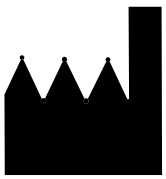
Eksigent MicroLC 200 Plus System

Operator Guide

eksigent®
part of AB SCIEX

RUO-IDV-05-1129-B
September 2014

For research use only. Not for use in diagnostic procedures.



AB Sciex Pte. Ltd.
Blk 33, #04-06
Marsiling Ind Estate Road 3
Woodlands Central Indus. Estate
SINGAPORE 739256

Contents


eksigent®
part of AB SCIEX

About this Guide

This guide is intended for laboratory technicians who are responsible for control and day-to-day maintenance of the Eksigent MicroLC 200 Plus system. It is assumed that the user of this guide is familiar with standard laboratory terminology.

Note: Read the safety instructions and the rest of this guide before using the Eksigent MicroLC 200 Plus system.

Symbols and Conventions

The following conventions are used throughout the guide.

Table 1-1 Symbols and Descriptions

Pictorial	Description
	The danger sign warns about a hazard. It calls attention to a procedure or practice which, if not adhered to, could results in injury or loss of life. Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.
	The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.
Caution:	The caution signal word denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment. Do not proceed beyond a caution sign until the indicated conditions are fully understood and met.
	The tip sign signals relevant information. Read this information, as it might be helpful.
	The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.
	This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an AB SCIEX field service employee for information concerning the decommissioning of equipment.

Safety Instructions

The following safety instructions apply to the Eksigent MicroLC 200 Plus system:

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

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

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

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

WARNING! Biohazard: When replacing tubing or fittings on the Eksigent MicroLC 200 Plus system, exposure to solvents may occur. It is therefore recommended that appropriate safety procedures be followed and personal protective equipment be used, according to the applicable Safety Data Sheets supplied by the solvent vendor.

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

WARNING! Electrical Shock Hazard: Do not turn the system on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and evaluate the system.

WARNING! Electrical Shock Hazard: Electrical damage may have occurred if any part of the system shows visible signs of damage, exposure to liquids or of having been transported under severe stress.

WARNING! Electrical Shock Hazard: Continue to exercise caution as capacitors inside the system may still be charged even after the system has been turned off.

WARNING! Electrical Shock Hazard: Disconnect power cords from the power supply before attempting any type of maintenance.

WARNING! Electrical Shock Hazard: The combination of the pump and autosampler with a LC/MS system may require additional safety measures as described by AB SCIEX. Refer to the mass spectrometer *Safety Guide* or *System User Guide* for instructions for the safe grounding on the LC/MS system.

WARNING! Electrical Shock Hazard: Use a grounding cable connected between the injection valve's sample loop and an appropriate grounding point at the LC/MS source. This supplementary grounding will reinforce the safety configuration specified by AB SCIEX.

Caution: Potential System Damage: Damage can result if the system is stored for prolonged periods under extreme conditions (for example, subjected to heat, water, etc.)

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

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

Caution: Potential System Damage: The system contains a number of sensitive electronic components that may be damaged if exposed to excessive line voltage fluctuations and/or power surges.

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

WARNING! Personal Injury Hazard: Use caution when working with any polymeric tubing under pressure:
– Always wear proper eye protection when near pressurized polymer tubing.
– Do not use polymer tubing that has been severely stressed or kinked.
– Do not use polymer tubing, in particular PEEK or DuPont Tefzel tubing, with tetrahydrofuran (THF), dimethylsulfoxide (DMSO), chlorinated organic solvents, concentrated mineral acids, such as nitric, phosphoric or sulfuric acids, or any related compounds.

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

Caution: Potential System Damage: An on-board lithium battery maintains the autosampler firmware when the instrument is turned off. It should only be replaced by a factory-authorized service engineer.

Caution: Potential Data Corruption: When you use the HTC-*xt* PAL autosampler for chromatographic analyses and observe a change in the retention of a particular compound, the resolution between two compounds or peak shapes, immediately determine the reason for the changes. Do not rely on the analytical results until the cause of the change is determined.

Qualified Personnel

After installing the system, the Field Service Employee (FSE) uses the *Customer Familiarization Checklist* to familiarize the customer on system operation, cleaning, and basic maintenance. Only AB SCIEX qualified personnel shall install, operate, and maintain the equipment. Equipment service shall only be conducted by AB SCIEX FSEs. Contact an AB SCIEX FSE for more information.

Equipment Use and Modification

Use the system indoors in a laboratory that complies with the environmental conditions recommended in the *Site Planning Guide*. If the system is used in an environment or in a manner not prescribed by AB SCIEX, the protection provided by the equipment can be impaired.

Unauthorized modification or operation of the system may cause personal injury and equipment damage, and may void the warranty. Contact an AB SCIEX representative for more information on servicing the instrument.

Regulatory Compliance

This system complies with the standards and regulations listed in this section. Applicable labels have been affixed to the system. For more information, see the Declaration of Conformance included with the system.

Canada

- **Safety**—CAN/CSA C22.2 No. 61010-1

Europe

- **Electromagnetic Compatibility**—EN 55011 Class A and EN 61326-1
- **Safety**—EN 61010-1
- **WEEE**—2002/96/EEC

USA

- **Safety**—UL 61010-1

International

- **Electromagnetic Compatibility**—CISPR 11 Class A, IEC 61326-1
- **Safety**—IEC 61010-1

Symbols and Labels

Table 1-2 Labels on the Eksigent MicroLC 200 Plus System

External Labels	Definition
	**WARNING:** Use only the original power supply.
	**Caution:** Risk of needle-stick puncture.
	Caution or Refer to the *Operator Guide*.
	**WARNING**: Flush Gas. Refer to the *Operator Guide* for instructions on using a purge gas with the stack cooler.
	Do not dispose of equipment as unsorted municipal waste (WEEE).
	Direct Current
A	Amperes (current)
V	Volts (voltage)
V-A	Volts - Amperes (power)

System Disposal (Waste Electrical and Electronic Equipment)

Do not dispose of system components or subassemblies, including computer parts, as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE (waste, electrical, and electronic equipment). To make sure that you safely dispose of this equipment, contact an FSE for instructions.

European Union customers: Contact a local AB SCIEX Customer Service office for complimentary equipment pick-up and recycling.

Related Documentation

- *PAL Installation and Operation User Manual*—contains detailed information about the autosampler. Printed and electronic copies are included.
- *Eksigent Control Software User Guide*—installed with the Eksigent control software
- *Analyst*® *Software Getting Started Guide* or *System User Guide*—installed with the Analyst software

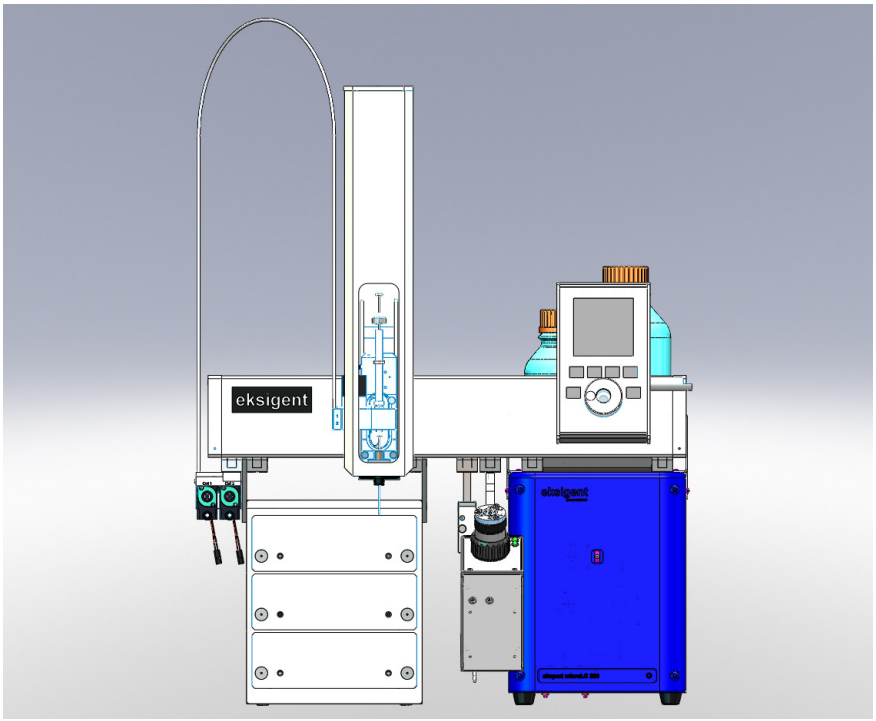
Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the web site at www.absciex.com.

The benefits of micro high-pressure liquid chromatography (HPLC) —high sensitivity, fast separations and reduced solvent consumption—can be achieved with careful attention to certain key operating procedures. There are subtle but important differences between liquid chromatography separations performed using 300 µm or 500 µm ID columns and those using 2.1 mm ID columns. This manual describes the basic operation and critical parameters to consider for routine and robust operation of the Eksigent MicroLC 200 Plus system.

System Description

Figure 2-1 Eksigent MicroLC 200 Plus System



The system includes:

- Binary gradient pumping system, with two flow rate configurations:
  - 5 µL/min to 50 µL/min
  - 20 µL/min to 200 µL/min
- Eksigent control software
- Eksigent system accessory kit, with three sample loops (2 µL, 5 µL, and 10 µL) a column, fittings, and other supplies (refer to System Accessory Kit on page 129)
- CTC Analytics HTC-*xt* PAL autosampler, including:
  - 3-drawer cooling stack holder (additional options available)

    - Dynamic load and wash feature (DLW) (refer to Dynamic Load and Wash on page 111)
    - PAL Loader software
    - Object Manager software
    - User documentation
- Column oven

Accessory Options

Depending upon the system you purchase, your system will have either:

- Column oven mounting kit (PN 5017397)

  or
- AB SCIEX mass spectrometer interface kit (PN 801-00084), including:
    - 65 µm stainless steel electrode (PN 5029342)
    - Column oven mounting kit (PN 5015996)
    - Clamp and rod assembly (PN 5016951)
    - Grounding kit (PN 5016941)
    - MS interface cable (PN 700-0049)

This chapter offers a brief tutorial on the use of the Eksigent MicroLC 200 Plus system, using either the Analyst® software or the Eksigent control software. The procedures described in this chapter assume the system has already been properly installed and initialized.

The Example Experiment

The experiment consists of two samples, each injected three times on the 5 cm HALO fused-core C18 column installed with the system. The sample runs are followed by one injection of a blank sample (50:50 water:acetonitrile) to flush the electrode. The instructions below assume a 2 µL sample loop is installed on the system and the column is heated to 35°C.

The mobile phases are water and acetonitrile. The aqueous channel for the pump (Channel A) will be filled with Mobile Phase A. The organic channel (Channel B) will be filled with Mobile Phase B, shown in Table 3-1.

Table 3-1 Mobile Phases for the Example Experiment

Mobile Phase	Mixture	Channel
Mobile Phase A	100% water:0.1% formic acid	Channel A
Mobile Phase B	100% acetonitrile:0.1% formic acid	Channel B

Each run is one minute and has a gradient from 90% to 10% water, except for the final run to flush the electrode, which is 20 minutes and is isocratic 50% water and 50% acetonitrile.

Guidelines for Sample Preparation

For the best results with the Eksigent MicroLC 200 Plus system, follow the guidelines below for sample preparation.

The flow path can clog if samples contain too much particulate matter.

- Use HPLC- or MS-grade solvents at all times.
- Avoid the use of non-volatile salts and buffers such as CHAPS, phosphate, TRIS, HEPES and perchlorates. These additives will foul the electrospray source and mass spectrometer orifice.
- Avoid overloading the column with sample.
  - For 0.3 mm and 0.5 mm ID columns—use <12 µg of material
  - For 1 mm ID columns—use <50 µg of material
- AB SCIEX recommends pre-filtering samples with 0.45 µm pore filters to avoid particulate contaminants which may cause clogging.
- If needed, centrifuge all samples at 10 000 RPM for 5 minutes to remove dust and particulates from the sample solution.
- If sample filtration is not sufficient, the following techniques can be used:
  - Protein precipitation (for biological samples)

- Liquid-liquid extraction
- Solid-phase extraction
- Addition of an in-line filter or guard column to the flow path (refer to Table C-4 on page 132 for part numbers)

Perform a Run Using the Analyst Software

The following instructions walk through the example experiment using the Analyst software. Use these instructions when using the mass spectrometer and the Eksigent MicroLC 200 Plus system as an integrated system.

These instructions assume you are familiar with the Analyst software. For more information, refer to the *Analyst® Software Getting Started Guide* or the *System User Guide*, available from the Start menu.

Complete the following steps in the order they appear:

1. Verify the Analyst Software Hardware Profile.
2. Load the Mobile Phases.
3. Flush the Injection Valve.
4. Enable the Column Oven.
5. Equilibrate the Eksigent MicroLC 200 Plus System.
6. Create the LC Methods.
7. Create the Acquisition Methods and the Batch.
8. Submit the Batch.
9. Monitor the Run.

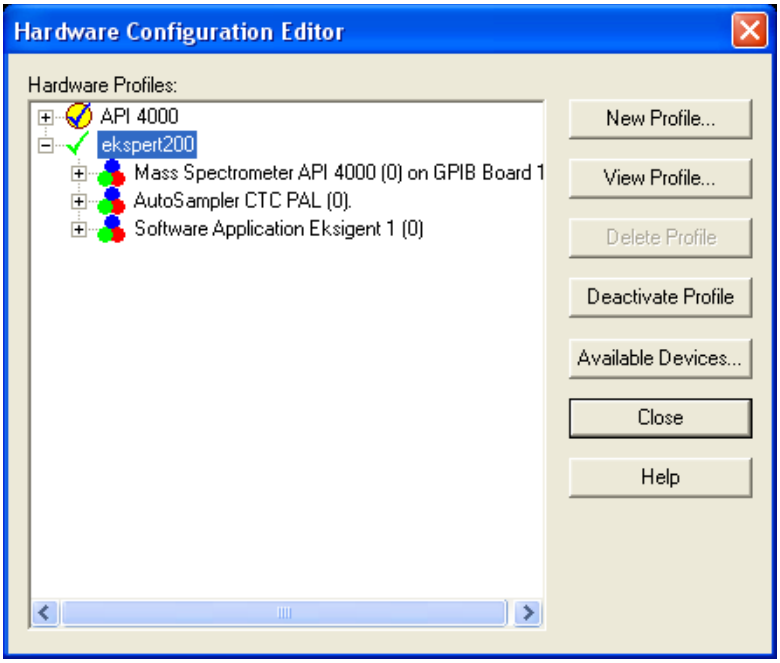
Verify the Analyst Software Hardware Profile

The active hardware profile must include the autosampler and the Eksigent control software to perform a run using the Analyst software. The FSE should configure your system for you, but if you are using a different computer or have uninstalled the Analyst software, the hardware profile may not be correct.

1. Start the Analyst software.
2. On the **Navigation** bar, under **Configure**, double-click **Hardware Configuration**.

3. In the **Hardware Configuration Editor** dialog, click each hardware profile to open it and locate a profile that contains a mass spectrometer, the autosampler, and the Eksigent control software as shown in Figure 3-1.

Figure 3-1 Hardware Profile Configured for the Eksigent MicroLC 200 Plus System

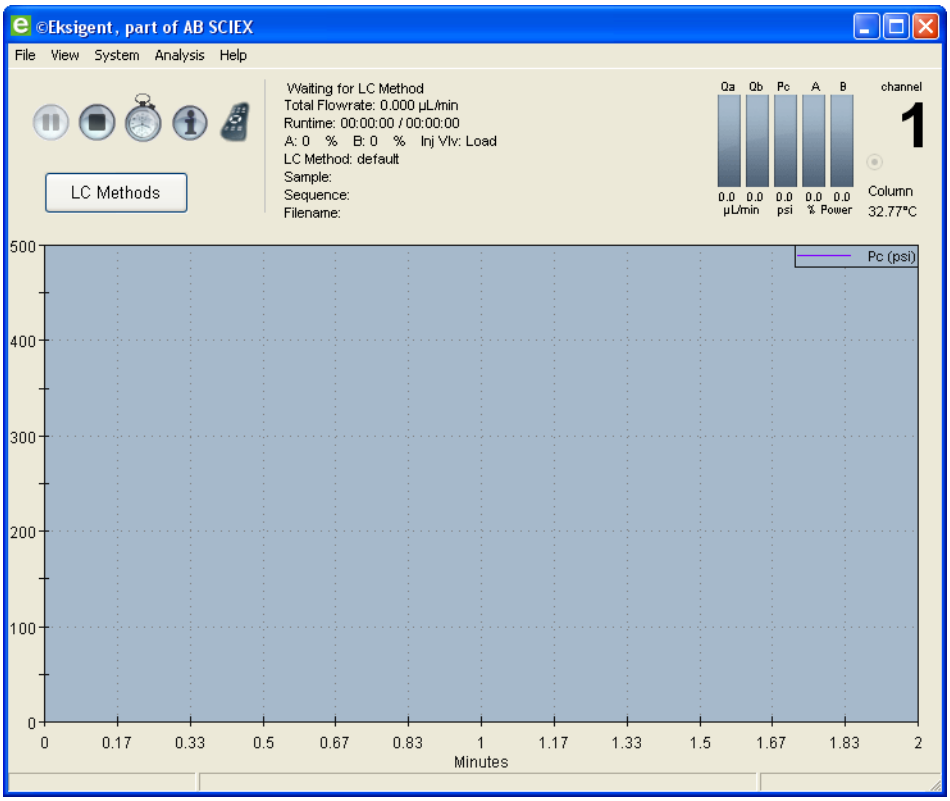


If an appropriate profile does not exist, create a profile as described in Create a Hardware Profile on page 19.

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

The active profile appears with a check, the Eksigent control software launches and the Eksigent control software **Acquisition** window appears (Figure 3-2).

Figure 3-2 Acquisition Window, Showing the LC Methods Button



If the **Acquisition** window does not appear (indicating that the Eksigent control software did not start) do the following:

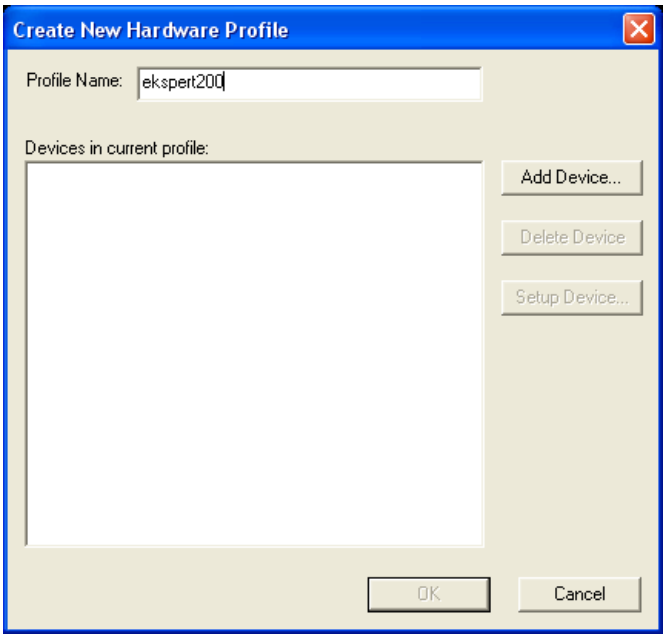
a. Close the Analyst software.
b. Select **Start > Programs > Eksigent > Driver Configuration**.
c. Uninstall, and then reinstall the Analyst software drivers.
d. Restart the Analyst software.

Note: Do not start the Eksigent control software manually. Instead, allow the Analyst software to start the Eksigent control software. (When the Eksigent control software is launched independently, the "**LC Methods**" button appears as "**Run Manager**".)

Create a Hardware Profile

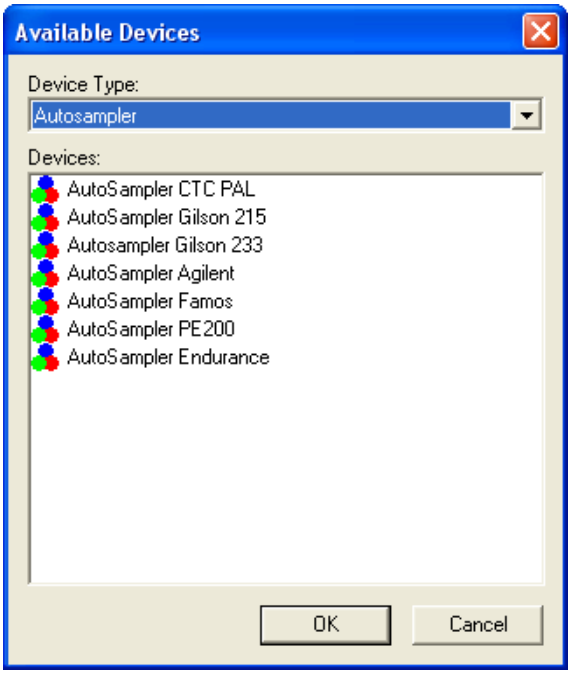
1. Click **New Profile** to open the **Create New Hardware Profile** dialog (Figure 3-3).

Figure 3-3 Create New Hardware Profile Dialog



2. Type a name for the profile in the **Profile Name** field.
3. Add the autosampler.
   a. Click **Add Device** to open the **Available Devices** dialog (Figure 3-4).

Figure 3-4 Available Devices Dialog, Showing Autosamplers

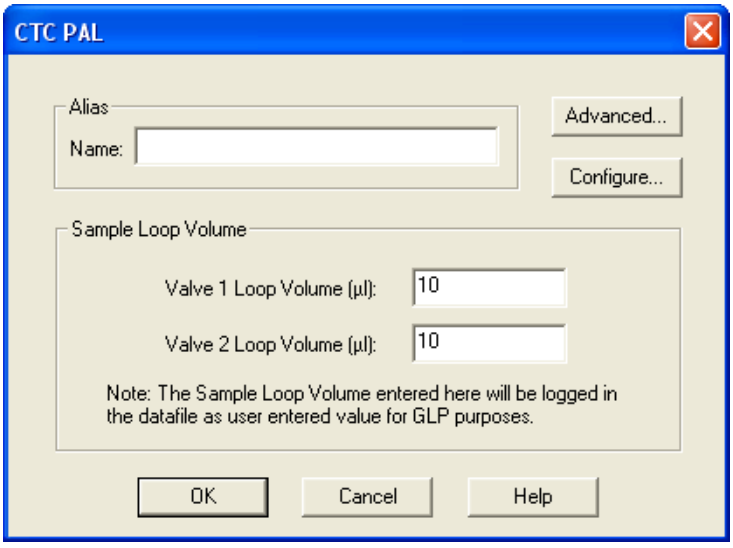


b. Select **Autosampler** in the **Device Type** list, click **AutoSampler CTC PAL** and then click **OK**.
c. Click **Setup Device** to open the **CTC PAL** dialog (Figure 3-5).
d. Type a volume in the **Valve Loop Volume** field.

Note: A 2 µL loop should be installed on the system. Enter a value between 5 µL and 100 µL in this field. The value is logged in the data file but does not affect the run. Future releases of the Analyst software will support smaller loop volumes (for example, 2 µL).

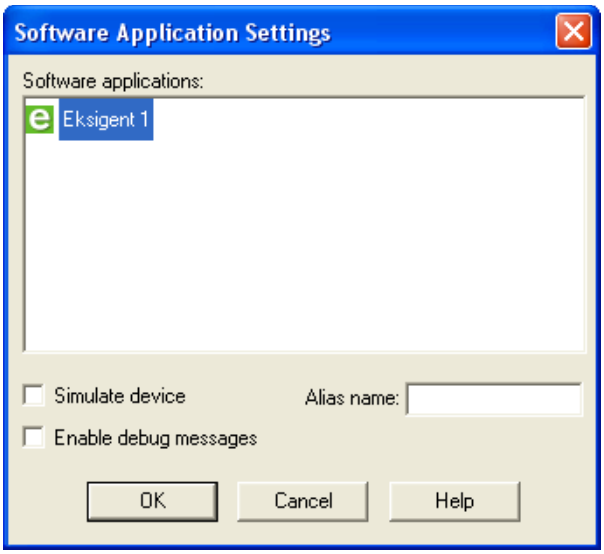
e. Click **Configure** and select the appropriate COM port.
f. Click **OK**.

Figure 3-5 CTC PAL Dialog



4. Click **Add Device** to add the Eksigent control software.
   a. Select **Software Application** in the **Device Type** list, then click **Software Application <not configured>**.
   b. Click **OK**.
   c. Click **Setup Device** to open the **Software Application Settings** dialog (Figure 3-6).
   d. Click **Eksigent 1** and then click **OK**.

Figure 3-6 Software Application Settings Dialog



5. Click **Add Device** to add the mass spectrometer.
   a. Select **Mass Spectrometer** in the **Device Type** list.
   b. Click the appropriate mass spectrometer in the list and then click **OK**.

Tip! The correct instrument is usually highlighted in the list.

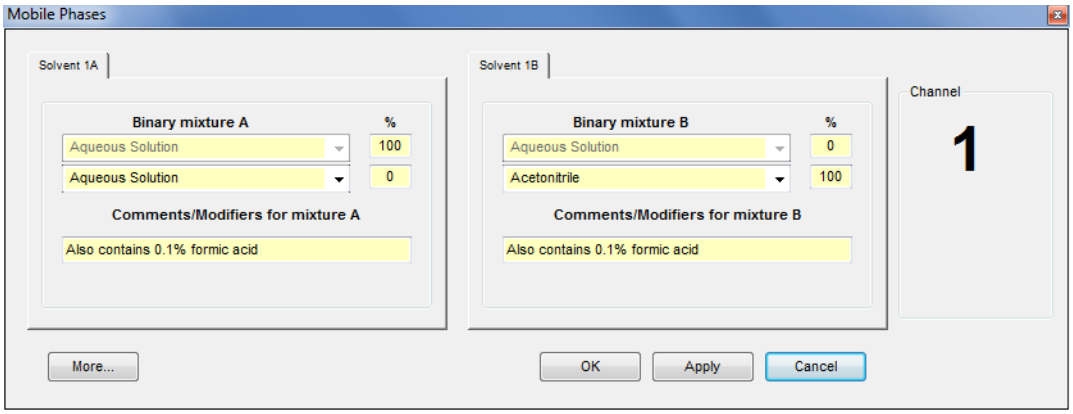
6. Click **OK** to save the profile and close the **Create New Hardware Profile** dialog

Load the Mobile Phases

For additional information about mobile phases for the Eksigent MicroLC 200 Plus system, refer to Recommendations for Mobile Phases on page 23.

1. Pour new mobile phase into the bottles, then insert the mobile phase transfer tubing and filters.
2. Specify the mobile phase information in the Eksigent control software.
   a. Click **System > Mobile Phases** to open the **Mobile Phases** dialog (Figure 3-7).
   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 lower list and type **100** in the **%** field.
   d. (Optional) Type any comments in the **Comments/Modifiers** fields.

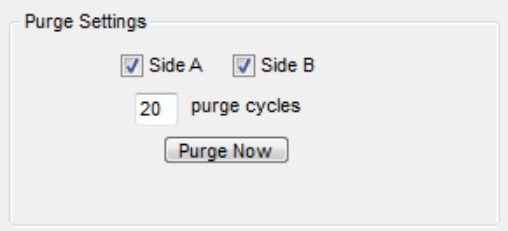
Figure 3-7 Mobile Phases Dialog—Settings for the Example Experiment



Note: If a mobile phase that is not listed in the **Mobile Phases** dialog is required for your experiment, either select a mobile phase from the list with a very similar viscosity or add a new one. Refer to Use a Custom Mobile Phase for instructions.

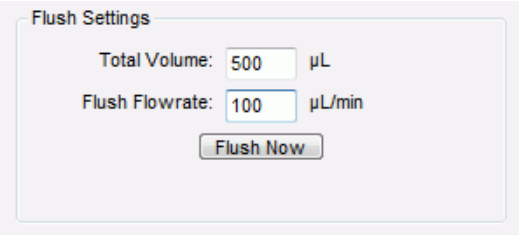
3. Purge the pumps a minimum of 20 times.
   a. Click **More** to display additional options in the dialog.
   b. In the **Purge Settings** section, select the pumps to be purged and set **purge cycles** to **20** (Figure 3-8).

Figure 3-8 Purge Settings Section of the Mobile Phases Dialog



   c. Click **Purge Now**.
      The pumps begin to execute purge cycles. While the pumps are purging, make sure the mobile phases are pulled through the mobile phase tubing to the pumps.
   d. Locate the waste tubing of the pumps being purged (the waste tubes are clear plastic tubing and emerge from the rear of the pump). After about 8 purges, the mobile phase should be purged through the waste tubing.
4. Flush the system.
   a. In the **Flush Settings** section, type **500** µL for the **Total Volume** (Figure 3-9).
   b. Set the **Flush Flowrate** based the configuration:
      – Type **50** µL/min for the low-flow configuration.
      – Type **100** µL/min for the high-flow configuration.

Figure 3-9 Flush Settings Section of the Mobile Phases Dialog—Settings for High-flow Configuration



c. Connect one end of a length of 1/32 inch OD tubing to the mobile phase outlet on the front of the pump and insert the other end in a waste vial.

Caution: Potential System Damage: Make sure that no LC columns are connected before proceeding with this operation. Flushing the system with a column connected could over-pressure the system and create leaks.

d. Click **Flush Now**.

e. When the flush sequence ends, click **OK** to close the dialog.

Recommendations for Mobile Phases

Mobile phases should be compatible with the following materials: 316L stainless steel, PTFE, FEP, PEEK, sapphire, glass, and fused silica. Some compatible solvents include water, acetonitrile, methanol, ethanol, n-propanol, isopropanol, hexane, heptane.

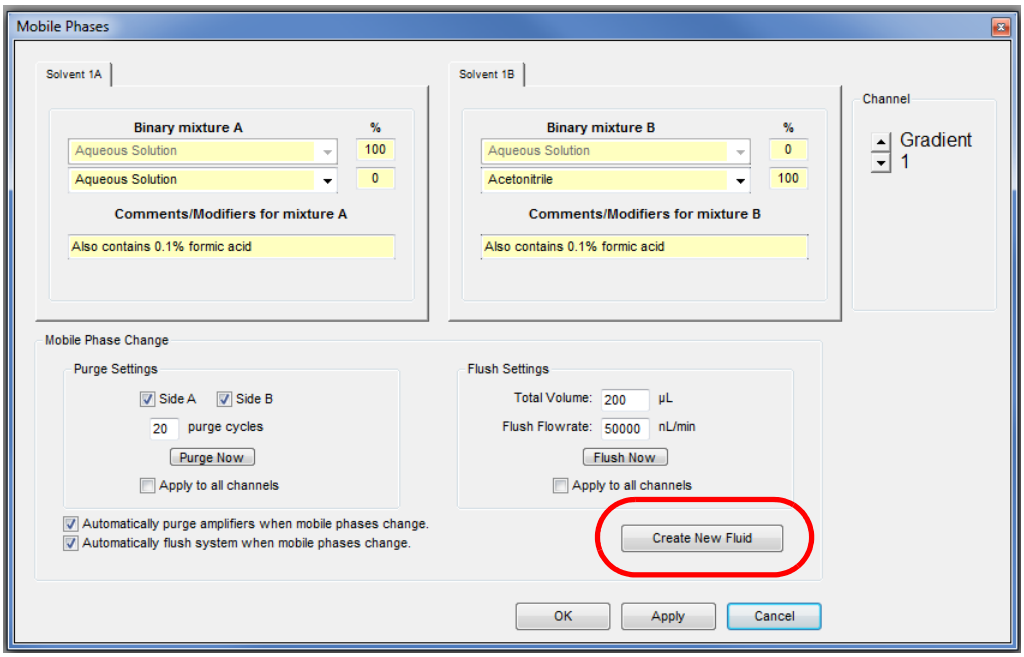
Use a Custom Mobile Phase

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, if you are using a mixture of two solvents in one bottle, create a custom mobile phase for the mixture.

Required Materials
• Calibration kit (with 100 µL and 200 µL pipettes) (PN 5018262)

1. Click **System > Mobile Phases** to open the **Mobile Phases** dialog.
2. Click **More** to show more options in the dialog (Figure 3-10).

Figure 3-10 Mobile Phases Dialog—Expanded



3. In the **Mobile Phase Change** section, click **Create New Fluid** to open the **Flowmeter Calibration** dialog.
4. Follow the steps in the **Flowmeter Calibration** dialog.

   Adding a custom mobile phase includes performing a flowmeter calibration. For the calibration, select the calibration pipette based on the system configuration:

   - Low-flow configuration—100 µL
   - High-flow configuration—200 µL

Flush the Injection Valve

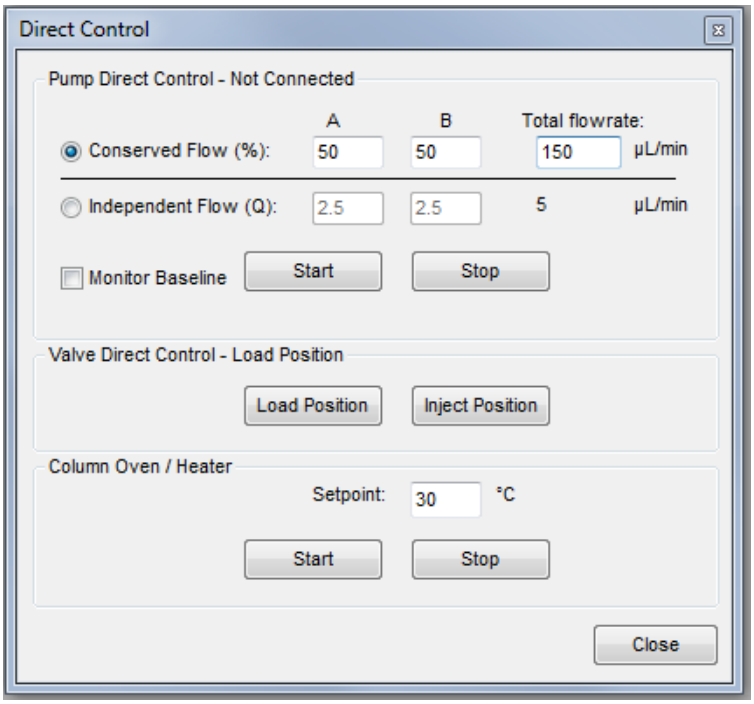
Flush the valve when the column is not connected to prevent introducing any contaminants from the valve to the column.

1. Make sure that the column is not connected.
2. Click **System > Direct Control** in the Eksigent control software to open the **Direct Control** dialog (Figure 3-11).

Tip! You can also open the **Direct Control** dialog by clicking  (Direct Control) in the **Acquisition** window.

3. In the **Pump Direct Control** section, select the **Conserved Flow** option and set both **A** (%) and **B** (%) to **50**.
4. Set the **Total flowrate** to **20 µL/min**.

Figure 3-11 Direct Control Dialog



5. Click **Start**.
6. In the **Valve Direct Control** section, alternate clicking **Load Position** and **Inject Position,** waiting ~ 10 seconds between each click.
7. Perform step 6 twice more.
8. Click **Load Position,** then click **Stop**.

Enable the Column Oven

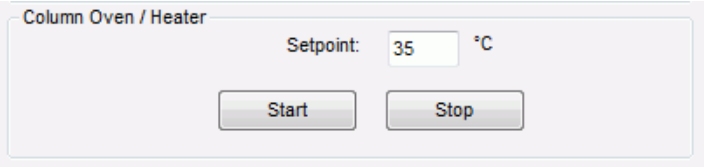
The temperature of the column compartment can be regulated, with a maximum temperature of 80°C.

1. Connect the column.

Tip! When reconnecting the column, follow the tips in Best Practices for Working with PEEKsil Tubing on page 62 to prevent crushing the PEEKsil tubing and potentially clogging the system.

2. Click **System > Direct Control** in the Eksigent control software to open the **Direct Control** dialog.
3. In the **Column Oven/Heater** section, type **35** in the **Setpoint** field (Figure 3-12).

Figure 3-12 Direct Control Dialog—Column Oven/Heater Section



4. Click **Start**.

   The oven will come to temperature quickly, however the column can take up to 30 minutes to stabilize to the oven temperature.

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

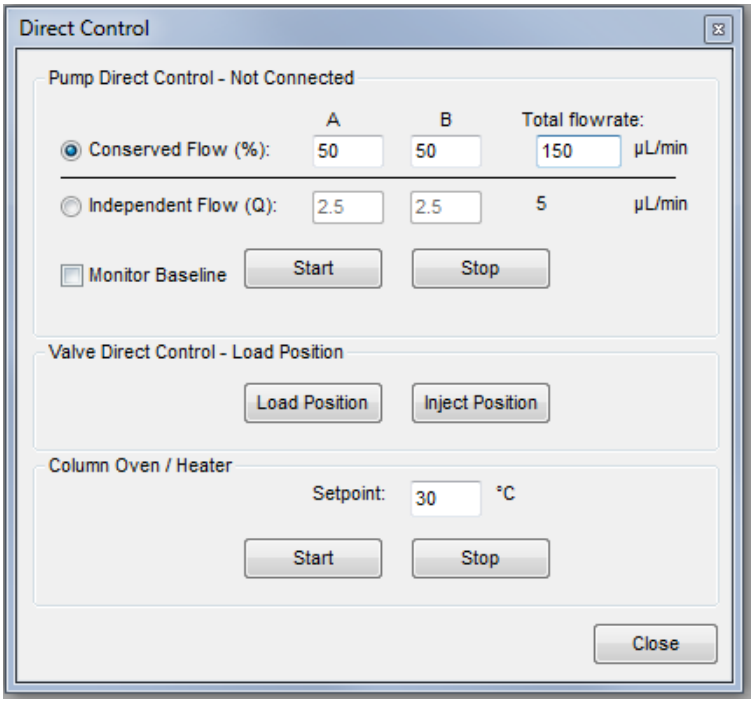
Equilibrate the Eksigent MicroLC 200 Plus System

1. Make sure that the LC column is connected.
2. Click **System > Direct Control** in the Eksigent control software to open the **Direct Control** dialog (Figure 3-13).
3. In the **Pump Direct Control** section, select the **Conserved Flow** option and set **A** (%) to **90** and **B** (%) to **10**.

   This is the mobile phase composition used for equilibration.
4. Set the **Total flowrate** based on the configuration:

- Type **50** µL/min for the low-flow configuration
- Type **150** µL/min for the high-flow configuration.

Figure 3-13 Direct Control Dialog—Settings for High-flow Configuration



5. Click **Start** to begin equilibration.
6. Allow the system to equilibrate for approximately 10 minutes, then click **Stop**.
7. Click **Close**.

Note: To avoid conflicts with the Analyst software after the system has equilibrated, be sure to click **Stop** to end pumping and then close the **Direct Control** dialog.

Create the LC Methods

An LC method contains the conditions used for separating the sample, including flow rate, flow mode, and mobile phase gradient.

The Eksigent MicroLC 200 Plus system has two flow modes:

- **Conserved**—you set the concentration and total flow rate and the system calculates the flow rate for each mobile phase.
- **Independent**—you set the flow rate for each mobile phase and the system calculates the total flow rate.

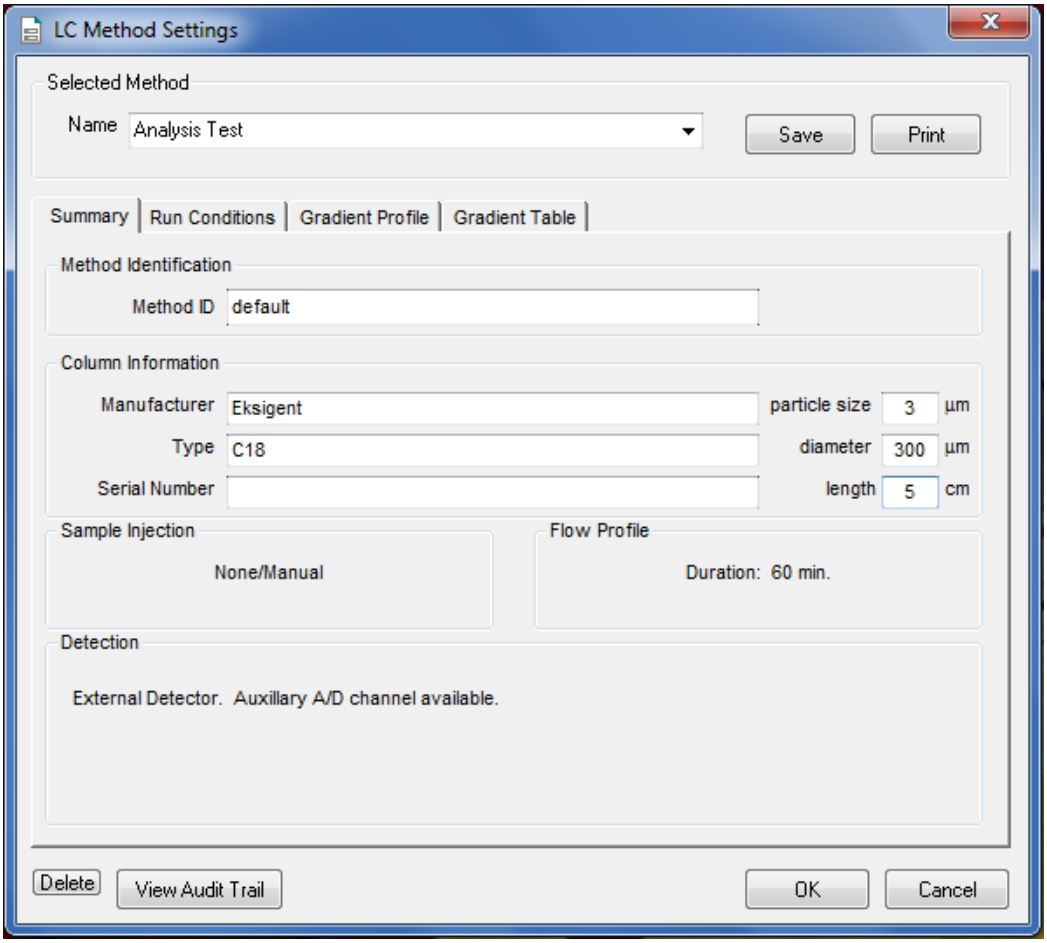
For the example experiment, two LC methods are required, a gradient for separating the sample and an isocratic method for flushing the system at the end of the batch or sequence.

Create the Separation Method

This section creates a gradient LC method called “Analysis Test”.

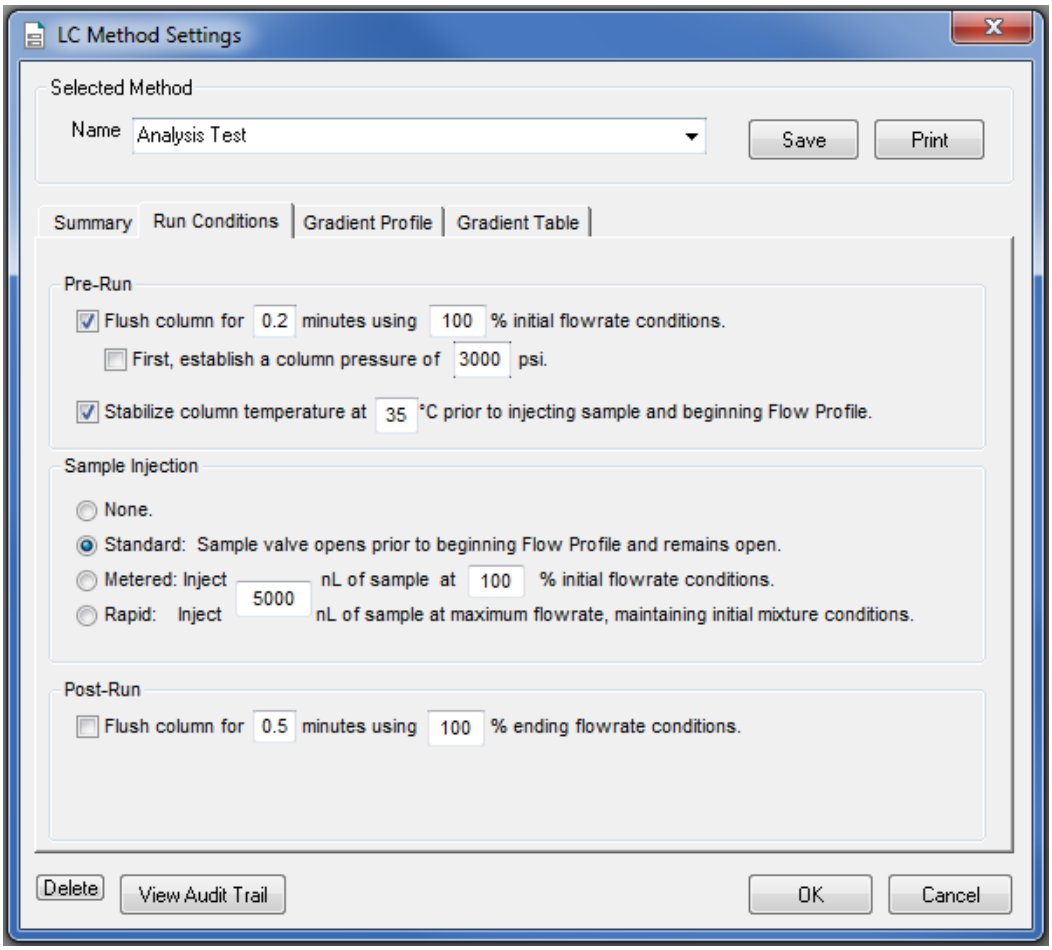
1. Click **LC Methods** to display the **LC Method Settings** dialog.
2. In the **Name** box, type “Analysis Test” as the name for the method, and then click **Save**.
3. In the Column Information section, specify the values as shown in Figure 3-14.

Figure 3-14 LC Methods Settings Dialog—Summary Tab



4. Click **Run Conditions** tab and specify the values shown in Figure 3-15.

Figure 3-15 LC Method Settings Dialog—Run Conditions Tab



The duration of the flow in the **Flush the column for** field should allow to 3 to 5 column volumes to pass through the system between runs. Depending on your experiment, a greater volume may be required.

When the **Stabilize column temperature** check box is selected, the run does not begin until the column reaches the specified temperature ±0.1°C.

There are four modes of sample injection. They differ in whether the sample loop stays in-line during the acquisition and how much of the contents of the sample loop is transferred to the column.

- **None**—the sample valve does not actuate during the acquisition.
- **Standard**—the sample valve moves to the inject position when acquisition begins and returns to the load position when acquisition ends. The sample loop remains in the flow path during acquisition.
- **Metered**—the valve switches to the inject position when acquisition starts and the specified volume of sample is delivered to the column by the LC pumps at the specified flow rate. After the specified volume is injected, the sample valve switches to the load position, removing the sample loop from the flow path.

The minimum injection volume (in nanoliters) is given by 2.5 x Q, where Q is the flow rate.

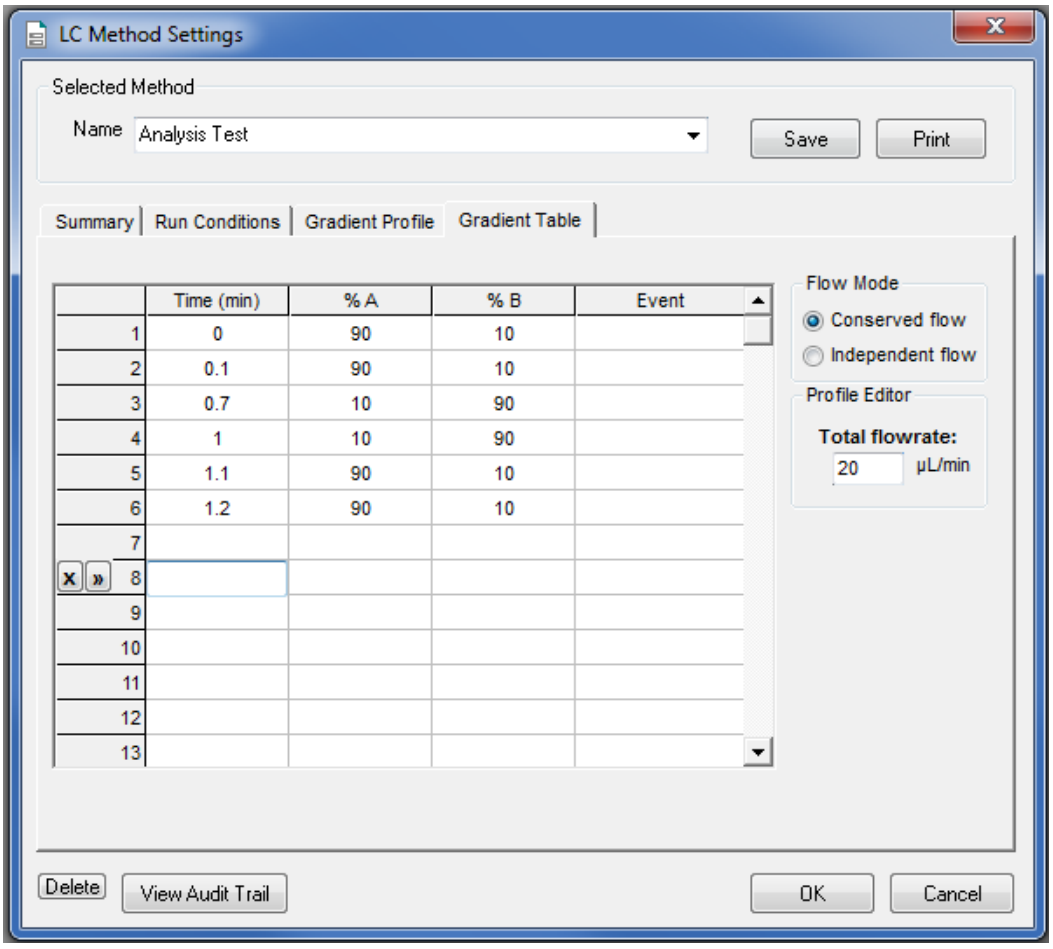
Use metered injection when the sample loop volume is larger than the volume to be injected on the column. With metered injection, the large sample loop doesn't add any extra dead-volume, preventing extra-column broadening.

- **Rapid**—the valve operates as during metered injection, except the LC pumps increase the flow rate during the injection in order to inject the sample quickly and prevent broadening.

Note: AB SCIEX does not recommend metered or rapid injection for quantitative experiments.

5. Click **Gradient Table** to set the flow mode, the gradient parameters, and the flow rate (Figure 3-16).

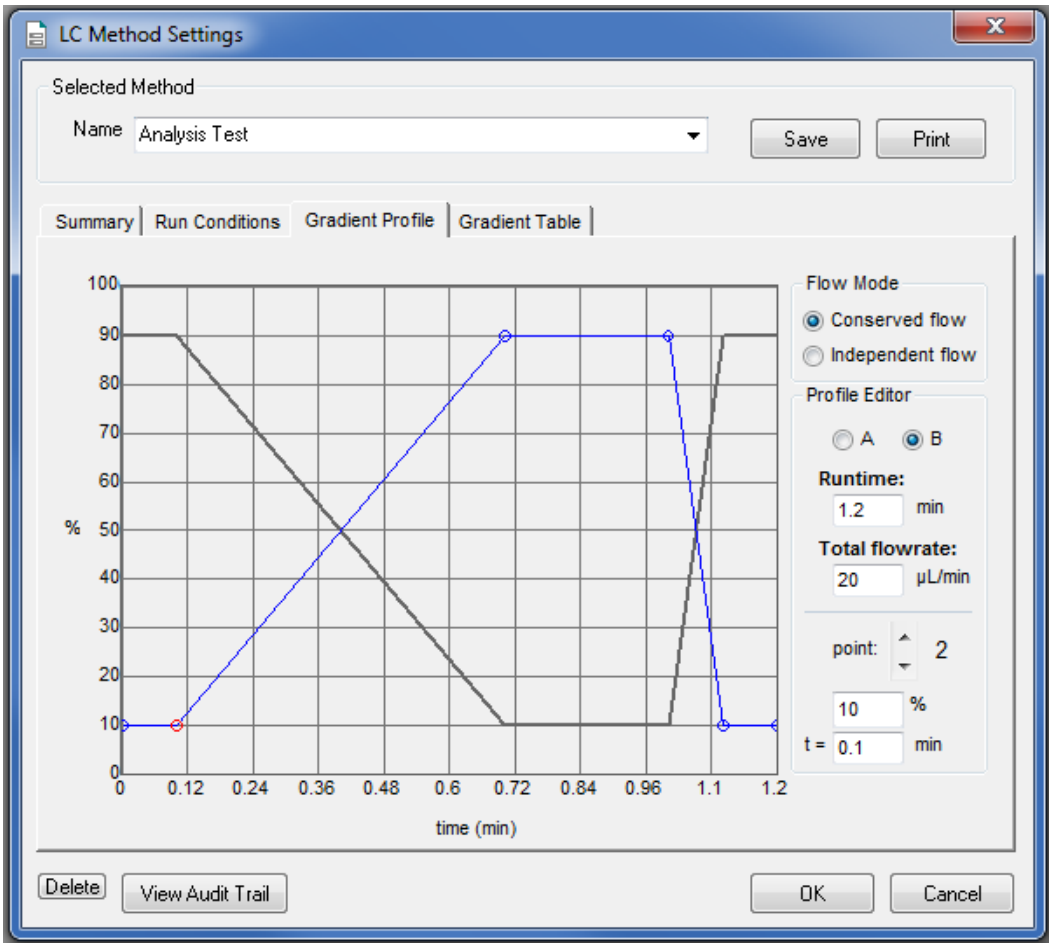
Figure 3-16 LC Method Settings Dialog—Gradient Table Tab



6. (Optional) To set additional events, such as valve switching and peak parking, type a **Time** and then select an event from the list in the **Event** cell.

7. Click **Gradient Profile** to view a graphical representation of the gradient (Figure 3-17).

Figure 3-17 LC Method Settings Dialog—Gradient Profile Tab



The last two steps in the method allow for the weaker solvent to flow through the sample loop before the next sample is injected.

Alternately, specify the gradient and event information in this tab:

a. Click the blue line to add a time point.
b. Drag the point to the mobile phase % for that time.
   The % for the other mobile phase adjusts automatically.
c. (Optional) Edit the **%** or **min** field to describe the change more exactly.
d. (Optional) Right-click the gradient profile to set additional events. Click and drag the event right or left to change its time.

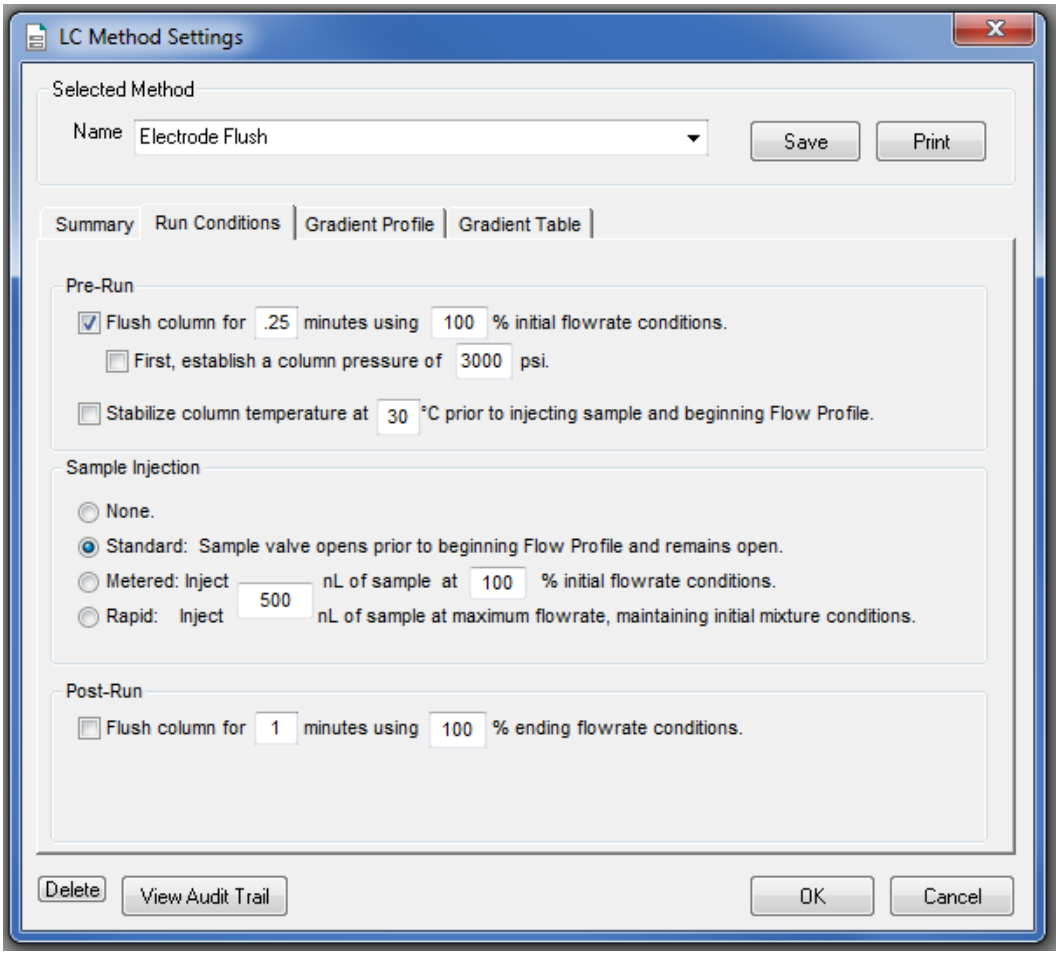
8. Click **Save,** then click **OK**.

Create the Electrode Flush Method

This section creates an LC method that is used to flush the ESI electrode after the samples have been run. The method flushes the system for 20 minutes with a 50:50 mixture of mobile phases A and B.

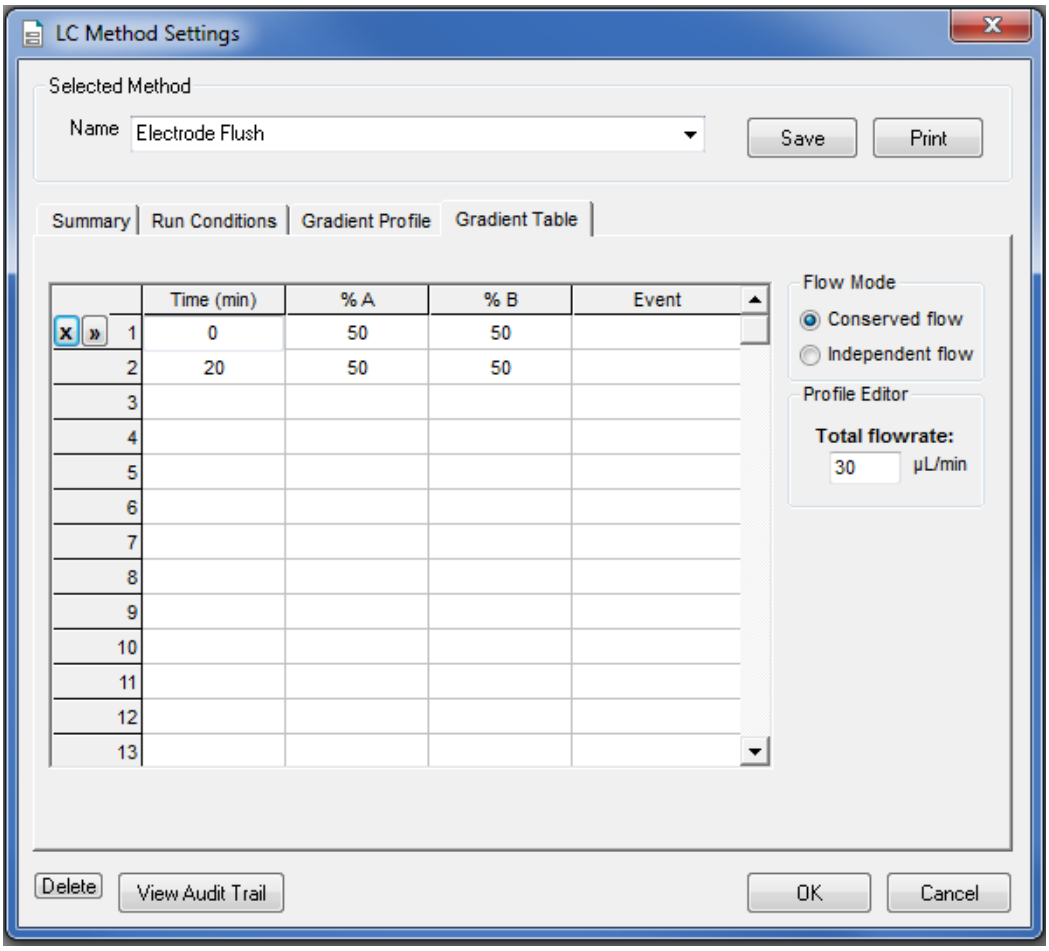
1. Click **LC Methods** to display the **LC Method Settings** dialog.
2. In the **Name** box, type a name for the method, such as “Electrode Flush” and then click **Save**.
3. Click **Run Conditions** tab and specify the values shown in Figure 3-18.

Figure 3-18 LC Method Settings Dialog—Run Conditions Tab



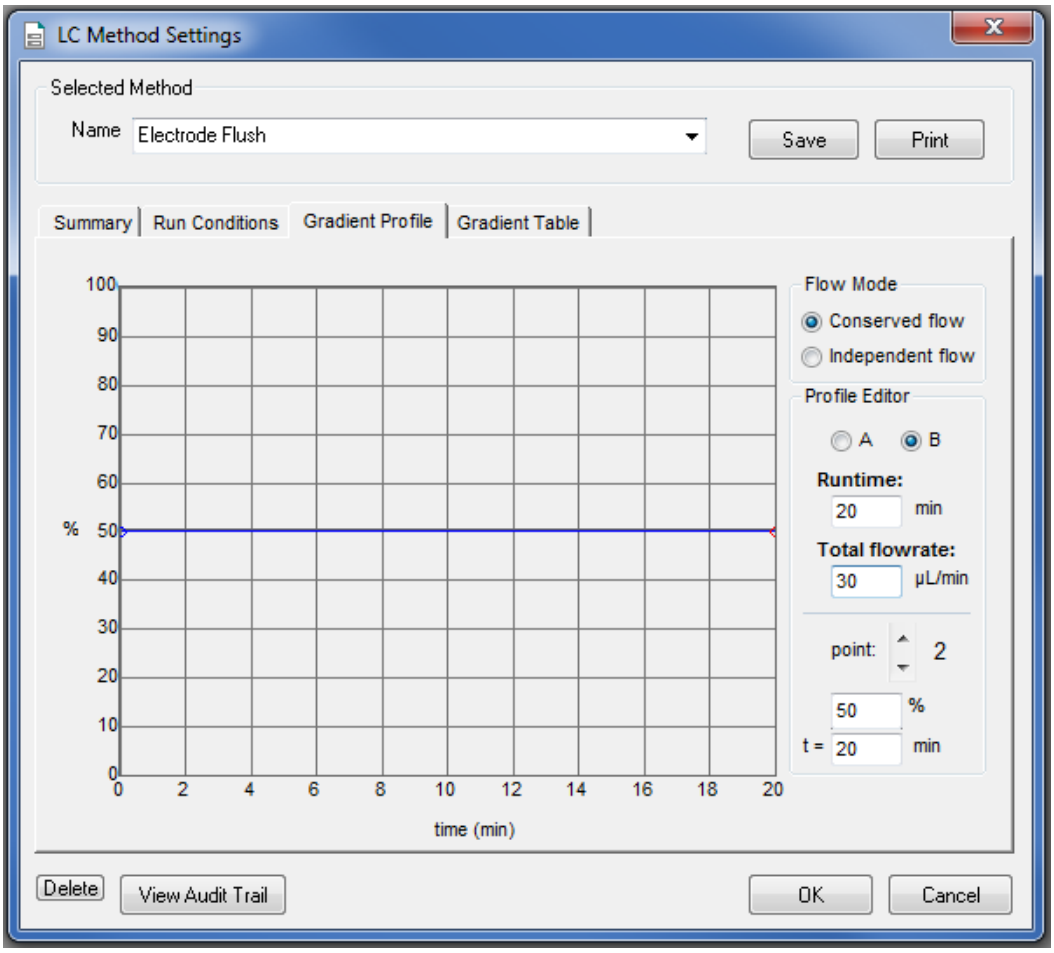
4. Click **Gradient Table** to set the flow mode, the gradient parameters, and the flow rate (Figure 3-19).

Figure 3-19 LC Method Settings Dialog—Gradient Table Tab



5. Click **Gradient Profile** to view a graphical representation of the gradient (Figure 3-20).

Figure 3-20 LC Method Settings Dialog—Gradient Profile Tab



6. Click **Save,** then click **OK**.

Create the Acquisition Methods and the Batch

The instructions below assume familiarity with the Analyst software. For more information, press **F1** to view the help or refer to the *Analyst® Software Getting Started Guide* or the *System User Guide* available from the **Start** menu or the *Customer Reference DVD*.

For the example experiment, two acquisition methods are needed, one for the samples and one to flush the electrode after the samples have been run.

Create the Acquisition Method for the Sample Data

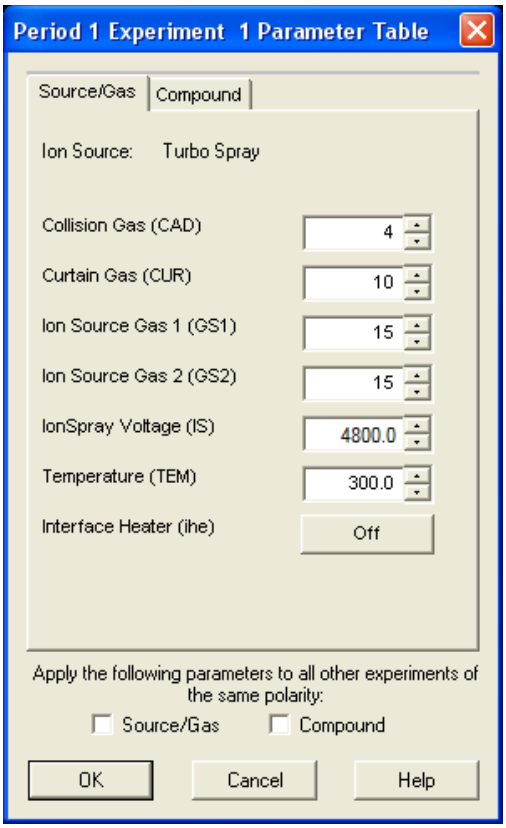
1. On the **Navigation** bar, under **Acquire**, double-click **Build Acquisition Method** to create the acquisition method.
2. Select the mass spectrometer method.
    a. In the **Acquisition Method** window, click **Mass Spec**.

b. Specify the parameters for the mass spectrometer acquisition.

c. Click **Edit Parameters** to set the **Source/Gas** parameters.

The parameters appropriate for micro-LC are different than for conventional liquid chromatography. Use the values in Figure 3-21 as a starting point and determine more optimal settings.

Figure 3-21 Initial Values for Source/Gas Parameters



For flow rates between 5 µL/min and 30 µL/min, these settings should be close to optimal. For higher flow rates, the temperature, curtain gas, GS1, and GS2 are typically higher, and the ion spray voltage is lower. Refer to Table 3-2 for suggested values.

Table 3-2 Source/Gas Parameters for 5 µL/min to 200 µL/min Flow Rates

Parameter	Suggested Ranges
Curtain Gas (CUR)	10 to 30
Ion Source Gas 1(GS1)	15 to 40
Ion Source Gas 2 (GS2)	15 to 50
Temperature (TEM)	150 to 400
Ion Spray Voltage (ISV)	4500 to 5000

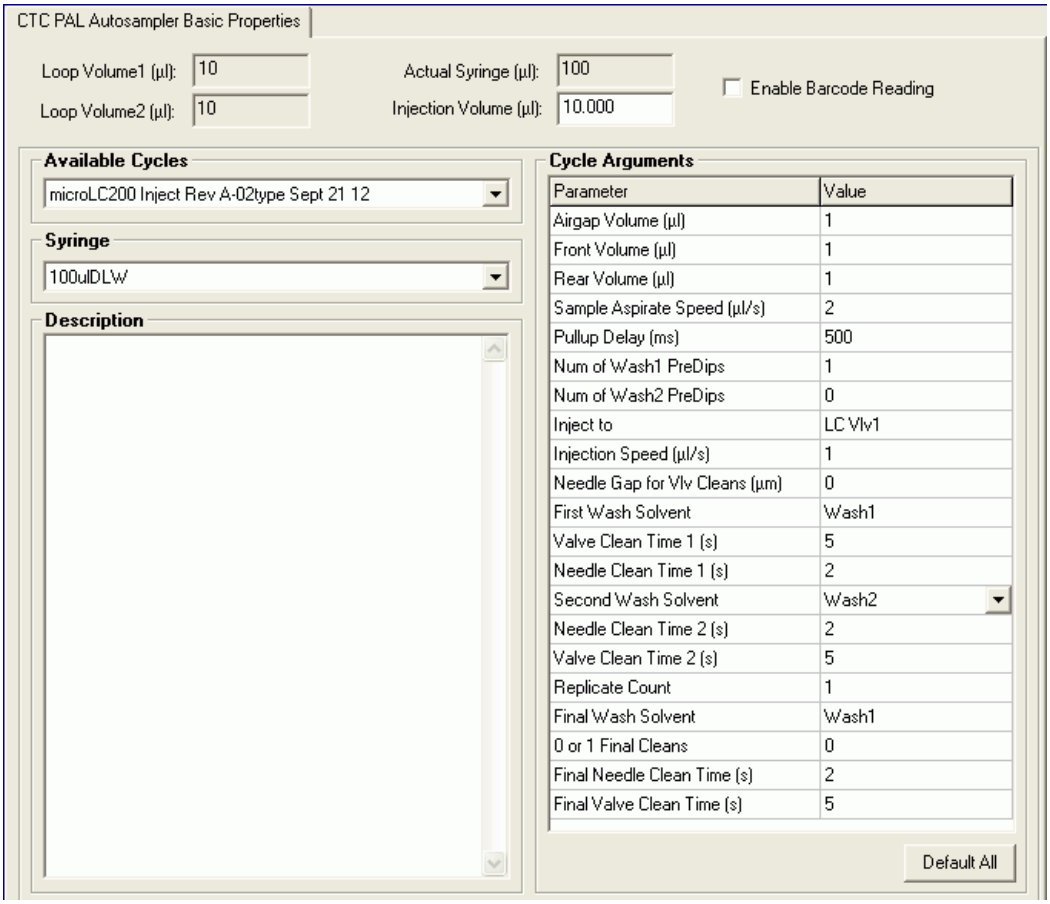
Tip! Higher temperatures can lead to clogged electrodes on the mass spectrometer. As appropriate, use lower temperatures for your experiments.

d. Click **OK** to save the source and gas parameters.

3. Select the autosampler method.

   a. In the **Acquisition Method** window, click **CTC PAL Autosampler**.

Figure 3-22 CTC Autosampler Basic Properties Tab in the Acquisition Method Window, Showing microLC200-Injection-Rev B



   b. Select **microLC200-Injection-Rev B** in the **Available Cycles** list.
   Refer to Autosampler Method on page 115 for more information about the method.

Note: The autosampler method installed with the Eksigent MicroLC 200 Plus system may have a different name than listed above. Use the most recent autosampler method supplied by AB SCIEX.

4. Set parameters for the autosampler method.

   a. Select **100ulDLW** in the **Syringe** list.
   b. In the **Injection Volume** field, type **10.**
   Refer to About the Volumes in the Autosampler Method on page 38.
   c. In the Cycle Arguments table, type **1** for both **Front Volume** and **Rear Volume**.
   d. In the Cycle Arguments table, select **Wash2** for **Second Wash Solvent.**

Note: When entering values less than 1, the Cycle Arguments table does not accept a zero before the decimal point. To enter a number less than 1, type a period and then the number.

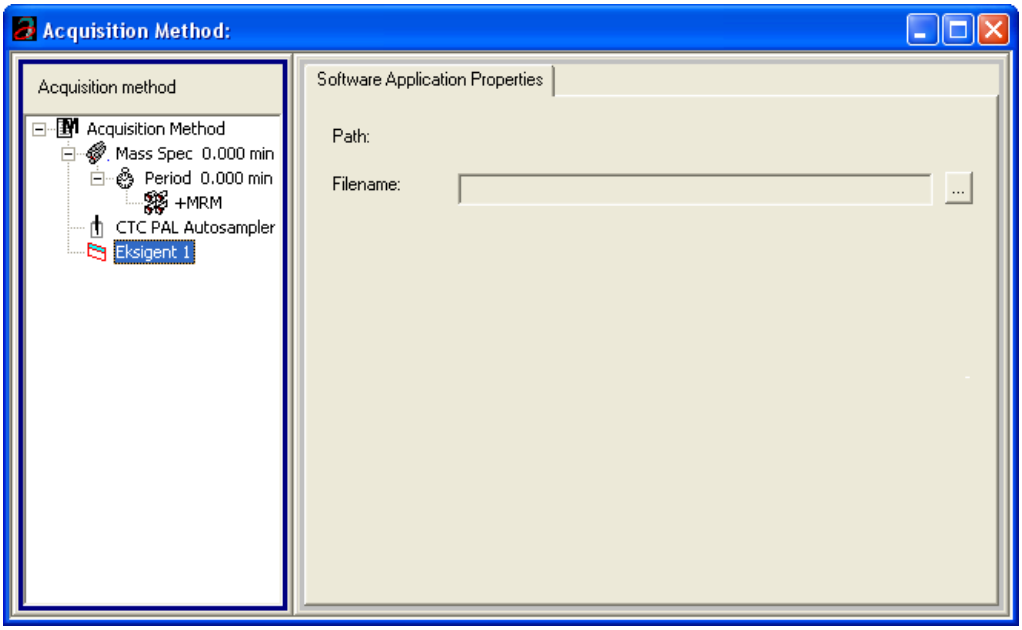
The parameters for this method (after making the edits above) are shown in Table 3-3.

Table 3-3 Parameters for the Autosampler Method

Parameter	Value	Parameter	Value
Airgap Volume (µL)	1	Valve Clean Time 1 (s)	5
Front Volume (µL)	1	Needle Clean Time 1 (s)	2
Rear Volume (µL)	1	Second Wash Solvent	Wash2
Sample Aspirate Speed (µL/s)	2	Needle Clean Time 2 (s)	2
Pullup Delay (ms)	500	Valve Clean Time 2 (s)	5
Num of Wash1 PreDips	1	Replicate Count	1
Num of Wash2 PreDips	0	Final Wash Solvent	Wash1
Inject to	LCVlv1	0 or 1 Final Cleans	0
Injection Speed (µL/s)	1	Final Needle Clean Time (s)	2
Needle Gap for Vlv Cleans (mm)	0	Final Valve Clean Time (s)	5
First Wash Solvent	Wash1		

5. Select the LC Method.
   a. In the **Acquisition Method** window, click **Eksigent 1** (Figure 3-23).

Figure 3-23 Acquisition Method—Selecting the LC Method



b. In the **Software Application Properties** tab, click **...** (Browse) to view the available LC methods.
c. Click **Analysis Test** and then click **Open**.

6. Click **File > Save**, type **Example Method** for the name of the method.

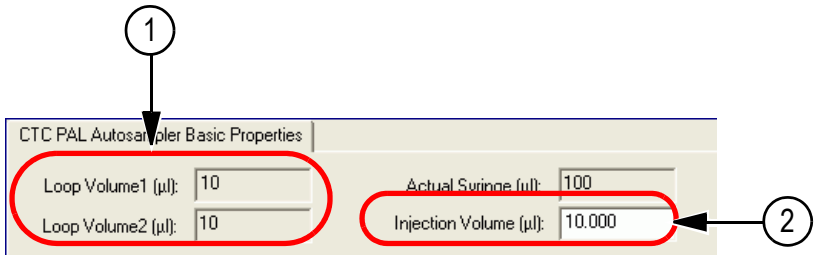
Create the Acquisition Method for Flushing the Electrode

This method is used at the end of the batch to flush the electrode. Use the method above as a starting point with the following changes:

1. Select the mass spectrometer method created above.
2. Click **File > Save As** and type **Electrode Flush Method** for the name of the method.
3. Edit the mass spec parameters to lower the source temperature.
   a. In the **Acquisition Method** window, click **Mass Spec**.
   b. Click **Edit Parameters** to set the **Source/Gas** parameters.
   c. In the **Temperature (TEM)** field, type **75**.
   d. Click **OK** to save the source and gas parameters.
4. Select the LC Method.
   a. In the **Acquisition Method** window, click **Eksigent 1**.
   b. Click **...** (Browse) to view the available LC methods.
   c. Click **Electrode Flush** and then click **Open**.
5. Click **File > Save**.

About the Volumes in the Autosampler Method

Figure 3-24 CTC Autosampler Basic Properties Tab, Detail



Item	Description
1	Loop Volume field
2	Injection Volume field

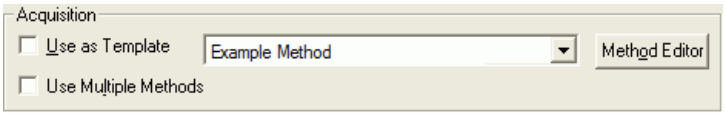
- The **Loop Volume** fields (item 1) indicates the volume of the sample loop installed on the injection valve. This number is entered during installation, when the Analyst software hardware profile is created. These volumes are not used in the method.
- The **Injection Volume** field (item 2) is the volume aspirated by the autosampler needle for loading in the loop. The recommended volume for this field is 2 to 5 times the sample loop volume.

- For standard injection mode, the volume of the sample injected on the column is determined by the volume of the sample loop.
- For other injection modes, the volume of the sample injected on the column is specified in the Sample Injection section of the **LC Method Settings** dialog (Figure 3-15).

Create the Acquisition Batch

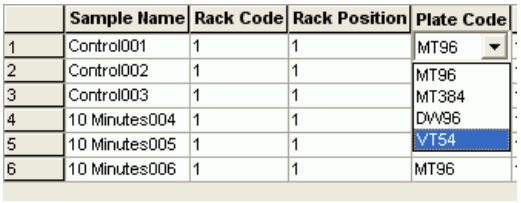
1. On the **Navigation** bar, under **Acquire**, double-click **Build Acquisition Batch** to create the Acquisition Batch.
2. Specify the required information in the **Samples** tab of the **Batch Editor** window.
   a. In the **Set** field, type **Test Table** and then click **Add Set**.
   b. Click **Add Samples** and add a sample named “Control” with **Number** set to 3.
   c. Repeat step b and add a second sample named “10 minutes”.
   d. Select **Example Method** (the acquisition method you created above) in the list in the **Acquisition** section (Figure 3-25).

Figure 3-25 Acquisition Section—Selecting the Acquisition Method



   e. For each sample in the table, click **Plate Code** and select **VT54** from the list (Figure 3-26).

Figure 3-26 Sample Table—Selecting the Plate Code

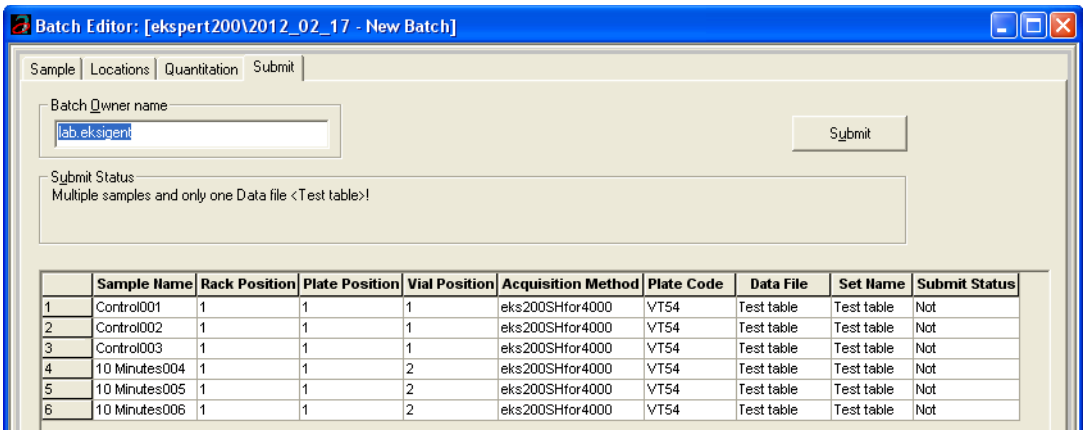


3. Add the blank sample for the flushing the electrode.
   a. Click **Add Samples** and add a sample named “Flush” with **Number** set to 1.
   b. Select **Electrode Flush Method** (the method created above) in the list in the **Acquisition** section.
   c. Click **Plate Code** and select **VT54** from the list.

Submit the Batch

1. Place the sample vials in positions 1 and 2 of tray 1 of the autosampler.
2. Place a vial containing the blank (50:50 water:acetonitrile) in position 3.
3. Click the **Submit** tab of the **Batch Acquisition** dialog, then click **Submit** to add the samples to the queue.

Figure 3-27 Batch Acquisition Dialog—Submit Tab



	Sample Name	Rack Position	Plate Position	Vial Position	Acquisition Method	Plate Code	Data File	Set Name	Submit Status
1	Control001	1	1	1	eks200SHfor4000	VT54	Test table	Test table	Not
2	Control002	1	1	1	eks200SHfor4000	VT54	Test table	Test table	Not
3	Control003	1	1	1	eks200SHfor4000	VT54	Test table	Test table	Not
4	10 Minutes004	1	1	2	eks200SHfor4000	VT54	Test table	Test table	Not
5	10 Minutes005	1	1	2	eks200SHfor4000	VT54	Test table	Test table	Not
6	10 Minutes006	1	1	2	eks200SHfor4000	VT54	Test table	Test table	Not

4. Click (View Queue) in the toolbar to open the **Queue Manager (Local)** dialog.
5. Click (Equilibrate) to equilibrate the Eksigent MicroLC 200 Plus system and the mass spectrometer.
6. When the equilibration is finished, click (Start Sample) to begin the run.

Monitor the Run

View the LC chromatogram and spectral data in Explore mode in the Analyst software.

View flow rate and pressure information in the **Acquisition** window of the Eksigent control software (Figure 3-35 and Figure 3-36).

Perform a Run Using the Eksigent Control Software

The following instructions demonstrate an example experiment using the Eksigent control software independently from the Analyst software.

Use these instructions when:

- the Eksigent MicroLC 200 Plus system is not connected to a mass spectrometer (you are using a different type of detector)

  or

- you are using the mass spectrometer as a standalone detector

Complete the following steps in the order they appear:

1. Turn on the System.
2. Prepare the Eksigent MicroLC 200 Plus System.
3. Create the LC Methods.
4. Create the Run Table.
5. Start the Run.
6. Monitor the Run.

For more information, refer to the *Eksigent Control Software User Guide*, installed with the software.

Turn on the System

1. If the system is not already on, turn on the power switch on the autosampler power supply.
2. Turn on the computer and start the Eksigent control software.

Tip! For more information about the Eksigent control software, click **Help > Help**.

Prepare the Eksigent MicroLC 200 Plus System

1. Load the mobile phases.

   Refer to Load the Mobile Phases on page 21.

2. Flush the injection valve.

   Refer to Flush the Injection Valve on page 24.

3. Enable the column oven.

   Refer to Enable the Column Oven on page 25.

4. Equilibrate the system.

   Refer to Equilibrate the Eksigent MicroLC 200 Plus System on page 26.

Create the LC Methods

1. In the **Acquisition** window of the Eksigent control software, click **Run Manager**.
2. In the **Run Manager** window, click **LC Methods**.
3. Follow the instructions in Create the Separation Method on page 28, starting with step 2 to create an LC method named “Analysis Test”.
4. Follow the instructions in Create the Electrode Flush Method on page 32 to create an LC method named “Electrode Flush”.

Create the Run Table

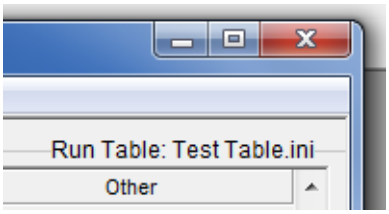
The run table links an autosampler method, an LC method, and an analysis method to a sample vial and tray. You can also add descriptive information related to the sample or analysis. Each row in the run table represents one sample injection (unless the Replicates cell is >1).

Autosampler methods contain the parameters used for loading the sample into the injection valve and for rinsing the autosampler syringe and injection port. The autosampler method used below is optimized for the DLW option on the autosampler.

1. Click **Edit > Erase Table** to create a new run table.
2. Click **File > Save As**, type **Test Table** in the **File name** field, and then click **Save** to create a template for a new run table.

   The name of the run table appears in the upper left corner of the window (Figure 3-28).

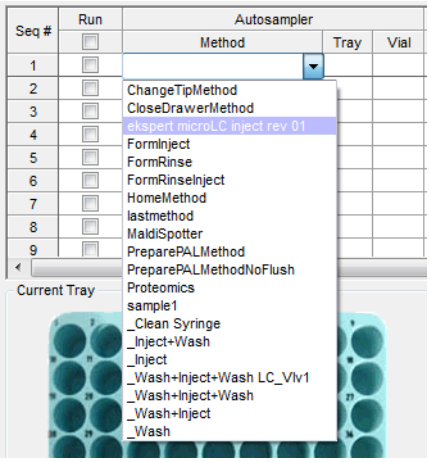
Figure 3-28 Run Manager Window, Showing Table Name



3. Specify the information for the first injection of the first sample.
   a. In the first line of the run table, double-click the **Autosampler Method** cell and select **microLC200-Injection-Rev B** from the list (Figure 3-29).

Note: The autosampler installed with the Eksigent MicroLC 200 Plus system may have a different name than listed above. Use the most recent autosampler method supplied by AB SCIEX.

Figure 3-29 Run Manager Window, Showing Autosampler Method Selection



The software automatically sets Tray and Vial to 1.

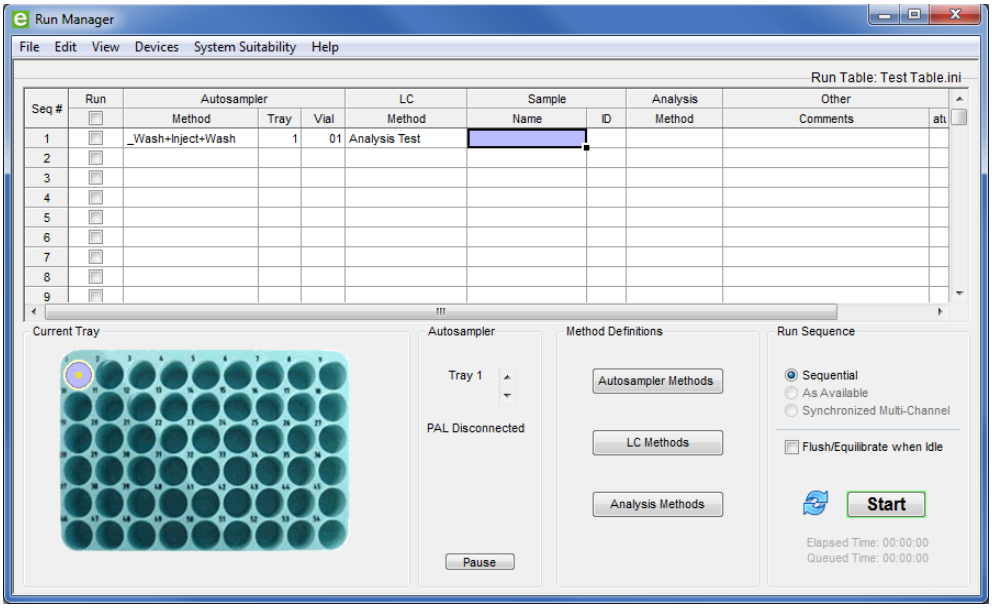
b. Double-click the **LC Method** cell and select **Analysis Test**.

c. Type **Control** in the **Sample Name** cell.

d. (Optional) Type information in the **Sample ID** or **Comments** cells.

The first line should look like Figure 3-30.

Figure 3-30 Run Manager Window, Showing One Injection



4. (Optional) Review the method.

a. Click **Autosampler Methods** to open the **Autosampler Method Editor** dialog.

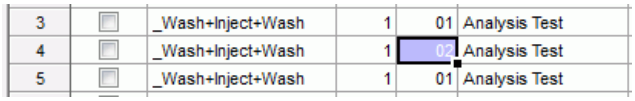
b. Select the method in the **Filename** list.

c. Click **Cancel** to close the dialog without saving changes.

Note: AB SCIEX recommends that you do *not* edit autosampler methods.

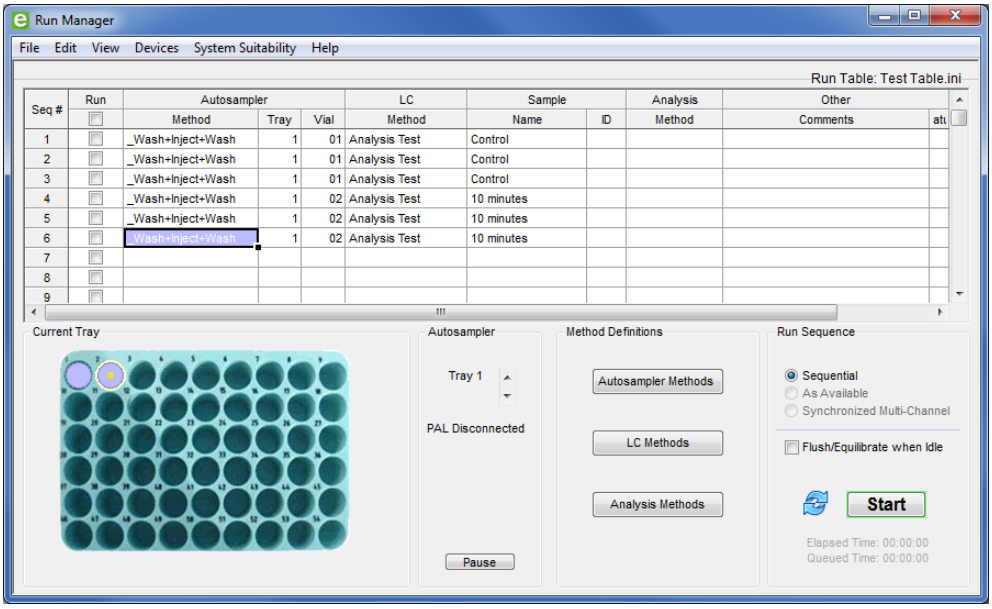
5. Create the other injections.
   a. Click **Seq #1**, and then right-click and select **Copy**.
   b. Highlight the rows 2 through 6 rows, right-click and select **Paste**.
6. Edit injections 3 to 6 to change the vial and sample information for the second sample.
   a. Double-click the **Vial** cell in row 4, then select 02 from the list.
   b. Click another cell, then click the **Vial** cell in row 4 again.
   c. Click and drag the small black box down two rows to change the vial from 01 to 02 (Figure 3-31).

Figure 3-31 Detail of Run Table, Showing Drag Handle



   d. Click the **Sample Name** cell in row 4 and type **10 minutes**.
   e. Click and drag the **Sample Name** cell to replace “Control” with “10 minutes”.
   The table should look like Figure 3-32.
   The yellow circle around vial 2 in the **Current Tray** section indicates the vial which is selected in the run table.

Figure 3-32 Run Manager Window, Showing Multiple Injections



7. Add a row for the blank.
   a. Copy the last row and paste it into row 7 in the table,
   b. Change the value in the **Vial** cell to **3**.

c. Double-click the **LC Method** cell and select **Electrode Flush**.
d. Type **Blank** in the **Sample Name** cell.

8. Click **File > Save** to save the run table.

Start the Run

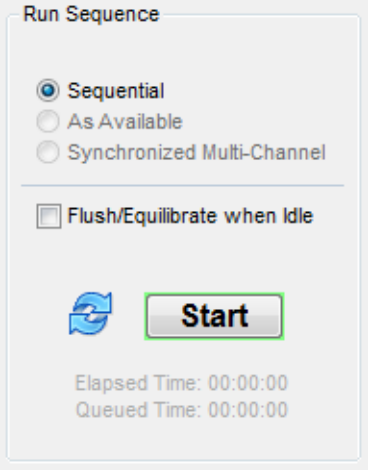
1. Place the sample vials in positions 1 and 2 of tray 1 of the autosampler.
2. Place a vial containing the blank (50:50 water:acetonitrile) in position 3.
3. In the run table, select the **Run** check box column header to queue all injections to be run (Figure 3-33).

   Clear the check box for any injection you do not want to run.

Figure 3-33 Run Manager Window—Run Column Header Check Box

4. In the **Run Sequence** section (Figure 3-34) click **Start**.

Figure 3-34 Run Manager Window—Run Sequence Section



After the flow rate has stabilized, sample injection begins.

After all of the samples have been injected, the system is flushed for 20 minutes.

Monitor the Run

View Run Status in the Run Manager Window

During a run, the color of the row in the run table indicates the status:

- White–the run can be started
- Light green–equilibrating
- Dark green–running

- Red–stopped
- Yellow–an error occurred
- Gray–completed

Stop a Run in the Run Manager Window

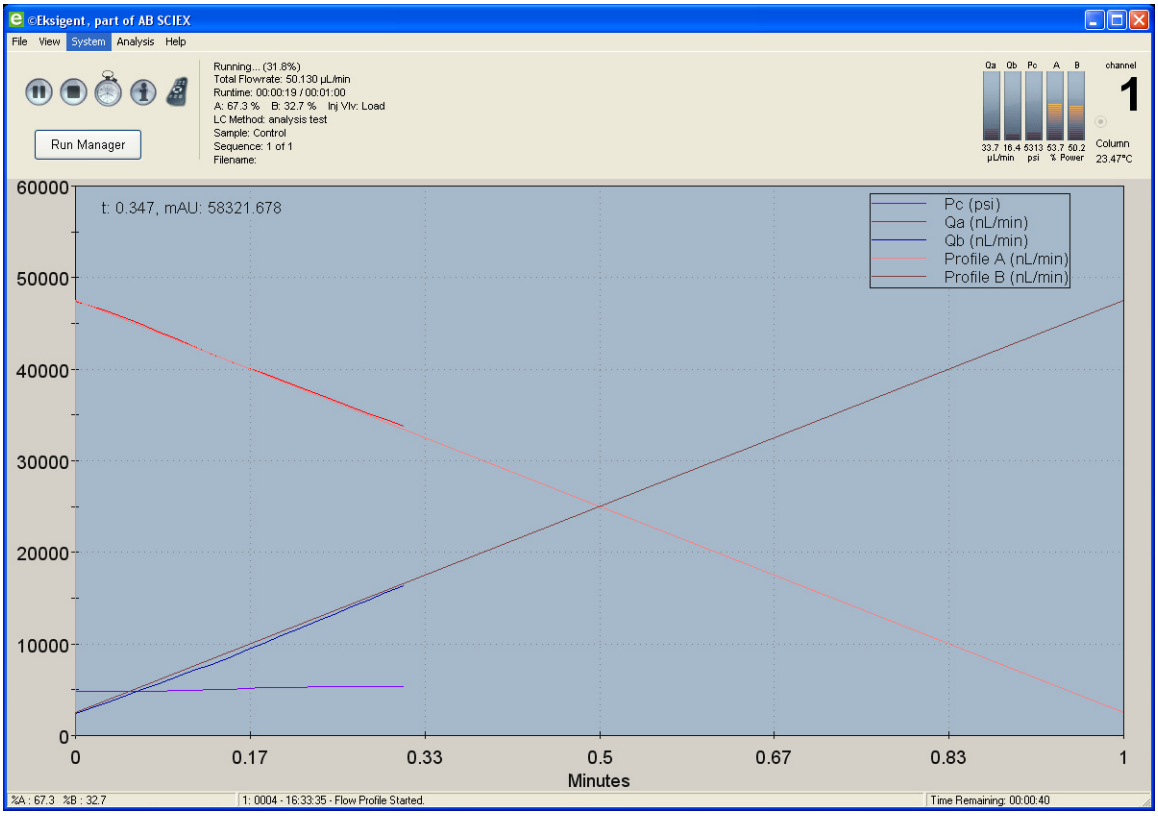
1. Click **Stop** to stop the run.
2. To start again, either:
   - Click (Reset) to clear the status of every row in the run table, and then click **Start** to start the run again at the first row in the run table

     or
   - Click **Start** to start the run again, beginning at the next row in the run table.

View Run Status in the Acquisition Window

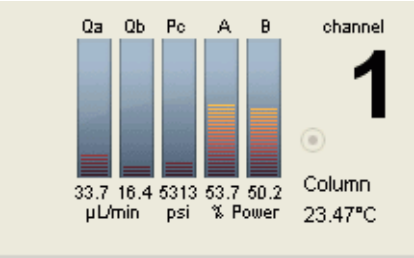
While the run is in progress, the **Acquisition** window can display the specified flow profiles for mobile phases A and B as well as their actual flow rates (Qa and Qb) (Figure 3-35).

Figure 3-35 Acquisition Window, Showing Flow Profiles



Status bars at the top right of the window display the actual flow rate for pump A (Qa) and pump B (Qb) in nL/min, and pressure for pump A (Pa), pump B (Pb) and the column (Pc) (Figure 3-36). Right-click the status bars to display a menu where you can customize the status bar display.

Figure 3-36 Status Bars for Flow Rate and Pressure



Status information such as %A, %B, and Time Remaining are also displayed at the bottom of the screen during the run.

Use the Control Buttons During a Run

Table 3-4 shows the buttons available in the **Acquisition** window.

Table 3-4 Acquisition Window Control Buttons

Click...	To...
	Pause the run. The system is maintained with its current conditions indefinitely until the run is stopped or resumed. To resume the run, click  (Resume).
	Change the run duration. Select the number of minutes to extend or shorten the run. You can add time indefinitely but you cannot shorten the run to less than the original run time in the LC method.
	Stop the run. The run stops and the pumps reset.
	View a dialog containing information about the sample run and other acquisition information saved with the data file.
	*(Not available during a run)* Open the **Direct Control** dialog. Use this dialog to change the mobile phase composition, flow rate, valve position, and column oven temperature when the system is not performing a run.

Zoom in on a Trace

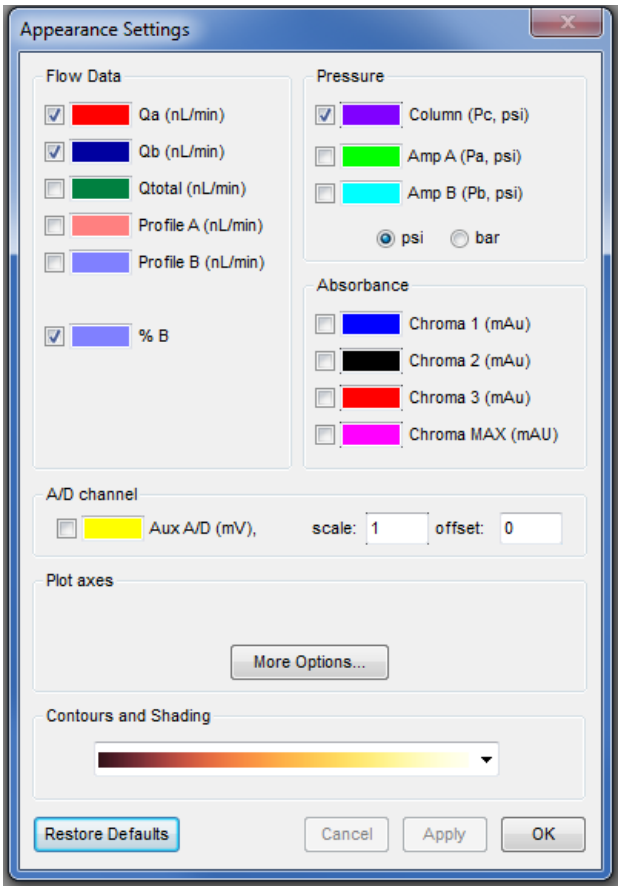
- To zoom in on a particular area of the plot, click and drag a box around the area of interest to enlarge that area of the plot.

  To zoom back out, right-click and select **Zoom Out** or **Back**.

Add or Remove Traces from the Acquisition Window

1. In the **Acquisition** window, click **System > Appearance Settings** to open the **Appearance Settings** dialog (Figure 3-37).

Figure 3-37 Appearance Settings Dialog



2. Select the items to view in the plot and, optionally, set the colors for the traces.
3. Click **OK** to save the changes and close the dialog.

Routine Maintenance 4

This chapter describes procedures to maintain the Eksigent MicroLC 200 Plus system.

- Basic Maintenance Schedule
- Dispose of Waste
- Inspect the System
- Maintenance Procedures for the Pump
- Maintenance Procedures for the Injection Valve
- Maintenance Procedures for the Turbo V™ Ion Source Electrode
- Maintenance Procedures for the Autosampler
- Configure the Autosampler
- Modify the Calibration Method for an AB SCIEX TripleTOF® System
- Transfer System Settings to a Different Computer
- Store the System

Caution: Potential System Damage: There are no user serviceable components or assemblies inside the Eksigent MicroLC 200 Plus pump. Service of any internal parts or assemblies should be completed by a trained Field Service Employee (FSE).

Basic Maintenance Schedule

To ensure reliable performance, the following procedures should be performed at the specified interval.

Table 4-1 Routine Maintenance for the Eksigent MicroLC 200 Plus System

Maintenance Procedure	Frequency
Inspect the System	Weekly
Re-initialize the Pressure Transducers	Weekly
Verify the DLW Actuator	Weekly
Replace the Injection Port Fitting	Quarterly
Backflush the Electrode	As needed
Calibrate Flowmeters	As needed
Dispose of Waste	As needed
Modify the Calibration Method for an AB SCIEX TripleTOF® System	As needed
Plumb the Injection Valve	As needed
Purge Mobile Phases	As needed
Replace the Autosampler Fuse	As needed

Table 4-1 Routine Maintenance for the Eksigent MicroLC 200 Plus System (Continued)

Maintenance Procedure	Frequency
Replace the Injection Valve Pod	As needed
Replace the Injection Valve Rotor Seal	As needed
Replace the Pump Seal Rinse	As needed
Replace the Sample Loop	As needed
Replace the Syringe Barrel	As needed
Replace the Syringe Needle	As needed
Replace the Syringe Plunger	As needed
Replace the Wash Solvents	As needed
Set the Temperature for the Stack Holder	As needed
Sonicate the Electrode	As needed
Transfer System Settings to a Different Computer	As needed
Test the DLW System	As needed

Dispose of Waste

Properly dispose of the contents of any effluent waste in an appropriate chemical waste container.

WARNING! Environmental Hazard: Always follow appropriate safety procedures and local requirements when handling or disposing of waste chemicals. Refer to the Safety Data Sheets for the mobile phases for more information.

Inspect the System

1. Inspect the waste and wash reservoirs for evidence of biological growth or precipitation.
2. Lower one of the mobile phase bottles to the table, then loosen the fitting at the rear of the pump to check for flow restriction in the DLW frit.

   If the liquid flows back into the bottle by gravity the frit is not plugged. You should be able to see the liquid/air interface move backwards through the clear tubing.
3. Repeat step 2 with the other mobile phase.
4. Check the injection port fitting and tighten or replace as needed (refer to Replace the Injection Port Fitting on page 57).
   - The fitting should be dry.
   - Check the fit by sliding a spare needle in and out of the fitting. It should be snug but able to move.
5. Check the tightness of the syringe plunger.

- If the plunger moves easily, replace it (refer to Replace the Syringe Plunger on page 74).

6. Visually inspect the system fluidics and electronic connectors.
   - Look for evidence of fluid leaks by inspecting all fluid connections.
   - Look for dried deposits that may indicate a slow leak.
   - As needed, tighten any loose connections.
7. Identify and correct the source of any leaks.
   - If a fluidic connection is broken, replace the fitting and re-flush the system.
   - Inspect the new connection to make sure that no leaks are present.

Maintenance Procedures for the Pump

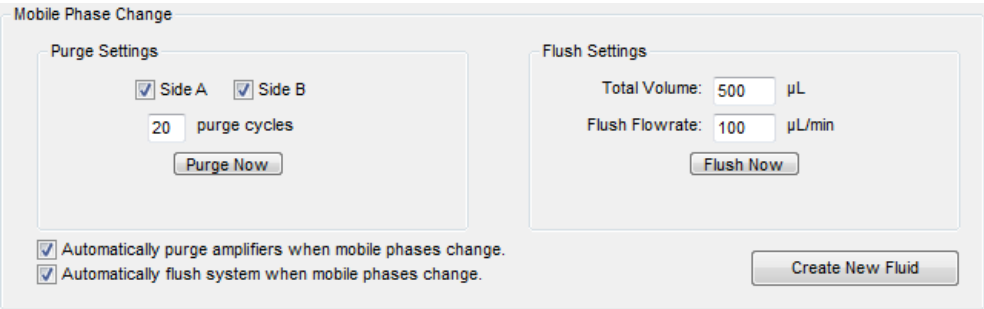
This section contains the following procedures for the pump:

Purge Mobile Phases

After changing the mobile phase bottle(s) or if the system has been idle for a week or more, purge the old mobile phase from the system.

1. Make sure the column is not connected.
2. In the Eksigent control software, click **System** > **Mobile Phases**, then click **More** to display additional options in the dialog.

Figure 4-1 Mobile Phase Change Section of the Mobile Phases Dialog—Settings for High-flow Configuration



3. Purge the mobile phases.
   a. In the **Purge Settings** section, select the **Side A** or **Side B** check box as appropriate.
   b. Specify a minimum of **20 purge cycles**.
   c. Click **Purge Now**.

4. Flush the system.
    a. In the **Flush Settings** section, type **500** µL for the **Total Volume.**
    b. Set the **Flush Flowrate** based the configuration:
    – Type **50** µL/min for the low-flow configuration.
    – Type **100** µL/min for the high-flow configuration.
    c. Connect one end of a length of 1/32 inch OD tubing to the mobile phase outlet on the front of the pump and insert the other end in a waste vial.

Caution: Potential System Damage: Make sure that no LC columns are connected before proceeding with this operation. Flushing the system with a column connected could over-pressure the system and create leaks.

    d. Click **Flush Now**.
5. Click **OK** to close the **Mobile Phases** dialog.
6. Reconnect the column.

Replace the Pump Seal Rinse

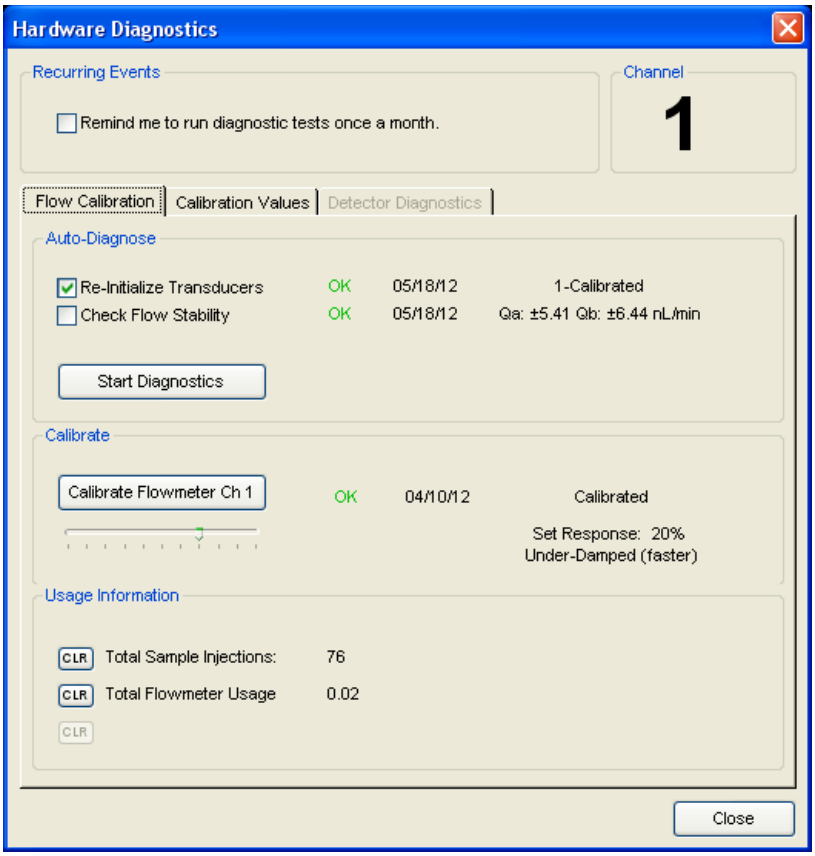
As needed, discard the pump seal rinse (in the small bottle on top of the pump) and replace with new solvents. Use a 50:50 mixture of water and a common alcohol such as methanol, ethanol, or propanol and fill the bottle 2/3 full.

Re-initialize the Pressure Transducers

Note: Before re-initializing the pressure transducers, open the pump outlet to ensure that there is no residual pressure on the outlet of the pump. Attempting to re-initialize the pressure transducers while there is still residual pressure on the pump will lead to inaccurate flow rates.

1. Stop the system flow.
2. Loosen the outlet fitting of the pump to release all of the residual pressure.
3. Click **System** > **Hardware Diagnostics**.
4. On the **Flow Calibration** tab, select the **Re-Initialize Transducers** check box.

Figure 4-2 Hardware Diagnostics Dialog—Flow Calibration Tab



5. Click **Start Diagnostics**.

   A warning appears that this procedure should only be performed if there is no residual pressure on the pump.

6. Make sure that the pump outlet is open, and then click **OK**.

   A status dialog indicates that re-initialization is in progress.

7. When the system displays that it is at ambient pressure, click **OK**.

8. When re-initialization is finished, exit the **Hardware Diagnostics** dialog and return to the **Acquisition** window.

Calibrate Flowmeters

Calibrating flowmeters consists of measuring the velocity of a liquid front in a tube of known diameter. Measure the flow rate to determine if the flowmeters need calibration.

Note: Make sure the system has been purged, flushed, and the pressure transducers re-initialized before proceeding with flowmeter calibration. Failure to do so will result in poor performance of the system.

Required Materials
• Calibration kit (with 100 µL and 200 µL pipettes) (PN 5018262)
• Autotuning assembly (PN 5018474)
• External timer

Different configurations of the Eksigent MicroLC 200 Plus system have different flow rates and require different pipettes for calibration (Table 4-2).

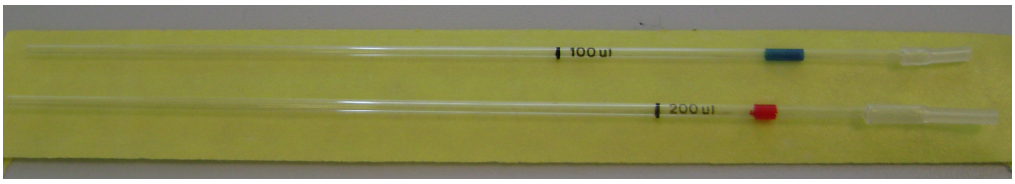
Table 4-2 Calibration Pipette Specifications

	High-flow Configuration (20-200 µL/min)	Low-flow Configuration (5-50 µL/min)
Calibrated pipette volume	200 µL	100 µL
Calibration flow rate	100 µL/min	25 µL/min

Measure the Flow Rate

1. Connect the flow calibration assembly to the pump using the autotuning assembly.
   a. Disconnect the tubing from the pump outlet.
   b. Connect the autotuning assembly to the pump.
   c. Insert the free end of the autotuning assembly into the silicone tubing on the calibration pipette (Figure 4-3).
      Refer to Table 4-2 to select the correct pipette for the system configuration.

Figure 4-3 Calibration Pipettes—100 µL (Top) and 200 µL (Bottom)



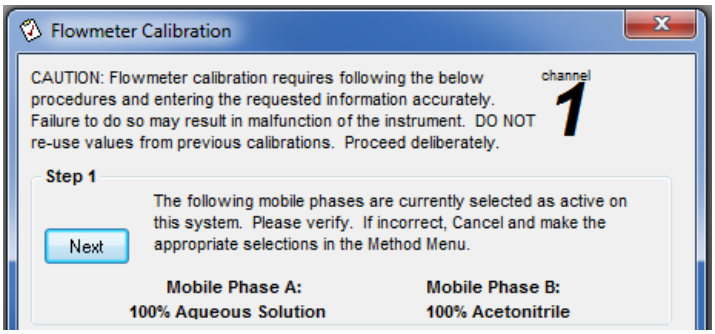
2. Using the **Direct Control** dialog, measure the time to fill the pipette with the volume specified in Table 4-2.
   For either configuration, the expected time is 240 seconds. A range of 230 to 250 seconds is acceptable.
3. Do one of the following:

- If the flow is within an acceptable range, the flowmeters do not need calibration.Disconnect the autotuning assembly and reconnect the original tubing between the pump and the injection valve.
- If the flow is not in the acceptable range, proceed to Calibrate the Flowmeters.

Calibrate the Flowmeters

1. If needed, connect the flow calibration assembly to the pump (refer to step 1 on page 54).
2. Click **System** > **Hardware Diagnostics**.
3. Click **Calibrate Flowmeter Ch 1** to open the **Flowmeter Calibration** wizard.
4. In Step 1, verify that the Mobile Phases are correct and click **Next** (Figure 4-4).

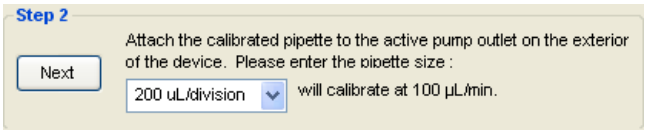
Figure 4-4 Flowmeter Calibration Wizard—Step 1



If the mobile phases are not correct, click **Cancel** and make the necessary changes in the **Mobile Phases** dialog. Repeat these steps.

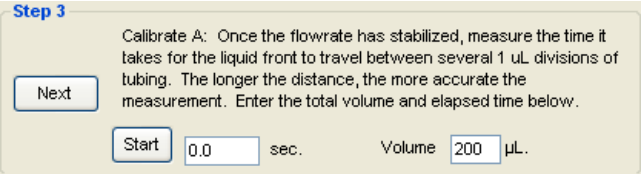
5. In the **Flowmeter Calibration** wizard, set the pipette size (Figure 4-5).
   - For the high-flow configuration—select **200 µL/division**.
   - For the low-flow configuration—select **100 µL/division**.

Figure 4-5 Set the Flowmeter Calibration Pipette Size—High-flow Configuration



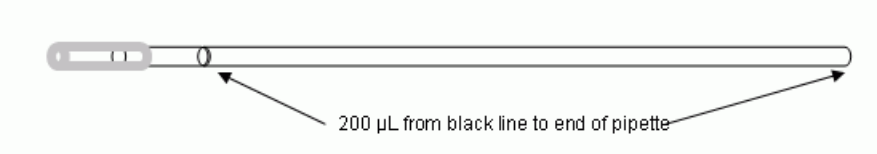
6. Click **Next** to start the flow in channel A.
7. In Step 3, specify the appropriate **Volume** (Figure 4-6).
   - For the high-flow configuration—type **200**.
   - For the low-flow configuration—type **100**.

Figure 4-6 Set the Flowmeter Calibration Volume



8. Bring the liquid front to the black line on the pipette and then click **Start** to begin the timer.

Figure 4-7 Example of Flow Direction—High-flow Pipette



9. When the fluid front reaches the end of the pipette, click **Stop**.
10. Click **Next**.
11. Repeat this procedure to calibrate the channel B flowmeter.
12. Click **Finish**.
13. Do one of the following:
    - If the calibration passed, repeat the Measure the Flow Rate procedure to determine if the flow rate is within acceptable bounds.
    - If the calibration failed, check for leaks, verify the settings in the Mobile Phases dialog are correct for the solvents in use, and purge and flush the system, then repeat the calibration. If the calibration fails again, contact AB SCIEX Technical Support.
14. Disconnect the autotuning assembly and reconnect the original tubing between the pump and the injection valve.

Check Flow Stability

The **Check Flow** option in the **Hardware Diagnostics** dialog should not be used. The test was developed for earlier versions of the Eksigent MicroLC 200 Plus system and the results are no longer meaningful.

Maintenance Procedures for the Injection Valve

This section contains the following procedures for the injection valve:

- Replace the Injection Port Fitting on page 57
- Replace the Sample Loop on page 57
- Replace the Injection Valve Rotor Seal on page 58
- Replace the Injection Valve Pod on page 59

- Plumb the Injection Valve on page 61
- Install the Electrode and Grounding Assembly for the Ion Source on page 64
- ESI Electrode Assemblies on page 65

Replace the Injection Port Fitting

Required Materials
• Injection port fitting (PN 5023797) • Spare needle (PN 5031383)

1. Remove the fitting in port 3.
2. Replace the fitting (Figure 4-8) and tighten until finger-tight.

Figure 4-8 Injection Port Fitting

3. Insert the spare needle into the fitting port 3 and slide it in and out.
4. Tighten the fitting until the needle is snug but can still move in and out of the fitting.
5. Follow the steps in Configure the Needle Penetration Depth on page 80 to check and, if necessary, set the needle penetration depth.

Replace the Sample Loop

The sample loop (2 µL) is pre-installed and is located between ports 1 and 4 on the HTC-*xt* PAL autosampler valve (Figure 4-11 on page 61). If you suspect a clog, you can change the sample loop.

Required Materials
• Sample loop (PN 5017800) • Tightening tool (PN 200-00356) • 2 nuts (PN 5024174) • 2 ferrules (PN 910-00087)

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

Replace the Injection Valve Rotor Seal

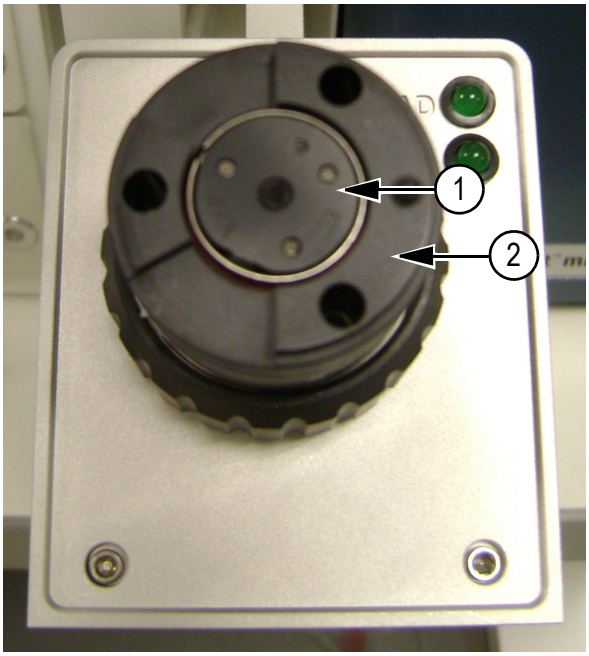
Required Materials
• 9/64 inch Allen key • Injection valve rotor seal (PN 200-00326)

Replace the valve rotor seal if the valve leaks or doesn't hold pressure.

1. (Optional) Remove the sample loop and any tubing connected to the injection valve:
2. Remove the valve stator and rotor seal.
    a. Use a 9/64 inch Allen key to remove the three Allen screws from the top of the valve stator.
    b. Lift off the stator and set it aside.
    c. (Optional) Lift off the black plastic alignment cylinder and set it aside.
    d. Remove the rotor seal.
    The rotor seal consists of a black disk in a silver case.

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

Figure 4-9 Injection Valve—Top View, with Stator Removed



Item	Description
1	Rotor seal
2	Alignment cylinder

3. Install the new rotor seal.
    a. Place the new rotor seal on the valve, seating it on the three pins.
    b. Replace the black plastic alignment cylinder on the valve, rotating as needed to seat it.
    c. Replace the stator and tighten the Allen screws.
    d. If needed, re-plumb the injection valve.
    Refer to Plumb the Injection Valve on page 61 for detailed instructions.

Replace the Injection Valve Pod

Required Materials
• Tightening tool (PN 200-00356) • Tightening tool (PN 200-00404) • Injection valve pod (PN 200-00452)

1. Remove the sample loop and any tubing connected to the injection valve,
2. Remove the pod from the acutator.
    a. Unscrew the black ribbed retaining nut that holds the pod in the actuator.
    Do not use a wrench. The retaining nut should only be tightened and loosened by hand.
    b. Pull the pod from the actuator.
3. Replace the pod.

Note: The position of the pod does not matter when installing.

    a. Insert the new pod into the actuator.
    The pod union will make contact with the spline in the actuator.
    b. Press lightly and rotate the pod until the pod slips further into the actuator and the pin contacts the actuator.
    c. Continue to rotate the pod until the pin is seated in the notch in the actuator, then push it in (Figure 4-10).
    d. Replace the retaining nut and tighten by hand.

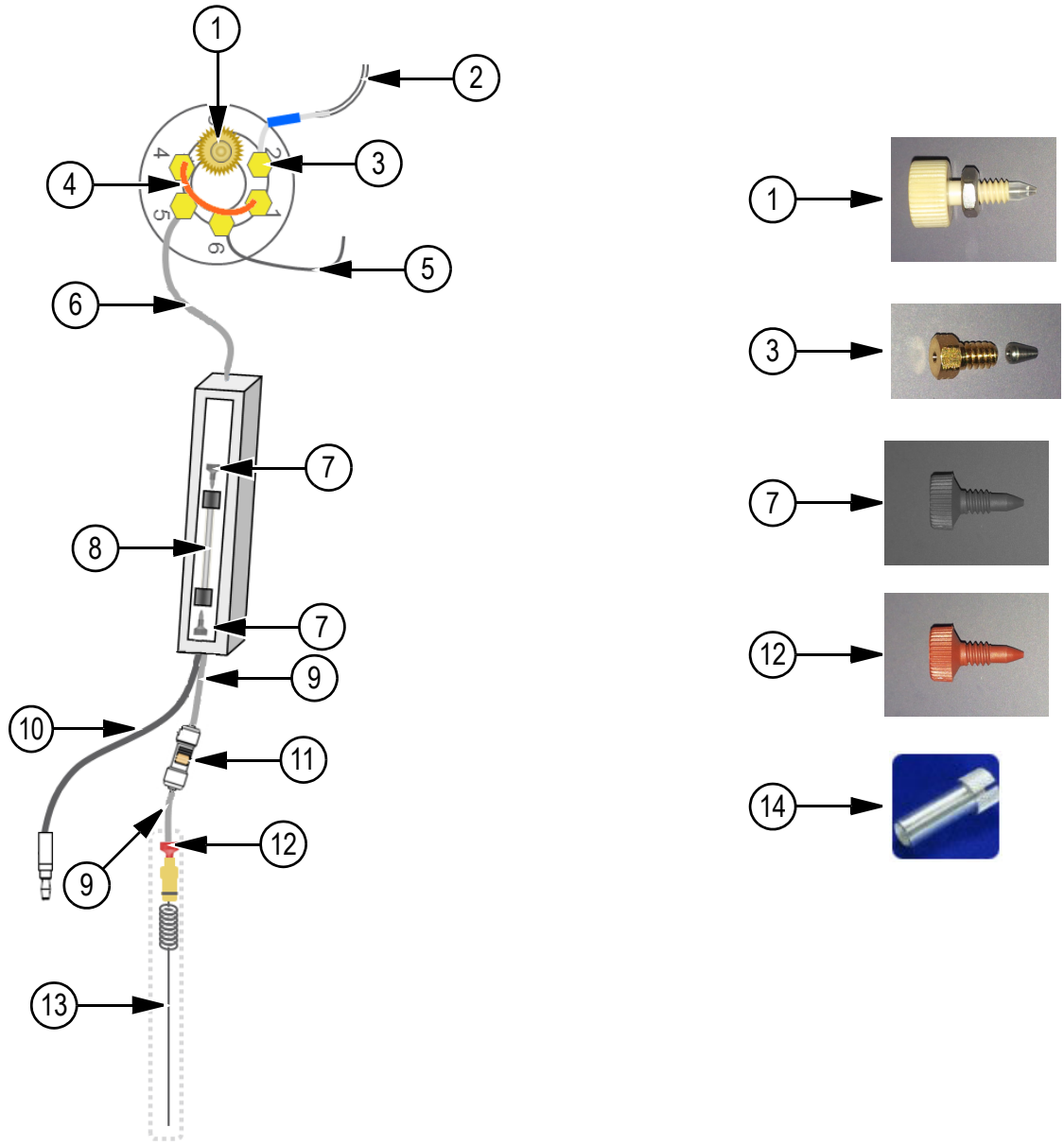
Figure 4-10 Valve Pod—Side View, Showing Pin in Notch

4. Re-plumb the injection valve.

   Refer to Plumb the Injection Valve on page 61 for detailed instructions.

Plumb the Injection Valve

Figure 4-11 Injection Valve Plumbing with Grounding Assembly (Left) and Fittings and Tools (Right)



Item	Description	Part Number
1	Injection port	5023797
2	Injection valve waste tube assembly	5017800
3	Gold-colored fittings (and ferrules) for all ports on injection valve except number 3 • Nuts • Ferrules	 5024174 910-00087

Item	Description	Part Number
4	One of the following: • 2 µL PEEKsil loop • 5 µL PEEKsil loop • 10 µL PEEKsil loop	 5017798 5017799 205-00054
5	Mixer-to-valve assembly	5017801
6	Gray PEEKsil tubing, 50 µm ID, 1/32 inch OD, 30 cm (If needed to accommodate laboratory layout, use 50 cm.)	205-00040 (30 cm) 205-00041 (50 cm)
7	Black PEEK fitting (If necessary, use tool PN 200-00356.)	200-00342
8	2.7 µm HALO fused C18 column, 0.5 mm x 50 mm	805-10100
9	Gray PEEKsil tubing, 50 µm ID, 1/32 inch OD, 5 cm or Orange PEEKsil tubing, 25 µm ID, 1/32 inch OD, 5 cm (Use for flows < ~20 µL/min) (For systems without the in-line filter, use 10 cm of tubing between the column and the electrode.)	205-00070 205-00089
10	Grounding assembly	5016941
11	In-line filter	200-00388
12	Red PEEK nonconducting fitting (use for <5000 psi only) (see note below)	200-00330
13	One of the following (see note below): • 65 µm ID electrode • (Available separately) 50 µm ID electrode • (Available separately) 25 µm ID electrode (Use for flows <~20 µL/min.)	 5029342 5028466 5028467
14	Tool for PEEK fittings (PN 200-00342)	200-00356

Note: When connecting the 65 µm ID electrode, use a red PEEK fitting (item 12) immediately before the electrode. For the 25 µm ID and 50 µm ID electrodes, use either the red fitting or a black PEEK fitting (item 7).

Best Practices for Working with PEEKsil Tubing

- Never cut PEEKsil tubing.

  Cutting PEEKsil tubing can result in small particles of cut glass entering the flow path, leading to plugged tubing and electrodes.

- When connecting PEEKsil tubing:

  a. Connect the tubing on the end farther from the mass spectrometer first.

b. Turn on the pump and allow liquid to flow through the tubing to flush out any particulate matter.
c. Allow flow for ~30 seconds before making the next connection.

- Don't overtighten connections to PEEKsil tubing.

  Overtightening can damage tubing and lead to plugged tubing. Instead, tighten fittings until finger-tight, turn on the pump and check for solvent at the fitting. If there is a leak, tighten the fitting about 1/16 turn at a time until there are no more leaks.

Step-by-Step Instructions for Plumbing the Injection Valve

1. Loosely install the fittings in the ports.
   - For port 3, use item 1 in Figure 4-11.
   - For all other ports, use nuts and ferrules (item 3).
2. Connect the waste tube assembly (item 2).
   a. Attach the tubing to port 2.
   b. Bend the metal around the valve spill collar so that it holds the clear tubing below the valve and will not get caught by movement of the autosampler.
   c. Connect the other end of the tubing to the upper port on the front of the wash station (Figure 4-11 on page 61).
3. Install the mixer-to-valve assembly (item 5) in port 6.

   Leave the other end of the assembly free, for installation to the pump outlet later.
4. Install the sample loop (item 4) into ports 1 and 4, using a 3/16 inch wrench.
5. Connect the column.
   a. Install 30 cm of 50 µm ID tubing, 1/32 inch OD PEEKsil tubing (item 6) in port 5.

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

   b. Connect the other end of the tubing to the column inlet using a black PEEK fitting (item 7).
6. Connect the column outlet to the in-line filter (item 11) and the in-line filter to the electrode.
   a. For each connection, use 5 cm of 1/32 inch OD PEEKsil tubing (item 9).
      - **Flow rates > ~20 µL/min**—50 µm ID tubing (PN 205-00070)
      - **Flow rates < ~20 µL/min**—25 µm ID tubing (PN 205-00089)
   b. Connect the other end of the tubing to the electrode, using the appropriate fitting:
      - **25 µm and 50 µm ID electrodes**—black PEEK fitting (PN 200-00342) (item 7).
      - **65 µm ID electrodes**—red PEEK fitting (PN 200-00330) (item 12).

WARNING! Electrical Shock Hazard: For the 65 µm ID electrode, use the red fitting to prevent the risk of an electrical shock. Do not use conductive fittings such as the high-pressure carbon-filled black fittings.

7. Place the column in the column oven.
8. Cut a piece of the foam block (PN 5023403, from the column oven kit) and place it in the column heater on top of the column.

Install the Electrode and Grounding Assembly for the Ion Source

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.

Based on the planned flow rates for the system, install the appropriate electrode assembly in the Turbo V™ ion source probe.

Table 4-3 Suggested Electrode, Based on Flow Rate

Flow Rate (µL/min)	Electrode
5 to 20	25 µm ID (PN 5028467)
20 to 50	50 µm ID (PN 5028466)
20 to 100	65 µm ID (PN 5029342)

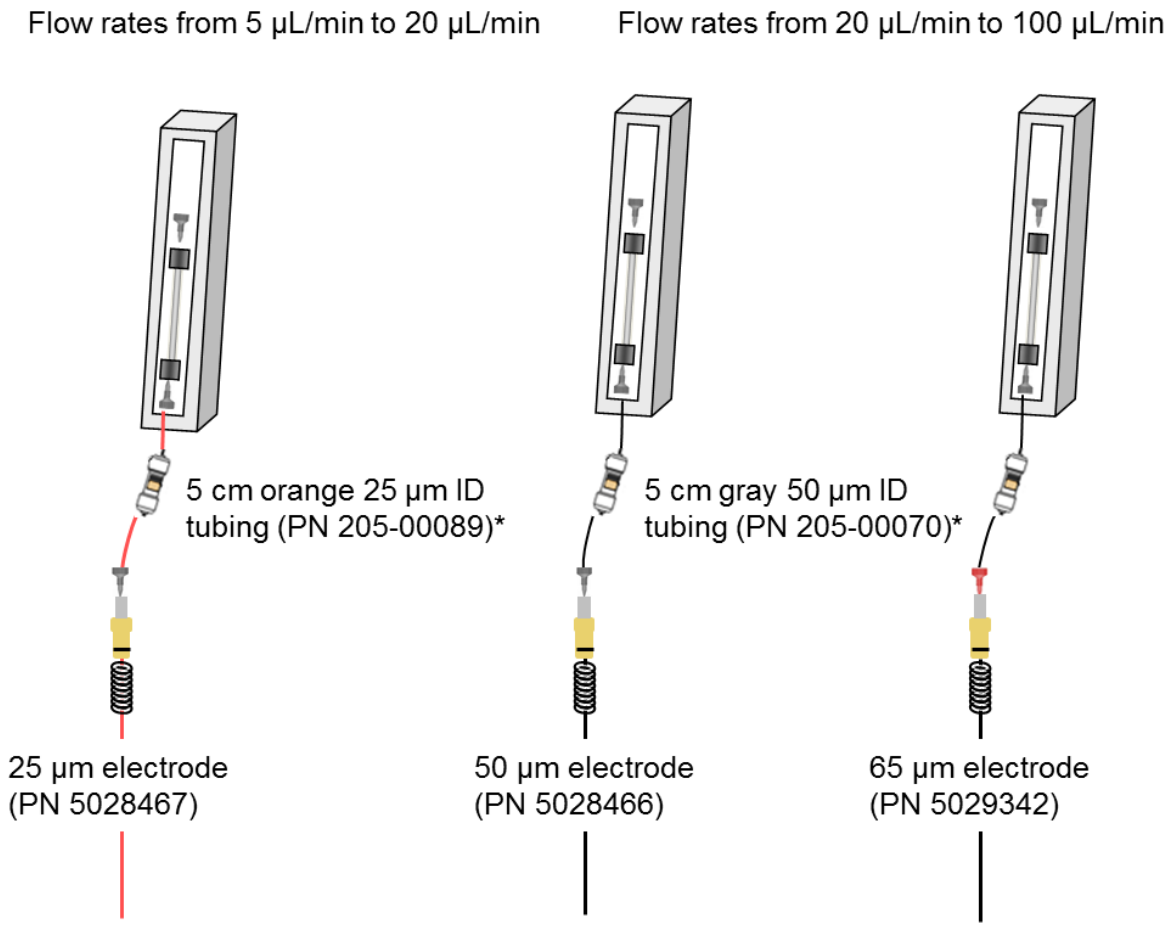
Note: The upper limit for flow rate is ultimately determined by the pressure limits of the system and the column.

1. Replace the existing spring with the one provided with the electrode.
2. Insert the electrode into the central shaft of Turbo V probe in the same manner as the standard larger ID Turbo V electrode.
3. Tighten the black screw cap on the probe and adjust as needed to extend the electrode tip 1 mm to 2 mm past the probe tip.
4. Clip one end of the black grounding cable (PN 5016941) to the grounding point on the ion source.
5. Clip the other end to the appropriate location for the electrode.
   - **25 µm and 50 µm ID electrodes**—clip to the grounding union on the probe
   - **65 µm ID electrode**—clip to the column in the column oven
6. Close the column oven.

ESI Electrode Assemblies

Figure 4-12 shows the plumbing and connections for ESI electrode assemblies.

Figure 4-12 ESI Probe Plumbing—25 µm ID Electrode (Left) and 50 µm and 65 µm ID Electrodes (Right)



Match the post-column tubing color to the electrode color.

*Without an in-line filter, use 10 cm of tubing between the column and the electrode: 25 µm ID – PN 205-00091 or 50 µm ID – PN 205-00070

Maintenance Procedures for the Turbo V™ Ion Source Electrode

This section contains the following procedures:

- Backflush the Electrode on page 66
- Sonicate the Electrode on page 69

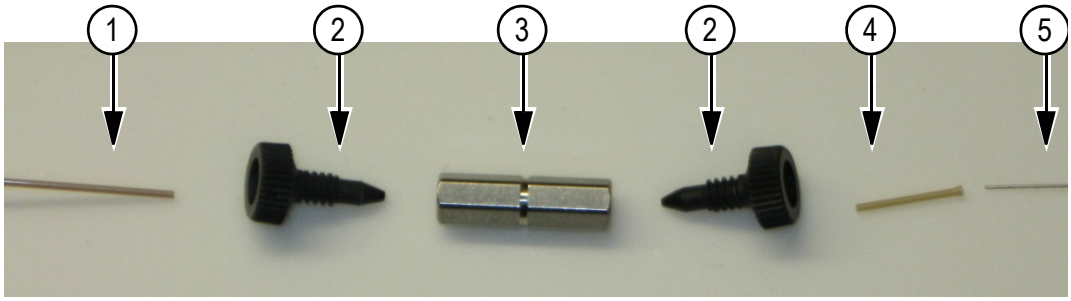
Backflush the Electrode

If the electrode is plugged, backflush the electrode using the Eksigent MicroLC 200 Plus pump.

Required Materials
• Stainless steel hex union (PN 5016413)
• Flared sleeve (PN 5025189)
• 2 PEEK fittings (PN 200-00342)

1. Remove the column from the flow path and connect the tubing with a union.
   a. Loosen the black PEEK fitting from the upstream end of the column.
   b. Remove the 1/32 in OD PEEKsil tubing from the column and connect it to the stainless steel hex union (item 3 in Figure 4-13) using the fitting.

Figure 4-13 Components for Connecting the System to Backflush the Electrode



Item	Description
1	1/32 in OD PEEKSil tubing
2	PEEK fittings
3	Stainless steel hex union
4	Flared sleeve
5	Electrode assembly

   c. Insert the sleeve (item 4) into a black PEEK fitting. Insert the sleeve with the flared end of the sleeve at the rear of the fitting.
   d. Insert the probe tip (item 5) into the sleeve using the flare as a guide.

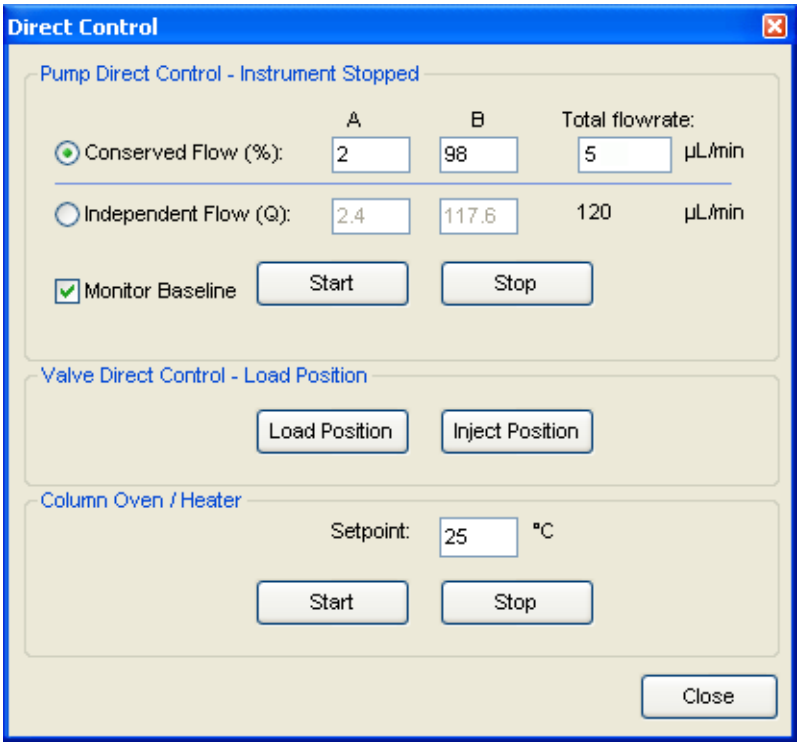
The first time the sleeve is used, make sure that the sleeve is pushed as far as possible into the union. After the first use, the sleeve will remain in the fitting.

e. Tighten the fitting into the union so that the probe tip is snug.

2. Start the pumps.

   a. Select **System > Direct Control** in the Eksigent control software to open the **Direct Control** dialog.

Figure 4-14 Direct Control Dialog



   b. Select **Monitor Baseline** to monitor pressure while backflushing.

   c. Specify the flowrate to be **5 µL/mL**.

   d. Specify **A** and **B** so the solvent pumped is mostly organic.

   e. Click **Start**.

Caution: Potential System Damage: Do not allow the pressure to exceed 10 000 psi. Stop the pump if the pressure is greater than 10 000 psi.

3. Monitor the baseline in the **Acquisition** window.

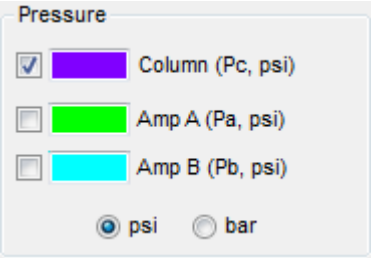
   If necessary, configure the window so that Pc appears in the plot.

   a. Select **System > Appearance Settings**.

   b. In the **Appearance Settings** dialog, select the **Column (Pc, psi)** check box in the **Pressure** section.

   c. Click **OK**.

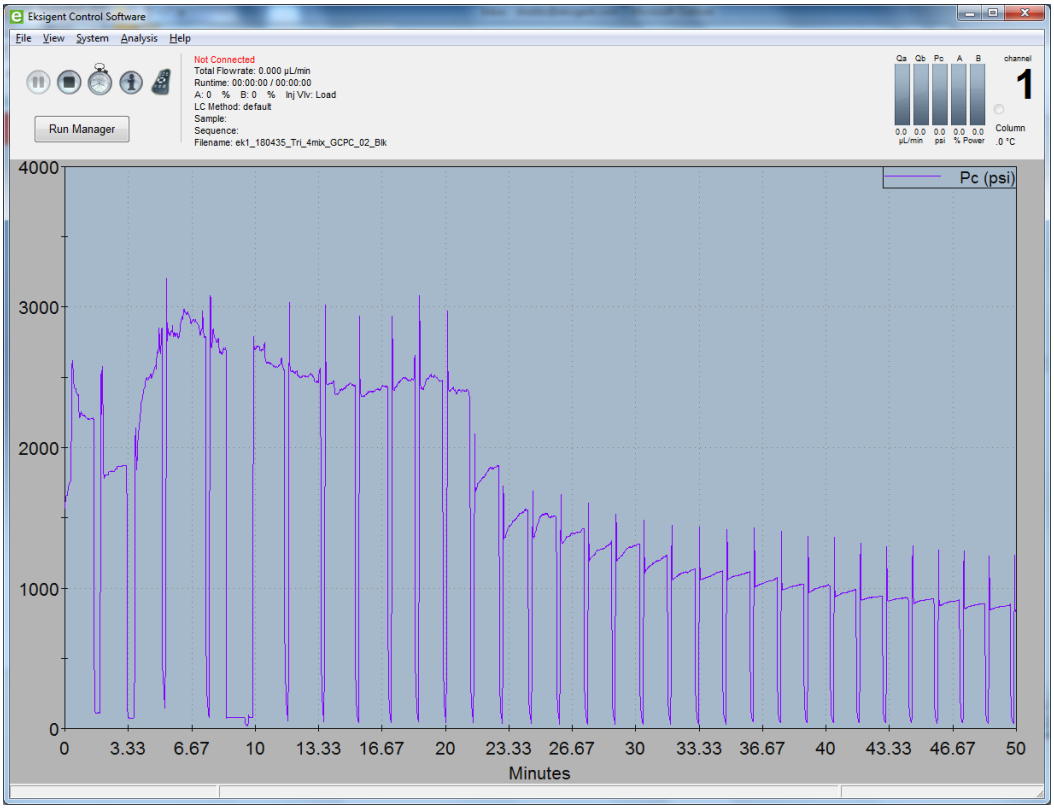
Figure 4-15 Appearance Settings Dialog—Pressure Section



4. Increase the flowrate, up to the maximum for the instrument's configuration (below) and pump until either the probe backpressure is reduced and stable or for 20 minutes, which ever comes first.
   - High-flow configuration—200 µL/min
   - Low-flow configuration—50 µL/min

Tip! Each time the pump restrokes, the Eksigent control software displays the error log. Minimize the window to hide it.

Figure 4-16 Acquisition Window, Showing Pressure Drop



The pump can be left unattended and be allowed to restroke repeatedly to dislodge the plug. The pressure fluctuations due to restroking or changing flow rate will aid in dislodging the blockage. If necessary the probe can be inserted into an ultrasonic bath while backflushing.

5. Click **Stop**.

   If the pressure dropped, the plug has been dislodged. Remove the union and reconnect the column into the flow path.

   If the pressure is still high, follow the procedure below to sonicate the electrode.

Sonicate the Electrode

Sonicate the electrode only if backflushing did not remove the plug.

Required Materials
• Sonicator • Beaker • Acetone, methanol, ethanol, or warm water

1. Remove the electrode from the system.
2. Place the tip of the electrode in the beaker then add enough solvent to cover the tip and then a bit more. Sonicate for 10 minutes.
3. Replace the electrode in the source and flush the electrode a second time.

Maintenance Procedures for the Autosampler

This section contains the following procedures for the autosampler and the DLW:

Replace the Wash Solvents

As needed, replenish the wash solvents in the 1 L glass bottles on top of the pump, using:

- Water with 0.1% formic acid
- Acetonitrile (or other organic solvent) with 0.1% formic acid

Set the Temperature for the Stack Holder

The temperature setting for the stack holder is shown on the front of the stack holder power supply. Change the temperature if the default is not applicable.

Figure 4-17 Stack Holder Power Supply Control Panel

1. Press **P** for less than two seconds.

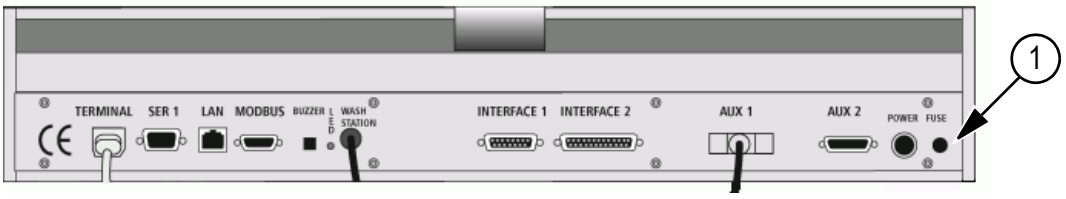
   "SP1" is displayed.
2. Press the **Up** and **Down** arrows to reach the desired temperature.
3. Press **P** again to set the temperature.

Replace the Autosampler Fuse

Required Materials
• 6.3 A 250 VAC 5 mm x 20 mm fuse

1. If the system is not already off, turn off the power switch on the autosampler power supply.
2. Remove the fuse from the back of the Z arm of the autosampler (Figure 4-18).

Figure 4-18 HTC-*xt* PAL Autosampler Z-Arm—Rear View



Item	Description
1	Fuse

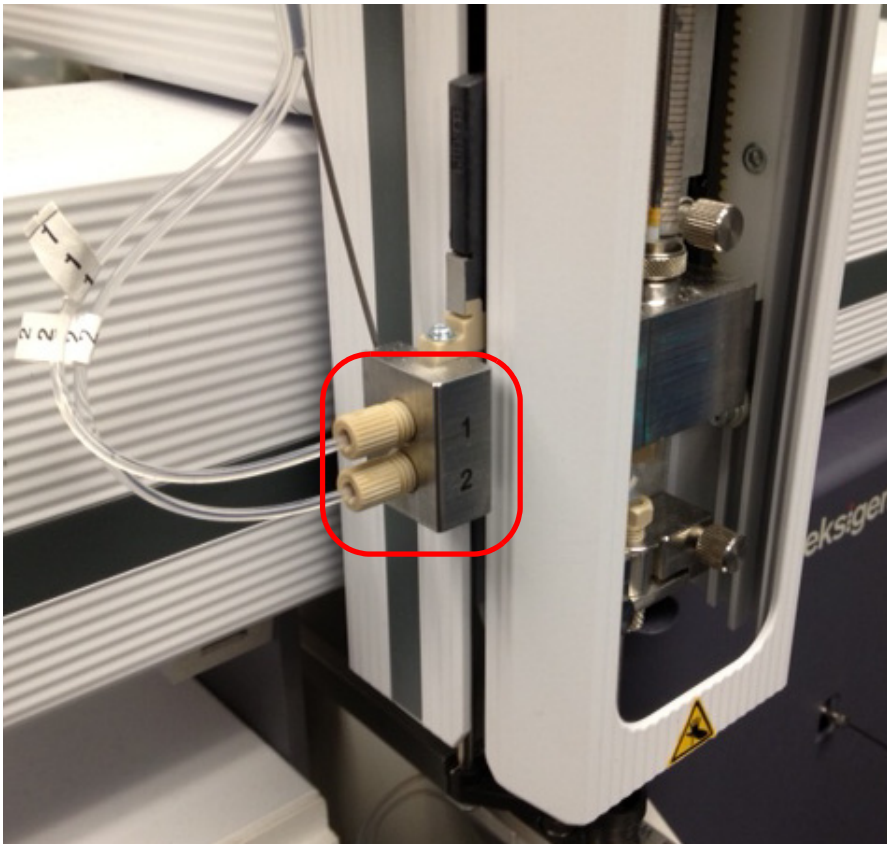
3. Replace the fuse.
4. Power up the system.

Test the DLW System

Required Materials
• Short beaker or other container to catch wash solvent during the test • 10 mL or 20 mL graduated cylinder

Note: This test procedure requires the use of water as the wash solvent. Organic solvents typically have lower viscosities and higher flow rates, resulting in final volumes that are different than those below.

Figure 4-19 Outlet Tubing to Z-arm Connection



1. Verify the wash solvent is delivered at the dynamic load and wash (DLW) pump outlet.
    a. Disconnect one of the two outlet tubings from the Z-arm (Figure 4-19) and place it into the beaker.
    b. Using the keypad terminal, select **Menu > Utilities > Wash Station > 1** (or **2**) **> MovtoWash (F3) > ActValve (F2)** to start the pump.
    c. Wait 30 seconds and measure the volume of the collected liquid using the graduated cylinder.

       The cylinder should contain approximately 10 mL.

If there is <10 mL in the cylinder, the pumps are not working or the solvent lines are restricted.

If there is >10 mL in the cylinder, go to step 2.

2. Verify the wash solvent is delivered at the needle.
   a. Reconnect the outlet tubing to the Z-arm.
   b. Disconnect the corrugated tubing from the barbed fitting on the bottom of the wash station.
   c. Place the beaker under the barbed fitting to catch the outflow.
   d. Using the keypad terminal, select **Menu > Utilities > Wash Station > 1** (or **2**) **> MovtoWash (F3) > ActValve (F2)** to start the pump.
   e. Wait 30 seconds, then press **Deact Valve (F3) > Esc** to stop the pump.
   f. Measure the volume of the collected fluid.

      The cylinder should contain approximately 8 mL.

      If there is >8 mL in the graduated cylinder, the DLW system is working correctly.

      If there is less than ~8 mL, the flow is restricted somewhere in the flow path. Check the following:

      - Verify there is no crimped or pinched tubing in the flow path and make sure that flow reaches the tan PEEK fitting at the DLW actuator.
      - Verify the connections to the sample loop are not leaking.
      - Verify the white Teflon needle seal is correctly inserted and the needle is installed correctly. If the needle has been previously bent and then straightened, install a new needle.

      Repeat step d through step f.
3. Repeat step 1 and step 2 for the tubing for the other outlet.

Verify the DLW Actuator

The DLW actuator is the valve associated with the DLW option for the HTC-*xt* PAL autosampler. It is located in the syringe holder assembly on the Z-arm.

1. Make sure the syringe needle is over the waste or in a wash position.
2. On the keypad terminal, select **Menu (F1)**.
3. Turn the outside circular button clockwise to position the cursor over **Utilities** and press **Enter**.

   This displays the service menu.
4. Select **Wash Station > Wash 1**.
5. Press **F2** to turn on wash pump 1 and open the valve.
6. Press **Enter**.

   Verify the blue LED on the syringe holder is lit.

   If liquid does not flow out of the syringe, check the fluidic and electronic connections. The blue LED to the right of the syringe indicates that power is supplied to the actuator.

Replace the Syringe Barrel

Note: It is critical that syringes be primed before beginning sample preparation. Prime every syringe manually before inserting it into the autosampler. After insertion, flush the syringe.

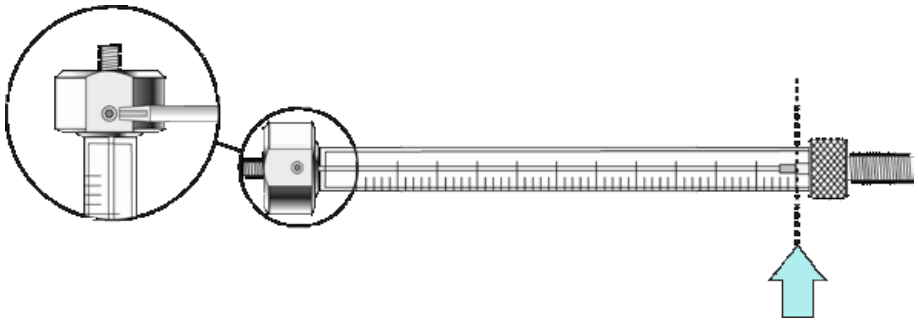
Required Materials
• Syringe barrel (PN 4460861) • T6 Torx driver

1. On the keypad terminal, select **Menu > F1 Chang Syr**.

   The injection unit moves to a location convenient for accessing the syringe and needle.

2. Remove the syringe barrel.
   a. Unscrew the knurled nuts at the top and bottom of the syringe
   b. Lift the syringe up and out to remove it.
3. Prepare the new syringe barrel (Figure 4-20).
   a. Manually move the plunger to the stop position.
   b. Pull the plunger backward slightly backwards to release a slight amount of pressure from the plunger tip.
   c. Install the plunger holder and tighten the Allen screw firmly.

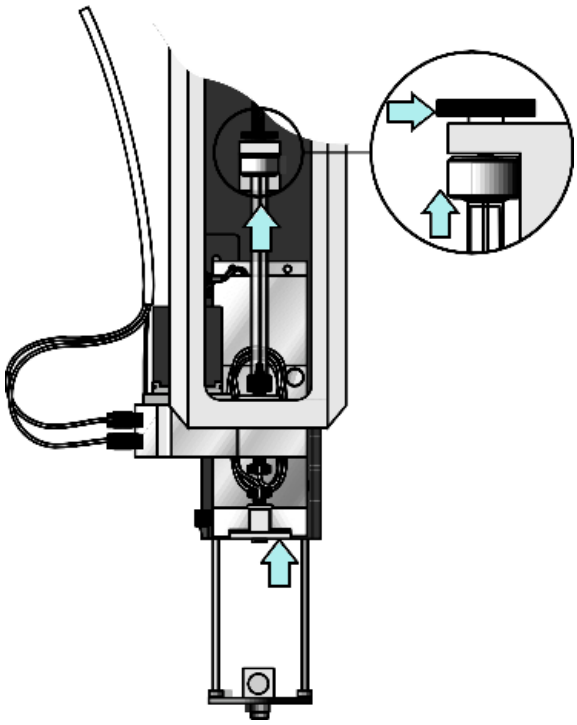
Figure 4-20 Inserting the Plunger Holder in the Syringe Barrel



4. Replace the syringe barrel (Figure 4-21).
   a. Screw the prepared syringe barrel into the holder.

      Hold the syringe barrel at the lower metal mount while tightening the syringe.
   b. Move the plunger up (plunger holder) until the thread of the screw catches the thread of the plunger bushing.
   c. Tighten the screw to fix the plunger holder.
   d. Tighten the holding screw to secure the syringe holder position.

Figure 4-21 Connecting the Syringe Plunger Holder



5. Insert the syringe needle tip into the lower needle guide, then move the needle back up to the needle holder and tighten firmly.
6. Press **F4 Home**.

   The plunger moves down until it hits the mechanical stop. This position is stored as the syringe's zero volume position. The injection unit then returns to the home position.
7. Select **Menu > Utilities > Syringe > F2 Clean Syr** to flush the syringe.

Replace the Syringe Plunger

Required Materials
• Syringe plunger (PN 4460827)

1. On the keypad terminal, select **Menu > F1 Chang Syr**.

   The injection unit moves to a location convenient for accessing the syringe and needle.
2. Remove plunger holder and syringe barrel.
3. Pull out the plunger.
4. Flush barrel with alcohol to remove debris and act as a lubricant for the plunger.
5. Carefully insert the new plunger into the syringe barrel.
6. Replace the plunger holder and syringe barrel.

Replace the Syringe Needle

After the needle is replaced, the needle penetration depth on the autosampler must be reset.

Required Materials
• Needle and Teflon seal (PN 5031383)

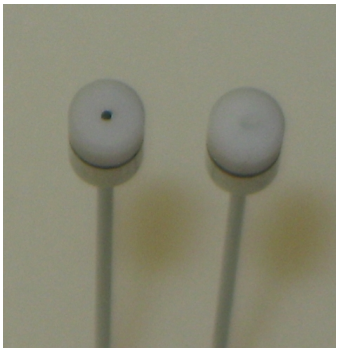
1. Select **Menu > F1 Chang Syr**.

   The injection unit moves to a location convenient for accessing the syringe and needle.

2. Loosen the knurled needle retaining nut and remove the needle.
3. Prepare the new needle.
   a. Insert the needle into the Teflon seal.

      Sometimes the hole in the seal contains a burr, Teflon residue from the seal (Figure 4-22).

Figure 4-22 Teflon Needle Seal—Clean Seal (Left) and Seal with Burr (Right)

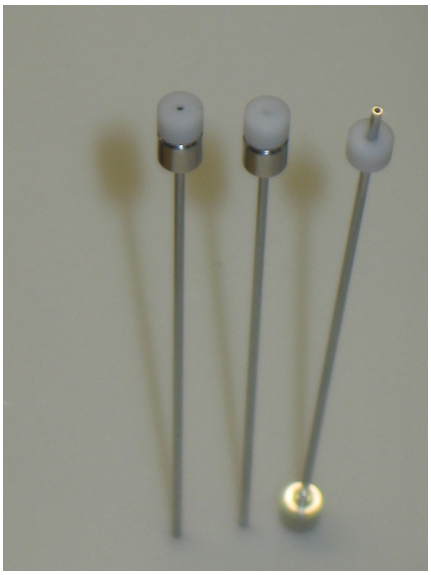


   b. If necessary, remove any burrs created by the insertion.

      Remove the seal from the needle and use the long end of the needle to push the burr out.

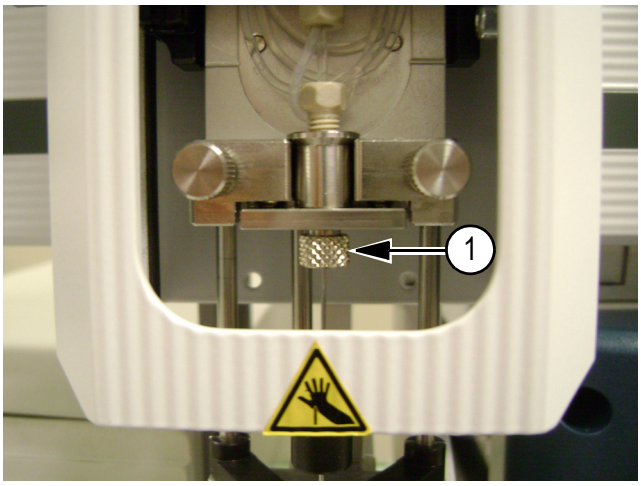
      Be careful not to scratch the seal.

Figure 4-23 Syringe Needles and Seals, Showing Cleaning the Needle Seal (Right)



4. Install the needle.
   a. With one hand, lift up the bottom needle guide until it touches the upper needle guide.
   b. With the other hand, guide the tip of the new needle into both guides and then release the bottom needle guide.
   c. Insert the top of the needle into the fitting and tighten the needle collar until finger tight (Figure 4-24).

Figure 4-24 Syringe Needle Collar



Item	Description
1	Needle collar

5. Gently slide the lower needle guide up and down to make sure the tip of the needle is near the bottom of the hole in the needle guide.

If the needle tip is above the hole at rest, the needle will probably hit the hole when compressed, bending the needle.

6. Follow the steps in Configure the Needle Penetration Depth on page 80.

Configure the Autosampler

If any of the components attached to the autosampler are moved, use the keypad terminal to adjust the X, Y, and Z positions of the injection unit. This section contains the following procedures:

Configure the Tray Holder Position

1. Open the top drawer in the stack holder.

   Make sure that the drawer is empty. The tray calibration hole should be visible.

2. On the keypad terminal:
   a. Press **Menu** (F1).
   b. Turn the outside circular button clockwise to position the cursor over **Setup**.
   c. Press **F3** and then press **Enter** (inside circular button) in quick succession.

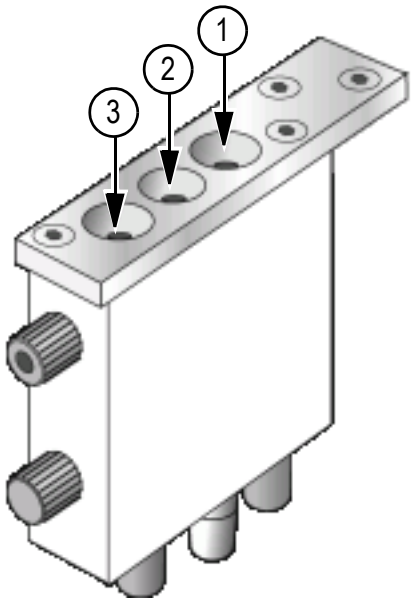
      This displays the service menu.
   d. Using the outside circular buttons to scroll, select **Objects > Tray Holders > CStack1**. Press **Enter** after each selection.
3. Select **Clear Position (F2)** and then select **Z**.
4. Select **Check Position (F1)** to move to the preset X and Y positions.
5. If needed, adjust the X, Y, and Z positions until the needle guide is accurately placed in the tray calibration hole.

   Make sure the bottom of the needle guide is flush with the bottom surface of the tray.
6. Select **Check Position (F1)** to verify the needle guide position.
7. Press **ESC** twice to return to the **Objects** menu.
8. Close the drawer.

Configure the Wash Station Position

1. On the keypad terminal, navigate to **Menu > Setup**.
2. Press **F3** then press **Enter**.
3. Select **Objects> Wash Stations** and then press **Enter**.
4. Select **Wash1** and press **Enter**.
5. Select **Clear Position (F2)** and then select **Z**.
6. Select **Check Position (F1)** to move to the preset position.
7. Adjust the X, Y, and Z positions until the needle guide is in the Wash1 port (Figure 4-25).

Figure 4-25 Wash Station—Top Ports



Item	Description
1	Wash1 port
2	Waste port
3	Wash2 port

8. Press **Enter**.
9. Select **Check Position (F1)** to verify the position.
10. Press **Esc**.
11. Repeat step 1 through step 10 for Wash2.

Configure the Injector Waste Position

1. On the keypad terminal, navigate to **Menu > Setup**.
2. Press **F3** then press **Enter**.
3. Select **Objects> Injectors** and then press **Enter**.
4. Select **Waste** and press **Enter**.
5. Select **Clear Position (F2)** and then select **Z**.
6. Select **Check Position (F1)** to move to the preset position.
7. Adjust the X, Y, and Z positions until the needle guide is seated in the Waste port (Figure 4-25).
8. Press **Enter**.
9. Select **Check Position (F1)** to verify the position.
10. Press **Esc** twice.

Configure the Injection Port Position

If the injection valve has been moved or the needle does not go to the correct location, reconfigure the injection port position. After you have reconfigured the port position, the needle penetration depth should be verified and reconfigured as needed.

The seal for the injection port needs to be tight to avoid leaks. Tighten the injection port as described below, then check the seal by doing a trial run. If solvent is appears at the port, tighten the fitting about 1/16 of a turn at a time until there are no more leaks.

1. Seat the injection port fitting.
   a. Using a spare syringe needle, manually insert the needle into the injection fitting in valve position 3.
   b. Tighten the fitting by hand until the needle is very snug.

Tip! Tighten the fitting until the needle can't be removed, then loosen the fitting a small amount. The fitting is tight enough when the fitting doesn't leak when injecting.

2. On the keypad terminal, navigate to **Menu >Setup**.
3. Press **F3** then press **Enter**.
4. Select **Objects > Injector** and then press **Enter**.
5. Select **LC Vlv1** and press **Enter**.
6. Select **Clear Position (F2)** and then select **Z**.

   Be sure to clear the Z position. If the Z position is not cleared, the needle may be damaged when you check the position.
7. Adjust the X, Y, and Z positions until the needle guide is centered over the injection fitting.
8. Press **Enter**.
9. Select **Check Position (F1)** to verify the position.

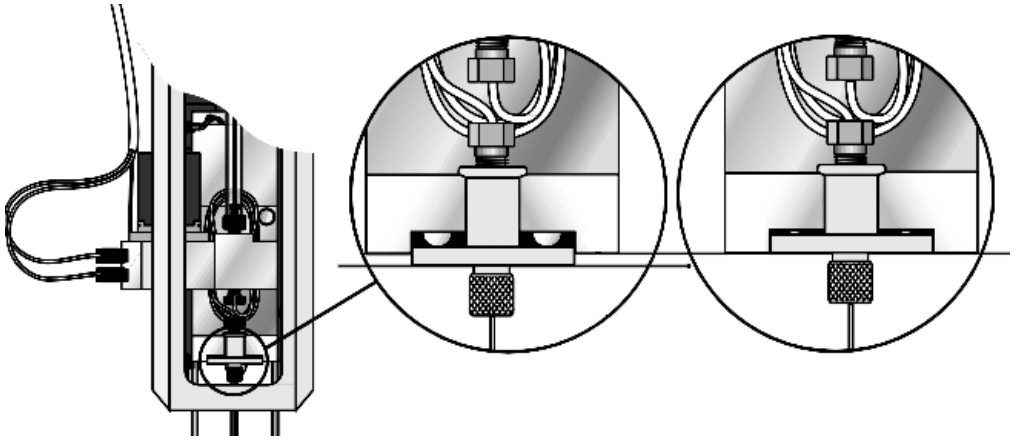
10. Configure the Needle Penetration Depth.
11. Press **Esc** five times to return to the main menu.

Configure the Needle Penetration Depth

1. Check the needle penetration depth.
    a. On the keypad terminal, navigate to **Menu > Setup**.
    b. Press **F3** then press **Enter**.
    c. Select **Objects > Injectors** and then press **Enter**.
    d. Select **LC Vlv1**.
    e. Select **Needle Penetr** and press **Enter**.

    The needle penetration depth is correct if the plate contacts the syringe holder assembly and the spring plate is fully compressed (Figure 4-26).

Figure 4-26 Needle Penetration Depth, Correct Compression Shown on Far Right



    If the penetration depth is not correct, continue to the steps below to set and verify the depth.

2. Set the needle penetration depth.
    a. Select **Clear Position (F2)** and then select **Z**.
    b. Select **Check Position (F1)** to move to the preset position.
    c. Slowly rotate the outer knob to adjust the needle penetration depth.

    The needle moves stepwise down into the injection port.
    d. When the needle tip enters the valve needle guide, slow down the Z movement.

    Move down stepwise until the moving plate contacts the syringe holder assembly and the spring plate is fully compressed (Figure 4-26).

    Always observe the needle during this step.

Note: This is more force than CTC recommends for conventional injection ports.

e. Rotate the outer knob two steps in the opposite direction and press **Enter** to save the value for needle penetration depth.

f. Press **F3 Movto Zero**.

3. Repeat step 1 to verify the needle penetration depth.

   If the needle bends, or the liquid appears at the fitting when doing a wash, repeat step 2.

Configure the Autosampler Tray Type

The default tray type is VT54. If the you want to use a different tray type, use this procedure to change the tray type.

1. Using the keypad terminal, select **Menu > Utilities > Tray > 1 > Tray Station**.
2. Scroll to the tray of interest and press **Select** to set the tray type.
3. Press **Esc**.
4. Repeat for tray positions 2 through 6.

Adjust the Needle Penetration into the Sample Vial

If the vial septa or vial cap changes from when the autosampler was first configured, or if vial inserts are being used, the depth at which the needle penetrates the vial may need to be changed.

Note: Changing this needle penetration will change the penetration for all trays of the selected type.

1. Place a vial in position 001 in Tray 1 in the stack cooler.

   Tip! Use a vial with a missing or torn septa so that it is easier to slide the vial up and down when you check the depth.

2. Close the drawer of the stack cooler.
3. Set the needle penetration depth.
   a. Using the keypad terminal, select **Menu > Utilities > Tray > 1 > F3** to move to vial 001.
   b. Select **OK** > **Needle Pentr**.

      The needle is inserted into the vial to the currently set depth.
   c. Hold the lower needle guide up and then pick up the vial or tray to see how far the needle is from the bottom.
   d. Scroll to set the needle depth and press **Select** to set the value.

      If you can't scroll to the required depth (the needle stops before the required needle penetration is reached) follow the instructions in step 4 to increase the allowed range for needle penetration.
4. If needed, increase the needle penetration range.
   a. Using the keypad terminal, select **Menu > Setup**.

b. Press **F3** and then press **Enter**.
c. Select **Setup > Objects >Tray Types > VT54** (or the tray in use) **> Max Needle Penetrat > Select**.
d. Scroll to adjust the value and press **Select** to set the value.
e. Repeat step 3 to set the depth.

Modify the Calibration Method for an AB SCIEX TripleTOF® System

For an AB SCIEX TripleTOF 4600 or 5600 system with CDS, the calibration method template must be modified so that the Eksigent MicroLC 200 Plus 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 AB SCIEX FSE during the installation of the Eksigent MicroLC 200 Plus system.

The calibration method template may need to be modified if:

- The Analyst software has been reinstalled or a different computer is connected to the system
- The column or other system plumbing will not support a 40 µL/min flow rate.
- The initial mobile phase composition for the run is very different from Table 3-1.
- The flow rate of the CDS is >500 µL/min.

Follow these steps to modify the calibration template:

1. Create the LC Method for the Calibration Method Template.
2. Update the Calibration Method Template.

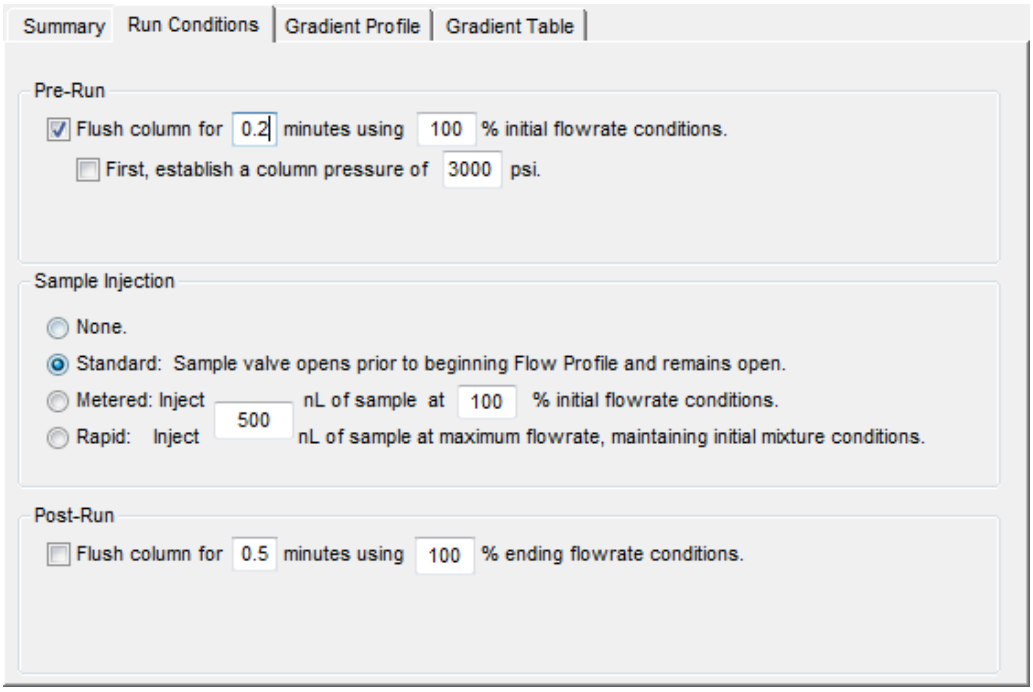
Create the LC Method for the Calibration Method Template

This method will run during the calibration run.

Caution: Potential System Damage: The LC method below is valid for the sample experiment described in Chapter 3. For other experimental conditions, the 40 µL/min flow rate may be too high for the column. In that case, edit the LC method and set the flow rate and the mobile phase composition as appropriate.

1. Click **LC Methods**.
2. In the **Name** box, type a name for the method, and then click **Save**.
3. On the **Run Conditions** tab, set the parameters as shown in Figure 4-27.

Figure 4-27 LC Method Settings Dialog—Run Conditions Tab

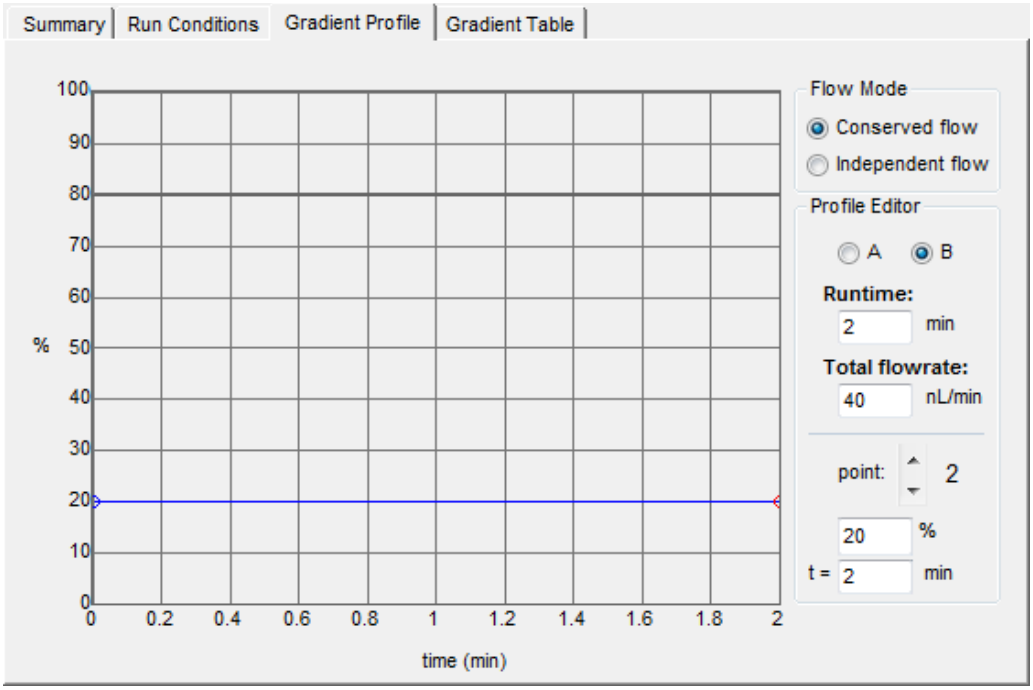


4. On the **Gradient Profile** tab, set the profile shown in Figure 4-28.

   As needed to support different experimental conditions or a different instrument configuration:

   a. Type the initial value for mobile phase B (for the beginning of the run) in the **%** field.
   b. Type the appropriate value for the current column and plumbing configuration in the **Total flowrate** field.
   c. If the flow rate for the CDS is greater than 500 µL/min, calculate the duration for the LC method: 1000/CDS flow rate.
   d. Type the new duration in the **t = min** field.

Figure 4-28 LC Method Settings Dialog—Gradient Profile Tab



5. Click **Save**.
6. Click **OK**.

Update the Calibration Method Template

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.

   a. In the Analyst software, activate the hardware profile that includes the Eksigent MicroLC 200 Plus system.
   b. In Windows, navigate to the location of the AutoCalPos.dam file.

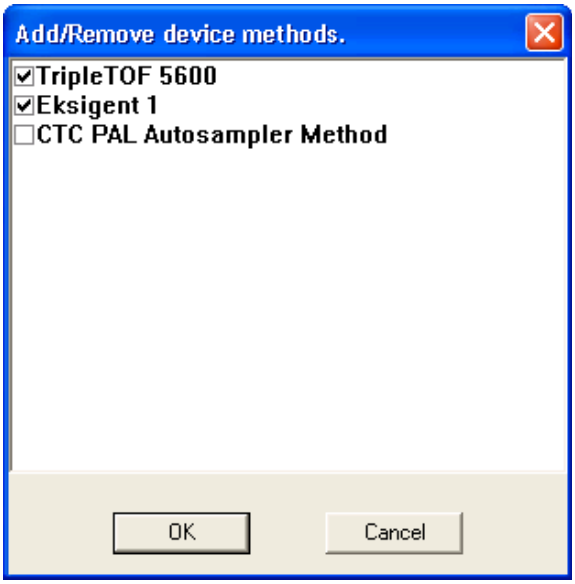
By default, this is D:\Analyst Data\Projects\API Instrument\Instrument Optimization\methods.

c. Double-click the file **AutoCalPos.dam** to open it in the Acquisition Method Editor in the Analyst software.

d. Right-click **Acquisition Method** and select **Add/remove device**.

e. In the **Add/Remove device methods** dialog, select **Eksigent 1** and then click **OK**.

Figure 4-29 Add/Remove device methods Dialog



3. Select the LC method.

   a. In the **Acquisition Method Browser** pane, click **Eksigent 1**.

   b. Click **...** (Browse) to view the available LC methods.

   c. Click the name of the method created previously and then click **Open**.

4. Save the calibration method with the same name (AutoCalPos) in the original location.

Transfer System Settings to a Different Computer

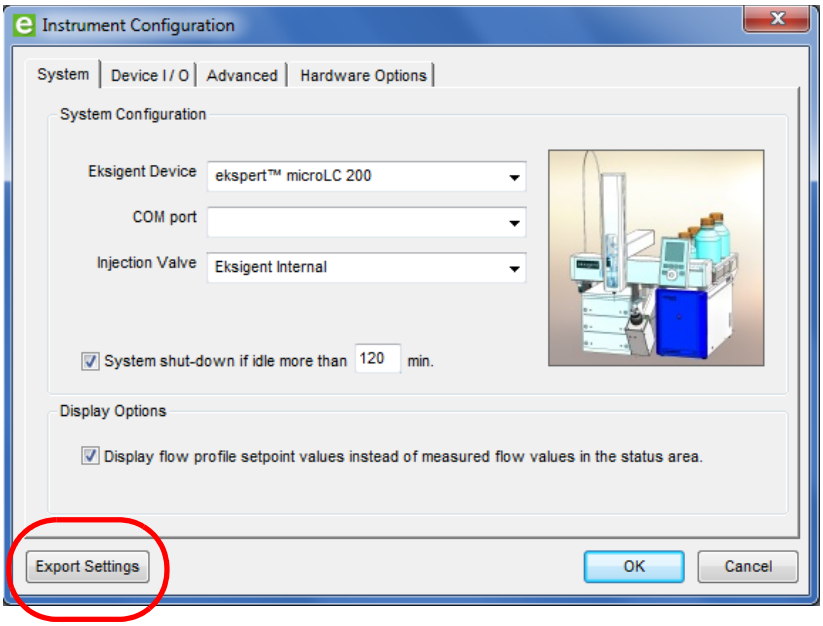
To use the Eksigent MicroLC 200 Plus system with a different computer, you must install the Eksigent control software on the new computer, transfer important files from the existing computer, and then configure the software on the new computer.

Required Materials
• Eksigent control software CD • Thumb drive

Note: Use the procedure below when you plan to use the same version of the Eksigent control software on the new 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. Copy the autosampler method from the current computer to the thumb drive.
    a. Navigate to the D:\Analyst Data\Projects\API Instrument\LC Devices\CTC PAL folder.
    b. Copy the latest version of the .cyx file (currently “microLC200-Injection-Rev B”) to the thumb drive.
2. Copy the Settings folder to the thumb drive.
    a. Navigate to the installation directory, either **C:\Program Files\EksigentNanoLC** on 32-bit operating systems or **C:\Program Files (x86)\ EksigentNanoLC** on 64-bit operating systems.
    b. Copy the Settings folder to the thumb drive.
3. Export the system settings .reg file on the current computer to the thumb drive.
    a. Start the Eksigent control software.
    b. Select **System > Instrument Configuration**.
    c. Click **Export Settings** in the lower left corner (Figure 4-30).
    All instrument settings are exported to C:\Program Files\Eksigent NanoLC\settings\EkSettings.reg.
    d. Copy the EkSettings.reg file to the thumb drive.

Figure 4-30 Instrument Configuration Dialog



4. Copy the Settings folder to the new computer.
   a. Insert the thumb drive into the new computer.
   b. Create the EksigentNanoLC folder on the new computer in the following directory:
      - For 32-bit operating systems—**C:\Program Files\EksigentNanoLC**
      - For 64-bit operating systems—**C:\Program Files (x86)\ EksigentNanoLC**

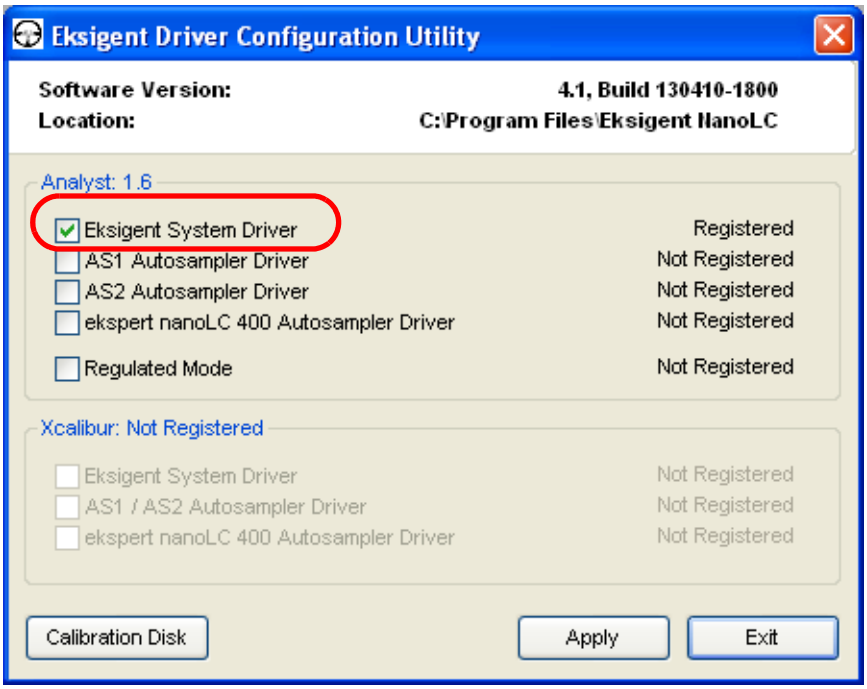
Note: If an EksigentNanoLC folder is already present on the new computer, it indicates the Eksigent control software is probably already installed. Back up the Settings folder on the new computer to a safe location before proceeding to the next step.

   c. Copy the Settings file from the thumb drive to the EksigentNanoLC folder.
5. Install the Eksigent control software on the new computer.
   a. Turn off the power to the Eksigent MicroLC 200 Plus system.
   b. Insert the Eksigent Control Software CD into the CD drive and follow the instructions to install the software.
6. Load the settings from the EKSettings.reg file.
   a. From the **Start** menu, select **Eksigent > Driver Configuration**.
      If the **User Account Control** dialog appears, click **Yes** to continue.
   b. Click **Calibration Disk** and navigate to the EkSettings.reg file on the thumb drive.
   c. In the **Analyst** section, select **Eksigent System Driver**.

Note: If the **Analyst** section is unavailable, this means the Analyst software is not installed. Install the Analyst software and repeat step 6.

d. Click **OK**.

Figure 4-31 Eksigent Driver Configuration Utility



7. Copy the .cyx file from the thumb drive to the D:\Analyst Data\Projects\API Instrument\LC Devices\CTC PAL folder.
8. Before using the system, Re-initialize the Pressure Transducers.

Troubleshooting a Move to a New Computer

Problems with the Hardware Profile in the Analyst Software

Sometimes the hardware profile in the Analyst software on the new computer does not activate due to the autosampler.

1. On the existing computer, locate the most recent version of the pal.pol file in the D:\Analyst Data\Projects\API Instrument\LC Devices\CTC PAL folder and copy it to a thumb drive.
2. On the new computer, copy the .pol file from the thumb drive to D:\Analyst Data\Projects\API Instrument\LC Devices\CTC PAL folder.
3. Follow the steps in Verify the Analyst Software Hardware Profile on page 16, to make sure the hardware profile specifies the correct COM port for the autosampler.

Problems with the Gain and Integral Settings for the Pump

If the flow is not stable or you hear the sound of gas venting when the pumps are on, this could be due to a problem with the integral and gain settings. Follow the instructions below to troubleshoot this problem.

1. On the new computer, in the Eksigent control software, select **System > Hardware Diagnostics** and then click the **Calibration Values** tab.
2. Record the gain and integral (int) settings in the **Control Parametres** section near the bottom of the tab.
3. Repeat the previous steps on the new computer and compare the values for the two computers.

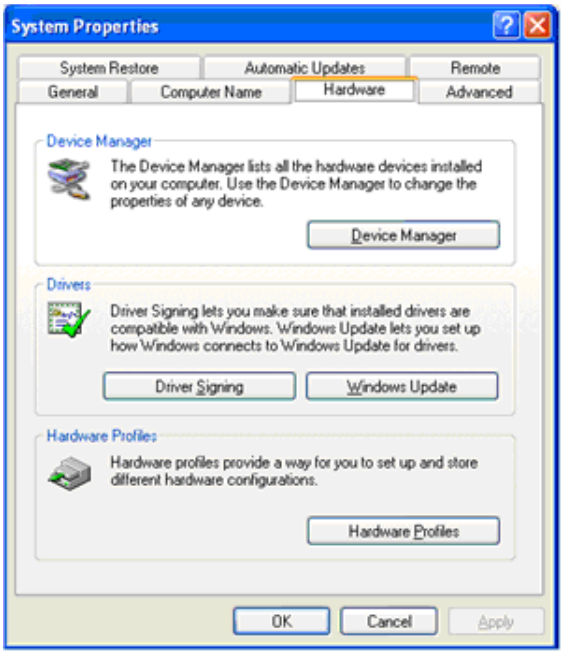
   The first two digits in each value should be the same. If the values are not the same, contact AB SCIEX Technical Support.

Problems with Recognizing the COM Port on the New Computer

If a message indicating an instrument is not connected appears when the Eksigent control software is started (Figure 4-34), the COM port setting may not be correct. To set the COM port:

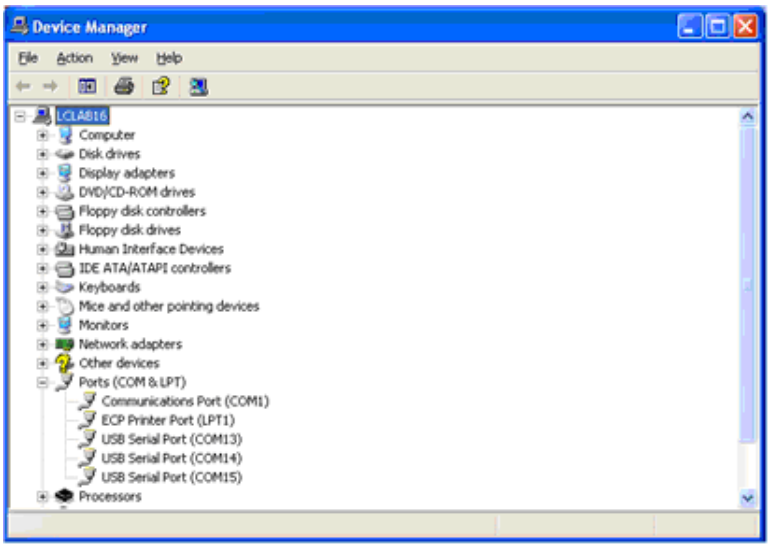
1. Turn on the Eksigent MicroLC 200 Plus system.
   a. Turn on the Eksigent power supply using the power switch on the back of the power supply.
   b. Turn on the Eksigent MicroLC 200 Plus system using the power switch on the back of the unit.
2. Wait for Windows to display the new device installed dialog, and then click **OK**.
3. Identify the COM port the system is connected to.
   a. Click **Control Panel > System > Hardware** (Figure 4-32).

Figure 4-32 System Properties Window—Hardware Tab



b. Click **Device Manager** to open the **Device Manager** window (Figure 4-33).

Figure 4-33 Device Manager Window



c. Expand the **Ports (COM & LPT)** section in the **Device Manager** window.

The three USB serial ports shown are the COM ports associated with the Eksigent MicroLC 200 Plus system. The number assigned to the serial port differ from PC to PC. The first USB serial port is assigned to the Eksigent MicroLC 200 Plus system The second and third serial ports are assigned to RS 232 (A) and RS 232 (B), respectively.

COM ports above 16 are not recognized. If this happens it is likely that lower COM port number assignments will have to be freed up from software.

Tip! AB SCIEX recommends that the autosampler be connected to RS 232 (A).

d. Note the number of the first port for use below.

4. Open the Eksigent control software.

The **No Instrument Connected** dialog appears (Figure 4-34).

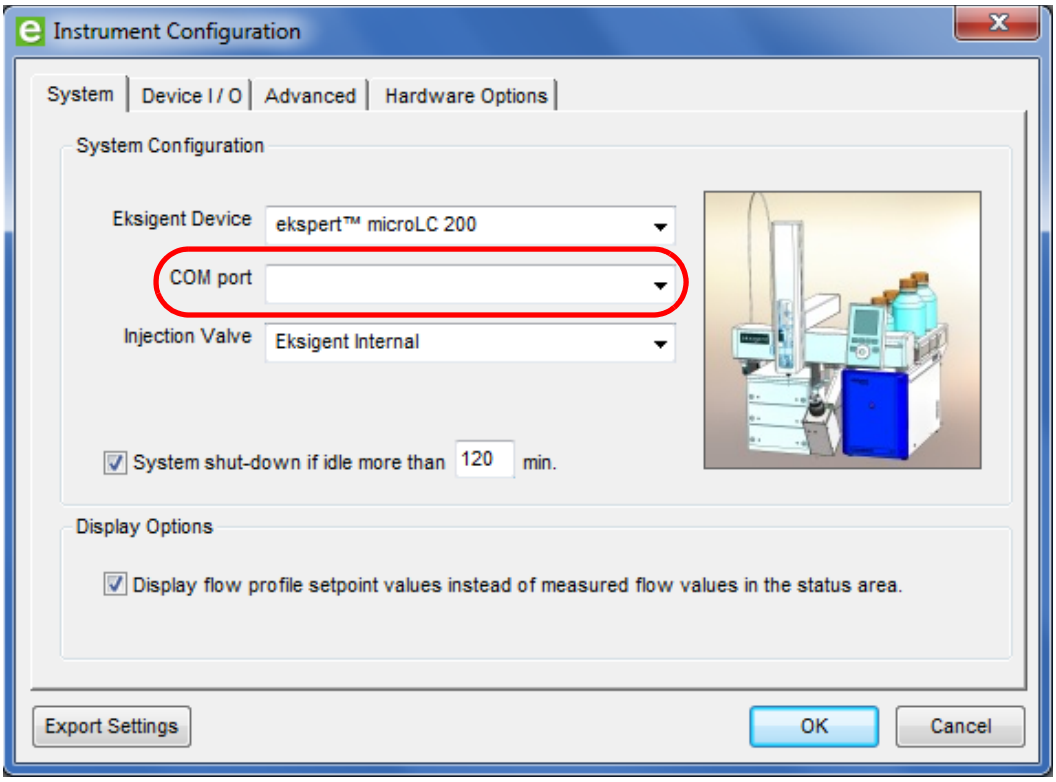
Figure 4-34 No Instrument Detected Dialog

5. Click **Cancel**.

The Eksigent control software **Acquisition** window appears.

6. In the **Acquisition** window, click **System** > **Instrument Configuration** to display the **Instrument Configuration** dialog (Figure 4-35).

Figure 4-35 Instrument Configuration Dialog—System Tab



7. In the **COM port** list, select the **COM** port that is connected to the system.
8. Click **OK**.

   The **Re-start Required** dialog appears.
9. Click **OK** to automatically quit the software.

Store the System

Before storing the system, do the following:

1. Change the mobile phases to an alcohol or acetonitrile.
2. Purge and flush the system to remove all water and mobile phases.


eksigent®
part of AB SCIEX

Troubleshooting Overview

When troubleshooting the Eksigent MicroLC 200 Plus system, follow these safety practices:

WARNING! Electrical Shock Hazard: To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the HTC-*xt* PAL autosampler. Once the power is turned off, wait approximately 30 seconds before disconnecting an assembly.

WARNING! Biohazard or Toxic Chemical Hazard: When replacing tubing or fittings on the Eksigent MicroLC 200 Plus system or the HTC-*xt* PAL autosampler, exposure to solvents may occur. Follow appropriate safety procedures and use personal protective equipment according to the applicable Safety Data Sheets supplied by the solvent vendor.

Caution: Potential System Damage: There are no user-serviceable components or assemblies inside the Eksigent MicroLC 200 Plus system. Service of any internal parts or assemblies requires an AB SCIEX trained Field Service Employee.

The basic steps for troubleshooting are:

1. Step back and look at the overall system. Is something obvious causing the problem? For example, is the instrument unplugged or improperly connected?
2. Compare 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 2500 psi for a certain method, is the system pressure currently in the same range, or drastically higher or lower?
3. In the order listed below, identify any symptoms which vary from normal system operation:
   - System power on and initialization (initialization fails)
   - System diagnostics (flow stability, controller tuning)
   - Flow rate in each channel (high, low, erratic)
   - Output pressure (high, low, erratic)
4. For each symptom, perform the corrective actions in the troubleshooting tables which follow.

   If this process does not correct the problem, contact AB SCIEX Technical Support.

Testing Fluid Connections

WARNING! Biohazard or Toxic Chemical Hazard: To avoid exposure to solvents when testing the fluid connections, be sure to have a vial or other container available to collect the solvent leaving the system.

Follow the instructions below to identify leaks or clogs in the system.

1. Disconnect all the exits in the flow path, then sequentially connect them, one by one, starting at the pump.
2. Set the flow conditions to 40 µL/min with a 50% A:50% B isocratic and start the pump.
3. Compare the pressure to the value in Figure 5-1.

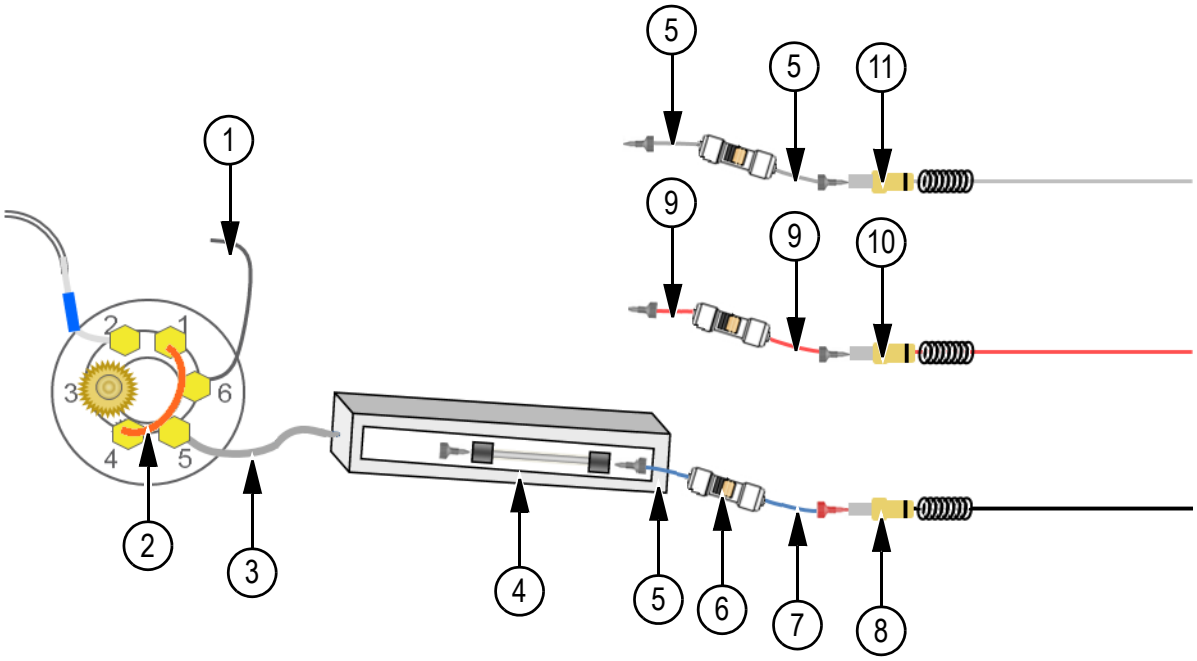
   Ensure that the vial is in place before staring the pump.

   - If the pressure is close to the value, the system is working as expected.
   - If the pressure is higher, there may be a clog.
   - If the pressure is low, there may be a leak.
4. Stop the flow.
5. Connect the next item in the flow path, start the pump, and measure the pressure.
   - If the pressure is close to the value, the system is working as expected.
   - If the pressure is higher, there may be a clog.
   - If the pressure is low, there may be a leak.

   Repeat for all the components in the flow path, including the column and the ion source.
6. Stop the flow.

Note: Column pressure (Pc) will vary with the composition of the mobile phase. More viscous mobile phases will result in higher pressure.

Figure 5-1 Expected Pressure Changes Across the Eksigent MicroLC 200 Plus system—Flow Rate 40 µL/min with a 50% A (Water):50% B (Acetonitrile)



Item	Description	Pressure (psi)	Total Pressure (Pc) (psi)
System Plumbed for 65 µm ID Electrode			
1	Mixer-to-valve assembly	37	37
2	2 µL PEEKsil sample loop	1	38
3	30 cm of 50 µm ID PEEKsil tubing	315	353
4	0.5 x 50 mm 2.7 µm HALO fused C18 column	3200	3553
5	5 cm of 50 µm ID PEEKsil tubing	32	3585
6	In-line filter	~0	3585
7	5 cm of 50 µm ID PEEKsil tubing	32	3617
8	65 µm ID electrode	44	3661
Other Components			
9	5 cm of 25 µm ID PEEKsil tubing	504	N/A
10	25 µm ID electrode	2017	N/A
11	50 µm ID electrode	126	N/A
(not shown)	5 µL PEEKsil sample loop	~0	N/A
(not shown)	10 cm of 50 µm ID PEEKsil tubing	63	N/A
(not shown)	10 cm of 25 µm ID PEEKsil tubing	1009	N/A

Troubleshooting Tables

Table 5-1 System Initialization

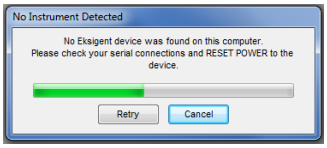
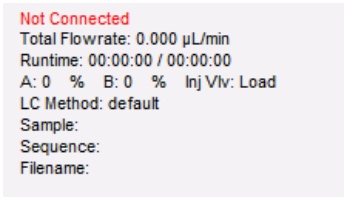
Symptom	Possible Cause	Corrective Action
Power LED on front panel is not on.	Power supply is not turned on	Turn on the power supply.
	No power at outlet	Repair the electrical outlet.
	Power LED has failed but system response OK	Contact AB SCIEX Technical Support for assistance.
No Instrument Detected dialog or “Not connected” in **Acquisition** window.  	Communication error between computer and LC system	Verify the instrument USB cable is securely connected to the computer USB port. Reboot the computer and cycle the power on the instrument.
		In the list of COM ports in the **Windows Device Manager** dialog, verify the number assigned to the COM port is less than 16. If not, re-assign the COM port with a lower number.
		Contact AB SCIEX Technical Support for assistance.
Loud hissing sound from the instrument.	Air leaks from the air inlet fitting	Verify the air tubing is properly connected to the gas fitting.
		Tighten the air inlet gas fitting.
		Contact AB SCIEX Technical Support for assistance.

Table 5-2 Flow Control System

Symptom	Possible Cause	Corrective Action
System pressure (Pc) and/or pump pressures (Pa & Pb) show pressure though the flow is off	Incorrect zero setting for pressure sensors	Re-initialize the pressure transducers (refer to Re-initialize the Pressure Transducers on page 52).

Table 5-2 Flow Control System (Continued)

Symptom	Possible Cause	Corrective Action
No liquid out of waste line when purging	Air trapped in the pump	Purge and flush the system (refer to Purge Mobile Phases on page 51).
	Internal filters are plugged	Contact AB SCIEX Technical Support for assistance.
	Leak in the system prior to the purge valve	Check all connections.
Pump restrokes frequently ("Pump has reached end of stroke" error message appears)	Air trapped in the pump	Purge the pump (refer to step 3 in Purge Mobile Phases on page 51).
	Pump remains on long enough to prompt a restroke	For the current flow rate, calculate the time to pump ~600 µL. Verify that the pump restrokes at approximately that time interval.
	Check valve is leaking	Contact AB SCIEX Technical Support for assistance.
Pump does not re-stroke at the end of a run.	Pump restroke delay is too short	Contact AB SCIEX Technical Support for assistance.
	Optical sensor not working correctly	
Pump flushes quickly but does not deliver ~600 µL per stroke	Leak in instrument	Contact AB SCIEX Technical Support for assistance.
Purge output drips slowly	Internal filters are plugged	Contact AB SCIEX Technical Support for assistance.
No flow rate with 100% power indicated. System pressure (Pc) and pump pressures (Pa and Pb) are all low.	No air to system	Connect 100 psi clean, dry air to the instrument air inlet.
	System not properly purged and flushed	Purge and flush the system (refer to Purge Mobile Phases on page 51).
Inability to reach desired flow rate	Internal filters are plugged	Contact AB SCIEX Technical Support for assistance.
	Flow rate setpoint too high for system back pressure	In the **Direct Control** dialog, decrease the flow rate.
	Air pressure too low	Verify that the air supply has an air pressure of 100 psi.

Table 5-2 Flow Control System (Continued)

Symptom	Possible Cause	Corrective Action
Flow rate will not initialize at start of run	Flow rate setpoint too high off system back pressure	In the **Direct Control** dialog, decrease the flow rate.
	Bubbles in the system causing erratic flow rate	Purge and flush the system (refer to Purge Mobile Phases on page 51).
	Unable to meet required flow rate within specified tolerance	Lower the flow stabilization limit in the **Advanced** tab of the **Instrument Configuration** dialog of the Eksigent control software.
	Internal filters are plugged	Purge the system (refer to Purge Mobile Phases on page 51) and inspect the flow through the waste tubing after ~8 purges. If the flow is very low or intermittent, the filter should be changed. Contact AB SCIEX Technical Support for assistance.
Flow rate will not stabilize during a run	Erratic flow rate due to bubbles in system	Purge and flush the system (refer to Purge Mobile Phases on page 51).
	Incorrect mobile phase setting	Check the settings in the **Mobile Phases** dialog and modify as needed.
	Pump controller out of tune	Contact AB SCIEX Technical Support for assistance.
	Flow temperature is not stable	Contact AB SCIEX Technical Support for assistance.
System responds sluggishly when changing flow rates	Incorrect mobile phase setting	Check the settings in the **Mobile Phases** dialog and modify as needed.
	Pump controller out of tune	Contact AB SCIEX Technical Support for assistance.
Inaccurate flow rate with no signs of leakage	Incorrect mobile phase setting	Check the settings in the **Mobile Phases** dialog and modify as needed.
	Incorrect k-values	Calibrate the flowmeters (refer to Calibrate Flowmeters on page 54).
System pressure (Pc) is unusually low but flow rate is OK	Loose connection after mixing tee	Check all connections for leaks.

Table 5-2 Flow Control System (Continued)

Symptom	Possible Cause	Corrective Action
System pressure (Pc) is low and flow rate is OK but pump pressures (Pa and Pb) are high	Incorrect k-values	Calibrate the flowmeters (refer to Calibrate Flowmeters on page 54).
	Flow module is plugged	Contact AB SCIEX Technical Support for assistance.
System pressure (Pc) is very high	Tubing or fitting is plugged	Starting at the waste tanks, remove each tube or fitting, one at a time, until the pressure drops dramatically. Replace the plugged item.
Excess flow noise	Trapped air in pump	Purge the pumps (refer to step 3 in Purge Mobile Phases on page 51).
	Pump controller out of tune	Contact AB SCIEX Technical Support for assistance.
Measured flow does not follow the flow profile	Pump controller out of tune	Contact AB SCIEX Technical Support for assistance.
	Pump time response is set incorrectly	Adjust the pump time response in the **Flow Metering and Control** section of the **Hardware Diagnostics** dialog.
Pump pressures (Pa and Pb) maximized to <12 000 psi at 100% pump power	Incorrect gain setting for pressure	Verify the pump pressures are 2800 psi/V in the **Calibration Values** tab of the **Hardware Diagnostics** dialog.
	Incorrect zero setting for pressure sensors	Re-initialize the pressure transducers (refer to Re-initialize the Pressure Transducers on page 52).
	In-line air pressure too low.	Verify that the air supply has an air pressure of 100 psi.

Table 5-3 Column Oven

Symptom	Possible Cause	Corrective Action
Column responds very slowly when changing temperature	Oven malfunction	Contact AB SCIEX Technical Support for assistance.
Temperature reads 47	Oven unplugged	Verify the column oven is plugged in.

Table 5-4 Autosampler

Symptom	Possible Cause	Corrective Action
Eksigent control software does not recognize HTC-*xt* PAL autosampler when Run Manager starts	Communication error between autosampler and computer	Verify the RS-232 cable is securely connected to the autosampler communication port.
	Software may be configured to use a different COM port than the autosampler is using	Determine which COM port is configured for the software in the **System** tab of the **Instrument Configuration** dialog of the Eksigent control software. If needed, select a different port.
"Autosampler not recognized" message when activating the hardware profile in the Analyst® software	Communication problem between autosampler and the Analyst software	Power cycle the autosampler and try again to reactivate the hardware profile.

Table 5-5 Injection Valve

Symptom	Possible Cause	Corrective Action
Injection valve does not switch positions	Valve is not configured in Eksigent control software	Select **Eksigent Internal** in the **Injection Valve** list in the System tab of the **Instrument Configuration** dialog of the Eksigent control software.
	Valve is not connected to the actuator	Contact AB SCIEX Technical Support for assistance.
	Actuator is faulty	
No flow coming out of the port	Valve is plumbed incorrectly	Verify the plumbing configuration and reconfigure if needed (refer to Plumb the Injection Valve on page 61).
	Ports are plugged	Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, contact AB SCIEX Technical Support for assistance.

Table 5-5 Injection Valve (Continued)

Symptom	Possible Cause	Corrective Action
When no column is connected, system pressure (Pc) is unusually high	Ports are plugged	Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, contact AB SCIEX Technical Support for assistance.
	Ends of tubing crushed	Replace tubing and do not over-tighten fittings.
Fluid leaking from the valve	Ferrule not properly seated in the port	Check the tubing connection and verify the ferrule is properly seated.
	Rotor seal is scratched	Replace the rotor seal (refer to Replace the Injection Valve Rotor Seal on page 58).
Inconsistent flow rate	Internal leakage in valve	Contact AB SCIEX Technical Support for assistance.
	Ports are plugged	Use a syringe to manually flush each port with cleaning solvent. If flushing does not clean the port, contact AB SCIEX Technical Support for assistance.
System does not initiate an injection	System flow is unstable	Purge the pumps and re-equilibrate the system.
	Flow stabilization set too low	Set the flow stabilization limit >100 nL/min in the **Advanced** tab of the **Instrument Configuration** dialog of the Eksigent control software.
	Autosampler configured to wait for injection but the Sample Injection setting in the Eksigent control software is “None”	In the **LC Method Settings** dialog, change **Sample Injection** to a value other than None.
Pressure drops at the beginning of each run	Air bubbles in sample loop	In the Analyst software, edit the autosampler method to make sure the loop is completely filled with sample. Either: • In the **Cycle Arguments** table, set the **Front Volume** and **Rear Volume** > 0. • Specify an **Injection Volume** greater than the volume of the sample loop.
High relative standard deviation between peak areas for successive runs		


eksigent®
part of AB SCIEX

Principles of Operation

A

The Eksigent MicroLC 200 Plus system is an ultra high-pressure liquid chromatography (UHPLC) system optimized for ultra-fast LC/MS analysis using 0.3 mm to 1.0 mm ID columns.The system incorporates microfluidic flow control (MFC) to generate precise LC gradients at microflow rates. The system also includes the HTC-*xt* PAL autosampler with dynamic load and wash (DLW), a sample injection system designed to minimize sample carryover.

This appendix contains the following sections:

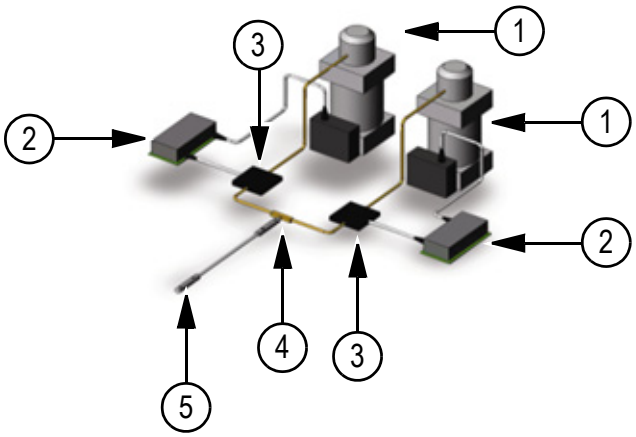
Microfluidic Flow Control

MFC has two primary benefits:

- precise gradients at microliter-per-minute rates without flow splitting
- extremely rapid response to setpoint changes enabling fast gradients and dynamic flow control

The components of a binary gradient MFC system are shown schematically in Figure A-1.

Figure A-1 Schematic Drawing of Microfluidic Flow Control (MFC) System Components



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

Item	Description
4	Mixing tee
5	Column

How the MFC System Works

For each mobile phase:

1. The flowmeter continuously monitors the flow rate and sends a signal to the PID controller (refer to Calculating the Flow Rate).

   A PID controller is a control loop feedback device that automatically adjusts one variable in a system in an attempt to hold another variable at a specified setpoint. For the Eksigent MicroLC 200 Plus system, the controller adjusts the pressure in order to maintain the specified flow rate.

2. The PID controller sends a voltage signal to the pressure source.

   The signal is proportional to the pressure required for the desired flow rate during the gradient (refer to Pressure Required to Generate a Gradient on page 105).

3. The pressure source changes the pressure to generate the required flow rate.

   Pressure in the system is generated by connecting laboratory air or nitrogen to a pneumatic amplifier. For example,100 psi incoming air pressure from the laboratory air system can be used to produce hydraulic pressure ranging from 0 psi to >10 000 psi.

Calculating the Flow Rate

The flow rate of each mobile phase is determined by measuring the differential pressure, ΔP, across a calibration module of known geometry (item 3 in Figure A-1).

Flow rate, Q, is given as:

$$Q = k \Delta P/\mu$$

where

k = flow conductance—determined by the flowmeter calibration

ΔP = differential pressure—measured at the flow module

μ = pressure-corrected viscosity of the mobile phase—from the Eksigent control software

The flow conductance (k) is measured and corrected using the flowmeter calibration in the Eksigent control software. This calibration is part of routine maintenance for the system (refer to Calibrate Flowmeters on page 54).

The flow rate measurement is determined for the individual mobile phases by measuring ΔP.

Viscosity for a given mobile phase is a function of pressure, temperature, and the composition of the mobile phase. The Eksigent control software contains mixture viscosity parameters for a wide variety of frequently used mobile phases. Because the temperature at the flowmeter is controlled, eliminating the need for temperature-correction.

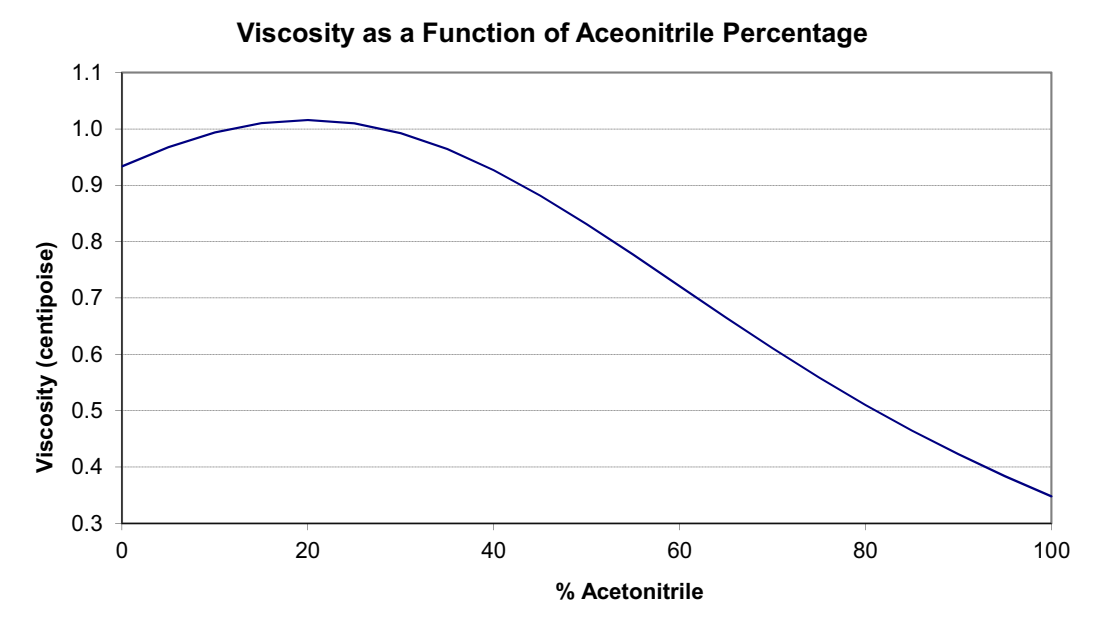
Pressure Required to Generate a Gradient

For a run, the total flow rate through the column, Q_{COL}, and the gradient profile are specified. From the gradient profile, the system can calculate the percentage of each mobile phase (and thus the required flow rate) at any point during the gradient.

Knowing the viscosities, flow conductances, and flow rates of each mobile phase, and the pressure at the head of the column (Pc), the setpoint pressures for mobile phase a (Pa) and mobile phase b (Pb) can be calculated. The microfluidic flow controllers maintain these setpoints and adjust the pressure as needed to change the flow rate of each mobile phase over the duration of the gradient.

For a typical water-acetonitrile gradient, Pc changes during the run due to the increased volume of acetonitrile as the gradient progresses. Figure A-2 shows how viscosity changes based on the percentage of acetonitrile.

Figure A-2 Viscosity as a Function of Acetonitrile % for a Water-acetonitrile Mixture



Pressure and flow rate for the system and each mobile phase can be viewed in the status area (Figure A-3) of the **Acquisition** window in the Eksigent control software (Figure A-4).

Figure A-3 Detail of Status Area in the Eksigent Control Software

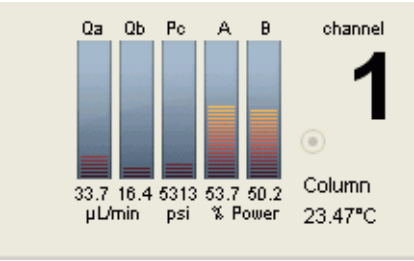
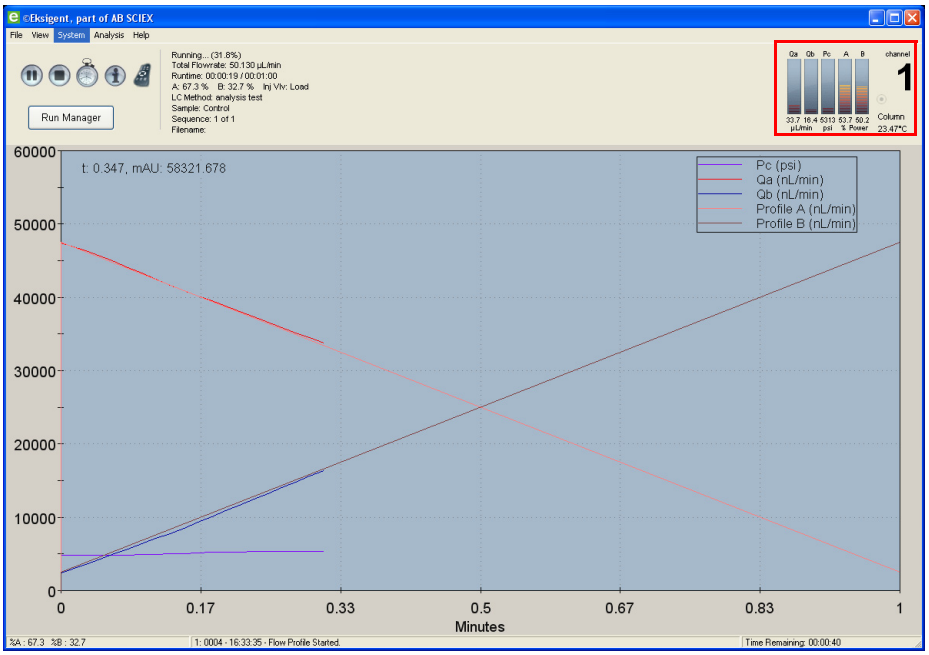
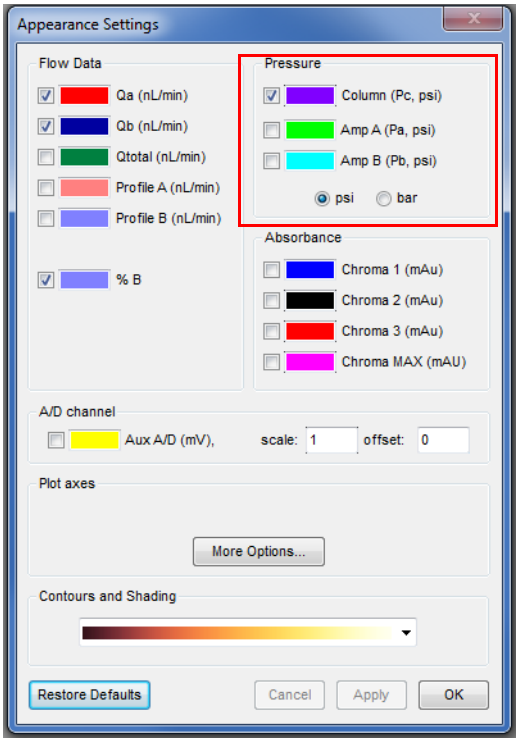


Figure A-4 Acquisition Window



Use the **Appearance Settings** dialog (accessed from **System > Appearance Settings** or by right-clicking the status bars) to select which parameters are displayed (Figure A-5).

Figure A-5 Appearance Settings Dialog

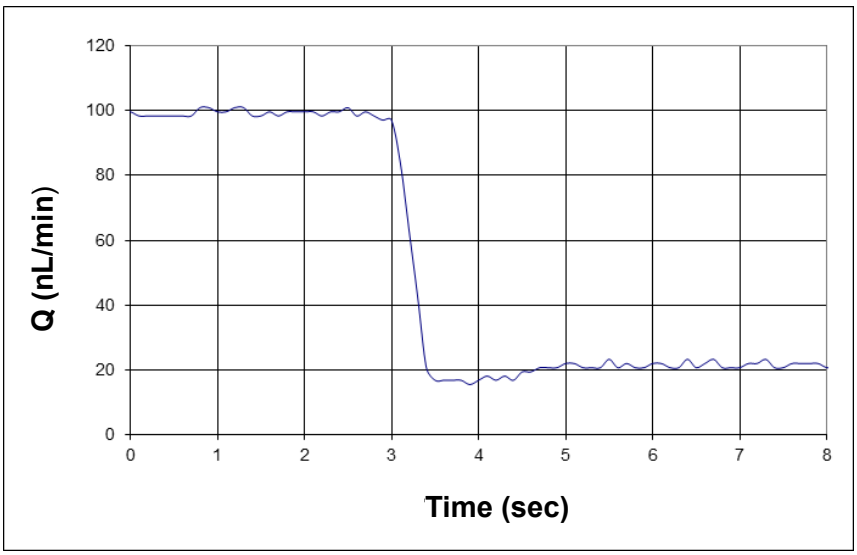


Dynamic Flow Control

In addition to precise control of gradients, the rapid pressure control of MFC also allows the flow rate to be changed dynamically during a gradient run.

Figure A-6 shows how flow rate (Q) drops from 100 nL/min to 20 nL/min in less than 1 second at the flowmeter. The column capacitance typical delays this effect for less than 2 seconds at the electrode tip.

Figure A-6 Plot of Flow Rate Versus Time, Showing Rapid Response



Guidelines for Micro UHPLC Methods

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

Flow Rate and Injection Volume

When converting a method from traditional UHPLC to micro UHPLC, flow velocity should be kept the same so that retention times do not change.

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

Q = flow rate

A = cross-sectional area of the column

Table A-1 demonstrates how flow rate varies by column diameter for (approximately) the same flow velocity. The table also gives appropriate injection volumes.

Table A-1 Equivalent Flow Rates and Injection Volumes for Micro UHPLC

Column Diameter (mm)	Flow Rate (µL/min)	Injection Volume (µL)	Cross-sectional Area (mm^2)
Traditional UHPLC			
4.6	2100	200	16.62
2.1	450	40	3.46
Micro UHPLC			
1.0	100	10	0.785
0.5	25	2.5	0.196
0.3	10	1.0	0.071

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

Determining Flow Rate for Different Columns

Consider converting a traditional UHPLC method to one for the Eksigent MicroLC 200 Plus system. Column a is used for traditional UHPLC, with a known flow rate (Q_a). Column b will be used on the Eksigent MicroLC 200 Plus system. What is the flow rate for column b?

Because the columns should have the same flow velocity, the relationship shown in Figure A-7 is true.

Figure A-7 Columns a and b with the Same Flow Velocity

$$FV = \frac{Q_a}{A_a} = \frac{Q_b}{A_b}$$

Solving for the flow rate for column b (Q_b) results in Figure A-8.

Figure A-8 Flow Rate for Column b

$$Q_b = \frac{Q_a}{A_a} \times A_b$$

Because columns are usually specified by internal diameter (ID), it is more useful to express the cross-sectional area in terms of the column diameter, as shown in Figure A-9, where D is the column diameter.

Figure A-9 Column Cross-sectional Area in Terms of Diameter

$$A = \pi \times \left[\frac{D}{2}\right]^2$$

Substituting the formula for column cross-sectional area into the equation for Q_b gives Figure A-10.

Figure A-10 Flow Rate for Column b, Showing Cross-sectional Area Explicitly

$$Q_b = \frac{Q_a}{\left(\pi \times \frac{D_a^2}{4}\right)} \times \left(\pi \times \frac{D_b^2}{4}\right)$$

Q_b can be simplified to Figure A-11. As can be seen below, the flow rate for column b is proportional to the square of the column diameter.

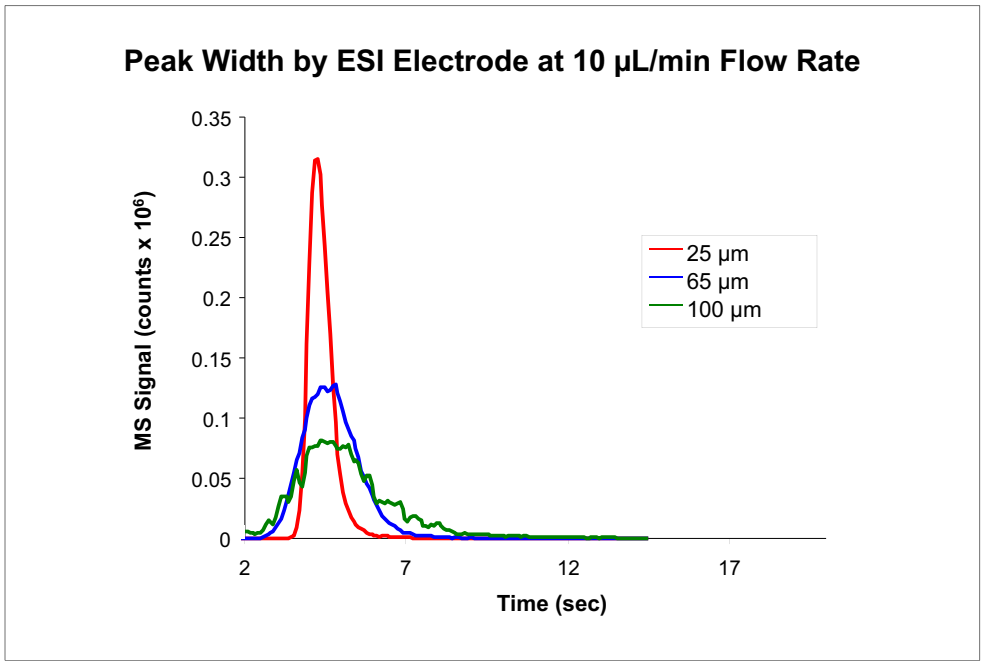
Figure A-11 Flow Rate for Column b, Showing Relationship to Column Diameter

$$Q_b = Q_a \times \frac{D_b^2}{D_a^2}$$

Electrodes and Tubing

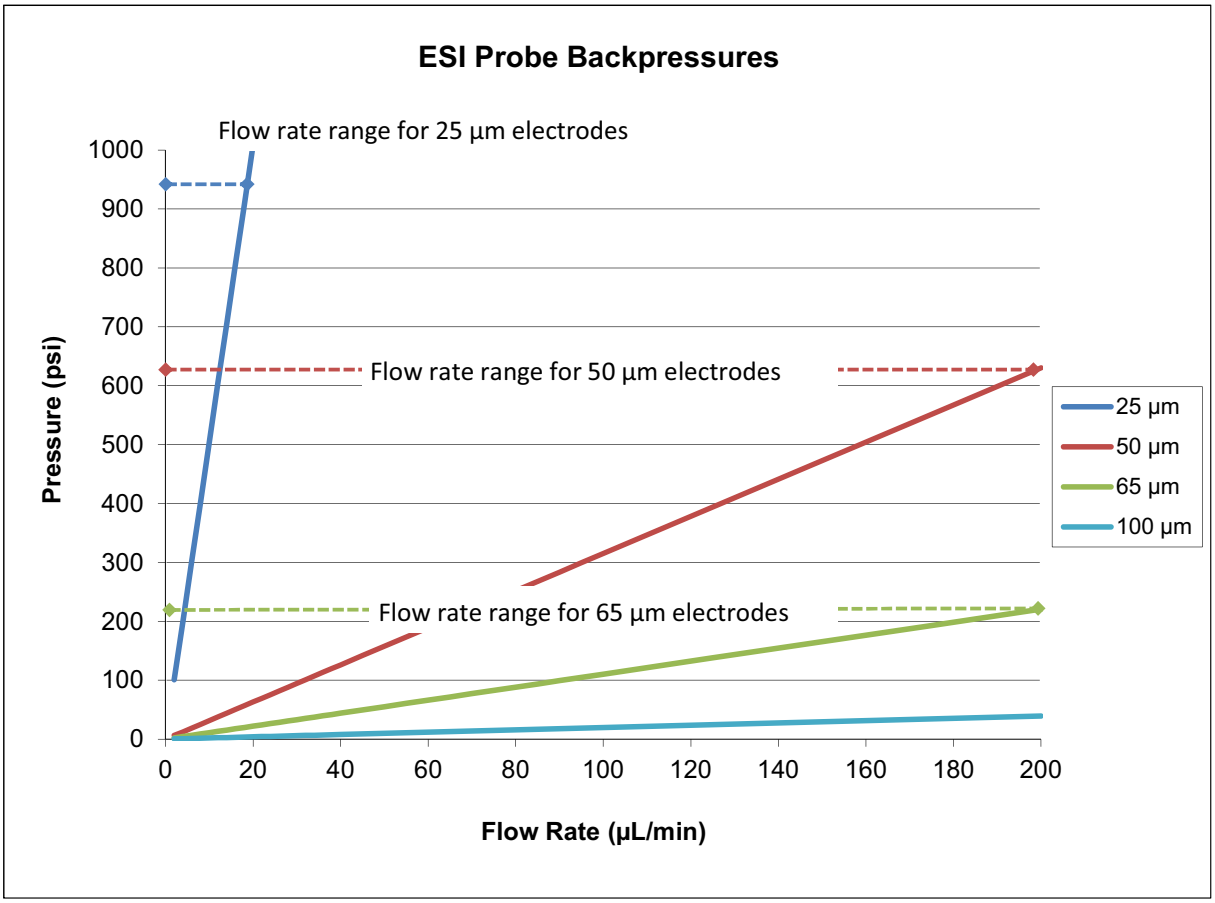
When selecting an electrode for use with the Eksigent MicroLC 200 Plus system, it is important to balance peak spreading with back pressure on the system. In general, the smaller the electrode, the narrower the peak width (Figure A-12).

Figure A-12 Mass Spectrometer Peak Width for Different ESI Electrodes



However, as flow rate increases so does the back pressure on the system. For example, at the relatively low flow rate of 20 µL/min, back pressure on a 25 µm diameter electrode can reach ~1000 psi (Figure A-13) (dashed lines indicate appropriate flow rate ranges for 25 µm (red) and 50 µm (brown) electrodes, green line shows pressure for a 100 µm diameter electrode for reference).

Figure A-13 Back Pressure Versus Flow Rate for Different ESI Electrodes



Use Table A-2 to select the appropriate electrode and tubing based on the column diameter. (Information for traditional UHPLC is given for reference.)

Table A-2 Tubing, Flow Rates, and Electrodes for Micro UHLPC

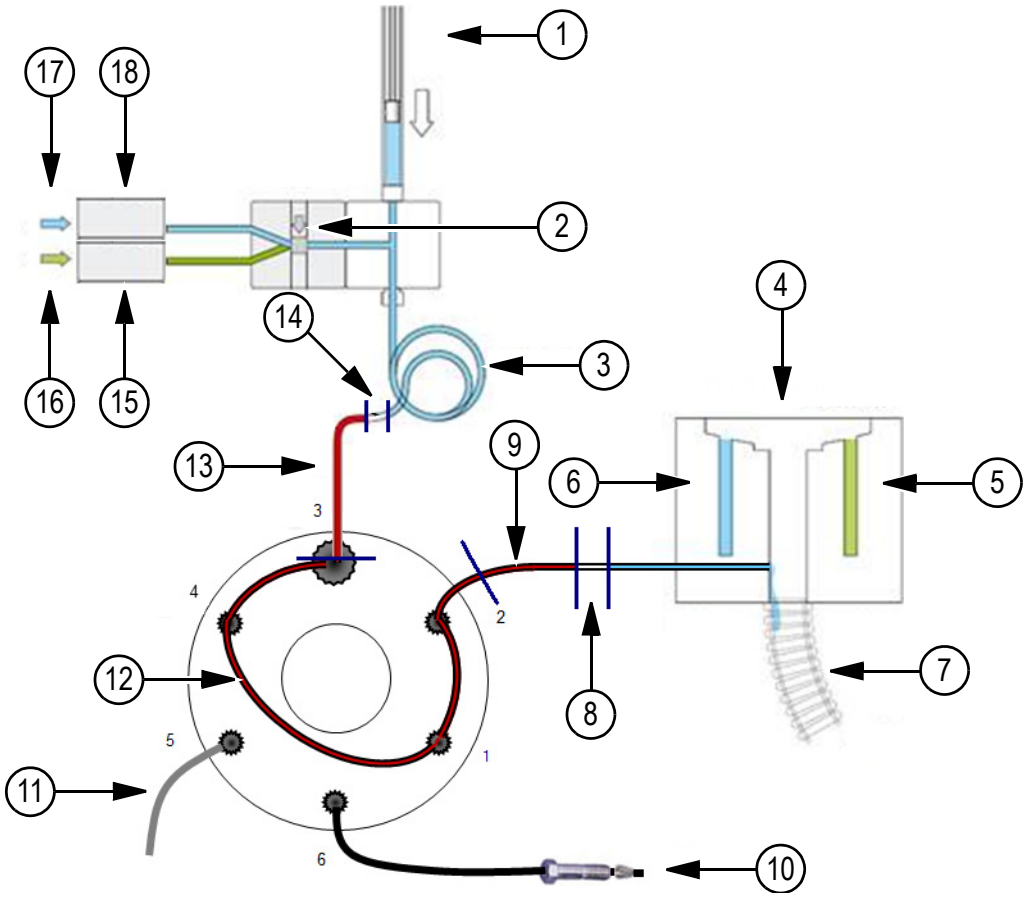
Column Diameter (mm)	Flow Rate (µL/min)	Pre-column Tubing Diameter (µm)	Post-column Tubing Diameter (µm)	Recommended ESI Electrode
Traditional UHPLC				
2.1	200 to 1000	~125 (0.005 inch)	~125 (0.005 inch)	Standard Turbo V electrode (100 µm ID)
Micro UHPLC				
0.3	4 to 20	50	25	25 µm or 50 µm ID hybrid PEEKsil/stainless steel
0.5	10 to 50	50	25 or 50	50 µm ID hybrid PEEKsil/ stainless steel
1.0	50 to 200	50	50	65 µm ID stainless steel or 50 µm ID hybrid PEEKsil/ stainless steel

Dynamic Load and Wash

The DLW (dynamic load and wash) feature on the HTC-*xt* PAL autosampler is designed to minimize sample carryover. Unlike conventional autosamplers, the sample is never in contact with the syringe. Instead, the sample only comes into contact with the needle and the holding loop. When the sample is aspirated, it is bracketed on both ends with a small volume of air which creates a barrier to prevent the diffusion of the sample into the wash solvent. The air also assists in cleaning the entire injection path. At the end of the injection cycle, all parts of the system which have been in contact with the sample are washed with both organic and aqueous wash solvents and are completely clean, resulting in near-zero carryover for most components.

Figure A-14 shows the DLW schematically, with sample indicated in red, wash solvent 1 indicated in blue, and wash solvent 2 indicated in green.

Figure A-14 Schematic Drawing of the DLW



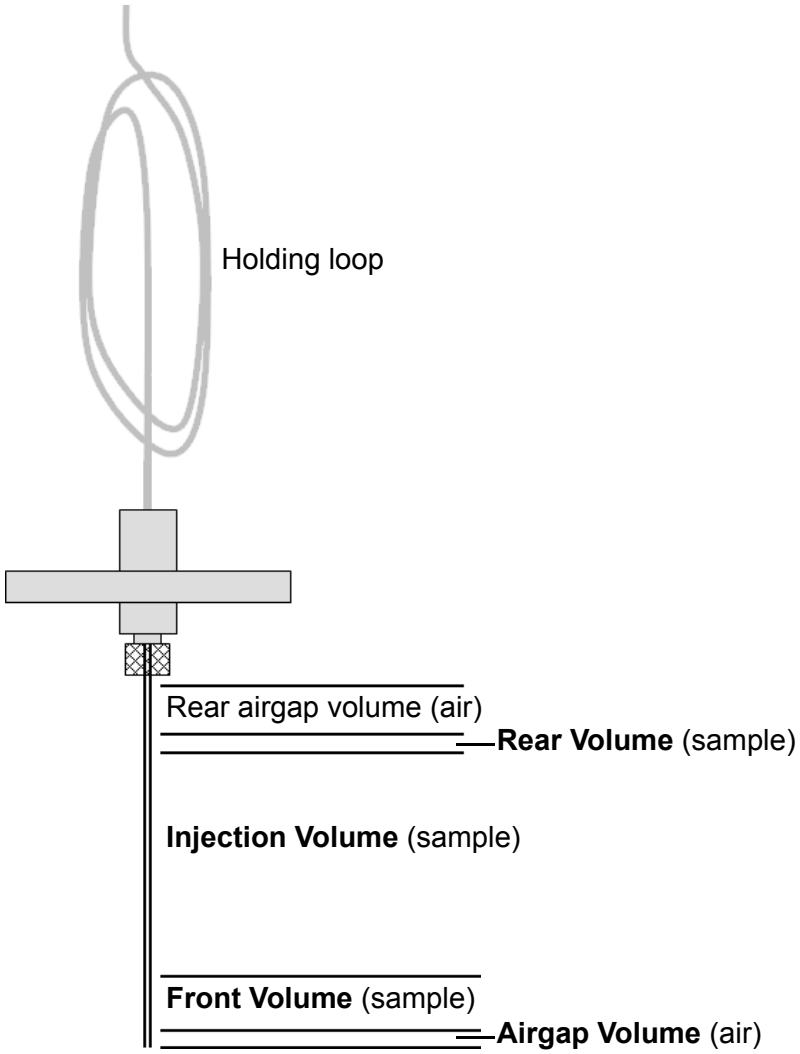
Item	Description	Item	Description
1	Syringe	10	From pump
2	Solenoid/actuator valve	11	To column
3	Holding loop	12	Sample—located between front sample (item 9) and rear sample (item 13); specified by Injection Volume
4	Wash station	13	Rear sample—specified by Rear Volume
5	Wash solvent 2 waste	14	Rear airgap—fixed volume of 0.5 µL
6	Wash solvent 1 waste	15	DLW pump 2
7	To waste container	16	Wash solvent 2 (aqueous)
8	Front airgap—specified by Airgap Volume in the autosampler method	17	Wash solvent 1 (organic)
9	Front sample—specified by Front Volume	18	DLW pump1

The DLW Process

1. Air and sample are aspirated in the needle and holding loop as shown in Figure A-15.

   Bold text indicates volumes which can be set by the user.

Figure A-15 Holding Loop and Syringe, Showing Location of Sample and Airgaps



2. The needle and holding loop, and their contents are moved to the injection port.
3. The front airgap and front sample is dispensed immediately.
4. When the Eksigent MicroLC 200 Plus pump is ready to start a run, the sample is dispensed and the valve is switched to inject the sample onto the column.
5. After the valve is switched, the entire sample path, including the valve and needle, is washed twice, first with the organic wash solvent and then with the aqueous solvent.

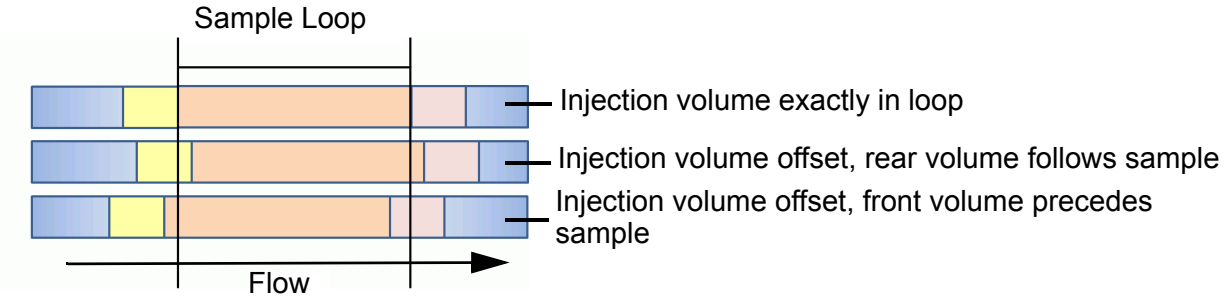
Sample Position in the Sample Loop

Ideally, the injection volume is injected exactly into the sample loop. In reality, the injection volume is often slightly offset. Therefore, the front and rear volume are used to ensure the loop is full with sample. This is illustrated schematically in Figure A-16, where:

- Blue—air (front air gap and rear air gap)
- Yellow—sample (rear volume)
- Orange—sample (injection volume)
- Pink—sample (front volume)

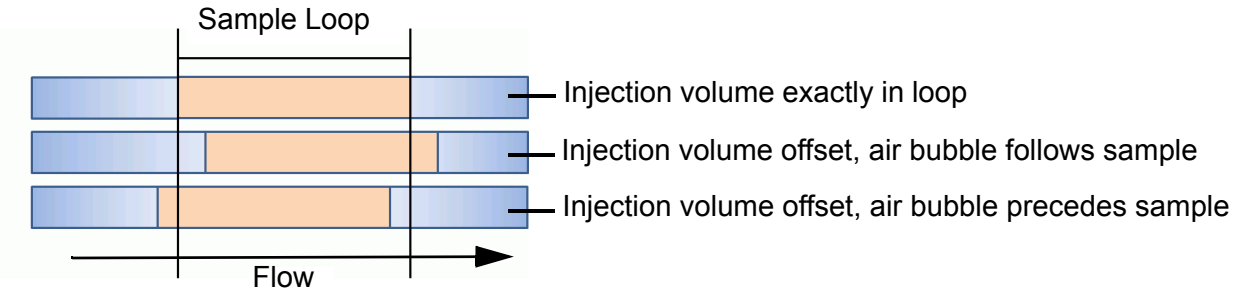
Even when the injection volume is not located exactly in the sample loop, the presence of the front and rear volume ensures that the entire volume injected onto the column contains sample.

Figure A-16 Possible Locations of Sample in Loop, Front and Rear Volume >0



Because the front and rear volumes can be set to 0 by the user in the autosampler method in the Analyst® software, it is possible that the volume injected onto the column can include an air bubble. This is illustrated schematically in Figure A-17, where blue represents air and orange represents the injection volume.

Figure A-17 Possible Locations of Sample in Loop, Front and Rear Volume = 0

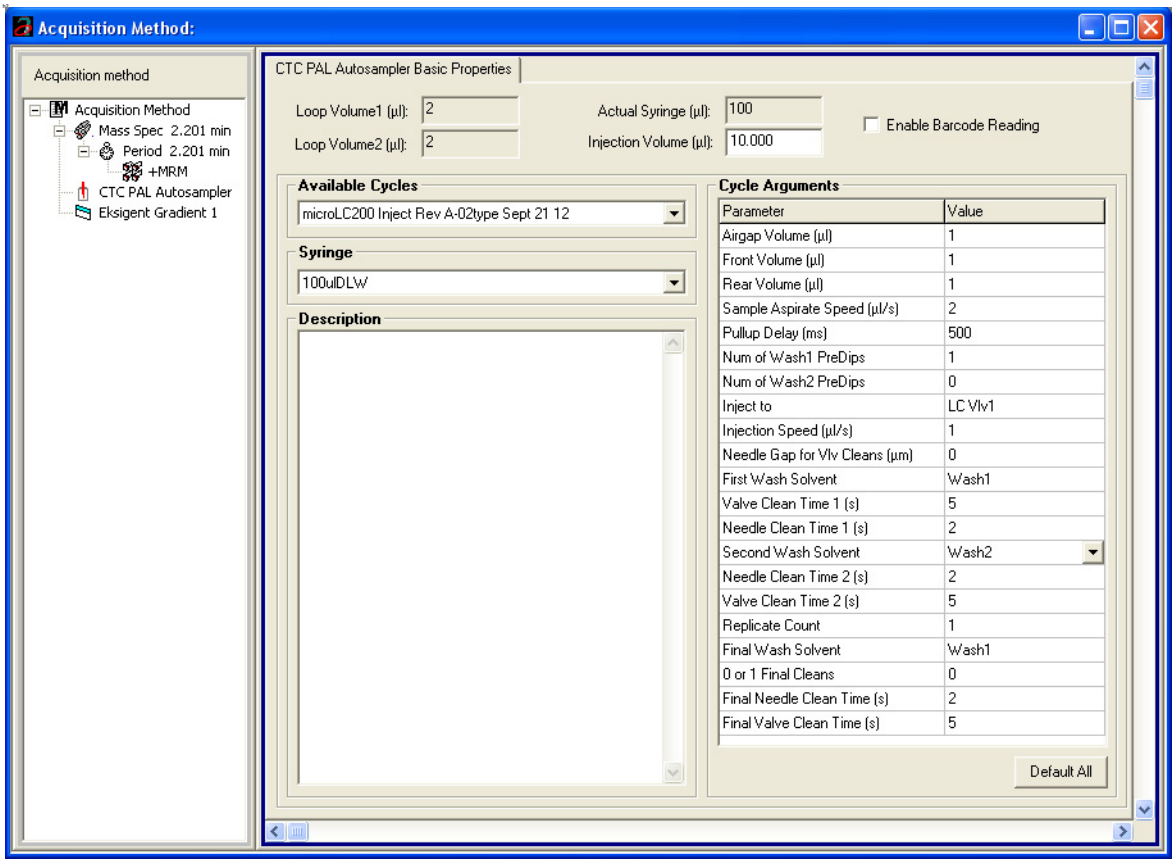


Communicating with the DLW

The Analyst® software uses the autosampler method to communicate with the HTC-*xt* PAL autosampler and the DLW. The autosampler method is part of the acquisition method.

The **Available Cycles** field in the **CTC PAL Autosampler Basic Properties** tab of the **Acquisition Method** window (Figure A-18) lists the available autosampler methods. The appropriate method for the HTC-*xt* PAL autosampler is “microLC200-Injection-Rev B” and is installed with the Eksigent MicroLC 200 Plus system.

Figure A-18 CTC PAL Autosampler Basic Properties Tab in the Acquisition Method Window, Showing microLC200-Injection-Rev B



Autosampler Method

The autosampler method consists of the steps below. In the method itself, some of the major steps are grouped together as routines so they can easily be repeated. The name of the routine is shown in parentheses after the step.

1. Lock the handheld terminal on the autosampler.
2. Wait for a signal from the mass spectrometer (Instrument Ready routine).
3. Aspirate the sample (Load Sample routine), then:
    a. Dip the needle in organic wash solvent 1 (Needle Dip 1 routine).
    b. Dip the needle in organic wash solvent 2 (Needle Dip 2 routine).
4. Move the needle into the injection port on the valve.
5. Load sample into the injection loop, then switch the valve to inject sample onto the column (Inject Sample routine).
6. Wash the system (Clean System routine):
    - Wash the injection valve and needle with the specified wash solvent (typically the organic wash solvent).

- Wash the needle and injection valve with specified wash solvent (typically the aqueous solvent).

7. Optionally, wash the injection valve and needle again with the specified wash solvent (typically the organic wash solvent) (Final Clean routine).
8. Move the needle to the home position.
9. Unlock the handheld terminal on the autosampler.

The autosampler method is used both when acquiring data and when equilibrating the mass spectrometer. During equilibration, the Instrument Ready, Get Sample, Inject Sample and Final Clean routines are not executed. When the mass spectrometer is acquiring data, the entire autosampler method is executed for each sample injection except for the Final Clean routine, which is omitted by default.

Parameters for the Autosampler Method

The volumes shown in bold in Figure A-15 on page 113, as well parameters influencing the timing of the DLW are set in **Cycle Arguments** table of the **Acquisition Method** window. The default values for most of the parameters, as well as their ranges, are listed in Table A-3.

Table A-4 and Table A-5 describe where the parameters are used.

Table A-3 Parameters in the Cycle Arguments Table

Parameter	Default Value	Minimum	Maximum
Airgap Volume (µL)	1	0.01	SYR.Max Volume
Front Volume (µL)	0	0	SYR.Max Volume
Rear Volume (µL)	0	0	SYR.Max Volume
Sample Aspirate Speed (µL/s)	2	SYR.Min Speed	SYR.Max Speed
Pullup Delay (ms)	500	0	20 000
Num of Wash1 PreDips	1	0	2
Num of Wash2 PreDips	0	0	2
Inject to	LCVlv1		
Injection Speed (µL/s)	1	SYR.Min Speed	SYR.Max Speed
Needle Gap for Vlv Cleans (mm)	0	0	35
First Wash Solvent	Wash1	Wash1 or Wash 2	
Valve Clean Time 1 (s)	5	0	100
Needle Clean Time 1 (s)	2	0	10
Second Wash Solvent	Wash1	Wash1 or Wash 2	
Needle Clean Time 2 (s)	2	0	10
Valve Clean Time 2 (s)	5	0	100
Replicate Count	1	0	10
Final Wash Solvent	Wash1	Wash1 or Wash 2	
0 or 1 Final Cleans	0	0	1
Final Needle Clean Time (s)	2	0	10

Table A-3 Parameters in the Cycle Arguments Table (Continued)

Parameter	Default Value	Minimum	Maximum
Final Valve Clean Time (s)	5	0	10

Note: Values prefixed with “SYR.” can be set in the autosampler using the keypad terminal, but the default values should be appropriate for the majority of situations.

For more details of the autosampler method, refer to the following sections.

Autosampler Method, Step-by-Step

All of the steps in the method are listed in Table A-4. Each step is described generally and with details specific to the microLC200-Injection-Rev B method. Routines begin with the REPEAT step and end with END. User-settable parameters (from the **CTC PAL Autosampler Basic Properties** tab) are shown in **bold** text.

For the actual parameters and values used by each step in the method, refer to Table A-5 on page 121.

Table A-4 microLC200-Injection-Rev B Autosampler Method

	Step	Step Name	General Description	Detailed Description
	1	LOCK_TERMINAL	Lock or unlock the handheld terminal	Terminal is locked.
Ready Routine	2	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, this routine is run once.
	3	WAIT_FOR_DS	Wait for response from mass spectrometer indicating it is ready	Allows the mass spectrometer to equilibrate for experiments which switch between positive and negative mode during a run.
	4	END	End a routine started with REPEAT	

Table A-4 microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	General Description	Detailed Description
Load Sample Routine	5	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, this routine is run once.
	6	WAIT_SYNC_SIG	Wait for a sync signal	Wait for the “Start” sync signal from the pump telling the autosampler to start. This is a software trigger.
	7	ASPIRATE_SYR	Raise syringe plunger to aspirate a specified volume	This aspirates the rear airgap, a fixed value of 0.5 µL.
	8	GET_SAMPLE	Fill syringe from a selected tray, vial or wash station	The **Rear Volume**, **Injection Volume, Front Volume**, and **Airgap Volume** enter the DLW holding loop at the specified **Filling Speed** and **Pullup Delay**.
	Needle Dip 1 Routine			
	9	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, **Num of Wash1 PreDips** specifies if this routine is run.
	10	MOVETO_OBJECT	Move to a specified position such as a vial, tray, wash station, or injector, with a specified needle penetration	Injection unit moves to Wash1 and dips the needle in wash solvent 1.
	11	END	End a routine started with REPEAT	Ends Needle Dip 1 routine.
	Needle Dip 2 Routine			
	12	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, **Num of Wash2 PreDips** specifies if this routine is run. By default, this routine is skipped.
	13	MOVETO_OBJECT	Move to a specified position such as a vial, tray, wash station, or injector, with a specified needle penetration	Injection unit moves to Wash2 and dips the needle in wash solvent 2.
	14	END	End a routine started with REPEAT	Ends Needle Dip 2 routine.
	15	END	End a routine started with REPEAT	Ends Load Sample routine.

Table A-4 microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	General Description	Detailed Description
	16	MOVETO_OBJECT	Move to a specified position such as a vial, tray, wash station, or injector, with a specified needle penetration	Injection unit moves to LC Vlv1.
Inject Sample Routine	17	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, the routine is run once.
	18	WAIT_EVENT	Wait until specified event has the specified state	Wait for the “TTL-In1” event to enter the “On” state. This is an input event when the pumps tell the autosampler that they are on. This is connected to “RDY OUT” on the rear of the LC pumps. The LC should be set to pull it low for “On”.
	19	DISPENSE_SYR	Lower plunger to dispense a specified volume	The **Injection Volume**, **Front Volume**, and **Airgap Volume** are dispensed through the injection loop to waste at the specified **Injection Speed**
	20	SWITCH_EVENT	Change the state of an output event	The event “SW-Out1” is set to “On” and pulsed for 500ms. For troubleshooting, “SW-Out1 NO” (red wire) and “SW-Out1 COM” (black wire) are connected to “RUN IN” and “CMN” respectively on the rear of the pump.
	21	SET_INJECTED	Fires an event after completion of a sample handling or injection step	This event allows other steps in the autosampler firmware to be synchronized to an injection.
	22	WAIT_EVENT	Wait until specified event has the specified state	Wait for the event “TTL-In2” to enter the “On” state. “TTL-In2” is connected by the blue wire to “VLV OUT” on the rear of the pump. “VLV OUT” is pulled low when the injection valve is in the Inject state.
	23	EJECT_SYR	Dispense the entire syringe volume	
	24	END	End a routine started with REPEAT	Ends Inject Sample routine.

Table A-4 microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	General Description	Detailed Description
Clean System Routine		REPEAT	Begin a routine to be executed a specified number of times	This routine rinses the valve and the syringe. **Replicates** specifies how many times the routine runs.
	26	RINSE_INJ	Rinse the injection valve with the specified wash solvent for the specified duration	Solvent is specified by **First Wash Solvent**, duration of rinse by **Valve Clean Time 1**.
	27	CLEAN_SYR	Rinse the syringe with the specified wash solvent for the specified duration	Solvent is specified by **First Wash Solvent**, duration of rinse by **Needle Clean Time 1**.
	28	CLEAN_SYR	Rinse the syringe with the specified wash solvent for the specified duration	Solvent is specified by **Second Wash Solvent**, duration of rinse by **Needle Clean Time 2**.
	29	RINSE_INJ	Rinse the injection valve with the specified wash solvent for the specified duration	Solvent is specified by **Second Wash Solvent**, duration of rinse by **Valve Clean Time 2**.
	30	END	End a routine started with REPEAT	Ends Clean System Routine.
Final Clean Routine	31	REPEAT	Begin a routine to be executed a specified number of times	During equilibration, this routine is skipped. During normal operation, **0 or 1 Final Cleans** specifies if this routine is run.By default, this routine is skipped.
	32	CLEAN_SYR	Rinse the syringe with the specified wash solvent for the specified duration	Solvent is specified by **Final Wash Solvent**, duration of rinse by **Final Needle Clean Time**.
	33	RINSE_INJ	Rinse the injection valve with the specified wash solvent for the specified duration	Solvent is specified by **Final Wash Solvent**, duration of rinse by **Final Valve Clean Time**.
	34	END	End a routine started with REPEAT	Ends Final Clean Routine.
	35	MOVETO_OBJECT	Move to a specified position such as a vial, tray, wash station, or injector, with a specified needle penetration	Injection unit moves to home position.
	36	LOCK_TERMINAL	Lock or unlock the handheld terminal	Terminal is unlocked.

Parameters and Values in the Autosampler Method

For each step in the autosampler method, Table A-5 shows the parameters and their values.

As before, the user-settable parameters are shown in **bold** text.

Table A-5 Parameters and Values for the microLC200-Injection-Rev B Autosampler Method

	Step	Step Name	Parameter	Value
	1	LOCK_TERMINAL	Terminal locking	On
Ready Routine	2	REPEAT	Count	1-SL.WashOnly
	3	WAIT_FOR_DS		
	4	END		

Table A-5 Parameters and Values for the microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	Parameter	Value
Load Sample Routine	5	REPEAT	COUNT	
	6	WAIT_SYNC_SIG	Sync Signal	Start
	7	ASPIRATE_SYR	Volume (µL)	**Airgap Volume**
			Overfill Rate (%)	0
			Fill Speed (µL/min)	**Sample Aspirate Speed**
			Pullup Delay (ms)	**Pullup Delay**
	8	GET_SAMPLE	Source	SL.Tray
			Index	SL.Index
			Sample Volume (µL)	**Rear Volume** + SL.Volume + **Front Volume** (SL.Volume corresponds to the **Injection Volume** set in the Analyst software)
			Air Volume (µL)	**Airgap Volume**
			Penetration (mm)	
			Fill Volume (µL)	
			Fill Speed (µL/min)	**Sample Aspirate Speed**
			Pullup Delay (ms)	**Pullup Delay**
			Eject Speed (µL/min)	
			Fill Strokes	0
			Needle Blocking	Off
			Wait for Timer	
			Wait Time (sec)	
	Needle Dip 1 Routine			
	9	REPEAT	Count	**Num of Wash1 PreDips**
	10	MOVETO_OBJECT	Object Name	Wash1
			Index	1
			Penetration (mm)	
	11	END		
	Needle Dip 2 Routine			
	12	REPEAT	Count	**Num of Wash2 PreDips**
	13	MOVETO_OBJECT	Object Name	Wash2
			Index	1
			Penetration (mm)	
	14	END		
	15	END		

Table A-5 Parameters and Values for the microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	Parameter	Value
	16	MOVETO_OBJECT	Object Name	**Inject to**
			Index	1
			Penetration (mm)	Object Name.Needle Penetr
Inject Sample Routine	17	REPEAT	Count	1-SL.WashOnly
	18	WAIT_EVENT	Event	TTL-In1
			Signal State	On
	19	DISPENSE_SYR	Volume (µL)	SL.Volume + **Front Volume** + **Airgap Volume**
			Eject Speed (µL/sec)	**Injection Speed**
	20	SWITCH_EVENT	Event	SW-Out 1
			Signal State	On
			Pulse Time (ms)	500
	21	SET_INJECTED		
	22	WAIT_EVENT	Event	TTL-In2
			Signal State	On
	23	EJECT_SYR	Eject Speed (µL/sec)	SYR.Eject Speed
	24	END		

Table A-5 Parameters and Values for the microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	Parameter	Value
Clean System Routine	25	REPEAT	Count	**Replicate Count**
	26	RINSE_INJ	Wash Station	**First Wash Solvent**
			Injector	**Inject to**
			Needle Gap (mm)	**Needle Gap for Vlv Cleans**
			Rinse Times (s)	**Valve Clean Time 1**
	27	CLEAN_SYR	Wash Station	**First Wash Solvent**
			Clean Cycles	0
			Clean Volume (%)	0
			Wash Penetration (mm)	
			Fill Speed	100
			Pullup Delay (ms)	0
			Eject Speed	100
			Rinse time (sec)	**Needle Clean Time 1**
	28	CLEAN_SYR	Wash Station	**Second Wash Solvent**
			Clean Cycles	0
			Clean Volume (%)	0
			Wash Penetration (mm)	
			Fill Speed	100
			Pullup Delay (ms)	0
			Eject Speed	100
			Rinse time (sec)	**Needle Clean Time 2**
	29	RINSE_INJ	Wash Station	**Second Wash Solvent**
			Injector	**Inject to**
			Needle Gap (mm)	**Needle Gap for Vlv Cleans**
			Rinse Times (s)	**Needle Clean Time 2**
	30	END		

Table A-5 Parameters and Values for the microLC200-Injection-Rev B Autosampler Method (Continued)

	Step	Step Name	Parameter	Value
Final Clean Routine	31	REPEAT	Count	**0 or 1 Final Cleans**
	32	CLEAN_SYR	Wash Station	**Final Wash Solvent**
			Clean Cycles	0
			Clean Volume (%)	0
			Wash Penetration (mm)	
			Fill Speed	100
			Pullup Delay (ms)	0
			Eject Speed	100
			Rinse time (sec)	**Final Needle Clean Time**
	33	RINSE_INJ	Wash Station	**Final Wash Solvent**
			Injector	**Inject to**
			Needle Gap (mm)	**Needle Gap for Vlv Cleans**
			Rinse Times (s)	**Final Valve Clean Time**
	34	END		
	35	MOVETO_OBJECT	Object Name	Home
			Index	1
			Penetration (mm)	
	36	LOCK_TERMINAL	Terminal locking	Off


eksigent®
part of AB SCIEX

System Specifications

B

Table B-1 Eksigent MicroLC 200 Plus system Specifications

Dimensions	71 cm x 64 cm x 59 cm (H x W x D) (28 inches x 25 inches x 20 inches)
Weight	25 kg (55 lbs)
Pump electrical	• Input line voltage:100 V to 240 V AC • Input line frequency: 50/60 Hz • Input current: 4 A
Autosampler electrical	• Input line voltage:100 V to 240 V AC • Input line frequency: 50/60 Hz • Input current: 4 A • Output voltage: 36 V DC • Output current: 4.16 A
Maximum pressure	10 000 psi
Flow rate range	5 µL/min to 200 µL/min
Flow rate precision	<0.5% RSD @ 150 µL/min (0.01 min for a 2 minute run)

Sample capacity	• 6 positions for trays or microtiter plates
	• 54 2 mL vials/tray
	• Microtiter plates:
	  • Standard depth 96-well
	  • Deep 96-well
	  • 384-well

Injection valve	• 1/32 inch connection with port-to-port volume <60 nL • Maximum pressure 10 000 psi • 316 stainless steel with proprietary coating
Injection volume	• Minimum volume: 15 nL (see note below) • Maximum volume: 10 µL (or loop volume)
Injection reproducibility	• Full loop: <1% RSD • Partial loop: <2% RSD
Sample carryover	0.01% to 0.005%
Sample cooling temperature range	4°C to 40°C
Gradient delay volume	1 µL to 3 µL
Column oven temperature range	+5°C to 80°C
Column length	3 cm to 10 cm

Table B-1 Eksigent MicroLC 200 Plus system Specifications (Continued)

Cycle time	<45 sec
Wetted parts	316 stainless steel, PEEK, PEEKsil, fused silica and FEP
Instrument control	Eksigent control software with plug-ins for: • AB SCIEX Analyst® software • Thermo Scientific Xcalibur software

Note: Minimum injection volume is dependent on the flow rate. For a metered injection, very small injection volumes require a slower flow rate. To calculate the minimum injection volume, multiply the flow rate by 2.5. The result is the injection volume expressed in nL.

View and Order Spare Parts

View the Parts List for the Eksigent MicroLC 200 Plus System

1. Go to www.eksigent.com/downloads/product-catalog.
2. Click **Download our parts and consumables catalog**.

Order Parts

1. Go to www.eksigent.com/company/contact-us.
2. Click your location on the map to view contact information for your region or country.

System Accessory Kit

Part numbers marked with * are also available individually.

Table C-1 Accessory Kit Contents (PN 5017802 Rev. H)

Part Number	Description	Quantity
5015766	Solvent pan	1
5016413*	Stainless steel union, hex, 6-32 FxF, 0.15 mm bore	2
5017651*	Tubing, mobile phase, 1/8 inch x 3 feet	3
5017797*	Bottle, seal rinse, 2 inch diameter	1
5017798*	Loop, 2 µL, 1/32 inch PEEKsil, no fittings	1
5017799*	Loop, 5 µL, 1/32 inch PEEKsil, no fittings	1
5017800*	Injection valve waste tube assembly	1
5017801*	Mixer-to-valve tube assembly	2
5018262*	Eksigent MicroLC Systems calibration kit	1
5018474*	Autotuning tube	2
5019820*	Adapter ferrule for 1/32 inch tubing in a 1/16 inch port, 5-pack	5
5019821*	Nut for adapter ferrule, 5-pack	5
5023797	Syringe needle guide	3
5024174	Nut, gold plated with 6-32 threads, 3/16 inch	10
5026646	Eksigent control software v. 4.1 CD	1
100-00549	Screw, stainless steel with 6-32 threads, 3/16 inch	4
100-00567*	Wrench for 1/32 inch and 1/16 inch nuts	2
200-00329	Mobile phase filter with 10 µm frit	3
200-00342*	Column fitting, 6-32 threads	10

Table C-1 Accessory Kit Contents (PN 5017802 Rev. H) (Continued)

Part Number	Description	Quantity
200-00356	Wrench, nut extender tool	1
200-00388	Microfilter	1
205-00040	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 30 cm	2
205-00041	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 50 cm	2
205-00049	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 75 cm	2
205-00054*	Loop, 10 µL, 1/32 inch PEEKsil, no fittings	1
205-00069	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 10 cm	4
205-00070	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 5 cm	2
205-00089	Tubing, PEEKsil, 25 µm ID, 1/32 inch OD, 5 cm	2
205-00091*	Tubing, PEEKsil, 25 µm ID, 1/32 inch OD, 10 cm	4
300-00019	Tubing, polyethylene 1/4 inch OD, 0.17 inch ID	10
300-00036	2 mL vials	10
300-00037	Snap caps for 2 mL vials	10
400-00465	3 meter USB cable	1
500-00779	Stop block	1
615-00025	Divided utility box	1
620-00071	Blue 54-vial plate	1
700-00050	Pump-autosampler (HT-CTC) interface cable	1
801-00067	Bottle, 250 mL with gray cap	2
805-10100	HALO fused C18 column, 5 cm, 2.7 µm, 500 µm ID	1
910-00070	Low pressure fittings, 5-pack	1
910-00087*	Ferrule, stainless steel 1/32 inch, 10-pack	1

Consumables Kit

Table C-2 Consumables Kit Contents (PN 5032078 Rev. A)

Part Number	Description	Quantity
5016413	Stainless steel union, hex, 6-32 FxF, 0.15 mm bore	2
5017798	Loop, 2 µL 1/32 inch PEEKsil, no fittings	1
5017799	Loop, 5 µL, 1/32 inch PEEKsil, no fittings	1
5017800	Injection valve waste tube assembly	1
5017801	Mixer-to-valve tube assembly	2
5018262	Eksigent MicroLC 200 Plus system calibration kit	1
5018474	Autotuning tube	2
5019820	Adapter ferrule for 1/32 inch tubing in a 1/16 inch port, 5-pack	1

Table C-2 Consumables Kit Contents (PN 5032078 Rev. A) (Continued)

Part Number	Description	Quantity
5019821	Nut for 1/32 inch to 1/16 inch adapter ferrule, 5-pack	1
5023797	Syringe needle guide	3
5024174	Nut, gold plated with 6-32 threads, 3/16 inch	10
5031383	Autosampler needles, 3-pack	1
100-00567	Wrench for 1/32 inch and 1/16 nuts	1
200-00329	Mobile phase filter with 10 µm frit	3
200-00342	Column fitting, 6-32 threads	10
200-00356	Wrench, nut extender tool	1
200-00373	Filter capsules, 2-pack	1
200-00388	Microfilter	1
205-00040	Tubing, PEEKsil, 50 µm ID,1/32 inch OD, 30 cm	2
205-00041	Tubing, PEEKsil, 50 µm ID,1/32 inch OD, 50 cm	2
205-00049	Tubing, PEEKsil, 50 µm ID, 75 cm	2
205-00069	Tubing, PEEKsil, 50 µm ID,1/32 inch OD, 10 cm	4
205-00070	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 5 cm	2
205-00089	Tubing, PEEKsil, 25 µm ID, 1/32 inch OD, 5 cm	2
205-00091	Tubing, PEEKsil, 25 µm ID,1/32 inch OD, 10 cm	4
615-00025	Divided utility box	1
910-00070	Low pressure fittings, 5-pack	1
910-00087	Ferrule, stainless steel 1/32 inch, 10-pack	1

Upgrade Kit

Contains fittings and other items to upgrade an older ekspert™ microLC 200 system to an Eksigent MicroLC 200 Plus system.

Table C-3 Upgrade Kit Contents (PN 5031861 Rev. D)

Part Number	Description	Quantity
5023797	Syringe needle guide	3
5024174	Nut, gold plated with 6-32 threads, 3/16 inch	10
5028466	50 µm ID electrode for AB SCIEX Turbo V™ ion source	1
5028467	25 µm ID electrode for AB SCIEX Turbo V ion source	1
5028658	C18 guard column	1
5031383	Autosampler needles, 3-pack	1
5036153	Front panel label	1
200-00388	Microfilter	1
205-00070	Tubing, PEEKsil, 50 µm ID, 1/32 inch OD, 5 cm	4

Table C-3 Upgrade Kit Contents (PN 5031861 Rev. D) (Continued)

Part Number	Description	Quantity
205-00089	Tubing, PEEKsil, 25 µm ID, 1/32 inch OD, 5 cm	4
910-00087	Ferrule, stainless steel 1/32 inch, 10-pack	2

Replacement Parts

Table C-4 Replacement Parts for the Eksigent MicroLC 200 Plus System and HTC-*xt* PAL Autosampler

Part Number	Description
Column Oven	
5015996	Mounting kit for mounting column oven on Turbo V and DuoSpray™ ion sources
5020904	Column oven
Electrodes and Related Accessories	
5028466	50 µm ID electrode for AB SCIEX Turbo V ion source
5028467	25 µm ID electrode for AB SCIEX Turbo V ion source
5029303	Union for grounding 65 µm ID electrode
5029342	65 µm ID electrode for AB SCIEX Turbo V ion source
800-00455	65 µm ID electrode for AB SCIEX API 2000 source
5032057	Replacement emitters for 50 µm ID electrodes
5032058	Replacement emitters for 25 µm ID electrodes
5028470	Tool for replacing electrode emitters
Guard Columns and In-line Filter Parts	
5028658	C18 guard column
5028659	C8 guard column
200-00373	Filter capsule (2-pack)
200-00388	In-line filter assembly (includes 5 filter capsules)
205-00089	Tubing, PEEKsil, 25 µm ID,1/32 inch OD, 5 cm
Injection Valve Parts	
5014843	Injection valve assembly
5019104	Injection valve assembly, without pod
5019105	Injection valve motor
200-00326	Injection valve rotor seal
200-00327	Circuit board for valve assembly
200-00417	6-port injection valve and fittings
200-00452	6-port injection valve pod and fittings

Table C-4 Replacement Parts for the Eksigent MicroLC 200 Plus System and HTC-*xt* PAL Autosampler (Continued)

Part Number	Description
Other Parts	
200-00327	PCB, Express Ultra valve
700-00028	Control cable
801-00084	AB SCIEX interface kit, includes electrode, MS interface cable, grounding kit, column oven mounting kit, clamp and rods
Sample Loops	
910-00081	Loop, 1 µL, PEEKsil
200-00367	Loop, 2 µL, stainless steel
5016578	Loop, 10 µL, 1/32 inch OD stainless steel, no fittings
5016279	Loop, 20 µL, 1/32 inch OD stainless steel, no fittings
SL50NW	Loop, 50 µL (do not use included fittings)
SL100NW	Loop, 100 µL (do not use included fittings)
Syringe and DLW Parts	
4460827	Replacement plungers for DLW syringe (PN 4460861) 10-pack
4460861	Syringe for HTC-*xt* PAL autosampler DLW option; removable needle not included.
4460863	Plunger holder for plunger for DLW syringe (PN 4460827)
4460866	DLW holding loop and needle adapter
4460868	Flow diverter
5031383	Autosampler needles, 3-pack
Tubing and Fittings	
5019621	Fitting, 6-40 threads, 1/32 inch, 10-pack
910-00089	Fitting, PEEK, red, non-conductive (PN 200-00330) 10-pack
200-00252	Fitting, polypropylene, flangeless tube-end, 1/16 inch OD
910-00085	Nut, stainless steel, 1/32 inch OD for 6-40 threaded columns, 10-pack (use with ferrule PN 910-00087)
5108305	Tubing, FEP, 3/100 inch ID, 1/16 inch OD
200-00061	Tubing, PEEKsil, 75 µm ID,1/32 inch OD, 30 cm
5018306	Tubing, stainless steel, 1/10 inch ID, 1/32 inch OD, 5 cm


eksigent®
part of AB SCIEX

Revision History

Revision	Reason for Change	Date
D5033433 A	First release of the document.	March 2012
D5033433 B	Revised to include higher resolution pictures.	March 2012
D5033433 C	Restructured and added information to the Routine Maintenance chapter. Added troubleshooting information.	September 2012
D5033433 D	Added procedures for adding a new mobile phase, moving to a new computer, flushing and sonicating electrodes, and configuring the autosampler. Also added tips for handling PEEKSil tubing, sample preparation guidelines, and explanation of injection modes. Updated example experiment to add method for flushing electrode. In Appendix A, made corrections to equations and added information about the sample position in the loop.	June 2013
D5033433 E	Updated part numbers for electrodes and guard columns. Updated image showing Eksigent control software driver utility. Deleted instructions for installing the Method Merger Tool software. Used consistent terminology for column oven.	October 2013
D5033433 F RUO-IDV-05-1129-A	Added procedure for testing the DLW. Updated plumbing instructions and figure. Updated procedure for calibrating flowmeters. Updated contents of accessory and consumables kits and added upgrade kit and additional spare parts.	February 2014
D5033433 G RUO-IDV-05-1129-B	Updated to incorporate MicroLC 200 Plus. Updated legal page and manufacturer's address. Corrected electrode part numbers in Figure 4-2 and Table C-4. Updated name of autosampler method throughout. Updated Table C-4.	September 2014


eksigent®
part of AB SCIEX

Index

G

H

I

L

M

N

P

R


eksigent®
part of AB SCIEX