MS/MS Library Creation of Q-TOF LC/MS Data for MassHunter PCDL Manager

Quick Start Guide

Use this guide to create a Personal Compound Database and Library (PCDL) as you acquire data from an Agilent 6400 Series Q-TOF LC/MS system.

This guide applies to these MassHunter programs:

- PCDL Manager B.04.00 and higher
- Data Acquisition for TOF/Q-TOF B.04.00 and higher

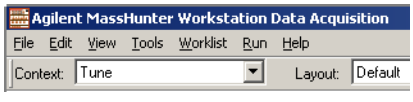
See the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide* for more information on data acquisition.

Agilent Technologies

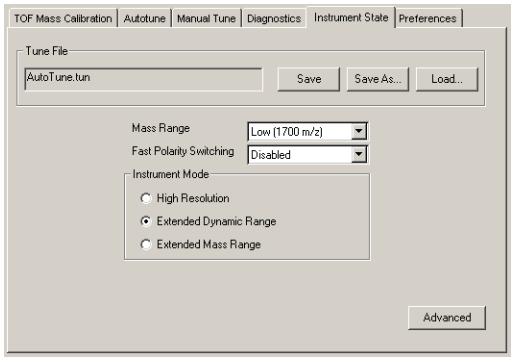
Step 1. Calibrate the Q-TOF LC/MS for low *m/z* ratios

See "Step 1. Calibrate the Q-TOF LC/MS for low m/z ratios" on page 24 for summary of these steps.

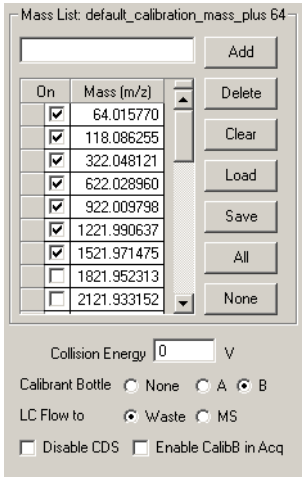
Do these steps in the MassHunter Data Acquisition program.



1 In the MassHunter Data Acquisition program, set the **Context** to **Tune**.



2 In the **Instrument State** tab, under **Instrument Mode**, click **Extended Dynamic Range**.



3 In the main window:

a Set **Calibrant Bottle** to **B**.

Make sure that Bottle B contains the ESI-L low concentration tuning mix (p/n G1969-85000).

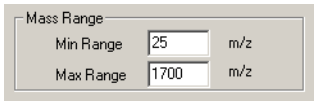
b Set **LC Flow** to **Waste**.

Do step 4 through step 11 twice, first for **Positive** ion polarity, and then again for **Negative** ion polarity.

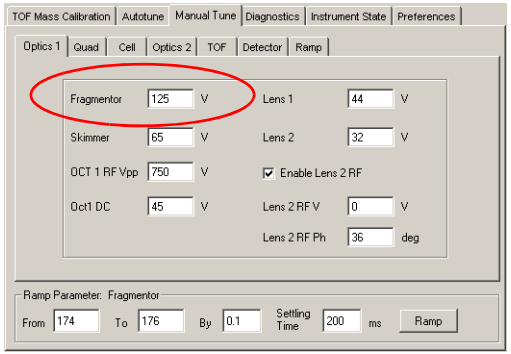
4 Set **Ion Polarity** to **Positive**. (Set to **Negative** the second time you do this step.)

5 To the **Mass List**, add the appropriate low *m/z* calibrant ions, depending on the ion polarity.

- Positive ion CH3CN-Na+ = 64.01577
- Negative ion CF3- = 68.99576

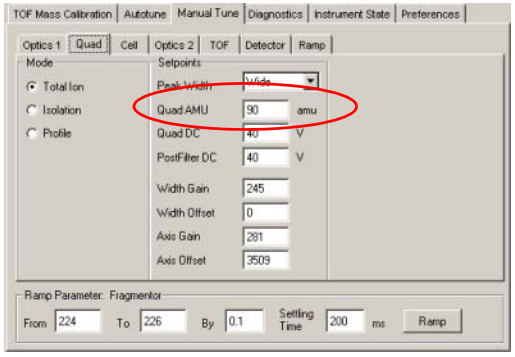


6 In **Manual Tune > TOF** tab, set **Min Range** to **25** m/z.



7 In the **Manual Tune > Optics 1** tab, reduce the **Fragmentor** voltage to approximately (±)**125 V** to increase the low *m/z* ion signals:

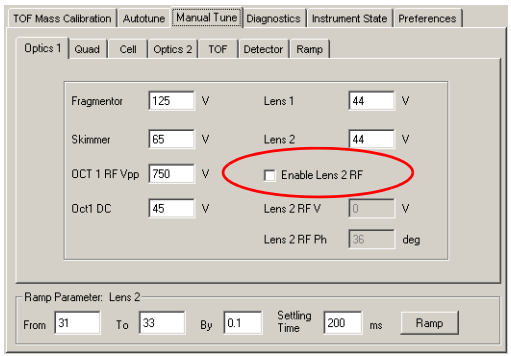
- *m/z* 64 for positive mode
- *m/z* 68 for negative mode



8 In the **Manual Tune > Quad** tab, reduce the Quad total transmission by setting **Quad AMU** to pass both *m/z* ratios with similar abundances:

- 64 and 1522 for positive mode
- 69 and 1634 for negative mode

Settings for positive mode is shown to the left.

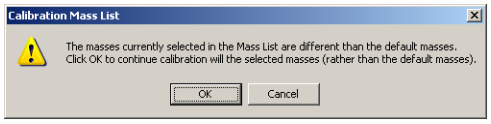


9 In the **Instrument State** tab, save the Tune file as **SmallTune.tun**.

10 In the **Manual Tune > Optics 1** tab, adjust the **Lens 2** voltage to reduce the abundance of the calibrant ions to 50-500K for mass calibration:

a Clear the **Enable Lens 2 RF** check box to turn off the Lens 2 RF.

b Set **Lens 2** voltage to a value greater than or equal to the **Lens 1** voltage.



11 In the **TOF Mass Calibration > Optics 1** tab, click **Calibration**.

If the Calibration Mass List warning appears, click **OK**.

A calibration graph appears. Good calibrations for measuring data for the PCDL and applications in which small molecules contribute to the MS/MS spectrum are seen in Figure 1.

12 Do step 4 through step 11 again for negative mode.

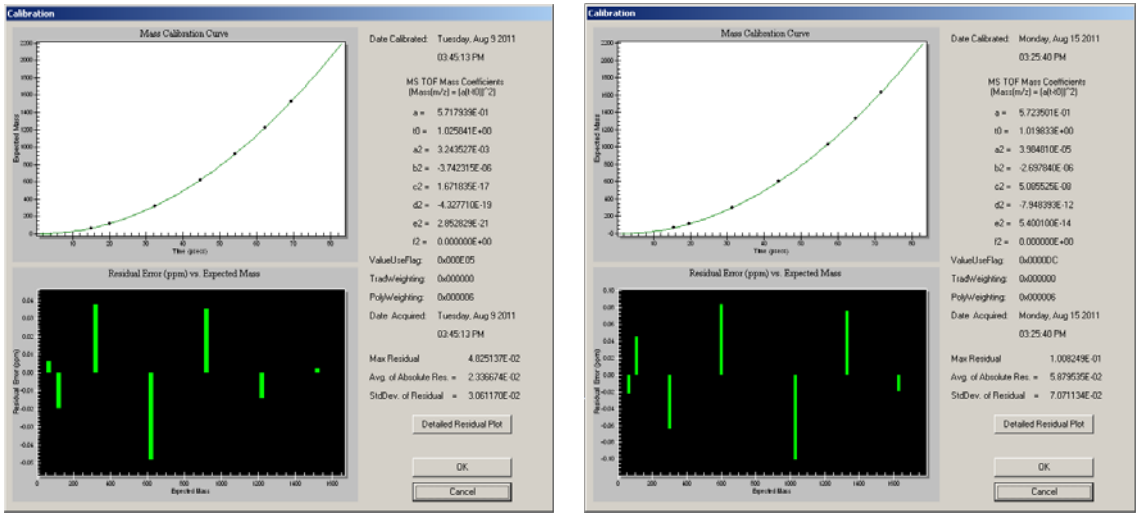
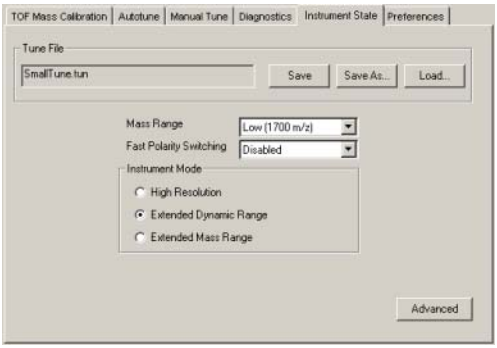
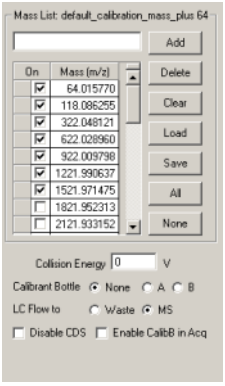


Figure 1 Good calibrations, positive mode (left) and negative mode (right)

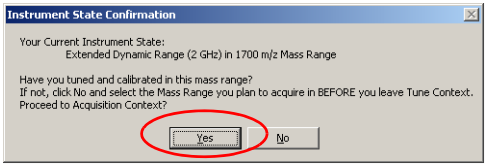


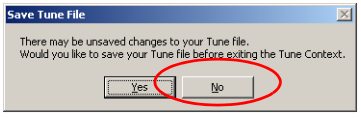
13 In the **Instrument State** tab, load **SmallTune.tun** again.



14 In the main window:

- **a** Set **Calibrant Bottle** to **None**.
- **a** Set **LC Flow** to **MS**.





15 Set **Context** to **Acquisition**.

If the **Instrument State Confirmation** warning appears, click **Yes**.

If the **Save Tune File** message appears, click **No**.

Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine

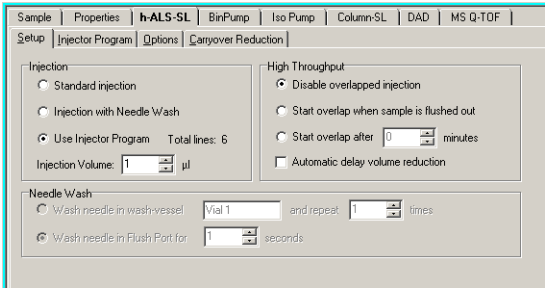
See "Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine" on page 26 for summary of these steps.

In this step, you create two new methods, one for Positive mode and one for Negative mode.

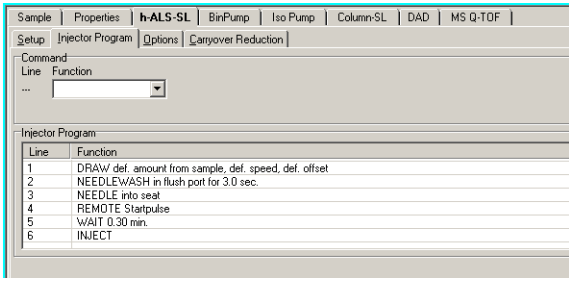
1 Bypass the column on the LC stack to do an FIA.

2 In the **Acquisition** context, use the default method as a template to create a new method.

Do the steps up to step 16 for Positive mode first, then repeat for Negative mode.

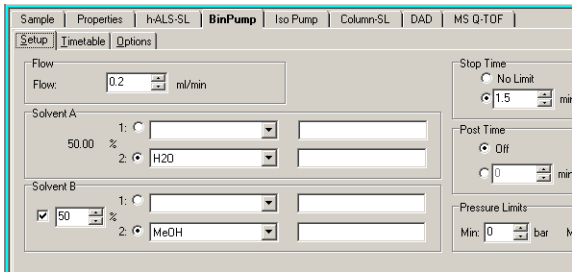


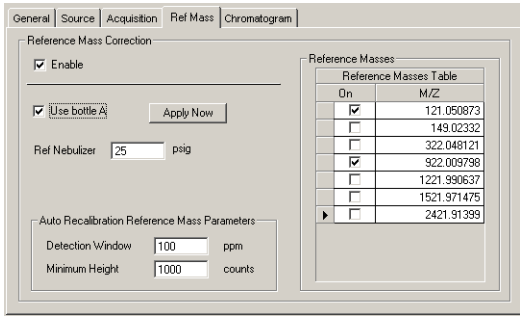
3 In the **h-ALS-SL > Setup** tab, click **Use Injector Program**.

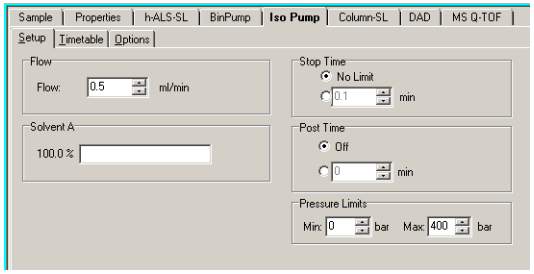


4 In the **h-ALS-SL > Injector Program** tab, create the list commands to acquire data with sufficient baseline before and after the LC peak.

- Draw sample
- Needle wash in flush port for 3.0 sec
- Needle into seat
- Remote Startpulse
- Wait 0.30 minute
- Inject







5 In the **BinPump > Setup** tab:

a Set **Flow** to **0.2 ml/min**.

b Set **Solvent B** to **50%**.

c Choose one of the following mobile phase combinations:

Water/Methanol containing 0.2% (v/v) acetic acid

Water/Acetonitrile containing 0.2% (v/v) acetic acid

d Set **Stop Time** to **1.5 min**.

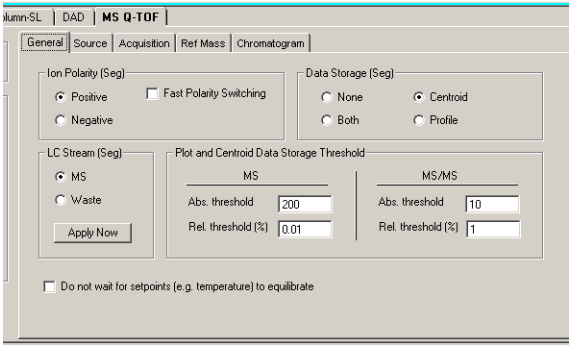
6 Include reference masses in the TOF spectra. Use *one* of these two methods:

- In the **MS Q-TOF > Ref Mass** tab, select **Use Bottle A**. Make sure that Bottle A contains the Reference Mass Solution prepared as described in the *Q-TOF Installation Guide*. Use the ES-TOF Reference Mass Solution Kit (p/n G1969-85001).

or

- Use the iso pump and a splitter (p/n G1607-60000) to infuse the Reference Mass Solution. In the **IsoPump > Setup** tab, set **Flow** to **0.5 ml/min**.

  This method minimizes analyte ion signal suppression.



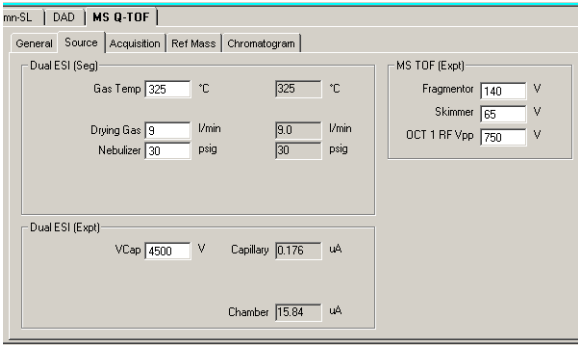


Figure 2 Positive mode

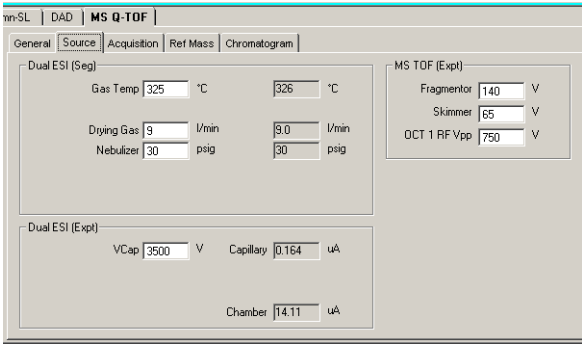


Figure 3 Negative mode

7 In the **MS Q-TOF > General** tab, under **Plot and Centroid Data Storage Threshold**:

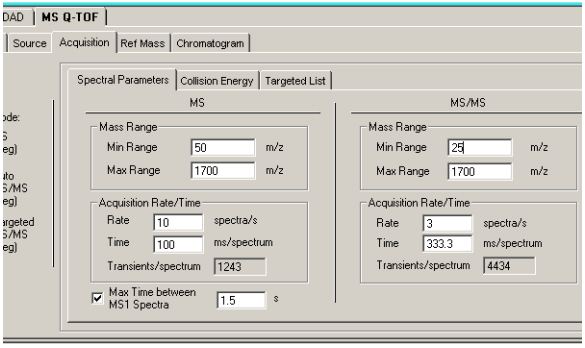
a Under **MS**, set **Abs. threshold** to **200** and **Rel. threshold (%)** to **0.01**.

b Under **MS/MS**, set **Abs. threshold** to **10** and **Rel. threshold (%)** to **1.0**.

8 In the **MS Q-TOF** tab, set **Ion Source** to **Dual ESI.** In the **Source** tab, set:

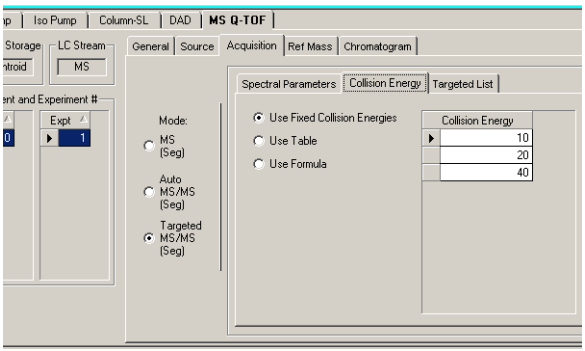
- **Gas Temp** to **325°C**
- **Drying Gas** to **9 L/min**
- **Nebulizer** to **30 psig**
- **VCap** to **4500 V** (positive) or **3500 V** (negative)
- **Fragmentor** to **140 V**
- **Skimmer** to **65 V**
- **Oct 1RF Vpp** to **750 V**

Do not change the default values for the Agilent Jet Stream and APCI sources.



9 In the **MS Q-TOF > Spectral Parameters** tab, set:

- **MS > Min Range** to **50 m/z**
- **MS > Rate** to **10 spectra/s**
- **MS > Time** to **100 ms/spectrum**
- **MS/MS > Min Range** to **25 m/z**
- **MS/MS > Rate** to **3 spectra/s**
- **MS/MS > Time** to **333 ms/spectrum**
- **Max Time between MS1 Spectra** to **1.5 s**

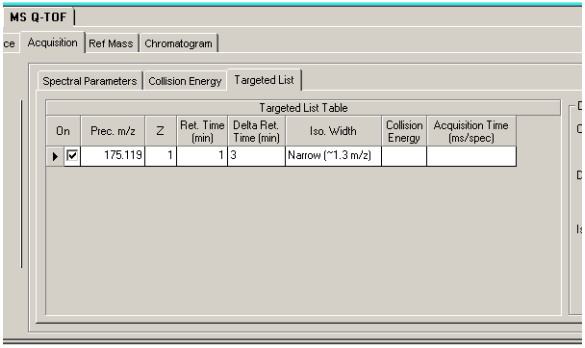


10 In the **MS Q-TOF > Acquisition** tab, click **Targeted MS/MS (Seg)**.

11 In the **MS Q-TOF > Acquisition > Collision Energy** tab:

a Click **Use Fixed Collision Energies**.

b Check that the **Collision Energies** are set to **10**, **20** and **40** eV.



12 In the **MS Q-TOF > Acquisition > Targeted List** tab, set:

- **Prec. m/z** to **175.1190** (positive) or **173.1044** (negative)
- **Z** to **1**
- **Ret.Time (min)** to **1**
- **Delta Ret. Time (min)** to **3**
- **Iso. Width** to **Narrow**
- **Collision Energy** to blank
- **Acquisition Time** to blank

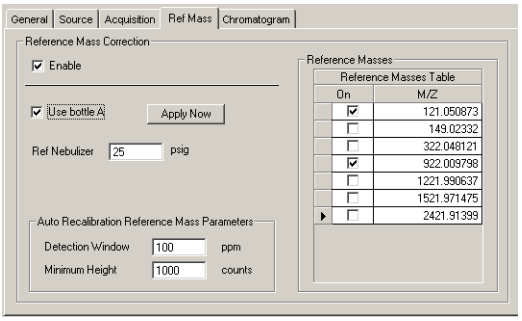


Figure 4 "Use Bottle A" method, positive mode

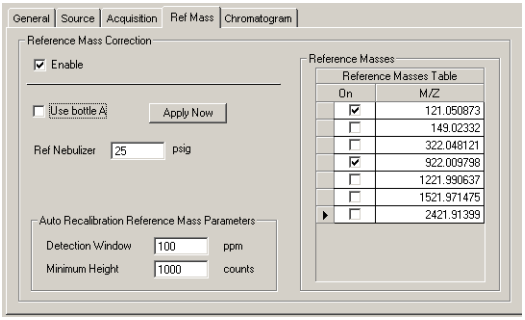


Figure 5 "Iso pump/splitter" method, positive mode

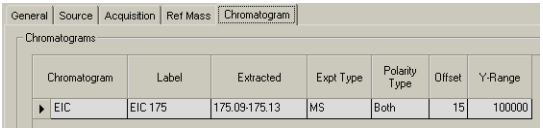


Figure 6 Positive mode

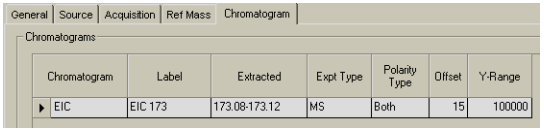


Figure 7 Negative mode

13 In **MS Q-TOF > Ref Mass** tab, select the following *m/z* ratios:

- **121.050873** and **922.009798** for positive mode
- **119.03632** and **980.016378** (acetate) for negative mode

14 Depending on which method you chose in step 6:

- "Use Bottle A" method – Mark the **Use bottle A** check box.
- "Iso pump/splitter" method – Clear the **Use bottle A** check box.

15 In the **MS Q-TOF > Chromatogram** tab, set:

- **Chromatogram** to **EIC**.
- **Extracted** to **175.09-175.13** (Positive mode) or **173.08-173.12** (Negative mode)
- **Y-range** to **100000**

16 Save the method:

- **Arginine FIA MSMS_pos.M** (Positive mode)
- **Arginine FIA MSMS_neg.M** (Negative mode)

17 Repeat step 2 through step 16 for Negative mode.

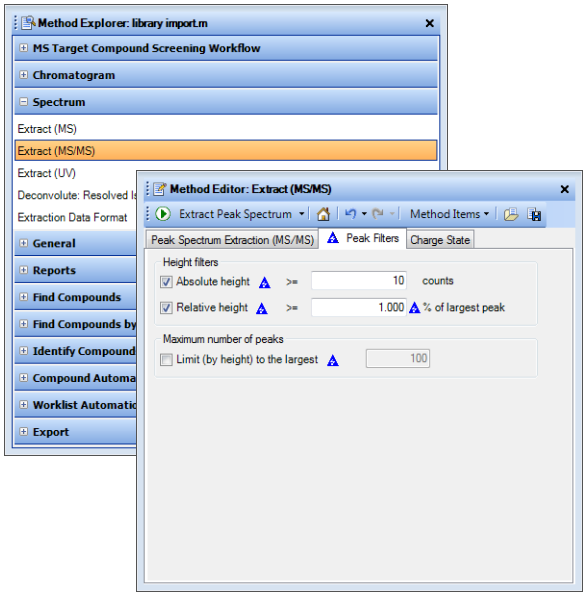
Step 3. Do an Instrument Quality Control Check before data acquisition

See “Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine” on page 29 for summary of these steps.

Do these steps in the MassHunter Qualitative Analysis program.

1 Open the data files in MassHunter Qualitative Analysis.

2 Create a new method using the default method as a template.

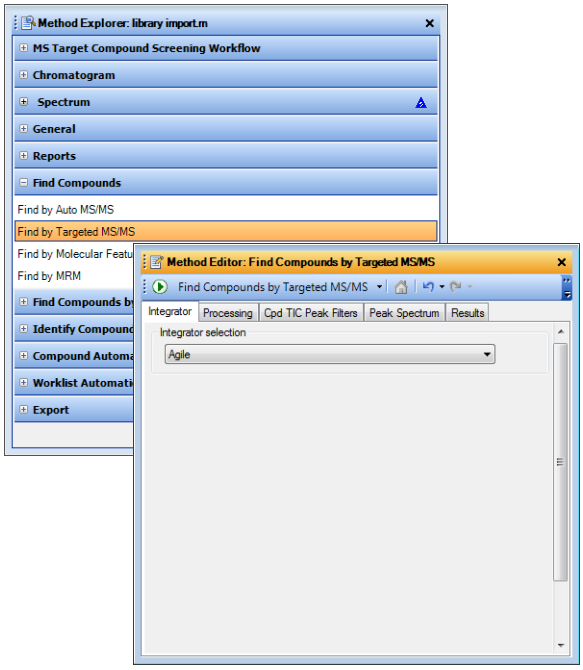


3 Set Extract (MS/MS) parameter:

a In **Method Explorer > Spectrum**, select **Extract (MS/MS)**.

b In the **Peak Filters** tab, set:

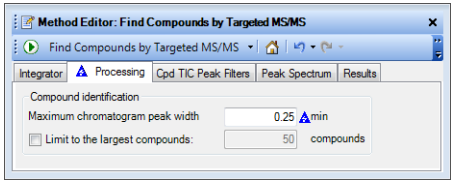
- **Absolute height** to **10 counts**
- **Relative height** to **1.000 % of largest peak**



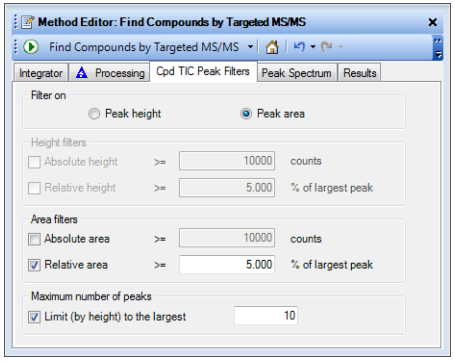
4 Set Find Compounds by Targeted MS/MS parameters:

a In **Method Explorer > Find Compounds**, select **Find by Targeted MS/MS**.

b In the **Integrator** tab, set **Integrator selection** to **Agile**.

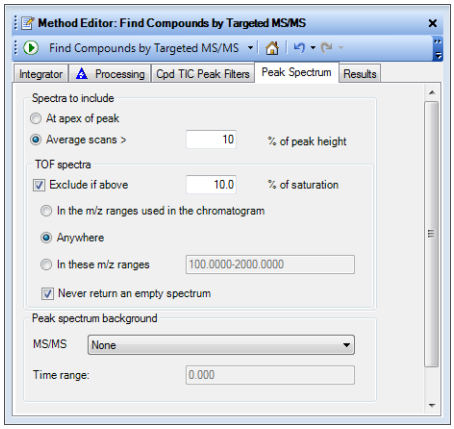


c In the **Processing** tab, set the **Maximum chromatogram peak width** to **0.25 min**.



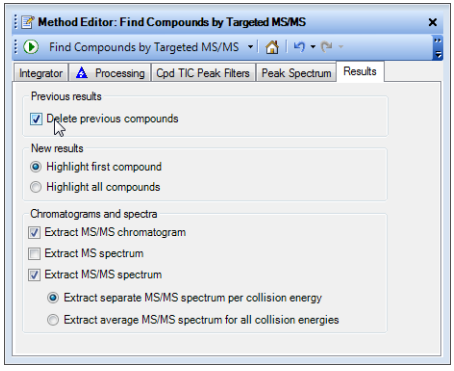
d In the **Cpd TIC Peak Filters** tab, set:

- **Relative area** to **5.000 % of largest peak**
- **Limit (by height) to the largest** to **10**



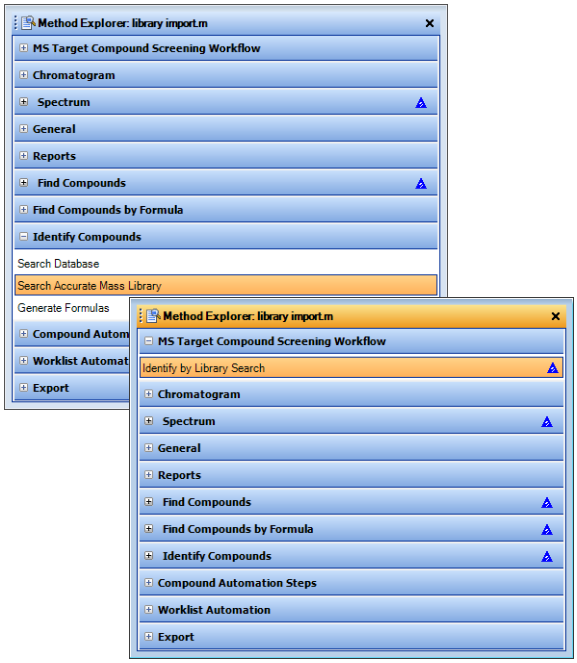
e In the **Peak Spectrum** tab, set:

- **Average scan** to **10 % of peak height**
- **Exclude if above** to **10% of saturation**



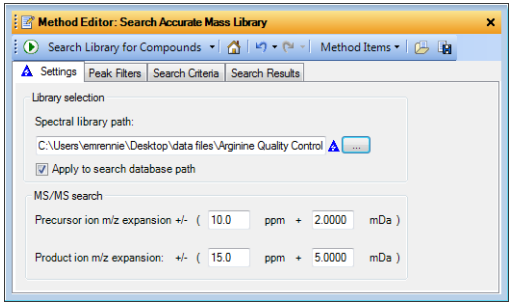
f In the **Results** tab, mark the check boxes for **Extract MS/MS chromatogram** and **Extract MS/MS spectrum**.

g Click the **green arrow** next to **Find Compounds by Targeted MS/MS**.



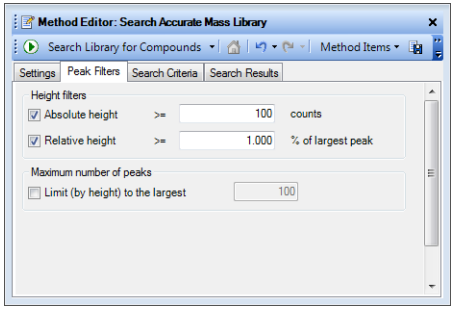
5 Set Search Accurate Mass Library parameters:

a In **Method Editor**, click either **Identify Compounds > Search Accurate Mass Library**, or click **MS Target Compound Screening Workflow > Identify by Library Search**.



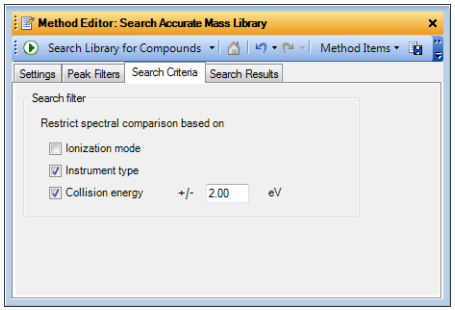
b In the Settings tab, set:

- **Spectral library path** to the library path. Use the browse button to select the path.
- **Precursor ion m/z expansion** to **10 ppm ± 2 mDa**
- **Product ion m/z expansion** to **15 ppm ± 5 mDa**

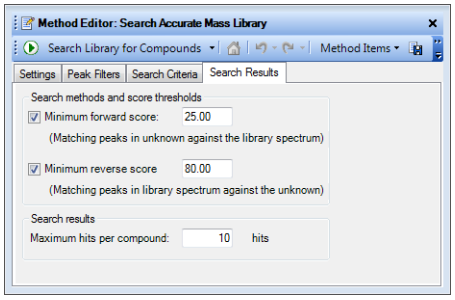


c In the **Peak Filters** tab, set:

- **Absolute height** to **100 counts**
- **Relative height** to **1.000 % of largest peak**



d In the **Search Criteria** tab, set **Collision energy** to **+/- 2.00 eV**.



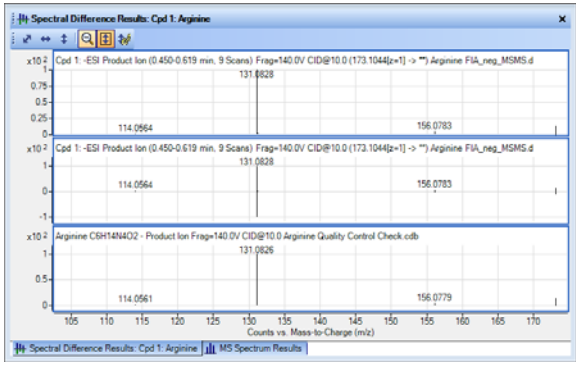
e In the **Search Results** tab, set:

- **Minimum forward score** to **25.00**
- **Minimum reverse score** to **80.00**

f Click the **green arrow** next to **Search Library for Compounds.** Expand the **Compound List** results.

6 Inspect the **Compound List** (see Figure 8) and make sure that:

- compound name is **Arginine**
- **Chromatogram Results** show only one LC peak
- **Forward** and **Reverse** scores are **98** or greater for all collision energies



7 Click the compound and make sure that the **Best** option is selected. Review the MS/MS library match using difference results.

Make sure all library ions line up with acquired data.

✔ If the quality check fails:

1 Check mass axis calibration.

2 Check mass range settings.

3 Review protocol.

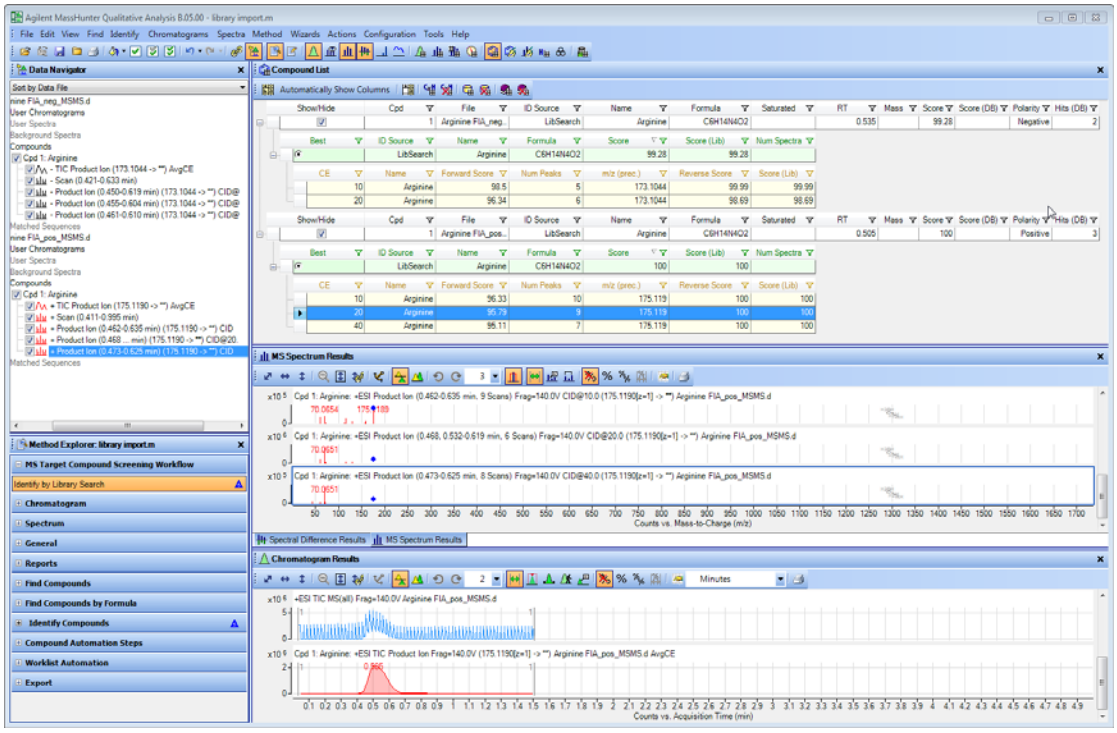


Figure 8 Compound list, Chromatogram and MS Spectrum Results

Step 4. Do a Quality Control Check on acquired data

1 In the MassHunter Qualitative Analysis program, open the data files and process the data using Find by Targeted MS/MS. Use the same steps that are described in “See “Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine” on page 26 for summary of these steps.” on page 6.

2 Make sure that:

- The Chromatogram results show only one LC peak.
- Each collision energy shows good signal/noise ratio.

The following conditions will result in bad spectra which will result in incorrect library matching.

- Base peaks that have less than 1000 counts.

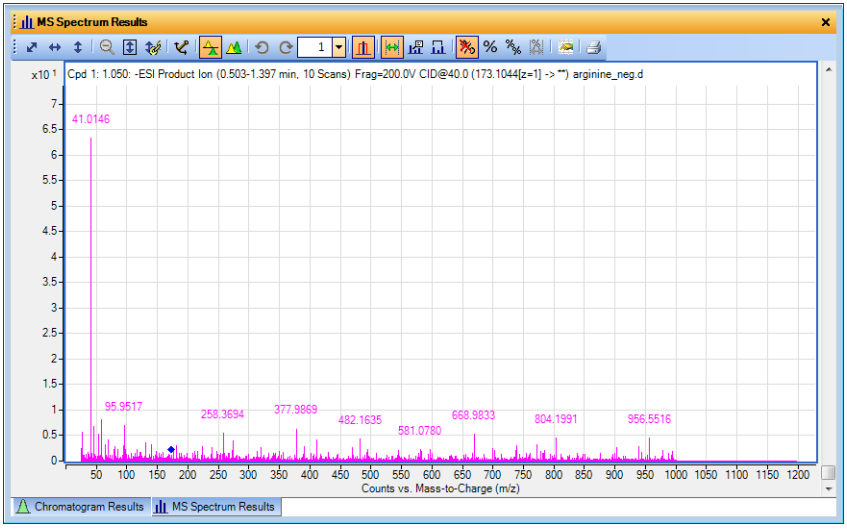


Figure 9 MS Spectrum Results for a low intensity spectrum

- Detector saturation on MS/MS base peaks. This situation occurs with high response compounds or high sample concentration. If the prior procedure are followed, TOF spectra with more than 10% saturation are not extracted. The resulting product ion spectra may then be of low intensity. The 10 eV CID spectrum is particularly affected by saturation, which can result in a lower intensity 10 eV spectrum along with good intensity 20 and 40 eV spectra. If the saturation is not severe, the data can still be used.

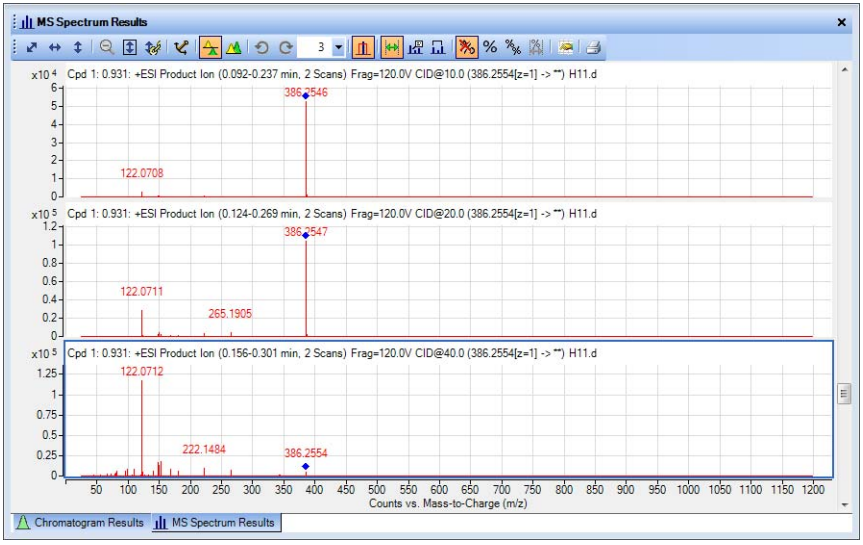


Figure 10 Usable extracted MS/MS spectra which show saturation effects.

Step 5. Create a custom PCDL

Custom PCDL creation is described in the *PCDL Manager Quick Start Guide*, in "Editing a custom PCDL" and "Spectral searching and editing". The topics in these sections are included here.

To edit compounds in a custom PCDL

Do this step to edit information for compounds, to add a new compound, or to remove a compound.

1 Open the custom PCDL of interest.

2 Enable editing by marking Allow Editing on the PCDL menu.

3 Do a search using either of the following (described in the *PCDL Manager Quick Start Guide*):

- "To find compounds"
- "To find compounds from a mass list"

4 When the search is complete, click the **Edit Compounds** tab.

5 To update information for a particular compound:

a Select the compound of interest by clicking on it in the **Search Results** table. Information for the selected compound is displayed in the **Edit Compounds** tab.

b Update information on the left side of the Edit Compounds tab, such as Name, Mass, RT, Formula, CAS or ChemSpider ID, and Ion type.

c Update structure information if desired in either of the following ways:

- Click on the MOL Text tab and paste in MOL file text that you have copied from a molecular drawing tool (**Ctrl+V**), or
- Click open file in the Structures area to open a .MOL file.

d Click **Update Selected**. The information for the compound is updated in the PCDL and the information you entered in Step 5b is reflected in the Results table.

6 To add a new compound to the PCDL:

a Click **Add New**. A new compound is created in the PCDL with a mass of 0 named “New Compound” and the new compound is displayed at the end of the Results table.

b Enter or change information for the new compound as described in step 5.

c Click **Update Selected**. The information you entered for the new compound is updated in the PCDL and the information is reflected in the Results table.

As an alternative, you can do these steps to add a new compound:

- Enter information for a new compound on the left side of the Edit Compounds tab, such as Name, Mass, RT, Formula, CAS ID, and Ion type, then click Save As New. A new compound is created in the PCDL using the specified values. The new compound is displayed at the end of the Results table.

7 To remove a compound from the PCDL:

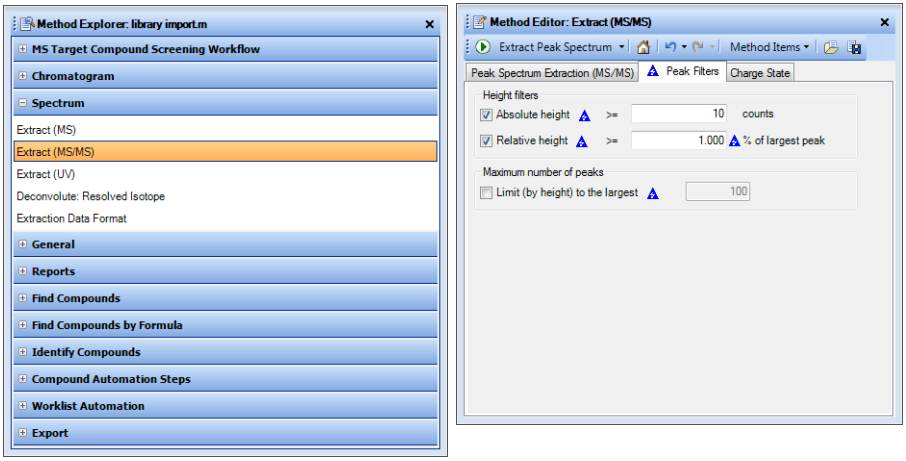
a Select the compound of interest by clicking on it in the Search Results table. Use **Ctrl+click** or **Shift+click** to select multiple compounds for deletion.

b Click **Delete Selected**.

To set the Extract MS/MS settings to reduce spectral noise

Do this step before you import the MS/MS spectra into PCDL Manager.

1 In Method Explorer, under **Spectrum**, select **Extract (MS/MS)**.



2 In the Peak filters tab, set:

- **Absolute height** to **10 counts**
- **Relative height** to **1.000 % of largest peak**
- **Maximum number of peaks** to no limit (clear the check box)

These parameters are recommended for the default setting to ensure that the library match is not affected by spectral noise. However, the parameters can be adjusted to vary the number of ions imported into the PCDL as required.

To import spectra from a .CEF file

Do this step to load spectra into MassHunter PCDL Manager that have been exported to a .CEF file from another program, such as MassHunter Qualitative Analysis program.

1 Click to display the **Spectral Search** or **Edit Spectra** tab.

2 Click **Load Spectra**.

3 Select the **.CEF** file of interest and click **Open**.

If profile spectra or non-MS/MS spectra are contained in the file, a message is displayed saying that they have been skipped. Only centroided MS/MS spectra are loaded for use in MassHunter PCDL Manager.

The spectra appear in the Acquired spectra table. See the following topics in the *PCDL Manager Quick Start Guide* for further use of imported spectra:

- "To search spectra in a PCDL"
- "To add, move, or remove spectra in a custom PCDL"
- "To clean up noisy spectra" (also included in this guide)

To copy spectra from MassHunter Qualitative Analysis

1 Open the data file of interest in MassHunter Qualitative Analysis software.

2 Run **Find by Targeted MS/MS** or **Find by Auto MS/MS**, depending on how the data was acquired.

3 Expand the compounds of interest in Data Navigator to see the spectral results.

4 Select the product ion spectra of interest.

5 If the data was acquired in Profile mode, right-click and select **Convert Profile to Centroid and Replace** from the shortcut menu.

6 Right-click the MS Spectrum Results window and select **Copy to Clipboard**. All spectra displayed in the MS Spectrum Results window are copied, not just the highlighted ones.

To paste spectra into MassHunter PCDL Manager

1 Copy spectra from MassHunter Qualitative Analysis as described in "To copy spectra from MassHunter Qualitative Analysis" on page 22.

2 Click to display the **Spectral Search** or **Edit Spectra** tab.

3 Right-click in the **Acquired spectra** table and select **Paste Spectra**.

If profile spectra or non-MS/MS spectra was copied from MassHunter Qualitative Analysis, a message is displayed saying that they have been skipped. Only centroided MS/MS spectra are pasted into MassHunter PCDL Manager. The spectra are listed in the Acquired spectra table.

NOTE Spectra cannot be pasted into the Library spectra table.

To clean up noisy spectra

Do this step to remove small noise peaks from spectra before or after you add them to a custom PCDL.

1 Select the spectrum of interest in either the Acquired spectra or Library spectra table on the Edit Spectra tab.

2 Click the **Mass Lists** tab in the **Spectral Plot** window. By default, the masses are sorted in descending order by relative abundance value. Click the **Rel Abund** column header if you want the lowest relative abundance values (likely noise peaks) to appear at the top of the table.

3 Select the masses to delete by clicking on them in the Acquired masses or Library masses list. Use **Ctrl-click** or **Shift-click** to select multiple masses.

4 Right-click in the **Acquired masses** or **Library masses** list and select **Delete Masses** from the shortcut menu.

5 Update the PCDL as follows, depending on whether the spectrum modified in step 4 was an Acquired or Library spectrum.

- Click **Add Spectra** to add an Acquired spectrum to the selected library compound.
- Click **Update Spectra** to update the selected library compound with a modified Library spectrum.

Summary of Steps

These tables summarize the steps that you need to do to set up to create a PCDL as you acquire Q-TOF LC/MS data. You must do the steps in the order listed.

Step 1. Calibrate the Q-TOF LC/MS for low m/z ratios

Summary for calibration setup

	In this location	Do this step	To this value
1	Toolbar	Set **Context**	Tune
2	**Instrument State** tab	Set **Instrument Mode**	Extended Dynamic Range
3	main window	Set **Calibrant Bottle**	B (use ESI-L tuning mix)
		Set **LC Flow**	Waste

Summary for calibration steps (do first for Positive mode and then for Negative mode)

			Positive Mode	Negative Mode
	In this location	Do this step	To this value	
4	**Ion Polarity**	Set	Positive	Negative
5	**Mass List** table	Add ion	$CH3CN\text{-}Na^+$ = 64.01577	$CF3^-$ = 68.99576
6	**Manual Tune > TOF** tab	Set **Min Range**	25 *m/z*	25 *m/z*
7	**Manual Tune > Optics 1** tab	Reduce **Fragmentor**	±125 V to increase *m/z* 64	±125 V to increase *m/z* 68
8	**Manual Tune > Quad** tab*	Set **Quad AMU**	to pass *m/z* 64 and 1522 with similar abundances	to pass *m/z* 69 and 1634 with similar abundances
9	**Instrument State** tab	Save Tune file	SmallTune.tun	SmallTune.tun
10	**Manual Tune > Optics 1** tab†	Set **Enable Lens 2 RF**	blank (clear check box)	blank (clear check box)
		Set **Lens 2**	≥ **Lens 1** value	≥ **Lens 1** value
11	**TOF Mass Calibration > Optics 1** tab	Click **Calibration**.		
	Calibration Mass List warning	Click **OK**		

Summary for calibration steps (do first for Positive mode and then for Negative mode) (continued)

			Positive Mode	Negative Mode
	In this location	**Do this step**	**To this value**	
12	Repeat for Negative mode			

* This step reduces Quad total transmission.

† This step reduces the abundance of the calibrant ions to 50K to 500K for mass calibration.

Summary for post-calibration steps

	In this location	**Do this step**	**To this value**
13	**Instrument State** tab	Load saved tune file	SmallTune.tun
14	main window	Set **Calibrant Bottle**	None
		Set **LC Flow**	MS
15	Toolbar	Set **Context**	Acquisition
	Instrument State Confirmation message	Click **Yes**	
	Save Tune File message	Click **No**	

Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine

Summary for instrument setup

	In this location	Do this step
1	LC stack	Bypass the column to do FIA

Summary for method creation (do first for Positive mode and then for Negative mode)

			Positive Mode	Negative Mode
	In this location	**Do this step**	**To this value**	
2	**Acquisition context**	Use default method to create a new method.		
3	**h-ALS-SL > Setup** tab	Click **Use Injector Program**		
4	**h-ALS-SL > Injector Program** tab	Create **Injector Program**	• Draw sample • Needle wash in flush port for 3.0 sec • Needle into seat • Remote Startpulse • Wait 0.30 minute • Inject	

5	**BinPump > Setup** tab	Reduce **Fragmentor**	• Set **Flow** to **0.2 ml/min**.
			• Set **Solvent B** to **50%**.
			• Choose one of the following mobile phase combinations:
			  • Water/Methanol containing 0.2% (v/v) acetic acid
			  • Water/Acetonitrile containing 0.2% (v/v) acetic acid
			• Set **Stop Time** to **1.5 min**.	

6	**MS Q-TOF > Ref Mass** tab* *or*	Select **Use Bottle A**. Use prepared Reference Mass Solution in bottle A.		
	IsoPump > Setup tab†	Set **Flow**	0.5 ml/min	0.5 ml/min

Summary for method creation (do first for Positive mode and then for Negative mode) (continued)

			Positive Mode	Negative Mode
	In this location	**Do this step**	**To this value**	
7	**MS Q-TOF > General** tab, under **Plot and Centroid Data Storage Threshold**	Set **MS > Abs. threshold**	200	200
		Set **MS > Rel. threshold (%)**	0.01	0.01
		Set **MS/MS > Abs. threshold**	10	10
		Set **MS/MS > Rel. threshold (%)**	1.0	1.0
8	**MS Q-TOF** tab	Set **Ion Source**	Dual ESI	Dual ESI
	MS Q-TOF > Source tab For AJI and APCI sources, use default settings.	Set parameters	• **Gas Temp** to **325°C** • **Drying Gas** to **9 L/min** • **Nebulizer** to **30 psig** • **VCap** to **4500 V** • **Fragmentor** to **140 V** • **Skimmer** to **65 V** • **Oct 1RF Vpp** to **750 V**	• **Gas Temp** to **325°C** • **Drying Gas** to **9 L/min** • **Nebulizer** to **30 psig** • **VCap** to **3500 V** • **Fragmentor** to **140 V** • **Skimmer** to **65 V** • **Oct 1RF Vpp** to **750 V**
9	**MS Q-TOF > Spectral Parameters** tab	Set parameters	• **MS > Min Range** to **50 m/z** • **MS > Rate** to **10 spectra/s** • **MS > Time** to **100 ms/spectrum** • **MS/MS > Min Range** to **25 m/z** • **MS/MS > Rate** to **3 spectra/s** • **MS/MS > Time** to **333 ms/spectrum** • **Max Time between MS1 Spectra** to **1.5 s**	
10	**MS Q-TOF > Acquisition**	Click **Targeted MS/MS (Seg)**		
11	**MS Q-TOF > Acquisition > Collision Energy** tab	Click **Use Fixed Collision Energies**		
		Set **Collision Energies**	**10**, **20** and **40**	**10**, **20** and **40**

Summary for method creation (do first for Positive mode and then for Negative mode) (continued)

			Positive Mode	Negative Mode
	In this location	**Do this step**	**To this value**	
12	**MS TOF > Acquisition > Targeted List** tab	Set parameters	• **Prec. m/z** to **175.1190** • **Z** to **1** • **Ret.Time** to **1** • **Delta Ret. Time** to **3** • **Iso. Width** to **Narrow** • **Collision Energy** to blank • **Acquisition Time** to blank	• **Prec. m/z** to **173.1044** • **Z** to **1** • **Ret.Time** to **1** • **Delta Ret. Time** to **3** • **Iso. Width** to **Narrow** • **Collision Energy** to blank • **Acquisition Time** to blank
13	**MS Q-TOF > Ref Mass** tab	Select **Reference Masses** values	**121.050873** and **922.009798**	**119.03632** and **980.016378**
14	**MS Q-TOF > Ref Mass** tab	Set **Use Bottle A**	Depends on how Reference Masses are included in the TOF spectra: • Selected (mark the check box)* or • Not selected (clear the check box)†	
15	**MS Q-TOF > Chromatogram** tab	Set **Chromatogram**	EIC	EIC
		Set **Extracted**	175.09-175.13	173.08-173.12
		Set **Y-range**	100000	100000
16		Save method	Arginine FIA MSMS_pos.M	Arginine FIA MSMS_neg.M

* Do this step if you choose to use Bottle A to include reference masses in the TOF spectra.

† Do this step if you choose to use the iso pump and a splitter to include reference masses in the TOF spectra.

Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine

Summary for Instrument Quality Control Check

	In this location	Do this step	To this value
1	MassHunter Qualitative Analysis	Open data files	
2	MassHunter Qualitative Analysis	Use default template to create new method	
3	**Method Explorer > Spectrum > Extract (MS/MS)**	Set **Peak Filters** parameters	• **Absolute height** to **10 counts** • **Relative height** to **1.000 % of largest peak**
4	**Method Explorer > Find Compounds > Find by Targeted MS/MS**	Set **Integrator** parameters	• **Integrator selection** to **Agile**
		Set **Processing** parameters	• **Maximum chromatogram peak width** to **0.25 min**
		Set **Cpd TIC Peak Filters** parameters	• **Relative area** to **5.000 % of largest peak** • **Limit (by height) to the largest** to **10**
		Set **Peak Spectrum** parameters	• **Average scan** to **10 % of peak height** • **Exclude if above** to **10% of saturation**
		Set **Results** parameters	Mark the check boxes for: • **Extract MS/MS chromatogram** • **Extract MS/MS spectrum**
		Click the green arrow.	

Summary for Instrument Quality Control Check

	In this location	Do this step	To this value
5	**Method Editor** > **Identify Compounds** > **Search Accurate Mass Library** *or* **Method Editor> MS Target Compound Screening Workflow > Identify by Library Search**	Set **Settings** parameters	• **Spectral Library path** to appropriate path • **Precursor ion m/z expansion** to **10 ppm ± 2 mDa** • **Product ion m/z expansion** to **15 ppm ± 5 mDa**
		Set **Peak Filters** parameters	• **Absolute height** to **100 counts** • **Relative height** to **1.000 % of largest peak**
		Set **Search Criteria** parameters	• Set **Collision energy** to **+/- 2.00 eV**.
		Set **Search Results** parameters	• **Minimum forward score** to **25.00** • **Minimum reverse score** to **80.00**
		Click the green arrow.	
6	Compound List	Inspect Compound List	Check that: • the name of the compound is Arginine • the Chromatogram results show only one LC peak • the Forward and Reverse scores are 98 or greater for all collision energies
7	Spectral Differences Result	Make sure all library ions line up with acquired data.	

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In This Book

This book contains instructions to set up MassHunter Data Acquisition and Qualitative Analysis methods that create libraries compatible with MassHunter PCDL Manager.

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