MassHunter Data Acquisition for LC/TOF and LC/Q-TOF

Familiarization Guide

Notices

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This guide is valid for the 11.0 revision or later of the MassHunter Data Acquisition for LC/TOF and LC/Q-TOF program and compatible MassHunter programs, until superseded.

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Manufactured for
Agilent Technologies
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

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In This Guide...

This guide teaches you how to use your MassHunter Data Acquisition for Agilent LC/TOF or LC/Q-TOF system.

Exercise 1 Set up acquisition methods

In 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 LC/TOF or LC/Q-TOF, 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 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 Exercise 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 shows an LC-based strategy where a calibration is used to calculate CCS values using a single-field method. The second task creates an infusion-based method where the field strengths are changed during one acquisition (multi-field method).

Before you begin...

This guide assumes that

- MassHunter Workstation has been installed
- LC modules and the LC/TOF or LC/Q-TOF have been configured
- The system has been turned on
- The performance has been verified, and.

If these actions have not yet been done, contact Agilent Technologies.

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 23, from the Electrospray LC Demo Sample, p/n 59987-20033
- For 6224, 6230, 6520, and 6530, Zorbax, Extend-C18 2.1mm x 50mm, 1.8um, 80Å, p/n 727700-902
- For all other instruments, Zorbax, RRHD Eclipse Plus C18, 2.1x50mm, 1.8um, p/n 959757-902
- Bradykinin, Sigma, B2359-1 mg
- Amino acid standard 10pmol/µL, Agilent, p/n 5061-3334

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.

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1 Set up acquisition methods

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In 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.

For more details on how to use these exercises, see **"Before you begin..."** on page 4.

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 data recording rates from 1 GHz to 4 GHz, as well as the mass range.
- If available for your TOF or Q-TOF, you can set **Fast Polarity Switching** to **Enabled** or **Disabled**. 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** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** In the Data Acquisition program on the ribbon in the **Context** group, click **Tune**. **g** Click the **Instrument State** tab.	• You may be asked whether or not to save the layout changes and whether or not to save any method changes when switching to the **Tune** context.
2 Select the following data collection settings. • Mass Range: Standard (3200 *m/z*) • Select to acquire data in High Resolution Mode.	**a** Set **Mass Range** to **Standard (3200 *m/z*)**. **b** If needed, select **High Resolution** for the Slicer Mode. **c** (optional) Set **Fast Polarity Switching** to **Disabled**. **d** For 6560, set **Acquisition Mode** to **QTOF-Only**. **e** Click **Apply**. **f** If you changed the **Mass Range**, tune the instrument. **g** Recalibrate the TOF mass axis.	• You need to click **Apply** to change the settings on the instrument. • The **Mass Range** can only be set to **High (20,000 *m/z*)** or **High (10,000 *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 a 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

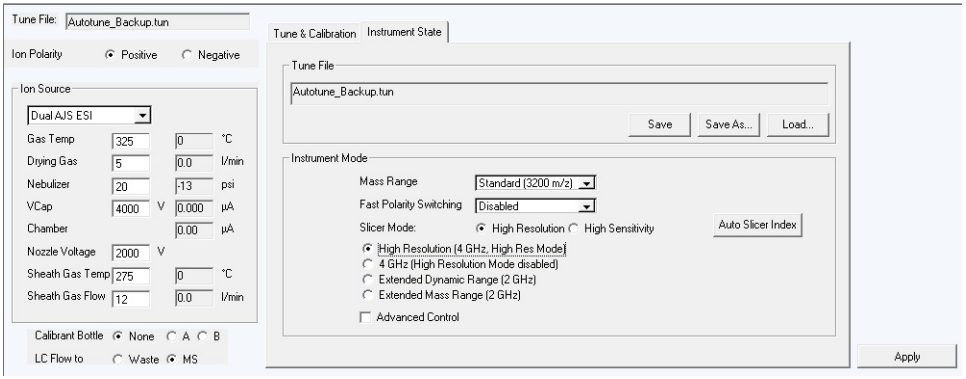


Figure 1. Instrument State tab for a 6550 iFunnel Q-TOF instrument

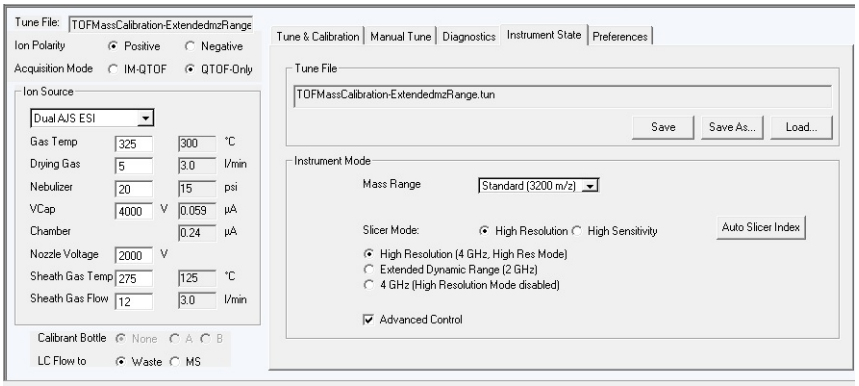


Figure 2. Instrument State tab for a 6560 Ion Mobility Q-TOF instrument with QTOF-Only chosen

3 Save the new settings to the tune file (Autotune.tun) and return to Acquisition.	**a** On the ribbon click **Acquisition**. **b** Click **Yes** in the Instrument State Confirmation message. **c** 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** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **g** If you have a 6560 Ion Mobility Q-TOF, click **QTOF-Only** for the **Acquisition Mode**.	• The MassHunter Data Acquisition window appears containing the Method Editor window. See **Figure 3**. • Your display will be different if the AJS source is not installed on your system.

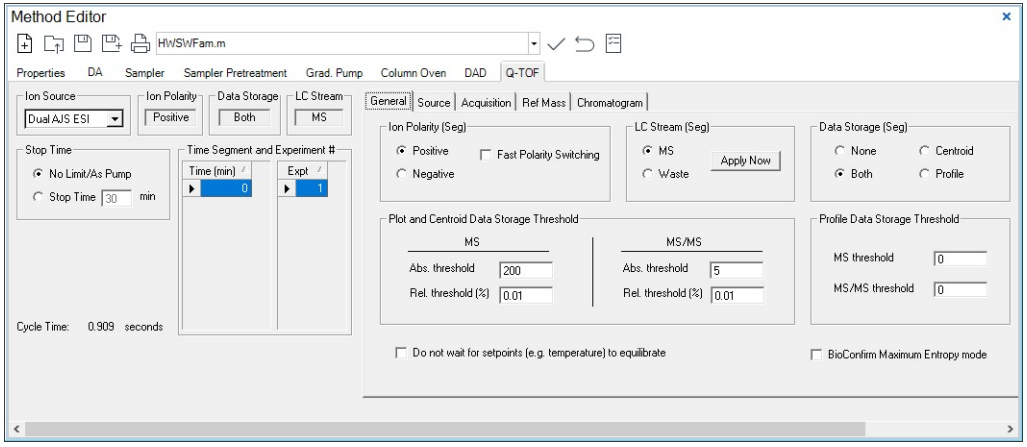


Figure 3. Method Editor window in the MassHunter Data Acquisition program

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

Steps	Detailed Instructions	Comments

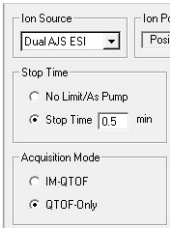


Figure 4. For a 6560 Ion Mobility Q-TOF, select QTOF-Only for the Acquisition Mode

2 Enter LC parameters appropriate for sulfa drug mix. See **Table 1**.	**a** In the Method Editor window, click each LC module tab to set parameter values. **b** Set the LC parameters listed in **Table 1**.	• LC parameters in each tab depend on the configuration of the LC attached to the mass spectrometer.

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
COL THERM	
• Temp	40° C

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 5**, if necessary.	**a** Click the **TOF** or **Q-TOF** tab. **b** On the **TOF** or **Q-TOF** tab, make sure the **General** tab is displayed. **c** Type the parameters as shown in **Figure 5**. (These are the default parameters.)	• The MS/MS parameters do not appear on the TOF General tab.

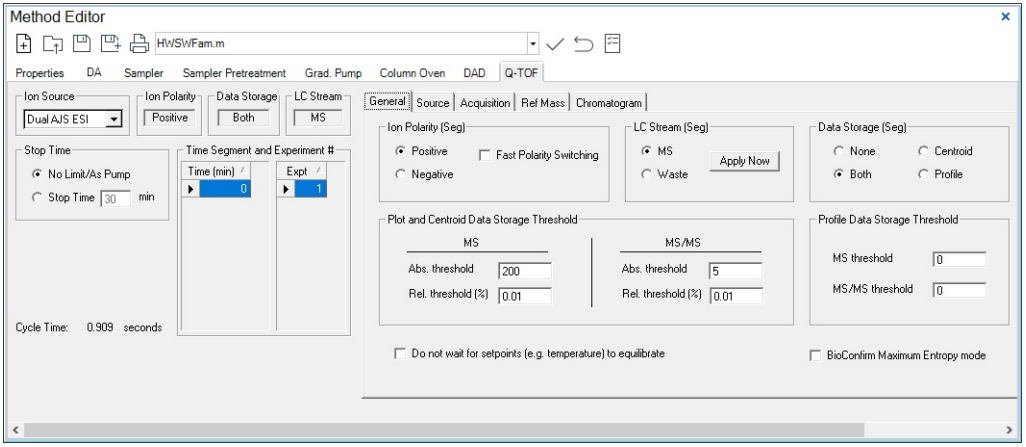
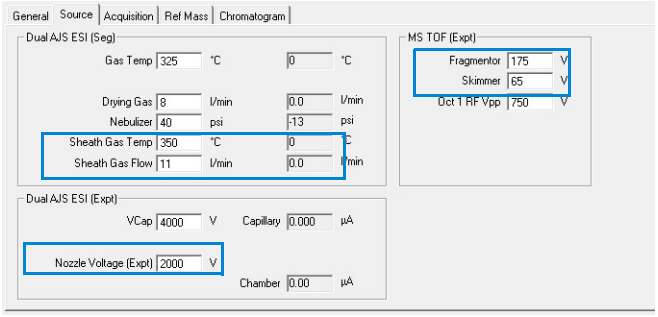


Figure 5. General tab for Q-TOF parameters for a 6546 Q-TOF

4 Enter ion source parameters as shown in **Figure 6**, if necessary.	**a** Click the **Source** tab. **b** Type the parameters as shown in **Figure 6**.	The name of the selected Ion Source is shown in this tab.



Sheath Gas Temp, Sheath Gas Flow, and Nozzle Voltage are visible if the source is an AJS ESI or a Dual AJS ESI.

For 6560 Q-TOF, the Fragmentor should be 400 V, and there is no Skimmer voltage.

Figure 6. Source tab for 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 7**.	**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 shown in **Figure 7**.	

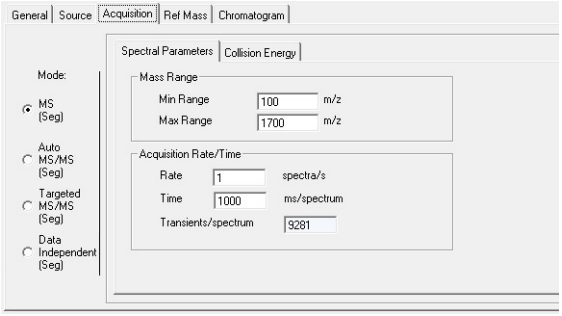


Figure 7. Acquisition tab for Q-TOF parameters (TOF uses the same parameters as MS Mode.)

6 Enter the reference mass parameters as shown in **Figure 8**.	**a** Click the **Ref Mass** tab. **b** Type the parameters as shown in **Figure 8**.	• If you have a 6560 Q-TOF in IM-QTOF mode, you cannot enable reference mass correction. Instead, the reference mass calibration for IM data is done during post-processing. See recalibration window in the online Help for BioConfirm or Qualitative Analysis.

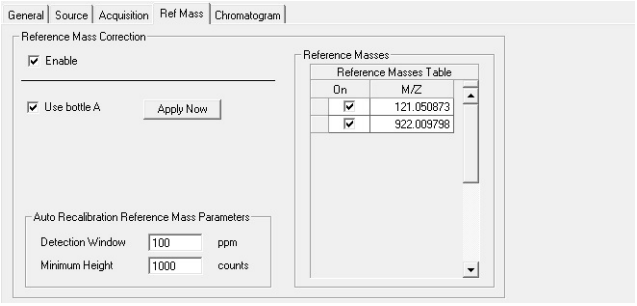


Figure 8. Reference Mass tab (Ref Mass) for TOF or Q-TOF parameters

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 9**.	**a** Click the **Chromatogram** tab. **b** Type the values in **Figure 9**.	• These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

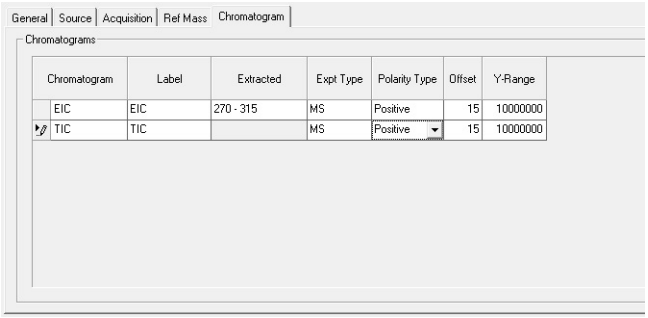


Figure 9. Chromatogram tab for TOF or Q-TOF parameters

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 parameter with a (Seg) next to it in a new Time Segment. • You can change a parameter with an (Expt.) next to it in a new Experiment. • See **Figure 6** for examples of parameters 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 or in the Method Editor toolbar. **b** Go to the **methods** folder in your project. **c** Type `iii`MS-only.m where *iii* are your initials. **d** Click **Save**. **e** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **f** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfhMS-only.m**. • The administrator can set up the project to prompt for a reason when saving the method. If that check box is marked, the administrator also marks whether or not to allow you to type your own reason, and the administrator can provide a list of possible reasons.

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

Task 3 shows you how to set up an acquisition method for the LC/Q-TOF 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 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 10.	**a** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click in the Method Editor toolbar. **c** Select *iii*MS-only.m, and click **Open**. **d** Click the **Q-TOF** tab. **e** Select the 0.5 minute **Time Segment**. **f** Right-click the selected **Time Segment** and click **Delete Time Segment**. **g** Click **MS** for the **LC Stream** **h** Click the **Acquisition** tab. **i** Click **Targeted MS/MS (Seg)** as the Mode. **j** 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.

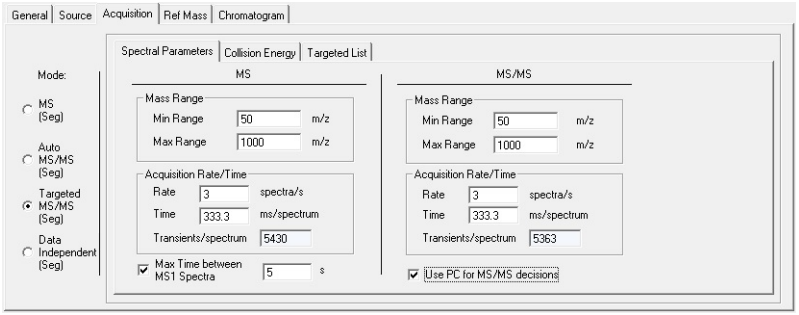


Figure 10. Acquisition Spectral Parameters tab for Targeted MS/MS mode

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

Steps	Detailed Instructions	Comments
2 Set up a fixed collision energy of 35 V. • Enter the parameters as shown in **Figure 11**.	**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.

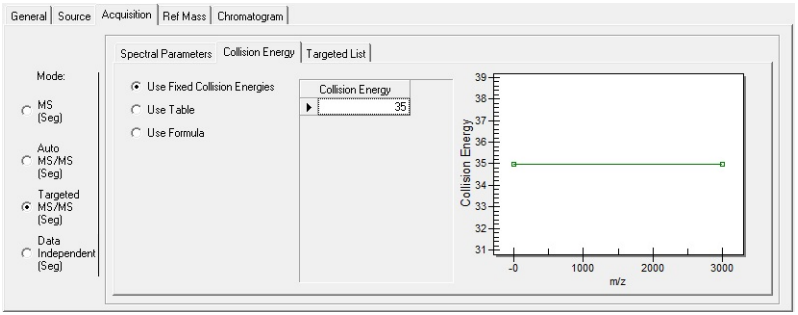


Figure 11. Acquisition Collision Energy tab for targeted MS/MS mode

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** on the shortcut menu. **c** Fill out the information for the **279.09102** ion. **d** Repeat **step b** and **step 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 on the previous tab (**Figure 11**). • 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.

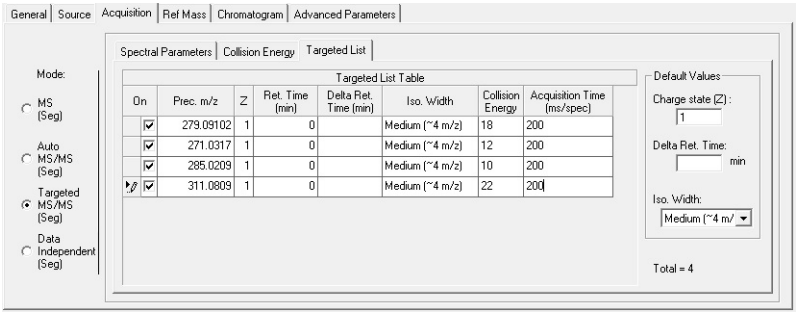


Figure 12. Acquisition Targeted List tab for Targeted MS/MS mode

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 in the Method Editor toolbar. **b** Type `iiitargetedMSMS`, and click **Save**. **c** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **d** Click **OK**.	• Be sure to use the folder **methods** in the project.

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 on the Acquisition tab, including these values on the Targeted List tab, may also be changed by using different time segments. See **Figure 3** on page 10.

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 *iii*targetedMSMS.m method for the MS Q-TOF, change to auto MS/MS mode and enter the spectral parameters below, if necessary. • If the *iii*targetedMSMS.m method is still displayed, begin with step c. • Enter the parameters as shown in Figure 13.	**a** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click in the Method Editor toolbar. **c** Select ***iii*targetedMSMS.m**, and click **Open**. **d** Click the **Q-TOF** tab. **e** Click the **Acquisition** tab. **f** Click **Auto MS/MS (Seg)** as the **Mode**. **g** 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.

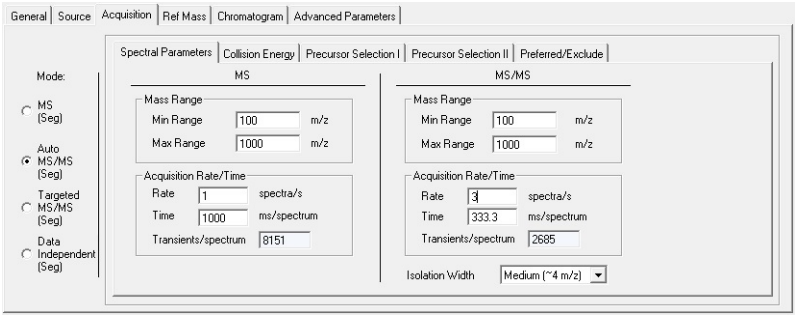


Figure 13. Acquisition Spectral Parameters tab for Auto MS/MS mode

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

Steps	Detailed Instructions	Comments
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.

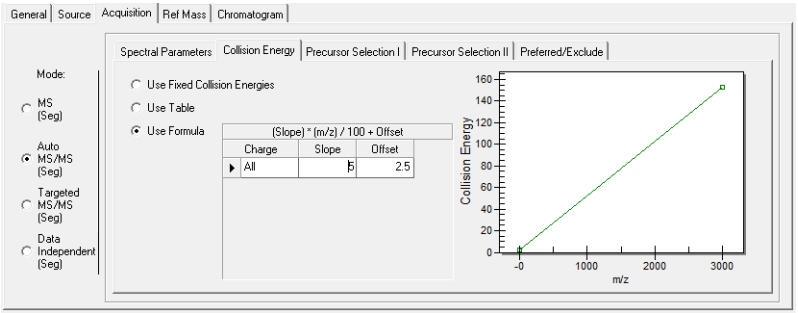


Figure 14. Acquisition Collision Energy tab for Auto MS/MS mode

3 Set **3** as the maximum number of precursor ions per cycle that the program will select in order of decreasing abundance. • Enter the other parameters in Precursor Threshold.	**a** Click the **Precursor Selection I** tab. **b** For **Max Precursor Per Cycle**, type `3`. **c** In the **Precursor Threshold** group, set the remaining parameters as needed.	• 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.

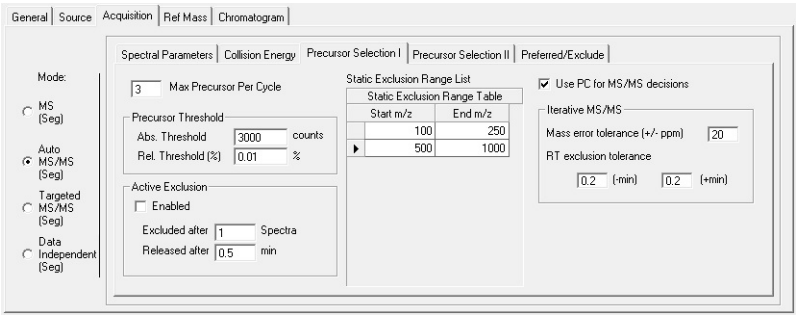
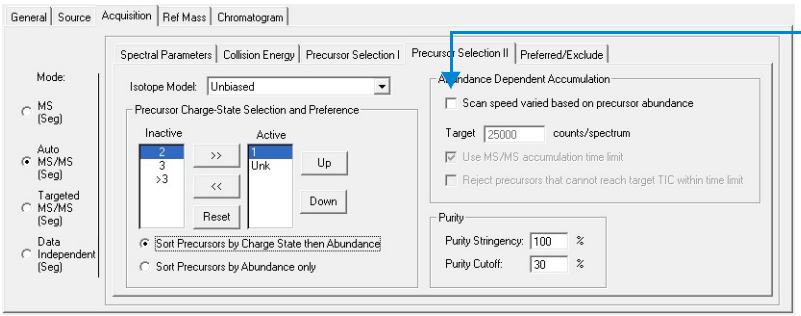


Figure 15. Acquisition Precursor Selection I tab for Auto MS/MS mode

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

Steps	Detailed Instructions	Comments
4 Change the parameters to see the 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 on the **Inactive** list and then click **>>**. **c** If necessary, click any values on the right that are not **1** or **Unk**, and then click **<<**. **d** Select **Unbiased** for the **Isotope Model**. **e** Click **Sort Precursor by Charge State then Abundance**.	• This setting means that if two precursors with charge state +1 are detected, the program selects the two of these with the highest abundance and no precursors with unknown charge state. • If no precursor with charge state of +1 are detected and three with unknown charge states are, then the program 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 16. 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 17**.	**a** Click the **Preferred/Exclude** tab. **b** Right-click the table area, and click **Add**. **c** Type all the values for **279.09102**. **d** Repeat steps b and c for the excluded ion, 311.08085.	

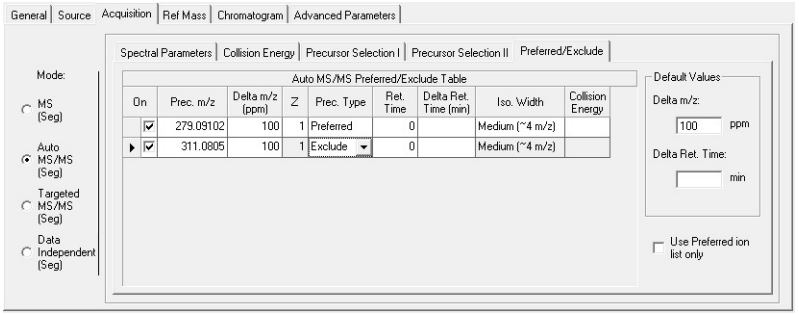


Figure 17. Acquisition Preferred/Exclude tab for Auto MS/MS mode

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

Steps	Detailed Instructions	Comments
6 Save the method as *iii*autoMSMS.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Type `iiiautoMSMS`, and click **Save**. **c** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **d** Click **OK**.	• Be sure use the Method folder.

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2 Set up and run single samples and worklists

Task 1. Set up and run a single sample 25
Task 2. Set up and run a worklist with multiple samples 27
Task 3. Set up and run a worklist to optimize parameters 31

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 LC/TOF or LC/Q-TOF, using the methods you created in Exercise 1.

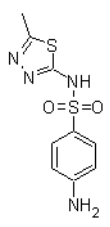
For more details on how to use these exercises, see **"Before you begin..."** on page 4.

Before you begin...

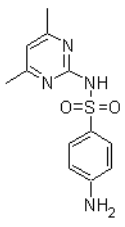
For this exercise you analyze a mixture of four sulfonamide compounds. This section gives instructions on how to prepare the demo sample.

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

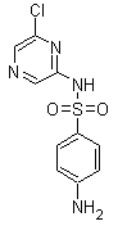
Name	Formula	Ion	*m/z*
sulfamethizole	$C_9H_{10}N_4O_2S_2$	$(M+H)^+$	271.03179
sulfamethazine	$C_{12}H_{14}N_4O_2S$	$(M+H)^+$	279.09102
sulfachloropyridazine	$C_{10}H_9ClN_4O_2S$	$(M+H)^+$	285.02075
sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	$(M+H)^+$	311.08085



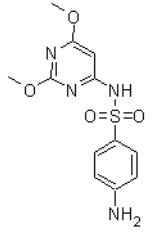
Sulfamethizole



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 LC/TOF or LC/Q-TOF (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.

- For 6224, 6230, 6520, and 6530, Zorbax, Extend-C18 2.1mm x 50mm, 1.8um, 80Å, p/n 727700-902
- For all other instruments, Zorbax, RRHD Eclipse Plus C18, 2.1x50mm, 1.8um, p/n 959757-902

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 the data 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** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click  in the Method Editor toolbar. **c** Select *one of the three methods*, and click **OK**. **d** On the ribbon click **Single Sample**. **e** Click the **Sample Run** window.	• The system stores the custom information with the data file.

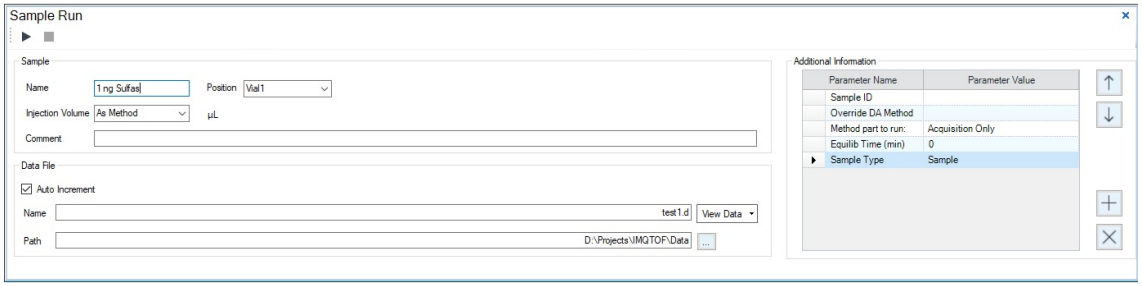


Figure 18. Sample Run window in the main window

	f For Sample **Name**, type `1 ng Sulfas`. **g** For Data File **Name**, type `test1.d`.	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 **Run** ▶ on the **Sample Run** toolbar .	• In locked mode, you cannot change the method while the sample is running. Also, you cannot overwrite these data files in the Data Acquisition program. • On the ribbon in the **Settings** group, if Locked Mode is highlighted, then Locked mode is on. Click to turn Locked mode on or off.

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 Create a new worklist.	**a** On the ribbon in the Layouts group, click **Worklist**.. **b** Click  in the Worklist toolbar.	• A new worklist by default has one sample. If your system has multiple worklist templates, you need to select a template to use.

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

Steps	Detailed Instructions	Comments
2 Set the file naming conventions and make sure that the worklist is set to run only data acquisition. • Data file: Sulfa 1-3.d to be saved to the Data folder in the YourInitials folder.	**a** Click in the Worklist toolbar. **b** Set **Method part to run** to **Acquisition Only**. **c** Click the **Data File Settings** tab. **d** Type `Sulfa` for the **User Text** under **File Naming**. **e** Change the directory path for the data files to **MassHunter\Data*YourInitials***.	• You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.

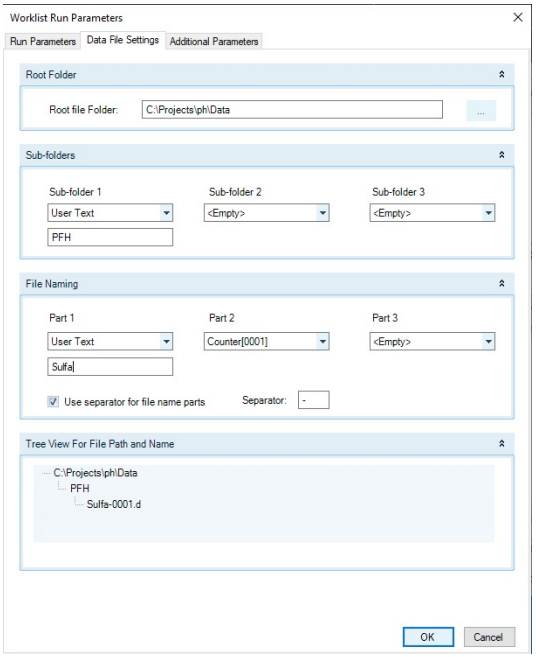


Figure 19. Worklist Run Parameters dialog box

f Click **OK**.

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

Steps	Detailed Instructions	Comments
3 Add three samples to the worklist - Sulfa 1, Sulfa 2, Sulfa 3 - with the following information: • 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** For **Sample Name**, type `Sulfa`. **d** Make sure that the **Append Counter** check box is marked and that all **Suffix Counter** boxes contain **1**. **e** Select the acquisition method from Exercise 1. **f** For **Injection Volume**, select **As Method**.	• A new worklist by default has one sample. If your system has multiple worklist templates, you need to select a template to continue.

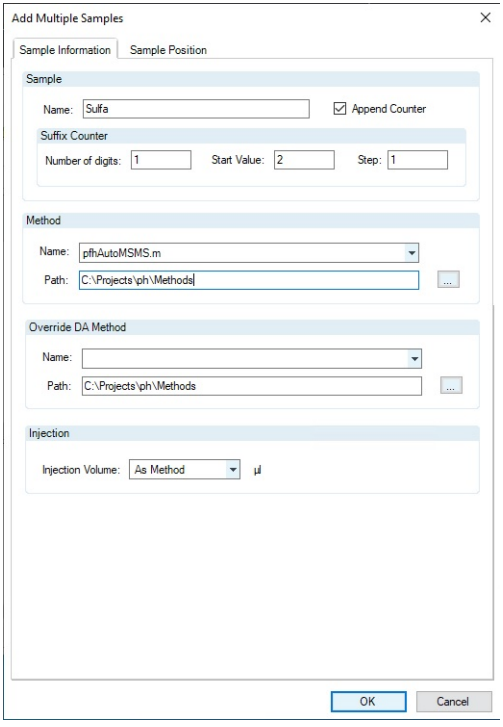


Figure 20. Add Multiple Samples dialog box

g Click the **Sample Position** tab.
h Select three positions in the graphic.
i Click **OK**.

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

Steps	Detailed Instructions	Comments
4 Delete the initial sample.	**a** Select the sample in the first row. **b** Right-click the selected row in the worklist spreadsheet. **c** Click **Delete Sample (s).** **d** Click **Yes** in the message box.	• If you right-click the upper-left-hand corner of the worklist instead, all rows are selected and deleted.
5 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 **Comment**.	• You are hiding these columns, not deleting them. The program recognizes their values even though they do not appear in the worklist.

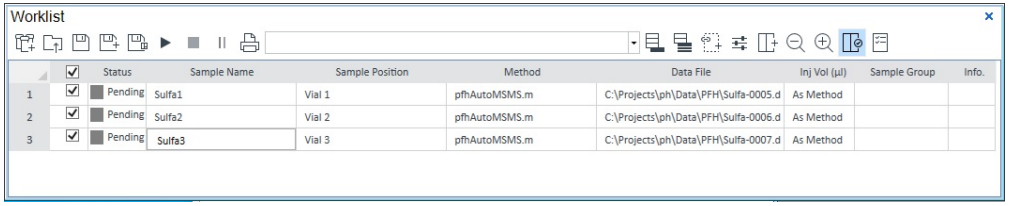


Figure 21. Worklist with three samples

6 Save the worklist as *iii*esdemo.	**a** Click in the Worklist toolbar. **b** Type the worklist **File name** and click **Save**. **c** If needed, enter information on the **Reason for Change** dialog box. You either select a reason from the **Select from list**, or you enter a reason in the **Own reason** box.	• The administrator decides whether or not you need to enter a reason when the project is set up. If the **Prompt for reason** when saving check box is marked on the MassHunter Workstation tab in the project, then the Reason for Change dialog box is opened.
7 Start the worklist. Hint: you must mark the check box at the beginning of each row to run the sample in the row.	• Click **Run** ▶ on the **Worklist** toolbar.	• You do not need to save the worklist in order to start it. • In locked mode, you cannot change the method or the worklist while the worklist is running. Also, you cannot overwrite these data files in the Data Acquisition program. • On the ribbon in the Settings group, if Locked Mode is highlighted, then locked mode is on. Click to turn locked mode on or off. • Each sample row turns blue as the program 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 Create a new worklist. • Data files: Frag 1- 4.d to be saved to the folder, MassHunter\Data*YourName*.	**a** Click in the Worklist toolbar. **b** Click in the Worklist toolbar. **c** Set **Method part to run** to **Acquisition Only**. **d** Click the **Data File Settings** tab. **e** Select **User Text** for **Sub-folder 1** and enter ***YourInitials***. **f** Type `Frag` for the **User Text** for **Part 1** under **File Naming**. **g** Click **OK**.	
2 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** For Sample **Name**, type `Frag.` **d** Make sure that the **Append Counter** check box is marked and that all **Suffix Counter** parameters contain a **1**. **e** Select the ***iii*MS-only.m** acquisition method. **f** For **Injection Volume** , type **As Method**. **g** Click the **Sample Position** tab. **h** Select 4 spots on the Well -plate/Tray. **i** Click **OK**. **j** Delete the original sample.	
3 Hide the following columns: • Override DA Method • 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 **Override DA Method**, **Sample Type**, **Level Name** and **Comment**, and click **OK**.	• You are hiding these columns, not deleting them. The program recognizes their values even though they do not appear in the worklist.

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

Steps	Detailed Instructions	Comments
4 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/Mody/Delete Column(s)**. **c** Select **MS Parameter**. **d** Select **Fragmentor**, and click **>>**. **e** Click **OK**. **f** Type the values into the column.	
5 Save the worklist as Fragwklst.	**a** Click  in the Worklist toolbar. **b** Type `Fragwklst`, and click **Save**. **c** If needed, enter information on the **Reason for Change** dialog box. You either select a reason from the **Select from list**, or you enter a reason in the **Own reason** box.	• Save the Fragwklst file into your project.
6 Start the worklist.	• Click **Run** ▶ on the **Worklist** toolbar.	

3 Set up and run IM-QTOF samples and worklists

Task 1. Configure the instrument for data collection in Ion Mobility mode **34**
Task 2. Set up an IM-MS method **36**
Task 3. Set up an IM-MS All Ions Method **41**

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 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 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.

For more details on how to use these exercises, see **"Before you begin..."** on page 4.

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** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** On the ribbon in the **Context** group, click **Tune**. **g** 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** Set **Mass Range** to **Standard (3200 *m/z*)**. **b** Click **Extended Dynamic Range Mode** if not already the default setting. **c** Click **IM-QTOF** for the **Acquisition Mode**. **d** Click **Advanced Control**. **e** Click **Apply**. **f** If you changed the **Mass Range**, tune the instrument. **g** Recalibrate the TOF mass axis.	• You need to click **Apply** to change the settings on the instrument. • The **Mass Range** can only be set to **High (20000 *m/z*)** (for the G6560A or G6560B) if the **Instrument Mode** is **Extended Mass Range (1 GHz)**. This requires MassHunter Acquisition B.09.00 or later with Firmware 723 or later (required for MH Acq B.09). The caveat is the 20,000 *m/z* requires 4 GHz Acquisition Board G1969-65216 (which has Logic version 0x8025). • If you change the **Instrument Mode,** or the **Mass Range**, you must recalibrate the TOF mass axis. • For a 6560 Ion Mobility Q-TOF, the two **Acquisition Modes** are **IM-QTOF** and **QTOF-Only**.

Task 1. Configure the instrument for data collection

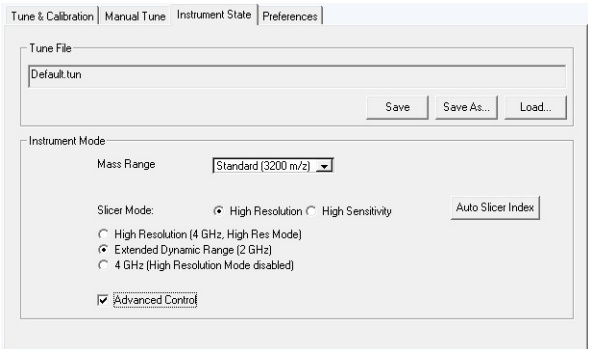
Steps	Detailed Instructions	Comments
		
3 In the Tune context, make sure drift tube gas pressure is stable at pressure 3.95 ± 0.03 Torr for Nitrogen buffer gas. Make sure that the trapping funnel pressure is 0.15 to 0.20 Torr less than the drift tube pressure.	**a** Click **Manual Tune > IM > Pressure & Actuals**. **b** Make sure that source temperature is stable at the temperature indicated in the method. **c** If the pressure difference is less than 0.15 Torr, then adjust the Drift gas pressure regulator valve. Locate the pressure valve labeled "Drift Cell" on the front of the instrument, next to the ion source. Adjust the valve until **Trap Funnel Pressure** shows a reading below **Drift Tube Pressure** by a difference of between 0.15 and 0.20 Torr, while **Drift Tube Pressure** remains close to 3.95 Torr.	• If the Manual Tune tab is not visible, then on the **Instrument State** tab, click **Advanced Control**.
4 Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**a** Click the **Instrument State** tab. **b** Click **Save**. **a** On the ribbon click **Acquisition**. **b** Click **Yes** in the Instrument State Confirmation message. **c** Click **Yes** in the Save Tune File message.	• You can save the tune settings to a new file name for safe-keeping. • You can only save a tune file if you have the Manual Tune permission. Permission are set by the OpenLab Control Panel program.

Figure 22. Instrument State tab for a 6560 Ion Mobility Q-TOF instrument

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** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** On the ribbon in the **Layouts** group, click **Method**. **g** In the Method Editor window, click the **Q-TOF** tab. **h** Set **Acquisition Mode** to **IM-QTOF**.	• The MassHunter Data Acquisition window appears containing the Method Editor window. See **Figure 23**. • 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**.

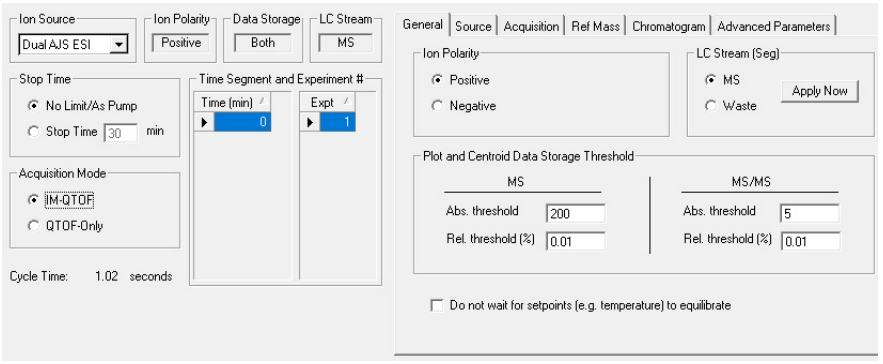


Figure 23. Method Editor window for a 6560 Ion Mobility Q-TOF in the Data Acquisition program

2 Enter LC parameters appropriate for sulfa drug mix. See **Table 2**.	**a** In the Method Editor window, click each LC module tab to set parameter values. **b** Enter LC parameters listed in **Table 2**.	• LC parameters 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
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 24**, if necessary.	**a** Click the **Q-TOF** tab. **b** Click the **General** tab. **c** Type the parameters as shown in **Figure 24**. (These are the default parameters.)	• The MS/MS parameters reflect the threshold for All Ions MS/MS experiments.

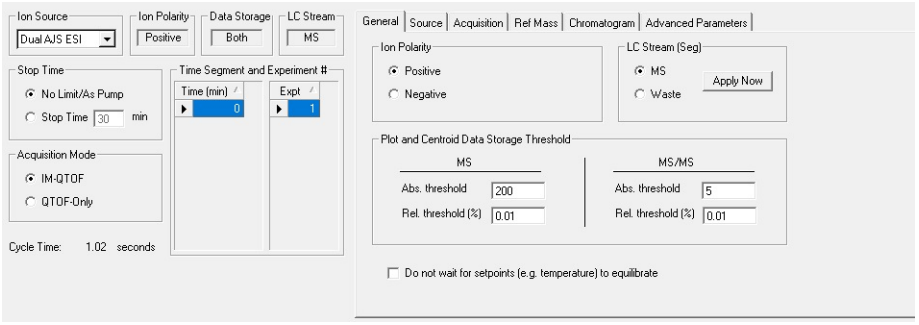


Figure 24. General tab for Q-TOF parameters for a 6560 IM-MS Q-TOF

4 Enter ion source parameters as shown in **Figure 25**, if necessary.	**a** Click the **Source** tab. **b** Type the parameters as shown in **Figure 25**.	The name of the selected Ion Source is shown in this tab. If you have an AJS source, set the **Sheath Gas Temp** to 350°C. Set the **Sheath Gas Flow** to 11 L/min.

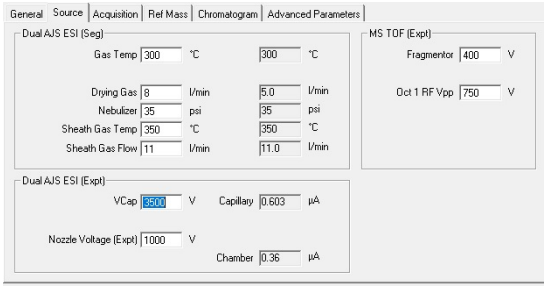


Figure 25. Source tab for IM-MS Q-TOF parameters

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

Steps	Detailed Instructions	Comments
5 Enter the acquisition spectral parameters for MS mode as shown in **Figure 26**.	**a** Click the **Acquisition** tab. **b** Set **Mode** to **MS**. **c** Type the IM-MS Spectra parameters as in **Figure 26**.	• 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.

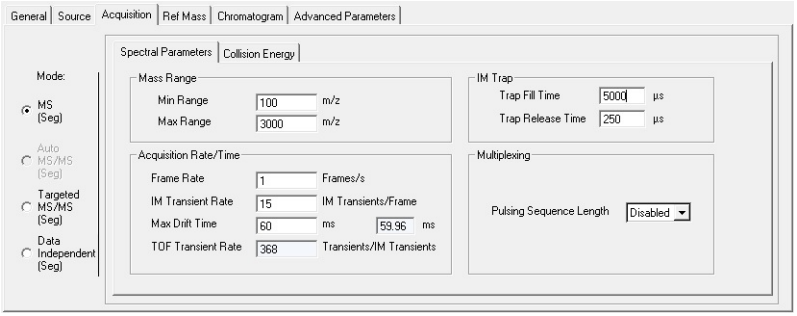


Figure 26. Acquisition tab for IM-MS Q-TOF parameters

6 Enter the reference mass parameters as shown in **Figure 27**.	**a** Click the **Ref Mass** tab. **b** Type the parameters as shown in **Figure 27**.	• 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**.

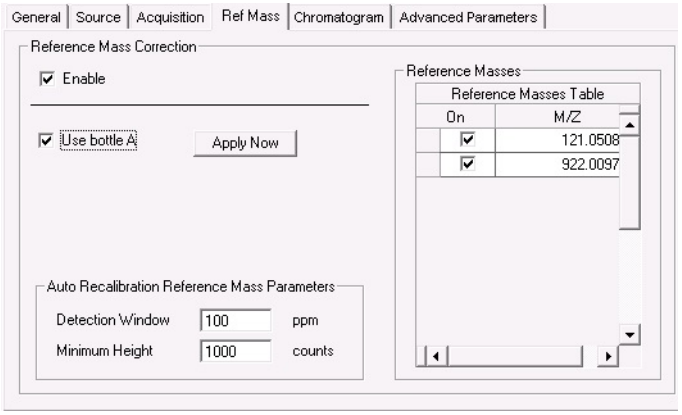


Figure 27. Ref Mass tab for Q-TOF parameters

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

Steps	Detailed Instructions	Comments
7 Enter the chromatogram plot settings as shown in **Figure 28**.	**a** Click the **Chromatogram** tab. **b** Type the values in **Figure 28**.	• These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

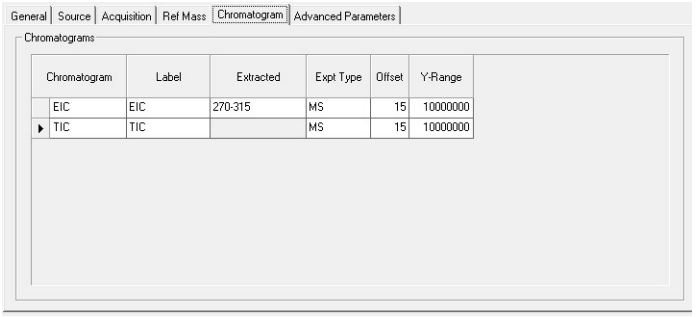


Figure 28. Chromatogram tab for Q-TOF parameters

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** Set **LC Stream** to **Waste**. **c** Right-click anywhere in the **Time segment** section, and click **Add Time Segment**. **d** Type `0.5` minutes. **e** Set **LC Stream** to **MS**.	• You can change a parameter with a (Seg) next to it with a new Time Segment. • See **Figure 24** on page 38 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.
9 Save the method as *iii*_IM-MS_only.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Go to the **methods** folder in your project. **c** Type `iii_IM-MS_only.m` where *iii* are your initials. **d** Click **Save**. **e** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **f** Click **OK**.	• 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

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 29.	**a** Click **Method > Open**. **b** Select *iii*_IM-MS-only.m, and click **Open**. **c** Click the **Q-TOF** tab. **d** Under **Acquisition Mode**, select **IM-QTOF**. **e** Click the Time Segment at 0.5 minutes. **f** Right-click the Time Segment table and click **Delete Time Segment**. **g** Click the General tab. **h** Set **LC Stream** to **MS**. **i** Click the **Acquisition** tab. **j** For **Frame rate**, type `1`. **k** Set **Pulsing Sequence Length** to **Disabled**.	• 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 1 Frames/s is usually sufficient to achieve this.

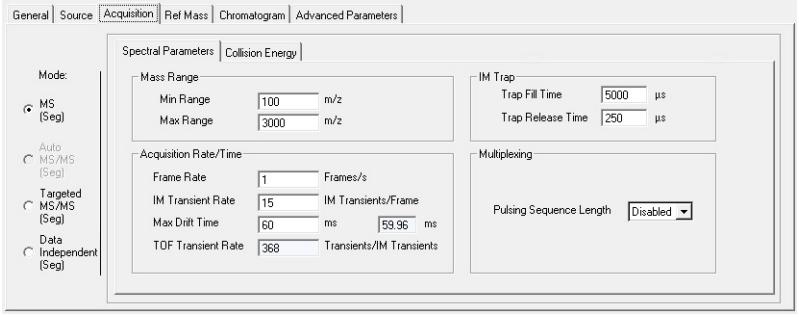


Figure 29. Acquisition Spectral Parameters tab for IM-MS All Ions MS/MS mode

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 30**.	**a** Click the **Collision Energy** tab. **b** Under **Alternating Frames**, click **Frame 2 Fixed** and type `35` for its collision energy.	• Frame 1 automatically is set to have a collision energy of 0 V when you set up alternating frames.

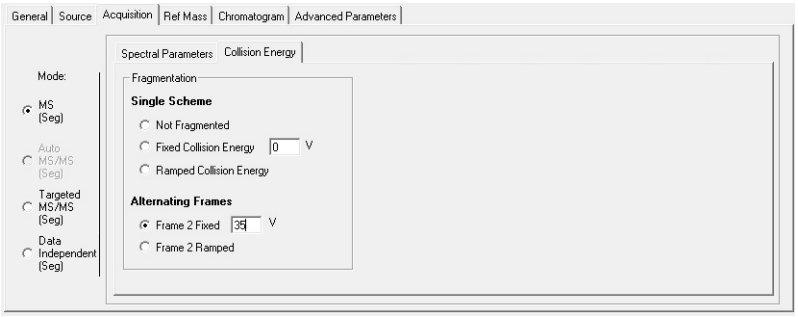


Figure 30. Acquisition Collision Energy tab for IM-MS All Ions mode

3 Save the method as *iii*_IM-MS-All_Ions.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Go to the **methods** folder in your project. **c** Type `iii_IM-MS-All_Ions`, and click **Save**. **d** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **e** Click **OK**.	

4 Optimize IM-MS Q-TOF Methods

Task 1. Set up and run an IM-MS method for Labile Compounds 45
Task 2. Set up IM-MS method for Small Compounds 49

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

For more details on how to use these exercises, see **"Before you begin..."** on page 4.

Before you begin...

This exercise introduces you to the parameters relevant to change for the analyses of different compound classes. 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 on the Manual Tune tab match the different parts of the instrument. Do not manually change these values in the Tune context. Change them in individual methods instead.

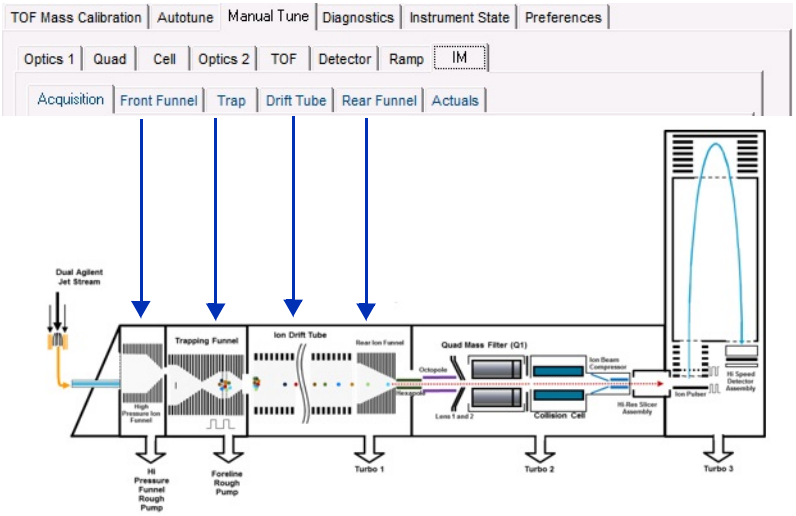


Figure 31. Manual Tune > IM tabs and the part of the instrument they affect

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 p/n 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 Data Acquisition to access the Instrument State tune parameters.	**a** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** On the ribbon in the **Context** group, click **Tune**. **g** Click the **Instrument State** tab.	
2 Select the following tune settings. • Mass Range: Low (1700 *m/z*) • Select to acquire data in Extended Dynamic Range Mode. • Select the IM-QTOF mode.	**a** Set **Mass Range** to **Low (1700 *m/z*)**. **b** Click **Extended Dynamic Range Mode** if not already the default setting. **c** Under **Acquisition Mode**, click **IM-QTOF**. **d** Click **Apply**. **e** Click the **Tune & Calibration** tab. **f** Mark **Positive**. **g** Click **Transmission Tune**. **h** Click **50-750 *m/z***. **i** Mark the **Fragile Ions** check box **j** Click **Start TOF Transmission Tune**.	• You need to click **Apply** to change the settings on the instrument. • If you change the **Instrument Mode,** or the **Mass Range**, you must recalibrate the TOF mass axis. • For a 6560 Ion Mobility Q-TOF, the two **Acquisition Modes** are **IM-QTOF** and **QTOF-Only**.

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

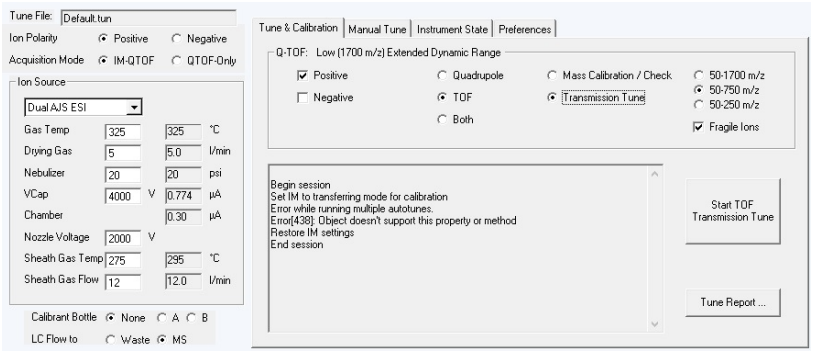
Steps	Detailed Instructions	Comments
		

Figure 32. Transmission tune for labile molecules

3 Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**a** Click the **Instrument State** tab. **b** Click **Save**. **c** On the ribbon in the **Context** group, click **Acquisition**. **d** Click **Yes** in the Instrument State Confirmation message. **e** 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.
4 Open the method for IM-QTOF created in **"Task 2. Set up an IM-MS method"** on page 36.	**a** Click in the Method Editor toolbar. **b** Select ***iii*_IM-MS_only.m**, and click **OK**. **c** Click the **Method Editor** window.	• If the Method Editor window is not visible, either you click **Method** in the Layouts group on the ribbon, or you click Method Editor in the **Windows** group on the ribbon.
5 Save the method as *iii*_IM-MS_labile.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Type `iii_IM-MS_labile.m`, where *iii* are your initials. **c** Click **Save**. **d** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **e** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_labile.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** On the ribbon in the Layouts group, click **Single Sample**. **b** For Sample **Name**, type `100 nM bradykinin`. **c** For Data File **Name**, type `bradykinin01.d`. **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.

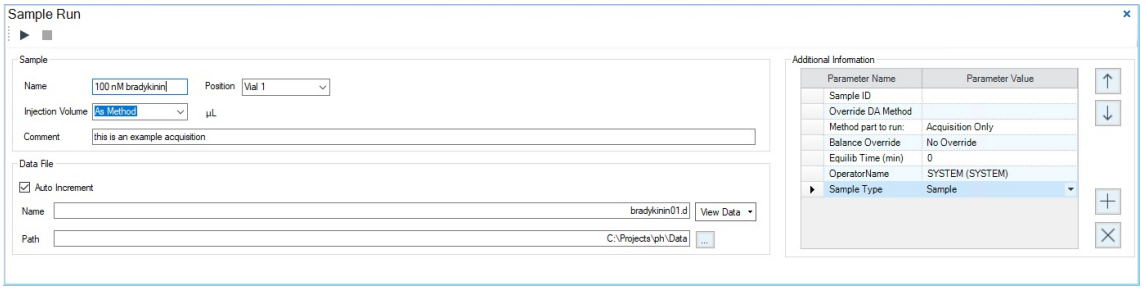


Figure 33. Sample Run window in the main window

7 Start the sample.	• Click **Run** ▶ on the Sample Run toolbar.	• In locked mode, you cannot change the method while the sample is running. Also, you cannot overwrite these data files in the Data Acquisition program. • On the ribbon in the **Settings** group, if **Locked Mode** is highlighted, then **Locked Mode** is on, and you can click to turn it on or off.
8 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.

To change the electric field across any of the above elements use the following parameters which are accessible in the Advanced Parameters tab in the Acquisition context. For positive mode experiments, use a positive value to reduce the voltage gradient.

- Collision cell delta -> collision cell delta delta
- IBC delta - > IBC delta delta
- IM Hex delta -> IM Hex delta delta

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 The information about the LC is the same, but the source conditions are slightly different, using a Nozzle voltage of 0V.

2 Enter sample and data file information for a single sample.

3 Acquire data.

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.

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

Steps	Detailed Instructions	Comments
1 Open Data Acquisition to access the Instrument State tune parameters.	**a** From the desktop, double-click the **OpenLab Control Panel** icon. **b** Click **Instruments** in the left pane of the Control Panel. **c** Select the instrument in the left pane. **d** Select the **Project**. **e** Click **Launch**. **f** On the ribbon in the **Context** group, click **Tune**. **g** Click the **Instrument State** tab.	
2 Select the following tune settings. • Mass Range: Low (1700 *m/z*) • Select to acquire data in Extended Dynamic Range Mode. • Select the IM-QTOF mode.	**a** Set **Mass Range** to **Low (1700 *m/z*)**. **b** If needed, select **Extended Dynamic Range Mode**. **c** Click **IM-QTOF** for the **Acquisition Mode**. **d** Click **Apply**. **e** Click the **Tune & Calibration** tab. **f** Mark **Positive**. **g** Click **Transmission Tune**. **h** Click **50-250 *m/z***. **i** Mark the **Fragile Ions** check box. **j** Click **Start TOF Transmission Tune**.	• You need to click **Apply** to change the settings on the instrument. • If you change the **Instrument Mode,** or the **Mass Range**, you must recalibrate the TOF mass axis. • For a 6560 Ion Mobility Q-TOF, the two **Acquisition Modes** are **IM-QTOF** and **QTOF-Only**.

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

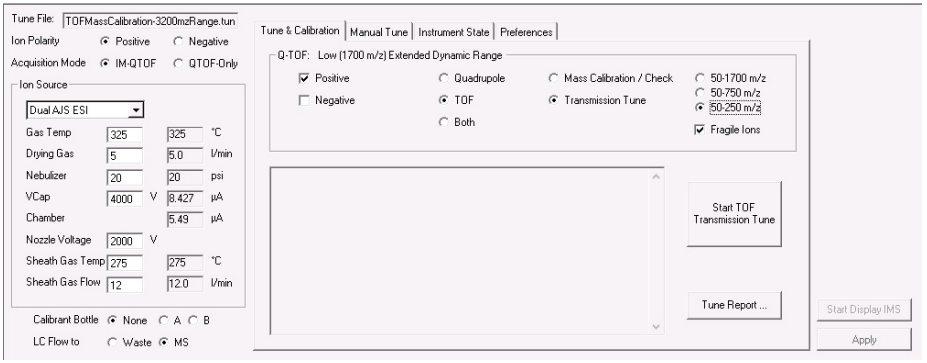
Steps	Detailed Instructions	Comments
		

Figure 34. Transmission tune for small compounds

3 Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**a** Click the **Instrument State** tab. **b** Click **Save**. **c** On the ribbon in the **Context** group, click **Acquisition**. **d** Click **Yes** in the Instrument State Confirmation message. **e** Click **Yes** in the Save Tune File message.	• You can save the tune settings to a new file name for safe-keeping. • You can only click **Save** or **Save As** if you have the Manual Tune permission. • 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.
4 Open the method for IM-QTOF created in **"Task 2. Set up an IM-MS method"** on page 36.	**a** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click  in the Method Editor toolbar. **c** Select ***iii*_IM-MS_only.m**, and click **OK**. **d** Click the **Method Editor** window.	
5 Change the Advanced Parameters:	**a** Click the **Q-TOF** tab. **b** Click the **Advanced Parameters** tab. **c** Clear 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 method is a first "walk-up" method and yields over the selection tab in a significantly reduced number of parameters to be optimized.

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

Steps	Detailed Instructions	Comments
6 Save the method as *iii*_IM-MS_small_molecules.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Go to the **methods** folder in your project. **c** Type `iii_IM-MS_small_molecules.m` where *iii* are your initials. **d** Click **Save**. **e** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **f** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_small_molecules.m**.
7 Enter this sample information: Name: **100 pg amino acid mix** Data file name: **aminoacid01.d**	**a** On the ribbon in the Layouts group, click **Single Sample**. **b** For Sample **Name**, type `100 pg amino acid mix`. **c** For Data File **Name**, type `aminoacid01.d`. **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.

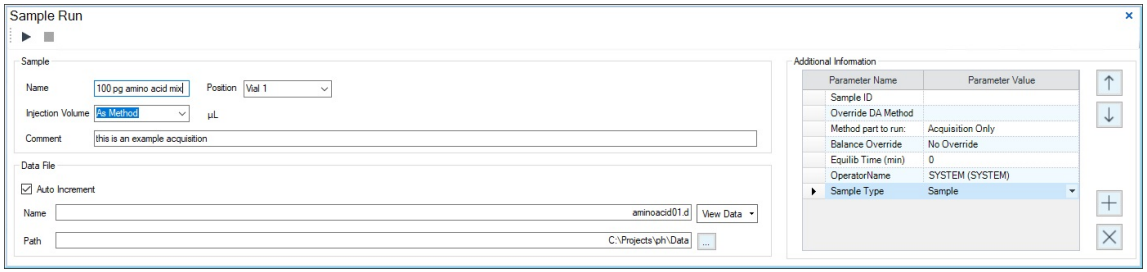


Figure 35. Sample Run window in the main window

8 Start the sample.	• Click **Run** ▶ on the Sample Run toolbar .	• In locked mode, you cannot change the method while the sample is running. Also, you cannot overwrite these data files in the Data Acquisition program.
9 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.

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5 Set up acquisition method for collision cross section calculation

Task 1. Set up LC method to calculate CCS using Single-Field ion mobility method **54**
Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility **63**

This exercise describes two strategies to acquire data for the calculation of collision cross sections. The first task shows an LC-based strategy where a calibration is used to calculate CCS values using a single-field method. The second task creates an infusion-based method where the field strengths are changed during one acquisition (multi-field method).

For more details on how to use these exercises, see **"Before you begin..."** on page 4.

Task 1. Set up LC method to calculate CCS using Single-Field ion mobility 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. Do run the tune mix experiment before and after the LC experiments.

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments
1 Open the method for IM-QTOF created in **"Task 2. Set up an IM-MS method"** on page 36.	**a** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click  in the Method Editor toolbar. **c** 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  in the Method Editor toolbar. **b** Type `iii_SulfaDrug_CCS.m` where *iii* are your initials, and click **Save**. **c** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **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.
3 Edit the method. • Set the Pump Flow Rate to 0.8 • Set the run time to 1.0 • Set Injection Volume to 5 • Set Needle Wash to Standard Wash • Set Draw speed to 100. • Set Eject speed to 400 • Set Injection Path Cleaning to Flush Port for 3 sectiond • Set temperature for column compartment to 40 °C.	**a** If needed, on the Ribbon in the Layouts group, click **Method**. **b** Click the **Quat. Pump** tab. **c** For **Channel A Pump Flow Rate**, type `0.8`. **d** For **Run Time**, type `1.0`. **e** Click the **Multisampler** tab. **f** For **Injection Volume**, type `5`. **g** Set **Needle Wash** to **Standard Wash**. **h** For **Draw speed**, type `100`. **i** For **Eject speed**, type `400`. **j** Set **Injection Path Cleaning** to **Flush Port** for **3** seconds. **k** If available, click the **Column Compartment** tab and type 40 °C for the temperature.	• 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**.

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments
4 Set values on the Q-TOF > General tab.	**a** Click the **Q-TOF** tab. **b** Click the **General** tab. **c** Click **IM-QTOF**. **d** Set **Ion Polarity** to **Positive**. **e** Under **MS**, for **Abs. Threshold** type `200` and for **Rel. Threshold (%)** type `0.01`. **f** Under **MS/MS**, for the **Abs. Threshold** type `5`, and for the **Rel. Threshold (%)** type `0.01`.	
5 Set values on the Q-TOF > Source tab.	**a** Click the **Source** tab. **b** For **Drying Gas**, type `350`. **c** For **Drying Gas Flow**, type `12`. **d** For **Nebulizer**, type `60`. **e** For **Sheath Gas Temp**, type `350`. **f** For **Sheath Gas Flow**, type `11`. **g** For **Capillary**, type `4000.` **h** For **Nozzle Voltage**, type `1000`. **i** For **Fragmentor**, type `365`. **j** For **Oct 1 RF Vpp**, type `750`.	
6 Set values on the Q-TOF > Acquisition tab.	**a** Click the **Acquisition** tab. **b** For **Minimum range**, type `100`. **c** For **Maximum range**, type `1700`. **d** For **Frame Rate**, type `1`. **e** For **Max Drift Time**, type `50`. **f** For **Trap Fill Time**, type `20000`. **g** For **Trap Release Time**, type `150`. **h** Set **Pulsing Sequence Length** to **Disabled**. **i** For all advanced parameters, use the values set from the autotune runs.	• This experiment should be run in 1700 *m/z* mass range.
7 Save the method as *iii*_6560_CCS_IM_SingleF.m, where *iii* are your initials.	**a** Click  in the Method Editor toolbar. **b** Type *`iii`*`_6560_CCS_IM_SingleF.m`, where *iii* are your initials. **c** Click **Save**. **d** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **e** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_6560_CCS_IM_SingleF.m**.
8 Change the Properties tab.	**a** Click the **Properties** tab. **b** Click  next to **Pre Run Script**. The **Select Script** dialog box opens. **c** Set **Script** to **SCP_AcquireCalibrantData**. **d** Click **OK**.	• This script does a run with Calibrant B on.

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments

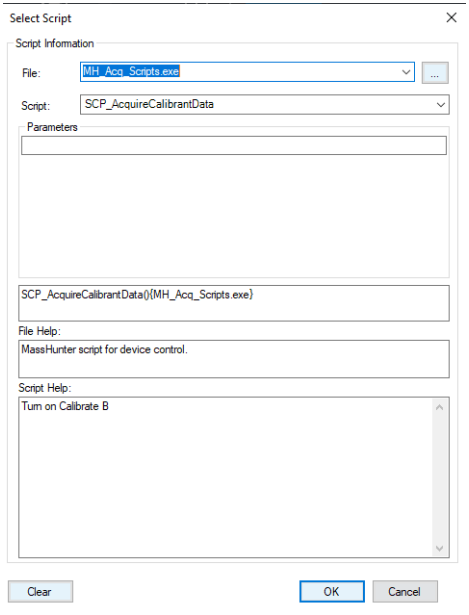


Figure 36. Select Script dialog box

9 Save the method as *iii*_6560_CCS_IM_SingleF_Calib.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Type `iii_6560_CCS_IM_SingleF_Calib.m` where *iii* are your initials **c** Click **Save**. **d** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **e** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_6560_CCS_IM_SingleF_Calib.m**.
10 Set up a worklist that acquires a tune calibrant data file and your sample file.	**a** On the ribbon in the **Layouts** group, click **Worklist**. **b** Add two samples with the following information.	

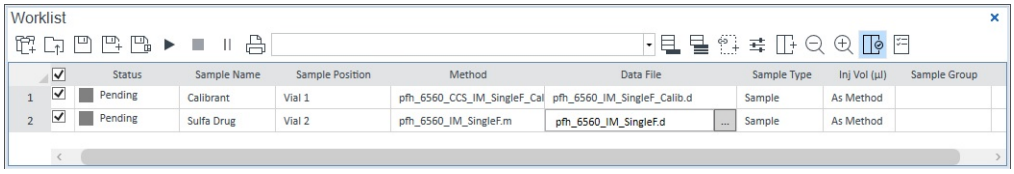


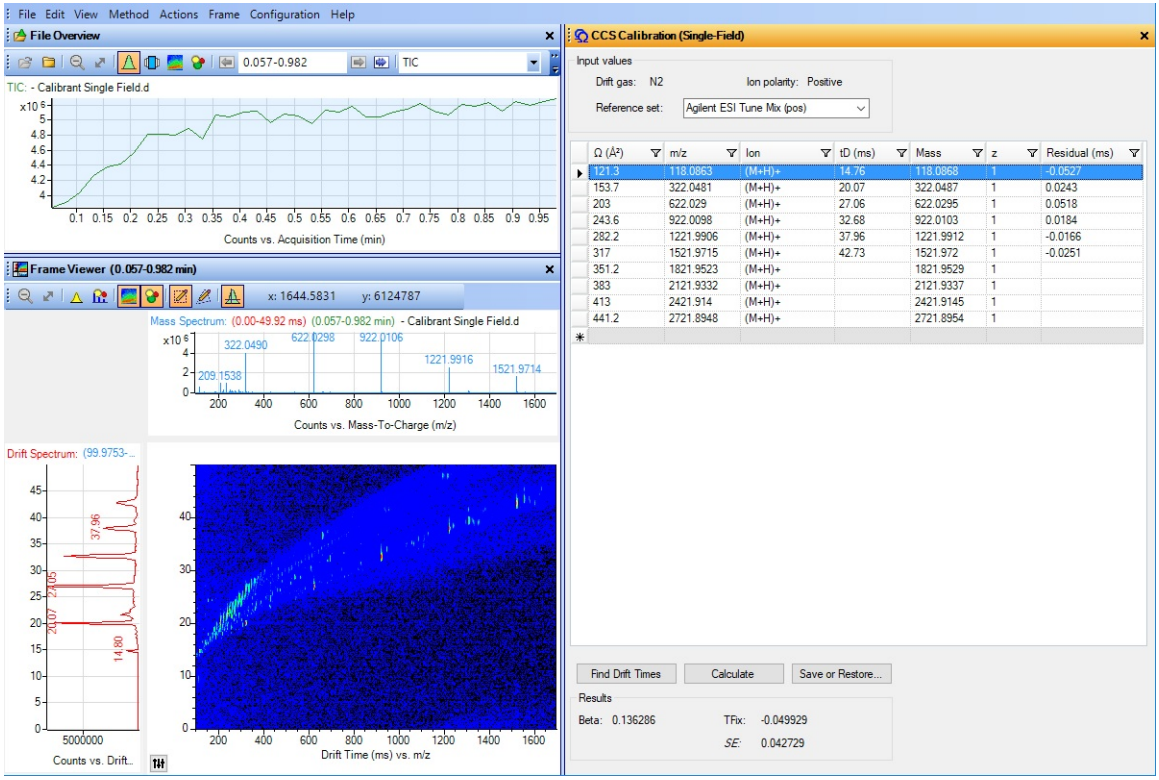
Figure 37. Worklist window with a calibrant and a sample

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments
11 Start the worklist.	• Click **Run** ▶ on the Worklist toolbar.	• You do not need to save the worklist to start it. • Each sample row turns blue as the program begins to acquire data for that worklist row.
12 Examine the data file in the IM-MS Browser program. The example data files Calibrant Single Field.d and Sulfa_Single Field_r001.d are used in this example.	**a** Start the IM-MS Browser program. **b** Open the **Calibrant Single Field.d** data or the file that you just acquired, ***iii*_6560_IM_SingleF_Calib.d**, where *iii* are your initials. **c** Select the entire run time in the File Overview window. **d** Right-click the File Overview window and click **Extract Field**. **e** Click **View > CCS Calibration (Single-Field)**. **f** Set **Reference set** to **Agilent ESI Tune Mix (pos)**. **g** Click **Find Drift Times** in the CCS Calibration (Single-Field) window.	• You can view cross section calculations in the IM-MS Browser program.

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments



Ω (Å²)	m/z	Ion	tD (ms)	Mass	z	Residual (ms)
121.3	118.0863	(M+H)+	14.76	118.0868	1	-0.0527
153.7	322.0481	(M+H)+	20.07	322.0487	1	0.0243
203	622.029	(M+H)+	27.06	622.0295	1	0.0518
243.6	922.0098	(M+H)+	32.68	922.0103	1	0.0184
282.2	1221.9906	(M+H)+	37.96	1221.9912	1	-0.0166
317	1521.9715	(M+H)+	42.73	1521.972	1	-0.0251
351.2	1821.9523	(M+H)+		1821.9529	1	
383	2121.9332	(M+H)+		2121.9337	1	
413	2421.914	(M+H)+		2421.9145	1	
441.2	2721.8948	(M+H)+		2721.8954	1	

Figure 38. IM-MS Browser with Calibrant Single Field.d open

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments
	h Save the CCS (Single-Field) coefficients. Click **Save or Restore**. The **CCS Calibration (Single-Field)** dialog box opens. **i** Click **Save to Multiple Files** and select **Sulfa_Single Field-r001.d**, **Sulfa_Single Field-r002.d**, and **Sulfa_SingleField_r003.d**. You can instead select ***iii*_6560_IM_SingleF.d**. **j** Click **Open**. **k** Click **OK**. **l** Click **File > Close**. **m** In IM-MS Browser, open **Sulfa_Single Field-r001.d.** **n** Click **Method > Find Features (IMFE)**. **o** Select the following parameters and click **Find Features**. **p** Click **View > Feature List**.	• 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. • If you need to remove calibration coefficients from the file, click **Restore Current File.**

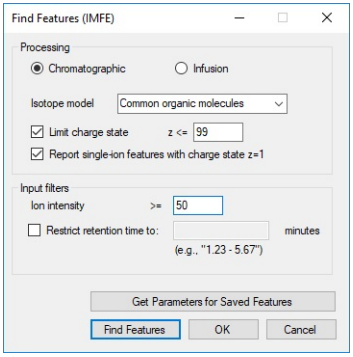


Figure 39. Find Features (IMFE) dialog box (Single Field Ion Mobility)

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments

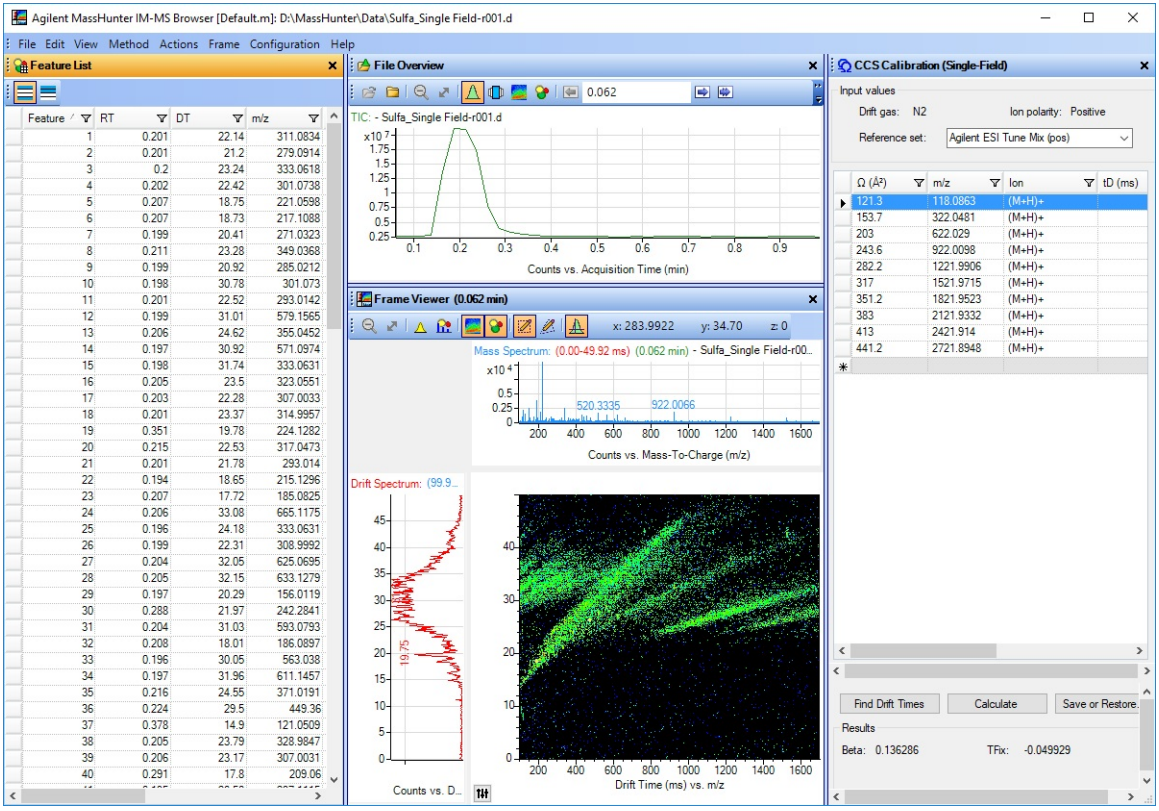
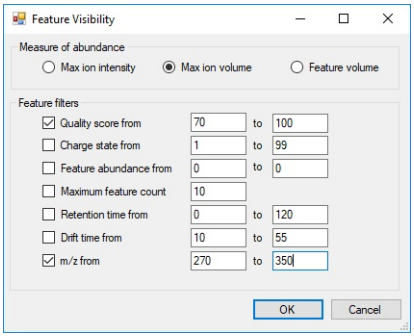


Figure 40. Find Features (IMFE) dialog box (Single Field Ion Mobility)

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments

13 Reduce the number of features.	**a** Click **Methods > Filter Features**.
	b Click **Max ion volume**.
	c Mark the **Quality score from** check box and for the limits, type `70` and `100`.
	d Mark the **m/z from** check box and for the limits, type `270` and `350`.
	e Clear other check boxes.
	f Click **OK**.
	 
	Figure 41. Feature Visibility dialog box (Single Field Ion Mobility)
		• **For the four sulfa drugs, here are the *m/z* values and the CCS (Å^2) values:**
		  • 311.0815 *m/z* -> 170.27 Å^2
		  • 285.0175 *m/z* -> 161.76 Å^2
		  • 279.0902 *m/z* -> 163.74 Å^2
		  • 271.0288 *m/z* -> 158.13 Å^2

Task 1. Set up an LC method to calculate CCS using Single Field method

Steps	Detailed Instructions	Comments

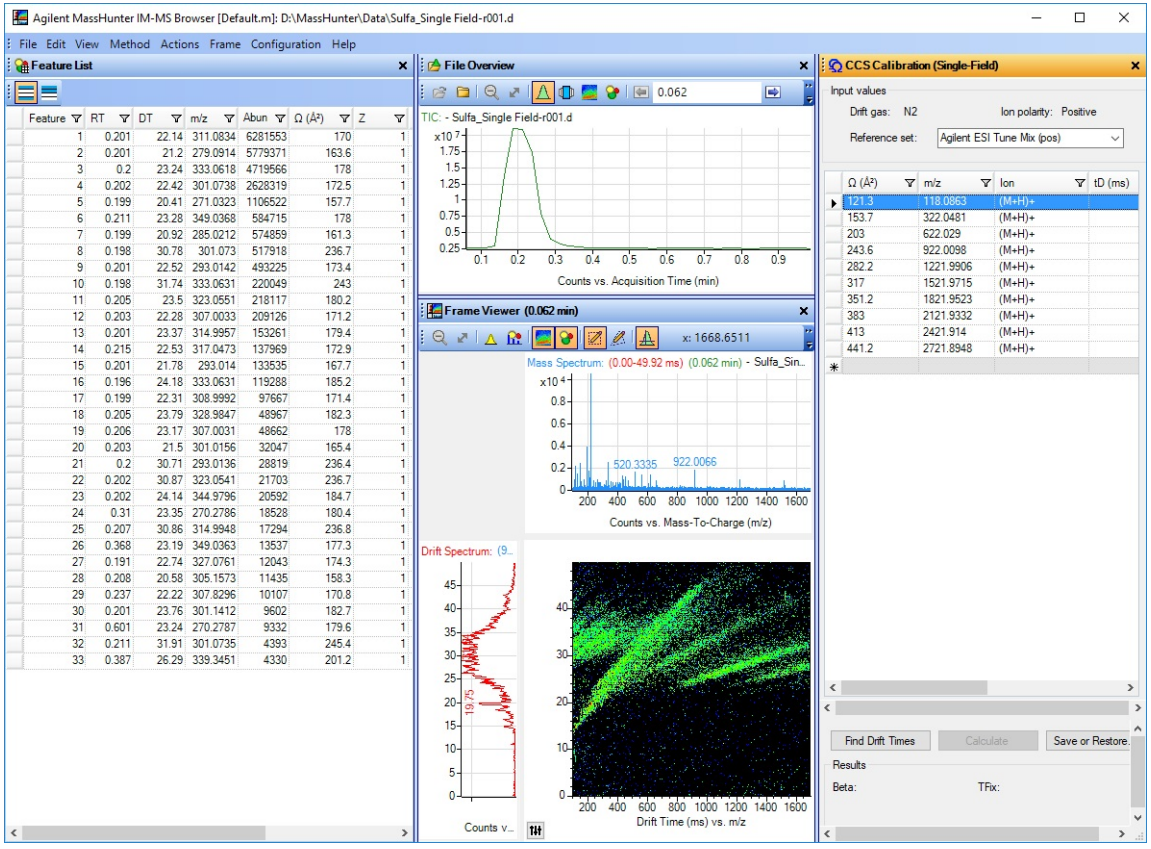


Figure 42. Filtered features in the IM-MS Browser program

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility

This task only applies if the alternate gas kit is installed.

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility

Steps	Detailed Instructions	Comments
1 Open the method ***iii*_6560_CCS_IM_SingleF_Calib.m**, where *iii* are your initials.	**a** Make sure that the Method Editor window is visible. On the ribbon in the Layouts group, click **Method** if the Method Editor window is not visible. **b** Click in the Method Editor toolbar. **c** Select ***iii*_6560_CCS_IM_SingleF_Calib.m**, where *iii* are your initials, and click **OK**. **d** Click the **Method Editor** window.	• This method was developed in **"Task 1. Set up LC method to calculate CCS using Single-Field ion mobility method"** on page 54

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility

Steps	Detailed Instructions	Comments
2 Change parameters in the acquisition method: • Extend the Run Time to 3.5 minutes in the Pump tab. • Extend the Max Drift Time to 70 msec. • Add six Time Segments and change advanced parameters. Change each time segment to increase the Drift Tube Entrance Voltage by 100 for each time segment. The method will have seven time segments.	**a** If needed, click **View > Method Editor** to open the Method Editor window. **b** On the Pump tab, for **Run Time**, type `3.5`. **c** Click the **Q-TOF** tab. **d** Click the **Acquisition** tab. **e** Click **No Limit/As Pump**. **f** For **Max Drift Time**, type `70`. **g** Add seven time segments lasting 30 seconds each. **h** Select the first Time Segment. **i** Click the **Advanced Parameters** tab. **j** For **Drift Tube Entrance Voltage**, mark the **Use Method** check box and set the value to `1074`. **k** For **Drift Tube Exit Voltage**, mark the **Use Method** check box and set the value to `224`. **l** For **Rear Funnel Entrance**, mark the **Use Method** check box and set the value to `217.5`. **m** For **Rear Funnel Exit**, mark the **Use Method** check box and set the value to `45`. **n** Mark the **Selected Items Only** check box. **o** Repeat **step j** through **step m** for each time segment. The only value that changes for each time segment is the **Drift Tube Entrance Voltage**. See the list under Comments.	• 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. • Drift Tube Entrance Voltage for each Time Segment ◦ Time 0 minutes: 1074 ◦ Time 0.5 minutes: 1174 ◦ Time 1.0 minutes: 1274 ◦ Time 1.5 minutes: 1374 ◦ Time 2.0 minutes: 1474 ◦ Time 2.5 minutes: 1574 ◦ Time 3.0 minutes: 1674

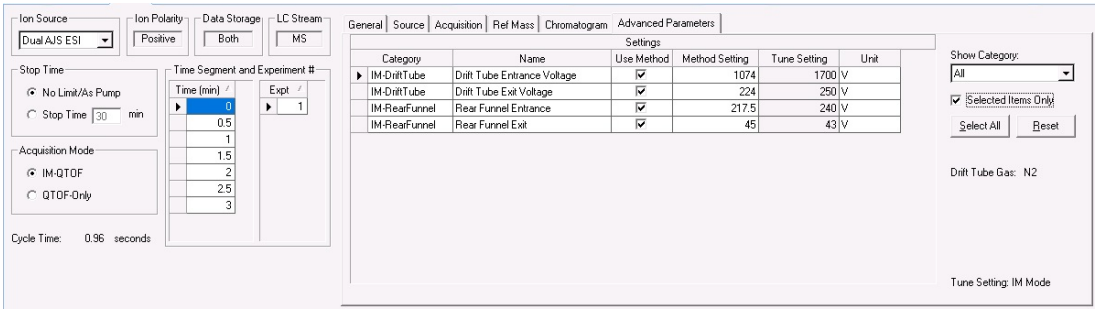


Figure 43. Advanced parameters for the first time segment

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility

Steps	Detailed Instructions	Comments
3 Save the method as *iii*_6560_CCS_IM_SteppedF.m, where *iii* are your initials.	**a** Click in the Method Editor toolbar. **b** Type `iii_6560_CCS_IM_SteppedF.m` where *iii* are your initials and click **Save**. **c** If needed, in the **Reason for Change** dialog box, either enter your own reason or select a reason from the list. **d** Click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_6560_CCS_IM_SteppedF.m**.
4 Enter this sample information: • Name: **Step Field** • Data file name: **TuneMix_SF000x.d**	**a** On the ribbon in the **Layouts** group, click **Single Sample** window. **b** For Sample **Name**, type `Step Field`. **c** For Data File **Name**, type `TuneMix_SF000x.d`. **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.

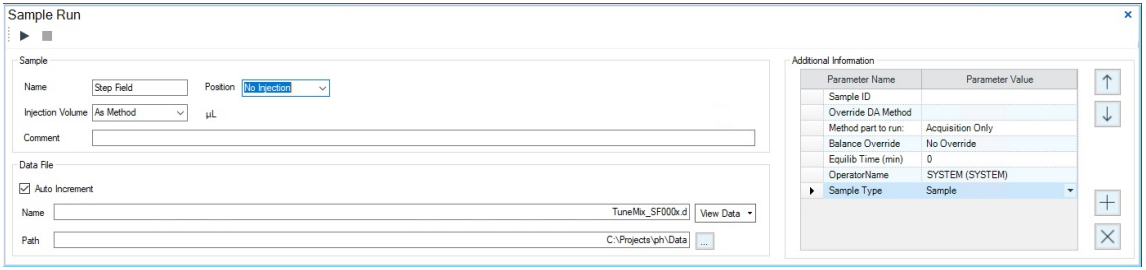


Figure 44. Sample Run window in the main window

5 Start the sample.	• Click **Run** ▶ on the Sample Run toolbar.	• In locked mode, you cannot change the method while the sample is running. Also, you cannot overwrite these data files in the Data Acquisition program.
6 View the data after the run. You can instead use the example files: Tunemix_SF001.d, Tunemix_SF002.d, and Tunemix_SF003.d	**a** Open the data file **Tunemix_SF001.d** in the IM-MS Browser program. **b** In the File Overview window, click .	• You can view cross section calculations in the IM-MS Browser program. • You can navigate the data file using chromatograms, frames, or the file abundance map.

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility

Steps	Detailed Instructions	Comments
	c Highlight the constant potential period in the fourth segment from **1.5** to **2.0** minutes. **d** Right-click and click **Extract Frame**. **e** In the Frame Viewer window, zoom the *m/z* **622** isotopic cluster. **f** Highlight the entire ***m/z*** cluster by clicking the left mouse button and dragging over the region. **g** Right-click that region, and click **Calculate CCS (Multi Field)**. **h** Click **View > CCS Calculator (Multi Field)**. **i** Review the results for the 622 *m/z* ion.	• Make sure the frame is within a single Time Segment.

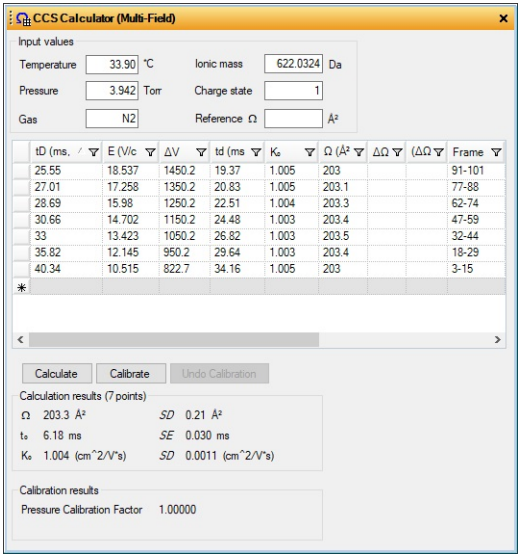


Figure 45. CCS Calculator (Multi-Field) window with ionic mass 622

In This Book

This guide teaches you how to use your MassHunter Data Acquisition for Agilent LC/TOF or LC/Q-TOF system.

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