NanoLC Ultra® Systems

Operator Guide

eksigent®
part of AB SCIEX

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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 Eksigent NanoLC® Ultra systems. Topics covered in this chapter include:

- NanoLC Ultra Systems Safety Practices (Section 1.1)
- NanoLC AS-2 Autosampler Safety Practices (Section 1.2)
- Site Requirements (Section 1.3)

1.1 NanoLC Ultra System Safety Practices

The following safety practices apply to the NanoLC Ultra system:

WARNING! Potential Operator Injury: Use of this equipment in a manner not approved by the manufacturer may inhibit its safety protection.

WARNING! Electrical Shock Hazard: Only use fuses of the type and current rating specified. Do not use repaired fuses or by-pass the fuse holder.

WARNING! Electrical Shock Hazard: The supplied power cord must be used with a power outlet containing a protective ground contact.

WARNING! Biohazard: When replacing tubing or fittings on the NanoLC Ultra 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.

WARNING! Environmental Hazard Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/or toxic solvents into a municipal sewage system.

1.2 NanoLC AS-2 Autosampler Safety Practices

The following safety practices apply to the optional NanoLC Ultra AS-2 autosampler:

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

Caution: When you use the NanoLC AS-2 autosampler, follow generally accepted procedures for quality control and methods development.

Caution: When you use the NanoLC AS-2 autosampler 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.

WARNING! Potential Operator Injury: Use of this equipment in a manner not approved by the manufacturer may inhibit its safety protection.

WARNING! Electrical Shock Hazard: Only use fuses of the type and current rating specified. Do not use repaired fuses or by-pass the fuse holder.

WARNING! Electrical Shock Hazard: The supplied power cord must be used with a power outlet containing a protective ground contact.

WARNING! Electrical Shock Hazard: 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 AS-2 autosampler. The instrument, as shipped, is properly grounded in accordance with normal safety regulations.

WARNING! Electrical Shock Hazard: The combination of a NanoLC AS-2 autosampler with a LC/MS system may require additional safety measures as described by the LC/MS system vendor. See the mass spectrometer vendor's operating/installation manual for detailed instructions for the safe grounding on the LC/MS system.

WARNING! Electrical Shock Hazard: Use 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.

WARNING! Potential Instrument Damage: Do not turn the autosampler on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact AB SCIEX Technical Support for a product evaluation. Do not attempt to use the instrument until it has been inspected and approved for use.

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

WARNING! Potential Instrument Damage: Damage can also result if the autosampler is stored for prolonged periods under extreme conditions (for example, subjected to heat, water, etc.).

WARNING! Electrical Shock Hazard: 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.

WARNING! Potential Instrument Damage: To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the NanoLC AS-2 autosampler. Once the power is turned off, wait approximately 30 seconds before disconnecting an assembly.

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

WARNING! Puncture Hazard: To avoid injury during NanoLC AS-2 autosampler operation, keep hands and loose objects away from the autosampler arm and syringe assembly.

WARNING! Puncture Hazard: Do not operate the NanoLC AS-2 autosampler without the safety shield properly installed.

WARNING! Biohazard: At all times, observe safe laboratory practices when handling solvents, changing tubing or operating the NanoLC AS-2 autosampler in order to avoid injury. Know the physical and chemical properties of the solvents you use. Refer to the solvent manufacturer's Material Safety Data Sheets (MSDS) for any solvent being used for information.

WARNING! Potential Operator Injury: Use caution when working with any polymeric 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.

Caution: 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 an AB SCIEX FSE.

1.3 Site Requirements

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

1.3.1 NanoLC Ultra System Power Requirements

The NanoLC Ultra instrument is powered by a 24 and 5 VDC external power supply. Only the Eksigent power supply provided with the instrument should be used.

The Eksigent power supply permits operation from any line voltage between 100–240 VAC, 47–63 Hz and requires 3.0 A at 115 VAC or 1.5 A at 230 VAC.

Fuses:

- For 115 VAC; two 3.0 A (5 x 20 mm) fuses
- For 230 VAC; two 1.5 A (5 x 20 mm) fuses
- All fuses are UL-listed and CSA-certified

1.3.2 NanoLC AS-2 Autosampler Power Requirements

The NanoLC AS-2 autosampler requires a line voltage between 95–240 VAC and 2 A.

1.3.3 Gas 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 6 m (20 ft) of the air/nitrogen regulator. When using compressed air, AB SCIEX strongly recommends an air supply having a dew point of less than 4.5°C (40°F). When using dry nitrogen or compressed air, AB SCIEX strongly recommends the use of air filtration to 5 µm (for compressed gas supplied at less than 150 psi, a Wilkerson F18 filter) and regulation to a working pressure of 100 psi (for example, for com-

pressed 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 (such as air supplied from an oil-based compressor), AB SCIEX strongly recommends the regulator be followed with a coalescing filter suitable for particle removal to 0.01 µm (for example, 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.

1.3.4 Bench Space Requirement

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

- 14 inches (36 cm) wide × 24 inches (61 cm) deep × 18 inches (46 cm) high (allow excess space for cables).

With the NanoLC AS-2 autosampler, the height requirement for both systems is 32 inches (81 cm).

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

1.3.5 Environment Requirements

The instrument is designed to operate in an environment with ambient temperatures between 15°C and 30°C (59°F to 86°F) and non-condensing humidity.

Chapter 2.
System Installation

Chapter 2 describes the recommended procedure for unpacking and installing the Eksigent NanoLC® Ultra system. Topics covered in this chapter include:

- NanoLC Ultra Systems Overview (Section 2.1)
- Unpack the NanoLC Ultra System (Section 2.2)
- Place the System (Section 2.3)
- Install the Software and Instrument Settings (Section 2.4)
- Connect to the Gas Supply (Section 2.5)
- Connect the NanoLC Ultra System to Power (Section 2.6)
- Connect the PC (Section 2.7)
- Configure the NanoLC Ultra System (Section 2.8)

2.1 NanoLC Ultra Systems Overview

Note: This guide is written for standard NanoLC Ultra systems. Some sections in the operator guide describe features which may not be included in a specific system.

The NanoLC Ultra systems are designed for HPLC applications that employ direct pumping at flow rates of several hundred nanoliters per minute. The fully integrated system includes binary gradient pumps, an additional pump or second binary gradient pump system, a column-switching valve, an operator guide, and temperature-controlled flow modules and column compartment. The system is compatible with the AS-1 and AS-2 autosamplers.

- The high flow rate channel is optimized to run from 1 µL/min to 10 µL/min.
- The low flow rate channel is optimized to run from 50 µL/min to 500 nL/min.
- The sample loading channel is optimized to run at 1 µL/min to30 µL/min.

This chapter introduces the hardware and software features of NanoLC Ultra systems.

2.2 Unpack the NanoLC Ultra System

1. Inspect the shipping cartons for damage or evidence of mishandling. If external damage is evident, notify the carrier before opening the cartons.
2. Cut the tape and open the flaps on the top of the NanoLC Ultra system shipping box.
3. Remove the foam from the box.
4. Grasp the sides of the cardboard cradle and carefully lift the NanoLC Ultra system out of the box.

   A second person may be needed to help lifting the system.
5. Place the instrument on the floor.
6. Grasp the sides of the instrument and place the instrument on a lab bench.
7. Cut and open the accessory kit shipping box.
8. Unpack the computer and monitor and verify that no parts are missing or damaged.

2.3 Placement of the System

Place the NanoLC Ultra 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 top of the instrument should be clear for placement of the mobile phase bottles; and the sides and back of the instrument should be clear to accommodate computer cables and fluidic tubing.

2.4 Install the Software and Instrument Settings

Required Materials
• CD with Eksigent control software • CD with system settings

1. Insert the CD with the control software into the CD drive and install the Eksigent control software (see software manual for additional information on installation).
2. After installing the software, but before starting it, insert the CD with the system settings (shipped with the new instrument).
3. Locate the file named “EkSettings.reg” in the Settings subfolder.
4. Double-click **EkSettings.reg** file to install the instrument settings into the registry of the computer.

A dialog appears, asking you if you want to write to the registry (Figure 2-1).

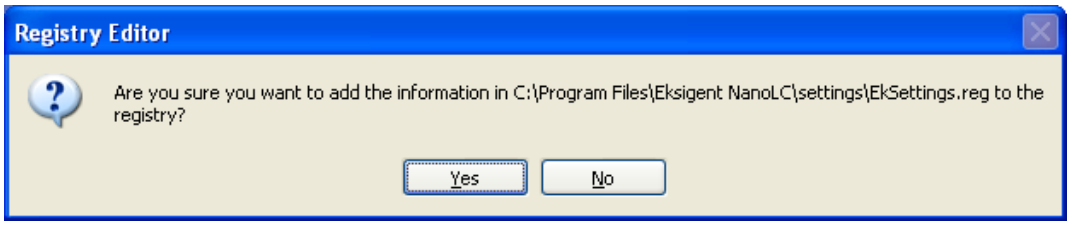


Figure 2-1 First Registry Dialog

5. Click **Yes**.

   A second dialog appears, indicating that you have successfully written to the registry (Figure 2-2).

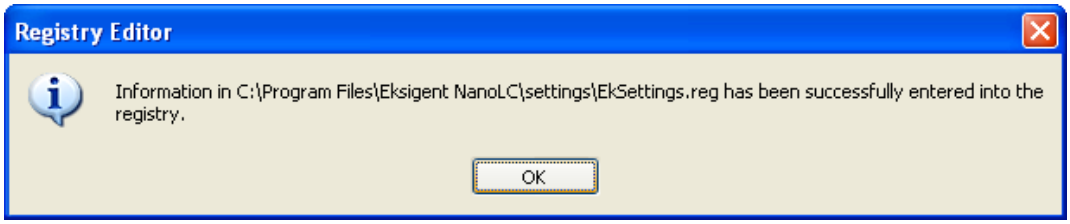


Figure 2-2 Second Registry Dialog

6. Click **OK**.

   The factory settings for your instrument should now be loaded into the registry. You should still configure the system (Section 2.8) in case the COM port or other settings need to be adjusted from the factory defaults.

2.5 Connect to the Gas 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 Section 1.3.3 (Gas Supply Requirements).

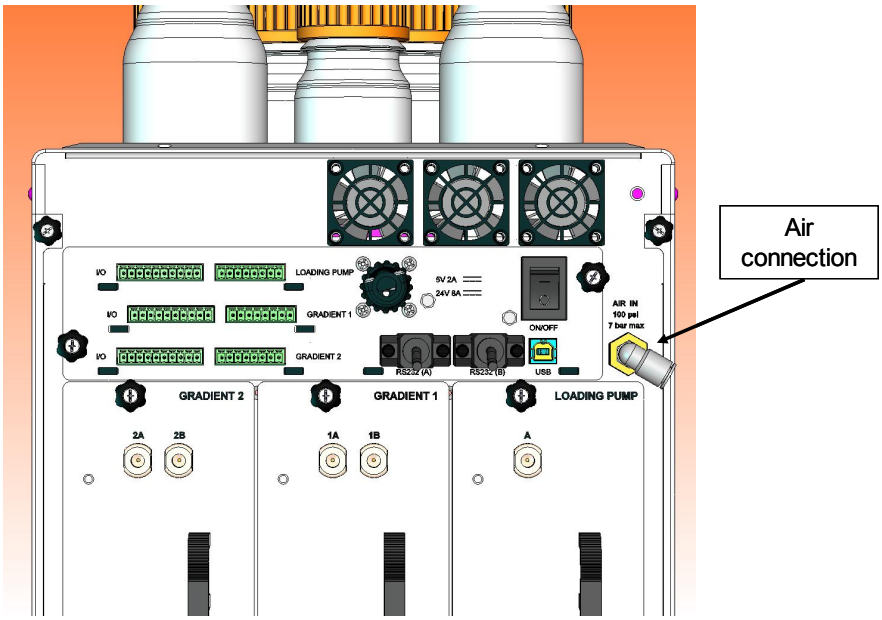


Figure 2-3 Rear panel—gas connection

Required Materials
• Clean, dry source of air or nitrogen at 100 psi (6.9 bar) • In-line moisture trap with ¼ inch end-fittings (recommended if an air compressor is used) • ¼ inch gas supply line (included) • Quick-connect adaptor to ¼ inch supply line (included)

1. Connect the supplied gas line to a source of 100 psi (6.9 bar) regulated clean, dry air or nitrogen using the supplied quick-connect adaptor.
2. Connect the other end of the gas line to the connection on the back of the NanoLC Ultra system (see Figure 2-3).
3. Turn on the gas source to the NanoLC Ultra system.
4. Test for leaks. Turn off gas supply and repair any leaks that are found.

2.6 Connect the NanoLC Ultra System to Power

Required Materials
• Eksigent combination 24 and 5 VDC power supply (included)

1. Insert the Eksigent power supply's plug into the connector located on the back of the instrument and the power supply (Figure 2-4 and Figure 2-5).

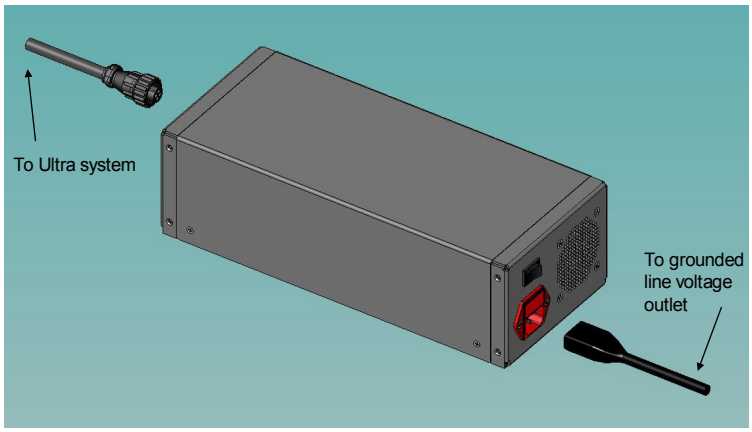


Figure 2-4 Eksigent Power Supply—Power Connection

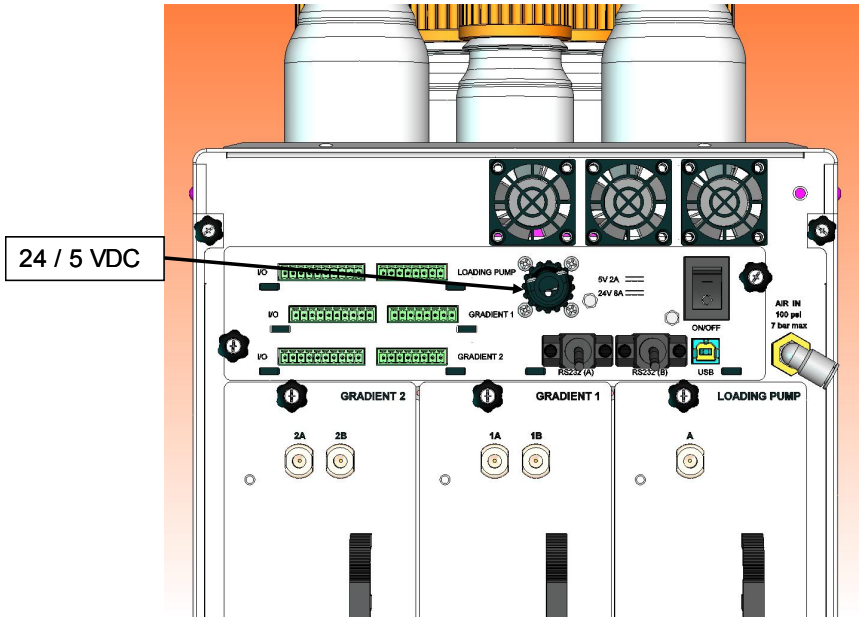


Figure 2-5 Rear Panel—Power Connection

2. Plug the line voltage cord into an appropriately grounded line voltage outlet.

3. Turn the Eksigent power supply on using the power switch on the back of the power supply.
4. Turn the NanoLC Ultra system on using the power switch on the back of the unit.

2.7 Connect the PC

Note: The Eksigent control software must be installed before connecting the instrument to the PC.

Required Materials
• Computer, keyboard and mouse • System USB cable (included)

1. Connect the Type A (Figure 2-6) connector of the USB cable to an available USB port on the computer.

Figure 2-6 USB Connectors—Type A (Left) and Type B (Right)

2. Connect the Type B (Figure 2-6) connector of the USB cable to the USB port labeled **USB** on the rear panel of the NanoLC Ultra system (Figure 2-7).

Make sure the connectors are securely connected.

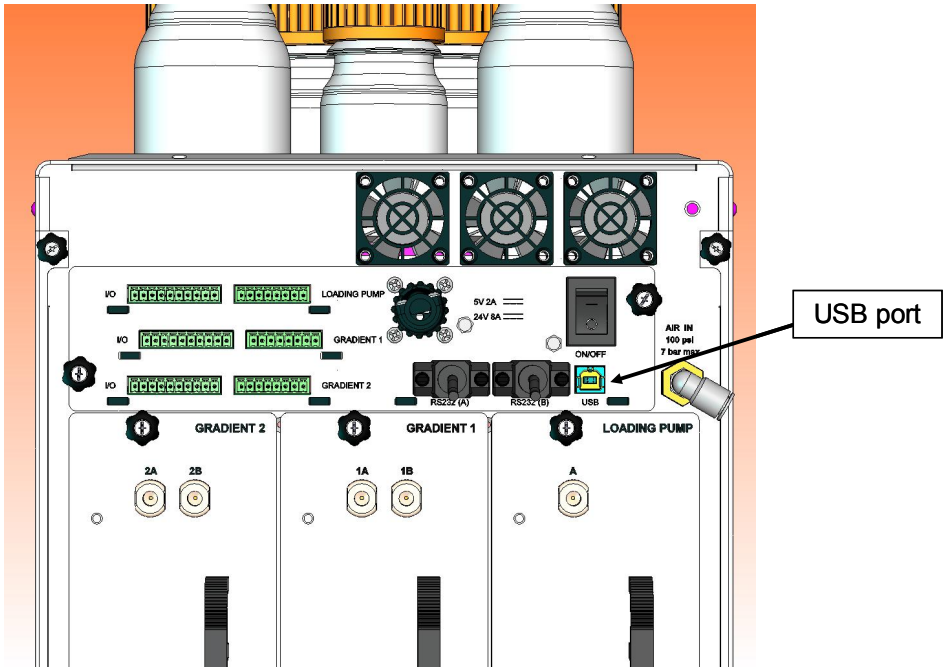


Figure 2-7 Rear Panel—USB Connector

2.8 Configure the NanoLC Ultra System

1. Power up the NanoLC Ultra system.
2. To determine which COM port the system is connected to, go to **Control Panel > System > Hardware** (Figure 2-8).

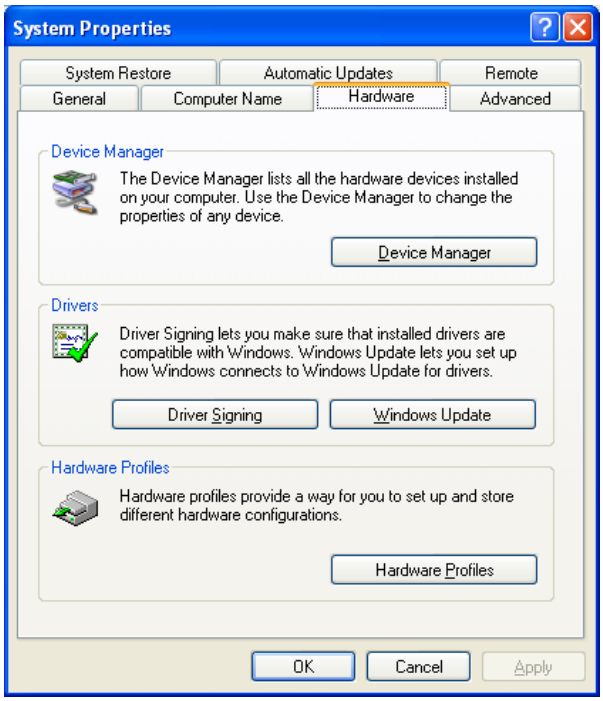


Figure 2-8 System Properties Dialog—Hardware Tab

3. Click **Device Manager** to display the Device Manager dialog (Figure 2-9).

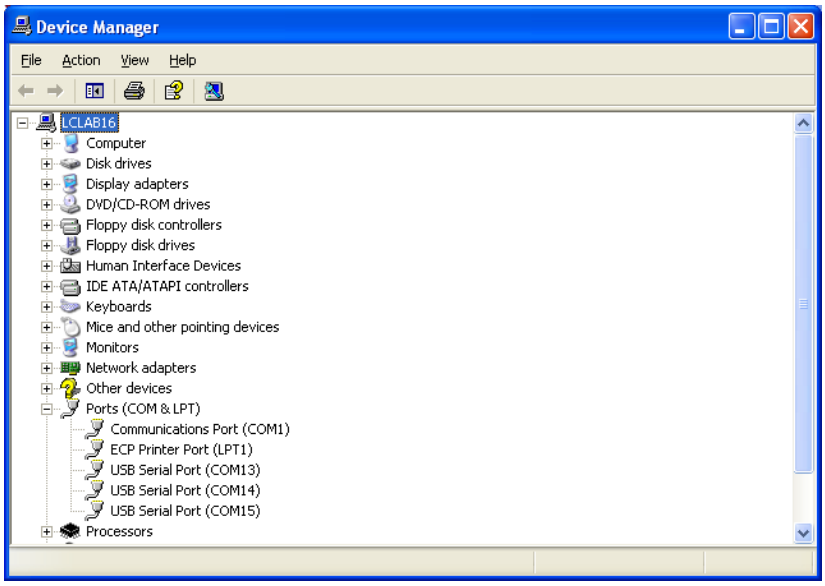


Figure 2-9 Device Manager Dialog

4. Expand the **Port (COMS and LPT)** in the Device Manager dialog.

   The three USB serial ports shown are the COM ports associated with the NanoLC Ultra system. The number assigned to the serial port will be different from PC to PC. The first USB serial port is assigned to the NanoLC Ultra instrument. The second and third serial ports are assigned to RS 232 (A) and RS 232 (B), respectively. It is recommended that NanoLC AS-2 autosampler be connected to RS 232 (A).

5. Launch the Eksigent control software from your computer's **Programs** menu or from the icon on the desktop.

   A COM Error dialog (Figure 2-10) will appear.

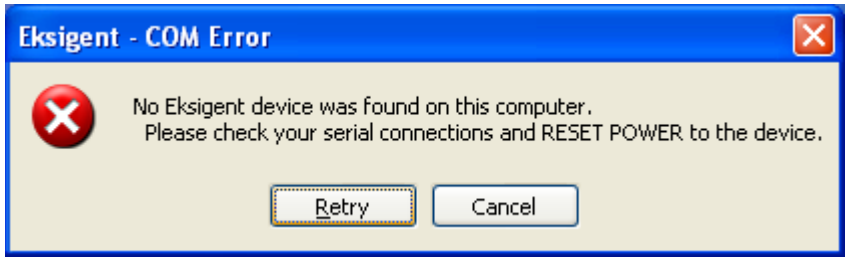


Figure 2-10 Serial Port Communications Error

6. Click **Cancel**.

A second window will appear (Figure 2-11).

Figure 2-11 Running the Software in Demo Mode

7. Click **OK** to enter the software in DEMO MODE and display the **Acquisition** window (Figure 2-12) .

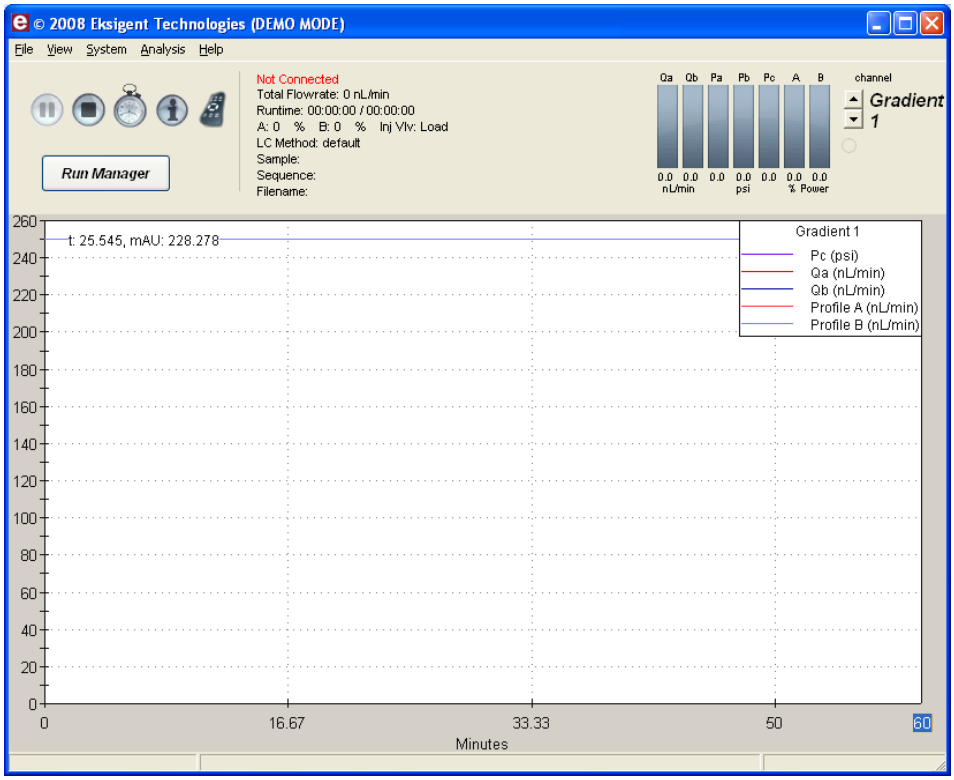


Figure 2-12 Acquisition Window

8. Select **System > Instrument Configuration** from the Eksigent control software's Acquisition window to access the **Instrument Configuration** dialog (Figure 2-13).

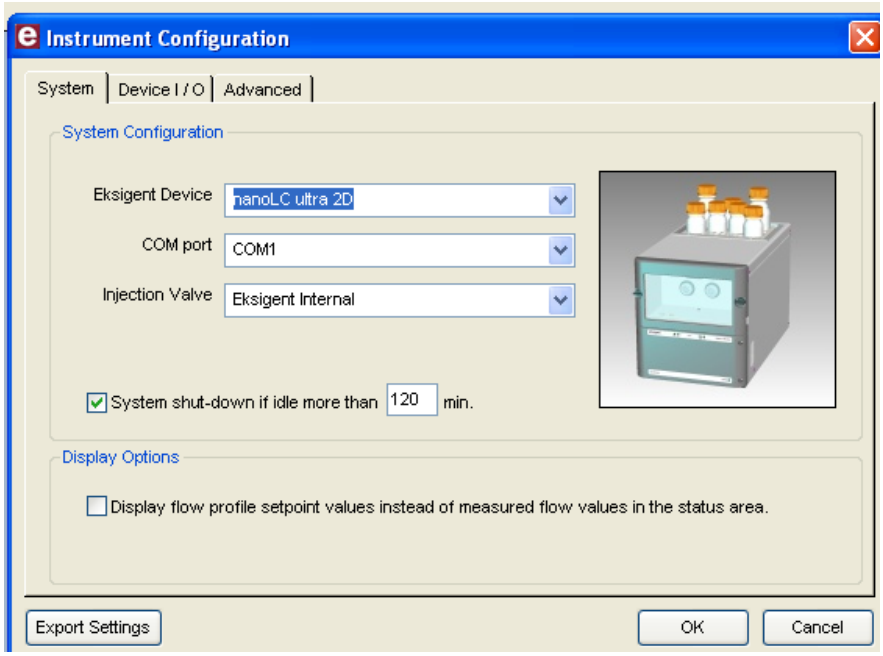


Figure 2-13 Instrument Configuration Dialog—System Tab

The Instrument Configuration dialog 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.

9. Make sure that one of the following systems is selected in the **Eksigent Device** field.
   - nanoLC Ultra-1D
   - nanoLC Ultra-1D+
   - nanoLC Ultra-2D
   - nanoLC Ultra-2D+
10. Select the COM port that is connected to the system. (Refer to step 4.)
11. In the **Injection Valve** field, select **Eksigent Internal**.
12. The system shut-down check box is an option to turn off the system after it is idle for the specified time.

13. If an auxiliary A/D input (such as a UV detector) is connected to the system, select the voltage range the external device will provide under **Input A/D Range**. (Figure 2-14).

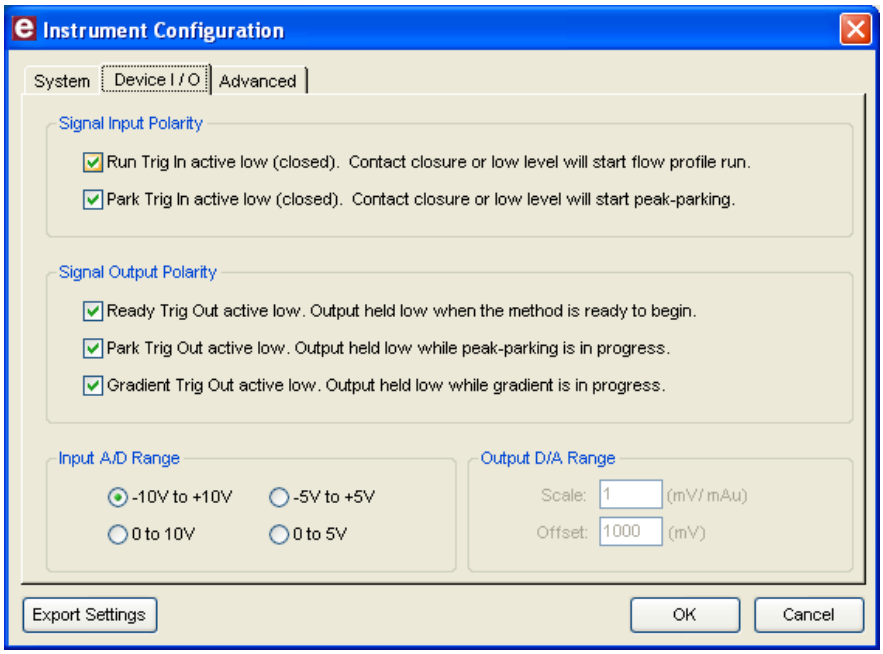


Figure 2-14 Instrument Configuration Dialog—Device I/O Tab

14. Click the **Advanced** tab **(Figure 2-15)** to set the **Flow Stabilization Limits** which specify the degree of flow rate stability that is required before a gradient will begin.

The recommended setting is 100 nL/min.

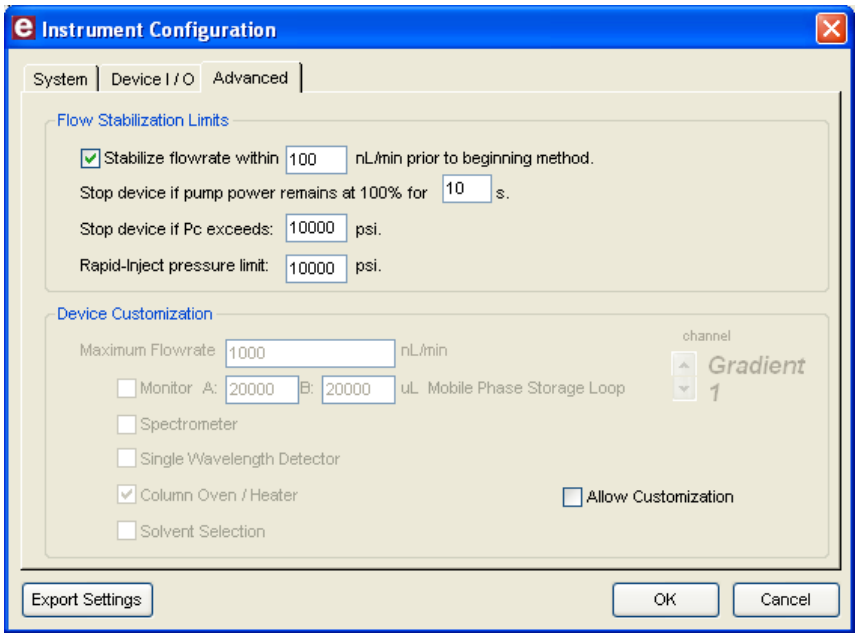


Figure 2-15 Instrument Configuration Dialog—Advanced Tab

15. The **Stop device if Pc exceeds** field (Figure 2-15) 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. For most applications a value of 10 000 psi is recommended.

16. Click **OK** to go back to the **Acquisition** window (Figure 2-12).

    The **Re-start Required** dialog will appear. (Figure 2-16)

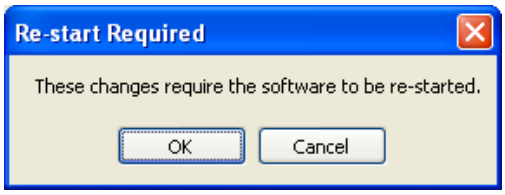


Figure 2-16 Re-start Required Dialog

17. Click **OK** to automatically end the software.
18. Launch the Eksigent control software.

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)
- Channel Assignment in the Eksigent Control Software (Section 3.2)
- Load the Mobile Phases (Section 3.3)
- Verify the Flow Rate (Section 3.4)
- Prepare the NanoLC AS-2 Autosampler (Section 3.5)
- Flush the Autosampler Syringe and Liquid Path (Section 3.6)
- Connect the NanoLC® Ultra System to the Autosampler (Section 3.7)

3.1 Hardware Components and Functions

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

Mobile phase outlets are located on the left side of the temperature-controlled column compartment in the NanoLC Ultra system (Figure 3-1). Each gradient pump has one mobile phase outlet which can be connected to the autosampler and a 10-port column-switching valve. The sample loading pump mobile phase outlet is located at the bottom-left corner of the lower front bezel (Figure 3-2).

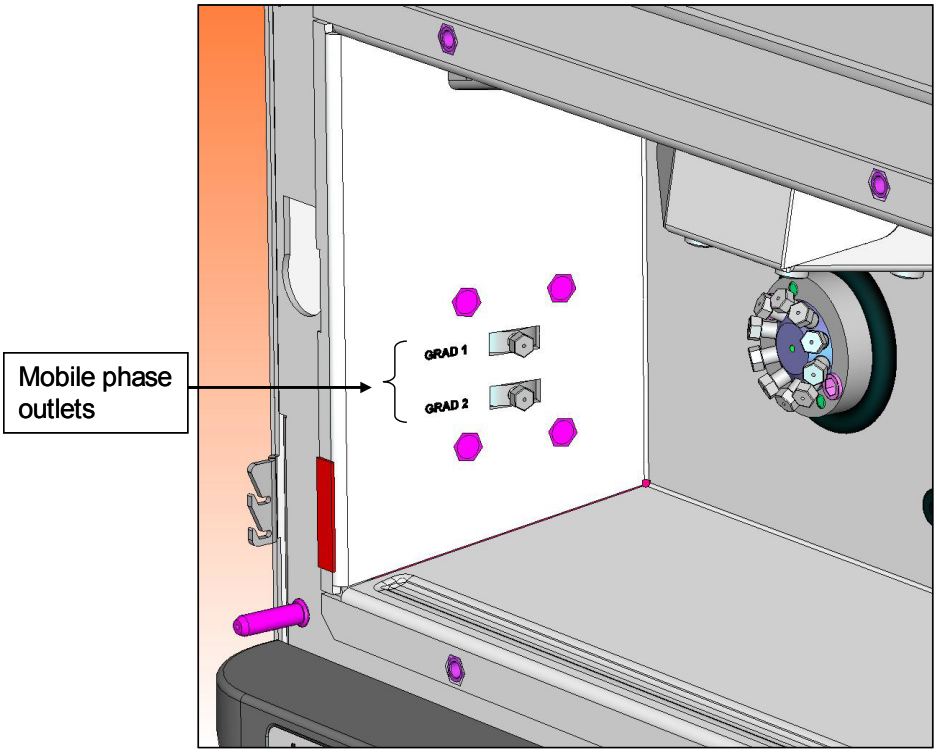


Figure 3-1 Mobile phase outlets

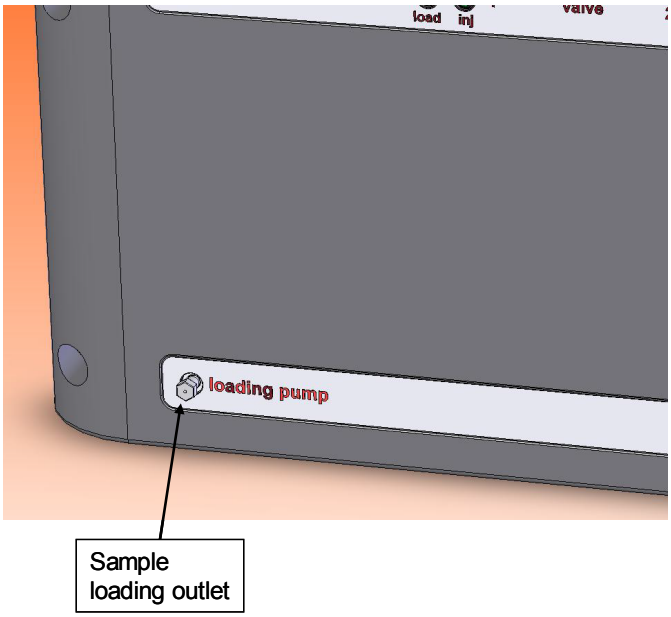


Figure 3-2 Sample loading outlet

The 10-port column-switching valve is used to switch between a trap column that is connected to the valve for rapid sample loading. The valve(s) is/are located in the temperature-controlled column compartment. Refer to Appendix C for different valve configurations.

3.2 Channel Assignment in the Eksigent Control Software

Table 3-1 shows the channel assignment in the Eksigent control software for the NanoLC Ultra series system for standard configurations.

Table 3-1 Channel Assignment in the Eksigent Control Software for the NanoLC Ultra Series System

System	Gradient 1	Gradient 2	Loading Pump
Ultra 1D	Nano flow (50 nL/min to 500 nL/min)	N/A	N/A
Ultra 1D+	Nano flow (50 nL/min to 500 nL/min)	N/A	Micro flow (1 µL/min to 30 µL/min)
Ultra 2D	Micro flow (1 µL/min to10 µL/min)	Nano flow (50 nL/min to 500 nL/min)	N/A
Ultra 2D+	Nano flow (50 nL/min to 500 nL/min)	Nano flow (50 nL/min to 500 nL/min)	Micro flow (1 µL/min to 30 µL/min)

3.3 Load the Mobile Phases

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

Note: 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.

Required Materials

- 250 mL mobile phase bottles (PN 300-00057)
- Clean, degassed HPLC-grade mobile phase A (for example, water with 0.1% formic acid)
- Clean, degassed HPLC-grade mobile phase B (for example, acetonitrile with 0.1% formic acid)
- Mobile phase tubing (PN 801-00062)
- Bottle filters (PN 200-00235)
- Super flangeless nuts (PN 200-00310)
- Super flangeless ferrules (PN 200-00269)

1. Clean all 250 mL mobile phase bottles with appropriate solvents.
2. Fill bottle A with mobile phase **A** and bottle **B** with mobile phase B.
3. Connect mobile phase tubing to the mobile phase connections in the back of the system. (Figure 3-3).

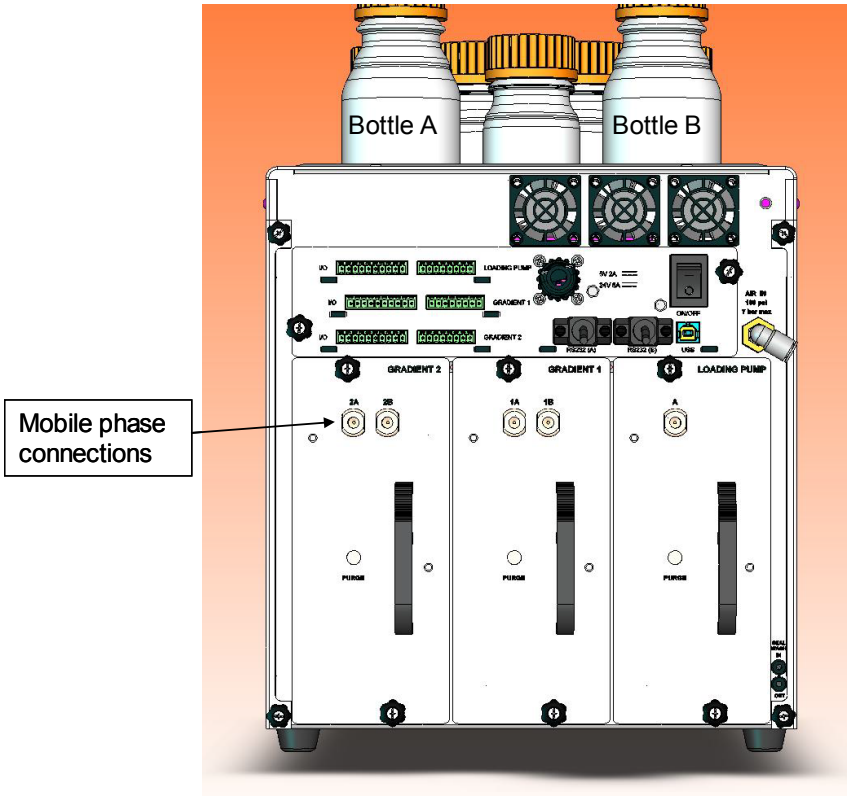


Figure 3-3 Back Panel—Bottles and Mobile Phase Connections for the NanoLC Ultra-2D System

4. Insert tubing through the pre-drilled bottles caps.
5. Install the bottle filters at the end of the tubing.
6. Insert the bottle filters in the bottle and place the caps onto the bottles.
7. Place the mobile phase bottles in the rack on top of the system.

8. Insert the waste tubing from the pumps to the provided waste bottle. Place the waste bottle on top of the bench.
9. Select **System > Mobile Phases** from the main window of the Eksigent control software to display the Mobile Phases dialog (Figure 3-4).

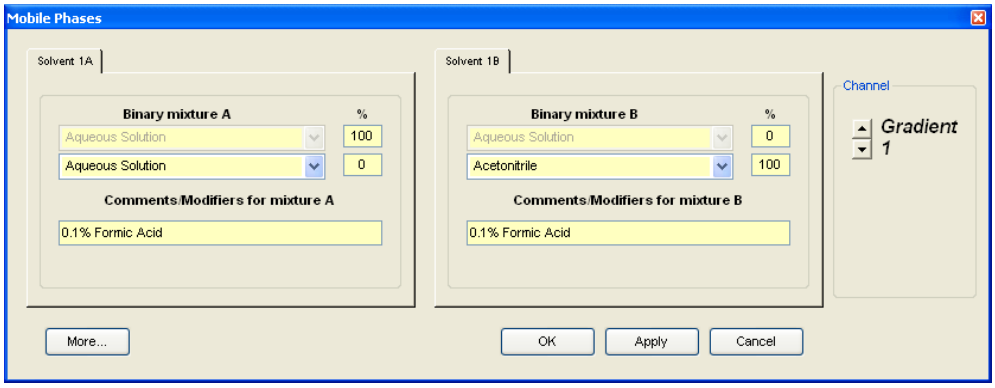


Figure 3-4 Mobile Phases Dialog

10. Set the composition for mobile phase A: Enter the correct solvent composition for **Mobile Phase A**.
11. Set the composition for mobile phase B: Enter the correct solvent composition for **Mobile Phase B**.
12. Click **More** to display the Purge and Flush settings groups (Figure 3-5).

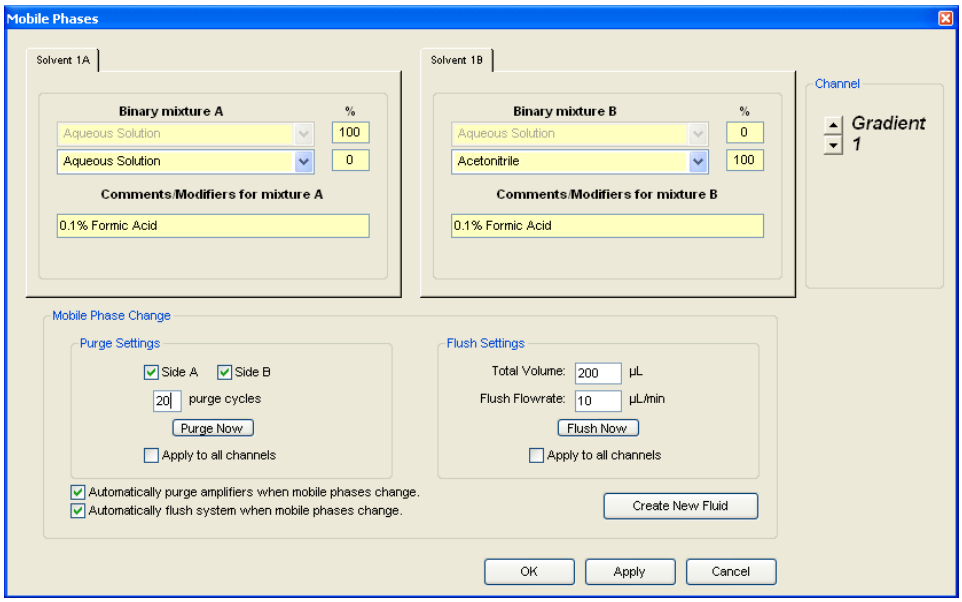


Figure 3-5 Mobile Phases Dialog—Advanced Options

13. Purge the bubbles from each pump using the following steps:

14. Under Purge Settings, select the pump to purge. Set the number of purge cycles to 20. Check the box **Apply to all channels** to purge all pumps simultaneously.
15. Click **Purge Now**. The pumps will begin to execute purge cycles.

    While the pumps are purging, make sure the mobile phases are pulled through the mobile phase tubing to the pumps.
16. Locate the waste tubing of the pumps being purged. After about 8 purges, the mobile phase should be purged through the waste tubing.
17. Enter 200 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 2000 for the nL/min Total Flow rate.
18. 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.
19. Click **Flush Now**.
20. After the flush sequence ends, click **OK** to close the **Mobile Phases** window.

3.4 Verify 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 Materials
• Flow calibration assembly (PN 801-00063) for high flow rate channel (includes 20 µL pipettes) • Flow calibration assembly (PN 801-00064) for low flow rate channel (includes 5 µL graduated pipettes)

1. Re-initialize the pressure transducers (refer to Section 4.5).
2. Attach the appropriate flow calibration assembly to the outlet of gradient 1 or 2.
3. Select **System > Direct Control** from the Eksigent control software **Acquisition** window to display the Direct Control dialog (Figure 3-6).

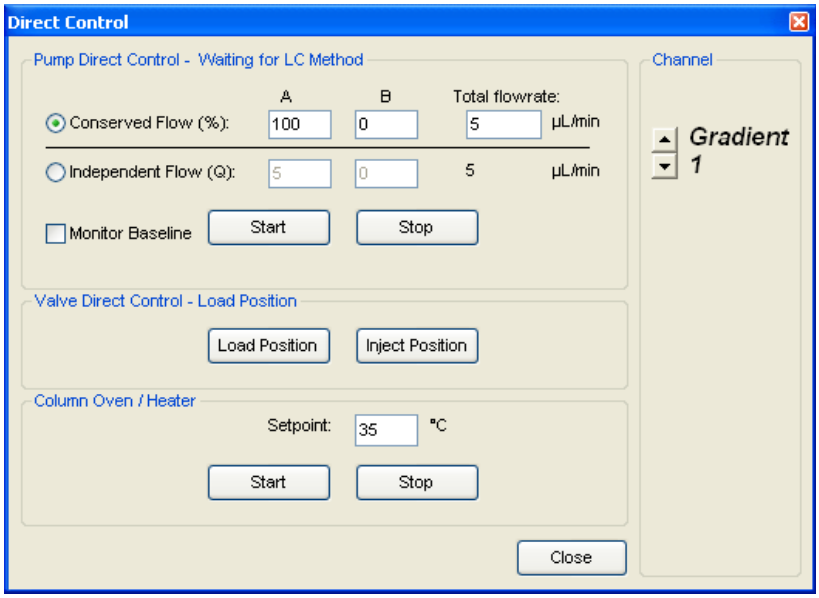


Figure 3-6 Direct Control Dialog—Flow Rate Check

4. Set channel **A** to 100% and an appropriate flow rate for that channel.
   - 5 µL/min for high flow channel
   - 500 nL/min for low flow channel
5. Click **Start** 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.

6. Click **Stop** when the fluid front reaches the end of the pipette.

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

7. Disconnect the calibration assembly and blow out the liquid inside the pipette using a pipette bulb or can of compressed air.
8. Set channel **B** to 100% and repeat steps 5 and 6 to verify the flow rate for pump **B**.
9. Disconnect the calibration assembly.

3.5 Prepare the NanoLC AS-2 Autosampler

The NanoLC AS-2 autosampler is the standard autosampler installed with the NanoLC Ultra system. Refer to the autosampler operator guide for a detailed description of its operation.

Required Materials
• Power cord (included) • RS-232 serial cable (included) • Clean HPLC-grade water • Clean HPLC-grade isopropanol

Figure 3-7 Eksigent NanoLC Ultra with NanoLC AS-2 autosampler

1. Place the NanoLC Ultra system on top of the NanoLC AS-2 autosampler (Figure 3-7). A second person might be needed to lift the system.
2. Connect the power cable to the back of the autosampler and a grounded line voltage outlet.
3. Connect one end of the RS-232 serial cable to the serial port labeled “communication” on the back of the autosampler.

4. Connect the other end to one of the RS 232 (A) COM port at the back of the NanoLC Ultra system (Figure 3-8).

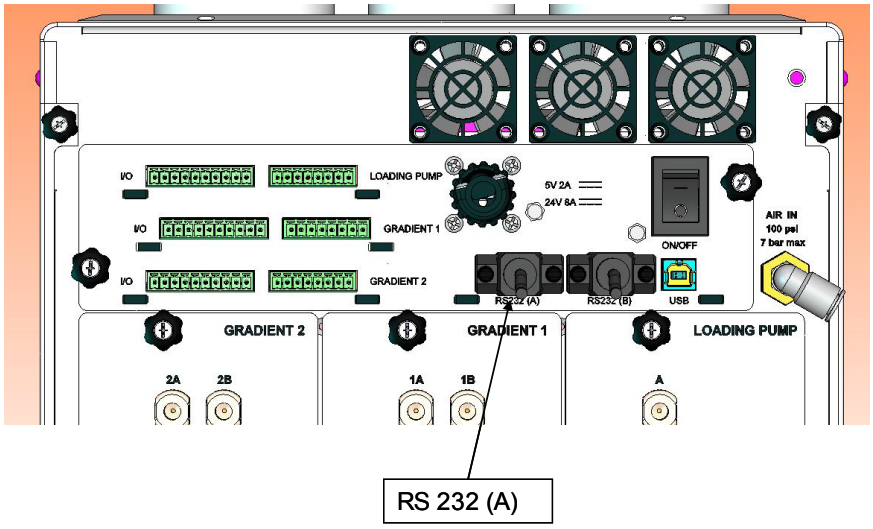


Figure 3-8 Back Panel–RS 232 (A) COM Port Connector Location

5. Fill the wash bottle with a 20:80 mixture of isopropanol/water that has been degassed.
6. Place the wash bottles on the wash station bracket and insert the Teflon tubing.
7. Power up the autosampler on using the power switch on the back of the autosampler.
8. Start the Eksigent control software.
9. Click **Run Manager** in the Acquisition window to display the Run Manager window (Figure 3-9).

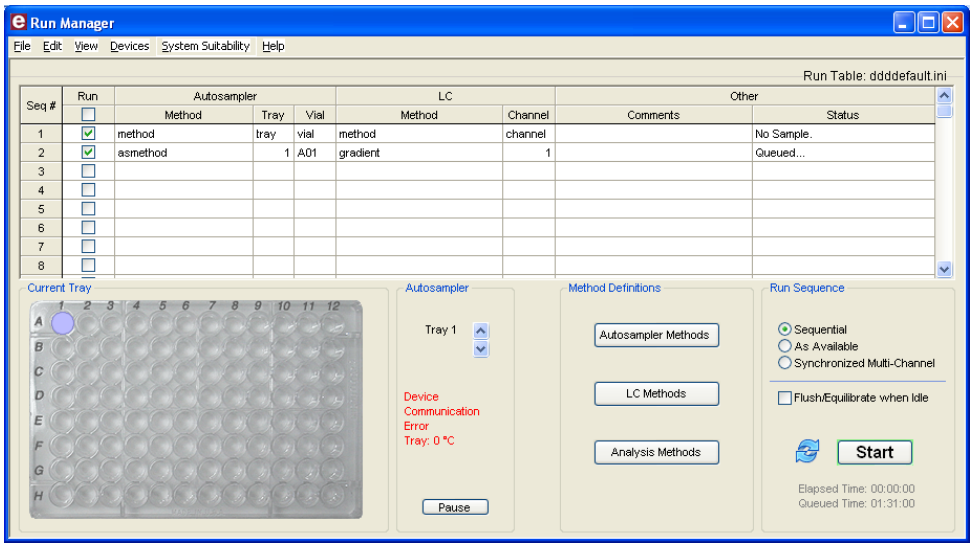


Figure 3-9 Run Manager Window

10. Select **Devices** > **Autosampler Type** > **Eksigent NanoLC AS-2**.

    The following message appears (Figure 3-10):

Figure 3-10 Warning: no autosampler detected Dialog

11. Click **Cancel**.
12. Select **Devices > Autosampler Device Settings** to display the Autosampler Configuration dialog (Figure 3-11)**.**
13. Set the COM port for the autosampler (refer to Section 2.8).

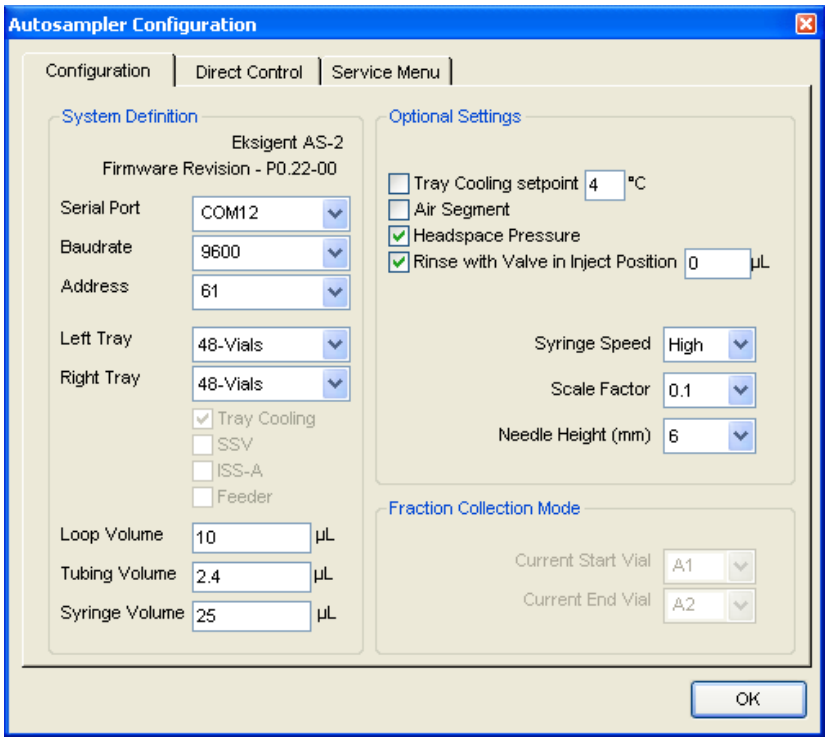


Figure 3-11 Autosampler Configuration Dialog

An initializing autosampler configuration dialog will appear (Figure 3-12).

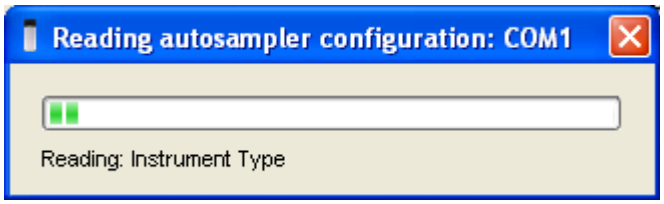


Figure 3-12 Initializing Autosampler Dialog

If the autosampler is successfully connected to the NanoLC Ultra system, the Run Manager window will show the NanoLC AS-2 state as idle.

14. In the Run Manager window, select **Devices** > **Autosampler Device settings**.
15. Click the **Direct Control** tab (Figure 3-13) and click **Exchange** in the Plate Position area.

    This will move the vial holders to a more accessible location to place the reagent and sample vials.

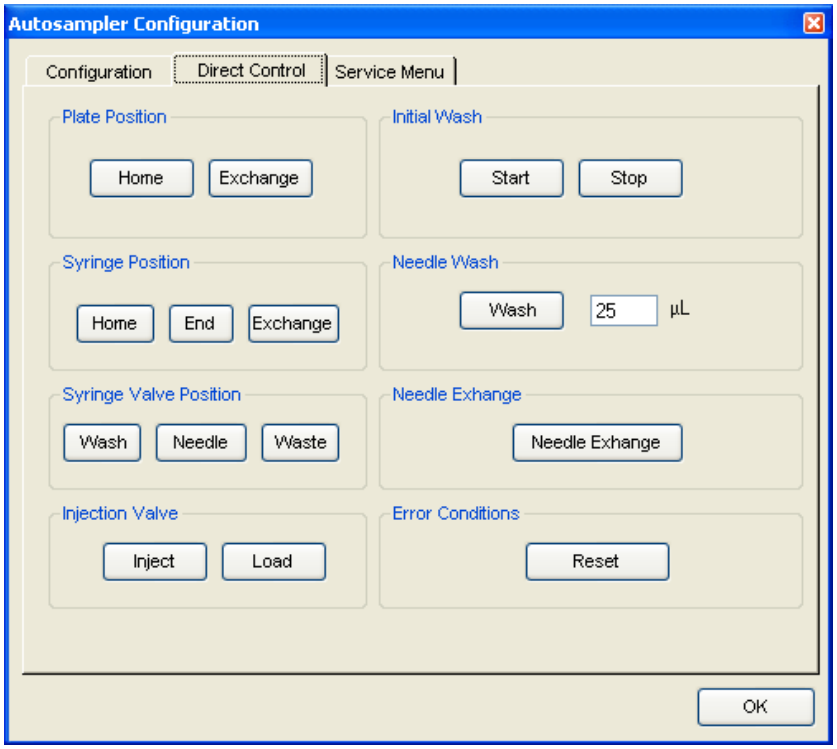


Figure 3-13 Autosampler Configuration Dialog—Direct Control Tab

16. Place the reagent and sample files in the autosampler, then click **Home**.

3.6 Flush the Autosampler Syringe and Liquid Path

1. In the Run Manager window, select **Devices** > **Autosampler Device Settings**.

2. In the **Direct Control** tab of the **Autosampler Configuration** dialog, click **Start** in the **Initial Wash** area.
3. Repeat step 2 until the syringe is full of liquid with no bubbles.

3.7 Connect the NanoLC Ultra System to the Autosampler

Required Materials
• Nut and ferrule for 1/32 inch fitting (PN 910-00085 and 910-00087) • Two pieces of 75 cm, 1/32 inch μm outside diameter (OD)/ 50 μm ID PEEKsil tubing (PN 205-00049) *or* Two meters of 360 μm OD/ 50 μm inside diameter (ID) fused silica (PN 910-00002) • Capillary cutter (PN 200-00096) • Natural PEEK sleeves (PN 910-00088) • Waste and seal rinse bottle and bracket (PN 300-00000 and 800-000321)

Note This section assumes connection of the NanoLC AS-2 autosampler using the high flow channel of the NanoLC Ultra instrument. If you are connecting with the low flow channel through the NanoLC AS-2 autosampler (for example, for direct loading experiments), use 20 µm ID PEEKsil or capillary for the connections to reduce delay volume. Two pieces of 75 cm, 1/32 inch OD / 20 μm ID PEEKsil (PN 205-00048) are included. **Do not attempt to cut PEEKsil tubing**.

Note: For NanoLC Ultra 1D+ and 2D+ systems, the loading pump channel will be connected to the autosampler for trap loading experiment.

1. Place the seal rinse and waste bottle of the NanoLC Ultra system beside the AS-2 autosampler.
2. Remove the screw that is located at the lower middle of the AS-2 side cover using an appropriate screw driver.
3. Secure the seal rinse and waste bottle by using the bottle bracket. Tighten the bracket to the screw hole located at the lower middle of the AS-2 side cover.
4. Insert the waste tubing from the pumps to the waste bottle.
5. Fill the seal rinse bottle with DI water and insert the seal rinse tubing (green) to the bottle. Make sure the tubing ends are submerged in the DI water.

6. Connect 50 µm ID PEEKsil tubing to the Gradient 1 outlet of the NanoLC Ultra using the ferrule, and nut. If using fused silica, cut the fused silica to an appropriate length. Make sure to use the capillary cutter to ensure a clean cut on the ends.
7. Connect the other end of the 50 µm ID PEEKsil tubing to the **Pump** connection on the injection valve of the AS-2 using the 1/32 inch nuts and ferrules. Make sure the PEEKsil tubing is fully seated in the valve fitting.
8. Connect the other piece of 50 µm ID PEEKsil to the **Column** port of the NanoLC AS-2 injection valve to the 10-port switching valve using one of the plumbing diagrams shown in Appendix C.
9. Open the **Direct Control** dialog (Figure 3-14) by clicking **System > Direct Control** from the Eksigent control software's **Acquisition** window.

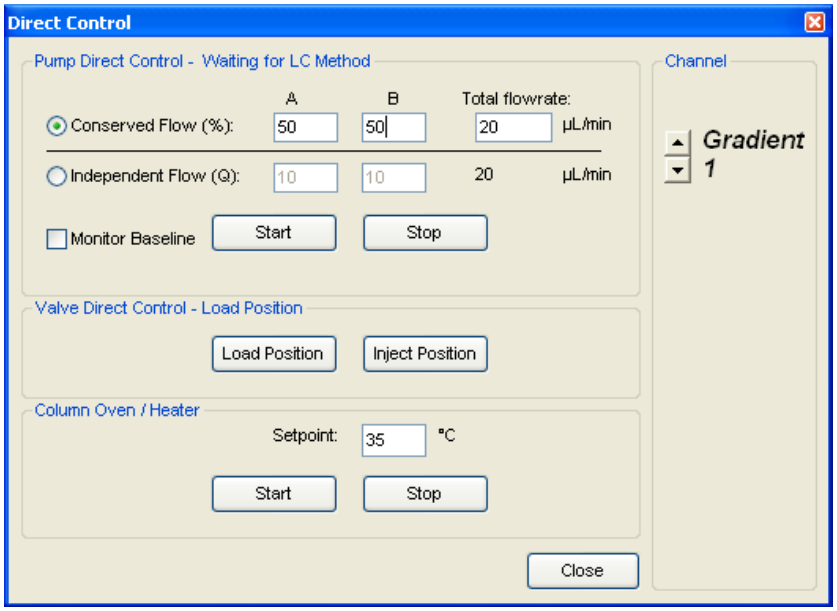


Figure 3-14 Direct Control Dialog—Flushing Gradient 1 Autosampler Connection

10. Set solvent **A and B** to 50/50 and **Total flow rate** to 20 µL/min. (for NanoLC Ultra-1D+ or 2D+, use 100%A).
11. Click **Start** to flush the valve ports and PEEKsil tubing. Flush for 10 minutes.
12. Stop the flow by clicking **Stop**.

Chapter 4.
Getting Started

Chapter 4 offers a brief tutorial which should be useful in understanding the normal operation of the NanoLC Ultra® system. The procedures described in this chapter presume the system has already been properly installed and initialized as described in chapters 2 and 3. Topics in this chapter include:

- Power Up the System (Section 4.1)
- Purge and Flush with New Solvents (Section 4.2)
- Equilibrate the System(Section 4.3)
- Using the temperature-controlled compartment (Section 4.4)
- Create an Autosampler Method (Section 4.5)
- Create an LC Method (Section 4.6)
- Create the Run Table (Section 4.7)
- Start the Run (Section 4.8)
- View the Collected Data (Section 4.9)

4.1 Power Up the System

1. If the system is not already on, turn on the NanoLC Ultra system power switch (on the rear panel).

   The green LED on the front of the instrument should illuminate.

2. If the autosampler is not already on, turn on the power switch on the NanoLC AS-2 autosampler.

3. Turn on the computer, log in to Windows and launch the Eksigent control software by double-clicking the software icon.

After initialization, the Acquisition window will be displayed (Figure 4-1).

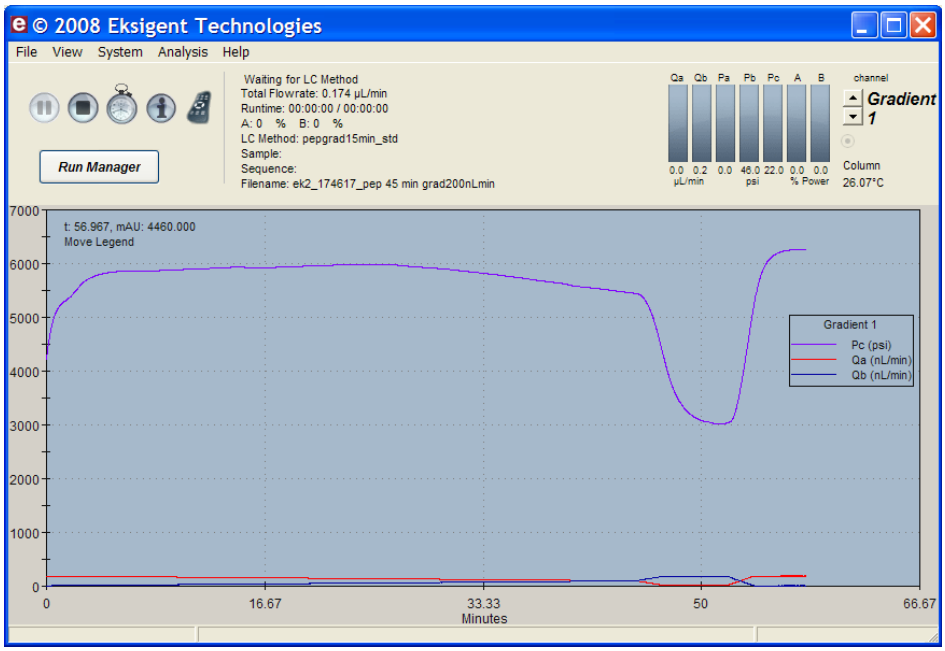


Figure 4-1 Acquisition Window

Note: On multichannel systems, The NanoLC Ultra-1D plus, 2D and 2D plus instrument have two or three channels of fluid control. They are denoted in this manual and in the Eksigent control software as gradient 1, 2, and loading pump (refer to Table 3-1 for channel assignment). Throughout the software common windows are used to display or control these channels. To select the channel appropriate to that window (when available) click the **Up** or **Down** arrows next to the channel number display in the upper right of the window.

4.2 Purge and Flush 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 Section 4.3.

To discard old solvent in the mobile phase bottles, remove the bottle and pour out the old solvent.

1. Pour new mobile phase into the bottle and insert the mobile phase transfer tubing and filters in the bottles. Purge the HPLC at least 10 times.

   Refer to Section 3.3 for a detailed procedure for purging the system.

2. After the pump is thoroughly purged, flush the system. Select **System > Mobile Phases** from the main control window.

3. Click **More** and flush the system using 200 µL for Gradient 1 and Gradient 2.

   Refer to Section 3.3 for a detailed procedure for flushing the system.

4.3 Equilibrate the System

The **Direct Control** dialog (Figure 4-2) 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.

1. Select **System > Direct Control** from the Acquisition window.

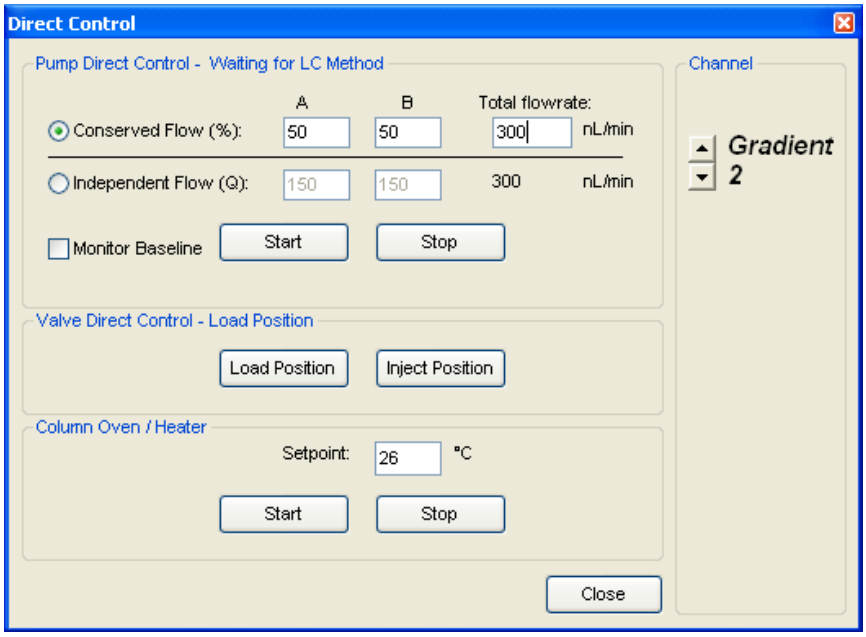


Figure 4-2 Direct Control Dialog—Equilibrating the System

2. Ensure that **Conserved Flow** is selected and set both **A** (%) and **B** (%) to 50.

   This will be the mobile phase composition used for equilibration.

3. Set the **Total flow rate** to **300 nL/min** or the appropriate flow rate.
4. Click **Start** to start the pumps and begin equilibration.
5. Flush the switching valve (or an injection valve connected directly to the NanoLC Ultra instrument) by alternately clicking the **Load Position** and **Inject Position** buttons in the **Valve Direct Control** area.
6. Select **Devices > Autosampler Device Settings** in the **Run Manager** window.

7. Click of the **Direct Control** tab, then alternately click the **Inject** and **Load** buttons in the **Injection Valve** area to switch the valve in the NanoLC AS-2 autosampler (Figure 4-3).

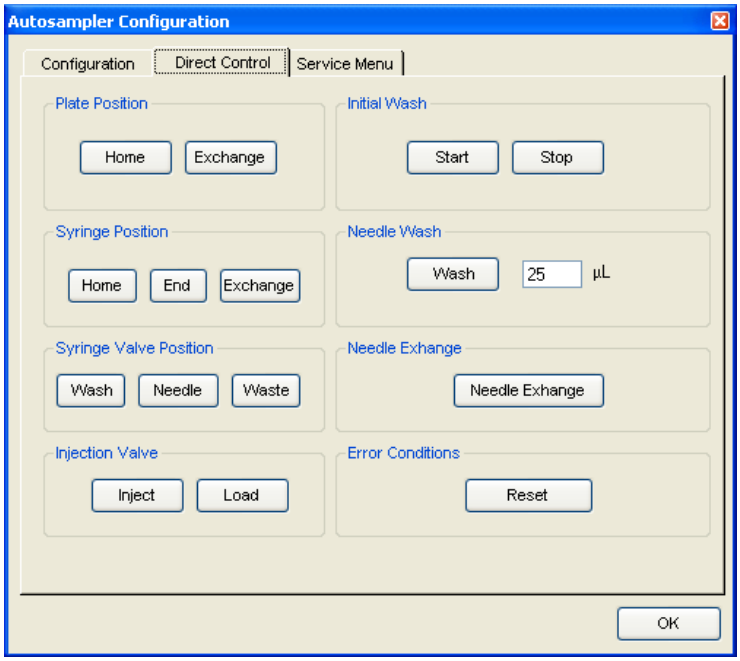


Figure 4-3 Autosampler Configuration Dialog

8. Allow the system to equilibrate for approximately 10 minutes.

4.4 Using the Temperature-Controlled Column Compartment

The 10-port column-switching valve is installed in the temperature-controlled column compartment (Figure 4-4). The column can be placed in the compartment to regulate the column temperature up to 40°C.

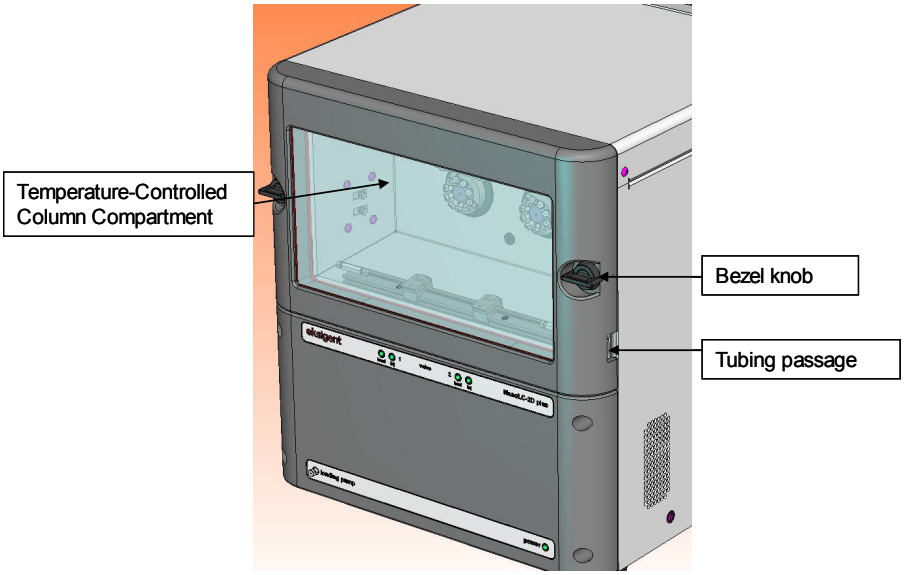


Figure 4-4 Front of the Instrument—Temperature-Controlled Column Compartment

1. Turn the bezel knobs counter clockwise to take off the top front bezel. Place the bezel on top of the instrument.
2. Make the capillary or PEEKsil connections from the NanoLC Ultra instrument to the NanoLC AS-2 autosampler and column.
3. Flip open the bracket clips and insert the column in the groove (Figure 4-5).

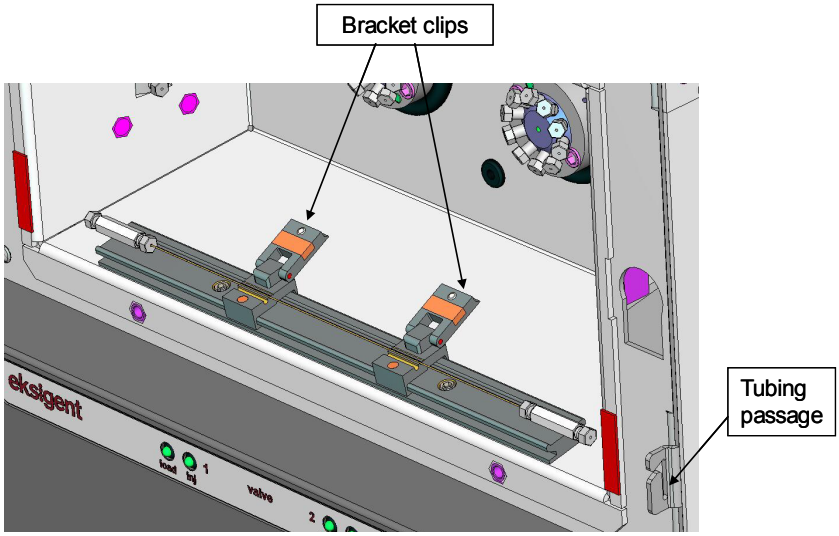


Figure 4-5 Column Mounting Bracket

4. When finished, place the clips back to their original position. Place the connecting capillaries or tubing through the tubing passage.

Note: Make sure all the connecting tubing is properly inserted in the tubing passage before closing the compartment. Failure to do so will damage or break the connecting tubing.

5. Place the bezel in front of the compartment to close the compartment. Make sure the latches are inserted properly to the chassis.
6. Turn the bezel knob clockwise to secure the top bezel to the instrument.
7. In the **Direct Control** dialog (Figure 4-6), set the **Setpoint** temperature. Click **Start**.

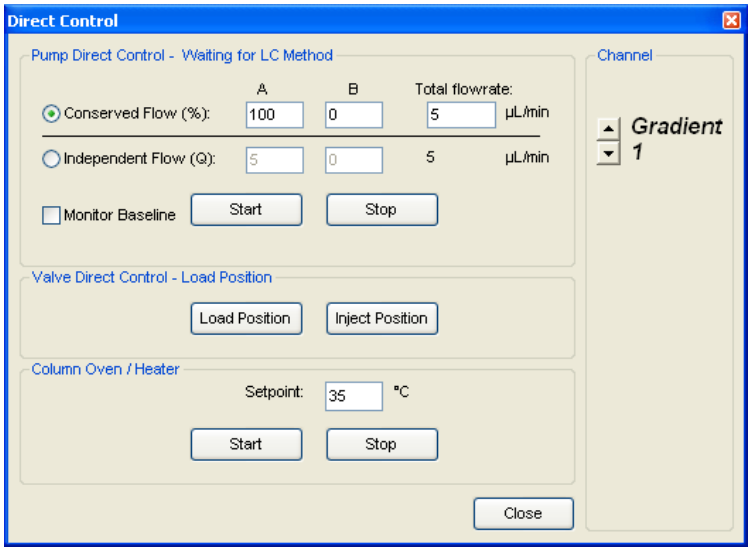


Figure 4-6 Direct Control Dialog—Setting Column Compartment Temperature

8. To disable or stop the temperature control, click **Stop**.

Note: Do not operate the oven for an extended period of time without closing the compartment.

4.5 Create an Autosampler Method

The parameters used for loading the sample into the 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 Gradient 1, and running a gradient with Gradient 2.

Note: Please read the following section carefully before proceeding to run the system with the AS-2 autosampler, especially if you have experience with the AS-1 autosampler, as there are important changes in vial assignment and the method.

1. Place the sample vial containing the standard test mixture in vial position B1 of the 48-vial tray.

   Vial positions A1 to A4 on the left tray are reserved for Reagent 1 to Reagent 4.

2. Click **Run Manager** in the **Acquisition** window to open the Run Manager window (Figure 4-7).

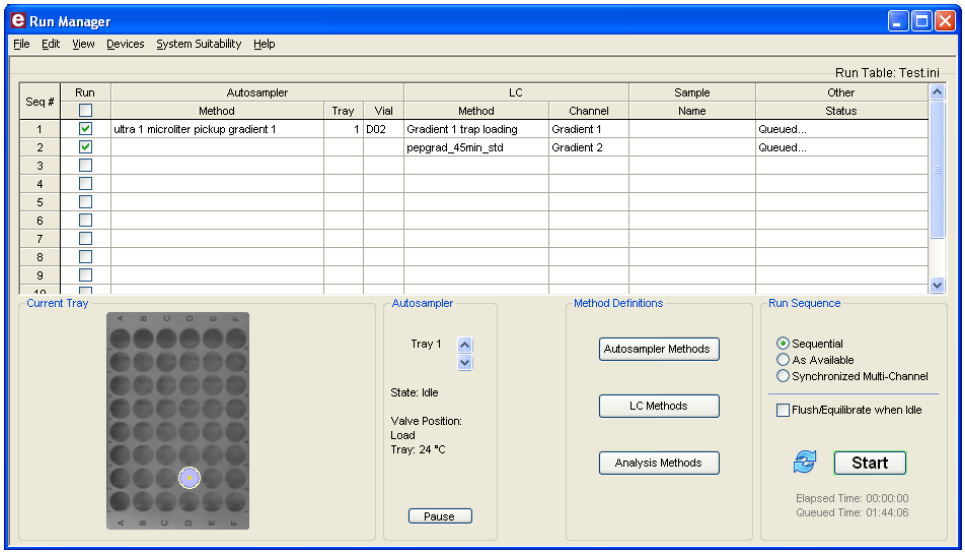


Figure 4-7 Run Manager Window

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

4. If you do not see the columns shown in Figure 4-7, select **Edit > Choose Column** and select the appropriate columns for display.

5. Click **Autosampler Methods** to display the **Autosampler Settings** dialog (Figure 4-8).

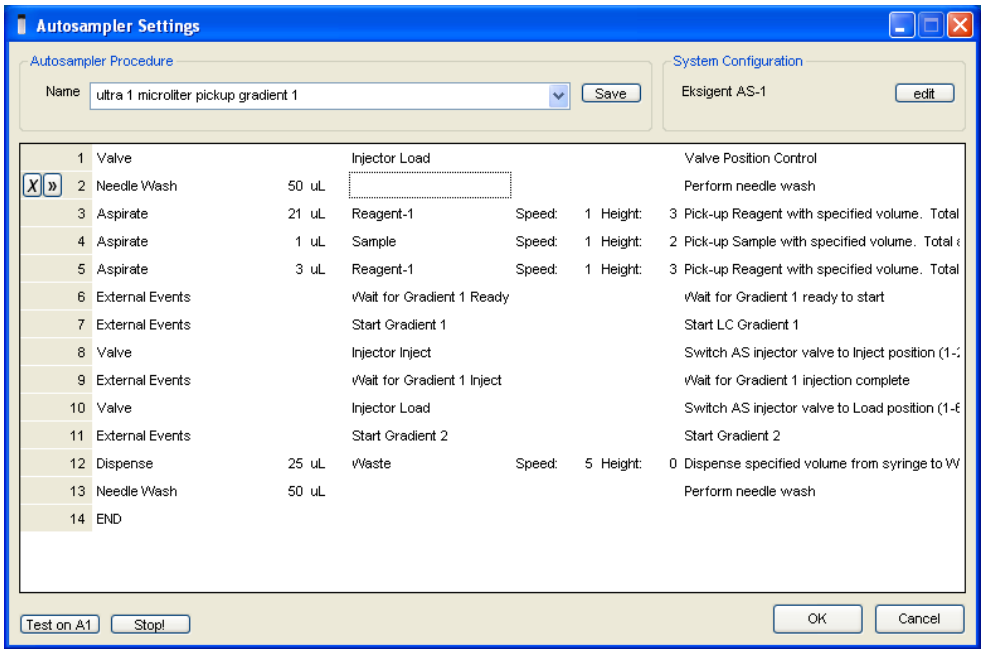


Figure 4-8 Autosampler Settings Dialog

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.

   All 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 4-1 Template for Micropickup Injection, Trap Loading Autosampler Method

Step #	Operation	Vol.	Parameter	Speed	Height	Description
1	Valve		Injector Load			Place autosampler valve in load position
2	Needle Wash	50µL				Perform 50µL needle wash
3	Aspirate	21µL	Reagent - 1	2	3	Aspirate from Reagent 1 vial position
4	Aspirate	1µL	Sample	2	(2-5)	Aspirate from Sample vial- use appropriate height for sample vial. 1= closest to bottom, 5= 5 mm from bottom of vial.

Step #	Operation	Vol.	Parameter	Speed	Height	Description
5	Aspirate	3µL	Reagent - 1	2	3	Aspirate from Reagent 1 vial position
6	External Events		Wait for Gradient 1 Ready			Wait for Gradient 1 ready to start (after equilibrating)
7	External Events		Start Gradient 1			Send signal to start loading method in Gradient 1
8	Valve		Injector Inject			Place autosampler valve in the inject position (1-2)
9	External Events		Wait for Gradient 1 Inject			Wait for the injection from Gradient 1 to be completed
10	Valve		Injector Load			Place autosampler valve in the load position (1-10)
11	External Events		Start Gradient 2			Send signal to start gradient method in Gradient 2
12	Dispense	25µL	Waste	5	0	Dispense aspirated volume to waste. Total aspirate and dispense volumes must be equal.
13	Needle Wash	50µL				Perform 50µL needle wash
14	End					Method end

7. As needed, modify method steps and parameters.
   - To modify method steps, click the step and change the volume by typing over it.
   - Modify the operation or parameter by clicking the step and choosing a new value in the drop-down menu.
8. As needed, add new steps by clicking **>>** to the left of the line.
9. As needed, delete steps by clicking the **X**.

Note: This method is for an autosampler configured with the standard 25 µL syringe. For an autosampler with a larger syringe, change steps 3 and 5 accordingly.

10. Click **Save** next to the name to store the method.
11. Click **OK** to close the Autosampler Settings dialog.

4.6 Create an LC Method—Gradient 1

The conditions used for separating the sample are stored in the LC method. LC methods are accessed for editing from the Run Manager window.

1. Click **LC Methods** in the **Run Manager** window to display the **LC Method Settings** dialog (Figure 4-9).

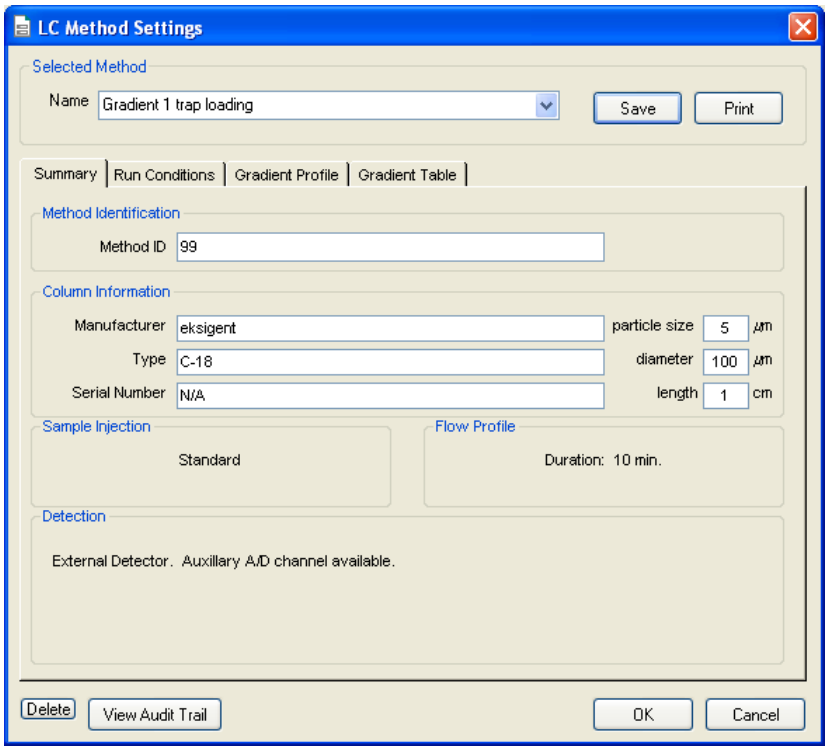


Figure 4-9 LC Method Settings Dialog—Summary Tab

2. To create a new method, type a name for the method and click **Save**.
3. Optionally, enter any column information appropriate for your experiment. This information is informational and stored with the LC method file.

4. Click the **Run Conditions** tab in the **LC Method Settings** dialog (Figure 4-10).

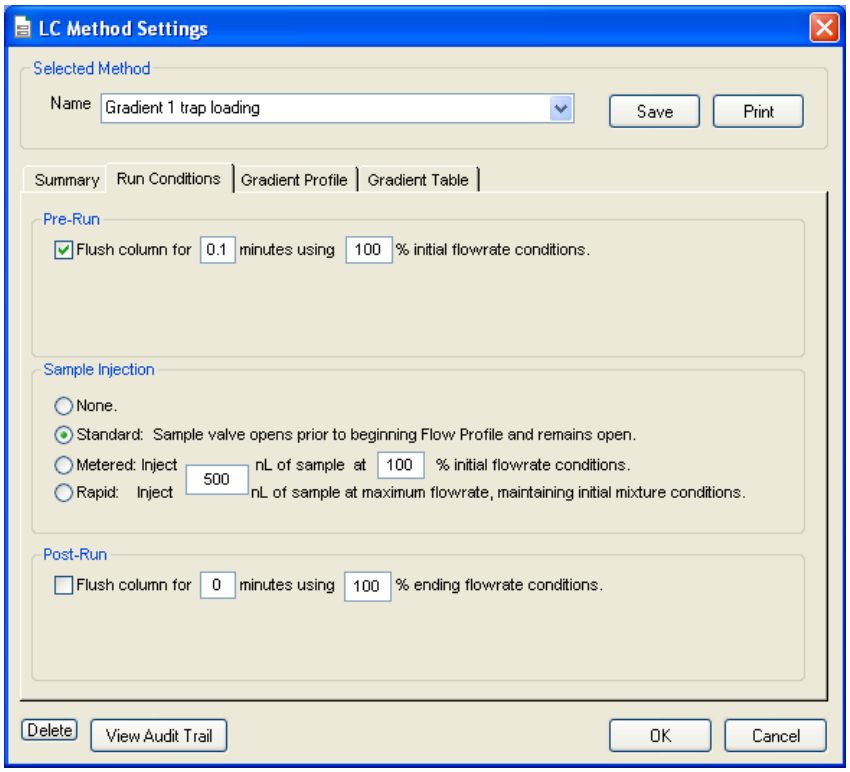


Figure 4-10 LC Method Settings Dialog—Run Conditions Tab

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% initial flow rate conditions.
6. Select **Standard:** in the **Sample Injection** region. This will signal the autosampler to place the column-switching valve in the inject position for the duration of the Gradient 1 run.
7. Leave the check box for **Post-Run Flush** column empty.
8. Click the **Gradient Table** tab to set the gradient parameters.
9. Enter the flow rate, time and percentages of A and B that are appropriate (Figure 4-11).
    - For a 2D system with the same mobile phase for Gradient 1 A and B, use 50% A and 50% B.

- For a 1D+ and 2D+ system, use 100% A.

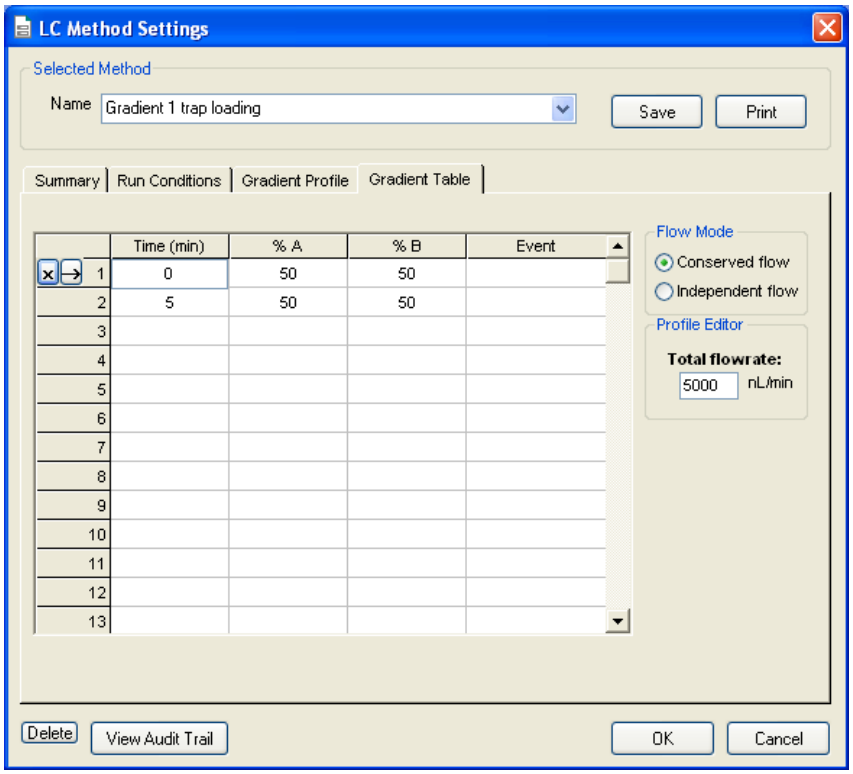


Figure 4-11 LC Method Settings Dialog—Gradient Table Tab

10. Click the **Gradient Profile** tab (Figure 4-12).

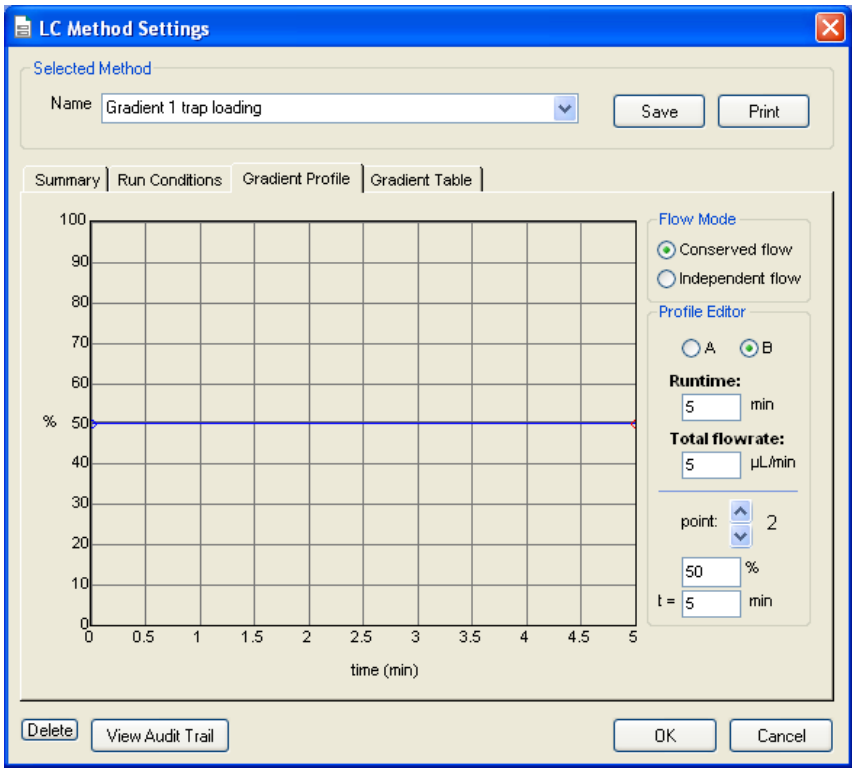


Figure 4-12 LC Method Settings Dialog—Gradient Profile Tab

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.

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

4.7 Create an LC Method—Gradient 2

1. Click **LC Methods** in the **Run Manager** window
2. To create a new method, type over the name of the method and click **Save**.
3. Optionally, enter any column information appropriate for your experiment. This information is informational and stored with the LC method file.
4. Click the **Run Conditions** tab in the **LC Method Settings** dialog to obtain the Run Conditions tab (Figure 4-13).

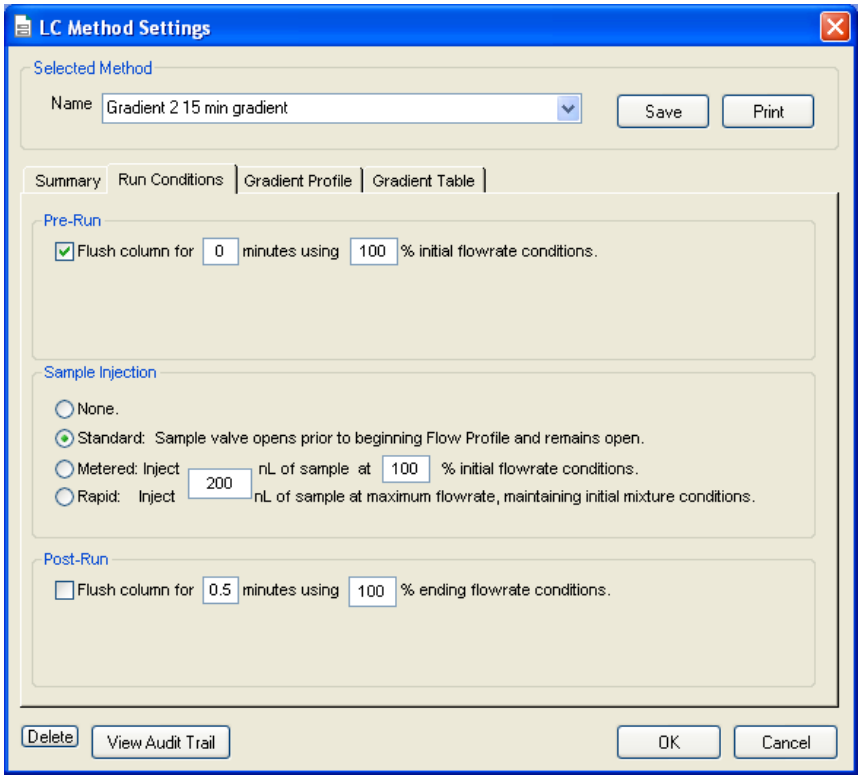


Figure 4-13 LC Method Settings Dialog—Run Conditions Tab

5. 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.
6. Select **Standard:** in the **Sample Injection** region. This will signal the 10-port valve to be placed in the inject position for the duration of the Gradient 2 run.
7. Leave the check box for **Post-Run Flush column** empty.
8. Click the **Gradient Table** tab to set the gradient parameters (Figure 4-14).
9. Enter the gradient parameters you wish to run.
   - Add new steps by clicking **>>** (on the left of the table).

- Delete steps by clicking on the **X**.
- Set the overall flow rate on the right side.

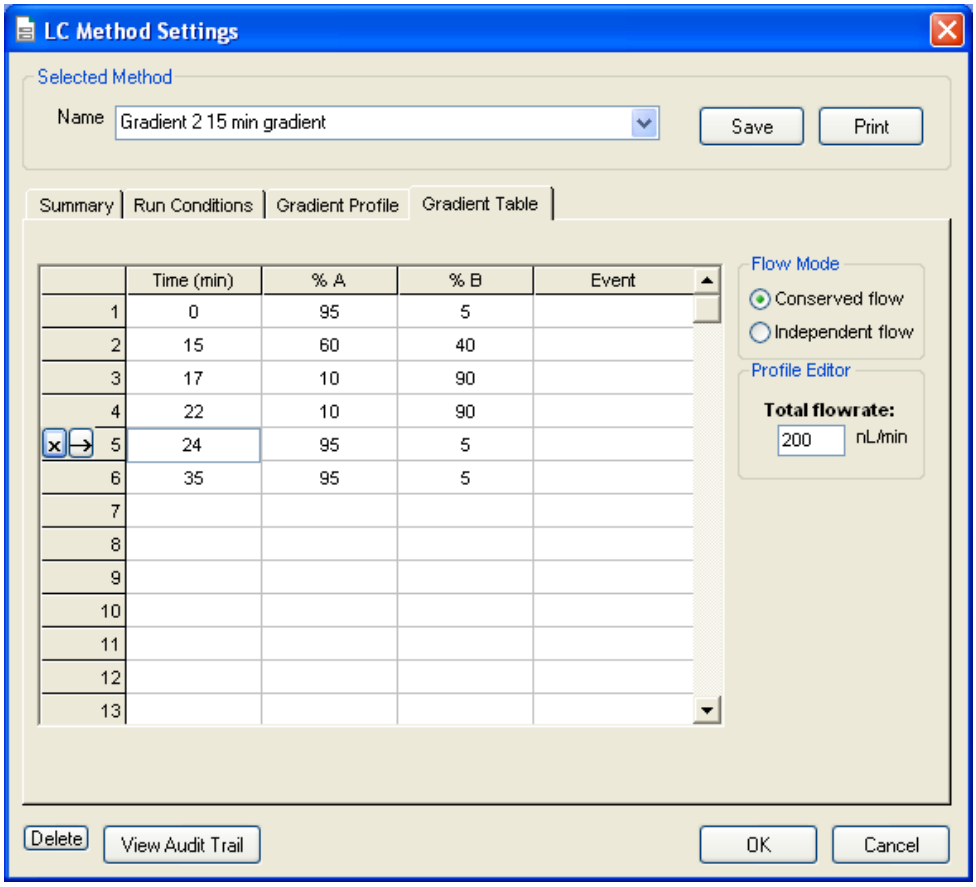


Figure 4-14 LC Method Settings Dialog—Gradient Table Tab

10. Select the Gradient Profile tab to display the Gradient Profile dialog (Figure 4-15).

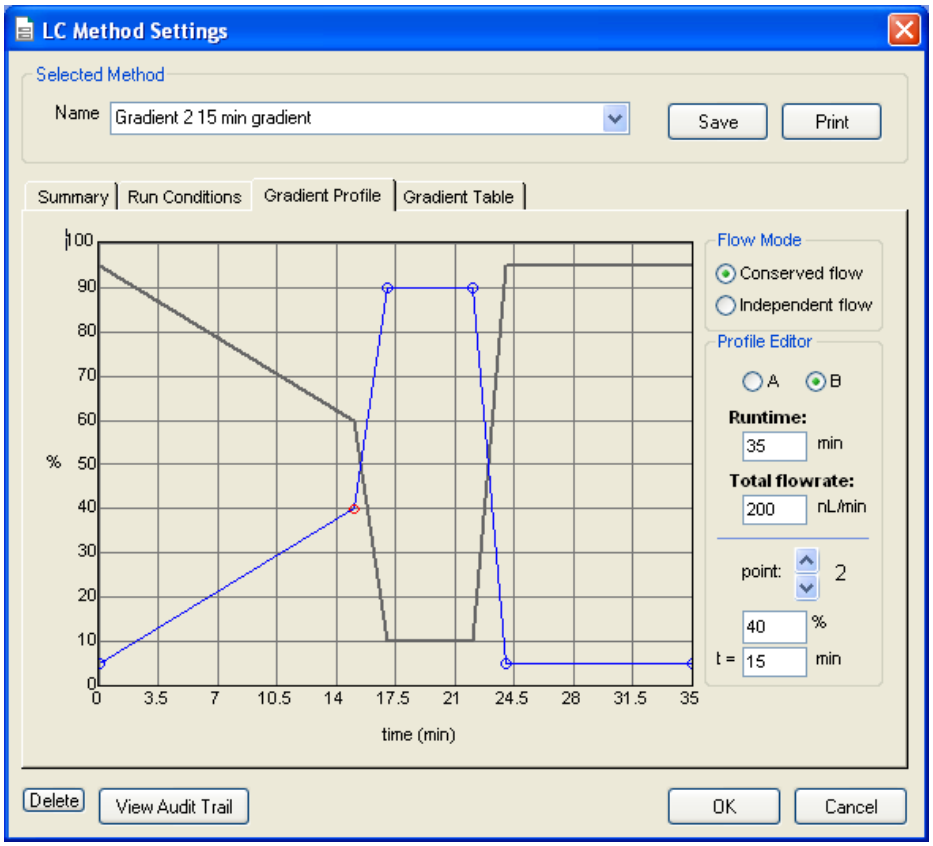


Figure 4-15 LC Method Settings Dialog—Gradient Profile Tab

11. Use the Gradient 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.

12. Once the method is complete, click **Save** to save the analysis method. Click **OK** to close the dialog.

4.8 Create the Run Table

The run table ties together an autosampler and one or more LC methods 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.

1. If not already in the Run Manager window, click **Run Manager** (in the Eksigent control software's **Acquisition** window**)**. (Figure 4-1).
2. Create a new, blank Run Table by selecting **Edit > Erase Table** (Figure 4-16).

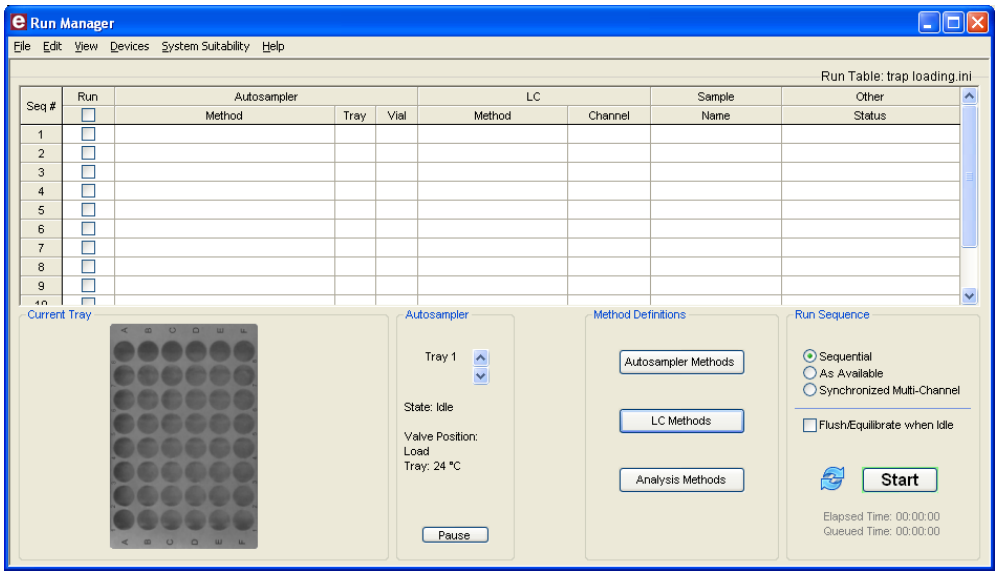


Figure 4-16 Creating a New Run Table

3. Select **File > Save As** and type **Trap Loading** in the **File name** field.
4. Click **Save**.
5. In the first line of the Run Table, double-click the **Autosampler Method** field and select the autosampler method **Ultra 1 microliter pickup Gradient 1**.
6. Enter **1** for the **Tray** and **D01** for the **Vial** location.

   Alternatively, enter the vial location by clicking the location of the vial on the picture of the vial tray in the **Run Manager** window.
7. Double-click **LC Method** and select **Gradient 1 Trap Loading**. Indicate **Gradient 1** in the **Channel** column.
8. In line 2, leave the autosampler method, tray and vial location blank. Double-click the **LC Method** field and select **Gradient 2 15 min gradient**. Indicate **Gradient 2** in the **Channel** column.
9. For each sample you wish to run, duplicate these two lines in succession.

In Figure 4-17, two sample injections are indicated.

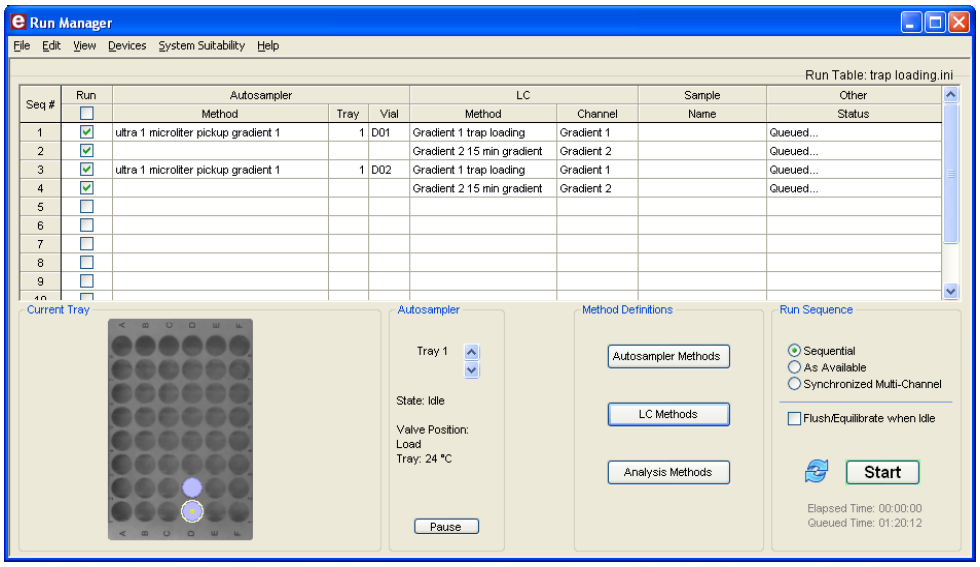


Figure 4-17 Run Manager Window—Two Samples

10. Check all the lines you wish to run in the **Run** column.
11. Run the sequence in **Sequential** mode.
12. Optionally, click **Flush/Equilibrate when Idle** to start all the pumps if you wish.

    If you need to edit the first two lines, un-check **Flush/Equilibrate**.
13. Now that the run table has been defined (Figure 4-16), save it by selecting **File > Save**.

4.9 Start the 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.

Select **Flush/Equilibrate when Idle** to initiate the pre-run flush for the first method. With this option 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. For the Trap loading run table created in Section 4.8, Gradient 1 will run first, then Gradient 2 will start at the conclusion of the Gradient 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 4-18) and selecting the desired items.

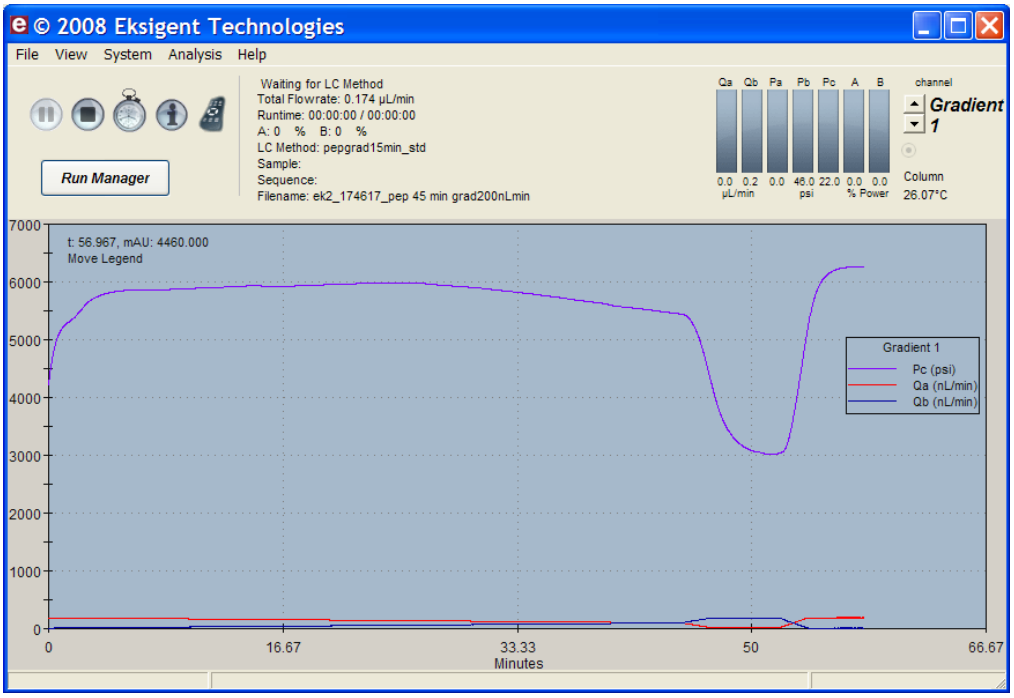


Figure 4-18 Acquisition window—flow profiles

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

Status information such as %A, %B and Time Remaining are also displayed at the bottom of the window 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).

4.10 View the Collected Data

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

To view the data file collected from the first chromatogram, click **File > Open** and then select the data file.

The NanoLC Ultra has an option to collect an external signal (such as a UV detector) through the A/D input on the 8-pin I/O connector. This data will be stored, along with the flow profile data, in the data file.

The Eksigent control software also includes a data analysis package and this analysis can be applied to the collected A/D signal. Refer to the *Eksigent Control Software User Guide* for instructions on analyzing data files with the software.

Chapter 5.
Routine Maintenance

Chapter 5 describes the general procedures used to properly maintain the NanoLC® Ultra system. Topics included in this chapter include:

- Recommended Maintenance (Section 5.1)
- Dispose of Waste (Section 5.2)
- Zero the Pressure Transducers (Section 5.3)
- Autotune Flow Controllers (Section 5.4)
- Calibrate the Flow Meters (Section 5.5)
- Check Flow Stability (Section 5.6)
- Clean and Inspect the Instrument (Section 5.7)

Note: For NanoLC AS-2 autosampler maintenance, refer to Appendix D.

5.1 Recommended Maintenance

The NanoLC Ultra 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 5-1 Recommended Maintenance

Task	Frequency	Refer To
Changing the Mobile Phase	As needed	Section 3.2
Zeroing pressure transducers	Monthly	Section 5.3
Checking flow stability	Quarterly	Section 5.6
Autotuning flow controllers	As needed	Section 5.4
Waste disposal	As needed	Section 5.2
Calibrating flow meters	Quarterly	Section 5.5
Cleaning and inspecting system	Quarterly	Section 5.7
Changing internal instrument filters	Annually	Contact AB SCIEX

5.2 Disposing of Waste

The user must properly dispose of the contents of any effluent waste in an appropriate chemical waste container. For typical 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 NanoLC AS-2 autosampler 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.

WARNING! Biohazard: Follow appropriate safety procedures and local requirements when handling or disposing of waste chemicals. See the solvent manufacturer's Material Safety Data Sheets (MSDS) for more information.

5.3 Zero 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.

Note: 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.

Note: For an autosampler configured with the standard 25 µL syringe. For an autosampler with a larger syringe, change steps 4 and 6 accordingly.

1. Select **System > Hardware Diagnostics** to initiate the pressure transducer zeroing procedure to display the Hardware Diagnostics dialog (Figure 5-1).

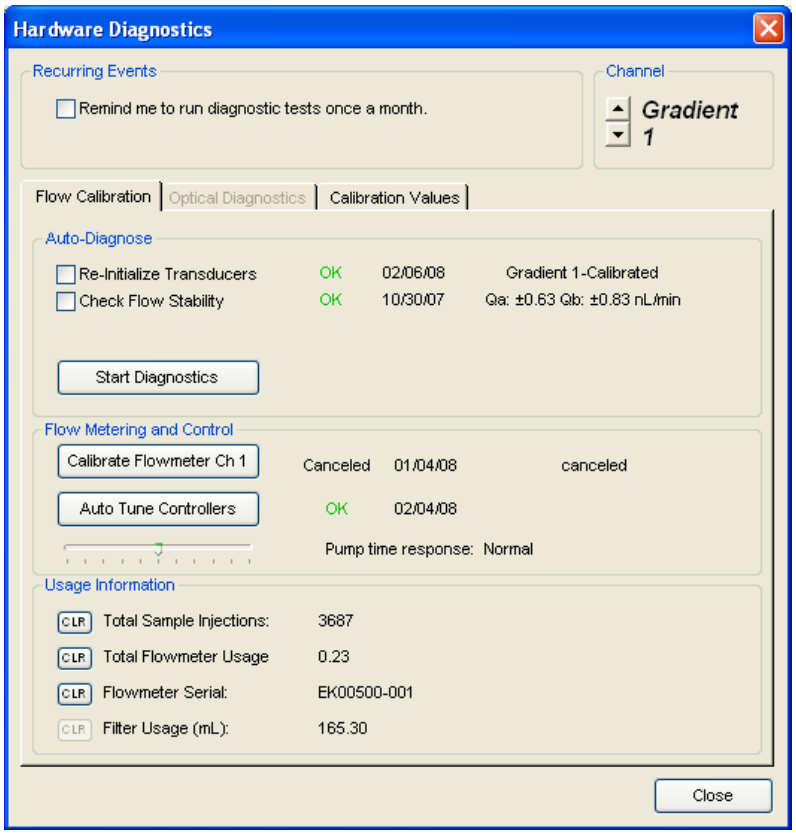


Figure 5-1 Hardware Diagnostics Dialog—Re-initialize Transducers

2. Stop the system flow.
3. Loosen the outlet fitting of the mixing tee to release all the residue pressure.
4. In **Auto-diagnose**, check **Re-Initialize Transducers**.
5. Click **Start Diagnostics**.

   A dialog will appear (Figure 5-2) warning that the procedure should only be performed if there is no residual pressure on the system.

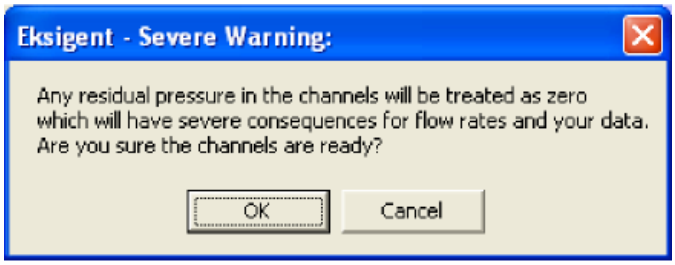


Figure 5-2 Residual Pressure Warning

6. Once the system indicates that it is at ambient pressure, click **OK** and a status window will indicate that auto-zeroing is in progress (Figure 5-3).

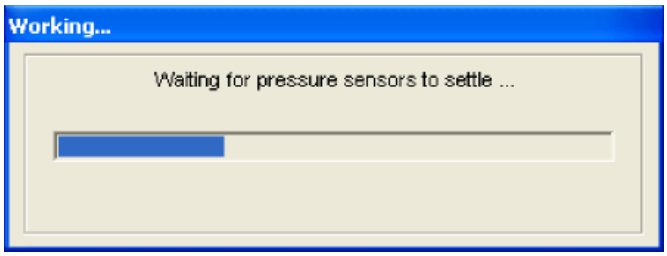


Figure 5-3 Auto-zero Status Window

7. When the auto-zeroing process is complete, exit the Hardware Diagnostics dialog and return to the **Acquisition** window.
8. Repeat for all channels (if not conducted simultaneously).

5.4 Autotune Flow Controllers

The flow controller autotune should be run when needed to optimize the performance of the system.

WARNING! Potential Instrument Damage: Damage can result from overpressure. Disconnect the capillary/PEEKsil tubing at the mixing tee prior to performing the auto tune procedure.

1. When the **Autotune** button is selected in the Hardware Diagnostics dialog, the system's response to changes in flow rate is monitored and adjustments are made to the controllers PID (proportional/integral/ derivative) loop.
2. A dialog warns the user to disconnect any devices which could be damaged by high pressure (Figure 5-4).

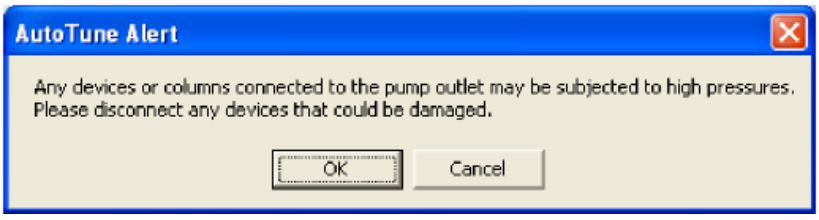


Figure 5-4 Autotune Alert Dialog

The dialog is followed by a status window (Figure 5-5) that alert you that the system is preparing to Auto-tune and then another window indicating that autotuning is occurring.

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

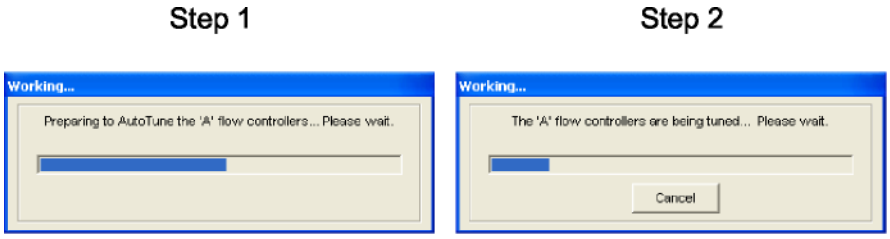


Figure 5-5 Autotune Status Windows—Pump A

3. Upon completion, close the Hardware Diagnostics dialog and return to the main screen.
4. Repeat for all 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).

5.5 Calibrate the Flow Meters

Calibration of the flow meters consists of measuring the velocity of a liquid front in a tube of known diameter. Selecting "Calibrate Flow meters test" will display a dialog with step-by-step instructions for performing the test.

Required Materials
• Flow calibration assembly (PN 801-00063) for high flow rate channel (includes 20 µL pipettes) • Flow calibration assembly (PN 801-00064) for low flow rate channel (includes 5 µL graduated pipettes)

Note: Make sure the system has been purged, flushed, and the pressure transducers are zeroed before proceeding to flowrate calibration. Failure to do so will result poor performance of the system. Refer to Sections 3.3 and 5.3.

Note: If calibrating the low flow channel using the 801-00063 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.

1. Select **System > Hardware Diagnostics** from the Eksigent control software **Acquisition** window.
2. Select **Calibrate Flowmeter** to display a dialog with step-by-step instructions for performing the test (Figure 5-6).

Figure 5-6 Flowmeter Calibration Dialog

3. Verify that mobile phases specified are correct.

   If incorrect, click **Cancel** to close the Flowmeter Calibration dialog. Make the necessary changes in the **Mobile Phases** window, repeat step 1 and then click **Next**.

4. 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 (refer to Table 5-2).

   - Use 20 µL/division in the pipette size to set the calibration flow rate to 5 µL/min for the high flow channel.
   - Use1 µL/division in the pipette size to set the calibration flow rate to 500 nL/min for the low flow channel.

5. Click **Next** to start the flow in pump A.
6. Enter the appropriate volume requested in step 3 of the **Flow Calibration Window** dialog (Figure 5-6). Wait until the liquid front travels to the black line mark on the pipette and press **Start** to begin timing.
   - For the high flow channel, time how long it takes for the liquid front to travel 20 µL.
   - For the low flow channel, time how long it takes for the liquid front to travel 2 µL.
7. Press **Stop** when the fluid front reaches the end of the pipette (or the appropriate mark) and then click **Next**. If necessary, disconnect the calibration assembly and dry out the liquid inside the capillary.
8. Repeat the measurement to calibrate the pump B flow meter.
9. Verify that the instructions were followed exactly and that all values entered are correct and then click **Finish**.

Use Table 5-2 to choose the proper calibration pipettes when calibrating the high flow and low flow channels of the NanoLC Ultra system.

Table 5-2 Calibration Pipette Guide

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

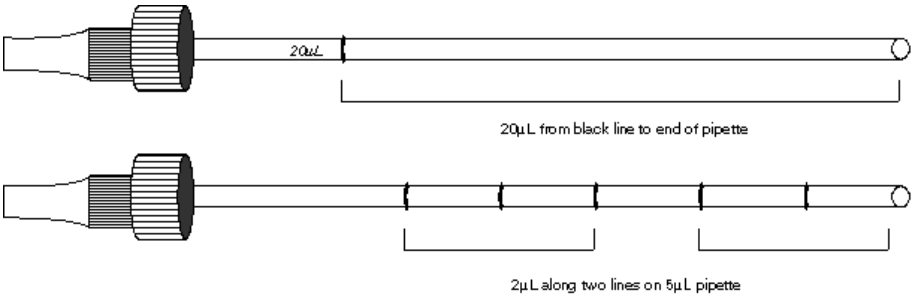


Figure 5-7 Flow calibration pipettes

5.6 Check Flow Stability

The flow stability of the A and B pumps can be determined in a similar fashion to the procedure used for zeroing pressure transducers (as described in Section 5.3) by selecting the **Check Flow Stability** diagnostic test. Two screens will be observed during the running of the test (Figure 5-8). 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

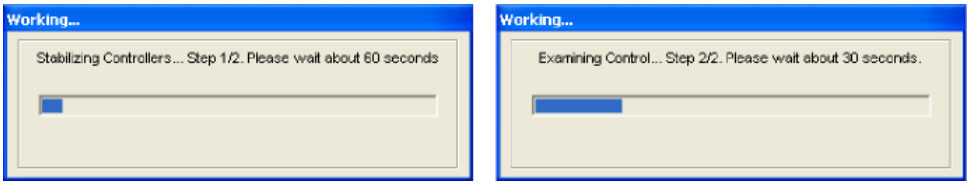


Figure 5-8 Checking flow stability progression

Repeat this for other channels.

5.7 Clean and Inspect the Instrument

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

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

- Overview of hardware diagnostics (Section 6.1)
- Calibration values (Section 6.2)
- General troubleshooting guidelines (Section 6.3)
- Troubleshooting checklist (Section 6.4)
- Error messages and system alerts (Section 6.5)

6.1 Overview of Hardware Diagnostics

The NanoLC Ultra system includes a number of diagnostic capabilities designed to maintain peak system performance. The status bars and the text displayed in the Eksigent 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 Sections 5.3 through 5.5 for details on running these diagnostics.

6.2 Calibration Values

The Calibration Values tab of the Hardware Diagnostics dialog 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 values should be documented before and after as part of maintaining a good instrument log and changes should be noted. These values should be very similar from diagnostic to diagnostic; large changes can indicate a problem.

6.3 General Troubleshooting Guidelines

When troubleshooting the NanoLC Ultra system, follow these safety practices:

WARNING! Potential Instrument Damage: To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the system. Once the power is turned off, wait approximately 30 seconds before disconnecting an assembly.

WARNING! Biohazard: When replacing tubing or fittings on the NanoLC Ultra system or the NanoLC AS-2 autosampler, 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.

WARNING! Potential Instrument Damage: There are no user serviceable components or assemblies inside the NanoLC Ultra system or the NanoLC AS-2 autosampler. Service of any internal parts or assemblies requires an AB SCIEX-trained Field Service Employee (FSE).

Caution: Potential Operator Injury: 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 you are using (refer to the Material Safety Data Sheets for the solvents in use).

The basic steps for NanoLC Ultra system troubleshooting are:

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

4. For each isolated symptom, identify a list of possible causes using the troubleshooting checklist below.

   The troubleshooting in Section 6.4 allows you to narrow down the possible causes of a symptom and find suggested corrective actions.

5. If this process does not correct the problem, contact AB SCIEX 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 supply is not turn on	Turn on the power supply.
	No power at outlet	Repair electrical outlet.
	Power LED has failed but system response OK	Contact AB SCIEX 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 USB cable is securely connected to the computer's USB port (refer to Section 2.7). Reboot computer and cycle power on instrument. Verify that the number assigned to the USB port is not greater than 16. If necessary, re-assign the USB port number in Control Panel. Contact AB SCIEX Technical Support for assistance.
Loud hissing sound from the instrument	Gas leaks from the gas inlet fitting	Verify the tubing is connected properly to the gas fitting. Tighten the gas inlet fitting. Contact AB SCIEX Technical Support for assistance.
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 (refer to Section 5.3).
No liquid out of waste line when purging	Air trap in the pump Internal filters are plugged	Prime and purge the pump (refer to Section 3.3).
	Leak in the system prior to the purge valve	Contact AB SCIEX Technical Support for assistance. Check all connections.

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Flow Control System (continued)		
Pump restrokes frequently-**Pump has reached end of stroke** error message occurs	Air trapped in the pump	Prime and purge the pump (refer to Section 3.3).
	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 AB SCIEX Technical Support for assistance.
Pump does not restroke at the end of a run	Pump restroke delay is too short	Contact AB SCIEX Technical Support for assistance.
	Optical sensor is not working properly	Contact AB SCIEX Technical Support for assistance.
Pump flushes out quickly but does not deliver ~600 µL per stroke.	Leak in instrument	Contact AB SCIEX Technical Support for assistance.
Purge output drips out slowly	Internal filters are plugged	Contact AB SCIEX Technical Support for assistance.
Inability to reach desired flow rate	Internal filters are plugged	Contact local AB SCIEX Technical Support to replace internal filters.
	Flow rate setpoint too high for system back pressure	Reset flow rate to a lower level.
	Air pressure too low	Establish correct pressure (100 psi).
No flow rate with 100% power indicated. System pressure (**Pc**) and pump pressures (**Pa** & **Pb**) are all low	No gas to system	Connect 100 psi clean, dry gas to the instrument's gas inlet.
	System not properly primed and flushed	Prime and flush system (refer to Section 3.3).

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Flow Control System (continued)		
System responds sluggishly when changing flow rates	Incorrect mobile phase setting in software	Check the Mobile Phases setting (refer to Section 3.3).
	Pump controller is out of tune	Autotune flow controllers (refer to Section 5.4)
Flow rate will not initialize at start of run	Flow rate setpoint 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 (refer to Section 3.3).
	Unable to meet required flow rate within specified tolerance	Lower flow stabilization limit in the Instrument Configuration dialog (refer to Section 3.3). Run the flow stability diagnostic to verify flow control (refer to Section 5.6). Autotune flow controllers (refer to Section 5.4).
	One or both of the Internal solvent filters may be plugged	Flush system if flow is unstable (refer to Section 3.3). If flow is still unstable, calibrate the flow meters (refer to Section 5.5). Contact AB SCIEX Technical Support for assistance.

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Flow Control System (continued)		
Flow rate will not stabilize during a run	Erratic flow rate due to bubbles in system	Prime and flush system (refer to Section 3.3).
	Incorrect mobile phase setting	Check the mobile phases setting (refer to Section 3.3).
	Pump controller is out of tune	Autotune flow controllers (refer to Section 5.4).
	Flow temperature is not stable	Contact AB SCIEX Technical Support for assistance.
Inaccurate flow rate with no signs of leakage	Incorrect mobile phase setting in software	Check the Mobile Phases setting (refer to Section 3.3).
	Incorrect k-values	Perform flow meter calibration (refer to Section 5.5).
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 (refer to Section 5.5).
	Flow module is plugged	Contact AB SCIEX Technical Support for assistance.
Excess flow noise	Trapped gas in the pump	Prime the pump (refer to Section 3.3).
	Pump controller is out of tune	Autotune flow controllers (refer to Section 5.4).

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Flow Control System (continued)		
Measured flow does not follow the flow profile	Pump controller is out of tune	Autotune flow controllers (refer to Section 5.4).
	Pump time response is set incorrectly	Adjust the pump time response in Hardware Diagnostics (refer to Section 4.7).
Pa and **Pb** maximized to < 12000 psi with 100% pump power.	Incorrect gain setting for pressure	Check the setting in the Calibration Values tab in Hardware Diagnostic menu (2800 for 14 000 psi transducers, refer to Section 6.3).
	Incorrect zero setting for pressure sensors	Check the setting in Service menu (should be 500, refer to Section 6.3).
	Inline gas pressure too low	Check the gas pressure (~100 psi).
Temperature-Controlled Column Compartment		
No air flowing out from the air deflector	Fan is not working.	Contact AB SCIEX Technical Support for assistance.
Compartment responds very slowly when changing temperature	Fan is not working. Thermistor is not secured at the right location Heater's PID parameters were incorrectly set Heater is not functioning properly	Contact AB SCIEX Technical Support for assistance.
Temperature does not rise at all but the fan is running	Heater does not function	Contact AB SCIEX Technical Support for assistance.
Faulty temperature reading	Thermistor is not secured at the right location Thermistor is not connected properly	Contact AB SCIEX Technical Support for assistance.

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Autosampler		
Software does not recognize NanoLC AS-2 autosampler when Run Manager is started	Communication error between computer and NanoLC AS-2 autosampler	Verify that the RS-232 cable is securely connected to the Communications port autosampler (refer to Section 3.4).
	Software may be configured to use a different COM port	Verify that the software is configured for autosampler to connect to the correct COM port (refer to Section 2.8).
The NanoLC AS-2 autosampler does not trigger instrument to start a run	Autosampler method does not trigger the correct channel	Verify the autosampler method. Make sure the correct LC channel is selected in the method. If the autosampler method is created correctly and the autosampler still does not trigger the LC to start a run, contact AB SCIEX Technical Support for assistance.
Software does not trigger the NanoLC AS-2 autosampler to switch valve position	Autosampler method does not include triggering the valve	Verify the autosampler method.

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Column-switching valve		
Valve does not switch positions	Valve is not configured in the Eksigent control software Valve is not connected to the actuator Valve is connected to the wrong channel	In the Instrument Configuration dialog, set valve as internal (refer to Section 2.8).
	Faulty actuator	Contact AB SCIEX Technical Support for assistance.
No flow coming out from the port	The valve is plumbed incorrectly	Verify the plumbing configuration and reconnect if needed (refer to Appendix C).
	The ports are plugged	Manually flush each port with cleaning solvent using a syringe. If flushing does not clean the port, contact AB SCIEX to replace the valve.
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 AB SCIEX 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 AB SCIEX Technical Support for assistance.

Table 6-1 Troubleshooting Checklist (Continued)

Symptom	Possible Cause	Corrective Action
Inconsistent flow rate	Internal leakage in the valve	Contact AB SCIEX 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 AB SCIEX FSE 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 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 (refer to Section 3.3).
	Flow stabilization is set too low	Set the Flow Stabilization Limit in the Instrument Configuration dialog to > 100 nL/ min (refer to Section 2.8).
	Autosampler is configured with wait for injection but the LC method is used with no injection	Change the LC method to with injection or change the autosampler method without wait for injection command (refer to 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 6-1) are reminders of an action that needs to be taken such as to run the diagnostic tests or refill the reagent storage loops. Clicking **Clear Alerts** will erase all alerts.

Note: Clicking **Clear Alerts** does not mean that the recommended alert action has been completed.

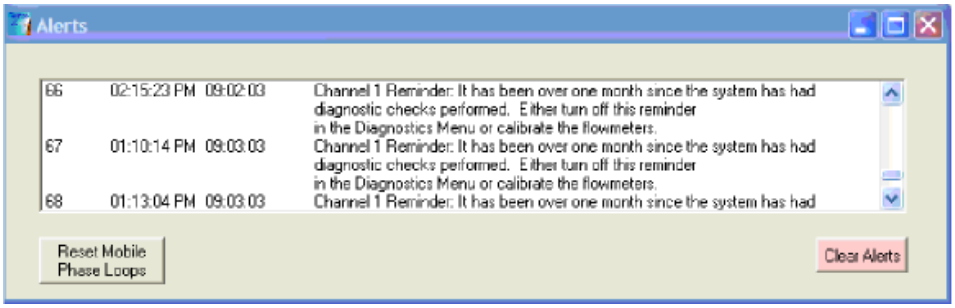


Figure 6-1 System Alerts Dialog

Appendix A
Spare Parts and Consumables

Table A-1 Consumables—NanoLC Ultra Systems

Part Number	Description
200-00301	1/32 inch SS union with short nuts and ferules
200-00319	1/32 inch microtight union (includes fittings)
200-00329	Mobile phase filter
205-00048	20 mm ID /1/32 inch OD x 75 cm PEEKsil tubing (black)
205-00049	50 mm ID /1/32 inch OD x 75 cm PEEKsil tubing (natural)
205-00052	20 mm ID /1/32 inch OD x 15 cm PEEKsil tubing (black)
801-00020	Replacement calibration pipettes, 20 μL, for 1-30 μL/min, 10/pkg
801-00063	Flow rate calibration kit with 20 μL calibrated pipettes (10) for 1-30 μL/min flow rate
801-00064	Flow rate calibration kit with 5 μL calibration pipettes (5) for 50-1000 nL/min flow rate
801-00065	Accessories kit for NanoLC Ultra systems. Includes mobile phase bottles, calibration kits, fittings, capillary tubing and cables.
910-00007	Replacement calibration pipettes, 5 μL, for 50-1000 nL/min, 10/pkg
910-00085	1/32 inch nut (long) 10/pkg
910-00086	1/32 inch nut (short) 10/pkg
910-00087	1/32 inch ferrule 10/pkg
910-00088	1/32 inch sleeve for 365 mm OD fused silica capillary 10/pkg
910-00089	1/32 inch microtight fitting 10/pkg
910-00090	1/8 inch Super flangeless nut 10/pkg
910-00091	1/8 inch Super flangeless ferrule 10/pkg

Table A-2 Replacement Parts—NanoLC Ultra Systems

Part Number	Description
100-00041	Fitting, air supply, 1/4 inch compression
200-00024	0.02 inch ID/1/16 inch OD x 8 inch SS tubing
200-00075	0.02 inch ID/1/16 inch OD x 4 inch SS tubing
200-00076	0.02 inch ID/1/16 inch OD x 5 1/4 inch SS tubing
200-00302	1/16 inch to 1/32 inch reducing adapter SS
200-00303	1/16 inch to 1/32 inch reducing union SS
200-00316	1/32 inch mixing Tee
200-00332	Rotor for 10 000 psi 10-port valve (1/32 inch ports)
200-00333	10 000 psi 10-port valve (1/32 inch ports) (does not include actuator)
205-00038	50 mm ID /1/32 inch OD x 15 cm PEEKsil tubing (natural)
205-00039	50 mm ID /1/32 inch OD x 20 cm PEEKsil tubing (natural)
205-00040	50 mm ID /1/32 inch OD x 30 cm PEEKsil tubing (natural)
205-00041	50 mm ID /1/32 inch OD x 50 cm PEEKsil tubing (natural)
205-00042	100 mm ID /1/32 inch OD x 75 cm PEEKsil tubing (red)
205-00043	100 mm ID /1/32 inch OD x 20 cm PEEKsil tubing (red)
205-00044	100 mm ID /1/32 inch OD x 30 cm PEEKsil tubing (red)
300-00000	Solvent waste/seal wash bottle, 250 mL
300-00007	Internal air line, black, polyethylene, 18 inch
300-00057	Solvent bottle 250 mL
300-00058	Solvent bottle 100 mL
400-00424	Pressure transducer Pa/Pb (14,000 psi)
400-00448	Pressure transducer Pc (14,000 psi)
400-00463	Connector, 10-pin electrical, I/O, green
400-00464	Connector, 8-pin electrical, I/O, green
800-00204	Purge valve
800-00205	Solenoid manifold gradient pump
800-00268	Pump sled gradient complete gradient 1
800-00269	Pump sled loading pump complete
800-00289	Solenoid manifold loading pump
800-00290	Flow module loading pump 1-30 mL/min

Part Number	Description
800-00294	Flow module gradient 50-500 nL/min
800-00310	EP controller
800-00323	Pump sled gradient complete gradient 2
800-00350	Flow module gradient 1-10 mL/min
800-00360	High pressure filter
801-00066	Check valve rebuild kit
910-00001	Line, air supply, 25 feet, 1/4 inch

Table A-3 Replacement Parts—NanoLC AS-2 Autosampler

Part Number	Description
5016577	Sample needle union, black
200-00334	Rotor seal for 1/32 inch 6-port injection valve (10 000 psi)
200-00335	1/32 inch 6-port injection valve (10 000 psi)
200-00072	Sample needle union, tan
620-00064	Syringe 25 µL
620-00147	Air needle
620-00148	Sample needle 2.4 mL
620-00149	Buffer tubing kit
620-00150	Syringe valve
620-00151	Wash bottle 250 mL
620-00152	Vial rack 12 vials
620-00153	Vial rack 48 vials
910-00081	PEEKsil sample loop, 1 µL
910-00082	PEEKsil sample loop, 10 µL
910-00083	PEEKsil sample loop, 20 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 connectors are located on the rear panel of the NanoLC® Ultra system.

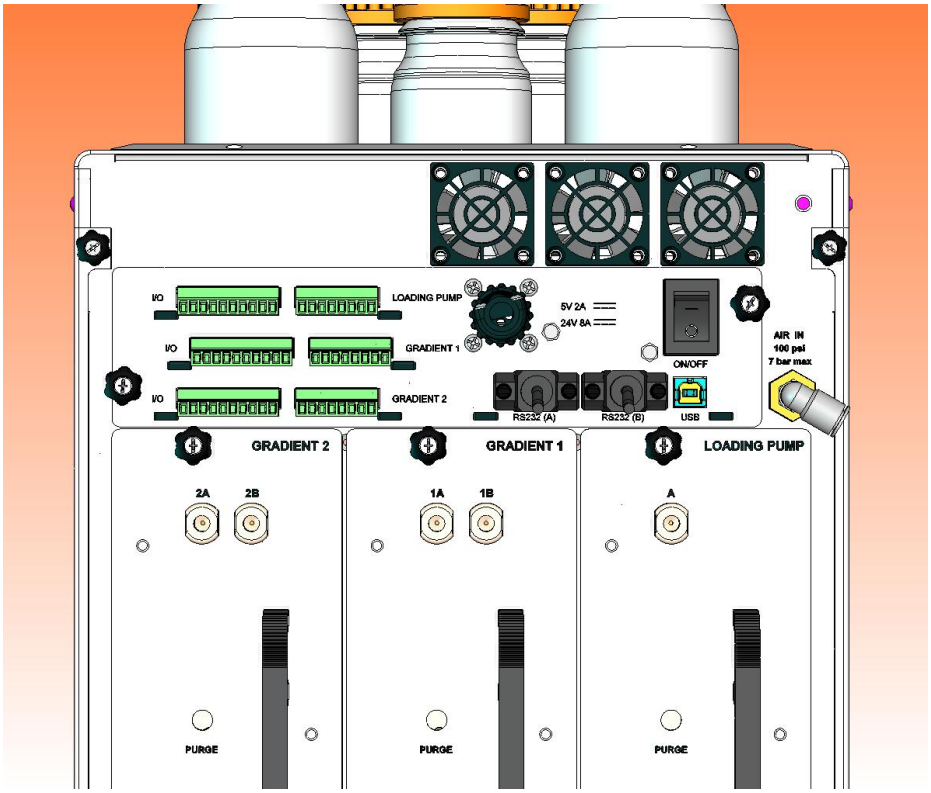


Figure B-1 Rear Panel—External I/O Interface

B.2 Remote Interface

An enlarged drawing of the External Interface Connectors (Figure B-2) shows the 10 lines that are available for the output and the 8 lines are available for the input. The active state for the output line (open or close) and input line (TTL high or TTL low) are defined in the Instrument Configuration dialog (Section 2.8).

The output lines are contact closures that share a common line. The CMN is available on pins O1 and O10. When connecting more than one external device, make sure they can share the CMN line.

Table B-1 Output Connections

Line	Name	Description
O1	Out CMN	The Out CMN contact is a common for the output interface.
O2	Not used	For future expansion.
O3	Not used	For future expansion.
O4	Not used	For future expansion.
O5	Not used	For future expansion.
O6	VLV out	The VLV out contact closes when the valve moves from the Load position to the Inject position. Its active state is NOT software configurable.
O7	Run out	The RUN out contact is used to trigger an external device at the time the gradient is started. Its active state is configurable from within the Eksigent control software. The logic state changes at the beginning of the gradient (end of injection when using metered injection mode) and stays in on-state during gradient.
O8	PRK out	The PRK out contact changes state when the peak parking function is actuated. Its polarity is configurable from within the Eksigent control software.
O9	RDY out	The RDY out contact changes state when the pump is ready to start run (for example at end of pre-run flush). Its active state is configurable from within the Eksigent control software.
O10	Out CMN	The Out CMN contact is a common for the output interface.

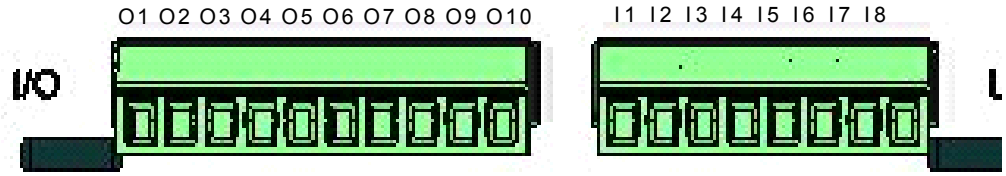


Figure B-2 External Interface Connectors –Output connector (left) and Input connector (right)

The input lines are TTL input that share a system GND on Pins I5 and I6. The lines are pulled up to 5V when not connected to an external device. The A/D in is a single-ended input that uses the same system GND. **All inputs need to be less than ±24V.**

Table B-2 Input Connections

Line	Name	Description
I1	Not used	For future expansion.
I2	Not used	For future expansion.
I3	PRK in	The PRK in contact allows remote triggering of the peak parking function. The instrument will remain in the peak parking state until the contact's state is changed. Its input polarity is configurable from within the Eksigent control software.
I4	RUN in	The RUN in contacts allows remote starting of a method. Its active state is configurable from within the Eksigent control software. The external trigger should be a pulse-type and does not need to be held in the trigger state.
I5	GND	The GND is a system ground connection used in combination with other input contacts.
I6	GND	The GND is a system ground connection used in combination with other input contacts.
I7	Not used	For future expansion.
I8	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 Ultra's data system. The reading can be plotted in the main acquisition window and saved to a data file. Please refer to Section 2.8 to change the input range.

B.3 Connecting to Other Instruments

For 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 dialog. It is important that the ground lead of the peripheral be connected to the ground connection on the NanoLC Ultra. In addition, use of the NanoLC AS-2 autosampler provides a number of additional I/O signals with additional programming flexibility.

Note: 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 Ultra 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 Ultra system described above. One suggested configuration is described here.

1. Set up the injection method and sample sequence using the autosampler interface (front panel or other software).
2. Set up a matching sequence of chromatographic methods in the Run Manager window of the NanoLC Ultra and make sure that **LC Wait for Contact Closure** is checked under the System menu in the **Run Manager** window.
3. Verify that the analysis time per sample (time between autosampler injections) in the autosampler method is long enough to allow for the NanoLC Ultra method run time plus any pre-run flush equilibration for the next run.
4. Connect the inject marker out from the autosampler to the Run Input (pin I4) and Ground (pin I5) of the NanoLC Ultra system.
5. 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 Ultra system to start its chromatographic run.

The NanoLC Ultra system also provides a Ready Out (pin O9) 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 AS-2 autosampler will, in general, not be available with other autosamplers. While the rapid inject or metered injection routines of the NanoLC Ultra 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 Ultra system using the Xcalibur, Analyst and Hystar software packages is available, other MS systems will allow synchronization of data collection through the use of contact closures. The following describes the general configuration for this synchronization.

1. 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 Eksigent control software with the corresponding sequence of methods.
2. Set the **Run Output** configuration to contact closure (box checked) in the NanoLC Ultra software (menu).
3. 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.
4. To synchronize MS data with the beginning of the gradient (after the injection), connect the **Run Out** (pin 07) 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 Ultra. For most applications, this connection will be made to the Gradient 2 (low flow gradient) side of the instrument.

   As an alternative, you can also choose to synchronize MS data with the beginning of the injection. To do this, connect the **Valve Out** (pin O6) and the **Ground** terminals to the MS contact closure.

B.3.3 Triggering Peak Parking

Peak parking on the NanoLC Ultra system can 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 I3). The **Park Out** terminal (pin O8) can also be used to monitor the state of Peak Parking. The Peak Parking Toolbox window in the Eksigent control software (**View > Toolboxes >PeakPark Toolbox**) 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 contact 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 AS-2 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 1** (red wire in the 4-wired cable) and **Gnd** (orange wire in the 4-wired cable) of the AS-2 I/O cable. Add the following line to the beginning of the AS-2 method.

'Wait for Input 1-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 1-HIGH.

The autosampler 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 autosampler method or to short In 3 to ground.

Appendix C
Quick Start Guides

C.1 Introduction

This document describes how to start up and run an installed NanoLC® Ultra system. Basic instrument operation is described, as well as some common configurations of the NanoLC Ultra system. It is not intended as a substitute for, but an addition to the training you will receive from your AB SCIEX Field Service Employee.

Note: Use the example methods provided for the NanoLC Ultra system you have.

C.2 Site Requirements

Gas Requirements:

- Air or nitrogen gas, clean and dry, regulated to 100 psi.
- 1/4 inch tubing connection for the gas line.

Power Requirements:

- Please provide surge protector or UPS outlets.
- 100-240V AC, 4 outlets necessary for the NanoLC-Ultra and autosampler, monitor and PC.

User-Supplied Computer Requirements:

- Windows XP Operating system
- Re-writable CD-ROM drive; 1.44 MB floppy disk drive.
- 2.8 GHz Pentium 4 processor; 512 MB RAM; 40 GB hard disk drive.
- 1 USB port

Bench Space:

- 14 inch x 24 inch x 14 inch (W x D x H)
- Up to 120 lbs with autosampler, depending on configuration

New, bottled UV-grade Mobile Phase:

- Water and acetonitrile with 0.1% formic acid are required

- IPA – 250 mL required

C.3 Starting Up

Follow this procedure to start a NanoLC Ultra system that has been idle or shut off a period of time.

1. Check gas supplies: ensure the gas is turned on, and set to 100 psi.
2. Check solvent level.

   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 4.

Note: Replacing mobile phase is a 2-step process. First purge and then flush.

To purge the pumps, click **System > Mobile Phases**.

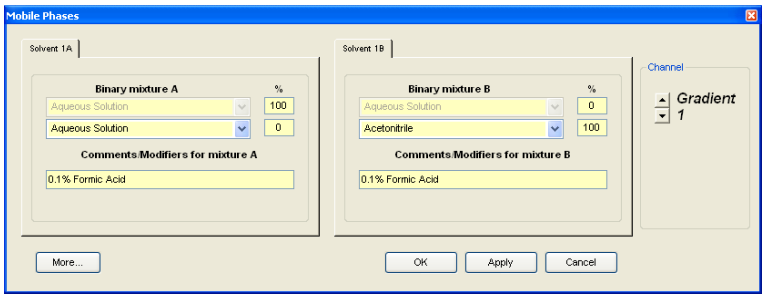
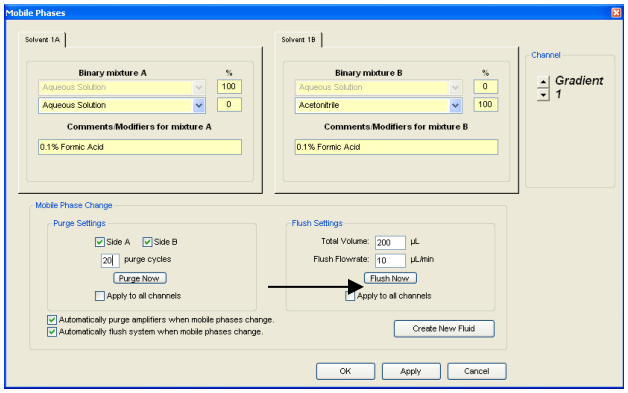


Figure C-1 Mobile Phases Dialog

Click **More** to reveal the purging menu.



All pumps can be purged at the same time

Figure C-2 Mobile Phases Dialog, Showing Additional Options

Purge all pumps at least 20 times to completely change the mobile phase.

3. Once new mobile phase is purged, flush the pump. Disconnect the pump outlet to prevent high pressure in the columns or traps. Flush all capillary flow rate pumps at 65 µL/min for 200 µL, and all nano flow rate pumps at 6500 nL/min for 200 µL.

Note: The flush is very important when changing mobile phase composition. Make sure to allow the pump to flush fully.

4. Re-initialize pressure transducers often to ensure reproducible gradient formation.

   This functions to establish the zero point of the pressure transducers, so make sure there is no backpressure on the pump outlet before beginning by turning the pump off and loosening the pump outlet.

   To reinitialize the transducers, click **System > Hardware Diagnostics**.

   Check the box and click **Start Diagnostics**.

   Click **Yes** to run on all channels.

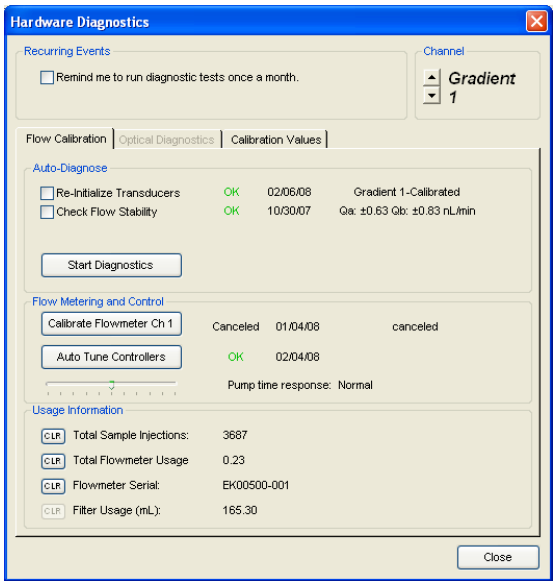


Figure C-3 Hardware Diagnostics Dialog

5. Reconnect pump outlets, and establish flow to the column and trap:
   - Click **System > Direct Control.**

- Set desired flow rate and click **Start**.

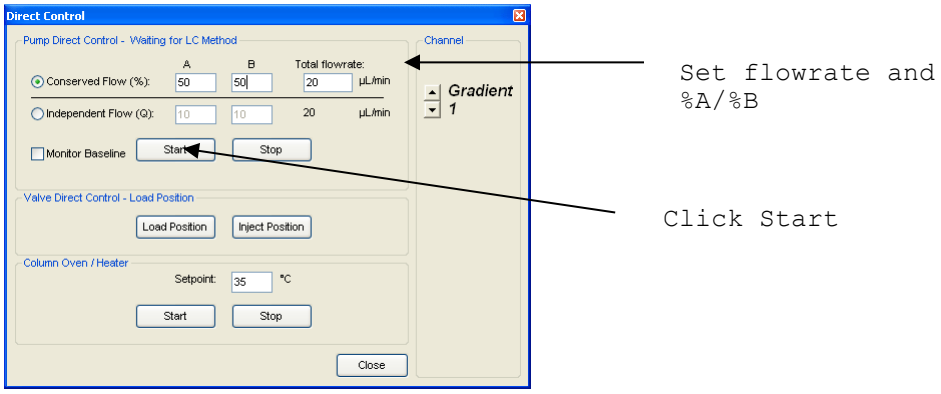


Figure C-4 Direct Control Dialog

C.4 NanoLC Ultra 1D+ System Methods

Note: Use the example methods provided for the NanoLC Ultra system you have.

C.4.1 Direct-to-Column Injection

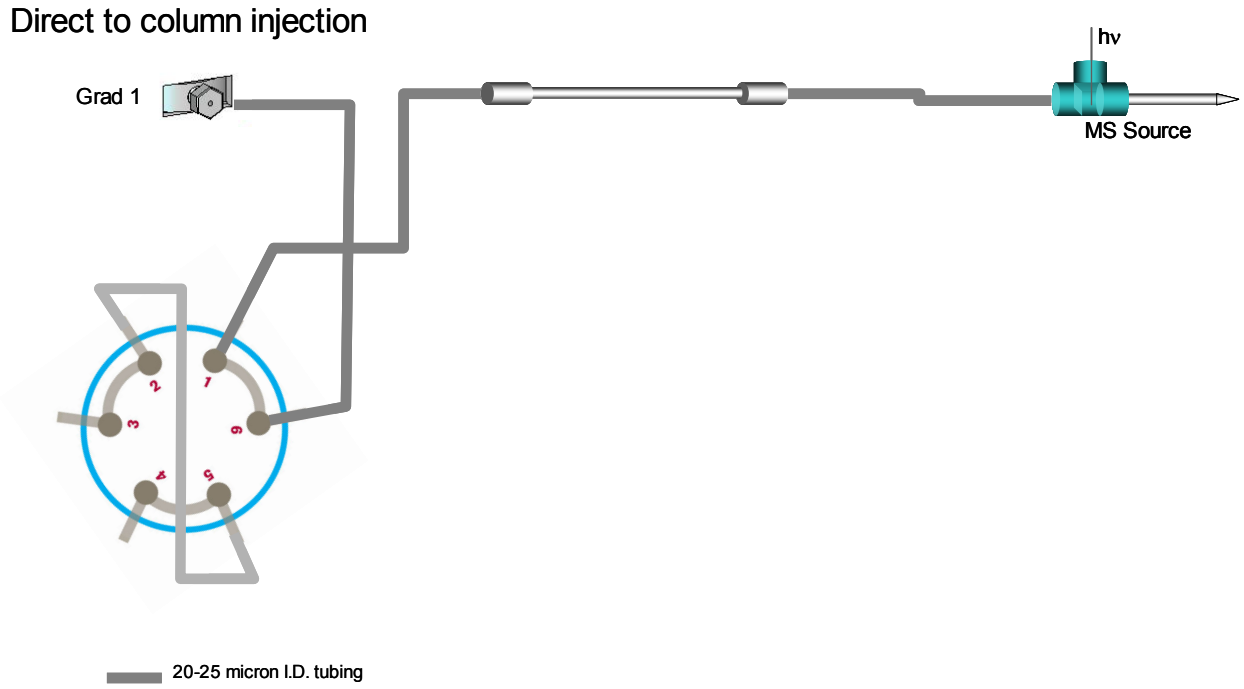


Figure C-5 Plumbing Diagram—Direct-to-column Injection for the NanoLC Ultra 1D+ System

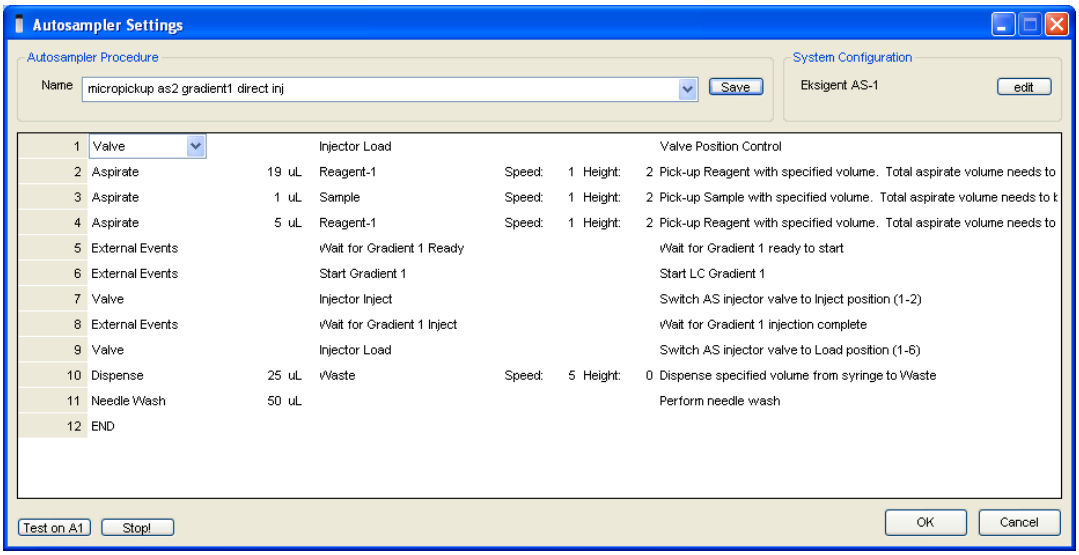


Figure C-6 Autosampler Method—Direct-to-column Injection for the NanoLC Ultra 1D+ System

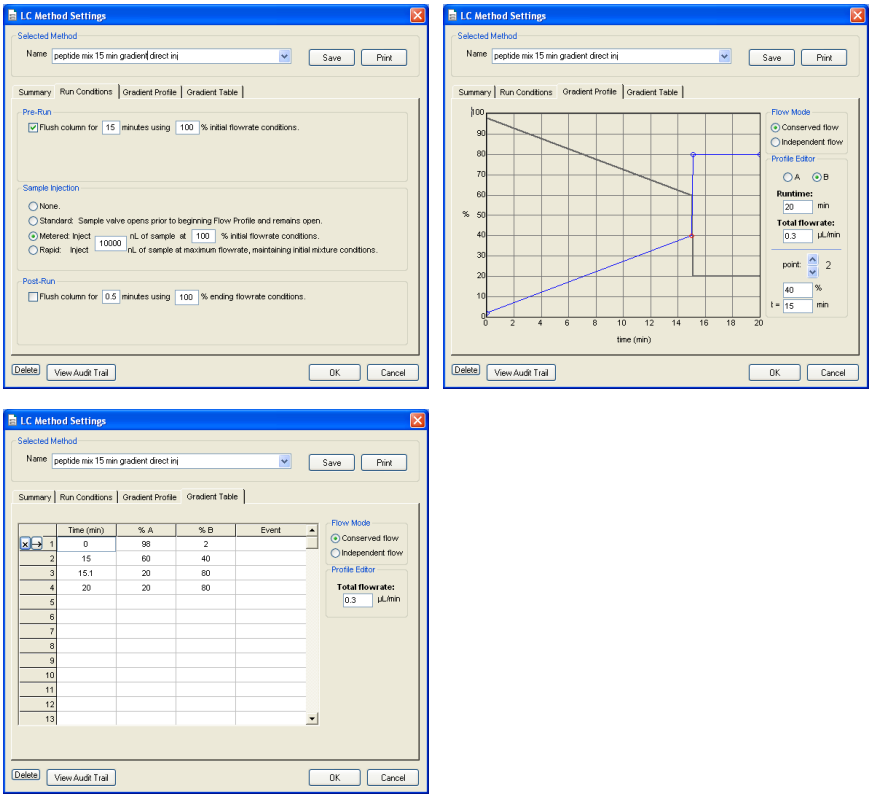


Figure C7 Gradient 1 Method—Direct-to-column Injection for the NanoLC Ultra 1D+ System

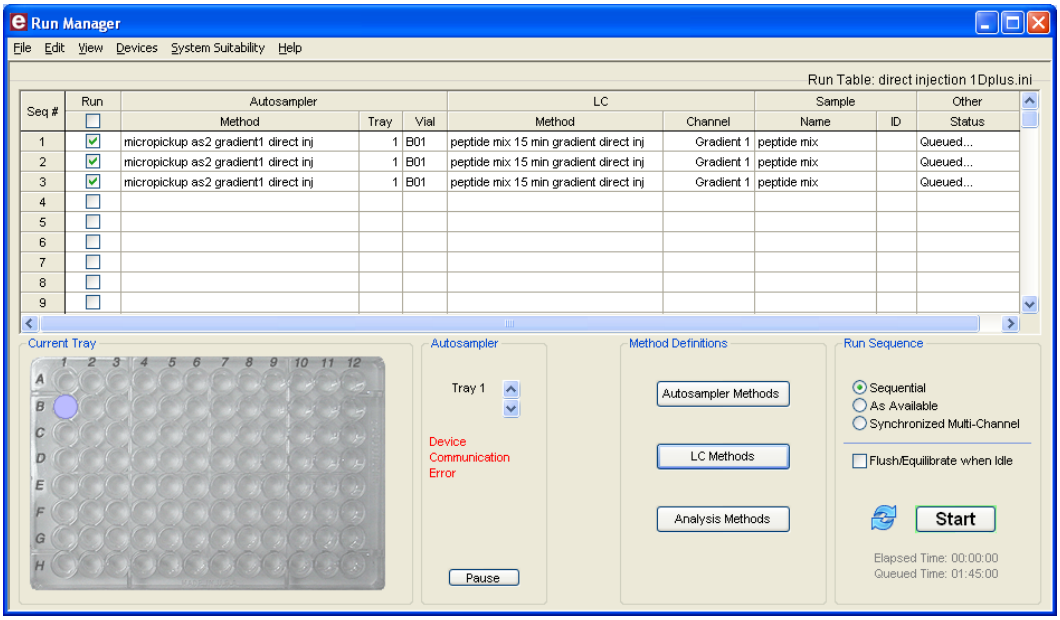


Figure C-8 Sequence Setup—Direct-to-column Injection for the NanoLC Ultra 1D+ System

C.4.2 Single Trap Loading

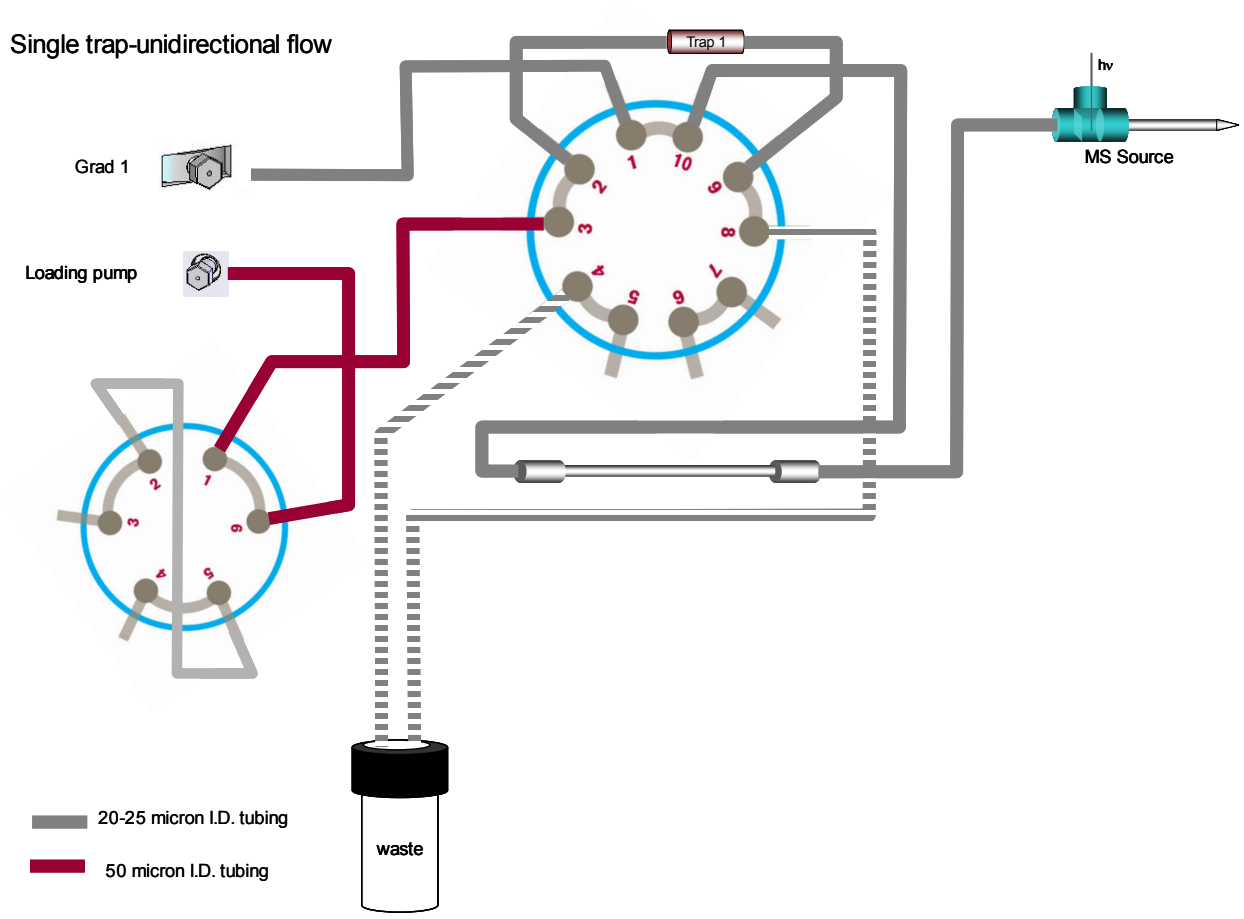


Figure C-9 Plumbing Diagram—Single Trap, Unidirectional Flow for the NanoLC Ultra 1D+ System

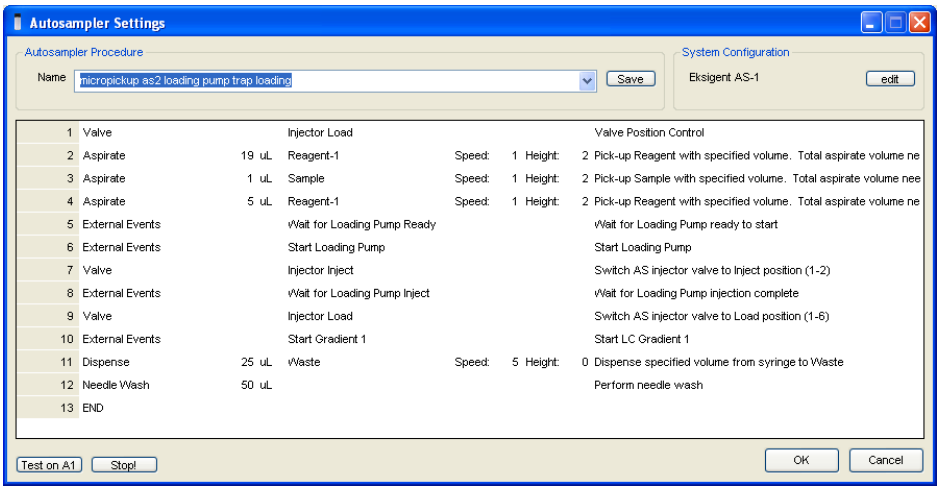


Figure C-10 Autosampler Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 1D+ System

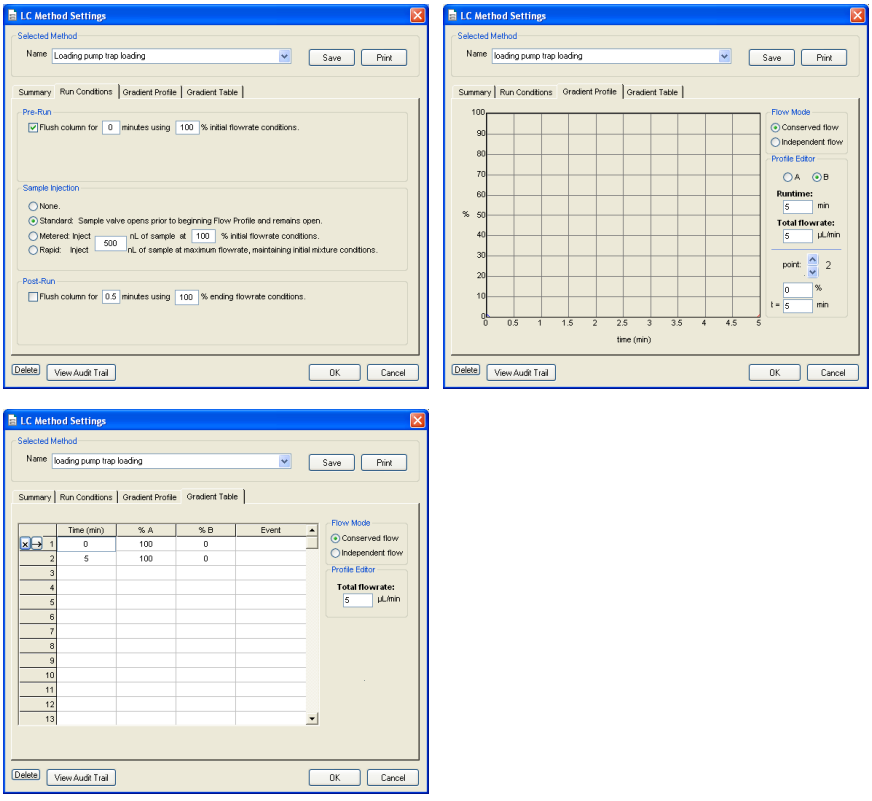


Figure C-11 Loading Pump Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 1D+ System

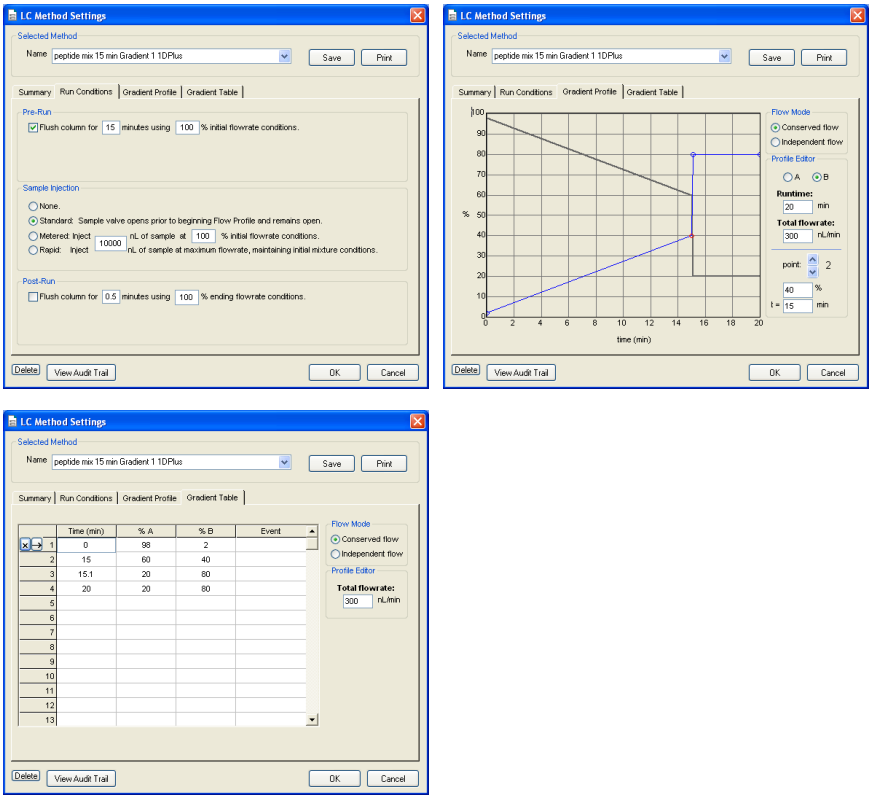


Figure C-12 Gradient 1 Method—Single trap, Unidirectional Flow for the NanoLC Ultra 1D+ System

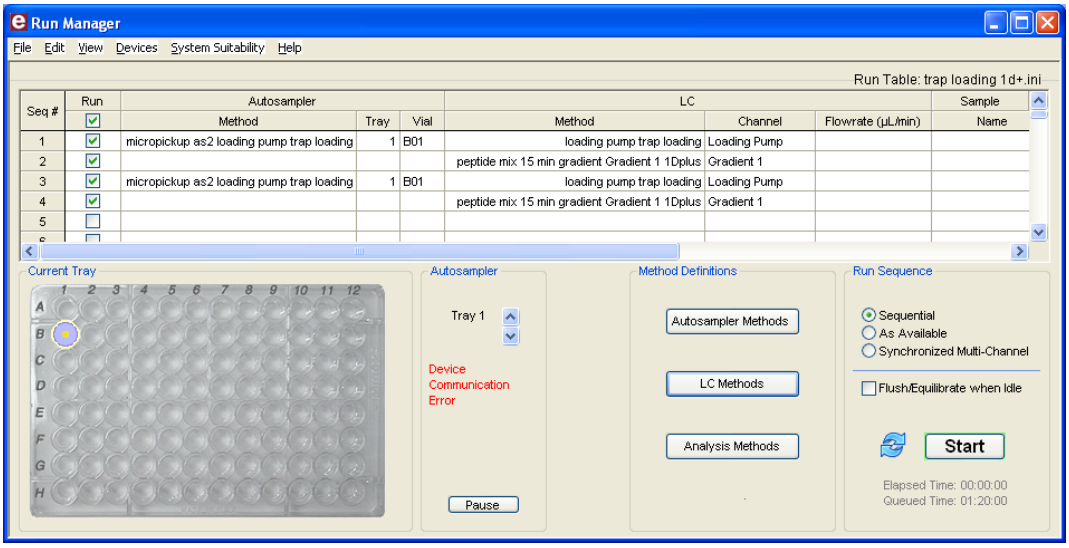


Figure C-13 Sequence Setup—Single Trap, Unidirectional Flow for the NanoLC Ultra 1D+ System

C.4.3 Single Trap Bidirectional Flow

Bidirectional trap and vented column method programming is the same as above trap loading methods.

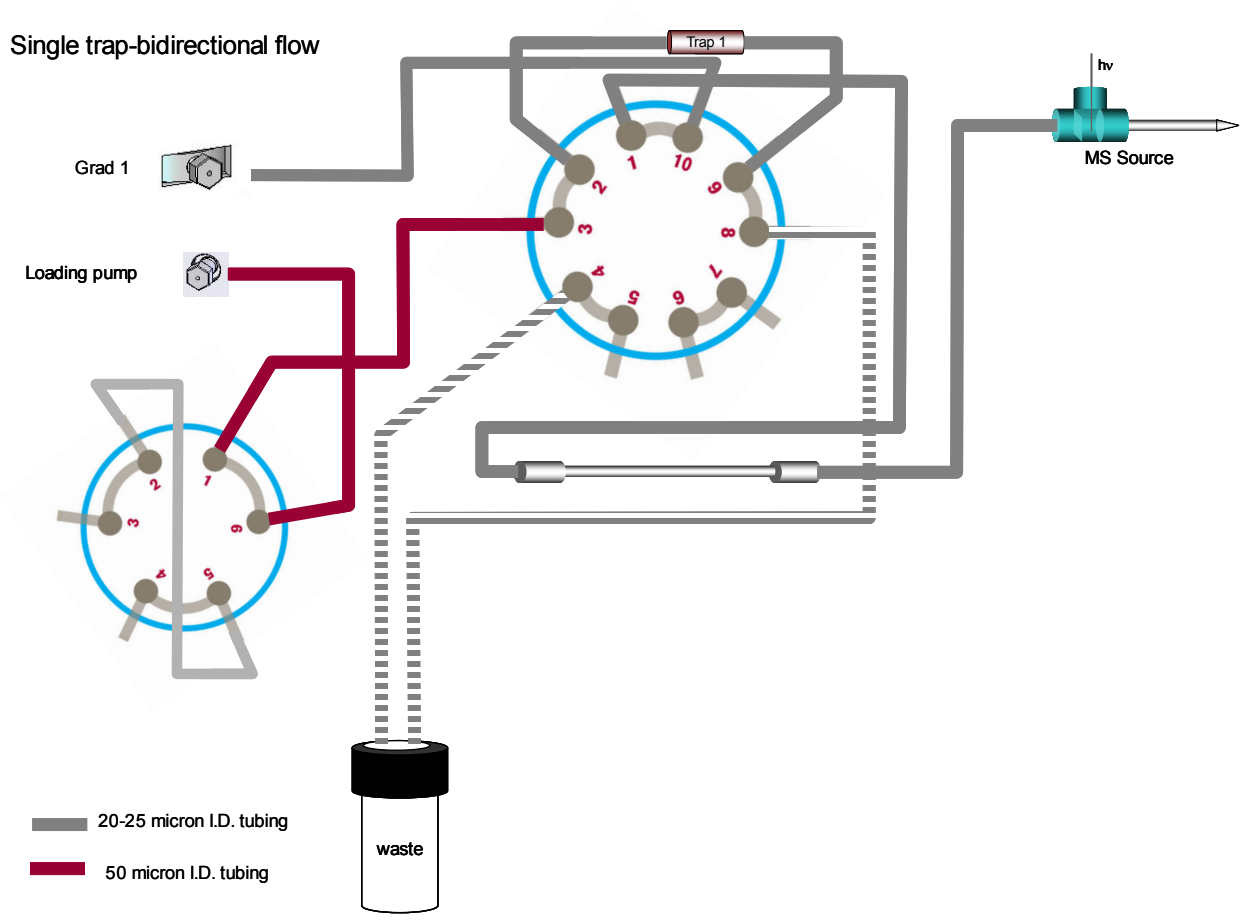


Figure C-14 Plumbing Diagram—Single Trap, Bidirectional Flow for the NanoLC Ultra 1D+ System

C.4.4 Vented Column

Bidirectional trap and vented column method programming is the same as above trap loading methods.

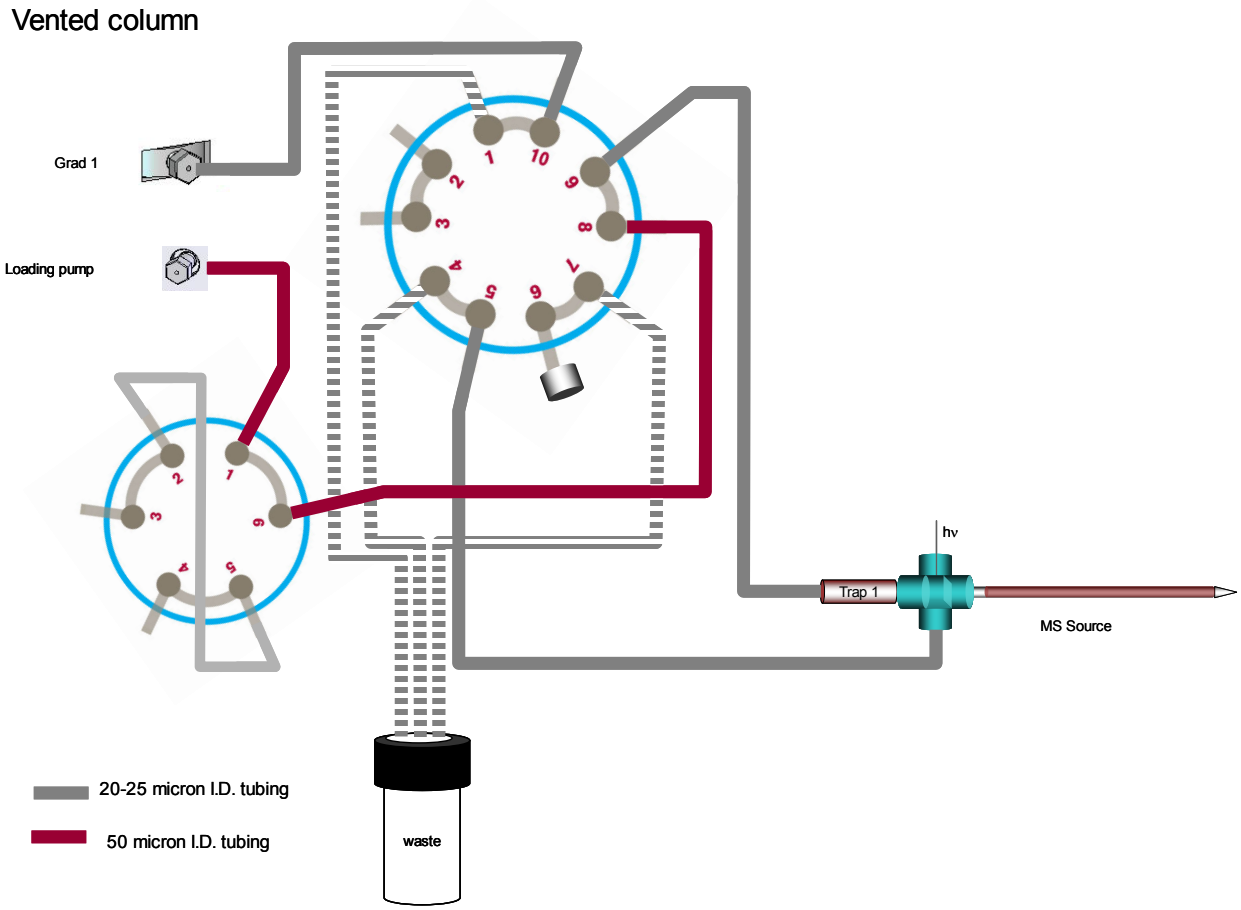


Figure C-15 Plumbing Diagram—Vented Column Injection for the NanoLC Ultra 1D+ System

C.4.5 Dual Trap

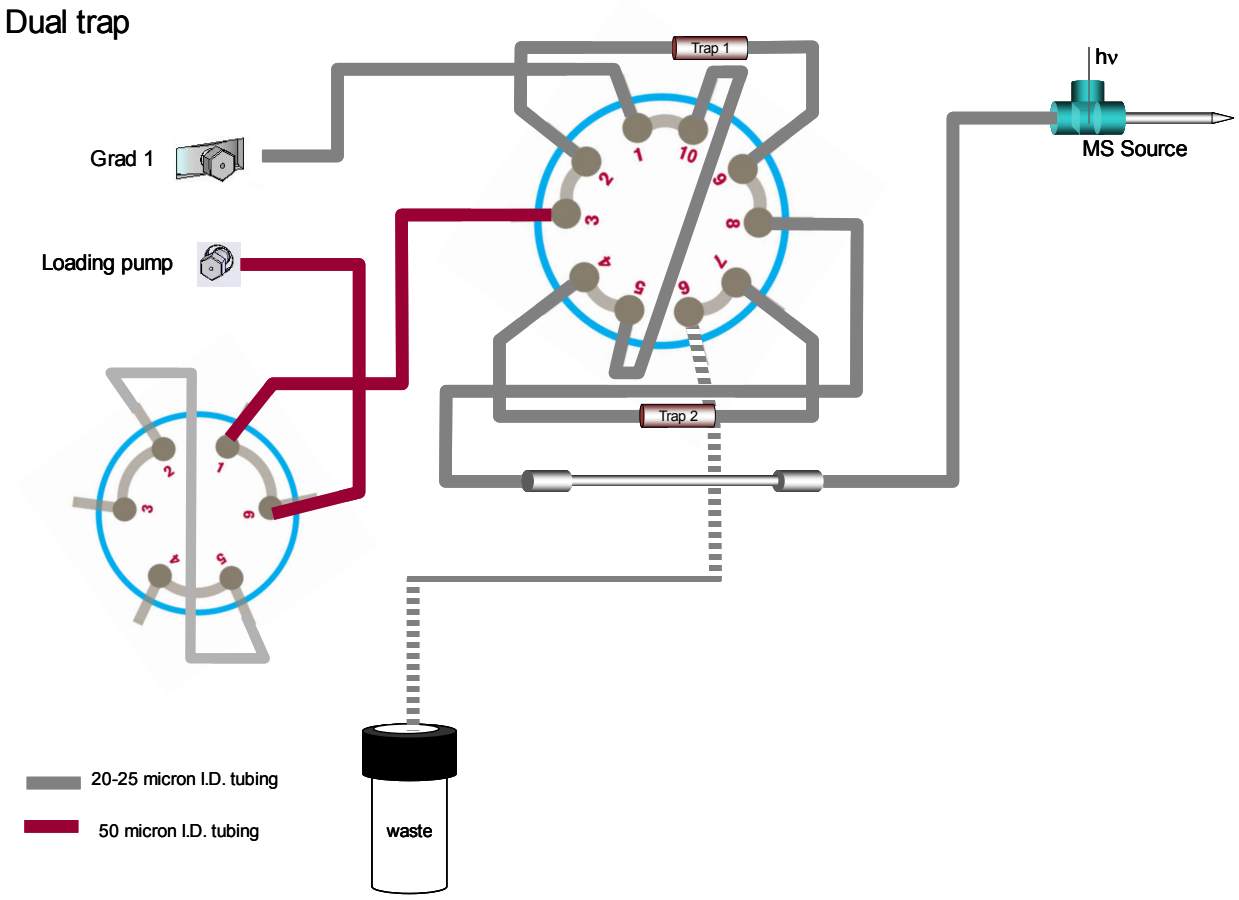


Figure C-16 Plumbing Diagram—Dual Trap Injection for the NanoLC Ultra 1D+ System

C.5 NanoLC Ultra 2D System Methods

Note: Use the example methods provided for the NanoLC Ultra system you have.

C.5.1 Direct-to-Column Injection

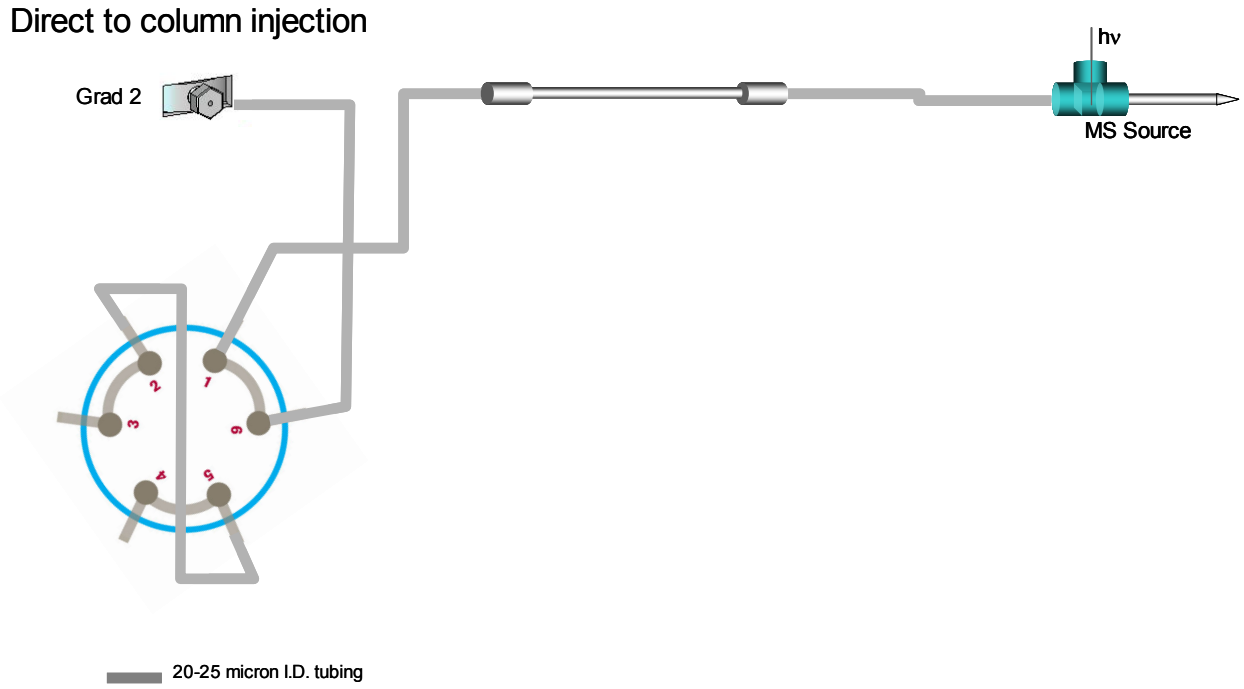


Figure C-17 Plumbing Diagram—Direct-to-column Injection for the NanoLC Ultra 2D System

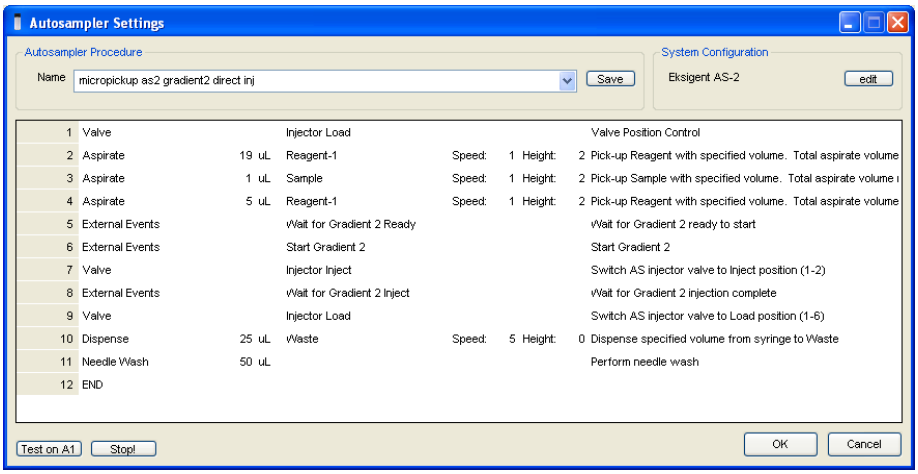


Figure C-18 Autosampler Method—Direct-to-column Injection for the NanoLC Ultra 2D System

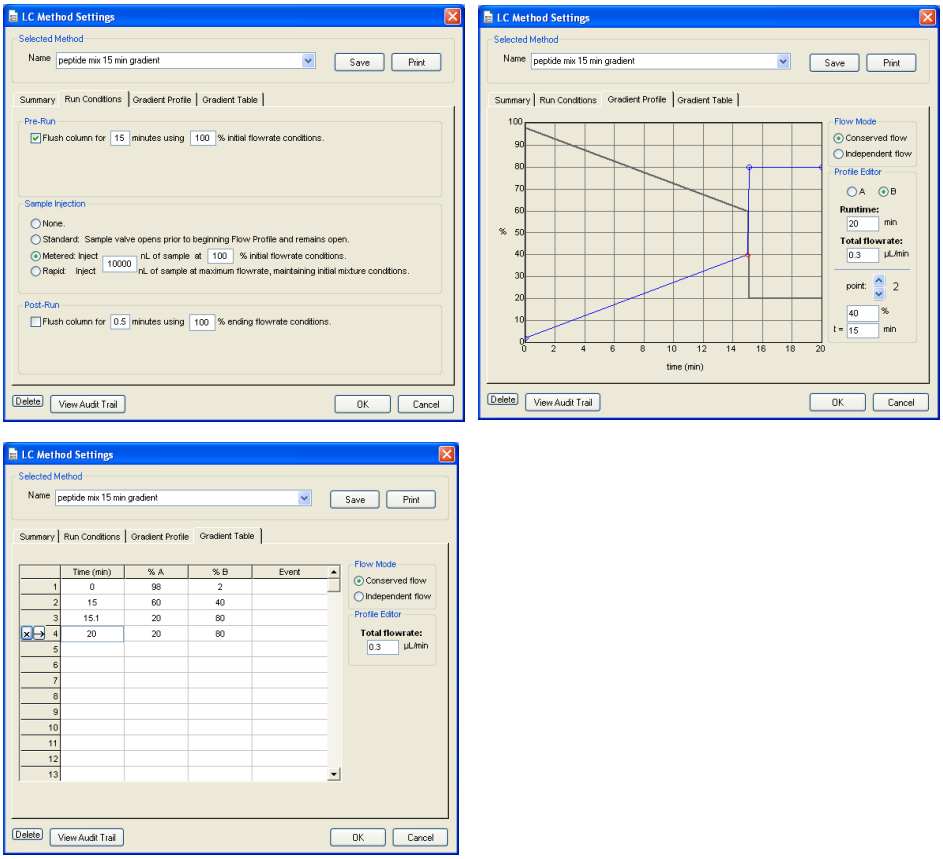


Figure C-19 Gradient 2 Method—Direct-to-column Injection for the NanoLC Ultra 2D System

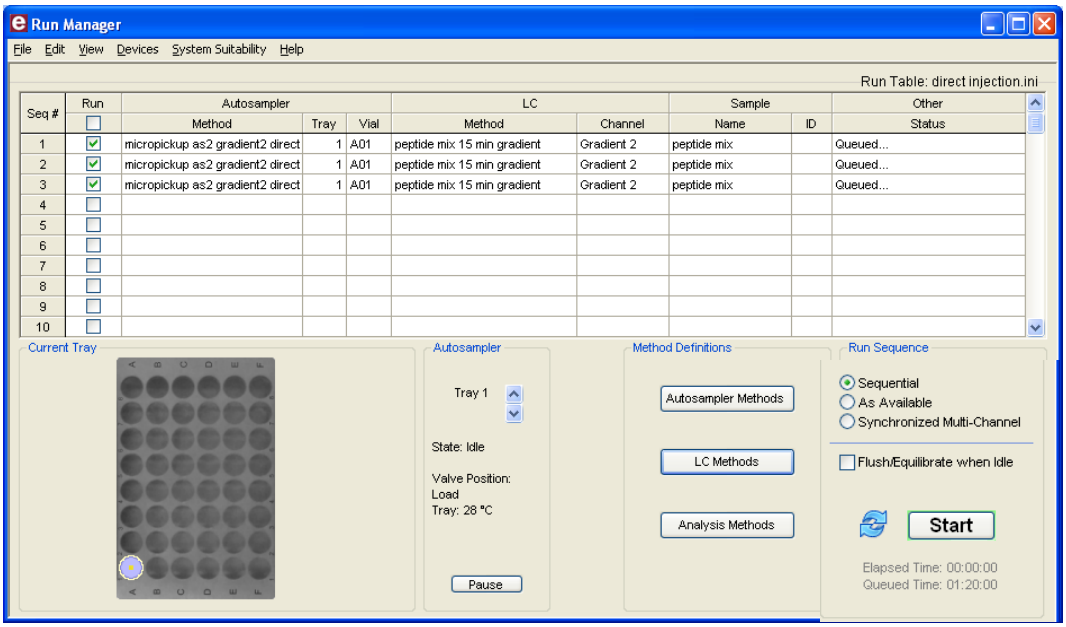


Figure C-20 Sequence Setup—Direct-to-column Injection for the NanoLC Ultra 2D System

C.5.2 Single Trap

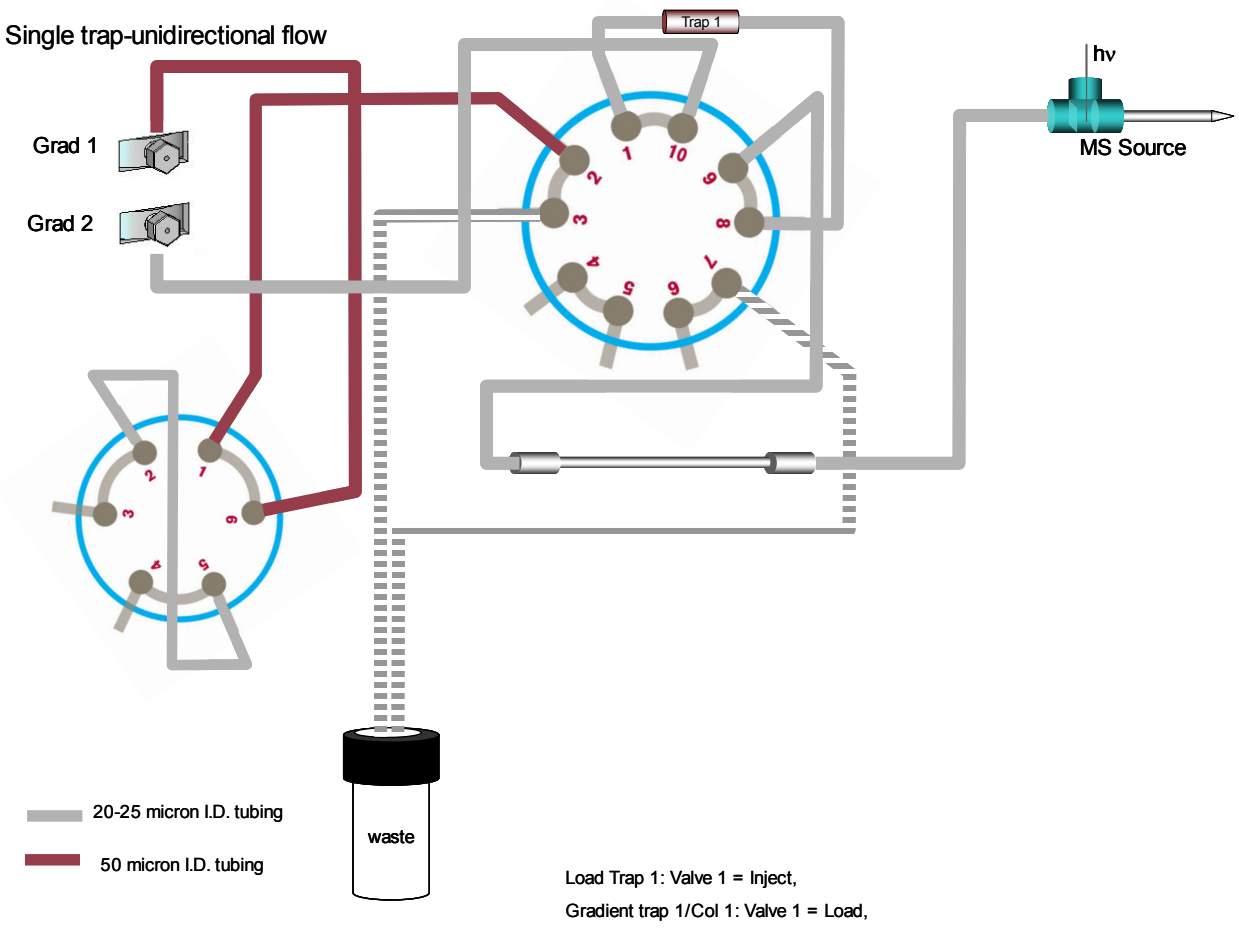


Figure C-21 Plumbing Diagram—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D System

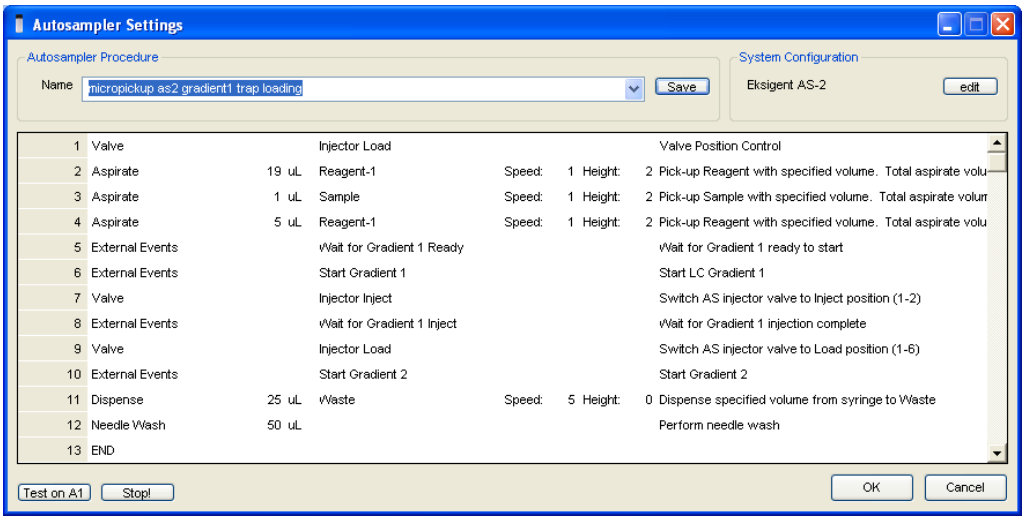


Figure C-22 Autosampler Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D System

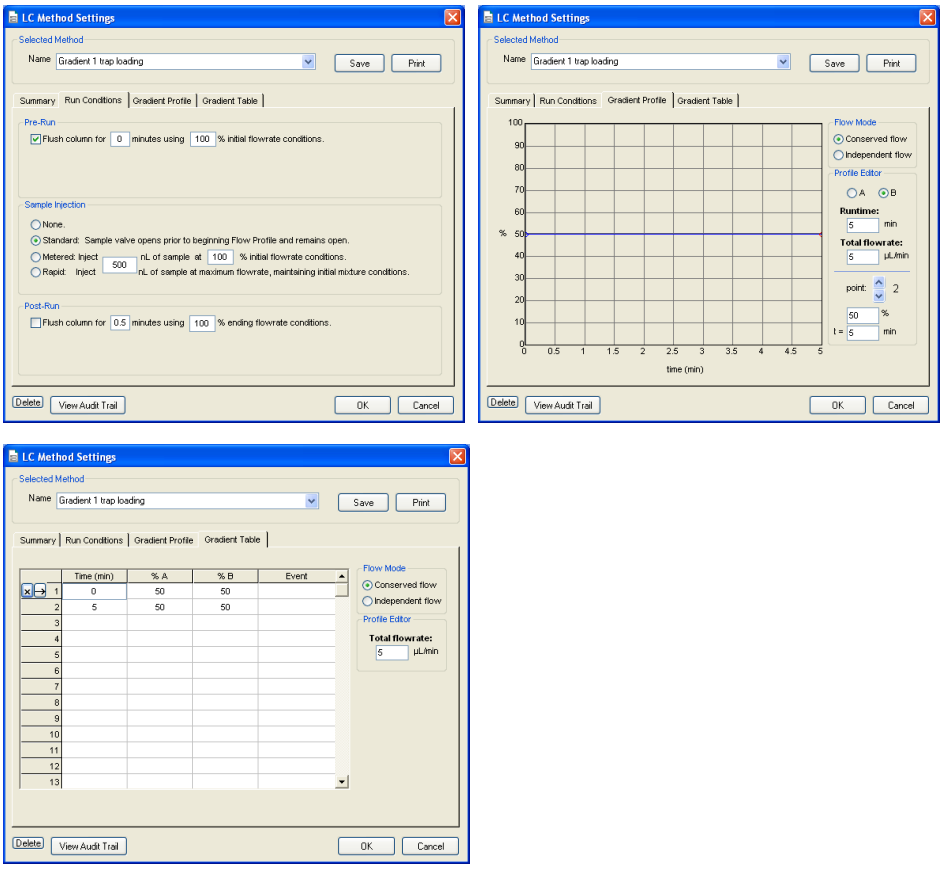


Figure C-23 Gradient 1 Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D System

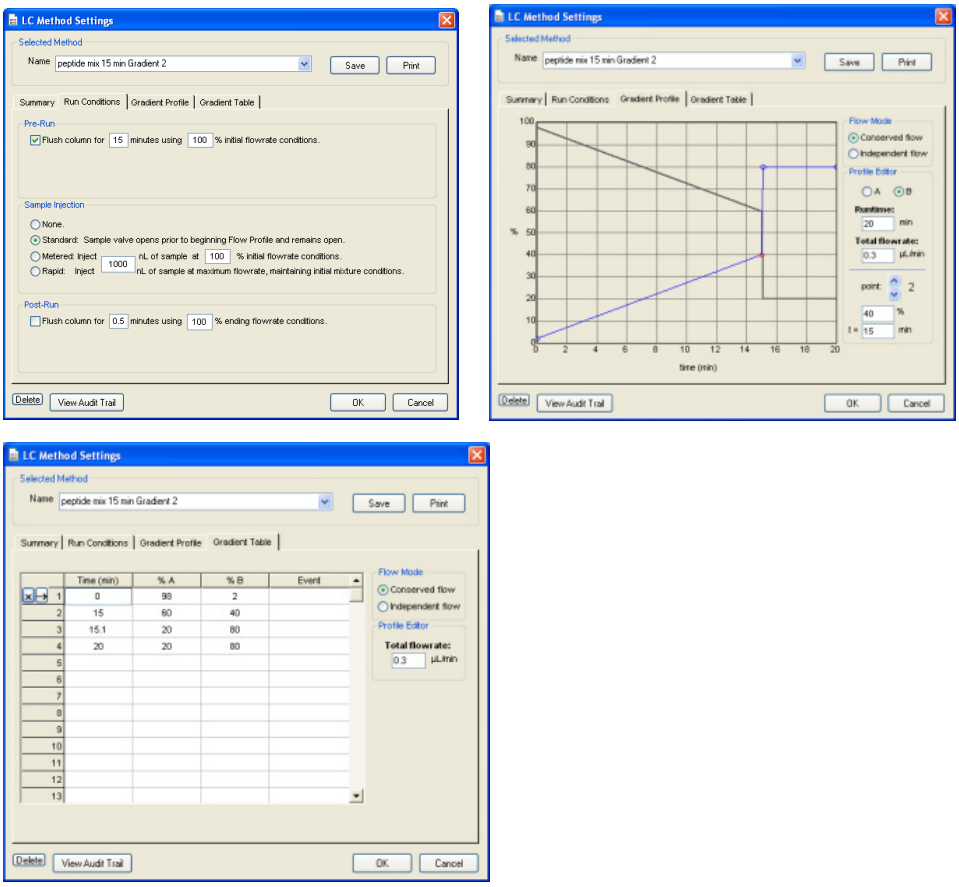


Figure C-24 Gradient 2 Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D System

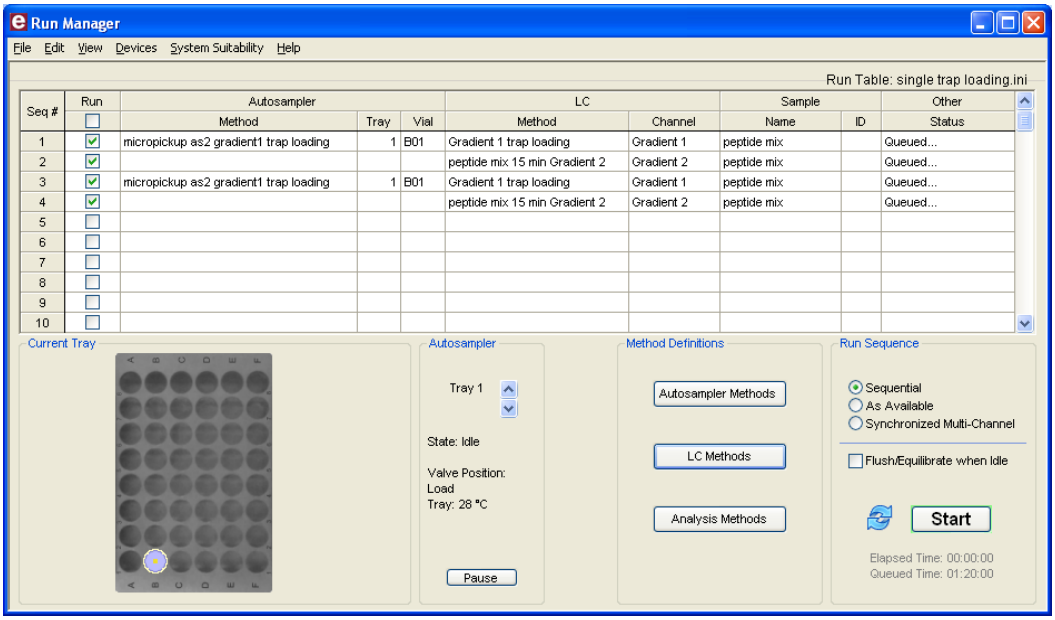


Figure C-25 Sequence Setup—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D System

C.5.3 Single Trap Bidirectional Flow

Bidirectional trap loading and vented column methods are the same as the above trap loading method.

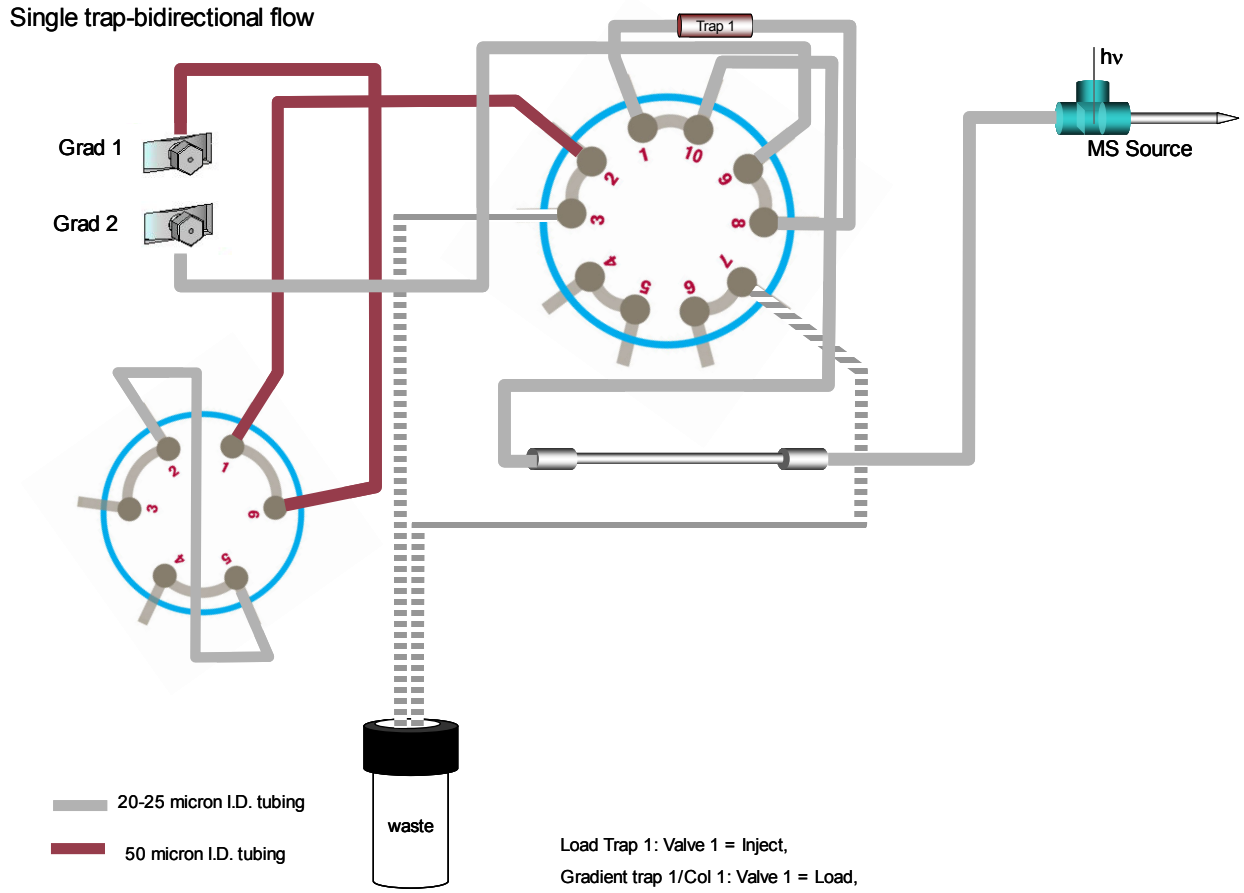


Figure C-26 Plumbing Diagram—Single Trap, Bidirectional Flow for the NanoLC Ultra 2D System

C.5.4 Vented Column

Bidirectional trap loading and vented column methods are the same as the above trap loading method.

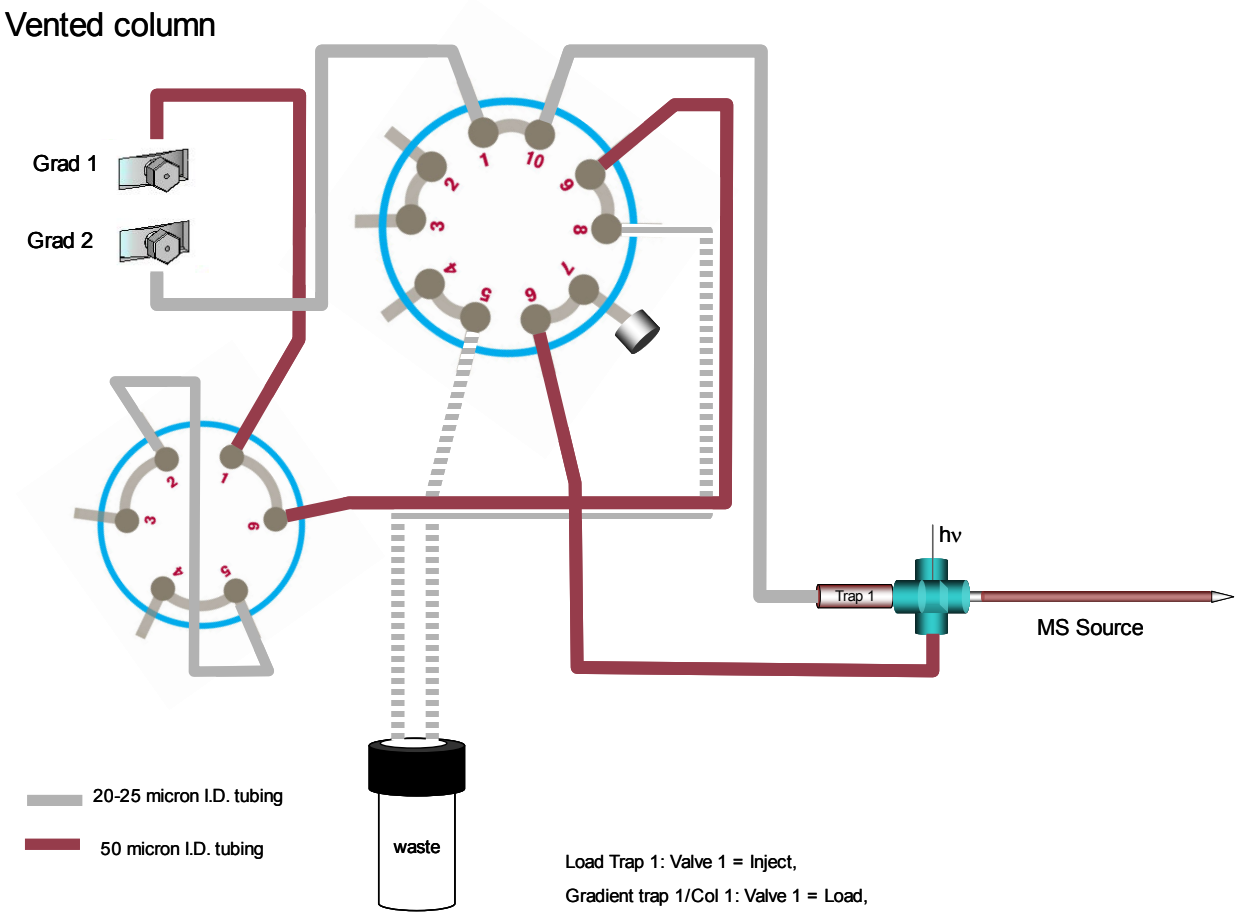


Figure C-27 Plumbing Diagram—Vented Column Injection for the NanoLC Ultra 2D System

C.5.5 Dual Trap

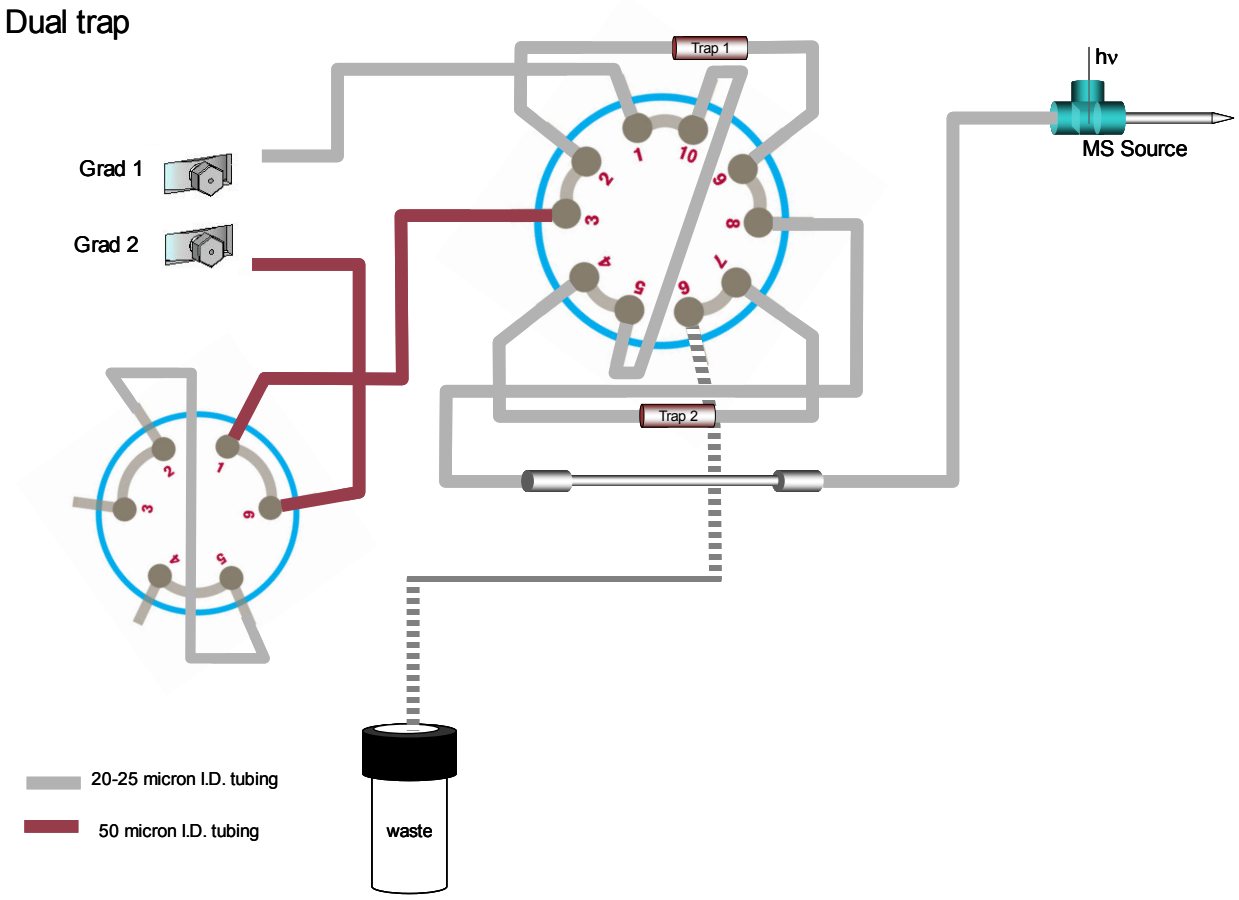


Figure C-28 Plumbing Diagram—Dual Trap Injection for the NanoLC Ultra 2D System

C.5.6 Dual Column

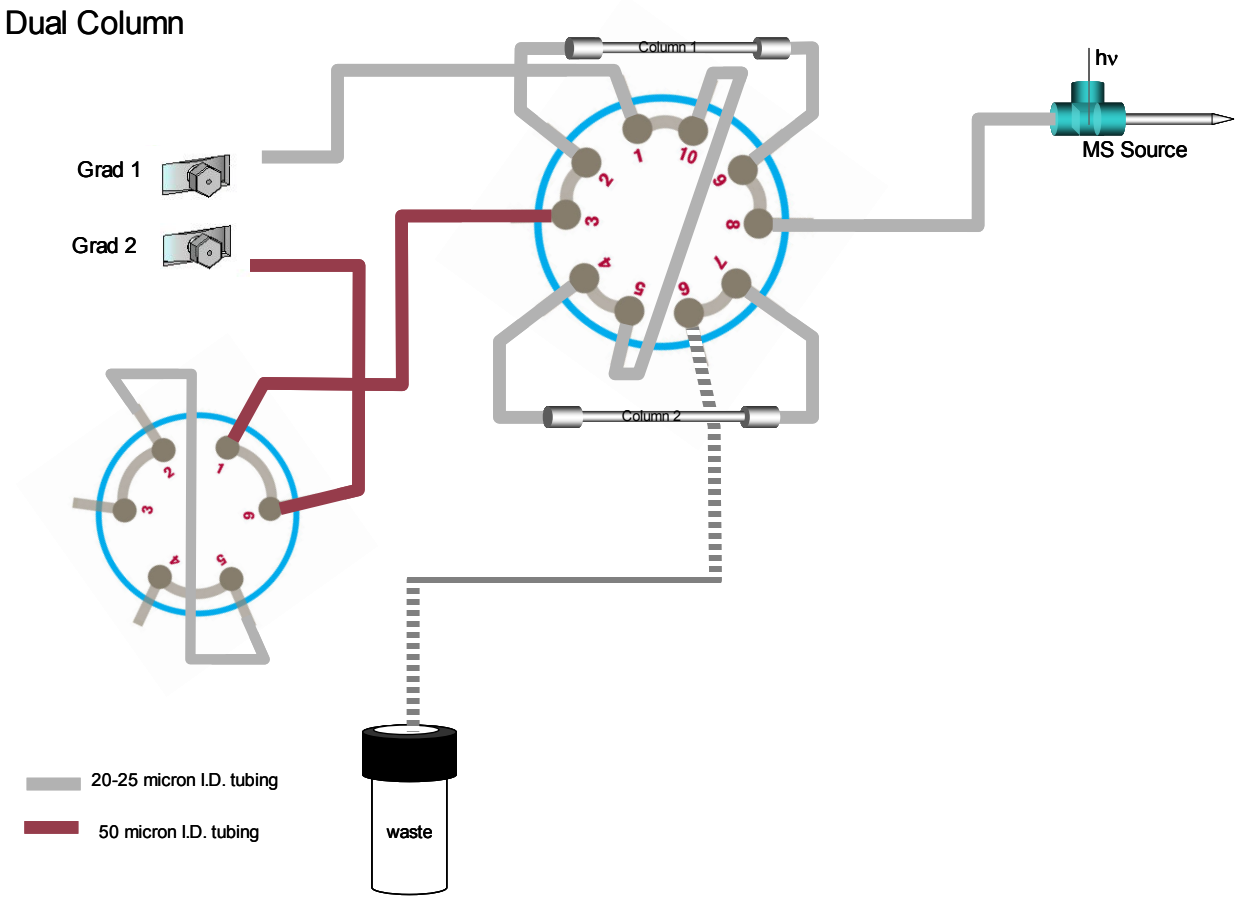


Figure C-29 Plumbing Diagram—Dual Column Injection for the NanoLC Ultra 2D System

C.6 NanoLC Ultra 2D+ System Methods

Note: Use the example methods provided for the NanoLC Ultra system you have.

C.6.1 Direct-to-Column Injection

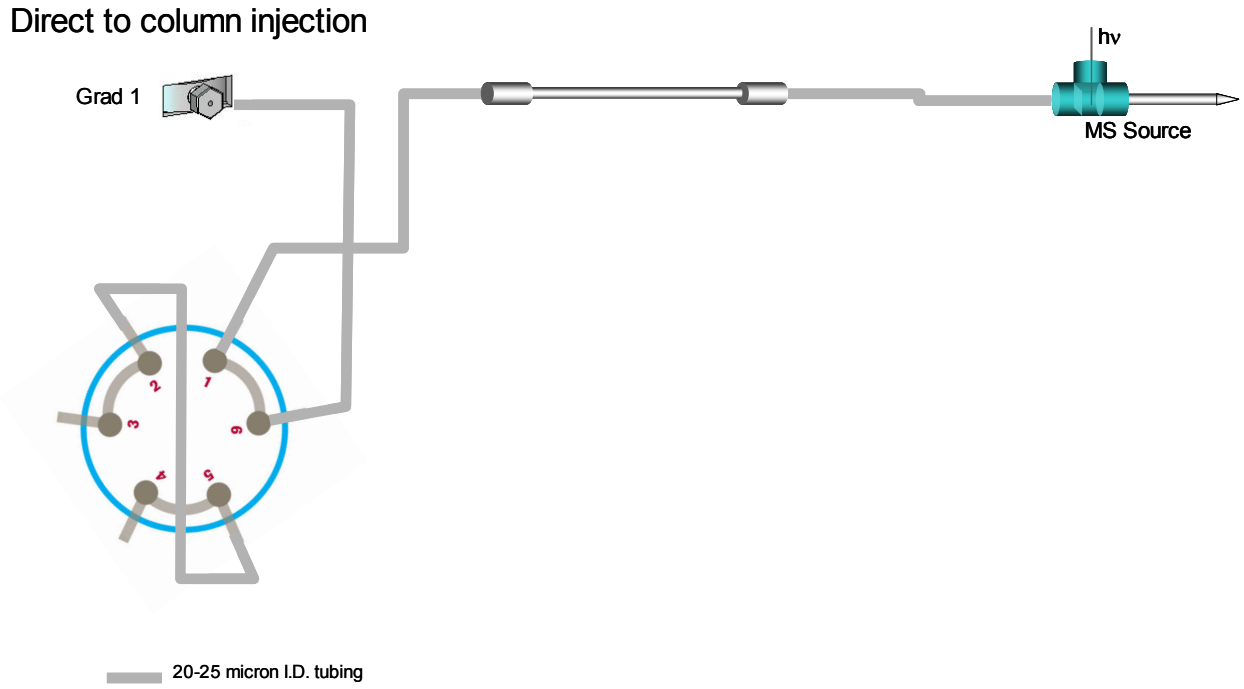


Figure C-31 Plumbing Diagram—Direct-to-column Injection for the NanoLC Ultra 2D+ System

Note: Depending on your configuration, direct injection can be done with gradient 1 or gradient 2.

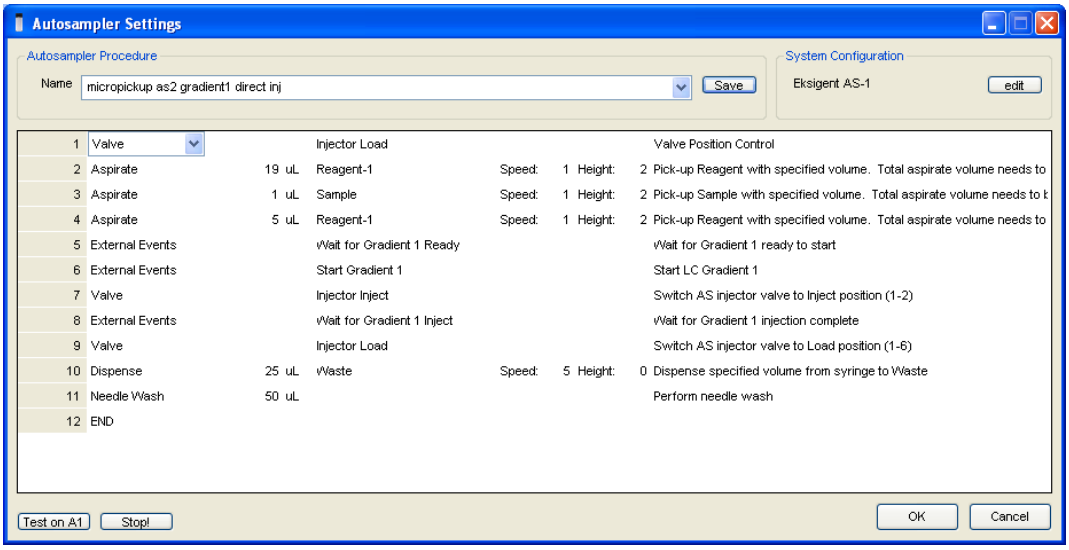


Figure C-32 Autosampler Method—Direct-to-column Injection for the NanoLC Ultra 2D+ System

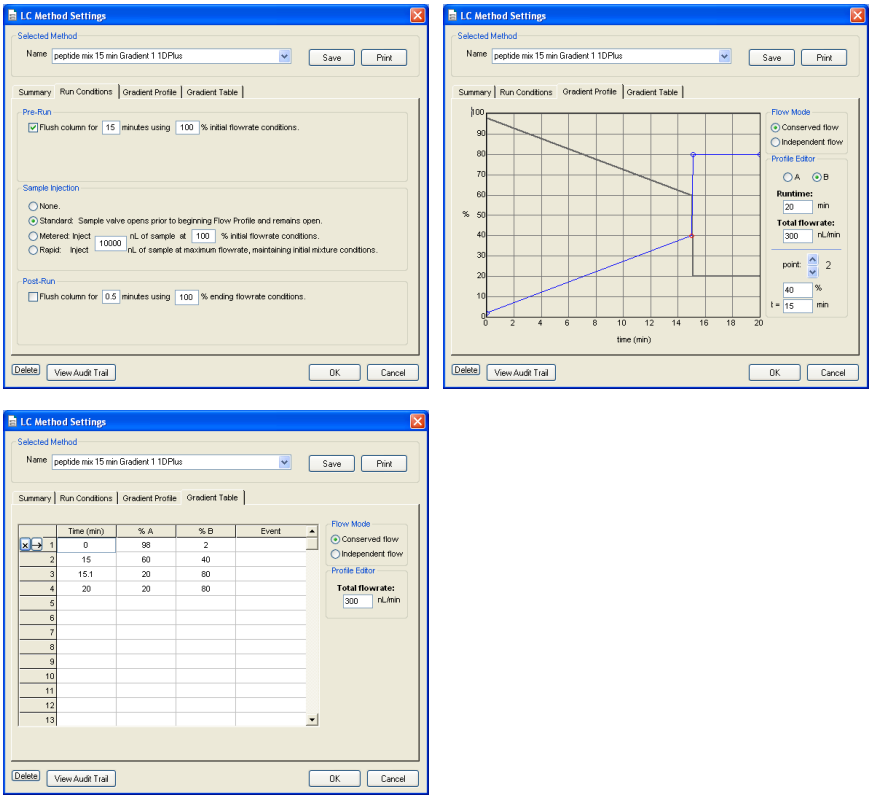


Figure C-33 Gradient 1 Method—Direct-to-column Injection for the NanoLC Ultra 2D+ System

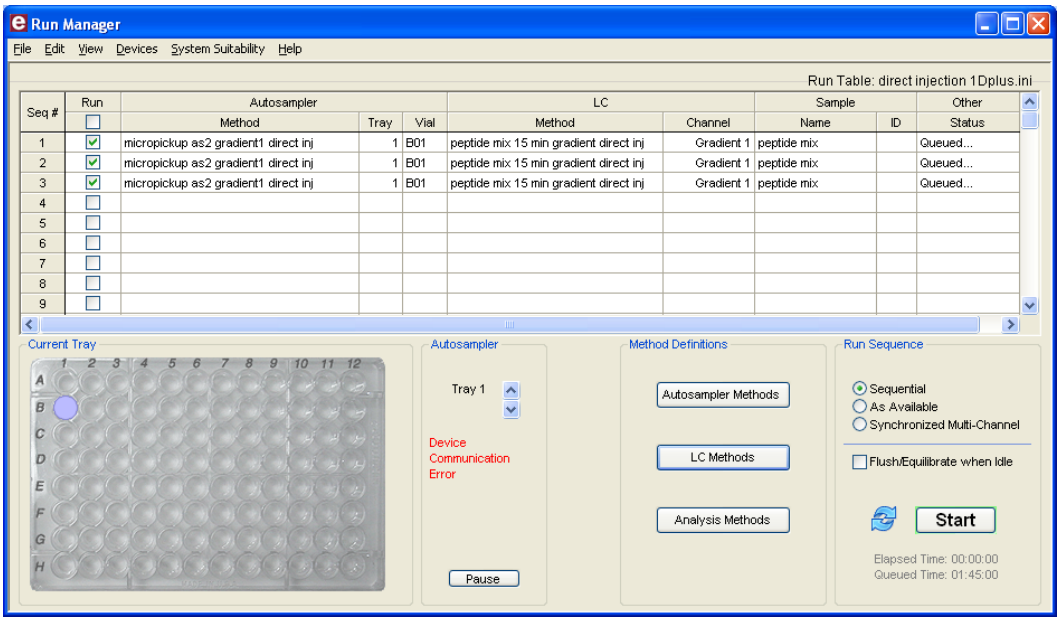


Figure C-34 Sequence Setup—Direct-to-column Injection for the NanoLC Ultra 2D+ System

C.6.2 Single Trap Loading

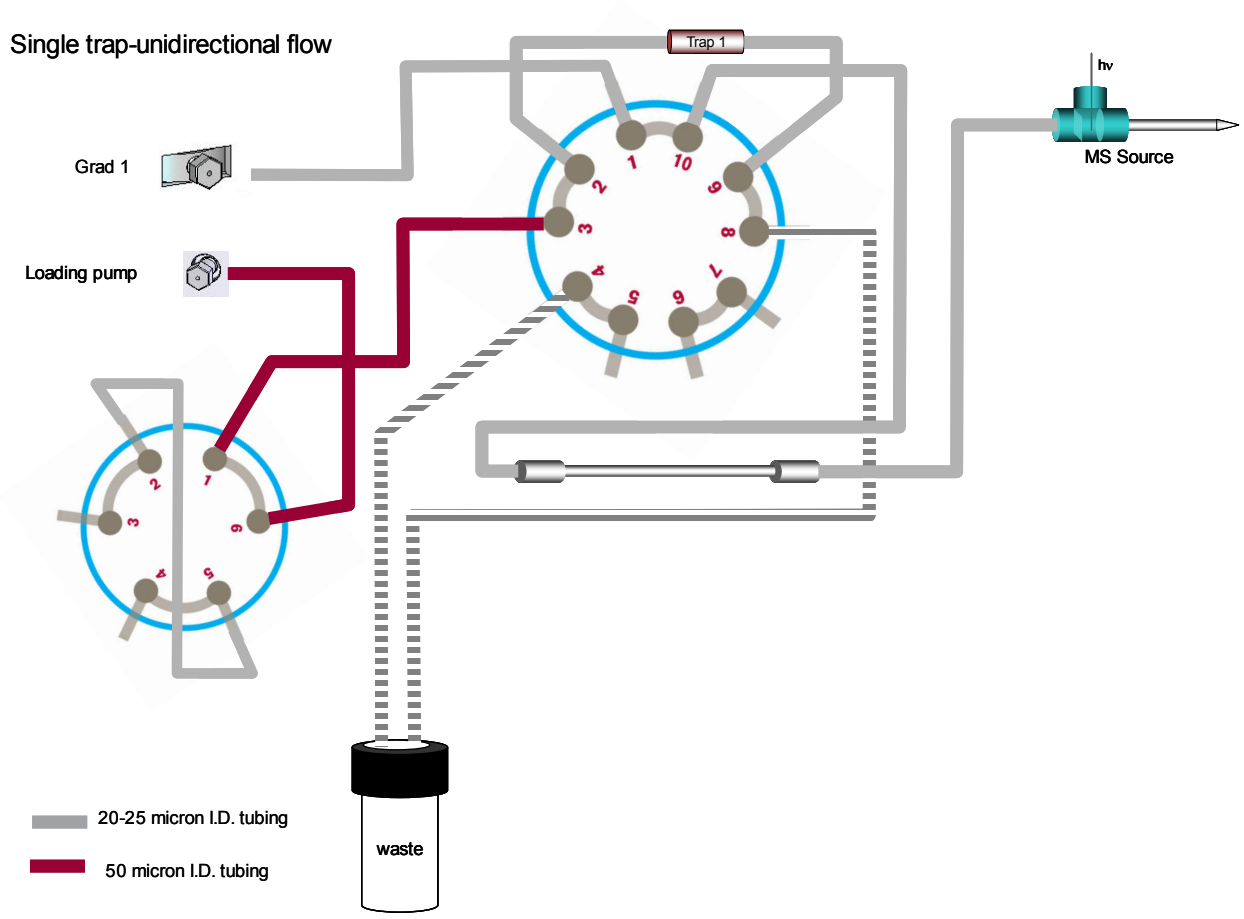


Figure C-35 Plumbing Diagram—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D+ System

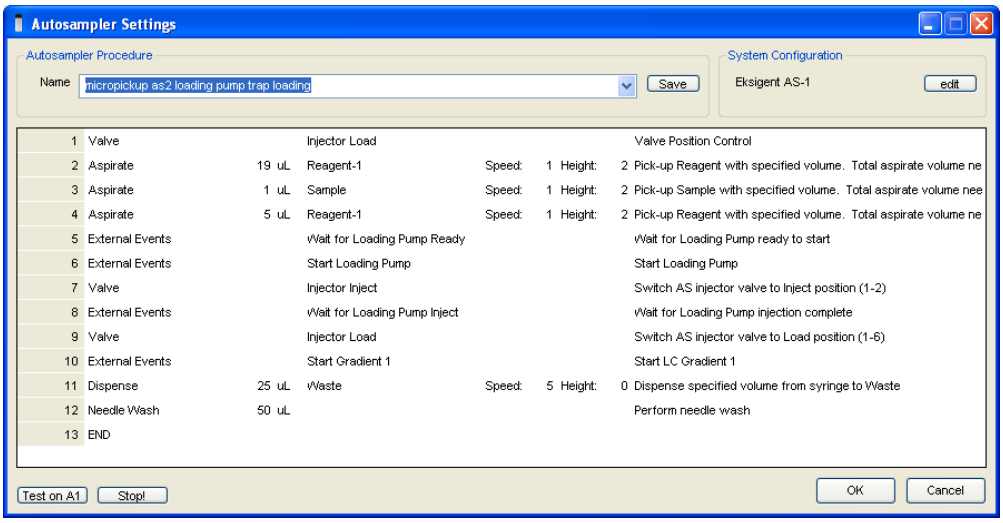


Figure C-36 Autosampler Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D+ System

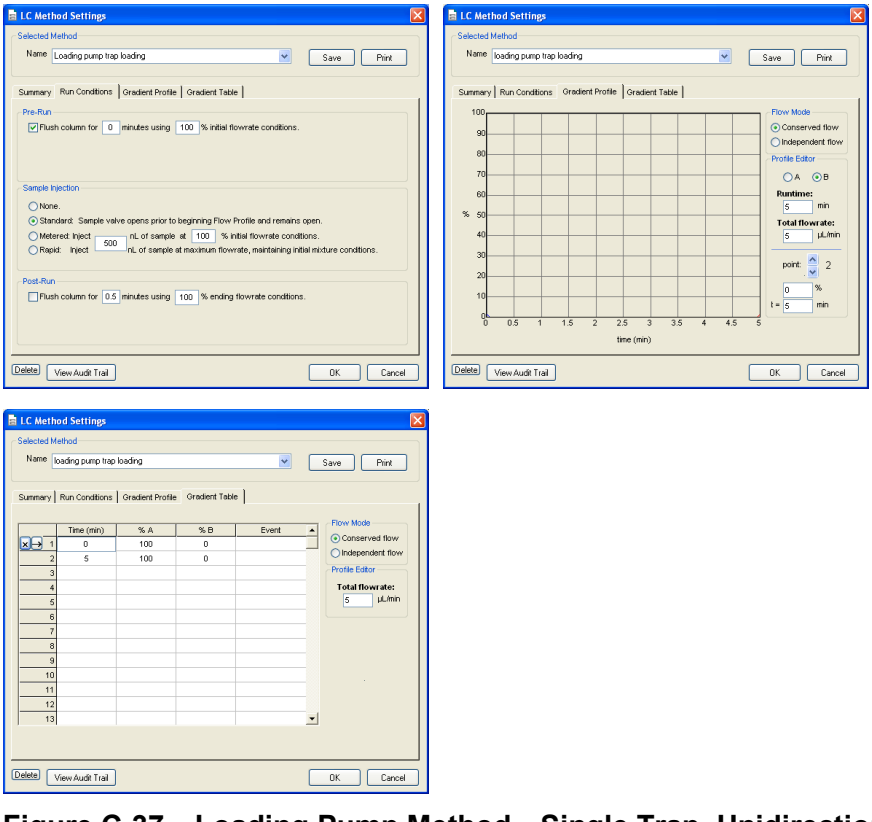


Figure C-37 Loading Pump Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D+ System

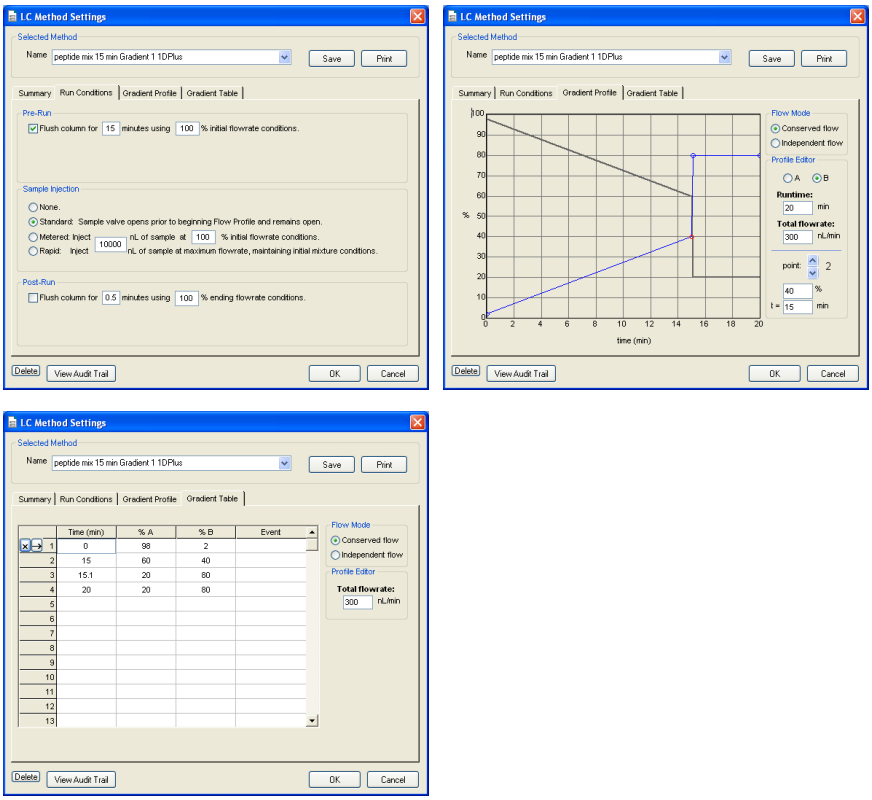


Figure C-38 Gradient 1 Method—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D+ System

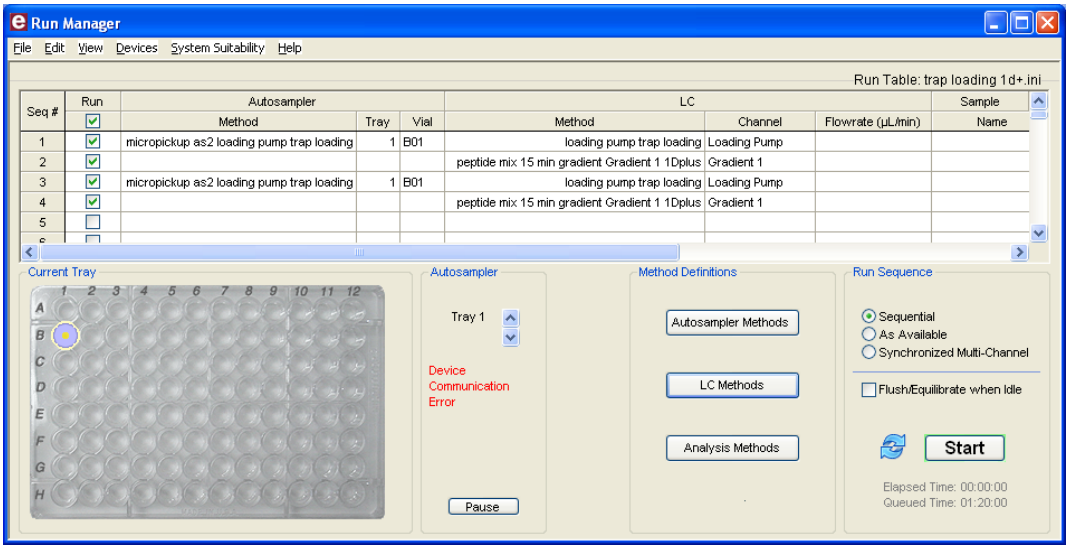


Figure C-39 Sequence Setup—Single Trap, Unidirectional Flow for the NanoLC Ultra 2D+ System

C.6.3 Single Trap Bidirectional Flow

Bidirectional trap and vented column method programming is the same as above trap loading methods.

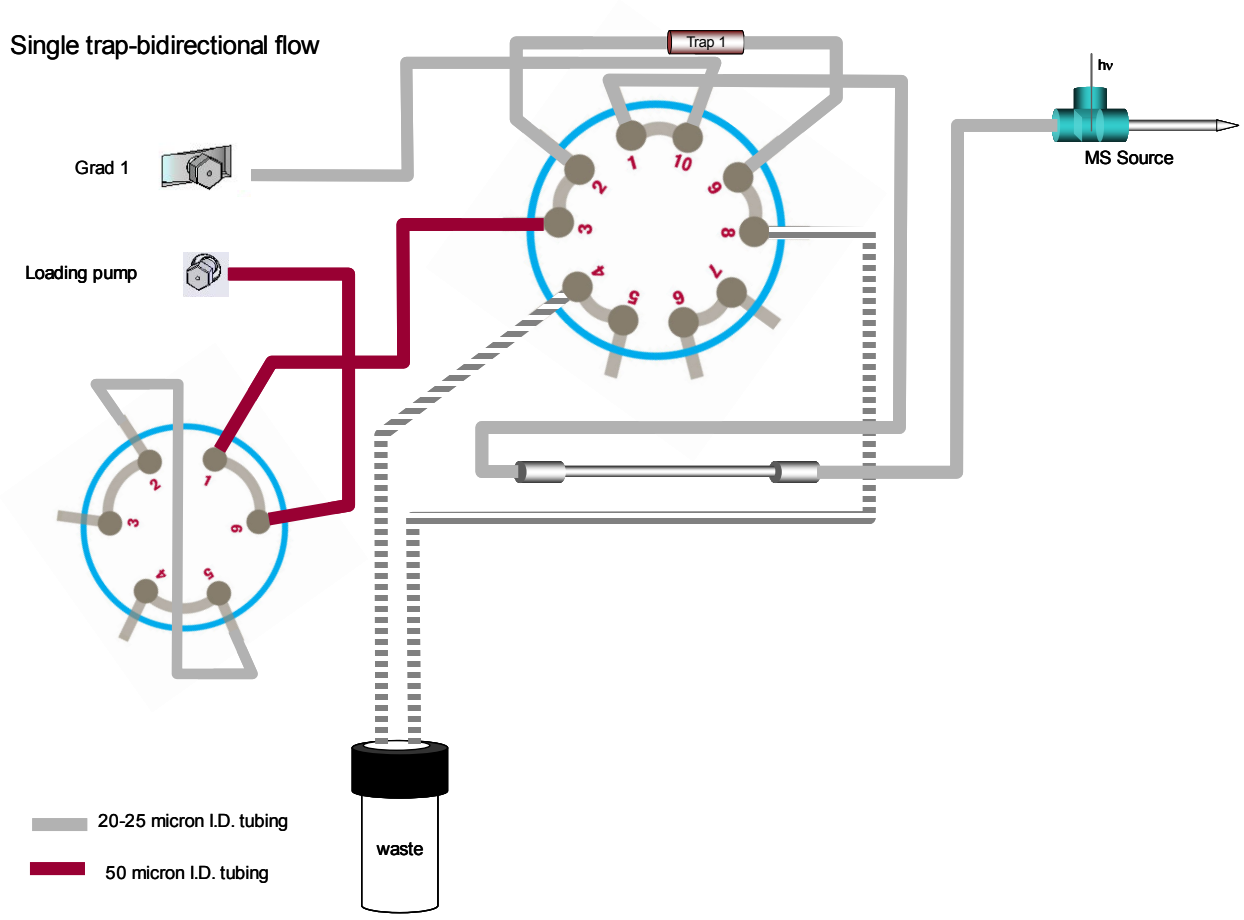


Figure C-40 Plumbing Diagram—Single Trap, Bidirectional Flow for the NanoLC Ultra 2D+ System

Bidirectional Trap

Bidirectional trap and vented column method programming is the same as above trap loading methods.

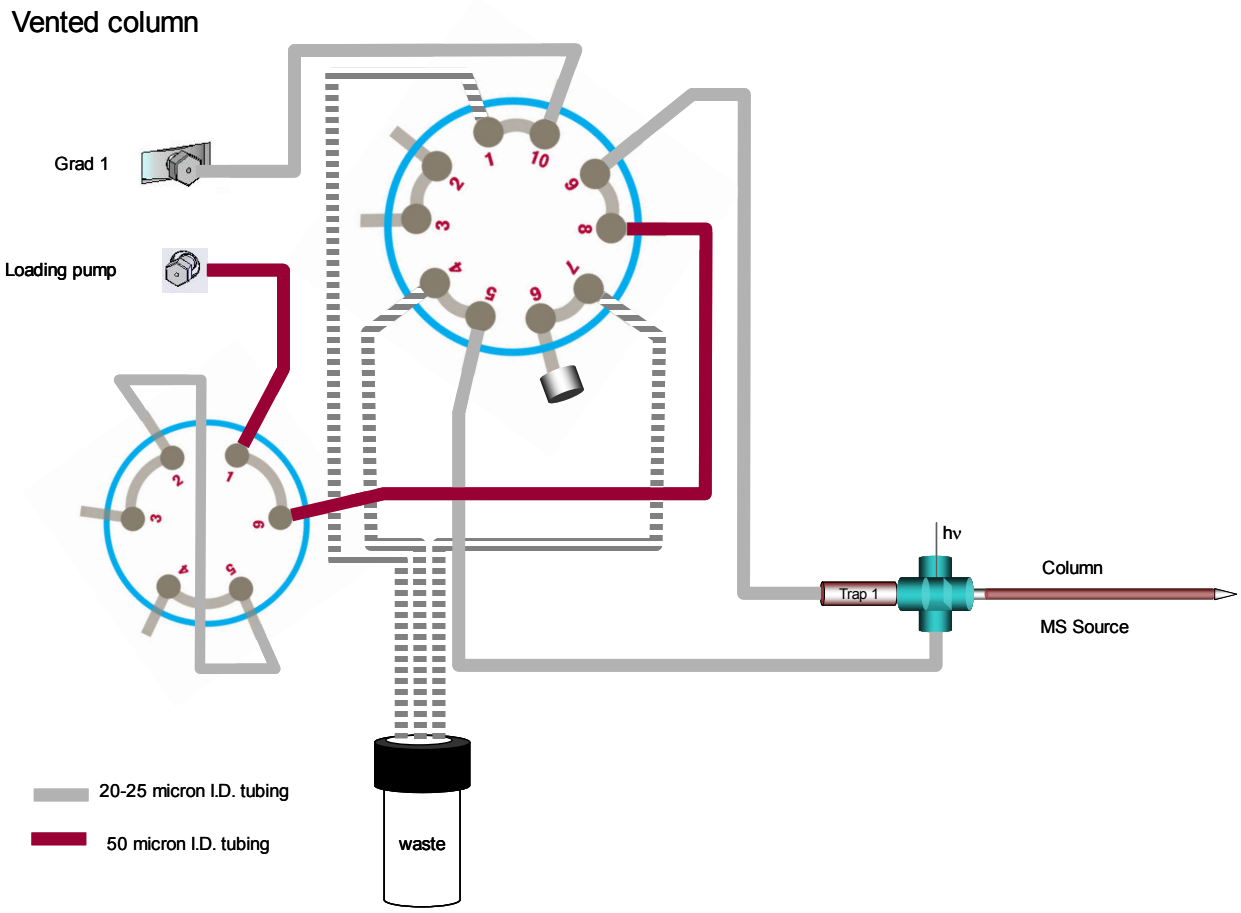


Figure C-41 Plumbing Diagram—Bidirectional Trap Injection for the NanoLC Ultra 2D+ System

C.6.4 Dual Trap

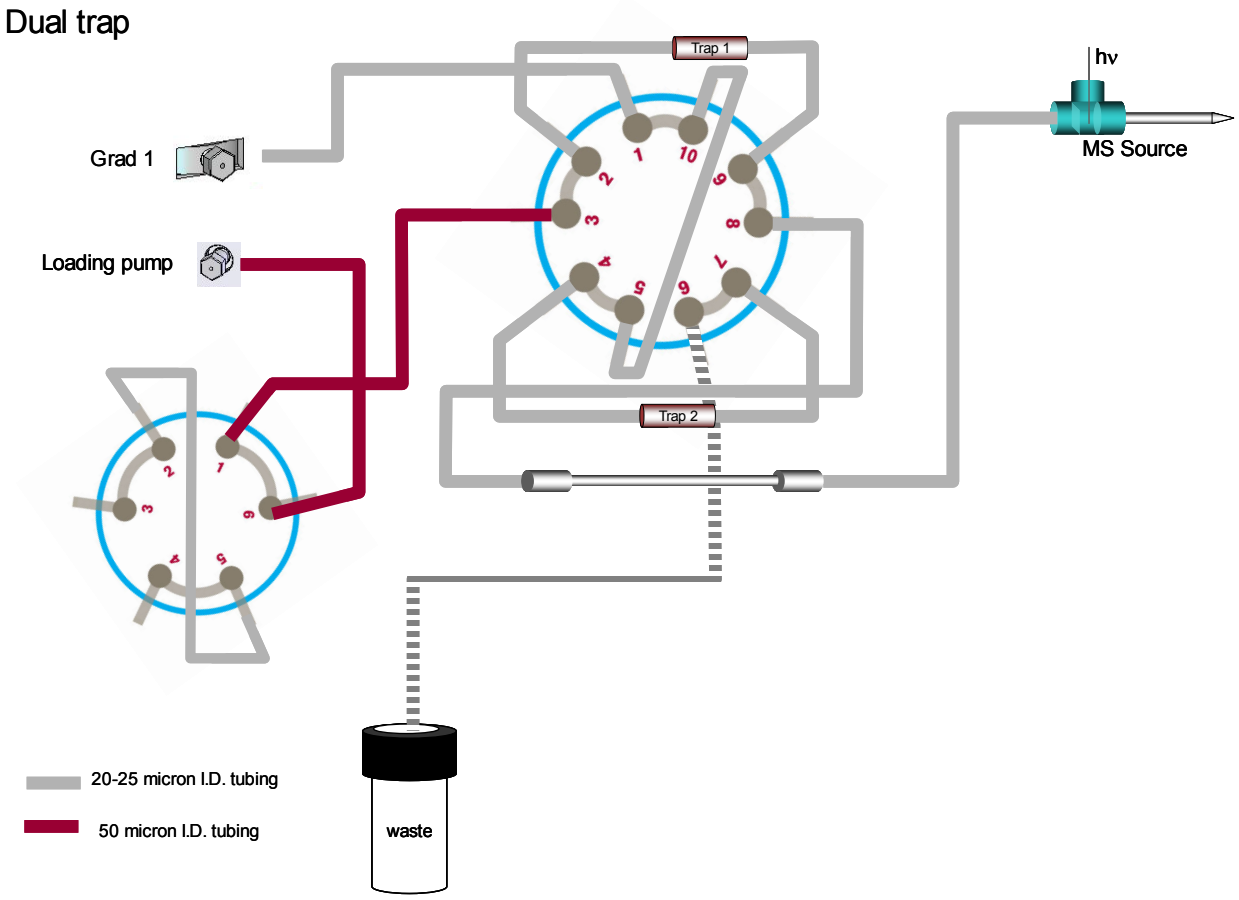


Figure C-42 Plumbing Diagram—Dual Trap Injection for the NanoLC Ultra 2D+ System

Appendix D
NanoLC™ AS-2 Autosampler Maintenance

Aspiration issues with the NanoLC AS-2 autosampler are usually caused by a restriction in the aspiration path. This chapter contains a maintenance schedule for the autosampler and describes how to test for and solve aspiration issues.

D.1 Maintenance Schedule

Table D-1 Maintenance Schedule

Maintenance Procedure	Frequency
Perform an initial wash, using the Autosampler Configuration dialog.	Daily
Change the Reagent-1 solution and refill to the proper level (on most vials, this level is indicated by blue wavy lines printed on the vial).	Weekly
Fill the wash solution with degassed 20% isopropanol in water.	Monthly

D.2 Test the Aspiration Path

The autosampler uses a syringe to aspirate liquid through the sample needle and into the sample loop. If a clog or damage to one of the components in the aspiration path occurs, then the autosampler may aspirate less than the programmed volume.

Symptoms of a restriction in the aspiration path include lower than expected signal intensity, air bubbles in the autosampler syringe or aspiration volumes less than the programmed volume. Each of these symptoms can result from a clog or restriction in the aspiration path.

When an aspiration issue is suspected, test the aspiration of the autosampler. During this test, a sample vial is weighed before and after aspiration and the volume aspirated is calculated and compared to a theoretical value. When the actual aspiration is less than the theoretical volume, there is an aspiration issue.

Required Materials
• Pipettor • Small vial • Water • Balance capable of accurately weighing 5 mg (0.005 g) or less

1. Pipette 25 µL water into a sample vial.
2. Weigh the vial.
3. Place the vial in the autosampler tray or rack.
4. Perform an initial wash to remove any air bubbles in the syringe:
   a. In the **Run Manager** window of the Eksigent control software, click **Devices > Autosampler Device Settings** to open the Autosampler Configuration dialog.
   b. In the **Autosampler Configuration** dialog, click the **Direct Control** tab.
   c. In the **Initial Wash** group, click **Start**.

   It may be necessary to replace the wash solution with 100% isopropanol to more effectively remove bubbles. Also, air may need to be removed from the syringe by removing the syringe and flushing it with clean wash solvent.
5. Run a method that aspirates 5 µL of sample from the vial.

   For an example, refer to Figure D-1.

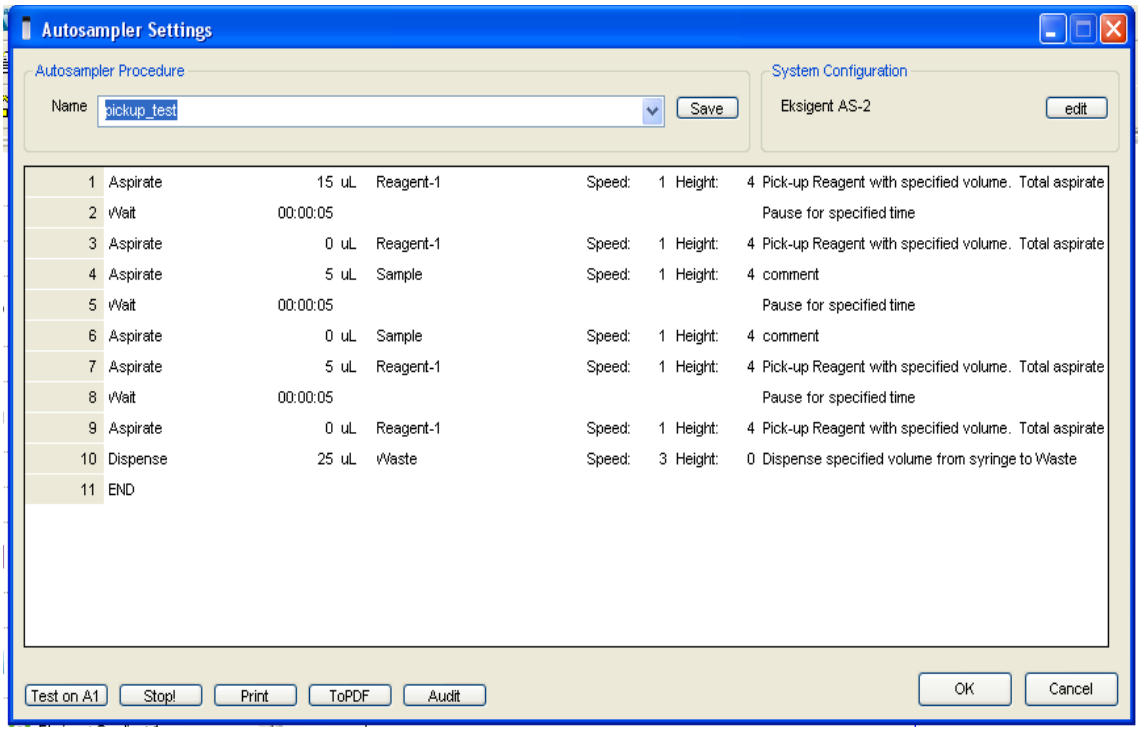


Figure D-1 Autosampler Settings Dialog–AS-2 Aspiration Test Method

6. When the aspiration is complete, weigh the vial.
7. Subtract the post-aspiration weight from the initial weight.

   5 µL of water weighs 5 mg (0.005 g) and a difference of 4.4 mg to 5.6 mg is acceptable.

   If the weight difference is less than 4.4 mg, then there may be a clog in the aspiration path. The most common sites for a restriction of the aspiration path are:

   - Sample needle, including union
   - Buffer tubing
   - Valve, including the stator and rotor seal
   - Sample loop
   - Syringe
   - Syringe valve

Follow the procedures below to test and, if needed, replace the components of the aspiration path.

D.3 Test the Sample Needle

The most common source of a clog in the aspiration path is in the sample needle or the sample needle union. This test describes how to assess the sample needle for a clog, and how to isolate the issue to the sample needle union, as appropriate.

1. Remove the sample needle from the autosampler (refer to “Replace the Sample Needle”).
2. Attach the sample needle to the outlet of the high-flow pump. Depending on the exact configuration of LC, this is most commonly the loading pump or Gradient 1.
3. In the Eksigent control software **Acquisition** window, select **System > Direct Control** to display the Direct Control dialog.
4. In the Direct Control dialog, start flow through the needle at 10 µL/min. If using a gradient channel, use 95% A.
5. In the Acquisition window, observe Pc.
   - If Pc is less than 5 psi, the sample needle and union are not clogged. If there is an aspiration issue, the issue is probably in another part of the aspiration path.
   - If Pc is greater than 5 psi, the sample needle or union may be clogged. Replace the sample needle union. Use either of the following unions:
     - Black 150 µM ID union, (PN 5016577)
     - Tan microtight union (PN 200-00072)
6. After the replacement union is installed, start flow and observe Pc.
   - If Pc is less than 5 psi, reinstall the sample needle and retest the aspiration.
   - If Pc is still greater than 5 psi, follow the instructions below to replace the sample needle.

D.3.1 Replace the Sample Needle

Follow this procedure to replace the sample needle.

Required Materials
• Sample needle (PN 620-00148)

1. In the **Run Manager** window of the Eksigent control software, click **Devices > Autosampler Device Settings** to open the Autosampler Configuration dialog.
2. Click the **Direct Control** tab.

3. Click **Needle Exchange** and follow the on-screen instructions (Figure D-2).

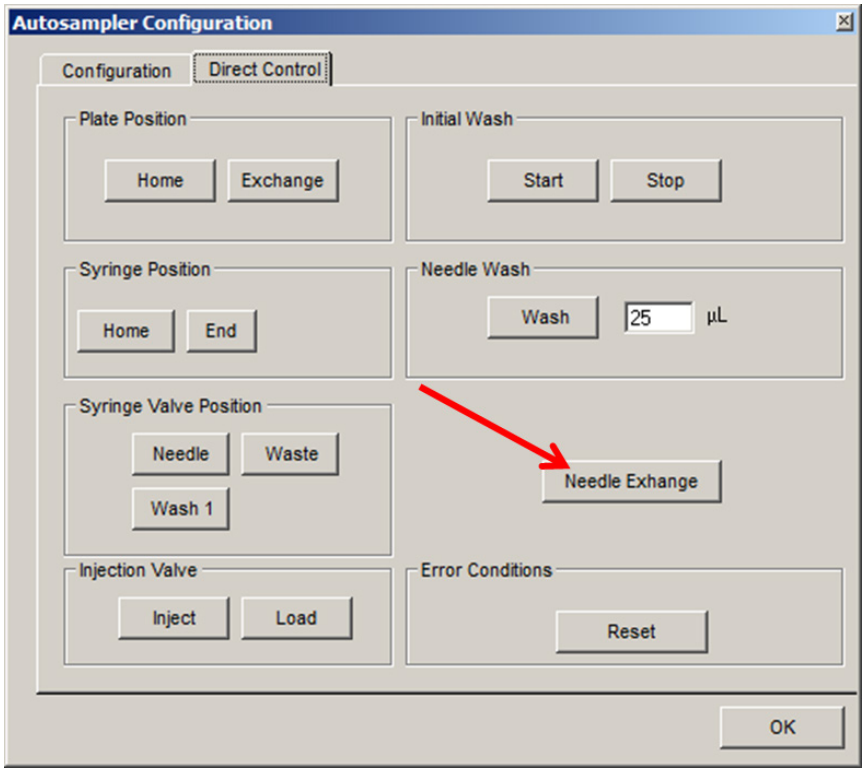


Figure D-2 Autosampler Configuration Dialog Indicating the Needle Exchange Button

4. Wait for the autosampler to move the needle to the exchange position.
5. Disconnect the sample needle from the 6-port valve.
6. Gently push the sample needle out of the retaining clip, if present (Figure D-3). (The retaining clip is not present on all NanoLC AS-2 autosamplers.)

7. Unscrew the air nut by rotating it counter-clockwise (Figure D-3).

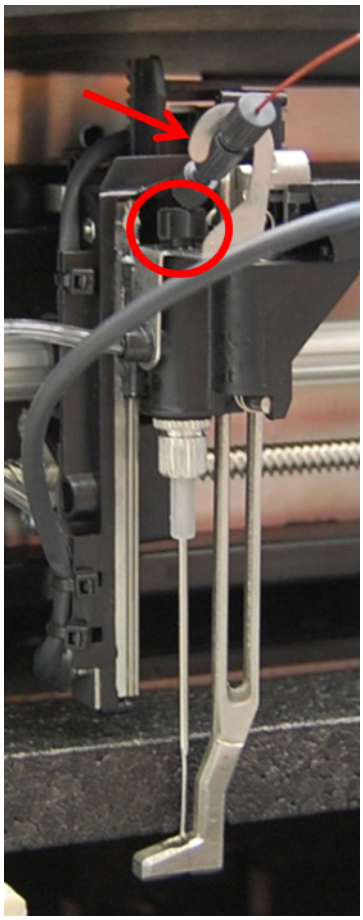


Figure D-3 Sample Needle Retaining Clip (Arrow) and the Air Nut (Circle)

8. Pull up on the sample needle to remove it from the sample needle assembly.
9. Insert a new needle into the sample needle assembly. There is a small hole into which the needle is inserted that is nearly impossible to see, so do this by feel.
10. Tighten the air nut finger tight. Take care to not cross-thread the nut.
11. Fasten the metal clip around the sample needle, if the clip is present.
12. Connect the sample needle to the 6-port valve.
13. Click **OK** after the needle has been exchanged.
14. Perform an initial wash to remove air from the new sample needle:
    a. In the **Autosampler Configuration** dialog, click the **Direct Control** tab.
    b. In the **Initial Wash** group, click **Start** (Figure D-9).
15. Click **OK** to close the Autosampler Configuration dialog.

16. Perform an aspiration test (refer to “Test the Aspiration Path”).

D.4 Replace the Buffer Tubing

The second most common place for a restriction in the aspiration path is in the buffer tubing. The buffer tubing runs between the 6-port valve and the syringe valve (located above the syringe). This tubing is very thin-walled and easy to damage by kinking, crimping, or bending.

Inspect the buffer tubing for any damage, paying special attention to the location where the tubing connects to the syringe valve and where it connects to the 6-port valve (Figure D-4).

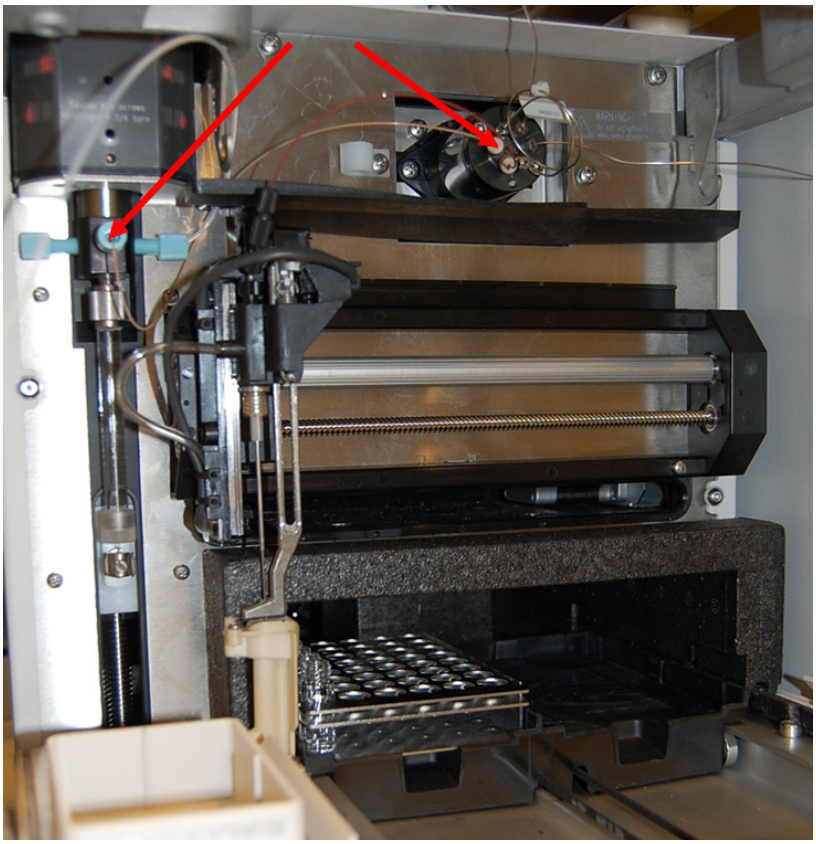


Figure D-4 Location of Buffer Tubing Connections

If the buffer tubing needs replacement, take care to not damage the replacement tubing.

Required Materials
• Replacement tubing set with fittings (PN 620-00149)

1. Remove the butter tubing.
   a. Remove the blue fitting for the buffer tubing from the front of the syringe valve.
   b. Remove the finger-tight fitting for the buffer tubing from the 6-port valve.

c. Remove the buffer tubing from the autosampler.

2. Place a finger-tight fitting one end of the buffer tubing.
3. Place a small PEEK ferrule on the tubing as shown in Figure D-5.

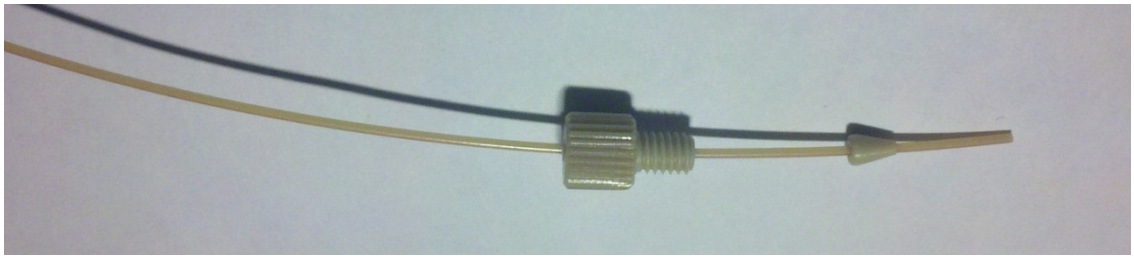


Figure D-5 Buffer Tubing Fittings at the 6-port Valve

4. Carefully place the tubing, ferrule, and fitting into the 6-port valve and tighten finger-tight.
5. Insert a blue fitting onto the other end of the new buffer tubing.
6. Insert the tubing carefully into the conical end of a new white ferrule as shown in Figure D-6.

   Make sure that the tubing does not pass through the ferrule and out of the flat end of the ferrule. Do not try to force the tubing through the ferrule; it does not fit and the tubing will be damaged.

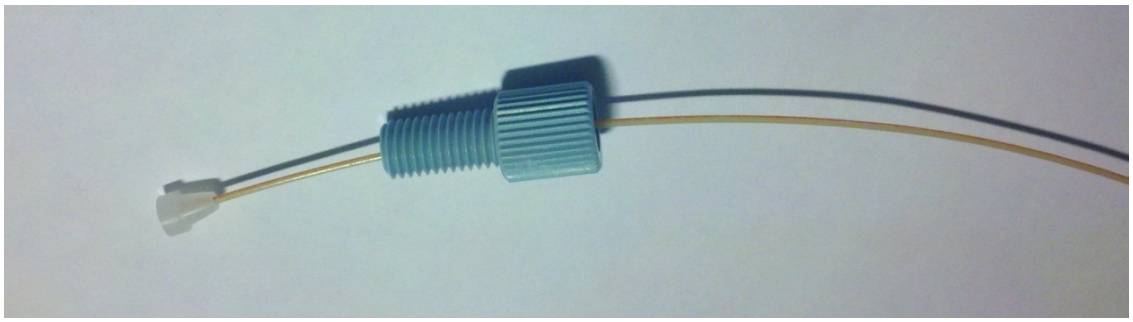


Figure D-6 Buffer Tubing Fittings at the Syringe Valve

7. Insert the fitting and ferrule into the front port of the syringe valve and tighten finger-tight.
8. Perform three Initial Wash cycles to clear the new buffer tubing of air.
9. Perform an aspiration test (refer to “Test the Aspiration Path”).

D.5 Replace the Rotor Seal and Stator

The stator is the front portion of the valve, containing the ports. The rotor seal is behind the stator and forms the liquid seal between the ports of the valve. If either of these parts is damaged, then aspiration or sample delivery issues can occur.

Required Materials

- To replace the seal and the stator: 6-port valve, includes valve body, rotor seal, and stator (PN 200-00335)

  or

- To replace only the rotor seal: rotor seal (PN 200-00334)
- Sonicator
- Methanol
- 9/64 inch hex wrench

1. Remove all fittings from the valve
2. Using a 9/64 in hex wrench, remove the two stator bolts.
3. Carefully remove the stator from the valve body (Figure D-7).
4. Carefully remove the rotor seal from the valve body (Figure D-7).

Figure D-7 Six-port Valve Components

5. Inspect the rotor seal and stator for damage or wear.

6. If the stator is not to be replaced, sonicate it in 100% methanol for 10 minutes with the sealing side (the side that contacts the rotor seal) up (refer to Figure D-7).
7. Replace the rotor seal with the new one.
   a. Position the rotor seal so that the sealing side faces out, away from the valve body (Figure D-8).
   b. Align the tabs on the rotor seal with the notches in the valve rotor and carefully press into place.

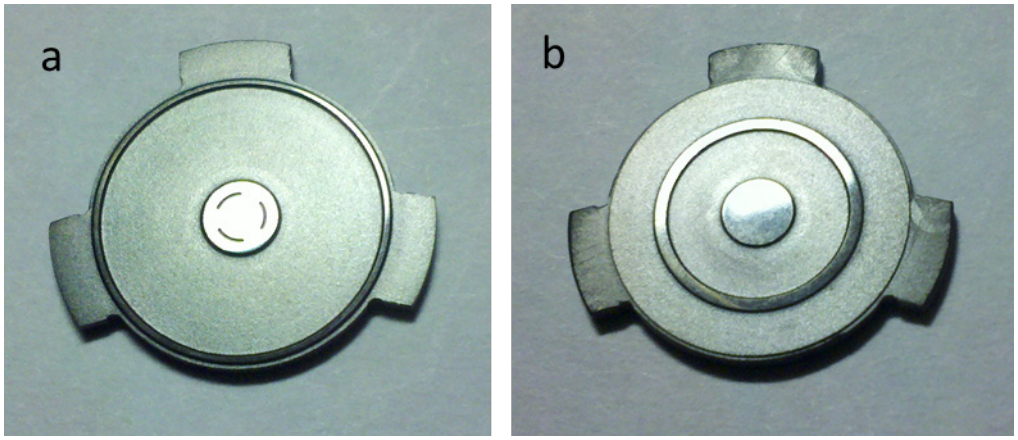


Figure D-8 Rotor Seal Details–Front, Sealing Side (a), and Back, Non-sealing Side (b)

8. Align the stator so that the bolt holes align and the pins fit into the pin holes and press the stator onto the valve body.
9. Place one of the bolts into the bolt hole and tighten until the bolt until slight resistance is felt.
10. Place the other bolt into the bolt hole and tighten until the bolt cannot go any further.
11. Tighten the first bolt completely. There are built-in stops so that the stator cannot be over-tightened.
12. Inspect the gap between the valve body and the stator. There should not be a gap.
13. Replace the fittings on the valve.
14. Switch the valve position between inject and load ten times.
    a. In the **Autosampler Configuration** dialog, click the **Direct Control** tab.

b. In the **Injection Valve** group, click **Inject** then **Load** (Figure D-9). Repeat until the valve has been switched ten times.

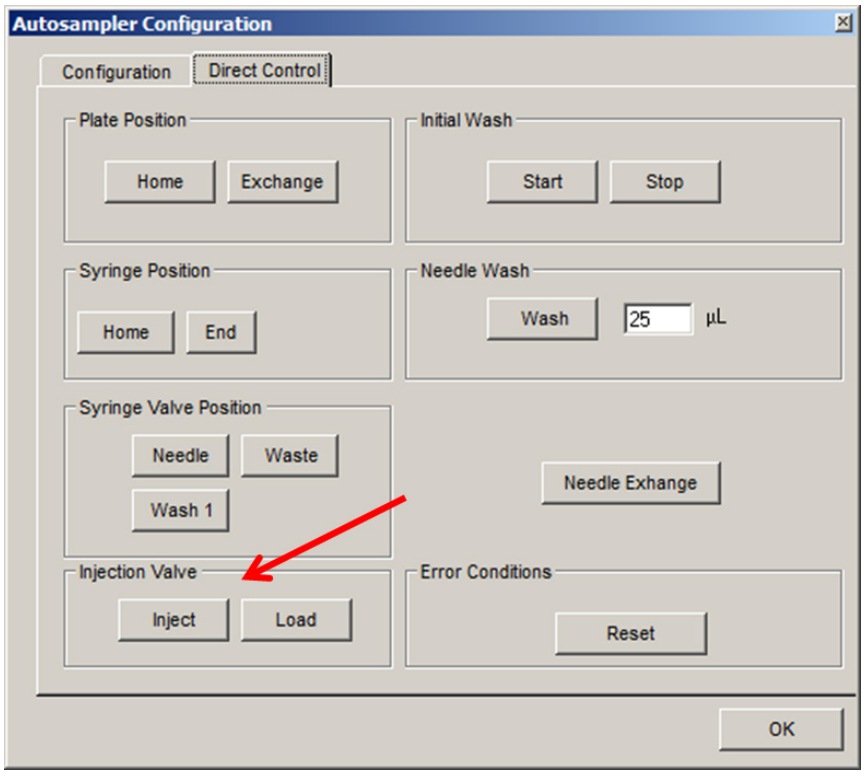


Figure D-9 Autosampler Configuration Dialog Indicating the Injection Valve Position Buttons

15. In the **Initial Wash** group, click **Start** (Figure D-9) to perform an initial wash.
16. Click **OK** to close the Autosampler Configuration dialog.
17. Perform an aspiration test (refer to "Test the Aspiration Path").

Note: If both the rotor seal and the stator were replaced, the new valve body will be left over.

D.6 Replace the Syringe

Another possible source of aspiration issues is the autosampler syringe, as this wears over time.

Note: Do not assume that the syringe needs replacement because there are air bubbles in the syringe. Air bubbles can mean that there is a restriction somewhere else in the aspiration path.

Required Materials
• 25 µL syringe (PN 620-00064) • Small screwdriver • Plastic pipette tip

1. Move the syringe to the end position.
   a. In the **Autosampler Configuration** dialog, click the **Direct Control** tab.
   b. In the **Syringe Position** group, click **End** (Figure D-10).

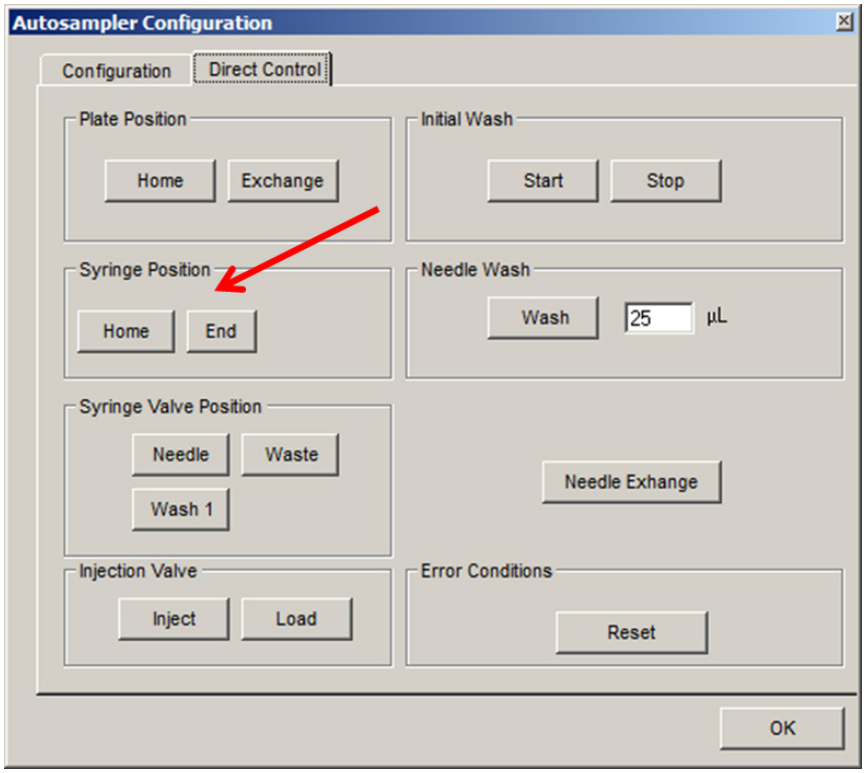


Figure D-10 Autosampler Configuration Dialog Indicating the Syringe Position Buttons

2. Remove the wash bottle from the front of the autosampler.
3. Carefully pull the plunger out of the plunger clip (Figure D-11).

A small slotted screwdriver can be used to help pry the plunger from the clip.

4. Remove the syringe from the syringe valve by rotating the top of the syringe clockwise (Figure D-11).

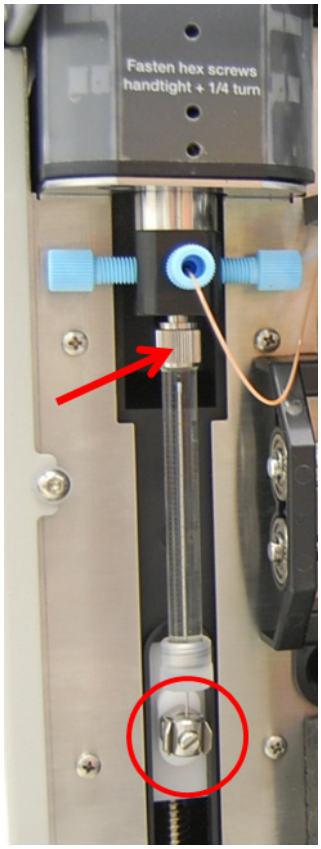


Figure D-11 Syringe Assembly Indicating the Plunger Clip (Circle) and Top of Syringe (Arrow)

5. Inside the syringe valve, use a plastic pipette tip to carefully remove the white Teflon seal. Be careful to not damage the syringe valve.
6. Fill the new syringe with the wash solvent, leaving a small amount of liquid on the tip of the syringe.
7. Clip the plunger into the plunger clip.
8. Place a new Teflon seal (included with the new syringe) on top of the syringe and carefully insert the syringe into the syringe valve.
9. Tighten the syringe finger-tight by rotating the top of the syringe counter-clockwise.
10. Perform several initial washes to clear any air in the syringe.
    a. In the **Autosampler Configuration** dialog, click the **Direct Control** tab.
    b. In the **Initial Wash** group, click **Start** (Figure D-10).

11. Perform an aspiration test (refer to “Test the Aspiration Path”).

D.7 Replace the Sample Loop

Another component of the aspiration path that can clog and lead to aspiration issues is the sample loop.

Required Materials
• 10 µL Sample loop (PN 910-00082)

1. Remove the sample loop from the 6-port autosampler valve.
2. Insert one end of the new sample loop into port 2 of the autosampler and tighten the nut. Leave the other end free.
3. Click **Inject** in the **Autosampler Configuration** dialog to switch the valve to the inject position (Figure D-9).
4. In the Direct Control dialog, start flow on the channel connected to port 1 of the autosampler (depending on the LC configuration, this is usually the loading pump or gradient 1). The flow rate depends on the channel:
   - For a micro-flow channel (such as the loading pump)–10 µL/min.
   - For a nano-flow channel–1000 nL/min.
5. Observe the free end of the sample loop for liquid. When liquid appears from the free end of the sample loop, lower the flow rate to a rate appropriate for any downstream connections, typically:
   - 3 µL/min for a trap
   - 300 µL/min for a column
6. Secure the free end of the sample loop into port 5 of the 6-port valve and tighten the fitting (refer to Figure D-12).
7. Perform an aspiration test (refer to “Test the Aspiration Path”).

D.8 Replace the Syringe Valve

The remaining element of the aspiration path is the syringe valve. If the sample needle, valve components, sample loop, buffer tubing, and syringe have been eliminated, then the syringe valve may need to be replaced by an AB SCIEX Field Service Employee. Contact AB SCIEX technical support to schedule an appointment.

D.9 Plumbing Diagram

A plumbing diagram for the basic autosampler configuration is shown in Figure D-12.

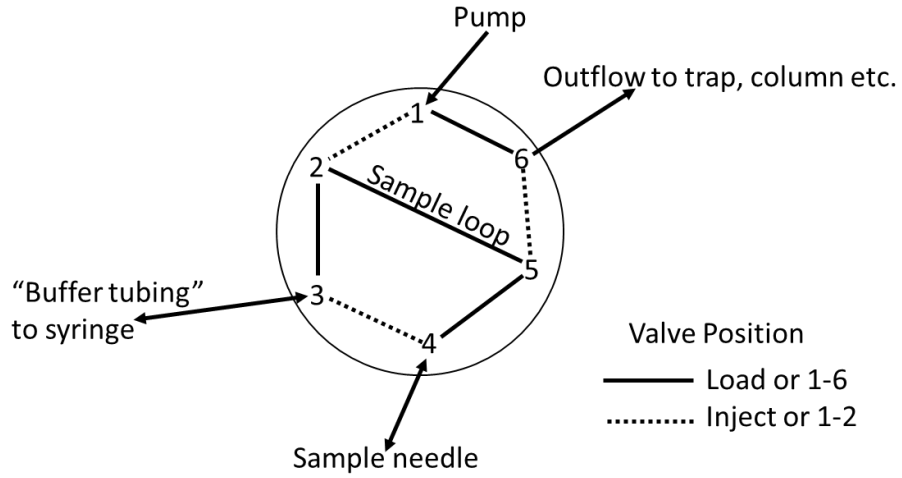


Figure D-12 Autosampler Plumbing Diagram

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