

Agilent MassHunter Workstation Unknowns Analysis

Familiarization Guide

Notices

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In this Guide...

In this Guide...

This guide presents step-by-step exercises to help you learn to use the Unknowns Analysis program. You can do these exercises with the demonstration analysis, method, and library files, shipped with the system installation disk, or with data you acquire.

Choosing Unknowns Analysis Desktop Icons

Quantitative Analysis B.09.00 offers Unknowns Analysis desktop icons for the **Classic** user interface and the **Quant-My-Way** user interface. The **Classic** user interface has a look and feel similar to the user interface offered in Quantitative Analysis B.08.00, with tools and options located in a menu bar. The **Quant-My-Way** user interface has a modern ribbon, with tools and options located on tabs and ribbons instead of in a menu bar. You can select to install the Classic user interface desktop icon, the **Quant-My-Way** user interface desktop icon, or both.

This Familiarization Guide follows the **Classic** user interface. However, where the **Quant-My-Way** user interface navigation differs, those steps are included and highlighted in orange.



Unknowns Analysis

(Quant-My-Way)

Analysis

Before you begin these exercises

Copy files from the installation media to your hard disk

- 1 Navigate to the \Data folder on the installation media.
- 2 If the folder is in a compressed format, extract the data files from their zip format.
- **3** Copy the **Data** folder from your installation media in uncompressed format to any location on your hard disk.

This folder contains all of the data, method, and library files needed for these exercises. Do not reuse the example data files on your system unless you know that they are identical to the originals on the media. If the example data files already on the system do not match the original ones of the media exactly, then the results obtained during these exercises will not match those shown in this guide.

Task 1: Identify Compounds with TIC Analysis

Task 1: Identify Compounds with TIC Analysis

Create a new analysis

1 Start Unknowns Analysis by double-clicking the desktop icon.

or

Click Start > Agilent > MassHunter Workstation > Unknowns Analysis.



When you open the program, the default layout appears.

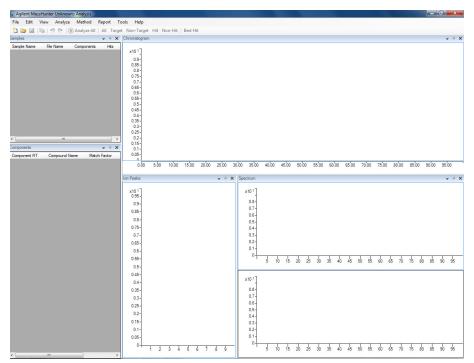


Figure 1.

Create a new analysis

If the default layout is not present, click **View > Preset Layout > Standard** to restore the default layout before creating a new analysis.

In the **Quant-My-Way** user interface, on the **View** tab, click **Preset Layout > Standard**.

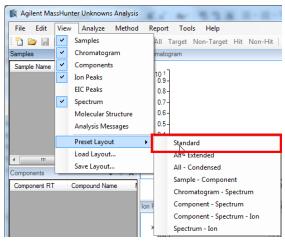


Figure 2.

2 Select File > New Analysis. In the Quant-My-Way user interface, on the Home tab, click New Analysis.

🛐 Ag	ilent Ma	assHunte	er Unknown	s Analysis	-		
File	Edit	View	Analyze	Method	Report	Tools	Help
Ð	New A	nalysis				Ctrl+N	
	Open /	Analysis				Ctri+O	
	Save A	nalysis				Ctrl+S	
	Save A	nalysis A	S				
	Close Analysis						
	Add Samples						
	Import Quantitative Analysis						
	Import Target Method (Quantitative Analysis Method)						
	Export.						
Â1	Page S	etup					
3	Print					Ctrl+P	
4	Print P	review					
	Exit						



- 3 Navigate to **MassHunter\Data\Evaldemo**, or the folder where the data file to be analyzed is stored.
- 4 Type the analysis name evaldemo for the analysis, and click Create.

🛐 New Analysis					X
Look in:	📔 Evaldemo	- 🧿 💋) 📂 🛄 -		
Recent Places Desktop Libraries Computer	Name	Analysis File	Data Ve S	ize Date Analyzed	Analyst
Network	III File name: evaldemo	•	III		► Create
	Files of type: Unknowns Analysis Files	: (*.uaf)		•	Cancel

Figure 4.

Add samples to the analysis

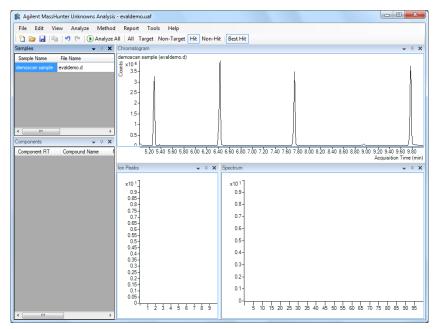
- 1 Select File > Add Samples. In the Quant-My-Way user interface, on the Home tab, click Add Samples.
- 2 Select the sample file(s) and click **OK** to add the sample to the batch.

Add samples to the analysis

A	dd Samples	×
	Batch Folder:	D:\MassHunter\Data\Eval
	File name	Sample name
	evaldemo.d	demoscan sample
	Browse to Co Translate MS Select All	ppy Samples

Figure 5.

The Analysis table is no longer empty. It now contains the demo sample.





Set up the method for the analysis

Select Method > Edit.

In the Quant-My-Way user interface, on the Home tab, click Edit Method.

🕅 Agilent MassHunter Unknowns Analysis - evaldemo.uaf						
File Edit Vi	ew Analyze	Met	hod	Report	Tools	Help
: 🛅 🗁 🛃 📭	n 🌱 🥲 💽	2	Edit.			F10
Samples	-		Load	d Method	to All Sar	nples
Sample Name File Name			Load	d Method.		
demoscan sample evaldemo.d			Save	Method	of Curren	t Sample
			Load	d Method.	•	t Sample

Figure 7.

The Method dialog box standard view appears. For this task, we will use the **Standard** view.

Method	8
Peak Detection Deconvolution Library Search	Compound Identification Target Match Blank Subtraction
Deconvolution	
Specify scan/signal for TIC Analysis	
Peak filter:	
Excluded m/z:	28
SNR threshold:	Example: 28,91,149 0
Maximum number of peaks	
Rank by:	▼
Maximum number of peaks:	
Area filters	
Absolute area >= 0	counts
Relative area >= 0	% of largest peak
Height filters	
Absolute height >= 0	counts
Relative height >= 0	% of largest peak
Advanced Apply to All Samples	Apply to Selected Sample Default Close

Figure 8.

Note that these are the default parameters for the method. You can click **Default** at the bottom of the Method dialog box to restore default parameters before creating a new method in the next step.

Set Peak Detection options

1 Select TIC Analysis from the Peak detection drop-down menu.

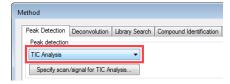


Figure 9.

- **TIC Analysis**: Identifies the chromatographic peaks using integration instead of deconvolution.
- **Deconvolution**: Deconvolutes the components in the chromatogram and extracts the 'clean' spectra from background noise based on both retention time and peak shape.
- 2 Click **Specify scan/signal for TIC Analysis** to process GC signals.

Method			
Peak Detection	Deconvolution	Library Search	Compound Identification
Peak detection			
TIC Analysis		-	
Specify scan	/signal for TIC Ar	nalysis	

Figure 10.

3 In the Maximum number of peaks section, select Area from the Rank by drop-down menu, and enter 5 for the Maximum number of peaks.

Maximum number of peaks	
Rank by:	Area 🔹
Maximum number of peaks:	5

Figure 11.

4 In the Area filters section, select Relative area and enter 1 for the % of largest peak.

Area filters						
Absolute area	>=	0	counts			
Relative area	>=	1	% of largest peak			

Figure 12.

Set Library Search options

1 Click Library Search.

thod	8
Peak Detection Deconvolution Libr	rary Search Compound Identification Target Match Blank Subtraction
- Libraries:	
C:\MassHunter\Library\demo.mslib	brary.xml Change Library Add Library
Move Up Move Down	m Open Library Remove Library
Search criteria:	Forward-Reverse Search:
Pre-search type:	Pure Weight Factor:
Nomal	• 0.7
Adjust Score	Example: 0.0 for reverse search
Remove Duplicate Hits	1.0 for forward search
Match factor:	
Use RT Match	
- RT penalty function:	RT mismatch penalty:
 Trapezoidal 	 Multiplicative
RT range:	6 sec Additive
Penalty-free RT range:	Max RT penalty: 20
Gaussian	
0	
Standard deviation:	6 sec
RT calibration file:	
	New Choose
	Ciluse
Advanced Apply to All	Samples Apply to Selected Sample Default Close

Figure 13.

Set Library Search options

	rary Search	Compound ic	enuncation	Target Match	Blank Subtraction
Libraries: Z:\GCMS Data\Libraries\RI-PESTI		0 11			
2:/GCMS Data/Libraries/RI-PEST	CIDES-MUL	2.mslibrary.x	ml		Change Library
					Add Library
Move Up Move Dov	vn		Op	en Library	Remove Library
Search criteria:		For	ward-Revers	e Search:	
Pre-search type:		Pun	e Weight Fa	ctor:	
Normal	•		0.7		
Adjust Score			Example: 0.0 for reve	rse search	
Remove Duplicate Hits			1.0 for forwa	ard search	
Match factor:					
Use RT Match					
RT penalty function:			CRT misma	tch penalty:	
Trapezoidal			Multip	licative	
RT range:	9	sec	Addition	/e	
Penalty-free RT range:	9	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
K I calibration file:					
					
				New	Choose

2 Click Change Library.

Figure 14.

3 Navigate to MassHunter\Data\Evaldemo\, or the relevant folder, select demo.L, and click Open.



Figure 15.

4 In the **Search criteria** section, select **None** from the **Pre-search type** drop-down menu.

Search criteria:	
Pre-search type:	
None	•
Adjust s None Normal	2
Remove Fast	

Figure 16.

There are 3 Pre-search types: **None, Normal**, and **Fast**. By default, Unknowns Analysis uses **Normal**.

- **None**: The library search is not subjected to a preliminary screening process.
- **Normal**: The screening algorithm uses the entire library as the list of candidates if the indexing scheme does not produce enough candidates. It is 50-100 times faster than no pre-search, with essentially zero false negatives rate for high-scoring hits (match score > 80).
- **Fast**: The screening algorithm uses whatever list of candidates it gets from the index and avoids the entire library-search even if there are not enough candidates found. The speed is 100-1000 times faster than no pre-search, with ≥1% false negatives rate for high-scoring hits.

Set Compound Identification options

1 1. Click Compound Identification.

fethod	2
Peak Detection Deconvolution Library Search	Compound Identification
Max hit count:	1
Min match factor:	50
Min MZ:	30
Library Search Type:	Spectral Search 🔹
Multi-Library Search Type:	Al
Advanced Apply to All Samples	Apply to Selected Sample Default Close

Figure 17.

For this task, we will use the default Compound Identification parameters.

2 Click Apply to All Samples, and then click Close.

Analyze and review results

1 Click Analyze All.

After the analysis is complete, the main view that appears should look like the example below. This is the default layout and contains the default column settings. If you see a different layout than the one in the example below, select **View > Preset Layout > Standard** to reset the standard layout.

In the **Quant-My-Way** user interface, on the **View** tab, select **Preset Layouts > Standard**.

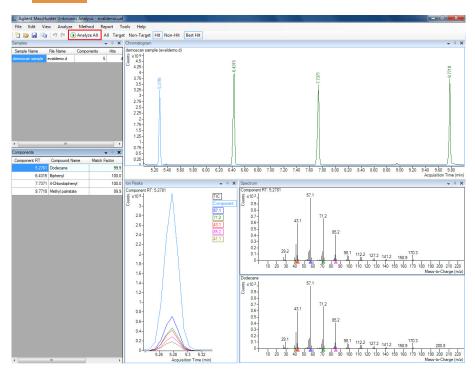


Figure 18.

2 Select View > Preset Layout > All-condensed.

The system displays the All-condensed view.

In the **Quant-My-Way user** interface, on the **View** tab, select **Preset Layouts > All-condensed**.

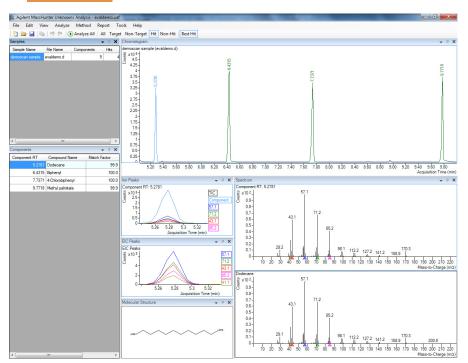


Figure 19.

3 Select the **demoscan sample** from the **Sample** table.

Click one of the following toolbar buttons to view the changes in the **Components** window.

In the **Quant-My-Way** user interface, click one of the following buttons on the **Home** tab.

- All: View all the peaks.
- Hit: View the peaks that are found in the library search.
- Non-Hit: View the peaks that are not found in the library search.

4 Right-click any column header in the **Components** window, and select **Add/Remove Columns**.

Components			→ ₽ X
Component RT	Compound Name	Match Factor	Add Remove Columns
5.2781	Dodecane	9	
6.4315	Biphenyl	10	
7.7371	4-Chlorobiphenyl	10	Set Best Hits
9.7718	Methyl palmitate	9	Show Alternate Hits
			Delete Hits Del

Figure 20.

5 Select **Component** from the **Select columns from** drop-down menu.

Select columns from:		
Component 👻		
Available columns:		Show these columns in the order:
Component Name Best Ht Comidden Component Area Component Find X Component Height Component Nat X Component Stat X Component Stat X Component Stat X Component Stat X Component Stat X Component Stat X Component Target Occonvolution Li Component_Targeted Deconvolution Li Component_Targeted Deconvolution Li	Add -> <- Remove Add All ->> <<- Remove All	Component RT Compound Name Match Factor Best Ht Formula
	OK B	Move Up Move Down



Select columns from:			
Component 🔹		0	
Available columns:		Show these columns in	the order:
Component Name Best Hit Candidate	Add ->	Component RT Compound Name	
Best Hit Overridden	<- Remove	Match Factor	
Component Area	<- Hemove	Best Hit	
Component End X		Formula	
Component Height	Add All ->>		
Component Is Accurate Mass			
Component RI	< Remove All		
Component Start X			
Component Shape Quality			
Deconvoluted Height			
Area %			
Area % Max.			
component_largetedDeconvolution_id			
Component_TargetedDeconvolution_Li			
Component_TargetedDeconvolution_Li			
		Move Up	Move Down

6 Select Area % and Area % Max from the Available columns list, and click Add.

Figure 22.

- Area %: Percentage of the peak area sum
- Area % Max: Percentage of the largest peak area
- 7 Verify that the selected columns are moved to the **Show these columns in the order** list, and click **OK**.
- 8 From the **Components** table, select a component in the **Component RT** column.

Components						→ ₽ X
Component RT	Compound Name	Match Factor	Best Hit	Formula	Area %	Area % Max.
5.2781	Dodecane	99.9	V	C12H26	19.158	63.44
6.4315	Biphenyl	100.0	V	C12H10	30.196	100.00
7.7371	4-Chlorobiphenyl	100.0	V	C12H9CI	25.572	84.69
9.7718	Methyl palmitate	99.9	V	C17H34O2	23.731	78.59

Figure 23.

View the Chromatogram, Spectrum, Ion Peaks, EIC Peaks, and Molecular Structure for the selected component.

In the **Spectrum** window, the top spectrum is from the component, and the bottom spectrum is from the library. The **Match Factor** in the **Components** table reflects how closely the two spectrum match.

To change to the Header-to-tail view, right-click inside the **Spectrum** window and select **Header-to-tail**.

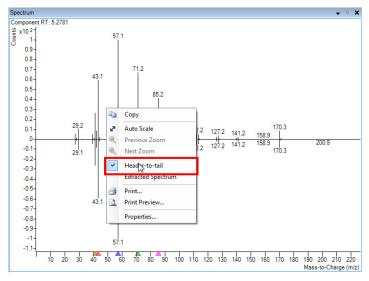


Figure 24.

The **Ion Peaks** and **EIC Peaks** windows show the extracted chromatograms of the selected ions. The EIC traces and their numeric identifiers to the right of the display are color-coded.

To interactively add the ion chromatogram traces in the **Ion Peaks** and **EIC Peaks** window to the display, click in any **Mass Spectral Display** area of the **Spectrum** window. If the selected m/z chromatogram is not already displayed, it will be added to the **Ion Peaks** and **EIC Peaks** window and symbol of the same color will be at the appropriate m/z position below the x-axis in the **Spectrum** window. To remove an ion chromatogram trace (and its numeric identifier) from the **Ion Peaks** and **EIC Peaks** window, click on its numeric identifier or on the corresponding m/z value position in the **Spectrum** window.

The **Molecular Structure** is from the library. If the searched library does not contain the structures for the entries, nothing will be displayed in the **Molecular Structure** window.

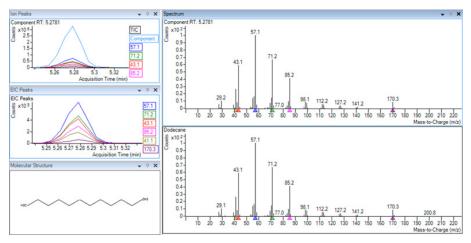


Figure 25.

9 To save the analysis, select File > Save Analysis.

In the Quant-My-Way user interface, on the Home tab, click Save Analysis.

10 . Click File > Exit.

Task 2: Identify Compounds with Deconvolution

Create a new analysis

1 Start **Unknowns Analysis** by double-clicking the desktop icon. or

Click Start > Agilent > MassHunter Workstation > Unknowns Analysis.



Way) 2 Select File > New Analysis.

In the Quant-My-Way user interface, on the Home tab, click New Analysis.

🛐 Ag	ilent Ma	assHunte	r Unknown	s Analysis			
File	Edit	View	Analyze	Method	Report	Tools	Help
1	New A	nalysis				Ctrl+N	
s 🖅	Open /	Analysis				Ctri+O	
	Save A	nalysis				Ctrl+S	
	Save A	nalysis A	S				
	Close A	Analysis					
	Add Sa	mples					
	Import	Quantit	ative Analy	sis			
	Import	Target I	Method (Qเ	antitative A	nalysis Me	ethod)	
	Export.						
印	Page S	etup					
3	Print					Ctrl+P	
4	Print P	review					
	Exit						

Figure 26.

- 3 Navigate to \Your Directory\RI-PEST-MATRIX\.
- 4 Type the analysis name demo, and click **Create**.

Add samples to the analysis

1 Select File > Import Quantitative Analysis.

In the **Quant-My-Way** user interface, on the **Home** tab, select **Import > Import Quantitative Analysis**.

2 Select TargetDemo.batch.bin, and click Open.

Verify the batch is imported. The **Sample** window now contains one matrix blank and five spiked samples at the different concentration levels. The **Chromatogram** shows the TIC of the sample selected in the **Sample** window.

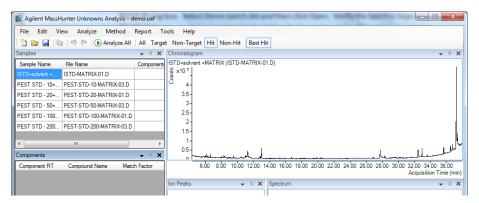


Figure 27.

Set up the method for the analysis

Press F10 or select Method > Edit.

In the Quant-My-Way user interface, on the Home tab, click Edit Method.

Set Peak Detection options

Select **Deconvolution** from the **Peak detection** drop-down menu, and click **Apply** to **All Samples**.

Method		?
Peak Detection Deconvolu	tion Library Search	Compound Identification Target Match Blank Subtraction
Peak detection		
Deconvolution	-	
Specify scan/signal for T	IC Analysis	
Peak filter		
Excluded m/z:		28
Excluded m/z.		20 Example: 28.91.149
SNR threshold:		0
Maximum number of peaks		
Rank by:		
Maximum number of peaks:		
· · · ·		
Area filters		
Absolute area	>= 0	counts
Relative area	- 0	% of largest peak
Height filters		
Absolute height :	- 0	counts
Relative height	= 0	% of largest peak
Advanced	oply to All Samples	Apply to Selected Sample Default Close
Advanced Ap	opry to Air Samples	Apply to Selected Sample Default Close

Figure 28.

Set Deconvolution options

1 Click **Deconvolution**.

Method	? ×
Peak Detection Deconvolution Jibrary Search	Compound Identification Target Match Blank Subtraction
Resolution:	
RT window size factor:	25,50,100,200
Extraction window:	
Left m/z delta:	0.3
Right m/z delta:	0.7
m/z delta units:	AMU 👻
Use integer m/z values	
Component shape:	
Use base peak shape	
Sharpness threshold:	25 %
lon peaks:	
Min # of ion peaks:	3
Max # of ion peak shapes to store:	10
Advanced Apply to All Samples	Apply to Selected Sample Default Close

Figure 29.

The default parameters for deconvolution display. By default, there are four values (25, 50, 100, 200) for the **RT window size factor**. Select any set of Window Size Factor (WSF) values in a comma-separated format.

	arch Compound Identification Target Match Blank Subtraction
Resolution: RT window size factor:	25,50,100,200
Extraction window:	
Left m/z delta:	0.3
Right m/z delta:	0.7
m/z delta units:	AMU 👻
Use integer m/z values	
Component shape:	
Use base peak shape	
Sharpness threshold:	25 %
lon peaks:	
Min # of ion peaks:	3
Max # of ion peak shapes to store:	10

Figure 30.

The WSF represents a dimensionless scale of the correlation window for grouping ion peaks into components, equivalent to Resolution and AMDIS. A smaller value (higher resolution) separates closely spaced peaks, finds more components, and runs longer. A larger value is used for wider peaks. Using multiple values covers all kinds of peaks without manual optimization.

2 In the Extraction Window section, select Use integer m/z values.

Extraction window:	
Left m/z delta:	0.3
Right m/z delta:	0.7
m/z delta units:	AMU 🔹
Use integer m/z values	

Figure 31.

Use integer m/z values runs the deconvolution with both integer and filtered m/z, and provides the best results.

3 Click Apply to All Samples.

Set Library Search options

1 Click Library Search.

Peak Detection Deconvolution U	orary Search	compound it	on third duoin	Target Match	Blank Subtraction
C:\MassHunter\Library\demo.msl	ibrary.xml				Change Library
					Add Library
Move Up Move Do	wn			en Library	Remove Library
Search criteria:			vard-Revers		
Pre-search type:		Pur	e Weight Fa	tor:	
Normal	•		0.7 Example:		
Adjust Score			0.0 for reve		
Remove Duplicate Hits			1.0 for forwa	ard search	
Match factor:					
Use RT Match					
RT penalty function:			RT misma	tch penalty:	
 Trapezoidal 			Multip	licative	
RT range:	6	sec	Addition	/e	
Penalty-free RT range:	0	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
K I calibration tile:					
				New	Choose

Figure 32.

2 Click Change Library.

Me	ethod ? ×
	Peak Detection Deconvolution Library Search Compound Identification Target Match
	Z:\GCMS Data\Libraries\RI-PESTICIDES-MOL2.mslibrary.xml Change Library
	Add Library
	Move Up Move Down Open Library Remove Library

Figure 33.

3 Navigate to the relevant folder, select **RI-PESTICIDESMOL2. mslibrary.xml**, and click **Open**.

4 In the **Search criteria** section, select **Normal** from the **Pre-search type** drop-down menu.

- Libraries: Z:\GCMS Data\Libraries\RI-PESTI	CIDES-MOL	.2.mslibrary.x	nl		Change Library
					Add Library
Move Up Move Dow	n		Op	en Library	Remove Library
Search criteria: Pre-search type: Normal	-	Pun	ward-Reverse Weight Fac 0.7		
 Adjust Score Remove Duplicate Hits 			Example: 0.0 for rever 1.0 for forwa		
Match factor: Use RT Match					
RT penalty function:			RT misma	tch penalty:	
 Trapezoidal 			Multipl		
RT range:	6	sec	Additiv	/e	
Penalty-free RT range:	0	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
				New	

Figure 34.

- Select Adjust Score to give the closest library match scores to NIST.
- Select **Remove Duplicate Hits** to remove duplicate hits that appear in the hit list for a given target spectrum. This deals with duplicate and highly similar library entries such as seen in NIST, and only returns the single library entry with the highest fit score.

Peak Detection Deconvolution Lib	rary Search	Compound Id	entification	Target Match	Blank Subtraction
Libraries:					
Z:\GCMS Data\Libraries\RI-PESTI	CIDES-MOL	2.mslibrary.xi	nl		Change Library
					Add Library
Move Up Move Dow	/n		Op	en Library	Remove Library
Search criteria:		Forv	vard-Revers	e Search:	
Pre-search type:		Pure	e Weight Fac	stor:	
Normal	•		0.7		
Adjust Score			Example: 0.0 for rever		
Remove Duplicate Hits			1.0 for forwa		
Match factor					
Match factor:					
RT penalty function:				tch penalty:	
Trapezoidal				licative	
RT range:	6	sec	Addition	-	
Penalty-free RT range:	0	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
				New	Choose

5 In the Match factor section, select Use RT Match.

Figure 35.

6 In the **RT penalty function** section, select **Trapezoidal** and enter the following: **RT range: 9 Penalty-free RT range: 9**

thod			1			?		
Peak Detection De	econvolution	Library Search	Compound Id	entification	Target Match	Blank Subtraction		
Libraries:								
Z:\GCMS Data\Li	braries\RI-PE	STICIDES-MOL	2.mslibrary.xr	ni		Change Library		
						Add Library		
Move Up	Move	Down		Op	en Library	Remove Library		
Search criteria:			Forv	vard-Reverse	e Search:			
Pre-search type:			Pure	e Weight Fac	tor:			
Norma	ł	•		0.7				
Adjust Score			E	Example: 0.0 for rever	za saarch			
Remove Duplic	cate Hits			1.0 for forwa				
Use RT Match RT penalty fur Trapezoida	nction:				tch penalty:			
RT range:		9	sec	Additive	/e			
Penalty-free	e RT range:	9	sec	Ma	x RT penalty:	20		
Gaussian				•				
Standard d	eviation:	6	sec					
RT calibration file								
					New.	. Choose		
dvanced Apply to All Samples Apply to Selected Sample Default Close								

Figure 36.

Libraries:					
Z:\GCMS Data\Libraries\RI-PE	STICIDES-MOL	2.mslibrary.xi	nl		Change Library
					Add Library
Move Up Move (Jown		Op	en Library	Remove Library
Search criteria:		Forv	vard-Revers	e Search:	
Pre-search type:		Pure	e Weight Fac	ctor:	
Normal	•		0.7		
Adjust Score		E	Example: 0.0 for rever	ree eearch	
Remove Duplicate Hits			1.0 for forwa		
Use RT Match RT penalty function: Trapezoidal RT range:	9	sec	 Multiplie Addition 		
Penalty-free RT range:	9	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
				New	Choose

7 In the RT calibration file section, click **Choose**.

Figure 37.

8 Navigate to the relevant folder, and select HCs-RTCAL1.rtc.

1ethod					? 🗾	
Peak Detection Deconvolution Libr	ary Search Co	impound k	lentification	Target Match	Blank Subtraction	
Libraries:						
Z:\GCMS Data\Libraries\RI-PESTIC	CIDES-MOL2.m	slibrary.x	ml		Change Library	
					Add Library	
Move Up Move Down	1		Op	en Library	Remove Library	
Search criteria:		For	ward-Revers	e Search:		
Pre-search type:		Pur	e Weight Fa	tor:		
Normal	-		0.7			
Adjust Score			Example: 0.0 for rever	re cearch		
Remove Duplicate Hits			1.0 for forwa			
Match factor: Ves RT Match RT penalty function: Trapezoidal RT range:	9	sec	 Multip Additiv 	-		
Penalty-free RT range:	9	sec	Ma	x RT penalty:	20	
Gaussian						
Standard deviation:	6	sec				
RT calibration file: D:\MassHunter\Data\RI-PEST-MATRIX-120810\RTCAL\HCs-RTCAL1.tc New						
Advanced Apply to All :	Samples	Apply to	Selected Sar	nple Def	ault Close	



RT/RI calculation is used with library matching to lower the false positive rate. The window is set to ± 9 seconds to qualify the hits from the Library Search.

9 In the Libraries section, click Add Library.

Method			? 💌
Peak Detection Deconvolution Library Search	Compound Identification	Target Match	Blank Subtraction
Libraries:			
Z:\GCMS Data\Libraries\RI-PESTICIDES-MO	L2.mslibrary.xml		Change Library
			Add Library
Move Up Move Down	Ор	en Library	Remove Library

Figure 39.

10 Navigate to the relevant folder, and select NIST11.L.

Multiple libraries can be used in Library Search. For this example, the target MS library contains 900+ pesticides with Retention Indexes (RI) information. NIST11.L can be used for the additional confirmation.

11 Select Fast from the Pre-search type drop-down menu.

ethod					?
Peak Detection Deconvolution Libr	ary Search	Compound Id	entification	Target Match	Blank Subtraction
Libraries:					
Z:\GCMS Data\Libraries\RI-PESTIC	CIDES-MOL	2.mslibrary.xr	ml		Change Library
Z:\GCMS Data\Libraries\NIST11.L	Add Library				
Move Up Move Dow	n		Ot	oen Library	Remove Library
Search criteria:			vard-Revers		
Pre-search type:		Pure	Weight Fa	ctor:	
Fast	•		U.7 Example:		
Adjust Score			0.0 for reve		
Remove Duplicate Hits			1.0 for forwa	ard search	
Match factor:					
Use RT Match					
RT penalty function:			RT misma	atch penalty:	
Trapezoidal		Multiplicative			
RT range:	9	sec	Additi-	ve	
Penalty-free RT range:	9	sec	Ma	x RT penalty:	20
Gaussian					
Standard deviation:	6	sec			
RT calibration file:					
D:\MassHunter\Data\RI-PEST-M/	ATRIX-1208	10\RTCAL\H0	s-RTCAL1.	tc	
				New.	. Choose
Advanced Apply to All	Samples	Apply to 1	Selected Sa	mple De	fault Close
/ ppy to / ii	o ampiloo	, apply to a			Citat

Figure 40.

12 Click Apply to All Samples.

You are able to set different Library Search parameters for different libraries.

Set Compound Identification options

Click Compound Identification.

Method	?
Peak Detection Deconvolution Library Search	Compound Identification arget Match Blank Subtraction
Max hit count:	1
Min match factor:	50
Min MZ:	30
Library Search Type:	Spectral Search 👻
Multi-Library Search Type:	Al
Advanced Apply to All Samples	Apply to Selected Sample Default Close



For this example, the **Min match factor** is set to 50 for the compound identification from the Library Search.

- **Max hit count**: The maximum number of Library Search hits to report per component.
- Min MZ: The lower m/z limit for library match score calculation.
- Library Search Type: Three search modes are available:
 - Spectral Search: searches Spectral data
 - Retention Time Match: searches GC or LC data
 - Accurate Mass Pattern Match: searches Accurate Mass LCMS data

- Ratio percent uncertainty: Only applicable when Pre-search type is selected in Library Search. The larger the value, the more Library Search candidates are generated, and the longer the library search process.
- **Multi-Library Search Type**: If multiple libraries were used, two search modes are available:
 - All: Search all libraries (default)
 - **StopWhenFound**: Stop searching the library when enough candidates are found

Set Target Match options

1 Click Target Match.

eak Detection	Deconvolution	Library Search	Compound Identification	Target Match	Blank Subtraction
Target requirem	ients:				
Final concer	ntration		Qualifier ion(s)		
Target resp	onse		Qualifier ion ra	tios	
Hit ion match c	riteria:				
Target ion			Qualifier ion(s)		
			Qualifier ion ra	tios	
Hit RT match c	riteria:				
Within targe	t RT window				
Additional targe	t hit match:				
Use comport	und name		Use CAS#		
Estimation resp	onse factor:				
Estimation:					
No estimation					-
Manual respons	se factor:				



Target Match identifies quantitation targets using the quantitation method. The goal of identifying non-target compounds is simplified by filtering out the target matches. RT window, compound name, and CAS# can be applied for **Target Match**.

Method				? 💌
Peak Detection Dec	onvolution Library S	earch Compound Identification	n Target Match	Blank Subtraction
Target requirements:				
Final concentratio	n	📃 Qualifier ior	(s)	
Target response		Qualifier ion	ratios	
Hit ion match criteria:				
Target ion		📃 Qualifier ior	(s)	
		Qualifier ion	ratios	
Hit RT match criteria:				
Additional target hit m	atch:			
Use compound na		Vise CAS#		
Estimation response f	actor:			
No estimation				•
Manual response fac	tor:			
Advanced	Apply to All Samp	Apply to Selected	Sample D	efault Close

2 In the Hit RT match criteria section, select Within target RT window.

Figure 43.

3 In the Additional target hit match section, select Use compound name and Use CAS#.

thod					8
Peak Detection	Deconvolution I	ibrary Search	Compound Identification	Target Match	Blank Subtraction
- Target requirem	ients:				
Final concer	ntration		Qualifier ion(s)		
Target respo	onse		Qualifier ion ratio	tios	
Ht ion match o	iteria:				
Target ion			Qualifier ion(s)		
			🔲 Qualifier ion ra	tios	
Hit RT match o	riteria:				
Within targe	t RT window				
- Additional targe	t hit match:				
Use compo	und name		Use CAS#		
Estimation resp	onse factor:			-	
Estimation:					
No estimation					-
Manual respons	se factor:				
Advanced	Apply to	All Samples	Apply to Selected Sar	nple De	fault Close

Figure 44.

4 In the Estimation response factor section, select Relative ISTD Estimation from the Estimation drop-down menu.

Method					? 💌
Peak Detection	Deconvolution	Library Search	Compound Identification	Target Match	Blank Subtraction
Target requirem	ents:				
Final conce	ntration		Qualifier ion(s)		
Target resp	onse		Qualifier ion rat	ios	
Hit ion match c	iteria:				
Target ion			Qualifier ion(s)		
			Qualifier ion rat	ios	
Hit RT match c	iteria:				
Within targe	t RT window				
- Additional targe	t hit match:				
Use compo	und name		Use CAS#		
 Estimation resp Estimation : 	onse factor:				
Relative ISTD					
Manual respons					•
Manual respons	e factor:				
Advanced	Apply to	All Samples	Apply to Selected San	nple De	fault Close

Figure 45.

Set up the method for the analysis

Concentration estimation leverages the Quant target **Response Factors** (RF), which are applied to Non-Target hits as well. Estimation of response factors is flexible, and can be adjusted to suit the particular analytical requirements.

- 5 Click Apply to All Samples, and then click Close.
- 6 To save the analysis, select File > Save Analysis.

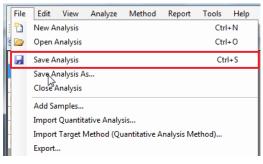


Figure 46.

Set Blank Subtraction options

Click Blank Subtraction.

Method
Peak Detection Deconvolution Library Search Compound Identification Target Mater Blank Subtraction
Perform Blank Subtraction
Retention Time Window O No retention time window
FWHM 5 times
Minutes
0.05 min.
Component Area
Sample <= 10 % of Blank (Example: 100)
Advanced Apply to All Samples Apply to Selected Sample Default Close

Figure 47.

- Perform Blank Subtraction: Select to perform blank subtraction.
- Retention Time Window: Select No retention time window, FWHM, or Minutes. If FWHM is selected, specify the number of time. If Minutes is selected, specify the number of minutes.
- **Peak Threshold**: Select **None**, **Component Area**, or **Estimated Concentration**. Enter a percentage.

Analyze and review results

- 1 In the Sample window, select the sample ISTD+solvent+MATRIX.
- 2 Select Analyze > Analyze Sample.

In the **Quant-My-Way** user interface, on the **Home** tab, select **Selected** Samples > Analyze Sample.

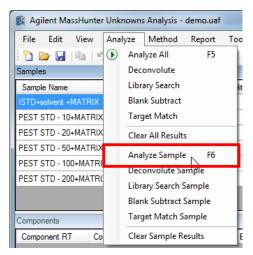


Figure 48.

To analyze the rest of the sample, click **Analyze All**. The analysis starts from where it left off and skips the sample(s) previously analyzed if no parameter in the method has been changed.



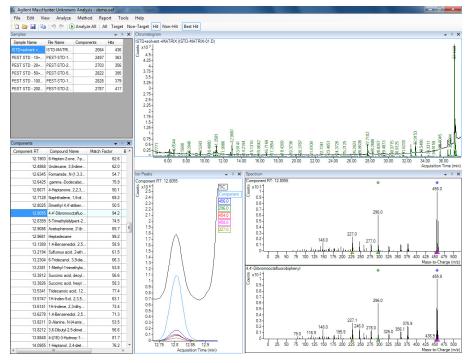


View validation information in the Analysis Messages.

Analysi	s Messages	→ # X
Туре	Target	Message
i	Sample ISTD+solvent +MATRIX	Deconvolution process has been already performed before. Skipping deconvolution process.
i	Sample ISTD+solvent +MATRIX	Library search process has been already performed before. Skipping library search process.
i	Sample ISTD+solvent +MATRIX	Blank subtraction process has been already performed before. Skipping blank subtraction process.
i	Sample ISTD+solvent +MATRIX	Target match process has been already performed before. Skipping target match process.

Figure 50.

After the analysis is complete, the main view that appears should look like the example below. This is the default layout and contains the default column settings.





Review best hit results

1 Right-click any column header in the **Samples** window, and select **Add/Remove Columns**.

Samples						🗸 🕂 🗙 Chroma
Sample Name	File Name	Compo	nents	Hits		
ISTD+solvent +	ISTD-MATRI		2064			Add/Remove Columns
PEST STD - 10+	PEST-STD-1		2497			Analyze Sample
PEST STD - 20+	PEST-STD-2		2703			Peak Detect Sample
PEST STD - 50+	PEST-STD-5		2822			Library Search Sample
PEST STD - 100	PEST-STD-1		2828			Blank Subtract Sample
PEST STD - 200	PEST-STD-2		2787			Target Match Sample
						Clear Sample Results
Components					×	Delete Sample
Component RT	Compound Na	ame	Match Fa	ctor		Format Column
4.419	4-Penten-2-one	e, 4-m		8	4	Print
4.4416	6 (·)-Methyl-3,3-d	limeth		5		Print Preview
4.4688	Pentanoic acid	ł		7	1.4	C5H100: 11 70

Figure 52.

2 Select Target Matches from the Available columns list and click Add.

Columns	10	? ×
Select columns from: <all> Available columns:</all>	•	Show these columns in the order:
Matrix Spike Group Plate Code Plate Pos. Ant. Info. Sample Group Pos. Type Sampling Date-Time Sampling Date-Time Sampling Date-Time Total Ant. Tray Name User Defined Val Code Code Tage Matches Work-raget Blank Subtracted	Add <> Add All >> Add All >> C< Remove All C <p< td=""><td>Sample Name File Name Components Hits Move Up Move Down et Default Cancel</td></p<>	Sample Name File Name Components Hits Move Up Move Down et Default Cancel
	UK Nes	

Figure 53.

3 Verify that the selected column is moved to the Show these columns in the order list, and click OK.

Sample Name	File Name	Components	Hits	Target Matches
	ISTD-MATRI	2064	436	2
PEST STD - 10+	PEST-STD-1	2497	363	39
PEST STD - 20+	PEST-STD-2	2703	356	43
PEST STD - 50+	PEST-STD-5	2822	395	51
PEST STD - 100	PEST-STD-1	2828	379	46
PEST STD - 200	PEST-STD-2	2787	417	48

Figure 54.

The Target Matches column is added to the **Samples** window.

4 Select the last sample in the **Samples** window.

Click one of the following toolbar buttons to view the changes in the **Components**, **Chromatogram**, **Ion Peaks**, and **Spectrum** windows.

In the **Quant-My-Way** user interface, click one of the following buttons on the **Home** tab.

- All: View all the peaks.
- Target: View the peaks that are also in the quantitation method.
- **Non-Target**: View the peaks that are not in the quantitation method.
- Hit: View the peaks that are found in the library search.
- Non-Hit: View the peaks that are not found in the library search.
- **Best Hit**: View the component with the highest library match score amongthe multiple hits of the same compound from different resolutions.

Analyze and review results

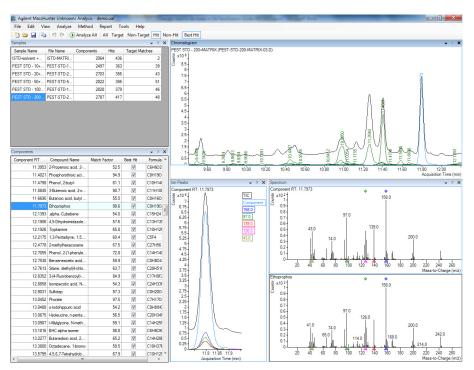


Figure 55.

Review blank hit subtraction results

- 1 Right-click any column header in the **Samples** window, and select **Add/Remove Columns**.
- 2 Select Type and Blank Subtracted from the Available columns list, and click Add.

Columns Select columns from: CAI> Available columns: ISTD Dilution	•		Show these colum	ns in the order:
Is To Judich Matrix Spike Group Plate Code Plate Pos. Art. Info. Sample Group Sample Group Sampling Time Gampling Time Tray Name User Defined Val Analysis State Non Target		Add All >> Add All >> <<- Remove All	Sanjue rvanie Tile Name Components Hits Target Matches	Move Down
		OK Re:	set Default	Cancel

Figure 56.

3 Verify that the selected columns are moved to the **Show these columns in the order** list, and click **OK**.

The Type and Blank Subtracted columns are added to the Samples window.

Samples							🚽 A 🗧
Sample Name	File Name	Components	Hits	Target Matches	Туре		Blank Subtracted
ISTD+solvent +	ISTD-MATRI	2064	436	2	Mat	•	0
PEST STD - 10+	PEST-STD-1	2497	363	39	Cal	-	155
PEST STD - 20+	PEST-STD-2	2703	356	43	Cal	-	153
PEST STD - 50+	PEST-STD-5	2822	395	51	Cal	•	142
PEST STD - 100	PEST-STD-1	2828	379	46	Cal	•	151
PEST STD - 200	PEST-STD-2	2787	417	48	Cal	-	139

Figure 57.

4 Note the list of available samples in the **Type** drop-down menu.

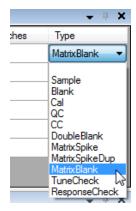


Figure 58.

The values shown in the **Blank Subtracted** column in the **Samples** window represent the number of hits that were blank subtracted from the samples.

Hits in a sample are marked as **Blank Subtracted Hits** when the same hit is found in the blank with RT±5FWHM. FWHM of a typical GC-MS peak is 1-2s. If we use 2s on this estimation, 5FWHM = 10s = 0.17min. You can see the **Blank Subtracted** hits only when you click **All** in the toolbar.

Blank Hit Subtraction is performed against the "blank" sample(s). The hit(s) in any sample(s) with Sample Type classified as Blank, DoubleBlank, or MatrixBlank will automatically get subtracted from all the standard samples during the process. You can designate the "blank" sample for blank subtraction purposes by changing the Sample Type in the Sample. No Blank Subtraction happens if there is no "blank" sample(s). Change the sample type to turn off Blank Subtraction.

Use Show Alternate Hits to evaluate results

- 1 Right-click any column header in the **Components** window, and select **Add/Remove Columns**.
- 2 Select Library File from the Available columns list, and click Add.

Select columns from: Library Search Method	~ · · · · · ·
Available columns: Library File Library Type Pre-search Enabled Pre-search Enabled Pre-search Type NIST Compatibility Pure Weight Factor Search Order RT Calibration RT Max Penalty RT Penalty Type RT Range RT Range RT Range No Penalty Spectrum Threshold Remove Duplicate Hits Accurate Mass Tolerance	Show these columns in the order:

Figure 59.

- 3 Verify that the selected columns are moved to the **Show these columns in the order** list, and click **OK**.
- 4 Select the **PEST STD-10+MATRIX** sample in the **Samples** window and click **Hit** in the toolbar to view the changes in the **Components** window.

In the	Quant-	·My-Way	use	r interface	e, or	ר ⁻	the Home i	tab, click I
😰 Agilent MassH	unter Unknowr	ns Analysis - dem	o.uaf					
	ew Analyze	,		ols Help	_	_		
i 눱 📂 🛃 💷	19010	Analyze All A	ll Target	Non-Target Hit	Non-H	lit	Best Hit	
Samples							🗕 🕂 🔶	
Sample Name	File Name	Components	Hits	Target Matches	Туре		Blank Subtracted	
ISTD+solvent +	ISTD-MATRI	2064	436	2	Mat	•	0	
PEST STD - 10+	PEST-STD-1	2497	363	39	Cal	•	155	
PEST STD - 20+	PEST-STD-2	2703	356	43	Cal	•	153	
PEST STD - 50+	PEST-STD-5	2822	395	51	Cal	•	142	
PEST STD - 100	PEST-STD-1	2828	379	46	Cal	•	151	
PEST STD - 200	PEST-STD-2	2787	417	48	Cal	-	139	

Figure 60.

Agilent MassH	unter Unknowns	Analysis - demo	o.uaf	-		-				-									- 0	x
	w Analyze		_	ools Help																
12 🗁 🖬 🕒						Hit Best Hit	1													
Samples	1-7 (- 107	analyze All Al	n narge		get The Ison			Chromato	ram										_	0
Sample				Target		Blank	~			RIX (PEST	-STD-10-MA	TRIX-	13 D)							
Name	File Name C	omponents	Hts	Matches	Туре	Subtracted		\$10 ⁶												
STD+solvent +	ISTD-MATRI	2064	436		2 Mat 💌	0		S 6.												
PEST STD - 10+	PEST-STD-1	2497	363		39 Cal 🔻	155		5.5-												
EST STD - 20+	PEST-STD-2	2703	356		43 Cal 💌	153		5.												
PEST STD - 50+	PEST-STD-5	2822	395		51 Cal 💌	142		4.5-									- 11			
	PEST-STD-1	2828	379		46 Cal 🝷	151		4.									- 11			
EST STD - 200	PEST-STD-2	2787	417		48 Cal 💌	139		3.5-									- 11			
																	11			
								3-	I.								- 11			
								2.5-	- 4								A . []			
								2.	1	٩			- 8				$(\Lambda N) \Lambda$	n		
								1.5-	A	1	۸.	٨	A		. 5			40	b .	
omponents						• 9	×	1-	/\	000	-grb	Ng	لي لي ا	-	118 B		10054	3825	Ana	St.
Component RT	Compound Nam	Match Fa	ctor	Best Hit	Formula	Library File	-	0.5-	8.1888	8.4986	9.045	1280.0	59.407	9.7685 9.8918 9.996 9	10 T	0.8414	11.2888	E.	11.20	1216
10.5295	7-Azaindole-3-car	bo	53.9	V	C8H6N2O	NIST11.L		<u> </u>	00 8.25		8.75 9.00	9.25	- Andrews	Marine	10.25 10.50	- int	- Same	11.50	11.75 12.00	12.25
10.8414	Diethyl 2,5-pyridin	edi	56.9	V	C11H13NO4	NIST11.L		· ·	00 0.25	0.50	0.75 5.00	0.20	0.00	3.75 10.00	10.20 10.00	10.75	1.00 11.20		Acquisition Tin	
10.9597	Decane, 2,3,8-tri	net	76.5	V	C13H28	NIST11.L		Ion Peaks				x S	pectrum							. ų)
10.9600	Nonane, 4,5-dime	thyl-	69.8		C11H24	NIST11.L			t RT: 11.80	017	_			RT: 11.8017		٠	80			
11.0465	Cyclobuta[1,2-d:3	.4	78.4	V	C6H8O4	NIST11.L		왕 ×10 6 1.1-	Δ		TIC	- ter	x10 ⁻²			15	0.80			
11.0954	1.3-Dioxol-2-one		65.1	V	C3H2O3	NIST11.L		1.05-	1	1	Componen	- 2	0.9-		97.0					
11.1416	DL-3-Aminoisobut	yri	50.5	V	C11H22N	NIST11.L	b	1-	V (_		158.0 97.0		0.7-		1					
11.1417	4H-1,3-Dioxin-4-o	ne,	50.4	V	C9H14O3	NIST11.L	E	0.95-	\mathbb{N}	1	126.0		0.6-	-	4.0	126.0				
11.2888	2-Butyl-5-pentylpy	lon	52.0	V	C13H27N	NIST11.L	Г	0.9-	~	(A)	74.0		0.5-	43.0		Tr				
11.3825	3-Hydroxy-7,8-dih	ydr	61.9	V	C13H20O2	NIST11.L		0.85-		~ \	139.0		0.4-	43.0			20	0.0		
11.3989	Phosphorothioic a	ici	73.5	V	C8H19O3	NIST11.L		0.75-			· · · · ·		0.2-	1 1		11				
11.4558	1H-3a,7-Methano	az	58.0	V	C15H24	NIST11.L		0.7-					0.1-				1 1		1	
11.7018	2-Cyclohexen-3-o	H1	63.5	V	C8H11NO2	NIST11.L	1	0.65-					0-		30 100	120 140				
11.8017	Ethoprophos		86.1		C8H19O2	RI-PESTICID		0.6-						20 40 60	80 100	120 140	160 180 20		240 260 280 Mass-to-Charo	
11.8717	3-Trifluoromethylb	en	58.3	V	C8H6F3NO	NIST11.L		0.55-				E	thoprophot			٠	~	_		
11.8908	5-Pyrimidinol, 2-m	sth	54.5		C6H8N2OS	NIST11.L	1	0.5-				e e	x10 ²			15	s <mark>8.0</mark>			
12.0120	tauMuurolol		51.9	V	C15H260	NIST11.L		0.45-				Ē								
12.1692	[1,3]Oxathiolane-	4-a	52.5	V	C9H14O4S	NIST11.L		0.35-					0.8-							
12.1954	DL-Tryptophan. N	I-m	59.2	V	C12H14N	NIST11.L	1	0.3-					0.7-		97.0					
12.4782	2,2-Dimethylpropa	ino	63.7	V	C21H42O2	NIST11.L		0.25-					0.5-			126.0				
12.5117	Cyclopropane, 1,	1.2	51.9	V	C10H20	NIST11.L		0.2-	Λ				0.4-	41.0			20	0.0		
12.7925	2,3-Dihydro-5-mel	hyl	56.2	V	C10H120	NIST11.L	1	0.15-					0.3-	1. 7	4.0	11		1	242.0	
12.8410	2-Fluorobenzoic a	d	71.8	V	C13H8FNO4	NIST11.L	1	0.1-		1			0.2-	- II I			L .		242.0	
12.9115	Sulfotep		53.9	V	C8H2005	RI-PESTICID	1	0.05-	1	1			0	لم الل	لشيل بال		h.	214.0		
13.0450	Phorate		76.4	V	C7H1702	NIST11.L	1	0.	11.8		-			20 40 60	80 100	120 140	160 180 20		240 260 280	
13 1774	Puttine 2 carbox	dia.	59.4	1	CENOCION	NIST111	Ŧ		Acquisition	n Time (mi		니니							Mass-to-Charg	je (m/z

Verify that the **Best Hits** are from different libraries.

Figure 61.

5 Right-click **Phosphorothioic acid** in the **Compounds** window and select **Show Alternate Hits**.

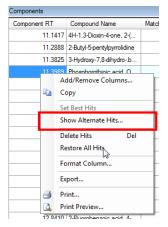
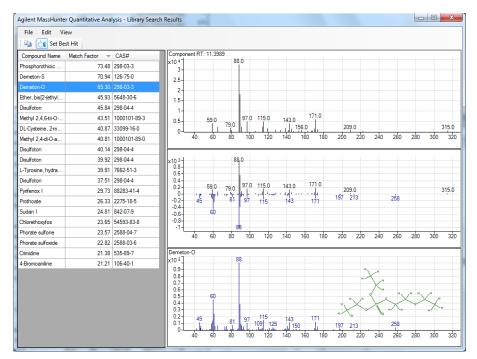


Figure 62.



The Library Search Results are displayed.

Figure 63.

6 Right-click any column header in the Library Search Results window, and select Add/Remove Columns.

Agilent MassHunte	r Quantitative Ana	lysis - Librai	y Search Results
File Edit Vi	ew		
i 🗈 试 Set Bes	st Hit		
Compound Name	Match Factor 🔍	CAS#	Add/Remove Columns
Phosphorothioic	73.48	298-03-3	~
Demeton-S	70.94	126-75-0	

Figure 64.

Available Columns:		Show these columns in the order:
Boil Point	Add ->	Compound Name Match Factor
Library	<- Remove	CAS#
Jbrary Retention Time Melt Point	Add All ->>	
Molecular Weight Overlap Count	< Remove All	
Retention Time Difference Farget Retention Index	< Remove All	
arget Retention Index		

7 Select Library from the Available Columns list, and click Add.

Figure 65.

8 Verify that the selected columns are moved to the **Show these columns in the order** list, and click **OK**.

File Edit Vi	ew			
🗄 🗈 🔀 Set Be	st Hit			
Compound Name	Match Factor -	CAS#	Library	Component RT: 11.3989
Phosphorothioic	73.48	298-03-3	Z:\GCMS Data\Libraries\NIST11.L	x10 ⁴ 88.0
Demeton-S	70.94	126-75-0	Z:\GCMS Data\Libraries\NIST11.L	2.5-
Demeton-O	65.30	298-03-3	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	2.5
Ether, bis[2-(ethyl	45.93	5648-30-6	Z:\GCMS Data\Libraries\NIST11.L	1.5
Disulfoton	45.84	298-04-4	Z:\GCMS Data\Libraries\NIST11.L	1-
Methyl 2,4,6-tri-O	43.51	1000101-89-3	Z:\GCMS Data\Libraries\NIST11.L	0.5 59.0 115.0 143
DL-Cysteine, 2-m	40.87	33099-16-0	Z:\GCMS Data\Libraries\NIST11.L	
Methyl 2,4-di-O-a	40.81	1000101-89-0	Z:\GCMS Data\Libraries\NIST11.L	40 60 80 100 120 140
Disulfoton	40.14	298-04-4	Z:\GCMS Data\Libraries\NIST11.L	
Disulfoton	39.92	298-04-4	Z:\GCMS Data\Libraries\NIST11.L	x10 ³ 88.0
L-Tyrosine, hydra	39.91	7662-51-3	Z:\GCMS Data\Libraries\NIST11.L	0.8-
Disulfoton	37.51	298-04-4	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	0.4-
Pyrifenox I	29.73	88283-41-4	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	0.2 59.0 115.0 143
Prothoate	26.33	2275-18-5	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	-0.2-45 81 97 115 14
Sudan I	24.81	842-07-9	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	-0.4-
Chlorethoxyfos	23.65	54593-83-8	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	-0.8-
Phorate sulfone	23.57	2588-04-7	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	
Phorate sulfoxide	22.82	2588-03-6	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	40 60 80 100 120 140
Crimidine	21.38	535-89-7	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	Demeton-O
4-Bromoaniline	21.21	106-40-1	Z:\GCMS Data\Libraries\RI-PESTICIDES-M	x10 ³ 88
			·	0.9-

The **Library** column is added to the table.

Figure 66.

9 Select Demeton-0 and click Set Best Hit.

A	gilent Ma	ssHunter Quant	itative Analy	sis - Librar	y Search Results
	File E	dit View			
	🖻 🙆	Set Best Hit			
Γ	Compound	Name Match	Factor 👻	CAS#	Component F

Figure 67.

Verify that the selected compound replaced the previous compound as the current **Best Hit** in the **Component** table.

Component RT	Compound Name	Match Factor	Best Hit	Formula	Library File
10.5295	7-Azaindole-3-carbo	53.9	V	C8H6N2O	NIST11.L
10.8414	Diethyl 2,5-pyridinedi	56.9	V	C11H13NO4	NIST11.L
10.9597	Decane, 2,3,8-trimet	76.5	V	C13H28	NIST11.L
10.9600	Nonane, 4,5-dimethyl-	69.8	V	C11H24	NIST11.L
11.0465	Cyclobuta[1,2-d:3,4	78.4	V	C6H8O4	NIST11.L
11.0954	1,3-Dioxol-2-one	65.1	V	C3H2O3	NIST11.L
11.1416	DL-3-Aminoisobutyri	50.5	v	C11H22N	NIST11.L
11.1417	4H-1,3-Dioxin-4-one,	50.4	V	C9H14O3	NIST11.L
11.2888	2-Butyl-5-pentylpyrrol	52.0	V	C13H27N	NIST11.L
11.3825	3-Hydroxy-7,8-dihydr	61.9	V	C13H20O2	NIST11.L
11.3989	Demeton-O	65.3	V	C8H19O3	RI-PESTICID
11.4558	1H-3a,7-Methanoaz	58.0	V	C15H24	NIST11.L
11.7018	2-Cyclohexen-3-ol-1	63.5	V	C8H11NO2	NIST11.L
11.8017	Ethoprophos	86.1	V	C8H19O2	RI-PESTICID
11.8717	3-Trifluoromethylben	58.3	V	C8H6F3NO	NIST11.L
11.8908	5-Pyrimidinol, 2-meth	54.5	V	C6H8N2OS	NIST11.L
12.0120	.tauMuurolol	51.9	V	C15H26O	NIST11.L
12.1692	[1,3]Oxathiolane-4-a	52.5	V	C9H14O4S	NIST11.L

Figure 68.

Review concentration estimation results

- 1 Right-click any column header in the **Components** window, and select **Add/Remove Columns**.
- 2 Select Base Peak Deconvoluted Area, Response Factor for Estimation, Target Multiplier, Estimated Conc., and Target Calc. Conc. from the Available columns list, and click Add.

Target Peak (from Quant) 🔹]	
Available columns:		Show these columns in the order:
Target Peak Area Target Calc. Conc. Final Conc.	Add >>	Component RT Compound Name Match Factor Best Hit
Target Peak FWHM Target Peak Height Target Peak Noise Target Peak Manually Integrated	Add All ->>	Formula Base Peak Deconvoluted Area Response Factor for Estimation
Target Peak Q Value Computed Target Peak RT Target Peak SNR Target Peak Symmetry	< Remove All	Target Multiplier Estimated Conc.
Target Resp. Target Peak Width Target Peak Ref. Lib. Match Score Target Peak Purity		
		Move Up Move Down

Figure 69.

- 3 Verify that the selected columns are moved to the **Show these columns in the order** list, and click **OK**.
- 4 Select the **PEST STD-200+MATRIX** sample in the **Samples** window, and click **Target** in the toolbar to view the changes in the **Components** window.

File Edit Vi	ew Analyze	Method Rep	ort To	ols Help			
🛅 🗁 🛃 🗎	1 1 ° C 10	Analyze All A	ll Target	Non-Target	Hit I	Non-	Hit Best H
Samples							- 4 ×
Sample Name	File Name	Components	Hits	Target Matches	Туре		Blank Subtracted
ISTD+solvent +	ISTD-MATRI	2064	436	2	Mat	-	0
PEST STD - 10+	PEST-STD-1	2497	363	39	Cal	-	155
PEST STD - 20+	PEST-STD-2	2703	356	43	Cal	-	153
PEST STD - 50+	PEST-STD-5	2822	395	51	Cal	-	142
PEST STD - 100	PEST-STD-1	2828	379	46	Cal	-	151
PEST STD - 200	PEST-STD-2	2787	417	48	Cal	-	139

Figure 70.

The estimated concentration results are listed in the **Estimated Conc**. column. For target compounds, you are able to compare with the Quant calculated concentrations.

Estimated Concentration is calculated using the following formula:

$EstimatedConcentration = \frac{BasePeakDeconvolutedArea}{RF for Estimation} \times Multiplier$

Component RT	Compound Name	Match Factor	Base Peak Deconvoluted Area	Response Factor for Estimation	Target Multiplier	Estimated Conc.	Target Calc. Conc.
11.402	Phosphorothioic aci	94.9	2721304.4	14882.6150	1.0	182.9	144.4
11.7973	Ethoprophos	98.6	2129122.9	23030.7854	1.0	92.45	80.33
12.903	Sulfotep	97.3	1015154.5	14085.4273	1.0	72.07	60.25
13.0452	Phorate	97.5	3420580.5	36958.8482	1.0	92.55	69.61
13.1810	BHC alpha isomer	98.8	1624103.4	30149.9823	1.0	53.87	50.11
13.7016	Pentachloroanisole	98.8	1731347.2	31680.1152	1.0	49.84	50.17
13.765	Dimethoate	96.0	2679103.0	27597.5245	1.0	97.08	79.42
14.3218	BHC beta isomer	98.5	1014024.0	16466.2678	1.0	59.17	49.7
14.584	Lindane	98.0	1123973.2	20441.2941	1.0	54.99	50.04
15.0133	Fonofos	97.4	2705421.8	35661.4284	1.0	75.86	60.31
15.588	Diazinon	98.2	2028613.6	19208.4327	1.0	98.63	79.78
15.678	Disulfoton	85.7	1203625.2	14752.9912	1.0	81.59	61.33
15.6842	BHC delta isomer	96.2	1201797.5	22136.8200	1.0	54.29	50.04
17.726	Methyl parathion	86.9	1539303.6	7893.1866	1.0	108	71.86
17.7336	Chloropyriphos-methyl	91.9	2612062.4	25588.5789	1.0	102.1	81.29
17.9452	Heptachlor	95.7	598014.1	9867.5025	1.0	60.6	50.24

Figure 71.

5 Click **Non-Target** in the toolbar to view the estimated concentrations for Non-Targets.

In the Quant-My-Way user interface, on the Home tab, click Non-Target.

File Edit Vi	ew Analyze	Method Rep	ort To	ools Help								
🔁 🗁 🖬 👒	0 9 0 10	Analyze All A	II Targe	t Non-Target	Hit No	n-Hit Best Hit						
Samples												– 4
Sample Name	File Name	Components	Hits	Target Matches	Туре	Blank Subtracted						
STD+solvent +	ISTD-MATRI	2064	436	2	Mat	• 0						
PEST STD - 10+	PEST-STD-1	2497	363	39	Cal	155						
PEST STD - 20+	PEST-STD-2	2703	356	43	Cal	• 153						
PEST STD - 50+	PEST-STD-5	2822	395	51	Cal	• 142						
PEST STD - 100	PEST-STD-1	2828	379	46	Cal	• 151						
	PEST-STD-2	2787	417	48	Cal	• 139						
Components												- 4
c	Compound Na	me Match Fa	inter	Best Hit	Formula	Base Peak		Response Factor	Target Multiplier	Estimated Conc.	Target Calc.	
RT 4	< compound Na	me Match Fa	ictor	Dest Hit	onnaid	Deconvoluted A	vea	for Estimation		Conc.	Conc.	
RT	9 8-Hydroxy-2-oc		71.8		8H16O2		krea 656768.6	for Estimation 7286050.8765	- and a second	0.2954	Conc.	
RT 4.417		tanone		I C							Conc.	
RT 4.417 4.419 4.419	9 8-Hydroxy-2-oc	tanone N-tetr	71.8	v c	8H16O2	2	656768.6	7286050.8765		0.2954	Conc.	
RT 4.4179 4.4190 4.5353	9 8-Hydroxy-2-oc 8 2-Butenamide,	tanone N-tetr	71.8 65.3		8H16O2 10H17NO	2	656768.6 79840.9	7286050.8765 7286050.8765		0.2954	Conc.	
4.417 4.419 4.535 4.548	9 8-Hydroxy-2-oc 8 2-Butenamide, 3 3-Methylpyridaz	tanone N-tetr	71.8 65.3 88.0		8H16O2 10H17NO 5H6N2	2	656768.6 79840.9 169459.6	7286050.8765 7286050.8765 7286050.8765		0.2954 0.03364 0.04268	Conc.	

Figure 72.

6 To save the analysis, select File > Save Analysis.

In the Quant-My-Way user interface, on the Home tab, click Save Analysis.

Task 3: Generate the Report

Task 3: Generate the Report

1 Select Report > Generate.

In the Quant-My-Way user interface, on the Home tab, click Generate Report.

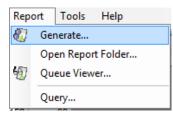


Figure 73.

2 Under Report method, click New.

Report	8 X
Report folder:	
D:\MassHunter\Data\RI-PEST-MATRIX\UnknownsReports\B482	Browse
Report method:	
Choose New	Edit
Samples:	
All samples	
Selected sample(s)	
Generate:	
Generate reports now	
Open report folder after reports generated	
Queue report task	
✓ Start Queue Viewer	
ОК	Cancel



3 Right-click in the window and select Add Template.

Report Method Edit (Unknowns Analysis)					
File Edit Tools					
1 🗁 🖬 🕺 📾 🛍 🛩 🧉 🚹					
Templates Graphics settings					
Template	Report mode	Destination file	Publish format	Language	Page
	Add Tem	olate			
	Remove T	emplate			
<					P.
Add Template Remove Template]			Edit Post Processe	s
			Save & Exit	Exit	

Figure 75.

4 Navigate to D:\MassHunter\Report Templates\ Quant\PDF-Report-Builder\ Unknowns, select LSR_NonTarget_Hits.template.xml, and click Open.

	nplates ▶ Quant ▶ PDF-ReportBuilder ▶ Unknowr	ns 👻 🗲		
Organize 🔻 New folder				- 🗆 🤇
🔆 Favorites	Name	Date modified	Туре	Size
	AreaPercent.template.xml	8/31/2017 9:44 AM	XML Document	50 KB
🧮 Desktop	LSR_NonTarget_Details.template.xml	8/31/2017 9:44 AM	XML Document	121 KB
	LSR_NonTarget_Hits.template.xml	8/31/2017 9:44 AM	XML Document	72 KB



Once the template(s) is selected, you can configure the **Report Publish Format** with *PDF*, *TEXT*, and *CSV*, **Language** with *English*, *Chinese*, *Japanese*, and *Russian*, **Page Size**, **Printer** with *A4* and *Letter*, and whether or not to **Open published file** after generating the report. The **Post Process** is also available to process the report further after finishing the report task.

Report Method Edit (Unknowns Analysis)			
File Edit Tools			
🖞 🗁 🛃 👗 🛍 🛍 🖌 🔛			
Templates Graphics settings			
Template	Report mode Destina	tion file Publish format	Language Page
D:\M\LSR_NonTarget_Hits.template.xml	Batch - LSR_No	on Target PDF	Content - Con
		PDF TEXT	
		CSV	
< [۱.
Add Template Remove Template			Edit Post Processes
		Save & Exit	Exit

Figure 77.

5 Click Graphics settings.

📅 Report Method Edit (Unknowns Analysis)	
File Edit Tools	
Template: Graphics settings	
Fixed range graphics:	
Fixed range graphics settings	
Sample chromatograms:	
Graphics settings	
Ion peaks chromatograms:	
Graphics settings	
Save & Exit	Exit



- Click Fixed range graphics settings to manipulate the way to want to present the graphics generated in your report by restricting the scale of your graphs.
- Click Sample chromatograms: Graphics settings to adjust the appearance of the sample chromatograms.
- Click **Ion peaks chromatograms: Graphics settings** to adjust the appearance of the ion peaks chromatograms.
- 6 Click Save & Exit to save the Report Method in a desired location.

📆 Repo	ort Method Edit (Unknowns Ana	Ilysis)				x
File	Edit Tools					
ት 🗁	🔒 👗 🗈 🐍 🔊 (° 🛉					
Templat	tes Graphics settings					
	Template	Report mode	Destination file	Publish format	Language Page	
•	D:\M\LSR_NonTarget_Hits.tem	plate.xml Batch	 LSR_NonTarget 	PDF 👻	<default> 🔻 Letter</default>	
•		111			4	
	Add Template Remo	ve Template			Edit Post Processes	
				Save & Exit	Exit	

Figure 79.

Report Methods have a .m extention.

📅 Save As						
🔾 🔾 🗸 Report Templates 🔸 Quant 🔸 PDF-ReportBuilder 🔸 Unknowns 🔹 🔹 😽				👻 🐓 Sea	rch Unknowns	٩
Organize 👻 Ner	w folder				!≡ ▼	0
🔆 Favorites	 Name 	^	Date modified	Туре	Size	
Desktop Desktop Libraries Homegroup Leah Parent Computer Network Control Panel Recycle Bin Recycle Bin	E		No items match your se	arch.		
File name	NonTargetHits.m					-
Save as type:	Methods (*.m)					-
Hide Folders					Save Cance	el



7 For samples, you can generate a report for **All samples** or the **selected Sample(s)**.

For Report Generating modes, you can select **Generate reports now** or **Queue report task**.

	Report ? X			
	Report folder: D\MassHurter\Data\RI-PEST-MATRIX\UnknownsReports\B482 Browse			
Report method:				
	D:\MassHunter\Report Templates\Quant\PDF-ReportBuilder\Unknowns\TargetHits Choose New Edit			
	Samples:			
	All samples			
	 Selected sample(s) 			
	Generate:			
	Generate reports now			
	Open report folder after reports generated			
	Queue report task If the second sec			
	OK Cancel			

Figure 81.

8 Click **OK** to begin generating reports.

The report folder opens automatically when the report generation is complete.

Alternatively, you can select **Menu > Open Report Folder** to view the newly generated report **LSR_NonTarget_Hits.pdf**. The report opens in Adobe Reader.

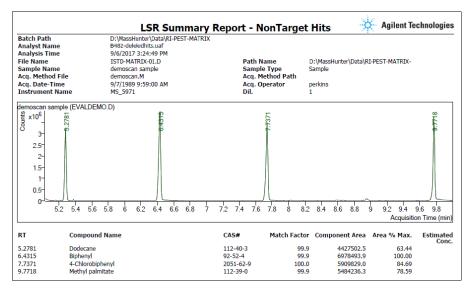


Figure 82.

- 9 Close the report.
- 10 To exit the program, select File > Exit.

Task 3: Generate the Report

Agilent MassHunter Workstation Unknowns Analysis Familiarization Guide

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September 2017 REVISION2 (use for SAP revision if different from REVISION)



G3335-90243 REVISION

