AB SCIEX

Calibrant Delivery System

Operator Guide

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

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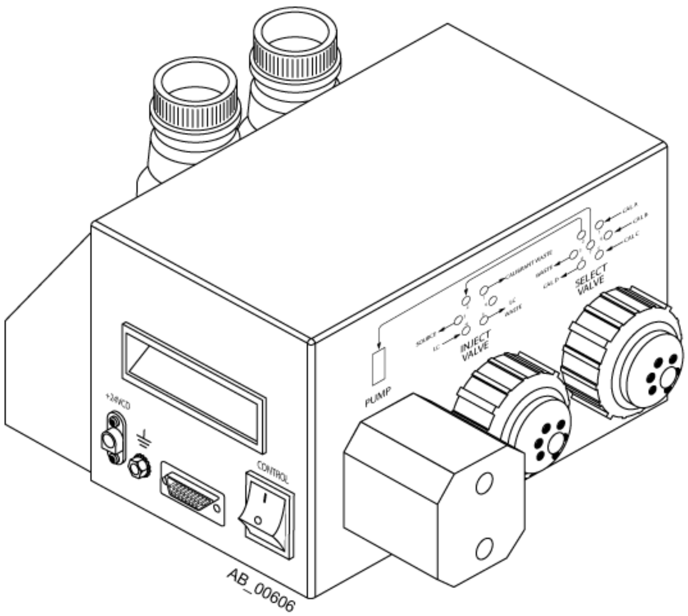
Overview 1

The calibrant delivery system (CDS) introduces calibration solution for automated mass calibration of the mass spectrometer, to ensure that the mass accuracy of the system is maintained throughout batch acquisition.

Because calibration takes only a few minutes, we recommend that you calibrate frequently. Calibrate at least hourly for MS/MS scans, and every two to three hours for TOF MS scans.

Figure 1-1 Calibrant Delivery System



The CDS is a separate component installed on top of the mass spectrometer. It connects to the gas and vacuum bulkhead on the left side of the mass spectrometer with a Control cable.

A rack holds a waste bottle and up to two calibrant bottles. The system supports up to four different calibrant solutions.

Operating Modes

The duration of each CDS operating mode depends on the flow rates and capacities of the pump and sample bottles.

Table 1-1 CDS Operating Modes

Mode	Description
Refill	Draws the calibrant into the pump. During refill, the **Detailed Status** tab for the CDS contains this information: • Status of Commands = Ready • Pump Status = Running BWD • Select Valve Position = Calibrant x • Inject Valve Position = LC where x is the calibrant port in use
Inject	Expels the calibrant from the pump and then routes it to the ion source. During injection, the Detailed Status tab for the CDS contains this information: • Status of Commands = Ready • Pump Status = Running FWD • Select Valve Position = Inject • Inject Valve Position = Calibrant
Purge	Cleans the line (typically, after a change in calibrant solution) and eliminates air bubbles from the system. Calibrant is drawn into the dispense pump and then pumped out to a waste port. The purge cycle consists of two inject and refill cycles. The amount of time for each inject and refill cycle is calculated as *pump volume / purge flow rate* * 1.1 For example, the pump volume is 1000 µl; if the purge flow rate is set to 1000 µl/min, the CDS will inject for 1.1 min and refill for 1.1 min. This cycle will be repeated two times, so the total Purge time will be about 4.4 minutes.

Fluidic Connections

Valve	Port	Label
Inject	1	CALIBRANT WASTE
	3	SOURCE
Select	1	CAL A
	4	CAL D
	5	CAL C

Valve	Port	Label
	6	CAL B
	7	Common: Select to pump

CDS Tubing Diagrams

Item	Description
	Calibrant in
	To and from pump
	To waste
	Inject valve to Select valve
	To the ion source
1	Pump
2	Inject valve
3	Select valve
4	To ion source
5	To waste
6	Calibrant D
7	Calibrant C
8	Calibrant B
9	Calibrant A

Figure 1-2 Refill Mode, Calibrant A

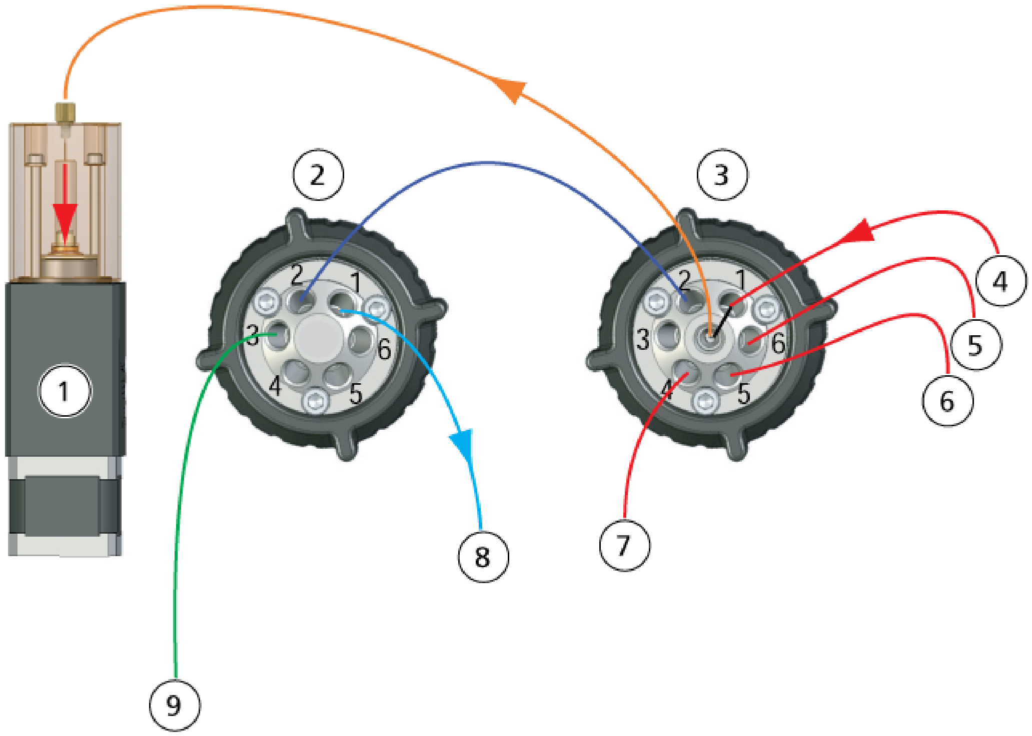


Figure 1-3 Inject Mode

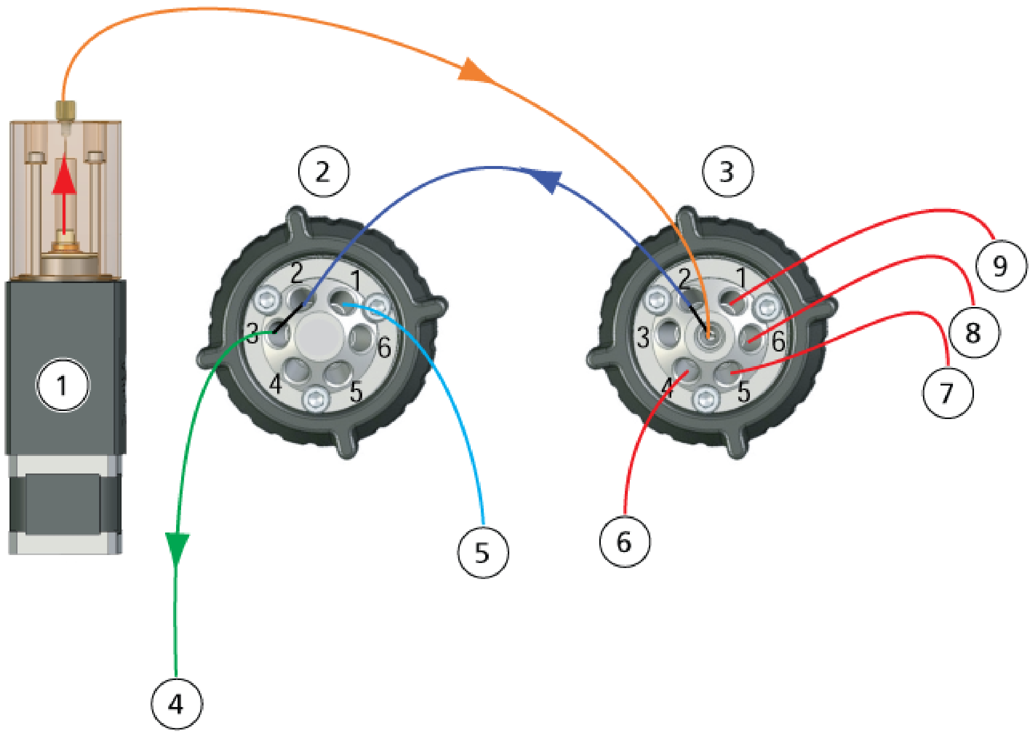


Figure 1-4 Purge Mode, Refill Cycle

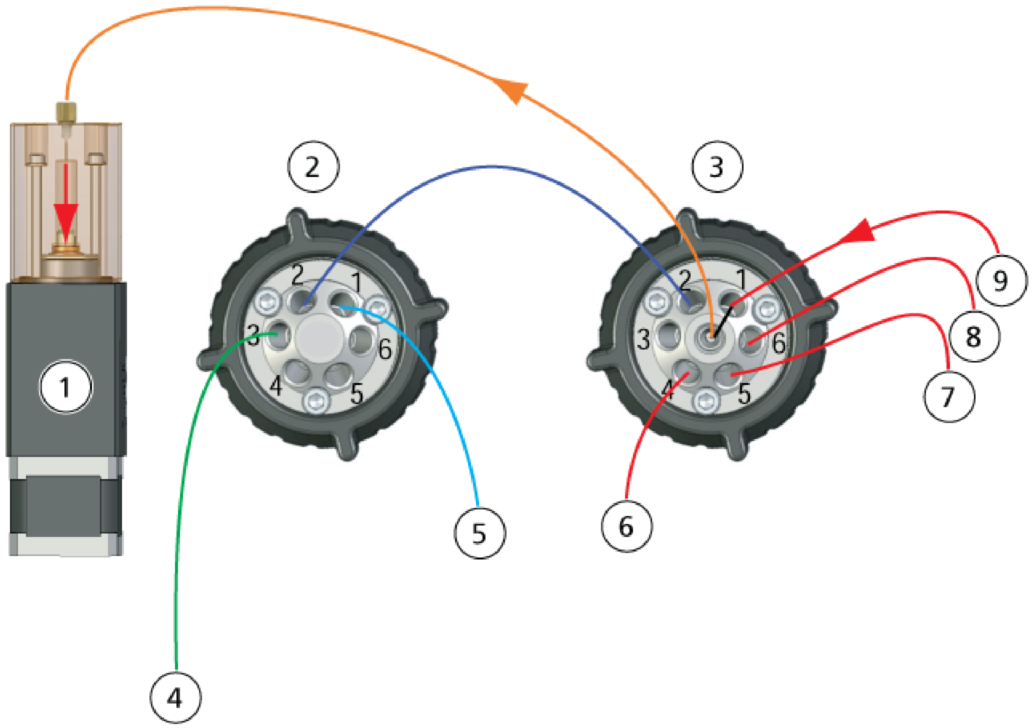
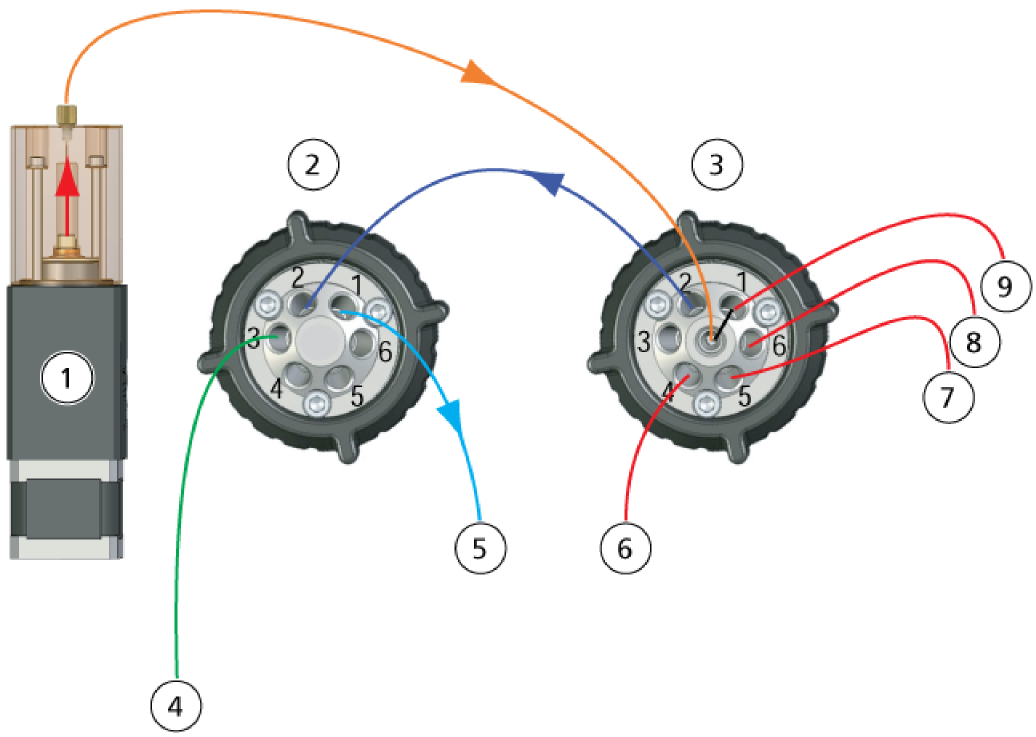


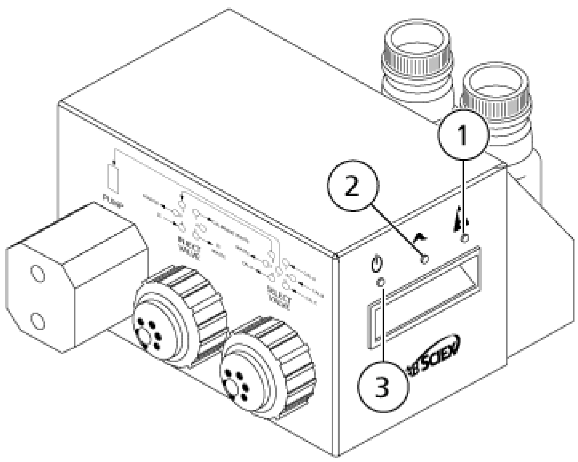
Figure 1-5 Purge Mode, Inject Cycle



LEDs

The CDS LEDs are located on the right side of the system.

Figure 1-6 CDS LEDs



Item	LED	Symbol	Description
1	Fault (Red)		Indicates a pump error.
2	Pump Status (Blue)		• Lit: The pump is stationery at the Home position. • Off: The pump is not at the Home position. • Flashing: The pump is moving backward or forward.
3	Power (Green)		Indicates that the CDS system is on.

CDS Configurations

When the CDS is used with the DuoSpray™ ion source, two configurations are available:

- Default configuration: The TurboIonSpray® probe is used for sample analysis, and the atmospheric pressure chemical ionization (APCI) probe for calibration.
- Alternative configuration: The APCI probe is used for sample analysis and the TurboIonSpray probe for calibration.

Using the CDS with the SelexION™ Technology

The CDS can be used with the SelexION technology. To use the SelexION technology with the CDS:

- Make sure that the CDS is installed on top of the SelexION modifier tray.
- Plumb the ion source and CDS as described in *Single-Probe Configuration on page 45*..
- Use the ESI calibration solution kits. Refer to *Consumables on page 43*.

CDS Installation 2

WARNING! Toxic Chemical Hazard: Follow all safety guidelines when handling, storing, and disposing of chemicals.

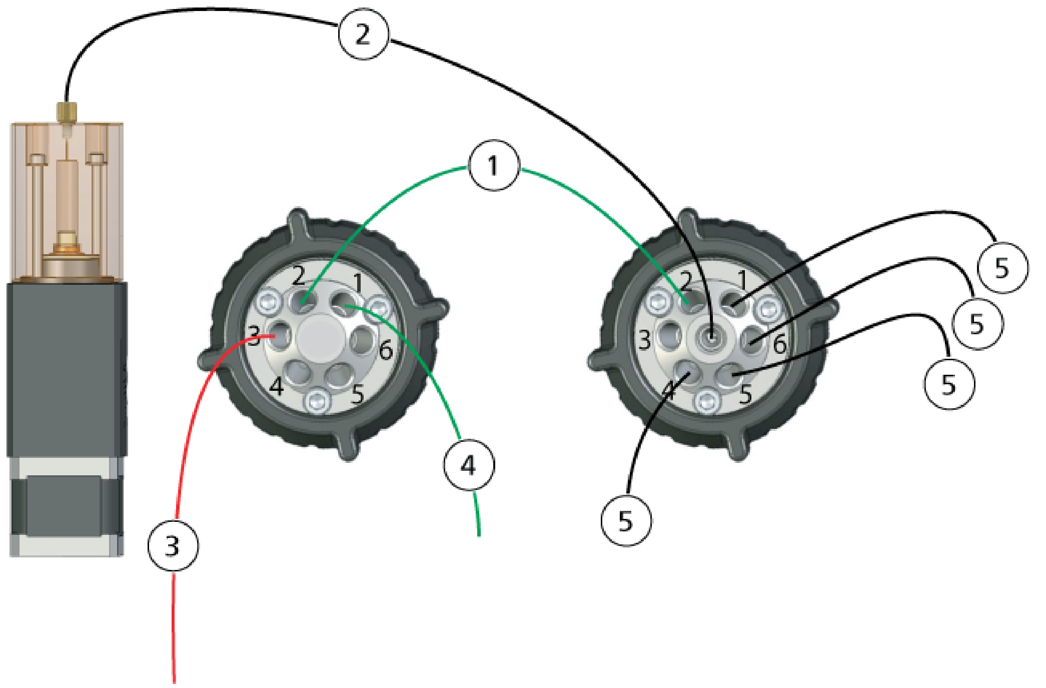
WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Do not operate the system if the tubing is not properly connected, or if there are leaks in the tubing.

This section provides instructions for installing the CDS for use with the DuoSpray™ ion source. To use the CDS with the Turbo V™ or IonDrive™ Turbo V ion source, refer to *Single-Probe Configuration on page 45*.

The CDS comes with these kits:

- Installation kit (PN 5008847): Contains the power and control cables for the CDS.
- Fittings and Tubings kit (PN 5011979): Contains the tubing, fittings, and bottles.
- Positive calibration solution kit (PN 4460131)
- Negative calibration solution kit (PN 4460134)

Figure 2-1 CDS Tubing

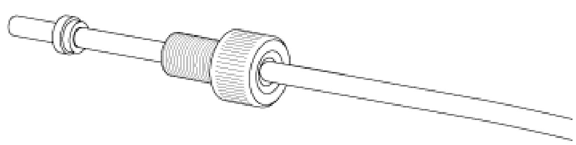


Item	Description
1	Transfer line: green PEEK tubing, 1/16 in. o.d., 0.03 in. i.d., 16 cm (PM1820G)
2	Pump line: clear FEP tubing, 1/16 in. o.d., 0.03 in. i.d., 17 cm (PM1000)
3	Ion source line: red PEEK tubing, 1/16 in. o.d., 0.005 in. (125 µm) i.d., 40 cm (PM-1945R)
4	Waste line: green FEP tubing, 1/16 in. o.d., 0.03 in. i.d., 60 cm (PM 1000G)
5	Calibrant lines: clear FEP tubing, 1/16 in. o.d., 0.03 in. i.d., 60 cm (PM1000)

Plumb the CDS Lines

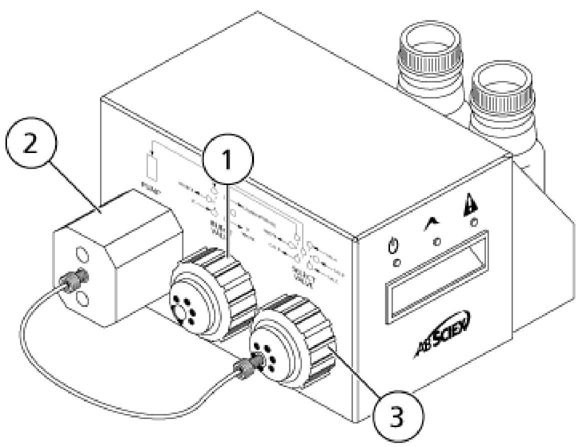
1. Assemble the short nut and ferrule on the 17 cm clear FEP tubing.

Figure 2-2 Tubing, Ferrule, and Nut



2. Insert the tubing into the pump as far as it will go, to prevent dead volumes from appearing in the clear FEP tubing, and then tighten the nut.

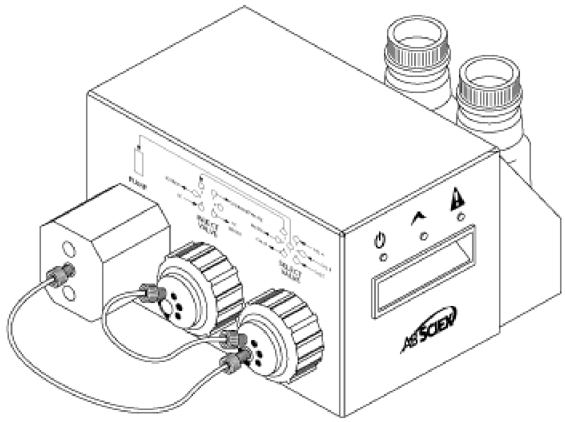
Figure 2-3 Clear Tubing from Pump to Select Valve



Item	Description
1	Inject valve
2	Pump
3	Select valve

3. Assemble the ferrule and Rheodyne fitting on the other end of the clear FEP tubing, insert the tubing into the Select valve port 7 as far as it will go, and then tighten the fitting.
4. Assemble the ferrule and Rheodyne fitting on the 17 cm green PEEK tubing, insert the tubing into the Inject valve port 2, and then tighten the fitting.
5. Assemble the ferrule and Rheodyne fitting on the other end of the green PEEK tubing, insert the tubing into the Select valve port 2, and then tighten the fitting.

Figure 2-4 Green PEEK Tubing



6. For the calibrant line, assemble the ferrule and Rheodyne fitting on the 60 cm clear FEP tubing, insert the tubing into the Select valve CAL A port as far as it will go, and then tighten the fitting.
7. **WARNING! Toxic Chemical Hazard: Install calibrant bottles inside the tray, to provide secondary containment in case of a spill.**

   Insert the other end of the clear FEP tubing through the lid of the calibrant bottle, into the bottle.

   Note: If you are using more than one calibrant, insert the tubing from the additional calibrant bottles into ports 4 (CAL D), 5 (CAL C), or 6 (CAL B) on the Select valve.

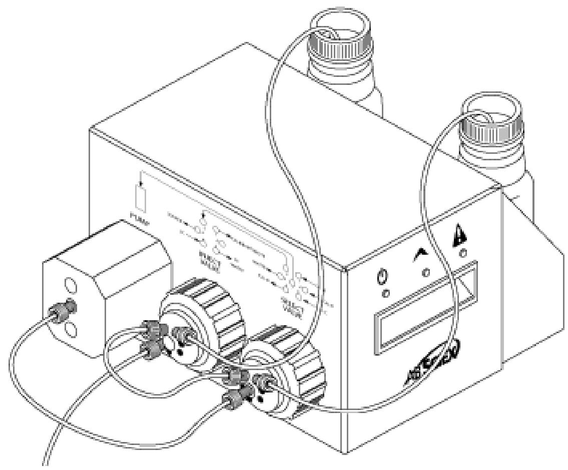
8. For the waste line, assemble the ferrule and Rheodyne fitting on the 60 cm green FEP tubing, insert the tubing into the Inject valve CALIBRANT WASTE port 1 as far as it will go, and then tighten the fitting.
9. Insert the other end of the green FEP tubing into the waste bottle on the rack.
10. Install a plug into the Inject valve port 6 to prevent leaks.

CAUTION: Potential System Damage: Make sure that the CDS is positioned far enough from the front of the mass spectrometer that the clear FEP tubing from the Select valve to the Pump is not above the ion source. If the tubing is above the ion source, it can become overheated and burst.

11. Cut a piece of red PEEK tubing that is long enough to reach from the CDS to the ion source probe (15 to 20 cm).

12. Assemble the ferrule and Rheodyne fitting on the red PEEK tubing, insert the tubing into the Inject valve port 3 as far as it will go, and then tighten the fitting.

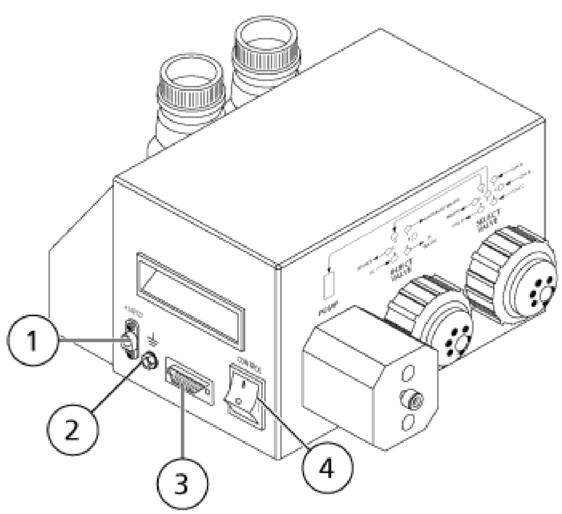
Figure 2-5 CDS Tubing Installed



Install the CDS

1. Put the CDS on top of the mass spectrometer.
2. Make sure that the power switch is off.

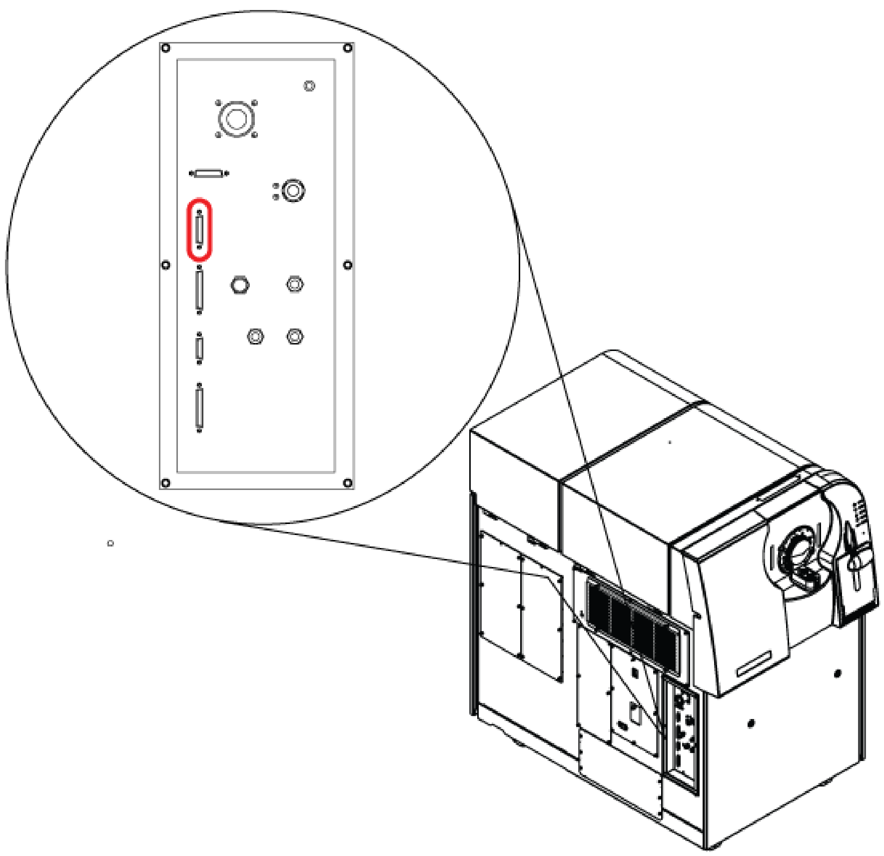
Figure 2-6 CDS Connections



Item	Description
1	Power cable connection
2	Ground connection indicator
3	Control cable connection
4	Power switch

3. Connect the cables to the connections on the CDS.
   a. Connect the gray cable with the red connector to the power cable connection.
   b. Connect the green-and-yellow striped cable to the ground connection.
   c. Connect the gray serial cable to the control cable connection.
4. Connect the control cable to the gas and vacuum bulkhead on the mass spectrometer.

Figure 2-7 CDS Connections on the Gas and Vacuum Bulkhead (TripleTOF 6600 System)



5. Turn on the CDS and confirm that the power LED is on. Refer to *LEDs on page 11*.

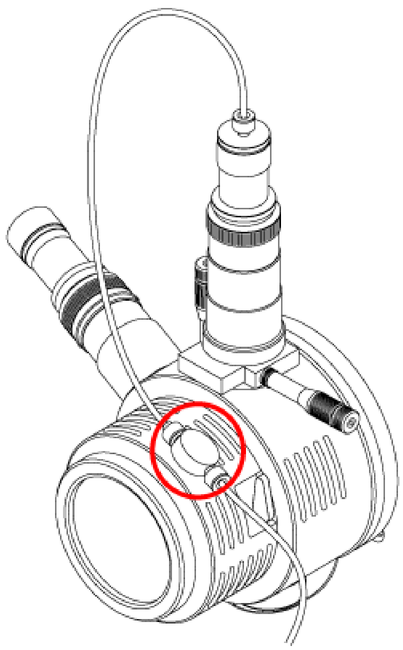
Plumb the Ion Source

Default Configuration

In this configuration, the APCI probe is used for calibration.

1. Insert the other end of the red PEEK tubing from Inject valve port 3 (refer to step *12* in *Plumb the CDS Lines on page 14*) into the sample tubing nut, install the sample tubing nut on the fitting at the top of the APCI probe, and then tighten the sample tubing nut until it is finger-tight.
2. Connect the red PEEK tubing from the sample supply device to the grounding union on the ion source.

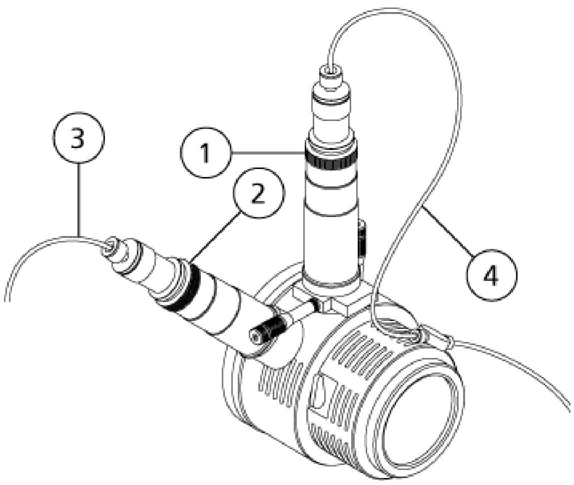
Figure 2-8 Grounding Union



3. Connect a 30 cm piece of red PEEK tubing to the grounding union.
4. Insert the other end of the 30 cm red PEEK tubing into the sample tubing nut, install the sample tubing nut on the fitting at the top of the TurboIonSpray® probe, and then tighten the sample tubing nut until it is finger-tight.

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the sample tubing nut is tightened properly before operating this equipment, to prevent leakage.

Figure 2-9 Default Configuration



Item	Description
1	TurboIonSpray probe
2	APCI probe
3	Calibrant stream, from the CDS
4	Sample stream, from the LC system

Alternative Configuration

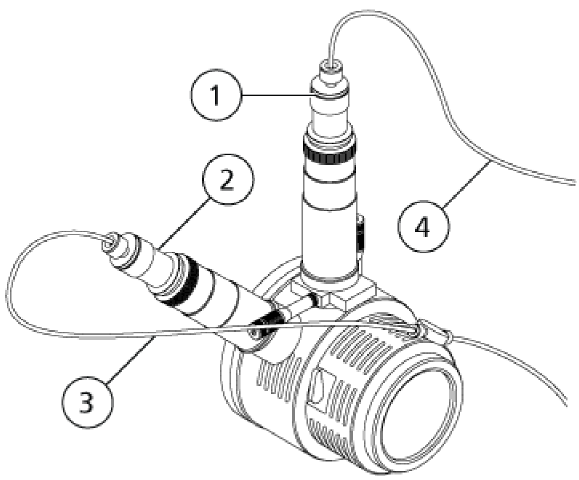
In this configuration, the TurboIonSpray® probe is used for calibration.

1. Insert the other end of the red PEEK tubing from the Inject valve port 3 (refer to step *12* in *Plumb the CDS Lines on page 14*) into the sample tubing nut, install the sample tubing nut on the fitting at the top of the TurboIonSpray probe, and then tighten the sample tubing nut until it is finger-tight.
2. Connect the red PEEK tubing from the sample supply device to the grounding union on the ion source.
3. Connect a 30 cm piece of red PEEK tubing to the grounding union.

4. Insert the other end of the 30 cm red PEEK tubing into the sample tubing nut, install the sample tubing nut on the fitting at the top of the APCI probe, and then tighten the sample tubing nut until it is finger-tight.

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the sample tubing nut is tightened properly before operating this equipment, to prevent leakage.

Figure 2-10 Alternate Configuration



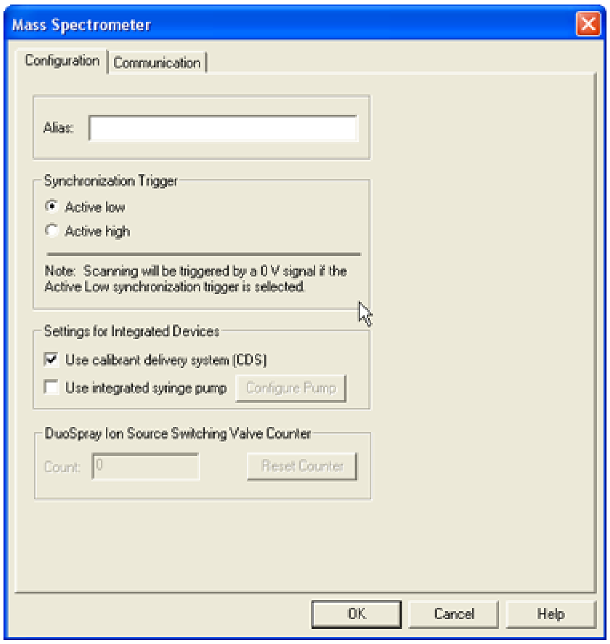
Item	Description
1	TurboIonSpray probe
2	APCI probe
3	Sample stream, from the LC system
4	Calibrant stream, from the CDS

Configure the Hardware Profile

1. In the Analyst® TF software, on the Navigation bar, double-click **Hardware Configuration**.
2. Create a hardware profile containing the mass spectrometer, or edit the hardware profile for the mass spectrometer.
3. In the **Devices** in current profile list, select the mass spectrometer and click **Setup Device**.

4. On the **Configuration** tab, select **Use calibrant delivery system (CDS)**.

Figure 2-11 Mass Spectrometer Dialog



5. Click **OK** two times, to return to the Hardware Configuration Editor dialog.
6. Activate the hardware profile.

Test the CDS Installation

1. Turn on the CDS.
2. Remove the tube from the APCI probe and then insert it into a container, such as the waste bottle.
3. In the Analyst® TF software, in the Navigation bar, double-click **Manual Tuning**.
4. Click **CDS Inject**. Listen for a click as the Inject valve moves, and then watch for droplets forming at the end of the tube.
5. Connect the tube to the APCI probe.
6. To test the calibrant connected to select valve A, click **CDS Refill**. Listen for a click as the Select valve moves.

Tip! Because the calibrant solution is clear, it is not visible as it moves through the tubing. To see if the solution is moving, lift the tube out of the calibrant solution in the bottle for a few seconds to introduce an air bubble. Click **Refill**. The air bubble should move through the tubing.

7. After the pump has refilled, make sure that it has returned to the Home position by double-clicking the mass spectrometer icon in the status bar, and then clicking the **Calibrant Delivery System (CDS) Status** tab.

   The Home field contains "Yes".

8. To test additional valves, edit the Reference table to select the valve. Refer to the Analyst TF software Help. Repeat step *6* and step *7*.

Prime the CDS

The lines are primed with the solution connected to the calibration valve port specified in the **Tuning Options** in the Analyst® TF software. Refer to the Analyst TF software Help.

Note: If this is the first time the system is being used, if the system has not been used for a long time, or if the calibration valve positions have changed, it may be necessary to purge the lines multiple times.

1. In the Analyst TF software, on the Navigation bar, double-click **Manual Tuning**.
2. From the list of method types, select **Calibrate**.
3. In the **Calibrant Reference Table** List field, select the reference table for the calibrant solution.
4. Click **CDS Purge**, inspecting the lines from the calibration solution bottle to the valve and from the pump to the valve for air bubbles.
5. Continue to click **CDS Purge** until no air bubbles are visible.

CDS Optimization 3

Optimize the Default CDS Configuration

In the default CDS configuration, the TurboIonSpray® probe is used for sample analysis, and the APCI probe is used for calibration.

Note: The IonSpray Voltage Floating (ISVF) is always applied to both the TurboIonSpray® probe and the APCI probe simultaneously, and the Temperature (TEM) is always applied to both the turbo and APCI heaters simultaneously.

Optimize the Sample Stream

At lower flow rates, the probe can be adjusted to its lowest position. For higher flow rates, position the probe higher than the curtain plate orifice. The curtain plate orifice should remain clear of solvent or solvent droplets at all times.

For multiply-charged proteins and peptides introduced at a few microliters per minute, position the sprayer nozzle higher than the curtain plate orifice.

Tip! It is easier to optimize signal and signal-to-noise with FIA or on-column injections.

Run the Method

1. Turn on the CDS.
2. Start the Analyst® TF software.
3. Activate a hardware profile containing the CDS.
4. In the Navigation bar, under **Tune and Calibrate** mode, double-click **Manual Tuning**.
5. If the ion source has been allowed to cool, then do the following.
   a. Set the **Temperature (TEM)** parameter to **450**.
   b. Let the ion source warm up for 30 minutes.

   The 30-minute warm-up stage prevents solvent vapors from condensing in the cold probe.
6. Start acquisition.

7. Run the method to be used to optimize the sample stream.

Set the Starting Conditions

1. On the **Source/Gas** tab in the Tune Method Editor, type a starting value for **Ion Source Gas 1 (GS1)**.

   For LC pumps, use a value between 40 and 60 for GS1.

2. Type a starting value for **Ion Source Gas 2 (GS2)**.

   For LC pumps, use a value between 30 and 50 for GS2.

   Note: Gas 2 is used with higher flow rates typical with an LC system and in conjunction with increased temperature.

3. Type **25** in the **Curtain Gas (CUR)** field.
4. Type **5500** in the **IonSpray Voltage Floating (ISVF)** field.

Optimize the TurboIonSpray® Probe Position

1. Look through the window of the ion source housing to view the position of the probe.
2. Use the previous horizontal and vertical micrometer settings or set them to **5** as a starting position.
3. Use FIA or a tee infusion to inject sample at a high flow rate.
4. Monitor the signal in the software.
5. Use the horizontal micrometer to adjust the probe position in small increments to achieve the best signal or signal-to-noise ratio.

   The probe can optimize slightly to either side of the aperture.

   Tip! It is easier to optimize signal and signal-to-noise with flow injection analysis or on-column injections.

6. Use the vertical micrometer to adjust the probe position in small increments to achieve the best signal or signal-to-noise ratio.

   Note: The vertical position of the probe depends on flow rate. At lower flow rates, the probe should be closer to the aperture. At higher flow rates, the probe should be farther from the aperture.

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the electrode protrudes beyond the tip of the probe, to prevent hazardous vapors from escaping from the source. The electrode must not be recessed within the probe.

7. Adjust the black electrode adjustment cap on the probe to extend the electrode tip. Typically, the optimum extension of the electrode is 0.5 mm to 1.0 mm beyond the end of the probe.

   After the probe is optimized, it needs only minor adjustment. If the probe is removed, or if the analyte, flow rate, or solvent composition change, repeat the optimizing procedure after installation.

   Tip! Direct the liquid spray from the TurboIonSpray probe away from the aperture in order to prevent contamination of the aperture, to prevent piercing of the Curtain Gas™ flow, which can create an unstable signal, and to prevent electrical shorting due to the presence of the liquid.

Optimize Source and Gas Parameters and Voltage

Optimize Gas 1 (nebulizer gas) for best signal stability and sensitivity. Gas 2 (heater gas) aids in the evaporation of solvent, which helps to increase the ionization of the sample.

Too high a temperature can cause premature vaporization of the solvent at the TurboIonSpray® probe tip, especially if the probe is too low, which will result in signal instability and a high chemical background noise. Similarly, a high heater gas flow could produce a noisy or unstable signal.

Use the lowest IonSpray™ source voltage possible without losing signal. Focus on signal-to-noise and not just signal. If the IonSpray source voltage is too high, then a corona discharge can occur. It is visible as a blue glow at the tip of the TurboIonSpray probe. This will result in decreased sensitivity and stability of the ion signal.

1. Adjust **GS1** and **GS2** in increments of 5 to achieve the best signal or signal-to-noise ratio.

   Note: To prevent contamination, use the highest value for CUR possible without sacrificing sensitivity. Do not set CUR lower than 20. This helps to prevent penetration of the Curtain Gas flow, which can produce a noisy signal, prevent contamination of the aperture and increase the overall signal-to-noise ratio.

2. Increase the value in the **CUR** field until the signal begins to decrease.
3. Adjust **ISVF** in increments of 500 V to maximize signal-to-noise.

   Note: If the **IonSpray Voltage Floating (ISVF)** is too high, then a corona discharge can occur. It is visible as a blue glow at the tip of the TurboIonSpray® probe. A corona discharge results in decreased sensitivity and stability of the ion signal.

Optimize the Turbo Heater Temperature

The optimal heater temperature is compound-dependent, flow rate-dependent, and mobile phase composition-dependent. The higher the flow rate and the higher the aqueous composition, the higher the optimized temperature.

When optimizing the source temperature, make sure that the ion source equilibrates to the new temperature setting.

- Adjust the **TEM** value in increments of 50°C to 100°C to achieve the best signal or signal-to-noise ratio.

Optimize the APCI Probe Position

The position of the APCI probe affects sensitivity of the sample signal.

1. Set the Y-axis adjustment knob to its maximum position.
2. Monitor the sample signal.
3. Use the Y-axis adjustment knob to retract the probe in small increments to achieve the best signal or signal-to-noise ratio.
4. Save the optimized method as a new method.

Optimize the Calibrant Stream

When optimizing the calibrant stream, use the GS1 and GS2 parameter values optimized for the TurboIonSpray® probe in *Optimize the Sample Stream on page 24*.

Run the Method

1. Turn on the CDS.
2. Start the Analyst® TF software.
3. Activate a hardware profile containing the CDS.
4. In the Navigation bar, under **Tune and Calibrate** mode, double-click **Manual Tuning**.
5. If the ion source has been allowed to cool, then do the following.
    a. Set the **Temperature (TEM)** parameter to **450**.
    b. Let the ion source warm up for 30 minutes.

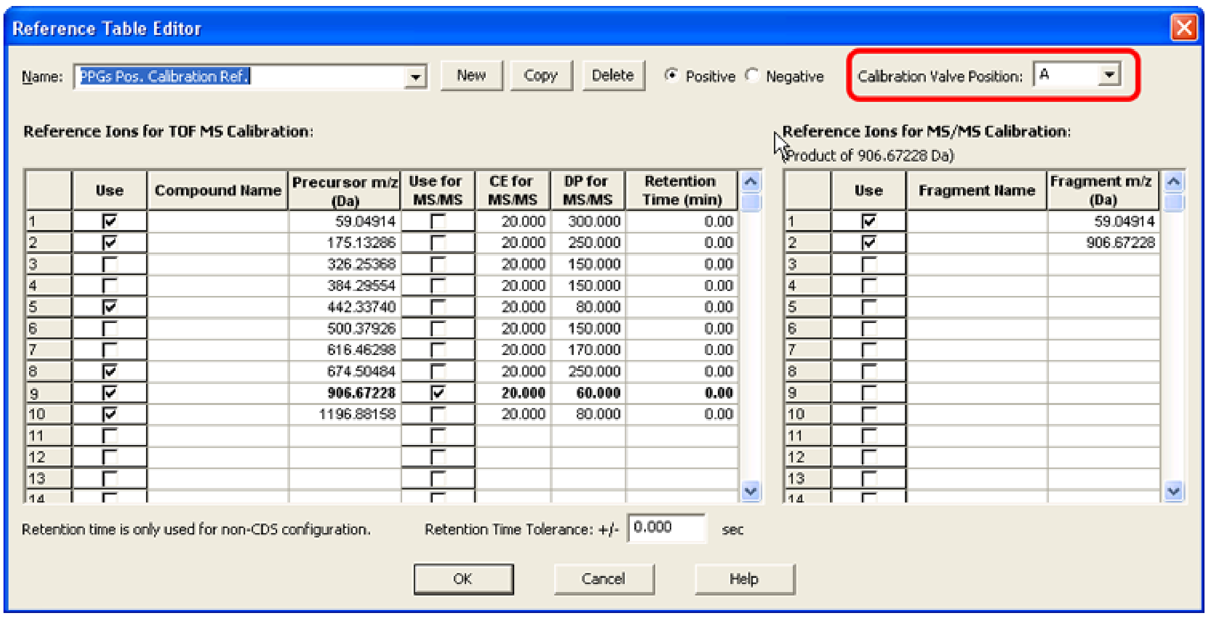
    The 30-minute warm-up stage prevents solvent vapors from condensing in the cold probe.
6. Start acquisition.
7. Run the method used to optimize the sample stream in *Optimize the Sample Stream on page 24*.

(Optional) Select the Calibrant Valve

By default, all reference tables are configured to use Cal A (valve 1). To use a different valve, follow these steps.

1. Click **Tools > Settings > Tuning Options.**
2. On the Tuning Options dialog, click **Reference**.
3. In the **Name** field, select the reference table.

Figure 3-1 Calibrant Valve Position Field in the Reference Table Editor



4. In the **Calibrant Valve Position** field, select the calibrant valve: Cal B (valve 6), Cal C (valve 5), or Cal D (valve 4).
5. Click **OK** to close the Reference Table Editor.
6. Click **OK** to close the Turning Options dialog.

Set the Starting Conditions

- Type **5500** in the **IonSpray Voltage Floating (ISVF)** field.

Adjust the Position of the Corona Discharge Needle

WARNING! Electrical Shock Hazard: Follow this procedure to avoid contact with the high voltages applied to the corona discharge needle, curtain plate, and turbo heaters.

When using the APCI probe, make sure that the corona discharge needle is pointing toward the aperture.

Required Materials
• Insulated flat-bladed screwdriver

1. Use an insulated flat-bladed screwdriver to rotate the corona discharge needle adjustment screw on the top of the needle.
2. Look through the glass window to make sure that the needle is aligned with the tip facing the aperture.
3. Save the optimized method as a new method.

Optimize the Flow Rate of the Calibrant Stream

- Increase the flow rate in steps of 50 µL/min, to a maximum of 1000 µL/min, to achieve a stable calibrant signal.

Optimize the Alternative CDS Configuration

In the alternative CDS configuration, the APCI probe is used for sample analysis, and the TurboIonSpray® probe is used for calibration.

Note: The IonSpray Voltage Floating (ISVF) is always applied to both the TurboIonSpray® probe and the APCI probe simultaneously, and the Temperature (TEM) is always applied to both the turbo and APCI heaters simultaneously.

Optimize the Sample Stream

Run the Method

1. Turn on the CDS.
2. Start the Analyst® TF software.
3. Activate a hardware profile containing the CDS.
4. In the Navigation bar, under **Tune and Calibrate** mode, double-click **Manual Tuning**.
5. If the ion source has been allowed to cool, then do the following.
   a. Set the **Temperature (TEM)** parameter to **450**.
   b. Let the ion source warm up for 30 minutes.

   The 30-minute warm-up stage prevents solvent vapors from condensing in the cold probe.
6. Start acquisition.

7. Run the method to be used to optimize the sample stream.

Set the Starting Conditions

1. On the **Source/Gas tab** in the Tune Method Editor, type **0** in the **Ion Source Gas 1 (GS1)** field.
2. Type **20** in the **Ion Source Gas 2 (GS2)** field.
3. Type **25** in the **Curtain Gas (CUR)** field.
4. Type **5500** in the **IonSpray Voltage Floating (ISVF)** field.

Optimize Gas 1, Gas 2 and Curtain Gas™ Flow

1. Adjust **GS2** in increments of five to achieve the best signal or signal-to-noise ratio.

Note: To prevent contamination, use the highest value for CUR possible without sacrificing sensitivity. Do not set CUR lower than 20. This helps to prevent penetration of the Curtain Gas flow, which can produce a noisy signal, prevent contamination of the aperture and increase the overall signal-to-noise ratio.

2. Increase **CUR** until the signal starts to decrease.

Optimize the IonSpray™ Voltage Floating

Note: If the **IonSpray Voltage Floating (ISVF)** is too high, then a corona discharge can occur. It is visible as a blue glow at the tip of the TurboIonSpray® probe. A corona discharge results in decreased sensitivity and stability of the ion signal.

- In positive mode, start at a value of 5500, and decrease in steps of 100 V to 500 V; in negative mode, start at a value of –4500, and increase in steps of 100 V to 500 V. Continue adjusting to achieve the best signal or signal-to-noise ratio.

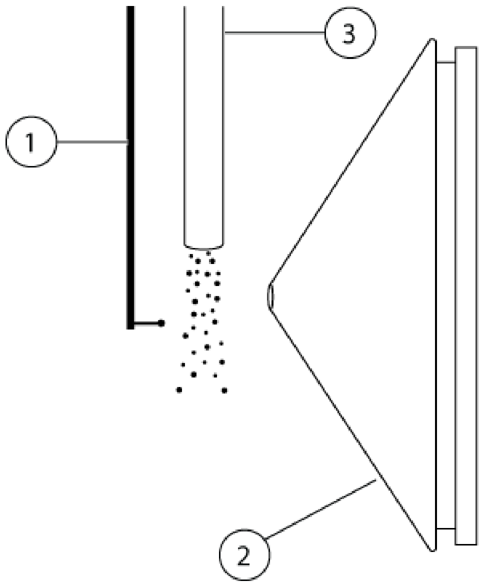
  This parameter usually optimizes around 5500 V in positive mode. If you observe no changes in signal with increasing ISVF, then leave the ISVF at the lowest setting that provides the best signal or signal-to-noise ratio.

Optimize the APCI Probe Position

Make sure that the curtain plate aperture remains clear of solvent or solvent droplets at all times.

The position of the sprayer nozzle affects sensitivity and signal stability. Adjust probe sensitivity in small increments only. At lower flow rates, position the probe closer to the aperture. For higher flow rates, position the probe farther away from the aperture.

Figure 3-2 Sprayer Nozzle Position



Item	Description
1	Corona discharge needle
2	Curtain plate
3	APCI probe

1. Use the previous setting or set it to 5 mm as an initial starting position.

Note: To avoid reducing the performance of the mass spectrometer, do not spray directly into the aperture.

2. Use FIA or a tee infusion to inject sample at a high flow rate.
3. Monitor the signal in the software.
4. Use the vertical micrometer to adjust the probe in small increments to achieve the best signal or signal-to-noise ratio.

   After the probe is optimized, it needs only minor adjustment. If the probe is removed, or if the analyte, flow rate, or solvent composition changes, repeat the optimizing procedure after installation.

Optimize the IonSpray™ Voltage Floating

Note: If the **IonSpray Voltage Floating (ISVF)** is too high, then a corona discharge can occur. It is visible as a blue glow at the tip of the TurboIonSpray® probe. A corona discharge results in decreased sensitivity and stability of the ion signal.

- In positive mode, start at a value of 5500, and decrease in steps of 100 V to 500 V; in negative mode, start at a value of –4500, and increase in steps of 100 V to 500 V. Continue adjusting to achieve the best signal or signal-to-noise ratio.

  This parameter usually optimizes around 5500 V in positive mode. If you observe no changes in signal with increasing ISVF, then leave the ISVF at the lowest setting that provides the best signal or signal-to-noise ratio.

Optimize the APCI Probe Temperature

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the mass spectrometer is properly vented and that good general laboratory ventilation is provided. Adequate laboratory ventilation is required to control solvent and sample emissions and to provide for safe operation of the mass spectrometer.

The quantity and type of solvent affects the optimal APCI probe temperature. At higher flow rates, the optimal temperature increases.

- Adjust the **TEM** value in increments of 50°C to 100°C to achieve the best signal or signal-to-noise ratio.

Adjust the Position of the Corona Discharge Needle

WARNING! Electrical Shock Hazard: Follow this procedure to avoid contact with the high voltages applied to the corona discharge needle, curtain plate, and turbo heaters.

When using the APCI probe, make sure that the corona discharge needle is pointing toward the aperture.

Required Materials
• Insulated flat-bladed screwdriver

1. Use an insulated flat-bladed screwdriver to rotate the corona discharge needle adjustment screw on the top of the needle.
2. Look through the glass window to make sure that the needle is aligned with the tip facing the aperture.
3. Save the optimized method as a new method.

Optimize the Calibrant Stream

When optimizing the calibrant stream, use the GS1 and GS2 parameter values optimized for the APCI probe in *Optimize the Sample Stream on page 29*.

The position of the TurboIonSpray® probe may impact the performance of the analytical stream. Verify the performance of the analytical stream after optimizing the calibrant stream.

Run the Method

1. Turn on the CDS.
2. Start the Analyst® TF software.
3. Activate a hardware profile containing the CDS.
4. In the Navigation bar, under **Tune and Calibrate** mode, double-click **Manual Tuning**.
5. If the ion source has been allowed to cool, then do the following.
   a. Set the **Temperature (TEM)** parameter to **450**.
   b. Let the ion source warm up for 30 minutes.

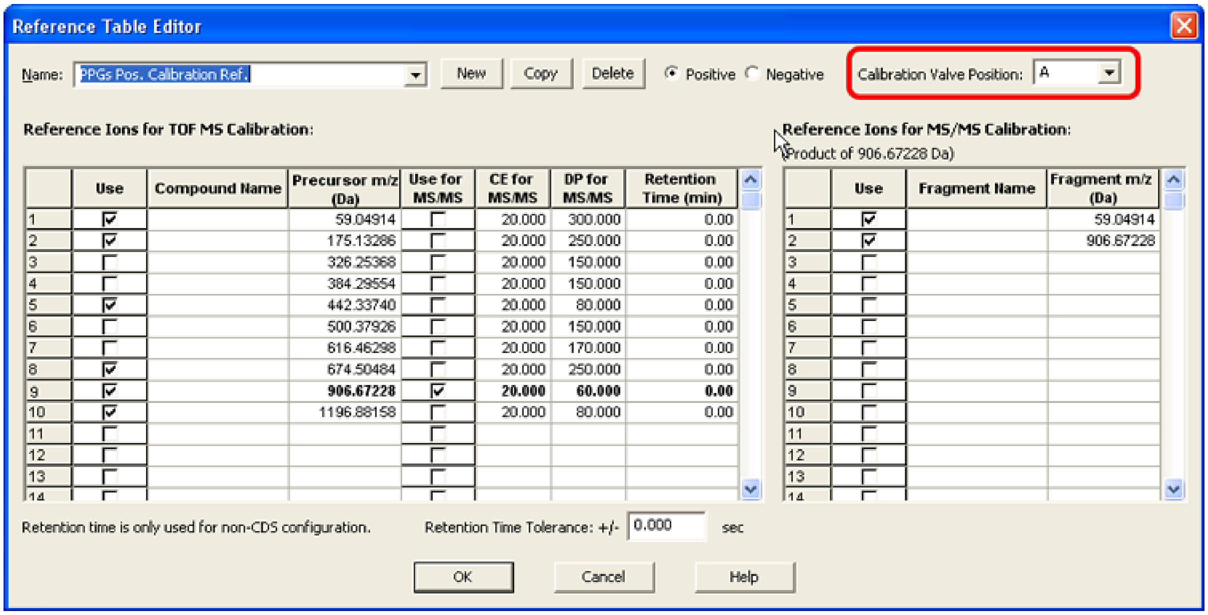
   The 30-minute warm-up stage prevents solvent vapors from condensing in the cold probe.
6. Start acquisition.
7. Run the method used to optimize the sample stream in *Optimize the Sample Stream on page 29*.

(Optional) Select the Calibrant Valve

By default, all reference tables are configured to use Cal A (valve 1). To use a different valve, follow these steps.

1. Click **Tools** > **Settings** > **Tuning Options.**
2. On the Tuning Options dialog, click **Reference**.
3. In the **Name** field, select the reference table.

Figure 3-3 Calibrant Valve Position Field in the Reference Table Editor



4. In the **Calibrant Valve Position** field, select the calibrant valve: Cal B (valve 6), Cal C (valve 5), or Cal D (valve 4).
5. Click **OK** to close the Reference Table Editor.
6. Click **OK** to close the Turning Options dialog.

Optimize the Flow Rate of the Calibrant Stream

- Increase the flow rate in steps of 50 µL/min, to a maximum of 1000 µL/min, to achieve a stable calibrant signal.

Optimize the TurboIonSpray®

1. Set **IonSpray Voltage (ISVF)** to **5500**.
2. Look through the window of the ion source housing to view the position of the probe.
3. Set the horizontal micrometer to 5 and the vertical micrometer to 0.
4. Click **CDS Inject**.
5. Monitor the signal in the software.
6. Use the horizontal micrometer to adjust the probe position in small increments to achieve the best signal or signal-to-noise ratio.

   The probe can optimize slightly to either side of the aperture.

Tip! It is easier to optimize signal and signal-to-noise with flow injection analysis or on-column injections.

7. Use the vertical micrometer to adjust the probe position in small increments to achieve the best signal or signal-to-noise ratio.

Note: The vertical position of the probe depends on flow rate. At lower flow rates, the probe should be closer to the aperture. At higher flow rates, the probe should be farther from the aperture.

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the electrode protrudes beyond the tip of the probe, to prevent hazardous vapors from escaping from the source. The electrode must not be recessed within the probe.

8. Adjust the black electrode adjustment cap on the probe to extend the electrode tip. Typically, the optimum extension of the electrode is 0.5 mm to 1.0 mm beyond the end of the probe.

   After the probe is optimized, it needs only minor adjustment. If the probe is removed, or if the analyte, flow rate, or solvent composition change, repeat the optimizing procedure after installation.

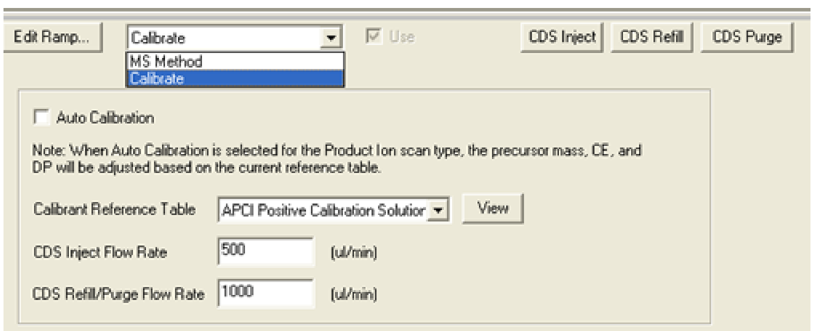
Tip! Direct the liquid spray from the TurboIonSpray probe away from the aperture in order to prevent contamination of the aperture, to prevent piercing of the Curtain Gas™ flow, which can create an unstable signal, and to prevent electrical shorting due to the presence of the liquid.

Calibration in Manual Tune Mode 4

Calibrate the Mass Spectrometer

1. Start the Analyst® TF software.
2. Make sure that the CDS is connected to the mass spectrometer and that it is turned on.
3. Activate a hardware profile containing the CDS.
4. In the Navigation bar, under **Tune and Calibrate**, click **Manual Tuning**.
5. In the **Tune Method Editor** window, select **Calibrate** from the list.

Figure 4-1 Tune Method Editor Window



6. In the **Calibrant Reference Table** field, select the required calibrant reference table.

Note: The calibrant valve position is configured in the calibrant reference table. By default, the system uses Cal A (valve 1). To use a different valve, follow the instructions in *(Optional) Select the Calibrant Valve(Optional) Select the Calibrant Valve on page 33*.

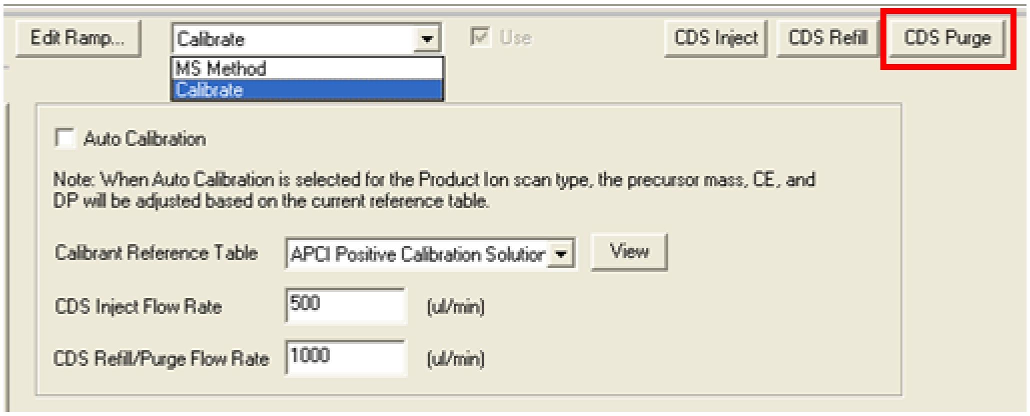
7. Click **View** to show the calibration ions and the calibrant valve position.

   Make sure that the correct ions for the experimental mass range, and the correct calibration valve position are selected. For information about editing reference tables, refer to the Analyst TF software Help.

8. Click **CDS Purge** to prime the CDS with the calibration solution.

While the CDS is filling or injecting, the blue LED blinks. When the purging of the system is complete, the CDS is in the Home position, filled with the calibration solution.

Figure 4-2 CDS Purge Button



9. In the **CDS Inject Flow Rate** field, type **200**.
10. From the list, select **MS Method**.
11. Create an MS method.
12. Set the ion source and mass spectrometric conditions to values that are typical for the application. For example, for a TOF MS method, and an LC flow rate of 200 µL/min, starting conditions may be as shown in *Table 4-1*.

Table 4-1 TOF MS Method

Parameter	Value
MS	
Scan Type	TOF MS
TOF Mass (Da)	Min= 100, Max=2000
Accumulation Time (seconds)	0.250
Source/Gas	
Ion Source Gas 1 (GS1)	40
Ion Source Gas 2 (GS2)	50
Curtain Gas (CUR)	25
Temperature (TEM)	550
IonSpray Voltage Floating (ISVF)	5500
Compound	

Table 4-1 TOF MS Method (continued)

Parameter	Value
Declustering Potential (DP)	80
Collision Energy (CE)	10

Tip! If **Auto Calibration** in the **Calibrate** tab is selected, the **Product of (Da)**, **Declustering Potential (DP)** and **Collision Energy (CE)** fields are automatically populated with the values specified in the Calibrant Reference table when **Product Ion** is selected as the scan type.

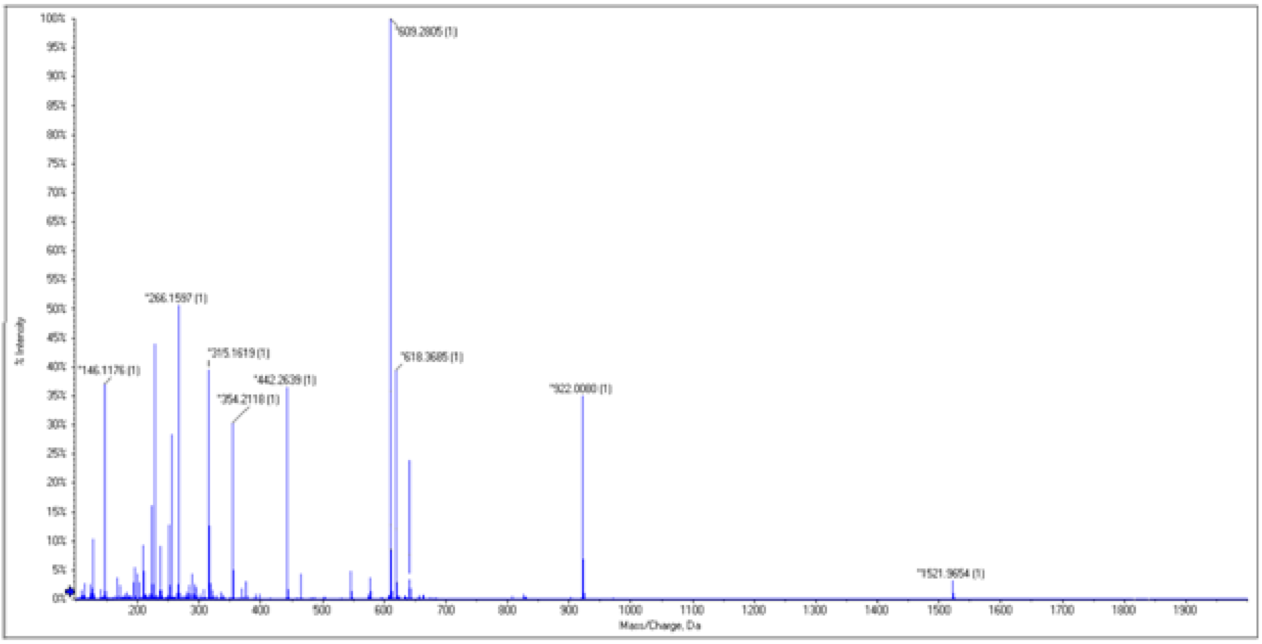
13. Introduce the LC flow into the TurboIonSpray® probe of the ion source at the initial starting conditions of the typical LC method. For example, in positive mode, the LC conditions might be 95:5 water:acetonitrile + 0.1% formic acid at 200 µL/min.
14. After the CDS has been primed, click **CDS Inject** to begin infusing the calibration solution into the APCI probe.
15. Click **Start** to begin acquiring data.

    The calibration ions should appear in the mass spectrum. Refer to the sample spectra in *Figure 4-3*.

    Note: If the CDS has not been used recently, or if another calibration solution was used previously, it may take a couple of minutes for the desired calibration ions to appear. To decrease the time, temporarily increase the CDS flow rate.

16. After the solution has been primed, use the CDS to automatically calibrate the mass spectrometer in Tune and Calibrate mode or in Acquire Mode.

Figure 4-3 Sample Spectra: TOF MS, APCI Positive Calibration Solution



Troubleshooting 5

Table 5-1 CDS Troubleshooting Tips

Issue	Possible Cause	Solution
The CDS is not communicating with the Analyst® TF software. (CDS statuses on the Detailed Status dialog are N/A, and the mass spec displays an error condition.)	The CDS is not configured in the hardware profile. CDS status on the **Detailed Status** dialog is **Not Used**.	Configure the hardware profile. Refer to *Configure the Hardware Profile on page 21*.
	The CDS is configured but not communicating with the Analyst TF software. CDS status on the **Detailed Status** dialog is **Disconnected**.	Inspect the communication cable connections.
	The communications (CCOMM) PCB is faulty.	The communications PCB may need to be replaced. Contact an AB SCIEX Field Service Employee (FSE).
Fittings are leaking.	A fitting or the tubing is loose or incorrectly installed	• Tighten fittings with a tool to the specified torque rating. Purge the pump head to waste and refill. • If the problem persists, remove the tubing and check for kinks or debris. Replace the tubing if required.
There is no flow.	A fitting or the tubing is loose or incorrectly installed	• Check for leaks or damaged tubing. • Tighten fittings with a tool to the specified torque rating. Purge the pump head to waste and refill. • If the problem persists, remove the tubing and check for kinks or debris. Replace the tubing if required.
	A blockage in the calibrant line is causing air to escape through the seal.	Make sure that the calibrant bottles are vented properly, and inspect the calibrant line for blockages.
	The calibrant tubing is not immersed in the calibrant solution.	Make sure that the tubing is in the solution.

Table 5-1 CDS Troubleshooting Tips (continued)

Issue	Possible Cause	Solution
	Priming is incomplete.	Refill and purge the CDS.
	The pump line is blocked.	Inspect the pump line for blockages.
	The ion source line is blocked.	Inspect the ion source line for blockages.
	The pump is faulty.	The pump may need to be replaced. Contact an FSE.
	There is electronic noise on the valve control line.	• Make sure the correct cables are installed. • Make sure that the grounding cable on the CDS is installed properly. • Make sure that the valve pods are grounded.
	The communications (CCOMM) PCB is faulty.	The communications PCB may need to be replaced. Contact an FSE.
A timeout error occurred while filling, homing, or purging the pump.	The refill/purge flow rate is set too high.	Make sure the flow rate is set to 1200 µL/min or less.
Calibrant ion intensity is weak.	A primary seal in the pump is leaking.	The pump may need to be replaced. Contact an FSE.

Table 5-2 APCI Calibration Solution Troubleshooting Tips

Issue	Possible Cause	Solution
Calibration ions are not observed in the spectrum.	The pump has not been primed properly.	Make sure that the calibrant tubing from CDS Select valve is in the calibration solution. Purge the CDS solution.
	The calibrant tubing contains air bubbles after purging or refilling the solution.	• Make sure that calibrant tubing from CDS Select valve is in the calibration solution. Purge the CDS solution. • Decrease the CDS Refill/Purge Flow rate.
	An incorrect Select valve position is selected for calibration solution.	In the Reference Table Editor, modify the Valve Position to the correct position.

Table 5-2 APCI Calibration Solution Troubleshooting Tips (continued)

Issue	Possible Cause	Solution
Calibration ion intensity is weak.	The CDS Inject Flow Rate is too low.	Increase the CDS inject Flow Rate.
Calibration Ion C or F are not observed.	Calibration ions C and F are temperature-dependent.	At high temperatures, do not include Calibration Ion F. At lower temperatures, do not include Calibration Ion C.
Batch Calibration fails.	An incorrect Calibrant Reference table is selected.	Choose the appropriate reference table for the calibration solution.
	Calibration ion intensity is too weak.	Increase the CDS Inject Flow Rate
Batch Calibration fails in MS/MS mode.	One or more fragment ions are not present, or weak in intensity.	• Increase the CDS Inject Flow Rate. • In the Reference table, clear the calibration ions that are low in intensity (three or more calibration ions are recommended for achieving good calibration).
Batch fails to submit. An error message indicates one or more calibration reference ions are out of the mass range.	One or more calibration reference ions are out of the mass range in the acquisition method selected for the batch.	Edit the reference table to include only the ions that are within the mass range of the acquisition method.

Consumables

A

Part Number	Description	Quantity	Upchurch Part Number
5008141	NUT, PEEK, SHORT, 1/4-28	1	LT-115
5008152	FERRULE, PEEK, SUPER FLANGELESS	2	P-260
5008157	FITTING, PEEK, NAT, LONG,	1	F130
5008232	PLUG, ACETAL, BLACK	1	U-467BLK
5008296	TUBING, PEEK, BLUE, 1/16x.010x40CM	1	PM-1960B
5002985	TUBING, PEEK, RED, 1/16x.125x40CM	1	PM-1945R
5008298	TUBING, PEEK, GREEN, 1/16x.030x16CM	1	PM1820G
	TUBING, FEP, NAT, 1/16x.030x60CM	5	PM1000
	TUBING, FEP, NAT, 1/16x.030x17CM	1	PM1000
5008302	TUBING, FEP, GREEN, 1/16x.030x60CM	2	PM1000G
5008303	FILTER ASSY, PEEK, BOB, 1/16,10um,GL-38	2	A-453
	CAP ASSY, BOTTLE, 1/16,GL-38	5	
	FITTINGS, KNOB, BLACK	11	
	FERRULE, PEEK	11	
	NUT, RHEFLEX	11	

Calibration Solutions

Part Number	Description	Quantity
4460131	APCI Positive Calibration Solution for the AB SCIEX TripleTOF® 5600 System	100 mL
4460134	APCI Negative Calibration Solution for the AB SCIEX TripleTOF® 5600 System	100 mL

Part Number	Description	Quantity
4460136	APCI Positive Calibration Solution for the AB SCIEX TripleTOF® 5600 System (5-pack)	5 × 100 mL
4460138	APCI Negative Calibration Solution for the AB SCIEX TripleTOF® 5600 System (5-pack)	5 × 100 mL
4463272	ESI Positive Calibration Solution for the AB SCIEX TripleTOF® 5600 System	100 mL
4463274	ESI Positive Calibration Solution for the AB SCIEX TripleTOF® 5600 System (5-pack)	5 × 100 mL
4463276	ESI Negative Calibration Solution for the AB SCIEX TripleTOF® 5600 System (5-pack)	5 × 100 mL
4463277	ESI Negative Calibration Solution for the AB SCIEX TripleTOF® 5600 System	100 mL

Single-Probe Configuration B

Use this configuration with the Turbo V™ or IonDrive™ Turbo V ion source and the SelexION™ technology. In this configuration, the LC stream is connected to the Inject valve. The stream is diverted to waste during calibration, and when calibration is finished, it is directed to the ion source.

Note: We recommend the use of the ESI calibration solution kits with the Turbo V or IonDrive Turbo V ion source and the SelexION technology. For part numbers, refer to *Consumables on page 43*.

Plumb the CDS Lines

1. Follow the instructions in *Plumb the CDS Lines on page 14*.
2. For the LC waste connection, assemble the ferrule and Rheodyne fitting on the 60 cm green FEP tubing, insert the tubing into the Inject valve LC WASTE port 5 as far as it will go, and then tighten the fitting.
3. Insert the other end of the green FEP tubing into the waste bottle on the rack.

Install the CDS

- Follow the instructions in *Install the CDS on page 17*.

Plumb the Ion Source

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Make sure that the sample tubing nut is tightened properly before operating this equipment, to prevent leakage.

1. Connect the other end of the red PEEK tubing from Inject valve port 3 to the probe on the ion source.
2. Insert the sample tubing into the sample tubing nut, install the sample tubing nut on the fitting at the top of the probe, and then tighten the sample tubing nut until it is finger-tight.
3. Connect the tubing from the sample supply device to the Inject valve port 4.

Configure the CDS in the Hardware Profile

- Follow the instructions in *Configure the Hardware Profile on page 21*.

Test the CDS Installation

- Follow the instructions in *Test the CDS Installation on page 46*.

Prime the CDS

The lines are primed with the solution connected to the calibration valve port specified in the **Tuning Options** in the Analyst® TF software. Refer to the Analyst TF software Help.

Note: If this is the first time the system is being used, if the system has not been used for a long time, or if the calibration valve positions have changed, it may be necessary to purge the lines multiple times.

1. In the Analyst TF software, on the Navigation bar, double-click **Manual Tuning**.
2. From the list of method types, select **Calibrate**.
3. In the **Calibrant Reference Table** List field, select the reference table for the calibrant solution.
4. Click **CDS Purge**, inspecting the lines from the calibration solution bottle to the valve and from the pump to the valve for air bubbles.
5. Continue to click **CDS Purge** until no air bubbles are visible.

Revision History

Document Number	Description of Change	Date
D5020801 A	First release of document.	January 2011
D5020801 B	Optimization procedure and troubleshooting chapter updated. New template applied.	June 2012
RUO-IDV-05-0810-A	Updated for SelexION ™ technology and TripleTOF® 6600 system. New template applied.	June 2014