Agilent

Agilent MassHunter Workstation Software - 7250 Accurate-Mass Quadrupole Time-of-Flight GC/MS

Familiarization Guide

Notices

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Contents

Familiarization Guide

This guide shows how to use the Agilent 7250 Q-TOF GC/MS System to acquire and analyze sample data. If you want to skip the data acquisition steps in this guide, use the demo data files shipped with MassHunter (See the **Reference material** on page 6).

In this guide, you learn how to determine the best acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a method to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Qualitative Analysis program to identify parameter values producing optimum signal response.

Before you Begin

Reference material

Available with the 7250 Q-TOF installation of the MassHunter Software User Information, the following documentation is delivered to you as part of your MassHunter Software.

- *MassHunter Qualitative Analysis Familiarization Guide for GC/MS* for an introduction to many Qualitative Analysis program features that are not covered in this guide including the Qualitative Analysis Navigator program.
- *Qualitative Analysis Training Videos* for those seeking visual and audio lessons presenting a comprehensive use of the MassHunter Qualitative Analysis Navigator and MassHunter Qualitative Workflows.
- *Online Help* for detailed information on how Qualitative Analysis works.
- *Demo data files and accurate mass library* that allow you to perform all the analysis steps demonstrated here using your own installation of Qualitative Analysis without acquiring compound data or owning a library license.
- *Quick Start Guide* explains what documentation is included in the applications and what information is in each document.

Available on the 7250 Q-TOF installation of the User Manuals and Tools DVD, the following documentation is delivered to you with your 7250 Q-TOF instrument.

- *Concepts Guide* to learn more about how the 7250 Q-TOF GC/MS System works.
- *Quick Start Guide* explains what documentation is included in the applications and what information is in each document.
- *Hardware manuals* to learn how to operate and perform maintenance on the 7250 Q-TOF.

Prepare your system

1 Check that:

- MassHunter Acquisition, MassHunter Qualitative Analysis, and MassHunter Quantitative Analysis are installed.
- Your system uses an Agilent 8890 or 7890 Series GC with a split/splitless or MultiMode (MMI) inlet and automatic liquid sampler.
- The acquisition uses a 10 uL ALS syringe tapered, fixed, with a 23-26s needle. A suitable syringe may be substituted.
- The 7250 Q-TOF GC/MS System is configured, and has a valid tune.
- The performance is verified.
- The system is turned on.
- A suitable column is installed. The J&W model 122-3832 DB-35MS: 30 m x 250 µm, 0.25 µm column is used for the examples in this guide.

2 Configure the GC for the installed column.

3 If needed, copy the *Demo data files and accurate mass library* noted in **Reference material** on page 6 to any location on your hard disk. The data file and accurate mass library file are needed for this exercise if you are not acquiring data and do not have an accurate mass library of the compounds shown in **Table 1** on page 8.

Prepare the samples required for data acquisition

If you do not intend to acquire data but want to learn how to use the Qualitative Analysis program, you can skip the sample preparation and actual acquisition and use the data file shipped with this guide. It is recommended that you read the **Exercise – Develop an acquisition method for the 7250** on page 9 to understand settings unique to the Agilent instrument.

Materials required for sample preparation:

- Sample (p/n 05970-60045 or p/n 5074-3025 Japan only)
- Isooctane for sample dilution
- Sample vials

The sample compounds are in an isooctane solvent contained in 1 mL ampules of 10 ng/µL, 100 ng/µL, and 100 pg/µL concentrations and are shown in **Table 1**.

Table 1 Sample Compound list

Compound	*m/z*	Formula
Dodecane	170.2029	$C_{12}H_{26}$
Biphenyl	154.0777	$C_{12}H_{10}$
4-Chlorobiphenyl (p/n 05970-60045 only)	188.0387	$C_{12}H_9Cl$
Methyl palmitate	270.2553	$C_{17}H_{34}O_2$

Prepare the Qualitative Analysis sample by emptying the contents of the 10 ng/µL ampoule into an ALS sample vial and cap the vial.

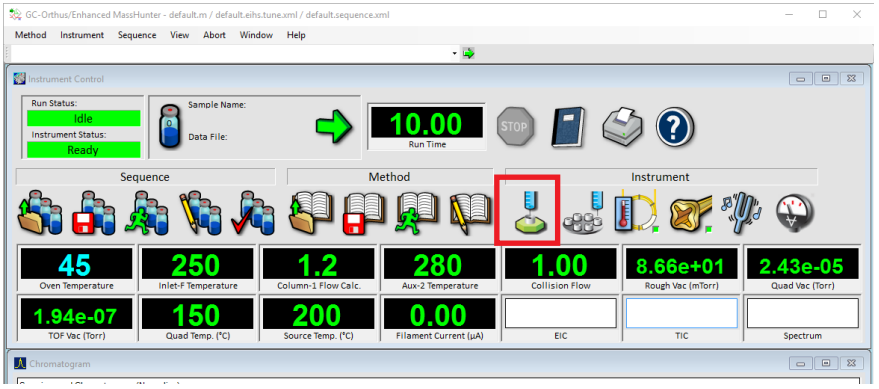
Fill an ALS wash vial with isooctane.

Exercise – Develop an acquisition method for the 7250

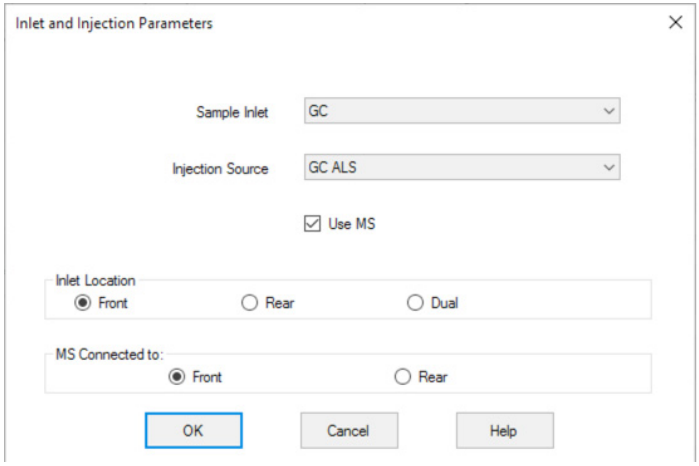
Task 1. Set the inlet and injection parameters

1 Double-click the **Data Acquisition** icon on the windows desktop.

2 Click the **Inlet and Injection Parameters** icon. Hover over an icon to display a tag identifying the icon.



The **Inlet and Injection Parameters** dialog box is displayed.



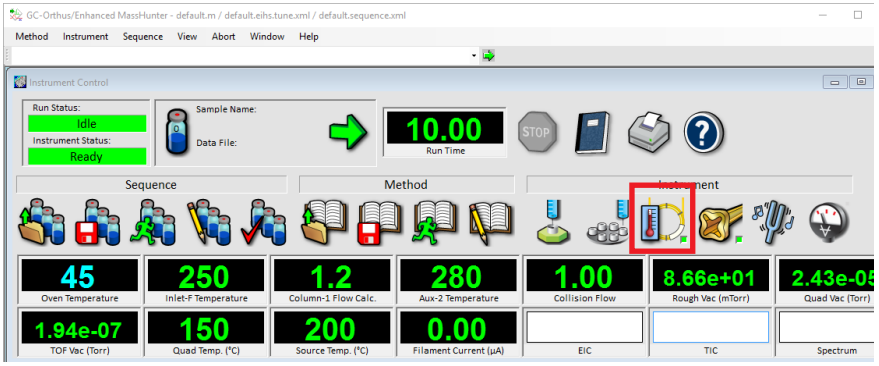
3 Select **GC** for the sample inlet and the installed ALS for the injection source.

4 Check the **Use MS** box.

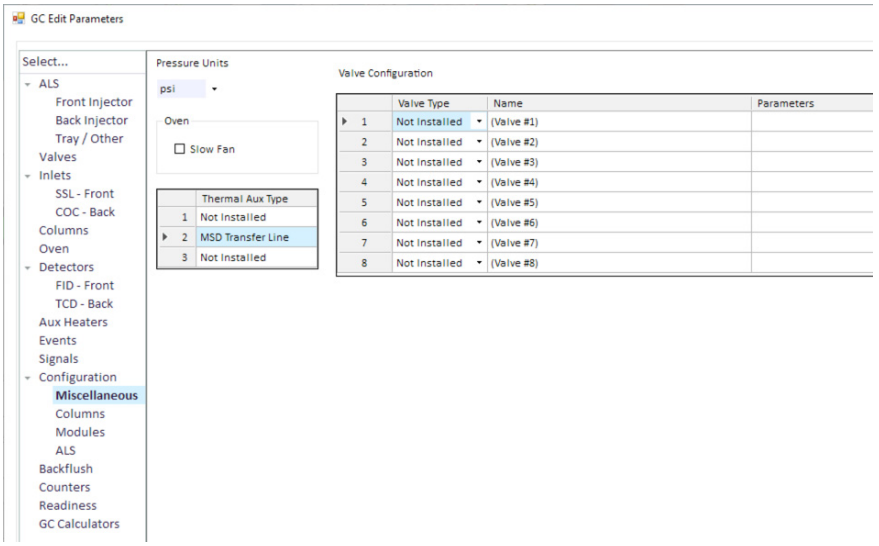
Task 2. Check the GC Configuration

In this exercise, you review the GC hardware setup for the analysis.

1 Click the **GC Edit Parameters** icon.



The **GC edit parameters** window is displayed.



2 In the navigation menu, select **Configuration > Miscellaneous**.

3 Set the **Pressure Units** to psi.

4 In the **Oven** area, the **Slow Fan** mode is unchecked.

5 In the navigation menu, select **Configuration > Columns**, and set **Column 1** to a J&W 122-3832 column or one that is similar. Set the **Inlet** to **Front (or Rear) Inlet** and the **Outlet** to **MSD**. **Heated By** is set to **Oven**.

When using a different column, you must adjust your GC parameter settings accordingly for acceptable chromatography.

6 In the navigation menu, select **Configuration > Modules**, and set the **SS inlet** gas to **He** and the **Collision Cell EPC** gas to **N2**.

7 In the navigation menu, select **Configuration > ALS**, and set the **Syringe Size** to 10 uL ALS syringe tapered, fixed, with 23-26s needle, and the **Solvent Wash Mode** to A, B.

A suitable syringe may be substituted.

8 Click **OK**.

The GC parameters are downloaded to the GC and the window closes.

Task 3. Optimize Base Ion Abundance and Perform a Mass Calibration

In this exercise you optimize the abundance of the base ion and perform a mass calibration. A mass calibration is completed in less than two minutes, and it is good practice to calibrate the instrument daily or even every couple of hours. A sequence table keyword allows automatic mass calibration between samples in a sequence. See the online Help for more information.

Step 1 Set the *m/z* range of acquired data and the range of that data to store for analysis.

1 In MassHunter Instrument Control view click the **MS tune** icon. The **GC/Q-TOF Tune** window is displayed.

2 Click the **Manual Tune** tab, then click the **Acquisition** tab.

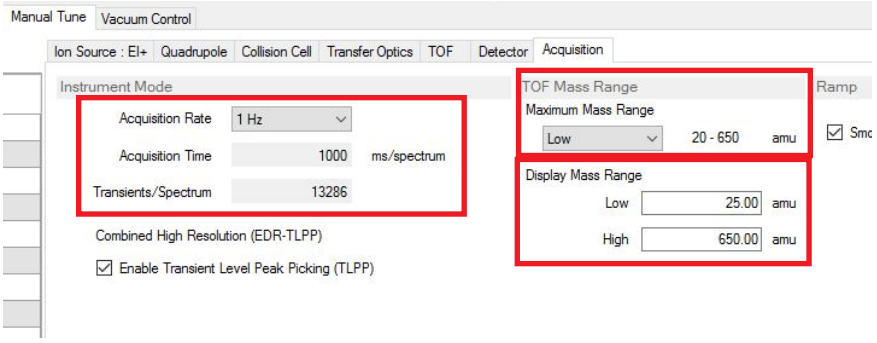
3 Select **1 Hz** from the **Acquisition Rate** dropdown. This is the rate used during calibration.

4 Select **Low** from the **Maximum Mass Range** dropdown.

Data will be scanned from 20 to 650 *m/z*. There are two other mass ranges available for scanning data. The **Standard** range for 20 to 1200 *m/z* and the **Extended** range for 20 to 3000 *m/z*. Here we select the **Low** range to get the highest sensitivity for our data.

5 Enter 25 for the **Low** end of the range and 650 for the **High**.

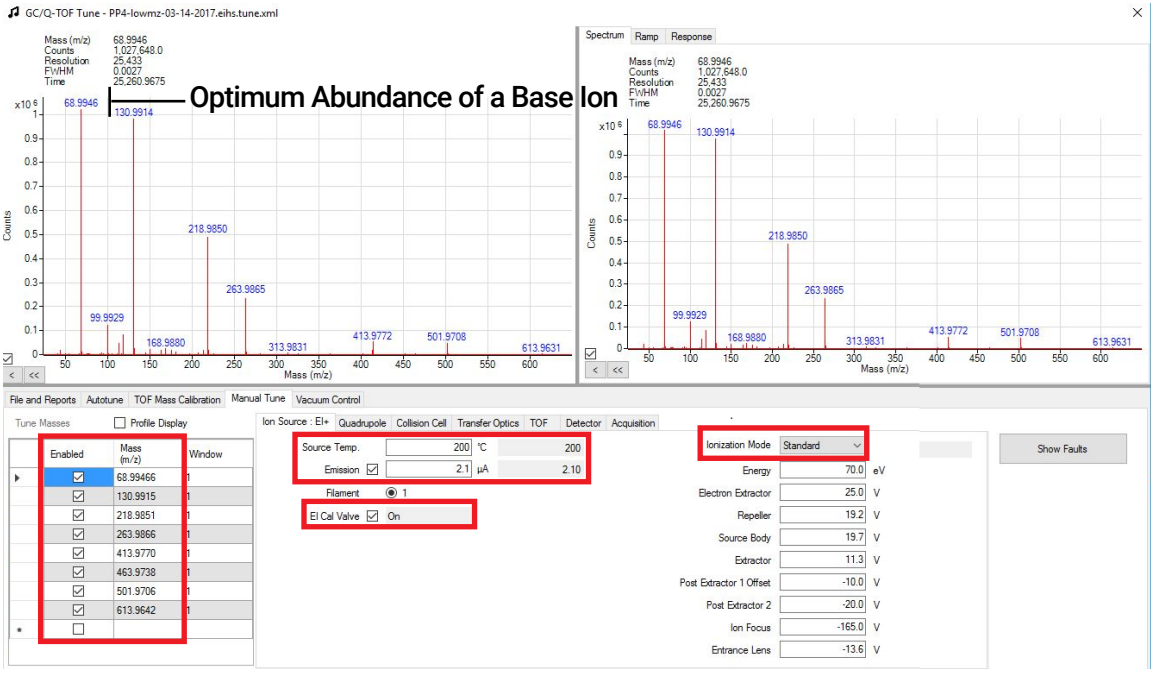
Acquired data between 25 and 650 *m/z* is displayed in the tune spectrum window.



Step 2 Optimize the base ion abundance.

This step is usually done before calibration is performed.

1 Click the **Ion Source** tab, and in the **Tune Masses** area select **Enabled**.

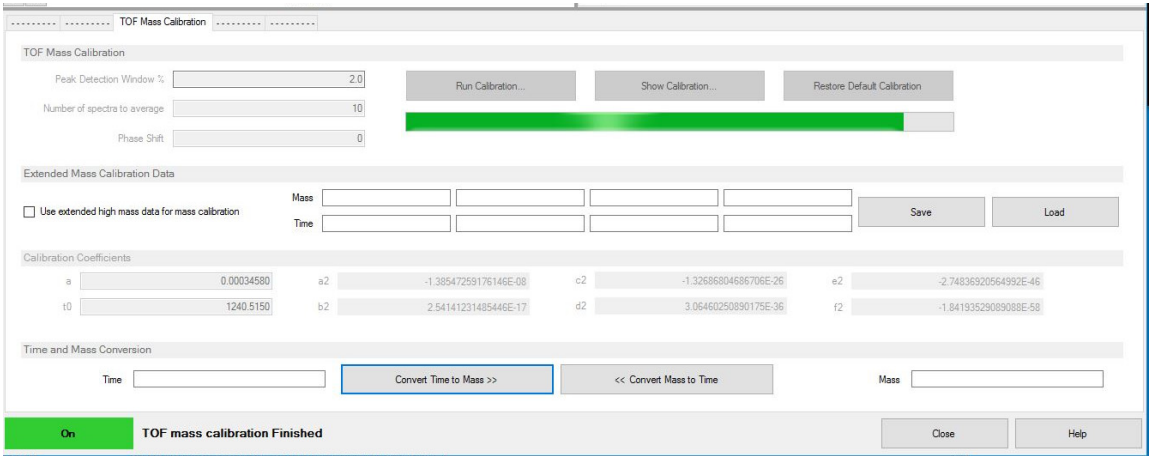


2 To enable calibrant flow ionization, enable the **Emission** and **EI Cal Valve**.

3 Adjust the **Emission** current so that the abundance of the ion of interest is ideally between 0.8 x 10^6 and 1.2 x 10^6 counts. Higher values will saturate the signal, and lower values will not provide sufficient ion statistics for optimal mass accuracy.

Step 3 Perform a mass calibration.

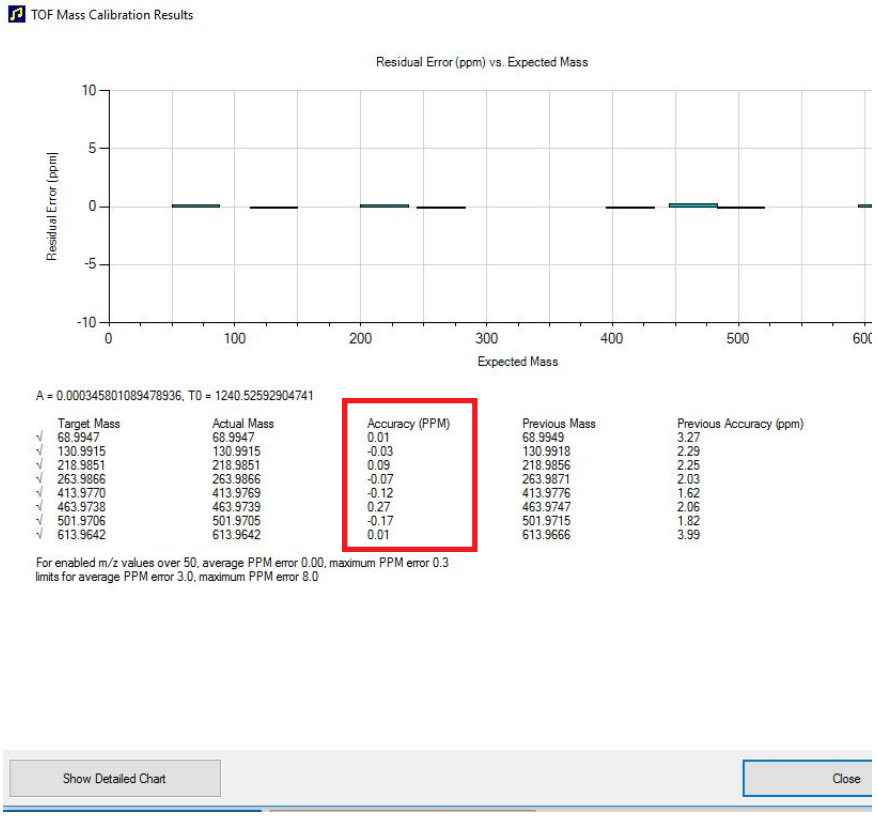
1 Select the **TOF Mass Calibration** tab from the **GC/Q-TOF Tune** window.



See **Task 6. Acquire MS scan data (Optional)** on page 24.

2 Click **Run Calibration**.

When the calibration completes the **TOF Mass Calibration Results** window displays. **Mass Accuracy (PPM)** should typically be below 1PPM for all ions used in calibration.



3 Click **Close**.

4 Click the **File and Reports** tab and save the tune file. Save the tune file as atune-lowmz_*date*.ei.tune.xml. Where *date* is today's date.

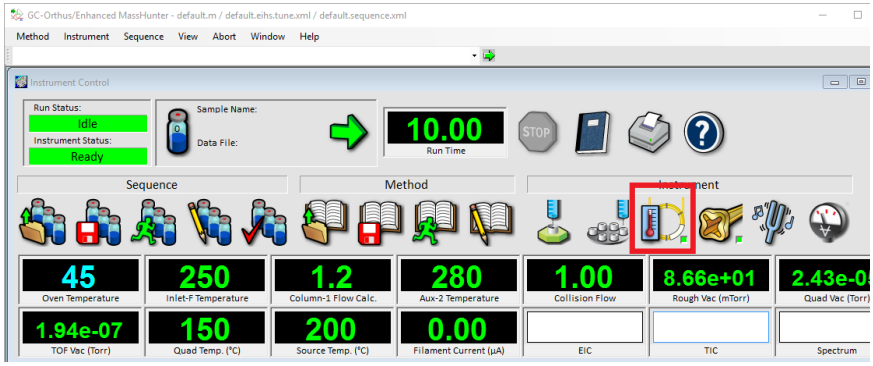
5 Click **Close**.

The **GC/Q-TOF Tune** window closes, and you are returned to Instrument Control view.

Task 4. Enter GC acquisition parameters

In this exercise, you enter the GC conditions for the analysis.

1 Click the **GC Edit Parameters** icon. With the window selected, mouse over the icons to identify the icon from the tool tip. The **GC edit parameters** window is displayed.



2 In the navigation menu, select **Columns** then select column 1 in the **Selection** column.

3 Select control mode **On** and then select **Constant Flow** mode. Enter 1.1 mL/min for the initial Flow.

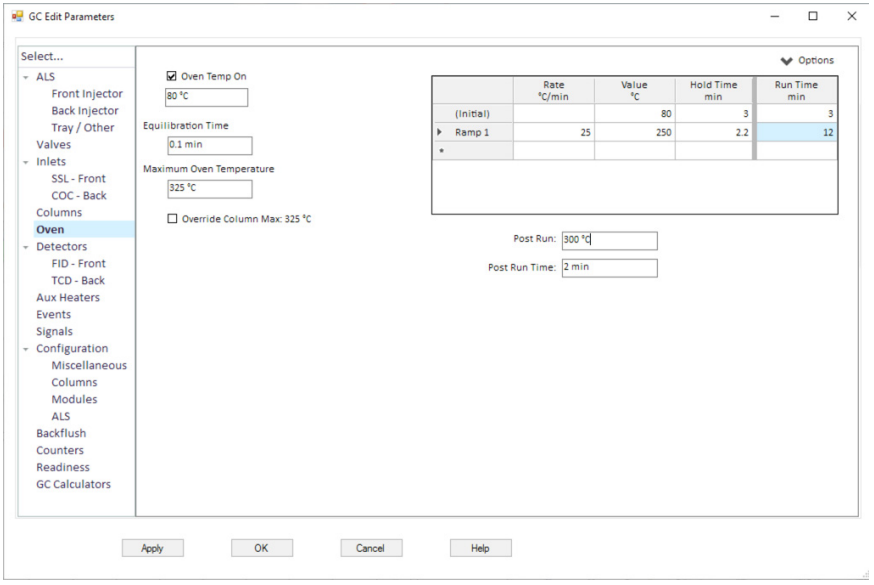
4 Select the **Collision Cell EPC** in the **Selection** column and then in the **Collision Cell EPC** area, set the **N2 Collision Gas** on at 1.5 mL/min.

If the current flow value of the collision cell N2 gas is not 1.5 mL/min and you change it to this value, an autotune will be required.

5 In the **Collision Cell EPC** area, uncheck the **He Quench Gas**.

6 In the navigation menu select **Inlets > SSL**, and enter the inlet parameters listed in **Table 2** on page 18.

7 Click the **Oven** icon, and enter the oven parameters listed in **Table 2** on page 18.



8 In the navigation menu select **ALS >Front Injector**, and enter the injector parameters listed in **Table 2** on page 18.

If your ALS is attached to the **Back Inlet**, select the **Back Injector** tab.

9 In the navigation menu, select **Aux Heaters**, enable, and set the temperature to 280 °C. This is the MSD transfer line heater.

10 Click **OK**. The GC parameters are downloaded to the GC and the window closes.

Table 2 GC Parameters for data acquisition method

Parameter	Value
Oven	
Equilibration Time	0.1 min
Oven Program	80 °C for 3 min, 25 °C/min to 250 °C, hold for 2.2 min
Run Time	12 min
Front SS Inlet	He

Table 2 GC Parameters for data acquisition method (continued)

Parameter	Value
Mode	Split
Heater	**On** 250 °C
Pressure	**On** Value automatically set with column flow
Septum Purge Flow	**On** 3 mL/min
Gas Saver	**On** 20 mL/min after 3 min
Split Flow	220 mL/min
Split Ratio	200:1
Thermal Aux 2 {MSD Transfer Line}	
Heater	**On**
Temperature	280 °C
Column # 1	J&W 122-3832 DB-35ms: 30 m x 250 µm, 0.25 µm
In	Front SS Inlet He
Out	Vacuum
(Initial)	80 °C
Flow	1.1 mL/min
Flow Program	**Off**
Front Injector	
Syringe Size	10 µL
Injection Volume	1 µL
Solvent A Washed (PreInj)	2
Solvent A Washes (PostInj)	2
Solvent A Volume	8 µL
Solvent B Washes (PreInj)	2
Solvent B Washes (PostInj)	2
Solvent B Volume	8
Sample Washes	0
Sample Wash Volume	8 µL
Sample Pumps	4

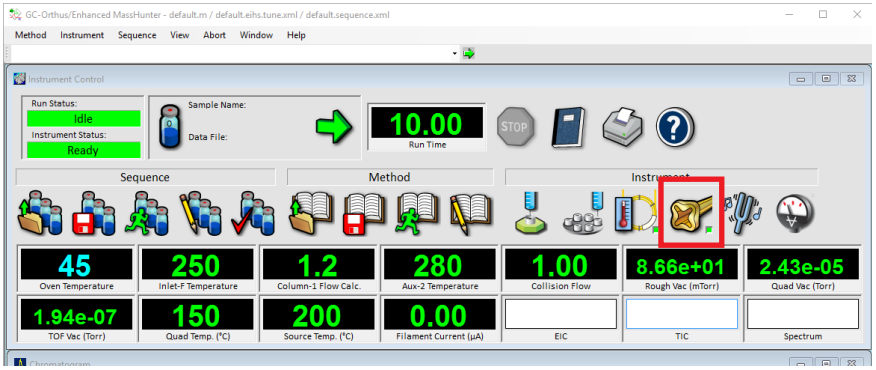
Table 2 GC Parameters for data acquisition method (continued)

Parameter	Value
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6,000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6,000 µL/min
Injection Dispense Speed	6,000 µL/min
Viscosity Delay	0 sec
Sample Depth	Disabled
Collision cell EPC Module	
Nitrogen	**On** 1.5 mL/min
Helium	**Off**

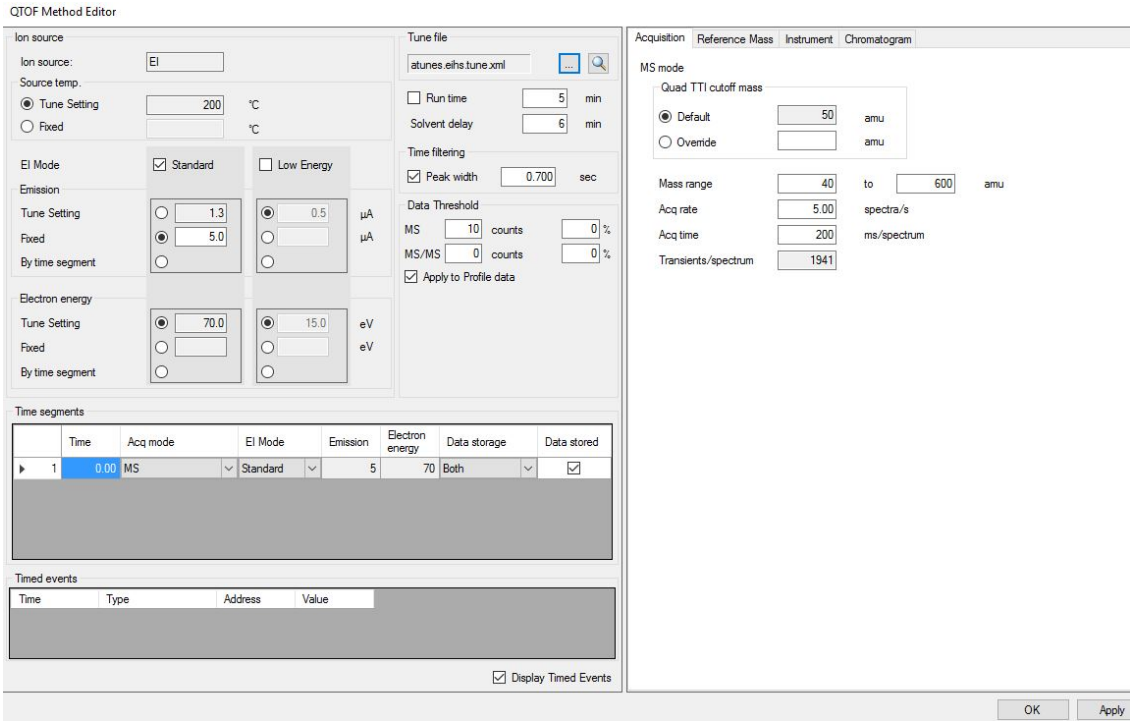
Task 5. Create a Qual acquisition method for scanning ions

This task starts with the GC parameters entered in the method from Task 4. In this task, you will enter the 7250 parameters for ion scanning and save to the method.

1 Click the **QTOF Method Editor** icon.



The **QTOF Method Editor** window opens.



2 In the **Tune file** area, click the … icon. Select the tune file created at the end of Task 3.

3 In the **Ion Source** area, set the **Source temperature** to 200 °C, set the **Emission** to **Fixed** with a value of 5.0 entered, and set the **Electron energy** to **Tune Setting**.

4 Set the **Solvent delay** to five minutes. The 7250 starts collecting data at five minutes due to the **Solvent delay** setting.

5 In the **Time Filtering** area, select **Peak width** and set it to 0.7 seconds. This filters out unwanted peaks to reduce data storage.

6 In the **Data Threshold** area, enter 10 for counts. This filters out unwanted noise to reduce data storage.

7 Select **Apply to Profile data**. This applies the **Data Threshold** filter to profile data to reduce storage.

8 In the **Time segment** area, select a **Scan Type** of **MS** from the **Acq mode** drop-down list.

   If we were doing an MS/MS acquisition we would enter the counts here to reduce data storage.

9 Select **Both** for **Data stored**.

   Selecting **Both** stores a peak's profile data and centroid data for data analysis.

10 In the **MS mode** section, for the **Mass range** enter 40 for the start mass, 600 for the end mass, and 5.00 **spectra/s** for the **Acq rate**

   All data up to 650 *m/z* is always acquired but only the data selected here (40 to 600) is saved to disk.

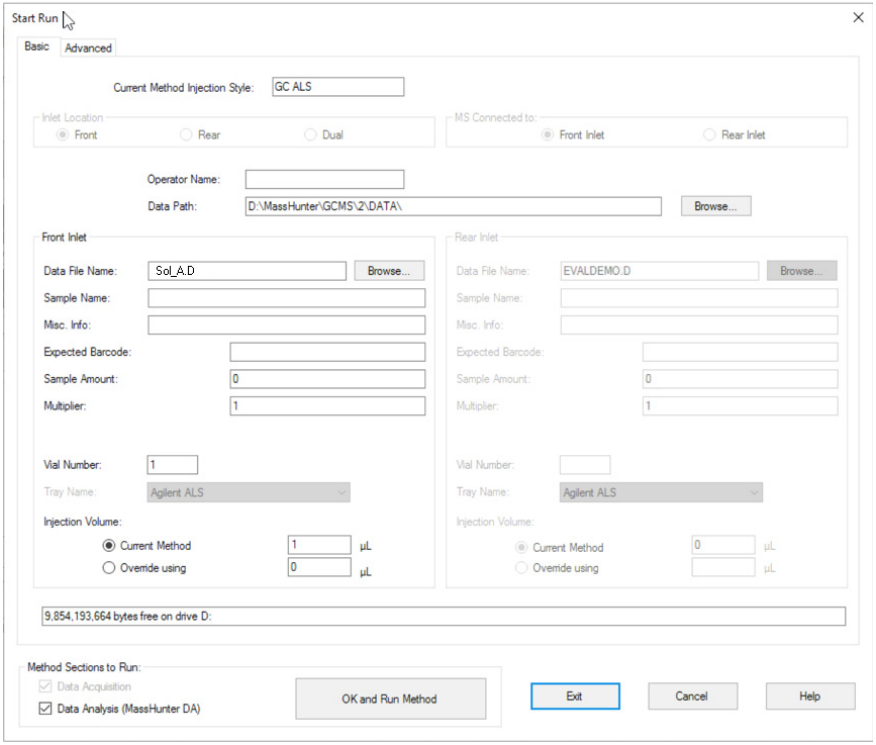
11 Click **OK** to close the window.

12 From the main window select **Method > Save Method As**, and save the method as **OFN EI 70eV.M**.

Task 6. Acquire MS scan data (Optional)

In this task, you acquire the scan data using the method developed in the previous tasks. This task is optional because you can perform the next task with an example data file that is provided with MassHunter in the location shown in **Reference material** on page 6. However, if you prefer, you can acquire your own data file as described in this task.

1 Click the **Start Run** (green arrow) icon. The **Start Run** dialog box is displayed.



2 In the **Data Path** enter the directory to save the data file that is acquired by this run.

3 In the Inlet section you are using, enter **Sol_A.D** for the **Data File Name**.

4 Enter the **Vial Number** location in the auto sampler tray.

5 Select **Current Method** for the **Injection Volume**. The **Injection volume** that you entered in Task 4 is used.

6 In the **Method Sections to Run** section, select **Data Acquisition**.

7 Click **OK and Run Method**.

The method is sent to the GC and the Q-TOF. When the instruments are ready, the sample is injected and the data is collected and sent to the data directory specified.

Task 7. Using a Sequence to Schedule Mass Calibrations

This automated procedure is used to schedule mass calibrations at the start of a sequence of sample runs and at timed intervals during those runs. It is recommended to do a mass calibration about every two hours when continuously running samples. This mass calibration only takes a couple of minutes, but allows you to maintain higher mass accuracy and immunity to drift.

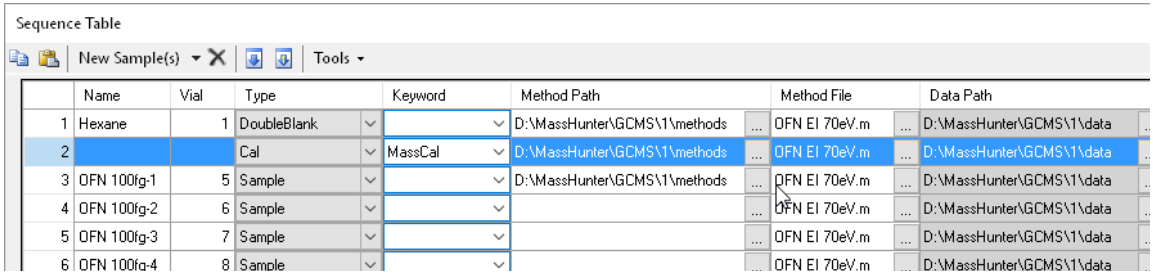
Step 1 Add a mass calibration at the start of sequence.

1 Insert an entry for the running of the mass calibration. This entry might follow the running of a sample blank.

2 Add the **MASSCAL** keyword to this entry.

3 Enter the **Method** used for processing samples that follow, and select **CAL** from the **Type** dropdown. This entry will also be used after running samples every two hours in our sequence.

4 Save the sequence.



Step 2 You can skip this step, and use the run time procedure shown in Step 3 instead.

Use this step to add a mass calibration entry at two hour intervals during sample runs by creating mass calibration entries in the sequence table.

1 Calculate the number of samples to run before performing the mass calibration.

- (120 min /*sample run time*) For a 10 minute sample run this means 12 samples can be processed before performing a mass calibration.
- 2 + 12 + 1 = entry 15 for our example. An empty entry is created.

2 Copy the mass calibration entry added in the above step.

3 Select the sample entry location for the mass calibration, and select **Insert sample** from the context menu.

4 Click **paste** with this entry selected.

5 Repeat step 2 as required.

6 Save the sequence.

Step 3 The same result for the mass calibration interval created in the above step can be obtained at run time as follows.

1 Select **Sequence > Run Sequence**.

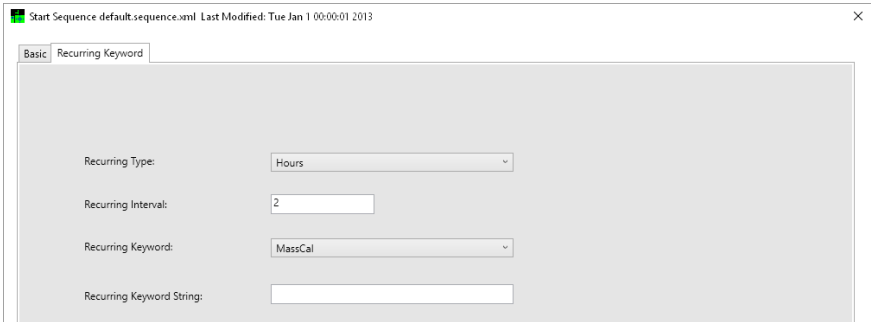
- The **Start Sequence** dialog opens.
- See online help for additional information.

2 Fill out the required information in the **Basic** tab.

3 Click the **Recurring Keyword** tab.

4 Select **Hours** as the **Recurring Type**.

You could also select **Method change or Hours** if you want to recalibrate when the method changes or at a timed interval.



5 Enter **2** hours for the **Recurring Interval**.

6 Select **MassCal** as the **Recurring Keyword** string. The mass calibration runs at the specified interval.

7 Click **Run Sequence**.

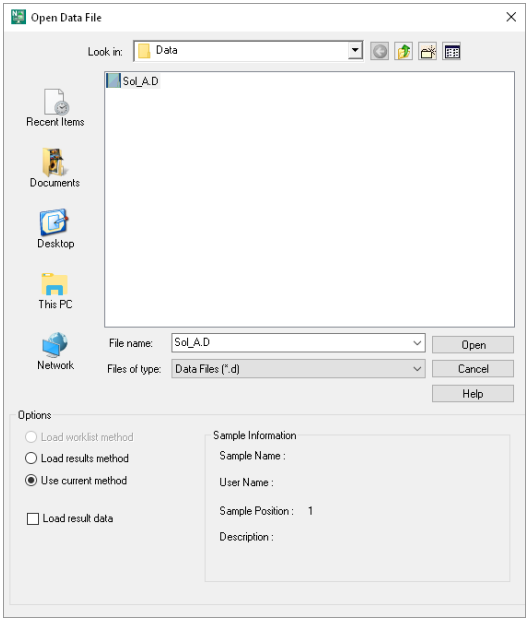
Exercise – Analyze data

In this exercise, you analyze data acquired from the previous exercises in this manual. If you did not acquire this data, you can use the example data file Sol_A.D provided in the location shown in **Reference material** on page 6. For additional details on using this program, see the *MassHunter Workstation Qualitative Analysis Familiarization Guide for GC/MS* and the *Qualitative Analysis Training Videos* that are provided with MassHunter in the location shown in **Reference material** on page 6.

Task 1. Open a data file in the Qualitative Navigator program

1 Double-click **Qualitative Navigator** shortcut on your desktop to start the Qualitative Analysis program.

The system displays the Open Data File dialog.

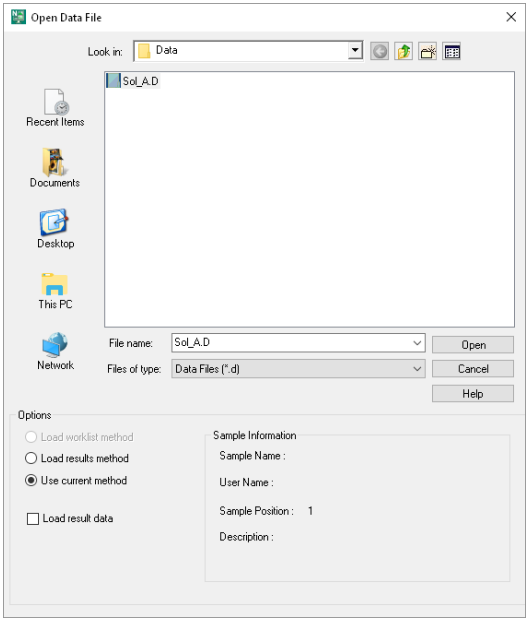


You can get Help by:

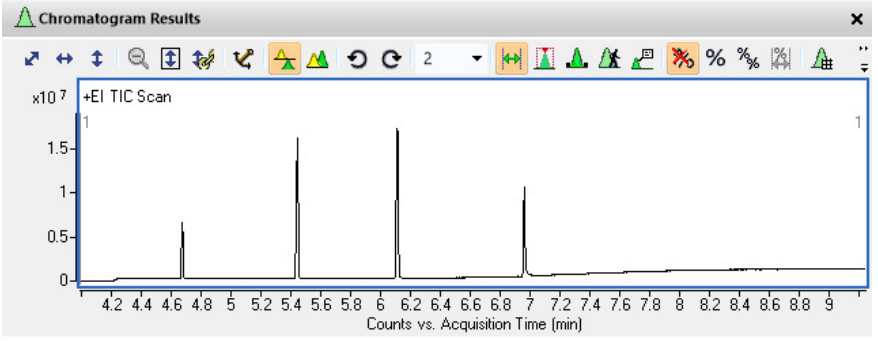
- Pressing the **F1** key when a window is active
- Selecting **Help > Contents** in the main menu
- Clicking the **Help** icon in the active window

2 Navigate to the location where your data file is located, and select your acquired data file or the demo file provided for this exercise **Sol_A.D**.

3 Under **Options**, select **Use current method** and clear **Load result data**.



4 Click **Open**. The data file is loaded and a TIC of the data is displayed.

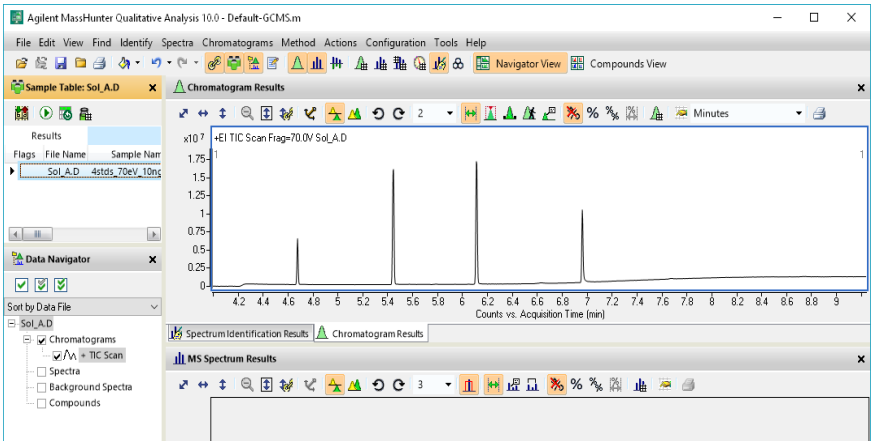


Task 2. Configure the qualitative analysis user interface

Step 1 Create a Qualitative Analysis method.

1 From the main menu, select **Method > Open**.

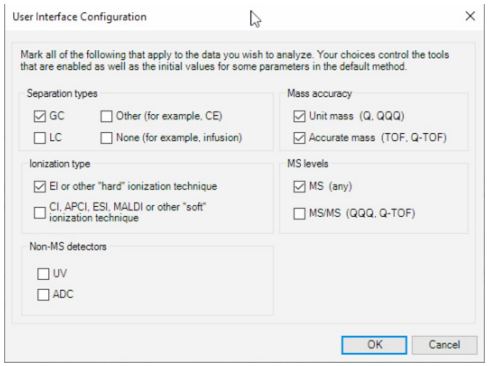
2 Select **default-GCMS.m** and click **Open**.



3 From the main menu, select **Configuration > Show Advanced Settings**.

4 From the main menu, select **Configuration > User Interface Configuration**.

The User Configuration dialog box opens. The settings selected here are based on the data file loaded and the default method previously selected. You may edit these if required.



5 Click **OK**.

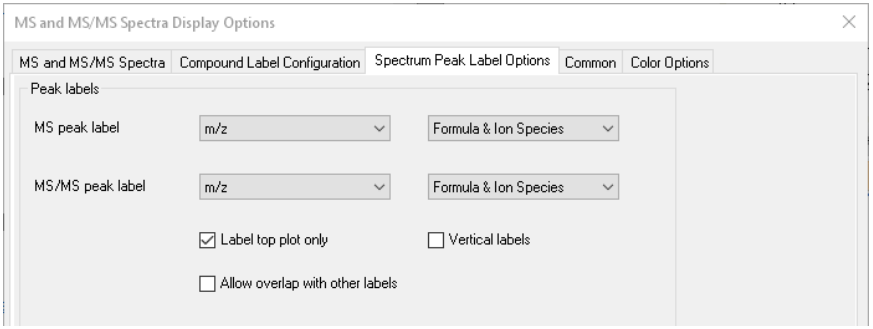
6 From the main menu, select **Method > Save As**. Rename the method since you can not overwrite the default method.

7 In the **File Name** enter `QTOF-GCMS.m` and click **OK**.

Step 2 Configure the MS and MS/MS Spectra Display options.

1 From the main menu, select **Configuration > MS and MS/MS Spectra Display Options**.

2 Click the **Spectrum Peak Label Options** tab, and set the values for the peak labels. Use the values displayed below. These values result in the horizontal display of *m/z* and Formula & Ion Species value above identified spectra.



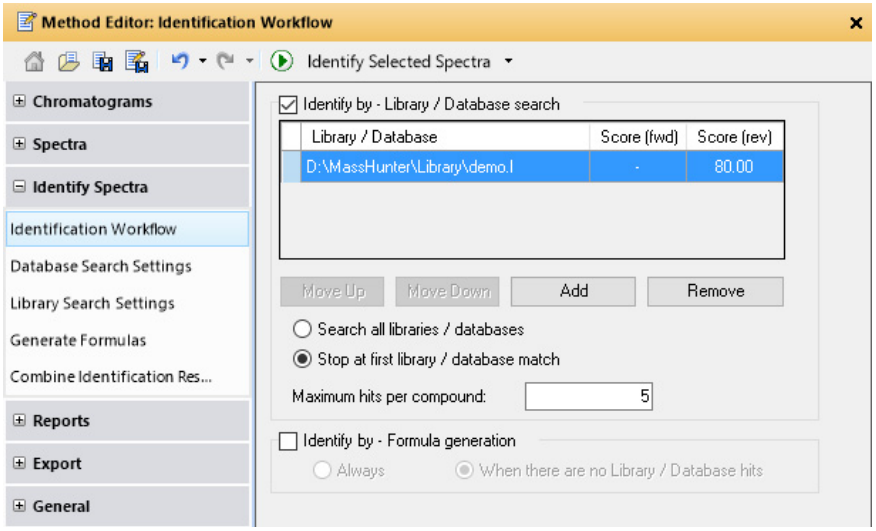
3 Save the method.

Step 3 Assign a library for identifying spectra.

1 Click the **Method Editor** icon to open the method editor and click on **Identification > Identification Workflow**.

For easier viewing, float the editor pane outside the Qualitative Analysis Navigator window.

2 Set the values for the library used to identify spectra. The Solo.cdb library is included with MassHunter and includes the four compounds used in our sample. Use the values displayed below.



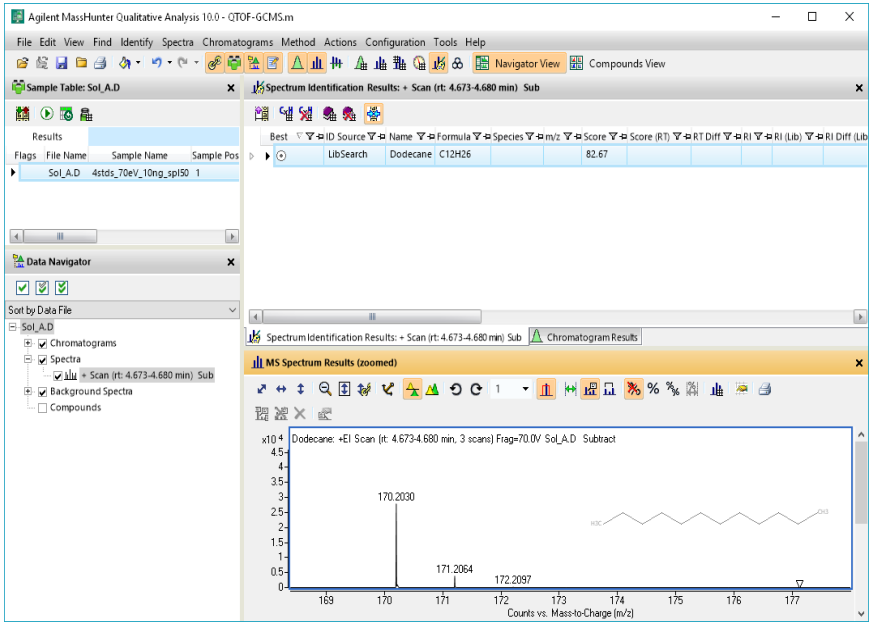
Task 3. Identify Peaks in Qualitative Navigator

1. In the **Chromatogram Results** pane, right-click and drag around the peak at 4.676 RT. The single zoomed peak is displayed.
2. With **Range Select**  selected, click and drag the mouse to select background from RT 4.7 to 4.71. This area selected includes background spectra.
3. Right-click inside the shaded area and select **Extract MS Spectrum to Background** from the context menu.

   The extracted spectrum displays in the **MS Spectrum Results** pane, and is also selected under **Background Spectra** in the **Data Navigator**.
   It will be automatically subtracted from any extracted MS spectrum that follows.
4. Click and drag the mouse to select an area containing the peak apex from RT 4.75 to 4.68.
5. Right-click inside the shaded area, and select **Extract MS Spectrum** from the context menu.

   This spectra is extracted and displayed in the **MS Spectrum Results** top pane and is also selected under **Spectra** in the **Data Navigator**. The background spectra was subtracted from it and noted by the **Subtract** label in the **MS Spectrum Results** top pane.
6. In the **MS Spectrum Results** tab, with **Autoscale Y-axis during Zoom**  and **Show Predicted Isotope Distribution**  selected, from the main menu select **Identify > Search Library/DB for Spectra**. The **Spectrum Identification Results** tab for this scan displays **Dodecane** as the compound found by the Library.

7 Zoom the *m/z* axis to display the range of values between 168 through 179. The predicted isotopes for Dodecane are displayed surrounded by a red outline indicating an isotope of Dodecane.



8 If desired, repeat this procedure to identify other peaks in this sample.

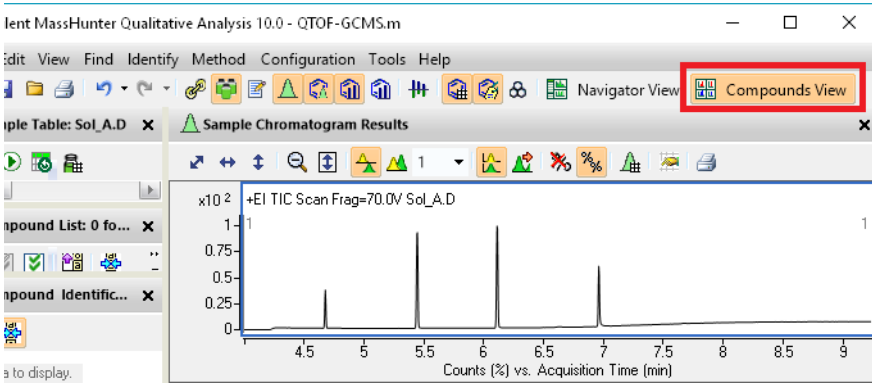
9 In the Method menu, select **Save**.

Task 4. Identify compounds using Qualitative Analysis Workflows

Step 1 Edit the Qualitative Analysis method settings in the Compound Identification workflow

This task begins with the Qualitative analysis program open and analysis on data file SOL_A.d is as completed at the end of Task 3.

1 Click the **Compound View** tab in the main toolbar area. The **Sol_A.D** data file is shown in the Sample Chromatogram.

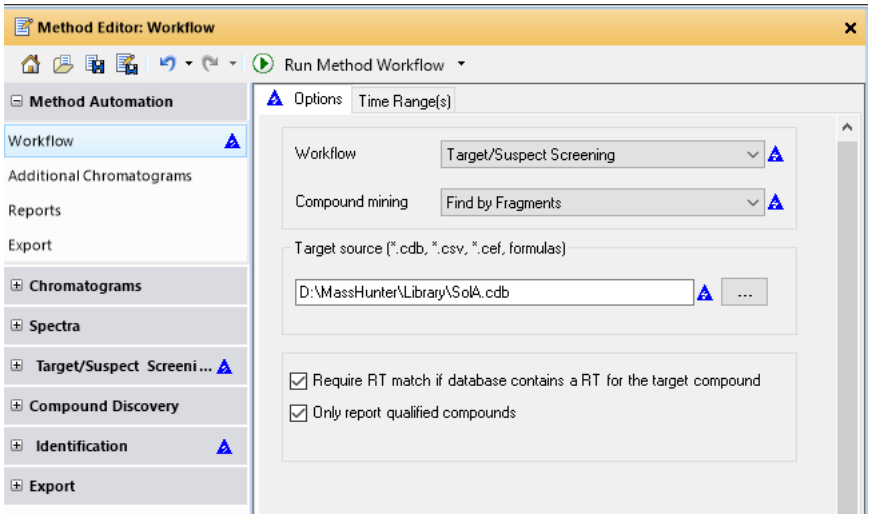


2 If the **Method Editor** icon in the main toolbar is not selected, open the Method Editor by selecting it.

For easier viewing, float the editor pane outside the Qualitative Analysis Navigator window.

3 In the Method Editor, click **Method Automation/Workflow**.The **Options** tab displays.

4 In the **Options** tab, select **Target/Suspect Screening** for the Workflow and select **Find by Fragments** for **Compound mining**.

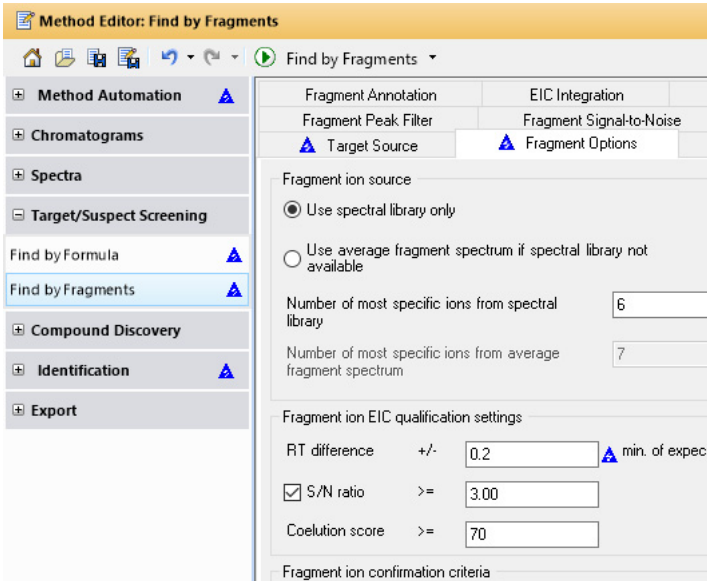


5 In the Method Editor, click **Target/Suspect Screening > Find by Fragments** and click the **Target Source** tab.

6 Click ... and select **SolA.cdb** as the target source. You can find this file in the location shown in **Reference material** on page 6.

Step 2 Configure the Find by Fragments algorithm.

1 In the Method Editor, click **Fragment Options** and set its values. Use the values shown. It is important to have these values set correctly. The default values in the other tabs are OK for processing this sample.



2 Click the **Find by Fragments** icon in the Method Editor toolbar.

The results are listed in the **Sample Table, Compound List, Sample Chromatogram Results, Compound Chromatogram Results**, and **Compound MS Spectrum Results** panes.

Step 3 Save the method.

1 In the Method Editor toolbar, click **Save Method** .

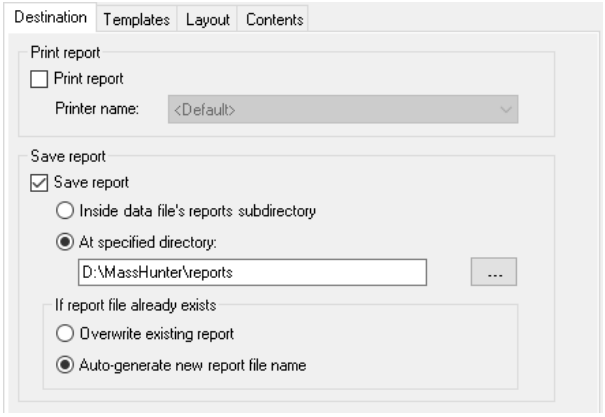
Task 5. Configure Method Automation Reports

You can print an analysis report interactively or generate it as part of a **Method Automation** workflow as we are doing here. An analysis report can contain the results from extracting and integrating chromatograms, extracting spectra, finding compounds, searching the database for peak spectra, or generating formulas from peak spectra.

Step 1 Edit the Qualitative Analysis method to include the Reports workflow.

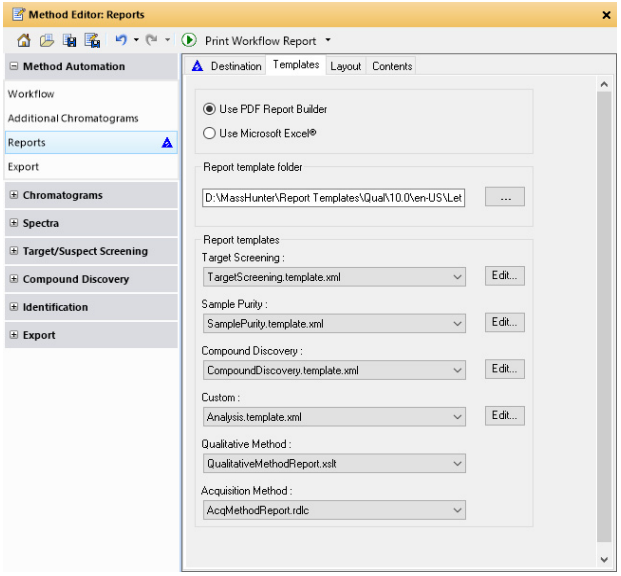
1 In the **Method Editor**, click **Method Automation** and select **Reports**.

2 Set the entries for the **Destination** tab to the values shown below.



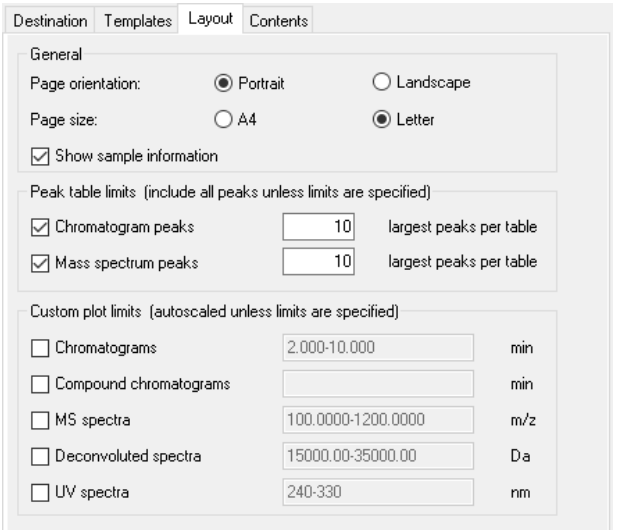
Select **Save Report**, and enter a folder location for the PDF-based report.

3 Set the entries for the **Templates** tab to the values shown below.

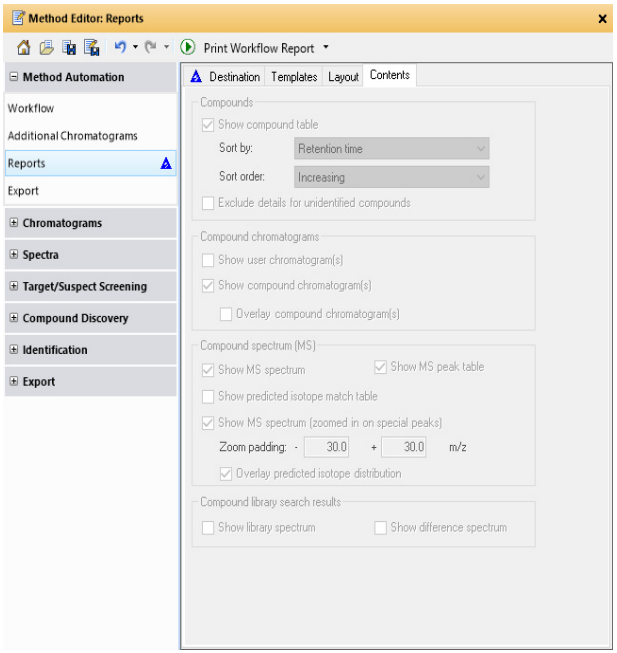


For this exercise, based on the c workflow selection, we are using the **Target screening report template**.

4 Set the entries for the **Layout** tab to the values shown below.



5 Set the entries for the **Contents** tab to the values shown below.



Select the compound tables, chromatograms, spectrum types, peak tables and library search results spectrum types to include.

Step 2 Save the method.

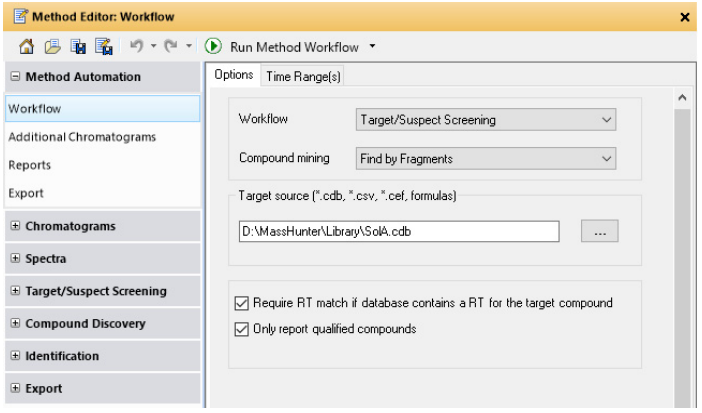
1 In the Method Editor toolbar, click **Save Method** .

Task 6. Generate the Method Automation workflow

This task automates the generation of the Find by Fragments and Reports workflows in a single saved method. Here we will perform the analysis based on the method created in the previous tasks. You can also use a saved data analysis method containing several workflows to generate the analysis automatically at the end of your sample data acquisition run.

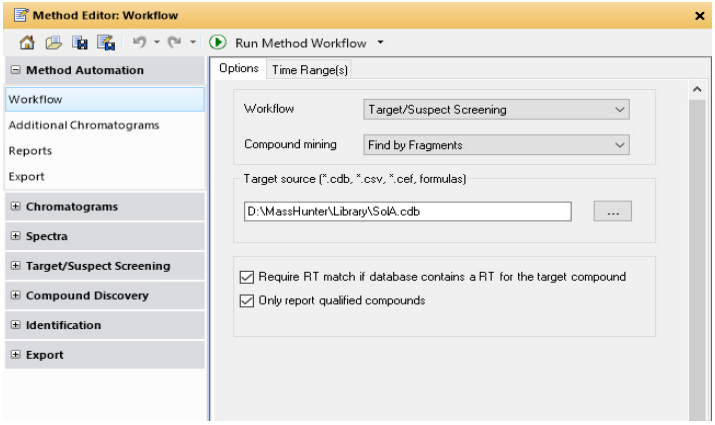
1 In the Method Editor, select **Method Automation > Workflow**.

This workflow was previously configured. See **Task 4. Identify compounds using Qualitative Analysis Workflows** on page 36.



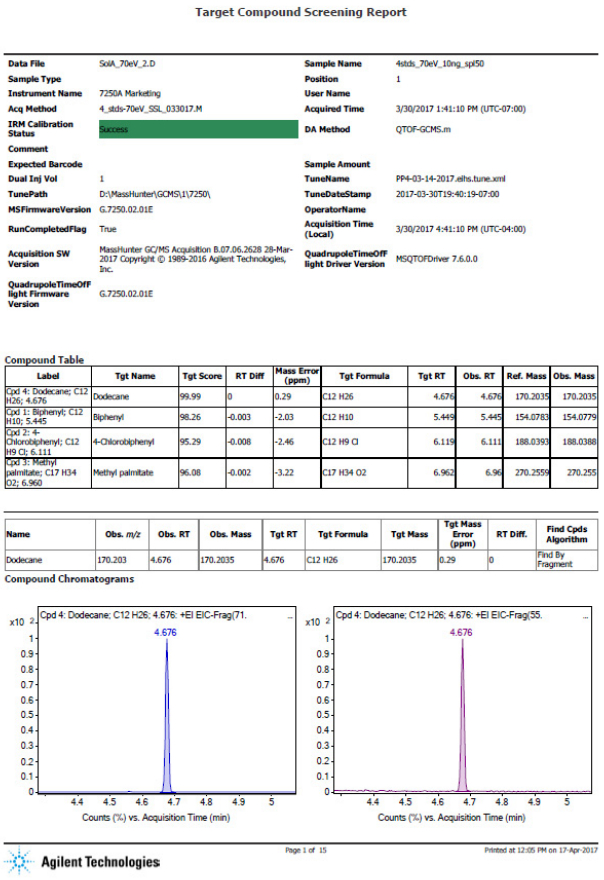
2 In the Method Editor, select **Method Automation > Workflow**.

This workflow was previously configured. See **Task 5. Configure Method Automation Reports** on page 39.



3 From the dropdown menu in the toolbar, select **Run Method Automation**

The compounds are found, identified, and a report is generated and saved as a pdf in the location specified.



Target Compound Screening Report

Data File	SolA_70eV_2.D	Sample Name	4stds_70eV_10ng_spl50
Sample Type		Position	1
Instrument Name	7250A Marketing	User Name	
Acq Method	4_stds-70eV_SSL_033017.M	Acquired Time	3/30/2017 1:41:10 PM (UTC-07:00)
IRM Calibration Status	Success	DA Method	QTOF-GCMS.m
Comment			
Expected Barcode		Sample Amount	
Dual Inj Vol	1	TuneName	PP4-03-14-2017.elhs.tune.xml
TunePath	D:\MassHunter\GCMS\1\7250\	TuneDateStamp	2017-03-30T19:40:19-07:00
MSFirmwareVersion	G.7250.02.01E	OperatorName	
RunCompletedFlag	True	Acquisition Time (Local)	3/30/2017 4:41:10 PM (UTC-04:00)
Acquisition SW Version	MassHunter GC/MS Acquisition B.07.06.2628 28-Mar-2017 Copyright © 1989-2016 Agilent Technologies, Inc.	QuadrupoleTimeOff light Driver Version	MSQTOFDriver 7.6.0.0
QuadrupoleTimeOff light Firmware Version	G.7250.02.01E		

Compound Table

Label	Tgt Name	Tgt Score	RT Diff	Mass Error (ppm)	Tgt Formula	Tgt RT	Obs. RT	Ref. Mass	Obs. Mass
Cpd 4: Dodecane; C12 H26; 4.676	Dodecane	99.99	0	0.29	C12 H26	4.676	4.676	170.2035	170.2035
Cpd 1: Biphenyl; C12 H10; 5.445	Biphenyl	98.26	-0.003	-2.03	C12 H10	5.449	5.445	154.0783	154.0779
Cpd 2: 4-Chlorobiphenyl; C12 H9 Cl; 6.111	4-Chlorobiphenyl	95.29	-0.008	-2.46	C12 H9 Cl	6.119	6.111	188.0393	188.0388
Cpd 3: Methyl palmitate; C17 H34 O2; 6.960	Methyl palmitate	96.08	-0.002	-3.22	C17 H34 O2	6.962	6.96	270.2559	270.255

Name	Obs. m/z	Obs. RT	Obs. Mass	Tgt RT	Tgt Formula	Tgt Mass	Tgt Mass Error (ppm)	RT Diff.	Find Cpds Algorithm
Dodecane	170.203	4.676	170.2035	4.676	C12 H26	170.2035	0.29	0	Find By Fragment

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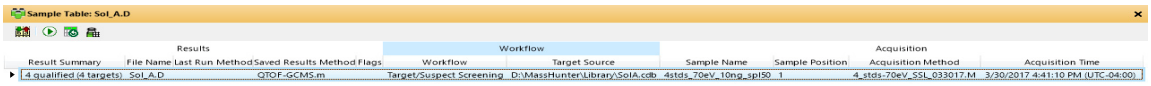
Printed at 12:05 PM on 17-Apr-2017

Task 7. Review the results

This task takes a brief look at the results shown in various windows of the Quantitative Analysis Workflow program. The first thing you will notice is that all windows are now populated with various results data.

Step 1 Determine the number of compounds found and how many of these were identified.

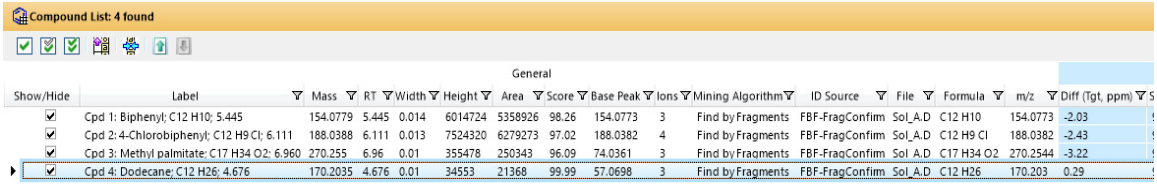
1 In the **Sample Table** section, the **Result Summary** column shows there were four qualified targets found.



2 Scroll to the right to review the data in the other columns.

Step 2 View the properties of the identified compounds in the Compound List.

1 In the **Compound List** toolbar, click **Hide any current empty columns**  .



- Saves time reviewing the parameters
- Eliminates adjusting columns.

2 In the **Compound List** toolbar, click **Auto Size All Columns**  .

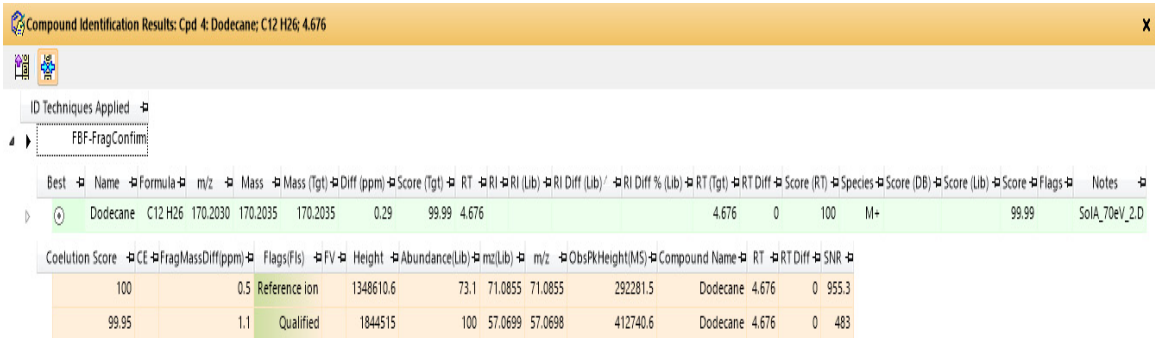
3 Scroll to the right, and review the results for the four identified compounds.

Step 3 View the Compound Identification Results.

1 Click on the **Compound Identification Results** tab at the bottom of the window.

2 In the **Compound List**, scroll right to view the **Compound Identification** section and select **Dodecane**.

Dodecane is the compound now displayed in the **Compound Identification Results** window.



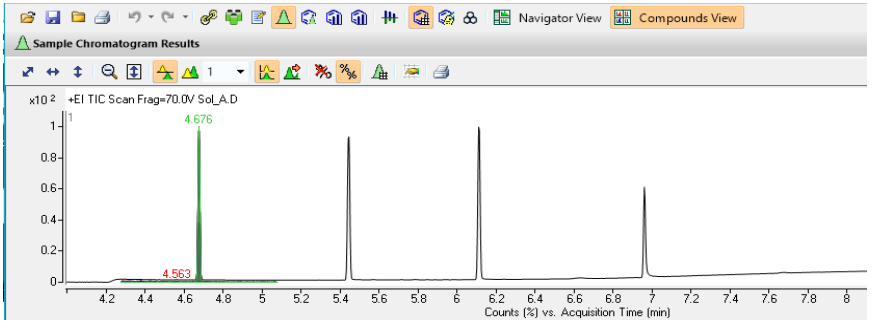
3 Scroll to the right, and review the results for Dodecane.

4 Select the other three compounds in this list, and see the view their plots in the results panes.

Step 4 Review the Sample Chromatogram Results window.

1 Click **Compound Overlaid mode**.

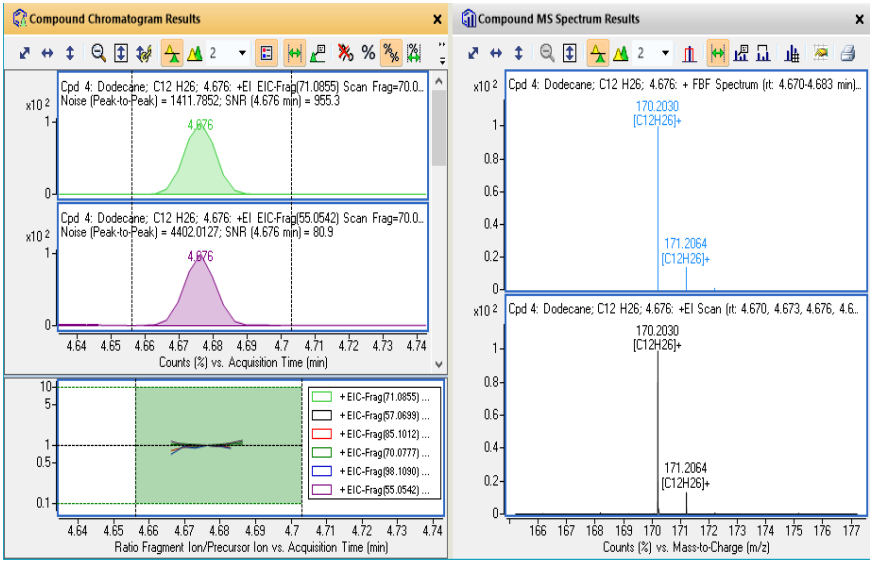
The Dodecane compound is prominently displayed in the TIC at a RT of 4.676 min.



Step 5 Review the Compound Chromatogram Results window and display its Overlaid mode.

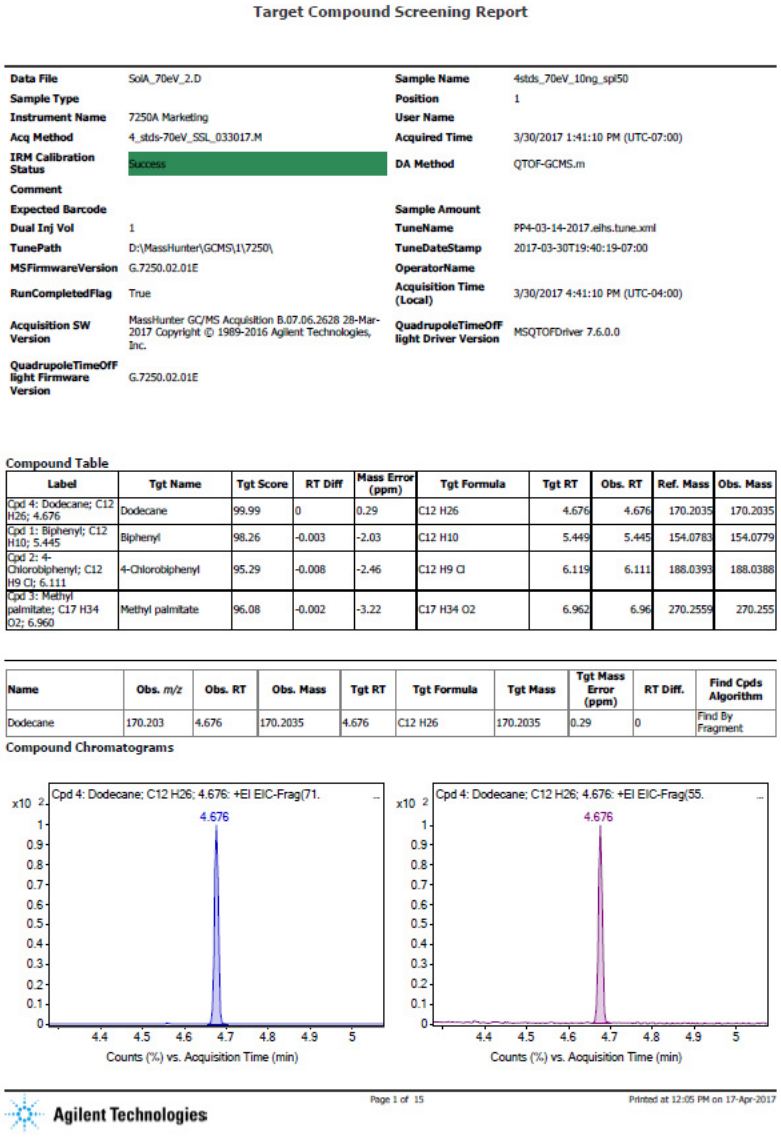
1 On the **Compound Chromatogram Results** window, click on  to change from List mode to Overlaid mode.

Overlaid mode shows the EIC as a peak outline and the ECC as a filled peak. In our example they are aligned.



Step 6 Review the compound identification report.

1 In file explorer, navigate to the folder containing the compound identification report pdf file and Open it. For our demo we specified to store it in D:\MassHunter\reports.



Target Compound Screening Report

Data File	SolA_70eV_2.D	**Sample Name**	4stds_70eV_10ng_spl50
Sample Type		**Position**	1
Instrument Name	7250A Marketing	**User Name**	
Acq Method	4_stds-70eV_SSL_033017.M	**Acquired Time**	3/30/2017 1:41:10 PM (UTC-07:00)
IRM Calibration Status	Success	**DA Method**	QTOF-GCMS.m
Comment			
Expected Barcode		**Sample Amount**	
Dual Inj Vol	1	**TuneName**	PP4-03-14-2017.elhs.tune.xml
TunePath	D:\MassHunter\GCMS\1\7250\	**TuneDateStamp**	2017-03-30T19:40:19-07:00
MSFirmwareVersion	G.7250.02.01E	**OperatorName**	
RunCompletedFlag	True	**Acquisition Time (Local)**	3/30/2017 4:41:10 PM (UTC-04:00)
Acquisition SW Version	MassHunter GC/MS Acquisition B.07.06.2628 28-Mar-2017 Copyright © 1989-2016 Agilent Technologies, Inc.	**QuadrupoleTimeOfFlight Driver Version**	MSQTOFDriver 7.6.0.0
QuadrupoleTimeOfFlight Firmware Version	G.7250.02.01E		

Compound Table

Label	Tgt Name	Tgt Score	RT Diff	Mass Error (ppm)	Tgt Formula	Tgt RT	Obs. RT	Ref. Mass	Obs. Mass
Cpd 4: Dodecane; C12 H26; 4.676	Dodecane	99.99	0	0.29	C12 H26	4.676	4.676	170.2035	170.2035
Cpd 1: Biphenyl; C12 H10; 5.445	Biphenyl	98.26	-0.003	-2.03	C12 H10	5.449	5.445	154.0783	154.0779
Cpd 2: 4-Chlorobiphenyl; C12 H9 Cl; 6.111	4-Chlorobiphenyl	95.29	-0.008	-2.46	C12 H9 Cl	6.119	6.111	188.0393	188.0388
Cpd 3: Methyl palmitate; C17 H34 O2; 6.960	Methyl palmitate	96.08	-0.002	-3.22	C17 H34 O2	6.962	6.96	270.2559	270.255

Name	Obs. m/z	Obs. RT	Obs. Mass	Tgt RT	Tgt Formula	Tgt Mass	Tgt Mass Error (ppm)	RT Diff.	Find Cpds Algorithm
Dodecane	170.203	4.676	170.2035	4.676	C12 H26	170.2035	0.29	0	Find By Fragment

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Task 8. Identify compounds using Unknowns Analysis

This task uses the Unknown Analysis application supplied with MassHunter Quantitative Analysis to find unknown compounds using SureMass.

Step 1 Start the Unknown Analysis application.

1 Double click the **Unknown Analysis** application shortcut on your desktop.

2 Select **File > New Analysis** from the main menu.

3 Enter `UnknownsDemo.uaf` for the **File name**. The application title bar displays this name for the analysis.

Step 2 Add samples to the new analysis.

1 Select **File > Add samples** from the main menu.

If the data file does not exist in the Quant batch folder, click **Browse to Copy Samples** and select the sample from another location. The sample is copied to the Quant batch folder.

2 Select the **Sol_A.D** file from the Quant batch folder, and click **OK**.

Step 3 Convert the sample data to SureMass format.

1 Select **Tools > Convert Accurate Mass Samples** from the main menu.

2 Browse to the Batch folder that contains the sample loaded above. See Step 2 **Add samples to the new analysis.**

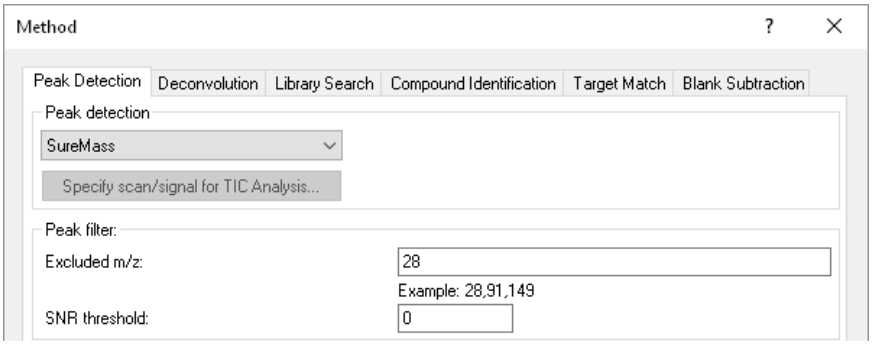
3 Select the **Sol_A.D** file from the batch folder and click **OK**.

4 In the **Convert** section of the dialog, select **Convert to SureMass format** and click **Convert**. The data file is converted to the SureMass format. Click **Close** when it finishes.

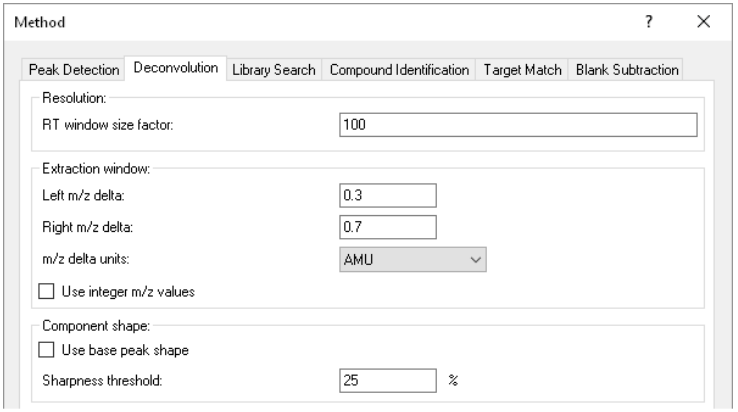
Step 4 Edit the analysis method.

1 Select **Method > Edit** from the main menu.

2 On the **Peak Detection** tab, select **SureMass** under **Peak Detection**.

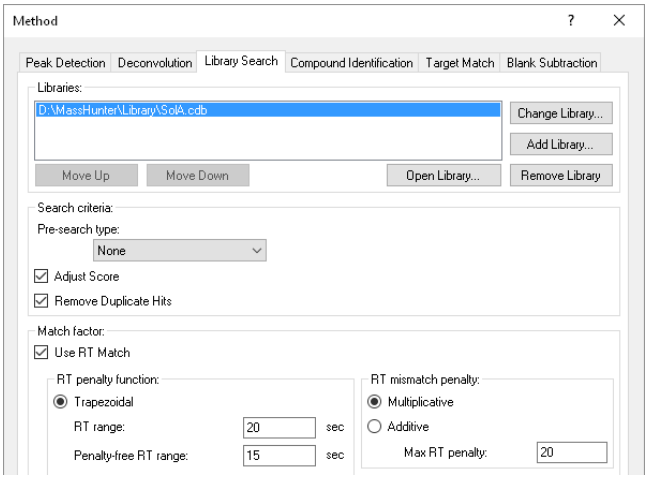


3 Click the **Deconvolution** tab, and under **Resolution** change the **RT windows size factor** to 100.



4 Under **Component shape** deselect **Use base peak shape**.

5 Click the **Library Search** tab, and click **Change Library**.



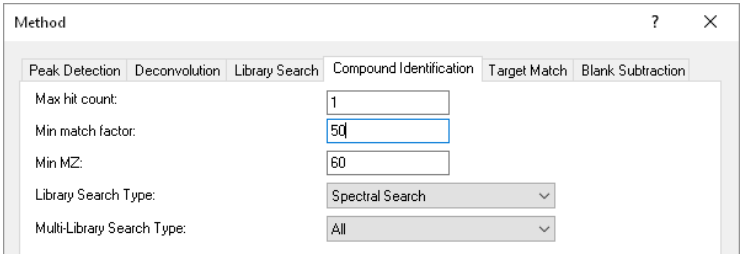
6 Browse to the location where the**SolA.cdb** file is located, and select the **SolA.cdb** file.

7 Under **Search criteria**, select **None** for the **Pre-search** type and select **Remove Duplicate Hits**.

8 Under **Match Factor**, select **Use RT Match**.

9 Under **RT penalty function**, select **Trapezoidal**, set the **RT range** to 20 sec and the **Penalty-free RT range** to 15 sec.

10 Click the **Compound Identification** tab, and set the **Min MZ** to 60.

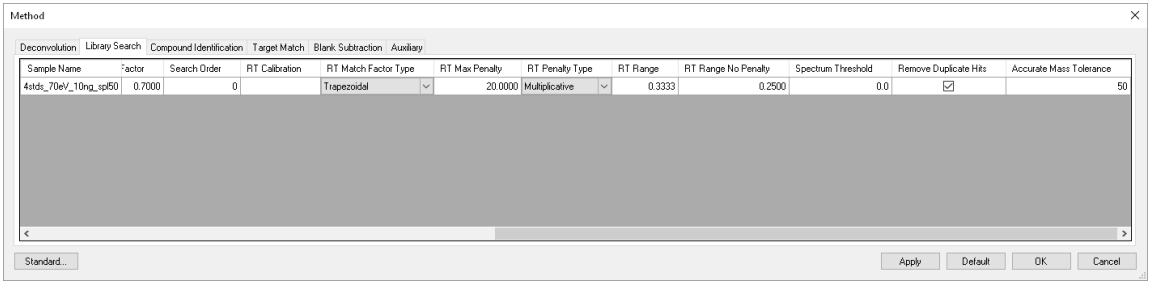


11 Click **Apply to All Samples**.

12 Click **Advanced**, and then click the **Library Search** tab.

13 Set the **Accurate Mass Tolerance** to 50, and click **OK**.

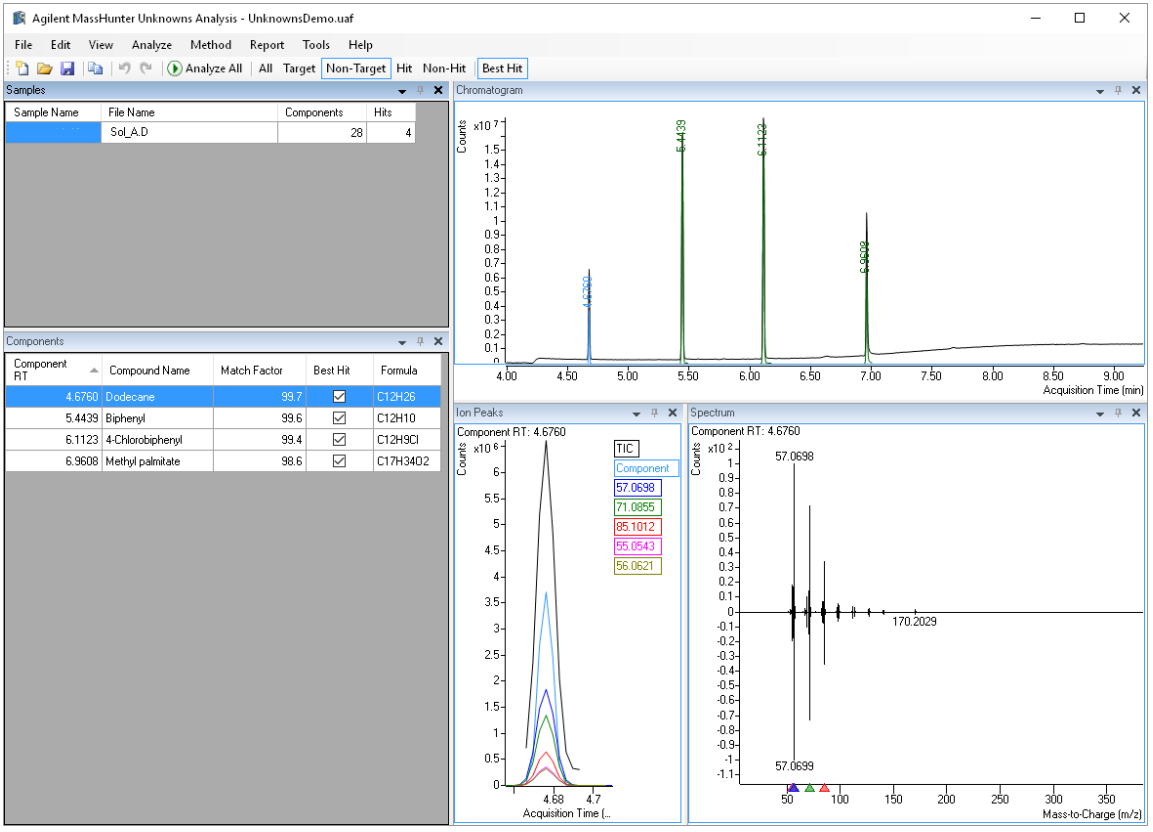
Scroll to the last column in the table. The Method Editor closes.



Step 5 Run the analysis.

1 In the **Unknowns Analysis** toolbar click the **Analyze All** icon. The sample file is analyzed according to the parameters set in the method.

2 Click **Non-Target** in the toolbar to display the identified compounds.



3 Right-click inside the Spectrum window and select **Header to Tail** if not already selected. This shows the Library spectra compared against the sample data.

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