



# **Agilent MassHunter Workstation Software Unknowns Analysis**

## **Familiarization Guide**



**Agilent Technologies**

# Notices

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## Manual Part Number

G3335-90109

## Edition

Second edition, February 2014

Printed in USA

Agilent Technologies, Inc.  
5301 Stevens Creek Boulevard  
Santa Clara, CA 95051 USA

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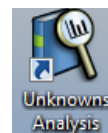
This guide presents step-by-step exercises to help you learn to use the Unknowns Analysis program. You can do these exercises with the demonstration analysis, method, and library files shipped with the system installation disk, or with data you acquire.

## Before You Begin These Exercises

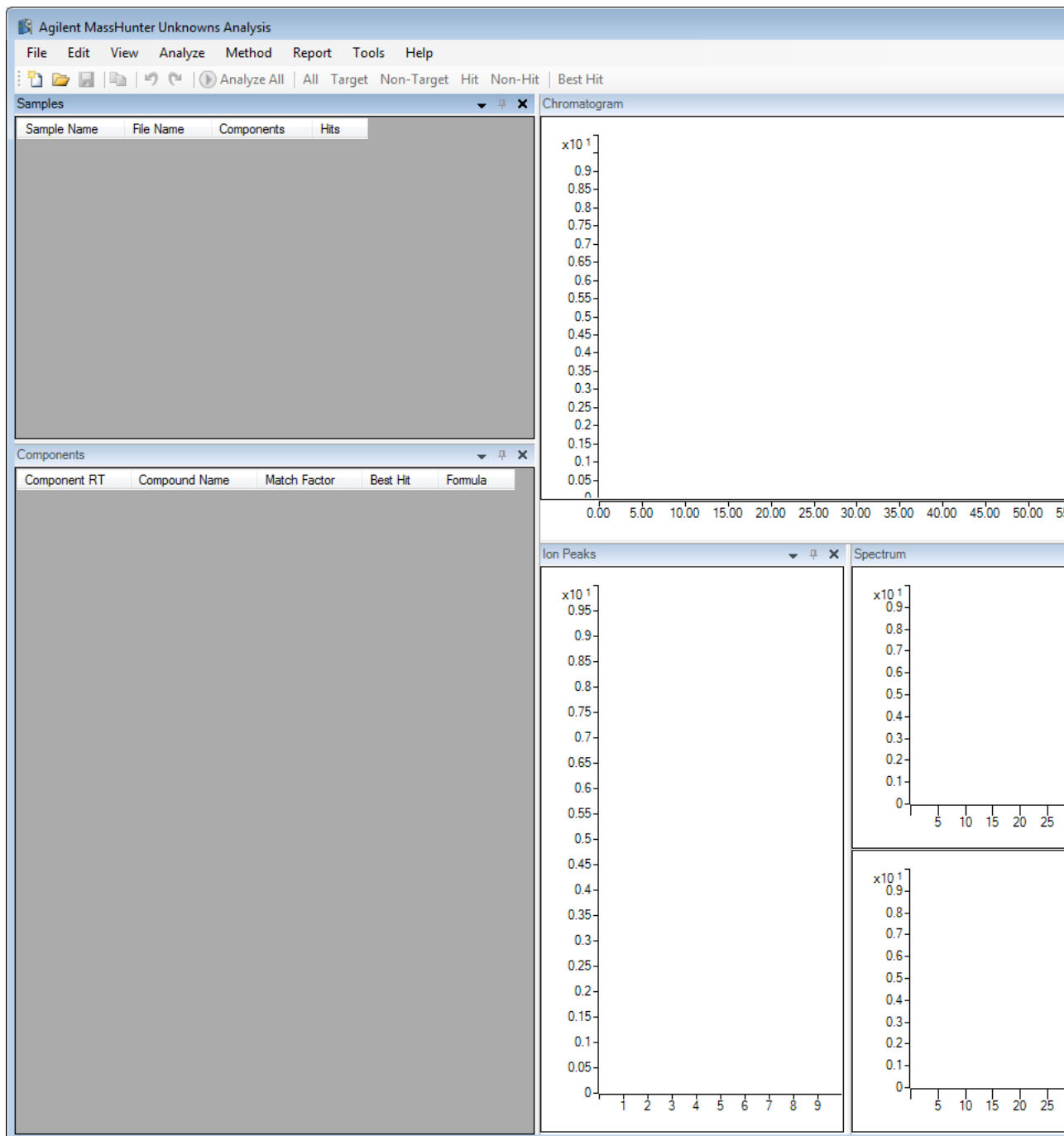
### Copy files from the installation disk to your hard disk

- 1 Insert the MassHunter Quantitative Analysis installation DVD into your computer.
- 2 Navigate to your DVD drive:\Data.
- 3 If the folder is in a compressed format, extract the data files from their zip format.
- 4 Copy the **Data** folder from your installation disk in uncompressed format to any location on your hard disk. This folder contains all of the data, method, and library files needed for these exercises. Do not reuse the example data files on your system unless you know that they are identical to the originals on the disk. If the example data files already on the system do not match the original ones on the disk exactly, then the results obtained during these exercises will not match those shown in this guide.

## Start the unknowns analysis software

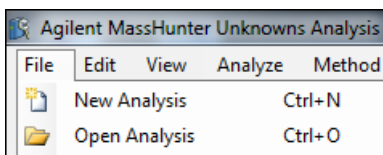


- 1 Select the **Unknowns Analysis** desktop icon, . Your start screen is blank as shown below.

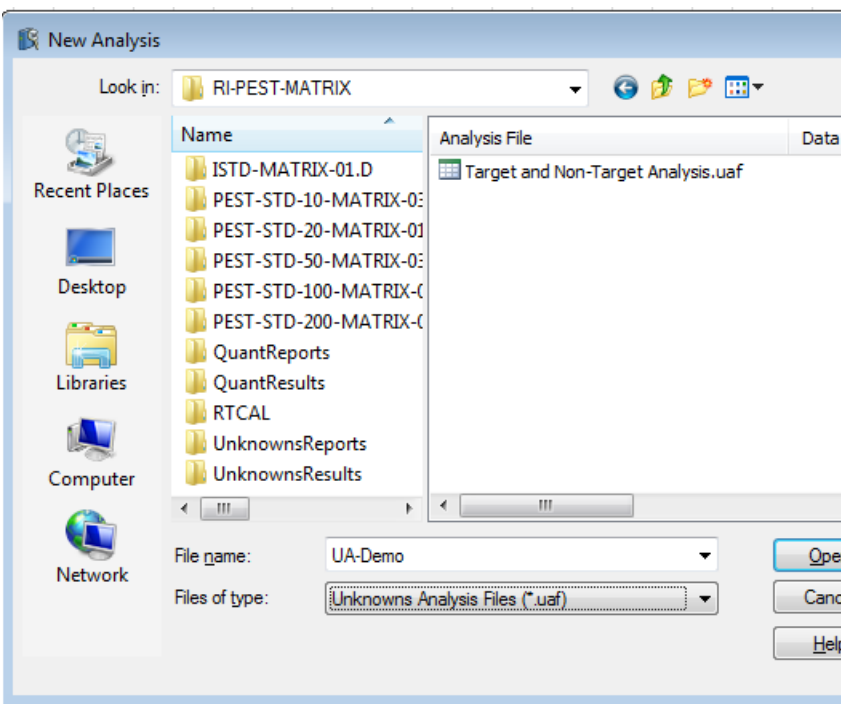


## Create a New Analysis

- 1 From the menu bar, select **File > New Analysis**.



- 2 In the **New Analysis** dialog box, navigate to the folder where you want to save the analysis. (**RI-PEST-MATRIX**)

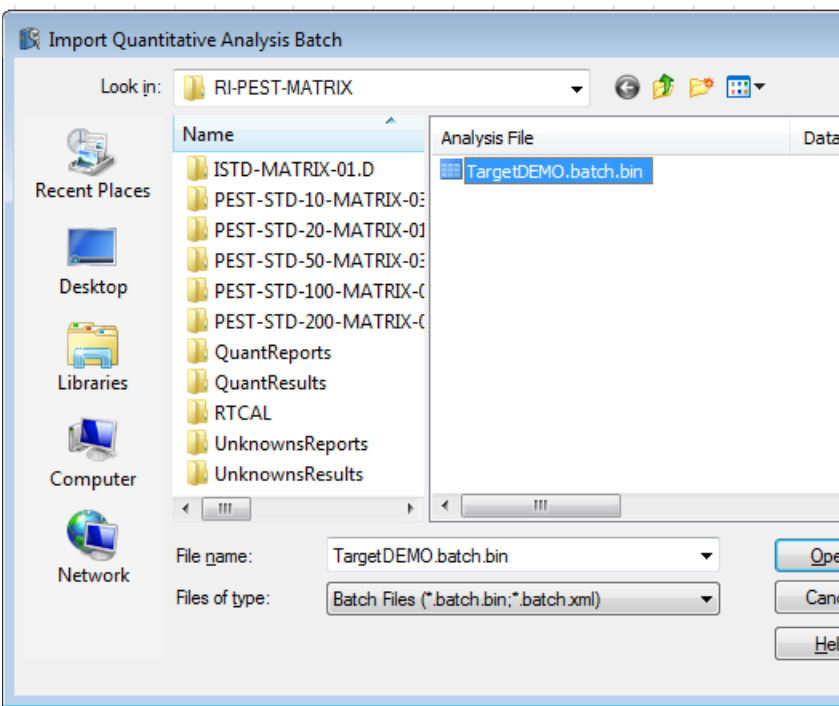


- 3 In the **File name** field, enter a name for your analysis. For this exercise use the file name, **UA-Demo.uaf**.
- 4 Select **Open**. The new analysis is created and opens with the name in the title bar.



## Import Data from a MassHunter Quantitative Analysis

- 1 From the menu bar, select **File > Import Quantitative Analysis**. The **Import Quantitative Analysis Batch** dialog box opens.
- 2 Navigate to **MassHunter/Data/R-PEST-MATRIX** and select **TargetDEMO.batch.bin**.



- 3 Select **Open**. The dialog box closes and the samples are added to the **Samples** table.

The screenshot displays the Agilent MassHunter Unknowns Analysis interface. The main window is titled "Agilent MassHunter Unknowns Analysis - UA-Demo.uaf". The menu bar includes File, Edit, View, Analyze, Method, Report, Tools, and Help. The toolbar contains icons for file operations and analysis controls, including "Analyze All", "All", "Target", "Non-Target", "Hit", "Non-Hit", and "Best Hit".

The **Samples** table is visible, listing the following entries:

Sample Name	File Name	Componen
ISTD+solvent +MATRIX	ISTD-MATRIX-01.D	
PEST STD - 10+MATRIX	PEST-STD-10-MATRIX-03.D	
PEST STD - 20+MATRIX	PEST-STD-20-MATRIX-01.D	
PEST STD - 50+MATRIX	PEST-STD-50-MATRIX-03.D	
PEST STD - 100+MATRIX	PEST-STD-100-MATRIX-01.D	
PEST STD - 200+MATRIX	PEST-STD-200-MATRIX-03.D	

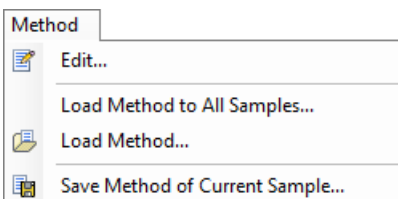
The **Chromatogram** panel shows the plot for "ISTD+solvent +MATRIX (ISTD-MATRIX-01.D)". The y-axis is labeled "Counts" and ranges from 0 to 4.5 (multiplied by  $10^7$ ). The x-axis represents retention time from 6.00 to 14.00 minutes. The plot shows a noisy baseline with several distinct peaks, notably around 11.5 and 13.0 minutes.

The **Components** table is currently empty, with columns for Component RT, Compound Name, Match Factor, and Best Hit.

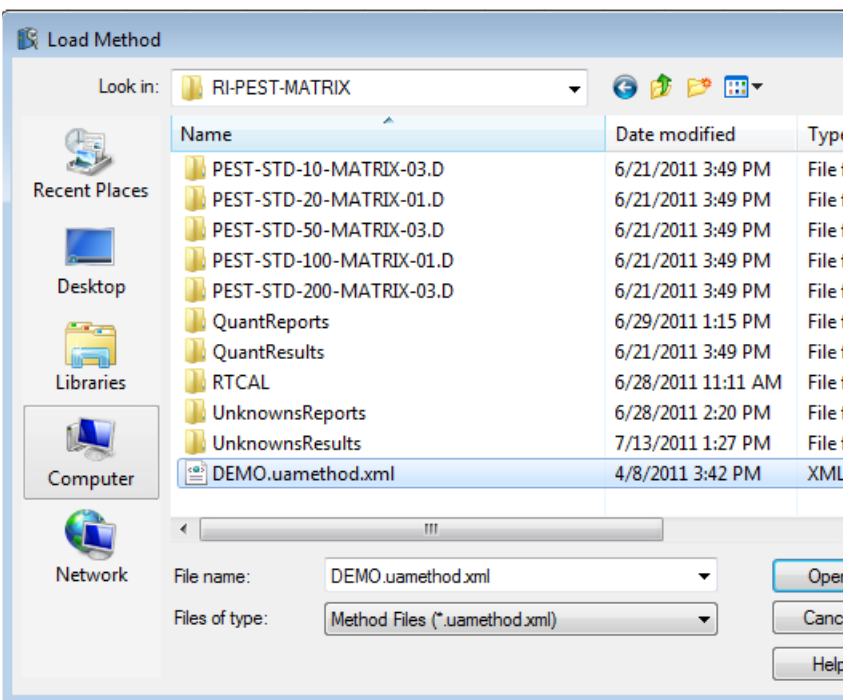
The **Ion Peaks** panel shows a plot with a y-axis ranging from 0.45 to 0.95 (multiplied by  $10^1$ ). This panel is currently empty, showing only the y-axis scale.

## Load the Method

- 1 From the menu bar, select **Method > Load Method to All Samples**.



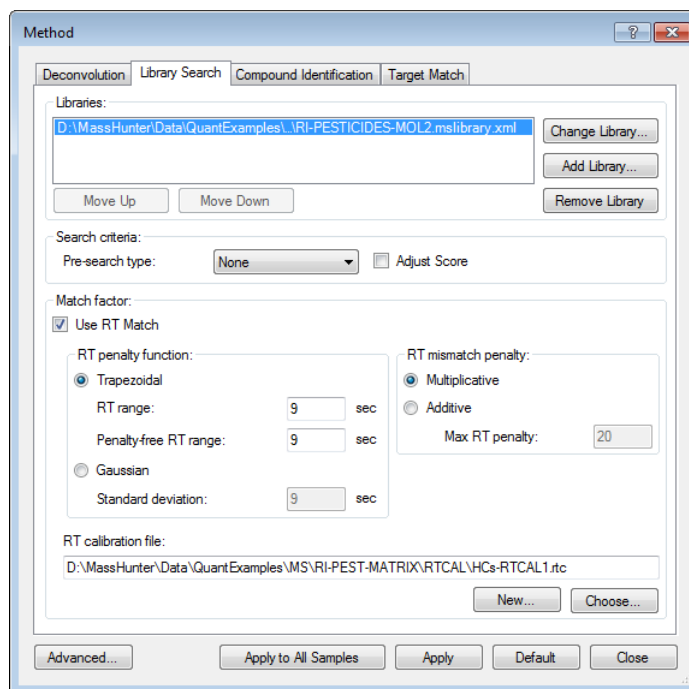
- 2 In the **Load Method** dialog box, navigate to **MassHunter/Data/RI-PEST-MATRIX** and select **DEMO.uamethod.xml**.



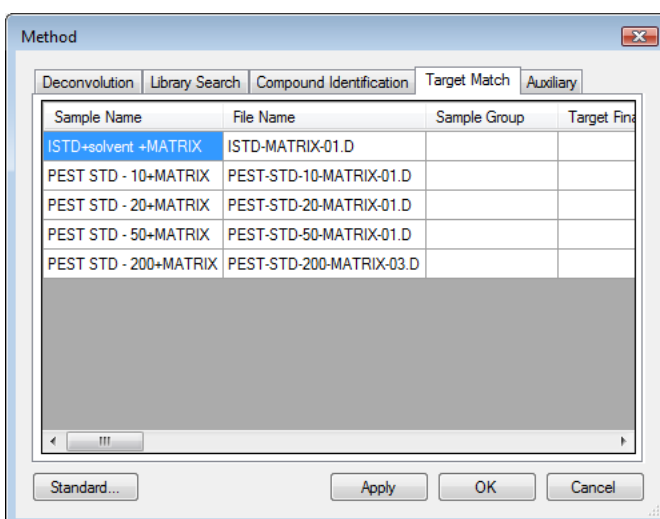
- 3 Select **Open**. The dialog box closes and the method is loaded to all the samples in your analysis.

## Select a Library to Use for the Unknowns Analysis

- From the menu bar, select **Method > Edit**. The **Method** dialog box opens in the view from which it was last closed. For this exercise, we will use the Standard view shown in [Figure 1](#) If the Advanced dialog box opened as shown in [Figure 2](#), select **Standard** to switch to the Standard view.



**Figure 1** The **Method** dialog box, standard view

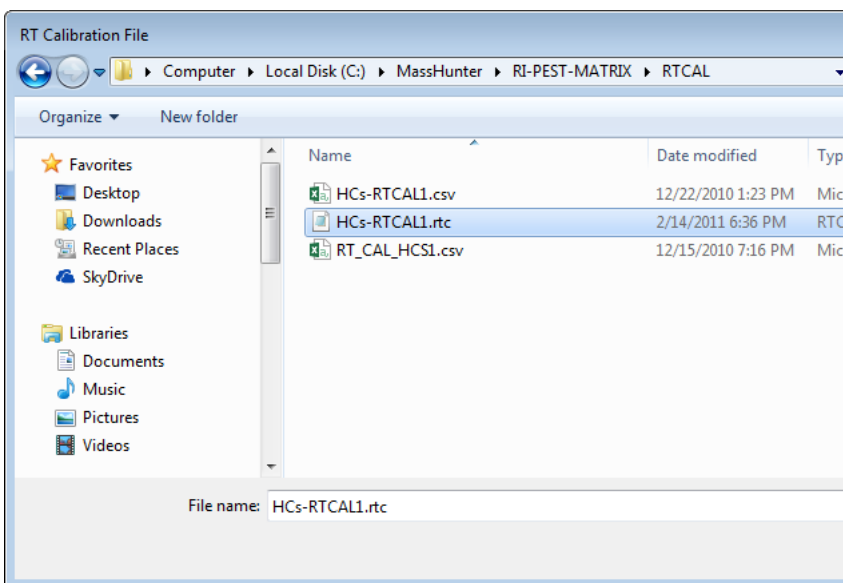


**Figure 2** The **Method** dialog box, advanced view

- 2 Select the **Library Search** tab.
- 3 In the **Libraries** area, select **Change Library**.
- 4 In the **Open** dialog box, navigate to your **Data** folder and select **RI-PESTICIDES\_MOL2.mslibrary.xml**.

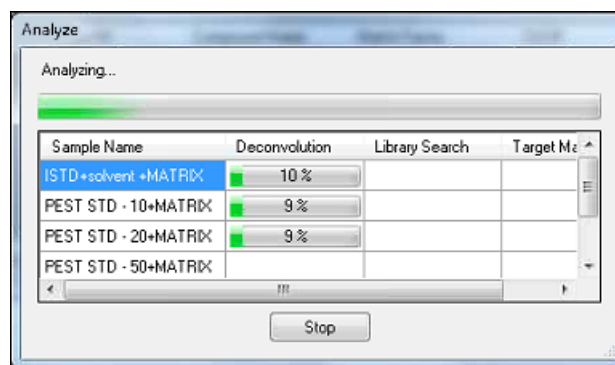
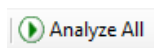
The Data folder was copied to your hard disk. See [step 4 on page 6](#).

- 5 Select **Open**. The dialog box closes and the library is added to the **Libraries** list in the **Method** dialog box. (see [Figure 1 on page 12](#)).
- 6 In the **RT Calibration File** area, select **Choose**.
- 7 In the **RT Calibration File** dialog box, navigate to your **DATA** folder and select **HCS-RTCal1.rtc**.
- 8 In the **Method** dialog box, select **Apply to All Samples** and then **Close**.



## Analyze and Review Results

- 1 From the menu bar, select **Analyze > Analyze All** or



- 2 From the menu bar, select **View > Preset Layout > All-Extended**.
- 3 Select a sample from the **Sample** table.
- 4 Select one of the following toolbar buttons:
  - **All** - Select to view all the peaks
  - **Target** - Select to view the peaks that are also in the quantitation batch
  - **Non Target** - Select to view the peaks that are not in quantitation batch
  - **Hit** - Select to view the peaks that are also in the library
  - **Non-Hit** - Select to view the peaks that are not in the library
  - **Best Hit**- Select to view the best match.
- 5 From the **Components** table, select a component from the **Component RT** column.
- 6 View the **Spectrum**, **Ion Peaks**, and **Molecular Structure** for the selected component.

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

The **Molecular Structure** is from the library.

The screenshot shows the Agilent MassHunter Unknowns Analysis - UA-Demo.uaf interface. Annotations point to various parts of the software:

- Toolbar buttons:** Located at the top of the interface, including File, Edit, View, Analyze, Method, Report, Tools, and Help.
- Sample:** A table listing sample names, file names, components, and hits.
- Components:** A table listing component retention times, compound names, match factors, best hit status, and formulas.
- Chromatogram:** A Total Ion Chromatogram (TIC) showing peaks at various retention times.
- Spectrum:** Mass spectra for the component at 9.1660 min, showing peaks at m/z 79.1, 151.1, and 44.0.
- Ion peaks:** A plot showing ion peaks for the component at 9.1660 min.
- EIC Peaks:** A plot showing extracted ion chromatogram (EIC) peaks for the component at 9.1660 min.
- Molecular structure:** The chemical structure of Tetrahydrophthalimide, cis-1,2,3,6- is displayed.

Sample Name	File Name	Components	Hits
ISTD+solvent+MATRIX	ISTD-MATRIX-01.D	214	11
PEST STD - 10+MATRIX	PEST-STD-10-MATRIX-03.D	241	32
PEST STD - 20+MATRIX	PEST-STD-20-MATRIX-01.D	255	42
PEST STD - 50+MATRIX	PEST-STD-50-MATRIX-03.D	247	51
PEST STD - 100+MATRIX	PEST-STD-100-MATRIX-01.D	267	54
PEST STD - 200+MATRIX	PEST-STD-200-MATRIX-03.D	258	55

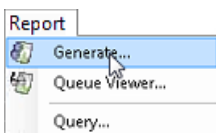
  

Component RT	Compound Name	Match Factor	Best Hit	Formula
6.3253	4-Isopropylaniline	65.1	<input checked="" type="checkbox"/>	C9H13N
7.2298	Promecarb artifact [...]	71.3	<input checked="" type="checkbox"/>	C10H14O
9.1660	Tetrahydrophthalimi...	93.7	<input checked="" type="checkbox"/>	C8H9NO2
9.5343	Cashmeran	63.7	<input checked="" type="checkbox"/>	C14H22O
10.9941	Diethyl phthalate	93.9	<input checked="" type="checkbox"/>	C12H14O4
12.8055	4,4'-Dibromooctaflu...	87.0	<input checked="" type="checkbox"/>	C12Br2F8
15.2633	Pyrimethanil	72.9	<input checked="" type="checkbox"/>	C12H13N3
21.6332	Cyprodinil	64.3	<input checked="" type="checkbox"/>	C14H15N3
28.2686	Triphenyl phosphate	95.2	<input checked="" type="checkbox"/>	C18H15O4P
30.0750	Bis(2-ethylhexyl)phth...	93.6	<input checked="" type="checkbox"/>	C24H38O4
33.5490	Decachlorobiphenyl	82.5	<input checked="" type="checkbox"/>	C12Cl10

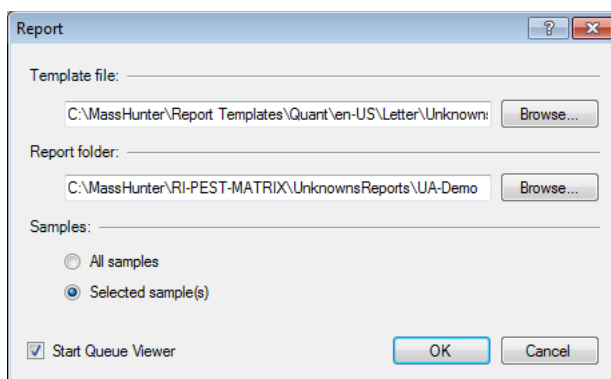
7 To save the analysis, select **File > Save Analysis**.

## Generate a Report

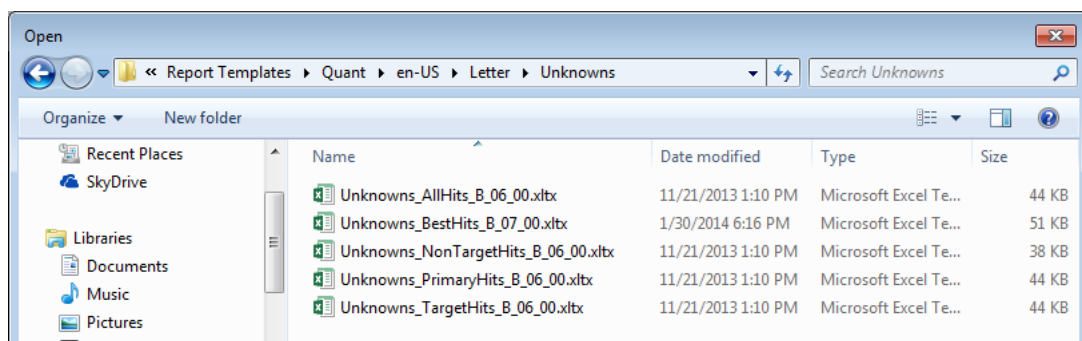
- 1 Select **File > Save Analysis**.
- 2 From the menu bar, select **Report > Generate**.



- 3 In the **Report** dialog box, under **Template file** select **Browse**.



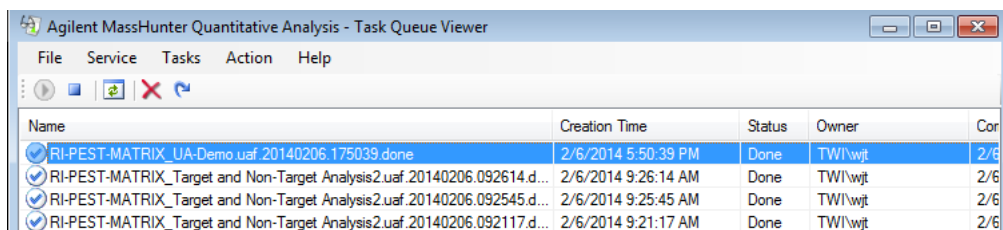
- 4 In the **Open** dialog box, browse to **MassHunter > Report Templates > Quant > en-US > Letter > Unknowns**. For this exercise select **Unknowns\_TargetHits\_B-06\_00.xltx**.



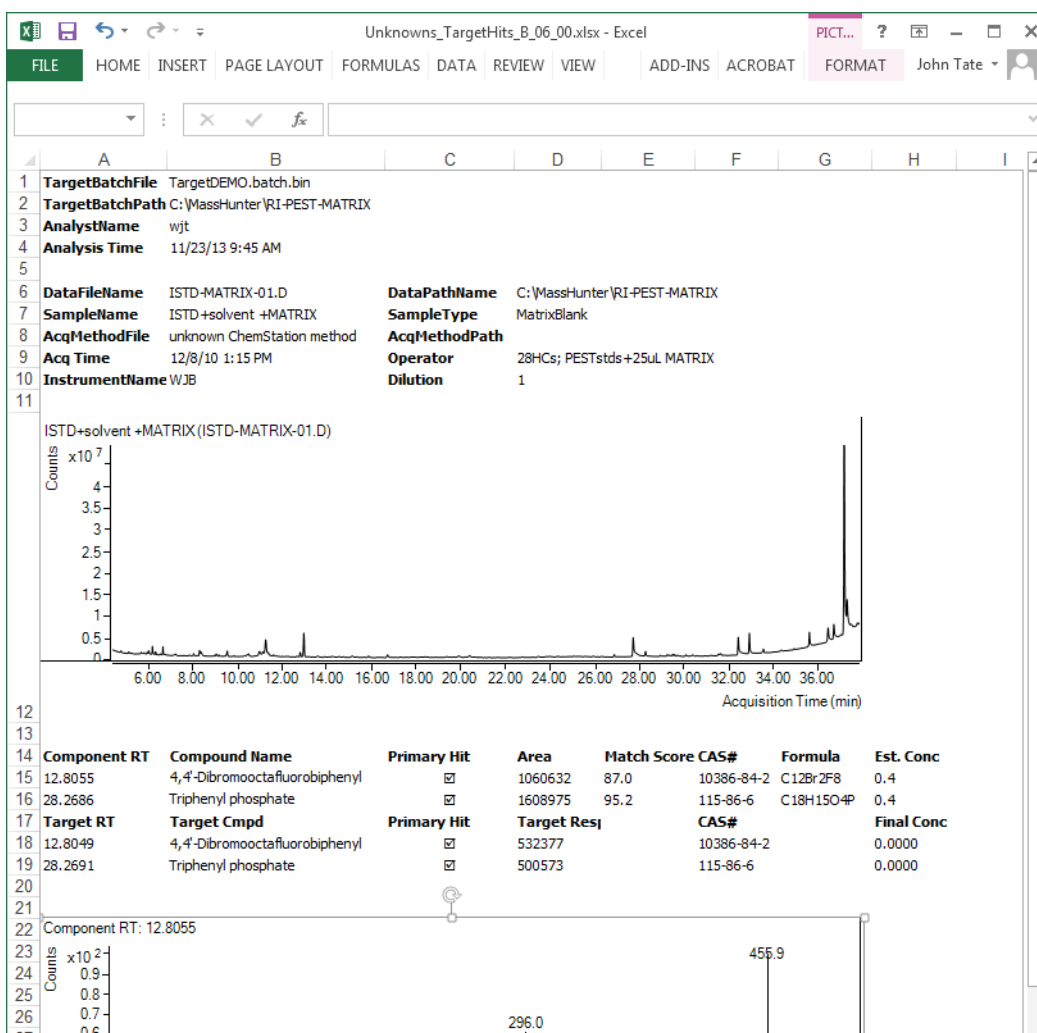
- 5 In the **Report** dialog box, under **Report folder** select **Browse**.
- 6 In the **Open** dialog box, browse to where you want to store your report and select **Open**. The default location is the same place the analysis is stored.
- 7 In the **Report** dialog box, select **OK**.



8 The **Task Queue Viewer** is displayed.



9 When the task is complete, in the **Task Queue** viewer select **Action > Open Unknowns TargetHits\_B\_06\_00.xlsx**. The report opens in Excel. You can use the normal Excel features to print, save, or modify the report.



10 Close the report.

11 To exit the program, select **File > Exit**.

## Unknowns Analysis



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Second edition, July 2014