

# **OptiMS Waters MS Adapter**

### For NanoLockSpray and NanoFlow 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 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
B85211	Adapter kit, containing:	1
	• Adapter	
	High-voltage cable	
	<ul> <li>OptiMS Waters MS Adapter for NanoLockSpray and NanoFlow Ion Sources Installation Guide (this document)</li> <li>Relay cable</li> </ul>	
B85396	Adapter stage	1

## **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 Waters MS Adapter for NanoLockSpray and NanoFlow 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.

⚠

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.

# Remove the Baffle and the Lockspray Capillary (NanoLockSpray Ion Sources Only)

Note: This procedure does not apply to mass spectrometers with a NanoFlow ion source.

#### **Required Materials**

- 2 mm hex key
- 1. Use the manufacturer's instructions to remove the ion source from the mass spectrometer. Put the ion source on the lab bench.
- 2. Remove the baffle and the lockspray capillary from the ion source.
  - a. Turn the ion source so that the back of the ion source points forward.
  - b. Use a 2 mm hex key to loosen the set screw on the baffle sufficiently to remove the baffle. Put the baffle in a safe place.





c. Loosen the fitting at the top of the lockspray capillary to remove it. Put the capillary in a safe place.



Figure 2-2 Fitting for the Lockspray Capillary

- 3. Install the ion source on the mass spectrometer.
- 4. Connect the top two Fischer connections (green and blue).

### Install the Adapter

Remove the existing stage from the ion source and install the adapter stage provided in the adapter kit.

R	Required Materials	
•	Adapter stage	
•	Adapter	
•	1/16 inch hex key	

1. Remove the ion source stage from the ion source.





ltem	Description
1	High-voltage port
2	Probe cover
3	Retaining screw
4	End stop

- a. Disconnect the high-voltage cable from the high-voltage port (Figure 2-3, item 1) on the mass spectrometer.
- b. Remove the probe cover (Figure 2-3, item 2) and put it in a safe place.
- c. Use a 1/16 inch hex key to loosen the set screws on the end stop only far enough so that the end stop can be removed.
- d. Remove the end stop (Figure 2-3, item 4) and put it in a safe place.
- e. Loosen the retaining screw (Figure 2-3, item 3).

f. Pull the stop screw out (circled in Figure 2-4) and continue to hold it out, then pull the ion source stage away from the ion source and remove the stage.



#### Figure 2-4 Ion Source Stage Removed from the Rails

- 2. Use the thumbscrews to adjust the position of the adapter stage. Refer to the figure: Figure 2-5.
  - a. Use thumbscrew 1 to move the vertical position to 4.5.
  - b. Use thumbscrew 3 to move the horizontal position to 13.5.
  - c. Use thumbscrew 5 to move the forward and backward position to 0, so that the adapter stage is as far back as possible.

#### Figure 2-5 Adapter Stage



ltem	Description
1	Thumbscrew for vertical position
2	Adapter bracket
3	Thumbscrew for horizontal position
4	Retaining screw
5	Thumbscrew for forward and backward position

- 3. Install the adapter stage on the ion source.
  - a. Install the adapter stage on the rails.
  - b. Pull the stop screw out (circled in Figure 2-4) and continue to hold it out, then push the adapter stage to the first detent position.
  - c. Install the end stop on the rails.
  - d. Use the 1/16 inch hex key to tighten the set screws. Do not overtighten the set screws.
- 4. Turn the adapter so that the lock icons are on top, and then push the adapter into the bracket until the adapter moves fully into position with a snap.



Figure 2-6 Adapter in the Bracket

- 5. Pull the stop screw out (circled in Figure 2-4) and continue to hold it out, then push the adapter stage toward the mass spectrometer until the adapter stage locks in position.
- 6. Tighten the retaining screw (Figure 2-5, item 4).

### **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 2-8, item 2) to the relay port on the CESI 8000 Plus system (labeled **I/O** in the following figure). Make sure that the connector is as far to the right as possible.



3. If required, then remove the green cap from the **External Connections 1** port on the back of the mass spectrometer. Connect the other end of the relay cable (Figure 2-8, item 2) to the port.



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

#### Figure 2-8 Relay Cable



ltem	Description
1	To External Connections 1 port on mass spectrometer
2	To CESI 8000 Plus system

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



Figure 2-9 High-Voltage Cable Connected

- b. On the right side panel of the CESI 8000 Plus system, open the door to the high-voltage connections panel (Figure 2-10).
- 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 2-10 High-Voltage Connections Panel

ltem	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.

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.

CAUTION: Potential System Damage. Move the adapter mounting stage as far away as possible from the inlet on the mass spectrometer. If the sprayer is too close to the inlet, then damage to the sprayer 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.
- 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 3-1 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.



Figure 3-2 Lock the Wheels of the Mobile Cart

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 3-3 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.
- 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 3-4 Sprayer in the Locked Position in the Adapter

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 4-1 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 μM	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.

- 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  $\mu$ L 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  $\mu L$  of beta-galactosidase stock solution and 50  $\mu L$  of 1  $\mu M$  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.
- Use a pipette to put 90 μL of the supernatant in a sample vial. Keep the sample at 2 °C to 8 °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  $\mu$ L 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 °C to 8 °C.
- 6. At the end of the day, discard the solution that was not used.

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

#### Table 5-1 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 HCI	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 5-1 Buffer Tray Layout





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 6-1 Cartridge Cover



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

#### Figure 6-2 Insertion Bar



3. Open the access panel.

#### Figure 6-3 Access Panel



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

Figure 6-4 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 6-5 Aligning Blocks

ltem	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 6-6 and Figure 6-7.
- 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 6-6 T Slider



ltem	Description
1	Align the T slider in the groove



#### Figure 6-7 Capillaries Below the Interface Block

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

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



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

#### Figure 6-9 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 6-10 Thumbscrews on the Interface Plate

ltem	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.





b. Tighten both thumbscrews.

#### Figure 6-12 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 6-13 Sprayer Immersed in Liquid



7. Run the CESI-MS conditioning method.

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

#### Figure 6-14 Dry the Sprayer



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

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

# Configure the Mass Spectrometer for Use with the CESI 8000 Plus System

1. In the MassLynx software, click the **Inlet Method** button to open the Inlet Method window.

#### Figure 6-15 Inlet Method Button



2. Click **Tools** > **Instrument Configuration** to open the Inlet Configuration dialog.

Inlet Configuration				×
	Pump	Autosampler	Detector 1	Detector 2
	Not Configured	Not Configured	Not Configured	Not Configured
Internet				
	Selected Config	guration Address	es	
	Pump	Autosampler	Detector 1	Detector 2
N Ö LA	-	-	-	
Inlet Configuration			Setup Instrument	Configure
C	Click a line in the list above to select an inlet channel. Click "Configure" to configure currently selected inlet channel.			
Click "Events & Trigger", to set triggering method and events. Events & Triggering Finish				

Figure 6-16 Inlet Configuration Dialog

- 3. Click **Configure** to open the Inlet Configuration Wizard.
  - a. In the Select Pump page, select None in the list of pumps, and then click Next.

Inlet Configuration Wizard	X
Select Pump Choose a pumping device.	<b>∜</b> ∎-
Choose a pump, and then press Next to continue.	
<ul> <li>None</li> <li>Waters 2695</li> <li>Waters 2795</li> <li>Waters 2796</li> <li>Waters ACQUITY BSM</li> <li>Waters ACQUITY Nano</li> <li>Waters ACQUITY QSM</li> <li>Waters CapLC</li> <li>Waters Pump Control</li> <li>External</li> <li>Gilson</li> <li>HP 1090</li> <li>HP 1050</li> <li>HP 1100</li> <li>HP 6890</li> <li>Jasco 900</li> </ul>	
K 🗌 🕨 🕨	
< Back Next > Cancel	Help

Figure 6-17 Select Pump Page—Inlet Configuration Wizard

b. In the **Select Auto Sampler** page, select **None** in the list of autosamplers, and then click **Next**.

Inlet Configuration Wizard	×
Select Auto Sampler Choose an autosampler.	
Choose an autosampler device, and then press Next to continue.	
<ul> <li>✓ None</li> <li>Waters 2700</li> <li>Waters 2747</li> <li>Waters 2767</li> <li>Waters 2777</li> <li>Cetac ASX500</li> <li>Cetac ASX100</li> <li>CTC A200S</li> <li>CTC A200SE</li> <li>External</li> <li>Famos</li> <li>Gilson</li> <li>CTC GC PAL (A200SE Emulation Mode)</li> <li>HP 1090</li> <li>HP 1050</li> <li>HP 1100</li> </ul>	
< Back Next > Cancel	Help

Figure 6-18 Select Auto Sampler Page—Inlet Configuration Wizard

c. In the **Select Detectors** page, select the first item in the **Configured Detectors** list, and then click the left arrow to remove the detector from the list.



Figure 6-19 Select Detectors Page—Inlet Configuration Wizard

d. Do step 3.c until the Configured Detectors list is empty. Then click **Next**. The Finished Configuring page opens.

Inlet Configuration V	Vizard			×
Finished Config	uring			-]∲]-
Configu	ration Su	ccessful		
Click Finish to	accept configu	ration.		
	< Back	Finish	Cancel	Help

Figure 6-20 Finished Configuring Page—Inlet Configuration Wizard

- e. Click **Finish** to close the wizard.
- 4. In the **Inlet Configuration** window, click **Events & Triggering** to open the Events and Triggering Wizard.
  - a. In the **Choose Events** page, in the **Event In** area, select **2** and then click **Next**.

Events and Tri	ggering Wizar	d			×	
Choose Events Choose input/output events.						
Selec	Select input/output events, and then press Next to continue.					
	Event In	<b>▼</b> 2	Г 3	<b></b> 4		
	Event Out				]	
	□ 1	2	Д 3	L 4		
		<	Back	Next >	Cancel	

Figure 6-21 Choose Events Page—Events and Triggering Wizard

- b. In the Choose Triggering Method page, in the Pump area, click Trigger by Contact Closure.
- c. In the Detector area, click Trigger by Contact Closure, and then click Next.

Events and Triggering Wizard				
Choose Triggering Method Choose software or contact closure triggering.				
Choose pump and/or detector trigger method, and then press Next to continue.				
Pump Trigger by Contact Closure C Trigger by Software				
Detector     Trigger by Contact Closure     Trigger by Software				
< Back Next > Cancel				

Figure 6-22 Choose Triggering Method Page—Events and Triggering Wizard

The Finished Configuring page opens.

d. Click **Finish** to close the wizard.

### **Create a Tune File**

Create a tune file to set the ESI voltage and other parameters for the mass spectrometer. This file is used as the starting point for two more tune files that are required for the test separation. The additional files are created in the section: Fine-Tune the Position of the Sprayer Tip.

1. In the MassLynx software, click the **MS Tune** button.

#### Figure 6-23 MS Tune Button



- $2. \quad \text{Put the mass spectrometer in } \text{Operate mode}. \\$ 
  - a. If necessary, manually turn on the gas supplies for the mass spectrometer.

b. In the bottom right corner of the Tune window, click **Operate**.

The system is removed from Standby mode.

Figure 6-24 Operate Button (Far Left) and System Status (Right)

Operate	

3. Open the Nanoflow+ tab, and type the values shown in the following figure.

TriWave DC	TriWave	Step Wave	Trapping	Quadrupole	Vacuum
Nanoflow+	Instrument	Fluidics	System 1	System 2	ADC
Source	Capillary (kV) Sampling Cone Source Offset	0.00 <b>]</b> 20 20			
Temperatures (	°C) Source	26 12	0		
Gas Flows Nano	Cone Gas (L/h) • Flow Gas (Bar) Purge Gas (L/h)	0 0 4.00 0.00 0 0	]		

#### Figure 6-25 Nanoflow+ Tab

4. To the right of the Nanoflow+ tab, type the values shown in the following figure.

#### Figure 6-26 Mass Range and Scan Conditions

	1SMS Mass: 450.00
Scan Conditions (sec) Scan Time: 0.5 - Data Type: Continuum -	

5. Click File > Save As, and type the name OptiMS\_OFF.

### **Establish a Stable Spray**

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

#### Figure 6-27 Pressure Settings Dialog to Fill the Conductive Liquid Capillary

Pressure Settings		×
Pressure 100 psi Duration: 2 min	Direction C Forward C Reverse	OK Cancel
Tray positions Inlet BI:A1 Outlet BO:A1	Pressure type Pressure Vacuum	Help

- 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 6-28 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.



#### Figure 6-29 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 6-30 Pressure Settings Dialog to Fill the Separation Capillary

Pressure Settings		×
Pressure     100     psi       Duration:     2.00     min       Tray positions	Direction Forward Reverse Pressure type Fressure Vacuum	OK Cancel Help

- 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 6-31 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.

Voltage Settings			×
Voltage 20 Duration: 30 Ramp time: 1	kV Voltage max: min Current max: min	30.0 kV 300.0 μA	OK Cancel Help
Tray positions Inlet: BI:A1 Outlet: B0:A1 Trays	Pressure With pressure With vacuum Pressure: 0.1 psi	Direction Forward C Reverse C Both	Polarity © Normal © Reverse

Figure 6-32 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  $\mu$ A to 3  $\mu$ A. If the BGE used is 50 mM AmAc buffer, pH 3.0, then the electrical current should be 0.5  $\mu$ A to 1  $\mu$ A.

- 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 6-33 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.

- 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 pl 7.0 or pl 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 6-34 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): Baseline fluctuation (%) = [(highest value average value)/average value] x 100.</li>
- 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 ≤ 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.

### 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.

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.

**Tip!** If the camera is available, use it to help position the tip. If not, look through the glass window on the front of the ion source as you move the sprayer tip.

- 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. In the 32 Karat software, use the Direct Control window to apply 20 kV voltage and 1 psi pressure in both directions.
  - 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.
- 5. Adjust the position of the sprayer tip to maximize the mass spectrometer signal intensity.
  - a. In the MassLynx software, open the Nanoflow+ tab and then type 0.5 in the **Capillary** (**kV**) field to set the ESI voltage.

	TriWave	Step Wave	Trapping	Quadrupole	Vacuum
Nanoflow+	Instrument	Fluidics	System 1	System 2	ADC
Source					
	Capillary (kV)	0.50			
	Sampling Cone	20 -	}		
	Source Offset	20 -	]		
– Temperatures (*	C) Source	26 12	0		
Gas Flows					
C	Cone Gas (L/h)	0 0			
Nano	Flow Gas (Bar)	4.00 0.00	]		
P	urge Gas (L/h)	0	]		

Figure 6-35 Nanoflow+ Tab in the MS Tune File

- b. Increase the value in the **Capillary (kV)** field in increments of 0.1 kV until electrospray ionization is detected. Record this voltage.
- c. Set ESI voltage to 0 kV and confirm that there is no electrospray ionization.
- d. Change the value in the **Capillary (kV)** field to 0.2 kV higher than the voltage found in step 5.b. Electrospray ionization should start. To make sure that the spray is stable, let it continue for 5 minutes.

**Note:** If a voltage of 20 kV and pressure of 1 psi are not sufficient to establish a stable signal, then increase the pressure at both ends of the cartridge, to a maximum of 3 psi. If pressure > 1.0 psi is required to maintain a stable electrospray ionization, then make sure to change the required pressure in the CESI-MS separation method.

e. Use the knobs on the adapter stage to adjust the position of the sprayer tip to maximize the signal intensity while minimizing fluctuations in signal intensity.
Make sure that the sprayer tip is 2 mm to 5 mm from the ion source inlet.



Figure 6-36 Sprayer Tip and Ion Source Inlet

6. Click File > Save As and type the name OptiMS RUN.

These are the settings used by the mass spectrometer during data acquisition after the sample has been separated by the CESI 8000 Plus system.

7. Change the value in the **Capillary (kV)** field to 0.4 kV higher than the voltage recorded in step 5.b, click **File** > **Save As**, and then type the name <code>OptiMS\_ON</code>.

These are the settings used to remove any rinse solution that collects on the sprayer tip.

- 8. In the MassLynx software, to open the OptiMS\_OFF MS tune file, click File > Open.
- 9. In the 32 Karat software, go to the Direct Control window, and then set the voltage to 1.0 kV with a ramp of 5.0 minutes and a duration of 5.0 minutes.

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 MS Methods and Sample List**

Use the MassLynx software to create the files that are required for the test separation. **Create the MS Methods** 

1. In the MassLynx software, click the **MS Method** button.

#### Figure 7-1 MS Method Button



- 2. Double-click the first row in the table to open the Function:1 MS dialog.
- 3. Type the values shown in the following figure, and then click **OK**.

unction:1 MS	? <mark>×</mark>
Acquisition TOF MS Tra	p CE Control Transfer CE Control
Acquisition Times	
Total time for this acqui	sition
Start Time	0 min
End Time	0.03 min
Source	
Source	ES
Acquisition Mode	
Polarity	Positive     Negative
Analyser Mode	
Analysei Mode	
Dynamic Range	Normal O Extended
Sensitivity	Normal
	Target Enhancement 200 Da
	OK Cancel Apply

Figure 7-2 Function: 1 MS Dialog for cap\_on MS Method

4. Click File > Save As and type cap\_on.

This method applies a high ESI voltage to remove any drops of buffer from the sprayer tip after the Rinse events.

5. Do the previous steps twice more to create methods with the time values and file names in the following table.

File Name	Start Time	End Time	Purpose
cap_off	0	0.03	Turn off high ESI voltage
bgal_250-2000	0	30	Collect MS data after CE separation

**Table 7-1 MS Method Parameters** 

### **Create the Sample List**

CAUTION: Potential System Damage. Always use the OptiMS\_OFF tune file for the last row in a sample list to turn off the ESI voltage at the end of the run. If voltage is applied when the capillary is empty, then the sprayer can break.

- 1. To open a new sample list, in the MassLynx software, click **File > New**.
- 2. Complete the first row in the sample table.
  - a. Click the File Name cell, and then type discard 1.
  - b. Double-click the **MS Tune File** cell, and then select the <code>OptiMS\_OFF</code> **MS** tune file.
  - c. Double-click the **MS File** cell, and then select the <code>cap\_off MS</code> file.
- 3. To complete the sample list, add the information in the following table to rows 2 through 5.

CAUTION: Potential System Damage. To keep the synchronization between the CESI 8000 Plus system and the mass spectrometer and prevent the introduction of solutions that are not compatible to the mass spectrometer, when multiple CESI-MS separations are done, always use rows that are equivalent to row 1 to row 4 in the following table.

Table 7-2	Samp	le List
-----------	------	---------

Row	File Name	MS Tune File	MS File
1	discard 1	Optims_OFF	cap_off
2	discard 2	OptiMS_ON	cap_on
3	discard 3	OptiMS_OFF	cap_off
4	bgal_1	OptMS_RUN	bgal_250-2000
5	discard 4	OptiMS_OFF	cap_off

- 4. To save the sample list, do this:
  - a. Click **File > Save**.
  - b. Type a name for the sample list, and then click **OK**.

### Manually Create the CESI-MS Separation Method

The cesi-ms\_Waters-ms.met method file is installed with the 32 Karat software 10.3 or later in the folder C: $\32$ Karat $\projectsCE$  MSMethod. 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.

👙 Initial Conditions 🛞 Time Progr	am
Auxiliary data channels         ✓ Noltage       max:       30.0       kV         ✓ Current       max:       10.0       μA         ✓ Power         ✓ Pressure	Temperature       Peak detect parameters         Cartridge:       25.0 °C         Sample storage:       10.0 °C         Trigger settings
Mobility channels	<ul> <li>Wait for external trigger</li> <li>Wait until cartridge coolant temperature is reached</li> <li>Wait until sample storage temperature is reached</li> </ul>
Apparent Mobility     Plot trace after voltage ramp     Analog output scaling     Factor:	Inlet trays     Outlet trays       Buffer:     36 vials       Sample:     48 vials         Sample:     No tray

#### Figure 7-3 Initial Conditions for the CESI-MS Separation Method

3. Use the time program in the following figure.

#### Figure 7-4 Time Program for the CESI-MS Separation Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
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 HCI rinse
3		Rinse - Pressure	100.0 psi	4.00 min	BI:C1	BO:A1	forward	Water rinse
4		Rinse - Pressure	100.0 psi	2.00 min	BI:B1	BO:A1	forward, In / Out vial inc 12	BGE fill
5		Relay On		1			1: 0.10 2: 0.10	
6		Rinse - Pressure	100.0 psi	2.00 min	BI:B1	BO:A1	forward, In / Out vial inc 12	BGE fill
7		Rinse - Pressure	75.0 psi	3.00 min	BI:B1	BO:A1	reverse, In / Out vial inc 12	Conductive Liquid Capillary fill
8		Relay On					1: 0.10 2: 0.10	
9		Inject - Pressure	5.0 psi	60.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic Injection
10		Inject - Pressure	0.5 psi	25.0 sec	BI:A1	BO:A1	No override, forward, In / Out vial inc 12	Push
11	0.00	Separate - Voltage	20.0 KV	30.00 min	BI:A1	BO:A1	1.00 Min ramp, normal polarity, both, In / Out vial inc 12	Separation
12	0.25	Relay On					1: 0.10 2: 0.10	
13	1.00	Relay On					1: 0.10 2: 0.10	
14	30.00	Separate - Voltage	1.0 KV	5.00 min	BI:A1	BO:A1	5.00 Min ramp, normal polarity, forward, In / Out vial inc 12	
15	35.00	End		]				
16	I							

4. In row 11 and row 14 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**.

Separate	×
Separation Type         Voltage         Current         Power         Pressure         Vacuum         Options:         With Pressure         With Vacuum         Polarity         Normal         Reverse	Pressure Direction       OK         C Forward       Cancel         Reverse       Help         At Time:       0.00         0.00       min         External Adapter

Figure 7-5 Separate Dialog: Pressure Settings for Rows 11 and 14

6. Click File > Method > Save As, and then save the method as cesi-ms Waters-ms.met.

### Load the Sample and Start the Run

- 1. Put the vial with beta-galactosidase test sample in position SI:A1 of the sample inlet tray.
- 2. In the 32 Karat software, open the CESI-MS Separation for Waters MS method file.
- 3. In the MassLynx software, open the sample list created in the section: Create the MS Methods and Sample List.
- 4. In the sample list, select all the rows in the table, and then click the **Start Run** icon.

#### Figure 7-6 Start Run Icon



The Start Sample List Run dialog opens.

Figure 7-7 Start Sample List Run Dialog

Start Sample List Run	<b>—</b>
E:\201606CESI.PRO	
□Pre-Run ✔Acquire Sample Data □Auto Process Samples □Auto Quantify Samples □Post-Run	Samples From To 4 Scheduling Priority Night Time Process
User Processes	OK Cancel

- 5. Make sure that the values in the Samples section show the rows in the sample list to be run and that the **Acquire Sample Data** check box is selected, and then click **OK**. Wait for the message Waiting for Inlet Start to show at the bottom of the MassLynx software window.
- 6. In the 32 Karat software tool bar, click the **Single Run** icon. The Single Run Acquisition dialog opens.
- 7. Make sure that the CESI-MS Separation for Waters MS method file shows in the Sequence name field.

If the correct method file does not show, then click the folder icon and browse to the CESI-MS Separation for Waters MS method file.

- 8. In the 32 Karat software, go to the Direct Control window, and then click **Load**.
- 9. 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.
- 10. Put the sample inlet tray in the back of the inlet carrier (left).
- 11. Click Start.

The run requires approximately 30 minutes to complete.

### Analyze the Data

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

1. Click File > Open Data File.

Data Browser			? <mark>x</mark>
File Name: 20160616_bgal	2.RAW	Directories: E:\201606CESI.PRO\Data\	<b>.</b>
20160616_bga 20160616_eco 20160616_eco 20160616_eco discard 1.raw discard 10.raw discard 11.raw discard 11.raw discard 11.raw discard 13.raw discard 14.raw discard 15.raw	12.raw i1.raw i2.raw i3.raw	<ul> <li>✓ E:\</li> <li>         ★ \$RECYCLE.BIN     </li> <li>         ⇒ 201606CESI.PRO     </li> <li>         ★ CurveDB     </li> <li>         ♥ ⇒ CurveDB     </li> </ul>	500.PR0
		Drives:	▼ Network
Information Sample Description:	bgal run		Chromatogram
Acquired: Function:	16-Jun-2016 07:19:10 TOF MS (250:2000) ES+	<b>_</b>	
	History Exp	periment Delete	OK Cancel

Figure 7-8 Data Browser Dialog

2. Select the bgal\_1 file from the test run, clear the **Spectrum** check box, and then click **OK**. The total ion chromatogram (TIC) opens in the Chromatogram window.



Figure 7-9 TIC for Test Run in Chromatogram Window

- 3. Extract the chromatograms for two of the peptides:
  - a. Click **Display > Mass**.

Mass Chromatogram	×
File:       20160616_bgal2         Description (m/z):       [714.8649, 729.3652         Function:       TOF MS (250:2000) ES+	OK Cancel File
Mass Chromatogram Window Parts per million 10.0 Abs Window (Da) 5.0000	<ul> <li>Add trace</li> <li>Replace trace</li> <li>New window</li> </ul>

Figure 7-10 Mass Chromatogram Dialog

- b. In the **Description (m/z)** field, do this:
  - For a NanoLockSpray ion source, type 714.8649, 729.3652.
  - For a NanoFlow ion source, type 714, 729.
- c. In the Abs Window (Da) field, type 5.0.
- d. Click OK.

A pane that shows the extracted ion chromatogram for each m/z opens in the Chromatogram window.



Figure 7-11 TIC and XICs in the Chromatogram Window

- 4. Calculate the signal-to-noise ratio (S/N) for the 729 Da peak.
  - a. Click the pane with the 729 Da peak to select it.
     When the pane is selected, a small square shows near the left edge. In the figure: Figure 7-11, the top pane is selected.
  - b. Click **Process > Signal To Noise**.

Signal To Noise		x
Ranges		ОК
Signal		Cancel
Noise		
Noise Processing		
Ignore Zeros		
NO Extra Processing	œ	
Ignore Worst 5% of Scans	0	
Ignore Scans Outside +/-1 SD	0	
Ignore Scans Outside +/-1.5 SD	0	
Ignore Scans Outside +/-2 SD	0	
Display		
RMS	œ	
Peak To Peak	0	
Calculations		
Use (n-1) for RMS	◄	
Use Underlying Raw Data		

Figure 7-12 Signal To Noise Dialog

- c. Click the **Signal** field, and then click-drag across the peak in the extracted ion chromatogram to select it.
- d. Click the **Noise** field, and then click-drag across an area in the extracted ion chromatogram with noise. The selected noise region should be approximately four times wider than the peak selected before.
- e. Click OK.

A new pane that shows the S/N ratio opens in the Chromatogram window.



Figure 7-13 TIC, XICs, and S/N in the Chromatogram Window

- 5. Examine the results.
  - The S/N for the 729 Da peak should be > 50.
  - Make sure that the two peaks are separated by at least 0.05 minutes.

If the results are not similar to these values, then set the home position on the mobile cart. Refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

### **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 8-1 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 8-2 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 8-3 Unlock the Sprayer from the Cartridge



ltem	Description
1	Sprayer in the Unlock position

#### Shut Down and Disconnect

ltem	Description
2	Turn to lock the sprayer from the adapter

9. Remove the sprayer from the adapter.

#### Figure 8-4 Sprayer Removal



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

#### Figure 8-5 Sprayer and Tubing Through the Access Panel



12. Remove the coolant tubing from the notched arm.



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.





**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.

- 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 2-10.
- 4. Disconnect the relay cable from the CESI 8000 Plus system and the mass spectrometer.
- 5. Attach the green cover to the **External Connections 1** port on the back of the mass spectrometer.
- 6. Disconnect the high-voltage input cable from the mass spectrometer.
- 7. Adjust the height of the mobile cart to the lowest position.
- 8. Disconnect the power cables from the CESI 8000 Plus system and the mobile cart.
- 9. To unlock the wheels on the mobile cart, lift the lever at each caster.

#### Figure 8-8 Unlock the Wheels of the Mobile Cart



- 10. Carefully move the CESI 8000 Plus system away from the mass spectrometer.
- 11. Remove the adapter from the ion source.
- 12. Keep the adapter and cables in a safe place.