Agilent

GC/Q-TOF PCDL

User Guide

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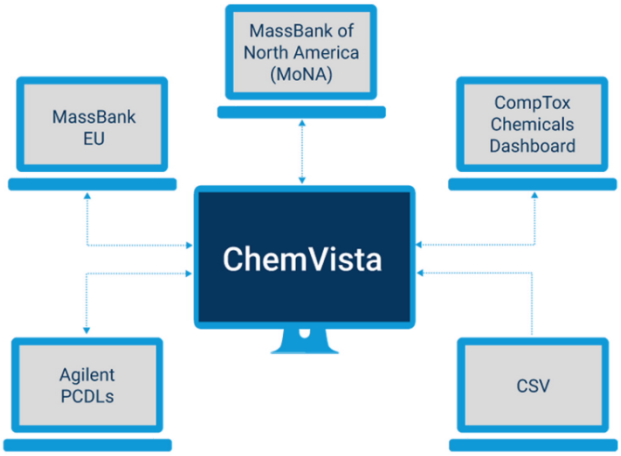

Notices

Document Identification

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is valid for the C.01.00 revision or higher of the GC/Q-TOF PCDL program and compatible GC/Q-TOF PCDL programs, until superseded.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

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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

This chapter provides an overview of 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 pertaining to the installed file location. When installed on a PC with a MassHunter home already defined and/or a fixed D:\ drive, files will be installed to the location above.

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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2 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

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:

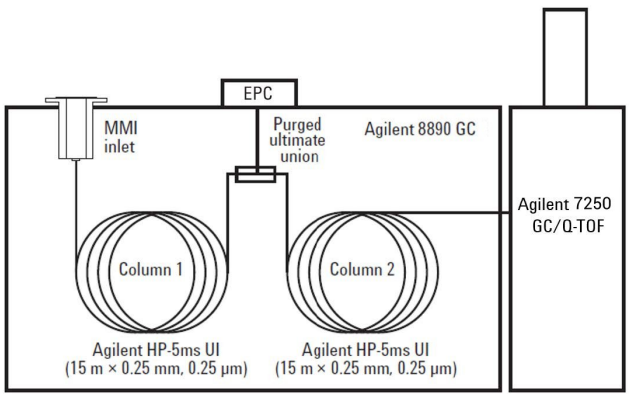
- Agilent 7693A Autoinjector Module (G4513A)
- Agilent 7693A Autosampler Tray Module (G4514A)

Parts and Consumables

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 µm, 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 µm film (two each)	Agilent HP-5ms UI, 15 m, 0.25 mm id, 0.25 µm 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 µL splitless	1 µL 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)

	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	EI	EI
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 µA	5 µA

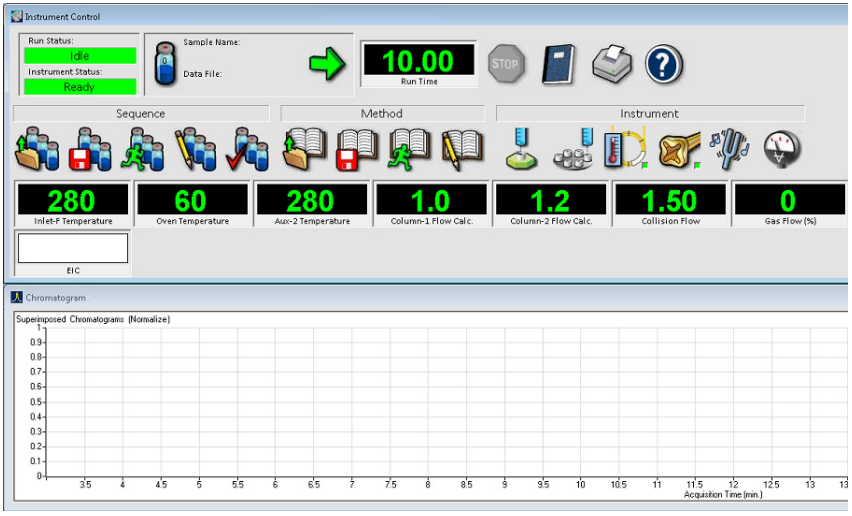
CSL Operating Conditions

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.

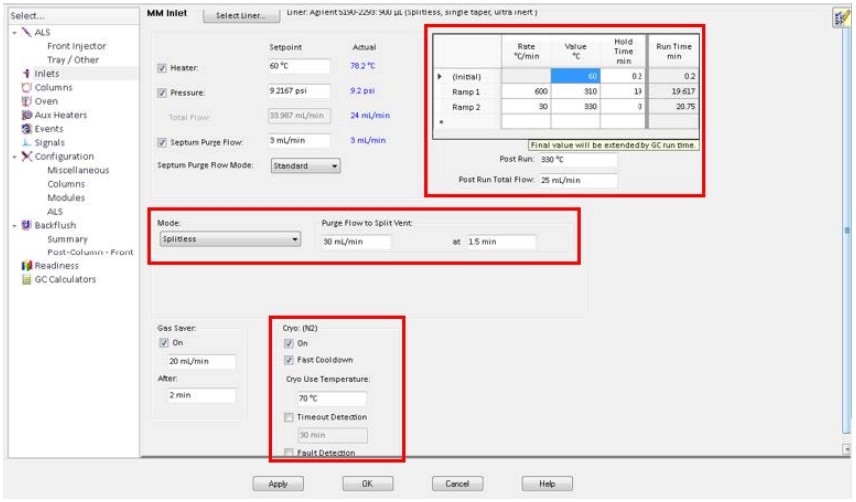
Injection volume	2 uL Cold splitless
MMI temperature program	60 °C for 0.2 minutes 600 °C/min to 300 °C, hold 330 °C, post run
Purge flow to split vent	30 min/min at 1.5 minutes
Cooling	On Fast cooldown 70 °C, Cyro use temperature

Set up a Cold Splitless Injection Method

1 Click **GC Edit Parameters**.



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



3 Click **OK.**

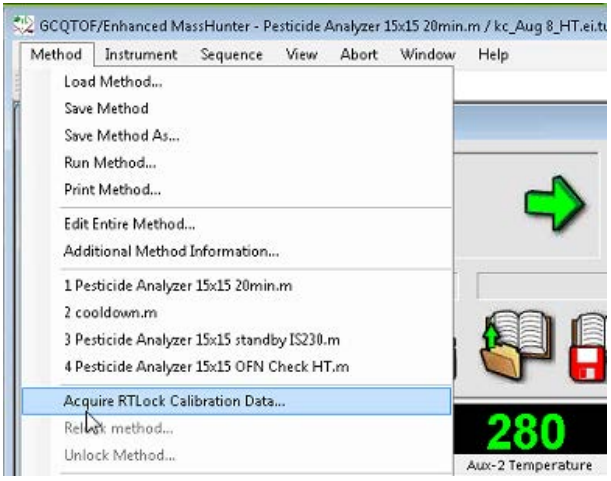
4 **Save** your method to a different name.

Retention Time Locking the Data Acquisition Method

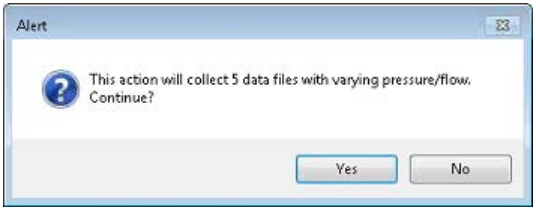
Perform Retention Time Locking (RTL) to Agilent-provided data acquisition methods before your sample analysis. Use the compound Chlorpyrifos-methyl as a locking compound.

Agilent recommends using the pesticide checkout standard (100 µg/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.

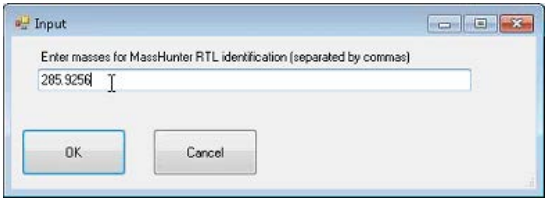
1 Click **Method > Acquire RTLock Calibration Data**.



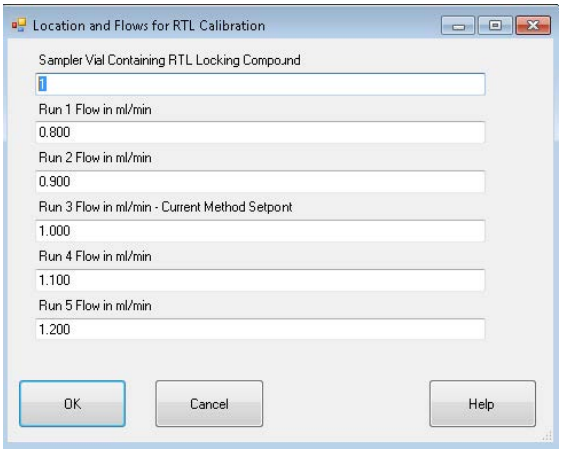
2 Click **Yes**.



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

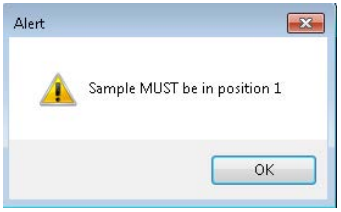


4 Ensure your checkout standard is placed in the correct vial position on the autosampler tray, as indicated in the **Sampler Vial Containing RTL Locking Compound** field.

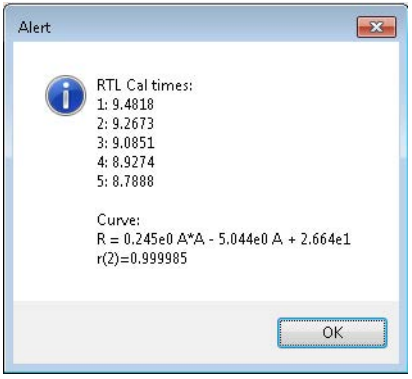


5 Click **OK** to start the retention time locking calibration.

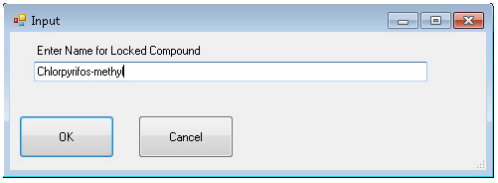
6 Click **OK**.



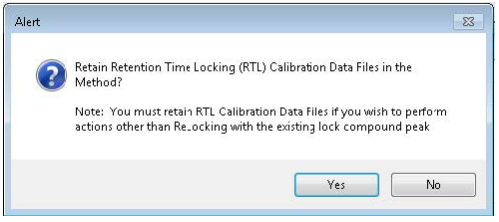
7 Once the calibration run completes, click **OK**.



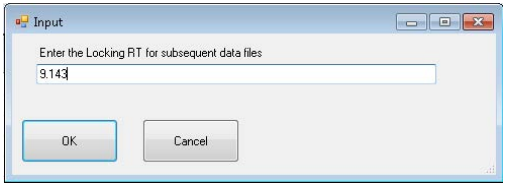
8 Enter the **name** of the locking compound, and click **OK**.



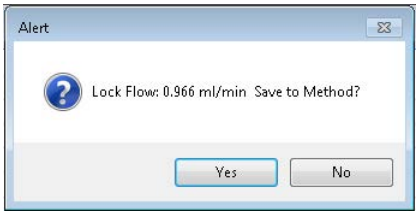
9 Select whether or not to retain **RTL Calibration Files** in the method.
Whether or not you save the original files, the calibration curve will be saved, allowing you to relock your method.



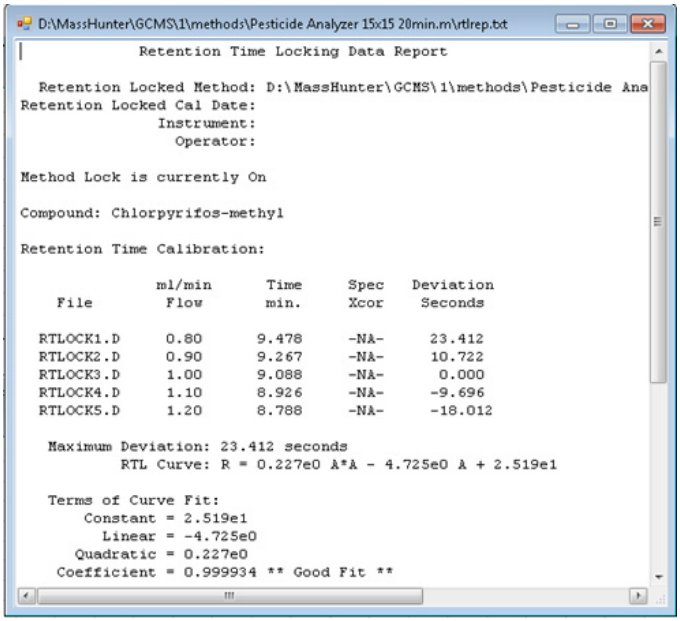
10 Enter the target retention **time** of the locking compound, and click **OK**.



11 Click **Yes** to save the flow to the method.



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

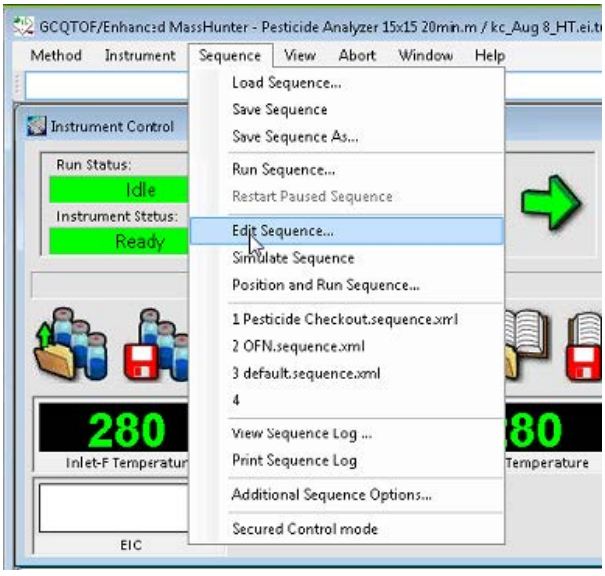


D:\MassHunter\GCMS\1\methods\Pesticide Analyzer 15x15 20min.m\rtlrep.txt

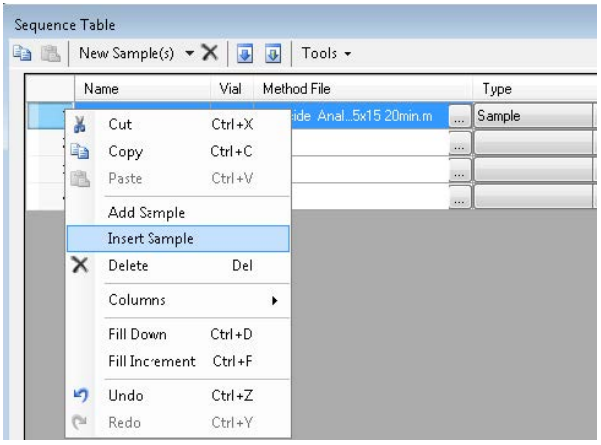
```
            Retention Time Locking Data Report

  Retention Locked Method: D:\MassHunter\GCMS\1\methods\Pesticide Ana
Retention Locked Cal Date:
               Instrument:
                 Operator:

Method Lock is currently On

Compound: Chlorpyrifos-methyl

Retention Time Calibration:

               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 Deviation: 23.412 seconds
           RTL Curve: R = 0.227e0 A*A - 4.725e0 A + 2.519e1

   Terms of Curve Fit:
       Constant = 2.519e1
         Linear = -4.725e0
      Quadratic = 0.227e0
    Coefficient = 0.999934 ** Good Fit **
```

Set up an Automatic Mass Calibration in your Sequence

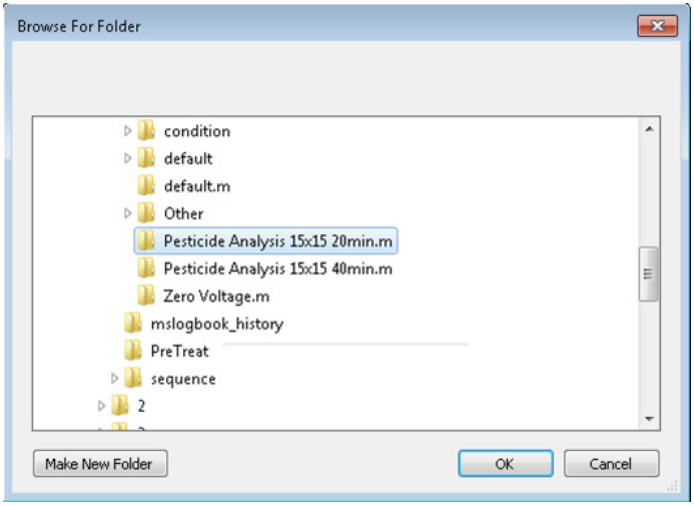
1 Click **Sequence > Edit Sequence**.



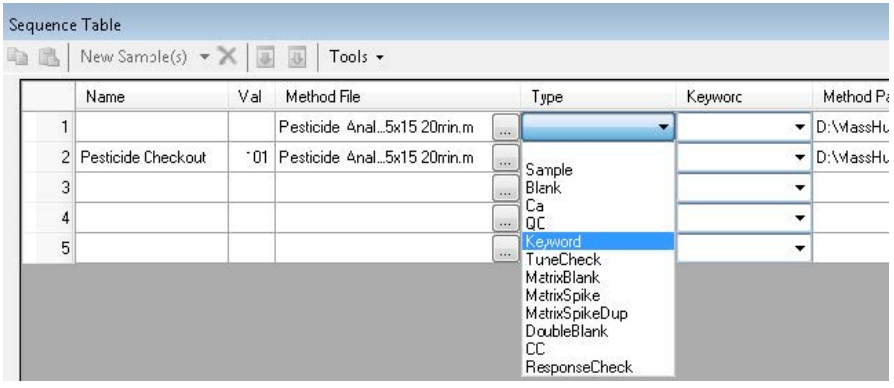
2 Right-click in the sequence table and select **Insert Sample**.



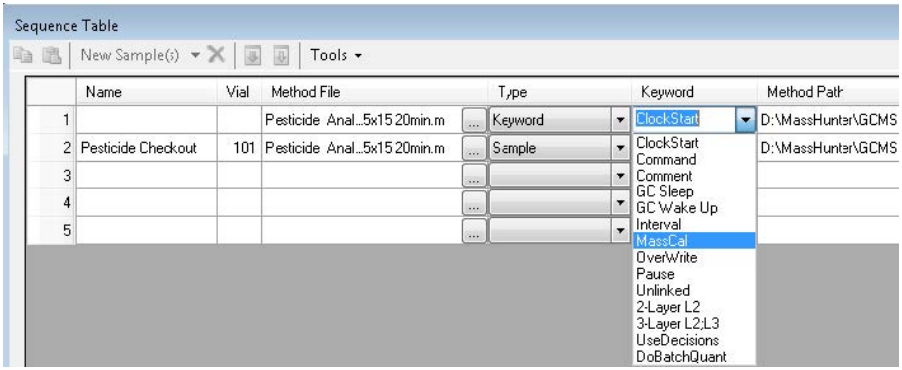
3 Select your **method**, and click **OK**. It is important to note that the method loaded for mass calibration should be the one chosen to perform your sample analysis.



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



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



6 Click **OK**.

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3 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

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.

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_8, C_9, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, and C_{30} 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 the following table.

Name	Concentration	RT (min)
Methyl caprylate C_8	100 µg/mL	7.812
Methyl perlargonate C_9	100 µg/mL	9.248
Methyl caprate C_{10}	100 µg/mL	10.647
Methyl laurate C_{12}	100 µg/mL	13.25
Methyl myristate C_{14}	100 µg/mL	15.597
Methyl palmitate C_{16}	100 µg/mL	17.723
Methyl stearate C_{18}	50 µg/mL	19.663
Methyl eicosanoate C_{20}	50 µg/mL	21.441
Methyl docosanoate C_{22}	50 µg/mL	23.082
Methyl linocerate C_{24}	50 µg/mL	24.603
Methyl hexacosanoate C_{26}	50 µg/mL	26.023

Name	Concentration	RT (min)
Methyl octacosanoate C_{28}	50 µg/mL	27.349
Methyl triacontanoate C_{30}	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 µL 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.

Trimethylsilylation

Add 90 µL 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.

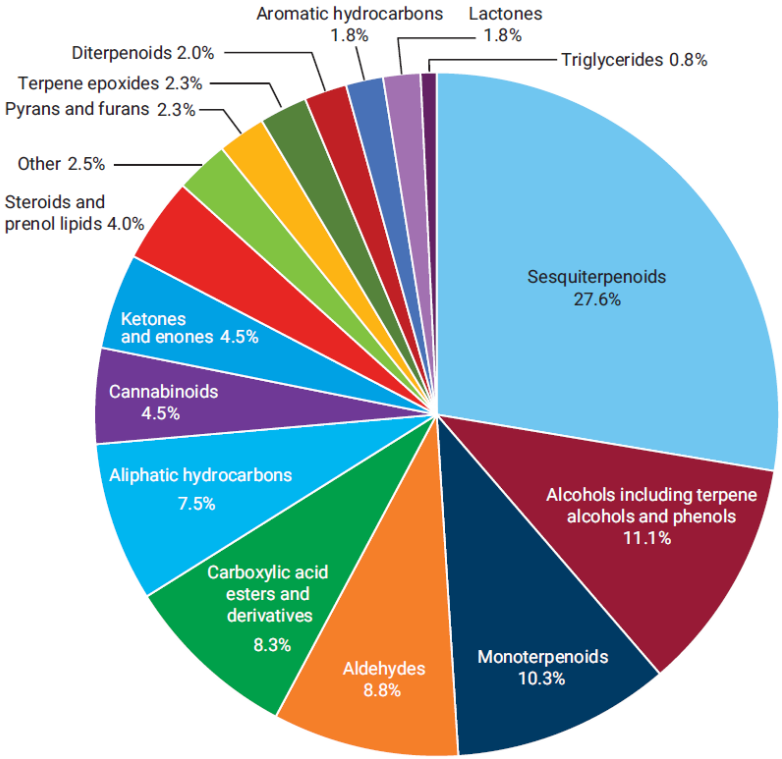
GC and MS Conditions	Q-TOF (7250)
GC	8890
Column	Agilent J&W DB-5MS UI, 30 m, 0.25 mm, 0.25 µm, DuraGuard, 10m
Inlet	SSL, 4-mm UI liner single taper
Injection volume	0.5 µL
Injection mode	Splitless
Inlet temperature	280°C
Oven temperature program	50°C for 0.5 min; 10°C/min to 325°C, 10 min hold
Carrier 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 µA
Spectral acquisition rate	5 Hz
Mass range	50 to 1200 m/z
Solvent delay	5.90 min

4 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

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

Suggested data acquisition parameters are described in the following table.

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 µL
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

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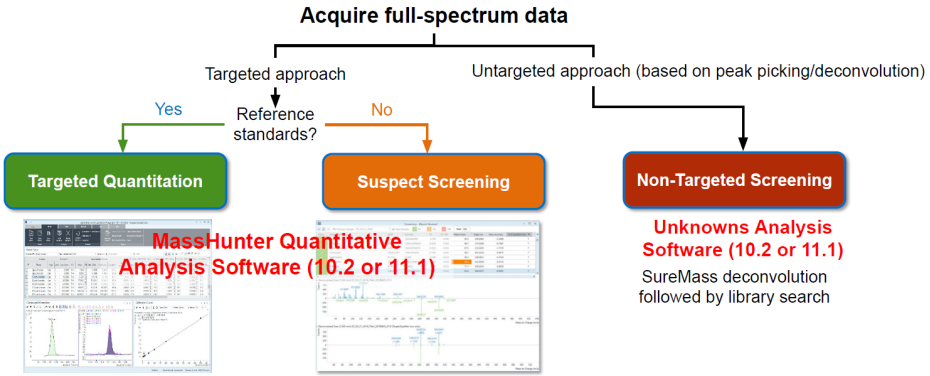
5 Data Analysis Workflows

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

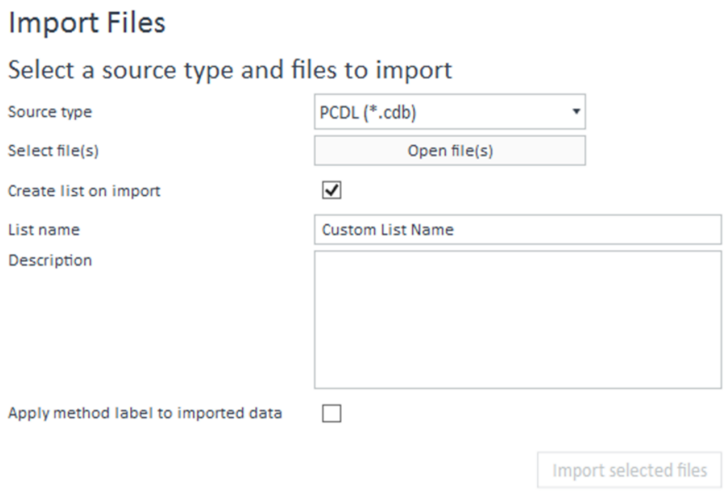
The following figure shows the GC/Q-TOF PCDL screening workflow for both targeted and non-targeted approaches.



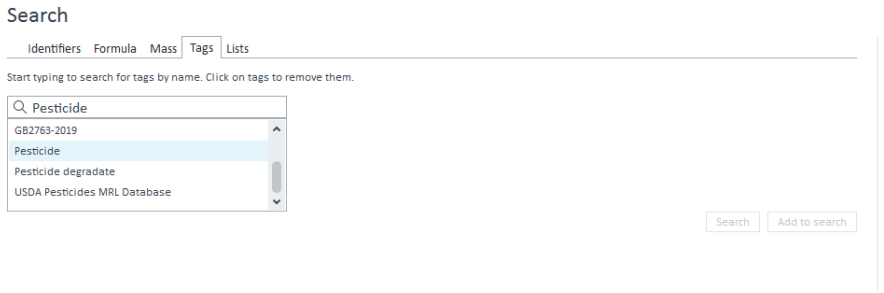
Managing the PCDL content

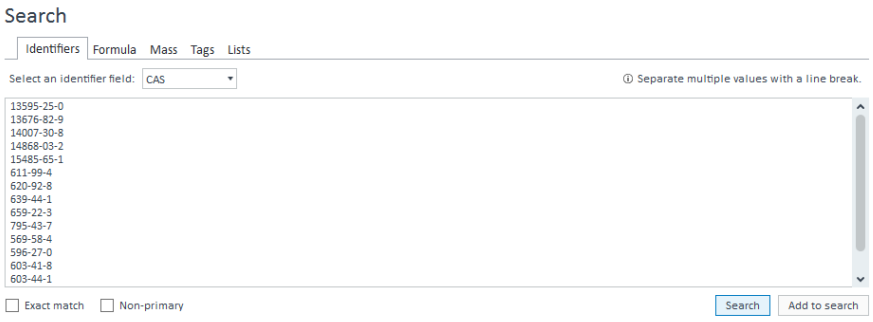
Use Agilent ChemVista to manage the content of your PCDL:

- 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.

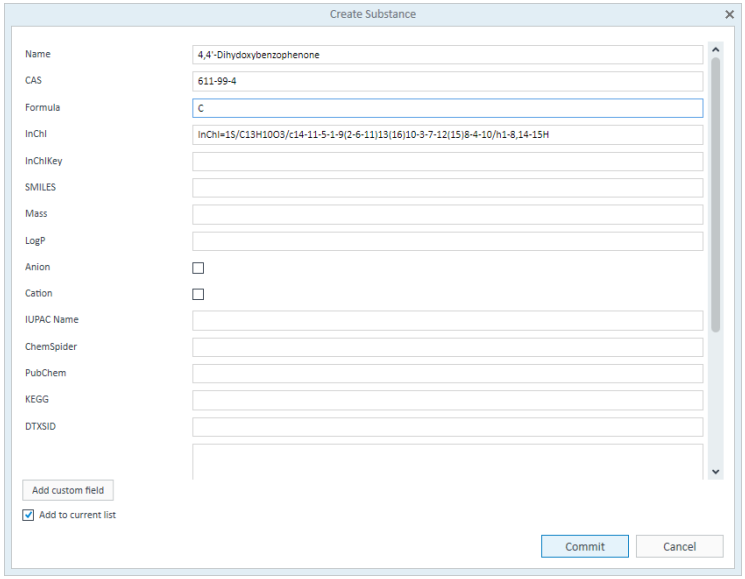


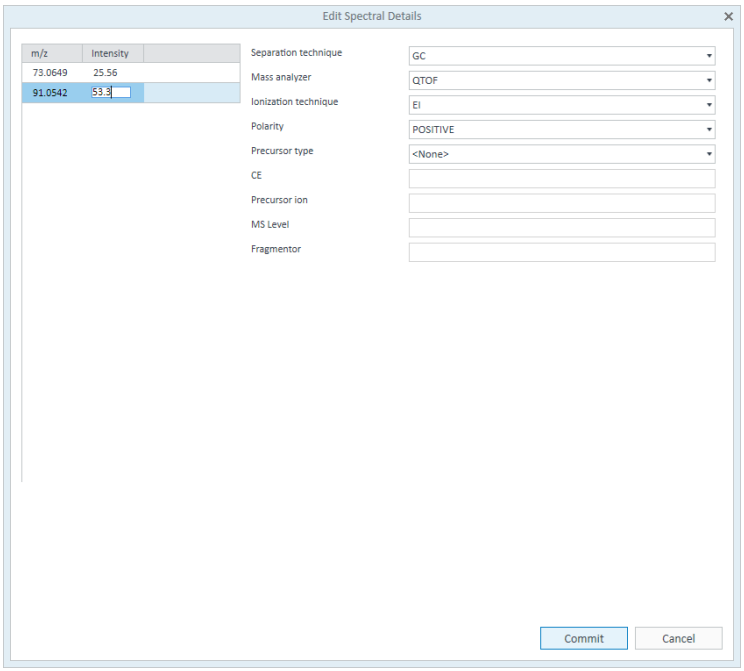
- Create custom screening lists specific to your analysis by searching for compound class groups and regulation tags as well as searches using compound name, formula, mass, CAS, InChIKey, etc.



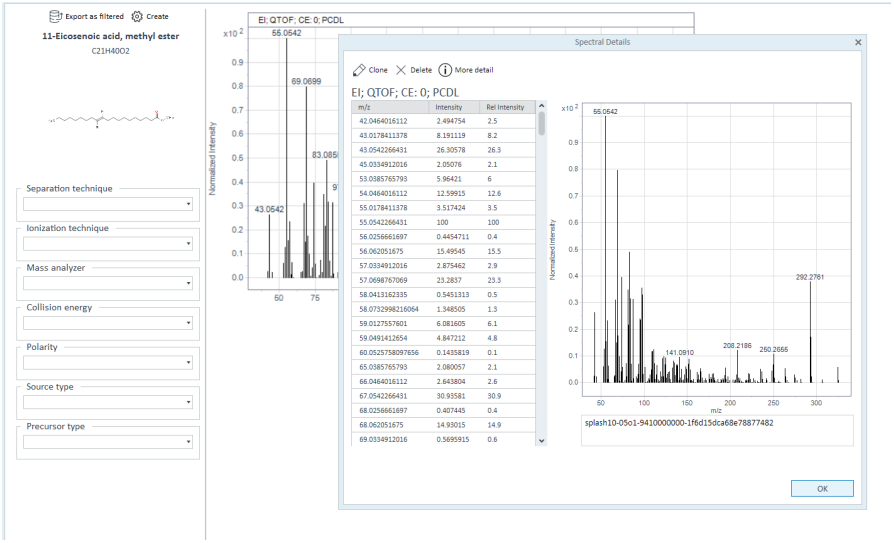


- Edit and add compounds, retention times, and spectra.





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



- 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*.

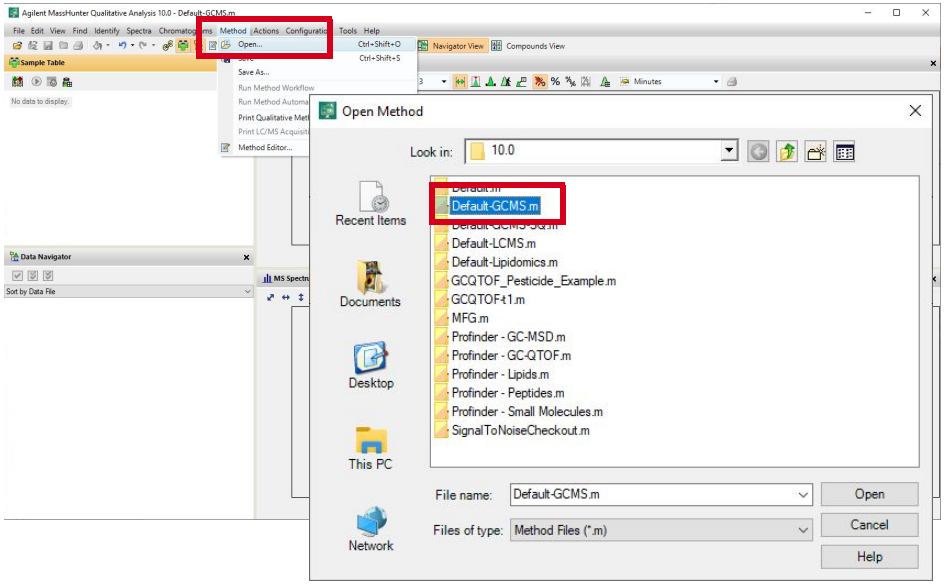
Adding GC/Q-TOF Spectra to a PCDL

Configuring MassHunter Qualitative Analysis 10.0

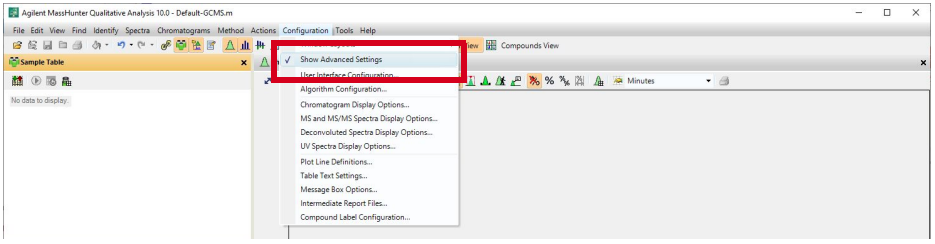
1 Acquire data in full spectrum acquisition mode.

2 Open **MassHunter Qualitative Analysis** software.

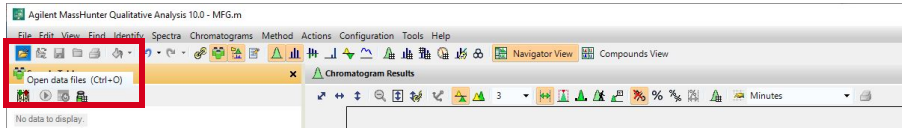
3 Under the **Method** tab**,** click **Open** then click **Default-GCMS.m** and open.



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

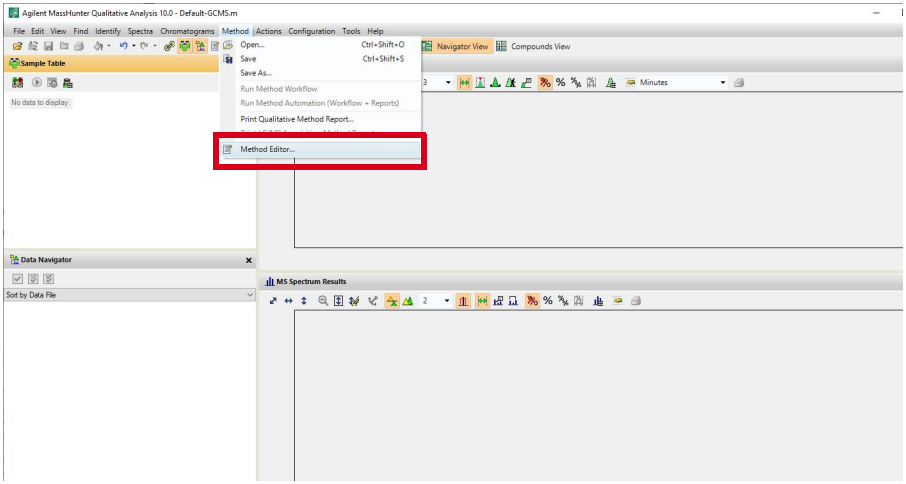


5 From the menu bar, load the **data file** (Ctrl+O).

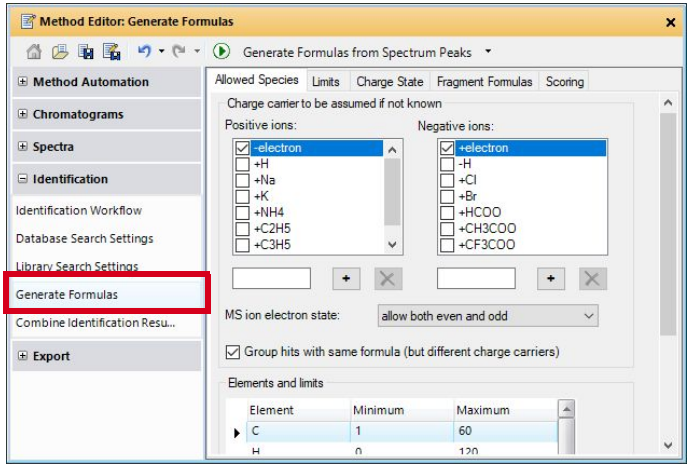


Setting up Molecular Formula Generation with Fragment Formula Annotation Tool in MassHunter Qualitative Analysis Software

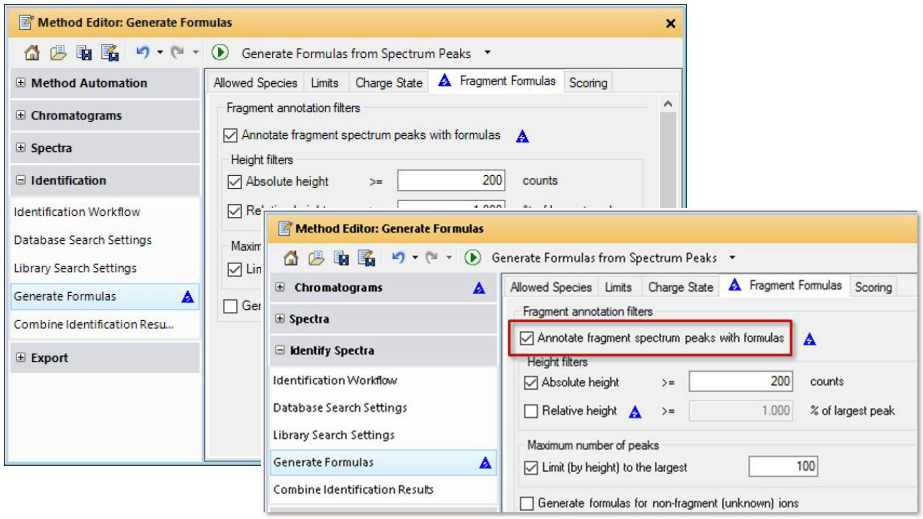
1 Under the **Method** tab, click **Method Editor.**



2 Navigate to **Generate Formulas** under the **Identification** tab.



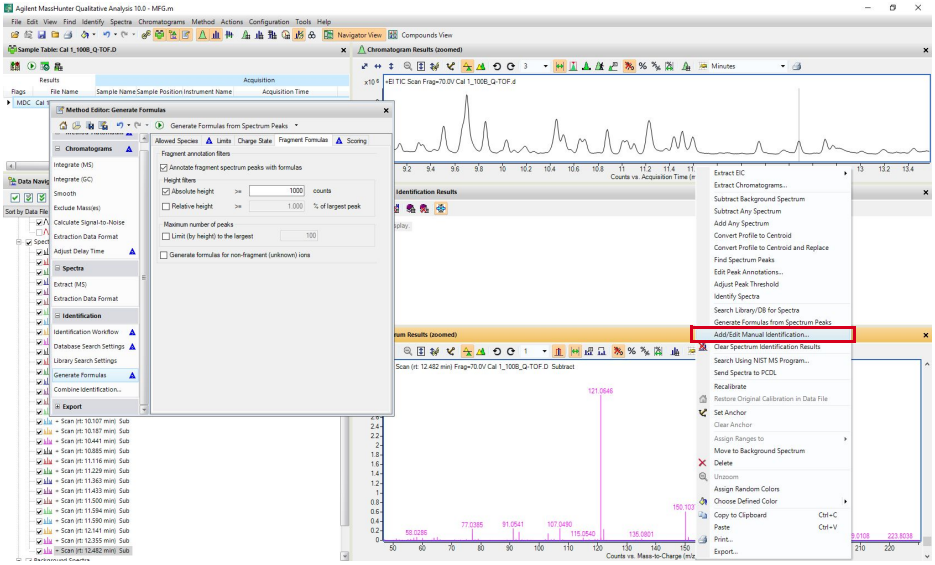
3 Under the **Fragment Formulas** tab, check **Annotate fragment spectrum peaks with formulas**. Change the filters parameters, if necessary.



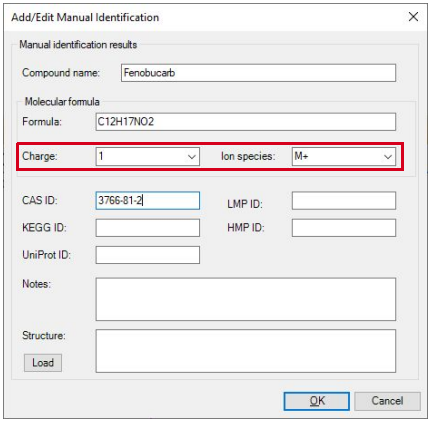
Annotation of a Spectrum with Fragment Formulas

1 Extract a background subtracted spectrum.

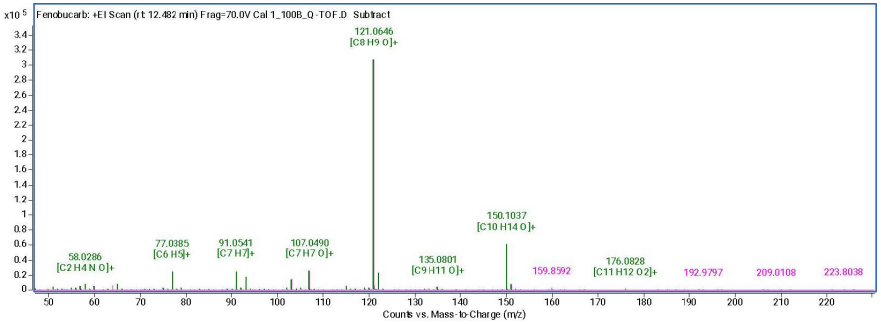
2 Right-click on the **spectrum**, and select **Add/Edit Manual Identification** from the right-click menu.



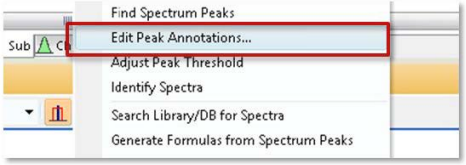
3 Enter **formula** and **compound name**. You can add other identifiers such as CAS #. Make sure that the Charge is 1 and M+ is selected in the **Ion species** window for EI.



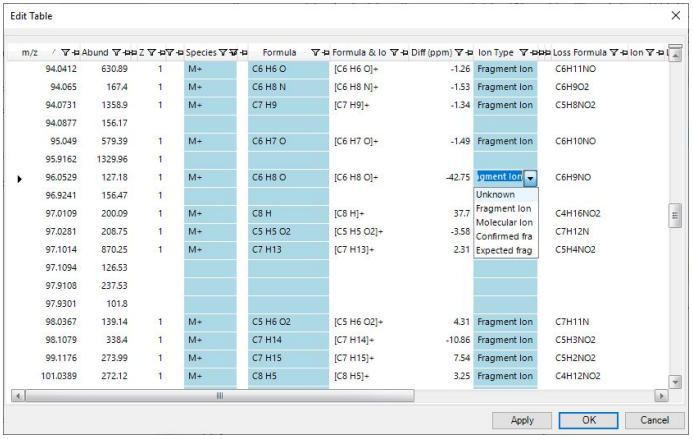
4 Check the **Fragment Formula Annotation Results**. All fragments in a spectrum are annotated with formulas which are subsets of a candidate formula for the molecular ion.



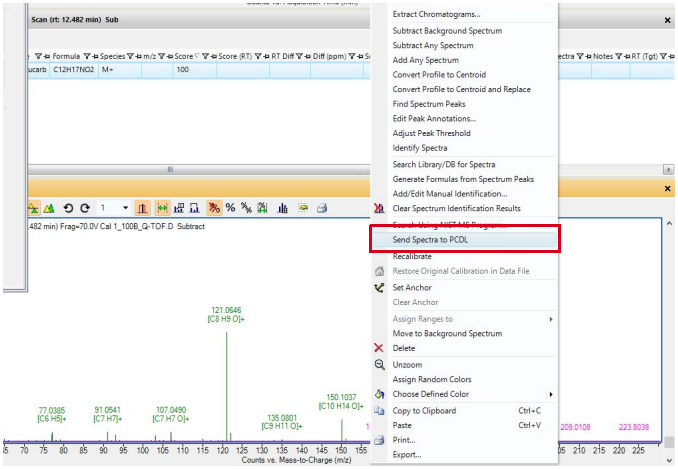
5 To edit Fragment Formula Annotation Results, from the right-click spectrum menu select **Edit Peak Annotations**.



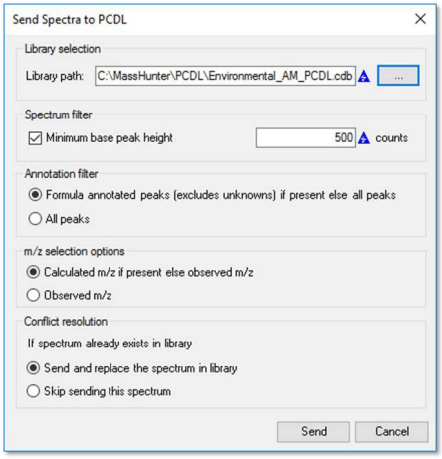
6 Species, Formula and Ion Type can be changed. To cancel annotation of an ion, select **Unknown** in the **Ion Type** column.



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



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.

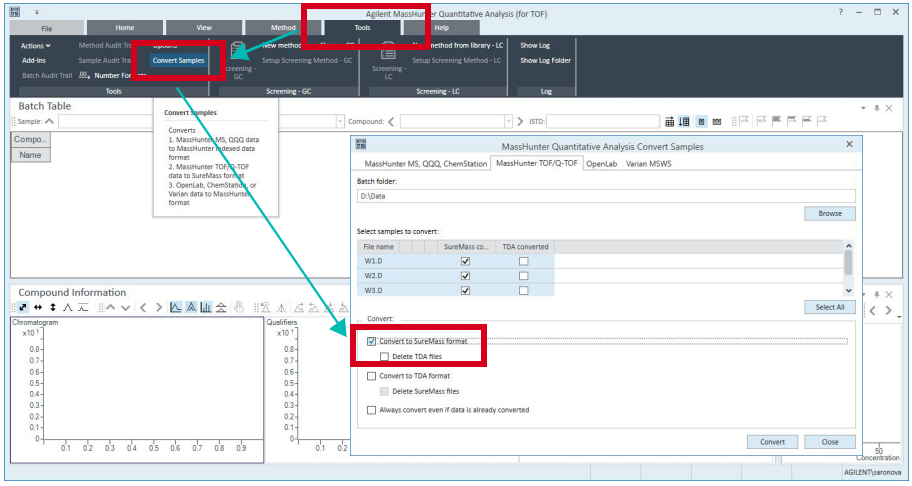


SureMass Overview

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_deconvolution_Suremass.pdf**

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



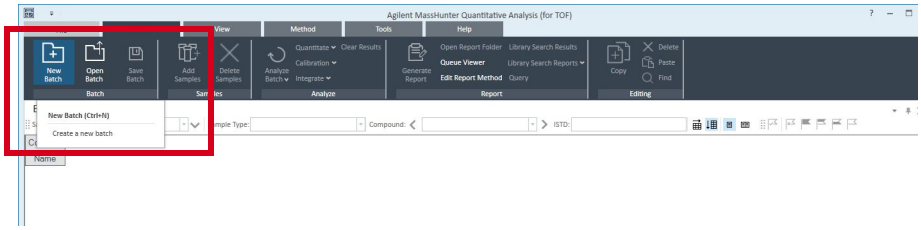
Suspect Screening and Target Quantitation Workflows

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

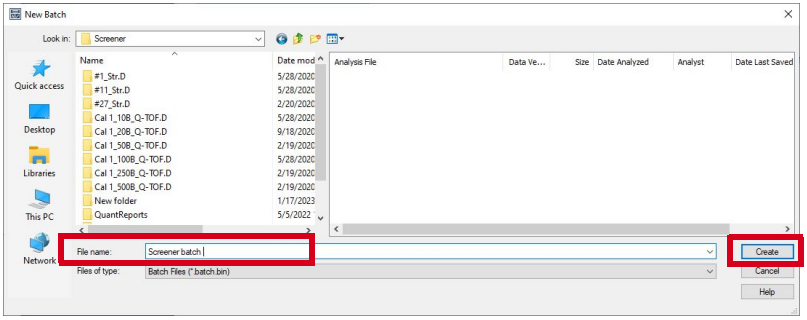
1. Use Agilent ChemVista or PCDL Manager to create a **subset PCDL** for Targets and another for non-targets (everything else).
2. Create a new **batch** in MassHunter Quantitative Analysis Software and add samples.
3. Create MassHunter Quantitative Analysis Software **method** from **GC library** using 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

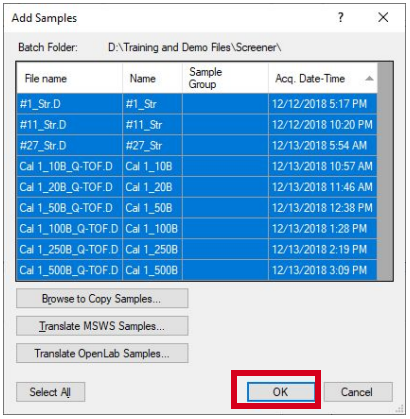
1. Open **Quantitative Analysis for TOF**.
2. Select **New Batch** and click **Create a new batch** (Ctrl+N).



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

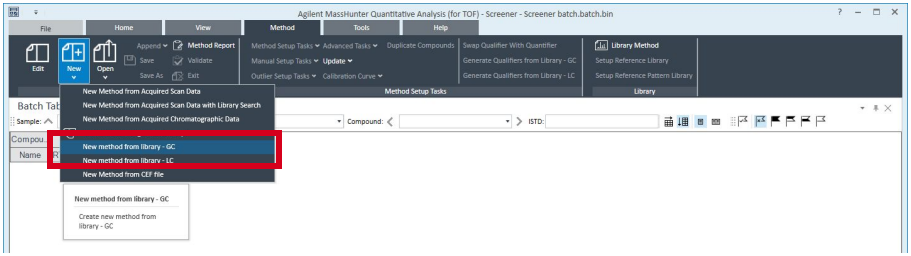


4 Add samples to the batch and click **OK**.

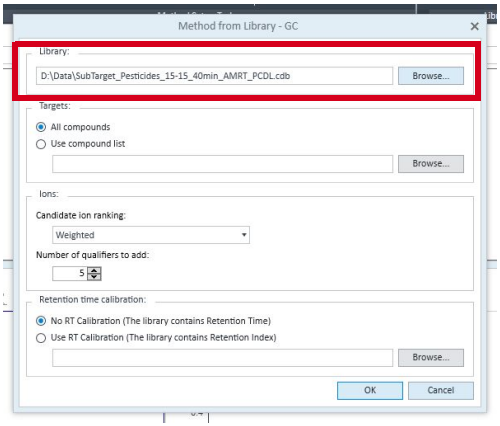


Target Quantitation Method Setup

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

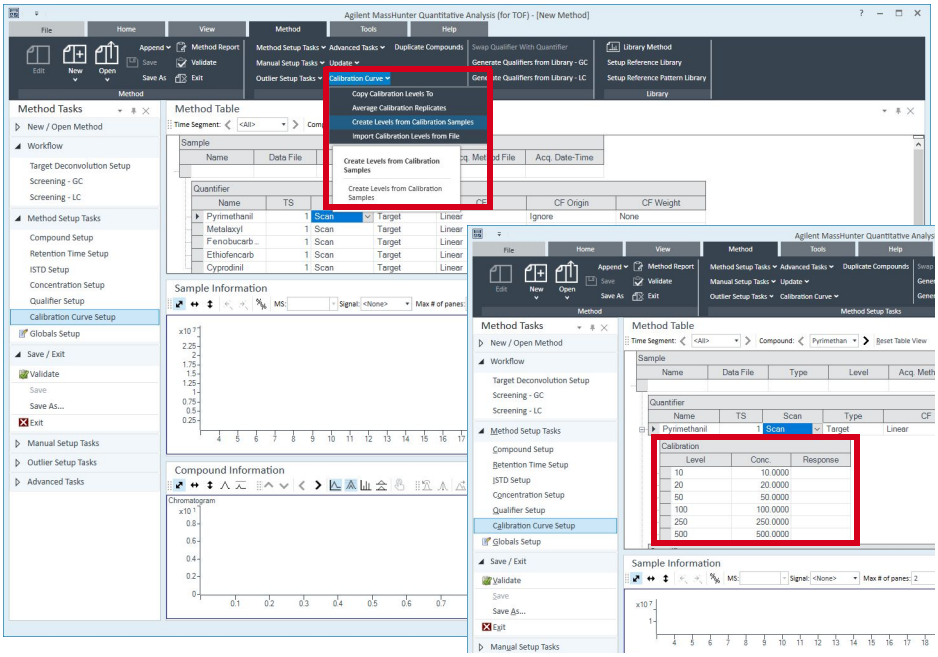


2 Select the **PCDL library** containing “targets” (compounds for which standards were run for quantitation).

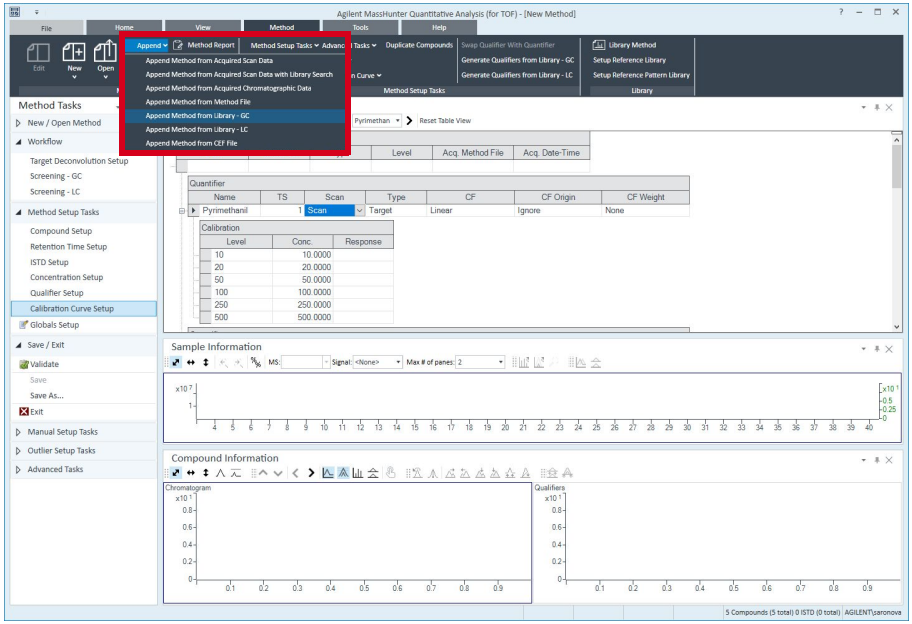


3 Choose **ion ranking algorithm** and the number of **qualifier ions**.

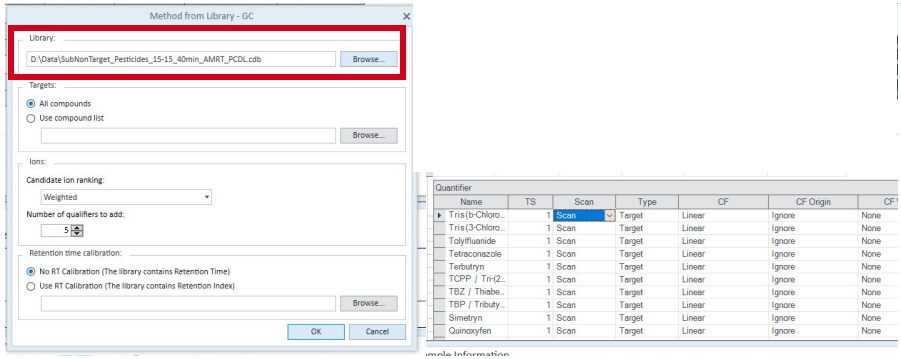
4 Select **Method > Calibration Curve**, and click **Create levels from Calibration Samples**.



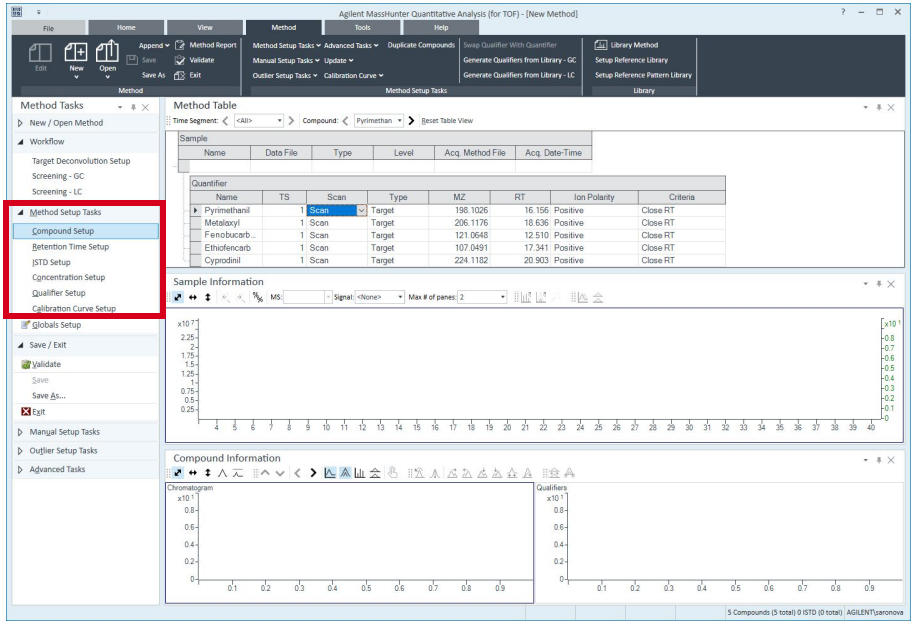
5 Select **Append** then click **Append method from Library-GC** to append with non-target compounds (for which no standards for quantitation will be run). This is using a subset PCDL containing spectra of these compunds.



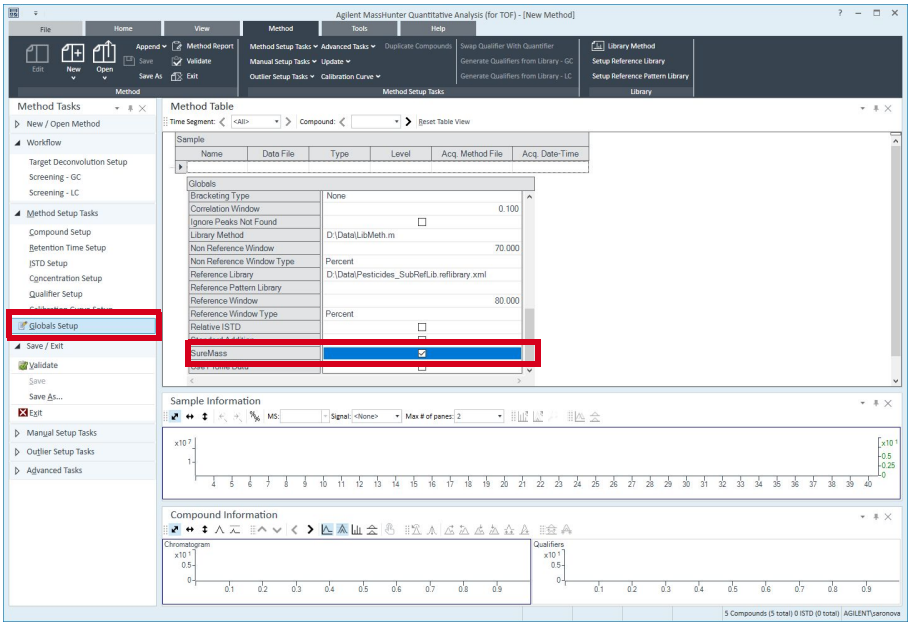
6 Browse and select the **Library**.



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.

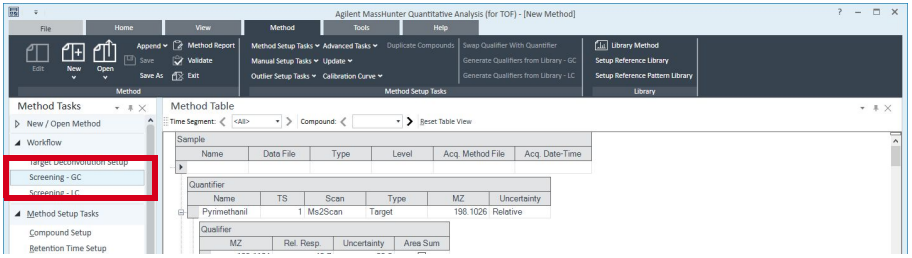


8 Click **Globals Setup** and check **SureMass** if you use profile data (recommended).

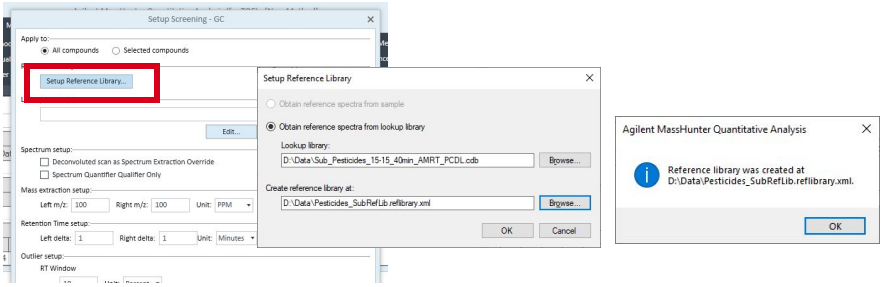


Suspect Screening Method Setup

1 Select **Screening-GC.**

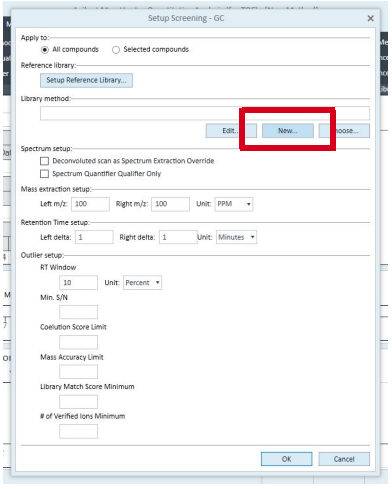


2 Click **Setup Reference Library** and browse for your library. This is based on the complete PCDL, which is your Lookup library.

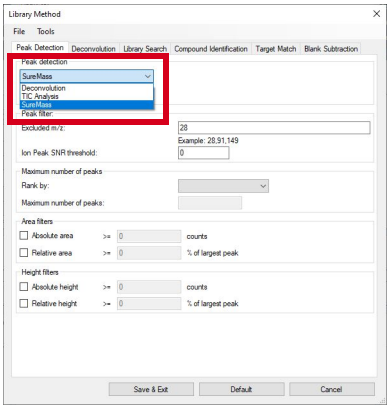


3 Click **OK** at library creation prompt.

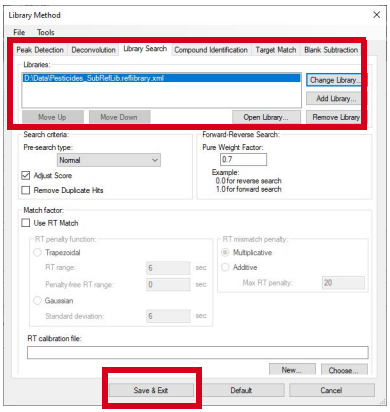
4 Click **New** to create a new library method.



5 Select **SureMass** peak detection.

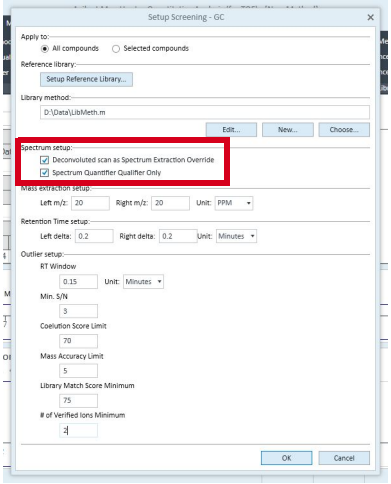


6 Select the newly created **reference library**.



7 Click **Save & Exit**.

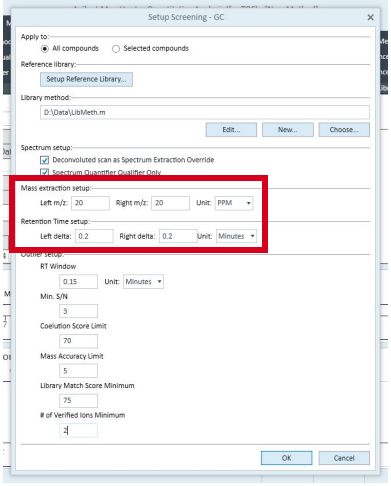
8 Check both boxes in **Spectrum setup**.



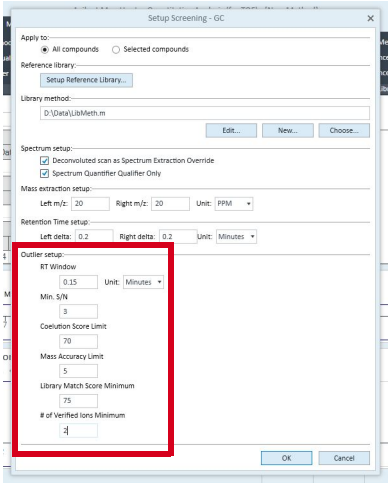
Deconvoluted scan as Spectrum Extraction Override: Sets which spectrum you want to see on mirror plot. If checked, a deconvoluted spectrum instead of spectrum extracted from the chromatographic region will be shown in mirror plot.

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.)



10 Set up **Outliers**.

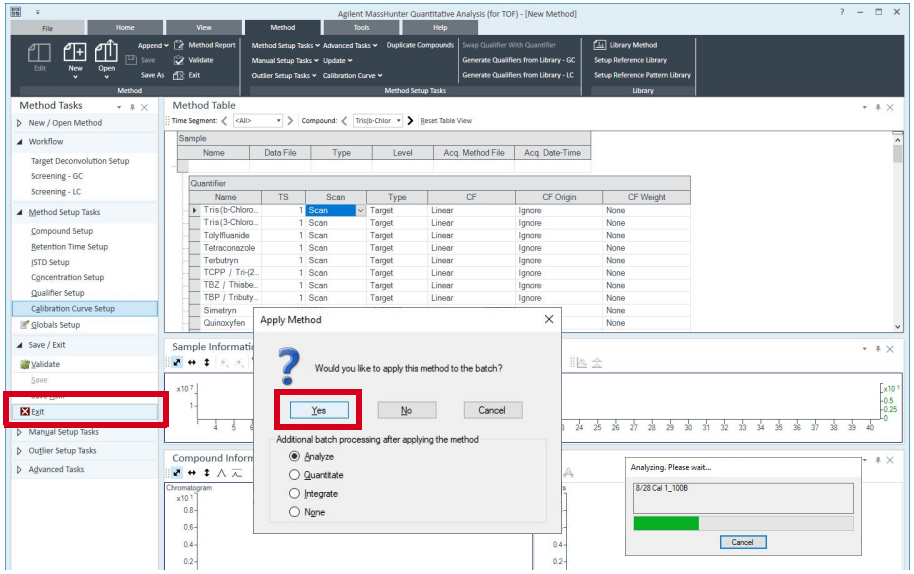


Outliers can be setup in Screener for all compounds for convenience to avoid Fill Down that takes time for large number of compounds.

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

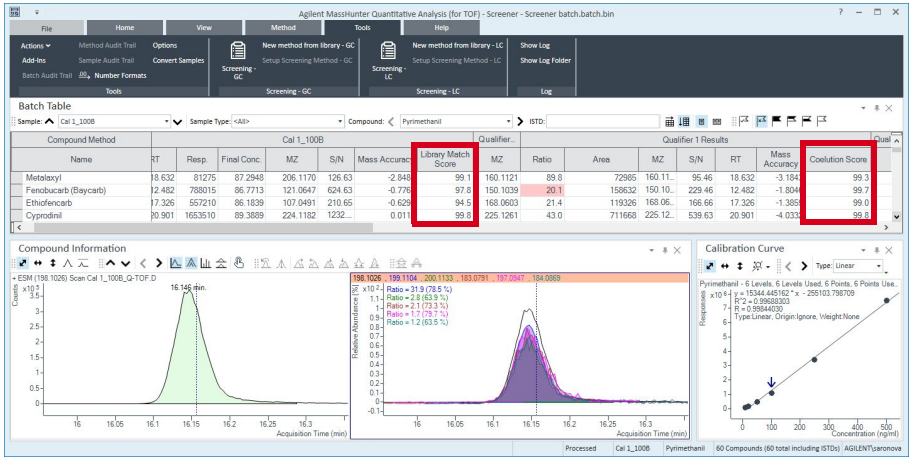
These Outliers parameters are recommended for screening for pesticides and environmental contaminants. Other applications may have different settings

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

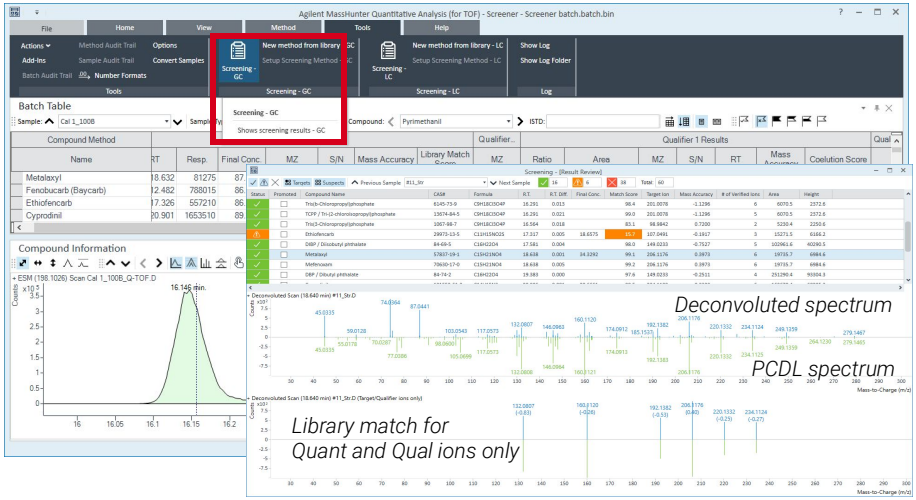


12 Wait for analysis to complete.

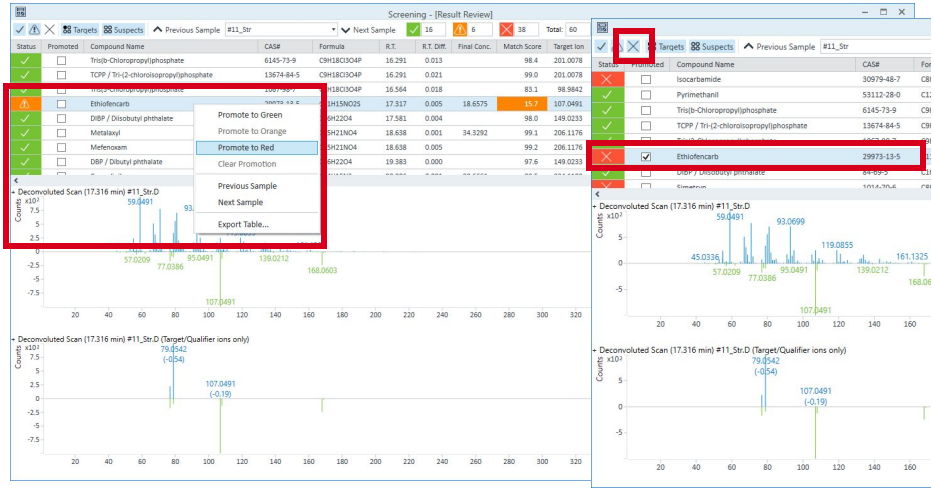
13 View **Target Quantitation Results Table**. You can view detailed results for each compound, including parameters related to the Screener such as Library Match Score and Coelution Score.



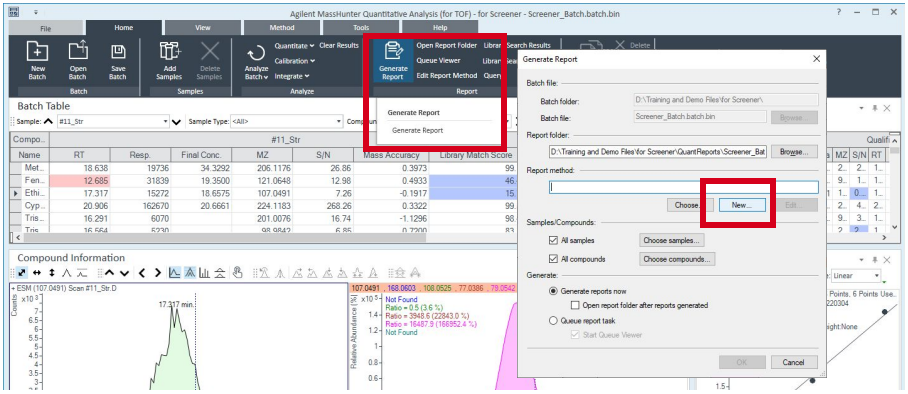
14 Click **Screening-GC** to view suspect screening results. The screening window is focused on the compounds identified in samples and only key parameters are displayed.



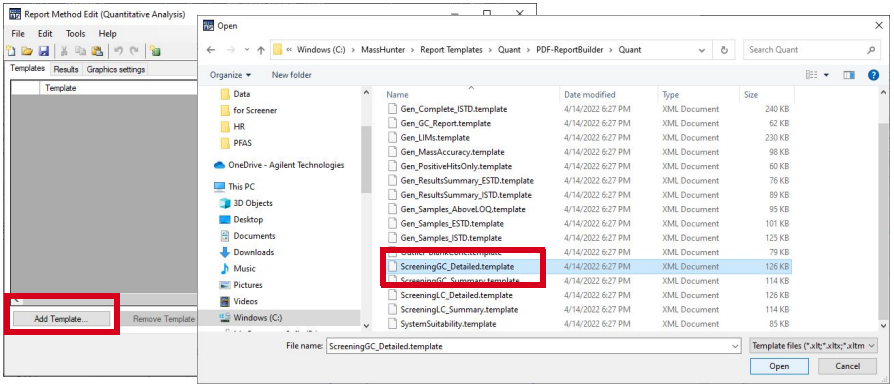
Orange color-labeled compounds are for user's review and can be moved to either "green" (confirmed hits) or "red" (rejected).



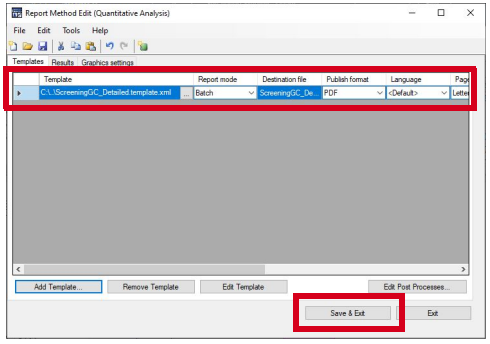
15 To create reports, select **Generate Report** and click **New** under Report method.



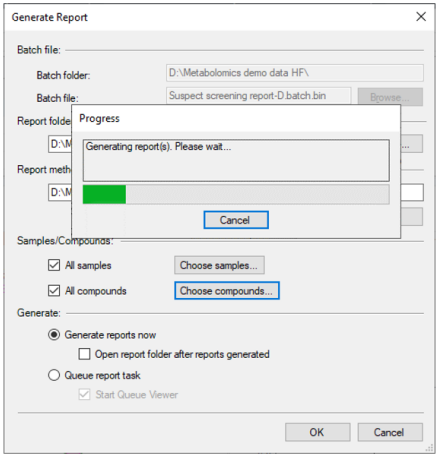
16 Click **Add Template** to select a report template.



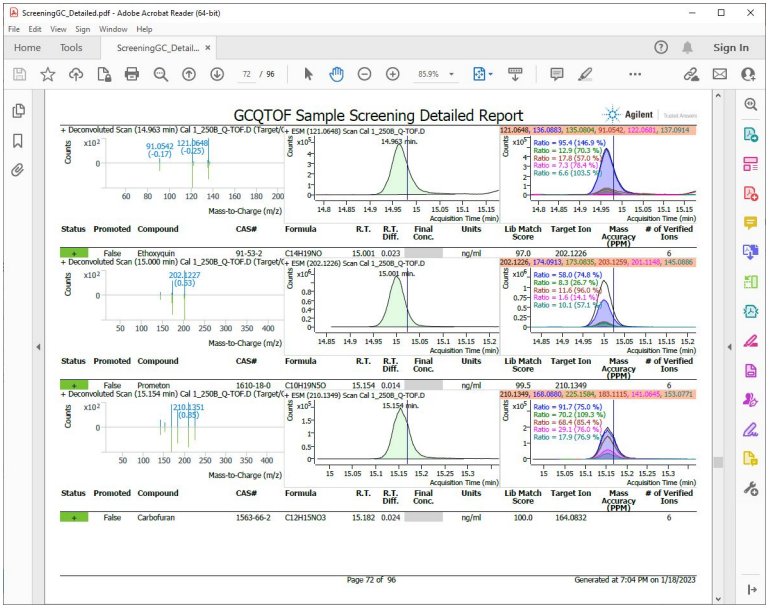
17 Select a template, then click **Save & Exit**.



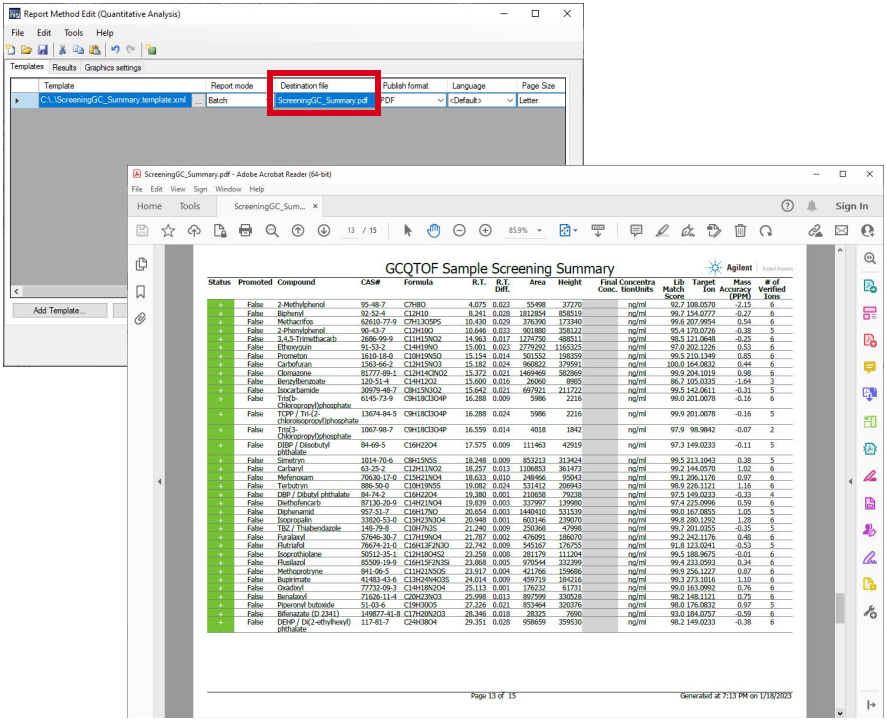
18 Click **OK** to generate the report.



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

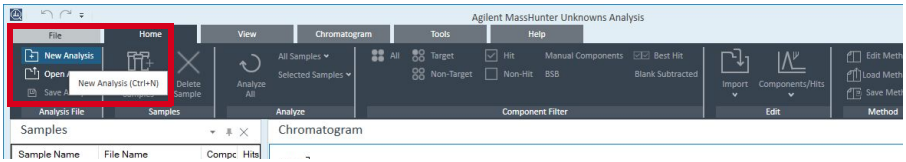


20 You can also generate and view a summary report.

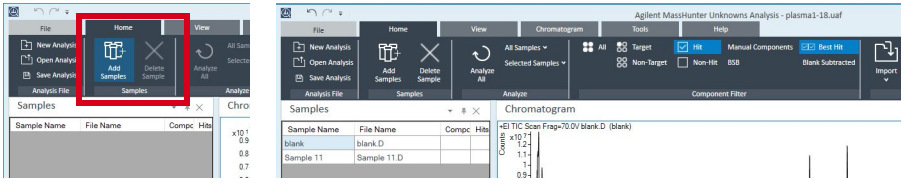


Non-targeted Screening in the Unknowns Analysis Software

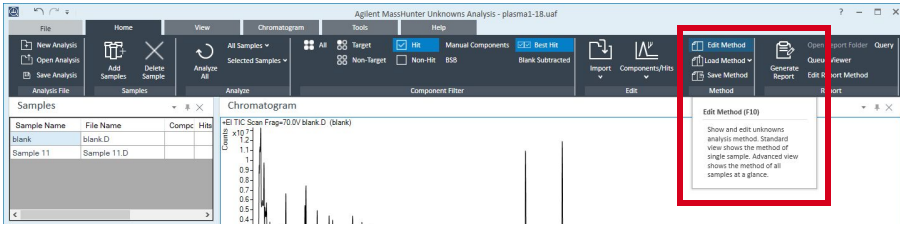
1 Click **New Analysis** (Ctrl+N) to create a new **batch**.



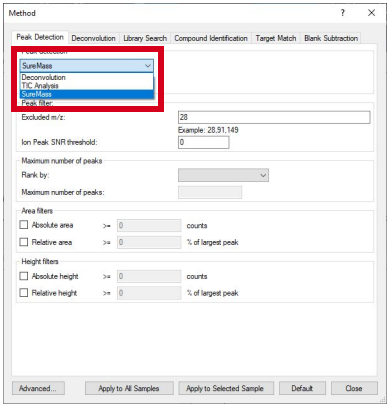
2 Click **Add Samples**.



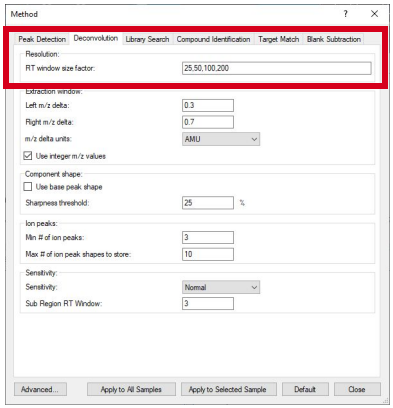
3 Click **Edit Method**.



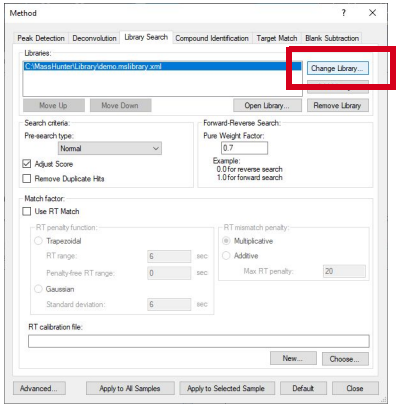
4 Under the **Peak Detection** tab, click **SureMass**.

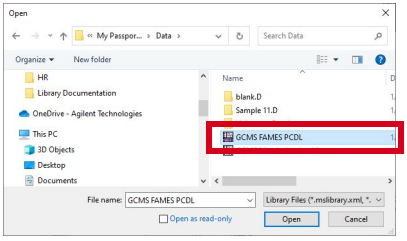


5 Under the **Deconvolution** tab, set the RT window size factor.

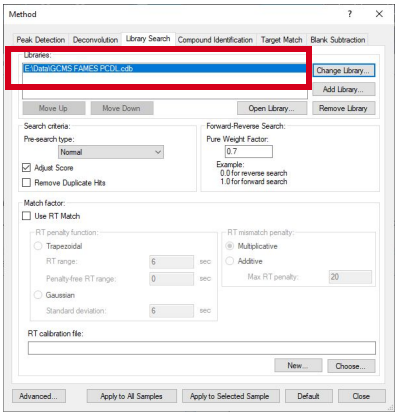


6 Under the **Library Search** tab, click **Change Library** to choose a **PCDL library**.

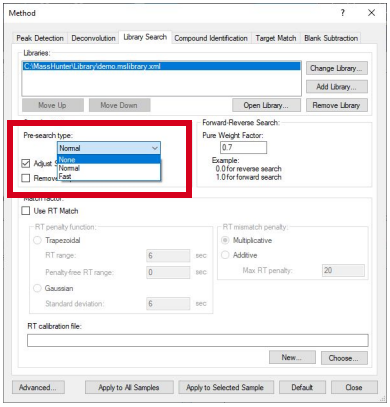




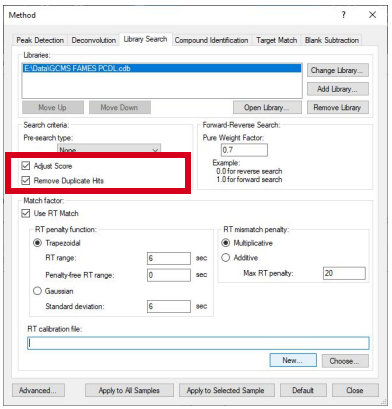
7 Choose a **Library**.



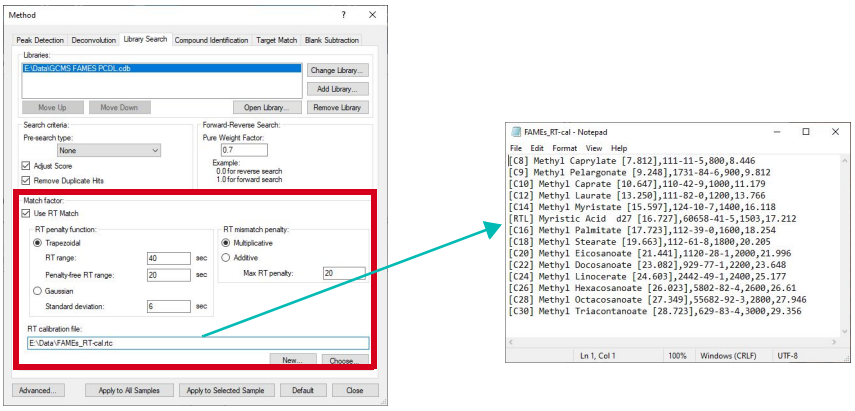
8 Use the **Pre-search type** pulldown to choose how fast and accurate you want the search.



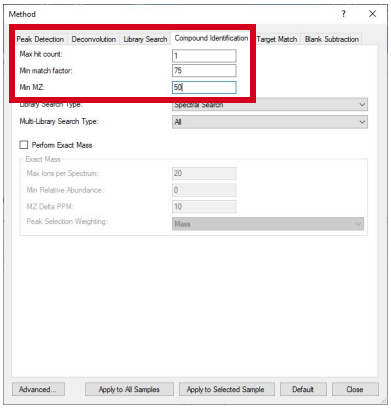
9 Check **Adjust Score** to make it similar to NIST scoring, and remove duplicates if you wish.



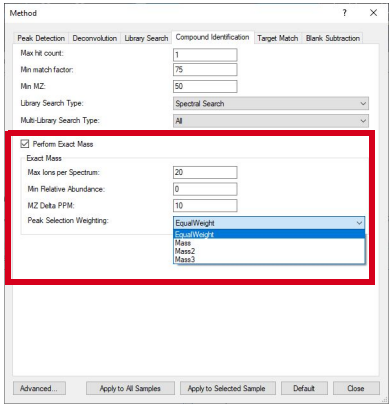
10 Check **Use RT Match** if you want the RT calibration file to match library's RTs. With the calibration file, it will match RIs.



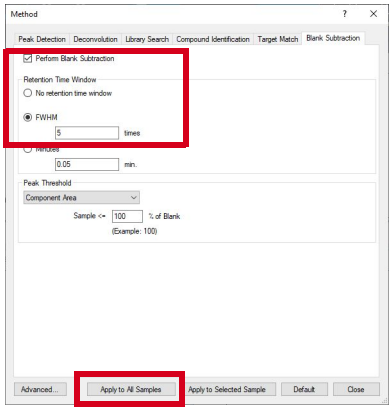
11 Under the **Component Identification** tab, set **Min m/z** so that it is no lower than the data acquisition mass range. Also select minimum **Library Match Factor** and **number of hits** per component.



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

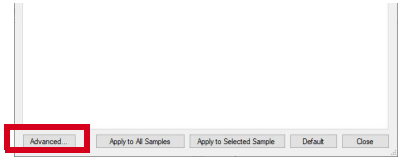


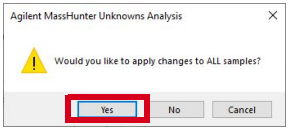
13 Under the **Blank Subtraction** tab, check **Perform Blank subtraction**.



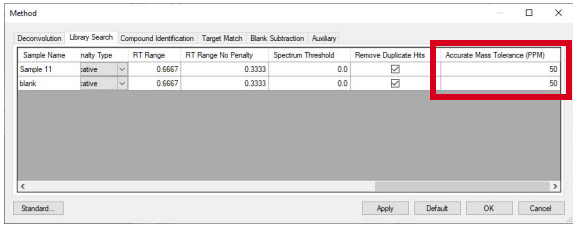
14 Click **Apply to All Samples**.

15 Click **Advanced** then click **Yes**.

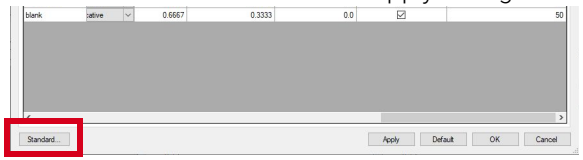


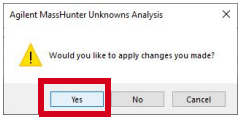


16 In **Accurate Mass Tolerance (PPM)**, increase the values so very small ions in the deconvoluted spectra do not get discarded due to increased mass accuracy error.

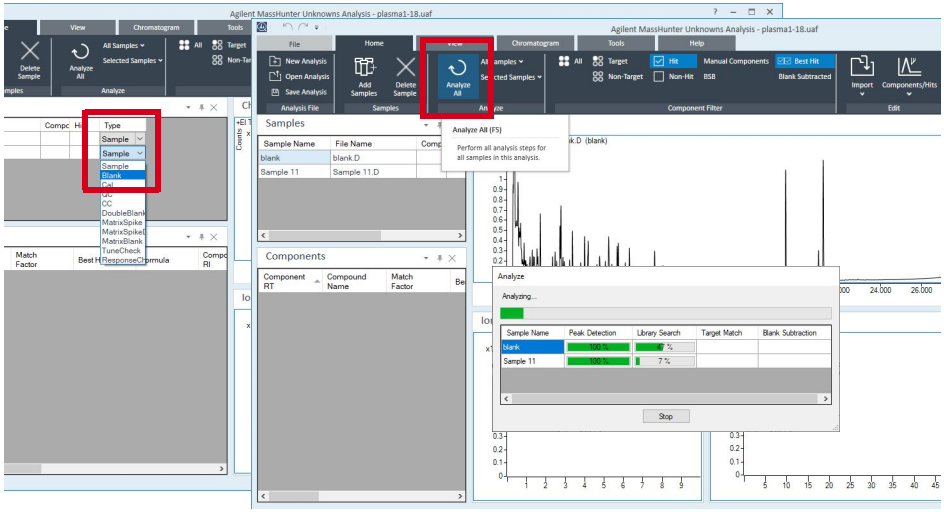


17 Click **Standard** then click **Yes** to apply changes.

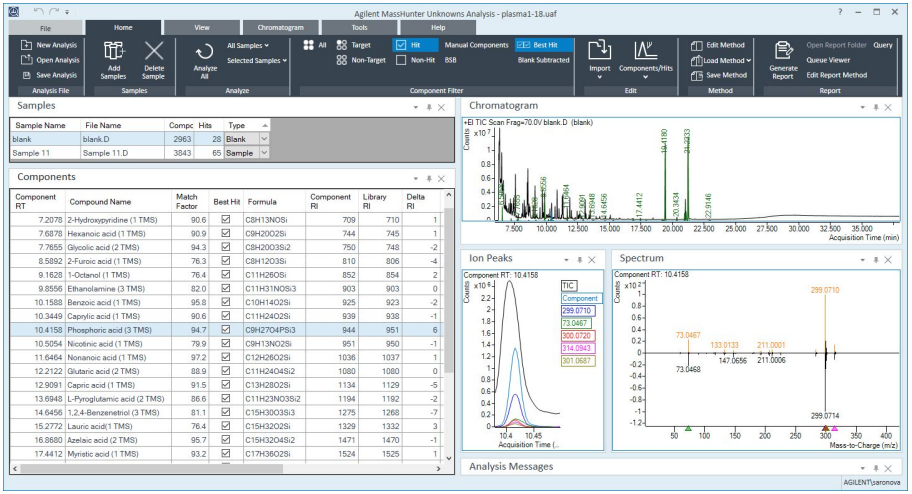




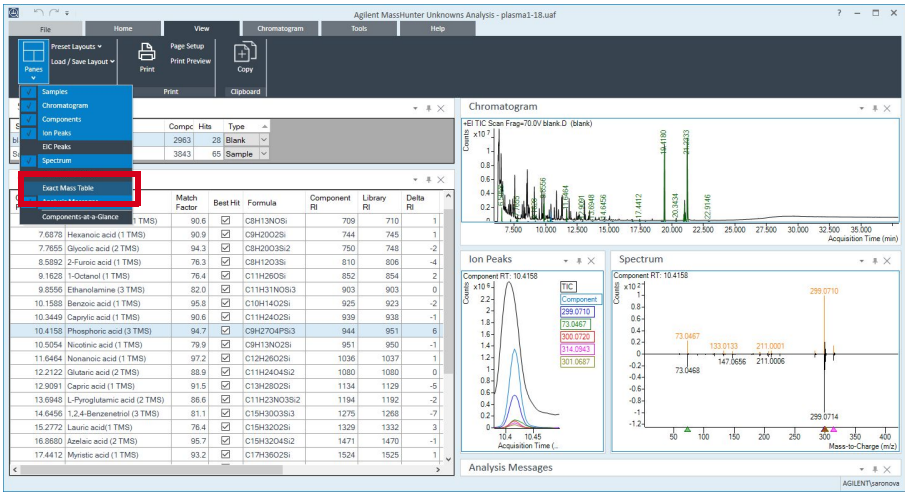
18 Select **Blank** sample type for subtraction, then click **Analyze All** to analyze the batch. An **Analyze** window will appear with a progress bar.



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.

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Index

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