SCIEX
The Power of Precision

Neutral OptiMS Cartridge

Instruction Guide

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Operational Precautions and Limitations 1

WARNING! Electrical Shock Hazard. To prevent a shock, do not touch an adapter on a mass spectrometer that is connected to the CESI 8000 Plus system when the separation voltage is on.

CAUTION: Potential System Damage. Use the lowest ESI voltage possible. If the ion source is exposed to high temperatures, then blockage in the emitter and damage can occur.

CAUTION: Potential System Damage. To prevent capillary blockage, use volatile buffers such as phosphate and borate buffers.

CAUTION: Potential System Damage. Do not expose the cartridge to temperatures that are < 2 °C. For storage, keep the cartridge at 2 °C to 8 °C.

CAUTION: Potential System Damage. Do not rinse the separation capillary with solutions that have a pH < 2 or > 9. The capillary is stable in the pH range from 2 to 9. Exposure to solutions with a pH that is < 2 and > 9 can cause damage to the neutral coating. The result of damage to the neutral coating can be poor separation and a shorter run life.

CAUTION: Potential System Damage. Do not use buffers that let the separation current become > 5 µA. A separation current that is > 5 µA can cause permanent damage to the coating of the separation capillary.

For instructions about how to use the CESI 8000 Plus system safely, refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*.

Neutral OptiMS Cartridge 2

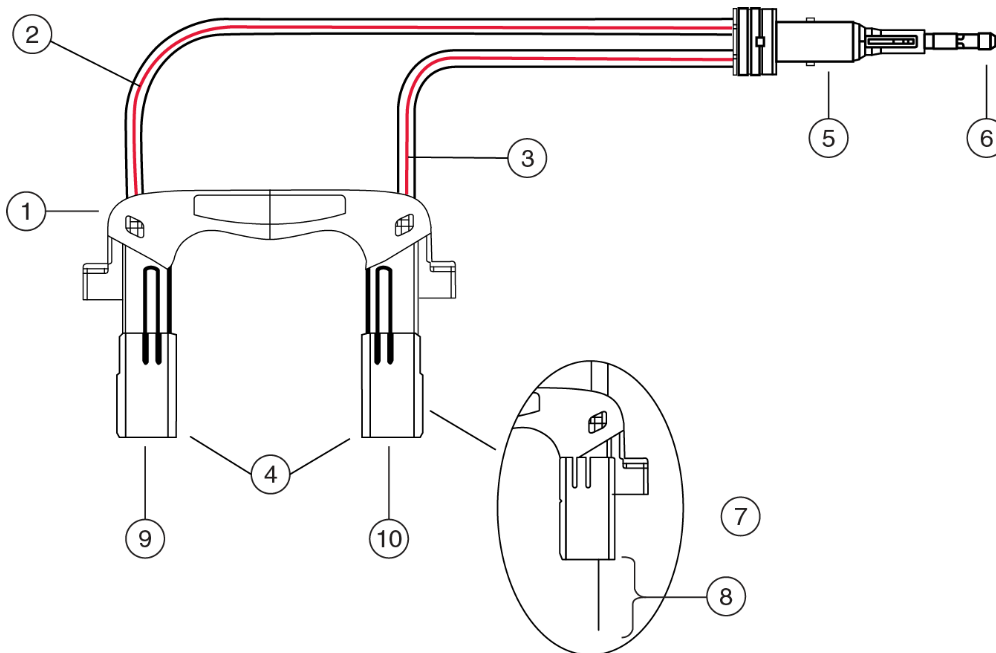
The cartridge assembly has a conductive liquid capillary and a separation capillary that ends in an ESI sprayer tip. The separation capillary and the conductive liquid capillary are different capillaries. The contents of the separation capillary never touch or mix with the contents of the conductive liquid capillary. Each capillary is inside a liquid-cooling tube.

The internal surface of the separation capillary is covalently coated with a neutral polymer to prevent the interaction between protein samples and the capillary surface, and to minimize electroosmotic flow. The separation of sample components (analytes) occurs in the separation capillary.

The conductive liquid capillary is filled with a conductive solution. The distal end of the capillary is porous, to let ion flow and electric contact occur.

Protective sleeves prevent damage to the inlet tips of the capillaries. The protective sleeves retract when the cartridge is installed in the system. When the cartridge is not installed in the system, locking mechanisms prevent the retraction of the protective sleeves.

Figure 2-1 OptiMS Cartridge

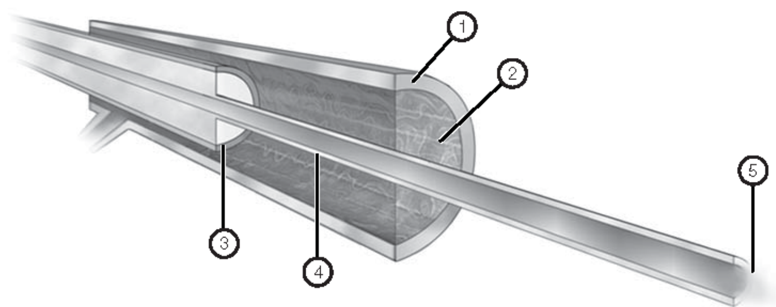


Item	Description
1	Cartridge body

Item	Description
2	Separation capillary in liquid-cooling tube
3	Conductive liquid capillary in liquid-cooling tube
4	Protective sleeves that retract when the cartridge is installed
5	Sprayer housing
6	Sprayer tip
7	Protective sleeve shown retracted
8	Exposed capillary when protective sleeve is retracted
9	Inlet end of separation capillary
10	Outlet end of cartridge body

The sprayer tip is made of polyether ether ketone (PEEK), and holds the ESI needle.

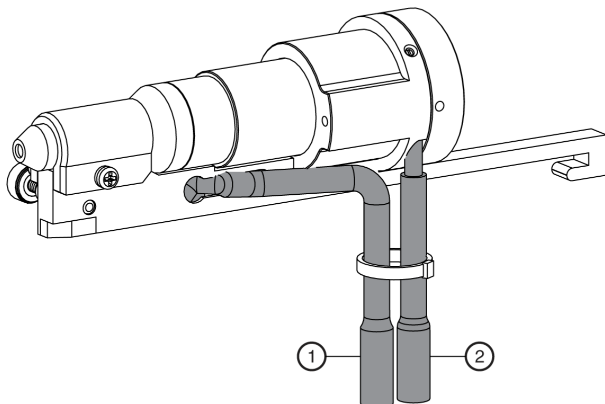
Figure 2-2 Conceptual View of Inside the Sprayer Tip



Item	Description
1	ESI needle
2	Conductive liquid
3	Separation capillary
4	Etched segment of separation capillary
5	Plume

The sprayer locks into an adapter that is made for the NanoSpray ion source on the mass spectrometer. The ESI needle in the sprayer tip closes the circuit between the CESI 8000 Plus system and the mass spectrometer to cause electrospray ionization. During the separation step, the spray of analytes goes into the mass spectrometer.

Figure 2-3 Adapter for the NanoSpray Ion Source



Item	Description
1	High-voltage input cable
2	High-voltage output cable

Note: Adapters for other mass spectrometers are available from SCIEX. For more information, go to store.sciex.com.

Prepare the Reagents 3

The following reagents are used with the neutral OptiMS cartridge.

Note: The preparation of every reagent is not required. Different experiments require different reagents. Prepare the reagents that are applicable to the experiment.

Table 3-1 Required Reagents for the Neutral OptiMS Cartridge

Reagent	Purpose
50 mM ammonium acetate (AmAc) buffer, pH 3.0	Used as the background electrolyte (BGE) and conductive liquid
100 mM AmAc buffer, pH 3.0	Used to make the 50 mM AmAc buffer, pH 3.0
10 mM AmAc buffer, pH 3.0	Used to make the Protein Test Mix sample
10% acetic acid, glacial (HAc)	Used as the BGE separation solution and conductive liquid
20% HAc	Used to adjust the pH for the 200-mM AmAc leading electrolyte (LE) buffer
200 mM AmAC LE buffer, pH 4.0	Used to make the sample become a concentrate during separation
Reconstituted Protein Test Mix	Used to make the Protein Test Mix sample
Protein Test Mix sample	Used to make sure that the cartridge operates correctly
Peptide test sample	Used to optimize the position of the capillary tip in front of the mass spectrometer to get the best signal intensity
PepCalMix test sample	Used to prepare for manual calibration and autocalibration

Table 3-2 Required Supplies from SCIEX

Material	Part Number
CE Grade water	C48034
cIEF Peptide Marker Kit	A58481

Table 3-2 Required Supplies from SCIEX (continued)

Material	Part Number
CESI Vials (100 Pack)	B11648
CESI Vial caps (100 Pack)	B24699
MS Synthetic Peptide Calibration Kit	5045759
nanoVials (100 Pack)	5043467
Neutral OptiMS Cartridge	B07368
PCR Microvials (100 Pack)	144709
Protein Test Mix	477436

Table 3-3 Other Required Reagents or Supplies

Reagent	Vendor	Part Number
Acetic acid, glacial (HAc)	Sigma-Aldrich	A6283
7.5 M Ammonium acetate (AmAc)	Sigma-Aldrich	A2706
Ammonium hydroxide (NH_4OH) (30%)	Sigma-Aldrich	05002
0.1 M Hydrochloric acid (HCl)	Sigma-Aldrich	1.09060

Initial Sample Concentrations

Use the following sample concentrations as initial conditions.

If required, then remove the salt from the sample to optimize the concentration for the sensitivity of the mass spectrometer and injection parameters.

Sample Type	Concentration
Protein Test Mix or intact proteins in pH 3.0 buffer	0.1 mg/mL in 10 mM AmAc buffer, pH 3.0
Peptide mixtures	3 µg/µL or 1.5 µM in 100 mM LE buffer, pH 4.0

Prepare the 50 mM AmAc Buffer, pH 3.0

1. In a 50 mL Falcon tube, add 10.0 mL of 100 mM AmAc buffer, pH 3.0, and 10.0 mL of CE Grade water.
2. To mix the contents, invert the Falcon tube three times.

3. Attach a label with the name `50 mM AmAc buffer, pH 3.0` and the preparation date to the Falcon tube.
4. When it is not in use, keep the buffer at 2 °C to 8 °C for as long as 3 months after preparation.

Prepare the 100 mM AmAc Buffer, pH 3.0

1. In a 500 mL volumetric flask, add 2.9 mL of HAc, and then add CE Grade water to make 500 mL of 100 mM HAc.
2. Transfer the solution to a 500 mL Nalgene bottle.
3. In a 50 mL volumetric flask, add 0.7 mL of 7.5 M AmAc, and then add CE Grade water to make 50 mL of 100 mM AmAc.
4. Use a calibrated pH meter to measure the initial pH of the 100 mM HAc solution.
5. At the same time, use a magnetic stirrer to mix the 100 mM HAc solution and add aliquots of 100 mM AmAc solution until the pH is 3.0 ± 0.1.
6. Attach a label with the name `100 mM AmAc buffer, pH 3.0` and the preparation date to the bottle.
7. When it is not in use, keep the buffer at 2 °C to 8 °C for as long as 1 year after preparation.

Prepare the 10 mM AmAc Buffer, pH 3.0

1. In a 15 mL Falcon tube, add 1.0 mL of 100 mM AmAc buffer, pH 3.0, and 9.0 mL of CE Grade water.
2. To mix the contents, invert the Falcon tube three times.
3. Attach a label with the name `10 mM AmAc buffer, pH 3.0` and the preparation date to the Falcon tube.
4. When it is not in use, keep the buffer at 2 °C to 8 °C for as long as 3 months after preparation.

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 AmAC 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 AmAc LE Buffer, pH 4.0

1. To prepare 50 mL of 400 mM AmAc 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 AmAC 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.

Reconstitute the Protein Test Mix

1. Add 2.0 mL of CE Grade water to a vial of Protein Test Mix.
2. Use a vortex mixer to mix the solution until all of the solid material is dissolved.
3. Divide the Protein Test Mix into aliquots of 20 µL.

4. When it is not in use, keep the reconstituted Protein Test Mix frozen at –35 °C to –15 °C for up to 1 year after preparation.

Prepare the Protein Test Mix Sample

1. If required, then let the vial of reconstituted Protein Test Mix thaw to ambient temperature.
2. In a 0.5 mL centrifuge tube, add 20 µL of reconstituted Protein Test Mix and 80 µL of 10 mM AmAc buffer, pH 3.0.
3. Attach a cap to the tube.
4. Use a vortex mixer to mix the sample for 3 seconds.
5. When it is not in use, keep the sample at 2 °C to 8 °C for as long as 1 year after preparation.

Prepare the Peptide Test Sample

1. In a centrifuge tube, add 100 µL of BGE and 5 µL of a peptide marker, such as pI 9.5.
2. Attach a cap to the tube.
3. Use a vortex mixer to mix the sample for 3 seconds.
4. Keep the sample at 2 °C to 8 °C.
5. At the end of the day, discard the solution that was not used.

Prepare the PepCalMix Test Sample

1. In a 0.5 mL centrifuge tube, add 10 µL of PepCalMix and 90 µL of 200 mM AmAC LE buffer, pH 4.0.
2. Mix the solution in a vortex mixer for 10 seconds.
3. To remove any precipitant, use a centrifuge to spin the solution at 12,000 × *g* for 5 minutes.

Prepare the Trays 4

Use only CESI vials and caps in the CESI 8000 Plus system buffer and sample trays.

Make sure that each buffer and sample vial is in the location identified in the tray layout. The locations of the buffer and sample vials are related to the method in the 32 Karat software. If a vial is put in a different position, then the position must be identified in the method in the 32 Karat software or manually in the sequence table.

About Vials

CAUTION: Potential System Damage. Do not overfill the vials. If the vials are too full, then liquid can go into the pressure system and cause damage.

CAUTION: Potential System Damage. Do not underfill the vials or let the liquid level get too low. If the liquid level in the vials is too low, then the separation capillary can fill with air and cause the vials to break if voltage is applied.

Three types of vials are used with the CESI 8000 Plus system:

- CESI vials are used for buffer and as holders for microvials and nanoVials.
- Microvials are used for sample volumes from 50 µL to 100 µL. These vials must be put in a CESI vial and then put in the sample tray.
- nanoVials are used for sample volumes from 5 µL to 50 µL. These vials are put in the sample tray.

Note: Always use a CESI vial cap.

Note: Do not use any vial or cap more than once.

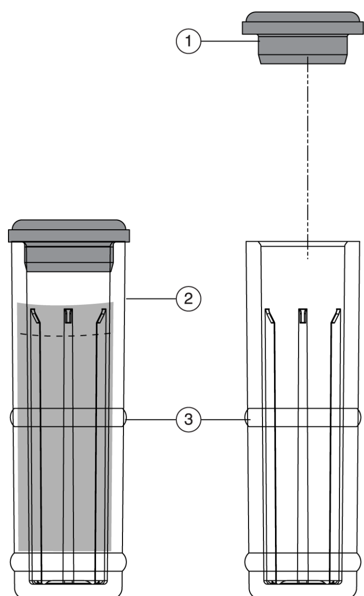
Fill a CESI Vial

Note: To prevent splashing, put the empty vials in the tray, and then add liquid and attach the caps.

1. Fill the CESI vial to the maximum fill line.

   Note: Do not put more than 1.5 mL in a CESI vial.

   Figure 4-1 CESI Vial

Item	Description
1	CESI Cap
2	Maximum fill line
3	CESI Vial

2. Attach a CESI cap.

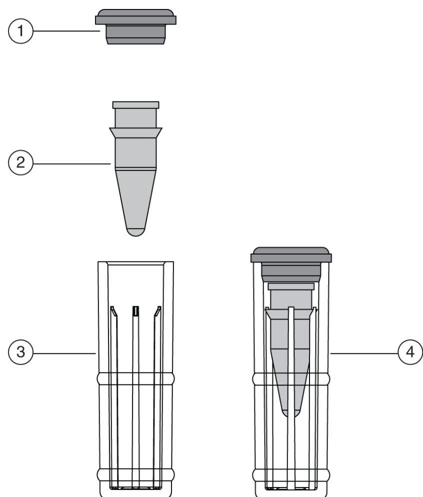
   Note: When filling the vials, do not make air bubbles. If there are air bubbles in the vial, then spin the vial in a centrifuge for a few seconds to remove the bubbles.

Fill a Microvial

Note: To prevent splashing, put the empty vials in the tray, and then add liquid and attach the caps.

1. Fill the microvial with at least 50 µL of sample.

Figure 4-2 Microvial



Item	Description
1	CESI Cap
2	Microvial
3	CESI Vial
4	Microvial inside CESI vial

2. Put the microvial inside the CESI vial.
3. Attach a CESI cap.

Note: When filling the vials, do not make air bubbles. If there are air bubbles in the vial, then spin the vial in a centrifuge for a few seconds to remove the bubbles.

Fill a nanoVial

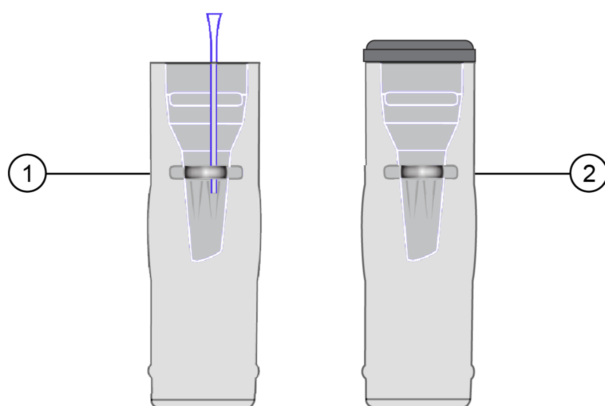
For sample volumes between 5 µL and 50 µL, use a nanoVial.

Note: To prevent splashing, put the empty vials in the tray, and then add liquid and attach the caps.

1. Use a thin pipette tip to transfer the sample to the deeper well of a nanoVial.

   When the tab points to the user, the deeper well is on the left side of the nanoVial.

   Figure 4-3 nanoVial

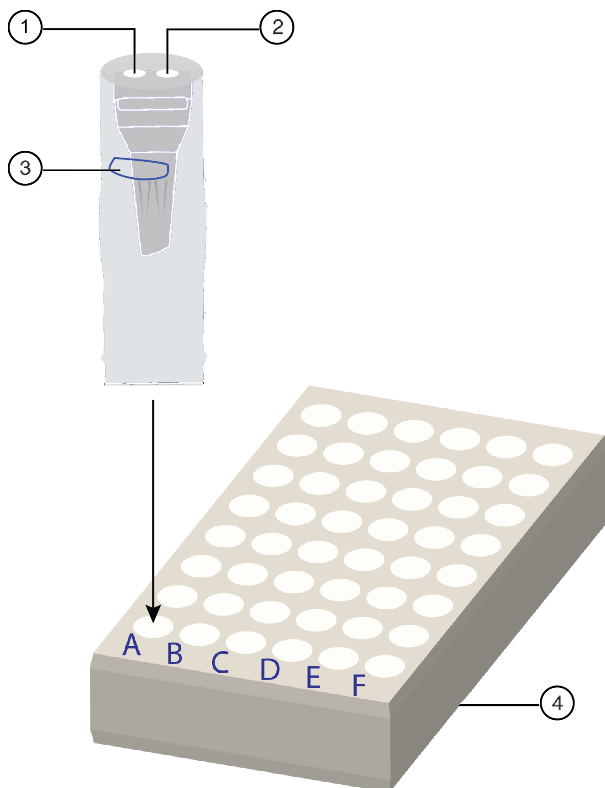
   

Item	Description
1	nanoVial inside the CESI vial with the thin pipette tip transferring sample
2	nanoVial inside the CESI vial with the cap attached

2. Attach a CESI cap.
3. Put the sample vial in the applicable position in the inlet sample tray.

   Note: Make sure that the tab on the outside of the nanoVial points to the front of the sample tray.

Figure 4-4 Orientation of the nanoVial Tab in the Sample Tray



Item	Description
1	Electrode at the left side of the nanoVial
2	Capillary at the right side of the nanoVial (for use in loading sample)
3	Tab outside of the nanoVial
4	Sample tray

Note: If the number of sample vials will not fill the tray, then keep every second column in the sample tray empty. This will make it easier to remove the nanoVials from the tray.

Note: When filling the vials, do not make air bubbles. If there are air bubbles in the vial, then spin the vial in a centrifuge for a few seconds to remove the bubbles.

Prepare the Buffer Trays

CAUTION: Potential System Damage. Use low-concentration buffers for separations with pH values > 7. High-concentration buffers can cause blockage in the separation capillary.

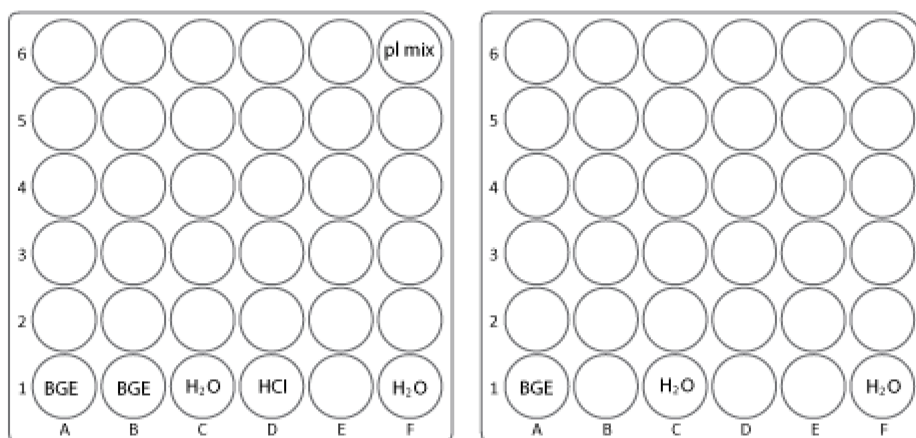
1. Use the following table to prepare the required reagents in CESI vials. Refer to the section: Fill a CESI Vial.

Table 4-1 Buffer Vial Contents by Sample Type

Sample Type	Position		
	BI:A1	**BI:B1**	**BO:A1**
Protein Test Mix or intact proteins in pH 3.0 buffer	50 mM AmAc buffer, pH 3.0	50 mM AmAc buffer, pH 3.0	50 mM AmAc buffer, pH 3.0
Peptide mixtures	10% HAc solution	10% HAc solution	10% HAc solution

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

Figure 4-5 Buffer Tray Layout



Prepare the System 5

To make sure that the system operates correctly, do the following procedures.

Condition the Capillaries

CAUTION: Potential System Damage. To prevent the sprayer tip from breaking, make sure that the sprayer tip is immersed in CE Grade water when the neutral CESI washing method is run. Remove the sprayer from the CE Grade water, and then install the sprayer in the adapter on the mass spectrometer before the neutral CESI electrical conditioning method is started.

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 both the neutral CESI washing method and the neutral CESI electrical conditioning method to condition the neutral capillaries.

- The neutral CESI washing method rehydrates the neutral coating of the capillaries.
- The neutral CESI electrical conditioning method establishes the electrical connection in the sprayer tip.

Refer to the section: Methods for the Neutral OptiMS Cartridge.

If both methods are not used, then earlier migration times, poor separation performance, loss of electrospray, and different electrical currents during separations can occur.

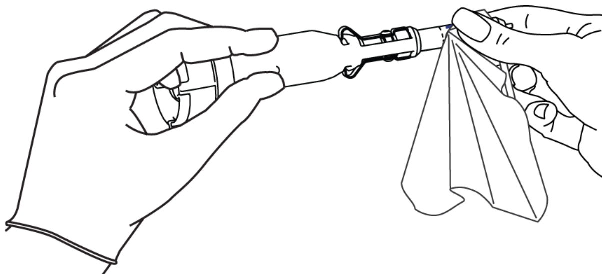
1. Remove the protective sleeve from the sprayer tip.
2. To make sure that the flow of liquid through the separation capillary is sufficient, do this:
    a. Do a forward rinse with CE Grade water at 100 psi for 3 minutes.
    b. When a liquid droplet shows at the end of the sprayer tip, stop the application 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 5 mL of CE Grade water in a 50 mL Falcon tube, and then put the Falcon tube in the holster on the side of the system.
6. Carefully immerse the sprayer tip in the Falcon tube.

Figure 5-1 Sprayer Immersed in Liquid

7. Run the neutral CESI washing 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 5-2 Dry the Sprayer



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

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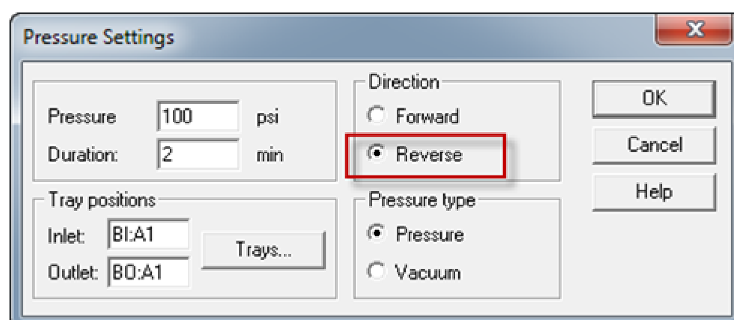
10. Make sure that the ESI voltage on the mass spectrometer is off.

11. Install the sprayer in the adapter on the mass spectrometer.
12. Run the neutral CESI electrical conditioning method.
    When the method is complete, the cartridge is ready to set a stable spray.
13. Remove the Falcon tube from the holster, and discard the contents.

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 Analyst TF 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.

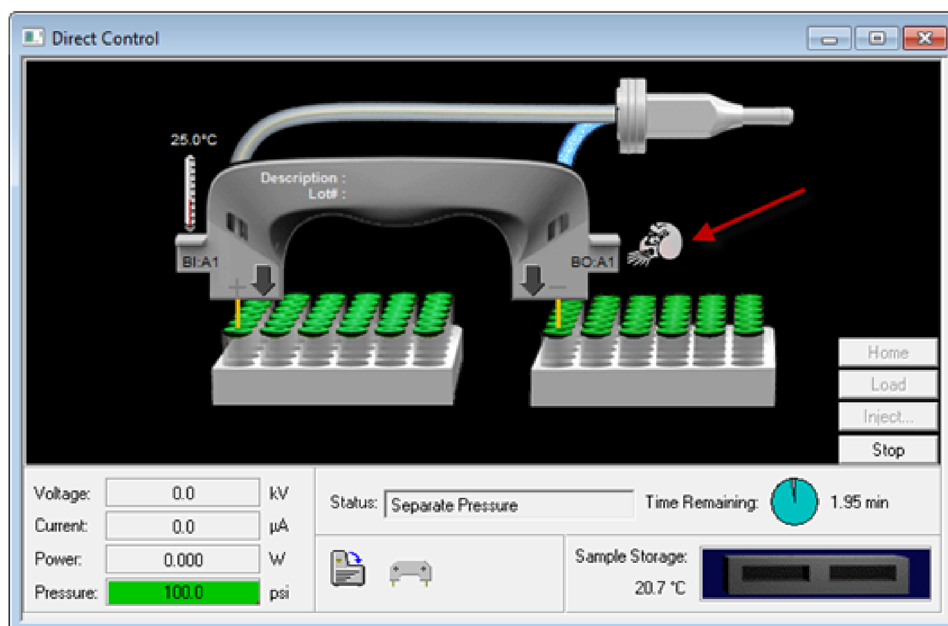
   Figure 5-3 Pressure Settings Dialog to Fill the Conductive Liquid Capillary

   c. In the **Pressure** field, type `100`.
   d. In the **Duration** field, type `5`.
   e. Click **Reverse**.
   f. Click **Pressure**.
   g. Click **OK**.

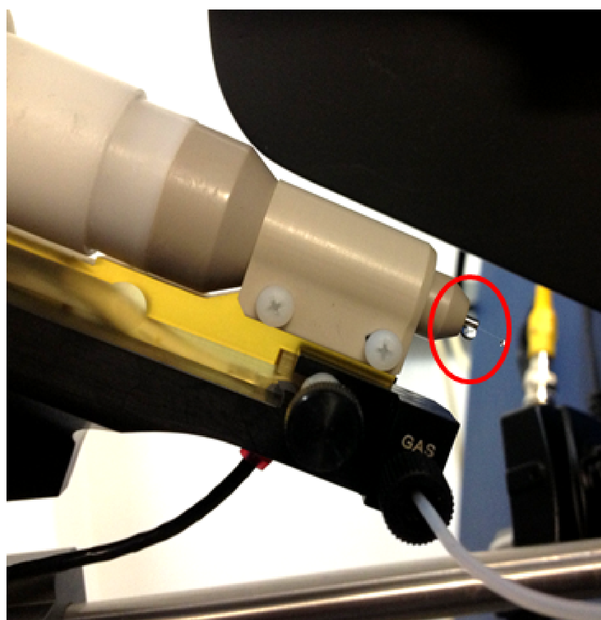
   To identify the direction of the pressure being applied, in the Direct Control window, look at the **Rinse Direction** icon (a blowing face identified by a red arrow in the following figure). The reverse direction refers to rinsing the conductive liquid capillary, and the blowing face shows on the right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

Figure 5-4 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 5-5 Droplet at the End of the Stainless Steel Needle

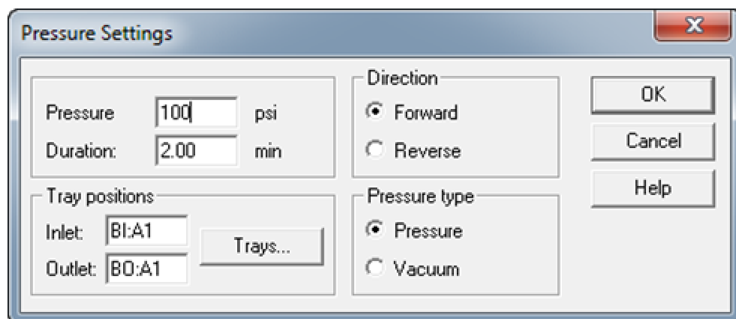


4. When the rinse is complete, to fill the separation capillary with BGE, do this:

a. Go to the Direct Control window.

b. Click the **Rinse** field.

Figure 5-6 Pressure Settings Dialog to Fill the Separation Capillary



c. In the **Pressure** field, type `100`.

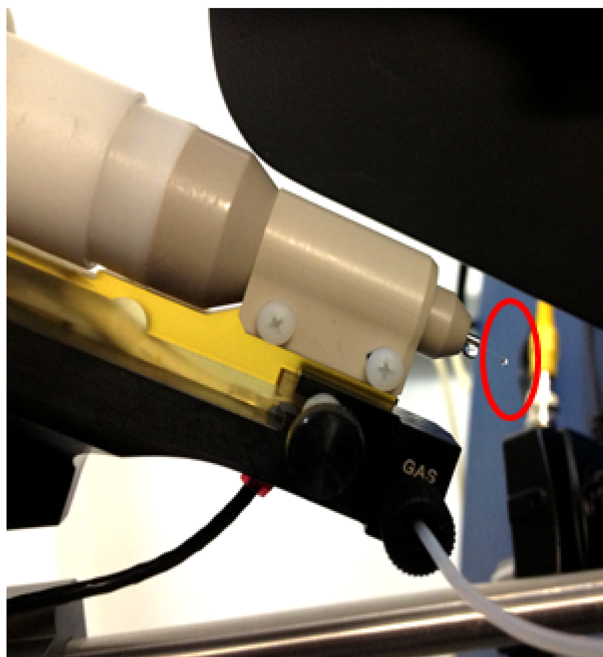
d. In the **Duration** field, type `5`.

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 5-7 Droplet at the End of the Sprayer Tip



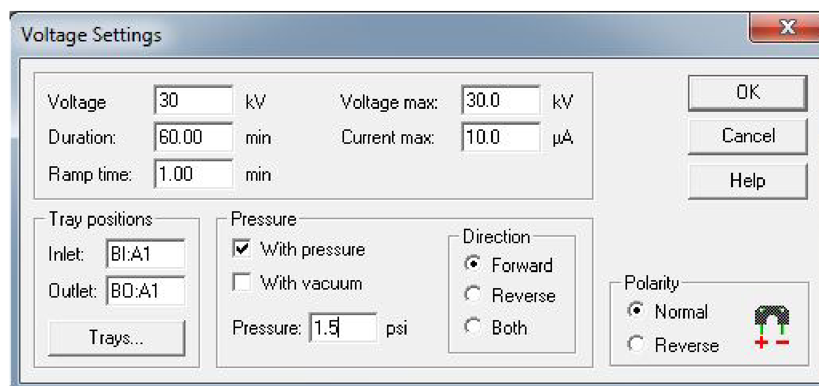
Note: If the separation capillary is empty, then the first droplet of solution might not show at the sprayer tip for as long as 7 minutes.

5. To find the minimum ESI voltage, refer to the section: Optimize the ESI Voltage.

Optimize the ESI Voltage

1. Fill the capillary with Protein Test Mix.
2. Make sure that the separation capillary and conductive liquid capillary are filled with BGE.
3. 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.

    Figure 5-8 Voltage Settings Dialog

    b. In the **Voltage** field, type `30`.
    c. In the **Duration** field, type `60`.
    d. In the **Ramp time** field, type `1`.
    e. Select the **With pressure** check box.
    f. In the **Pressure** field, type `1.5`.
    g. Click **Normal**.
    h. 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.

4. In the Analyst TF software, in the Navigation bar, under Tune and Calibrate, double-click **Manual Tuning**.
5. 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. To change the setting for gas for the Curtain Gas interface, use the Curtain Gas patch application that is available on the computer for the mass spectrometer.

b. In the **IonSpray Voltage Floating (ISVF)** field, type `1.0`.

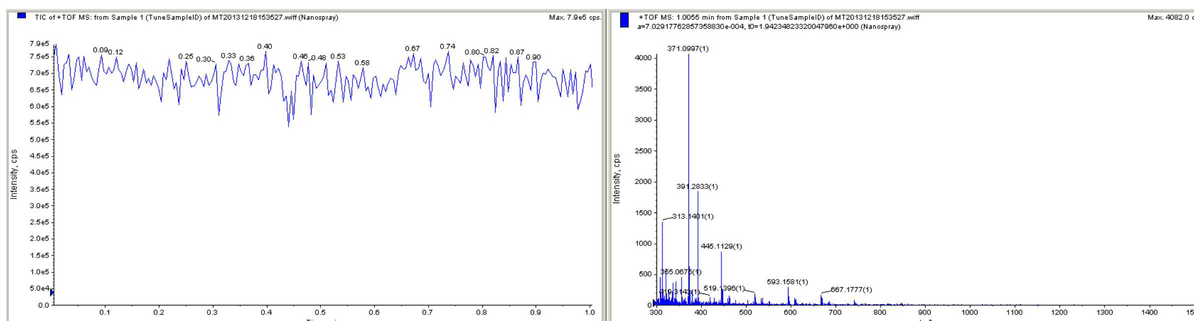
The **IonSpray Voltage Floating (ISVF)** parameter sets the ESI voltage.

c. In the **Interface Heater Temperature (IHT)** field, type `50.0`.

6. 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`.
7. Click **Start**.
8. 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 *m/z* 200 to *m/z* 2000.
   c. Click **Start**.
9. 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 5-9 Typical Spray Profile of a Cartridge that Uses 10% HAc Solution as the BGE



10. In the Direct Control window, click **Stop**.
11. 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.

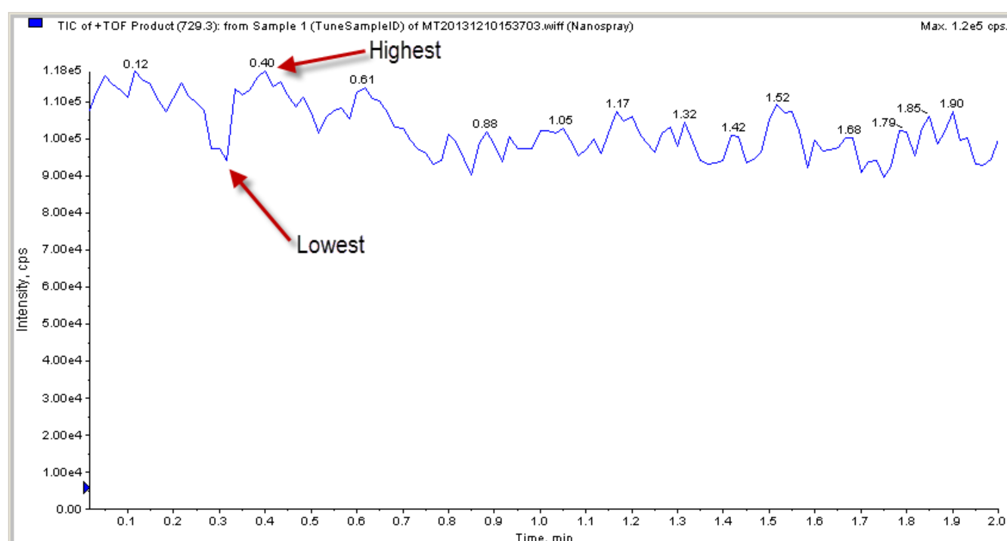
12. In the **IonSpray Voltage Floating (ISVF)** field, type the minimum ESI voltage, and then click **OK**.
    Make sure that electrospray starts again.
13. In the **IonSpray Voltage Floating (ISVF)** field, increase the value by 100 V.
14. To find the best position for the sprayer in relation to the 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.

15. To check the stability of the spray, continue to apply 20 kV (normal polarity) for 20 minutes.
16. 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`.

17. Click **Acquire**, and then type a file name (for example, `Baseline`).
18. 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.
19. Use the Y-axis alignment knob to maximize the baseline fluctuations.

Figure 5-10 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.
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.

20. 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.

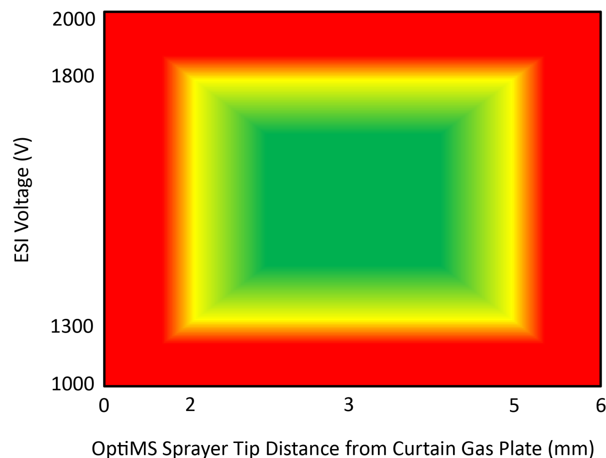
    b. Turn off the electrospray voltage on the mass spectrometer.

Fine-Tune the Position of the Sprayer Tip

CAUTION: Potential System Damage. Make sure that the sprayer tip is in position outside of the curtain gas plate. If the sprayer tip is too close, then rinse solution can drip onto the curtain gas plate and cause damage.

Before a separation is run, it is critical to optimize the position of the sprayer tip in front of the curtain gas plate to get the correct ESI voltage. If the sprayer tip is too far away from the curtain gas plate, 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 5-11 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

1. Fill the separation capillary and the conductive liquid capillary with BGE.

2. Use the XYZ-axes adjustment knobs to move the sprayer tip 3 mm from the curtain gas plate.
3. In the 32 Karat software, use the Direct Control window to apply 30 kV voltage and 1.5 psi forward pressure with a ramp of 1 minute.
    - 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 1,000 V and then increase the value in increments of 100 V until electrospray is detected.
5. Increase the ESI voltage by 200 V.
6. Use the XYZ-axes adjustment knobs to move the sprayer tip and maximize the signal intensity of the mass spectrometer.

   Make sure that the sprayer tip is approximately 3 mm from the curtain gas plate.
7. When the position of the sprayer is optimized, set the ESI voltage to zero.
8. Set the ESI voltage to 1,000 V and then increase the value in increments of 100 V 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 200 V.

   Note: This is the optimal ESI voltage. Use this voltage with the mass spectrometer method to provide a stable electrospray during separation.
10. Set the ESI voltage to zero.
11. Turn off the separation voltage.

Manual Calibration

To get the most accurate mass determination, manually calibrate the mass spectrometer.

Note: The following procedures were made for the SCIEX 6600 system. For other SCIEX mass spectrometers or mass spectrometers from other manufacturers, use the manufacturer's recommendations.

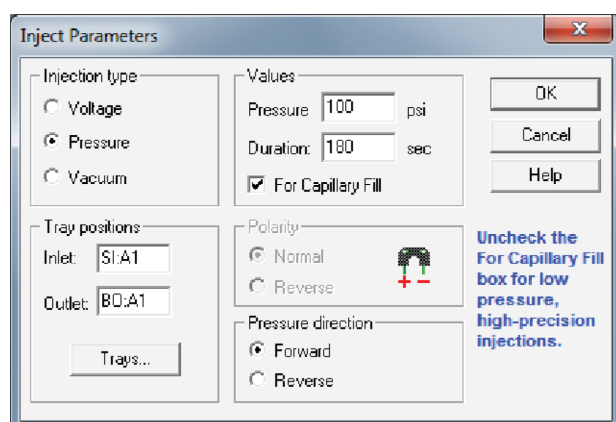
After the mass spectrometer has been manually calibrated, include autocalibration in the sequence when data is acquired for samples. Refer to the section: About Autocalibration.

For more information about calibration, refer to the document: *AB SCIEX Mass Calibration Tutorial*. To open the document, click **Start Menu** > **All Programs** > **AB SCIEX** > **Analyst TF 1.7** > **Hardware and Software Guides**.

Prepare for Manual Calibration

1. In a sample vial, add 90 µL of PepCalMix and 10% HAc solution to make a 1:1 solution.
2. Put the vial in position A1 (SI:A1) in the sample tray.
3. In the 32 Karat software, go to the Direct Control window, and then click **Inject**.

Figure 5-12 Inject Parameters Dialog

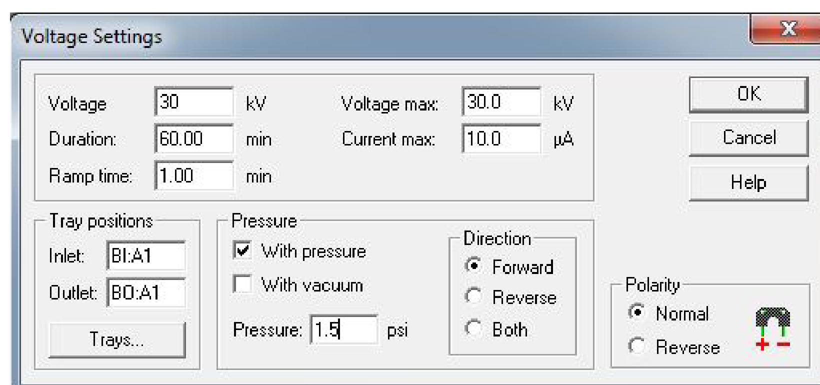


4. Select the injection parameters.

Note: To inject at 100 psi for 180 seconds, make sure that the **For Capillary Fill** check box is selected.

5. When the capillary is filled, go to the Direct Control window, and then click **Voltage**.

Figure 5-13 Voltage Settings Dialog



6. In the **Voltage** field, type `30`.
7. In the **Duration** field, type `20`.
8. Select the **With pressure** check box.
9. In the **Pressure** field, type `1.5`.
10. Make sure that the capillary inlet is in position BI:A1 and the outlet is in position BO:A1.
11. Click **OK**.

Create a Reference Table

1. In the Analyst TF software, in the Navigation bar, click **Acquire**.
2. Click **Tools** > **Settings** > **Tuning Options**, and then click **Reference**.

Figure 5-14 Reference Table Editor Window

Reference Table Editor

Name: MixSCIEX-AgI | New | Copy | Delete | Positive | Negative | Calibration Valve Position: A

Reference Ions for TOF MS Calibration:

	Use	Compound Name	Precursor m/z (Da)	Use for MS/MS	CE for MS/MS	DP for MS/MS	Retention Time (min)
6	☑	amino-dPEG 8-acid	442.26467	☐	20.000	50.000	0.00
7	☑	**Reserpine**	**609.28066**	☑	**20.000**	**50.000**	**0.00**
8	☑	Ion 3	622.02896	☐	42.000	80.000	0.00
9	☑	ALILTLVS	829.53933	☐	20.000	50.000	0.00
10	☑	Ion 4	922.00980	☐	42.000	80.000	0.00
11	☑	ALILTLVS + Cs	961.43696	☐	20.000	50.000	0.00
12	☑	Ion 5	1221.99064	☐	42.000	80.000	0.00
13	☑	Heptakis(2,3,6-tri-O-	1446.73224	☐	20.000	50.000	0.00
14	☑	Ion 6	1521.97148	☐	42.000	80.000	0.00
15	☑	Heptakis(2,3,6-tri-O-	1561.60332	☐	20.000	50.000	0.00
16	☑	Ion 7	1821.95231	☐	42.000	80.000	0.00
17	☑	Tryaceyl-b-cyclode	2034.62545	☐	20.000	50.000	0.00

Reference Ions for MS/MS Calibration:
(Product of 609.28066 Da)

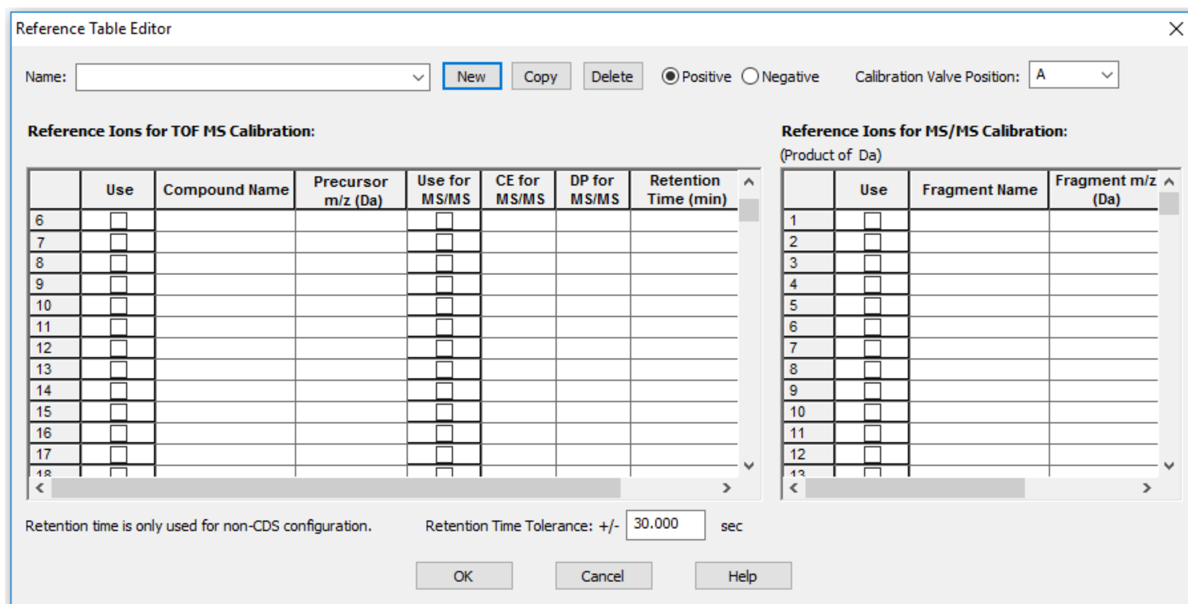
	Use	Fragment Name	Fragment m/z (Da)
1	☑	y1	174.09130
2	☑	y3	195.06520
3	☑	y5	236.12810
4	☐		365.18600
5	☑	y8	397.21220
6	☑	y10	448.19660
7	☑	y12	609.28070
8	☑		609.28066
9	☐		
10	☐		
11	☐		
12	☐		

Retention time is only used for non-CDS configuration. Retention Time Tolerance: +/- 30.000 sec

OK | Cancel | Help

3. To open a new, empty reference table, click **New**.

Figure 5-15 New Reference Table in the Reference Table Editor Window



4. In the **Name** field, type a name that identifies the calibration solution (in this example, `PepCalMix`).
5. Make sure that **Positive** is clicked.
6. In the Reference Ions for TOF MS Calibration table, type the following values.

Table 5-1 Reference Ions for TOF MS Calibration

Compound Name	Precursor *m/z* (Da)	CE for MS/MS	DP for MS/MS
AETSELHTSLK	408.55010	40	80
GAYVEVTAK	473.26020	40	80
IGNEQGVSR	485.25302	40	80
LVGTPAEER	491.26559	40	80
LDSTSIPVAK	519.79969	40	80
AGLIVAEGVTK	533.32333	40	80
LGLDFDSFR	540.27342	40	80
GFTAYYIPR	549.28633	40	80
SGGLLWQLVR	569.83398	40	80
AVGANPEQLTR	583.31360	40	80

Table 5-1 Reference Ions for TOF MS Calibration (continued)

Compound Name	Precursor *m/z* (Da)	CE for MS/MS	DP for MS/MS
SAEGLDASASLR	593.80053	40	80
VFTPLEVDVAK	613.34955	40	80
VGNEIQYVALR	636.35273	40	80
YIELAPGVDNSK	657.34499	40	80
DGTFAVDGPGVIAK	677.85827	40	80
YDSINNTEVSGIR	739.36148	40	80
SPYVITGPGVVEYK	758.91050	40	80
ALENDIGVPSDATVK	768.90340	40	80
AVYFYAPQIPLYANK	883.47380	40	80
TVESLFPEEAETPGSAVR	964. 97741	40	80

7. For each compound, click the **Use** check box.
8. In the Reference Ions for MS/MS Calibration table, type the following values.

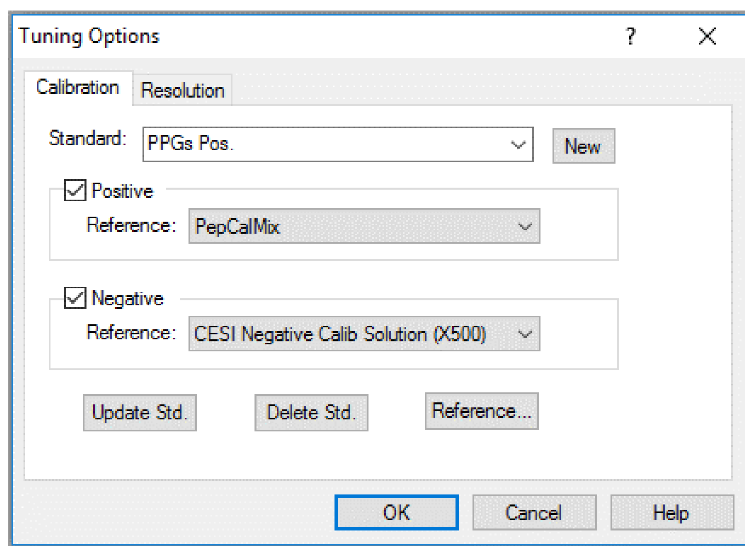
Table 5-2 Reference Ions for MS/MS Calibration

Fragment Name	Fragment *m/z* (Da)
b2	185.09207
b3	348.15540
b4	560.30788
b5	661.35555
N/A	758.91050
y7	799.44398
y8	856.46544
y9	957.51312
y10	1070.59719
y11	1169.66560
y12	1332.72893

9. For each fragment, click the **Use** check box.

10. In the **Use for MS/MS** column, select the check box for the applicable compound in the calibration solution (in this example, the peptide at *m/z* 758.91).
11. Make sure that the value in the **Retention time tolerance +/-** field is **30**.
12. Click **OK**.

Figure 5-16 Tuning Options Dialog



13. Make sure that the name of the new reference table (in this example, **PepCalMix**) shows in the **Positive Reference** field.
14. Click **OK**.

Manually Calibrate in TOF MS Mode

1. In the Analyst TF software, in the Navigation bar, under Tune and Calibrate, double-click **Manual Tuning**.

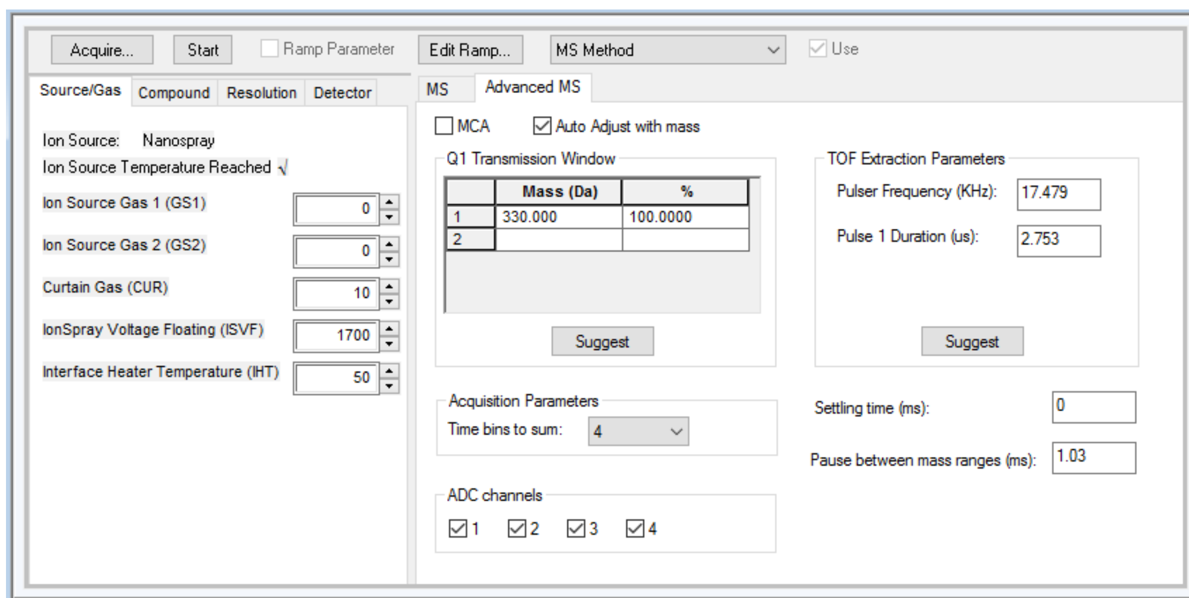
Figure 5-17 Tune Method Editor Window

2. On the Source/Gas tab, do this:
   a. In the **Ion Source Gas1 (GS1)** field, type `0` (zero).
   b. In the **Ion Source Gas1 (GS2)** field, type `0` (zero).
   c. In the **Curtain Gas (CUR)** field, keep the default value, or type `10`.

   Note: To change the setting for gas for the Curtain Gas interface, use the Curtain Gas patch application that is available on the computer for the mass spectrometer.

   d. In the **Interface Heater Temperature (IHT)** field, type `50`.
3. On the MS tab, do this:
   a. In the **Scan type** list, select **TOF MS**.
   b. In the **Accumulation time** field, type `1.000`.
   c. In the TOF Masses (Da) **Min** field, type `350`.
   d. In the TOF Masses (Da) **Max** field, type `1000`.
   e. In the Period **Duration** field, type `1000`.
4. Open the Advanced MS tab.

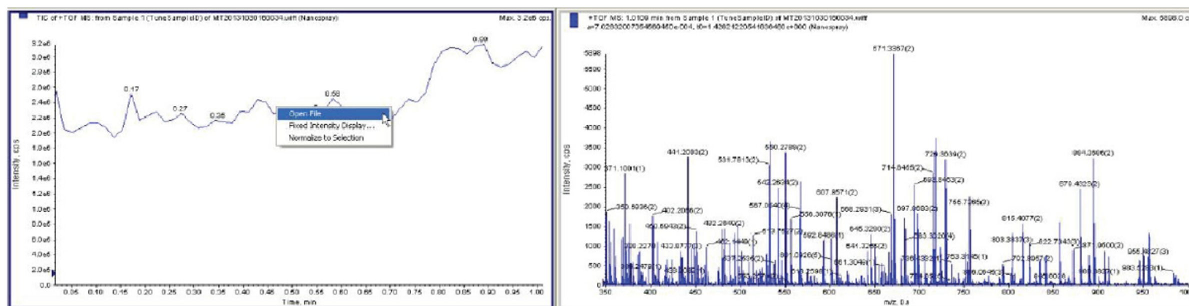
Figure 5-18 Tune Method Editor Window: Advanced MS Tab



5. Make sure that the **MCA** check box is cleared.
6. In the **IonSpray Voltage Floating (ISVF)** field, type a value.

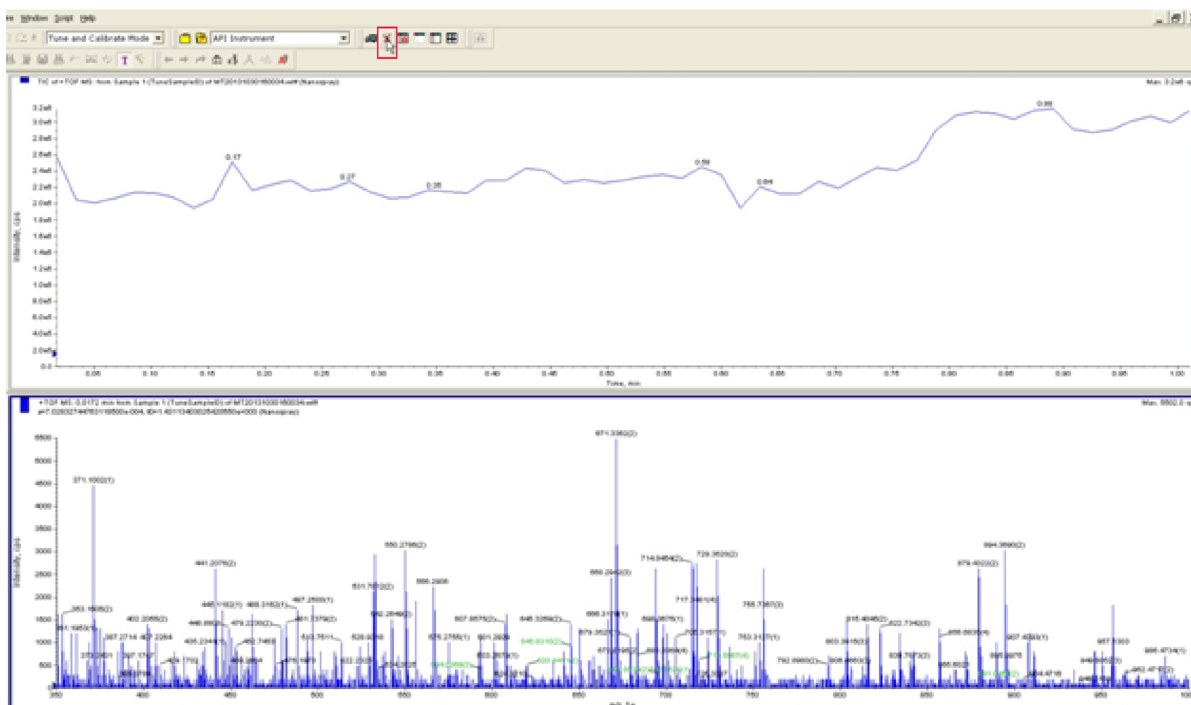
   The **IonSpray Voltage Floating (ISVF)** parameter sets the ESI voltage.
7. Adjust the position of the sprayer until the spray is stable.
8. Click **Start**.
   After approximately 1 minute, windows open to show the total ion chromatogram (TIC) and mass spectrum.

Figure 5-19 TIC and Mass Spectrum Windows



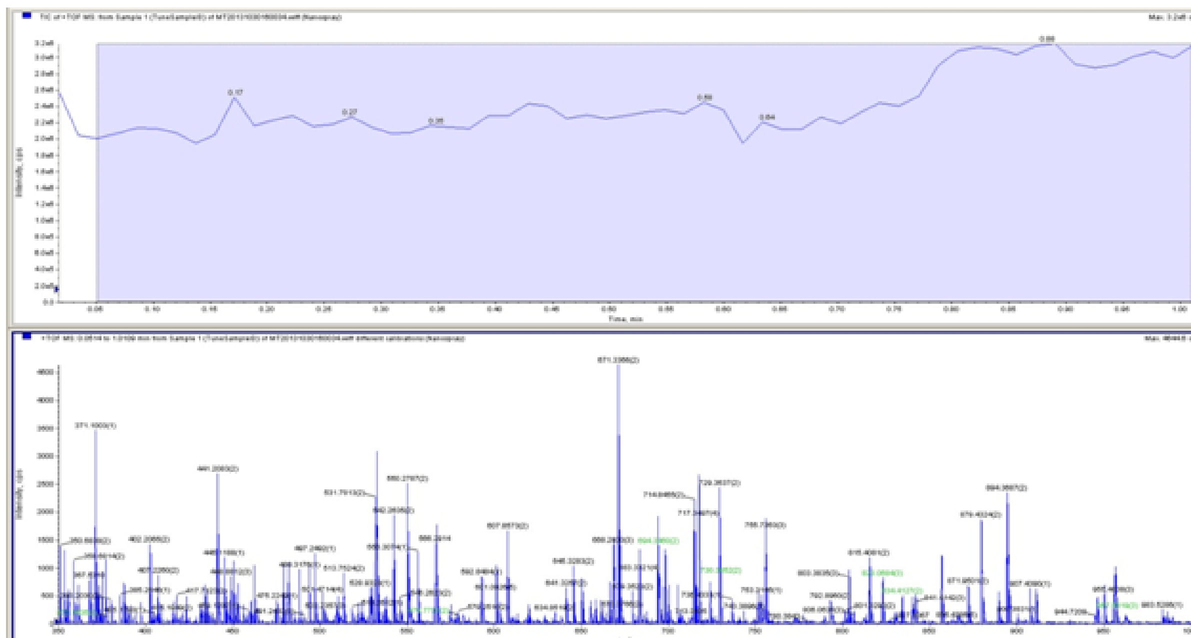
9. To open windows that show the TIC and mass spectrum for the data file that was acquired, right-click the TIC window, and then click **Open File**.

Figure 5-20 TIC and Mass Spectrum Windows from the Acquired Data File



10. To delete the mass spectrum pane, click the pane, and then on the toolbar, click the **Delete** icon.
11. To get an average mass spectrum, click and drag the cursor to highlight the TIC window, and then double-click the TIC window.

Figure 5-21 Average Mass Spectrum Pane



12. Right-click the mass spectrum pane, and then click **Re-Calibrate TOF**.

Figure 5-22 TOF Calibration Window

TOF Calibration

Reference Table PepCalMix tolerance 0.2 Da

	Experimental Mass	Theoretical Mass
1	408.550675	408.550100
2	473.260049	473.260200
3	485.252907	485.253020
4	491.265683	491.265590
5	519.799530	519.799690

Calculate new calibrations

Average Error: 0.317107 ppm

External Calibration...

SAVE CURRENT CALIBRATION

Selected Range — Calibration is applied to selected range of scans

Whole Sample — Calibration is applied to all scans in current sample

Entire File — Calibration is applied to all samples in the file

Set As Instrument Default

CALIBRATION VALUES

	a	t0
Current	7.02161077082593320e-004	-1.38574153765218070e+001
New	---	---

Calibrate spectrum

Close

Help

13. From the **Reference Table** list, select the reference table that was created for the calibrant (in this example, **PepCalMix**).

    Note: If the correct reference file does not show in the list, then the reference table has not been created. Refer to the section: Create a Reference Table.

14. In the **Tolerance** field, type `0.2`.
15. To calculate the average error for this new calibration, click **Calculate new calibrations**.
16. Make sure that the value for the Average Error is within the routine operating standards for the mass spectrometer being calibrated.
17. Click **Calibrate spectrum**.
    The new calibration values show.
18. Make sure that the **Set As Instrument Default** check box is selected.

    Note: If an ion is not found during calibration, then right-click the missing ion in the reference table, and then click **Delete**. Click **Calculate new calibrations**.

19. To apply this calibration to all samples in the file, click **Entire File**.
20. Click **Save**.
21. Click **OK** twice.
22. To close the TOF Calibration window, click **Close**.
23. Close the TIC and mass spectrum panes.

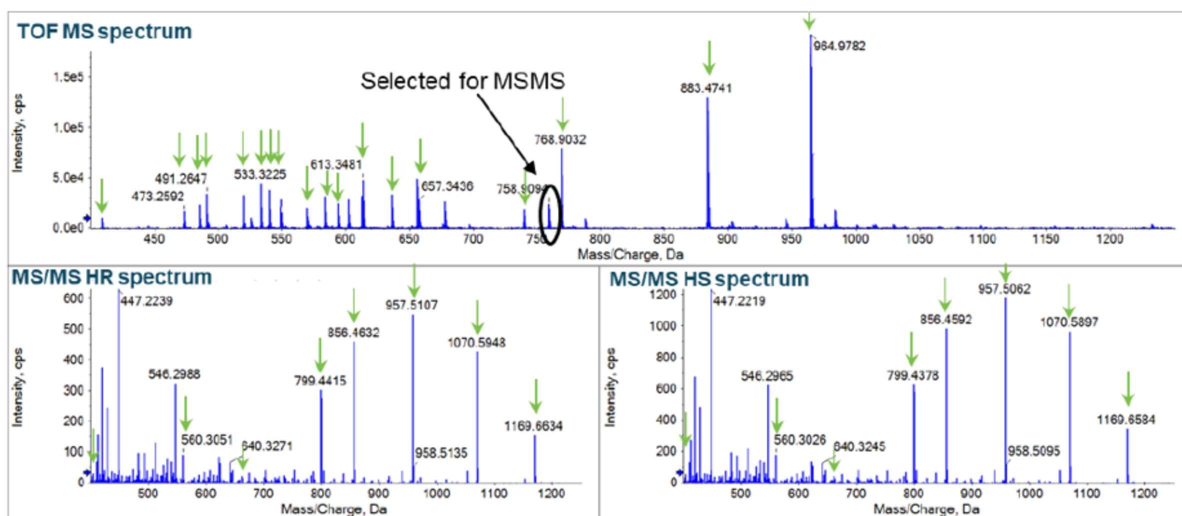
Manually Calibrate in Product Ion Mode

1. In the Analyst TF software, in the Navigation bar, under Tune and Calibrate, double-click **Manual Tuning**.

Figure 5-23 Tune Method Editor Window: Compound Tab

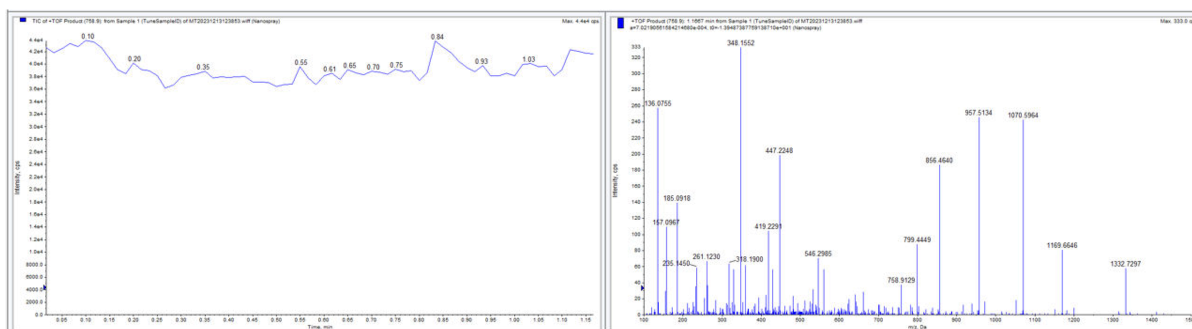
2. Open the Compound tab, and then do this:
   a. In the **Collision Energy** field, type `42`.
   b. In the **Collision Energy Speed (CES)** field, type `5`.
3. On the MS tab, do this:
   a. In the **Scan type** list, select **Product Ion**.
   b. In the **Product Of** field, type `758.91`.
   c. In the TOF Masses (Da) **Min** field, type `100`.
   d. In the TOF Masses (Da) **Max** field, type `1500`.
   e. Make sure that **High Sensitivity** is selected.
4. Click **Start**.
   Windows open to show the total ion chromatogram (TIC) and mass spectrum generated during the product ion calibration.

Figure 5-24 TIC and Mass Spectrum Windows



5. Right-click the TIC window, and then click **Open File**.
   Windows open to show the TIC and mass spectrum for the product ion calibration data file that was acquired.

Figure 5-25 TIC and Mass Spectrum Windows from the Product Ion Calibration Data File

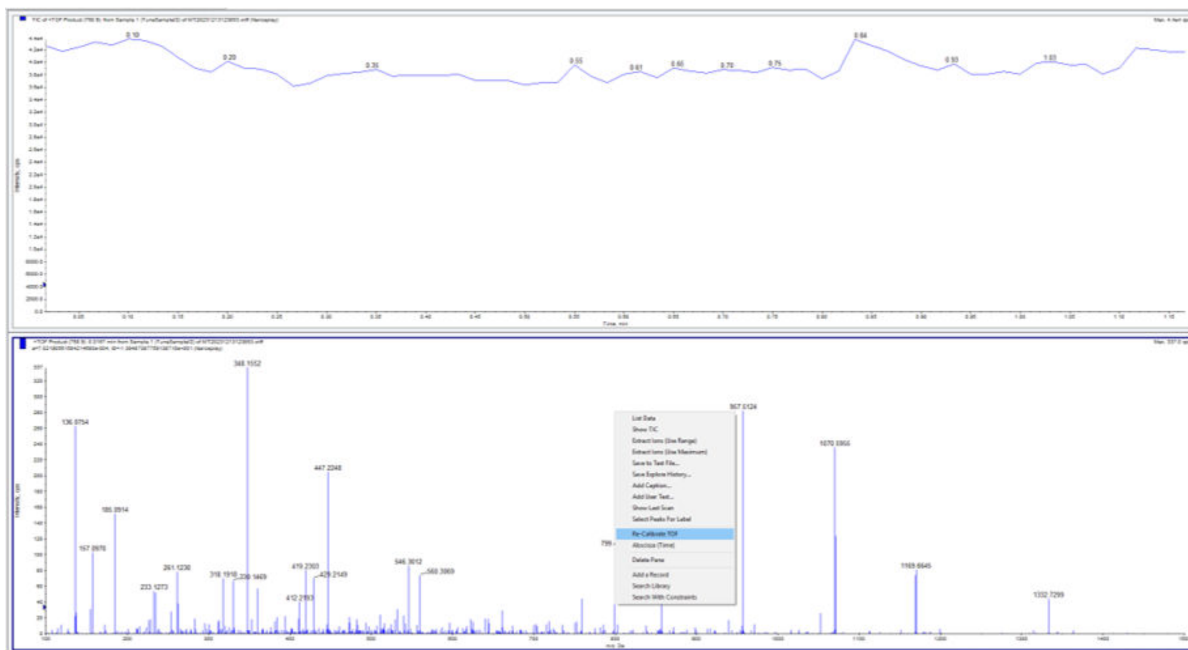


Note: The spectrum that is shown cannot be used for calibration.

---

6. Click the mass spectrum window, and then on the toolbar, click the **Delete** icon.
7. To get an average mass spectrum, click and drag the cursor to highlight the TIC window, and then double-click the TIC window.

Figure 5-26 Re-Calibrate TOF



8. Right-click the mass spectrum window, and then click **Re-Calibrate TOF**.

Figure 5-27 TOF Calibration Window

TOF Calibration

Reference Table PepCalMix tolerance 0.2 Da

	Experimental Mass	Theoretical Mass
1	185.091436	185.092070
2	560.306908	560.307880
3	661.353623	661.355550
4	758.911622	758.910500
5	799.441851	799.443980

Calculate new calibrations — Average Error: 1.884388 ppm

External Calibration...

SAVE CURRENT CALIBRATION

Selected Range — Calibration is applied to selected range of scans

Whole Sample — Calibration is applied to all scans in current sample

Entire File — Calibration is applied to all samples in the file

☑ Set As Instrument Default

CALIBRATION VALUES

	a	t0
Current	7.02190561584214680e-004	-1.39487387759138710e+001
New	---	---

Calibrate spectrum

Close Help

9. From the **Reference Table** list, select the reference table that was created for the calibrant (in this example, **PepCalMix**).

   Note: If the correct reference file does not show in the list, then the reference table has not been created. Refer to the section: Create a Reference Table.

10. In the **Tolerance** field, type `0.2`.
11. To calculate the average error for this new calibration, click **Calculate new calibrations**.
12. Make sure that the Average Error is within the routine operating standards for the mass spectrometer being calibrated.
13. Click **Calibrate spectrum**.
    The new calibration values show.
14. Make sure that the **Set As Instrument Default** check box is selected.
15. To apply this calibration to all samples in the file, click **Entire File**.
16. Click **Save**.
17. Click **OK** twice.
18. To close the TOF Calibration window, click **Close**.
19. Close the TIC and mass spectrum windows.

Methods for the Neutral OptiMS Cartridge 6

The methods and sequences for the neutral OptiMS cartridge have been updated for robustness. Save the methods to the `C:\32karat\projects\CEMS\Methods` folder.

Table 6-1 Methods for the Neutral OptiMS Cartridge

File Name	Method Description
Neutral CESI electrical conditioning method	Used to create the electrical path in the sprayer of a new cartridge or a cartridge that has been in long-term storage.
Neutral CESI peptide separation method	Used to inject and separate a peptide mixture.
Neutral CESI protein separation method	Used to inject and separate a protein sample.
Neutral CESI rest method	Used to clean the separation capillary and the conductive liquid capillary at the end of a sequence before the cartridge is removed for long-term storage.
Neutral CESI washing method	Used to rehydrate the neutral coating of a new cartridge or a cartridge that has been in long-term storage.
CLC conditioning method	Used to condition the conductive liquid capillary

If the methods are missing, then use the following parameters on the Initial Conditions and Time Program tabs to create them manually.

Initial Conditions Tab

All of the methods for the neutral OptiMS cartridge use the initial conditions in the following figure.

Figure 6-1 Initial Conditions Tab

Auxiliary data channels
- [x] Voltage max: 30.0 kV
- [x] Current max: 5 µA
- [ ] Power
- [x] Pressure

Mobility channels
- [ ] Mobility
- [ ] Apparent Mobility
- [x] Plot trace after voltage ramp

Analog output scaling
Factor: 1

Temperature
Cartridge: 20.0 °C
Sample storage: 10.0 °C

Peak detect parameters
Threshold 2
Peak width: 9

Trigger settings
- [ ] Wait for external trigger
- [x] Wait until cartridge coolant temperature is reached
- [x] Wait until sample storage temperature is reached

Inlet trays
Buffer: 36 vials
Sample: 48 vials

Outlet trays
Buffer: 36 vials
Sample: No tray

Time Program Tab

Each of the methods for the neutral OptiMS cartridge uses a different time program.

Figure 6-2 Neutral CESI Washing Method

Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:D1	BO:A1	forward	0.1 M HCl Separation capillary rinse
2		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:A1	forward	BGE-Separation capillary fill
3		Rinse - Pressure	100.0 psi	5.00 min	BI:C1	BO:C1	reverse	DDI water-Conductive Liquid Capillary fill
4		Rinse - Pressure	100.0 psi	30.00 min	BI:C1	BO:C1	forward	DDI water-Separation capillary rinse
5								

For the neutral CESI electrical conditioning method, use the following parameters:

- In steps 1 and 2, use 50 mM AmAc buffer, pH 3.0 for the BGE.
- In steps 3 and 4, use a pressure of 5 psi at both ends.

Figure 6-3 Neutral CESI Electrical Conditioning Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	3.00 min	BI:A1	BO:A1	reverse	Fill CLC with BGE.
2		Rinse - Pressure	100.0 psi	5.00 min	BI:A1	BO:A1	forward	Fill separation capillary with BGE.
3	0.00	Separate - Voltage	30.0 KV	60.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both	30 kV voltage application with 5 psi pressure at both ends.
4	60.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, normal polarity, both	Ramp down with 5 psi pressure at both ends.
5	65.00	End						

For the neutral CESI protein separation method, use the following parameters:

- In step 7, use a forward pressure of 0.5 psi and a reverse pressure of 0.5 psi to prevent the conductive line needle from drying.

- In step 9, use a forward pressure of 1.5 psi and a reverse pressure of 1.5 psi to prevent the conductive line needle from drying.
- In step 10, use a forward pressure of 50 psi and a ramp time of 5 minutes to decrease the voltage.

Note: The ramp down in step 10 can be omitted. Line 11 would be at time 21.

Figure 6-4 Neutral CESI Protein Separation Method

Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	3.00 min	BI:D1	BO:A1	forward, In / Out	0.1M HCl rinse
2		Rinse - Pressure	75.0 psi	3.00 min	BI:A1	BO:A1	reverse, In / Out	BGE Conductive Liquid Capillary fill
3		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:A1	forward, In / Out	BGE separation capillary fill
4		Inject - Pressure	2.5 psi	15.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic injection of sample
5		Wait		0.00 min	BI:C1	BO:A1	In / Out vial inc 1	Water dip
6		Inject - Pressure	2.5 psi	10.0 sec	BI:B1	BO:A1	No override, forw	Hydrodynamic injection of BGE plug
7	0.00	Separate - Voltage	30.0 KV	10.00 min	BI:B1	BO:A1	1.00 Min ramp, n	Separation at low flow
8	1.00	Relay On					1: 0.10 2: 0.10	Trigger MS
9	10.00	Separate - Voltage	30.0 KV	11.00 min	BI:B1	BO:A1	1.00 Min ramp, n	Separation at high flow
10	21.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, n	voltage ramp down to 1kV with 50 psi FWD pressure
11	26.00	End						
12								

For the neutral CESI peptide separation method, use the following parameters:

- In step 7, use a forward pressure of 0.5 psi and a reverse pressure of 0.5 psi to prevent drying.
- In step 9, use a forward pressure of 2.0 psi and a reverse pressure of 2.0 psi to prevent drying.
- In step 10, use the values in the Separate dialog to decrease the voltage. This step is mandatory for the method.

Note: The ramp down in step 10 can be omitted.

Figure 6-5 Neutral CESI Peptide Separation Method: Separate Dialog

Separate

Separation Type: (•) Voltage, ( ) Current, ( ) Power, ( ) Pressure, ( ) Vacuum

Options: [x] With Pressure, [ ] With Vacuum

Polarity: (•) Normal, ( ) Reverse

Values: Voltage 1.0 KV; Pressure: 50.0 psi; Duration: 5.00 min; Ramp Time: 5.00 min

Tray Positions: Inlet: BI:B1; Outlet: BO:A1

Increment: [x] Inlet [x] Outlet

Increment Every 6 Cycles

Trays...

Pressure Direction: (•) Forward, ( ) Reverse, ( ) Both

[x] At Time: 80.00 min

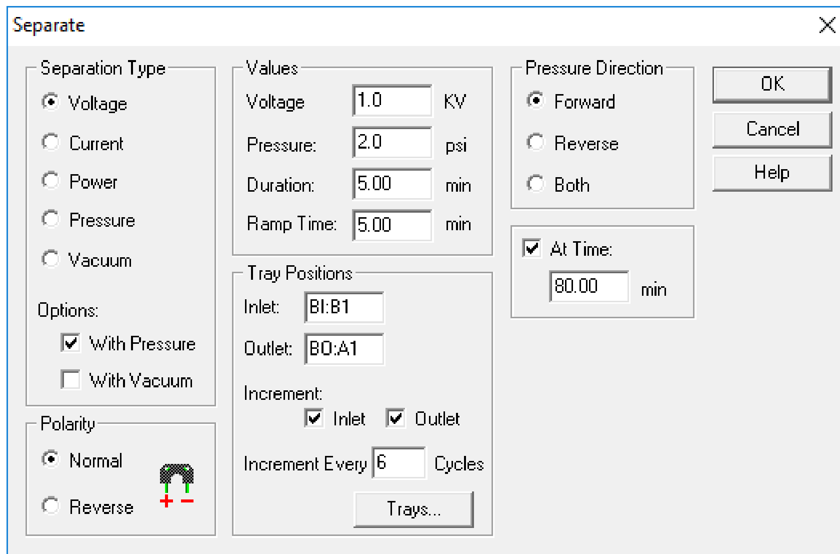
OK | Cancel | Help

Figure 6-6 Neutral CESI Peptide Separation Method

Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:D1	BO:A1	forward, In / Out	0.1M HCl rinse
2		Rinse - Pressure	75.0 psi	3.00 min	BI:A1	BO:A1	reverse, In / Out	BGE Conductive Liquid Capillary fill
3		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:A1	forward, In / Out	BGE separation capillary fill
4		Inject - Pressure	5.0 psi	60.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic injection of sample with LE
5		Wait		0.00 min	BI:C1	BO:A1	In / Out vial inc 6	Water dip
6		Inject - Pressure	2.5 psi	15.0 sec	BI:B1	BO:A1	No override, forw	Hydrodynamic injection of BGE
7	0.00	Separate - Voltage	30.0 KV	25.00 min	BI:B1	BO:A1	1.00 Min ramp, n	Separation at low flow
8	1.00	Relay On					1: 0.10 2: 0.10	Trigger MS
9	25.00	Separate - Voltage	30.0 KV	56.00 min	BI:B1	BO:A1	1.00 Min ramp, n	Separation at high flow
10	81.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, n	Voltage ramp down to 1kV with 50 psi FWD pressure
11	86.00	End						
12								

Figure 6-7 Neutral CESI Peptide Separation Method: Separate Dialog with Parameters to Ramp Down the Separation Voltage



If the cartridge will be used again within 24 hours, then to prevent blockage in the sprayer tip, in step 3 of the neutral CESI rest method, change the rinse pressure to 5 psi and the duration to 999 min.

Figure 6-8 Neutral CESI Rest Method

Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:D1	BO:F1	forward	0.1 M HCl rinse
2		Rinse - Pressure	75.0 psi	5.00 min	BI:C1	BO:C1	reverse	Conductive Liquid Capillary water rinse
3		Rinse - Pressure	100.0 psi	10.00 min	BI:C1	BO:C1	forward	Separation line water rinse
4								

Do a Cartridge Performance Test 7

After the mass spectrometer and CESI 8000 Plus system are set up, do the following procedure to make sure that the neutral OptiMS cartridge operates correctly.

1. In a microvial, add 100 µL of Protein Test Mix sample.
2. Put the sample vial in the inlet sample tray in position S1:A1.
3. If required, then update the sample location in the sequence, and then save the sequence.
4. Do a check of the coolant level in the CESI 8000 Plus system.
   If required, then add coolant. Refer to the section: Add Capillary Cartridge Coolant.
5. Start the mass spectrometer batch.
6. Make sure that the mass spectrometer is ready to acquire.
7. Start the neutral CESI protein separation method.

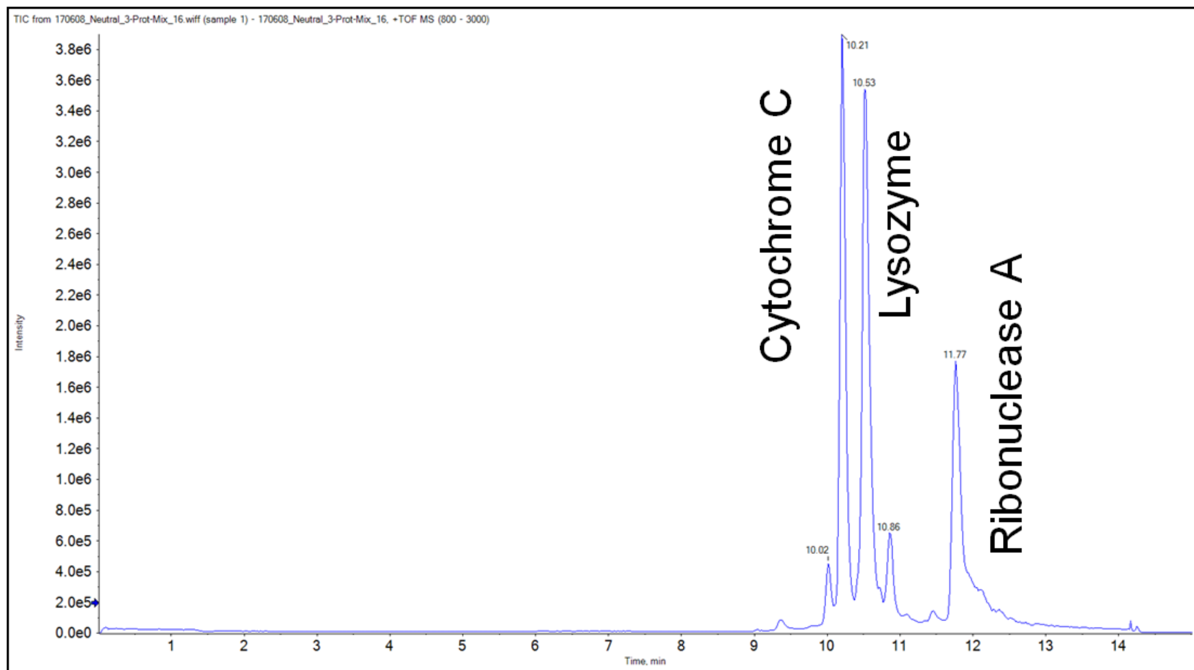
Analyze the Data (Protein Test Mix Sample)

When the separation of the Protein Test Mix is complete, look at the detection times to make sure that all three proteins are in the 8 minute to 15 minute range.

If the proteins are not separated, then refer to the section: Troubleshooting.

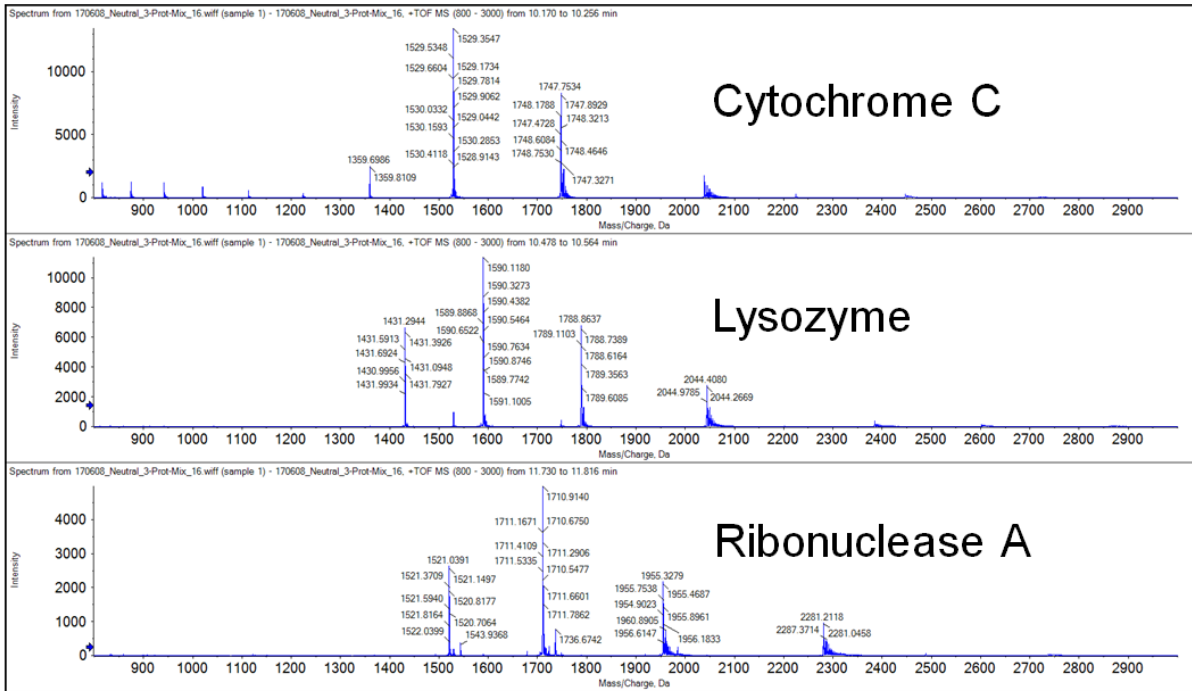
The following figure shows a total ion electropherogram (TIE) for a typical separation of Protein Test Mix and 50 mM AmAc buffer, pH 3.0.

Figure 7-1 TIE of Protein Test Mix in 50 mM AmAc, pH 3.0 Buffer



The following figure shows the mass spectrum for each protein in the Protein Test Mix.

Figure 7-2 Mass Spectra of Cytochrome C, Lysozyme, and Ribonuclease A Protein Test Mix



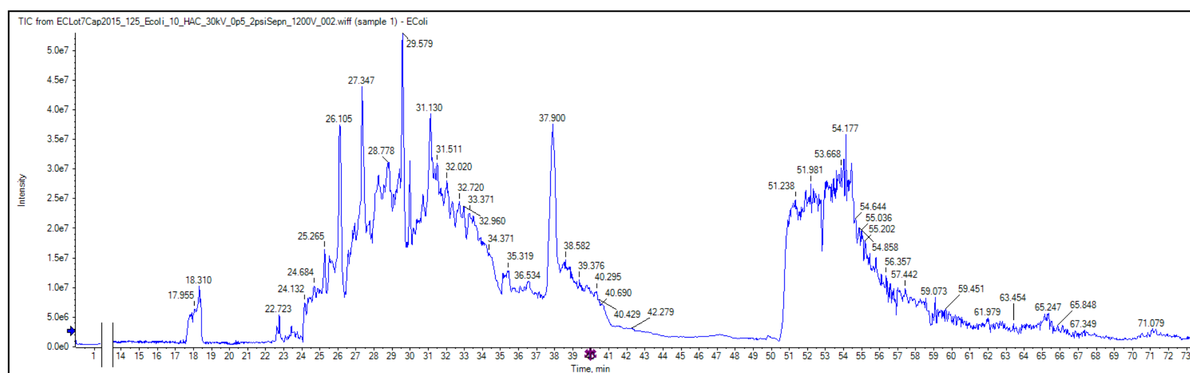
Separation of Peptide Mixtures 8

For a separation run with the neutral OptiMS cartridge, do this:

- Use the neutral CESI peptide separation method with 10% HAc solution.
- If required, then optimize the neutral CESI peptide separation method.
- Make sure that the sample has 100 mM LE buffer, pH 4.0.

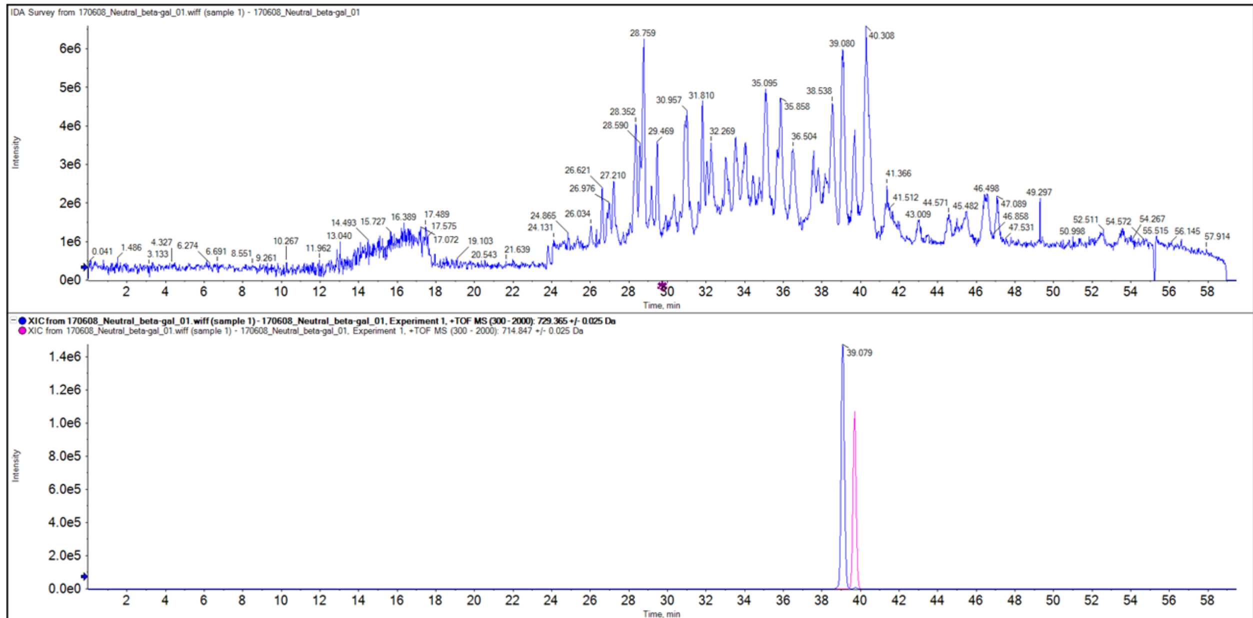
The following figure is an example of a total ion electropherogram (TIE) for a separation of an E. coli cell lysate digest. The protein concentration was 3 µg/µL. The sample was injected at 5 psi for 60 seconds, and then separated with the neutral CESI peptide separation method.

Figure 8-1 TIE of E. coli Cell Lysate Digest



The following figure is an example of a TIE for a separation of beta-galactosidase digest. The protein concentration was 1 µM.

Figure 8-2 TIE of 1 µM Beta-Galactosidase Digest Diluted 50:50 in 200 mM AmAC LE buffer, pH 4.0 with 10% HAc Solution as the BGE



About Autocalibration 9

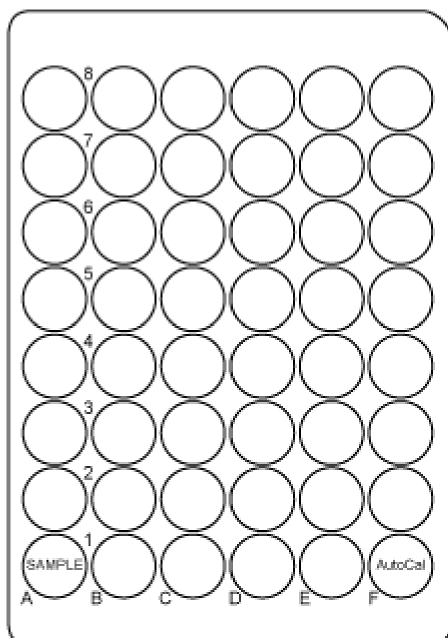
Small variations in temperature that occur during usual operation can cause incorrect masses to be reported. To increase the mass accuracy, we recommend that autocalibration be done every 5 hours of data acquisition.

Note: Do manual calibration before autocalibration is done. If the reference ions are outside of the 100 ppm tolerance for peak identification, then autocalibration will not be successful.

Note: The following procedures are for the SCIEX 5600 and 6600 systems. For other SCIEX mass spectrometers or mass spectrometers from other manufacturers, use the manufacturer's recommendations.

The following figure shows the sample tray layout for autocalibration.

Figure 9-1 Sample Tray Layout for Autocalibration



Autocalibration uses the CESI-MS autocalibration method and the following time program.

Figure 9-2 Time Program for Autocalibration

Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	3.00 min	BI:A1	BO:A1	reverse	BGE Conductive Liquid Capillary fill
2		Rinse - Pressure	100.0 psi	1.00 min	BI:A1	BO:A1	forward	BGE rinse
3		Inject - Pressure	100.0 psi	120.0 sec	SI:F1	BO:A1	Override, forward	Separation line fill with calibration mixture
4	0.00	Separate - Pressure	10.0 psi	1.70 min	BI:A1	BO:A1	forward	Infusion of the calibration mixture into the MS
5	0.20	Relay On					1: 0.10 2: 0.10	Contact closure trigger
6	1.70	End						
7								

Analyze the autocalibration data to make sure that the peaks were identified and their calculated masses are satisfactory. Refer to the section: Analyze the Autocalibration Data.

Create a Reference Table

1. In the Analyst TF software, in the Navigation bar, click **Acquire**.
2. Click **Tools** > **Settings** > **Tuning Options**, and then click **Reference**.

   Figure 9-3 Reference Table Editor Window

   Reference Table Editor

   Name: MixSCIEX-AgI | New | Copy | Delete | ◉ Positive ○ Negative | Calibration Valve Position: A

   Reference Ions for TOF MS Calibration:

	Use	Compound Name	Precursor m/z (Da)	Use for MS/MS	CE for MS/MS	DP for MS/MS	Retention Time (min)
6	☑	amino-dPEG 8-acid	442.26467	☐	20.000	50.000	0.00
7	☑	**Reserpine**	**609.28066**	☑	**20.000**	**50.000**	**0.00**
8	☑	Ion 3	622.02896	☐	42.000	80.000	0.00
9	☑	ALILTLVS	829.53933	☐	20.000	50.000	0.00
10	☑	Ion 4	922.00980	☐	42.000	80.000	0.00
11	☑	ALILTLVS + Cs	961.43696	☐	20.000	50.000	0.00
12	☑	Ion 5	1221.99064	☐	42.000	80.000	0.00
13	☑	Heptakis(2,3,6-tri-O-	1446.73224	☐	20.000	50.000	0.00
14	☑	Ion 6	1521.97148	☐	42.000	80.000	0.00
15	☑	Heptakis(2,3,6-tri-O-	1561.60332	☐	20.000	50.000	0.00
16	☑	Ion 7	1821.95231	☐	42.000	80.000	0.00
17	☑	Tryaceyl-b-cyclode	2034.62545	☐	20.000	50.000	0.00
18	☑	Ion 8	2121.93315	☐	42.000	80.000	0.00

   Reference Ions for MS/MS Calibration:
   (Product of 609.28066 Da)

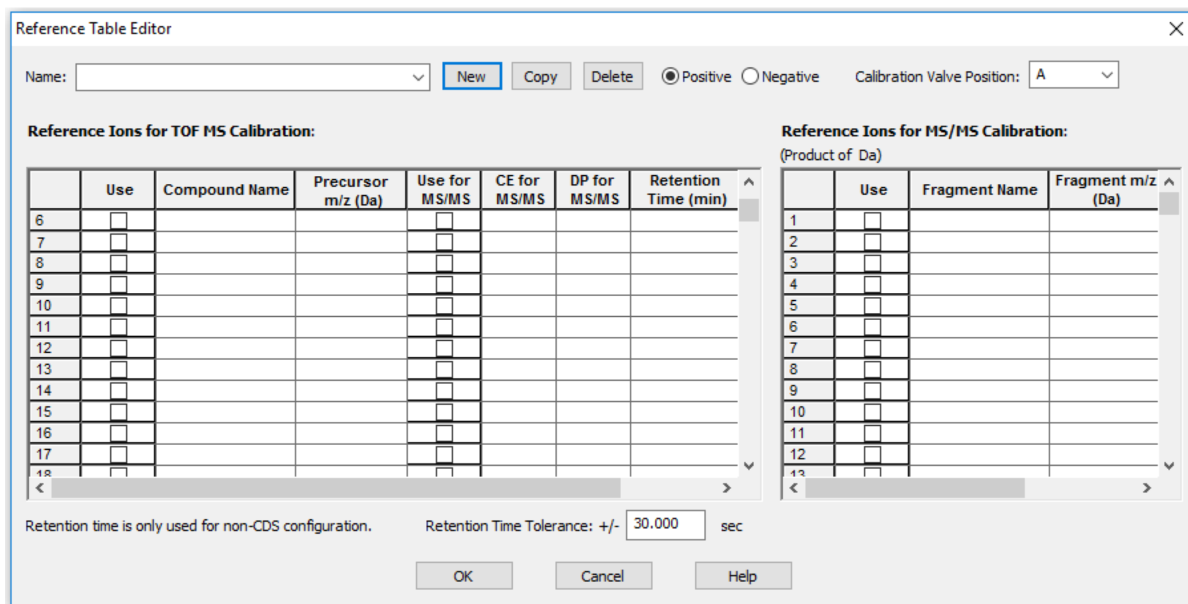
	Use	Fragment Name	Fragment m/z (Da)
1	☑	y1	174.09130
2	☑	y3	195.06520
3	☑	y5	236.12810
4	☐		365.18600
5	☑	y8	397.21220
6	☑	y10	448.19660
7	☑	y12	609.28070
8	☑		609.28066
9	☐		
10	☐		
11	☐		
12	☐		
13	☐		

   Retention time is only used for non-CDS configuration. Retention Time Tolerance: +/- 30.000 sec

   OK | Cancel | Help

3. To open a new, empty reference table, click **New**.

Figure 9-4 New Reference Table in the Reference Table Editor Window



4. In the **Name** field, type a name that identifies the calibration solution (in this example, `PepCalMix`).
5. Make sure that **Positive** is clicked.
6. In the Reference Ions for TOF MS Calibration table, type the following values.

Table 9-1 Reference Ions for TOF MS Calibration

Compound Name	Precursor *m/z* (Da)	CE for MS/MS	DP for MS/MS
AETSELHTSLK	408.55010	40	80
GAYVEVTAK	473.26020	40	80
IGNEQGVSR	485.25302	40	80
LVGTPAEER	491.26559	40	80
LDSTSIPVAK	519.79969	40	80
AGLIVAEGVTK	533.32333	40	80
LGLDFDSFR	540.27342	40	80
GFTAYYIPR	549.28633	40	80
SGGLLWQLVR	569.83398	40	80
AVGANPEQLTR	583.31360	40	80

Table 9-1 Reference Ions for TOF MS Calibration (continued)

Compound Name	Precursor *m/z* (Da)	CE for MS/MS	DP for MS/MS
SAEGLDASASLR	593.80053	40	80
VFTPLEVDVAK	613.34955	40	80
VGNEIQYVALR	636.35273	40	80
YIELAPGVDNSK	657.34499	40	80
DGTFAVDGPGVIAK	677.85827	40	80
YDSINNTEVSGIR	739.36148	40	80
SPYVITGPGVVEYK	758.91050	40	80
ALENDIGVPSDATVK	768.90340	40	80
AVYFYAPQIPLYANK	883.47380	40	80
TVESLFPEEAETPGSAVR	964. 97741	40	80

7. For each compound, click the **Use** check box.
8. In the Reference Ions for MS/MS Calibration table, type the following values.

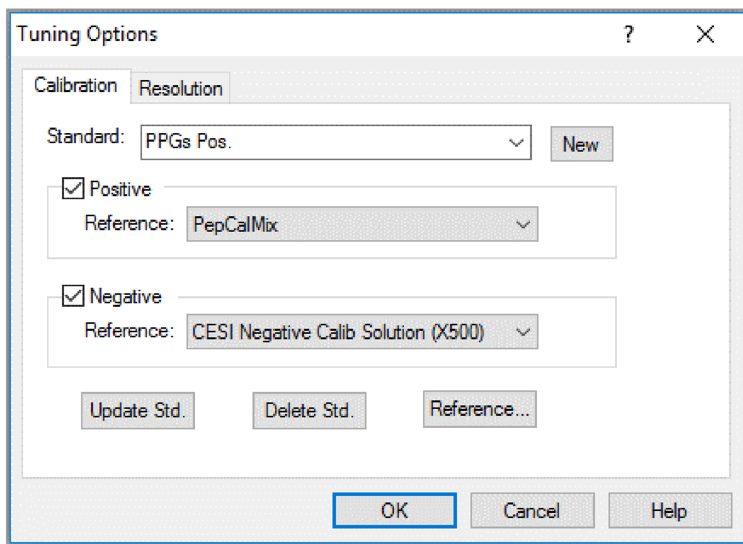
Table 9-2 Reference Ions for MS/MS Calibration

Fragment Name	Fragment *m/z* (Da)
b2	185.09207
b3	348.15540
b4	560.30788
b5	661.35555
N/A	758.91050
y7	799.44398
y8	856.46544
y9	957.51312
y10	1070.59719
y11	1169.66560
y12	1332.72893

9. For each fragment, click the **Use** check box.

10. In the **Use for MS/MS** column, select the check box for the applicable compound in the calibration solution (in this example, the peptide at *m/z* 758.91).
11. Make sure that the value in the **Retention time tolerance +/-** field is **30**.
12. Click **OK**.

Figure 9-5 Tuning Options Dialog

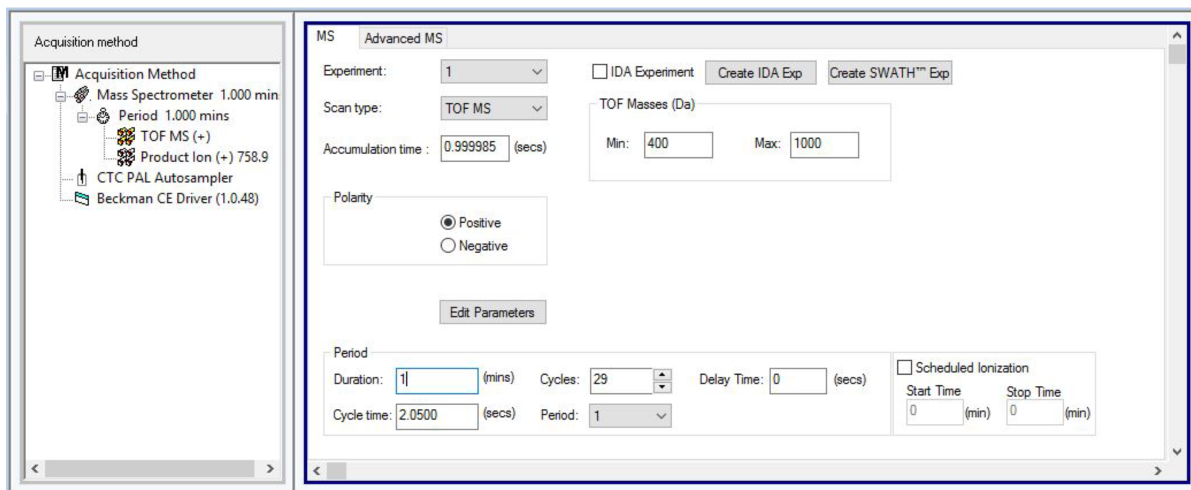


13. Make sure that the name of the new reference table (in this example, **PepCalMix**) shows in the **Positive Reference** field.
14. Click **OK**.

Create the MS Acquisition Method for Autocalibration

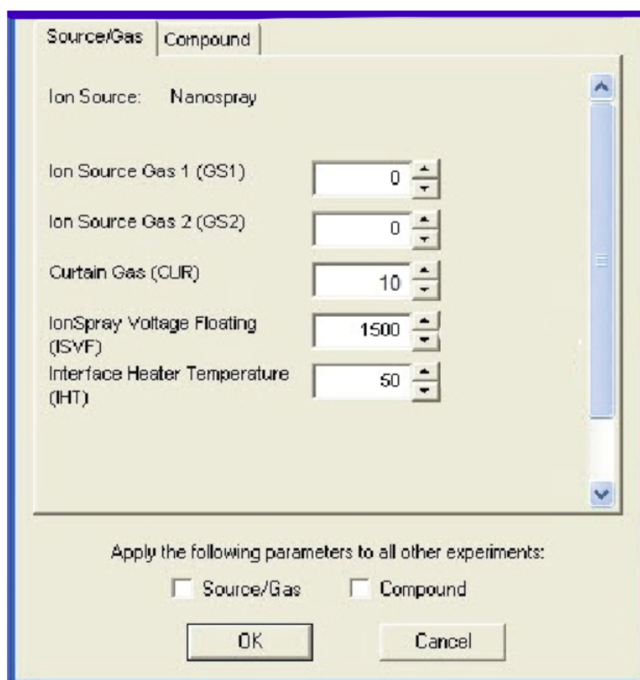
1. In the Analyst TF software, in the Navigation bar, under Acquire, double-click **Build Acquisition Method**.
2. In the Acquisition method pane, click **TOF MS (+)**.

Figure 9-6 Acquisition method Pane: TOF MS



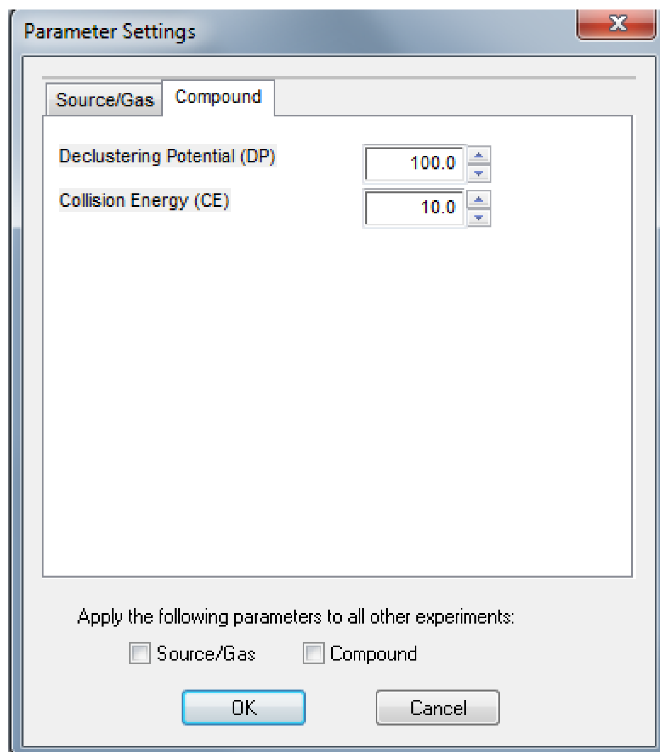
3. On the MS tab, click **Edit Parameters**.
   The Parameter Settings dialog opens.
4. On the Source/Gas tab, do this:
   a. In the **Curtain Gas (CUR)** field, type `10`.
   b. In the **IonSpray Voltage Floating (ISVF)** field, type `1500` or the optimized voltage.
      The ISVF parameter sets the ESI voltage.
   c. In the **Interface Heater Temperature (IHT)** field, type `50.0`.

Figure 9-7 TOF MS (+) Parameter Settings Dialog: Source/Gas Tab



5. Open the Compound tab, and then do this:

Figure 9-8 TOF MS (+) Parameter Settings Dialog: Compound Tab

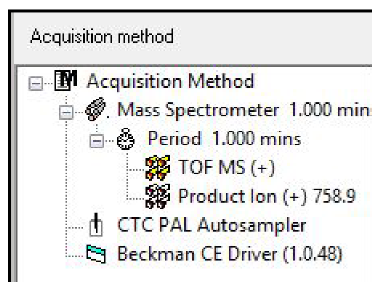


a. In the **Declustering Potential (DP)** field, type `100`.

b. In the **Collision Energy (CE)** field, type `10`.

6. In the Acquisition method pane, click **Product Ion (+) 758.9** (item 1).

Figure 9-9 Acquisition method Pane: Product Ion



7. On the MS tab, click **Edit Parameters**.
   The Parameter Settings dialog opens.

8. On the Source/Gas tab, do this:

a. In the **Curtain Gas (CUR)** field, type `10`.

   b. In the **IonSpray Voltage Floating (ISVF)** field, type `1600`.
      The ISVF parameter sets the ESI voltage.
   c. In the **Interface Heater Temperature (IHT)** field, type `50.0`.
9. Open the Compound tab, and then do this:
   a. In the **Declustering Potential (DP)** field, type `100`.
   b. In the **Collision Energy (CE)** field, type `42`.
   c. In the **Collision Energy Spread (CES)** field, type `5`.
   d. In the **Ion Release Delay (IRD)** field, type `67`.
   e. In the **Ion Release Width (IRW)** field, type `25`.

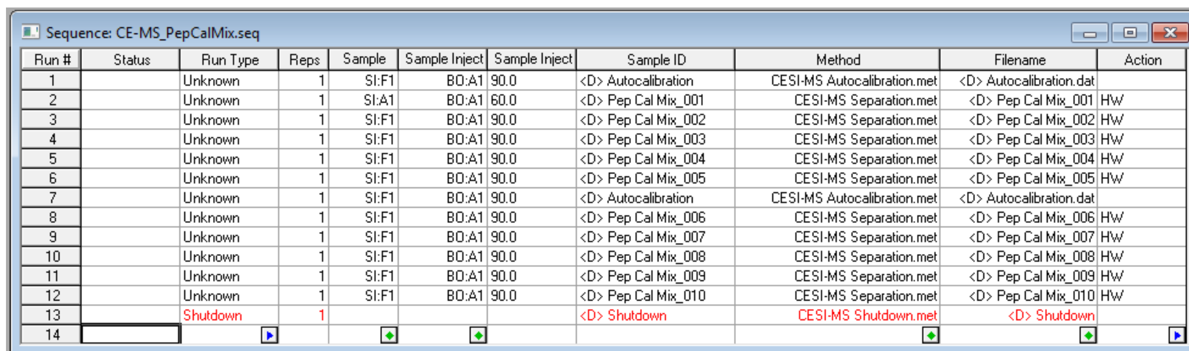
Add Autocalibration to the Sequence

Use this procedure to edit the sequence table to include autocalibration.

Note: The sequences in the 32 Karat software and on the mass spectrometer must be the same.

1. In the 32 Karat software, open the sequence table.
2. Click to highlight the first run, right-click the highlighted run, and then click **Insert Line**.
3. At the bottom of the **Method** column, click the green arrow icon and then click `CESI-MS Auto-calibration_ABSciex.met`.
4. In the **Sample ID** and **File name** fields, type the applicable values.
5. To add another line for autocalibration, click to highlight the run on line 7, right-click the highlighted run, and then click **Insert Line**.
6. Do steps 4 and 5 again.
   The following figure shows an example of the sequence.

Figure 9-10 Sample Sequence Example

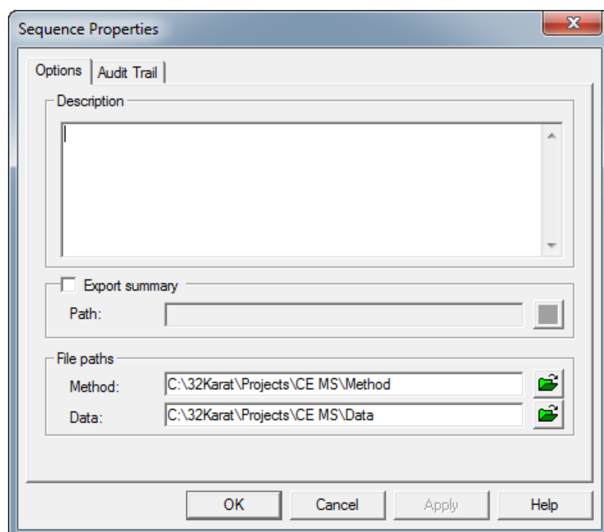


Sequence: CE-MS_PepCalMix.seq

Run #	Status	Run Type	Reps	Sample	Sample Inject	Sample Inject	Sample ID	Method	Filename	Action
1		Unknown	1	SI:F1	BO:A1	90.0	<D> Autocalibration	CESI-MS Autocalibration.met	<D> Autocalibration.dat	
2		Unknown	1	SI:A1	BO:A1	60.0	<D> Pep Cal Mix_001	CESI-MS Separation.met	<D> Pep Cal Mix_001	HW
3		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_002	CESI-MS Separation.met	<D> Pep Cal Mix_002	HW
4		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_003	CESI-MS Separation.met	<D> Pep Cal Mix_003	HW
5		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_004	CESI-MS Separation.met	<D> Pep Cal Mix_004	HW
6		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_005	CESI-MS Separation.met	<D> Pep Cal Mix_005	HW
7		Unknown	1	SI:F1	BO:A1	90.0	<D> Autocalibration	CESI-MS Autocalibration.met	<D> Autocalibration.dat	
8		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_006	CESI-MS Separation.met	<D> Pep Cal Mix_006	HW
9		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_007	CESI-MS Separation.met	<D> Pep Cal Mix_007	HW
10		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_008	CESI-MS Separation.met	<D> Pep Cal Mix_008	HW
11		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_009	CESI-MS Separation.met	<D> Pep Cal Mix_009	HW
12		Unknown	1	SI:F1	BO:A1	90.0	<D> Pep Cal Mix_010	CESI-MS Separation.met	<D> Pep Cal Mix_010	HW
13		Shutdown	1				<D> Shutdown	CESI-MS Shutdown.met	<D> Shutdown	
14										

7. On the toolbar, click **Sequence** > **Properties**.

Figure 9-11 Sequence Properties Dialog



8. Make sure that the File paths for **Method** and **Data** show the correct folder to save the data, and then click **OK**.

Note: If required, then create a folder.

9. Click **File** > **Sequence** > **Save As**.

   In this example, the sequence name is PepCalMix_Installation.

Analyze the Autocalibration Data

For the autocalibration to be successful, the following criteria must be met:

- The intensity of the reference ions is at least 10 counts per second (cps) in the mass spectrometer data and 3.3 cps in the MS/MS data.
- The reference ions are within a mass tolerance of 100 ppm.
- There are ≥ 80% of the selected ions in the reference table.

If autocalibration is successful, then a green check mark is shown. If the sample was acquired but the calibration failed because one or more of the reference ions did not meet the calibration criteria, then a red circle with a diagonal line through it is shown. To see information about the failure, double-click the circle icon.

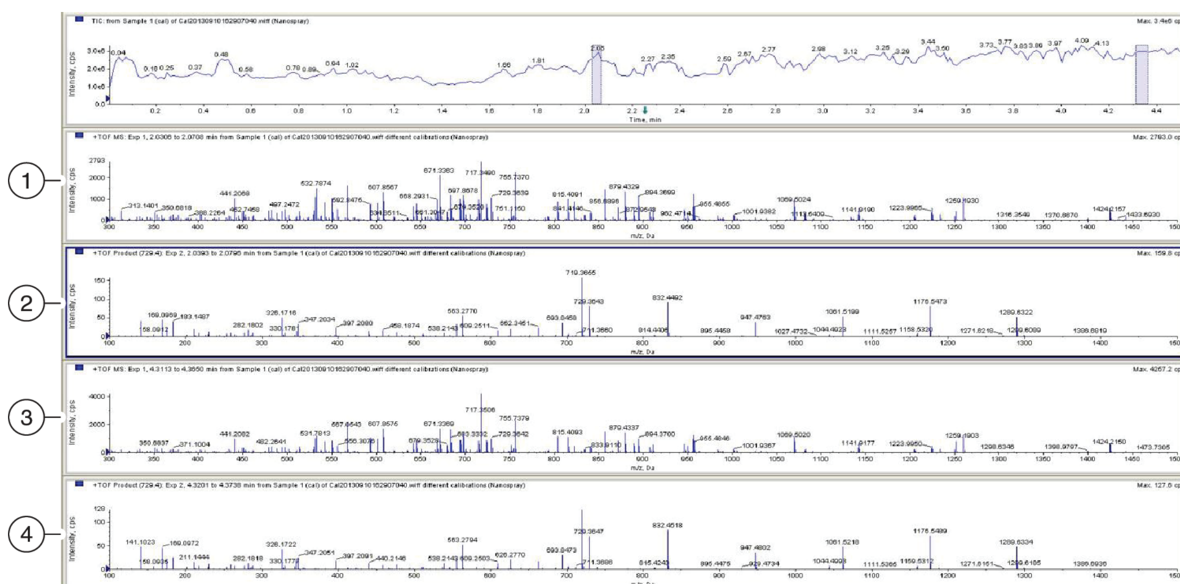
If autocalibration fails, then an error message is shown in the Sample Details dialog. Click **OK**, and then refer to the section: Troubleshoot an Autocalibration Failure.

1. In the Analyst TF software, in the Navigation bar, under Explore, double-click **Open Data File**.

   If the **Keep calibration data file** check box was selected in the Queue Options dialog, then data for each autocalibration is saved as a separate data file in the `Cal Data` subfolder. The autocalibration data file names start with `Cal` and include the time stamp and calibration sample index. For example, `Cal20130910162907040.wiff`.

2. To extract the mass spectra from the TIC, highlight the region and then double-click the region. Extract one mass spectrum between 2 minutes to 2.5 minutes (MS before calibration) and another between 4 minutes to 4.5 minutes (MS after calibration).

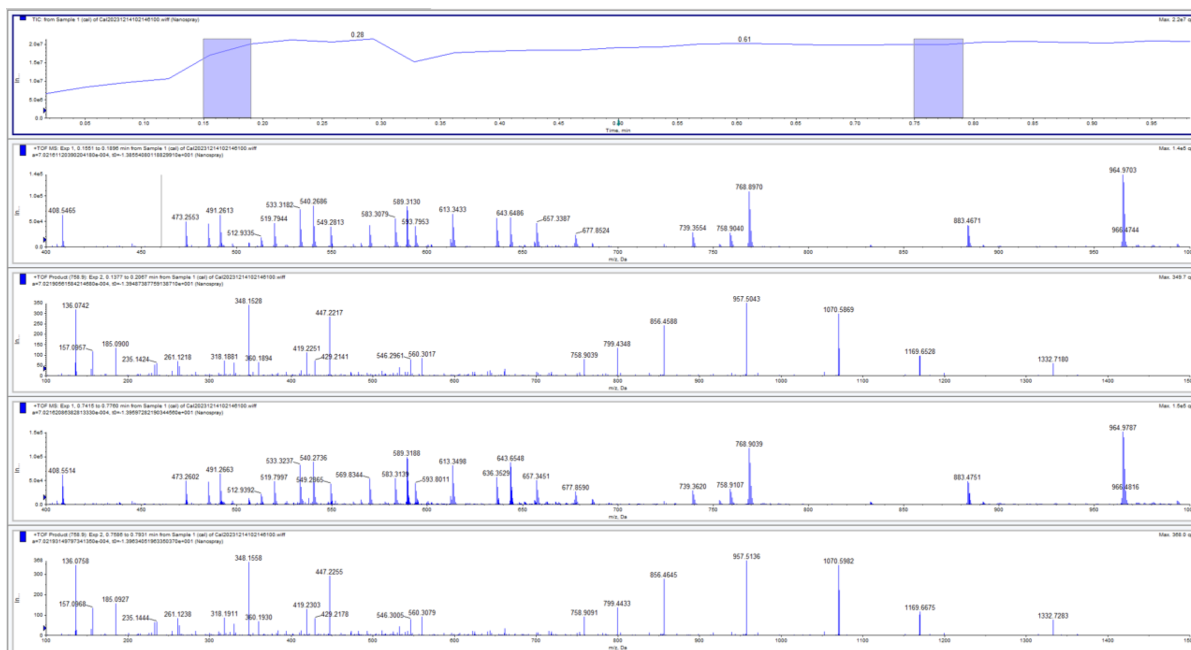
Figure 9-12 Data Analysis



Item	Description
1	MS before calibration
2	MS/MS before calibration
3	MS after calibration
4	MS/MS after calibration

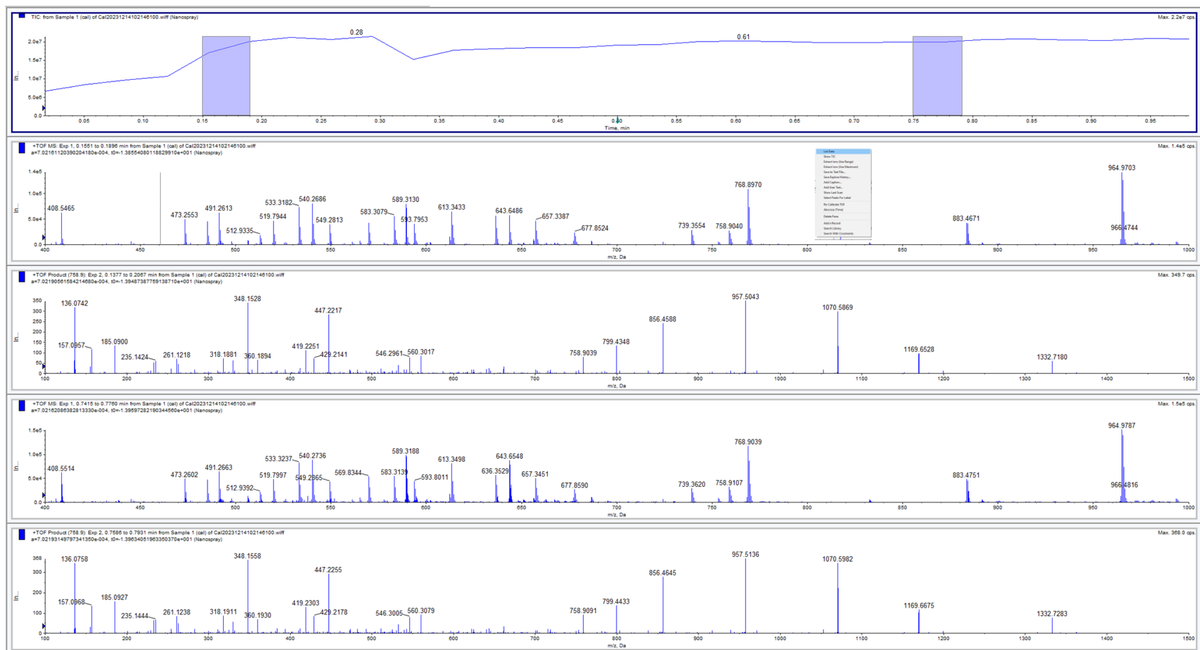
3. To get a list of ions in the mass spectrum, right-click the mass spectrum and then click **List data**. Do this for both MS before calibration and MS after calibration.

Figure 9-13 Obtaining List Data



4. To show all the reference ions used for calibration, in each data list table, open the Calibration Peak List tab.

Figure 9-14 Reference Ions



5. To make sure that the correct reference table has been selected, right-click the reference table, click the **PepCalMix_CE-MS_Calibration Ref** reference table, and then click **Use as reference**.

Figure 9-15 Reference Table Verification for MS Before Calibration

	Target Mass (Da)	Found At (Da)	Intensity (cps)	Width (Da)	Mass Shift (ppm)
1	408.55010	408.54647	6.34510e4	0.01874	-8.89295
2	473.26020	473.25528	5.03555e4	0.02048	-10.39865
3	485.25302	485.24858	4.68445e4	0.02042	-9.15009
4	491.26559	491.26129	6.34940e4	0.02160	-8.75499
5	519.79969	519.79440	4.76360e4	0.02334	-10.17151
6	533.32333	533.31817	7.43020e4	0.02230	-9.67837
7	540.27342	540.26855	8.10030e4	0.02242	-9.01083
8	549.28633	549.28134	4.02555e4	0.02413	-9.08802

	Target Mass (Da)	Found At (Da)	Intensity (cps)	Width (Da)	Mass Shift (ppm)
1	408.55010	408.55137	6.22725e4	0.01974	3.11812
2	473.26020	473.26017	4.94930e4	0.02131	-0.06598
3	485.25302	485.25330	4.75160e4	0.02085	0.58447
4	491.26559	491.26629	6.45585e4	0.02161	1.43152
5	519.79969	519.79972	4.85890e4	0.02314	0.05462
6	533.32333	533.32366	8.24835e4	0.02194	0.62220
7	540.27342	540.27360	8.89190e4	0.02317	0.33366
8	549.28633	549.28654	4.29780e4	0.02299	0.38665
9	569.83398	569.83441	5.40475e4	0.02364	0.75614

Figure 9-16 MS After Calibration Analysis

	Target Mass (Da)	Found At (Da)	Intensity (cps)		Mass Shift (ppm)
1	408.55010	408.54647	6.34510e4	0.01874	-8.89295
2	473.26020	473.25528	5.03555e4	0.02048	-10.39865
3	485.25302	485.24858	4.68445e4	0.02042	-9.15009
4	491.26559	491.26129	6.34940e4	0.02160	-8.75499
5	519.79969	519.79440	4.76360e4	0.02334	-10.17151
6	533.32333	533.31817	7.43020e4	0.02230	-9.67837
7	540.27342	540.26855	8.10030e4	0.02242	-9.01083
8	549.28633	549.28134	4.02555e4	0.02413	-9.08802

	Target Mass (Da)	Found At (Da)	Intensity (cps)		Mass Shift (ppm)
1	408.55010	408.55137	6.22725e4	0.01974	3.11812
2	473.26020	473.26017	4.94930e4	0.02131	-0.06598
3	485.25302	485.25330	4.75160e4	0.02085	0.58447
4	491.26559	491.26629	6.45585e4	0.02161	1.43152
5	519.79969	519.79972	4.85890e4	0.02314	0.05462
6	533.32333	533.32366	8.24835e4	0.02194	0.62220
7	540.27342	540.27360	8.89190e4	0.02317	0.33366
8	549.28633	549.28654	4.29780e4	0.02299	0.38665
9	569.83398	569.83441	5.40475e4	0.02364	0.75614

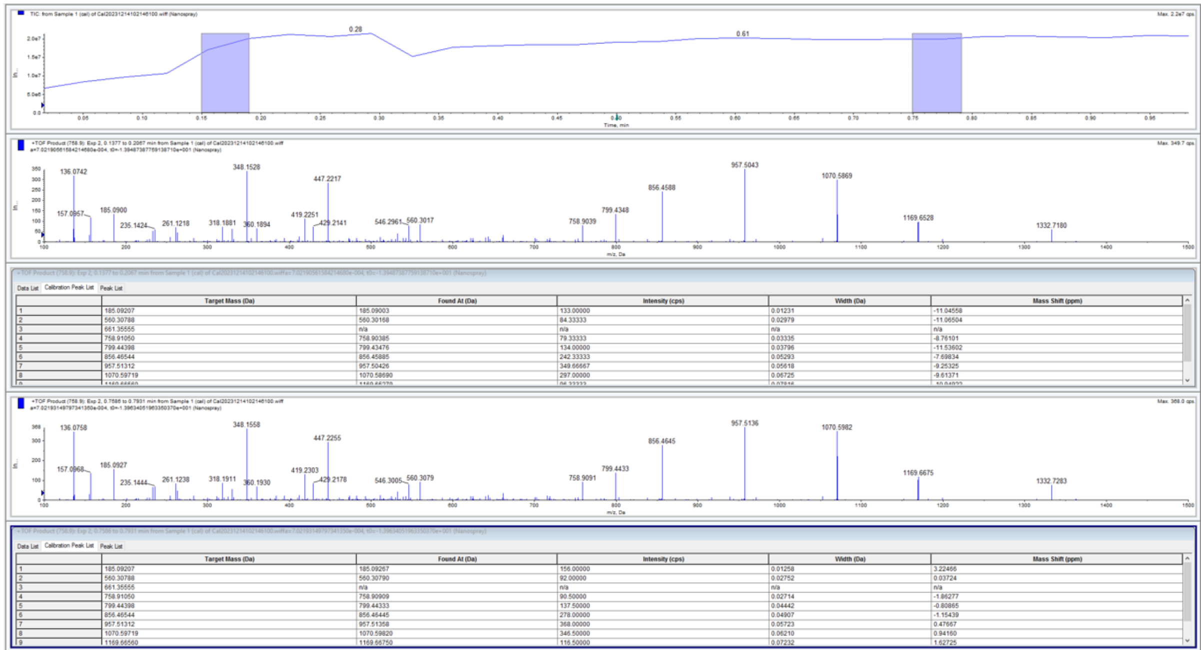
The tables show the Target Mass (or theoretical mass) and the Found At mass (or experimental mass) for all eight reference ions selected for autocalibration. The mass shift between the Target Mass and the Found At mass is given as Mass Shift (ppm).

The mass shift is higher before calibration and lower after calibration for all ions.

6. To evaluate the MS/MS mass accuracy, do steps 3 and 4 for MS/MS before and after calibration.

   For the MS/MS experiment, the mass shift after calibration is lower than it was before calibration.

Figure 9-17 Evaluation of Peak Data in MS/MS Experiment



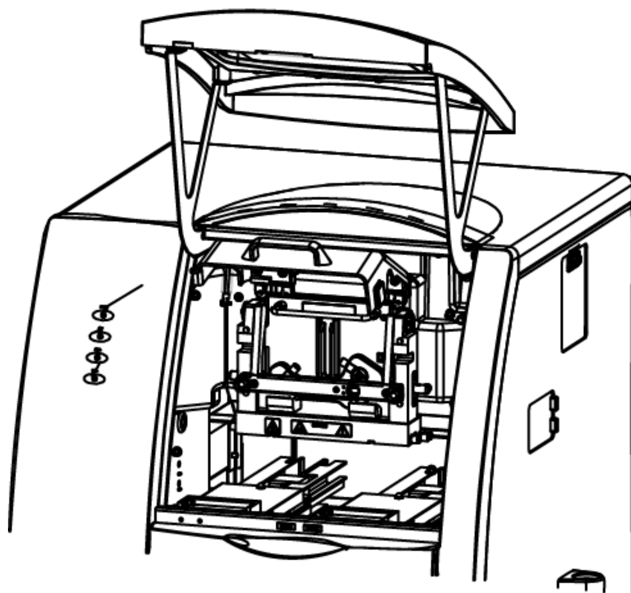
Shut Down and Disconnect 10

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: Stow the Cartridge.
3. In the 32 Karat software, go to the Direct Control window, and then click **Load**.
4. Open the cartridge cover.

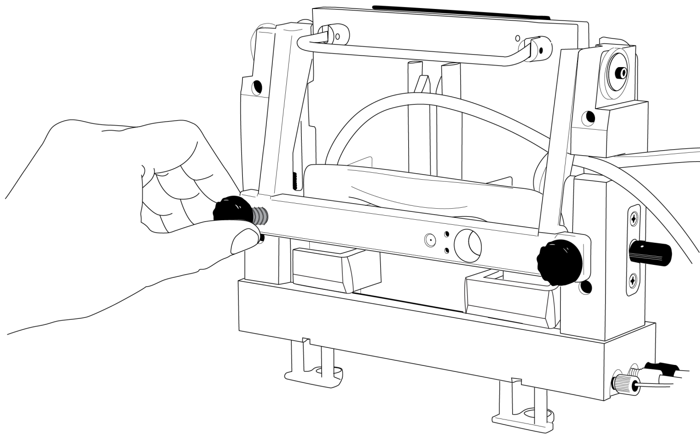
   Figure 10-1 Cartridge Cover

   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.

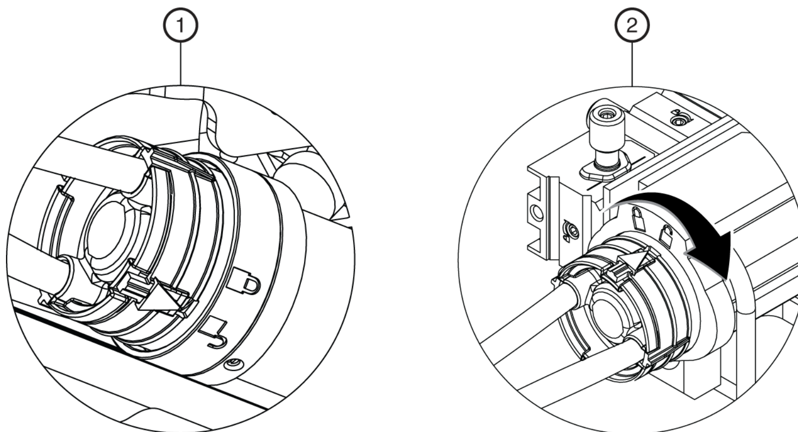
Figure 10-2 Thumbscrews on the Insertion Bar



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 10-3 Unlock the Sprayer from the Cartridge

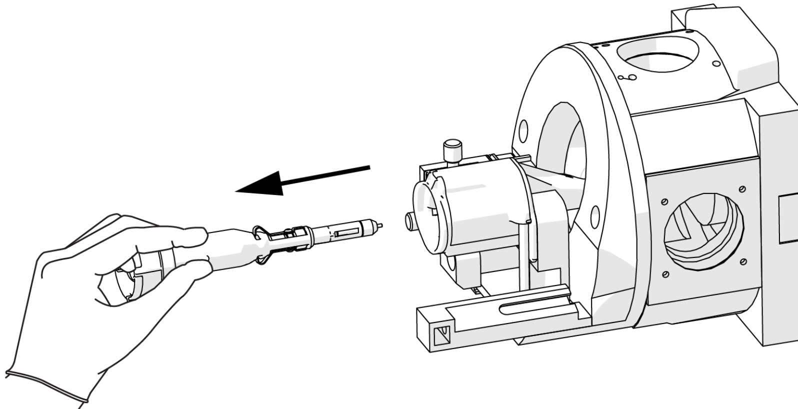


Item	Description
1	Sprayer in the Unlock position

Item	Description
2	Turn to lock the sprayer from the adapter

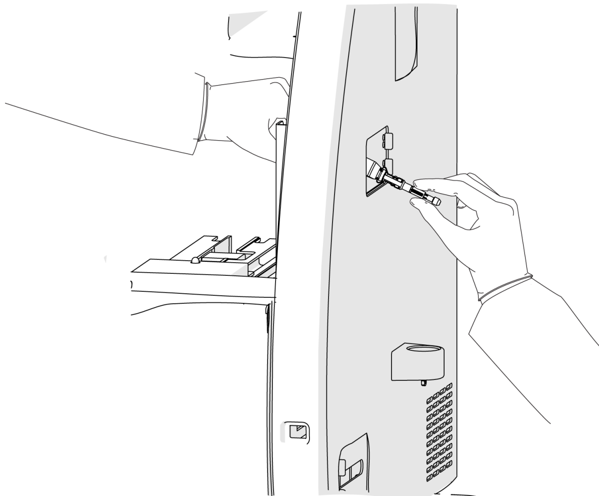
9. Remove the sprayer from the adapter.

Figure 10-4 Sprayer Removal



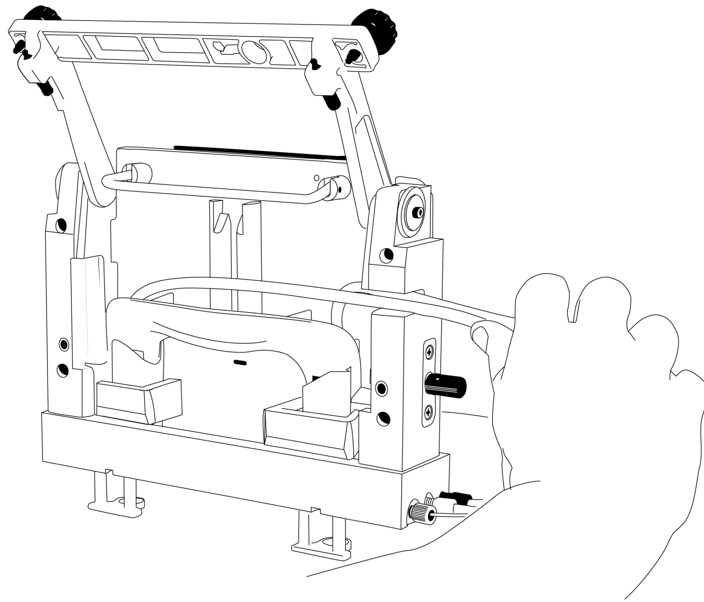
10. Put the protective sleeve on the sprayer tip.
11. Put the tubing with the sprayer end through the access panel.

Figure 10-5 Sprayer and Tubing Through the Access Panel



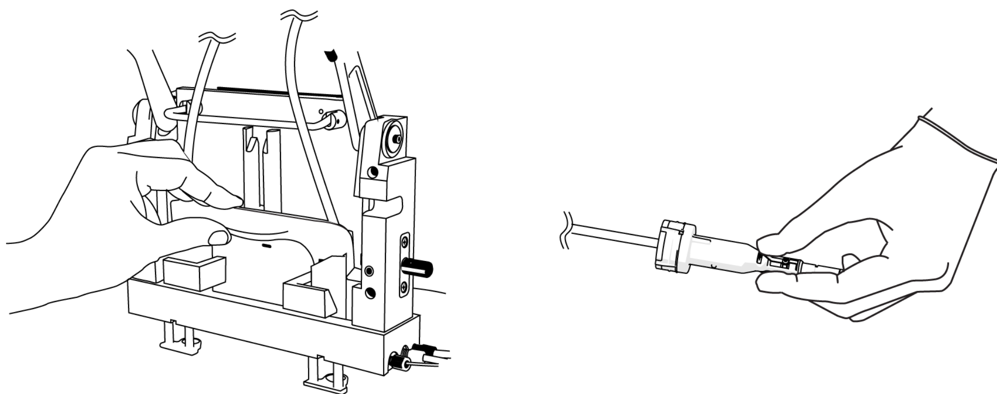
12. Remove the coolant tubing from the notched arm.

Figure 10-6 Coolant and Capillary Tubing



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

Figure 10-7 Remove the Cartridge



Note: As the cartridge moves up, the protective sleeves on the inlet and outlet sides go down over the capillary tips.

Note: Drops of liquid coolant usually fall from the cartridge tips, and do not cause damage to the hardware.

14. Refer to the section: Stow the Cartridge.

Stow the Cartridge

WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips 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 stowed for less than 3 days, do this:
    a. Run the neutral CESI rest method.
    b. Keep the cartridge in the system for as long as 3 days.
2. To prepare the cartridge to be stowed for more than 3 days, do this:
    a. Run the neutral CESI rest method.
    b. Disconnect the sprayer from the ion source.
    c. Remove the cartridge from the system.
    d. Put 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 a film cover.
    g. Keep the cartridge in a safe area at 2 °C to 8 °C.

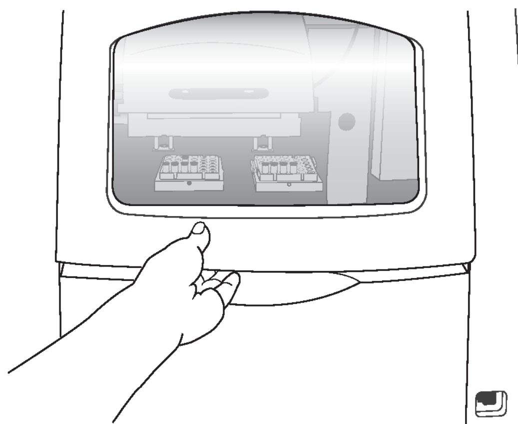
Add Capillary Cartridge Coolant 11

Required Materials
• Capillary cartridge coolant (PN 359976) • Coolant fill tool (PN 144647)

CAUTION: Potential System Damage. To prevent damage, do not use the plunger in the coolant fill tool when coolant is added. Gravity supplies sufficient force to pull the coolant into the system.

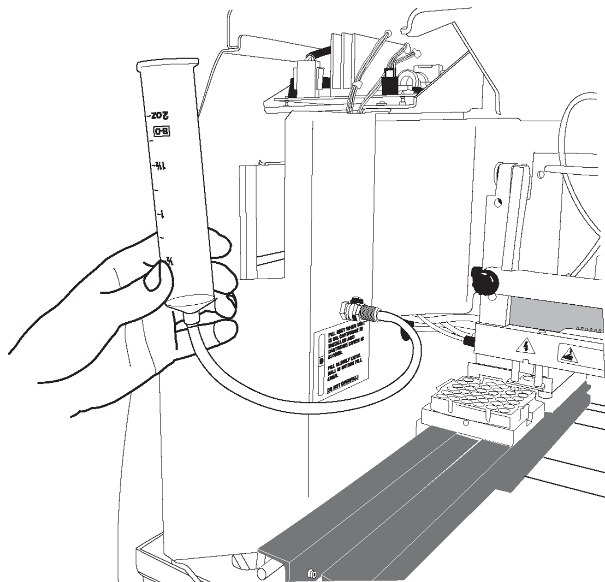
1. Open the sample cover.

   Figure 11-1 Sample Cover (Outer Door)

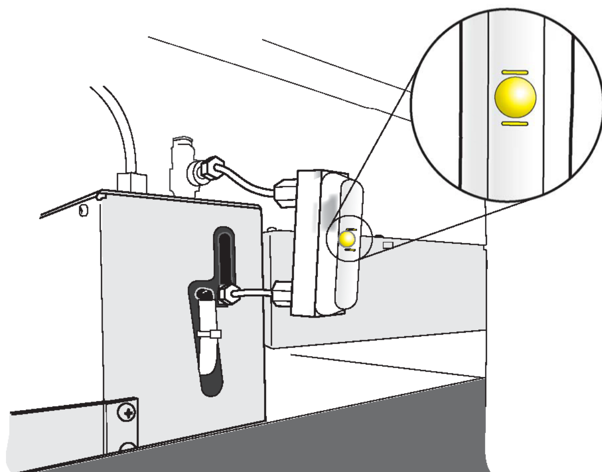
2. Connect the coolant fill tool to the coolant fill port.

Figure 11-2 Coolant Fill Tool Connected to the Coolant Fill Port



3. Fill the syringe with 120 mL of coolant.
4. Make sure that the CESI 8000 Plus system is on and a cartridge is installed.
5. Slowly add coolant until the fill indicator is between the yellow lines in the coolant sight glass.

Figure 11-3 Coolant Sight Glass



6. Remove the coolant fill tool, and then close the sample cover.

Troubleshooting 12

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

Symptom	Possible Cause	Corrective Action
Electrospray is detected when the ESI voltage is zero and separation voltage is applied with forward pressure	1. A cable is missing or is not fully connected. 2. The adapter on the mass spectrometer does not touch the cartridge. 3. The cartridge capillaries are not filled with separation buffer.	1. Make sure that all of the required cables are attached fully. Refer to the document: *CESI 8000 Plus High Performance Separation-ESI Module User Guide*. 2. Remove the sprayer from the adapter and pull up on the metal contact for the sprayer. Install the sprayer in the adapter, and make sure that the sprayer is in the locked position. 3. To make sure that the separation capillary and conductive liquid capillary are filled with separation buffer, rinse each one at 100 psi for 3 minutes.

Symptom	Possible Cause	Corrective Action
No flow through the separation capillary	1. The capillary is blocked. 2. The source temperature is too high, which causes the precipitation of protein at the emitter. 3. The capillary is broken.	1. Refer to the section: Clean Blockage from the Sprayer Tip. If the blockage is at the inlet side, then apply a vacuum. 2. To increase solubility, add an organic such as 10% isopropanol. To remove the blockage, run the neutral CESI washing method with 10% IPA while the sprayer tip is immersed in a 50 mL Falcon tube filled with 10 mL of 10% IPA. 3. Replace the cartridge.

Symptom	Possible Cause	Corrective Action
Electrospray is not stable	1. The ESI voltage is not optimized. 2. The sprayer position is not optimized in front of the inlet on the mass spectrometer. 3. The forward pressure in the separation capillary is < 1.5 psi. 4. The sprayer needle is not fully filled. 5. The buffer concentration and pH are not optimized. 6. The value for curtain gas is too high. 7. The cartridge was exposed to temperatures < 2 °C.	1. Find the minimum ESI voltage, and then increase its working value by 0.2 kV. 2. At the same time, optimize the sprayer position, and apply separation voltage and forward pressure at 1.5 psi. 3. Increase the pressure to 1.5 psi or more. 4. Make sure that the conductive liquid capillary is filled fully with solution. 5. Optimize the buffer concentration and pH. 6. Decrease the curtain gas to the recommended value. 7. Condition the capillaries. If the electrospray is still not stable, then replace the cartridge.

Symptom	Possible Cause	Corrective Action
Carryover occurs between runs	1. The buffer vials are contaminated with sample. 2. The separation capillary was not rinsed sufficiently between separations.	1. Replace the buffer vials. To prevent contamination of the buffer vials, make sure that a water dip step is included after sample injection in the separation method. 2. Do this: a. Do a rinse with 0.1 M hydrochloric acid (HCl) at the start of each separation. b. Increase the rinse times between separations.
Sample peaks are tailing in the TIE	1. The separation capillary was not rinsed sufficiently between separations. 2. The buffer concentration and pH are not optimized, and high electro-migration dispersion occurred. 3. The forward pressure value is too high, which caused a distorted peak shape. 4. The neutral coating is damaged.	1. Do a rinse with 0.1 M HCl at the start of each separation. 2. Do this: a. Increase the rinse times between separations. b. Optimize the buffer concentration and pH for the sample that is analyzed. 3. Decrease the forward pressure to 1.5 psi. 4. Run a Protein Test Mix sample to make sure that the separation is correct. If the separation fails, then replace the cartridge.

Symptom	Possible Cause	Corrective Action
No sample is detected	1. The sample vial is in an incorrect position. 2. The sample volume is not sufficient. 3. The injection plug is too short. 4. The sample is too dilute. 5. An incorrect polarity is used in the method. 6. The separation method is too short. 7. The mass spectrometer settings are not optimized.	1. Make sure that the sample vial is in the correct position in the sample tray, sequence, and method. 2. Increase the sample volume to > 50 µL for microvials or > 5 µL for nanoVials. 3. Increase the injection time and/or injection pressure. 4. Use a more concentrated sample. 5. Use the normal polarity. 6. Increase the separation time in the CE and mass spectrometer methods. 7. Make sure that the mass spectrometer settings are correct and the ionization parameters have been optimized for the sample that is analyzed.
Low sensitivity due to highly oxidized proteins	1. The ESI voltage is too high. 2. The sprayer tip is too close to the inlet on the mass spectrometer. 3. The entrance temperature for the mass spectrometer is too high.	1. Decrease the ESI voltage and optimize the position of the sprayer tip. 2. Move the sprayer tip away from the inlet on the mass spectrometer. Refer to the section: Fine-Tune the Position of the Sprayer Tip. 3. Decrease the entrance temperature for the mass spectrometer.

Symptom	Possible Cause	Corrective Action
Capillary coating is frozen	The cartridge was exposed to temperatures < 2 °C.	Run the neutral CESI washing method to rehydrate the neutral coating, and then keep the cartridge in the instrument for 24 hours. Continued flushing is not required. After 24 hours, run the neutral CESI electrical conditioning method.

Troubleshoot an Autocalibration Failure

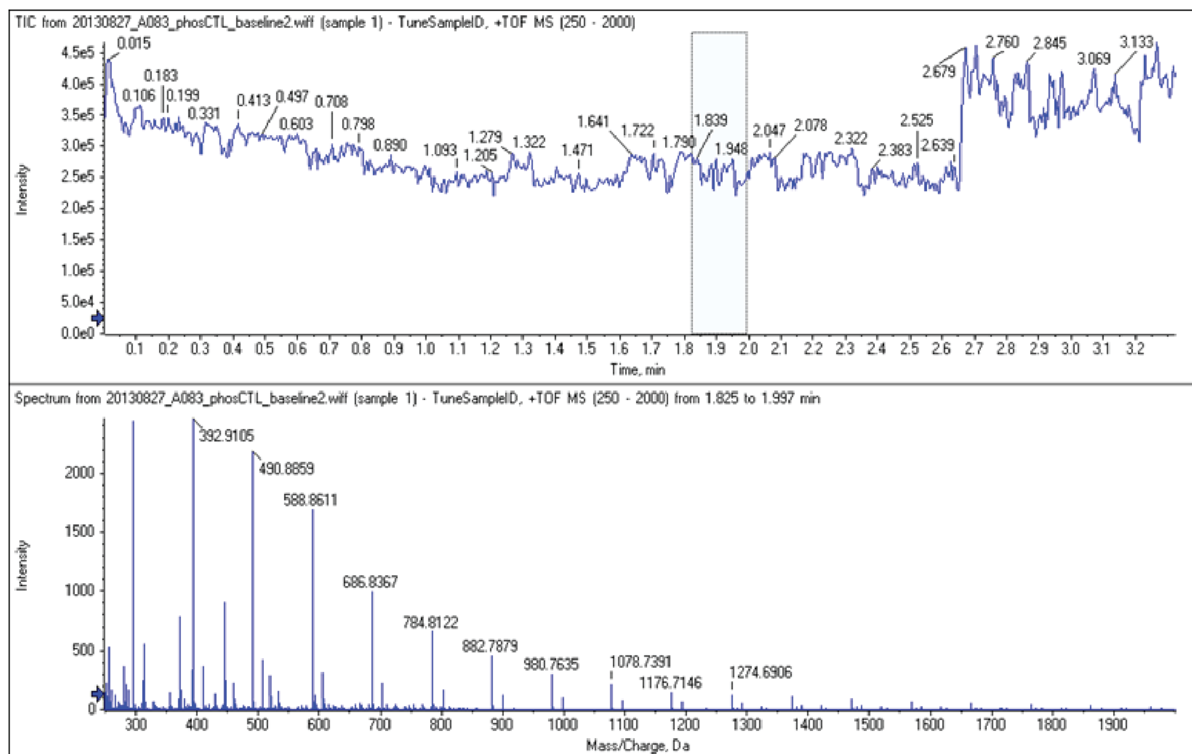
1. Do the procedure in the section: Analyze the Autocalibration Data.
2. Make sure that the intensity of the reference ions is correct:
   - Mass spectrometer data: > 10 cps
   - MS/MS data: > 3.3 cps
3. In the Calibration peak list table, if the value in the **Mass shift (ppm)** field is > 100 ppm, then do manual calibration.

   Refer to the section: Manual Calibration.
4. Make sure that the threshold for peak detection is 1% in the spectrum.
   a. In the Navigation bar, under Tune and Calibrate, click **Tools** > **Settings** > **Appearance Options** > **Other Graph**.
   b. In the **Default Threshold for the Spectrum** field, type `1`.

Conductive Liquid Capillary Contamination

If the baseline mass spectrum shows a series of peaks with a difference in *m/z* of 98, then the conductive liquid capillary might be contaminated with phosphate. To remove phosphate contamination, condition the conductive liquid capillary.

Figure 12-1 Profile of Phosphate Contamination



Condition the Conductive Liquid Capillary

1. Use the time program in the following figure.

Figure 12-2 Time Program for the Conductive Liquid Capillary Conditioning Method

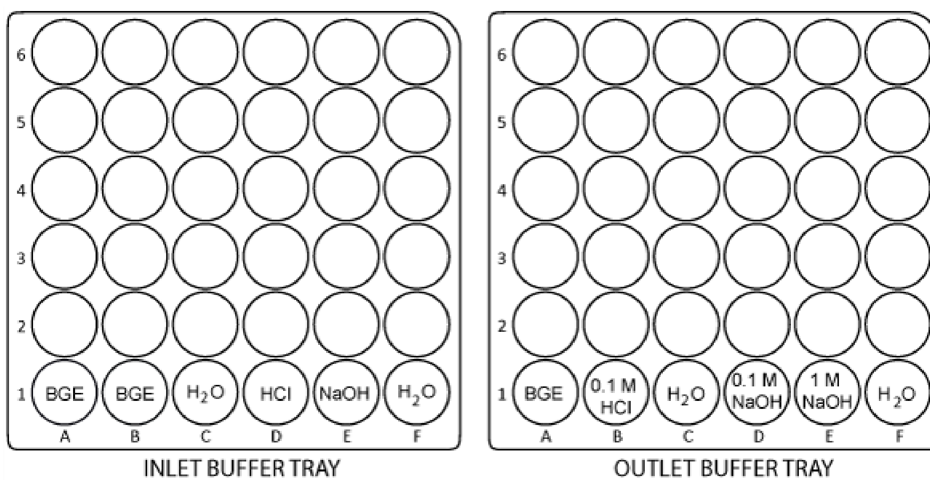
Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	10.00 min	BI:E1	BO:E1	reverse	1 M NaOH rinse
2		Rinse - Pressure	100.0 psi	10.00 min	BI:E1	BO:D1	reverse	0.1 M NaOH rinse
3		Rinse - Pressure	100.0 psi	10.00 min	BI:C1	BO:C1	reverse	0.1 M HCl rinse
4		Rinse - Pressure	100.0 psi	10.00 min	BI:C1	BO:B1	reverse	DDI water rinse
5		Rinse - Pressure	100.0 psi	5.00 min	BI:A1	BO:A1	reverse	10% HAc rinse
6		Rinse - Pressure	100.0 psi	5.00 min	BI:A1	BO:A1	forward	10% HAc rinse
7								

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

Note: This method uses reverse rinses. Only one vial is required in the inlet buffer tray, at position A1.

Figure 12-3 Tray Layout for the Conductive Liquid Capillary Conditioning Method

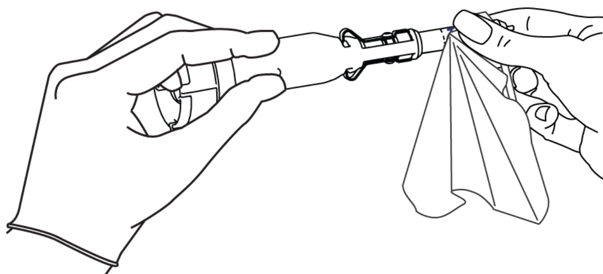


Clean Blockage from the Sprayer Tip

CAUTION: Potential System Damage. Do not put more than 10 mL of CE Grade water in the 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.

1. Put 10 mL of CE Grade water in a 50 mL Falcon tube, and then put the tube in the holster on the side of the system.
2. Carefully immerse the sprayer tip in the CE Grade water.
3. In the 32 Karat software, go to the Direct Control window.
4. Do a forward rinse with BGE at 100 psi for 5 minutes.
5. If required, then run the neutral CESI washing method and then the neutral CESI electrical conditioning method to condition the capillaries.
6. After 5 minutes, remove the sprayer from the CE Grade water.
7. Use lint-free wipes to dry the sprayer carefully.

Figure 12-4 Dry the Sprayer



8. Install the sprayer in the adapter on the mass spectrometer.
9. Remove the Falcon tube of CE Grade water from the holster, and discard the contents.

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Customer Training

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- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

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- sciex.com/contact-us
- sciex.com/request-support

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This version of the document supersedes all previous versions of this document.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the documentation that comes with the system or component.

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