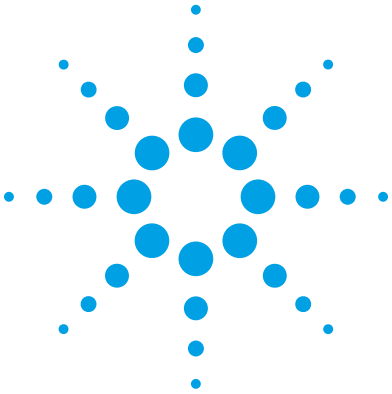

Agilent MassHunter Workstation Software

Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

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Software Revision

This guide applies to the Agilent MassHunter Workstation Software -Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF version B.06.00 or higher until superseded.

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In This Guide...

This guide contains information to learn to use your Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS system.

Exercise 1 Set up acquisition methods

With this exercise, you learn how to set up and run a series of three acquisition methods that help you in different application situations. You create these three acquisition methods for a mixture of four sulfa drugs.

Exercise 2 Set up and run single samples and worklists

This chapter provides familiarization exercises to help you learn how to set up and run single samples and sequences of samples through worklists on your Agilent TOF or Q-TOF LC/MS, using the methods you created in Exercise 1.

Exercise 3 Set up and run IM-QTOF samples and worklists

In this exercise, you learn how to acquire data in Ion Mobility mode. You learn how to set up and run a series of two acquisition methods that help you in different application situations. You create these two acquisition methods for a mixture of four sulfa drugs. This exercise is based on the methods established in Exercise 1, but the method is modified for the IM-QTOF parameters.

Exercise 4 Optimize IM-MS Q-TOF Methods

This chapter provides familiarization exercises to help you learn how to optimize methods for different compound classes, using the methods you created in Chapter 3.

Exercise 5 Set up acquisition method for collision cross section calculation

This exercise describes two strategies to acquire data for the calculation of collision cross sections. The first task creates an infusion based method where the field strengths are changed during one acquisition. The second task shows an LC based strategy where multiple LC runs are performed under different field strengths.

Before you start...

This guide assumes that the Agilent MassHunter Workstation software has been installed, and the LC modules and the 6200 Series TOF or 6500 Series Q-TOF LC/MS have been configured. Also, the performance has been verified, and the system has been turned on. If these actions have not yet been done, see the *Installation Guide* for your instrument.

The exercises in this guide use this equipment and materials:

- Agilent 1100/1200/1260/1290 LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- A 1 ng/µL sulfa mix sample, prepared as directed in "Before you begin..." on page 26, from the Electrospray LC Demo Sample, p/n 59987-20033
- Zorbax, Extend-C18 2.1mm x 50mm, 1.8um, 80Å, p/n 727700-902
- Bradykinin, Sigma, B2359-1 mg
- IgG 1, Sigma, I5154-1MG
- Amino acid standard 10pmol/µL, Agilent, p/n 5061-3334

Content

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1 Set up acquisition methods

With this exercise, you learn how to set up and run a series of three acquisition methods that help you in different application situations. You create these three acquisition methods for a mixture of four sulfa drugs.

These instructions help you understand how to do these tasks:

- Set up and run an MS-only method (TOF or Q-TOF).

  Use this type of method when you need only accurate mass MS data with the TOF or Q-TOF instruments, or intend to determine precursor ion masses for a subsequent MS/MS analysis.

- Set up and run a targeted MS/MS method (Q-TOF).

  Use this type of method when you need MS/MS data and know the precursor masses of interest. This is also the preferred type of method for quantitation work.

- Set up and run an auto MS/MS method (Q-TOF).

  Use this type of method when you need MS/MS data and don't know what precursors to choose, or the sample is complex enough that a targeted MS/MS method would be tedious to implement.

  In general, you would not use this type of method for quantitative MS/MS work because the start/stop retention times for MS/MS operation are determined by the data and instrument, not by you.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Task 1. Configure the instrument for data collection

Before you run samples with one of the methods you just created, you must select the data collection parameters for your run. You set these parameters on the **Instrument State** tab in the Tune window.

- If the TOF or Q-TOF has 4 GHz data collection capability, you can select storage sizes from 1 GHz to 4 GHz, as well as the mass range.
- If the TOF or Q-TOF allows **Fast Polarity Switching**, then you can select **Enabled** or **Disabled** in this list box. These exercises are run in Positive polarity, so you select **Disabled**.

Task 1. Configure the instrument for data collection

Steps	Detailed Instructions	Comments
1 Open Data Acquisition to access the Instrument State tune parameters.	**a** Click the **Agilent Data Acquisition** icon. **b** From the **Context** list in the main toolbar, select **Tune**. **c** Click the **Instrument State** tab.	
2 Select the following data collection settings. • Mass Range: Standard (3200 m/z) • Select to acquire data in High Resolution Mode.	**a** From the **Mass Range** list, click the **Standard (3200 m/z)** setting. **b** Click **High Resolution (4 GHz, High Res Mode)** if it's not the default setting for a 4 GHz instrument. **c** (optional) Select **Disabled** in the **Fast Polarity Switching** combo box. **d** For Agilent 6560, click **QTOF-Only** for the **Acquisition Mode**. **e** Click **Apply**. **f** If you changed the **Mass Range**, tune the instrument. **g** Recalibrate the TOF mass axis.	• You have to click the **Apply** button to change the settings on the instrument. • The **Mass Range** can only be set to **High (20000 m/z)** if the **Instrument Mode** is **Extended Mass Range (1 GHz)**. • If you change the **Instrument Mode, the Fast Polarity Switching mode** or the **Mass Range**, you must recalibrate the TOF mass axis. • For an Agilent 6560 Ion Mobility Q-TOF, the two **Acquisition Modes** are **IM-QTOF** and **QTOF-Only**.

Task 1. Configure the instrument for data collection

Steps	Detailed Instructions	Comments

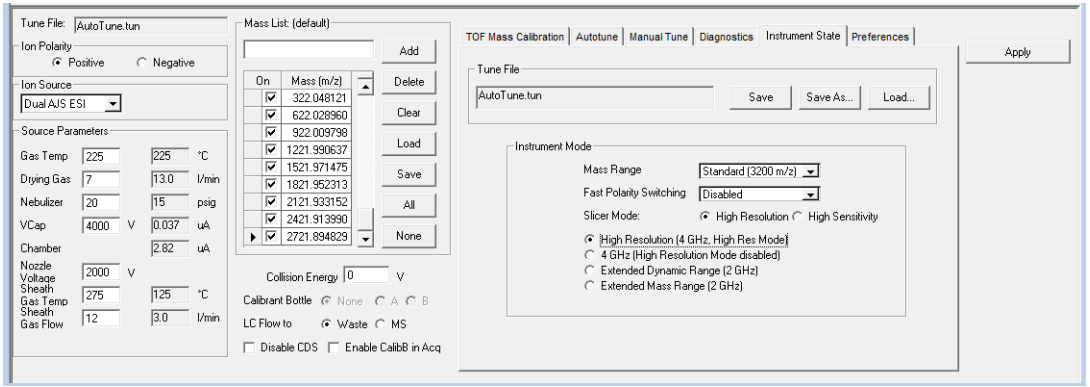


Figure 1 Instrument State tab for a 6550 iFunnel Q-TOF instrument

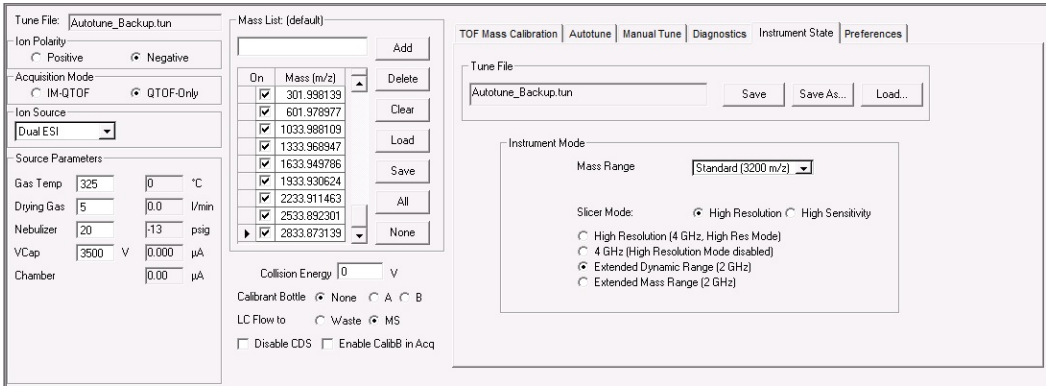


Figure 2 Instrument State tab for a 6560 Ion Mobility Q-TOF instrument with QTOF-Only chosen

3 Save the new settings to the tune file (Autotune.tun) and return to Acquisition.

a Click **Save**.
b From the **Context** list, select **Acquisition**.
c Click **Yes** in the Instrument State Confirmation message.
d Click **Yes** in the Save Tune File message.

- You can save the tune settings to a new file name for safe-keeping.
- To use the settings in the new file for a run, you must load the file and resave the settings to the default Autotune.tun file.

Task 2. Set up an MS-only method (TOF or Q-TOF)

In this exercise, you enter the LC and TOF MS conditions to analyze a sulfa drug mix, or Q-TOF MS-only conditions to identify precursor ions in the mix.

Task 2. Set up an MS-only method (TOF or Q-TOF)

Steps	Detailed Instructions	Comments
1 Open Data Acquisition to access the window for editing methods.	**a** Double-click the **Agilent Data Acquisition** icon. **b** Make sure that Acquisition appears as the selection in the **Context** box in the main toolbar. If Tune is the selection, click **Acquisition** from the **Context** list. **c** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **d** If you have an Agilent 6560 Ion Mobility Q-TOF, click **QTOF-Only** for the **Acquisition Mode**.	• The Agilent MassHunter Workstation Data Acquisition window appears containing the Method Editor window. See Figure 3. • Your display will be different if the Agilent Jet Stream Technology is not installed on your system.

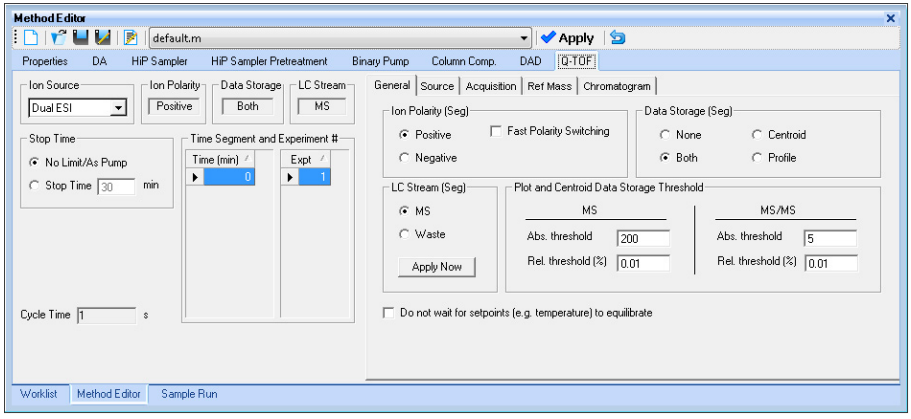


Figure 3 Method Editor window in the Agilent MassHunter Workstation Data Acquisition software

Task 2. Set up an MS-only method (TOF or Q-TOF) (continued)

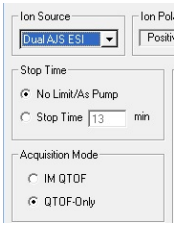
Steps	Detailed Instructions	Comments
		
2 Enter LC parameters appropriate for sulfa drug mix. See Table 1.	**e** In the Method Editor window, click each LC module tab to type parameter values. **f** Enter LC parameters listed in Table 1.	• LC fields in each tab depend on the configuration of the LC attached to the mass spectrometer. • See Figure 5.

Figure 4 For an Agilent 6560 Ion Mobility Q-TOF, select QTOF-Only for the Acquisition Mode

Table 1 LC parameters for sulfa drug mix

Parameter	Value for all instruments
PUMP	
• Flowrate	600 µL/min
• Solvent A	Water with 0.1% Formic Acid
• Solvent B	Acetonitrile with 0.1% Formic Acid
• Gradient (minutes - %B)	Initial Conditions: 90% Channel A and 10% Channel B 0 minutes - 10% B 5.0 minutes - 90% B
• Stop Time	5 minutes
• Post Time	3 minutes
INJECTOR	
• Inj. Vol.	1 µL
• Injection	Standard
• Draw Position	3.0 mm

Table 1 LC parameters for sulfa drug mix

Parameter	Value for all instruments
UV DETECTOR	
• Ch A	272 nm (100 nm BW on DAD)
• REF A (DAD only)	360 nm (100 nm BW)
COL THERM	
• Temp	40° C

Task 2. Set up an MS-only method (TOF or Q-TOF) (continued)

Steps	Detailed Instructions	Comments

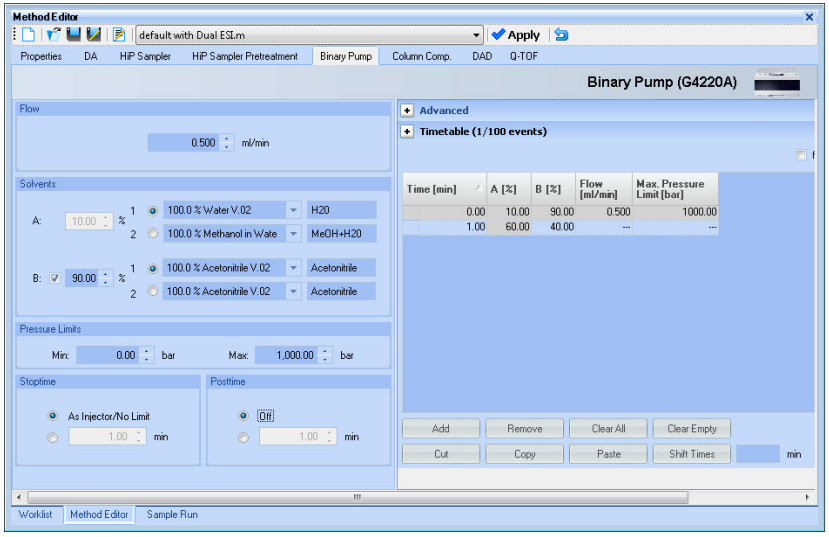


Figure 5 LC Timetable for sulfa mix analysis

Task 2. Set up an MS-only method (TOF or Q-TOF) (continued)

Steps	Detailed Instructions	Comments
3 For TOF and Q-TOF parameters, make sure the General tab is displayed. • Enter the parameters as shown in Figure 6, if necessary.	**a** Click the **TOF** or **Q-TOF** tab. **b** In the **TOF** or **Q-TOF** tab, make sure the **General** tab is displayed. **c** Type the parameters as shown in Figure 6. (These are the default parameters.)	• Of course, the MS/MS fields do not appear in the TOF General tab.

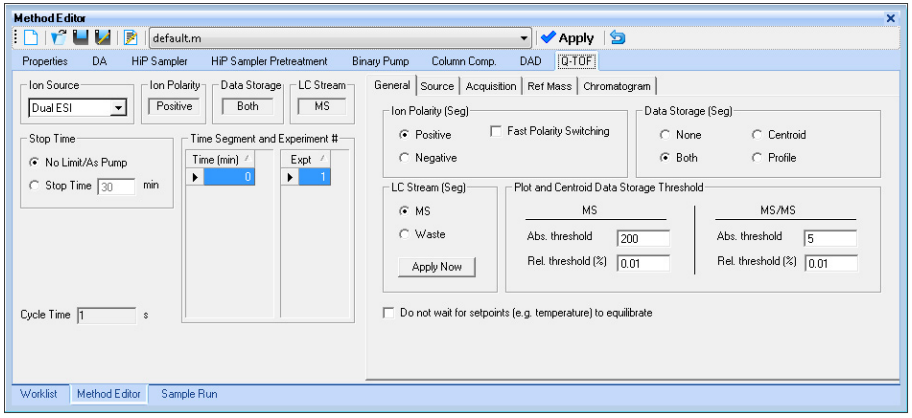
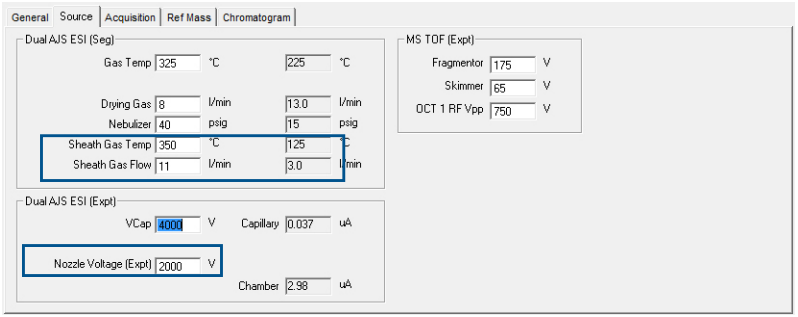


Figure 6 General tab for Q-TOF parameters for a 6530 Q-TOF

4 Enter ion source parameters as shown in Figure 7, if necessary.	**a** Click the **Source** tab. **b** Type the parameters as shown in Figure 7.	The name of the selected Ion Source is shown in this tab.



These options appear on a 6530 Q-TOF and a 6230 TOF mass spectrometer equipped with the Agilent Jet Stream Technology.

Figure 7 Source tab for MS Q-TOF parameters

Task 2. Set up an MS-only method (TOF or Q-TOF) (continued)

Steps	Detailed Instructions	Comments
5 Enter the acquisition spectral parameters for MS mode as shown in Figure 8.	**a** Click the **Acquisition** tab. For the TOF, skip to step c. **b** Click **MS** as the **Mode**. **c** Type the **TOF Spectra** parameters as in Figure 8.	

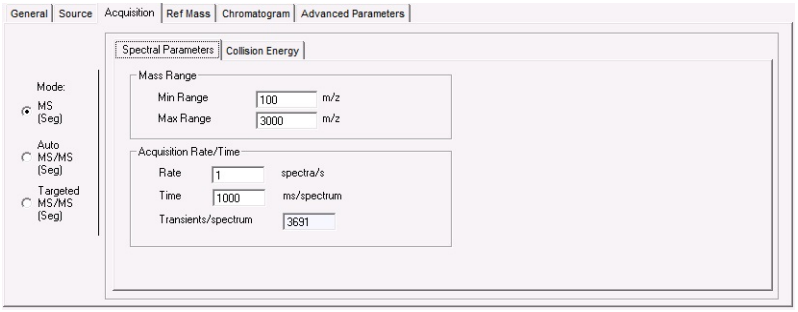


Figure 8 Acquisition tab for MS Q-TOF parameters (MS TOF uses the same parameters as MS Mode.)

6 Enter the reference mass parameters as shown in Figure 9.	**a** Click the **Ref Mass** tab. **b** Type the parameters as shown in Figure 9.	

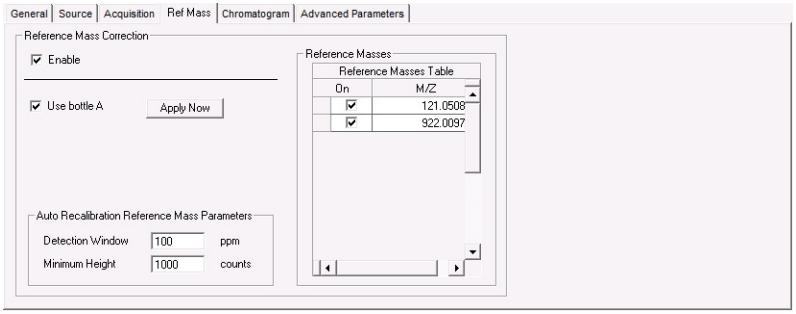


Figure 9 Ref Mass tab for MS TOF or MS Q-TOF parameters

Task 2. Set up an MS-only method (TOF or Q-TOF) (continued)

Steps	Detailed Instructions	Comments
7 Enter the chromatogram plot settings as shown in Figure 10.	**a** Click the **Chromatogram** tab. **b** Type the values in Figure 10.	• These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

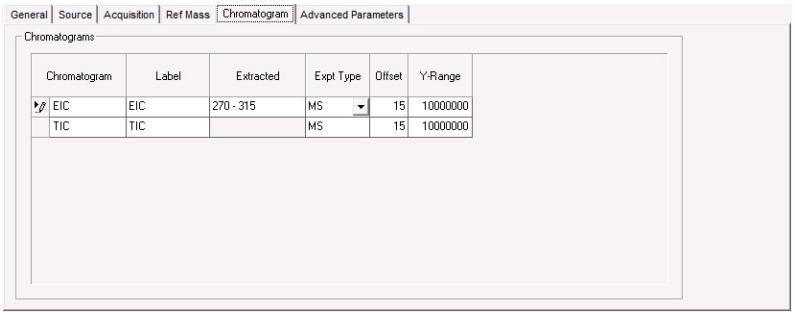


Figure 10 Chromatogram tab for TOF or Q-TOF parameters

8 Set up to change MS parameters during run: • Time Segment of 0 min. - Make sure you have selected an LC Stream of Waste. • Time Segment of 0.5 min. - Change LC Stream to MS.	**a** Click the **General** tab. **b** Click **Waste** for the **LC Stream**. **c** Right-click anywhere in the Time segment section, and click **Add Time Segment**. **d** Type `0.5` minutes. **e** Click **MS** for the **LC Stream**.	• You can change a field with a (Seg) next to it with a new Time Segment. • You can change a field with an (Expt.) next to it with a new Experiment. • See Figure 7 for examples of fields that can change with time segments and those changeable with experiments. • When you create a new time segment, the initial values are copied from the time segment that is selected.
9 Save the method as ***iii*MS-only.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as `iii`MS-only.m, where ***iii*** are your initials. **d** Click **Save**.	For example, if your initials are PFH, then the method name is **pfhMS-only.m**.

Task 3. Set up a targeted MS/MS method (Q-TOF)

Task 3 shows you how to set up an acquisition method for the Q-TOF LC/MS when you know what you're looking for, but you're not sure if the compounds are present in your mixture. In this task you also learn about the importance of collision energy.

Task 3. Set up a targeted MS/MS method (Q-TOF)

Steps	Detailed Instructions	Comments
1 Using the ***iii*MS-only.m** method for the MS Q-TOF, change to targeted MS/MS mode and enter the spectral parameters below, if necessary. • If the ***iii*MS-only.m** method is still displayed, begin with step c. • Delete the 0.5 min Time Segment. • Enter the parameters as shown in Figure 11.	**a** Click **Method > Open**. **b** Select *iii***MS-only.m**, and click **Open**. **c** Click the **Q-TOF** tab. **d** Select the 0.5 minute **Time Segment**. **e** Right-click the selected **Time Segment** and click **Delete Time Segment**. **f** Click the **Acquisition** tab. **g** Click **Targeted MS/MS (Seg)** as the **Mode**. **h** Type the spectral parameters below.	• The LC, General, Source, Ref Mass and Chromatogram parameters remain the same as in ***iii*MS-only.m** for this method.

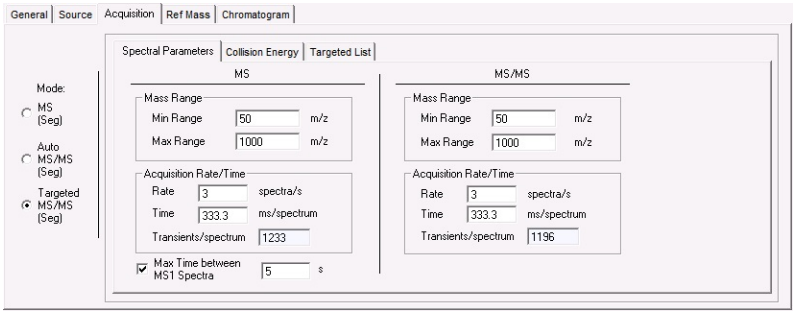


Figure 11 Acquisition Spectral Parameters tab for targeted MS/MS mode

2 Set up a fixed collision energy of 35 V. • Enter the parameters as shown in Figure 12.	**a** Click the **Collision Energy** tab. **b** Click **Use Fixed Collision Energy**. **c** Type `35`.	• For this type of method, the precursor ions and collision energy are usually known, although you can have the system determine the "best guess" collision energy for each mass. See the next task for how to do this.

Task 3. Set up a targeted MS/MS method (Q-TOF) (continued)

Steps	Detailed Instructions	Comments

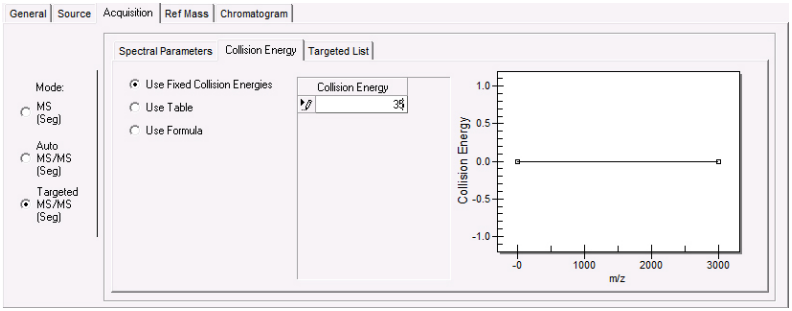


Figure 12 Acquisition Collision Energy tab for targeted MS/MS mode

3 Set up a targeted list of precursor ions so the resulting chromatogram shows peaks for only these ions.
- Enter 279.09102, 311.08085, 271.0317 and 285.0290 as the precursor ions.
- Use 0 minute for the Delta and Medium for the **Iso. width**.

a Click the **Targeted List** tab.
b Right-click the table and click **Add** from the shortcut menu.
c Fill out the information for the 279.09102 ion.
d Repeat steps b and c for the 311.08085 ion, the 271.0317 ion, and the 285.0209 ion.

- You can also enter a Collision Energy and Acquisition Time for each precursor ion. If you do, these values override the ones entered in the previous tab (Figure 12).
- You can enter the retention times also.
- In general, use accurate mass values (at least four decimal places) for the precursor values in this table, as some of the data processing routines in Qualitative Analysis and Quantitative Analysis make use of this information.

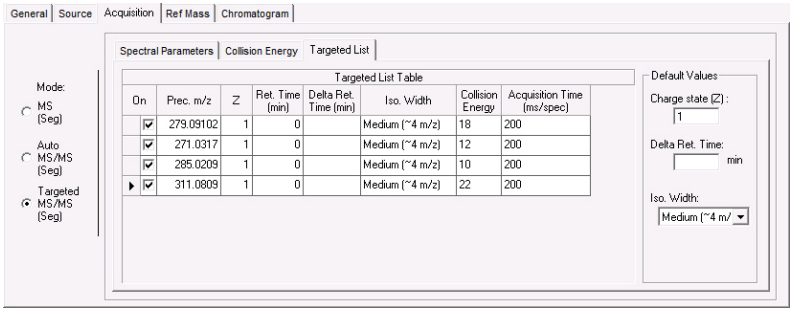


Figure 13 Acquisition Targeted List tab for targeted MS/MS mode

Task 3. Set up a targeted MS/MS method (Q-TOF) (continued)

Steps	Detailed Instructions	Comments
4 Save the method as ***iii*targetedMSMS.m**, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Type `iiitargetedMSMS`, and click **Save**.	• Be sure the folder you use is \MassHunter\methods.

NOTE If the retention time and the delta retention time for a precursor in Figure 10 are zero, then the program performs targeted MS/MS on this precursor for the entire time segment. Alternatively, you can specify an expected retention time (for example 5 minutes) and a delta retention time (for example 1 minute) in which case targeted MS/MS will be performed on this precursor from 4.5 to 5.5 minutes.

NOTE The parameters in the Acquisition tab, including these values in the Targeted List tab, may also be changed by using different time segments. See Figure 3 on page 11

NOTE See the *Concepts Guide* to learn more about why the collision energy voltages are important.

Task 4. Set up an auto MS/MS method (Q-TOF)

In this part of learning Q-TOF method development, you set up an auto MS/MS method because you are not sure what you are looking for and want the instrument to determine which precursor *m/z* values to examine "on the fly" according to criteria you select prior to the start of the run.

Task 4. Set up an auto MS/MS method (Q-TOF)

Steps	Detailed Instructions	Comments
1 Using the ***iit*targetedMSMS.m** method for the MS Q-TOF, change to auto MS/MS mode and enter the spectral parameters below, if necessary. • If the ***iit*targetedMSMS.m** method is still displayed, begin with step c. • Enter the parameters as shown in Figure 14.	**a** Click **Method > Open**. **b** Select ***iit*targetedMSMS.m**, and click **Open**. **c** Click the **Q-TOF** tab. **d** Click the **Acquisition** tab. **e** Click **Auto MS/MS(Seg)** as the **Mode**. **f** Type the spectral parameters shown below.	• For this method, the LC, General, Source, Ref Mass and Chromatogram parameters will remain the same as in MS-only.m.

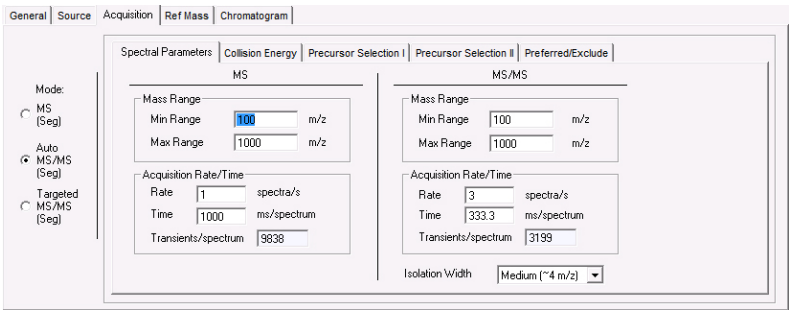


Figure 14 Acquisition Spectral Parameters tab for Auto MS/MS mode

2 Set up a linear equation for the collision energy so that the slope times the *m/z* value divided by 100 plus the offset equals the collision energy. • Use 5 for the slope and 2.5 for the offset.	**a** Click the **Collision Energy** tab. **b** Click **Use Formula**. **c** For the **Slope**, type `5.` **d** For the **Offset**, type `2.5`.	• For this type of method, you have the system determine the collision energy for each *m/z* value, because the optimal collision energy for each precursor ion is not known. • These values for slope and offset work well for these sulfa drugs but may not work as well for other compounds and charge states.

Task 4. Set up an auto MS/MS method (Q-TOF) (continued)

Steps	Detailed Instructions	Comments

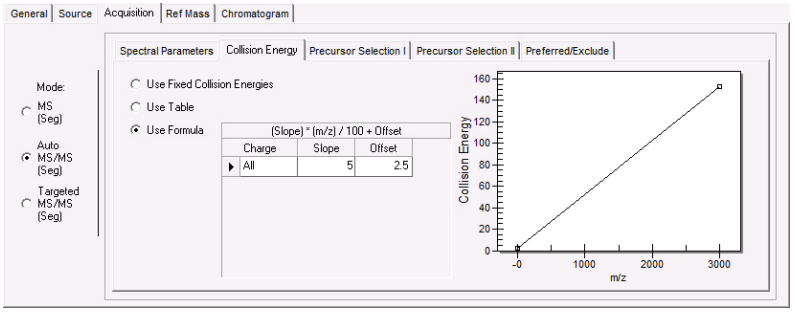


Figure 15 Acquisition Collision Energy tab for Auto MS/MS mode

3 Set **3** as the maximum number of precursor ions per cycle that the software will select in order of decreasing abundance.

- Enter the other parameters in Precursor Threshold.

a Click the **Precursor Selection I** tab.

b Type 3 as the **Max Precursor Per Cycle**.

c Type the other parameters in the **Precursor Threshold** group box.

- Active exclusion of precursor ions is used for complex samples. These settings specify the time during which a previously selected precursor ion will be excluded from selection.
- Static Exclusion Range lets you set the range of ions to be excluded.

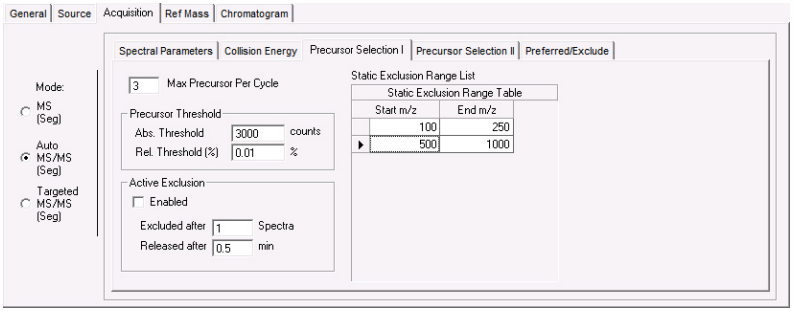


Figure 16 Acquisition Precursor Selection I tab for Auto MS/MS mode

Task 4. Set up an auto MS/MS method (Q-TOF) (continued)

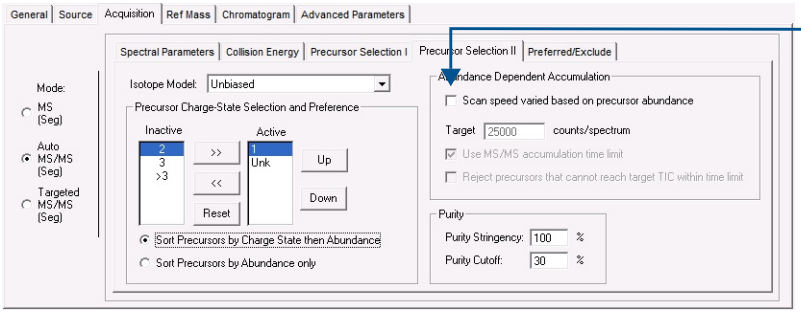
Steps	Detailed Instructions	Comments
4 Modify the parameters to see the masses of charge 2 first, then masses of charge 1 and then masses of unknown charge.	**a** Click the **Precursor Selection II** tab. **b** If necessary, click **1** and **Unk** in that order from the **Inactive** list and then click the **>>** button. **c** If necessary, click any values on the right that are not **1** or **Unk,** and then click the **<<** button.	• This setting means that if there are two precursors detected with charge state +1, the software selects the two of these with the highest abundance and no precursors with unknown charge state. • If there is no precursor with charge state of +1, and three with unknown charge states, then the software selects the precursor with charge state +1 and the most abundant precursor with unknown charge state.
		If you have a complex sample, you can mark the **Scan speed varied based on precursor abundance** check box. See the online Help for more information.

Figure 17 Acquisition Precursor Selection II tab for auto MS/MS mode

5 Set up to monitor the 279.09102 precursor ion as a preferred ion and exclude the 311.08085 ion. • Use the other entries in Figure 18.	**a** Click the **Preferred/Exclude** tab. **b** Right-click the table area, and click **Add** from the menu. **c** Type all the values for 279.09102 **d** Repeat steps b and c for the excluded ion, 311.08085.	For this example, you do not need to mark the **Scan speed varied based on precursor abundance** check box.

Task 4. Set up an auto MS/MS method (Q-TOF) (continued)

Steps	Detailed Instructions	Comments

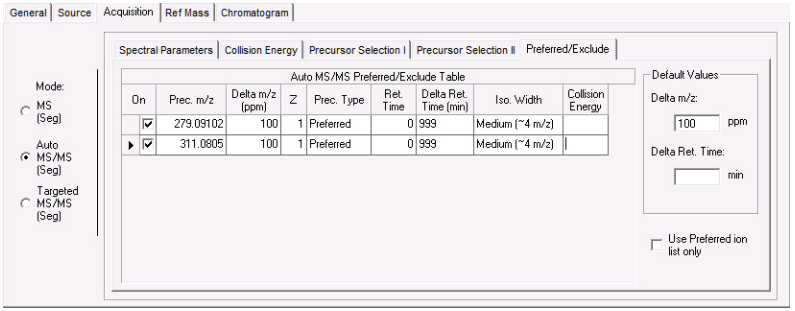


Figure 18 Acquisition Preferred/Exclude tab for Auto MS/MS mode

6 Save the method as ***iii*autoMSMS.m**, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Type `iiiautoMSMS`, and click **Save**.	• Be sure the folder you use is \MassHunter\methods.

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2
Set up and run single samples and worklists

This chapter provides familiarization exercises to help you learn how to set up and run single samples and sequences of samples through worklists on your Agilent TOF or Q-TOF LC/MS, using the methods you created in Exercise 1.

Each exercise is presented in a table with three columns:

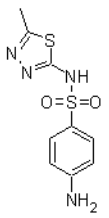
- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin...

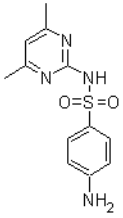
For this exercise you analyze a mixture of four sulfonamide compounds. This section gives instructions on how to prepare the demo sample.

The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampoules with 100 ng/µL each of:

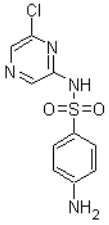
Name	Formula	Ion	m/z
sulfamethizole	$C_9H_{10}N_4O_2S_2$	$(M+H)^+$	271.03179
sulfamethazine	$C_{12}H_{14}N_4O_2S$	$(M+H)^+$	279.09102
sulfachloropyridazine	$C_{10}H_9ClN_4O_2S$	$(M+H)^+$	285.02075
sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	$(M+H)^+$	311.08085



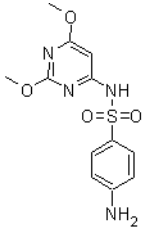
Sulfamethizole



Sulfamethazine



Sulfachlorpyridazine



Sulfadimethoxine

NOTE

The instrument must be tuned using the ESI tune calibrant solution before proceeding with the rest of the exercise. Make sure you have used Checktune or Autotune for the instrument you have, either the TOF LC/MS or the Q-TOF LC/MS (both TOF and Quad components), to verify that each of the calibrant ions has the proper mass assignment, peak width, and signal intensity.

See the *Quick Start Guide* for instructions on tuning the instrument.

1 Put on protective gloves.

2 Prepare the LC solvent.

In 1-liter reservoirs of HPLC-grade water and acetonitrile, add 1.0 mL of 99% LC-MS Reagent Grade Formic Acid (HCOOH) each to make 0.1% (v/v) Solvent A and Solvent B, respectively.

3 Prepare the sample.

a Add 10 µL sulfa mix from one of the ampoules (500 µL) to 990 µL of solvent A in an autosampler vial so that the final concentration is 1 ng/µL. Seal with the appropriate cap (crimp or snap).

b Place the sample vial in the autosampler.

4 Set up the LC column.

- Zorbax, Extend-C18 2.1mm x 50mm, 1.8 µm, 80Å, p/n 727700-902

5 Set the column temperature.

Agilent suggests a column temperature of 40°C when using this column in this exercise.

Task 1. Set up and run a single sample

This task shows you how to enter sample and data file information for a single sample and then begin to acquire data north sample.

Task 1. Set up and run a single sample

Steps	Detailed Instructions	Comments
1 Open one of the three methods you created in Exercise 1, and enter this sample information: • Name: *same as method* • Position of sample in your sampler • Data file name: *same as method*.d	**a** Click **Method > Open**. **b** Select one of the three methods, and click **OK**. **c** Click the **Sample Run** window.	• The system stores the custom information with the data file.

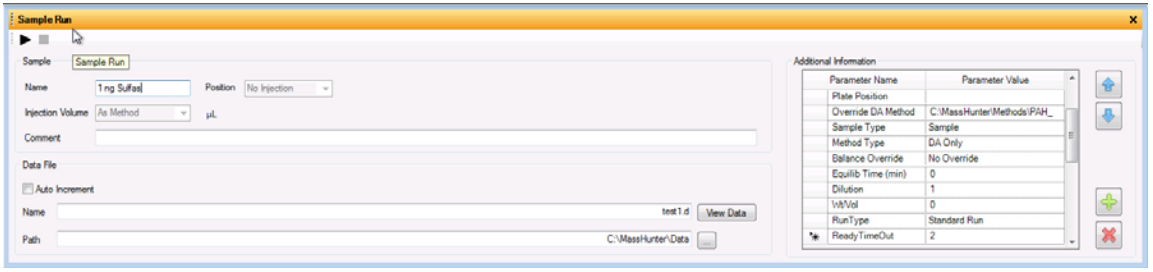


Figure 19 Sample Run window in the main window

	d Type `1 ng Sulfas` as the sample **Name**. **e** Type `test1.d` as the Data File **Name**.	You can type any number at the end of the Name field. This value is incremented for each new data file.
2 Start the sample.	• Click the Run button, ▶, in the Sample Run toolbar or the Run button, , in the main toolbar.	• If you have clicked the Lock icon in the toolbar, you cannot modify the method while the sample is running. Also, you cannot overwrite this data file in the Data Acquisition program. • The button, , in the main toolbar indicates that locked mode is on.

Task 1. Set up and run a single sample

Steps	Detailed Instructions	Comments
3 View the data after the run.	• After the run is complete, click **View Data** in the **Sample Run** window.	• When you click **View Data**, the Qualitative Analysis program automatically opens and loads the data file that is specified in the Sample Results window.

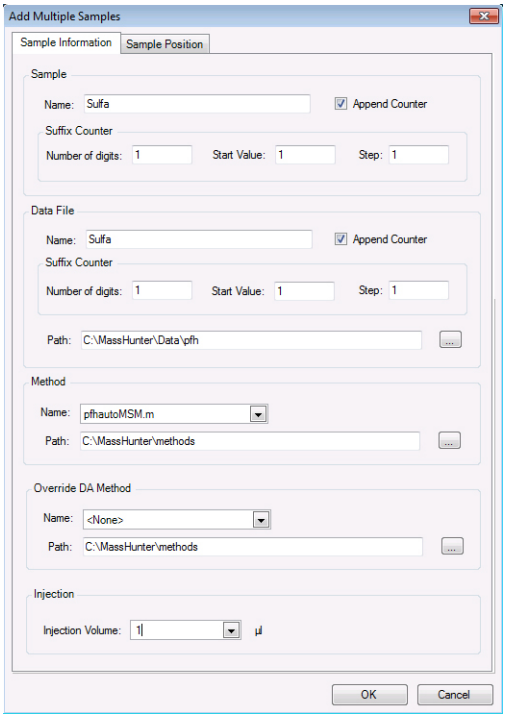
Task 2. Set up and run a worklist with multiple samples

This task shows you how to enter sample and data file information for multiple samples in a worklist and then begin to acquire data.

Task 2. Set up and run a worklist with multiple samples

Steps	Detailed Instructions	Comments
1 Add three samples to the worklist - Sulfa 1, Sulfa 2, Sulfa 3 - with the following information: • Data file: Sulfa 1-3.d to be saved to the folder, **MassHunter****Data*****YourName***. • Acquisition method: *any of the three you created in Exercise 1* • Injection volume: 1 • Sample position: any three positions convenient for your sampler	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Click **Add Multiple Samples**. The **Add Multiple Samples** dialog box opens. **c** Type the **Sample Name** as `Sulfa` and the **Data File Name** as `Sulfa`. **d** Make sure that the **Append Counter** check boxes are marked and that all **Suffix Counter** boxes contain a 1 for the **Sample** and the **Data File** names. **e** Change the folder path for the data files to **MassHunter****Data*****YourName***. **f** Select the acquisition method from Exercise 1. **g** Type an Injection Volume of `1`.	• If another worklist already exists in the Worklist window, click **Worklist > New** to create this worklist.

Task 2. Set up and run a worklist with multiple samples

Steps	Detailed Instructions	Comments
		
	Figure 20 Add Multiple Samples dialog box	
	h Click the **Sample Position** tab. **i** Select **None** for the Autosampler. **j** For the **Number of Samples**, type 3. **k** Click **OK**.	
2 Hide the following columns: • Sample Type • Level Name • Comment	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Click **Show/Hide/Order Columns**. **c** Clear the check boxes for **Sample Type**, **Level Name** and **Comments**.	• You are hiding these columns, not deleting them. The software recognizes their values even though they do not appear in the worklist.

Task 2. Set up and run a worklist with multiple samples

Steps	Detailed Instructions	Comments

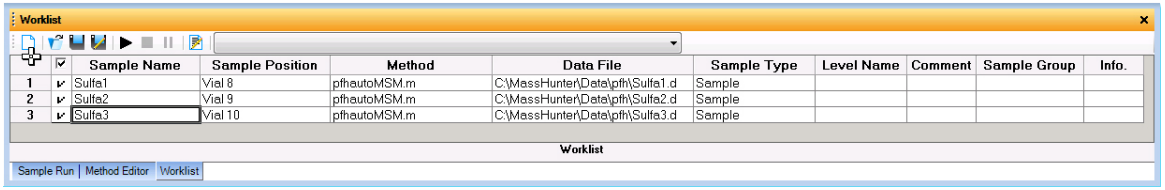


Figure 21 Worklist with three samples

3 Save the worklist as *iii*esdemo.	**d** Click **Worklist > Save As.** Then, type the worklist **File name** and click **Save**.	
4 Make sure that the worklist is set to run only data acquisition.	**a** Right-click the upper-left-hand cell of the worklist spreadsheet. **b** Select **Worklist Run Parameters**. **c** Select **Acquisition Only** from the **Part of method to run** list. **d** Change the directory path for the data files to **MassHunter\Data** ***YourName***.	• You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.

Task 2. Set up and run a worklist with multiple samples

Steps	Detailed Instructions	Comments

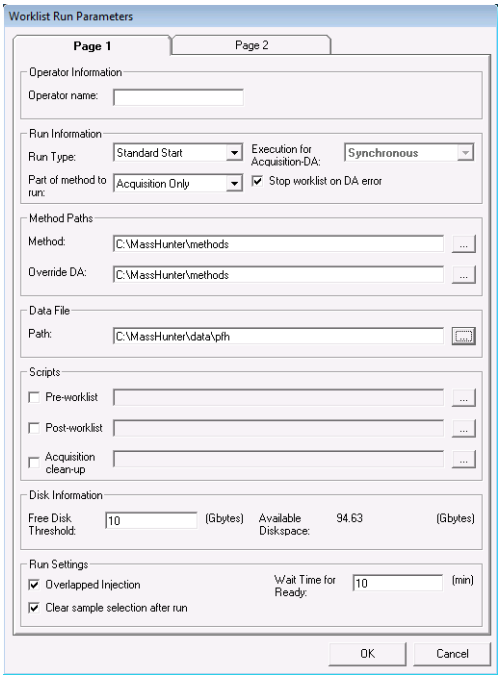


Figure 22 Worklist Run Parameters dialog box

e Click **OK**.

5 Start the worklist.

Hint: you must mark the check box at the beginning of each row to run the sample in the row.

- Click the Run button, ▶ , in the Worklist toolbar or the Run Worklist button, , in the main toolbar.

- You do not have to save the worklist in order to start it.
- If you have clicked the Lock icon in the toolbar, you cannot modify the method or the worklist while the worklist is running. Also, you cannot overwrite these data files in the Data Acquisition program.
- The button, , in the main toolbar indicates that locked mode is on.
- Each sample row turns blue as the software begins to acquire data for that worklist row.

Task 3. Set up and run a worklist to optimize parameters

You can also optimize acquisition parameters with a worklist. This task shows you how to set up a worklist to evaluate the signal as the fragmentor voltage changes. You can then use the Qualitative Analysis program to compare the chromatographic signals at the different fragmentor voltages.

Task 3. Set up and run a worklist to optimize parameters

Steps	Detailed Instructions	Comments
1 Add four samples to the worklist - Frag 1, Frag 2, Frag 3, Frag 4 - with the following sample information: • Sample position: any four positions convenient for your sampler • Data files: Frag 1- 4.d to be saved to the folder, MassHunter\Data*YourName*. • Acquisition method: ***iii*ms-only.m** • Injection volume: 1	**a** Right-click the upper-left-hand corner of the worklist. **b** Click **Add Multiple Samples**. **c** Type the **Sample Name** as `Frag` and the **Data File Name** as `Frag`. **d** Make sure that the **Append Counter** check boxes are marked and that all **Suffix Counter** fields contain a 1. **e** Change the folder for the data files to **MassHunter\Data*YourName***. **f** Select the ***iii*ms-only.m** acquisition method. **g** Type an **Injection Volume** of `1`. **h** Click the **Sample Position** tab. **i** Select **None** for the Autosampler. **j** For the **Number of Samples**, type `4`. **k** Click **OK**.	• Click **Worklist > New** to create a new worklist.
2 Hide the following columns: • Sample Type • Level Name • Comment	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Click **Show/Hide/Order Columns**. **c** Clear the check boxes for **DA Method**, **Sample Type**, **Level Name** and **Comment**, and click **OK**.	• You are hiding these columns, not deleting them. The software recognizes their values even though they do not appear in the worklist.
3 For all four samples, add a column for the fragmentor parameter, and enter these values: • Frag 1: 225 • Frag 2: 200 • Frag 3: 175 • Frag 4: 150	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Click **Add Column(s)**. **c** Select **MS Parameter**. **d** Select **Fragmentor**, and click the **>** button. **e** Click **OK**. **f** Type the values into the column.	

Task 3. Set up and run a worklist to optimize parameters

Steps	Detailed Instructions	Comments
4 Save the worklist as Fragwklst.	**g** Click **Worklist > Save As**. **h** Type `Fragwklst`, and click **Save**.	• Save the Fragwklst file into your own folder.
5 Make sure that the worklist is set to run only data acquisition.	**a** Right-click the upper-left-hand cell of the worklist spreadsheet. **b** Click **Worklist Run Parameters**. **c** Select **Acquisition Only** from the **Part of method to run** list. **d** Change the folder for the data files to **MassHunter\Data*YourName***. **e** Click **OK**.	• You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.
6 Start the worklist.	• Click the Run button, ▶ , in the Worklist toolbar or the Run Worklist button,  , in the main toolbar.	

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3 Set up and run IM-QTOF samples and worklists

In this exercise, you learn how to acquire data in Ion Mobility mode. You learn how to set up and run a series of two acquisition methods that help you in different application situations. You create these two acquisition methods for a mixture of four sulfa drugs. This exercise is based on the methods established in Exercise 1, but the method is modified for the IM-QTOF parameters.

These instructions help you understand how to do these tasks:

- Set up and run an IM-MS only method.

  You use this type of method when you need Ion Mobility accurate mass MS data with the Agilent 6560, or intend to determine precursor ion masses for a subsequent All Ions MS/MS analysis.

- Set up and run an All Ions MS/MS method.

  You use this type of method when you need MS/MS data and do not know what precursors to choose, or the sample is complex enough that a targeted MS/MS method would be tedious to implement. You can also use this method if you have known fragments belonging to a specific precursor or compound class and want to align these via the drift time.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Task 1. Configure the instrument for data collection in Ion Mobility mode

Before you run samples with one of the methods you just created, you must select the data collection parameters for your run. You set these parameters on the Instrument State tab in the Tune window.

Task 1. Configure the instrument for data collection

Steps	Detailed Instructions	Comments
1 Open Data Acquisition to access the Instrument State tune parameters.	**a** Click the **Agilent Data Acquisition** icon. **b** From the **Context** list in the main toolbar, select **Tune**. **c** Click the **Instrument State** tab.	
2 Select the following data collection settings. • Mass Range: Standard (3200 m/z) • Select to acquire data in Extended Dynamic Range Mode. • Select the IM-QTOF mode.	**a** From the **Mass Range** list, click the **Standard (3200 m/z)** setting. **b** Click **Extended Dynamic Range Mode** if it is not the default setting. **c** Click **IM-QTOF** for the **Acquisition Mode**. **d** Click **Apply**. **e** If you changed the **Mass Range**, tune the instrument. **f** Recalibrate the TOF mass axis.	• You have to click the **Apply** button to change the settings on the instrument. • The **Mass Range** can only be set to **High (10000 m/z)** if the **Instrument Mode** is **Extended Mass Range (1 GHz)**. • If you change the **Instrument Mode,** or the **Mass Range**, you must recalibrate the TOF mass axis. • For an Agilent 6560 Ion Mobility Q-TOF, the two **Acquisition Modes** are **IM-QTOF** and **QTOF-Only**.

Task 1. Configure the instrument for data collection

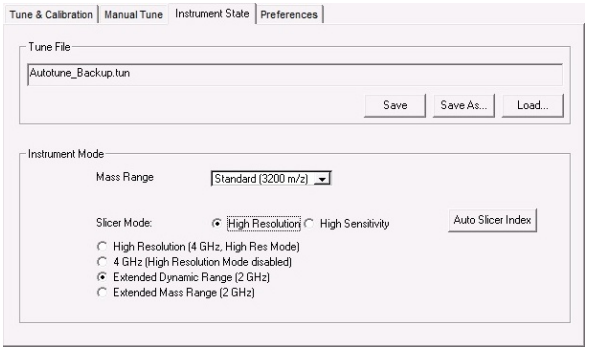
Steps	Detailed Instructions	Comments
		

Figure 23 Instrument State tab for a 6560 Ion Mobility Q-TOF instrument

3	Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**a** Click **Save**. **b** From the **Context** list, select **Acquisition**. **c** Click **Yes** in the Instrument State Confirmation message. **d** Click **Yes** in the Save Tune File message.	• You can save the tune settings to a new file name for safe-keeping. • To use the settings in the new file for a run, you must load the file and resave the settings to the default Autotune.tun file.

Task 2. Set up an IM-MS method

This task shows you how to edit an IM-MS method.

Task 2. Set up an IM-MS method in **MS (Seg)** mode

Steps	Detailed Instructions	Comments
1 Open Data Acquisition to access the window for editing methods.	**a** Double-click the **Agilent Data Acquisition** icon. **b** Make sure that Acquisition appears as the selection in the **Context** box in the main toolbar. If **Tune** is the selection, select **Acquisition** from the **Context** list. **c** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **d** Click **IM-QTOF** for the **Acquisition Mode**.	• The Agilent MassHunter Workstation Data Acquisition window appears containing the Method Editor window. See Figure 24. • Tune values are saved for positive and negative mode, as well as for Q-TOF and IM-MS mode. • Your display will be different if you have a different **Ion Source**.

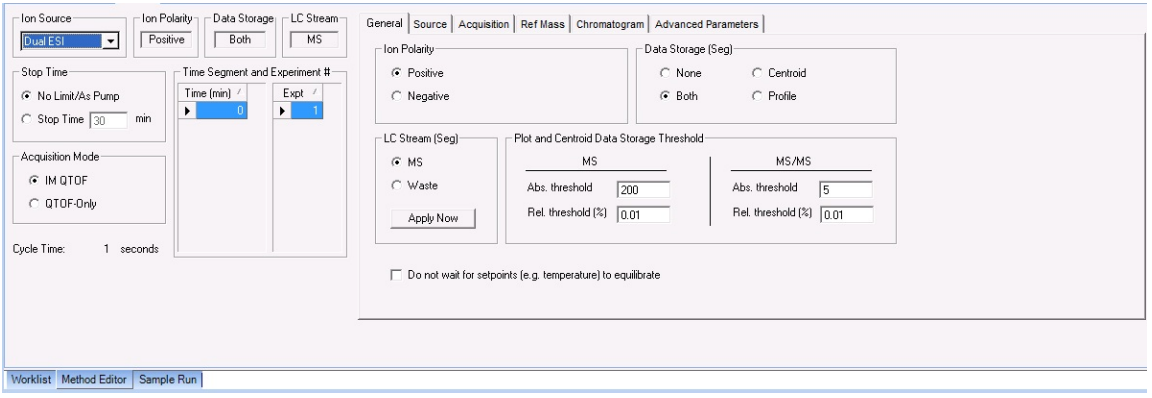


Figure 24 Method Editor window for a 6560 Ion Mobility Q-TOF in the Data Acquisition software

2 Enter LC parameters appropriate for sulfa drug mix. See Table 2.	**e** In the Method Editor window, click each LC module tab to type parameter values. **f** Enter LC parameters listed in Table 2.	• LC fields in each tab depend on the configuration of the LC attached to the mass spectrometer.

Table 2 LC parameters for sulfa drug mix

Parameter	Value for all instruments
Instruments PUMP	
• Flowrate	600 µL/min
• Solvent A	Water with 0.1% Formic Acid
• Solvent B	Acetonitrile with 0.1% Formic Acid
• Gradient (minutes - %B)	Initial Conditions: 90% Channel A and 10% Channel B 0 minutes - 10% B 5.0 minutes - 90% B
• Stop Time	5 minutes
• Post Time	3 minutes
INJECTOR	
• Inj. Vol.	1 µL
• Injection	Standard
• Draw Position	3.0 mm
UV DETECTOR	
• Ch A	272 nm (100 nm BW on DAD)
• REF A (DAD only)	360 nm (100 nm BW)
COL THERM	
• Temp	40° C

Task 2. Set up an IM-MS method in **MS (Seg)** mode (continued)

Steps	Detailed Instructions	Comments
3 For the 6560 IM-MS Q-TOF parameters, make sure the General tab is displayed. • Enter the parameters as shown in Figure 25, if necessary.	**a** Click the **Q-TOF** tab. **b** In the **Q-TOF** tab, make sure the **General** tab is displayed. **c** Type the parameters as shown in Figure 25. (These are the default parameters.)	• The MS/MS parameters reflect the threshold for All Ions MS/MS experiments.

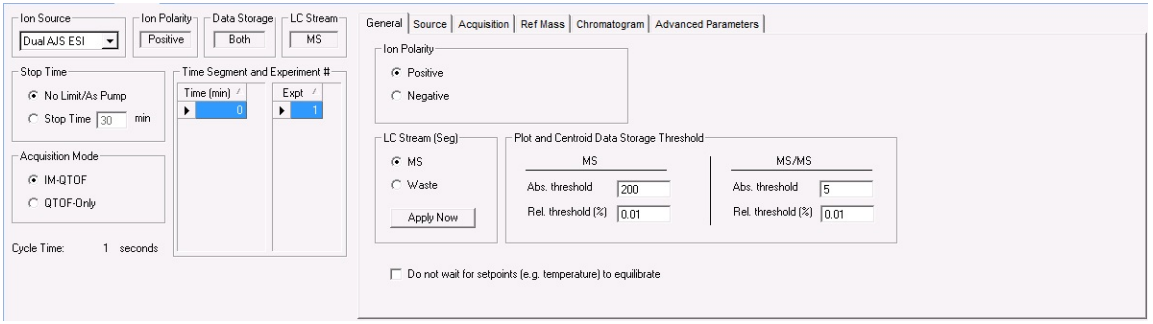
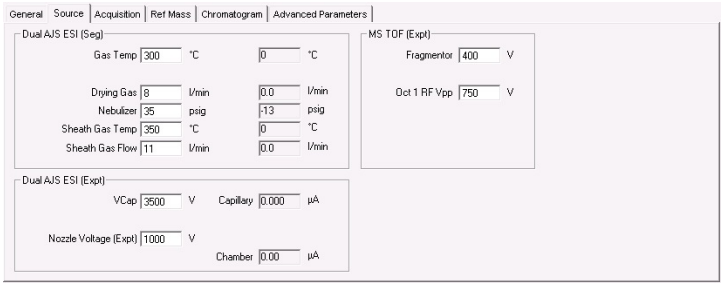


Figure 25 General tab for Q-TOF parameters for a 6560 IM-MS Q-TOF

4 Enter ion source parameters as shown in Figure 26, if necessary.	**a** Click the **Source** tab. **b** Type the parameters as shown in Figure 26.	The name of the selected Ion Source is shown in this tab.



If you have an Agilent Jet Stream, set the **Sheath Gas Temp** to 350°C. Set the **Sheath Gas Flow** to 11 L/min.

Figure 26 Source tab for IM-MS Q-TOF parameters

Task 2. Set up an IM-MS method in **MS (Seg)** mode (continued)

Steps	Detailed Instructions	Comments
5 In the Tune context, adjust the drift tube pressure to be 3.95 ± 0.03 Torr for Nitrogen buffer gas. Make sure that the trapping funnel pressure is 0.10 to 0.15 Torr less than the drift tube pressure.	**a** Change the **Context** to **Tune**. **b** Click **Manual Tune > IM > Actuals**. **c** Make sure that source temperature is stable at the temperature indicated in the method. **d** Locate the pressure valves on the front of the instrument, next to the ion source. **e** Turn the drift tube valve until **Drift Tube Pressure** shows 3.95 ± 0.03 Torr. **f** Continue to adjust the two valves until **Trap Funnel Pressure** shows a reading below **Drift Tube Pressure** by a difference of between 0.10 and 0.15 Torr, while **Drift Tube Pressure** remains close to 3.95 Torr.	
6 Enter the acquisition spectral parameters for MS mode as shown in Figure 27.	**a** Change **Context** to **Acquisition**. **b** Click the **Acquisition** tab. **c** Click **MS** as the **Mode**. **d** Type the **IM-MS Spectra** parameters as in Figure 27.	• A drift time of 60 ms is suitable for most applications. With an acquisition rate of 1 frame/sec, 16 consecutive IM-MS experiments are performed (1000/60) per frame.

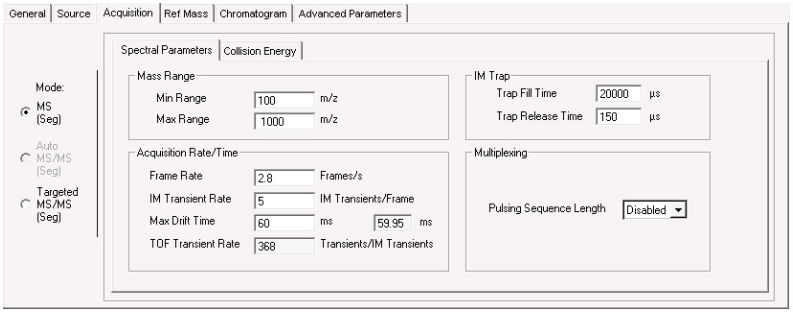


Figure 27 Acquisition tab for IM-MS Q-TOF parameters

Task 2. Set up an IM-MS method in **MS (Seg)** mode (continued)

Steps	Detailed Instructions	Comments
7 Enter the reference mass parameters as shown in Figure 28.	**a** Click the **Ref Mass** tab. **b** Type the parameters as shown in Figure 28.	• This version requires a manual recalibration of the data after the acquisition is completed. To start the recalibration program, click **All Programs > Agilent > MassHunter Workstation > IM-MS Reprocessor**.

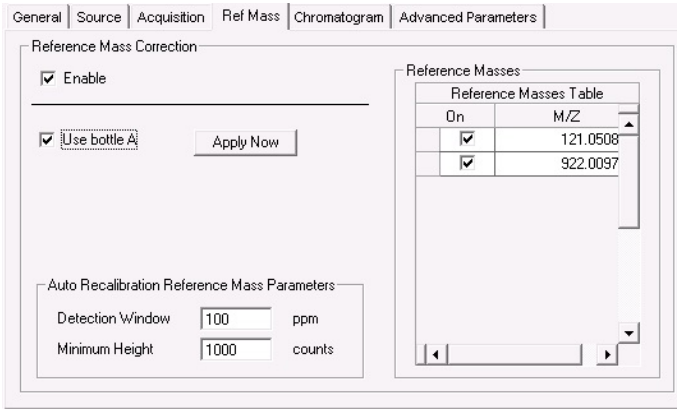


Figure 28 Ref Mass tab for Q-TOF parameters

Task 2. Set up an IM-MS method in **MS (Seg)** mode (continued)

Steps	Detailed Instructions	Comments
8 Enter the chromatogram plot settings as shown in Figure 29.	**a** Click the **Chromatogram** tab. **b** Type the values in Figure 29.	• These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

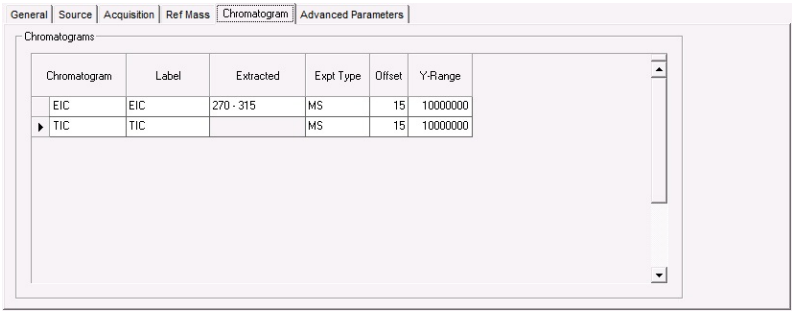


Figure 29 Chromatogram tab for Q-TOF parameters

9 Set up to change MS parameters during run: • Time Segment of 0 min. - Make sure you have selected an LC Stream of Waste. • Time Segment of 0.5 min. - Change LC Stream to MS.	**a** Click the **General** tab. **b** Click **Waste** for the **LC Stream**. **c** Right-click anywhere in the Time segment section, and click **Add Time Segment**. **d** Type `0.5` minutes. **e** Click **MS** for the **LC Stream**.	• You can change a field with a (Seg) next to it with a new Time Segment. • See Figure 25 on page 43 for examples of parameters that can change with time segments. • When you create a new time segment, the initial values are copied from the time segment that is selected.
10 Save the method as ***iii*_IM-MS_only.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as *`iii_IM`*`-MS_only.m`, where ***iii*** are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_only.m**.

Task 3. Set up an IM-MS All Ions Method with one time segment

This task shows you how to set up an acquisition method for the Q-TOF LC/MS when you know what you are looking for, but you are not sure if the compounds are present in your mixture. In this task, you learn how to alternate collision energy by frame. The IM-MS Browser program has special features to work with All Ions data files with frames with alternating collision energy. When you alternate collision energy in a method, the method can only have one **Time Segment** and **Multiplexing** has to be disabled.

Task 3. Set up an IM-MS All Ions method

Steps	Detailed Instructions	Comments
1 Using the ***iii*_IM-MS-only.m** method for the IM-MS Q-TOF, set the collision energy to alternating. • If the ***iii*_IM-MS-only.m** method is still displayed, begin with step c. • Delete the 0.5 min Time Segment. • Enter the parameters as shown in Figure 30.	**a** Click **Method > Open**. **b** Select *iii*_**IM-MS-only.m**, and click **Open**. **c** Click the **Q-TOF** tab. **d** Click the **IM-QTOF** button under Acquisition Mode. **e** Select the 0.5 minute **Time Segment**. **f** Right-click the selected **Time Segment** and click **Delete Time Segment**. **g** Click the **Acquisition** tab. **h** Type 3 frames/sec as **Frame rate**. **i** Select **Disabled** for the **Pulsing Sequence Length**.	• The LC, General, Source, Ref Mass and Chromatogram parameters remain the same as in ***iii*_IM-MS-only.m** for this method. • A minimum of 12 data points over a chromatographic peak is required for quantitative work. A **Frame rate** of 3 Frames/sec is usually sufficient to achieve this.

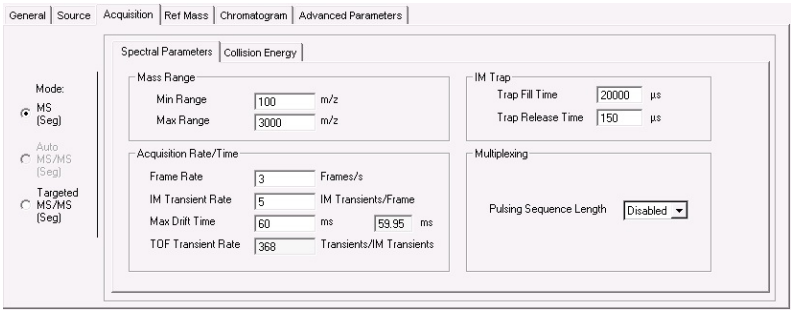


Figure 30 Acquisition Spectral Parameters tab for IM-MS All Ions MS/MS mode

Task 3. Set up an IM-MS All Ions method

Steps	Detailed Instructions	Comments
2 Set the collision energy to alternate between 0 and 35. • Enter the parameters as shown in Figure 31.	**a** Click the **Collision Energy** tab. **b** Click Frame 2 Fixed under Alternating Frames. **c** Type 35 for the collision energy for Frame 2.	• Frame 1 automatically is set to have a collision energy of 0 V when you set up alternating frames.

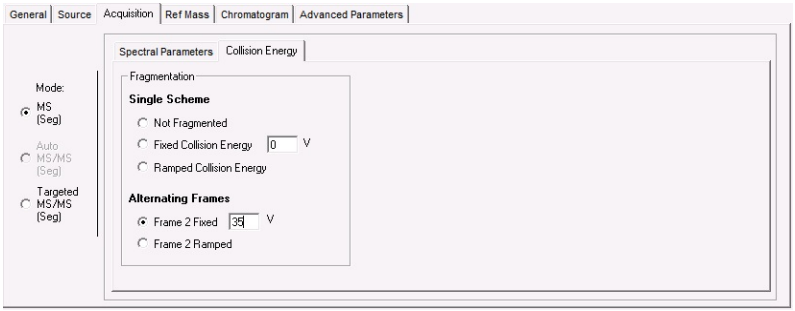


Figure 31 Acquisition Collision Energy tab for IM-MS All Ions mode

3 Save the method as ***iii*_IM-MS-All_Ions.m**, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Type `iii_IM-MS-All_Ions`, and click **Save**.	• Be sure the folder you use is \MassHunter\methods.

4
Optimize IM-MS Q-TOF Methods

This chapter provides familiarization exercises to help you learn how to optimize methods for different compound classes, using the methods you created in Chapter 3.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin...

This exercise introduces you to the parameters relevant to change for the analyses of different compound classes. For all three classes, individual methods are placed in the **MassHunter\methods** folder and allows an easy access to most of the relevant parameters. This guide has a focus on most commonly changed parameters, which allows you to measure samples under predefined conditions.

For the optimization and understanding the optical elements to be changed, the next few images show how the tabs in the Manual Tune tab match the different parts of the instrument. It is not recommended to change these values in the Tune context, but instead you make these changes in individual methods. For more information on the Ion Mobility Q-TOF, see the *Concepts Guide*.

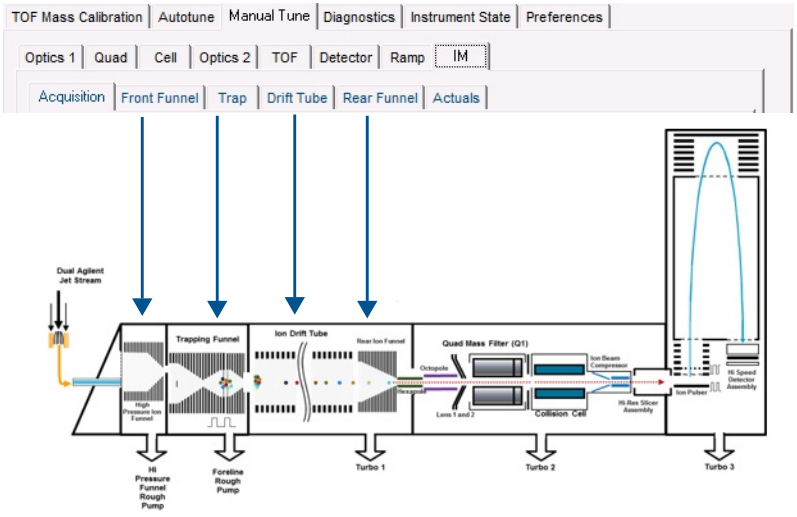


Figure 32 Manual Tune > IM tabs and the part of the instrument they affect

Task 1. Set up and run an IM-MS method for Labile Compounds

This task shows you how to set up a method for the analysis of bradykinin as an example of a labile/heat-sensitive molecule.

Experimental set up

1. Re-suspend bradykinin (1 mg, Sigma, B3259) in 1 mL H_20 as a stock solution. The final concentration based on peptide content will be 883.29 µM.
2. Dilute 11.3 µL of the stock with 88.7 µL 50% MeOH, 0.1% formic acid (FA) to get a 100 µM solution with a volume of 100 µL.
3. Dilute this solution further with 50% MeOH, 0.1% FA to obtain a final solution of 100 nM with a volume of 100 µL.
4. Use 1 mL syringe and appropriate tubing and fittings to connect to the Dual AJS ESI source, adjusting the flow rate of the syringe pump to 50 µL/min.
5. Enter sample and data file information for a single sample and begin to acquire data.

Task 1. Set up and run a method for labile molecules

Steps	Detailed Instructions	Comments
1 Open the method for labile molecules: IM-MS_bradykinin.m	**a** Click **Method > Open**. **b** Select **IM-MS_bradykinin**, and click **OK**. **c** Click the **Method Editor** window.	• Example methods are included on the installation disk.
2 Change the Advanced Parameters: • Make the relative changes as shown in Figure 33, as necessary.	**a** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **b** Click the **Q-TOF** tab. **c** Click the **Advanced Parameters** tab. **d** Clear the **Selected Items Only** check box. **e** Make the relative changes to the Advanced Parameters marked in Figure 33. **f** Mark the **Selected Items Only** check box.	• You are overriding the values in the tune file with the values that you enter in the table. The values are only used if you mark the **Use Method** check box. • The most critical parameter is the **Trap RF** which needs to be optimized for each application and instrument. Typically, values below 90V have a trade-off with signal abundance. The provided default method is a first "walk-up" method and yields over the selection tab in a significantly reduced number of parameters to be optimized.

General | Source | Acquisition | Ref Mass | Chromatogram | Advanced Parameters

Settings

Category	Name	Use Method	Method Setting	Tune Setting	Unit
IM-FrontFunnel	High Pressure Funnel RF	☑	150	150	V
IM-FrontFunnel	Trap Funnel RF	☑	50	150	V
IM-Trap	Trap Entrance Grid Delta	☑	7	12	V
IM-Trap	Trap Exit Grid 2 Delta	☑	10	15	V
IM-DriftTube	Drift Tube Entrance Voltage	☑	1700	1700	V
IM-RearFunnel	Rear Funnel RF	☑	120	200	V

Figure 33 Advanced parameters for bradykinin

3 Save the method as ***iii*_bradykinin.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as *iii_bradykinin*.m, where ***iii*** are your initials. **d** Click **Save**.	For example, if your initials are PFH, then the method name is **pfh_bradykinin.m**.

Task 1. Set up and run a method for labile molecules

Steps	Detailed Instructions	Comments
6 Enter this sample information: • Name: *100 nM bradykinin* • Data file name: *bradykinin01.d*	**a** Click the **Sample Run** window. **b** Type 100 nM bradykinin as the sample **Name**. **c** Type bradykinin01.d as the Data File **Name**. **d** Mark the **Auto Increment** check box.	• The system stores the custom information with the data file. • You can type any number at the end of the Name parameter. This value is incremented for each new data file.

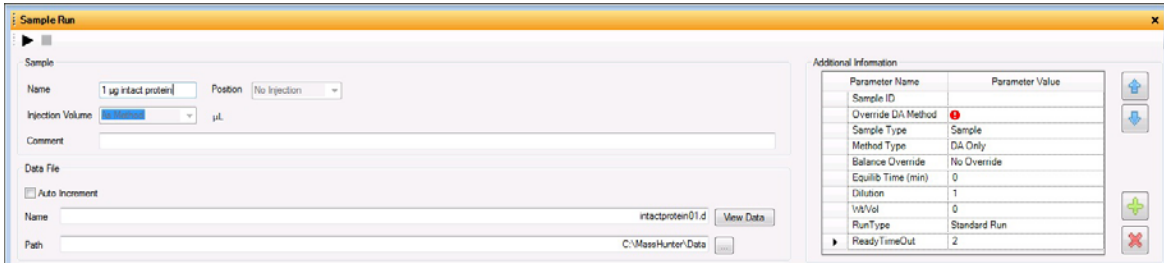


Figure 34 Sample Run window in the main window

4 Start the sample.	• Click the Run button (▶) in the Sample Run toolbar or the Run button ( ) in the main toolbar.	• If you have clicked the Lock icon in the toolbar, you cannot modify the method while the sample is running. Also, you cannot overwrite this data file in the Data Acquisition program. • The button, , in the main toolbar indicates that locked mode is on.
5 View the data after the run.	• After the run is complete, click **View Data** in the **Sample Run** window. • Open the data file in the IM-MS Browser program to display Drift data.	• When you click **View Data**, the Qualitative Analysis program automatically opens and loads the data file that is specified in the Sample Run window.

Evaluation for bradykinin parameters

You open the data file in the IM-MS Browser program. Then, you sum all spectra (for details, refer to the online Help in the IM-MS Browser program). Finally, you examine the final spectrum. The criteria for the successful usage of operating conditions are

- Charge state 3+ (354.1944) has a higher abundance than 2+ (530.7880)
- Minimal abundance of the water loss of 3+ charge state (348.1909)
- Two IMS peaks in front of the most dominant peak

Other parameters for labile compounds

This task describes how to reduct the most relevant voltages for bradykinin in the IM-MS domain.

In a few cases, heating/fragmentation can occur after the drift tube. You can visualize this in the IM-MS Browser. If fragments occur at the same drift time as the analyte, this is indicative of post drift tube fragmentation. To reduce post drift tube fragmentation, do the following:

- Reduction of the collision cell delta
- Reduction of the IBC delta
- Reduction of the IM Hex delta

All of these will have a negative impact on IM-MS resolution, as ions are slowed down post drift-separation, and the diffusion leads to a spread of the ion packet. Nevertheless, for some purposes as collision cross section calculation, a lower resolution is still preferable to a dissociated structure, and within the Tune and Acquisition context, these deltas can be minimized.

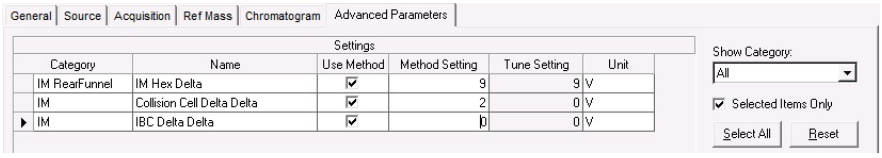


Figure 35 Delta Delta display in Advanced Parameters tab

In this example, the default **Collision Cell Delta** of -9V (not shown, it is part of the tune file) was in the delta reduced by 2V via the **Collision Cell Delta Delta** setting. The resulting delta over the cell is therefore now -7V, which reduces fragmentation of labile compounds. Values below a collision cell delta of -2V (delta delta 7V) will lead to loss in signal abundance, as well as the previously mentioned IM-MS resolution. The **IBC Delta Delta** should not be greater than 2V, as otherwise ions are not completely transferred through the IBC. It is suggested to do the reduction stepwise in 1V increments, finding an optimum between maintaining structure, abundance and IM-MS resolution.

Task 2. Set up IM-MS method for Small Compounds

This tasks show you how to set up a method for the analysis of amino acids as an example of small molecules separated in IM-MS.

Experimental set up

1. Use the example method provided for small compounds: IM-MS_small_molecules.m.
2. The information about the LC is the same, but the source conditions are slightly different, using a Nozzle voltage of 0V.
3. Enter sample and data file information for a single sample.
4. Acquire data.

The most relevant parameters for small molecules are displayed in Figure 36 on page 56. The most critical parameter is the **Trap RF**, which needs to be optimized for each application and instrument. The values for other parameters are similar to the values for the labile compounds, but they are reduced further, as lowering RF and DC voltages still allows good transmission of these low *m/z* species.

An increase in trap time of up to 20 milliseconds can increase the abundance of small molecules significantly. In order to trap these species efficiently, a reduction of the delta between trap entrance and trap exit to 1V showed also better signal abundance.

Task 2. Set up IM-MS method for Small Compounds

Steps	Detailed Instructions	Comments
1 Open the method for small molecules. • IM-MS_small molecules	**a** Click **Method > Open**. **b** Select **IM-MS_small_molecules.m**, and click **OK**. **c** Click the **Method Editor** window.	• Example methods are included on the installation disk.
2 Change the Advanced Parameters: • Enter the parameters as shown in Figure 36, if necessary.	**a** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **b** Click the **Q-TOF** tab. **c** Click the **Advanced Parameters** tab. **d** Clear the **Selected Items Only** check box. **e** Enter the parameters as shown in Figure 36.	• You are overriding the values in the tune file with the values that you enter in the table. The values are only used if you mark the **Use Method** check box. • The provided method is a first "walk-up" method and yields over the selection tab in a significantly reduced number of parameters to be optimized.

General | Source | Acquisition | Ref Mass | Chromatogram | Advanced Parameters

Settings					
Category	Name	Use Method	Method Setting	Tune Setting	Unit
IM-FrontFunnel	High Pressure Funnel Delta	☑	150	150	V
IM-FrontFunnel	High Pressure Funnel RF	☑	120	150	V
IM-Trap	Trap Entrance Grid Delta	☑	7	12	V
IM-Trap	Trap Exit Grid 2 Delta	☑	10	15	V
IM-DriftTube	Drift Tube Entrance Voltage	☑	1700	1700	V
IM-RearFunnel	Rear Funnel RF	☑	90	200	V

Figure 36 Advanced parameters for amino acid mix

3 Save the method as ***iii*_IM-MS_small_molecules.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as `iii_IM-MS_small_molecules.m`, where ***iii*** are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_small_molecules.m**.
4 Enter this sample information: Name: *100 pg amino acid mix* Data file name: *aminoacid01.d*	**a** Click the **Sample Run** window. **b** Type 100 pg amino acid mix as the sample **Name**. **c** Type aminoacid01.d as the Data File **Name**. **d** Mark the **Auto Increment** check box.	• The system stores the custom information with the data file. • You can type any number at the end of the Name parameter. This value is incremented for each new data file.

Task 2. Set up IM-MS method for Small Compounds

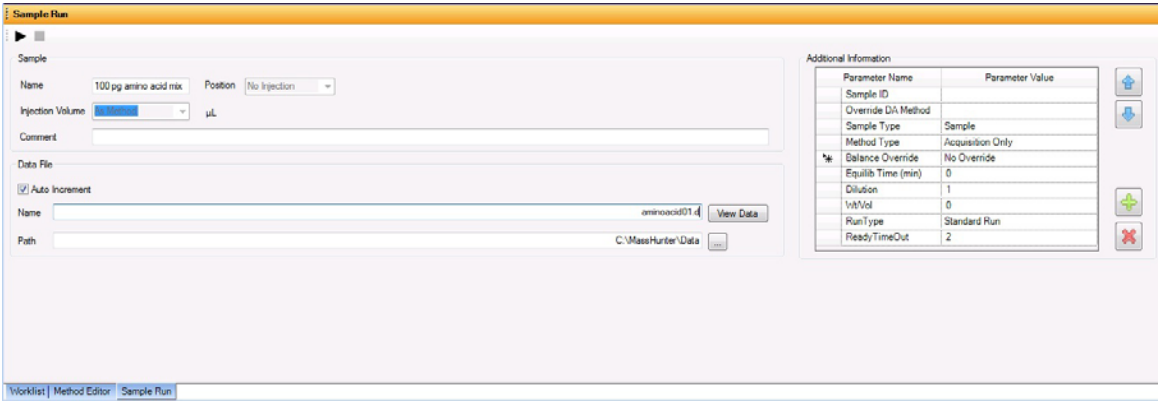
Steps	Detailed Instructions	Comments
		

Figure 37 Sample Run window in the main window

5 Start the sample.	• Click the Run button (▶) in the Sample Run toolbar or the Run button ( ) in the main toolbar.	• If you have clicked the Lock icon in the toolbar, you cannot modify the method while the sample is running. Also, you cannot overwrite this data file in the Data Acquisition program. • The button, , in the main toolbar indicates that locked mode is on.
6 View the data after the run.	• After the run is complete, click **View Data** in the **Sample Run** window. • Open the data file in the IM-MS Browser program to display Drift data.	• When you click **View Data**, the Qualitative Analysis program automatically opens and loads the data file that is specified in the Sample Results window.

Task 3. Set up IM-MS method for Intact Proteins

This tasks shows you how to set up a method for the analysis of intact proteins as an example of large molecules separated in IM-MS.

Experimental set up

1. Use the example method provided for intact proteins: *IM-MS_intact_proteins.m*.
2. The information about the source conditions are the same, using a Nozzle voltage of 2000V.
3. The LC conditions are changed according to the instructions below.
4. Enter sample and data file information for a single sample.
5. Acquire data.

In contrast to labile molecules and small molecules, the emphasis for intact proteins is on the transmission of these molecules. In general, the RF voltages need to be increased, with the **Trap RF** having the most effect.

Task 3. Set up IM-MS method for intact proteins

Steps	Detailed Instructions	Comments
1 Open the method for intact proteins.	**a** Click **Method > Open**. **b** Select **IM-MS_intact_proteins**, and click **OK**. **c** Click the **Method Editor** window.	• Example methods are included on the installation disk.
2 Change the column temperature to 60°C.	**a** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **b** Click the **Column** tab. **c** Set the Column temperature to 60°C.	• For intact proteins, the column temperature is higher than for small molecules and labile molecules.

Task 3. Set up IM-MS method for intact proteins

Steps	Detailed Instructions	Comments
3 Change the Advanced Parameters: • Modify the parameters as shown in Figure 38, with values appropriate to your instrument.	**a** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **b** Click the **Q-TOF** tab. **c** Click the **Advanced Parameters** tab. **d** Clear the **Selected Items Only** check box. **e** Modify the parameters shown in Figure 38 with the relative values appropriate for your instrument. **f** Mark the **Selected Items Only** check box.	• You are overriding the values in the tune file with the values that you enter in the table. The values are only used if you mark the **Use Method** check box. • For intact proteins, the emphasis is on the transmission of these molecules. In general, the RF voltages need to be increased, with the Trap RF having the most effect. An increase in trap time of up to 5 milliseconds can increase the abundance of intact proteins significantly.

General | Source | Acquisition | Ref Mass | Chromatogram | Advanced Parameters

Settings

Category	Name	Use Method	Method Setting	Tune Setting	Unit
IM-FrontFunnel	High Pressure Funnel RF	☑	200	150	V
IM-FrontFunnel	Trap Funnel RF	☑	200	150	V
IM-Trap	Trap Entrance Grid Delta	☑	12	12	V
IM-Trap	Trap Exit Grid 2 Delta	☑	15	15	V
IM-DriftTube	Drift Tube Entrance Voltage	☑	1700	1700	V
IM-RearFunnel	Rear Funnel RF	☑	200	200	V

Figure 38 Advanced Parameters for intact proteins

4 Save the method as ***iii*_IM-MS_intact_proteins.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as *iii_IM-MS_intact_proteins*.m, where ***iii*** are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_intact_proteins.m**.
6 Enter this sample information: • Name: *1 µg intact protein* • Data file name: *intactprotein01.d*	**a** Click the **Sample Run** window. **b** Type 1 µg intact protein as the sample **Name**. **c** Type intactprotein01.d as the Data File Name. **d** Mark the **Auto Increment** check box.	• The system stores the custom information with the data file. • You can type any number at the end of the Name parameter. This value is incremented for each new data file.

Task 3. Set up IM-MS method for intact proteins

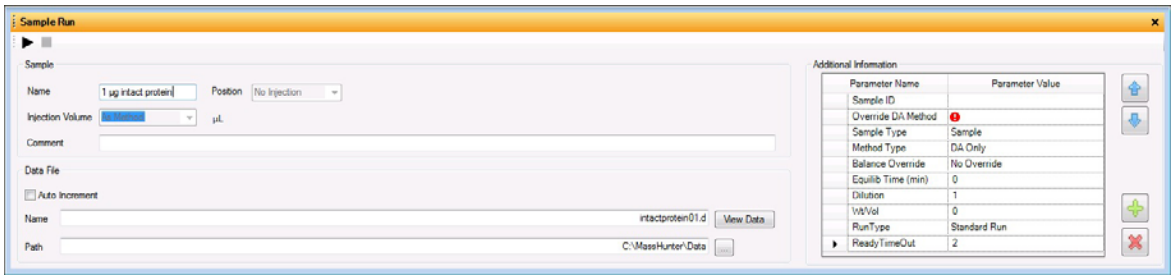
Steps	Detailed Instructions	Comments
		

Figure 39 Sample Run window in the main window

5 Start the sample.	• Click the Run button, ▶ , in the Sample Run toolbar or the Run button, , in the main toolbar.	• If you have clicked the Lock icon in the toolbar, you cannot modify the method while the sample is running. Also, you cannot overwrite this data file in the Data Acquisition program. • The button, , in the main toolbar indicates that locked mode is on.
6 View the data after the run.	• After the run is complete, click **View Data** in the **Sample Run** window.	• When you click **View Data**, the Qualitative Analysis program automatically opens and loads the data file that is specified in the Sample Results window. • Open the data file in the IM-MS Browser program to display Drift data.

5 Set up acquisition method for collision cross section calculation

This exercise describes two strategies to acquire data for the calculation of collision cross sections. The first task creates an infusion based method where the field strengths are changed during one acquisition. The second task shows an LC based strategy where multiple LC runs are performed under different field strengths.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Task 1. Set up and run an infusion method to calculate CCS using traditional Multi-Field method

Task 1. Set up and run an infusion method to calculate CCS using traditional Multi-Field method

Steps	Detailed Instructions	Comments
1 Open the method for labile molecules that you modified in "Task 1. Set up and run an IM-MS method for Labile Compounds" on page 51: *iii*_IM-MS_bradykinin.m, where *iii* are your initials.	**a** Click **Method > Open**. **b** Select ***iii*_IM-MS_bradykinin**, and click **OK**. **c** Click the **Method Editor** window.	• The Advanced Parameters for the Q-TOF are changed in the method that you created.
2 Change the Advanced Parameters: • Make the relative changes as shown in Figure 40, as necessary.	**a** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **b** Click the **Q-TOF** tab. **c** Click the **Advanced Parameters** tab. **d** Clear the **Selected Items Only** check box. **e** Make the relative changes to the Advanced Parameters marked in Figure 40. **f** Mark the **Selected Items Only** check box.	• You are overriding the values in the tune file with the values that you enter in the table. The values are only used if you mark the **Use Method** check box. • The provided default method is a first "walk-up" method and yields over the selection tab in a significantly reduced number of parameters to be optimized.

General | Source | Acquisition | Ref Mass | Chromatogram | Advanced Parameters

Settings

Category	Name	Use Method	Method Setting	Tune Setting	Unit
IM-FrontFunnel	High Pressure Funnel RF	☑	150	150	V
IM-FrontFunnel	Trap Funnel RF	☑	50	150	V
IM-Trap	Trap Entrance Grid Delta	☑	7	12	V
IM-Trap	Trap Exit Grid 2 Delta	☑	10	15	V
IM-DriftTube	Drift Tube Entrance Voltage	☑	1700	1700	V
IM-RearFunnel	Rear Funnel RF	☑	120	200	V

Figure 40 Advanced parameters for bradykinin

Task 1. Set up and run an infusion method to calculate CCS using traditional Multi-Field method

Steps	Detailed Instructions	Comments
3 Add seven time segments. Modify each time segment to decrease the Drift Tube Entrance Voltage by 100 for each time segment. The method will have eight time segments.	**a** Right-click the Time Segment table and click **Add Time Segment**. **b** Enter `1` for the **Time (min)** for this new time segment. **c** Enter `1600` for the Method Setting for **Drift Tube Entrance Voltage**. **d** Right-click the Time Segment table and click **Add Time Segment**. **e** Enter `2` for the **Time (min)** for this new time segment. **f** Enter `1500` for the Method Setting for **Drift Tube Entrance Voltage**. **g** Continue to add five more time segments, adjusting the **Time (min)** for each and the **Drift Tube Entrance Voltage** for each. **h** Change the **Stop Time** to 8 minutes.	• You are overriding the values in the tune file with the values that you enter in the table. The values are only used if you mark the **Use Method** check box.

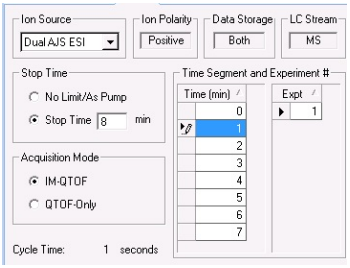


Figure 41 Eight time segments for bradykinin

4 Save the method as ***iii*_bradykinin_CSS.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as *`iii_bradykinin_CSS`*`.m`, where ***iii*** are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_bradykinin_CSS.m**.

Task 1. Set up and run an infusion method to calculate CCS using traditional Multi-Field method

Steps	Detailed Instructions	Comments
5 Enter this sample information: • Name: *100 nM bradykinin* • Data file name: *bradykin_CCS01.d*	**a** Click the **Sample Run** window. **b** Type 100 nM bradykinin as the sample **Name**. **c** Type `bradykinCSS01.d` as the Data File **Name**. **d** Mark the **Auto Increment** check box.	• The system stores the custom information with the data file. • You can type any number at the end of the Name parameter. This value is incremented for each new data file.

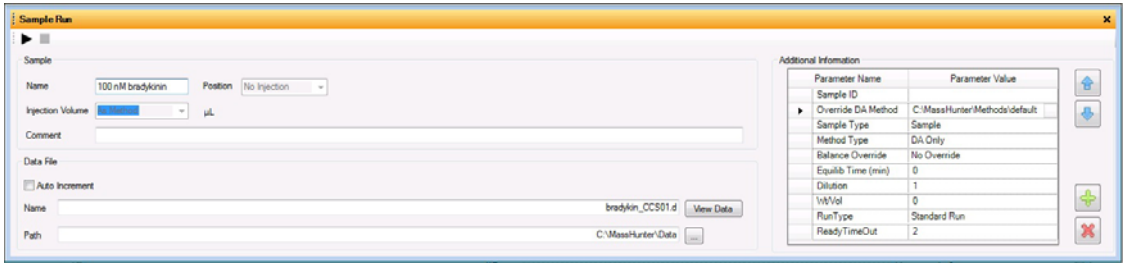


Figure 42 Sample Run window in the main window

6 Start the sample.	• Click the Run button, ▶ , in the Sample Run toolbar or the Run button, , in the main toolbar.	• If you have clicked the Lock icon in the toolbar, you cannot modify the method while the sample is running. Also, you cannot overwrite this data file in the Data Acquisition program. • The button, , in the main toolbar indicates that locked mode is on.
7 View the data after the run.	• After the run is complete, open the data in the IM-MS Browser.	• You can view cross section calculations in the IM-MS Browser program.

Task 2. Set up an LC method to calculate CCS using Single-Field method

In this task, you set up a worklist to run an infusion experiment and an LC experiment. Data from the infusion experiment is used to generate calibration coefficients to calculate CCS for the compounds from the LC experiment. The conditions for the tune mix run (a direct infusion run for about 0.5 minutes) should be exactly the same as the LC experiment (method settings as well as the drift tube pressure). If the instrument parameters or the instrument conditions are different between the two experiments (LC and tune mix), then this method will not work properly. It is recommended to run the tune mix experiment before and after the LC experiments.

Task 2. Set up an LC method to calculate CCS using SingleField method

Steps	Detailed Instructions	Comments
1 Open an IM-QTOF method. For this example, open the method ***iii*_IM-MS_only.m**, where ***iii*** are your initials. This method was created previously for the Sulfa drug mix analysis.	**a** Click **Method > Open**. **b** Select ***iii_IM-MS_only.m***, and click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_only.m**.
2 Save the method as *iii*_SulfaDrug_CCS.m.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type ***iii_SulfaDrug_CCS.m***, where ***iii*** are your initials. **d** Click **OK**.	• You save the method with a new name to make the example clearer to read. You will use this method to acquire the sample.

Task 2. Set up an LC method to calculate CCS using SingleField method

Steps	Detailed Instructions	Comments
3 Open Data Acquisition to access the window for editing methods.	**a** Make sure that Acquisition appears as the selection in the **Context** box in the main toolbar. If Tune is the selection, click **Acquisition** from the **Context** list. **b** Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. **c** Click the **Quat. Pump** tab. **d** Under Hip Sampler, set the injection volume to be 0.00 µL. **e** Click the **Q-TOF** tab. **f** Click the **General** tab. **g** Click **Stop Time** and type 0.5 for the time.	• You acquire a short infusion run including the reference ions to be used for the calibration. The Agilent tune mix is perfectly adequate for this purpose, as the cross sections for these ions are all known. • Change the injection volume for the pump that is installed with your instrument. • Verify that you clicked **IM-QTOF** for the **Acquisition Mode**.
4 Change the Properties tab.	**a** Click the **Properties** tab. **b** Click the … button. The **Select Script** dialog box opens. **c** Select **SCP_AcquireCalibrantData** as the **Script**. **d** Click **OK**.	• This script does a run with Calibrant B on.

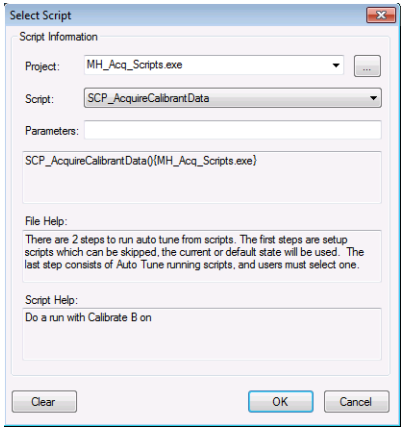


Figure 43 Select Script dialog box

Task 2. Set up an LC method to calculate CCS using SingleField method

Steps	Detailed Instructions	Comments
5 Save the method as ***iii*_IM-QTOF_PosTuneMethod.m**, where ***iii*** are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type the name of the method as *iii_IM-QTOF_PosTuneMethod*.m, where ***iii*** are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_IM-QTOF_PosTuneMethod.m**.
6 Set up a worklist that acquires a tune calibrant data file and your sample file.	**a** Click the **Worklist** window. **b** Add two samples with the following information.	

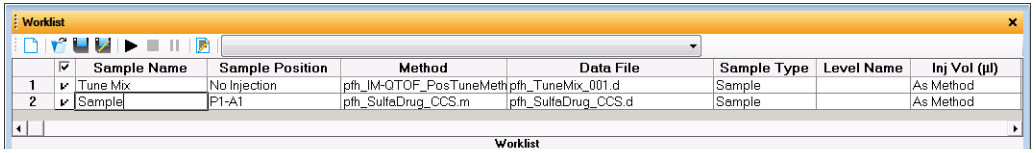


Figure 44 Worklist window with a tune sample and a sample

Task 2. Set up an LC method to calculate CCS using SingleField method

Steps	Detailed Instructions	Comments
7 Start the worklist.	• Click the Run button, ▶ , in the Worklist toolbar or the Run Worklist button,  , in the main toolbar.	• You do not have to save the worklist in order to start it. • If you have clicked the Lock icon in the toolbar, you cannot modify the method or the worklist while the worklist is running. Also, you cannot overwrite these data files in the Data Acquisition program. • The button,  , in the main toolbar indicates that locked mode is on. • Each sample row turns blue as the software begins to acquire data for that worklist row.
8 Examine the data file in the IM-MS Browser program.	**a** Open the *iii*_TuneMix_001.d data file that you just acquired, where ***iii*** are your initials. **b** Click **View > CCS Calibration (Single-Field)**. **c** Select **Agilent ESI Tune Mix (pos)** as the **Reference set**. **d** Click **Find Drift Times**. **e** Save the CCS (Single-Field) coefficients. Click **Save or Restore**. The **CCS Calibration (Single-Field)** dialog box opens. **f** Click **Save to Multiple Files** and select ***iii*_SulfaDrug_CCS.m**. **g** In IM-MS Browser, open ***iii*_SulfaDrug_CCS.m.** **h** Go to **Method > Find Features (IMFE)** to get CCS values for Sulfa Drugs. **i** If you need to remove calibration coefficients from the file, click **Restore Current File**.	• You can view cross section calculations in the IM-MS Browser program. • You can save the coefficients in one or more already acquired data files or as the instrument default. If you set these values as the instrument default, then these values are copied into any new data files acquired after the coefficients are saved. Whenever feature finding is done on any of those files, CCS values are automatically computed.

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In this Book

This guide contains information to learn to use your Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS system.

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