

# GC/Q-TOF PCDL

# **User Guide**

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### **Software Revision**

This guide is valid for the C.01.00 revision or higher of the GC/Q-TOF PCDL program and compatible GC/Q-TOF PCDL programs, until superseded.

### Software Manufacturing

Manufactured for Agilent Technologies 5301 Stevens Creek Blvd Santa Clara, CA 95051

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## In This Book

This book describes the GC/Q-TOF PCDLs. It explains the system configuration and/or method setup information pertaining to subset PCDLs and provides a general data analysis workflow.

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### Content

### 1 Overview

What is the GC/Q-TOF PCDL? 8 Software Requirements 8 Installation file locations 8 Glossary 9

7

### 2 Pesticides PCDL for GC/Q-TOF 11

GC/Q-TOF System Setup 12

Parts and Consumables 13

### Data Acquisition Methods 14

HSL Operating Conditions 14 CSL Operating Conditions 15 Set up a Cold Splitless Injection Method 16

### Retention Time Locking the Data Acquisition Method 17

Set up an Automatic Mass Calibration in your Sequence 21

### 3 Metabolomics PCDL for GC/Q-TOF 25

#### Sample Preparation and Derivatization 26

Biological sample extracts 26 Metabolites 26 Internal standard 26 Retention index markers 27 Derivatization 28

#### Content

#### Configuration and Acquisition Method 30

4 Natural Products PCDL for GC/Q-TOF 31

PCDL Description 32

Configuration and Data Acquisition Method 33

#### 5 Data Analysis Workflows 35

# Overview of Target and non-target screening workflows for high-resolution accurate mass GC/Q-TOF 36

Managing the PCDL content 36

#### Adding GC/Q-TOF Spectra to a PCDL 40

Configuring MassHunter Qualitative Analysis 10.0 40 Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software 42 Annotation of a Spectrum with Fragment Formulas 44

#### SureMass Overview 47

#### Suspect Screening and Target Quantitation Workflows 48

Summary of a Typical Workflow for Suspect Screening and Target Quantitation with Large Number of Targets 48 Suspect Screening and Target Quantitation Method Setup 48 Target Quantitation Method Setup 50 Suspect Screening Method Setup 55

#### Non-targeted Screening in the Unknowns Analysis Software 64

# 1 Overview

This chapter provides an overview of the GC/Q-TOF PCDL.

What is the GC/Q-TOF PCDL? 8 Software Requirements 8 Installation file locations 8 Glossary 9 1

Overview What is the GC/Q-TOF PCDL?

### What is the GC/Q-TOF PCDL?

The GC/Q-TOF Personal Compound Database and Library (PCDL) is a collection of accurate mass spectral libraries that let you screen for a wide range of analytes. This document describes configuration and method setup for data acquisition when using Pesticides, Metabolomics, or Natural Product PCDLs. It also provides a generic data analysis workflows.

### **Software Requirements**

The following software is required:

- Agilent MassHunter GC/MS Acquisition software B.10.2
- Agilent MassHunter Qualitative Data Analysis software B.10.0
- Agilent MassHunter Quantitative Data Analysis software B.10.2 or B.11.1
- Use Agilent ChemVista to manage compound and spectral data, including creating subset target lists

### Installation file locations

Folder location for files after installation from the Agilent-provided solution disc are described in the following table.

Item	Folder location for files
GC/Q-TOF Metabolomics PCDL (2 .cdb files)	D:\MassHunter\PCDL\GCQTOF PCDLs\Metabolomics
GC/Q-TOF Natural Products PCDL (2 .cdb files)	D:\MassHunter\PCDL\GCQTOF PCDLs\Natural Products
GC/Q-TOF Pesticides PCDL (3 .cdb files)	D:\MassHunter\PCDL\GCQTOF PCDLs\Pesticides
See the Installation Guide for more details pertai with a MassHunter home already defined and/o	ning to the installed file location. When installed on a PC r a fixed D:\ drive, files will be installed to the location above.

Overview Glossary

# Glossary

Item	Description
CI	Chemical Ionization
CSL	Cold splitless mode
FAME	Fatty Acid Methyl Esters
EI	Electron Ionization
GC	Gas Chromatography
HSL	Hot splitless mode
MS	Mass spectrometry
PCDL	Personal Compound Database and Library
Q-TOF	Quadrupole Time-of-Flight mass spectrometer
SureMass	Agilent signal processing algorithm for chemical component detection designed specifically for high-resolution profile MS data
RTL	Retention Time Locking

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# Pesticides PCDL for GC/Q-TOF

This chapter describes the system configuration and recommended data acquisition methods to implement the Pesticide PCDL and Workflow for the GC/Q-TOF solution. Three PCDLs are included for pesticides, each with a different set of RTs based on different gradients with specific GC oven ramps described below.

The GC/Q-TOF system is configured with a midcolumn backflushing setup. After the hardware is properly installed, perform the Retention Time Locking the Data Acquisition Method procedure.

GC/Q-TOF System Setup 12

Parts and Consumables 13

Data Acquisition Methods 14

HSL Operating Conditions 14

CSL Operating Conditions 15

Set up a Cold Splitless Injection Method 16

Retention Time Locking the Data Acquisition Method 17

Set up an Automatic Mass Calibration in your Sequence 21

2

GC/Q-TOF System Setup

# GC/Q-TOF System Setup

The Agilent 7250 Quadrupole Time-of-Flight GC/Q-TOF MS System is shown in the following figure.



#### The Agilent 8890 GC system includes:

- Fast oven power supply, where applicable (G3540 Option 002 or 003)
- Split-Splitless or Multimode G3540 Inlet (G3540 Option 112, 150, or 151) The Split/Splitless inlet (Option 112) only allows for a hot split or splitless injection method. For cold splitless or large volume injection methods, use a Multimode Inlet cooled with air or liquid N2 (Option 151). If a Multimode Inlet cooled with liquid  $CO_2$  is preferred, choose options 150.
- Mass Spectrometer Detector Interface (G3450-60599)
- Pre-Installed Purged Ultimate Union (G3440-60604)

#### The Agilent 7693 Series system includes:

- Agilent 7693A Autoinjector Module (G4513A)
- Agilent 7693A Autosampler Tray Module (G4514A)

Parts and Consumables

# Parts and Consumables

Required Parts and Consumables and described in the following table.

Part number	Description	Quantity
19091S-431U	HP-5ms Ultra Inert, 15 m, 0.25 mm, 0.25 $\mu$ m, 7-inch cage	2
5183-4757	Agilent septa, bleed and temperature optimized (BTO), nonstick, 11 mm, 50/pk	1
5190-2293	Agilent liner, Ultra Inert, splitless, single taper, glass wool	1
5190-2297	Agilent liner, Ultra Inert, splitless, dimpled, 2 mm id	1
5190-6194	Self-tightening column nut, for Agilent inlet and detector fittings	1
5190-5233	Self-tightening column nut, for Agilent mass spec interface transfer line	1
5181-3323	Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 0.1 to 0.25 column, 10/pk	1
1460-1914	Column hanger	1
G3440-80217	Column install pre-swaging tool, graphite	1

The GC/Q-TOF hardware setup is shown in the following figure.



# Data Acquisition Methods

This section describes the HSL and CSL operating conditions.

### **HSL Operating Conditions**

The operating conditions for the two recommended Hot Splitless (HSL) methods are described in the following table.

	Hot splitless, 20 minutes	Hot splitless, 40 minutes
GC		
Columns	Agilent HP-5ms UI, 15 m, 0.25 mm id, 0.25 $\mu$ m film (two each)	Agilent HP-5ms UI, 15 m, 0.25 mm id, 0.25 $\mu$ m film (two each)
Carrier gas	Helium	Helium
Column 1 flow	1.0 mL/min	1.0 mL/min
Column 2 flow	1.2 mL/min	1.2 mL/min
Injection volume	1 $\mu$ L splitless	1 $\mu$ L splitless
Inlet liner	4 mm id Ultra Inert Liner Single Taper w wool (p/n 5190-2293)	4 mm id Ultra Inert Liner Single Taper w wool (p/n 5190-2293)
MMI temperature	280 °C	280 °C
Septum purge flow	3 mL/min	3 mL/min
Purge flow to split vent	50 min/min at 1 minute	50 min/min at 1 minute
Gas saver	On, 20 mL/min after 2 minutes	On, 20 mL/min after 2 minutes
Oven temperature program	60 °C for 1 minute 40 °C/min to 170 °C, 0 minutes 10 °C/min to 310 °C, 3 minutes	60 °C for 1 minute 40 °C/min to 120 °C, 0 minutes 5 °C/min to 310 °C, 0 minutes
Run time	20.75 minutes	40.5 minutes
Backflush conditions	5 minutes (post run) 310 °C (oven temperature) 50 psi (Aux EPC pressure) 2 psi (Inlet pressure)	5 minutes (post run) 310 °C (oven temperature) 50 psi (Aux EPC pressure) 2 psi (Inlet pressure)

#### Pesticides PCDL for GC/Q-TOF

**CSL Operating Conditions** 

	Hot splitless, 20 minutes	Hot splitless, 40 minutes
Retention time locking	Chlorpyrifos-methyl locked to 9.143 minutes	Chlorpyrifos-methyl locked to 18.111 minutes
Transfer line temperature	280 °C	280 °C
Q-TOF MS		
Ionization mode	El	El
Source temperature	280 °C	280 °C
Quadrupole temperature	180 °C	180 °C
Mass range	45 to 550 m/z	45 to 550 m/z
Spectral acquisition rate	3-5 Hz, collecting both in centroid and profile modes	3-5 Hz, collecting both in centroid and profile modes
Emission current	5 <i>µ</i> A	5 <i>µ</i> A

### **CSL** Operating Conditions

Cold Splitless (CSL) mode is also an option to introduce samples. To implement CSL, modify your data acquisition method with the MMI parameters specified in the following table.

Injection volume	2 uL Cold splitless
MMI temperature program	60 °C for 0.2 minutes 600 °C/min to 300 °C, hold 330 °C, post run
Purge flow to split vent	30 min/min at 1.5 minutes
Cooling	On Fast cooldown 70 °C, Cyro use temperature

### Set up a Cold Splitless Injection Method

1 Click GC Edit Parameters.

	5:	Sampl	e Name:					$\frown$		n				
Instrumer	Idle nt Status:	Data F	ile:			<u> 10.</u>	00	STOP		2	$(\mathbf{?})$			
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Chromato perimposed 0.9- 0.8- 0.7- 0.6- 0.5- 0.4- 0.3- 0.2- 0.1-	EIC	maize)												

2 Enter the **method parameters** as shown in the figure.

Vertextin	Select Lin	et	c 5190-2293: 900 µL (5p	intress, :	single taper,	uitra inert )				
ALS Front Injector		Setpoint	Actual	Г		Rate	Value	Hold Time	Run Time	
Inay / Other	[2] Heater:	60 °C	78.2 °C			4	-	min		
Columns	V Pressure:	9.2167 psi	9.2 psi	ľ	(initial) Ramp1	600	310	19	19.617	
Aux Heaters	Total Flow	33.987 mL/min	24 mL/min		Ramp 2	30	330	0	20.75	
L Signals	Septum Purge Flow:	3 mL/min	3 mL/min			Finals	value will be	extended by	GC run time	
Configuration Miscellaneous Columns Modules	Septurn Purge Row Mode	Standard •	1		Post Run 1	Post Run: 330 fotal Flow: 25 r	P°C mL∕min			
ALS Backflush	Mode:	Purj	ge Flow to Split Vent							
ALS Backflush Summary Post-Column - Front	Mode: Splittess	• Purj 30	ge Flow to Split Vent: mL/min		at 15 min					
ALS Backflush Summary Post-Column - Front Readiness GC Calculators	Mode: Splitless	• 9urg 30 1	ge Flow to Split Vent mL/min		at 15 min					
ALS Backflush Summary Post-Column - Front Readiness GC Calculators	Mode: Splittess	• Purj 30	ze Flow to Split Vent mL/min		at 15 min					
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ALS Backfluch Summary Summary Readiness GC Calculators	Mode: Splittess Gas Saver: ☑ On 20 mJ/min Ater:	Cryo: (N2) On Cryo Use Tem	ge Flow to Split Vent. mL/min town persture:		at 15 min					
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- 3 Click OK.
- **4** Save your method to a different name.

# Retention Time Locking the Data Acquisition Method

Perform Retention Time Locking (RTL) to Agilent-provided data acquisition methods before your sample analysis. Use the compound Chlorpyrifos-methyl as a locking compound.

Agilent recommends using the pesticide checkout standard ( $100 \mu g/mL$ ) for the retention locking process (p/n 5190-0494). The RTL procedure performs several calibration runs and saves the data in your method file. You may choose to acquire only centroid data to reduce the RTL file size. The method must be changed after RTL to acquire Both files (profile and centroid) for your sample analysis.



#### 1 Click Method > Acquire RTLock Calibration Data.

2 Click Yes.



3 Enter an accurate mass value for the selected ion, and click OK.



4 Ensure your checkout standard is placed in the correct vial position on the autosampler tray, as indicated in the **Sampler Vial Containing RTL Locking Compound** field.

Fiun T Flow in mi/mi	n	
0.800		
Run 2 Flow in ml/mi	n	
0.900		
Run 3 Flow in ml/mi	n - Current Method Setpont	
1.000		
Run 4 Flow in ml/mi	n	
1.100		
Run 5 Flow in ml/mi	n	
1.200		

- 5 Click **OK** to start the retention time locking calibration.
- 6 Click OK.

Alert	<b>×</b>
<u>^</u>	Sample MUST be in position 1
	ОК

7 Once the calibration run completes, click OK.



8 Enter the name of the locking compound, and click OK.

🚽 Input		
Enter Name for Loc	ked Compound	
Chlorpyrifos-methy		
OK	Cancel	
OK	Carlos	

**9** Select whether or not to retain **RTL Calibration Files** in the method. Whether or not you save the original files, the calibration curve will be saved, allowing you to relock your method.



10 Enter the target retention time of the locking compound, and click OK.

🗜 Input		
Enter the Locking	RT for subsequent data files	
9.143		
OK	Cancel	

11 Click Yes to save the flow to the method.



12 A Retention Time Locking Data Report is available to review the RTL results.

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Method Lock is	currently	On				
Compound: Chic	prpyrifos-n	ethyl				E
Retention Time	Calibrati	on:				
	ml/min	Time	Spec	Deviation		
File	Flow	min.	Xcor	Seconds		
RTLOCK1.D	0.80	9.478	-NA-	23.412		
RTLOCK2.D	0.90	9.267	-NA-	10.722		
RTLOCK3.D	1.00	9.088	-NA-	0.000		
RTLOCK4.D	1.10	8.926	-NA-	-9.696		
RTLOCK5.D	1.20	8.788	-NA-	-18.012		
Maximum Dev RTI	viation: 23 , Curve: R	.412 secor = 0.227e0	nds Å*Å - 4.	725e0 A + 2.5	19e1	
Terms of Cu	rve Fit:					
Constan	nt = 2.519e	1				
Lines	ar = -4.725	e0				
Quadrat	c = 0.227e	0				
Coefficien	nt = 0.9999	34 ** Good	I Fit **			-
						E

Set up an Automatic Mass Calibration in your Sequence

# Set up an Automatic Mass Calibration in your Sequence

1 Click Sequence > Edit Sequence.



2 Right-click in the sequence table and select Insert Sample.

Name		Vial	/ial Method File			Туре		
X	Cut	Ctrl+X		ide Anal5x15 20min.m		Sample		
-	Сору	Ctrl+C				[		
	Paste	Ctrl+V				[		
	Add Sample							
	Insert Sample							
×	Delete	Del						
	Columns		•	-				
	Fill Down	Ctrl+D		-				
	Fill Increment	Ctrl+F						

Set up an Automatic Mass Calibration in your Sequence

**3** Select your **method**, and click **OK**. It is important to note that the method loaded for mass calibration should be the one chosen to perform your sample analysis.

Browse For Folder		×
Image:		^
🛛 🔐 default		
📔 default.m		
> 🏭 Other		
Pesticide Analysis 15x15 20min.m		
Pesticide Analysis 15x15 40min.m		=
🕌 Zero Voltage.m		
mslogbook_history		
🌗 PreTreat		
Image: Sequence in the sequence is a sequence in the sequence is a sequence in the sequence is a		
Þ 👪 2		
		+
Make New Folder	OK Cance	1

4 From the Type drop-down list, select Keyword.

	Name	Val	Method File	Туре	Keyworc	Method F
1			Pesticide Anal5x15 20min.m		-	D:\MassHi
2	Pesticide Checkout	101	Pesticide Anal5x15 20rrin.m	 Sample	•	D:\MassHi
3				 Blank		
4				 QC	-	
5				 Keyword TuneCheck MatrixBlank MatrixSpike MatrixSpikeDup DoubleBlank CC	•	

2

Set up an Automatic Mass Calibration in your Sequence

5 From the Keyword drop-down list, select MassCal.

	Name	Vial	Method File	Туре		Keyword	Method Path
1			Pesticide Anal5x1520min.m	 Keyword	-	ClockStart	D:\MassHunter\GCMS
2	Pesticide Checkout	101	Pesticide Anal5x1520min.m	 Sample	-	ClockStart	D:\MassHunter\GCMS
3				 1	-	Comment	
4		j.			-	GC Wake Up	
5				 1	-	Interval MassCal	
						OverWrite Pause Unlinked 2-Layer L2 3-Layer L2;L3 UseDecisions	

6 Click OK.

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### Metabolomics PCDL for GC/Q-TOF

This chapter describes sample preparation, system configuration, and recommended data acquisition methods when using Metabolomics PCDL for GC/Q-TOF. Two PCDLs are included for metabolomics, each with a different set of RI values based on the FAMES and Alkanes indices, respectively.

Sample Preparation and Derivatization 26 Biological sample extracts 26 Metabolites 26 Internal standard 26 Retention index markers 27 Derivatization 28

Configuration and Acquisition Method 30

3

## Sample Preparation and Derivatization

### **Biological sample extracts**

The preparation of optimal extracts from biological samples is very much dependent on the type of matrix analyzed and the class of compound that is of interest for the study. The exact procedures have to be carefully examined and are not part of this guide.

### **Metabolites**

Metabolites are generally small molecules, and most primary metabolites bear hydrophilic functional groups such as carboxyl, hydroxyl, or amino groups. According to the functional groups, these molecules are often classified into amino acids, carbohydrates, fatty acids and organic acids.

The presence of a variety of hydrophilic functional groups enables cells to use metabolites for a variety of cellular purposes, including transport in the aqueous cellular environment or between compartments and organs.

The presence of these functional groups in extracts of biological samples causes a significant rise in boiling points, rendering most primary metabolites unsuitable for GC separations.

### Internal standard

The RTL locking compound should be included in every sample. Examining the locking compound in a data file from an RTL method determines if a re-locking needs to be performed. The retention time or RI is a very important part of the analysis. This measurement is used in combination with the EI spectra to identify a metabolite.

**Retention index markers** 

RTL locking compound: Myristic acid d27. RT of the locking standard is 16.752 minutes.

### **Retention index markers**

The FAME markers used for calculating retention indices are not required in a sample. It is required that a FAME marker calibration sample is run and correctly associated with a sample. Acquiring data for FAMEs markers daily can guarantee that a correct RI calibration can be performed.

Fatty acid methyl esters (FAME) are C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub>, C<sub>22</sub>, C<sub>24</sub>, C<sub>26</sub>, C<sub>28</sub>, and C<sub>30</sub> linear chain length. The inclusion of these markers in a sample chromatogram can be used as a QC check. If identical chromatographic conditions as those employed to generate the Agilent Fiehn Library were used, the RT values match.

The Agilent Fiehn GC/MS Metabolomics Standards Kit (p/n 400505) contains the RTL locking compound d27 myristic acid, a mix of FAMEs with d27 myristic acid, pyridine as well as MSTFA/1 % TMCS mix.

FAME marker	concentrations and RT	values are described in	n the following table.

Name	Concentration	RT (min)
Methyl caprylate C <sub>8</sub>	100 µg/mL	7.812
Methyl perlargonate C <sub>9</sub>	100 µg/mL	9.248
Methyl caprate C <sub>10</sub>	100 µg/mL	10.647
Methyl laurate C <sub>12</sub>	100 µg/mL	13.25
Methyl myristate C <sub>14</sub>	100 µg/mL	15.597
Methyl palmitate C <sub>16</sub>	100 µg/mL	17.723
Methyl stearate C <sub>18</sub>	50 µg/mL	19.663
Methyl eicosanoate C <sub>20</sub>	50 µg/mL	21.441
Methyl docosanoate C <sub>22</sub>	50 µg/mL	23.082
Methyl linocerate C <sub>24</sub>	50 µg/mL	24.603
Methyl hexacosanoate C <sub>26</sub>	50 µg/mL	26.023

#### Metabolomics PCDL for GC/Q-TOF

Derivatization

Name	Concentration	RT (min)
Methyl octacosanoate C <sub>28</sub>	50 µg/mL	27.349
Methyl triacontanoate C <sub>30</sub>	50 µg/mL	28.723

### Derivatization

The hydrophilic functional groups must be derivatized to remove hydrogen bond formations to increase volatility. This also reduces interaction with the column phase that can cause tailing peaks, poor sensitivity, and poor chromatographic separation. The Agilent Fiehn 2013 GC/MS Metabolomics RTL Library uses a two step derivatization procedure routinely used in most published literature in metabolite profiling by GC/MS.

Key points to consider:

- Thoroughly dry metabolite samples before derivation. Reagents are not compatible with water or protic solvents.
- Use enough reagent to completely derivatize all metabolites.
- Derivatized samples have a 24-hour shelf life.

Before doing a large study, conduct a test to determine the maximum sample size that is completely derivatized with the recommended protocol.

#### Preparing the biological extract

An aliquot of 5  $\mu$ L of myristic acid d27 stock solution (0.75 mg/mL) previously diluted 50x is added to the biological extracts. The sample is then evaporated to dryness.

#### Methoxyamination

Add 10 μL of a 40 mg/mL solution of methoxyamine hydrochloride (Sigma-Aldrich; Cat. No. 226904) in pyridine. This mixture is gently shaken at 30 °C for 90 minutes. Derivatization

#### Trimethylsilylation

Add 90  $\mu$ L of N-Methyl-N- trimethylsilyltrifluoroacetamide with 1% Trimethylchlorosilane (MSTFA +1% TMCS) to the methoxyaminated samples. The mixture is incubated at 37 °C for 30 minutes. The derivatized samples are cooled to room temperature before being transferred into GC vials.

The vials are injected for GC/MS analysis under the conditions given in the "Acquisition Method" section. When analyzing multiple samples, randomize the injection order to reduce the affects of variable reaction times on statistical analysis.

# Configuration and Acquisition Method

The following method was used to acquire the retention and spectral data used in the library.

Equipment: Agilent 8890 GC/7250 Q-TOF

GC/Q-TOF Acquisition Parameters are described in the following table.

GC and MS Conditions	Q-TOF (7250)
GC	8890
Column	Agilent J&W DB-5MS UI, 30 m, 0.25 mm, 0.25 $\mu$ m, DuraGuard, 10m
Inlet	SSL, 4-mm UI liner single taper
Injection volume	0.5 µL
Injection mode	Splitless
Inlet temperature	280°C
Oven temperature program	50°C for 0.5 min; 10°C/min to 325°C, 10 min hold
Carrier gas	Helium
Column flow	1 mL/min (The actual flow of the system is determined during the RTL procedure.)
Transfer line temperature	280°C
Quadrupole temperature	150°C
Source temperature	200°C
Electron energy	70 eV
Emission current	5 <i>µ</i> A
Spectral acquisition rate	5 Hz
Mass range	50 to 1200 m/z
Solvent delay	5.90 min

# Natural Products PCDL for GC/Q-TOF

This chapter describes the Natural Products PCDL as well as a recommended data acquisition method. Please note that while this PCDL includes RTs based on the GC method described below, it also includes Kovats RIs for non-polar phase. Additionally, two PCDLs are included: one containing only compounds with spectra and another which includes compounds without spectra.

PCDL Description 32

4

Configuration and Data Acquisition Method 33

**PCDL** Description

### PCDL Description

The Natural Products PCDL for GC/Q-TOF is a user-contributed PCDL that has been created from 2D GCxGC hemp CBD oil data and currently includes compound classes shown in the following figure. It is considered user-contributed because it is not solely generated from single standards and contains unidentified compounds with spectra.



# Configuration and Data Acquisition Method

GC and MS Conditions	Description
MS	Agilent 7250 GC/Q-TOF
GC	Agilent 8890 GC
Inlet	Multimode inlet, 4 mm Agilent Ultra Inert inlet liner, single taper with wool
Inlet Temperature	280 °C
Injection Volume	1 <i>µ</i> L
Columns	Agilent J&W DB-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 μm (p/n 122-5532UI)
Oven Temperature Program	60 °C for 5 min; 4 °C/min to 300 °C, 7 min hold
Carrier Gas	Helium
Column Flow	1 mL/min constant flow
Transfer Line Temperature	280 °C
Quadrupole Temperature	150 °C
Source Temperature	200 °C
Electron Energy	70 eV
Emission Current	5 µA
Spectral Acquisition Rate	5 Hz
Mass Range	m/z 40 to 650

Suggested data acquisition parameters are described in the following table.

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# 5 Data Analysis Workflows

This chapter describes workflows that are typically used with the accurate mass GC/MS PCDL with GC/Q-TOF data.

Overview of Target and non-target screening workflows for high-resolution accurate mass GC/Q-TOF **36** 

Managing the PCDL content 36

Adding GC/Q-TOF Spectra to a PCDL 40

Configuring MassHunter Qualitative Analysis 10.0 40
Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software 42
Annotation of a Spectrum with Fragment Formulas 44

SureMass Overview 47

Suspect Screening and Target Quantitation Workflows 48

Summary of a Typical Workflow for Suspect Screening and Target Quantitation with Large Number of Targets 48
Suspect Screening and Target Quantitation Method Setup 48
Target Quantitation Method Setup 50

Suspect Screening Method Setup 55

Non-targeted Screening in the Unknowns Analysis Software 64

#### **Data Analysis Workflows**

Overview of Target and non-target screening workflows for high-resolution accurate mass GC/Q-TOF

### Overview of Target and non-target screening workflows for high-resolution accurate mass GC/Q-TOF

The following figure shows the GC/Q-TOF PCDL screening workflow for both targeted and non-targeted approaches.



### Managing the PCDL content

Use Agilent ChemVista to manage the content of your PCDL:

 Import your PCDL into the standalone library manager to manage data in a compound-centric fashion. Note that in order to keep derivatized and non-derivatized compound and spectral data properly organized, it may be desirable to turn off or edit the classification feature in ChemVista prior to importing data. This may also apply for stereoisomers where structure information is undefined. See the ChemVista Online Help for more details.
#### Import Files

Calasta			ام مر م	<u>e</u> 1	+ -		
Select a	source	type	and	mes	ιο	import	

Source type	PCDL (*.cdb)	•
Select file(s)	Open file(s)	
Create list on import	$\checkmark$	
List name	Custom List Name	
Description		
Apply method label to imported data		
		Import selected files

• Create custom screening lists specific to your analysis by searching for compound class groups and regulation tags as well as searches using compound name, formula, mass, CAS, InChIKey, etc.

Identifiers Formula Mass Tags Lists	
Start typing to search for tags by name. Click on tags to remove them	
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USDA Pesticides MRL Database	
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	Search Add to search
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Identifiers Formula Mass Tags Lists Letet an identifier field: CAS U007-30-8	General Ard to search

• Edit and add compounds, retention times, and spectra.

## Data Analysis Workflows Managing the PCDL content

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• Search, browse, and store spectra acquired on a Q-TOF instrument.

Managing the PCDL content



- Merge compounds from your PCDL with compounds and spectra from MassBank, MassBank of North America (MoNA), and the EPA CompTox Chemicals Dashboard. For more information, see the Agilent ChemVista Introduction Workbook, introductory videos, and Online Help.
- Send spectra to your customized PCDL directly from the Qualitative Analysis
  program to create your own custom library. Choose from options to filter
  spectral noise and/or to correct the product ions to their theoretical accurate
  mass.
- Import the customized PCDL into Agilent ChemVista.
- Load spectra from either a .CEF file or by copy-and-pasting mass spectra from MassHunter Qualitative.
- For more information, see the MassHunter Personal Compound Database and Library Manager Quick Start Guide, PCDL Manager Online Help, and MassHunter Qualitative Analysis Help.

## Adding GC/Q-TOF Spectra to a PCDL

### **Configuring MassHunter Qualitative Analysis 10.0**

- 1 Acquire data in full spectrum acquisition mode.
- 2 Open MassHunter Qualitative Analysis software.
- 3 Under the Method tab, click Open then click Default-GCMS.m and open.



#### 4 Make sure Show Advanced Settings is checked in the Configuration tab.

Agilent MassHunter Qualitative Analysis 10.0 - Default-GCMS.m				-	×
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Data Analysis Workflows Configuring MassHunter Qualitative Analysis 10.0

5 From the menu bar, load the **data file** (Ctrl+O).

🛐 Agilent MassHunter Qualitat	ive Analysis 10.0 - MFG.m		
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Open data files (Ctrl+O)	×	<u>∧</u> Chromatogram Results	
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No data to display.			

Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software

#### Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software

1 Under the Method tab, click Method Editor.

🛐 Agilent MassHunter Qualitative Analysis 10.0 - Default-GCM	MS.m	
File Edit View Find Identify Spectra Chromatograms	Method Actions Configuration Tools Help	
양 또 교 다 과 가 가 가 가 가 가 가 가 가 가 가 가 가 가 가 가 가 가	Opn. Chri-Shift-O     Sheve Chri-Shift-O     Sheve Chri-Shift-O     Sheve Chri-Shift-O     Ram Methodo Automation (Workflow = Report)     Print Qualitative Vethodo Report.     Methodo Editor:     Me	
ିଳ୍ଲ Data Navigator ୧୮ ଓ ୪	X // MS Spectrum Results	
Sort by Data File	アサキ 9月194 ビ 44 A 2 ・11 日 25 第 9 次回 由 第 回	

2 Navigate to Generate Formulas under the Identification tab.



#### Data Analysis Workflows

Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software

**3** Under the **Fragment Formulas** tab, check **Annotate fragment spectrum peaks with formulas**. Change the filters parameters, if necessary.

Method Editor: Generate For	nulas	×
🕼 🕒 🖬 🙀 🤊 • (° •	Generate Formulas from Spectrum Peaks *	
Method Automation	Allowed Species Limits Charge State A Fragment F	omulas Scoring
	Fragment annotation filters	<u>^</u>
	Annotate fragment spectrum peaks with formulas Height filters	<b>A</b>
Identification	Absolute height >= 200	counts
Identification Workflow Database Search Settings Library Search Settings	Maxin Maxin	erate Formulas from Spectrum Peaks 👻
Generate Formulas	Chromatograms	llowed Species Limits Charge State 🛦 Fragment Formulas Scoring
Combine Identification Resu	Ger 🕀 Spectra	Fragment annotation filters
⊞ Export	🖙 klentify Spectra	Annotate fragment spectrum peaks with formulas
	Identification Workflow	Absolute height >= 200 counts
	Database Search Settings	□ Relative height 🛕 >= 1.000 % of largest peak
	Library Search Settings	Maximum number of peaks
	Generate Formulas 🔺	Limit (by height) to the largest
	Combine Identification Results	Generate formulas for non-fragment (unknown) ions

## Annotation of a Spectrum with Fragment Formulas

- 1 Extract a background subtracted spectrum.
- 2 Right-click on the **spectrum**, and select **Add/Edit Manual Identification** from the right-click menu.

Agilent Ma	ssHunter Qualitative Analysis 10.0	0 - MFG.m			- ø ×
File Edit V	New Find Identify Spectra Ch	promatograms Method Actions Configuration Tools Help			
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Sample Tai	ible: Cal 1_1008_Q-TOF.D	× ∆Chr	romatogram Results (zoomed)		×
	品	2.4	н ‡ 🔍 🗄 🐙 🗲 🗛 Э С 3 🕞 🖬 ք 🖄 % ¾ 🖄 👍 🦉	🛎 Minutes 🛛 👻 🎯	
Ret	sults	Acquisition x10	+ELTIC Scan Frag-70.0V Cal 1 100B Q-TOF.d		
Flags	File Name Sample Name Sam	mple Position Instrument Name Acquisition Time			
MDC Call	Method Editor: Generate Fo	ormalas	×		
	0 6 H 6 9 . C	Generate Formulas from Spectrum Peaks			
	B Chromatograms	Allowed Species 🛕 Linits Charge State Fragment Formulas 🛕 Scoring Fragment annotation filters	hall hand hall have have have have have have have have	mm	
4	Integrate (MS)	Annotate fragment spectrum peaks with formulas	92 94 96 98 10 102 104 106 108 11 112 114 11		13 132 134
PA Data Navid	Integrate (GC)	Height filters	Counts vs. Acquisition Time (m	Extract DL F	
CD (21 (21	Smooth	Absolute height >= 1000 counts	Identification Results	Extract Unromatograms	×
	Enstante Manaries)	Relative bright as 1,000 % of largest peak		Subtract Background Spectrum	
Sort by Data File	2 COLUCE MARRIES		2 52 W2 🛣	Subtract Any Spectrum	
-20	V Calculate Signal-to-Noise	Maximum number of peaks	splay.	Add Any Spectrum	
E Spec	Extraction Data Format	Limit (by height) to the largest 100		Convert Profile to Centroid	
- V 1	Adjust Delay Time 🔺	Concepts from the fee are frammed (unbrown) into		Convert Profile to Centroid and Replace	
- <b>W</b>				Find Spectrum Peaks	
- VI	a spectra			Edit Peak Annotations	
	Extract (MS)			Adjust Peak Threshold	
NH.	Extraction Data Format			Identify Spectra	
-21				Search Library/DB for Spectra	
	Identification			Generate Formulas from Spectrum Peaks	
- W1	Identification Workflow		rum Results (zoomed)	Add/Edit Manual Identification	×
×1	Database Search Settings			Gear Spectrum Identification Results	
×1	Library County Continue		○回参え来すのの1、1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	County Holes AND AND Descents	
	Contry search sectings		Scan (rt: 12.482 min) Frag=70.0/ Cal 1_1008_Q-TOF.D Subtract	Search Garrier to DCDI	^
- 21	Generate Formulas			Send Spectra to PCDC	
21	Combine Identification		121.0646	Recalibrate	
- <b>2</b> b	# Expert	-	6	Restore Original Calibration in Data File	
-21			u v	Set Anchor	
	u - Scan (rt: 10.107 min) Sub	2	4-	Clear Anchor	
	w + Scan int: 10.441 min) Sub	2	2-	Assign Ranges to >	
-91	u + Scan (rt: 10.885 min) Sub		2-	Move to Background Spectrum	
E	u - Scan (rt: 11.116 min) Sub	11	s	C Delete	
-91	u + Scan (rt: 11.229 min) Sub	1.	4-	a Hannan	
- V 1	u + Scan (rt: 11.363 min) Sub	13	2-	Assian Resident Colors	
×1	tu = scan (rt: 11,433 min) Sub			Assign Services	
- MH	In + Scan (rt: 11.500 min) Sub	0.1	150.103	, Undose Defined Color +	
	w + Scan Irt: 11.590 min) Sub	0.		Copy to Clipboard Ctrl+C	
	w + Scan (rt: 12.141 min) Sub	0.	2- 58.0286 91.0041 107.0490 116.0640 116.0640	Paste Ctrl+V	
- 21	u + Scan (rt: 12.355 min) Sub			j Print	223.8038
- 1 L	+ Scan (rt: 12,482 min) Sub	<b>a</b>	50 80 70 80 90 100 110 120 130 140 150 Counts vs. Mass-to-Charge (m/z	Export	210 220

3 Enter formula and compound name. You can add other identifiers such as CAS #. Make sure that the Charge is 1 and M+ is selected in the **Ion species** window for El.

Compound na	ame: Fenob	ucarb			
Molecular for	mula				
ormula:	C12H17NO2	2			
Charge:	1	~	lon species:	M+	~
CAS ID:	3766-81-2		LMP ID:		
EGG ID:			HMP ID:		
JniProt ID:					
Notes:					

4 Check the **Fragment Formula Annotation Results**. All fragments in a spectrum are annotated with formulas which are subsets of a candidate formula for the molecular ion.



**5** To edit Fragment Formula Annotation Results, from the right-click spectrum menu select **Edit Peak Annotations**.



6 Species, Formula and Ion Type can be changed. To cancel annotation of an ion, select **Unknown** in the **Ion Type** column.

/z /	V+	bund V + PP	7.97.	Species V V +	Formula	⊽ 🖶 Formula & lo 🔽 🖶 Diff (ppm) 🟹 🕇	Ion Type 🖓 🛱	😕 Loss Formula 🏹 🕫 Ion 🏹
94	0412	630.89	1	M+	C6 H6 O	[C6 H6 O]+ -1.26	Fragment Ion	C6H11NO
9	4.065	167.4	1	M+	C6 H8 N	[C6 H8 N]+ -1.53	Fragment Ion	C6H9O2
94	.0731	1358.9	1	M+	C7 H9	[C7 H9]+ -1.34	Fragment Ion	C5H8NO2
94	.0877	156.17						
9	5.049	579.39	1	M+	C6 H7 O	[C6 H7 O]+ -1.49	Fragment Ion	C6H10NO
95	9162	1329.96	1					
96	.0529	127.18	1	M+	C6 H8 O	[C6 H8 O]+ -42.75	igment ion 👻	C6H9NO
96	9241	156.47	1				Unknown	
97	.0109	200.09	1	M+	C8 H	[C8 H]+ 37.7	Fragment Ion	C4H16NO2
97	.0281	208.75	1	M+	C5 H5 O2	[C5 H5 O2]+ -3.58	Confirmed fra	C7H12N
97	1014	870.25	1	M+	C7 H13	[C7 H13]+ 2.31	Expected frag	C5H4NO2
97	1094	126.53						
97	9108	237.53						
97	9301	101.8						
98	.0367	139.14	1	M+	C5 H6 O2	[C5 H6 O2]+ 4.31	Fragment Ion	C7H11N
98	1079	338.4	1	M+	C7 H14	[C7 H14]+ -10.86	Fragment Ion	C5H3NO2
99	1176	273.99	1	M+	C7 H15	[C7 H15]+ 7.54	Fragment Ion	C5H2NO2
101	0389	272.12	1	M+	C8 H5	[C8 H5]+ 3.25	Fragment Ion	C4H12NO2

Annotation of a Spectrum with Fragment Formulas

7 To send Fragment Formula Annotated Spectrum to the PCDL, from the right-click spectrum menu select **Send Spectra to PCDL**.

Scan	(rt: 12.4	482 min	) Sub										Extract Chromatograms				
V⇔ ucarb	C12H	ila ♥∔ 17NO2	Species 7 M+	7 += m/z ♥ +	Score⊽ 100	<b>⊽</b> +⊨ Sco	re (RT)	₹4	RT Dif	7₽	Diff (ppm) 🍸 🖶	54	Subtract Background Spectrum Subtract Any Spectrum Add Any Spectrum Convert Profile to Centroid Convert Profile to Centroid and Replace Find Spectrum Peaks		ectra ♥+a Ne	otes <b>V →</b> RT (Tg	rt) *
													Edit Peak Annotations Adjust Peak Threshold Identify Spectra				
													Search Library/DB for Spectra Generate Formulas from Spectrum Peaks Add/Edit Manual Identification				
▲ 4 0 C 1 × <u>1</u> H 品 3 % % % 単 ※ ④						3	X	Clear Spectrum Identification Results									
482 m	iin) Frag	g=70.0\	Cal 1_10	0B_Q-TOF.0	) Subtrac	:t							Send Spectra to PCDL				
												ି ଏ ଏ	Recalibrate Restore Original Calibration in Data File Set Anchor		-		
						121.0 [C8 H9	646 0]+					×	Assign Ranges to Move to Background Spectrum Delete	}			
											150.1037	Q ()	Unzoom Assign Random Colors Choose Defined Color	1.			
77. [C6	0385 H5]+	I	91.0641 27 H7]+	107. [C7 ⊨	0490 17 O]+			ICS	35.080 H11 (	1 0]+	[C10 H14 O]+		Copy to Clipboard Ctrl Paste Ctrl	C V	209.0108	223.8038	
	00	0°E	00 05	100 100	110 1	15 120	126	120	100	110	140 100 10	1.0			Ar 010 01	5 220 225	

8 Select Library Path for the PCDL you want to update. Select Formula annotated peaks and Calculated m/z... if you would like only theoretical m/z of annotated peaks present in the PCDL.

Library selection		
Library path: C:\MassHunter\PCDL\Envir	onmental_AM_PCDL.cdb	
Spectrum filter		
🖂 Minimum base peak height	500 🛕 cour	nts
Annotation filter		
Formula annotated peaks (excludes un	knowns) if present else all peaks	
○ All peaks		
○ All peaks m/z selection options		
<ul> <li>All peaks</li> <li>m/z selection options</li> <li>Calculated m/z if present else observed</li> </ul>	m/z	
<ul> <li>All peaks</li> <li>m/z selection options</li> <li>Calculated m/z if present else observed</li> <li>Observed m/z</li> </ul>	m/z	
All peaks m/z selection options Calculated m/z if present else observed Observed m/z Conflict resolution	m/z	
All peaks x/z selection options Calculated m/z if present else observed Observed m/z Conflict resolution If spectrum already exists in library	m/z	
All peaks  All peaks  Z selection options  Calculated m/z if present else observed  Dubserved m/z  Conflict resolution  If spectrum already exists in library  Send and replace the spectrum in library	m/z	
All peaks  M/2 selection options  Calculated m/2 if present else observed  Observed m/2  Conflict resolution  If spectrum already exists in library  Send and replace the spectrum in library  Skip sending this spectrum	m/z	

5

## SureMass Overview

SureMass key takeaways:

- Signal processing and feature extraction algorithm for accurate mass data in MassHunter Quant
- Requires profile data
- · Allows improvement of mass accuracy and linearity
- Used in suspect screening/target quantitation as well as non-target accurate mass deconvolution (SureMass deconvolution) workflows
- For more information, see the following: https://www.msconsult.dk/wp-content/uploads/5991-8048EN-High\_res\_de convolution\_Suremass.pdf

The SureMass Conversion is in both MassHunter Quantitative Analysis Software and Unknowns Analysis (Tools > Convert Samples > Convert to SureMass format).



Suspect Screening and Target Quantitation Workflows

# Suspect Screening and Target Quantitation Workflows

## Summary of a Typical Workflow for Suspect Screening and Target Quantitation with Large Number of Targets

- 1 Use Agilent ChemVista or PCDL Manager to create a **subset PCD**L for Targets and another for non-targets (everything else).
- 2 Create a new **batch** in MassHunter Quantitative Analysis Software and add samples.
- **3** Create MassHunter Quantitative Analysis Software **method** from **GC library** using a target PCDL subset.
- 4 Set up quantitation method for these compounds as you normally do.
- 5 Create calibration levels.
- 6 Append method from GC Library for non-target compounds.
- 7 Finish setting up MassHunter Quantitative Analysis Software method.
- 8 Set up GC screening method.
- 9 Validate and run.

## Suspect Screening and Target Quantitation Method Setup

- 1 Open Quantitative Analysis for TOF.
- 2 Select New Batch and click Create a new batch (Ctrl+N).

			View	Method	Agile Tools	nt MassHunte	r Quantitativ Help	e Analysis (for TOF)				?	-	
New Open Batch Batch		Add Samples	Delete Samples	Analyze Batch V Analyze	Clear Results	Ciper Senerate Report Edit I	i Report Folder ie Viewer Report Method Report	Library Search Results Library Search Reports 🛩 Query	÷) copy	C Delete				
E New Batch (Ctri+h Create a new bat	I) ch		mple Type:		- Compound	<		✓ > ISTD:			i II a a	3	Ť	*
Name														

Suspect Screening and Target Quantitation Method Setup

**3** Name the batch and click **Create**.

New Batch									,
Look in:	Screener		- 🔮 🙆 - 🔁						
4	Name	^	Date mod ^	Analysis File	Data Ve	Size	Date Analyzed	Analyst	Date Last Save
<b>X</b>	#1_Str.D		5/28/2020						
Quick access	#11_Str.D		5/28/2020						
	#27_Str.D		2/20/2020						
· · · ·	Cal 1_10B	Q-TOF.D	5/28/2020						
Desktop	Cal 1_20B	_Q-TOF.D	9/18/2020						
_	Cal 1_50B	_Q-TOF.D	2/19/2020						
<b>C</b>	Cal 1_100	B_Q-TOF.D	5/28/2020						
Libraries	Cal 1_250	B_Q-TOF.D	2/19/2020						
	Cal 1_500	B_Q-TOF.D	2/19/2020						
	New folde	er	1/17/2023						
This PC	QuantRep	ports	5/5/2022						
	<		>	<					
<b>.</b>	File name:	Screener batch		2				~	Create
Network	Files of type:	Batch Files (* batch bin	)					~	Cancel
									Hole
									neip

4 Add samples to the batch and click **OK**.

File name	Name	Sample Group	Acq. Date-Time 🔺
#1_Str.D	#1_Str		12/12/2018 5:17 PM
#11_Str.D	#11_Str		12/12/2018 10:20 PM
#27_Str.D	#27_Str		12/13/2018 5:54 AM
Cal 1_10B_Q-TOF.D	Cal 1_10B		12/13/2018 10:57 AM
Cal 1_20B_Q-TOF.D	Cal 1_20B		12/13/2018 11:46 AM
Cal 1_50B_Q-TOF.D	Cal 1_50B		12/13/2018 12:38 PM
Cal 1_100B_Q-TOF.D	Cal 1_100B		12/13/2018 1:28 PM
Cal 1_250B_Q-TOF.D	Cal 1_250B		12/13/2018 2:19 PM
Cal 1_500B_Q-TOF.D	Cal 1_500B		12/13/2018 3:09 PM
Browse to Copy S	amples		
Translate MSWS	Samples		
Translate OpenLab	Samples		

1 Select New and click New method from library-GC.



2 Select the **PCDL library** containing "targets" (compounds for which standards were run for quantitation).

Library:	
D:\Data\SubTarget_Pesticides_15-15_40min_AMRT_PCDL.cdb	Browse
Targets:	
All compounds	
O Use compound list	
	Browse
lons:	
Candidate ion ranking:	
Weighted *	
Number of qualifiers to add:	
5 🗢	
Retention time calibration:	
No RT Calibration (The library contains Retention Time)	
Use RT Calibration (The library contains Retention Index)	
	Browse

3 Choose ion ranking algorithm and the number of qualifier ions.

- ? – 🗆 X s (for TOF) - [New Me பியி Manual Setup Tasks ~ 1 s from Library - GC New Method Tasks Method Table Average Calib ration Replicates • #× + 8 Time Segment: 🗶 <All: • > ci New / Open Method Sample ▲ Workflow Name Data File q. Mel od File Acq. Date-Time Create Levels from Calibration Target Deconvolution Setup Screening - GC Quantifier Create Levels from Calibration Screening - LC Name TS Pyrimethanil Metalaxyl CF Origin CF Weight ore None Method Setup Tasks Target Ignore Target Linear 👪 🗧 Scar Compound Setup Fenobucarb Target Target Scar Linea Retention Time Setup Ethiofencarb ap Tasks 🛩 Advi ISTD Setup Cyprodinil ⊞൘ Target Linear Validate ual Setup Tasks 🛩 Update 🛩 Concentration Setup Sample Information New 0pe Qualifier Setup Max # of pa 2 + + + + + + MS: Signal: <None> Calibration Curve Setup Method Table Method Tasks ×107 \* \* > Clobals Setup New / Open Method Time Segment: 🗶 <All> ▼ > Compound: < Pyrimethan ▼ > Reset Table View 2.25-2-1.75-1.5-1.25-1.25-1.25-0.75-0.5-0.25-▲ Save / Exit ▲ Workflow Sample Data File Type Level Acg. Met Validate Name Target Deconvolution Setup Screening - GC Save As... Quantifie Screening - LC TS Туре Exit ✓ Tan Method Setup Tasks 4 5 6 7 8 9 10 11 12 13 14 15 16 Manual Setup Tasks Compound Setup Calibration Conc. 10.0000 ~10 Dutlier Setup Tasks Response Retention Time Setup Compound Information Advanced Tasks ISTD Setup 10.0000 20.0000 50.0000 100.0000 250.0000 🛛 🕂 🕈 ヘ 元 🛛 ヘ 🗸 🔺 📐 🛝 山 会 🖏 🖄 Concentration Setup Qualifier Setup ×10<sup>1</sup> Calibration Curve Set 0.6-0.4-0.2-Globals Setup ▲ Save / Exit Sample Information 🛃 🕂 💲 🖉 🥳 MS Signal: <None> Max # of panes: 2 0-0.2 0.3 0.4 0.5 0.6 ×107 Save As... Exit 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 Manual Setup Tasks
- 4 Select Method > Calibration Curve, and click Create levels from Calibration Samples.

**5** Select **Append** then click **Append method from Library-GC** to append with non-target compounds (for which no standards for quantitation will be run). This is using a subset PCDL containing spectra of these compunds.



6 Browse and select the Library.

Method from Library - GC	×							
Ubrary:								
D:\Data\SubNonTarget_Pesticides_15-15_40min_AMRT_PCDL.cdb Browse								
Targets:								
All compounds								
Use compound list								
Browse								
lons:								
lons:								
lons:		Quantifier		D.	ц	1		L.
lons: 2andidate ion ranking: Weighted •		Quantifier Name	TS	Scan	Туре	CF	CF Origin	
ions: 2andidate ion ranking: Weighted umber of qualifiers to add:		Quantifier Name Tris (b-Chloro	TS 1	Scan	Type Target	CF	CF Origin Ignore	Non
lon:: anddate ion ranking: Weigned ▼ wumber of qualifiers to add: 5 5⊕		Quantifier Name Tris (b-Chloro Tris (3-Chloro	TS 1 1	Scan Scan V Scan	Type Target Target	CF Linear Linear	CF Origin Ignore Ignore	Non
lon:: Anddate ion ranking: Weighted * Under of qualifiers to add: 5 @		Quantifier Name Tris (b-Chloro Tris (3-Chloro Tolylfluanide	TS 1 1 1	Scan Scan Scan Scan	Type Target Target Target	CF Linear Linear	CF Origin Ignore Ignore	None
Ion: andate ion ranking: Weighted • whites of qualifiers to add: 5 States Freetroon meric calibration:		Quantifier Name ► Tris (b-Chloro Tris (3-Chloro Tolylfluanide Tetraconazole	TS 1 1 1 1	Scan Scan Scan Scan Scan	Type Target Target Target Target	CF Lineer Lineer Lineer Lineer	CF Origin Ignore Ignore Ignore Ignore	None None None
lon:: Andiate ion ranking: Weighted * where of qualifies to add: 5 State Retention mer calibration: Mod 50 Calibration:		Quantifier Name Tris (b-Chloro Tris (3-Chloro Tolyffluanide Tetraconazole Tetrouzzole Terbutryn	TS 1 1 1 1 1 1	Scan Scan Scan Scan Scan Scan	Type Target Target Target Target Target	CF Linear Linear Linear Linear	CF Origin Ignore Ignore Ignore Ignore Ignore	Non Non Non Non
Ions: Candidate ion marking: Weighted Visite of qualifiers to add:		Quantifier Name Tris (b-Chloro Tris (3-Chloro Tolylfluanide Tetraconazole Terbutryn TCPP / Tri-(2	TS 1 1 1 1 1 1 1	Scan Scan Scan Scan Scan Scan	Type Target Target Target Target Target Target	CF Linear Linear Linear Linear Linear	CF Origin Ignore Ignore Ignore Ignore Ignore Ignore	None None None None None
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lon: Candidate lon ranking: Weighted * Weighted function of the state of the sta		2uantifier Name Tris (b-Chloro Tris (3-Chloro Tolyfflannide Tetraconazole Terbutryn TCPP / Tri-(2 TBP / Tributy TBP / Tributy TBP / Tributy	TS 1 1 1 1 1 1 1 1 1 1 1	Scan Scan Scan Scan Scan Scan Scan Scan	Type Target Target Target Target Target Target Target Target Target	CF Linear Linear Linear Linear Linear Linear Linear	CF Origin Ignore Ignore Ignore Ignore Ignore Ignore Ignore Ignore	None None None None None None None

7 From **Method Setup Tasks**, click **Compund Setup** to set up the Quant method. Note that many Quant method parameters will be set up in the Screener part of the method.



8 Click **Globals Setup** and check **SureMass** if you use profile data (recommended).

The Home	Agilent MassHunter Quantitative Analysis (for TOF) - [New Method]	? – 🗆 ×
Edit New Open Save A	Y         Method Report         Method Setup Table *         Advanced Table *         Opticate Composition         Setup Counting Counting Table With Quenting         Counting Co	
Method Tasks + # ×	Method Table	* # ×
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	0- 01 02 03 04 05 06 07 08 09 0- 01 02 03 04 05 06 07 0	ola ola
	S Compounds (5 total) 0 ISTD (0 to	tal) AGILENT\saronova

1 Select Screening-GC.



2 Click **Setup Reference Library** and browse for your library. This is based on the complete PCDL, which is your Lookup library.

N oc	Setup Screening - GC Apply to:      ① Selected compounds	×	
a	Setup Reference Library	Setup Reference Library X	
	Edit	Obtain reference spectra from lookup lbray     Lookup lbray:	Agilent MassHunter Quantitative Analysis X
	Deconvoluted scan as Spectrum Extraction Override     Spectrum Quantifier Qualifier Only     Mass extraction setup:	D-Data-Sub-Pesticides_15-15_40min_AMRT_PCDL.cdb Bowse Create reference library at:	Reference library was created at D:\Data\Pesticides_SubRefLib.reflibrary.xml.
	Left m/z: 100 Right m/z: 100 Unit: PPM • Retention Time setup: Left delta: 1 Right delta: 1 Unit: Minutes •	Dr. Ludia (resticices_sub her Lib restorary xml OK Cancel	ОК
4	Outlier setup: RT Window	=	

- 3 Click OK at library creation prompt.
- 4 Click New to create a new library method.

Apply to:						
All compo	unos O selecter	compound	15			
Reference library:						
Setup Refer	ence Library					
Library method						
						1.0
				EGR.	wew	oose
Spectrum setup:						
Deconvolu	ited scan as Spectrur	n Extraction	Override			
Spectrum	Quantifier Qualifier (	Dnly				
Mass extraction setu	ip:					
Left m/z: 10	Right m/z	100	Unit:	PPM •		
Retention Time setu						
Left dalta: 1	Right dat	1	Unit	Minutes +		
centoens.	Togrit der		orne.	minutes		
Outlier setup:						
RT Window						
10	Unit: Percent					
Min. S/N						
Coelution Sco	re Limit					
Mass Accuracy	Limit					
Library Match	Score Minimum					
H of Lincidiand In	and Miniature					
+ or vermed to	milerana an					

5 Select SureMass peak detection.



6 Select the newly created reference library.



7 Click Save & Exit.

8 Check both boxes in **Spectrum setup**.



**Deconvoluted scan as Spectrum Extraction Override**: Sets which spectrum you want to see on mirror plot. If checked, a deconvoluted spectrum instead of spectrum extracted from the chromatographic region will be shown in mirror plot.

**Spectrum Quantifier Qualifier Only**: Sets how you want the Library Match Score calculated. If checked, Library Match Score will be based on the selected (quantifier and qualifier) ions.

**9** Set up **Mass Extraction** and **Retention Time**. (Note: Both mz and RT windows can be set up in Screener for convenience to avoid Fill Down that takes time for large number of compounds.)

Apply to:			
Air compounds     O delected com	Inpoditus		
Satua Defensera Library			
betap nere ence borary			
Library method:			
D:\Data\LibMeth.m			
	Edit	New	Choose.
Spectrum setup:			
Deconvoluted scan as Spectrum Ex	traction Override		
Spectrum Quantifier Qualifier Only	1		
Mass extraction setup:			
Left m/z: 20 Right m/z: 20	0 Unit: PPM		
Retention Time setup:		_	
Left delta: 0.2 Right delta:	0.2 Unit: Minute		
BT Window			
0.15 Holt Minuter a			
Min S/M			
Cost day Score Limit			
20			
Afass Accuracy Limit			
Mass Accuracy Limit			
o Mahak Paras Maharan			
Library Match score Minimum			
/5			
# of Verified Ions Minimum			

10 Set up Outliers.

Apply to: All compounds      Selected compounds	ts
Reference library	
Setup Reference Library	
(here we had	
DAData) Linkfath m	
D. (Osta/Liowietr.in	
	Loit New Choo
Spectrum setup:	1 percent and
Deconvoluted scan as Spectrum Extraction	Override
Spectrum Quantitier Qualitier Univ	
Mass extraction setup:	1
Left m/z: 20 Right m/z: 20	Unit: PPM +
Retention Time setup:	
Left delta: 0.2 Right delta: 0.2	Unit: Minutes •
Outlier setup:	
RT Window	
0.15 Unit: Minutes *	
Min. S/N	
3	
Coelution Score Limit	
70	
Mass Accuracy Limit	
5	
Library Match Score Minimum	
75	
# of Verified Ions Minimum	
2	

Outliers can be setup in Screener for all compounds for convenience to avoid Fill Down that takes time for large number of compounds.

Outliers can then be adjusted in Quant part of the method for individual compounds if necessary.

5

These Outliers parameters are recommended for screening for pesticides and environmental contaminants. Other applications may have different settings



11 Exit Method editor and click Yes to analyze the batch.

- 12 Wait for analysis to complete.
- 13 View Target Quantitation Results Table. You can view detailed results for each compound, including parameters related to the Screener such as Library Match Score and Coelution Score.



**14** Click **Screening-GC** to view suspect screening results. The screening window is focused on the compounds identified in samples and only key parameters are displayed.



Orange color-labeled compounds are for user's review and can be moved to either "green" (confirmed hits) or "red" (rejected).



- ? 🗆 X 110 Ľî D T) Ð Ē. J nerate Report New Batch Generate Report Batch file: D:\Training and Demo Riles\for Batch folder Batch Table rate Report ar\_Batch batc Batch file: Browse ... Sample: 🔨 #11\_Str Sample Type: Generate Report Report folder D:\Training and Demo Files\for Sc Name Met.. Fen.. Final Conc. RT MZ MZ S/N RT sp. 19736 31839 18.638 26.86 12.98 7.26 268.26 16.74 6.85 206.1176 121.0648 34.3292 19.3500 0.3973 107.0491 224.1183 201.0076 98.9842 18.6575 17.317 20.906 15272 0.1917 0 Cyp. Tris. 162670 20.6661 0.3322 Chor New 16.291 16.564 6070 5230 -1.1296 n 7200 Al samples Choose samples Compound Information ▼ ◆ ↑ ∧ 元 Ⅱ▲ ✓ <> ▷ ▲ 교 숲 왕 비장 ▲ 조 조 초 쇼 쇼 Ⅱ술 A ESM(107:0431) Som #11\_SH: D 107:0491, 166:0603 All compounds Choose compounds... Generate reports now pints, 6 Points Not Found Ratio = 0.5 (3.6 %) Ratio = 3948.6 (22843.0 %) Ratio = 16487.9 (166952.4 %) Not Found Open report folder after 1.4-O Queue report task 6.5-5.5-4.5-3.5-3.5-1.2-0.8 OK Cancel 0.6-1.5-
- 15 To create reports, select Generate Report and click New under Report method.

16 Click Add Template to select a report template.

Report Method Edit (Quantitative Analysis)	_	- n x				
File Edit Tools Help	Open					×
🖬 🤊 ए 🔊 🖉 🐇 🔜 😋 🖞	← → · ↑	MassHunter > Report Templates > Quant > PD	F-ReportBuilder > Quant	~ (	Search Quant	م
Templates Results Graphics settings	Organize - New folder					BE - T 0
Template	Data	^ Name	Date modified	Type	Size	^
	for Screener	Gen Complete ISTD.template	4/14/2022 6:27 PM	XML Document	240 KB	
	LIP.	Gen GC Report.template	4/14/2022 6:27 PM	XML Document	62 KB	
	DEAC	Gen_LIMs.template	4/14/2022 6:27 PM	XML Document	230 KB	
	PIAS	Gen_MassAccuracy.template	4/14/2022 6:27 PM	XML Document	98 KB	
	<ul> <li>OneDrive - Agilent Technologies</li> </ul>	Gen_PositiveHitsOnly.template	4/14/2022 6:27 PM	XML Document	60 KB	
	This DC	Gen_ResultsSummary_ESTD.template	4/14/2022 6:27 PM	XML Document	76 KB	
	Ins PC	Gen_ResultsSummary_ISTD.template	4/14/2022 6:27 PM	XML Document	89 KB	
	JD Objects	Gen_Samples_AboveLOQ.template	4/14/2022 6:27 PM	XML Document	95 KB	
	Desktop	Gen_Samples_ESTD.template	4/14/2022 6:27 PM	XML Document	101 KB	
	Documents	Gen_Samples_ISTD.template	4/14/2022 6:27 PM	XML Document	125 KB	
	Downloads	- outre our content of plate	4/14/2022 6:27 PM	XML Document	79 KB	
	h Music	ScreeningGC_Detailed.template	4/14/2022 6:27 PM	XML Document	126 KB	
	Pictures	ScreeningGC Summary template	4/14/2022 6:27 PM	XML Document	114 KB	
	Videor	ScreeningLC_Detailed.template	4/14/2022 6:27 PM	XML Document	126 KB	
		ScreeningLC_Summary.template	4/14/2022 6:27 PM	XML Document	114 KB	
Add Template Remove Template	windows (C:)	<ul> <li>SystemSuitability.template</li> </ul>	4/14/2022 6:27 PM	XML Document	85 KB	~
	File name: Screenin	gGC_Detailed.template			✓ Template files	$\sim \text{mit}_*x\text{it}_*x\text{it}_*$
					Open	Cancel

17 Select a template, then click Save & Exit.



18 Click OK to generate the report.



#### 19 View the detailed report saved in the Quant Report folder.



20 You can also generate and view a summary report.



## Non-targeted Screening in the Unknowns Analysis Software

1 Click New Analysis (Ctrl+N) to create a new batch.

1	<u>@</u>				Ą	gilent MassHunter Unknow	ns Analysis		
	File Home			Chromatogram	Tools	Help			
	New Analysis     Open /     New Analysis (Ctri+N)     Save A	Delete Sample	Analyze All	All Samples   Selected Samples	All 👸 Target		ponents I Best Hit Blank Subtracted	Import Components/Hits	Edit Meth
1	Analysis File Sam	ples		Analyze		Component Filter		Edit	Method
	Samples		- # ×	Chromatogram					
	Sample Name File Name	(	Compc Hits						

#### 2 Click Add Samples.

Ele ℃ ↔	Home		View		₩ ∽ ···	Home		View	Chromato	ram	Agilent M Tools	lassHunter Uni He	knowns Analysis - pl:	asma1-18.uaf	
<ul> <li>New Analysis</li> <li>□<sup>†</sup> Open Analysis</li> <li>□ Save Analysis</li> </ul>	Add Samples			All Sam Selecte	New Analysis     Open Analysis     Save Analysis	Add Samples	Delete Sample	Analyze All	All Samples ¥ Selected Samples ¥	<b>33</b> All	88 Target 88 Non-Target	Hit Non-Hit	Manual Components BSB	Best Hit Blank Subtracted	Import
Analysis File	Sam	ples		Analyze	Analysis File	Sar	nples		Analyze			Compone	int Filter		
Samples			* ×	Chro	Samples			* # X	Chromatogram	n					
Sample Name	File Name	Cor	mpc Hits	×10 1 0.9 0.8 0.7	Sample Name blank Sample 11	File Name blank.D Sample 11.D		Compc Hits	+EI TIC Scan Frag=70 2 ×10 7 0 1.2- 0 1.1- 1- 0.9-	OV blank.D	(blank)			11	

#### 3 Click Edit Method.

■ ∩ <						Agilent M	assHunter Un	knowns Analysis - pl	asma1-18.uaf							? - 🗆	×
File	Home		View	Chromatog	ram	Tools	н	elp							-		
New Analysis     Open Analysis     Save Analysis	Add Samples	Delete Sample	ی Analyze All	All Samples ¥ Selected Samples ¥	<b>#</b> AI	88 Target 88 Non-Target	Hit Non-Hit	Manual Components BS8	Blank Subtracted	iniônt C-J	Components/Hits	1 1 1	Edit Method	Generate Report	Open Queu Edit R	eport Folder Quer Viewer Kort Method	1
Analysis File	Sampl	les		Analyze			Compon	ent Filter			Edit		Method		R	ion	1
Sample Name F blank b Sample 11 S	File Name Ilank D Sample 11.D	C	# × smpc Hits	Chromatogram +EI TIC Scan Frag-70 22 x1077 12- 0.1- 0.9- 0.8- 0.7- 0.6- 0.5- 0.4-	n OV blank D	(blank)							Edit Method (F10) Show and edit uni analysis method. S view shows the me single sample. Adv shows the method samples at a gland	nowns itandard ethod of anced view I of all e.		* * ×	

4 Under the **Peak Detection** tab, click **SureMass**.

Park Detection					
Teak Detection Deco	Wolution	Library Search	Compound identification	Target Match	Blank Subtraction
a at					
Sure Mass		~			
TIC Analysis					
SureMass Peak filter					
Excluded m/z:			28		
			Example: 28.91,149		
Ion Peak SNR thresho	id:		0		
Maximum number of pe	aks				
Bank by:				~	
Maximum number of pr	Naks:				
Area filters					
Absolute area	>=	0	counts		
Relative area	>=	0	% of largest peak		
Height filters					
Absolute height	>=	0	counts		
Relative height	>=	0	% of largest peak		

5 Under the **Deconvolution** tab, set the RT window size factor.



6 Under the Library Search tab, click Change Library to choose a PCDL library.

C1MassHunterlLibraryIdemo.mslibrary.cml		Change Library			
Move Up Move Down	Open Library	Remove Library			
Search criteria: Pre-search type: Normal ~	Forward-Reverse Search: Pure Weight Factor: 0.7				
Adjust Score	Example: 0.0 for reverse search 1.0 for forward search		Open		
Match factor			← → × ↑ 📙 « My Passpor → Dat	ta > 、 で	Search Data
Use RT Match			Organize  Vew folder		III • 🔟
RT penalty function:     Trapezoidal     RT range:     6	RT mismatch penalty: Multiplicative Sec Additive		HR Library Documentation	A Name blank	
Penalty-free RT range: 0 Gaussian Standard deviation: 6	sec Max RT penalty:	20	<ul> <li>OneUrive - Agirent rectinologies</li> <li>This PC</li> <li>3D Objects</li> </ul>	GCM	S FAMES PCDL
RT calibration file:			Desktop	~ <	
	Nat	0	File name: GCMS FAM	IFS PCDI	Library Files (".mslibrary.sml."
	100	un			

7 Choose a Library.



8 Use the **Pre-search type** pulldown to choose how fast and accurate you want the search.

	passing institute	ry.xml				Change Library.
Move Up	Move Down			Open Library		Remove Library
			For	ward-Reverse Search		
Pre-search type:		_	Pur	e Weight Factor:		
Normal		~		0.7		
Adjust Nomal			- 8	Example: 0.0 for reverse search		
RemoveFast				1.0 for forward search		
		_				
Use RT Match						
- RT penalty functio	n:			- RT mismatch penal	tv:	
O Trapezoidal				Multiplicative		
RT range:		6	sec	<ul> <li>Additive</li> </ul>		
	range:	0	BEC.	Max RT pen	alty:	20
Penalty-free R1						
Penaty-free R1						
Penalty-free R1 Gaussian Standard devia	tion:	6	sec			
Penalty-free R1 Gaussian Standard devia	tion:	6	sec			

**9** Check **Adjust Score** to make it similar to NIST scoring, and remove duplicates if you wish.

Peak Detection Deconvolution L	ibrary Search	Compound lo	entification	Target Match	Blank Subtraction	
Libraries:						
E1/Data1GCMS FAMES PCDL ed	Ь				Change Library	
					Add Library	
Move Up Move Do	nwo		Op	oen Library	Remove Library	
Search criteria:		For	ward-Reverse	e Search:		
Pre-search type:		Pun	e Weight Fac	ctor:		
None	×		0.7			
Adjust Score		1	Example:			
Remove Duplicate Hits			1.0 for forwa	ard search		
Match factor:						
Use RT Match						
RT penalty function:			RT misma	stch penalty:		
Trapezoidal			Multiple	licative		
RT range:	6	900	O Addith	ve		
Penalty-free RT range:	0	sec	Ma	sx RT penalty:	20	
() Gaussian						
Standard deviation:	6	sec				
RT calibration file:						
1						1
2 C				New	Changes	
					010000	

**10** Check **Use RT Match** if you want the RT calibration file to match library's RTs. With the calibration file, it will match RIs.

Libraries:								
E1Data1GCMS FAMES PCDL.edb		Change Library.	-					
		Add Library						
Move Up Move Down	Open Library	Remove Library	<i>i</i>					
Search criteria:	Forward-Reverse Search:			/ (i)	AMEs_RT-cal - Notepad		- 0	5
None V	0.7			File	Edit Format View Help			
Address Second	Example:			[C8]	Methyl Caprylate [7.812],	111-11-5,800,8.446		
C Deserve Destante Une	0.0 for reverse search			[C9]	Methyl Pelargonate [9.248	3],1731-84-6,900,9.81	2	
<ul> <li>Nemove Dupicase Hits</li> </ul>	1.010 Tormard search			[C16	] Methyl Caprate [10.647],	110-42-9,1000,11.179		
Match factor:				IC12	1] Methyl Myristate [15.59]	1.124-10-7.1400.16.1	18	
Use RT Match				(RTL	Myristic Acid d27 [16.]	27],60658-41-5,1503,	17.212	
RT penalty function:	RT mismatch penalty:			[C16	] Methyl Palmitate [17.72]	3],112-39-0,1600,18.2	54	
<ul> <li>Trapezoidal</li> </ul>	Multiplicative			[C18	3] Methyl Stearate [19.663]	,112-61-8,1800,20.20	5	
RT range: 40 s	ec O Additive			[C2e	] Methyl Eicosanoate [21.4	41,1120-28-1,2000,2	649	
Penalty-free RT range: 20 s	ec Max RT penalty:	20		[C24	Methyl Linocerate [24.66	31,2442-49-1,2400,25	.177	
Gaussian		/		[C26	] Methyl Hexacosanoate [26	.023],5802-82-4,2600	,26.61	
Standard deviation: 6	er -			[C28	3] Methyl Octacosanoate [2]	.349],55682-92-3,280	ð,27.946	
				[C36	] Methyl Triacontanoate [3	28.723],629-83-4,3000	,29.356	
RT calibration file:								
E:\Data\FAMEs_RT-cal.rtc				<				
		E COMPANY			Lo 1 Col 1	100% Windows (CRLF)	UTE-8	

11 Under the **Component Identification** tab, set **Min m/z** so that it is no lower than the data acquisition mass range. Also select minimum **Library Match Factor** and **number of hits** per component.

Peak Detection Deconvolution Library Search	Compound Identification	Target Match	Blank Subtraction	
Max hit count:	1			
Min match factor:	75			
Min MZ:	50			
Library Search Type:	spectral search			~
Multi-Library Search Type:	Al			~
Perform Exact Mass				
Exact Mass				
Max lons per Spectrum:	20			
Min Relative Abundance:	0			
MZ Deka PPM:	10			
Peak Selection Weighting:	Mass			I

12 Click Perform Exact Mass box, and select algorithm for ion Peak Selection Weighting.

Pask Detection Deconvolution Library Search	Compound Identification	Turnet Match	Black Subtraction	
Marchit count:		Target Hasart	Durit Subsection	
Min match factor	75			
Mar M7.	50			
Min MZ:	50			
Library Search Type:	Spectral Search		~	
Multi-Library Search Type:	Al		~	
Perform Exact Mass				1
Exact Mass				
Max lons per Spectrum:	20			
Min Relative Abundance:	0			
MZ Deta PPM:	10			
Peak Selection Weighting:	EauniMoista			ı
	EqualWeight			l
	Mass Mass2 Mass2			
	(mass)			1
				4

13 Under the Blank Subtraction tab, check Perform Blank subtraction.



14 Click Apply to All Samples.

15 Click Advanced then click Yes.



16 In Accurate Mass Tolerance (PPM), increase the values so very small ions in the deconvoluted spectra do not get discarded due to increased mass accuracy error.

Sample Name	naity Type		RT Range	RT Range No Penalty	Spectrum Threshold	Remove Duplicate Hits	Accurate Mass Tolerance (PPM)
Sample 11	ative	~	0.6667	0.3333	0.0		
blank	ative	~	0.6667	0.3333	0.0		

17 Click Standard then click Yes to apply changes.



**18** Select **Blank** sample type for subtraction, then click **Analyze All** to analyze the batch. An **Analyze** window will appear with a progress bar.



**19** Analysis results will be displayed. Columns can be configured to include Component and Library RI.



#### 20 Click Exact Mass Table to display Exact Mass Results.



All highlighted ions in the component spectrum and shown in the ExactMass Table correspond to the hit with regards to the accurate mass.

This page intentionally left blank.
# Index

# A

Accurate Mass Tolerance, 69 Adding GC/Q-TOF Spectra to a PCDL, 40 Adding GC/Q-TOF Spectra to a PCDL Workflow, 40

# В

Biological sample extracts, 26

#### С

Calculated m/z, 46 Configuring MassHunter Qualitative Analysis 10.0, 40 CSL Operating Conditions, 15

#### D

Derivatization, 28 detailed report, 62

# F

Formula annotated peaks, 46 Fragment, 45 Fragment Formula Annotation, 45 Fragment Formula Annotation Tool, 42 Fragment Formulas, 43

# G

GC/Q-TOF System Setup, 12 Generate Formulas, 42, 43 Glossary, 9

# Н

HSL Operating Conditions, 14

#### 

Installation file locations, 8 Internal standard RTL, 26 Ion species window, 44

# Μ

Managing the PCDL content, 36 Mass Extraction, 58 Metabolites, 26 Method Editor, 42 Methoxyamination, 28

# Ν

Non-targeted Screening in the Unknowns Analysis Software, 64 Non-targeted Screening in the Unknowns Analysis Software Workflow, 64

# 0

Overview of Target and non-target screening workflows, 36

#### Ρ

Parts and Consumables, 13 PCDL Description, 32 Preparing the biological extract, 28

# R

Report generate, 61 Retention index markers, 27 Retention Time, 58 Retention Time Locking the Data Acquisition Method, 17

# S

Set up Cold Splitless Injection Method, 16 Setting up Automatic Mass Calibration, 21 Fragment Formula Annotation Tool, 44 Molecular Formula Generation, 42 Setup a Reference Library, 55 Software Requirements, 8 summary report, 63 SureMass features, 47 SureMass Overview, 47 SureMass peak detection, 56 Suspect Screening and Target Quantitation, 48 Suspect Screening and Target Quantitation Method Setup, 48 Suspect Screening Method Setup, 55

# Т

Target Quantitation Method Setup, 50 Target Quantitation Results Table, 59 Trimethylsilylation, 29 Typical Workflow for Suspect Screening, 48

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