

OptiMS Thermo MS Adapter

For Nanospray II, Nanospray Flex, and Nanospray Flex NG Ion Sources

Installation Guide



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This document includes instructions to install an adapter for the mass spectrometer, connect the CESI 8000 Plus system to the mass spectrometer, and perform a test separation to verify correct system operation.

Note: For instructions about how to use the system safely, refer to the document: *User Guide*.

Bill of Materials

Part Number	Description	Quantity
B10249	Adapter	1
B83710	Adapter mounting bracket	1
B83380	High-voltage cable	1
B86096	<i>OptiMS Thermo MS Adapter for NanoSpray II, NanoSpray Flex, and NanoSpray Flex NG Ion Sources Installation Guide</i> (this document)	1
B83383	Relay cable	1
B82395	Thumbscrews	2

Regulatory Compliance

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

International Standards

The OptiMS Thermo MS Adapter for NanoSpray II, NanoSpray Flex, and NanoSpray Flex NG Ion Sources has been evaluated as part of the CESI 8000 Plus High Performance Separation-ESI Module and found to be in compliance with the International Electrotechnical Commission standard IEC 61010-1, 2nd edition: 2010 Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.

Decommissioning and Disposal



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

Before decommissioning, obey local regulations to decontaminate the entire system.

When the system is removed from service, obey national and local environmental regulations to divide and recycle different materials.

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

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

Hazard Symbols

This section lists the hazard symbols and conventions used in the laboratory environment, on the system, and in the documentation.

Occupational Health and Safety Symbols

This section describes some occupational health and safety symbols found in the documentation and laboratory environment.

Table 1-1 General Hazard Symbols


Safety Symbol	Description
	Personal Injury Hazard

Table 1-2 Chemical Hazard Symbols


Safety Symbol	Definition
	Biohazard

Table 1-2 Chemical Hazard Symbols (continued)




Safety Symbol	Definition
	Explosion Hazard
	Toxic Chemical Hazard
	Fire Hazard

Table 1-3 Electrical Hazard Warning Symbols



Safety Symbol	Definition
	Electrical Shock Hazard

Table 1-4 Mechanical Hazard Symbols

Safety Symbol	Definition
	Hot Surface Hazard

Symbols, Indicators, and Labels

Note: If any of the labels used to identify a component become detached, then contact a SCIEX service representative.

Documentation Symbols and Conventions

The following symbols and conventions are used throughout the guide.



DANGER! Danger identifies an action that can cause severe injury or death.

Overview



WARNING! Warning identifies an action that can cause personal injury if precautions are not obeyed.

CAUTION: Caution identifies an operation that can cause damage to the system or corruption or loss of data if precautions are not obeyed.

Note: Notes supply important information in a procedure or description.

Tip! Tips supply information that helps to apply the techniques in a procedure or gives a shortcut, but that is not essential to the completion of a procedure.

Install the Adapter

2

Install the Adapter (Nanospray Flex and Nanospray Flex NG)



WARNING! Electrical Shock Hazard. Before the adapter is installed, make sure that the ESI voltage is zero, the mass spectrometer is idle, and the separation voltage from the CESI 8000 Plus system is off.

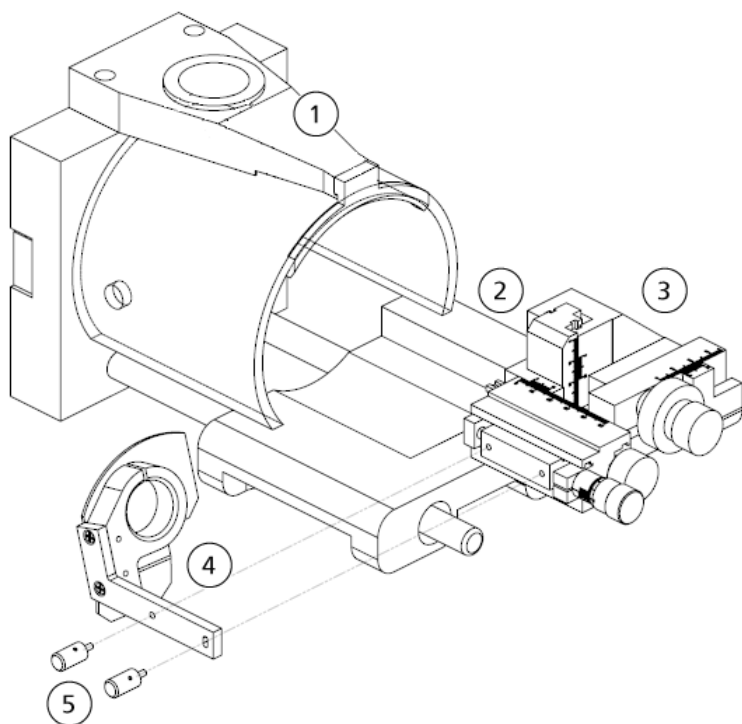


WARNING! Hot Surface Hazard. Beware of burns. The ion sweep cone becomes hot during system operation. Do not touch the part until it has had sufficient time to cool.

1. Use the manufacturer's instructions to remove the ion sweep cone from the mass spectrometer.
2. Use the manufacturer's instructions to install the ion source on the mass spectrometer.
3. Pull the ion source drawer fully out, away from the inlet on the mass spectrometer.

Install the Adapter

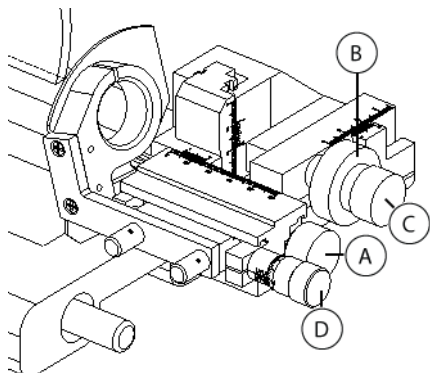
Figure 2-1 Ion Source Drawer Components Without Mounting Bracket



Item	Description
1	Ion source
2	Ion source drawer
3	XYZ manipulator
4	Mounting bracket
5	Thumbscrews

4. If applicable, then remove the direct junction adapter from the mass spectrometer.
5. Use the thumbscrews to attach the mounting bracket to the ion source.
The top of the arm on the mounting bracket should be parallel with the horizontal plane of the XYZ manipulator.
6. Use the alignment knobs on the XYZ manipulator to do an initial alignment:

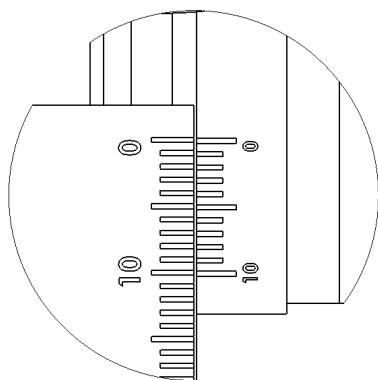
Figure 2-2 Alignment Knobs on the XYZ Manipulator



Item	Description
A	Moves toward the inlet on the mass spectrometer (coarse)
B	Moves horizontally
C	Moves vertically
D	Moves toward the inlet on the mass spectrometer (fine)

- a. Use knob **A** to move the stage as far as possible away from the inlet on the mass spectrometer. Make sure that the **0** marks are aligned.

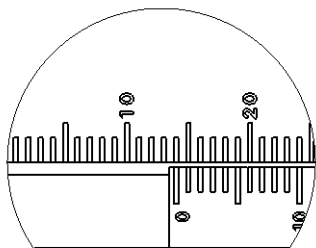
Figure 2-3 Alignment of Rulers: Knob A



- b. Use knob **B** to align **14** on the moveable ruler with **0** on the static ruler.

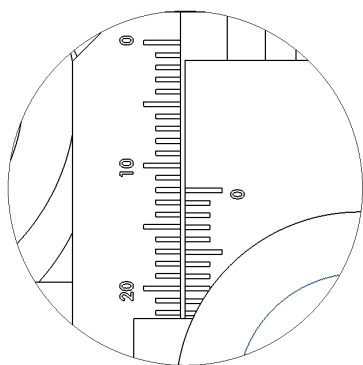
Install the Adapter

Figure 2-4 Alignment of Rulers: Knob B



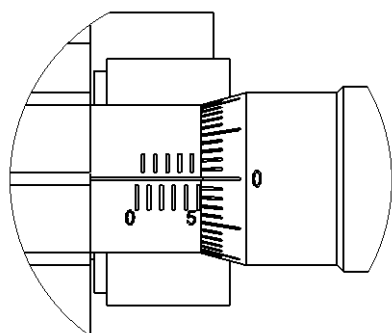
- c. Use knob **C** to align **12** on the moveable ruler with **0** on the static ruler.

Figure 2-5 Alignment of Rulers: Knob C



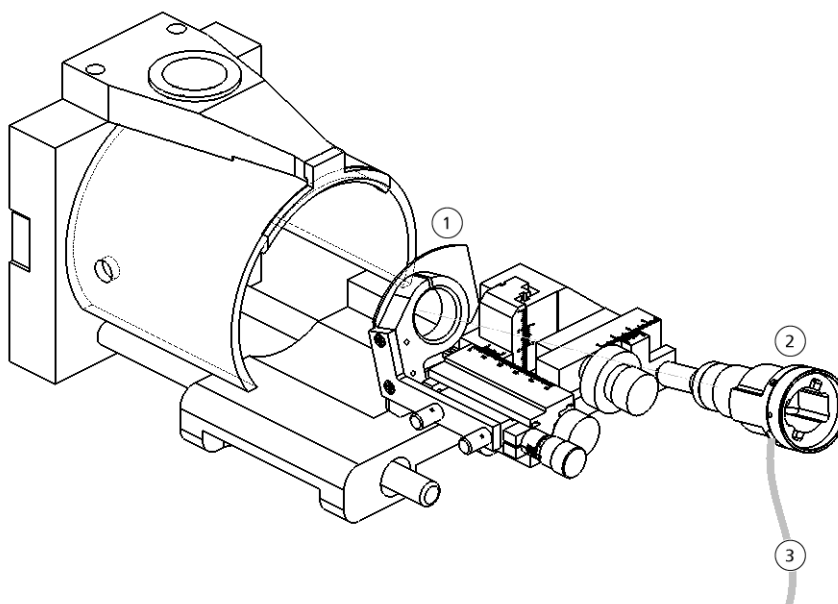
- d. Use knob **D** to align **0** on the rotating ring with **5** on the static ruler.

Figure 2-6 Alignment of Rulers: Knob D



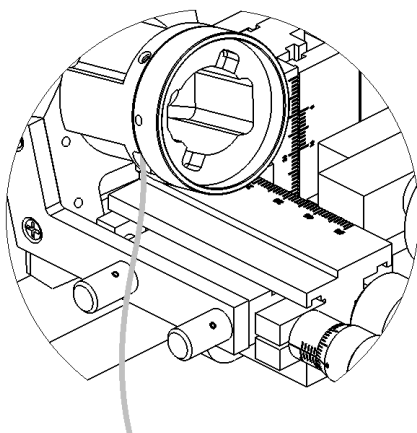
7. Install the adapter in the mounting bracket until the adapter is tight against the bracket. Make sure that the high-voltage cable hangs freely from the adapter. If required, then turn the adapter.

Figure 2-7 Adapter and Mounting Bracket



Item	Description
1	Mounting bracket
2	Adapter
3	High-voltage cable

Figure 2-8 Adapter Installed in the Mounting Bracket



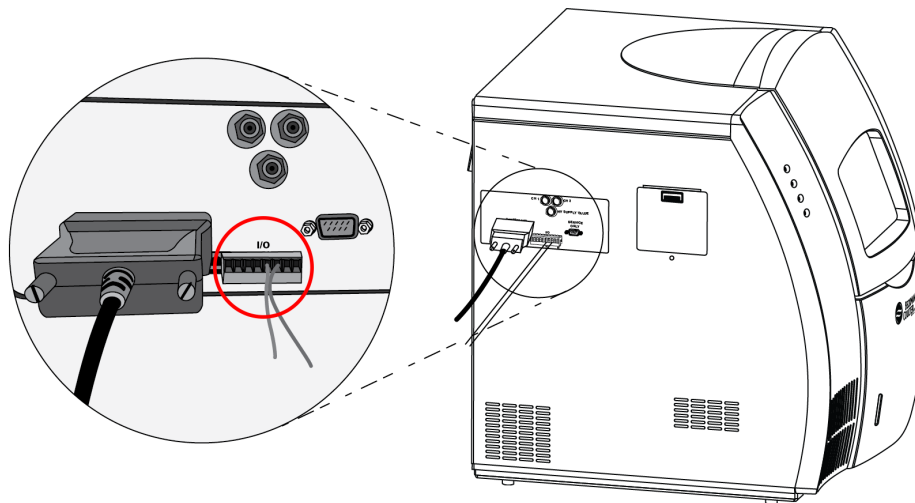
8. Push the ion source drawer toward the inlet on the mass spectrometer.

Install the Adapter

Connect the Cables

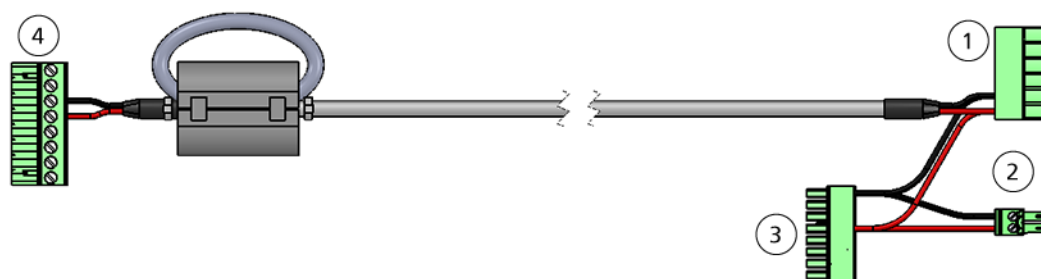
1. Put the CESI 8000 Plus system approximately 18 cm to 20 cm (7.1 inches to 7.9 inches) away from the ion source.
2. Connect the single connector on the end of the relay cable (Figure 2-10, item 4) to the relay port on the CESI 8000 Plus system (labeled I/O in the following figure).

Figure 2-9 Relay Cable Connection



3. Connect the other end of the relay cable (Figure 2-10, item 1) to the mass spectrometer.

Figure 2-10 Relay Cable



Item	Description
1	To Peripheral Control port on mass spectrometer Connector for: <ul style="list-style-type: none"> • LCQ Deca XP MAX, LTQ-series, and Velos-series ion trap • LTQ Orbitrap-series and Orbitrap Velos-series hybrid ion trap-Orbitrap
2	To leftmost ports of Input section of Peripheral Control area on mass spectrometer (labeled Digital Start In) Connector for: <ul style="list-style-type: none"> • Exactive-series Orbitrap • Q Exactive-series hybrid quadrupole-Orbitrap • TSQ Quantum-series and TSQ Vantage triple-stage quadrupole
3	Connects to Peripheral Control connector on mass spectrometer Connector for: <ul style="list-style-type: none"> • Orbitrap Fusion Tribrid • TSQ Endura triple-stage quadrupole
4	To CESI 8000 Plus system

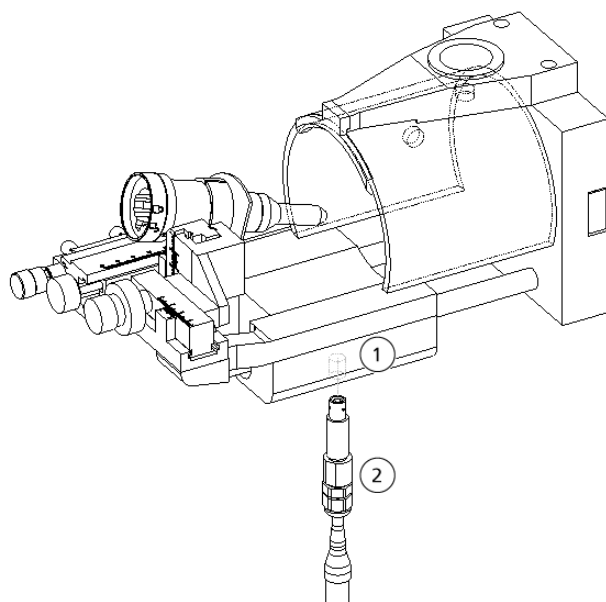


WARNING! Personal Injury Hazard. Route the relay cable where users cannot trip on it.

4. Connect the input cable between the ion source and the CESI 8000 Plus system.
 - a. Connect the high-voltage input cable to the port on the ion source.

Install the Adapter

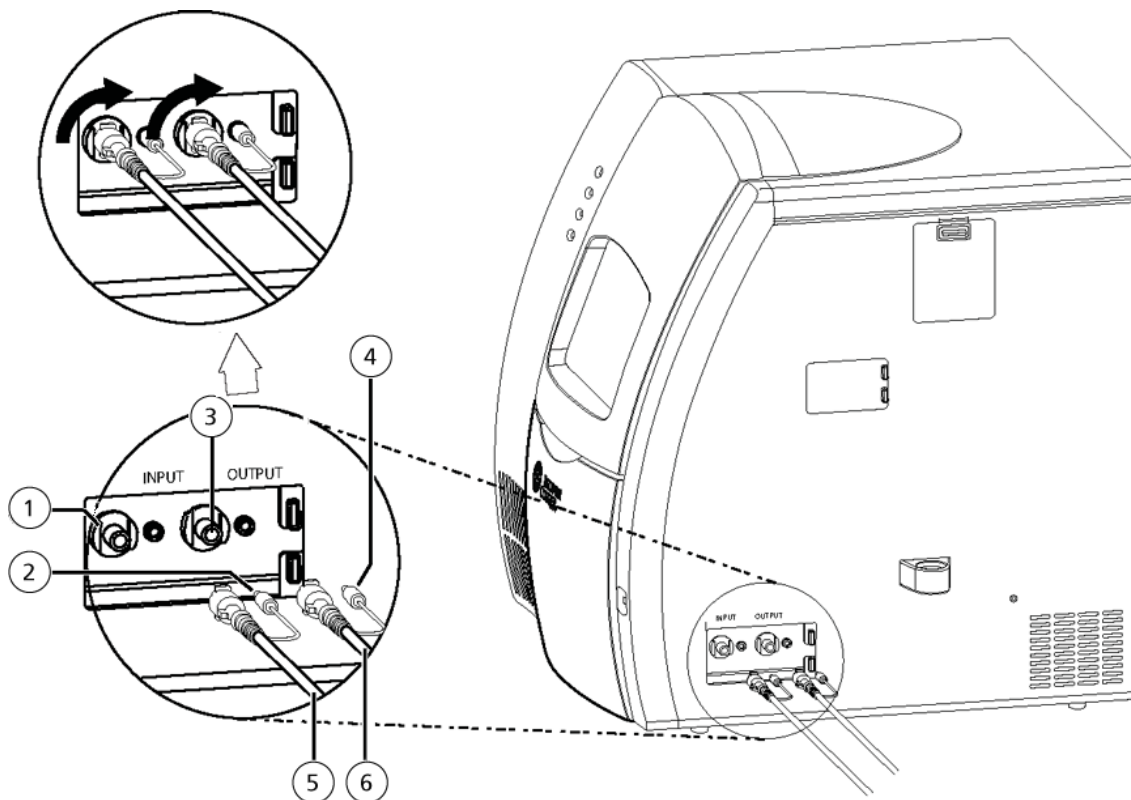
Figure 2-11 High-Voltage Input Cable for the Adapter



Item	Description
1	Ion source port
2	High-voltage input cable

- b. On the right side panel of the CESI 8000 Plus system, open the door to the high-voltage connections panel ([Figure 2-12](#)).
- c. Connect the white high-voltage cable to the **Input** port on the connections panel, and then turn the cable clockwise to lock it in position.
- d. Connect the red banana plug to the red port on the connections panel.

Figure 2-12 High-Voltage Connections Panel



Item	Description
1	Input (red) port on connections panel
2	Red banana plug
3	Output (black) port on connections panel
4	Black banana plug
5	High-voltage input cable from mass spectrometer
6	High-voltage output cable to adapter

5. Connect the high-voltage output cable (attached to the adapter) to the CESI 8000 Plus system.
 - a. Connect the white high-voltage cable to the **Output** port on the connections panel, and then turn the cable clockwise to lock it in position.
 - b. Connect the black banana plug to the black port on the connections panel.

Install the Adapter (Nanospray II)



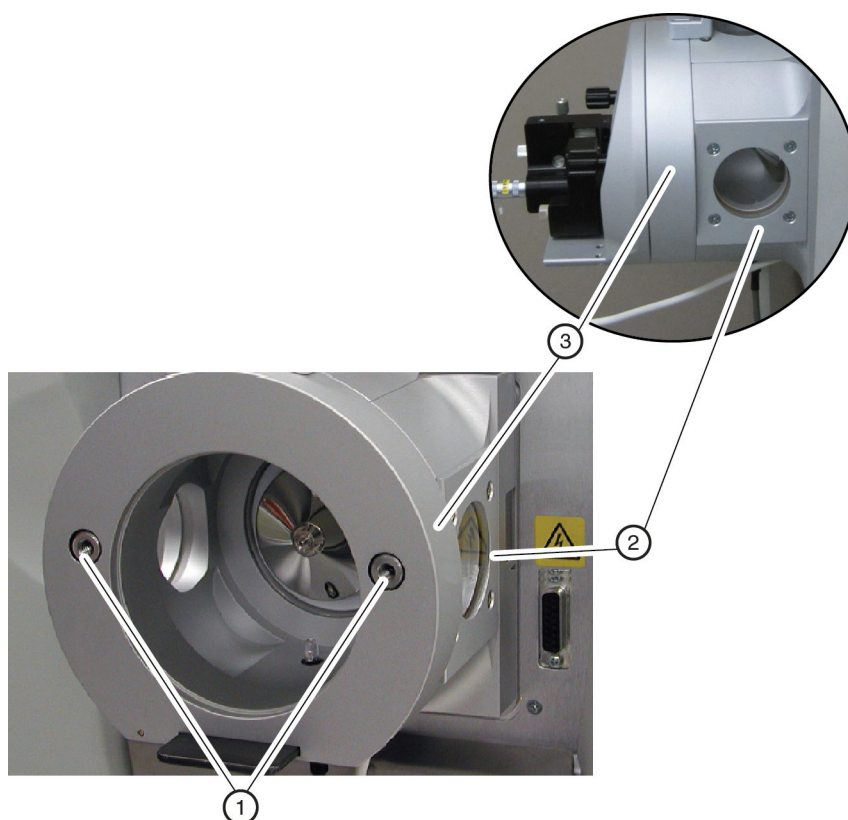
WARNING! Electrical Shock Hazard. Make sure that the mass spectrometer is idle, the ESI voltage is zero, and the separation voltage on the CESI 8000 Plus system is off.



WARNING! Hot Surface Hazard. Beware of burns. The ion sweep cone becomes hot during system operation. Do not touch the part until it has had sufficient time to cool.

1. Make sure that the 1-inch adapter ring is not installed.

Figure 2-13 Adapter Ring

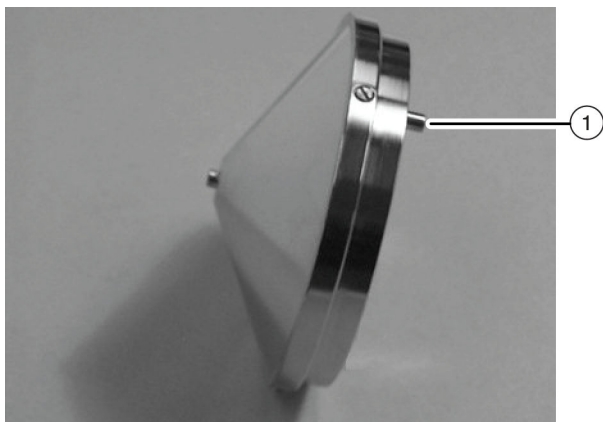


Item	Description
1	Socket head screws
2	Ion max adapter
3	1-inch (deep) adapter ring

If necessary, then use the manufacturer's instructions to remove 1-inch adapter ring.

2. Use the manufacturer's instructions to remove the ion sweep cone from the mass spectrometer.

Figure 2-14 Ion Sweep Cone

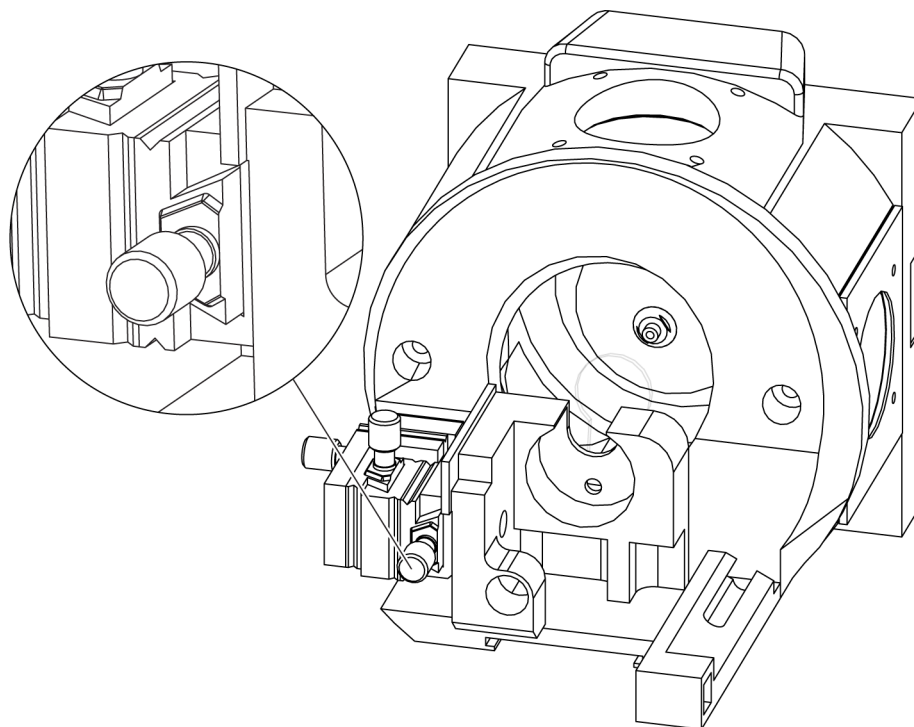


Item	Description
1	Gas inlet

3. Use the manufacturer's instructions to install the ion source on the mass spectrometer.
4. Use the alignment knob to move the ion source stage as far away as possible from the inlet on the mass spectrometer.

Install the Adapter

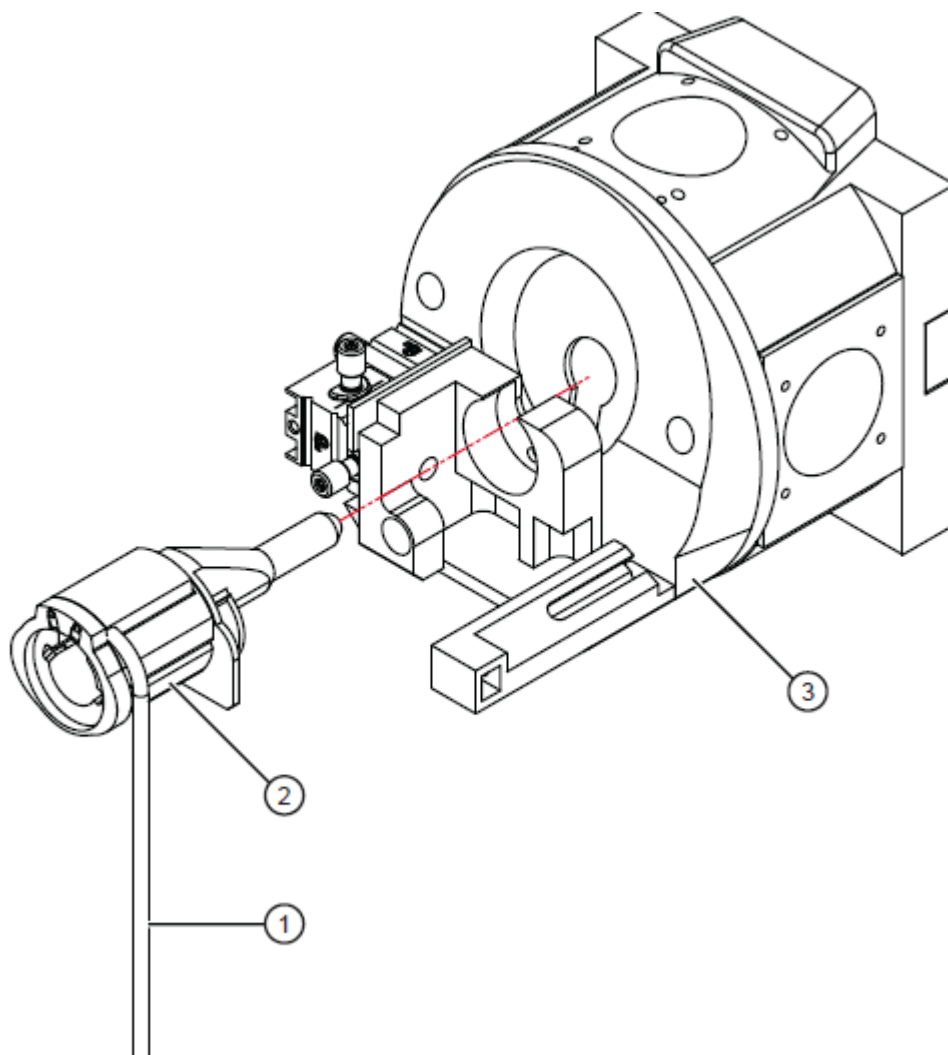
Figure 2-15 Alignment Knob



CAUTION: Potential System Damage. Move the stage before the adapter is installed on the mass spectrometer. During the installation of the sprayer assembly, the glass tip of the sprayer is exposed. If the ion source stage is too close to the inlet on the mass spectrometer, then damage to the sprayer tip can occur.

5. Install the adapter in the holder on the ion source.

Figure 2-16 Orientation of the Adapter Before Installation

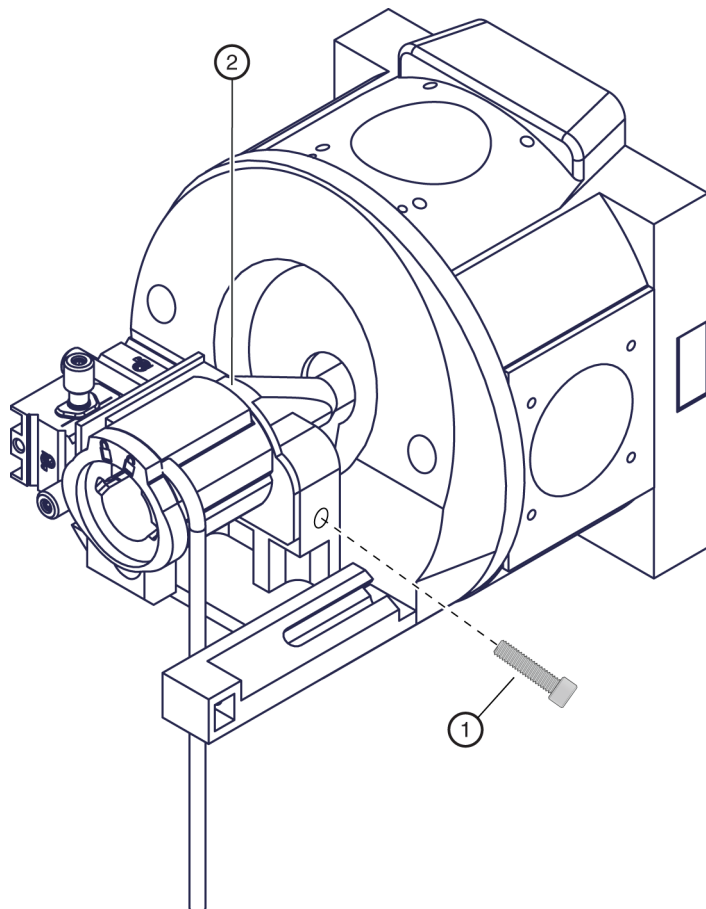


Item	Description
1	High-voltage output cable
2	Adapter
3	Ion source

6. If the ion source has a metal set screw on the right side of the holder, then replace the metal set screw with the plastic thumbscrew supplied in the adapter kit.
7. Make sure that the position of the adapter is tight, and then tighten the thumbscrew.

Install the Adapter

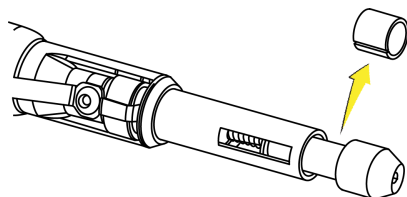
Figure 2-17 Tighten the Thumbscrew



Item	Description
1	Thumbscrew
2	Adapter installed in the ion source

8. Remove the protective sleeve from the sprayer tip.

Figure 2-18 Protective Sleeve and Sprayer Tip

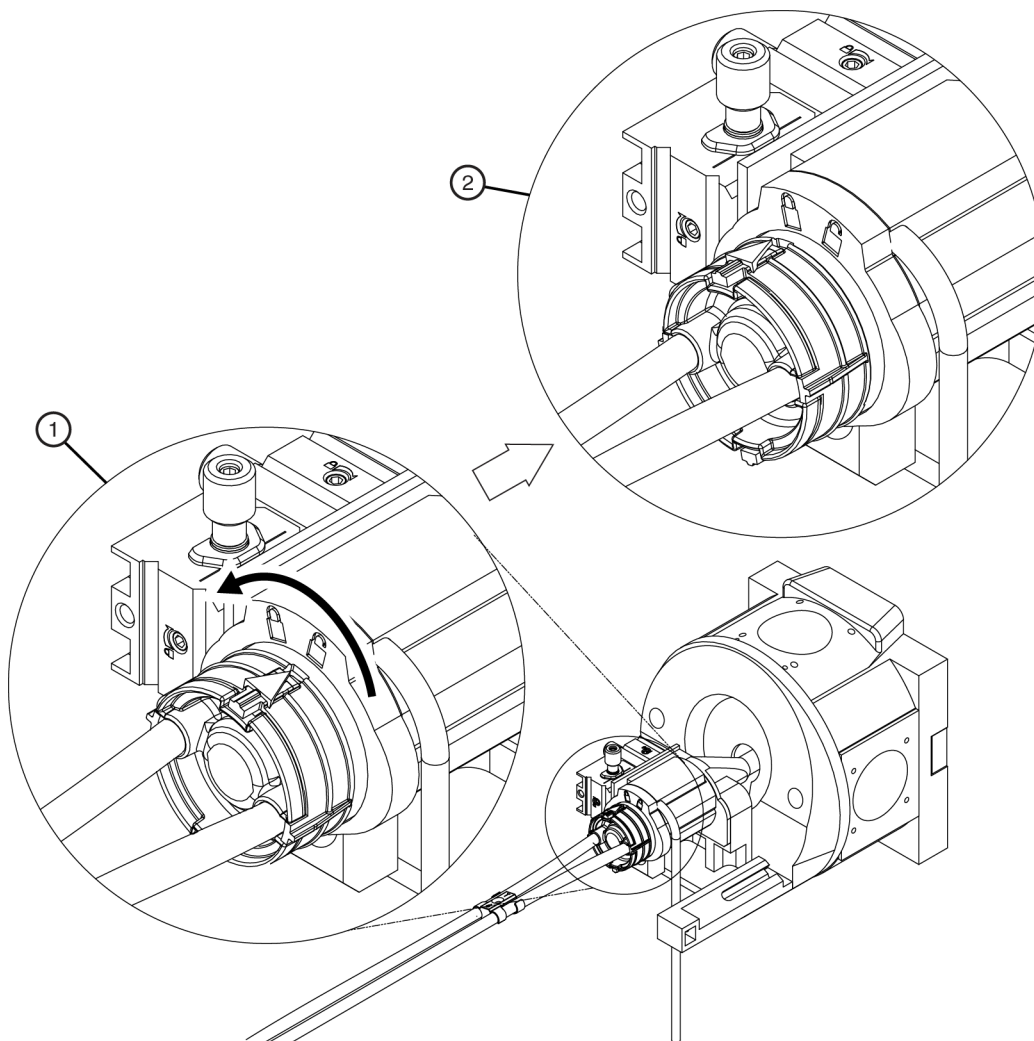


Note: Keep the protective sleeve for later use.

CAUTION: Potential System Damage. When the protective sleeve is removed, the protective guard on the tip can retract. During the installation of the sprayer assembly, if the ion source stage is too close to the inlet on the mass spectrometer, then damage to the sprayer tip can occur.

9. To install the sprayer assembly, carefully align the arrow with the Unlock position on the adapter. Do not let the sprayer tip touch any surfaces.
10. Turn the sprayer assembly counterclockwise to lock it in the adapter.

Figure 2-19 Lock the Sprayer Assembly in the Adapter



Install the Adapter

Item	Description
1	Sprayer assembly in the Unlock position
2	Sprayer assembly in the Lock position

Connect the Cables



WARNING! Electrical Shock Hazard. Make sure that the mass spectrometer is idle, the ESI voltage is zero, and the separation voltage on the CESI 8000 Plus system is off.

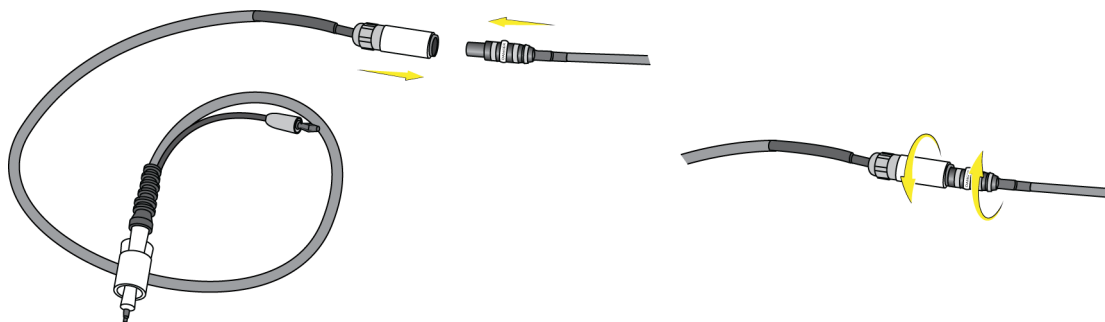


WARNING! Electrical Shock Hazard. Before turning on and operating the CESI 8000 Plus system, make sure that the high-voltage input and output cables are fully connected at both ends.

The high-voltage cables connect the mass spectrometer adapter to the power supply on the CESI 8000 Plus system.

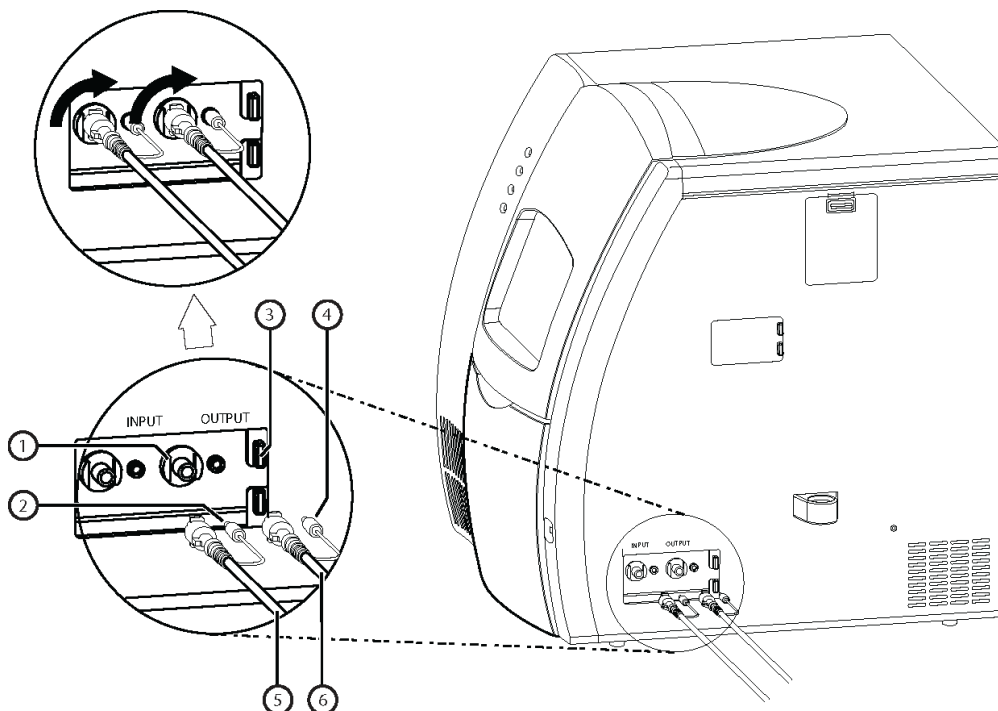
1. To connect the input cable for the adapter to the input cable for the mass spectrometer, push the connectors together and then twist to lock them in position.

Figure 2-20 High-Voltage Input Cable-to-Cable Connection



2. Put the CESI 8000 Plus system approximately 4 inches (10 cm) away from the ion source stage.
3. On the right side of the CESI 8000 Plus system, open the access door to show the high-voltage connections panel.

Figure 2-21 High-Voltage Connections Panel



Item	Description
1	Input (red) port on connections panel
2	Red banana plug
3	Output (black) port on connections panel
4	Black banana plug
5	High-voltage input cable from mass spectrometer to Input (red)
6	High-voltage output cable to adapter to Output (black)

4. Connect the black banana plug to the black port on the connections panel.

Note: If the black banana plug is not installed, then EMI from the high-voltage cables can occur.

5. Connect the high-voltage output cable from the adapter to the **Output** port on the connections panel, and then turn the cable 90 degrees clockwise to lock it in position.
6. Connect the red banana plug to the red port on the connections panel.

Install the Adapter

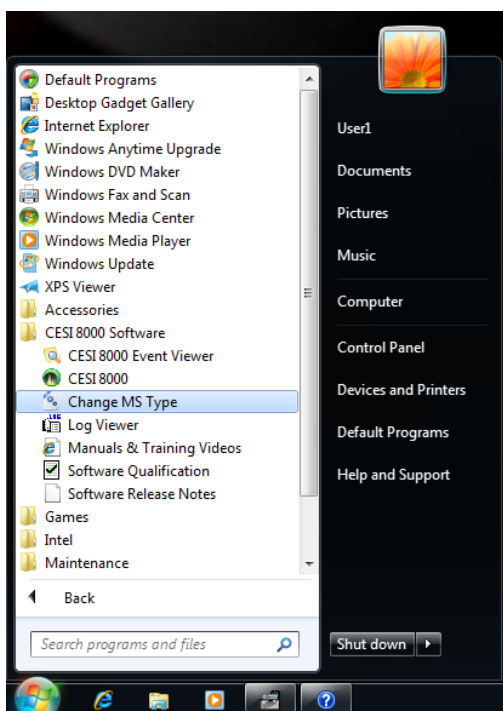
Note: If the red banana plug is not installed, then EMI from the high-voltage cables can occur.

7. Connect the high-voltage input cable from the mass spectrometer to the **Input** port on the connections panel, and then turn the cable 90 degrees clockwise to lock it in position.

Change the Mass Spectrometer Type 3

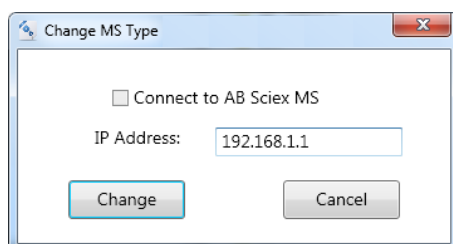
1. Turn on power to the CESI 8000 Plus system.
2. Click **Start > CESI 8000 Software**, and then click **Change MS Type**.

Figure 3-1 Change MS Type Option



3. Make sure that the **Connect to AB SCIEX MS** check box is cleared.

Figure 3-2 Change MS Type Dialog



Change the Mass Spectrometer Type

Note: The IP address in the dialog should be the one that was preconfigured for the mass spectrometer. Do not change the IP address unless it is known that the IP address is in use by another network connection. If the IP address that shows needs to be changed, then contact sciex.com/request-support.

4. Click **Change**.

Align the System with the Mass Spectrometer

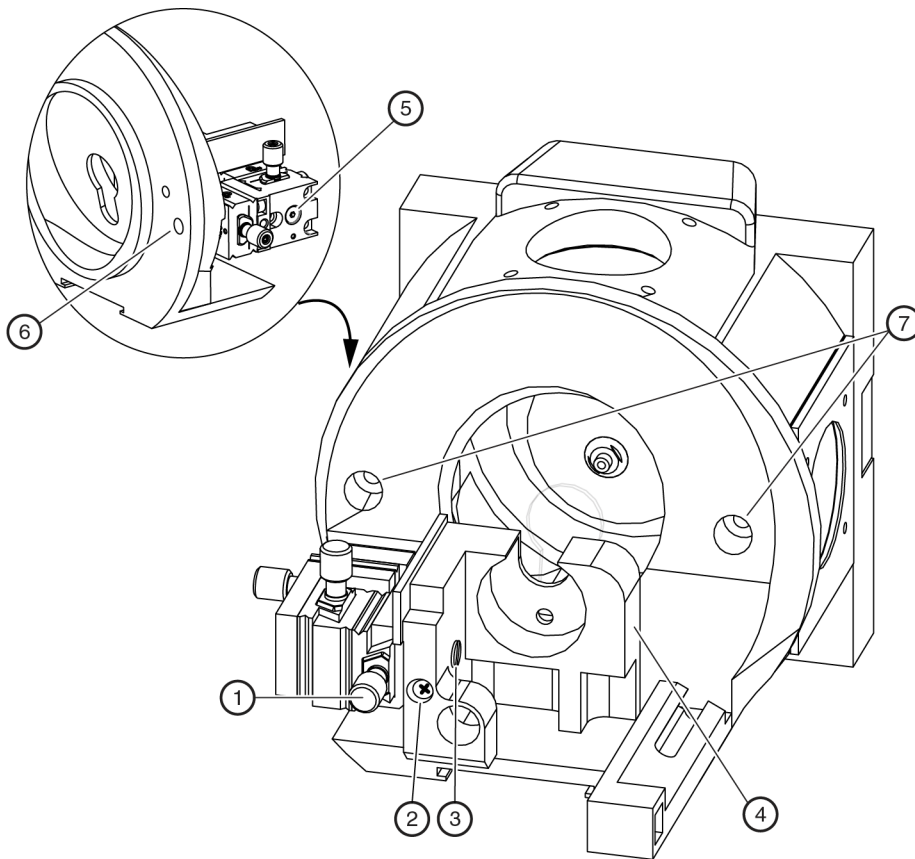
4

To prevent hydrodynamic (siphoning) flow in the capillaries and to let the coolant drain from the cartridge before the cartridge assembly is removed, align the sprayer tip installed in the mass spectrometer adapter with the height indicator on the CESI 8000 Plus system.

Align the System with the Mass Spectrometer (Nanospray II)

1. If required, then use the alignment knob to move the ion source stage and adapter fully away from the curtain gas plate. Make sure that the capillary will not touch the mass spectrometer when the stage moves forward.

Figure 4-1 Nanospray II Alignment Knob



Align the System with the Mass Spectrometer

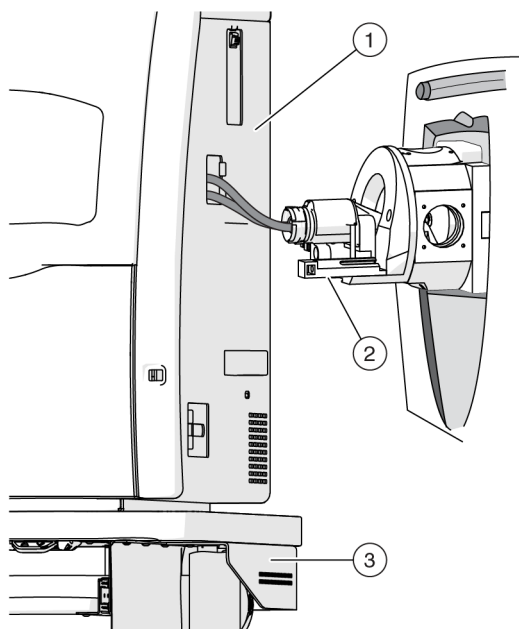
Item	Description
1	Alignment knob
2	Screw for the holder screw
3	Screw for the holder with the stage
4	Holder
5	Screw that tightens the stage
6	Screw that tightens the stage
7	Thumbscrews for the faceplate

Note: The stage and stage holder become loose with use. Frequently tighten all of the screws.

CAUTION: Potential System Damage. Move the stage before the adapter is installed on the mass spectrometer. During the installation of the sprayer assembly, the glass tip of the sprayer is exposed. If the ion source stage is too close to the inlet on the mass spectrometer, then damage to the sprayer tip can occur.

2. Make sure that the CESI 8000 Plus system is approximately 4 inches (10 cm) away from the ion source stage so that the coolant tubes can be extended fully.

Figure 4-2 System Near the Ion Source Stage

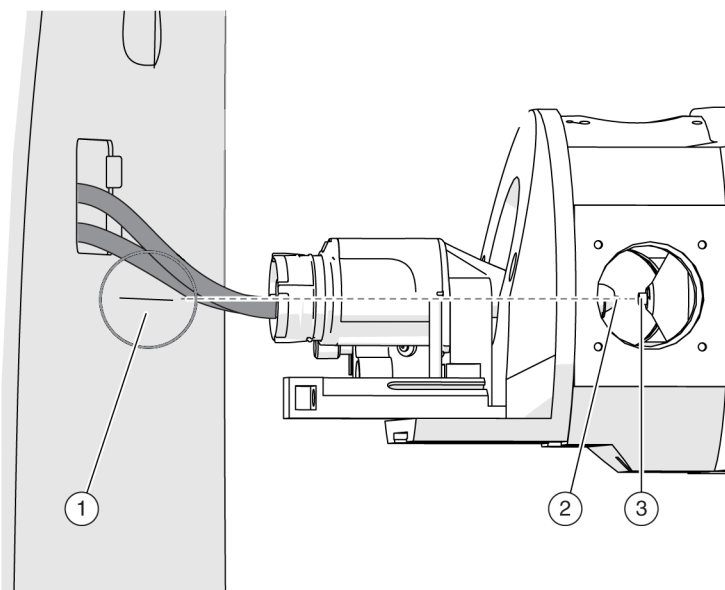


Align the System with the Mass Spectrometer

Item	Description
1	CESI 8000 Plus system
2	Ion source stage
3	Mobile cart

3. At the same time, align the sprayer tip with the height indicator on the CESI 8000 Plus system and the center of the inlet on the mass spectrometer.

Figure 4-3 Alignment of the Sprayer Tip, Height Indicator, and Inlet on the Mass Spectrometer



Item	Description
1	Height indicator on the CESI 8000 Plus system
2	Sprayer tip
3	Inlet on the mass spectrometer

4. Use the **Up** and **Down** buttons on the front of the mobile cart to adjust the height of the cart until the height indicator on the CESI 8000 Plus system aligns with the sprayer tip.

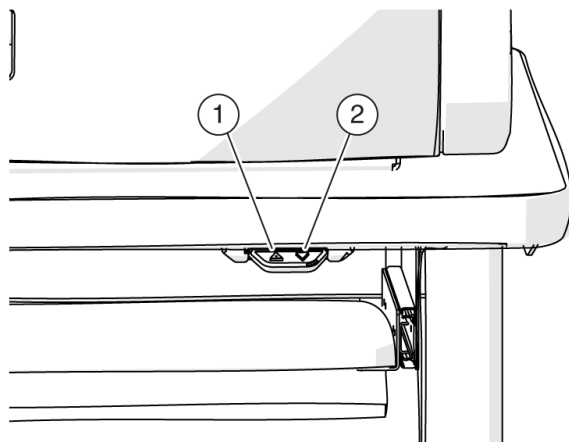


WARNING! Personal Injury Hazard. Remove consumables from the mobile cart before the height is adjusted.

Align the System with the Mass Spectrometer

Note: Do not press both the **Up** and **Down** buttons at the same time.

Figure 4-4 Buttons for Height Adjustment on the Mobile Cart

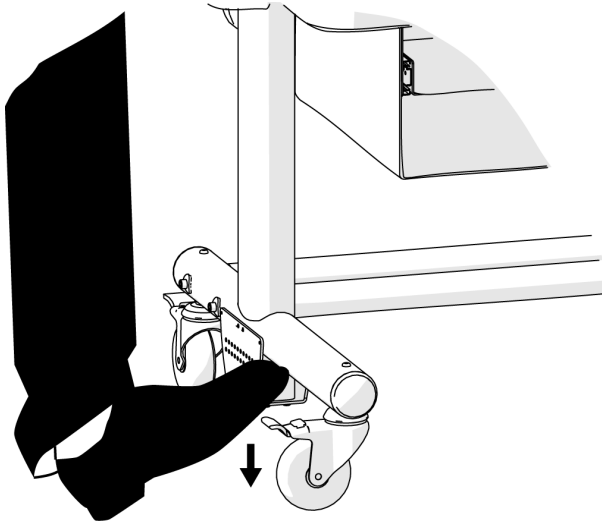


Item	Description
1	Up button
2	Down button

Note: If the cart cannot move to its lowest height or if it becomes locked in position, then the cart must be programmed again. To set the home position for the mobile cart, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

5. When the CESI 8000 Plus system is in visual alignment with the mass spectrometer, step on the locking lever of each caster to lock each wheel of the mobile cart in position.

Figure 4-5 Lock the Wheels of the Mobile Cart



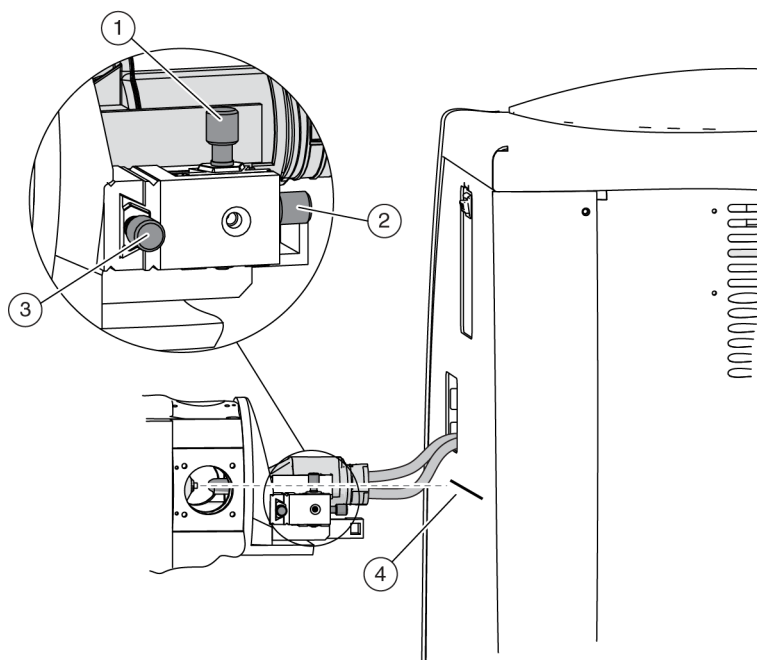
Note: When the alignment is correct, the tubing from the sprayer assembly will not have dips or pockets.

6. Use the other adjustment knobs on the mass spectrometer to fine-tune the alignment of the sprayer tip with the center of the inlet on the mass spectrometer.

CAUTION: Potential System Damage. Keep the sprayer tip at least 2 mm away from the inlet on the mass spectrometer. If the sprayer tip is too close to the inlet, then the accidental suction of rinse solution into the mass spectrometer can occur and cause damage.

Align the System with the Mass Spectrometer

Figure 4-6 Alignment Knobs on the Nanospray II Ion Source



Item	Description
1	Y-axis (movement up and down)
2	X-axis (movement toward and away from the inlet on the mass spectrometer)
3	Z-axis (movement side to side)
4	Height indicator

Align the System with the Mass Spectrometer (Nanospray Flex and Nanospray Flex NG)

CAUTION: Potential System Damage. Before the adapter is installed and aligned, push the ion source drawer fully toward the inlet on the mass spectrometer. If the sprayer is too close to the inlet on the mass spectrometer, then damage can occur.

1. Make sure that the CESI 8000 Plus system is approximately 18 cm to 20 cm (7.1 inches to 7.9 inches) away from the ion source so that the coolant tubes can be extended fully.
-

Align the System with the Mass Spectrometer

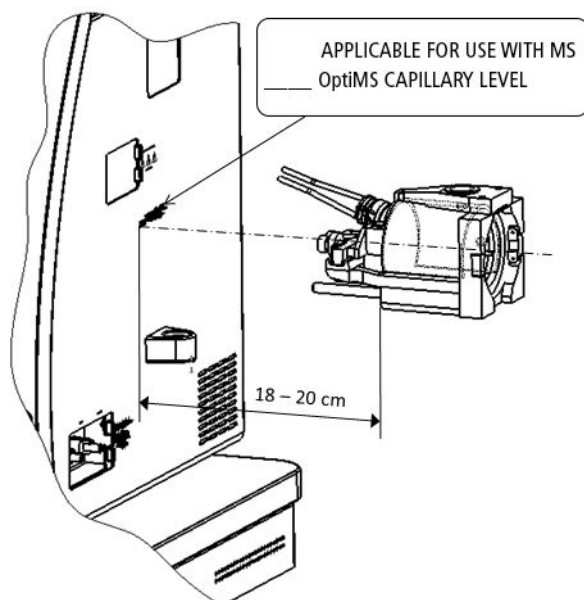
2. To adjust the height of the cart, press the buttons on the front of the cart until the indicator line on the CESI 8000 Plus system is aligned with the inlet on the mass spectrometer.



WARNING! Personal Injury Hazard. Remove consumables from the mobile cart before the height is adjusted.

Note: Do not press both the **Up** and **Down** buttons at the same time.

Figure 4-7 Alignment of the CESI 8000 Plus System and the Ion Source Inlet

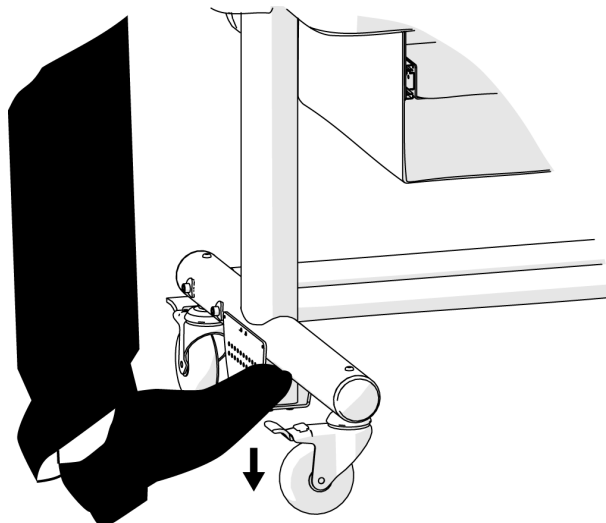


Note: If the cart cannot move to its lowest height or if it becomes locked in position, then the cart must be programmed again. To set the home position for the mobile cart, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

3. When the CESI 8000 Plus system is in visual alignment with the mass spectrometer, step on the locking lever of each caster to lock each wheel of the mobile cart in position.

Align the System with the Mass Spectrometer

Figure 4-8 Lock the Wheels of the Mobile Cart



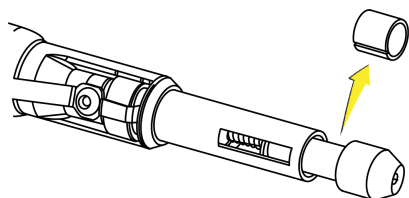
Note: When the alignment is correct, the tubing from the sprayer assembly will not have dips or pockets.

4. Install the sprayer tip in the adapter.

CAUTION: Potential System Damage. Do not move the sprayer tip too close to the inlet on the mass spectrometer. If the sprayer tip is < 2 mm from the inlet, then the accidental suction of rinse solution into the mass spectrometer can occur and cause damage.

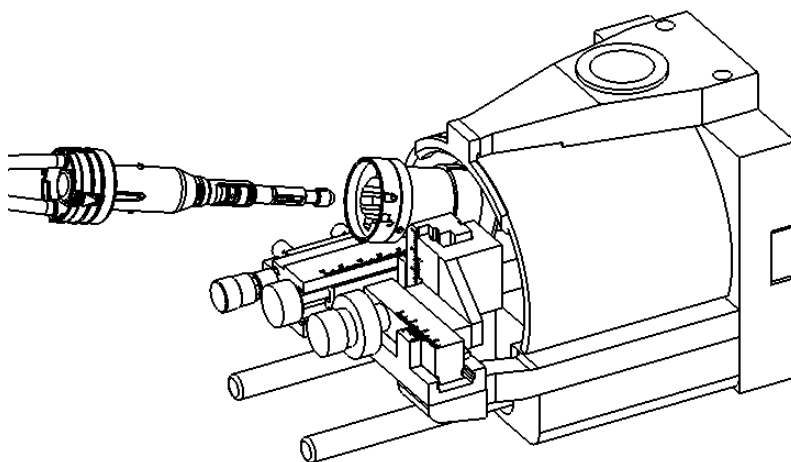
- a. Remove the protective sleeve from the sprayer tip.

Figure 4-9 Protective Sleeve and Sprayer Tip



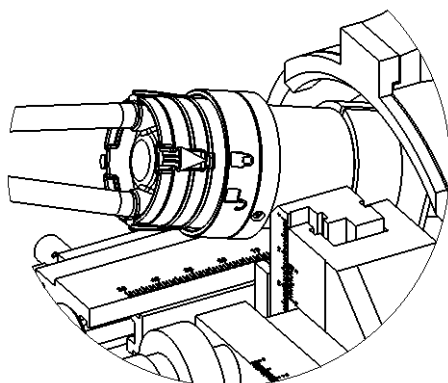
- b. While holding the body of the adapter to prevent it from moving towards the inlet, install the sprayer in the adapter, aligning the arrow on the cartridge with the unlock position on the adapter.

Figure 4-10 Sprayer Tip Approaching the Adapter



- c. Push the sprayer into the adapter and turn it counterclockwise to lock it in position. The triangle on the sprayer should line up with the lock icon on the adapter.

Figure 4-11 Sprayer in the Locked Position in the Adapter



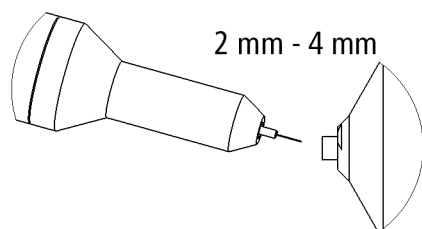
5. Put the sprayer tip in position relative to the ion source inlet.

CAUTION: Potential System Damage. Do not move the sprayer tip too close to the inlet on the mass spectrometer. If the sprayer tip is < 2 mm from the inlet, then the accidental suction of rinse solution into the mass spectrometer can occur and cause damage.

- a. While watching the position of the sprayer tip on the monitor, slowly turn knob A clockwise to move the sprayer tip forward. Refer to the figure: [Figure 2-2](#).
- b. Move the sprayer tip until it is approximately 2 mm to 4 mm away from the ion source inlet.

Align the System with the Mass Spectrometer

Figure 4-12 Sprayer Tip at the Ion Source Inlet



Prepare the Reagents

5

The following reagents are used with the silica surface OptiMS cartridge. The sample type controls which reagents are required to do a separation.

Note: The examples in this guide use beta-galactosidase stock solution. For information about how to prepare and use PepCalMix, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

Table 5-1 Required Reagents for the Silica Surface OptiMS Cartridge

Reagent	Purpose
10% Acetic acid (HAc)	Background electrolyte (BGE) and conductive liquid
20% HAc	pH adjustment of leading electrolyte (LE)
LE buffer, 200 mM ionic strength in ammonium acetate (AmAc), pH 4.0	Sample and system suitability preparation
Beta-galactosidase stock solution, 1 μ M	Test sample
(Optional) PepCalMix	Test sample
Peptide test sample	Optimize electrospray ionization (ESI) voltage

Prepare the 10% HAc Solution

Use this solution as the background electrolyte (BGE). Prepare new 10% HAc solution every day.

1. In a clean 20 mL glass vial, add 18 mL of CE Grade Water.
2. Inside a fume hood, add 2 mL of HAc to the vial.
3. Invert the vial three times to mix the contents.
4. Keep the 10% HAc solution at room temperature.
5. At the end of the day, discard the solution that was not used.

Prepare the 20% HAc Solution

Only prepare new 20% HAc solution when the 200 mM LE buffer, pH 4.0 is prepared.

1. In a 100 mL glass bottle, add 80 mL of CE Grade Water.

Prepare the Reagents

2. Inside a fume hood, add 20 mL of HAc to the bottle.
3. Invert the bottle three times to mix the contents.
4. Attach a label with the name 20% HAc solution and the preparation date to the bottle.
5. Keep the 20% HAc solution at room temperature.
6. At the end of the day, discard the solution that was not used.

Prepare the 200 mM LE Buffer, pH 4.0

1. To prepare 50 mL of 400 mM LE buffer, pH 4.0, do this:
 - a. In a 50 mL glass volumetric flask, add 20 mL of CE Grade Water.
 - b. Inside a fume hood, add 2.7 mL of 7.5 M AmAc to the flask.
 - c. To increase the volume to 50 mL, add CE Grade Water.
 - d. Invert the flask three times to mix the contents.
2. Pour the 50 mL of 400 mM AmAc solution into a 100 mL beaker.
3. Use a calibrated pH meter to measure the initial pH of the solution.
4. Add aliquots of newly prepared 20% HAc solution until the pH of the solution is 4.0.
5. Transfer the solution to a 100 mL volumetric flask.
6. To increase the volume to 100 mL, add CE Grade Water.
7. Invert the flask three times to mix the contents.
8. Attach a label with the name 200 mM LE buffer, pH 4.0 and the preparation date to the flask.
9. When it is not in use, keep the buffer at 2 °C to 8 °C for as long as 2 years after preparation.

Prepare the Beta-Galactosidase Stock Solution



WARNING! Toxic Chemical Hazard. When handling, storing, and disposing of chemicals, refer to the chemical product safety data sheets. Obey all of the recommended safety procedures. For health and safety precautions, refer to the document: *Overview Guide*.

1. Add 625 µL of CE Grade Water to the vial with beta-galactosidase stock solution.
2. Mix the solution in a vortex mixer for at least 30 seconds.
3. Use a centrifuge to spin the vial and move the liquid to the bottom.
4. Divide the beta-galactosidase stock solution into aliquots of 50 µL.
5. Keep the beta-galactosidase stock solution at –35 °C to –15 °C for as long as 1 year.

Prepare the Beta-Galactosidase Test Sample

1. If required, then remove 50 μL of beta-galactosidase stock solution from the freezer and let it thaw at room temperature.
2. In a 0.5 mL centrifuge tube, add 50 μL of beta-galactosidase stock solution and 50 μL of 1 μM 200 mM LE buffer, pH 4.0 to make a 1:1 solution.
3. Mix the solution in a vortex mixer for at least 30 seconds.
4. To remove any precipitant, use a centrifuge to spin the solution at 12,000 g for 5 minutes.
5. Use a pipette to put 90 μL of the supernatant in a sample vial.
Keep the sample at 2 $^{\circ}\text{C}$ to 8 $^{\circ}\text{C}$ for as many as 3 days.

Prepare the Peptide Test Sample

1. In a centrifuge tube, add 100 μL of BGE.
2. Add 5 μL of a peptide marker, such as pl 9.5.
3. Put the cap on the tube.
4. Mix the solution in a vortex mixer for 3 seconds.
5. Keep the sample at 2 $^{\circ}\text{C}$ to 8 $^{\circ}\text{C}$.
6. At the end of the day, discard the solution that was not used.

Prepare the Buffer Trays

6

1. Use the following table to prepare the required reagents.

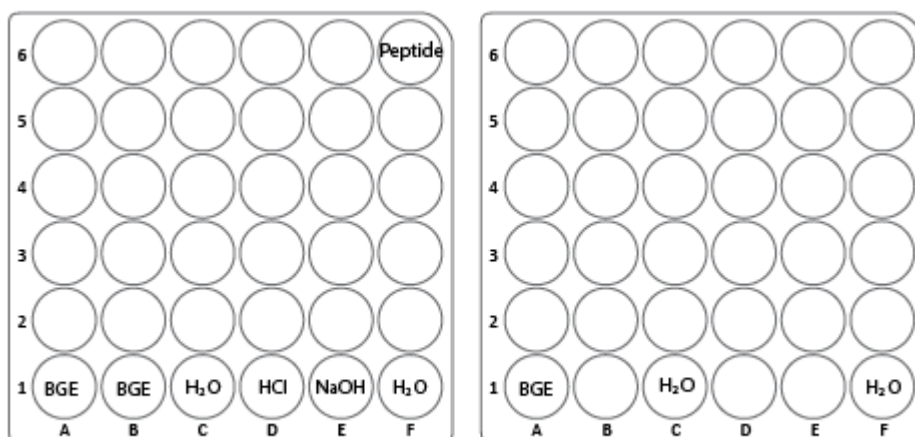
Table 6-1 Required Reagents

Content	Quantity	Volume
BGE (such as 10% HAc solution or 50 mM AmAc buffer, pH 3.0)	3 vials	1.5 mL
Peptide test sample	1 vial	1.5 mL
CE Grade Water	4 vials	1.5 mL
0.1 M NaOH	1 vial	1.5 mL
0.1 M HCl	1 vial	1.5 mL

2. Use the following figure to put each reagent vial in the correct position in the buffer inlet and outlet trays.

Note: This layout is the same for all five methods in the CESI 8000 Plus software.

Figure 6-1 Buffer Tray Layout



Prepare the System

7

This section describes how to verify that the CESI 8000 Plus system and the mass spectrometer can communicate.



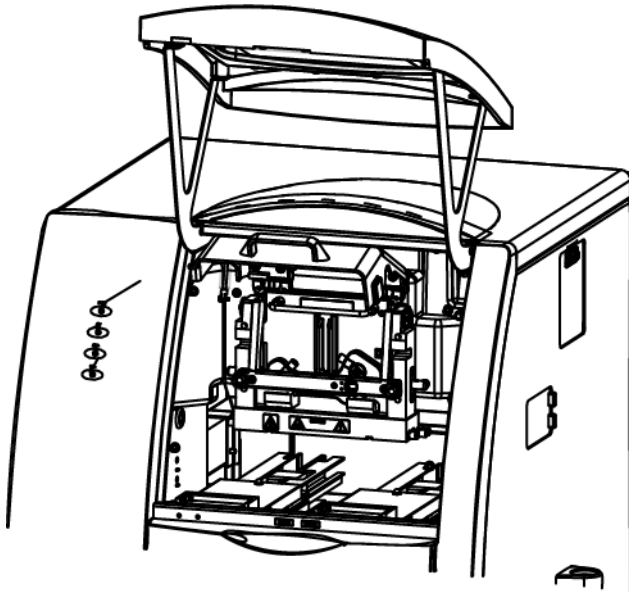
WARNING! Electrical Shock Hazard. Do not touch the adapter when it is connected to the CESI 8000 Plus system and the separation voltage is on.

Install the Cartridge

Before a cartridge can be installed, the interface plate must be installed on the CESI 8000 Plus system. Refer to the section: [Remove and Install the Interface Plate](#).

1. Open the cartridge cover.

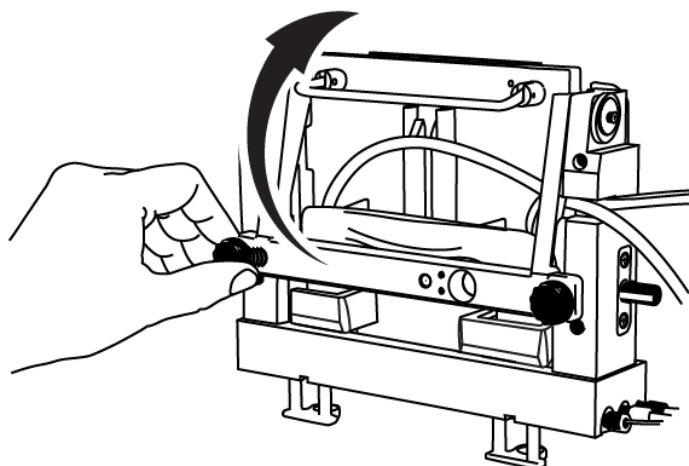
Figure 7-1 Cartridge Cover



2. Loosen the thumbscrews on the insertion bar, and then lift the insertion bar.

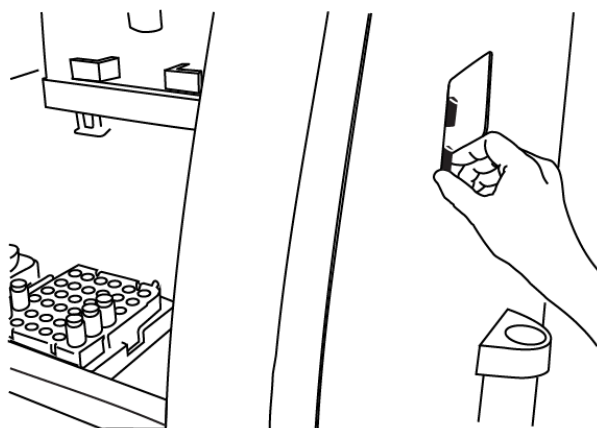
Prepare the System

Figure 7-2 Insertion Bar



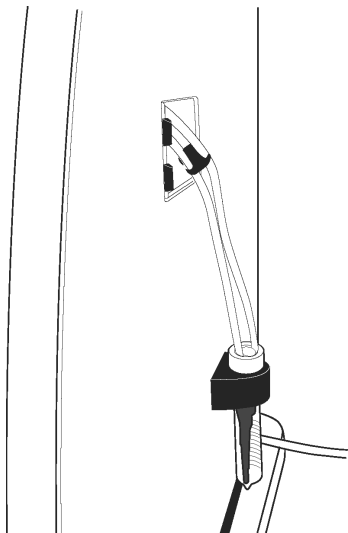
3. Open the access panel.

Figure 7-3 Access Panel



4. Put the sprayer housing through the side panel, and then put the sprayer in the holster.

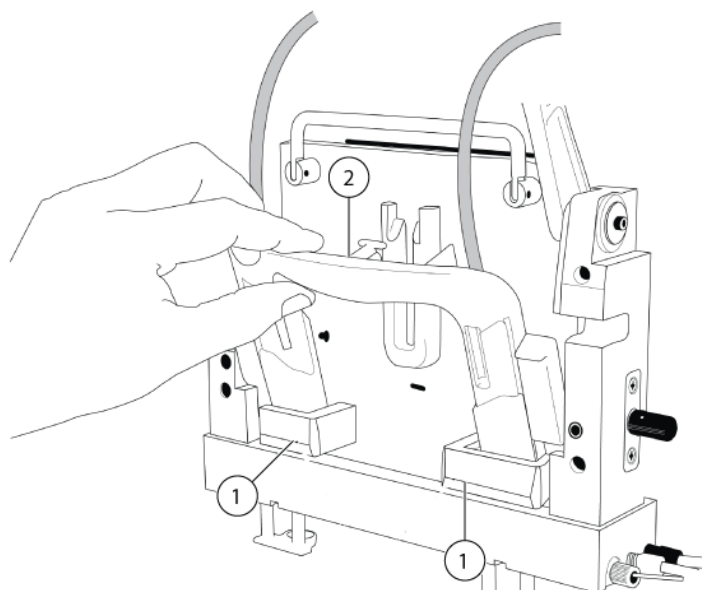
Figure 7-4 Sprayer in the Holster



Note: To prevent damage, make sure that the protective sleeve stays on the sprayer tip. Do not let the sprayer tip touch any surfaces.

5. To install the cartridge body, put the ends of the cartridge on the aligning blocks of the interface plate at a 45-degree angle, and lightly push the cartridge in.

Figure 7-5 Aligning Blocks

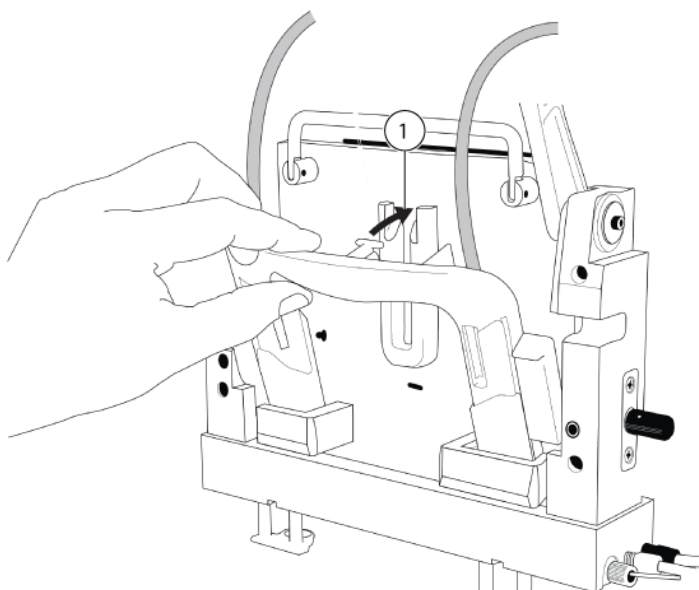


Prepare the System

Item	Description
1	Aligning blocks
2	T slider

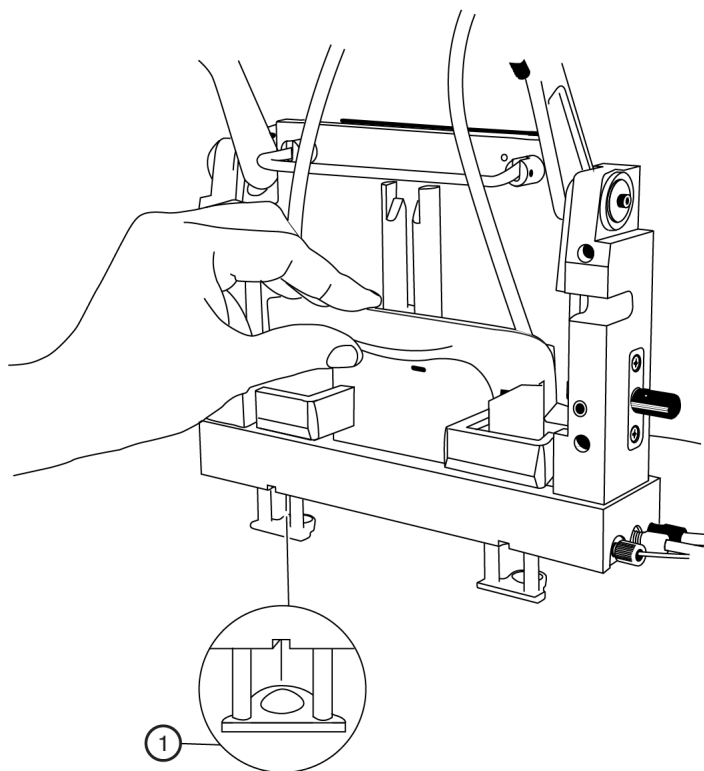
6. Turn the cartridge up until it is parallel with the interface plate.
7. Put the upper handles in the position shown in the figures: [Figure 7-6](#) and [Figure 7-7](#).
8. Align the T slider in the groove of the interface plate and move the cartridge down. As the cartridge moves down, the covers on the inlet and outlet sides retract and the capillaries can be seen below the interface block within the ejectors.

Figure 7-6 T Slider



Item	Description
1	Align the T slider in the groove

Figure 7-7 Capillaries Below the Interface Block

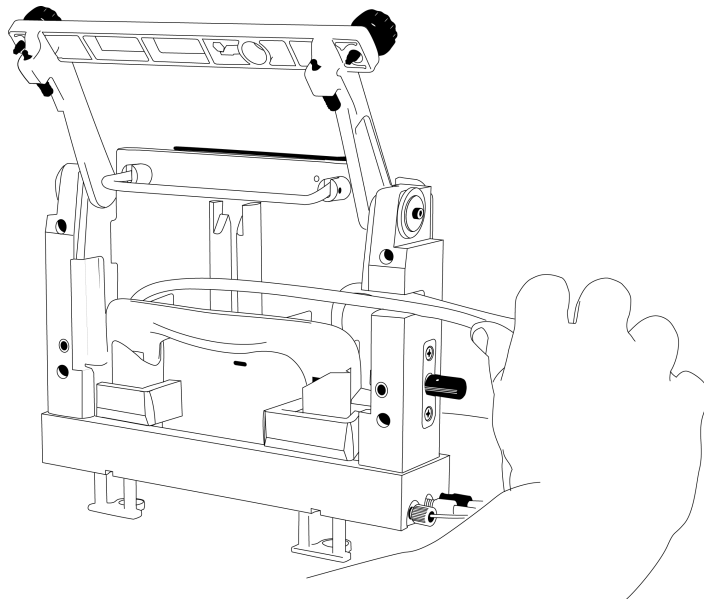


Item	Description
1	Capillaries can be seen below the interface block within the ejectors

9. Put the coolant and capillary tubing through the notched arm.

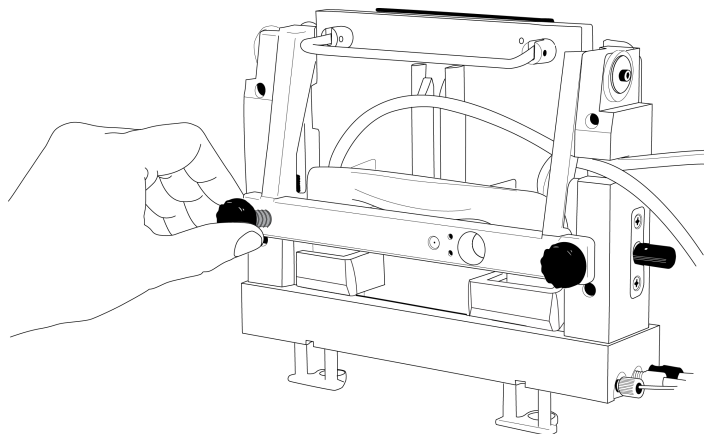
Prepare the System

Figure 7-8 Coolant and Capillary Tubing



10. Lower the insertion bar, and then tighten the thumbscrews.

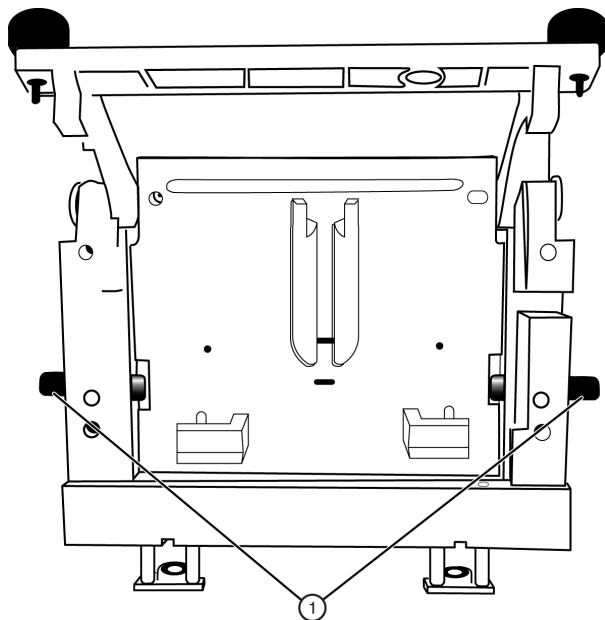
Figure 7-9 Thumbscrews on the Insertion Bar



Remove and Install the Interface Plate

1. To remove the interface plate, do this:
 - a. Remove the cartridge. Refer to the section: [Remove the Cartridge](#).
 - b. Turn off the power to the CESI 8000 Plus system.
 - c. Loosen the thumbscrews on the sides of the interface plate.

Figure 7-10 Thumbscrews on the Interface Plate

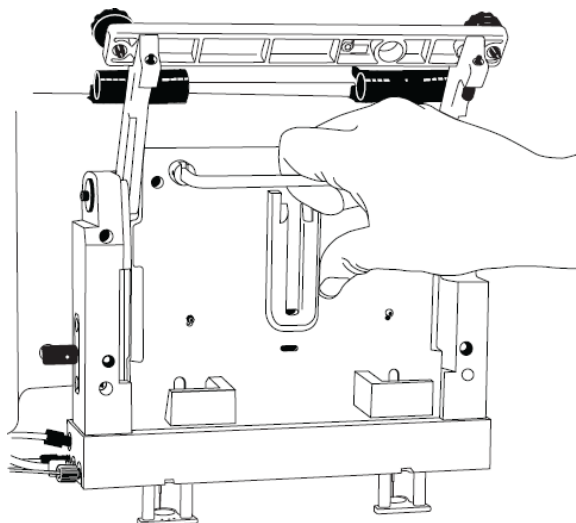


Item	Description
1	Thumbscrews

- d. Hold the handle on the top of the interface plate and pull it out.
 - e. Keep the interface plate in a safe place.
2. To install the interface plate, do this:
- a. Hold the handle on the top of the interface plate and install it. Make sure that the interface plate is flush with the mounting surface of the CESI 8000 Plus system.

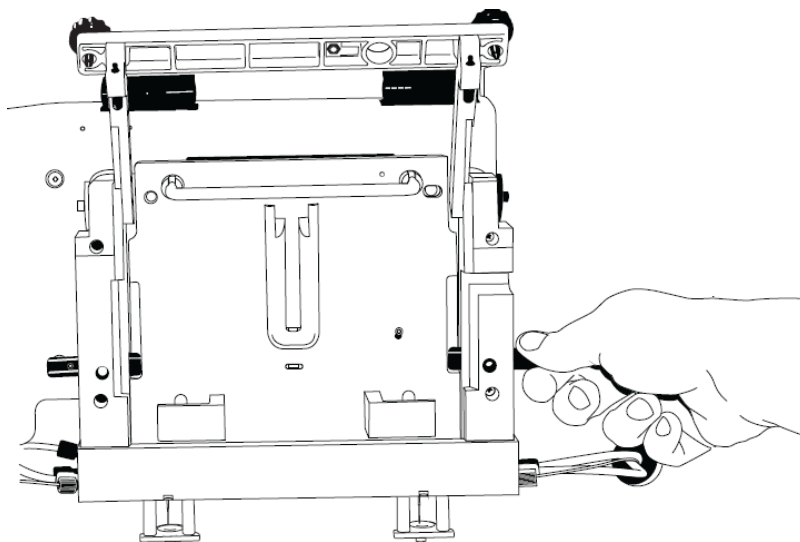
Prepare the System

Figure 7-11 Install the Interface Plate



- b. Tighten both thumbscrews.

Figure 7-12 Tighten the Thumbscrews



Condition the Capillaries

Condition the capillaries before a new cartridge is used for the first time and before a cartridge that has been in storage is used again.

Use the CESI-MS conditioning method to rehydrate the coating of the bare fused-silica capillaries and to establish the electrical connection in the sprayer tip.

1. Remove the protective sleeve from the sprayer tip.
2. To make sure that the flow of liquid through the separation capillary is sufficient, do this:
 - a. Do a forward rinse with CE Grade Water at 100 psi for 3 minutes.
 - b. When a liquid droplet shows at the end of the sprayer tip, stop the application of pressure.
3. To make sure that the flow of liquid through the conductive liquid capillary is sufficient, do this:
 - a. Do a reverse rinse with CE Grade Water at 100 psi for 5 minutes.
 - b. When a liquid droplet shows at the end of the stainless steel needle, stop the application of pressure.
4. Install the protective sleeve on the sprayer.
5. Put 10 mL of MeOH in a 50 mL Falcon tube, and then put the Falcon tube in the holster on the side of the system.

CAUTION: Potential System Damage. Do not put more than 10 mL of MeOH in the Falcon tube. If there is more than 10 mL in the tube, then the liquid can splash onto the metal components of the sprayer and cause damage.

6. Carefully immerse the sprayer tip in the Falcon tube.

Figure 7-13 Sprayer Immersed in Liquid

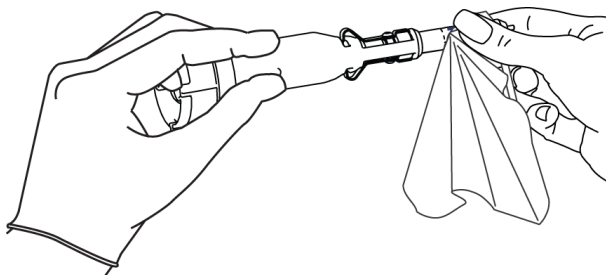


7. Run the CESI-MS conditioning method.

Prepare the System

- When the method is complete, remove the sprayer from the Falcon tube.
- Use lint-free wipes to dry the sprayer carefully.

Figure 7-14 Dry the Sprayer



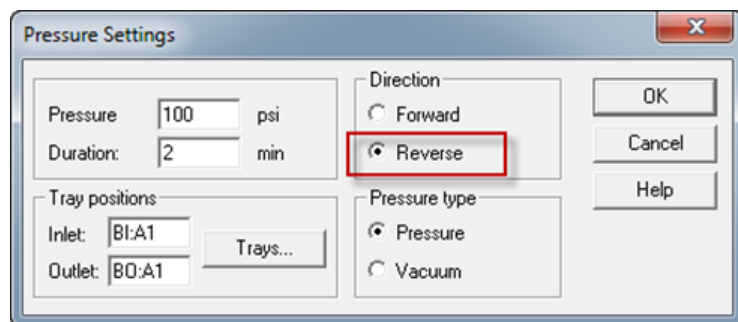
CAUTION: Potential System Damage. To prevent the sprayer tip from breaking, do not move the retractable protective guard during drying.

- Make sure that the ESI voltage on the mass spectrometer is off.
- Install the sprayer in the adapter on the mass spectrometer. The cartridge is ready to set a stable spray.
- Remove the Falcon tube from the holster, and discard the contents.

Establish a Stable Spray

- Make sure that the sprayer tip is in the correct position for the inlet on the mass spectrometer.
- In the Xcalibur software, make sure that the ESI voltage is zero.
- To fill the conductive liquid capillary with BGE, do this:
 - In the 32 Karat software, go to the Direct Control window.
 - Click the **Pressure** field.

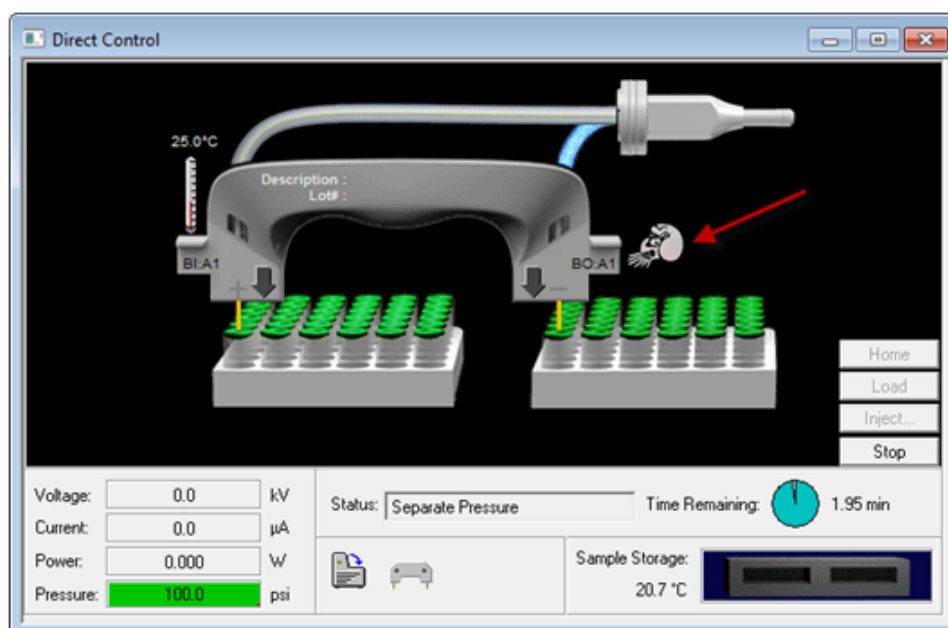
Figure 7-15 Pressure Settings Dialog to Fill the Conductive Liquid Capillary



- c. In the **Pressure** field, type 100.
- d. In the **Duration** field, type 2.
- e. Click **Reverse**.
- f. Click **Pressure**.
- g. Click **OK**.

To identify the direction of the pressure being applied, in the Direct Control window, look at the **Rinse Direction** icon (a blowing face identified by a red arrow in the following figure). The reverse direction refers to rinsing the conductive liquid capillary, and the blowing face shows on the right side of the window.

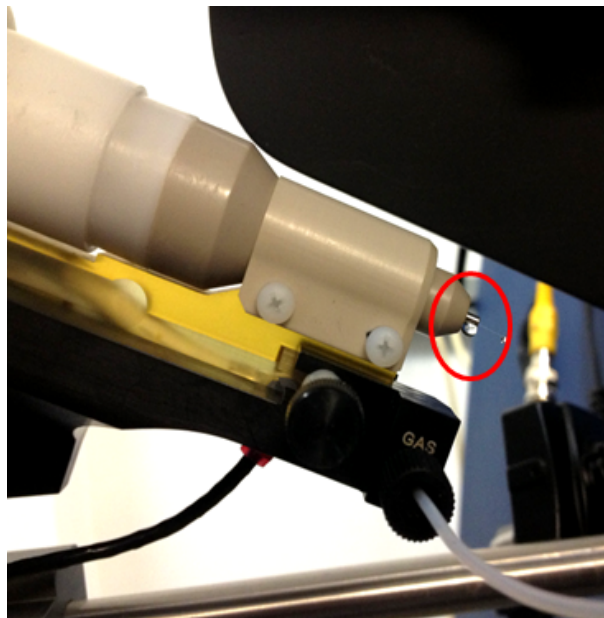
Figure 7-16 Rinse Direction (Blowing Face) Icon



When the conductive liquid capillary is filled with BGE, a droplet will show at the end of the stainless steel needle.

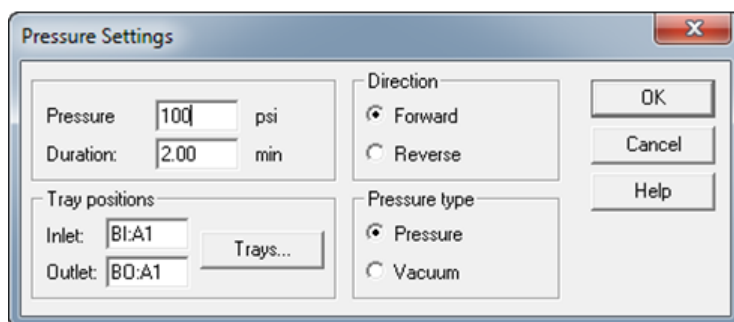
Prepare the System

Figure 7-17 Droplet at the End of the Stainless Steel Needle



4. When the rinse is complete, to fill the separation capillary with BGE, do this:
 - a. Go to the Direct Control window.
 - b. Click the **Rinse** field.

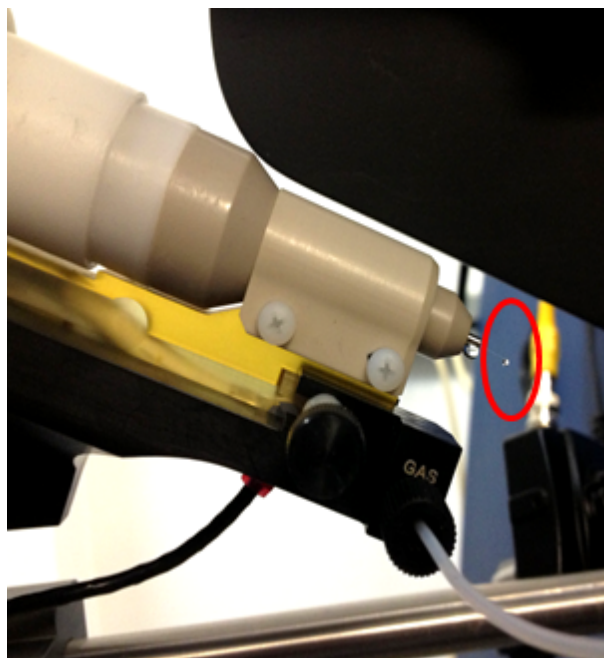
Figure 7-18 Pressure Settings Dialog to Fill the Separation Capillary



- c. In the **Pressure** field, type 100.
- d. In the **Duration** field, type 2.
- e. Click **Forward**.
- f. Click **Pressure**.
- g. Click **OK**.

When the separation capillary is filled with BGE, a droplet will show at the end of the sprayer tip.

Figure 7-19 Droplet at the End of the Sprayer Tip



Note: If the separation capillary is empty, then the first droplet of solution might not show at the sprayer tip for as long as 7 minutes.

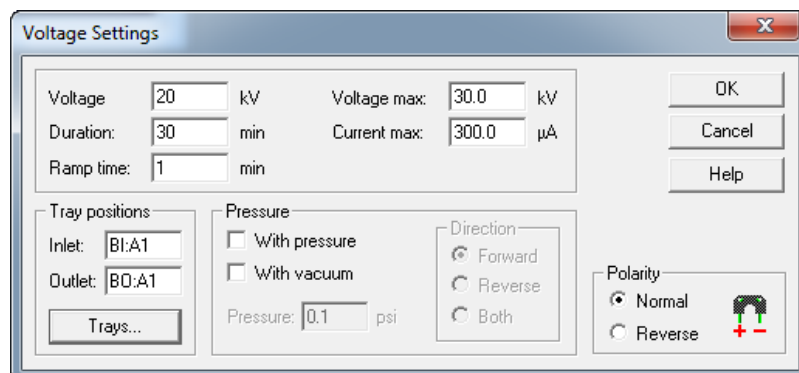
5. To find the minimum ESI voltage, refer to the section: [Optimize the ESI Voltage](#).

Optimize the ESI Voltage

1. Make sure that the separation capillary and conductive liquid capillary are filled with BGE.
2. To set the values for voltage in the 32 Karat software, do this:
 - a. Go to the Direct Control window, and then double-click the **Voltage** field.

Prepare the System

Figure 7-20 Voltage Settings Dialog



- b. In the **Voltage** field, do this:
 - For the silica surface OptiMS cartridge, type 20.
 - For the neutral OptiMS cartridge, type 30.
- c. In the **Duration** field, type 60.
- d. In the **Ramp time** field, type 1.
- e. Click **Normal**.
- f. Click **OK**.

Note: If the BGE used is 10% HAc solution, then the electrical current should be 2 µA to 3 µA. If the BGE used is 50 mM AmAc buffer, pH 3.0, then the electrical current should be 0.5 µA to 1 µA.

3. In the Analyst TF software, in the Navigation bar, under Tune and Calibrate, double-click **Manual Tuning**.
4. On the Source/Gas tab, do this:
 - a. In the **Curtain Gas (CUR)** field, type 10.

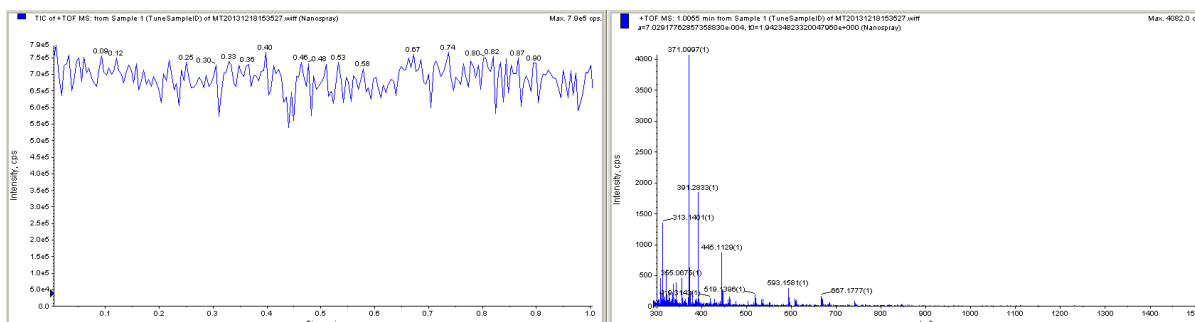
Note: In the Analyst TF software, the minimum value for curtain gas is 10. CESI-MS runs use a value of 5 for curtain gas. If the mass spectrometer is connected to the CESI 8000 Plus system, then the factory setting of 5 for curtain gas is applied.

- b. In the **IonSpray Voltage Floating (ISVF)** field, type 1.0.
The **IonSpray Voltage Floating (ISVF)** parameter sets the ESI voltage.
 - c. In the **Interface Heater Temperature (IHT)** field, type 50.0.
5. On the MS tab, do this:
 - a. In the **Scan type** field, select **TOF MS**.

- b. In the **Accumulation time** field, type 500.
 - c. In the TOF Masses (Da) **Min** field, type 200.
 - d. In the TOF Masses (Da) **Max** field, type 2000.
 - e. In the Period **Duration** field, type 10.
6. Click **Start**.
7. If a non-SCIEX mass spectrometer is in use, then do this on the mass spectrometer acquisition computer:
- a. Set the ESI voltage to zero.
 - b. Set the scan range from 200 m/z to 2000 m/z .
 - c. Click **Start**.
8. Increase the value in the **IonSpray Voltage Floating (ISVF)** field in increments of 0.1 kV until a continuous signal shows in the mass spectrum window.

Note: This is the minimum ESI voltage.

Figure 7-21 Typical Spray Profile of a Cartridge that Uses 10% HAc Solution as the BGE



9. In the Direct Control window, click **Stop**.
10. On the Source/Gas tab, in the **IonSpray Voltage Floating (ISVF)** field, type 0.0, and then click **OK**.
Make sure that there is no spray or background mass spectrum.

Note: If there is still spray when **IonSpray Voltage Floating (ISVF)** is 0.0, then there might be an issue with the connection. Refer to the section: [Fine-Tune the Position of the Sprayer Tip](#).

11. In the **IonSpray Voltage Floating (ISVF)** field, type the minimum ESI voltage, and then click **OK**.
Make sure that electrospray starts again.

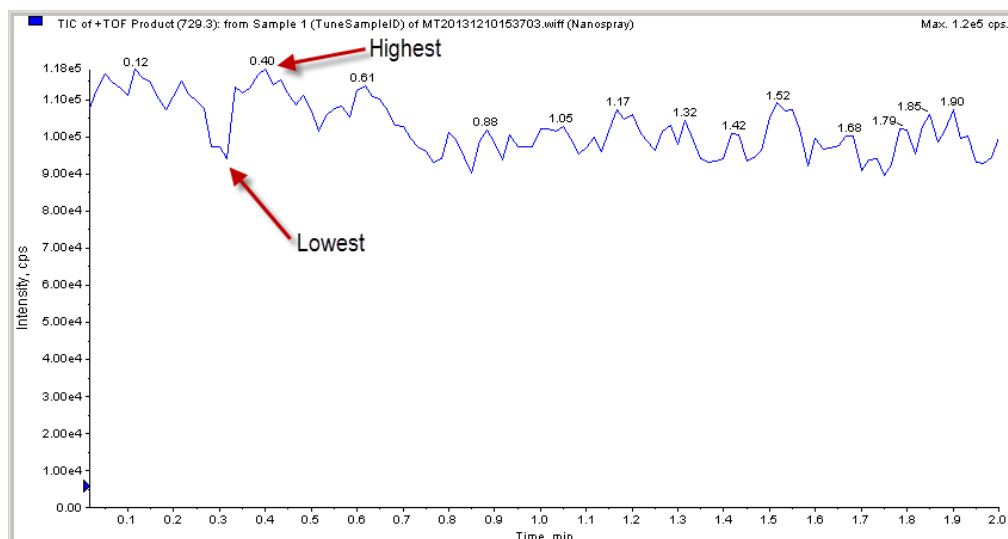
Prepare the System

12. In the **IonSpray Voltage Floating (ISVF)** field, increase the value by 100 V.
13. To find the best position for the sprayer in relation to the inlet on the mass spectrometer, use the XYZ-axes adjustment knobs to get a maximum total ion current signal.

Note: Keep the sprayer tip at least 2 mm away from the curtain gas plate.

14. To check the stability of the spray, continue to apply 20 kV (normal polarity) for 20 minutes.
15. In the Analyst TF software, do this:
 - a. Open the Advanced MS tab, and then click **MCA**.
 - b. Open the MS tab.
 - c. In the **Scan type** field, select **TOF MS**.
 - d. In the **Accumulation time** field, type 1.
 - e. In the TOF Masses (Da) **Min** field, type 70.
 - f. In the TOF Masses (Da) **Max** field, type 2000.
 - g. In the Period **Duration** field, type 5.
16. Click **Acquire**, and then type a file name (for example, *Baseline*).
17. If a non-SCIEX mass spectrometer is in use, then do this on the mass spectrometer acquisition computer:
 - a. Set the ESI voltage to 1.0 kV and continue to acquire data.
 - b. Increase the ESI voltage in increments of 0.1 kV until there is a continuous signal in the mass spectrum window.
 - c. To find the best position for the sprayer in relation to the inlet on the mass spectrometer, maximize the XIC (eXtracted Ion Electropherogram) signal for the pI 7.0 or pI 10 marker in use while minimizing its fluctuations.
 - d. When the position of the sprayer is optimized, decrease the ESI voltage in increments of 0.1 kV until the spray stops.
 - e. Increase the ESI voltage in increments of 0.1 kV until continuous spray is detected.
 - f. Record this value as the minimum ESI voltage.
 - g. Increase the minimum ESI voltage by 0.2 kV.
18. Use the Y-axis alignment knob to maximize the baseline fluctuations.

Figure 7-22 Find the Baseline Fluctuation



- a. If the baseline fluctuation is < 40% within 2 minutes to 5 minutes, then make a note of the ESI voltage for the mass spectrometer methods (ion collection ESI voltage):

$$\text{Baseline fluctuation (\%)} = \frac{[\text{highest value} - \text{average value}]/\text{average value}}{\text{average value}} \times 100.$$
 - b. If the baseline fluctuation is > 40%, then do this procedure again until a good baseline is set. If a good baseline cannot be set, then refer to the section: [Fine-Tune the Position of the Sprayer Tip](#).
19. If a non-SCIEX mass spectrometer is in use, then do this:
- a. Monitor the spray stability for 20 minutes to 30 minutes to make sure that the baseline fluctuation is $\leq 40\%$.
 If the baseline fluctuation is > 40%, then condition the capillaries. Refer to the section: [Condition the Capillaries](#).
 - b. Turn off the electrospray voltage on the mass spectrometer.

About Tune Files

Make sure that the mass spectrometer system is calibrated correctly before a test separation is done.

Note: The customer must calibrate the mass spectrometer. Refer to the manufacturer's documentation for calibration procedures and the recommended performance frequency.

As a general guideline, any known peptide can be used as a tuning mix. For metabolomic applications, use a metabolite-type compound to generate the tuning. Make sure that the tune mix buffer is in 10% acetic acid.

Prepare the System

Two tune files must be created. The first tune file is set with a spray voltage of 0 kV. The second tune file is set with a spray voltage that has been found to supply a stable spray. Refer to the sections: [Establish a Stable Spray](#) and [Optimize the ESI Voltage](#).

Both tune files are used to do the test separation. The tune file with 0 kV is used for 1 minute, which relates to the interval when the CE voltage gradually increases to 20 kV. When the separation voltage gets to the applicable magnitude of 20 kV, the second tune file with the optimal spray voltage is applied. This is to make sure that the correct voltage is applied during the sample separation.

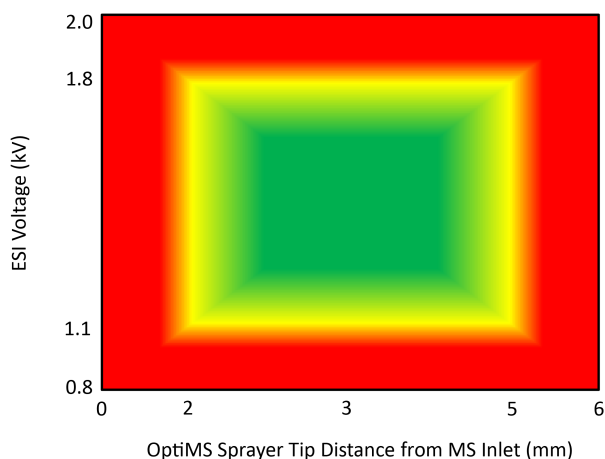
When the tune files have been acquired and saved successfully, continue with the section: [Do a Test Separation](#).

Fine-Tune the Position of the Sprayer Tip

CAUTION: Potential System Damage. Do not move the sprayer tip too close to the inlet on the mass spectrometer. If the sprayer tip is < 2 mm from the inlet, then the accidental suction of rinse solution into the mass spectrometer can occur and cause damage.

Before a separation is run, it is critical to optimize the position of the sprayer tip in front of the inlet on the mass spectrometer to get the correct ESI voltage. If the sprayer tip is too far away from the inlet on the mass spectrometer, then a high ESI voltage can be required, which causes analyte fragmentation. For intact proteins, electrochemical reactions such as oxidation can occur. The green area in the following figure shows the recommended distance of the sprayer tip and the recommended ESI voltage values. Avoid the red area.

Figure 7-23 Sprayer Tip Distance and ESI Voltage Correlation



Use this procedure to adjust the position of the sprayer tip and find the required ESI voltage for mass spectrometer acquisition:

- After installing a cartridge

- If the signal separation voltage is changed
- If a different BGE is used

For troubleshooting information, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

1. Fill the separation capillary and the conductive liquid capillary with BGE.
2. Put the sprayer tip 2 mm to 5 mm from the inlet on the mass spectrometer.
3. In the 32 Karat software, use the Direct Control window to apply 20 kV voltage.
 - Make sure that the cartridge does not spray when the ESI voltage is 0 V during mass spectrometer scanning.
 - Monitor the Direct Control window to make sure that the electrical current is stable.
4. Set the ESI voltage to 0.5 kV, and then increase the value in increments of 0.1 kV until electrospray is detected.
5. Increase the ESI voltage by 0.2 kV.
6. Use the XYZ-axes adjustment knobs to move the sprayer tip and maximize the signal intensity of the mass spectrometer while minimizing the ion trap time.
Make sure that the sprayer tip is approximately 3 mm from the inlet on the mass spectrometer.
7. When the position of the sprayer is optimized, set the ESI voltage to zero.
8. Set the ESI voltage to 0.5 kV, and then increase the value in increments of 0.1 kV until electrospray is detected.

Note: This is the minimum ESI voltage. This voltage is not high enough to maintain an effective spray during separation.

9. Increase the minimum ESI voltage by 0.2 kV.

Note: This is the optimal ESI voltage. Use this voltage with the mass spectrometer method to supply a stable electrospray during separation.

10. Set the ESI voltage to zero.
11. Turn off the separation voltage.

Do a Test Separation

8

This section gives the instructions to do a test separation with beta-galactosidase test sample to make sure that the CESI 8000 Plus system and the mass spectrometer operate correctly.

As an alternative, PepCalMix can be used as the test sample. PepCalMix has 20 different stable isotope-labeled peptides and is available from SCIEX. For instructions about how to use PepCalMix, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

Note: Before a test separation is run, make sure that a stable spray has been established and the optimum ESI voltage has been set. Refer to the section: [Fine-Tune the Position of the Sprayer Tip](#).

Create the Mass Spectrometer Method

Use the Xcalibur software to create an MS method with two segments.

1. Create one method with two segments.
 - The following figure shows the first segment of the tune method that was created when the mass spectrometer was tuned, with a duration of 1 minute and an ESI voltage set to zero.

Figure 8-1 First Segment of a Method Used to Run Beta-Galactosidase Test Sample

MS Detector Setup | Mass Lists | Syringe Pump | Divert Valve | Contact Closure | Summary

Run settings
 Acquire time (min): 45.00 Segments: 2 Start delay (min): 0.00

To display a chromatogram here, use LTQ XL/Open raw file...

Segment 2

Retention time (min)

Segment 1 settings
 Segment time (min): 1.00 Scan events: 1 Tune method: C:\Xcalibur\methods\Beta gal_20kV_zeroESI.LTQTu

Scan Event 1

Scan event 1 settings

Scan Description

Mass Range: Normal
 Scan Rate: Normal
 Scan Type: Full
 Polarity: Positive
 Data type: Profile

Source Fragmentation

Qn Energy (V): 35.0
 Dependent scan Settings...

FAIMS

CY (V): 0.00

MSn Settings

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	35.0	0.250	30.000

Scan Ranges

#	First Mass (m/z)	Last Mass (m/z)
1	150.00	1200.00

Input: From/To

Supplemental Activation
 Wideband Activation SA Charge State: 2
 Use MS/MS Mass List APCI Corona On APPI Lamp On

- The following figure shows the second segment of the tune method, with a duration of 44 minutes and an the optimum ESI voltage set for a stable spray.

Do a Test Separation

Figure 8-2 Second Segment of the Method Used to Run Beta-Galactosidase Test Sample

The screenshot displays the MS Detector Setup software interface with the following configuration:

- Run settings:** Acquire time (min): 45.00, Segments: 2, Start delay (min): 0.00.
- Segment 2 settings:** Segment time (min): 44.00, Scan events: 1, Tune method: C:\Xcalibur\methods\Beta gal_20kV_wESI.LTQTune.
- Scan event 1 settings:**
 - Scan Description:** Mass Range: Normal, Scan Rate: Normal, Scan Type: Full, Polarity: Positive, Data type: Centroid.
 - Source Fragmentation:** Qn Energy (V): 35.0, Dependent scan: Settings...
 - FAIMS:** CV (V): 0.00
- MSn Settings Table:**

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	35.0	0.250	30.000
- Scan Ranges Table:**

#	First Mass (m/z)	Last Mass (m/z)
1	150.00	1200.00
- Other settings:** Supplemental Activation: ; Wideband Activation: ; SA Charge State: 2; Use MS/MS Mass List: ; APCI Corona On: ; APPI Lamp On: ; Input: From/To.

2. In the Scan Description section, from the **Data type** list, select **Profile**.
3. In the Scan Ranges section, in the **First Mass (m/z)** field, type 300.00, and in the **Last Mass (m/z)** field, type 1500.00.

Figure 8-3 Scan Event Settings

Scan event 1 settings

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: Full

Polarity: Positive

Data type: Centroid

MSn Settings

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	35.0	0.250	30.000

Scan Ranges

#	First Mass (m/z)	Last Mass (m/z)
1	300.00	1500.00

4. Save the method.

Note: We recommend that the temperature of the ion transfer capillary be set to 200 °C for the analysis of the beta-galactosidase stock solution test sample. If the sample is different, then the temperature might be different.

Create and Start a Sequence with the Xcalibur Software

Use the Xcalibur software to include the MS method in a sequence.

Note: Make sure that the system will go into standby mode when the sequence completes.

1. In the Xcalibur software, open the New Sequence Template window.

Do a Test Separation

Figure 8-4 New Sequence Template Window

New Sequence Template

General

Base File Name: BetaGal_test_sequence Starting Number: 1

Path: C:\Xcalibur\Data\ Browse...

Instrument Method: C:\Xcalibur\methods\BetaGal_test Browse...

Processing Method: Browse...

Calibration File: Browse...

Samples

Number of Samples: 1 Tray Type: [v]

Injections per Sample: 1 Initial Vial Position: [v] Re-Use Vial Positions

Base Sample ID: [v]

Bracket Type

None Open Non-Overlapped Overlapped

Calibration

Add Standards
Number of brackets: 1
Injections per Level: 1

Add Blanks

Fill in Sample ID for Standards

QC

Add QC's
 After First Calibration Only
 After Every Calibration

Add Blanks

Fill in Sample ID for QC's

OK Cancel Save As Default Help


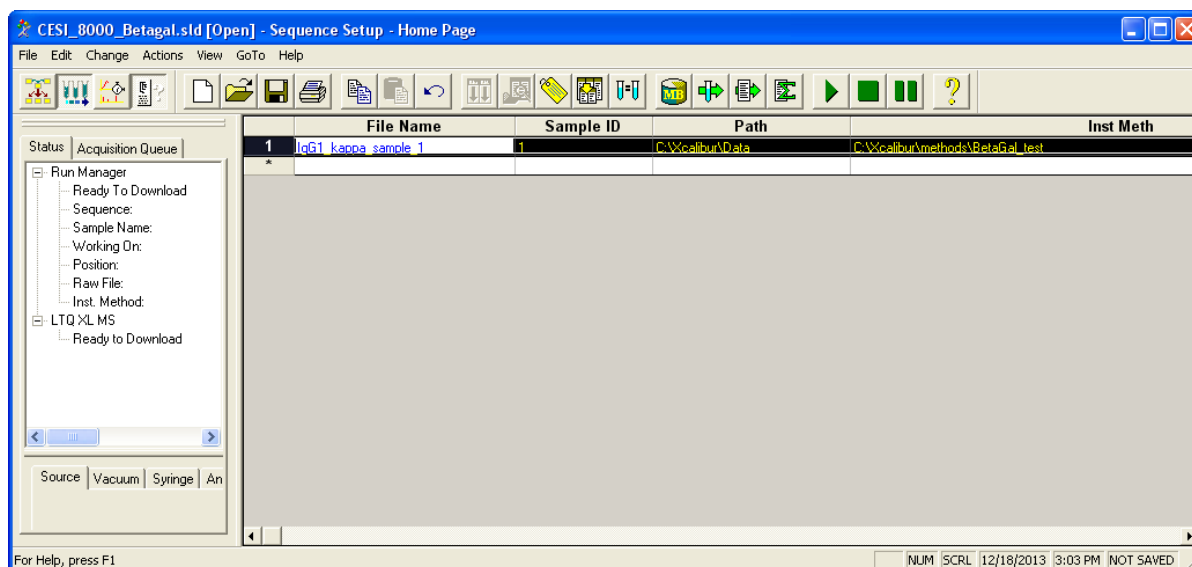
2. Click **OK**.
3. On the Sequence Setup-Home Page, highlight the row for the sample to run, and then click the **Sequence Run** icon .

Figure 8-5 Sequence Setup-Home Page



4. When the sequence finishes, in the Run Sequence dialog, set the instrument to standby, and then click **OK**. Otherwise, the spray voltage will stay on. The Acquisition Queue tab shows the sequence that is running and the sequence row being acquired. The Status tab shows the status of the sequence. As the sequence is downloaded and queued, the status will change from Ready to Downloading to Waiting for contact closure.
5. Load the sample and buffer trays in the CESI 8000 Plus system.

Note: Before starting a sequence on the CESI 8000 Plus system, make sure that the status on the Xcalibur software is Waiting for contact closure.

Manually Create the CESI-MS Separation Method

The CESI-MS Separation for Thermo MS.met method file is installed with the 32 Karat software 10.3 or later in the folder C:\32Karat\projects\CE MS\Method. If the method is missing, then use this procedure to create it manually.

1. In the 32 Karat software, click **File > Method > New**.
2. Use the initial conditions in the following figure.

Do a Test Separation

Figure 8-6 Initial Conditions for the CESI-MS Separation Method

3. Use the time program in the following figure.

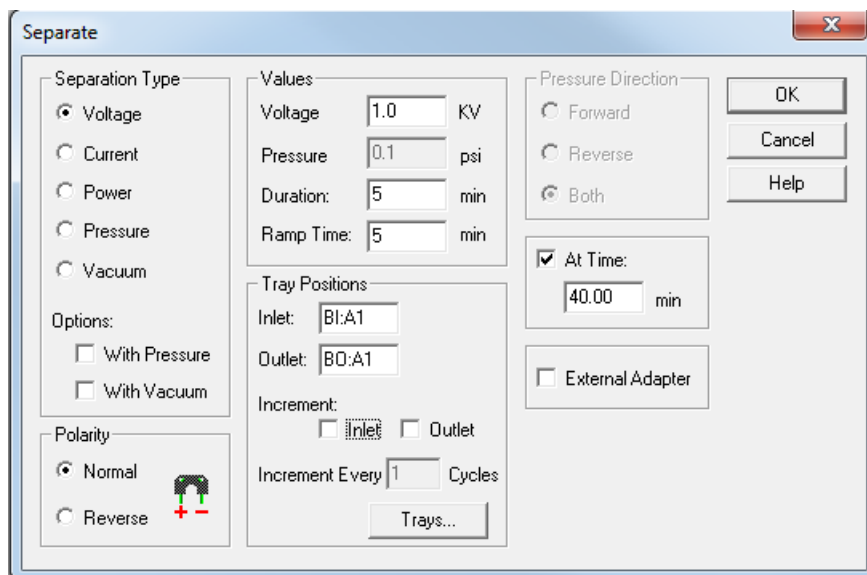
Figure 8-7 Time Program for the CESI-MS Separation Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	2.50 min	BI:E1	BO:A1	forward	0.1 N NaOH rinse
2		Rinse - Pressure	100.0 psi	2.50 min	BI:D1	BO:A1	forward	0.1 N HCl rinse
3		Rinse - Pressure	100.0 psi	4.00 min	BI:C1	BO:A1	forward	Water rinse
4		Rinse - Pressure	75.0 psi	3.00 min	BI:B1	BO:A1	reverse, In / Out vial inc 12	Conductive Liquid Capillary fill
5		Rinse - Pressure	100.0 psi	4.00 min	BI:B1	BO:A1	forward, In / Out vial inc 12	BGE fill
6		Inject - Pressure	5.0 psi	60.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic Injection
7		Inject - Pressure	0.5 psi	25.0 sec	BI:A1	BO:A1	No override, forward, In / Out vial inc 12	Push
8	0.00	Separate - Voltage	20.0 KV	45.00 min	BI:A1	BO:A1	1.00 Min ramp, normal polarity, In / Out vial inc 12	Separation
9	0.10	Relay On					1: 0.10 2: 0.10	
10	45.00	Separate - Voltage	1.0 KV	5.00 min	BI:A1	BO:A1	5.00 Min ramp, normal polarity, In / Out vial inc 12	Voltage Ramp Down Step
11	50.00	End						
12								

Note: For a new method, make sure that the value for the duration of the **Relay On** event is at least 0.05 minutes.

4. In row 10 of the sequence table, click the **Event** cell, then select **Separate**.
5. Set the parameters that show in the following figure, and then click **OK**.

Figure 8-8 Voltage Ramp Down



6. Click **File > Method > Save As**, and then save the method as `CESI-MS Separation for Thermo MS.met`.


Create a Sequence in the 32 Karat Software

1. In the 32 Karat software, click **File > Sequence > Sequence Wizard**.

Do a Test Separation

Figure 8-9 Sequence Wizard - Method Page

Sequence Wizard - Method

Method : 

Data File Type

For acquisition

From existing data files

Amount values

Sample amount :

Internal standard amount :

Multiplication factors :

Dilution factors :

? Cancel < Back Next > Finish





2. To the right of the **Method** field, click , and then select the CESI-MS separation method.
3. Click **Next**.

Figure 8-10 Sequence Wizard - Unknowns Page

Sequence Wizard - Unknowns

Sample ID : 

Data path : 

Data file : 

Number of unknown runs in sequence :

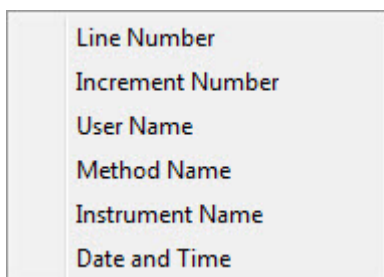
Repetitions per run :

Create a separate row in the sequence for each repetition

? Cancel < Back Next > Finish

4. In the **Sample ID** field, type a text string, and then click the blue arrow icon to the right of the **Sample ID** field.

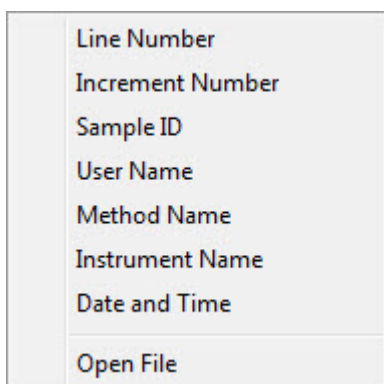
Figure 8-11 Sample ID Options Menu



If an option is selected from this menu, then a related parameter symbol is included in the **Sample ID** field. When the sequence is run, the parameter is added to the sample ID automatically. Any combination of parameters can be used.

5. Select the options to include in the sample ID automatically when the sequence is run. A symbol shows in the **Sample ID** field for the parameters selected.
6. To the right of the **Data path** field, click the green folder icon, and then select the file path to the data files.
7. In the **Data file** field, type a text string, and then click the blue arrow icon to the right of the **Data file** field.

Figure 8-12 Data File Options Menu



If an option is selected from this menu, then a related parameter code is included in the **Sample ID** field. When the sequence is run, the parameter is added to the data file name automatically.

Note: The **Open File** option is used to reprocess data files, not to acquire data.

8. Select the applicable options.

Do a Test Separation

A symbol shows in the **Data file** field for the parameters selected.

- In the **Number of unknown runs in sequence** field, type the number of sample runs. This number does not include autocalibration runs.
- In the **Repetitions per Run** field, type the number of times to run each line in the sequence. When this option is used, an identifier is added to the file name for each repetition automatically.
- Click **Next**.

Figure 8-13 Sequence Wizard - Vials Page

Sequence Wizard - Vials

32 Karat™
SCIEX

First unknown vials of sequence :

Inlet : BI:C2 Trays Advance

Outlet : BO:C1 Trays Advance

First calibration vials of sequence :

Inlet : BI:C2 Trays Advance

Outlet : BO:C1 Trays Advance

Injection duration: 10.0 sec

Advance direction: Row major Column major

? Cancel < Back Next > Finish

- If the **Allow Override** check box has been selected for the method, then, in the **Inlet** and **Outlet** fields, type the vials positions to use in the sequence table.

The **Allow Override** option is set in the Inject event. For more information, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

- To set the advance direction for the sequence, click **Advance direction**.

Note: The advance direction is different than vial incrementing. The advance direction helps to simplify the planning and organization of the sequence table. The values for the vial positions can be changed in the sequence table, and the values are not saved with any method. The values are saved in the sequence.

Note: If the **Advance** check box is selected for the inlet, outlet, or both vials, then the values for the vial positions are filled down automatically in the **Sample Inject Inlet/Outlet** columns in the sequence table.

- To fill down vial positions automatically and increase them by rows in the same tray (for example, A1, A2, A3, A4, A5, A6, B1, B2, B3, B4 for buffer tray configuration), click **Row major**.
- To fill down vial positions automatically and increase them by columns in the same tray (for example, A1, B1, C1, D1, E1, F1, A2, B2, C2, D2 for buffer tray configuration), click **Column major**.

A set of calibration vials can also be identified. For information about calibration through the sequence table, refer to the document: *CESI 8000 Software Online Help*.

14. Click **Next**.
15. Keep all of the check boxes on the Sequence Wizard - Reports page cleared.
None of the settings apply to CESI-MS.
16. Click **Finish**.

Figure 8-14 Sequence Table: Part A

Run #	Status	Run Type	Reps	Sample Inject Inlet	Sample Inject Outlet	Sample Inject Duration	Sample ID	Method	Filename
1	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal001.dat
2	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal002.dat
3	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal003.dat
4	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal004.dat
5	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal005.dat
6	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal006.dat
7	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal007.dat
8	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal008.dat
9	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal009.dat
10	Unknown	1	1	SI:A2	BO:A1	60.0	Betagal	CESI-MS Separation for AB Sciex MS.met	Betagal010.dat
11									
12									

Load the Sample

1. Put the reagents in the following table in CESI-MS vials.

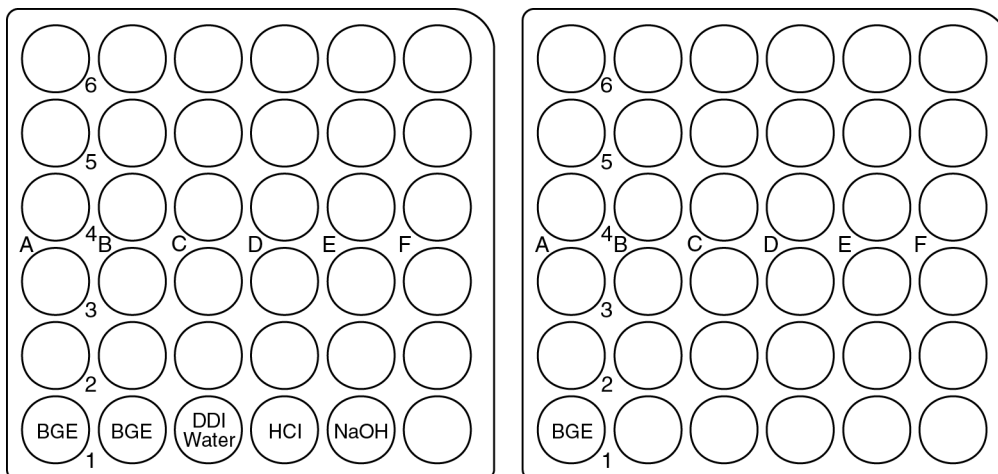
Table 8-1 Reagent Requirements

Content	Quantity	Volume
BGE	3 vials	1.5 mL
CE Grade Water	1 vial	1.5 mL
0.1 N NaOH	1 vial	1.5 mL
0.1 N HCl	1 vial	1.5 mL

2. Put the reagents in the following positions in the buffer tray.


Do a Test Separation

Figure 8-15 Buffer Inlet and Outlet Tray Layout for Sample Separation Method



3. Put the beta-galactosidase stock solution test sample in a microvial.
4. Put the microvial inside a CESI-MS vial, and then attach a cap.
5. Put the CESI-MS vial in position SI:A1 of the sample inlet tray.
6. In the 32 Karat software, go to the Direct Control window, and then click **Load**.
7. When the carriers have moved forward, open the front cover of the CESI 8000 Plus system and put the inlet and outlet buffer trays in the correct carrier.
8. Put the sample inlet tray in the back of the inlet carrier (left).

Start the Sequence in the 32 Karat Software

1. On the toolbar, click the **Sequence Run** icon .
2. Make sure that the value in the **Sequence name** field is correct, or click the yellow folder icon to browse to the correct file.
3. Click **Start** to start the sequence.

When the method starts, the status in the Queue Server on the mass spectrometer changes from *Ready* to *PreRun*.

When the signal is sent from the 32 Karat software to the mass spectrometer, the status in the Queue Server changes from *PreRun* to *Acquiring*.

As the batch run continues, a green check mark shows for each run that is acquired successfully and the run status changes to *Acquired*.

4. To see the status of the first autocalibration run, monitor the Bgal_Installation sequence. The first autocalibration run in the following figure is being acquired.

Figure 8-16 Monitor the Sequence Run

Run #	Status	Run Type	Reps	Sample Inject Inlet	Sample Inject Outlet	Sample Inject Duration	Sample ID	Method	Filename
1	Acquiring	Unknown	1					CESI-MS Auto-calibration_ABSciex.met	
2		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal001.dat
3		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal002.dat
4		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal003.dat
5		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal004.dat
6		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal005.dat
7		Unknown	1					CESI-MS Auto-calibration_ABSciex.met	
8		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal006.dat
9		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal007.dat
10		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal008.dat
11		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal009.dat
12		Unknown	1	SI:A2	BD:A1	60.0	Betagal	CESI-MS Separation for AB Scieux MS.met	Betagal010.dat
13									

Analyze the Data

Use the Xcalibur software to analyze the data from the test run.

Efficient system performance can be gauged by the identification and clear separation of the beta-galactosidase peaks 714.8469 m/z and 729.3652 m/z. The following table shows the mass assignment for the two fragments of interest.

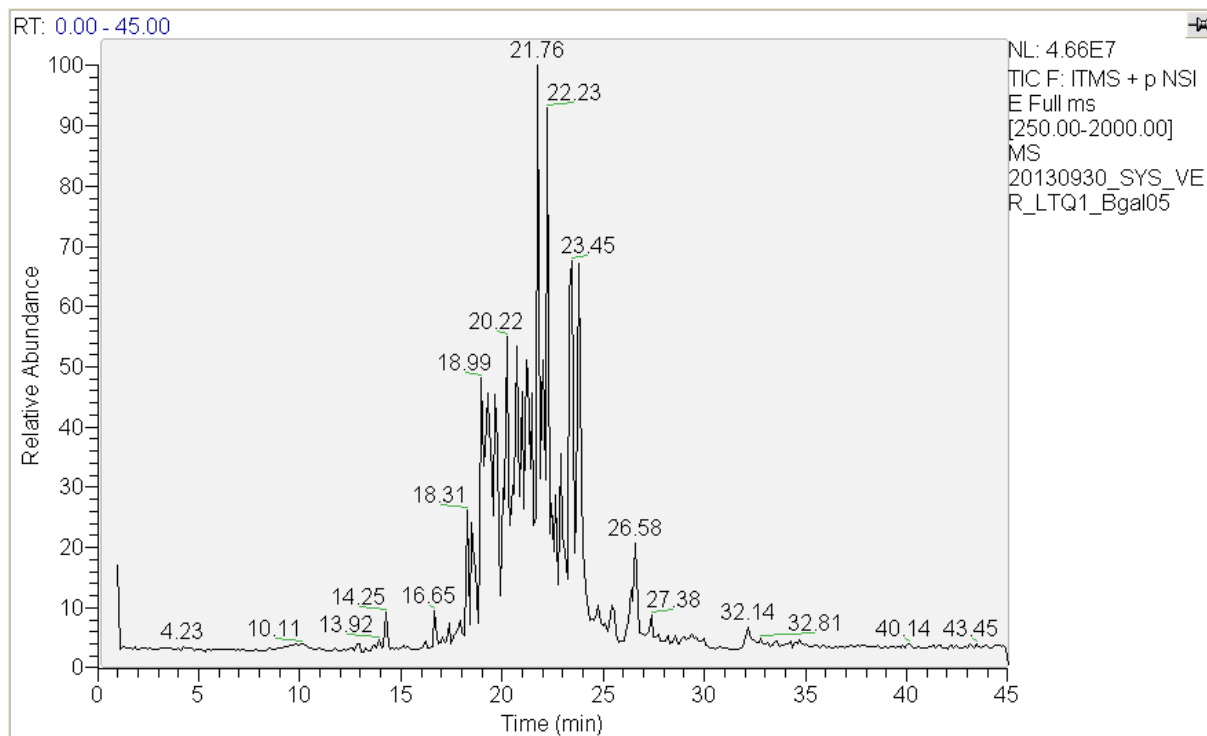
Table 8-2 Beta-Galactosidase 729 m/z and 714 m/z Mass Assignment

m/z	Peptide Sequence	Monoisotopic Molecular Weight (Da)	Charge State
714	DWENPGVTQLNR	1427.6793	2
729	APLDNDIGVSEATR	1456.7158	2

1. Click **File > Open**, and then browse to the location where the beta-galactosidase data file was saved.
2. Click **Open**.

Do a Test Separation

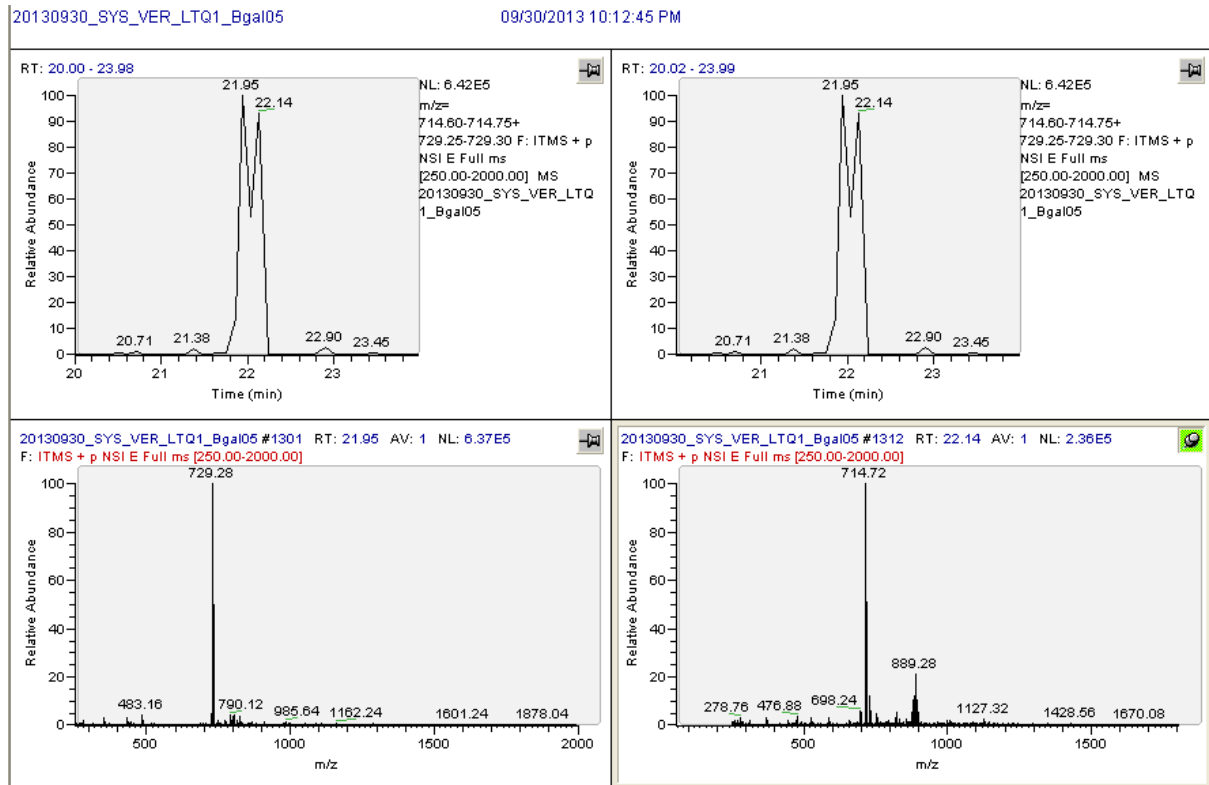
Figure 8-17 Example of a Typical Beta-Galactosidase TIC



3. Change the view to Base Peak Chromatogram.
4. Configure the base peak chromatogram to see peaks 714 and 729.
5. Apply the following settings to the base peak chromatogram:
 - Set the peak ranges to 714.5 to 714.7 and 729.2 to 729.4.

Note: If required, then use the mass spectrometer mass calibration to adjust the peak ranges 714.5 to 714.7 and 729.2 to 729.4.

- Set automatic processing to include smoothing with a smoothing value of 5.

Figure 8-18 Identification and Clear Separation of the Beta-Galactosidase 714 and 729 m/z Fragments

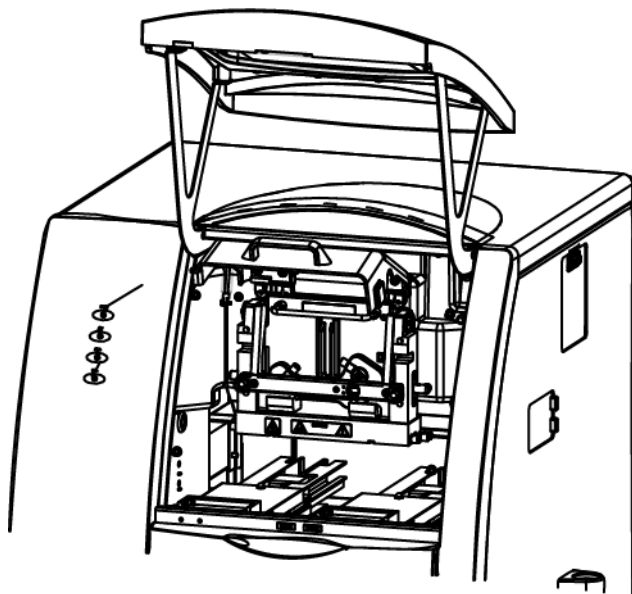
6. Calculate the migration time of each peak.
7. Make sure that the two peaks are separated by at least 0.05 minutes.

Remove the Cartridge

If the CESI 8000 Plus system will be shut down and the power turned off, then remove the cartridge.

1. Make sure that the ESI voltage on the mass spectrometer is off.
2. Do the applicable procedure in the section: [Store the Cartridge](#).
3. In the 32 Karat software, go to the Direct Control window, and then click **Load**.
4. Open the cartridge cover.

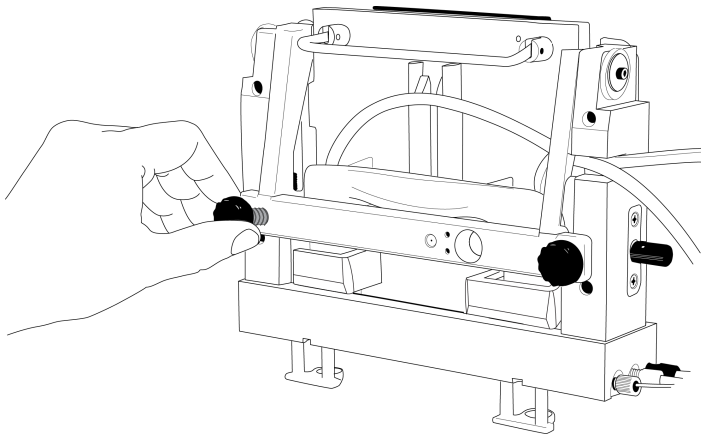
Figure 9-1 Cartridge Cover



The coolant pump for the CESI 8000 Plus system turns on and releases the coolant from the cartridge coolant lines. Approximately 30 seconds are required. Wait for the pump to turn off.

5. Loosen the thumbscrews on the insertion bar.

Figure 9-2 Thumbscrews on the Insertion Bar

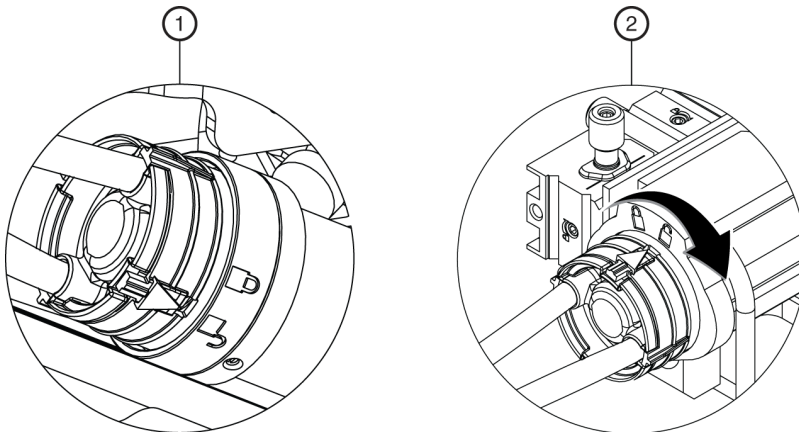


6. Lift the insertion bar fully.
7. To prevent damage to the sprayer tip, retract the stage as far away from the inlet on the mass spectrometer as possible.
8. To loosen the end of the sprayer from the adapter, turn the arrow on the sprayer to the Unlock position.



WARNING! Hot Surface Hazard. The surfaces of the ion source become hot during operation. Let the ion source cool for at least 30 minutes before the sprayer is removed from the adapter on the mass spectrometer.

Figure 9-3 Unlock the Sprayer from the Cartridge



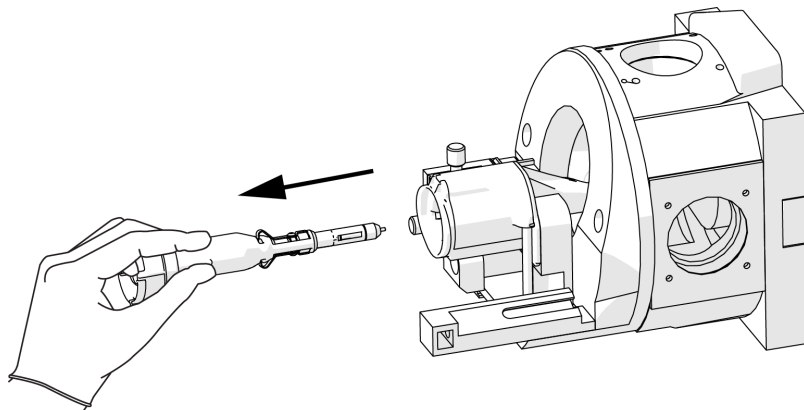
Item	Description
1	Sprayer in the Unlock position

Shut Down and Disconnect

Item	Description
2	Turn to lock the sprayer from the adapter

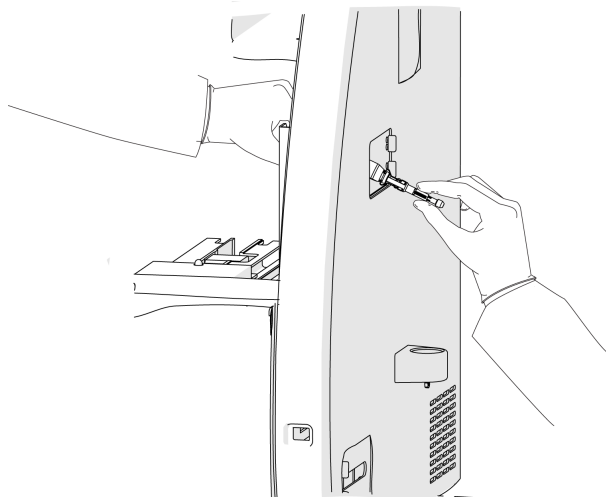
9. Remove the sprayer from the adapter.

Figure 9-4 Sprayer Removal



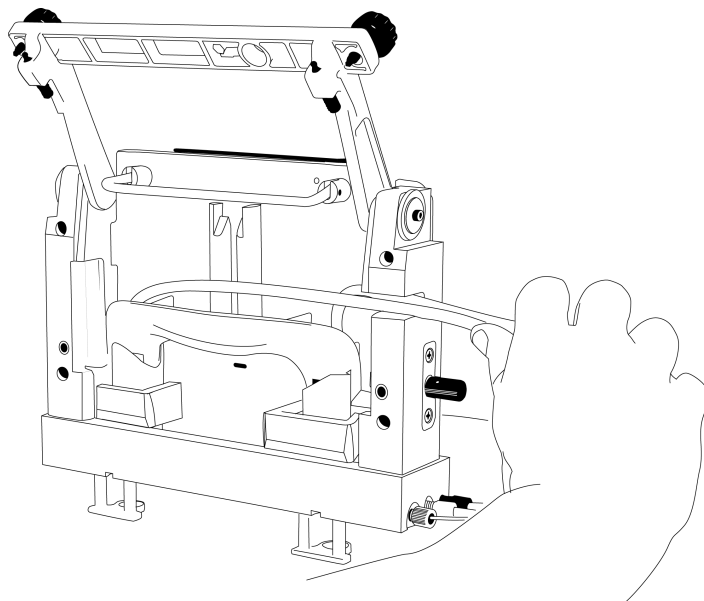
10. Install the protective sleeve on the sprayer tip.
11. Put the tubing with the sprayer end through the access panel.

Figure 9-5 Sprayer and Tubing Through the Access Panel



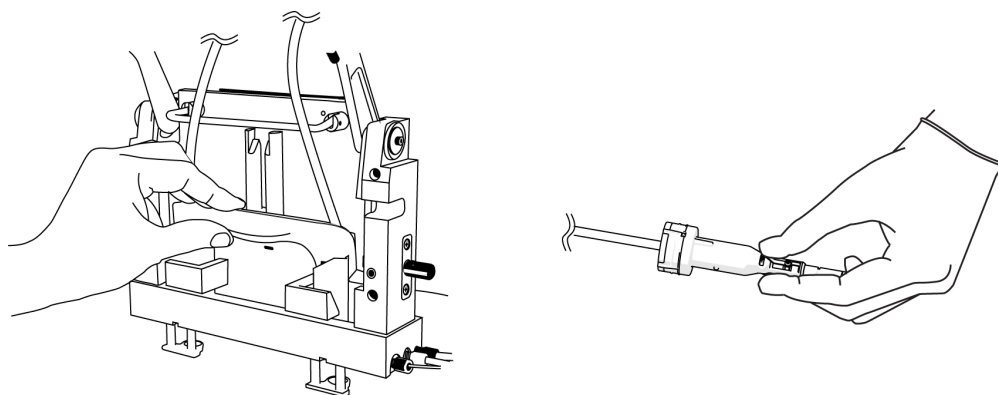
12. Remove the coolant tubing from the notched arm.

Figure 9-6 Coolant and Capillary Tubing



13. Hold the cartridge sprayer with one hand and the midsection of the cartridge in the other, then lift the cartridge up and pull it out.

Figure 9-7 Remove the Cartridge



Note: As the cartridge is moved up, the sheaths on the inlet and outlet sides go down over the capillary ends.

Note: Drops of liquid coolant that might fall from the cartridge ends are normal and do not cause damage to the hardware.

14. Refer to the section: [Store the Cartridge](#).

Store the Cartridge



WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.

CAUTION: Potential System Damage. To prevent contamination of the internal surface of the separation capillary, do not use the pressure rinse function to blow air through the capillaries. Use the vacuum function to dry the capillaries.

1. To prepare the cartridge to be stored for less than 3 days, do this:
 - a. Run the CESI-MS cleaning method.
 - b. Store the cartridge in the system for as long as 3 days.
2. To prepare the cartridge to be stored for more than 3 days, do this:
 - a. Run the CESI-MS storage method.

Note: The CESI-MS storage method cannot be run as part of a sequence because methanol is volatile and can evaporate before the sequence is complete. Vials filled with methanol must be used immediately. The ESI needle can stay in air but not in liquid. The ESI needle can be dried by vacuum.

- b. Disconnect the sprayer from the ion source.
- c. Remove the cartridge from the system.
- d. Install the protective sleeve on the sprayer tip.
- e. Put the sprayer tip in a 2 mL microcentrifuge tube of water.
- f. Loosely seal the tube with Parafilm.
- g. Keep the cartridge in a safe place at room temperature.

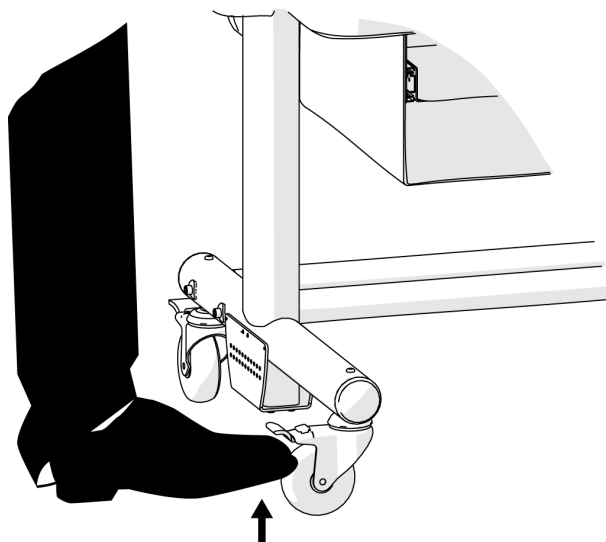
Disconnect the CESI 8000 Plus System from the Mass Spectrometer

Disconnect the CESI 8000 Plus system from the mass spectrometer when:

- The mass spectrometer will be used without the CESI 8000 Plus system.
 - The mass spectrometer or the CESI 8000 Plus system will be moved.
1. Make sure that the cartridge has been removed.
Refer to the section: [Remove the Cartridge](#).
 2. Turn off the CESI 8000 Plus system.

3. On the CESI 8000 Plus system high-voltage connections panel, disconnect the input and output cables and the black and red banana plugs.
Refer to the figure: [Figure 2-21](#).
4. Disconnect the relay cable from the CESI 8000 Plus system and the mass spectrometer.
5. Disconnect the high-voltage input cable from the mass spectrometer.
6. Adjust the height of the mobile cart to the lowest position.
7. Disconnect the power cables from the CESI 8000 Plus system and the mobile cart.
8. To unlock the wheels on the mobile cart, lift the lever at each caster.

Figure 9-8 Unlock the Wheels of the Mobile Cart



9. Carefully move the CESI 8000 Plus system away from the mass spectrometer.
10. Loosen the thumbscrew on the adapter, and then move the adapter fully off the stage.
11. Remove the adapter from the ion source. If required, then remove the adapter mounting bracket.
12. Keep the adapter, adapter mounting bracket and thumbscrews, and cables in a safe place.