

Chapter 3

Setting-Up and Tuning for APCI

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

3.1 Setting-Up

3.1.1 Removing the ESI Probe

You may need to remove the ESI probe from the instrument before fitting the APCI probe. Figure 3-1 shows the ESI 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 ESI probe may be biohazardous and/or toxic. Always wear nitrile gloves when working with these items.

2. Disconnect the LC system from the ESI probe.
3. Wait for three minutes to allow the desolvation gas flow to cool the probe and source.
4. In the MassLynx window, click  to turn off 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.

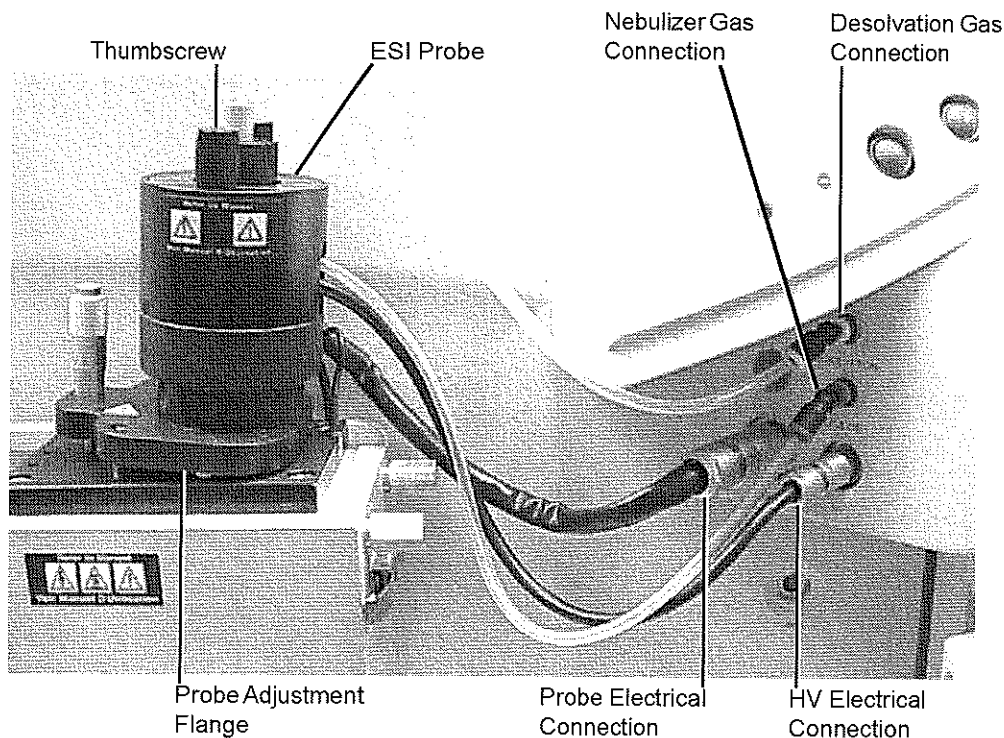


Figure 3-1 ESI Probe Mounted on the Source Enclosure

6. Disconnect the Probe and HV electrical connections on the instrument front panel.
7. Disconnect the PTFE tubes at the Nebuliser 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.

3.1.2 Installing the APCI Corona Discharge Pin



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

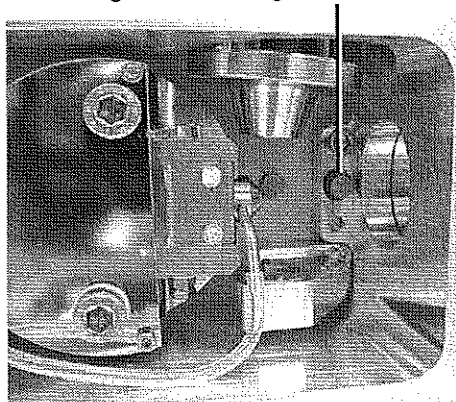
1. In the MassLynx Tune window, click Press for Standby and confirm that the adjacent instrument status indicator shows red.



Warning: The source components may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while performing this procedure.

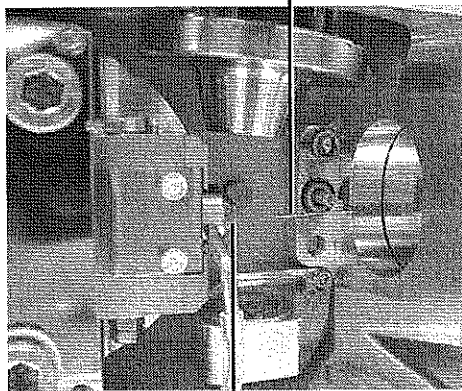
2. Unfasten the source enclosure door's securing clips and open the door.
3. Use needle-nose pliers to remove the blanking plug from the corona discharge pin mounting contact. Store the blanking plug in a safe location.

Corona Discharge Pin Mounting Contact Blanking Plug



4. Use the needle-nose pliers to fit the corona discharge pin to the mounting contact. Ensure that the corona discharge pin tip aligns with the sample cone tip.

Corona Discharge Pin



Sample Cone Tip

5. Close the source enclosure door and fasten the securing clips.

3.1.3 Installing the APCI Probe



Warning: To avoid electric shock, ensure that the instrument is in Standby when installing the APCI probe.

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



Warning: The probe may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while performing this procedure.

2. Carefully slide the APCI probe into the hole in the probe adjustment flange.
3. Secure the probe by tightening the two thumbscrews (Figure 3-2).
4. Connect the probe PTFE tubes to the nebulizer and desolvation gas connections on the front panel.
5. Connect the APCI probe electrical lead to the Probe connection on the front panel.

Note: The probe adjustment flange electrical lead is not connected when the APCI probe is fitted.

6. Close the instrument's access door.

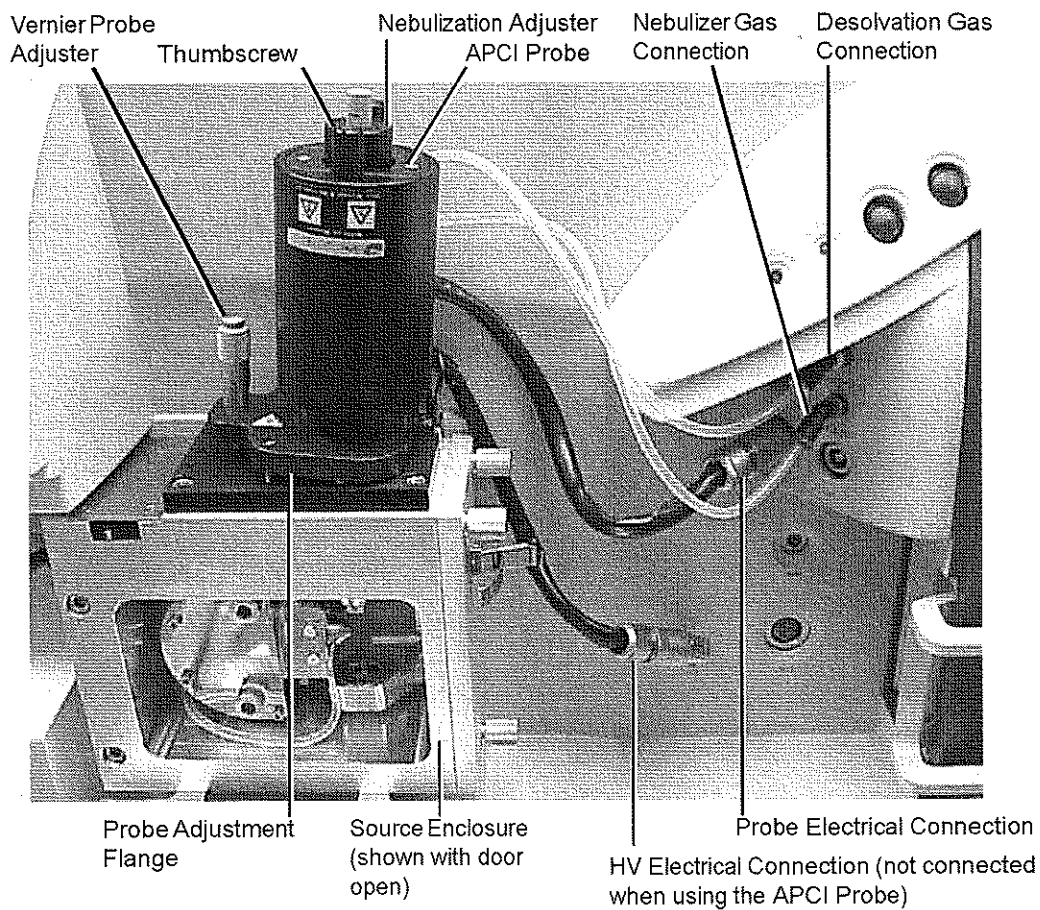


Figure 3-2 APCI Probe Mounted on the Source Enclosure

3.2 Tuning via Sample Infusion

Note: This example procedure specifies 17- α -hydroxyprogesterone 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.

3.2.1 Tuning for MS Operation



Warning: The liquid passing through the HPLC pump, LC column, syringe pump, and APCI 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 APCI 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 HPLC pump, LC column, syringe pump, and APCI probe as shown in Figure 3-3.

The column used in this example is a Waters Symmetry C₁₈ 2.1 × 100 mm, 3.5 μ m. The mobile phase is 70:30 acetonitrile/water.

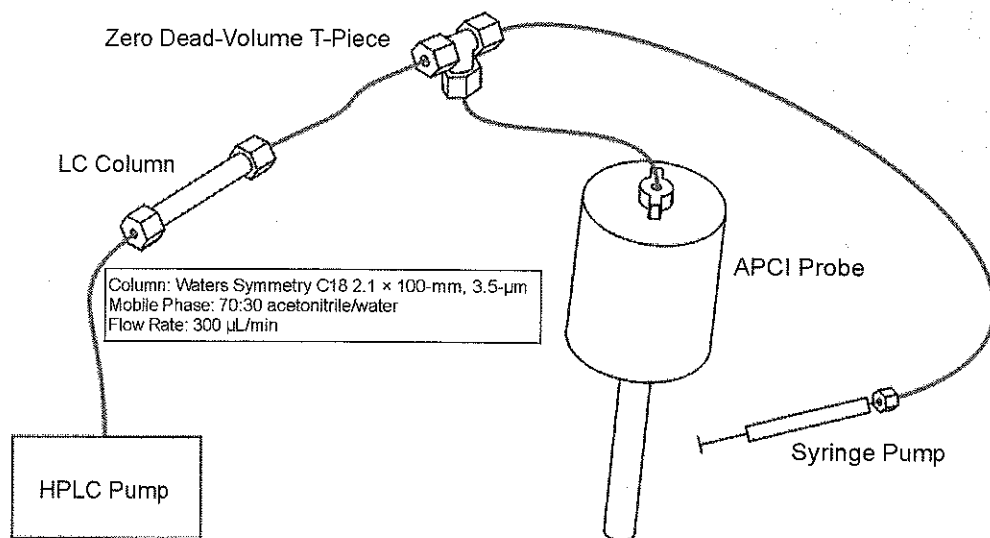


Figure 3-3 Pump and Column Connections to the APCI Probe

3. Look through the source's view port and confirm that the isolation valve lever is fully to the left (that is, the valve is open) (see Figure 2-4 on page 23).



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.
- 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 > APCI+ to display the APCI+ Source page (Figure 3-4).

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

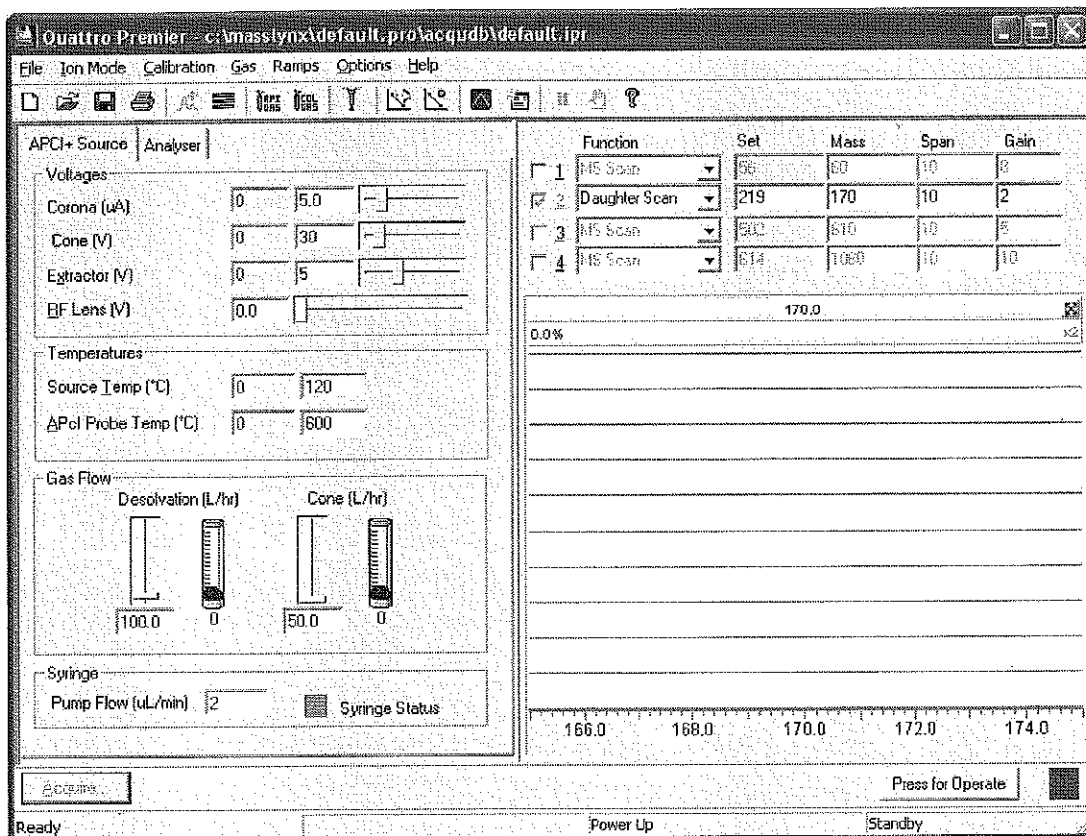


Figure 3-4 Tune Window APCI+ Source Page

5. Set the parameters to the recommended values shown in Table 3-1.
6. Click the Analyser tab.
7. Set the parameters to the recommended values shown in Table 3-2.


Table 3-1 Recommended APCI+ Source Page Parameter Values

Parameter	Recommended Value
Voltages	
Corona (μ A)	5.0
Cone (V)	30
Extractor (V)	5
RF Lens (V)	0.0
Temperatures	
Source Temp ($^{\circ}$ C)	120
APCI Probe Temp ($^{\circ}$ C)	600
Gas Flow	
Desolvation (L/hr)	100.0
Cone (L/hr)	50.0

Table 3-2 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	1.0
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 APCI+ Source page, observe the Desolvation and Cone gas flows; confirm they are stabilized and correct.
10. Confirm that the APcI Probe Temp (°C) readback shows approximately room temperature.
11. Click Press for Operate and confirm that the adjacent instrument status indicator shows green.
12. Confirm that the APcI Probe Temp (°C) readback reaches and stabilizes at the temperature set in step 5 on page 42.
13. Turn on the LC system at a flow rate of 300 $\mu\text{L}/\text{min}$ and confirm that its pressure is stable.
14. Allow 15 minutes for the LC column to equilibrate.
15. Load the syringe pump with sample. In this example, 17- α -hydroxyprogesterone (concentration 1 ng/ μL , in 70:30 acetonitrile/water) is used.
16. 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.
17. On the APCI+ Source page, set Syringe, Pump Flow ($\mu\text{L}/\text{min}$) to 10.
18. In the Tune window, select the Function 1 box.
19. Select MS Scan from the adjacent drop-down list.
20. Enter Mass 331.2, Span 3, and Gain 150.
Note: The above are recommended values and can vary from instrument to instrument.
21. Observe the 17- α -hydroxyprogesterone peak at m/z 331.2 ($\text{M}+\text{H}^+$) in the Tune window (see Figure 3-5).
22. Starting with the probe tip midway between the cone and the end of the corona pin, use the vernier probe adjuster (see Figure 3-2) to maximize the displayed peak intensity.

23. Use the vernier probe adjuster to move the probe tip as far away from the cone as possible, without losing more than 20% of the maximum displayed peak intensity obtained in step 22 on page 44. This minimizes source contamination.
24. Use the nebulization adjuster on the probe (see Figure 3-2) to give the best displayed peak intensity and stability.
25. On the Tune window's APCI+ Source page, optimize the Corona (μA) current to give the maximum displayed peak intensity. Do this by looking for the maximum peak intensity while performing the following steps:
 - a. Starting with a value of 5, decrease the value to 1, in decrements of 1.
 - b. Decrease the value to 0, in decrements of 0.1.
 - c. Reset the value to 5, and then increase the value in increments of 5.
 - d. Set the Corona (μA) current to the value giving the maximum displayed peak intensity.
26. 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.
27. Starting from a value of 15, increase the Cone (V) voltage in increments of 5. 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. Reduce the APCI Probe Temp ($^{\circ}\text{C}$) to 300, and then increase its value in increments of 50. Allow the temperature readback to stabilize after each adjustment. Set the temperature to the highest value that does not reduce the displayed peak intensity.

If the probe temperature is too low, the mobile phase may not be efficiently desolvated. This can result in chromatographic peak tailing.
31. Confirm that the displayed peaks have the correct resolution and the isotopes are resolved as shown in Figure 3-5. If necessary, adjust the Analyser page LM Resolution 1, HM Resolution 1, and Ion Energy 1 slider bars to achieve optimum resolution.
32. In the Tune window, determine the m/z value of the peak's center to one decimal place (Figure 3-5). Record this value.

33. MS tuning is now complete, Perform the MS/MS tuning procedure (see Section 3.2.2).

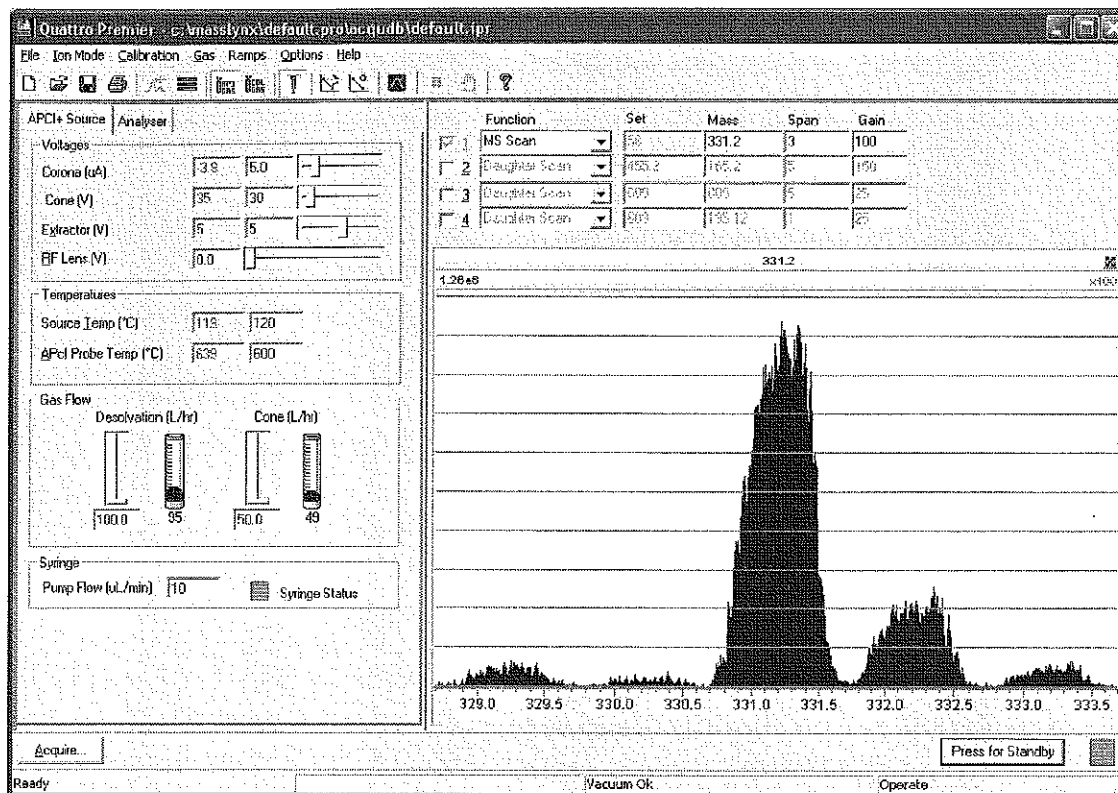



Figure 3-5 Tune Window with a 17- α -Hydroxyprogesterone Peak

3.2.2 Tuning for MS/MS (Daughter Ion) Operation

1. In the Tune window, select the Function 2 box. (The Function 1 box may be cleared, as shown in Figure 3-6.)
2. Select Daughter Scan in the adjacent drop-down list box.
3. Enter Set 331.2 (i.e., the 17- α -hydroxyprogesterone peak), Mass 109.1 (daughter ion), Span 5, and Gain 600.
4. Set Entrance to 0, Collision to 30, and Exit to 1.0. Leave the other parameters at their current settings.
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 3.2.1.

6. Click  to start 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 109.1, 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 the Collision (i.e., collision energy) in increments of 2. Set the collision energy 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 (Figure 3-6). Record this value.

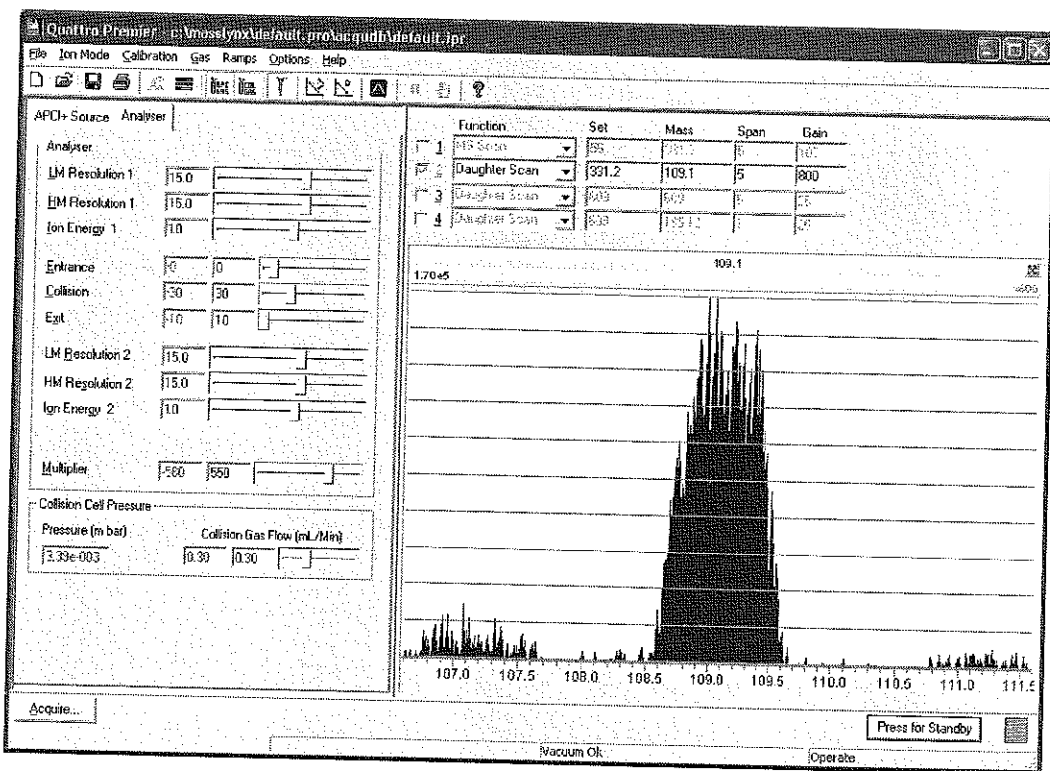


Figure 3-6 Tune Window with a Daughter Ion Peak

13. MS/MS tuning is now complete. Create an MRM MS method file (see Section 3.2.3).

3.2.3 Creating the MRM MS Method File

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

1. In the MassLynx window, click the MS Method icon to open the MS Method Editor (Figure 3-7).

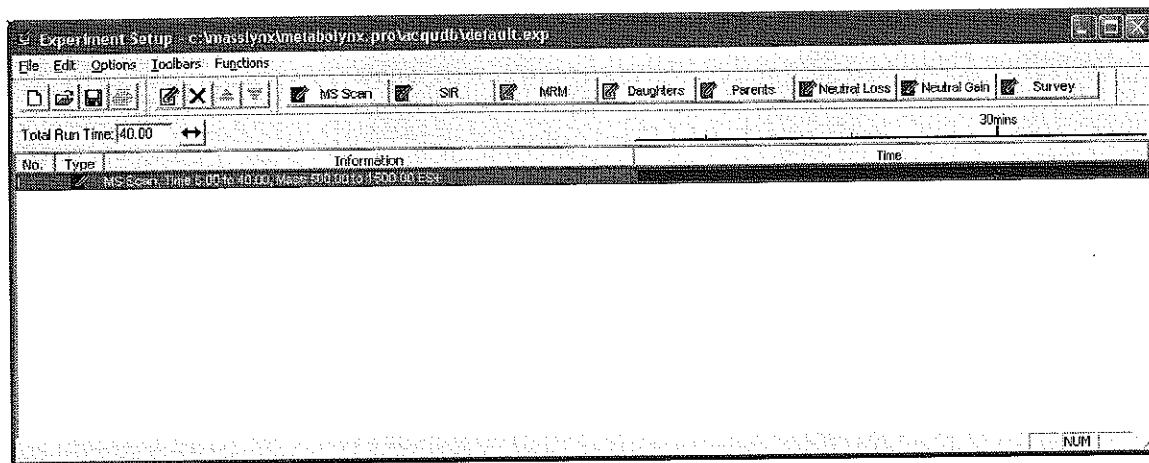


Figure 3-7 MS Method Editor

2. Click to delete the current entry from the MS Method Editor.
3. Click to open the MRM Function Editor (Figure 3-8).
4. Enter the value recorded in Section 3.2.1, step 32 on page 45, in the Parent (m/z) box.
5. Enter the value recorded in Section 3.2.2, step 12 on page 47, in the Daughter m/z) box.
6. Enter 0.1 in the Dwell (Secs) box.
7. Enter the value recorded in Section 3.2.1, step 27 on page 45, in the Cone (Volts) box.
8. Enter the value recorded in Section 3.2.2, step 10 on page 47, in the Coll Energy (eV) box.

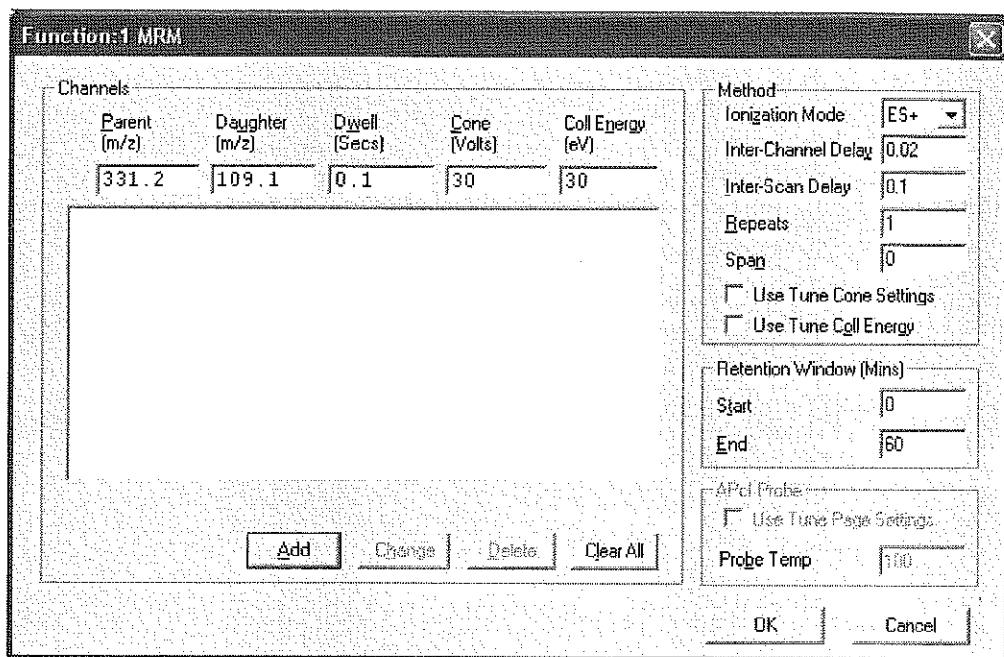



Figure 3-8 MRM Function Editor

9. Click Add. The values entered above are added to the Function List.
10. Select API+ from 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 hydroxyprogesterone peak_1.exp.
The instrument is now ready for data acquisition in APCI mode (see Section 3.3).

3.3 Preparing the Instrument for Data Acquisition

3.3.1 Reconfiguring the Connection to the Probe




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.



Warning: To avoid high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, and APCI 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 APCI 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 3-9).

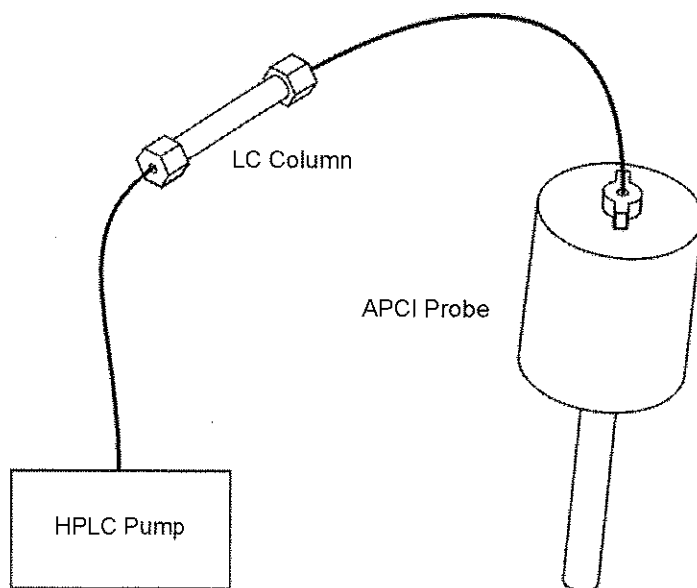


Figure 3-9 Column Connection to the APCI Probe


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 was too high. Clean the source (see Section 7.9), then repeat the tuning procedures, using a suitable sample concentration.

3.3.2 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.

3.3.3 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 hydroxyprogesterone peak_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 further details).

3.3.4 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.*