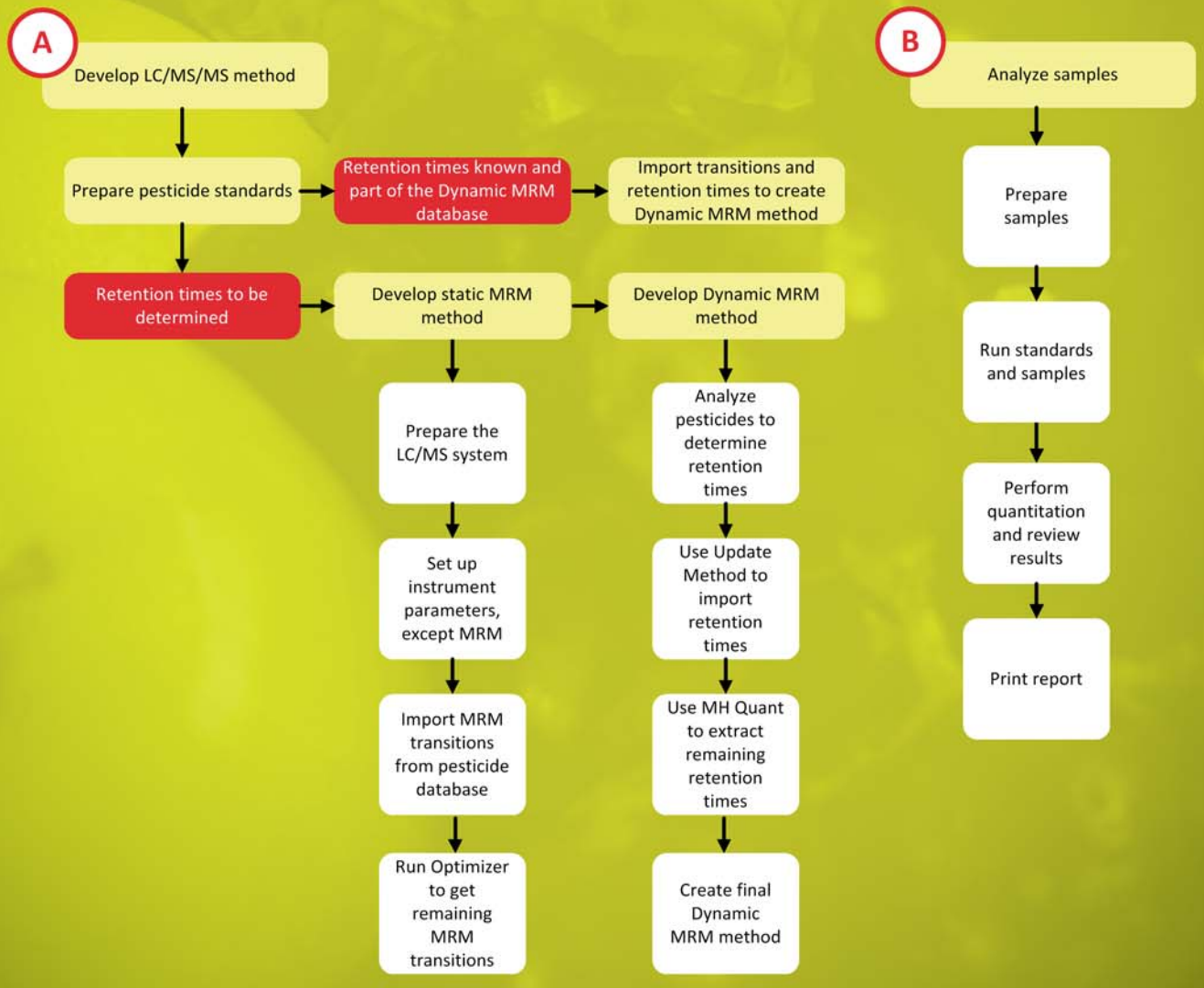
Agilent Triple Quadrupole LC/MS Quantitation of Pesticides

Workflow Guide



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Notices

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

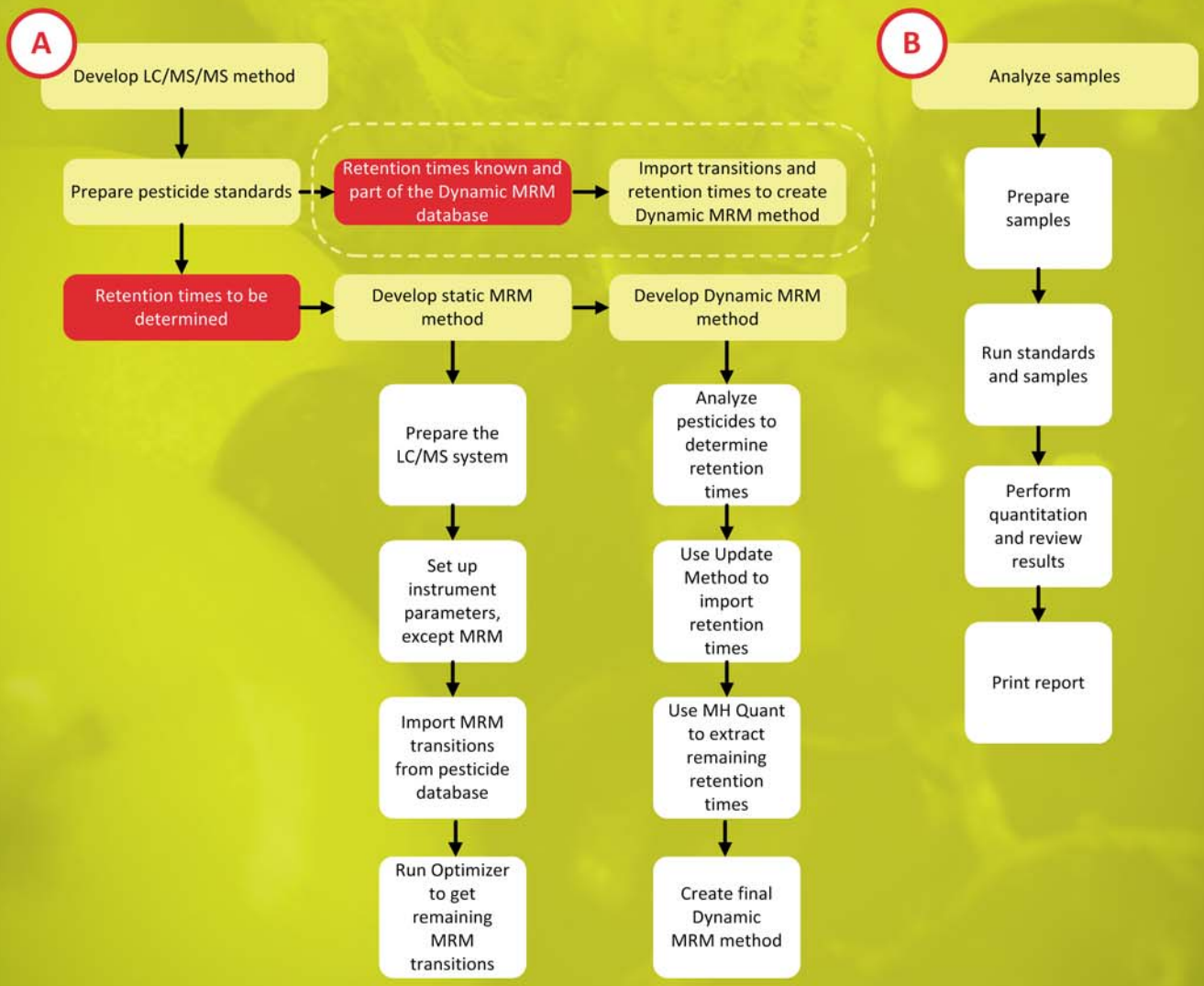
WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

Before You Begin

Make sure you read and understand the information in this chapter and have the necessary instrumentation, software, solvents, and lab supplies before you start the analysis.



Introduction

Pesticide analysis in food and environmental samples is a challenge because you need low detection limits and you often must monitor large numbers of compounds. Triple quadrupole mass spectrometry (MS) systems are frequently used for this analysis because of their high sensitivity and specificity.

This manual outlines an efficient way to do pesticide quantitation by liquid chromatography (LC) with triple quadrupole MS detection. It describes use of Agilent LC/MS systems that deliver robust, sensitive analyses, as well as Agilent software tools that accelerate method development and sample analyses.

Overview of the workflow

This guide describes the workflow (shown on the previous page) to use an Agilent 6400 Series Triple Quadrupole LC/MS System with Agilent MassHunter Workstation software to do high-sensitivity multi-residue pesticide quantitation. The manual describes development of multiple reaction monitoring (MRM) and Dynamic MRM methods (part A of the workflow), as well as sample analysis (part B).

Once you have developed a Dynamic MRM method, you can use Agilent MassHunter Optimizer to save the retention times and compound-specific triple quadrupole settings to a database. The next time you need to analyze the same pesticides under the same LC conditions, you can import the settings from the database, which saves significant time. These steps are highlighted within the dashed line in the diagram.

Similarly, Agilent provides a pesticide database for triple quadrupole analyses that you can use to eliminate some of the method development steps shown in the workflow.

While you can use this guide to set up an LC/MS/MS analysis of many types of pesticides in many matrices, it is not a compendium of sample preparation and LC methods. It focuses primarily on setup of the triple quadrupole MS analysis.

You can use this workflow as a roadmap for any analysis that requires multi-residue quantitation using Dynamic MRM on the Agilent 6400 Series Triple Quadrupole LC/MS System. While written specifically for pesticides, most of the concepts apply to other types of analyses.

What are MRM and Dynamic MRM?

During an MRM analysis, the first quadrupole (MS1) in the triple quad selectively passes a precursor ion from the analyte of interest. The precursor ion fragments in the hexapole collision cell. The second quadrupole (MS2) passes to the detector only the selected product ions produced by collisions of the precursor ion.

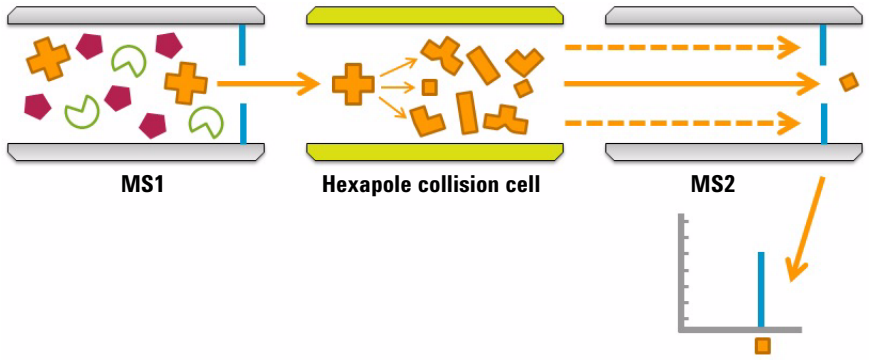


Figure 1 *MRM analysis with an Agilent triple quadrupole MS*

To confirm the presence of a pesticide, you typically monitor two MRM transitions (precursor-product ion combinations) for each analyte of interest. You then confirm the pesticide by a combination of retention time and correct ion ratios.

As you increase the number of analytes that the instrument monitors by MRM, the sensitivity decreases because the system must cycle among more signals. When you need to analyze hundreds of pesticides, you can set up time segments and monitor a subset of the analytes in each time segment. However, this can be tricky because you must ensure that each analyte is fully eluted within a time segment. A shift in retention time may cause you to repeat the analysis. Also, the sensitivity is reduced when peaks are eluted close together, forcing you to monitor a large number of analytes within a given time segment.

Dynamic MRM solves these problems. With Dynamic MRM, the triple quadrupole monitors each analyte only in a short window (for example, 1 min) around the retention time of the analyte. Throughout the run, the Agilent MassHunter Data Acquisition program constantly updates the list of analytes that it monitors.

Dynamic MRM makes it easier to develop methods, and to modify them to add more analytes. The instrument spends more time monitoring each analyte, which increases sensitivity.

A key advantage of Dynamic MRM is that you set the cycle time and the program sets dwell times accordingly. Cycle time determines the number of points across a peak—a very important parameter for good quantitation and peak representation.

More information

If you need a general introduction to triple quadrupole (QQQ) mass spectrometry before you begin, see the *Agilent 6400 Series Triple Quad LC/MS System Concepts Guide* (Agilent publication G3335-90069, Sixth Edition, May 2009). The following sections are especially useful:

- "How a triple quadruple mass spectrometer works"
- "How Dynamic MRM works"

You may also view an online video that describes how a triple quad works.

For more information about Dynamic MRM, see the following:

- "Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS – Fast and Effective Method Development

Using an Application Kit and a Pesticides Compound Parameter Database" (Agilent application note 5990-4253EN, October 2009)
- "New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses" (Agilent application note 5990-3595EN, June 2009)

NOTE

This manual gives links to most references. If you have an electronic copy of this manual, you can easily download the documents from the Agilent literature library. Look for and click the blue hypertext; for example, you can click the library link in the previous sentence.

If you have a printed copy, go to the Agilent literature library at **www.agilent.com/chem/library** and type the publication number in the **Keywords** box. Then click **Search**.

Chapter 4 contains a complete list of references.

Advantages of this workflow

If you need to quantitate pesticides, this workflow has many benefits:
- Extremely high sensitivity
- Very selective analysis, with only a small chance of false positive results
- Ability to monitor hundreds of pesticides in a single LC run
- Ability to add new pesticides to the method, to meet emerging needs
- Excellent quantitative results (provided that you run appropriate standards, matrix-matched standards, internal standards, and quality control samples)

NOTE

Many food matrices cause ion suppression, and matrix-matched standards compensate for this phenomenon. For environmental analyses, it is not possible to have matrix-matched standards.

What you cannot do with the workflow

This workflow helps you quantitate the pesticides you analyze; however, it does not give you information about other sample components. If you need a pesticide screen that gives full sample characterization and lets you reanalyze the data for new pesticides weeks or years later, then a time-of-flight (TOF) or quadrupole-TOF (Q-TOF) LC/MS analysis may be a better choice for you. Agilent offers two types of MS systems for this purpose:
- Agilent 6200 Series Accurate-Mass TOF LC/MS systems
- Agilent 6500 Series Accurate-Mass Q-TOF LC/MS systems

Safety Notes

WARNING **Always take proper precautions when you use and dispose of solvents, pesticides, and other chemicals. Read the material data safety sheets supplied by the vendors.**

WARNING **When you disconnect LC columns or fittings, solvents may leak. Use appropriate safety procedures (for example, goggles, safety gloves and protective clothing), especially when you use toxic or hazardous solvents. Read the material data safety sheets supplied by the solvent vendors.**

WARNING **Read, understand, and meet conditions of all warnings in the *Agilent 6400 Series Triple Quad LC/MS Maintenance Guide*.**

CAUTION Read, understand, and meet conditions of all cautions in the *Agilent 6400 Series Triple Quad LC/MS Maintenance Guide*.

Required Items

Required hardware and software

Figure 2 *The workflow requires an Agilent LC and an Agilent 6400 Series Triple Quadrupole LC/MS System.*

To do this workflow, you need:

- One of the following LCs:
  - Agilent 1220 Infinity LC
  - Agilent 1260 Infinity LC
  - Agilent 1290 Infinity LC
  - Agilent 1200 Series LC system
  - Agilent 1200 Series Rapid Resolution LC system
- Agilent 6400 Series Triple Quadrupole LC/MS System
- Agilent MassHunter software:
  - Agilent MassHunter Data Acquisition for QQQ version B.04.00
  - Agilent MassHunter Optimizer version B.04.00 (installed with MassHunter Data Acquisition for QQQ version B.04.00)
  - Agilent MassHunter Quantitative Analysis software version B.04.00

NOTE

The retention times in the Agilent Triple Quadrupole LC/MS Pesticide Application Kit (G1733AA) were determined with the Agilent 1200 Series Rapid Resolution LC (RRLC) system and the Agilent 1290 Infinity LC System. If you want to use the retention times, then you need one of these LCs. However, you may use the Agilent 1260 Infinity LC System in place of the 1200 Series RRLC, because Agilent engineered the 1260 Infinity LC for full backwards compatibility.

These LCs operate at higher pressure and enable better resolution, which allows you to analyze more pesticides in a single method and helps to separate pesticides from matrix compounds that can cause ion suppression.

The exercises in the next two chapters assume that:

- All instruments have already been installed and are working to specifications.
- You have some experience with pesticide analysis.
- You have been trained on the instrumentation and software. For example, you have taken an operator course at an Agilent training center or you have been trained on-site by an Agilent instructor (Application Engineer or consultant).

Optional kit

The Agilent Pesticide Dynamic MRM Database—available with the Agilent Triple Quadrupole LC/MS Pesticide Application Kit (G1733AA)—is optional for this workflow. However, this kit speeds method development, especially when you need to analyze for a large number of pesticides.

The database in the kit contains more than 600 pesticides and 150 forensic compounds monitored throughout the world. It includes compound names, optimized MRM transitions, fragmentor voltages, collision energies, and retention times. A support disk contains the methods that were used to produce the retention times.

The database kit has the following advantages:

- Rapid set-up of methods for hundreds of pesticides
- Ability to add new compounds to the database, to meet changing needs

For more information, read "Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems" (Agilent application note 5990-4255EN, April 2010).

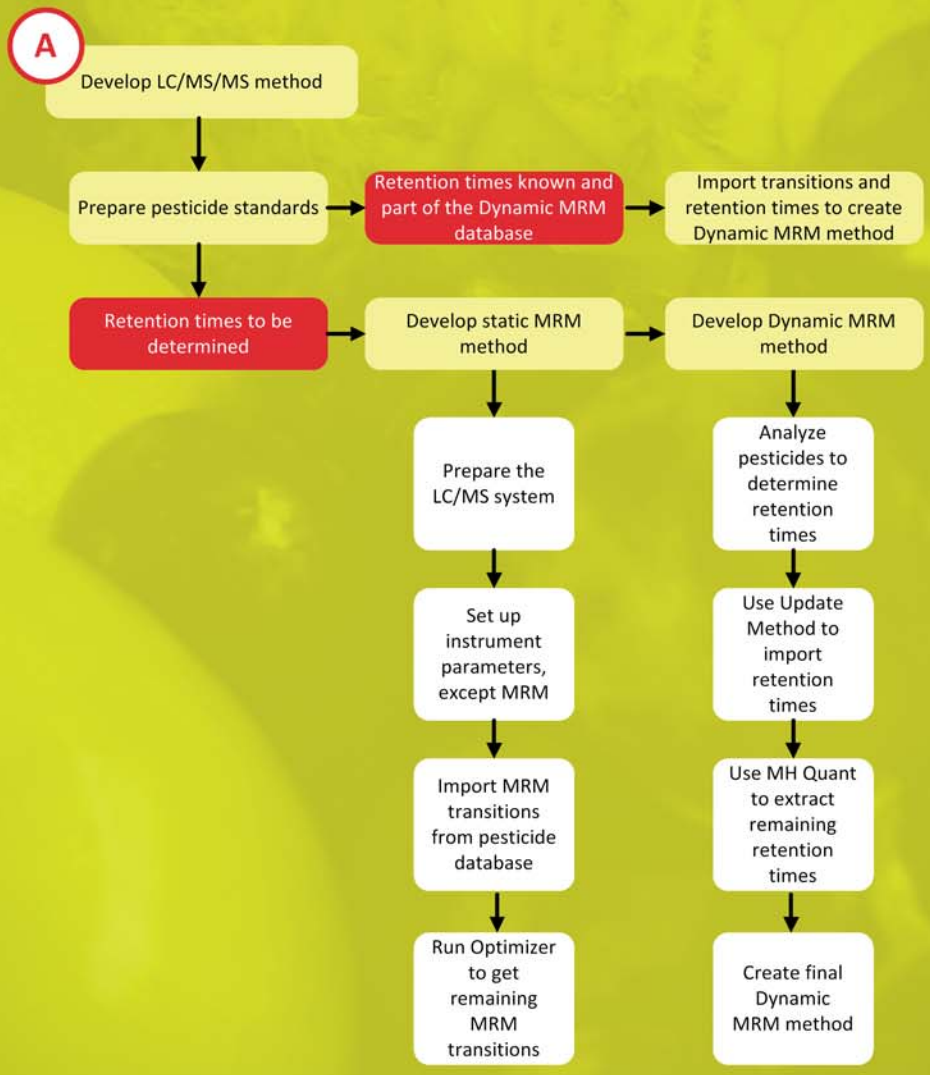
CAUTION Use of the Agilent Pesticide Dynamic MRM Database does not free you from the need to run standards, do method validation, and use a good quality assurance/quality control (QA/QC) program.

Additional items

See "Required supplies and chemicals" on page 50.

Developing the Data Acquisition Method

These exercises show you how to set up a Dynamic MRM method for multi-residue pesticide quantitation with an Agilent 6400 Series Triple Quadrupole LC/MS System and MassHunter Workstation software.



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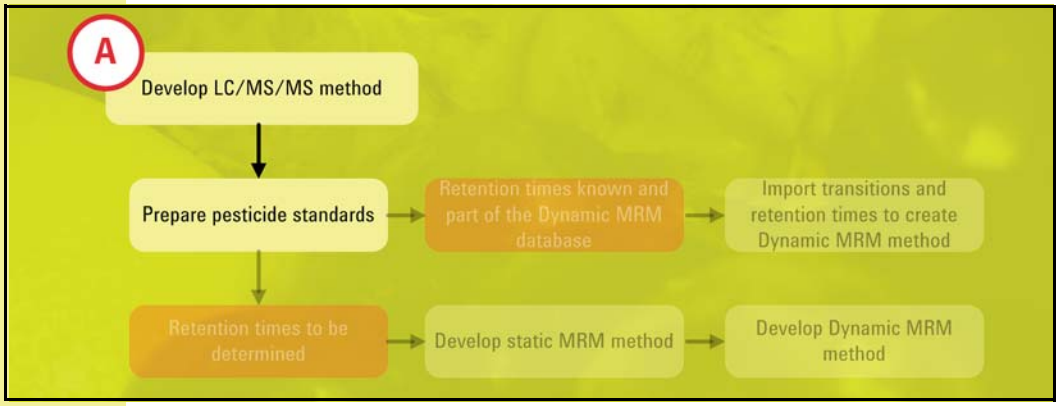
Prepare pesticide standards

In this exercise, you choose an initial LC/MS/MS method. Then you prepare all the pesticide standards you need for method development and sample analysis.



Do preliminary work

1. Choose an initial LC/MS/MS method for data acquisition.

   - Do one of the following:
     - If you have the Agilent Pesticide Dynamic MRM Database, search the database for the pesticides you will analyze. If they exist in the database and retention times are listed, Agilent suggests to use the LC/MS method referenced in the database. (Retention times are LC-specific. See note on page 10.)
     - Choose one of the methods from the appendix of Agilent application note 5990-4253EN, "Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS – Fast and Effective Method Development Using an Application Kit and a Pesticides Compound Parameter Database."
     - Choose an LC/MS method from another source, or create one yourself.

   If you choose the first option, be aware that multiple methods were used to collect the retention times in the database. Retention times apply only to the method for which they were collected.

   The *MassHunter Pesticide Dynamic MRM Database Kit Support Disk* contains the methods referenced in the database, as both MassHunter method files and Adobe® PDF files.

   To learn about searches of the database and import of compounds into methods, see "Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent LC/MS Triple Quadrupole Systems" (Agilent application note 5990-4255EN, April 2010).

2. Purchase standards and solvents, if you have not already done so.

   a Read the all of the steps in the next task to determine which standards you need to purchase.

   b Purchase the standards and solvents.

Prepare the standards

1. Prepare stock standards of individual pesticides.

2. Prepare working standards of individual pesticides, for use with the Agilent MassHunter Optimizer program.

   a Prepare individual standards at a concentration of about 1 ng/µL. You will inject 2 µL, for a total of about 2 ng on-column. (If you have the Agilent 6490 Triple Quadrupole LC/MS System, prepare the standards at 100 pg/µL and inject 2 µL.)

   b You will use these standards for "Use MassHunter Optimizer to determine remaining MRM transitions" on page 22.

   If you have the Agilent Pesticide Dynamic MRM Database, you only need to prepare this set of standards for pesticides that are *not* in the database. (You still need to prepare the other standards in this exercise, for example, those in step 4.)

   Some pesticides may need higher or lower concentrations. The concentration should give you a good response, but not be so high that you see carry-over.

3. Prepare standard(s) that you will use to determine (or confirm) retention times for the Dynamic MRM method.

   a Note any isobaric or nearly isobaric pesticides, and prepare them in separate mixtures.

   b Prepare pesticide mixture(s) such that the concentration of each pesticide is about 100 ng/mL (100 pg/µL) in acetonitrile. You will inject 1 to 5 µL. (For the 6490 Triple Quadrupole, inject 1 µL.)

   c You will use these standards for "Analyze pesticides to determine retention times" on page 29.

   It is important to prepare a standard that gives a good response for *all* MRM transitions, because that allows you to set up the Dynamic MRM method more efficiently.

4. Prepare additional standards required for your protocol.

   Additional standards may include:
   - Calibration standards
   - Internal standards
   - Standards used to spike samples/determine recoveries

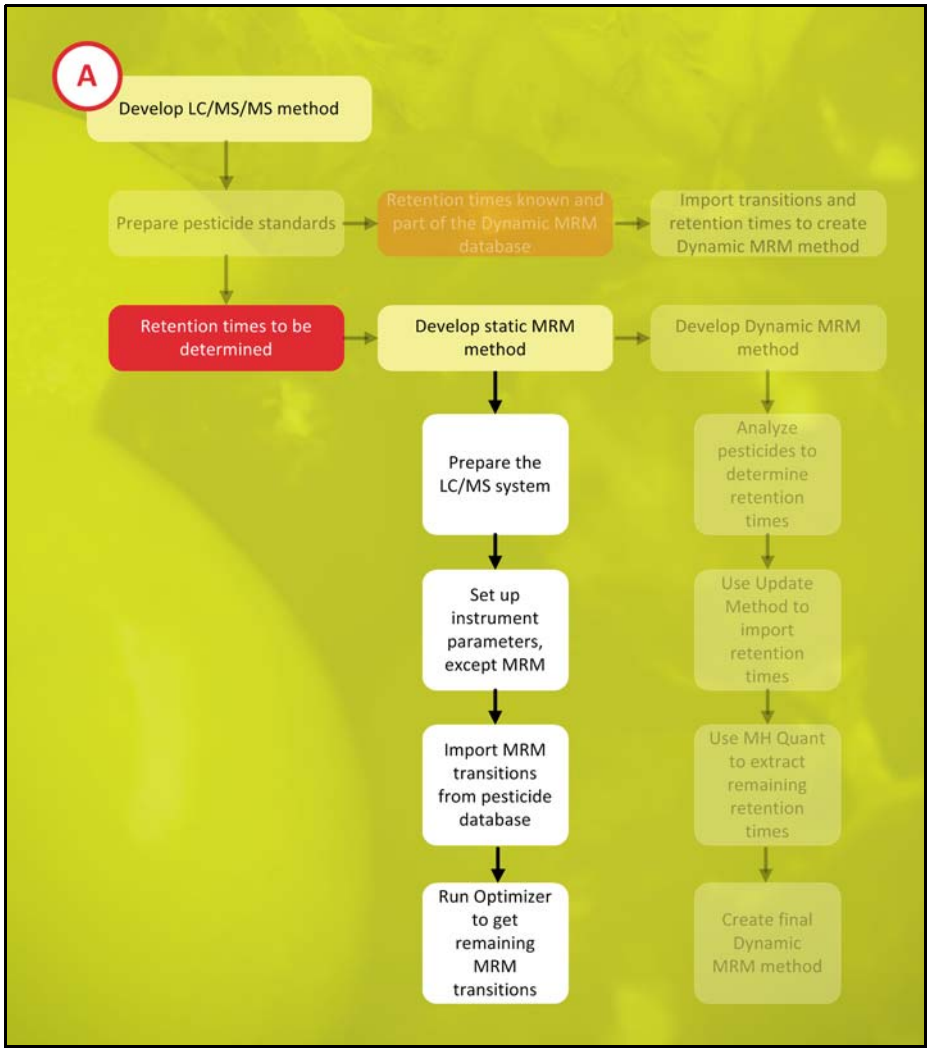
   You will use these standards when you analyze samples (Chapter 3).

   Some pesticides precipitate if diluted in solvent that contains a high percentage of water. In these cases, Agilent recommends that you use an autosampler program to dilute the sample before injection onto the column. For example, you may use a stacked ("sandwich") injection to successively draw aliquots of water, nonaqueous standard, and more water.

5. Refrigerate the standards.

Develop the preliminary static MRM method

In this exercise, you develop one or more preliminary static MRM methods with single time-segments. You will use these static MRM methods as a starting point to develop the Dynamic MRM method that you will use to analyze samples.



Prepare the LC/MS system

1. Prepare LC solvents.

   a Prepare the aqueous and organic mobile phases for the method you chose under "Prepare pesticide standards" on page 14.

   b Put the solvent bottles on the LC.

2. Start the MassHunter Data Acquisition program.

- Double-click the MassHunter Data Acquisition icon.

If you need help, see Step 1 in the "Getting Started" section of the *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide* (Agilent publication G3335-90077, Eighth Edition, January 2010).

3. Prepare the LC modules.

a Switch the LC stream to waste (or disconnect it from the MS).

b Purge the LC pump.

c Install the column and condition it as described in the column instructions included in the column package.

d Set up to view real-time parameter values (actuals).

e Set up to display real-time plots.

If you need help, see Step 2 in the "Getting Started" section of the *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide*.

It is very important to purge solvent channels A and B because trapped air causes irreproducible retention times for analytes.

4. Prepare the Agilent 6400 Series Triple Quadrupole LC/MS System.

a Do a Checktune, or if necessary do an Autotune.

b Switch the LC stream to MS.

c Start the flow at initial method conditions.

d Monitor the MS baseline and spectral displays.

If you need help, see Step 3 in the "Getting Started" section of the *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide*.

Set up LC/MS parameters, except for MRM settings

This task gives you the basic steps to set up an LC/MS method with the MassHunter Data Acquisition program. If you need more details and practice, see the *Agilent MassHunter Workstation Software – Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide* (Agilent publication B3335-90059, Third Edition, May 2009).

1. Enter values for all the LC modules.

- In the MassHunter Data Acquisition program, click each LC tab and enter values from the method you chose in step 1 on page 14.
- If you have the Agilent Pesticide Dynamic MRM Database, and you want to use the retention times for your Dynamic MRM method, enter the LC settings from the method that is listed in the database, or load the method listed in the database. (Methods are on the *Support Disk* for the kit.)

If you need help, see Step 4 in the "Getting Started" section of the *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide* (Agilent publication G3335-90077, Eighth Edition, January 2010).

2. Enter triple quadrupole MS parameters.

a Click the **MS QQQ** tab and enter values from the method you chose in step 1 on page 14.

b For Scan Type (under Time segments), select **MRM**.

If you need help, see Step 4 in the "Getting Started" section of the *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide*.

A Dynamic MRM method allows only a single time-segment. If you need to divert the LC flow to waste at the beginning of the analysis, activate the "SCP_MSDivertValveToWaste" script in the Properties tab. The script diverts the LC flow to waste until the start time specified for the Dynamic MRM segment.

3. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

c If you need to analyze more than 50 pesticides (or 100 transitions), save multiple copies of the method.

At this point, the method has no MRM transitions. You will add those in the next two tasks.

You will add up to 50 pesticides (100 transitions) to each copy of the method.

NOTE

The 50-compound limit is based on 5-second peak widths and the desire to have a maximum cycle time of 0.5 sec (10 points across a peak).

- If your peaks are narrower, reduce the number of compounds in your method.
- If your peaks are wider, you may increase the number of compounds in your method.

Import MRM transitions from a pesticide database

When you analyze pesticides by MRM with the Agilent 6400 Series Triple Quadrupole LC/MS System, you can use Autotune settings for most of the MS parameters. However, on any triple quadrupole instrument, some parameters are compound-specific. On the Agilent systems, they are:

- MRM transitions (precursor ion/product ion pairs)
- Fragmentor voltage that gives best sensitivity for the precursor ion (except for the 6490 Triple Quadrupole, where fragmentor voltage is not compound-dependent)
- Collision energy (in the hexapole collision cell) that gives greatest response for each product ion

You can determine these parameters experimentally, by analysis of each pesticide. However, it is much easier to simply import the correct settings from a database.

In this exercise, you import MRM transitions, fragmentor voltages, and collision energies from the Agilent Pesticide Dynamic MRM Database, which is available when you purchase the Agilent Triple Quadrupole LC/MS Pesticide Application Kit. If you do not have this kit, go to "Use MassHunter Optimizer to determine remaining MRM transitions" on page 22.

The database also includes pesticide retention times, and shows the method that produced each retention time. If you reproduce the LC method exactly, you can import and use these retention times. However, you still need to run standards to confirm that you get the same retention times. If the database lacks retention times for some pesticides of interest, you will determine those in the next task.

NOTE

Retention times in the database were collected with multiple methods, and they apply only for the method for which they were collected. If you choose to replicate method A from the database, you may import MRM transitions, fragmentor voltages, and collision energies for pesticides that were analyzed using method B, but do not import the retention times for method B.

More information is available in "Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems" (Agilent application note 5990-4255EN, April 2010).

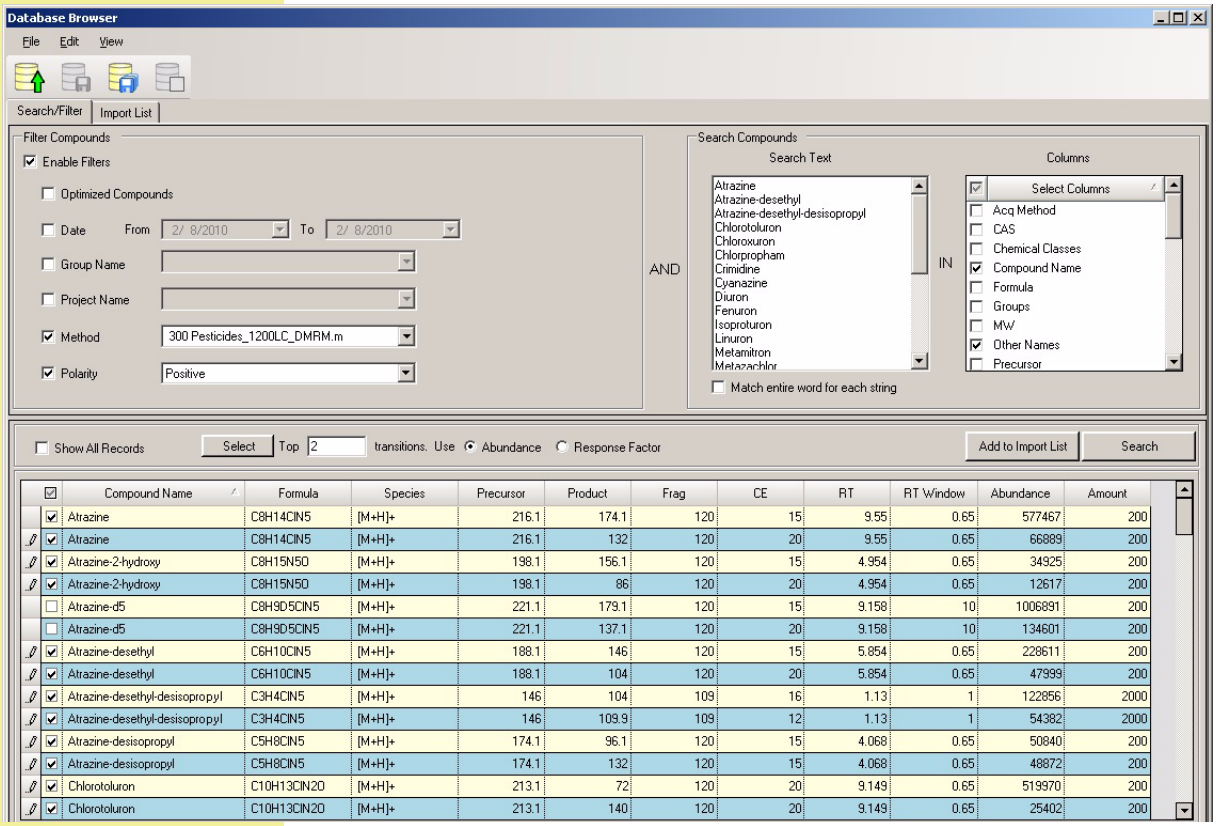


Figure 3 *The Agilent Pesticide Dynamic MRM Database contains the compound-specific settings for Agilent triple quadrupole LC/MS analysis. Note that the* ***Frag*** *column does not appear for the 6490 Triple Quadrupole.*

1. Open MassHunter Data Acquisition, if it is not already open.

2. Load your method.

   - Load the method you created in "Set up LC/MS parameters, except for MRM settings" on page 17.

3. Import MRM transitions.

   If you have the Agilent Pesticide Dynamic MRM Database, import MRM transitions. You can import as many as you want, but assuming 5-sec chromatographic peak widths, Agilent recommends importing up to 50 pesticides (or 100 total transitions). See the note on page 18.

   a In the MassHunter Data Acquisition program, click the **MS QQQ** tab.

   b Click the **Acquisition** tab.

   c In the MS QQQ tab, make sure the Scan Type is set to **MRM**. (Set it to **Dynamic MRM** *only* if you want to import the retention times as well as transitions.)

   d Right-click an empty area on the Acquisition tab, then click **Import from Optimizer**.

e In the Database Browser, open the MassHunter Pesticide Dynamic MRM Database.

f Import the required MRM transitions from the database. If you want to import all pesticides that were acquired with a particular method (so you can use all their retention times), filter the database by method, as shown below.

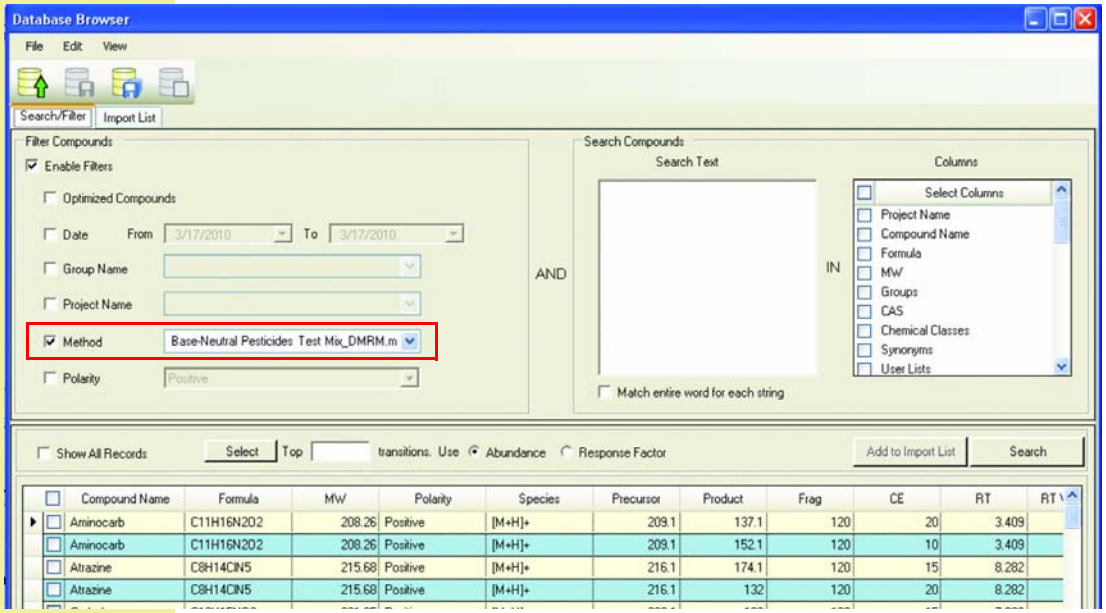


Figure 4 *To import all retention times associated with a particular method, first filter the database by method. Note that the* ***Frag*** *column does not appear for the 6490 Triple Quadrupole.*

For details on importing MRM transitions, see "To create an MRM method to run your own sample" in the "Getting Started" section of the *Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide* (Agilent publication 5990-5742EN, Second Edition, May 2010).

To filter the database and search for compounds, see the "Setting Up Compounds and Working with Databases" section in the *Agilent G3793AA MassHunter Optimizer Quick Start Guide* (Agilent publication G3793-90003, Third Edition, January 2010).

For more information about import of MRM transitions, read technical note 5990-4255EN.

4. Set the dwell time.

- For 50 compounds with 2 transitions each, use a 2 msec dwell time. For peaks that are 5 sec wide, this gives about 10 points across the peak and a cycle time of 500 msec.
- If you have fewer compounds or wider peaks, set a longer dwell time (for example, 5 msec) because that will give better ion statistics.

For a static MRM experiment (such as the one you set up in this task), you set the dwell time and the program calculates the cycle time.

For a Dynamic MRM experiment (such as the one you set up in a later task), you set the cycle time and the program calculates the dwell times.

5. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

6. If necessary, repeat this task with additional methods.

Because you do not know the compound retention times, each method has a single time segment and should have no more than about 100 MRM transitions (or 50 pesticides with two transitions each). See the note on page 18.

7. If you imported retention times from the database, analyze pesticide standards to confirm the retention times.

a Use the method you saved in step 5 to run a standard at a concentration such that all pesticides are detected (typically 100 pg/µL, with a 1- to 5-µL injection).

b If the retention times differ by a small amount, use the **Update Method** command to update the retention times. See "Import retention times to create the Dynamic MRM method (Update Method)" on page 30.

c If pesticides are not detected, increase the retention time window and rerun the standard to determine the shift in retention time.
- A window of 5 min is sufficient for this experiment.
- Check the method with the Dynamic MRM Viewer to make sure you still have a minimum dwell time of 2 msec. The viewer will highlight in red any problem.
- If all pesticides are detected, use the **Update Method** command with this data file to update the retention times.

If any of the pesticides you imported did *not* have a retention time in the database, in the Acquisition tab of MassHunter Data Acquisition, you will see **Ret Time (min) = 0** and **Delta Ret time = 0**, which means they are measured for the entire time. In this case, do the steps in "Develop the final Dynamic MRM method" on page 28 for these pesticides.

Use MassHunter Optimizer to determine remaining MRM transitions

In this exercise, you use the MassHunter Optimizer software to determine the best triple quad settings for MRM analysis of any remaining pesticides that you need to analyze. You do this task under these conditions:
- You do *not* have the Agilent Pesticide Dynamic MRM Database
- You do have the database, but either
  - You need to determine compound-specific settings for additional pesticides, or
  - You wish to reoptimize the MRM analysis for some pesticides because you need utmost sensitivity or you have changed your mobile phase or modifiers (which can change the predominant precursor ion)

MassHunter Optimizer software provides a way to automatically optimize the MRM parameters for each compound analyzed on the Agilent 6400 Series Triple Quadrupole LC/MS System. Specifically, it automates the:
- Selection of the best precursor ion
- Optimization of the fragmentor voltage for each precursor ion (except for the 6490 Triple Quadrupole, where the fragmentor voltage is *not* compound-specific)
- Selection of the best product ions

- Optimization of collision energies for each transition

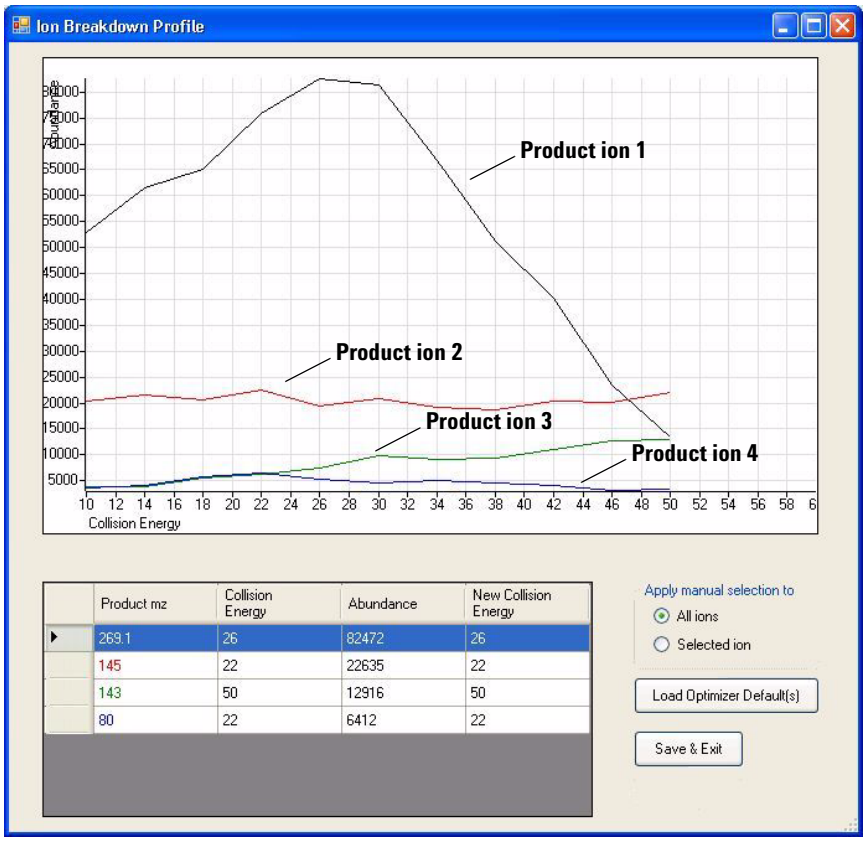


Figure 5 *MassHunter Optimizer software quickly finds the best parameters, such as collision energy, for a given pesticide. This figure shows the ion breakdown profile from a single-compound optimization.*

For more information, see "MassHunter Optimizer Software for Automated MRM Method Development Using the Agilent 6400 Series Triple Quadrupole Mass Spectrometers" (Agilent 5990-5011EN, February, 2010).

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1. Prepare for on-column or flow injection analysis.

a Switch the LC stream to waste.

b Do one of the following:
- Install and condition a short column that will give you a peak width baseline-to-baseline of about 12 to 15 sec.
- Install and flush a 5-meter length of .010"-id PEEK tubing, which will give a 30-sec peak at a typical LC flow rate of 0.3 mL/min.

For examples of short columns and cartridges, see **Optimizer-recommendations.pdf** in the **Reference** folder on your Optimizer software disk.

Some reasons to use PEEK tubing:
- Compound is not eluted from column in a reasonable time
- Peak is too sharp on-column

2. Make sure that you have individual standards of each compound you need to optimize.

a Get the pesticide standard(s) that you prepared in step 2 of "Prepare pesticide standards" on page 14.

b Make sure that the concentrations are about 1 ng/µL (100 pg/µL for the 6490 Triple Quadrupole).

3. Copy MassHunter Optimizer method files to your MassHunter Workstation installation.

- If you have not already done so, copy the methods from the **Reference\Optimizer Methods** and **Reference\Sample\Methods** folders on your Optimizer software disk to the **\MassHunter\Methods** folder on your computer.

4. Update any Optimizer methods that you plan to use.

a Open MassHunter Data Acquisition.

b Open a method.

c Review settings and if necessary, change them to make them compatible with your system.

d Set the injection volume to 2 µL.

e Set the method to use 50:50 buffer:organic and the flow rate you plan to use when you analyze pesticides. Flows between 0.2 and 0.4 mL/min work well if you use the 5-meter length of .010"-id PEEK tubing.

f Save the method.

5. Open MassHunter Optimizer.

- Double-click the **Optimizer** icon on the desktop. If you do not see it, double-click the folder icon for **Agilent MassHunter Workstation** and then double-click the **Optimizer** icon within the folder.

6. Create or open a project.

- Click the **New Project** button or the **Open Project** button on the toolbar.

For details, see the "Using Projects" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide* (Agilent publication G3793-90003, Third Edition, January 2010).

7. Enter settings on the Optimizer Setup tab.

a Click the **Optimizer Setup** tab.

b Enter the settings.

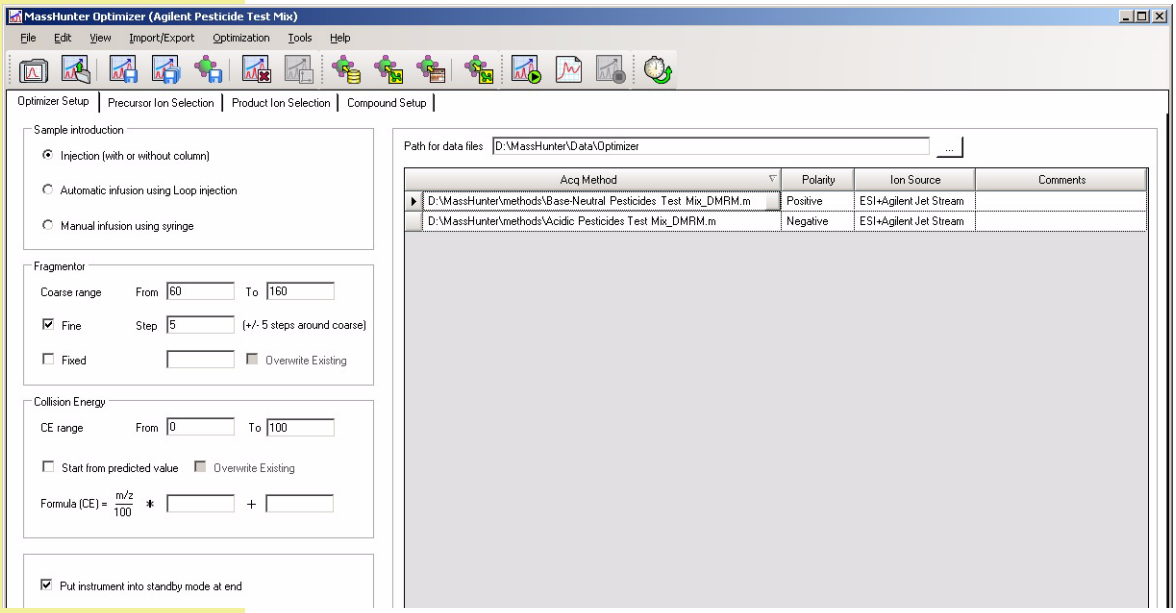


Figure 6 *Optimizer Setup tab. Note that fragmentor settings are not applicable for the 6490 Triple Quadrupole because they are set in Autotune.*

For details on this and the next two steps, see the "Setting Optimization Parameters" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide*.

8. Enter settings on the Precursor Ion Selection tab.

a Click the **Precursor Ion Selection** tab.

b Enter the settings for **Positive ions** and **Negative ions**. Be sure to specify the correct adduct ions. For example:
- If the compound is likely to lose water, for **Positive ions** add **+H** and **-OH**.
- With ammonium formate or ammonium acetate as buffer, for **Positive ions**, add **+H** and **+NH_4**.
- In negative ion mode with acetate as buffer, for **Negative ions** add **+$O_2C_2H_3$** and **-H**.

c Enter the other settings.

The system selects the most abundant precursor ion based on the list of adducts you provide. If you select only one adduct (for example, **+H** for positive ion mode or **-H** for negative ion mode), then the program looks only for the precursor ion that results from that adduct.

9. Enter settings on the Product Ion Selection tab.

a Click the **Product Ion Selection** tab.

b Enter the settings.

10. Enter settings on the Compound Setup tab.

- To establish the list of pesticides you need to optimize, do one of the following:
  - Manually set up a compound list.
  - Import compounds from a database.
  - Import compounds from a MassHunter Data Acquisition method.
  - Import compounds from a Microsoft® Excel file.

For details, see the "Setting Up Compounds and Working with Databases" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide*.

11. Save the project.

- Click the **Save Project** button on the toolbar.

For details, see the "Using Projects" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide*.

12. Load the pesticide standards.

a Load the autosampler or well-plate sampler with standards of individual pesticides.

b Make sure the sample positions match the vial numbers in the MassHunter Optimizer software, **Compound Setup** tab.

13. Start the optimization.

- Click the **Start Optimization** button on the toolbar.

For details on this and the next three steps, see the "Optimization and Results" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide*.

If you need to stop the optimization, click the **Stop Optimization** button.

14. Review the results.

a Click the **Compound Setup** tab.

b Mark the **Show results summary** check box above the table.

c Review the fragmentor and collision energy values for each compound. (Review only the collision energy if you have a 6490 Triple Quadrupole.)

d Review the printed optimization report.

Guideline: Use 1000 counts as the minimum signal for a product ion in MRM mode for 1 ng of pesticide on-column. If the signal is below this value, inspect the product ion spectra to be sure you are monitoring the correct ions.

If you are monitoring the correct ions, but your abundances are too low and the graphs are noisy, try one of the following:

- Increase the injection volume from 2 µL to 5 µL (but be aware that peak shape may suffer).
- Prepare and inject a standard at a higher concentration.

15. Save the compounds and results to a database.

- Click the **Save Project** button on the toolbar.

Once you save the compounds to a database, you can then import them into MRM and Dynamic MRM methods, as described in "Import MRM transitions from a pesticide database" on page 18.

If you wish to save the compounds to the Agilent Pesticide Dynamic MRM Database, first save that database with a new name. Then save the compounds.

16. Rerun optimizations, if necessary.

a If any standards gave poor response, rerun them at higher concentration or larger injection volume.

b Save the compounds to the database you used in step 15.

17. Import the MRM transitions into a MassHunter Data Acquisition method.

a Open MassHunter Data Acquisition.

b Load one of the methods you created in "Set up LC/MS parameters, except for MRM settings" on page 17 and possibly modified in "Import MRM transitions from a pesticide database" on page 18.

c Follow the directions in "To import optimization results to Acquisition for MRM time segments" in the "Optimization and Results" section of the *Agilent G3793AA MassHunter Optimizer Quick Start Guide*. Because you do not yet know the retention time of the pesticides you just optimized, keep a single time segment.

Make sure that each method with a single time segment has no more than about 100 MRM transitions (or 50 pesticides with two transitions each). See the note on page 18.

18. Set the dwell time.

- For 50 compounds with 2 transitions each, set a 2 msec dwell time. For peaks that are 5 sec wide, this gives about 10 points across the peak and a cycle time of 500 msec.
- If you have fewer compounds or wider peaks, set a larger dwell time (for example, 5 msec) because that will give better ion statistics.

19. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

20. Prepare additional methods, if necessary.

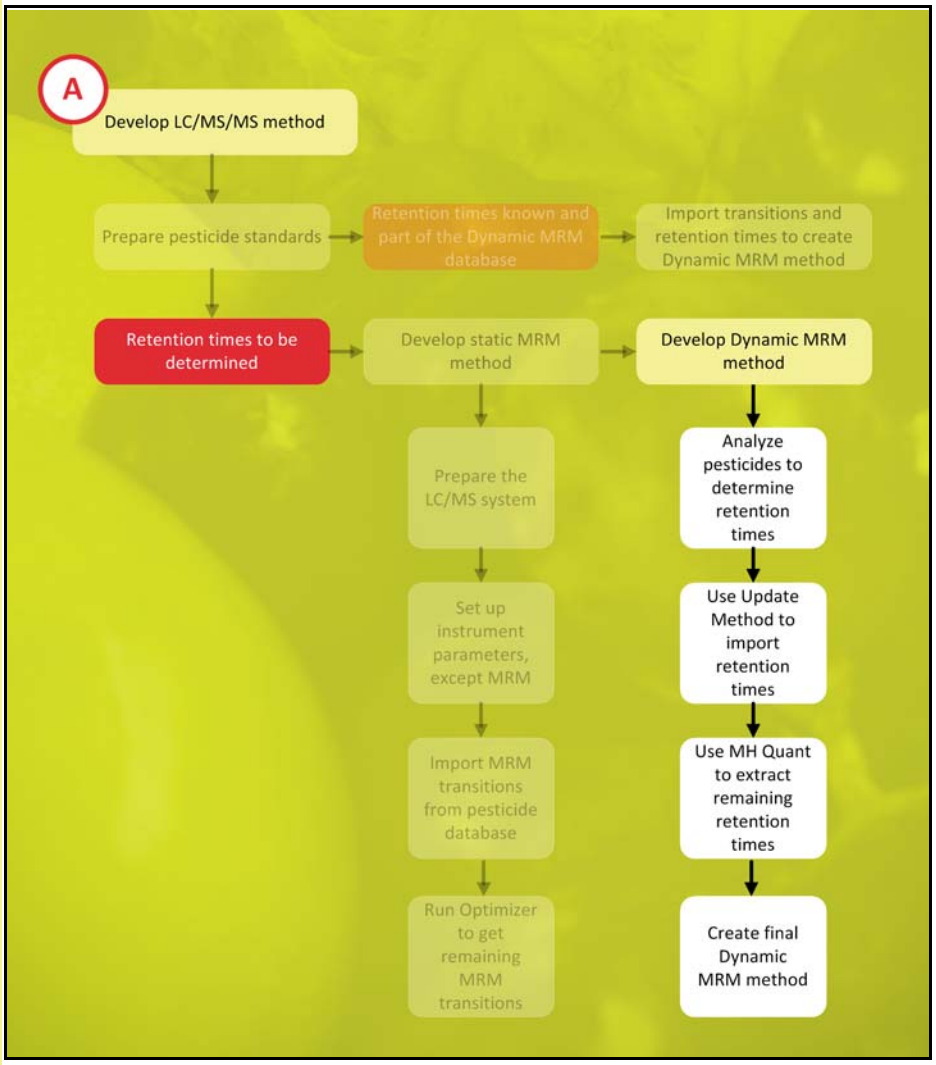
- If necessary, repeat step 17 through step 19 with additional methods, until you have a method for each pesticide of interest.

NOTE

It is recommended to add stable isotope internal standards to your methods. However, *do not mark the check box that identifies them as internal standards.* Currently, the **Update Method** command does not recognize them if you do. You can mark the check box that identifies them as internal standards in the final step of creating and saving your Dynamic MRM method.

Develop the final Dynamic MRM method

This exercise shows you the final steps of method development—the conversion of your static MRM method to a Dynamic MRM method. With Dynamic MRM, the program builds MRM transition segments "on-the-fly" during the LC separation, based on a retention time window for each analyte. That allows the triple quad to monitor each transition for only the period of time that is necessary, so you get better quantitation and sensitivity, even when you analyze hundreds of compounds.



Compound Name	Precursor Ion	Product Ion	Retention Time
Cinosulfuron	414.1	183	5.579
Cinosulfuron (Q)	414.1	157	5.579
Chlorotoluron	213.1	72	5.642
Chlorotoluron (Q)	213.1	140	5.642
Atrazine	216.1	174	5.682
Atrazine (Q)	216.1	132	5.682
Carbaryl	202.1	145	5.736
Carbaryl (Q)	202.1	117	5.734
Carboxin	236.1	143	5.836
Carboxin (Q)	236.1	87	5.836
Chlorsulfuron	358.0	167	5.896
Chlorsulfuron (Q)	358.0	141	5.896
Ethiofencarb	226.1	107	5.937
Ethiofencarb (Q)	226.1	164	5.936
Dodemorph	282.3	116	6.073
Dodemorph (Q)	282.3	98	6.074
Diuron	233.0	72	6.101
Diuron (Q)	233.0	160	6.101
Cyprodinil	226.1	108	6.245
Cyprodinil (Q)	226.1	93	6.246
Difenoxurone	287.1	123	6.509
Difenoxurone (Q)	287.1	72	6.509

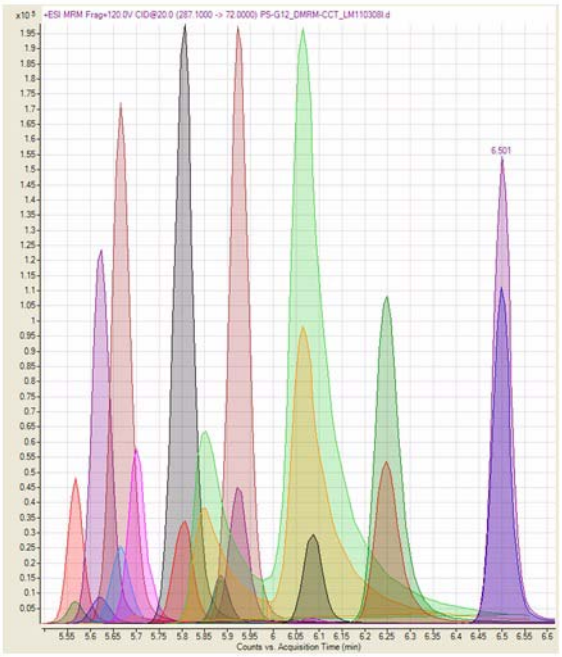


Figure 7 *Dynamic MRM maximizes the number of pesticides you can analyze in a single run. The extracted ion chromatogram of 11 pesticides and 11 qualifier ions shows significant coelution in this one-minute window, but well-chosen MRM transitions allow for accurate quantitation of all sample components.*

NOTE

If you have the Agilent Pesticide Dynamic MRM Database, you may skip the tasks in this exercise if:

- All the pesticides that you need to analyze have retention times in a single method in the database, *and*
- Your analysis will use exactly the same LC method as was used to generate those retention times, *and*
- You have analyzed standards to confirm the retention times.

Analyze pesticides to determine retention times

In this task, you analyze all the pesticides with your static MRM method(s), so that you can learn their retention times. You need the retention times to create a Dynamic MRM method.

1. Condition the analytical column.

If you have not already done so, condition the analytical column that you will use for pesticide analysis.

a Install the column.

b Switch the LC stream to waste (or disconnect it from the MS).

c Condition the column.

d Switch the LC stream to MS.

e Make sure that you have a stable MS baseline at your initial LC conditions.

2. Run the pesticide standard(s) at about 100 pg/µL to get retention times.

a Get the pesticide standard(s) that you prepared in step 3 of "Prepare pesticide standards" on page 14.

b Run them with the static MRM method(s) that you prepared in "Develop the preliminary static MRM method" on page 16.

For the steps in this task, see the first step under "To create a Dynamic MRM method" in the "Getting Started" section of the *Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide* (Agilent publication 5990-5742EN, Second Edition, May 2010).

Import retention times to create the Dynamic MRM method (Update Method)

In this task, you use the **Update Method** command (available in Agilent MassHunter Data Acquisition for QQQ version B.03.01 or later) to import the retention times and retention time windows from the data files you created in the last task, into a Dynamic MRM method that you create. If the following situation applies to you, skip this task and go to the next one:

- You have isomeric compounds that have the same transitions. (**Update Method** extracts only the most abundant peak).

1. In MassHunter Data Acquisition, open your most up-to-date method for this analysis.

- Open the latest method that you created in "Develop the preliminary static MRM method" on page 16.

2. Save the method with a new name.

3. Open the Dynamic MRM Update Options dialog box.

a Click the **MS QQQ > Acquisition** tab.

b Right-click the **Scan segments** table or the gray area to the right or below it.

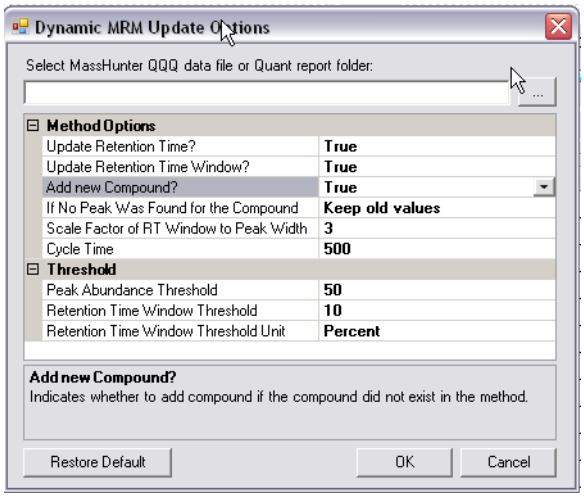
c Click **Update Method**.

For details on this and the rest of the steps in this task, see "To create a Dynamic MRM method" in the "Getting Started" section of the *Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide* (Agilent publication 5990-5742EN, Second Edition, May 2010).

4. Update the method with each data file.

a Select the data file you wish to use to update the method.

b Change the options in the Dynamic MRM Update Options dialog box as needed:
- Set the first three parameters under **Method Options** to **True**.
- Set **If No Peak Was Found for the Compound** to **Keep old values**.
- Set **Scale Factor of RT Window to Peak Width** to at least **2**.



c Click **OK**.

d Repeat these steps for each data file.

If any data files give errors, skip them for now. You will process them in the next task.

Note that there will be no errors if *all* transitions collected in the data files are *detected*. That is why you need to use a standard that gives a good response for all pesticide transitions.

When you choose a data file and update the method, the Scan Type in the method is automatically converted to Dynamic MRM.

5. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

6. Open the Dynamic MRM Viewer.

a Click the **MS QQQ > Acquisition** tab.

b Right-click the **Scan segments** table or the gray area to the right or below it.

c Click **View Method**.

7. Adjust the cycle time for appropriate integration and dwell time.

Adjust the cycle time so that the following criteria are met:

- MS-MS integrator (if used) has 64 data points. (The new Agile integrator in MassHunter Quantitative Analysis software version B.04.00 requires only 15 data points.)
- Minimum dwell time is 2 msec
- Peaks have sufficient data points for good integration

Note that you see the effect of changing the cycle time in the viewer, but that does not change the cycle time in the method. To change the cycle time or the retention time window in the method, you must exit the viewer and make the changes to the acquisition method, as described in step 8.

To adjust the cycle time in the viewer:

a If any compounds appear in red, decrease the cycle time or increase the retention time window so that the integrator has the correct number of data points.

b If any dwell times are less than 2 msec, increase the cycle time.

c Make sure that the cycle time is set such that at least 10 data points are measured across each chromatographic peak. Decrease the cycle time if necessary.

d Note the adjusted values. You will need them for step 8.

With Dynamic MRM, you set the cycle time, and the program automatically calculates the dwell time.

8. Type the new cycle time and retention time windows into the method.

a Click the **MS QQQ > Acquisition** tab.

b Under Dynamic MRM Parameters, type a **Cycle Time**.

c Under Scan Segments, change the **Delta Ret Time** for any compounds for which you need to change the retention time window.

d (Optional) Return to the viewer to verify that the changes are acceptable.

Changes to **Delta Ret Time** affect the average dwell time of peaks that overlap.

9. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

10. (Optional) Save compounds and settings to a database.

If you have not already saved your compounds and settings to a database, use MassHunter Optimizer to do that now:

a Open MassHunter Optimizer.

b Click the **Import from Acquisition Methods** button on the toolbar.

c Select the MassHunter Data Acquisition methods that contain the compounds of interest, then click the **Open** button.

d Click the **Save Compounds** button on the toolbar.

Once you save the compounds to a database, you can then import them into MRM and Dynamic MRM methods, as described in "Import MRM transitions from a pesticide database" on page 18.

If you wish to save the compounds to the Agilent Pesticide Dynamic MRM Database, first save that database with a new name. Then you may save the compounds.

Use MassHunter Quant to extract remaining retention times

Do this task in these situations:

- You saw an error when you used the **Update Method** function to create a Dynamic MRM method directly from a data file. Do this task only for the data file(s) that gave error(s).
- You need to analyze isomeric pesticides that share transitions, which means you must review and assign the correct retention times.

If neither of these situations applies to you, you are done with method development, so you may skip to the next chapter.

In this task, you use MassHunter Quantitative Analysis to extract the retention times of pesticides from data file(s). You create a batch file and a report for each data file you process. If you need to process multiple data files, you create a separate batch file and report for each one. Then in the next task, you use the reports to create or update a single Dynamic MRM method for all of your pesticides.

Figure 8 *MassHunter Quantitative Analysis extracts the pesticide retention times to report(s), which you then use to add retention times to the Dynamic MRM method.*

1. Open the MassHunter Quantitative Analysis program.

2. Create a new batch.

   - Create a new batch in the folder that contains the MRM data that you collected in "Analyze pesticides to determine retention times" on page 29.

For details of all steps in this task, see "To update a Dynamic MRM method to include data files with errors" in the "Getting Started" section of the *Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide* (Agilent publication 5990-5742EN, Second Edition, May 2010).

3. Load the single-time-segment MRM data from a standard that you still need to process.

4. Create a new quantitation method from the acquired MRM data.

If you have isomers in the sample, make sure the retention times are correct.

5. Click **Validate** and correct the errors.

- If you see the error "missing qualifier ratio," set the qualifier ratio to any number other than 0.
- If you see the error "retention time cannot be zero," delete the compound.

Two errors cause **Update Method** to fail:
- "Missing qualifier ratio" means the qualifier ion was not detected.
- "Retention time cannot be zero" means no transitions were detected.

6. Apply the method to the batch.

7. Save the batch and analyze it.

8. Clear all errors that you see in the "Quantitation message summary."

9. Save the batch again, now that the results are processed.

10. Generate a report for the data file. Use the template **DMRM_Method_Gen.xltx**.

This report template is available in the Agilent Triple Quadrupole LC/MS Pesticide Application Kit (G1733AA).

You must copy it to **\MassHunter\ReportTemplates\Quant**.

11. Repeat the steps in this task for every mix of standards that you still need to include in the Dynamic MRM method.

Create the final Dynamic MRM method

In this task, you import the MRM settings and retention times from the reports you created in the last task, into your Dynamic MRM method. If you did *not* need to do "Use MassHunter Quant to extract remaining retention times" on page 33, then you do not need to do this task, either. You may skip to the next chapter.

1. In MassHunter Data Acquisition, open your latest method for this analysis.

   - Do one of the following:
     - If you created a method in "Import retention times to create the Dynamic MRM method (Update Method)" on page 30, open that one.
     - If not, open the latest method that you created in "Develop the preliminary static MRM method" on page 16.

2. (Optional) Save the method with a new name.

3. Open the Dynamic MRM Update Options dialog box.

   a Click the **MS QQQ > Acquisition** tab.

   b Right-click the **Scan segments** table or the gray area to the right or below it.

   c Click **Update Method**.

   For this and the rest of the steps in this task, see "Create final Dynamic MRM method" under "To update a Dynamic MRM method to include data files with errors" in the "Getting Started" section of the *Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide* (Agilent publication 5990-5742EN, Second Edition, May 2010).

4. Update the method using the Quant report folders you generated in the previous task.

   a In the Dynamic MRM Update Options dialog box, click the **Browse** button and select the **Quant** folder (inside the data folder) for the report.

   b Click **OK**.

   c Repeat these steps as necessary for any additional reports that you generated in "Use MassHunter Quant to extract remaining retention times" on page 33.

5. Save the method.

   a Click **File > Save As > Method**.

   b Give the method a name and click **OK**.

6. Open the Dynamic MRM Viewer.

a Click the **MS QQQ > Acquisition** tab.

b Right-click the **Scan segments** table or the gray area to the right or below it.

c Click **View Method**.

7. Adjust the cycle time for appropriate integration and dwell time.

Adjust the cycle time so that the following criteria are met:

- MS-MS integrator has 64 data points, or Agile integrator has 15 data points
- Minimum dwell time is 2 msec
- Peaks have sufficient data points for good integration

Note that you see the effect of changing the cycle time in the viewer, but that does not change the cycle time in the method. To change the cycle time or the retention time window in the method, you must exit the viewer and make the changes to the acquisition method, as described in step 8.

To adjust the cycle time in the viewer:

a If any compounds appear in red, decrease the cycle time or increase the retention time window so that the integrator has the correct number of data points.

b If any dwell times are less than 2 msec, increase the cycle time.

c Make sure that the cycle time is set such that at least 10 data points are measured across each chromatographic peak. Decrease the cycle time if necessary.

d Note the adjusted values. You will need them for step 8.

With Dynamic MRM, you set the cycle time, and the program automatically calculates the dwell time.

8. Type the new cycle time and retention time windows into the method.

a Click the **MS QQQ > Acquisition** tab.

b Under Dynamic MRM Parameters, type a **Cycle Time**.

c Under Scan Segments, change the **Delta Ret Time** for any compounds for which you need to change the retention time window.

d (Optional) Return to the viewer to verify that the changes are acceptable.

Changes to **Delta Ret Time** affect the average dwell time of peaks that overlap.

9. Save the method.

a Click **File > Save As > Method**.

b Give the method a name and click **OK**.

10. (Optional) Save your compounds and settings to a database.

If you have not already saved your compounds and settings to a database, use MassHunter Optimizer to do that now.

a Open MassHunter Optimizer.

b Click the **Import from Acquisition Methods** button on the toolbar.

c Select the MassHunter Data Acquisition methods that contain the compounds of interest, then click the **Open** button.

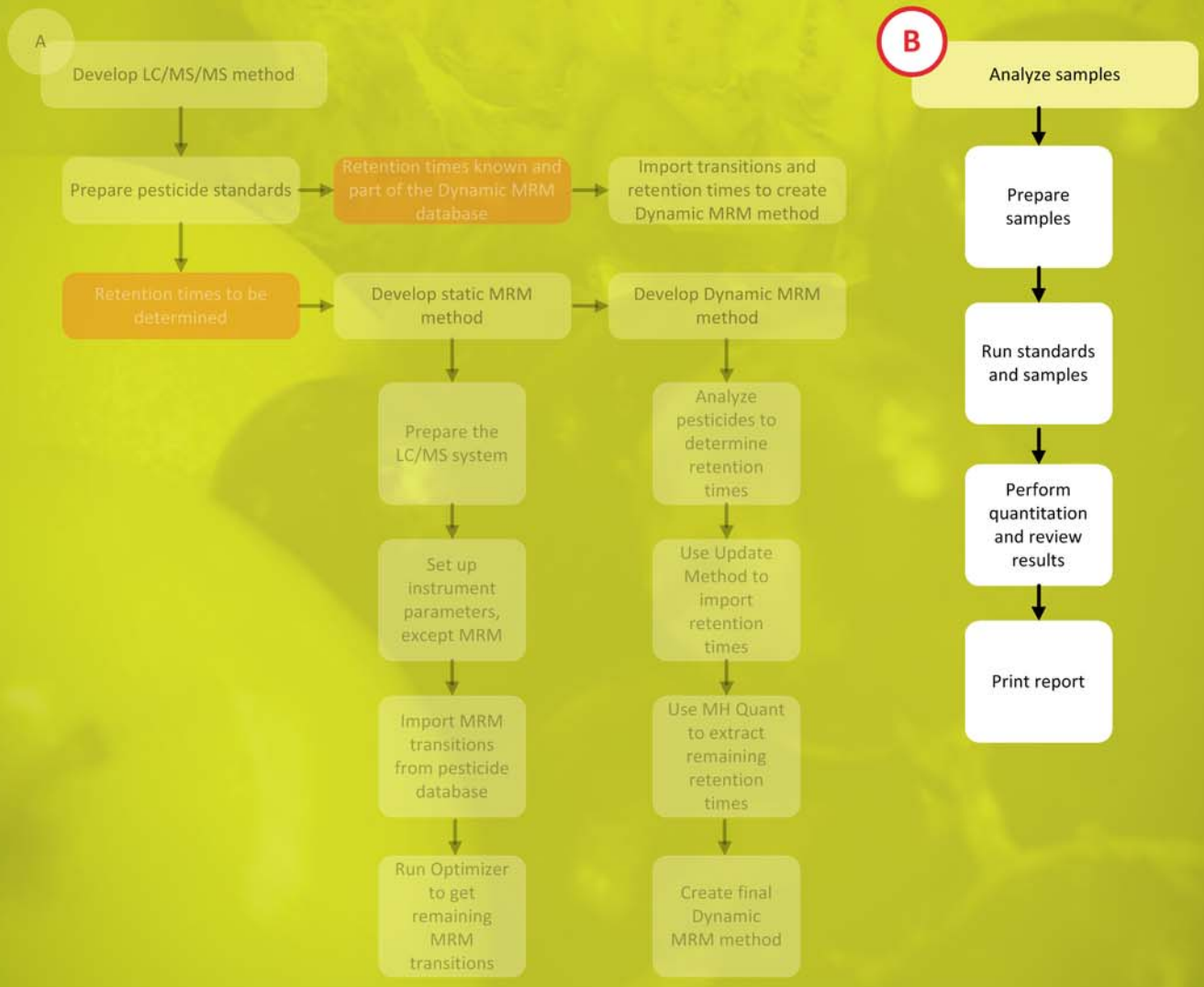
d Click the **Save Compounds** button on the toolbar.

Once you save the compounds to a database, you can then import them into MRM and Dynamic MRM methods, as described in "Import MRM transitions from a pesticide database" on page 18.

If you wish to save the compounds to the Agilent Pesticide Dynamic MRM Database, first save that database with a new name. Then you may save the compounds.

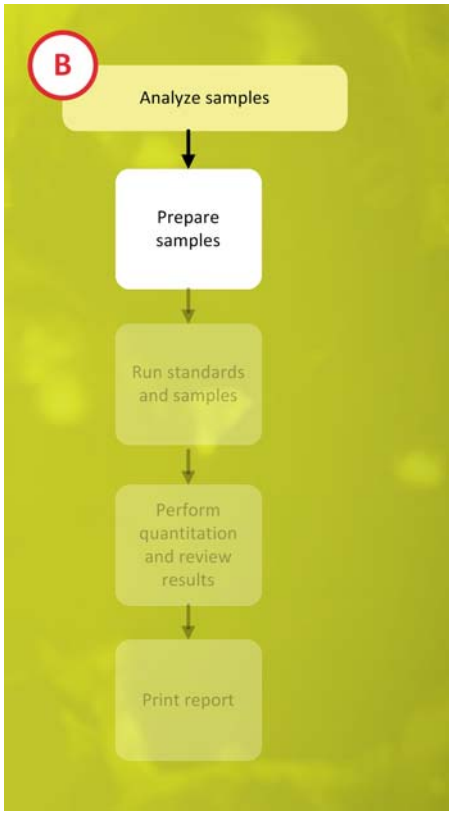
Analyzing Samples

This chapter describes how to prepare samples and analyze them for pesticides, using the Dynamic MRM method you developed in the last chapter. The sample preparation method is specific to spinach, but the rest of the chapter about the LC/MS/MS analysis applies to any sample matrix.



Prepare samples

In this exercise, you prepare a spinach sample using Agilent SampliQ QuEChERS extraction and sample cleanup kits. If you analyze other foods, your sample preparation may be similar. However, it is important to select the proper kit for each type of food sample. See "Agilent SampliQ QuEChERS Kits" (Agilent publication number 5990-3562EN, February, 2010) for guidance. This publication also gives examples of sample preparation methods.



You can get more information about SampliQ QuEChERS on the Agilent Web site, including a standard operating procedure and a demo video to help you get started. An application compendium (5990-4977EN) describes food safety applications, with an emphasis on pesticide analysis.

If you analyze other matrices, such as soil or water, you need a different sample preparation method than the one given here.

Be sure to prepare any necessary QC samples and blanks along with your other samples. For examples, see the list to the right of step 1 on page 43.

Figure 9 *QuEChERS stands for **qu**ick, **e**asy, **ch**eap, **e**ffective, **r**ugged and **s**afe. These adjectives describe this sample preparation technique for multi-residue pesticide analysis in fruits and vegetables.*

1. Weigh samples.

- Weigh 15 g (±0.1 g) of homogenized spinach sample.

2. Spike samples.

- Spike samples as necessary with:
  - Standards to determine recoveries
  - Standards of targeted pesticides at the desired detection limit (to ensure that they are detected)
  - Internal standard solution

3. Vortex 30 sec.

4. Add 15 mL of 1% acetic acid in acetonitrile.

5. Add extraction packet.

- To each tube, add an Agilent SampliQ QuEChERS AOAC buffered extraction salt packet (from Agilent p/n 5982-5755).

Each packet contains 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous sodium acetate.

6. Cap and hand-shake vigorously for 1 min.

Some Agilent SampliQ QuEChERS kits contain ceramic homogenizers, which make the shaking step easier and more consistent.

7. Centrifuge at 4000 rpm for 5 min.

8. Transfer sample extract to cleanup kit.

- Transfer upper layer to dispersive SPE kit for highly pigmented fruits and vegetables:
  - 1 mL to p/n 5982-5321 *or*
  - 8 mL to p/n 5982-5356)

9. Vortex 1 min.

10. Centrifuge.

- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15-mL tubes at 4000 rpm for 5 min.

11. Transfer upper layer.

- Transfer 200 µL of the upper layer to an autosampler vial.

12. Add water.

- Add 800 µL of water or appropriate standard spiking solution.

13. Cap and vortex.

- Cap the sample and vortex 1 min, to prepare for LC/MS analysis.

Acquire data for standards and samples

In this exercise, you learn to do triple quadrupole LC/MS analyses of samples and standards.

1. Make sure you have all the standards and samples you need.

- Make sure you have the following prepared in the appropriate containers for your LC autosampler:
  - Calibration standards for all pesticides
  - Sample extracts
  - QC samples to verify method recoveries
  - Solvent blanks
  - Double blanks (no internal standard)
  - Zero control samples (samples that are free of pesticides), to look for possible contamination in the sample preparation process

Analyze appropriate standards and QC samples to meet requirements of regulatory agencies and standard operating procedures for your lab. Make sure that you validate your method.

2. Put the standards and samples into the LC autosampler.

3. Start the MassHunter Data Acquisition program.

- Double-click the Data Acquisition icon on your desktop.

4. Set up a worklist.

a If necessary, click the **Worklist** button on the toolbar to display the Worklist pane. 

b To set up the worklist run, click **Worklist > Worklist Run Parameters**.

c Set the **Data File Path**, verify that the other parameters are set properly, and click **OK**.

d Click **Worklist > Add Multiple Samples**.

e Type or select the necessary information in the Sample Information tab. Be sure to select the method you developed in Chapter 2.

f Click the **Sample Position** tab.

g Drag to select the sample positions, then click **OK** (Figure 10).

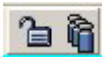
h Verify that the worklist has been populated with samples (Figure 10).

i Edit the worklist table as necessary.

If you need practice to run a worklist, see the following:

- *Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide* (Agilent publication G3335-90077, Eighth Edition, January 2010). For example, see the end of "Step 4. Set up and run an acquisition method."
- *Agilent MassHunter Workstation Software – Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide* (Agilent publication G3335-90059, Third Edition, May 2009). For example, see the end of Exercise 1, Task 1.

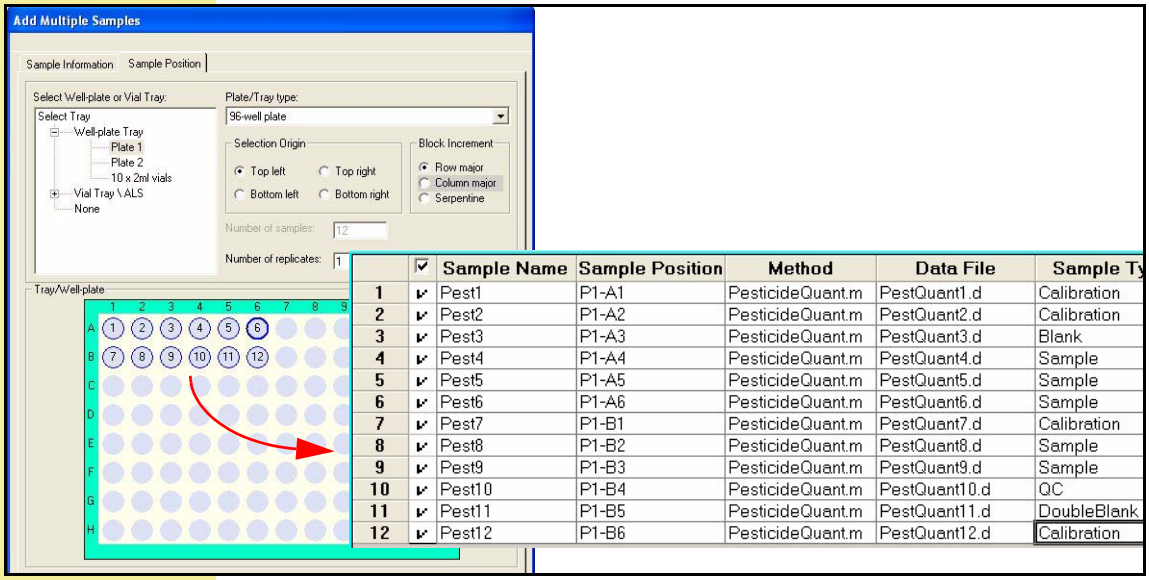
5. Run the worklist.

a To start the run, click the **Run Worklist** button on the toolbar. 

b Wait for the worklist to finish.

c Verify that your analysis was successful. See Figure 11 as an example.

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running.



		Sample Name	Sample Position	Method	Data File	Sample Ty
1	✓	Pest1	P1-A1	PesticideQuant.m	PestQuant1.d	Calibration
2	✓	Pest2	P1-A2	PesticideQuant.m	PestQuant2.d	Calibration
3	✓	Pest3	P1-A3	PesticideQuant.m	PestQuant3.d	Blank
4	✓	Pest4	P1-A4	PesticideQuant.m	PestQuant4.d	Sample
5	✓	Pest5	P1-A5	PesticideQuant.m	PestQuant5.d	Sample
6	✓	Pest6	P1-A6	PesticideQuant.m	PestQuant6.d	Sample
7	✓	Pest7	P1-B1	PesticideQuant.m	PestQuant7.d	Calibration
8	✓	Pest8	P1-B2	PesticideQuant.m	PestQuant8.d	Sample
9	✓	Pest9	P1-B3	PesticideQuant.m	PestQuant9.d	Sample
10	✓	Pest10	P1-B4	PesticideQuant.m	PestQuant10.d	QC
11	✓	Pest11	P1-B5	PesticideQuant.m	PestQuant11.d	DoubleBlank
12	✓	Pest12	P1-B6	PesticideQuant.m	PestQuant12.d	Calibration

Figure 10 *The worklist is filled in automatically from your sample selection. You can then edit it.*

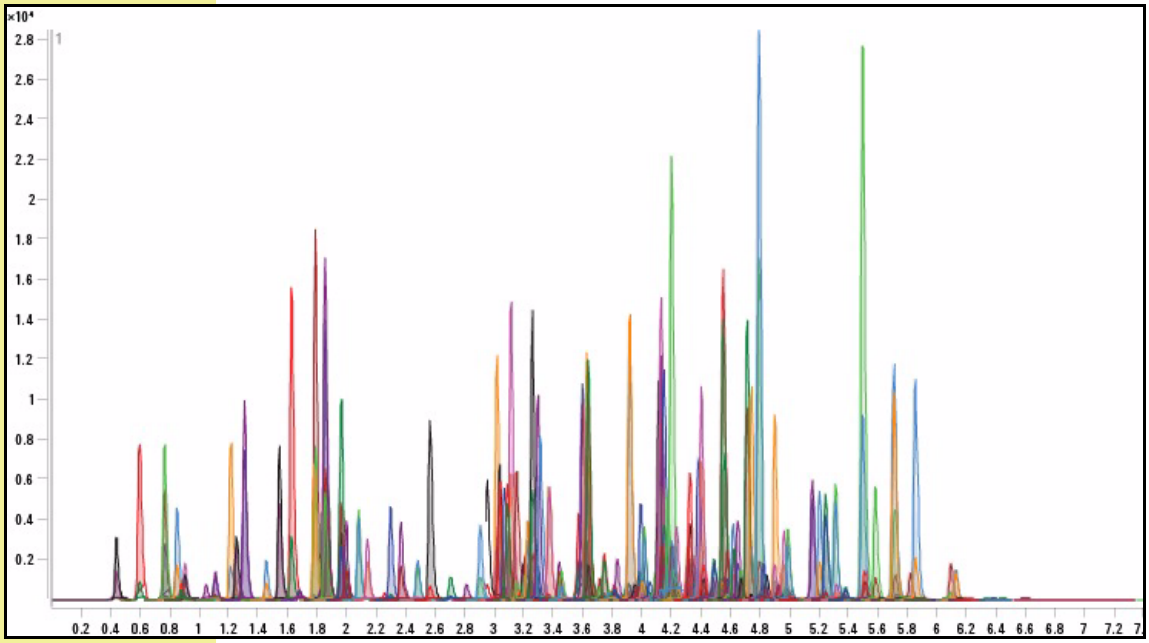
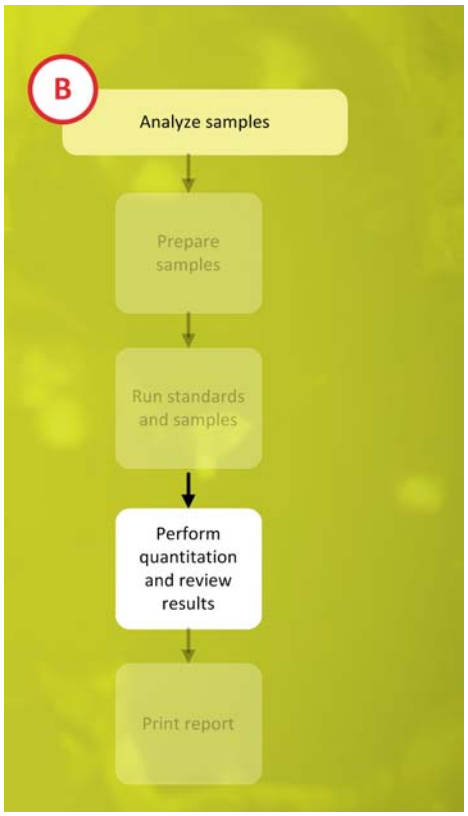


Figure 11 *This example shows the Dynamic MRM analysis of 224 pesticides in less than seven minutes with the Agilent 1290 Infinity LC System, the Agilent 6460 Triple Quadrupole LC/MS System, and an Agilent ZORBAX Rapid Resolution High Definition column.*

Perform quantitation and review results

In this exercise, you use MassHunter Quantitative Analysis to set up calibration curves and determine the concentrations of pesticides in your samples.



1. Quantitate the Dynamic MRM data files.

- Follow directions in the *Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide*, except use the data files you generated in "Acquire data for standards and samples" on page 43.

See "Set Up and Quantitate a Batch of Acquired MRM Data Files" in the *Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide* (Agilent publication G3335-90061, Fourth Edition, April 2010).

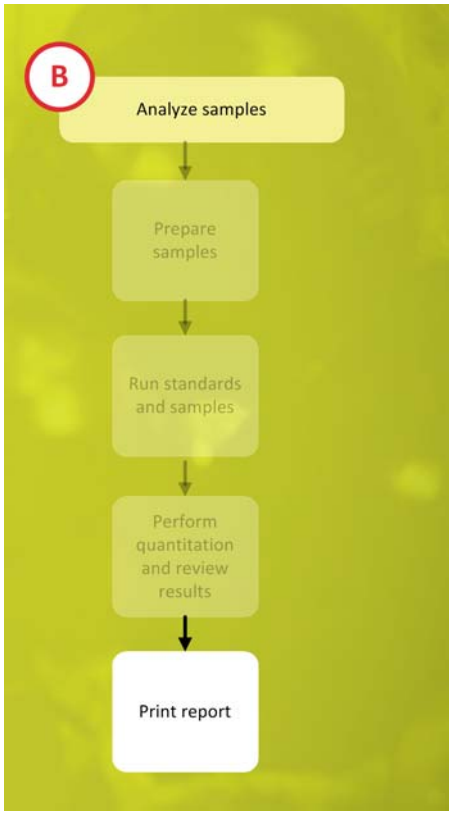
2. Review the quantitation results.

- Follow directions in the *Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide*, except use the data files you generated in "Acquire data for standards and samples" on page 43.

See "Review Quantitation Results" and "Use Three Tools to Evaluate Results" in the *Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide*.

Print a report

In this exercise, you print a report of your results.



1. Set up to print a quantitative analysis report.

- Follow directions in the *Agilent MassHunter Workstation Software Reporting Familiarization Guide*, except use the results you generated in "Perform quantitation and review results" on page 46.
- As you do the exercises, choose the report template that gives the results format you need. Use the names of the templates as a guide. You may need to experiment until you get the correct template.

See the following sections in the *Agilent MassHunter Workstation Software Reporting Familiarization Guide* (Agilent publication G3335-90062, Second Edition, March 2010). All are in the "Creating Reports" chapter:

- Task 5. Open a batch in the Quantitative Analysis program
- Task 6. Generate quantitation reports using the standard dialog box
- Task 7. Generate quantitation reports using the advanced Report dialog boxes

Note that Tasks 6 and 7 provide alternative ways to generate a report, so you do not need to do it both ways.

2. Print a hard copy of the report, if necessary.

If you did not initially print a hard copy of the report, and you wish to do so now:

a Navigate to the folder that contains the report, for example **\MassHunter\Data\Quant\ <BatchName>\QuantReports\ <ReportFolder>**.

b Open the Excel or PDF file.

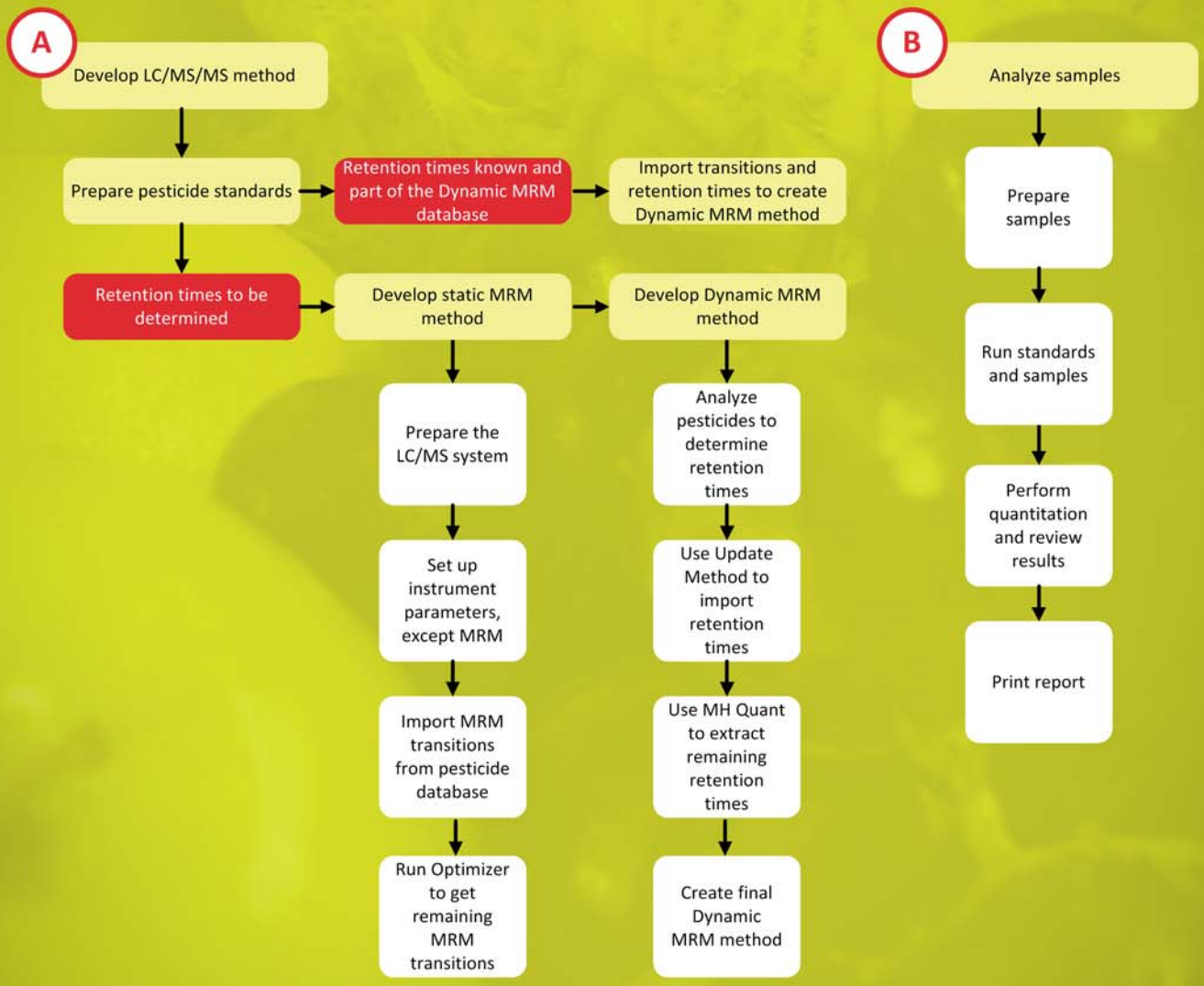
c Print the report.

3. Create a custom report, if desired.

- See the following for instructions:
  - *Agilent MassHunter Workstation Software Reporting Familiarization Guide* (Agilent publication G3335-90062, Second Edition, March 2010)
  - *Agilent MassHunter Reporting Training DVD* (Agilent publication G6845-60005, February, 2009). You received this DVD with your MassHunter Workstation software.

Reference Information

This chapter lists the lab equipment, lab supplies, and chemicals you need to do these analyses, as well as manuals, application and technical notes, and other reference materials that will help you to be successful with your analyses.



Required supplies and chemicals

The lab equipment, solvents, and chemicals that you need will depend on the sample matrix and pesticides to be analyzed. The following sections give general guidelines.

Required equipment and lab supplies

Description	Vendor and part number
Analytical balance	
Spatula, to weigh standards	
Weigh boats or paper	
Protective gloves	
Safety glasses	
Lab coat or other protective clothing	
Fume hood	
Volumetric flasks, for standard preparation	
Pipetman micropipettors, (P-10, P-20, P-200, P-1000) or equivalent	•Gilson
Vials to store pesticide standards, for example: •2-mL amber vials, 100/pk •Blue screw caps (for 2-mL vials), 100/pk, PTFE/silicone/PTFE septa	 •Agilent p/n 5182-0716 •Agilent p/n 5182-0723
Refrigerator for flammables storage (to store pesticide standards)	
Containers for LC autosampler	
Analytical LC column, for example: •Agilent ZORBAX Eclipse Plus C18 Rapid Resolution High Throughput (RRHT) column, 2.1 × 100 mm, 1.8 µm •Agilent ZORBAX Eclipse Plus C18 Rapid Resolution High Definition (RRHD) column, 2.1 × 100 mm, 1.8 µm •Agilent ZORBAX Eclipse Plus C18 RRHD column, 2.1 × 150 mm, 1.8 µm **Note:** If you want to use the retention times from the Agilent Pesticide Dynamic MRM Database, use the column from the method listed in the database	 •Agilent p/n 959764-902 •Agilent p/n 959758-902 •Agilent p/n 959759-902
Container to capture LC waste (for example, old solvent bottle in secondary containment)	
SampliQ QuEChERS Buffered Extraction Kit, AOAC Method (used for analysis of pesticides in spinach)	•Agilent p/n 5982-5755
Centrifuge (used for analysis of pesticides in spinach)	
Vortex mixer	
SampliQ QuEChERS Dispersive SPE Cleanup Kit, EN Method (used for analysis of pesticides in spinach, not for use with planar pesticides)	•Agilent p/n 5982-5321 *or* 5982-5356
Nitrogen gas, for MS	

Required chemicals – solvents, reagents, and standards

Description	Vendor and part number
Pesticide standards	•For example, Agilent Pesticide Test Mix, p/n 5190-0469
Solvents to prepare pesticide standards, pesticide or LC/MS grade recommended	
LC solvents (for example, acetonitrile and/or methanol), pesticide or LC/MS grade recommended	
Milli-Q water or equivalent	
Glacial acetic acid, 99.9% (highest purity)	
Formic acid (highest purity)	
Ammonium acetate (highest purity)	
Ammonium formate (highest purity), for example: •5 M ammonium formate	 •Agilent p/n G1946-85021

Optional equipment and chemicals

Description	Vendor and part number
5-meter length of .010"(.25 mm) id PEEK tubing, used when finding optimum settings with MassHunter Optimizer	•Agilent p/n 5042-6463
Short LC column or cartridge, used when finding optimum settings with MassHunter Optimizer. Examples: •Agilent ZORBAX SB-C8 guard cartridge, 2.1 × 12.5 mm, 5 µm particles •Agilent ZORBAX SB-C18 guard cartridge, 2.1 × 12.5 mm, 3.5 µm particles	 •Agilent p/n 821125-915 (4/pk) •Agilent p/n 821125-936 (4/pk)
Guard hardware kit	•Agilent p/n 820888-901
Ultrasonic bath	

References

The references in this list give valuable information that will help you set up multi-residue analyses of pesticides with the Agilent 6400 Series Triple Quadrupole LC/MS System. When the documents are available in the online Agilent Literature Library, links are provided.

Manuals

Agilent 6400 Series Triple Quad LC/MS System Concepts Guide (Agilent publication G3335-90069, Sixth Edition, May 2009 or G3335-90091, November 2010)

Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide (Agilent publication G3335-90077, Eighth Edition, January 2010 or G3335-90090, November 2010)

Agilent 6400 Series Triple Quad LC/MS Maintenance Guide (Agilent publication G2571-90035, Fourth Edition, September 2008 or G2571-90140, October 2010)

Agilent MassHunter Workstation Software – Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide (Agilent publication G3335-90059, Third Edition, May 2009 or G3335-90092, November 2010)

Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide (Agilent publication 5990-5742EN, Second Edition, May 2010)

Agilent G3793AA MassHunter Optimizer Quick Start Guide (Agilent publication G3793-90003, Third Edition, January 2010 or G3793-90005, October 2010)

Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide (Agilent publication G3335-90061, Fourth Edition, April 2010)

Agilent MassHunter Workstation Software Reporting Familiarization Guide (Agilent publication G3335-90062, Second Edition, March 2010)

Note: All MassHunter software includes online Help, in addition to manuals. See the online Help for details about the software.

Application and technical notes

"Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS – Fast and Effective Method Development Using an Application Kit and a Pesticides Compound Parameter Database" (Agilent application note 5990-4253EN, October 2009)

"New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses" (Agilent application note 5990-3595EN, June 2009)

"Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems" (Agilent application note 5990-4255EN, April 2010)

"MassHunter Optimizer Software for Automated MRM Method Development Using the Agilent 6400 Series Triple Quadrupole Mass Spectrometers" (Agilent application note 5990-5011EN, February, 2010)

"Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection" (Agilent application note 5990-4248EN, August 2009)

Other references

"How It Works Video – 6400 Series Triple Quadrupole LC/MS Systems," (available on the Agilent Web site)

MassHunter Pesticide Dynamic MRM Database Kit Support Disk – available with the Agilent Pesticide Dynamic MRM Database Kit (G1733AA) – contains the methods referenced in the database

"Innovative Approaches for today's food analysis challenges – Agilent SampliQ QuEChERS Food Safety Applications Notebook," Volume 2, (Agilent publication 5990-4977EN, December, 2009)

"Agilent SampliQ QuEChERS Kits" (Agilent publication number 5990-3562EN, February, 2010)

QuEChERS information is available on the Agilent Web site, including a standard operating procedure and a demo video to help you get started.

Agilent MassHunter Reporting Training DVD (Agilent publication G6845-60005, February, 2009). You received this DVD with your MassHunter Workstation software.

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