eksigent

operator's manual

nanoLC 1D™ plus system

nanoLC 2D™ system

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Printed in the United States of America

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Chapter 1.
Safety and Site Requirements

Chapter 1 describes safety conventions, safety procedures and site requirements necessary for proper operation of the nanoLC-1D plus and nanoLC-2D systems. Topics covered in this chapter include:

- safety conventions (Section 1.1)
- nanoLC safety practices (Section 1.2)
- nanoLC Autosampler safety practices (Section 1.3)
- site requirements (Section 1.4)

1.1 Safety Conventions

The following symbols are used in the manual:

This label calls attention to a procedure, which, if not correctly executed, could result in injury or loss of life. Do not proceed beyond a 'DANGER' sign until the indicated conditions are fully understood and met.

This label calls attention to a procedure, which, if not correctly executed, could result in personal injury. Do not proceed beyond a 'WARNING' sign until the indicated conditions are fully understood and met.

This label calls attention to a procedure, which, if not correctly executed, could result in damage to the equipment. Do not proceed beyond a 'CAUTION' sign until the indicated conditions are fully understood and met.

This label calls attention to important information. Read this information before continuing.

1.2 nanoLC Safety Practices

The following safety practices apply to the nanoLC system:

Use of this equipment in a manner not approved by Exsigent Technologies may inhibit its safety protection.

Perform periodic leak checks on all lines and fittings.

When replacing capillaries or fittings on the nanoLC system, exposure to solvents may occur. It is therefore recommended that appropriate safety procedures be followed and personal protective equipment be used, according to the applicable material safety data sheets supplied by the solvent vendor.

Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal pro gram. Never dispose of flammable and/or toxic solvents into a municipal sewage system.

1.3 nanoLC Autosampler Safety Practices

The following safety practices apply to the optional nanoLC AS1 Autosampler system:

Changes or modifications to this unit not expressly approved by Exsigent Technologies could void the instrument warranty and render the system inoperable.

Use of this equipment in a manner not approved by Exsigent Technologies may inhibit its safety protection.

When you use the nanoLC Autosampler system, follow generally accepted procedures for quality control and methods development.

When you use the nanoLC Autosampler system for chromatographic analyses and observe a change in the retention of a particular compound, the resolution between two compounds or peak shapes, immediately determine the reason for the changes. Do not rely on the analytical results until the cause of the change is determined.

Only use fuses of the type and current rating specified. Do not use repaired fuses or by-pass the fuse holder.

The supplied power cord must be used with a power outlet containing a protective ground contact.

Do not change the external or internal grounding connections. Tampering with or disabling these connections could create a safety hazard and/or damage the nanoLC Autosampler system. The instrument, as shipped, is properly grounded in accordance with normal safety regulations.

The combination of a nanoLC Autosampler system with a LC/MS system may require additional safety measures as described by the LC/MS system vendor. Detailed instructions for the safe grounding on the LC/MS system are outlined in the corresponding vendor's operating/installation manual.

Exsigent Technologies recommends using a grounding cable connected between the injection valve's sample loop and an appropriate grounding point at the LC/MS source. This supplementary grounding will reinforce the safety configuration specified by the LC/MS system vendor.

Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage.

Instead, disconnect the power cord and contact an Exsigent Technologies representative for a product evaluation. Do not attempt to use the instrument until it has been inspected and approved for use.

Electrical damage may have occurred if the system shows visible signs of damage, exposure to liquids or of having been transported under severe stress.

Damage can also result if the instrument is stored for prolonged periods under extreme conditions (e.g. subjected to heat, water, etc.).

Disconnect the power cord from its power supply before attempting any type of maintenance.

Continue to exercise caution as capacitors inside the instrument may still be charged even after the instrument has been turned off.

To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the nanoLC Autosampler system. Once the power is turned off, wait approximately 30seconds before disconnecting an assembly.

This instrument contains a number of sensitive electronic components that may be damaged if exposed to excessive line voltage fluctuations and/or power surges.

There are no operator-serviceable or replaceable parts inside the nanoLC Autosampler system or its power supply. If either unit is not functioning, contact an Eksigent Technologies representative.

Never try to repair or replace any of the instrument's components not described in the manual without the assistance of an Exsigent Technologies representative.

To avoid injury during nanoLC Autosampler system operation, keep hands and loose objects away from the autosampler arm and syringe assembly.

Do not operate the nanoLC Autosampler system without the safety shield properly installed.

At all times, observe safe laboratory practices when handling solvents, changing tubing or operating the nanoLC Autosampler system in order to avoid injury. Know the physical and chemical properties of the solvents you use. See the solvent manufacturer's Material Safety Data Sheets for any solvent being used.

Use caution when working with any polymer tubing under pressure:

- Always wear proper eye protection when near pressurized polymer tubing.
- Do not use polymer tubing that has been severely stressed or kinked.
- Do not use polymer tubing, in particular PEEK or DuPont Tefzel tubing, with tetrahydrofuran (THF), dimethylsulfoxide (DMSO), chlorinated organic solvents, concentrated mineral acids, such as nitric, phosphoric or sulfuric acids, or any related compounds.

An on board lithium battery maintains the autosampler firmware when the instrument is turned off. Because it is hard-wired in place, it should only be replaced a factory authorized service engineer.

1.4 Site Requirements

This section describes the requirements for power, air, space and environment for operation of your instrument.

1.4.1 nanoLC Power Requirements

The nanoLC is powered by a 24 VDC external power supply. Only the universal AC/DC adapter and power cord supplied with the instrument should be used.

The external adapter permits operation from any line voltage between 100–240 VAC, 47–63 Hz and 4A.

1.4.2 nanoLC Autosampler Power Requirements

Line voltage:

- 115 VAC; + 15/-20 %; 50 Hz/60 Hz; 250 VA
- 230 VAC; + 15/-20 %; 50 Hz/60 Hz; 250 VA

Fuses:

- For 115 VAC; two 5.0 AT-fuses
- (¼" x 1¼", UL/CSA)
- For 230 VAC; two 2.5 AT-fuses
- (5 x 20 mm, IEC 127)
- All fuses UL-listed and CSA-certified

1.4.3 Air Supply Requirements

Operation of the instrument requires connection to a source of 100 psi (6.9 bar) regulated clean, dry air or nitrogen. The instrument site should be within about 6 m (20 ft) of the air/nitrogen regulator. When using compressed air, Exsigent Technologies strongly recommends an air supply having a dew point of less than 4.5 C (40 °F). When using dry nitrogen or compressed air, Exsigent Technologies strongly recommends the use of air filtration to 5 µm (e.g. for compressed gas supplied at less than 150 psi, a Wilkerson F18 filter) and regulation to a working pressure of 100 psi (e.g. for compressed gas supplied at less than 150 psi, a Wilkerson R18 regulator or a Wilkerson B18 combination regulator/filter). If hydrocarbons are suspected in the air supply (i.e. air supplied from an oiled compressor). Exsigent Technologies strongly recommends the regulator be followed with a coalescing filter suitable for particle removal to 0.01 µm (e.g. a Wilkerson M18 coalescing filter).

Note: Always follow manufacturer's specifications in selecting and operating gas filters and regulators.

Note: Always follow manufacturer's specifications for connecting, mounting and orienting gas filters and regulators.

Note: Always perform proper maintenance of traps, filters and coalescing filters per manufacturer's specifications. Liquids collected in filters and coalescing filters must be drained before the liquid level exceeds the manufacturer's specifications.

1.4.4 Bench Space Requirement

The nanoLC system requires clear bench space of at least the following dimensions.

- nanoLC-1D plus™: 21” (53 cm) wide × 20” (51 cm) deep × 19” (48 cm) high (allowing excess space for cables).
- nanoLC-2D™: 21” (53 cm) wide × 24” (61 cm) deep × 19” (48 cm) high (allowing excess space for cables).

With the nanoLC Autosampler the height requirement for both systems is 27” (69 cm).

This bench space requirement does not accommodate the computer, keyboard, mouse and monitor.

1.4.5 Environment Requirements

The instrument is designed to operate in an environment with ambient temperatures between 20 and 30 °C (68–86 °F) and non-condensing humidity.

Chapter 2.
System Installation

Chapter 2 describes the recommended procedure for unpacking and installing the nanoLC system. Topics covered in this chapter include:

- nanoLC system overview (Section 2.1)
- unpacking the nanoLC system (Section 2.2)
- placement of the system (Section 2.3)
- connecting to the air supply (Section 2.4)
- connecting the PC (Section 2.5)
- installing the nanoLC Autosampler (Section 2.6)
- connecting the nanoLC to power (Section 2.7)
- installing software and instrument settings (Section 2.8)
- configuring the nanoLC system (Section 2.9)
- configuring the nanoLC Autosampler (Section 2.10)

2.1 nanoLC System Overview

The nanoLC systems are designed for HPLC applications that employ direct pumping at flow rates of several hundred nL/min. The fully integrated system includes binary gradient pumps, an additional pump or second binary gradient pump system, temperature-controlled flow modules, and a column switching valve. The system is compatible with the nano LC Autosampler. This chapter introduces the hardware and software features of the nanoLC systems.

2.2 Unpacking the nanoLC System

step 1 Inspect the shipping cartons for damage or evidence of mishandling. If external damage is evident, notify the carrier before opening the cartons.

step 2 Cut the tape and open the flaps on the top of the nanoLC system shipping box. Remove the outer shipping box.

step 3 Open the inner shipping box and remove the foam packing material from the top of the nanoLC system.

step 4 Carefully lift the nanoLC system out of the box. Grasp the sides of the instrument; do not use the protective bag to lift the instrument. A second person may be needed to help slide the carton and foam inserts off the instrument.

step 5 Place the instrument on a lab bench.

step 6 Check the contents of the accessory kit against the contents checklist in Appendix A to confirm that all items are included.

step 7 The checklist for parts included with the nanoLC Autosampler is included in the nanoLC Autosampler manual.

step 8 Unpack the computer and monitor and verify that no parts are missing or damaged.

2.3 Placement of the System

Place the nanoLC system on a lab bench in a location with convenient access to power and a source of 100 psi (6.9 bar) regulated clean, dry air or nitrogen. The front of the instrument should be accessible at all times. The sides and back of the instrument should be clear to accommodate mobile phase reservoir placement and attachment, and computer cable attachments

2.4 Connecting to the Air Supply

Operation of the instrument requires connection to a source of 100 psi (6.9 bar) regulated clean, dry air or nitrogen as described in the section on Site Requirements.

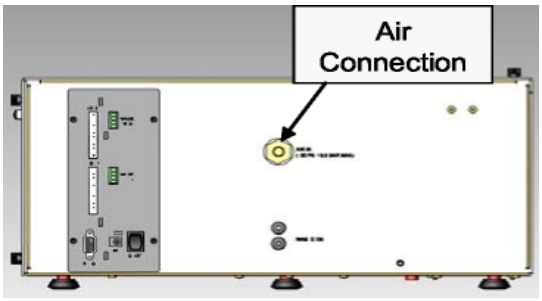


Figure 2-1. Rear Panel - Air Connection

Required tools and materials:

- Clean, dry source of air or nitrogen at 100 psi (6.9 bar)
- In-line moisture trap with ¼" end-fittings
- 6 mm or ¼" air supply line (included)
- Quick-connect adaptor to 6 mm or ¼" supply line (included)

step 1 Connect the supplied air line to a source of 100 psi (6.9 bar) regulated clean, dry air or nitrogen using the supplied quick-connect adaptor. Connect the other end of the air line to the air connection on the back of the nanoLC system (see Figure 2-1).

step 2 Turn on the air source to the nanoLC system.

step 3 Test for air leaks. Turn off air supply and repair any air leaks that are found.

2.5 Connecting the PC

Required materials:

- Computer, keyboard and mouse
- nanoLC system serial cable (included)

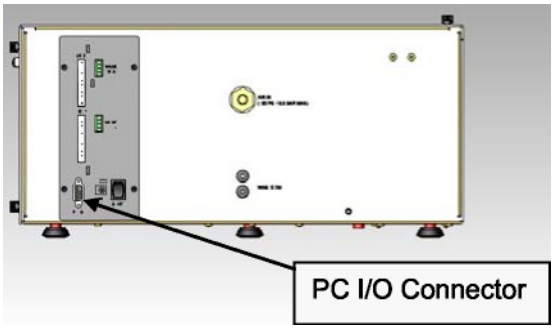


Figure 2-2. Rear Panel – PC I/O Connector

Connect one end of the RS-232 cable to an available COM port connector on the computer. COM1 is recommended as it is selected as the default in the software configuration.

The other end of the cable should be connected to the 9-pin connector labeled **PCI/O** on the rear panel of the nanoLC system (see Figure 2-2). Tighten the retaining screws at both ends to secure the cable in place.

2.6 Connecting the nanoLC to Power

Required materials:

- 24 VDC power supply (included)

Insert the supplied 24 VDC power supply's plug into the connector located on the back of the instrument (Figure 2-3). Plug the line voltage cord into an appropriately grounded line voltage outlet. Turn the nanoLC on using power switch on the back of the unit.

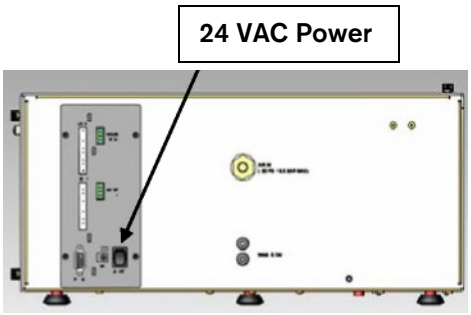


Figure 2-3. Rear Panel – Power Connection

2.7 Installing Software and Instrument Settings

Required materials:

- CD with control software
- disk with system settings

step 1 Insert the CD into the CD drive and install the Control Software (see software manual for additional information on installation).

step 2 After installing software, but before starting the software, insert the disk with the system settings (shipped with new instrument).

step 3 Locate the file titled 'EKsetting.reg' in the **Settings** subfolder.

step 4 Double click on the file 'EKsettings.reg' to install the instrument settings into the registry of the computer. A window will pop up asking you if you really want to write to the registry (Figure 2-4).

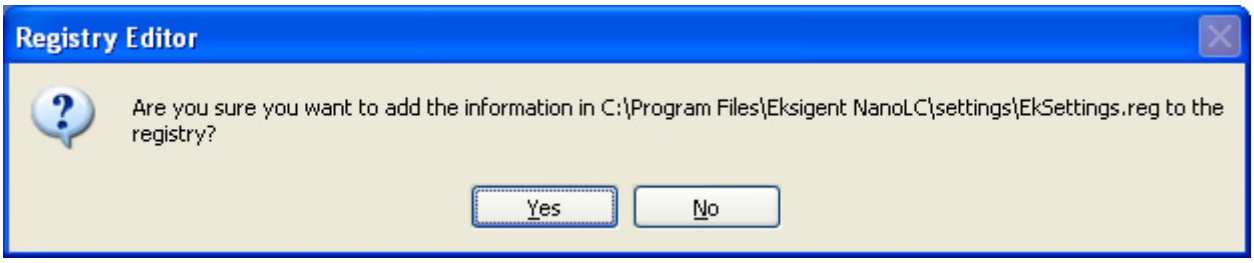


Figure 2-4. First Registry Prompt Window

step 5 Click **Yes.** A second window will popup indicating that you have successfully written to the registry (Figure 2-5).

step 6 Click **OK**.

step 7 The factory settings for your instrument should now be loaded into the registry. You should still configure the system (section 2.9) in case the **COM** port or other settings need to be adjusted from the factory defaults.

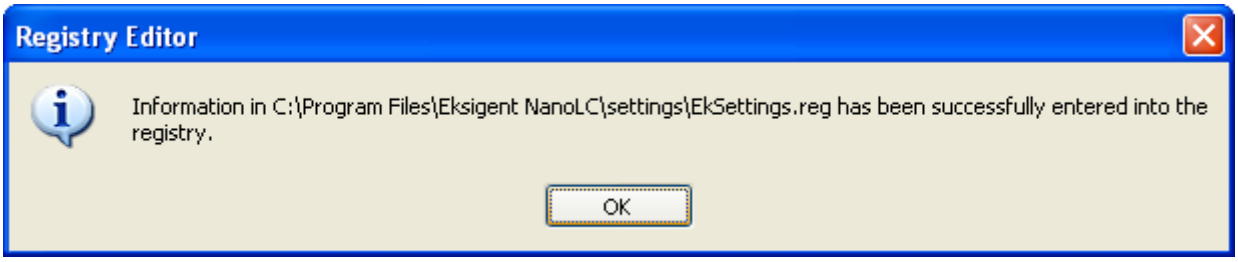


Figure 2-5. Second Registry Prompt Window

2.8 Configuring the nanoLC System

Launch the Control Software from your computer's **Programs** menu or from the Control Software icon on the desktop.

If you did not connect the serial cable to **COM1**, or the instrument power is not turned on, a COM Error dialog box (Figure 2-6) will appear:

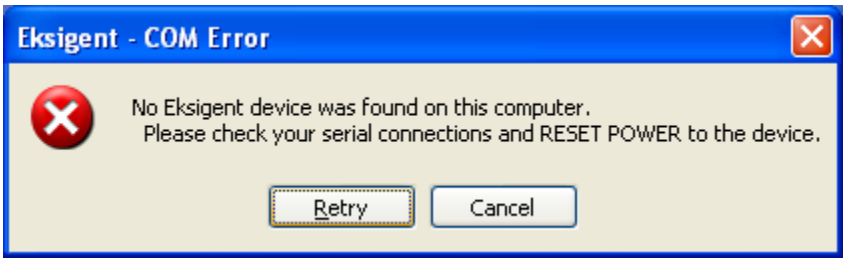


Figure 2-6. Serial Port Communications Error

Note: If the nanoLC system is connected to the PC's COM1 port and the error message still appears, re-boot the PC and re-start the software.

If the nanoLC is connected to a communications port other than COM 1, the instrument configuration will need to be changed.

step 1 Launch the control software. The COM error in Figure 2-8 will appear. Click on 'Cancel.' A second window will appear (see Figure 2-7). Click on 'OK' to enter the software in DEMO MODE.

Figure 2-7. Running the Software in Demo Mode

step 2 Select **System > Instrument Config…** from the Control Software's Acquisition Window to access the **Instrument Configuration Window** (see Figure 2-8).

The **Instrument Configuration Window** (Figure 2-8) is used to indicate which components are installed. It also sets the communications protocol and configures the system to work with other connected devices. Several instrument performance parameters are also set in this window.

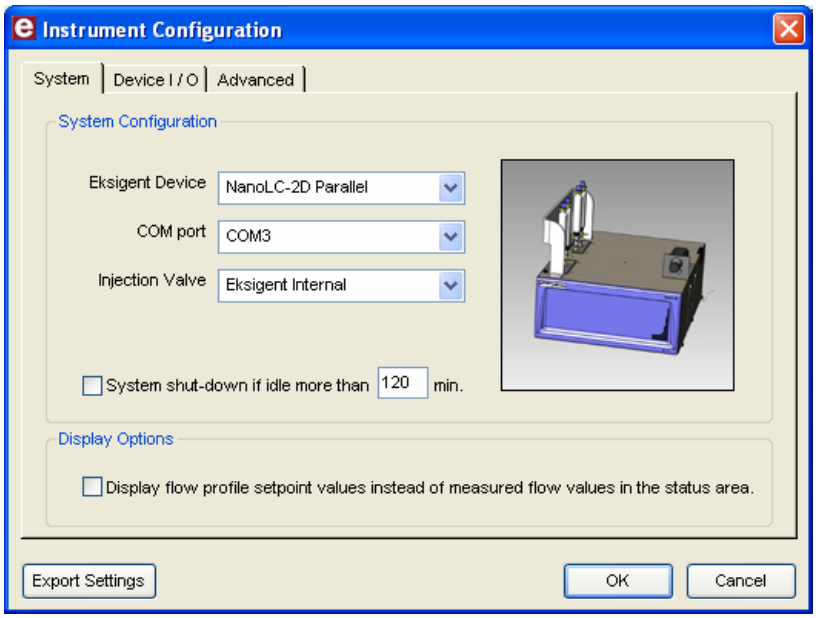


Figure 2-8. Instrument Configuration Window

step 3 Select the appropriate COM port for the nanoLC from the dropdown list.

step 4 Exit from the Control software.

step 5 Launch the control software.

The **Instrument Configuration Window** (Figure 2-8) is used to indicate which components are installed. It also sets the communications protocol and configures the system to work with other connected devices. Several instrument performance parameters are also set in this window.

Select **System > Instrument Config…** from the Control Software's Acquisition Window to access the **Instrument Configuration Window**.

Make sure that one of the following systems is selected in the **Device** field.

- nanoLC-1D plus
- nanoLC-2D

The computer is configured at the factory to use **COM1** to communicate with the nano LC system. If the serial cable is connected to a different serial port, change the setting in the on this page to indicate the correct **COM** port.

In the **Injection Valve** field, select **Eksigent Internal**.

If an auxiliary A/D input (such as a UV detector) is connected to the system, specify the voltage range the external device will provide under **Aux A/D**.

In the **Advanced Options** box, type an acceptable idle time after which the system will be shut-down and then check the box.

Select the appropriate signal input and output settings based on the requirements of the other instruments connected to the Eksigent nano LC system.

Flow Stabilization Limits specifies the degree of flow rate stability required before a gradient will begin. For most applications, a setting of 100 nL/min should be sufficient. However for high sensitivity applications, this parameter should be set closer to 20 nL/min.

The **Pressure Limits** box allows for the specification of a maximum system pressure. If the column pressure exceeds this limit, the run will be automatically stopped and no further injections will take place. There is no loss of sample if the system shuts down. For most applications a value of 3500 psi is recommended.

Click **OK** to go back to the **Acquisition Window**.

2.9 Configuring the nanoLC Autosampler

Click on the **Run Manager** button to bring up the **Run Manager Window** for configuring the autosampler (Figure 2-9).

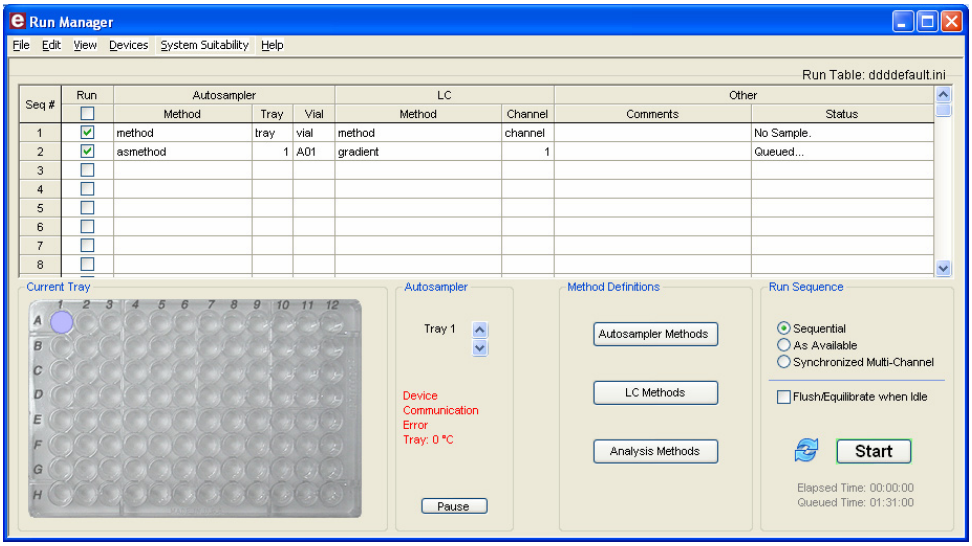


Figure 2-9. Run Manager Window

Select **Devices > Autosampler Type > NanoLC-AS1** and the following message will appear:

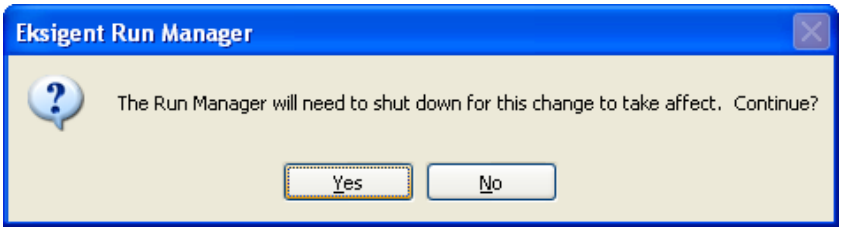


Figure 2-10. Run Manager shutdown prompt

Click **Yes** to close the program.

Click on Run Manager again, and Select Devices > Autosampler Device Settings. Set the COM port for the Autosampler

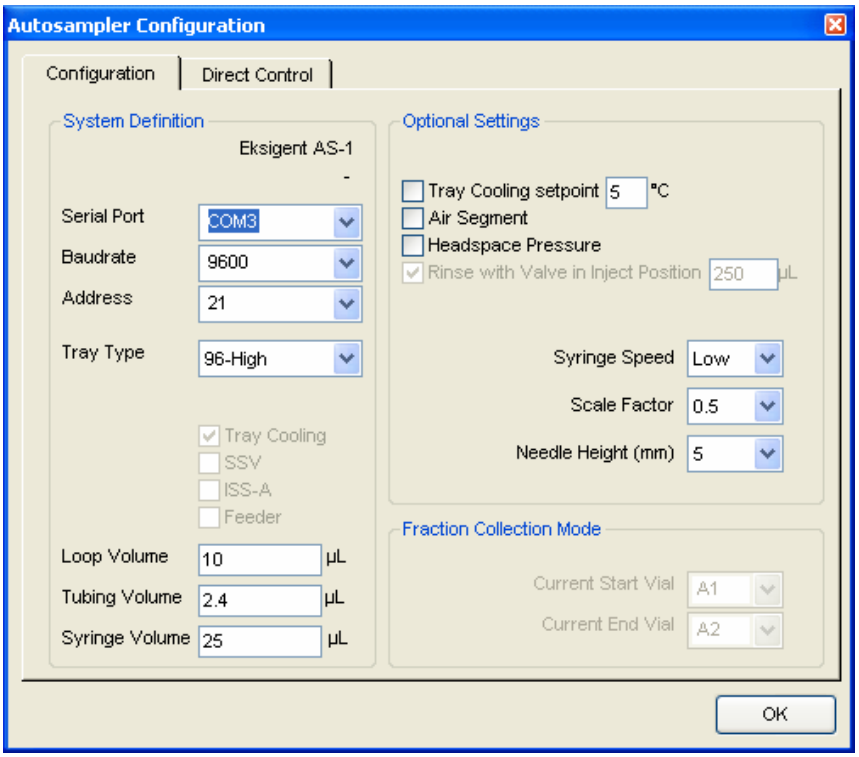


Figure 2-11. Autosampler Device Settings

The system is not yet configured for operation. You must first complete the instructions in **System Initialization** *prior to system operation.*

Chapter 3.
System Initialization

Chapter 3 describes procedures used to prepare a system for initial operation or for operation following an extended period of non-use. Topics covered in this chapter include:

- hardware components and functions (Section 3.1)
- loading mobile phases (Section 3.2)
- preparing the nanoLC Autosampler (Section 3.3)
- flushing the sample loop and sample needle (Section 3.4)
- connecting the nanoLC to the nanoLC Autosampler (Section 3.5)
- verifying the flow rate (Section 3.6)

3.1 Hardware Components and Functions

This section provides a general description of the key components of the nanoLC systems and their various functions.

Mobile phase outlets are located on the right side of the nanoLC system. Each channel has one mobile phase outlet which can be connected to the autosampler and a 10-port column switching valve.

The **10-port Column Switching Valve** is used to switch between reverse phase traps that are connected to the valve for rapid sample loading. Refer to Appendix D for different valve configurations.

An **Optional 6-port injection valve** is available instead of the nano LC Autosampler for manual sample loading. Refer to Appendix D for valve configurations.

3.2 Loading Mobile Phases

The procedure for loading mobile phases will be described for a single binary gradient system for reverse phase chromatography.

If you have a NanoLC-2D system, complete this procedure for both channels 1 and 2. It is suggested that you use mobile phase **A** described below in both reservoirs of channel 1 and use the suggested **A** and **B** mobile phases for the low flow gradient in channel 2.

If you have a nanoLC-1D system, complete this procedure for channel 1 using the mobile phases described below. It is suggested that you use mobile phase **A** in the single reservoir for channel 2 for high flow sample loading.

Required tools and materials:

- Mobile phase reservoirs (p/n 800-00105)
- Clean, degassed HPLC-grade mobile phase A (water with 2% acetonitrile and 0.1% formic acid suggested).
- Clean, degassed HPLC-grade mobile phase B (acetonitrile with 2% water and 0.1% formic acid suggested).
- nanoLC priming tool (p/n 801-00003)
- 15 mL centrifuge tube

step 1 Clean all 50 mL mobile phase reservoirs with appropriate solvents.

step 2 Close the orange valve on the reservoir by turning it to the perpendicular position.

step 3 Fill reservoir A with mobile phase **A** and reservoir **B** with mobile phase B.

step 4 Install both reservoirs and open each valve by rotating to a vertical position.

Purging and flushing the pumps are critical operations to get maximum performance from a new instrument. Purging rapidly replaces the solvent in the pumps while flushing replaces the solvent in the capillaries connecting the pumps to the sample injector.

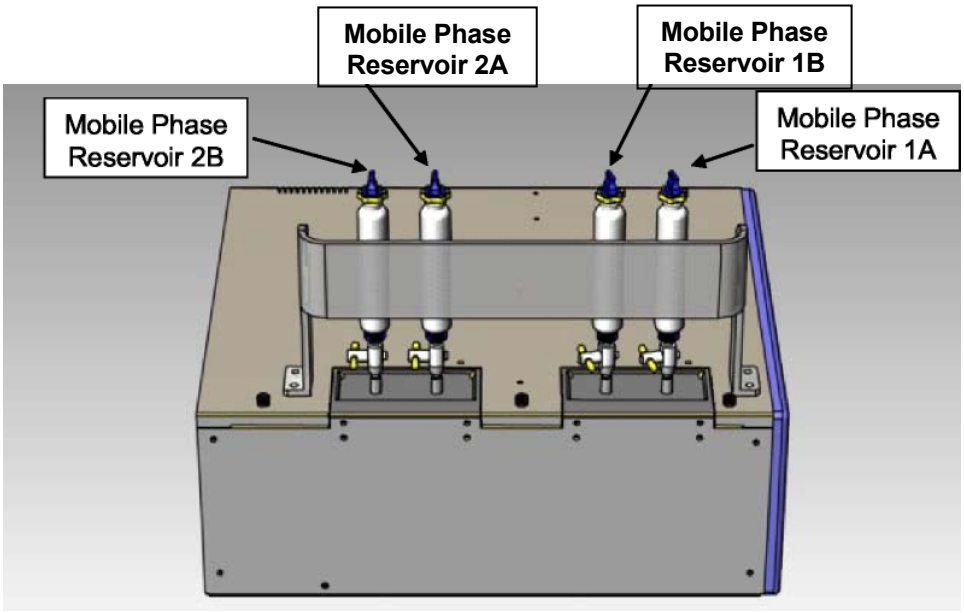


Figure 3-1. Top Panel – Reservoirs for the NanoLC-2D System

step 5 Select **System > Mobile Phases** from the main window of the control software to present the Mobile Phases dialog box (Figure 3-2).

step 6 Set the composition for mobile phase A: Enter the correct solvent composition for **Mobile Phase A**. the A mobile phase will generally be mostly water.

step 7 Set the composition for mobile phase B: Enter the correct solvent composition for **Mobile Phase B**. the B mobile phase will generally be mostly acetonitrile.

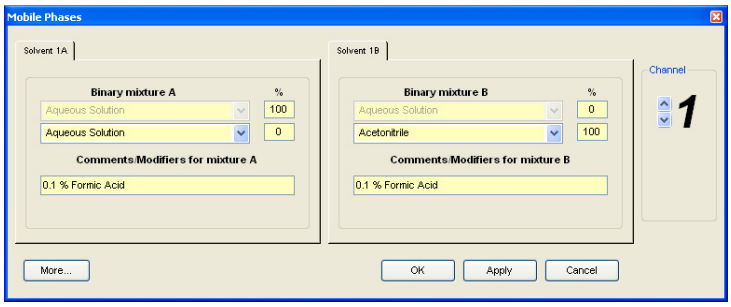


Figure 3-2. Mobile Phases Window

step 8 Click the **More…** button to present the purge and flush settings (Figure 3-3).

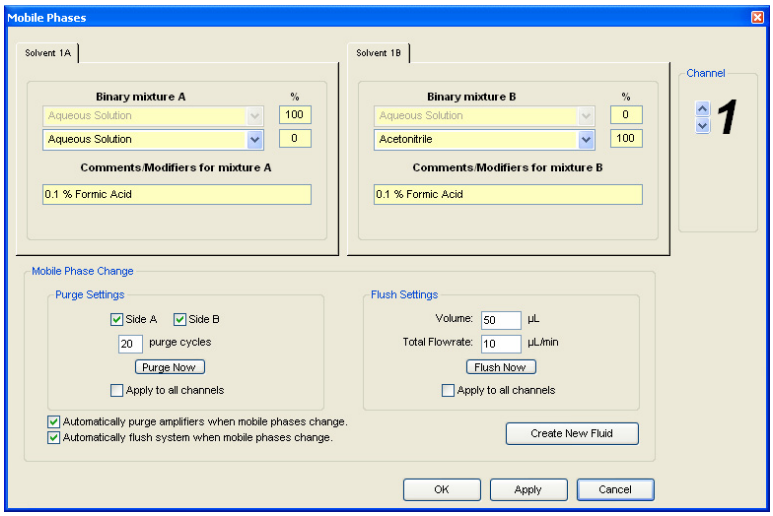


Figure 3-3. Mobile Phases Window – Advanced

step 9 Purge the bubbles from each pump using the priming tool using the following steps:

a. Under Purge Settings, select only one pump to purge, i.e. channel 1 A. Set the number of purge cycles to 20.

b. Click Purge Now. The pump will begin to execute purge cycles. While the pump is purging, insert the priming tool all the way into the pump and depress the check valve.

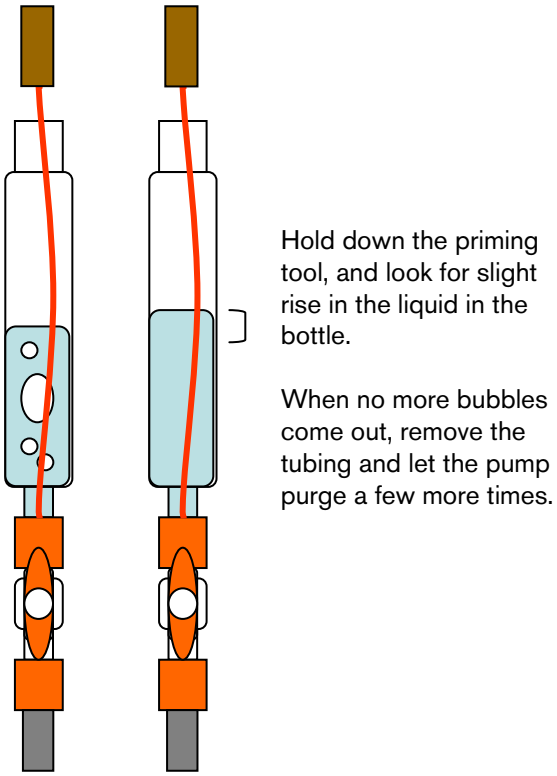


Figure 3-3. Priming Tool Usage

c. After bubbles cease to come up into the bottle, allow the pump to continue purging for the rest of the 20 cycles.

d. Repeat steps a-c for all the pumps separately to ensure each pump is adequately primed.

step 10 Enter 10 for **purge cycles**, select **Side A** and de-select **Side B**.

step 11 Click **Purge Now** to purge the **A** pump.

step 12 Collect the purge output in a 15 mL graduated centrifuge tube. The pump should deliver a total of approximately 6 mL of solvent (600 µL per purge cycle × 10 cycles).

step 13 Next de-select **Side A** and select **Side B**.

step 14 Click the **Purge Now** button to purge the B pump.

step 15 Collect the purge output for pump B and verify that the total volume is approximately 6 mL.

step 16 Enter 100 for the **µL Flush volume** and select a flow rate appropriate for the maximum flow of the channel. For high flow channels, select 10 for the **µL/min Total Flow rate**. For low flow channels, select 6500 for the nL/min Total Flow rate. **Ensure that the outlets of the pump are disconnected before proceeding. Flushing the system with a column connected could over-pressure the system and create leaks.**

step 17 Click the **Flush Now** button.

step 18 Repeat the above steps for the other channel.

step 19 After the flush sequence ends, click **OK** to close the **Mobile Phases** window.

3.3 Preparing the nanoLC Autosampler

The nanoLC Autosampler is the standard autosampler installed with nanoLC system. The autosampler should be pre-assembled and aligned by a qualified Eksigent Technologies service representative. Refer to the nanoLC Autosampler User Manual for a detailed description on its operation.

Required tools and materials:

- Clean HPLC-grade water
- Clean HPLC-grade isopropanol

Figure 3-4. Eksigent nanoLC-1D+ Shown with Nano LC Autosampler

step 1 Fill the wash bottle with a 20/80 mixture of isopropanol/water that has been degassed.

step 2 Place the wash bottles on the wash station bracket and insert the Teflon tubing.

3.4 Flushing the Autosampler Syringe and Liquid Path

step 1 On the front panel of the autosampler, select SYRINGE

step 2 Select SYRINGE END and SYRINGE HOME repeatedly until the syringe is full of liquid with no bubbles. Once no further bubbles are observed, click ESC and return to the main menu.

step 3 Press WASH on the keypad to execute an initial wash. Repeat twice for a dry liquid flow path.

step 4 Press [menu] SERIAL to return the Autosampler to Serial Mode.

step 5 Open the Run Manager and verify the AS-1 state is Idle. This verifies the serial communication is active and the autosampler is connected.

3.5 Connecting the nanoLC to the nanoLC Autosampler

Required tools and materials:

- Two orange 1/16" PEEK sleeves (p/n 910-00024)
- Two 1/16" PEEK fittings and ferrules (p/n 920-00006 and 910-00023)
- Green PEEK sleeve (p/n 910-00025)
- Nut and ferrule for 0/025" fitting (p/n 920-00002 and 920-00003)
- Two meters of 360mm OD / 50mm ID silica capillary (p/n 910-00002)
- Capillary cutter (p/n 200-00096).

Note: This section assumes connection of the nanoLC Autosampler using the high flow channel of the nanoLC instrument. If you are connecting with the low flow channel through the nanoLC Autosampler (e.g. for direct loading experiments) use 25 µm ID capillary for the connections to reduce delay volume. Two meters of 360mm OD / 25mm ID silica capillary (p/n 910-00008) are provided.

step 1 Use the capillary cutter to ensure a clean cut on one end of the 50 µm ID capillary and connect it to the CH1 outlet of the nanoLC using the 0.025" green PEEK sleeve, ferrule, and nut.

step 2 Cut the capillary at an appropriate length (approximately 40 cm) to connect from the CH1 outlet to the **Pump** connection on the injection valve of the nano LC Autosampler. Using an 1/ 16" orange PEEK sleeve and two-piece connector attach the capillary to the nanoLC Autosampler. Please make a clean cut at the end of the capillary and to fully seat the capillary and sleeve in the fitting.

step 3 Connect the remaining piece of 50 µm ID capillary to the **Column** port of the nanoLC Autosampler injection valve using a second 1/16" orange PEEK sleeve and two-piece fitting.

step 4 Cut the 50 µm capillary to a length appropriate to your experiment. This will most likely be a connection to port 1 or 2 of the 10-port switching valve using one of the plumbing diagrams shown in Appendix D.

step 5 Open the **Direct Control Window** (Fig. 3-5) by clicking **System > Direct Control** from the Control software's **Acquisition Window**.

step 6 Set solvent **A and B** to 50/50 and **Total flow rate** to 20 µL/min. (for NanoLC-1D+, use 100%A).

step 7 Click **Start** to flush the valve ports and capillary. Flush for 10 minutes.

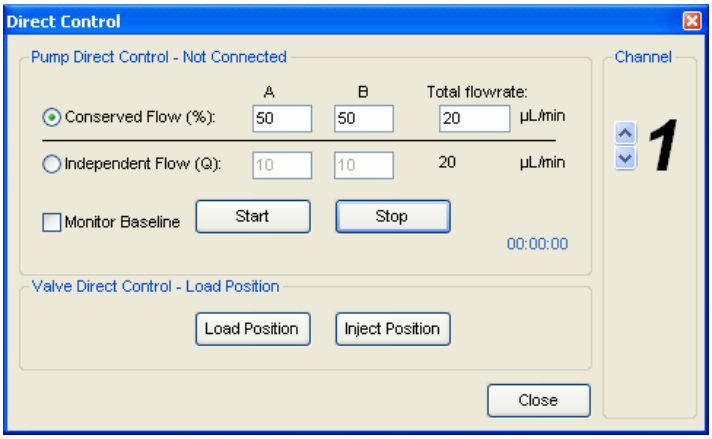


Figure 3-5. Direct Control Window – Flushing CH1 Autosampler Connection

step 8 Stop the flow by clicking **Stop**.

3.6 Verifying the Flow Rate

Before operating the system it is suggested that you verify that the flow rate is properly calibrated. This is done by measuring the time it takes to move a liquid front through a graduated capillary of known volume.

Required tools:

- Flow calibration assembly (p/n 801-00002) for high flow rate channel (includes 20 µL pipettes)
- Flow calibration assembly (p/n 910-00006) for low flow rate channel (includes 5 µL graduated pipettes)

step 1 Re-initialize the pressure transducers (see section 4.5).

step 2 Attach the appropriate flow calibration assembly to the outlet of channel 1 or 2.

step 3 Select **System > Direct Control** from the Eksigent Control Software **Acquisition Window**.

step 4 Set channel **A** to 100% and an appropriate flow rate for that channel (Figure 3-6).

- 5 µL/min for high flow channel
- 500 nL/min for low flow channel

step 5 Click the **Start** button and begin timing. With the high flow calibration assembly, the time it takes for the meniscus or an air bubble to transit from the black stripe to the end of the capillary should be 4 minutes. With the low flow calibration assembly, the time it takes for the meniscus or an air bubble to transit across two segments of the capillary (2 µL) should be 4 minutes. Press **Stop** when the fluid front reaches the end of the pipette

step 6 If the flow rate falls outside of the acceptable range (> ±5%) , re-calibrate the flow meters per the procedure found in Section 4.8

step 7 Disconnect the calibration assembly and blow out the liquid inside the pipette using a pipette bulb or can of compressed air.

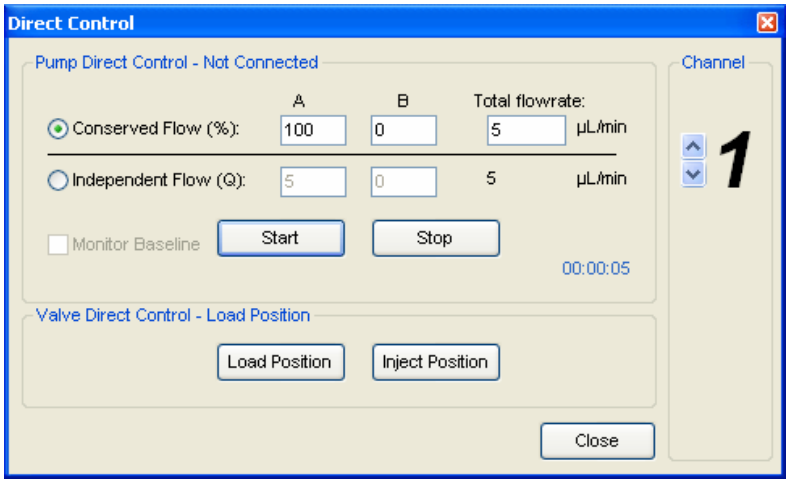


Figure 3-6. Direct Control Window – Flow Rate Check

step 8 Set channel **B** to 100% and repeat steps 5 and 6 to verify the flow rate for pump **B**.

step 9 Disconnect the calibration assembly.

Chapter 4.
Routine Maintenance

Chapter 4 describes the general procedures used to properly maintain the nanoLC system. Topics included in this chapter include:

- recommended maintenance (Section 4.1)
- disposing of waste (Section 4.2)
- changing the sample loop (Section 4.3
- replacing capillary connections (Section 4.4)
- zeroing the pressure transducers (Section 4.5)
- checking flow stability (Section 4.6)
- autotuning flow controllers (Section 4.7)
- calibrating flow meters (Section 4.8)
- cleaning and inspecting the instrument (Section 4.9)
- replacing internal instrument filters (Appendix E)

4.1 Recommended Maintenance

The nanoLC system is designed and built for long-term, robust use in an active laboratory environment. To ensure reliable performance, the following procedures should be performed at the specified interval.

Table 4-1. Recommended Maintenance

Procedure	Frequency	Instructions
Changing Mobile Phase	As needed	Section 3.2
Zero pressure transducers	Monthly	Section 4.5
Checking flow stability	Quarterly	Section 4.6
Autotune flow controllers	As needed	Section 4.7
Waste disposal	As needed	Section 4.2
Replace interconnecting capillaries	As needed	Section 4.4
Change sample loop	As needed	Section 4.3
Calibrate flow meters	Quarterly	Section 4.8
Clean and inspect system	Quarterly	Section 4.9
Change internal instrument filters	Annually	Appendix E

4.2 Disposing of Waste

The user will need to properly dispose of the contents of any effluent waste in an appropriate chemical waste container. For typical nanospray experiments, waste from the 10-port column switching valve due to high flow sample loading will be collected in a waste vial.

The pump purge waste container located on the left side of the instrument will also need to be emptied periodically. Unscrew the thumbscrew holding the clamp around the waste bottle. Remove the bottle from the clamp and unscrew the cap from the bottle. Pour the contents into an appropriate chemical waste container.

Always follow appropriate safety procedures when handling or disposing waste chemicals. See the solvent Material Safety Data Sheets for more information.

4.3 Changing the Sample Loop

Required tools and materials:

- Laboratory wipes
- Clean HPLC-grade methanol
- 1/4" open end wrench
- Sample loop
- Two 1/16" HPLC fittings (p/n 920-00006 and 910-00023)

Note: This sample loop may be on the optional 6-port sample injection valve or on the nano LC Autosampler.

step 1 Use the 1/4" wrench (or fingertight fittings) to remove the installed sample loop.

step 2 Insert one end of the new sample loop through a 1/16" fitting and ferrule and into the injection valve port. Make sure the end of sample loop is flat and fully inserted into the valve port. Do not reuse fittings from other valves as the port depth can vary from valve to valve.

step 3 Attach the other end of the sample loop to the opposite valve port.

step 4 Open the **Direct Control Window** by clicking **System > Direct Control** from the Control software's **Acquisition Window**.

step 5 Set solvent **A** to 50, solvent **B** to 50 and **Total flow rate** to 6 µL/min (Figure 4-1).

step 6 Click **Start**.

step 7 Toggle the injection valve between the load and the inject position several times to flush out any loose particles.

step 8 Look for any signs of leakage indicating a loose connection. Remake any connections that indicate a leak.

step 9 Click **Stop**.

4.4 Replacing Capillary Connections

Required tools and materials:

- Laboratory wipes
- Clean HPLC-grade methanol
- 1/4” Allen wrench
- Transfer capillary
    - For the high flow channel, use 50 µm ID (p/n 910-00002)
    - For the low flow channel, use 25 µm ID (p/n 910-00008)
- Microtight fitting
- Green PEEK sleeve (p/n 910-00025)
- Black microtight ferrule (p/n 920-00002)
- Tan microtight nut (p/n 920-00003)
- 1/16” sleeved fitting
- 1/16” OD Orange PEEK sleeve (p/n 910-00024)
- 1/16” PEEK fitting nut (p/n 920-00006)
- 1/16” PEEK fitting ferrule (p/n 910-00023)

step 1 Use the 1/4” Allen wrench or your fingers to remove the current fitting, ferrule, and sleeve.

step 2 Remove the fitting and ferrule from the capillary and discard the old capillary.

step 3 Cut the capillary to the appropriate length. Ensure a clean end cut and clean with methanol.

step 4 Insert one end of a new capillary through the appropriate PEEK sleeve.

step 5 Slide the end of the capillary and the PEEK sleeve through the compression fitting and ferrule. Make sure the sleeve extends through the ferrule.

step 6 Slide the capillary, sleeve and fitting into the port until both bottom out.

step 7 While lightly pressing the capillary and sleeve into the fitting with one hand, use the other hand or the 1/4” wrench to tighten the fitting.

step 8 Open the **Direct Control** window by clicking **System > Direct Control** from the Control software’s **Acquisition Window** (Figure 4-1).

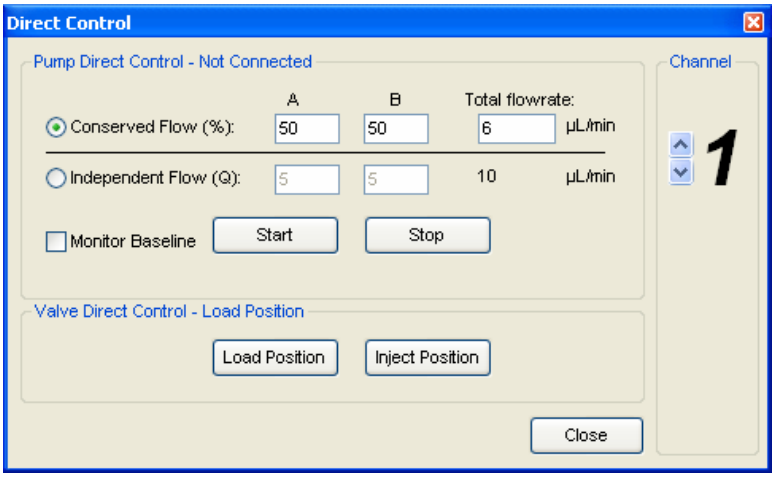


Figure 4-1. Direct Control Window

step 9 Set solvent **A** to 50, solvent **B** to 50 and **Total flow rate** to an appropriate value for the channel you are connecting.

step 10 Click the **Start** button and flush the capillary for 5 minutes.

4.5 Zeroing the Pressure Transducers

Before zeroing the pressure transducers, it is advisable to open the outlet fittings from the mixing tees on all channels. This will ensure there is no residual pressure on the outlet of the system.

Zeroing the pressure transducers should be performed on a monthly basis. To initiate the pressure transducer zeroing procedure, start the Control software and access the diagnostics screen by selecting **System > Hardware Diagnostics**.

It is very important that the instructions below are followed precisely. Attempting to zero the pressure transducers while there is still residual pressure on the system will lead to inaccurate flow rates.

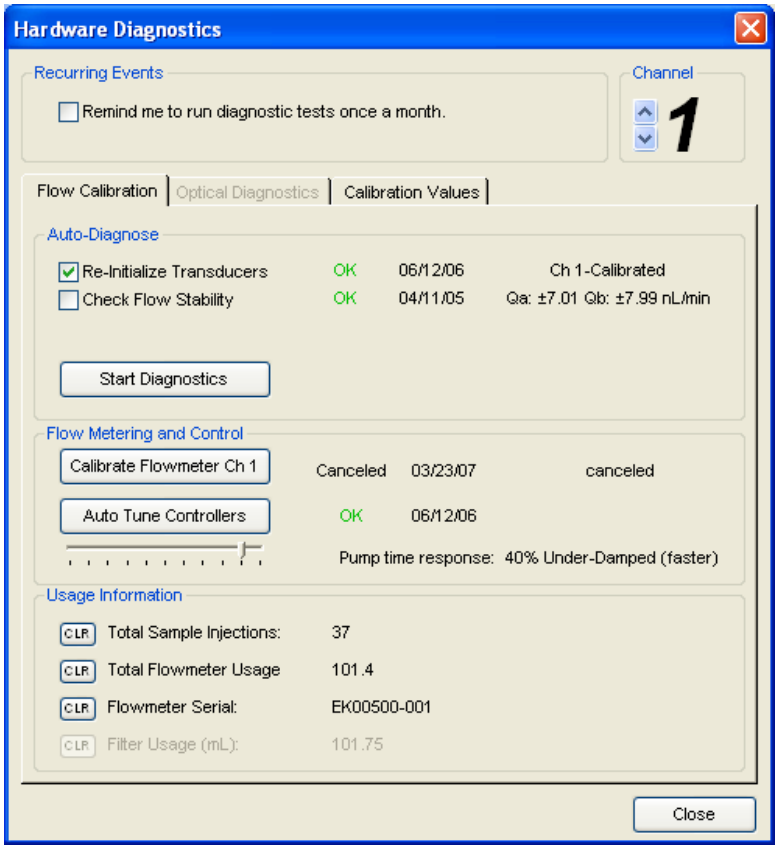


Figure 4-2. Hardware Diagnostics Window -Reinitialize Transducers

step 1 In diagnostics, check the **Re-Initialize Transducers** box.

step 2 Click **Start Diagnostics**. A message will appear (Fig. 4-3) warning that the procedure should only be performed if there is no residual pressure on the system.

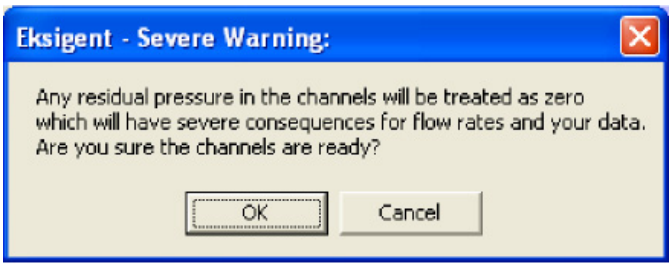


Figure 4-3. Residual Pressure Warning

step 3 Once the system indicates that it is at ambient pressure, click **OK** and a status window will indicate that auto-zero is in progress (Figure 4-4).

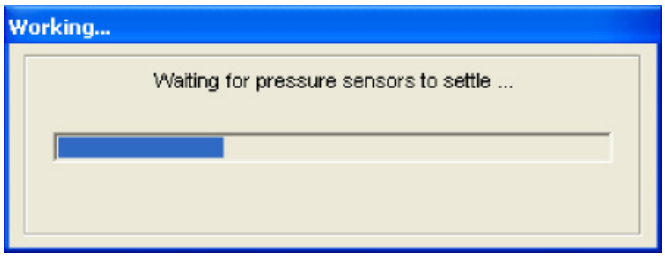


Figure 4-4. Auto-zero Status Window

step 4 When the auto-zeroing process is complete, exit the diagnostics window and return to the **Acquisition Window**.

step 5 Repeat for both channels (if not conducted simultaneously).

4.6 Checking Flow Stability

The flow stability of the A and B channels can be determined in a similar fashion to the procedure used for zeroing pressure transducers as described in Section 4.5 by selecting the **Check Flow Stability** diagnostic test. Two screens will be observed during the running of the test (Figure 4-5). Initially the process requires the controllers to stabilize for 60 seconds. This is then followed by a 30 second examination of the control.

Step 1 **Step 2**

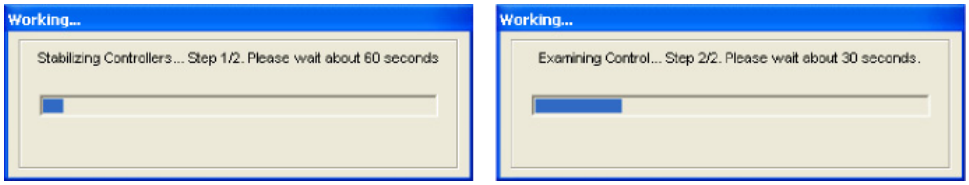


Figure 4-5. Checking Flow Stability Progression

Repeat this for both channels.

4.7 Autotuning Flow Controllers

The flow controller autotune should be run on a monthly basis to optimize the performance of the system.

CAUTION

To avoid the possibility of overpressure, disconnect the capillary at the mixing tee fitting prior to performing the autotune procedure.

step 1 When the **Autotune** button is selected in the diagnostic window, the system's response to changes in flow rate is monitored and adjustments are made to the controllers PID (proportional/integral/derivative) loop.

step 2 A prompt window alerts you that the autotune is about to begin (Figure 4-6).

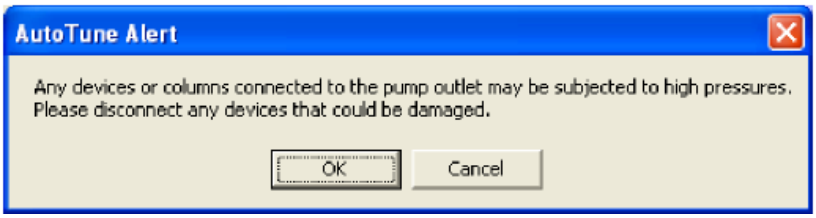


Figure 4-6. Autotune Prompt

step 3 This is followed by status windows (Figure 4-7) which alert you that the system is preparing to Autotune and then performing the autotune.

These adjustments improve the system's response time and flow rate accuracy.

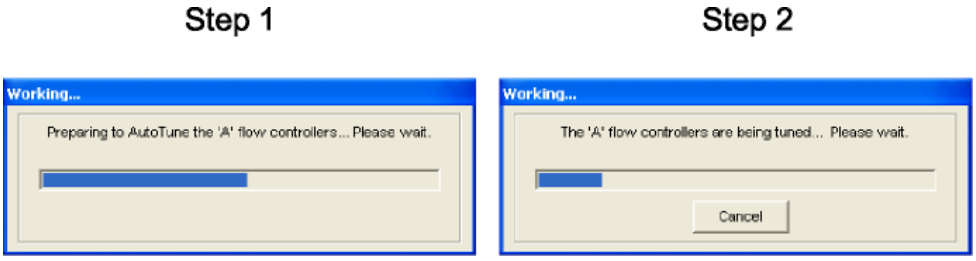


Figure 4-7. Autotune Status Bars – Channel A

step 4 Upon completion, close the diagnostics window and return to the main screen.

step 5 Repeat for both channels (if not conducted simultaneously).

Note: The slider below the Auto Tune Controllers button is used to change the pump time response. Moving the slider to the right will change the pump to respond faster (under-damped). Moving the slider to the left will change the pump to respond slower (over-damped).

4.8 Calibrating the Flow Meters

The flow meters should be calibrated quarterly or when the gradient separation performance seems to be drifting. Calibration of the flow meters consists of measuring the velocity of a liquid front in a tube of known diameter. Selecting the Calibrate Flow meters test will bring up a dialog box with step-by-step instructions for performing the test.

Required tools:

- flow calibration assembly (p/n 801-00002 or 801-00006)

Note: If calibrating the low flow channel using the 801-00002 assembly, you can save time by connecting the pipette to the system and prefilling the pipette to approximately 5 µm before the first black line using either Direct Control or the Mobile Phases > Flush Now feature.

step 1 Select **System > Hardware Diagnostics** from the Control software **Acquisition Window**. Selecting the **Calibrate Flow meter** test will bring up a dialog box with step-by-step instructions for performing the test.

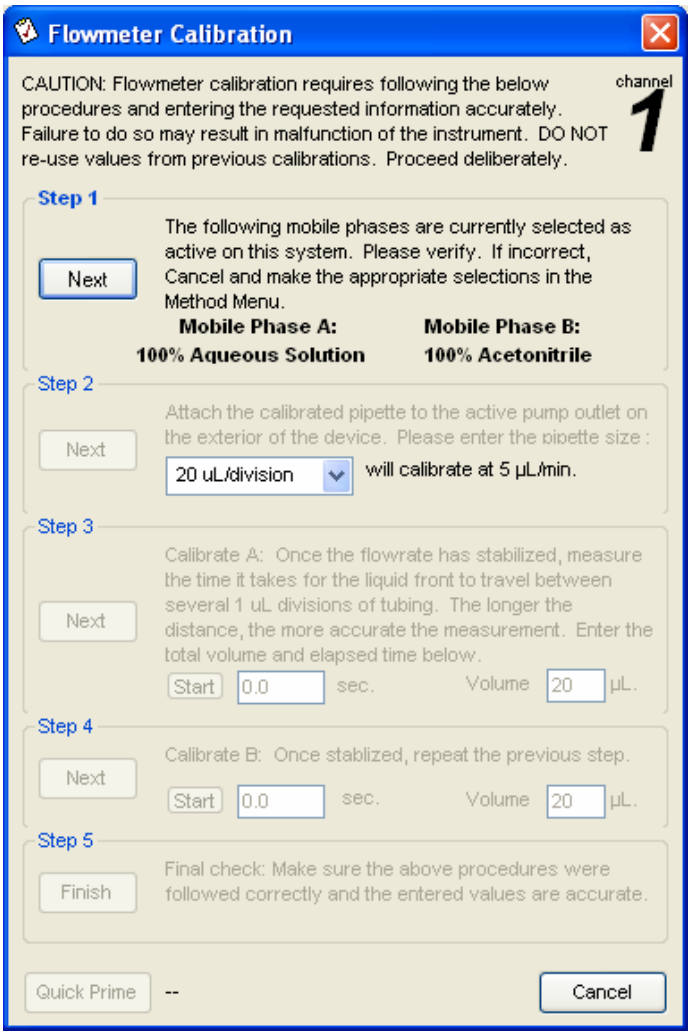


Figure 4-8. Flow Calibration Window

step 2 Verify that mobile phases specified are correct. If incorrect, click **Cancel** to close the **Flow meter Calibration Window**. Make the necessary changes in the **Mobile Phases Window**, perform step 1 again and then click **Next**.

step 3 Attach the flow calibration assembly, with appropriate pipette to either the mixing tee or after the valve or column. Select the appropriate **pipette size** and flow rate for calibrating that channel (see table 4-2).20 µL/division in the **pipette size** to set the calibration flow rate to 5 µL/min for the high flow channel or 1 µL/division in the **pipette size** to set the calibration flow rate to 500 nL/min for the low flow channel.

step 4 Click **Next** to start the flow in channel A. Enter the appropriate volume requested in step 3 of the **Flow Calibration Window** dialog (Fig. 4-8). Wait until the liquid front travels to the black line mark on the pipette and press **Start** to begin timing. For Channel 1, time how long it takes for the liquid front to travel 20 µL. For Channel 2, time how long it takes for the liquid front to travel 2 µL. Press **Stop** when the fluid front reaches the end of the pipette, or the appropriate mark. Then click **Next**. If necessary disconnect the calibration assembly and dry out the liquid inside the capillary.

step 5 Repeat the measurement for calibration of the **B** channel flow meter.

step 6 Verify that the instructions were followed exactly and that all values entered are correct and then click finish.

step 7 Use Table 4-2 to choose the proper calibration pipettes when calibrating the high flow and low flow channels of the nanoLC.

Table 4-2. Calibration Pipette Guide

	High Flow Channel	Low Flow Channel
Calibrated Pipette Size	20 µL	1 µL/division (5µL total)
Calibration Flow Rate	5 µL/min	500 nL/min
Calibration Volume	20 µL/side	2 µL/side

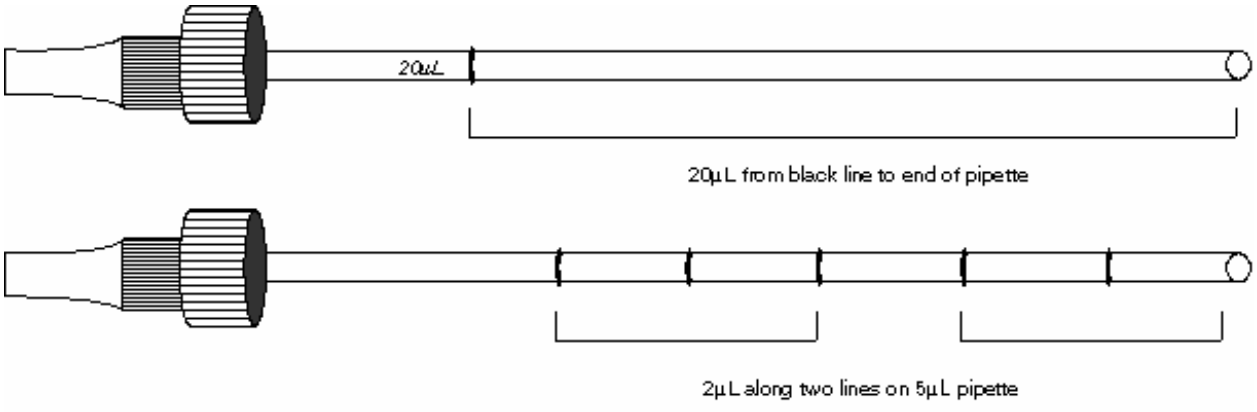


Figure 4-9. Flow Calibration Pipettes

4.9 Cleaning and Inspecting the Instrument

Unplug the external power supply from the power source before cleaning and internal inspection.

step 1 Clean the outside of the instrument by wiping down with a cloth slightly dampened with water and a small amount of liquid dish soap.

step 2 Visually inspect the system fluidics and electronic connectors on a quarterly basis. Look for evidence of fluid leaks by checking all fluid connections. Also look for dried deposits that may indicate a slow leak.

step 3 Identify and correct the source of any leaks if found. If a fluidic connection is broken, replace the fitting and re-flush the system. Inspect the new connection to ensure that no leaks are present. Dabbing a laboratory wipe around fluid connections is a good method to identify slow leaks.

Chapter 5.
Quick-start Guide

Chapter 5 offers a brief tutorial which should be useful in understanding the normal operation of the Eksigent nano LC. The procedures described in this chapter presume that the system has already been properly installed and initialized as described in chapters 2 and 3. Topics in this chapter include:

- powering-up the system (Section 5.1)
- purging and flushing with new solvents (Section 5.2)
- equilibrating the system (Section 5.2)
- creating an autosampler method (Section 5.3)
- creating an LC method (Section 5.4)
- creating a run table (Section 5.5)
- starting a run (Section 5.6)
- viewing the collected data file (Section 5.7)

5.1 Powering-up the System

If the system is not already on, turn on the nanoLC power switch mounted on the rear panel. The green LED on the front of the instrument should illuminate and the injection valve should initialize.

If the autosampler is not already on, turn on the power switch on the nanoLC Autosampler.

Turn on the computer, log-in to Windows and launch the Control Software by double clicking the software icon.

After initialization, the Control software's **Acquisition Window** of the Control software will be displayed (Figure 5-1).

Note: On multichannel systems: Both the nanoLC-1D plus and the NanoLC-2D instrument have two channels of fluid control. They are denoted in this manual and in the software as channel numbers 1 and 2. Throughout the software common windows are used to display or control these channels. To select the channel appropriate to that window (when available) simply click on the up or down arrows next to the channel number display in the upper right of the window.

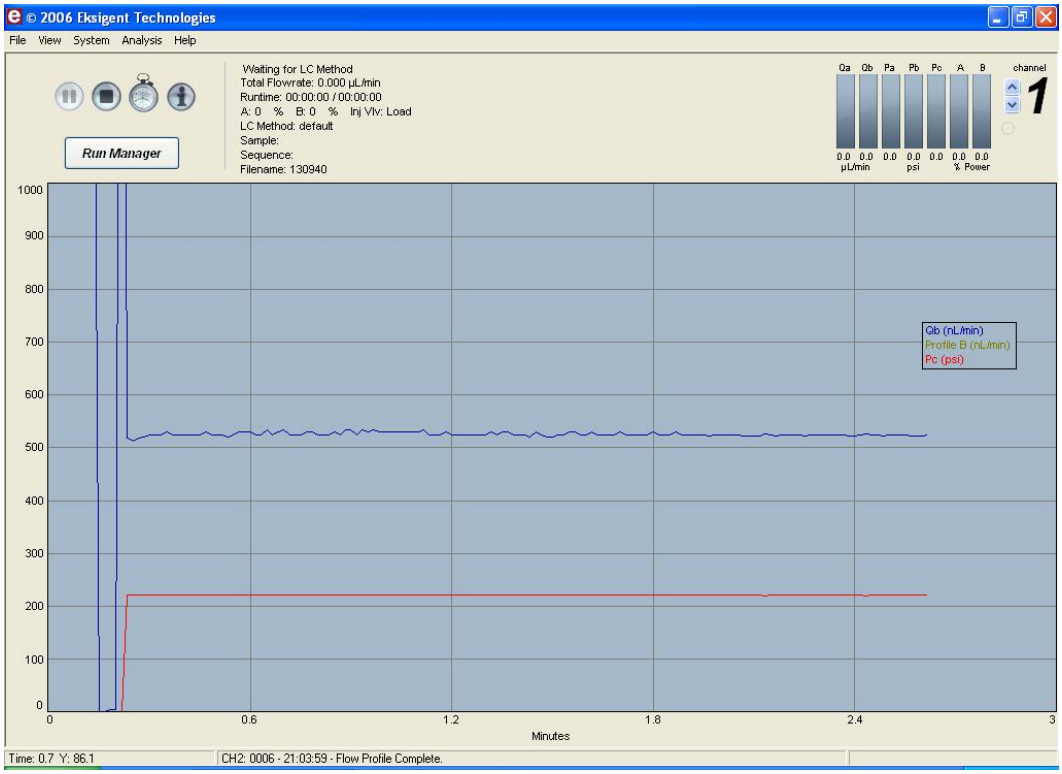


Figure 5-1. Acquisition Window

5.2 Purging and Flushing with New Solvents

If solvent has been sitting for more than 2 weeks, the solvent should be replaced with fresh solvent, then purged and flushed. If the solvent is less than two weeks old, proceed to step 5.3.

step 1 To discard old solvent in the reservoir bottles, one of two methods can be used: remove the bottle and pour out the old solvent, or purge the old solvent through to waste (Figure 5-2).

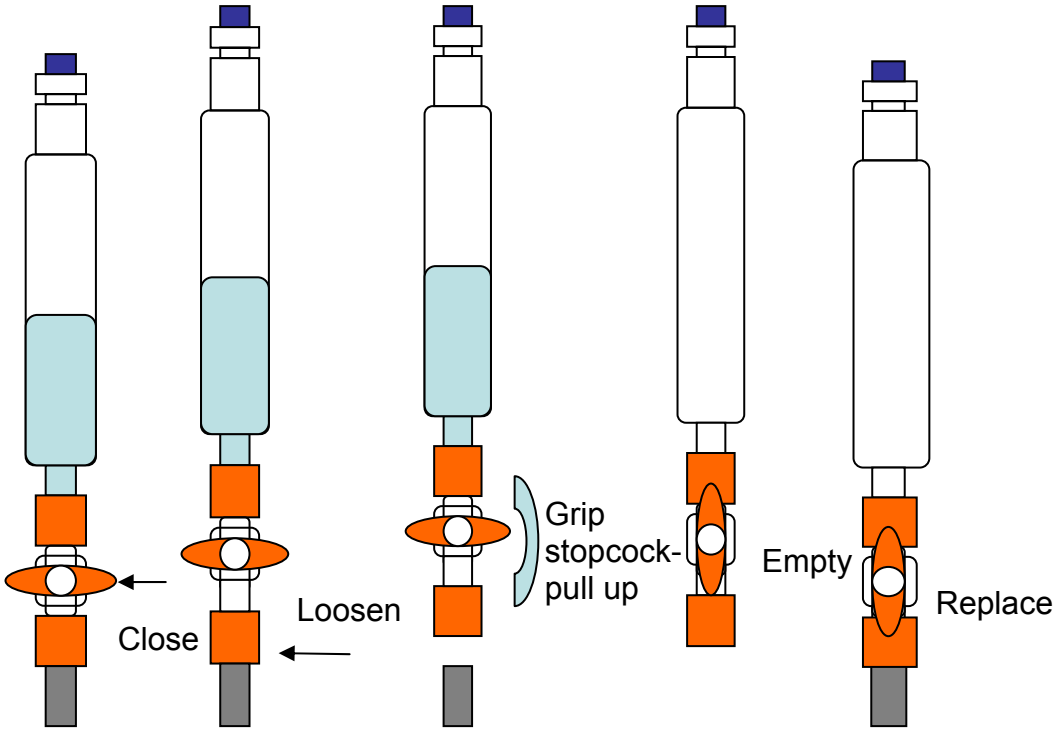


Figure 5.2- Emptying Solvent Reservoirs

step 2 Pour new mobile phase into the bottle. Purge the HPLC at least 10 times. Depress the check valve while purging to dislodge bubbles in the pump head, as shown in figure 3.3.1.

step 3 After the pump is thoroughly purged, flush. Select **System > Mobile Phases** from the main control window. Click **More** and flush the system 100uL on Channel 1 and Channel 2. See section 3.2 for more information on flushing.

5.3 Equilibrating the System

The Control software's **Direct Control** window (Figure 5-3) can be used to equilibrate the system following system power-up, a change of solvent or change of column. The injection valve can also be toggled between load and inject positions to flush the injection valve loop and interconnecting ports. Select **System > Direct Control** from the main Control software Acquisition Window.

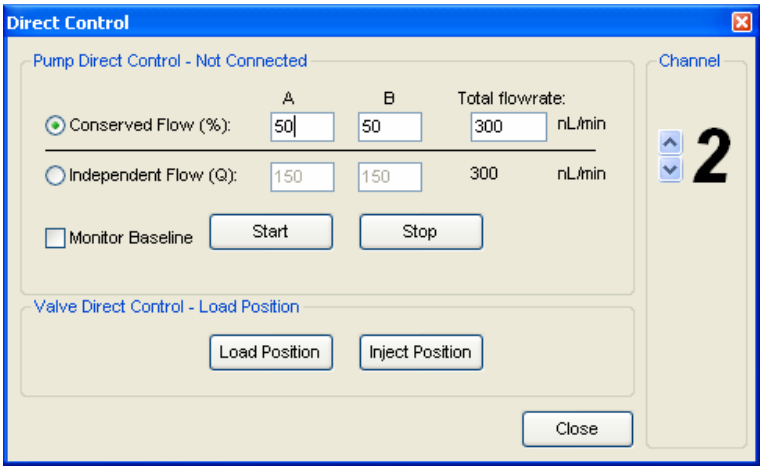


Figure 5-3. Direct Control Window

step 1 Ensure that the **Conserved Flow** radio button is selected, set **A** (%) to 50 and **B** (%) to 50. This will be the mobile phase composition used for equilibration.

step 2 Set the **Total flow rate** to 300 (nL/min).

step 3 Click on **Start** to start the pumps and begin equilibration.

step 4 Flush the switching valve (or an injection valve connected directly to the Eksigent nano LC instrument) by alternately clicking on the **Load Position** and **Inject Position** buttons in the **Valve Manual Control** area. To switch the valve in the nano LC Autosampler, use the Autosampler direct control in the run manager under **Devices > Autosampler Device Settings > Direct Control Tab** (Figure 5-5).

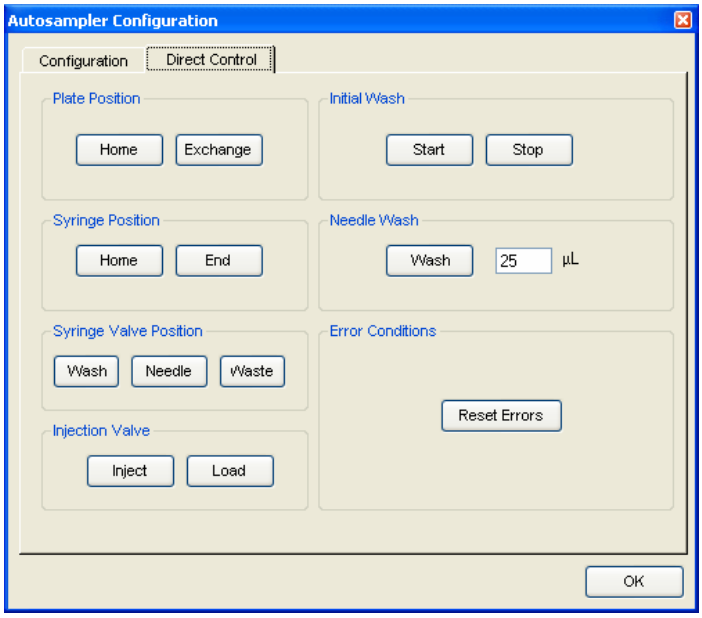


Figure 5-5. Autosampler Configuration Window

step 5 Allow the system to equilibrate for approximately 10 minutes.

5.4 Creating an Autosampler Method

The parameters used for loading the sample into the injection valve and for rinsing the autosampler syringe and sample needle are stored in the autosampler method. This section will review an autosampler method appropriate for loading a trap with Channel 1, and running a gradient with Channel 2.

step 1 Place the sample vial containing the standard test mixture in vial position A1 of autosampler 48-vial tray.

step 2 Click **Run Manager** in the **Acquisition Window** to open the **Run Manager** display (Figure 5-6).

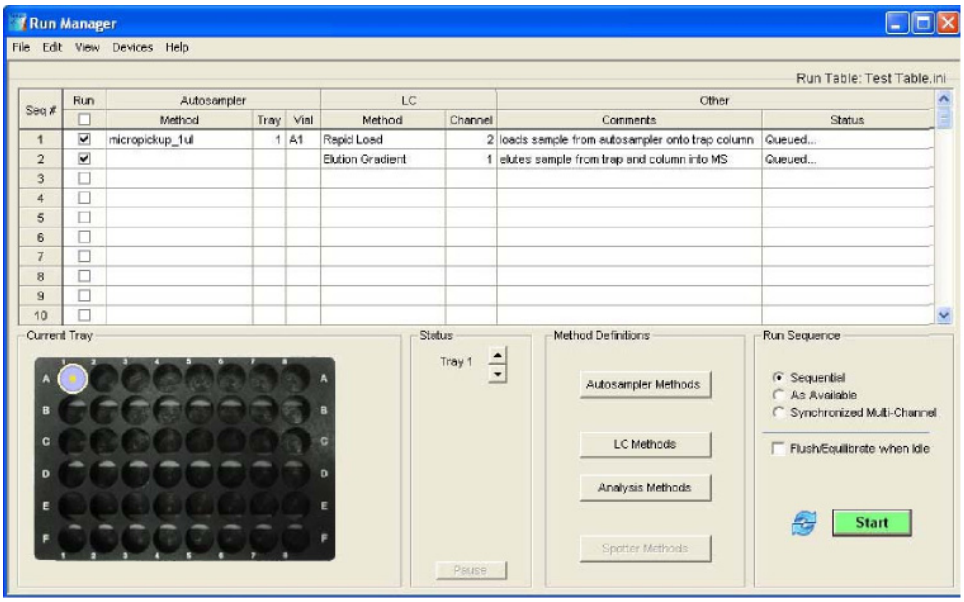


Figure 5-6. Run Manager Window

step 3 If you do not see a picture of the 48-vial tray in the **Run Manager** Window select the **Devices > Autosampler Device Settings** menu to set the tray type. You will need to restart the **Run Manager** after changing the tray type.

step 4 If you do not see the columns shown in Figure 5-6, choose **Edit > Choose Column…** from the menu and select the appropriate columns for display.

step 5 Click **Autosampler Methods** to bring up the **Autosampler Method Editor Window** (Figure 5-7).

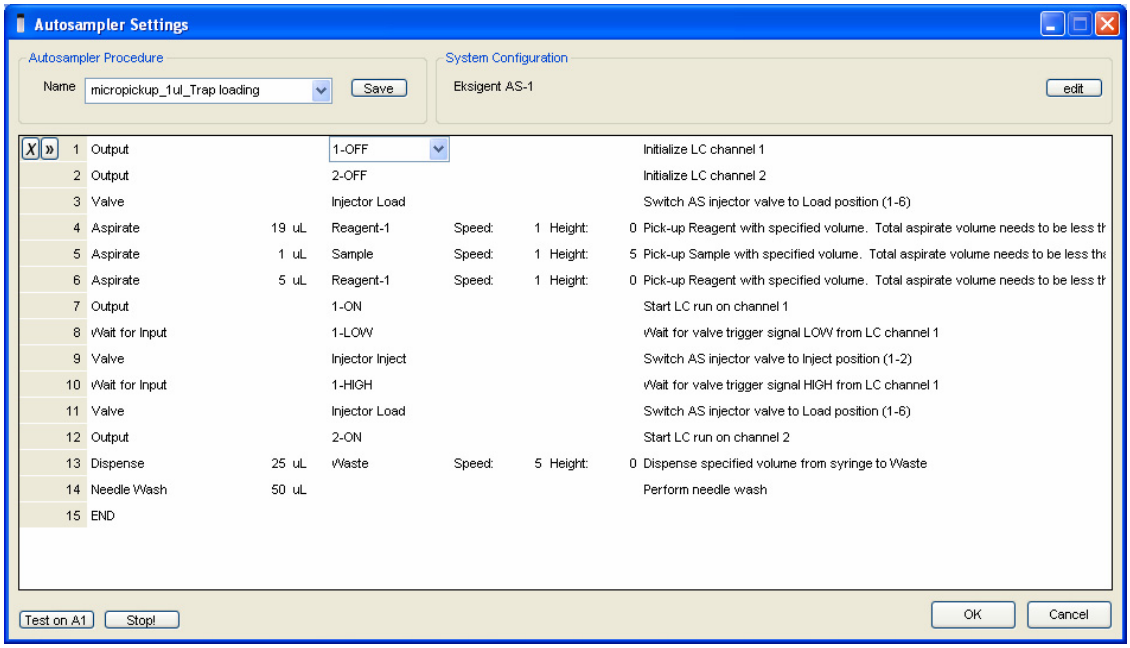


Figure 5-7. Autosampler Method Editor Window

step 6 Select a method from the drop down method similar to the one above. Highlight the name of the method, rename it, and click **Save** to create a new method.

step 7 All Eksigent autosampler methods should contain the following steps in the same order. The volumes can be modified but the general format should remain the same for a given method type. In this example, a trap loading autosampler method is indicated:

Table 5.1. Template for Micropickup Injection, Trap Loading Autosampler Method

Step #	Operation	Volume	Parameter	Speed	Height	Description
1	Output		1- Off			Ensure output 1 relay is in off state
2	Output		2- Off			Ensure output 2 relay is in off state
3	Valve		Injector Load			Place autosampler valve in load position
4	Aspirate	19uL	Reagent -1	1	0	Aspirate from Reagent 1 vial position
5	Aspirate	1uL	Sample	1	(1-5)	Aspirate from Sample vial- use appropriate height for sample vial. 1= closest to bottom, 5= 5 mm from bottom of vial.
6	Aspirate	5uL	Reagent -1	1	0	Aspirate from Reagent 1 vial position
7	Output		1-On			Send Output 1 relay signal to LC Channel 1, signaling LC Channel 1 to start the method
8	Wait for input		1 - Low			Wait for an input signal (injection signal) from Channel 1 before continuing autosampler method.
9	Valve		Injector Inject			Place autosampler valve in the inject position
10	Wait for input		1- High			Wait for injection signal from LC Channel 1 to turn off, indicating the loop injection is complete.
11	Valve		Injector Load			Place autosampler valve in the load position
12	Output		2- On			Send Output 2 relay signal to LC Channel 2, signaling LC Channel 2 to start the gradient method.
13	Dispense	25uL	Waste	5	0	Dispense aspirated volume to waste. Total aspirate and dispense volumes must be equal.
14	Needle wash	50uL				Perform 50uL needle wash
15	End					Method end

step 8 To modify method steps, click on the step and change the volume by typing over. Modify the operation or parameter by clicking on the step and choosing a new value in the drop-down menu.

step 9 Add new steps by clicking on the **>>** arrows to the left of the line. Delete a step by clicking on the **X**.

Note: for an autosampler configured with the standard 25uL syringe,

step 10 Change the Needle Wash volume to 100 µL.

step 11 Needle Wash 100 µL.

step 12 Type Autosampler Test in the **Name** field and click on **Save** next to the name to store the method.

step 13 Click **OK** button to close the Autosampler Method Editor Window

5.5 Creating an LC Method- Channel 1

The conditions used for separating the sample are stored in the LC method. This section will create an analysis method called LC Test. LC methods are accessed for editing by clicking on **LC Method Editor** located in the **Run Manager** window.

Note: For the NanoLC-2D. If 'NanoLC-2D Parallel' is chosen in the System > Instrument Configuration menu, then the LC Method editor appears as described in this section. If 'NanoLC-2D is chosen, then the 2D Editor will appear. This editor allows for the easy and rapid con-figuration of multi-step, on-line 2D methods. Each multi-step experiment is saved as a single method file and can be programmed on a single line of the Run Table. Please see both the 2D section of the software manual as well as Appendix C for further description of the 2D valve configurations.

step 1 Click **LC Methods** on the **Run Manager** window to display the **Method Settings** dialog box (Figure 5-8).

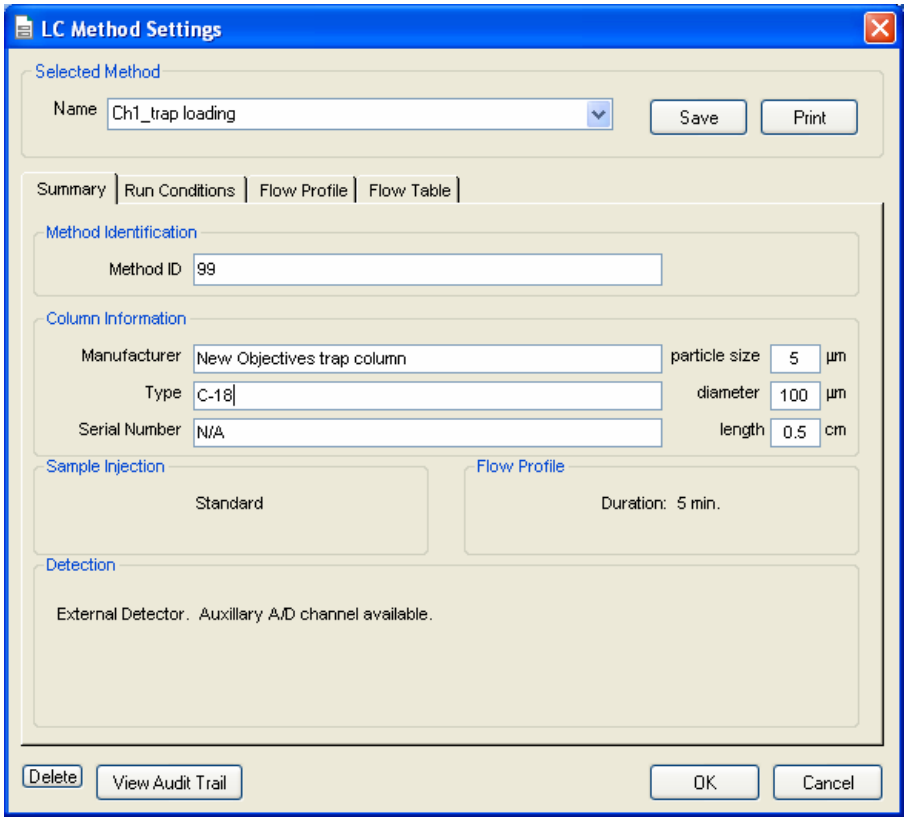


Figure 5-8. LC Method Editor-Summary Tab

step 2 To create a new method, type over the name of the method and click **Save**.

step 3 If you wish, enter any column information appropriate for your experiment. This information is information-al and stored with the LC method file.

step 4 Click on the **Run Conditions** tab of the **LC Method Editor** window to obtain the Run Conditions tab (Figure 5-9).

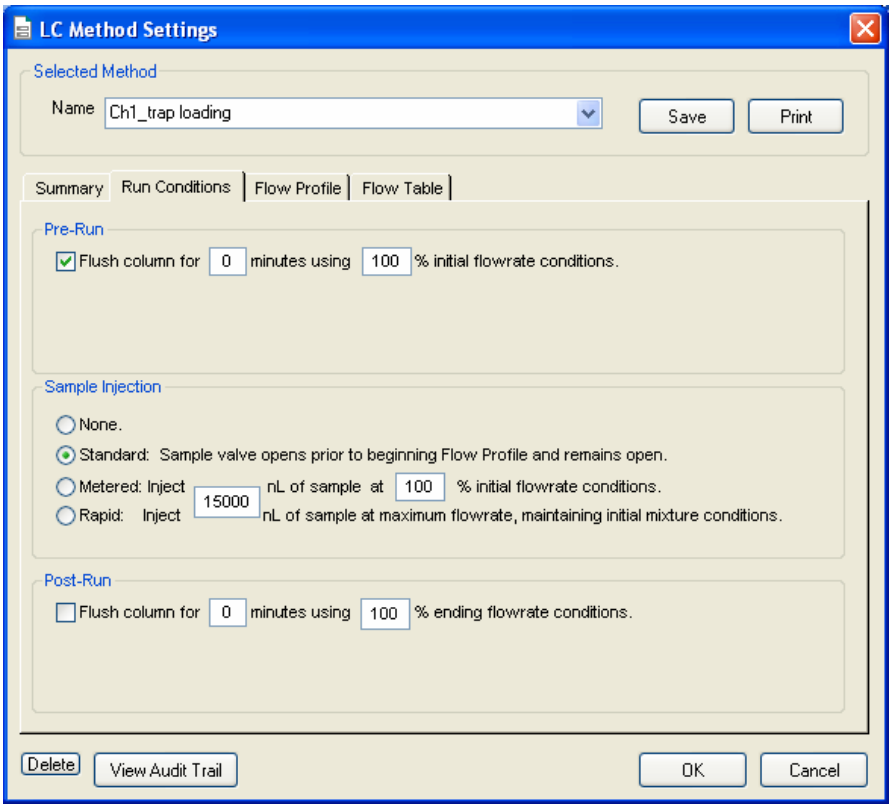


Figure 5-9. LC Method Editor – Run Conditions Tab

step 5 Put a check mark in the **Pre-Run Flush** column check box and specify a time of 0.1 minutes to flush the column using 100% of the initial flow rate conditions.

step 6 Select **Standard:** in the **Sample Injection** region. This will cause the injection valve to be placed in the inject position for the duration of the Channel 1 run.

step 7 Leave the check mark box for **Post-Run Flush** column empty.

step 8 Click on the **Flow Table** tab to set the gradient parameters.

step 9 Enter the flow rate, time and percentages of A and B that are appropriate. For a 2D system that has the same mobile phase present for Channel 1 A and B, use 50% A and 50%B. For a 1D+ system, use 100%A.

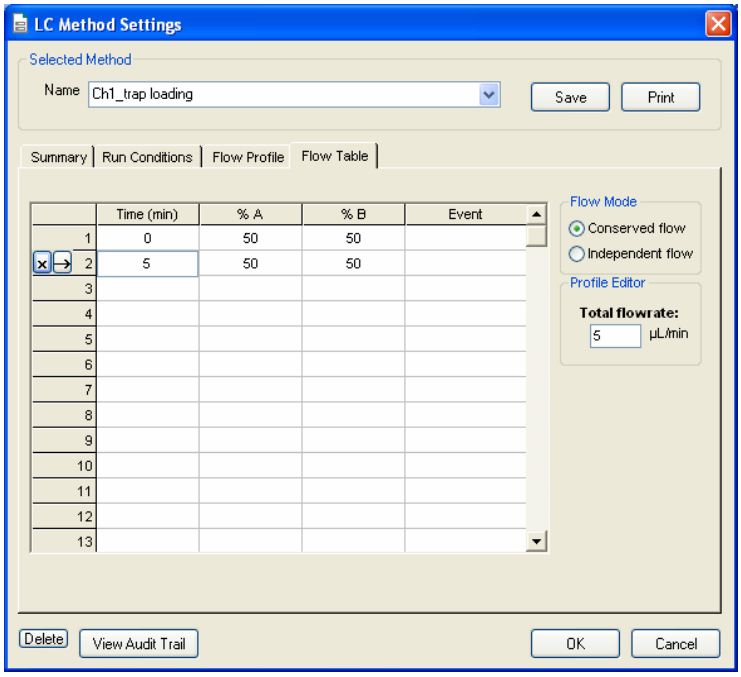


Figure 5-10. LC Method Editor – Flow Table Tab

step 10 Select the Flow Profile tab to present the *Flow Profile* dialog box (Figure 5-8).

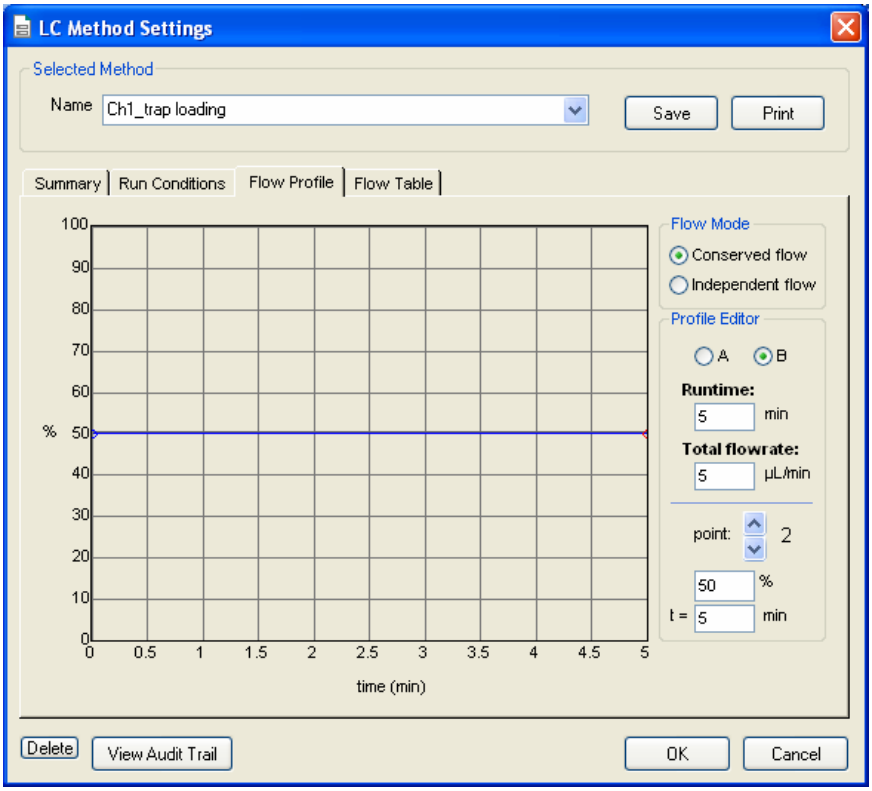


Figure 5-11. LC Method Editor – Flow Profile Tab

step 11 The mobile phase composition can also be set by clicking and dragging points on the graph or by setting the % of **A** or B in the **Profile Editor** region.

step 12 Once the method is complete, click **Save** to save the analysis method.

5.6 Creating an LC Method- Channel 2

step 1 Open the **LC Method** editor and type over the name of the method to create a new method. Click **Save**.

step 2 If you wish, enter any column information appropriate for your experiment. This information is information-al and stored with the LC method file.

step 3 Click on the **Run Conditions** tab of the **LC Method Editor** window to obtain the Run Conditions tab (Figure 5-12).

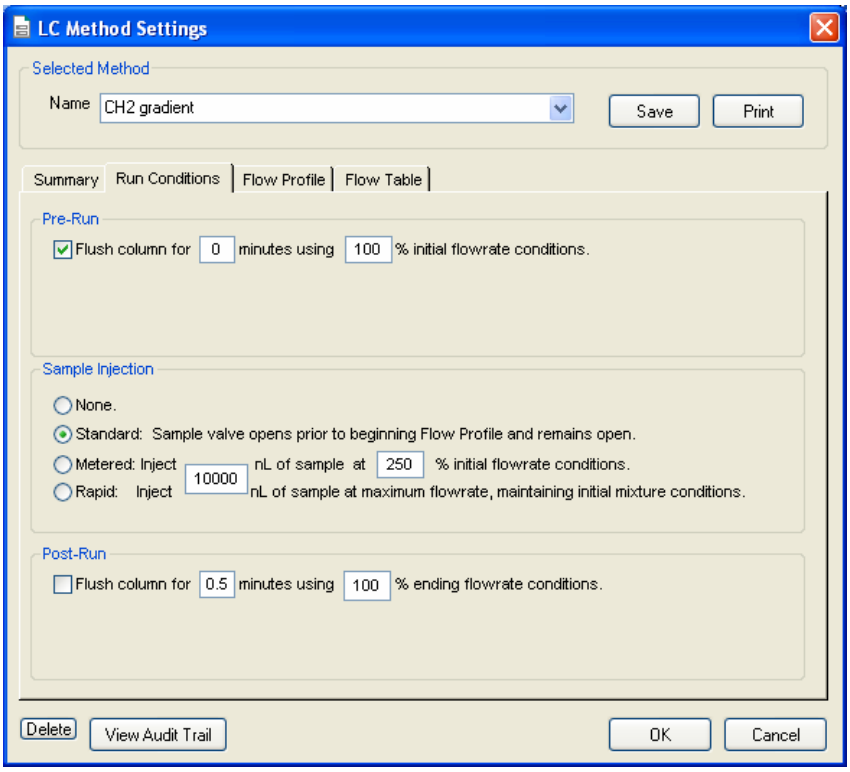


Figure 5-12. LC Method Editor – Run Conditions Tab

step 4 Put a check mark in the **Pre-Run Flush** column check box and specify a time of 0 minutes to flush the column using 100% of the initial flow rate conditions.

step 5 Select **Standard:** in the **Sample Injection** region. This will cause the 10-port valve to be placed in the inject position for the duration of the Channel 2 run.

step 6 Leave the check mark box for **Post-Run Flush** column empty.

step 7 Click on the **Flow Table** tab to set the gradient parameters.

step 8 Enter the gradient parameters you wish to run. Add new steps by clicking on the **>>** to the left of the table. Delete steps by clicking on the **X**. Set the overall flow rate on the right side.

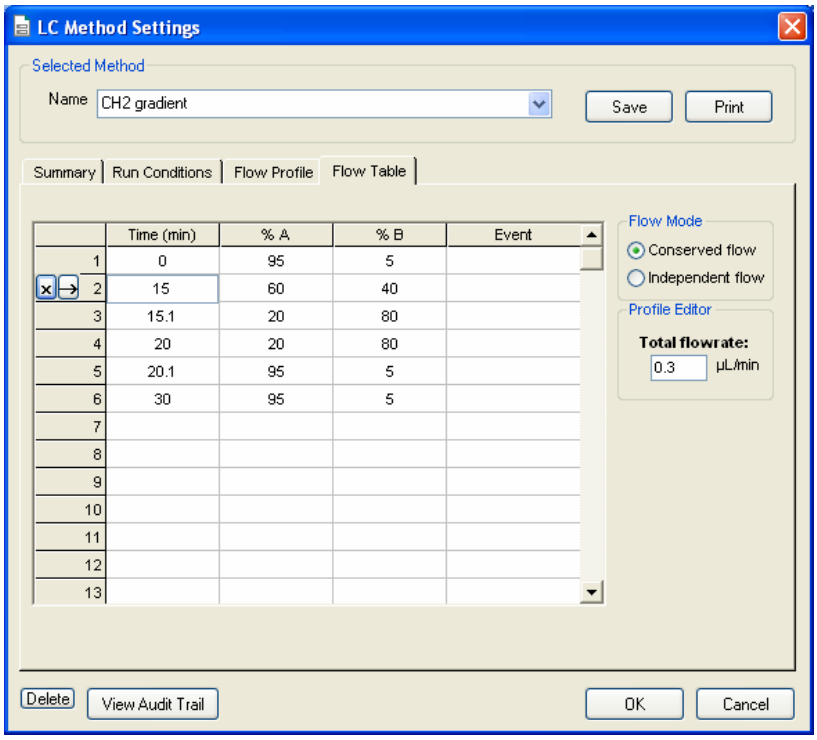


Figure 5-13. LC Method Editor – Flow Table Tab

step 9 Select the Flow Profile tab to present the Flow Profile dialog box (Figure 5-8)

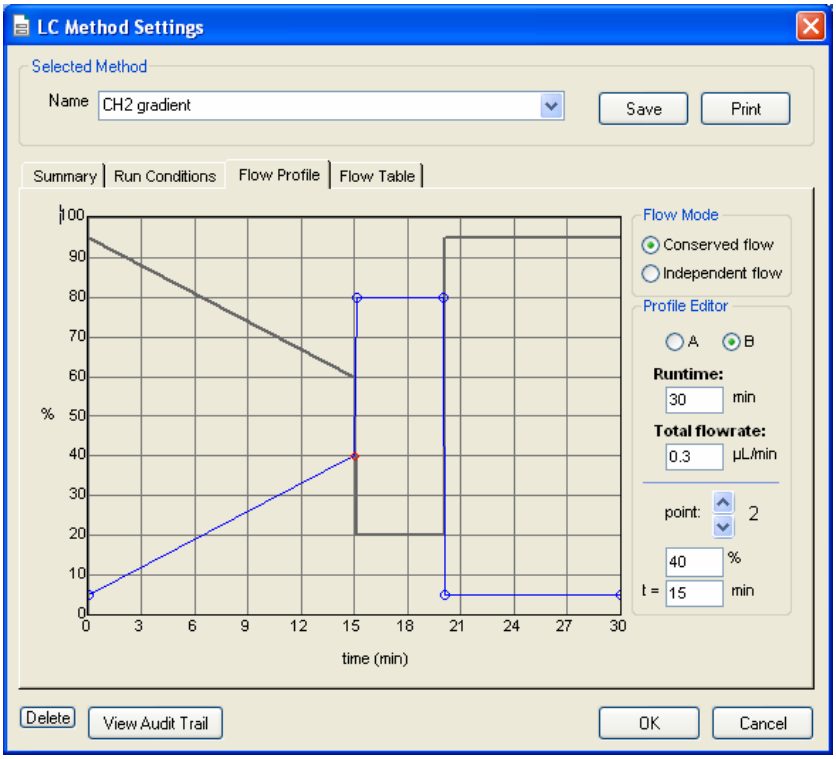


Figure 5-14. LC Method Editor – Flow Profile Tab

step 10 Use the flow profile tab to survey the gradient you created to make sure it is correct. The mobile phase composition can also be set by clicking and dragging points on the graph or by setting the % of **A** or **B** in the **Profile Editor** region.

step 11 Once the method is complete, click **Save** to save the analysis method. Click **OK** to close the editor.

5.7 Creating the Run Table

The **Run Table** ties together an autosampler and one or more LC method with a sample vial and tray position. You can also enter descriptive information related to the sample or analysis. This section will create a **Run Table** to run two samples with a trap and elute method.

step 1 If not already in the Run Manager, click the **Run Manager** button located on the Eksigent Control Software's **Acquisition Window**. (Figure 5-1).

step 2 Create a new, blank **Run Table** by selecting **Edit > Erase Table** (Figure 5-15).

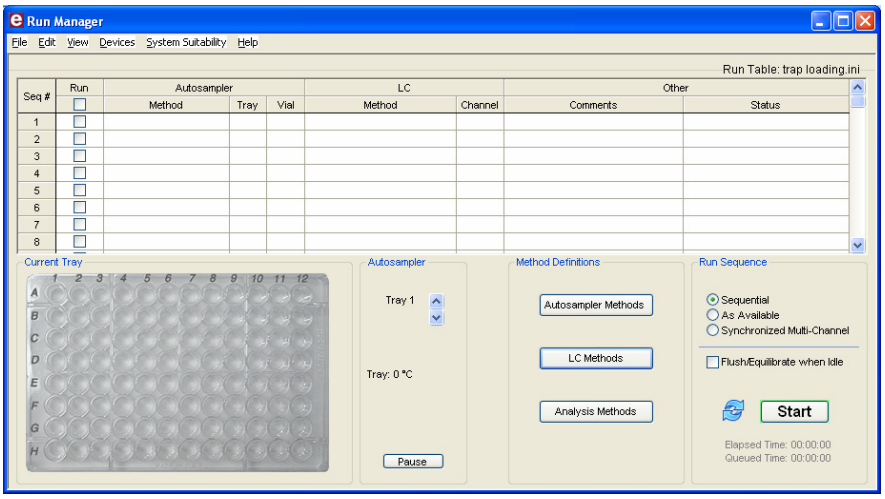


Figure 5-15. Creating a New Run Table

step 3 Select **File > Save As…** and type trap loading in the **File name** field.

step 4 Click **Save**.

step 5 In the first line in the **Run Table** double click on the **Autosampler Method** field and select the autosampler method micropickup_1uL_trap loading from the drop down menu.

step 6 Next, enter 1 for the tray and A1 for the sample **Vial** location. Alternatively, vial location can be entered by clicking at the vial on the picture of the vial tray in the **Run Manager** window.

step 7 Double click on the **LC Method** field and select CH1 Trap Loading. Indicate Channel **1** under the Channel column.

step 8 In line 2, leave the autosampler method, tray and vial location blank. Double click on the **LC Method** field and choose CH2 gradient. Indicate Channel **2** under the channel column.

step 9 For each sample you wish to run, duplicate these two lines in succession. In Figure 5-16, two sample injections are indicated.

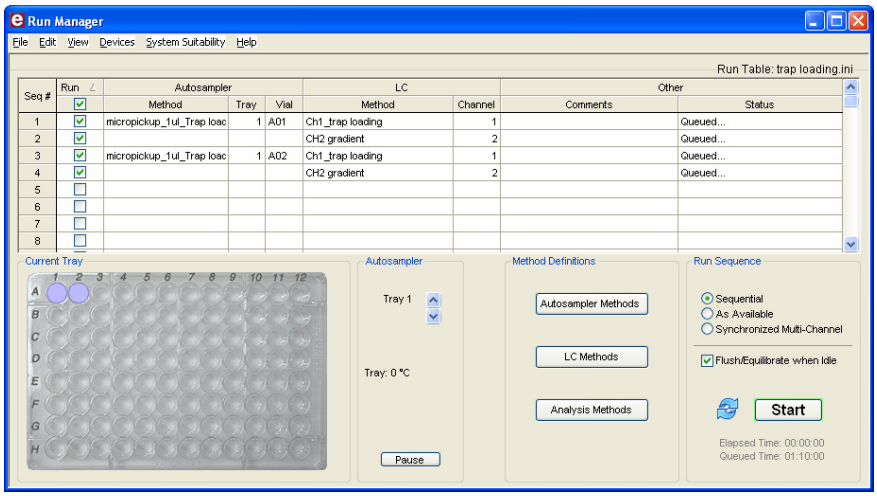


Figure 5-16. Run Manager Window – Two Samples

step 10 Check all the lines you wish to run under the **Run** column.

step 11 Run the sequence in Sequential mode. Click Flush/Equilibrate when Idle to start all the pumps if you wish. *Note: If you need to edit the first two lines, un-check flush/equilibrate to do so.*

step 12 Now that the run table has been defined (Figure 5-16), save it by selecting **File > Save**.

5.8 Starting a Run

Samples to be analyzed are selected by placing a check mark in the box to the right of the appropriate row numbers in the **Run Table**. For this series of runs make sure there are check marks in the boxes for the first four rows of the Test Table created in the previous section.

Checking the **Flush/Equilibrate when Idle** will initiate the pre-run flush for the first method. With this options selected, the system will continue to flush at the end of the sequence.

Initiate the sequence of analyses by clicking on **Start**. After the start button has been pressed, it will change to a red 'Stop' button that can be used to abort the run at any point during the analysis.

Once the flow rate has stabilized, the sample injection process will begin. Channel 1 will run first, then Channel 2 will start at the conclusion of the Channel 1 run.

While the run is in progress, the **Acquisition Window** can display the specified flow profiles for solvents A and B as well as their actual flow rates (Qa and Qb). Traces can be added or deleted from the display by clicking on **System > Appearance** in the **Acquisition Window** (Figure 5-1) and selecting the desired items.

To zoom in on a particular area of the chromatogram, click on the display and drag a box around the area of interest. This will enlarge that area of the chromatogram. To zoom back out, right click and select **Zoom Out** or **Back**.

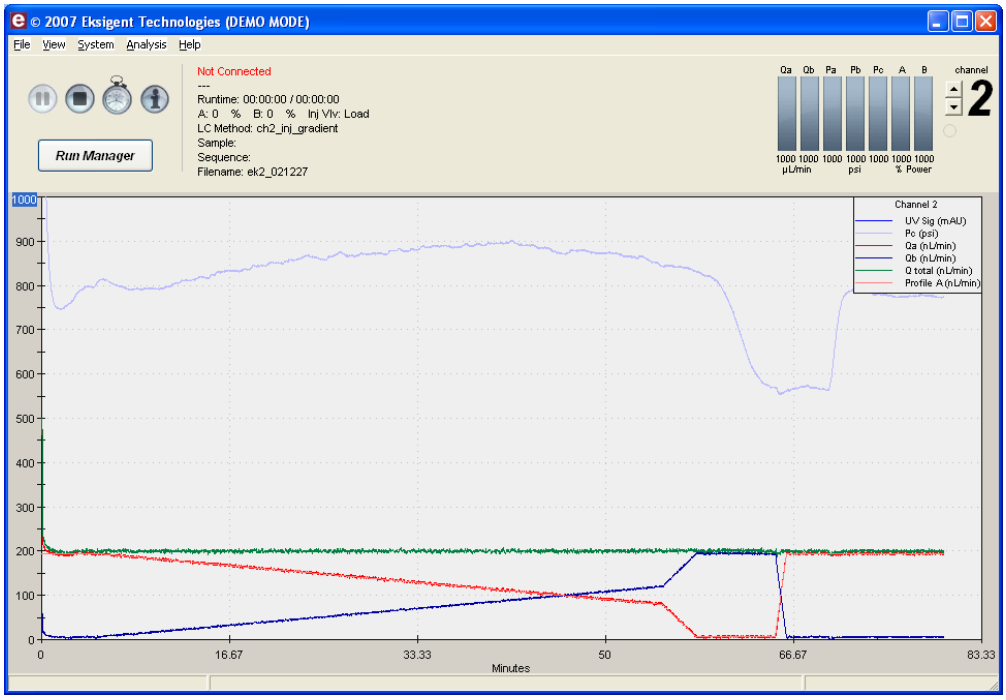


Figure 5-17. Acquisition Window – Flow Profiles

Status information such as %**A**, %**B** and **Time Remaining** are also displayed at the bottom of the screen during the run. Status bars at the top of the screen display the actual flow rate for pump A (Qa) and pump B (Qb) in nL/min, and pressure, in psi, for pump A (Pa), pump B (Pb) and column (Pc).

5.9 Viewing the Collected Data Files

Previously collected data files can be re-opened, reviewed and re-processed.

step 1 To view the data file collected from the first chromatogram, click on **File > Open…** then select the data file. The nanoLC has an option to collect an external signal (e.g. a UV detector) through the A/D input on the 12-pin I/O connector. This data will be stored, along with the flow profile data, in the data file. The Control software also includes a data analysis package and this analysis can be applied to the collected A/D signal. See the software manual for further instructions on analyzing data files with the software.

Chapter 6.
Diagnostics and Troubleshooting

Chapter 6 describes the built-in diagnostic capabilities of the nanoLC system along with the most common troubleshooting procedures. Topics covered in this chapter include:

- overview of hardware diagnostics (Section 6.1)
- calibration values (Section 6.3)
- general troubleshooting guidelines (Section 6.4)
- troubleshooting checklist (Section 6.5)
- error messages and system alerts (Section 6.6)

6.1 Overview of Hardware Diagnostics

The nanoLC system includes a number of diagnostic capabilities designed to maintain peak system performance. The status bars and the text displayed in the Control software acquisition window provide general diagnostic capability for routine operation. It is a good practice to keep track of **Pc** pressure readings for the desired chromatographic method. Running the Hardware diagnostics is part of routine instrument maintenance and should be performed in the following schedule:

- Every Month: Re-initialize pressure transducers
- Every three months: Autotune the controllers
- Every three months: Calibrate the flow meters

Refer to section 4.5, 4.7 and 4.8 for details on running these diagnostics.

6.2 Calibration Values

The Calibration Values tab of the Hardware Diagnostics Window summarizes the current K values of the flow meter, the gain and zero offsets of pressure transducers and the PID parameters for the pumps. The following settings are typical for a nanoLC and are shown for example only. The window on the left is for channel 1 and the window on the right is for channel 2,

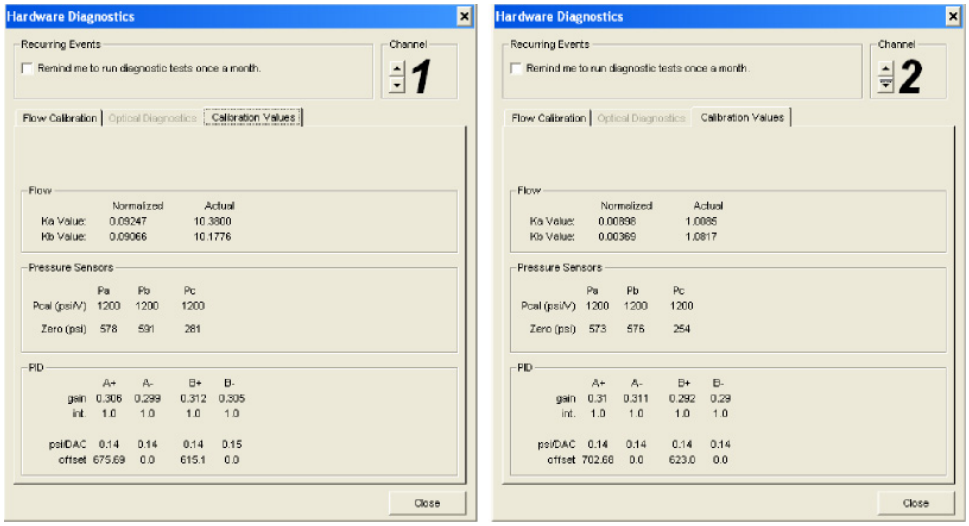


Figure 6-1. Hardware Diagnostics – Calibration Values Tab

The values should be documented before and after as part of maintaining a good instrument log, and noting changes. These values should be very similar from diagnostic to diagnostic; large changes can indicate a problem.

6.3 General Troubleshooting Guidelines

To avoid the possibility of electric shock, never disconnect an electrical assembly while power is applied to the system. After turning power off, wait about ten seconds before disconnecting an assembly.

To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the system. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.

There are no user serviceable components or assemblies inside the nanoLC. Service or any internal parts or assemblies requires a Factory Qualified Service Technician.

When troubleshooting the nanoLC system, follow these safety practices:

The basic steps for nanoLC system troubleshooting are:

step 1 Step back and look at the overall system. Is something obvious causing the problem? For example, is an instrument unplugged or improperly connected?

step 2 Compare current system operation with the way the system operated before the problem started. Identify conditions (pressures, power settings, flow rates) that are different than they were when the system was operating normally.

For example, if the output pressure is usually 500 psi with a certain method, is the system pressure currently in the same range, or drastically higher (possibly caused by a plug) or lower (possibly caused by a leak)?

step 3 Identify in the order listed below the symptom that varies from normal system operation:

- System power on and initialization (initialization fails)
- System diagnostics (flow stability, controller tuning)
- Flow rate in each channel (high, low, erratic)
- Output pressure (high, low, erratic)

step 4 For each isolated symptom, identify a list of possible causes using the troubleshooting checklist below. The troubleshooting in section 6.5 allows you to narrow down the possible causes of a symptom and find suggested corrective actions.

step 5 If this process does not correct the problem, contact Eksigent Technologies Technical Support.

6.4 Troubleshooting Checklist

Table 6-1. Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
System Initialization…		
Power LED on front panel is not on.	Power cord not connected	Connect power cord.
	No power at outlet	Repair electrical outlet.
	Power LED has failed but system response OK	Contact Eksigent Technologies Technical Support for assistance.
Front panel power LED is on but software fails to recognize the instrument's presence.	Communication error between computer and an LC system	Verify that the instrument's serial cable is securely connected to the computer's COM1 serial port. (see Section 2.5) Reboot computer and cycle power on instrument.
		Contact Eksigent Technologies Technical Support for assistance.
Liquid leaking at the bottom of the mobile phase reservoirs	The reservoirs were not placed in the pump adapter properly	Verify the stopcock is placed properly and tightly screwed in.
Loud hissing sound from the instrument	Air leaks from the air inlet fitting	Verify the air tubing is connected properly to the gas fitting.
		Tighten the air inlet gas fitting.
		Contact Eksigent Technologies Technical Support for assistance.

Table 6-1. (cont.) Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
Flow Control System…		
*System pressure (***Pc***) and/or pump pressures (***Pa** & **Pb***) show pressure even though the flow is off*	Pressure transducers zero offsets are incorrect	Zero pressure transducers (Section 4.5)
No liquid out of waste line when purging	Air trap in the pump	Prime the pump (Section 3.2).
	Internal filters are plugged	Contact Eksigent Technologies Technical Support for assistance.
	Leak in the system prior to the purge valve	Check all connections.
Pump restrokes frequently- ***Pump has reached end of stroke*** *error message occurs*	Air trapped in the pump	Prime the pump (Section 3.2).
	Pump remains on long enough to prompt a re-stroke	Check the duration of time between re-strokes to see if the pump re-stroke was appropriate.
	Check valve is leaking	Contact Eksigent Technologies Technical support for assistance.
Pump does not restroke at the end of a run	Pump restroke delay is too short	Contact Eksigent Technologies Technical Support for assistance.
Pump flushes out quickly but does not deliver ~600 µL per stroke.	Leak in instrument	Contact Eksigent Technologies Technical Support for assistance.
Purge output drips out slowly	Internal filters are plugged	Contact Eksigent Technologies Technical Support for assistance.
*No flow rate with 100% power indicated. System pressure (***Pc***) and pump pressures (***Pa** & **Pb***) are all low*	No air to system	Connect 100 psi clean, dry air to the instrument's air inlet.
	System not properly primed and flushed	Prime and flush system (Section 3.2).
Inability to reach desired flow rate	Internal filters are plugged	Contact local Eksigent Technologies Technical Support to replace internal filters.
	Flow rate set point too high for system back pressure	Reset flow rate to a lower level.
	Air pressure too low	Establish correct air pressure (100 psi).

Table 6-1. (cont.) Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
Flow rate will not initialize at start of run	Flow rate set point too high for system back pressure	Reset flow rate to a lower level.
	Erratic flow rate due to bubbles in system	Prime and flush system (Section 3.2).
	Unable to meet required flow rate within specified tolerance	Relax flow tolerance setting (section 3.2).
		Run the flow stability diagnostic to verify flow control (Section 4.6)
		Autotune flow controllers. [**System > Hardware Diagnostics > Auto Tune Controllers**] (Section 4.7)
		Flush system if flow is unstable (Section 3.2).
		If flow is still unstable, calibrate flow-meters (Section 4.8)
	One or both of the Internal solvent filters may be plugged	Contact Eksigent Technologies Technical Support for assistance.
Flow rate will not stabilize during a run	Erratic flow rate due to bubbles in system	Prime and flush system (Section 3.2).
	Incorrect mobile phase setting	Check the Mobile Phases setting (Section 3.2).
	Pump controller is out of tune	Autotune flow controllers (Section 4.7).
	Flow temperature is not stable	Contact Eksigent Technologies Technical Support for assistance.
System responds sluggishly when changing flow rates	Incorrect mobile phase setting	Check the Mobile Phases setting (section 3.2).
	Pump controller is out of tune Flow temperature is not stable	Autotune flow controllers. [**System > Hardware Diagnostics > Auto Tune Controllers**] (see section 4.7)
Inaccurate flow rate with no signs of leakage	Incorrect mobile phase setting	Check the Mobile Phases setting (section 3.2).
	Incorrect k-values	Perform flow meter calibration. [**System > Hardware Diagnostics > Calibrate Flow meter**] (see Section 4.8)
*System pressure (***Pc***) is unusually low but flow rate is OK*	Loose connection after mixing tee	Check all connections for leaks.
*System pressure (***Pc***) is low and the flow rate is OK but pump pressures (***Pa** & **Pb***) are high*	Incorrect k-values	Perform flow meter calibration. [System > Hardware Diagnostics > Calibrate Flow meter] (Section 4.8)
	Flow module is plugged	Contact Eksigent Technologies Technical Support for assistance.

Table 6-1. (cont.) Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
Excess flow noises	Trapped air in the pump	Prime the pump (Section 3.2).
	Pump controller is out of tune	Autotune flow controllers. [**System > Hardware Diagnostics > Auto Tune Controllers**] (Section 4.7)
Measured flow does not follow the flow profile	Pump controller is out of tune	Autotune flow controllers. [**System > Hardware Diagnostics > Auto Tune Controllers**] (Section 4.7)
	Pump time response is set incorrectly	Adjust the pump time response in Hardware Diagnostics.
Pa *and* **Pb** *maximized to < 5300 psi with 100% pump power.*	Incorrect gain setting for pressure	Check the setting in Calibration **Values Window** in **Hardware Diagnostic** menu (1200 for 6000 psi transducers, see Section 6.3).
	Incorrect zero setting for pressure sensors	Check the setting in Service menu (should be 500±200, see Section 6.3).
	Inline Air pressure too low	Check the air pressure (~100 psi).

Table 6-1. (cont.) Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
Autosampler…		
Software does not recognize nano LC Autosampler when **Run Manager** *is started*	Communication error between computer and nano LC Autosampler	Verify that the RS-232 cable is securely connected to the S2 In port on nano LC Autosampler (see Section 2.6).
	Software may be configured to use a different COM port	Verify that the software is configured for nano LC Autosampler to connect to the correct COM port (see Section 2.6).
	nano LC Autosampler is not in serial mode	Using the front panel of nano LC Autosampler, set it to serial mode. [**Menu > Serial**] (see Section 2.6).
nano LC Autosampler does not trigger instrument to start a run	Output cable is not connected properly	Verify the output cable is connected to P1 connector at the back of nano LC Autosampler.
	Loose wire connection at the instrument I/O plug	Make sure the wire ends are securely screwed in the I/O plug.
Software does not trigger nano LC Autosampler to switch valve position	Input cable is not connected properly	Verify the input cable is connected to P6 connector at the back of nano LC Autosampler
	Loose wire connection at the instrument I/O plug	Make sure the wire ends are securely screwed in the I/O plug.
Injection Valve…		
Injection valve does not switch positions	Valve is not connected to the actuator	Connect the cable from valve to actuator at the back of the instrument.
	Valve is connected to the wrong channel	Check and reconnect cable from actuator to the correct channel
	Valve is not configured in nanoLC software	In Instrument Configuration, set injection valve as internal (Section 2.9).
	Faulty actuator (LED is not on)	Replace actuator.
No flow coming out from the port	The valve is plumbed incorrectly	Verify the plumbing configuration and reconnect if needed (see Appendix C).
	The ports are plugged	Manually flush each port with cleaning solvent using a syringe. If flushing does not clean the port, contact Eksigent Technologies Service Representative to replace the valve.

Table 6-1. (cont.) Troubleshooting Checklist

Symptom	Possible Cause	Corrective Action
System pressure (Pc) with no column connected is unusually high	The ports are plugged	Manually flush each port with cleaning solvent using a syringe. If flushing does not clean the port, contact Eksigent Technologies Service Representative to replace the valve.
	The ends of fused silica connected to the port might be crushed or not cut properly	Check the ends under a microscope. Cut the ends if needed.
Fluid leaking from the valve	Ferrule was not seated properly in the port	Check the tubing connection and make sure the ferrule is seated properly.
	Rotor seal is scratched	Contact Eksigent Technologies Technical Support for assistance.
Inconsistent flow rate	Internal leakage in the valve	Contact Eksigent Technologies Technical Support for assistance
	Valve tubing connections are not made correctly (gap/dead volume between sleeves and port)	Check tubing connections. Make sure the cuts at the end of the capillaries are square.
	The ports are plugged	Manually flush each port with cleaning solvent using a syringe, or contact your Eksigent Technologies Service Representative to replace the valve.
Bubbles in the flow stream	Bubbles trapped in the valve	Attach a high restrictor at the port to flush out the trapped air bubbles.
	Valve tubing connections are not made correctly (gap/dead volume between sleeves and port)	Check tubing connection.
System does not initiate an injection	System flow is unstable	Purge both pumps and re-equilibrate system (Section 3.2).
	Flow stabilization is set too low	Set the Flow Stabilization Limit in instrument configuration to > 100 nL/min. [**System > Instrument Configuration**] (Section 2.9)
	Autosampler is configured with wait for injection but the LC method is used with no injection	Change LC method to with injection or change the AS method without wait for injection command (see Sections 5.3 and 5.4).

6.5 Error Messages and System Alerts

System alerts can be displayed by selecting View > System Logs > Alerts. These alerts (Figure 6-4) are reminders of an action that needs to be taken; such as to run the diagnostic tests or refill the reagent storage loops. Pressing Clear Alerts will erase all alerts. Note: that this does not mean that the recommended alert action has been completed.

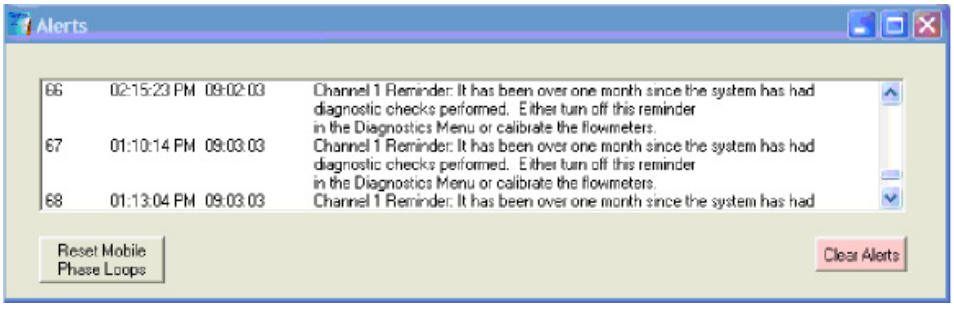


Figure 6-4. System Alerts Window

Appendix A
Spare Parts and Consumables

A.1 Consumables: nanoLC-1D+ and nanoLC-2D

part number	description
800-00006	Accessories kit for 1D. Includes calibration kit, syringe, fittings, capillary tubing and cables.
801-00025	Accessories kit for 1Dplus and 2D. Includes mobile phase reservoirs, calibration kits, priming tool and syringe, fittings, capillary tubing and cables.
801-00002	Flow rate calibration kit with 20 µL calibrated pipettes (10) for 1 – 30 µL/min flow rate
801-00020	Replacement calibration pipettes, 20 µL, for 1-30 µL/min, 10/pkg
801-00006	Flow rate calibration kit with 5 µL calibration pipettes (5) for 50 – 1000 nL/min flow rate
910-00007	Replacement calibration pipettes, 5 µL, for 50 – 1000 nL/min, 10/pkg
801-00003	Pump priming tool kit, includes 10 mL syringe (1D)
910-00020	Fitting, FingerTight, 1/16”, PEEK, 10/pkg
910-00021	Fitting, hex, 1/16”, SS, 0.43” long, 10/pkg
920-00006	PEEK hex nut, 10/pkg
910-00023	Ferrule, 1/16”, PEEK, 10/pkg
910-00026	Ferrule, 1/16”, SS, 10/pkg
920-00004	Fitting, hex, 1/16", SS, 3/4" long, 10/pkg
920-00005	Fitting, hex, 1/16”, PEEK, ¾” long, 10/pkg
920-00008	Sleeve, FEP, green, 395 µm ID, 1/16" OD, 10/pkg
200-00165	Fitting, NanoTight 1/16”, PEEK, 0.90” long, with Tefzel ferrule
910-00022	Fitting, MicroTight, 0.025”, PEEK, 10/pkg
920-00002	Ferrule, MicroTight, for 0.025", 10/pkg
200-00002	MicroTight union, PEEK
200-00044	Plug, MicroTight, PEEK, black
920-00003	Nut, female, MicroTight, 10/pkg
910-00043	Sleeve, PEEK, yellow, 180 µm ID, 0.025" OD, 10/pkg
910-00024	Sleeve, PEEK, orange,405 µm ID, 1/16" OD, 10/pkg
910-00025	Sleeve, PEEK, green, 395 µm ID, 0.025" OD, 10/pkg
200-00138	Mixing tee ferrule for 365 µm OD capillary, 1/pkg

A.2 Replacement Parts: nanoLC-1D, nanoLC-1D+ and nanoLC-2D

part number	description
200-00020	Internal pump filter element
200-00032	Internal pump filter holder and element
200-00047	Replacement rotor for 6 port PEAK injection valve (0.25 mm)
200-00048	Replacement stator 6 port PEAK injection valve (0.25 mm)
200-00058	Syringe adapter fill port, PEEK, NanoLC-1D injection valve
200-00061	PEEK sample loop, 2 µL
200-00092	PEEK sample loop, 5 µL
200-00164	PEEK sample loop, 10 µL
200-00093	PEEK sample loop, 20 µL
200-00063	PEEK sample loop, 50 µL
200-00074	Syringe adapter fill port, SS, NanoLC-2D injection valves
200-00083	10-port valve, titanium stator
200-00130	Replacement stator for Titanium 10-port valve
200-00212	Replacement stator for PAEK 10-port valve
200-00131	Replacement stator for Titanium 6-port valve
200-00159	Replacement rotor for Titanium 10-port valve
200-00211	Replacement rotor for PAEK 10-port valve
200-00160	Replacement rotor for Titanium 6-port valve
200-00167	Front panel mixing tee with fittings and ferrules
300-00000	Solvent waste/seal wash bottle, 250 mL
400-00104	Power supply, input 100 – 240VAC /3A, output 24VDC /4A, NanoLC-1D plus & NanoLC-2D
620-00060	Power cable, North America
400-00078	Power cable, European
920-00020	1.25 A fuses 5/pkg
800-00020	Flow meter module, standard for NanoLC-1D
800-00223	Flow meter module 1Dplus channel 2, 1-20 µL/min
800-00054	Flow meter module, standard for NanoLC-2D channel 1A & 1B
800-00055	Flow meter module, standard for NanoLC-2D channel 2A & 2B
800-00076	Flow meter module, standard for NanoLC-1D+ channel 2A & 2B
800-00077	Flow meter module, standard for NanoLC-1D+ channel 1A & 1B
910-00066	Replacement capillaries kit 1D Plus. Includes all SS/fused silica capillaries used in a 1D Plus, with fittings.
910-00067	Replacement capillaries kit 2D. Includes all SS and fused silica capillaries used in a 2D, with necessary fittings.
910-00063	Replacement capillaries kit 1D Plus. Includes all SS capillaries used in a 1D Plus, with necessary fittings.
910-00064	Replacement capillaries kit 2D. Includes all SS capillaries used in a 2D, with necessary fittings.
800-00058	Waste reservoir clamp and thumbscrew
800-00105	Solvent reservoir, 50mL, clear

800-00141	Solvent reservoir, 50 mL, amber
800-00194	Solvent reservoir, 100 mL, clear
800-00200	Connector, 12-pin electrical, I/O, green

400-00196	Connector, 4-pin electrical, switching valve, green
400-00106	Serial cable for instrument control and data collection
800-00056	External control synchronization cable
910-00001	Line, air supply, 25', 1/4"
300-00007	Internal air line, black, polyethylene, 18"
100-00041	Fitting, air supply, 1/4" compression
920-00088	Luer-lock fitting for mobile phase valve (set of 2) (1D)

A.3 Replacement Parts: AS-1 Autosampler

part number	description
801-00044	PM kit for AS-1 with Titanium valve. Includes pre-puncturing needle, fused silica needle, 25 µL syringe and rotor
801-00045	PM kit for AS-1 with PAEK valve. Includes pre-puncturing needle, fused silica needle, 25 µL syringe and rotor
620-00005	Wash bottle bracket
620-00021	Air/pre-puncturing needle
800-00044	Fused silica needle 2.4 µL Bio
620-00139	PEEKsil needle 2.5 µL

800-00045	Fused silica needle 5.4 µL Bio
620-00027	Wash solvent vial with cap & screws
200-00208	PEEKSil sample loop, 1 µL
620-00039	PEEKSil sample loop, 10 µL
620-00052	Belt 25.0 x 1.2
620-00053	Syringe valve #5-4 & #5

620-00059	Power cable Europe
620-00060	Power cable USA
620-00064	Syringe 25 µL
620-00140	Teflon seal between syringe and syringe valve
910-00045	Replacement tip for 25 µL syringe, 5/pkg
620-00065	Syringe 100 µL
620-00072	48 vial adapter
800-00059	NanoLC-AS1 P6 input cable
800-00060	NanoLC-AS1 P1 output cable
800-00063	Buffer tubing, 500 µL
800-00064	Buffer tubing, 50 µL

Appendix B
External Interface

B.1 Interface Connections

Appendix B describes the external interface to other instrumentation in order to synchronize sample injection with data collection. The connector pin assignments are described below. The connector is located on the rear panel of the nanoLC.

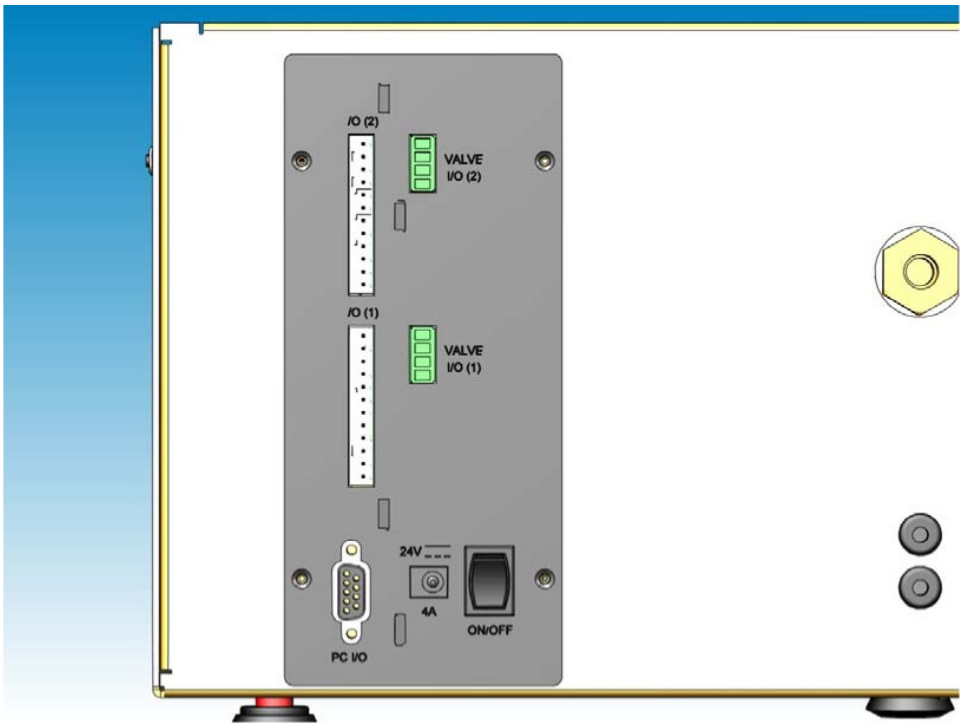


Figure B-1. Rear Panel – External Interface and Valve Connectors

B.1.1 Remote Interface

An enlarged drawing of the External Interface Connector shows that 12 lines are available (Figure B-2). These will be described in turn.

A/D in — The A/D in is a 24-bit A/D input for collecting the signal from an external detector using the nanoLC's data system.

GND — GND is a system ground connection used in combination with other contacts such as RUN out.

Run out — The RUN out contact is used to trigger an external device at the time the gradient is started. Its polarity is configurable from within the Control software. The logic state changes at the be-ginning of the gradient (end of injection when using metered injection mode) and stays in on-state during gradient.

PRK out — The PRK out contact changes state when the peak parking function is actuated. Its polarity is configurable from within the Control software.

RDY out — The RDY out contact changes state when the pump is ready to start run (e.g. at end of prerun flush). Its polarity is configurable from within the Control software.

VLV out — The VLV out contact goes to 0V (closes) when the injection valve moves from the Load position to the Inject position. Its polarity is NOT software configurable.

ABS out — The ABS out contact outputs an analog signal corresponding to the nanoLC's detector absorbance. The wavelength monitored and the voltage- per-absorbance unit is configurable from within the Control software.

RUN in — The RUN in contacts allows remote starting of a method. Its polarity is configurable from within the Control software. The external trigger should be a pulse-type and does not need to be held in the trigger state.

PRK in — The PRK in contact allows remote triggering of the peak parking function. The instrument will re-main in the peak parking state until the contact's state is changed. Its input polarity is configurable from within the Control software.

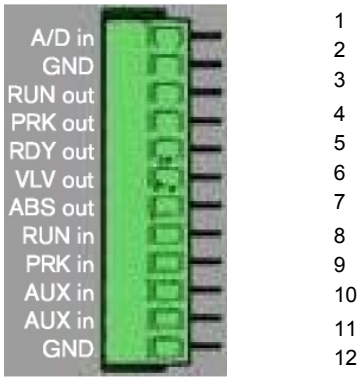


Figure B-2. External Interface Connector

AUX in — The first AUX in contact is included for future expansion but is not currently used. AUX in The second AUX in contact is also included for future expansion.

GND — The GND contact is a system ground connection used in combination with other contacts such as ABS out.

B.2 Valve Connectors

B.2.1 Remote Interface

The rear panel also features a Valve connector used as an electrical interface between the nanoLC and various valves (Figure B-3).

24 V The 24V contact supplies the 24V power for the valve actuator.

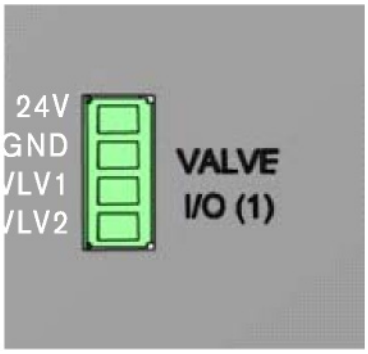


Figure B-3. External Valve Connector

GND The GND contact is a system ground for the valve connection. It is shared between the power sup-ply for the valve actuator and the trigger lines.

VLV1 The VLV1 contact is the trigger line to move the valve to position 1 (the LOAD position by default). It is held low (0 V) momentarily to trigger valve actuation to this position.

VLV2 The VLV2 contact is the trigger line to move the valve to position 2 (the INJECT position by de-fault). It is held low (0 V) momentarily to trigger valve actuation to this position.

B.3 Connecting to Other Instruments

Other Autosamplers The active state (TTL low = 0 V or TTL high = 5 V) can be set for most I/O connections in the Instrument Configuration menu. It is important that the ground lead of the peripheral be connected to the ground connection on the nano LC. In addition, use of the nanoLC Autosampler provides a number of additional I/O signals with additional programming flexibility.

Note: For all of the methods provided with the nanoLC and nanoLC Autosampler, and for all of the configurations discussed in this document, it is assumed that all of the inputs and outputs are configured as contact closures that are active in the closed state.

B.3.1 Other Autosamplers

The nanoLC can be integrated with autosamplers from other vendors through the use of contact closures. A variety of configurations are possible using the inputs and outputs of the nanoLC described above. One suggested configuration is described here.

Setup up the injection method and sample sequence using the autosampler interface (front panel or other software). Setup a matching sequence of chromatographic methods in the Run Manager of the nanoLC. Also make sure that LC Wait for Contact Closure is checked under the System menu in the Run Manager Window. Make sure that the analysis time per sample (time between autosampler injections) in the autosampler method is long enough to allow for the nanoLC method run time plus any pre-run flush equilibration for the next run.

Connect the inject marker out from the autosampler to the Run Input (pin 8) and Ground (pin 7) of the nanoLC. Start the sequence by starting the Run Manager sequence and then the autosampler. After the autosampler initiates its own sequence for dispensing sample it will trigger the nanoLC to start its chromatographic run.

The nanoLC also provides a Ready Out (pin 5) which can be used as an input to other autosamplers to synchronize runs and error check operation.

Note: The optimized injection routines that can be utilized with the nanoLC Autosampler will, in general, not be available with other autosamplers. While the rapid inject or metered injection routines of the nanoLC method can be used to increase the flow rate during sample loading, one will not be able to switch the sample loop out of the flow path during the gradient. The may lead to significantly increased gradient delay times and decreased response times of the system.

B.3.2 Triggering MS Data Collection

While integrated control of the nanoLC system through the Xcalibur and Analyst software packages will be available, most MS systems will allow synchronization of data collection through the use of contact closures. The following describes the general configuration for this synchronization.

In the MS software package, choose the option to start data collection with an external contact closure. This can be applied for a single MS run or to a sequence or batch of data collection methods. When conducting multiple runs in a sequence table, please configure the **Run Manager** of the nanoLC software with the corresponding sequence of methods.

Set the **Run Output** configuration to contact closure (box checked) in the nanoLC soft-ware (menu).

One must then identify the hardware interface for the MS contact closure input. This is typically either a terminal block mounted on the side of the MS instrument, or a pair of wires in an interface cable supplied with the MS.

To synchronize MS data with the beginning of the gradient (after the injection), connect the **Run Out** (pin 3) and the **Ground** terminals to the MS contact closure. If one lead of the MS trigger is ground, make sure to connect it to the Ground connection of the nanoLC. For most applications, this connection will be made to the channel 2 (low flow gradient) side of the instrument.

One can also choose to synchronize MS data with the beginning of the injection. To do this, connect the **Valve Out** (pin 6) and the **Ground** terminals to the MS contact closure.

B.3.3 Triggering Peak Parking

Peak parking on the nanoLC system can also be triggered externally using the back panel I/O connectors. Connect the ground of the triggering contact closure to a **Ground** terminal on the back panel and connect the second wire of the contact closure to the **Park In** terminal (pin 9). The **Park Out** terminal (pin 4) can also be used to monitor the state of Peak Parking. The **Peak Parking Toolbox** in the nanoLC software is used to configure the Peak Parking flow rate, flow rate reference (typically 'column'), and provide for hold and lockout times on triggering.

Peak Parking can be configured on ThermoFinnigan MS systems that provide a configurable con-tact closure output. This variable contact closure can be configured in the Xcalibur software to be activated based on selected criteria including ion intensities and ion inclusion lists. A number of systems may also allow a fixed time or duration contact closure that can be configured in the MS software to trigger peak parking. These may be useful for well defined experiments that will benefit from peak parking at a known time (not MS-dependent signals).

B.3.4 Ready Out

When using the nanoLC Autosampler, one can also handshake with other components, such as a MS detector, using the **Ready Out** from that device. This will allow the LC queue to stop if the peripheral is not ready. To do this, connect the **Ready Out** from the peripheral to the **In 3** and **Gnd** of the AS1 Input (P6) cable. Add the following line to the beginning of the AS1 method.

'Wait for Input 3-LOW'

Note: If the Ready Out from the peripheral is a TTL high trigger instead of TTL low or contact closure, change the line to 3-HIGH.

The AS method will not proceed beyond the first line until it has received a ready signal. If you disconnect the peripheral, be sure to remove this line from the AS method or to short In 3 to ground.

Appendix C
Valve Configuration Diagrams

This Appendix will show several possible configurations for the nanoLC. These sketches can be used as guides when configuring the plumbing for your own instrument.

Figure C-1 shows an automated 2D configuration with 2 parallel traps. The flow on the traps is unidirectional.

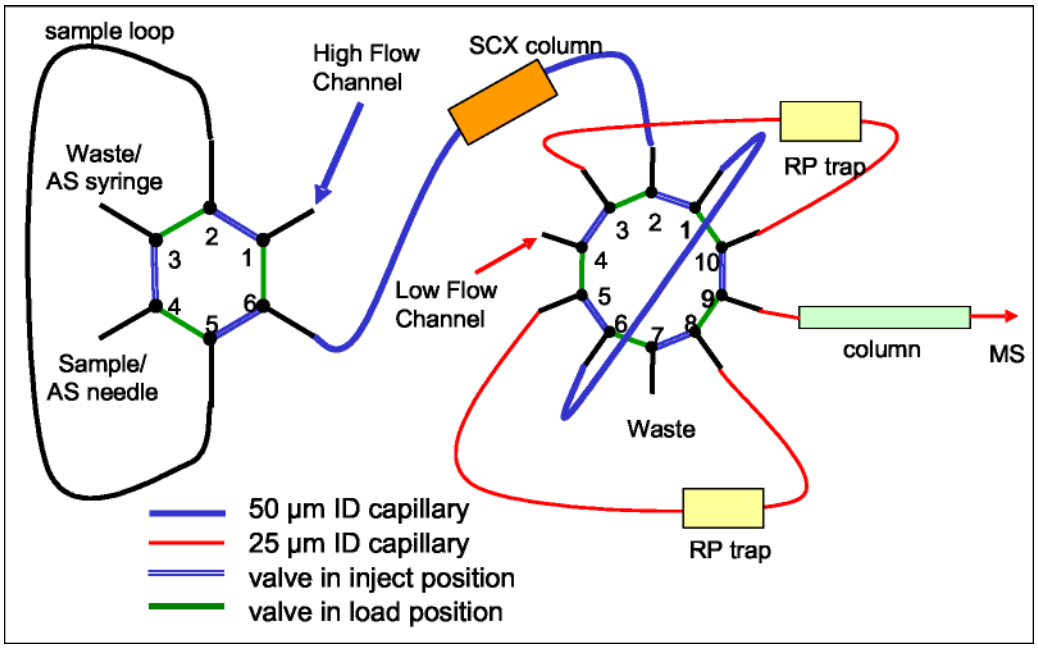


Figure C-1. 2D Configuration with 2 Parallel Traps

Figure C-2 shows a rapid sample loading configuration with 2 parallel traps. The flow on the traps is unidirectional.

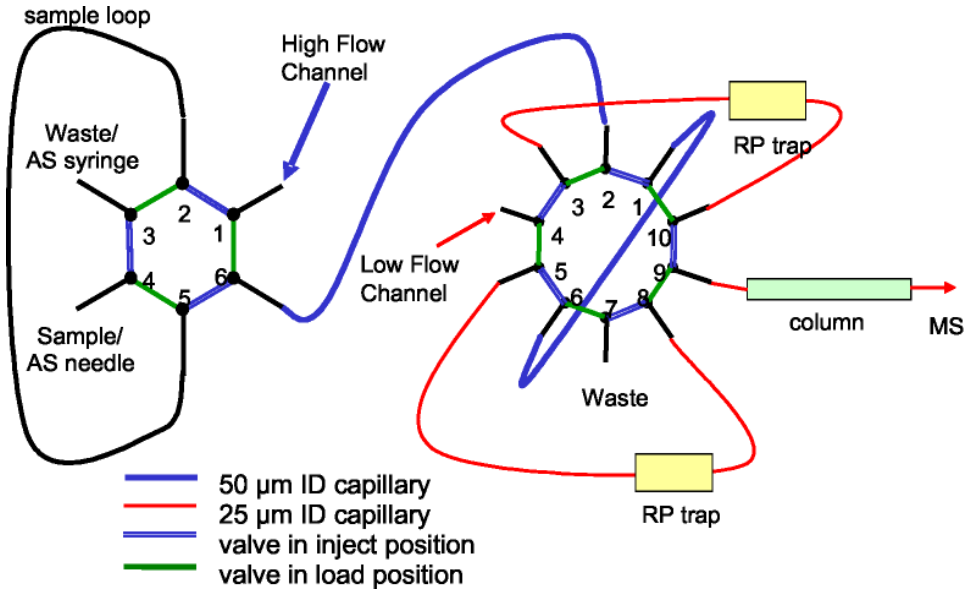


Figure C-2. Loading Configuration with 2 Parallel Traps

Figure C-3 shows a rapid sample loading configuration with 1 trap. The flow on the traps is unidirectional.

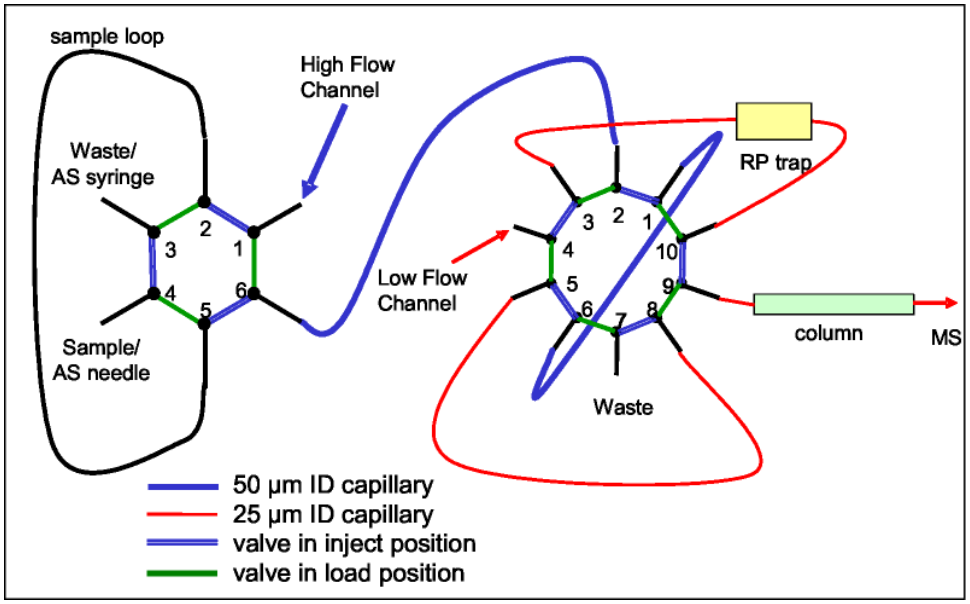


Figure C-3. Loading configuration with 1 trap – unidirectional

Figure C-4 shows a rapid sample loading configuration with 1 parallel trap. The flow on the traps is bidirectional.

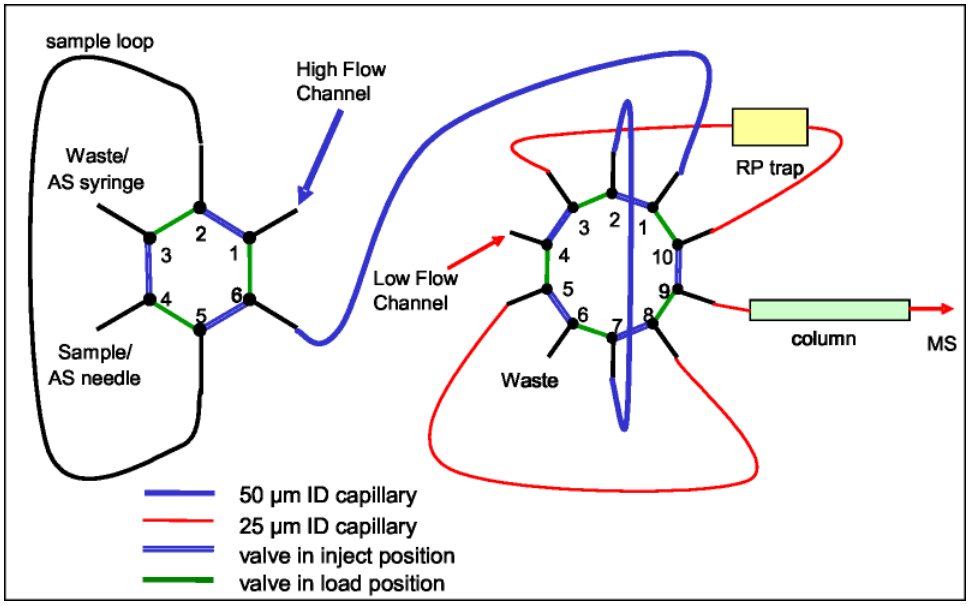
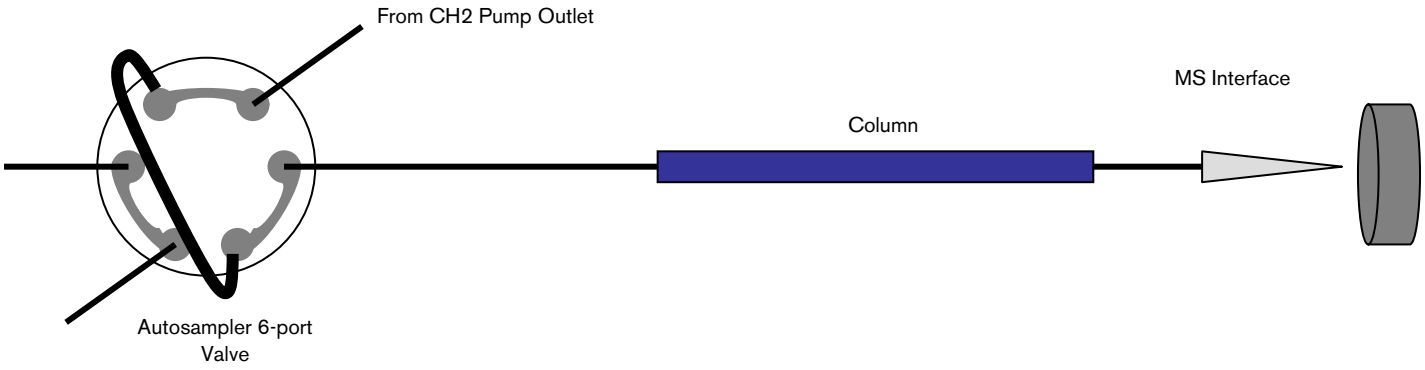


Figure C-4. Loading configuration with 1 trap – bidirectional

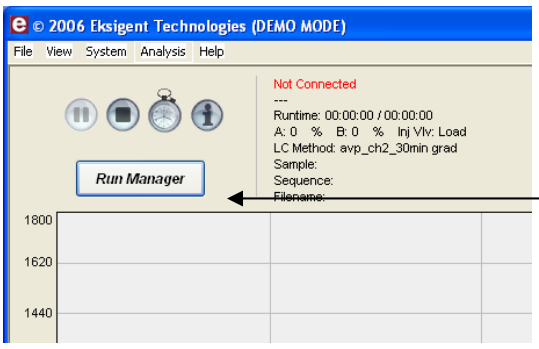
Appendix D
Quick Start Guides

D.1 Quick Start guide: Direct to Column Injection

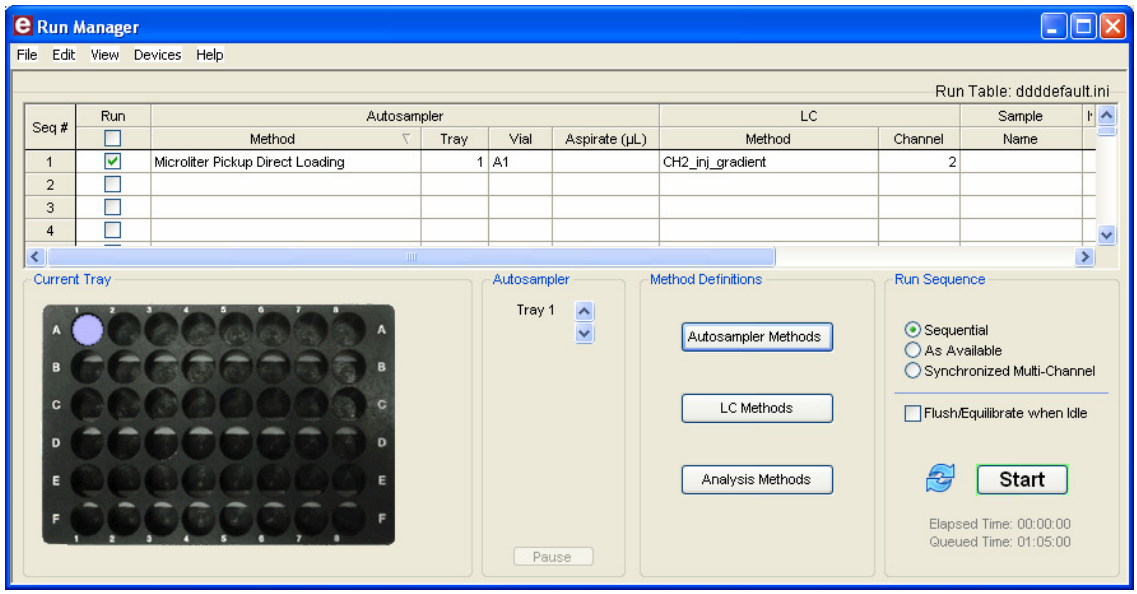
Direct to column sample injection: Plumbing schematic



To start a run in Eksigent software, open the Run Manager:

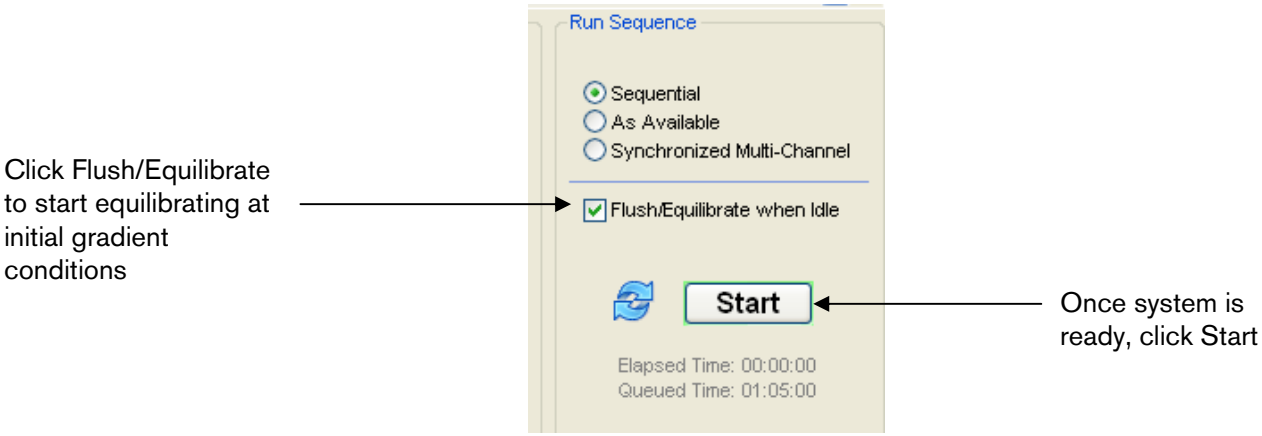


To start a sample run, click Run Manager.



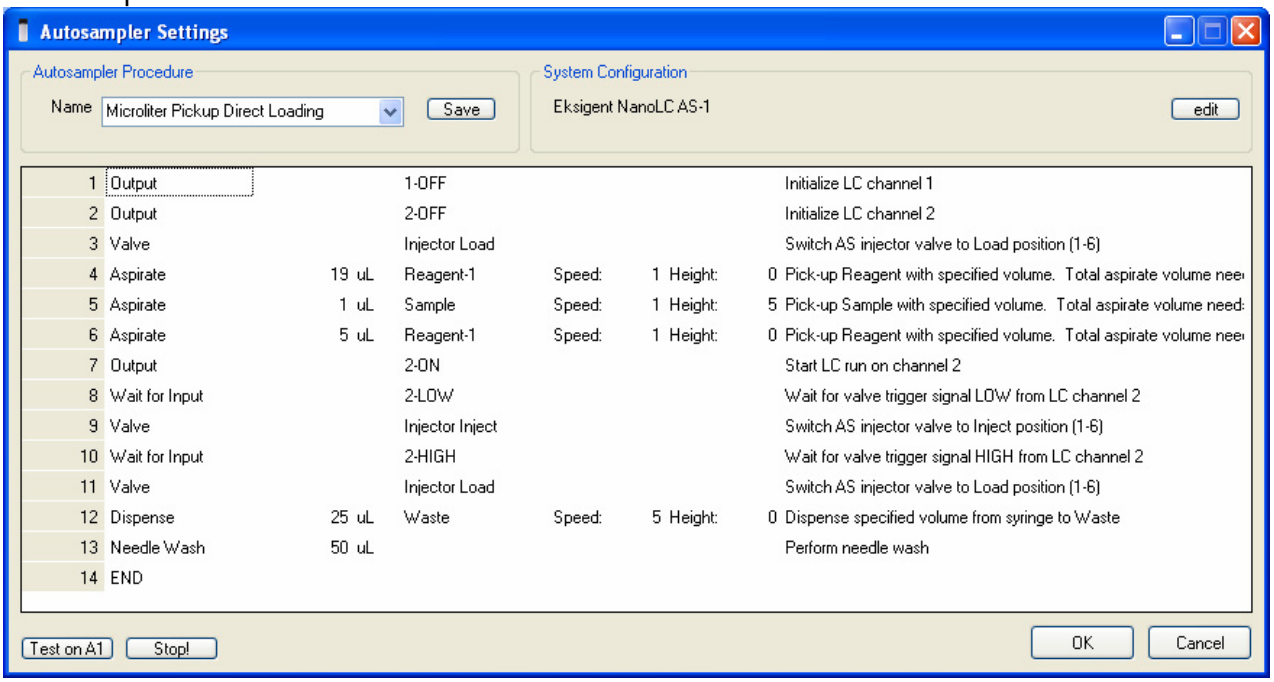
Select: Autosampler method, Tray, Vial, LC Method and Channel 2.

Click on Flush/Equilibrate when Idle to start equilibrating pumps at initial gradient conditions.

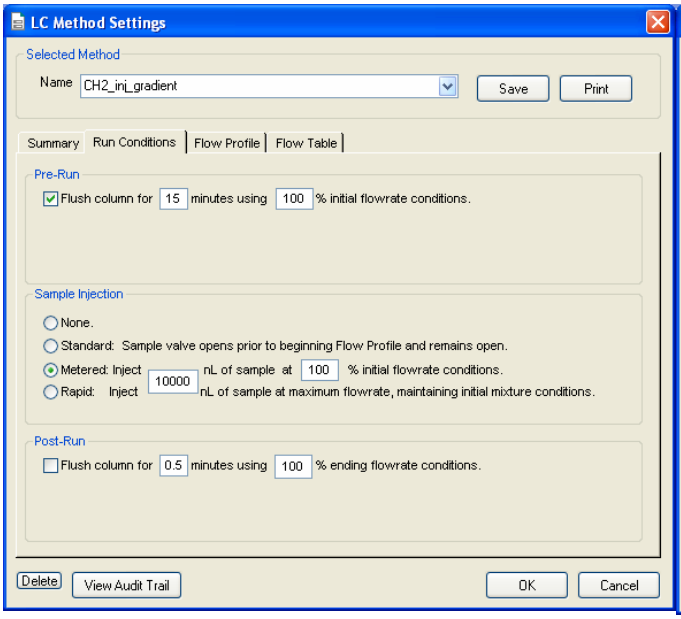


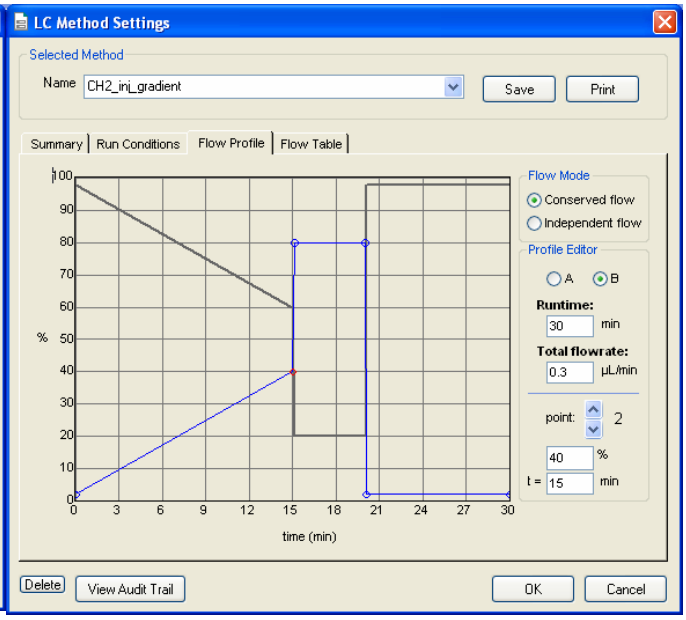
Default methods: Direct injection to column with Channel 2.

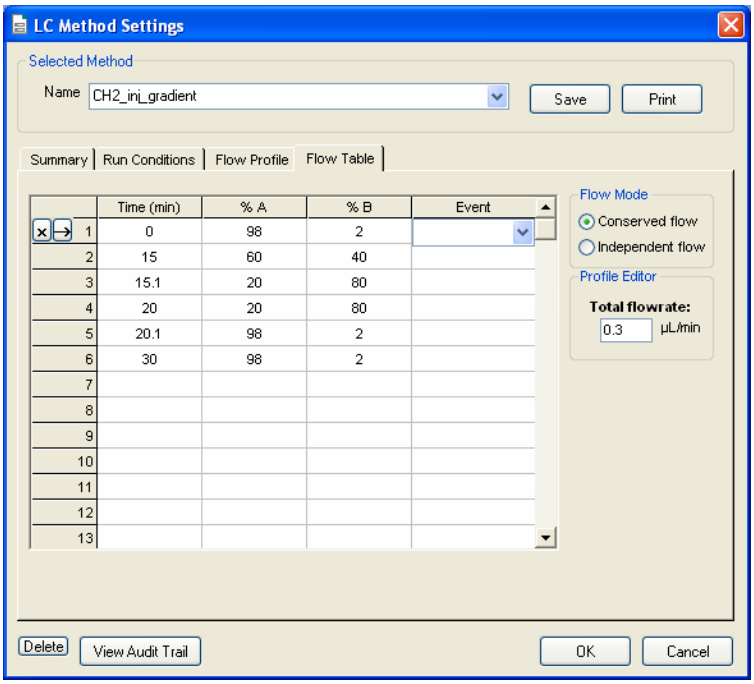
Autosampler:



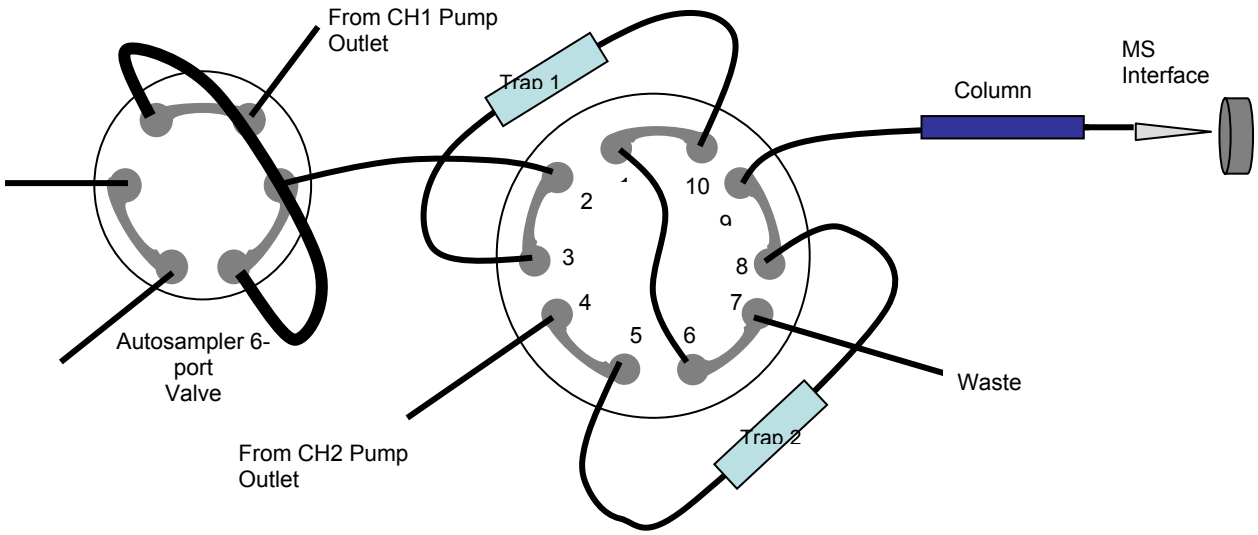
Default methods: Direct injection to column with Channel 2.
Pump: Channel 2



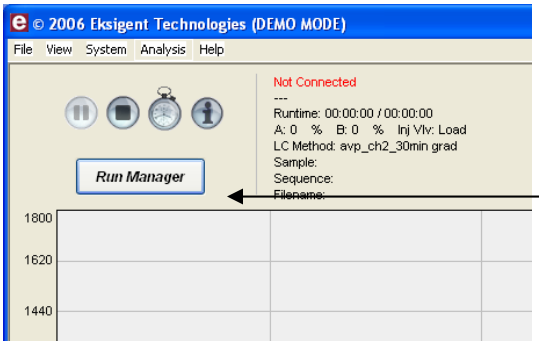




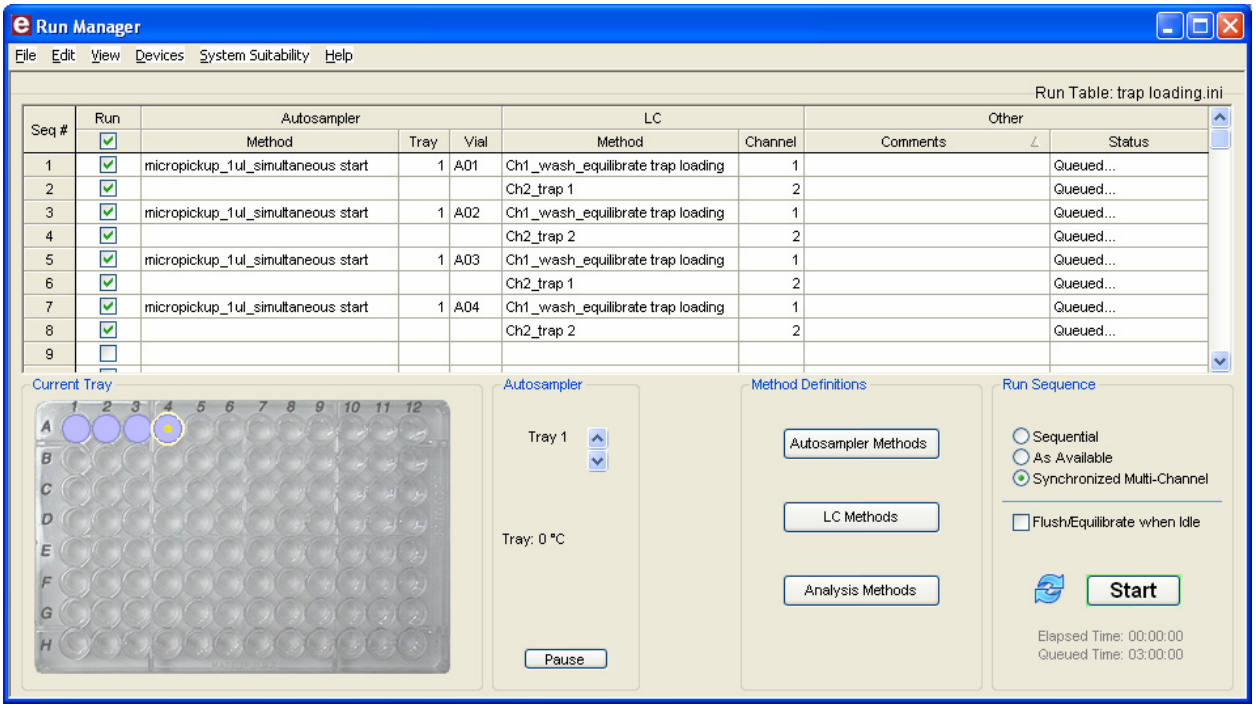
D.2 Quick Start guide: Dual Trap Column Method



To start a run in Eksigent software, open the Run Manager:



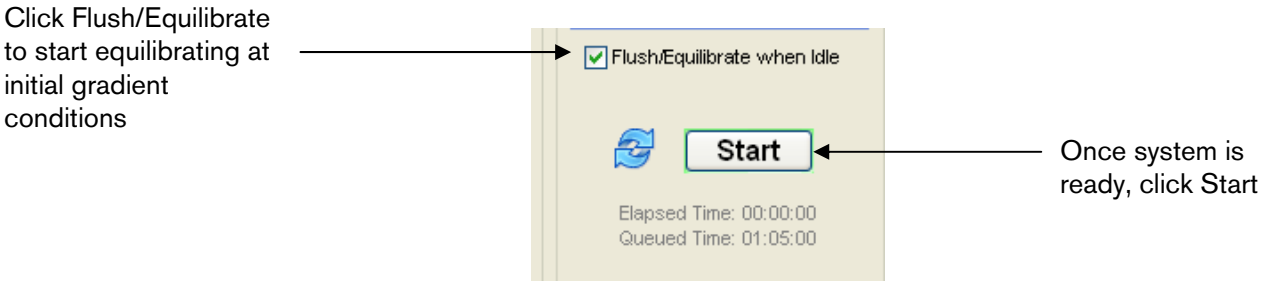
To start a sample run, click Run Manager.



Run in Synchronized Multi-Channel mode.

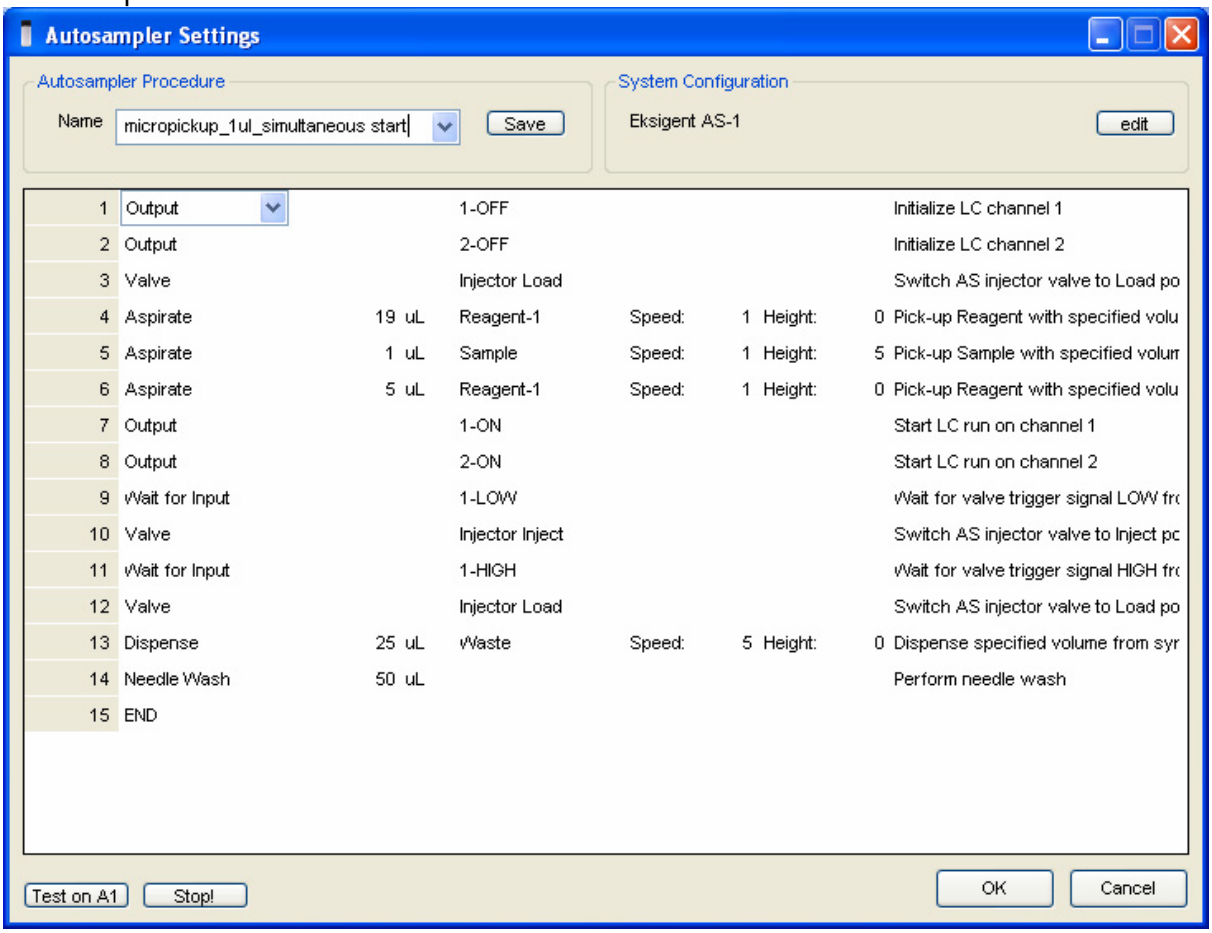
Sequence line 1: Load trap 2
Line 2: Gradient Trap 1
Line 3: Load trap 1
Line 4: Gradient trap 2

Click on Flush/Equilibrate when Idle to start equilibrating pumps at initial gradient conditions.



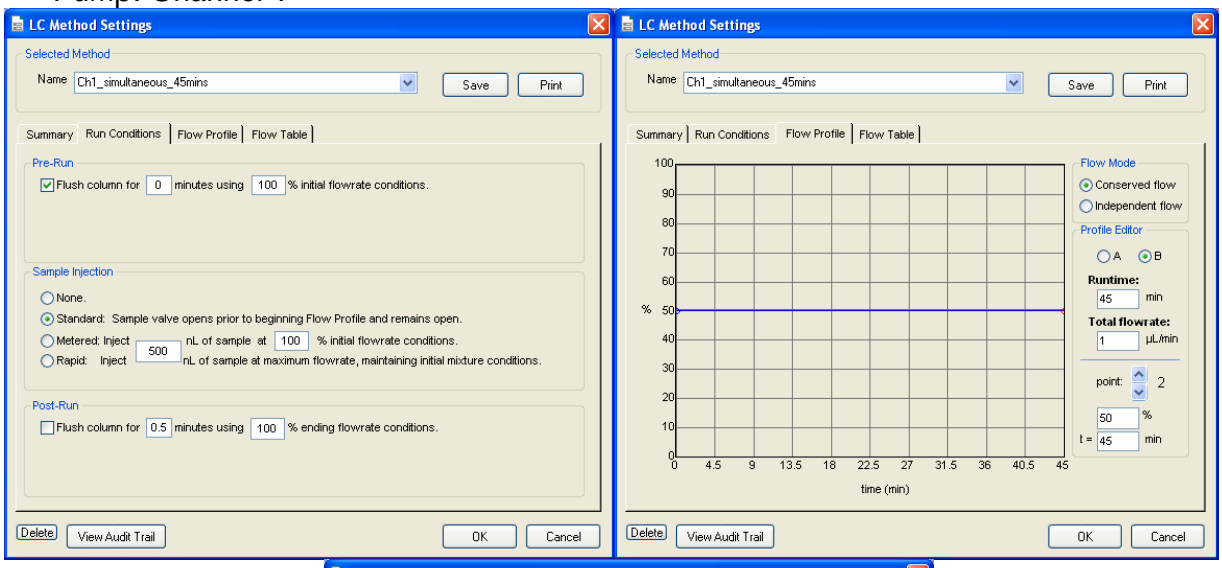
Default methods: Dual Trap column configuration

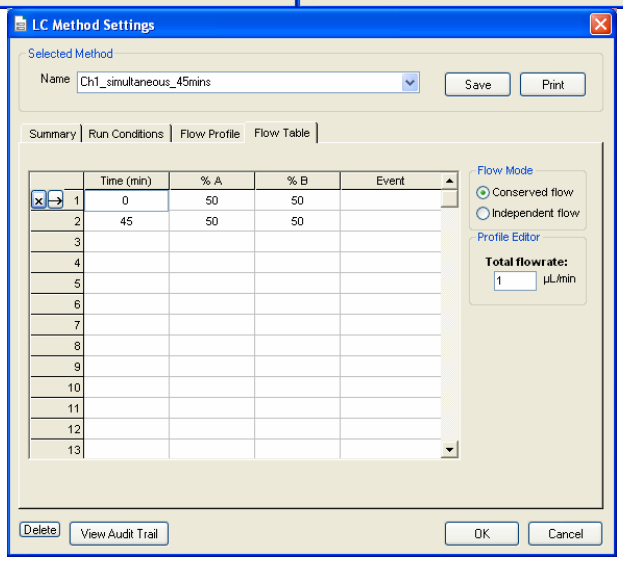
Autosampler:



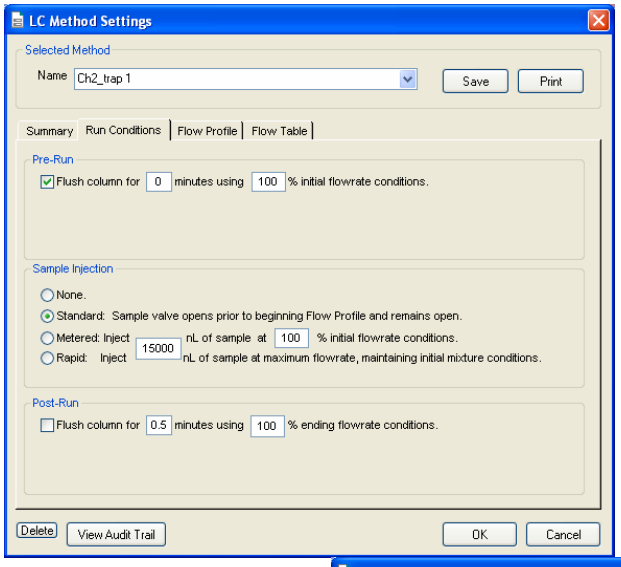
Default methods: Dual Trap Column Configuration:

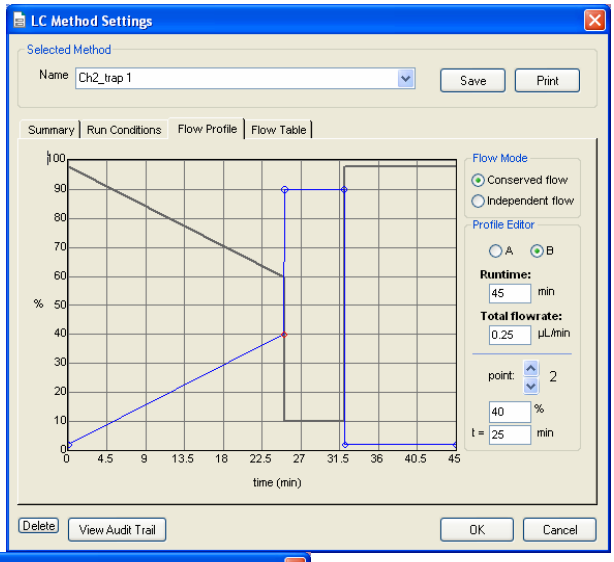
Pump: Channel 1

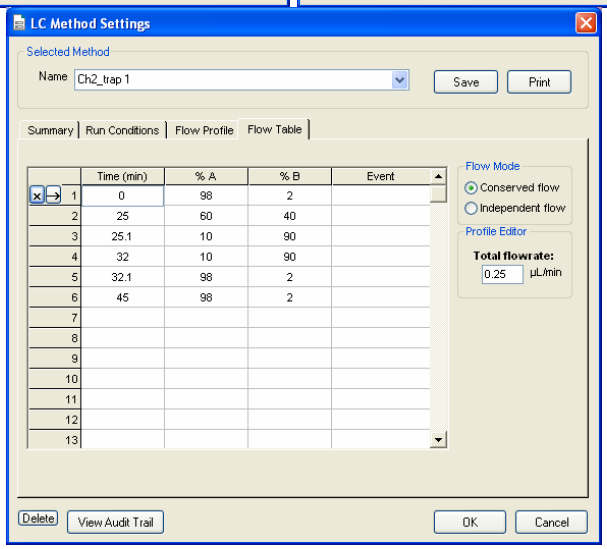




Default methods: Dual Trap Column Configuration:
Pump: Channel 2, Trap 1



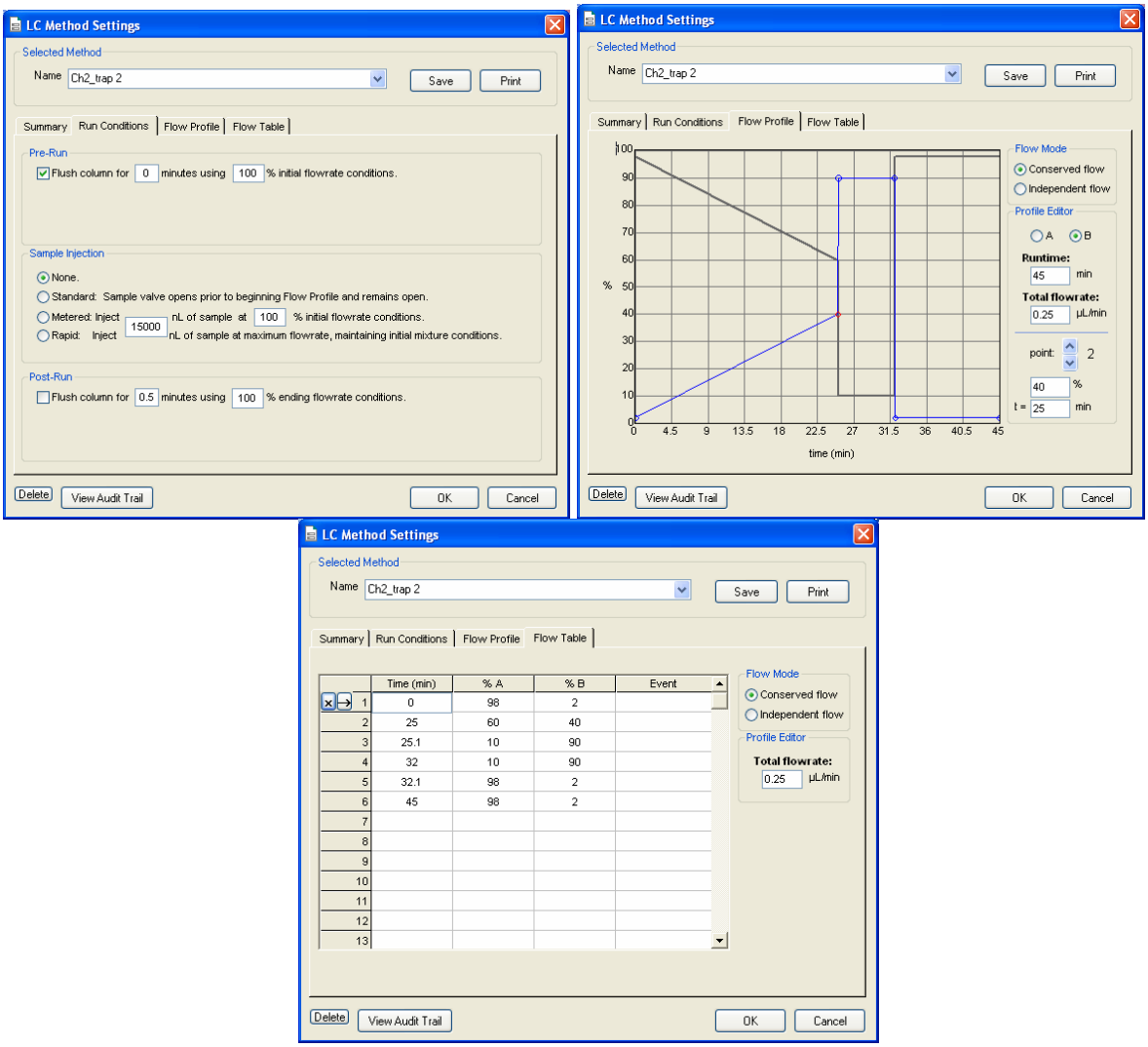




Default methods: Dual Trap Column Configuration:

Pump: Channel 2, Trap 1

Same as previous method- except injection = NONE instead of injection = Standard.



Hints for Dual Trap columns:

1. Channel 1 and 2 methods should run the same length of time.
2. If pre-washing traps is desired, change Channel 1 sample injection type to NONE and use timed events:
    a. VLV TTL low starts the injection
    b. VLV TTL high ends the injection
3. Contact Eksigent Technologies Support for guidance with complex HPLC methods.

Appendix E

Replacing Internal Instrument Filters

Replace solvent filters and check for leaks

step 1 Power off AS-1 autosampler and remove output, input, serial and power cables. Disconnect tubing connections from the autosampler valve, and remove the waste tubing from the waste container. Remove the autosampler from the top of the nanoLC and set aside.

step 2 Power off the nanoLC.

step 3 Open the cover of the nanoLC and visually check for any signs of leaks, corrosion, etc. If any of the pumps appear to have leaked, follow the procedure to replace the pump seals found in Appendix A of this document.

step 4 Remove the internal instrument filter assemblies with a 9/16" and ¼" wrench.

step 5 Open the filter assemblies and remove the filter frits. Rinse the filter assemblies with methanol and replace with new filter frits. Tighten the filter frits in place finger tight + ¼ turn with two 7/16" wrenches.

step 6 Re-install the filter assemblies and tighten the stainless steel tubing connections into the filter assemblies.

step 7 Turn on the power to the nanoLC and establish communication.

step 8 Purge out or remove the old solvent from the mobile phase reservoirs. Refill with new solvent and purge at least 20 times on each pump.

step 9 Disconnect any tubing at the outlet of Channel 1 and Channel 2.

step 10 Flush 100uL through Channel 1 and Channel 2.

step 11 Test the nanoLC for leaks:

a. Plug the outlet of CH1 and CH2
b. In the service menu, ramp the power in steps: 25%, 50%, 100%. Check system for leaks in fittings inside the box. Repair any leaks, then ramp the power again to test.
c. Hold the system at 100% power for 10 minutes. Repair any leaks and then stop the flow.

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