

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

## **Quick Start Guide**

Step 1. Calibrate the Q-TOF LC/MS for low m/z ratios 2
Step 2. Set up a Flow Injection Analysis (FIA) method for Arginine 6
Step 3. Do an Instrument Quality Control Check before data acquisition 11
Step 4. Do a Quality Control Check on acquired data 17
Step 5. Create a custom PCDL 19
To edit compounds in a custom PCDL 19
To set the Extract MS/MS settings to reduce spectral noise 21
To import spectra from a .CEF file 22
To copy spectra from MassHunter Qualitative Analysis 22
To paste spectra into MassHunter PCDL Manager 23
To clean up noisy spectra 23

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.



## 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.

🚟 Agilent Mass	Hunter Worksta	ition Data	Acquis	ition	
<u>File E</u> dit <u>V</u> iew	<u>T</u> ools <u>W</u> orklist	<u>R</u> un <u>H</u> elp	)		
Context: Tune		🛨 L	ayout:	Default	
TOF Mass Calibration	Autotune Manual Tune	Diagnostics	Instrumer	nt State Pr	eferences
Tune File					
AutoTune.tun		S	ave !	Save As	Load
Ľ,					
	Mass Range	Low (1700 r	n/z)	-	
	Fast Polarity Switching	Disabled		-	
	Instrument Mode				
	C High Resolution				
	Extended Dynami     Extended Mass B	ic Hange			
	C Extended mass in	ango			
					Advanced

-Mass List: default_calibration_mass_plus 64-				
			Add	
On	Mass (m/z)		Delete	
	64.015770			
	118.086255		Clear	
	322.048121			
	622.028960		Load	
V	922.009798		Sava	
	1221.990637			
	1521.971475		AIL	
	1821.952313			
	2121.933152	-	None	
Collision Energy 0 V				
Calibrant Bottle 🔿 None 🔿 A 💿 B				
LC Flow to 📀 Waste C MS				
🔲 Disable CDS 📄 Enable CalibB in Acq				

- **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

Mass Range Min Range 25 Max Range 1700	m/z m/z	
TOF Mass Calibration Autotune	fanual Tune Diagnostics I	nstrument State Preferences
Optics 1 Quad Cell Optics	2 TOF Detector Ran	qr
	<u> </u>	
Fragmentor 125	V Lens 1	44 V
Skimmer 65	V Lens 2	32 V
OCT 1 RF Vpp 750	V 🔽 Enable	Lens 2 RF
Oct1 DC 45	V Lens 2 RF	V 0 V
	Lens 2 BF	Ph 36 deg
		,
- Dama Darameter, Fragmenter		
Ramp Parameter: Fragmentor	Settling	
From 174 10 176	By 0.1 Time	200 ms hamp
TOF Mass Calibration   Autotune	Manual Tune Diagnostics	Instrument State Preferences
Optics 1 Quad Cell Optics	s 2   TOF   Detector   Ra	mp
Mode Setp	oints	-
Total Ion Peak	Wide 2	4
C Isolation Quad	JAMU 90 ami	·
C Profile Quad	IDC 40 V	
Post	-itter DC 40 V	
Widt	h Gain 245	
Width	h Offset 0	
Axis I	Gain 281	
Axis I	Offset 3509	
Ramp Parameter: Fragmentor	a di sanaan	
From 224 To 226	By 0.1 Setting Time	200 ms Ramp
-		
TOF Mass Calibration Autotune	anual Tune Diagnostics	Instrument State Preferences
Optics 1 Quad Cell Optics	s 2   TOF   Detector   Ran	np
Fragmentor 125	V Lens 1	44 V
Skimmer 65	V Lens 2	44 V
OCT 1 RF Vpp 750	V 🗆 Enable	Lens 2 RF
Oct1 DC 45		
	V Lens 2 RF	V 0 V

- 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.

By 0.1

Setting 200 ms Ramp

Ramp Parameter: Lens 2

To 33

From 31

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

Calibratio	on Mass List 🔀
<u>.</u>	The masses currently selected in the Mass List are different than the default masses. Click OK to continue calibration will the selected masses (rather than the default masses).
	OK Cancel

#### 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)



Save Tune File	×
There may be unsaved changes to your Tune file. Would you like to save your Tune file before exiting the T	fune Context.
<u>Yes</u> <u>N</u> o	

#### 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.

 $\times$ 

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

## 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.

Sample )	Properties h-ALS-SL E	BinPump   Iso Pump   Column-SL   DAD   MS Q-TOF			
Setup Injector Program Options Carryover Reduction					
Injection O Sta O Inje	) andard injection jection with Needle Wash	High Throughput C Disable overlapped injection C Start overlap when sample is flushed out			
ତ Use Injector Program Total lines: 6     C Start overlap after     □ minutes       Injection Volume:     1 µl     □ Automatic delay volume reduction					
Needle Wash C Wash needle in washwessel Vial 1 and repeat T times					
Sample   Properties   h-ALS-SL   BinPump   Iso Pump   Column-SL   DAD   MS Q-TOF   Setup   Iriector Program   Options   Carryover Reduction					
Sample )	Properties   <b>h-ALS-SL</b>   E	SinPump   Iso Pump   Column-SL   DAD   MS Q-TOF			
Sample ] Setup !n Command Line Fur 	Properties   <b>h-ALS-SL</b>   E jector Program   <u>Options</u>   <u>C</u> arry Inclion	SimPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction			
Sample ] Setup In Command Line Fur 	Properties   <b>h-ALS-SL</b>   E jector Program   <u>Options</u>   <u>Carry</u> Inction	SinPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction			
Sample ) Setup In Command Line Fur 	Properties   h-ALS-SL   E jector Program   Options   Carry I ction f f f ogram Function	SinPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction			
Sample ] Setup In Command Line Fur - Injector Pr Line 1	Properties h-ALS-SL E jector Program Options Carry nction rogram Function DRAW def. amount from samp	SimPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction   sile, def. speed, def. offset			
Sample ] Setup In Command Line Fur ]	Properties h-ALS-SL E jector Program Options Cerry f cotion rogram Function DRAW det, smouth from samp DRAW det, smouth from samp	SinPump ] Iso Pump ] Column-SL ] DAD ] MS Q-TDF ] over Reduction ] sle, def. speed, def. offset ar 3.0 sec.			
Sample ] Setup In Command Line Fur 	Properties h-ALS-SL E jector Program Options Carry to cogram DFAnction DFANV def. amount from samp NEEDLE vito seat BEMOTE Standale	inPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction   			
Sample Setup In Command Line Fur 	Properties h-ALS-SL E jector Program Options Carry notion Function DRAW del. amount from samp NEEDLEWASH in flush port fn NEEDLEWASH in flush port fn	SinPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction   			
Sample ) Setup In Command Line Fur  Injector Pr Line 1 2 3 4 5 6	Properties h-ALS-SL E jector Program Options Carry f notion Pranction PRAW def. amount from same NEEDLEWASH in flush poot fi NEEDLE into seat REMOTE Starbulie WAIT 0.30 min. INJECT	SinPump   Iso Pump   Column-SL   DAD   MS Q-TOF   over Reduction   			

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

	Sample Properties h-ALS-SL BinPump Iso Pump Column-SL DAD	MS Q-TOF
	Setup Imetable Options	
	Flow Flow 0.2 in m/min	Stop Time O No Limit I.5 mi
	Soverk A 1: C 50.00 % 2: C H20	Post Time © Off © 0 = min
	Solvent B 1: C 50 1 2: C 2: C MeDH	Pressure Limits
I		

eterence Mass Correction		
✓ Enable	Reference Mass	es
	Reference	e Masses Table
	On	M/Z
Use bottle A Apply Now	<b>V</b>	121.050873
		149.02332
Ref Nebulizer 25 DSig		322.048121
	V	922.009798
		1221.990637
		1521.971475
Auto Recalibration Reference Mass Parameters		2421.91399
Detection Window 100 ppm		

Sample Properties h-ALS-SL BinPump	Iso Pump Column-SL DAD MS Q-TOF
Setup Timetable Options	
Flow: 0.5 and ml/min	Stop Time ● No Limit C 0.1      min
Solvent A	Post Time
100.0.%	● Off
	C 💭 🚎 min
	Pressure Limits
	Min: 0 🛋 bar Max 400 🛋 bar

#### **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.

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

Ion Polarity (Seg)	olarity Switching	Data Storage ( C None	Seg) © Centroi	d
C Negative		C Both	C Profile	
MS     Plot	and Centroid Data St MS	orage Threshold -	MS/MS	
C Waste	Abs. threshold	0	Abs. threshold	10
Apply Now	Hel. threshold (%)	01	Hel. threshold (%)	1
Do not wait for setpoints (e.g	. temperature) to equili	orate		
,,				

General Source Acquisition Ref M	tass Chromato	igram	
Dual ESI (Seg)			MS TOF (Expt)
Gas Temp 325	°C	325 °C	Fragmentor 140 V
			Skimmer 65 V
Drying Gias 9	i/min psia	9.0 1/min	OCT 1 RF Vpp 750 V
Nebulizer 130	poig	100 1009	
- Dual ESI (Euro)			
VCap 4500	V Capillary	0176 uA	
		10.110	
	Chamber	15.84 uA	



mn-SL ÌI	DAD MSQ-TOF					
General	Source Acquisition Ref	Mass	Chromato	igram		
Dual	ESI (Seg)					MS TOF (Expt)
	Gas Temp 325	°C		326	°C	Fragmentor 140 V
						Skimmer 65 V
	Drying Gas 9	1/min		9.0	1/min	0CT 1 RF Vpp 750 V
	Nebulizer 30	psig		30	psig	
Dual	ESI (Expt)					
	VCap 3500	v	Capillary	0.164	uA	
				,		
			Chamber	14.11	uA	



- 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.

DAD ) MS	S Q-TOF	
Source Seg) ito S7MS eg) argeted S7MS eg)	Acquisition Ref Mass Chromatogram Spectral Parameters Collision Energy Targeted List MS Mass Range Min Range 50 m/2 Max Range 1700 m/2 Acquisition Rate/Time Rate 10 spectra/s Time 100 ms/spectrum Transients/spectrum 1243	MS/MS Mass Range Min Range 25 m/z Max Range 1700 m/z Acquisition Rate/Time Rate 3 spectra/s Time 333.3 ms/zpectrum Transients/spectrum 4434
	Max Time between 1.5 s	



#### MS Q-TOF

pectral	Parameters	Collisi	on Energy	Targeted L	ist		
				Targe	eted List Table		
On	Prec. m/z	z	Ret. Time (min)	Delta Ret. Time (min)	Iso. Width	Collision Energy	Acquisition Time (ms/spec)
• 🔽	175.119	1	1	3	Narrow (~1.3 m/z)		

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

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

E Fuchie	- Reference Masse	\$
IV Enable	Reference	Masses Table
	On	M/Z
Use bottle A Apply Now	<b>V</b>	121.050873
		149.02332
Bef Nebulizer 25 Psig		322.048121
	<b>V</b>	922.009798
		1221.990637
		1521.971475
Auto Recalibration Reference Mass Parameters		2421.91399
Detection Window 100 ppm		
Minimum Height 1000 ocumte		

Figure 4 "Use Bottle A" method, positive mode

E Fachle	- Reference Masse	\$
Chable	Reference	Masses Table
	On	M/Z
Use bottle A Apply Now	<b>V</b>	121.050873
		149.02332
Bef Nebulizer 25 Psig		322.048121
120	<b>V</b>	922.009798
		1221.990637
		1521.971475
Auto Recalibration Beference Mass Parameters		2421.91399
Detection Window         100         ppm           Minimum Height         1000         counts		

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

Gen	eral	Source Acqu	uisition Ref Mass	Chromatogram				
	hroi	matograms						
		Chromatogram	Label	Extracted	Expt Type	Polarity Type	Offset	Y-Range
	۲	EIC	EIC 175	175.09-175.13	MS	Both	15	100000

#### Figure 6 Positive mode

Ger	ieral	Source Acq	uisition Ref Mass	Chromatogram				
	.hroi	matograms						
		Chromatogram	Label	Extracted	Expt Type	Polarity Type	Offset	Y-Range
	•	EIC	EIC 173	173.08-173.12	MS	Both	15	100000

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.

Method Explorer: li MS Target Compour Chromatogram Spectrum	brary import.m X dd Screening Workflow
Extract (MS) Extract (MS/MS)	
Extract (UV) Deconvolute: Resolved Is Extraction Data Format	Method Editor: Extract (MSMS)     ×       D Extract Peak Spectrum     C
General     General     General     Find Compounds     Find Compounds by     Identify Compound     Compound Automa     Worklist Automatic     Export	Peak Spectrum Extraction (MS/MS)       ▲ Peak Filters       Charge State         Height filters       ☑ Absolute height ▲ >=       10       counts         ☑ Relative height ▲ >=       1.000 ▲ % of largest peak         Maximum number of peaks       …       1000         ☑ Limit (by height) to the largest ▲       100

- **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

MC Townsh Com	- d Canada - Washilan
MS Target Compour	nd Screening Workflow
Chromatogram	
Spectrum	<b>A</b>
General	
Reports	
Find Compounds	
nd by Auto MS/MS	
nd by Targeted MS/MS	
nd by Molecular Featu	Method Editor: Find Compounds by Targeted MS/MS
nd by MRM	Find Compounds by Targeted MS/MS V 🖓 V 🕅 V
Find Compounds by	Integrator Processing Cod TIC Peak Filters Peak Spectrum Results
Identify Compound	Integrator selection
Compound Automa	Agile
Worklist Automati	
Export	
Method Editor: Fin	d Compounds by Targeted MSMS ×
Method Editor: Fine	d Compounds by Targeted MSMS × by Targeted MS/MS 이 값 바가 이야 ~
Method Editor: Fin Find Compounds egrator A Processin	d Compounds by Targeted MSMS × by Targeted MS/MS × A P • P • P 9 Cpd TiC Peak Fitters Peak Spectrum Results
Method Editor: Fine Find Compounds tegrator A Processin Compound identification	Id Compounds by Targeted MSMS     X       by Targeted MS/MS     Image: Compound to the second secon
Method Editor: Fine Find Compounds tegrator A Processin Compound identification Maximum chromatogram	d Compounds by Targeted MSMS × by Targeted MS/MS × A M × C · · · · · · · · · · · · · · · · · ·
Method Editor: Fin Find Compounds segretor A Processin Compound identification Maximum chromatogram Limit to the largest of	d Compounds by Targeted MSMS × by Targeted MS/MS ↓ △ (*) • (* - * * * * * * * * * * * * * * * * *
Method Editor: Fini Find Compounds egator A Processin Compound detrification Maximum chromatogram Limit to the largest of	d Compounds by Targeted MS/MS × by Targeted MS/MS ▼ ( △ ↓ ▷ ▼ < ▷ ▪ © Cpd TilC Peak Fitters   Peak Spectrum   Results m peak width 0.25 ↓min compounds: 50 compounds
Method Editor: Fini Find Compounds egrator A Processin Compound identification Maximum chromatogram Limit to the largest of	d Compounds by Targeted MSMS × by Targeted MS/MS ▼
Method Editor: Fini Find Compounds agrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini	Id Compounds by Targeted MSMS     X       Iby Targeted MS/MS     Iby Targeted MS/MS     X       In peak width     0.25 Amin compounds     0.25 Amin 50 compounds
Method Editor: Fini Find Compounds egrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds	d Compounds by Targeted MSMS × by Targeted MS/MS ▼ (△) ▷ ♀ • ▷ • · · · · · · · · · · · · · · · · ·
Method Editor: Fini Find Compounds segrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds segrator A Processin	d Compounds by Targeted MSMS     X       by Targeted MS/MS     Image: Compounds       Cpd Tit C Peak Rites     Peak Spectrum       m peak width     0.25 Amin       compounds     50 compounds       d Compounds by Targeted MSMS     X       by Targeted MS/MS     Image: Compounds       g Cpd Tit C Peak Rites     Peak Spectrum       Parageted MS/MS     Image: Compounds
Method Editor: Fin Find Compounds agrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fin Find Compounds agrator A Processin Filter on	d Compounds by Targeted MSMS     ×       by Targeted MS/MS     (1)       ''''''''''''''''''''''''''''''''''''
Method Editor: Fin Find Compounds agrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds egrator A Processin Fiter on Peak h	d Compounds by Targeted MSMS     ×       by Targeted MS/MS     i       '''     ''''''''''''''''''''''''''''''''''''
Method Editor: Fin Find Compounds egrator Find Compound identification Maximum chromatogra in timit to the largest o Method Editor: Fin Find Compounds egrator Find Compounds egrator Piter o Peak h Height filens	ad Compounds by Targeted MSMS     X       by Targeted MS/MS     Imoustic Compounds       amoust of Compounds     Imoustic Compounds       ad Compounds by Targeted MSMS     X       by Targeted MS/MS     Imoustic Compounds       ad Compounds by Targeted MSMS     X       by Targeted MS/MS     Imoustic Compounds       by Targeted MS/MS     X       by Targeted MS/MS     X       by Targeted MS/MS     X       by Targeted MS/MS     Imoust of Compounds
Method Editor: Fini Find Compounds egator A Processin Compound identification Maximum chromatograu iii Limit to the largest of Method Editor: Fini Find Compounds egrator A Processin Fiker O Peak h Height fiken Absolute height	d Compounds by Targeted MSMS     X       by Targeted MS/MS     Image in the im
Method Editor: Fini Find Compounds egatar A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds egrator A Processin Filter on Peak to Height filter Absolute height Relative height	d Compounds by Targeted MSMS     X       by Targeted MS/MS     Impact MS/MS       m peak width     0.25 min       compounds     50       compounds by Targeted MSMS     X       by Targeted MS/MS     Impact MSMS       compounds by Targeted MSMS     Impact MSMS       by Targeted MS/MS     Impact MSMS
Method Editor: Fini Find Compounds segator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds segator A Processin Filter on Peak th Height filters Height filters Relative height Area filters	d Compounds by Targeted MSMS     X       by Targeted MS/MS     Image in the im
Method Editor: Fini Find Compounds agrator A Processin Compound detentioation Maximum chromatogram Limit to the largest of Method Editor: Fini Find Compounds agrator A Processin Fiter on Peak the Height fiters Absolute area	d Compounds by Targeted MSMS × by Targeted MS/MS
Method Editor: Fin Find Compounds agrator A Processin Compound identification Maximum chromatogram Limit to the largest of Method Editor: Finn Find Compounds agrator A Processin Filter on Peak In Height filten Absolute height Absolute area Pelative area	d Compounds by Targeted MSMS     X       by Targeted MS/MS     Impose Spectrum       by Targeted MS/MS     Impose Spectrum       m peak width     0.25 min       compounds     50       compounds     50       compounds     50       compounds     50       compounds     50       d Compounds by Targeted MSMS     X       by Targeted MS/MS     Impose Spectrum       g Cod TIC Peak Filter     Peak Spectrum       paint     Impose Spectrum <tr< td=""></tr<>

- **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

🖉 Method Editor: Find Con	npounds by Targeted MS/MS	×
Find Compounds by Ta	rgeted MS/MS 💌 🚮 🛛 🖛 🍽 👻	
Integrator 🛕 Processing Cp	od TIC Peak Filters Peak Spectrum Results	
Spectra to include		<u>^</u>
At apex of peak		
Average scans >	10 % of peak height	
TOF spectra		
Exclude if above	10.0 % of saturation	
In the m/z ranges used	in the chromatogram	
<ul> <li>Anywhere</li> </ul>		E
In these m/z ranges	100.0000-2000.0000	
Never return an empty	spectrum	
Peak spectrum background		
MS/MS None		
-		
Time range:	0.000	
		-
Method Editor: Find Con	2M2M batenatic water	~
Find Compounds by Ta		~
Integration A Descention Co	d TIC Dark Diver Dark Constant Results	Ŧ
Processing   C	a no reak niters   reak spectrum   results	
Delete previous compour	de	
- Sector provides composition		
New results		
<ul> <li>Highlight all compounds</li> </ul>		
Chromotograme and energies		
Extract MS/MS chromatog	ram	
Extract MS spectrum		
Extract MS/MS spectrum		
Extract separate MS/I	IS spectrum per collision energy	
Extract average MS/M	IS spectrum for all collision energies	

- 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.

Method Explorer:	library import.m X	
MS Target Compo	und Screening Workflow	
Chromatogram		
Spectrum	۵	
🗉 General		
Reports		
Find Compounds	۵	
• Find Compounds b	y Formula	
Identify Compound	İs	
Search Database		
Search Accurate Mass L	ibrary	
Generate Formulas	Method Explorer: library import.m	3
Compound Autom	MS Target Compound Screening Workflow	
Worklist Automat	Identify by Library Search	A
Export	Chromatogram	
	Spectrum	۵
	General	
	Reports	
	Find Compounds	۵
	Find Compounds by Formula	۵
	Identify Compounds	۵
	Compound Automation Steps	
	Worklist Automation	
	• Export	
l		

🚰 Method Editor: Search Accurate Mass Library 🗙 🗙 🗙
🕑 Search Library for Compounds 🔹 🚮 🖃 🕶 📜 Method Items 🔹 😕 🙀
A Settings Peak Filters Search Criteria Search Results
Library selection
Spectral library path:
C:\Users\emrennie\Desktop\data files\Arginine Quality Control 🛕 🔜
Apply to search database path
MS/MS search
Precursor ion m/z expansion +/- ( 10.0 ppm + 2.0000 mDa )
Production m/z expansion: +/- ( 15.0 ppm + 5.0000 mDa )

Method Editor: Search Accurate Mass Library						
: 🕩 Search Library	for Compou	nds 🔹 🚮 🛛 🖛	🖓 🐨 🗌 Method Items 🕶	lin į		
Settings Peak Filters	Search Crit	eria Search Result	S			
Height filters				-		
Absolute height	>=	100	counts			
Relative height	>= [	1.000	% of largest peak			
Maximum number o	f peaks			E		
Limit (by height	to the larges	t	100			
				-		

- **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

Search Library for Compounds      A	
Settings Peak Filters Search Criteria Search Results Search filter Restrict spectral comparison based on Control comparison based on Control comparison mode	
Search filter Restrict spectral comparison based on Inization mode	
Restrict spectral comparison based on	
lonization mode	
Instrument type	
Collision energy +/- 2.00 eV	
제 Method Editor: Search Accurate Mass Library	×
출 Method Editor: Search Accurate Mass Library (): Search Library for Compounds ▼1 △ 1 2 - 0 - 1 Method Items ▼ 12	×
Method Editor: Search Accurate Mass Library Search Library for Compounds	×
Method Editor: Search Accurate Mass Library  Search Library for Compounds   A Search Results  Bearch Results  Search Results  Search Results  Method Items *   Search Results   ×	
Method Editor: Search Accurate Mass Library  Search Library for Compounds  Add Search Results  Search Results  Search methods and score thresholds  Minimum forward score: 25.00	×
Method Editor: Search Accurate Mass Library  Search Library for Compounds	×
Method Editor: Search Accurate Mass Library  Search Library for Compounds  Gamma Compounds	×
Method Editor: Search Accurate Mass Library  Search Library for Compounds  Settings Peak Riters Search Results  Search Results  Minimum forward score: 25.00 (Matching peaks in unknown against the library spectrum)  Minimum reverse score 80.00	×
Method Editor: Search Accurate Mass Library Search Library for Compounds < A to be a search results Settings Peak Filters Search Criteria Search Results Search methods and score thresholds Ø Minimum forward score: 25.00 (Matching peaks in unknown against the library spectrum) Ø Minimum reverse score 80.00 (Matching peaks in library spectrum against the unknown)	×
Method Editor: Search Accurate Mass Library  Search Library for Compounds  Search Results Search Results Search Results Minimum forward score: 25.00 (Matching peaks in unknown against the library spectrum) (Matching peaks in library spectrum against the unknown) Search results	×



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).

Method Explorer: library import.m	i 🔄 Method Editor: Extract (MS/MS) 🗙
MS Target Compound Screening Workflow	🗄 💽 Extract Peak Spectrum 🔹 🚮 🖃 🕶 🖓 Hethod Items 🔹 😕 👔
Chromatogram	Peak Spectrum Extraction (MS/MS)
Spectrum	Height filters
Extract (MS)	Absolute height A >= 10 counts
Extract (MS/MS)	
Extract (UV)	Maximum number of peaks
Deconvolute: Resolved Isotope	Limit (by height) to the largest 🛕 100
Extraction Data Format	
General	
• Reports	
Find Compounds	
Find Compounds by Formula	
Identify Compounds	
Compound Automation Steps	
Worklist Automation	
Export	

- **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.

Spectra cannot be pasted into the Library spectra table.

#### To clean up noisy spectra

NOTE

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

	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 setup

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-Na <sup>+</sup> = 64.01577	CF3 <sup>-</sup> = 68.99576
6	Manual Tune > TOF tab	Set Min Range	25 <i>m/z</i>	25 <i>m/z</i>
7	Manual Tune > Optics 1 tab	Reduce Fragmentor	$\pm 125$ V to increase $m/z$ 64	$\pm 125$ V to increase $m/z$ 68
8	Manual Tune > Quad tab <sup>*</sup>	Set Quad AMU	to pass <i>m/z</i> 64 and 1522 with similar abundances	to pass <i>m/z</i> 69 and 1634 with similar abundances
9	Instrument State tab	Save Tune file	SmallTune.tun	SmallTune.tun
10	<b>Manual Tune &gt; Optics 1</b> $tab^{\dagger}$	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 <b>OK</b>		

Summarv	for calibration	steps (do	o first for	Positive m	ode and then	for Nega	tive mode)	(continued)	۱
cannar,	for ounstation	01000 (4	0 11101 101		ouo una mon	101 110 90	iento iniouoj	loonanaoa	,

			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 <b>Yes</b>	
	Save Tune File message	Click <b>No</b>	

## 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 <b>Use Injector</b> Program		
4	h-ALS-SL > Injector Program tab	Create Injector Program	<ul> <li>Draw sample</li> <li>Needle wash in flush por</li> <li>Needle into seat</li> <li>Remote Startpulse</li> <li>Wait 0.30 minute</li> <li>Inject</li> </ul>	rt for 3.0 sec
5	BinPump > Setup tab	Reduce <b>Fragmentor</b>	<ul> <li>Set Flow to 0.2 ml/min.</li> <li>Set Solvent B to 50%.</li> <li>Choose one of the follow combinations: <ul> <li>Water/Methanol con</li> <li>Water/Acetonitrile con acid</li> </ul> </li> <li>Set Stop Time to 1.5 min</li> </ul>	ving mobile phase taining 0.2% (v/v) acetic acid ontaining 0.2% (v/v) acetic I.
6	MS Q-TOF > Ref Mass tab <sup>*</sup> or	Select <b>Use Bottle A</b> . Use prepared Reference Mass Solution in bottle A.		
	IsoPump > Setup tab <sup>†</sup>	Set Flow	0.5 ml/min	0.5 ml/min

			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
	<b>MS Q-TOF &gt; Source</b> tab For AJI and APCI sources, use default settings.	Set parameters	<ul> <li>Gas Temp to 325°C</li> <li>Drying Gas to 9 L/min</li> <li>Nebulizer to 30 psig</li> <li>VCap to 4500 V</li> <li>Fragmentor to 140 V</li> <li>Skimmer to 65 V</li> <li>Oct 1RF Vpp to 750 V</li> </ul>	<ul> <li>Gas Temp to 325°C</li> <li>Drying Gas to 9 L/min</li> <li>Nebulizer to 30 psig</li> <li>VCap to 3500 V</li> <li>Fragmentor to 140 V</li> <li>Skimmer to 65 V</li> <li>Oct 1RF Vpp to 750 V</li> </ul>
9	MS Q-TOF > Spectral Parameters tab	Set parameters	<ul> <li>MS &gt; Min Range to 50 f</li> <li>MS &gt; Rate to 10 spectra</li> <li>MS &gt; Time to 100 ms/s</li> <li>MS/MS &gt; Min Range to</li> <li>MS/MS &gt; Rate to 3 spe</li> <li>MS/MS &gt; Time to 333 f</li> <li>Max Time between MS1</li> </ul>	n/z a/s pectrum o 25 m/z ectra/s ns/spectrum I 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)

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	<b>MS TOF &gt; Acquisition &gt; Targeted List</b> tab	Set parameters	<ul> <li>Prec. m/z to 175.1190</li> <li>Z to 1</li> <li>Ret.Time to 1</li> <li>Delta Ret. Time to 3</li> <li>Iso. Width to Narrow</li> <li>Collision Energy to blank</li> <li>Acquisition Time to blank</li> </ul>	<ul> <li>Prec. m/z to 173.1044</li> <li>Z to 1</li> <li>RetTime to 1</li> <li>Delta Ret. Time to 3</li> <li>Iso. Width to Narrow</li> <li>Collision Energy to blank</li> <li>Acquisition Time to blank</li> </ul>
13	MS Q-TOF > Ref Mass tab	Select <b>Reference</b> 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 TOF spectra: • Selected (mark the check • Not selected (clear the cl	Masses are included in the ( box) <sup>*</sup> or heck box) <sup>†</sup>
15	MS Q-TOF > Chromatogram tab	Set Chromatogram	EIC	EIC
		Set Extracted	175.09-175.13	173.08-173.12
		Set <b>Y-range</b>	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.

<sup>†</sup> 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 <b>Peak Filters</b> parameters	<ul> <li>Absolute height to 10 counts</li> <li>Relative height to 1.000 % of largest peak</li> </ul>
4	Method Explorer > Find Compounds > Find by Targeted MS/MS	Set <b>Integrator</b> parameters	<ul> <li>Integrator selection to Agile</li> </ul>
		Set <b>Processing</b> parameters	<ul> <li>Maximum chromatogram peak width to 0.25 min</li> </ul>
		Set <b>Cpd TIC Peak</b> Filters parameters	<ul> <li>Relative area to 5.000 % of largest peak</li> <li>Limit (by height) to the largest to 10</li> </ul>
		Set <b>Peak Spectrum</b> parameters	<ul> <li>Average scan to 10 % of peak height</li> <li>Exclude if above to 10% of saturation</li> </ul>
		Set <b>Results</b> 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 <b>Settings</b> parameters	<ul> <li>Spectral Library path to appropriate path</li> <li>Precursor ion m/z expansion to 10 ppm ± 2 mDa</li> <li>Product ion m/z expansion to 15 ppm ± 5 mDa</li> </ul>
		Set <b>Peak Filters</b> parameters	<ul> <li>Absolute height to 100 counts</li> <li>Relative height to 1.000 % of largest peak</li> </ul>
		Set <b>Search Criteria</b> parameters	<ul> <li>Set Collision energy to +/- 2.00 eV.</li> </ul>
		Set <b>Search Results</b> parameters	<ul> <li>Minimum forward score to 25.00</li> <li>Minimum reverse score to 80.00</li> </ul>
		Click the green arrow.	
6	Compound List	Inspect Compound List	<ul> <li>Check that:</li> <li>the name of the compound is Arginine</li> <li>the Chromatogram results show only one LC peak</li> <li>the Forward and Reverse scores are 98 or greater for all collision energies</li> </ul>
7	Spectral Differences Result	Make sure all library ions line up with acquired data.	

**Summary of Steps** 

## www.agilent.com

## 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.

 ${\ensuremath{\mathbb C}}$  Agilent Technologies, Inc. 2012

Revision A, March 2012



5991-0076EN

