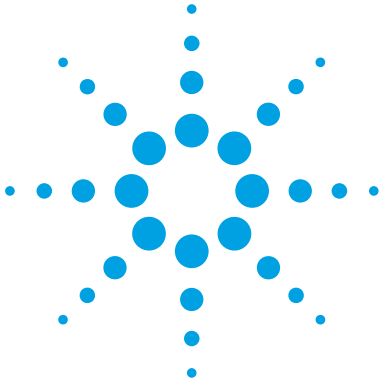


MassHunter PCDL for Qualitative Analysis

Familiarization Guide

Agilent Technologies

Use the exercises in this guide to learn how to use your Personal Database and Library (PCDL) with the Mass Hunter Qualitative Analysis program. You use the example Checkout Mix data, method files, and PCDL to learn how to find and identify compounds in a data file. The Checkout Mix data files, methods, and PCDL are based on the Pesticides Checkout Test Mix, which contains a wide variety of compound classes.

As an optional step, you can run the LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) to acquire your own data for use with this guide.

These Familiarization Files are included on the PCDL media:

- Checkout Mix PCDL
  - **Checkout_TestMix_Std.cdb**
- Checkout Mix example methods
  - **Checkout_TestMix_MS.m**

    TOF/Q-TOF acquisition method for MS-only analysis (positive mode)
  - **Checkout_TestMix_MS_neg.m**

    TOF/Q-TOF acquisition method for MS-only analysis (negative mode)
  - **Checkout_TestMix_MS_DA.m**

    TOF/Q-TOF data analysis method for MS-only analysis
  - **Checkout_TestMix_TMSMS.m**

    Q-TOF acquisition method for targeted MS/MS analysis
  - **Checkout_TestMix_TMSMS_DA.m**

    Q-TOF data analysis method for targeted MS/MS analysis
  - **Checkout_TestMix_AMSMS.m**

    Q-TOF acquisition method for auto MS/MS analysis
  - **Checkout_TestMix_AMSMS_DA.m**

    Q-TOF data analysis method for auto MS/MS analysis
- Checkout Mix example data files
  - **Checkout_TestMix_MS.d**
  - **Checkout_TestMix_TMSMS.d**
  - **Checkout_TestMix_AMSMS.d**
- Checkout Mix example reports

Before you begin

To do the exercises in this guide, you can use the Familiarization Checkout Mix example data files that are included with the PCDL. Or you can acquire your own data.

To prepare for the familiarization exercises

1 Install the Checkout Mix PCDL (**Checkout_TestMix_Std.cdb**):

   a From the **Contents and Information** page, click **MassHunter PCDL Familiarization Files** to expand the topic.

      The Contents and Information page appears when you insert the PCDL media into media drive. If the page does not appear, double-click **start.htm** from the PCDL media.

   b Click **Install the Checkout Mix PCDL**.

   Follow the instructions that are displayed to complete the installation.

2 Click **Open the Familiarization Files Folder**, then:

   a Copy the files from the **Checkout Mix Example Data** folder to the **\MassHunter\Data** folder on your computer.

   b Copy the files from the **Checkout Mix Example Methods** folder to the **\MassHunter\Methods** folder on your computer.

   The example data files were acquired with the Checkout Mix on a system with the LC/MS system configured as described in "To run the Checkout Mix" on page 6. The Checkout Mix PCDL media also includes the methods that were used to acquire the data files.

To prepare to run the Checkout Mix

1 Make sure that you have these required parts and reagents:

- LC/MS grade acetonitrile, and water
- Glacial acetic acid 99.9% (highest purity)
- ZORBAX LC Column (p/n 959758-902), Eclipse Plus C18, 2.1 mm × 100 mm

2 Check that the Agilent 1200 Series Infinity LC is properly installed and verified.

3 On the Agilent 1200 Series Binary Pump SL, check that the mixer and damper are bypassed. See "To bypass mixer and damper" on page 8 for details.

4 Check that *one* of the following instruments is properly installed and verified:

- Agilent 6200 Series Time-of-Flight LC/MS (TOF), *or*
- Agilent 6500 Series Quadrupole Time-of-Flight (Q-TOF)

NOTE The 6200 Series TOF LC/MS cannot acquire MS/MS data, so you can only do the exercises in "Familiarization Exercises - Compound Search" on page 10 with data acquired on a 6200 Series TOF LC/MS.

5 Check that MassHunter Data Acquisition B.05.00 or higher is properly installed.

6 To use a system configuration that is different from the one described in "To run the Checkout Mix" on page 6, then create or edit a method for your system configuration and the Checkout Mix method parameters. The Checkout Mix parameters are in the Checkout Mix example data acquisition method.

Use the MassHunter Data Acquisition program to open and view these method files:

- **Checkout_TestMix_MS.m** for compound searches
- **Checkout_TestMix_TMSMS.m** (targeted MS/MS), or **Checkout_TestMix_AMSMS.m** (auto MS/MS) for library searches (Q-TOF only)

These methods include Data Acquisition settings for the Checkout Mix:

- Data Acquisition method information

- Q-TOF LC/MS settings
- Wellplate sampler settings
- Binary pump settings
- Thermostatted column compartment settings

7 Prepare the reference ion solution as recommended in the installation guide for your instrument. *Do not use the trifluoracetic acid (TFA) found in the reference kit*.

The Checkout Mix methods use two reference ions, which are dispensed from reference bottle A of the calibration delivery system. The two compounds used are from the API-TOF Reference Mass Solution (p/n G1969-85001) and are purine and HP-0921.

If you previously used TFA in your calibrant, make sure little or no TFA signal remains.

Use the same reference solution for positive and negative ion analysis. If you do not get a usable negative ion signal for purine at *m/z* 119.06320, clean your ion source.

Alternative configuration

The Checkout Mix example methods and data files are all based on the configuration described in the installation instructions. Any Agilent Q-TOF LC/MS instrument configuration can be used for library search screening and identification, but not all configurations have been tested. No retention times are provided with the library. You can create as many custom libraries as you need for your use. These libraries can be named to distinguish your chromatographic conditions and the matrices for which they are intended.

Running the Checkout Mix

To run the Checkout Mix

1 Do a check tune to verify that the instrument operates properly.

Refer to the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide* for instructions to tune the instrument.

2 Prepare the Checkout Mix.

The concentration of the Checkout Mix stock solution is 100 ppm for both positive and negative mixes.

a Dilute 100 µL of the stock solution to 10.0 mL with acetonitrile to create Working Solution 1(1 ppm).

Use Working Solution 1 for systems with no iFunnel and no Agilent Jet Stream source. The examples in this guide were produced in this way, with the Q-TOF LC/MS operating in the **Low (1700 m/z) mass range** and **2 GHz Extended Dynamic Range** mode.

b Take 1 mL of Working Solution 1 and dilute it to 10.0 mL with 10:90 acetonitrile:water to create Working Solution 2 (100ppb).

Use Working Solution 2 for systems with an Agilent Jet Stream source, or for systems with iFunnel optics. The examples in this guide were produced in this way, with the Q-TOF LC/MS operating in the **Low (1700 m/z) mass range** and **2 GHz Extended Dynamic Range** mode. On some instruments, or when operating the Q-TOF LC/MS in the **4 GHz High Resolution** mode, dilute this solution again (to make a 10 ppb Working Solution 3) if needed.

c Transfer an aliquot of the Working Solution 1, 2, or 3 to a standard 2-mL sample vial for analysis.

Do this step separately for the positive and negative Checkout Mix.

NOTE

For some instrument configurations, this sample concentration is too high. If you consistently see "saturated" warnings listed for some compounds, or if "*" indicators routinely appear above mass peaks in spectra, dilute the sample again by a factor of 10 or more, and inject the diluted sample.

3 Prepare mobile phases A and B.

- A= 5 mM acetic acid in water (286-µL glacial acetic acid in 1 L water)
- B= 100% acetonitrile

These mobile phases are suitable for both acidic and basic Checkout Mix.

The examples in this guide were run in positive mode only, using a different mobile phase optimized for basic analytes. The elution order of the basic analytes differs slightly from the examples in the guide when you use this composition.

4 Verify the system configuration.

The Checkout method uses the system configuration listed in the next table. If your system deviates from this configuration, adjust the method as needed.

Column	ZORBAX LC Column (p/n 959758-902), Eclipse Plus C18, 2.1 mm × 100 mm
Wellplate Sampler	h-ALS-SL+, model# G1367D
Pump	Binary Pump – G4220A configured with damper and mixer bypassed. See "To bypass mixer and damper" on page 8.
Column Compartment	Column – SL, Model G1316B

For Database Searches

5 Load the Checkout method **Checkout_TestMix_MS.m** or **Checkout_TestMix_MS_neg.m**.

6 Check that the method is set up to make a 1-µL injection.

7 Click **Sample > Run** to do a single sample run, or create a worklist to make multiple injections.

8 If you do not see all the peaks after you process your data:

a Extend your **Stop time** in the method to 15 minutes.

b Check that you detect reference ions between 10,000 and 250,000 counts, and that their *m/z* values are within a few millidaltons of the expected *m/z* values.

c Make sure that your system is tuned and calibrated correctly.

d Run the Checkout Mix again.

This step does not affect your results. It shows if retention times are different on your system. A number of reasons can change your retention times from the retention times determined by Agilent, such as different instrument delay volume, dead volumes, or configuration.

For Library Searches (6500 Series LC/MS only)

9 Run the Checkout Mix again with the methods **Checkout_TestMix_TMSMS.m** and **Checkout_TestMix_AMSMS.m**.

When you run the Checkout Mix with this method, a workflow is simulated for the screening and identification of analytes using library searching. Refer to the application notes that are included on your PCDL media.

To bypass mixer and damper

Bypass the mixer and damper only if you have a G1312B Agilent 1260 Infinity Binary Pump.

The Binary Pump SL is delivered in standard configuration (damper and mixer connected). This step shows how to bypass the damper and mixer and convert the pump to low delay volume mode.

Agilent does not support configurations in which only the damper or the mixer is disconnected while the other part is still in-line.

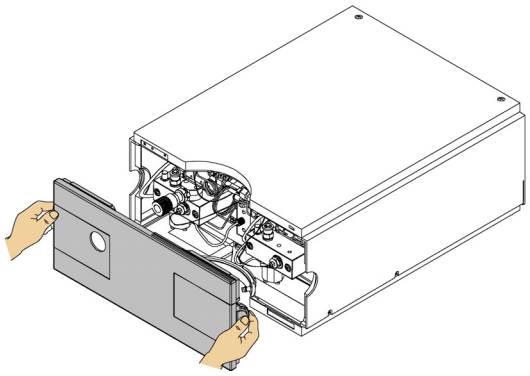
Tools required

- wrench, 1/4-inch x 5/16-inch (p/n 8710-0510)
- wrench, open end, 14-mm (p/n 8710-1924)
- hex driver, 1/4-inch, slitted (p/n 5023-0240)

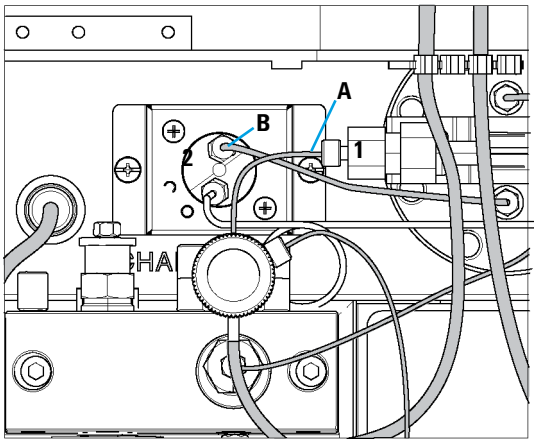
Preparations for this procedure

- Flush the system (water if buffers were used, otherwise IPA).
- Turn off the flow.

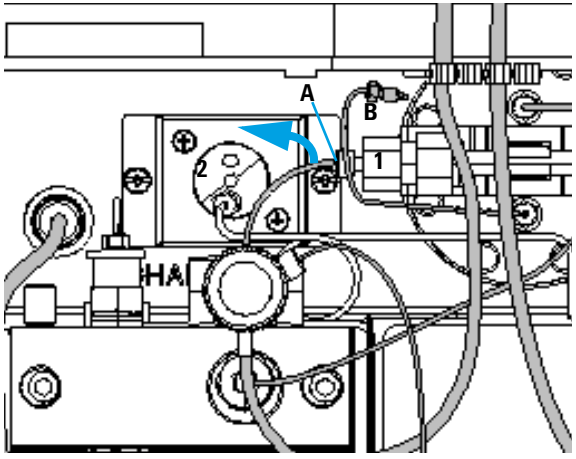
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



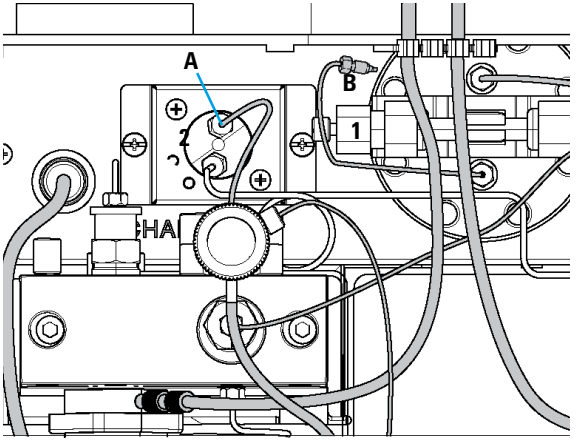
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



3 Fold capillary end **B** away. It remains unconnected. Disconnect fitting **A** from outlet **1** of the mixer.



4 Connect fitting **A** to port **2** of the pressure sensor. Seal port **1** of the mixer with a plastic blank nut.



Familiarization Exercises - Compound Search

Use the Checkout Mix example data files that are included with the PCDL. You can also use the data that you acquired from the Checkout Mix and Checkout Mix method.

Two exercises are described in this topic to do a compound search.

For screening workflow, use Find by Formula. See "Exercise 1. Process and interpret data with Find by Formula" on page 11. In Find by Formula, the PCDL is used as the formula source. Compounds that are present but are not in the PCDL are not identified.

For unknown/discovery workflow, use Find by Molecular Feature Extraction (MFE). See "Exercise 2. Process and interpret data with Find by Molecular Feature Extractor" on page 18. Find by MFE finds all compounds, which you can then search against the PCDL.

The elution order of the compounds in the Checkout Mix have been determined using the Eclipse Plus C18 column and mobile phases specified in the "To run the Checkout Mix" on page 6. The expected elution order is:

- Aminocarb
- Imazapyr
- Thiabendazole
- Dimethoate
- Imazalil (Enilconazole)
- Metoxuron
- Carbofuran
- Atrazine
- Metosulam
- Metazachlor
- Molinate
- Malathion
- Pyraclostrobin
- Diazinon (Dimpylate)

Depending on the delay volume the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.

The example data used in this guide were measured with a slightly different mobile phase composition and the resulting elution order in the example figures is different.

Exercise 1. Process and interpret data with Find by Formula

Before you begin, make sure that **Checkout_TestMix_Std.cdb** was copied to **\MassHunter\PCDL** on your computer.

If you acquired data from the Checkout Mix, you can use the data file that you acquired. Your results can differ slightly.

Steps	Detailed Instructions	Comments
1 Process the data file for the positive ion Checkout Mix. Open the data file.	**a** Open the Agilent MassHunter Qualitative Analysis program. Click **Cancel** if you are asked to open a data file. **b** Process the data file for the positive ion Checkout Mix: • Load the method **Checkout_TestMix_MS_DA.m**. • Open the data file **Checkout_TestMix_MS.d**. See Figure 1.	

Steps	Detailed Instructions	Comments

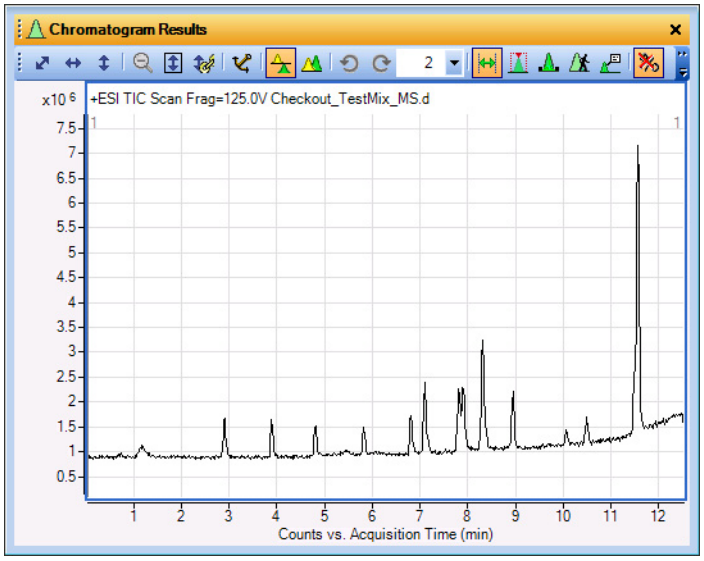


Figure 1 Example Checkout Mix Total Ion Chromatogram

2 Review the method to become familiar with the settings for Find by Formula. Use the database **Checkout_TestMix_Std.cdb**.	**a** Locate the **Find Compounds by Formula > Options** section in the Method Explorer. **b** Select the custom database **Checkout_TestMix_Std.cdb** . See Figure 2. **c** Review the settings in this method to become familiar with peak detection, mass tolerances, and other settings. If needed, adjust for specific matrices.	**Checkout_TestMix_Std.cdb** does not contain retention times or isomers, therefore all compounds are easily identified using the Mass option. To identify easily the isomeric compounds, add retention times to your custom PCDL and select one of the Mass and retention time options.

Steps	Detailed Instructions	Comments

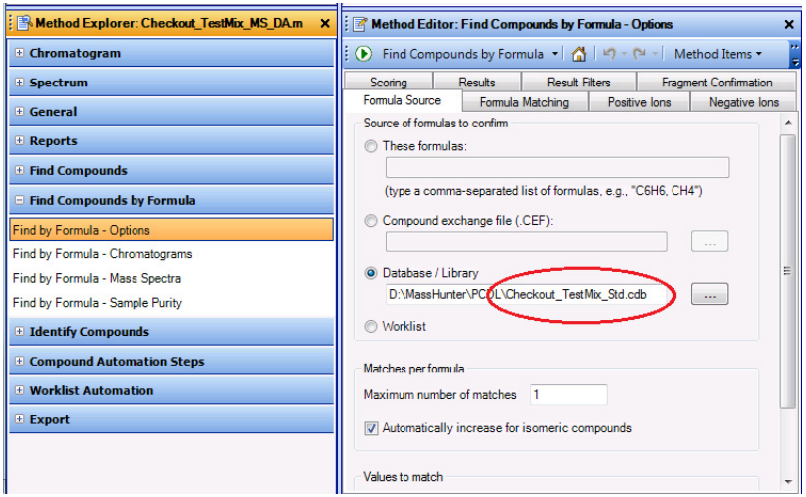


Figure 2 Find by Formula Method Editor Options (Custom Database)

3 Check that the desired ion species are present.	**a** In the **Positive Ions** tab, check that the desired ion species are present. See Figure 3. For example, make sure that the adduct m/z is not shown if only the protonated species is desired.	In this exercise, you look only for protonated species, to minimize data processing time. Typical data files contain multiple adduct species. When you create or edit a data analysis method for your own data, select the ion species that are appropriate to the mobile phase and sample preparation.

Steps	Detailed Instructions	Comments

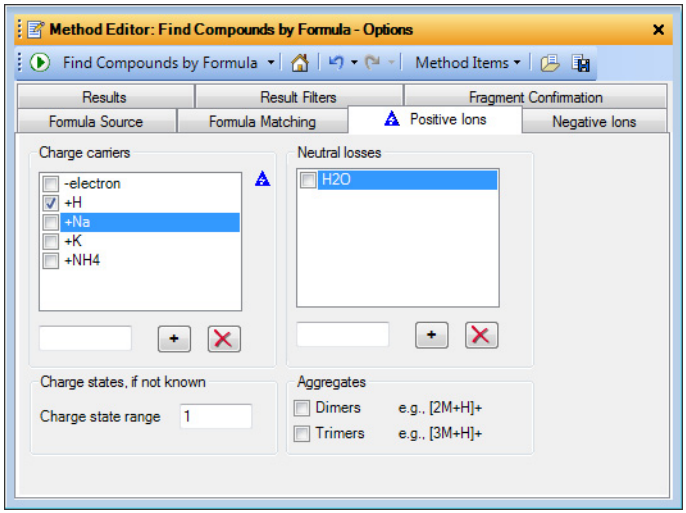


Figure 3 Positive Ions tab.

4 Use the Checkout Mix PCDL to find compounds in the data file **Checkout_TestMix_MS.d**	**a** Click the green arrow ( ) in the Method Editor toolbar.	The Qualitative Analysis program searches each entry in the Checkout Mix PCDL (**Checkout_TestMix_Std.cdb**) to find compounds in the data file.

Steps	Detailed Instructions	Comments

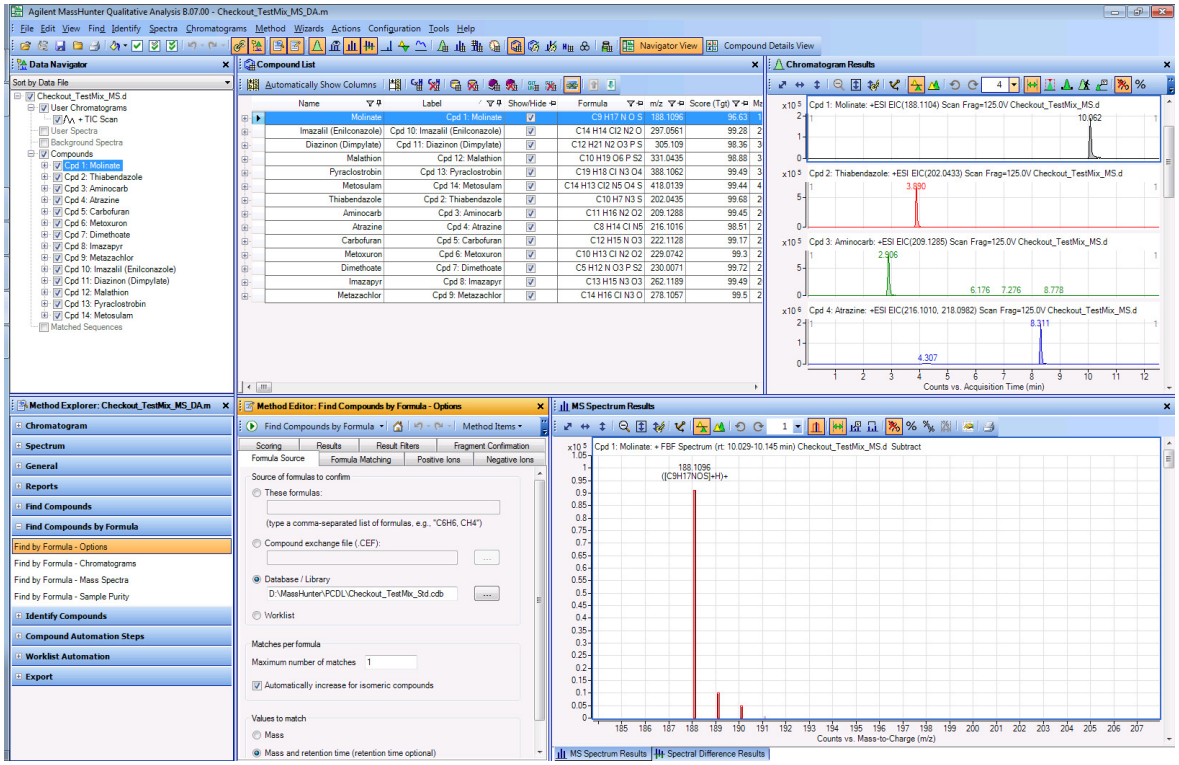


Figure 4 Find By Formula Results using the Checkout Mix PCDL (**Checkout_TestMix_Std.cdb**).

5 Review the Compound Table. Return to the Navigation view when you are done.	**a** Click **Compound Details View** to switch views. See Figure 5. **b** Click or use the arrow keys to move through the Compound Table to review one compound at a time. **c** Click Navigator View.	

Steps	Detailed Instructions	Comments

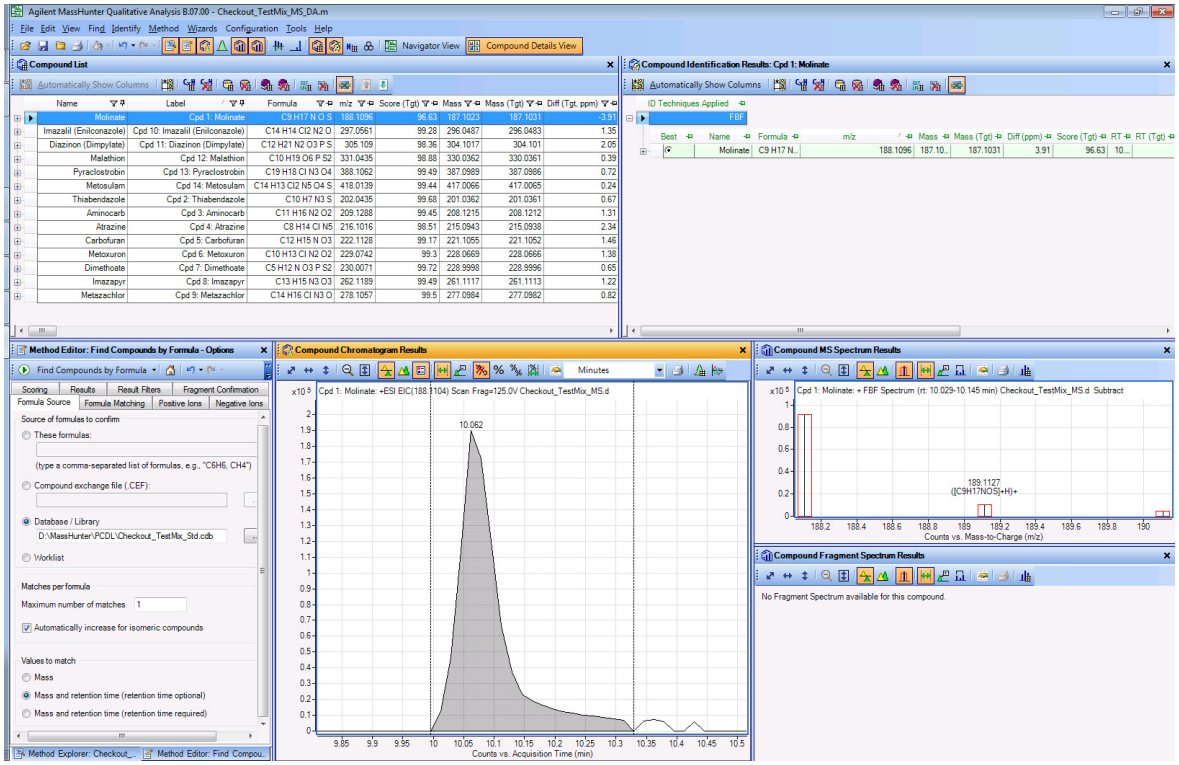


Figure 5 Compound Details view.

6 Export the compound list as a spreadsheet in text format.

- **a** In the Compound List table, select all rows.
- **b** Right-click anywhere in the compound list and select **Export**. See Figure 6.
- **c** For **File type**, select **Data as Text file (*.txt; *.csv)**.
- **d** Click **OK**.

The spreadsheet file appears in the data file folder with the same name as the data file.

You use this file in a later exercise for Targeted MS/MS analysis.

The **Checkout_TestMix_MS.csv** data file in Excel format is included in the **Example Reports** folder on the PCDL media.

Steps	Detailed Instructions	Comments

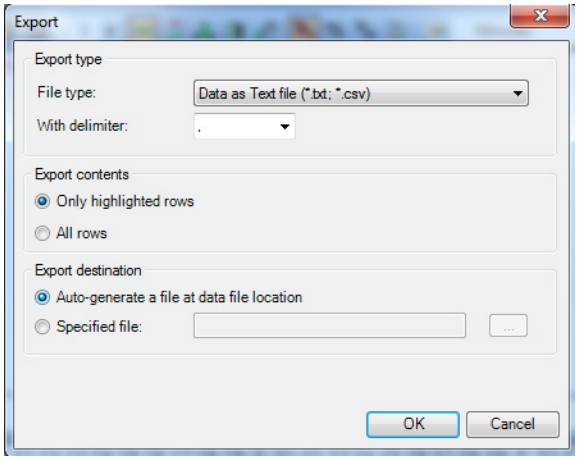


Figure 6 Export Find by Formula results to a Text file.

7 Remove the results before you do the next exercise and close the Compound List.	**a** Click **Find >Delete Find Compound Results** to remove the results **b** Close the Compound List to free up display space.	

Exercise 2. Process and interpret data with Find by Molecular Feature Extractor

Steps	Detailed Instructions	Comments
1 Review the settings for Find by Molecular Feature. Make sure that only protonated species are selected.	**a** Locate the **Find Compounds/Find by Molecular Feature** section in the Method Explorer. **b** In the Method Editor, review all settings in the Find Compounds by Molecular Feature tabs. Adjust these settings per sample type and according to sample matrices. Click **Find by Molecular Feature > Ion Species** and make sure that only the protonated species is checked. If multiple adduct ion species are checked, the compound result list becomes long. See Figure 7.	In this exercise, you look only for protonated species, to minimize data processing time. Typical data files contain multiple adduct species. When you create or edit a data analysis method for your own data, select the ion species that are appropriate to the mobile phase and sample preparation.

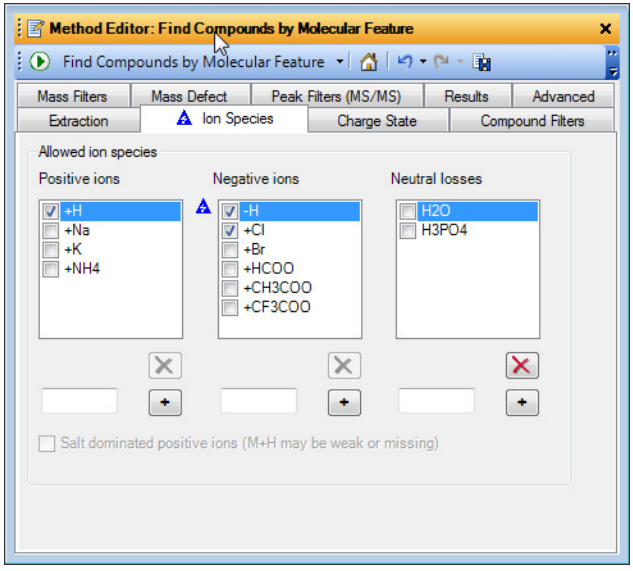


Figure 7 Ion Species tab.

Steps	Detailed Instructions	Comments
2 Search the data file to generate a compound list. Use the model settings.	**a** Click the green arrow () in the Method Editor toolbar.	The Molecular Feature Extractor (MFE) "mines" the data file for all possible compounds and uses a "first principle" approach. Once the possible compounds have been separated and identified from probable background interferences, a compound list is generated. All possible analytes according to the method settings are extracted. Figure 8 illustrates the results for Find by Molecular Feature.
3 Search the PCDL for the selected compounds.	**a** In the Data Navigator, click the **Compounds** line to select all compounds that MFE generated and which are shown. **b** When all the compounds are selected, right-click the selected compounds and click **Search Database for Compounds** from the shortcut menu (Figure 8).	If the Advanced tab is not visible in the Method Editor, click **Configuration > User Interface Configuration**. Then mark the **Accurate mass (TOF, Q-TOF)** and **Show advanced parameters** check boxes.

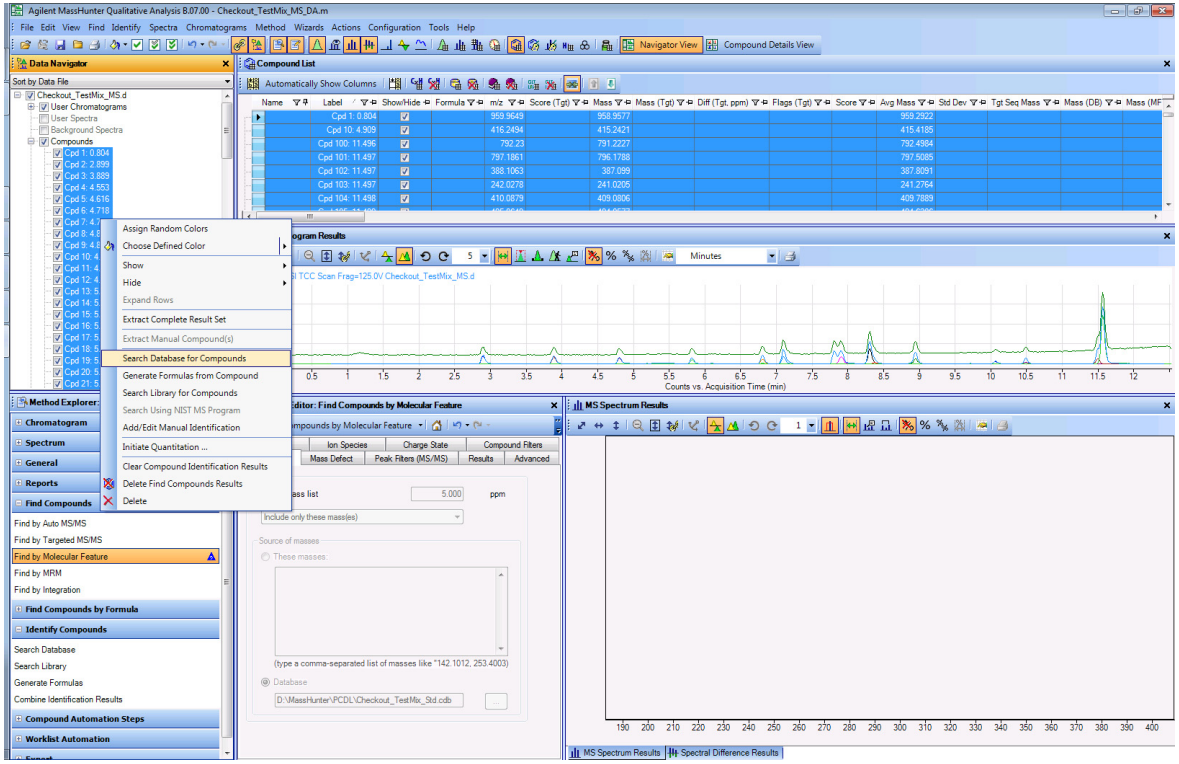


Figure 8 Database Search Results on Find by Molecular Feature compounds. To get the overlaid chromatograms in the display, use the **Overlaid** tool at the top of the Chromatogram Results window.

The custom database is searched against each MFE result. Figure 9 shows the compound identification results obtained from a search on the Checkout Mix PCDL.

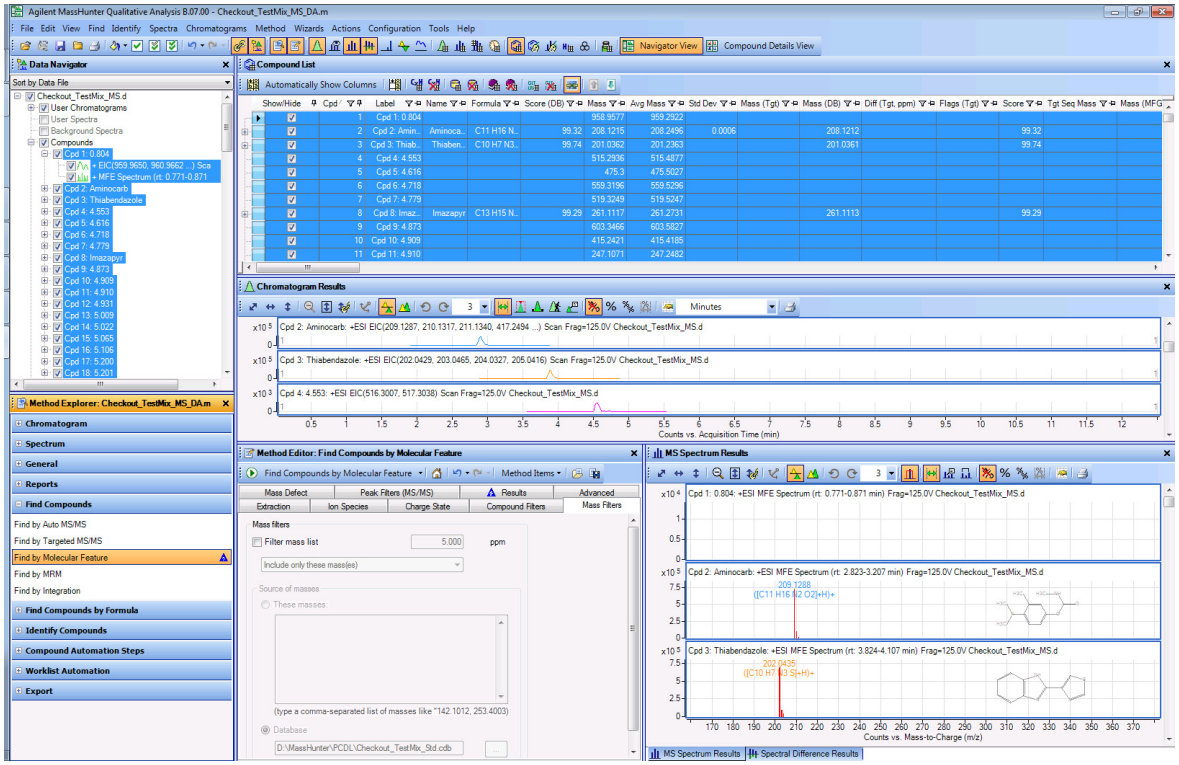


Figure 9 Find by Molecular Feature Database Search. Use the tools at the top of the Compound List window to hide columns, auto-size the column widths, and sort the list.

Exercise 3. Process data automatically using Worklist Automation

After you decide the correct settings for all aspects of the Find Compounds algorithms and Search Database algorithms, you can save these settings to one Qualitative Analysis method for repetitive and consistent data manipulation over time. (Refer to the application notes included on the PCDL media for example workflows.)

The Worklist Automation feature of the MassHunter Qualitative Analysis program lets you take advantage of the ability to save reprocessing options. This topic describes how you can set up Worklist Automation to:

- process a data file automatically with the Find by Molecular Feature algorithm
- search the Checkout Mix PCDL
- send the report of results to a specific printer or data file location.

Steps	Detailed Instructions	Comments
1 Open the automation worklist.	**a** In the Method Explorer, click **Worklist Automation > Worklist Actions**.	The Method Editor shows a list of automatic Qualitative Analysis actions that are executed in the order shown.
2 Add actions to the worklist.	**a** Copy the actions that you want the method to do from the **Available actions** list to the **Actions to be run** list. See Figure 10.	If Search Database for Compounds is selected as an action to be run, then make sure that in the **Find Compounds by Molecular Feature > Results** tab, the **Highlight All Compounds** option is selected.

Steps	Detailed Instructions	Comments

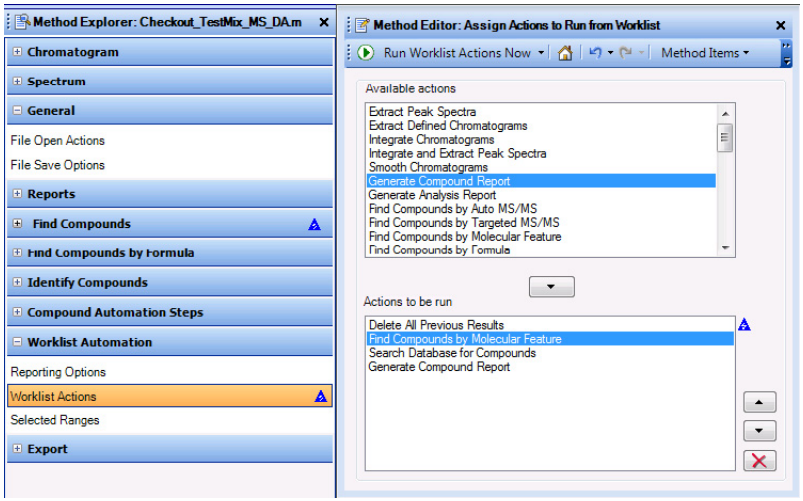


Figure 10 Method Editor with list of selected actions

3 If you chose **Generate Compound Report**, then modify the reporting options.	**a** From the **Worklist Automation** list, click **Reporting Options**. **b** In the Method Editor, in the Reporting Options section, set your reporting options. See Figure 11.	

Steps	Detailed Instructions	Comments

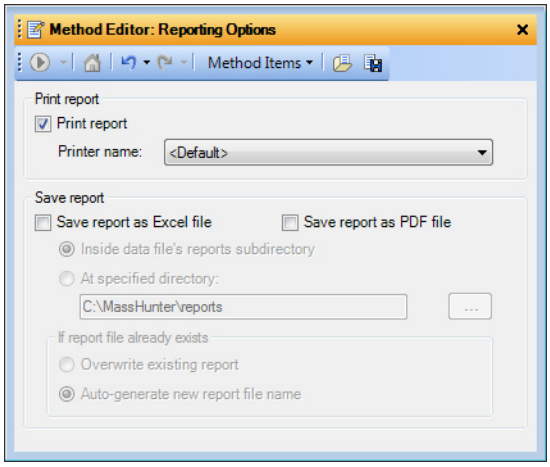


Figure 11 Reporting Options

4 Save the method settings to an acquisition method.

a In the MassHunter Qualitative Analysis program, click **Method > Save As**.
b Browse to the folder on your system that contains the Data Acquisition method that you want to automate.
c Click the name of the Data Acquisition method that you want to automate and click **Save**.

The Qualitative Analysis method is now attached and is a part of the Data Acquisition method.

5 Create a Data Acquisition worklist, and then run the worklist.

a In the MassHunter Data Acquisition program, click **Worklist > Worklist Run Parameters**.
b For **Part of method to run**, select **Both Acquisition and DA**.
c Select whether **Execution for Acquisition-DA** is to be **Synchronous** or **Asynchronous**.
d Save the worklist.
e Run the worklist.

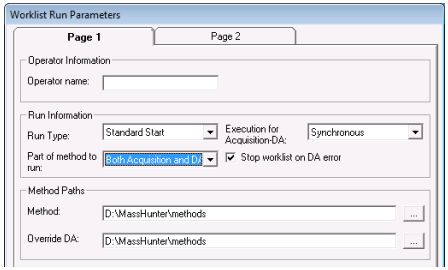


Figure 12 Worklist Run Parameters window

The Qualitative Analysis steps defined and set up under **Actions to be Run** in the Method Editor runs automatically during the sample acquisition without any user intervention.

Using worklist automation, features of the MassHunter Data Acquisition program for TOF and Q-TOF with the MassHunter Qualitative Analysis program and in combination with the Checkout Mix PCDL, samples can be screened for and reported automatically.

You can create smaller and more focused custom user databases from the MassHunter master PCDL for specific industry needs, such as work-place drug testing or environmental contaminate analysis. See "To develop a custom PCDL" on page 26.

NOTE Some compounds in the database only ionize using specific LC/MS sources, such as electrospray or APCI.

To develop a custom PCDL

The use of a smaller and more focused database and library to screen samples can be a powerful tool to detect and identify specific analytes.

1 In MassHunter PCDL Manager, create a custom user PCDL. You can do either of these steps:

- Create a new PCDL. Use the MassHunter master PCDL as the starting file.

  See "To create a custom PCDL" in the *Agilent MassHunter PCDL Manager Quick Start Guide*.

- Open the MassHunter master PCDL, select compounds of interest, and create a subset PCDL.

  See "To create a subset PCDL from selected compounds" in the *Agilent MassHunter PCDL Manager Quick Start Guide*.

2 Use a standard chromatography method to run standards of targeted compounds to identify compound retention times. Add the retention times to your custom user PCDL.

  See "To update retention time data" in the *Agilent MassHunter PCDL Manager Quick Start Guide*.

3 For compounds that do not contain the library spectra that you need, add the spectra from the Qualitative Analysis program.

  See "To send spectra from Qualitative Analysis" in the *Agilent MassHunter PCDL Manager Quick Start Guide*.

These technical notes describe how to create a custom user PCDL, and to add retention times to the custom user PCDL.

- *Pesticide Personal Compound Database for Screening and Identification* (p/n 5990-3976EN)
- *Forensics and Toxicology Personal Compound Database and Library for Screening and Identification: the Broecker, Herre and Pragst PCDL Accurate Mass Spectral Library* (p/n 5990-6450EN)

This application note describes the addition of Checkout Mix retention times to a custom user PCDL:

- *An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database* (p/n 5990-4251EN).

These technical notes and application note are include on the PCDL media.

Familiarization Exercises - Targeted MS/MS Analysis with Identification by Library Search

The use of Targeted MS/MS has many advantages.

Refer to the MassHunter Data Acquisition online Help and user guides to learn more about how Targeted MS/MS works.

- Only one run is needed both to screen for compounds using accurate mass database searching and to perform a library search for identification.
- Targeted MS/MS always performs MS/MS acquisition at exactly the specified *m/z* value over the specified time range in the run. If the target is present, even in a complex matrix and of low abundance, the precursor of the target compound is fragmented and an MS/MS spectrum is obtained. If you use Auto MS/MS mode instead, MS/MS acquisition occurs only if the incoming MS ion peak data meets the criteria defined in the acquisition method. (See "Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search" on page 46.) Under some conditions of high sample complexity and low precursor intensity, or if multiple adducts are formed, Auto MS/MS operation can miss desired precursors.
- The number of precursors that can be examined in any cycle is limited. If the number of targets is too large, or the chromatography too fast for good integration or peak detection, divide the target list over multiple methods and inject the sample repetitively.
- To acquire spectra of compounds that are not listed in the acquisition method or are not present in the database/library, use Auto MS/MS. Targeted MS/MS operation does not acquire MS/MS spectra on unexpected targets, only on what is on the precursor list in the method.

In these exercises, you process the data file **Checkout_TestMix_TMSMS.d.** Use the Checkout Mix example data file. If you acquired data from the Checkout Mix, you can use the data file that you acquired. Your results can differ slightly.

Exercise 1. Set up the targeted MS/MS method

In this exercise, you use the compound information found in the previous exercises using Find by Formula.

You have screened the compounds by match to the accurate MS mass and isotope pattern in the library. You now confirm the identifications with an MS/MS experiment.

Exercise 1. Set up the targeted MS/MS method

Step	Detailed Instructions	Comments
1 Create a template file in .csv format. See Figure 14. Then open the template in Excel.	**a** Open the MassHunter Data Acquisition program. **b** In the Method Editor pane, right-click the table in the **Targeted List** tab and click **Add** to add a row. **c** Change the **Iso. Width** to **Narrow (~1.3 m/.z)**. **d** For **Delta Ret. Time window**, type 0.5. **e** Right-click the table in the Targeted List tab and select **Export**. See Figure 13. **f** For **File type**, select **text (*.csv)**. **g** Select a file name and location. **h** Click **OK**. **i** In Excel, open the template .csv file that you created. See Figure 14.	

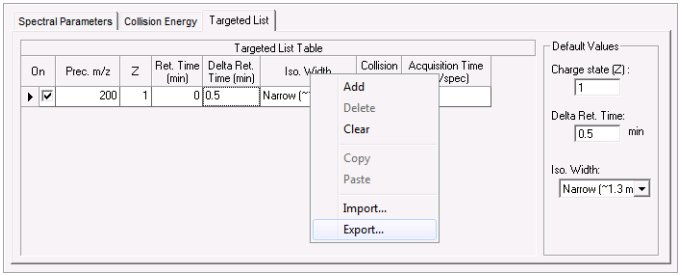


Figure 13 Targeted List tab, Export listed in shortcut menu

Exercise 1. Set up the targeted MS/MS method (continued)

Step	Detailed Instructions	Comments

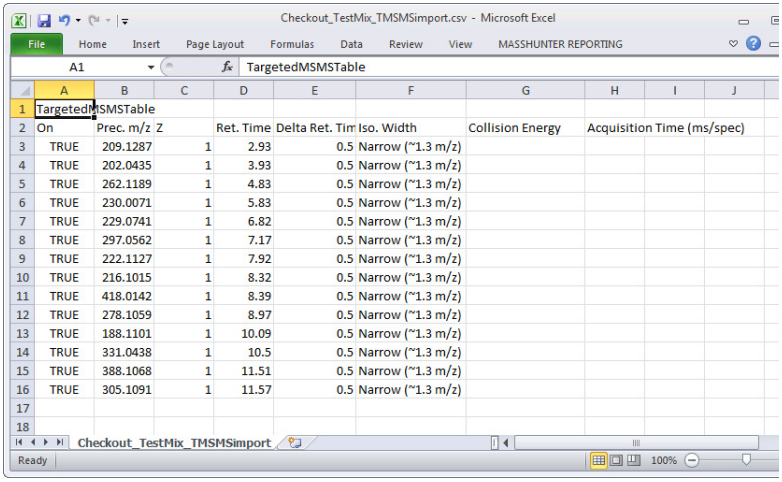
TargetedMSMSTable							
On	Prec. m/z	Z	Ret. Time	Delta Ret.	Iso. Width	Collision Energy	Acquisition Time (ms/spec)
TRUE	200	1	0	0.5	Narrow (~1.3 m/z)		

Figure 14 Template .csv file

Step	Detailed Instructions	Comments
2 Create exact mass column in the Compounds List results file that you saved previously, and add to the template file. See Figure 15.	**a** Start the Excel program, and open the spreadsheet file that you exported from the MassHunter Qualitative Analysis program in "Exercise 1. Process and interpret data with Find by Formula" on page 11. **b** Add a column called **Prec. m/z**. **c** Set the formula for this column to be the **Mass(tgt)** value plus 1.00727645 (the mass of hydrogen minus an electron). This value represents the exact mass of the protonated compound found in the library. **d** Copy all **Prec. m/z** values to the template .csv file.	The base peak column in the compound list table is the measured *m/z* of the largest mass peak in the spectrum for this "found" compound. However, in samples with matrix, the base peak can sometimes not be the protonated ion. Using the calculated exact mass for the targeted MS/MS analysis is by far a better approach.

Exercise 1. Set up the targeted MS/MS method (continued)

Step	Detailed Instructions	Comments
	e From the compound list Excel file, copy: • the Z values • the retention times • the delta retention times • the iso widths The template .csv file now looks similar to Figure 15. **f** Save the template .csv file. The compound list Excel file and the template .csv file used in these examples can be found on the PCDL media under **Example Reports**, as **Checkout_TestMix_MS.csv** and **Checkout_TestMix_TMSMSimport .csv**.	The collision energy values are expected to be the same as the three energies in the library (10 eV, 20 eV, and 40 eV), as described in the application notes that are on your PCDL media. However, for real samples, the duty cycle of the Q-TOF LC/MS can be negatively affected if you measure at 2 or 3 collision energies. The alternative is to use a collision energy calculation which is calculated from a linear fit of the collision energy to the *m/z* of the precursor ion as described in "Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search" on page 46.



	A	B	C	D	E	F	G	H	I	J
1	TargetedMSMSTable									
2	On	Prec. m/z	Z	Ret. Time	Delta Ret. Tim	Iso. Width	Collision Energy	Acquisition Time (ms/spec)		
3	TRUE	209.1287	1	2.93	0.5	Narrow (~1.3 m/z)				
4	TRUE	202.0435	1	3.93	0.5	Narrow (~1.3 m/z)				
5	TRUE	262.1189	1	4.83	0.5	Narrow (~1.3 m/z)				
6	TRUE	230.0071	1	5.83	0.5	Narrow (~1.3 m/z)				
7	TRUE	229.0741	1	6.82	0.5	Narrow (~1.3 m/z)				
8	TRUE	297.0562	1	7.17	0.5	Narrow (~1.3 m/z)				
9	TRUE	222.1127	1	7.92	0.5	Narrow (~1.3 m/z)				
10	TRUE	216.1015	1	8.32	0.5	Narrow (~1.3 m/z)				
11	TRUE	418.0142	1	8.39	0.5	Narrow (~1.3 m/z)				
12	TRUE	278.1059	1	8.97	0.5	Narrow (~1.3 m/z)				
13	TRUE	188.1101	1	10.09	0.5	Narrow (~1.3 m/z)				
14	TRUE	331.0438	1	10.5	0.5	Narrow (~1.3 m/z)				
15	TRUE	388.1068	1	11.51	0.5	Narrow (~1.3 m/z)				
16	TRUE	305.1091	1	11.57	0.5	Narrow (~1.3 m/z)				
17										
18										

Figure 15 Template .csv after retention time and accurate mass are added

Exercise 1. Set up the targeted MS/MS method (continued)

Step	Detailed Instructions	Comments
3 Open the Compounds List results file that you saved in "Exercise 1. Process and interpret data with Find by Formula" on page 11, and then import the values from the template .csv file that you created. Run the newly saved Targeted MS/MS method.	**a** Use Excel to open the spreadsheet file that you saved in "Exercise 1. Process and interpret data with Find by Formula" on page 11. This spreadsheet file is in the same folder as the data file that was processed in that exercise. **b** In the Data Acquisition program, right-click the **Targeted Mass** tab and select **Import**. **c** Import the values from the template .csv file that you created. **d** Save this Targeted MS/MS method as the method to use to identify the compounds found by library search. **e** Run the sample again with the newly saved Targeted MS/MS method.	

Figure 16 shows the total ion chromatogram of the targeted MS/MS data. The alternation of single-MS to MS/MS is seen in the signal intensity change across peaks that are targeted. This acquisition was done with a delta retention time window of 0.5 minutes. The data shows that this setting causes the acquisition program to collect MS/MS spectra from 0.25 minutes before the peak to 0.25 minutes after the peak. If chromatographic reproducibility is excellent, this window can be reduced, which increases the duty cycle by reducing overlapping peaks.

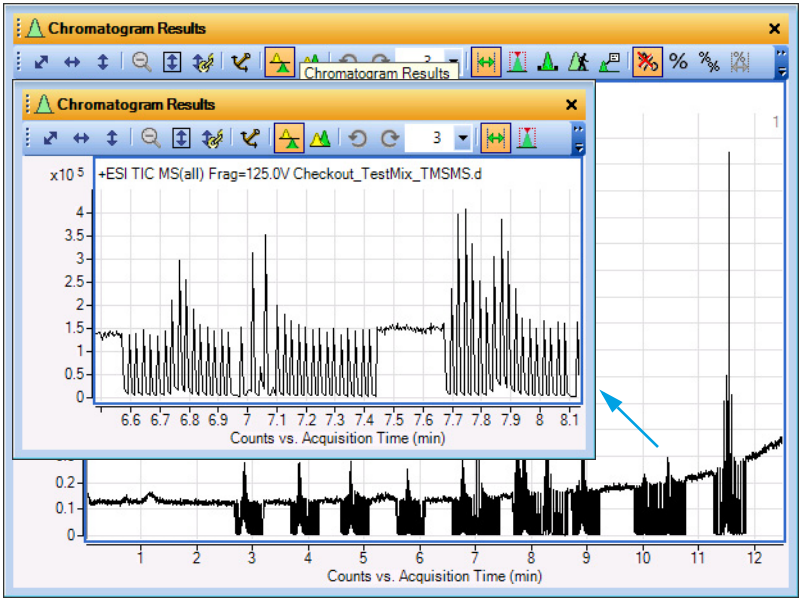


Figure 16 Total ion chromatogram from a typical targeted MS/MS data shows sawtooth pattern from alternating MS and MS/MS scans.

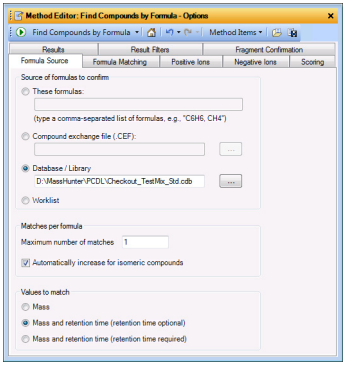
Exercise 2. Process the data

You can process the data in one of several ways. The steps used in this exercise support automated data processing. Processing the data file consists of these steps:

- Find compound using “Find Compounds by Formula”
- Identify compounds using “Search Accurate Mass Library”
- Generate Compound Report
- Print Compound Report

You find the best match for the single-MS precursor ion, based on accurate mass and isotope information. Then you search the MS/MS library to find the best match for the MS/MS spectrum.

Exercise 2. Process the data

Step	Detailed Instructions	Comments
1 Update settings for Find Compounds by Formula so that all compounds are found.	**a** Start the MassHunter Qualitative Analysis program **b** Open the Method Editor. **c** Open the data analysis method **Checkout_TestMix_TMSMS_DA.m**. **d** Click **Find Compounds by Formula > Options**, and then on the **Formula Source** tab, set the **Database/Library path** to the Checkout Mix PCDL. See Figure 17. **e** On the **Results** tab, select **Extract MS/MS spectrum** and **Separate MS/MS spectrum per CE**. See Figure 18.	 **Figure 17** Formula Source tab Make sure that in the **Find by Formula - Chromatograms > EIC Integration** tab, the integration option is set to either **Agile** or **MS/MS**. For each new analysis or matrix, do a compound search with each of these integrators before you select the integrator that gives you the best results.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

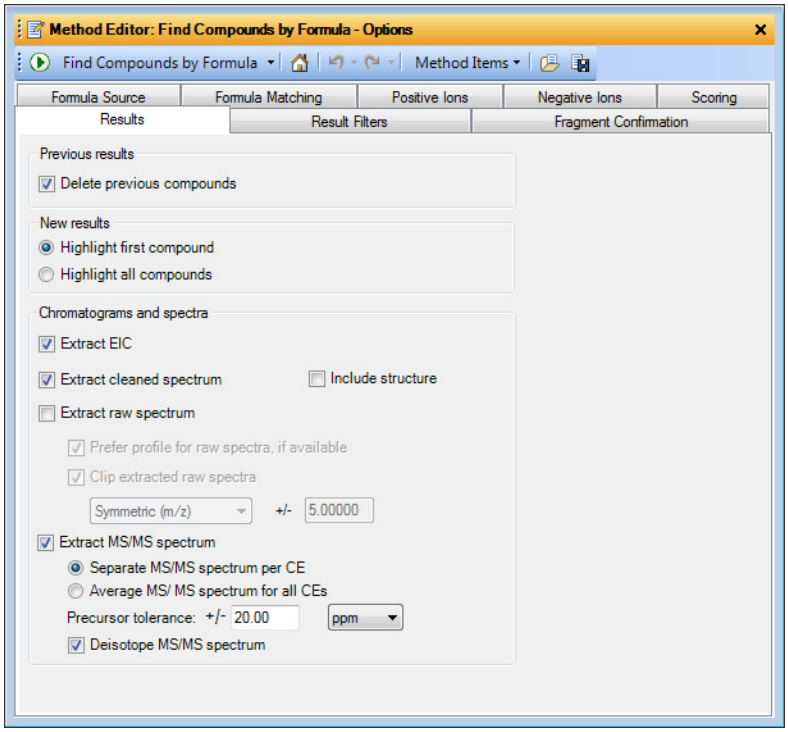
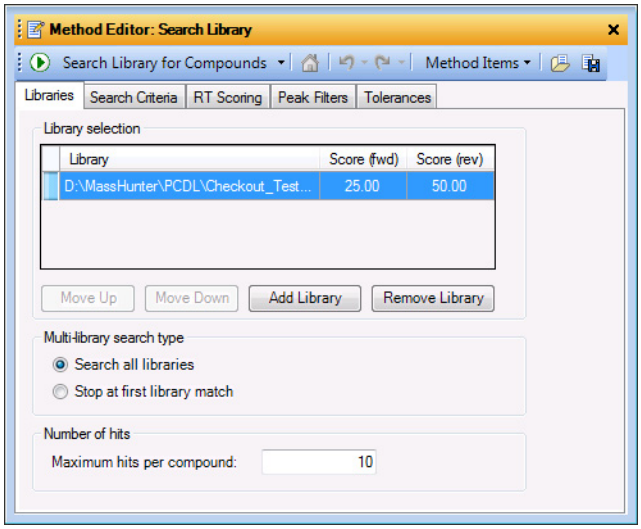


Figure 18 Results tab

2 Search the **Checkout_TestMix_Std.cdb** library. As search criteria: • Add collision energy. • Set to use both a minimum forward score and a minimum reverse score.	**a** In the Method Explorer, click **Identify Compounds > Search Library**. **b** In the **Libraries** tab, click **Add Library** to add **Checkout_TestMix_Std.cdb**. See Figure 19. **c** In the **Libraries** tab, click the current **Score (fwd)** and **Score (rev)** values. Set the forward score to **25** and reverse score to **50**. See Figure 19.	See "Forward vs. Reverse Library Search" on page 66 for more details. The score settings can seem too low, but these settings let you detect any issues that can occur as you become familiar with these techniques. For real methods, a forward score of 50 and a reverse score of 70 are typical. For each analysis and matrix type, review and update the Matching criteria settings in the Results filters tab in the Find by Formula Options.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments
		
	Figure 19 Libraries tab	
	d In the **Search Criteria** tab, mark the check boxes for **Collision energy** and **Exclude precursor ion from Reverse Score**. See Figure 20. **e** In the Peak Filters tab, set the **Absolute height** to **5** counts and the **Relative height** to **1% of largest peak**. See Figure 21.	If you do not see **Exclude precursor ion from Reverse Score**, make sure that **Show advanced parameter** is selected in the MassHunter Qualitative Analysis program. See step 3 on page 19.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

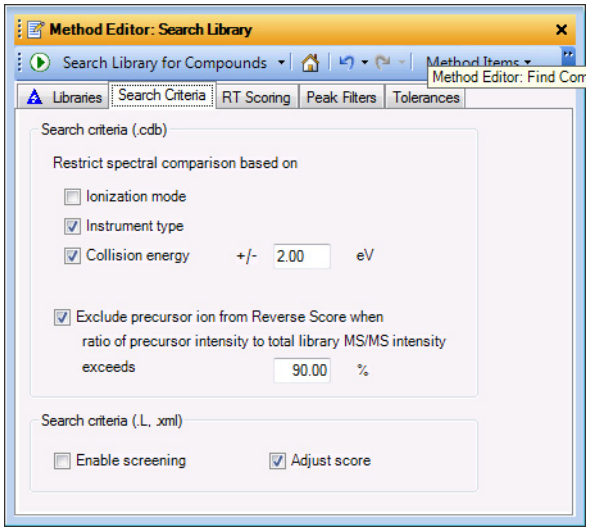


Figure 20 Search Criteria tab.

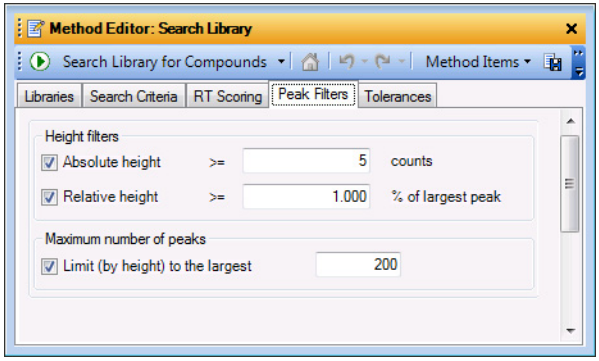


Figure 21 Peak Filters tab.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments
3 Set up the method to: • Find all of the compounds in the Checkout Mix by Find by Formula. • Do a library search.	**a** In the Method Explorer, click **Compound Automation Steps > Find and Identify**. **b** In the **Options** tab, select these options as shown in Figure 22: • **Find by Formula** • **Search a library for each compound** • **Show only identified compounds**	If they are not, make sure that the mix is prepared fresh and run within 4 hours of preparation, and that your system background has been minimized. Setting the Matching criteria in the Results filters tab in the Find by Formula options can prevent small impurities from being reported.

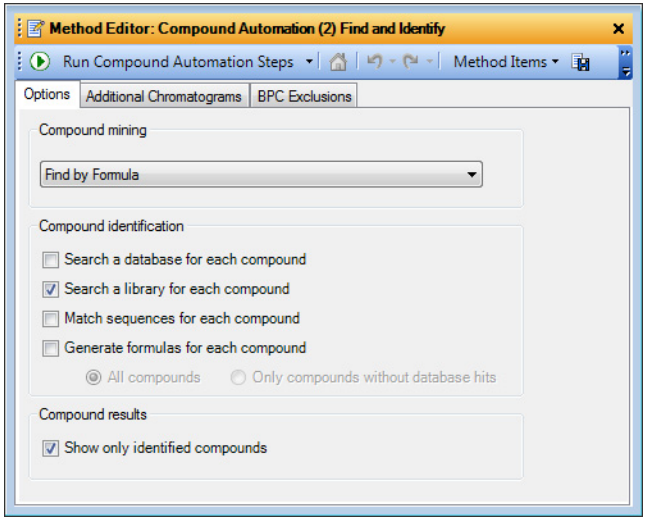


Figure 22 Options tab for Compound Automation Find and Identify

4 Set up report options to produce a report that shows the MS/MS peak table and spectra.	**a** In the Method Editor, click **Reports > Common Reporting Options**. **b** For **Compound report template**, select **CompoundReport.xltx**. See Figure 23. **c** In the Method Explorer, click **Compound Automation Steps > Compound Report**. **d** Under **Compound spectrum (MS/MS)**, mark the check boxes for **Show MS/MS spectrum** and **Show MS/MS peak table**. See Figure 24. **e** Save the method.	Figure 25 and Figure 26 shows the first two pages from the report for the Targeted MS/MS analysis on the **Checkout_TestMix_TMSMS.d** (found on the PCDL media). A copy of this report is also available in the report folder as a PDF file.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

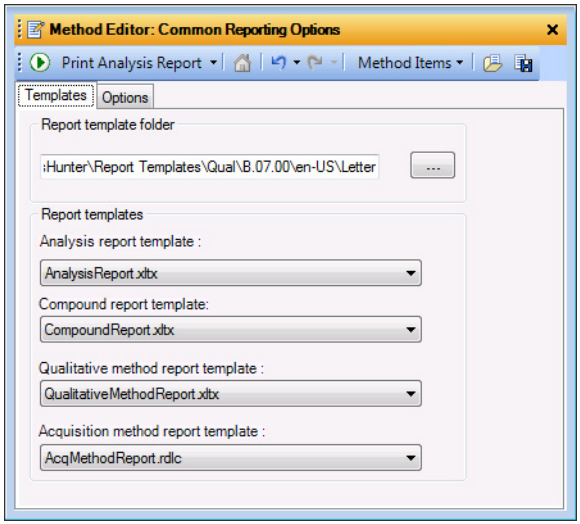


Figure 23 Template tab in Common Reporting Options.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

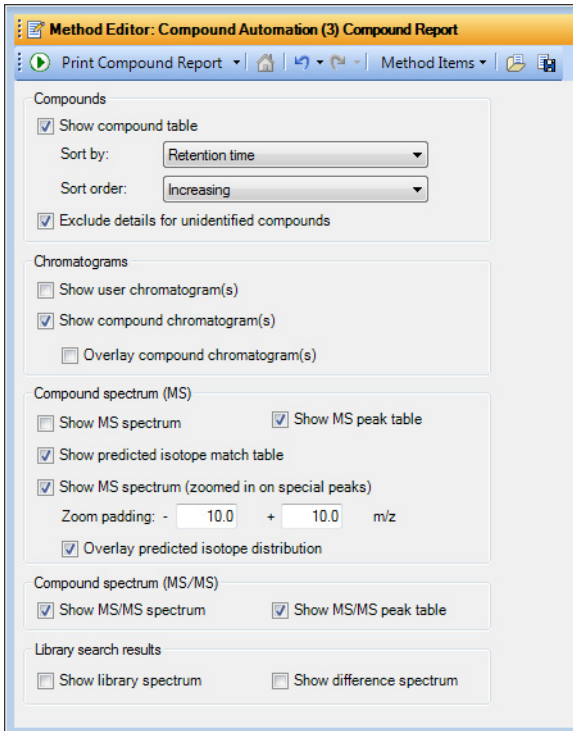
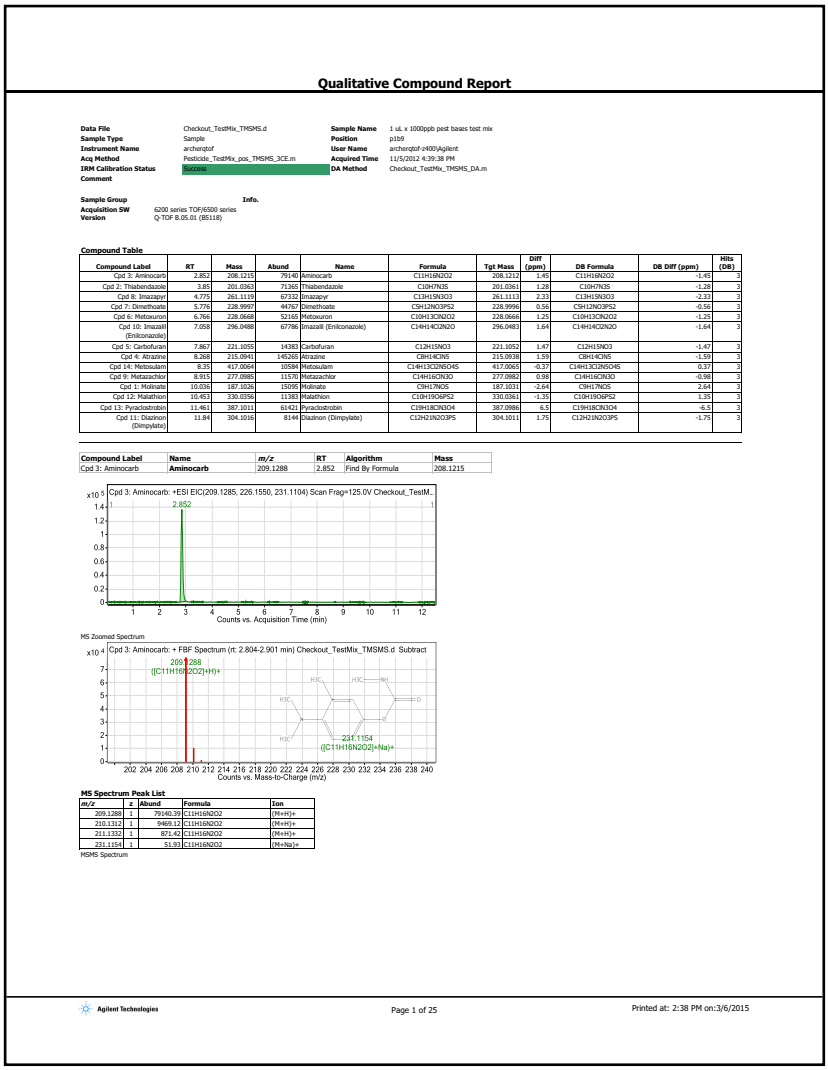


Figure 24 Compound Report dialog box.

When the method is run, a report is generated that includes a summary (Figure 25) as well as details for each compound found in the library (Figure 26). The isotope abundance and mass accuracy are taken from the single-MS spectra in the data and not the MS/MS. These values (isotope abundance and mass accuracy) come from molecular formula generation. In addition, Figure 26 shows the mass accuracy of each precursor. Again the MFG Diff (ppm) comes from the single-MS spectra and the DB Diff (ppm) comes from the precursor ion in the MS/MS spectrum.

You can use these reports to determine the presence of a specific compound in your sample. Manual inspection of the data file can help you determine whether any information in the data is not being reported.



Qualitative Compound Report

Data File	Checkout_TestMix_TMSMS.d	**Sample Name**	1 uL x 1000ppb pest bases test mix
Sample Type	Sample	**Position**	p1b9
Instrument Name	archerqtof	**User Name**	archerqtof-z400\Agilent
Acq Method	Pesticide_TestMix_pos_TMSMS_3CE.m	**Acquired Time**	11/5/2012 4:39:38 PM
IRM Calibration Status	Success	**DA Method**	Checkout_TestMix_TMSMS_DA.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5118)

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	Diff (ppm)	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 3: Aminocarb	2.852	208.1215	79140	Aminocarb	C11H16N2O2	208.1212	1.45	C11H16N2O2	-1.45	3
Cpd 2: Thiabendazole	3.85	201.0363	71365	Thiabendazole	C10H7N3S	201.0361	1.28	C10H7N3S	-1.28	3
Cpd 8: Imazapyr	4.775	261.1119	67332	Imazapyr	C13H15N3O3	261.1113	2.33	C13H15N3O3	-2.33	3
Cpd 7: Dimethoate	5.776	228.9997	44767	Dimethoate	C5H12NO3PS2	228.9996	0.56	C5H12NO3PS2	-0.56	3
Cpd 6: Metoxuron	6.766	228.0668	52165	Metoxuron	C10H13ClN2O2	228.0666	1.25	C10H13ClN2O2	-1.25	3
Cpd 10: Imazalil (Enilconazole)	7.058	296.0488	67786	Imazalil (Enilconazole)	C14H14Cl2N2O	296.0483	1.64	C14H14Cl2N2O	-1.64	3
Cpd 5: Carbofuran	7.867	221.1055	14383	Carbofuran	C12H15NO3	221.1052	1.47	C12H15NO3	-1.47	3
Cpd 4: Atrazine	8.268	215.0941	145265	Atrazine	C8H14ClN5	215.0938	1.59	C8H14ClN5	-1.59	3
Cpd 14: Metosulam	8.35	417.0064	10584	Metosulam	C14H13Cl2N5O4S	417.0065	-0.37	C14H13Cl2N5O4S	0.37	3
Cpd 9: Metazachlor	8.915	277.0985	11570	Metazachlor	C14H16ClN3O	277.0982	0.98	C14H16ClN3O	-0.98	3
Cpd 1: Molinate	10.036	187.1026	15095	Molinate	C9H17NOS	187.1031	-2.64	C9H17NOS	2.64	3
Cpd 12: Malathion	10.453	330.0356	11385	Malathion	C10H19O6PS2	330.0361	-1.35	C10H19O6PS2	1.35	3
Cpd 13: Pyraclostrobin	11.461	387.1011	61421	Pyraclostrobin	C19H18ClN3O4	387.0986	6.5	C19H18ClN3O4	-6.5	3
Cpd 11: Diazinon (Dimpylate)	11.84	304.1016	8144	Diazinon (Dimpylate)	C12H21N2O3PS	304.1011	1.75	C12H21N2O3PS	-1.75	3

Compound Label	Name	m/z	RT	Algorithm	Mass
Cpd 3: Aminocarb	**Aminocarb**	209.1288	2.852	Find By Formula	208.1215

MS Zoomed Spectrum

MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
209.1288	1	79140.39	C11H16N2O2	(M+H)+
210.1312	1	9469.12	C11H16N2O2	(M+H)+
211.1332	1	871.42	C11H16N2O2	(M+H)+
231.1154	1	51.93	C11H16N2O2	(M+Na)+

MSMS Spectrum

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Figure 25 Page 1 of the Checkout Mix Compound report.

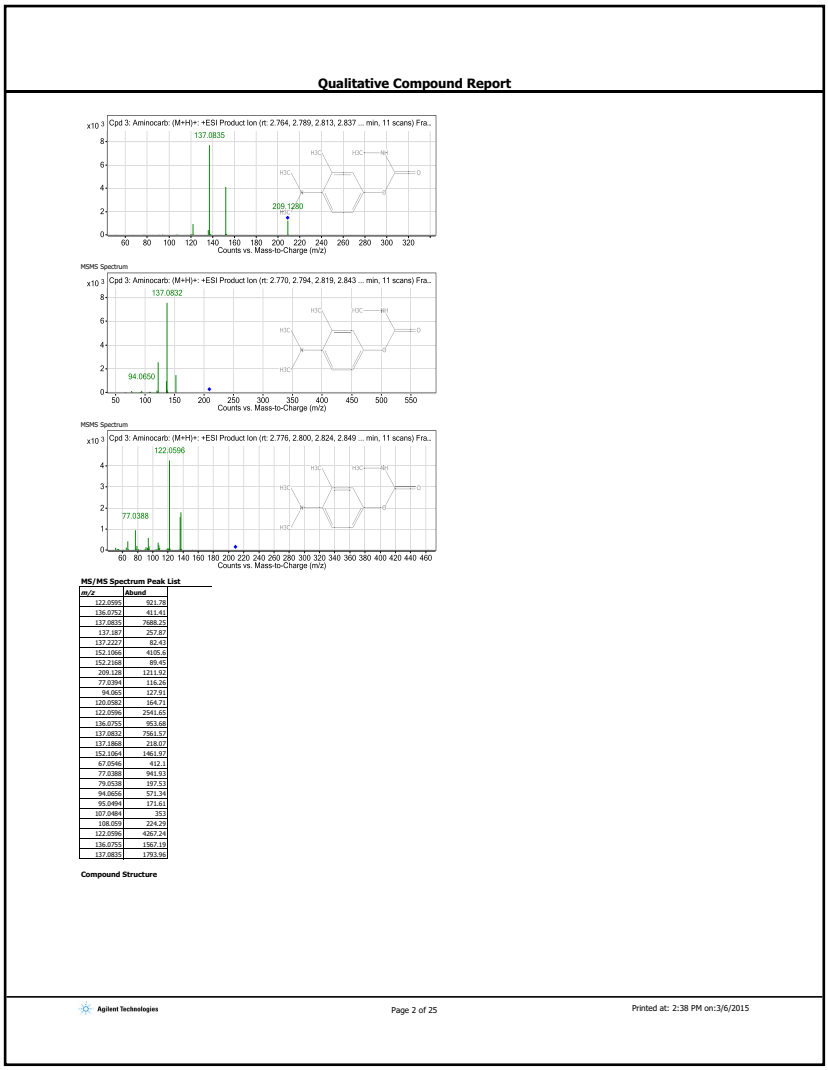


Figure 26 Page 2 of the Checkout Mix Compound report

Exercise 3. Automate the process with worklist actions

The ability to automate the process and run these steps in a workflow can be useful, especially when you need to analyze many samples.

Automation is done by the use of worklist actions.

Exercise 3. Automate the process with worklist actions

Step	Detailed Instruction	Comments
1 Set up a worklist to create a compound report.	**a** In Method Explorer, click **Worklist Automation > Worklist Actions**. **b** Select these **Actions to be run**: • **Compound Automation without Report** • **Generate Compound Report**	The **Compound Automation without Report** action includes most of the other available actions, so they do not need to be selected. Some data files can require long processing time. You can do the compound automation and report generation in separate steps.

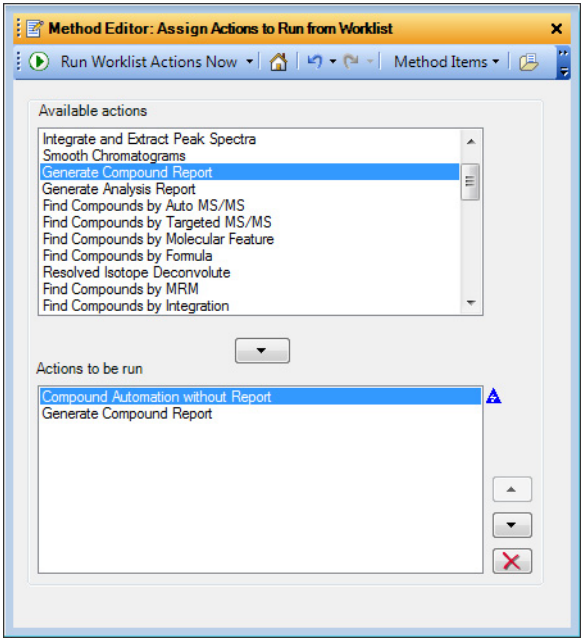
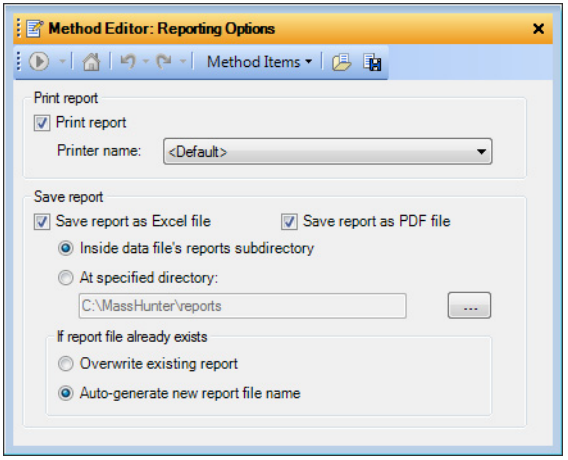


Figure 27 Assign Actions to Run from Worklist dialog box.

Exercise 3. Automate the process with worklist actions (continued)

Step	Detailed Instruction	Comments
2 Set print options.	**a** In the Method Explorer, click **Worklist Automation > Reporting Options**. **b** Select whether to print the report, save to a file (Excel file or PDF), or both. See Figure 28. **c** Save the method.	
		
	Figure 28 Reporting Options dialog box.	
3 Attach the method to an acquisition method.	**a** In the MassHunter Qualitative Analysis program, click **Method > Save As**. **b** Browse to the folder on your system that contains the Data Acquisition method that you want to automate. **c** Click the name of the Data Acquisition method that you want to automate and click **Save**. The Qualitative Analysis method is now attached and is an integral part of the Data Acquisition method.	

Exercise 3. Automate the process with worklist actions (continued)

Step	Detailed Instruction	Comments
4 Check that the method will run correctly when you use it within a worklist.	**a** In Method Explorer, click **Worklist Automation > Worklist Actions**. **b** Click the green arrow to run the worklist actions. **c** Check the report to make sure that the method options are correctly set.	

When you set up a worklist in Data Acquisition, add the data analysis method you created under the column **Override DA Method**. Refer to the MassHunter Data Acquisition user guides and online Help for more information.

If you do not see the column for **Override DA Method** in the worklist, it can be hidden between the Method and Data File columns. Move the mouse pointer to the boundary between these two columns. When the pointer changes to a double-sided arrow, move the column boundary to the right until you see the **Override DA Method** column.

Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search

The use of Auto MS/MS has many advantages.

- Only one run is needed to both screen for compounds using accurate mass database search, and do a library search for identification.
- For a complex sample, a large database can result in a high number of hits, which are difficult for Targeted MS/MS to handle because of the burden on the duty cycle for the instrument, especially as two or three collision energies (10 eV and 20 eV, or 10 eV, 20 eV and 40 eV) are collected for each MS/MS peak. Auto MS/MS eliminates this problem because false positives are removed with the library search. However, lower library scores are expected because the collision energies do not exactly match the collision energies of the library spectra, which are measured at 10 eV, 20 eV and 40 eV.
- Auto MS/MS can collect MS/MS spectra of potentially important compounds that are not currently in the PCDL. The ability to archive and retrieve these spectra can be useful, for example, in contaminate analysis where time has passed and another analyte is now suspected to be present.

Refer to the MassHunter Data Acquisition online Help and user guides to learn more about how Auto MS/MS works.

Use the example data file **Checkout_TestMix_AMSMS.d** found in the **Example Data** folder on the PCDL media. If you ran the Checkout Mix, you can use the data file that you acquired. Your results can differ slightly.

Exercise 1. Learn about the content of an Auto MS/MS data file

In this step, you use Find Compounds by Formula to screen the compounds by match to the accurate MS mass and isotope pattern in the PCDL.

Exercise 1. Learn about the content of an Auto MS/MS data file

Step	Detailed Instructions	Comments
1 Open the **Checkout_TestMix_AMSMS.d** file.	**a** Open the Agilent MassHunter Qualitative Analysis program. Click **Cancel** if you are asked to open a data file. **b** Load the data analysis method **Checkout_TestMix_AMSMS_DA.m** . **c** Open the data file **Checkout_TestMix_AMSMS.d.** See Figure 30.	This chromatogram is different than for Targeted MS/MS. In Auto MS/MS mode, single-MS data is collected in a survey scan. When an ion meets the criteria that you set, an MS/MS analysis is done under the conditions specified in the method. In this example, the collision energy uses a collision energy calculation described below. For an example of Auto MS/MS results, see **Checkout_TestMix_AMSMS.d** on the PCDL media. It was run with a linear fit of the collision energy to the *m/z* of the precursor ion. Figure 29 shows the Collision Energy tab for Auto MS/MS. In this example, the actual collision energy is calculated as 6 * the *m/z* of the precursor ion divided by 100 plus the offset voltage. If the precursor is *m/z* 300, then the collision energy is 6*300/100 + 4 = 22 eV. The precursor *m/z* value is taken from the Auto list and both that value and the charge are recorded with the data file. Therefore, if z=2, the nominal mass of the compound is 598 (for a di-protonated molecule), but the collision energy would still be 22 eV. The graph in Figure 29 reflects the last available settings for the Use Table function, and does not reflect the Use Slope function as marked in the figure.

Exercise 1. Learn about the content of an Auto MS/MS data file (continued)

Step	Detailed Instructions	Comments

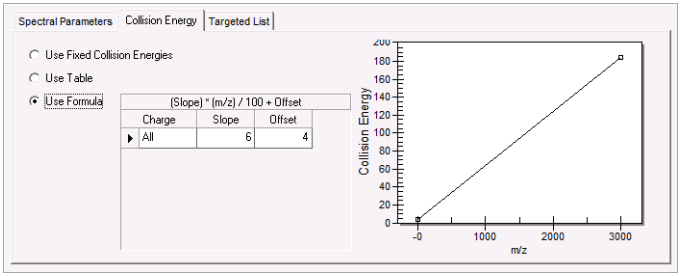


Figure 29 Collision Energy tab showing calculated values

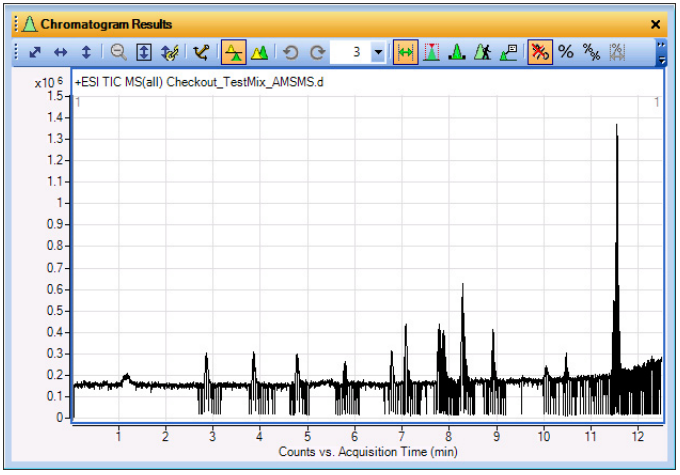
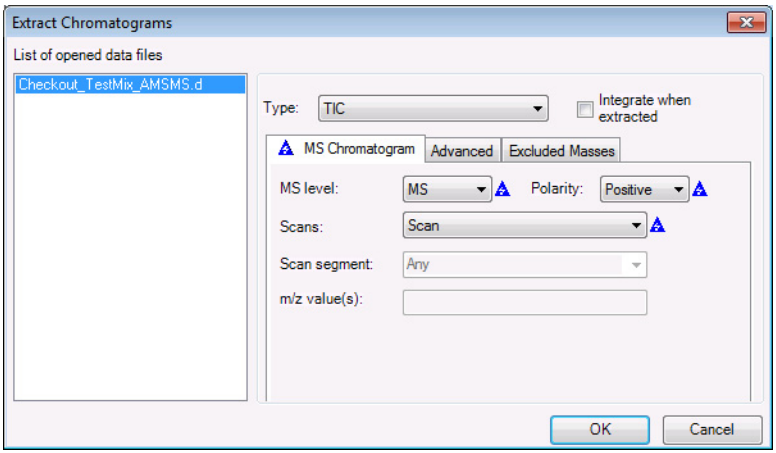


Figure 30 Total ion chromatogram of the Checkout Mix with auto MS/MS settings.

Exercise 1. Learn about the content of an Auto MS/MS data file (continued)

Step	Detailed Instructions	Comments
2 Extract chromatograms to get a clearer picture of the data.	**a** Right-click the chromatogram window, then click **Extract Chromatograms**. **b** For **Type**, select **TIC**. **c** In the MS Chromatogram tab, for **MS level**, select **MS**. **d** For **Polarity**, select **Positive**. **e** For **Scans**, select **Scan**. See Figure 31. **f** Click **OK**.	
		
	Figure 31 Extract Chromatograms setting for MS.	
3 Extract MS/MS data.	**a** Repeat step 2, but change the **MS level** to **MS/MS**. See Figure 32	When you compare the MS and MS/MS chromatograms, you can see that in MS mode, data across the peak is collected, while in MS/MS mode, data across specific points of the peak based on the acquisition settings are collected. See Figure 33.

Exercise 1. Learn about the content of an Auto MS/MS data file (continued)

Step	Detailed Instructions	Comments

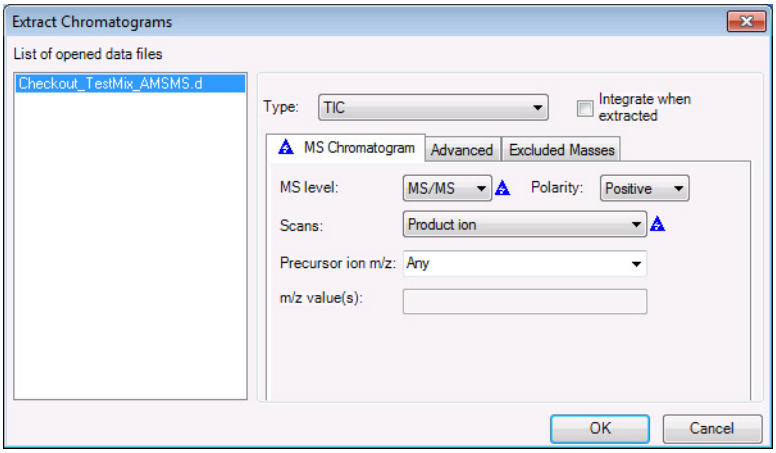


Figure 32 Extract Chromatograms setting for MS/MS.

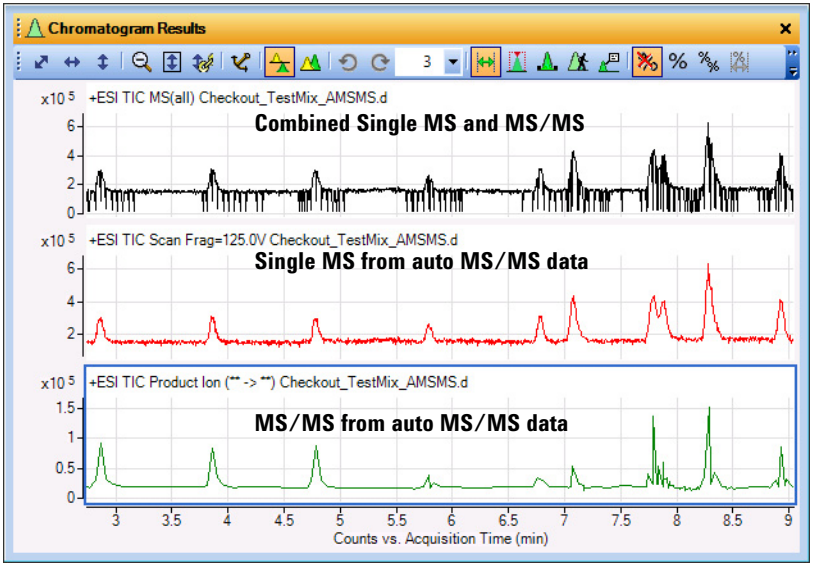


Figure 33 The top chromatogram shows all of the data points for single-MS and MS/MS. MS/MS data points have lower total signal because ions in a narrow mass range are isolated for fragmentation. The middle chromatogram shows the single-MS only and it is clear that the Q-TOF LC/MS is collecting mostly single-MS data. The bottom chromatogram is created by connecting all points where MS/MS spectra were acquired.

Exercise 2. Optimize the number of data points

The number of data points for the single-MS and the MS/MS in Auto MS/MS mode depend on the acquisition settings. The more spectra per second that are collected, the fewer transients per spectrum, and the lower the signal. Spectral parameters can be adjusted in the MassHunter Data Acquisition program, in the Acquisition tab. You want to find the balance between missing compounds due to low sensitivity, or missing compounds because of slow cycle time.

Figure 34 shows the spectra parameters that are typically used for Auto MS/MS.

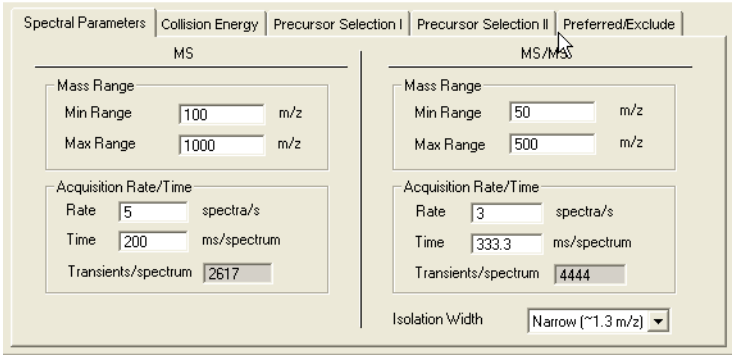


Figure 34 Spectral parameters for Auto MS/MS

1 In the Data Acquisition program, click the **Acquisition** tab.

2 In the **Precursor Selection I** tab, select the conditions for acquisition of MS/MS spectra. See Figure 35.

- **Max Precursor Per Cycle** determines how many co-eluting ions are selected for MS/MS. Too many ions negatively affect the cycle time. Too few ions cause ions to be missed.
- **Precursor Threshold** selection depends on the background of the system and how sensitive you want the analysis to be. Lower settings find more spectra, but compounds can be missed because the system is burdened with MS/MS collection for low-level ions while an ion of interest is eluting. Also, lower settings can increase the collection of lower quality spectra because of weak precursor ion signal.

- **Active Exclusion** causes the ions to be selected as a peak elutes only *n* times (in Figure 35, *n* = 2). If *not* enabled, lower-level ions can be missed. If enabled with too long a time before release, spectra near the top of the peak can be missed. The quality of the MS/MS can suffer.
- **Static Exclusion Range List** excludes the range of ions that you specify. In Figure 35, reference ions and *m/z* above 600 are excluded. Use this setting if you expect only smaller molecules to be in your sample.

Refer to the Data Acquisition program online Help and user guides for detailed explanation of these parameters.

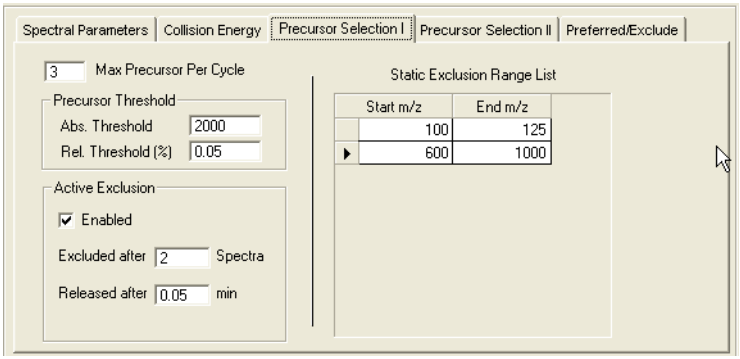


Figure 35 Precursor Selection I tab

3 In the **Precursor Selection II** tab, select the charge states to include.

The inclusion of only charge state of 1 is used for the Checkout Mix and applies to most small molecules. The other parameters in this tab are useful for more advanced data-dependent operation. See the MassHunter Data Acquisition online Help and user guides for more information.

4 In the **Preferred/Exclude** tab, define the ions that you want to include or exclude in the search.

The ions in the list of preferred or excluded ions must have an associated mass window (in ppm), retention time, and retention time window. For example, if you have peaks that elute in your blank, exclude them when collecting MS/MS. No ions were preferred or excluded for the Checkout Mix analysis.

Exercise 3. Process the data and automate

Before you finalize the data processing method to run as an automated worklist, you manually process the data first.

Data processing for Auto MS/MS is the same as data processing for Targeted MS/MS.

The steps for Auto MS/MS analysis include:

- Find compounds by “Find by Formula”.
- Identify compounds by “Search Accurate Mass Library”.
- Generate Compound Report.
- Print Compound Report.

Exercise 3. Process the data and automate

Steps	Detailed Instructions	Comments
1 Process data for Auto MS/MS as you would for Targeted MS/MS, but omit the collision energy as a library search option: • Check that the settings for Search Library are the same for Find by Formula as they are in the Targeted MS/MS exercise. • Update the library search parameters to disable the collision energy filter.	**a** Start the MassHunter Qualitative Analysis program. **b** Open the Method Editor. **c** In **Compound Automation Steps > Find and Identity**, select only these options: • **Find by Formula** • **Search a library for each compound** • **Show only identified compounds** See Figure 36. **d** In **Identify Compounds > Search Library**, in the **Search Criteria** tab, *clear* the check box for **Collision energy**. See Figure 37. To automate the process, do the steps in "Exercise 3. Automate the process with worklist actions" on page 43.	MS/MS peaks triggered on adduct ion species produce spectra that do not match to the library spectra, as these spectra are not present in Checkout_TestMix_Std.cdb, and results in a library score of zero. An auto MS/MS acquisition by its very nature is an untargeted process. It can examine only a relatively few precursors at any one instant, and can select adducts which do not fragment well under the conditions selected. As a result, an auto MS/MS analysis can produce library search results in which some compounds are missed in certain circumstances. For these cases, place entries on the auto MS/MS preferred/exclude list during specific elution time ranges to increase the chances of selecting the desired precursors or to exclude unwanted precursors. Refer to the MassHunter Q-TOF Acquisition documentation or online Help for more information. The first two pages form the results report for the Auto MS/MS analysis on Checkout_TestMix_AMSMS.d (found on the PCDL media) is shown in Figure 38 and Figure 39. A copy of this report is also available in the Example Reports folder as a PDF file.

Exercise 3. Process the data and automate (continued)

Steps	Detailed Instructions	Comments

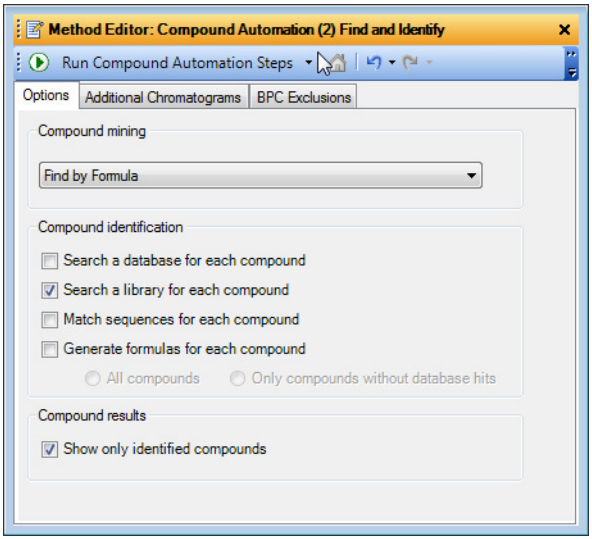


Figure 36 Find and Identify options for Auto MS/MS.

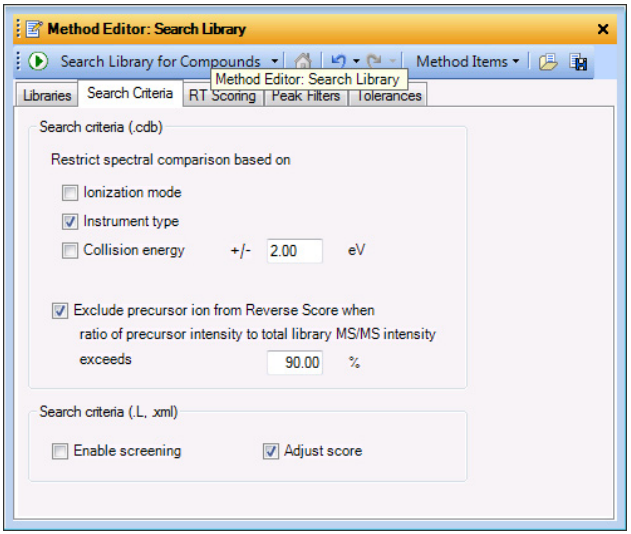
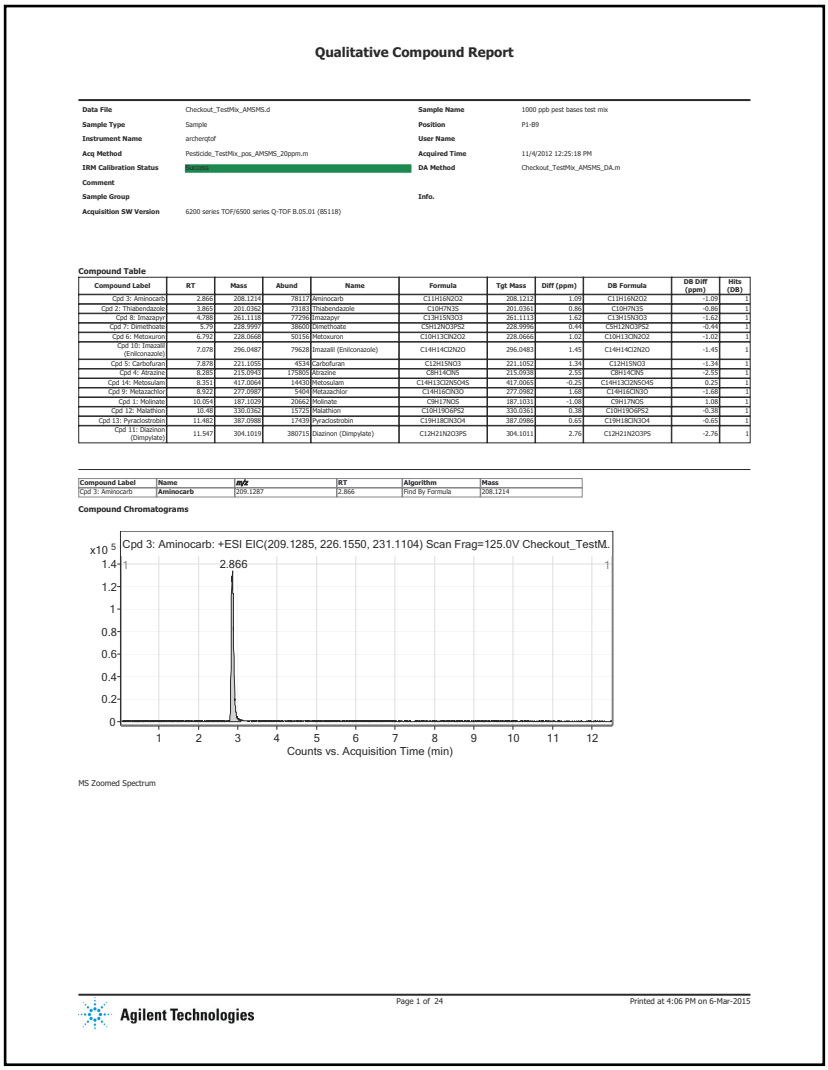


Figure 37 Search Criteria tab with Collision energy check box cleared.



Qualitative Compound Report

Data File	Checkout_TestMix_AMSMS.d	**Sample Name**	1000 ppb pest bases test mix
Sample Type	Sample	**Position**	P1-B9
Instrument Name	archerqtof	**User Name**	
Acq Method	Pesticide_TestMix_pos_AMSMS_20ppm.m	**Acquired Time**	11/4/2012 12:25:18 PM
IRM Calibration Status	Success	**DA Method**	Checkout_TestMix_AMSMS_DA.m
Comment			
Sample Group		**Info.**	
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5118)		

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	Diff (ppm)	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 3: Aminocarb	2.866	208.1214	78117	Aminocarb	C11H16N2O2	208.1212	1.09	C11H16N2O2	-1.09	1
Cpd 2: Thiabendazole	3.865	201.0362	73183	Thiabendazole	C10H7N3S	201.0361	0.86	C10H7N3S	-0.86	1
Cpd 8: Imazapyr	4.788	261.1118	77296	Imazapyr	C13H15N3O3	261.1113	1.62	C13H15N3O3	-1.62	1
Cpd 7: Dimethoate	5.79	228.9997	38606	Dimethoate	C5H12NO3PS2	228.9996	0.44	C5H12NO3PS2	-0.44	1
Cpd 6: Metoxuron	6.792	228.0668	50156	Metoxuron	C10H13ClN2O2	228.0666	1.02	C10H13ClN2O2	-1.02	1
Cpd 10: Imazalil (Enilconazole)	7.078	296.0487	79628	Imazalil (Enilconazole)	C14H14Cl2N2O	296.0483	1.45	C14H14Cl2N2O	-1.45	1
Cpd 5: Carbofuran	7.878	221.1055	4534	Carbofuran	C12H15NO3	221.1052	1.34	C12H15NO3	-1.34	1
Cpd 4: Atrazine	8.285	215.0943	175805	Atrazine	C8H14ClN5	215.0938	2.55	C8H14ClN5	-2.55	1
Cpd 14: Metosulam	8.351	417.0064	14430	Metosulam	C14H13Cl2N5O4S	417.0063	0.25	C14H13Cl2N5O4S	0.25	1
Cpd 9: Metazachlor	8.922	277.0987	5404	Metazachlor	C14H16ClN3O	277.0982	1.68	C14H16ClN3O	-1.68	1
Cpd 1: Molinate	10.054	187.1029	20662	Molinate	C9H17NOS	187.1031	-1.08	C9H17NOS	1.08	1
Cpd 12: Malathion	10.48	330.0362	15725	Malathion	C10H19O6PS2	330.0361	0.38	C10H19O6PS2	-0.38	1
Cpd 13: Pyraclostrobin	11.482	387.0988	17439	Pyraclostrobin	C19H18ClN3O4	387.0986	0.65	C19H18ClN3O4	-0.65	1
Cpd 11: Diazinon (Dimpylate)	11.547	304.1019	380715	Diazinon (Dimpylate)	C12H21N2O3PS	304.1011	2.76	C12H21N2O3PS	-2.76	1

Compound Label	Name	m/z	RT	Algorithm	Mass
Cpd 3: Aminocarb	**Aminocarb**	209.1287	2.866	Find By Formula	208.1214

Compound Chromatograms

MS Zoomed Spectrum

Figure 38 Page 1 of Auto MS/MS analysis report

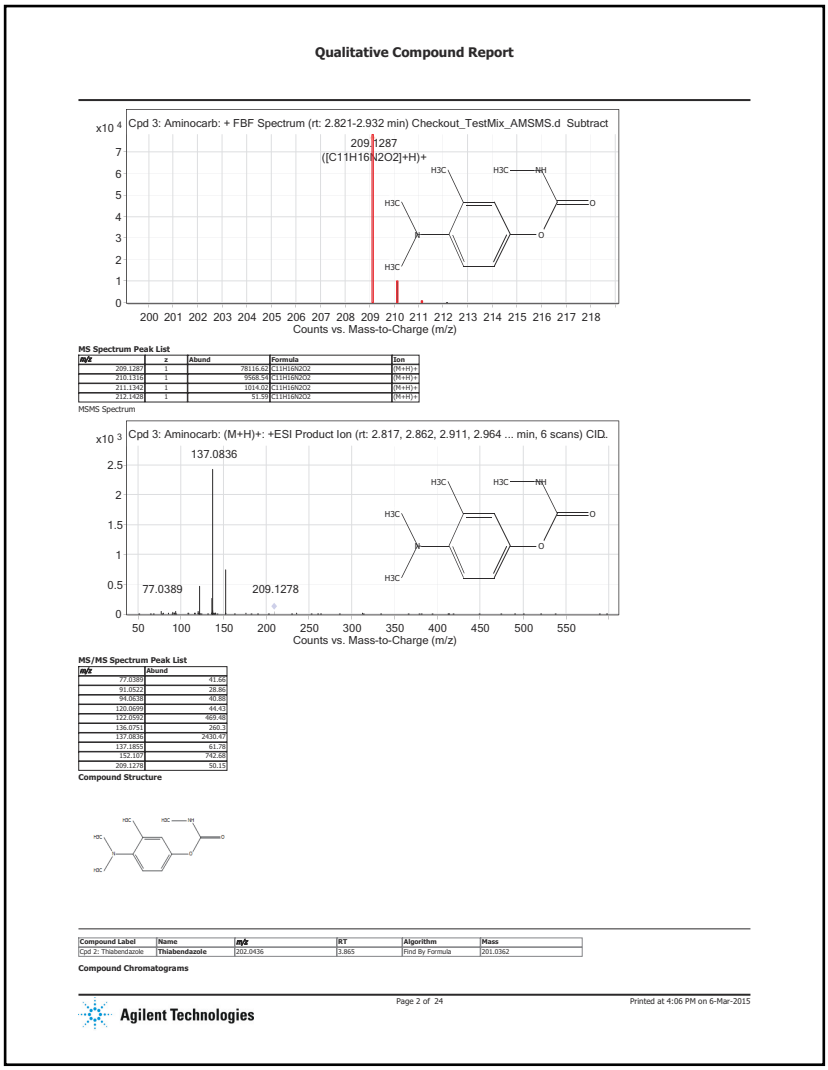


Figure 39 Page 2 of Auto MS/MS analysis report

Reference

Checkout Mix Content

The content of the Checkout Mix is listed here.

Table 1 Checkout Mix (p/n 5190-0469) Basic Compounds

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Aminocarb/2032-59-9	100.2 µg/mL	0.5 µg/mL	$C_{11}H_{16}N_2O_2$	208.1211777698
2	Atrazine/1912-24-9	100.4 µg/mL	0.5 µg/mL	$C_8H_{14}ClN_5$	215.0937731936
3	Carbofuran/1563-66-2	100.2 µg/mL	0.5 µg/mL	$C_{12}H_{15}NO_3$	221.1051933528
4	Diazinon (Dimpylate)/333-41-5	100.4 µg/mL	0.5 µg/mL	$C_{12}H_{21}N_2O_3PS$	304.1010497716
5	Dimethoate/60-51-5	100.2 µg/mL	0.5 µg/mL	$C_5H_{12}NO_3PS_2$	228.9996212071
6	Imazalil (Enilconazole)/35554-44-0	100.4 µg/mL	0.5 µg/mL	$C_{14}H_{14}Cl_2N_2O$	296.0483185037
7	Imazapyr/81334-31-1	100.2 µg/mL	0.5 µg/mL	$C_{13}H_{15}N_3O_3$	261.1113413676
8	Malathion/121-75-5	100.4 µg/mL	0.5 µg/mL	$C_{10}H_{19}O_6PS_2$	330.0360662899
9	Metazachlor/67129-08-2	100.2 µg/mL	0.5 µg/mL	$C_{14}H_{16}ClN_3O$	277.0981898649
10	Metosulam/139528-85-1	100.4 µg/mL	0.5 µg/mL	$C_{14}H_{13}Cl_2N_5O_4S$	417.0065300909
11	Metoxuron/19937-59-8	100.2 µg/mL	0.5 µg/mL	$C_{10}H_{13}ClN_2O_2$	228.0665553841
12	Molinate/2212-67-1	100.4 µg/mL	0.5 µg/mL	$C_9H_{17}NOS$	187.103084902
13	Pyraclostrobin/175013-18-0	100.2 µg/mL	0.5 µg/mL	$C_{19}H_{18}ClN_3O_4$	387.0985837956
14	Thiabendazole/148-79-8	100.4 µg/mL	0.5 µg/mL	$C_{10}H_7N_3S$	201.0360679755
	Acetonitrile	Solvent		C_2H_3N	41.0265

Table 2 Checkout Mix (p/n 5190-0469) Mixture 2 Acidic Compounds

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Acifluorfen/50594-66-6	100.2 µg/mL	0.5 µg/mL	$C_{14}H_7ClF_3NO_5$	360.9964846522
2	2,4,5-T/93-76-5	100.4 µg/mL	0.5 µg/mL	$C_8H_5Cl_3O_3$	253.9304271564
3	Bentazone/25057-89-0	100.2 µg/mL	0.5 µg/mL	$C_{10}H_{12}N_2O_3S$	240.0568629945
4	Dinoseb (Subitex)/88-85-7	100.4 µg/mL	0.5 µg/mL	$C_{10}H_{12}N_2O_5$	240.0746215091
5	2,4,5-TP (Silvex) (Fenoprop)/93-72-1	100.2 µg/mL	0.5 µg/mL	$C_9H_7Cl_3O_3$	267.9460772202
6	Hexaflumuron/86479-06-3	100.4 µg/mL	0.5 µg/mL	$C_{16}H_8Cl_2F_6N_2O_3$	459.9816167569
	Acetonitrile	Solvent		C_2H_3N	41.0265

Checkout Mix LC Parameters

HiP Sampler

Name: HiP Sampler	Model: G1367D
Auxiliary	
Draw Speed	200.0 µL/min
Eject Speed	200.0 µL/min
Draw Position Offset	0.0 mm
Wait Time After Drawing	0.0 s
Sample Flush Out Factor	5.0
Vial/Well bottom sensing	No
Injection	
Injection Mode	Injection with needle v
Injection Volume	1.00 µL
Needle Wash	
Needle Wash Location	Flush Port
Wash Time	5.0 s
High throughput	
Automatic Delay Volume Reduction	No
Overlapped Injection	
Enable Overlapped Injection	No
Valve Switching	
Valve Movements	0
Valve Switch Time 1	
Switch Time 1 Enabled	No
Valve Switch Time 2	
Switch Time 2 Enabled	No
Valve Switch Time 3	
Switch Time 3 Enabled	No
Valve Switch Time 4	
Switch Time 4 Enabled	No
Stop Time	
Stoptime Mode	As pump/No limit
Post Time	
Posttime Mode	Off

Binary Pump

The mobile phase listed in "Running the Checkout Mix" on page 6 is suitable for both basic and acidic analytes. The example data in this guide was run using the mobile phase shown below and is suited to basic analytes only. As a result, the elution order of the basic analytes will differ from the example data.

Name: Binary Pump **Model:** G1312B

Flow	0.300 mL/min
Use Solvent Types	Yes
Low Pressure Limit	0.00 bar
High Pressure Limit	600.00 bar
Maximum Flow Gradient	100.000 mL/min²
Stroke A	
Automatic Stroke Calculation A	Yes
Stroke B	
Automatic Stroke Calculation B	Yes
Compress A	
Compressibility Mode A	Compressibility Value Set
Compressibility A	50 10e-6/bar
Compress B	
Compressibility Mode B	Compressibility Value Set
Compressibility B	115 10e-6/bar
Stop Time	
Stoptime Mode	Time set
Stoptime	12.50 min
Post Time	
Posttime Mode	Time set
Posttime	4.50 min

Timetable

Timetable

	Time	Function	Parameter
1	12.00 min	Change Solvent Composition	Solvent composition A: 5.0 % B:95.0 %

Solvent Composition

	Channel	Solvent 1	Name 1	Solvent 2	Name 2	Selected	Used	Percent
1	A	H20	0.1% formic acid	H20		Ch. 1	Yes	95.0 %
2	B	H20	100 % ACN	H20		Ch. 1	Yes	5.0 %

Column Compartment parameters

Name: Column Comp.	Model: G1316B
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	35.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Right temperature Control Mode	Combined
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.80 °C
Stop Time	
Stoptime Mode	As pump/injector
Post Time	
Posttime Mode	Off

Checkout Mix LC/MS Parameters

Source parameters

Source Parameters

Parameter	Value
Gas Temp (°C)	250
Gas Flow (l/min)	7
Nebulizer (psig)	40

Scan Segments

Scan Seg #	Ion Polarity
1	Positive

Scan Segment 1

Scan Source Parameters

Parameter	Value
VCap	3500
Fragmentor	125
Skimmer1	65
OctopoleRFPeak	500

ReferenceMasses

Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer (psig)	5

AutoRecalibration

Average Scans	1
Detection Window (ppm)	50
Min Height (counts)	500

Reference Masses
<Positive>
121.05087300
922.00979800

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	40000000

LC/MS parameters for MS acquisition

Component Name	MS Q-TOF	**Component Model**	G6530A
Ion Source	Dual ESI	**Stop Time (min)**	No Limit/As Pump
Can wait for temp.	Enable	**Fast Polarity**	N/A
MS Abs. threshold	200	**MS Rel. threshold(%)**	0.010
MS/MS Abs. threshold	5	**MS/MS Rel. threshold(%)**	0.010
Tune File	Autotune.tun		

Time Segments

Time Segment #	Start Time (min)	Diverter Valve State	Storage Mode	Ion Mode
1	0	MS	Centroid	Dual ESI

Time Segment 1

Acquisition Mode MS1

Min Range (m/z)	100
Max Range (m/z)	1000
Scan Rate (spectra/sec)	1.00

Forward vs. Reverse Library Search

The forward search compares the Target spectrum to the library. The reverse search compares the library spectra to the Target spectrum. Scores depend on which search is done. High scores are achieved when the bulk of the ion signal is assigned.

In a *forward* search, peaks in Target spectrum are compared to peaks in Library spectrum. Forward search penalizes peaks that are in Target but not in Library AND the peaks that are in Library but not in Target.

A low score for a forward search indicates noise and/or impurities.

In a *reverse* search, peaks in Library spectrum are compared to peaks in Target spectrum. Reverse search only penalizes peaks that are in Library but not in Target.

A reverse search works well for weak or noisy signals if all library ions are included at the approximate correct abundance.

A low reverse search indicates a bad match. Table 3 shows examples of product ion conditions and results.

The **Exclude ion from Reverse Score when ratio of precursor intensity to total library MS/MS intensity exceeds (percent)** check box prevents a very high intensity precursor ion from distorting the reverse score (Score (Rev)). The default value for this check box has been set to 90%. See Figure 40.

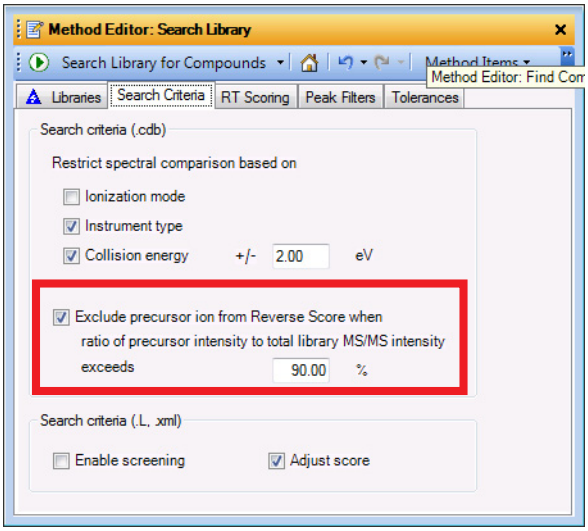


Figure 40 Search Criteria tab with the **Exclude precursor...** check box marked.

If you mark this check box:

- A high intensity precursor ion will not distort the reverse score (Score (Rev).
- The reverse score is calculated as usual unless the precursor ion is more than the given percentage of the total MS/MS intensity. If the precursor ion is the only ion in the spectrum, the hit is reported but the reverse score is blank and is not rolled into the Score (Lib). If the score is blank, then the Flags column is set to Precursor ion only match.

Table 3 Example product ion conditions and search results

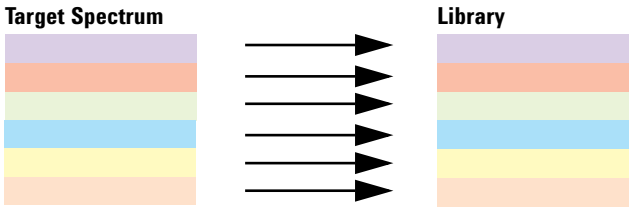
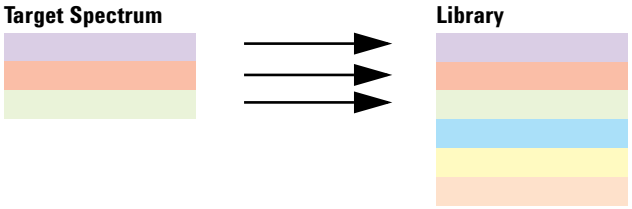
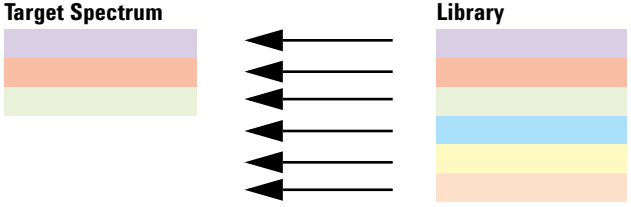
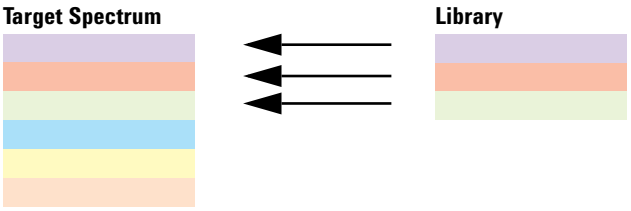
Search	Condition	Score
Forward	 All of the product ions in the sample spectrum are found in the library and vice versa.	High
Forward	 All of the product ions in the sample spectrum are found in the library, but only some of the product ions in the library are found in the sample spectrum.	Low
Reverse	 Only some of the product ions in the library are found in the sample spectrum.	Low

Table 3 Example product ion conditions and search results (continued)

Search	Condition	Score
Reverse	 All of the product ions in the library are found in the sample spectrum.	High

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In This Guide

This Quick Start Guide describes how to use the Checkout Mix PCDL as a learning tool.

This guide is valid for the B.07.00 revision or higher of the Checkout Mix PCDL, until superseded.

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