



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

Familiarization Guide



Notices

Manual Part Number

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Software Revision

This guide is valid for the C.09.00 and 10.0 revisions or later of the Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF program and compatible Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF programs, until superseded.

Software Manufacturing



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Safety Notices

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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

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 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 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

- Agilent MassHunter Workstation has been installed
- LC modules and the 6200 Series TOF or 6500 Series Q-TOF LC/MS have been configured
- The system has been turned on
- The performance has been verified, and.

If these actions have not yet been done, see 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
- Zorbax, Extend-C18 2.1mm x 50mm, 1.8um, 80Å, p/n 727700-902
- 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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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.	<ol style="list-style-type: none"> Click the Agilent Data Acquisition icon. On the main toolbar, set Context to Tune. Click the Instrument State tab. 	
2 Select the following data collection settings. <ul style="list-style-type: none"> • Mass Range: Standard (3200 m/z) • Select to acquire data in High Resolution Mode. 	<ol style="list-style-type: none"> Set Mass Range to Standard (3200 m/z). If needed, select High Resolution (4 GHz, High Res Mode). (optional) Set Fast Polarity Switching to Disabled. For 6560, set Acquisition Mode to QTOF-Only. Click Apply. If you changed the Mass Range, tune the instrument. Recalibrate the TOF mass axis. 	<ul style="list-style-type: none"> • 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 Click **Save**.
 - b Set **Context** to **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	<p>Open Data Acquisition to access the window for editing methods.</p> <p>a Click the Agilent Data Acquisition icon.</p> <p>b On the main toolbar, set Context to Acquisition.</p> <p>c Make sure that the Method Editor window is visible. Click View > Method Editor if the Method Editor window is not visible.</p> <p>d If you have a 6560 Ion Mobility Q-TOF, click QTOF-Only for the Acquisition Mode.</p>	<ul style="list-style-type: none"> The MassHunter Data Acquisition window appears containing the Method Editor window. See Figure 3. Your display will be different if the AJ5 source is not installed on your system.

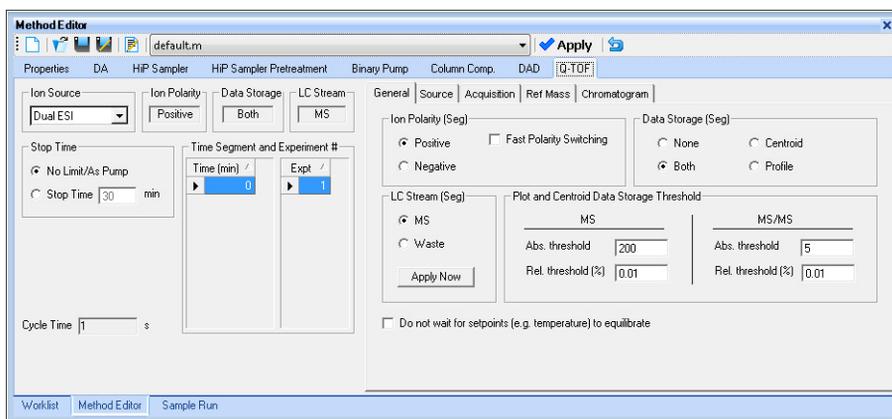


Figure 3. Method Editor window in the MassHunter Data Acquisition program

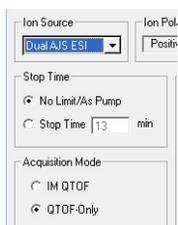


Figure 4. For a 6560 Ion Mobility Q-TOF, select QTOF-Only for the Acquisition Mode

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.	<p>a In the Method Editor window, click each LC module tab to set parameter values.</p> <p>b Set the LC parameters listed in Table 1.</p>	<ul style="list-style-type: none"> 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	<p>For TOF and Q-TOF parameters, make sure the General tab is displayed.</p> <ul style="list-style-type: none"> Enter the parameters as shown in Figure 5, if necessary. 	<p>a Click the TOF or Q-TOF tab.</p> <p>b On the TOF or Q-TOF tab, make sure the General tab is displayed.</p> <p>c Type the parameters as shown in Figure 5. (These are the default parameters.)</p>	<ul style="list-style-type: none"> Of course, the MS/MS fields do not appear on the TOF General tab.

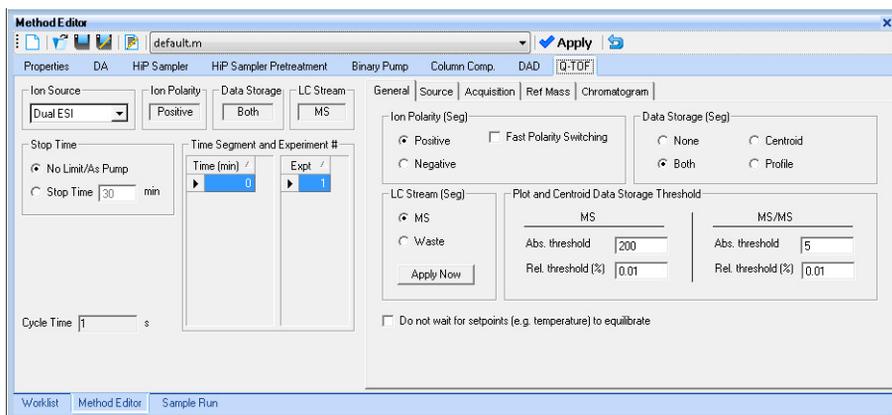


Figure 5. General tab for Q-TOF parameters for a 6530 Q-TOF

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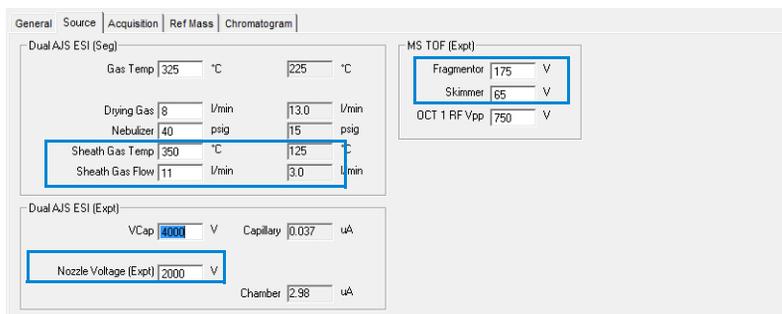


Figure 6. Source tab for Q-TOF parameters

These options appear on a 6530 Q-TOF and a 6230 TOF mass spectrometer equipped with the AJS source.

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

1

Set up acquisition methods

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

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 .	<p>a Click the Acquisition tab.</p> <p>For the TOF, skip to step c.</p> <p>b Click MS as the Mode.</p> <p>c Type the TOF Spectra parameters as shown in Figure 7.</p>

The screenshot shows the 'Acquisition' tab with the following settings:

- Mode:** MS (Seg) (selected)
- Auto MS/MS (Seg):** (unselected)
- Targeted MS/MS (Seg):** (unselected)
- Spectral Parameters:**
 - Min Range: 100 m/z
 - Max Range: 3000 m/z
- Acquisition Rate/Time:**
 - Rate: 1 spectra/s
 - Time: 1000 ms/spectrum
 - Transients/spectrum: 3691

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 .	<p>a Click the Ref Mass tab.</p> <p>b Type the parameters as shown in Figure 8.</p>
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The screenshot shows the 'Ref Mass' tab with the following settings:

- Reference Mass Correction:**
 - Enable
 - Use bottle A (with 'Apply Now' button)
- Auto Recalibration Reference Mass Parameters:**
 - Detection Window: 100 ppm
 - Minimum Height: 1000 counts
- Reference Masses:**

On	M/Z
<input checked="" type="checkbox"/>	121.0508
<input checked="" type="checkbox"/>	922.0097

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

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

Steps	Detailed Instructions	Comments
7	<p>Enter the chromatogram plot settings as shown in Figure 9.</p> <p>a Click the Chromatogram tab. b Type the values in Figure 9.</p>	<ul style="list-style-type: none"> These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

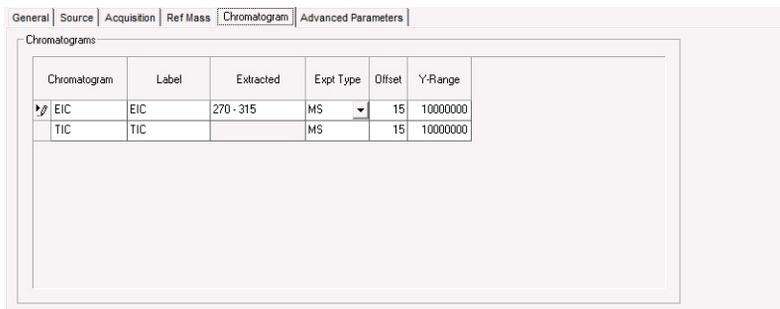


Figure 9. Chromatogram tab for TOF or Q-TOF parameters

8	<p>Set up to change MS parameters during run:</p> <ul style="list-style-type: none"> 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. 	<p>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.</p>	<ul style="list-style-type: none"> You can change a parameter with a (Seg) next to it with a new Time Segment. You can change a parameter with an (Expt.) next to it with 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	<p>Save the method as <i>iii</i>MS-only.m, where <i>iii</i> are your initials.</p>	<p>a Click Method > Save As. b Go to the MassHunter\methods folder. c Type <i>iii</i>MS-only.m where <i>iii</i> are your initials. d Click Save.</p>	<ul style="list-style-type: none"> 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
<p>1 Using the <i>iiiMS-only.m</i> method for the Q-TOF, change to targeted MS/MS mode and enter the spectral parameters below, if necessary.</p> <ul style="list-style-type: none"> • If the <i>iiiMS-only.m</i> method is still displayed, begin with step c. • Delete the 0.5 min Time Segment. • Enter the parameters as shown in Figure 10. 	<p>a Click Method > Open.</p> <p>b Select <i>iiiMS-only.m</i>, and click Open.</p> <p>c Click the Q-TOF tab.</p> <p>d Select the 0.5 minute Time Segment.</p> <p>e Right-click the selected Time Segment and click Delete Time Segment.</p> <p>f Click the Acquisition tab.</p> <p>g Click Targeted MS/MS (Seg) as the Mode.</p> <p>h Type the spectral parameters below.</p>	<ul style="list-style-type: none"> • The LC, General, Source, Ref Mass and Chromatogram parameters remain the same as in <i>iiiMS-only.m</i> for this method.

The screenshot shows the 'Acquisition Spectral Parameters' tab in a software interface. On the left, the 'Mode' is set to 'Targeted MS/MS (Seg)'. The main area is divided into two columns: 'MS' and 'MS/MS'. Each column has a 'Mass Range' section with 'Min Range' and 'Max Range' input fields (both set to 50 and 1000 m/z respectively) and an 'Acquisition Rate/Time' section with 'Rate' (3 spectra/s), 'Time' (333.3 ms/spectrum), and 'Transients/spectrum' (1233 for MS, 1196 for MS/MS) fields. A checkbox labeled 'Max Time between MS1 Spectra' is checked, with a value of 5 s.

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. <ul style="list-style-type: none"> Enter the parameters as shown in Figure 11. 	<p>a Click the Collision Energy tab.</p> <p>b Click Use Fixed Collision Energy.</p> <p>c Type 35 .</p>	<ul style="list-style-type: none"> 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.

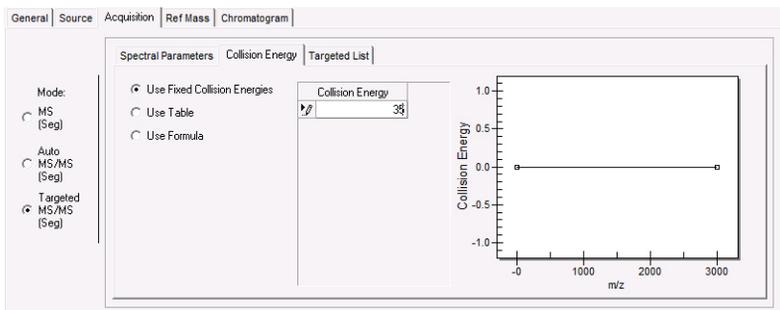


Figure 11. Acquisition Collision Energy tab for targeted MS/MS mode

3 Set up a targeted list of precursor ions so the resulting chromatogram shows peaks for only these ions. <ul style="list-style-type: none"> 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. 	<p>a Click the Targeted List tab.</p> <p>b Right-click the table and click Add on the shortcut menu.</p> <p>c Fill out the information for the 279.09102 ion.</p> <p>d Repeat step b and step c for the 311.08085 ion, the 271.0317 ion, and the 285.0209 ion.</p>	<ul style="list-style-type: none"> 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.
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Task 3. Set up a targeted MS/MS method (Q-TOF) (continued)

Steps	Detailed Instructions	Comments

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

- 4 Save the method as *iii*targetedMSMS.m, where *iii* are your initials.
- a Click **Method > Save As**.
- b Type *iii*targetedMSMS, and click **Save**.
- Be sure to use the folder **\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 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	<p>Using the <i>iii</i>targetedMSMS.m method for the MS Q-TOF, change to auto MS/MS mode and enter the spectral parameters below, if necessary.</p> <ul style="list-style-type: none"> If the <i>iii</i>targetedMSMS.m method is still displayed, begin with step c. Enter the parameters as shown in Figure 13. 	<p>a Click Method > Open.</p> <p>b Select <i>iii</i>targetedMSMS.m, and click Open.</p> <p>c Click the Q-TOF tab.</p> <p>d Click the Acquisition tab.</p> <p>e Click Auto MS/MS(Seg) as the Mode.</p> <p>f Type the spectral parameters shown below.</p>	<ul style="list-style-type: none"> For this method, the LC, General, Source, Ref Mass and Chromatogram parameters will remain the same as in MS-only.m.

The screenshot shows the 'Acquisition Spectral Parameters' tab in a software interface. On the left, the 'Mode' is set to 'Auto MS/MS (Seg)'. The main area is divided into two sections: 'MS' and 'MS/MS'. The 'MS/MS' section is active and contains the following parameters:

- Mass Range:** Min Range: 100 m/z, Max Range: 1000 m/z
- Acquisition Rate/Time:** Rate: 3 spectra/s, Time: 333.3 ms/spectrum, Transients/spectrum: 3199
- Isolation Width:** Medium (~4 m/z)

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	<p>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.</p> <ul style="list-style-type: none"> Use 5 for the slope and 2.5 for the offset. 	<p>a Click the Collision Energy tab.</p> <p>b Click Use Formula.</p> <p>c For the Slope, type 5.</p> <p>d For the Offset, type 2.5.</p>	<ul style="list-style-type: none"> 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.

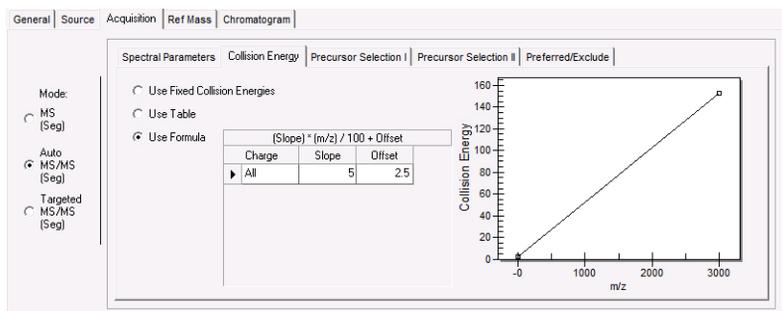


Figure 14. Acquisition Collision Energy tab for Auto MS/MS mode

3	<p>Set 3 as the maximum number of precursor ions per cycle that the program will select in order of decreasing abundance.</p> <ul style="list-style-type: none"> Enter the other parameters in Precursor Threshold. 	<p>a Click the Precursor Selection I tab.</p> <p>b For Max Precursor Per Cycle, type 3.</p> <p>c In the Precursor Threshold group, set the remaining parameters as needed.</p>	<ul style="list-style-type: none"> 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.
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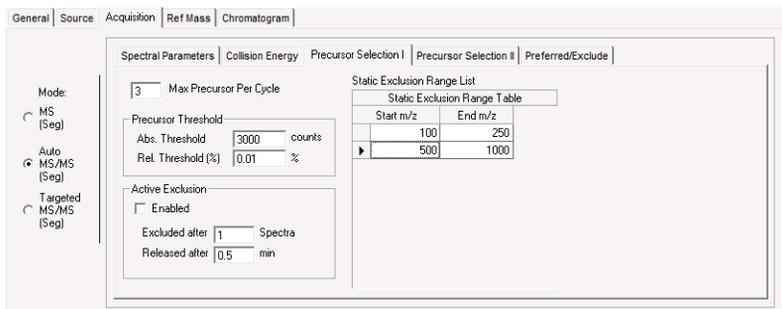
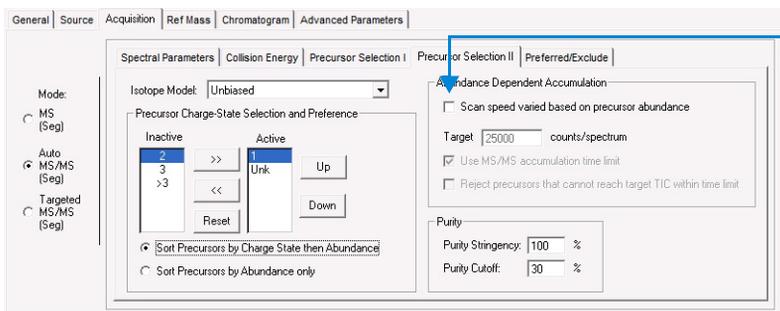


Figure 15. Acquisition Precursor Selection I tab for Auto MS/MS mode

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

Steps	Detailed Instructions	Comments
4	<p>Change the parameters to see the masses of charge 2 first, then masses of charge 1 and then masses of unknown charge.</p> <p>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 <<.</p>	<ul style="list-style-type: none"> 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	<p>Set up to monitor the 279.09102 precursor ion as a preferred ion and exclude the 311.08085 ion.</p> <ul style="list-style-type: none"> Use the other entries in Figure 17. 	<p>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.</p>	<ul style="list-style-type: none"> For this example, you do not need to mark the Scan speed varied based on precursor abundance check box.
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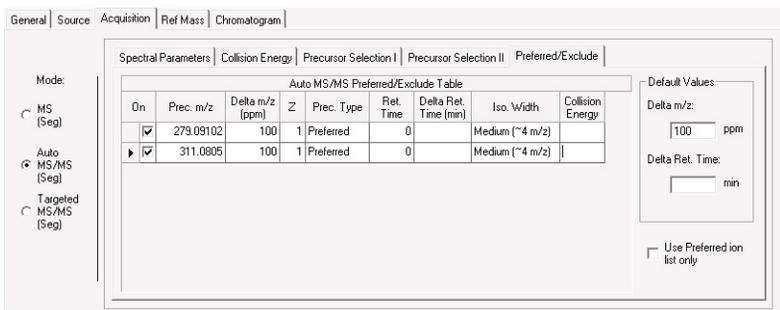


Figure 17. Acquisition Preferred/Exclude tab for Auto MS/MS mode

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

Steps	Detailed Instructions	Comments
6 Save the method as <i>iii</i> autoMSMS.m, where <i>iii</i> are your initials.	<p>a Click Method > Save As.</p> <p>b Type <i>iii</i>autoMSMS, and click Save.</p>	<ul style="list-style-type: none">• Be sure use the folder \MassHunter\methods.

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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 26
Task 3. Set up and run a worklist to optimize parameters 30

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.

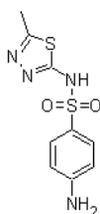
For more details on how to use these exercises, see **“Before you begin...”** on page 4.

Before you begin...

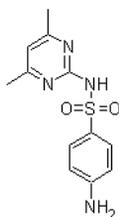
For this exercise you analyze a mixture of four sulfonamide compounds. This section gives instructions on how to prepare the demo sample.

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

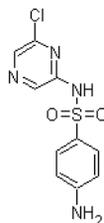
Name	Formula	Ion	m/z
sulfamethizole	C ₉ H ₁₀ N ₄ O ₂ S ₂	(M+H) ⁺	271.03179
sulfamethazine	C ₁₂ H ₁₄ N ₄ O ₂ S	(M+H) ⁺	279.09102
sulfachlorpyridazine	C ₁₀ H ₉ ClN ₄ O ₂ S	(M+H) ⁺	285.02075
sulfadimethoxine	C ₁₂ H ₁₄ N ₄ O ₄ S	(M+H) ⁺	311.08085



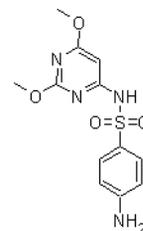
Sulfamethizole



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 the data sample.

Task 1. Set up and run a single sample

Steps	Detailed Instructions	Comments
1	<p>Open one of the three methods you created in Exercise 1, and enter this sample information:</p> <ul style="list-style-type: none"> Name: <i>same as method</i> Position of sample in your sampler Data file name: <i>same as method.d</i> 	<p>a Click Method > Open.</p> <p>b Select <i>one of the three methods</i>, and click OK.</p> <p>c Click the Sample Run window.</p> <ul style="list-style-type: none"> The system stores the custom information with the data file.

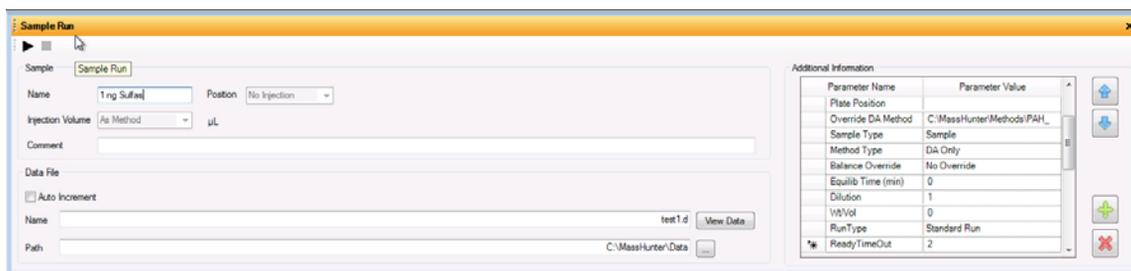


Figure 18. Sample Run window in the main window

	<p>d For Sample Name, type 1 ng Sulfa.s.</p> <p>e For Data File Name, type test1.d.</p>	<p>You can type any number at the end of the Name field. This value is incremented for each new data file.</p>
2	<p>Start the sample.</p> <ul style="list-style-type: none"> Click Run on the Sample Run toolbar or Run Sample on the main toolbar. 	<ul style="list-style-type: none"> 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. The Lock button on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
3	<p>View the data after the run.</p> <ul style="list-style-type: none"> After the run is complete, click View Data in the Sample Run window. 	<ul style="list-style-type: none"> 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
<p>1 Add three samples to the worklist - Sulfa 1, Sulfa 2, Sulfa 3 - with the following information:</p> <ul style="list-style-type: none"> Data file: Sulfa 1-3.d to be saved to the folder \MassHunter\Data\YourName. Acquisition method: <i>any of the three you created in Exercise 1</i> Injection volume: 1 Sample position: any three positions convenient for your sampler 	<p>a Right-click the upper-left-hand corner of the worklist spreadsheet.</p> <p>b Click Add Multiple Samples. The Add Multiple Samples dialog box opens.</p> <p>c For Sample Name, type <i>Su1fa</i>. For Data File Name, type <i>Su1fa</i>.</p> <p>d Make sure that the Append Counter check boxes are marked and that all Suffix Counter boxes contain 1 for the Sample and the Data File names.</p> <p>e Change the folder path for the data files to \MassHunter\Data\YourName.</p> <p>f Select the acquisition method from Exercise 1.</p> <p>g For Injection Volume, type 1.</p>	<ul style="list-style-type: none"> 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**

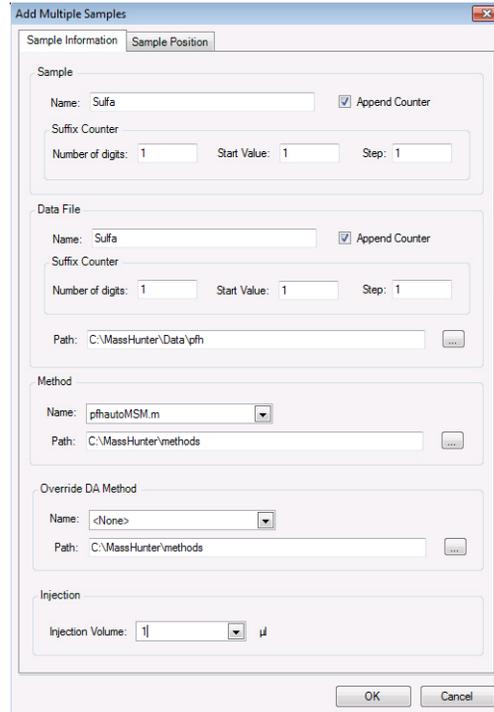


Figure 19. Add Multiple Samples dialog box

- h** Click the **Sample Position** tab.
- i** Set **Autosampler** to **None**.
- 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 program recognizes their values even though they do not appear in the worklist.

	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment	Sample Group	Info.
1	Sulfa1	Vial 8	pfhautoMSM.m	C:\MassHunter\Data\pht\Sulfa1.d	Sample				
2	Sulfa2	Vial 9	pfhautoMSM.m	C:\MassHunter\Data\pht\Sulfa2.d	Sample				
3	Sulfa3	Vial 10	pfhautoMSM.m	C:\MassHunter\Data\pht\Sulfa3.d	Sample				

Figure 20. Worklist with three samples

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

Steps	Detailed Instructions	Comments
3 Save the worklist as <i>iiiesdemo</i> .	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.	<p>a Right-click the upper-left-hand cell of the worklist spreadsheet.</p> <p>b Select Worklist Run Parameters.</p> <p>c Set Part of method to Acquisition Only.</p> <p>d Change the directory path for the data files to MassHunter\Data\YourName.</p>	<ul style="list-style-type: none"> You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.

Figure 21. Worklist Run Parameters dialog box

e Click **OK**.

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

Steps	Detailed Instructions	Comments
<p>5 Start the worklist.</p> <p>Hint: you must mark the check box at the beginning of each row to run the sample in the row.</p>	<ul style="list-style-type: none"> Click Run  on the Worklist toolbar or Run Worklist  on the main toolbar. 	<ul style="list-style-type: none"> 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. The Lock button  on the main toolbar indicates that 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
<p>1 Add four samples to the worklist - Frag 1, Frag 2, Frag 3, Frag 4 - with the following sample information:</p> <ul style="list-style-type: none"> • 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: <i>iiims-only.m</i> • Injection volume: 1 	<p>a Right-click the upper-left-hand corner of the worklist.</p> <p>b Click Add Multiple Samples.</p> <p>c For Sample Name, type <i>Frag</i> . For Data File Name, type <i>Frag</i>.</p> <p>d Make sure that the Append Counter check boxes are marked and that all Suffix Counter fields contain a 1.</p> <p>e Change the folder for the data files to \MassHunter\Data\YourName.</p> <p>f Select the <i>iiims-only.m</i> acquisition method.</p> <p>g For Injection Volume , type 1.</p> <p>h Click the Sample Position tab.</p> <p>i Set Autosampler to None.</p> <p>j For the Number of Samples, type 4.</p> <p>k Click OK.</p>	<ul style="list-style-type: none"> • Click Worklist > New to create a new worklist.
<p>2 Hide the following columns:</p> <ul style="list-style-type: none"> • Sample Type • Level Name • Comment 	<p>a Right-click the upper-left-hand corner of the worklist spreadsheet.</p> <p>b Click Show/Hide/Order Columns.</p> <p>c Clear the check boxes for DA Method, Sample Type, Level Name and Comment, and click OK.</p>	<ul style="list-style-type: none"> • You are hiding these columns, not deleting them. The program recognizes their values even though they do not appear in the worklist.
<p>3 For all four samples, add a column for the fragmentor parameter, and enter these values:</p> <ul style="list-style-type: none"> • Frag 1: 225 • Frag 2: 200 • Frag 3: 175 • Frag 4: 150 	<p>a Right-click the upper-left-hand corner of the worklist spreadsheet.</p> <p>b Click Add Column(s).</p> <p>c Select MS Parameter.</p> <p>d Select Fragmentor, and click >.</p> <p>e Click OK.</p> <p>f Type the values into the column.</p>	
<p>4 Save the worklist as Fragwklst.</p>	<p>g Click Worklist > Save As.</p> <p>h Type <i>Fragwklst</i>, and click Save.</p>	<ul style="list-style-type: none"> • Save the Fragwklst file into your own folder.

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

Steps	Detailed Instructions	Comments
5 Make sure that the worklist is set to run only data acquisition.	<ul style="list-style-type: none"> a Right-click the upper-left-hand cell of the worklist spreadsheet. b Click Worklist Run Parameters. c Set Part of method to run to Acquisition Only. d Change the folder for the data files to \MassHunter\Data\YourName. e Click OK. 	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • Click Run ▶ on the Worklist toolbar or Run Worklist  on the main toolbar. 	

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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 42

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.	<ol style="list-style-type: none"> Click the Agilent Data Acquisition icon. Set Context on the main toolbar to Tune. Click the Instrument State tab. 	
2 Select the following data collection settings. <ul style="list-style-type: none"> Mass Range: Standard (3200 m/z) Select to acquire data in Extended Dynamic Range Mode. Select the IM-QTOF mode. 	<ol style="list-style-type: none"> Set Mass Range to Standard (3200 m/z). Click Extended Dynamic Range Mode if not already the default setting. Click IM-QTOF for the Acquisition Mode. Click Apply. If you changed the Mass Range, tune the instrument. Recalibrate the TOF mass axis. 	<ul style="list-style-type: none"> 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 Ox8025). 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.

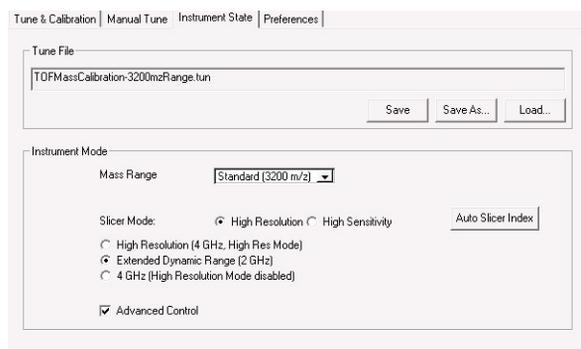


Figure 22. Instrument State tab for a 6560 Ion Mobility Q-TOF instrument

Task 1. Configure the instrument for data collection

Steps	Detailed Instructions	Comments
3 Save the new settings to the tune file (<i>Autotune.tun</i>) and return to Acquisition.	a Click Save . b Set Context to Acquisition . c Click Yes in the Instrument State Confirmation message. d Click Yes in the Save Tune File message.	<ul style="list-style-type: none"> 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 <i>Autotune.tun</i> 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	<p>Open Data Acquisition to access the window for editing methods.</p> <p>a Click the Agilent Data Acquisition icon.</p> <p>b Set Context to Tune.</p> <p>c If the Method Editor window is not visible, click View > Method Editor.</p> <p>d Set Acquisition Mode to IM-QTOF.</p>	<ul style="list-style-type: none"> 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.

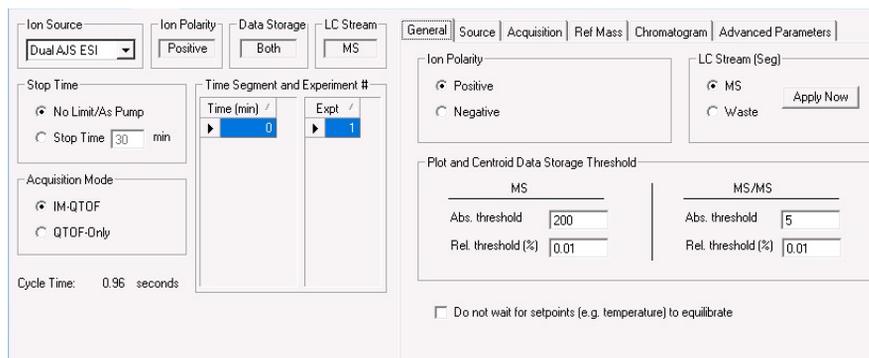


Figure 23. Method Editor window for a 6560 Ion Mobility Q-TOF in the Data Acquisition program

2	<p>Enter LC parameters appropriate for sulfa drug mix.</p> <p>See Table 2.</p>	<p>e In the Method Editor window, click each LC module tab to set parameter values.</p> <p>f Enter LC parameters listed in Table 2.</p>	<ul style="list-style-type: none"> 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 μ L/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 μ L
• 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. <ul style="list-style-type: none"> Enter the parameters as shown in Figure 24, if necessary. 	<ul style="list-style-type: none"> The MS/MS parameters reflect the threshold for All Ions MS/MS experiments.

The screenshot shows the 'General' tab of the instrument's software interface. Key settings include:

- Ion Source:** Dual AJS ESI
- Ion Polarity:** Positive
- Data Storage:** Both
- LC Stream:** MS
- Acquisition Mode:** IM-QTOF
- MS/MS Parameters:**
 - Abs. threshold: 200
 - Rel. threshold (%): 0.01

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.	<ul style="list-style-type: none"> The name of the selected Ion Source is shown in this tab.
---	--------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------

The screenshot shows the 'Source' tab with the following parameters:

- Dual AJS ESI (Seg):**
 - Gas Temp: 300 °C
 - Drying Gas: 8 l/min
 - Nebulizer: 35 psi
 - Sheath Gas Temp: 350 °C
 - Sheath Gas Flow: 11 l/min
- MS TOF (E-apt):**
 - Fragmentor: 400 V
 - Oct 1 RF Vpp: 750 V
- Dual AJS ESI (E-apt):**
 - VCap: 3500 V
 - Capillary: 6.845 µA
 - Nozzle Voltage (E-apt): 1000 V
 - Chamber: 6.27 µA

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

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

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

Steps	Detailed Instructions	Comments
5	<p>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.</p> <p>a Set Context to Tune. b Click Manual Tune > IM > Pressure & Actuals. c Make sure that source temperature is stable at the temperature indicated in the method. d 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.</p>	<ul style="list-style-type: none"> If the Manual Tune tab is not visible, then on the Instrument State tab, click Advanced Control.
6	<p>Enter the acquisition spectral parameters for MS mode as shown in Figure 26.</p> <p>a Set Context to Acquisition. b Click the Acquisition tab. c Set Mode to MS. d Type the IM-MS Spectra parameters as in Figure 26.</p>	<ul style="list-style-type: none"> 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.

The screenshot shows the 'Acquisition' tab in the software interface. On the left, the 'Mode' is set to 'MS (Seg)'. The main area is divided into several sections:

- Mass Range:** Min Range is 100 m/z, Max Range is 3000 m/z.
- Acquisition Rate/Time:** Frame Rate is 1 Frames/s, IM Transient Rate is 16 IM Transients/Frame, Max Drift Time is 60 ms (with a secondary value of 60.06 ms), and TOF Transient Rate is 366 Transients/IM Transients.
- IM Trap:** Trap Fill Time is 5000 μs, Trap Release Time is 250 μs.
- Multiplexing:** Pulsing Sequence Length is set to 'Disabled'.

Figure 26. 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 27 .	<p>a Click the Ref Mass tab.</p> <p>b Type the parameters as shown in Figure 27.</p>	<ul style="list-style-type: none"> 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.

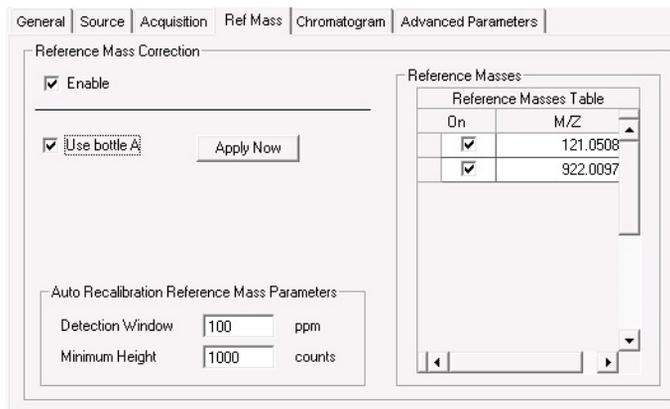


Figure 27. Ref Mass tab for Q-TOF parameters

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

Steps	Detailed Instructions	Comments
8	Enter the chromatogram plot settings as shown in Figure 28 .	<ul style="list-style-type: none"> • These settings show that the base peak chromatogram will be displayed in the Real-time Plot.

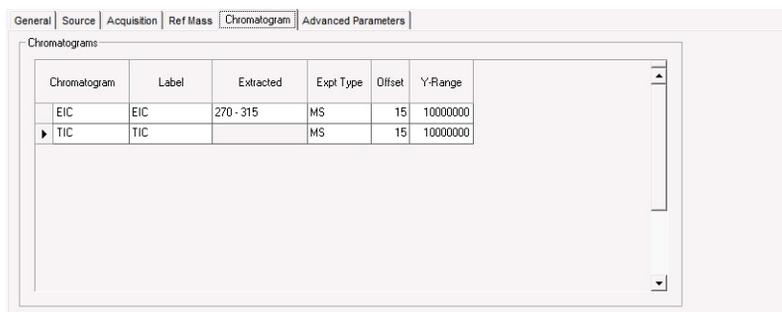


Figure 28. Chromatogram tab for Q-TOF parameters

9	Set up to change MS parameters during run: <ul style="list-style-type: none"> • 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. 	<ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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.
10	Save the method as <i>iii</i> _IM-MS_only.m, where <i>iii</i> are your initials.	<ul style="list-style-type: none"> a Click Method > Save As. b Go to the \MassHunter\methods folder. c Type <i>iii</i>_IM-MS_only .m where <i>iii</i> are your initials. d Click Save. 	<ul style="list-style-type: none"> • 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
<p>1 Using the <i>iii_IM-MS-only.m</i> method for the IM-MS Q-TOF, set the collision energy to alternating.</p> <ul style="list-style-type: none"> If the <i>iii_IM-MS-only.m</i> method is still displayed, begin with step c. Delete the 0.5 min Time Segment. Enter the parameters as shown in Figure 29. 	<p>a Click Method > Open.</p> <p>b Select <i>iii_IM-MS-only.m</i>, and click Open.</p> <p>c Click the Q-TOF tab.</p> <p>d Under Acquisition Mode, select IM-QTOF.</p> <p>e Select the 0.5 minute Time Segment.</p> <p>f Right-click the selected Time Segment and click Delete Time Segment.</p> <p>g Click the Acquisition tab.</p> <p>h For Frame rate, type 1.</p> <p>i Set Pulsing Sequence Length to Disabled.</p>	<ul style="list-style-type: none"> The LC, General, Source, Ref Mass and Chromatogram parameters remain the same as in <i>iii_IM-MS-only.m</i> 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.

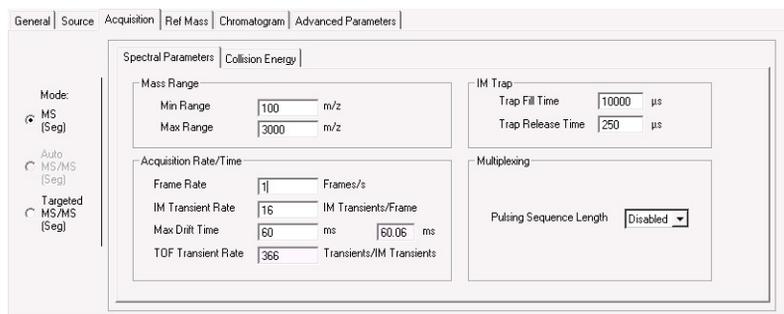


Figure 29. Acquisition Spectral Parameters tab for IM-MS All Ions MS/MS mode

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

Steps	Detailed Instructions	Comments
2	<p>Set the collision energy to alternate between 0 and 35.</p> <ul style="list-style-type: none"> Enter the parameters as shown in Figure 30. 	<ul style="list-style-type: none"> Frame 1 automatically is set to have a collision energy of 0 V when you set up alternating frames.

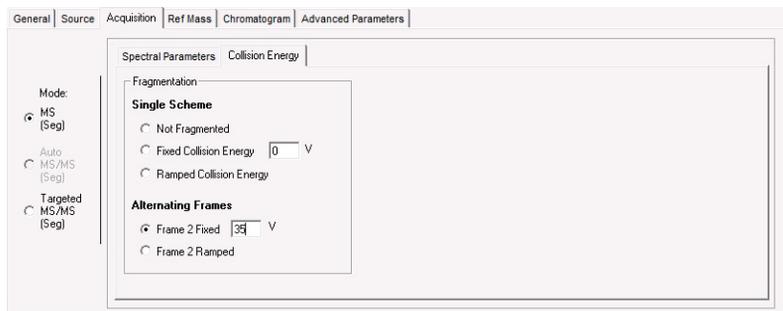


Figure 30. Acquisition Collision Energy tab for IM-MS All Ions mode

3	<p>Save the method as <i>iii</i>_IM-MS-All_Ions.m, where <i>iii</i> are your initials.</p>	<p>a Click Method > Save As.</p> <p>b Type <i>iii</i>_IM-MS-All_Ions, and click Save.</p>	<ul style="list-style-type: none"> Be sure to use the folder \MassHunter\methods.
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4

Optimize IM-MS Q-TOF Methods

Task 1. Set up and run an IM-MS method for Labile Compounds 47

Task 2. Set up IM-MS method for Small Compounds 51

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.

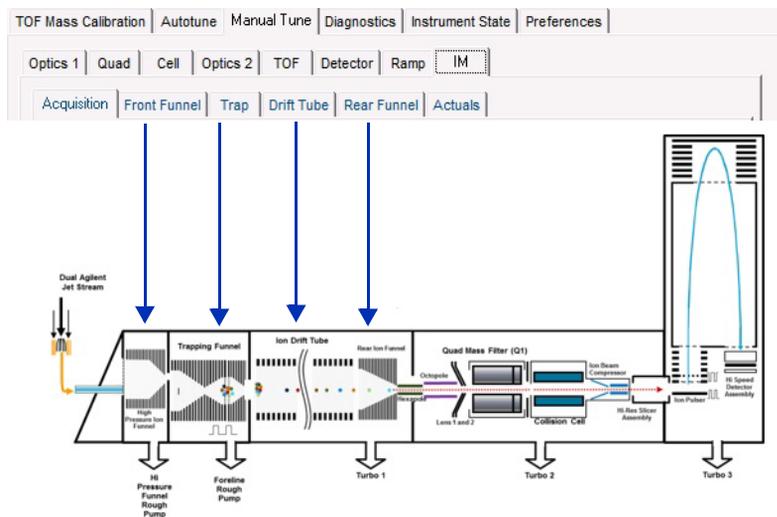


Figure 31. Manual Tune > IM tabs and the part of the instrument they affect

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 H₂O 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.	<ol style="list-style-type: none"> a Click the Agilent Data Acquisition icon. b Set Context on the main toolbar to Tune. c Click the Instrument State tab. 	
2 Select the following tune settings. <ul style="list-style-type: none"> • Mass Range: Standard (3200 m/z) • Select to acquire data in Extended Dynamic Range Mode. • Select the IM-QTOF mode. 	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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

- | | | |
|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>3 Save the new settings to the tune file (<i>Autotune.tun</i>) and return to Acquisition.</p> | <p>a Click Save.
 b Set Context on the main toolbar to Acquisition.
 c Click Yes in the Instrument State Confirmation message.
 d Click Yes in the Save Tune File message.</p> | <ul style="list-style-type: none"> 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 <i>Autotune.tun</i> file. |
| <p>4 Open the method for IM-QTOF created in “Task 2. Set up an IM-MS method” on page 36.</p> | <p>a Click Method > Open.
 b Select <i>iii_IM-MS_only.m</i>, and click OK.
 c Click the Method Editor window.</p> | |
| <p>5 Save the method as <i>iii_IM-MS_labile.m</i>, where <i>iii</i> are your initials.</p> | <p>a Click Method > Save As.
 b Go to the <i>\MassHunter\methods</i> folder.
 c Type <i>iii_IM-MS_labile.m</i>, where <i>iii</i> are your initials.
 d Click Save.</p> | <ul style="list-style-type: none"> 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	<p>Enter this sample information:</p> <ul style="list-style-type: none"> Name: 100 nM bradykinin Data file name: bradykinin01.d <p>a Click the Sample Run window.</p> <p>b For Sample Name, type 100 nM bradykinin.</p> <p>c For Data File Name, type bradykinin01.d.</p> <p>d Mark the Auto Increment check box.</p>	<ul style="list-style-type: none"> 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.

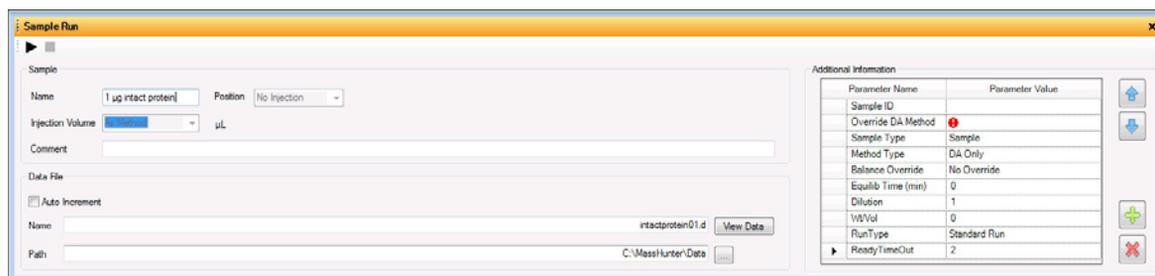


Figure 33. Sample Run window in the main window

7	<p>Start the sample.</p> <ul style="list-style-type: none"> Click Run  on the Sample Run toolbar or Run Sample  on the main toolbar. 	<ul style="list-style-type: none"> 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. The Lock button  on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
8	<p>View the data after the run.</p> <ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> 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 reduce 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.	<ol style="list-style-type: none"> a Click the Agilent Data Acquisition icon. b Set Context on the main toolbar to Tune. c Click the Instrument State tab. 	
2 Select the following tune settings. <ul style="list-style-type: none"> • Mass Range: Standard (3200 m/z) • Select to acquire data in Extended Dynamic Range Mode. • Select the IM-QTOF mode. 	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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

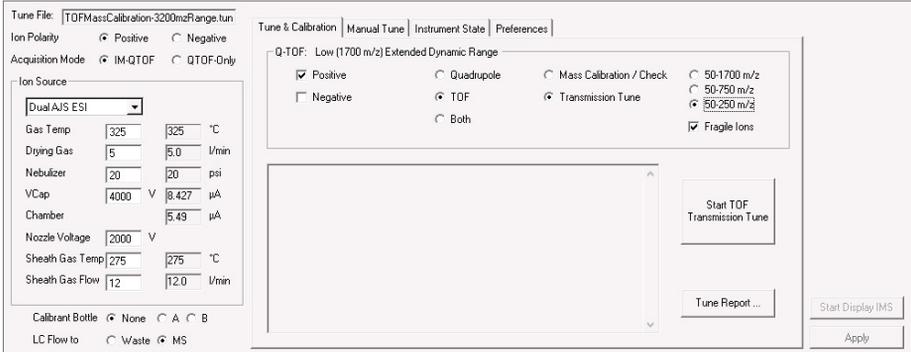
Steps	Detailed Instructions	Comments
		

Figure 34. Transmission tune for small compounds

- | | | |
|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>3 Save the new settings to the tune file (<i>Autotune.tun</i>) and return to Acquisition.</p> | <p>a Click Save.
 b Set Context to Acquisition.
 c Click Yes in the Instrument State Confirmation message.
 d Click Yes in the Save Tune File message.</p> | <ul style="list-style-type: none"> • 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 <i>Autotune.tun</i> file. |
| <p>4 Open the method for IM-QTOF created in “Task 2. Set up an IM-MS method” on page 36.</p> | <p>a Click Method > Open.
 b Select <i>iii_IM-MS_only.m</i>, and click OK.
 c Click the Method Editor window.</p> | |
| <p>5 Change the Advanced Parameters:</p> | <p>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.</p> | <ul style="list-style-type: none"> • 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. |
| <p>6 Save the method as <i>iii_IM-MS_small_molecules.m</i>, where <i>iii</i> are your initials.</p> | <p>a Click Method > Save As.
 b Go to the <code>\MassHunter\methods</code> folder.
 c Type <i>iii_IM-MS_small_molecules.m</i> where <i>iii</i> are your initials.
 d Click Save.</p> | <ul style="list-style-type: none"> • For example, if your initials are PFH, then the method name is pfh_IM-MS_small_molecules.m. |

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

Steps	Detailed Instructions	Comments
7	<p>Enter this sample information:</p> <p>Name: 100 pg amino acid mix</p> <p>Data file name: aminoacid01.d</p> <p>a Click the Sample Run window.</p> <p>b For Sample Name, type 100 pg amino acid mix.</p> <p>c For Data File Name, type aminoacid01.d.</p> <p>d Mark the Auto Increment check box.</p>	<ul style="list-style-type: none"> 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.

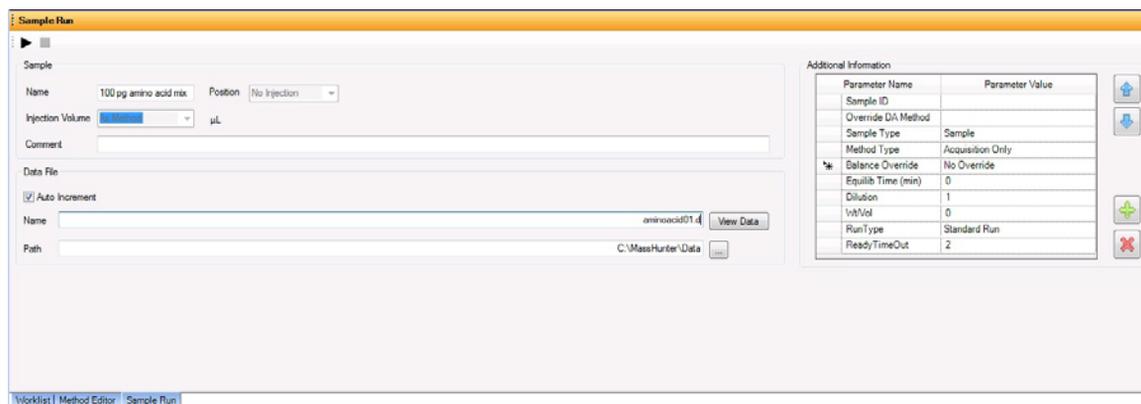


Figure 35. Sample Run window in the main window

8	<p>Start the sample.</p> <ul style="list-style-type: none"> Click Run ► on the Sample Run toolbar or the Run Sample  on the main toolbar. 	<ul style="list-style-type: none"> 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. The Lock button  on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
9	<p>View the data after the run.</p> <ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> 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 **56**

Task 2. Set up and run a method to calculate CCS using Step Field Ion Mobility **65**

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.	<p>a Click Method > Open.</p> <p>b Select <i>iii_IM-MS_only.m</i>, and click OK.</p>	<ul style="list-style-type: none"> For example, if your initials are PFH, then the method name is pfh_IM-MS_only.m.
2 Save the method as <i>iii_SulfaDrug_CCS.m</i> .	<p>a Click Method > Save As.</p> <p>b Go to the \MassHunter\methods folder.</p> <p>c Type <i>iii_SulfaDrug_CCS.m</i> where <i>iii</i> are your initials.</p> <p>d Click OK.</p>	<ul style="list-style-type: none"> 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 Open Data Acquisition to access the window for editing methods.	<p>a Set Context on the main toolbar to Acquisition.</p> <p>b If needed, click View > Method Editor to open the Method Editor window.</p> <p>c Click the Quat. Pump tab.</p> <p>d For Channel A Pump Flow Rate, type 0.8.</p> <p>e For Run Time, type 1.0.</p> <p>f Click the Multisampler tab.</p> <p>g For Injection Volume, type 5.</p> <p>h Set Needle Wash to Standard Wash.</p> <p>i For Draw speed, type 100.</p> <p>j For Eject speed, type 400.</p> <p>k Set Injection Path Cleaning to Flush Port for 3 seconds.</p> <p>l If available, click the Column Compartment tab and type 40 °C for the temperature.</p>	<ul style="list-style-type: none"> 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.

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

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.	<p>a Click the Q-TOF tab.</p> <p>b Click the General tab.</p> <p>c Click IM-QTOF.</p> <p>d Set Ion Polarity to Positive.</p> <p>e Under MS, for Abs. Threshold type 200 and for Rel. Threshold (%) type 0.01.</p> <p>f Under MS/MS, for the Abs. Threshold type 5, and for the Rel. Threshold (%) type 0.01.</p>	
5 Set values on the Q-TOF > Source tab.	<p>a Click the Source tab.</p> <p>b For Drying Gas, type 350.</p> <p>c For Drying Gas Flow, type 12.</p> <p>d For Nebulizer, type 60.</p> <p>e For Sheath Gas Temp, type 350.</p> <p>f For Sheath Gas Flow, type 11.</p> <p>g For Capillary, type 4000.</p> <p>h For Nozzle Voltage, type 1000.</p> <p>i For Fragmentor, type 365.</p> <p>j For Oct 1 RF Vpp, type 750.</p>	
6 Set values on the Q-TOF > Acquisition tab.	<p>a Click the Acquisition tab.</p> <p>b For Minimum range, type 100.</p> <p>c For Maximum range, type 1700.</p> <p>d For Frame Rate, type 1.</p> <p>e For Max Drift Time, type 50.</p> <p>f For Trap Fill Time, type 20000.</p> <p>g For Trap Release Time, type 150.</p> <p>h Set Pulsing Sequence Length to Disabled.</p> <p>i For all advanced parameters, use the values set from the autotune runs.</p>	<ul style="list-style-type: none"> This experiment should be run in 1700 <i>m/z</i> mass range.
7 Save the method as <i>iii_6560_CCS_IM_SingleF.m</i> , where <i>iii</i> are your initials.	<p>a Click Method > Save As.</p> <p>b Go to the \MassHunter\methods folder.</p> <p>c Type <i>iii_6560_CCS_IM_SingleF.m</i>, where <i>iii</i> are your initials.</p> <p>d Click Save.</p>	<ul style="list-style-type: none"> For example, if your initials are PFH, then the method name is pfh_6560_CCS_IM_SingleF.m.
8 Change the Properties tab.	<p>a Click the Properties tab.</p> <p>b Click . The Select Script dialog box opens.</p> <p>c Set Script to SCP_AcquireCalibrantData.</p> <p>d Click OK.</p>	<ul style="list-style-type: none"> This script does a run with Calibrant B on.

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

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

Steps	Detailed Instructions	Comments
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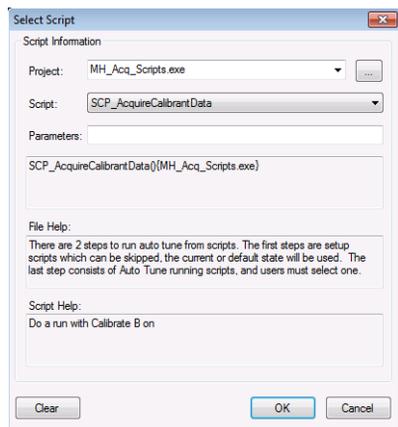


Figure 36. Select Script dialog box

- | | | |
|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>9 Save the method as <i>iii_6560_CCS_IM_SingleF_Calib.m</i>, where <i>iii</i> are your initials.</p> | <p>a Click Method > Save As.</p> <p>b Go to the <code>\MassHunter\methods</code> folder.</p> <p>c Type <i>iii_6560_CCS_IM_SingleF_Calib.m</i> where <i>iii</i> are your initials.</p> <p>d Click Save.</p> | <ul style="list-style-type: none"> For example, if your initials are PFH, then the method name is pfh_6560_CCS_IM_SingleF_Calib.m. |
| <p>10 Set up a worklist that acquires a tune calibrant data file and your sample file.</p> | <p>a Click the Worklist window.</p> <p>b Add two samples with the following information.</p> | |



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.	<ul style="list-style-type: none"> Click Run  on the Worklist toolbar or Run Worklist  on the main toolbar. 	<ul style="list-style-type: none"> You do not need to save the worklist 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. The Lock button  on the main toolbar indicates that 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.
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.	<ol style="list-style-type: none"> Start the IM-MS Browser program. Open the Calibrant Single Field.d data or the file that you just acquired, iii_6560_IM_SingleF_Calib.d, where <i>iii</i> are your initials. Select the entire run time in the File Overview window. Right-click the File Overview window and click Extract Field. Click View > CCS Calibration (Single-Field). Set Reference set to Agilent ESI Tune Mix (pos). Click Find Drift Times in the CCS Calibration (Single-Field) window. 	<ul style="list-style-type: none"> You can view cross section calculations in the IM-MS Browser program.

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

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

Steps	Detailed Instructions	Comments																																																																													
	<p>The screenshot displays the IM-MS Browser interface. The top window shows the Total Ion Chromatogram (TIC) for 'Calibrant Single Field.d' with a y-axis of $\times 10^5$ and an x-axis of 'Counts vs. Acquisition Time (min)'. Below it, the 'Frame Viewer' shows a 'Mass Spectrum (0.00-49.92 ms) (0.057-0.982 min) - Calibrant Single Field.d' with peaks at m/z 209, 1538, 322, 0490, 622, 0298, 922, 0106, 1221, 9916, and 1521, 9714. The bottom left shows a 'Drift Spectrum (99.9753-...)' with peaks at m/z 14, 80, 21, 07, 27, 05, and 37, 96. The bottom right shows a 2D CCS plot of 'Drift Time (ms) vs. m/z'. On the right side, the 'CCS Calibration (Single-Field)' window is open, showing input values (Drift gas: N2, Ion polarity: Positive, Reference set: Agilent ESI Tune Mix (pos)) and a table of calibration data.</p> <table border="1"> <thead> <tr> <th>Ω (\AA^2)</th> <th>m/z</th> <th>Ion</th> <th>tD (ms)</th> <th>Mass</th> <th>z</th> <th>Residual (ms)</th> </tr> </thead> <tbody> <tr><td>121.3</td><td>118.0863</td><td>(M+H)+</td><td>14.76</td><td>118.0668</td><td>1</td><td>-0.0527</td></tr> <tr><td>153.7</td><td>322.0481</td><td>(M+H)+</td><td>20.07</td><td>322.0487</td><td>1</td><td>0.0243</td></tr> <tr><td>203</td><td>622.029</td><td>(M+H)+</td><td>27.06</td><td>622.0295</td><td>1</td><td>0.0518</td></tr> <tr><td>243.6</td><td>922.0098</td><td>(M+H)+</td><td>32.68</td><td>922.0103</td><td>1</td><td>0.0184</td></tr> <tr><td>282.2</td><td>1221.9906</td><td>(M+H)+</td><td>37.96</td><td>1221.9912</td><td>1</td><td>-0.0166</td></tr> <tr><td>317</td><td>1521.9715</td><td>(M+H)+</td><td>42.73</td><td>1521.972</td><td>1</td><td>-0.0251</td></tr> <tr><td>351.2</td><td>1821.9523</td><td>(M+H)+</td><td></td><td>1821.9529</td><td>1</td><td></td></tr> <tr><td>383</td><td>2121.9332</td><td>(M+H)+</td><td></td><td>2121.9337</td><td>1</td><td></td></tr> <tr><td>413</td><td>2421.914</td><td>(M+H)+</td><td></td><td>2421.9145</td><td>1</td><td></td></tr> <tr><td>441.2</td><td>2721.8948</td><td>(M+H)+</td><td></td><td>2721.8954</td><td>1</td><td></td></tr> </tbody> </table> <p>Buttons: Find Drift Times, Calculate, Save or Restore... Results: Beta: 0.136286, TFix: -0.049929, SE: 0.042729</p>	Ω (\AA^2)	m/z	Ion	tD (ms)	Mass	z	Residual (ms)	121.3	118.0863	(M+H)+	14.76	118.0668	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		
Ω (\AA^2)	m/z	Ion	tD (ms)	Mass	z	Residual (ms)																																																																									
121.3	118.0863	(M+H)+	14.76	118.0668	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
	<p>h Save the CCS (Single-Field) coefficients. Click Save or Restore. The CCS Calibration (Single-Field) dialog box opens.</p> <p>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.</p> <p>j Click Open.</p> <p>k Click OK.</p> <p>l Click File > Close.</p> <p>m In IM-MS Browser, open Sulfa_Single Field-r001.d.</p> <p>n Click Method > Find Features (IMFE).</p> <p>o Select the following parameters and click Find Features.</p> <p>p Click View > Feature List.</p>	<ul style="list-style-type: none"> 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.

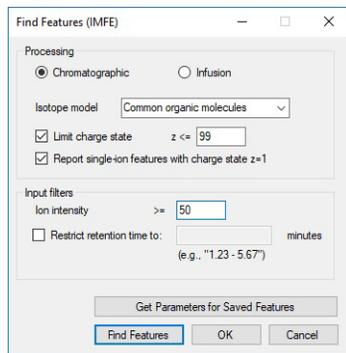


Figure 39. Find Features (IMFE) dialog box (Single Field Ion Mobility)

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

Steps

Detailed Instructions

Comments

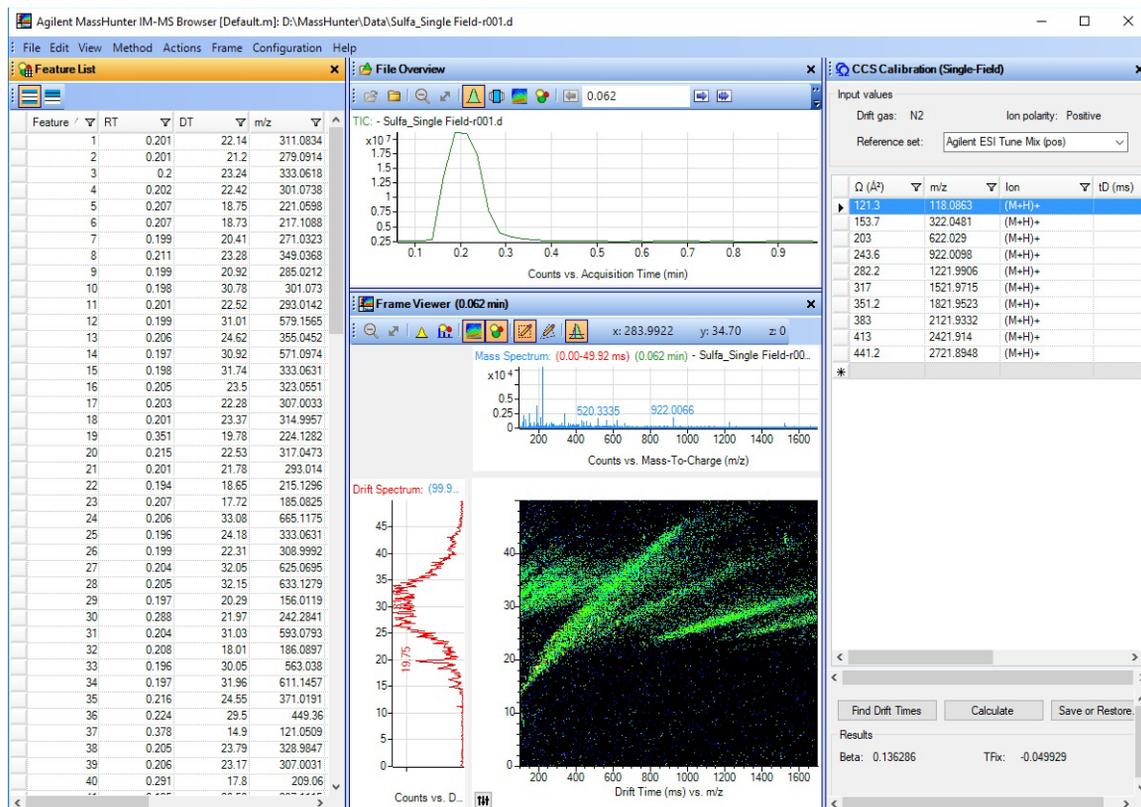


Figure 40. Find Features (IMFE) dialog box (Single Field Ion Mobility)

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

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

Steps	Detailed Instructions	Comments
13 Reduce the number of features.	<p>a Click Methods > Filter Features.</p> <p>b Click Max ion volume.</p> <p>c Mark the Quality score from check box and for the limits, type 70 and 100.</p> <p>d Mark the m/z from check box and for the limits, type 270 and 350.</p> <p>e Clear other check boxes.</p> <p>f Click OK.</p>	<ul style="list-style-type: none"> For the four sulfa drugs, here are the m/z values and the CCS (\AA^2) values: <ul style="list-style-type: none"> 311.0815 m/z -> 170.27 \AA^2 285.0175 m/z -> 161.76 \AA^2 279.0902 m/z -> 163.74 \AA^2 271.0288 m/z -> 158.13 \AA^2

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

Steps

Detailed Instructions

Comments

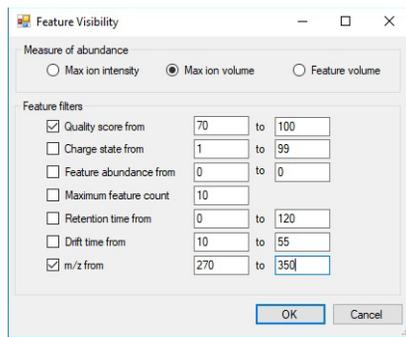


Figure 41. Feature Visibility dialog box (Single Field Ion Mobility)

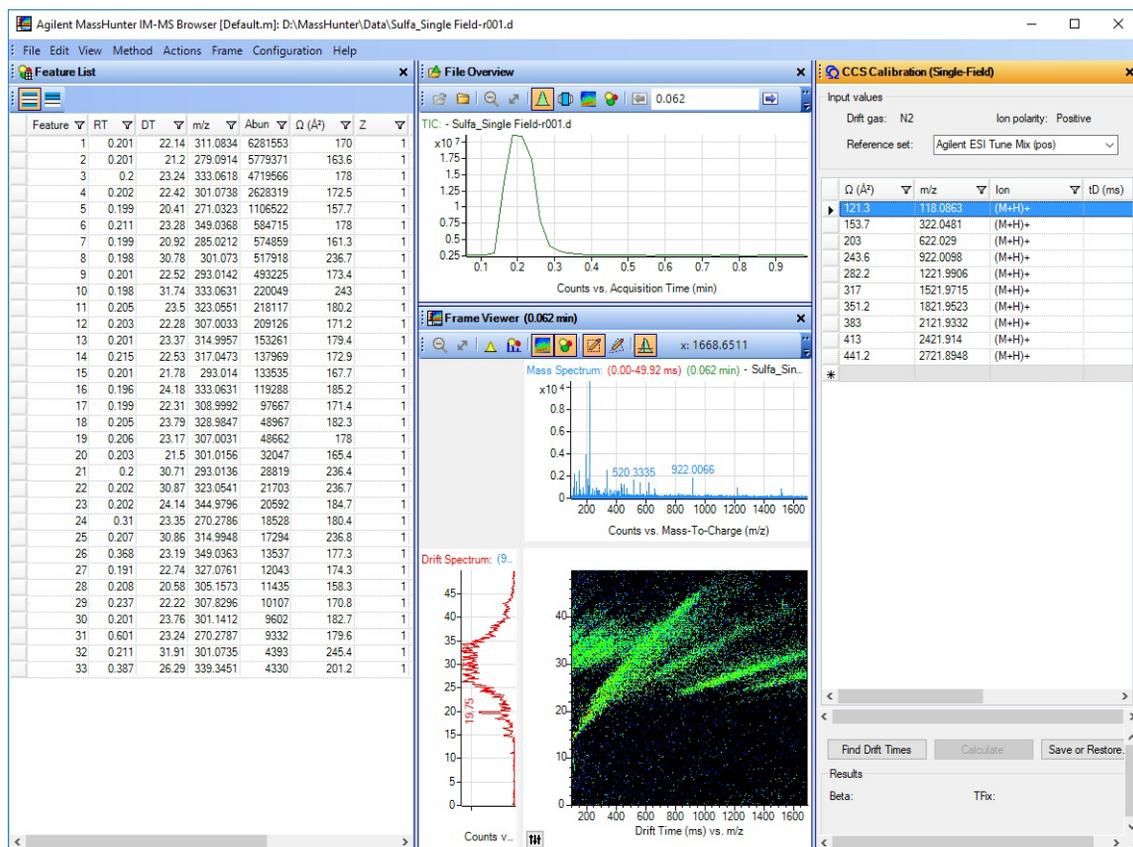


Figure 42. Filtered features in the IM-MS Browser program

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 <i>iii</i> are your initials.	<ol style="list-style-type: none"> a Click Method > Open. b Select iii_6560_CCS_IM_SingleF_Calib.m, where <i>iii</i> are your initials, and click OK. c Click the Method Editor window. 	<ul style="list-style-type: none"> • This method was developed in “Task 1. Set up LC method to calculate CCS using Single-Field ion mobility method” on page 56
2 Change parameters in the acquisition method: <ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 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

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

Steps	Detailed Instructions	Comments

Figure 43. Advanced parameters for the first time segment

- Save the method as `iii_6560_CCS_IM_SteppedF.m`, where *iii* are your initials.

 - Click **Method > Save As**.
 - Go to the `\MassHunter\methods` folder.
 - Type `iii_6560_CCS_IM_SteppedF.m` where *iii* are your initials.
 - Click **Save**.
 - For example, if your initials are PFH, then the method name is **pfh_6560_CCS_IM_SteppedF.m**.
- Enter this sample information:

 - Name: **Step Field**
 - Data file name: **TuneMix_SF000x.d**
 - Click the **Sample Run** window.
 - For Sample **Name**, type `step Field`.
 - For Data File **Name**, type `TuneMix_SF000x.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

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

Steps	Detailed Instructions	Comments
5 Start the sample.	<ul style="list-style-type: none"> Click Run  on the Sample Run toolbar or Run Sample  on the main toolbar. 	<ul style="list-style-type: none"> 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. The Lock button  on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
6 View the data after the run. You can instead use the example files: Tunemix_SF001.d, Tunemix_SF002.d, and Tunemix_SF003.d	<ol style="list-style-type: none"> Open the data file Tunemix_SF001.d in the IM-MS Browser program. In the File Overview window, click . 	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> Make sure the frame is within a single Time Segment.
d	Right-click and click Extract Frame .	
e	In the Frame Viewer window, zoom the <i>m/z</i> 622 isotopic cluster.	
f	Highlight the entire <i>m/z</i> 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 <i>m/z</i> ion.	

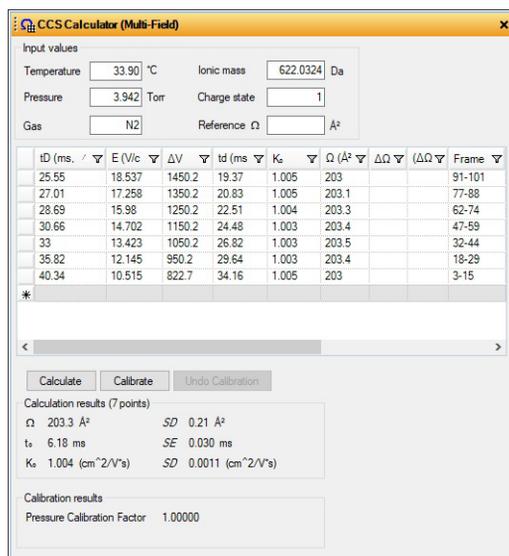


Figure 45. CCS Calculator (Multi-Field) window with ionic mass 622

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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