

# Chapter 4

## Tuning with Your Analyte in LC/ESI/MS Mode

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This chapter provides information on tuning the MS detector in the LC/ESI/MS mode using your analyte. You optimize the sensitivity of your analyte in the MS detector through an automatic procedure.

The customized Tune Methods contained in your LCQ Advantage data system are optimized for a wide range of applications, and they can often be used without further tuning of your MS detector. However, for certain applications you might need to tune and optimize several MS detector parameters.

For instance, the most important parameters that interact with the ESI interface and signal quality are as follows:

- Electrospray voltage
- Ion transfer capillary temperature
- Tube lens offset voltage
- Capillary voltage
- Sheath gas flow rate
- AUX/Sweep gas flow rate

The settings for these parameters depend on the solvent flow rate and target analyte composition. In general, you should fine tune your MS detector whenever you change the solvent flow rate conditions of your particular application. In this procedure, you use the ESI low-flow Tune Method *ESImyTune.LCQTune* as a starting point, and then further optimize the MS detector parameters using an automatic procedure. The automatic procedure adjusts the tube lens offset voltage, capillary voltage, and voltages applied to the ion optics until the ion transmission of your analyte is maximized.

The capillary is heated to maximize the ion transmission to the MS detector. For ESI only, you set the ion transfer capillary temperature proportional to the infusion flow rate of your solution. See Table 1-2. For this procedure, the ion transfer capillary temperature is set to 350 °C, and sheath gas is set to 30.

**Note.** If your experiment is performed at a flow rate below 10  $\mu\text{L}/\text{min}$ , and the results you want can be obtained without optimizing the MS detector on your particular analyte, you can go to **Chapter 5: Acquiring ESI Sample Data Using the Tune Plus Window** to acquire sample data.

**Note.** Before you begin any of the procedures in this chapter, ensure that you have completed the procedures in the chapter: **Tuning and Calibrating Automatically in the ESI/MS Mode.**

To tune the MS detector in the ESI/MS (high-flow) mode using your analyte, you do the following:

- Set up the MS detector for your specific analyte from the Tune Plus window.
- Infuse your analyte into the MS detector using a syringe pump connected to the LC with a Tee union.
- Optimize the MS detector parameters for your analyte while the solution flows into the MS detector.

This chapter contains the following topics:

- Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC

Setting Up to Tune the MS Detector with Your Analyte

Optimizing the MS Detector Tune Automatically with Your Analyte

Saving the ESI/MS Tune Method

## 4.1 Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC

Set up to introduce your analyte by syringe pump into solvent flow from an LC. The plumbing connections for ESI/MS sample introduction from the syringe pump into solvent flow from an LC are shown in Figure 4-1

To make the plumbing connections for ESI/MS sample introduction from the syringe pump into solvent flow from an LC, do the following.

1. Connect a 4 cm (1.5 in.) segment of Teflon tubing with a (brown) Fingertight fitting and a (brown) ferrule to the (black) LC union.
2. Fill a clean, 500- $\mu$ L Unimetrics syringe (P/N 00301-19012) with the 1 ng/ $\mu$ L solution of reserpine or your analyte of interest. (Refer to **Appendix B: Sample Formulations** for a procedure for making the reserpine tuning solution.)

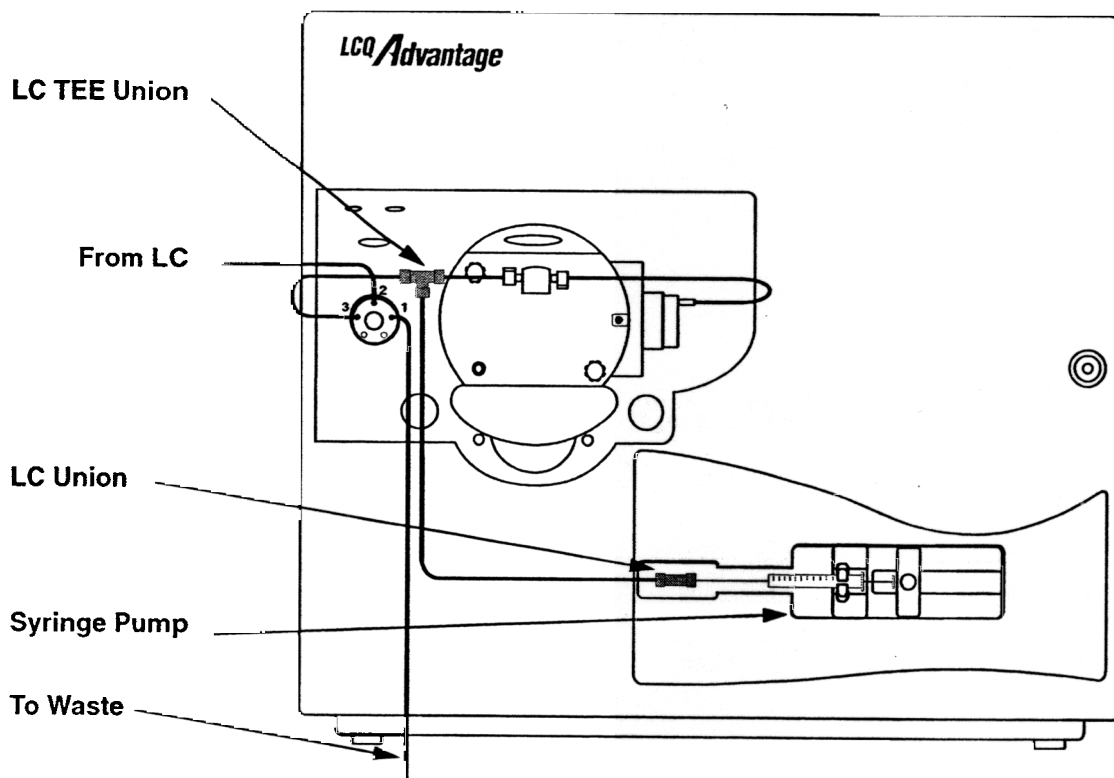


Figure 4-1. ESI/MS plumbing connections for sample introduction from the syringe pump into solvent flow from an LC

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 pressing the release button on the handle, push the handle forward until it just contacts the syringe plunger.
6. Connect the fused-silica infusion line from the (black) LC union to the (black) LC Tee union, as follows. See Figure 4-2.
  - a. 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.
  - b. Connect the other end of the infusion line with a (red) Fingertight fitting and a (brown) ferrule to the side arm of the LC Tee union.

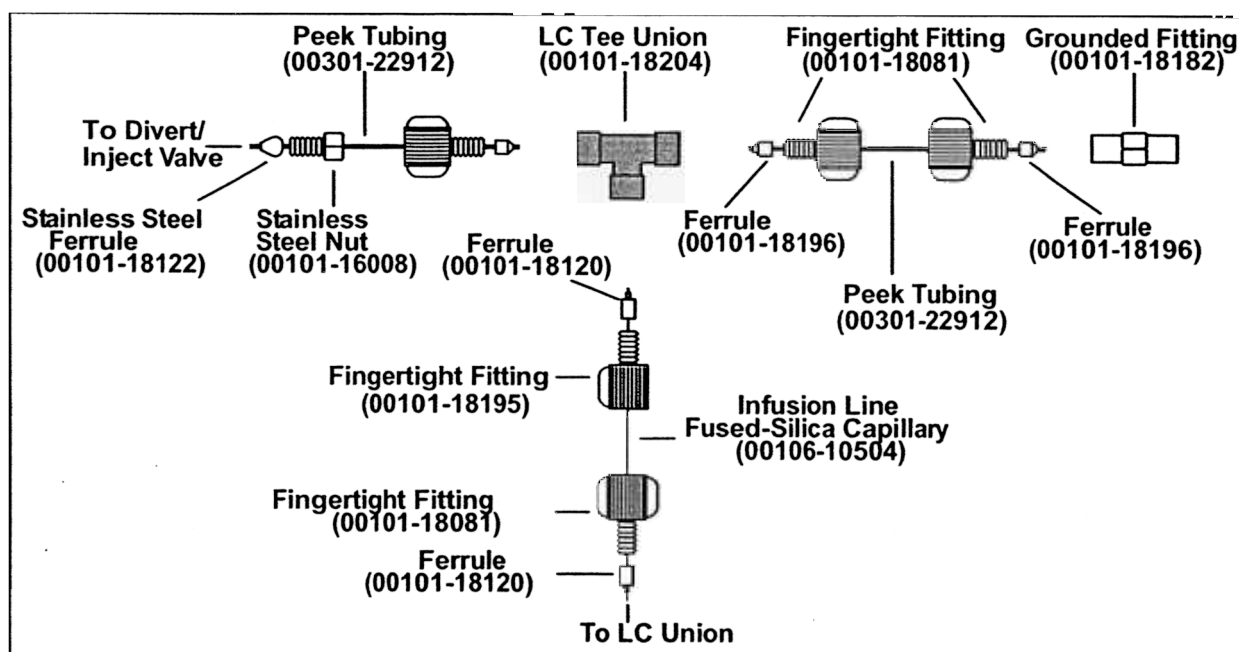


Figure 4-2. ESI/MS plumbing connections for the LC Tee union

7. Connect an appropriate length of (red) PEEK tubing from the (stainless steel) grounded fitting to the (black) LC Tee union, as follows. (See Figure 4-2.)
  - a. Use a PEEK tubing cutter to cut a 4 cm (1.5 in.) length of the PEEK tubing.
  - b. Connect the PEEK tubing with a (brown) Fingertight fitting and a (brown) ferrule to the grounded fitting.

- c. Connect the other end of the PEEK tubing with a (brown) Fingertight fitting and a (brown) ferrule to the LC Tee union.
8. If you have not already done so, connect the PEEK safety sleeve and fused-silica sample tube from the grounded fitting to the ESI probe sample inlet following the procedure in the topic: **Connecting the PEEK Safety Sleeve and Fused-Silica Sample Tube to the ESI Probe.**

If you have installed the stainless steel needle in the ESI probe, connect the PEEK safety sleeve and fused-silica capillary tube from the grounded fitting to the ESI probe sample inlet following the procedure in the topic: **Installing the Stainless Steel Needle and Connecting the PEEK Safety Sleeve and Fused-Silica Capillary Tube to the ESI Probe in LCQ Advantage Getting Connected.**

When the connections between the grounded fitting and the LC Tee union are complete, they should appear as in Figure 4-3.

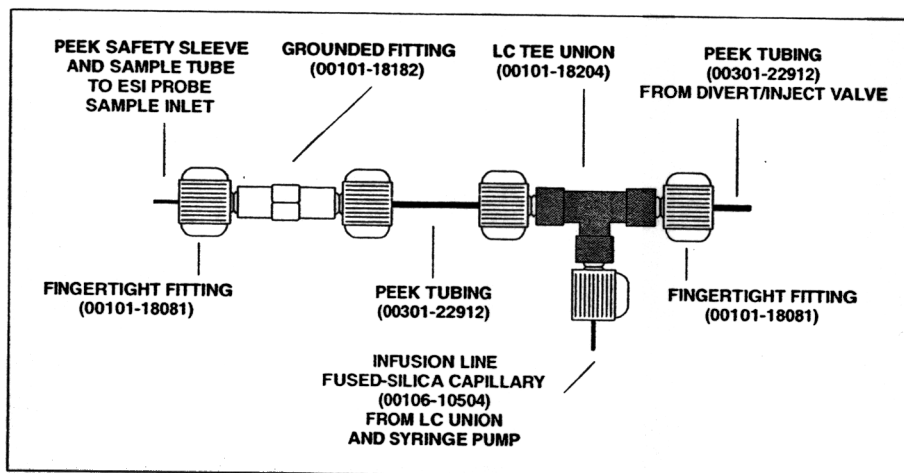


Figure 4-3. ESI/MS plumbing connections for the LC Tee union (shown fully connected)

9. Connect an appropriate length of PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the free end of the (black) LC Tee union, as follows. See Figure 4-4.
  - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve.
  - b. Connect the other end of the PEEK tubing with a (brown) Fingertight fitting and a (brown) ferrule to the free end of the LC Tee union.

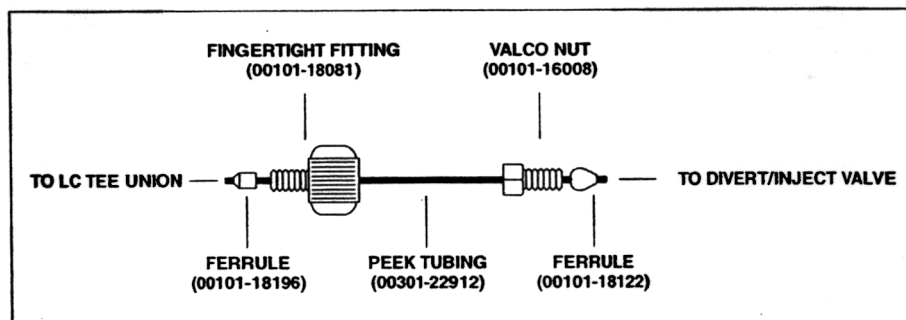


Figure 4-4. Plumbing connections for the divert/inject valve and LC Tee union

10. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC, as follows:
  - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 2 of the divert/inject valve.
  - b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.
11. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container, as follows:
  - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 1 of the divert/inject valve.
  - b. Insert the other end of the PEEK tubing in a suitable waste container.

**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.

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

Go on to the next topic: **Setting Up to Tune the MS Detector with Your Analyte.**

## 4.2 Setting Up to Tune the MS Detector with Your Analyte

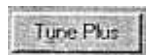
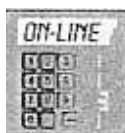
Use the following procedure to set up the MS detector to tune automatically on your analyte in ESI/MS mode. (In this example, you can use the reserpine solution described in **Appendix B: Sample Formulations**, or you can use a solution of your analyte of interest.)

**Caution.** Do not use calibration solution at flow rates above 10  $\mu\text{L}/\text{min}$ . Ultramark 1621 can contaminate your system at high concentrations.

**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 and/or LCQ Advantage Hardware Manual.

1. If you have not already done so, open the Tune Plus window from the Start button on your Windows 2000 Desktop, as follows:
  - a. Choose **Start | Programs | Xcalibur | Xcalibur**. The Xcalibur Roadmap – Home Page appears.
  - b. Click on the Instrument Setup button. The Instrument Setup window appears.
  - c. Click on the LCQ Advantage MS button. The New Method page is displayed.
  - d. Click on the Tune Plus button. The Tune Plus window appears.
2. In Tune Plus, take the MS detector out of Standby mode and turn it On, as follows:

Click on the On/Standby button in 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.



On



Standby



3. Open the *ESImyTune.LCQTune* Tune Method, the Tune Method you saved in Chapter 3, as follows:

- a. Display the Open dialog box from the File/Display toolbar:  
Click on the Open File icon.
- b. Select the file *ESImyTune.LCQTune* in the directory *C:\Xcalibur\methods*:  
Scroll down in the File Name combo box until you see *ESImyTune.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, and the title bar in the Tune Plus window should read as follows:

*C:\Xcalibur\methods\ESImyTune.LCQTune – Tune Plus*

4. Define the scan parameters for tuning with your analyte in the ESI/MS mode, as follows:



- a. Open the Define Scan dialog box:  
Click on the Define Scan button in the Instrument Control toolbar. See Figure 4-5.
- 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 MS<sup>n</sup> power to 1.
- c. Select the SIM scan type:  
Click on the Scan Type: SIM option button. Note that LCQ Advantage sets the Total Scan Ranges to 1.
- 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. For this example, leave the maximum injection time set to its default value of 200.00 ms.
- f. Specify that the ion source fragmentation option is turned off:  
Confirm that the Turn On check box is not selected .
- g. Set the center mass for the scan range to *m/z* 609.2 (for reserpine) in the Scan Ranges group box:  
Double-click in the Center Mass text box, then type 609.2.
- h. Set the width of the scan range to *m/z* 2:  
Double-click in the Width text box, then type 2.
- i. Ensure that the settings in your Define Scan dialog box are the same as those shown in Figure 4-5.
- j. Save the MS detector scan parameters, and close the Define Scan dialog box:  
Click on **OK**.



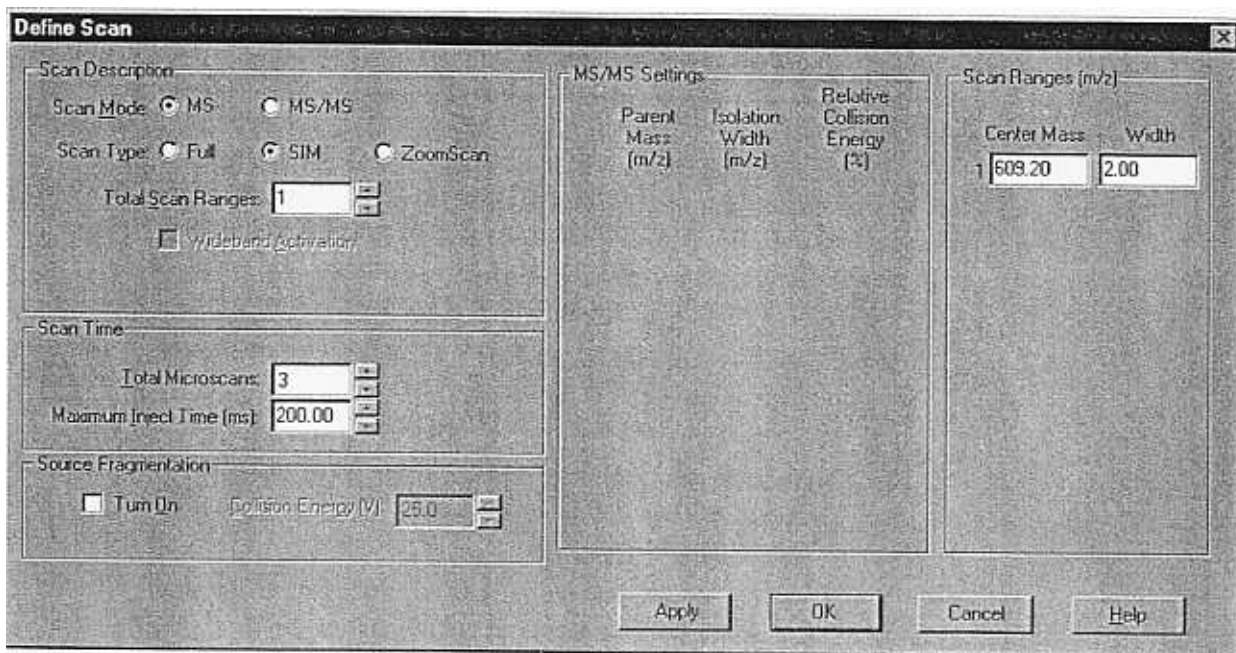


Figure 4-5. Define Scan dialog box, showing typical settings for acquiring reserpine data of the SIM type

5. Select the profile data type from the Control/Scan Mode toolbar, as follows:  
Click on the Centroid/Profile button to toggle the data type to profile. (The picture on the button should be the same as that shown here).
6. Select the positive ion polarity mode, as follows:  
Click on the Positive/Negative button in the to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

You have completed setting up to tune your MS detector with your analyte in ESI/MS mode. Go to the next topic: **Optimizing the MS Detector Tune Automatically with Your Analyte.**

## 4.3 Optimizing the MS Detector Tune Automatically with Your Analyte

Optimize the MS detector tune automatically to maximize the ion transmission of reserpine, or your analyte of interest, for a high-flow experiment. It is recommended that you begin optimizing after you have successfully passed an automatic tuning procedure and an automatic calibration procedure with the calibration solution infused at 5  $\mu\text{L}/\text{min}$ .

Use the following procedure to tune the MS detector automatically on the reserpine  $m/z$  609.2 at your particular flow rate, for example, 1 mL/min. (See Table 1-2 for guidelines about flow rates and temperatures.)

1. Display the automatic tuning page in the Tune dialog box, as follows:  
Click on the Tune button on the Control/Scan Mode toolbar. Then, click on the Automatic tab.
2. Optimize your tune on the peak at  $m/z$  609.2, as follows:  
Double-click on the Mass spin box, then type **609.2**.
3. Ensure that the Divert/Inject valve is in the Detector position, as follows:
  - a. Click on the Divert/Inject button to open the Divert/Inject Valve dialog box. See Figure 4-6.
  - b. Select the Detector option button.
  - c. Click on **Close**.

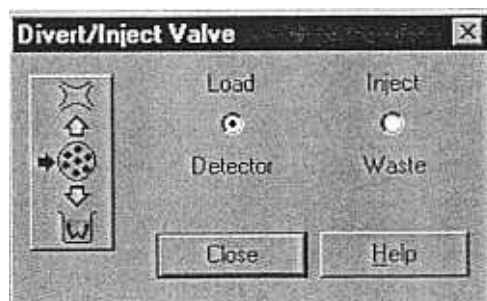


Figure 4-6. Divert/Inject Valve dialog box

4. Start the automatic tuning procedure from the Tune dialog box, as follows:
  - a. Click on **Start**. A message box displays the following message:  
*Please ensure that the 500 microliter syringe is full.*  
Ensure the syringe pump contains at least 420  $\mu\text{L}$  of the 1 ng/ $\mu\text{L}$  reserpine tuning solution.



- b. Close the message box, and return to the Tune Plus window:  
Click on **OK**.
5. Display the Graph view, as follows:  
Click on the Graph View button in the File/Display toolbar.
6. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, LCQ Advantage displays various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box. Your Tune Plus window should now look like the one shown in Figure 4-7

**Note.** The most important parameters that affect the signal quality during ESI/MS operation are the ion transfer capillary temperature, capillary voltage, tube lens offset voltage, gases, and solution flow rate. If any one of these parameters is changed, you need to reoptimize MS detector parameters. You can use the Semi-Automatic tune procedure to tune the MS detector on individual parameters.

You have now successfully tuned the MS detector in ESI/MS mode for the compound reserpine (or your analyte of interest). Go on to the next topic: **Saving the ESI/MS Tune Method.**

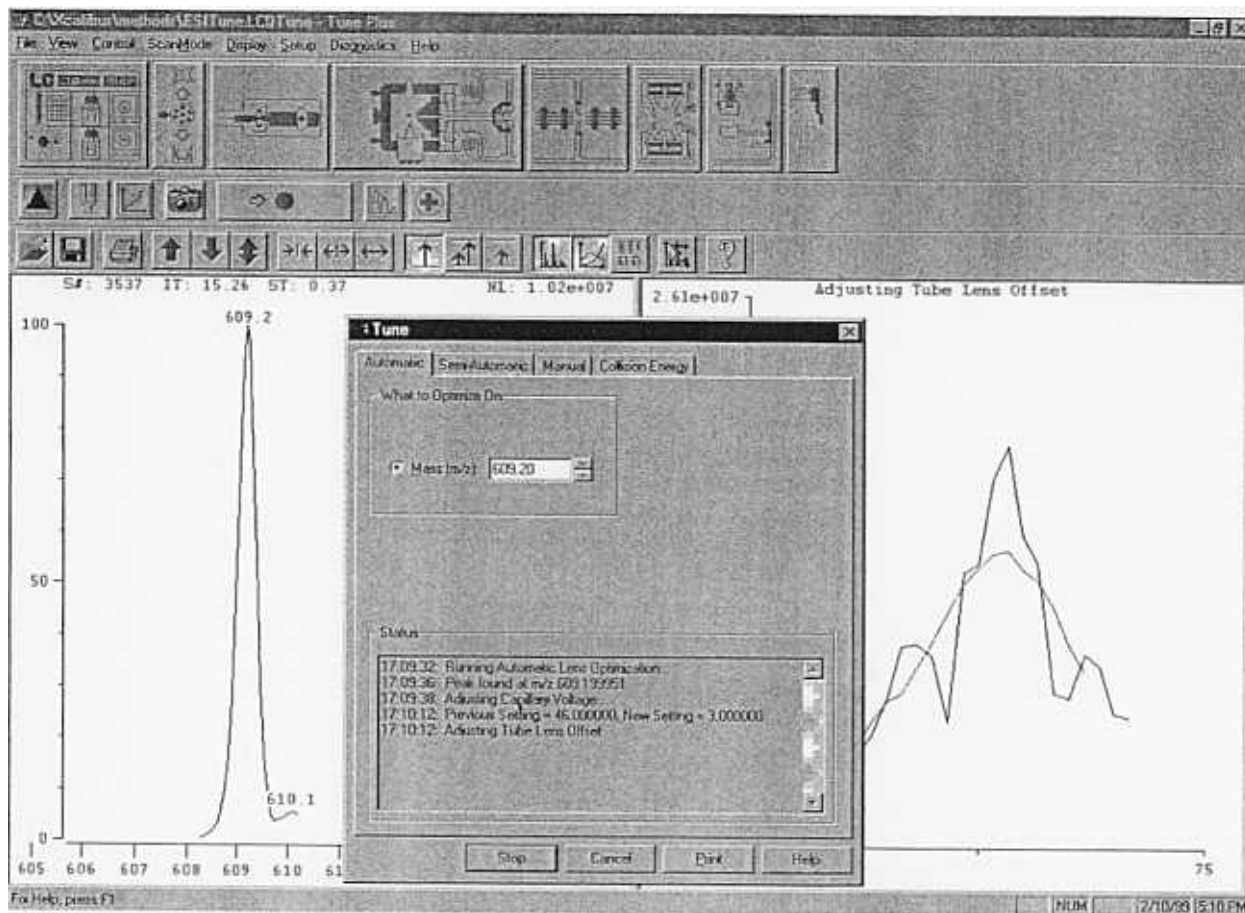


Figure 4-7. Tune Plus window with the Tune dialog box, showing the Automatic page

## 4.4 Saving the ESI/MS Tune Method

Save your ESI/MS tune method (for a high-flow experiment using your analyte) when automatic tuning is complete, as follows:

**Note.** Save the Tune Method while the MS detector is On, if any of the ion source parameters are different from those with which you started.

Display the Save As dialog box, as follows:  
Choose **File | Save As**. See Figure 4-8.

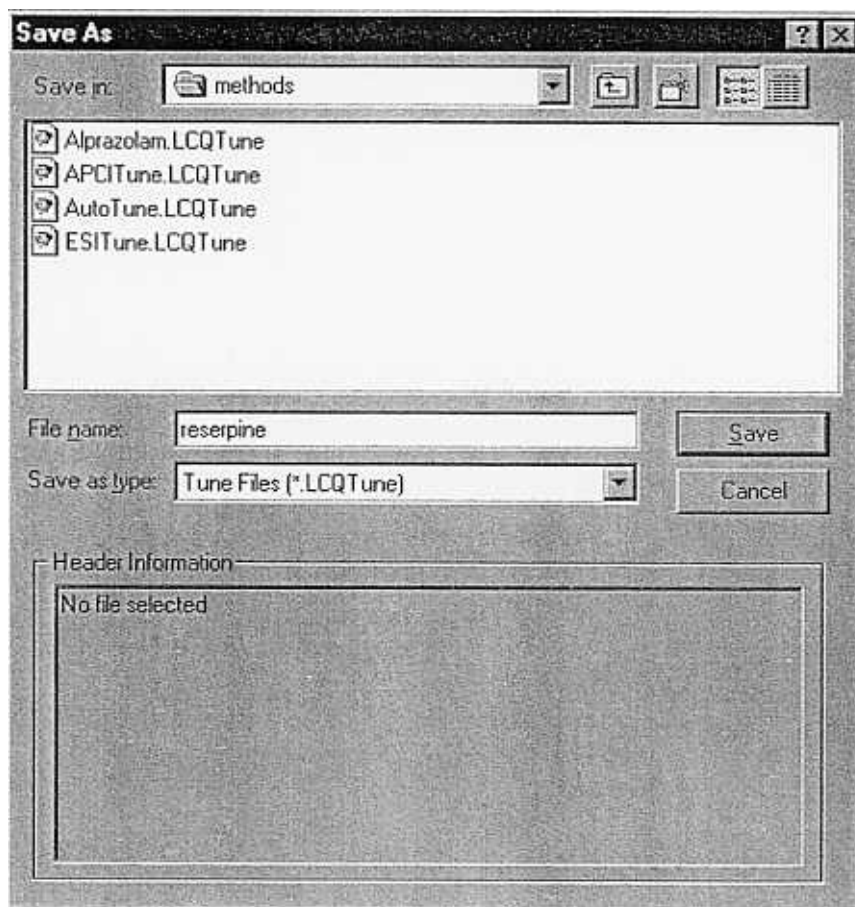


Figure 4-8. Save As dialog box, showing files in the directory *C:\Xcalibur\methods*

2. Select the *C:\Xcalibur\methods* directory.
3. Name the Tune Method for the analyte of interest, as follows:  
Click on the File Name text box, and type **reserpine**.
4. Save the Tune Method, close the dialog box, and return to the Tune Plus window, as follows:  
Click on **Save**. Note that the Tune Method is named *reserpine.LCQTune*.

Once you have optimized the tune of the MS detector, you are ready to perform your ESI/MS experiment in MS scan modes. Go on to the next chapter: **Acquiring ESI Sample Data using the Tune Plus Window**.