

# **MassHunter Pesticides PCD or PCDL**

# **Quick Start Guide**

What is the MassHunter Pesticides PCD or PCDL? 3 Kit Contents 4 Where to find more information 6 Before You Begin 7 Installation 7 Required reagents and parts (to run test mix) 8 Alternative configuration 8 Running the Test Mix 9 To run the test mix 10 To bypass mixer and damper 13 Using MassHunter Qualitative Analysis to Identify Compounds 15 To identify compounds using the MassHunter Qualitative Analysis program 15 To identify spectrum peaks using the MassHunter Qualitative Analysis program (PCDL only) 15 Familiarization Exercises - Compound Search 16 Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms 16 Exercise 2. Process and interpret data with Find by Formula 20 Exercise 3. Process and interpret data with Find by Molecular Feature Extractor 25 Exercise 4. Process data automatically using Worklist Automation 28 To develop a custom database 31



Familiarization Exercises - Targeted MS/MS Analysis with Identification by Library Search 32
Exercise 1. Confirm identification by search of the MS/MS Library 34
Exercise 2. Process the data 38
Exercise 3. Automate the process with worklist actions 47
Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search 50
Exercise 1. Learn about the content of an Auto MS/MS data file 51
Exercise 2. Optimize the number of data points 56
Exercise 3. Process the data and automate 59
Reference 64
Test Mix Content 64
Forward vs. Reverse Library Search 66

### What is the MassHunter Pesticides PCD or PCDL?

The MassHunter Pesticides PCD or PCDL lets you screen over 1,600 pesticides with accurate mass measurement, all in a single LC/MS analysis.

The MassHunter Pesticides PCD or PCDL helps minimize method development time for your analysis. The database stores accurate mass values, retention time values, and other information for compounds existing in the database at purchase and for compounds added to the database after purchase. 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 instruments 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 pesticide screen, or required (targeted screen only). What is the MassHunter Pesticides Drugs PCD or PCDL? Kit Contents

# **Kit Contents**

Quick StartMassHunter Pesticides PCD or PCDL Quick Start GuideThe Quick Start GuideGuidesgives an overview of the MassHunter Pesticides PCD or PCDL and tells you<br/>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<br/>SupplementalEach kit includes the MassHunter Personal Compound Database and<br/>Library Manager disc. Each kit also contains either the MassHunterDiscsMassHunter 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 note on accurate mass database *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)
- technical note on compound database *Pesticide Personal Compound* Database for Screening and Identification (p/n 5990-3976EN)
- 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)
- other related 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
- file that contains a list of the MassHunter Pesticides PCD or PCDL compounds

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

#### What is the MassHunter Pesticides PCD or PCDL?

Where to find more information

**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 "Test Mix Content" on page 64.

## 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 13 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.05.00 or higher
  - MassHunter Quantitative Analysis B.05.02 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, methanol and water
- ZORBAX LC Column (p/n 959758-902) (100 mm)
- Glacial acetic acid 99.9% (highest purity)
- Formic acid (highest purity)
- Ammonium formate (highest purity)
- Ammonium acetate (highest purity)
- Ammonium hydroxide (highest purity)

# **Alternative configuration**

The sample methods and data files from the test mix are all based on the configuration described in the installation instructions. A different system configuration will do the library search screening and identification just as well. 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 7. 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 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 test mixes.

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

**a** Dilute 100  $\mu$ L of the stock solution to 10.0 mL with acetonitrile to create the interim solution (1 ppm).

Use this solution for systems with no iFunnel and no Agilent Jet Stream source. The examples in this guide were produced in this way, with the instrument operating in 1700 maximum mass mode and 2 GHz extended dynamic range mode.

**b** Take 1 mL of the interim solution and dilute it to 10.0 mL with 10:90 acetonitrile:water. This final solution is 100 ppb concentration.

Use this solution for systems with an Agilent Jet Stream source, or for systems with iFunnel optics. On some instruments, or when operating the instrument in the 4 GHz high resolution mode, dilute this solution again (to make a 10 ppb working solution) if needed.

**c** Transfer an aliquot of the interim or final solution to a standard 2 mL sample vial for analysis.

Do this separately for the positive and negative 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.

To run the test mix in positive mode, use:

- A= 0.1% (vol/vol) formic acid in water
- B=100% acetonitrile

The examples in this guide were run in positive mode only. If you want to run the negative mode analysis also, prepare the alternative mobile phase below. Your results in positive mode with this composition will differ somewhat from the examples in the guide. The negative mode method **Pesticides TestMix MS neg.m** is included in the methods folder on the disc.

To run the test mix in negative mode, use:

- A= 5 mM acetic acid in water (286 µL glacial acetic acid in 1 L water)
- B= 100% acetonitrile
- **4** Verify the system configuration.

Load the method **Pesticides\_TestMix\_MS.m**. This method uses the system configuration as listed below. Systems that deviate from this configuration may not work with this method.

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 – SL, Model 1312B configured with damper and mixer bypassed. See "To bypass mixer and damper" on page 13.
Column Compartment	Column – SL, Model G1316B

- **5** Check that your method is set up to make a  $1 \mu L$  injection.
- 6 Click **Run > Interactive Sample** to do a single sample run, or create a worklist to make multiple injections.
- 7 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)

8 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

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.

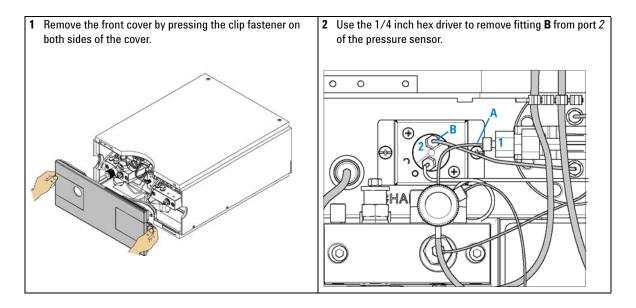
Configurations where only the damper or the mixer is disconnected while the other part is still in line are not supported by Agilent Technologies.

**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)

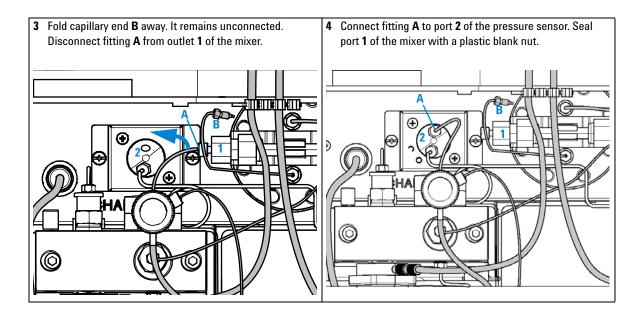
Preparations for this procedure

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



#### **Running the Test Mix**

To bypass mixer and damper



# **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**.

Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms

# **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 2. Process and interpret data with Find by Formula" on page 20.

# Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms

In this exercise, you process the data file **Pesticides\_TestMix\_MS.d.** 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 test mix" on page 7), 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.	а	Open the Agilent MassHunter Qualitative Analysis program.	A list of the exact <i>m/z</i> values of the compounds in the mixture is displayed in the	
			Click <b>Cancel</b> if you are asked to open a data file.	<b>Chromatograms &gt; Define Chromatograms</b> section.	
		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.		
		e	In Method Explorer, click <b>Chromatogram</b> > <b>Define Chromatograms</b> . See Figure 2.		

Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms

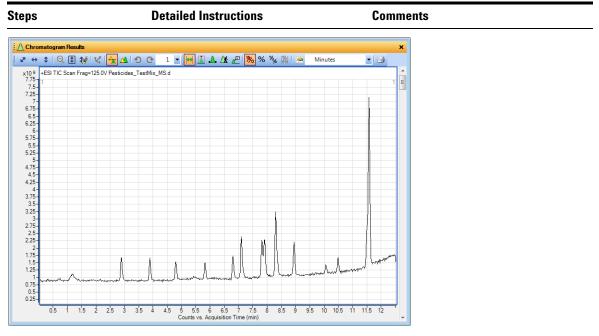


Figure 1 Example Test Mix Total Ion Chromatogram

Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms

Steps	Detailed Instructions Comments	
Method Explorer: Pesticides_Tes	tMix_MS_DA_ X	
Chromatogram	🖆 💽 Extract Defined Chromatogram 🔹 🚮 🖃 🕶 🖬 Hethod Items 🔹 🙀 🦉	
Integrate (MS)	Defined chromatograms	
Integrate (MS/MS)	EIC (209.1288 m/z) MS	
Integrate (UV)	EIC (262.1190 m/z) MS EIC (202.0435 m/z) MS	
Integrate (ADC)	EIC (230.0070 m/z) MS EIC (297.0560 m/z) MS	
Smooth	EIC (229.0741 m/z) MS EIC (222.1128 m/z) MS	
Exclude Mass(es)		
Calculate Signal-to-Noise	Chromatogram definition	
Define Chromatograms	E Type: EIC   Integrate with extracted	
Adjust Delay Time	MS Chromatogram Advanced Excluded Masses	
Extraction Data Format	MS level: MS  Polarity: Both	
Spectrum		
🗄 General	Scans: All single stage scan types	
	m/z of interest: Any -	
• Reports	m/z value(s): 209.1288	
Find Compounds	Do cycle sum	
• Find Compounds by Formula	Merge multiple masses into one chromatogram	
Identify Compounds		
Search Database		
"	not a way a section calculated. Click the super away (simpled) to a streat the impa	
igure 2 Define Chro	natograms section selected. Click the green arrow (circled) to extract the ions.	•
Extract the ions.	<ul> <li>a Click the green arrow in the Method Editor toolbar.</li> <li>After the chromatograms are extract are displayed in the Chromatogram R window, as seen in Figure 3, if the vie</li> </ul>	Resul

List Mode. In Figure 3, you can see the major

peak in each EIC.

Exercise 1. Process and interpret data with Defined Extracted Ion Chromatograms



Figure 3 Extracted Ion Chromatograms

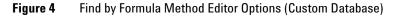
**Exercise 2. Process and interpret data with Find by Formula** 

# **Exercise 2. 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.

Steps		Detailed Instructions		Comments	
bec sett	view the method to come familiar with the tings for Find by		Locate the <b>Find Compounds by Formula</b> section in the Method Explorer. Select the custom database	The <b>Pesticides_Std.cdb</b> does not contain retention times or isomers, therefore all compounds are easily identified using the	
data	mula. Use the abase <b>sticides_Std.cdb</b> .	C	<b>Pesticides_Std.cdb</b> . See Figure 4. Review the settings in this method to become familiar with peak detection,	Mass option. To easily identify isomeric compounds, add retention times to your custom PCDL and select one of the Mass	
	_		mass tolerances and other settings. If needed, adjust for specific matrices.	retention time options.	

🖺 Method Explorer: Pesticides_TestMix_MS_DA_ 🗙	🛛 🔄 Method Editor: Fi	ind Compound	ds by Formula - Option	ns	×
• Chromatogram	🗄 💽 Find Compound	ls by Formula	•   🚮   🔊 • (* -	Method Items 🔹 🙀	••
± Spectrum	Negative lons	Results	Result Filters	Fragment Confirmation	
± General	Formula Source		Formula Matching	Positive lons	
± Reports	Source of formulas to These formulas:	confirm			Â
• Find Compounds					
∃ Find Compounds by Formula			f formulas, e.g., "C6H6	5, CH4")	
Find by Formula - Options	Compound exchange	ange file (.CEF)	):		
Find by Formula - Chromatograms					
Find by Formula - Mass Spectra	<ul> <li>Database / Librar</li> </ul>				Е
Find by Formula - Sample Purity	C:\MassHunter\	PODL\Pesticide	es_Std.cdb		
* Identify Compounds	⊘ Worklist				
Compound Automation Steps	Matches per formula				
Worklist Automation	Maximum number of	matches 1			
Export	Automatically inc	rease for isom	eric compounds		
	Values to match				~



2 Check that the desired ion species are present.
 a In the Positive lons tab, check that the desired ion species are present. See Figure 5.
 For example, make sure that the adduct m/z is not shown if the protonated species is desired.

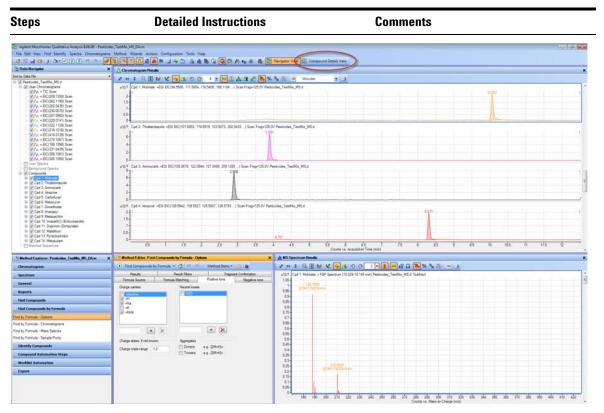
Exercise 2. Process and interpret data with Find by Formula

Method Editor: Find Compounds by Formula - Options
😧 🕟 🛛 Find Compounds by Formula 🔹 🚮 🖉 🕶 🖉 Method Items 🔹 💋 🏢
Results Result Filters Fragment Confirmation
Formula Source Formula Matching A Positive lons Negative lons
Charge carriers Neutral losses
Image: Here       Image: Here <t< th=""></t<>
Charge state range 1 Dimers e.g., [2M+H]+
Figure 5 Positive lons tab.

- 3 Use the MassHunter Pesticides Standard PCDL to find compounds in the data file Pesticides\_TestMix\_MS .d
- Open the data file Pesticides\_TestMix\_MS.d.
- PCDL to find compounds<br/>in the data filebClick the green arrow () in the<br/>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.

**Exercise 2. Process and interpret data with Find by Formula** 



#### Figure 6 Find By Formula Results using MassHunter Pesticides Standard PCDL. (Pesticides\_Std.cdb)

4 Review the Compound Table. Return to the Navigation view when you are done.

- a Click **Compound Details View** to switch views. See Figure 7.
- b Click or use the arrow keys to move through the Compound Table to review one compound a a time.
- c Click Navigator View.

Exercise 2. Process and interpret data with Find by Formula

teps	<b>Detailed Instructions</b>	Comments
Agilent MassHunter Qualitative Analysis 8.06.00 - Pesticides_1	estMix_MS_DA.m	
ile Edit View Find Identify Method Wizards Configura	tion Tools Help	
s , = 3   a -   • • • - B C C A G G	👭 🔟 🕼 🗞 🐘 & 🔝 Navigator View 🐼 Compound Details View	
Compound List		x Compound Identification Results: Cpd 1: Mainate 🛛 🗙 🕹
🖉 Automatically Show Columns 🛛 🎇 🖓 🚱 👧	8. 9. 🐼 🗊 I	🔛 Automatically Show Columns 🔛 🗐 🗐 🧐 🆓 🖓 🖏 🔊
Label V <sup>2</sup> Cod <sup>7</sup> V <sup>2</sup>		
Cpd 1. Molimate		187 1031 -2.9 FEF
		2010361 0.7 Best    Name
Cpd 3: Aminocarb	3 Aminocarb C11 H16 N2 O2 99.44 208.1215 208.1212	208.1212 1.3 m kg   Malanta C0.017.00 107.1005 107.1005 200 00.05 00 00.05
		215.0938 2.3
		2211052 1.2
		220.0666 1.2
Spart. Serversere		228 9996 0.3
Cpd 8: Imazapyr		261.1113 1.2 277.0882 0.7
		277.092 0.7 296.043 1.3
	1 Diazinon (Dimpylate) C12 H21 N2 O3 P S 98.56 304.1017 304.101	209.0463 1.3 304.101 2.0
		300.001 0.3
		387,0996 0.5
		4770065 0
Hethod Editor: Find Compands by Formula - Options     Find Compands by Formula - Options     Find Compands by Formula - (A) 49 - (B)	K Compound Chromatogram Results	× jr · · · · · · · · · · · · · · · · · ·
	📱 🔹 🕂 ‡ 🔍 🗄 📥 🛤 🔛 👐 🥷 % % 🎘 🖮 Minutes	x Constant MS Spectrum Results x
) Find Compounds by Formula 🔹 🖄 🖃 🕫 🖓		x Constant MS Spectrum Results x
Find Compounds by Formula	X ↔ \$ Q E ★ 44 Q E ★ 20 00 00 00 00 00 00 00 00 00 00 00 00	X         Compound MS Spectrum Reads         X           V         A         B         X         X           V         A         B         X         X           V         Sectors Transfer         X         X         X           V         Sectors Transfer         X         X         X         X           V         Packades         Testicides
Find Compounds by Formula		x Constant MS Spectrum Results x
Find Compounds by Formula      Compounds by Formula     Compounds by Formula     Source Result Rees Fragment Confination     Missioner Result Matching Posttive Ione Negative Ione     Source of formulas to confirm	Image: Processing of the second sec	X         Compound MS Spectrum Reads         X           V         A         B         X         X           V         A         B         X         X           V         Sectors Transfer         X         X         X           V         Sectors Transfer         X         X         X         X           V         Packades         Testicides
Find Compounds by Formula      Compounds by Formula     Compounds by Formula     Source Result Rees Fragment Confination     Missioner Result Matching Posttive Ione Negative Ione     Source of formulas to confirm	*****         2         0         E         2         0         0         %         %         %         Minutes           1002         Cat1         Mainutes         -ESI IEC(54 5588, 111 5564, 116 5468, 118 1104 - 1). See Frage-125 C         -2.4         -2.4         -2.2         -	X         Compound N3 Spectrum Results         X           *
Find Compounds by Formula • (2) +1 + (2) - (3)     Some Resda Rese Regime Confirmation     minds Source (Founda Matching Pastave lone Jones formulas     These formulas:     (1) the formulas:     (2) the some assessment of sixt of formulas. e.g.: (CDH, CHH)	*****         1         0         1         2         0         2         0 <td>X         Compound MS Spectrum Result         X           *        </td>	X         Compound MS Spectrum Result         X           *
Find Compounds by Formula	Image: Control of the state         Image: Control of the state <t< td=""><td>Image: Control (Control (Contro((Control (Control (Control (Control (Control (Control (Control (C</td></t<>	Image: Control (Control (Contro((Control (Control (Control (Control (Control (Control (Control (C
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Pind Compounds by Fornula          (a) In In IC          (b) In IC          (c) In I	***         2         0         2         2         2         2         3         5         %	X         Compound MS Spectrum Results         X           V         A         B         A         B         A         Compound MS Spectrum Results         X           V         A         B         A         B         A         B         A         B         A         B         A         B         A         B         A         B         A         B         A         B         B         A         B         B         A         B         B         A         B         B         B         A         B
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Pind Compounds by Fornula          (a) In In IC          (b) In IC          (c) In I	***         2         0         2         2         2         2         2         2         3         5         %	Image: Compound WS Sepacture Reach     X       Image: Compound WS Sepacture Reach     X       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach       Image: Compound WS Sepacture Reach     Image: Compound WS Sepacture Reach  <

Figure 7 Compound Details view.

5	Export the compound list	а	Right-click anywhere in the compound list	The spreadsheet file appears in the data file
	as a spreadsheet in text		and select <b>Export</b> . See Figure 8.	folder with the same name as the data file.
	format.	b	For File type, select Data as Text file (*.txt; *.csv).	You will use this file in a later exercise for Targeted MS/MS analysis.
		C	Click <b>OK</b> .	The <b>Pesticides_TestMix_MS.csv</b> test mix
				data file in Excel format is included.

Exercise 2. Process and interpret data with Find by Formula

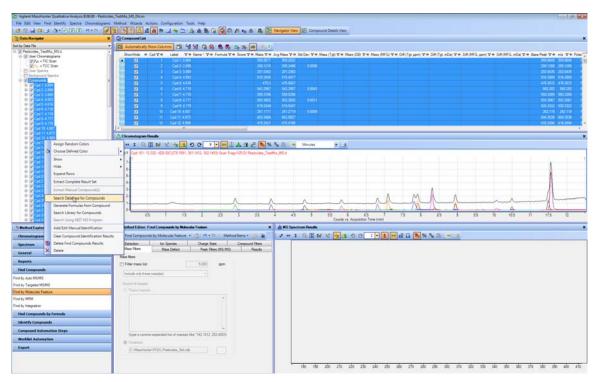
Steps	<b>Detailed Instructions</b>	Comments
	Export	×
	Export type File type: Data as Text file (*txt; * With delimiter:	*.csv) ▼
	Export contents	
	Export destination <ul> <li>Auto-generate a file at data file location</li> <li>Specified file:</li> </ul>	
	<u></u>	OK Cancel

- **6** Remove the results prior to the next exercise and
- a Click Find >Delete Find Compound Results to remove the results
- close the Compound List. **b** Close the Compound List to free up display space.

# **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.	<ul> <li>a Locate the Find Compounds by Molecular Feature section in the Method Explorer.</li> <li>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.</li> </ul>	
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 9 illustrates the results for Find by Molecular Feature.
3	Search the PCD or PCDL for the selected compounds.	<ul> <li>a In the Data Navigator, click the Compounds line to select all compounds that were generated by MFE and which are shown.</li> <li>b When all the compounds are selected, right-click the selected compounds and click Search Database for Compounds from the shortcut menu (Figure 9).</li> </ul>	If the Advanced tab is not visible, click Configuration > User Interface Configuration and then mark the Accurate mass (TOF, Q-TOF) and Show advanced parameters check boxes.

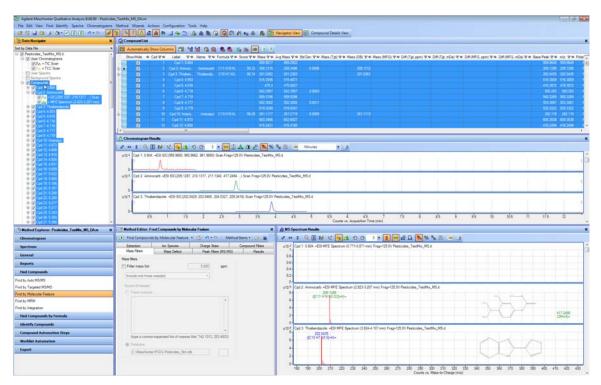
Exercise 3. Process and interpret data with Find by Molecular Feature Extractor



**Figure 9** 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 10 shows the compound identification results obtained from a search on the MassHunter Pesticides Standard PCDL.

Exercise 3. Process and interpret data with Find by Molecular Feature Extractor



**Figure 10** 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.

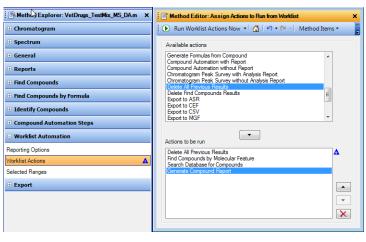
**Exercise 4. Process data automatically using Worklist Automation** 

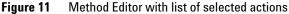
## **Exercise 4. Process data automatically using Worklist Automation**

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 11.	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.	





Exercise 4. Process data automatically using Worklist Automation

Steps		Detailed Instructions	Comments
3	If you chose <b>Generate</b> <b>Compound Report</b> , then modify the reporting options.	<ul> <li>a From the Worklist Automation list, click Reporting Options.</li> <li>b In the Method Editor, in the Reporting Options section, set your reporting options. See Figure 12.</li> </ul>	
		Method Editor: Reporting Options	×
		Print report  Print report  Printer name:  CDefault>	
		Save report Save report as Excel file Save report as Excel file Save report as PE C:\MassHunter\reports If report file already exists Overwrite existing report Auto-generate new report file name	)F file
		Figure 12 Reporting Options	
4	Save the method settings to an acquisition method.	<ul> <li>a In the MassHunter Qualitative Analysis program, click Method &gt; Save As.</li> <li>b Browse to the folder on your system that contains the Data Acquisition method that you want to automate.</li> <li>c Click the name of the Data Acquisition method that you want to automate and click Save.</li> </ul>	The Qualitative Analysis method is now attached and is an integral part of the Data Acquisition method.

**Exercise 4. Process data automatically using Worklist Automation** 

Steps	Detailed Instructions	Comments
5 Create a Data Acquisition worklist, and then run the worklist.	<ul> <li>a In the MassHunter Data Acquisition program, click Worklist &gt; Worklist Run Parameters.</li> <li>b For Part of method to run, select Both Acquisition and DA.</li> <li>c Select whether Execution for Acquisition-DA is to be Synchronous or Asynchronous.</li> <li>d Save the worklist.</li> <li>e Run the worklist.</li> </ul>	Worklist Run Parameters         Deviator name:         Run Information         Run Type:         Standard Start         Pat of method to         Both Acquilition and D?         Veride Data         Dr.MassHunter/methods         Dremide DA:         Dr.MassHunter/methods         method Paths         Figure 13       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 focussed custom databases from the larger MassHunter Pesticides PCD or PCDL for a specific industry needs such as work-place drug testing.

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

NOTE

### To develop a custom database

The use of a smaller and more focussed database to screen samples can be a powerful tool to detect and identify specific analytes that are required by various regulatory agencies, such as governmental work-place drug testing. 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 note Pesticide Personal Compound Database for Screening and Identification (p/n 5990-3976EN) 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.

(Note that the MassHunter Personal Compound Database program described in Pesticide Personal Compound Database for Screening and Identification (p/n 5990-3976EN) is an earlier version of the MassHunter Personal Compound Database and Library (PCDL) Manager.

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.
- A great advantage of Targeted MS/MS is that it 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. This is different from Auto MS/MS acquisition (see later section). For Auto MS/MS mode, 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, desired precursors can be missed during Auto MS/MS operation.
- A drawback of Targeted MS/MS operation is that it has a limit on the number of precursors that can be examined in any cycle and still sample the peaks fast enough for good integration and peak detection. For a target list with a large number of targets, or under conditions of very fast chromatography, it is not possible to examine all targets in a single run. The solution is to divide the target list over multiple methods and inject the sample repetitively, searching for different targets in different runs.
- A disadvantage of Targeted MS/MS operation is that it never performs MS/MS on unexpected targets, only on what is on the precursor list in the method. This is opposed to one of the advantages of Auto MS/MS operation (later section), that Auto MS/MS operation produces MS/MS spectra of compounds that are not on the list in the acquisition method or even are not present in the database/library.

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. Confirm identification by search of the MS/MS Library
- Exercise 2. Process the data
- Exercise 3. Automate the process with worklist actions

# Exercise 1. Confirm identification by search of the MS/MS Library

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. Confirm identification by search of the MS/MS Library

Step	Detailed Instructions	Comments
1 Create a template file in .csv format. See Figure 15. Then open the template in Excel.	<ul> <li>a Open the MassHunter Data Acquisition program.</li> <li>b In the Method Editor pane, right-click the table in the Targeted List tab and select Export. See Figure 14.</li> <li>c For File type, select text (*.csv).</li> <li>d Select a file name and location.</li> <li>e Click OK.</li> <li>f In Excel, open the template .csv file that you just created. See Figure 15.</li> </ul>	

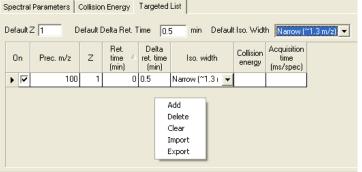


Figure 14	Targeted List tab, Export listed in shortcut menu
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TargetedMSMSTable							
On	Prec. m/z	Ζ	Ret. time (min)	Delta ret. time (min)	Iso. width	Collision energy	Acquisition time (ms/spec)
TRUE	100	1	0		Narrow (~1.3 m/z)		

	Figure	15	Template	.csv file
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Exercise 1. Confirm identification by search of the MS/MS Library

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 16.	<ul> <li>a Start the Excel program, and open the spreadsheet file that you exported from the MassHunter Qualitative Analysis program in "Exercise 2. Process and interpret data with Find by Formula" on page 20.</li> <li>b Add a column called Prec. m/z.</li> <li>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.</li> <li>d Copy all Prec. m/z values to the template .csv file.</li> </ul>	The base peak column in the compound list table is the measured <i>m/z</i> 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.
	<ul> <li>e From the compound list Excel file, copy: <ul> <li>the Z values</li> <li>the retention times</li> <li>the delta retention times</li> <li>the iso widths</li> </ul> </li> <li>The template .csv file now looks similar to Figure 16.</li> <li>f Save the template .csv file.</li> </ul> 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_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 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). 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 <i>m/z</i> of the precursor ion as described in "Familiarization Exercises - Auto MS/MS Analysis with Identification by Library Search" on page 50.

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

**Exercise 1. Confirm identification by search of the MS/MS Library** 

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pec)	on Time (ms/s	Acquisitio	Collision Energy	Iso. Width	elta Ret. I	Ret. Time I	z	Prec. m/z	On	2 0
				Narrow ("1.3 m/z)	0.5	2.93	1	209.1286813	TRUE	з
				Narrow (~1.3 m/z)	0.5 1	3.93	1	202.0435483	TRUE	4
				Narrow (~1.3 m/z)	0.5 1	4.83	1	262.1188907	TRUE	5
				Narrow (~1.3 m/z)	0.5	5.83	1	230.0070903	TRUE	6
				Narrow (~1.3 m/z)	0.5 1	6.82	1	229.0741344	TRUE	7
-				Narrow (~1.3 m/z)	0.5 1	7.17	1	297.0561567	TRUE	8
				Narrow (~1.3 m/z)	0.5 1	7.92	1	222.1127415	TRUE	9
				Narrow (~1.3 m/z)	0.5 1	8.32	1	216.1015124	TRUE	10
				Narrow (~1.3 m/z)	0.5 1	8.39	1	418.0141719	TRUE	11
				Narrow (~1.3 m/z)	0.5 1	8.97	1	278.1058788	TRUE	12
				Narrow (~1.3 m/z)	0.5 1	10.09	1	188.1101488	TRUE	13
				Narrow (~1.3 m/z)		10.5	1	331.0437881	TRUE	14
				Narrow (~1.3 m/z)	0.5 1	11.51	1	388.1067889	TRUE	15
				Narrow ("1.3 m/z)	0.5	11.57	1	305.1090545	TRUE	16
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#### Exercise 1. Confirm identification by search of the MS/MS Library (continued)

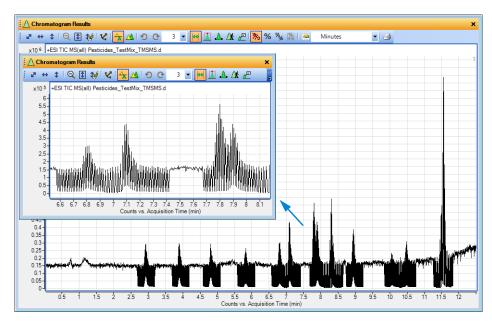
Figure 16 Template .csv after retention time and accurate mass are added

3 Open the Compounds List results file that you saved in "Exercise 2. Process and interpret data with Find by Formula" on page 20, 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 2. Process and interpret data with Find by Formula" on page 20. 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 17 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 17** 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** 

## **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	
<ol> <li>Update settings for Find Compounds by Formula so that all compounds will be found.</li> </ol>	<ul> <li>a Start the MassHunter Qualitative Analysis program</li> <li>b Open the Method Editor.</li> <li>c Open the data analysis method Pesticides_TestMix_TMSMS_DA.m.</li> <li>d Click Find Compounds by Formula &gt; Options, and then on the Formula Source tab, set the Database/Library path to the Pesticides Standard Library. See Figure 18.</li> <li>e On the Results tab, select Extract MS/MS spectrum and Separate MS/MS spectrum per CE. See Figure 19.</li> </ul>	Image: Intercomparate to Format - Weak         Image: Intercomparate to Format - Weak	

a compound search with each of these integrators before you select the integrator that gives you the best results.

#### Familiarization Exercises - Targeted MS/MS Analysis with Identification by Library Search Exercise 2. Process the data

Step	Detailed Instr	ructions	Ca	omments					
	Find Compounds by F	Method Editor: Find Compounds by Formula - Options       X         O       Find Compounds by Formula + ()         Method Items * ()       ()         Method Items * ()       ()							
	Formula Source Results	Fomula Matching Positive Ions Result Filters	Negative lons Fragment Confirmatio	Scoring					
	Previous results  Previous composition  New results  Highlight first compound  Highlight first compound  Chromatograms and spectra  Chromatograms and spectr	m □ Include structure w spectra, if available spectra v +/- 5.00000 n peetrum for all CEs t/- 20.000 pppm ▼							

#### Exercise 2. Process the data (continued)

#### Figure 19 Results tab

2	Search the <b>Pesticides_Std.cdb</b> library. As search criteria:	a	In the Method Explorer, click Identify Compounds > Search Accurate Mass Library.	lf you customized your newly created PCDL, you can select it instead.
	<ul> <li>Add collision energy.</li> <li>Set to use both a minimum forward score and a minimum reverse score.</li> </ul>	C	In the Settings tab, browse to select Pesticides_Std.cdb. In the Peak Filters tab, set the Absolute height to 5 counts and the Relative height to 1% of largest peak. See Figure 20. In the Search Criteria tab, mark the check hox for Collision energy. See Figure 21.	If you do not see the Search Criteria tab, make sure that <b>Show advanced parameters</b> is selected in the MassHunter Qualitative Analysis program. See step 3 on page 25.

box for **Collision energy**. See Figure 21.

**MassHunter Pesticides PCD or PCDL Quick Start Guide** 

**Exercise 2. Process the data** 

ep	Detailed Instructions	Comments		
	E Method Editor: Search Accurate Mass Library	×		
	🗄 💽 Search Library for Compounds 🔹 🚮 🖃 🤟	(* - 1)		
	Settings Peak Filters Search Criteria Search Results			
	Height filters         Image: Absolute height       >=       5         Image: Relative height       >=       1.000	=		
	Maximum number of peaks           Imit (by height) to the largest	200		
		-		

#### Exercise 2. Process the data (continued)

Figure 20 Peak Filters tab.

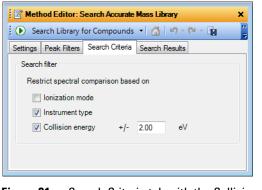
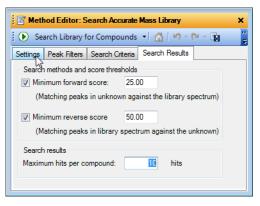


Figure 21 Search Criteria tab with the Collision energy check box marked.

**Exercise 2. Process the data** 

Step	Detailed Instructions	Comments
	e In the Search Results tab, mark the Minimum forward score and Minimum	See "Forward vs. Reverse Library Search" or page 66 for more information.
	<b>reverse score</b> check boxes and set the forward score to <b>25</b> and reverse score to <b>50</b> .	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)



#### Figure 22 Search Results tab

- 3 Set up the method to:Find all of the
  - compounds in the text 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 23:
  - 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.

**Exercise 2. Process the data** 

Step	Detailed Instructions	Comments
	Method Editor: Compound Automation (2) F	🔊 - 🍽 -   Method Items - 👔 🦉
	Options Additional Chromatograms BPC Exclusion Compound mining Find by Fomula	s 
	Compound identification Search a database for each compound Search a library for each compound	
	Match sequences for each compound Generate formulas for each compound G All compound Only compoun Compared partite	ds without database hits
	Compound results	

#### Exercise 2. Process the data (continued)

Figure 23 Options tab for Compound Automation Find and Identify

4 Set up report options to a In the Method Editor, click Reports > Figure 26 and Figure 27 shows the first two produce a report that **Common Reporting Options.** pages from the report for the Targeted shows the MS/MS peak b For Compound report template, select MS/MS analysis on the CompoundReport.xltx. Pesticides TestMix TMSMS.d (found on table and spectra. the MassHunter Pesticides PCD or PCDL See Figure 24. disc). A copy of this report is also available in c In the Method Explorer, click Compound the report folder as a PDF file. 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 25. e Save the method.

#### Familiarization Exercises - Targeted MS/MS Analysis with Identification by Library Search Exercise 2. Process the data

Step	Detailed Instructions	Comments
	Method Editor: Common Reporting Options	×
	Print Analysis Report	
	Templates Options	
	Report template folder	
	Hunter\Report Templates\Qual\B.06.00\en-US\Lett	etter
	Report templates	
	Analysis report template :	
	Analysis Report xbx	▼
	Compound report template:	
	Compound Report xltx	▼
	Qualitative method report template :	
	Qualitative Method Report xltx	<b>-</b>
	Acquisition method report template :	
	AcqMethod Report.rdlc	▼

#### Exercise 2. Process the data (continued)

**Exercise 2. Process the data** 

Step	Detailed Instructions Comments
	F Method Editor: Compound Automation (3) Compound Report
	🗄 💽 Print Compound Report 🔹 🚮 🖃 🕶 🖓 Hethod Items 🔹 😕 🌆
	Compounds          Image: Sort by:       Retention time         Sort order:       Increasing         Image: Sort Compounds       Image: Sort Compounds
	Chromatograms Show user chromatogram(s) Show compound chromatogram(s) Overlay compound chromatogram(s)
	Compound spectrum (MS) Show MS spectrum I Show MS peak table Show MS spectrum (zoomed in on special peaks) Zoom padding: - 10.0 + 10.0 m/z I Overlay predicted isotope distribution
	Compound spectrum (MS/MS) Show MS/MS spectrum Show MS/MS peak table
	Library search results           Show library spectrum           Show difference spectrum

#### Exercise 2. Process the data (continued)

Figure 25 Compound Report dialog box.

When the method is run, a report is generated that includes a summary (Figure 26) as well as details for each compound found in the library (Figure 27). 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 27 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.

				Qua	litative Comp	ound R	eport			
Data Rie Sample Type Instrument Name Acq Nethold IRN Calibration Status Comment	Sampja anchangtof	الل مريغ Hind		Sample Name Position User Name Acquired Time O.A.Nethod	2 o.L.o. 2000 gab port boson to p. 200 a school of e-MD (Vergent 2.2 (P.(M. 22. e-20 c) P.M. Port (P.(e-2) e-1M (PM. SMS_					
Sample Group Acquiation SW 5200 Version Q-TO	m rjen TO P/6501 F 8.85.01   8.511	Darfo. U norijen 18								
Compound Table	RT	Nasa	Abund	Name	Formula	Tgt Nass	D(# (ppm)	NFG formula	DB Formula	DS D(ff (ppm) (D
Cpd 3; Anjeoux th Cpd 2; Thjebend acoje Cpd 5; Imazapyt	2.8.52 3.8.5 42.75	288.1215 281.2351 281.112	71315	Arrijecent k Thije he ndazelje Irrazapy:	C11 H15 N202 C11H7N35 C11 H2 5N303	288.1212 281.8381 281.1113	1,47	C11H18N202 C18H7N05 C13H15N303	C11H14N202 C11H7N55 C13H15N303	-1.47 -1.31 -1.37
Cpd 7; Djewikowie Cpd 5; Metowa xon	5276	228,3997	44717 52115	Direction Melocator	C5H32N03F52 C31H33C[N202	228,9995	1.51	C3H12N01752 C10H13C[N002	C3H12N03F52 C10H13CN202	4.51
Cpd 10; Imaza () ()   Injkonazoja (	7,2,53	235.3.485	17785	(maza) (()   telkom zoje)	C14H14CPN20	255.0483	13.4	C14H34C[2N20	C14H14CP 100	-3.5.4
Cpd: 5; Carbofs w.e. Cpd: 4; Atradjee Cpd: 14; Mietonajeer	7.817 1.213 1.33	221,1055 215,0340 407,0154	1405 1 14625 5 11554	Carbofa wa Afrazijia Metosalam	C12H15N03 C1H14CNS C14H13CDN5045	221,1852 215,8938 417,8855	1,45 1,58 	C12 H15ND 3 C1 H14QNS C14H13CE N5045	C12H15 N23 C8H14CINS C14H13CPN5045	-0.45 -0.51 -0.51
Cpd 9 ; Matazachijor Cpd 3 ; Maljasta	8 3 15 11 3 15	27 7,8 985	11574	Metonolom Metonoch (or Moljicate	CI 4H 16 CH 10 CI HI 7ND S	277,0982	0.0 0 -2.0 7	C14H18CH10 C5H17NOS	C14H16CN10 CH12NOS	2.3.8
Cpd 32 ; Majothjon Cpd 33; Pyracijostrob je	11.451 11.451	338 J 358 38 7, 3833	1118 1 8142 1	Maljeth jon Pyracijost sobije	C10H1905752 C19H18C(N104	338,8383	-0.34 5.53	C10 H30 05752 C10 H30 (NO 4	C10 H0505 P52 C19H18C (\$30.4	1.14
Cpd (11) Operation (Operation)	11,8 4	314,3131	1144	Djestnon (Djengyleta (	C12H21 N20 3PS	314,111	1.75	CI 2H21N2O3FS	C12 H2 1N20175	4.75
Compound Label I Cpd 3: Am Inocerb J	Name Aminocarb		<b>m /z</b> 209.1288	RT Algorithm 2.852 Find By For	M ass mult 208.1215					
Spectrum Peak List	+ FBF Spectr 2021288 H6F 2021+H1 208 210 212 208 210 212	P 214 216 211 Counts vs. W	101 min) Pes HJC HJC 1 220 222 2 I 220 222 2		n 					
211,1312 1 5465	.33 C 33 H58 N2 .1 2 C 33 H58 N2 .42 C 33 H58 N2	102	X+H +   X+H +   X+H +							
	33 C13 H18 N2 (M+H)+: +ES 137.08	I Product Ion	(X+Na)+ (2.764, 2.78)	3, 2,813, 2,837 min, 11	Scans) Frage					
X5MS Spectrum x10 3 Cpd 3: Aminocarb: 8 4 4 2 0 0 80 10	0 120 14	0 160 18	209,128							
x10 3 Cpd 3. Amnocarb 8 4 4 2 0 0 0 0 0 0 10 10 10 10 10	(M+H)+: +E8 832	10 160 150 Counts vs. M Il Product Ion 220 5 Counts vs. M	12.770, 2.79	0 240 260 280 3 (mx) 260 280 3 5, 2, 2, 819, 2, 843 min, 11	Scans) Frage_					

Figure 26 Page 1 of the Test Mix Compound report.

Exercise 2. Process the data

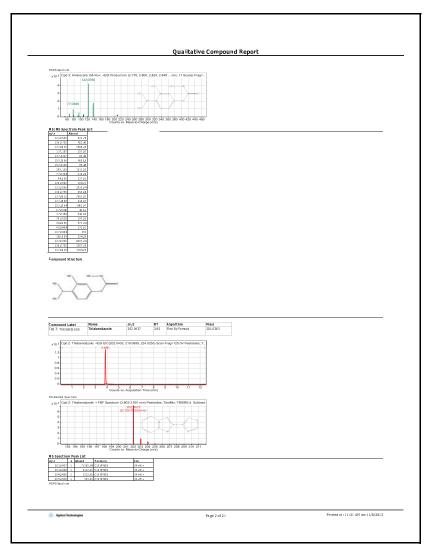


Figure 27 Page 2 of the Test Mix Compound report

## 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.

Step	Detailed Instruction Co	
<ol> <li>Set up a worklist to create a compound report.</li> </ol>	<ul> <li>a In Method Explorer, click Worklist Automation &gt; Worklist Actions.</li> <li>b Select these Actions to be run:         <ul> <li>Compound Automation without Report</li> <li>Generate Compound Report</li> </ul> </li> </ul>	The <b>Compound Automation without</b> <b>Report</b> 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.

vailable actions	
ntegrate and Extract Peak Spectra Smooth Chromatograms	<u>^</u>
Generate Compound Report Generate Analysis Report	E
ind Compounds by Auto MS/MS ind Compounds by Targeted MS/MS	
ind Compounds by Molecular Feature ind Compounds by Formula	
Resolved Isotope Deconvolute Find Compounds by MRM	
ind Compounds by Integration	<b>.</b>
ctions to be run	
Compound Automation without Report Generate Compound Report	A
senerale Compound Nepon	
	-
	×

Exercise 3. Automate the process with worklist actions

Exercise 3. Automate the process with worklist actions (continued)
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Step	<b>Detailed Instruction</b>	Comments
<b>1</b> Set print options.	<ul> <li>a In the Method Explorer, click Worklist Automation &gt; Report Options.</li> <li>b Select whether to print the represented on the save to a file (Excel file or PDF both. See Figure 29.</li> <li>c Save the method.</li> </ul>	port,

🖀 Method Editor: Reporting Options 🛛 🗙					
🕟 🕞 🚮 🖃 ~ 🍽 🖌 Method Items 🔹 📴					
Print report					
Save report					
Save report as Excel file Save report as PDF file					
Inside data file's reports subdirectory					
At specified directory:					
C:\MassHunter\reports					
If report file already exists					
Overwrite existing report					
Auto-generate new report file name					

**Figure 29** Reporting Options dialog box.

2	Attach the method to an acquisition method.	a	In the MassHunter Qualitative Analysis program, click <b>Method &gt;</b> <b>Save As</b> .
			Browse to the folder on your system that contains the Data Acquisition method that you want to automate. Click the name of the Data Acquisition method that you want to automate and click <b>Save</b> . The Qualitative Analysis method is now attached and is an integral part of the Data Acquisition method.

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

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.

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 which 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

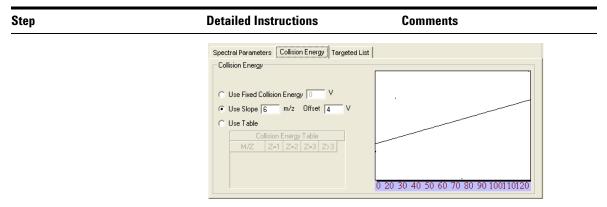
In this step, you use Find Compounds by Formula to screen the compounds by match to the accurate MS mass and isotope pattern in the PCDL.

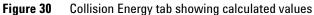
Step	Detailed Instructions	Comments
1 Open the Pesticides_TestMix_AMSMS.d file.	<ul> <li>a Open the Agilent MassHunter Qualitative Analysis program.</li> <li>Click Cancel if you are asked to open a data file.</li> <li>b Load the data analysis method Pesticides_TestMix_AMSMS_DA.</li> <li>m.</li> <li>c Open the data file Pesticides_TestMix_AMSMS.d. See Figure 31.</li> </ul>	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 <b>Pesticides_TestMix_AMSMS.d</b> 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. <b>Figure 30</b> 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 30 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

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

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





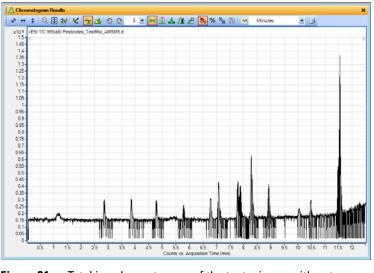


Figure 31 Total ion chromatogram of the test mix run with auto MS/MS settings.

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

Step	Detailed Instructions	Comments
2 Extract chromatograms to get a clearer picture of the data.	<ul> <li>a Right-click the chromatogram window, then click Extract Chromatograms.</li> <li>b For Type, select TIC.</li> <li>c In the MS Chromatogram tab, for MS level, select MS.</li> <li>d For Polarity, select Positive.</li> <li>e For Scans, select Scan. See Figure 32.</li> <li>f Click OK.</li> </ul>	

Extract Chromatograms	
List of opened data files	
Pesticides_TestMix_AMSMS.d	Type: TIC
	OK Cancel

**Figure 32** Extract Chromatograms setting for MS.

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

Step	Detailed Instructions	Comments
	Extract Chromatograms List of opened data files Pesticides_TestMix_AMSMS.d	Type: TIC      Integrate when     extracted      MS Chromatogram Advanced Excluded Masses      MS level: MS/MS      MS/MS      Polarity: Positive      Scans: Product ion      Precursor ion m/z: Any      m/z value(s):      OK Cancel

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

Figure 33 Extract Chromatograms setting for MS/MS.

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

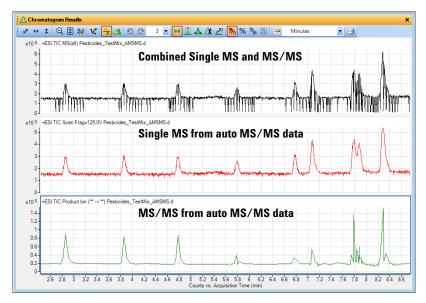


Figure 34 The top chromatogram shows all of the data points for single-MS and MS/MS. Note that MS/MS data points have lower total signal because ions in a narrow mass range are isolated for fragmentation. The middle chromato-gram shows the single-MS only and it is clear that the Q-TOF LC/MS is collecting 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

## 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 35 shows the spectra parameters that are typically used for Auto MS/MS.

Figure 35 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 36.
  - **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 36, 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 36, 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.

Spectral Parameters Collision Energy Preci	ursor Selection I Precursor Selection II Preferred/Exclude
3     Max Precursor Per Cycle       Precursor Threshold     2000       Abs. Threshold     2000       Rel. Threshold (%)     0.05   Active Exclusion       ✓     Enabled       Excluded after     2       Released after     0.05	Static Exclusion Range List Stat m/z End m/z 100 125 ▶ 600 1000

Figure 36 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.

**Exercise 2. Optimize the number of data points** 

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	
<u>S</u> í 1	teps 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.	<ul> <li>Detailed Instructions</li> <li>a Start the MassHunter Qualitative Analysis program.</li> <li>b Open the Method Editor.</li> <li>c Select only these options: <ul> <li>Find by Formula</li> <li>Search a library for each compound</li> <li>Show only identified compounds See Figure 37.</li> </ul> </li> <li>d In the Search Criteria tab, <i>clear</i> the check box for Collision energy. See Figure 38.</li> <li>To automate the process, do the steps in "Exercise 3. Automate the process with worklist actions" on page 47.</li> </ul>	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	
			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 39 and	
			Figure 40. A copy of this report is also available on the report disc as a PDF file.	

#### Exercise 3. Process the data and automate

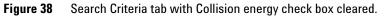
Exercise 3. Process the data and automate

Exercise 3. Process the data and automate (continued)

Steps	Detailed Instructions	Comments
	E Method Editor: Compound Automation (	2) Find and Identify
	: 💽 Run Compound Automation Steps 🔹	Xa   ⊔າ • (≃
	Options Additional Chromatograms BPC Exclusion	sions
	Compound mining	
	Find by Formula	
	Conserved the other time	
	Compound identification	
	Search a database for each compound	
	Search a library for each compound	
	Match sequences for each compound	
	Generate formulas for each compound	
	Only compounds	ounds without database hits
	Compound results	
	V Show only identified compounds	

**Figure 37** Find and Identify options for Auto MS/MS.

Method Editor: Search Accurate Mass Library	×
🗄 💽 Search Library for Compounds 🔹 🚮 🛛 🛩 🍽 🗠 🖬	••• =
Settings Peak Filters Search Criteria Search Results	
Search filter	
Restrict spectral comparison based on	
Ionization mode	
☑ Instrument type	
Collision energy +/- 2.00 eV	
L	



Exercise 3. Process the data and automate

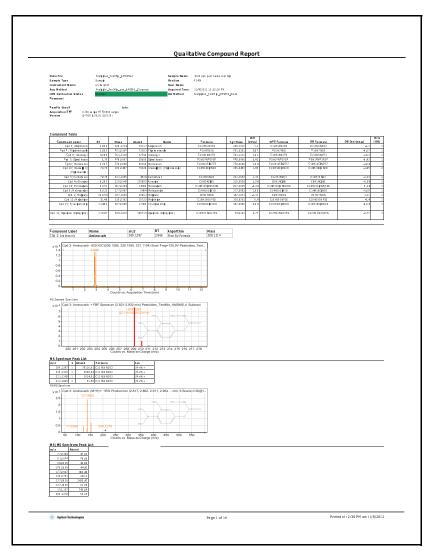


Figure 39 Page 1 of Auto MS/MS analysis report

Exercise 3. Process the data and automate

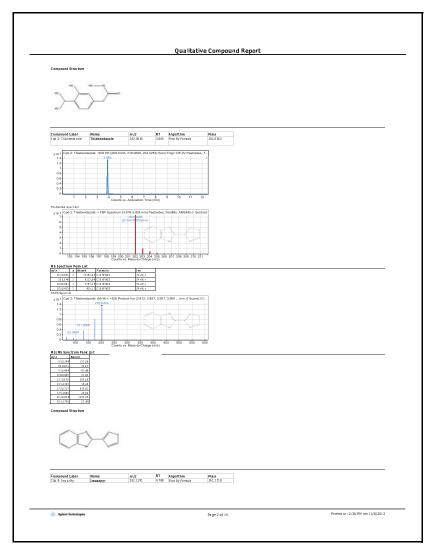


Figure 40 Page 2 of Auto MS/MS analysis report

## Reference

## **Test Mix Content**

The content of the checkout test mix is listed here.

In addition to standard MRM parameters, the retention time and retention window settings are listed for each compound. This allows longer dwell time, better signal stability, and higher data quality compared to traditional MRM method.

 Table 2
 LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) Mixture 1 Basic Compounds

#	Chemical Name/CAS #	Neat Material Chemical Purity (%)	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Aminocarb/2032-59-9		100.0 µg/mL		$C_{11}H_{16}N_2O_2$	208.1211777698
2	Atrazine/1912-24-9		100.0 µg/mL		$C_8H_{14}CIN_5$	215.0937731936
3	Carbofuran/1563-66-2		100.0 µg/mL		$C_{12}H_{15}NO_{3}$	221.1051933528
4	Diazinon (Dimpylate)/333-41-5		100.0 µg/mL		$C_{12}H_{21}N_2O_3PS$	304.1010497716
5	Dimethoate/60-51-5		100.0 µg/mL		$C_5H_{12}NO_3PS_2$	228.9996212071
6	Imazalil (Enilconazole)/35554-44-0		100.0 µg/mL		$\mathrm{C_{14}H_{14}Cl_2N_2O}$	296.0483185037
7	Imazapyr/81334-31-1		100.0 µg/mL		$C_{13}H_{15}N_3O_3$	261.1113413676
8	Malathion/121-75-5		100.0 µg/mL		$C_{10}H_{19}O_6PS_2$	330.0360662899
9	Metazachlor/67129-08-2		100.0 µg/mL		$C_{14}H_{16}CIN_3O$	277.0981898649
10	Metosulam/139528-85-1		100.0 µg/mL		$C_{14}H_{13}CI_2N_5O_4S$	417.0065300909
11	Metoxuron/19937-59-8		100.0 µg/mL		$C_{10}H_{13}CIN_2O_2$	228.0665553841
12	Molinate/2212-67-1		100.0 µg/mL		C <sub>9</sub> H <sub>17</sub> NOS	187.103084902
13	Pyraclostrobin/175013-18-0		100.0 µg/mL		$C_{19}H_{18}CIN_{3}O_{4}$	387.0985837956
14	Thiabendazole/148-79-8		100.0 µg/mL		$C_{10}H_7N_3S$	201.0360679755

#### Table 2 LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) Mixture 1 Basic Compounds

Acetonitrile	Solvent	C <sub>2</sub> H <sub>3</sub> N	41.0265	

#### Table 3 LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469) Mixture 2 Acidic Compounds

#	Chemical Name/CAS #	Neat Material Chemical Purity (%)	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Acifluorfen/50594-66-6		100.0 µg/mL		$C_{14}H_7CIF_3NO_5$	360.9964846522
2	2,4,5-T/93-76-5		100.0 µg/mL		$C_8H_5CI_3O_3$	253.9304271564
3	Bentazone/25057-89-0		100.0 µg/mL		$C_{10}H_{12}N_2O_3S$	240.0568629945
4	Dinoseb (Subitex)/88-85-7		100.0 µg/mL		$C_{10}H_{12}N_2O_5$	240.0746215091
5	2,4,5-TP (Silvex) (Fenoprop)/93-72-1		100.0 µg/mL		$C_9H_7CI_3O_3$	267.9460772202
6	Hexaflumuron/86479-06-3		100.0 µg/mL		$C_{16}H_8CI_2F_6N_2O_3$	459.9816167569
	Acetonitrile		Solvent		$C_2H_3N$	41.0265

The acquisition method parameters for the negative ion test mix are in the test mix method **Acid Pesticides Test Mix\_DMRM.m**.

## 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 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 4 shows some examples of product ion conditions and results.

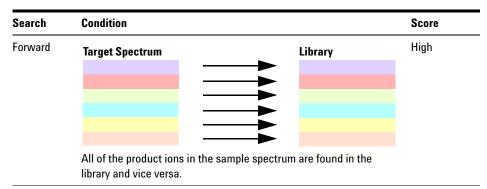


 Table 4
 Example product ion conditions and search results

Search	Condition		Score
Forward	Target Spectrum	Library	Low
	All of the product ions in the sam library, but only some of the produ the sample spectrum.		n
Reverse	Target Spectrum	Library Library the library are found in the samp	Low
Reverse	spectrum.	Library	High
	All of the product ions in the libra spectrum.	ry are found in the sample	

 Table 4
 Example product ion conditions and search results (continued)

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## In This Guide

This Quick Start Guide describes how to use the MassHunter Pesticides PCD or PCDL.

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