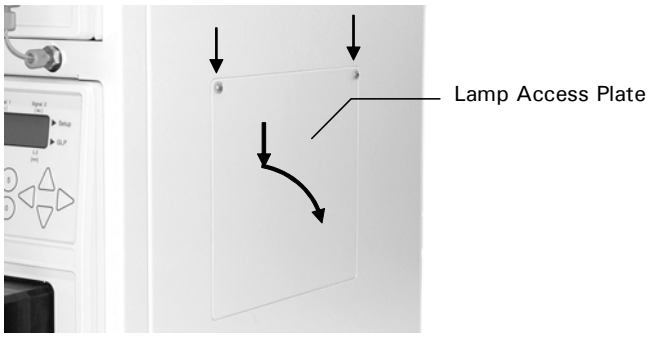


FIGURE 5-17. Lamp Access Plate

b) Unplug the 3 pin connector of the lamp (item 1, FIGURE 5-18), remove the two screws of the lamp (item 2, FIGURE 5-18) and pull the lamp out carefully.

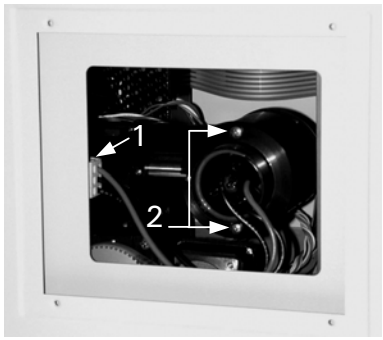


FIGURE 5-18. Replacing D_2 Lamp

c) When putting in a new lamp make sure that it is correctly seated in the guiding slot (FIGURE 5-18).

d) Check the lamp intensity (Section 5.4.1).

Note: When replacing the lamp, take care that you do not touch any optical components. If you get fingerprints on an optical component, they should be removed with a lint free cloth saturated with methanol.

e) Tighten the screws and plug the 3 pin connector in.

f) Increment the lamp counter in the LAMP Screen (the working time counter will automatically be reset).

Note: To obtain optimum performance and minimum noise, a new lamp should be allowed to burn in for at least 24 hours (Section 5.4.3).

To replace the D_2 Lamp of the stand-alone UV Detector:

a) Remove the 3 screws on the right side, the 3 screws on the left side of the instrument and remove the Top Cover.

b) Follow the procedure described above.

5.5.4 Replacing the Tungsten Lamp

To Replace the Tungsten Lamp:

a) Unplug the 2 pin connector of the lamp, (FIGURE 5-19), loosen the pin screw in the socket and pull out the Tungsten Lamp).

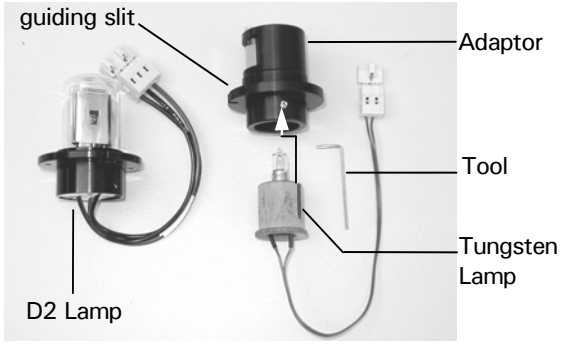


FIGURE 5-19. D2 Lamp/Tungsten Lamp Assembly

b) When installing a new lamp, make sure that it is correctly seated in the guiding slit, (FIGURE 5-19) and tighten the pin screw.

c) Install the socket with Tungsten Lamp.

d) Increment the lamp counter in the LAMP Screen (the working time counter will automatically be reset)

5.5.5 Replacing the Main Fuse

WARNING **Warning: Disconnect the instrument from the electrical supplies before inspecting/changing the fuse.**

To change the Fuse:

a) Pull out the fuse holder (item 1, FIGURE 5-20).

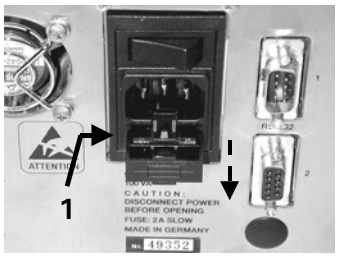


FIGURE 5-20. The Fuse Compartment

b) Replace the blown fuse by a fuse of identical rating (2 A, Slo-Blo Fuse).

c) Close the fuse compartment.

5.6 Troubleshooting

TABLE 5-3 contains troubleshooting information relating to problems of an electronic/mechanical nature, while TABLE 5-4 presents troubleshooting information relating to problems of a chromatographic nature.

TABLE 5-3. Electronic/Mechanical Troubleshooting

Problem	Probable Cause	Solution
Error "Wavelength calibration failed" or calibration does not finish	Intensity values too low	Check Lamp, check 'Integration Time' setting (Sect. 5.4.1 and 5.4.4)
	Wrong lamp type selected	Check/change settings
Intensity too low on both channels (dummy cell installed)	Old lamp	Replace lamp
	'Integration Time' too low	Check/Adjust 'Integration Time' (Sect. 5.4.1 and 5.4.4)
Intensity value on reference channel OK, but on signal channel it is much too low.	Dirty Flow Cell	Clean Flow Cell
	Booster Flow Cell installed	-
	Faulty Beamsplitter	Contact LC Packings
Intensity Overflow	No flow cell installed	Install flow cell
	Overflow of Signal or Reference channel	Check 'integration time' (Sect. 5.4.1 and 5.4.4) Tungsten Lamp: check voltage
D2 lamp does not ignite	'Halogen' option selected in SETUP Mode	Change settings
	Faulty lamp	Change lamp
Baseline has spikes (dummy cell installed)	Old or faulty Lamp	Check/Replace Lamp
	Faulty PCB/connections	Contact LC Packings
No sound of Cut-off filter when pressing AUTOZERO key (a)	AUTOZERO has been pressed within the last 60 sec.	Wait 60 sec and try again
	Faulty/sticking Cut-off filter	Contact LC Packings
No sound of Cut-off filter during the calibration (a)	Firmware Version higher than V2.20	-
	Faulty/sticking Cut-off filter	Contact LC Packings
Detector does not start, no power-up message, LCD backlight does not illuminate.	Blown fuse(s)	Check fuse
	Faulty PCB/connections	Contact LC Packings
Detector does not power up properly, no power-up message, LCD backlight lights up.	Contrast not adjusted properly	Contact LC Packings
	Corrupted KERNEL or firmware	Contact LC Packings
Motor does not rotate	Measurement at a single wavelength	-
	Faulty Main PCB	Contact LC Packings
λ1 and λ2 display looks like: -.0020 -*0020 -*0020	Wrong data presentation formats	Change format (Sect. 5.3.4)

(a) Firmware versions lower V2.20 only

TABLE 5-4. Chromatographic Troubleshooting

Problem	Probable Cause	Solution
Baseline is not stable	Light is entering the cell compartment	Use light protective tape to cover flow cell compartment
	Strongly retained materials are eluting from the column	Increase the organic component of mobile phase to remove the material that is adsorbed on the column.
	Pump Check Valves not working properly	Clean/Replace check valves
Extra Peaks in Chromatogram (especially very broad peaks at unexpected retention times)	Strongly retained materials are eluting from the column	Increase the organic component of mobile phase to remove strongly retained material from the column. If necessary, replace column
No flow through cell	Inlet or outlet is clogged	Backflush flow cell with a high flow rate (P_{max} 200 bar).
		Cut a few mm off the capillary (Nano or Capillary Cells only)
	Cell is leaking	Replace flow cell
Decreased Sensitivity	Diminished Lamp output	Check/Replace Lamp
	Cell is dirty	Clean cell (Section 5.5.2)
Spikes in baseline	Air in flow cell	Increase backpressure on flow cell (see caution note below). Nano or Cap cells: Place the end of the capillary into a septum and pump for a few seconds Micro cells: Place a plug or cap on outlet of flow cell and increase pressure for a few seconds.
Noise at wavelength below 380 nm is too high	Faulty/Sticking Cut-off filter	Contact LC Packings
	Faulty Tungsten Lamp	Check Tungsten Lamp
Intensity values below 380 nm too low	Faulty/Sticking Cut-off filter	Contact LC Packings
	Faulty Tungsten lamp	Check Tungsten Lamp
Baseline has spikes (flow cell installed)	Air bubbles in flow cell	Degas solvent Check with dummy cell
Baseline has spikes (dummy cell installed)	Faulty Lamp	Check Lamp
	Faulty PCB/connections	Contact LC Packings



Caution: Use care when increasing the backpressure. If the backpressure is significantly increased, it is possible that the cell can be destroyed.

5.7 Error Messages

The error messages in TABLE 5-5 may be presented on the display.

TABLE 5-5. Error Messages

No.	Error Message	Note	Probable Cause	Solution
9	**ERROR signal detected**	G,N,E	Error In input is activated	Check device driving the input
14	**Net error**	E	Network is not established on broken	Check Connections, Cables
46	**Instrument operates in SLAVE mode**	-	An attempt to enter data was made during instrument operation	Operate instrument in stand-alone mode
49	**D_2-Lamp does not start**	G,N,E	Faulty Connection	Check Cables, Connectors
			Tungsten Lamp Installed	Replace Lamp with D_2 Lamp
			Faulty D_2 Lamp	Replace Lamp
			Faulty Lamp Power Supply	Contact LC Packings
50	**Lamp switched off**	N	Faulty Connection	Check Cables, Connectors
			Faulty D_2 Lamp	Replace Lamp
			Faulty Lamp Power Supply	Contact LC Packings
51	**No Scan Data**	-		
52	**Calibration Failed**	N	Cut-off filter sticks	Contact LC Packings
			Faulty Reference, Main or Signal PCB	Contact LC Packings
			Lamp Intensity too low	Replace Lamp
53	**Wavelength check failed**	N	Lamp Intensity too low	Replace Lamp

Notes: G – stored as GLP information in RAM
N – send via network
E – ERROR OUT signal enabled

5.8 Service Codes

The codes in TABLE 5-6 are entered by the service engineer when an adjustment or replacement of a part is performed. These values can be viewed via the GLP menu, but cannot be edited by the user.

TABLE 5-6. Service Codes

Code	Work carried out
0	No code entered (default setting when shipped)
99	non listed service
100	Replacement/maintenance of flow cell.
101	Replacement of D_2 Lamp
102	Replacement of Tungsten Lamp
110	Replacement of Optical Bench
111	Replacement of Cut-Off Filter ASSY
112	Replacement of Lens
113	Replacement of Grating
114	Replacement of Mirror
120	Adjustment of the Optical Bench
130	Replacement of Lamp Power Supply
131	Replacement of 24 V Power Supply
132	Replacement of Main PCB
133	Replacement of CPU PCB
134	Replacement of Signal PCB
135	Replacement of Reference PCB
140	Software Update

5.9 Spare Parts Lists

5.9.1 Major Items

Description	P/N	Note
UltiMate UV Detector (without flow cell)	160008	

5.9.2 Lamps

Description	P/N	Note
Deuterium lamp	160063	
Tungsten lamp	160064	
Tungsten lamp with socket	160065	Required only if replacing a D_2 lamp for the first time. Contact LC Packings for assistance.

5.9.3 Flow Cells

Description	P/N	Note
Micro LC (10-100 µL/min), 10 mm U-Z view	160011	Type UZ-M10
Capillary LC (1-10 µL/min), 10 mm U-Z view	160013	Type UZ-C10
Nano LC (0.1-1.0 µL/min),10 mm U-Z view	160015	Type UZ-N10
Micro LC (10-100 µL/min), 30 mm High Sensitivity UV-Booster™	160012	Type UZ-M30
Capillary LC (1-10 µL/min), 30 mm High Sensitivity UV-Booster™	160014	Type UZ-C30
Nano LC (0.1-1.0 µL/min),30 mm High Sensitivity UV-Booster™	160016	Type UZ-N30
Nano LC Flow Cell for Monolithic Capillary Columns, I.D. 20 µm / O.D. 365 µm (0.1-3.0 µL/min)	161719	Type UZ-MON

5.10 Specifications

5.10.1 General

Power Requirements	90-260 V, 47-63 Hz, 100 VA Maximum
Fuse	2 Slo-Blo, 2A
Dimensions (WxDxH)	160 mm (6.4 in) x 340 mm (13.4 in) x 185 mm (7.3 in).
Weight	6.1 kg (13.3 lb.).
Production Quality	ISO 9001:2000 certified manufacturing. CE certified (LVD and EMC).
Operating Temperature	10-40°C, 20-80% relative humidity.

5.10.2 Detection Characteristics

Detection Type	Rapid scanning monochromator
Noise	1 x 10^{-5} AU (254 nm, 1.0 sec)
Drift	1.5 x 10^{-4} AU/h
Time Constants	0.1/0.2/1/2/5/10 sec
Wavelength Range	190-740 nm, up to four wavelengths can be monitored simultaneously
Sources	D_2, Tungsten
Wavelength Accuracy	+/- 1 nm
Scans per run	Number is limited only by speed of data transfer (time programmed or manually launched)
Scan Ranges	190-380, 381-740, 190-740 nm
Scan Autozero	Full wavelength range
Bandwidth	< 7 nm
Scan Speed	~ 100 nm/sec

5.10.3 Instrument Control

User Interface	Keypad, 16 Keys, Liquid Crystal Display 2 x 24 characters
Instrument Control and Data Evaluation	2 x RS-232C terminals for remote control operation via CHROMELEON® Software
Data Rate	1-10 Hz
GLP Features	Continuous tracking of lamp burn time, instrument usage, electronic records of installation, maintenance, error codes

5.10.4 Inputs/Outputs

Digital Output	2 RS-232 2 EVENT: (Open Collector, Relay) START-, ERROR IN: Optocoupler START-, ERROR OUT: Open Collector
Analog Input	1 V (1V/100 nm)
Analog Output	2 ±0.1 V/ ±1 V / ±10 V (for recorder or integrator)

5.10.5 Flow Cells, 10 mm UZ-View™

Application	Micro LC	Capillary LC	Nano LC
Type	UZ-M10	UZ-C10	UZ-N10
P/N	160011	160013	160015
Flow rate range	10-100 µL/min	1-10 µL/min	0.1-1 µL/min
Illuminated volume	180 nL	45 nL	3 nL
Capillary ID	150 µm	75 µm	20 µm
Path length	10 mm		
Noise	< 0.05 mAU (a)		
Max. Pressure	400 bar (6000 psi)		

(a) @245 nm, Acetonitrile/water (70/30), TC 2 s

5.10.6 Flow Cells, 30 mm UZ-View™

Application	Micro LC	Capillary LC	Nano LC
Type	UZ-M30	UZ-C30	UZ-N30
P/N	160012	160014	160016
Flow rate range	10-100 µL/min	1-10 µL/min	0.1-1 µL/min
Illuminated volume	540 nL	135 nL	10 nL
Capillary ID	150 µm	75 µm	20 µm
Path length	30 mm		
Noise	< 0.05 mAU (a)		
Max. Pressure	400 bar (6000 psi)		

(a) @245 nm, Acetonitrile/water (70/30), TC 2 s

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APPENDIX A

Optional Configurations

A.1 Overview

This appendix provides information about the various available system configurations and (factory installed) options of the UltiMate™ system. It describes how to install these systems or components and how to use them.

The following configurations and options are discussed:

- UltiMate system without UV Detector – Section A.2
- Manual Injection Valve (factory installed option) – Section A.3
- Flow Sensor (factory installed option) – Section A.4

For more details about the common installation procedures and the installation of the system in conjunction with other LC Packings system components (e.g. the FAMOS Microautosampler and the Switchos Advanced Microcolumn Switching Unit) refer to Chapter 2 and the documentation provided with these instruments.

A.2 UltiMate System without UV Detector

The following sections describe the installation of the network connections and the CHROMELEON setup needed to operate the UltiMate system without a UV Detector. Refer to Chapter 2 for additional details about other electrical connections that need to be made (e.g. the LPG VALVES connection).

A.2.1 Communication Ports

Because there is no UV Detector in the system, only the two communication ports of the Micropump are to be connected. To control the Micropump, connect the Y-Cable between a free COM port on the PC and the RS 232-1 port and the RS 232-2 port on the Micropump (item a, FIGURE A-1).

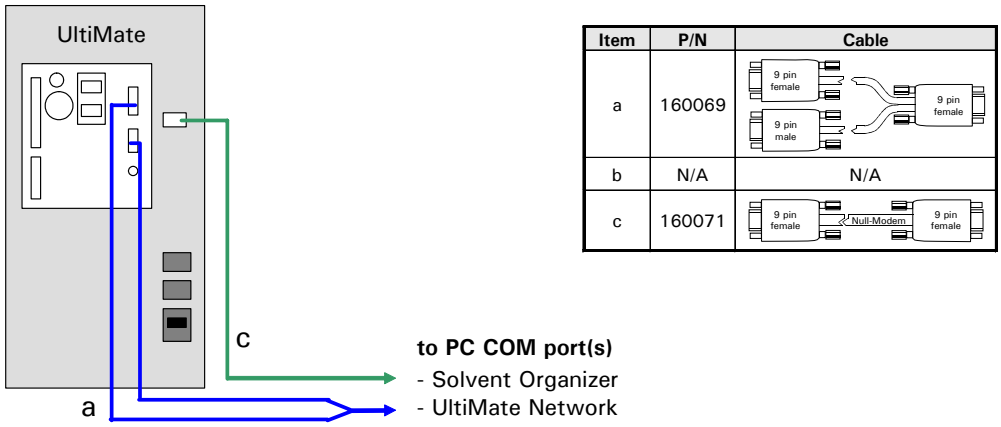


FIGURE A-1. Setting up the RS-232 Connections- UltiMate Micropump

To install an UltiMate system without UV Detector in conjunction with the Switchos 2 unit, connect the COM ports of the UltiMate Micropump, Switchos Loading Pump and the PC as presented in FIGURE A-2.

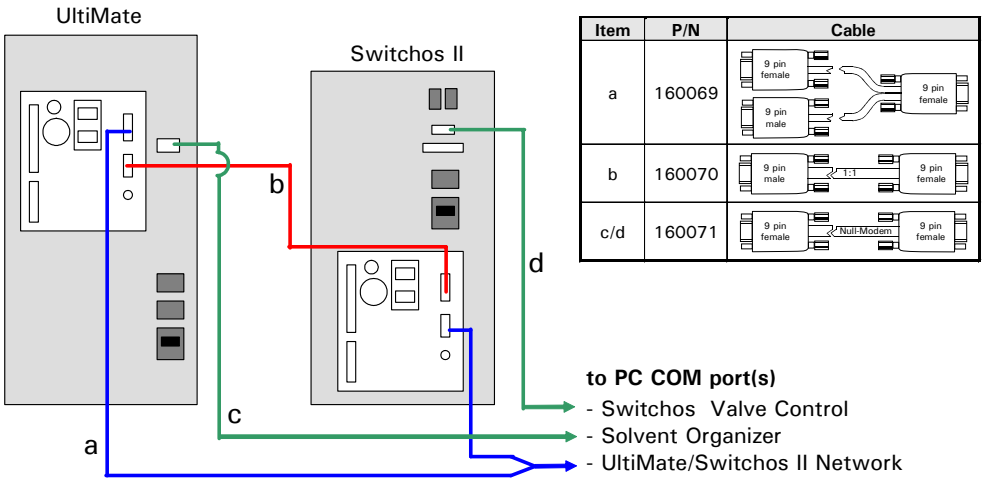


FIGURE A-2. Setting up the RS-232 Connections - UltiMate and Switchos

Note: If your system includes a flow sensor, please refer to Section A.4 for details about how to connect the communication port of the flow sensor.

A.2.2 CHROMELEON Setup – UltiMate without UV Detector

Check **UltiMate Pump**, **Switchos Pump** and the **Inject Valve** (if installed) in the *LC Packings UltiMate/ Switchos* box of the server configuration (FIGURE A-3).

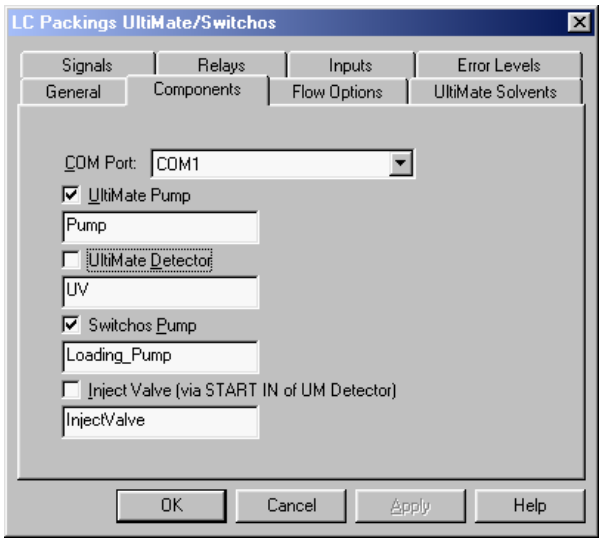


FIGURE A-3. The LC Packings UltiMate Switchos box

The following server configuration check message will appear (FIGURE A-4). Click on **Close** to confirm the warning and to close the window.

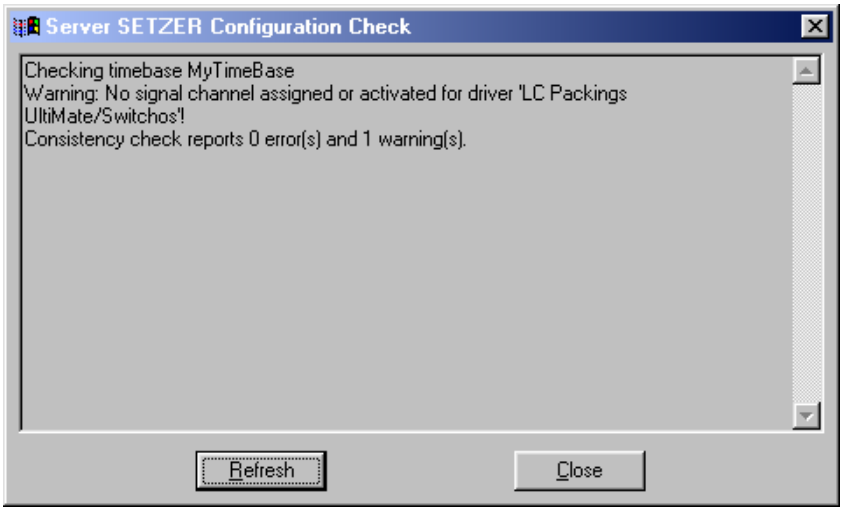


FIGURE A-4. Server Configuration Check Result

A.3 The Manual Injection Valve

A.3.1 Overview

The Valco Cheminert® Model C1 low dispersion manual injection valve is located in the top front panel (FIGURE A-5). The valve is equipped with a contact closure that is activated in Inject position.

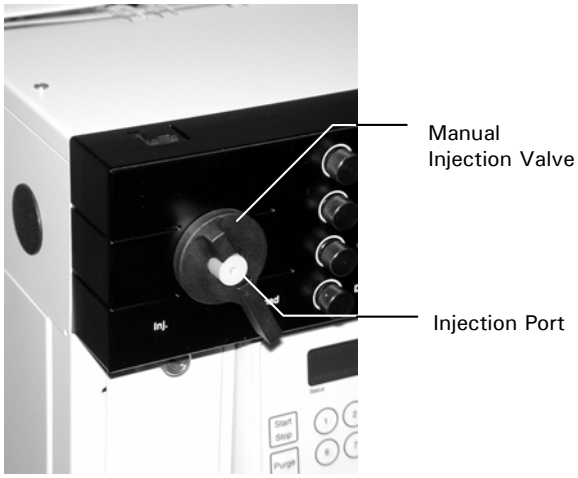


FIGURE A-5. The Manual Injection Valve

In order to start data acquisition (e.g. of the CHROMELEON® software), this contact closure signal must be connected to the start input of the *UltiMate* UV Detector (if installed) or the *UltiMate* Micropump (if no UV Detector is installed).

The connection tubing is preinstalled. When changing the system configuration (e.g. converting from a CAP to a NAN application) this tubing must be replaced (Section A.3.3).

A.3.2 Electrical Connection

A.3.2 A UltiMate System with UV Detector

To start a sequence by the manual injection valve, connect the contact closure cable (black cable) that comes out of the *UltiMate* Solvent Organizer to the START IN on the *UltiMate* UV-Detector (FIGURE A-6).



FIGURE A-6. START IN Port on the UV-Detector

A.3.2 B UltiMate System without UV Detector

If there is no UV Detector installed in your system (option), the START IN input of the *UltiMate* Micropump is used to start a sequence by the manual injection valve.

To connect the contact closure cable (black cable) to the START IN port on the *UltiMate* Micropump:

a) Remove the 4-way connector that is attached to the contact closure cable.

b) Attach a 3-way connector (which is supplied with the *UltiMate* system) to the cable as presented in FIGURE A-7.

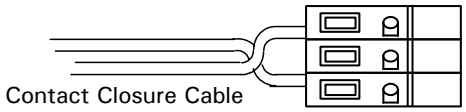


FIGURE A-7. Connecting the Contact Closure Cable

c) Connect the contact closure cable of the manual injection valve to the START IN of the Micropump (FIGURE A-8).

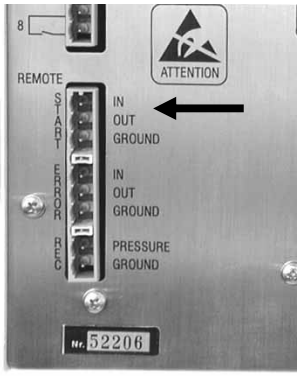


FIGURE A-8. START IN Port on the Micropump

A.3.3 Fluidic Connection

The connecting tubing that is required to connect the manual injection valve to the Nano/Micro flow outlet (upper T-Piece, FIGURE A-9) and the manual injection valve to the column is presented in TABLE A-1.

TABLE A-1 Connecting Tubing of the Manual Injection Valve

Part Number	Description
160074	Connecting tubing, calibrator – injection valve, 75 µm I.D.
160078	Connecting tubing, calibrator – injection valve, 20 µm I.D.
160076	Connecting tubing, manual injection valve - column, 75 µm I.D.
160079	Connecting tubing, manual injection valve - column, 20 µm I.D.
161040	Connecting tubing, calibrator – injection valve, 75 µm I.D., INERT
161044	Connecting tubing, calibrator – injection valve, 20 µm I.D., Nano, INERT
161042	Connecting tubing, manual injection valve - column, INERT
161045	Connecting tubing, manual injection valve - column, Nano, INERT

A.3.3 A Bypass the Manual Injection Valve

To bypass the manual injection valve of the *UltiMate* system (e.g. if you connect the system to the FAMOS™ Microautosampler):

a) Remove the fluidics access plate from the solvent organizer (Section 3.7.5).

b) Disconnect the connection tubing from the Nano/Micro flow outlet (upper T-Piece) to the manual injection valve (FIGURE A-9).

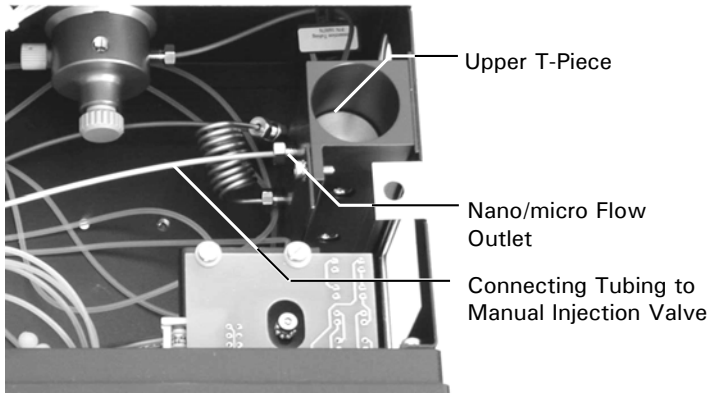


FIGURE A-9. The Nano/Micro Flow Outlet of the Solvent Organizer

c) Remove the connection tubing from the manual injection valve to the column oven.

d) Remove the black cap from the hole that is located in the left side panel of *UltiMate.*

e) Connect the appropriate tubing to the Nano/Micro flow outlet (e.g. if you want to connect the FAMOS Microautosampler, refer to Section 2.5.2, TABLE 2-3).

f) Guide the capillary through the hole in the side panel of the *UltiMate* and connect it to the instrument that is to be connected to the *UltiMate* system.

g) Replace the fluidics access plate.

A.3.4 CHROMELEON Setup

To start a sequence by the manual injection valve, the Server Configuration must be configured as follows:

a) Open the Server Configuration, select your Timebase and open the 'LC Packings UltiMate/Switchos' device in the Server Configuration box. The *LC Packings UltiMate/Switchos* box will appear.

b) Click on the **Components** tab and check the **Inject Valve (via START IN ...)** box (FIGURE A-10).

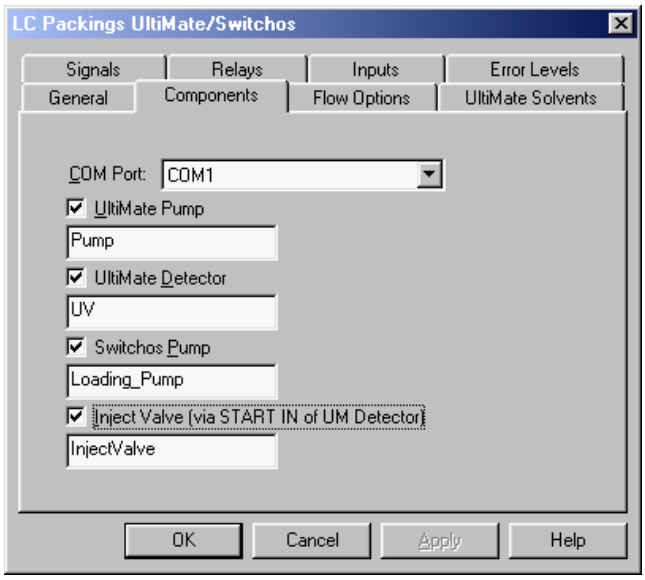


FIGURE A-10. The Components Tab

c) Click on the **Inputs** tab and check the name of the instrument to which the manual injection valve is connected to. As an example, check **Detector_StartIn** if the start input of the UV Detector is used (item 1, FIGURE A-11) or check **Pump_StartIn** if the input of the Micropump is used (item 2, FIGURE A-11).

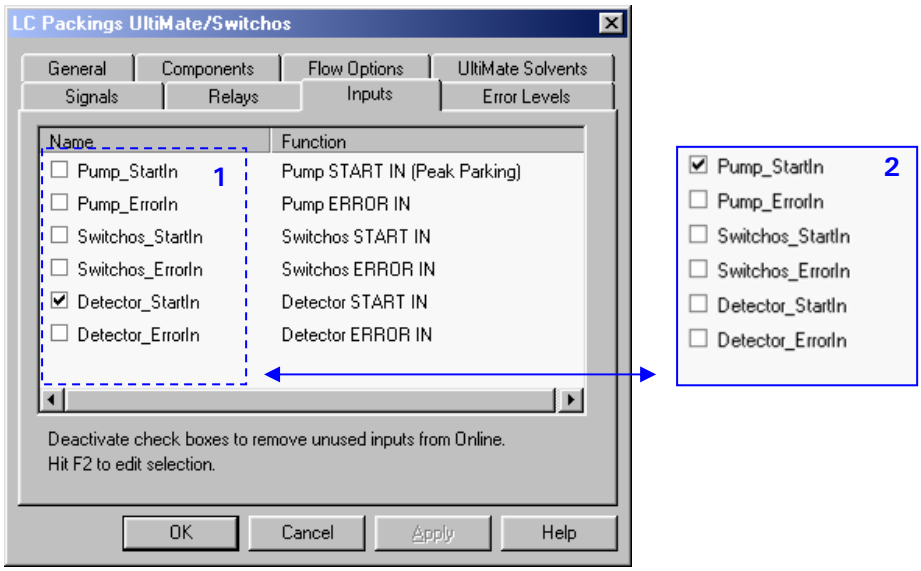


FIGURE A-11. The Input Tab

d) Close the *LC Packings UltiMate/Switchos* box and save the Timebase.

e) If a FAMOS Microautosampler is also configured in the same Timebase, the following configuration check message will appear (FIGURE A-12). Click on **Close** to confirm the warning and to close the window.

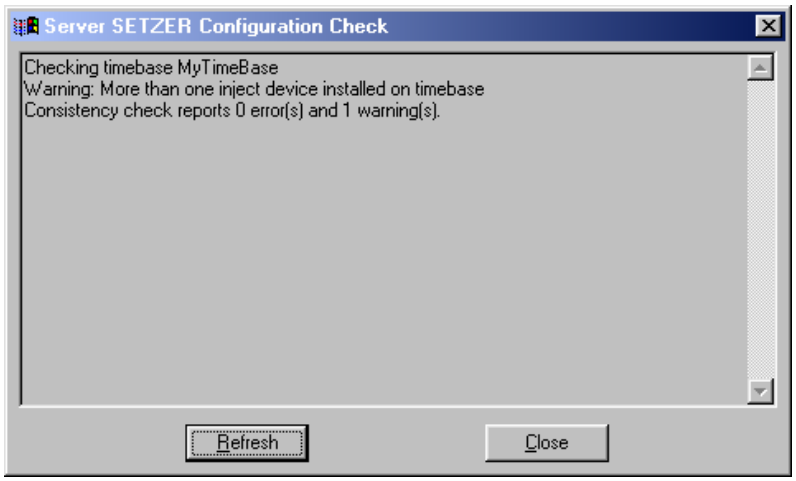


FIGURE A-12. Server Configuration Check Result

When you are setting up the CHROMELEON program, it will be necessary to indicate how to start the sequence at some point. Select the inject source you want to use and click on **Next** to continue.

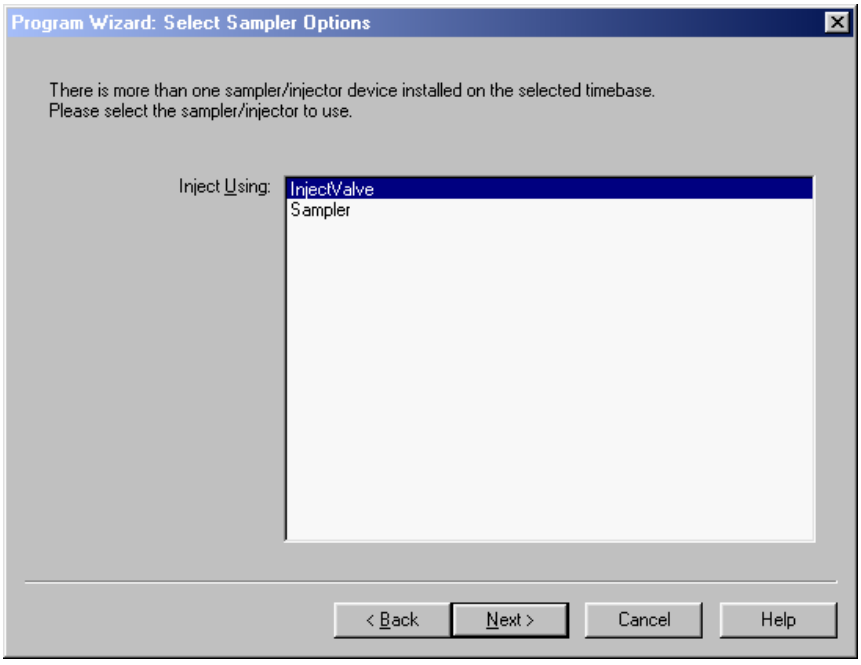


FIGURE A-13. The Program Wizard – Select Sampler Options Box

A.4 The Flow Sensor

A.4.1 Overview

The flow sensor (factory installed option) is located on the top of the proportioning valves in the left part of the fluidic compartment (item 1, FIGURE A-14, top cover plate removed).

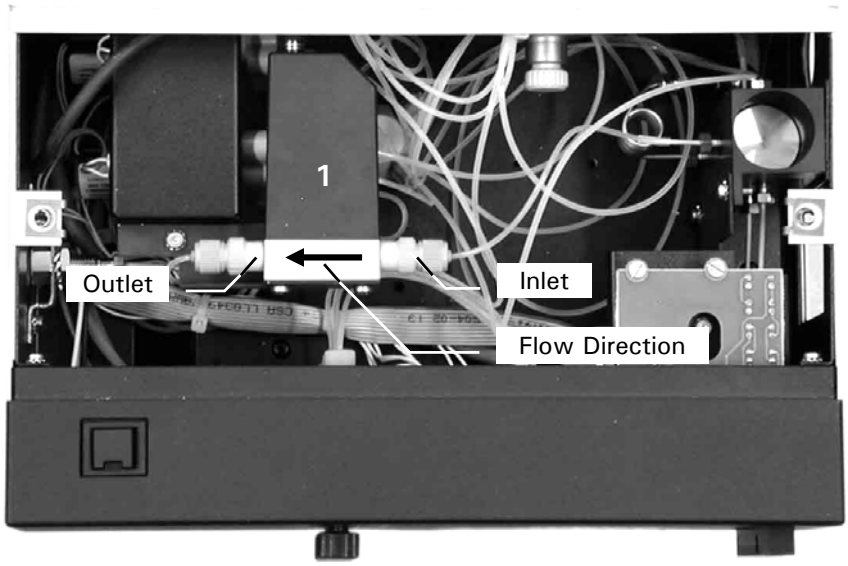


FIGURE A-14. The Fluidic Compartment with the Nano Flow Sensor

The flow sensor measures and corrects the flow rate according to the settings in the CHROMELEON Server Configuration and in the Program File (Section A.4.4).

Different types of flow sensors are available for the different system configurations as indicated in TABLE A-2.

TABLE A-2 Flow Sensor Type vs. Flow Rate and Calibrator Type

Flow Sensor	Maximum Flow Rate	Calibrator Type
Nanoflow Sensor	1.5 µL/min	NAN-nnn
Capflow Sensor	7 µL/min	MON-nnn
		CAP-nnn
Micflow Sensor	*Contact your local LC Packing/Dionex sales office about availability of a MIC flow sensor*	

CAUTION **Caution: Do not use the flow sensor at flow rates higher than specified.**

A.4.2 Electrical Connection

Use a 'Solvent Organizer Cable' (P/N 160071) to connect the flow sensor communication port to a free COM port on the PC (FIGURE A-15).

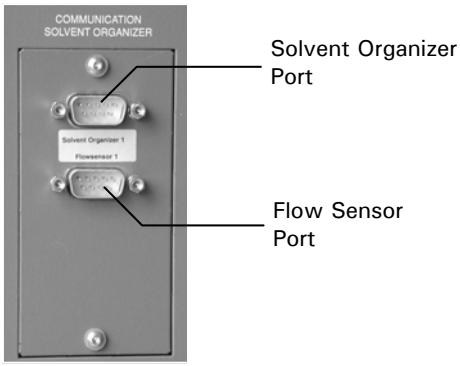


FIGURE A-15. Communication Ports on the Rear Panel

A.4.3 Fluidic Connections

All internal connections are already pre-installed. The Nano/Micro flow outlet is located on the left side of the instrument (FIGURE A-16).

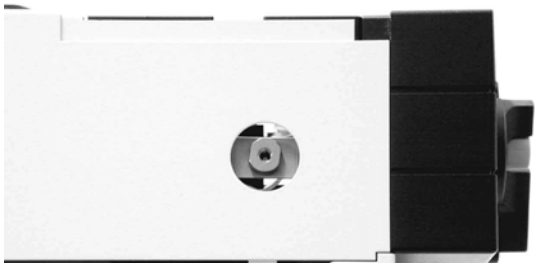


FIGURE A-16. The Nano/Micro Flow Outlet

To connect the UltiMate system to the injection system (e.g. of the FAMOS Microautosampler), select the proper tubing as indicated in TABLE A-3.

TABLE A-3 Connecting Tubing from Nano/Micro Flow Outlet to Injection System

Calibrator Type	**Flow Sensor Type**	**I.D.** (L = 50 cm)	**Connecting Tubing from Nano/Micro Flow Outlet to Injection System (FAMOS)**	
			Standard Version	Inert Version
NAN-nnn	NAN	20 µm	P/N 162203	P/N 162273
CAP-nnn	CAP	50 µm	P/N 162277	P/N 162275
MON-nnn				

A.4.4 CHROMELEON Setup

The flow sensor is programmed and controlled by the CHROMELEON software. In order to be able to control the flow sensor, the option **Flow Sensor** must be configured in the CHROMELEON Server Configuration (FIGURE A-17). The flow sensor is activated if the COM port to be used for data transfer is specified.

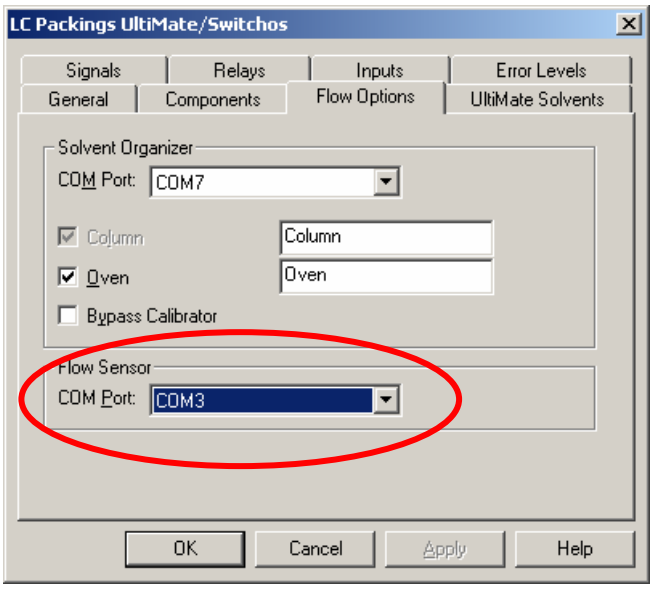


FIGURE A-17. CHROMELEON Server Configuration for the Flow Sensor

The flow sensor is now ready for use. Save and close the server configuration (if all parameters for the other instruments are correct) and start the CHROMELEON client program.

A.4.5 Using the Flow Sensor

The flow sensor can be used in 3 different modes:

- **Calibrate CRP.... Before the First Sample of Each Sequence** (default setting) – measures the flow rate and then adjusts the CRP value (**C**olumn **R**esistance **P**arameter) to compensate for the measured resistance. This step is performed before the injection of the first sample (of a sequence) and is used for all samples in the sequence.

- **Calibrate CRP.... Before Each Sample** – measures the flow rate and then adjusts the CRP value before each sample injection.

- **Calibrate CRP.... Never** – does not measure or adjust the CRP value.

Once the flow sensor has been configured in the Server Configuration, the operating mode can be selected in the *UltiMate Pump Options* box (FIGURE A-18).

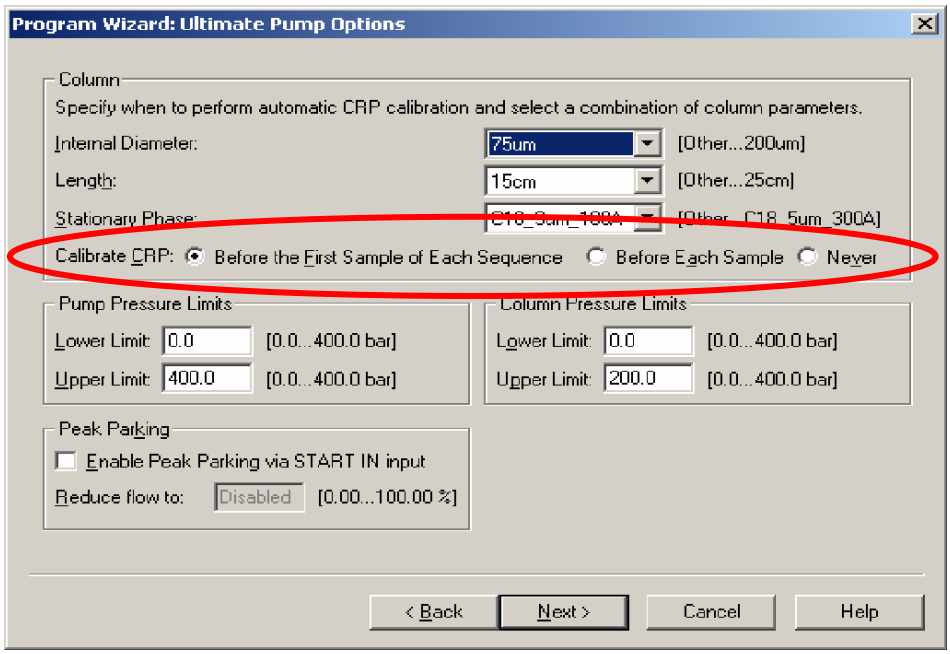


FIGURE A-18. Flow Sensor Setting in the UltiMate Pump Options Box.

A.4.6 Bypassing the Flow Sensor

The following section describes how to bypass the flow sensor (e.g. if the desired flow rate exceeds the sensor limit). Refer also to Section A.4.7 for information about how to replace the flow sensor (e.g. when changing the configuration).

CAUTION **Caution: Do not use the flow sensor at flow rates higher than specified.**

To bypass the flow sensor:

f) Stop flow delivery.

g) Disconnect the connecting tubing that connects the upper T-Piece (item 2, FIGURE A-19) and the flow sensor inlet. Protect the inlet capillary with a plug that is provided to prevent clogging of the tubing.

h) Disconnect the connecting capillary from the injection system (e.g. the FAMOS Microautosampler) and protect the capillary with a plug that is provided to prevent clogging.

i) Connect the UltiMate to the injection system using the same connecting tubing as discussed in Section 2.4 and Section 2.5.

j) If the system is controlled by CHROMELEON software, follow the instructions provided in Section A.4.4 to reset the **Flow Sensor COM Port** to '<None>'.

A.4.7 Replacing the Flow Sensor

If the UltiMate system is used in a configuration where the maximum flow rate range of the installed flow sensor is exceeded (e.g. a CAP-300 calibrator in conjunction with a NAN flow sensor), the flow sensor must be replaced by one that fits the flow rate range. Another option is to bypass the flow sensor to avoid any damage due to a flow rate that is too high (Section A.4.6).

CAUTION **Caution: Do not use the flow sensor at flow rates higher than specified.**

Note: A combination of a flow sensor for a higher flow rate range and a calibrator for a lower range (e.g. a NAN-75 calibrator in conjunction with a CAP flow sensor) may lead to a gradient delay that may not be suitable for the current application.

To replace the flow sensor:

a) Stop flow delivery.

b) Disconnect the connecting tubing that connects the nano/micro flow outlet and the flow sensor outlet (item 1, FIGURE A-19).

c) Disconnect the connecting tubing that connects the upper T-Piece and the flow sensor inlet (item 2, FIGURE A-19).

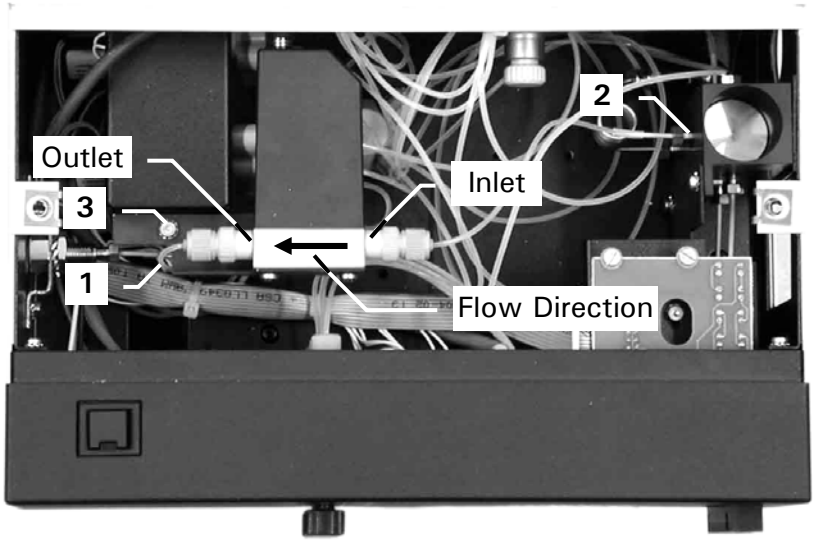


FIGURE A-19. Connecting Tubing of the Flow Sensor

d) Protect the inlet and outlet capillary with the plugs that are provided to prevent clogging of the tubing.

e) Unscrew the mounting bracket and remove the sensor from the fluidic compartment (item 3, FIGURE A-19).

f) Remove the two screws that attach the flow sensor to the mounting bracket and replace the flow sensor.

g) Replace the sensor and connect the inlet and outlet tubing.

h) Start flow delivery and check for flow and for any leakage.

A.4.8 Replacing the Connecting Tubing of the Flow Sensor

The two internal connections are already pre-installed (item 1 and 2, FIGURE A-19). If the connecting tubing needs to be replaced, follow carefully the instructions provided in this section.

To replace the connecting tubing:

a) Disconnect the inlet and outlet capillaries and remove the sensor from the fluidics compartment as discussed in Section A.4.7, steps a) – f).

b) Select the replacement tubing according to TABLE A-4.

TABLE A-4 Flow Sensor Connecting Tubing

Part Number	Connecting Tubing
162278	Inlet tubing 20 µm I.D. x 14 cm for Nanoflow Sensor
162274	Outlet tubing 20 µm I.D. x 10 cm for Nanoflow Sensor
162276	Inlet tubing 50 µm I.D. x 14 cm for Capflow Sensor
162279	Outlet tubing 50 µm I.D. x 10 cm for Capflow Sensor

c) Install a PEEK nut and ferrule that are provided as presented in FIGURE A-20. The fused silica capillary must extend the ferrule by approximately 3 mm.

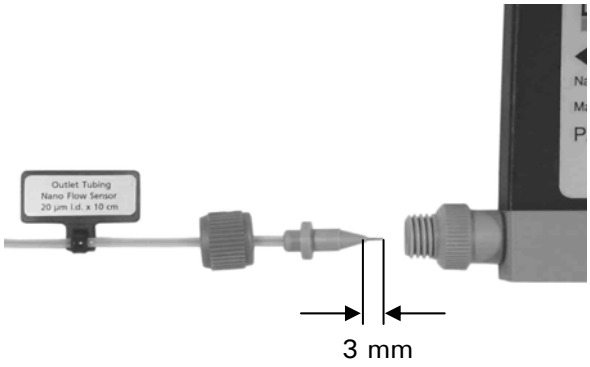


FIGURE A-20. Installing the PEEK Nut and Ferrule

d) Connect the capillary to the flow sensor. To avoid the possibility of dead volume, make certain that the fused silica capillary is long enough to reach the bottom of the flow sensor port when tightening the nut.

Note: It is essential that the fused silica is in contact with the bottom of the flow sensor port for a proper operation of the flow sensor.

e) Connect the second connecting tubing in the same way.

f) Replace the sensor and reconnect the inlet and outlet tubing.

g) Start flow delivery and check for flow and for any leakage.

A.4.9 Troubleshooting and Frequently-Asked-Questions

Troubleshooting refers to the determination of the cause of a problem. Since the flow sensor is incorporated into the HPLC system, the first step is to determine if the problem is due to this module. The flow sensor should be bypassed (Section A.4.6) and an injection should be performed. Compare the results from the two runs; if the observed results without the unit present acceptable data, the problem is most likely due to the flow sensor.

Analytical problems may be caused by external influences, such as a change in the temperature of the laboratory. In the same vein, it is possible that the compounds to be separated are affected by a change in the temperature, or are light sensitive.

When you are troubleshooting, it is worthwhile to verify that no changes have been made to the analytical protocol since it was last performed successfully. In some cases, seemingly small changes in the overall protocol can have a dramatic impact on the separation (e.g. changing the supplier of a buffer salt can have a significant impact on the overall separation).

The following tables will help to identify and diagnose operating problems and instrument failures.

TABLE A-5 Troubleshooting the Flow Sensor

Problem	Possible Cause	Solution
No communication with the flow sensor (a)	Wrong COM port selected in CM server configuration.	Check Server Configuration (Flow Options Box), restart CHROMELEON Server
	Faulty, wrong communication cable	Check, replace cable
The sequence is aborted after CRP calibration	The new calculated CRP value differs by more than 30% from the previous value.	Change initial CRP on CM panel. Check for possible leak or column blockage.
Unstable CRP value, changes significantly with each new calibration	The column flow rate is not stable during the calibration procedure (e.g. 1 min before the injection).	Extend the CHROMELEON method to for a longer equilibration time. Check Micropump.
It is not possible to set a particular CRP value in the program even if the option 'Calibrate CRP.... Never' is selected.	It is not possible to modify the CRP value in the program.	Adjust the CRP value in the CHROMELEON panel. This CRP will also be used in the program.
Note: a) In this case there is typically also no communication with the Micropump.		

TABLE A-6 Frequently Asked Questions

FAQ	Answer
What is the typical CRP value of a standard 'NAN' column (C18 PepMap, 75 μm I.D., 3 μm, 15 cm length)?	625 ± 10%
What is the typical CRP value of a standard 'CAP' column (C18 PepMap, 300 μm I.D., 3 μm, 15 cm length)?	50 ± 10%
What is the typical CRP value of a standard 'SCX' column (Poros 10S, 300 μm I.D., 15 cm length)?	50 ± 10%
What is the maximum flow rate for the different flow sensors?	Nanoflow sensor: 1.5 μl/min Capflow sensor: 7 μl/min
Where are the calibrated CRP values recorded?	They can be found in the audit trail of each sample injection.

A.4.10 Spare Parts List

Part Number	Description
	Major Items
162174	Nanoflow Sensor for UltiMate Nano LC System
162202	Capflow Sensor for UltiMate Capillary LC System
	Connecting Tubing
162203	Connecting tubing, 20 μm I.D. x 50 cm, for UltiMate with flow sensor
162273	Connecting tubing, 20 μm I.D. x 50 cm, for UltiMate INERT with flow sensor
162275	Connecting tubing, 50 μm I.D. x 50 cm, for UltiMate INERT with flow sensor
162277	Connecting tubing, 50 μm I.D. x 50 cm, for UltiMate with flow sensor
162278	Inlet tubing, 20 μm I.D. x 14 cm, for Nanoflow Sensor
162274	Outlet tubing, 20 μm I.D. x 10 cm, for Nanoflow Sensor
162276	Inlet tubing, 50 μm I.D. x 14 cm, for Capflow Sensor
162279	Outlet tubing, 50 μm I.D. x 10 cm, for Capflow Sensor
162282	Yellow Tefzel end cap for 1/16" nut

APPENDIX B

The UltiMate™ Dual Gradient System

B.1 Overview

This appendix describes how to install the UltiMate Dual Gradient System in conjunction with the CHROMELEON® Chromatographic Management System. In addition, it shows as an example of a typically application how to setup the system in the Parallel Nano-LC configuration (including some information about the installation of the LC Packings Nano Switching Valve).

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. For more details about the instruments, refer to the manuals provided with the instruments.

It is also assumed that the user is familiar with basic procedures and features in the CHROMELEON software. For more details about the CHROMELEON software, refer to the manual provided or to the On-Line Help (F1 key).

B.2 General Design

The UltiMate Dual Gradient System (FIGURE B-1) consists of two identical solvent delivery systems (gradient pump, flow splitting unit, low pressure mixing system, etc.). By selecting the appropriate dual calibrator cartridge, the system can be configured for different applications like Parallel Nano LC (e.g. a NAN/NAN calibrator cartridge is installed) or Comprehensive 2D Nano-LC (e.g. the NAN/CAP calibrator cartridge is installed).

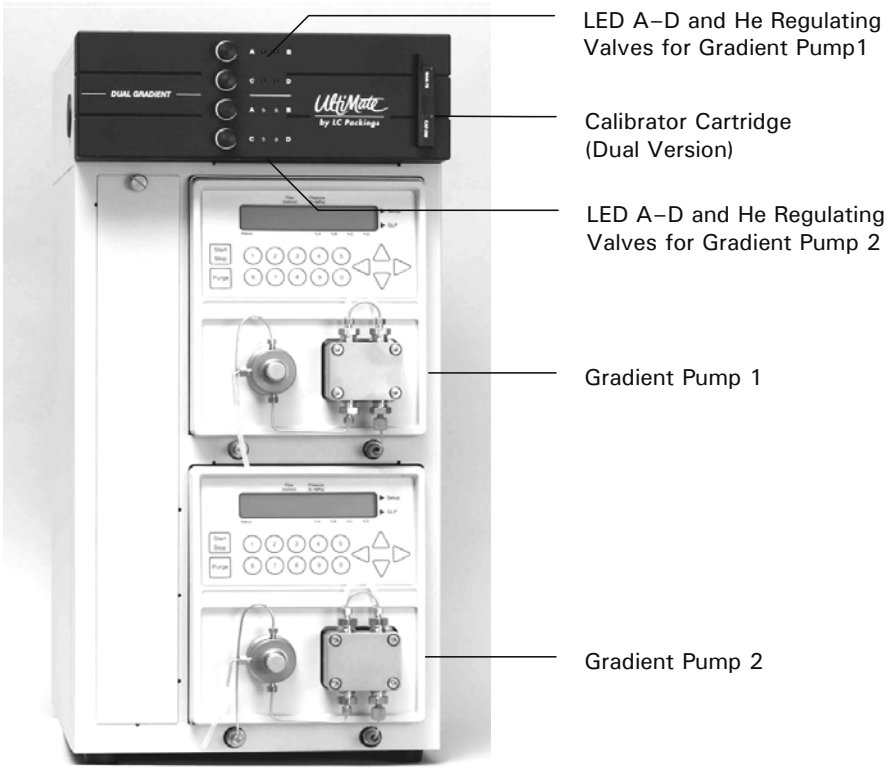


FIGURE B-1 Front View UltiMate Dual Gradient System

The system incorporates the following (main) modules:

- **Solvent Bottles and Degassing System** - provides mobile phase to the system. The Helium degassing system is provided to improve check valve reliability and diminish baseline noise (the solvent bottles and degassing system are located in the Solvent Organizer module).
- **Low Pressure Mixing System** – Two groups of 3 rapid response solenoid valves that are controlled by the two micropumps are used to generate the desired mobile phase compositions. Either isocratic or gradient mobile phases can be readily generated via the CHROMELEON software. The two low pressure mixing systems are located in the Solvent Organizer module.
- **Gradient Pump 1 and 2** - deliver the mobile phase that is generated by the two 3-channel low pressure mixing systems to the flowsplitting unit. The micropumps are controlled by the CHROMELEON software via a token-ring network.
- **Flowsplitting Unit** - consists of the dual calibrator cartridge and 2 waste restrictors (located in the Solvent Organizer). Flow rates from 50 nL/min to 500 μL/min can be provided. The different flow rates through the

microcolumns is determined by the flow rate of the Micropumps and the type of the calibrator cartridge.

- **Dual Calibrator Cartridge** - is located in the Solvent Organizer. A variety of calibrator cartridges are available and are readily interchanged as described in B.3.3-B.

- **Flow Sensor1 and 2 (Option)** - are located in the Solvent Organizer. Two different types of flow sensor are available (Nanoflow / Capflow) and are readily interchanged as described in Appendix A.

FIGURE B-2 presents a flow diagram of the system and FIGURE B-3 shows the fluidics compartment of the UltiMate Dual Gradient System (flow sensor option installed).

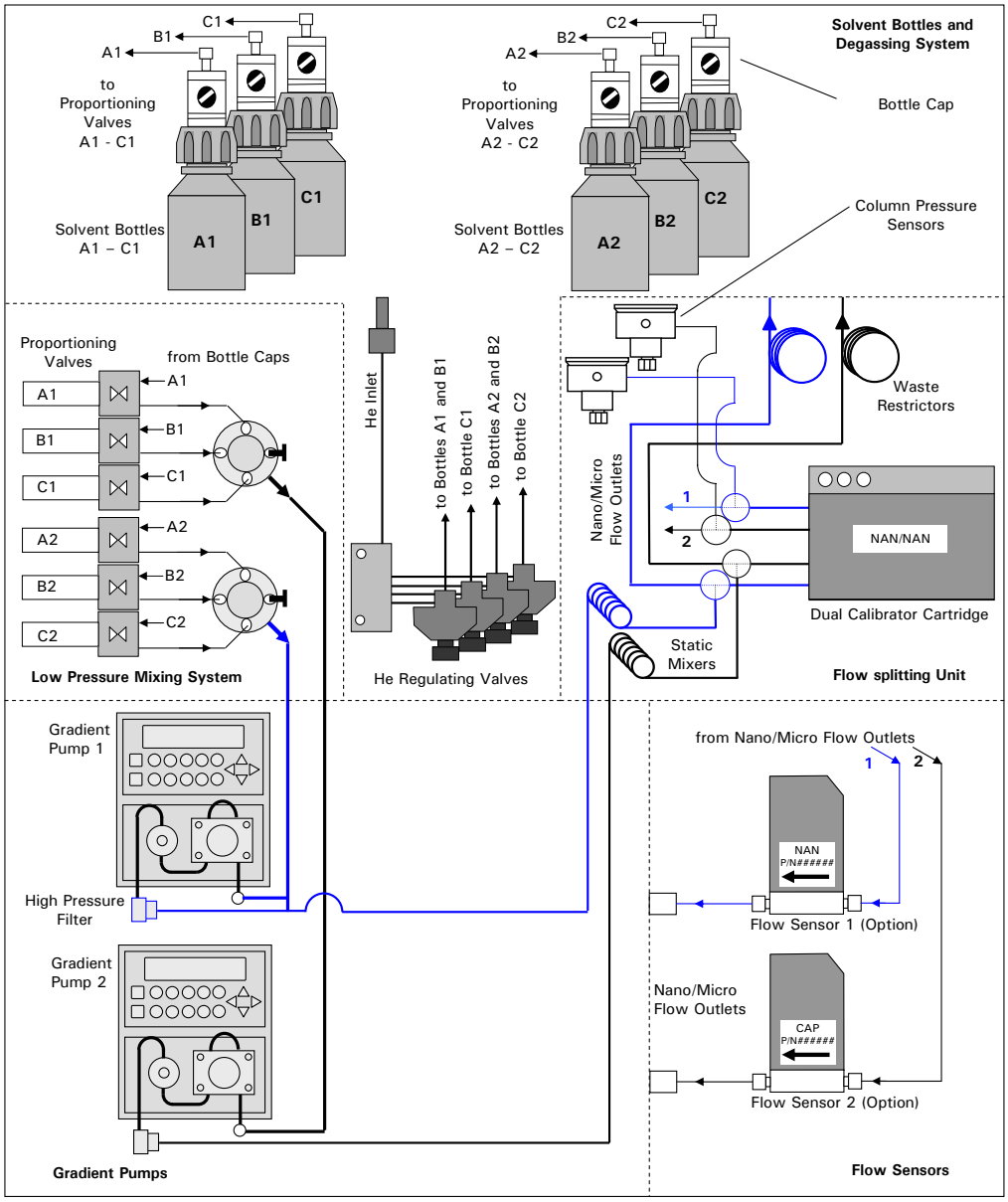
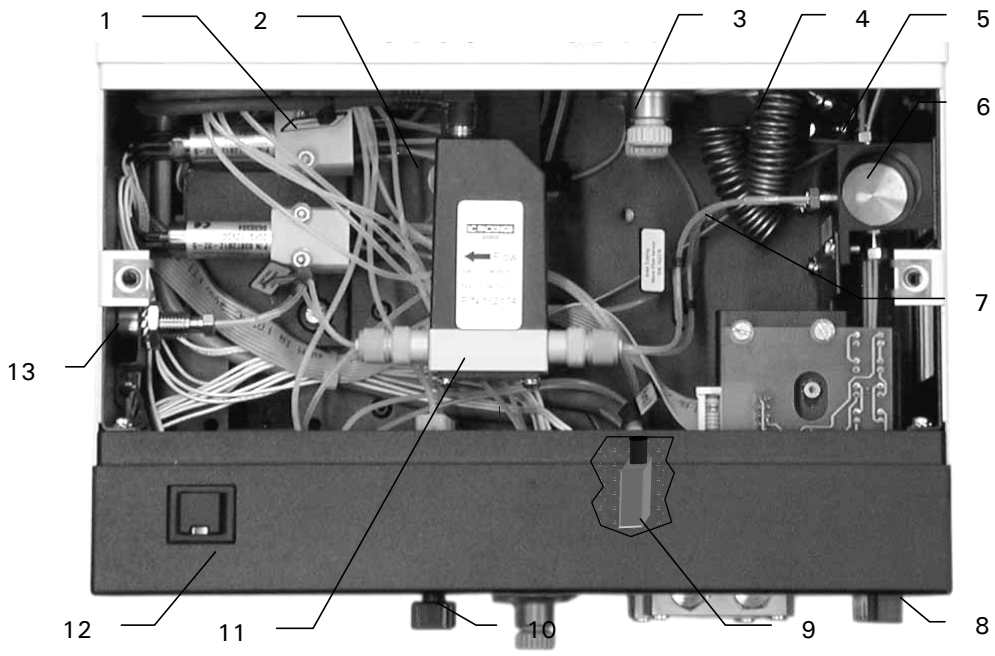


FIGURE B-2 Flow Diagram of the UltiMate Dual Gradient System



Item	Description	P/N Standard System	P/N Inert System
1	Proportioning Valves (A1–C1, A2–C2)	162297	162297
2	5 Port Mixing Manifold	160089	160089
3	Column Pressure Sensor	-	-
4	Static Mixer	160684	161059
5	Waste Restrictor	160077	161043
6	T-Pieces, Flow Splitter, Upper Lower	 160694 160695	 162299 162298
7	Connection Capillary to Flow Sensor	see Appendix A.4	
8	Calibrator		
9	5 Port He Manifold	160087	160087
10	He Regulating Valves	160086	160086
11	Flow Sensor (Option)	see Appendix A.4	
12	ON/Standby Switch	-	-
13	Nano/Micro Flow Outlet	-	-

FIGURE B-3 The Fluidic Compartment of the UltiMate Dual Gradient (with Flow Sensor Option)

B.3 Installation

B.3.1 PC Requirements

A minimum of 6 RS-232 (serial communication ports) is required. Either a Meilhaus or an Equinox serial COM port extension card is recommended (e.g. to add 8 COM ports, use a Meilhaus 8-Channel PCI Card, Dionex P/N 5906.2095).

B.3.2 Electrical Connections

All electrical connections are made via the rear panel of the UltiMate system (FIGURE B-4). The necessary cables are included with the instrument.

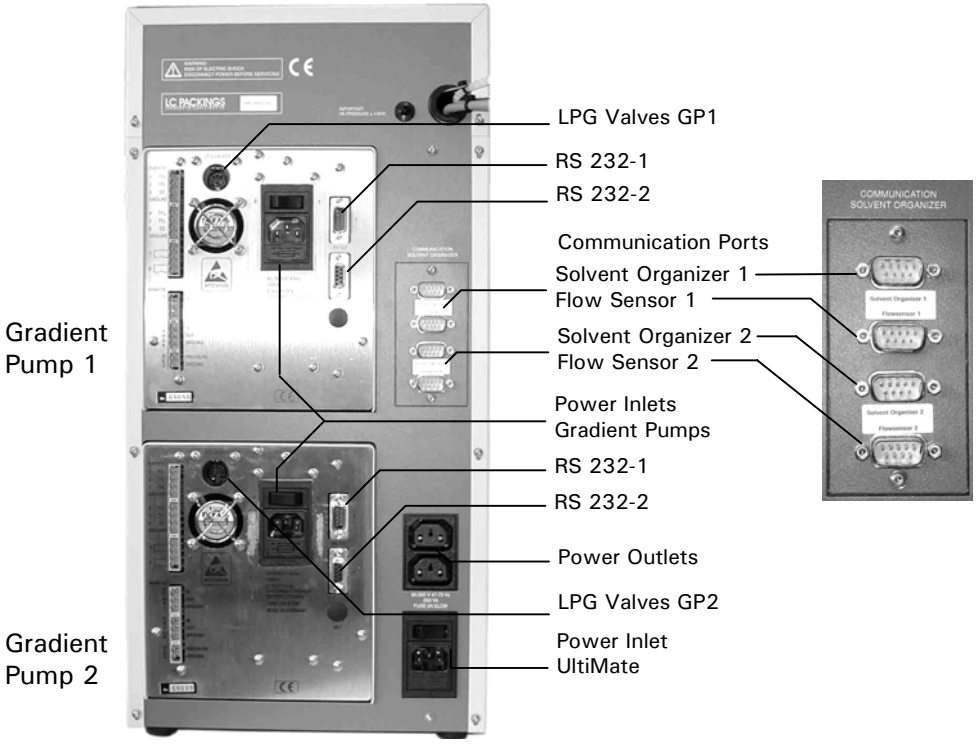


FIGURE B-4 Rear View of the UltiMate Dual Gradient System

The installation of the UltiMate Dual Gradient system requires the PC connections presented in FIGURE B-5.

Note: If the UltiMate system is to be installed in conjunction with the CHROMELEON software, it is recommended that you use the COM ports of the extension card only.

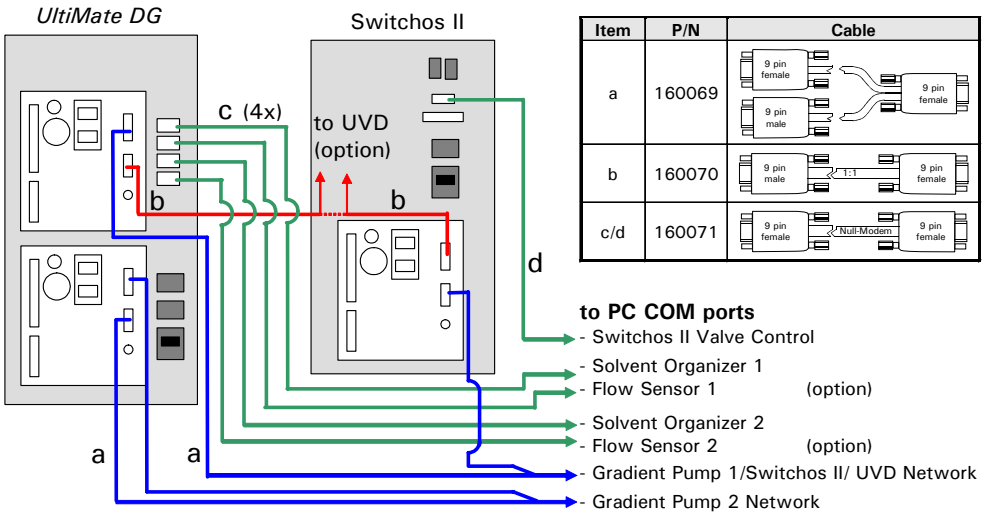


FIGURE B-5 PC Connections of the UltiMate Dual Gradient System (without Flow Sensors)

B.3.2-A RS-232 Network Connections (Y-Cable, Serial Communication Cable)

Connect the two Gradient Pumps as follows (FIGURE B-5):

- Connect the Gradient Pump 1, the Switchos Loading Pump (if available in the configuration) and the UV Detector (if available in the configuration) to a free COM port of the PC using a Y-Cable and (a) Serial Communication Cable(s).
- Connect the Gradient Pump 2 to a free COM port of the PC using a Y-Cable.

B.3.2-B COM Port-to-COM Port Connections

- Connect the Solvent Organizer 1 (of Gradient Pump 1) to a free COM port of the PC using the Solvent Organizer Cable (0-Modem-Cable).
- Connect the Solvent Organizer 2 (of Gradient Pump 2) to a free COM port of the PC using the Solvent Organizer Cable (0-Modem-Cable).
- Connect the Flow Sensor 1 (if available in the configuration) to a free COM port of the PC using the Solvent Organizer Cable (0-Modem-Cable).
- Connect the Flow Sensor 2 (if available in the configuration) to a free COM port of the PC using the Solvent Organizer Cable (0-Modem-Cable).
- Connect the Communication port of the Switchos II (if available in the configuration) to a free COM port of the PC using the Solvent Organizer Cable (0-Modem-Cable).
- Connect the FAMOS (if available in the configuration) to a free COM port of the PC using the FAMOS communication cable.

B.3.2-C The Solvent Organizer Cables

Carefully insert the black Solvent Organizer cables in the LPG VALVES connectors on the rear panel of the Gradient Pumps according to the labels attached to these cables (e.g. insert the cable labeled ‘Pump 1’ in the Gradient Pump 1, which is the upper pump).

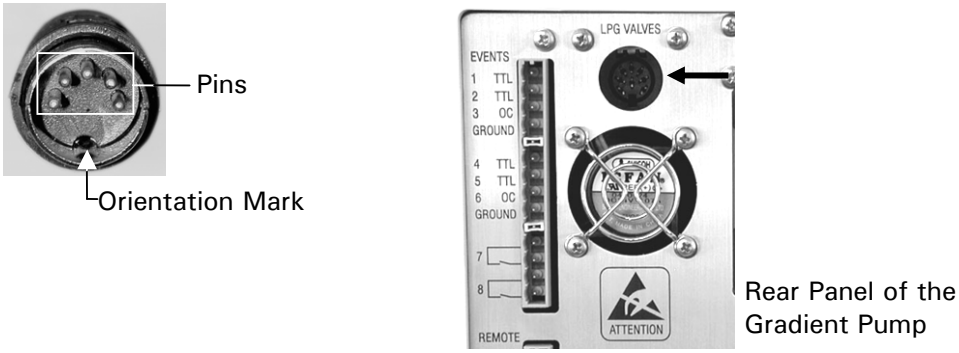


FIGURE B-6 Connector Plug to Solvent Organizer Module

Note: Make certain that the pins are positioned properly and the orientation mark on the plug is at the bottom before inserting it into the Gradient Pump

B.3.2-D Additional Input/Output Cables

The I/O Cable to control the valve position of 6-Port Nano Switching Valve is connected to the Switchos Loading Pump event output 7 (relay).

Additional cables for starting the mass spectrometer (MS) or other external devices (e.g. Probot™ Micro Fraction Collector) are needed. The MS accepts either the P5 Auxiliaries output of the FAMOS (contact closure signal) or one of the Ultimate Gradient Pumps Event outputs (depending on the type of input).

To clarify what type of the start signal is needed with your MS, refer to the user's manual of the MS system or contact your local MS support.

All electrical connections are made on the rear panels of the UltiMate Dual Gradient system components (including FAMOS and Switchos).

B.3.2-E He Connection

Connect the Helium line (1/4" O.D.) that is supplied to the Helium inlet on the back of the UltiMate (directly above the Gradient Pump 1). The Helium pressure should be set to approximately 1 bar.

CAUTION **Caution: Do not operate the He lines at a pressure greater than 4 bar (60 PSI).**

B.3.3 Fluidic Connections

B.3.3-A Nano/micro flow outlets

To connect the nano/micro flow outlets of the Gradient Pump 1 and the Gradient Pump 2 of the UltiMate system:

a) Remove the fluidics access plate from the solvent organizer (Section 3.7.5). The top view of the fluidics compartment is shown in (FIGURE B-3).

b) Remove the black cap from the hole that is located in the left side panel of UltiMate.

c) Connect the appropriate tubing to the nano/micro flow outlets (e.g. if you want to connect the FAMOS Microautosampler, refer to Section 2.5.2, TABLE 2-3).

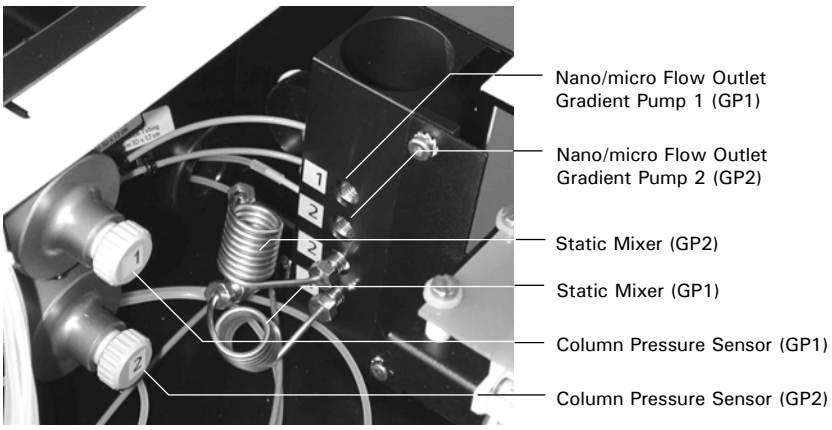


FIGURE B-7 The Nano/micro Flow Outlets of the two Gradient Pumps

d) Guide the capillaries through the hole in the side panel of the UltiMate and connect it to the instrument(s) that is(are) to be connected to the UltiMate system.

e) Replace the fluidics access plate.

Note: Section 2.5 provides details how to connect the UltiMate to a FAMOS Microautosampler and Section 2.6 shows how to connect the system to Switchos II. In addition, Appendix B provides instructions how to install the different Nano and Micro columns.

B.3.3-B Calibrator Installation

The UltiMate Dual Gradient system is shipped with the calibrator cartridge (FIGURE B-8) installed. Follow the instructions below if a different calibrator cartridge needs to be installed or replaced.

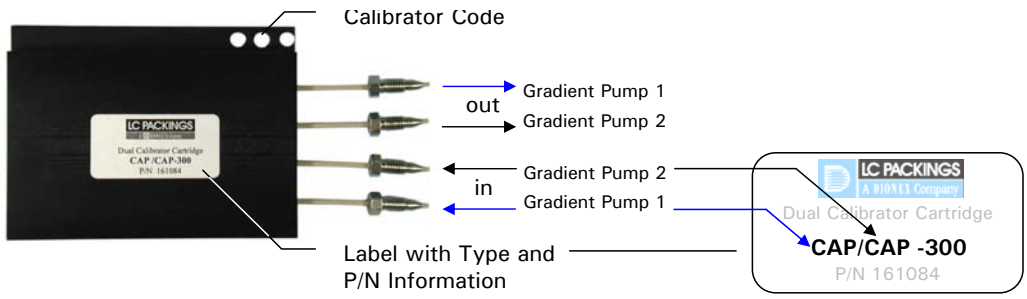


FIGURE B-8 The Dual Calibrator Cartridge (CAP/CAP-300)

The system is provided in different configurations and a variety of calibrators are available (FIGURE B-8 and TABLE B-1). The calibrator(s) that is(are) provided for a given system is dependent on the application, the column sizes and flow rates that are to be used (see the shipping list for details).

TABLE B-1 Dual Gradient Calibrator Cartridge Selection Guide

Calibrator Type	Flow Rate Gradient Pump 1 (a)	Flow Rate Gradient Pump 2 (a)	P/N
NAN/NAN-75	300 nL/min [625]	300 nL/min [625]	161082
CAP/CAP-300	4 µL/min [50]	4 µL/min [50]	161084
NAN/CAP-75/300	300 nL/min [625]	4 µL/min [50]	161083

a) typical flow rate at the specified CRP value [nnn]

The appropriate calibrator should be installed by placing it in the slot on the upper front of the UltiMate and carefully tightening the nuts (do not overtighten).

B.3.3-C Waste Bottle

Insert the waste lines (rear side) into a waste reservoir of sufficient volume. The reservoir should be able to hold at least 1 L of mobile phase.

B.4 Application Example – Parallel Nano-LC Fluidic Setup

The fluidic setup that is required for the Parallel Nano-LC application is presented in FIGURE B-9.The figure shows the flow path and the positions of the three valves during the elution of the first precolumn (performing the first solvent gradient) and the loading of the second precolumn. TABLE B-2 lists the required port connections and tubing dimensions.

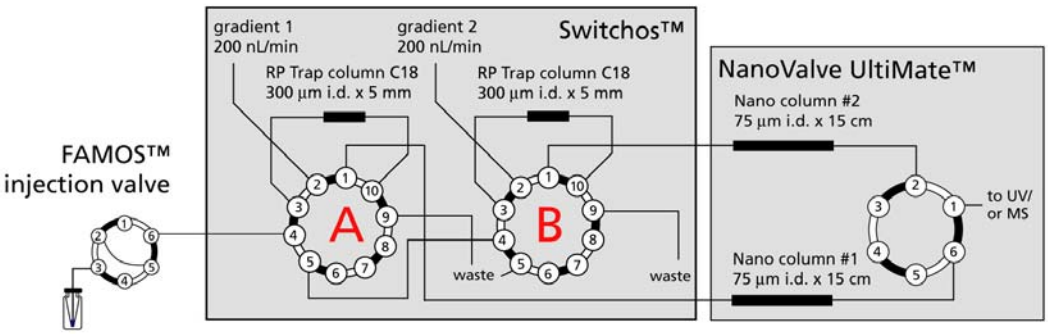


FIGURE B-9 Fluidic Connections for the Parallel Nano-LC Application

B.4.1 Fluidic Connections – Switchos Valves

Connect the switching valve A and the switching valve B of the Switchos unit as presented in TABLE B-2.

TABLE B-2 Description of the Fluidic Connections for the Parallel Nano-LC Application

Switchos [Valve. Port No.]	**Connected to**
A.1	1st PepMap Nano Column
A.2	1st Ultimate Gradient
A.3	1st Trap Column
A.4	FAMOS Injection Valve ; 130µm x 40cm
A.5	Valve B, Port 4; 130µm x 20cm
A.6	(not in use)
A.7	(not in use)
A.8	(not in use)
A.9	Waste
A.10	1st Trap Column
B.1	2nd PepMap Nano Column
B.2	2nd Ultimate Gradient
B.3	2nd Trap Column
B.4	Valve A, Port 5 ; 130µm x 20cm
B.5	Waste
B.6	(not in use)
B.7	(not in use)
B.8	(not in use)
B.9	Waste
B.10	2nd Trap Column

B.4.2 Fluidic Connections – 6-port Nano Valve

Connect the Nano Switching Valve as presented in TABLE B-3. Use the supplied 280 µm O.D. / 20 µm I.D. fused silica capillary (P/N 160475) and a 300 µm PEEK sleeve (P/N 162139). Follow the instructions provided in the users' manual of the Nano Switching Valve.

TABLE B-3 Description of the Fluidic Connections for the Parallel Nano-LC Application

Port Number	Connected to
1	Mass spectrometer
2	Nano column 2
3	Waste
4	Not used
5	Waste
6	Nano column 1



Note: For optimal performance it is important to keep all connections as short as possible. The length of fused silica tubing that is required is dependent on the location of your Mass spectrometer, the UltiMate system and the Nano Switching Valve.

B.5 CHROMELEON Setup – Parallel Nano LC

This section provides the specific settings for UltiMate Dual Gradient (DG) systems only. In addition, the setup of a 'Comprehensive Two-dimensional Separation' will be discussed as an application example.

Note: To control the UltiMate Dual Gradient system, CHROMELEON 6.50 SP2 or higher is required.

Note: The programs and panels as described below are provided with the CM6.5/SP2 CD ROM and can be found in the folder 'UltiMateDualGradient Examples'.

B.5.1 Server Configuration

To operate the UltiMate DG in conjunction with CHROMELEON, two 'LC Packings UltiMate/Switchos' devices (drivers) must be configured in the CHROMELEON Timebase. In order to use the programs and panels provided with the CHROMELEON CD, it is required that you change component names as described below. If you use different names, CHROMELEON may generate an error message and predefined programs or panels can not be used. The Gradient Pump 1 is configured as described in Chapter 2.

B.5.1-A Configuring the Gradient Pump 2

To configure the Gradient Pump 2 in the CHROMELEON Timebase:

f) Add a second 'LC Packings UltiMate/Switchos' device to the Timebase as shown in FIGURE B-10.

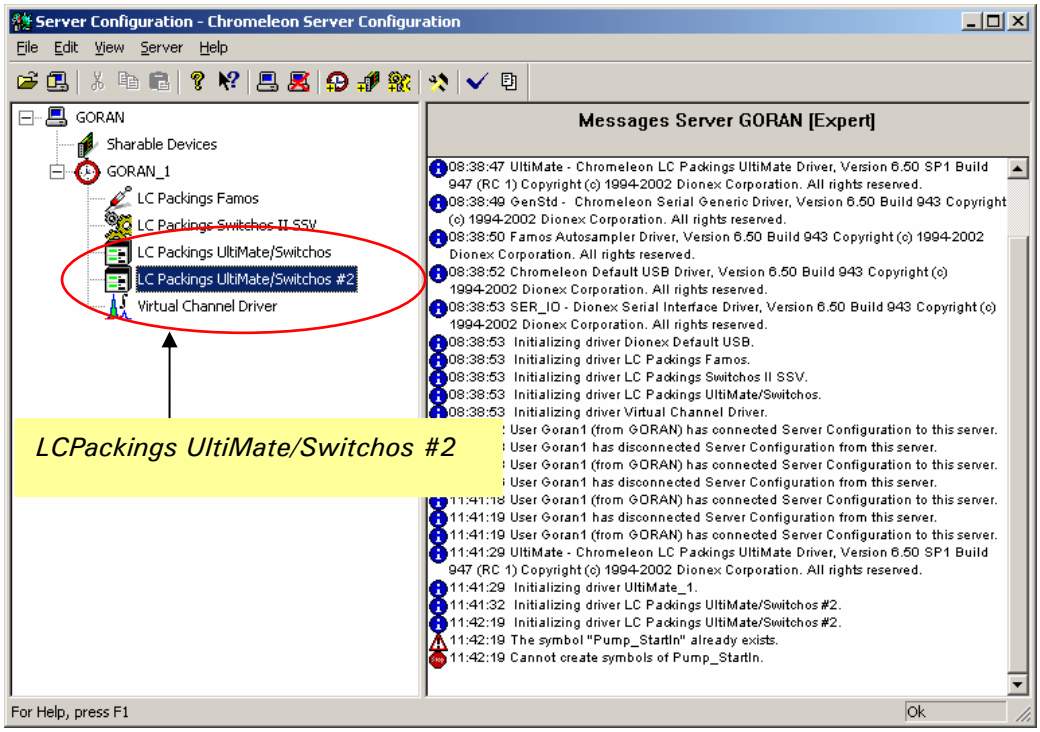


FIGURE B-10 Server configuration for UltiMate Dual Gradient

g) Rename the **Device Name** to 'UtliMate_System2' (FIGURE B-11).

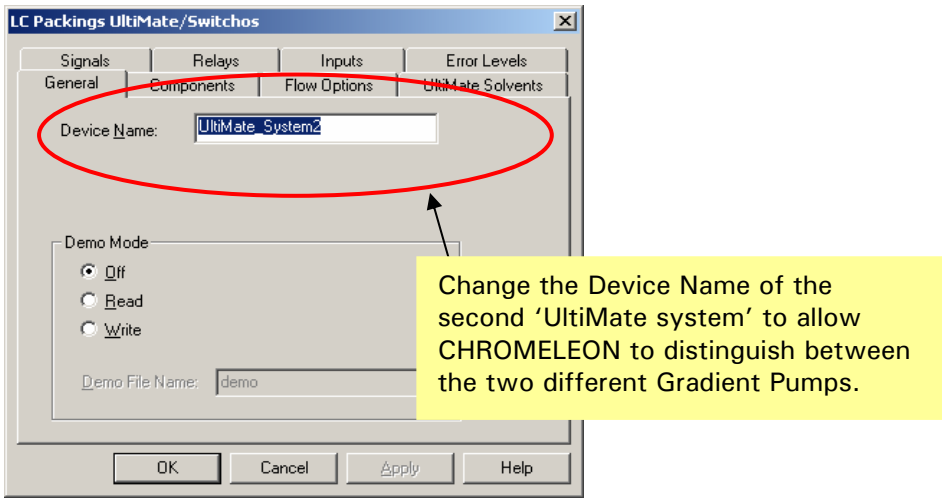


FIGURE B-11 Configuration of the 'LC Packings UltiMate/Switchos#2' Device

h) Rename the names all *Components* used with the second 'Ultimate system' by adding the suffix '2' (FIGURE B-12).

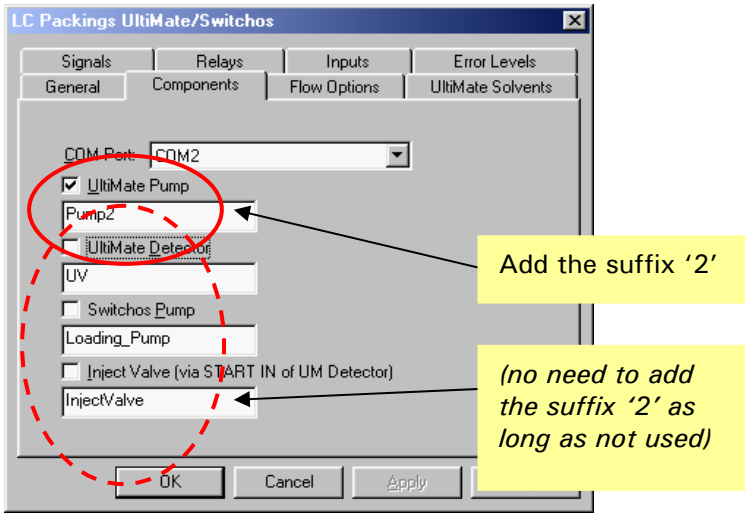


FIGURE B-12 Configuration of the UltiMate/Switchos Components

i) Rename the column name on the *Flow Options* tab to 'column2'. Uncheck the **Oven** box, there is no second oven supported (FIGURE B-13).

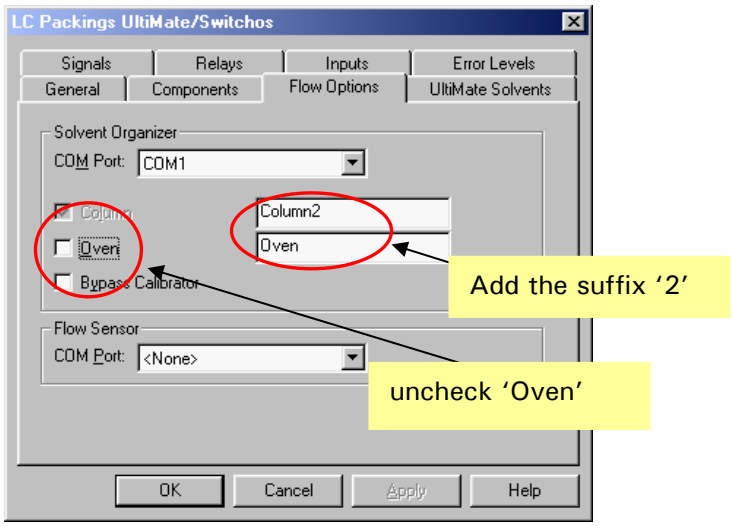


FIGURE B-13 Configuration of the Flow Options Tab

j) Rename the START IN input on the *Inputs* tab to 'Pump2_StartIn' (FIGURE B-14).

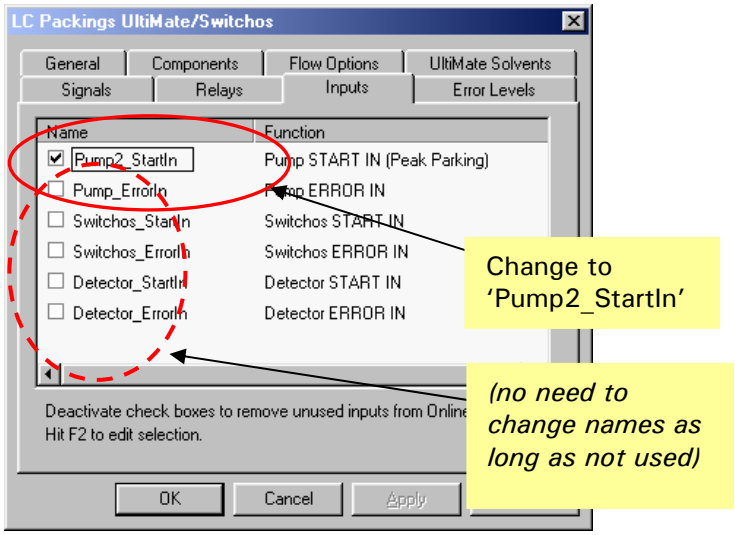


FIGURE B-14 Configuration of the UltiMate/Switchos components

In order to acquire all significant signals from both systems, add channels to the Virtual Channel Driver as shown in (FIGURE B-15).

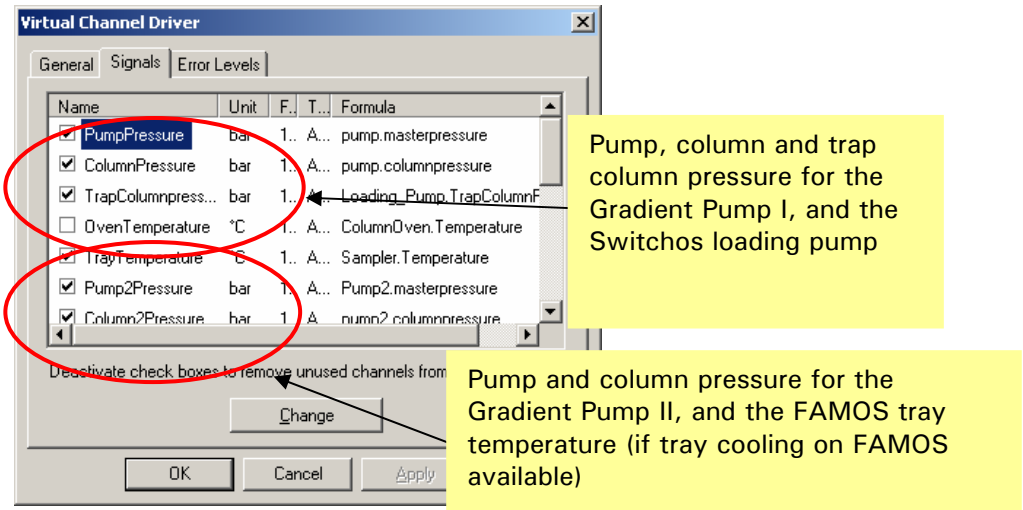


FIGURE B-15 Signals Tab for Virtual Channel Drivers

k) Use the signal names and formulas indicated in TABLE B B-4 to record the pressure signals:

TABLE B B-4 Formulas to record the different Signals – Parallel LC

Signal Name	CHROMELEON Formula
PumpPressure	pump.masterpressure
Pump2Pressure	pump2.masterpressure
ColumnPressure	pump.columnpressure
Column2Pressure	pump2.columnpressure
TrapColumnPressure	loading_pump.trapcolumnpressure
TrayTemperature	Sampler.Temperature
ColumnOvenTemperature	oven.temperature

l) To disable the acquiring of UV data, uncheck the appropriate boxes in the *Signals* tab of the 'LCPackings UltiMate/Switchos#2' driver.

Depending on the hardware requirements of the MS, use either 'Pump Output_1(TTL)' or 'Pump Output_7 (Relay)' to trigger the MS. Rename the event as 'Start_MS' as shown in FIGURE B-16.

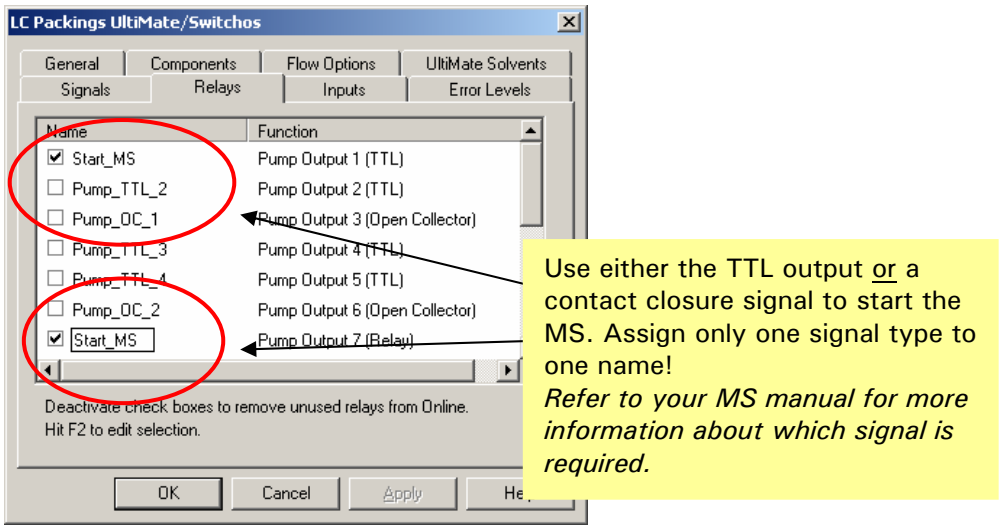


FIGURE B-16 Relay tab with configured Pump Outputs

B.5.1-B Controlling the Nano Switching Valve

To control the 6-port Nano switching Valve use event 7 (relay) of the UltiMate Gradient Pump 1. Make sure that the Nano Switching Valve is configured that an open relay contact drives the valve into position 1-2, while a closed contact will drive it into position 6-1.

Check the option Switchos_Relay_1 on the *Relay* tab and rename the signal to 'Nanovalve' (FIGURE B-17).

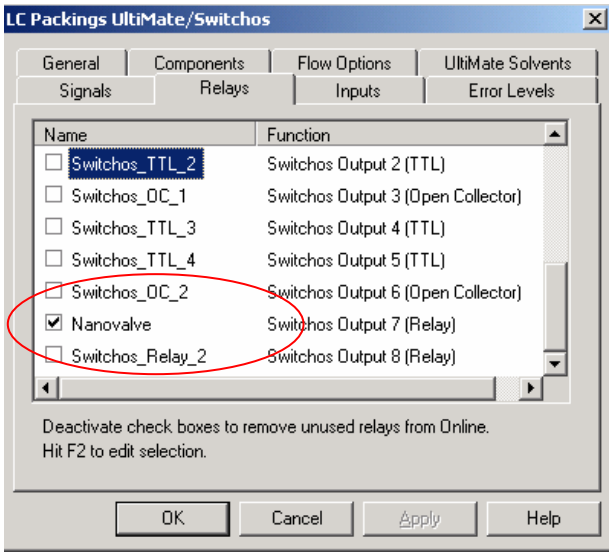


FIGURE B-17 Relay Tab with configured Switchos Outputs

B.6 The Control Panel

The UltiMate Dual Gradient system for the Parallel Nano-LC application is controlled via the Control Panel as shown in FIGURE B-18.

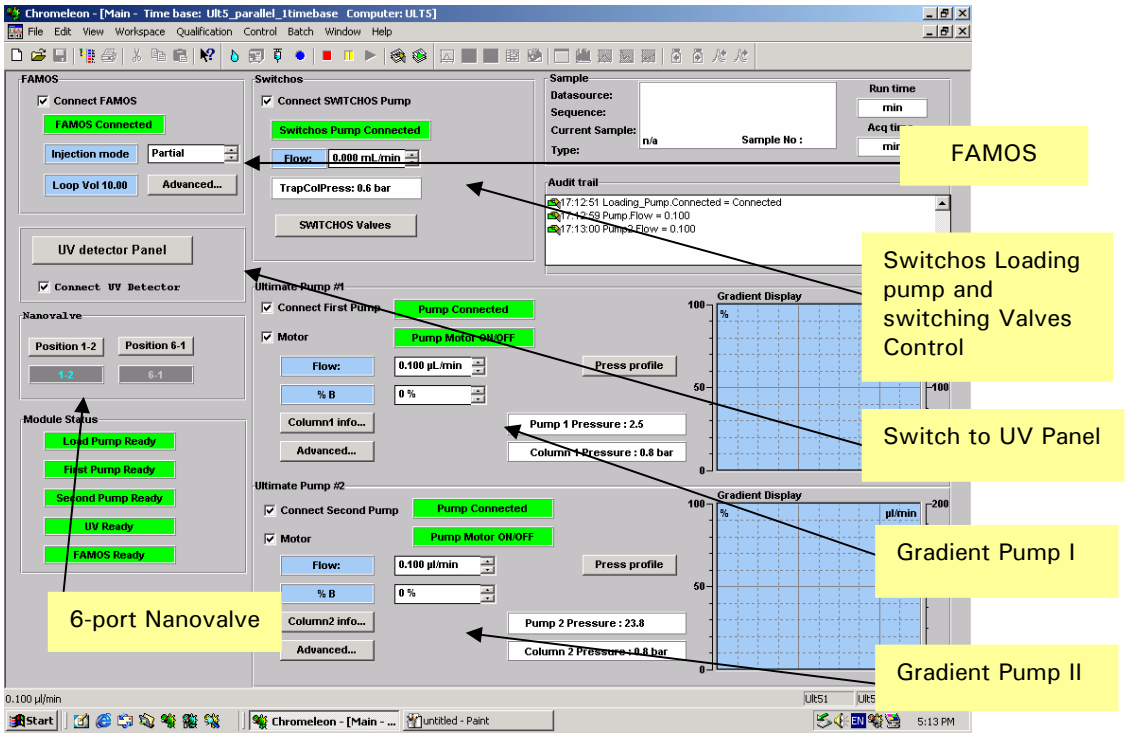


FIGURE B-18 CHROMELEON Control Panel for Parallel Nano-LC

The UV Detector (if installed) is controlled from a second control panel, which can be opened by clicking the UV Detector button (FIGURE B-19).

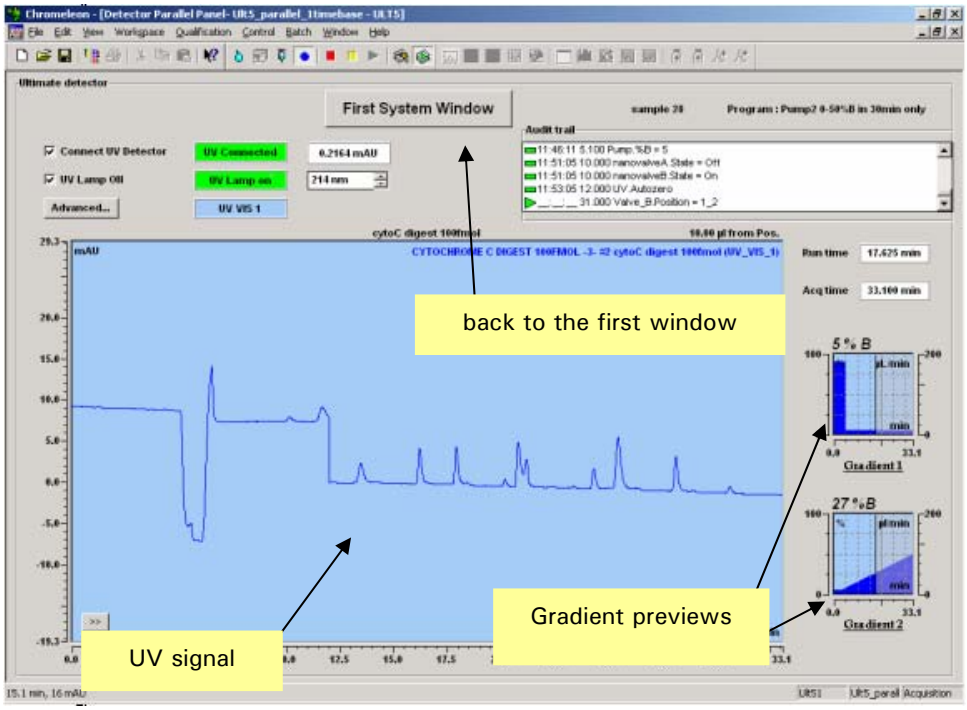


FIGURE B-19 Control Panel for the UV Detector

B.7 CHROMELEON Setup – 2-D Nano LC

The configuration of a the UltiMate Dual Gradient system for a 2-D Nano LC application is identical to the steps a) to e) as discussed in Section B.3.3 except that some signal names of the Virtual Channel Driver are different (TABLE B-5). The following section describes the setup of the Virtual Channel Driver.

Note: To control the UltiMate Dual Gradient system, CHROMELEON 6.50 SP2 or higher is required.

Note: The programs and panels as described below are provided with the CM6.5/SP2 CD ROM and can be found in the folder 'UltiMateDualGradient Examples'.

TABLE B-5 Formulas to record the different Signals – 2-D Nano LC

Signal Name	CHROMELEON Formula
PumpPressure	pump.masterpressure
Pump2Pressure	pump2.masterpressure
ColumnPressure	pump.columnpressure
SCX_ColumnPressure	pump2.columnpressure
TrapColumnPressure	loading_pump.trapcolumnpressure
TrayTemperature	sampler.temperature
ColumnOvenTemperature	oven.temperature

To setup the Virtual Channel Driver for a 2-D Nano CL application:

a) In order to acquire all significant signals from both systems, add 7 channels to the Virtual Channel Driver.

b) Rename the signals using the signal names and formulas indicated in TABLE B-5.

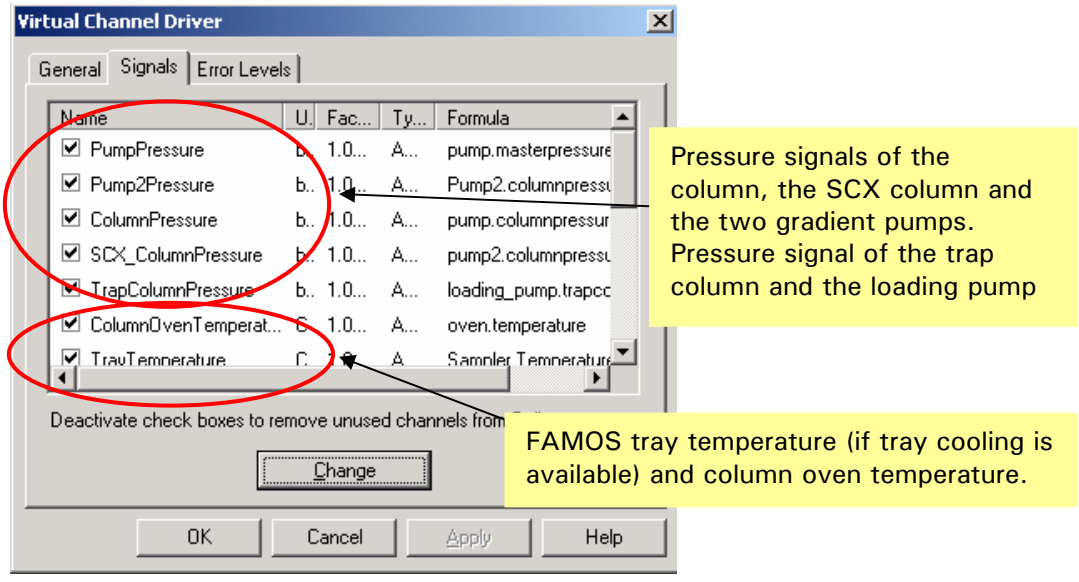


FIGURE B-20 Signals Tab for Virtual Channel Drivers

c) To disable the acquiring of UV data, uncheck the appropriate boxes in the *Signals* tab of the 'LCPackings UltiMate/Switchos#2' device.

Depending on the hardware requirements of the MS, use either 'Pump Output_1(TTL)' or 'Pump Output_7 (Relay)' to trigger the MS. Rename the event as 'Start_MS' as shown in FIGURE B-21.

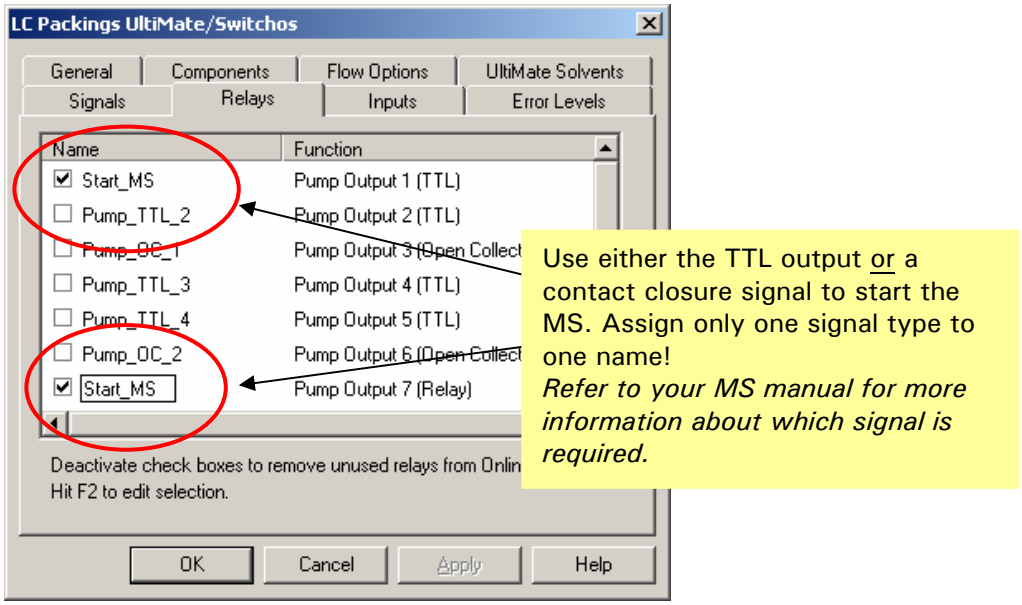


FIGURE B-21 Relay Tab with configured Pump Outputs

The UltiMate Dual Gradient system for the 2-D LC application is controlled via the **Control Panel** as shown in FIGURE B-22.

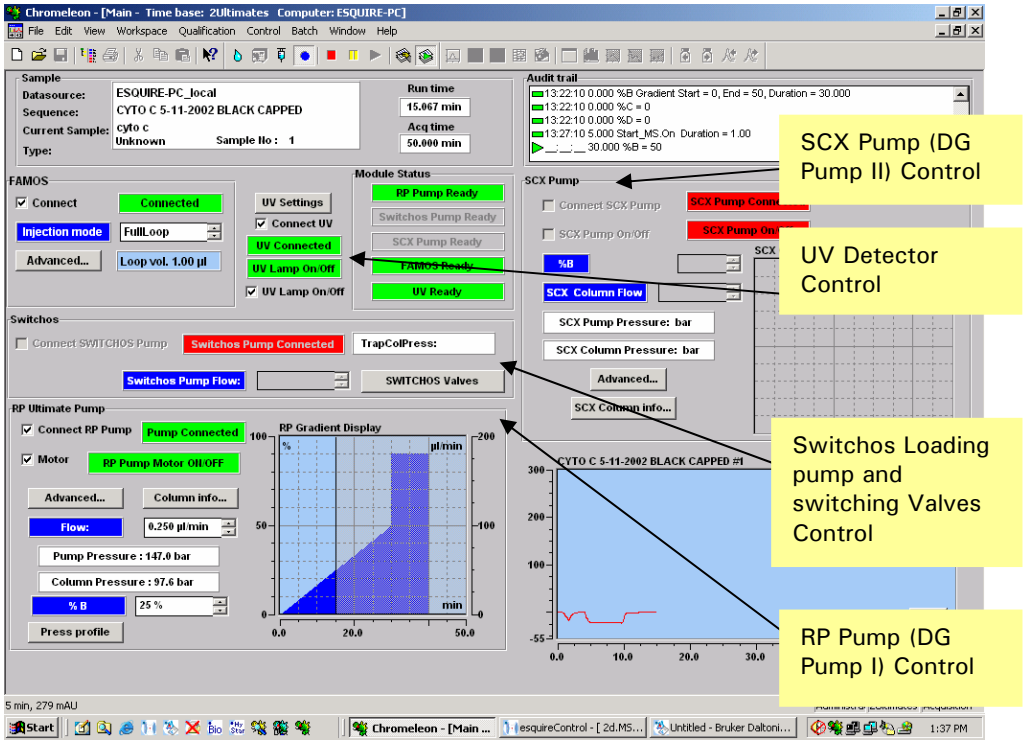


FIGURE B-22 CHROMELEON Control Panel for 2-D LC

B.8 Spare Parts List

The following table lists spare parts which are different to those used in the standard UltiMate Capillary HPLC system (e.g. the dual calibrator cartridges). Please refer to Chapter 3 for solvent organizer parts (e.g. a waste restrictor), to Chapter 4 for gradient pump spare parts (e.g. a check valve cartridge) and to Appendix A if you are looking for flow sensor spare parts.

TABLE B-6 Spare Parts UltiMate Dual Gradient

Part Number	Description
	Solvent Organizer
162297	Proportioning Valve for UltiMate Dual Gradient
161082	Dual Gradient calibrator cartridge for Nano/Nano LC
161084	Dual Gradient calibrator cartridge for Capillary/Capillary LC
161083	Dual Gradient calibrator cartridge for Capillary/Nano LC
	Nanoflow Sensor
162174	Nanoflow Sensor for UltiMate Nano LC System
162278	Inlet tubing, 20 µm I.D. x 14 cm, for Nanoflow Sensor
162274	Outlet tubing, 20 µm I.D. x 10 cm, for Nanoflow Sensor
	Capflow Sensor
162202	Capflow Sensor for UltiMate Capillary LC System
162276	Inlet tubing, 50 µm I.D. x 14 cm, for Capflow Sensor
162279	Outlet tubing, 50 µm I.D. x 10 cm, for Capflow Sensor
	Connecting Tubing (e.g. to FAMOS)
162203	Connecting tubing, 20 µm I.D. x 50 cm, for UltiMate with flow sensor
162273	Connecting tubing, 20 µm I.D. x 50 cm, for UltiMate INERT with flow sensor
162275	Connecting tubing, 50 µm I.D. x 50 cm, for UltiMate INERT with flow sensor
162277	Connecting tubing, 50 µm I.D. x 50 cm, for UltiMate with flow sensor
162282	Yellow Tefzel end cap for 1/16" nut

APPENDIX C

Column Installation Instructions

C.1 Overview

Nano Series™, Pico Series™ and Microbore/Microprep™ and 1 mm I.D. Micro columns are especially suited for Capillary- or Nano LC. Their unique column design and packing procedures result in high efficiency and resolution, as well as ease of installation in the UltiMate Capillary HPLC System or other micro HPLC systems.

The easy-to-handle trap column cartridge system is used for concentration of diluted samples in Capillary- or Nano LC as well as for desalting of proteins and peptides prior to MS analysis and in multidimensional chromatography.

In order to achieve the highest performance, the guidelines provided in this appendix must be observed. In addition, please refer the instruction sheet that is provided with the product for latest information.

C.2 Installation of the Column

When installing the column in the oven compartment, guide the appropriate connecting capillary (the dimensions depend on your application, see Sections 2.5.2 and 3.10) through the foam material on top of the oven into the fluidic compartment. Then guide the capillary through the hole on the left side and connect it to the FAMOS injection valve or to the manual injection valve (if installed).

When installing a Nano column, use one of the slots around the oven cover plate to keep the connection as short as possible (e.g. item 1, FIGURE C-1).

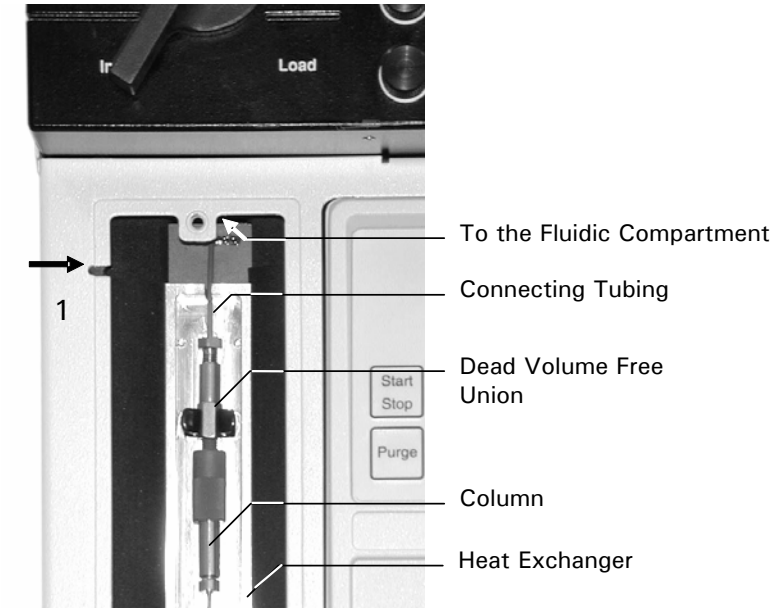


FIGURE C-1. Installing of a 180 µm – 800 µm Column in the UltiMate Oven

Note: When you install a column, make certain that the connection does not have any dead (void) volume. Use appropriate connecting tubing (Sections 2.5.2) and a calibrator cartridge (Section 2.4.3).

CAUTION

Caution: Use the columns only in the flow direction indicated by the arrow on the column label (FIGURE C-2).

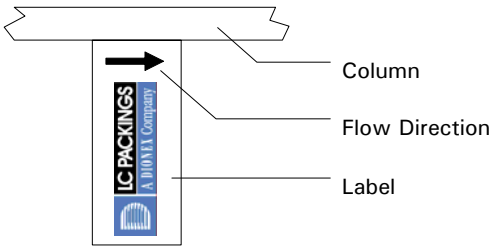


FIGURE C-2. Column Label Indicating Flow Direction

Note: After the column is installed, equilibrate the entire system by flushing with a minimum of 5 times the column volume of the mobile phase.

The following sections provide detailed installation procedures for various columns:

- 1 mm I.D. Micro Columns (Section A)
- 800 µm I.D. Microbore/Microprep™ Columns (Section B)
- 300 µm I.D. Capillary Column/180 µm I.D. Pico Series Columns (Section C)
- 50, 75, 100 µm I.D. Nano Series Columns (Section D)

For additional information about the installation, care and use of the column, refer to the instruction sheet supplied with the column.

Note: When installing the column in the UltiMate System use appropriate connecting tubing (Sections 2.5.2) and a calibrator cartridge (Section 2.4.3).

A. 1 mm I.D. Micro Columns

To install a 1 mm I.D. Micro Column:

a) Remove the yellow protective cap from the column inlet.

b) Connect the PEEK inlet tubing with the universal 1/16" fingertight fitting or a stainless steel nut and ferrule directly to the injection valve (e.g. the FAMOS injection valve) or to the union of the connecting tubing, FIGURE C-3).

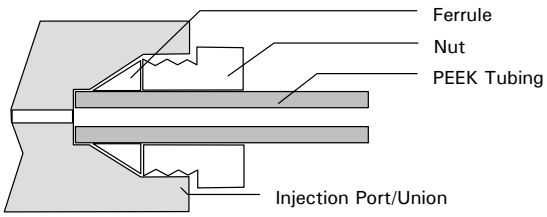


FIGURE C-3. PEEK tubing with Nut and Ferrule

c) To avoid the formation of dead volumes, make certain that the column inlet enters all the way to the bottom of the injector port or the union, respectively.

The 1 mm I.D. Micro Column can be directly connected to the 140 nL micro flow cell of the UltiMate UV Detector (or any kind of detector equipped with the U-Z View™ Capillary Flow Cells).

To connect the column outlet to the flow cell of the detector, use the same type of connection as described in steps a) and c).

Note: When connecting the 1 mm I.D. Micro Column to a flow cell other than the LC Packings 140 nL U-Z View™ Capillary Flow Cell, make certain that the cell volume does not exceed 200 nL to avoid extra band broadening (that will result in peak tailing, etc.).

CAUTION **Caution: Cut the 60 µm I.D. PEEK inlet or outlet tubing according the PEEK tubing cutting instructions as described in Section C.4.**

B. 800 µm I.D. Microbore/Microprep™ Columns

To install a Microbore/Microprep column:

a) Remove the yellow protective cap.

b) Connect the column inlet with the universal 1/16" fingertight fitting directly to the injection valve (e.g. the FAMOS injection valve) or to the union of the connecting tubing.

c) To minimize the dead volume, make certain that the column inlet goes all the way to the bottom of the injector port or the union, respectively.

Note: It is highly recommended that a µ-Guard™ column (e.g. P/N 160662) be employed to protect the analytical column. These columns are available with a variety of packings (for additional information, please refer to the LC Packings price list).

The column can be directly connected to the flow cell of the UltiMate UV Detector (or any kind of detector equipped with the U-Z View™ Capillary Flow Cells) using the Teflon® connectors provided with the column.

To connect a Microbore/Microprep column to the flow cell of the detector:

d) For optimal connection, make certain that both capillaries (column outlet and flow cell inlet) present a clean square cut.

e) Connect the capillary ends using the Teflon connector (FIGURE C-4). The connection can withstand pressures up to 10 bar (150 psi).

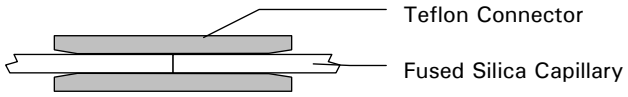


FIGURE C-4. Connecting Fused Silica Capillaries

f) To minimize the chance of clogging, push first one capillary all the way through the Teflon connector.

g) Cut 5 mm off the end of the capillary, and then pull the capillary end back to the middle of the Teflon connector. Connect it to the other capillary.

For connections with microflow cells other than the U-Z View Flow Cell, use the PEEK sleeve provided with the column. Use the PEEK sleeve and mount a nut and ferrule corresponding to the type of fitting (FIGURE C-5).

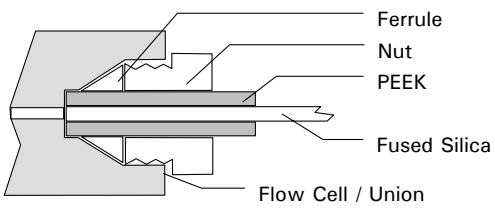


FIGURE C-5. Using a PEEK Sleeve

C. 300 µm I.D. Capillary Column/180 µm I.D. Pico Series Columns

To connect the 300 µm I.D. capillary column or the 180 µm I.D. Pico Series column to the injection valve (e.g. the FAMOS injection valve) or to the union of the connecting tubing:

- Follow instructions a) – c) in Section C.2, part B.

To connect the 300 µm I.D. capillary column or the 180 µm I.D. Pico Series column to the flow cell of the UltiMate UV Detector:

- Follow the instructions d) - g) in Section C.2, part B.

Note: For connections with capillaries other than 280 µm O.D. (± 20 µm), a Teflon tubing kit is available (P/N 160490). This kit allows connection to nearly any kind of fused silica tubing with an O.D. between 180 – 320 µm O.D. Advantages of connections made with transparent Teflon tubing include the possibility of visual inspection, the reduction of dead volume and ease to use.

For high pressure applications, use either the high pressure connecting kit (P/N 160705) or a Microtight® union (P/N 161497) in conjunction with the appropriate sleeves (e.g. 280 µm O.D. sleeve, P/N 161498). Section C.3 provides details about how to install the Microtight union.

D. 50, 75, 100 µm I.D. Nano Series Columns

To connect the Nano Series column to the injection valve (e.g. the FAMOS injection valve) or to the union of the connecting tubing:

a) Insert one of the column inlet filters (P/N160497) into the injection valve/union. Make certain that the filter comes to rest at the bottom of the port (FIGURE C-3).

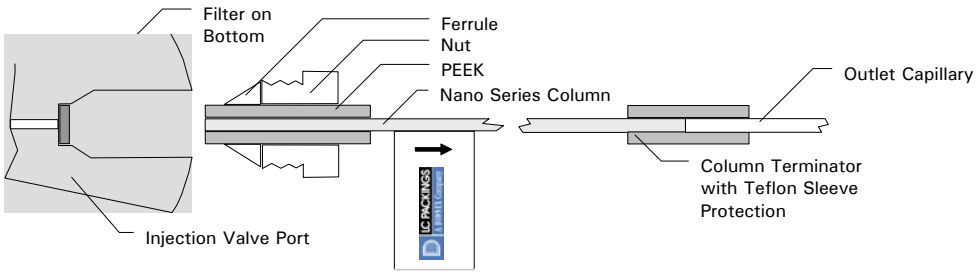


FIGURE C-6. Installation Inlet Filter/Nano Column

b) Connect the column with a PEEK sleeve connector directly to the port.

CAUTION **Caution: Make certain that you do not turn the column and the sleeve while tightening the fitting. If the column inlet filter is turned, it could get twisted and may lead to clogging.**

Note: Replace the column inlet filter (P/N160497) on a regular basis to prevent clogging. The outlet terminator is protected by a Teflon sleeve that should not be removed.

To connect the Nano Series column to the UZ View flow cell of the UltiMate UV Detector:

c) Use a Teflon connector (FIGURE C-4) and follow the instructions d) - g) in Section C.2, part B.

A major advantage of the UV-View Nano Series flow cells is the capability of on-line UV/MS detection (e.g. the outlet capillary of the flow cell allows for direct connection to the mass spectrometer interface with virtually no dispersion). For extension of the connecting line use fused silica tubing with an I.D. equal or smaller the 20 µm only (e.g. P/N 160475).

It is also possible to directly connect the outlet capillary of the Nano Series column to the MS interface.

Note: For connections with capillaries other than 280 µm O.D. (± 20 µm), a Teflon tubing kit is available (P/N 160490). This kit allows connection to nearly any kind of fused silica tubing with an O.D. between 180 – 320 µm O.D. Advantages of connections made with transparent Teflon tubing include the possibility of visual inspection, the reduction of dead volume and easy to use.

For high pressure applications, use either the high pressure connecting kit (P/N 160705) or a Microtight® union (P/N 161497) in conjunction with the appropriate sleeves (e.g. 280 µm O.D. sleeve, P/N 161498). Section C.3 provides details how to install the Microtight union.

CAUTION **Caution: It is not recommended to back flush Nano Series column, packing material may be flushed from the column.**

C.3 High Pressure Connection

In a typical application, the column outlet does not have to withstand high pressure (typically less than 5 bar) and therefore a standard Teflon sleeves is used. If a high pressure connection is required, use a Microtight® union (P/N 161497) in conjunction with the appropriate sleeves.

Note: For optimal connection, always make certain that all capillaries present a clean square cut.

To install the Microtight union:

a) Screw the gauge plug in the Microtight union (FIGURE C-7).

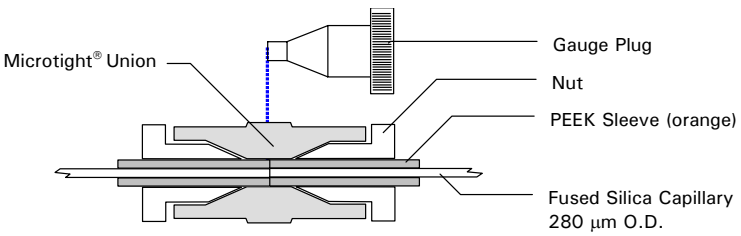


FIGURE C-7. High Pressure Connection using a Microtight union.

b) Put in the capillary using an orange sleeve (P/N 161498) and tighten the nut (FIGURE C-7).

c) Remove the gauge plug and attach the second capillary in the same way.

Note: Make certain that you use the right sleeve (a 280 µm O.D. capillary requires an orange sleeve).

C.4 PEEK Tubing Cutting Instructions

In order to cut the PEEK tubing (e.g. the 60 µm tubing of the 1 mm I.D. column) to make a flat 90 degree, burr-free cut that will not clog and will maintain the bore in the center of the tube, the following steps should be used.

Note: Use a 'guillotine' type cutter with a 1/16" guide hole and sufficient spring tension (P/N 160484) only to hold the tubing in a fixed position.

a) Open the cutter by loosening the spring tension and insert the tubing in the 1/16" guide hole.

b) Rotate the cutter 6- 8 times around the tubing.

c) Remove the cutter by loosening the spring tension.

d) Pull at the both ends to break the tubing into two parts.

C.5 Column Removal

CAUTION **Caution: Never remove a column before the decompression is fully completed.**

To prepare the column for removal:

a) Stop the flow and wait until decompression is completed. This usually takes 5-10 minutes.

b) Never remove the column if the column pressure is higher than 5 bar (70 psi). This may damage the column.

c) Remove the column by disconnecting the column outlet first.

d) Avoid unnecessary column removal, keep it installed whenever possible.

C.6 Column Storage

For prolonged storage, it is necessary to use the appropriate mobile phase to avoid any damage of the column.

To prepare a reversed phase column for storage:

a) Flush the column with a mobile phase of 60-80% acetonitrile or methanol in water.

b) Keep capped to prevent drying out.

c) When not in use, store the column in the protective shipping box.

C.7 Backflushing a Clogged Column

Clogged Microbore/Microprep, Pico Series and 1 mm Micro Columns can be backflushed. However, backflushing of micro columns must be done with the utmost care.



Caution: It is not recommended to backflush Nano Series column as packing material may be flushed from the column.

To backflush a Microbore/Microprep, Pico Series or other micro column:

a) Connect the outlet capillary of the column to the column bulkhead of the UltiMate system (Micropump) or to the outlet of the Acurate™ by using a PEEK sleeve connector (FIGURE C-5, P/N 160492).

b) Backflush with mobile phase.

c) Do not backflush at pressures higher than 200 bar (3,000 psi).

d) Start and stop the flow delivery gradually in order to avoid pressure shocks.

e) Backflush for a maximum of 10 - 15 minutes.



Caution: Use care when increasing the backpressure. If the backpressure is significantly increased, it is possible that the column can be destroyed.

C.8 Trap Column Installation

The trap column cartridge system consists of a cartridge holder (item 2), tubing (item 1) and a set of disposable cartridges (item 3, FIGURE B-8). It is used for concentration of diluted samples in Capillary- or Nano LC. Other typical applications are desalting of proteins and peptides prior to MS analysis and in multidimensional chromatography.



FIGURE B-8. The Trap Column Cartridge System

TABLE C-1 presents the various trap column cartridge, cartridge holder and tubing configurations.

TABLE C-1 Trap Column Cartridge, Holder and Tubing Configurations

Cartridge Holder	Trap Column Length	Holder Length	O-ring Thickness (a)	Connecting Tubing
P/N 160461	1 mm	26 mm	2.2 mm	30 µm I.D. PEEKsil (b)
P/N 160431	5 mm	26 mm	4.5 mm	30 µm I.D. PEEKsil (b)
P/N 160432	15 mm	36 mm	4.5 mm	60 µm I.D. PEEK (c)

(a) Note: The O-rings are used for proper positioning of the cartridge in the holder.
(b) Replacement part: P/N 160182, 15 cm, 1 each
(c) Replacement part: P/N 160472, length 1m, must be cut to the proper length

Note: Use the configurations indicated in TABLE C-1 only.

CAUTION **Caution: In order to avoid damage of the cartridge, do not replace the PEEK fittings by stainless steel fittings.**

CAUTION **Caution: Do not cut PEEKsil tubing.**

C.8.1 Installing a Micro- or Nano-Trap Column in the Holder

To install the cartridge:

a) Remove the connecting tubing from one side of the holder (FIGURE C-9).

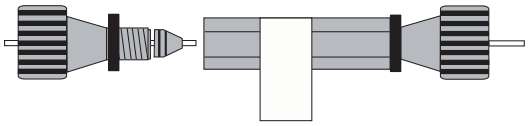


FIGURE C-9. Removing the Connecting Tubing from the Cartridge Holder

b) Replace the Micro- or Nano trap column cartridge.

c) Insert the connecting tubing. While tightening, push the tubing all the way into the holder to prevent any dead volume (FIGURE B-10).

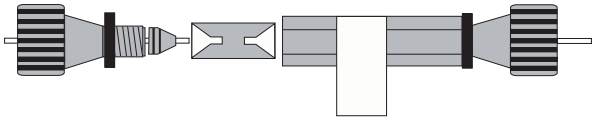


FIGURE B-10. Inserting a Cartridge into the Cartridge Holder

d) Install the trap column assembly in your LC system.

Note: Make certain that the proper O-rings are used (TABLE C-1).

CAUTION **Caution: In order to avoid damage of the cartridge, do not replace the PEEK fittings by stainless steel fittings.**

CAUTION **Caution: Do not cut PEEKsil tubing.**

C.8.2 Replacing the Micro- or Nano-Trap Column from the Holder

To remove the cartridge from the holder:

a) Remove the connecting tubing from one side of the holder.

b) To remove the cartridge, turn the holder upside down; usually, this way the cartridge falls out of the holder easily.

c) Install a new cartridge (see step b) – d) above).

If the cartridge is lodged in the holder:

a) Loosen the second fingertight fitting by approximately ½ turn.

b) Push the connection tubing into the holder, this way the cartridge falls out of the holder easily (FIGURE C-11).

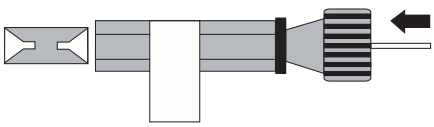


FIGURE C-11. Removing the Cartridge from the Holder

C.9 Troubleshooting

C.9.1 Sample and Mobile Phase Considerations

To optimize performance of the system, we recommend that all samples and mobile phases are free of particulate matter. Samples and mobile phases should be filtered through a 0.22 μm membrane filter. The filter should be checked to ensure that extractable materials are not present.



Caution: It is strongly recommend that you use bottled HPLC-grade water and solvents only. If water from water purification systems is used, polymeric contamination may damage the flow cell (e.g. coating of the capillary walls).

If a gradient is used, make certain that the sample and the buffer are soluble in all compositions of the mobile phase that will be used in the separation. This test should be run in a beaker or test tube so that particulate matter does not enter the system. If any cloudiness is observed in the test, the gradient should be adjusted and repeated.

After you have finished using the system, flush the system with a water/methanol or water/acetonitrile mobile phase before shutting it down.

The solvents must be degassed via the He degassing technique described before. If other techniques are used (e.g. vacuum degassing) the performance of the system will be seriously degraded and the performance specifications will not be obtained.



The pump head of the Micropump should be backflushed with propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the piston may result; this will dramatically shorten the life of these components.

C.9.2 Trap Cartridge System

It is important that the delay ('dead') volume of the trap cartridge and the tubing connections is as low as possible. For example, if the volume is 500 nL only it will take 2 ½ minutes to flush this volume at a nanoflow rate of 200 nL/min. The sample could be diluted too much and you may even 'lose' the injected sample (e.g. no peaks in the chromatogram).

FIGURE C-12 shows 4 chromatograms of a standard cytochrome c separation, two runs with a proper installed trap column cartridge system and two runs with a far too high delay volume.

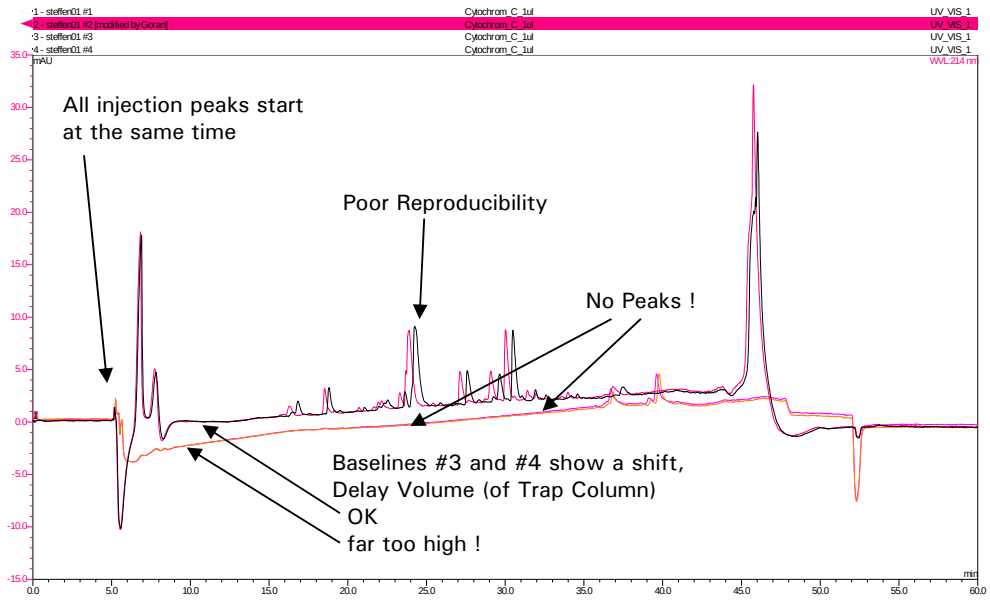


FIGURE C-12. Troubleshooting the Trap Column

In addition, FIGURE C-12 shows a problem with the reproducibility of the retention times. A possible reason could be temperature fluctuations or gradient composition variations. Flow rate fluctuations are not very likely because all injection peaks start at the same time. Refer to Section C.9.3 for more help on identifying and diagnosing operating problems and instrument failures.

C.9.3 General Hints

The troubleshooting section will help to identify and diagnose operating problems and instrument failures.

Problem	Possible Cause	Solution
Peaks too broad/ bad separation	• No peak focussing • Dead volume in system • Bad column	- Check sample solvent - Check connections - install new column
Reproducibility > 0.5 % RSD	• Column temperature not stable • Column (pump) pressure not stable	- Use column oven - Purge pump - Check degassing - Replace check valves - Check/replace injection valve
Sensitivity low	• Injected amount too low • Flow cell contaminated	- Check injection valve/autosampler - Clean flow cell
No flow through column - no column pressure	• Solvent Bottle(s) empty • Air in column pressure sensor • Leakage between Calibrator and column • Calibrator clogged	- Purge column pressure sensor - Check for leakage
Flow through column too low - low column pressure	• CRP value too low • Calibrator partially clogged • Waste restrictor broken (pump pressure too low) • Leakage between Calibrator and column	- Check for leakage - Change CRP value - Replace Calibrator - Replace waste restrictor
Flow through column too low - column pressure high	• Column or connecting capillary partially clogged	- Replace capillaries or column - Replace column
Flow through column too high	• CRP value too high • waste restrictor partially clogged (pump pressure too high)	- Change CRP value - Replace waste restrictor
Back pressure on Micropump is too high.	• Contamination of the high pressure in-line filter • Clogged the waste restrictor • Pressure sensor of the pump needs to be calibrated	- Check filter - Replace waste restrictor - Adjust pressure sensor
Back pressure on Micropump is too low/zero.	• Leaking/broken waste restrictor • pump problems	- Check for leakage - Replace waste restrictor - Check pump
Peaks eluting too fast	• Solvent filter A clogged	- Check/ replace solvent filter

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APPENDIX D

Maintenance of the Manual Sample Injector

The Valco Cheminert® Model C1 Manual Sample Injector, which (if this option is installed) is mounted in the upper left corner of the Solvent Organizer is a 6-port, through the handle external loop injector manufactured by Valco Instruments, Co. Inc. It is designed to be used for either the partial filling method, in which the injection volume is determined by a syringe and full loop injection, in which the volume is determined by the size of the loop. The design prevents any contact between the needle and the stator and rotor faces.



Note: A detailed discussion on the Installation, Use and Maintenance of the valve is presented in Technical Note 802 from Valco Instruments, Co. Inc. and can be obtained at the Valco website (www.Valco.com).

D.1 Removing the Valve from the Solvent Organizer

To remove the valve from the Solvent Organizer:

a) Remove the fittings from the valve

b) Unscrew the Syringe Injection Port from the knob

c) Remove the knob

d) Remove the two screws which attach the valve to the Solvent Organizer



Note: When re-installing the valve, make certain that the proper tubing is attached to the appropriate fitting. The loop is connected to ports 1 and 4, the pump is connected at port 2 and the column is connected at port 3.

D.2 Maintenance

In most instances, the only maintenance that is required is cleaning of the valve. Cleaning can often be accomplished by flushing all the lines with appropriate solvent(s). The selection of the solvent is dependent on the nature of the sample and the mobile phases that are used. Typically solvents such as methanol, acetonitrile, methanol/water (80/20) or acetonitrile/water (80/20) should be used.

D.3 Disassembly/Reassembly of the Valve

Note: Do not disassemble the valve unless system malfunction is definitely isolated to the valve.

D.3.1 Disassembly of the Valve

To disassemble the valve:

a) Use a 9/64" hex driver to remove the socket head screws which secure the cap to the valve (FIGURE D-1)

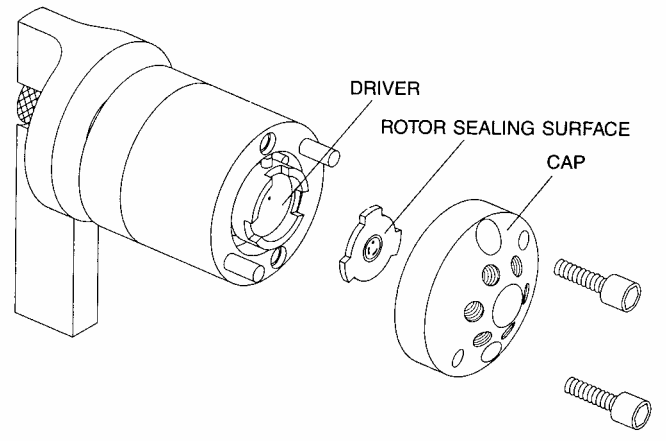


FIGURE D-1 Exploded View of the Valco Model C1 Valve

b) To insure that the sealing surface of the cap is not damaged, rest it on its outer face. If the tubing is still attached, leave it suspended by the tubing.

c) Gently pry the rotor away from the driver with your fingers or a small screwdriver.

d) Examine the rotor sealing surface for scratches.

- If scratches are visible to the naked eye, the rotor must be replaced.
- If no scratches are visible, clean all parts thoroughly with an appropriate solvent. Take care that no surfaces get scratched while you are cleaning the components.

Note: The most common problem in the use of the valve with HPLC is the formation of buffer crystals, which are usually water soluble. After cleaning, it is not necessary to dry the rotor.

D.3.2 Reassembly of the Valve

To reassemble the valve:

a) Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.

b) Replace the cap Insert the two socket head screws and tighten them gently until both are snug.



Caution: Do not overtighten the screws-they simply hold the assembly together and do not affect the sealing force, which is automatically set as the screws close the cap against the valve body.

c) Test the valve by pressurizing the system. If the valve does not hold pressure it should be returned for repair.

D.3.3 Spare Parts List

Part Number	Description
	Standard Version
160068	Low dispersion injection valve for UltiMate™
160066	Replacement rotor for low dispersion injection valve
160067	Replacement stator for low dispersion injection valve
160028	Injection Loop, 1 µl
160029	Injection Loop, 5 µl
160074	Connecting tubing, calibrator – injection valve, 75 µm I.D.
160078	Connecting tubing, calibrator – injection valve, 20 µm I.D.
160076	Connecting tubing, manual injection valve - column, 75 µm I.D.
160079	Connecting tubing, manual injection valve - column, 20 µm I.D.
	Inert Version
161047	Low dispersion injection valve for UltiMate™ INERT
161046	Replacement rotor for low dispersion injection valve, INERT
161004	Replacement stator for low dispersion injection valve
161015	Injection Loop, 1 µl
161016	Injection Loop, 5 µl
161040	Connecting tubing, calibrator – injection valve, 75 µm I.D., INERT
161044	Connecting tubing, calibrator – injection valve, 20 µm I.D., Nano, INERT
161042	Connecting tubing, manual injection valve - column, INERT
161045	Connecting tubing, manual injection valve - column, Nano, INERT
	Common Parts
160090	PEEK needle guide for manual injection valve
160088	3-Port waste manifold, including finger tights

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APPENDIX E

CHROMELEON® - Additional Program Examples

E.1 Overview

This appendix provides a number of programs that the user can prepare for activities that are commonly performed with the UltiMate™ Capillary HPLC System. In addition to the direct use of these programs, they can be used to provide an understanding of the logical processes that are used in generating a program.

The following programs are included in this appendix:

- Example1 - Standard Full Loop Injection – Section E.2
- Example 2 - Pre-concentration on Switchos II, standard 'Partial Loop Fill' injection – Section E.3
- Example 3 - Pre-concentration on Switchos II, modified 'µL-Pickup' injection, UDP – Section E.4
- Example 4 - Manual Injection – Section E.5

For more details about the common installation procedures and the installation of the system in conjunction with other LC Packings system components (e.g. the FAMOS Microautosampler and the Switchos Advanced Microcolumn Switching Unit) refer to Chapter 2 and the documentation provided with these instruments.

E.2 Example1 - Standard Full Loop Injection

```
Sampler.TempCtrl =                      On
        Sampler.Temperature.Nominal =   8
        Sampler.Temperature.LowerLimit = 4
        Sampler.Temperature.UpperLimit = 40
        Oven.TempCtrl =                 Off
        %A.Equate =                     "%A"
        %B.Equate =                     "%B"
        %C.Equate =                     "%C"
        %D.Equate =                     "%D"
        Diameter =                      75um
        Length =                        15cm
        StationaryPhase =               C18_3um_100A
        CRP =                           625
        ParkPercentage =                Disabled
        MasterPressure.LowerLimit =     0.0
        MasterPressure.UpperLimit =     300
        ColumnPressure.LowerLimit =     0.0
        ColumnPressure.UpperLimit =     200.0
        Data_Collection_Rate =          5
        Wait                            Sampler.Ready
        InjectMode =                    FullLoop
        LowDispersionMode =             Off
        LowDispersionFlow =             0.2
        LowDispersionFactor =           1.00
        UseAirSegment =                 Off
        UseHeadSpace =                  Off
        SyringeSpeed =                  Low
        SyringeSpeedFactor =            0.2
        SampleHeight =                  5
        FlushVolume =                   5.0
        WashVolume =                    50
        RinseBetweenReinjections =      no
        UV_VIS_1.Wavelength =           214
        UV_VIS_1.Step =                 0.50
        UV_VIS_1.Average =              ON
        UV_VIS_2.Wavelength =           280
        UV_VIS_2.Step =                 0.50
        UV_VIS_2.Average =              ON
        PumpPressure.Formula            Formula=Pump.MasterPressure
        PumpPressure.Type =             Analog
        ColumnPressure.Formula          Formula=Pump.ColumnPressure
        ColumnPressure.Type =           Analog

0.000   Flow =                         0.300
        %B =                            0
        %C =                            0
        %D =                            0
        Wait                            Sampler.Ready and UV.Ready and
                                         Pump.Ready
        Inject
        UV.Autozero
        UV_VIS_1.AcqOn
        UV_VIS_2.AcqOn
        PumpPressure.AcqOn
        ColumnPressure.AcqOn
        Flow =                          0.300
        %B =                            0
        %C =                            0
        %D =                            0
```

```
30.000   %B =                                  50

31.000   %B =                                  90

36.000   %B =                                  90

37.000   %B =                                  0

55.000   UV_VIS_1.AcqOff
         UV_VIS_2.AcqOff
         PumpPressure.AcqOff
         ColumnPressure.AcqOff
         Flow =                                0.300
         %B =                                  0
         %C =                                  0
         %D =                                  0

         End
```

E.3 Example 2 - Pre-concentration on Switchos II, standard 'Partial Loop Fill' injection

```
TempCtrl =                                     Off
         Loading_Pump.TrapColumnPressure.LowerLimit =          0.0
         Loading_Pump.TrapColumnPressure.UpperLimit =          400.0
         Pump.%A.Equate =                      "98%H2O, 2%AcN, 0.1% FA"
         %B.Equate =                           "20%H2O, 80%AcN, 0.08%FA"
         %C.Equate =                           "water, 0.1%FA"
         %D.Equate =                           "%D"
         Diameter =                            75um
         Length =                              15cm
         StationaryPhase =                     C18_3um_100A
         CRP =                                 625
         MasterPressure.LowerLimit =           0.0
         MasterPressure.UpperLimit =           400.0
         Pump.ColumnPressure.LowerLimit =      0.0
         Pump.ColumnPressure.UpperLimit =      200.0
         Data_Collection_Rate =                2
         Wait                                  Sampler.Ready
         InjectMode =                          PartialLoop
         LowDispersionMode =                   Off
         UseAirSegment =                       Off
         UseHeadSpace =                        Off
         SyringeSpeed =                        Normal
         SyringeSpeedFactor =                  0.2
         SampleHeight =                        1.5
         WashVolume =                          100
         RinseBetweenReinjections =            Yes

         UV_VIS_1.Wavelength =                 214
         UV_VIS_1.Step =                       0.50
         UV_VIS_1.Average =                    Off
         PumpPressure.Formula                  Formula=pump.masterpressure
         PumpPressure.Type =                   Analog
         ColumnPressure.Formula                Formula=pump.columnpressure
         ColumnPressure.Type =                 Analog
         TrapColumnPressure.Formula
         Formula=loading_pump.trapcolumnpressure
         TrapColumnPressure.Type =             Analog

 0.000   UV.Autozero
```

```
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0
            Wait                            Loading_Pump.Ready and UV.Ready and
Pump.Ready and Sampler.Ready
            Inject
            UV_VIS_1.AcqOn
            PumpPressure.AcqOn
            ColumnPressure.AcqOn
            TrapColumnPressure.AcqOn
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0
            Loading_Pump.Flow =             0.030
            Valve_A.Position =              1_2
            SSV.Position =                  A

 5.000      Start_MS.On                     Duration=1.00
            Valve_A.Position =              10_1
            SSV.Position =                  A

30.000      %B =                            60

30.100      %B =                            90

35.000      %B =                            90

35.100      %B =                            0

45.000      Valve_A.Position =              1_2
            SSV.Position =                  A

50.000      UV_VIS_1.AcqOff
            PumpPressure.AcqOff
            ColumnPressure.AcqOff
            TrapColumnPressure.AcqOff
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0

            End
```

E.4 Example 3 - Pre-concentration on Switchos II, modified 'µL-Pickup' injection, UDP

```
TempCtrl =                                  Off
         Loading_Pump.TrapColumnPressure.LowerLimit =      0.0
         Loading_Pump.TrapColumnPressure.UpperLimit =      400.0
         Pump.%A.Equate =                   "98%H2O, 2%AcN, 0.1% FA"
         %B.Equate =                        "20%H2O, 80%AcN, 0.08%FA"
         %C.Equate =                        "water, 0.1%FA"
         %D.Equate =                        "%D"
         Diameter =                         75um
         Length =                           15cm
         StationaryPhase =                  C18_3um_100A
         CRP =                              625
         MasterPressure.LowerLimit =        0.0
         MasterPressure.UpperLimit =        400.0
         Pump.ColumnPressure.LowerLimit =   0.0
         Pump.ColumnPressure.UpperLimit =   200.0
         Data_Collection_Rate =             2
         Wait                               Sampler.Ready
         InjectMode =                       UserProg
         ReagentAVial =                     1
         ReagentBVial =                     2
         ReagentCVial =                     3
         ReagentDVial =                     4
         PrepVial =                         F8
         SyringeValve                       Position = Needle
         InjectValve                        Position = Load
         Draw                               From = ReagentAVial, Volume = 2.5,
                                            SyringeSpeed = 1,
                                            SampleHeight = 4
         MixWait                            Duration = 2
         Draw                               From = ReagentAVial, Volume = 0.0,
                                            SyringeSpeed = Low,
                                            SampleHeight = 4
         Draw                               From = SampleVial, Volume = 5.0,
                                            SyringeSpeed = 1, SampleHeight = 4
         MixWait                            Duration = 2
         Draw                               From = SampleVial, Volume = 0.0,
                                            SyringeSpeed = Low,
                                            SampleHeight = 4
         Draw                               From = ReagentAVial, Volume = 2.5,
                                            SyringeSpeed = 1, SampleHeight = 4
         MixWait                            Duration = 2
         Draw                               From = ReagentAVial, Volume = 0.0,
                                            SyringeSpeed = Low,
                                            SampleHeight = 4
         InjectValve                        Position = Inject
         InjectMarker
         Dispense                           To = Waste, Volume = 10.0,
                                            SyringeSpeed = Low,
                                            SampleHeight = 4
         MixNeedleWash                      Volume = 400
         UV_VIS_1.Wavelength =              214
         UV_VIS_1.Step =                    0.50
         UV_VIS_1.Average =                 Off
         PumpPressure.Formula               Formula = pump.masterpressure
         PumpPressure.Type =                Analog
         ColumnPressure.Formula             Formula = pump.columnpressure
         ColumnPressure.Type =              Analog
         TrapColumnPressure.Formula
         Formula = loading_pump.trapcolumnpressure
```

```
            TrapColumnPressure.Type =       Analog

0.000       UV.Autozero
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0
            Wait                            Loading_Pump.Ready and UV.Ready
                                            and Pump.Ready and
                                            Sampler.Ready

            Inject
            UV_VIS_1.AcqOn
            PumpPressure.AcqOn
            ColumnPressure.AcqOn
            TrapColumnPressure.AcqOn
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0
            Loading_Pump.Flow =             0.030
            Valve_A.Position =              1_2
            Valve_B.Position =              1_2
            SSV.Position =                  A

5.000       Start_MS.On                     Duration = 1.00
            Valve_A.Position =              10_1
            Valve_B.Position =              1_2
            SSV.Position =                  A

30.000      %B =                            60

30.100      %B =                            90

35.000      %B =                            90

35.100      %B =                            0

45.000      Valve_A.Position =              1_2
            Valve_B.Position =              1_2
            SSV.Position =                  A

50.000      UV_VIS_1.AcqOff
            PumpPressure.AcqOff
            ColumnPressure.AcqOff
            TrapColumnPressure.AcqOff
            Pump.Flow =                     0.300
            %B =                            0
            %C =                            0
            %D =                            0

            End
```

E.5 Example 4 - Manual Injection

```
Sampler.TempCtrl =                      Off
        Oven.TempCtrl =                 Off
        pump.%A.Equate =                "%A"
        %B.Equate =                     "%B"
        %C.Equate =                     "%C"
        %D.Equate =                     "%D"
        Diameter =                      75um
        Length =                        15cm
        StationaryPhase =               C18_3um_300A
        CRP =                           625
        ParkPercentage =                Disabled
        MasterPressure.LowerLimit =     0.0
        MasterPressure.UpperLimit =     400.0
        Pump.ColumnPressure.LowerLimit = 0.0
        Pump.ColumnPressure.UpperLimit = 400.0
        Data_Collection_Rate =          2
        CalculatedCRP.Step =            0.50
        CalculatedCRP.Average =         Off
        MeasuredFlow.Step =             0.50
        MeasuredFlow.Average =          Off
        UV_VIS_1.Wavelength =           254
        UV_VIS_1.Step =                 0.50
        UV_VIS_1.Average =              Off
        Flow =                          0.300
        %B =                            0
        %C =                            0
        %D =                            0

 0.000  UV.Autozero
        Wait                            UV.Ready and Pump.Ready
        Inject
        UV_VIS_1.AcqOn
        PumpPressure.AcqOn
        ColumnPressure.AcqOn
        Pump.Flow =                     0.300
        %B =                            0
        %C =                            0
        %D =                            0

30.000 %B =                             50

30.100 %B =                             90

35.000 %B =                             90

35.100 %B =                             0

50.000  UV_VIS_1.AcqOff
        PumpPressure.AcqOff
        ColumnPressure.AcqOff
        Pump.Flow =                     0.300
        %B =                            0
        %C =                            0
        %D =                            0
        End
```

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