

Agilent Quadrupole Ion Trap SL Infusion Mode

Glossary

ESI = electrospray ionization CID = collisionally-induced dissociation

Who Should Use These Instructions

Use these instructions if you want to take a quick mass spectrum or manual MS/MS spectrum using direct infusion with standard conditions. Separate instructions cover in-source CID and LC/MS and automatic MS/MS. Please read the introduction to “Electrospray Ion Trap Mass Spectrometry.”

Before you start

Determine a good wash solvent or solvent combination. Make sure your sample concentration is 1×10^{-4} M or less. Higher concentrations require more time to clean the source. Choose a solvent and any additives to enhance ionization for your sample. For example, 50% isopropanol, acetonitrile, or methanol in water are good solvents. The goal is to minimize surface tension and enhance volatility. Formic acid or acetic acid are useful for enhancing ionization for positive ion mode. Concentrations in the range of 0.02-1% are common, but 10% may be necessary for some compounds. 10-20 mM ammonium formate, ammonium acetate, or ammonium bicarbonate are also often used for positive and negative ion mode.

Determine which spray needle is installed, and adjust your flow rates accordingly. The flow rate range for the standard source with the stainless steel needle is roughly 4-1000 $\mu\text{L}/\text{min}$. 10-100 $\mu\text{L}/\text{min}$ is a good starting range. If the source is fitted with the taper-tip, the usable flow rate maximum is decreased to around 25 $\mu\text{L}/\text{min}$ for infusion.

Start filling out the Log sheet before you start, including information on your sample, a good wash solvent and the concentration.

General Principles

Make sure the source gas flows and the drying gas temperature are properly set before infusing any solvent or sample.

In the control section, when you make a change you must click on the APPLY button to make the change active. The new numbers that you type into dialog boxes will be in green to remind you that the changes haven't been made yet. After you click APPLY the changes will be made and your new values will be listed in black in the dialog boxes. The system requires some time to stabilize the new settings. Many dialog boxes have a second list box to the right. The dialog box with the white background is where you type in the set point. The list box with the gray background gives the actual value for the parameter.

After you have finished, do not exit or click the go-away-box to switch to another application. The MSD Trap Control must always be running.

MS Startup

1. If the MSD Trap Control application isn't visible, select its icon at the bottom of the screen.

2. Pull down the Option menu and select the Vacuum System option. Check that the pressures are within 3.0-6.0 mbar for the fore pressure and less than 2×10^{-5} mbar for the high vacuum pressure. Click the close button.
2. Select the Tune tab in the control section.
3. Observe the Source ESI control check boxes at the lower-left hand side of the control section. Make sure the Nebulizer, Dry gas, and Dry heat check boxes are checked.
4. For normal operation for infusion at ~ 10 $\mu\text{L}/\text{min}$ set the nebulizer pressure to 15 psi. Set the Dry Gas flow to 6 L/min. Set the Dry Temperature to 300°C. Click Apply. If your flow rates are lower or higher use the table below. Flows above 25 $\mu\text{L}/\text{min}$ will not be accessible with the TaperTip fused silica spray needle.

Flow rate $\mu\text{L}/\text{min}$	Nebulizer Pressure	Drying gas Flow L/min	Drying gas Temp °C
1-10	10-15	4	325
10-50	15-20	5	325
50-200	20-40	8	350
200-500	30-50	8-10	350
500-1000	50-70	10-12	350

5. Wait until the actual dry gas temperature reaches the set value.
6. Choose the Expert mode for the Tune mode. Enter 2000 V for the capillary voltage.
7. Switch the MS to Operate by clicking the check box in the Status box in the upper-left side of the control section.
8. Set the maximum accumulation time to 300 msec and the Trap Averages to 5. Choose a scan range of 100-2000 m/z. This average is done before the data is sent to the computer for display.
9. In the Rolling Averages section, in the No. dialog box type in 2. Make sure the “On” check box is selected. These averages are calculated between successive displayed spectra.
10. Return to the Smart mode. Enter 1000 m/z for the Target mass and 30% for the Compound Stability. Click on the Wide range button. Press Apply.

Setting up the syringe pump

1. The off/on switch is on the back of the pump in the upper right corner near the power cord.
2. Fill a ~ 100 μL syringe with reagent grade methanol.
3. The pusher plate on the syringe pump is moved by depressing the brass button on the front of the pusher plate and sliding the plate. Move the pusher plate to the left to allow the syringe to be placed into the pump. Place the syringe in the V-shaped block at the right of the pump. Secure the syringe in place by lifting on the restraining clamp and rotating the clamp over the syringe.
4. Loosen the knurled fitting injection port adapter. Insert the syringe needle into the injection port and tighten. Test the connection by pulling gently backwards on the injection port. If the syringe needle pulls out, loosen the knurled fitting injection port and try again.

5. Make sure the barrel of the syringe is pushed as far to the right as possible so that the collar on the syringe touches the V-block.
6. Move the pusher plate into contact with the syringe plunger.
7. Turn on the light so that you can see the tip of the ESI spray needle.
8. Very slowly, manually slide the pusher plate on the syringe pump to inject methanol into the ESI source until you can see the spray.
9. Press the select button on the syringe pump control panel. Press the \uparrow and \downarrow keys until the display reads Table, and press select. In Table mode use the \uparrow and \downarrow keys to choose the manufacturer of your syringe. Press select. Then use the \uparrow and \downarrow keys to choose the volume of the syringe. The diameter of the syringe should then be displayed. For example, the diameter of a Hamilton 100 μL syringe is 1.46 mm. Press select.
10. The syringe pump keeps track of the actual volume of the solution the syringe to avoid destroying the syringe by pushing on an empty syringe. However, you need to tell the pump what the actual volume of the solution is. The Volume screen should be displayed at this point. Use the \uparrow and \downarrow keys to set the volume of the solution that you have in the syringe. Press select.
11. The Rate screen should now be displayed. If not, press select and use the use the \uparrow and \downarrow keys until the display reads Rate, and press select. Use the \uparrow and \downarrow keys to set the delivery rate. Note that the rate is in $\mu\text{L}/\text{hr}$, so 10 $\mu\text{L}/\text{min}$ corresponds to 600 $\mu\text{L}/\text{hr}$. Press select.
12. To start the syringe press the run/stop button. While the pump is running an \rightarrow arrow will blink at the far right of the display. The pump will run until the volume of solution that you entered above is delivered or until you press the run/stop button again.

Testing for a clean source

1. Infuse HPLC grade methanol at 10 $\mu\text{L}/\text{min}$ (600 $\mu\text{L}/\text{hr}$) from the syringe pump.
2. Let the system stabilize and observe the accumulation time. The accumulation time should be greater than 100 msec and the scale for the intensity axis on the mass spectrum should be 5×10^4 or less. You should see many background peaks with comparable intensity (i.e. a forest of peaks). If you see just a few tall mass peaks, then the ESI source is contaminated and must be cleaned; see the cleaning instructions below.

Running your sample

1. After you have tested for a clean source the syringe will contain methanol. If your sample isn't soluble in methanol, rinse the syringe with a good solvent for your sample. Place your sample in the syringe and the syringe in the syringe pump.
2. In the Mode tab, in the control section, choose the ion mode. Positive ion mode is the usual starting point. If you don't see anything, switching to negative ion mode can be helpful.
3. Return to the Tune tab, and select the Expert mode. Set the capillary voltage. 2000 V is a good starting value. However, capillary voltages from -3000 to -4000 V are quite common for positive ion electrospray. For negative ion ESI, the voltages are usually lower, in the $+1000$ to $+2000$ V range.

3. Choose the Smart mode. If you know the mass of your analyte, choose the Narrow range and enter the analyte mass as the target mass. If you are screening for many possible masses use the Wide range and a guess for the average mass of your analytes. Start with a Compound Stability of 30%.
4. Set the desired mass range into the Trap Scan range dialog boxes in the upper right of the control section.
5. Choose 2 for the Rolling Averages. Make sure the On check box is checked. Press Apply.
6. Very slowly, manually slide the pusher plate on the syringe pump to inject methanol into the ESI source until you can see the spray. On the syringe pump, press select and use the use the ↑ and ↓ keys until the display reads Volume, and press select. Use the ↑ and ↓ keys to set the volume of the solution that you have in the syringe. If the Rate screen is not displayed, press select and use the use the ↑ and ↓ keys until the display reads Rate, and press select. Use the ↑ and ↓ keys to set the delivery rate. Then, press run/stop on the syringe pump to infuse your sample at the chosen flow rate.
7. Note the electrospray current, which is listed at the top of the Capillary section. This current should be in the range of 1-20 namp. If you see an excessive electrospray current, or large spikes in the spectrum, the capillary voltage is set too high.
8. Once the signal has stabilized, change the averages setting to 10 to get better signal to noise.
9. Pull down the Acquisition menu and choose Sample Info.... Enter a name for the data file in the Filename dialog box. Remember to use 8 characters or less, letters and numbers only with no spaces or punctuation. Click on Apply and then Close.
10. If the mass spectrum shows good signal to noise you can use the snap shot mode to collect a single mass spectrum. The Snap Shot icon is a green arrow with a small mass spectrum and is located in the top icon bar. Press the Snap Shot arrow icon to capture the current mass spectrum.
11. If the signal to noise of the mass spectrum is poor, you will need to do some additional signal averaging. To save a series of spectra for this purpose, press the Run Method icon. The run method icon is a green arrow icon in the top icon bar. When the syringe is empty, press the Stop icon.

MS/MS

CID, collisionally induced dissociation can be used to fragment ions. This process produces mass spectra that are similar to the spectra obtained using electron impact ionization, but in a more controlled manner. CID can also be used to break up gas phase complexes. In the first stage of MS/MS experiments an ion of interest is first isolated in the trap, while all other ions are expelled. Then extra radiofrequency irradiation is supplied to cause the trapped ions to move faster, which causes more energetic collisions with the background He gas. These collisions fragment the ions. In the second stage of the MS/MS experiment the mass spectrum of the fragmented ions is determined.

12. To do CID and MS/MS, start by clicking on the MS(n) tab in the control section.
13. Click on the “mouse maximum cursor” icon in the top icon bar. This icon shows a MS peak with a red dot above the peak and a black arrow. Click on the peak that you want to fragment. A small, white, vertical arrow should appear on the chosen peak.

14. Click right just to the right of the chosen peak and choose Isolate/Fragment.
15. In the MS(n) window, the mass of the chosen peak should be listed in the first "Isolation mass" dialog box. The strength of the CID is determined by the value in the Ampl dialog box at the right hand side of this same line. Ampl is short for the amplitude of the added radiofrequency. Change the Ampl value, typically in the range of 0.2-0.8 to obtain the desired level of fragmentation. A typical setting would be large enough to decrease the isolated mass peak to about 20% of its starting value, but not so large as to completely remove the isolated mass peak.
16. Click on the All Off button when finished with MS/MS.

MS Cleaning and Shutdown

17. Rinse the source well with a compatible solvent or even better a series of compatible solvents, ending with isopropanol and then methanol. Usually it is best to use several solvents for washing instead of many washes with the same solvent. Inject the wash solvents by hand so that you can use a high flow rate (that is, don't use the syringe pump).
18. Test for a Clean Source, as instructed above. If the source isn't clean, continue injecting wash solvents. If plasticizer peaks are a problem, inject several syringe-fulls of the Agilent "magic solvent mix." This mix is 25:25:50 cyclohexane:acetonitrile:isopropanol. Use the methanol wash bottle to spray the outside of the nebulizer. Test for a Clean Source again. Record your final results in the Log sheet.
19. Set the nebulizer pressure at 4 psi, the dry gas flow at 2 L/min, and the source temperature at 300°C. Click on the Standby button.
20. Finish off filling out the Log sheet and place it in the three-ring binder.

Data Analysis

General Principles

To learn the use of any icon, position the mouse over the icon and the icon label will be printed in the message bar at the bottom of the application window.

There are four possible windows where data will be displayed. These windows are controlled by four icons in the second to last icon group in the upper-right hand corner of the Data Analysis application window. Left to right they are:

Chromatogram Window: If you took multiple spectra by using the HPLC method application or the Run Method command from the MSD Trap Control, the total ion current plot will be displayed in this window.

Mass Spectrum View Window: Clicking or dragging the mouse in the Chromatogram window will display a mass spectrum in this window. The "spectrum picking mode" icon is a cursor arrow with a small mass spectrum. If this icon is not selected, dragging the mouse in the Chromatogram window selects a range of mass spectra for averaging. Clicking on the Σ icon will average this range and display the result in this window. If the spectrum picking mode icon is selected, clicking with a mouse in the chromatogram window selects a single spectrum to display in this window.

Compound Mass Spectra Window: The MS spectra in the Mass Spectrum View and the Mass Spectrum Window are not available for processing. For processing

operations the mass spectrum must be loaded into this window. To load a mass spectrum into this window, right click with the mouse on the Mass Spectrum View or the Mass Spectrum Window and choose "Load Compound."

Mass Spectrum Window: This window is used for displaying Snap-Shot mode mass spectra only.

You can display or remove any of the above windows by clicking on its icon in the icon bar.

Spectrum Scaling: The total ion chromatogram and the mass spectra can be rescaled to zoom in on important features. The easiest way to do this is with the mouse. Position the mouse over the x or y-axis scale of the plot of interest. The mouse icon will change. The mouse buttons will act as follows:

Left mouse button: shifts the axis center

Right mouse button: expands and contracts the scale (zooms)

By alternating between the left and right mouse buttons you can rapidly zoom in on any region of the spectrum. To return to full scale, click the check boxes in the lower left-hand corner of each window.

Printing: To print your spectrum or the total ion chromatogram, pull down the File menu and choose Print Preview. Select the Display Report option from the pull down list and click on OK.

Closing Spectral Data Files: To close your data file, click on the data file name, which is listed in the data tree in the upper-left corner of the display. The data tree looks like a series of cascaded file folders. Pull down the file menu and choose Close.

Leaving the Data Analysis Application: After you have finished, do not exit or click the go-away-box to switch to another application. The Data Analysis application must always be running. Instead, minimize the window, or just switch to the MSD Trap Control or Instrument 1 window using the icon bar at the bottom of the screen.

Data Analysis Startup

1. Click on the Bruker Daltonics Data Analysis icon at the bottom of the screen. If you have not loaded a file into the Data Analysis application, this icon will be listed as "untitled Bruker Daltonics Data Analysis." If there is a file loaded, the name of this file will be listed before Bruker Daltonics Data Analysis instead of "untitled."
2. Pull down the File menu and choose Open... Click on the file name for your run. Press Open.

Data Analysis for a Snap-Shot

Use this section if you acquired your data in the Snap-Shot Mode. Your spectrum will be loaded in to the Mass Spectrum Window. If other windows are showing, you can close them by clicking on their icons in the top icon bar. You can zoom in to see the details of your spectrum.

Data Analysis for a series of Spectra

Use this section if you acquired your data by running an MS method. The total ion chromatogram will be loaded into the Chromatogram window.

1. Make sure the “spectrum picking mode” icon is selected; it will appear to be depressed. Click the mouse on the Chromatogram window at various points to load individual mass spectra into the Mass Spectrum View window.
2. Make sure the “spectrum picking mode” icon is not selected. Drag the mouse in the Chromatogram window to select a range of mass spectra for averaging. Click the Σ icon to average this range and display the result in the Mass Spectrum View window. Use the mouse left and right buttons to zoom in on interesting parts of the spectrum.