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

Familiarization Guide

Agilent Technologies

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 section below).

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.

Reference material

Available on the 7250 QTOF installation of the MassHunter Software User Information. This documentation application 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 than 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.
- *On-line 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 you 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 on each document.

Available on the 7250 QTOF installation of the User Manuals and Tools DVD. This documentation application is delivered to you with your 7250 QTOF 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 on each document.
- *Hardware manuals* to learn how to operate and perform maintenance on the 7250 Q-TOF.

Before you begin

In this manual, each task is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin, you need to check that your system is ready. If you plan to acquire data, you also need to set up the instrument.

Prepare your system

1. Check that:
   - MassHunter Acquisition, MassHunter Qualitative Analysis, and MassHunter Quantitative Analysis are installed.
   - Your system uses an Agilent 7890 GC with split/splitless or MultiMode (MMI) inlet and automatic liquid sampler.
   - The acquisition uses a 10 uL ALS syringe tapered, fixed, with 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 2 to any location on your hard disk. This 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 “Sample Compound list” on page 4.

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 5 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

Steps	Detailed instructions	Comments
1 Set up the inlet, injection source, and enable the 7250.	**a** Double-click the **Data Acquisition** icon on the windows desktop. **b** Click the **Inlet and Injection Parameters** icon. **c** Select **GC** for the sample inlet and the installed ALS for the injection source. **d** Select the **Use MS** check box.	• The **Data Acquisition** window shown in Figure 1 is displayed. • Hover over an icon to display a tag identifying the icon. • The **Inlet and Injection Parameters** dialog box shown in Figure 2 on page 6 is displayed.

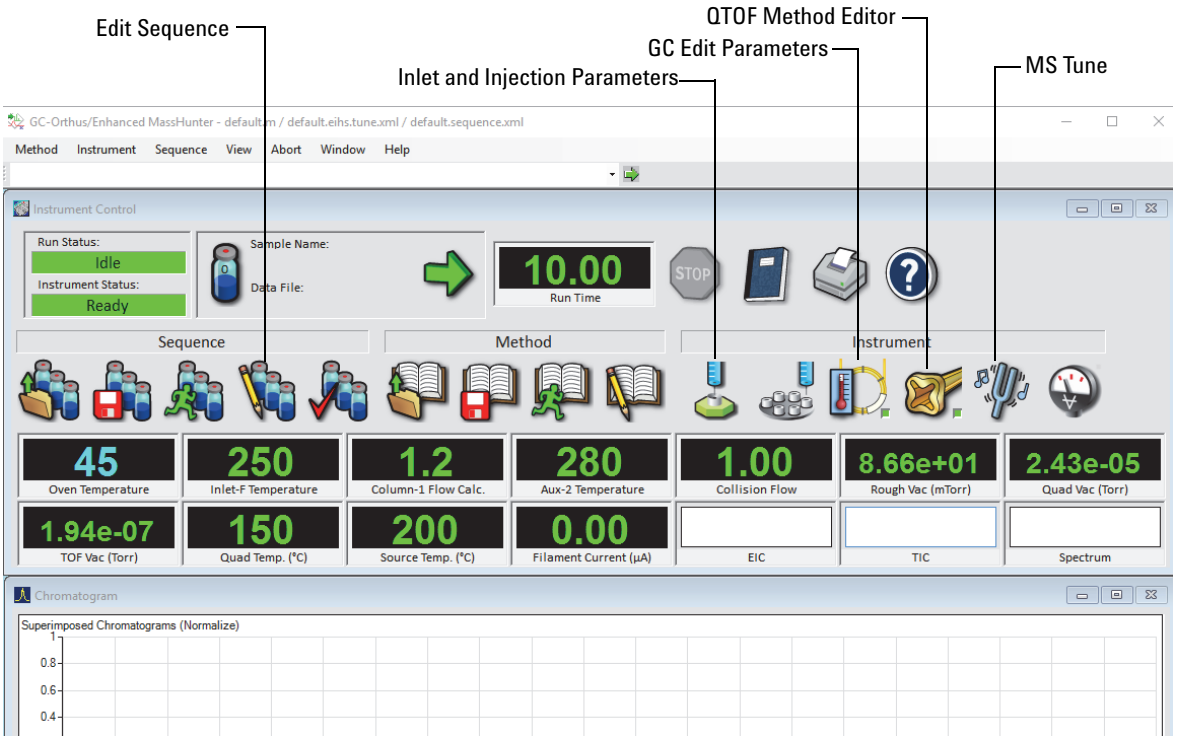


Figure 1 Agilent MassHunter Workstation Software – Data Acquisition window

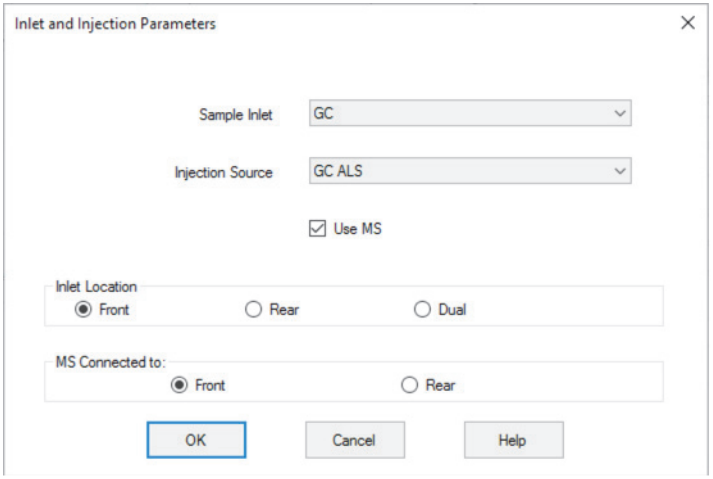


Figure 2 Inlet and Injection Parameters

Task 2. Check the GC Configuration

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

Steps	Detailed instructions	Comments
1 Check that the GC hardware configuration is suitable for the analysis.	**a** Click the **GC Edit Parameters** icon. **b** In the navigation menu select **Configuration > Miscellaneous**. **c** Set the **Pressure Units** to **psi**. **d** In the **Oven** area the **Slow Fan** mode is unchecked. **e** 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**. **f** In the navigation menu select **Configuration > Modules** and set the **SS inlet** gas to **He** and the **Collision Cell EPC** gas to **N2**. **g** In the navigation menu select **Configuration > ALS** and set the **Syringe Size** to **10 uL** and the **Solvent Wash Mode** to **A, B**. **h** Select the **OK** button.	• See Figure 1. The **GC edit parameters** window shown in Figure 3 is displayed. • If using a different column you must adjust your GC parameter settings accordingly for acceptable chromatography. • 10 uL ALS syringe tapered, fixed, with 23-26s needle. A suitable syringe may be substituted. • The GC parameters are downloaded to the GC and the window closes.

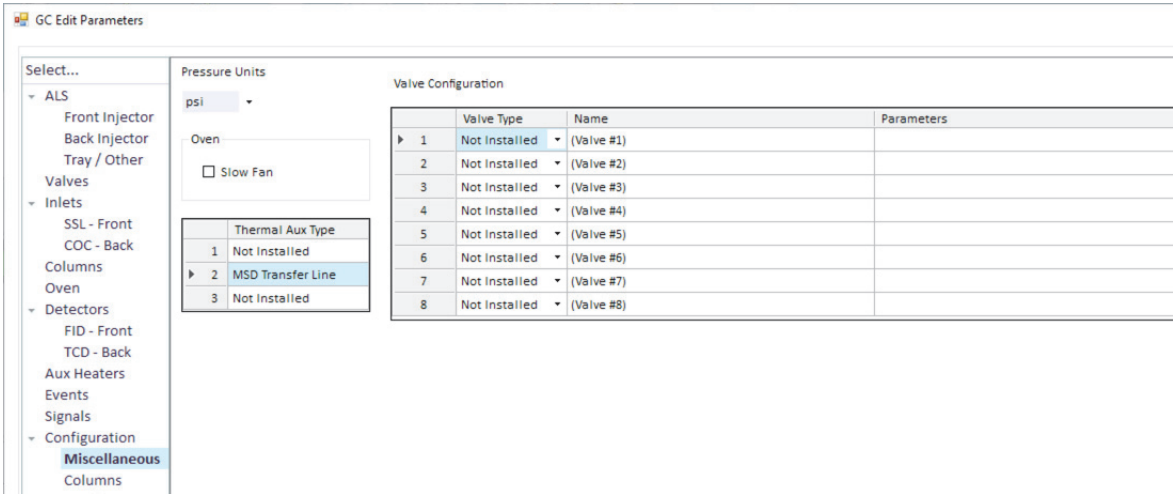


Figure 3 The Configuration Settings

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 on-line help for more information.

Steps	Detailed instructions	Comments
1 Set the *m/z* range of acquired data and the range of that data to store for analysis.	**a** In MassHunter Instrument Control view click the **MS tune** icon.	• The **GC/Q-TOF Tune** window is displayed.
	b Click the **Manual Tune** tab, then click the **Acquisition** tab.	• See Figure 4 below.
	c Select **1 Hz** from the **Acquisition Rate** dropdown.	• This is the rate used during calibration.
	d Select **Low** from the **Maximum Mass Range** dropdown.	• Data will be scanned from 20 to 650 *m/z*. There are 2 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.
	e 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.

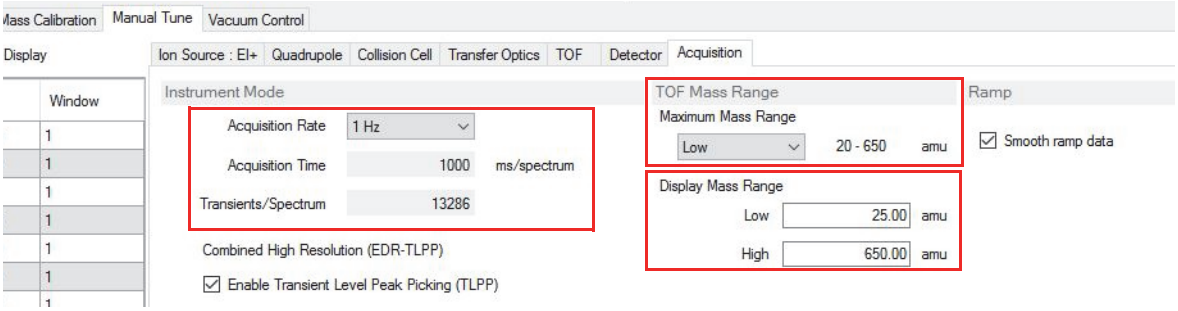


Figure 4 Selecting the m/z range of acquired data

Steps	Detailed instructions	Comments
2 Optimize the base ion abundance. This step is usually done before calibration is performed.	**a** Click the **Ion Source** tab and in the **Tune Masses** area select **Enabled**. **b** Enable the **Emission** and **EI Cal Valve**. **c** 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.	• See Figure 5 on page 10. • To enable calibrant flow ionization. • Higher values will saturate the signal and lower values will not provide sufficient ion statistics for optimal mass accuracy.

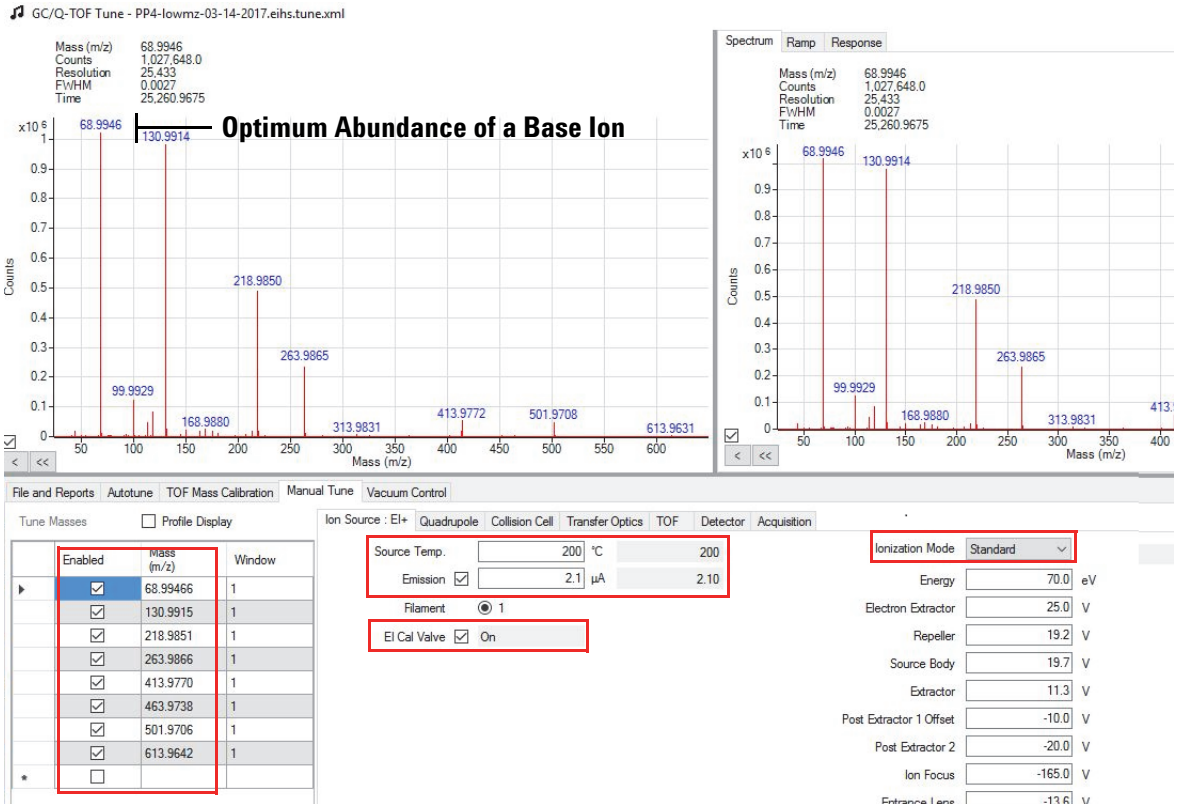


Figure 5 Optimizing base ion abundance

Steps	Detailed instructions	Comments
3 Perform a mass calibration.	**a** Select the **TOF Mass Calibration** tab from the **GC/Q-TOF Tune** window.	• See Figure 6 on page 11. Also see "Task 6. Acquire MS scan data (Optional)" on page 19.
	b Click the **Run Calibration** button.	• 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. See Figure 7 on page 12.
	c Select the **Close** button.	• The **TOF Mass Calibration Results** closes.
	d Click the **File and Reports** tab and save the tune file. **e** Select the **Close** button.	• Save the tune file as atune-lowmz_*date*.ei.tune.xml. Where *date* is today's date. • The **GC/Q-TOF Tune** window closes and you are returned to Instrument Control view.

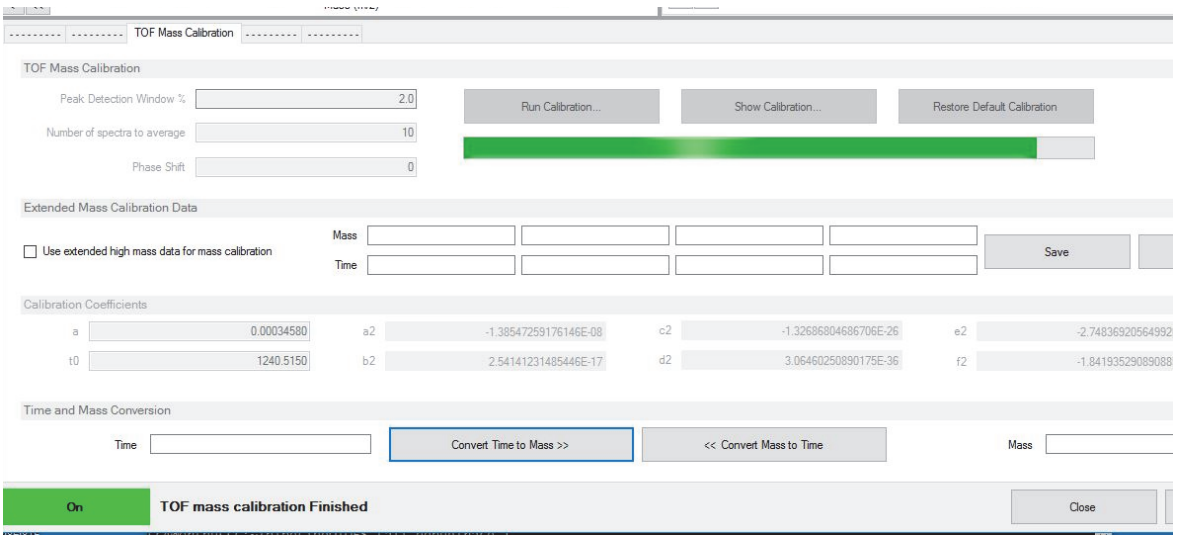


Figure 6 TOF Mass Calibration tab

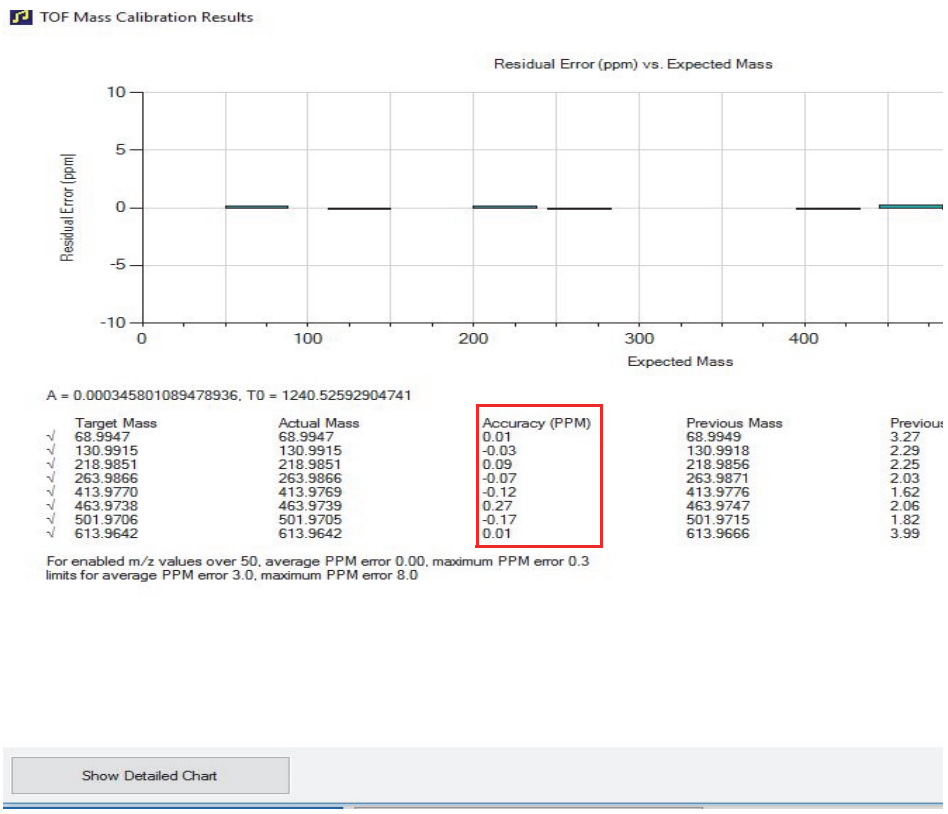


Figure 7 TOF Mass Calibration Results

Task 4. Enter GC acquisition parameters

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

Steps	Detailed instructions	Comments
1 Enter GC parameters appropriate for the sample. See Table 2.	**a** Click the **GC Edit Parameters** icon (Figure 1).	• The **GC edit parameters** window shown in Figure 8 on page 14 is displayed. • With the window selected, mouse over the icons to identify the icon from the tool tip.
	b In the navigation menu select **Columns** then select column **1** in the **Selection** column. **c** Select control mode **On** and then select **Constant Flow** mode. Enter 1.1 mL/min for the initial **Flow**. **d** 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. **e** In the **Collision Cell EPC** area, uncheck the **He Quench Gas**. **f** In the navigation menu select **Inlets** > **SSL** and enter the inlet parameters listed in Table 2. **g** Select the **Oven** icon and enter the oven parameters listed in Table 2.	• 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.
	h In the navigation menu select **ALS** >**Front Injector** and enter the injector parameters listed in Table 2.	• If your ALS is attached to the **Back Inlet** select the **Back Injector** tab.
	i In the navigation menu select **Aux Heaters**, enable, and set the temperature to 280 °C.	• This is the MSD transfer line heater.
	j Select the **OK** button.	• The GC parameters are downloaded to the GC and the window closes.

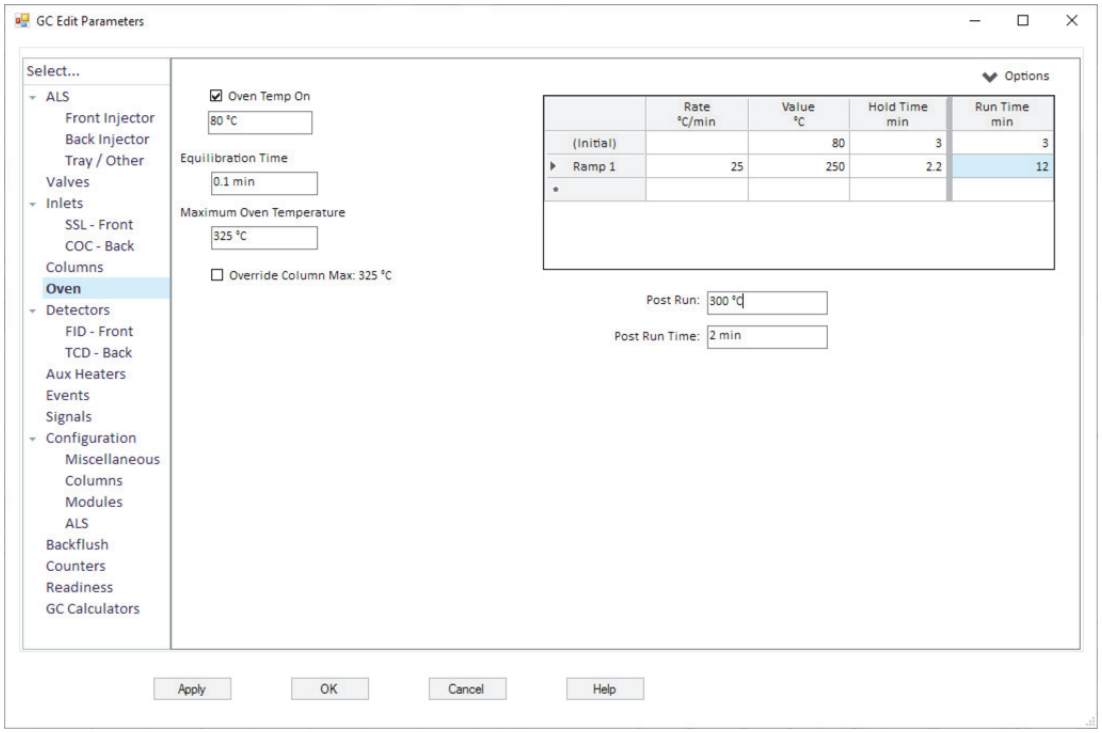


Figure 8 GC Edit Parameters window with **Oven** icon selected

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
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 Washes (PreInj)	2

Parameter	Value
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
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	6000 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 exercise 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.

Steps	Detailed instructions	Comments
1 Enter MS parameters appropriate for the sample and save the method as **OFN EI 70eV.M**.	**a** Click the **QTOF Method Editor** icon (Figure 1).	• The **QTOF Method Editor** window shown in Figure 9 on page 18 opens.
	b In the **Tune file** area, click the  icon.	• Select the tune file created in
	c 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**.	• Select the tune file created at the end of Task 3.
	d Set the **Solvent delay** to 5 minutes.	• The 7250 starts collecting data at 5 minutes due to the **Solvent delay** setting.
	e In the **Time Filtering** area select **Peak width** and set it to 0.7 seconds.	• Filter out unwanted peaks to reduce data storage.
	f In the **Data Threshold** area enter 10 for **counts**.	• Filter out unwanted noise to reduce data storage.
	g Select **Apply to Profile data**.	• To apply the **Data Threshold** filter to profile data to reduce storage.
	h 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.
	i Select **Both** for **Data stored**.	• Selecting **Both** stores both a peak's profile data and centroid data for data analysis.
	j 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.
	k Click **OK** to close the window.	
	l From the main window select **Method > Save Method As** and save the method as **OFN EI 70eV.M**.	

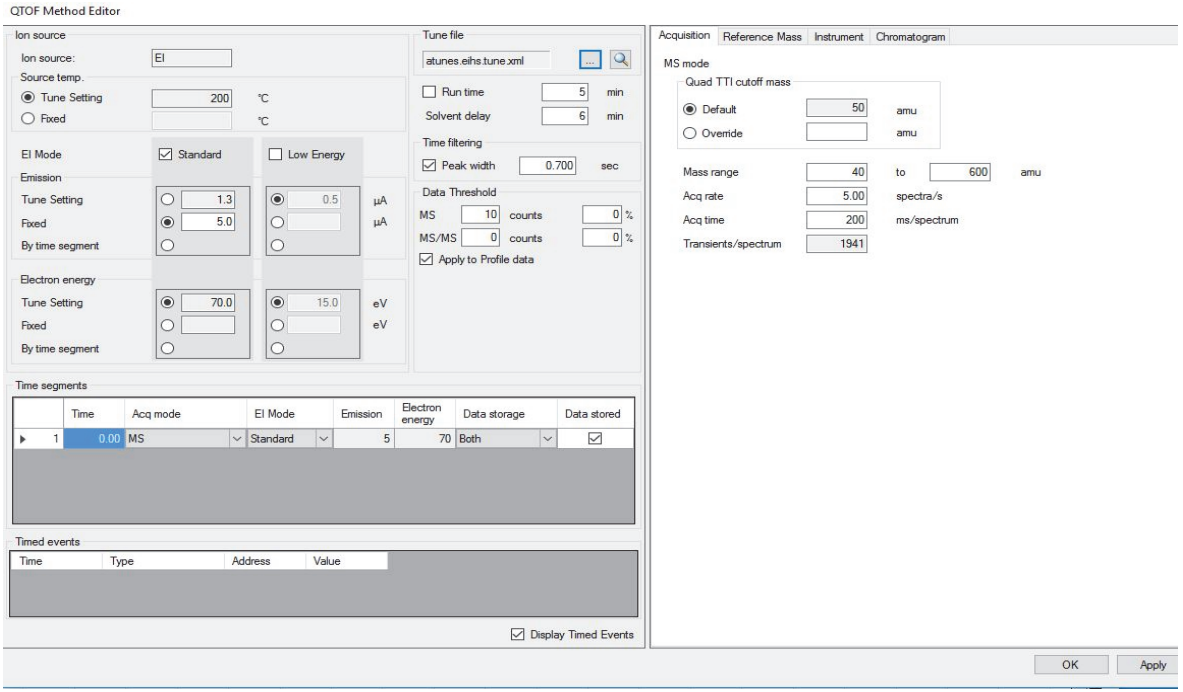


Figure 9 QTOF Method Editor

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 2. However, if you prefer, you can acquire your own data file as described in this task.

Steps	Detailed instructions	Comments
1 Acquire data (optional). • Name the data file **Sol_A.D**. • Designate a directory path to hold your data files and method.	**a** Click the **Start Run** (green arrow) icon. **b** In the **Data Path** enter the directory to save the data file that is acquired by this run. **c** In the Inlet section you are using, enter **Sol_A.D** for the **Data File Name**. **d** Enter the **Vial Number** location in the auto sampler tray. **e** Select **Current Method** for the **Injection Volume**. **f** In the **Method Sections to Run** section, select **Data Acquisition**. **g** Click the **OK and Run Method** button.	• The **Start Run** dialog box shown in Figure 10 on page 20 is displayed. • The **Injection volume** that you entered in Task 4 is used. • 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.

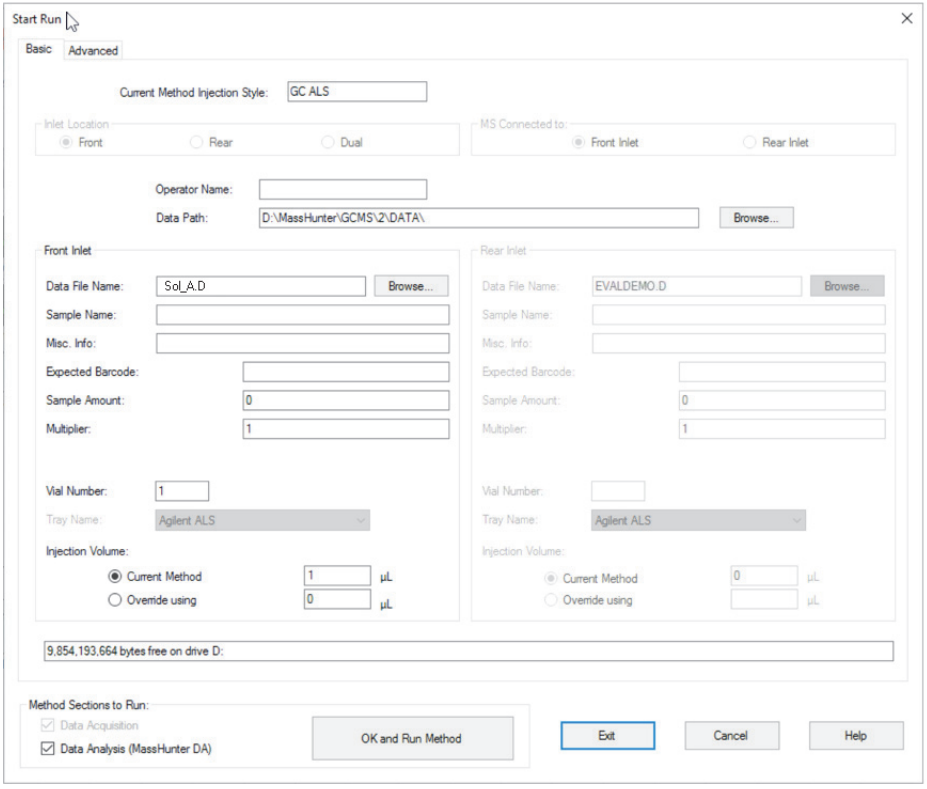


Figure 10 **Start Run** dialog box

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 2 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.

Steps	Detailed instructions	Comments
1 Add a mass calibration at the start of sequence.	**a** Insert an entry for the running of the mass calibration. **b** Add the **MASSCAL** keyword to this entry. **c** Enter the **Method** used for processing samples that follow and select **CAL** from the **Type** dropdown. **d** Save the sequence.	• This entry might follow the running of a sample blank. • See Figure 11 below. • This entry will also be used after running samples every 2 hours in our sequence.

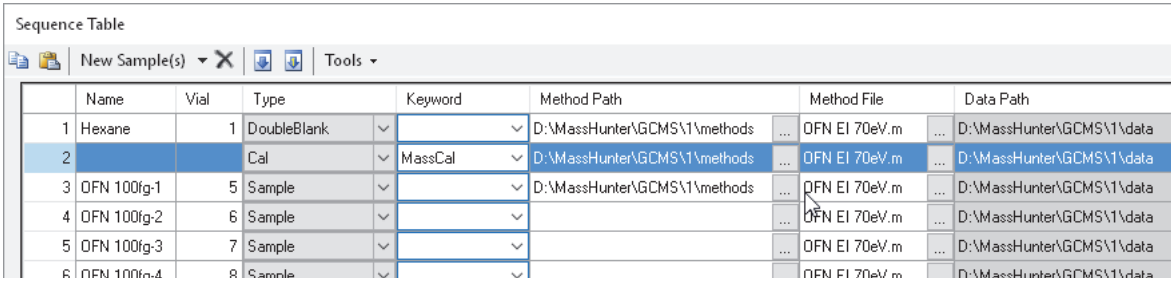


Figure 11 Sequence table setup for automated mass calibration

Steps	Detailed instructions	Comments
2 You can skip this step and use the run time procedure shown in the step below instead. Use this step to add a mass calibration entry at 2 hour intervals during sample runs by creating mass calibration entries in the sequence table.	**a** Calculate the number of samples to run before performing the mass calibration. **b** Copy the mass calibration entry added in the above step. **c** Select the sample entry location for the mass calibration and select **Insert sample** from the context menu. **d** Click paste with this entry selected. **e** Repeat step 2 as required. **f** Save the sequence.	• (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. • The copied calibration is entered.
3 The same result for the mass calibration interval created in the above step can be obtained at run time as follows.	**a** Select **Sequence > Run Sequence**. **b** Fill out the required information in the **Basic** tab. **c** Click the **Recurring Keyword** tab. **d** Select **Hours** as the **Recurring Type**. **e** Enter **2** hours for the **Recurring Interval**. **f** Select **MassCal** as the **Recurring Keyword** string. **g** Click **Run Sequence**.	• The **Start Sequence** dialog opens. • See online help for additional information. • You could also select **Method change or Hours** if you want to recalibrate when the method changes or at a timed interval. • The mass calibration runs at the specified interval. • The loaded sequence runs.

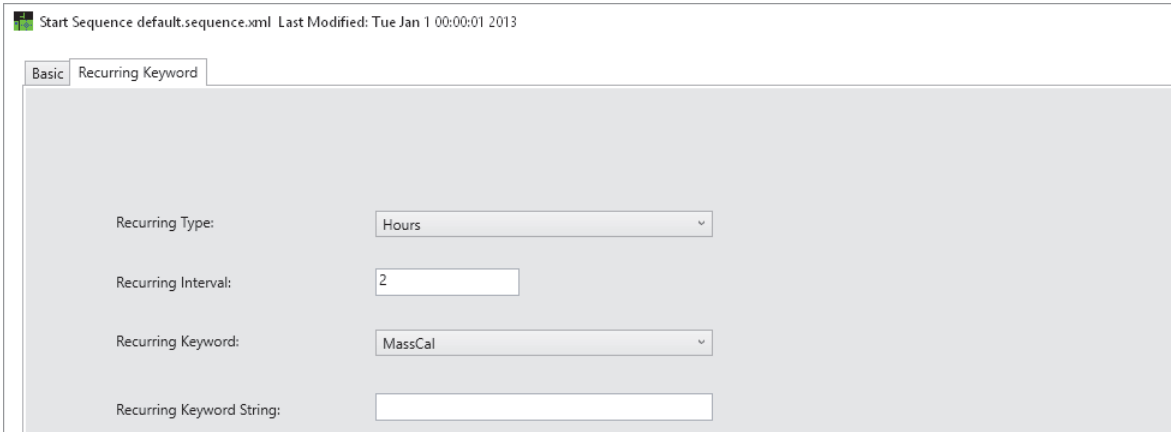


Figure 12 Using the Recurring Keyword feature

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 2.

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 2.

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

Steps	Detailed instructions	Comments
1 Start the Qualitative Analysis program.	**a** Double-click **Qualitative Navigator** shortcut on your desktop. 	• The system displays the Open Data File dialog. See Figure 13 on page 24. You can get help by: • Pressing the F1 key when a window is active • Selecting **Help > Contents** in the main menu • Selecting the **Help** icon in the active window
2 Load a Data file.	**b** Navigate to the location where your data file is located and then select your acquired data file or the demo file provided for this exercise **Sol_A.D**. **c** Under **Options**, select **Use current method** and clear **Load result data**. **d** Click **Open**.	• See Figure 13 on page 24. • The data file is loaded and a TIC of the data is displayed. See Figure 14 on page 24.

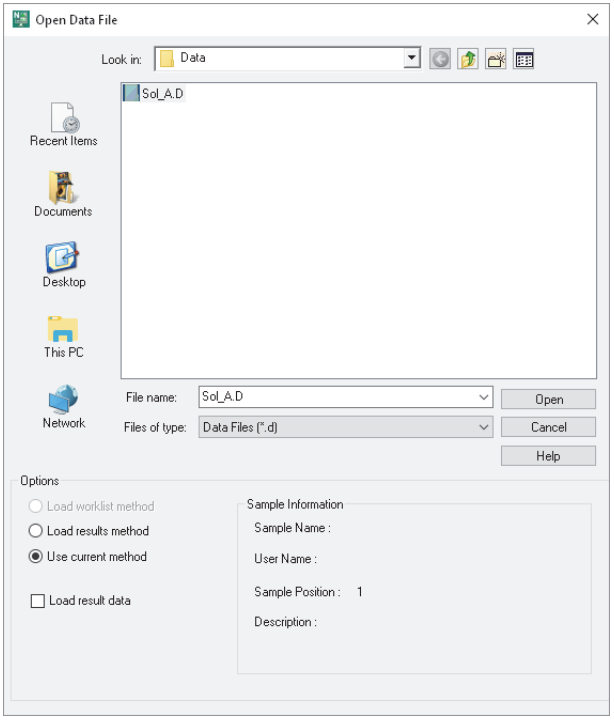


Figure 13 Opening the data file

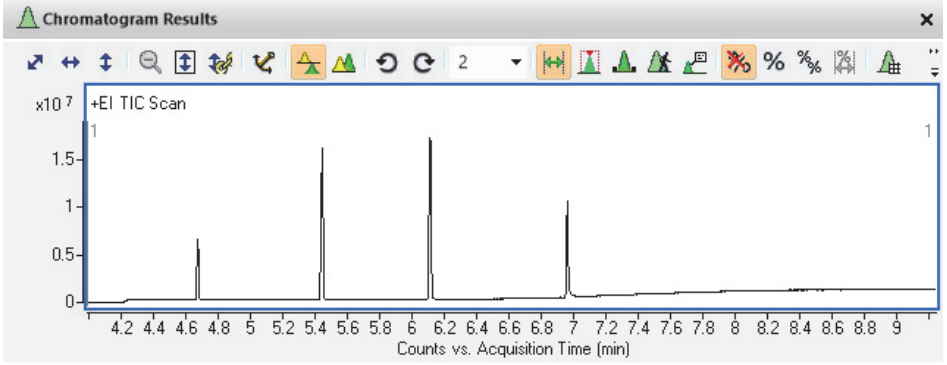


Figure 14 Sample Chromatogram for Sol_A.d loaded

Task 2. Configure the qualitative analysis user interface

Steps	Detailed instructions	Comments
1 Create a Qualitative Analysis method.	**a** From the main menu, select **Method > Open**. **b** Select default-GCMS.m and click **Open**. **c** From the main menu, select **Configuration > Show Advanced Settings**. **d** From the main menu, select **Configuration > User Interface Configuration**. **e** Click **OK**. **f** From the main menu, select **Method > Save As**. **g** In the **File Name** enter QTOF-GCMS.m and click **OK**.	• The **Open Method** dialog is displayed. • The default method for a GC QTOF is loaded. • Advanced menu items are now available. • The User Configuration dialog box opens. See Figure 16 on page 26. The settings selected here are based on the data file loaded and the default method previously selected. You may edit these if required. • To close the dialog.The **Save Method** dialog is displayed. • Rename the method since you can not overwrite the default method.
2 Configure the MS and MS/MS Spectra Display options.	**a** From the main menu, select **Configuration > MS and MS/MS Spectra Display Options**. **b** Click the **Spectrum Peak Label Options** tab and set the values for the peak labels. **c** Save the method.	• The **MS and MS/MS Spectra Display Options** dialog is displayed. • Use the values displayed in Figure 17 on page 27. These values result in the horizontal display of m/z and Formula & Ion Species value above identified spectra.
3 Assign a library for identifying spectra.	**a** Click the Method Editor icon to open the method editor and click on **Identify Spectra > Identification Workflow**. **b** Set the values for the library used to identify spectra. The demo.L library is included with MassHunter and includes the 4 compounds used in our sample	• For easier viewing, float the editor pane outside the Qualitative Analysis Navigator window, • Use the values displayed in Figure 18 on page 27.

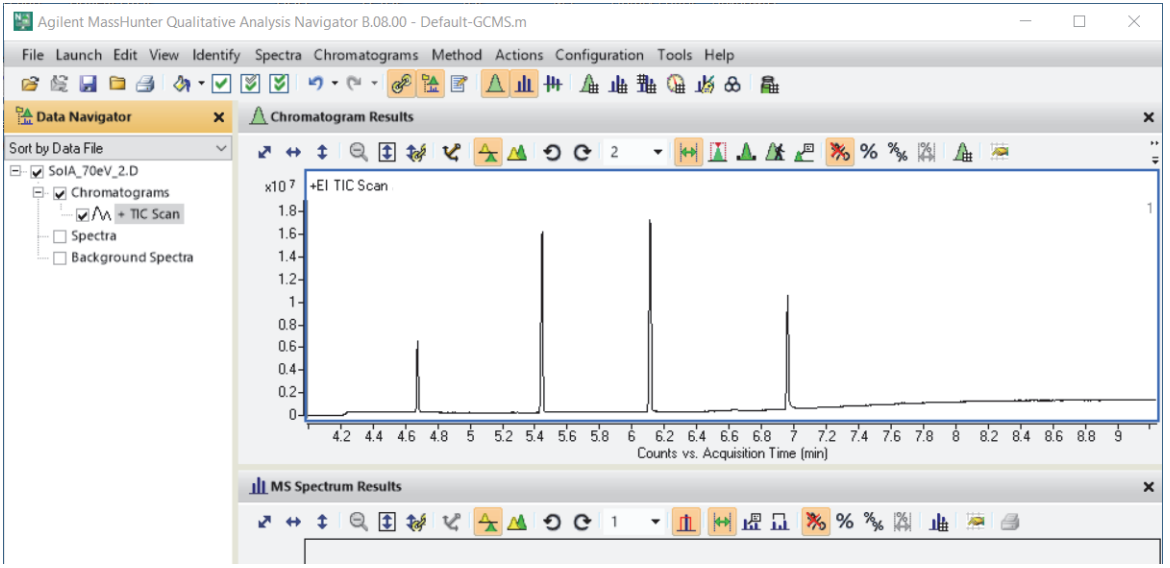


Figure 15 Default layout for a GC QTOF

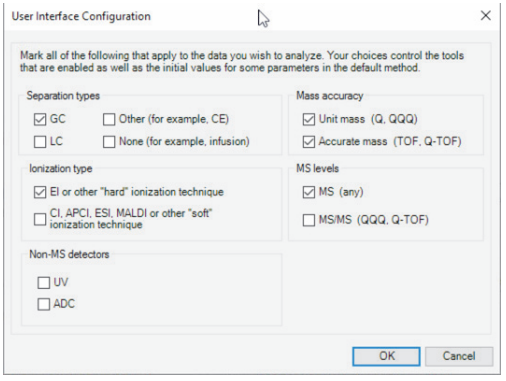


Figure 16 Configuring the User Interface

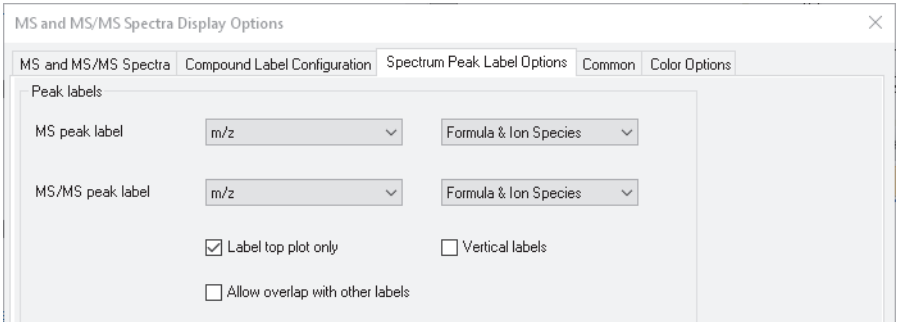


Figure 17 Configuring spectrum peak labels

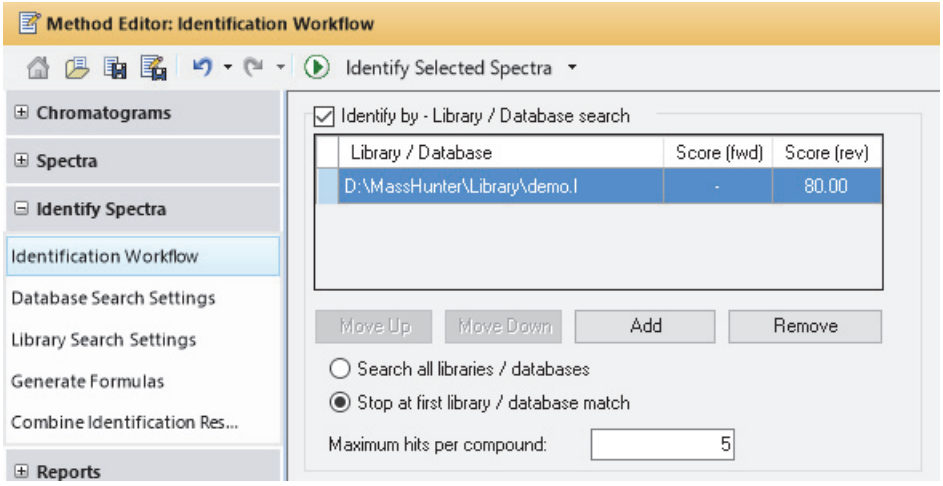


Figure 18 Assign a library for identifying spectra

Task 3. Identify Peaks in Qualitative Navigator

Steps	Detailed instructions	Comments
1 Identify the fist major peak in the Chromatogram.	**a** In the **Chromatogram Results** pane, right-click and drag around the peak at 4.676 RT.	• The single zoomed peak is displayed.
	b 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.
	c 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.
	d Click and drag the mouse to select an area containing the peak apex from RT 4.675 to 4.68.	
	e 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.
	f 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.
	g 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. See Figure 19 on page 29.
	h If desired, repeat this procedure to identify other peaks in this sample.	
	i In the Method menu select **Save**.	

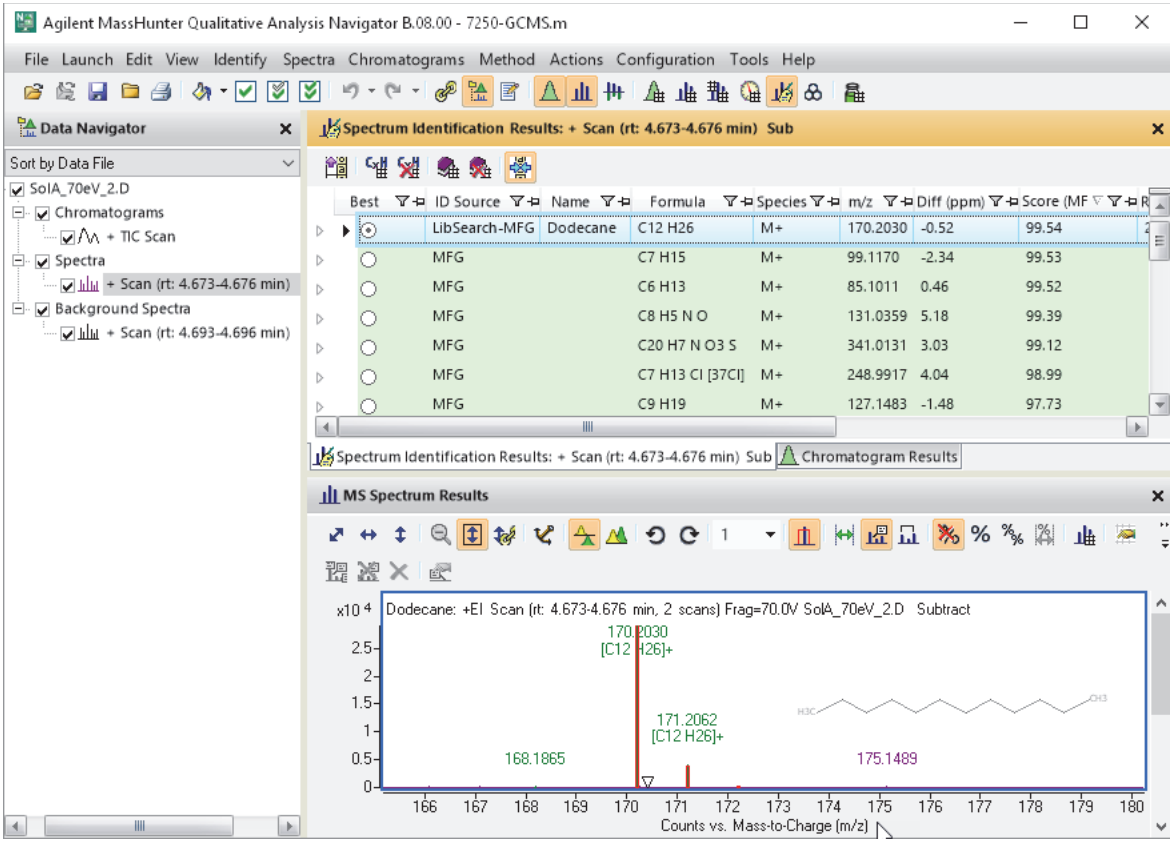
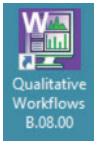


Figure 19 MS Spectrum Results showing predicted isotopes

Task 4. Identify compounds using Qualitative Analysis Workflows

Steps	Detailed instructions	Comments
1 Edit the Qualitative Analysis method settings in the **Compound Identification** workflow.	**a** Double-click **Qualitative Workflows** shortcut on your desktop. 	• The **Open Data File** dialog displays. If you are in the Navigator you can also select **Launch > Qualitative Workflows** from the main menu.
	b Select the SolA_70eV_2.D data file.	• This is the same data file loaded in the Qualitative Navigator in the previous task.
	c In the Method Editor, click **Method Automation/Workflow**.	• The **Options** tab displays.
	d In the Options tab select **Target/Suspect Screening** for the Workflow.	• **Find by Fragments** is automatically selected for **Compound mining** and is the only type available here.
	e In the Method Editor, click **Target/Suspect Screening** > **Find by Fragments** and click the T**arget Source** tab.	
	f Click ... and select SolA.cdb as the target source.	• You can find this file in the location shown in “Reference material” on page 2.
2 Configure the **Find by Fragments** algorithm.	**a** In the Method Editor click **Fragment Options** and set its values.	• Use the values shown in Figure 20 on page 31. It is important to have these values set correctly. The default values in the other tabs are OK for processing this sample.
	b 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.
3 Save the method.	**a** In the Method Editor toolbar click Save Method .	

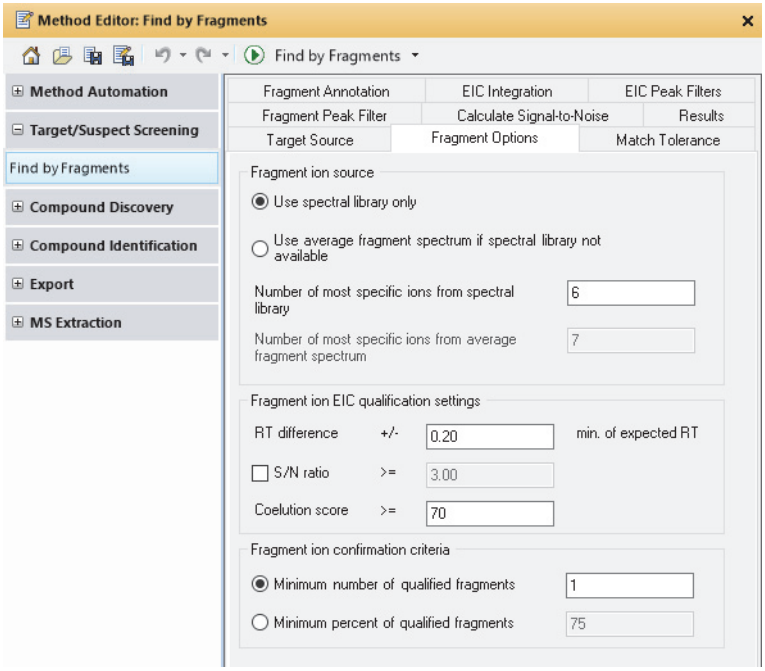
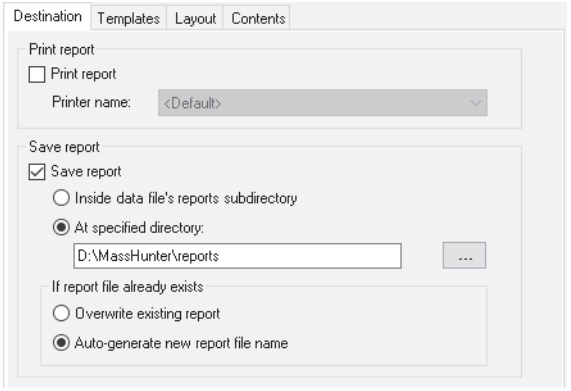


Figure 20 Setting values in the Fragments Options tab

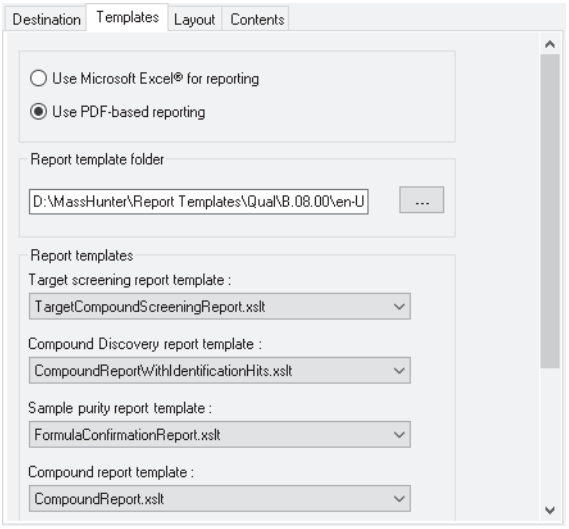
Task 5. Configure Method Automation Reports

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.

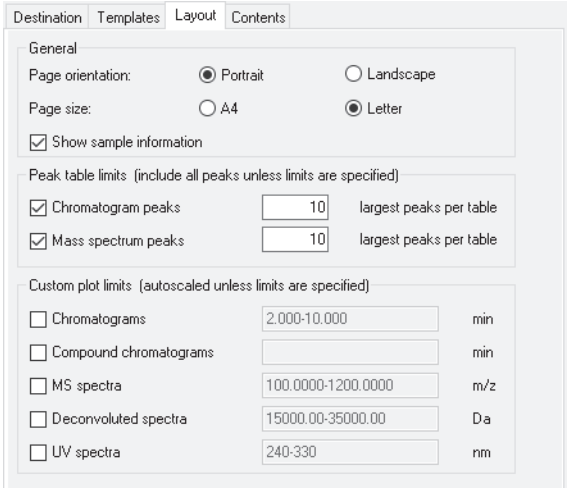
Steps	Detailed instructions	Comments
1 Edit the Qualitative Analysis method to include the **Reports** workflow.	**a** In the Method Editor, click **Method Automation** and select **Reports**.	• See Figure 21 on page 33.
	b Set the entries for the **Destination** tab to the values shown in Figure 21 on page 33. **c** Set the entries for the **Templates** tab to the values shown in Figure 21 on page 33. **d** Set the entries for the **Layout** tab to the values shown in Figure 21 on page 33. **e** Set the entries for the **Contents** tab to the values shown in Figure 21 on page 33.	• Select **Save Report** and enter a folder location for the PDF-based report. • For this exercise, based on the **c** workflow selection, we are using the **Target screening report template**. • Select the compound tables, chromatograms, spectrum types, peak tables and library search results spectrum types to include.
2 Save the method.	**f** In the Method Editor toolbar click Save Method  . **g**	



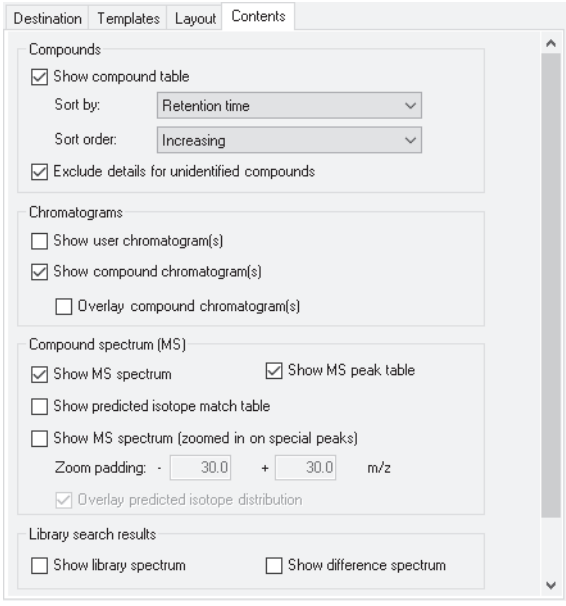
Destination tab



Templates tab



Layout tab



Contents tab

Figure 21 Reports tab settings

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.

Steps	Detailed instructions	Comments
1 Review the **Method Automation Workflow** for finding compounds.	**a** In the Method Editor select **Method Automation > Workflow**.	• See Figure 22 on page 35. This workflow was previously configured. See "Task 4. Identify compounds using Qualitative Analysis Workflows" on page 30.
2 Review the **Method Automation Reports** for generating a report of the results.	**a** In the Method Editor select **Method Automation > Reports**.	• This workflow was previously configured. See "Task 5. Configure Method Automation Reports" on page 32.
3 Run the Automation Workflow.	**a** 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. See Figure 29 on page 39.

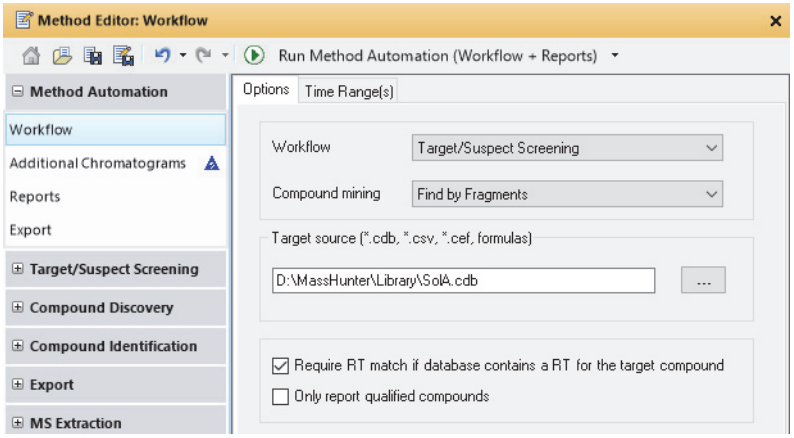


Figure 22 Setting values in Method Automation Workflow

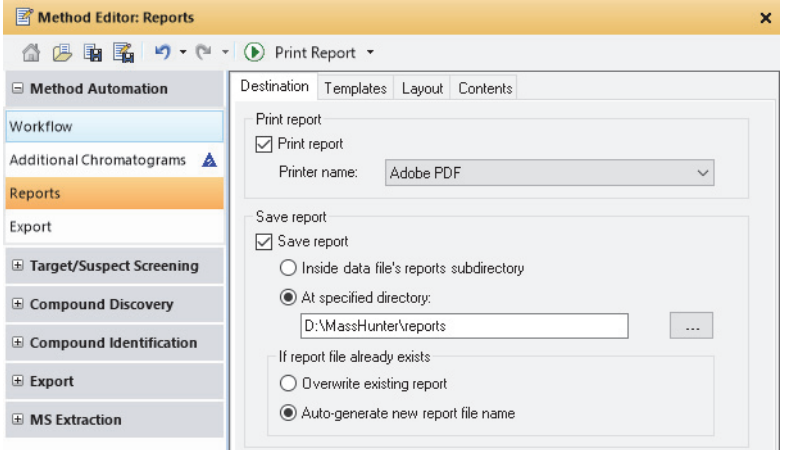


Figure 23 Setting values in Method Automation Reports

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.

Steps	Detailed instructions	Comments
1 Determine the number of compounds found and how many of these were identified.	**a** In the **Sample Table** section, the **Result Summary** column shows there were 4 qualified targets found.	• (See Figure 24 on page 36). • Scroll to the right to review the data in the other columns.
2 View the properties of the identified compounds in the **Compound List**.	**a** In the **Compound List** toolbar click **Hide any current empty columns** . **b** In the **Compound List** toolbar click **Auto Size All Columns** . **c** Scroll to the right and review the results for the four identified compounds.	• (See Figure 25 on page 37) Saves time reviewing the parameters • Eliminates adjusting columns.
3 View the **Compound Identification Results**.	**a** Click on the **Compound Identification Results** tab at the bottom of the window. **b** In the **Compound List** scroll right to view the **Compound Identification** section and select **Dodecane**. **c** Scroll to the right and review the results for Dodecane. **d** Select the other three compounds in this list and see the view their plots in the results panes.	• The **Compound Identification Results pane** is displayed. • Dodecane is the compound now displayed in the **Compound Identification Results** window. (See Figure 26 on page 37).

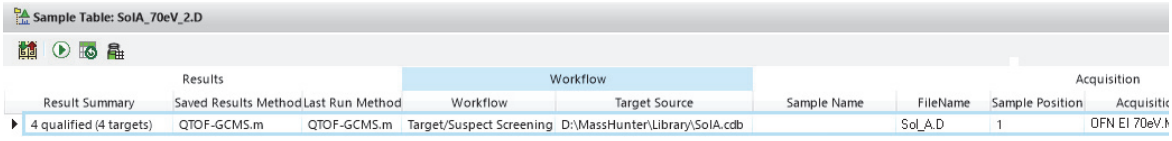
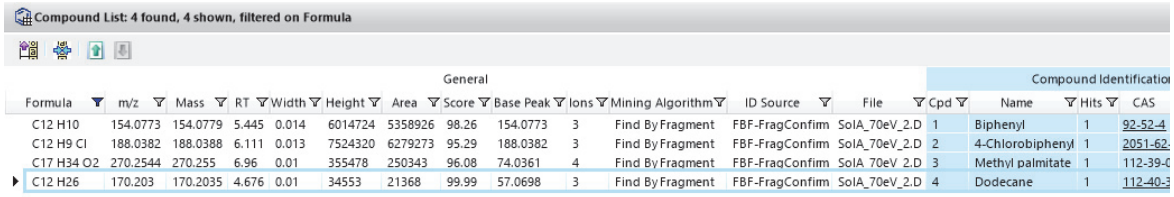


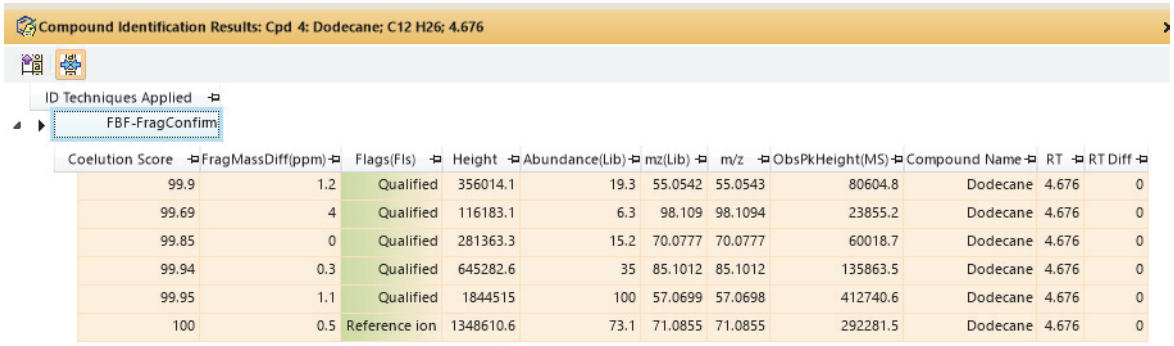
Figure 24 Sample Table results



Compound List: 4 found, 4 shown, filtered on Formula

	General												Compound Identificatio			
Formula	m/z	Mass	RT	Width	Height	Area	Score	Base Peak	Ions	Mining Algorithm	ID Source	File	Cpd	Name	Hits	CAS
C12 H10	154.0773	154.0779	5.445	0.014	6014724	5358926	98.26	154.0773	3	Find By Fragment	FBF-FragConfirm	SolA_70eV_2.D	1	Biphenyl	1	92-52-4
C12 H9 Cl	188.0382	188.0388	6.111	0.013	7524320	6279273	95.29	188.0382	3	Find By Fragment	FBF-FragConfirm	SolA_70eV_2.D	2	4-Chlorobiphenyl	1	2051-62-
C17 H34 O2	270.2544	270.255	6.96	0.01	355478	250343	96.08	74.0361	4	Find By Fragment	FBF-FragConfirm	SolA_70eV_2.D	3	Methyl palmitate	1	112-39-0
C12 H26	170.203	170.2035	4.676	0.01	34553	21368	99.99	57.0698	3	Find By Fragment	FBF-FragConfirm	SolA_70eV_2.D	4	Dodecane	1	112-40-3

Figure 25 Compound List results



Compound Identification Results: Cpd 4: Dodecane; C12 H26; 4.676

ID Techniques Applied

FBF-FragConfirm

Coelution Score	FragMassDiff(ppm)	Flags(FIs)	Height	Abundance(Lib)	mz(Lib)	m/z	ObsPkHeight(MS)	Compound Name	RT	RT Diff
99.9	1.2	Qualified	356014.1	19.3	55.0542	55.0543	80604.8	Dodecane	4.676	0
99.69	4	Qualified	116183.1	6.3	98.109	98.1094	23855.2	Dodecane	4.676	0
99.85	0	Qualified	281363.3	15.2	70.0777	70.0777	60018.7	Dodecane	4.676	0
99.94	0.3	Qualified	645282.6	35	85.1012	85.1012	135863.5	Dodecane	4.676	0
99.95	1.1	Qualified	1844515	100	57.0699	57.0698	412740.6	Dodecane	4.676	0
100	0.5	Reference ion	1348610.6	73.1	71.0855	71.0855	292281.5	Dodecane	4.676	0

Figure 26 Compound Identification Results for Dodecane

Steps	Detailed instructions	Comments
4 Review the **Sample Chromatogram Results** window	**a** Click **Compound Overlaid mode.**	• The Dodecane compound is prominently displayed in the TIC at a RT of 4.676 min.(See Figure 27 on page 38).
5 Review the **Compound Chromatogram Results** window and display its Overlaid mode.	**a** 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. (See Figure 28 on page 38).

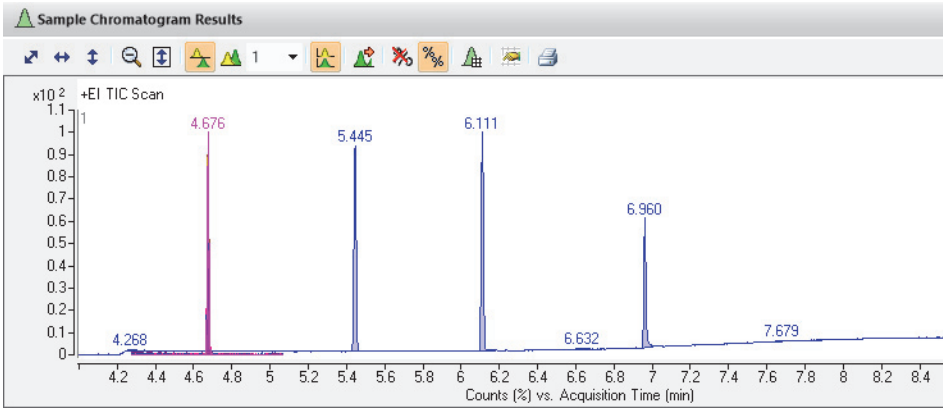


Figure 27 Sample Chromatogram Results for Dodecane in compound overlaid mode

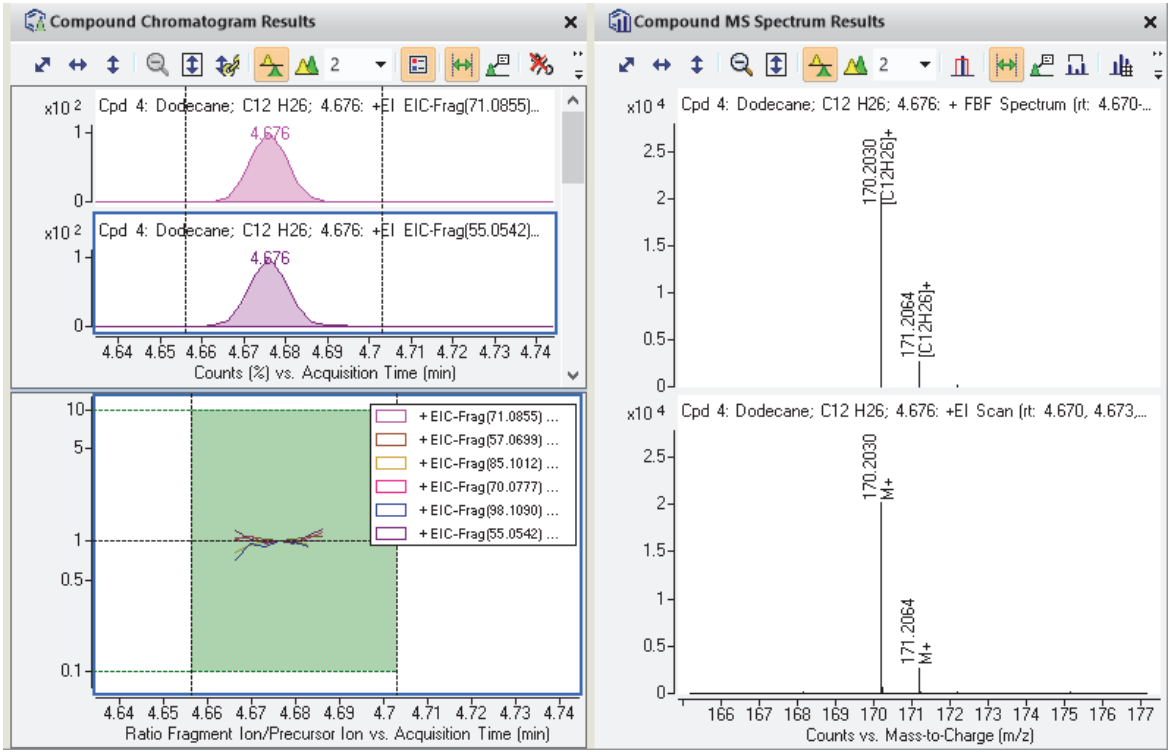
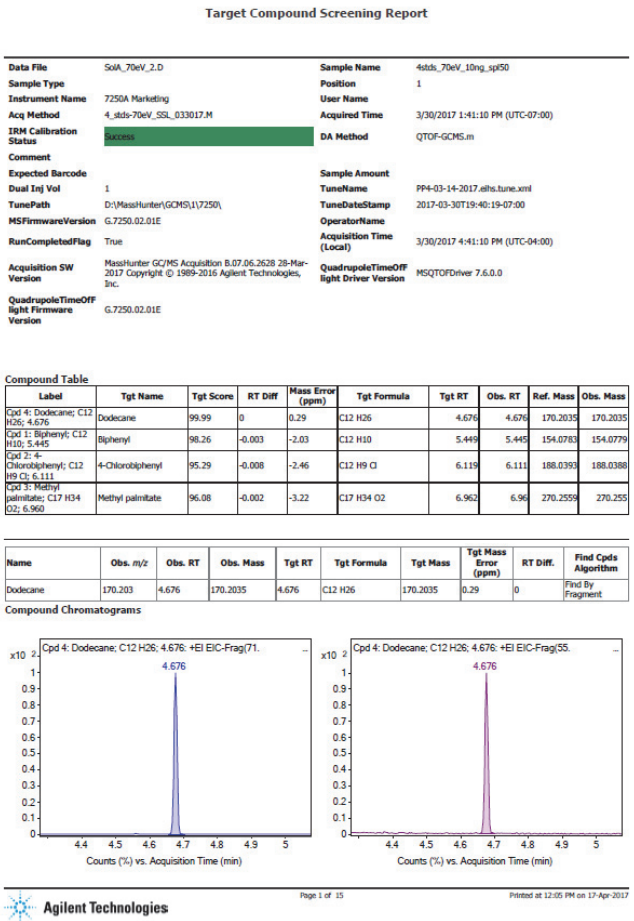


Figure 28 Compound Chromatogram and MS Spectrum Results for Dodecane

Steps	Detailed instructions	Comments
6 Review the compound identification report.	**a** 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. (See Figure 29 on page 39)



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

Compound Chromatograms

Agilent Technologies

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

Figure 29 Target Compound Screening Report

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.

Steps	Detailed instructions	Comments
1 Start the Unknown Analysis application.	**a** Double click the Unknown Analysis application shortcut on your desktop. 	• The application opens.
	b Select **File > New Analysis** from the main menu.	• The **New Analysis** dialog opens.
	c Enter UnknownsDemo.uaf for the **File name**.	• The application title bar displays this name for the analysis.
2 Add samples to the new analysis.	**a** Select **File > Add samples** from the main menu.	• The **Add Samples** dialog opens.
	b Select the Sol_A.D file from the Quant batch folder and click **OK**.	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.
3 Convert the sample data to SureMass format.	**a** Select **Tools > Convert Accurate Mass Samples** from the main menu.	• The **Convert Samples** dialog opens.
	b Browse to the Batch folder that contains the sample loaded above.	• See step 2 above.
	c Select (highlight) the Sol_A.D file from the batch folder and click **OK**.	
	d 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.

Steps	Detailed instructions	Comments
4 Edit the analysis method.	**a** Select **Method > Edit** from the main menu.	• The **Method** editor dialog opens.
	b On the **Peak Detection** tab select **SureMass** under **Peak Detection**.	• See Figure 30 on page 42.
	c Click on the **Deconvolution** tab and under **Resolution** change the **RT windows size factor** to 100.	• See Figure 31 on page 42.
	d Under **Component shape** deselect **Use base peak shape**.	
	e Click on the **Library Search** tab and click on **Change Library**.	• See Figure 32 on page 43. • The **Open** dialog opens.
	f Select the SolA.cdb file.	• Browse to the location where the SolA.cdb file is located.
	g Under **Search criteria** select **None** for the **Pre-search** type and select **Remove Duplicate Hits**.	
	h Under **Match Factor** select **Use RT Match**.	
	i Under **RT penalty function** select **Trapezoidal** , set the **RT range** to 20 sec and the **Penalty-free RT** range to 15 sec.	
	j Click on the **Compound Identification** tab and set the **Min MZ** to 60.	• See Figure 33 on page 44.
	k Click **Apply to All Samples**.	
	l Click **Advanced** and then click on the **Library Search** tab.	
	m Set the **Accurate Mass Tolerance** to 50 and click OK.	• Scroll to the last column in the table. The Method Editor closes. See Figure 34 on page 44.

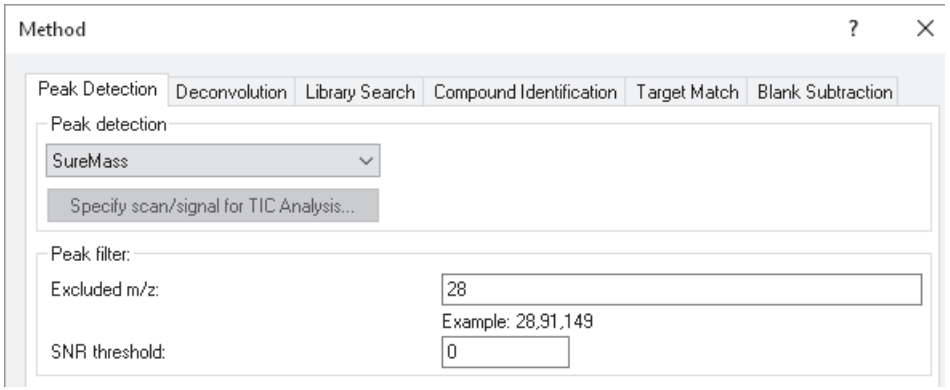

Figure 30 Method Editor Peak Detector tab parameter changes

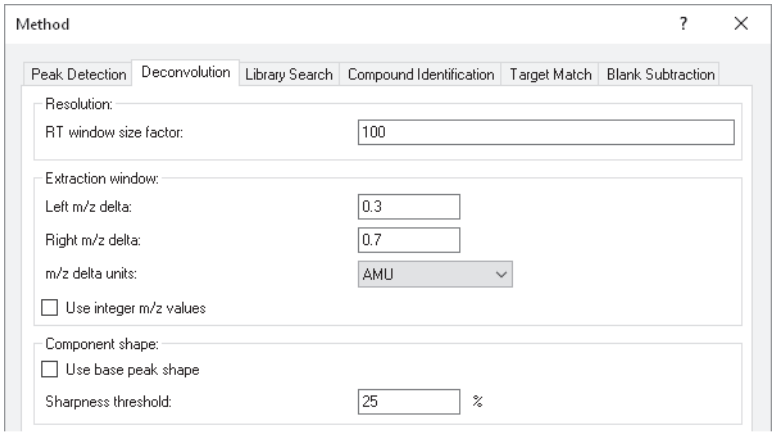

Figure 31 Method Editor Deconvolution tab parameter changes

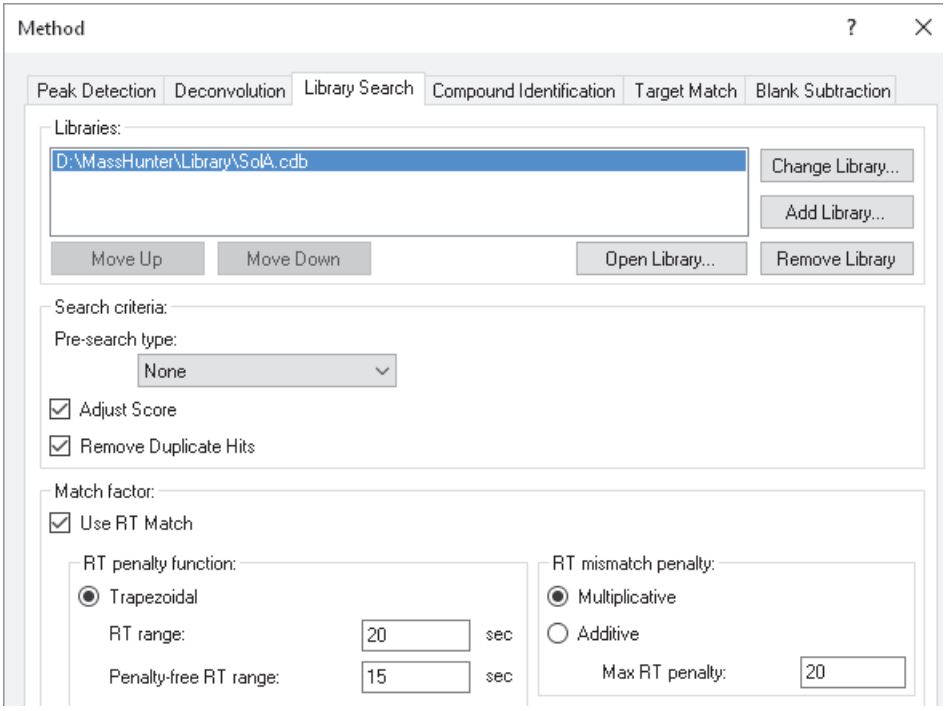

Figure 32 Method Editor Library Search tab parameter changes

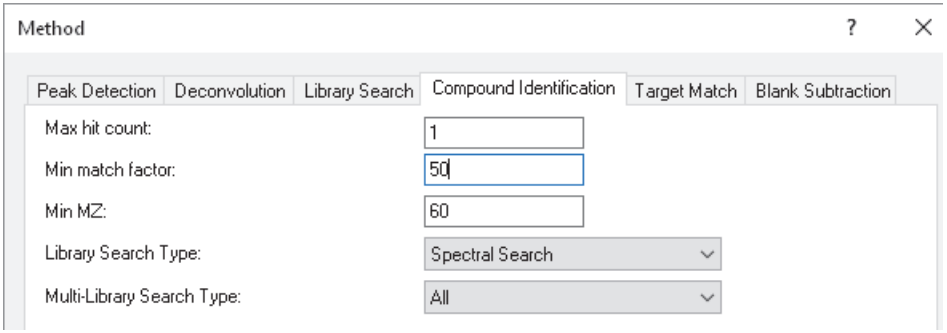

Figure 33 Method Editor Compound Identification tab parameter changes

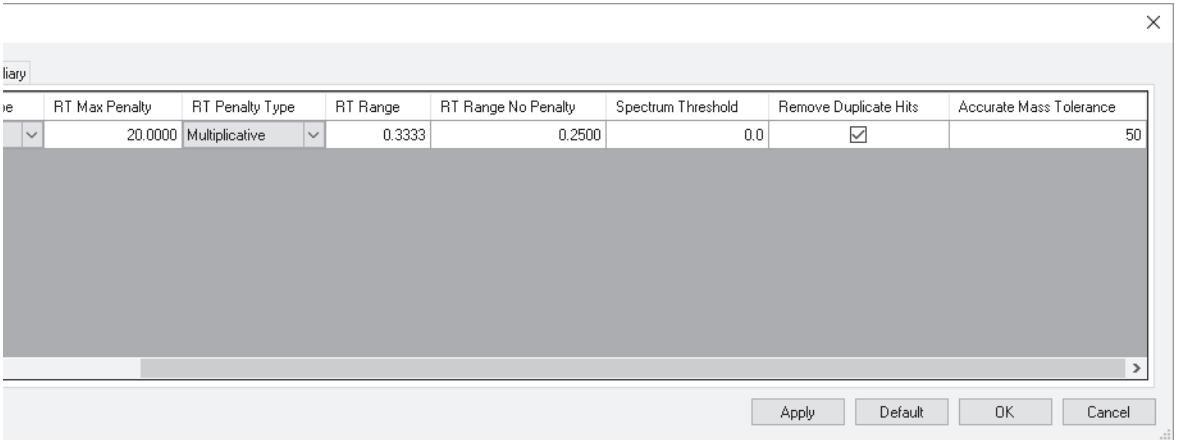

Figure 34 Method Editor Advanced Library Search parameter changes

Steps	Detailed instructions	Comments
5 Run the analysis.	**a** In the Unknowns Analysis toolbar click the **Analyze All** icon. **b** Click on **Non-Target** in the toolbar to display the identified compounds. **c** Right-click inside the Spectrum window and select **Header to Tail** if not already selected.	• The sample file is analyzed according to the parameters set in the method. • Results are displayed in Figure 35 on page 45. • This shows the Library spectra compared against the sample data.

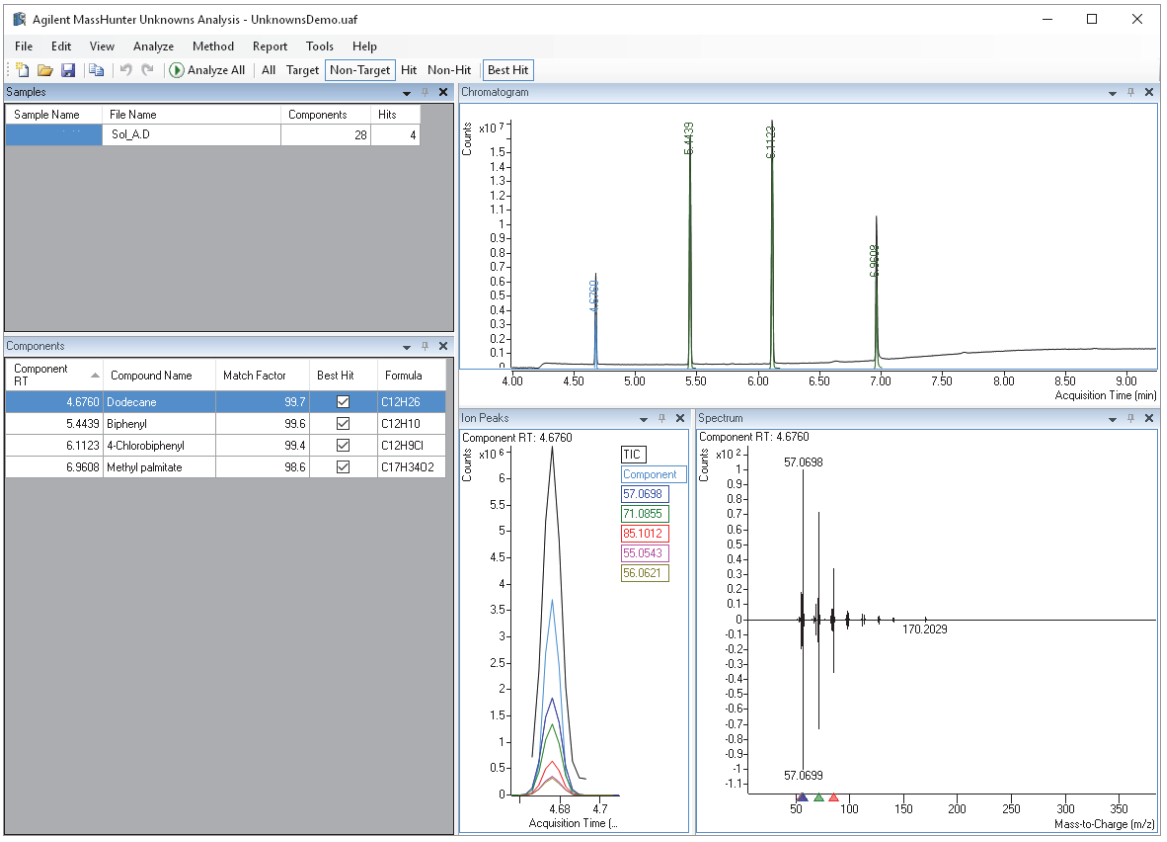

Figure 35 Unknown Analysis results

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