

Chapter 2

Setting Up for Tuning and Calibrating the MS Detector in ESI/MS Mode

You tune and calibrate your LCQ Advantage in ESI mode before you acquire data in either the ESI or the APCI mode.

This chapter contains the following topics:

- Removing the APCI Probe Assembly
- Connecting the PEEK Safety Sleeve and Sample Tube to the ESI Probe
- Installing the ESI Probe Assembly
- Setting Up the Syringe Pump for Tuning and Calibrating
- Setting Up the MS Detector for Tuning and Calibrating

Note. If you have not yet configured Xcalibur for your LCQ Advantage, do so by following the instructions in **Appendix A: Instrument Configuration**.

If the ESI probe and the PEEK safety sleeve are installed on the API source, go to the topic: **Setting Up the Syringe Pump for Tuning and Calibrating**.

If no probe is installed, or if the ESI probe is installed without the PEEK safety sleeve, go to the topic: **Connecting the PEEK Safety Sleeve and Sample Tube to the ESI Probe**.

If the APCI probe is installed on the API source, go on to the next topic: **Removing the APCI Probe Assembly**.

2.1 Removing the APCI Probe Assembly

To remove the APCI probe assembly, proceed as follows. See Figure 2-1 for the location of the components of the APCI probe assembly.

Note. The following procedures assume that you are familiar with your LCQ Advantage instrument. If you need assistance, refer to LCQ Advantage online Help, LCQ Advantage Getting Connected and/or LCQ Advantage Hardware Manual.

Ensure that the MS detector is in Standby mode before removing the APCI probe assembly, as follows:

Open the Tune Plus window from the Start button on your Windows® 2000 Desktop, as follows:



- a. Choose **Start | Programs | Xcalibur | Xcalibur** to display the Xcalibur Home Page — Roadmap view.
- b. Click on the Instrument Setup button to display the window.
- c. Click on the LCQ Advantage MS button to display the New Method page.
- d. Click on the Tune Plus button to display the window. See Figure 2-1

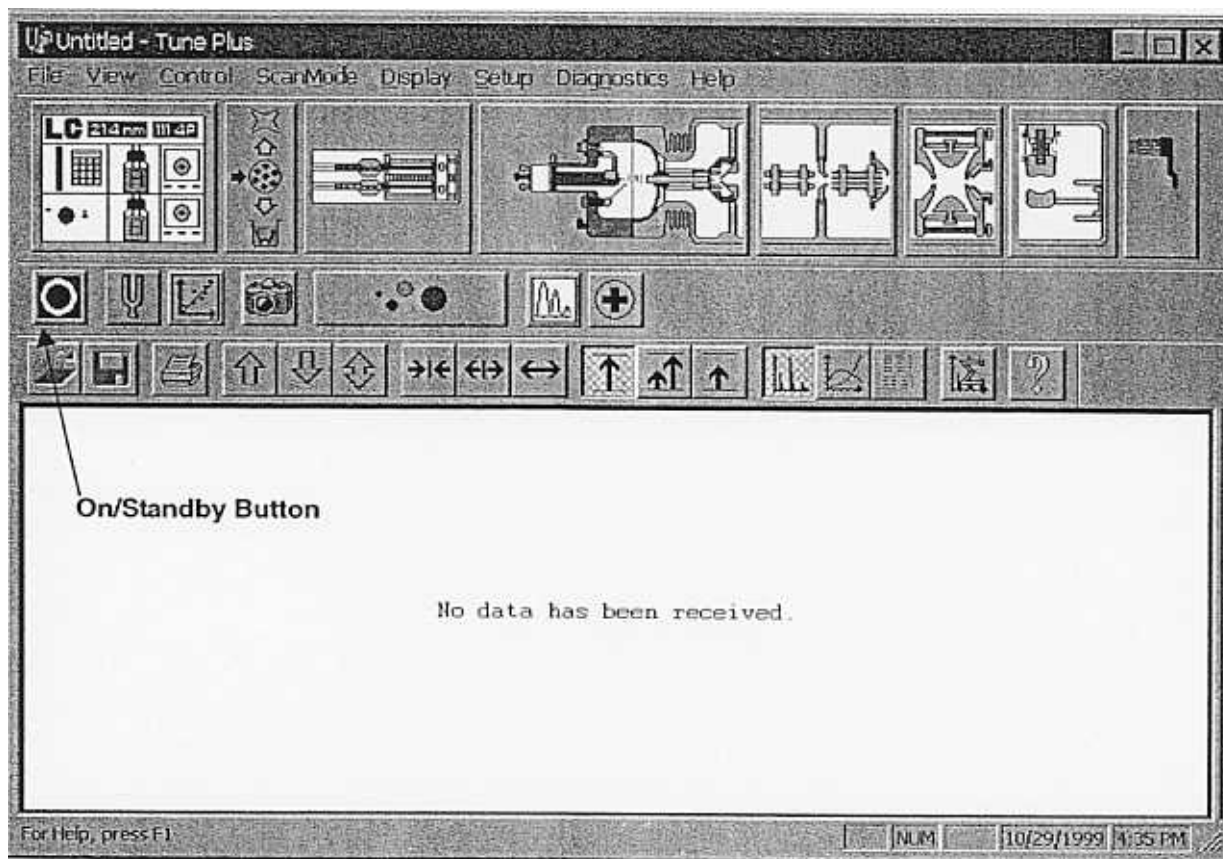


Figure 2-1. Tune Plus window, showing the MS detector in the Standby mode



On



Standby

1. If the MS detector is On, put it in Standby mode, as follows:
Click on the On/Standby button. When the MS detector is in Standby, the LCQ Advantage turns Off the sheath gas, Sweep/AUX gas, and high voltage.
2. Loosen the two flange retainer bolts that secure the APCI probe assembly to the API spray shield. See Figure 2-2.
3. Pull back the APCI probe assembly from the spray shield.
4. Disconnect the corona needle high voltage cable from the corona needle high voltage connector. To disconnect the cable, turn the locking ring on the cable counterclockwise until you can pull the cable free.
5. Disconnect the vaporizer heater cable from the connector on the probe, and then allow it to hang down.
6. Disconnect the sample transfer line from the APCI probe by turning the sample transfer line fitting counterclockwise until you can pull the transfer line and fitting free from the probe.

7. Disconnect the sheath gas line from the APCI probe by turning the sheath gas line fitting counterclockwise until you can pull the sheath gas line and fitting free from the probe.
8. Disconnect the AUX/Sweep gas line from the APCI probe by turning the auxiliary gas line fitting counterclockwise until you can pull the auxiliary gas line and fitting free from the probe.

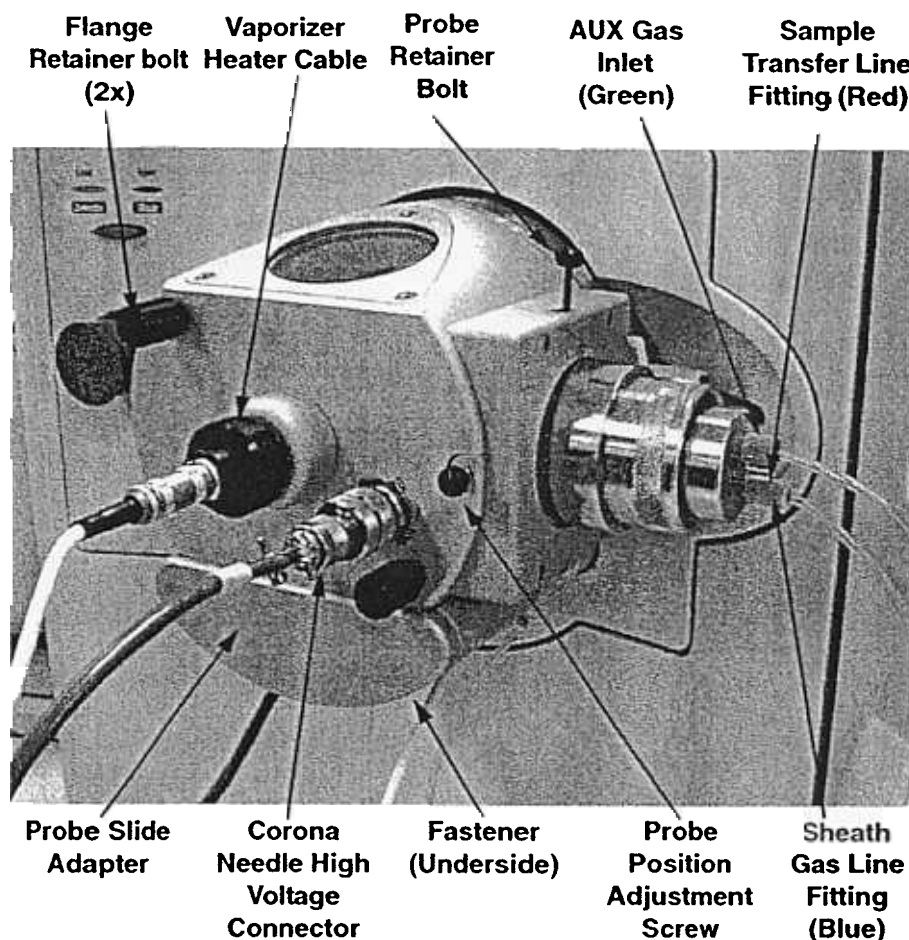


Figure 2-2. APCI probe assembly, showing AUX gas connected



CAUTION. AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 °C. Always allow the APCI probe to cool to ambient temperature, for approximately 20 min, before handling or removing the APCI probe from the APCI flange.

9. With one hand holding the APCI flange, loosen the knurled fastener that secures the APCI flange to the probe slide adapter.
10. Remove the APCI probe assembly from the probe slide adapter by sliding the probe off the slide adapter. Place the APCI probe assembly on a lint-free tissue and allow it to cool to ambient temperature (approximately 20 min).
11. Remove the corona discharge needle from the APCI probe assembly by pulling it free from the corona discharge needle assembly. Store the corona discharge needle by inserting it into one of the foam walls of the APCI probe assembly storage container.
12. Store the APCI probe assembly in its foam storage container. (Make sure that the APCI probe assembly is at ambient temperature before you place it in its storage container.)

2.2 Connecting the PEEK Safety Sleeve and a New Fused-Silica Sample Tube to the ESI Probe

Before you operate your LCQ Advantage, connect the PEEK safety sleeve and fused silica sample tube to the ESI probe.



CAUTION. AVOID ELECTRICAL SHOCK. When you are operating your instrument in the ESI Mode, there are two situations in which you could receive an electrical shock unless you install the safety kit discussed below. When you are using the Metal Needle Kit (P/N 7001-62217 or 7005-62013), you might receive an electrical shock if you touch the fused-silica capillary tube. You might receive an electrical shock if the fused-silica capillary tube breaks during ESI operation, with or without the metal needle installed. Therefore, for your safety and in compliance with international safety standards, you **must** cover the fused-silica capillary tube with the PEEK safety sleeve (P/N 00301-22806) and associated PEEK ferrules (P/N 00101-18119) provided in the Safety Sleeve Kit (P/N 70005-62015) before you operate the instrument. Installation instructions (P/N 70005-97009) are included in the kit. Operation of the instrument without the safety sleeve impairs the safety protection provided by the instrument and, thus, could lead to serious injury.

Connect the PEEK safety sleeve and sample tube to the ESI probe, as follows. See Figure 2-3.

Use a fused-silica cutting tool to cut a 12-in. (30 cm) piece of sample tube (P/N 00106-10499). Ensure that you squarely cut the ends of the sample tube.

2. Insert the sample tube through the exit end of the ESI needle and into the ESI probe.
3. Push the sample tube through the ESI probe until approximately 3.5 cm (1.5-in.) is left protruding from the exit end of the ESI needle. The remaining length of sample tube should exit the ESI probe sample inlet.
4. Slide the (brown) 10-32 × 1/4-28 PEEK fitting adapter over the sample tube and tighten the fitting in the ESI probe sample inlet.
5. Slide the 25.4 cm (10.0-in.) precut (brown) PEEK safety sleeve over the sample tube.
6. Slide a (brown) ferrule (P/N 00101-18119), narrow end first, over the PEEK safety sleeve and up to the 10-32 × 1/4-28 PEEK fitting.

- Slide a (red) fingertight fitting onto the PEEK safety sleeve and push it to the 10-32 x 1/4-28 PEEK fitting. Tighten the fingertight fitting slightly, but not completely.

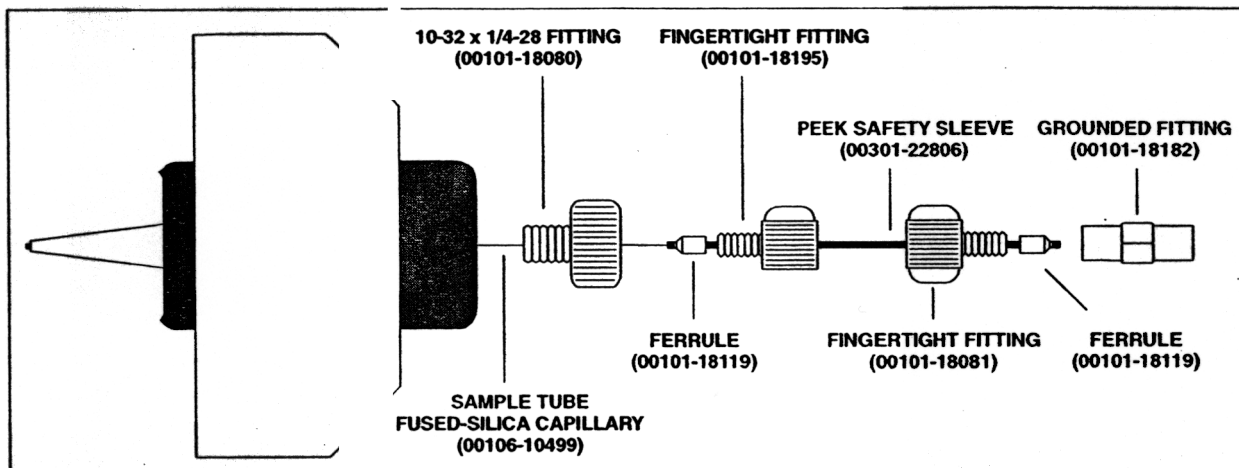


Figure 2-3. ESI/MS plumbing connections for the PEEK safety sleeve and sample tube

- Push the PEEK safety sleeve over the sample tube and into the ESI probe until it stops against the Teflon[®] needle seal inside the ESI probe.
- Pull the sample tube (from the ESI needle end) until the sample tube is flush with the precut square end of the PEEK safety sleeve.
- Slide a (brown) fingertight fitting and (brown) ferrule (P/N 00101-18119), wide end first, over the free end of the PEEK safety sleeve.
- Connect the PEEK safety sleeve and ferrule to the grounded fitting by tightening the fingertight fitting. Ensure that the fingertight fitting is securely tightened around the PEEK safety sleeve, otherwise the sample stream might enter between the sample tube and the PEEK safety sleeve.
- Ensure the sample tube is held tightly in the grounded fitting; check by pulling the sample tube from the exit end of the ESI needle.
- Use a fused-silica cutting tool to cut the sample tube at the ESI needle so that only 2.5 cm (1-in.) remains protruding from the exit end of ESI needle.
- From the ESI sample inlet carefully pull the PEEK safety sleeve back until the exit end of the sample tube is recessed inside the ESI needle (approximately 1 mm).
- Tighten the (red) fingertight fitting to securely hold the PEEK safety sleeve and sample tube in place in the ESI sample inlet.

2.3 Installing the ESI Probe Assembly

To install the ESI probe assembly, proceed as follows. See Figure 2-4 for the location of the components of the ESI probe assembly.

1. If your ESI probe assembly does not already contain a sample tube (fused-silica capillary), you need to follow the procedure for installing a sample tube that is outlined in the previous topic: **Connecting the PEEK Safety Sleeve and a New Fused-Silica Sample Tube to the ESI Probe.**
2. With one hand holding the ESI flange, align the ESI flange with the probe slide adapter, and place it onto the guide rails.

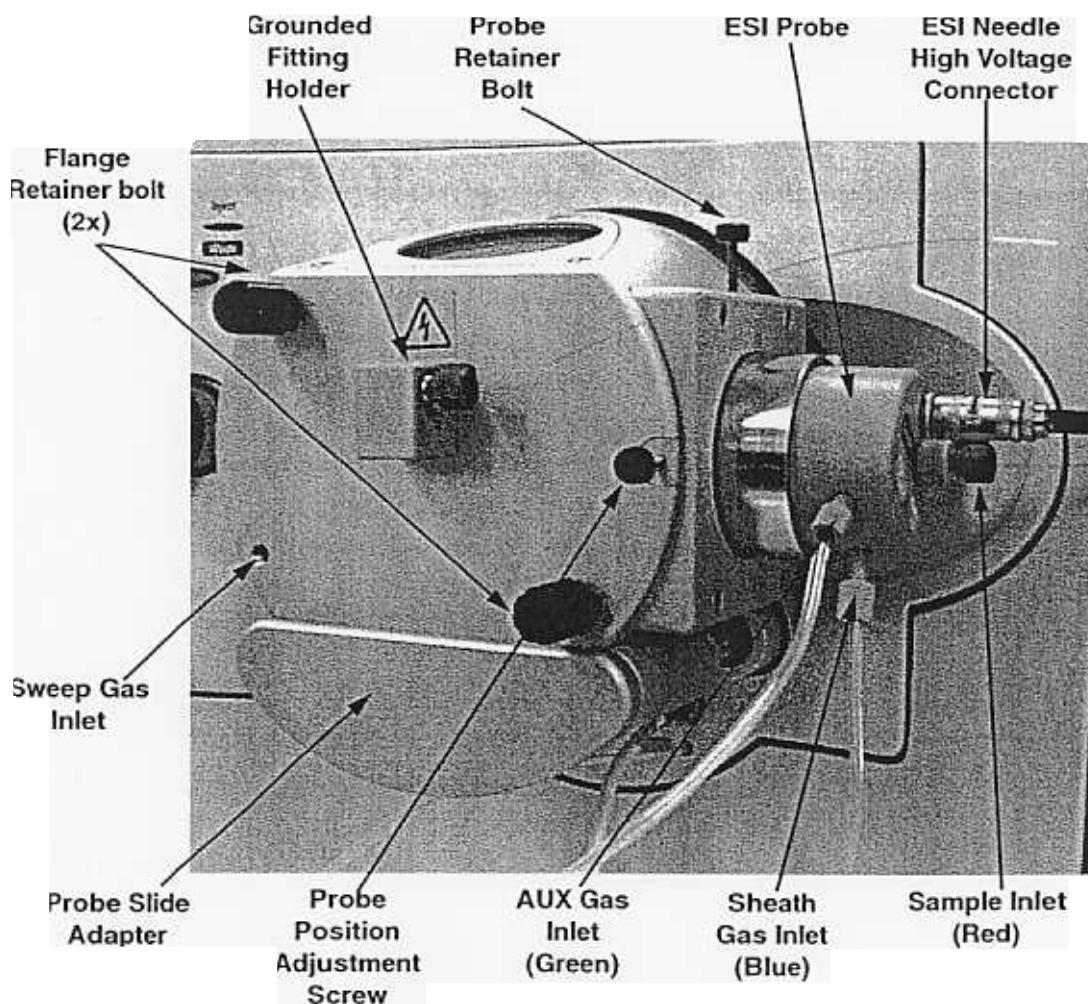


Figure 2-4. ESI probe assembly, showing AUX gas connected

3. Slide the ESI probe assembly onto the probe slide adapter. Secure the ESI probe assembly to the probe slide adapter with the knurled fastener that is located on the underside of the probe slide adapter.
4. Remove the Teflon coated septum from the entrance end of the ion transfer capillary.
5. With one hand, hold the ESI probe on the nozzle side of the probe to keep it from moving in the flange. With the other hand, connect the high voltage power cable to the connector labeled *HV* on the ESI probe. Turn the locking-ring on the cable clockwise to secure the cable.
6. Push the ESI probe assembly against the spray shield.
7. Secure the ESI flange to the spray shield with the two (black) flange retainer bolts.
8. Set the probe position to 3B for a 5- μ L/min experiment, as follows.
 - a. Loosen the probe retainer bolt to free the ESI probe. See Figure 2-4.
 - b. Grasp the probe firmly, and gently pull the probe $\frac{1}{4}$ in. away from the rest of the assembly. Notice the numbered position markers on top of the probe.
 - c. Gently move the probe in and out so that the metal sleeve around the probe aligns with position 3 on the probe.
 - d. Turn the probe position adjustment screw to align the mark on the metal sleeve with position B (the second mark from the right) on the instrument.
 - e. Tighten the probe retainer bolt to stabilize the probe on position 3B.
9. Connect the sheath gas line and (blue) fitting to the inlet labeled *Sheath Gas* on the ESI probe.
10. Connect either AUX or Sweep gas as follows:
 - To connect AUX gas: Connect the auxiliary gas line and (green) fitting to the inlet labeled *Aux Gas* on the ESI probe.
 - To connect sweep gas: Connect the auxiliary gas line and (green) fitting to the inlet labeled *Sweep Gas* on the ESI probe.
11. Connect the sample transfer line (coming from the syringe) to the grounded fitting.

Note. The ESI needle inside the ESI probe is positioned on a Teflon needle seat to prevent the loss of gas from inside the probe. Ensure that the needle is seated properly when you reassemble the ESI probe. Over time, the needle seat becomes compressed and needs to be replaced.

2.4 Setting Up the Syringe Pump for Tuning and Calibrating

The inlet that you use for ESI tuning and calibrating is a syringe infusion pump. A syringe pump allows you to infuse a sample solution into the ESI source for extended periods of time.

The syringe pump and syringe are located on the front panel of your LCQ Advantage MS detector. The plumbing connections for ESI/MS sample introduction from the syringe pump are shown in Figure 2-5. To infuse solution for tuning and calibrating, you install on the pump a 500- μ L Unimetrics syringe containing the calibration solution.

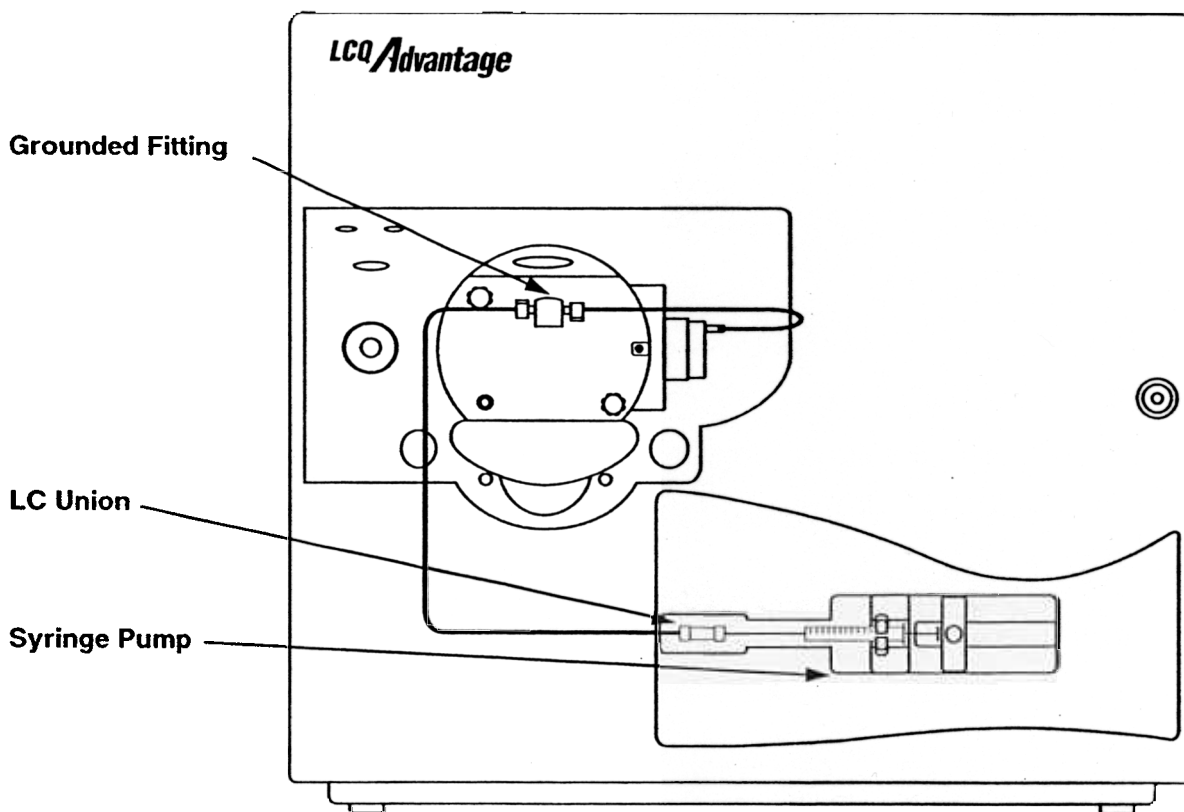


Figure 2-5. ESI/MS plumbing connections for sample introduction from the syringe pump

Use the following procedure to set up the syringe pump for infusion.

1. Connect a 4 cm (1.5 in.) segment of Teflon tube (00301-22803) with a (brown) Fingertight fitting and a (brown) ferrule to the (black) LC union. See Figure 2-6

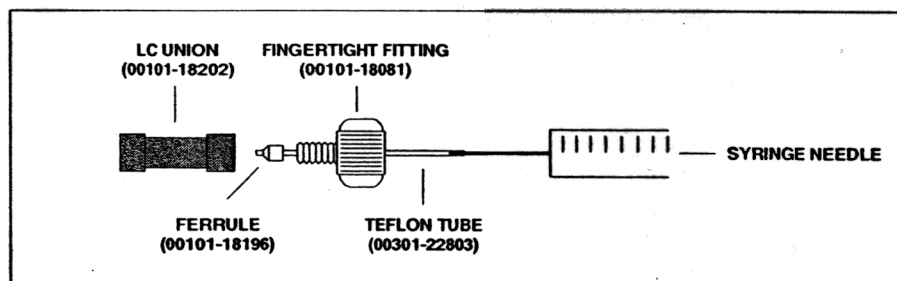


Figure 2-6. Plumbing connections for the syringe

2. Load a clean, 500- μ L Unimetrics syringe (P/N 00301-19012) with 420 μ L of the calibration solution. (Refer to **Appendix B: Sample Formulations** for a procedure for making the calibration solution.)
3. Insert the needle of a syringe into the segment of Teflon tube. Check that the needle tip of the syringe fits readily into the opening in the free end of the Teflon tubing. If necessary, you can enlarge the opening in the end of the tubing slightly.
4. Place the syringe into the syringe holder of the syringe pump.
5. While squeezing the silver release button on the syringe pump handle, push the handle forward until it just contacts the syringe plunger.
6. Connect a fused-silica infusion line from the LC union to the (stainless steel) grounded fitting, as follows. See Figure 2-7.
7. Connect the infusion line (P/N 00106-10504) with a (brown) Fingertight fitting and a (brown) ferrule to the free end of the LC union.
8. Connect the other end of the infusion line with a (red) Fingertight fitting and a (brown) ferrule to the grounded fitting.

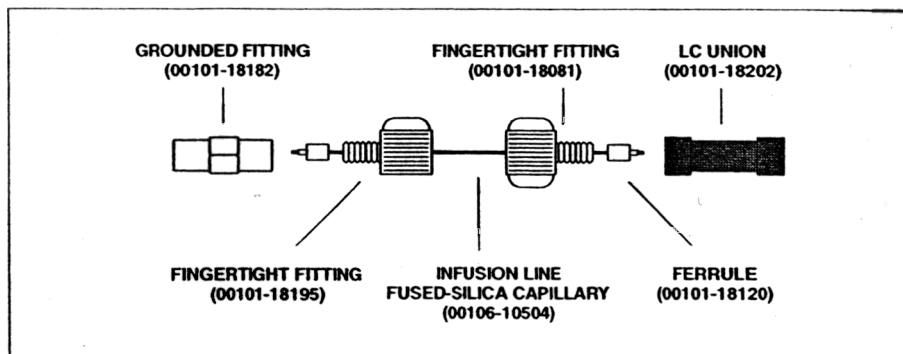


Figure 2-7. ESI/MS plumbing connections for the fused-silica infusion line

Caution. Prevent solvent waste from backing up into the API ion source and MS detector. Always ensure that the PVC drain hose is above the level of liquid in the waste container.

9. Connect an appropriate length of (clear) 3/8 in. ID PVC hose to the ESI probe outlet drain. Insert the end of the hose into a suitable container (such as a gallon jug). Ideally, the container should be vented to a fume exhaust system.

2.5 Setting Up the MS Detector for Tuning and Calibrating

Use the following procedure to set up the MS detector for tuning and calibrating in the ESI/MS mode.

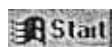
You tune first with calibration solution to ensure detection of a sufficient quantity of ions on which to calibrate. You then calibrate automatically some of the LCQ Advantage instrument parameters with calibration solution. The parameters that are optimized during calibration need to be updated only occasionally, unless your experiments involve high resolution mass assignment. Then, you might want to calibrate frequently.

Note. The following procedures assume that you are familiar with your LCQ Advantage instrument and the Tune Plus window. If you need assistance, refer to: LCQ Advantage online Help, LCQ Advantage Getting Connected, and/or LCQ Advantage Hardware Manual.



CAUTION. Before you begin normal operation each day, ensure that you have sufficient nitrogen for your API source. If you run out of nitrogen, LCQ Advantage automatically turns the MS detector Off to prevent the possibility of atmospheric oxygen from entering the ion source. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (In addition, if LCQ Advantage turns Off the MS detector during an analytical run, you could lose data.)

Open the Tune Plus window from the Start button on your Windows® 2000 Desktop, as follows:



- a. Choose **Start | Programs | Xcalibur | Xcalibur** to display the Xcalibur Home Page — Roadmap view.
- b. Click on the Instrument Setup button to display the window.
- c. Click on the LCQ Advantage MS button to display the New Method page.
- d. Click on the Tune Plus button to display the window.

2. Set the probe position to 3B as follows:
 - a. Loosen the probe retainer bolt to free the ESI probe. See Figure 2-4. ESI probe assembly
 - b. Grasp the probe firmly, and gently pull the probe ¼ in. away from the rest of the assembly. Notice the numbered position markers on top of the probe.
 - c. Gently move the probe in and out so that the metal sleeve around the probe aligns with position 3 on the probe.
 - d. Turn the probe position adjustment screw to align the mark on the metal sleeve with position B (the second mark from the right) on the instrument.
 - e. Tighten the probe retainer bolt to stabilize the probe on position 3B.



On



Standby

3. In Tune Plus, take the MS detector out of Standby mode and turn it On, as follows:

Click on the On/Standby button on the Control/Scan Mode toolbar. The MS detector begins scanning, LCQ Advantage applies high voltage to the ESI probe, and LCQ Advantage shows a real-time display in the Spectrum view.

Note. The LCQ Advantage data system contains customized tune files for different applications in the directory *C:\Xcalibur\methods*, including an ESI low flow LC/MS experiment.

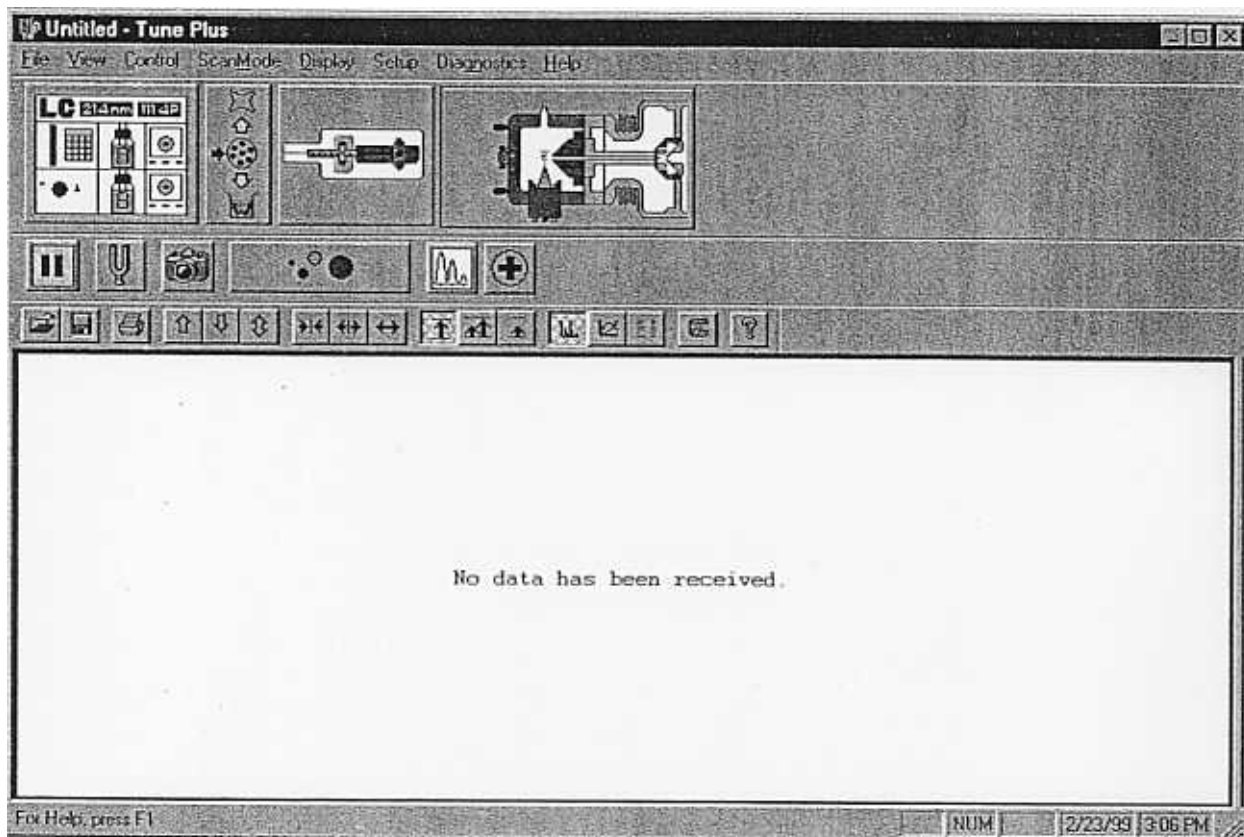


Figure 2-8. Tune Plus window, showing the MS detector in the Standby mode. (This Tune Plus window shows abbreviated toolbars. Refer to the Note on page 3-4 for more information.)

4. Open the Tune Method file that stores the factory default tune settings for low-flow ESI operation, as follows:
 - a. Display the Open dialog box:
Choose **File | Open**.
 - b. Select the file *AutoTune.LCQTune* in the directory *C:\Xcalibur\methods*:
Scroll down in the File Name combo box until you see *AutoTune.LCQTune*. Then, click on the file name.
 - c. Open the file, and close the dialog box:
Click on **Open**. LCQ Advantage downloads the Tune Method parameters to the MS detector.
5. Examine the pre-tune ESI source settings, as follows:
 - a. If necessary, specify the ESI source:
Choose **Setup | Change API Source Type**. Then, in the Change Source Type dialog box, which appears, select the ESI option button.

- b. Return to the Tune Plus window:
Click on **OK**.
- c. Open the ESI Source dialog box from the Instrument Setup toolbar:
Click on the API Source button. See Figure 2-9, and verify that the settings in your dialog box are the same as those shown in the figure.
- d. Return to the Tune Plus window:
Click on **OK**.

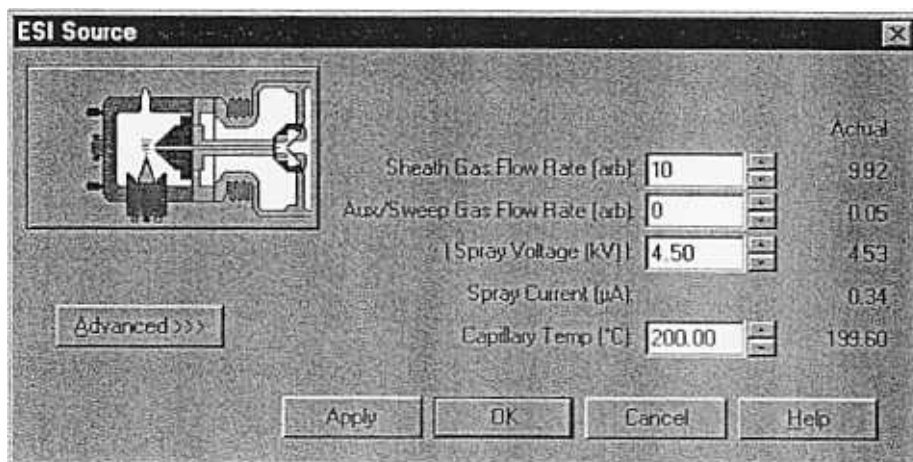
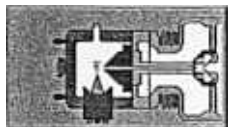


Figure 2-9. ESI Source dialog box, showing the settings to start a typical low flow experiment

6. Set the scan parameters for tuning and calibration, as follows:
 - a. Open the Define Scan dialog box:
Click on the Define Scan button on the Control/Scan Mode toolbar. See Figure 2-10.
 - b. Select the MS scan mode in the Scan Description group box:
Click on the Scan Mode: MS option button. Note that LCQ Advantage sets the MSⁿ power to 1.
 - c. Select the Full scan type:
Click on the Scan Type: Full option button.
 - d. Set the total number of microscans to 3 in the Scan Time group box:
Double-click in the Total Microscans spin box, then type 3.
 - e. Set the maximum injection time to 200.00 ms:
Double click in the Maximum Inject Time spin box, then type 200.
 - f. Specify that the ion source fragmentation option is turned off:
Confirm that the Turn On check box is not selected ()
 - g. Set the first mass for the scan range to m/z 150 in the Scan Ranges group box:
Double-click in the First Mass text box, then type 150.



- h. Set the last mass for the scan range to m/z 2000:
 Double-click in the Last Mass text box, then type 2000.
 - i. Ensure that the settings in your Define Scan dialog box are the same as those shown in Figure 2-10.
 - j. Save the MS detector scan parameters, and return to the Tune Plus window:
 Click on **OK**.
7. Select the profile data type, as follows:
 Click on the Centroid/Profile button in the Control/Scan Mode toolbar to toggle the data type to profile. (The picture on the button should be the same as that shown here.)
8. Select the positive ion polarity mode, as follows:
 Click on the Positive/Negative button in the Control/Scan Mode toolbar to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here)

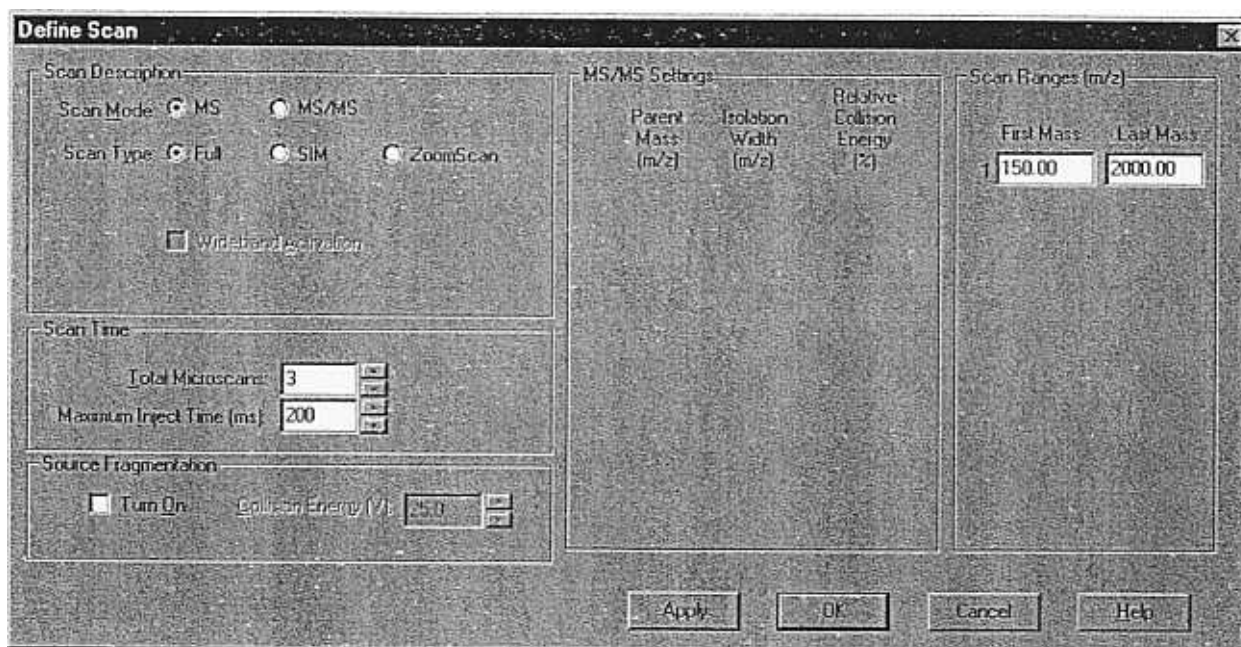


Figure 2-10. Define Scan dialog box, showing the typical settings for ESI/MS operation