Agilent

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 B.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and 6500 Series Q-TOF program and compatible Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF programs, until superseded.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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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.

Content

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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.

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.

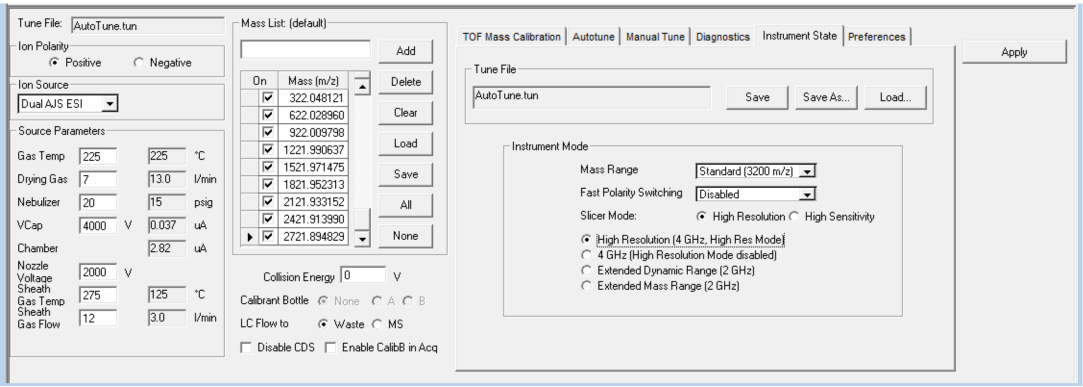
- If the TOF or Q-TOF has 4 GHz data collection capability, you can select data recording rates from 1 GHz to 4 GHz, as well as the mass range.
- If available for your TOF or Q-TOF, you can set **Fast Polarity Switching** to **Enabled** or **Disabled**. These exercises are run in Positive polarity, so 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** Click the **Agilent Data Acquisition** icon. **b** On the main toolbar, set **Context** to **Tune**. **c** Click the **Instrument State** tab.	
2 Select the following data collection settings. • Mass Range: Standard (3200 m/z) • Select to acquire data in High Resolution Mode.	**a** Set **Mass Range** to **Standard (3200 m/z)**. **b** If needed, select **High Resolution (4 GHz, High Res 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


Tune File: AutoTune.tun

Ion Polarity: ● Positive ○ Negative

Ion Source: Dual AJS ESI

Source Parameters

Gas Temp	225	225	°C
Drying Gas	7	13.0	l/min
Nebulizer	20	15	psig
VCap	4000 V	0.037	uA
Chamber		2.82	uA
Nozzle Voltage	2000 V		
Sheath Gas Temp	275	125	°C
Sheath Gas Flow	12	3.0	l/min

Mass List: (default)

On	Mass (m/z)
☑	322.048121
☑	622.028960
☑	922.009798
☑	1221.990637
☑	1521.971475
☑	1821.952313
☑	2121.933152
☑	2421.913990
☑	2721.894829

Collision Energy 0 V

Calibrant Bottle ● None ○ A ○ B

LC Flow to ● Waste ○ MS

☐ Disable CDS ☐ Enable CalibB in Acq

Tune File: AutoTune.tun

Instrument Mode

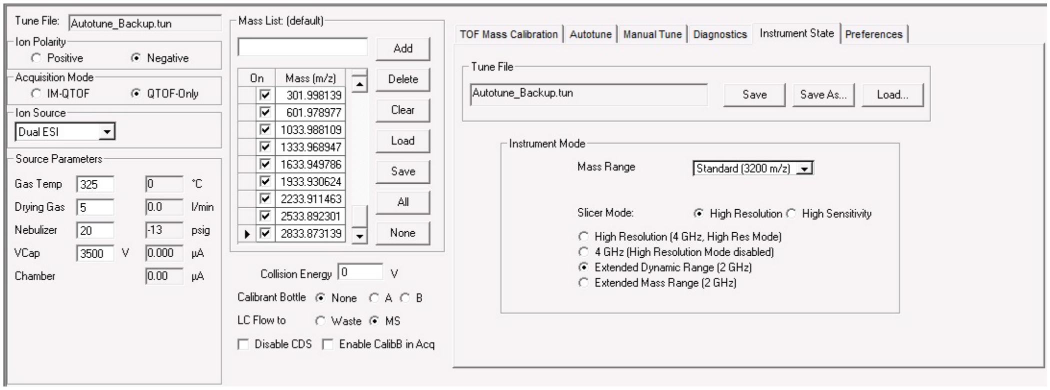
Mass Range: Standard (3200 m/z)

Fast Polarity Switching: Disabled

Slicer Mode: ● High Resolution ○ High Sensitivity

● High Resolution (4 GHz, High Res Mode)
○ 4 GHz (High Resolution Mode disabled)
○ Extended Dynamic Range (2 GHz)
○ Extended Mass Range (2 GHz)

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


Tune File: Autotune_Backup.tun

Ion Polarity: ○ Positive ● Negative

Acquisition Mode: ○ IM-QTOF ● QTOF-Only

Ion Source: Dual ESI

Source Parameters

Gas Temp	325	0	°C
Drying Gas	5	0.0	l/min
Nebulizer	20	-13	psig
VCap	3500 V	0.000	µA
Chamber		0.00	µA

Mass List: (default)

On	Mass (m/z)
☑	301.998139
☑	601.978977
☑	1033.988109
☑	1333.968947
☑	1633.949786
☑	1933.930624
☑	2233.911463
☑	2533.892301
☑	2833.873139

Collision Energy 0 V

Calibrant Bottle ● None ○ A ○ B

LC Flow to ○ Waste ● MS

☐ Disable CDS ☐ Enable CalibB in Acq

Tune File: Autotune_Backup.tun

Instrument Mode

Mass Range: Standard (3200 m/z)

Slicer Mode: ● High Resolution ○ High Sensitivity

○ High Resolution (4 GHz, High Res Mode)
○ 4 GHz (High Resolution Mode disabled)
● Extended Dynamic Range (2 GHz)
○ Extended Mass Range (2 GHz)

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

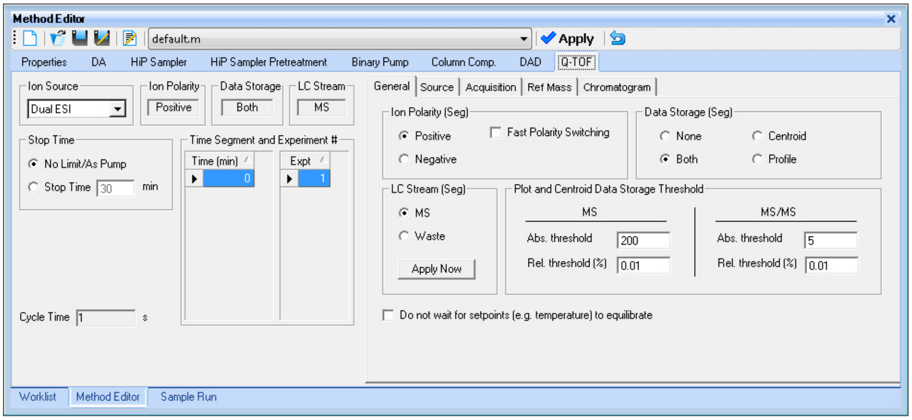
Steps	Detailed Instructions	Comments
3 Save the new settings to the tune file (Autotune.tun) and return to Acquisition.	**a** Click **Save**. **b** Set **Context** to **Acquisition**. **c** Click **Yes** in the Instrument State Confirmation message. **d** Click **Yes** in the Save Tune File message.	• 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 Open Data Acquisition to access the window for editing methods.	a Click the **Agilent Data Acquisition** icon. b On the main toolbar, set **Context** to **Acquisition**. c Make sure that the Method Editor window is visible. Click **View > Method Editor** if the Method Editor window is not visible. d If you have 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.



default.m

Ion Source: Dual ESI

Ion Polarity: Positive

Data Storage: Both

LC Stream: MS

Stop Time: ● No Limit/As Pump ○ Stop Time 30 min

Time Segment and Experiment #: Time (min) 0, Expt 1

Cycle Time 1 s

Ion Polarity (Seg): ● Positive ○ Negative ☐ Fast Polarity Switching

Data Storage (Seg): ○ None ● Both ○ Centroid ○ Profile

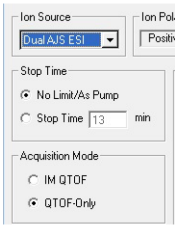
LC Stream (Seg): ● MS ○ Waste

Plot and Centroid Data Storage Threshold:

	MS	MS/MS
Abs. threshold	200	5
Rel. threshold (%)	0.01	0.01

☐ Do not wait for setpoints (e.g. temperature) to equilibrate

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



Ion Source: Dual AJS ESI

Ion Pol: Positi

Stop Time: ● No Limit/As Pump ○ Stop Time 13 min

Acquisition Mode: ○ IM QTOF ● QTOF-Only

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)

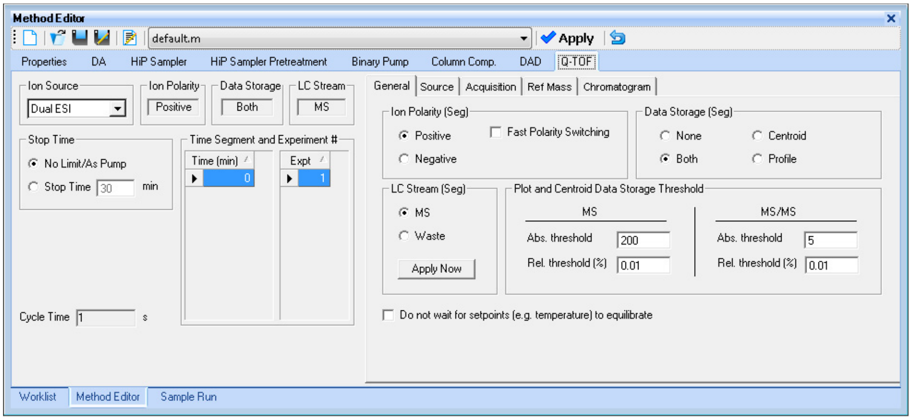
Steps	Detailed Instructions	Comments
2 Enter LC parameters appropriate for sulfa drug mix. See **Table 1**.	**a** In the Method Editor window, click each LC module tab to set parameter values. **b** Set the LC parameters listed in **Table 1**.	- LC parameters in each tab depend on the configuration of 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.)	• Of course, the MS/MS fields do not appear on the TOF General tab.



default.m

Ion Source: Dual ESI
Ion Polarity: Positive
Data Storage: Both
LC Stream: MS

Stop Time
● No Limit/As Pump
○ Stop Time 30 min

Time Segment and Experiment #
Time (min): 0
Expt: 1

Cycle Time 1 s

Ion Polarity (Seg)
● Positive
○ Negative
☐ Fast Polarity Switching

Data Storage (Seg)
○ None
● Both
○ Centroid
○ Profile

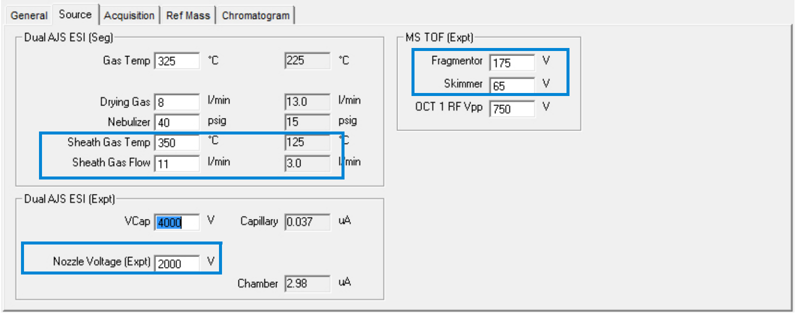
LC Stream (Seg)
● MS
○ Waste

Plot and Centroid Data Storage Threshold
MS: Abs. threshold 200; Rel. threshold (%) 0.01
MS/MS: Abs. threshold 5; Rel. threshold (%) 0.01

☐ Do not wait for setpoints (e.g. temperature) to equilibrate

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

Steps	Detailed Instructions	Comments
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.



Dual AJS ESI (Seg)
Gas Temp 325 °C; 225 °C
Drying Gas 8 l/min; 13.0 l/min
Nebulizer 40 psig; 15 psig
Sheath Gas Temp 350 °C; 125 °C
Sheath Gas Flow 11 l/min; 3.0 l/min

Dual AJS ESI (Expt)
VCap 4000 V
Capillary 0.037 uA
Nozzle Voltage (Expt) 2000 V
Chamber 2.98 uA

MS TOF (Expt)
Fragmentor 175 V
Skimmer 65 V
OCT 1 RF Vpp 750 V

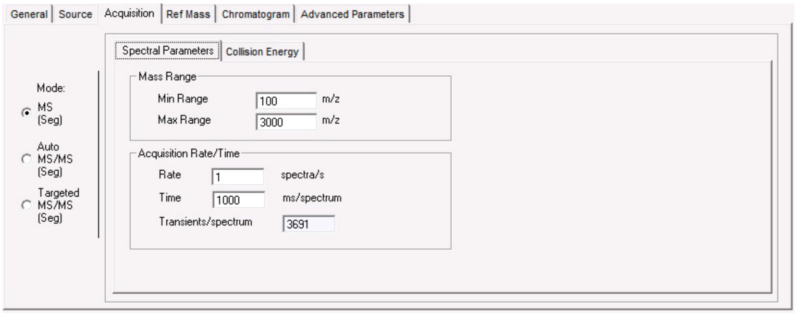
These options appear on a 6530 Q-TOF and a 6230 TOF mass spectrometer equipped with the AJS source.

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

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.	



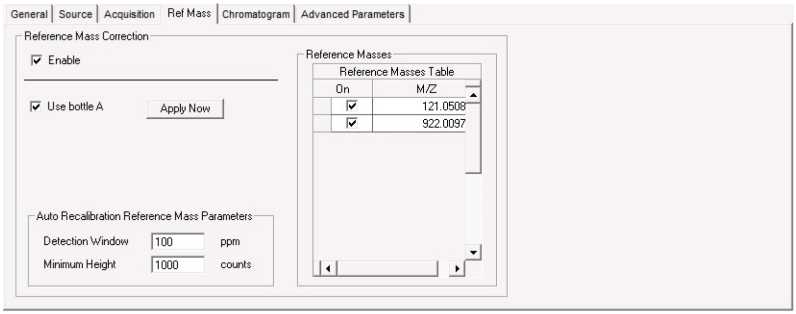
Mode:
● MS (Seg)
○ Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Mass Range
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.	a Click the **Ref Mass** tab. b Type the parameters as shown in Figure 8.	



Reference Mass Correction
☑ Enable
☑ Use bottle A

Auto Recalibration Reference Mass Parameters
Detection Window 100 ppm
Minimum Height 1000 counts

Reference Masses

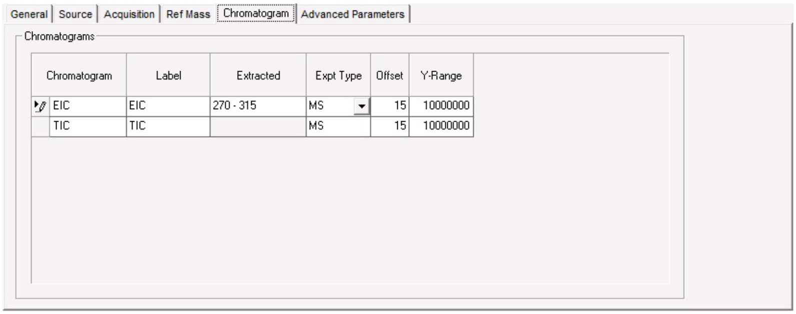
Reference Masses Table

On	M/Z
☑	121.0508
☑	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 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.



Chromatogram	Label	Extracted	Expt Type	Offset	Y-Range
EIC	EIC	270 - 315	MS	15	10000000
TIC	TIC		MS	15	10000000

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

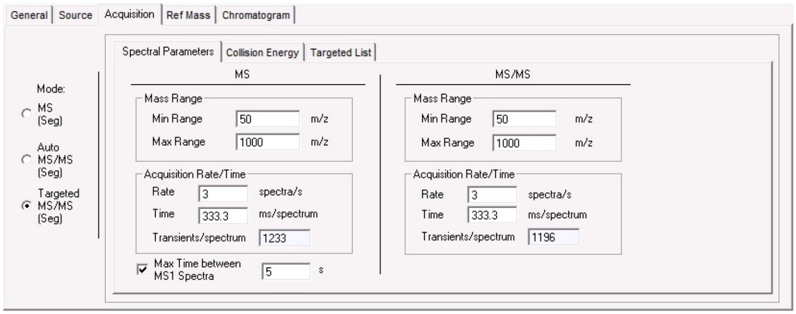
Steps	Detailed Instructions	Comments
8 Set up to change MS parameters during run: • Time Segment of 0 min. - Make sure you have selected an LC Stream of Waste. • Time Segment of 0.5 min. - Change LC Stream to MS.	**a** Click the **General** tab. **b** Click **Waste** for the **LC Stream**. **c** Right-click 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 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 Save the method as *iii*MS-only.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **MassHunter\methods** folder. **c** Type `iiiMS-only.m` where *iii* are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfhMS-only.m**.

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

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

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

Steps	Detailed Instructions	Comments
1 Using the *iii*MS-only.m method for the 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** Click **Method > Open**. **b** Select *iii*MS-only.m, and click **Open**. **c** Click the **Q-TOF** tab. **d** Select the 0.5 minute **Time Segment**. **e** Right-click the selected **Time Segment** and click **Delete Time Segment**. **f** Click the **Acquisition** tab. **g** Click **Targeted MS/MS (Seg)** as the Mode. **h** Type the spectral parameters below.	• The LC, General, Source, Ref Mass and Chromatogram parameters remain the same as in *iii*MS-only.m for this method.



Mode:
○ MS (Seg)
○ Auto MS/MS (Seg)
● Targeted MS/MS (Seg)

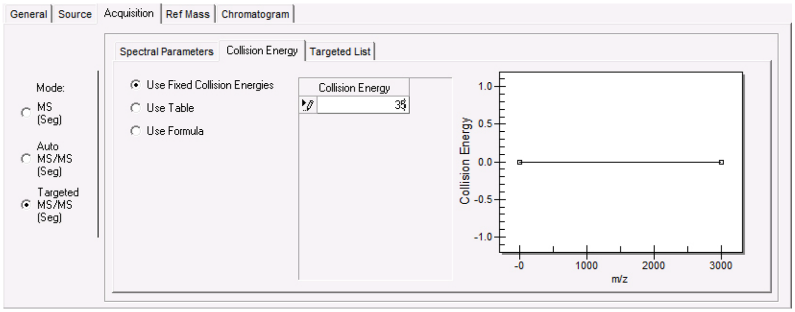
MS
Mass Range
Min Range 50 m/z
Max Range 1000 m/z
Acquisition Rate/Time
Rate 3 spectra/s
Time 333.3 ms/spectrum
Transients/spectrum 1233
☑ Max Time between MS1 Spectra 5 s

MS/MS
Mass Range
Min Range 50 m/z
Max Range 1000 m/z
Acquisition Rate/Time
Rate 3 spectra/s
Time 333.3 ms/spectrum
Transients/spectrum 1196

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.
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.



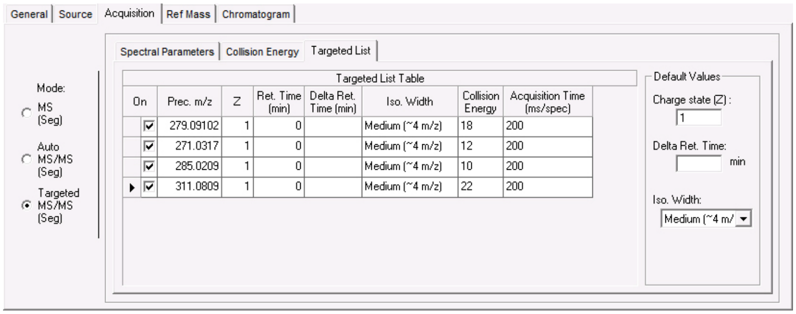
Mode:
○ MS (Seg)
○ Auto MS/MS (Seg)
● Targeted MS/MS (Seg)

● Use Fixed Collision Energies
○ Use Table
○ Use Formula

Collision Energy: 35

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

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



Mode:
○ MS (Seg)
○ Auto MS/MS (Seg)
● Targeted MS/MS (Seg)

Targeted List Table

	On	Prec. m/z	Z	Ret. Time (min)	Delta Ret. Time (min)	Iso. Width	Collision Energy	Acquisition Time (ms/spec)
	☑	279.09102	1	0		Medium (~4 m/z)	18	200
	☑	271.0317	1	0		Medium (~4 m/z)	12	200
	☑	285.0209	1	0		Medium (~4 m/z)	10	200
▶	☑	311.0809	1	0		Medium (~4 m/z)	22	200

Default Values
Charge state (Z) : 1
Delta Ret. Time: min
Iso. Width: Medium (~4 m/

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

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

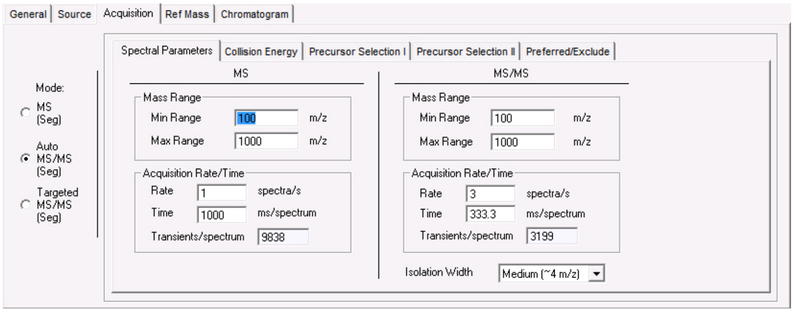
NOTE The parameters on the Acquisition tab, including these values on the Targeted List tab, may also be changed by using different time segments. See **Figure 3** on page 10.

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 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 Click **Method > Open**. b Select ***iii*targetedMSMS.m**, and click **Open**. c Click the **Q-TOF** tab. d Click the **Acquisition** tab. e Click **Auto MS/MS(Seg)** as the **Mode**. f Type the spectral parameters shown below.	• For this method, the LC, General, Source, Ref Mass and Chromatogram parameters will remain the same as in MS-only.m.



Mode:
○ MS (Seg)
● Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

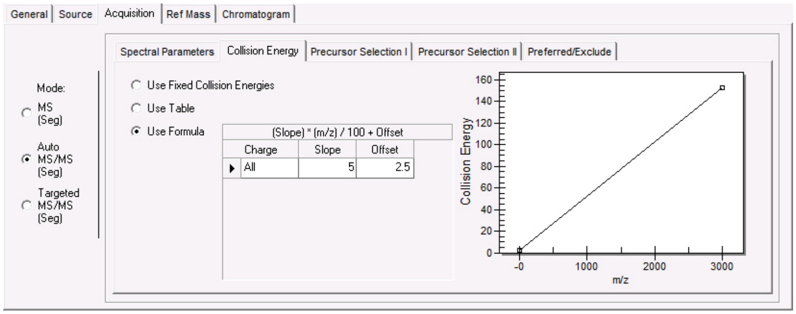
MS
Mass Range
Min Range 100 m/z
Max Range 1000 m/z
Acquisition Rate/Time
Rate 1 spectra/s
Time 1000 ms/spectrum
Transients/spectrum 9838

MS/MS
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 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.



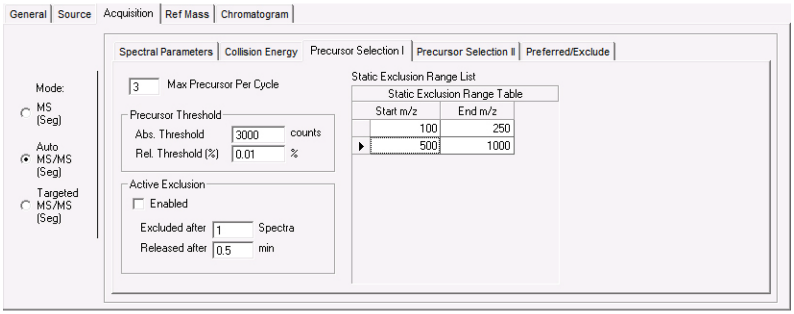
Mode:
○ MS (Seg)
● Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

○ Use Fixed Collision Energies
○ Use Table
● Use Formula

(Slope) * (m/z) / 100 + Offset		
Charge	Slope	Offset
All	5	2.5

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

Steps	Detailed Instructions	Comments
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.



Mode:
○ MS (Seg)
● Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

3 Max Precursor Per Cycle

Precursor Threshold
Abs. Threshold 3000 counts
Rel. Threshold (%) 0.01 %

Active Exclusion
☐ Enabled
Excluded after 1 Spectra
Released after 0.5 min

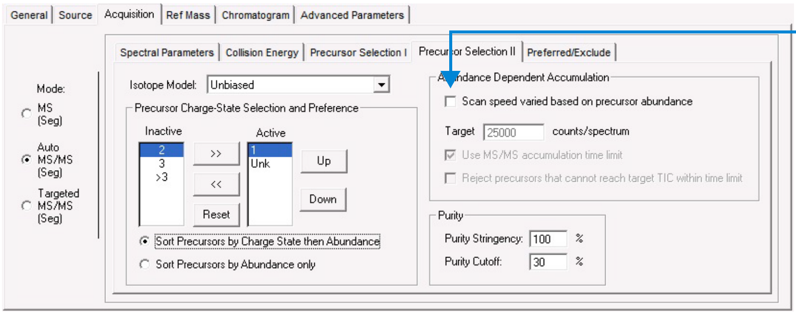
Static Exclusion Range List

Static Exclusion Range Table	
Start m/z	End m/z
100	250
500	1000

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 2 first, then masses of charge 1 and then masses of unknown charge.	**a** Click the **Precursor Selection II** tab. **b** If necessary, click **1** and **Unk** in that order 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 **<<**.	• 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.



Mode:
○ MS (Seg)
● Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Isotope Model: Unbiased

Precursor Charge-State Selection and Preference

Inactive: 2, 3, >3
Active: 1, Unk

● Sort Precursors by Charge State then Abundance
○ Sort Precursors by Abundance only

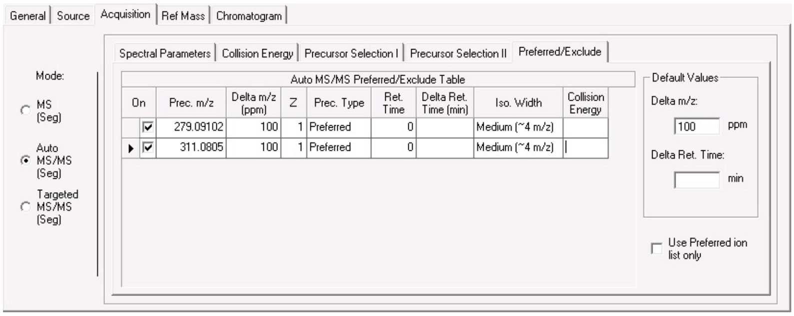
Abundance Dependent Accumulation
☐ Scan speed varied based on precursor abundance
Target 25000 counts/spectrum
☑ Use MS/MS accumulation time limit
☐ Reject precursors that cannot reach target TIC within time limit

Purity
Purity Stringency: 100 %
Purity Cutoff: 30 %

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

Steps	Detailed Instructions	Comments
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.	• For this example, you do not need to mark the **Scan speed varied based on precursor abundance** check box.



Mode:
○ MS (Seg)
● Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Auto MS/MS Preferred/Exclude Table

On	Prec. m/z	Delta m/z (ppm)	Z	Prec. Type	Ret. Time	Delta Ret. Time (min)	Iso. Width	Collision Energy
☑	279.09102	100	1	Preferred	0		Medium (~4 m/z)	
☑	311.0805	100	1	Preferred	0		Medium (~4 m/z)	

Default Values
Delta m/z: 100 ppm
Delta Ret. Time: min

☐ Use Preferred ion list only

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 **Method > Save As**. **b** Type `iiiautoMSMS`, and click **Save**.	- Be sure use the folder **\MassHunter\methods**.

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2 Set up and run single samples and worklists

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

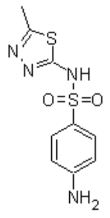
For more details on how to use these exercises, see **"Before you begin..."** on page 4.

Before you begin...

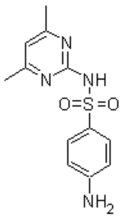
For this exercise you analyze a mixture of four sulfonamide compounds. This section gives instructions on how to prepare the demo sample.

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

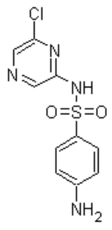
Name	Formula	Ion	m/z
sulfamethizole	$C_9H_{10}N_4O_2S_2$	$(M+H)^+$	271.03179
sulfamethazine	$C_{12}H_{14}N_4O_2S$	$(M+H)^+$	279.09102
sulfachloropyridazine	$C_{10}H_9ClN_4O_2S$	$(M+H)^+$	285.02075
sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	$(M+H)^+$	311.08085



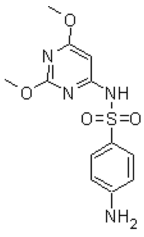
Sulfamethizole



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 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.

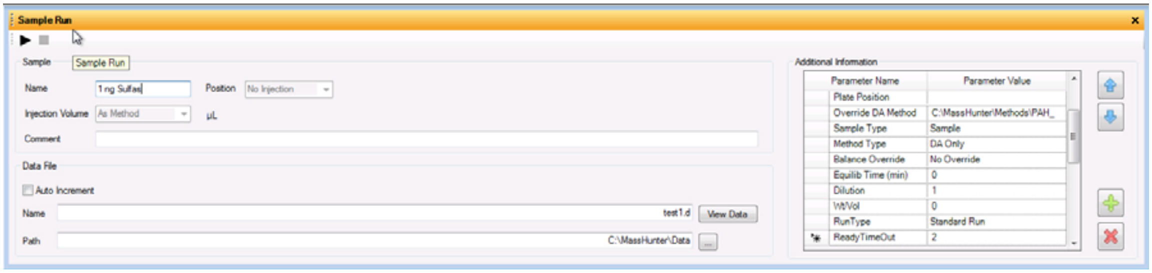
Agilent suggests a column temperature of 40ºC when using this column in this exercise.

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** Click **Method > Open**. **b** Select *one of the three methods*, and click **OK**. **c** Click the **Sample Run** window. **d** For Sample **Name**, type `1 ng Sulfas`. **e** For Data File **Name**, type `test1.d`.	- The system stores the custom information with the data file. 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 or **Run Sample** on the main 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. - The Lock button 🔒 on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
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.



Sample
Name: 1 ng Sulfas
Position: No Injection
Injection Volume: As Method µL
Comment:

Data File
☐ Auto Increment
Name: test1.d
Path: C:\MassHunter\Data

Additional Information

Parameter Name	Parameter Value
Plate Position	
Override DA Method	C:\MassHunter\Methods\PAH_
Sample Type	Sample
Method Type	DA Only
Balance Override	No Override
Equilib Time (min)	0
Dilution	1
Wt/Vol	0
RunType	Standard Run
ReadyTimeOut	2

Figure 18. Sample Run window in the main window

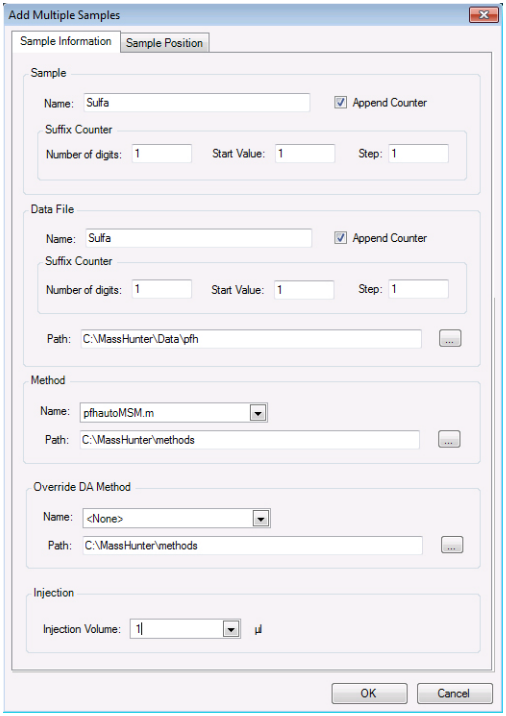
Task 2. Set up and run a worklist with multiple samples

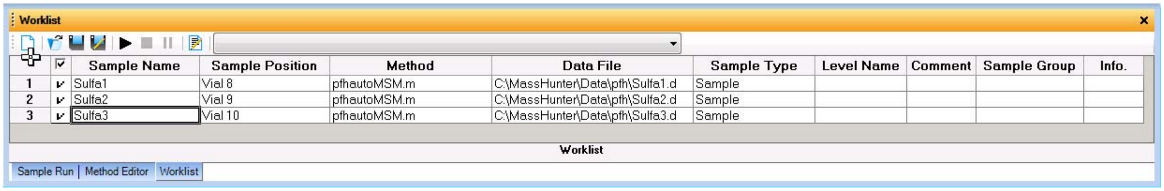
This task shows you how to enter sample and data file information for multiple samples in a worklist and then begin to acquire data.

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

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

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

Steps	Detailed Instructions	Comments
	 Add Multiple Samples Sample Information | Sample Position Sample Name: Sulfa ☑ Append Counter Suffix Counter Number of digits: 1 Start Value: 1 Step: 1 Data File Name: Sulfa ☑ Append Counter Suffix Counter Number of digits: 1 Start Value: 1 Step: 1 Path: C:\MassHunter\Data\pfh Method Name: pfhautoMSM.m Path: C:\MassHunter\methods Override DA Method Name: <None> Path: C:\MassHunter\methods Injection Injection Volume: 1 µl OK Cancel **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.

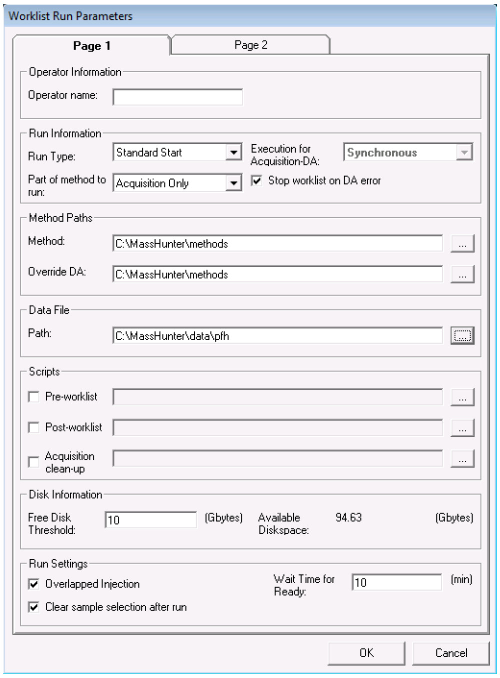


Worklist

	☑	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment	Sample Group	Info.
1	✓	Sulfa1	Vial 8	pfhautoMSM.m	C:\MassHunter\Data\pfh\Sulfa1.d	Sample				
2	✓	Sulfa2	Vial 9	pfhautoMSM.m	C:\MassHunter\Data\pfh\Sulfa2.d	Sample				
3	✓	Sulfa3	Vial 10	pfhautoMSM.m	C:\MassHunter\Data\pfh\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 *iii*esdemo.	**d** Click **Worklist > Save As.** Then, type the worklist **File name** and click **Save**.	
4 Make sure that the worklist is set to run only data acquisition.	**a** Right-click the upper-left-hand cell of the worklist spreadsheet. **b** Select **Worklist Run Parameters**. **c** Set **Part of method** to **Acquisition Only**. **d** Change the directory path for the data files to **MassHunter\Data*YourName***.  Worklist Run Parameters Page 1 / Page 2 Operator Information Operator name: Run Information Run Type: Standard Start Execution for Acquisition-DA: Synchronous Part of method to run: Acquisition Only ☑ Stop worklist on DA error Method Paths Method: C:\MassHunter\methods Override DA: C:\MassHunter\methods Data File Path: C:\MassHunter\data\pfh Scripts ☐ Pre-worklist ☐ Post-worklist ☐ Acquisition clean-up Disk Information Free Disk Threshold: 10 (Gbytes) Available Diskspace: 94.63 (Gbytes) Run Settings ☑ Overlapped Injection ☑ Clear sample selection after run Wait Time for Ready: 10 (min) OK Cancel **Figure 21.** Worklist Run Parameters dialog box **e** Click **OK**.	- You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.

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

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

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.	**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**.	• You can run a method that contains both acquisition and qualitative analysis parameters in a worklist. See the online Help for more information.
6 Start the worklist.	• Click **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

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.

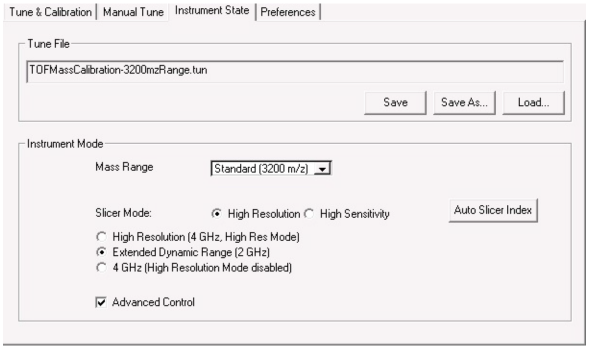
For more details on how to use these exercises, see **"Before you begin..."** on page 4.

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** 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 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 **Apply**. **e** If you changed the **Mass Range**, tune the instrument. **f** 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**.



Tune File: TOFMassCalibration-3200mzRange.tun

Mass Range: Standard (3200 m/z)

Slicer Mode: ● High Resolution ○ High Sensitivity

○ High Resolution (4 GHz, High Res Mode)
● Extended Dynamic Range (2 GHz)
○ 4 GHz (High Resolution Mode disabled)

☑ Advanced Control

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

Task 1. Configure the instrument for data collection

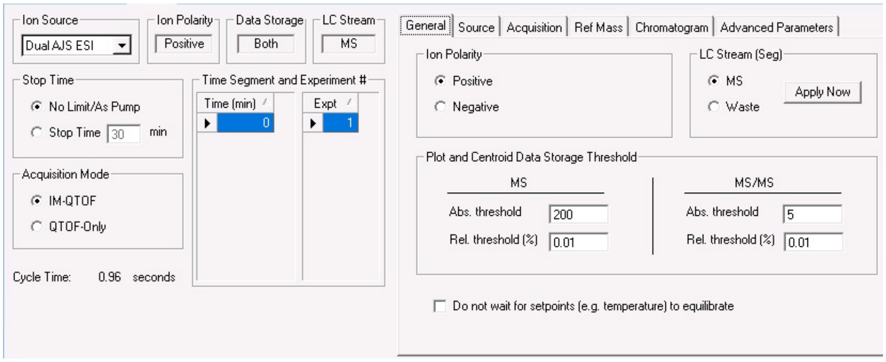
Steps	Detailed Instructions	Comments
3 Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**a** Click **Save**. **b** Set **Context** to **Acquisition**. **c** Click **Yes** in the Instrument State Confirmation message. **d** Click **Yes** in the Save Tune File message.	• 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 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** Click the **Agilent Data Acquisition** icon. **b** Set **Context** to **Tune**. **c** If the Method Editor window is not visible, click **View > Method Editor**. **d** 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**.
2 Enter LC parameters appropriate for sulfa drug mix. See Table 2.	**e** In the Method Editor window, click each LC module tab to set parameter values. **f** Enter LC parameters listed in Table 2.	• LC parameters in each tab depend on the configuration of the LC attached to the mass spectrometer.



Ion Source: Dual AJS ESI
Ion Polarity: Positive
Data Storage: Both
LC Stream: MS

Stop Time
● No Limit/As Pump
○ Stop Time 30 min

Time Segment and Experiment #
Time (min): 0
Expt: 1

Acquisition Mode
● IM-QTOF
○ QTOF-Only

Cycle Time: 0.96 seconds

Ion Polarity
● Positive
○ Negative

LC Stream (Seg)
● MS
○ Waste

Plot and Centroid Data Storage Threshold

	MS	MS/MS
Abs. threshold	200	5
Rel. threshold (%)	0.01	0.01

☐ Do not wait for setpoints (e.g. temperature) to equilibrate

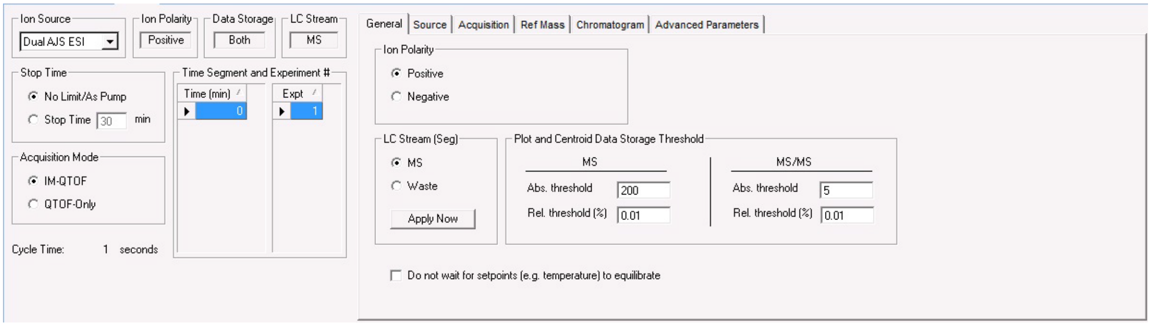
Figure 23. Method Editor window for a 6560 Ion Mobility Q-TOF in the Data Acquisition program

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.



Ion Source: Dual AJS ESI

Ion Polarity: Positive

Data Storage: Both

LC Stream: MS

Stop Time: ● No Limit/As Pump ○ Stop Time 30 min

Time Segment and Experiment #: Time (min) 0; Expt 1

Acquisition Mode: ● IM-QTOF ○ QTOF-Only

Cycle Time: 1 seconds

Ion Polarity: ● Positive ○ Negative

LC Stream (Seg): ● MS ○ Waste

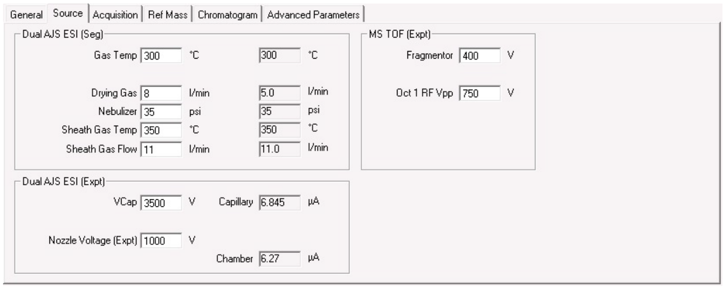
Plot and Centroid Data Storage Threshold

	MS	MS/MS
Abs. threshold	200	5
Rel. threshold (%)	0.01	0.01

☐ Do not wait for setpoints (e.g. temperature) to equilibrate

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

Steps	Detailed Instructions	Comments
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.



Dual AJS ESI (Seg)

Gas Temp	300	°C	300	°C
Drying Gas	8	l/min	5.0	l/min
Nebulizer	35	psi	35	psi
Sheath Gas Temp	350	°C	350	°C
Sheath Gas Flow	11	l/min	11.0	l/min

MS TOF (Expt)

Fragmentor 400 V

Oct 1 RF Vpp 750 V

Dual AJS ESI (Expt)

VCap 3500 V

Capillary 6.845 µA

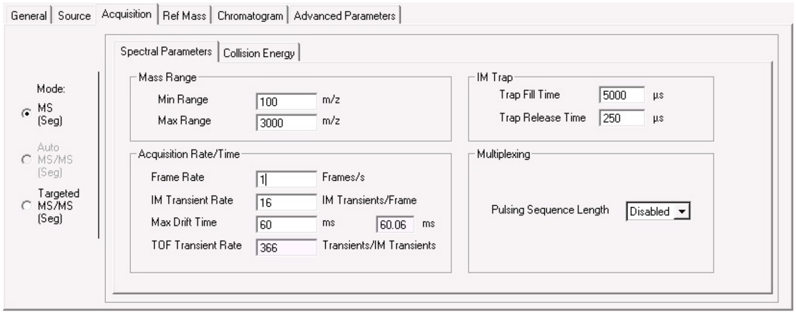
Nozzle Voltage (Expt) 1000 V

Chamber 6.27 µA

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 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** 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.	• If the Manual Tune tab is not visible, then on the **Instrument State** tab, click **Advanced Control**.
6 Enter the acquisition spectral parameters for MS mode as shown in Figure 26.	**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.	• 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.



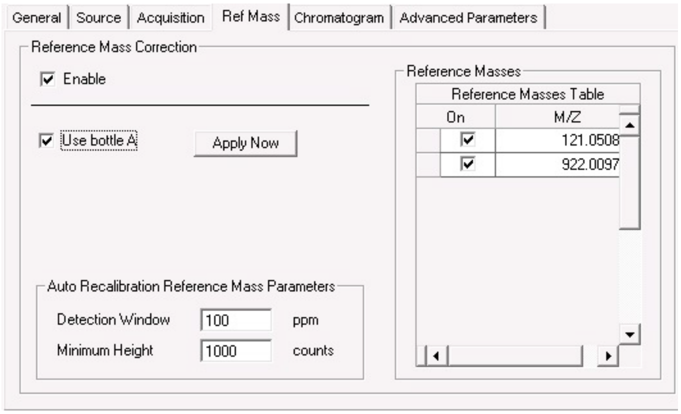
Mode:
● MS (Seg)
○ Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Min Range 100 m/z
Max Range 3000 m/z
Frame Rate 1 Frames/s
IM Transient Rate 16 IM Transients/Frame
Max Drift Time 60 ms 60.06 ms
TOF Transient Rate 366 Transients/IM Transients
Trap Fill Time 5000 µs
Trap Release Time 250 µs
Pulsing Sequence Length 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.	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**.



Reference Mass Correction

☑ Enable

☑ Use bottle A

Auto Recalibration Reference Mass Parameters

Detection Window 100 ppm

Minimum Height 1000 counts

Reference Masses

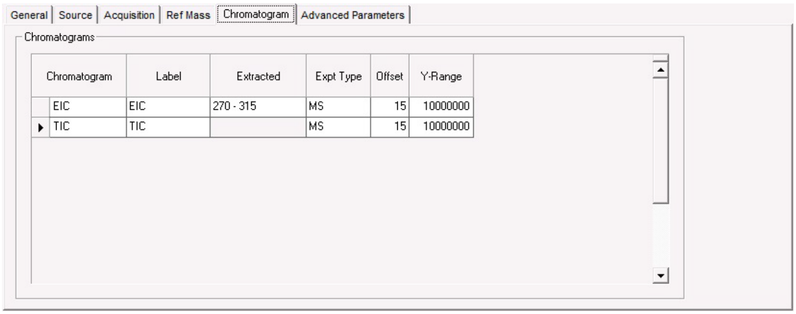
Reference Masses Table

On	M/Z
☑	121.0508
☑	922.0097

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.	**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.
9 Set up to change MS parameters during run: • Time Segment of 0 min. - Make sure you have selected an LC Stream of Waste. • Time Segment of 0.5 min. - Change LC Stream to MS.	**a** Click the **General** tab. **b** 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.
10 Save the method as *iii*_IM-MS_only.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_IM-MS_only.m` where *iii* are your initials. **d** Click **Save**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_only.m**.



Chromatogram	Label	Extracted	Expt Type	Offset	Y-Range
EIC	EIC	270 - 315	MS	15	10000000
TIC	TIC		MS	15	10000000

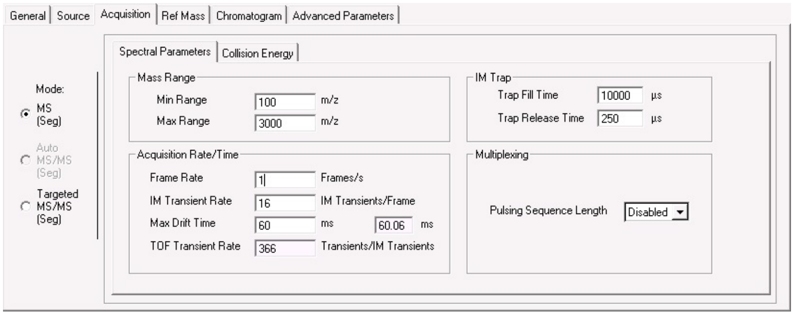
Figure 28. Chromatogram tab for Q-TOF parameters

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** Select the **0.5** minute **Time Segment**. **f** Right-click the selected **Time Segment** and click **Delete Time Segment**. **g** Click the **Acquisition** tab. **h** For **Frame rate**, type `1`. **i** 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.



Mode:
● MS (Seg)
○ Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Mass Range
Min Range 100 m/z
Max Range 3000 m/z

IM Trap
Trap Fill Time 10000 µs
Trap Release Time 250 µs

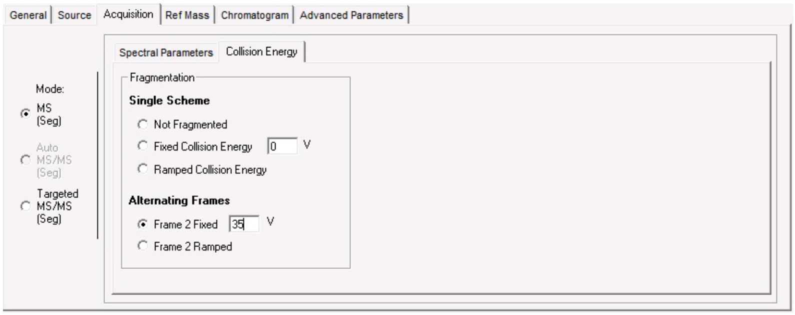
Acquisition Rate/Time
Frame Rate 1 Frames/s
IM Transient Rate 16 IM Transients/Frame
Max Drift Time 60 ms 60.06 ms
TOF Transient Rate 366 Transients/IM Transients

Multiplexing
Pulsing Sequence Length Disabled

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.



Mode:
● MS (Seg)
○ Auto MS/MS (Seg)
○ Targeted MS/MS (Seg)

Fragmentation

Single Scheme
○ Not Fragmented
○ Fixed Collision Energy 0 V
○ Ramped Collision Energy

Alternating Frames
● Frame 2 Fixed 35 V
○ Frame 2 Ramped

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

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

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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.

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.

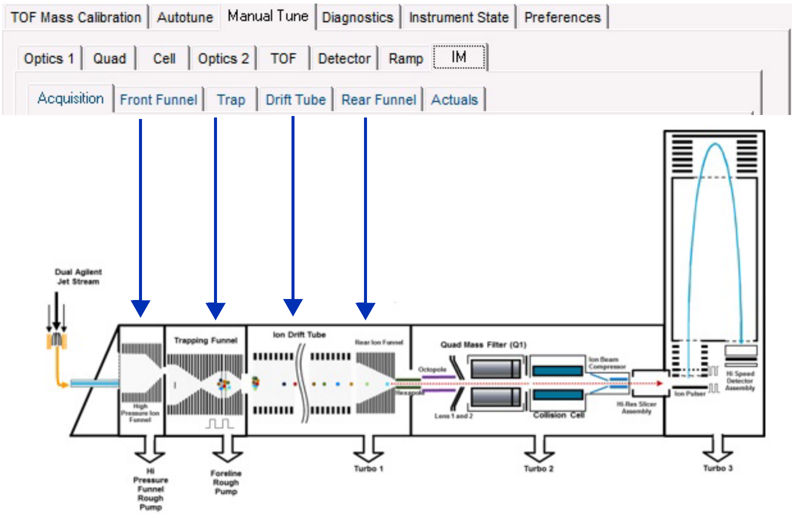


Figure 31. Manual Tune > IM tabs and the part of the instrument they affect

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.

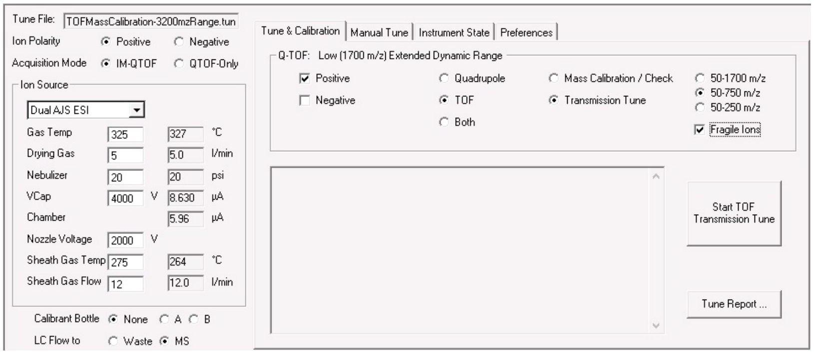
Experimental set up

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** 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. - 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 **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



Tune File: TOFMassCalibration-3200mzRange.tun

Ion Polarity ● Positive ○ Negative

Acquisition Mode ● IM-QTOF ○ QTOF-Only

Ion Source: Dual AJS ESI

Gas Temp	325	327	°C
Drying Gas	5	5.0	l/min
Nebulizer	20	20	psi
VCap	4000 V	8.630	µA
Chamber		5.96	µA
Nozzle Voltage	2000 V		
Sheath Gas Temp	275	264	°C
Sheath Gas Flow	12	12.0	l/min

Calibrant Bottle ● None ○ A ○ B

LC Flow to ○ Waste ● MS

Q-TOF: Low (1700 m/z) Extended Dynamic Range

☑ Positive ☐ Negative

○ Quadrupole ● TOF ○ Both

○ Mass Calibration / Check ● Transmission Tune

○ 50-1700 m/z ● 50-750 m/z ○ 50-250 m/z

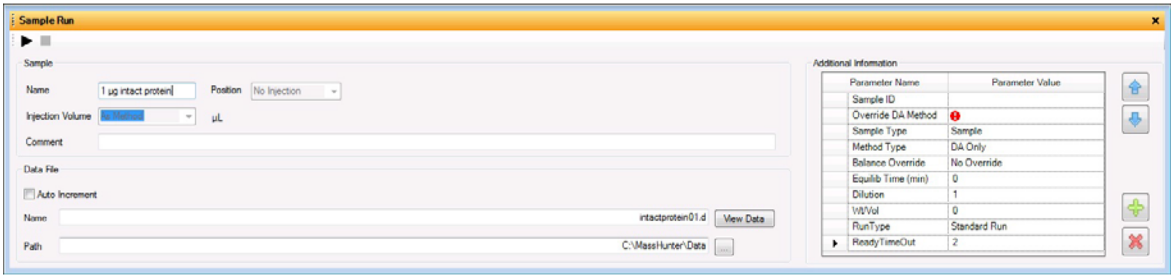
☑ Fragile Ions

Figure 32. Transmission tune for labile molecules

Steps	Detailed Instructions	Comments
3 Save the new settings to the tune file (*Autotune.tun*) and return to Acquisition.	**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.	- 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 **Method > Open**. **b** Select ***iii*_IM-MS_only.m**, and click **OK**. **c** Click the **Method Editor** window.	
5 Save the method as *iii*_IM-MS_labile.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_IM-MS_labile.m`, where *iii* are your initials. **d** Click **Save**.	- 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 Click the **Sample Run** window. 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.



Sample

Name: 1 µg intact protein

Position: No Injection

Injection Volume: As Method µL

Comment:

Data File

☐ Auto Increment

Name: intactprotein01.d

Path: C:\MassHunter\Data

Additional Information

Parameter Name	Parameter Value
Sample ID	
Override DA Method	❗
Sample Type	Sample
Method Type	DA Only
Balance Override	No Override
Equilib Time (min)	0
Dilution	1
W/Vol	0
RunType	Standard Run
ReadyTimeOut	2

Figure 33. Sample Run window in the main window

Steps	Detailed Instructions	Comments
7 Start the sample.	• Click **Run** ▶ on the Sample Run toolbar or **Run Sample** on the main 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. • The Lock button 🔒 on the main toolbar indicates that locked mode is on. Click to turn locked mode 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.

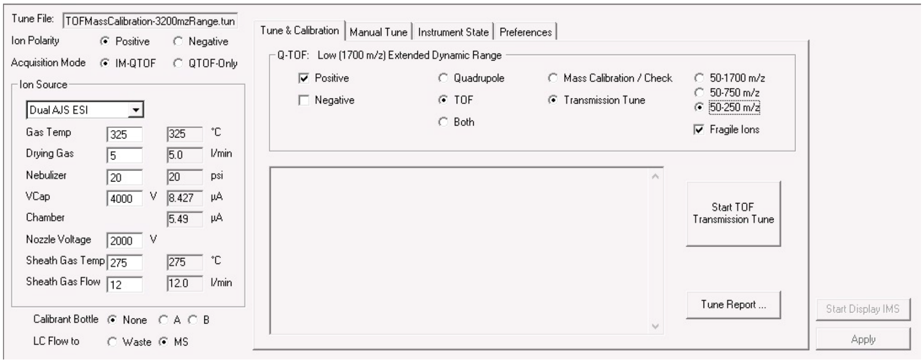
3 Acquire data.

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 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. • 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 **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



Tune File: TOFMassCalibration-3200mzRange.tun
Ion Polarity ● Positive ○ Negative
Acquisition Mode ● IM-QTOF ○ QTOF-Only

Ion Source: Dual AJS ESI

Gas Temp	325	325	°C
Drying Gas	5	5.0	l/min
Nebulizer	20	20	psi
VCap	4000 V	8.427	µA
Chamber		5.49	µA
Nozzle Voltage	2000 V		
Sheath Gas Temp	275	275	°C
Sheath Gas Flow	12	12.0	l/min

Calibrant Bottle ● None ○ A ○ B
LC Flow to ○ Waste ● MS

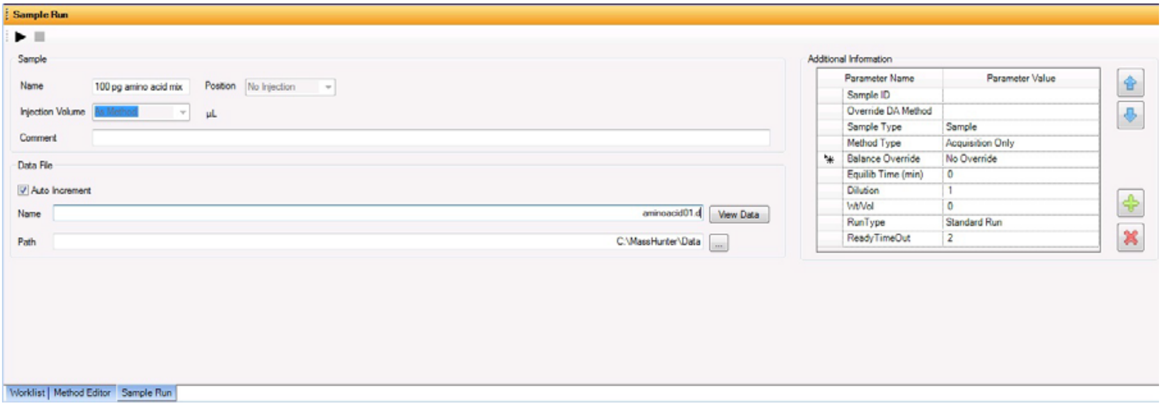
Q-TOF: Low (1700 m/z) Extended Dynamic Range
☑ Positive
☐ Negative
○ Quadrupole ● TOF ○ Both
○ Mass Calibration / Check ● Transmission Tune
○ 50-1700 m/z ○ 50-750 m/z ● 50-250 m/z
☑ Fragile Ions

Figure 34. Transmission tune for small compounds

Steps	Detailed Instructions	Comments
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.
4 Open the method for IM-QTOF created in **"Task 2. Set up an IM-MS method"** on page 36.	**a** Click **Method > Open**. **b** Select ***iii*_IM-MS_only.m**, and click **OK**. **c** Click the **Method Editor** window.	
5 Change the Advanced Parameters:	**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.	- 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.
6 Save the method as *iii*_IM-MS_small_molecules.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_IM-MS_small_molecules.m` where *iii* are your initials. **d** Click **Save**.	- For example, if your initials are PFH, then the method name is **pfh_IM-MS_small_molecules.m**.

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

Steps	Detailed Instructions	Comments
7 Enter this sample information: Name: **100 pg amino acid mix** Data file name: **aminoacid01.d**	**a** Click the **Sample Run** window. **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.



Sample
Name 100 pg amino acid mix
Position No Injection
Injection Volume As Method µL
Comment

Data File
☑ Auto Increment
Name aminoacid01.d
Path C:\MassHunter\Data

Additional Information

Parameter Name	Parameter Value
Sample ID	
Override DA Method	
Sample Type	Sample
Method Type	Acquisition Only
Balance Override	No Override
Equilib Time (min)	0
Dilution	1
Wt/Vol	0
RunType	Standard Run
ReadyTimeOut	2

Figure 35. Sample Run window in the main window

Steps	Detailed Instructions	Comments
8 Start the sample.	• Click **Run** ▶ on the Sample Run toolbar or the **Run Sample** on the main 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. • The Lock button 🔒 on the main toolbar indicates that locked mode is on. Click to turn locked mode on or off.
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

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

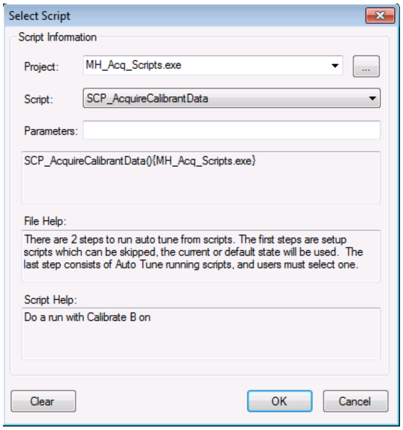
Steps	Detailed Instructions	Comments
1 Open the method for IM-QTOF created in **“Task 2. Set up an IM-MS method”** on page 36.	**a** Click **Method > Open**. **b** Select ***iii*_IM-MS_only.m**, and click **OK**.	• For example, if your initials are PFH, then the method name is **pfh_IM-MS_only.m**.
2 Save the method as *iii*_SulfaDrug_CCS.m.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_SulfaDrug_CCS.m` where *iii* are your initials. **d** Click **OK**.	• You save the method with a new name to make the example clearer to read. You will use this method to acquire the sample.
3 Open Data Acquisition to access the window for editing methods.	**a** Set **Context** on the main toolbar to **Acquisition**. **b** If needed, click **View > Method Editor** to open the Method Editor window. **c** Click the **Quat. Pump** tab. **d** For **Channel A Pump Flow Rate**, type `0.8`. **e** For **Run Time**, type `1.0`. **f** Click the **Multisampler** tab. **g** For **Injection Volume**, type `5`. **h** Set **Needle Wash** to **Standard Wash**. **i** For **Draw speed**, type `100`. **j** For **Eject speed**, type `400`. **k** Set **Injection Path Cleaning** to **Flush Port** for **3** seconds. **l** 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 **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type *`iii`*`_6560_CCS_IM_SingleF.m`, where *iii* are your initials. **d** Click **Save**.	- 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 ... . 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



Script Information

Project: MH_Acq_Scripts.exe

Script: SCP_AcquireCalibrantData

Parameters:

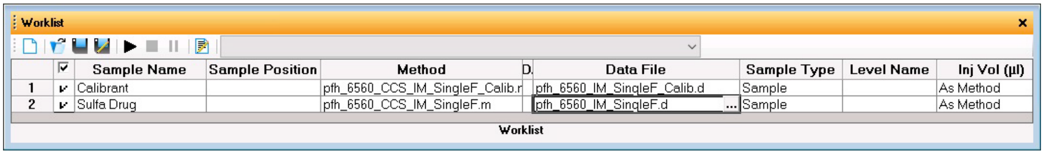
SCP_AcquireCalibrantData(){MH_Acq_Scripts.exe}

File Help:
There are 2 steps to run auto tune from scripts. The first steps are setup scripts which can be skipped, the current or default state will be used. The last step consists of Auto Tune running scripts, and users must select one.

Script Help:
Do a run with Calibrate B on

Figure 36. Select Script dialog box

Steps	Detailed Instructions	Comments
9 Save the method as *iii*_6560_CCS_IM_SingleF_Calib.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_6560_CCS_IM_SingleF_Calib.m` where *iii* are your initials. **d** Click **Save**.	• 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** Click the Worklist window. **b** Add two samples with the following information.	



	☑	Sample Name	Sample Position	Method	D.	Data File	Sample Type	Level Name	Inj Vol (µl)
1	✓	Calibrant		pfh_6560_CCS_IM_SingleF_Calib.r		pfh_6560_IM_SingleF_Calib.d	Sample		As Method
2	✓	Sulfa Drug		pfh_6560_CCS_IM_SingleF.m		pfh_6560_IM_SingleF.d	Sample		As Method

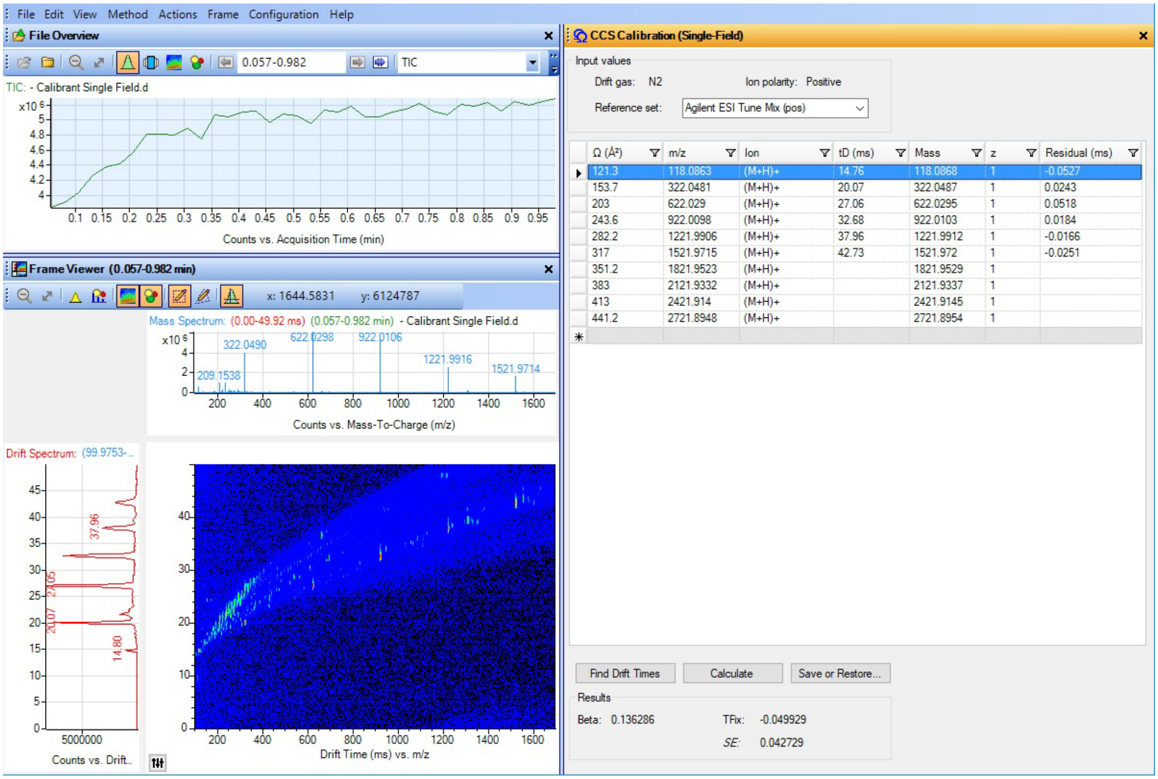
Figure 37. Worklist window with a calibrant and a sample

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 or **Run Worklist** on the main toolbar.	• 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.	**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



Input values

Drift gas: N2

Ion polarity: Positive

Reference set: Agilent ESI Tune Mix (pos)

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	

Results

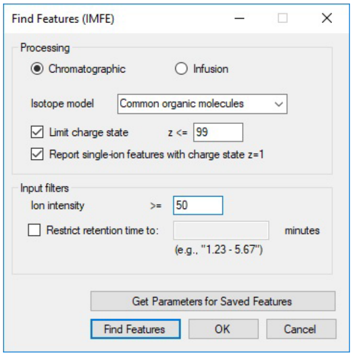
Beta: 0.136286

TFix: -0.049929

SE: 0.042729

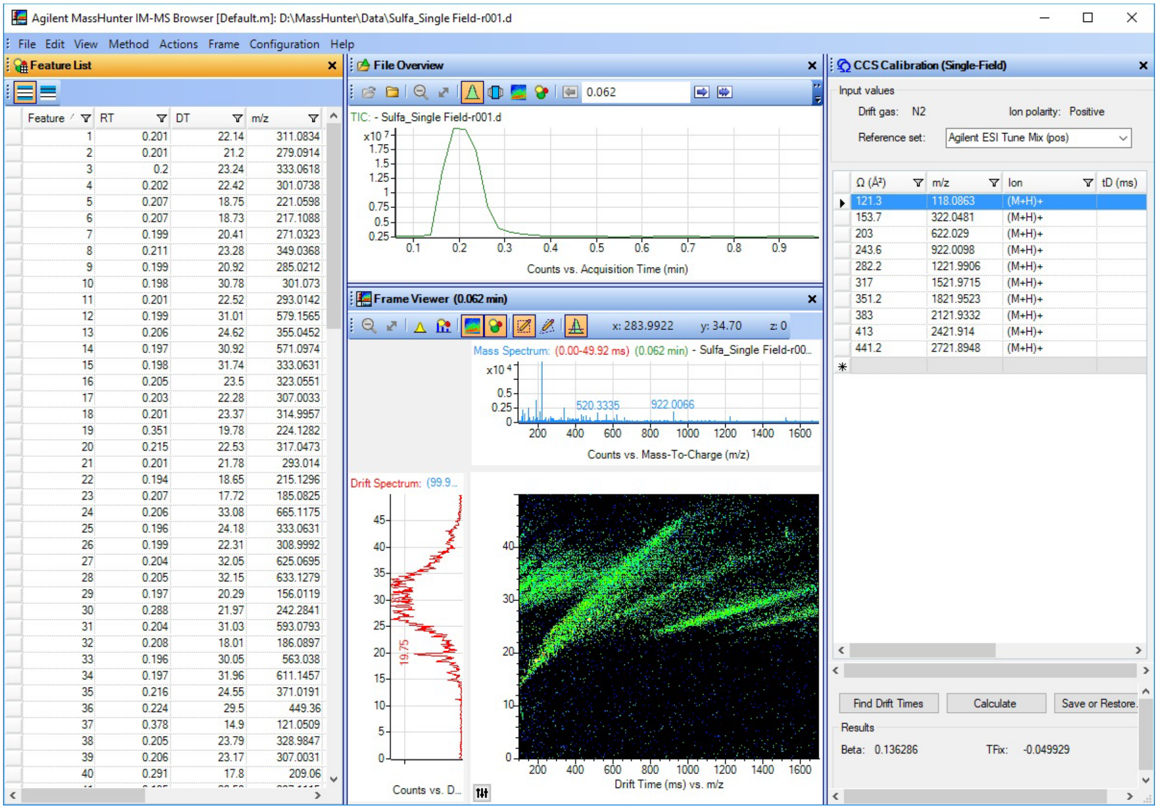
Figure 38. IM-MS Browser with Calibrant Single Field.d open

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**.  Processing ● Chromatographic ○ Infusion Isotope model: Common organic molecules ☑ Limit charge state z <= 99 ☑ Report single-ion features with charge state z=1 Input filters Ion intensity >= 50 ☐ Restrict retention time to: minutes (e.g., "1.23 - 5.67") **Figure 39.** Find Features (IMFE) dialog box (Single Field Ion Mobility)	• 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.**

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

Steps	Detailed Instructions	Comments



Feature	RT	DT	m/z
1	0.201	22.14	311.0834
2	0.201	21.2	279.0914
3	0.2	23.24	333.0618
4	0.202	22.42	301.0738
5	0.207	18.75	221.0598
6	0.207	18.73	217.1088
7	0.199	20.41	271.0323
8	0.211	23.28	349.0368
9	0.199	20.92	285.0212
10	0.198	30.78	301.073
11	0.201	22.52	293.0142
12	0.199	31.01	579.1565
13	0.206	24.62	355.0452
14	0.197	30.92	571.0974
15	0.198	31.74	333.0631
16	0.205	23.5	323.0551
17	0.203	22.28	307.0033
18	0.201	23.37	314.9957
19	0.351	19.78	224.1282
20	0.215	22.53	317.0473
21	0.201	21.78	293.014
22	0.194	18.65	215.1296
23	0.207	17.72	185.0825
24	0.206	33.08	665.1175
25	0.196	24.18	333.0631
26	0.199	22.31	308.9992
27	0.204	32.05	625.0695
28	0.205	32.15	633.1279
29	0.197	20.29	156.0119
30	0.288	21.97	242.2841
31	0.204	31.03	593.0793
32	0.208	18.01	186.0897
33	0.196	30.05	563.038
34	0.197	31.96	611.1457
35	0.216	24.55	371.0191
36	0.224	29.5	449.36
37	0.378	14.9	121.0509
38	0.205	23.79	328.9847
39	0.206	23.17	307.0031
40	0.291	17.8	209.06

Drift gas: N2

Ion polarity: Positive

Reference set: Agilent ESI Tune Mix (pos)

Ω (Å²)	m/z	Ion	tD (ms)
121.3	118.0863	(M+H)+	
153.7	322.0481	(M+H)+	
203	622.029	(M+H)+	
243.6	922.0098	(M+H)+	
282.2	1221.9906	(M+H)+	
317	1521.9715	(M+H)+	
351.2	1821.9523	(M+H)+	
383	2121.9332	(M+H)+	
413	2421.914	(M+H)+	
441.2	2721.8948	(M+H)+	

Beta: 0.136286

TFix: -0.049929

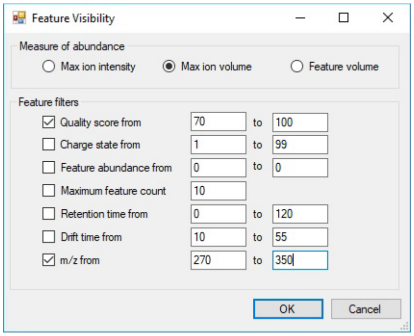
Figure 40. Find Features (IMFE) dialog box (Single Field Ion Mobility)

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**.	• 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



Feature Visibility

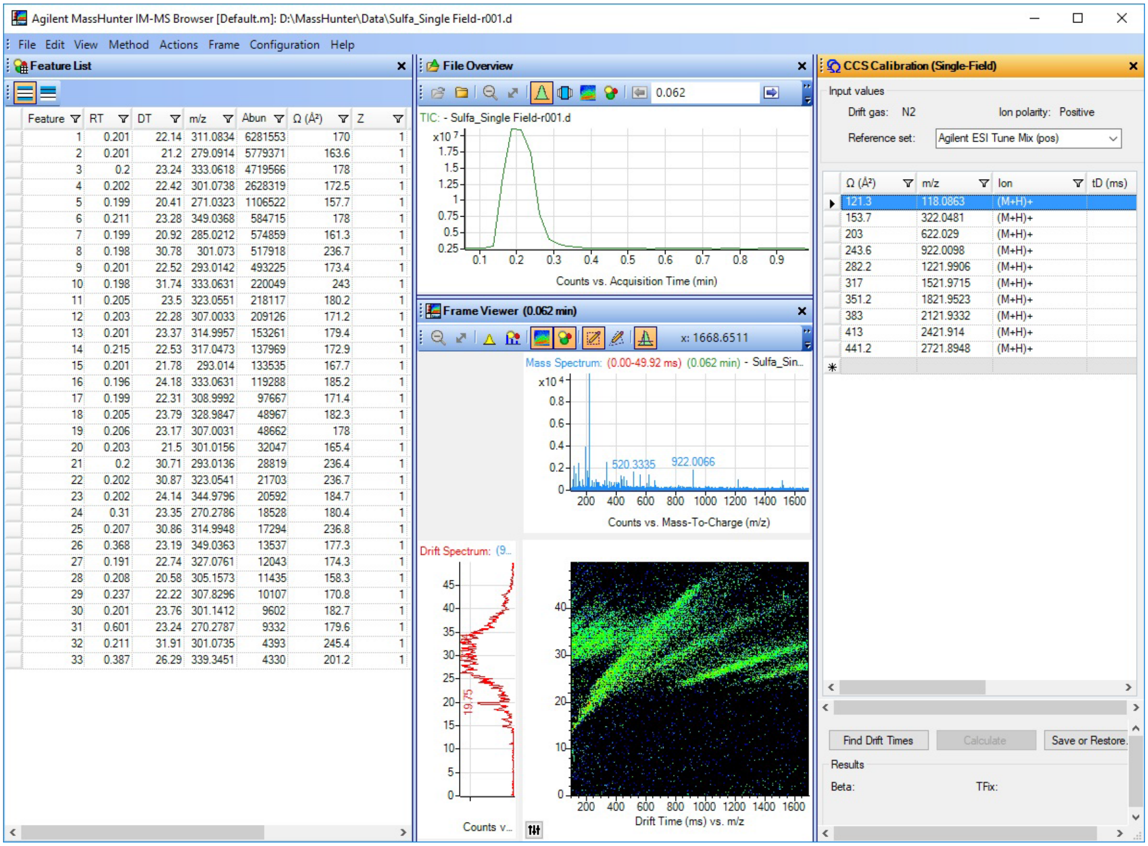
Measure of abundance

○ Max ion intensity ● Max ion volume ○ Feature volume

Feature filters

☑ Quality score from	70	to	100
☐ Charge state from	1	to	99
☐ Feature abundance from	0	to	0
☐ Maximum feature count	10		
☐ Retention time from	0	to	120
☐ Drift time from	10	to	55
☑ m/z from	270	to	350

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



Agilent MassHunter IM-MS Browser [Default.m]: D:\MassHunter\Data\Sulfa_Single Field-r001.d

Feature	RT	DT	m/z	Abun	Ω (Å²)	Z
1	0.201	22.14	311.0834	6281553	170	1
2	0.201	21.2	279.0914	5779371	163.6	1
3	0.2	23.24	333.0618	4719566	178	1
4	0.202	22.42	301.0738	2628319	172.5	1
5	0.199	20.41	271.0323	1106522	157.7	1
6	0.211	23.28	349.0368	584715	178	1
7	0.199	20.92	285.0212	574859	161.3	1
8	0.198	30.78	301.073	517918	236.7	1
9	0.201	22.52	293.0142	493225	173.4	1
10	0.198	31.74	333.0631	220049	243	1
11	0.205	23.5	323.0551	218117	180.2	1
12	0.203	22.28	307.0033	209126	171.2	1
13	0.201	23.37	314.9957	153261	179.4	1
14	0.215	22.53	317.0473	137969	172.9	1
15	0.201	21.78	293.014	133535	167.7	1
16	0.196	24.18	333.0631	119288	185.2	1
17	0.199	22.31	308.9992	97667	171.4	1
18	0.205	23.79	328.9847	48967	182.3	1
19	0.206	23.17	307.0031	48662	178	1
20	0.203	21.5	301.0156	32047	165.4	1
21	0.2	30.71	293.0136	28819	236.4	1
22	0.202	30.87	323.0541	21703	236.7	1
23	0.202	24.14	344.9796	20592	184.7	1
24	0.31	23.35	270.2786	18528	180.4	1
25	0.207	30.86	314.9948	17294	236.8	1
26	0.368	23.19	349.0363	13537	177.3	1
27	0.191	22.74	327.0761	12043	174.3	1
28	0.208	20.58	305.1573	11435	158.3	1
29	0.237	22.22	307.8296	10107	170.8	1
30	0.201	23.76	301.1412	9602	182.7	1
31	0.601	23.24	270.2787	9332	179.6	1
32	0.211	31.91	301.0735	4393	245.4	1
33	0.387	26.29	339.3451	4330	201.2	1

Input values

Drift gas: N2 Ion polarity: Positive

Reference set: Agilent ESI Tune Mix (pos)

Ω (Å²)	m/z	Ion	tD (ms)
121.3	118.0863	(M+H)+	
153.7	322.0481	(M+H)+	
203	622.029	(M+H)+	
243.6	922.0098	(M+H)+	
282.2	1221.9906	(M+H)+	
317	1521.9715	(M+H)+	
351.2	1821.9523	(M+H)+	
383	2121.9332	(M+H)+	
413	2421.914	(M+H)+	
441.2	2721.8948	(M+H)+	

Results

Beta: TFix:

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

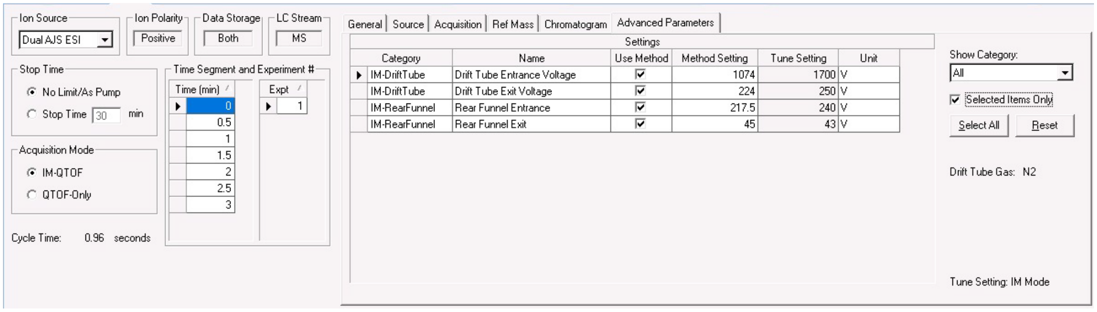
This task only applies if the alternate gas kit is installed.

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** Click **Method > Open**. **b** Select ***iii*_6560_CCS_IM_SingleF_Calib.m**, where *iii* are your initials, and click **OK**. **c** 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 56
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

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

Steps	Detailed Instructions	Comments



Ion Source: Dual AJS ESI
Ion Polarity: Positive
Data Storage: Both
LC Stream: MS

Stop Time
● No Limit/As Pump
○ Stop Time 30 min

Acquisition Mode
● IM-QTOF
○ QTOF-Only

Cycle Time: 0.96 seconds

Time Segment and Experiment #

Time (min)	Expt
0	1
0.5	
1	
1.5	
2	
2.5	
3	

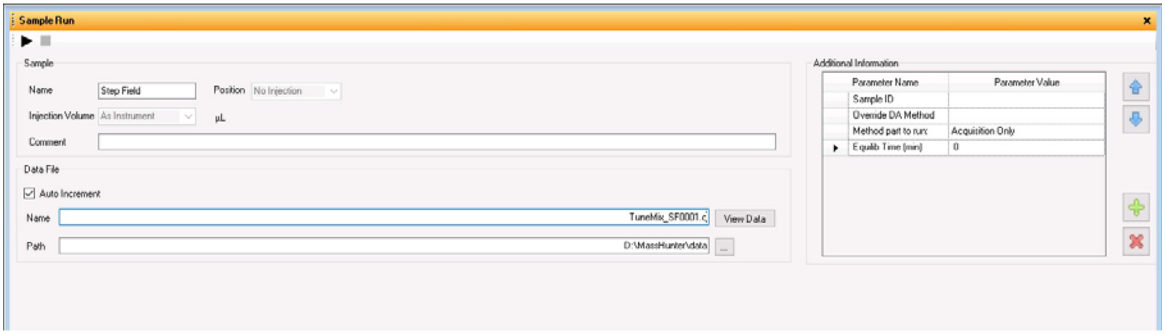
Settings

Category	Name	Use Method	Method Setting	Tune Setting	Unit
IM-DriftTube	Drift Tube Entrance Voltage	☑	1074	1700	V
IM-DriftTube	Drift Tube Exit Voltage	☑	224	250	V
IM-RearFunnel	Rear Funnel Entrance	☑	217.5	240	V
IM-RearFunnel	Rear Funnel Exit	☑	45	43	V

Show Category: All
☑ Selected Items Only
Drift Tube Gas: N2
Tune Setting: IM Mode

Figure 43. Advanced parameters for the first time segment

3 Save the method as *iii*_6560_CCS_IM_SteppedF.m, where *iii* are your initials.	**a** Click **Method > Save As**. **b** Go to the **\MassHunter\methods** folder. **c** Type `iii_6560_CCS_IM_SteppedF.m` where *iii* are your initials. **d** Click **Save**.	- 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** Click the **Sample Run** 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.



Sample
Name: Step Field
Position: No Injection
Injection Volume: As Instrument µL
Comment:

Data File
☑ Auto Increment
Name: TuneMix_SF0001.d
Path: D:\MassHunter\data

Additional Information

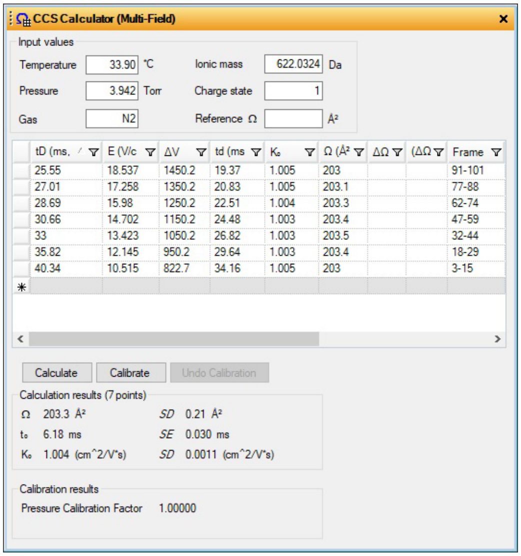
Parameter Name	Parameter Value
Sample ID	
Override DA Method	
Method part to run:	Acquisition Only
Equilib Time (min)	0

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.	• Click **Run** ▶ on the Sample Run toolbar or **Run Sample** ▶ on the main 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. • 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	**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.  **Figure 45.** CCS Calculator (Multi-Field) window with ionic mass 622	- Make sure the frame is within a single Time Segment.

Temperature 33.90 °C
Pressure 3.942 Torr
Gas N2
Ionic mass 622.0324 Da
Charge state 1
Reference Ω Å²

tD (ms,	E (V/c	ΔV	td (ms	K₀	Ω (Å²	ΔΩ	(ΔΩ	Frame
25.55	18.537	1450.2	19.37	1.005	203			91-101
27.01	17.258	1350.2	20.83	1.005	203.1			77-88
28.69	15.98	1250.2	22.51	1.004	203.3			62-74
30.66	14.702	1150.2	24.48	1.003	203.4			47-59
33	13.423	1050.2	26.82	1.003	203.5			32-44
35.82	12.145	950.2	29.64	1.003	203.4			18-29
40.34	10.515	822.7	34.16	1.005	203			3-15

Calculation results (7 points)
Ω 203.3 Å² *SD* 0.21 Å²
t₀ 6.18 ms *SE* 0.030 ms
K₀ 1.004 (cm^2/V*s) *SD* 0.0011 (cm^2/V*s)

Calibration results
Pressure Calibration Factor 1.00000

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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