Chapter 2
Setting-Up and Tuning for ESI

Note: *For full details of the Tune window, see Appendix C.*

2.1 Setting-Up

2.1.1 Removing the APCI Probe

You may need to remove the APCI probe and corona discharge pin from the instrument before fitting the ESI probe. Figure 2-1 shows the APCI probe mounted on the instrument.

Warning: *To avoid electric shock, ensure that the instrument is in Standby before starting this procedure.*

1. In the MassLynx Tune window, click Press for Standby and confirm that the adjacent instrument status indicator shows red.

Warning: *The liquid passing through the HPLC pump, LC column, and APCI probe may be biohazardous and/or toxic. Always wear nitrile gloves when working with these items.*

2. Disconnect the LC system from the APCI probe.
3. Wait for three minutes to allow the desolvation gas flow to cool the probe and source.
4. In the MassLynx Tune window, click  to stop the nitrogen flow.

Warning: *The probe and source may be hot. To avoid burns, take great care while working with the instrument's access door open.*

5. Open the instrument's access door.
6. Disconnect the probe electrical connection on the instrument front panel.

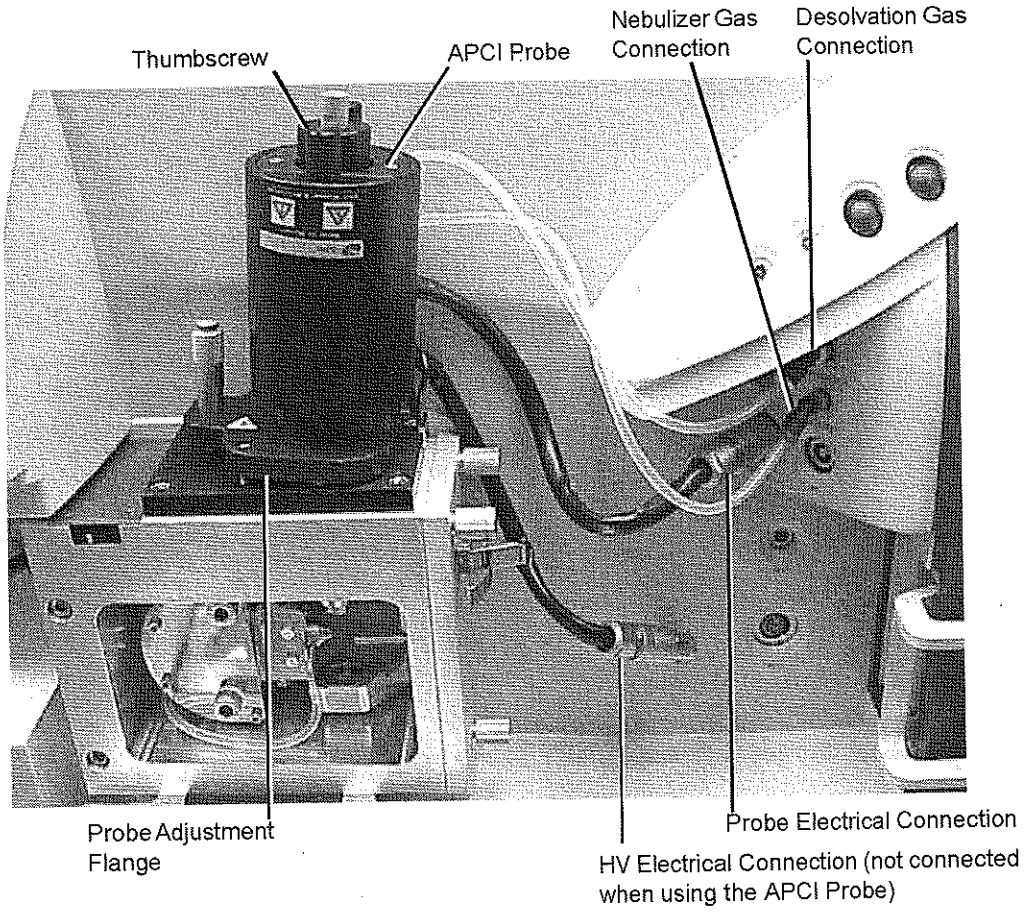


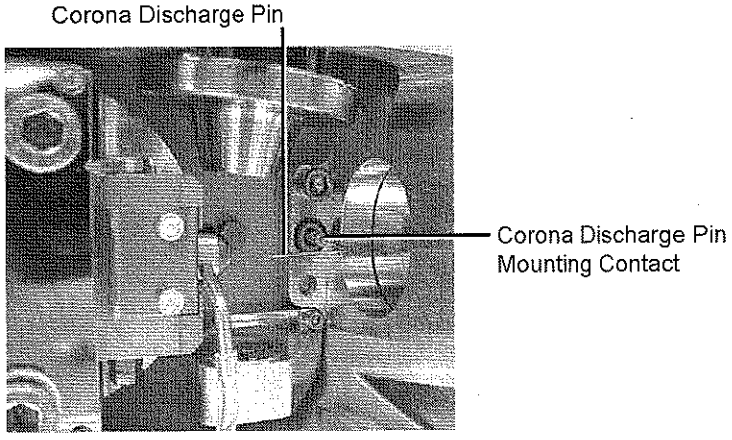
Figure 2-1 APCI Probe Mounted on the Source Enclosure

7. Disconnect the PTFE tubes at the nebulizer and desolvation gas connections on the front panel.
8. Undo the two thumbscrews securing the probe to the probe adjustment flange.
9. Carefully remove the probe from the probe adjustment flange.

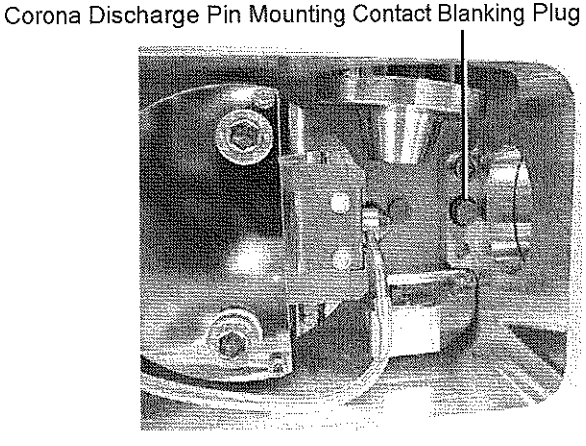
2.1.2 Removing the APCI Corona Discharge Pin

Warning: *To avoid electric shock, ensure that the instrument is in Standby when installing the corona discharge pin.*

1. In the MassLynx Tune window, click Press for Standby and confirm that the adjacent instrument status indicator shows red.
2. Unfasten the source enclosure door's securing clips and open the door.
3. Use needle-nose pliers to remove the corona discharge pin from its mounting contact. Store the corona discharge pin in a safe location.



4. Use the needle-nose pliers to fit the blanking plug to the corona discharge pin mounting contact.



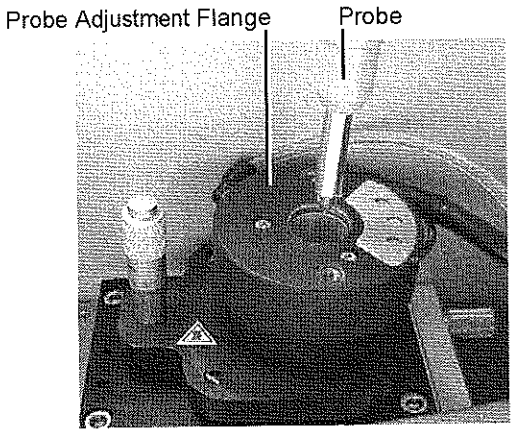
2.1.3 Installing the ESI (Electrospray) Probe

Warning: *The probe and source may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while handling these components.*

Warning: *The probe and source may be hot. To avoid burns, take great care while working with the instrument's access door open.*

Warning: *To avoid electric shock, ensure that the instrument is in Standby before commencing this procedure.*

1. In the MassLynx Tune window, click Press for Standby and confirm that the adjacent instrument status indicator shows red.
2. Open the instrument's access door.
3. Remove the protective sleeve, if fitted, from the electrospray probe tip.
4. Carefully slide the probe into the hole in the probe adjustment flange.



5. Secure the probe by tightening the two thumbscrews (Figure 2-2).

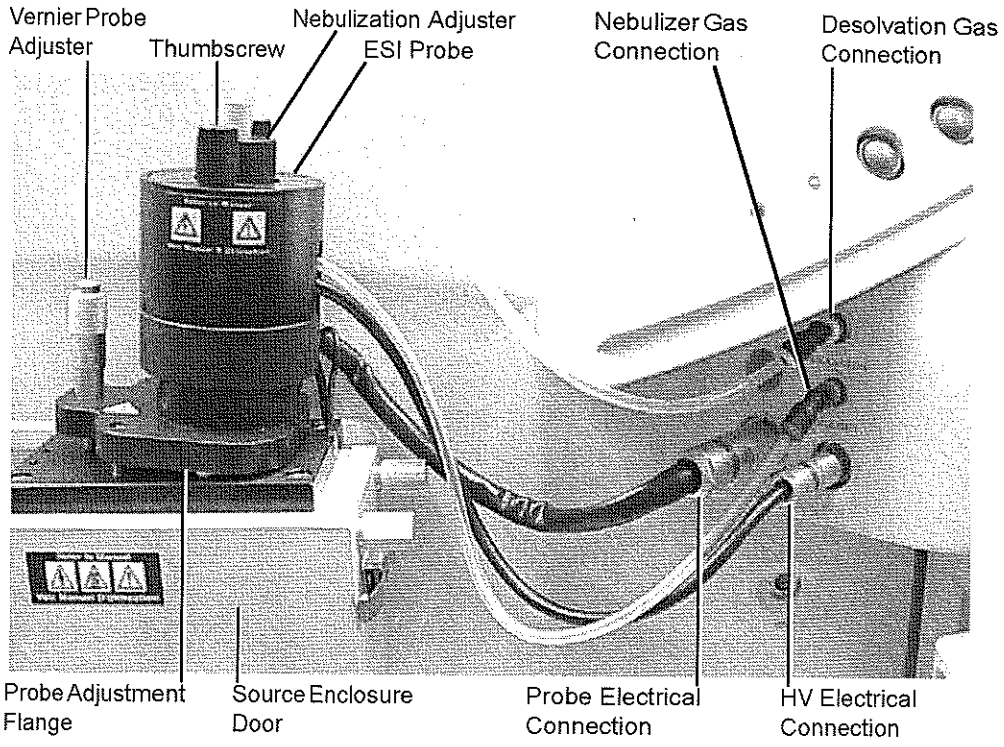


Figure 2-2 ESI Probe Mounted on the Source Enclosure, Showing the Connections to the Front Panel

6. Connect the probe adjustment flange electrical cable to the Probe connection.
7. Connect the probe adjustment flange PTFE tube to the Desolvation gas connection.
8. Connect the probe PTFE tube to the Nebuliser gas connection.
9. Connect the probe electrical lead to the HV connection.
10. Close the instrument's access door.

2.2 Tuning via Sample Infusion

Note: *This example procedure specifies verapamil as the infused sample. Therefore, the choice of HPLC column and some parameter settings may be specific to that compound. You may tune the instrument using a different sample compound, however you may need to use a different type of column, and parameter settings may differ from the ones given here.*

2.2.1 Tuning for MS Operation

Warning: *The liquids passing through the HPLC pump, LC column, syringe pump, and ESI probe may be biohazardous and/or toxic. Always wear nitrile gloves when working with these items.*

Warning: *To avoid high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, syringe pump, and ESI probe.*

Warning: *To avoid electric shock, ensure that the instrument is in Standby before commencing this procedure.*

1. In the MassLynx Tune window, click Press for Standby, and confirm that the adjacent instrument status indicator shows red.
2. Complete the connections between the HPLC pump, syringe pump, and ESI probe as shown in Figure 2-3.

   The column used in this example is a Waters Symmetry® C_{18} 2.1 × 100-mm, 3.5-µm. The mobile phase used is 70:30 acetonitrile/water.
3. Look through the source's view port and confirm that the isolation valve lever is fully to the left (i.e., the valve is open) (Figure 2-4).

Warning: *The source may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while handling this component.*

Warning: *The source may be hot. To avoid burns, take great care while working with the instrument's access door open.*

If the isolation valve lever is in the wrong position:

a. Open the instrument's access door.

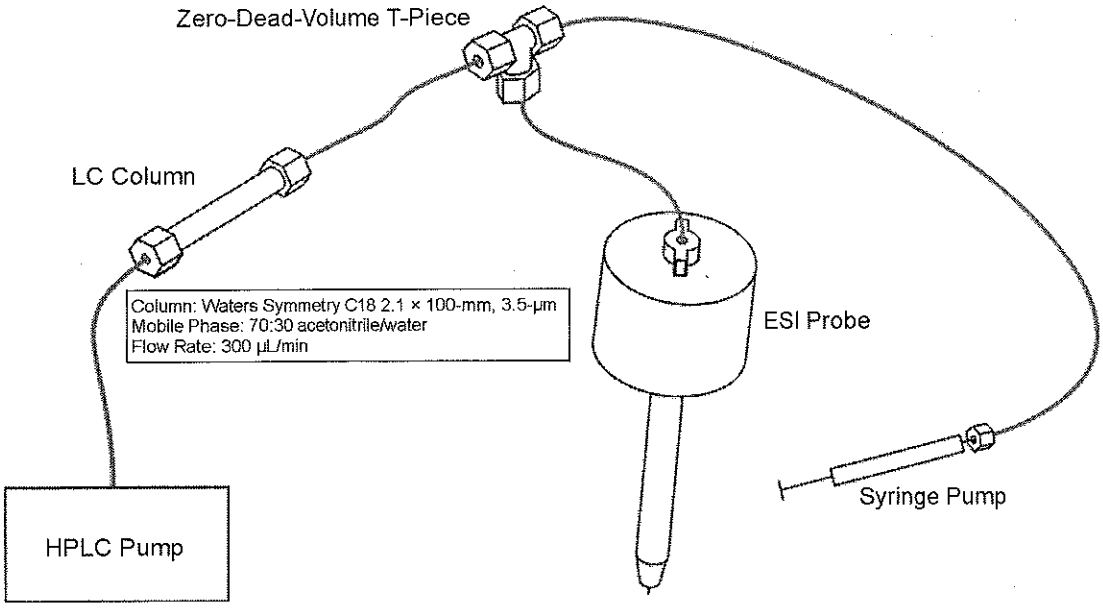


Figure 2-3 Syringe and Column Connections to the ESI Probe

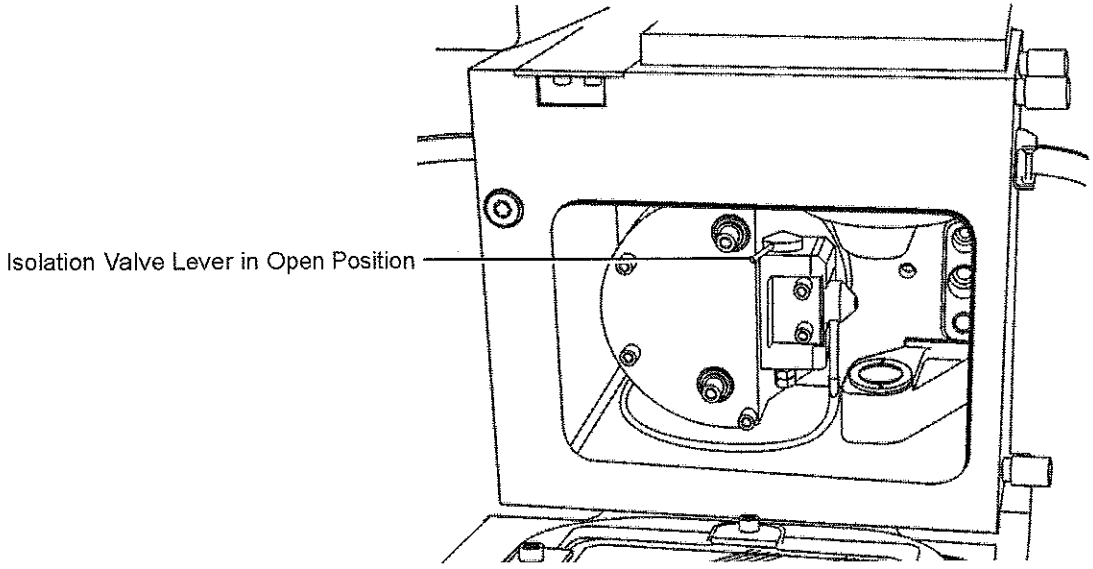


Figure 2-4 Isolation Valve in the Open Position (Source Enclosure Door Shown Open for Clarity)

b. Unfasten the source enclosure door's securing clips, and open the door.

c. Move the lever to the open position.

d. Close the source enclosure door, and fasten the securing clips.

e. Close the instrument's access door.

4. In the MassLynx Tune window, select Ion Mode > Electrospray+ to display the ES+ Source page (Figure 2-5).

Note: *The instrument can also be tuned via the EasyTune Source page (see Section C.13).*

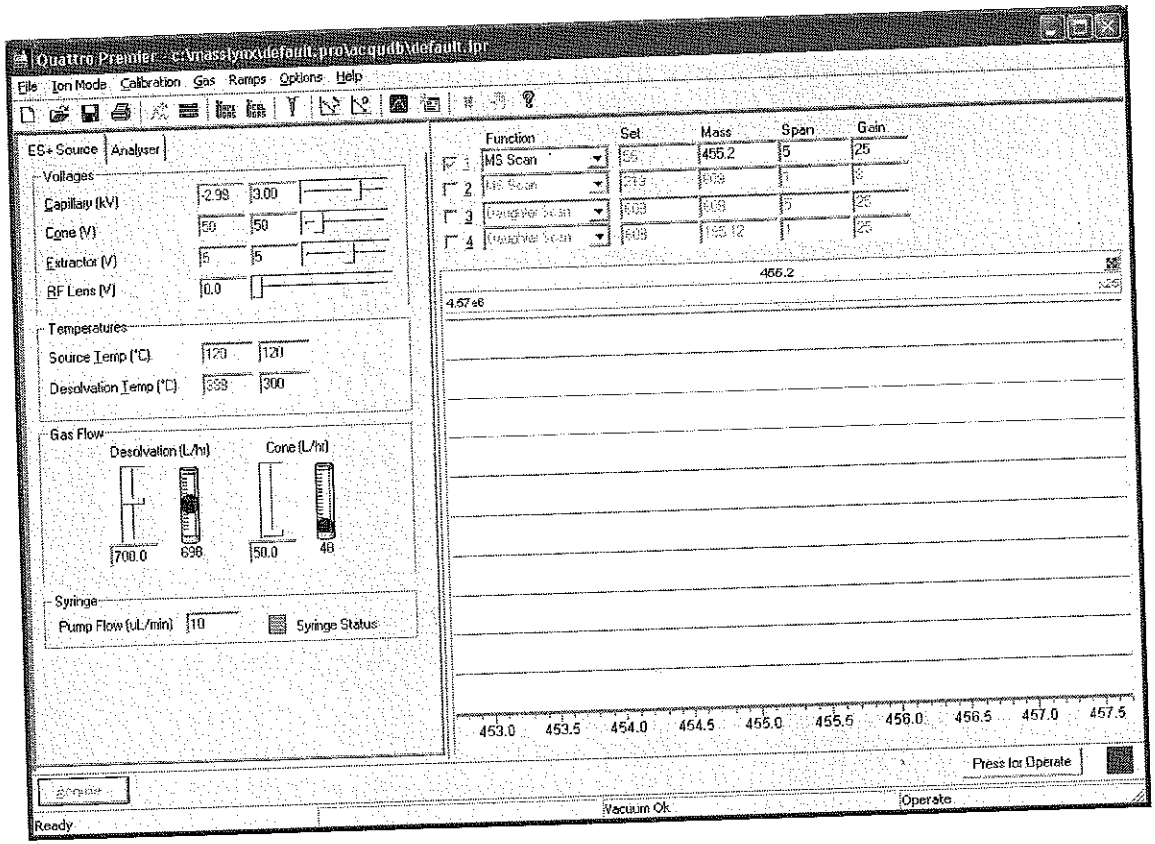


Figure 2-5 Tune Window ES+ Source Page

5. Set the parameters to the recommended values shown in Table 2-1.

6. Click the Analyser tab.

7. Set the parameters to the recommended values shown in Table 2-2.

Table 2-1 Recommended ES+ Source Page Parameter Values

Parameter	Recommended Value
Voltages	
Capillary (kV)	3.00
Cone (V)	50
Extractor (V)	5
RF Lens (V)	0.0
Temperatures	
Source Temp (°C)	120
Desolvation Temp (°C)	300
Gas Flow	
Desolvation (L/hr)	700.0
Cone (L/hr)	50.0

Table 2-2 Recommended Analyser Page Parameter Values

Parameter	Recommended Value
Analyser	
LM Resolution 1	15.0
HM Resolution 1	15.0
Ion Energy 1	0.5
Entrance	50
Collision	2
Exit	50.0
LM Resolution 2	15.0
HM Resolution 2	15.0
Ion Energy 2	3.0
Multiplier*	550

* The value stated for Multiplier is typical; in practice, you should use the value determined by the Waters Field Service Engineer during installation of the instrument.

8. Click  to start the nitrogen flow.
9. On the ES+ Source page, observe the Desolvation and Cone gas flows; confirm they are stabilized and correct.
10. Click Press for Operate, and confirm that the adjacent instrument status indicator shows green.
11. Turn on the LC system at a flow rate of 300 µL/min, and confirm that its pressure is stable.
12. Allow 15 minutes for the LC column to equilibrate.
13. Load the sample syringe with sample. In this example, verapamil (concentration 50 pg/µL, in 70:30 acetonitrile/water) is used.
14. Select the correct syringe type using this procedure:
    a. Select Options > Syringe Type to open the Syringe Selection dialog box.
    b. Choose the required syringe type from the drop-down list.

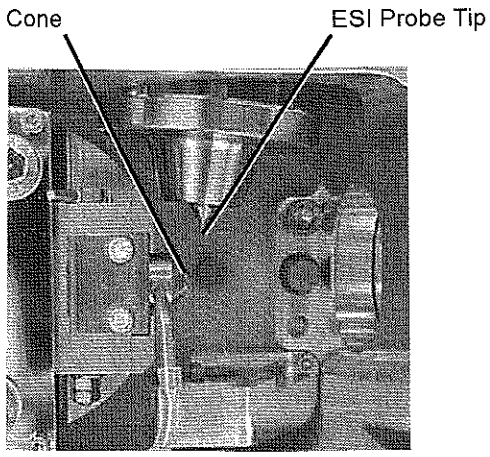
    Note: *If the syringe type is not listed, click Edit, and add the relevant details.*

    c. Click OK.
15. On the ES+ Source page, set Syringe, Pump Flow (µL/min) to 10.
16. Click  to start the syringe pump.
17. In the Tune window, select the Function 1 box.
18. Choose MS Scan from the adjacent drop-down list.
19. Enter Mass 455.2, Span 5, and Gain 25.

    Note: *The above are recommended values and can vary from instrument to instrument.*

20. Observe the verapamil peak at m/z 455.2 ($M+H^+$) in the Tune window (see Figure 2-6).
21. Use the vernier probe adjuster, on the probe mounting flange (see Figure 2-2), to maximize the displayed peak intensity.

22. Use the vernier probe adjuster to move the probe tip as far away from the sample cone as possible without losing more than 20% of the maximum displayed peak intensity obtained in step 21. This minimizes source contamination.



23. Use the nebulization adjuster, on the probe (see Figure 2-2), to give the best displayed peak intensity and stability.
24. In the Tune window ES+ Source page, adjust the Desolvation (L/hr) gas flow, in increments of 100; allow the pressure to stabilize after each adjustment. Set the gas flow to the value giving the highest displayed peak intensity.
25. Starting from a value of 0, increase the Cone (L/hr) gas flow in increments of 50; allow the pressure to stabilize after each adjustment. Set the gas flow to the highest value that does not significantly reduce the peak intensity. This minimizes solvent ion cluster formation.
26. Starting from a value of 2.6, increase the Capillary (kV) voltage in increments of 0.2. Set the capillary voltage to the value giving the highest displayed peak intensity.
27. Starting from a value of 15, increase the Cone (V) voltage in increments of 2. Set the cone voltage to the value giving the highest displayed peak intensity. Record this value.
28. Starting from a value of 0, increase the Extractor (V) voltage in increments of 1. Set the extractor voltage to the value giving the highest displayed peak intensity.
29. Adjust the RF Lens (V) voltage to the minimum value that maintains the highest peak intensity, without loss of resolution.
30. Confirm that the displayed peaks have the correct resolution (the isotopes are resolved as shown in Figure 2-6). If necessary, adjust the Analyser page LM

Resolution 1, HM Resolution 1, and Ion Energy 1 slider bars to achieve optimum resolution [typically 0.75 Da full width at half maximum (FWHM)].

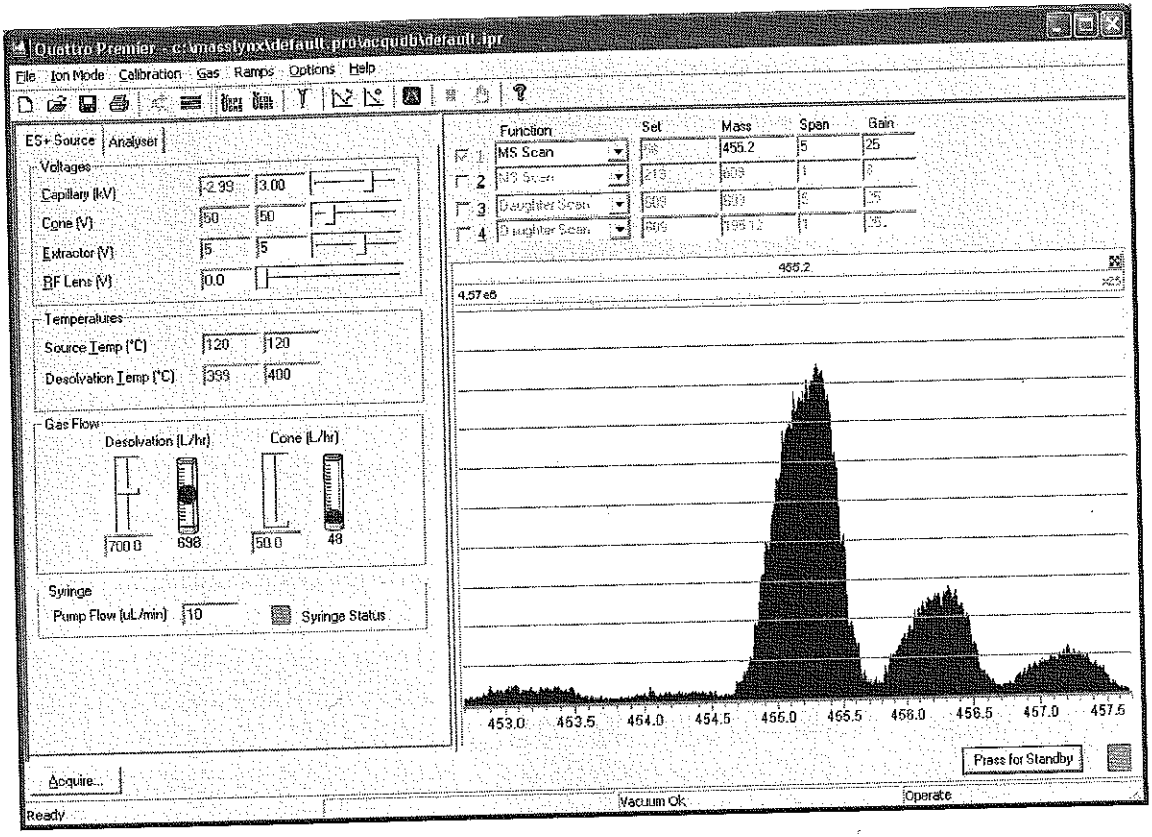


Figure 2-6 Tune Window with a Verapamil Peak

31. In the Tune window, determine the m/z value of the peak's center, to one decimal place (Figure 2-6). Record this value.
32. MS tuning is now complete. Perform the MS/MS tuning procedure (Section 2.2.2).

2.2.2 Tuning for MS/MS (Daughter Ion) Operation

1. In the Tune window, select the Function 2 box. The Function 1 box may now be cleared (Figure 2-7).

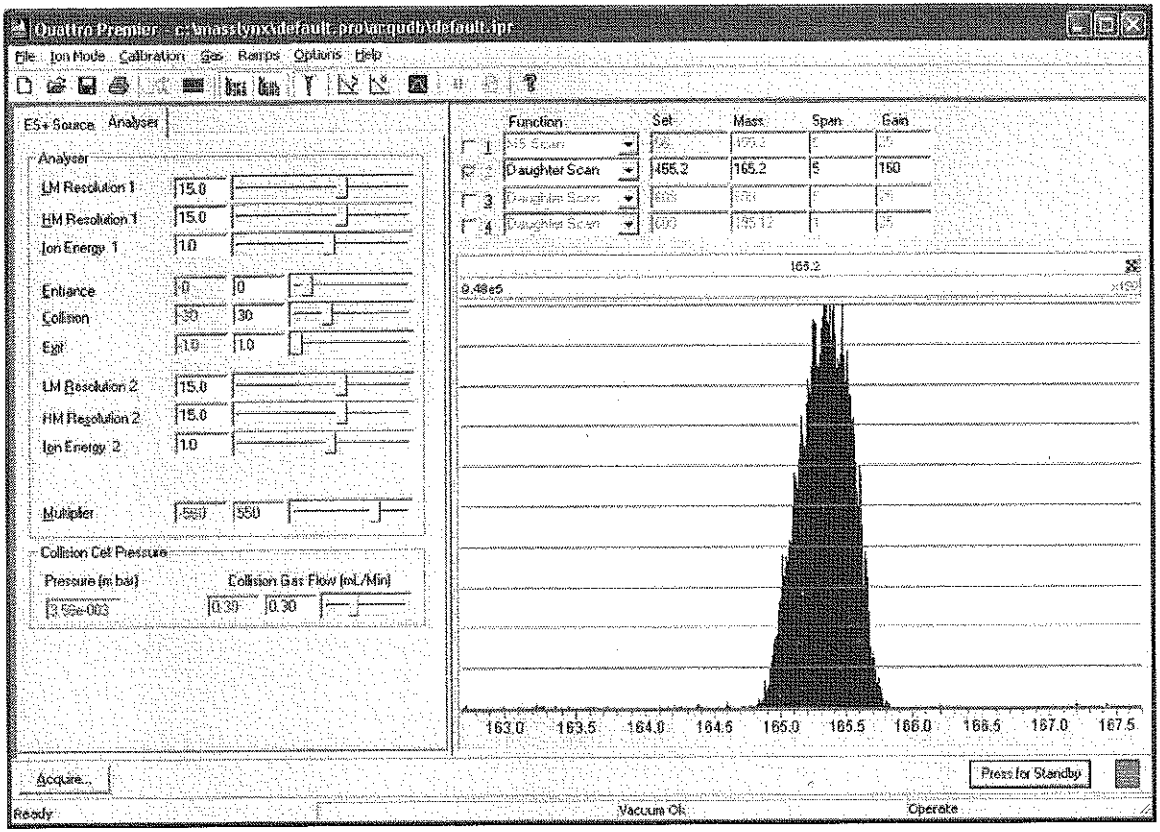


Figure 2-7 Tune Window with a Daughter Ion Peak

2. Select Daughter Scan from the adjacent drop-down list.
3. Enter Set 455.2 (i.e. the verapamil peak), Mass 165.2 (daughter ion), Span 5, and Gain 150.
4. Set Entrance to 0, Collision to 30, and Exit to 1.0.
5. On the Analyser page, set Ion Energy 2 to 1.0.

   Note: *Do not change the optimal Ion Energy 1 value obtained in Section 2.2.1.*

6. Click  to turn on the collision gas flow.

7. On the Analyser page, set the Collision Gas Flow (mL/min) to 0.3. This sets the Collision Cell Pressure to approximately 3 to 4 × 10^{-3} mbar.
8. Observe the daughter ion peak, at m/z 165.2, displayed in the Tune window.
9. If necessary, adjust the LM Resolution 2, HM Resolution 2, and Ion Energy 2 slider bars to achieve optimum resolution (typically <1 Da FWHM).
10. Starting at a value of 10, increase Collision (i.e., collision energy) in increments of 2. Set Collision to the value giving the highest displayed peak intensity. Record this value.
11. Optimize the Collision Gas Flow (mL/min) using increments of 0.05. Allow the Collision Cell Pressure readback to stabilize after each adjustment. Set the gas flow to the value giving the highest displayed peak intensity.
12. In the Tune window, determine the m/z value of the daughter ion peak's center to one decimal place (see Figure 2-7). Record this value.
13. MS/MS tuning is now complete. Create a Multiple Reaction Monitoring (MRM) MS method file (see Section 2.2.3).

2.2.3 Creating the MRM MS Method File

An MRM MS method file, containing the information obtained during the instrument tuning process, must now be created.

1. In the MassLynx window, click the MS Method icon to open the MS Method Editor (Figure 2-8).

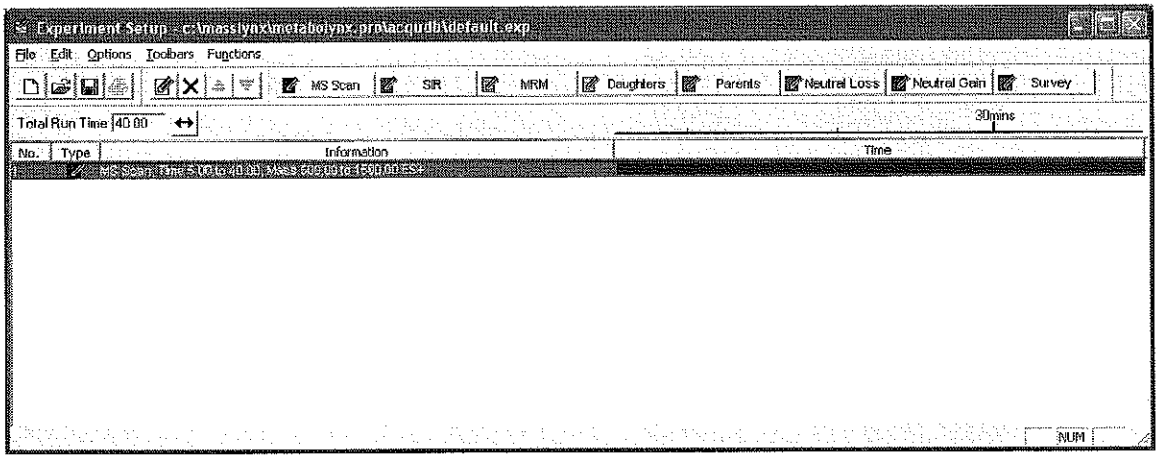


Figure 2-8 MS Method Editor

2. Click ✕ to delete the current entry from the MS Method Editor.
3. Click MRM to open the MRM Function Editor.
4. Enter the value recorded in Section 2.2.1, step 31 on page 28, in the Parent (m/z) box (Figure 2-9).

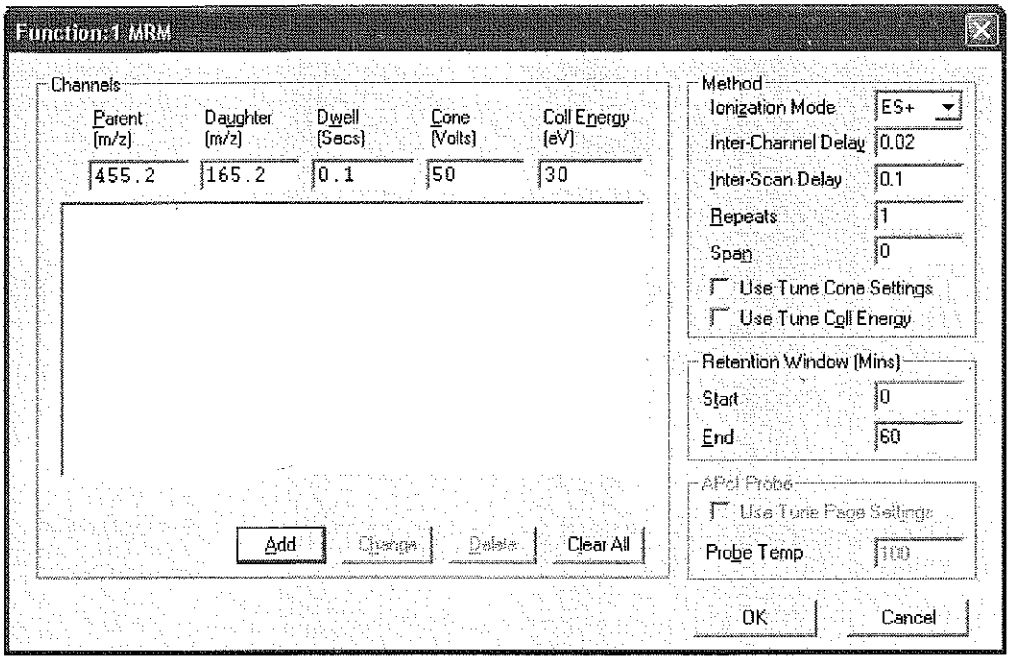


Figure 2-9 MRM Function Editor

5. Enter the value recorded in Section 2.2.2, step 12 on page 30, in the Daughter (m/z) box.
6. Enter 0.1 in the Dwell (Secs) box.
7. Enter the value recorded in Section 2.2.1, step 27 on page 27, in the Cone (Volts) box.
8. Enter the value recorded in Section 2.2.2, step 10 on page 30, in the Coll Energy (eV) box.
9. Click Add; the values entered above are added to the Function List.
10. Select ES+ in the Ionization Mode drop-down list.
11. Set Span to 0.

12. Enter a correct LC run time in the Retention Window (Mins), End text box.
13. Click OK. The MRM Function Editor is closed and the values are included in the MS Method Editor.
14. Click  to open the Save As dialog box.
15. Save the experiment file as verapamil_1.exp.

    The instrument is now ready for data acquisition in ESI mode (see Section 2.3).

2.3 Preparing the Instrument for Data Acquisition

2.3.1 Reconfiguring the Connection to the Probe

Warning: *The liquid passing through the HPLC pump, LC column, and ESI probe may be biohazardous and/or toxic. Always wear nitrile gloves when working with these items.*

Warning: *To avoid high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, and ESI probe.*

Warning: *To avoid electric shock, ensure that the instrument is in Standby before commencing this procedure.*

1. Click  to stop the syringe pump.
2. Reconfigure the connections to the ESI probe, so that the LC column is connected directly to the probe; i.e., remove the zero-dead-volume T-piece and syringe pump connection (Figure 2-10).
3. In the Tune window, confirm that the daughter ion peak of interest has disappeared.

   If the peak does not disappear, consider two possible causes:

   - You tuned the instrument using a large background ion peak. Repeat the tuning procedure using a more suitable sample.
   - The source is contaminated with the sample compound. This may occur if the tuning standard sample concentration is too high. Clean the source (see Section 7.9), then repeat the tuning procedures, using a suitable sample concentration.

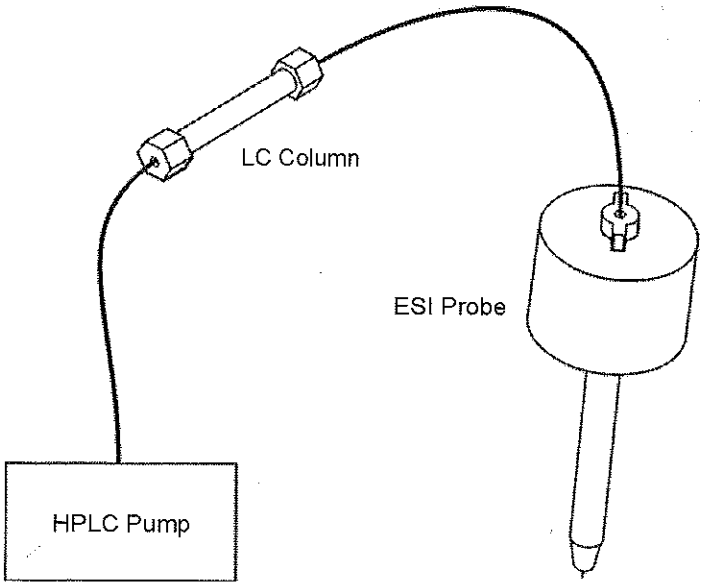


Figure 2-10 Column Connection to the ESI Probe

2.3.2 Configuring the Inlet for LC Operation

1. Click the MassLynx window Instrument shortcut bar Inlet Method icon to open the Inlet Method dialog box.
2. Select Tools > Instrument Configuration to open the Inlet Configuration dialog box.
3. Click Configure to open the Inlet Configuration Wizard.
4. Click Next.
5. Follow the on-screen instructions.
6. When the Inlet Configuration Wizard has finished, click Finish, Finish, and then close the Inlet Configuration dialog box.

2.3.3 Creating a Sample List and Starting Data Acquisition

Note: *For comprehensive information on creating a MassLynx Sample List and starting data acquisition, see the MassLynx User's Guide.*

1. In the MassLynx window, insert the required number of samples in the Sample List.
2. Enter the required file name(s) in the File Name column.
3. Enter the required text in the File Text column.

4. In the MS File column, select verapamil_1.exp.
5. Create a suitable inlet method file (see the *MassLynx NT Inlet Control Guide).*
6. Enter the inlet method file name in the Sample List Inlet File column.
7. Enter the bottle number(s) in the Bottle column.
8. Enter the injection volume(s) in the Inject Volume column.
9. Save the Sample List.
10. To start data acquisition, click  (see Chapter 5 for details).

2.3.4 Viewing and Printing the Tuning Parameters Associated with a Data File

The tuning parameters associated with a data file are stored with the file as part of the experimental record. You can view or print these tuning parameters from the MassLynx Data Browser dialog box. See the *MassLynx User's Guide* for more information.

Note: *The readbacks incorporated in the experimental record are for indication purposes only. They are not true (calibrated) records of the actual voltages that were on the instrument during data acquisition.*