M5 MicroLC Systems

Operator Guide

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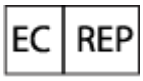

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Contents

Operational Precautions and Limitations 1

Note: Before operating the system, carefully read all of the sections of this guide.

This section contains general safety-related information and provides regulatory compliance information. It also describes potential hazards and associated warnings for the system and the precautions that should be taken to minimize the hazards.

In addition to this section, for information about the symbols used in the laboratory environment, on the system, and in this documentation, refer to the section: Glossary of Symbols. For site requirements, refer to the document: *Site Planning Guide*.

General Safety Information

To prevent personal injury or system damage, read, understand, and obey all of the safety precautions and warnings in this document, the manufacturer chemical safety data sheets (SDSs), and product label information. Labels are shown with internationally recognized symbols. Failure to heed these warnings could result in serious injury.

This safety information is intended to supplement federal, state, provincial, and local environmental health and safety (EHS) regulations. The information provided covers system-related safety information applicable to the operation of the LC system. It does not cover every safety procedure that should be practiced. Ultimately, the user and the organization are responsible for compliance with federal, state, provincial, and local EHS regulations and for maintaining a safe laboratory environment.

Refer to the correct laboratory reference material and standard operating procedures.

Regulatory Compliance

This system complies with the regulations and standards listed in this section. For dated references, refer to the declaration of conformity included with the system and the individual system components. Applicable labels have been affixed to the system.

Australia and New Zealand

- **Electromagnetic Compatibility (EMC):** Radio Communications Act 1992 as implemented in these standards:
  - Electromagnetic Interference—AS/NZS CISPR 11/ EN 55011/ CISPR 11 (Class A). Refer to the section: Electromagnetic Interference.

- **Safety:** AS/NZ 61010-1 and IEC 61010-2-081

Canada

- **Electromagnetic Interference (EMI):** CAN/CSA CISPR11. This ISM device complies with Canadian ICES-001. Refer to the section: Electromagnetic Interference.
- **Safety:**
  - CAN/CSA C22.2 No. 61010-1

Europe

- **Electromagnetic Compatibility (EMC):** Electromagnetic Compatibility Directive 2014/30/EU as implemented in these standards:
  - EN 61326-1
  - EN 55011 (Class A)

  Refer to the section: Electromagnetic Compatibility.
- **Safety:**
  - EN 61010-1
- **Waste Electrical and Electronic Equipment (WEEE):** Waste Electrical and Electronic Equipment Directive 2012/96/EEC, as implemented in EN 40519. Refer to the section: Waste Electrical and Electronic Equipment.
- **Packaging and Packaging Waste (PPW):** Packaging and Packaging Waste Directive 94/62/EC
- **RoHS Restriction of Hazardous Substances:** RoHS Directive 2011/65/EU and 2015/863/EU

United States

- **Radio Emissions Interference Regulations:** 47 CFR 15, as implemented in FCC Part 15 (Class A)
- **Safety:** Occupational Safety and Health Regulations, 29 CFR 1910, as implemented in these standards:
  - UL 61010-1

International

- **Electromagnetic Compatibility (EMC):**
  - IEC 61326-1

- IEC CISPR 11 (Class A)
- IEC 61000-3-2
- IEC 61000-3-3

Refer to the section: Electromagnetic Compatibility.

- **Safety:**
  - IEC 61010-1

Electrical Precautions

WARNING! Electrical Shock Hazard. Do not remove the covers. Removing the covers might cause injury or malfunctioning of the system. The covers need not be removed for routine maintenance, inspection, or adjustment. Contact a SCIEX Field Service Employee (FSE) for repairs that require the covers to be removed.

- Follow required electrical safe work practices.
- Use cable management practices to control electrical cables. This will decrease the chance of a tripping hazard.

For information about system electrical specifications, refer to the document: *Site Planning Guide*.

Mains Supply

Connect the system to a compatible mains supply as instructed in this guide.

WARNING! Electrical Shock Hazard. Use only qualified personnel for the installation of all of the electrical supplies and fixtures, and make sure that all of the installations adhere to local regulations and safety standards.

WARNING! Electrical Shock Hazard. Use only the mains supply cables supplied with the system. Do not use mains supply cables that are not properly rated for the operation of this system.

CAUTION: Potential System Damage. Do not unpack or connect any system components. The FSE will unpack, connect, and configure the system for the correct operating voltage.

Protective Earth Conductor

The mains supply must include a correctly installed protective earth conductor. The protective earth conductor must be installed or examined by a qualified electrician before the system is connected.

WARNING! Electrical Shock Hazard. Do not intentionally interrupt the protective earth conductor. Any interruption of the protective earth conductor creates an electrical shock hazard.

WARNING! Electrical Shock Hazard. Connect the protective earth connector to the mass spectrometer carefully. Refer to the mass spectrometer *Safety Guide* or *System User Guide* for instructions. The combination of the pump and the integrated autosampler with a mass spectrometer might require additional safety measures as described by SCIEX.

Chemical Precautions

WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Identify whether decontamination is required before cleaning or maintenance. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.

WARNING! Environmental Hazard. Do not dispose of system components in municipal waste. Follow local regulations when disposing of components.

CAUTION: Potential System Damage. Do not submerge the end of the drain tubing in the waste liquid in the waste container.

CAUTION: Potential System Damage. Make sure that there are no loops in the drain tubing that goes to the waste container.

- Identify which chemicals have been used in the system before service and regular maintenance. For the health and safety precautions that must be followed for a chemical, refer to the safety data sheet (SDS). For storage information, refer to the certificate of analysis. To find a SCIEX safety data sheet or certificate of analysis, go to sciex.com/tech-regulatory.
- Always wear assigned personal protective equipment, including powder-free gloves, safety glasses, and a laboratory coat.

  Note: Nitrile or neoprene gloves are recommended.
- Work in a well-ventilated area or fume hood.
- Avoid ignition sources when working with flammable materials, such as isopropanol, methanol, and other flammable solvents.
- Take care in the use and disposal of any chemicals. There is a potential risk of personal injury if correct procedures for handling and disposal of chemicals are not followed.

- Avoid skin contact with chemicals during cleaning, and wash hands after use.
- Make sure that all exhaust hoses are connected correctly and that all connections are functioning as designed.
- Collect all spent liquids and dispose of them as hazardous waste.
- Comply with all of the local regulations for the storage, handling, and disposal of biohazardous, toxic, and radioactive materials.

System Safe Fluids

The following fluids can safely be used with the system.

Mobile phases should be compatible with the following materials: 316L stainless steel, PTFE, FEP, PEEK, sapphire, glass, and fused silica. The pH of the mobile phases should be between pH 1 and pH 10.

CAUTION: Potential System Damage. Do not use any other fluid until confirmation is received from SCIEX that it does not present a hazard. This is not an exhaustive list.

Note: Use only new, freshly prepared LC-MS-grade or better solvents for the LC mobile phases.

- **Organic Solvents**
  - LC-MS-grade acetonitrile; up to 100%
  - LC-MS-grade methanol; up to 100%
  - LC-MS-grade isopropanol; up to 100%
  - LC-MS-grade or higher water; up to 100%
- **Buffers**
  - Ammonium acetate; less than 100 mM
  - Ammonium formate; less than 100 mM
- **Acids and Bases**
  - Formic acid; less than 1%
  - Acetic acid; less than 1%
  - Trifluoroacetic acid (TFA); less than 1%
  - Heptafluorobutyric acid (HFBA); less than 1%
  - Ammonia/ammonium hydroxide; less than 1%

Environmental Precautions

Use qualified personnel for the installation of electrical mains, heating, ventilation, and plumbing supplies and fixtures. Make sure that all of the installations comply with local bylaws and biohazard regulations. For information about the required environmental conditions for the system, refer to the document: *Site Planning Guide*.

Allow access space around the equipment when setting up the system.

WARNING! Biohazard. For biohazardous material use, always comply with local regulations for hazard assessment, control, and handling. This system or any part is not intended to act as a biological containment.

WARNING! Environmental Hazard. Follow established procedures for disposal of biohazardous, toxic, radioactive, and electronic waste. The customer is responsible for disposal of hazardous substances, including chemicals, waste oils, and electrical components, in accordance with local laws and regulations.

Electromagnetic Environment

Electromagnetic Compatibility

Basic Electromagnetic Environment: Environment existing at locations characterized by being supplied directly at low voltage from the public mains network.

Performance Criteria A (Criteria A): Equipment shall operate as intended with no degradation of performance and no loss of function during or after the test.

Performance Criteria B (Criteria B): Equipment may experience loss of function (one or more) during the test but shall operate as intended with some degradation of performance and functions self-recoverable after the test.

Performance Criteria C (Criteria C): Equipment may experience loss of function (one or more) during the test but shall operate as intended with some degradation of performance and functions recoverable by an operator after the test.

The equipment is intended for use in a basic electromagnetic environment.

Make sure that a compatible electromagnetic environment for the equipment can be maintained so that the device will operate as intended. If the power supply line is subject to high electrical noise, then install a surge protector.

Electromagnetic Interference

Group 1 Equipment: This equipment is classified as industrial, scientific, and medical (ISM) equipment that might use RF energy for internal operation.

Class A Equipment: Equipment which is suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes. [Derived from CISPR 11:2009, 5.3] Class A equipment shall meet Class A limits.

CAUTION: Potential Radio Interference. This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC (Federal Communications Commission) Compliance Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the operator's manual, can cause harmful interference to radio communications.

Operation of this equipment in a residential area is likely to cause harmful interference in which case you will be required to correct the interference, at your own expense. Changes or modifications not expressly approved by the manufacturer could void your authority to operate the equipment.

Decommissioning and Disposal

WARNING! Environmental Hazard. Follow established procedures for disposal of biohazardous, toxic, radioactive, and electronic waste. The customer is responsible for disposal of hazardous substances, including chemicals, waste oils, and electrical components, in accordance with local laws and regulations.

Before decommissioning, decontaminate the entire system following local regulations.

When removing the system from service, separate and recycle different materials according to national and local environmental regulations. Refer to the section: Storage and Handling.

Note: SCIEX will not accept any system returns without a completed *Decontamination Form*. Contact an FSE to obtain a copy of the form.

Do not dispose of system components or subassemblies, including computer parts, as unsorted municipal waste.

Waste Electrical and Electronic Equipment

Follow local municipal waste ordinances for proper disposal provisions to decrease the environmental impact of waste, electrical, and electronic equipment (WEEE). To safely dispose of this equipment, contact a local Customer Service office for complimentary equipment pick-up and recycling.

Laboratory Conditions

Safe Environmental Conditions

The system is designed to operate safely under these conditions:

- Indoors
- Altitude: Up to 2,000 m (6,560 ft) above sea level
- Ambient temperature: 15 °C (59 °F) to 30 °C (86 °F)
- Relative humidity: 20% to 80%, non-condensing
- Mains supply voltage fluctuations: ± 10% of the nominal voltage
- Transient overvoltages: Up to the levels of Overvoltage Category I
- Temporary overvoltages on the mains supply
- Pollution Degree 2

Equipment Use and Modification

WARNING! Personal Injury Hazard. Contact the SCIEX representative if product installation, adjustment, or relocation is required.

WARNING! Electrical Shock Hazard. Do not remove the covers. Removing the covers might cause injury or malfunctioning of the system. The covers need not be removed for routine maintenance, inspection, or adjustment. Contact a SCIEX Field Service Employee (FSE) for repairs that require the covers to be removed.

WARNING! Personal Injury Hazard. Use SCIEX-recommended parts only. Use of parts not recommended by SCIEX or use of parts for any purpose other than their intended purpose can put the user at risk of harm or negatively impact system performance.

WARNING! Crushing Hazard. Wear protective footwear when moving heavy objects.

WARNING! Puncture Hazard. Keep hands and loose objects away from the autosampler arm and syringe assembly during operation.

Use the system indoors in a laboratory that complies with the environmental conditions recommended in the document: *Site Planning Guide* or contact an FSE.

If the system is used in an environment or in a manner not prescribed by the manufacturer, then the performance and protection provided by the equipment might be impaired.

Contact an FSE for information on servicing the system. Unauthorized modification or operation of the system might cause personal injury and equipment damage, and might void the warranty. Erroneous data might be generated if the system is operated outside the recommended environmental conditions or with unauthorized modifications.

Qualified Personnel

Only qualified SCIEX personnel shall install, examine, and service the equipment. After installing the system, the Field Service Employee (FSE) uses the *Customer Familiarization Checklist* to orient the customer on system operation, cleaning, and basic maintenance. SCIEX might not cover the damage to a system under warranty if it is serviced by personnel not authorized by SCIEX.

Documentation Symbols and Conventions

The following symbols and conventions are used throughout the guide.

DANGER! Danger signifies an action that leads to severe injury or death.

WARNING! Warning signifies an action that could cause personal injury if precautions are not followed.

CAUTION: Caution signifies an operation that could cause damage to the system or corruption or loss of data if precautions are not followed.

Note: Note emphasizes significant information in a procedure or description.

Tip! Tip provides useful information that helps apply the techniques and procedures in the text for a specific need and provides shortcuts, but is not essential to the completion of a procedure.

Introduction 2

This guide describes the basic operation and critical parameters to consider for routine and robust operation of the SCIEX M5 MicroLC systems.

System Description

The M5 MicroLC system can do direct injection experiments. It has these components:

- Binary gradient pumping system with one of three flow rate configurations:
  - Low-flow: 1 µL/min to 10 µL/min
  - Micro-flow: 5 µL/min to 50 µL/min
  - High-flow: 20 µL/min to 200 µL/min
- 6-port stainless steel injection valve
- Solvent rack with multiple mounting options
- System accessory kit, with sample loops, a column, fittings, and other supplies
- Integrated CTC Analytics CTC PAL 3 autosampler, including:
  - Three cooled sample drawers
  - LCMS tool for high throughput with low carryover

The M5 MicroLC-TE system can do trap-and-elute or direct injection experiments. It includes all of the above, as well as:

- A second binary gradient pump configured for 20 µL/min to 200 µL/min flow rates
- A 6-port stainless steel auxiliary valve
- A trap column

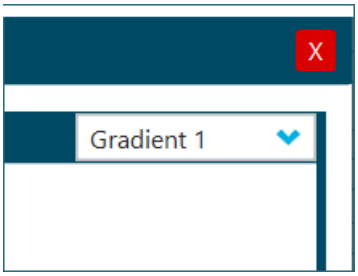
Channel Assignments in the Eksigent Control Software or the M5 MicroLC SCIEX OS Driver

The M5 MicroLC and M5 MicroLC-TE systems have one and two pump channels, respectively. The pumps are identified in this guide and in the software as Gradient 1 and Gradient 2.

In the software, select the channel in an active dialog or window by clicking the arrow beside the channel. The channel is usually shown in the top right corner of a dialog or window. Refer to the figure: Figure 2-1 or Refer to the figure: Figure 2-2.

Figure 2-1 Channel Selection Controls for Eksigent Control Software

Figure 2-2 Channel Selection Controls for M5 MicroLC SCIEX OS Driver



Theory of Operation

The M5 MicroLC system is a high-pressure liquid chromatography (HPLC) system optimized for analysis at flow rates from 1 µL/min to 200 µL/min. The system incorporates the Microfluidic Flow Control (MFC) system technology to generate precise LC gradients at microflow rates. The system also includes the CTC PAL 3 autosampler with the LCMS tool designed to minimize sample carryover.

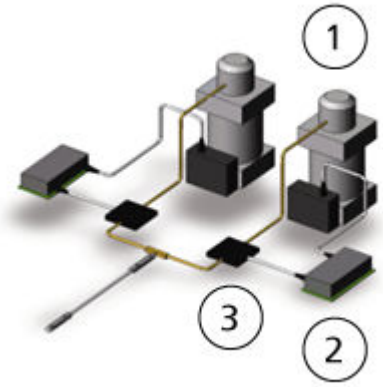
Microfluidic Flow Control System Technology

The Microfluidic Flow Control (MFC) system technology has two primary benefits:

- Precise gradients at microliter-per-minute rates without flow splitting
- Extremely rapid response to changes in flow enabling fast gradients and dynamic flow control

The components of a binary gradient MFC system are shown schematically in the following figure.

Figure 2-3 Microfluidic Flow Control System Components



Item	Description
1	Electronically controlled pressure source
2	Proportional-integral-derivative (PID) controller
3	Flowmeter

How the Microfluidic Flow Control System Technology Works

For each mobile phase:

1. The flowmeter continuously monitors the flow rate and sends signals to the PID controller, a control loop feedback device.
2. The PID controller automatically adjusts the pump pressure to deliver the required flow rate.
3. The air pressure to each pump is controlled separately to provide accurate flow rates at pressures up to 10,000 psi.

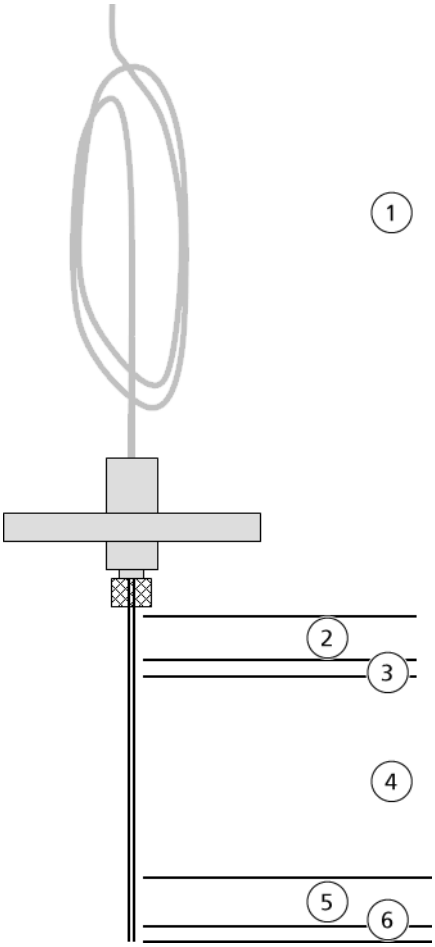
The LCMS Tool on the CTC PAL 3 Autosampler

The LCMS tool is part of the CTC PAL 3 autosampler and consists of the syringe and syringe needle, a holding loop, two pumps, two wash solvents, and associated tubing. The LCMS tool helps prevent carryover in three ways:

- The system is designed so that the sample only comes in contact with the needle and the holding loop.
- Small volumes of air can be used to bracket both sides of the sample plug, preventing the sample from diffusing into the solvent.
- At the end of the injection, all parts of the system that have been in contact with the sample are washed with both organic and aqueous wash solvents.

The LCMS Tool Syringe Needle and Holding Loop

Figure 2-4 Holding Loop and Syringe Needle, Showing Location of Sample and Air Gap



Item	Description
1	Holding loop
2	Rear airgap volume (air)
3	Rear volume (sample)
4	Injection volume (sample)
5	Front volume (sample)
6	Front airgap volume (air)

For the Analyst software, do the following:

- Set the injection volume in the acquisition batch in the Analyst software.
- Set the front and rear airgap volumes in one of the three of the autosampler scripts supplied with the M5 MicroLC system.
- Set the front and rear volumes in the M5_Advanced script.

For SCIEX OS software, do the following:

- Set the injection volume in the acquisition batch in SCIEX OS.
- Set the front and rear airgap volumes in the autosampler scripts supplied with the M5 MicroLC system under the Autosampler tab in the LC method window in the M5 MicroLC driver section.
- If Show Advanced Parameters is enabled on the Autosampler tab in the LC method window in the M5 MicroLC driver section, then set the front and rear volumes in the M5_Advanced script.

About Partial and Full-loop Injections

Depending on the sample loop that is installed on the system and the volume of sample available, the sample loop can either be partially or completely filled.

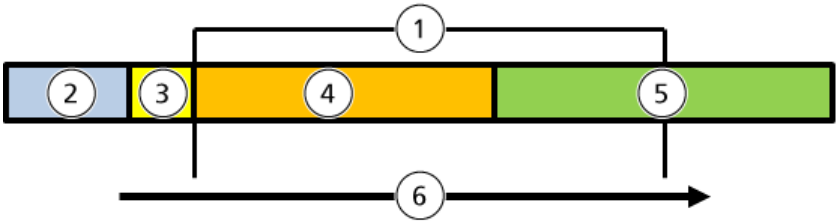
Full loop injections typically give the best quantitative results but require overfilling of the sample loop and therefore result in some sample waste. In full loop injections, the volume of the sample loop controls the sample volume. Partial loop injections allow for variable injection volumes without changing the sample loop, and the autosampler parameters identify injection volume. Partial loop injections might have slightly higher variability but can be conducted with minimal sample waste.

In the M5 MicroLC system, the default position of the sample loop on the injection valve at the start of a new method is the load position. The loop must be full of a solvent that will allow the sample to be retained on the trap or column (typically, this is the starting composition of the LC method), particularly for a partial-loop injection where the front volume is 0. The final steps of the Gradient 1 LC method return the sample loop to the initial conditions. Refer to the figures: Figure 4-21 and Figure 4-29.

Partial-loop Injections

For a partial-loop injection, the autosampler script specifies that the autosampler will pick up a rear airgap (air), a rear volume (sample), and the specified volume of sample. Refer to the figure: Figure 2-5. The rear airgap isolates the sample from the transfer liquid of the autosampler, and the rear volume prevents the airgap from entering the sample loop. (Airgap volumes are typically between 1 µL to 3 µL and the rear volume is typically 1 µL of the sample.) The sample volume is the amount of sample to be injected in the experiment and is less than the volume of the sample loop.

Figure 2-5 Schematic Drawing of Partial-Loop Injection



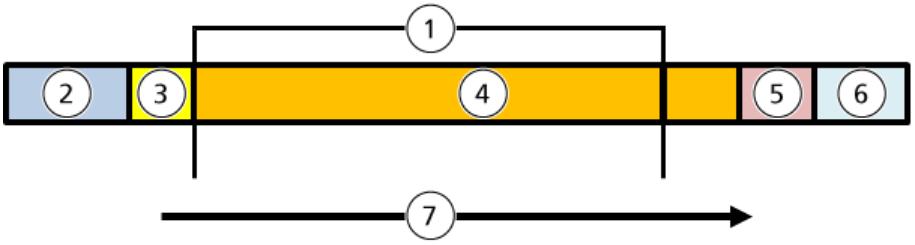
Item	Description
1	Sample loop
2	Rear air gap
3	Rear volume
4	Sample volume
5	Mobile phase
6	Direction of flow

In a partial-loop injection, the amount of sample dispensed is less than the total volume of the sample loop. As a result, the sample loop is only partially filled with the sample, with the remainder of the loop filled with the mobile phase.

Full-loop Injections

In a full-loop injection, the autosampler script specifies a rear airgap, a rear volume, the sample volume, a front volume and a front airgap. Refer to the figure: Figure 2-6. The front volume and front airgap help to limit the sample mixing with the liquid originally in the loop, and the rear airgap and rear volume act as in a partial-loop injection.

Figure 2-6 Schematic Drawing of Full-Loop Injection



Item	Description
1	Sample loop
2	Rear air gap
3	Rear volume
4	Sample volume
5	Front volume
6	Front air gap
7	Direction of flow

In a full-loop injection, the amount of sample dispensed by the autosampler into the valve is more than the total volume of the sample loop.

Analyst software: For either type of injection, the parameters in the autosampler script identifies the volumes and the airgaps. For more information, refer to the section: Parameters for the Autosampler Script.

SCIEX OS: For either type of injection, the settings under Autosampler tab in the LC method window in the M5 MicroLC driver section identifies the volumes and the airgaps. Refer to the section: Parameters for the Autosampler Script.

Injection Modes

Partial-loop and full-loop injections, have four modes of sample injection. These modes differ in whether the sample loop stays inline during the acquisition and how much of the content of the sample loop is transferred to the column. For the Ekisgent Control software, the injection mode is selected in the Run Conditions tab in LC Method Settings dialog. Refer to the figure: Figure 2-7. For the M5 MicroLC driver in SCIEX OS, the injection mode is selected in the **Run Conditions** tab in the LC Method Settings dialog, Refer to the figure: Figure 2-8.

Figure 2-7 LC Method Settings Dialog: Sample Injection Section for Eksigent Control Software

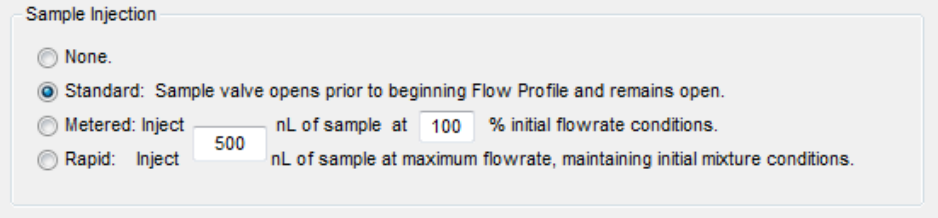
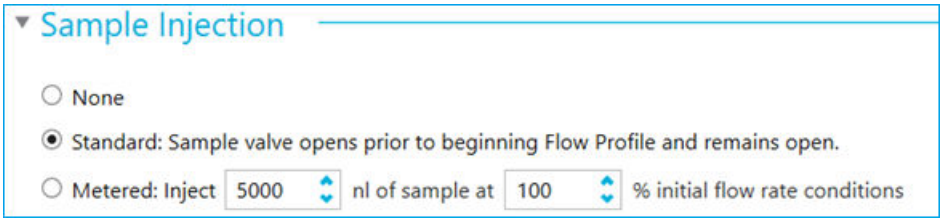


Figure 2-8 LC Method Settings Dialog: Sample Injection Section for M5 MicroLC SCIEX OS Driver



- None: The sample valve does not switch during the acquisition.
- Standard: The sample valve switches to the inject position when acquisition begins and returns to the load position when acquisition ends. The sample loop stays in the flow path during acquisition.
- Metered: The valve switches to the inject position when acquisition starts, and then the specified volume of sample is delivered to the column. After the specified volume is injected, the sample valve switches to the load position, removing the sample loop from the flow path.

  Use metered injection when the sample loop volume is larger than the volume to be injected on the column. This prevents the large sample loop from adding extra dead-volume and peak broadening.

  The minimum injection volume (in nanoliters) is 2.5 x Q, where Q is the flow rate. To inject a smaller volume, decrease the flow rate in the LC method.
- (Applicable to the Analyst software) Rapid: The valve operates as for a metered injection, except that the flow rate increases during the injection to inject the sample quickly and prevent extra-column broadening.

Note: The sample injection mode in SCIEX OS supports only the None, Standard, and the Metered modes.

About the Autosampler Script

The Analyst software uses the autosampler script to communicate with the autosampler. The autosampler script is part of the acquisition method.

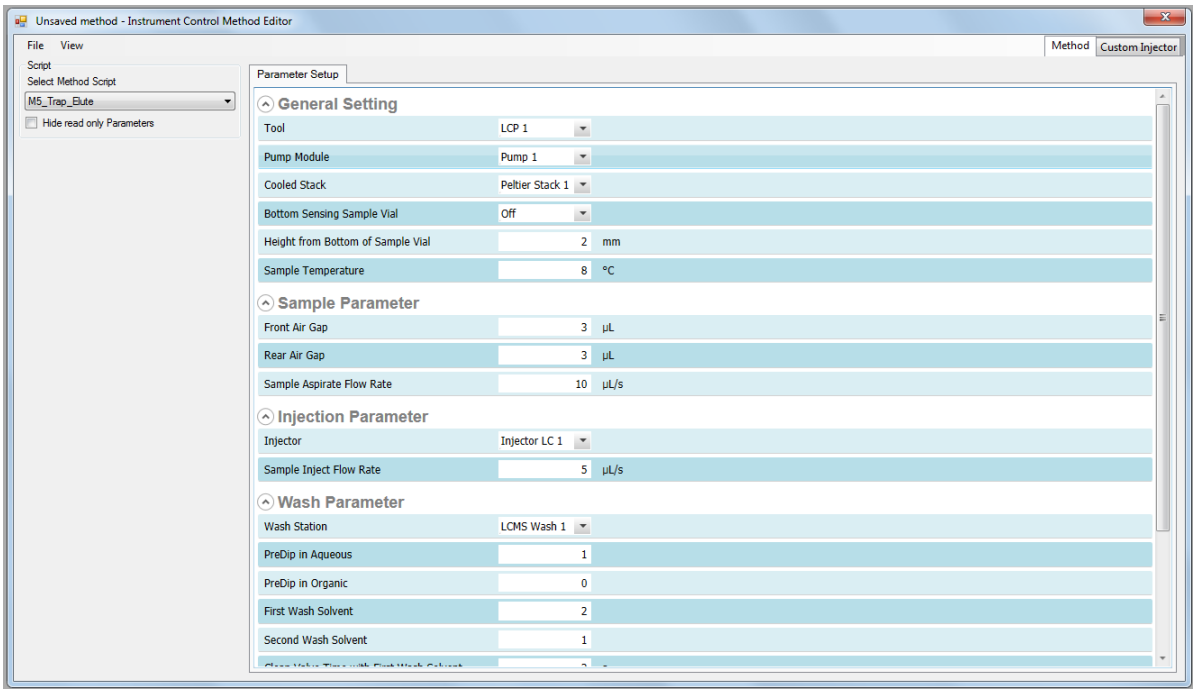
The applicable script depends on the type of experiment to be performed:

- For a direct injection experiment: M5_Direct Inject
- For a trap-and-elute experiment: M5_TrapElute

By default, both of these scripts are for partial-loop injections. For any necessay full-loop injection changes, refer to the table: Table 2-1.

Note: For the Analyst software: An additional autosampler script is supplied with the system, M5_Advanced. This script gives more control over more autosampler functions. Refer to the section: Parameters for the Advanced Autosampler Script. For most situations, M5_Direct Inject or M5_TrapElute are sufficient.

Figure 2-9 Instrument Control Method Editor Window: Parameter Setup Tab



Steps in the Autosampler Script

The autosampler script consists of the following steps:

1. Aspirate the sample, then, optionally, dip the needle in wash solvent 1 and wash solvent 2.
2. Move the needle to the injection port on the valve.
3. Load the sample on the injection loop, then switch the valve to inject the sample on to the column.
4. Wash the system:
    a. Wash the injection valve and needle with the specified wash solvent, typically the organic wash solvent.
    b. Wash the injection valve and needle with the specified wash solvent, either the aqueous wash solvent or the organic wash solvent, for a second time.
5. Optionally, wash the injection valve and needle again with the specified wash solvent, typically the aqueous wash solvent.

Note: If the first two wash steps use the organic wash solvent, then a final aqueous wash step is recommended. (Wash solvents are selected in the Instrument Method Editor window in Analyst Device Driver. The parameters are **First Wash Solvent** and **Second Wash Solvent**, and the options are **1** and **2**, where the numbers correspond to the labels on the tubing from the LCMS tool pumps.

6. Move the needle to the home position.

Parameters for the Autosampler Script

Analyst software: Parameters for the autosampler script are set in the Parameters Setup tab in the Instrument Control Method Editor window in the Analyst Device Driver (ADD) software.

SCIEX OS: Parameters for the autosampler script are set in the **Autosampler** tab under the LC method window of the M5 MicroLC driver

For recommended values for the parameters and their ranges, refer to the table: Table 2-1.

Note: The values with an asterisk in the following table are mandatory. Use the values in the Recommended Value column.

Table 2-1 Parameters in the Parameter Setup Tab: Direct Injection and Trap and Elute Autosampler Scripts

Parameter Name	Recommended Value	Minimum Value	Maximum Value
General Setting Section			
Tool*	LCP1	LCP1	
Pump Module*	Pump 1	Pump 1	
Cooled Stack*	Peltier Stack 1	Peltier Stack 1	
Bottom Sensing Sample Vial (see note)	Off	Off or On	
Height from Bottom of Sample Vial (mm)	2	0	32
Sample Temperature (°C)	8	4	40
Sample Parameter Section			
Front Air Gap (µL)	3	0	3
Rear Air Gap (µL)	3	1	3
Sample Aspirate Flow Rate (µL/s)	10	0.1	50
Injection Parameter Section			
Injector*	Injector LC 1	Injector LC 1	

Table 2-1 Parameters in the Parameter Setup Tab: Direct Injection and Trap and Elute Autosampler Scripts (continued)

Parameter Name	Recommended Value	Minimum Value	Maximum Value
Sample Injector Flow Rate (µL/s)	5	0.1	10
Wash Parameter Section			
Wash Station*	LCMS Wash 1	LCMS Wash 1	
PreDip in Aqueous	1	0	2
PreDip in Organic	0	0	2
First Wash Solvent	2	1	2
Second Wash Solvent	1	1	2
Clean Valve Time with First Wash Solvent (s)	2	2	10
Clean Valve Time with Second Wash Solvent (s)	2	2	10
Clean Syringe Cycle with First Wash Solvent	1	0	10
Clean Syringe Cycle with Second Wash Solvent	1	0	10
Final Clean	Off	Off or On	
Final Clean with Wash Solvent	1	1	2

Note: The **Bottom Sensing Sample Vial** option might not be compatible with some vials, particularly glass inserts. If a different type of vial is to be used, then use a new vial to run a test sample. Make sure that the results are acceptable before a complete run is set up.

Set Up the System to Do an Experiment with SCIEX OS 3

This chapter gives the steps to prepare the M5 MicroLC system to use SCIEX OS to do an experiment. The procedures are similar for direct injection and trap-and-elute experiments with any differences noted.

The procedures in this chapter assume that the system has already been correctly installed and initialized.

Do the following steps in the order in which they are given:

- Configure the Settings for M5 MicroLC Systems
- Plumb the Injection Valve
- (For the M5 MicroLC-TE Systems Only) Configure the System Configuration
- Load the Mobile Phases
- Flush the Injection Valve
- Turbo V, DuoSpray, and IonDrive Turbo V Ion Sources
- Test the LC System Connections with SCIEX OS
- Create the LC Methods

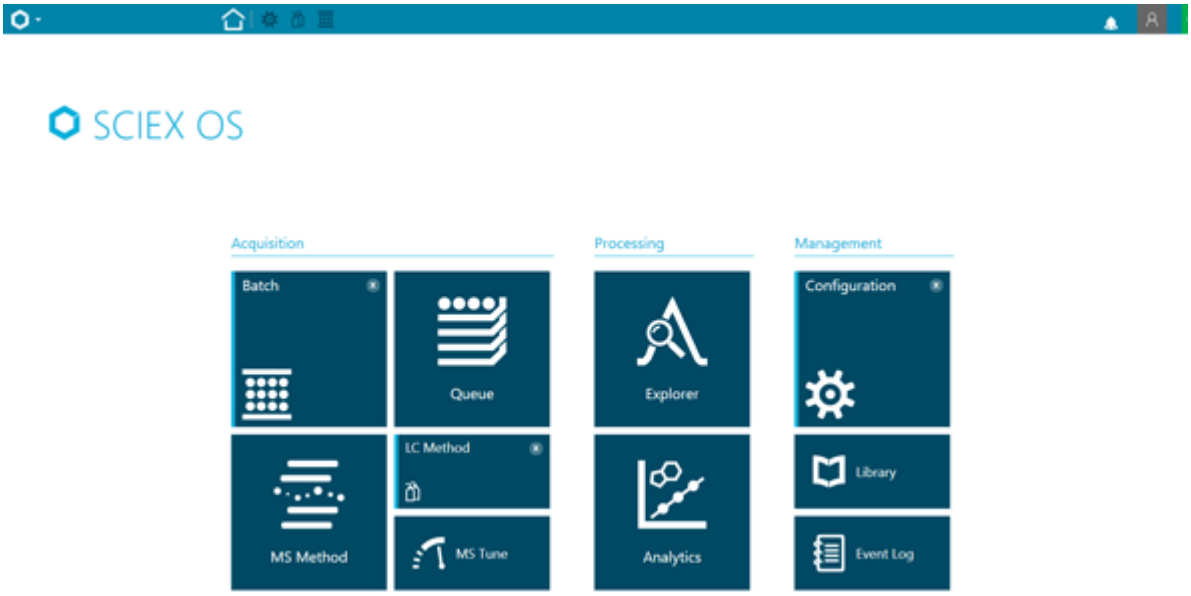
Note: The screen captures shown in this chapter are for the M5 MicroLC-TE system. For an M5 MicroLC system, the screens are different.

Configure the Settings for M5 MicroLC Systems

The active hardware configuration must include the mass spectrometer and the M5 MicroLC system. If either of the devices is not available, then add the device.

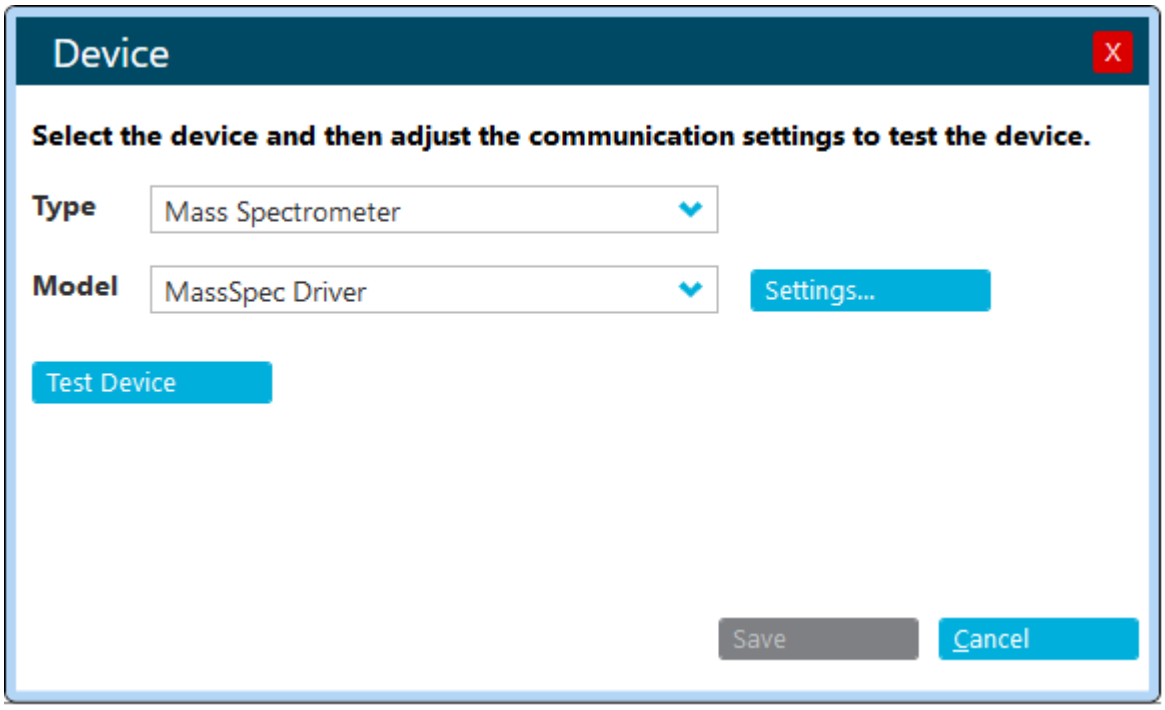
1. Close all of the open software.
2. Open SCIEX OS.
3. Open the Configuration workspace.

Figure 3-1 Home Page



4. Click **Devices**.
5. Add the mass spectrometer to the profile.
   a. Click **Add**.

Figure 3-2 Device

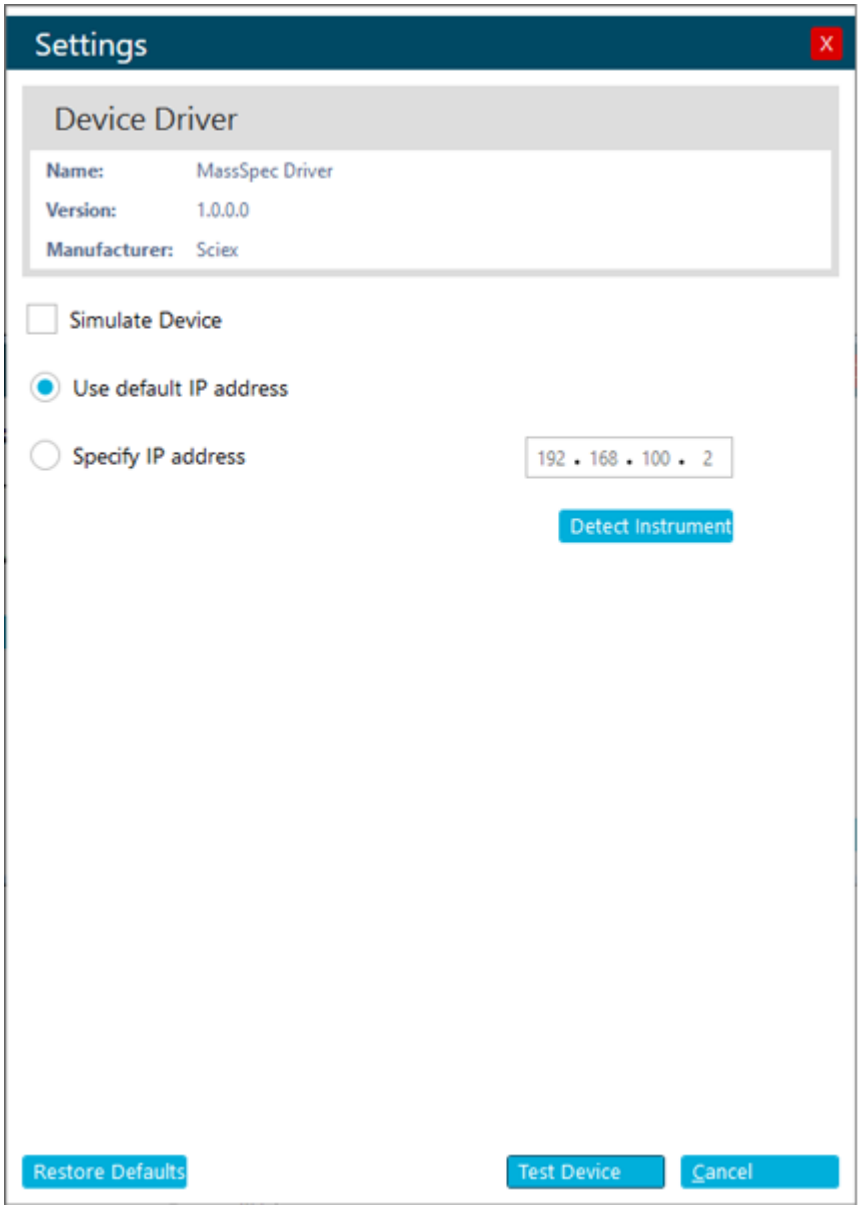


a. In the **Type** list, select **Mass Spectrometer**.

b. In the **Model** list, select **MassSpec Driver**.

c. Click **Settings**.

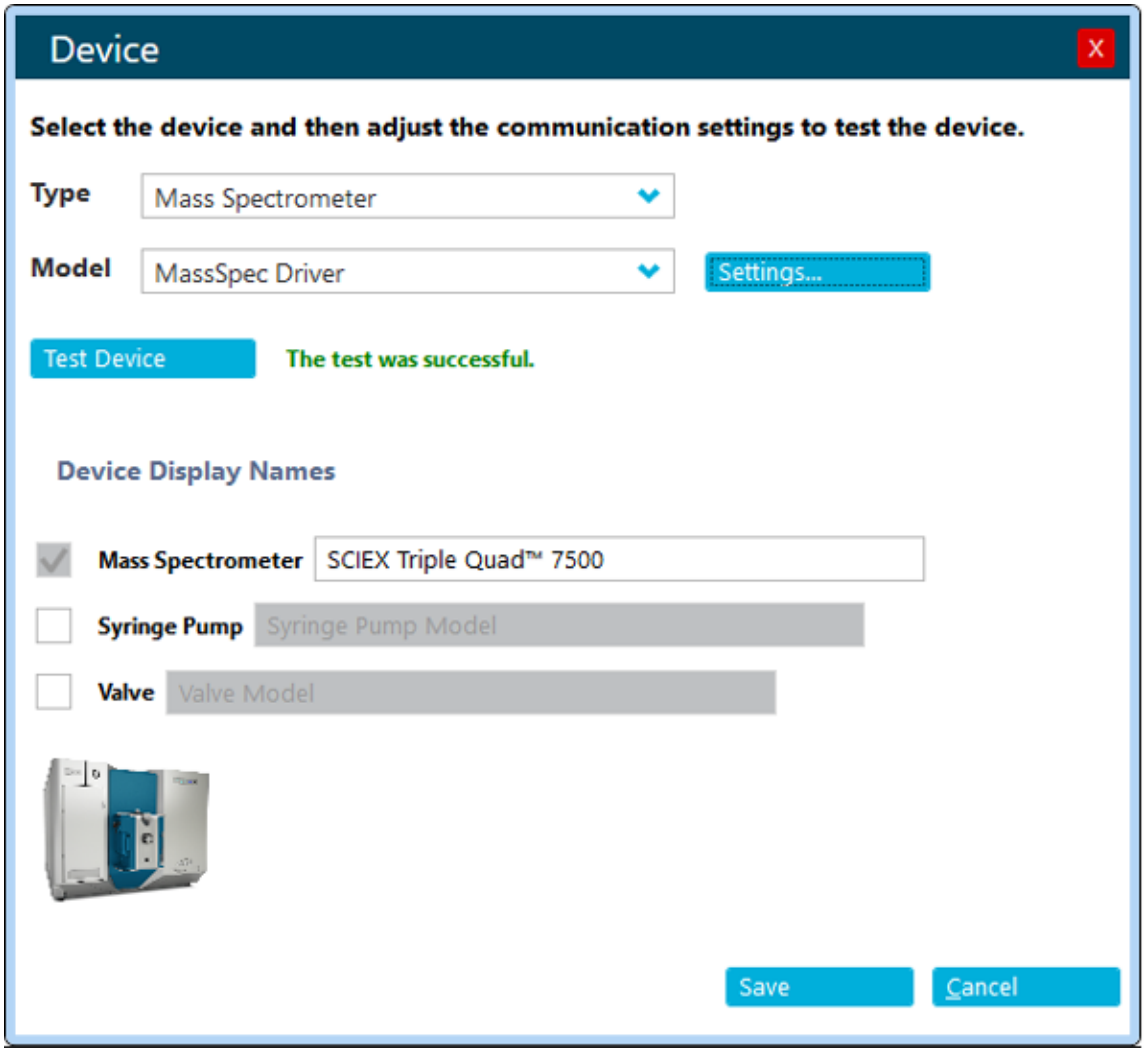
Figure 3-3 Settings



d. Click **Detect Instrument**.

e. Click **Test Device** to make sure that the device is configured correctly and available for use.

Figure 3-4 Device

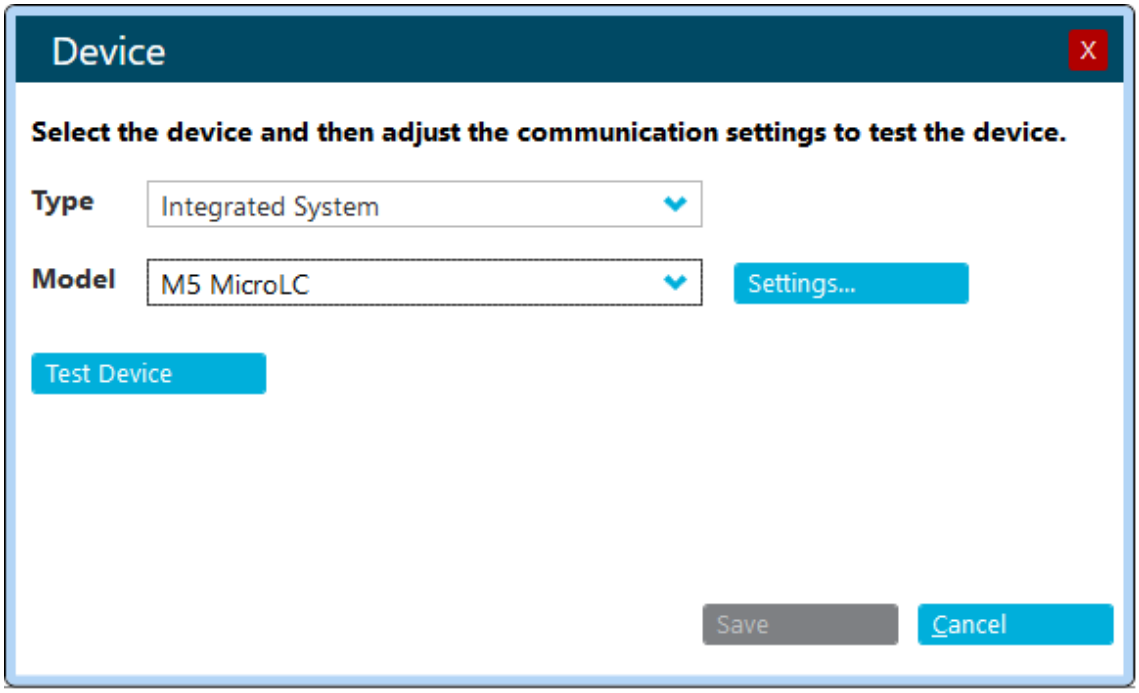


f. After the message `The test was successful` is shown, click **Save**.

6. Add the intergrated system to the profile:

   a. Click **Add**.

Figure 3-5 Device

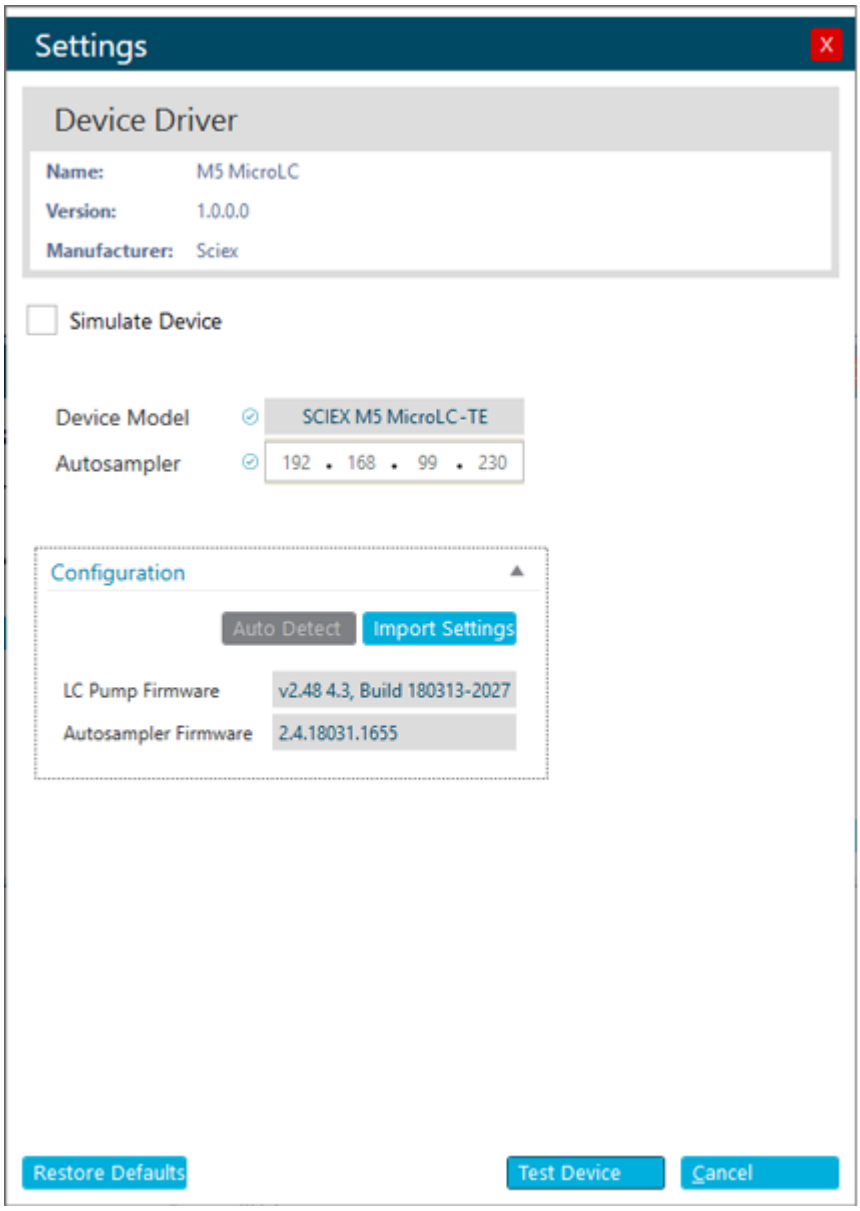


b. In the **Type** list, select **Integrated System**.

c. In the **Model** list, select **M5 MicroLC**.

d. Click **Settings**.

Figure 3-6 Settings

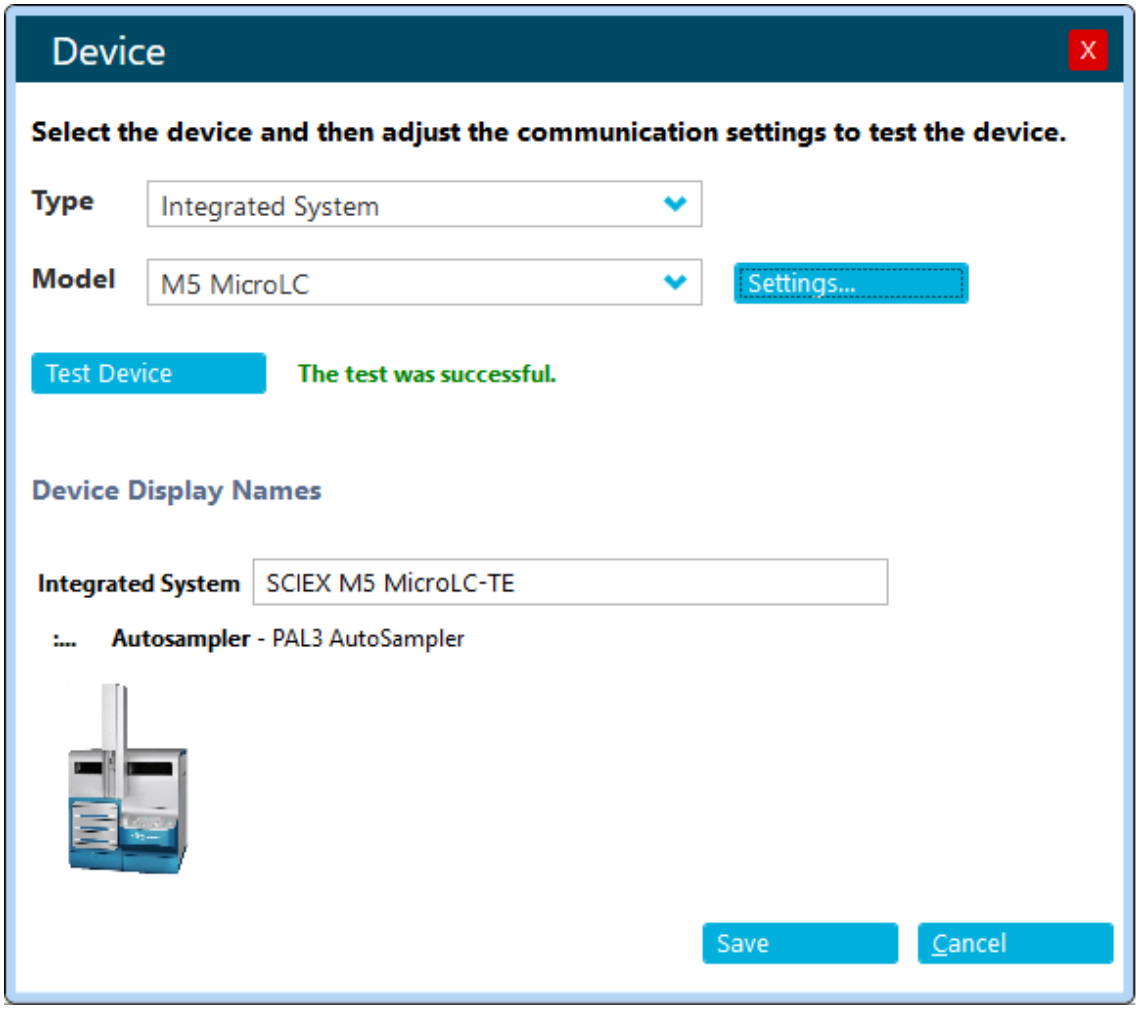


e. Click **Import Settings** and then browse to the CD to find and select the `EkSettings_xx-xx-xx-xxxx.reg` file, where *xx-xx-xx-xxxx* is the serial number.

f. Click **Auto Detect**.

g. Click **Test Device** to make sure that the device is configured correctly and available for use.

Figure 3-7 Device



7. After the message, test was successful is shown, click **Save**.

Plumb the Injection Valve

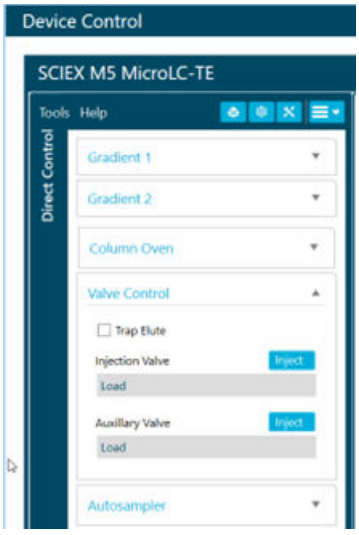
The M5 MicroLC system is plumbed according to the type of experiment. Refer to the section: Plumb the Injection Valve.

(For the M5 MicroLC-TE Systems Only) Configure the System Configuration

Make sure that the system is set up correctly for the type of experiment to be performed.

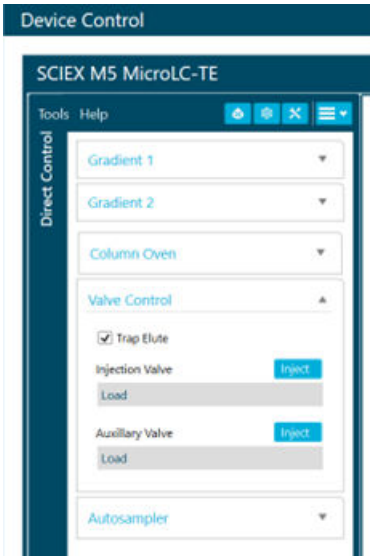
1. Make sure that the valve is correctly plumbed for the type of experiment to be performed.
   - Direct Injection Plumbing Diagram
   - Trap-and-Elute Plumbing Diagram
2. On the status panel, click **Direct device control** ( ) to the right of the device name.
3. Click the arrow to expand the **Valve Control** section.
4. For a direct injection experiment with Gradient 1, make sure that the **Trap-Elute Mode** check box is cleared.

Figure 3-8 Direct Control Dialog: Direct Injection with Gradient 1



5. For a trap-and-elute experiment or direct injection experiment with Gradient 2, make sure that the **Trap-Elute Mode** check box is selected.

Figure 3-9 Direct Control Dialog: Trap-and-Elute Mode or Direct Injection with Gradient 2



6. Click **Close**.

Load the Mobile Phases

Note: We do not recommend the use of Milli-Q water because the quality is not satisfactory for use in LC-MS systems.

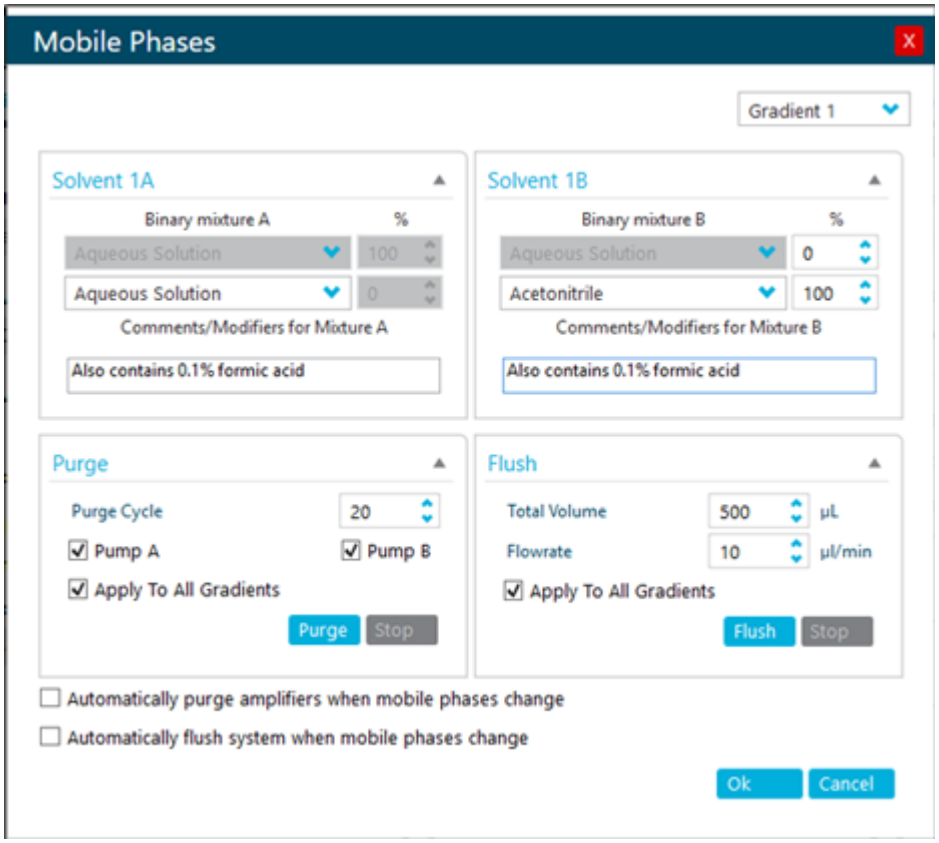
This procedure assumes that the mobile phases are water and acetonitrile, that water goes into the pump at the Mobile Phase A inlet (labeled **1A** or **2A** on the back of the system), and that acetonitrile goes into the pump through the Mobile Phase B inlet (labeled **1B** or **2B** on the back of the system). Usually, the more organic mobile phase should use the B inlet.

Before using different mobile phases, refer to the section: System Safe Fluids.

1. If required, then discard any old solvents in the mobile phase bottles, and then clean the bottles with the correct solvents.
2. Put new mobile phases in the bottles, and then install the mobile phase tubing and filters.
    a. Fill the Mobile Phase A bottle with water with 0.1% formic acid.
    b. Fill the Mobile Phase B bottle with acetonitrile with 0.1% formic acid.
3. Identify the mobile phases.
    a. On the status panel, click **Direct device control** ( ) at the right of the device name.

b. Click **Mobile Phase** ( ).

Figure 3-10 Mobile Phases Dialog



c. For Binary mixture A (mobile phase A), do not make any changes.

d. For Binary mixture B (mobile phase B), select **Acetonitrile** in the second list, and then type `100` in the **%** field.

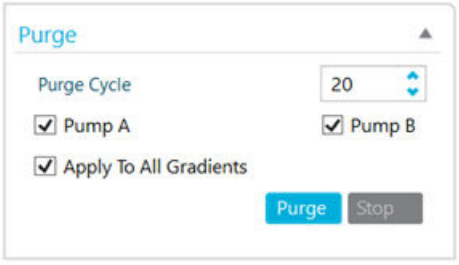
e. (Optional) Type comments in the **Comment/Modifiers** fields. For example, type the modifier and concentration.

f. Click **Ok**.

4. Purge the pumps a minimum of 20 times.

a. Click **Mobile Phase**( ).

Figure 3-11 Purge Section



a. In the Purge section, select the pumps to be purged and then type `20` in the **Purge Cycle** field.

b. M5 MicroLC-TE system: Select the **Apply To All Gradients** check box.

c. Click **Purge**.
The pumps begin to purge. While the pumps purge, make sure that the mobile phases are pulled through the mobile phase tubing to the pumps.

d. Examine the waste tubing of the pumps that have started to purge. The waste tubes are clear plastic tubes and extend from the back of the pump. After about eight purges, the mobile phase should be purged through the waste tubing.

5. Flush the system.

a. Disconnect the tubing that comes from the G1 pump outlet.

b. Connect one end of a length of 1/32 inch o.d. tubing to the G1 pump outlet and put the other end in the waste drain.

c. In the Flush section, set the parameters for the specified system.

Figure 3-12 Flush Section

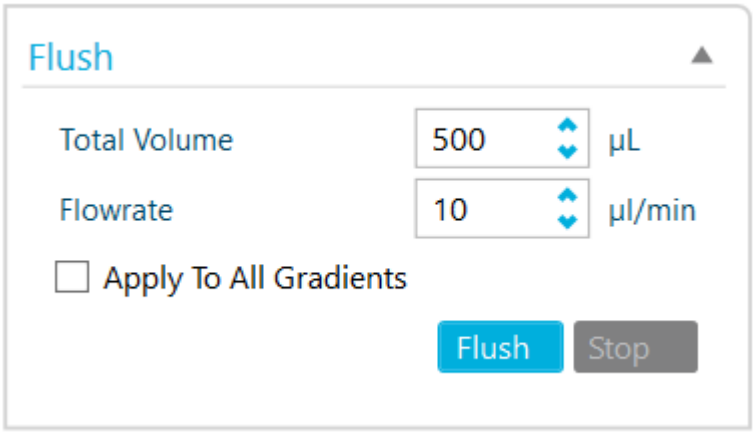


Table 3-1 Flush Settings Parameters

System	Total Volume (µL)	Flush Flow Rate (µL/min)
Low-flow system	100	10
Micro-flow system	500	50
High-flow system and all Gradient 2 pumps	500	50

d. Click **Flush**.

e. After the flush squence is completed, click **Stop**.

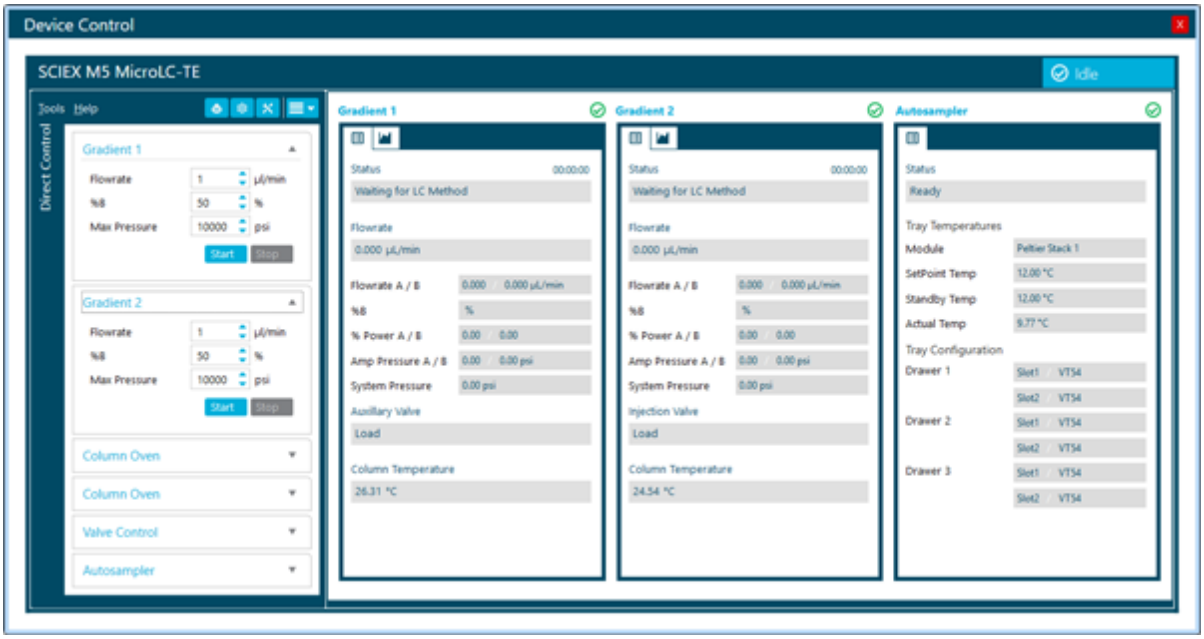
6. Do step 5 again for the G2 pump outlet. Install the 1/32 inch o.d. tubing from the G1 outlet in the G2 outlet.
7. For each pump, connect the original tubing between the pump outlet and the valve.

Flush the Injection Valve

Flush the valve when the column is not connected to prevent the introduction of contaminants from the valve to the column.

1. Disconnect the tubing from the column inlet.
2. On the status panel, click **Direct device control** () to the right of the device name.
3. For an M5 MicroLC-TE system, click the **Channel** buttons to select **Gradient 2**.

Figure 3-13 Device Control Dialog



4. Set the Direct Control parameters and then start the pump.
   a. In the **B (%)** field, type `50`.
   b. Set the **Total flowrate** for the specified configuration:
      - For a low-flow system, type `10`.
      - For a micro-flow or high-flow system, type `20`.
   c. Click **Start**.
5. In the **Injection Valve** section, click **Load**, wait 10 seconds, and then click **Inject**. Do this three times.
6. Click **Load**, then click **Stop**.
7. Connect the tubing to the column inlet.

Pre-Heat the Column Oven

Turbo V, DuoSpray, and IonDrive Turbo V Ion Sources

WARNING! Hot Surface Hazard. Beware of burns. The column can become hot during operation. Allow the column to cool before removing it or replacing the PEEK clad fused silica tubing.

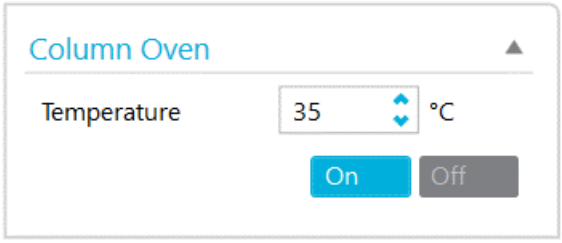
The temperature of the column can be regulated. The column heater in use controls the maximum temperature.

- For the OptiFlow Turbo V ion source, refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.
- For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, the maximum temperature is 60 °C.

Note: The column oven reaches the correct temperature quickly, but the column can take as long as 30 minutes to fully equilibrate.

1. Connect the column.
2. On the status panel, click **Direct device control** ( ) to the right of the device name.
3. For the trap-and-elute system, select **Gradient 1**.
4. In the Column oven section, type `35` in the **Setpoint** field, and then click **Start**.

Figure 3-14 Direct Control Dialog: Column Oven Section



5. Close the compartment so that the oven can get to the specified temperature.

OptiFlow Pro Ion Source

WARNING! Hot Surface Hazard. Beware of burns. The column can become hot during operation. Allow the column to cool before removing it or replacing the PEEK clad fused silica tubing.

The temperature of the column can be regulated. The maximum temperature depends on the column heater in use.

- For the OptiFlow Pro ion source, refer to the document: *OptiFlow Pro Ion Source Operator Guide*.

Note: The column oven reaches the correct temperature quickly, but the column can take as long as 30 minutes to fully equilibrate.

Note: The OptiFlow Pro ion source column is controlled by the mass spectrometer.

1. On the status panel, click **Direct device control** () to the right of the device name.
2. Click the **Settings** > **Hardware Options** tab and then clear the **Column oven option** check box.
3. Click **OK**.
4. Connect the OptiFlow Pro column oven.
5. Click Equilibrate.
6. In the MS Method list, select the **7500 system ms method column oven temp of 35 °C** method.
7. In the Column temperature field, type `35`.
8. Click **OK**.
9. Close the OptiFlow Pro oven compartment so that the oven can reach the specified temperature.

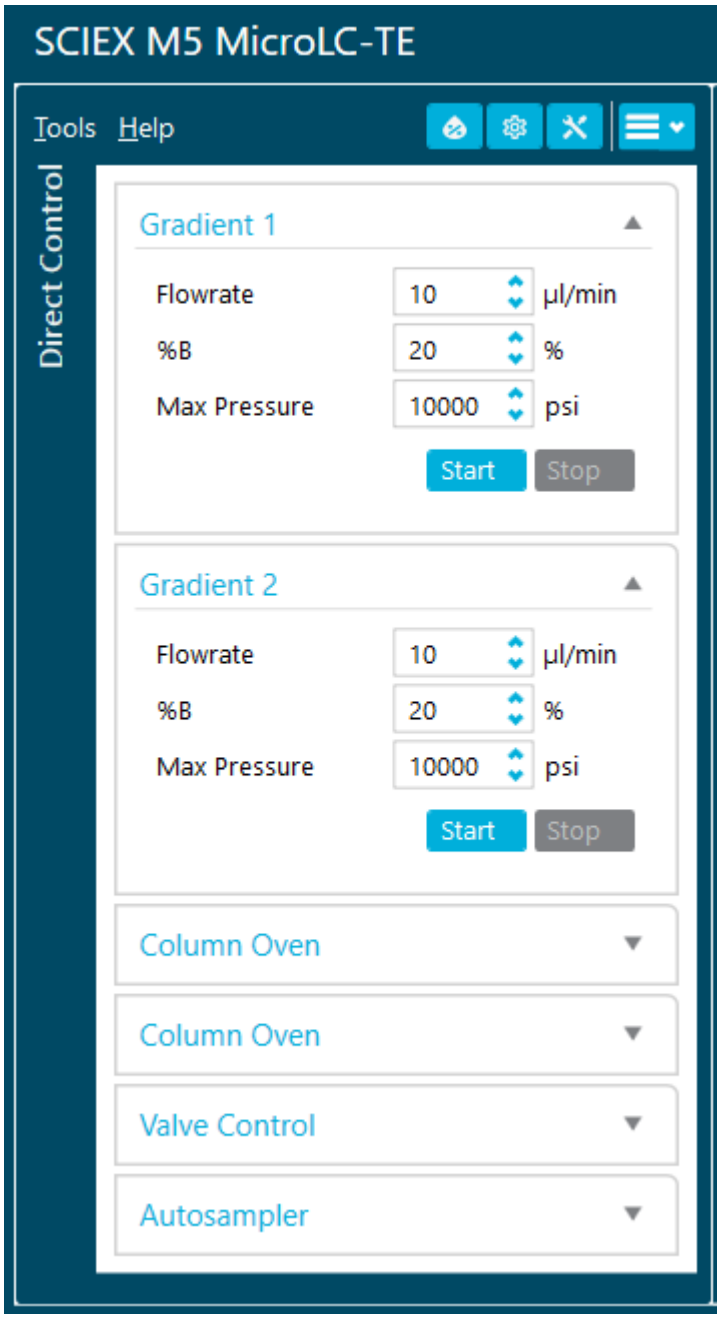
Test the LC System Connections with SCIEX OS

1. Make sure that the column is connected.
2. On the status panel, click **Direct device control** () to the right of the device name.
3. Set the Pump Direct Control parameters.
   a. Type `20` in the **%B** field.

      Note: For other experiments, set **%B** to be the same as the initial conditions in the LC method to be used in the experiment.

   b. Set the **Flowrate** (µL/min) for the specified column diameter.

Figure 3-15 Direct Control Dialog: Total Flowrate



- For a 0.3 mm i.d. column, type `10`.
- For a 0.5 mm i.d. column, type `40`.

4. In the Pump Direct Control section, click **Start** to start the pump.

5. Let the system pump for approximately 2 minutes, examine the connections for leaks, and then click **Stop**.
6. For a trap-and-elute experiment, click and then expand **Gradient 2** and then do step 3 to step 5 again to test the connections for the other pump. Set the **Flowrate** to `40`.
7. Click **Close**.

Create the LC Methods

In SCIEX OS, the LC method template is predefined in the direct injection method or trap-elute method under LC Method dialog.

Note: Make sure to create the method that is correct for the flow rate configuration of the system in use.

Note: For other LC methods, it is recommended to set a minimum of 3% for the mobile phase A or mobile phase B.

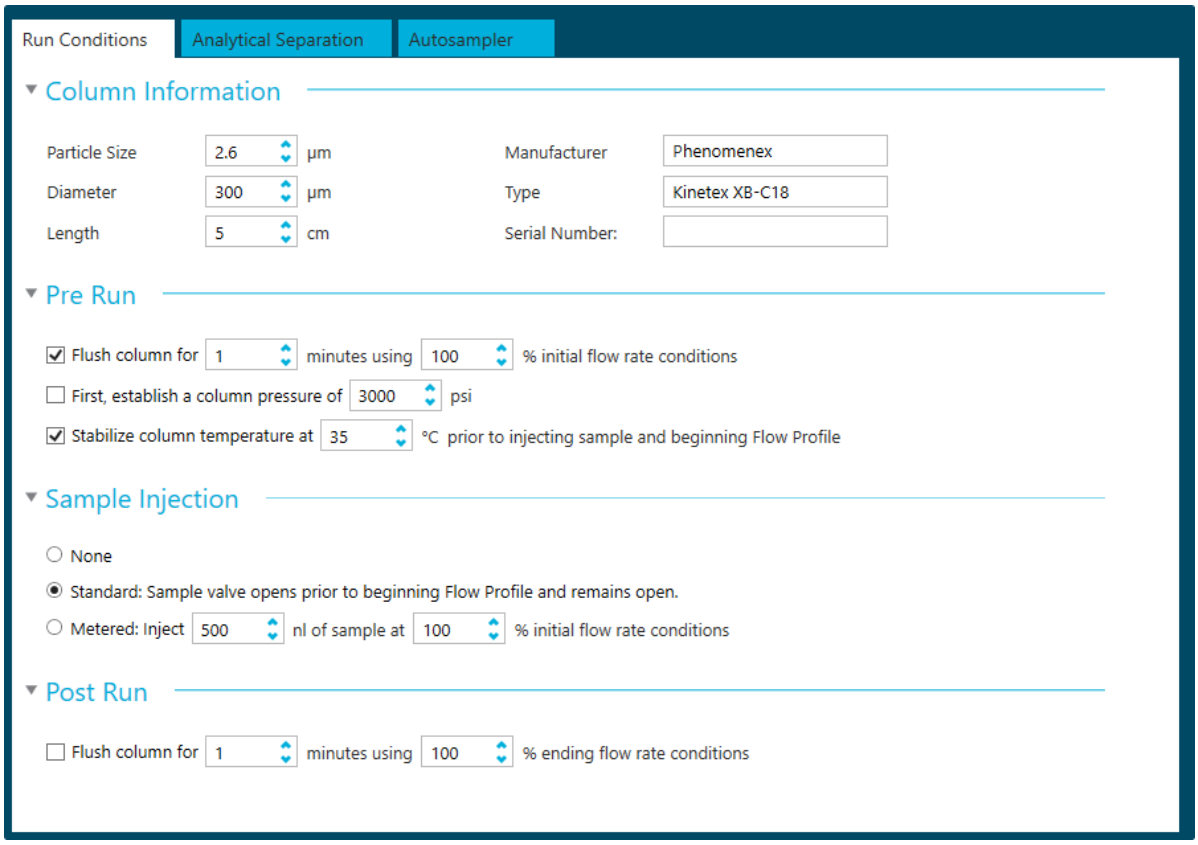
Table 3-2 LC Methods

Type of Experiment	Low-Flow System	Micro-Flow or High-Flow System
Direct Injection	Create the Direct Injection Method for a Low-flow System	Create the Direct Injection Method for Micro-flow and High-flow Systems
Trap-and-elute	Create the Trap-and-Elute Method for a Low-flow System	Create the Trap-and-Elute Method for Micro-flow and High-flow Systems

Create the Direct Injection Method for a Low-flow System

1. Click **LC Method**.
2. Click **New** > **Direct Inject**.
3. Open the Run Conditions tab and then set the values as shown in the following figure.

Figure 3-16 Run Conditions Tab



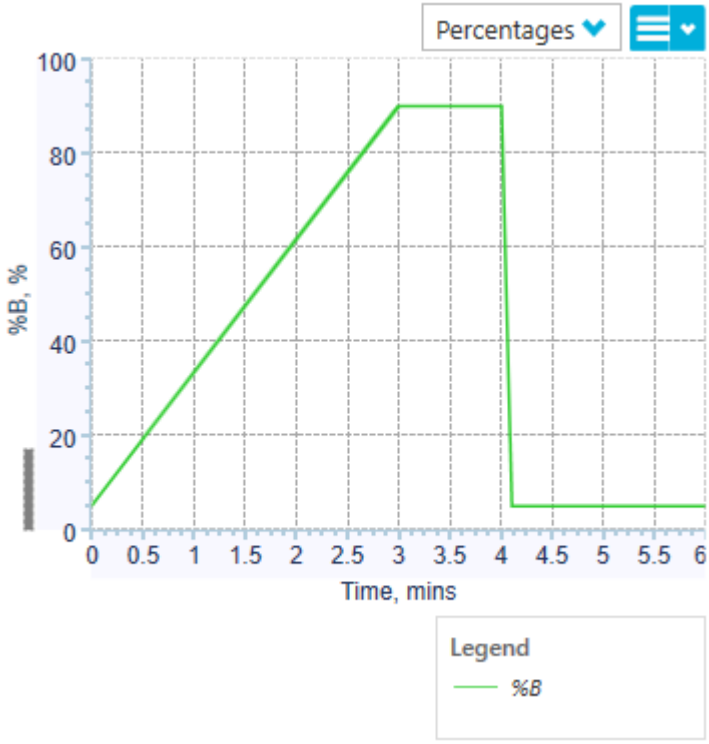
4. Open the Analytical Separation tab and then complete the Gradient Table as shown in the following figure.

Figure 3-17 Analytical Separation Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	10.000	5.00	
3.00	10.000	90.00	
4.00	10.000	90.00	
4.10	10.000	5.00	
6.00	10.000	5.00	

When the table is complete, the graphical representation should be the same as the following figure.

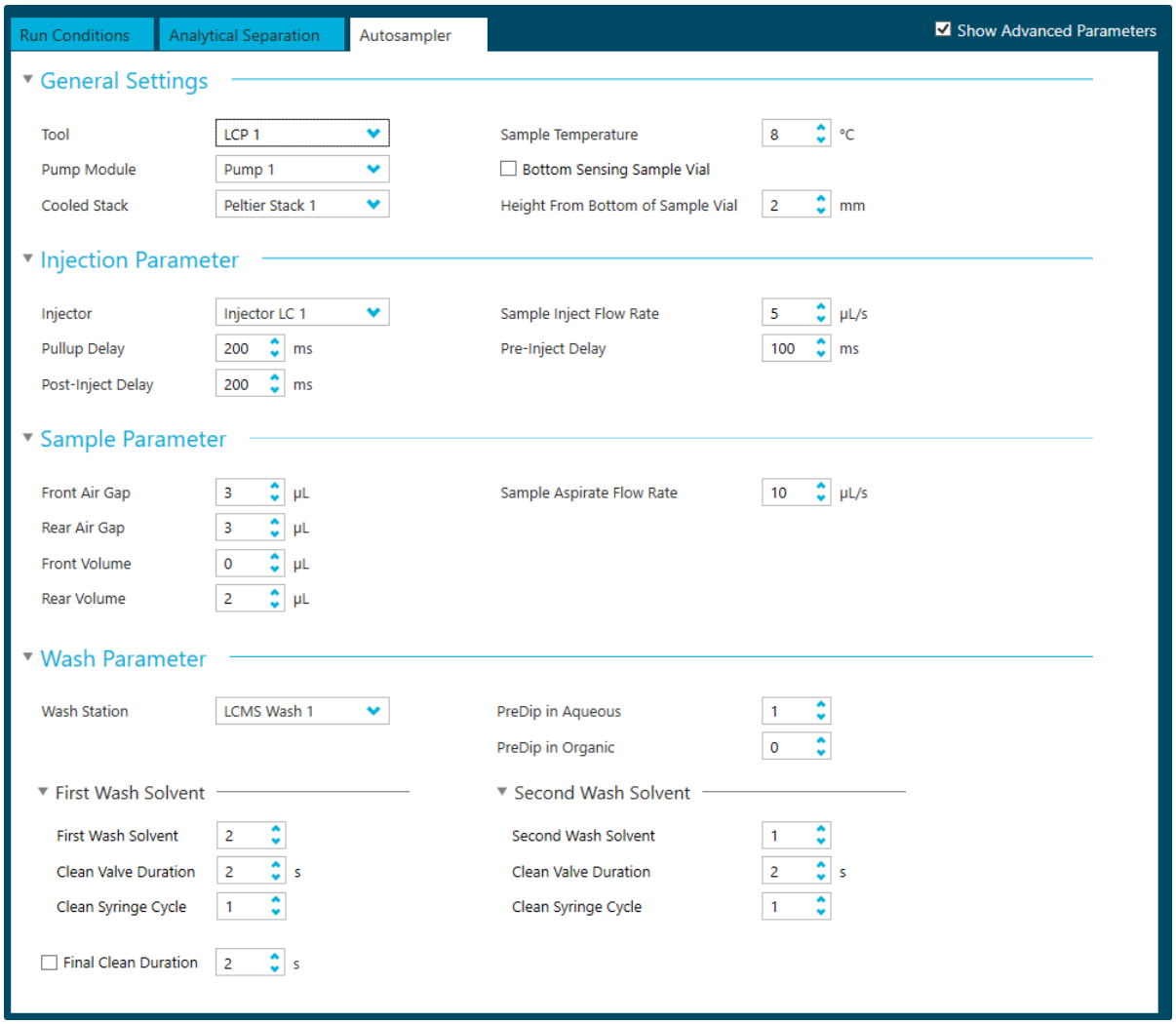
Figure 3-18 Analytical Separation Tab: Gradient Graph



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

5. Open the Autosampler tab and then set the values as shown in the following figure.

Figure 3-19 Autosampler Tab

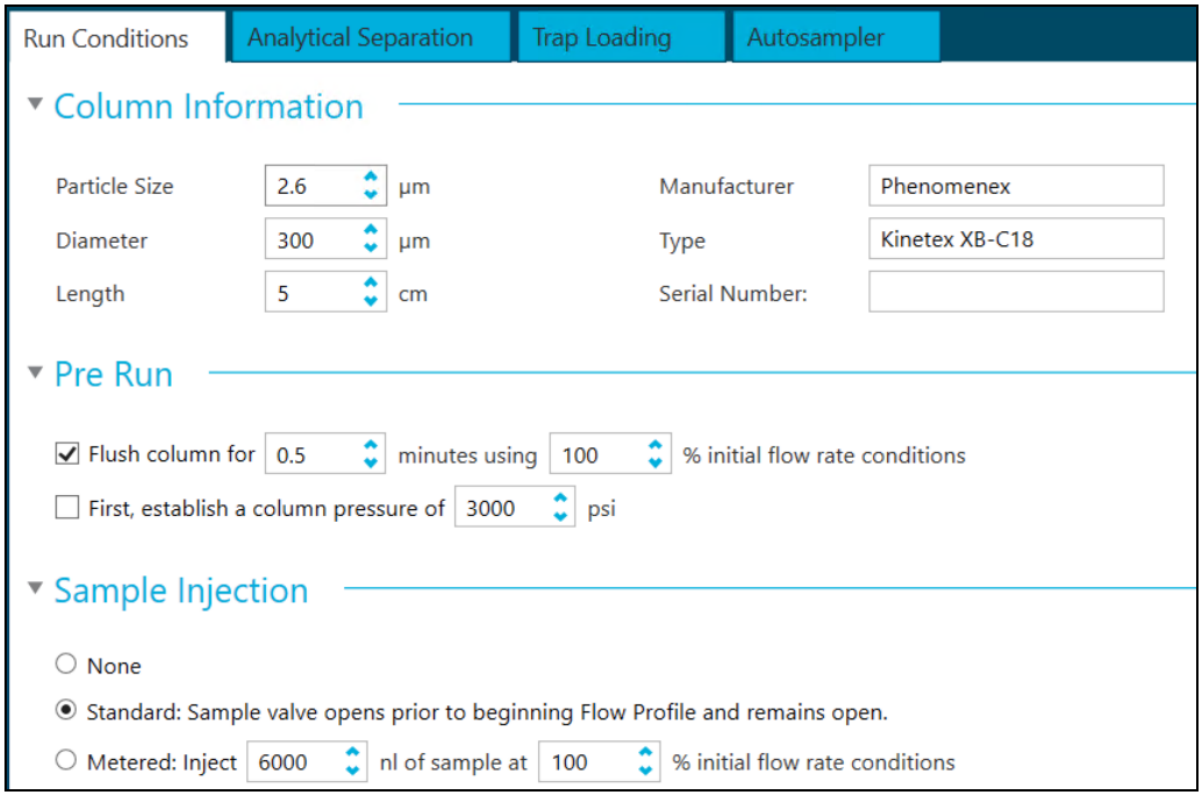


6. Save the method as `Low-flow Direct Inject Method`.

Create the Trap-and-Elute Method for a Low-flow System

1. Click **LC Method**.
2. Click **New** > **Trap Elute**.
3. Open the Run Conditions tab and then set the values as shown in the following figure.

Figure 3-20 Run Conditions Tab



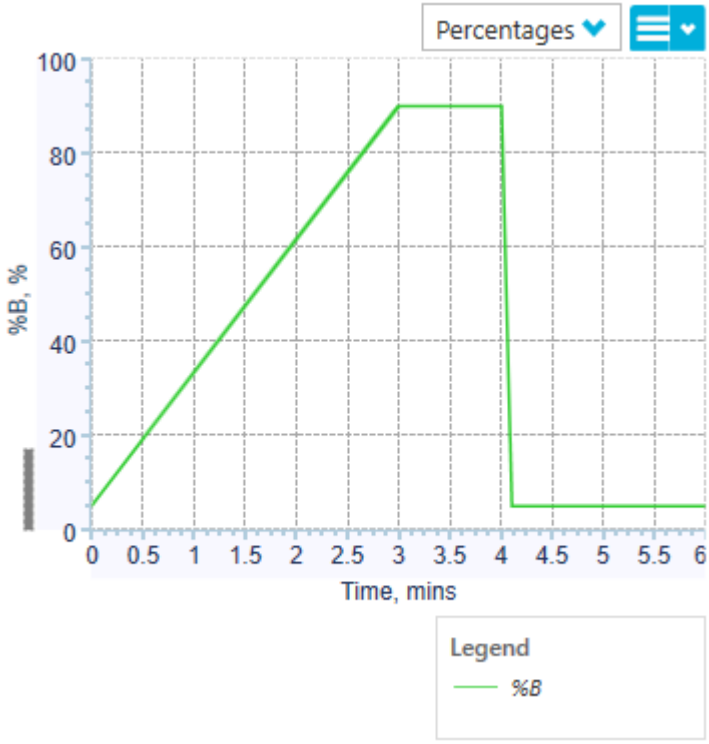
4. Open the Analytical Separation tab and then complete the Gradient Table as shown in the following figure.

Figure 3-21 Analytical Separation Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	10.000	5.00	
3.00	10.000	90.00	
4.00	10.000	90.00	
4.10	10.000	5.00	
6.00	10.000	5.00	

When the table is complete, the graphical representation should be the same as the following figure.

Figure 3-22 Analytical Separation Tab: Gradient Graph



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

5. Open the Trap Loading tab and then complete the Gradient Table as shown in the following figure.

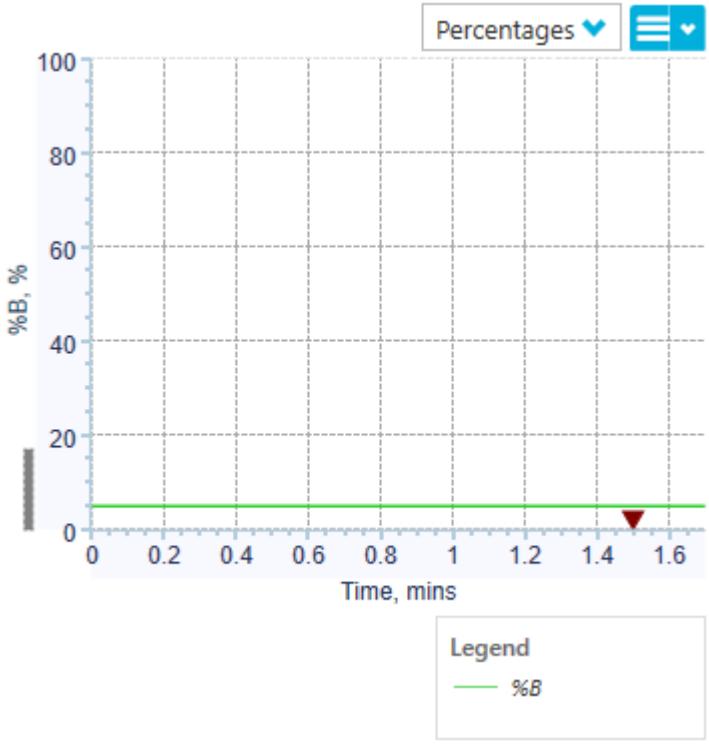
   In the **Event** column, at 1.5 minutes, make sure to select **Start Gradient 1**.

Figure 3-23 Trap Loading Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	50.000	5.00	
1.50	50.000	5.00	Start Gradient 1
1.70	50.000	5.00	

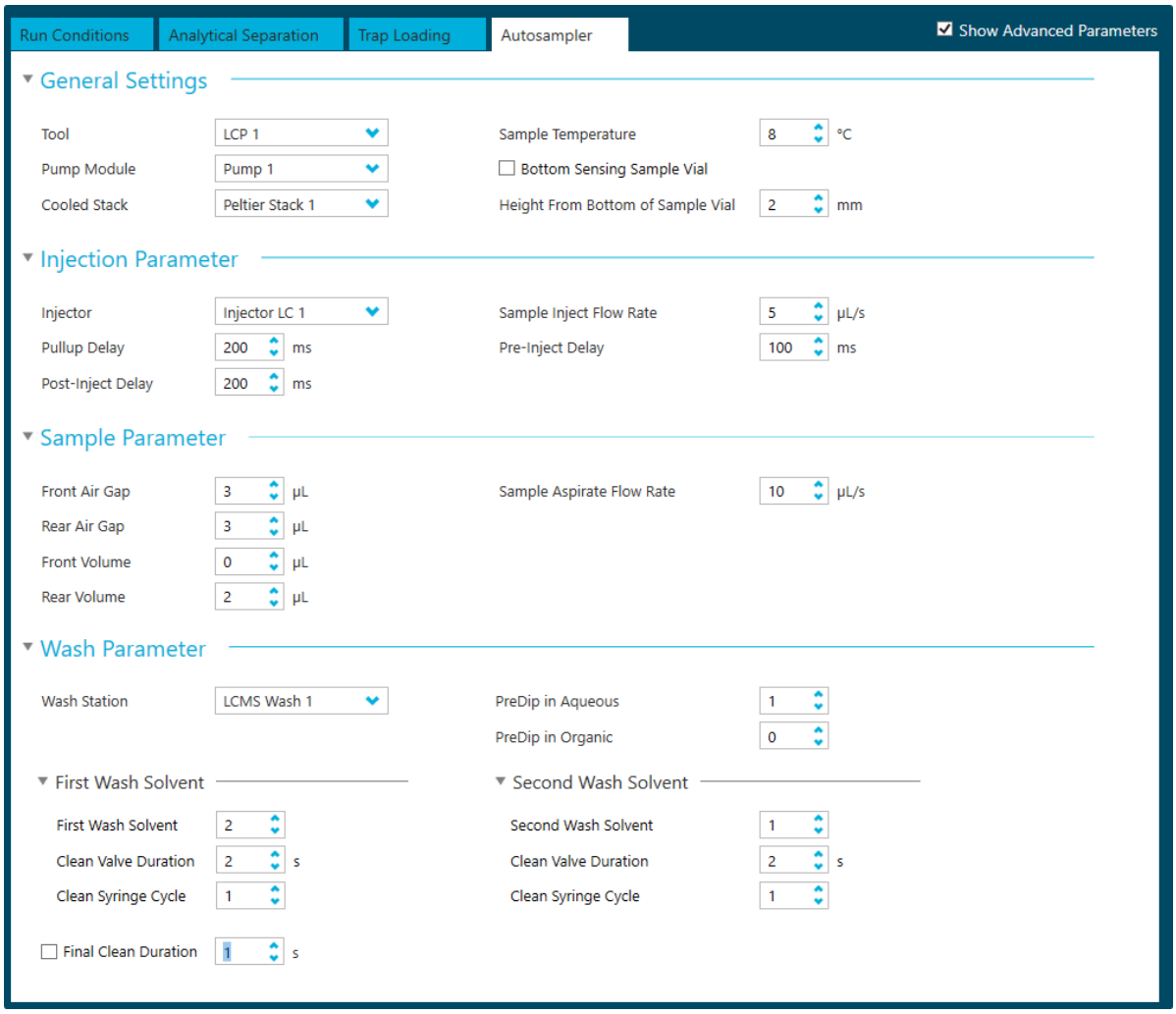
When the table is complete, the graphical representation should match the following figure.

Figure 3-24 Trap Loading Tab: Gradient Graph



6. Open the Autosampler tab and then set the values as shown in the following figure.

Figure 3-25 Autosampler Tab

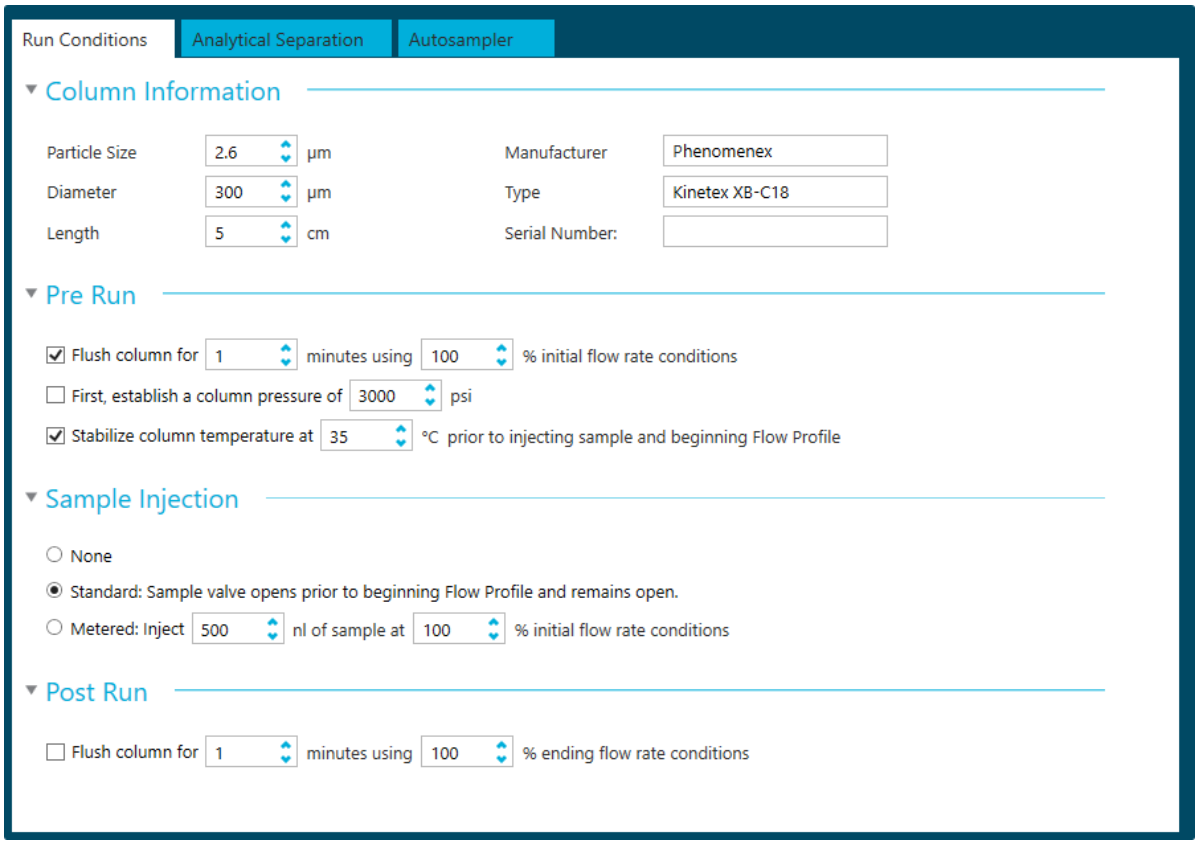


7. Save the method as `Low-flow Trap Elute Method`.

Create the Direct Injection Method for Micro-flow and High-flow Systems

1. Click **LC Method**.
2. Click **New** > **Direct Inject**.
3. Open the Run Conditions tab and then set the values as shown in the following figure.

Figure 3-26 Run Conditions Tab



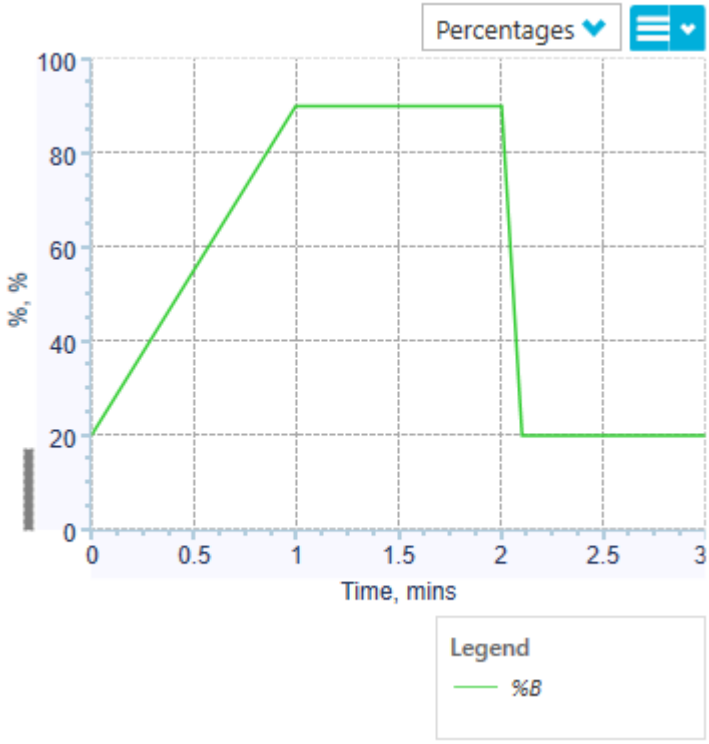
4. Open the Analytical Separation tab and then complete the Gradient Table as shown in the following figure.

Figure 3-27 Analytical Separation Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	50.000	5.00	
3.00	50.000	90.00	
4.00	50.000	90.00	
4.10	50.000	5.00	
6.00	50.000	5.00	

When the table is complete, the graphical representation should be the same as the following figure.

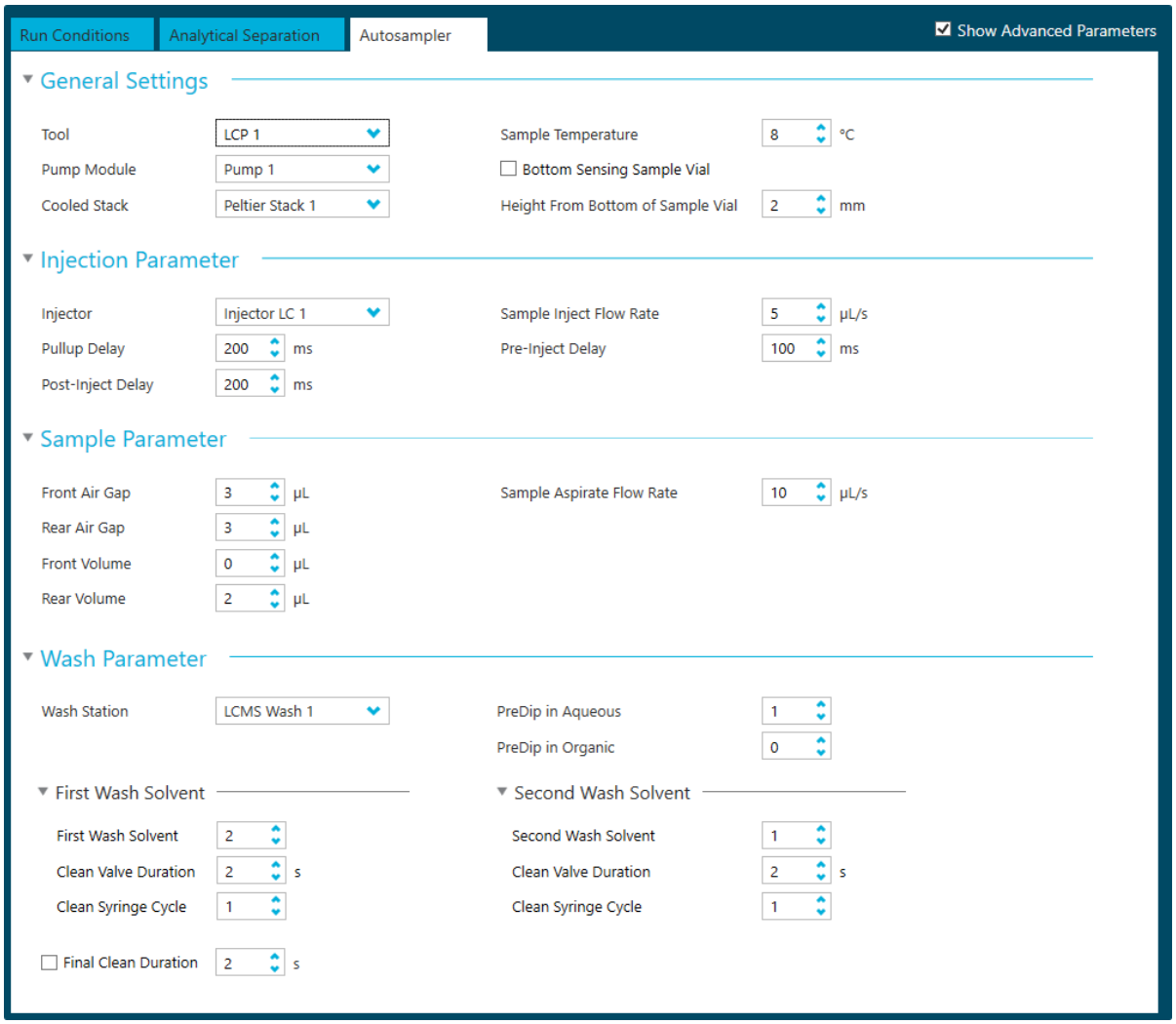
Figure 3-28 Analytical Separation Tab: Gradient Graph



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

5. Open the Autosampler tab and then set the values as shown in the following figure.

Figure 3-29 Autosampler Tab

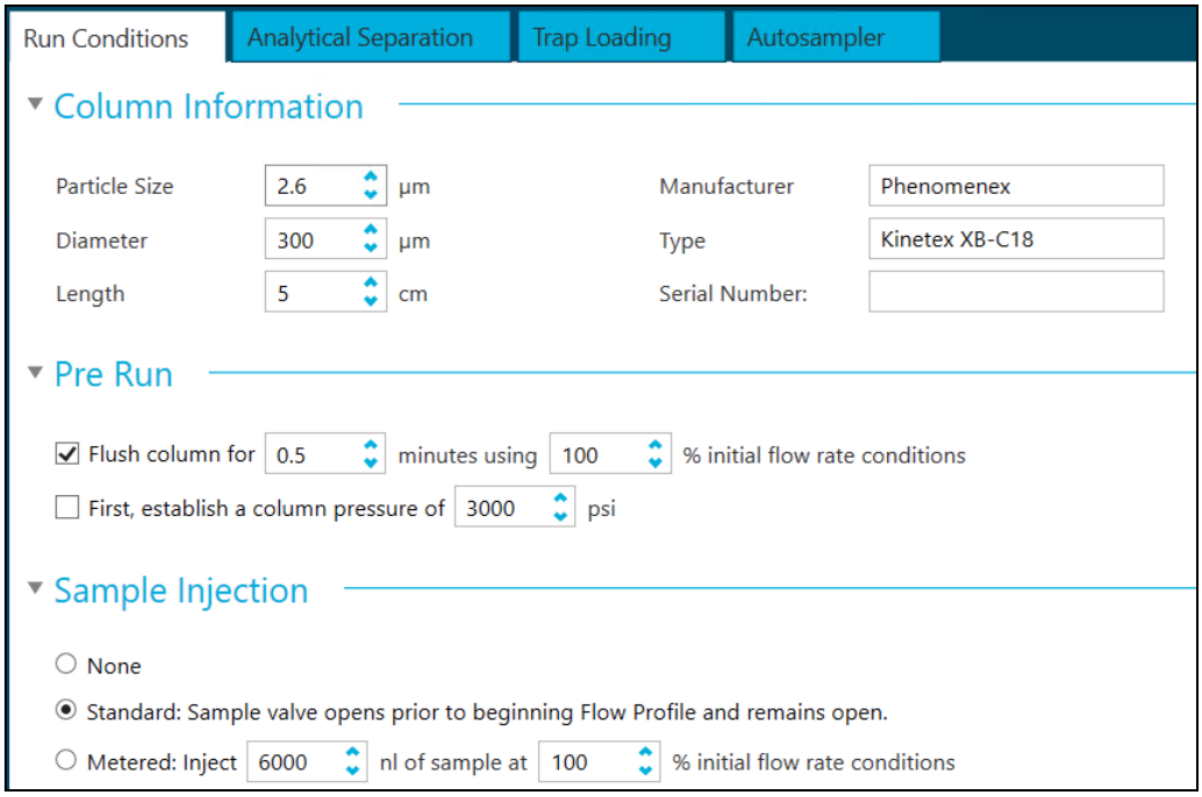


6. Save the method as `Micro-flow and High-flow Direct Inject Method`.

Create the Trap-and-Elute Method for Micro-flow and High-flow Systems

1. Click **LC Method**.
2. Click **New** > **Trap Elute**.
3. Open the Run Conditions tab and then set the values as shown in the following figure.

Figure 3-30 Run Conditions Tab



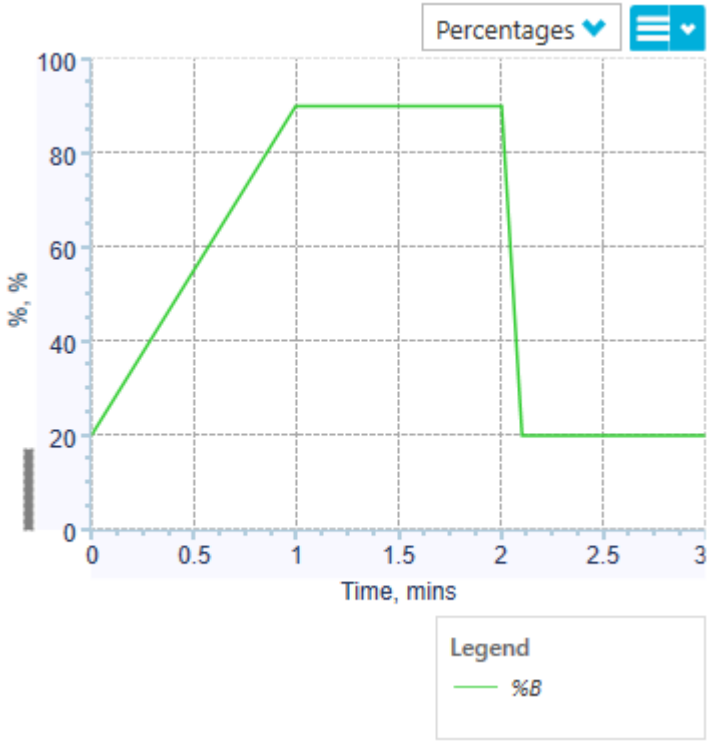
4. Open the Analytical Separation tab and then complete the Gradient Table as shown in the following figure.

Figure 3-31 Analytical Separation Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	50.000	5.00	
3.00	50.000	90.00	
4.00	50.000	90.00	
4.10	50.000	5.00	
6.00	50.000	5.00	

When the table is complete, the graphical representation should be the same as the following figure.

Figure 3-32 Analytical Separation Tab: Gradient Graph



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

5. Open the Trap Loading tab and then complete the Gradient Table as shown in the following figure.

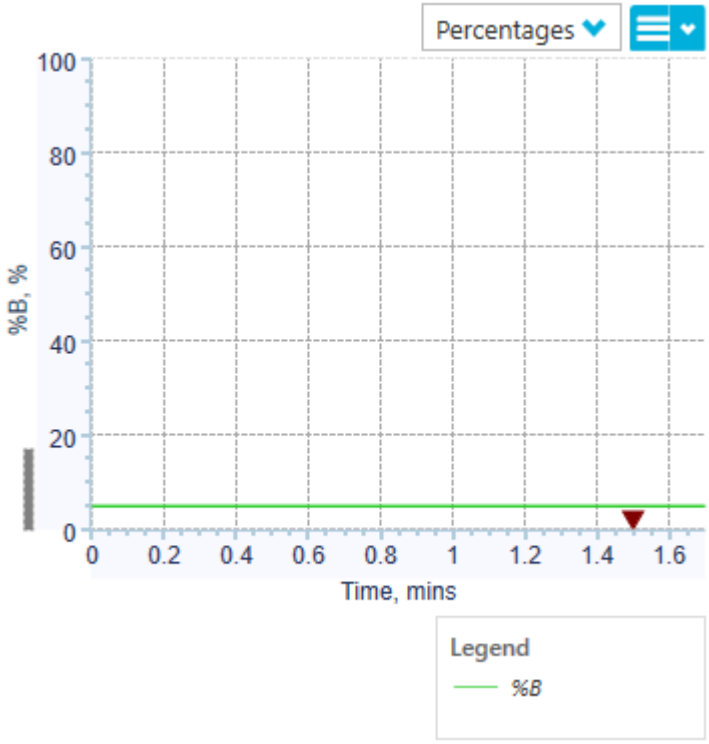
   In the **Event** column, at 1.5 minutes, make sure to select **Start Gradient 1**.

Figure 3-33 Trap Loading Tab: Gradient Table

Time (mins)	Flowrate (µL/min)	%B	Event
0.00	50.000	5.00	
1.50	50.000	5.00	Start Gradient 1
1.70	50.000	5.00	

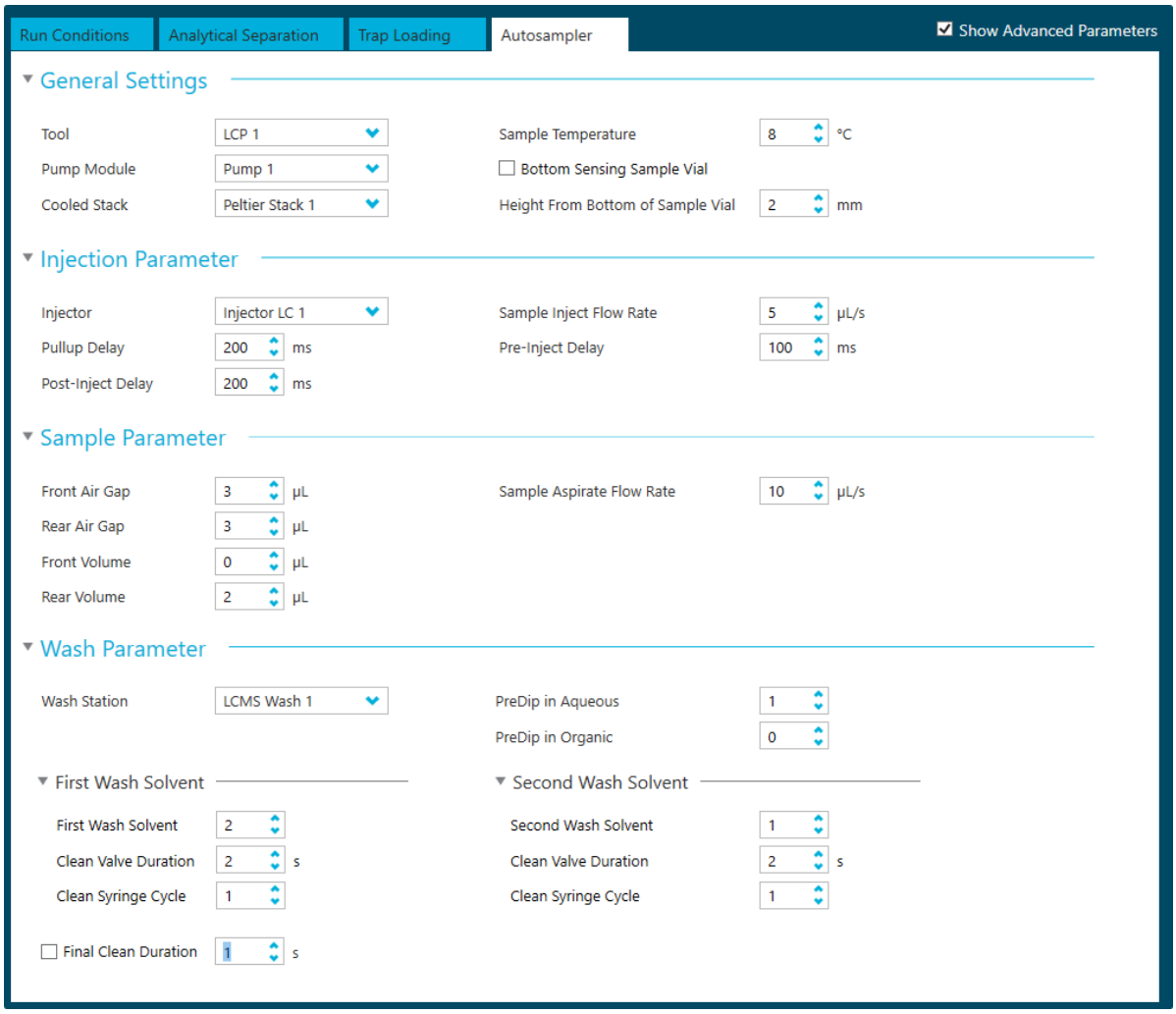
When the table is complete, the graphical representation should match the following figure.

Figure 3-34 Trap Loading Tab: Gradient Graph



6. Open the Autosampler tab and then set the values as shown in the following figure.

Figure 3-35 Autosampler Tab



7. Save the method as `Micro-flow and High-flow Trap Elute Method`.

Set Up the System to Do an Experiment with the Analyst Software

4

This chapter describes the steps to prepare the M5 MicroLC system to run an experiment using the Analyst software. The procedures are similar for direct injection and trap-and-elute experiments with any differences noted.

The procedures described in this chapter assume the system has already been correctly installed and initialized.

Complete the following steps in the order in which they appear:

- Verify the Hardware Profile
- Plumb the Injection Valve
- (M5 MicroLC-TE Systems Only) Examine the System Configuration
- Load the Mobile Phases
- Flush the Injection Valve
- Let the Column Oven Pre-Heat
- Test the LC System Connections with the Analyst Software
- Create the LC Methods

Note: The screen captures shown in this chapter are for the M5 MicroLC-TE system. For an M5 MicroLC system, the screens are different.

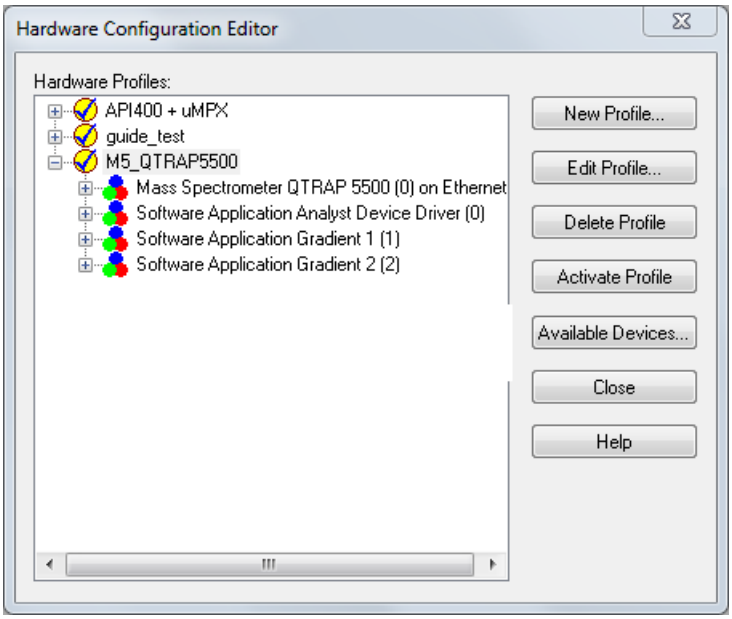
Verify the Hardware Profile

The active hardware profile in the Analyst software must include the autosampler, the Analyst Device Driver (ADD) software and the M5 MicroLC pump. For an M5 MicroLC-TE system, there are two pumps. However, if a different computer is being used or the Analyst software has been uninstalled, then the hardware profile might not be correct. Use these steps to verify the hardware profile.

1. Close the Eksigent Control software.
2. Open the Analyst software.
3. On the Navigation bar, under **Configure**, double-click **Hardware Configuration**.

4. In the Hardware Configuration Editor dialog, click each hardware profile to open it and find a profile that contains a mass spectrometer, the ADD software for the autosampler, and the Eksigent Control software.

Figure 4-1 Hardware Configuration Editor Dialog: Configured for M5 MicroLC-TE System



If an appropriate hardware profile is not available, then create one. Refer to the section: Create a Hardware Profile with the Analyst Software.

Note: For a TripleTOF system with a CDS, additional steps are required after creating the hardware profile. Refer to the section: Change the Calibration Method for a SCIEX TripleTOF System with the Analyst Software.

5. If the profile does not have a green check mark to the left, then click **Activate Profile.** The active profile is shown with a check mark, the Eksigent Control software starts, and the Acquisition window opens. If the window does not open (indicating that the Eksigent Control software did not start), then close the Analyst software.
6. Close the Hardware Profile window.

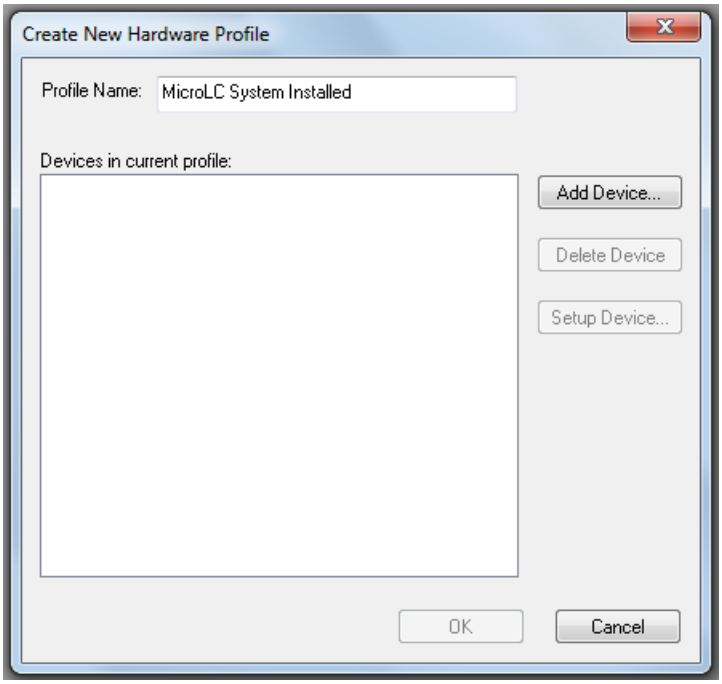
Note: Do not start the Eksigent Control software manually. Instead, let the Analyst software start the Eksigent Control software. (When the Eksigent Control software is started manually, the **LC Methods** button is replaced with the **Run Manager** button.)

Create a Hardware Profile with the Analyst Software

The active hardware profile must include the autosampler, the Analyst Device Driver (ADD) software and the M5 MicroLC pump. For an M5 MicroLC-TE, there are two pumps. If an applicable hardware profile is not available, then create one.

1. Close the Eksigent Control software or SCIEX OS.
2. Open the Analyst software.
3. On the Navigation bar, under **Configure**, double-click **Hardware Configuration.**
4. Click **New Profile**.

Figure 4-2 Create New Hardware Profile Dialog



5. Type a name in the **Profile Name** field.
6. Add the mass spectrometer to the profile.
   a. Click **Add device**.
   b. Select **Mass Spectrometer** in the **Device Type** list.
   c. Click the applicable mass spectrometer in the list and then click **OK**.

   Tip! The correct mass spectrometer is usually highlighted in the list.
7. Configure the hardware profile for the ADD software.

a. Click **Software Application <not configured>.**

b. Click **Setup Device**.
The Software Application Settings dialog opens.

c. Click **Analyst Device Driver** and then click **OK**.

8. Configure the profile for the Gradient 1 pump.

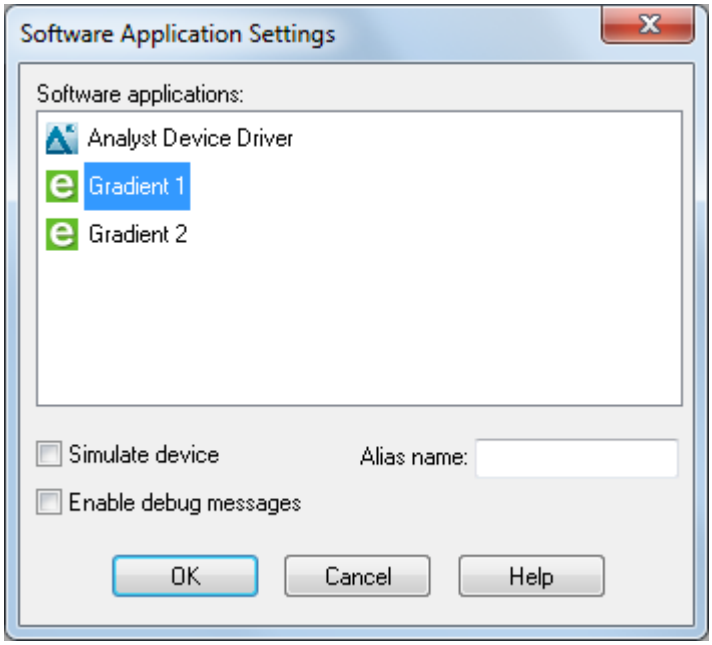
a. Click **Software Application <not configured>**.

b. Click **Setup Device**.
The Software Application Settings dialog opens.

Note: If nothing is listed in the Software applications list in the Software Application Settings dialog, then the Eksigent driver for the Analyst software is not installed. To install the driver, follow the instructions in the section: Install the Eksigent Control Software and Transfer the Settings . Omit the step for the settings.

c. Click **Gradient 1** and then click **OK**.

Figure 4-3 Software Application Settings Dialog: M5 MicroLC-TE System



9. M5 MicroLC-TE system: Do step 8 again to configure the second pump. Click **Gradient 2**.

10. Click **OK** to save the profile.

11. If the profile does not have a green check to the left, then click **Activate Profile.**

12. Click **Close**.

The active profile is shown with a check and the ADD Device Driver and Eksigent Control software both start. In the Eksigent Control software, the Acquisition window opens.

Figure 4-4 Analyst Device Driver (ADD)

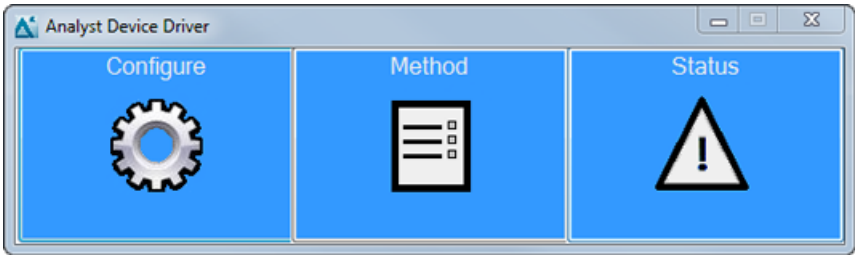
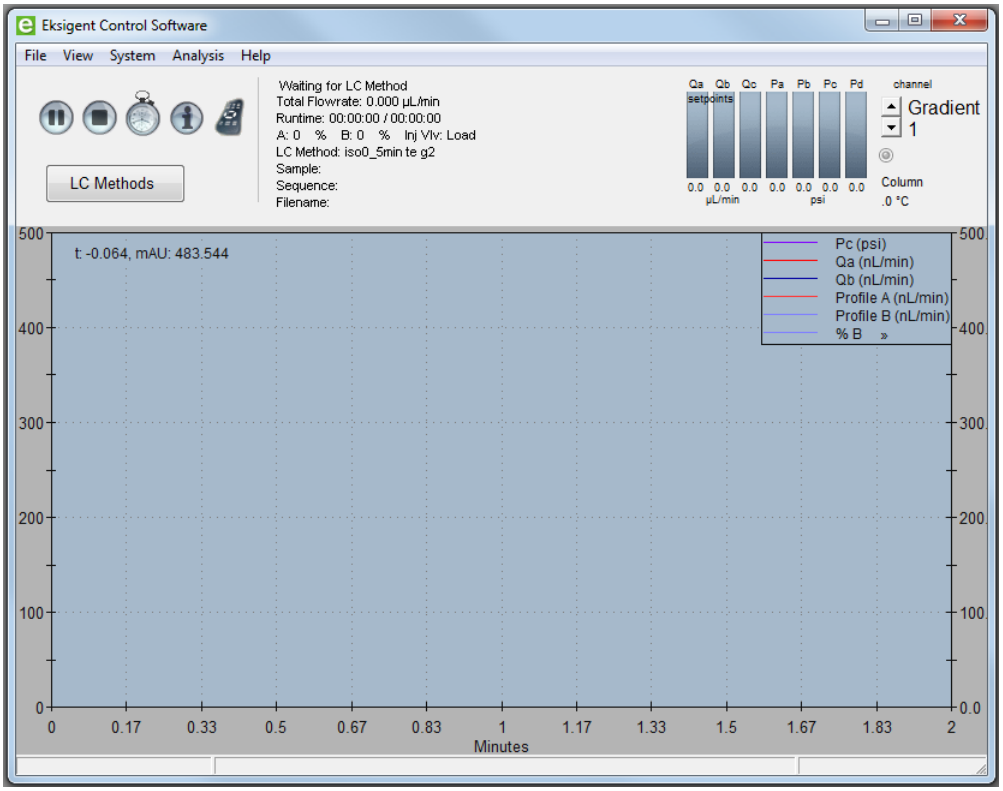


Figure 4-5 Eksigent Control Software Acquisition Window Started from Analyst Software



Note: Do not start the Eksigent Control software manually. Instead, let the Analyst software start the Eksigent Control software. (When the Eksigent Control software is started manually, the **LC Methods** button is replaced with the **Run Manager** button.)

Plumb the Injection Valve

The experiment type controls how the M5 MicroLC system is plumbed.

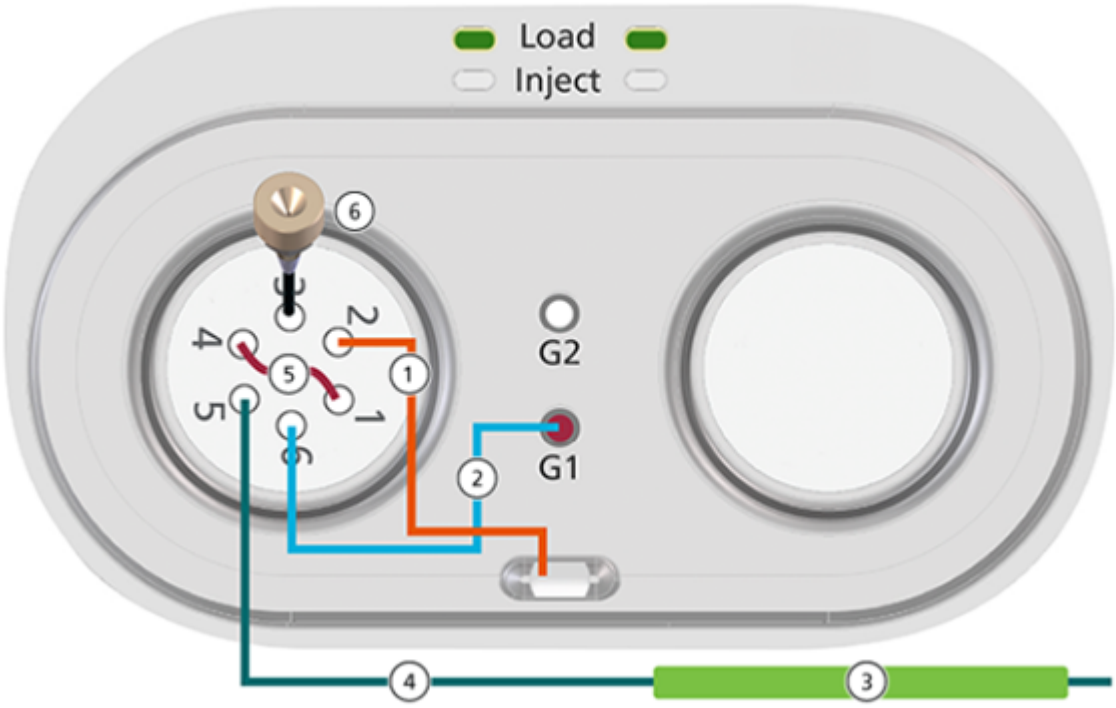
- For a direct injection experiment, refer to the section: Direct Injection Plumbing Diagram.
- For a trap-and-elute experiment, refer to the section: Trap-and-Elute Plumbing Diagram.

For either type of experiment, a different column, trap column, or sample loop can be installed as applicable for the experimental conditions.

Direct Injection Plumbing Diagram

Direct injection experiments can be performed on either an M5 MicroLC system or an M5 MicroLC-TE system.

Figure 4-6 Injection Valve Plumbing for Direct Injection Experiments



Item	Description	Part Number
1	Stainless steel tubing, 250 µm i.d., 1/32 inch o.d., 10 cm	200-00367
2	Gray tubing, 50 µm i.d., 1/32 inch o.d., 10 cm	205-00069
3	Low- or micro-flow systems: Kinetex 2.6 µm 100 Å XB-C18 0.3 mm i.d. x 5 cm column Micro- or high-flow systems: Kinetex 2.6 µm 100 Å XB-C18 0.5 mm i.d. x 5 cm column	00B-4496-AC 00B-4496-AF
4	SecurityLINK tubing, 75 cm (or longer as required)	AJ1-2294

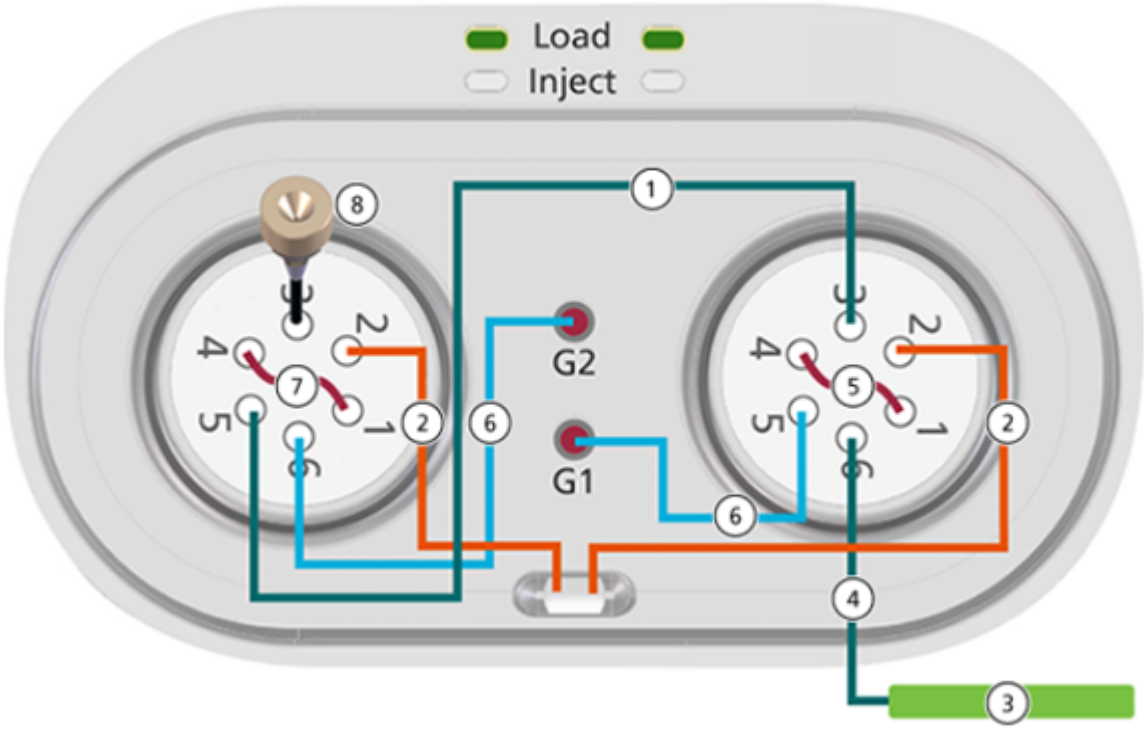
Item	Description	Part Number
5	5 µL sample loop (loops with other volumes can be used)	5017799
6	Injection port	5052374

For all of the connections before the analytical column, use gold nuts (PN 5024174) and ferrules (PN 910-00087).

Trap-and-Elute Plumbing Diagram

Trap-and-elute experiments can only be performed on the M5 MicroLC-TE system.

Figure 4-7 Injection Valve Plumbing for Trap-and-Elute Experiments



Item	Description	Part Number
1	Gray tubing, 50 µm i.d., 1/32 inch o.d., 20 cm	205-00039
2	Stainless steel tubing, 250 µm i.d., 1/32 inch o.d., 10 cm	200-00367
3	Low- or micro-flow systems: Kinetex 2.6 µm 100 Å XB-C18 0.3 mm i.d. x 5 cm column Micro- or high-flow systems: Kinetex 2.6 µm 100 Å XB-C18 0.5 mm i.d. x 5 cm column	00B-4496-AC 00B-4496-AF

Item	Description	Part Number
4	SecurityLINK tubing, 75 cm (or longer as required)	AJ1-2294
5	Luna C18 (2) 5 µm 100 Å 0.3 mm i.d. x 2 cm trap column Two pieces of SecurityLINK tubing, 50 µm i.d., 15 cm	03M-4252-AC AJ1-2224
6	Gray tubing, 50 µm i.d., 1/32 inch o.d., 10 cm	205-00069
7	50 µL stainless steel sample loop	5040770
8	Injection port	5052374

For all of the connections before the analytical column, use gold nuts (PN 5024174) and ferrules (PN 910-00087).

Post-Column Plumbing

Note: The following instructions apply only to mass spectrometers that use the Turbo V, DuoSpray, and IonDrive Turbo V ion sources. For mass spectrometers that use the OptiFlow Turbo V ion source, refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.

Note: For mass spectrometers using the OptiFlow Pro ion source, refer to the document: *OptiFlow Pro Ion Source Operator Guide*.

Note: The electrodes, fittings, and tubing required for the Turbo V, DuoSpray, and IonDrive Turbo V ion source are not included with the M5 MicroLC system and must be purchased separately from SCIEX. Refer to the section: Order Parts.

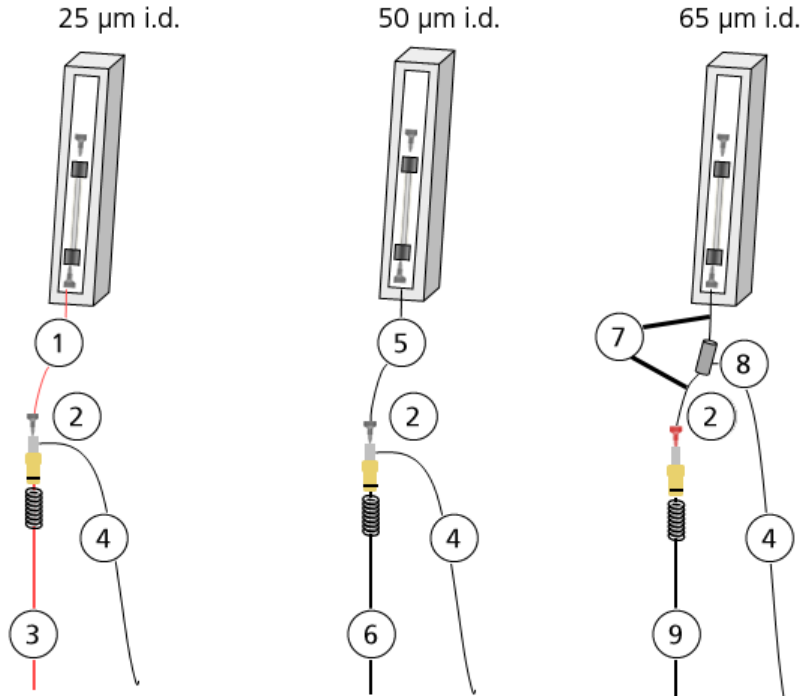
Plumbing details after the column vary based on the ion source electrode in use. To select the correct electrode for the planned flow rate, refer to the table: Table 4-1.

WARNING! Electrical Shock Hazard: For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, use a red fitting at the ion source electrode to decrease the risk of electrical shock. Do not use conductive fittings such as the high-pressure carbon-filled black fittings.

WARNING! Electrical Shock Hazard. Do not bypass the grounding union connection. The grounding union provides grounding between the mass spectrometer and the sample introduction device.

All tubing is 1/32 inch o.d.

Figure 4-8 Connections: Ion Source Electrodes



Item	Description	Part Number
1	Orange 25 µm i.d. tubing, 10 cm	205-00091
2	Red PEEK fitting	200-00330
3	25 µm i.d. electrode	5028467
4	Grounding cable	5016435
5	Gray 50 µm i.d. tubing, 10 cm	205-00069
6	50 µm i.d. electrode	5028466
7	Gray 50 µm i.d. tubing, 5 cm	205-00070
8	Stainless steel grounding union	5016413
9	65 µm i.d. electrode	5029342

Plumb the Valves Step-by-Step Instructions

WARNING! Electrical Shock Hazard: For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, use a red fitting at the ion source electrode to decrease the risk of electrical shock. Do not use conductive fittings such as the high-pressure carbon-filled black fittings.

WARNING! Hot Surface Hazard. Beware of burns. The column oven becomes hot during operation.

Before plumbing the valves, read the section: Work with PEEK-clad Fused Silica Tubing.

Unless otherwise noted, all connections are made with gold nuts and ferrules.

1. Plumb port 2 on the injection valve with 10 cm of 0.010 inch (250 µm) i.d., 1/32 inch o.d. stainless steel tubing and then put the other end in the waste drain.
2. Install the sample loop in port 1 and port 4 on the injection valve using a 3/16 inch wrench.
   - For a direct injection experiment, use a 5 µL sample loop.
   - For a trap-and-elute experiment, use a 50 µL sample loop.

   Note: Do not use the fittings that are included with the loop. Use the gold nuts and ferrules.
3. For a direct injection experiment, plumb the G1 pump outlet to port 6 on the injection valve with 10 cm of 50 µm i.d., 1/32 inch o.d. tubing.
4. For a trap-and-elute experiment, use 10 cm of 50 µm i.d., 1/32 inch o.d. tubing is correct and plumb the pump outlets as follows:
   - G2 pump outlet to port 6 on the injection valve.
   - G1 pump outlet to port 5 on the auxiliary valve.
5. For a trap-and-elute experiment, install the trap column and then plumb the auxiliary valve.
   a. Install the trap column in port 1 and port 4 on the auxiliary valve. For each connection, use 15 cm SecurityLINK tubing and a gold nut and ferrule. Tighten the fitting on the SecurityLINK tubing until the first audible click.
   b. Put the trap column in the trap column holder on the valve basin.
   c. Plumb port 2 on the auxiliary valve with 10 cm of 0.010 inch (250 µm) i.d., 1/32 inch o.d. stainless steel tubing and put the other end in the waste drain.
   d. Connect port 5 on the injection valve to port 3 on the auxiliary valve with 20 cm of 50 µm i.d., 1/32 inch o.d. tubing.

6. Connect the valve to the column inlet using 75 cm SecurityLINK tubing. Tighten the fitting on the SecurityLINK tubing until the first audible click. The valve and port depend on the type of experiment:

   - For a direct injection experiment, use port 5 on the injection valve.
   - For a trap-and-elute experiment, use port 6 on the auxiliary valve.

   Tip! If required to accommodate the laboratory layout, then longer tubing can be used, but make the length of the tubing from the valve to the column as short as possible.

7. If required, then install the electrode in the ion source probe. For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, refer to the section: Install the Electrode. For the OptiFlow Turbo V ion source, refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.
8. For the OptiFlow Turbo V ion source, install the column.
   a. Connect the column to the ion source probe. Tighten the fitting until it is finger tight.
   b. Install the column oven on the ion source. Refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.
9. For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, install the column and then ground the electrode.
   a. Connect the column to the ion source electrode using a black PEEK fitting at the column outlet, two pieces of tubing connected by a grounding union after the column, and a red PEEK fitting at the electrode. Refer to the section: Post-Column Plumbing.
   b. Attach one end of the grounding cable to the grounding point on the ion source.
   c. Cut the other end of the grounding cable as follows:
      - 25 µm and 50 µm i.d. electrodes: Cut to the grounding union on the probe.
      - 65 µm i.d. electrodes: Cut to the grounding union installed on the tubing after the column.
   d. Put the column in the column oven.
   e. Cut a piece of the foam block from the column oven kit, and then put the foam on top of the column to hold the column securely against the metal surface of the oven for good thermal contact.
   f. Close the column oven.

Install the Electrode

Note: The following instructions apply only to mass spectrometers that use the Turbo V, DuoSpray, and IonDrive Turbo V ion sources. For mass spectrometers that use the OptiFlow Turbo V ion source, refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.

The smaller columns used in micro ultra-high performance liquid chromatography require lower flow rates and smaller electrodes than traditional liquid chromatography. Depending on the planned flow rates for the system, install the correct electrode in the ion source probe.

Table 4-1 Suggested Electrode by Flow Rate

Flow Rate (µL/min)	Electrode
5 to 20	25 µm i.d.
20 to 50	50 µm i.d.
20 to 100	65 µm i.d.

Note: The upper limit for the flow rate is ultimately determined by the pressure limits of the system and the column.

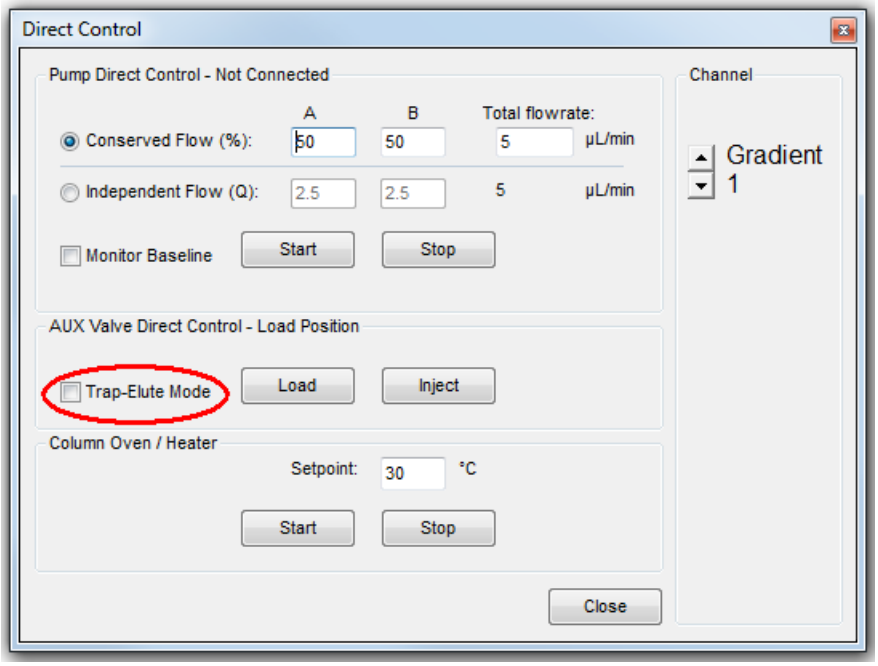
1. Replace the existing spring with the one provided with the electrode.
2. Install the electrode in the ion source probe in the same manner as the standard larger i.d. electrode.
3. Tighten the black screw cap on the probe and then adjust it as required to extend the electrode tip 1 mm to 2 mm past the probe tip.

(M5 MicroLC-TE Systems Only) Examine the System Configuration

For M5 MicroLC-TE systems, the plumbing and settings in the Eksigent Control software depend on the type of experiment. Make sure that the system is set up correctly for the type of experiment to be performed.

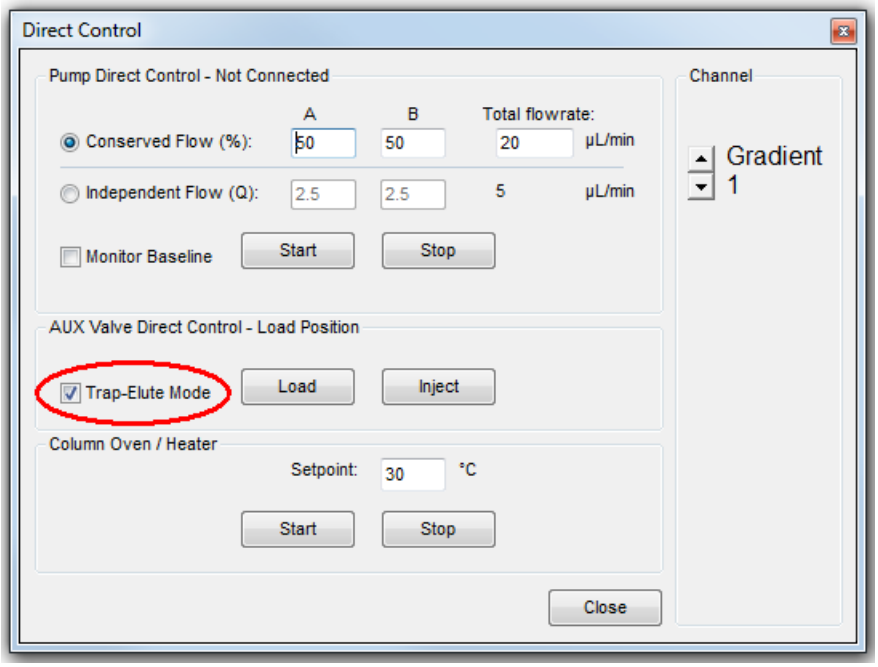
1. Make sure that the valve is correctly plumbed for the type of experiment to be performed.
   - Direct Injection Plumbing Diagram
   - Trap-and-Elute Plumbing Diagram
2. Set the mode in the Direct Control dialog:
   a. Select **System** > **Direct Control**.
   The Direct Control dialog opens.
   b. For a direct injection experiment, make sure that the **Trap-Elute Mode** check box is cleared.

Figure 4-9 Direct Control Dialog: Direct Injection Mode



c. For a trap-and-elute experiment, make sure that the **Trap-Elute Mode** check box is selected.

Figure 4-10 Direct Control Dialog: Trap-and-Elute Mode



d. Click **Close**.

Load the Mobile Phases

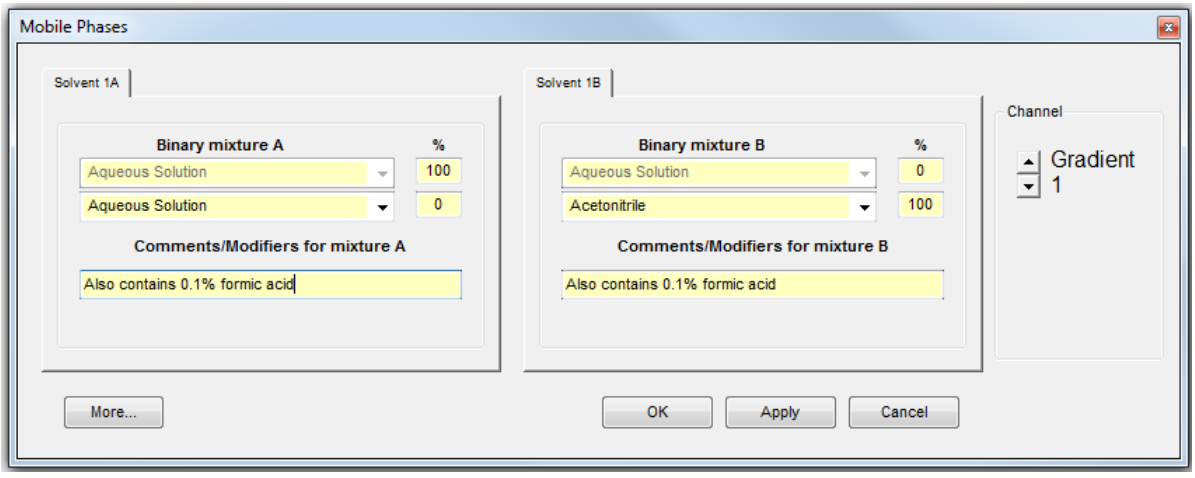
Note: We do not recommend the use of Milli-Q water because the quality is not satisfactory for use in LC-MS systems.

This procedure assumes that the mobile phases are water and acetonitrile, that water goes into the pump at the Mobile Phase A inlet (labeled 1A or 2A on the back of the system), and that acetonitrile goes into the pump through the Mobile Phase B inlet (labeled **1B** or **2B** on the back of the system). In general, the more organic mobile phase should use the B inlet.

Before using different mobile phases, refer to the section: System Safe Fluids.

1. If required, then discard any old solvents in the mobile phase bottles, then clean the bottles with the correct solvents.
2. Put new mobile phases in the bottles, then install the mobile phase tubing and filters.
3. Supply the mobile phase information in the Eksigent Control software.
   a. Click **System** > **Mobile Phases**.
   b. For Binary mixture A (mobile phase A), do not make any changes.
   c. For Binary mixture B (mobile phase B), select **Acetonitrile** in the second list and then type `100` in the **%** field.
   d. (Optional) Type comments in the **Comment/Modifiers** fields. For example, type the modifier and concentration.

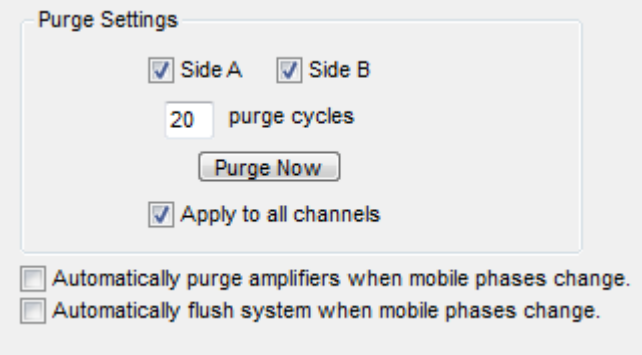
Figure 4-11 Mobile Phases Dialog



Note: If a mobile phase that is not listed in the Mobile Phases dialog is required, then either select a mobile phase with a very similar viscosity from the list or add a new mobile phase. For more instructions, refer to the section: Create a Custom Mobile Phase.

4. Purge the pumps a minimum of 20 times.
   a. Click **More** to show additional options in the dialog.
   b. In the Purge Settings section, select the pumps to be purged and then type `20` in the **purge cycles** field.

Figure 4-12 Purge Settings Section



   a. M5 MicroLC-TE system: Select the **Apply to all channels** check box.
   b. Click **Purge Now**.
      The pumps begin to execute purge cycles. While the pumps are purging, make sure that the mobile phases are pulled through the mobile phase tubing to the pumps.
   c. Examine the waste tubing of the pumps being purged. The waste tubes are clear plastic tubing and emerge from the back of the pump. After about 8 purges, the mobile phase should be purged through the waste tubing.
5. Flush the system.
   a. Disconnect the tubing that comes from from the G1 pump outlet.
   b. Connect one end of a length of 1/32 inch o.d. tubing to the G1 pump outlet and put the other end in the waste drain.
   c. In the Flush Settings section, set the parameters based on the system.

Table 4-2 Flush Settings Parameters

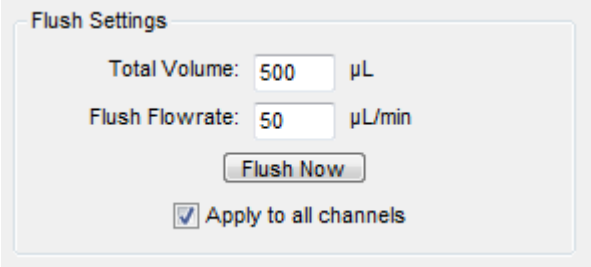
System	Total Volume (µL)	Flush Flowrate (µL/min)
Low-flow system	100	10
Micro-flow system	500	50

Table 4-2 Flush Settings Parameters (continued)

System	Total Volume (µL)	Flush Flowrate (µL/min)
High-flow system and all Gradient 2 pumps	500	50

d. Click **Flush Now**.

Figure 4-13 Flush Settings Section: Micro-flow and High-flow



e. When the flush sequence ends, click **OK**.

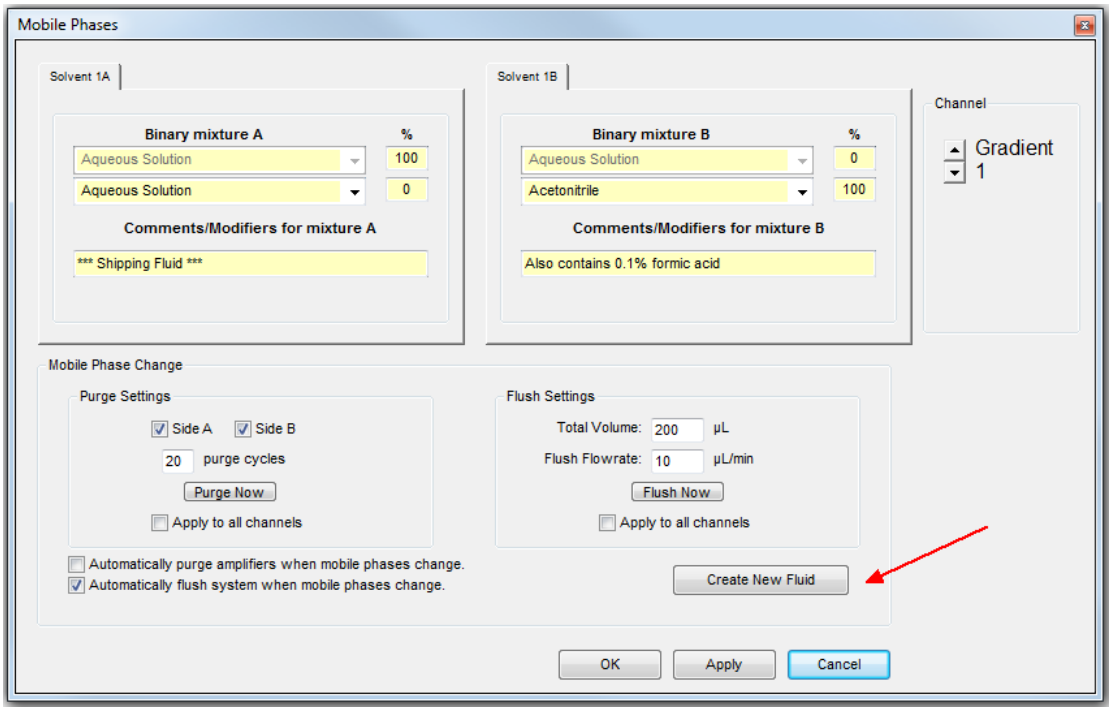
6. Do the flush step again for the G2 pump outlet. Install the 1/32 inch o.d. tubing from the G1 outlet in the G2 outlet.
7. For each pump, connect the original tubing between the pump outlet and the valve.

Create a Custom Mobile Phase

Some experiments require a mobile phase other than those available in the Eksigent Control software. Add a custom mobile phase in the Mobile Phases dialog. Also, create a custom mobile phase for a mixture of two solvents in one bottle.

1. Click **System** > **Mobile Phases**.

Figure 4-14 Mobile Phases Dialog: Expanded



2. For an M5 MicroLC-TE system, click the **Channel** buttons to select the pump that will use the new mobile phase.
3. Click **More** to show more options in the dialog.
4. In the Mobile Phase Change section, click **Create New Fluid** to open the Flowmeter Calibration dialog. (Adding a custom mobile phase includes performing a flowmeter calibration.)
5. Follow the steps in the Flowmeter Calibration dialog.

   Select the calibration pipette based on the system:

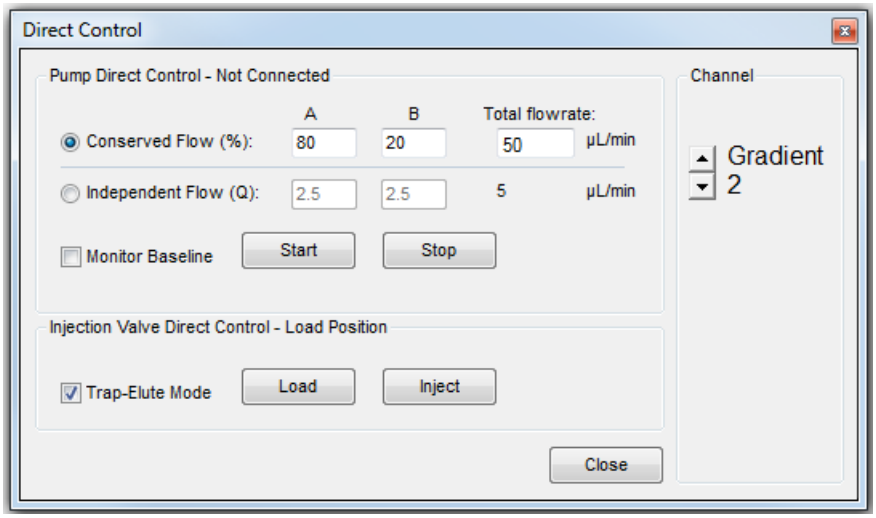
   - Low-flow system: 20 µL
   - Micro-flow system: 100 µL
   - High-flow system: 200 µL

Flush the Injection Valve

Flush the valve when the column is not connected to avoid introducing any contaminants from the valve to the column.

1. Disconnect the tubing from the column inlet.
2. Click **System** > **Direct Control**.

Figure 4-15 Direct Control Dialog



3. For an M5 MicroLC-TE system, click the **Channel** buttons to select **Gradient 2**.
4. Set the Pump Direct Control parameters and then start the pump.
   a. Select the **Conserved Flow** option.
   b. Set both **A (%)** and **B (%)** to `50`.
   c. Set the **Total flowrate (µL/min)** based on the system configuration:
      - For a low-flow system, type `10` .
      - For micro-flow or high-flow system type `20` .
   d. Click **Start**.
5. In the Valve Direct Control section, alternate clicking **Load** and **Inject**, waiting approximately 10 seconds between each click, for a total of 3 times.
6. Click **Load**, then click **Stop**.
7. Reconnect the tubing to the column inlet.

Let the Column Oven Pre-Heat

The temperature of the column can be regulated. The maximum temperature depends on the column heater in use.

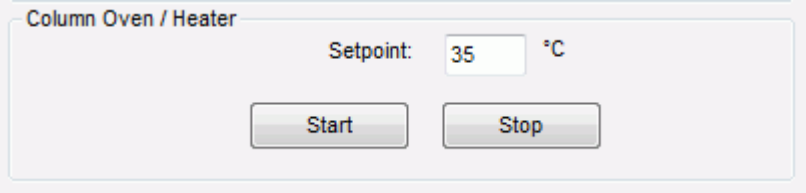
- For the OptiFlow Turbo V ion source, refer to the document: *OptiFlow Turbo V Ion Source Operator Guide*.
- For the Turbo V, DuoSpray, and IonDrive Turbo V ion sources, the maximum temperature is 60 °C.

Note: The column oven reaches the correct temperature quickly, but the column can take as long as 30 minutes to fully equilibrate.

WARNING! Hot Surface Hazard. Beware of burns. The column can become hot during operation. Allow the column to cool before removing it or replacing the PEEK clad fused silica tubing.

1. Connect the column.
2. In the Eksigent Control software, click **System > Direct Control**.
3. For a trap-and-elute system, click the **Channel** buttons to select **Gradient 1**.
4. In the **Column Oven/Heater** section, type `35` in the **Setpoint** field, and then click **Start**.

Figure 4-16 Direct Control Dialog: Column Oven/Heater Section



5. Close the compartment so that the oven can reach the specified temperature.

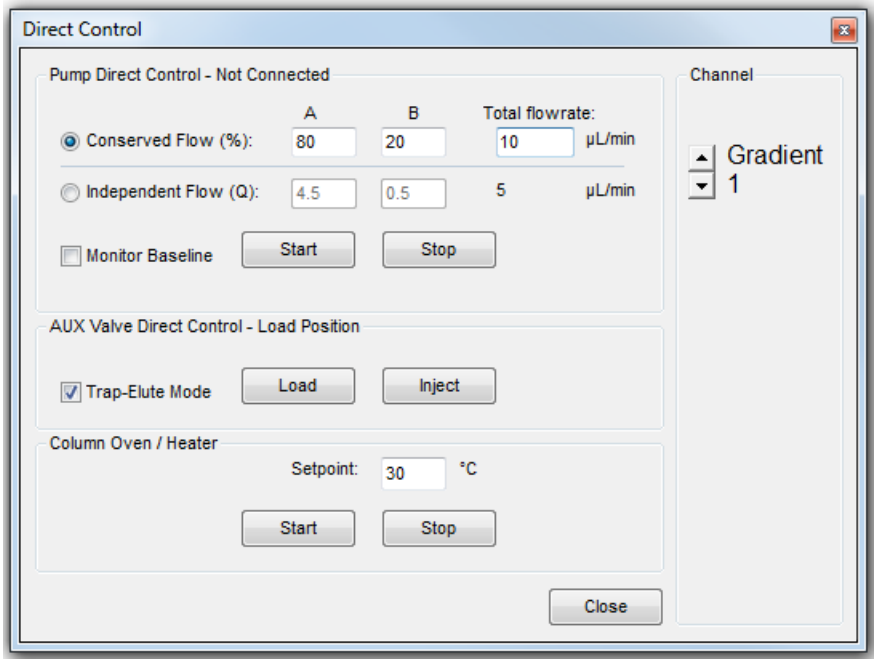
Test the LC System Connections with the Analyst Software

1. Make sure that the column is connected.
2. Click **System** > **Direct Control** .
   The Direct Control dialog opens.
3. Set the Pump Direct Control parameters.
   a. Select the **Conserved Flow (%)** option.
   b. Type `80` in the **A** field and `20` in the **B** field.

      Note: For other experiments, set **A** and **B** to match the initial conditions in the LC method to be used in the experiment.

   c. Set the **Total flowrate** (µL/min) for the specified column diameter.
      - For a 0.3 mm i.d. column, type `10` .
      - For a 0.5 mm i.d. column, type `40` .

Figure 4-17 Direct Control Dialog



4. In the **Pump Direct Control** section, click **Start** to start the pump.
5. Allow the system to pump for approximately 2 minutes, inspecting the connections for any leaks, and then click **Stop**.
6. For a trap-and-elute experiment, click the **Channel** arrow buttons to select **Gradient 2** and then do steps 3 through 5 again to test the connections for the other pump. Set the **Total flowrate (µL/min)** to `40`.
7. Click **Close**.

Create the LC Methods

An LC method contains the conditions used for separating the sample, including the flow rate, flow mode, and mobile phase gradient. For a direct injection experiment, one method is required. For a trap-and-elute experiment, two methods are required. Refer to the table: Table 4-3.

Note: Make sure to create the method that is correct for the flow rate configuration of the system in use.

Table 4-3 LC Methods

Type of Experiment	Low-flow System	Micro-flow or High-flow System
Direct injection	• Create the Gradient Method for a Low-flow System	• Create the Gradient Method for Micro-flow and High-Flow Systems
Trap-and-elute	• Create the Gradient Method for a Low-flow System • Create the LC Method to Load the Trap Column for a Low-flow System	• Create the Gradient Method for Micro-flow and High-Flow Systems • Create the LC Method to Load the Trap Column for Micro-flow and High-flow Systems

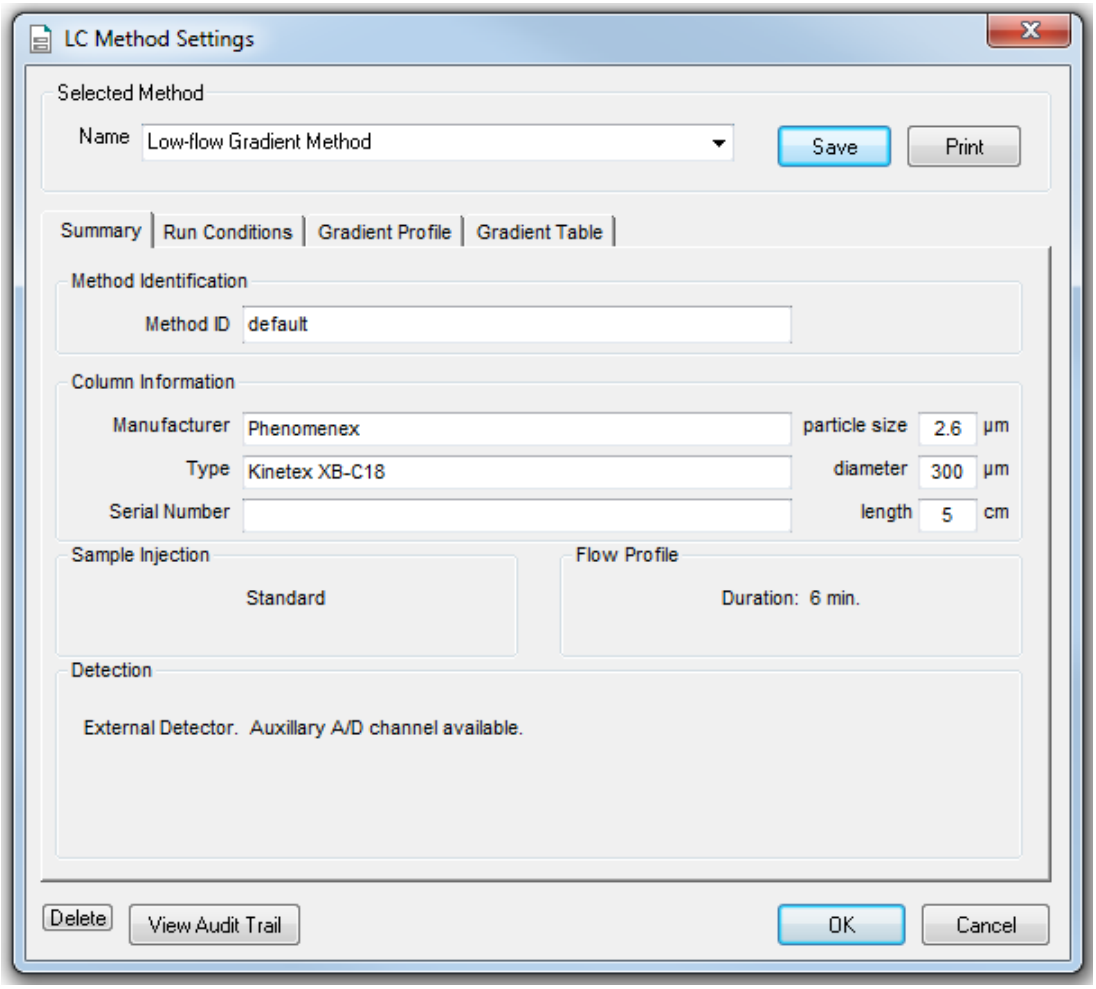
For other LC methods, SCIEX recommends setting a minimum of 3% for mobile phase A.

Create the Gradient Method for a Low-flow System

This method is used to separate the samples with the analytical column on a low-flow system.

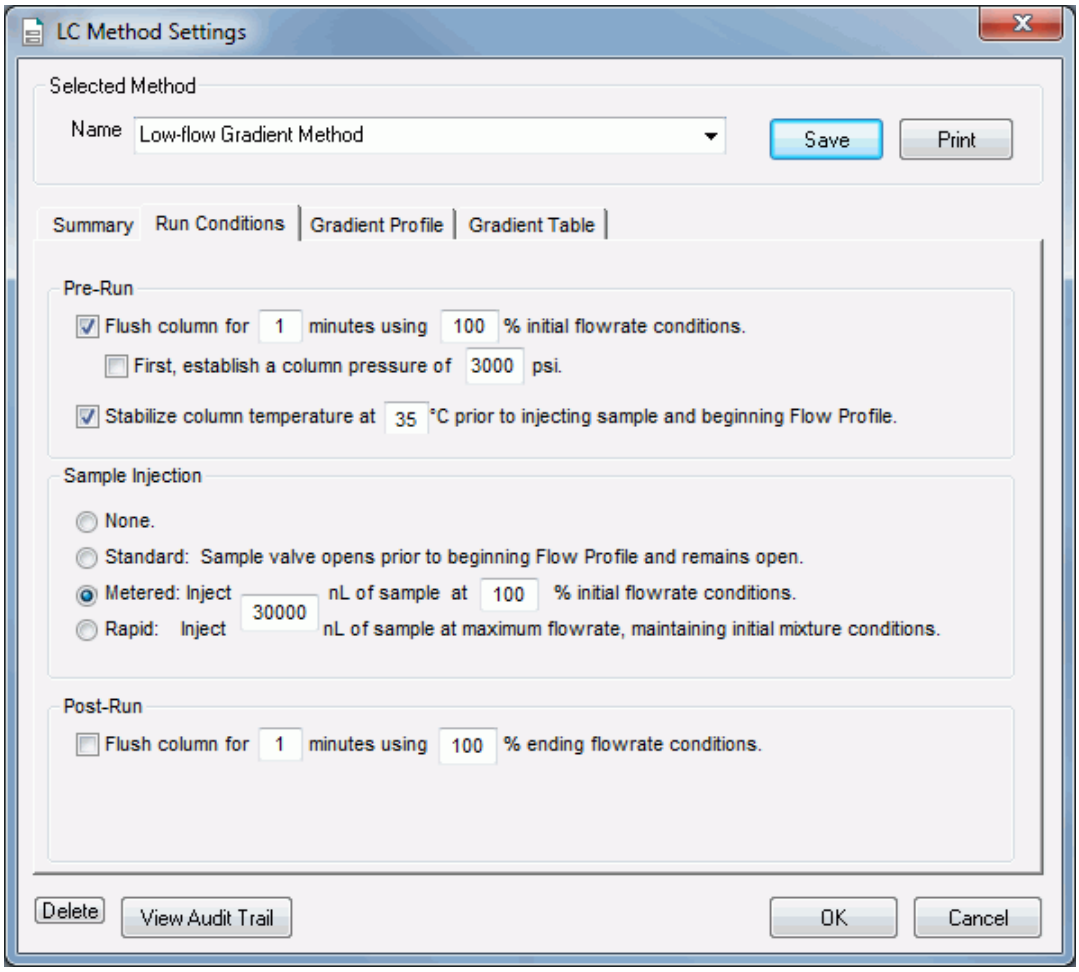
1. Click **LC Methods**.
   The LC Method Settings dialog opens.
2. In the **Name** field, type `Low-flow Gradient Method`, and then click **Save**.
3. In the Column Information section, set the values as shown in the following figure.

Figure 4-18 Low-flow Gradient Method: Summary Tab



4. Open the Run Conditions tab, and then set the values as shown in the following figure.

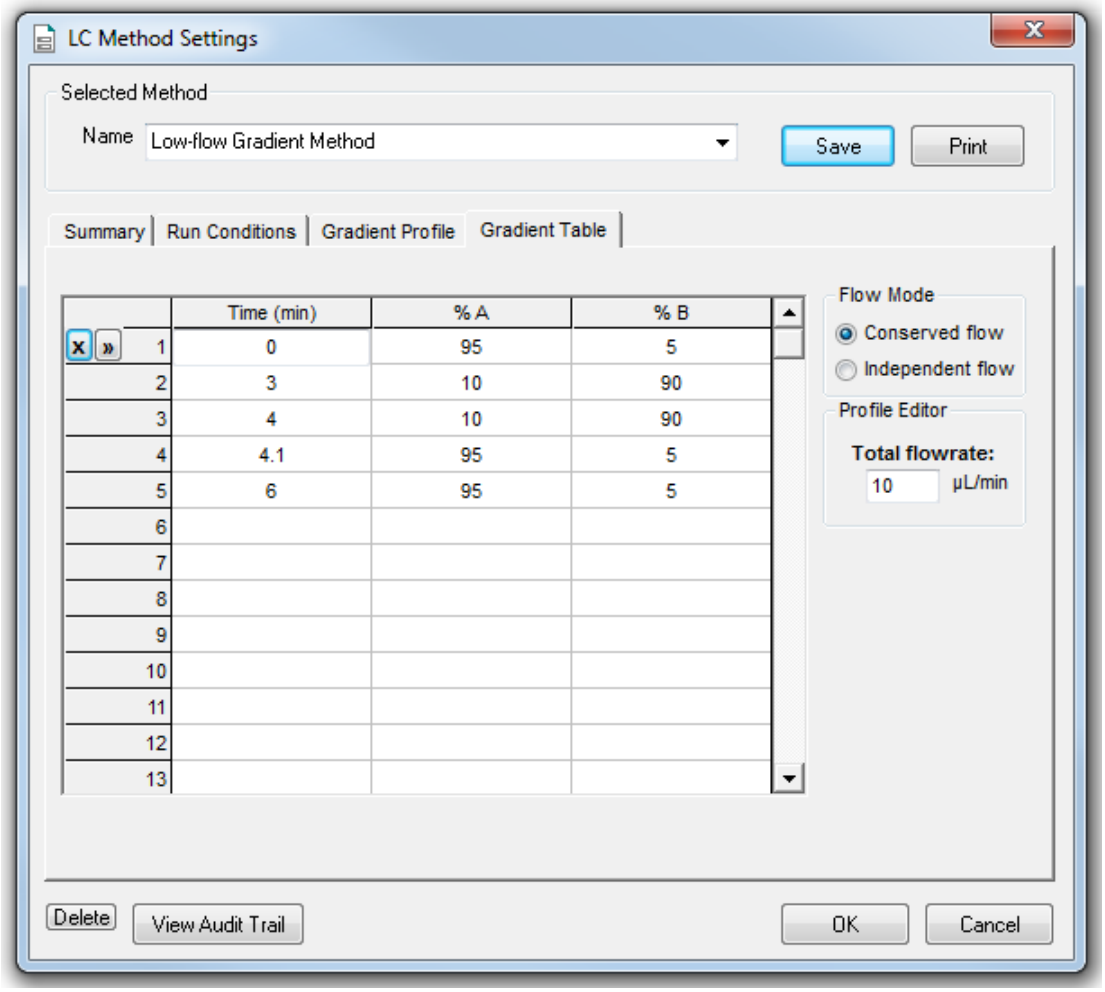
Figure 4-19 Low-flow Gradient Method: Run Conditions Tab



5. Open the Gradient Table tab, and then set the flow mode, the gradient parameters, and the flow rate as shown in the following figure.

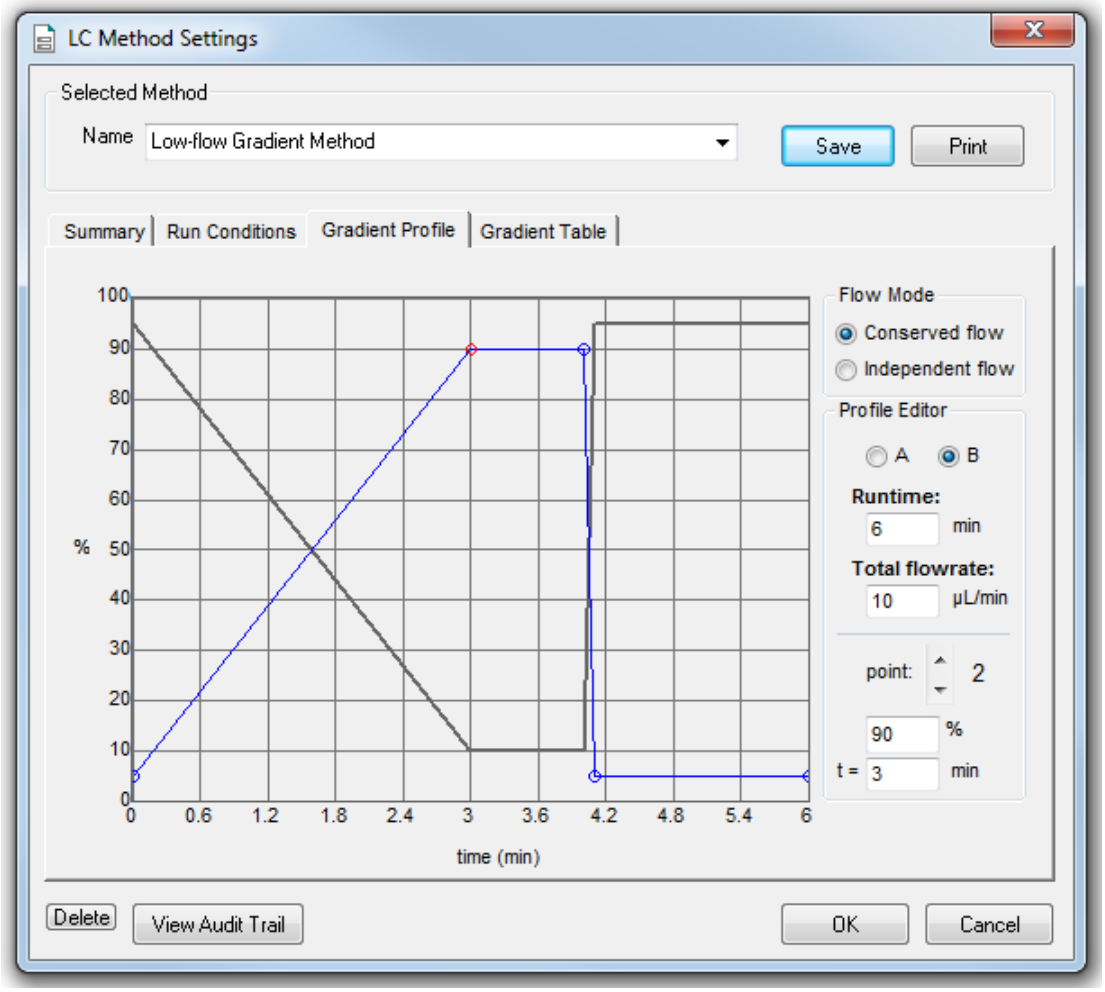
   For most experiments, select **Conserved** for the **Flow Mode**. In Conserved mode, the system uses the composition and total flow rate to calculate the flow rate for each mobile phase.

Figure 4-20 Low-flow Gradient Method: Gradient Table Tab



6. Open the Gradient Profile tab to see a graphical representation of the gradient.

Figure 4-21 Low-flow Gradient Method: Gradient Profile Tab



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

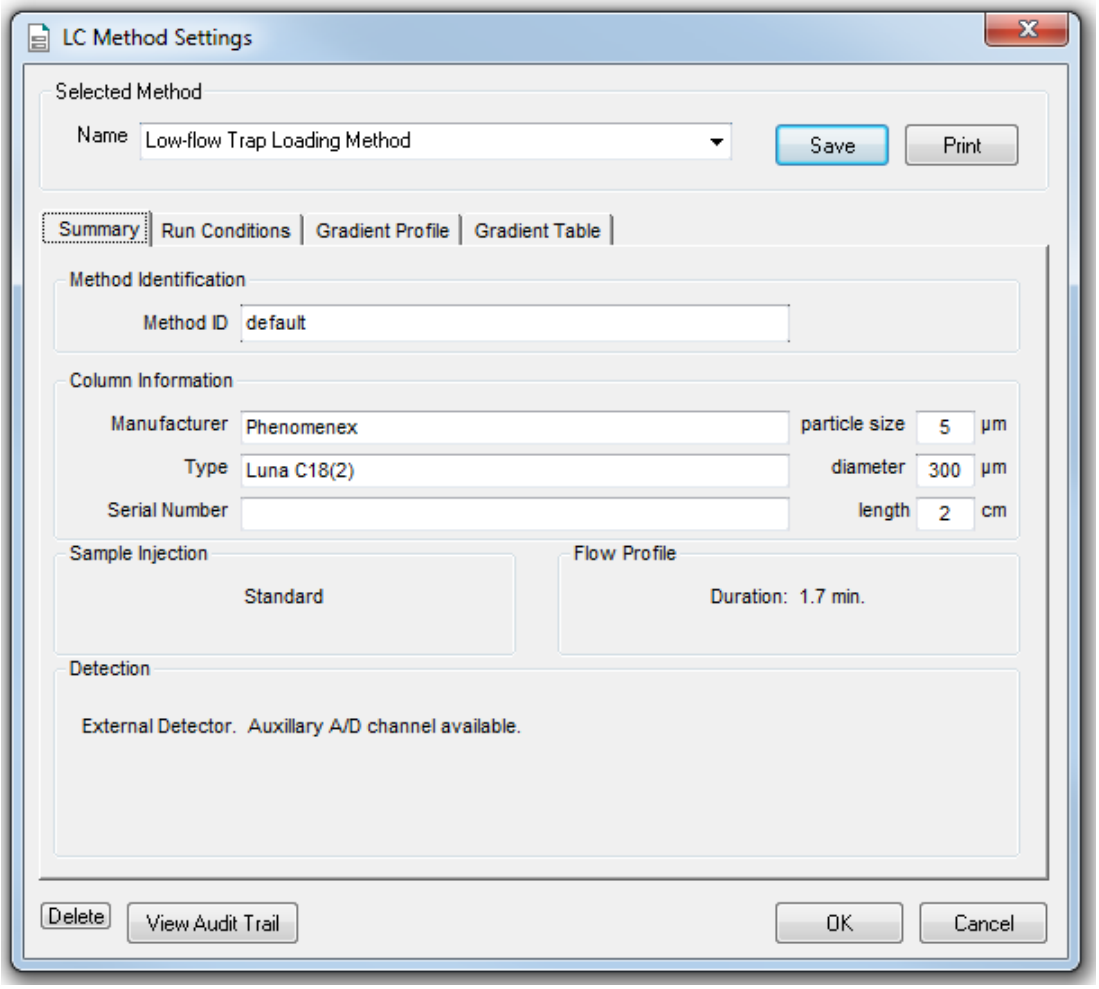
7. Click **Save**, and then click **OK**.

Create the LC Method to Load the Trap Column for a Low-flow System

This method is used to load the sample on to the trap column.

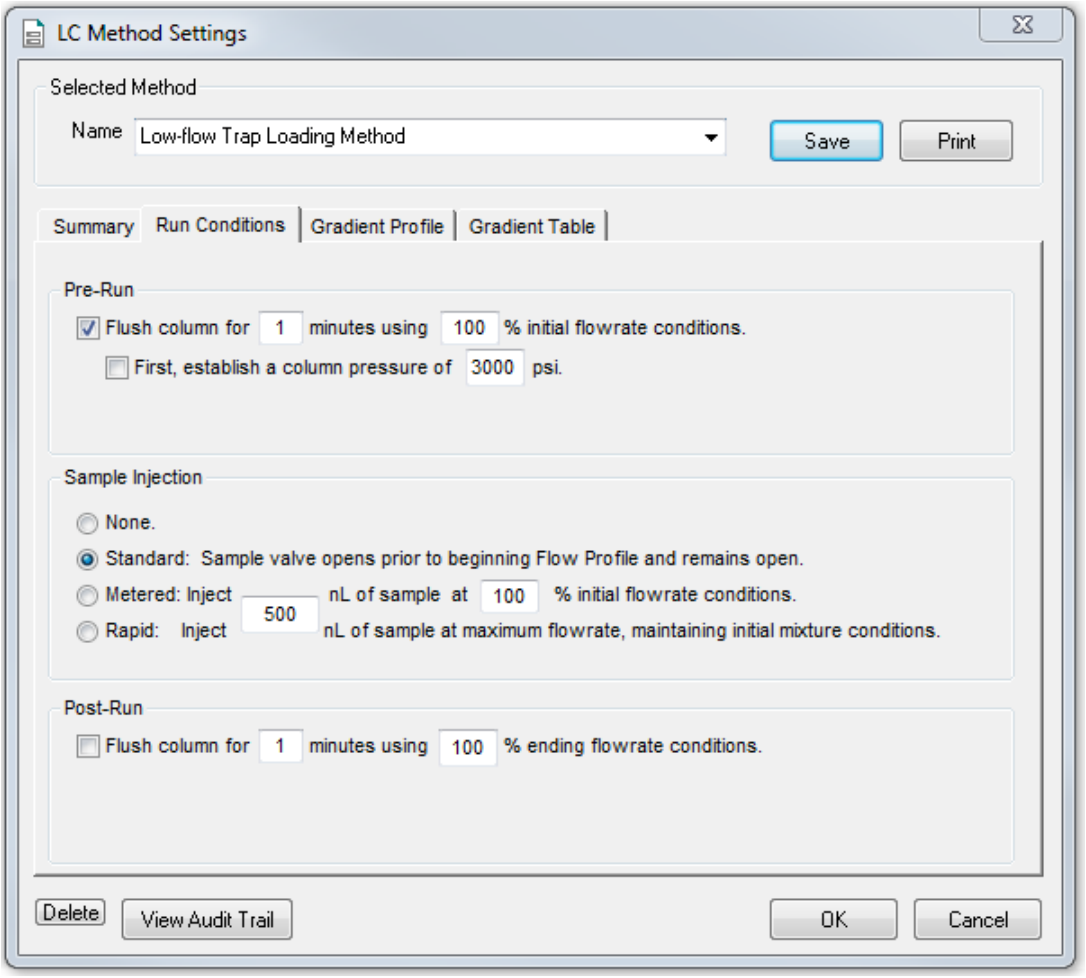
1. In the Acquisition window of the Eksigent Control software, click the arrows to select **Gradient 2** in the Channel section.
2. Click **LC Methods**.
   The LC Method Settings dialog opens.
3. In the **Name** field, type `Low-flow Trap Loading Method`, and then click **Save**.
4. In the Column Information section, set the values shown in the following figure.

Figure 4-22 Low-flow Trap Loading Method: Summary Tab



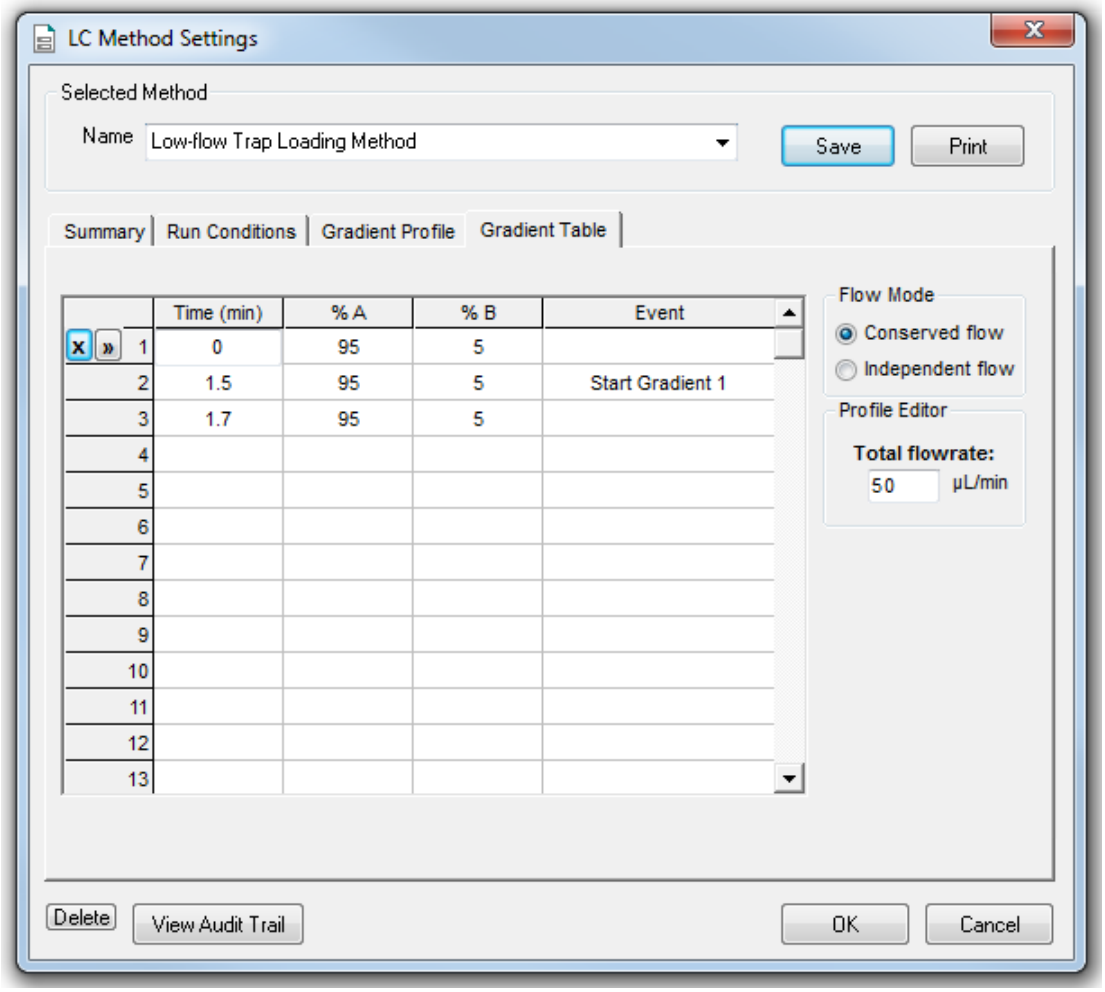
5. Open the Run Conditions tab, and then set the values shown in the following figure.

Figure 4-23 Low-flow Trap Loading Method: Run Conditions Tab



6. Open the Gradient Table tab, and then set the flow mode, the gradient parameters, and the flow rate as shown in the following figure.

Figure 4-24 Low-flow Trap Loading Method: Gradient Table Tab



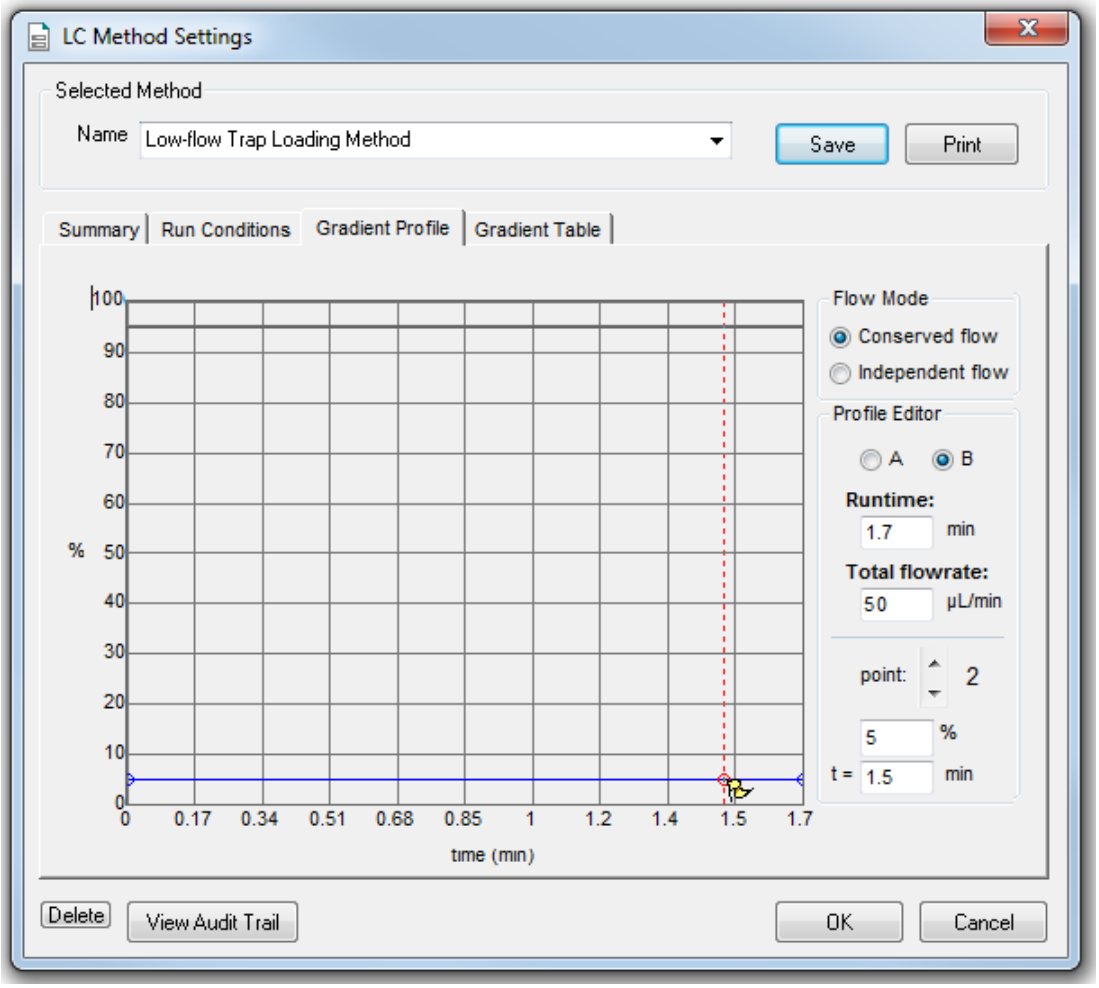
Note: If there is carryover from one sample to the next, then add a step at the end of the method to wash the sample loop with the organic mobile phase.

7. At 1.5 minutes, click the **Event** cell and then select **Start Gradient 1**.

   This event starts the Gradient 1 pump, which switches the trap column inline with the analytical column. The sample will be eluted from the trap column on to the analytical column.

8. Open the Gradient Profile tab to see a graphical representation of the gradient.

Figure 4-25 Low-flowTrap Loading Method: Gradient Profile Tab



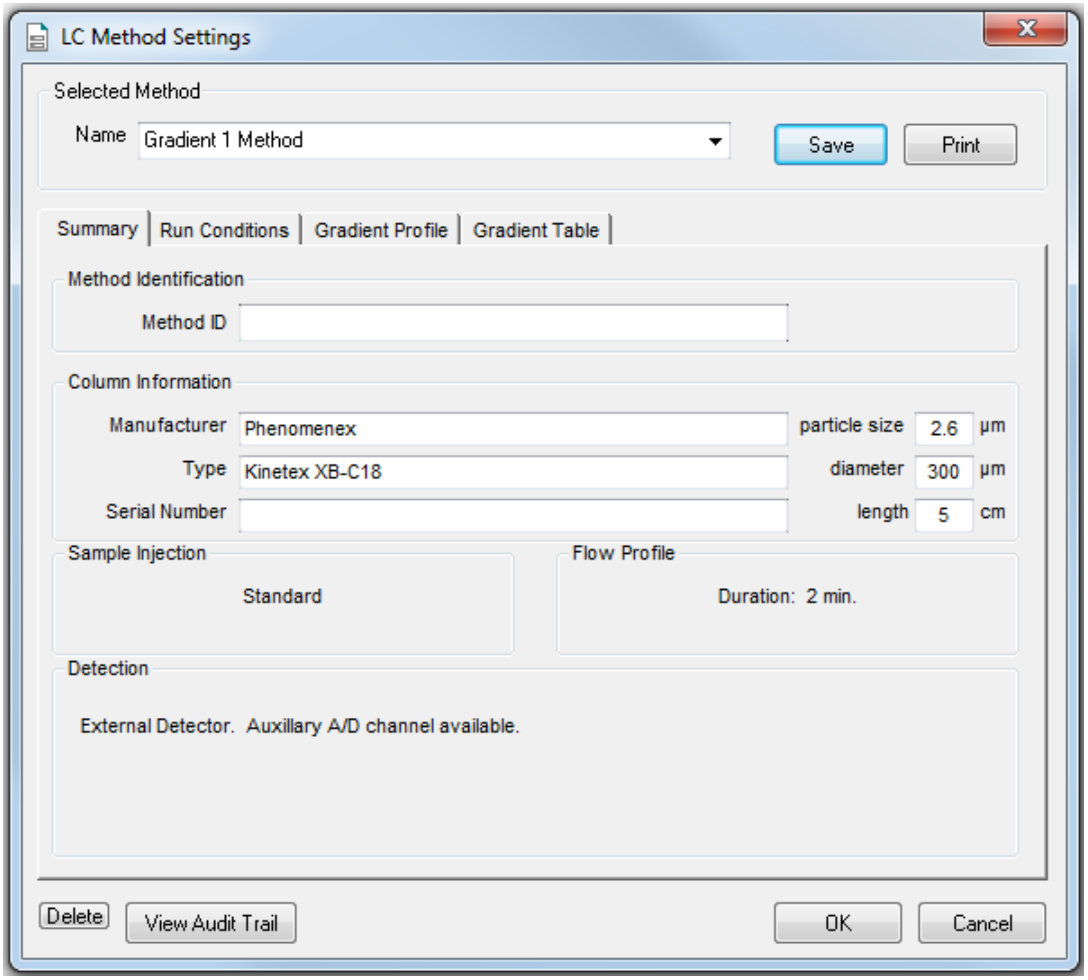
9. Click **Save**, and then click **OK**.

Create the Gradient Method for Micro-flow and High-Flow Systems

This method is used to separate the samples with the analytical column on a micro-flow or high-flow system.

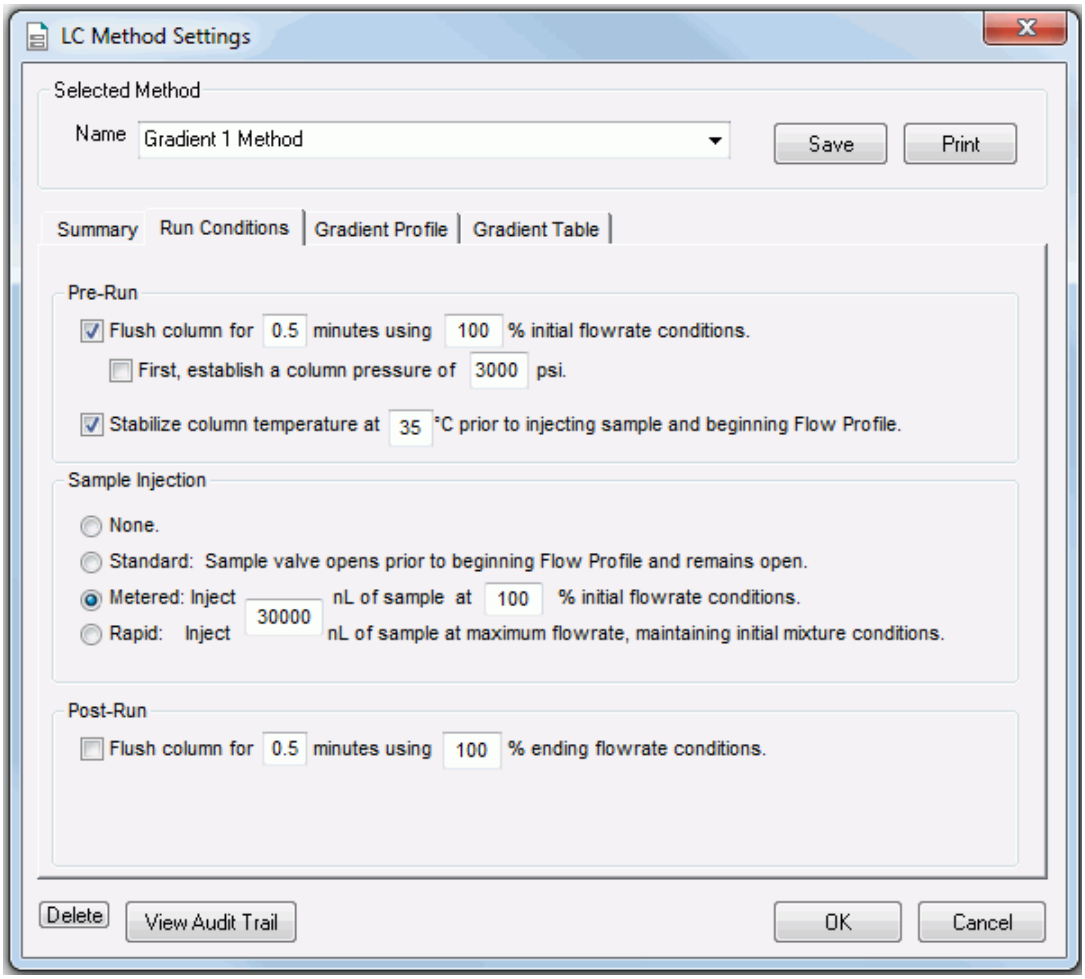
1. Click **LC Methods**.
   The LC Method Settings dialog opens.
2. In the **Name** field, type `Gradient 1 Method`, and then click **Save**.
3. In the **Column Information** section, set the values as shown in the following figure.

Figure 4-26 Gradient 1 Method: Summary Tab



4. Open the Run Conditions tab and set the values as shown in the following figure.

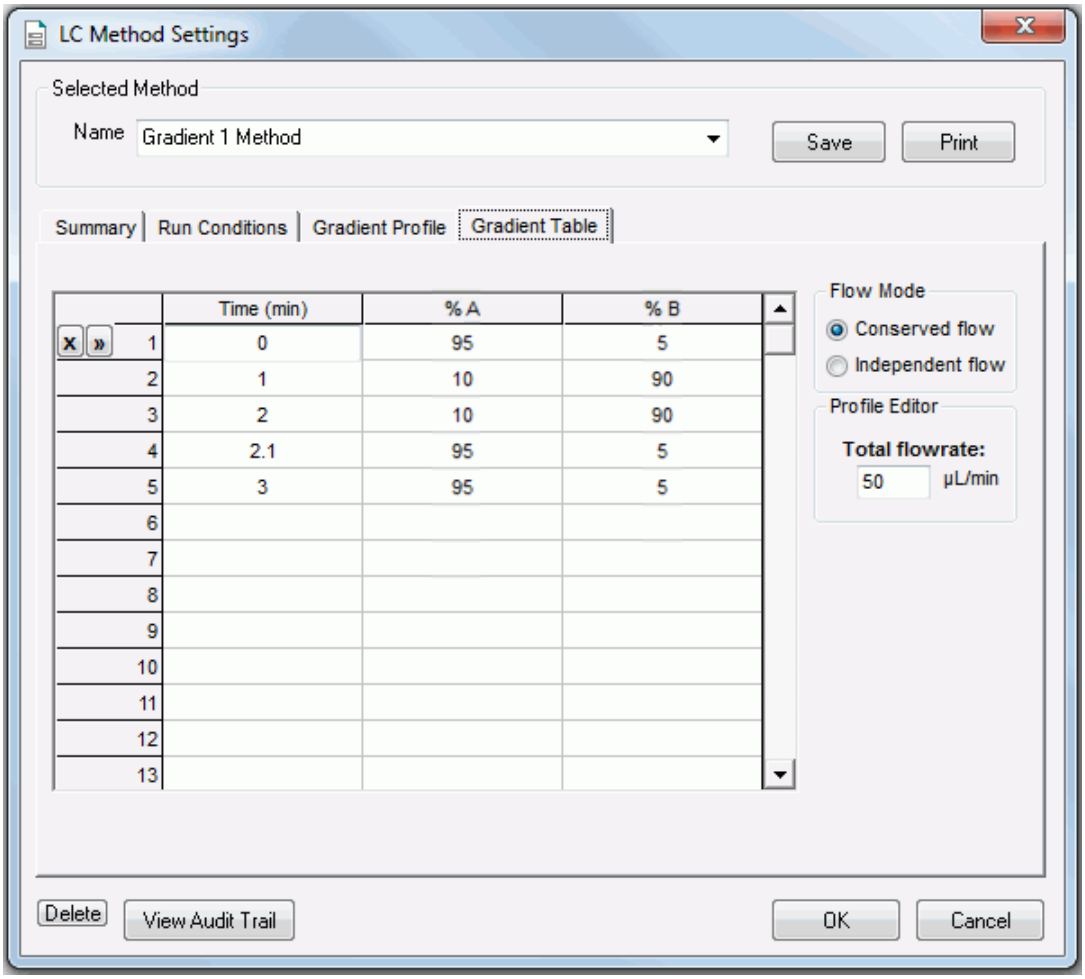
Figure 4-27 Gradient 1 Method: Run Conditions Tab



5. Open the Gradient Table tab, and then set the flow mode, the gradient parameters, and the flow rate as shown in the following figure.

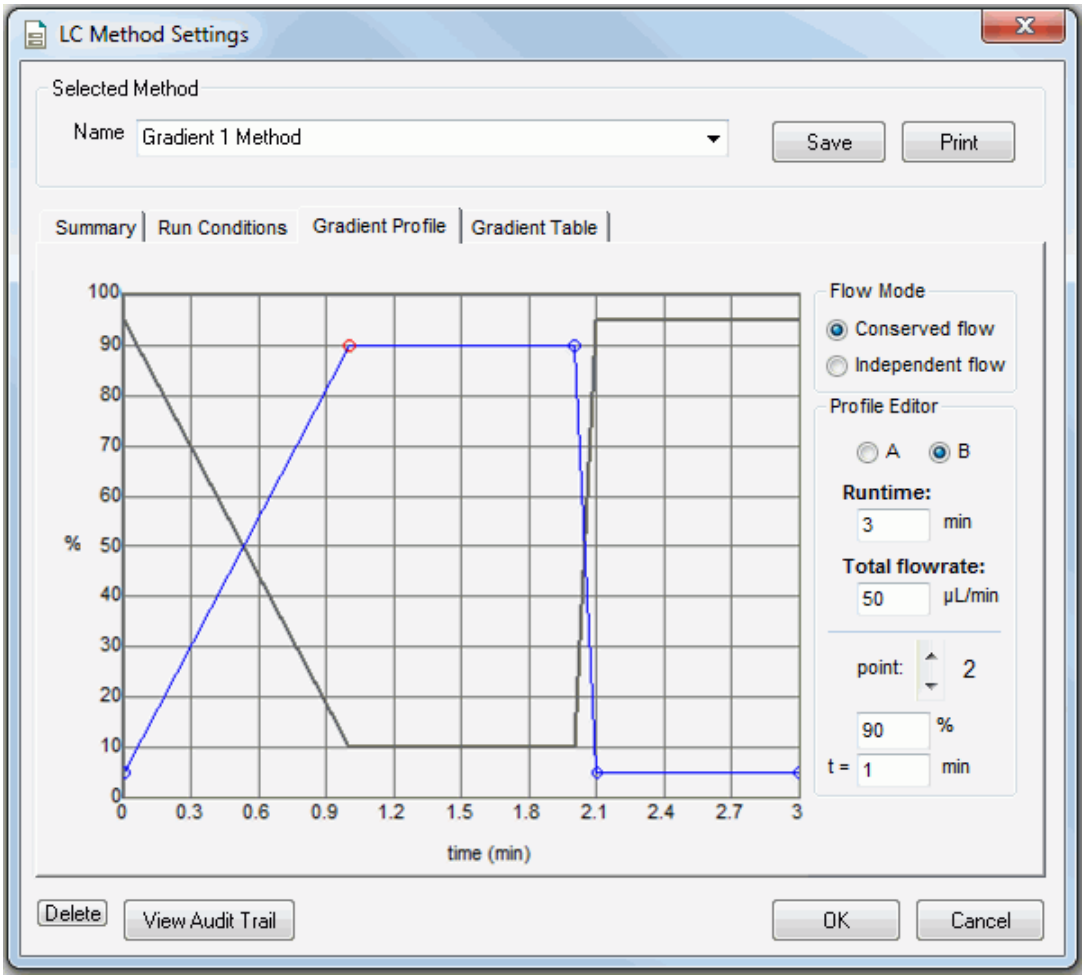
   For most experiments, select **Conserved** for the **Flow Mode**. In Conserved mode, the system uses the composition and total flow rate to calculate the flow rate for each mobile phase.

Figure 4-28 Gradient 1 Method: Gradient Table Tab



6. Open the Gradient Profile tab to see a graphical representation of the gradient.

Figure 4-29 Gradient 1 Method: Gradient Profile Tab



The last two steps in the method let the aqueous solvent flow through the sample loop before the next sample is injected.

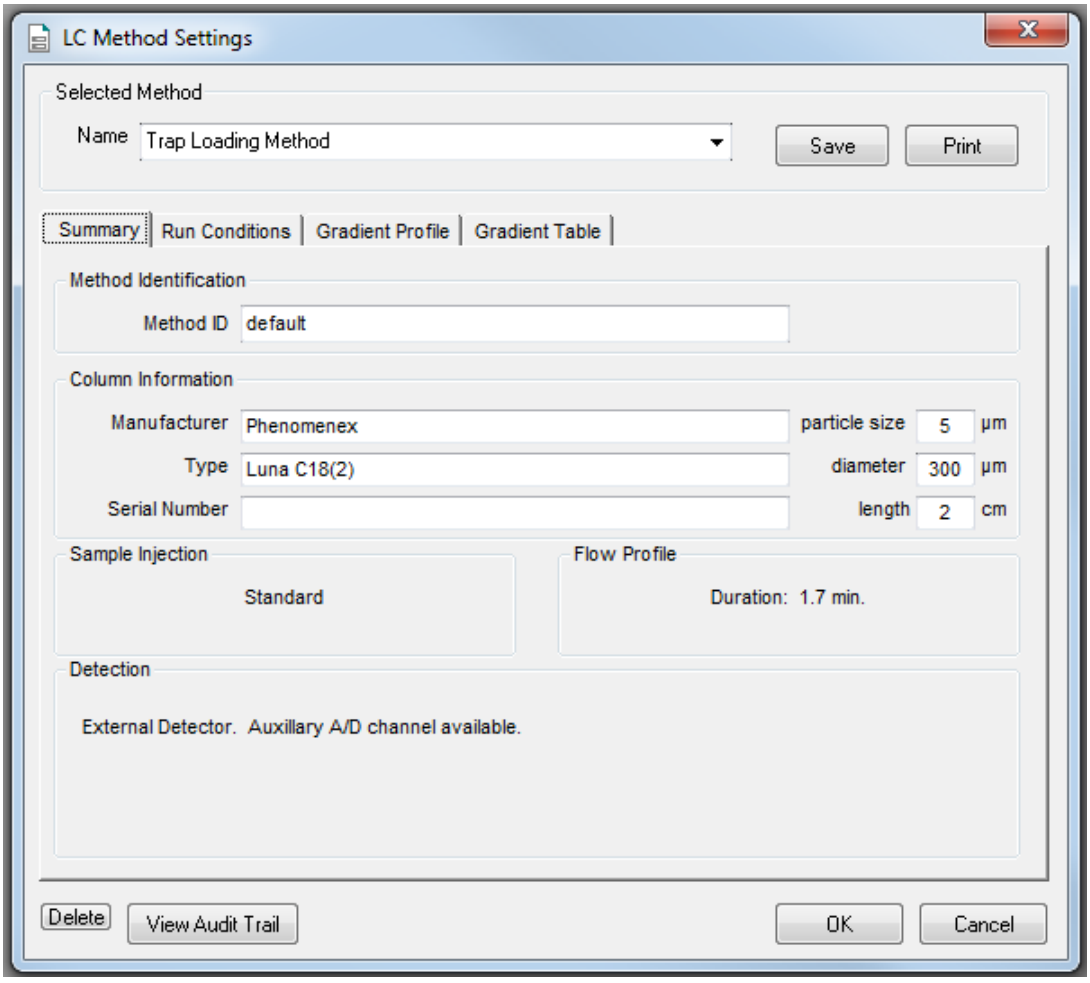
7. Click **Save**, and then click **OK**.

Create the LC Method to Load the Trap Column for Micro-flow and High-flow Systems

This method is used to load the sample on to the trap column on a micro-flow or high-flow system.

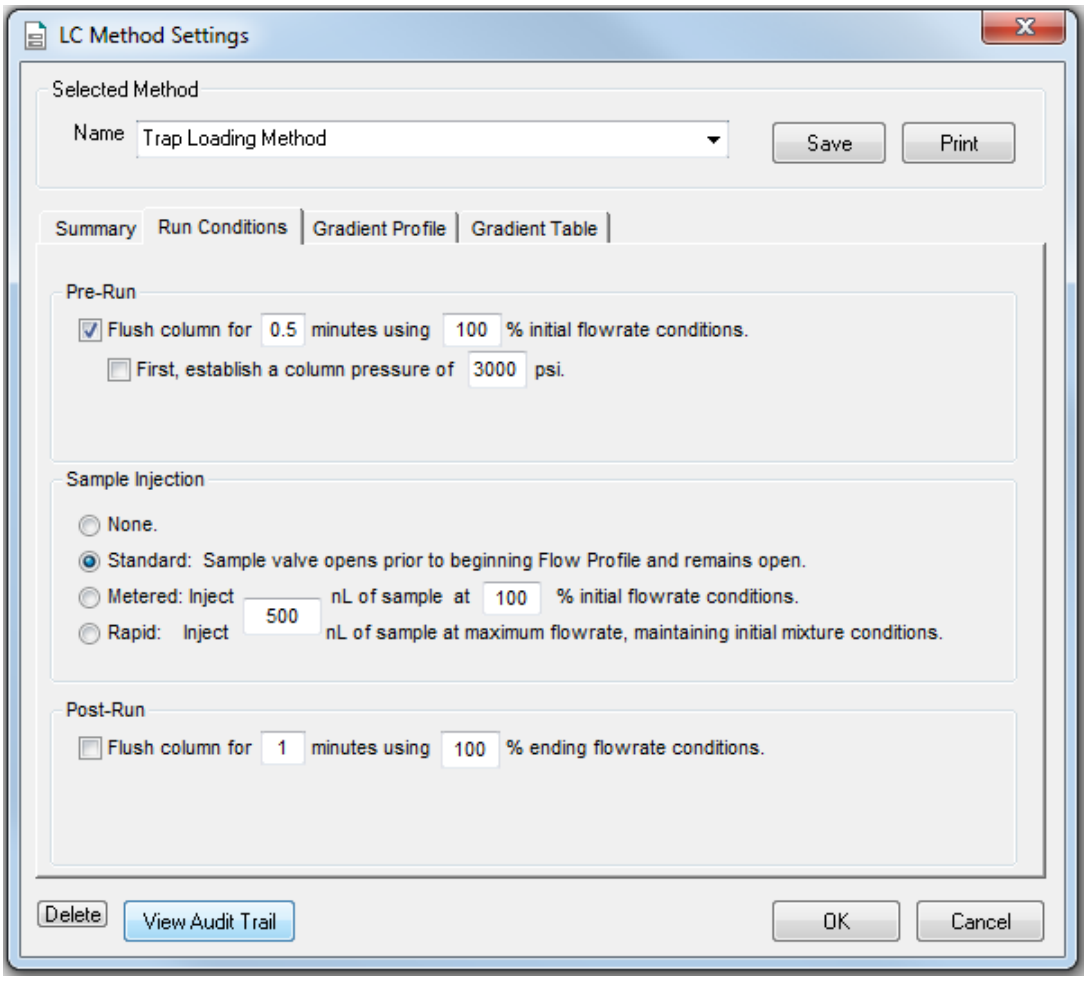
1. In the Eksigent Control software Acquisition window, click the arrows to select **Gradient 2** in the Channel section.
2. Click **LC Methods**.
   The LC Method Settings dialog opens.
3. In the **Name** field, type `Trap Loading Method` and then click **Save**.
4. In the Column Information section, type the values shown in the following figure.

Figure 4-30 Trap Loading Method: Summary Tab



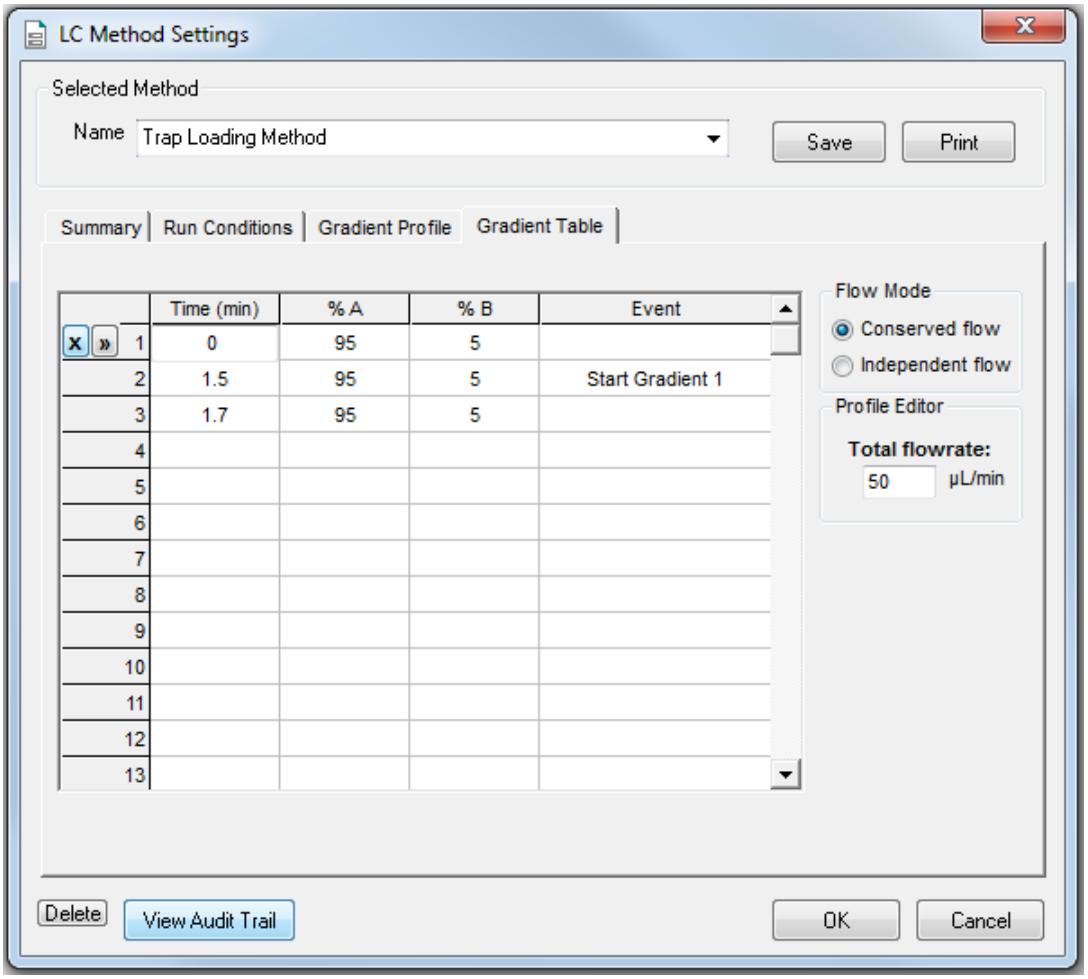
5. Open the Run Conditions tab, and then type the values shown in the following figure.

Figure 4-31 Trap Loading Method: Run Conditions Tab



6. Open the Gradient Table tab, and then set the flow mode, the gradient parameters, and the flow rate shown in the following figure.

Figure 4-32 Trap Loading Method: Gradient Table Tab



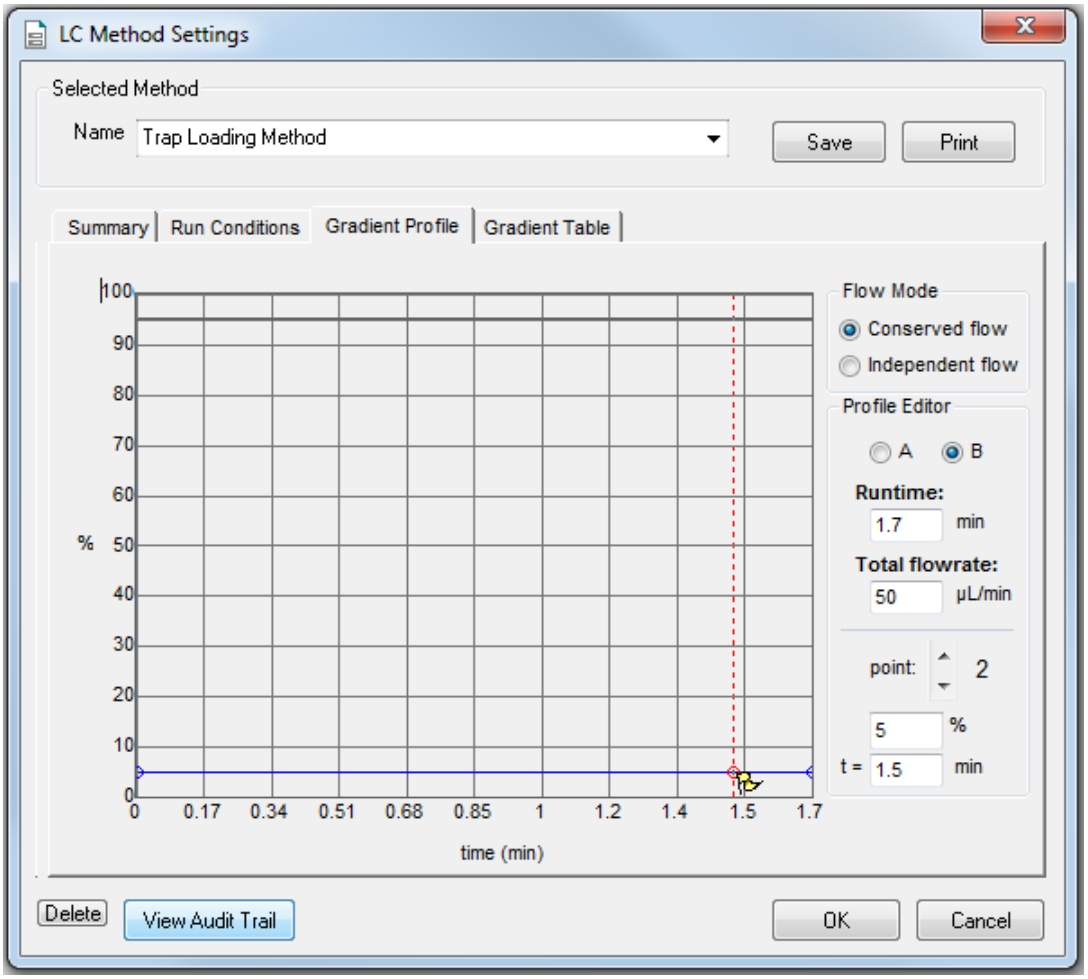
Note: If there is carryover from one sample to the next, then add a step at the end of the method to wash the sample loop with the organic mobile phase.

7. At 1.5 minutes, click the **Event** cell and then select **Start Gradient 1**.

   This event starts the Gradient 1 pump, which switches the trap column inline with the analytical column. The sample will be eluted from the trap column on to the analytical column.

8. Open the Gradient Profile tab to see a graphical representation of the gradient.

Figure 4-33 Trap Loading Method: Gradient Profile Tab



9. Click **Save**, and then click **OK**.

Do a Direct Injection Experiment with SCIEX OS 5

This section gives a brief tutorial on the use of the M5 MicroLC system to do a direct injection experiment with SCIEX OS. In a direct injection experiment, the sample is loaded into the injection loop and then injected directly on the analytical column.

In the example experiment that follows, a 5 µL sample loop with a full-loop injection is used. For more information about partial-loop and full-loop injections, refer to the section: About Partial and Full-loop Injections.

Direct injection experiments can be done with either an M5 MicroLC system or an M5 MicroLC-TE system.

Note: These instructions assume familiarity with SCIEX OS. For more information, refer to the document: *Software User* Guide. The Software User Guide is included the software.

Create the Direct Injection MS Method

The acquisition method sets the parameters for each device in the hardware profile.

1. Open the MS Method workspace.
2. Do the following:
   - For the SCIEX Triple Quad systems: Click **New** > **MRM**.
   - For the QTRAP systems: Click **New** > **MRM**.
   - For the TOF systems: Click **New** > **TOF MS**.
3. In the **Source and Gas Parameters** section, set the source and gas parameters as shown in the following table.

Table 5-1 LC Methods

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Curtain Gas (CUR)	30 (or as optimized)	30 (or as optimized)
CAD Gas	Medium	Medium
Temperature (TEM)	200 (or as optimized)	350 (or as optimized)
Ion Source Gas 1 (GS1)	15 (or as optimized)	25 (or as optimized)

Table 5-1 LC Methods (continued)

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Ion Source Gas 2 (GS2)	75 (or as optimized)	75 (or as optimized)

Note: For higher flow rates, the temperature (TEM) and ion source gas 1 (GS1) are typically higher.

Tip! Higher temperatures can cause a blockage in the electrodes on the mass spectrometer. As applicable, use lower temperatures.

4. In the **Experiment** section in the **Mass Table**, set the parameters as shown for the mass spectrometer.

Table 5-2 Suggested Compound Parameters by Mass Spectrometer

Mass Spectrometer	Declustering Potential (DP)	Collision Energy (CE)
SCIEX Triple Quad systems and QTRAP systems	70	30
TripleTOF systems	80	10

Table 5-3 Suggested Experiment Parameters by LC System

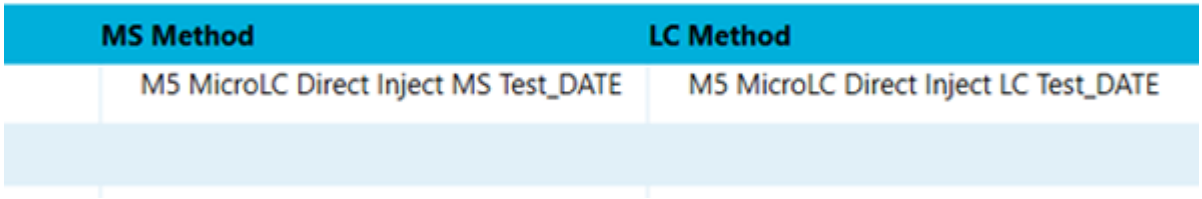
Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
IonSpray Voltage (IS) (V)	4,500 (or as optimized)	4,500 (or as optimized)

5. Click **Save as**.
6. In the **File name** field, type `M5 MicroLC Direct Inject MS Test_DATE`, where DATE is todays date.

Create the Batch

1. Open the Batch workspace.
2. On the Batch Editor window, supply the required information.
3. Select the acquisition method that was created previously.

Figure 5-1 Method Section: Select the MS and LC Method



	MS Method	LC Method
	M5 MicroLC Direct Inject MS Test_DATE	M5 MicroLC Direct Inject LC Test_DATE

4. In the **Plate Type** column, select **VT54** for all samples in the table.

Note: VT54 refers to the type of autosampler tray that is used for the vials.

Figure 5-2 Sample Table

	Sample Name	Rack Position	Plate Type	Plate Position
1	Blank001	Drawer 1	VT54	Slot1
2	Blank002	Drawer 1	VT54	Slot1
3	Blank003	Drawer 1	VT54	Slot1
4	0.01 ng/mL triazine mix_1	Drawer 1	VT54	Slot1
5	0.01 ng/mL triazine mix_2	Drawer 1	VT54	Slot1
6				

5. Scroll to the right side of the Sample Table and then type `6` in the **Inj. Volume (µl)** field for all of the samples in the table.

Note: For other experiments, set the volume as appropriate, but the maximum volume must be less than 80 µL.

Figure 5-3 Sample Table: Injection Volume

Vial Position	Injection Volume (ul)	Data File
1	6.00	System Integration Test
1	6.00	System Integration Test
1	6.00	System Integration Test
2	6.00	System Integration Test
2	6.00	System Integration Test

Submit the Batch

1. Put the sample vials in the correct positions in the cooled sample drawers.
2. Click **Submit** to add the samples to the queue.
3. Click **Queue Manager** ().
4. To equilibrate the LC system and the mass spectrometer, click **Direct Control** > **Equilibrate**.
5. When the equilibration is completed, click **Acquisition** > **Queue** and then click **Start**.

Monitor the Run

1. See the LC chromatogram and spectral data in the Data Acquisition window.
2. See the flow rate and pressure information in the Detail Status window of the M5 MicroLC system.

(M5 MicroLC-TE Systems Only) Direct Injection with the Gradient 2 Pump

A low-flow or micro-flow M5 MicroLC-TE system can be used to do a high-flow direct injection experiment with the Gradient 2 pump. Make the following changes:

- Plumb the valve for a direct injection experiment. Refer to the section: Direct Injection Plumbing Diagram.
- Select the **Trap-Elute Mode** check box in the Direct Control dialog. Refer to the figure: Figure 3-9.
- Plumb the G2 pump outlet to port 6 on the injection valve.
- To create an LC method, refer to the section: Create the Direct Injection Method for Micro-flow and High-flow Systems.

Do a Direct Injection Experiment with the Analyst Software 6

This section gives a brief tutorial on the use of the M5 MicroLC system to do a direct injection experiment, with the Analyst software. In a direct injection experiment, the sample is loaded into the injection loop and then injected directly on the analytical column.

In the example experiment that follows, a 5 µL sample loop and full-loop injection are used. For more information about partial-loop and full-loop injections, refer to the section: About Partial and Full-loop Injections.

Direct injection experiments can be done with either an M5 MicroLC or an M5 MicroLC-TE system.

Note: The screen captures shown in this chapter are for the M5 MicroLC-TE system. For an M5 MicroLC system, the screens are different.

Note: These instructions assume familiarity with the Analyst software. For more information, refer to the document: *Analyst Software User Guide*.

Create the Direct Injection Acquisition Method

The acquisition method sets the parameters for each device in the hardware profile.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
2. Click **Acquisition Method** in the left pane, and then set **Synchronization Mode** to **LC Sync**.
3. In the Acquisition Method Browser pane, click **Mass Spec** and then supply the applicable parameters for the experiment.
4. Click **Edit Parameters** to set the **Source/Gas** parameters.

   The parameters for micro LC are different than those for conventional liquid chromatography. Use the following values as a starting point and then set the optimal settings as required.

   Note: Make sure to use the correct parameters for the flow rate configuration of the LC system in use.

Table 6-1 Source/Gas Parameters by LC System

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Curtain Gas (CUR)	30 (or as optimized)	30 (or as optimized)
CAD Gas	Medium	Medium
IonSpray Voltage (IS) (V)	4,500 (or as optimized)	4,500 (or as optimized)
Temperature (TEM)	200 (or as optimized)	350 (or as optimized)
Ion Source Gas 1 (GS1)	15 (or as optimized)	25 (or as optimized)
Ion Source Gas 2 (GS2)	75 (or as optimized)	75 (or as optimized)

Note: For higher flow rates, the temperature (TEM) and ion source gas 1 (GS1) are typically higher.

Tip! Higher temperatures can lead to clogged electrodes on the mass spectrometer. As appropriate, use lower temperatures.

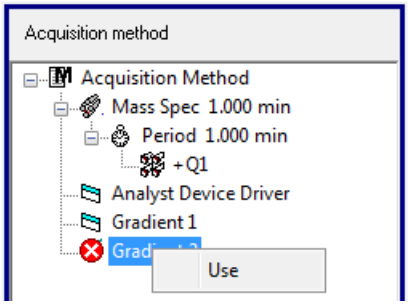
5. Open the Compound tab and then set the parameters as shown for the mass spectrometer.

Table 6-2 Suggested Compound Parameters by Mass Spectrometer

Mass Spectrometer	Declustering Potential (DP)	Collision Energy (CE)
SCIEX Triple Quad and QTRAP systems	70	30
TripleTOF systems	80	10

6. Click **OK** save the parameters.
7. Select the LC method for the Gradient 1 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 1**.
   b. Browse to the available LC methods.
   c. Click **Gradient 1 Method** and then click **Open**.
8. For an M5 MicroLC-TE system, right-click **Gradient 2** and then select **Use** to disable the Gradient 2 pump.

Figure 6-1 Disabling Gradient 2



9. Click **File** > **Save**, and then type `Direct Inject Example Method` for the name of the method.
10. Select the autosampler script.
    a. To open the Instrument Control Method Editor window, in the Analyst Device Driver window click **Method**.
    b. In the **Select Method Script** list, click **M5_Direct Inject**.

    Note: The autosampler script installed with the system might have a different name than listed previously. Use the most recent autosampler script supplied by SCIEX.
11. In the Parameter Setup tab, set the parameters for the autosampler script.

    Tip! Required fields are outlined in red. If a parameter is cannot be seen in the software, then it might be in a panel that is closed. Click ⌄ to open a closed panel.

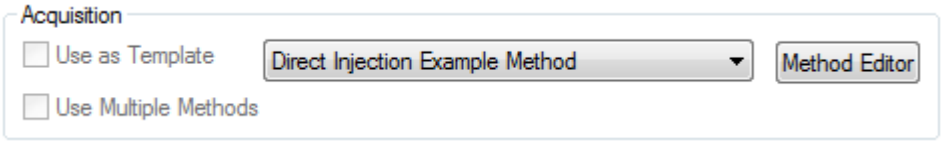
    a. In the **Tool** row, click **LCP 1**.
    b. In the **Pump Module** row, click **Pump 1**.
    c. In the **Cooled Stack 1** row, click **Peltier Stack 1**.
    d. In the **Injector** row, click **Injector LC1**.
    e. In the **Wash Station** row, click **LCMS Wash 1**.
    f. Click **File** > **Save to Analyst .dam file**, and then select the acquisition method that was previously created.
    g. Click **Yes** when prompted to overwrite the acquisition method and then close the Instrument Control Method Editor window.
12. Click **File** > **Save** to save the changes to the acquisition method.

Create the Acquisition Batch

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Batch**.

2. Supply the required information on the Samples tab of the Batch Editor window.
3. Select the acquisition method that was previously created. (**Direct Injection Example Method**).

Figure 6-2 Acquisition Section: Selecting the Acquisition Method



4. In the **Plate Code** column, select **VT54** for all of the samples in the table.

Note: VT54 refers to the type of autosampler tray used for the vials.

Figure 6-3 Sample Table

	Sample Name	Rack Code	Rack Position	Plate Code
1	Blank001	Drawer	1	VT54
2	Blank002	Drawer	1	VT54
3	Blank003	Drawer	1	VT54
4	0.01 ng/mL triazine mix_1	Drawer	1	VT54
5	0.01 ng/mL triazine mix_2	Drawer	1	VT54

5. Scroll to the right side of the **Sample Table** and type `6` in the **Inj. Volume (µl)** field for all of the samples in the table.

Note: For other experiments, set the volume as applicable, but the maximum volume must be less than 80 µL.

Figure 6-4 Sample Table: Injection Volume

Vial Position	Data File	Inj.Volume (µl)
1	System Integration Te	6
1	System Integration Te	6
1	System Integration Te	6
2	System Integration Te	6
2	System Integration Te	6

Submit the Batch

1. Put the sample vials in the correct positions in the cooled sample drawers.

2. Open the Submit tab of the Batch Acquisition dialog, and then click **Submit** to add the samples to the queue.
3. Click **View** > **Sample Queue**.
   The Queue Manager (Local) dialog opens.
4. To equilibrate the LC system and the mass spectrometer, click **Acquire** > **Equilibrate**.
5. When the equilibration is finished, click **Acquire** > **Start Sample** to start the batch.

Monitor the Run

1. See the LC chromatogram and spectral data in the Explore workspace.
2. See the flow rate and pressure information in the Acquisition window of the Eksigent Control software.

(M5 MicroLC-TE Systems Only) Direct Injection with the Gradient 2 Pump

A low-flow or micro-flow M5 MicroLC-TE system can be used to do a high-flow direct injection experiment with the Gradient 2 pump. Make the following changes:

- Plumb the valve for a direct injection experiment. Refer to the section: Direct Injection Plumbing Diagram.
- Clear the **Trap-Elute Mode** check box in the Direct Control dialog. Refer to the figure: Figure 4-9.
- Plumb the G2 pump outlet to port 6 on the injection valve.
- Remove the MS interface cable from the I/O G1 port and connect it to the I/O G2 port.
- Delete the **Start Gradient 1** event. To create an LC method, refer to the section: Create the LC Method to Load the Trap Column for a Low-flow System or Create the LC Method to Load the Trap Column for Micro-flow and High-flow Systems.
- Select the autosampler script to be used in the acquisition method.

  1. In the Acquisition Method window, click **Analyst Device Driver**.
  2. In the Analyst Device Driver window, click **Method** to open the Instrument Control Method Editor window.
  3. In the **Select Method Script** list, click **M5_TrapElute** (or the most current version installed on the system). The method contains instructions required to operate the Gradient 2 pump, no matter what type of experiment is performed.

- In the Acquisition Method Browser pane, disable the Gradient 1 pump and enable the Gradient 2 pump.

Do a Trap-and-Elute Experiment with SCIEX OS 7

This section gives a brief tutorial on the use of the M5 MicroLC-TE system to do a trap-and-elute experiment with SCIEX OS. In a trap-and-elute experiment, the sample is loaded into the injection loop and then transferred to the trap column by one of the pumps. Sample components are concentrated on to the trap column, while any non-retained impurities like salts are washed away. After the sample has been loaded, the trap column is switched inline with the analytical column, and the gradient for the analysis is started.

In the example experiment that follows, a 50 µL sample loop and full-loop injection are used. For more information about partial-loop and full-loop injections, refer to the section: About Partial and Full-loop Injections.

Trap-and-elute experiments can only be done with an M5 MicroLC-TE system.

Note: These instructions assume familiarity with the *SCIEX OS Software User Guide*.

Create the Trap-and-Elute MS Method

The acquisition method sets the parameters for each device in the hardware profile.

1. Open the MS Method workspace.
2. Do the following:
   - For the SCIEX Triple Quad systems: Click **New** > **MRM**.
   - For the QTRAP systems: Click **New** > **MRM**.
   - For the TOF systems: Click **New** > **TOF MS**.
3. In the **Source and Gas Parameters** section, set the source and gas parameters as shown in the following table.

Table 7-1 LC Methods

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Curtain Gas (CUR)	30 (or as optimized)	30 (or as optimized)
CAD Gas	Medium	Medium
Temperature (TEM)	200 (or as optimized)	350 (or as optimized)
Ion Source Gas 1 (GS1)	15 (or as optimized)	25 (or as optimized)

Table 7-1 LC Methods (continued)

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Ion Source Gas 2 (GS2)	75 (or as optimized)	75 (or as optimized)

Note: For higher flow rates, the temperature (TEM) and ion source gas 1 (GS1) are typically higher.

Tip! Higher temperatures can lead to clogged electrodes on the mass spectrometer. As applicable, use lower temperatures.

4. In the **Experiment** section in the **Mass Table**, set the parameters as shown for the mass spectrometer.

Table 7-2 Suggested Compound Parameters by Mass Spectrometer

Mass Spectrometer	Declustering Potential (DP)	Collision Energy (CE)
SCIEX Triple Quad systems and QTRAP systems	70	30
TripleTOF systems	80	10

Table 7-3 Suggested Experiment Parameters by LC System

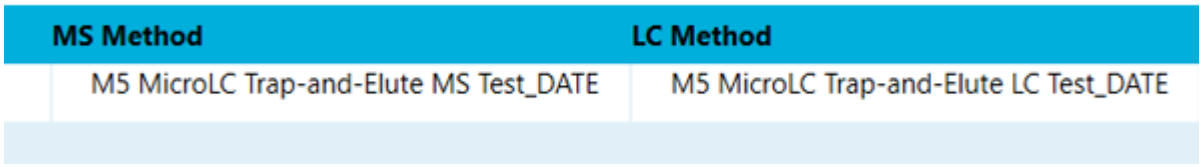
Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
IonSpray Voltage (IS) (V)	4,500 (or as optimized)	4,500 (or as optimized)

5. Click **Save as**.
6. In the **File name** field type, `M5 MicroLC Trap-and-Elute MS Test_DATE`, where DATE is todays date.

Create the Batch

1. Open the Batch workspace.
2. On the Batch Editor window, supply the required information.
3. Select the MS method that was created previously.

Figure 7-1 Method Section: Select the MS and LC Method

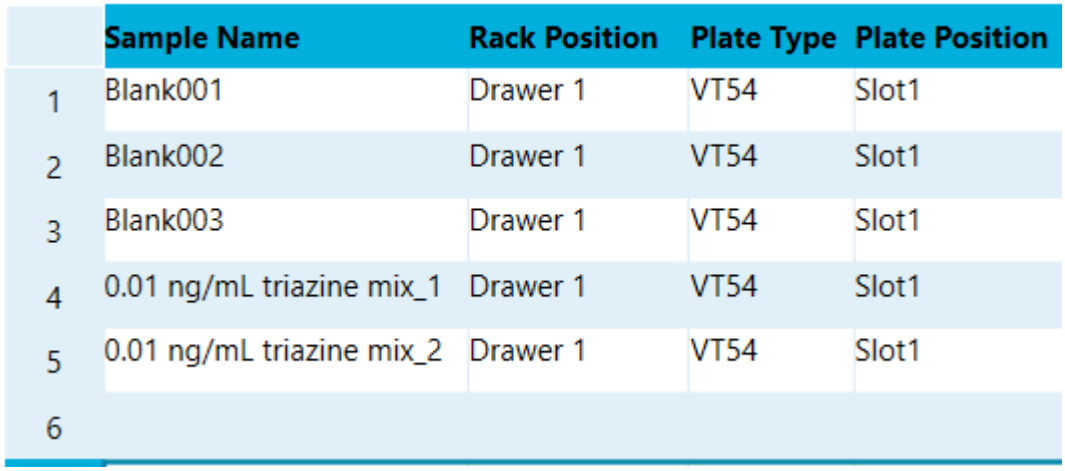


MS Method	LC Method
M5 MicroLC Trap-and-Elute MS Test_DATE	M5 MicroLC Trap-and-Elute LC Test_DATE

4. In the Plate Type column, select **VT54** for all samples in the table.

Note: VT54 refers to the type of autosampler tray that is used for the vials.

Figure 7-2 Sample Table



	Sample Name	Rack Position	Plate Type	Plate Position
1	Blank001	Drawer 1	VT54	Slot1
2	Blank002	Drawer 1	VT54	Slot1
3	Blank003	Drawer 1	VT54	Slot1
4	0.01 ng/mL triazine mix_1	Drawer 1	VT54	Slot1
5	0.01 ng/mL triazine mix_2	Drawer 1	VT54	Slot1
6				

5. Scroll to the right side of the Sample Table and then type `6` in the **Inj. Volume (µl)** field for all of the samples in the table.

Note: For other experiments, set the volume as appropriate, but the maximum volume must be less than 80 µL.

Figure 7-3 Sample Table: Injection Volume

Vial Position	Injection Volume (ul)	Data File
1	6.00	System Integration Test
1	6.00	System Integration Test
1	6.00	System Integration Test
2	6.00	System Integration Test
2	6.00	System Integration Test

Submit the Batch

1. Put the sample vials in the correct positions in the cooled sample drawers.
2. Click **Submit** to add the samples to the queue.
3. Click **Queue Manager** ().
4. To equilibrate the LC system and the mass spectrometer, click **Direct Control** > **Equilibrate**.
5. When the equilibration is completed, click **Acquisition** > **Queue** and then click **Start**.

Monitor the Run

1. View the LC chromatogram and spectral data in the Data Acquisition window.
2. View the flow rate and pressure information in the Detail Status window of the M5 MicroLC-TE system.

Do a Trap-and-Elute Experiment with the Analyst Software 8

This section gives a brief tutorial on the use of the M5 MicroLC-TE system to do a trap-and-elute experiment with the Analyst software. In a trap-and-elute experiment, the sample is loaded into the injection loop and then transferred to the trap column by one of the pumps. Sample components are concentrated on to the trap column, while any non-retained impurities like salts are washed away. After the sample has been loaded, the trap column is switched inline with the analytical column, and the gradient for the analysis is started.

In the example experiment that follows, a 50 µL sample loop with a full-loop injection is used. For further information about partial-loop and full-loop injections, refer to the section: About Partial and Full-loop Injections.

Trap-and-elute experiments can only be done with an M5 MicroLC-TE system.

Note: These instructions assume familiarity with the Analyst software. For more information, refer to the document: *Analyst Software User Guide*.

Create the Trap-and-Elute Acquisition Method

The acquisition method specifies the parameters for each device in the hardware profile.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
2. Click **Acquisition Method** in the left pane, and then set **Synchronization Mode** to **LC Sync**.
3. In the Acquisition Method Browser pane, click **Mass Spec** and then supply the applicable parameters for the experiment.
4. Click **Edit Parameters** to set the **Source/Gas** parameters.

   The parameters for micro LC are different than those for conventional liquid chromatography. Use the following values as a starting point and then set the optimal settings as required.

   Note: Make sure to use the correct parameters for the flow rate configuration of the LC system in use.

Table 8-1 Source/Gas Parameters by LC System

Parameter	Low-flow M5 MicroLC System	Micro-flow or High-flow M5 MicroLC System
Curtain Gas (CUR)	30 (or as optimized)	30 (or as optimized)
CAD Gas	Medium	Medium
IonSpray Voltage (IS) (V)	4,500 (or as optimized)	4,500 (or as optimized)
Temperature (TEM)	200 (or as optimized)	350 (or as optimized)
Ion Source Gas 1 (GS1)	15 (or as optimized)	25 (or as optimized)
Ion Source Gas 2 (GS2)	75 (or as optimized)	75 (or as optimized)

Note: For higher flow rates, the temperature (TEM) and ion source gas 1 (GS1) are typically higher.

Tip! Higher temperatures can lead to clogged electrodes on the mass spectrometer. As appropriate, use lower temperatures.

5. Open the Compound tab and then set the parameters as shown for the mass spectrometer.

Table 8-2 Suggested Compound Parameters by Mass Spectrometer

Mass Spectrometer	Declustering Potential (DP)	Collision Energy (CE)
SCIEX Triple Quad and QTRAP systems	70	30
TripleTOF systems	80	10

6. Click **OK** save the parameters.
7. Select the LC method for the Gradient 1 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 1**.
   b. Browse to the available LC methods.
   c. Click **Gradient 1 Method** and then click **Open**.
8. Select the LC method for the Gradient 2 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 2**.
   b. Browse to see the available LC methods.
   c. Click **Trap Loading Method**, and then click **Open**.

9. Click **File** > **Save**, and then type `Trap-and-Elute Example Method` for the name of the method.
10. Select the autosampler script.
    a. To open the Instrument Control Method Editor window, in the Analyst Device Driver window click **Method**.
    b. In the **Select Method Script** list, click **M5_Direct Inject**.

    Note: The autosampler script installed with the system might have a different name than listed previously. Use the most recent autosampler script supplied by SCIEX.
11. In the Parameter Setup tab, set the parameters for the autosampler script.

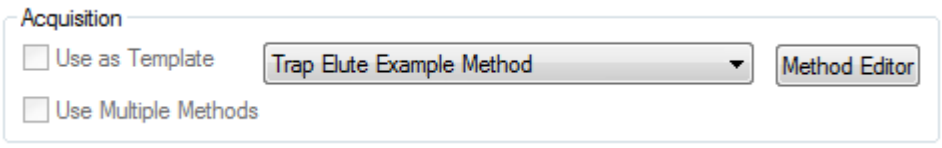
    Tip! Required fields are outlined in red. If a parameter is cannot be seen in the software, then it might be in a panel that is closed. Click ⌄ to open a closed panel.

    a. In the **Tool** row, click **LCP 1**.
    b. In the **Pump Module** row, click **Pump 1**.
    c. In the **Cooled Stack 1** row, click **Peltier Stack 1**.
    d. In the **Injector** row, click **Injector LC1**.
    e. In the **Wash Station** row, click **LCMS Wash 1**.
    f. Click **File** > **Save to Analyst .dam file**, and then select the acquisition method that was previously created.
    g. Click **Yes** when prompted to overwrite the acquisition method and then close the Instrument Control Method Editor window.
12. Click **File** > **Save** to save the changes to the acquisition method.

Create the Acquisition Batch

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Batch**.
2. Supply the required information on the Samples tab of the Batch Editor window.
3. Select the acquisition method that was previously created (**Trap Elute Example Method**).

Figure 8-1 Acquisition Section: Selecting the Acquisition Method



4. In the **Plate Code** column, select **VT54** for all of the samples in the table.

Note: VT54 refers to the type of autosampler tray used for the vials.

Figure 8-2 Sample Table

	Sample Name	Rack Code	Rack Position	Plate Code
1	Blank001	Drawer	1	VT54
2	Blank002	Drawer	1	VT54
3	Blank003	Drawer	1	VT54
4	0.01 ng/mL triazine mix_1	Drawer	1	VT54
5	0.01 ng/mL triazine mix_2	Drawer	1	VT54

5. Scroll to the right side of the **Sample Table** and type `60` in the **Inj. Volume (µl)** field for all samples in the table.

Note: For other experiments, set the volume as applicable, but the maximum volume must be less than 80 µL.

Figure 8-3 Sample Table: Injection Volume

Vial Position	Data File	Inj.Volume (µl)
1	System Integration Te	60
1	System Integration Te	60
1	System Integration Te	60
2	System Integration Te	60
2	System Integration Te	60

Submit the Batch

1. Put the sample vials in the correct positions in the cooled sample drawers.
2. Open the Submit tab of the Batch Acquisition dialog, and then click **Submit** to add the samples to the queue.
3. Click **View** > **Sample Queue**.
   The Queue Manager (Local) dialog opens.
4. To equilibrate the LC system and the mass spectrometer, click **Acquire** > **Equilibrate**.
5. When the equilibration is finished, click **Acquire** > **Start Sample** to start the batch.

Monitor the Run

1. See the LC chromatogram and spectral data in the Explore workspace.

2. See the flow rate and pressure information in the Acquisition window of the Eksigent Control software.

Maintenance 9

WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Identify whether decontamination is required before cleaning or maintenance. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.

Storage and Handling

WARNING! Environmental Hazard. Do not dispose of system components in municipal waste. Follow local regulations when disposing of components.

The environmental requirements for the storage and transport of the M5 MicroLC system are as follows:

- Ambient temperature between 15 °C and 30 °C (59 °F and 86 °F).
- Altitude not exceeding 2,000 m (6,562 feet) above sea level.
- For ambient temperatures between 15 °C and 30 °C, a non-condensing relative humidity of between 20% and 80% is required.

Note: Do not install the system adjacent to heaters or cooling ducts, or in direct sunlight.

Maintenance Schedule

Do the following procedures at the specified interval.

Table 9-1 Routine Maintenance

Procedure	Frequency
Waste Disposal	As needed
Replace the Autosampler Wash Solvents and Load the Mobile Phases	As needed
If the system is idle for more than a week, Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS	As needed
Clean the Surfaces	As needed
Replace the Syringe Needle	As needed

Table 9-1 Routine Maintenance (continued)

Procedure	Frequency
Replace the Syringe	As needed
Examine the System	Weekly
Re-initialize the Pressure Transducers with the Analyst Software or Re-initialize the Pressure Transducers with SCIEX OS	Weekly
Replace the Pump Seal Rinse	Quarterly
Install the Valve Rotor Seal	Yearly (or as needed)
Install the Injection Port	Yearly (or as needed)

Examine the System

1. Examine all solvent reservoirs for evidence of biological growth or precipitation.

   If they are present, replace the solvent and filter, then purge the mobile phases. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS.

2. Examine the system tubing and fittings.

   Look for broken fittings and dried deposits that might show a slow leak.

   a. Tighten any loose connections.

   b. If a fluidic connection is broken, replace the fitting and then flush the system. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS.

Waste Disposal

Correctly dispose of any effluent waste in a correct chemical waste container. After disposing of the waste liquid, make sure that the waste tubing has no loops and that the end of the tubing protrudes from the waste bottle cap, where about 1 inch (2.5 cm) of the tubing is shown.

WARNING! Biohazard or Toxic Chemical Hazard. Follow local directives when disposing of chemicals and the remains of the prepared samples, if applicable. They might contain regulated compounds and biohazardous agents.

Clean the Surfaces

Clean the external surfaces of the system after a spill or when they become dirty.

Required Materials
• Soft cloth

1. Use a soft, damp cloth to clean the surfaces of the system.
2. Use a soft, dry cloth to remove any moisture from the surfaces.

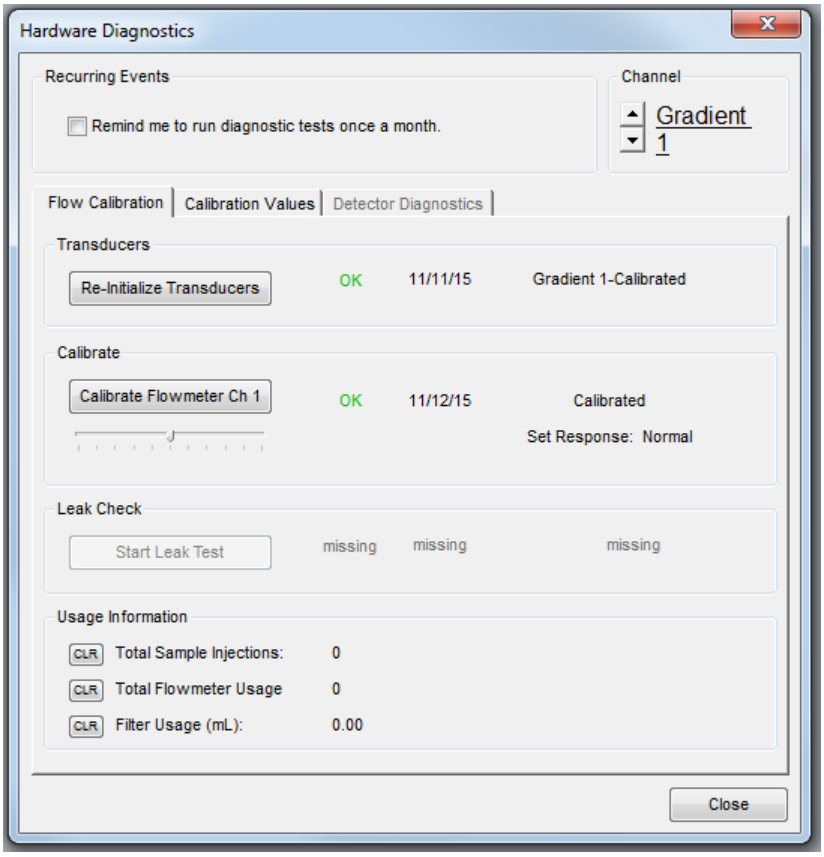
Maintenance Procedures for the Pumps

Re-initialize the Pressure Transducers with SCIEX OS

CAUTION: Potential System Damage. Open the pump outlet to make sure that there is no residual pressure on the outlet of the pump before initializing the pressure transducers. If the pressure transducers are initialized while there is residual pressure, then incorrect flow rates damage to the LC system can occur.

1. Stop the sample flow.
2. Loosen the fittings in the pump outlets on the valve panel to release all of the residual pressure.
3. On the status panel, click **Direct device control** () to the right of the device name.
4. Click **Maintenance** ().
   The Hardware Diagnostics dialog opens.
5. On the Flow Calibration tab, click **Re-Initialize Transducers**.

Figure 9-1 Hardware Diagnostics Dialog: Flow Calibration Tab



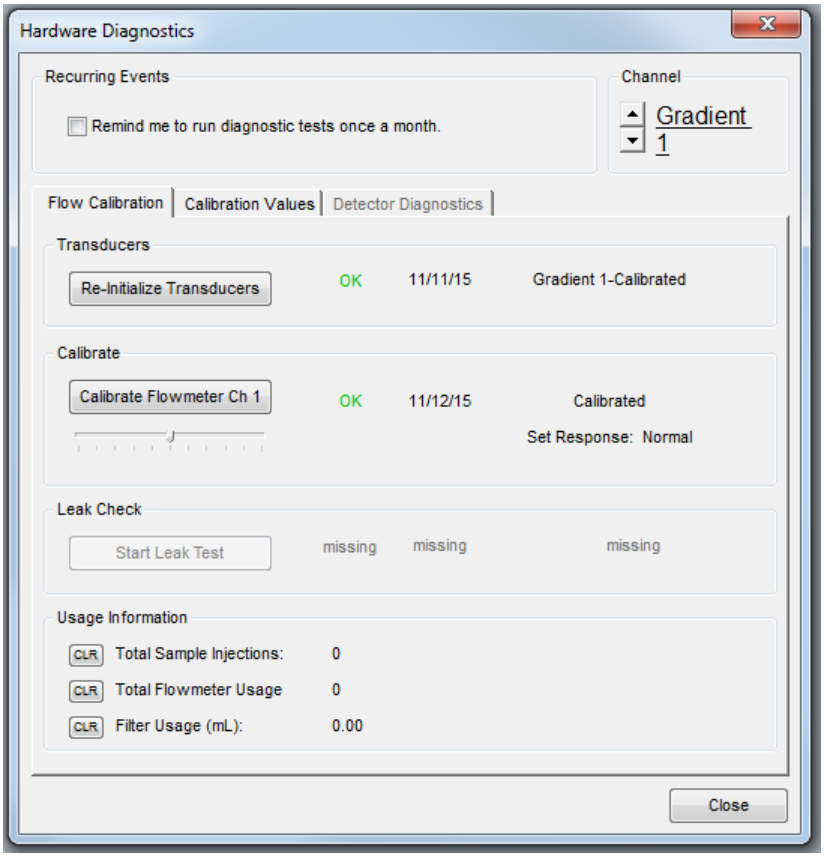
6. M5 MicroLC-TE systems: A message is shown asking if the calibration should be performed for both channels. Click **Yes**. A warning is shown that this procedure should only be done if there is no residual pressure on the pump.
7. Make sure that the pump outlets are open, and then click **OK**.
   A status dialog shows that the re-initialization is in progress.
8. When the system shows a message that the transducers are re-initialized, click **OK**.
9. Click **Close**.
   The Hardware Diagnostics dialog closes. The Direct Control page shows.

Re-initialize the Pressure Transducers with the Analyst Software

CAUTION: Potential System Damage. Open the pump outlet to make sure that there is no residual pressure on the outlet of the pump before initializing the pressure transducers. Attempting to initialize the pressure transducers while there is still residual pressure leads to inaccurate flow rates and possibly damage to the LC system.

1. Open Eksigent Control software.
2. Stop the sample flow.
3. Loosen the fittings in the pump outlets on the valve panel to release all of the residual pressure.
4. Click **System** > **Hardware Diagnostics**.
5. On the Flow Calibration tab, click **Re-Initialize Transducers**.

Figure 9-2 Hardware Diagnostics Dialog: Flow Calibration Tab



6. For M5 MicroLC-TE systems, a message is shown asking if the calibration should be performed for both channels. Click **Yes**. A warning is shown that this procedure should only be done if there is no residual pressure on the pump.
7. Make sure that the pump outlets are open, and then click **OK**.
   A status dialog shows that the re-initialization is in progress.
8. When the system shows a message that the transducers are re-initialized, click **OK**.
9. Click **Close**.
   The Hardware Diagnostics dialog closes. The Acquisition window shows.

Replace the Pump Seal Rinse

Required Materials
• Alcohol such as methanol, ethanol, or propanol

- As required, discard the pump seal rinse (in the bottle with the green tubing) and then replace it with new solvents.
  Use a 1:1 mixture of water and a common alcohol such as methanol, ethanol, or propanol and fill the bottle two-thirds full.

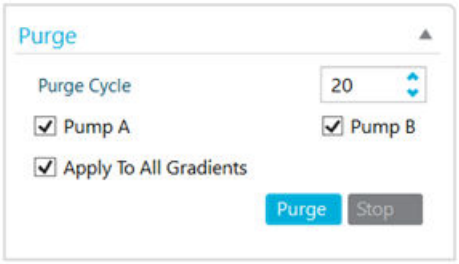
Purge the Mobile Phases and Flush the System with SCIEX OS

After changing the mobile phase bottles or if the system has been idle for a week or more, then purge the old mobile phases from the system.

Required Materials
• 1/32 inch o.d. tubing

1. Make sure that the column is not connected.
2. Connect one end of a length of 1/32 inch o.d. tubing to the mobile phase outlet on the front of the pump and put the other end into the waste bottle.
3. In SCIEX OS, click **Direct Control** > **Mobile Phases**.
4. Purge the mobile phases.
   a. In the Purge Settings section, select the **Side A** check box, the **Side B** check box, or both check boxes as applicable.
   b. (Optional) For the M5 MicroLC-TE system, select the **Apply to all channels** check box to purge both channels at once.
   c. Type a minimum of `20` in the **purge cycles** field.
   d. Click **Purge** and wait until all of the purge cycles have completed.

Figure 9-3 Mobile Phase Dialog: Settings for Purging

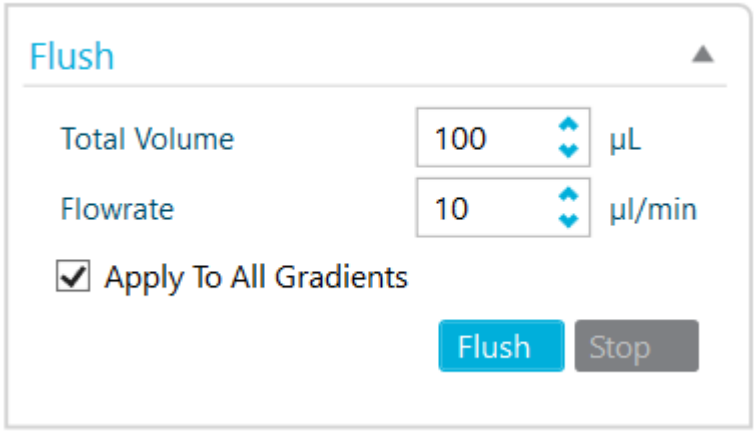


5. Flush the system.
   a. In the Flush Settings section, type `100 µL` for the **Total Volume**.
   b. In the **Flush Flowrate** based on the system configuration:
      - For a low-flow system, type `10` µL/min.
      - For a micro-flow or high-flow system, type `50` µL/min.
   c. (Optional) For the M5 MicroLC-TE system, select the **Apply to all channels** check box to flush both channels at once.

   CAUTION: Potential System Damage. Make sure that the LC column is not connected before proceeding with this operation. Flushing the system with a column connected might result in over-pressure in the system and create leaks.

   d. Click **Flush Now**.

Figure 9-4 Mobile Phase Dialog: Settings for Flushing for Micro-flow and High-flow Systems



The system flushes 100 µL through the system.

6. Click **OK**.

7. Remove the tubing from the pump outlet and then reconnect the column.

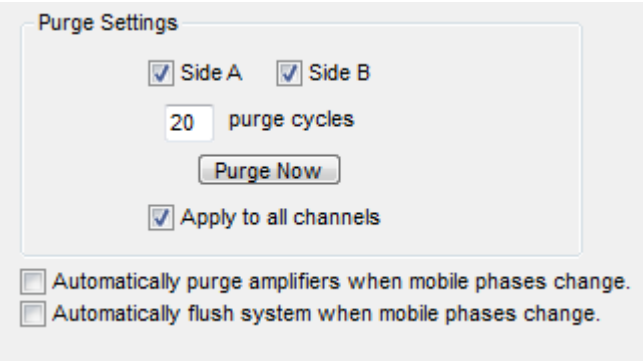
Purge the Mobile Phases and Flush the System with the Analyst Software

After changing the mobile phase bottles or if the system has been idle for a week or more, then purge the old mobile phases from the system.

Required Materials
• 1/32 inch o.d. tubing

1. Make sure that the column is not connected.
2. Connect one end of a length of 1/32 inch o.d. tubing to the mobile phase outlet on the front of the pump and put the other end into the waste bottle.
3. In the Eksigent Control software, click **System** > **Mobile Phases**, and then click **More** to show additional options in the dialog.
4. Purge the mobile phases.
   a. In the Purge Settings section, select the **Side A** check box, the **Side B** check box, or both, check box as applicable.
   b. (Optional) For the M5 MicroLC-TE system, select the **Apply to all channels** check box to purge both channels at once.
   c. Type a minimum of `20` in the **purge cycles** field.
   d. Click **Purge Now** and wait until all of the purge cycles have completed.

Figure 9-5 Mobile Phase Dialog: Settings for Purging



5. Flush the system.
   a. In the Flush Settings section, type `100 µL` for the **Total Volume**.

b. Set the **Flush Flowrate** based on the system configuration:

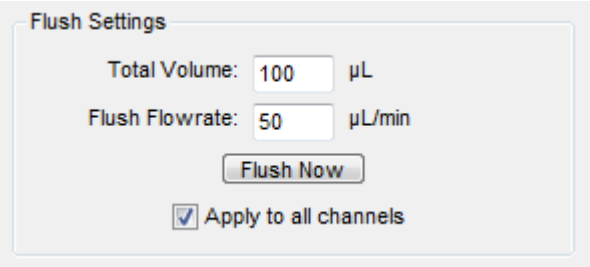
- For a low-flow system, type `10`.
- For a micro-flow or high-flow system, type `50`.

c. (Optional) For the M5 MicroLC-TE system, select the **Apply to all channels** check box to flush both channels at once.

CAUTION: Potential System Damage. Make sure that the LC column is not connected before proceeding with this operation. Flushing the system with a column connected might result in over-pressure in the system and create leaks.

d. Click **Flush Now**.

Figure 9-6 Mobile Phase Dialog: Settings for Flushing for Micro-flow and High-flow Systems



The system flushes 100 µL through the system.

6. Click **OK**.
7. Remove the tubing from the pump outlet and then reconnect the column.

Measure the Flow Rate with SCIEX OS

Required Materials

• The applicable calibration kit for the system.
  • For the low-flow M5 MicroLC-TE system, both calibration kits are required.
  • For any other system, use the correct kit for the flow rate of the system.
  • External timer

Table 9-2 Flow Rate and Calibration Specifications

System Configuration	Calibration Pipette Volume	Calibration Flow Rate
Low-flow (1 µL/min to 10 µL/min)	20 µL	5 µL/min
Micro-flow (5 µL/min to 50 µL/min)	100 µL	25 µL/min
High-flow (20 µL/min to 200 µL/min)	200 µL	100 µL/min

1. Connect the flow calibration pipette to the pump using the 25 µm i.d. PEEK-clad fused silica tubing.
   a. Disconnect the tubing from the pump outlet.
   b. Connect the 25 µm i.d. PEEK-clad fused silica tubing to the pump.
   c. Select the correct pipette for the system configuration. Refer to the table: Table 9-2.
   d. Install the free end of the 25 µm i.d. PEEK-clad fused silica tubing in the silicon tubing on the calibration pipette.
2. Measure the time to fill the pipette with the volume specified. Refer to the table: Table 9-2.
   a. In the Direct Control dialog, set the **Flowrate** option as specified in the table .
   b. Set the **%B** to `50`.
   c. Click **Start.**

   The expected time varies by system configuration:

   - For the low-flow configuration, the expected time is 240 seconds. A range of 230 to 240 seconds is required.
   - For the micro-flow configuration, the expected time is 240 seconds. A range of 230 to 250 seconds is required.
   - For the high-flow configuration, the expected time is 120 seconds. A range of 115 to 125 seconds is required.
3. Do one of the following:
   - If the flow is within the acceptable range, then the flowmeters need not be calibrated. Disconnect the 25 µm i.d. PEEK-clad fused silica tubing and then reconnect the original tubing between the pump and the injection valve.
   - If the flow is outside of the acceptable range, then calibrate the flow meters. Refer to the section, Calibrate the Flowmeters in the document: *M5 MicroLC Systems Operator Guide*.

Measure the Flow Rate with the Analyst Software

Required Materials

• The applicable calibration kit for the system.
  • For the low-flow M5 MicroLC-TE system, both calibration kits are required.
  • For any other system, use the correct kit for the flow rate of the system.
• External timer

Table 9-3 Flow Rate and Calibration Specifications

System Configuration	Calibration Pipette Volume	Calibration Flow Rate
Low-flow (1 µL/min to 10 µL/min)	20 µL	5 µL/min
Micro-flow (5 µL/min to 50 µL/min)	100 µL	25 µL/min
High-flow (20 µL/min to 200 µL/min)	200 µL	100 µL/min

1. Connect the flow calibration pipette to the pump using the 25 µm i.d. PEEK-clad fused silica tubing.
   a. Disconnect the tubing from the pump outlet.
   b. Connect the 25 µm i.d. PEEK-clad fused silica tubing to the pump.
   c. Select the correct pipette for the system configuration. Refer to the table: Table 9-3.
   d. Install the free end of the 25 µm i.d. PEEK-clad fused silica tubing in the silicon tubing on the calibration pipette.
2. Measure the time to fill the pipette with the volume specified. Refer to the table: Table 9-3.
   a. In the Direct Control dialog, select the **Conserved Flow** option.
   b. Set the mobile phase composition to `50` **A** and `50` **B.**
   c. Set the **Total flowrate** as specified. Refer to the table: Table 9-3.
   d. Click **Start.**

   The expected time varies by system configuration:

   - For the low-flow configuration, the expected time is 240 seconds. A range of 230 to 240 seconds is required.
   - For the micro-flow configuration, the expected time is 240 seconds. A range of 230 to 250 seconds is required.

- For the high-flow configuration, the expected time is 120 seconds. A range of 115 to 125 seconds is required.

3. Do one of the following:
   - If the flow is within the acceptable range, then calibration for the flowmeters is not required. Disconnect the 25 µm i.d. PEEK-clad fused silica tubing and then reconnect the original tubing between the pump and the injection valve.
   - If the flow is outside of the acceptable range, then calibrate the flowmeters. Refer to the section: "Calibrate the Flowmeters" in the document: *Operator Guide*.

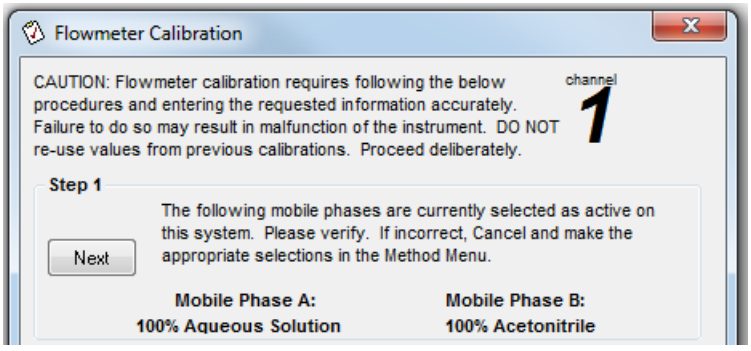
Calibrate the Flowmeters with SCIEX OS

Required Materials

• The applicable calibration kit for the system.
  • For the low-flow M5 MicroLC-TE system, both calibration kits are required.
  • For any other system, use the correct kit for the flow rate of the system.

1. If required, then use 25 µm i.d. tubing to connect the flow calibration assembly to the pump.
2. On the status panel, click **Direct device control** () to the right of the device name.
3. Click **Maintenance** ().
   The Hardware Diagnostic dialog opens.
4. Click **Calibrate Flowmeter**.
   The Flowmeter Calibration wizard opens.

Figure 9-7 Flowmeter Calibration Wizard: Step 1

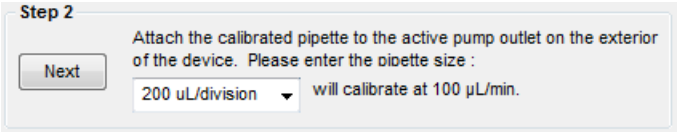


5. In the Step 1 section, make sure that the mobile phases are correct, and then click **Next**.

Note: If the mobile phases are not correct, then click **Cancel** and then make the required changes in the Mobile Phases dialog. Refer to the section: Load the Mobile Phases.

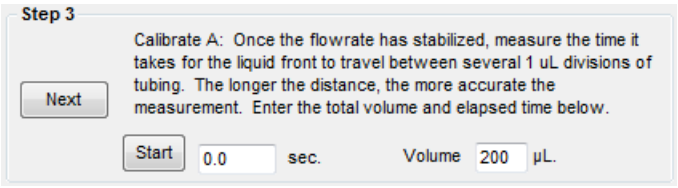
6. In the Step 2 section, set the pipette size.
   - For a low-flow system, select **20 µL/division**.
   - For a micro-flow system, select **100 µL/division**.
   - For a high-flow system, select **200 µL/division**.

Figure 9-8 Set the Flowmeter Calibration Size: High-flow System



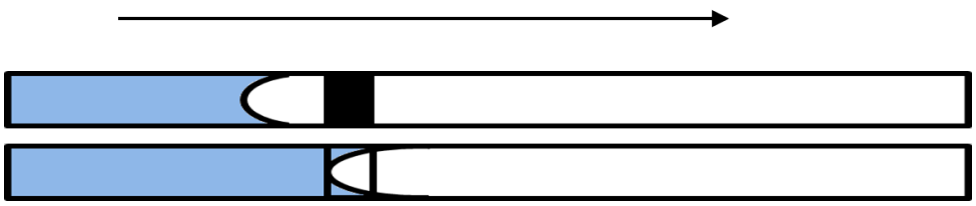
7. To start the flow on side A, click **Next**.
8. In the Step 3 section, set the correct volume.
   - For a low-flow system, type `20`.
   - For a micro-flow system, type `100`.
   - For a high-flow system, type `200`.

Figure 9-9 Set the Flowmeter Calibration Volume: High-flow System



9. Move the meniscus to the black line on the pipette and then click **Start** to start timing.

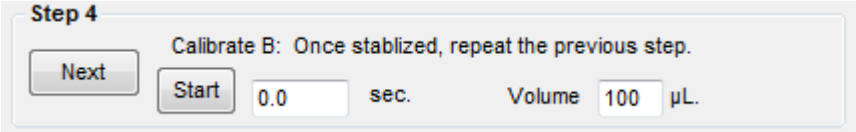
Figure 9-10 Calibration Pipette, Meniscus Before (Top) and At (Bottom) Black Line (Arrow Identifies Direction of Flow)



10. When the fluid gets to the end of the pipette, click **Stop**.

11. Click **Next** and then do the procedure again to calibrate the side B flowmeter.

Figure 9-11 Calibrate Side B



12. Click **Finish**.
13. Do one of the following:
    - If the calibration passed, then do the section: Measure the Flow Rate with SCIEX OS again to find whether the flow rate is within acceptable bounds.
    - If the calibration failed, then examine for leaks, make sure that the settings in the Mobile Phases dialog are correct for the solvents in use, and then purge and flush the system. Do the calibration again. If the calibration fails again, contact SCIEX Technical Support at sciex.com/request-support.
14. M5 MicroLC-TE system: Do the calibration for Gradient 2 again.
    a. Close the Flowmeter Calibration dialog.
    b. In the Hardware Diagnostics dialog, click the arrows in the top right corner to select **Gradient 2**.
    c. Click **Calibrate Flowmeter Ch 2** to do the calibration again.
    d. In the Step 2 section, select **200 µL/division** for the pipette size.
    e. In the Step 3 section, type `200` in the **Volume** field.
15. Disconnect the 25 µm i.d. tubing and then connect the original tubing between the pump and the injection valve.

Calibrate the Flowmeters with the Analyst Software

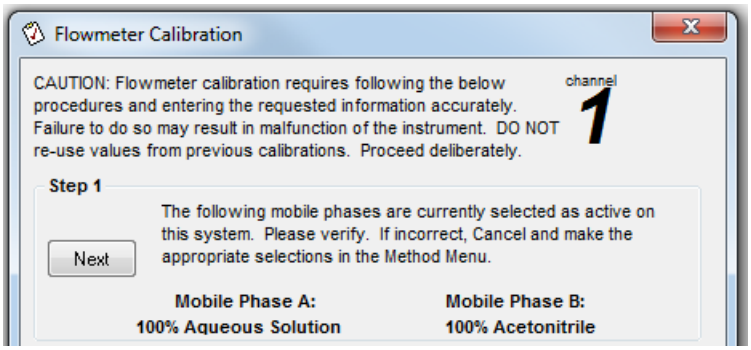
Required Materials

• The applicable calibration kit for the system.
  • For the low-flow M5 MicroLC-TE system, both calibration kits are required.
  • For any other system, use the correct kit for the flow rate of the system.

1. If required, then connect the flow calibration assembly to the pump using the 25 µm i.d. tubing.
2. Click **System** > **Hardware Diagnostics**.

3. Click **Calibrate Flowmeter** .
   The Flowmeter Calibration wizard opens.

Figure 9-12 Flowmeter Calibration Wizard: Step 1

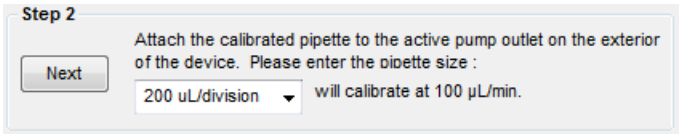


4. In the Step 1 section, make sure that the mobile phases are correct, and then click **Next**.

Note: If the mobile phases are not correct, then click **Cancel** and then make the required changes in the Mobile Phases dialog. Refer to the section: Load the Mobile Phases.

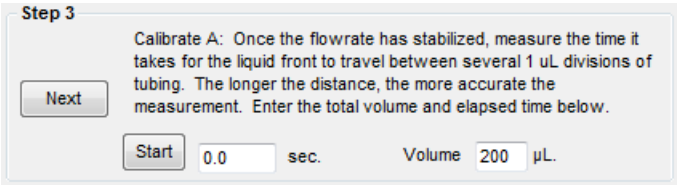
5. In the Step 2 section, set the pipette size.
   - For a low-flow system, select **20 µL/division**.
   - For a micro-flow system, select **100 µL/division**.
   - For a high-flow system, select **200 µL/division**.

Figure 9-13 Set the Flowmeter Calibration Size: High-flow System



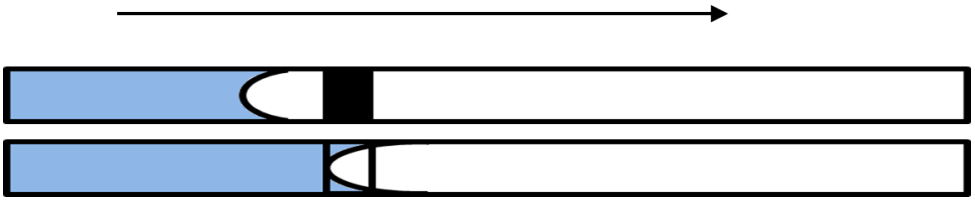
6. Click **Next** to start the flow in side A.
7. In the Step 3 section, set the correct volume.
   - For a low-flow system, type `20`.
   - For a micro-flow system, type `100`.
   - For a high-flow system, type `200`.

Figure 9-14 Set the Flowmeter Calibration Volume: High-flow System



8. Move the meniscus to the black line on the pipette and then click **Start** to start timing.

Figure 9-15 Calibration Pipette, Meniscus Before (Top) and At (Bottom) Black Line (Arrow Identifies Direction of Flow)



9. When the fluid gets to the end of the pipette, click **Stop**.
10. Click **Next** and then do the procedure again to calibrate the side B flowmeter.

Figure 9-16 Calibrate Side B

11. Click **Finish**.
12. Do one of the following:
    - If the calibration passed, then do the section: Measure the Flow Rate with the Analyst Software again to find whether the flow rate is within acceptable bounds.
    - If the calibration failed, then examine for leaks, make sure that the settings in the Mobile Phases dialog are correct for the solvents in use, and then purge and flush the system. Do the calibration again. If the calibration fails again, contact SCIEX Technical Support at sciex.com/request-support.
13. M5 MicroLC-TE system: Do the calibration for Gradient 2 again.
    a. Close the Flowmeter Calibration dialog.
    b. In the Hardware Diagnostics dialog, click the arrows in the top right corner to select **Gradient 2**.
    c. Click **Calibrate Flowmeter Ch 2** to do the calibration again.

d. In the Step 2 section, select **200 µL/division** for the pipette size.

e. In the Step 3 section, type `200` in the **Volume** field.

14. Disconnect the 25 µm i.d. tubing and then connect the original tubing between the pump and the injection valve.

Maintenance Procedures for the Valve

Install the Injection Port

Required Materials
• Injection port

1. Remove the injection port from port 3 on the valve.
2. Put the injection port in port 3 on the injection valve and then tighten the injection port until it is finger tight.

Figure 9-17 Injection Port

Install the Sample Loop

The sample loop is located between port 1 and port 4 on the injection valve. Change the sample loop to inject a different sample volume or to clear a blockage in the loop.

Required Materials
• Sample loop
• Wrench for 1/32 inch and 1/16 inch nuts (in the system accessory kit)
• 2 nuts
• 2 ferrules

1. Remove the loop.

2. Connect the new loop to port 1 on the injection valve using the wrench and one of the ferrules.
3. Connect the other end of the loop to port 4 with the other nut and ferrule.

Install the Valve Rotor Seal

If the valve leaks, then replace the valve rotor seal.

Required Materials
• Long Phillips screwdriver • Wrench for 1/32 inch and 1/16 inch nuts (in the system accessory kit) • 9/64 inch hex key • T20 star screwdriver • Injection valve rotor seal

1. Close the Eksigent Control software or SCIEX OS.
2. Use the switch on the back of the system to turn off the power to the system, and then disconnect the mains supply cable.
3. Remove the valve stator.
    a. Use the 9/64 inch hex key to remove the three hex screws from the top of the valve stator.
    b. Lift off the stator and then set it aside.

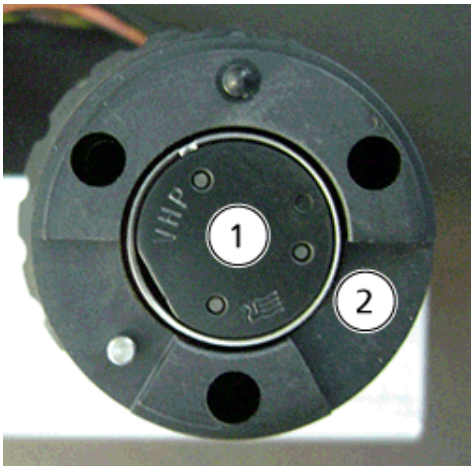
Figure 9-18 Remove the Screws in the Valve Stator

4. Remove the rotor seal.
   a. (Optional) Lift off the black plastic alignment cylinder and then set it aside.
   b. Lift the rotor seal out of the valve.

   The rotor seal consists of a black disk in a silver case.

Note: It might be possible to lift the rotor seal without removing the alignment cylinder.

Figure 9-19 Injection Valve: Top View, With Stator Removed



Item	Description
1	Rotor seal
2	Alignment cylinder

5. Install the new rotor seal.
   a. Put the new rotor seal on the valve. Make sure that the valve is installed fully on the three pins.
   b. Install the black plastic alignment cylinder on the valve, rotating as necessary to install it fully.
   c. Install the stator, and then tighten the hex screws.
6. Plumb the injection valve.
7. Connect the mains supply cable, and then use the switch on the back of the system to turn on the power.
8. Turn on the power switch on the front of the system.

Maintenance Procedures for the Autosampler

Replace the Autosampler Wash Solvents

1. Refill the aqueous wash solvent in the 1 L glass bottle. The aqueous solvent is typically water with 0.1% formic acid.
   Make sure that the solvent tubing labeled W1 is attached to the Wash 1 pump and that the other end is in the glass bottle.
2. Refill the organic wash solvent in the 1 L glass bottle. The organic solvent is typically acetonitrile (or other organic solvent) with 0.1% formic acid.
   Make sure that the solvent tubing labeled W2 is attached to the Wash 2 pump and that the other end is in the glass bottle.
3. After replacing the wash solvents, prime the LCMS tool pumps. Refer to the section: Prime the LCMS Tool Wash Solvent Tubing.

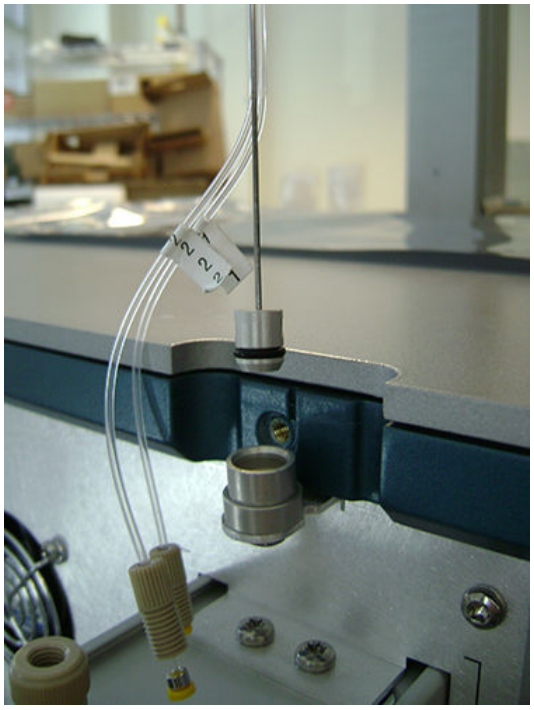
Replace the LCMS Tool Wash Solvent Tubing

CAUTION: Potential Operator Injury. To avoid the possibility of exposure to solvents, be sure to wear gloves.

Required Materials
• LCMS tool tubing kit • T6 star screwdriver

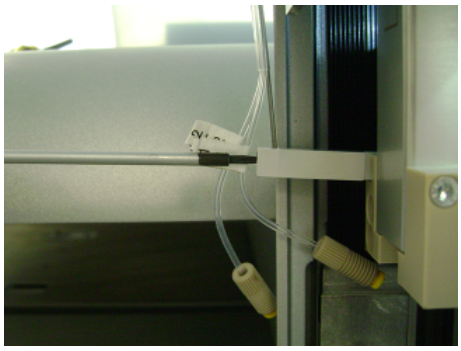
1. Remove the LCMS tool wash solvent tubing from the system.
   a. Disconnect the tubing from the LCMS tool pumps on the back of the system and then pull up on the guide wire to remove the barrel from the bracket.

Figure 9-20 Location for LCMS Tool Guide Wire: Back of System



   b. Disconnect the tubing from the LCMS tool on the front of the system.
   c. Using the T6 star driver, loosen (but do not remove) the set screw that holds the guide wire, and then lift the solvent tubing away from the system.

Figure 9-21 Location of LCMS Tool Guide Wire: Front of System



2. Reverse step 1 to connect the guide wire and wash solvent tubing. Tighten the fittings until they are finger tight.
3. Prime the wash solvent tubing. Refer to the section: Prime the LCMS Tool Wash Solvent Tubing.

Replace the LCMS Tool Pump Module

CAUTION: Potential Operator Injury. To avoid the possibility of exposure to solvents, be sure to wear gloves.

Required Materials
• LCMS tool pump module • T20 star driver • Gloves

1. Press the power switch on the front of the system to turn off the system.
2. (Optional) If the solvent tray is installed on the same side of the system as the LCMS tool pump module, then remove it for better access to the pump.
3. Remove the tubing from the back of the system.
   a. Disconnect the tubing from the top of the pump module on the back of the system.
   b. Remove the check valves.
   c. Pull the tubing from the wash solvent bottles up, out of the solvent, to prevent additional solvent from filling the tubing, and then disconnect the tubing from the bottom of the pump module.
   d. Put the ends of the tubing up so that the solvent does not drain out.
4. Remove the pump module.

a. Use the T20 star screwdriver, remove the screws at the top of the pump module.

b. Pull the pumps away from the system, and then disconnect the cable from the back of the pump module.

c. Disconnect the cable from the back of the system. Gently pull the cable away so that it does not fall inside the case.

5. Reverse steps 4 and 3 to replace the pump module and the check valves,and then attach the tubing.

   Make sure that the number on the tubing is the same as the number on the pump.

6. Turn on the power switch on the front of the system.

Prime the LCMS Tool Wash Solvent Tubing

1. Start the the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.
2. Click **LCP1**.
3. Select **Options** > **Prime LC-Tool**.
4. Click **Filling Stroke**, and then set the number of strokes to `10`.

   Tip! If the wash solvent containers are far from the system, then increase the number of strokes.

5. Click **Washsource**, and then select **1**.
6. Start the priming. Watch the tubing and make sure that liquid is pulled through the tubing.
7. If required, do the steps again until the liquid is flowing through the tubing.

   Tip! As an alternative, remove the check valve from the LCMS tool pump, connect the tubing directly to the pump, and then prime the pump. When liquid is flowing, install the check valve, connect the tubing, and then prime the pump again.

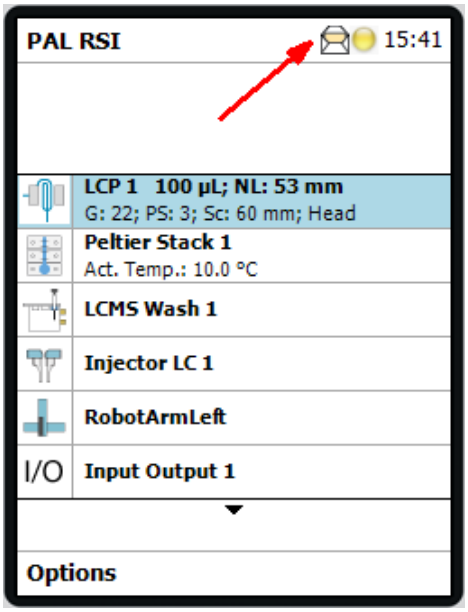
8. Do again for the other solvent tubing, selecting **2** for the **Washsource**.

Configure the LCMS Tool Pumps with SCIEX OS

The LCMS Tool pump module has an ID that is saved in the CTC PAL 3 autosampler firmware. If the pump module is replaced, then the new ID will not match the existing ID in the firmware and the autosampler must be configured to use the new pump module.

1. Start the the PAL Virtual Terminal software, and then make sure that Access level is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level. The status indicator in the upper right corner is yellow, indicating a problem with the autosampler.

Figure 9-22 Autosampler Message and Status Icons

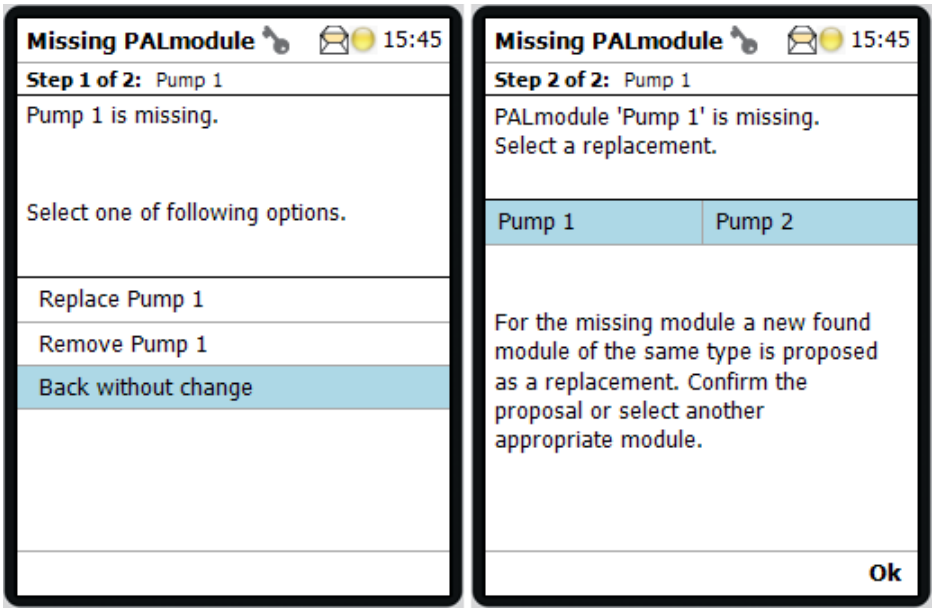


2. In the PAL Virtual Terminal software, replace the old pump with the new pump and then rename it.

   a. Click **Options** > **Pending Message**.

   b. Click **OK** in the message.

Figure 9-23 Replace Missing PAL Module Steps



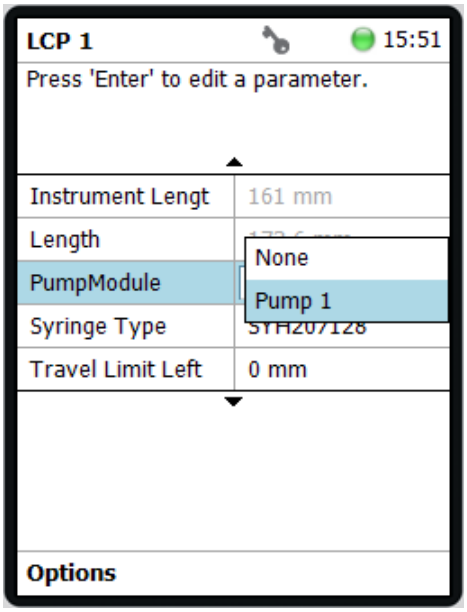
c. In step 1, click **Replace Pump 1** and then press **Enter**.

d. In step 2, click **OK** to replace the old pump with the new pump.

e. Click **LCP1** and then click the arrow button to scroll to PumpModule.

f. Double-click **PumpModule** and then click **Pump 1**.

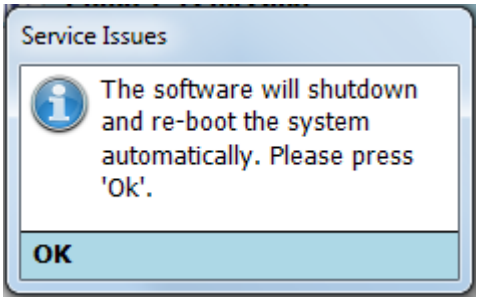
Figure 9-24 Rename the Pump



g. Click **Back**.

h. Click **OK** in the message to restart the autosampler.

Figure 9-25 Restart Message



The PAL Virtual Terminal software closes and the CTC PAL 3 autosampler power cycles.

3. In the SCIEX OS software, deactivate the hardware profile.

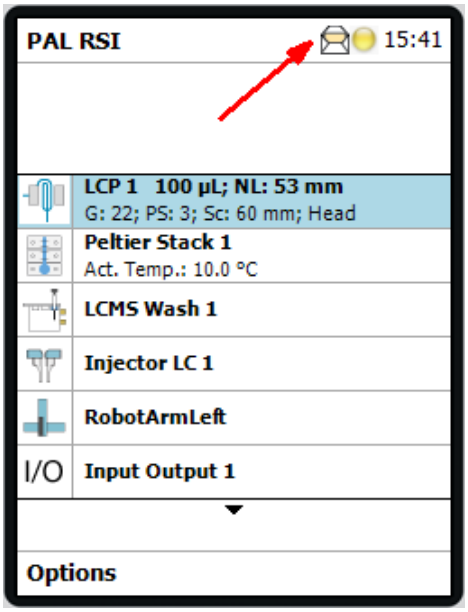
4. Clicking the **Auto Detect** button in the M5 MicroLC device settings to retrieve the autosampler configuration. Refer to the section: Configure the Settings for M5 MicroLC Systems.
5. Prime the wash solvent tubing. Refer to the section: Prime the LCMS Tool Wash Solvent Tubing.
6. Activate the device profile in the SCIEX OS software and then update the acquisition methods. For each method:
   a. In the LC Method window, open a **LC method** file.
   b. Click **Save**.
7. After all the methods have been updated, click **Close**.

Configure the LCMS Tool Pumps with the Analyst Software

The LCMS Tool pump module has an ID that is saved in the CTC PAL 3 autosampler firmware. If the pump module is replaced, then the new ID will not match the existing ID in the firmware and the autosampler must be configured to use the new pump module.

1. Start the the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level. The status indicator in the upper right corner is yellow, indicating a problem with the autosampler.

Figure 9-26 Autosampler Message and Status Icons

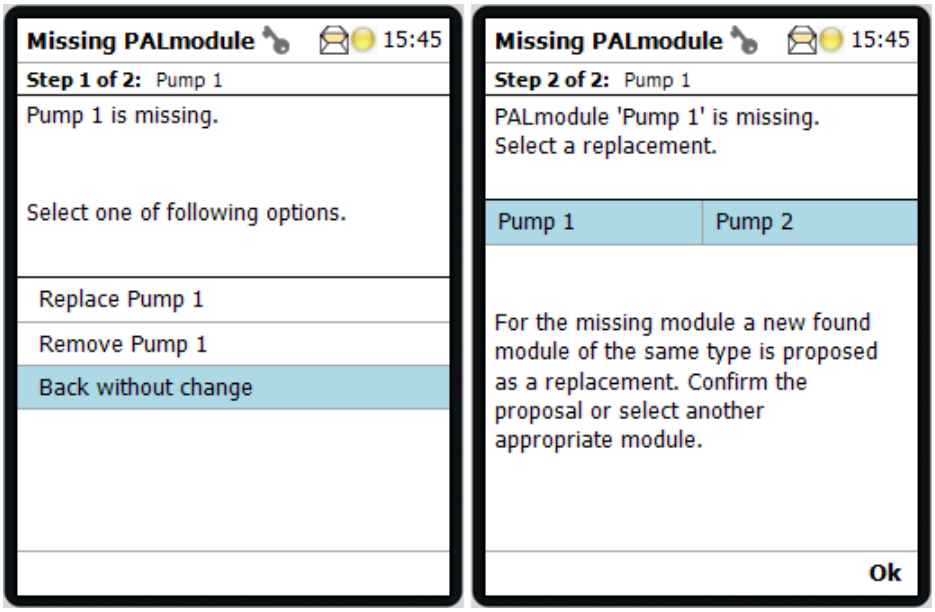


2. In the PAL Virtual Terminal software, replace the old pump with the new pump and then rename it.

a. Click **Options** > **Pending Message**.

b. Click **OK** in the message.

Figure 9-27 Replace Missing PAL Module Steps



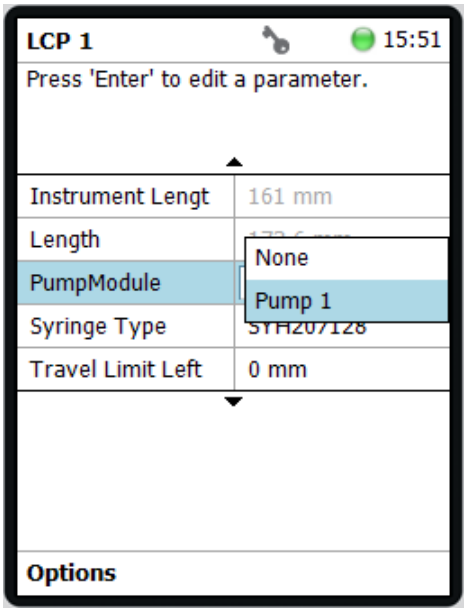
c. In step 1, click **Replace Pump 1** and then press **Enter**.

d. In step 2, click **OK** to replace the old pump with the new pump.

e. Click **LCP1** and then click the arrow button to scroll to PumpModule.

f. Double-click **PumpModule** and then click **Pump 1**.

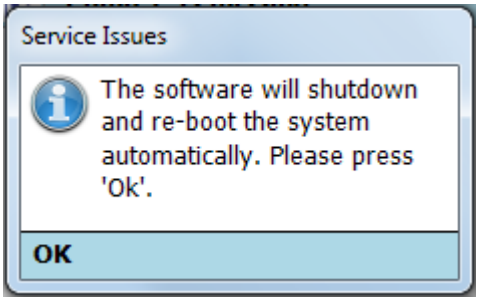
Figure 9-28 Rename the Pump



g. Click **Back**.

h. Click **OK** in the message to restart the autosampler.

Figure 9-29 Restart Message



The PAL Virtual Terminal software closes and the CTC PAL 3 autosampler power cycles.

3. In the Analyst software, deactivate the hardware profile.
4. Using the Analyst Device Driver (ADD) software, retrieve the autosampler configuration. Refer to step 7 in the section: Install the CTC PAL 3 Driver and Configure the Device.
5. Prime the wash solvent tubing. Refer to the section: Prime the LCMS Tool Wash Solvent Tubing.
6. Activate the hardware profile in the Analyst software and then update the acquisition methods. For each method:

a. In the Acquisition Method window, double-click **Analyst Device Driver**.

b. In the Analyst Device Driver window, click **Method** to open the Instrument Control Method Editor window.

c. Click **File** > **Open from Analyst.dam file** and then select an acquisition method.

d. Click **Save**.

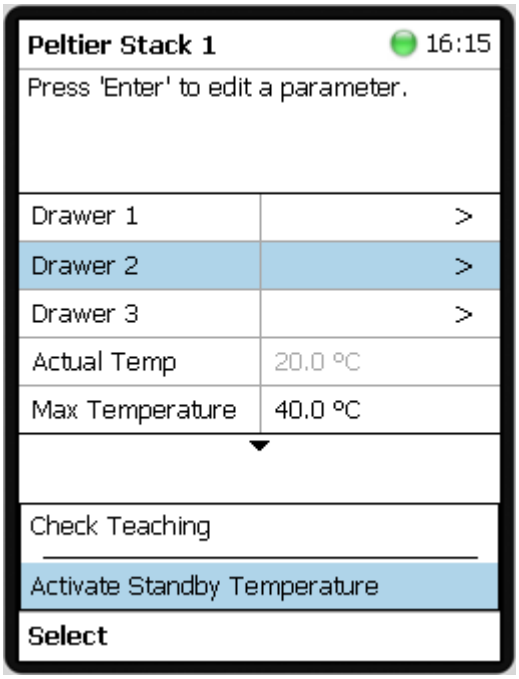
7. After all the methods have been updated, click **File** > **Exit**.
The Instrument Control Method Editor window closes.

Set the Temperature of the Sample Drawers

1. Start the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.

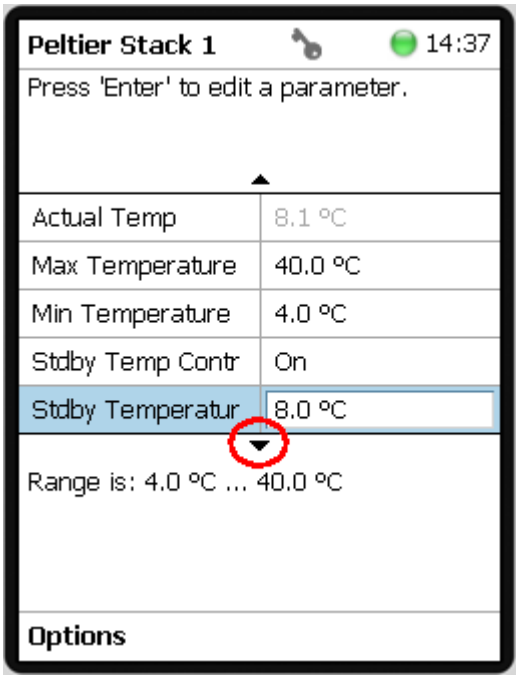
2. Click **Peltier Stack 1** and then click **Options** > **Activate Standby Temperature**.

Figure 9-30 Selecting Activate Standby Temperature



3. Click the black arrow to scroll through the list of parameters until **Stdby Temperature** is shown. Double-click **Stdby Temperature**, press the up and down arrow keys to change the temperature, and then press **Enter** to save the changes.

Figure 9-31 Peltier Stack 1 Parameters



4. Click **Back**, and then click to close the Remote Terminal window.

Replace the Syringe Needle

WARNING! Puncture Hazard. Handle the needle with care. The tip of the needle is extremely sharp.

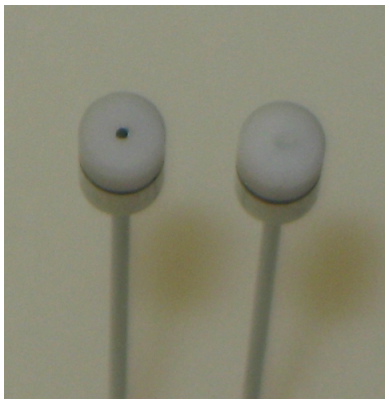
1. Start the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.
2. Move the Z-arm to a location with better access to the LCMS tool.
   a. Click **LCP1** > **Options** > **Change Syringe**.
   b. Click **Move**.

   The Z-arm moves down and away from the system.
3. Remove the syringe needle.
   a. Loosen the needle collar and then move the needle down. Make sure that the PTFE seal is attached to the needle.
   b. Holding the needle with one hand, push the lower needle guide up with the other hand.
   c. Tilt the needle to the side so it can be removed from the needle guides.
   d. Put the needle collar and the washer in a safe place.

4. Prepare the needle.

   a. Install the needle in the PTFE seal.
   Sometimes the hole in the seal has a burr (PTFE residue from the seal), as shown in the following figure.

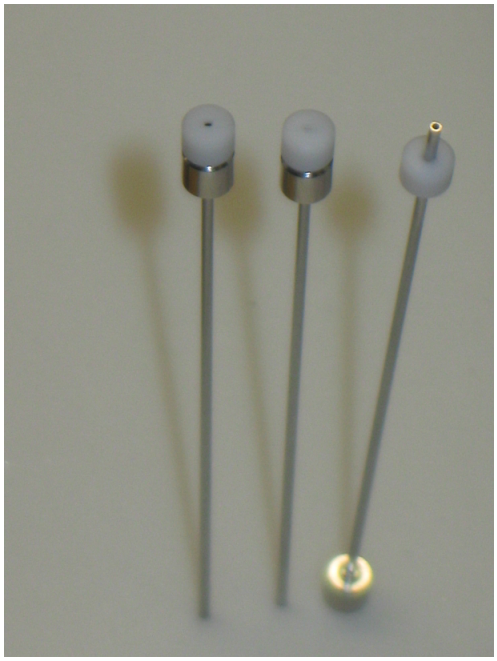
   Figure 9-32 PTFE Needle Seal: Clean (Left) and with Burr (Right)

   b. If required, remove any burrs.
   Remove the seal from the needle and then use the long end of the needle to push the burr out. Refer to the figure: Figure 9-33.

   Be careful not to scratch the seal.

   Figure 9-33 Cleaning the PTFE Needle Seal

c. Install the washer on the needle, and then put the needle collar on the needle.

5. Install the needle.

a. Push the clear safety shield on the Z-arm up to give access to the syringe.

b. With one hand, lift the bottom needle guide until it touches the upper needle guide.

c. With the other hand, guide the tip of the new needle into both guides and then release the lower needle guide.

d. Install the top of the needle in the fitting, and then tighten the needle collar until it is finger tight.

Figure 9-34 Syringe Needle Collar

6. Gently move the needle guides up and down to make sure that the tip of the needle is near the bottom of the hole in the needle guide.
   If the needle guide is above the hole at rest, then the needle will probably hit the hole when compressed and bend the needle.

Figure 9-35 Correctly Installed Syringe Needle

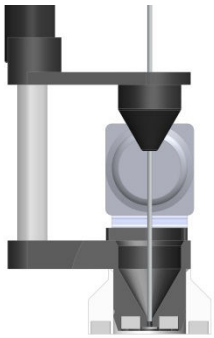
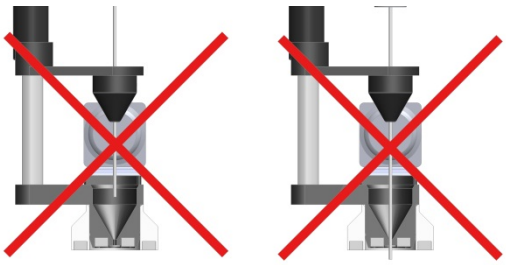


Figure 9-36 Incorrectly Installed Syringe Needle



7. In the Remote Terminal window, click **Next** to move the Z-arm to the original position.

Replace the Syringe

Use this procedure to replace the syringe.

Required Materials
• Syringe barrel

WARNING! Puncture Hazard. Handle the needle with care. The tip of the needle is extremely sharp.

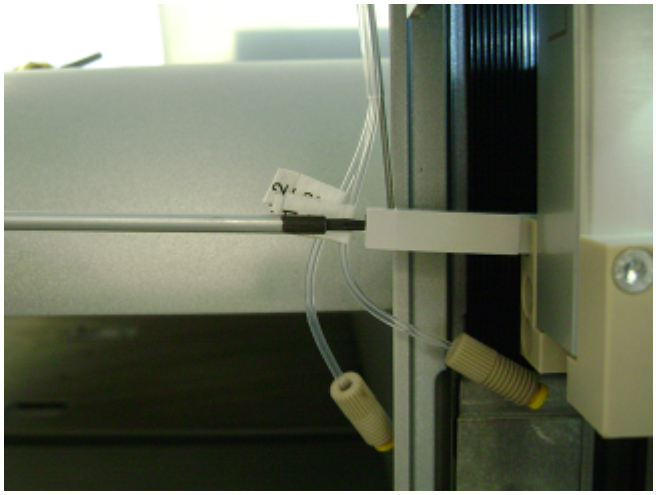
1. Start the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.

2. Move the Z-arm to a location with better access to the LCMS tool.
   a. Click **LCP1** > **Options** > **Change Syringe**.
   b. Click **Move**.

   The Z-arm moves down and away from the system.
3. Remove the LCMS tool from the Z-arm.
   a. Remove the syringe needle from the syringe. Be careful not to lose the washer.
   b. Using a T6 Torx driver, loosen the set screw from the metal arm until the wire can be pulled free.

   Tip! After removing the wire, tighten the set screw to prevent it from getting lost.

   Figure 9-37 Location for Guide Wire, Front of System

   c. Disconnect the tubing from the front of the tool.

Figure 9-38 LCMS Tool Tubing, Front of System

d. Lift the silver latch on the plunger coupling adapter to free the plunger.

e. Hold the bottom of the LCMS tool with one hand and then pull the black latch to the right with other hand until the tool is free from the Z-arm.

4. Remove the syringe barrel.

   a. Turn the black ribbed nut at the top of the LCMS tool to loosen it and then pull the nut and the plunger away from the tool.

   b. Turn the metal nut at the bottom of the syringe barrel to loosen it and then lift the barrel out of the tool.

5. Install the plunger in the LCMS tool.

   a. Install the plunger in hole in the ribbed nut and then in the syringe.

   b. Put the syringe through the hole in the top of the LCMS tool.

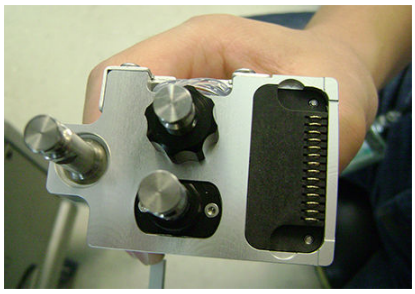
   c. Align the hole in the syringe barrel with the hole in the bottom of the LCMS tool and tighten the metal retaining nut.

   d. At the top of the LCMS tool, tighten the retaining nut to secure the mechanical stop. Do not overtighten.

6. Install the LCMS tool on the Z-arm.

   a. Turn the LCMS tool so that the black portion points away from the system.

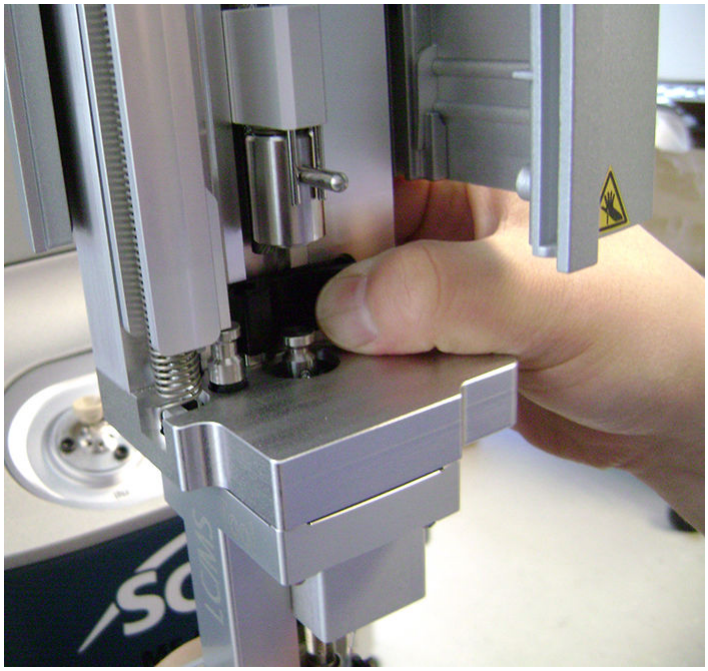
Figure 9-39 LCMS Tool: Top View



b. Align the three cylinders on the top of the LCMS tool with the holes in the Z-arm platform and then move the tool up until the cylinders protrude from the upper side of the Z-arm platform.

c. Hold the tool in place with one hand, push the black latch to the right with the other hand, and then release the latch.

Figure 9-40 LCMS Tool Latch



7. Connect the syringe plunger to the LCMS tool.

   a. Pull the plunger partially up from the black retaining nut.

   b. Lower the plunger coupling adapter and the plunger coupling block over the syringe plunger until it clicks.

Figure 9-41 LCMS Tool: Plunger Coupling Adapter

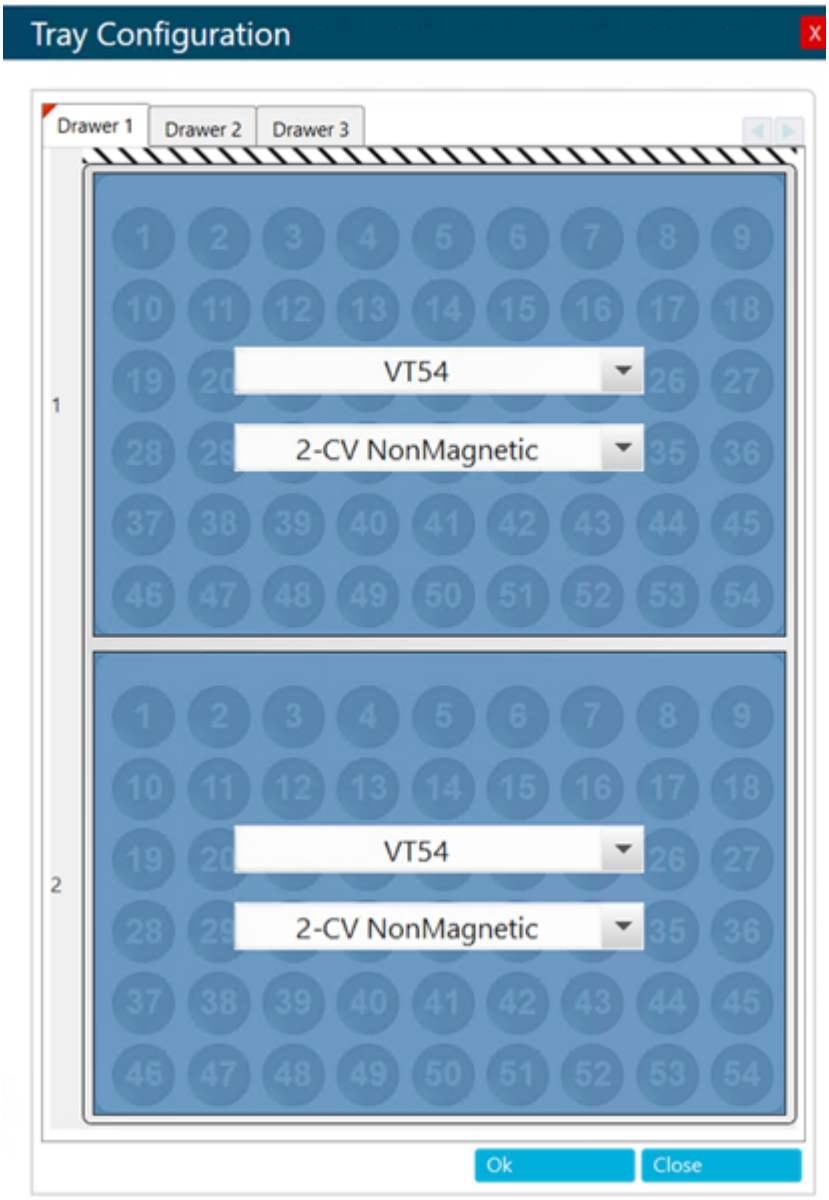
8. Connect the guide wire and the tubing to the front of the system.
9. Tilt the syringe needle to install the needle tip in the lower needle guide, then move the needle back and up to the needle holder and tighten it firmly.
10. In the Remote Terminal window, click **Next** to move the Z-arm to the original position.
11. Prime the wash solvent tubing. Refer to the section: Prime the LCMS Tool Wash Solvent Tubing.

(Optional) Configure the Autosampler to Use Microtiter Plates with SCIEX OS

By default, the system is configured to use VT54 vial plates. The system can also use 96- or 384-well plates.

1. Remove the VT54 plates from both slots in drawer 1 and then replace them with empty microtiter plates.
2. On the status panel, click **Direct device control** () at the right of the devices name.
3. Expand the Autosampler section.
4. Click **Tray Configuration**.
   The Tray Configuration dialog opens.

Figure 9-42 Tray Configuration Dialog



5. Click the upper list and select the type of plate or rack. Do not change the lower list.
   - **MTP96** for a 96-well plate
   - **MTP384** for a 384-well plate
   - **DPW96** for a deep-well 96-well plate
6. If necessary, change the plate or tray in slot 2 in Drawer 1.
7. If necessary, update Drawers 2 and 3.

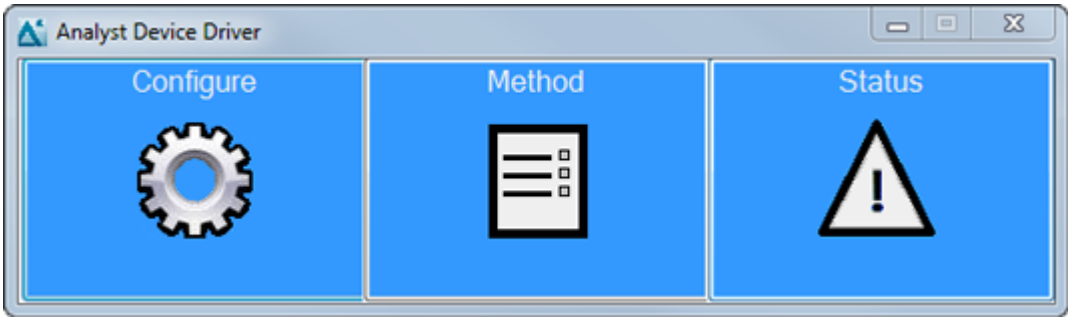
8. Click **OK**.
9. After changing the tray configuration, open the Configuration workspace, deactivate the M5 MicroLC device and then activate the M5 MicroLC to see the changes in the Batch dialog.

(Optional) Configure the Autosampler to Use Microtiter Plates with the Analyst Software

By default, the system is configured to use VT54 vial plates. The system can also use 96- or 384-well plates.

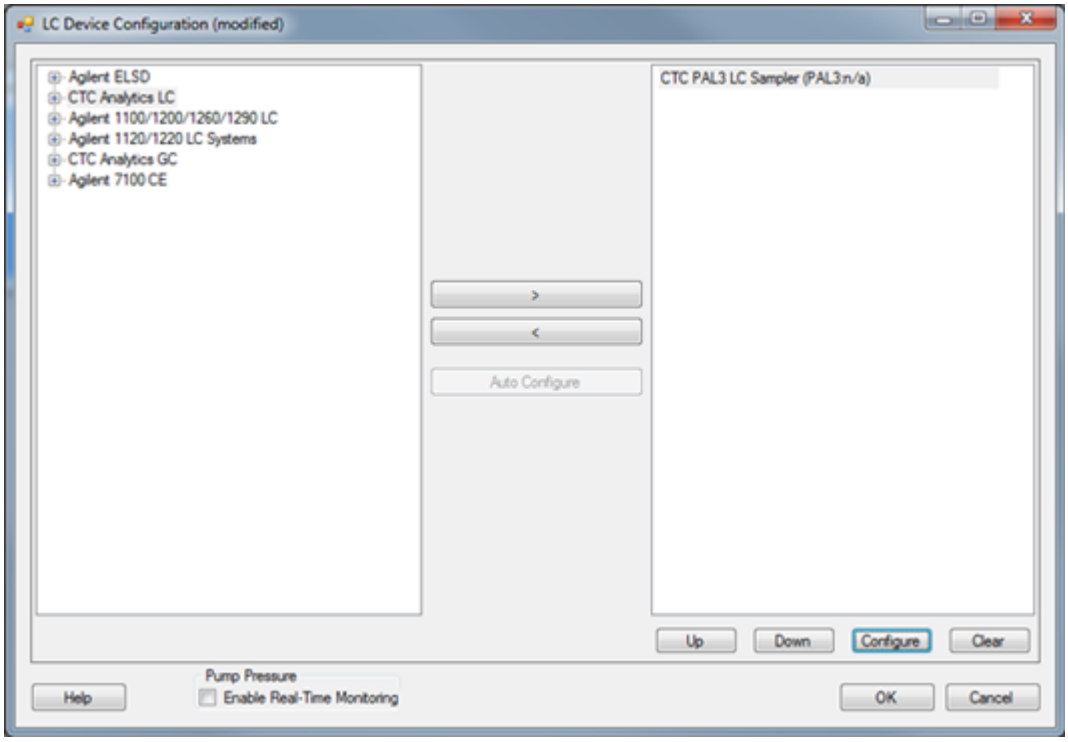
1. Remove the VT54 plates from both slots in drawer 1, and then replace them with empty microtiter plates.
2. Retrieve the autosampler configuration in Analyst Device Driver (ADD) software.
   a. Make sure that the hardware profile is deactivated.
   b. On the Navigation bar, under **Companion Software**, double-click **Analyst Device Driver**.

Figure 9-43 Analyst Device Driver



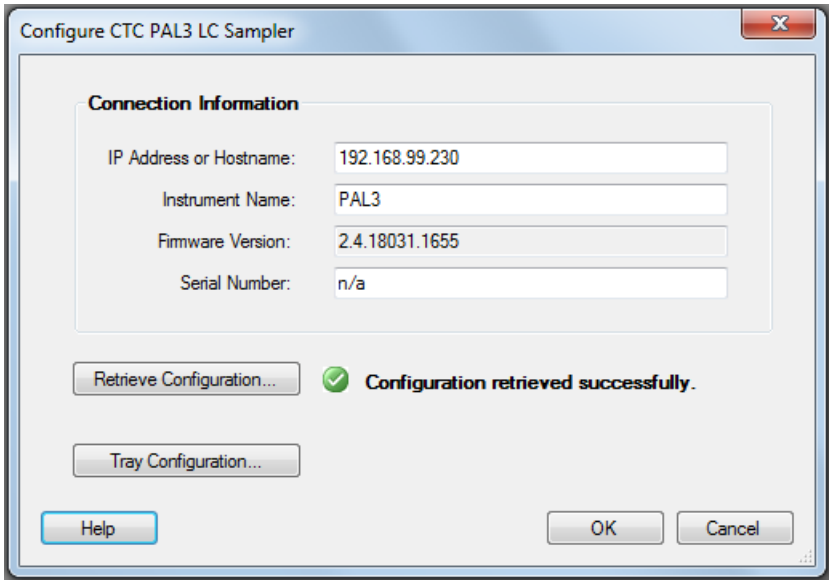
   c. In the Analyst Device Driver window, click **Configure**.

Figure 9-44 LC Device Configuration Dialog with the CTC PAL3 Autosampler Selected



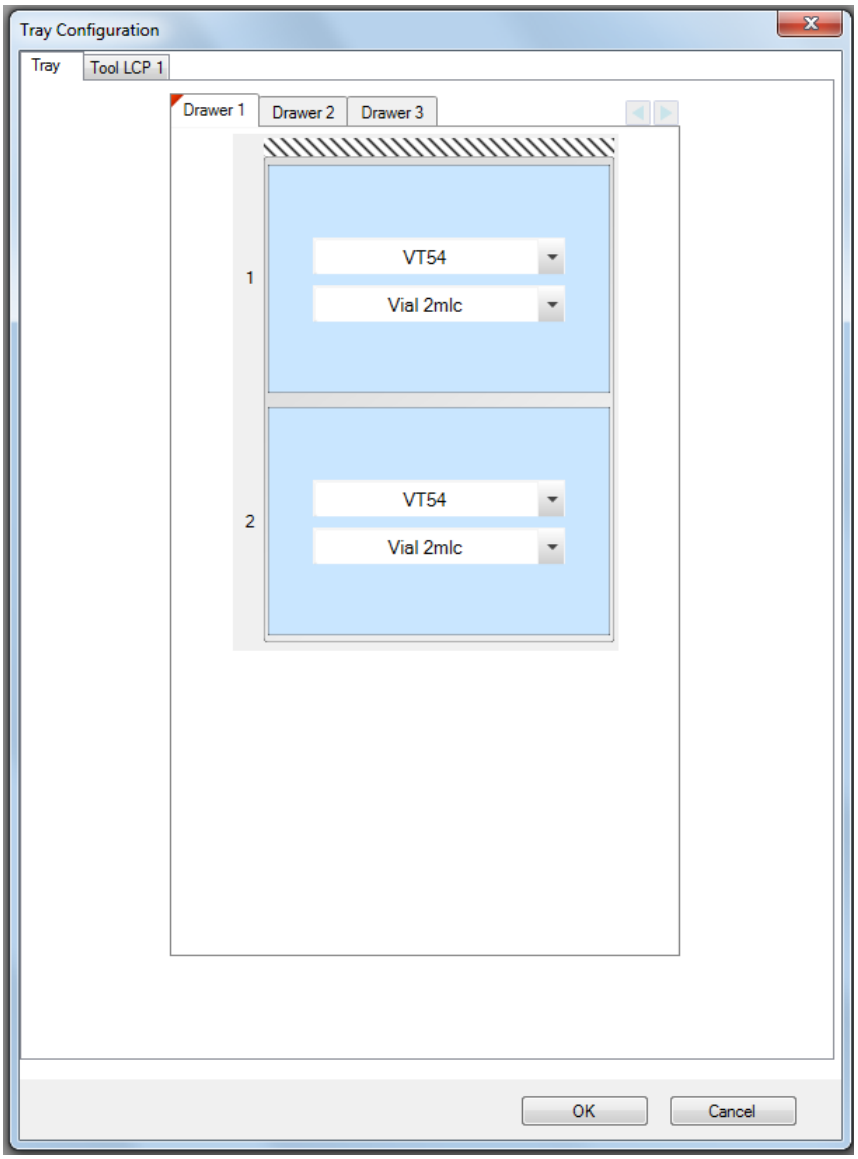
d. Click **Retrieve Configuration**.

Figure 9-45 Configure CTC PAL3 LC Sampler Dialog with Configuration Retrieved



3. Click **Tray Configuration**.

Figure 9-46 Tray Configuration Dialog



4. Click the first list, and then select the type of plate or rack. Do not change the second list.
   - **MTP96** for a 96-well plate
   - **MTP384** for a 384-well plate
   - **DPW96** for a deep-well 96-well plate
5. If required, change the plate or tray in slot 2 in Drawer 1.

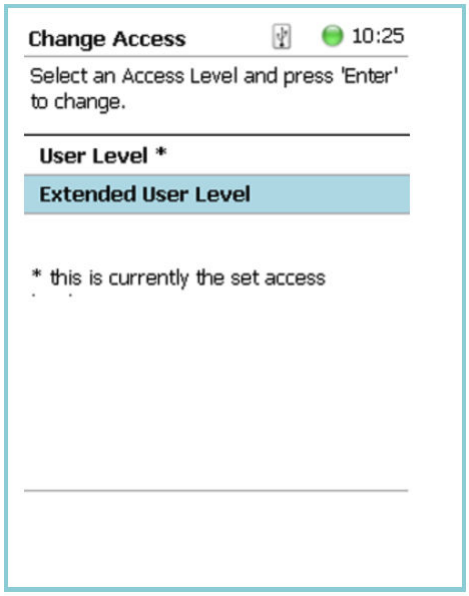
6. If required, update Drawers 2 and 3.
7. Click **OK** to close the Tray Configuration dialog.
   ADD retrieves the configuration from the autosampler.
8. Click **OK** to close the Configure CTC PAL3 LC Sampler dialog and then click **OK** to close the LC Device Configuration dialog.
9. In the Analyst software, activate the hardware profile.

Change the Access Mode to Extended User Level

By default, the PAL3 starts in User Level access mode. The procedures in this guide require that the system be in Extended User Level access mode.

1. On the keyboard, press **A** and **B** simultaneously.

Figure 9-47 Change Access Screen



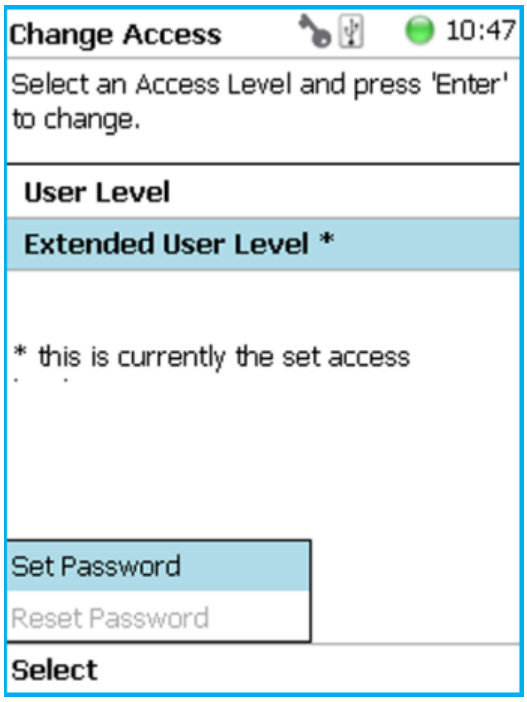
The asterisk indicates that the access mode is set to **User Level**.

2. Select **Extended User Level** and then press **Enter**.

Note: The key icon ( ) indicates the system is in Extended User Level access mode.

Note: If the Remote Terminal window is inactive for 60 minutes, then the software automatically reverts to User Level access mode.

Figure 9-48 Extended Access Level

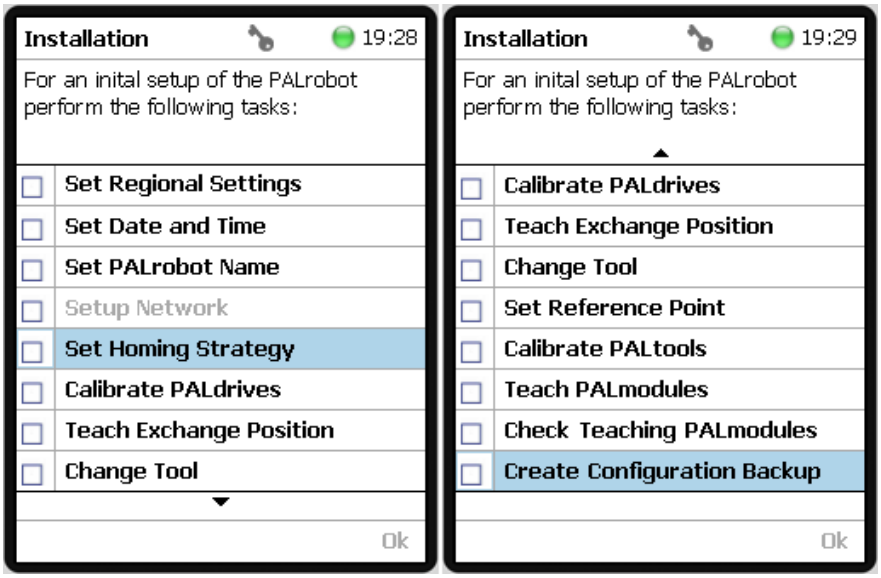


Complete the CTC PAL 3 Installation Wizard

CAUTION: Potential System Damage. Be sure to do all of the steps in the Installation Wizard. The PAL drives and tools must all be calibrated before operation of the system.

1. Turn on the M5 MicroLC system.
2. Remove any racks from the first sample drawer.
3. Start the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.
4. Click **Options** > **Service** > **Installation**.

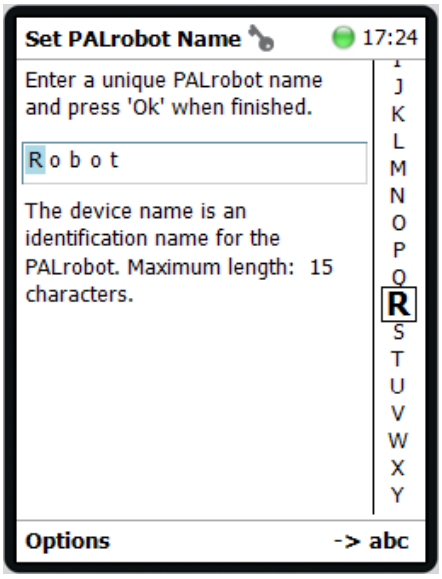
Figure 9-49 Installation Wizard: All Steps Shown



5. Follow the instructions in the Remote Terminal window to do each step of the installation wizard. The Setup Network step is not available and does not need to be performed.

   - To edit a number, press the up and down arrow keys and then press **Enter**. To scroll quickly, hold down the arrow key. To save the value, double-click the field.
   - To edit text, press **Enter** and then use the up and down arrows to scroll through the alphabet. Press **Enter** to select the letter. To save the value, double-click the field.

Figure 9-50 Editing Text in the PAL Virtual Terminal Software



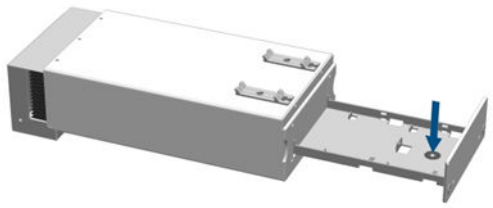
6. At the **Set Homing Strategy** step, accept the default parameters.
7. At the **Teach Exchange Position** step, manually move the Z-arm forward, away from the system, and then down.

   Select a location with convenient access to the black latch that attaches the LCMS tool to the Z-arm.

   CAUTION: Potential System Damage. Move the Z-arm in a controlled manner until it reaches the reference or teaching position. Do not hold the needle guide to move the Z-arm. This part is mechanically flexible and might become misaligned.
8. At the **Change Tool** step, accept the default parameters.
9. At the **Set Reference Point** step, open the top sample drawer, move the Z-arm to the reference position, and then click **Save**. Write down the coordinates of the reference position.

   The reference position is identified with a lunette, a disc with two concentric rings. The lower needle guide should touch the drawer.

   Figure 9-51 Reference Position (Only Top Drawer is Shown)



10. At the **Calibrate PALtools** step, move the Z-arm to the reference position and then click **Save**.
11. Fine tune the reference position by following these steps.
    a. Press **Enter**.
    b. Click **X**, press **Enter**, and then edit the value to match the value recorded in step 9. Press **Enter** to save the value.
    c. Do step 11.b again to edit the values for Y and Z so that they match the values recorded in step 9.
    d. Click **Next**.
12. At the **Teach PAL Modules** step, move the Z-arm to the teaching position for each module. As required, click **Next** or **Save** to go to the next step.

There are three modules: Injector LC 1, Peltier Stack 1, and LCMS Wash 1.

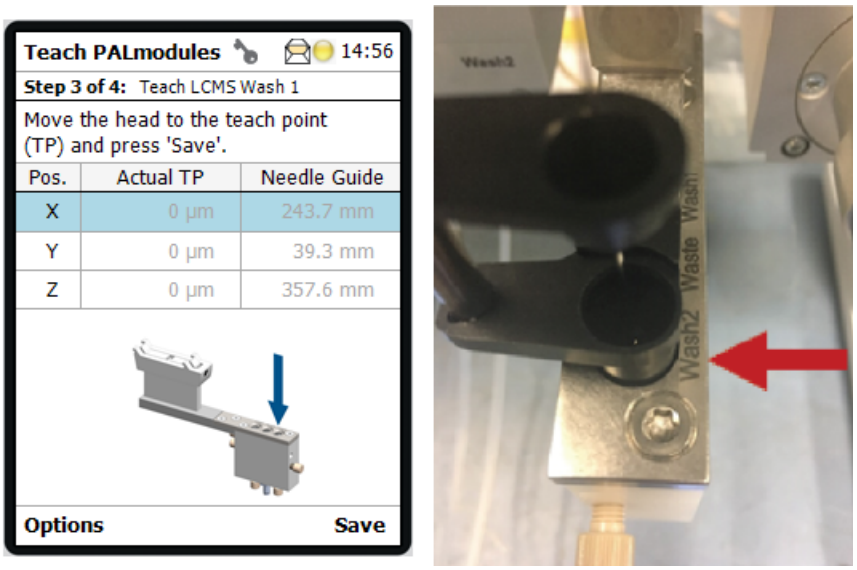
a. For **Step 1**, Injector LC 1, move the Z-arm to the injection port on the valve. The lower needle guide should touch the injection port.

b. For **Steps 2 and 3**, Peltier Stack 1, the teaching position is the same as the reference position. Fine tune it by repeating step 11.

c. For **Step 4**, LCMS Wash 1, move the Z-arm to Wash2, the wash station port that is farthest from the system. The lower needle guide should touch the injection port.

Note: When teaching the LCMS Wash 1, do not confuse the tool identifier, as shown in the wizard, and the physical port number. In the following example, the tool is LCMS Wash 1, and the port is Wash2. Use Wash2 as the teaching point. If Wash1 is used, then the wash cycle will not execute correctly, and the wash will be bypassed.

Figure 9-52 Teaching the LCMS Wash



13. For the **Check Teaching PALmodules** step, visually examine the location of each module to make sure that the lower needle guide is directly over the teaching position. As required, click **Next** or **Save** to go to the next step.

14. Back up the settings. Refer to the section: Create Configuration Backup.

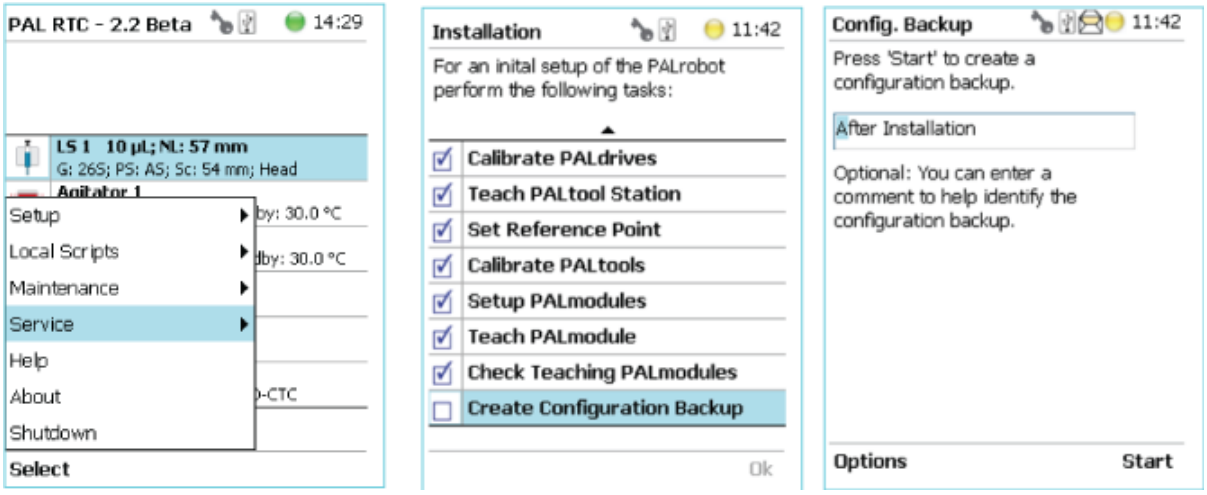
Create Configuration Backup

CAUTION: Potential System Damage. Do not turn off the power to the M5 MicroLC system. If the power is interrupted during this process, the boot commands might become corrupted. If this happens, the system can no longer be started. The autosampler control board will have to be returned to the factory, so that the boot loader can be reconfigured.

1. Select **Options** > **Service** > **Installation Wizard** or **Create Configuration Backup**.

   Tip! Alternatively, a backup can be created with the **Options** > **Maintenance** > **Create Diagnostic Backup** or **Create Configuration Backup** option.

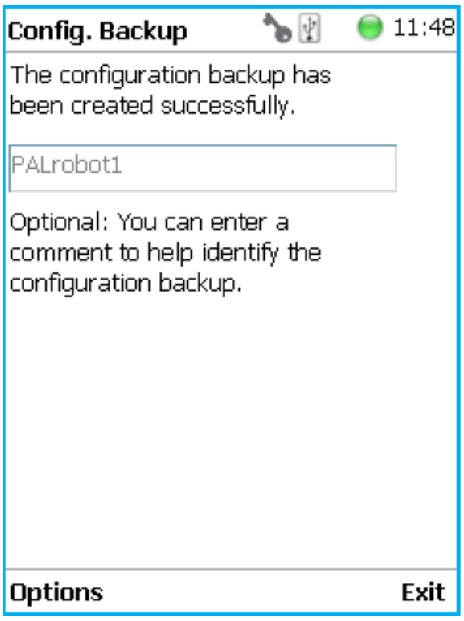
Figure 9-53 Create Backup



2. Press **Start** to start the process.

   The blue LED blinks shows that the backup is in process. The backup takes several minutes. When it is complete, a message is shown.

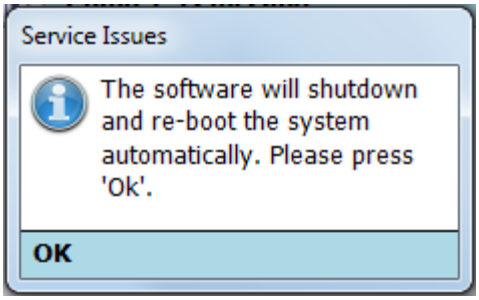
Figure 9-54 Backup Complete Message



Restore the CTC PAL 3 Autosampler Settings

1. Start the PAL Virtual Terminal software, and then make sure that **Access level** is set to **Extended User**. Refer to the section: Change the Access Mode to Extended User Level.
2. Click **Options** > **Maintenance** > **Restore Backup**.
3. At the prompt to remove the USB flash drive, click **Yes** to restart. Wait until the system restarts. This can take several minutes.

Figure 9-55 Prompt to Restart



4. Start the PAL Virtual Terminal software and make sure that the status icon is green and the envelope icon is not shown.

   If the envelope icon is shown, then click **Options** > **Pending Messages** or **Options** > **Service Messages**, and then follow the instructions to resolve the issue.

Figure 9-56 Status Icon

Change the Calibration Method for a SCIEX TripleTOF System with the Analyst Software

For a TripleTOF system with a calibrant delivery system (CDS), the calibration method template must be modified so that the pump will continue to flow during the calibration run on the mass spectrometer. Without this modification, the signal stability on the mass spectrometer will be poor. This modification is initially performed by the SCIEX FSE during the installation of the system.

The calibration method template might need to be modified if:

- The Analyst TF software has been reinstalled or a different computer is connected to the system.
- The column or other system plumbing does not support a 40 µL/min flow rate.
- The initial mobile phase composition for the run is very different from that for the example experiment.
- The flow rate of the CDS is more than 500 µL/min.

Update the Calibration Method Template with the Analyst Software

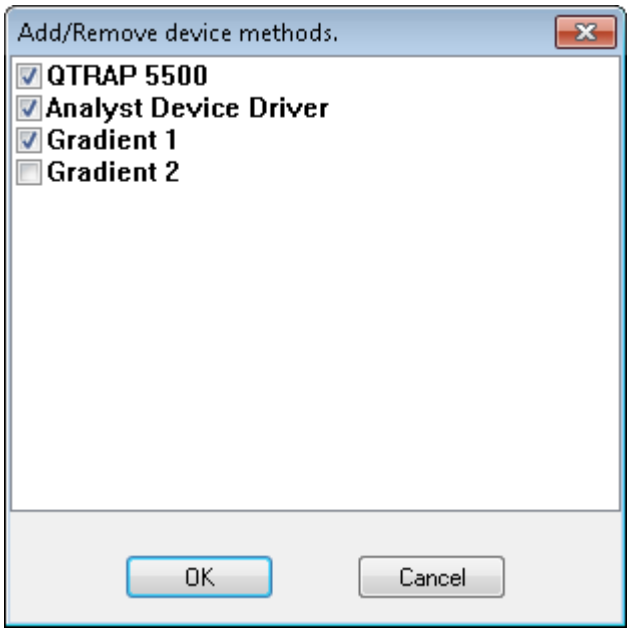
Add the LC device and the LC method to the calibration method template.

1. Back up the `AutoCalPos.dam` file in a safe location.
   By default, the file is found in `D:\Analyst Data\Projects\API Instrument\Instrument Optimization\methods`.
2. Add the LC device to the AutoCalPos method in the Analyst TF software.
   a. Activate the hardware profile that includes the M5 MicroLC system.
   b. In Windows Explorer, browse to the location of the AutoCalPos.dam file.
      By default, the file is found in `D:\Analyst Data\Projects\API Instrument\Instrument Optimization\methods`.
   c. Double-click the `AutoCalPos.dam` file to open it in the Acquisition Method Browser Editor.
   d. Right-click **Acquisition Method** and then select **Add/remove device**.

The Add/Remove device methods dialog opens.

e. Select **Gradient 1** and then click **OK**.

Figure 9-57 Add/Remove device methods Dialog: M5 MicroLC-TE System



3. Select the LC method for the Gradient 1 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 1**.
   b. Click ... (Browse) to show the available LC methods.
   c. Click the name of the method that was created previously, and then click **Open**.
4. Save the calibration method with the same name (`AutoCalPos`) in the original location.

Best Practices 10

This chapter contains best practices for using the M5 MicroLC systems.

Guidelines for Sample Preparation

Sample preparation methods commonly used for conventional HPLC are suitable for micro LC, but the flow path can become blocked if samples contain too much particulate matter.

For best results, follow these guidelines:

- Use HPLC- or LCMS-grade solvents at all times.
- Avoid the use of non-volatile salts and buffers such as CHAPS, phosphate, TRIS, HEPES, and perchlorates. These additives foul the ion source and the mass spectrometer orifice.
- Avoid overloading the column, and the trap column, if present, with sample.
    - For 0.3 mm and 0.5 mm i.d. columns: Use <12 µg of material
    - For 1 mm i.d. columns: Use <50 µg of material
- If required, use a centrifuge to spin all of the samples at 10,000 RPM for 5 minutes to remove dust and particulates from the sample solution. Use the supernatant as the sample.
- Add a guard column before the analytical column to protect the analytical column from impurities in the sample. Guard columns can be purchased from SCIEX.

Work with PEEK-clad Fused Silica Tubing

- Never cut PEEK-clad fused silica tubing.

  Cutting PEEK-clad fused silica tubing results in small particles of cut glass entering the flow path, leading to blocked tubing, valves, and electrodes.
- For all connections, seat the tubing at the bottom of the fitting.
- When connecting PEEK-clad fused silica tubing, do this:

  1. Connect the tubing on the end farther from the mass spectrometer first.
  2. Turn on the pump and let liquid flow through the tubing to flush out any particulate matter.
  3. Let liquid flow for approximately 30 seconds before making the next connection.
- Do not overtighten connections to PEEK-clad fused silica tubing.

  Overtightening can cause blockage and damage to the tubing. Instead, tighten fittings until they are finger tight, turn on the pump, and then examine the fitting for the presence of

solvent. If there is a leak, tighten the fitting about 1/16 turn at a time until there are no more leaks.

- When connecting the fitting on a SecurityLINK tubing, tighten until the first audible click.

Guidelines for Micro HPLC Methods

The smaller columns used in micro high performance liquid chromatography (HPLC) require lower flow rates, smaller injection volumes, and different electrodes and tubing than those used in traditional HPLC.

Flow Rate

When converting a method from traditional HPLC to micro HPLC, keep the flow velocity the same so that retention times do not change.

Flow velocity, FV, is given by FV = Q / A, where:

Q = flow rate

A = cross-sectional area of the column

The following table shows how flow rate varies by column diameter for (approximately) the same flow velocity. Refer to the table: Table 10-1.

For other column diameters or other flow rates, a general guideline is that flow rate scales with the square of the column diameter.

Table 10-1 Equivalent Flow Rates for Micro HPLC

Column Diameter (mm)	Flow Rate (µL/min)	Cross-sectional Area (mm^2)
Traditional HPLC		
4.6	2,100	16.62
2.1	450	3.46
Micro HPLC		
1.0	100	0.785
0.5	25	0.196
0.3	10	0.071
0.2	5	0.031

Flush the Electrode at the End of the Batch

The smaller diameter electrodes used for micro HPLC can become blocked. To decrease the risk of this occurring, add a sample at the end of the acquisition batch to flush the electrode.

1. Create an LC method to flush the electrode.
2. Create an acquisition method that includes the LC method to flush the electrode.
3. Load a vial containing a 50:50 mixture of Mobile Phase A and Mobile Phase B in the cooled sample drawer.
4. Add the sample to the batch, assigning the acquisition method.

Troubleshooting 11

Troubleshooting Steps

1. Step back and look at the overall system. Is something obvious causing the problem? For example, is the instrument disconnected from the mains supply, or incorrectly connected?
2. Compare the current system operation with the way the system operated before the problem started. Identify conditions such as pressures, power settings, or flow rates that are different from when the system was operating normally.
   For example, if the output pressure is usually 2,500 psi for a method, is the system pressure currently in the same range, or drastically higher or lower?
3. In the following order, identify any symptoms which vary from normal system operation:
   a. System power on and initialization (initialization fails)
   b. System diagnostics, such as flow stability
   c. Flow rate in each channel (high, low, erratic)
   d. Output pressure (high, low, erratic)
4. For each symptom, refer to the section: Troubleshooting Tables and then do the applicable corrective actions.
   If this process does not correct the problem, then contact SCIEX Technical Support at sciex.com/request-support.

Troubleshooting Tables

System Initialization

Table 11-1 System Initialization

Symptom	Possible Cause	Corrective Action
The power button on the front of the system is not illuminated.	1. The mains supply cable is not connected. 2. There is no power at the outlet. 3. The light failed but the system response is OK. 4. The power button on the back of system is in the off position. 5. The power button on the front of the system is in the off position.	1. Make sure that the cable is connected to the system and the electrical outlet. 2. Repair the electrical outlet. 3. Contact SCIEX Technical Support at sciex.com/request-support. 4. Press the power button on the back of the system. 5. Press the power button on the front of the system.
The No Instrument Detected dialog is shown or the Not connected message is shown in the Acquisition window.	There is a communication error between the computer and the LC system.	1. Do the following: a. Make sure that the instrument USB cable between the system and the computer is tightly connected to the USB port. Restart the computer and cycle the power on the system. b. Make sure that the IP address of the autosampler is correct. Refer to the section: Assign the IP Address to the Autosampler. If the address is not correct, then assign the COM port with a lower number. c. Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-1 System Initialization (continued)

Symptom	Possible Cause	Corrective Action
A loud hissing sound is coming from the instrument.	1. There is an air leak from the inlet fitting.	1. Do the following: a. Make sure that the air tubing is correctly connected to the gas fitting. b. Tighten the air inlet gas fitting.
The hardware profile in the Analyst software is not activated.	The hardware profile and the system hardware do not match.	Retrieve the configuration from Analyst Device Driver (ADD) software. If the configuration does not exist, then delete the hardware profile and create a new one. Refer to the section: Create a Hardware Profile with the Analyst Software.
The Analyst Device Driver (ADD)window does not close when a hardware profile is deactivated.	The ADD service is still in operation.	1. Do the following: a. Close the Analyst software. b. Click **Control Panel** > **All Control Panel Items** > **All Control Panel Items** > **Administrative Tools** > **Services**. c. Click **AnalystService** and then click **Stop**. d. Click **AnalystDeviceDriverService** and then click **Stop**. e. Click **AnalystDeviceDriverService** and then click **Restart**. f. Open the Analyst software. If the problem continues, then turn the system off and on.

Valves

Table 11-2 Valves

Symptom	Possible Cause	Corrective Action
The injection valve does not change positions.	1. The valve is not configured in the Eksigent Control software. 2. The valve is not connected to the actuator. 3. The actuator is defective. 4. The LC Method is not correct.	1. In the Eksigent Control software, click **System** > **Instrument Configuration** to open the Instrument Configuration dialog. Open the System tab and then select **Eksigent Internal** in the **Injection Valve** list. 2. Contact SCIEX Technical Support at sciex.com/request-support. 3. Contact SCIEX Technical Support at sciex.com/request-support. 4. Review the LC method.
The auxiliary valve does not change positions.	1. The valve is not configured in the Eksigent Control software. 2. The electronics are defective. 3. The actuator is defective. 4. The LC Method is not correct.	1. In the Eksigent Control software, click **System** > **Instrument Configuration** to open the Instrument Configuration dialog. Open the System tab and then select **Eksigent Internal** in the **Injection Valve** list. 2. Contact SCIEX Technical Support at sciex.com/request-support. 3. Contact SCIEX Technical Support at sciex.com/request-support. 4. Review the LC method.

Table 11-2 Valves (continued)

Symptom	Possible Cause	Corrective Action
No flow comes out of the port.	1. The valve is not plumbed correctly. 2. The ports are blocked.	1. Make sure that the plumbing configuration is correct. If required, then reconfigure the plumbing. Refer to the section: Plumb the Injection Valve. 2. Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, then contact SCIEX Technical Support at sciex.com/request-support.
Fluid is leaking from the valve.	1. A ferrule is not correctly seated in the port. 2. The rotor seal has scratches.	1. Examine the tubing connection and make sure that the ferrule is correctly seated. 2. Replace the rotor seal. Refer to the section: Install the Valve Rotor Seal.
When no column is connected, the system pressure (Pc) is unusually high.	1. The ports are blocked. 2. The ends of the tubing are damaged. 3. The outlet union is blocked.	1. Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, then contact SCIEX Technical Support at sciex.com/request-support. 2. Replace the tubing. Do not overtighten the fittings. 3. Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-2 Valves (continued)

Symptom	Possible Cause	Corrective Action
The system does not initiate an injection.	1. The system flow is not stable. 2. The flow stabilization is set too low. 3. The autosampler is configured to wait for injection but the **Sample Injection** setting in the Eksigent Control software is **None**.	1. Purge and flush the pump. Refer to the section:Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. After the pump is flushed, equilibrate the system. 2. In the Eksigent Control software, click **System** > **Instrument Configuration** to open the Instrument Configuration dialog. Open the Advanced tab and then set the flow stabilization limit to >100 nL/min. 3. In the LC Method Settings dialog, change **Sample Injection** to a value other than **None**.

Table 11-2 Valves (continued)

Symptom	Possible Cause	Corrective Action
The system does not initiate an injection (continued).	1. The column oven is not at the specified temperature. 2. After a module on the autosampler was replaced, the acquisition methods were not opened and saved.	1. Do the following: • In the Direct Control dialog, decrease the required temperature for the column oven. Refer to the section: Let the Column Oven Pre-Heat or Turbo V, DuoSpray, and IonDrive Turbo V Ion Sources . • Monitor the column oven temperature in the upper right corner of the Acquisition window in the Eksigent Control software and wait until the oven gets to the specified temperature. If the column oven does not get to the specified temperature within 15 minutes, then the column oven might be defective. Contact SCIEX Technical Support at sciex.com/request-support. 2. If a module is replaced on the autosampler, then each acquisition method must be saved again in the Analyst Device Driver (ADD) software. Refer to the section: Configure the LCMS Tool Pumps with the Analyst Software.

Table 11-2 Valves (continued)

Symptom	Possible Cause	Corrective Action
The flow rate is inconsistent.	1. The valve has an internal leak. 2. The ports are blocked. 3. The outlet union is blocked.	1. Replace the rotor seal. Refer to the section: Install the Valve Rotor Seal. If the issue continues, then contact SCIEX Technical Support at sciex.com/request-support. 2. Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, then contact SCIEX Technical Support at sciex.com/request-support. 3. Contact SCIEX Technical Support at sciex.com/request-support.
• The pressure decreases at the start of each run. • The relative standard deviation (RSD) between peak areas for successive runs is high.	The sample loop contains air bubbles.	In the Sample Table in the Batch Editor window, set an **Inj. Volume (µL)** that is more than the volume of the sample loop to make sure that the sample loop is completely filled with sample.
For a trap-and-elute experiment, there is carryover from one run to the next.	The sample loop retains a small amount of the sample.	In the LC Method Settings dialog, edit the Trap Loading Method to add a step that washes the sample loop with the organic mobile phase between injections.

Column Oven

Table 11-3 Column Oven

Symptom	Possible Cause	Corrective Action
The column responds very slowly when changing temperature.	1. The oven is malfunctioning. 2. An invalid temperature is entered in the software.	1. Contact SCIEX Technical Support at sciex.com/request-support. 2. Enter a value between room temperature + 5 °C and 80 °C in the software.
The temperature reads 47.	The oven is not connected to the mains supply.	Make sure that the column oven is connected to the mains supply.

Autosampler

Table 11-4 Autosampler

Symptom	Possible Cause	Corrective Action
The sample drawers are occasionally damp.	The sample drawers contain condensation.	If the dampness is intermittent, then open the drawers 1 cm to 5 cm to let air circulate when the system is not in use.
The sample drawers are continuously damp.	The sample drawers contain condensation.	If the dampness is continuous, then blow clean, dry air or nitrogen through the sample drawers. Connect the gas line to the 1/8 inch fitting labeled **Flush Gas**, at the bottom right on the back of the system. Let the gas flow at 300 mL/min to 400 mL/min.
Peak areas are not reproducible from run to run.	The incorrect sample volume is aspirated because the syringe needle is too close to the bottom of the vial.	1. Do the following: a. Click **Method** in the Analyst Device Driver (ADD) window to open the Instrument Control Method Editor window. b. Make sure that the value for **Height from Bottom of Sample Vial** is more than or equal to 2 mm.

Table 11-4 Autosampler (continued)

Symptom	Possible Cause	Corrective Action
The CTC PAL 3 Virtual Terminal window is unresponsive.	1. The IP address for the Remote Terminal window is incorrect. 2. The IP address for the LAN connection to the autosampler is incorrect. 3. The Ethernet cable between the M5 MicroLC system and the acquisition computer is not connected. 4. The autosampler initialization did not complete.	1. Do the following: a. Close the Remote Terminal window and then double-click the **PAL VT** icon to start the software. b. In the Connecting Remote Terminal dialog, type `192.168.99.230`. 2. Open the CTC PAL 3 Properties dialog and then make sure that the IP address is 192.168.99.231. Refer to the section: Assign the IP Address to the Autosampler. 3. Connect the cable. 4. Do the following: a. Close the Remote Terminal window. b. Turn the M5 MicroLC system power off and then on. Wait for the movement of the Z-arm to stop. c. Double-click the **PAL VT** icon to start the software.
The autosampler script is missing from the **Script** list in the Instrument Control Method Editor window.	The autosampler script is missing from Analyst Device Driver (ADD)software.	Import the script again using the Script Manager in ADD. Refer to the section: Map the Rack Positions and Import the Autosampler Scripts with the Analyst Software.
Parameters for the autosampler cannot be edited in the Remote Terminal window.	The access mode is set to **User Level**.	Change the access mode to Extended User Level. Refer to the section: Change the Access Mode to Extended User Level.

Table 11-4 Autosampler (continued)

Symptom	Possible Cause	Corrective Action
When the Z-arm stops moving at start up, it is located at the left side of the sample drawers instead of the home position above the wash station.	The autosampler initialization did not complete.	1. Do the following: a. Close the Remote Terminal window. b. Turn the M5 MicroLC system power off and then on. Wait for the movement of the Z-arm to stop. c. Double-click the **PAL VT** icon to start the software. d. If the problem continues, then complete the CTC PAL 3 installation wizard. Refer to the section: Complete the CTC PAL 3 Installation Wizard.

Pumps

Table 11-5 Pumps

Symptom	Possible Cause	Corrective Action
The system pressure (Pc) or pump pressures (Pa and Pb) show pressure but flow is off.	The zero setting for the pressure sensors is incorrect.	Initialize the pressure transducers. Refer to the section: Re-initialize the Pressure Transducers with the Analyst Software or Re-initialize the Pressure Transducers with SCIEX OS.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
No liquid comes out of the waste tubing during a purge.	1. The pump contains trapped air. 2. The internal filters are blocked. 3. There is a leak in the system before the purge valve.	1. Purge and flush the pump. Refer to the section:Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. 2. Purge the pumps and inspect the flow through the waste tubing after approximately eight purges. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. If the flow is very low or intermittent, then the filter should be changed. Contact SCIEX Technical Support at sciex.com/request-support. 3. Contact SCIEX Technical Support at sciex.com/request-support.
The pump restrokes frequently (`Pump has reached end of stroke` error message is shown).	1. The pump stays on long enough to cause a restroke. 2. The check valve leaks.	1. For the current flow rate, calculate the time to pump approximately 600 µL. Make sure that the pump re-strokes at approximately that time interval. 2. Contact SCIEX Technical Support at sciex.com/request-support.
The pump does not refill at the end of a run.	1. Operation of the optical sensor is not correct.	1. Contact SCIEX Technical Support at sciex.com/request-support.
The pump flushes quickly but does not deliver approximately 600 µL per stroke.	The system has a leak.	Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The purge output drips slowly.	The internal filters are blocked.	Purge the pumps and inspect the flow through the waste tubing after approximately eight purges. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. If the flow is very low or intermittent, then the filter should be changed. Contact SCIEX Technical Support at sciex.com/request-support.
The flow rate is 0 with 100% power indicated. The system pressure (Pc) and the pump pressures (Pa and Pb) are all low.	1. There is no gas supplied to the system. 2. The system was not correctly purged and flushed.	1. Connect 100 psi clean, dry air or nitrogen to the instrument gas inlet. 2. Purge and flush the pump. Refer to the section:Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS.
The system responds slowly when the flow rates are changed.	1. The mobile phases are incorrect. 2. The pump controller is out of tune. 3. There is no flow through the flow module.	1. Verify the settings in the Mobile Phases dialog. 2. Contact SCIEX Technical Support at sciex.com/request-support. 3. Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The system does not get to the specified flow rate.	1. The internal filters are blocked. 2. The flow rate is too high for the system back pressure. 3. The gas pressure is too low. 4. The system does not get to the required flow rate within the specified tolerance. 5. The system has a leak.	1. Purge the pumps and inspect the flow through the waste tubing after approximately eight purges. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. If the flow is very low or intermittent, then the filter should be changed. Contact SCIEX Technical Support at sciex.com/request-support. 2. In the Direct Control dialog, decrease the flow rate. 3. Make sure that the gas supply has a pressure of 100 psi. 4. Increase the flow stabilization limit on the Advanced tab of the Instrument Configuration dialog of the Eksigent Control software. 5. Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The flow rate does not initialize at the start of the run.	1. The system has a leak. 2. The internal filters are blocked. 3. The flow rate stabilization is set too low.	1. Contact SCIEX Technical Support at sciex.com/request-support. 2. Purge the pumps and inspect the flow through the waste tubing after approximately eight purges. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. If the flow is very low or intermittent, then the filter should be changed. Contact SCIEX Technical Support at sciex.com/request-support. 3. Increase the flow stabilization limit on the Advanced tab of the Instrument Configuration dialog of the Eksigent Control software.
The flow rate is incorrect but there are no signs of leakage.	1. The mobile phases are incorrect. 2. The k-values are incorrect.	1. Verify the settings in the Mobile Phases dialog. 2. Calibrate the flowmeters. Refer to section: Calibrate the Flowmeters with the Analyst Software or Calibrate the Flowmeters with SCIEX OS.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The flow rate does not stabilize during a run.	1. Tubing or a fitting is blocked. 2. The pump contains trapped air. 3. The mobile phases are incorrect. 4. The pump controller is out of tune. 5. The column temperature is not stable.	1. From as far downstream as possible from the pump outlet, remove each tube or fitting, one at a time, until the pressure decreases dramatically. Replace the blocked item. Refer to the section: Test the Fluid Connections with the Analyst Software or Test the Fluid Connections with SCIEX OS. 2. Purge and flush the pump. Refer to the section:Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. 3. Verify the settings in the Mobile Phases dialog. 4. Contact SCIEX Technical Support at sciex.com/request-support. 5. Do the following: a. Monitor the column oven temperature in the upper right corner of the Acquisition window in the Eksigent Control software. b. If the temperature fluctuates more than 2 °C, then contact SCIEX Technical Support at sciex.com/request-support.
The system pressure (Pc) is unusually low but the flow rate is OK.	There is a loose connection after the mixing Tee.	Examine all connections for leaks.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The system pressure (Pc) is low and the flow rate is OK but the pump pressures (Pa and Pb) are high.	1. The k-values are incorrect. 2. A flow module is blocked.	1. Calibrate the flowmeters. Refer to section: Calibrate the Flowmeters with the Analyst Software or Calibrate the Flowmeters with SCIEX OS. 2. Contact SCIEX Technical Support at sciex.com/request-support.
The system pressure (Pc) is very high.	1. Tubing or a fitting is blocked. 2. The trap column is blocked.	1. From as far downstream as possible from the pump outlet, remove each tube or fitting, one at a time, until the pressure decreases dramatically. Replace the blocked item. Refer to the section: Test the Fluid Connections with the Analyst Software or Test the Fluid Connections with SCIEX OS. 2. Replace the trap column.
The flow noise is excessive.	1. The pump contains trapped air. 2. The pump controller is out of tune.	1. Purge and flush the pump. Refer to the section:Purge the Mobile Phases and Flush the System with the Analyst Software or Purge the Mobile Phases and Flush the System with SCIEX OS. 2. Contact SCIEX Technical Support at sciex.com/request-support.
The measured flow does not follow the flow profile.	1. The pump controller is out of tune.	1. Contact SCIEX Technical Support at sciex.com/request-support.

Table 11-5 Pumps (continued)

Symptom	Possible Cause	Corrective Action
The pump pressures (Pa and Pb) are maximized to < 12,000 psi at 100% pump power.	1. The gas pressure is too low. 2. The zero setting for the pressure sensors is incorrect. 3. The gain setting for pressure is incorrect.	1. Make sure that the gas supply has a pressure of 100 psi. 2. Initialize the pressure transducers. Refer to the section: Re-initialize the Pressure Transducers with the Analyst Software or Re-initialize the Pressure Transducers with SCIEX OS. 3. On the Calibration Values tab of the Hardware Diagnostics dialog, make sure that the pump pressures (in the **Scale Parameter** field) are approximately 2,800 psi/V. If the value is not 2,800, then contact SCIEX Technical Support at sciex.com/request-support.

Test the Fluid Connections with SCIEX OS

CAUTION: Potential Operator Injury. To prevent the possibility of solvent exposure, make sure sure to have a vial or other container available to collect the solvent that comes from the system.

1. Disconnect all of the exits in the flow path except for the tubing that comes from the G1 pump.
2. In the Direct Control dialog, set the **% B** to 20% (acetonitrile), and set the **Total flowrate** for the specified system configuration.
   - For a low-flow system, type `5 µL/min`.
   - For a micro-flow or high-flow system, type `40 µL/min`.
3. Click **Start**.
4. Use the following table to calculate the approximate pressure for the items in the flow path. Initially, the only component is the 10 cm of 50 µm i.d., 1/32 inch o.d. tubing that comes from the pump.

   Table 11-6 Approximate Pressure Changes for Tubing and Other Components at 40 µL/min

Item	Pressure (psi)
5 µL sample loop	0
50 µL sample loop	0
Gray PEEKsil tubing, 50 µm i.d., 1/32 inch o.d., 10 cm	60
Gray PEEKsil tubing, 50 µm i.d., 1/32 inch o.d., 20 cm	120
Orange PEEKsil tubing, 25 µm i.d., 1/32 inch o.d., 10 cm	1,000
SecurityLINK tubing, 50 µm i.d, 15 cm	100
SecurityLINK tubing, 50 µm i.d, 75 cm	475
SecurityLINK tubing, 50 µm i.d, 100 cm	650

   Note: The composition of the mobile phase controls the calculated pressures in the table: depend on the mobile phase composition. For mobile phases other than 80% A (water):20% B (acetonitrile), the values in the table: Table 11-6 are not correct.

5. Compare the calculated pressure to the system pressure (Pc) in the Direct Control window of the M5 MicroLC device.
   - If the pressure is close to the value, then the system operation is correct.

- If the pressure is higher than the value, then there might be a blockage. Replace the part.
- If the pressure is lower than the value, then there might be a leak. Tighten the connection or replace the part.

6. Stop the flow, and then connect the next item in the flow path.
7. Start the flow again, and then monitor the pressure and compare it to the calculated value.
8. Do this procedure again for the remaining components in the flow path, including the column and the ion source.
9. Stop the flow.

Test the Fluid Connections with the Analyst Software

CAUTION: Potential Operator Injury.To prevent the possibility of solvent exposure, make sure sure to have a vial or other container available to collect the solvent that comes from the system.

1. Disconnect all of the exits in the flow path except for the tubing that comes from the G1 pump.
2. In the Direct Control dialog, set the flow conditions to 80% A (water):20% B (acetonitrile), and set the **Total flowrate** for the specified configuration.
   - For a low-flow system, type `5`.
   - For a micro-flow or high-flow system, type `40`.
3. Click **Start**.
4. Use the following table to calculate the approximate pressure for the items in the flow path. Initially, the only component is the 10 cm of 50 µm i.d., 1/32 inch o.d. tubing coming from the pump.

   Table 11-7 Approximate Pressure Changes for Tubing and Other Components at 40 µL/min

Item	Pressure (psi)
5 µL sample loop	0
50 µL sample loop	0
Gray PEEKsil tubing, 50 µm i.d., 1/32 inch o.d., 10 cm	60
Gray PEEKsil tubing, 50 µm i.d., 1/32 inch o.d., 20 cm	120
Orange PEEKsil tubing, 25 µm i.d., 1/32 inch o.d., 10 cm	1,000
SecurityLINK tubing, 50 µm i.d, 15 cm	100
SecurityLINK tubing, 50 µm i.d, 75 cm	475
SecurityLINK tubing, 50 µm i.d, 100 cm	650

   Note: The composition of the mobile phase controls the calculated pressures in the table: depend on the mobile phase composition. For mobile phases other than 80% A (water):20% B (acetonitrile), the values in the table: Table 11-7 are not correct.

5. Compare the calculated pressure to the actual pressure (Pc) in the Acquisition window of the Eksigent Control software.

- If the pressure is close to the value, then the system operation is correct.
- If the pressure is higher, than the value then there might be a blockage. Replace the part.
- If the pressure is lower than the value, then there might be a leak. Tighten the connection or replace the part.

6. Stop the flow, and then connect the next item in the flow path.
7. Start the flow again, and then monitor the pressure and compare it to the calculated value.
8. Do this procedure again for the remaining components in the flow path, including the column and the ion source.
9. Stop the flow.

Recover if the Analyst Software Does Not Respond

Note: If the SCIEX OS driver is in use, then this does not apply.

If the Analyst software does not respond, then use this procedure to stop any related processes or services.

1. From the desktop, open the Task Manager dialog.
2. Stop the services.
   a. In the Task Manager dialog, open the Services tab.
   b. On the Services tab, right-click **AnalystDeviceDriverService** and then click **Stop**.
   c. Stop the **AnalystService** service.
   d. Stop the **EksigentProcessMonitorService** service.
3. Stop the processes.
   a. Open the Processes tab.
   b. Click **Analyst Device Driver (ADD)** and then click **End Task**.
   c. Stop the **Analyst** process.
   d. Stop the **Eksigent Control** process.
4. Start the services.
   a. Open the Services tab.
   b. Right-click **AnalystDeviceDriverService** and then click **Start**.
   c. Start the **AnalystService** service.
   d. Start the **EksigentProcessMonitorService** service.

5. Close the Task Manager dialog.
6. Open the Analyst software.

Show Pressure and Flow Data in SCIEX OS

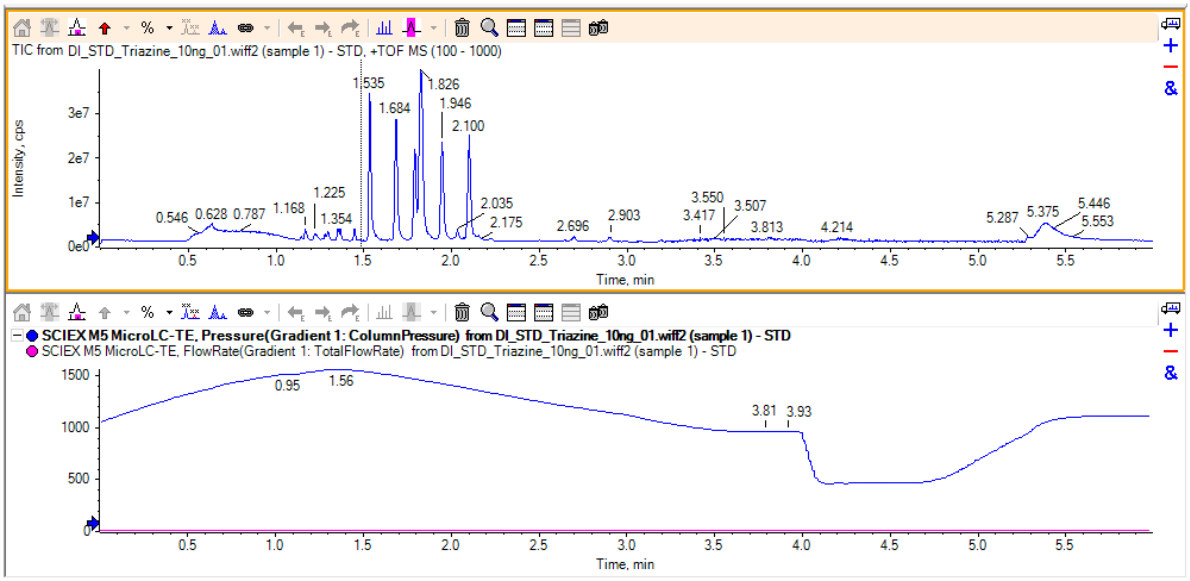
Pressure and flow data is saved to the wiff data file during data acquisition and can be seen in SCIEX OS.

1. Open a sample.
   a. Click the **Explorer** tile.
   b. Click **File** > **Open Sample**.
   The Select Sample dialog opens.
   c. Browse to and then select the sample to be opened.
   d. Click **OK**.
2. Click **Show** > **LC Traces**.
3. In the Select Trace(s) dialog, click the type of data to be shown and then click **OK**.

Tip! Use **Shift+click** or **Ctrl+click** to select multiple traces.

The selected traces open in a pane below the graph.

Figure 11-1 Pressure and Flow Data in SCIEX OS



4. To view the different types of LC trace data, at the top of the pane, click the line for the type of data.

Move the System with SCIEX OS 12

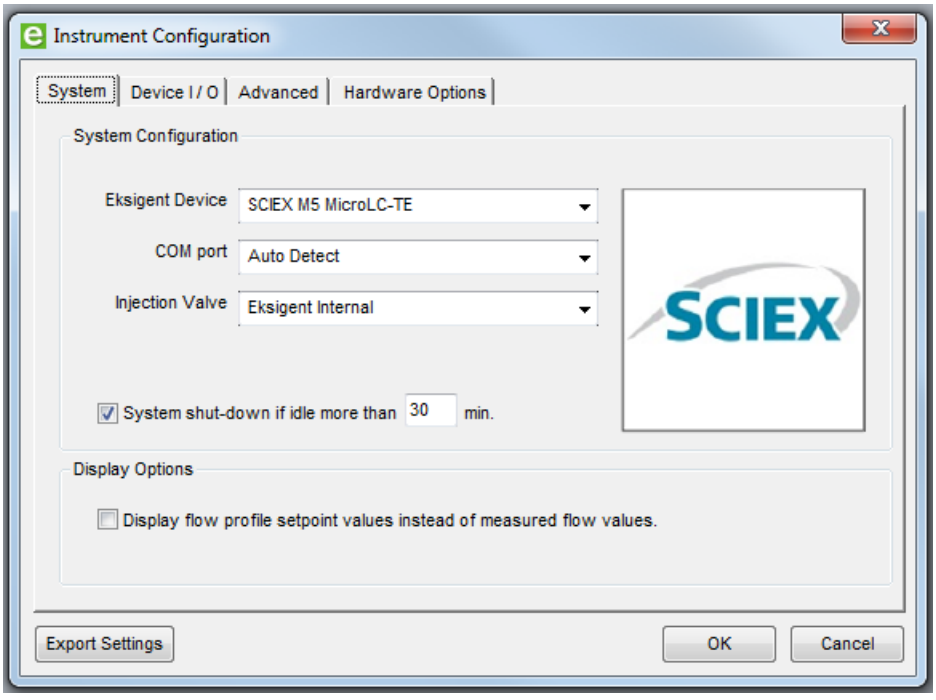
This section identifies how to move the M5 MicroLC system from one location to another. It assumes that the new location includes a mass spectrometer and that the system is on a wheeled cart. Complete disassembly of the system is not required.

Instructions for setting up the system to use a different acquisition computer are also given, as well as instructions for storing the system.

Disconnect the System at the Original Location

1. Export the system settings `reg` file from the current computer and copy them to the USB flash drive.
    a. Start the Eksigent Control software.
    b. Click **System** > **Instrument Configuration**.

Figure 12-1 Instrument Configuration Dialog System Tab



    c. Click **Export Settings** in the lower left corner.

The instrument settings are exported and a dialog showing the location of the backup file opens.

d. Write down the location for use in the following step and then click **OK**.

2. In Window Explorer, copy the `settings` folder, that has the `Eksettings.reg` file from the current computer to the USB flash drive.
   a. Browse to the installation directory.
      - For 32-bit operating systems: `C:\Program Files\Eksigent NanoLC`
      - For 64-bit operating systems: `C:\Program Files (x86)\Eksigent NanoLC`
   b. Copy the `settings` folder to the USB flash drive.
3. Close SCIEX OS.
4. Use the switch on the back of the system to turn off the power to the system, and then disconnect the mains supply cable.
5. Turn off the gas supply.
6. Disconnect the system from the gas.
   - For the M5 MicroLC system: Push in the red plastic ring while pulling out the tubing to remove the gas tubing from the back of the pump.
   - For the M5 MicroLC-TE system: Disconnect the gas supply at the Tee.

   The system is still pressurized, so it might be difficult to remove the tubing.
7. Disconnect the USB cable between the LC system and the acquisition computer.
8. Disconnect the Ethernet cable between the LC system and the acquisition computer.
9. Disconnect the tubing from the column at the ion source.

Install the System at the New Location

WARNING! Lifting Hazard. Make sure that at least four people or a lifting device are available to lift the LC system. Follow established safe lifting procedures. For the weights of system components, refer to the document: *Site Planning Guide*.

WARNING! Crushing Hazard. Wear protective footwear when moving heavy objects.

CAUTION: Potential System Damage. Make sure to use a bench or table that will support the system. If the bench collapses, then the system could be damaged.

1. Carefully move the system to the new location.

2. Connect the USB cable to the port labeled **USB 2.0** on the back of the LC system and to the acquisition computer.
3. Connect the LAN cable to the port labeled **LAN** on the back of the LC system and to an Ethernet port on the acquisition computer.
4. Connect the gas tubing to the pump.
   - For the M5 MicroLC system: Push the tubing straight in the fitting on the back of the pump.
   - For the M5 MicroLC-TE system: Connect the gas supply to the Tee.
5. Connect the gas tubing to a source of gas (clean, dry, compressed air or nitrogen, regulated to 100 psi).
6. Connect the column outlet tubing to the ion source.
7. Connect the system to the mains supply.
8. Connect the mains supply cable, and then use the switch on the back of the system to turn on the power.
9. If the acquisition computer was not moved, then set up the new acquisition computer. Refer to the section: Set Up a New Computer for Use with the System.

Set Up a New Computer for Use with the System

The following procedures describe how to set up a new acquisition computer for use with the M5 MicroLC system.

Note: The SCIEX OS must be installed on the acquisition computer first. Refer to the *SCIEX OS Software Installation Guide*.

Install the Eksigent Control Software and Transfer the Settings

To use the M5 MicroLC system with a different acquisition computer, install the Eksigent Control software on the new computer in the default location, `C:\Program Files (x86)\Eksigent NanoLC`, transfer important files from the existing computer, and then configure the software on the new computer.

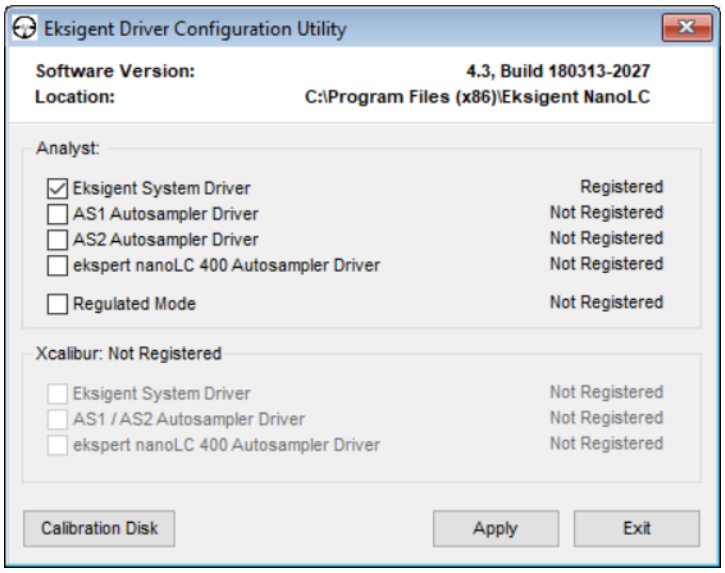
Required Materials
• Eksigent Control software, downloaded from sciex.com/software-support/software-downloads. If the new computer does not have internet access, then save the installer to a USB flash drive. • USB flash drive

Note: The following instructions assume that SCIEX OS is already installed on the new computer.

Note: Use the following procedure to use the same version of the Eksigent Control software on the new acquisition computer. To update the Eksigent Control software as part of the move to a new computer, follow the instructions provided with the software update.

1. Install the Eksigent Control software on the new computer.
    a. Use the switch on the front of the system, to turn off the M5 MicroLC system.
    b. Double-click the downloaded file for the Eksigent Control software and then follow the prompts to install the software.
2. Copy the `settings` folder to the new computer.
    a. Put the USB flash drive into a USB port on the new computer.
    b. Copy the `settings` folder from the USB flash drive to the Eksigent NanoLC folder.
        - For 32-bit operating systems: `C:\Program Files\EksigentNanoLC`
        - For 64-bit operating systems: `C:\Program Files (x86)\EksigentNanoLC`
3. Load the settings from the `EKSettings.reg` file.
    a. From the **Start** menu, select **Eksigent** > **Driver Configuration.** If the User Account Control dialog appears, then click **Yes** to continue.

Figure 12-2 Eksigent Driver Configuration Utility



b. In the Analyst section, select **Eksigent System Driver** and then click **Yes.**

c. To load the settings, click **Calibration Disk** and browse to the `EKSettings.reg` file that is located in the same folder as in step 2.

d. Click **Apply** and then **Exit.**

4. Before using the system, refer to the section: Re-initialize the Pressure Transducers with SCIEX OS.

Assign the IP Address to the Autosampler

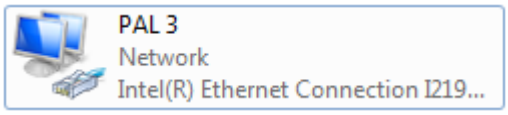
1. Click **Start** > **Control Panel** > **Network and Sharing Center**.
2. Click **Change adapter settings**.
   The Network Connections window opens.
3. (Optional) Rename the connection for easier identification.

   a. Right-click **Local Area Connection X**, where X is a number, and then select **Rename**.

   Tip! To identify the correct Local Area Connection, disconnect the Ethernet cable that comes from the autosampler and then wait a few seconds. The correction connection will show `"Not connected"`. Make sure to connect the Ethernet cable.

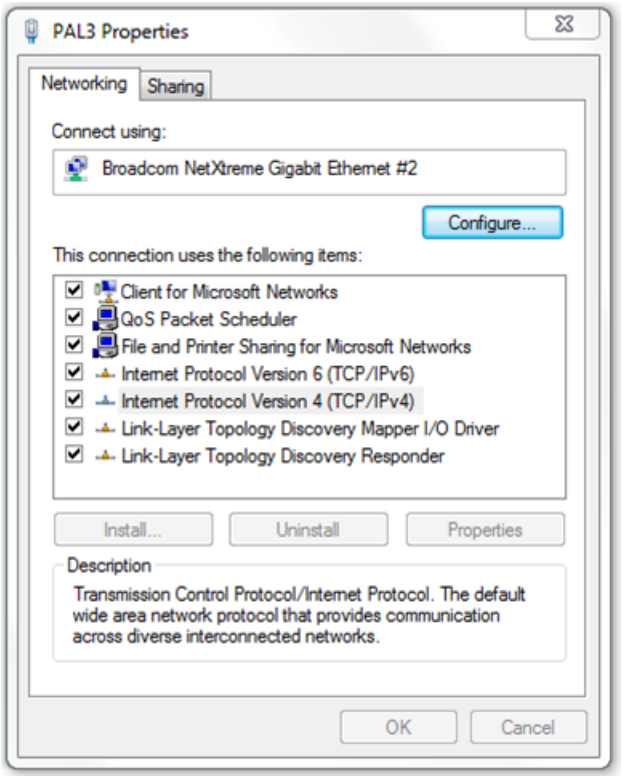
   b. Type `PAL 3` and then press **Enter**.

Figure 12-3 Local Area Connection after Renaming



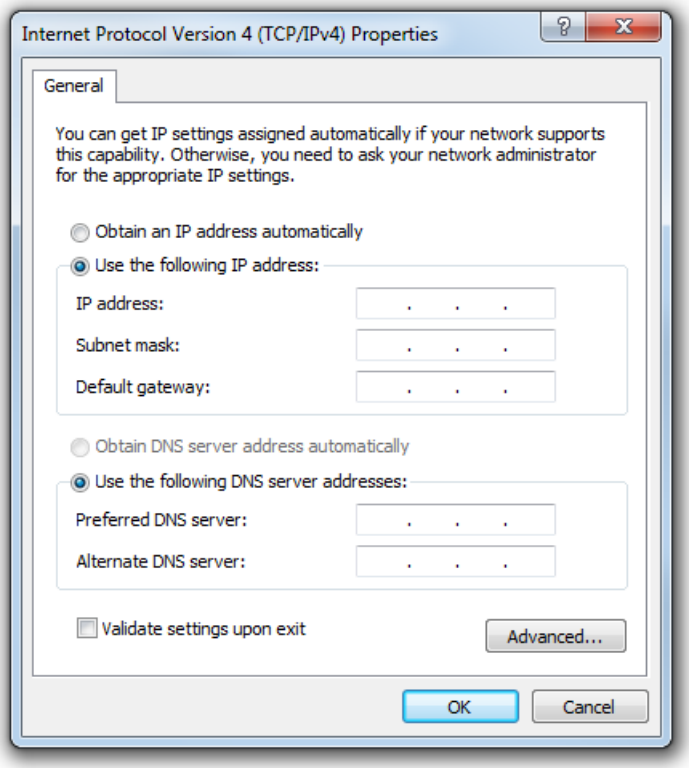
4. Right-click **PAL3** and then select **Local Area Connection** > **Properties** to open the Local Area Connection Properties dialog.

Figure 12-4 PAL3 Properties



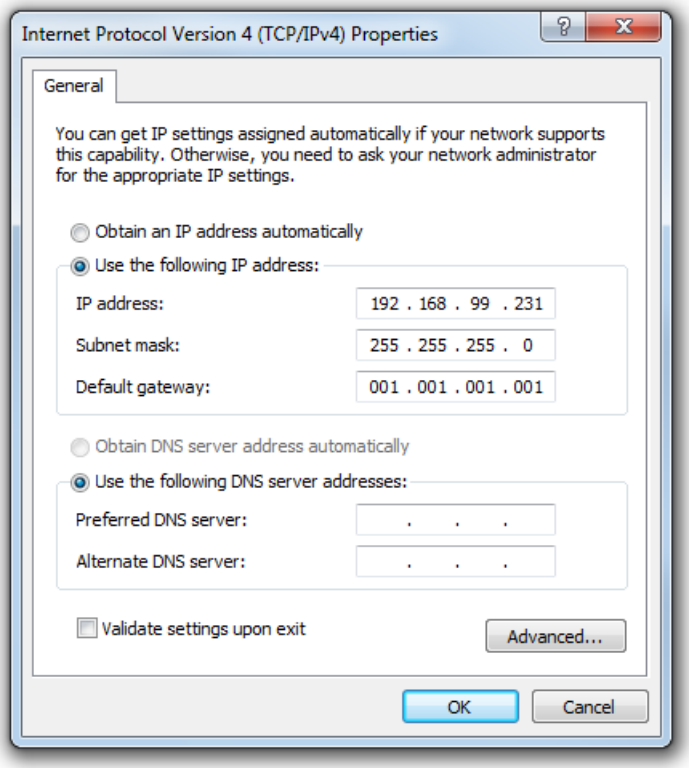
5. On the Networking tab, click **Internet Protocol Version 4 (TCP/IPv4)** and then click **Properties**.

Figure 12-5 Internet Protocol Version 4 (TCP/IPv4) Properties



6. Select **Use the following IP address** and then type the following:
   - For the **IP address**, type `192.168.99.231`.
   - For the **Subnet mask**, type `255.255.255.0`.
   - For the **Default gateway**, type `1.1.1.1`.

Figure 12-6 IP Address



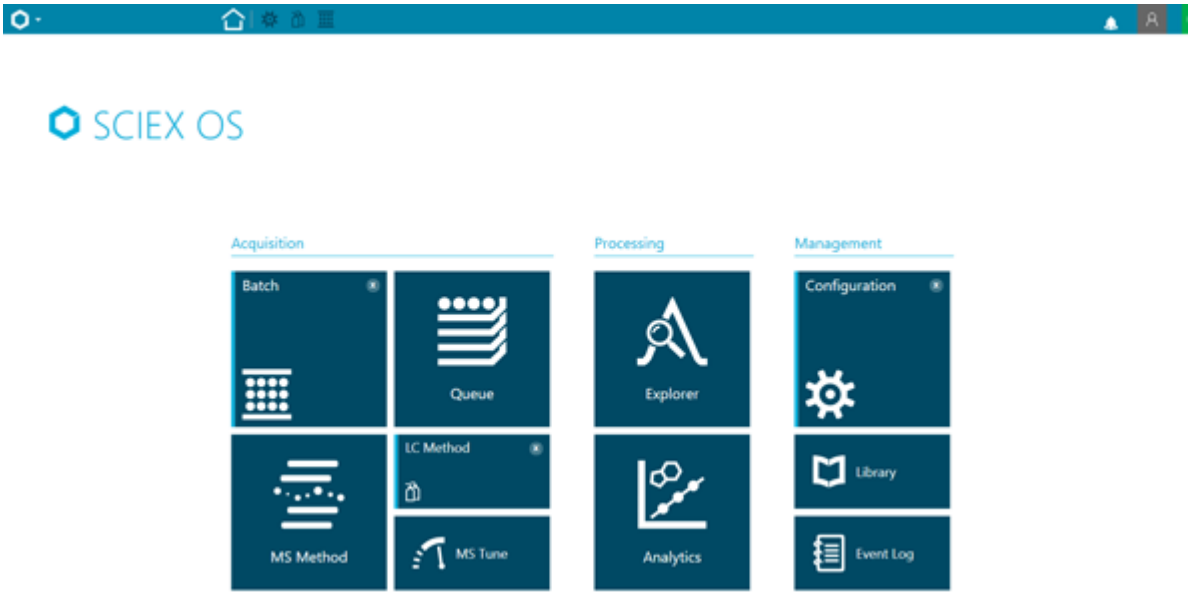
7. Click **OK** and then click **Close**.

Configure the Settings for M5 MicroLC Systems

The active hardware configuration must include the mass spectrometer and the M5 MicroLC system. Add the device if any of the two devices does not already exist.

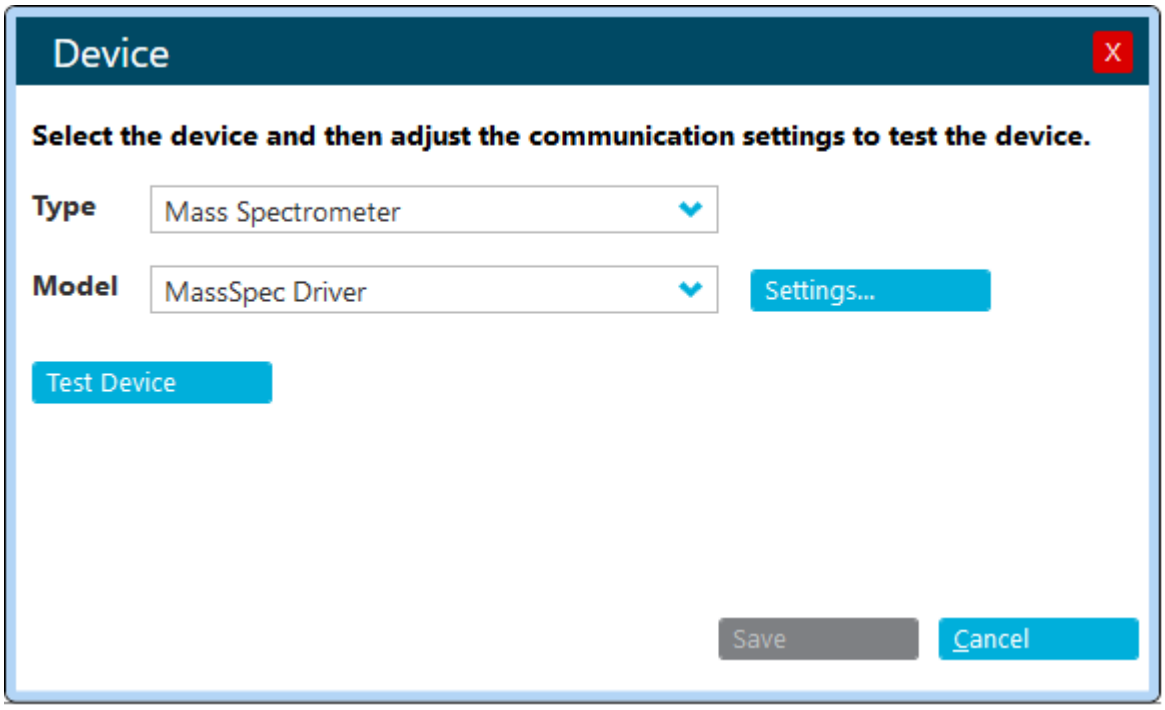
1. Close all open software.
2. Open SCIEX OS.
3. Open the Configuration workspace.

Figure 12-7 Home Page



4. Click **Devices**.
5. Add the mass spectrometer to the profile.
   a. Click **Add**.
      The Device dialog opens.

Figure 12-8 Device

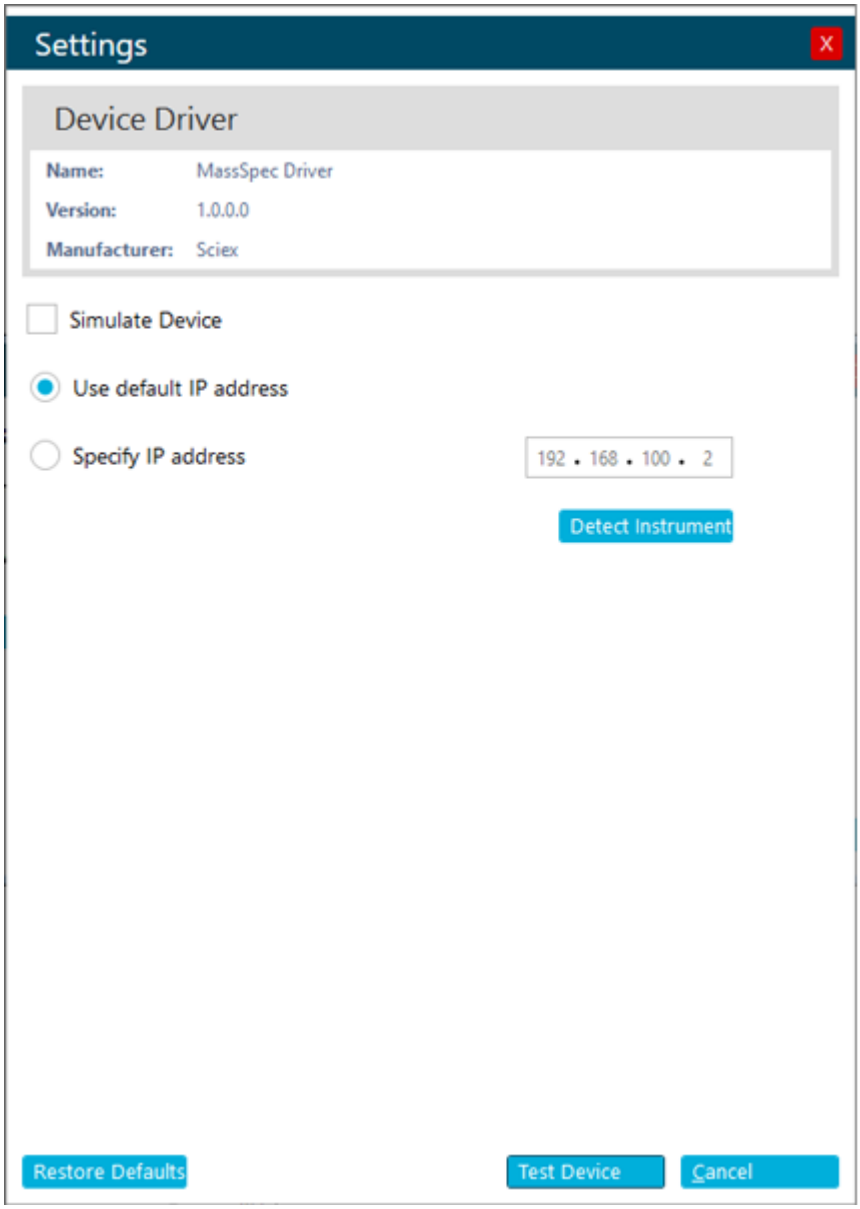


a. In the **Type** list, select the **Mass Spectrometer**.

b. In the **Model** list, select **MassSpec Driver**.

c. Click **Settings**.

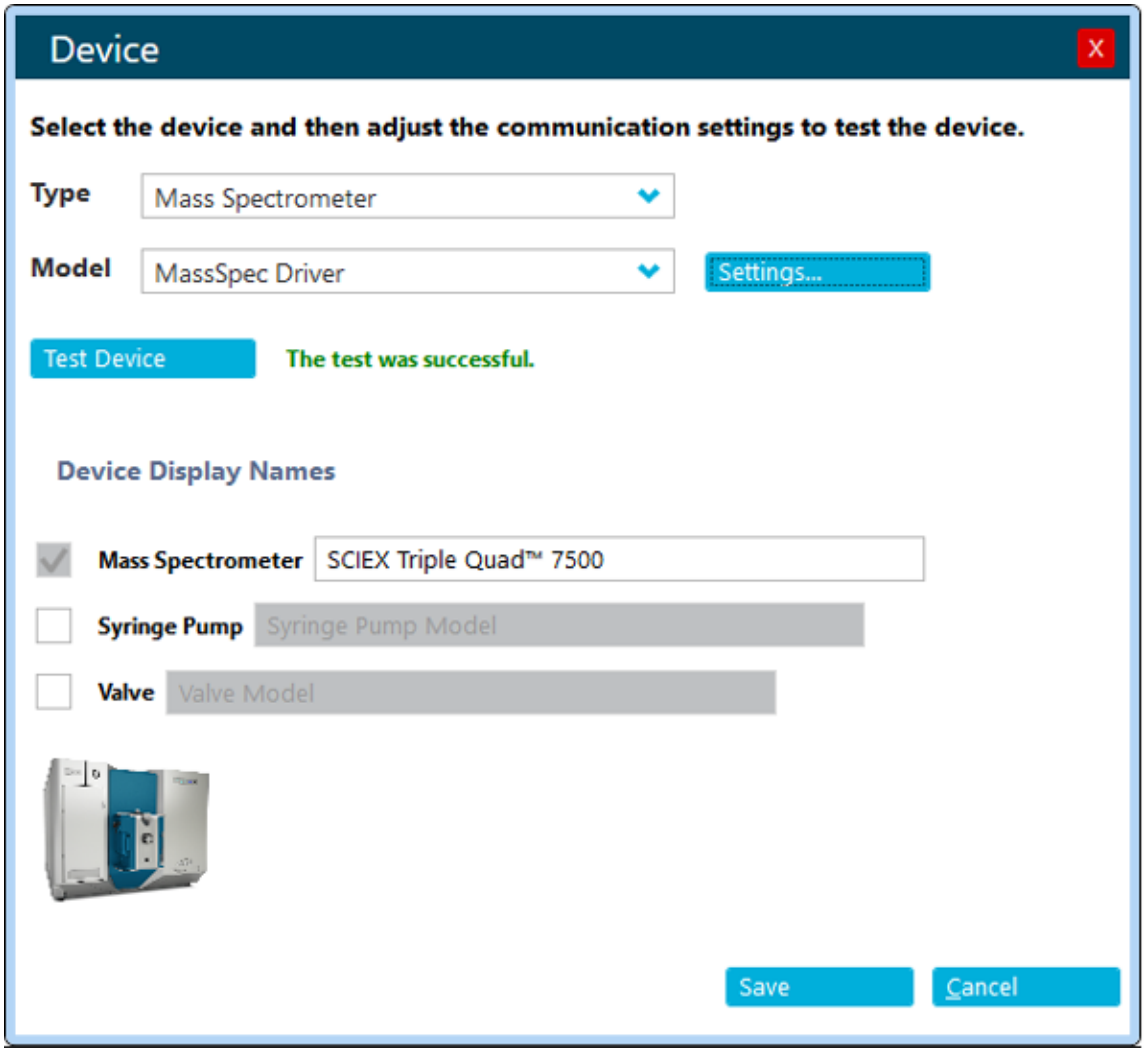
Figure 12-9 Settings



d. Click **Detect Instrument**.

e. Click **Test Device** to make sure that the device is configured correctly and available for use.

Figure 12-10 Device

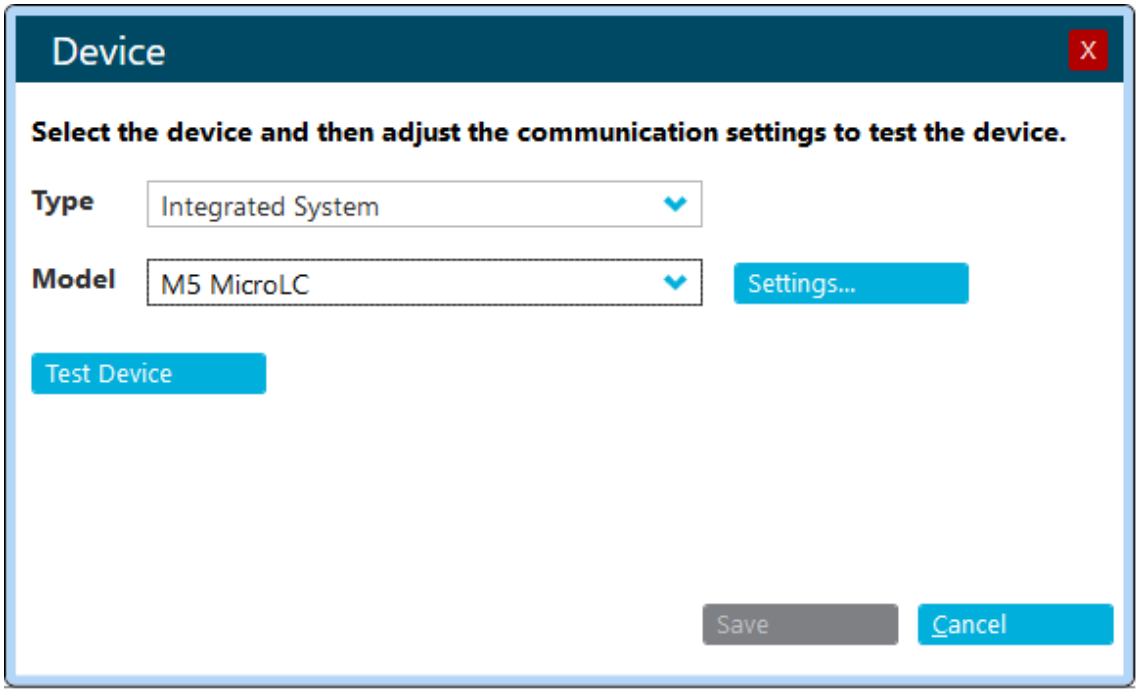


f. After the message, test was successful is shown, click **Save**.

6. Add the M5 MicroLC system to the profile:

   a. Click **Add**.

Figure 12-11 Device

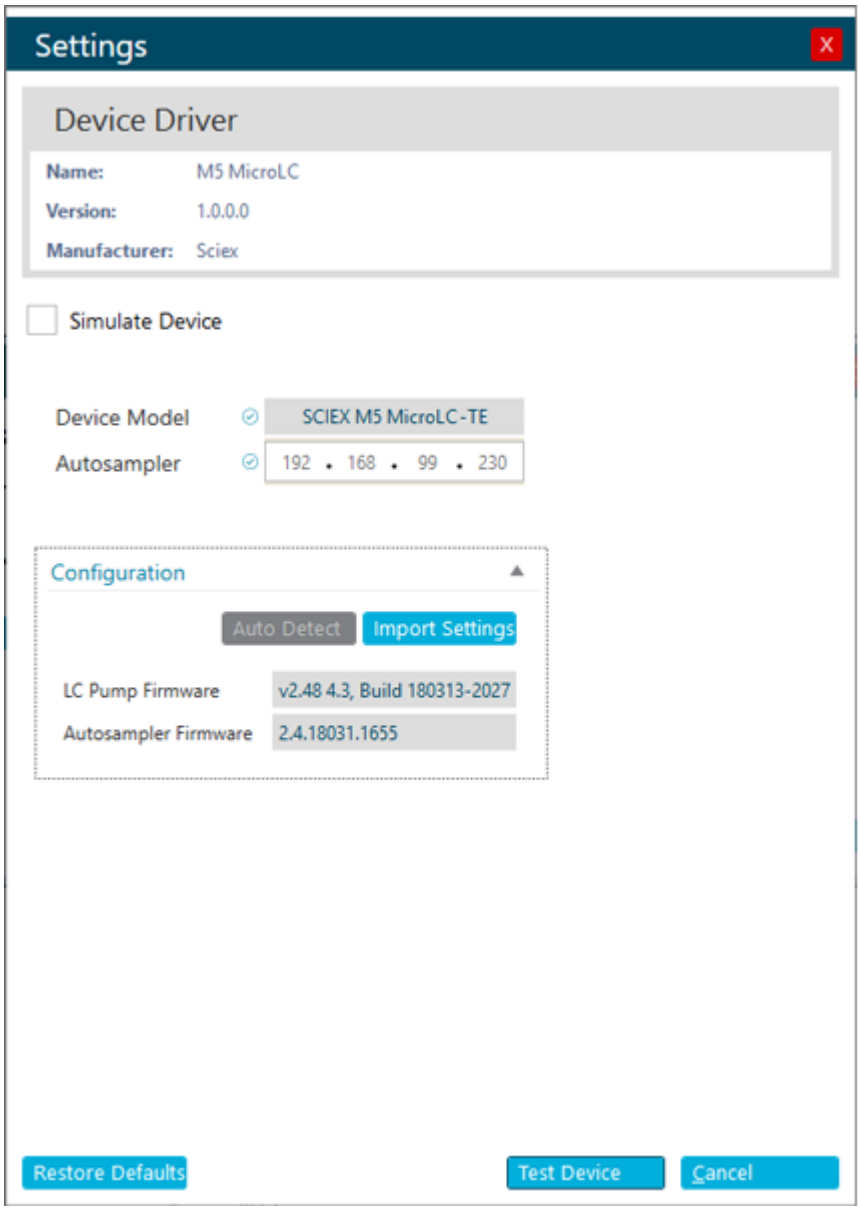


b. In the **Type** list, select the **Integrated System**.

c. In the **Model** list, select **M5 MicroLC**.

d. Click **Settings**.

Figure 12-12 Settings



e. Click **Auto Detect**.

f. Click **Import Settings** and then browse to the `Settings` folder in the paths below to locate the `EKSettings.reg` file:

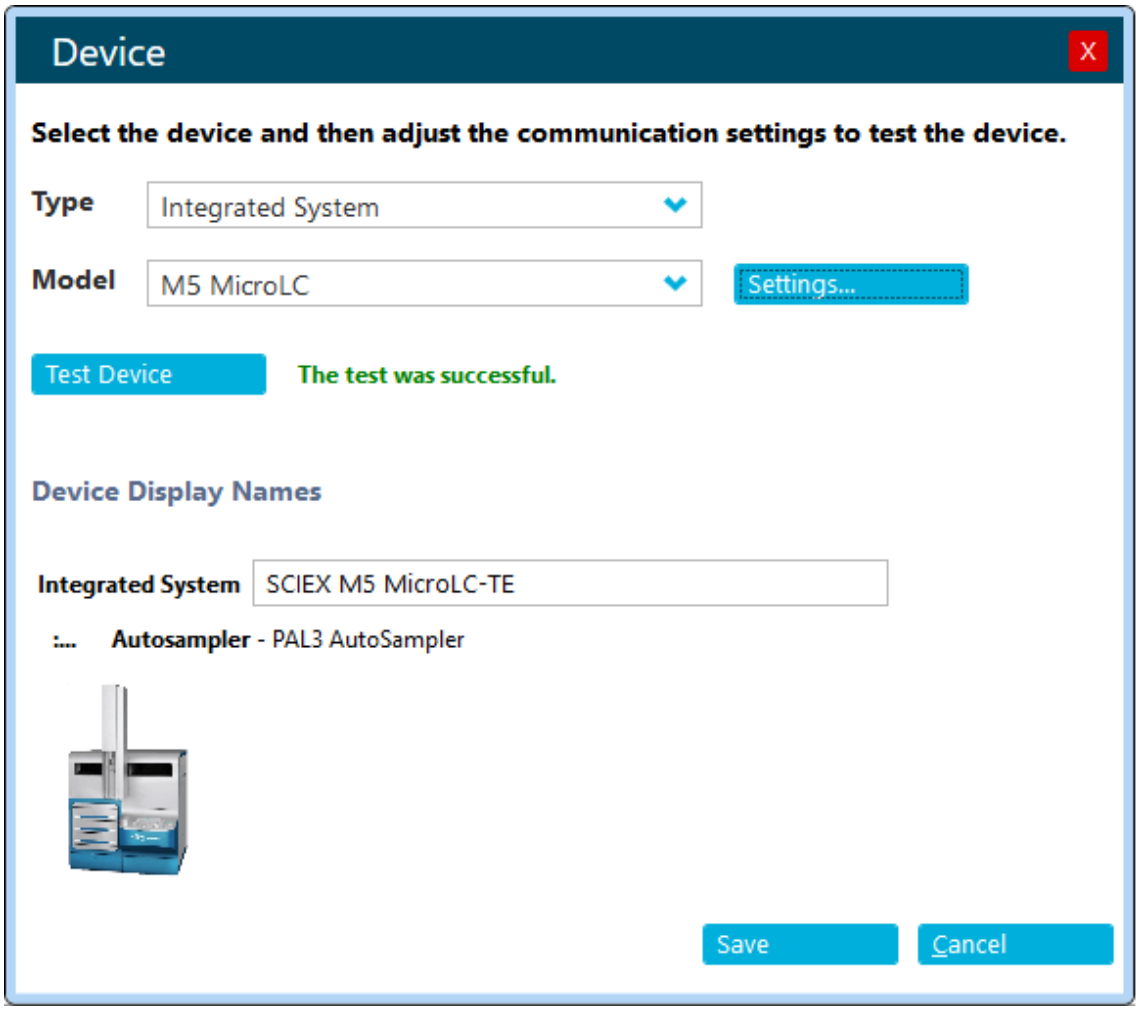
- For 32-bit operating systems: `C:\Program Files\Eksigent NanoLC`
- For 64-bit operating systems: `C:\Program Files (x86)\Eksigent NanoLC`

g. Select the Reg file, then click **Open**.

h. Click **Yes** to confirm import settings.

i. After the reg file is imported, click **Test Device** to make sure that the device is configured correctly and available for use.

Figure 12-13 Device

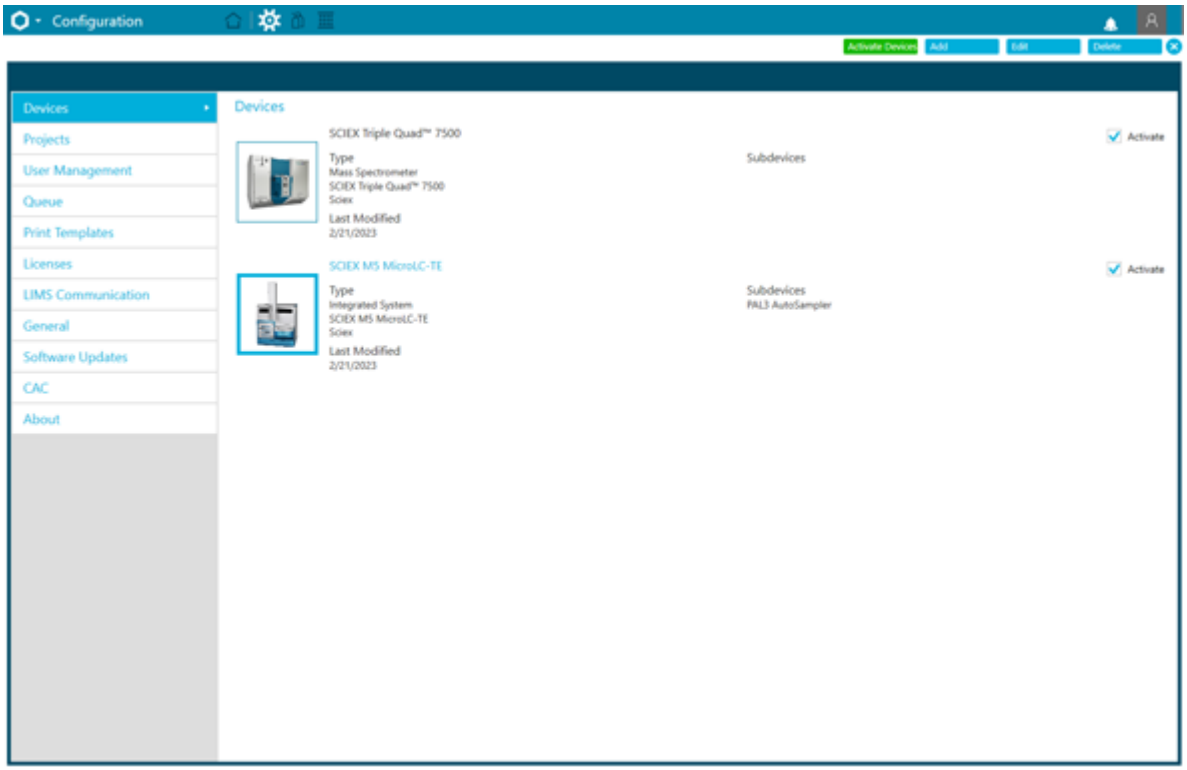


7. After the message, test was successful is shown, click **Save**.

Activate the M5 MicroLC Systems

- Select the **Activate** checkbox beside each device to be activated, and then click **Activate Devices**(Activate Devices).

Figure 12-14 Configuration: Devices



Configure the M5 MicroLC Driver Settings

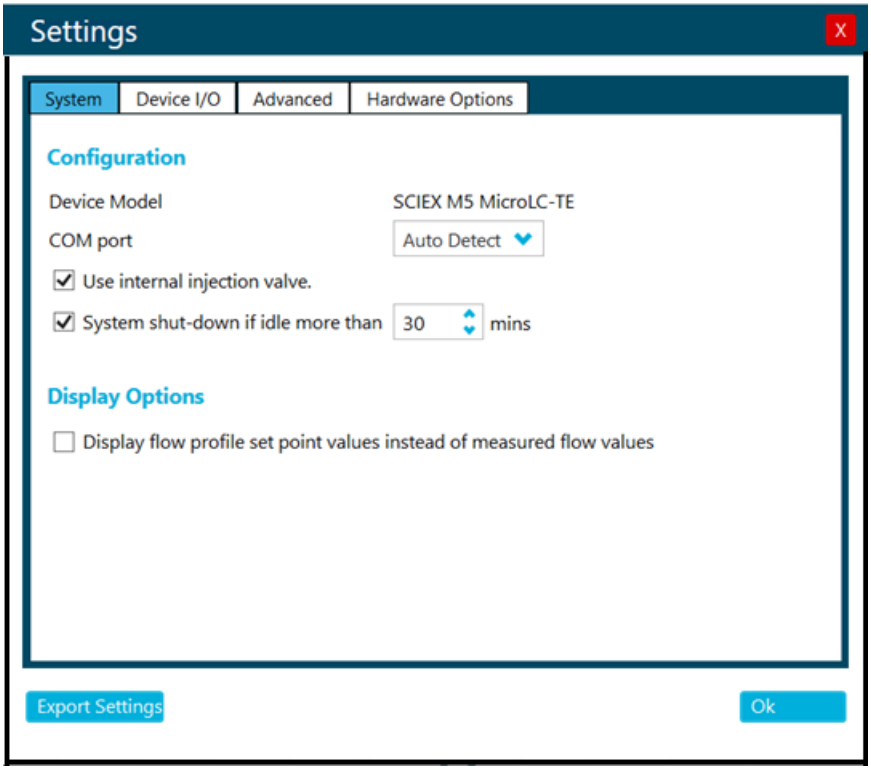
Note: Make sure that the settings are correct and if required, make any changes to the settings.

1. Click **Ready** (

).
The right side panel opens.
2. On the status panel, click **Direct device control** () to the right of the device name.
3. Click **Settings** ().
4. Make sure that the parameters are set as shown in the following figure.

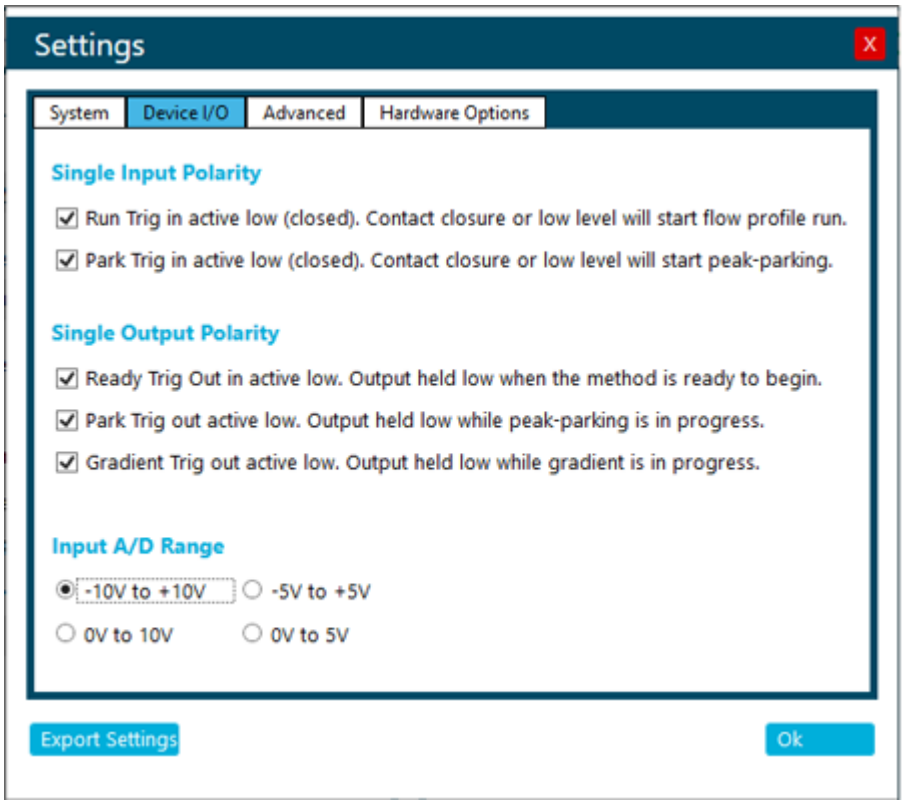
Note: If the system is not a trap-and-elute system, then the name in the **M5 MicroLC Devices** list will be different than in the figure.

Figure 12-15 Instrument Setting Dialog: System Tab



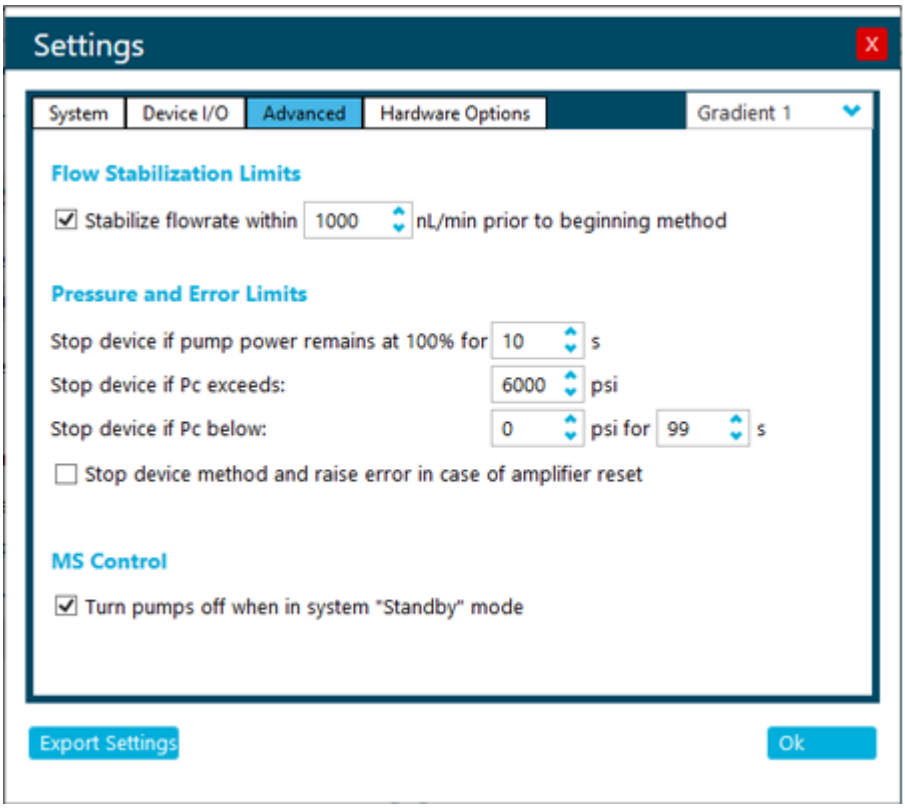
5. If necessary, set the parameters in the System tab.
   a. Make sure that the correct system shows in the **Devices Model** field, either **SCIEX M5 MicroLC-TE** or **SCIEX M5 MicroLC**.
   b. Select **Use internal injection valve** checkvbox.
   c. Select the **System shut-down if idle more than** check box and then type `30` to automatically shut down the system after it is idle for the specified time.
6. Open the Device I/O tab and then make sure that all of the check boxes are selected, as shown in the following figure.

Figure 12-16 Instrument Setting Dialog: Device I/O Tab



7. Open the Advanced tab and make sure that the parameters are set. Refer to step 8.

Figure 12-17 Instrument Setting Dialog: Advanced Tab



8. If required, set the flow stabilization limits.
   This limit sets the degree of flow rate stability that is required before a gradient will start.
   a. In the Flow Stabilization Limits section, type the applicable value from the following table in the **Stabilize flowrate within nnnn nL/min prior to beginning method** field.

      Table 12-1 Flow Stabilization Limit by Pump Flow Rate

Flow Rate Range	Recommended Setting
1 µL/min to 10 µL/min	100 nL/min
5 µL/min to 50 µL/min	500 nL/min
20 µL/min to 200 µL/min	1000 nL/min

   b. M5 MicroLC-TE system: Click the **Channel** arrow buttons to select **Gradient 2**, and then type `1000` in the **Stabilize flowrate within nnnn nL/min prior to beginning method** field.
9. If required, set the remaining parameters in the Advanced tab.

a. In the Pressure and Error Limits section, type `6000` in the **Stop device if Pc exceeds: nnnn psi** field.

b. In the MS Control section, select **Turn pumps off when in system "Standby" Mode.**

c. M5 MicroLC-TE system: Click the **Channel** arrow buttons to select **Gradient 2**, and then select **Turn pumps off when in system "Standby" Mode.**

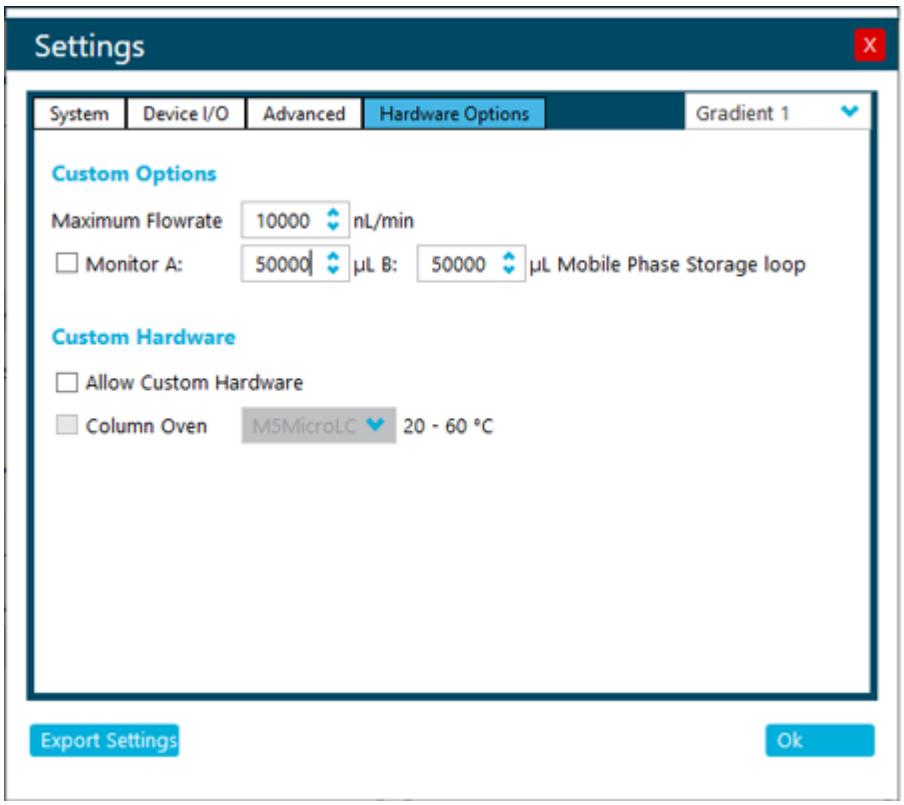
10. Click the Hardware Options tab and then make sure that the **Column Heater** check box is selected.

a. If a column heater will not be installed, then click the **Allow Custom Hardware** check box and then clear the **Column Oven/Heater** check box.

Note: The **Column Oven/Heater** check box should not be selected if the column heater is not installed.

b. M5 MicroLC-TE system: Click the **Channel** arrow buttons to select **Gradient 2** and then make sure that the **Column Oven/Heater** check box is cleared for Gradient 2.

Figure 12-18 Instrument Setting Dialog: Hardware Options Tab

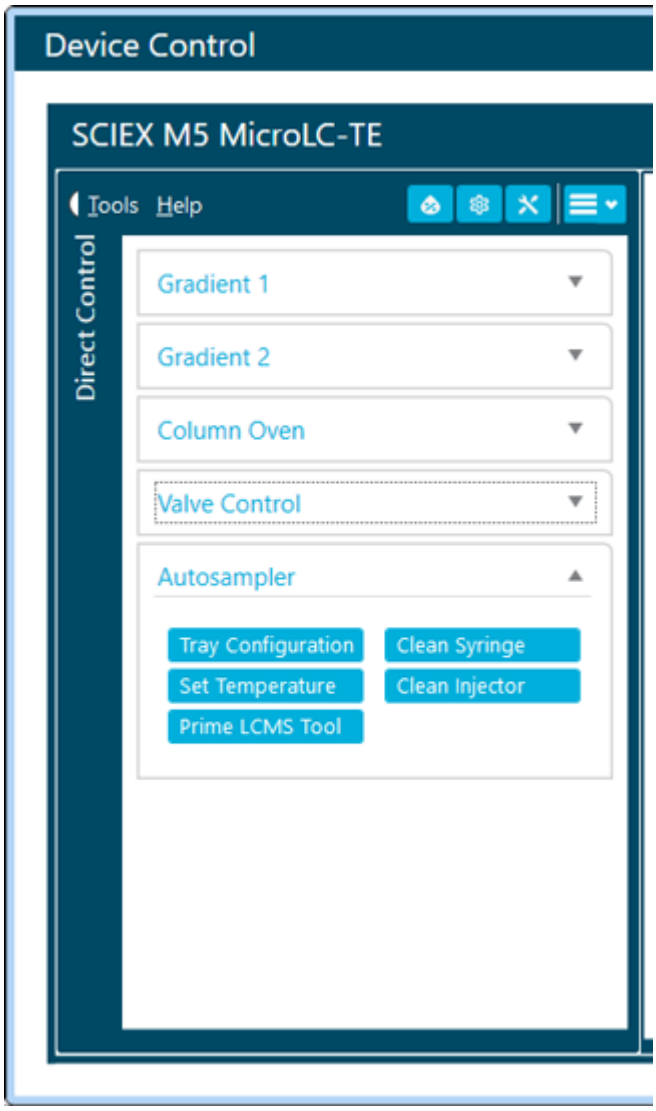


11. Click **OK**.

Configure the Trays in the Cooled Sample Drawers

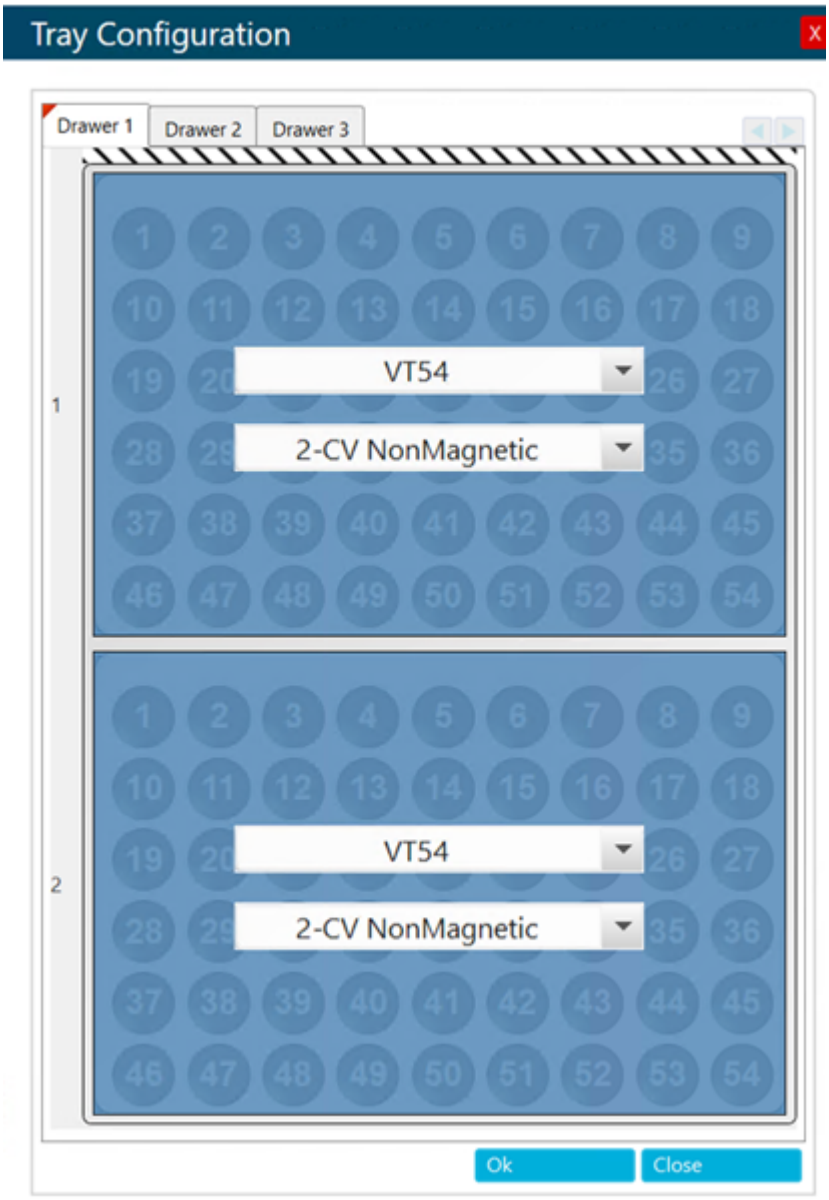
1. On the status panel, click **Direct device control** ( ) to the right of the device name.
2. In the Direct Control section, expand the **Autosampler** section.

Figure 12-19 Device Control: Autosampler



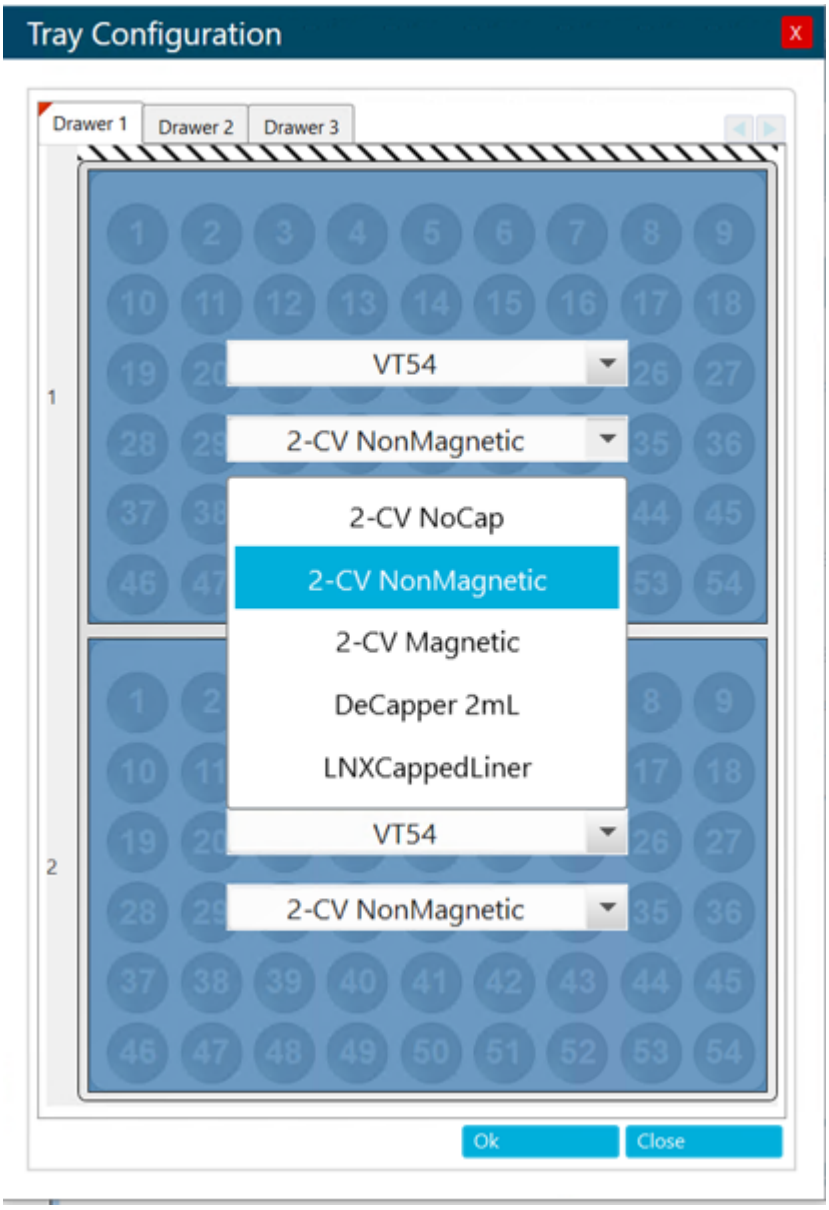
3. Click **Tray Configuration** (Tray Configuration).
   The Tray Configuration dialog opens.

Figure 12-20 Tray Configuration Dialog



4. In the **1** list, select **VT54**.
5. In the **2** list, select **Vial 2-CV NoMagnetic**.

Figure 12-21 Selecting the Vial for Slot 1 in Drawer 1

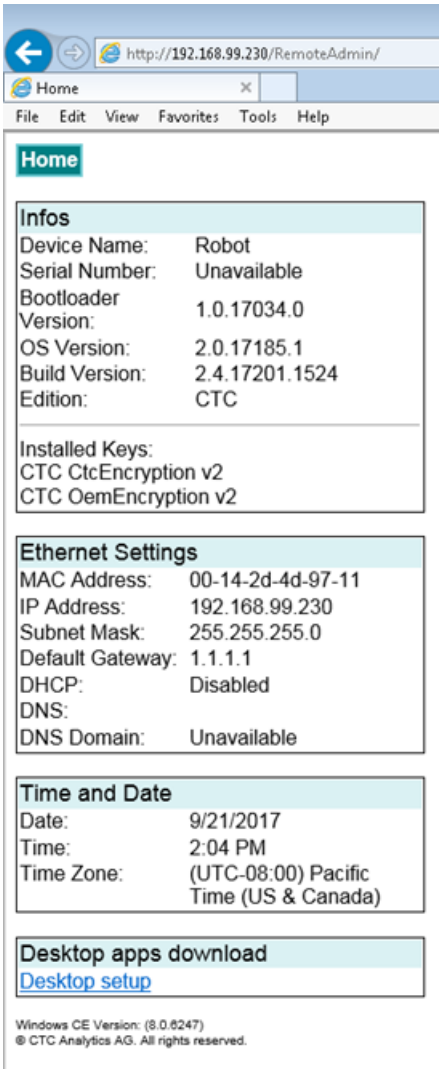


6. Do step 4 to step 5 again to configure the other slot for **Drawer 1**.
7. Click **OK**.
   The M5 MicroLC driver gets the configuration from the autosampler.
8. Click **Close**.
9. After changing the tray configuration, open the Configuration workspace, deactivate the M5 MicroLC device and then activate the M5 MicroLC to see the changes in the Batch dialog.

Install and Configure the PAL Virtual Terminal Software

1. Install the PAL Virtual Terminal software.
   a. Open a web browser window and type `http://192.168.99.230` and then press **Enter**.
   The web browser page updates with information about the autosampler.

   Figure 12-22 Autosampler Connection in Web Browser



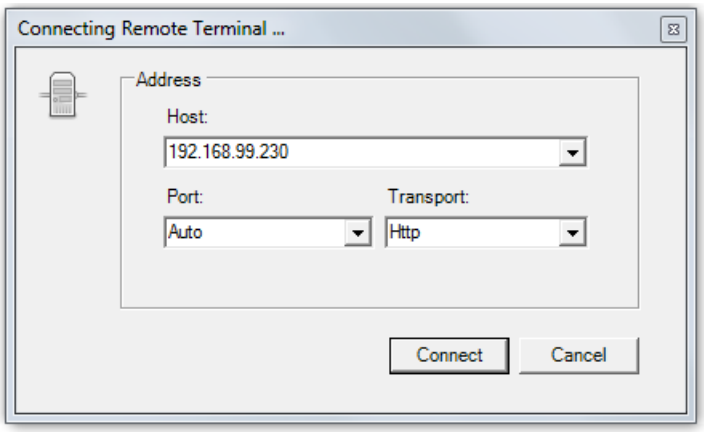
   b. Click **Desktop setup** at the bottom of the window to download the installer.
   c. Click `Pal3DesktopSetup.exe` and then click **Run** to install the Remote Terminal software.

d. Browse to `C:\Program Files\PAL3 System\Integration\Applications\2.4.51`, right-click `TerminalDesktop.exe` and then create a shortcut on the Windows desktop. The PAL VT icon appears on the desktop.

Figure 12-23 PAL VT Icon

2. Double-click the **PAL VT** icon.
3. In the **Connecting Remote Terminal** dialog, set the parameters for the remote terminal.

Figure 12-24 Connecting Remote Terminal Dialog

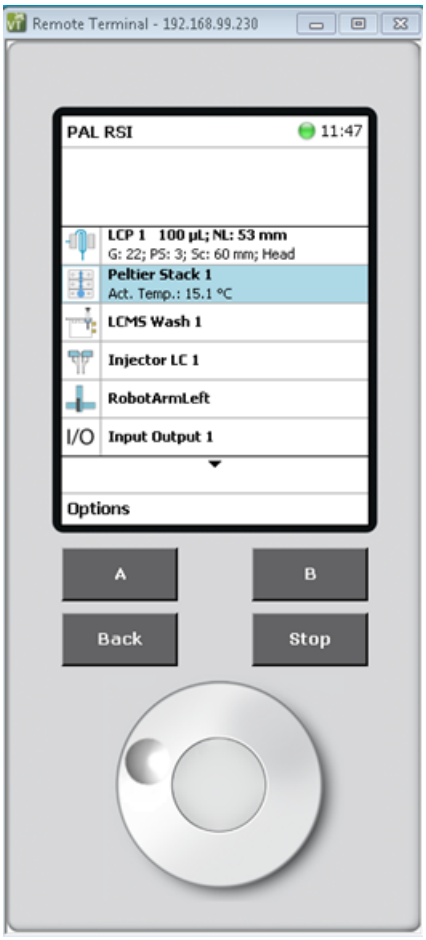


a. In the **Port** list, select **Auto**.

b. In the **Transport** list, select **Http**.

c. Click **Connect**.

Figure 12-25 Remote Terminal Window



Troubleshoot the Move to a New Computer

When using a different computer than the one on which the M5 MicroLC system was originally installed, the following procedure might be helpful in resolving any issues.

Issues with Gain and Integral Settings for the Pump

Incorrect integral or gain settings might cause flow instability or gas venting when the pumps are on. Use the following instructions to troubleshoot this issue.

1. On the status panel, click **Direct device control** ( ) to the right of the device name.
2. Click **Maintenance** ( ).
   The Hardware Diagnostics dialog opens.

3. Click Calibration Values tab.
4. Write down the **gain** and **int** values found in the Control Parameters (Field Service) section near the bottom of the tab.
5. Do the preceding steps again on the old computer and then compare the values for the two computers.
   The first two digits in each value should be the same. If they are not, then contact sciex.com/contact-us.

Prepare the System for Storage

1. Change the mobile phases in the solvent bottles to isopropanol (IPA).

   Note: Removing the aqueous mobile phases is required to decrease the possibility of bacterial growth.

2. Purge and flush the system. Refer to the section: Purge the Mobile Phases and Flush the System with SCIEX OS.
3. Plug all pump inlets and outlets.
4. Label the system for storage to make sure that the next user is aware that the system contains IPA.

Move the System with the Analyst Software 13

This section describes how to move the M5 MicroLC system from one location to another. It assumes that the new location includes a mass spectrometer and that the system is on a wheeled cart. Complete disassembly of the system is not required.

Instructions for setting up the system to use a different acquisition computer are also given, as well as instructions for storing the system.

Disconnect the System at the Original Location

1. Close the Eksigent Control software and the mass spectrometer software, if it is open.
2. Use the switch on the back of the system to turn off the power to the system, and then disconnect the mains supply cable.
3. Turn off the gas supply.
4. Disconnect the system from the gas.
   - For the M5 MicroLC system: Push in the red plastic ring while pulling out the tubing to remove the gas tubing from the back of the pump.
   - For the M5 MicroLC-TE system: Disconnect the gas supply at the Tee.

   The system is still pressurized, so it may be difficult to remove the tubing.
5. Disconnect the USB cable between the LC system and the acquisition computer.
6. Disconnect the MS interface cable between the LC system and the mass spectrometer.
7. Disconnect the Ethernet cable between the LC system and the acquisition computer.
8. Disconnect the tubing from the column at the ion source.

Install the System at the New Location

WARNING! Lifting Hazard. Make sure that at least four people or a lifting device are available to lift the LC system. Follow established safe lifting procedures. For the weights of system components, refer to the document: *Site Planning Guide*.

WARNING! Crushing Hazard. Wear protective footwear when moving heavy objects.

CAUTION: Potential System Damage. Make sure to use a bench or table that will support the system. If the bench collapses, then the system could be damaged.

1. Carefully move the system to the new location.
2. Connect the USB cable to the port labeled **USB** on the back of the LC system and to the acquisition computer.
3. Connect the MS interface cable to the port labeled **I/O G1** on the back of the LC system and to the mass spectrometer.
4. Connect the LAN cable to the port labeled **LAN** on the back of the LC system and to an Ethernet port on the acquisition computer.
5. Connect the gas tubing to the pump.
   - For the M5 MicroLC system: Push the tubing straight in the fitting on the back of the pump.
   - For the M5 MicroLC-TE system: Connect the gas supply to the Tee.
6. Connect the gas tubing to a source of gas (clean, dry, compressed air or nitrogen, regulated to 100 psi).
7. Connect the column outlet tubing to the ion source.
8. Connect the system to the mains supply.
9. Connect the mains supply cable, and then use the switch on the back of the system to turn on the power.
10. If the acquisition computer was not moved, then set up the new acquisition computer. Refer to the section: Set Up a New Computer for Use with the System.

Set Up a New Computer for Use with the System

The following procedures describe how to set up a new acquisition computer for use with the M5 MicroLC system.

Note: The Analyst software must be installed on the acquisition computer first. Refer to the *Analyst Software Installation Guide*.

Install the Analyst Device Driver Software

Install the latest available version of the Analyst Device Driver (ADD) software. The software can be downloaded from sciex.com/software-support/software-downloads.

Note: Make sure to use the PAL3 RC.NET driver recommended for the ADD version.

- Install the ADD by following the instructions in the *Release Notes*.

Note: Admin privileges are required to install the ADD, and the Analyst software must be closed.

Install the Eksigent Control Software and Transfer the Settings

To use the M5 MicroLC system with a different acquisition computer, install the Eksigent Control software on the new computer, transfer important files from the existing computer, and then configure the software on the new computer.

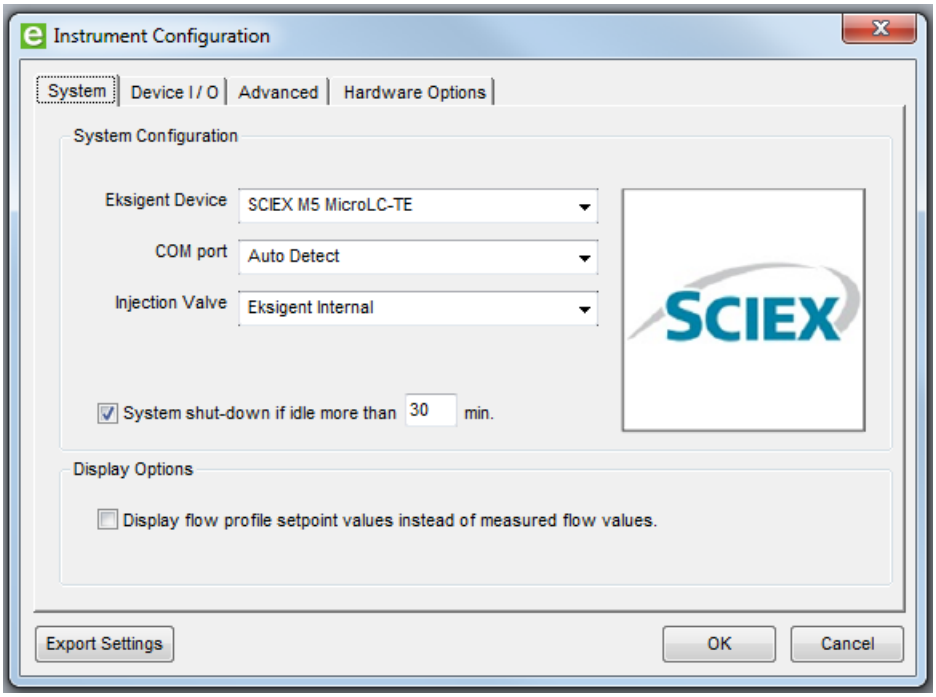
Required Materials
• Eksigent Control software, downloaded from sciex.com/software-support/software-downloads. If the new computer does not have internet access, then save the installer to a USB flash drive. • USB flash drive.

Note: The following instructions assume that the Analyst software is already installed on the new computer.

Note: Use the following procedure to use the same version of the Eksigent Control software on the new acquisition computer. To update the Eksigent Control software as part of the move to a new computer, follow the instructions supplied with the software update.

1. Export the system settings `reg` file from the current computer and copy them to the USB flash drive.
    a. Start the Eksigent Control software.
    b. Click **System** > **Instrument Configuration**.

Figure 13-1 Instrument Configuration Dialog: System Tab



c. Click **Export Settings** in the bottom left corner.
The instrument settings are exported and a dialog that shows the location of the backup file opens.

d. Write down the location for use in the following step and then click **OK**.

e. In Windows Explorer, browse to the location of the `Eksettings.reg` file and then copy the file to the USB flash drive.

2. In Window Explorer, copy the `settings` folder from the current computer to the USB flash drive.

   a. Browse to the installation directory.

      - For 32-bit operating systems: `C:\Program Files\EksigentNanoLC`
      - For 64-bit operating systems: `C:\Program Files (x86)\EksigentNanoLC`

   b. Copy the `settings` folder to the USB flash drive.

3. Install the Eksigent Control software on the new computer.

   a. Use the switch on the front of the system to turn off the M5 MicroLC system.

   b. Double-click the downloaded file for the Eksigent Control software and then follow the instructions to install the software.

4. Copy the `settings` folder to the new computer.

a. Install the USB flash drive in a USB port on the new computer.

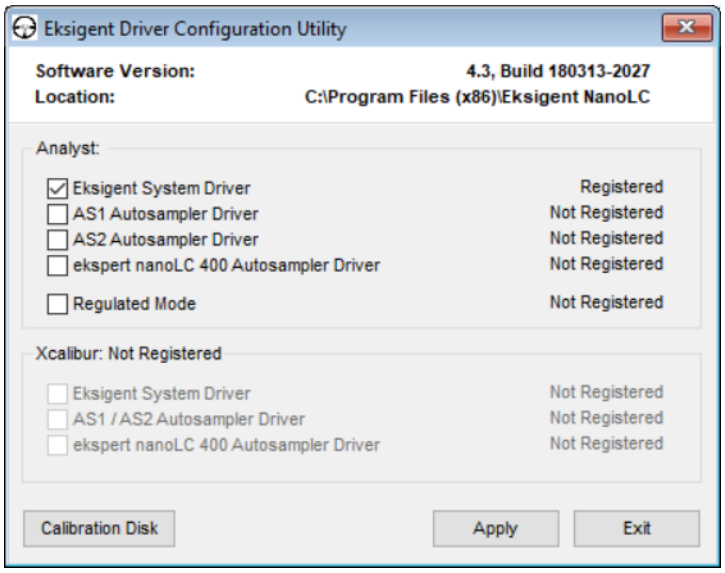
b. Copy the `settings` folder from the USB flash drive to the Eksigent NanoLC folder.

- For 32-bit operating systems: `C:\Program Files\EksigentNanoLC`
- For 64-bit operating systems: `C:\Program Files (x86)\EksigentNanoLC`

5. Install the driver for the Analyst software and then load the settings from the `EKSettings.reg` file.

a. From the **Start** menu, select **Eksigent** > **Driver Configuration.** If the User Account Control dialog opens, then click **Yes** to continue.

Figure 13-2 Eksigent Driver Configuration Utility Dialog



b. In the Analyst section, select **Eksigent System Driver** and then click **Apply.**

Note: If the Analyst section is unavailable, then the Analyst software is not installed. Install it, and then do this step again.

c. To load the settings, click **Calibration Disk** and browse to the `EKSettings.reg` file on the USB flash drive.

d. Click **Apply** and then **Exit.**

6. Before using the system, refer to the section: Re-initialize the Pressure Transducers with the Analyst Software.

Assign the IP Address to the Autosampler

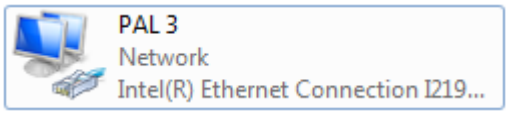
1. Click **Start** > **Control Panel** > **Network and Sharing Center**.

2. Click **Change adapter settings**.
   The Network Connections window opens.
3. (Optional) Rename the connection for easier identification.
   a. Right-click **Local Area Connection X**, where X is a number, and then select **Rename**.

   Tip! To identify the correct Local Area Connection, disconnect the Ethernet cable that comes from the autosampler and then wait a few seconds. The correction connection will show `"Not connected"`. Make sure to connect the Ethernet cable.

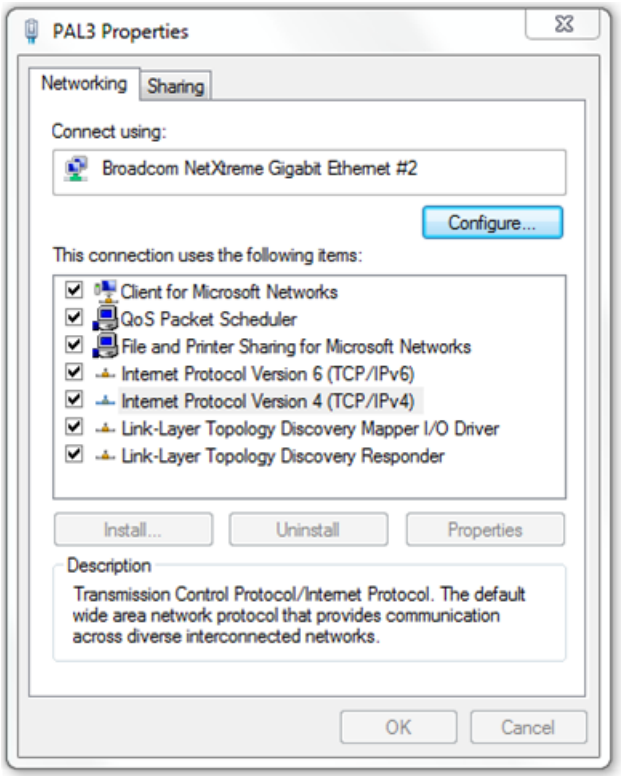
   b. Type `PAL 3` and then press **Enter**.

Figure 13-3 Local Area Connection after Renaming



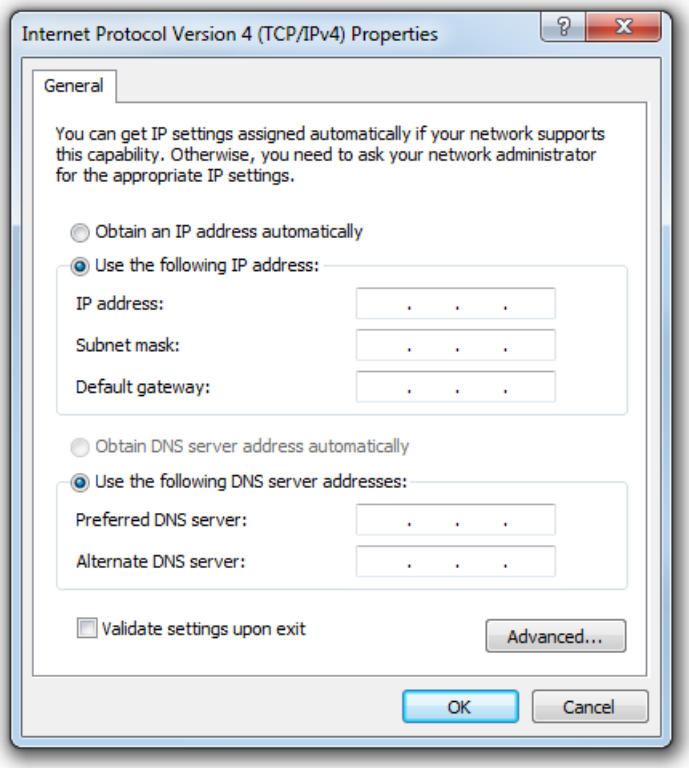
4. Right-click **PAL3** and then select **Local Area Connection** > **Properties** to open the Local Area Connection Properties dialog.

Figure 13-4 PAL3 Properties



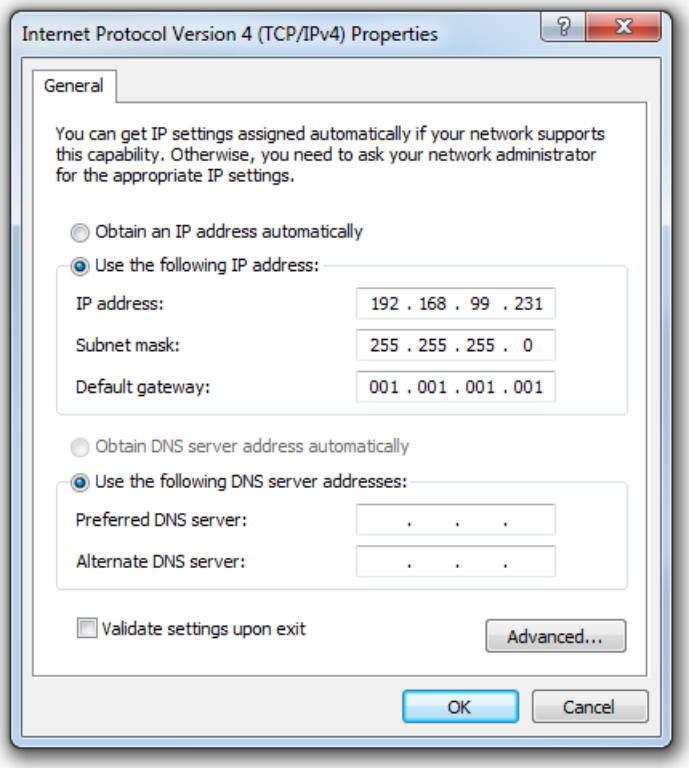
5. On the Networking tab, click **Internet Protocol Version 4 (TCP/IPv4)** and then click **Properties**.

Figure 13-5 Internet Protocol Version 4 (TCP/IPv4) Properties



6. Select **Use the following IP address** and then type the following:
   - For the **IP address**, type `192.168.99.231`.
   - For the **Subnet mask**, type `255.255.255.0`.
   - For the **Default gateway**, type `1.1.1.1`.

Figure 13-6 IP Address



7. Click **OK** and then click **Close**.

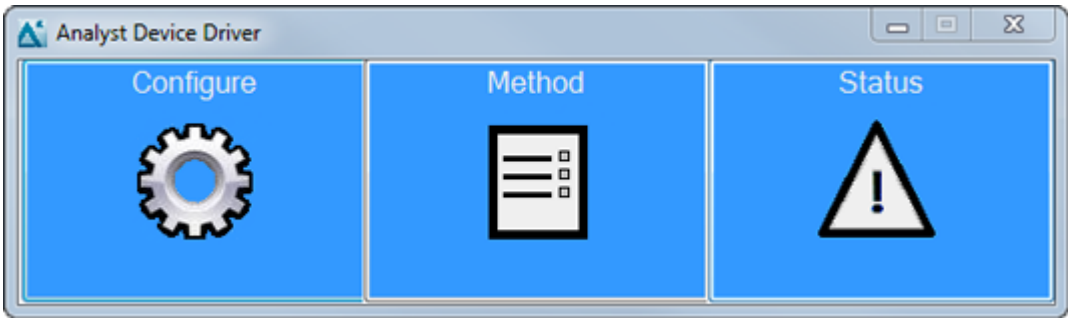
Install the CTC PAL 3 Driver and Configure the Device

Note: The CTC PAL 3 driver is included with the Analyst Device Driver (ADD) installer.

The following procedure includes configuring the cooled sample drawers with V54 trays. The drawers can also be configured with microtiter plates. Refer to the section: (Optional) Configure the Autosampler to Use Microtiter Plates with the Analyst Software.

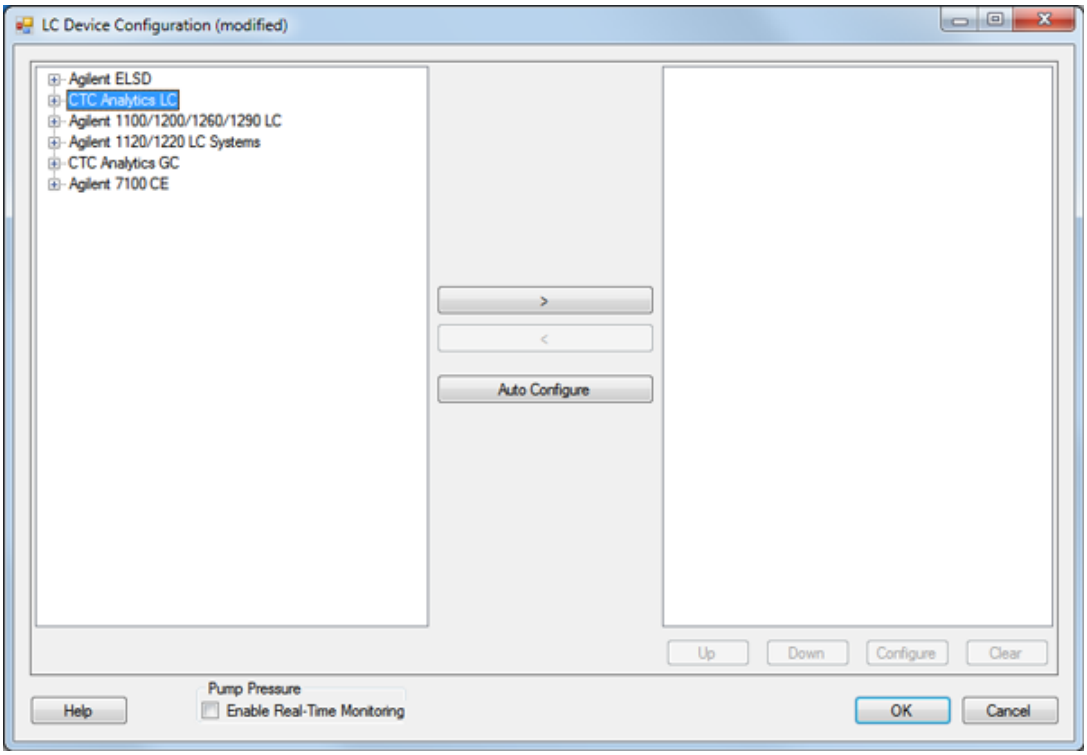
1. Open the Analyst software.
2. Make sure that the hardware profile is deactivated.
3. On the Navigation bar, under **Companion Software**, double-click **Analyst Device Driver**.

Figure 13-7 Analyst Device Driver



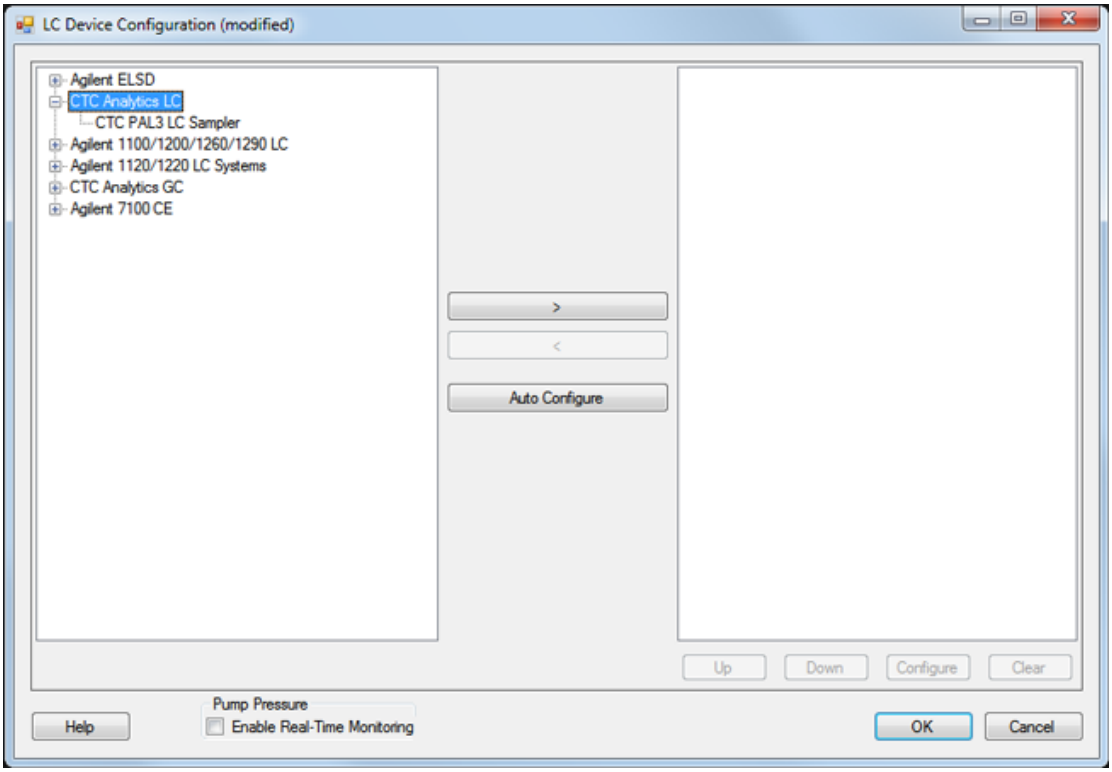
4. In the Analyst Device Driver window, click **Configure**.

Figure 13-8 LC Device Configuration Dialog



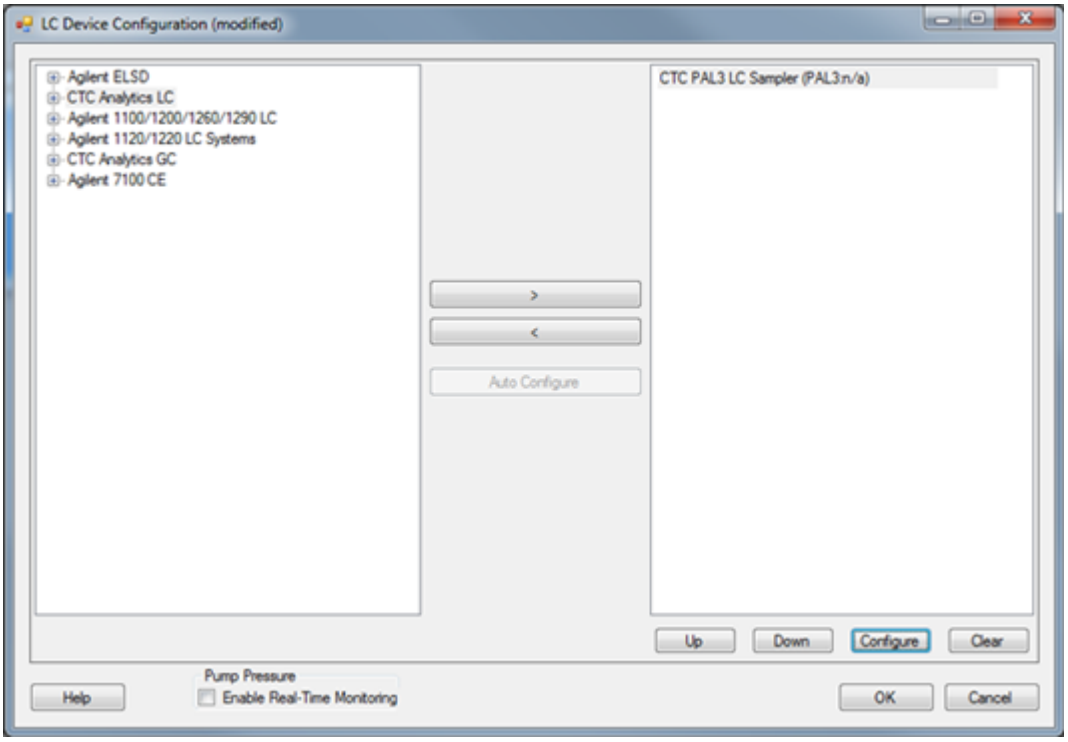
5. If required, click **CTC Analytics LC** system in the left panel and then expand the CTC Analytics LC group to see the peripheral devices for that group.

Figure 13-9 LC Device Configuration Dialog



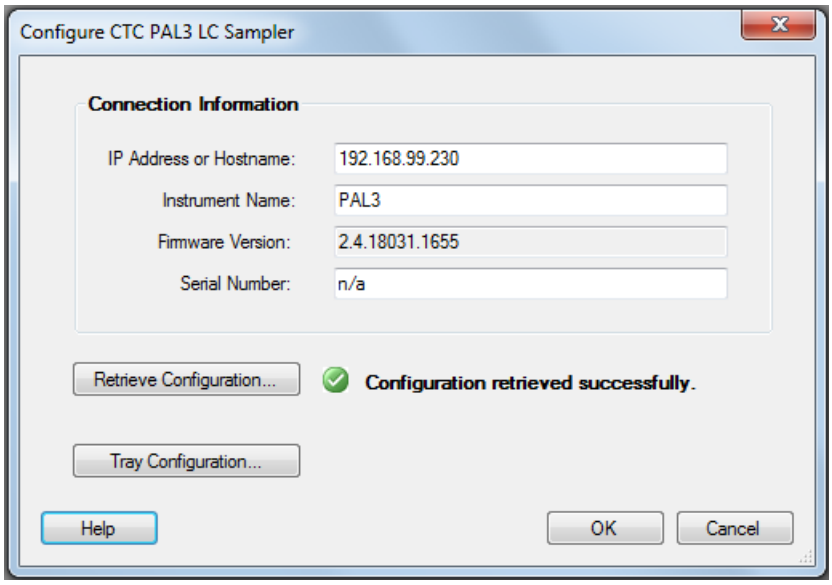
6. Click **CTC PAL3 LC Sampler** and then click **>**.

Figure 13-10 LC Device Configuration Dialog with the CTC-PAL3 Autosampler Selected



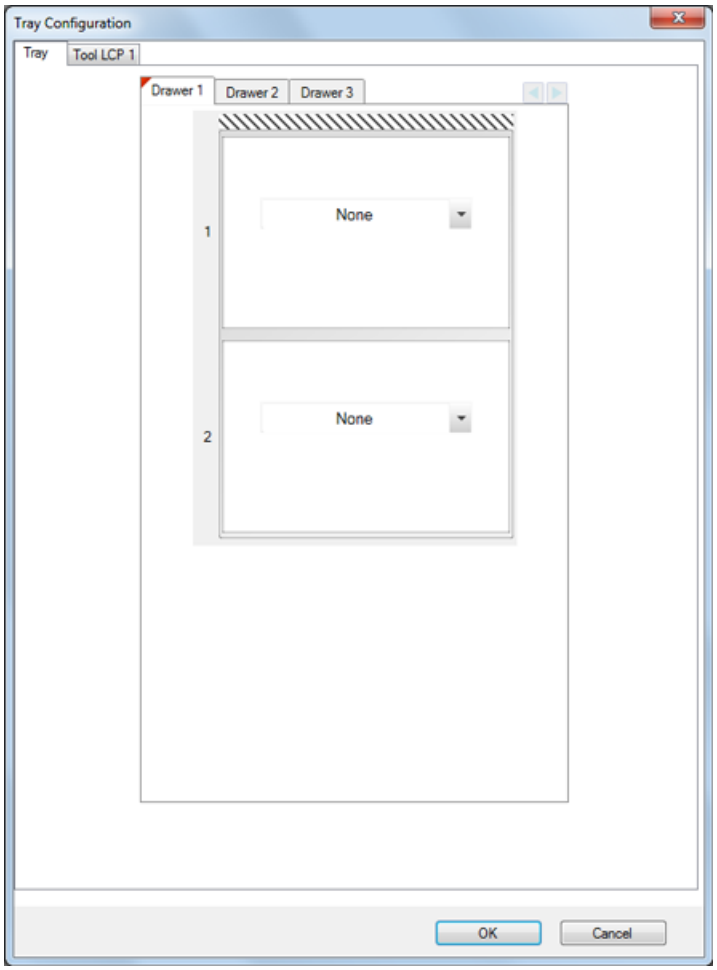
7. Click **Configure**.
8. Type `192.168.99.230` in the **IP Address or Hostname** field and then click **Retrieve Configuration**.

Figure 13-11 Configure CTC PAL3 LC Sampler Dialog with Configuration Retrieved



9. Configure the trays in the cooled sample drawers.
   a. Click **Tray Configuration** to open the Tray Configuration dialog.

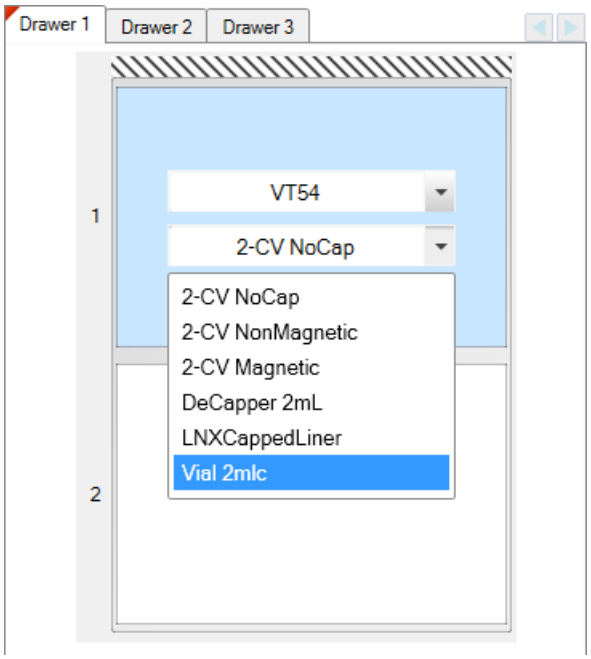
Figure 13-12 Tray Configuration Dialog



b. Click the list in **1** and select **VT54**.

c. Click the lower list and select **Vial 2mlc**.

Figure 13-13 Selecting Vial for Slot 1 in Drawer 1



d. Do the step 9.b and 9.c again to configure the other slot for Drawer 1.

e. Configure the other slot and then configure the other drawers.

f. Click **OK** to close the Tray Configuration dialog.

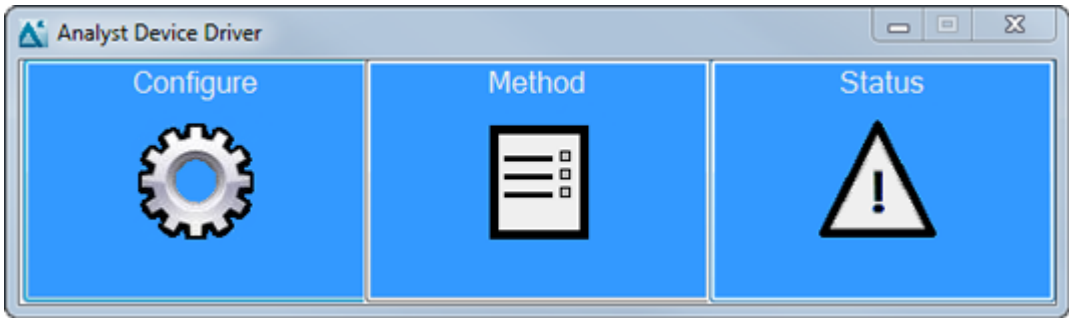
The ADD retrieves the configuration from the autosampler.

10. Click **OK** to close the Configure CTC PAL3 LC Sampler dialog and then click **OK** to close the LC Device Configuration dialog.

Map the Rack Positions and Import the Autosampler Scripts with the Analyst Software

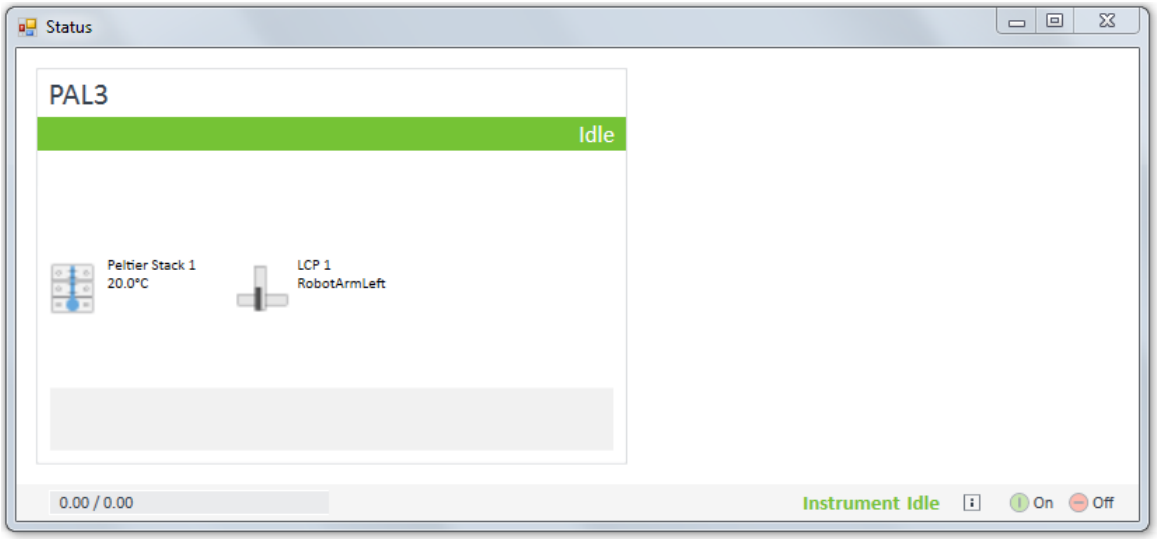
1. Click **Status** in the Analyst Device Driver window.

Figure 13-14 Analyst Device Driver



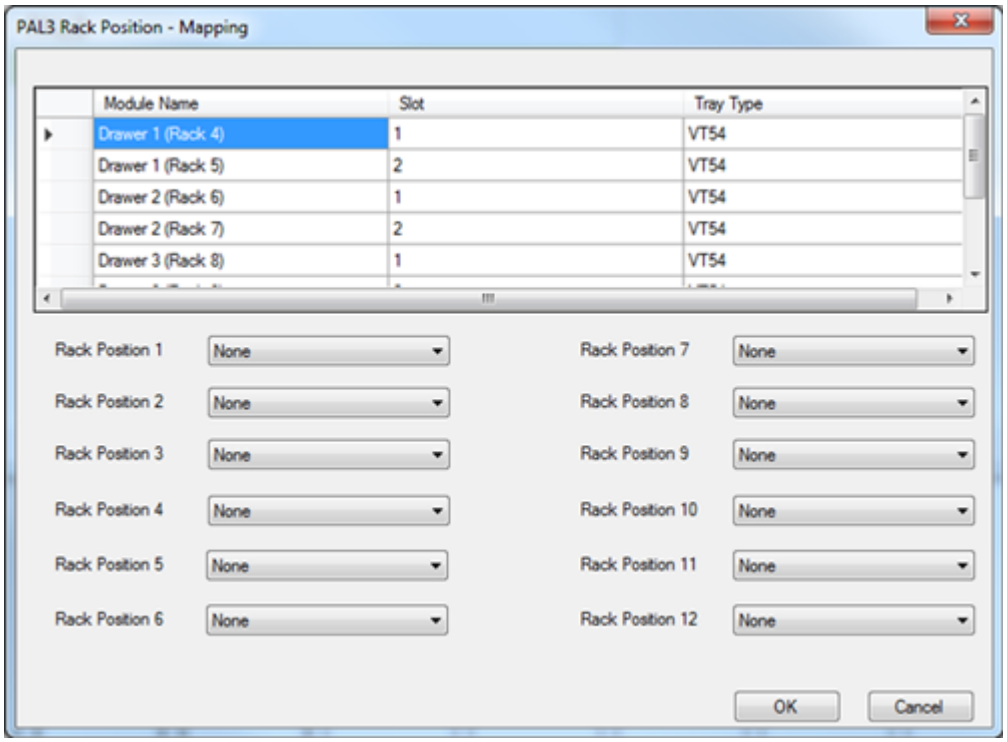
The Status window to configure the PAL3 autosampler opens.

Figure 13-15 Status Window for PAL3 Autosampler



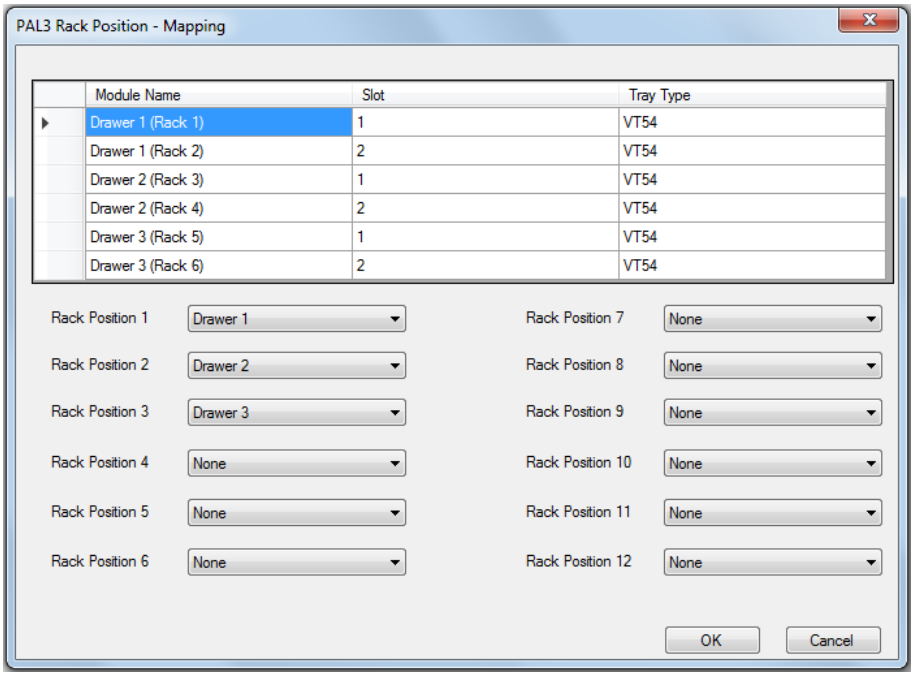
2. Right-click in the **PAL3** box and then click **Show Rack Position Mapping**.

Figure 13-16 PAL3 Rack Position: Mapping Dialog, Before Mapping



3. Map the first three rack positions in the dialog to the rack positions in the autosampler. Refer to the figure: Figure 13-17.

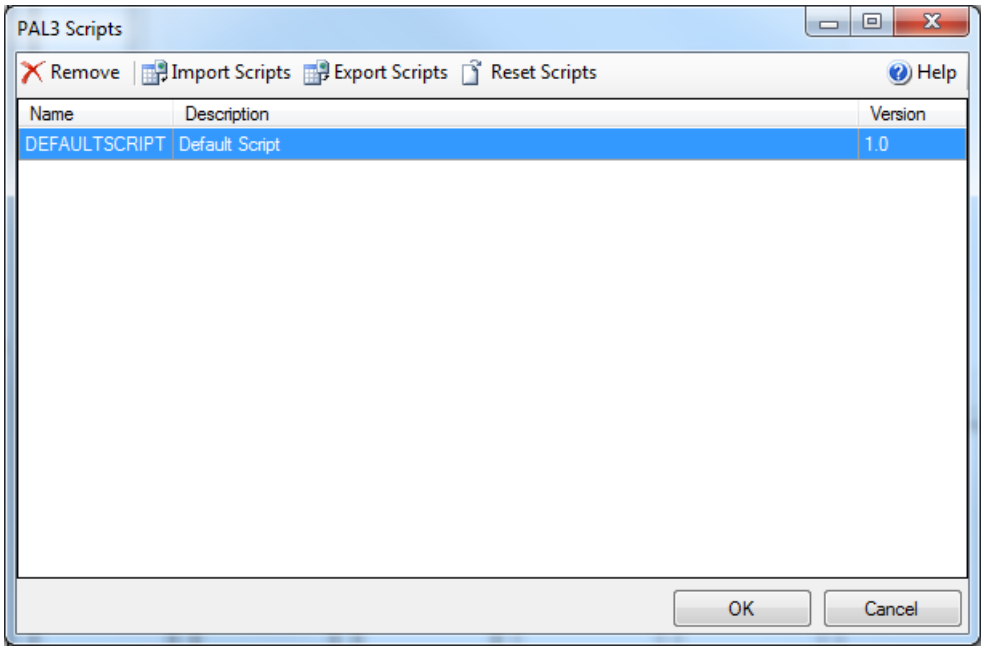
Figure 13-17 PAL3 Rack Position: Mapping Dialog, After Mapping



Note: Make sure to use the same mapping in the Sample tab and Locations tab when a batch is created in the Batch Editor in the Analyst software.

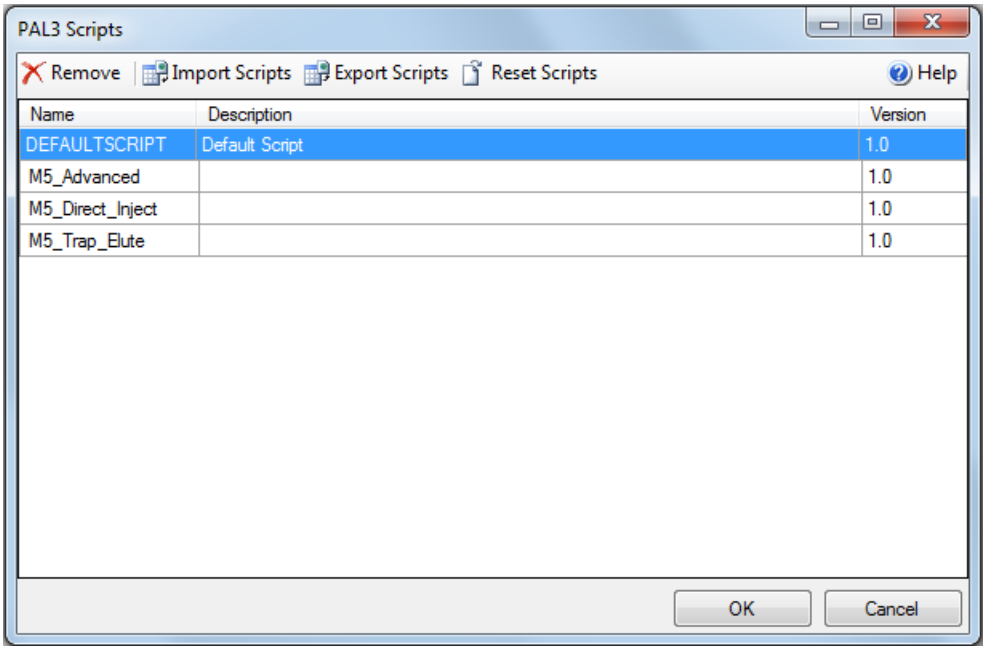
4. Click **OK**.
5. Right-click in the **PAL3** box in the Status window and then click **Show Script Manager**.

Figure 13-18 PAL3 Scripts Dialog, Before Importing



6. Click **Import Scripts**.
7. Browse to the folder where PAL3 scripts are stored. By default, the scripts are found in `C:\Program Files(x86)\EksigentNanoLC\settings\EKPAL3` folder. The folder contains the scripts M5_Direct Inject, M5_TrapElute, and M5_Advanced.
   The folder contains the scripts M5_Direct Inject, M5_TrapElute, and M5_Advanced.
8. Ctrl-click all of the script files, and then click **Open**.
   The imported scripts are shown in the PAL3 Scripts dialog.

Figure 13-19 PAL3 Scripts Dialog, After Importing

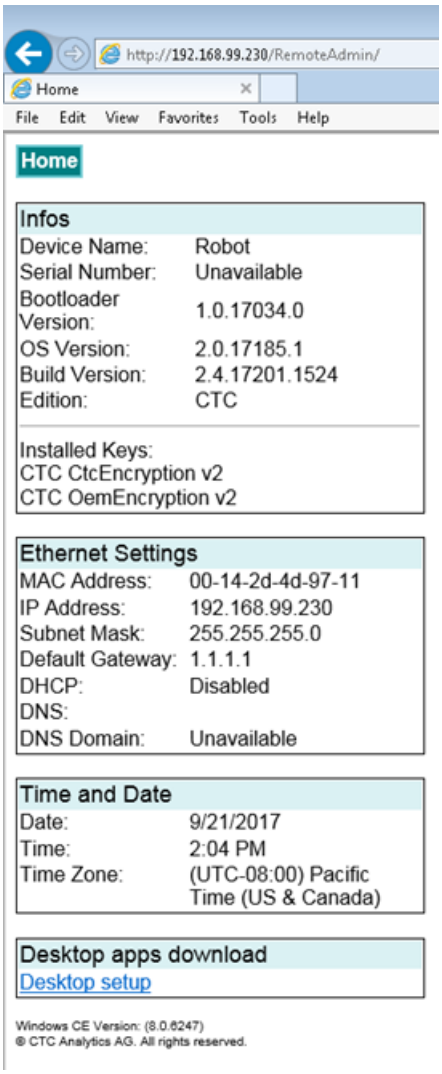


9. Click **OK** in the PAL3 Scripts dialog.

Install and Configure the PAL Virtual Terminal Software

1. Install the PAL Virtual Terminal software.
   a. Open a web browser window and type `http://192.168.99.230` and then press **Enter**.
   The web browser page updates with information about the autosampler.

Figure 13-20 Autosampler Connection in Web Browser



b. Click **Desktop setup** at the bottom of the window to download the installer.

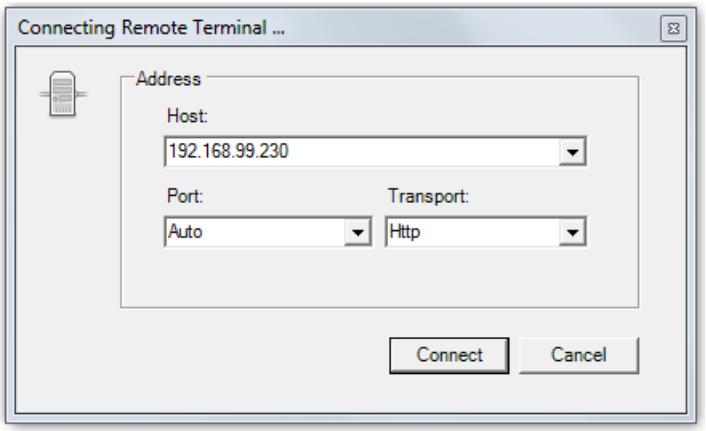
c. Click `Pal3DesktopSetup.exe` and then click **Run** to install the Remote Terminal software.

d. Browse to `C:\Program Files\PAL3 System\Integration\Applications\2.4.51`, right-click `TerminalDesktop.exe` and then create a shortcut on the Windows desktop. The PAL VT icon appears on the desktop.

Figure 13-21 PAL VT Icon

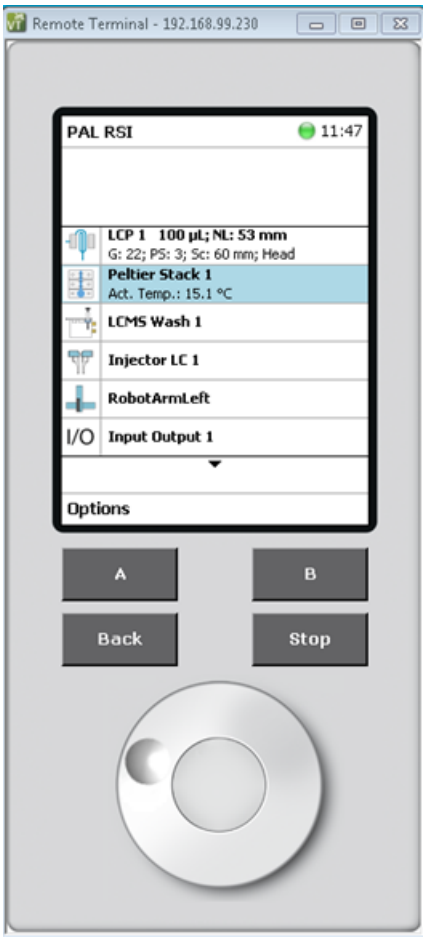
2. Double-click the **PAL VT** icon.
3. In the **Connecting Remote Terminal** dialog, set the parameters for the remote terminal.

Figure 13-22 Connecting Remote Terminal Dialog



a. In the **Port** list, select **Auto**.
b. In the **Transport** list, select **Http**.
c. Click **Connect**.

Figure 13-23 Remote Terminal Window



Troubleshoot the Move to a New Computer

When using a different computer than the one on which the M5 MicroLC system was originally installed, the following procedure might be helpful in resolving any issues.

Issues with Gain and Integral Settings for the Pump

Incorrect integral or gain settings might cause flow instability or gas venting when the pumps are on. Use the following instructions to troubleshoot this issue.

1. On the new computer, in the Eksigent Control software, select **System** > **Hardware Diagnostics** and then click the **Calibration Values** tab.
2. Write down the **gain** and **int** values found in the Control Parameters (Field Service) section near the bottom of the tab.
3. Do the preceding steps again on the old computer and then compare the values for the two computers.

The first two digits in each value should be the same. If they are not, then contact SCIEX Technical Support.

Prepare the System for Storage

1. Change the mobile phases in the solvent bottles to isopropanol (IPA).

   Note: Removing the aqueous mobile phases is required to decrease the possibility of bacterial growth.

2. Purge and flush the system. Refer to the section: Purge the Mobile Phases and Flush the System with the Analyst Software.
3. Plug all pump inlets and outlets.
4. Label the system for storage to make sure that the next user is aware that the system contains IPA.

System Specifications A

System	
Dimensions (L × W × D)	98 cm × 58 cm × 70 cm (38.6 in. × 22.8 in. × 27.6 in.) For a side-mounted solvent tray, add 14 cm (5.6 in.) to width.
Weight	• M5 MicroLC system: 54 kg (119 pounds) • M5 MicroLC-TE system: 68 kg (150 pounds)
Electrical	• Input line voltage: 100 V to 240 V AC • Input line frequency: 50Hz or 60 Hz • Input current: 2.5 A
Instrument control	Eksigent Control software with driver for the Analyst software
I/O	• Communication: USB 2.0 • TTL: Run in • Contact closure: Ready out/run out/Valve out/2 programmable auxiliary • LAN
Working environment	• Altitude: ≤2,000 m (6,562 feet) above sea level • Humidity: 20% to 80%, non-condensing • Temperature: 15 °C to 30 °C (59 °F to 86 °F)
Pump	
Flow rate range	Analytical gradient: 1 µL/min to 10 µL/min, 5 µL/min to 50 µL/min, or 20 µL/min to 200 µL/min (M5 MicroLC-TE only) Loading gradient: 20 µL/min to 200 µL/min
Gradient delay volume	<3 µL

Maximum pressure	10,000 psi
Retention time reproducibility	<0.5% RSD at 20 µL/min for the 5 µL/min to 50 µL/min configuration
Wetted parts	Stainless steel, PEEK, fused silica, titanium, FEP, PTFE, and ceramic
Autosampler	
Injection reproducibility	• Full loop: <1% RSD • Partial loop: <2% RSD
Injection valves	• 6 ports • 1/32 inch connections • Port-to-port volume <60 nL • Maximum pressure: 10,000 psi • 316 stainless steel with proprietary coating
Injection volume	2 µL to 50 µL
Sample carryover	<0.005% (benzophenone)

Sample capacity	• 6 positions for trays or microtiter plates
	• 54 2 mL vials per tray
	• Microtiter plates:
	  • Standard depth 96-well
	  • Deep 96-well
	  • 384-well

Sample cooling temperature	Minimum 4 °C (room temperature –20 °C)
Syringe volume	100 µL
Wetted parts	Stainless steel, PEEK, FEP, glass

Consumables and Accessories B

Order Parts

1. Order parts from SCIEX in any of the following ways:
   - **Telephone:** (877) 740-2129, Option 1 (toll-free, United States only), or go to sciex.com/contact-us to find a local office.
   - **E-mail:** Sales.Americas@sciex.com
   - **Fax:** (800) 343-1346
   - **Internet:** For customers in the United States, United Kingdom, and Germany order from store.sciex.com.
2. Order columns and SecurityLINK tubing from Phenomenex in any of the following ways:
   - **Telephone:** (310) 212-0555, or visit phenomenex.com
   - **E-mail:** info@phenomenex.com
   - **Fax:** (310) 328-7768

Consumables and Accessories

Table B-1 Consumables and Accessories-Ordered by Description

Part Number	Description
Autosampler	
5052374	Injection port
5043351	LCMS tool pump module
5043354	LCMS tool, tubing kit
5052374	Needle Seal 22 Gauge for Rheodyne 1/32
5031383	Syringe needle kit, with hubs and nuts for autosampler LCMS tool (3-pack)
4460861	Syringe, 100 µL
4460827	Syringe, replacement plungers (10-pack)
4371037	Tray, 54 well sample holder
4460859	Tubing, CTC DLW wash station waste tubing

Table B-1 Consumables and Accessories-Ordered by Description (continued)

Part Number	Description
Column Oven and Accessories	
5023403	Column oven
5017449	Column oven mounting kit, clamps and levers
5028230	Column oven mounting kit for Turbo V, DuoSpray, and IonDrive Turbo V ion sources
5041604	Column oven support rod kit (attaches to rear of system), PN 5028230 also required
Consumables	
200-00388	Inline filter assembly (includes 5 filter capsules)
5027467	Trap column cartridge holder, required for ChromXP cartridges
5028897	Trap column cartridge, ChromXP C18 3 µm, 120 Å, 0.3 mm i.d. × 1 cm (5-pack)
5028898	Trap column cartridge, ChromXP C18 3 µm, 120 Å, 0.5 mm i.d. × 1 cm (5-pack)
910-00103	Vial caps, snap caps with split septa (100-pack)
800-00209	Vials, 2 mL (100-pack)
Fittings and Ferrules	
5019820	Ferrule, 1/32 inch to 1/16 inch 10-32 port (5-pack)
910-00091	Ferrule, 1/8 inch, super flangeless (10-pack)
910-00087	Ferrule, stainless steel 1/32 inch (10-pack)
5019821	Fitting, 1/32 inch to 1/16 inch 10-32 port (5-pack)
200-00342	Fitting, column 6-32 threading
5019301	Fitting, drain collar
200-00252	Fitting, flangeless tube end 1/16 inch ferrule
200-00418	Fitting, headless PEEK, 1/32 inch o.d.
5016413	Fitting, union straight hex 6-32 F × 6-32 F
910-00085	Nut, 1/32 inch o.d., 0.45 inches long (10-pack)
910-00090	Nut, 1/8 inch super-flangeless (10-pack)
200-00446	Nut, PEEK, 1/8 Inch

Table B-1 Consumables and Accessories-Ordered by Description (continued)

Part Number	Description
5024174	Nut, gold, 6-32 thread 3/16 (1 nut)
200-00413	Union, stainless steel, 1/32 inch o.d. ports, 0.50 mm
Turbo V, DuoSpray, and IonDrive Turbo V Ion Source Accessories	
5016941	Grounding cable, for mass spectrometers made before 2018
Miscellaneous	
801-00075	Bottle, 1 L with drilled cap
801-00067	Bottle, 250 mL with drilled cap
5017797	Bottle, seal rinse
400-00465	Cable, USB, 3 m
700-00020	Cable, power supply to instrument
801-00063	Calibration kit for low-flow (1 µL/min to 10 µL/min) configuration
5018262	Calibration kit for micro-flow (5 µL/min to 50 µL/min) and high-flow (20 µL/min to 200 µL/min) configurations
801-00020	Calibration pipette, 20 µL
200-00396	Calibration pipette, 100 µL
200-00383	Calibration pipette, 200 µL
200-00329	Mobile phase filter with 10 µm frit
100-00567	Wrench, for 1/32 inch and 1/16 inch nuts
Tubing (Order from Phenomenex)	
AJ1-2224	Tubing, SecurityLINK PEEK-clad fused silica, 50 µm i.d, 15 cm
AJ1-2274	Tubing, SecurityLINK PEEK-clad fused silica, 50 µm i.d, 50 cm
AJ1-2294	Tubing, SecurityLINK PEEK-clad fused silica, 50 µm i.d, 75 cm
AJ1-22A4	Tubing, SecurityLINK PEEK-clad fused silica, 50 µm i.d, 100 cm
Tubing	
205-00089	Tubing, PEEK-clad fused silica, 25 µm i.d., 1/32 inch o.d., 5 cm
205-00091	Tubing, PEEK-clad fused silica, 25 µm i.d., 1/32 inch o.d.,10 cm
205-00038	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d.,15 cm
205-00069	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 10 cm

Table B-1 Consumables and Accessories-Ordered by Description (continued)

Part Number	Description
205-00039	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 20 cm
205-00040	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 30 cm
205-00070	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 5 cm
205-00041	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 50 cm
205-00049	Tubing, PEEK-clad fused silica, 50 µm i.d., 1/32 inch o.d., 75 cm
205-00061	Tubing, PEEK-clad fused silica, 75 µm i.d., 1/32 inch o.d., 30 cm
5019620	Tubing, mobile phase, 5 ft
300-00054	Tubing, waste 5/16 inch i.d.
Valve Parts	
5017798	Sample loop, 2 µL PEEK-clad fused silica (no fittings included)
5017799	Sample loop, 5 µL PEEK-clad fused silica (no fittings included)
205-00054	Sample loop, 10 µL PEEK-clad fused silica (no fittings included)
5040770	Sample loop, 50 µL stainless steel
200-00452	Valve pod and fittings
200-00326	Valve rotor seal

Parameters for the Advanced Autosampler Script

C

Use this script for finer control over more autosampler functions.

Recommended values for the parameters and their ranges are listed in Table C-1.

Note: For a partial-loop injection, change the value for Rear Volume to 0.

Note: The values with an asterisk (*) in the following table are mandatory. Use the values in the Recommended Value column.

Table C-1 Parameters in the Parameter Setup Tab: Advanced Autosampler Script

Parameter Name	Recommended Value	Minimum Value	Maximum Value
General Setting			
Tool*	LCP1	LCP1	
Pump Module*	Pump 1	Pump 1	
Cooled Stack*	Peltier Stack 1	Peltier Stack 1	
Bottom Sensing Sample Vial (see note)	Off	Off or On	
Height from Bottom of Sample Vial (mm)	2	0	32
Sample Temperature (°C)	8	4	40
Sample Parameter			
Front Air Gap (µL)	3	0	3
Front Volume (µL)	0	0	5
Rear Air Gap (µL)	3	1	3
Rear Volume (µL)	2	0	5
Sample Aspirate Flow Rate (µL/sec)	15	0.1	50
Injection Parameter			
Injector*	Injector LC 1	Injector LC 1	
Sample Injector Flow Rate (µL/sec)	5	0.1	10
Pre-inject Delay (ms)	100	0	1,000

Table C-1 Parameters in the Parameter Setup Tab: Advanced Autosampler Script (continued)

Parameter Name	Recommended Value	Minimum Value	Maximum Value
Post-inject Delay (ms)	200	0	2,000
Pullup Delay (ms)	200	0	2,000
Wash Parameter			
Wash Station*	LCMS Wash 1		
PreDip in Aqueous	1	0	2
PreDip in Organic	0	0	2
First Wash Solvent	2	1 or 2	
Second Wash Solvent	1	1 or 2	
Clean Valve Time with First Wash Solvent (s)	2	2	10
Clean Valve Time with Second Wash Solvent (s)	1	0	10
Clean Syringe Cycle with First Wash Solvent	1	0	10
Clean Syringe Cycle with Second Wash Solvent	1	0	10
Final Clean*	Off	Off or On	
Final Clean with Wash Solvent	1	1	2
Final Clean Valve Time (s)	2	1	10
Handshake Parameter			
Input Signal 1 (Pump Ready)*		Gr1Ready, GR 1Running, Gr2Ready, GR2Running	
Input Signal 2 (Pump Running)*		Gr1Ready, GR 1Running, Gr2Ready, GR2Running	
Output Signal (Start Gradient)*		StartGr1, StartG2	

Note: The **Bottom Sensing Sample Vial** option might not be compatible with some vials, particularly glass inserts. If a different type of vial is to be used, then use a new vial to run a test sample. Make sure that the results are acceptable before a complete run is set up.

External Interface Connections D

This section shows the external interface to other components. The external interface connections synchronize sample injection with data collection. The connector pin assignments are also described.

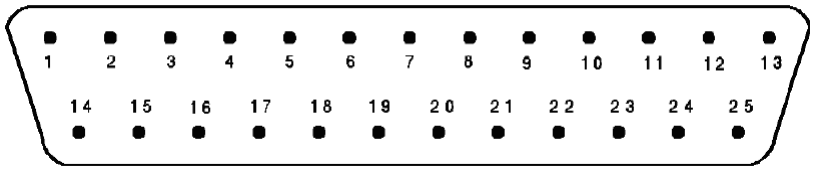
Table D-1 Pin Assignments for DB-25 Connector

Pin Number	Function	Pin Number Mate
Top Row (large side)		
1	Aux out	14
2	Divert valve trigger	15
3	Not used	16
4	Not used	17
5	Valve out	18
6	Run out	19
7	TE mode	20
8	Rdy out	21
9	A/D ground	
10	Not used	
11	A/D input	
12	Prk in	
13	Run in	
Bottom Row (small side)		
14	Aux out	1
15	Divert valve trigger	2
16	Not used	3
17	Not used	4
18	Valve out	5
19	Run out	6
20	TE mode	7

Table D-1 Pin Assignments for DB-25 Connector (continued)

Pin Number	Function	Pin Number Mate
21	Rdy out	8
22	Common ground	
23	Common ground	
24	Common ground	
25	Common ground	

Figure D-1 25-pin DB Connector Male Fitting



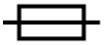
The 25-pin DB connector in the previous figure is a male connector viewed end on (that is, from the exposed male pin side and not from the hidden solder post side). D connectors have very small numbers inscribed on them indicating the pin numbers—a high power magnifier is often required to observe the numbers. Identify the numbers before to creating or modifying connectors to the pump. When the connector cover is removed to solder new connections, the location of the pins might appear reversed. Verify the orientation before soldering. The common grounds are all tied together and can be used interchangeably.

Glossary of Symbols E

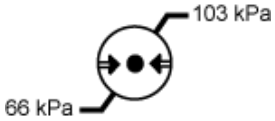
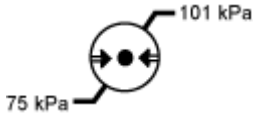
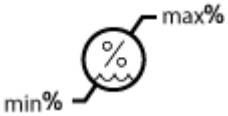
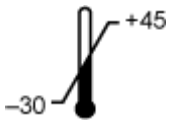
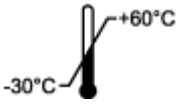
Note: Not all of the symbols in the following table are applicable to every instrument.

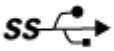
Symbol	Description
	Australian Regulatory Compliance Mark. Indicates that the product complies with Australian Communications Media Authority (ACMA) EMC Requirements.
	Alternating current
A	Amperes (current)
	Asphyxiation Hazard
EC REP	Authorized representative in the European community
	Biohazard
CE	CE Marking of Conformity
C US	cCSAus mark. Indicates electrical safety certification for Canada and USA.
REF	Catalog number

Symbol	Description
	Caution. Consult the instructions for information about a possible hazard. **Note:** In SCIEX documentation, this symbol identifies a personal injury hazard.
	China RoHS Caution Label. The electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.
	China RoHS logo. The device does not contain toxic and hazardous substances or elements above the maximum concentration values and it is an environmentally-friendly product that can be recycled and reused.
	Consult instructions for use.
	Crushing Hazard
	cTUVus mark for TUV Rheinland of North America
	Data Matrix symbol that can be scanned by a barcode reader to obtain a unique device identifier (UDI)
	Environmental Hazard
	Ethernet connection

Symbol	Description
	Explosion Hazard
	Eye Injury Hazard
	Fire Hazard
	Flammable Chemical Hazard
	Fragile
	Fuse
Hz	Hertz
	International safety symbol "Caution, risk of electric shock" (ISO 3864), also known as High Voltage symbol If the main cover must be removed, then contact a SCIEX representative to prevent electric shock.
	Hot Surface Hazard
	In Vitro Diagnostic Device
	Ionizing Radiation Hazard

Symbol	Description
	Keep dry. Do not expose to rain. Relative humidity must not exceed 99%.
	Keep upright.
	Lacerate/Sever Hazard
	Laser Radiation Hazard
	Lifting Hazard
	Magnetic Hazard
	Manufacturer
	Moving Parts Hazard
	Pacemaker Hazard. No access to people with pacemakers.
	Pinching Hazard

Symbol	Description
	Pressurized Gas Hazard
	Protective Earth (ground)
	Puncture Hazard
	Reactive Chemical Hazard
SN	Serial number
	Toxic Chemical Hazard
	Transport and store the system within 66 kPa to 103 kPa.
	Transport and store the system within 75 kPa to 101 kPa.
	Transport and store the system within the specified minimum (**min**) and maximum (**max**) levels of relative humidity, noncondensing.
	Transport and store the system within –30 °C to +45 °C.
	Transport and store the system within –30 °C to +60 °C.

Symbol	Description
	USB 2.0 connection
	USB 3.0 connection
	Ultraviolet Radiation Hazard
UK CA	United Kingdom Conformity Assessment Mark
VA	Volt Ampere (apparent power)
V	Volts (voltage)
	WEEE. Do not dispose of equipment as unsorted municipal waste. Environmental Hazard
W	Watts (power)
	*yyyy-mm-dd* Date of manufacture

Glossary of Warnings F

Note: If any of the labels used to identify a component become detached, then contact a Field Service Employee (FSE).

Label	Translation (if applicable)
FCC Compliance. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.	FCC Compliance. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.	FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

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To find software product documentation, refer to the release notes or software installation guide that comes with the software.

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