ANTEC
LEYDEN

ALEXYS
installation guide
LC connection kit, DCC I-I, CS

Edition 2, 2006 180.7018

Symbols

The following pictogram is used in this installation guide:

Caution

General precautions

Execute periodic leak checks on LC tubing and connections. Do not allow flammable and/or toxic solvents to accumulate. Do not close or block drains. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.

Use proper eye and skin protection when working with solvents.

Use of this product outside the scope of this guide may present a hazard.

Spare parts and service availability

Manufacturer provides operational spare parts of instruments and current accessories for a period of five years after shipment of the final production run of the instrument. Spare parts will be available after this five years period on an ‘as available’ basis.
Manufacturer provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Contact your local supplier for servicing. Technical support and training can be provided by qualified chemists on both contractual or as-needed basis.

Copyright ©2005 All rights reserved. Contents of this publication may not be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from the copyright of the owner.
The information contained in this document is subject to change without notice. The software and the information provided herein is believed to be reliable. However, Antec Leyden shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this software or this manual. All use of the software shall be entirely at the user’s own risk.
Whatman (word and device) and Whatrnan (word only) are trademarks of Whatman International Ltd. SOLVENT IFD and AQUEOUS IFD are trademarks of Arbor Technologies, Inc. USA

Table of contents

Symbols 1
General precautions 1
Spare parts and service availability 2

Installation guide 5
Introduction 5
Unpacking 6
Tools 6
Schematic drawing of all ALEXYS LC connections 7
Important installation information 8
Installation of tubing assemblies 9
Back pressure adjustment procedure 23

Accessories 31

Operating instructions Whatman IFD 32
Specifications 32
Operating instructions 33
Filter installation and priming 34
Trouble shooting 35

APPENDIX A

Calculations Pressure Adjustment Procedure 37

C H A P T E R 1

Installation guide

Introduction

The LC connection kit (p/n 180.0164) is a tailor-made kit containing all tubing assemblies to make the necessary LC connections in a complete ALEXYS 100 LC-EC system with a DECADE II DCC in a column-switching configuration. This procedure specifically describes the installation of these assemblies. It is assumed that:

Equipment has been unpacked and checklists are verified.
Installation procedure of ALEXYS is followed as described in manuals/installation guides, in the order: OR 100, AC 100, LC 100, AS 100, DECADE II and CS valve option.

Follow the installation instructions in the ALEXYS installation checklist and user manuals and keep this document at hand. Sections describing installation of liquid tubing are referring to this document.

Order	Part no.	User manual / installation guide
	185.0010	ALEXYS data system
1	184.0010	OR 100 organizer rack
2	183.0010	AC 100 acquisition controller
3	182.0010	LC 100 pump
4	181.0010	AS 100 autosampler
5	171.0010	DECADE II
6	250.7006	Installation guide CS valve option

Before complete installation, passivation of all metal parts in the HPLC system is required. The passivation step is described in step 3 of the installation procedure.

For optimal performance all metal parts in the system should be passivated with a 15% nitric acid solution for 20 minutes.

Unpacking

Inspect the *transport box* for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise she may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. Carefully unpack the instrument and inspect it for completeness and possible damage. Contact your supplier in case of damage or if not all marked items on the checklist are included. Prior to shipment, your ALEXYS LC connection kit has been inspected and tested to ensure the best possible performance.

Tools

The following tools are necessary for the installation of the LC connection kit:

- 5/16" - 1/4" wrench
- Small flat head screw driver
- PEEK tubing cutter

Figure 1 *Photograph of listed tools. From left-to-right: 5/16" - 1/4" wrench, flathead screwdriver and tubing cutter.*

A small glass botle/ beaker which fits in the oven compartment and a ruler is necessary for the pressure adjustment procedure.

Schematic drawing of all ALEXYS LC connections

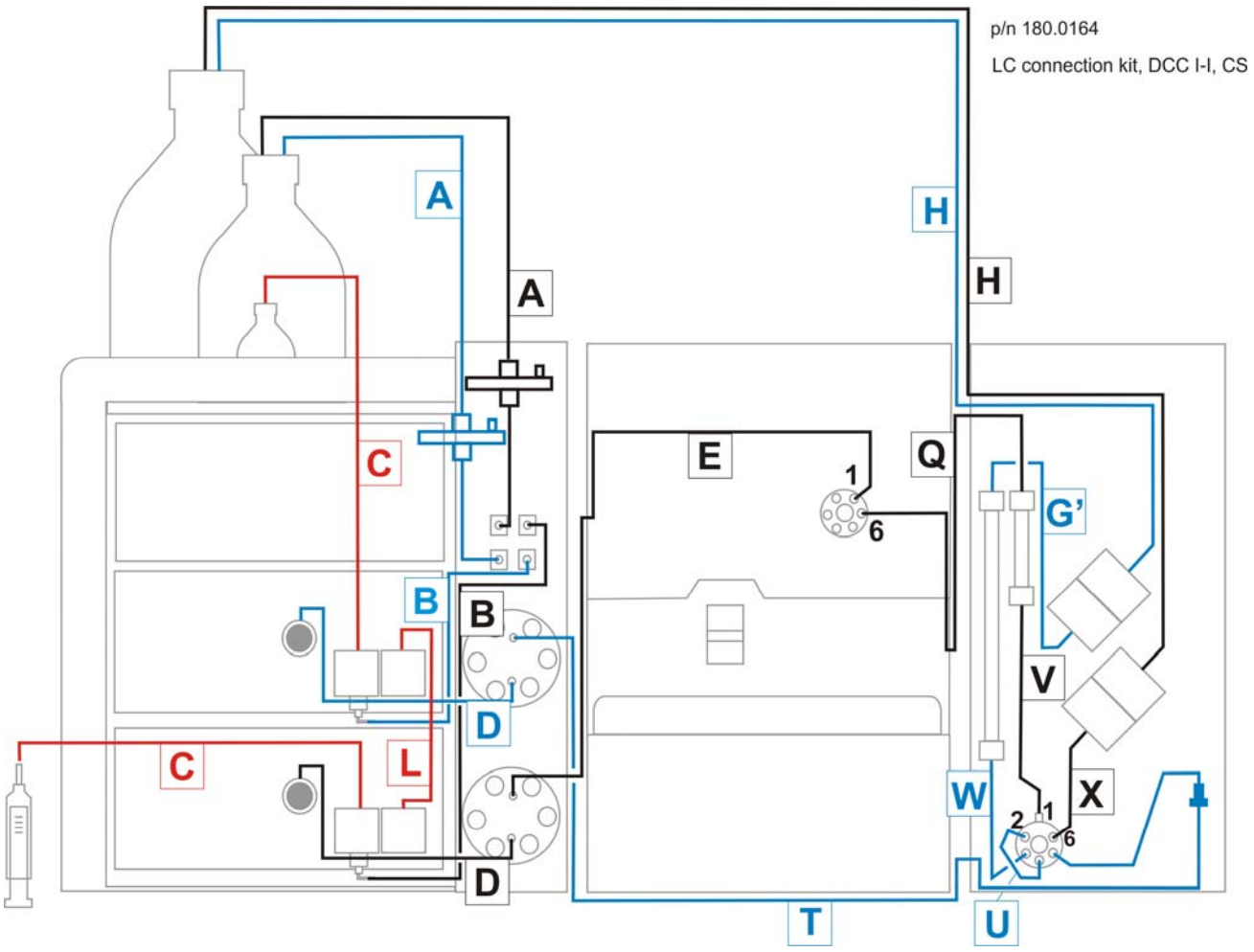

Figure 2 *Schematic drawing of all LC connections available in the LC connection kit, DCC I-I, CS. [a] The numbers refer to the part numbers on the plastic bags in which the assemblies are shipped. [b] For identification each individual assembly has a vinyl label with letter code (A, B etc.) attached.*

Important installation information

For the installation of the ALEXYS DCC I-I system with column switching configuration it is necessary to match the pressure of the different LC channels in order to prevent pressure fluctuations during column switching steps. This in order to prevent damage to column, pulse damper and to ensure good system stability. To achieve equal pressure levels it is necessary to cut the supplied restriction capillaries in a controlled way to match the pressure of the different LC channels. For this purpose a specific pressure adjustment procedure is described in this installation section.

Two restriction capillary assemblies are provided in the kit:

Part no	assembly	Description
180.0242	T	OR 100 pd outlet assembly, DCC, CS -R
180.0250	X	CS valve-to-cell connection -R
250.0901*		Tubing PEEK 1/16", 0.0025" ID, 1 m

*) 2 spare pieces of restriction capillary (p/n 250.0901) are supplied in the kit.

The pressure alignment procedure is described in a separate section after the installation procedure. Note: that during the pressure adjustment procedure temporary rearrangement of the tubing assemblies is necessary in order to perform the pressure measurements of the different flow channels of the set-up.

Please follow the specific steps in the pressure adjustment procedure very carefully to prevent damage to analytical columns and pulse dampers.

Do not switch the CS valve before the pressure adjustment procedure is completed to prevent damage to the analytical columns.

All PEEK tubing assemblies are provided with PEEK fingertights. Please ensure that <u>all</u> PEEK fingertights of the LC connections are securely tightened before switching on the pumps. Not properly secured fingertights can results in tubing slide off at high pressure and subsequent severe pressure drops. Severe pressure drops can lead to damage to analytical columns and pulse dampers.

Installation of tubing assemblies

The installation procedure of the LC connections consist of the following steps:

1. **Assembly D**, *OR 100 pulse damper inlet assembly (p/n 180.0210):* For both pumps an assembly D is provided. Connect the pre-shaped stainless steel tubing to the high-pressure outlet of the pump and the inlet of the pulse damper with the supplied nuts and ferrules (p/n 250.1564 and 250.1562).

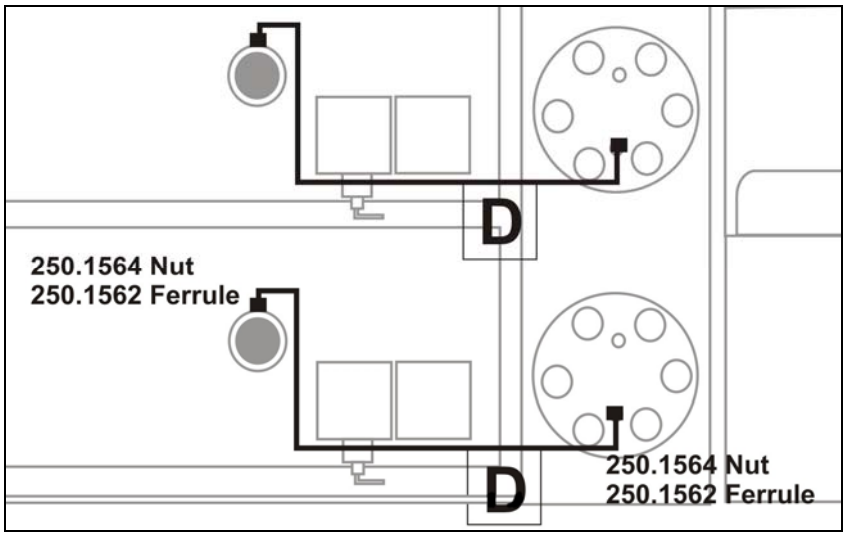

Figure 3. *Assembly D: Connection between pump – pulse damper.*

2. ***Assembly E**, OR 100 pulse damper outlet assembly (p/n 180.0212):* Connect the pre-shaped stainless steel tubing to the outlet of the pulse damper and port 1 of the AS 100 injection valve. The supplied nuts and ferrules for the connection of tubing to the pulse damper and auto-sampler are not the same.

Making a tubing connection with the wrong type of nut and ferrule can damage the port or result in large dead volumes

The autosampler valve has Valco-type ports the pulse damper SSI-type. Use parts 250.1562 and 250.1564 to connect the outlet of the pulse damper. For the connection on the injector on the autosampler use parts 250.1558 and 250.1560.

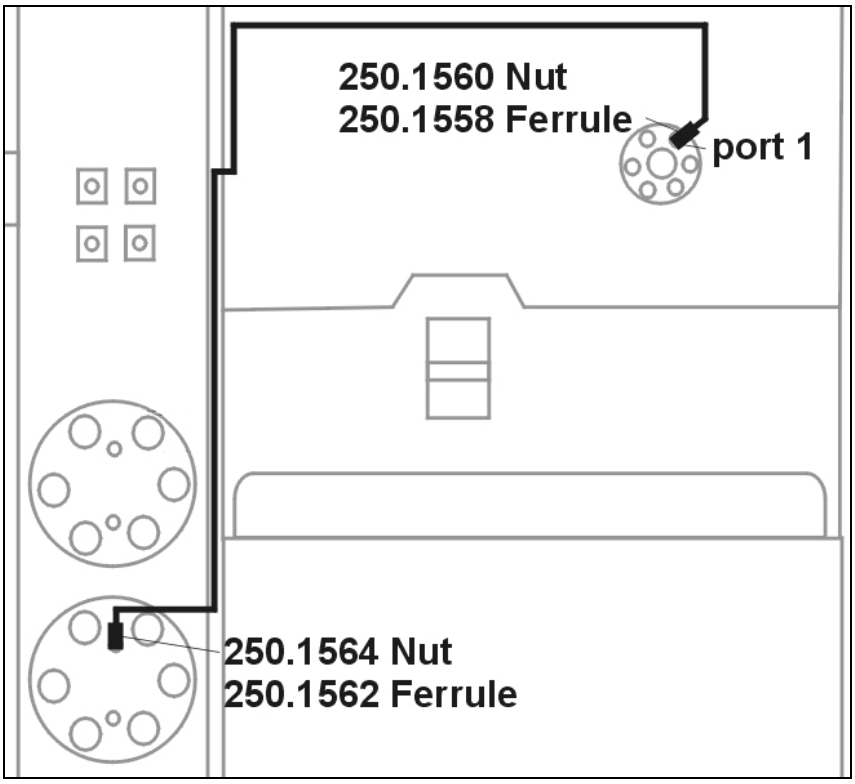

***Figure 4** Assemby E: Connection between pulse damper – AS 100.*

3. Passivation of metal parts

For optimal performance always passivate all metal parts in the system with a 15% nitric acid solution for 20 minutes.

Make sure that all parts that are not acid-resistant such as: nylon inlet filters, column and flow cell are not connected during this step.

Make the connections as depicted in figure 23. Use the Teflon tubing (p/n 182.0400) supplied in the ship kit to deliver the nitric acid solution to the pump. Use one tubing assembly H, DECADE II outlet assembly (180.0218), temporary to flush the acid from port 6 of the AS 100 to waste. The acid should be flushed through the pump, the pump tubing, the dampener, the injector and to waste**. Flush the injector in both the load and the inject position!** After flushing with nitric acid, the system must be thoroughly flushed with demi-water. Make sure that no traces of nitric acid are left in the tubing or pulse dampener (check with pH paper).

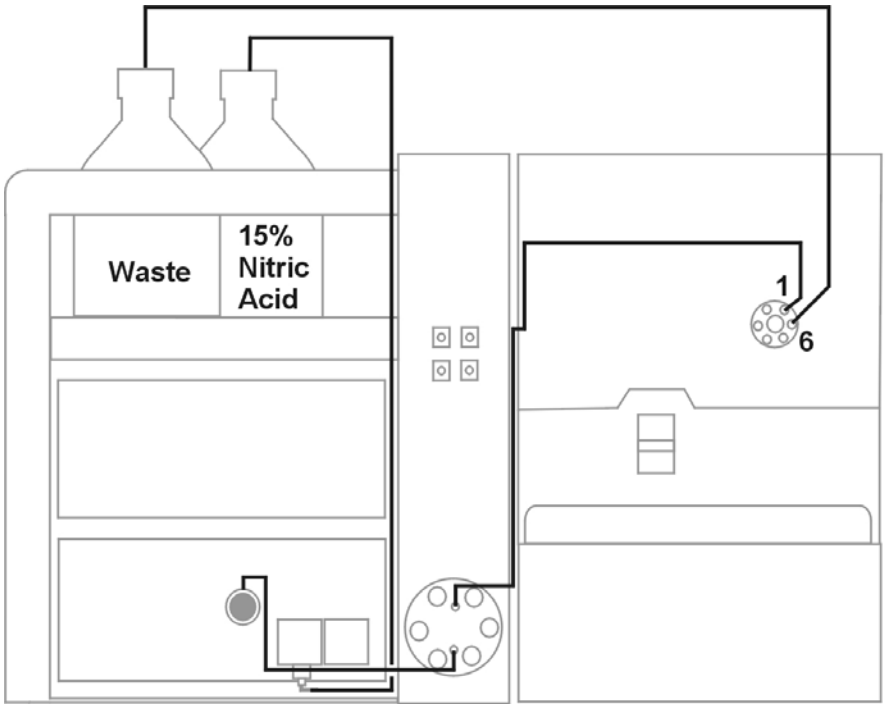

Figure 5 *System passivation*.

4. **Assemblies A**, *OR 100 degasser inlet assembly (p/n 180.0204):*
Two assemblies A are provided consisting of two pieces of FEP tubing and a 0.2 μm IFD in-line filter. Place the end of the FEP tubing A in the mobile phase bottle as depicted in figure 6.

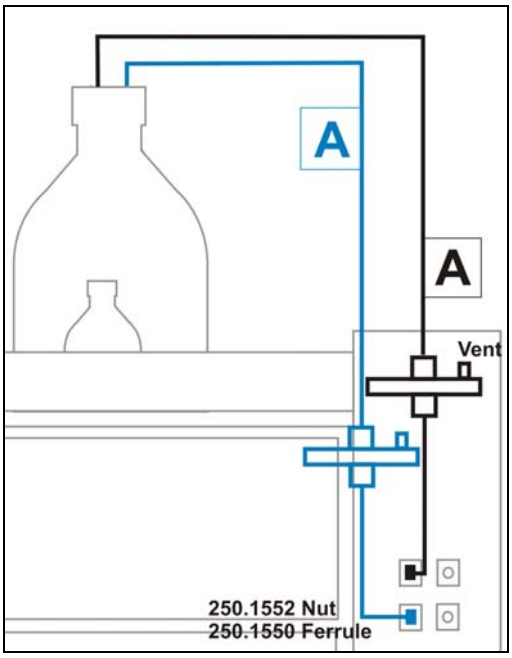

Figure 6. *Assemblies A: Connection between bottle - degasser.*

Make sure that the vent of the in-line filter is facing towards the solvent bottle. Prepare a solution of 200 mL 10% v/v Methanol/Water to initially flush the system. Open the filter (vent) and draw the flushing solution through the tubing using a syringe until the top is completely wetted and the upper compartment is air bubble free. Close the vent.
Connect the syringe to the outlet tubing and gently draw the plunger to fill the outlet tubing with solvent. Remove the syringe and hold the outlet tubing well below the liquid level in the flushing solution bottle. The outlet tubing should spontaneously siphon solvent. If this is not the case consult the trouble-shooting section in chapter 3. The inlets of both assemblies should be placed in the same mobile phase bottle.

For detailed operating instructions of the Whatman IFD in-line filters see chapter 3 in this installation guide.

When all tubing is filled with mobile phase connect the outlet to the inlet of the degasser using the supplied nut and ferrule as depicted in figure 7.

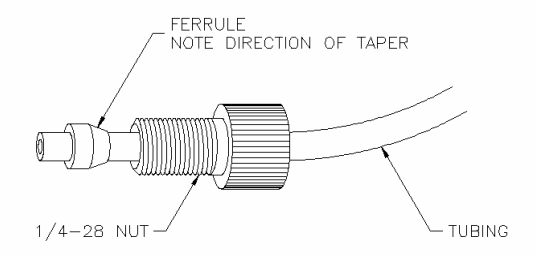

Figure 7. *Configuration of ¼-28 Nut, Ferrule and Tubing.*

5. **Assemblies B**, *OR 100 degasser outlet assembly (p/n 180.0206)*: For the LC connections between the degasser and the pumps two assemblies B are provided.

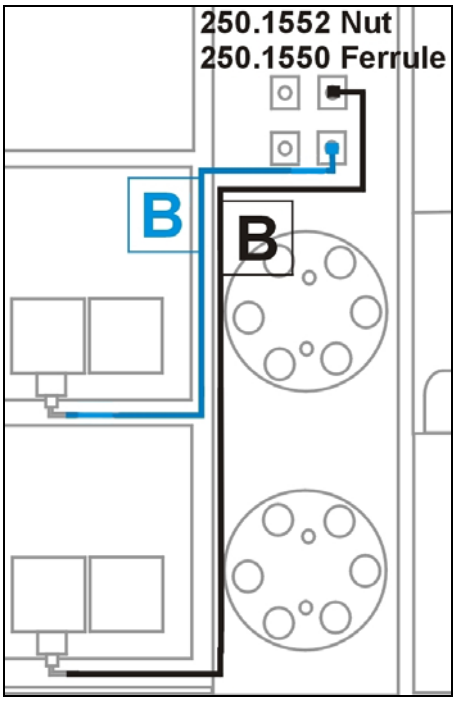

Figure 8. *B: Connection between degasser – pump.*

Connect the end of the FEP tubing without label ("LC 100 side") to the degasser output using the supplied nut and ferrule (Fig. 4, p/n 250.1552 and 250.1550). Connect the other end of the tubing to the low-pressure inlet of the pump (bended metal tubing on bottom of

pump head). Apply force to push the FEP tubing **all the way up** the bended metal tubing. Otherwise leakage will occur at this point! The second assembly B (depicted in blue) should be used for the delivery of mobile phase to pump 2. For priming instructions of the pump please consult the manual of the LC 100 (p/n 182.0010).

Never <u>push</u> the solvent through the channel of the degasser.

6. **<u>Assembly C</u>**, *LC 100 piston back flush assembly (p/n 180.0208) and* **<u>Assembly L</u>**, *Piston back flush interconnection (p/n 180.0209):* Connect the two pieces of 1/16" FEP tubing (C) to the piston wash connections on top of the pump heads on the left side. Use the supplied stainless steel nuts and ferrules (p/n 250.1564 and 250.1562).

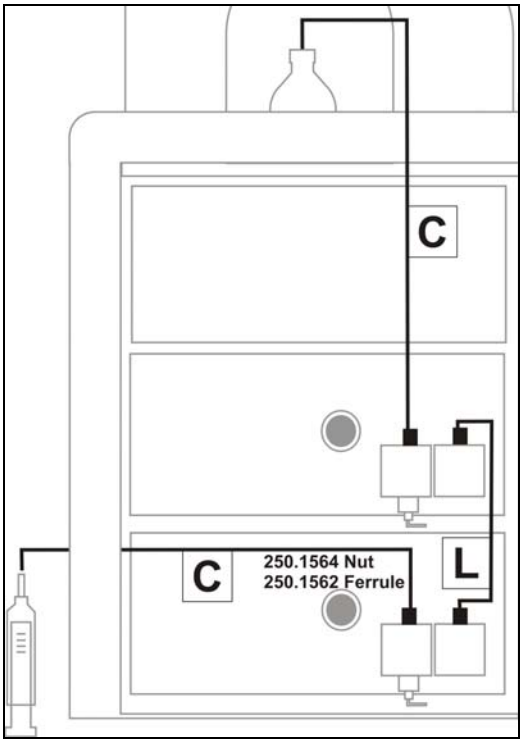

Figure 9. *Assemblies C & L: Connection of piston wash of the two LC 100 pumps.*

Place one tubing end in the bottle with wash solvent as depicted in figure 6. Connect the syringe (p/n 182.0408) supplied in the LC 100 ship kit to the other tubing. Install the interconnection tubing (L) and fill the piston wash with wash solvent by withdrawal of the syringe.

7. **Assemby Q**, *AS 100 outlet assembly micro (p/n 180.0230):*
   Assembly Q is provided to connect the 6-port valve to the 50 mm (I) HPLC column. Connect the PEEK tubing to port 6 of the injection valve of the autosampler using the supplied PEEK fingertights (p/n 250.1570). Guide the tubing via the metal tubing holder on the right-side of the AS 100 towards the DECADE II. Guide the tubing through the upper tubing hole in the DECADE II. **Switch on the flow of pump 1 and make a "wet" connection (dripping tubing) with the column to prevent the introduction of air bubbles into the column.** Connect the tubing to the inlet of the HPLC column using the supplied PEEK fingertights (p/n 250.1570).

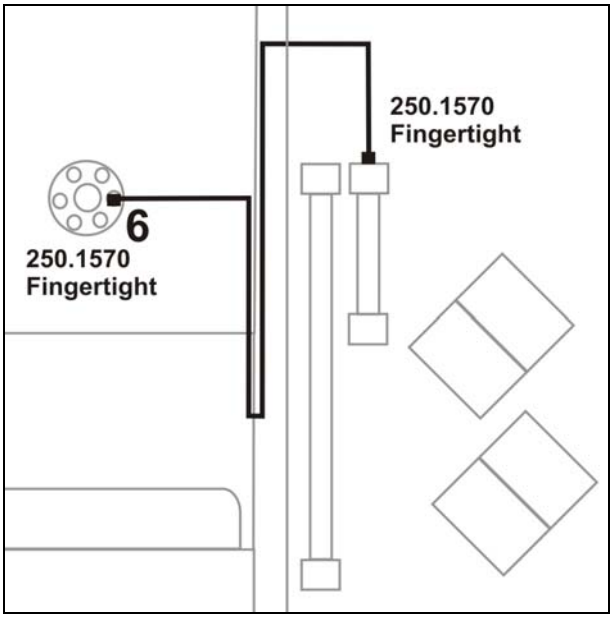

Figure 10. *Assembly Q: Connection between AS 100 – column*.

8. **Assembly V**, *Column-to-CS valve connection (p/n 180.0246):*
   Assembly V is provided to connect the first HPLC column to port 1 of the CS valve located inside the oven compartment of the DECADE II. See figure 10. Connect one tubing end to the outlet of the column using the supplied PEEK fingertight (p/n 250.1570). Connect the other free tubing end to port 1 of the CS valve using the narrow head PEEK fingertight (p/n 250.1570).

Before connecting the tubing:
- Make sure that the CS valve is installed as described in the CS valve option installation guide (p/n 250.7006).

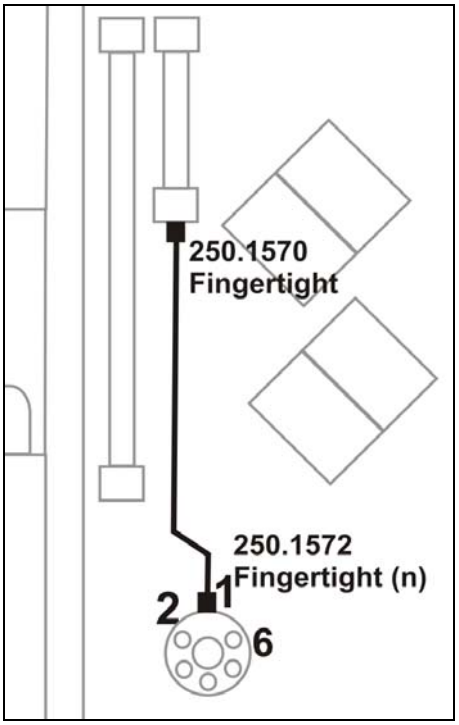

Figure 11. *Assembly V: Connection between first column - CS valve*.

9. **Assembly X**, *CS valve-to-cell connection -R (p/n 180.0250):*
   Assembly X is provided to connect the CS valve to the first VT-03 flow cell as depicted in figure 11.

Before installation of assembly X measure the exact length ($L_{res-205}$) of the restrictor by means of a ruler and write the value down. The length will be used for calculations during the pressure adjustment procedure.

Assembly X consists of narrow-bore tubing and is used as a restrictor to match the pressure in the LC system. Use the narrow head PEEK fingertight (p/n 250.1570) to connect the tubing to the CS valve and the VT-03 fingertight (p/n 110.1045) for the connection with the flow cell.

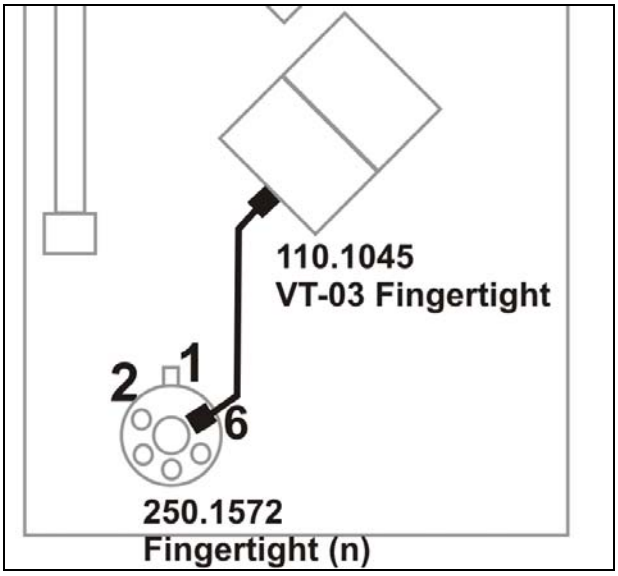

Figure 12. *Assembly X: Connection between CS valve - flow cell*.

Detailed information about the installation of the flow cell can be found in the manual of the DECADE II (p/n 171.0010). Make sure that the flow cell connected to system 1 is electrically connected to the connector marked "cell 1" on the left-side of the oven compartment.

10. **Assembly T**, *OR 100 pd outlet assy, DCC, CS -R (p/n 180.0242):* Assembly T is provided to connect the pulse damper outlet of pump 2 to port 5 of the CS valve in the oven compartment of the DECADE II as depicted in figure 13B . Assembly T consists partly of narrow-bore tubing, and is also used as a restrictor to match the pressure in the LC system. Install the PEEK bulk head assembly (p/n 250.1860) as shown in figure 13A.

Before installation of assembly T measure the exact length ($L_{res-215}$) of the restrictor by means of a ruler and write the value down. The length will be used for calculations during the pressure adjustment procedure.

First remove the plastic cover to free the screw hole in the backplane of the oven compartment. Fix the bulkhead mounting frame in the backplane with the supplied M3 screw and subsequently fix the bulkhead into the mounting frame.

Figure 13A. *PEEK bulkhead assembly in DECADE II.*

Connect one end of the orange-striped PEEK tubing to pulse damper 2 using the supplied PEEK fingertight (p/n 250.1570) and guide the tubing through the lower tubing hole in the DECADE II located on the left side. The orange-striped tubing (part from pulse damper to DECADE II) can be pushed under the AS 100 tray-box. Connect the other end with a PEEK fingertight (p/n 250.1570) to the PEEK bulkhead union mounted on the right side of the oven compartment. The PEEK resistor tubing (Pink striped) should be connected to the other end of the bulk head using a PEEK fingertight (p/n 250.1570) and to port 5 of the CS valve using a narrow head PEEK fingertight (p/n 250.1572).

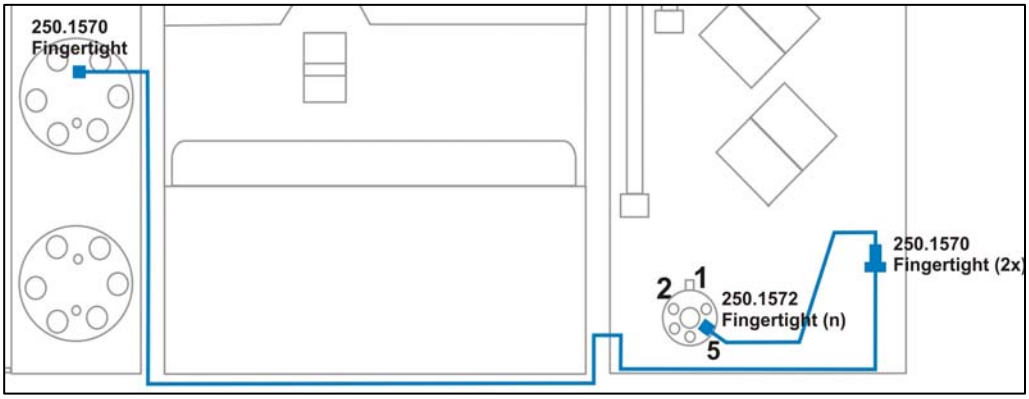

Figure 13B. *Assembly V: connection pulse damper 2 - CS valve.*

11. **Assembly U**, *Valve port interconnection (p/n 180.0244):*
Assembly U is provided to interconnect port 2 and 4 of the column switching (CS) valve located inside the oven compartment of the DECADE II). See figure 13. Connect the PEEK tubing to both ports using the supplied narrow-head PEEK fingertights (p/n 250.1572).

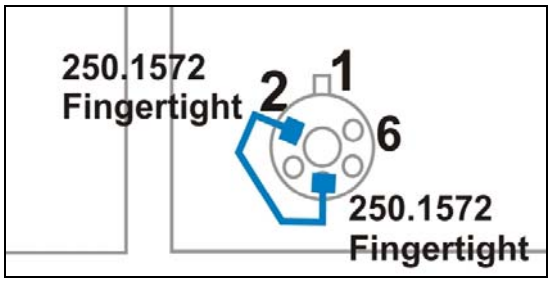

Figure 14. *Assembly U: Valve port inter connection*.

12. **Assembly W**, *CS valve-to-column connection (p/n 180.0248):*
Assembly W is used to connect the second HPLC column to port 3 of the CS valve located inside the oven compartment of the DECADE II , as depicted in figure 15. Connect one tubing end to port 3 of the CS valve using the narrow-head fingertight (p/n 250.1572).

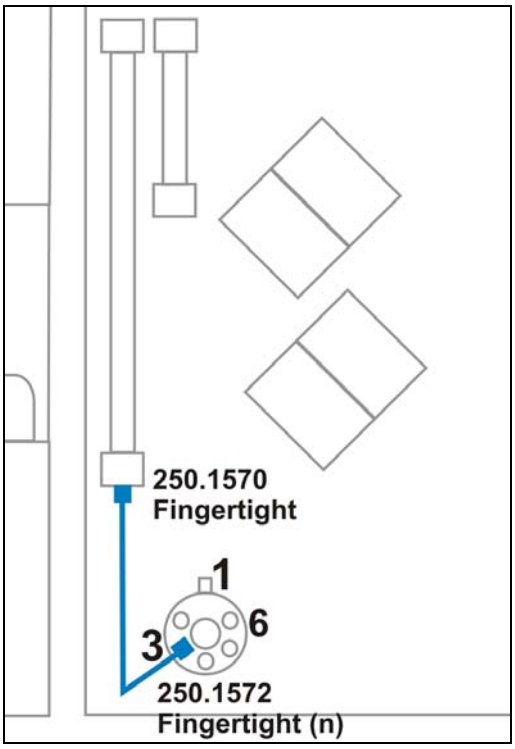

Figure 15. *Assembly W: Connection between valve - 2nd column*.

Switch on the flow of pump 2 and make a "wet" connection (dripping tubing) with the column to prevent the introduction of air bubbles into the column. Connect the tubing end to the inlet of the column using the supplied PEEK fingertight (p/n 250.1570).

13. **Assembly G'** *DECADE II inlet assembly micro (p/n 180.0232):* assembly G' is used to connect the second HPLC columns to the corresponding flow cell as depicted in figure 15. Connect one end of the PEEK tubing to the outlet of the HPLC column using the supplied PEEK finger tight (p/n 250.1570).

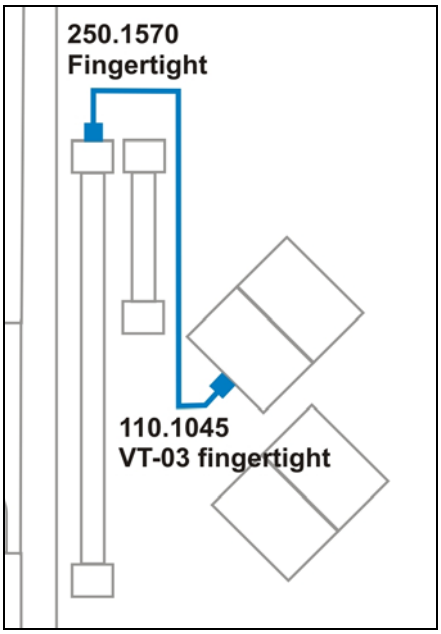

Figure 16 *Assemby G': Connection between column – VT-03 cell*.

Connect the other end of the tubing to the inlet of VT-03 flow cell using the KEL-F fingertight (p/n 110.1045). Detailed information about the installation of the flow cell can be found in the manual of the DECADE II (p/n 171.0010). Make sure that the flow cell connect to system 2 is electrically connected to the connector marked "cell 2" on the right-side of the oven compartment.

14. **Assemby H** *DECADE II outlet assembly (180.0218):* Two assemblies H are provided to connect the flow cells to the waste bottle(s). Connect the PEEK tubing to the outlet of the VT-03 flow cell using the KEL-F fingertights (p/n 110.1045). Guide the tubing through the tubing hole in the DECADE II into the waste bottle as depicted in figure 17.

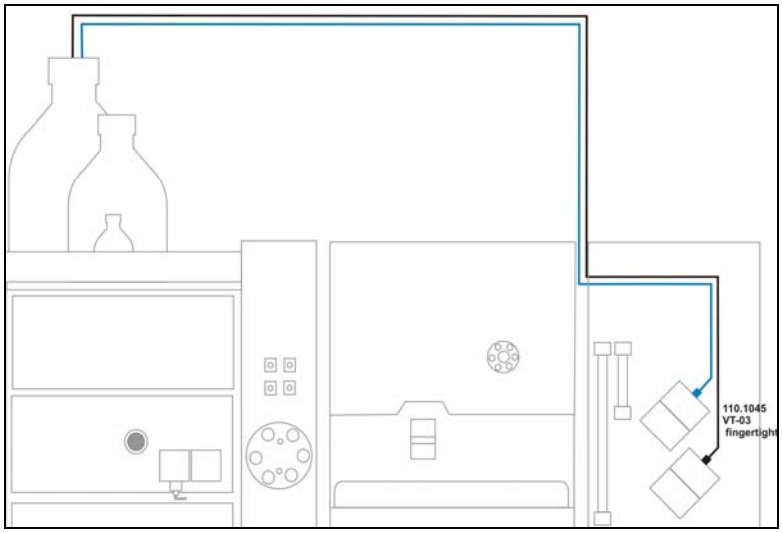

Figure 17. *Assembly H: Connection between cell – waste bottle.*

This completes the installation of all tubing and connectors of the ALEXYS 100 LC-EC 100 kit. After successful installation a number of parts in the individual ship kits will be superfluous.

For reference all tubing dimensions of the assemblies in the LC connection kit are listen in the table below:

Table I. Tubing dimensions and material .

assembly	Material	I.D. (Inch)	O.D (Inch)
A	FEP	1/16	1/8
B	FEP	1/16	1/8
C	FEP	0.03	1/16
L	FEP	0.03	1/16
D	Stainless steel	0.02	1/16
E	Stainless steel	0.02	1/16
Q	PEEK (Red)	0.005	1/16
G'	PEEK (Red)	0.005	1/16
T	PEEK (Orange)	0.02	1/16
	PEEK (Restrictor)	0.0025	1/16 or 1/32*
U	PEEK	0.0025	1/16
V	PEEK (Red)	0.005	1/16
W	PEEK (Red)	0.005	1/16
X	PEEK (Restrictor)	0.0025	1/16 or 1/32*
H	PEEK (Orange)	0.02	1/16

*) Systems delivered from second half of 2006 will be equipped with 1/32" OD restrictors.

Back pressure adjustment procedure

- **In the following section the back pressure adjustment procedure is described. Please follow the specific steps in the procedure very carefully to prevent damage to columns and dampers.**
- **Do not switch the CS valve in a running LC system, before the pressure adjustment procedure is completed.**
- **Please ensure that <u>all</u> PEEK fingertights of the LC connections are securely tightened before starting the procedure.**
- **ALWAYS perform the pressure adjustment procedure using a mobile phase with exactly the same composition as used for the analysis. Prior to the adjustment procedure flush your system with mobile phase.**
- **The following Excel document "CS_valve_adjustment_revxx.xls" is necessary to calculate the length of tubing which has to be removed from the pressure restrictors. It can be downloaded from the Antec Leyden support site at www. antecleyden.com.**

<u>System preparations</u>

Perform the following steps prior to starting the back pressure adjustment procedure:

- Check if the flow rates of pump 1 and 2 and the DECADE II oven temperature corresponds with that mentioned in the application note. If not please set these parameters manually to the correct value. Do not yet switch the pumps on.
- Make sure that the CS valve is in the right position (position A) .The valve position can be manually changed by means of the supplied manual control box. Connect this control box to the actuator and check if the valve is switched to position A.
- Install the AC 100 analog input cable for DECADE II (p/n 183.0508). Plug the 9 pins sub-D connector into "Channel 1/2" on the back of the AC 100. Connect the DIN plugs "Analog 1" and "Analog 2" to the pressure monitor output of pump 1 and 2, respectively.
- Open the system file "DCC_I-I_ CS_tuning_xxxyy.smt" in the ALEXYS HPLC data system software. This ALEXYS system file is

used to monitor the system pressure during the back pressure adjustment procedure.

- Let the LC system run for at least 30 minutes under the mobile phase conditions specified in the relevant application note used in connection with the system, before continuing with the pressure alignment procedure. Make sure that the DECADE II oven is stabilized at the specified temperature. Do not switch on the flow cells yet.

Brief outline of back pressure adjustment procedure

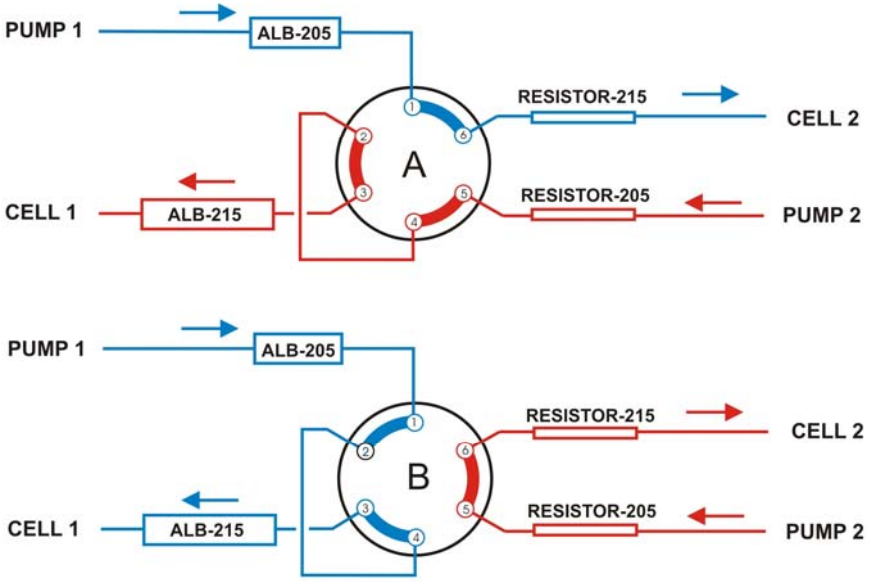

Figure 18. *Schematic representation of the two valve positions for column switching. Position A: 5 and 15 cm column in parallel, position B: 5 + 15 cm column in series.* $P_{red} = P_{blue}$

In case of the ALEXYS DCC I-I, CS system it is necessary to balance the back pressure of the different LC channels in order to minimize pressure fluctuations during column switching steps. The pressure of the red LC channels should match that of the blue LC channels in figure 18, in order to prevent damage to column, pulse damper and to ensure good system stability. To achieve equal pressure levels it is necessary to cut the supplied restriction capillaries (restrictor 215 & restrictor 205) in a controlled way to match the pressure of the different LC channels.

The back pressure adjustment procedure consist of the following steps:

1. Measurement of the back pressure of both LC channels with the CS valve in position A.
2. Measurement of the back pressure of both LC channels with the CS valve in position B.
3. Calculation of the length of tubing which has to be removed from restrictor 205 (assembly X) and 215 (assembly T) to balance the back pressure of both LC channels.
4. Final check of residual back-pressure fluctuations in the final configuration during column switching experiment.

In the following section a detailed description is given of all adjustment steps:

1. *Measurement of back pressures with CS-valve in position A.*

   - Open the system file "ALX_CS-tuning_xxxyy.smt" in ALEXYS to recording the pressure profiles of both pump 1 (flow path A1) and pump 2 (A2). See figure 19.

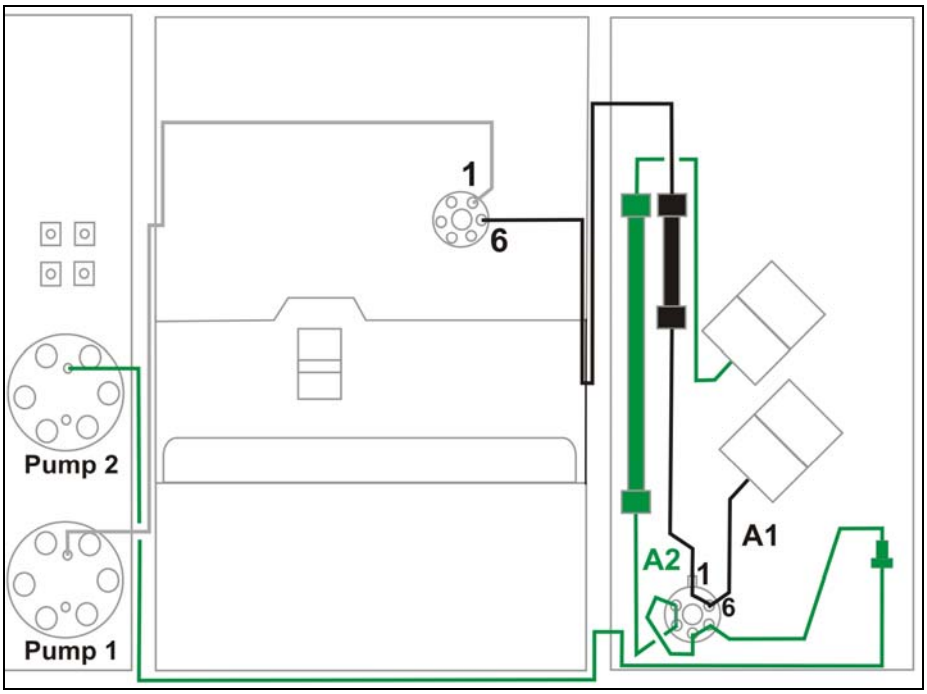

Figure 19. *Pressure measurement of both flow paths with CS valve in position A.*

- Make sure that the DECADE II oven temperature is stabile and the pumps are switched off. Open the purge valve for 10 seconds to depressurize the LC system completely (Only open the purge valve when the pump is not delivering flow and at the moment the pressure approaches 0 MPa).

The back pressure adjustment procedure will fail if the back pressure of the different flow paths are determined in a system which is not stabilized yet.

- Record the pressure traces of pump 1 and 2 for two minutes with the flow of both pump switched-off to determine the zero-point. After 2 minutes start pump 1 and 2 manually.

- Record the pressure profile for at least 20 minutes at the relevant flow rate (see example trace in figure 20). Check after 20 minutes if the back pressure is stabile (pressure line running horizontally). Continue recording the pressure signal in case equilibrium is not yet reached.

- Determine the back pressure (with two decimals) of the flow paths A1 and A2 from the recorded pressure traces (1 mV = 1 MPa). $P_{flowpath} = P_{at\ x\ mL/min} - P_{at\ 0\ mL/min}$.

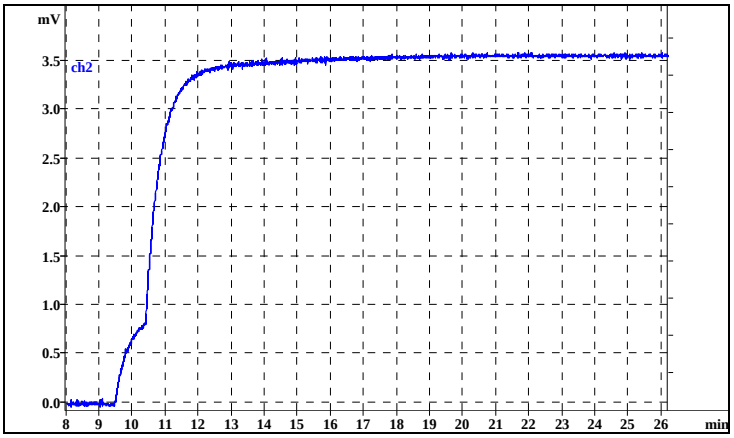

Figure 20. *Example of a pressure trace recorded with ALEXYS data system software.*

2. Measurement of back pressures with CS-valve in position B

- Switch of the flow of both pumps manually and wait till the pressure drops to 0 MPa. Subsequently open the purge valve for 10 seconds to depressurize the LC system completely

- Switch the CS valve to position B by means of the manual valve control box.

- Record the pressure traces of pump 1 (flow path B1) and 2 (B2) for two minutes with the flow of both pump switched-off to determine the zero-point. After 2 minutes start pump 1 and 2 manually.

- Record the pressure profile for at least 20 minutes at the relevant flow rate (see example trace in figure 20). Check after 20 minutes if the back pressure is stabile (pressure line running horizontally). Continue recording the pressure signal in case equilibrium is not yet reached.

- Determine the back pressure (with two decimals) of the flow paths B1 and B2 from the recorded pressure traces (1 mV = 1 MPa). $P_{flowpath} = P_{at\ x\ mL/min} - P_{at\ 0\ mL/min}$.

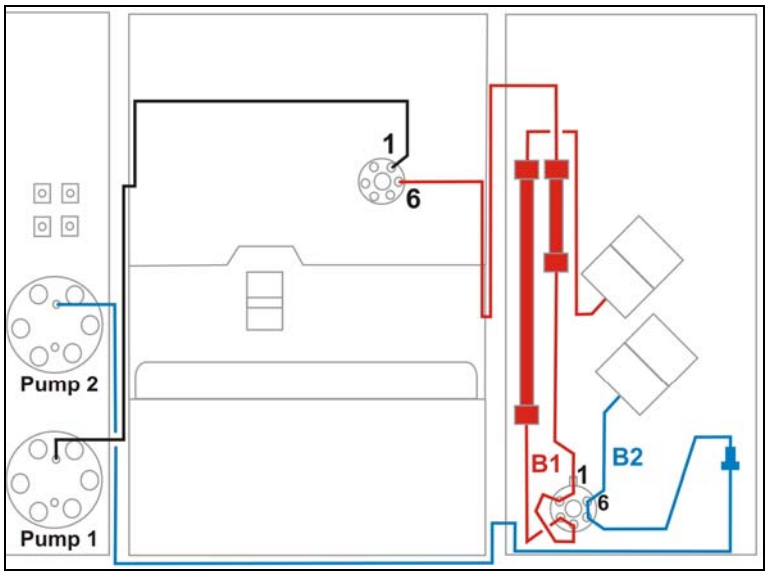

Figure 21. *Pressure measurement of both flow paths with CS valve in position B.*

3. Balancing back pressure of restrictor 205 and 215

- Open the Excel spreadsheet “CS_valve_adjustment_revxx.xls“ Fill in the required parameters in the designated cells:
  1. Restrictor length $L_{res\text{-}205}$ and $L_{res\text{-}215}$
  2. Pressure of CH1 and CH2 with valve in position A.
  3. Pressure of CH1 and CH2 with valve in position B.

- Take into account a safety margin of 3.0 cm tubing (step 6 of excel document) in order to prevent cutting the restrictor to a too short length.

- Based on the values entered in step 2 – 6, the length of tubing which has to be removed from the restrictors res-205 and res-215 is reported in step 7 and 8. For reference the calculation of the cut-back length are described in appendix A at the end of this manual.

- Switch of the flow of both pumps manually and wait till the pressure drops to 0 MPa. Remove exactly the reported lengths of tubing from the restrictors, using the supplied PEEK tubing cutter.

- Repeat step 1 and 2 of the pressure adjustment procedure again. So measure the pressure again of both LC channels (CH1 and CH2) with the CS valve in position A and B to determine the residual pressure deviation.

- Fill in the required parameters again in the designated cells (2 – 5) in the excel sheet.

- Reduce the safety margin to 0.2 cm (step 6 of excel sheet).

- Switch of the flow of both pumps manually and wait till the pressure drops to 0 MPa. Remove exactly the reported lengths of tubing (step 7 -8 of excel sheet) from the restrictors, using the supplied PEEK tubing cutter.

- Repeat step 1 and 2 of the pressure adjustment procedure again. So measure the pressure again of both LC channels (CH1 and CH2) with the CS valve in position A and B to determine the residual pressure deviation.

In the case one of the restrictors were cut to a too short length repeat the procedure using the spare restrictor tubing supplied in the kit (p/n 250.0901). If the pressure is balanced (average pressure difference < 1 bar), continue with step 4 of the pressure adjustment procedure otherwise repeat step 3.

4. *Final check of back pressure fluctuations during column switching*

- Restore the original system configuration as shown in figure 2.
- Check if the CS valve is in position A..
- Switch on both pumps with the flow rates specified in the application note.
- Start recording the pressure profile of both pump 1 and 2 using the system file "ALX_DCC_I-I_ CS_tuning_xxxyy.smt".
- Determine the back pressure of both channels with CS valve in postion A, when the system is stabilised.
- Switch the CS valve to position B.
- Determine the back pressure of both channels with CS valve in postion B, when the system is stabilised.
- Fill in the restrictor length and pressure values in the excel spreadsheet.
- When the pressure difference between pos A and B for CH1 and CH2 is < 0.1 MPa, the system is ready for use!

C H A P T E R 2

Accessories

The LC connection kit is shipped with a number of parts. The listing in the table below may not be complete, see checklist of delivery for complete listing.

Table II. Accessories LC connection kit.

Part no	assembly	Description
180.0204	A	OR 100 degasser inlet assembly
180.0206	B	OR 100 degasser outlet assembly
180.0208	C	LC 100 piston back flush assembly
180.0209	L	LC 100 piston back flush interconnection
180.0210	D	OR 100 pulse damper inlet assembly
180.0212	E	OR 100 pulse damper outlet assembly
180.0218	H	DECADE II outlet assembly
180.0230	Q	AS 100 outlet assembly (micro)
180.0232	G'	DECADE II inlet assembly (micro)
180.0242	T	OR 100 pd outlet assembly, DCC, CS -R
180.0244	U	Valve port interconnection
180.0246	V	Column to CS valve connection
180.0248	W	CS valve to column connection
180.0250	X	CS valve-to-cell connection -R
250.0901*		Tubing PEEK 1/16" or 1/32" * 0.0025" ID, 1 m

*) Systems delivered from second half of 2006 will be equipped with 1/32" OD restrictors.

C H A P T E R 3

Operating instructions Whatman IFD

Specifications

The information listed below is compiled from the original Whatman data sheet (reference number 90600A):

Table III. Specifications of Whatman AQUEOUS IFD Disposable Filters.

Parameter	Specification
Dimensions:	53 mm (2.1 in.) x 44.5mm (1.75 in.)
Weight:	11.5 grams (20 grams with ferrule nuts)
Filtration Area:	16 cm2
Maximum Pressure:	
Housing Burst	4.1 bar (60 psi)
Operating	2.1 bar (30 psi)
Housing	Polypropylene
Vent	On Inlet with Luer Lock Cap
Volume "Hold Up"	Full housing 1.0 ml, with Air Purge < 0.1 ml
Filter Media	Nylon
Flow Direction	Flow should enter from the inlet
Operating Flow Rate	< 2.5 mL/min
Connectors	5/16-24 Threads + 1/18" O.D. Ferrule Nuts
Biosafe	All Materials Pass USP Class VI

Table III. Chemical compatibility summary.*

Classes of Substances 20°C	PolypropylenelNylon Guide for use
Acids, dilute	Usable
Acids, concentrated	Not usable
Alcohols (selected)	Usable
Aldehydes	Not usable
Bases	Usable
Esters	Short term use
Hydrocarbons, aromatic	Not usable
Hydrocarbons, halogenated (selected)	Short term use
Ketones	Not usable

*) This chemical compatibility chart is intended as a general guide only. This guide has been compiled from results of inhouse studies, material supplier

studies and currently available technical literature. Because of solvent condition variabilities, which may exist from lab to lab, component compatibility cannot be guaranteed. In order to verify chemical compatibility, studies on individual chemicals of interest should be undertaken.

The AQUEOUS IFD, product number 6726-5002A, is designed to work with aqueous mobile phases. Whatman recommends the SOLVENT IFD product number 6725-5002A for organically based mobile phases (organic modifier concentrations > 30%).

Operating instructions

Safety: Considering the special factors of your application consult the table of Technical Data to determine the correctness of use. Do not exceed the pressure, temperature or chemical compatibility recommendations.

High pressures are easily obtained when using syringes. Care should be taken not to exceed the recommended pressures. Hold the filter to the syringe when pressure is applied to prevent disengaging the filter from the syringe. This could occur if excessive pressure is applied.

Filter Media Considerations: The 0.2 µm nylon membrane filter media provides an excellent means of filtering aqueous based HPLC mobile phases. It provides high flow rates and throughput. For specific solutions see the Chemical Cornpatihility Summary. "Wetted" media will not allow gas to easily pass through the media. The pressure required for gas to pass through wetted media (bubble point) is dependent on the media's pore size. Air entrained on the upstream side of wetted media blocks the flow path and reduces or stops flow.

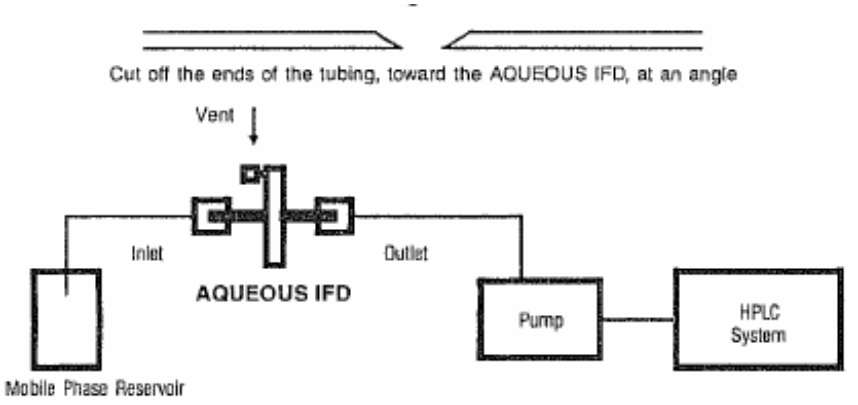

Figure 14 *Schematic drawing of filter Installation in HPLC set-up.*

Filter installation and priming

- Establish continuous, bubble free flow from mobile phase reservoir to pump by aspirating with a syringe through the pump bleed valve.
- Cut, at an angle, an appropriate length of 118" O.D. tubing to reach comfortably from the mobile phase reservoir to the inlet of the AQUEOUS IFD. Slide a ferrule nut over the cut tubing. Connect tubing to the inlet of the AQUEOUS IFD, angled end toward the AQUEOUS IFD, by tightening the nut firmly.

Gently insert cutted tubing ends into filter to prevent damage of the filter media.

- Plug the inlet tubing, or seal the end by attaching and clamping off a short length of flexible tubing.
- Fill a 10 ml syringe with the mobile phase, remove vent cap and secure the syringe to the vent.
- With outlet pointed up SLOWLY push the syringe plunger completely wetting out the filter media and filling the AQUEOUS IFD housing.
- Connect outlet to 1/18" 0.D. pump inlet tubing (cut at an angle) with a ferrule nut, as in step 2.
- Unplug or unclamp AQUEOUS IFD inlet tubing and place it in the mobile phase reservoir.
- Making sure the vent is on the upper side of the AQUEOUS IFD, fill the tubing leading to the mobile phase reservoir by pushing slowly on the syringe plunger.
- Slowly pull on the syringe plunger to withdraw a few ml of the mobile phase into the syringe. Note: This should remove any remaining entrapped air from the inlet side of the AQUEOUS IFD housing.
- Maintaining the AQUEOUS IFD at the same level as the mobile phase in the reservoir, remove the syringe and replace the vent cap on the vent.
- Pump mobile phase through system, bypassing the column, for 15 minutes to purge any remaining entrapped air in the tubing between the AQUEOUS IFD and the pump.

Trouble shooting

- To check the AQUEOUS IFD connections for air tightness: plug the tubing at the mobile phase reservoir. Remove vent cap and secure an empty syringe to the vent. Pull back on the plunger. If there are any air leaks, air bubbles will be observed.
- Air present in the inlet side of the AQUEOUS IFD during operation: The air may be evacuated by holding the AQUEOUS IFD level with the mobile phase in the mobile phase reservoir, removing the vent cap, securing an empty syringe to the vent and pulling back on the syringe plunger. Then remove the syringe and replace the vent cap. Normally a small bubble of air will remain in the vent. This will not interfere with mobile phase flow.
- Trouble with priming: Follow steps 7 through 10 exactly. Check for mobile phase leaks and or air leaks (bubbles), step 1 of Trouble Shooting section.
- No flow immediately after Installation:
    - Check for air blocking the inlet side of the AQUEOUS IFD by repeating steps 7 through 10.
    - To determine if the mobile phase is flowing from the mobile phase reservoir to the inlet side of the AQUEOUS IFD; secure a syringe filled with mobile phase to the vent and push the syringe plunger. Mobile phase should flow back from the AQUEOUS IFD to the mobile phase reservoir with a small amount of pressure on the syringe plunger.
    - To determine if the mobile phase is flowing through the AQUEOUS IFD to the pump; plug the tubing to the mobile phase reservoir, secure a syringe filled with mobile phase to the vent and push the syringe plunger. mobile phase should flow easily through the AQUEOUS IFD and the tubing to the pump.
- Slow or no flow after use: Check for air blockage and clear any entrapped air by following steps 7 through 10. If problem persists, the AQUEOUS IFD is probably clogged with particulates and should be replaced.
- Air appears to be passing through the AQUEOUS IFD: Check for air leaks by following Trouble Shooting step 1. If no air leaks are observed on the outlet side, replace the AQUEOUS IFD, the media may have ruptured. Operating Considerations: Proper operation of the system requires flow rates of < 2.5 ml/min. The filter unit should

always be changed when changing from one mobile phase to another.

- Integrity Testing: Bubble Point (BP) Test: Flush filter with 10 ml or more of an appropriate solution. After the media is completely wet, with outlet pointed upward, apply air under controlled pressure to the inlet until air breaks through the media and bubbles from the outlet. The pressure where air begins to pass through the media is the BP.

A P P E N D I X A

Calculations Pressure Adjustment Procedure

- The back-pressure per cm restrictor can be calculated in the following way:

$$\text{Pressure/cm restrictor (MPa/cm)} = \frac{P_{B2}}{(L_{res\text{-}205} + L_{res\text{-}215})}$$

- The length of tubing (cm) to be remove from restrictor 205 can be calculated in two ways:

$$L_{cut\text{-}back\ I,\ res\text{-}205} = \frac{(P_{A2} - P_{B1})}{P_{B2} / (L_{res\text{-}205} + L_{res\text{-}215})}$$

$$L_{cut\text{-}back\ II,\ res\text{-}205} = \frac{(P_{B2} - P_{A1})}{P_{B2} / (L_{res\text{-}205} + L_{res\text{-}215})}$$

- The length of tubing (cm) to be remove from restrictor 215 can be calculated in two ways:

$$L_{cut\text{-}back\ I,\ res\text{-}215} = \frac{(P_{A1} - P_{B1})}{P_{B2} / (L_{res\text{-}205} + L_{res\text{-}215})}$$

$$L_{cut\text{-}back\ II,\ res\text{-}215} = \frac{(P_{B2} - P_{A2})}{P_{B2} / (L_{res\text{-}205} + L_{res\text{-}215})}$$