MassHunter Pesticides PCD or PCDL

Quick Start Guide

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What is the MassHunter Pesticides PCD or PCDL?

The MassHunter Pesticides Personal Compound Database (PCD) and the accurate mass Personal Compound Database and Library (PCDL), along with the included methods and example test mix data, lets you screen pesticide analytes in a single LC/MS analysis.

The G6854AA or G3878AA Pesticides PCD or PCDL Kit also includes the column and test mix to acquire example data. The search is done using the MassHunter Pesticides PCD or the MassHunter Pesticides PCDL.

The PCDL contains accurate mass MS/MS spectra for some compounds, in addition to accurate mass information for all compounds in the PCD.

PCD

The PCD lets you screen over 1600 analytes all in a single LC/MS analysis.

The MassHunter Pesticides PCD kit helps minimize method development time for your analysis. The database stores accurate mass values, as well as retention time values and other information that you add to compounds in the database. Subsets of the database can also be created. These subsets can contain different lists of compounds which have different retention times associated with them, allowing the database collection to be tailored to the specific needs of your laboratory.

The high mass accuracy of the Agilent time-of-flight (TOF) and tandem quadrupole time-of-flight (Q-TOF) LC/MS instrument provides the capability to screen and identify all compounds in the database that are detected by their exact mass and retention time (if known). Retention times can be a search criterion specified as not required (non-targeted screen), as optional providing a targeted and non-targeted pesticides screen, or required (targeted screen only).

PCDL

The Pesticides PCDL lets you screen 1600 analytes with accurate mass database and/or perform a compound library search for over 700 compounds. The MassHunter Pesticides PCDL kit helps minimize method development time for your analysis.

The master Pesticides PCDL can be used as is, or as the basis of your own customized PCDL. Your customized PCDL can store the retention times for compounds you analyze. You can add, remove and change the compounds in your PCDL to meet the specific needs of your laboratory and your analyses. You can also add your own spectra to your customized PCDL, in addition to those provided in the master PCDL. With MassHunter Qualitative Analysis B.07.00, you can run a database search to identify compounds, and then send the MS/MS spectra to your customized PCDL. You can also filter spectral noise and correct the product ions to their theoretical accurate mass.

The high mass accuracy of the Agilent tandem quadrupole time-of-flight (Q-TOF) LC/MS instrument provides the capability to screen all compounds in the library that are detected by their exact mass and retention time (if known). Searching the library can then identify the compounds found by comparison to their accurate product ion mass spectra. Retention times can be a search criterion specified as not required (non-targeted screen), as optional providing a targeted and non-targeted pesticides screen, or required (targeted screen only). With the Q-TOF, detection of unknowns (compounds not in the library), and identification using the MS/MS spectra, is also possible.

Kit Contents

Quick Start Guides

MassHunter Pesticides PCD or PCDL Quick Start Guide The Quick Start Guide gives an overview of the MassHunter Pesticides PCD or PCDL and tells you how to use it.

MassHunter Personal Compound Database and Library Manager Quick Start Guide The Quick Start Guide gives you an overview of the MassHunter Personal Compound Database and Library Manager and tells you how to use it with the MassHunter Pesticides PCD or PCDL.

Installation and Supplemental Discs

Each kit includes the **MassHunter Personal Compound Database and Library Manager** disc. Each kit also contains either the **MassHunter MassHunter Pesticides PCD** disc or the **MassHunter Pesticides PCDL** disc.

MassHunter Pesticides PCD or PCDL disc This disc contains:

- MassHunter Pesticides PCD (**Pesticides_AM_PCD.cdb**) or MassHunter Pesticides PCDL (**Pesticides_AM_PCDL.cdb**)
- Test Mix database:
  - **Pesticides_Std.cdb**
- *MassHunter Pesticides PCD or PCDL Quick Start Guide* (PDF)
- Technical notes
- Application notes

- TOF/Q-TOF LC/MS methods to run and analyze the test mix:
  - Pesticides_TestMix_MS.m

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

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

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

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

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

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

    Q-TOF data analysis method for auto MS/MS analysis
- Example data files:
  - Pesticides_TestMix_MS.d
  - Pesticides_TestMix_TMSMS.d
  - Pesticides_TestMix_AMSMS.d
- Example reports
- MassHunter Pesticides PCD or PCDL Comprehensive Test Mix *Method Setup Guide*

MassHunter Personal Compound Database and Library Manager disc This disc contains:

- MassHunter Personal Compound Database and Library Manager
- *MassHunter Personal Compound Database and Library Manager Quick Start Guide* (PDF)
- Software license agreements
- Example data

Other Parts If you purchase the G6854AA or G3878AA Pesticides PCD or PCDL Kit, you also receive these parts.

ZORBAX LC Column (p/n 959758-902) Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm.

ZORBAX LC Column (p/n 959759-902) Eclipse Plus C18, 2.1 mm × 150 mm, 1.8 µm.

Poroshell 120 Column (p/n 695775-902) EC-C18, 2.1 mm × 100 mm, 2.7 µm.

LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) Test mix containing 20 analytes of interest for your test runs. The contents are listed in "Checkout Mix Content" on page 67.

Where to find more information

Application Notes and Publications You can find information about the MassHunter Pesticides PCD or PCDL in the application notes and publications included on the MassHunter Pesticides PCD or PCDL disc.

Go to http://www.chem.agilent.com/ for the most current information on Agilent products.

Before You Begin

Installation

To run the test mix

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

2 On the Agilent 1200 Series Binary Pump SL, check that the mixer and damper are bypassed. See "To bypass mixer and damper" on page 14 for details.

3 Check that the 6500 Series LC/MS (PCD or PCDL) or 6200 Series LC/MS (PCD only) is properly installed and verified.

To do compound and library searches

1 Check that the following programs are properly installed:

- MassHunter Data Acquisition B.05.00 or higher
- MassHunter Qualitative Analysis B.07.00 or higher

2 Install the MassHunter Personal Compound Database and Library Manager. Refer to the *MassHunter Personal Compound Database and Library Manager Quick Start Guide*.

3 Install the MassHunter Pesticides PCD or PCDL:

a Insert the database disc into the disc drive.

b In the welcome screen, click **Pesticides PCD** (or **PCDL**) **Installation**.

c Read the instructions to install the database, then click the command to install the MassHunter Pesticides PCD or PCDL and the Test Mix PCDL.

4 Copy the methods from the MassHunter Pesticides PCD or PCDL disc to the **MassHunter\Methods** folder on your computer.

Required reagents and parts (to run test mix)

- LC/MS grade acetonitrile, and water
- Glacial acetic acid 99.9% (highest purity)
- ZORBAX LC Column (p/n 959758-902)

Alternative configuration

The sample methods and data files from the test mix 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 Test Mix

Do the steps in this section if you purchased the G6854AA or G3878AA Pesticides PCD or PCDL Kit, and you want to run the test mix to collect example data. Otherwise, use the example data that is included with the PCD or PCDL disc to do the exercises in this guide.

The sample data files provided in the MassHunter Pesticides PCD or PCDL disc were acquired with the test mix on a system with the LC/MS system configured as described in "Installation" on page 8. Along with the sample data files are the methods with which these data files were acquired. If you review the acquisition method and sample data, you will get a sense of the data acquisition, data processing, and result interpretation you will encounter while using the MassHunter Pesticides PCD or PCDL.

To review the Data Acquisition methods, use the MassHunter Data Acquisition program to open these method files:

- Pesticides_TestMix_MS.m for compound searches
- Pesticides_TestMix_TMSMS.m (targeted MS/MS), or Pesticides_TestMix_AMSMS.m (auto MS/MS) for library searches (Q-TOF only)

The following Data Acquisition settings for the test mix are listed:

- Data Acquisition method information
- Q-TOF LC/MS settings
- Wellplate sampler settings
- Binary pump settings
- Thermostatted column compartment settings

Note that the method uses 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. 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.*

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.

To run the Checkout Test Mix

Run the LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) to get a better idea of how the database kit will work for you.

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 Test Mixes.

The concentration of the Checkout Test 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 separately for the positive and negative Checkout Test Mixes.

NOTE

For some instrument configurations, this sample concentration is too high. If you consistently see “saturated” warnings listed for some compounds, or if “*” indicators appear routinely 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 Test Mixes.

The examples in this guide were run in positive mode only, using a different mobile phase optimized for basic pesticides. The elution order of the basic pesticides will differ 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, 1.8 µm
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 14.
Column Compartment	Column – SL, Model G1316B Column - SL, Model G1316C

5 Load the Checkout Test Mix method Pesticides_TestMix_MS.m or Pesticides_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 your system is tuned and calibrated correctly.

d Run the test mix again.

This will not affect your results but will show if retention times are different on your system. There are a number of reasons your retention times can change from those determined by Agilent, such as different instrument delay volume, dead volumes or configuration.

For Library Searches (with PCDL)

9 Run the test mix again with the methods **Pesticides_TestMix_TMSMS.m** and **Pesticides_TestMix_AMSMS.m**.

When you run the test mix with this method, a workflow is simulated for the screening and identification of pesticides using library searching. See the application note *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).

To bypass mixer and damper

You only need to bypass the mixer and damper 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.

Configurations where only the damper or the mixer is disconnected while the other part is still in line are not supported by Agilent Technologies.

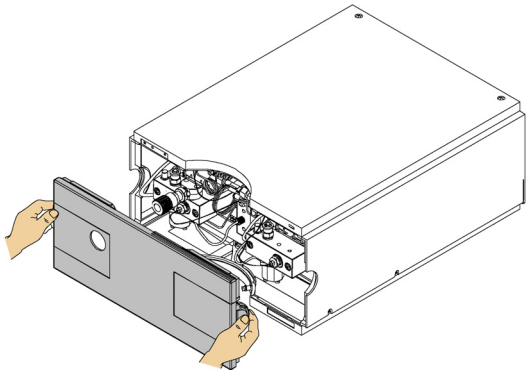
Tools required

- 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)

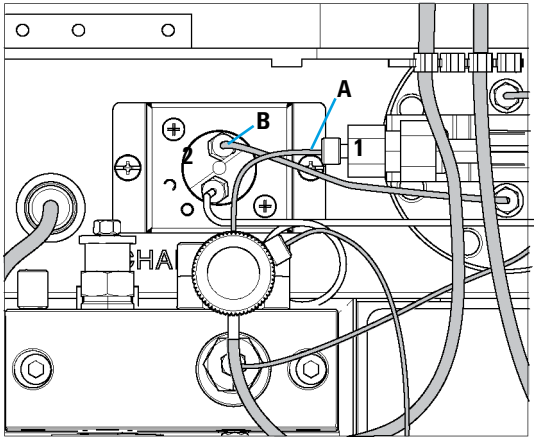
Preparations for this procedure

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

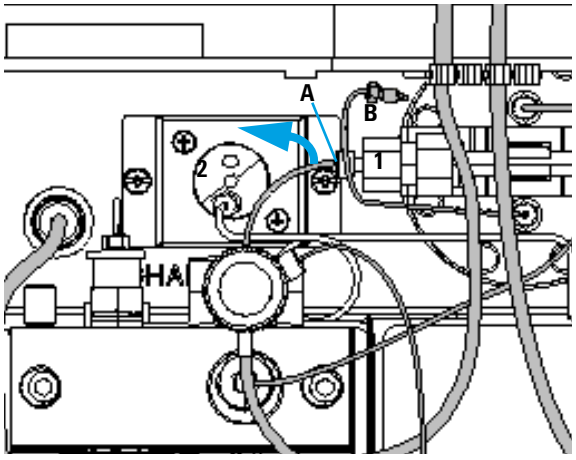
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



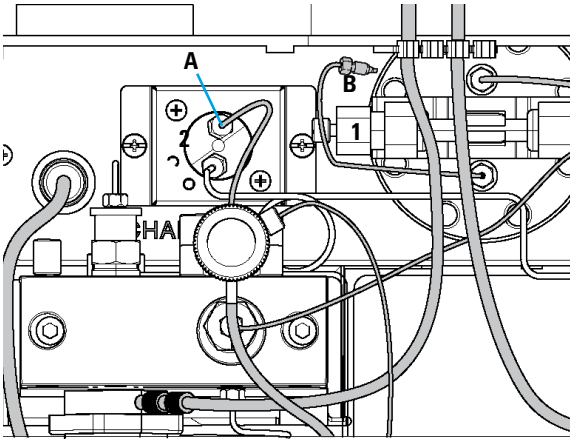
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



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.



Using MassHunter Qualitative Analysis to Identify Compounds

To identify compounds using the MassHunter Qualitative Analysis program

- To search the PCD or PCDL to identify compounds (with or without retention times), refer to the online Help for **Identifying Compounds > Search database for a compound**.
- To search the PCD or PCDL to identify compounds from spectrum peaks, refer to the online Help for **Spectrum Tasks > Search database from a spectrum**.

To identify spectrum peaks using the MassHunter Qualitative Analysis program (PCDL only)

- To search the PCDL to identify compounds, refer to the online Help for **Identifying Compounds > Search accurate mass library for compounds**.
- To search the PCDL for spectra, refer to the online Help for **Spectrum Tasks > Search accurate mass library for spectra**.

Familiarization Exercises - Compound Search

The exercises in this section can be done with a TOF or Q-TOF LC/MS, with the MassHunter Pesticides PCD or PCDL.

Three exercises are described in this topic to do a compound search. The recommended process is described in "Exercise 1. Process and interpret data with Find by Formula" on page 18.

The elution order of the compounds in the Checkout Test Mix have been determined using the Eclipse Plus C18 column and mobile phases specified in the "To run the Checkout Mix" on page 11. The expected elution order is:

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

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

Note the example data 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, copy the custom database **Pesticides_Std.cdb** to **D:\MassHunter\PCDL**, or wherever MassHunter databases are stored.

Use the data file found in the **Example Data** folder on the MassHunter Pesticides PCD or PCDL disc. If you have the G6854AA or G3878AA Pesticides PCD or PCDL Kit and you ran the test mix (see "To run the Checkout Test Mix" on page 11), you can use the data file that you acquired. Your results may differ slightly.

Steps	Detailed Instructions	Comments
1 Process the data file for the positive ion test 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 test mix: **c** Load the method **Pesticides_TestMix_MS_DA.m**. **d** Open the data file **Pesticides_TestMix_MS.d**. See Figure 1.	

Steps	Detailed Instructions	Comments

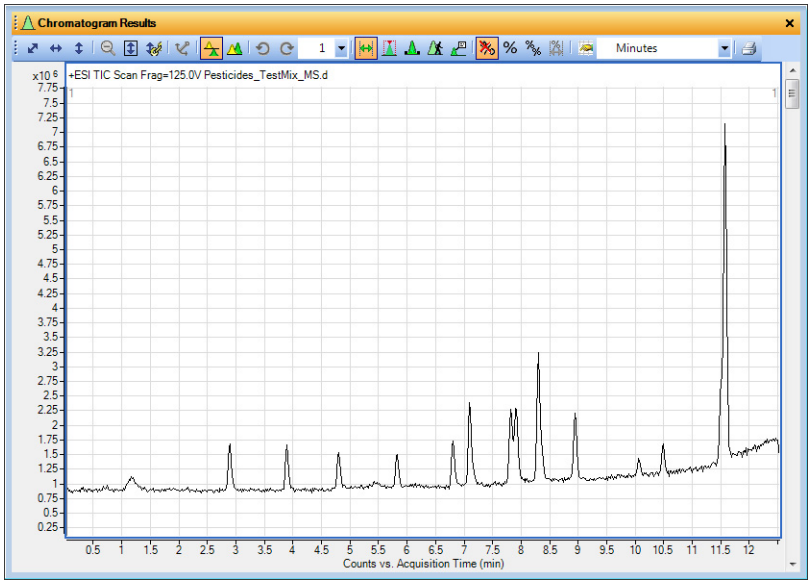


Figure 1 Example Test Mix Total Ion Chromatogram

2 Review the method to become familiar with the settings for Find by Formula. Use the database **Pesticides_Std.cdb**.	**a** Locate the **Find Compounds by Formula > Options** section in the Method Explorer. **b** Select the custom database **Pesticides_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.	The **Pesticides_Std.cdb** does not contain retention times or isomers, therefore all compounds are easily identified using the Mass option. To easily identify isomeric compounds, add retention times to your custom PCDL and select one of the Mass and retention time options.

Steps	Detailed Instructions	Comments

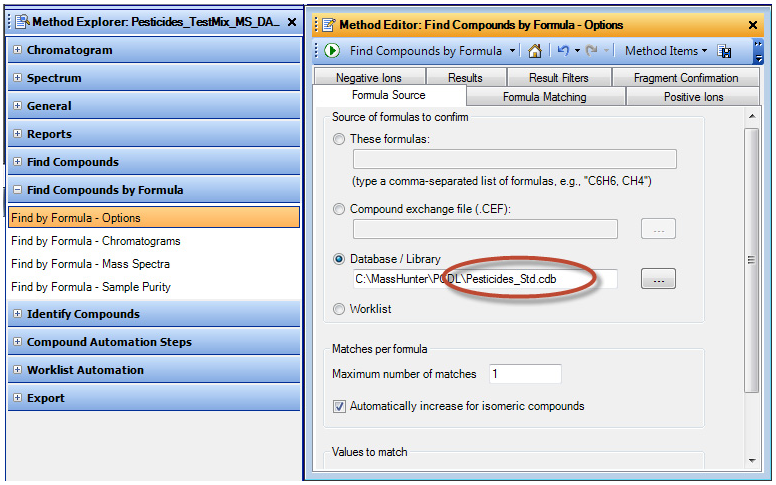


Figure 2 Find by Formula Method Editor Options (Custom Database)

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.	

Steps	Detailed Instructions	Comments

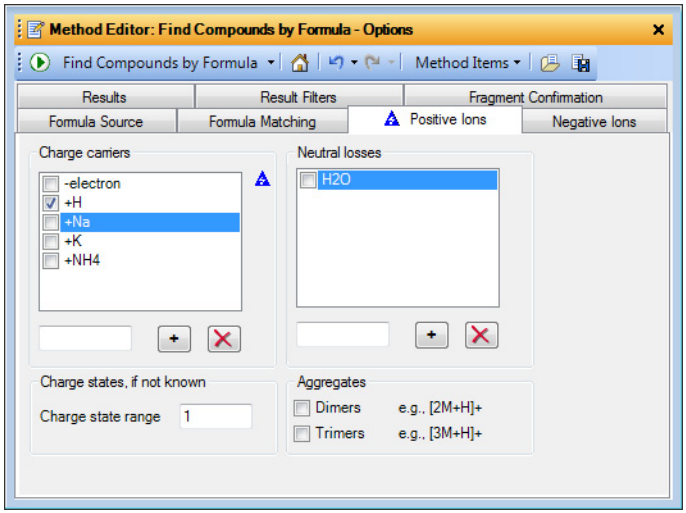


Figure 3 Positive Ions tab.

4	Use the MassHunter Pesticides Standard PCDL to find compounds in the data file **Pesticides_TestMix_MS.d**	**a** Click the green arrow ( ) in the Method Editor toolbar.	The Qualitative Analysis program searches each entry in the MassHunter Pesticides Standard PCDL (**Pesticides_Std.cdb**) to find compounds in the data file.

Steps	Detailed Instructions	Comments

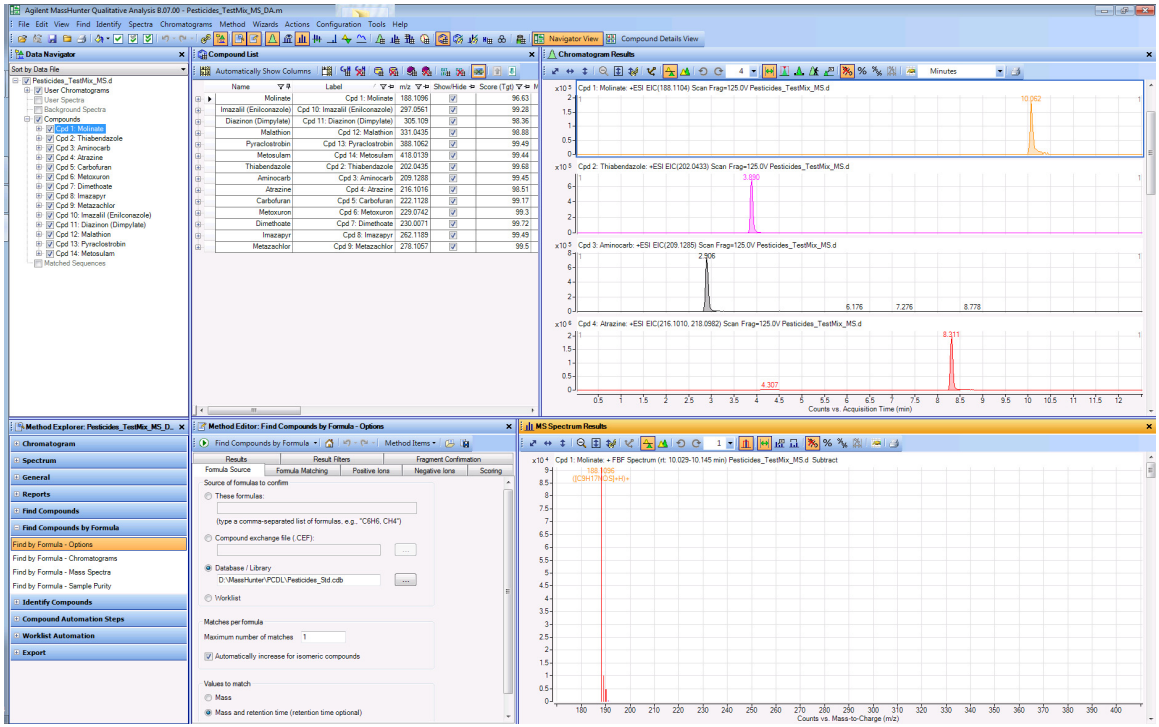


Figure 4 Find By Formula Results using MassHunter Pesticides Standard PCDL. (**Pesticides_Std.cdb**)

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 a a time. **c** Click Navigator View.	

Steps	Detailed Instructions	Comments

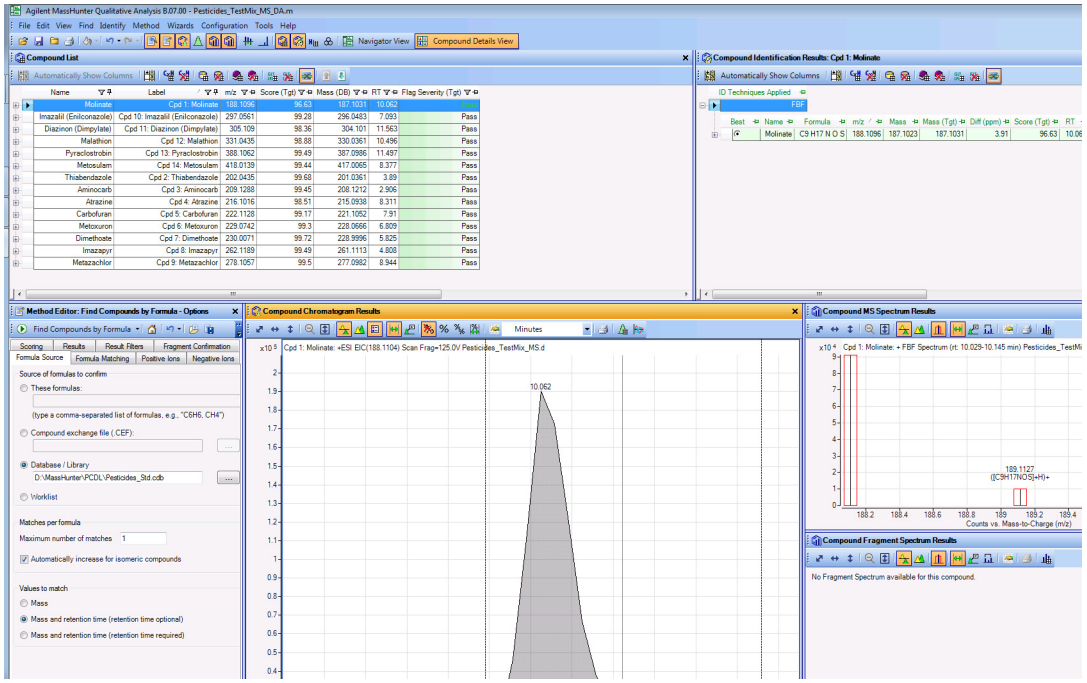


Figure 5 Compound Details view.

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 will use this file in a later exercise for Targeted MS/MS analysis. The **Pesticides_TestMix_MS.csv** test mix data file in Excel format is included in the **Example Reports** folder on the installation disc.

Steps	Detailed Instructions	Comments

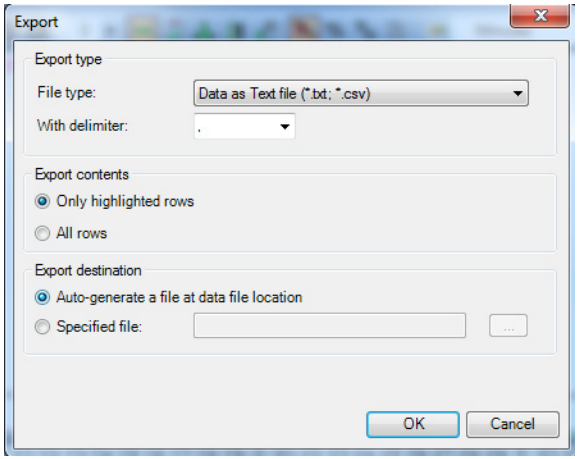


Figure 6 Export Find by Formula results to a Text file.

7 Remove the results prior to 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 Defined Extracted Ion Chromatograms

In this exercise, you process the data file **Pesticides_TestMix_MS.d.**

Steps	Detailed Instructions	Comments
1 Process the data file for the positive ion test mix.	**a** In Method Explorer, click **Chromatogram > Define Chromatograms**. See Figure 8.	A list of the exact *m/z* values of the compounds in the mixture is displayed in the **Chromatograms > Define Chromatograms** section.

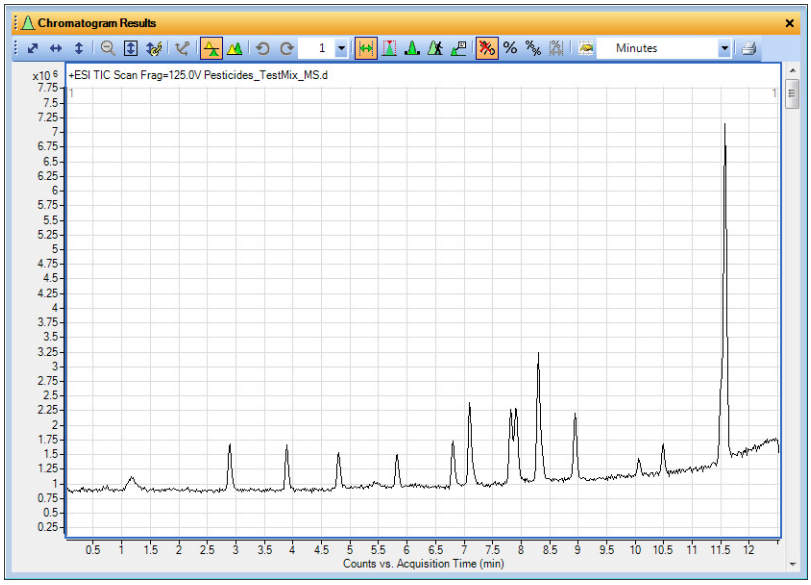


Figure 7 Example Test Mix Total Ion Chromatogram

Steps	Detailed Instructions	Comments

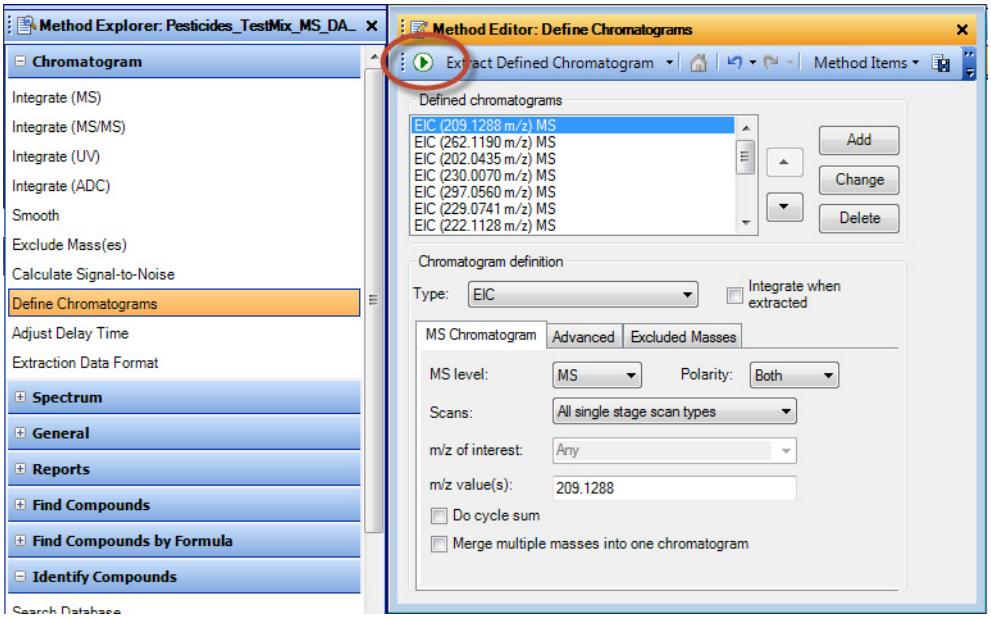


Figure 8 Define Chromatograms section selected. Click the green arrow (circled) to extract the ions.

2 Extract the ions.	**a** Click the green arrow in the Method Editor toolbar.	After the chromatograms are extracted, they are displayed in the Chromatogram Results window, as seen in Figure 9, if the view is in List Mode. In this figure, you can see the major peak in each EIC.

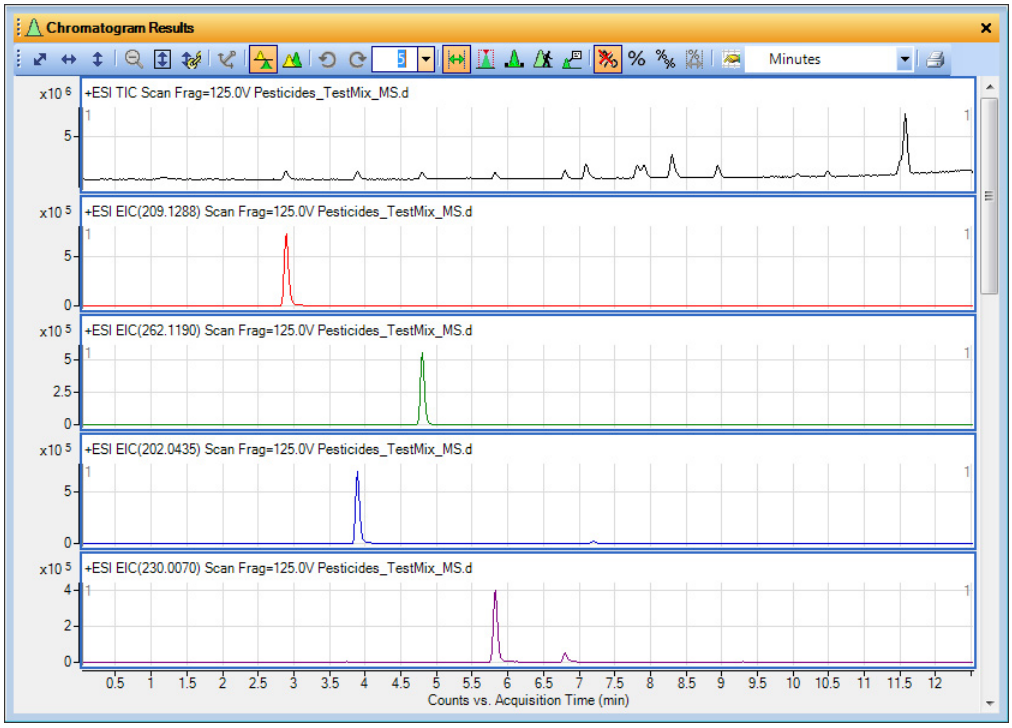


Figure 9 Extracted Ion Chromatograms

Exercise 3. 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. These will have to be adjusted 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 unnecessarily long. See Figure 10.	

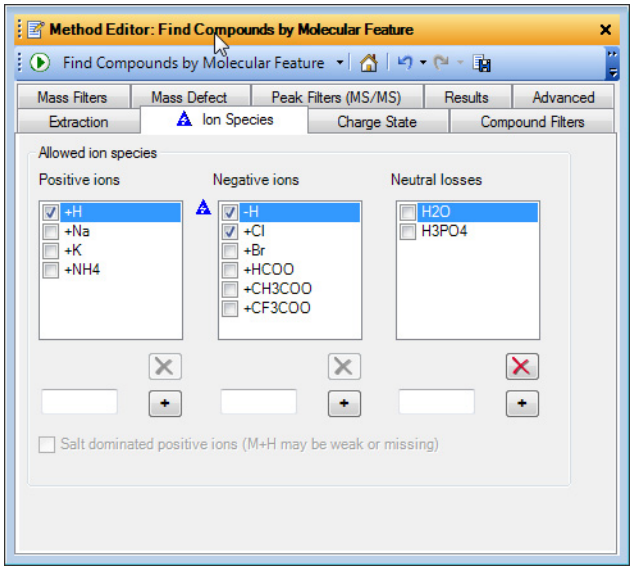


Figure 10 Ion Species tab.

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 will be extracted. Figure 11 illustrates the results for Find by Molecular Feature.
3 Search the PCD or PCDL for the selected compounds.	**a** In the Data Navigator, click the **Compounds** line to select all compounds that were generated by MFE 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 11).	If the Advanced tab is not visible in the Method Editor, click **Configuration > User Interface Configuration** and then mark the **Accurate mass (TOF, Q-TOF)** and **Show advanced parameters** check boxes.

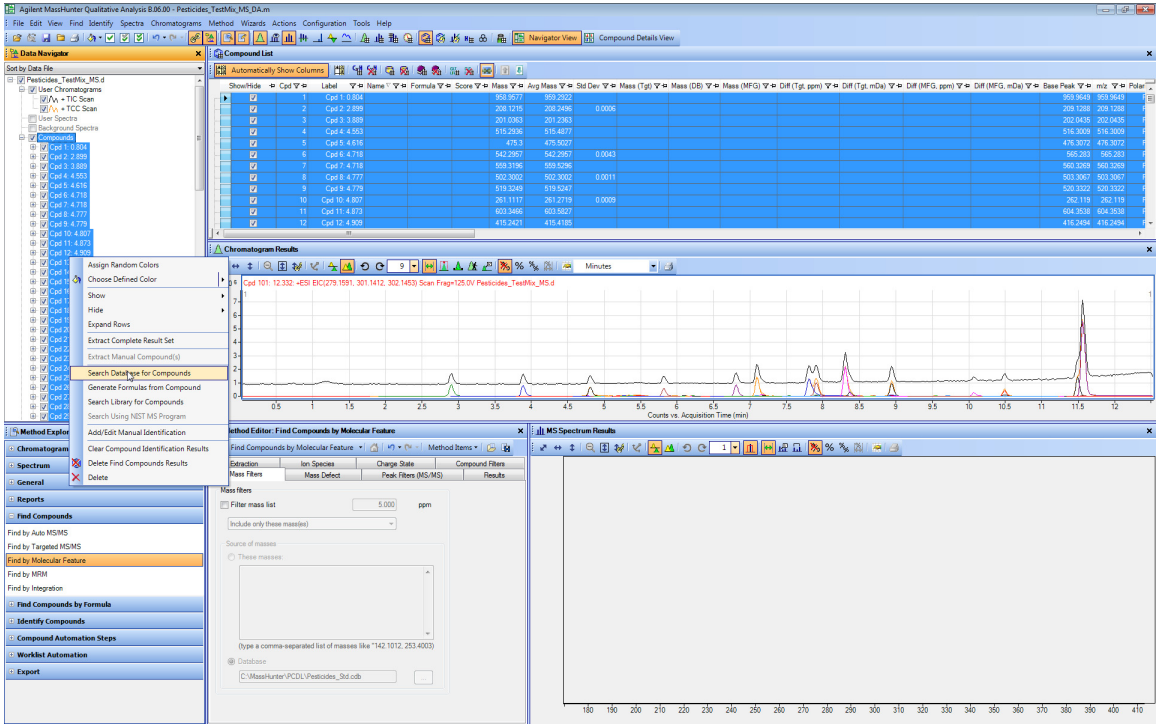


Figure 11 Database Search Results on Find by Molecular Feature compounds. To get the overlaid chromatograms in the display, use the **Overlaid** tool at the top of the Chromatogram Results window.

The custom database is searched against each MFE result. Figure 12 shows the compound identification results obtained from a search on the MassHunter Pesticides Standard PCDL.

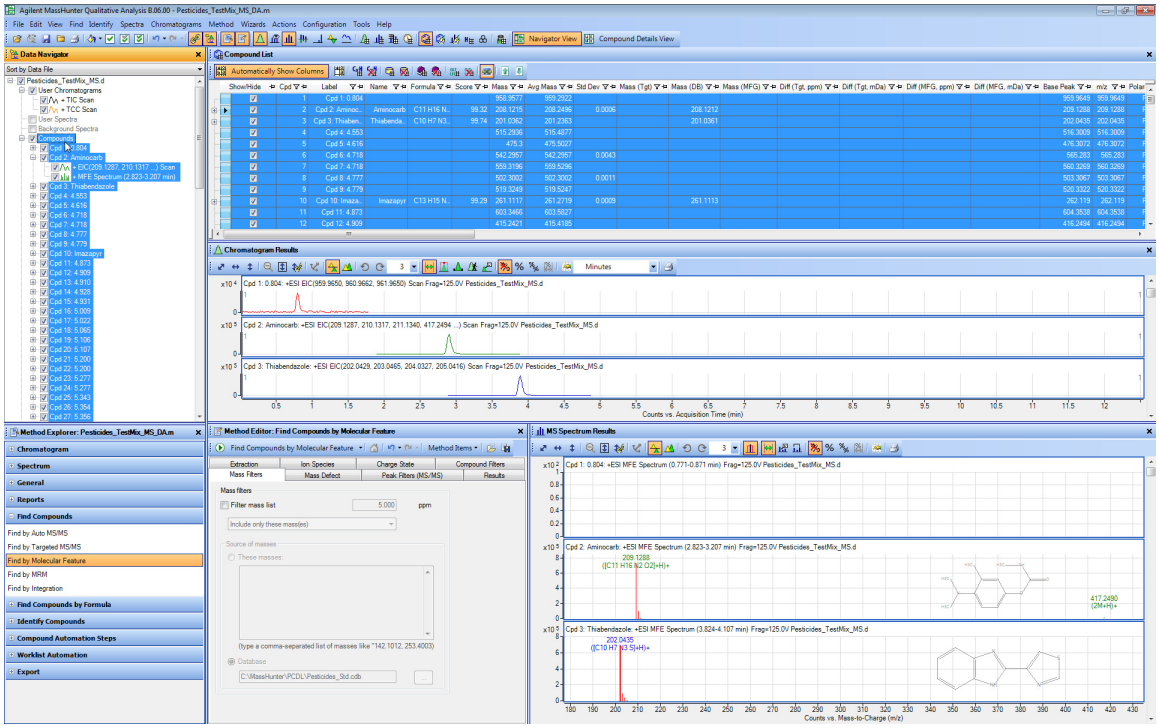


Figure 12 Find by Molecular Feature Database Search. Use the tools at the top of the Compound List window to hide columns, auto-size the column widths, and sort the list.

Exercise 4. Process data automatically using Worklist Automation

After you decide the correct settings for all aspects of the Find Compounds algorithms and Search Database algorithms (such as those described in the application note 5990-4252EN), you can save these settings to one convenient Qualitative Analysis method for repetitive and consistent data manipulation from week to week.

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 automatically process a data file with the Find by Molecular Feature algorithm, search the MassHunter Pesticides PCD or PCDL, and 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 will be 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 13.	Note that 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.

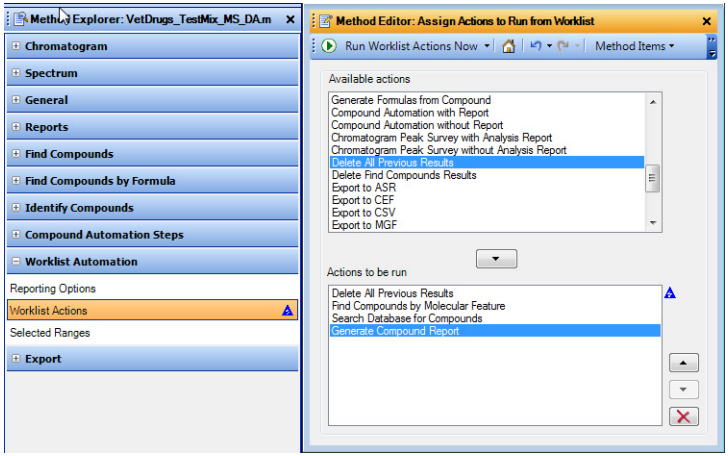


Figure 13 Method Editor with list of selected actions

Steps	Detailed Instructions	Comments
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 14.	

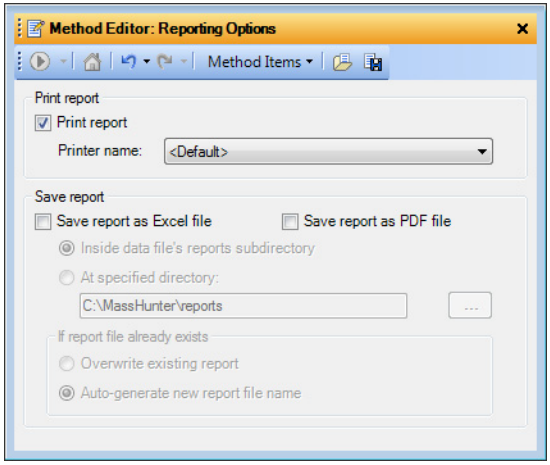
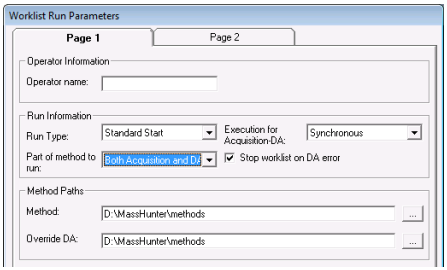


Figure 14 Reporting Options

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 an integral part of the Data Acquisition method.

Steps	Detailed Instructions	Comments
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 15** Worklist Run Parameters window

The Qualitative Analysis steps defined and set up under **Actions to be Run** in the Method Editor will run 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 MassHunter Pesticides PCD or PCDL, samples can be screened for and reported automatically.

You can create smaller and more focused custom databases from the larger MassHunter Pesticides PCD or PCDL for a specific industry needs such as work-place drug testing.

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

To develop a custom PCD or PCDL

The use of a smaller and more focused database to screen samples can be a powerful tool to detect and identify specific analytes that are required by various regulatory agencies. After a custom database of targeted compounds is created, single standards of those compounds must be analyzed using a standard chromatography method, retention times recorded, and detection limits determined.

- Run standards of targeted compounds and create custom databases from the MassHunter Pesticides PCD or PCDL.

  The technical notes *Pesticide Personal Compound Database for Screening and Identification* (p/n 5990-3976EN) and *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) included on the MassHunter Pesticides PCD or PCDL disc describes how to create a custom database, and to add retention times for your compounds and chromatographic conditions to the database.

  An example of the addition of retention times to a custom database for a negative ion test mix is given in the application note *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).

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 will be fragmented and an MS/MS spectrum will be obtained. If you use Auto MS/MS mode instead (see "Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search" on page 54), the precursor in the mass spectrum must satisfy certain "on-the-fly" rules in order to be chosen for fragmentation. 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 **Pesticides_TestMix_TMSMS.d.** Use the example data file found in the **Example Data** folder on the MassHunter Pesticides PCD or PCDL disc. If you have the G3878AA MassHunter Pesticides PCDL Kit and you ran the test mix, you can use the data file that you acquired. Your results can differ slightly.

This section consists of three exercises:

- Exercise 1. Set up the targeted MS/MS method
- Exercise 2. Process the data
- Exercise 3. Automate the process with worklist actions

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 17. 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 16. **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 just created. See Figure 17.	

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

Step	Detailed Instructions	Comments

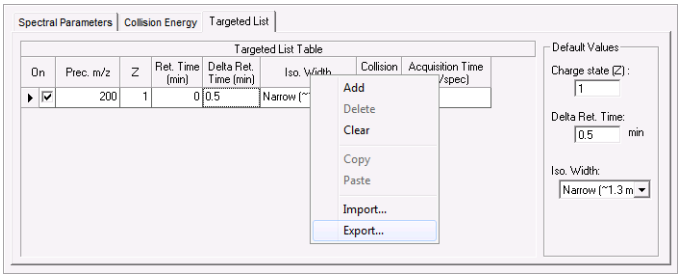


Figure 16 Targeted List tab, Export listed in shortcut menu

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 17 Template .csv file

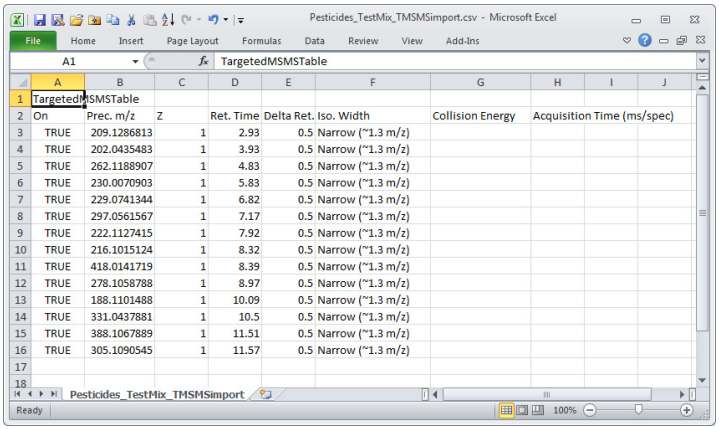
2 Create exact mass column in the Compounds List results file that you saved previously, and add to the template file. See Figure 18.	**a** Start the Excel program, and open the spreadsheet file that you exported from the MassHunter Qualitative Analysis program in "Exercise 1. Process and interpret data with Find by Formula" on page 18. **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 may 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 18. **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 MassHunter Pesticides PCDL disc under **Example Reports**, as **Pesticides_TestMix_MS.csv** and **Pesticides_TestMix_TMSMSimport .csv**.	The collision energy values should be the same as the three energies in the library (10, 20 and 40 eV), as described in the application notes *Toxicological Screening with the Agilent LC/MS-QTOF and the Personal Compound Database and Library using the "Broecker, Herre and Pragst" Accurate Mass Spectral Library* (p/n 5590-6419EN) and *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). 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 54.

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

Step	Detailed Instructions	Comments



	A	B	C	D	E	F	G	H	I	J
1	TargetedMSMSTable									
2	On	Prec. m/z	Z	Ret. Time	Delta Ret.	Iso. Width	Collision Energy	Acquisition Time (ms/spec)		
3	TRUE	209.1286813	1	2.93	0.5	Narrow (~1.3 m/z)				
4	TRUE	202.0435483	1	3.93	0.5	Narrow (~1.3 m/z)				
5	TRUE	262.1188907	1	4.83	0.5	Narrow (~1.3 m/z)				
6	TRUE	230.0070903	1	5.83	0.5	Narrow (~1.3 m/z)				
7	TRUE	229.0741344	1	6.82	0.5	Narrow (~1.3 m/z)				
8	TRUE	297.0561567	1	7.17	0.5	Narrow (~1.3 m/z)				
9	TRUE	222.1127415	1	7.92	0.5	Narrow (~1.3 m/z)				
10	TRUE	216.1015124	1	8.32	0.5	Narrow (~1.3 m/z)				
11	TRUE	418.0141719	1	8.39	0.5	Narrow (~1.3 m/z)				
12	TRUE	278.1058788	1	8.97	0.5	Narrow (~1.3 m/z)				
13	TRUE	188.1101488	1	10.09	0.5	Narrow (~1.3 m/z)				
14	TRUE	331.0437881	1	10.5	0.5	Narrow (~1.3 m/z)				
15	TRUE	388.1067889	1	11.51	0.5	Narrow (~1.3 m/z)				
16	TRUE	305.1090545	1	11.57	0.5	Narrow (~1.3 m/z)				
17										
18										

Figure 18 Template .csv after retention time and accurate mass are added

3 Open the Compounds List results file that you saved in "Exercise 1. Process and interpret data with Find by Formula" on page 18, and then import the values from the template .csv file that you just 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 18. 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 just 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 19 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.

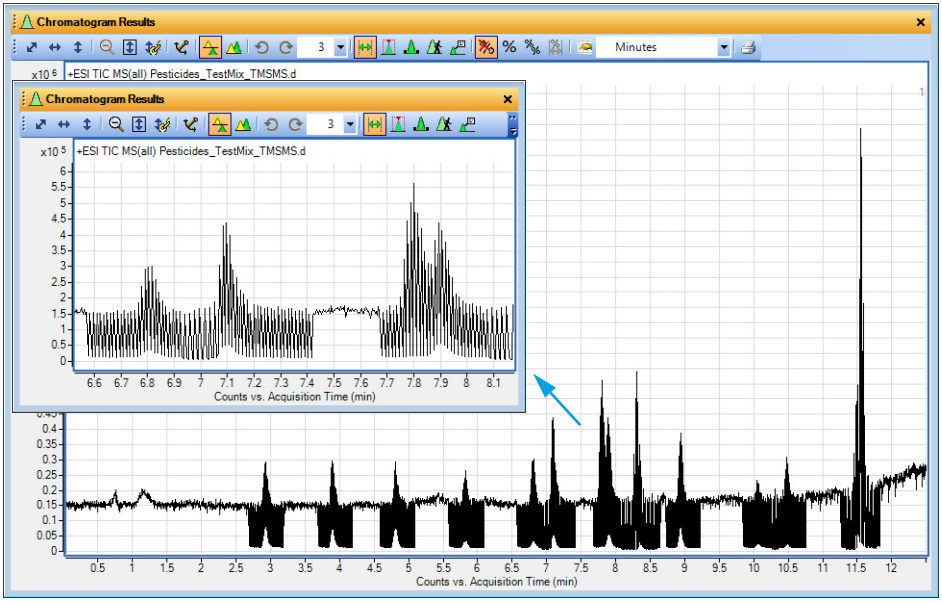


Figure 19 Total ion chromatogram from a typical targeted MS/MS data shows sawtooth pattern from alternating MS and MS/MS scans.

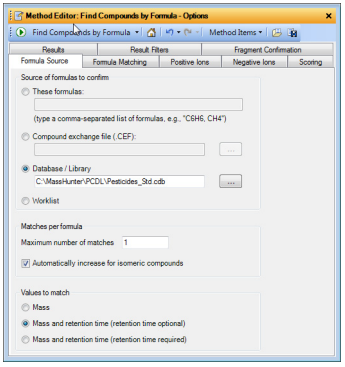
Exercise 2. Process the data

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

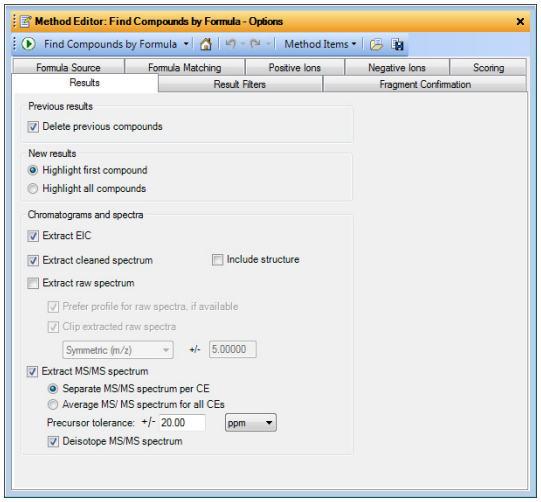
- Find compound using “Find Compounds by Formula”
- Identify compounds using “Search Accurate Mass Library”
- Generate Compound Report
- Print Compound Report

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 will be found.	**a** Start the MassHunter Qualitative Analysis program **b** Open the Method Editor. **c** Open the data analysis method **Pesticides_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 Pesticides Standard Library. See Figure 20. **e** On the **Results** tab, select **Extract MS/MS spectrum** and **Separate MS/MS spectrum per CE**. See Figure 21.	 **Figure 20** 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 21 Results tab	
2 Search the **Pesticides_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 **Pesticides_Std.cdb**. See Figure 22. **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 22.	See "Forward vs. Reverse Library Search" on page 74 for more information. 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

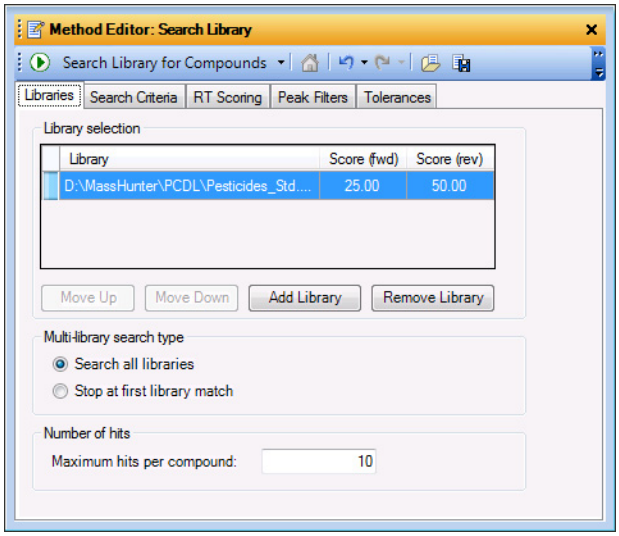


Figure 22 Libraries tab

d	In the **Search Criteria** tab, mark the check boxes for **Collision energy** and **Exclude precursor ion from Reverse Score**. See Figure 23.	If you do not see **Exclude precursor ion from Reverse Score**, make sure that **Show advanced parameters** is selected in the MassHunter Qualitative Analysis program. See step 3 on page 29.
e	In the Peak Filters tab, set the **Absolute height** to **5** counts and the **Relative height** to **1% of largest peak**. See Figure 24.	

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

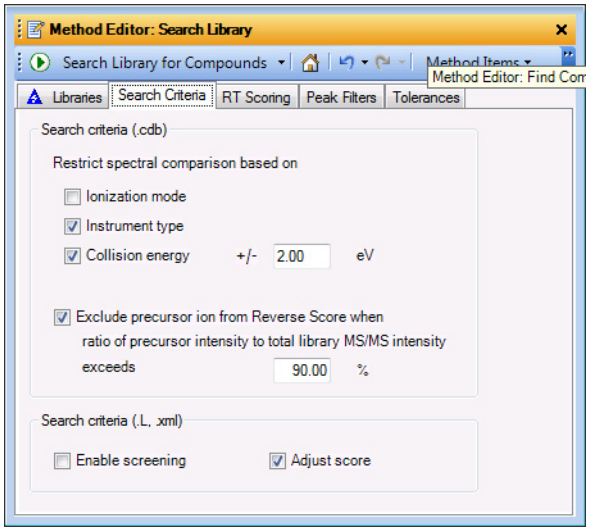


Figure 23 Search Criteria tab.

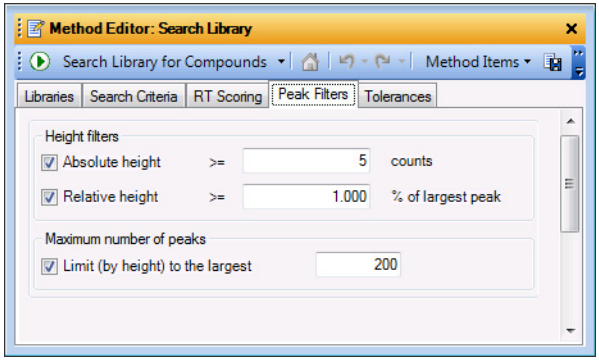


Figure 24 Peak Filters tab.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments
3 Set up the method to: • Find all of the compounds in the Checkout Test 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 25: • **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 reduced as much as possible. Note that setting the Matching criteria in the Results filters tab in the Find by Formula options can prevent small impurities from being reported.

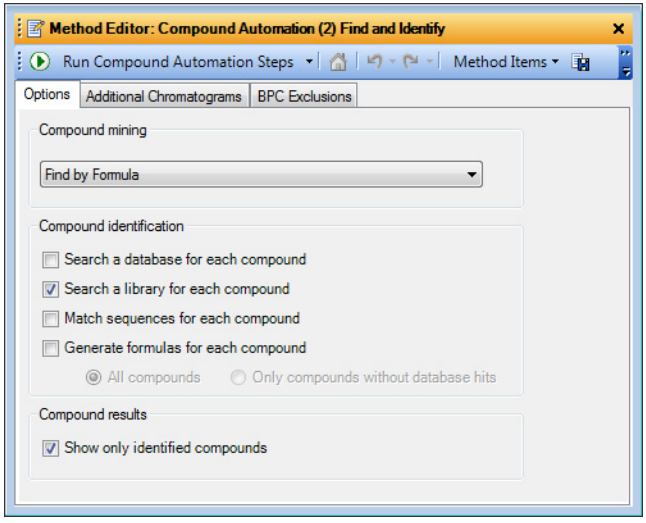


Figure 25 Options tab for Compound Automation Find and Identify

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments
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 26. **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 27. **e** Save the method.	Figure 28 and Figure 29 shows the first two pages from the report for the Targeted MS/MS analysis on the **Pesticides_TestMix_TMSMS.d** (found on the MassHunter Pesticides PCD or PCDL disc). A copy of this report is also available in the report folder as a PDF file.

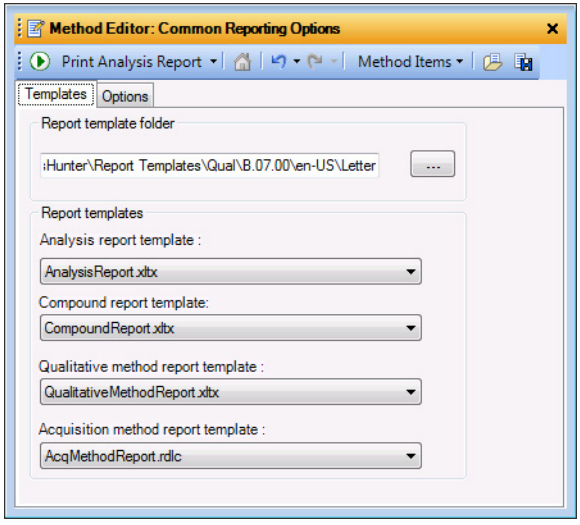


Figure 26 Template tab in Common Reporting Options.

Exercise 2. Process the data (continued)

Step	Detailed Instructions	Comments

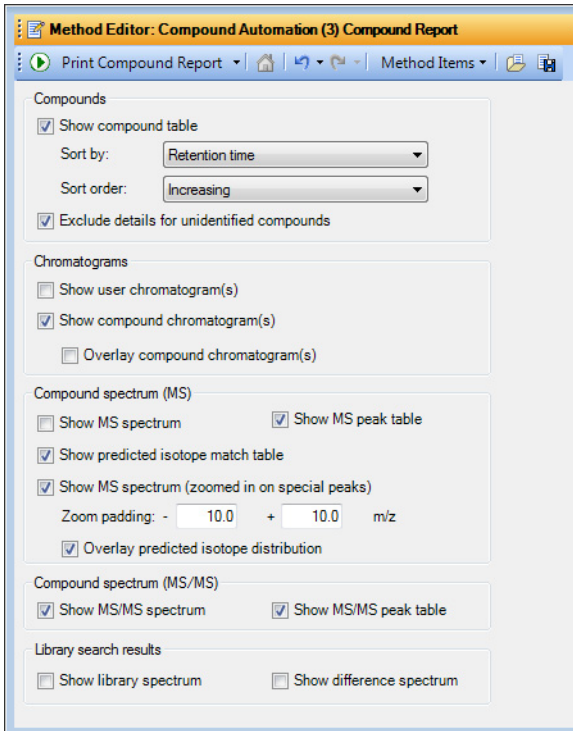
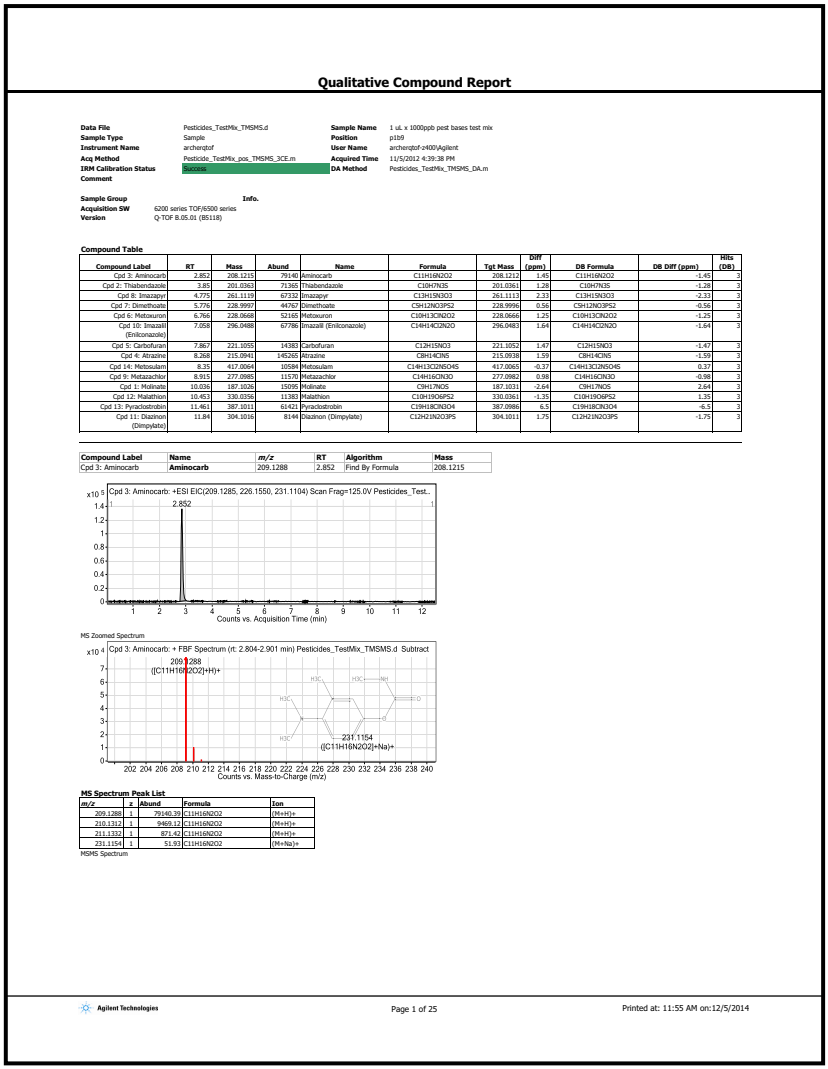


Figure 27 Compound Report dialog box.

When the method is run, a report is generated that includes a summary (Figure 28) as well as details for each compound found in the library (Figure 29). Note that 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 29 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. The data file can be inspected manually as well as to determine if anything was missed, or to get further supporting information that may be in the data but is not being reported.



Qualitative Compound Report

Data File	Pesticides_TestMix_TMSMS.d	**Sample Name**	1 uL x 1000ppb pest bases test mix
Sample Type	Sample	**Position**	p1b9
Instrument Name	archerqtof	**User Name**	archerqtof-p400\Agilent
Acq Method	Pesticide_TestMix_pos_TMSMS_3CE.m	**Acquired Time**	11/5/2012 4:39:38 PM
IRM Calibration Status	Success	**DA Method**	Pesticides_TestMix_TMSMS_DA.m
Comment			

Sample Group	**Info.**
Acquisition SW	6200 series TOF/6500 series
Version	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)+
211.1322	1	871.42	C11H16N2O2	(M+H)+
231.1154	1	51.93	C11H16N2O2	(M+Na)+

MSMS Spectrum

Agilent Technologies Page 1 of 25 Printed at: 11:55 AM on:12/5/2014

Figure 28 Page 1 of the Test Mix Compound report.

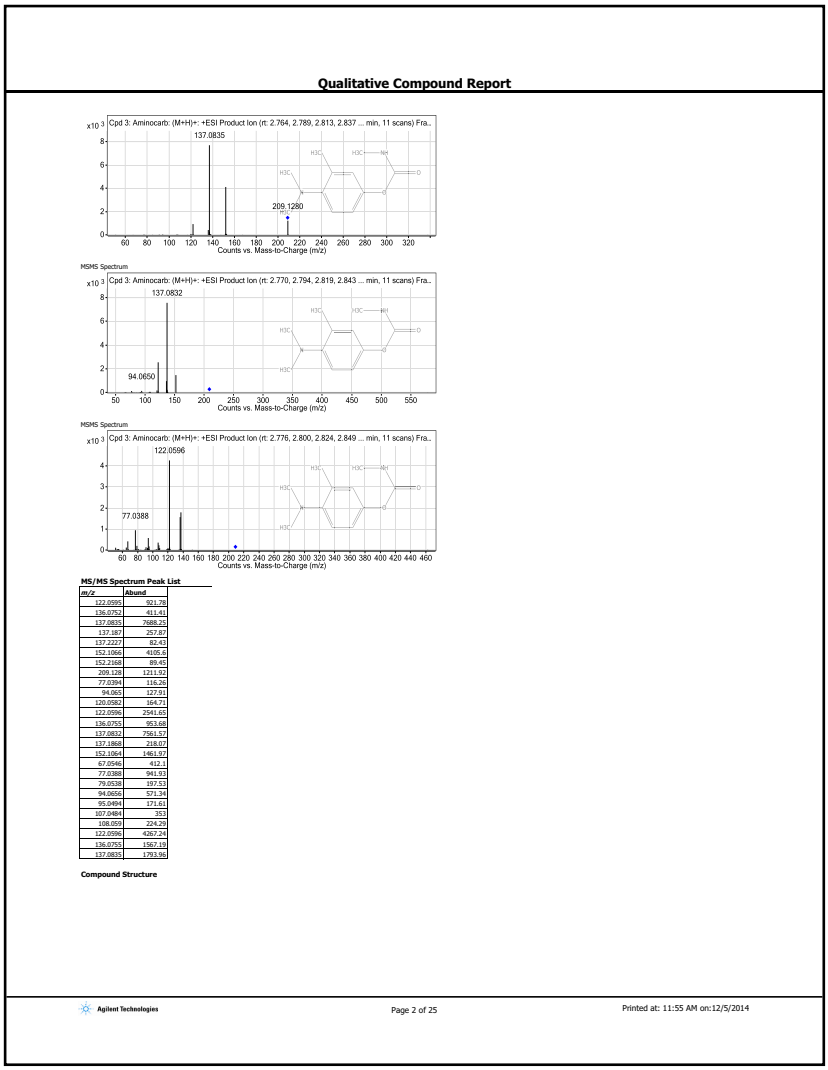


Figure 29 Page 2 of the Test Mix Compound report

Exercise 3. Automate the process with worklist actions

The ability to automate the process and run these steps in a workflow can be very 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, so you may want to do the compound automation and report generation in separate steps.

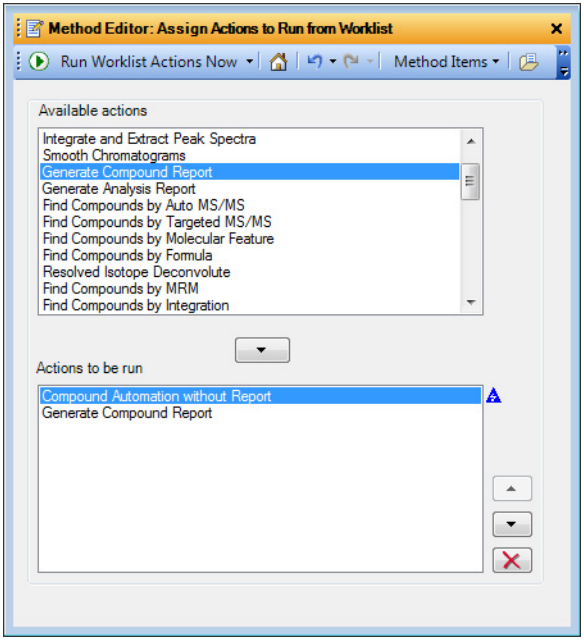
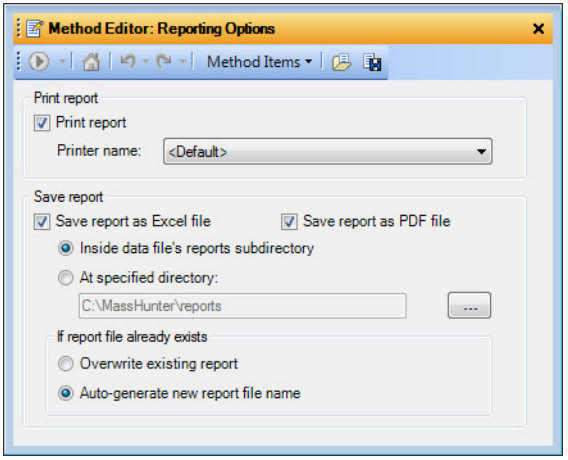


Figure 30 Assign Actions to Run from Worklist dialog box.

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 31. **c** Save the method.	
		
	Figure 31 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 just 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 may 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 is 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 and 20 or 10, 20 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 those of the library spectra, which are measured at 10, 20 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 environmental analysis where time has passed and another pesticide 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 **Pesticides_TestMix_AMSMS.d** found in the **Example Data** folder on the MassHunter Pesticides PCDL disc. If you have the G3878AA MassHunter Pesticides PCDL Kit and you ran the test 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 **Pesticides_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 **Pesticides_TestMix_AMSMS_DA.m**. **c** Open the data file **Pesticides_TestMix_AMSMS.d.** See Figure 33.	This chromatogram is different than for Targeted MS/MS. In Auto MS/MS mode, single-MS data is collected in a survey scan, and 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 **Pesticides_TestMix_AMSMS.d** on the MassHunter Pesticides PCDL disc. It was run with a linear fit of the collision energy to the *m/z* of the precursor ion. Figure 32 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. Note that the graph in Figure 32 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

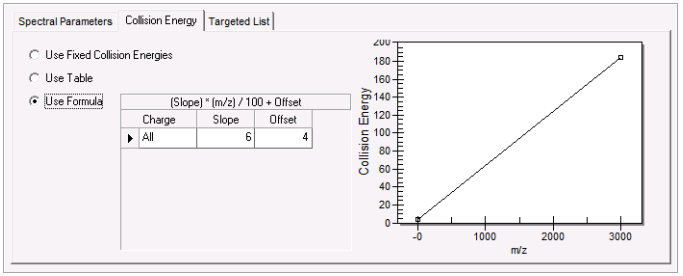


Figure 32 Collision Energy tab showing calculated values

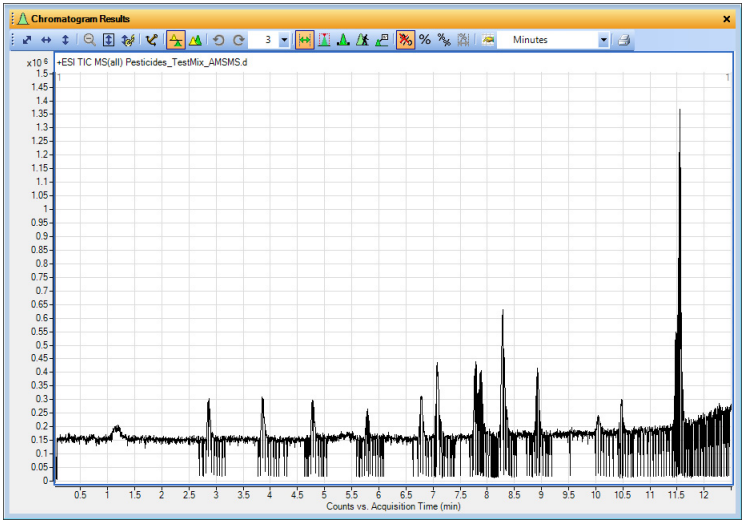
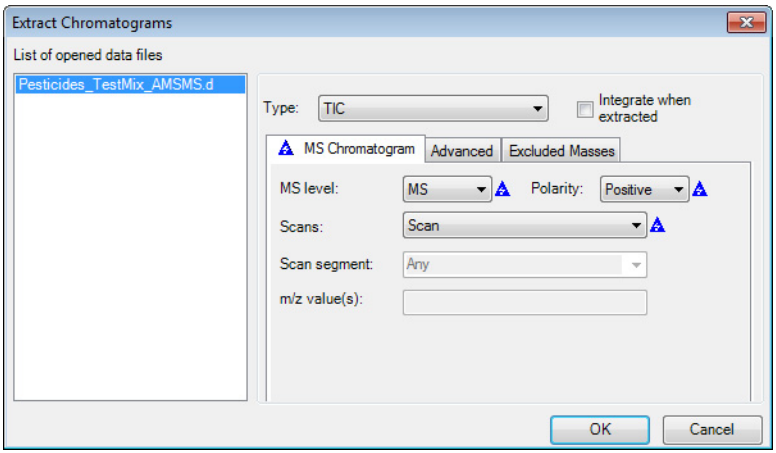


Figure 33 Total ion chromatogram of the test mix run with auto MS/MS settings.

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 34. **f** Click **OK**.	
	 **Figure 34** Extract Chromatograms setting for MS.	
3 Extract MS/MS data.	**a** Repeat step 2, but change the **MS level** to **MS/MS**. See Figure 35	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 36.

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

Step	Detailed Instructions	Comments

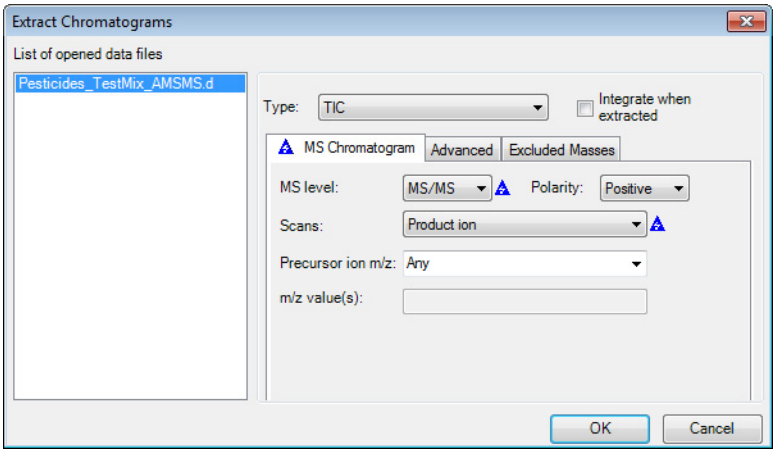


Figure 35 Extract Chromatograms setting for MS/MS.

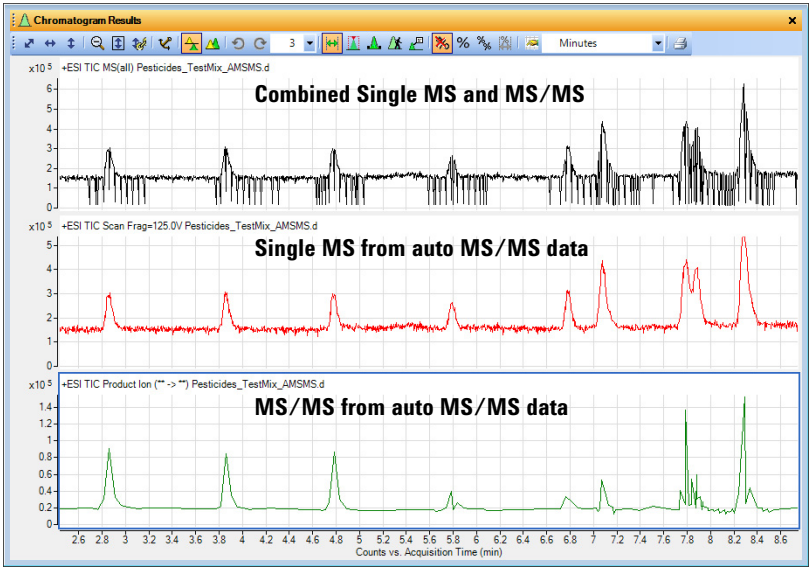


Figure 36 The top chromatogram shows all of the data points for single-MS and MS/MS. Note that MS/MS data points have lower total signal because ions in a narrow mass range are isolated for fragmentation. The middle chromatogram shows the single-MS only and it is clear that the Q-TOF LC/MS is collecting 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 37 shows the spectra parameters that are typically used for Auto MS/MS.

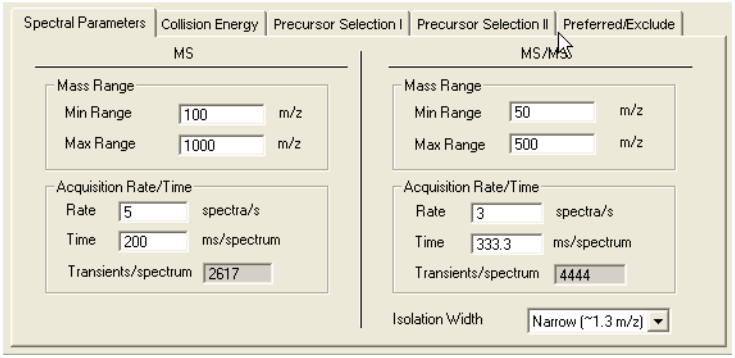


Figure 37 Spectral parameters for Auto MS/MS

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 38.

- **Max Precursor Per Cycle** determines how many co-eluting ions are selected for MS/MS. Too many will negatively affect the cycle time. Too few will 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 will 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 38, *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 and the quality of the MS/MS can suffer.
- **Static Exclusion Range List** excludes the range of ions that you specify. In Figure 38, 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.

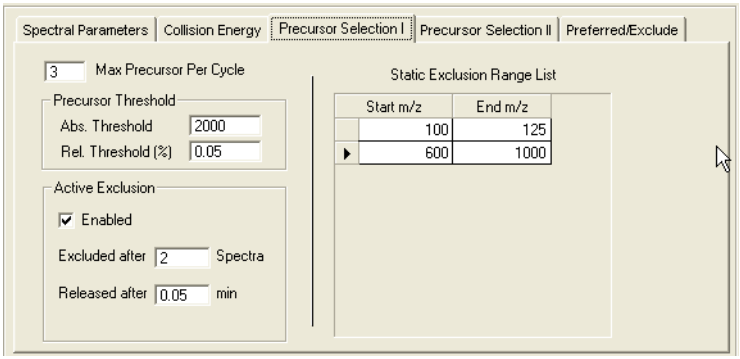


Figure 38 Precursor Selection I tab

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 test mix and applies to most small molecule drugs and pesticides. The other parameters in this tab are useful for more advanced data-dependent operation. Please 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, you may want to exclude them when collecting MS/MS. No ions were preferred or excluded for the test 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 for that of 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, except that you omit the collision energy in the library search options. Update settings for Find Compounds by Auto MS/MS so that all compounds will be found.	**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 39. **d** In **Identify Compounds > Search Library**, in the **Search Criteria** tab, *clear* the check box for **Collision energy**. See Figure 40. To automate the process, do the steps in "Exercise 3. Automate the process with worklist actions" on page 51.	Note that MS/MS peaks triggered on adduct ion species will produce spectra that will not match to the library spectra, as these spectra are not present in Pesticides_Std.cdb, and will result 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 Pesticides_TestMix_AMSMS.d (found on the MassHunter Pesticides PCD or PCDL disc) is shown in Figure 41 and Figure 42. A copy of this report is also available on the report disc as a PDF file.

Exercise 3. Process the data and automate (continued)

Steps	Detailed Instructions	Comments

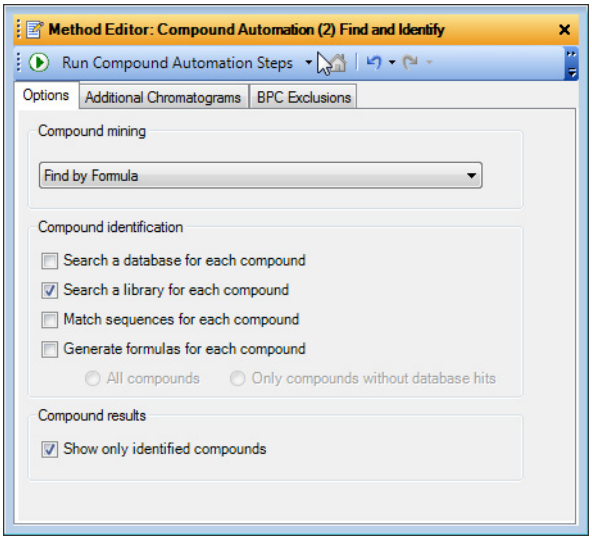


Figure 39 Find and Identify options for Auto MS/MS.

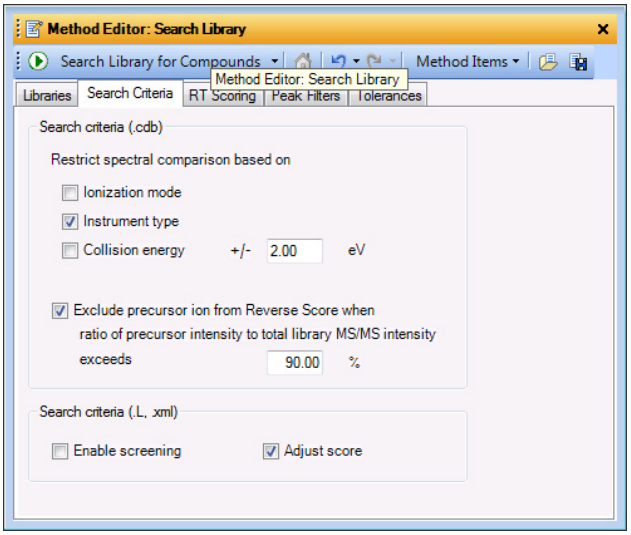
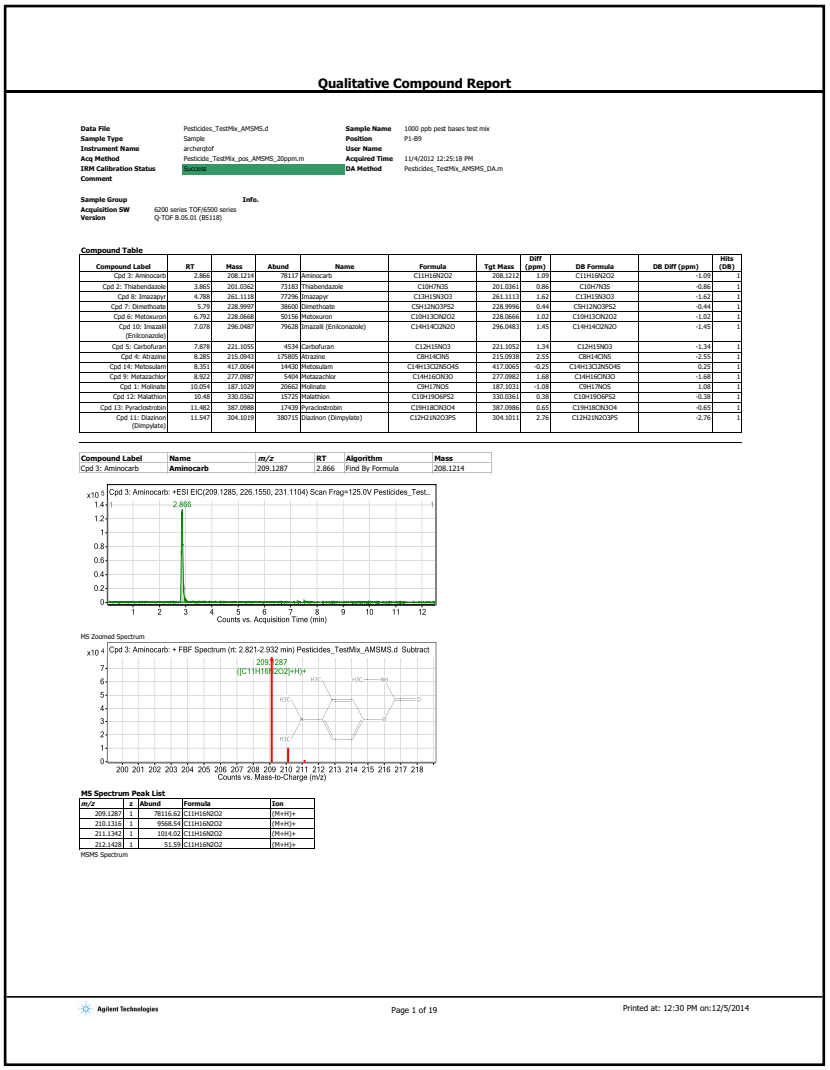


Figure 40 Search Criteria tab with Collision energy check box cleared.



Qualitative Compound Report

Data File	Pesticides_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**	Pesticides_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	38600	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	417.0065	-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

MS Zoomed Spectrum

MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
209.1287	1	78116.62	C11H16N2O2	(M+H)+
210.1316	1	9568.54	C11H16N2O2	(M+H)+
211.1342	1	1014.02	C11H16N2O2	(M+H)+
212.1428	1	51.59	C11H16N2O2	(M+H)+

MSMS Spectrum

Figure 41 Page 1 of Auto MS/MS analysis report

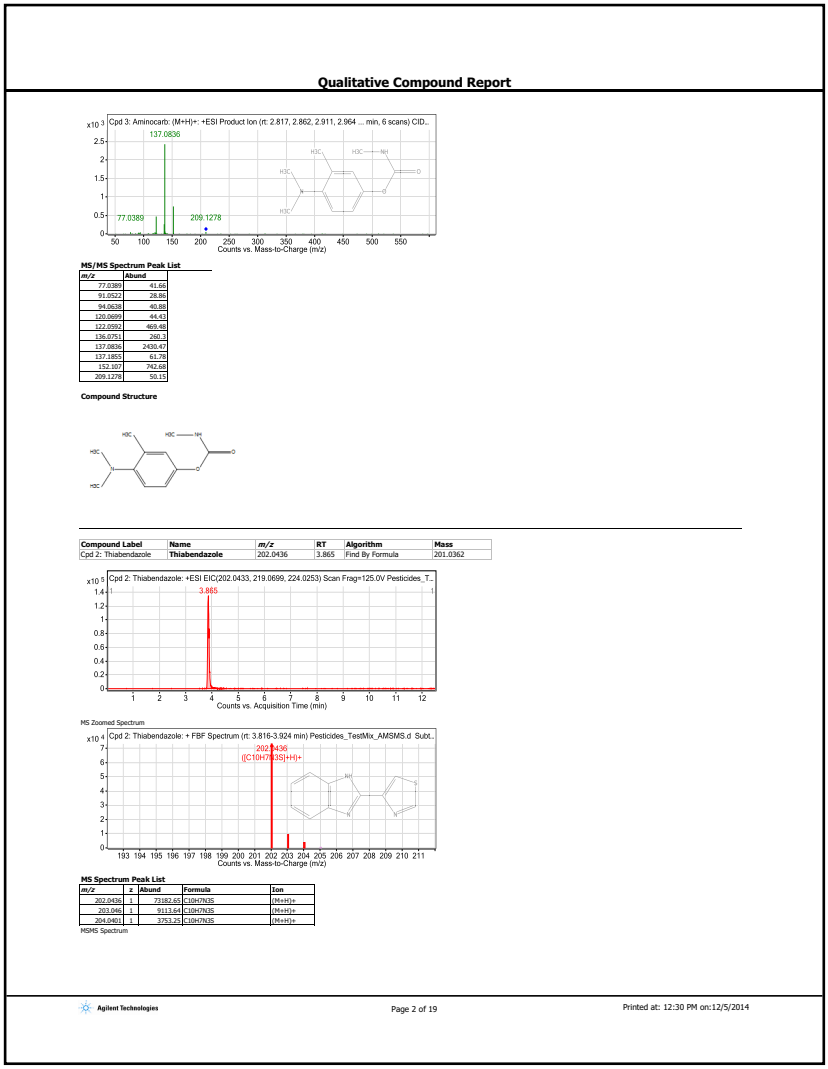


Figure 42 Page 2 of Auto MS/MS analysis report

Reference

Checkout Mix Content

The content of the Checkout Mix is listed here.

Table 1 Pesticide Checkout Test 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 Pesticides Checkout Test 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

Pesticides 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 Test Mix" on page 10 is suitable for both basic and acidic pesticides. The example data in this guide was run using the mobile phase shown below and is suited to basic pesticides only. As a result, the elution order of the basic pesticides 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

Pesticides 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 43.

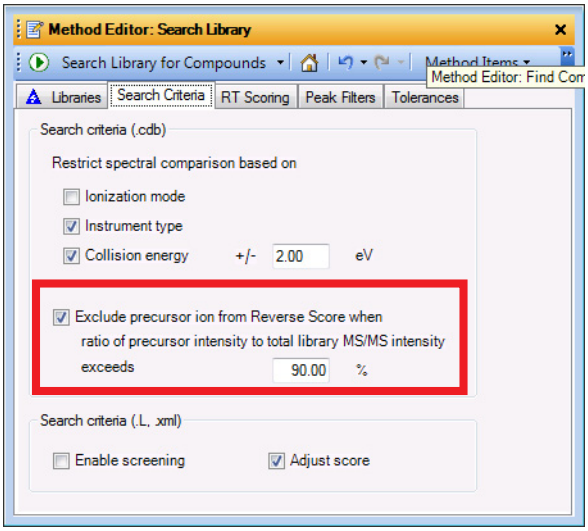


Figure 43 Search Criteria tab with the **Exclude precursor...** check box marked.

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

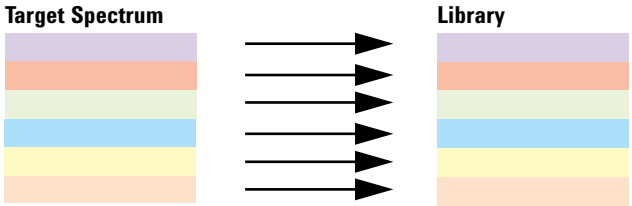
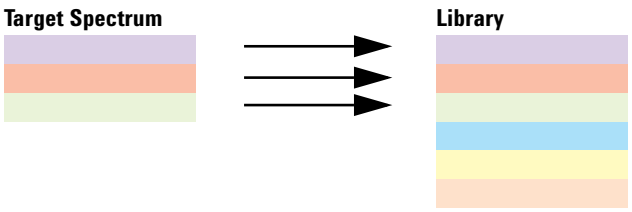
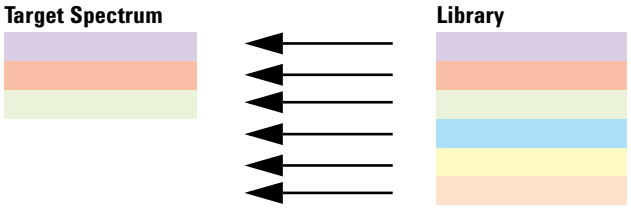
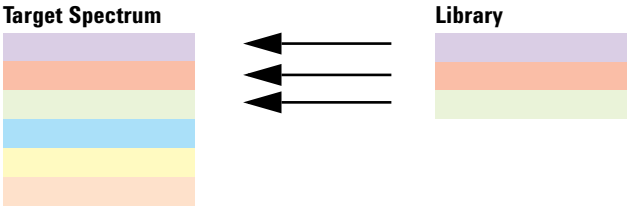
Search	Condition	Score
Forward	 All of the product ions in the sample spectrum are found in the library and vice versa.	High
Forward	 All of the product ions in the sample spectrum are found in the library, but only some of the product ions in the library are found in the sample spectrum.	Low

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

Search	Condition	Score
Reverse	 Only some of the product ions in the library are found in the sample spectrum.	Low
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 MassHunter Pesticides PCD or PCDL.

This guide is valid for the B.07.00 revision or higher of the MassHunter Pesticides PCD or PCDL, until superseded.

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