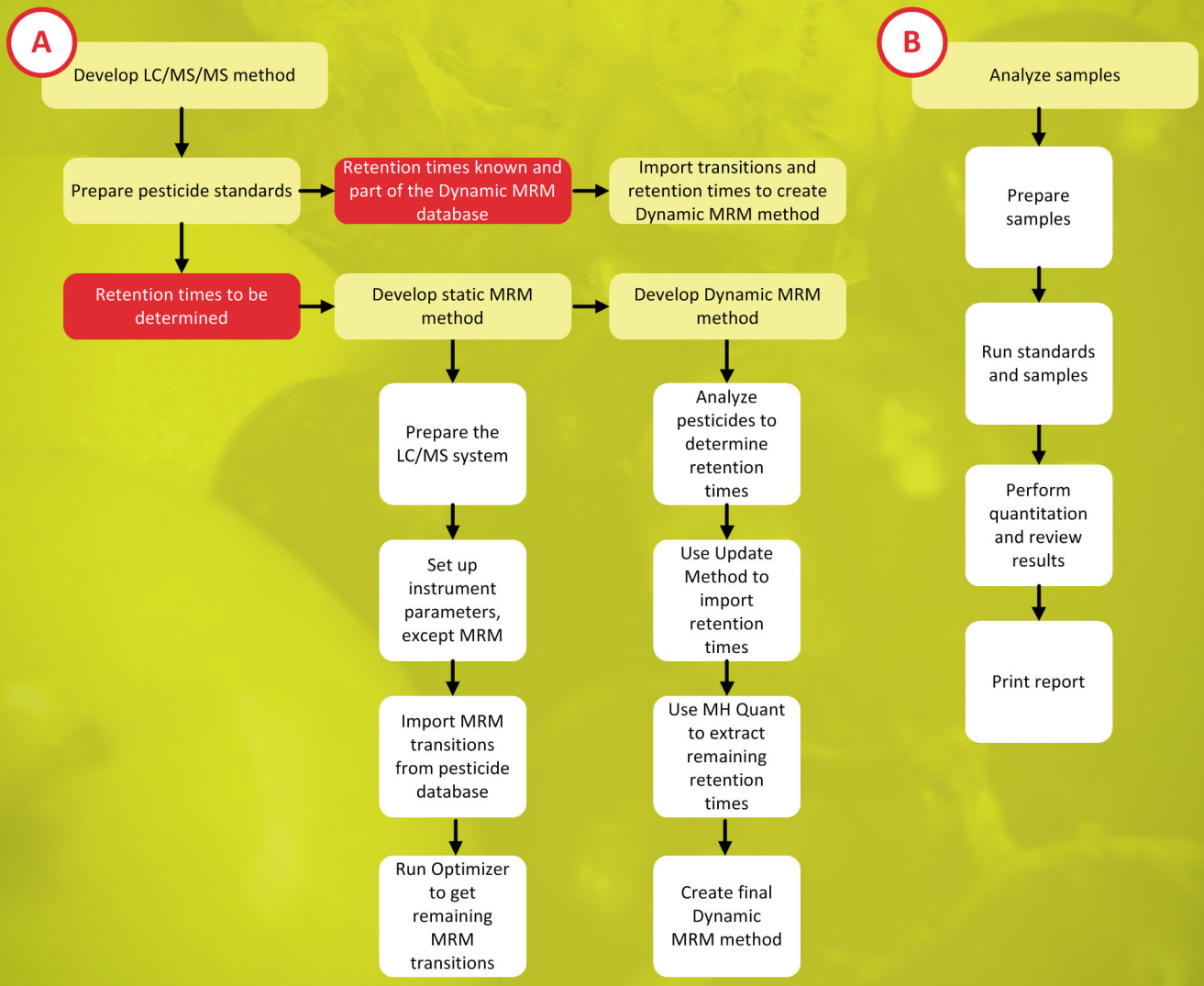
Agilent Triple Quadrupole LC/MS Quantitation of Pesticides

Workflow Overview



Agilent Technologies

Required Items

Required hardware and software

Figure 1 *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 Data Acquisition)
  - Agilent MassHunter Quantitative Analysis software version B.04.00

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.

Developing the Data Acquisition Method

The workflow includes method development and sample analysis. See the *Agilent Triple Quadrupole LC/MS Quantitation of Pesticides Workflow Guide* for details about the steps in this *Workflow Overview*.

Prepare pesticide standards

1. Do preliminary work.

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

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

2. Prepare the standards.

   a Prepare stock standards of individual pesticides.

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

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

   d Prepare additional standards required for your protocol, including calibration standards, internal standards, and standards used to spike samples/determine recoveries.

   e Refrigerate the standards.

Develop the preliminary static MRM method

1. Prepare the LC/MS system.

   a Prepare LC solvents.

   b Start the MassHunter Data Acquisition program.

   c Prepare the LC modules.

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

2. Set up LC/MS parameters, except for MRM settings.

   a Enter values for all the LC modules.

   b Enter triple quadrupole MS parameters.

   c Save the method.

3. Import MRM transitions from a pesticide database.

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

   b Load the method you created in "Set up LC/MS parameters, except for MRM settings."

c 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.)

d Set the dwell time.

e Save the method.

f If necessary, repeat this task with additional methods.

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

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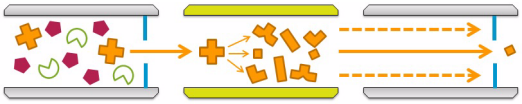
4. Use MassHunter Optimizer to determine remaining MRM transitions.



a Prepare for on-column or flow injection analysis.

b Make sure that you have individual standards of each compound you need to optimize, at a concentration of about 1 ng/µL (100 pg/µL for the 6490 Triple Quad).

c Copy MassHunter method files to your MassHunter Workstation installation.

d Update any Optimizer methods that you plan to use.

e Open MassHunter Optimizer.

f Create or open a project.

g Enter settings on the following tabs:
- Optimizer Setup
- Precursor Ion Selection
- Product Ion Selection
- Compound Setup

h Save the project.

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

j Start the optimization.

k Review the results.

l Save the compounds and results to a database.

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

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

o Set the dwell time.

p Save the method.

q If necessary, repeat step n through step p with additional methods, until you have a method for each pesticide of interest.

Develop the final Dynamic MRM method

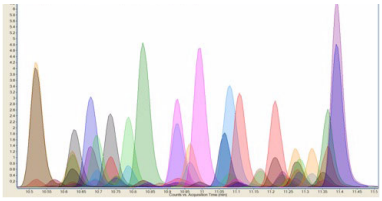
1. Analyze pesticides to determine retention times.

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

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

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

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



b Save the method with a new name.

c Open the Dynamic MRM Update Options dialog box.

d Update the method with each data file that contains pesticide retention times.

e Save the method.

f Open the Dynamic MRM Viewer.

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

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

i Save the method.

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

3. Use MassHunter Quant to extract remaining retention times.

Do this procedure only if you saw errors when you imported retention times directly from the data files, or if you have isomeric pesticides.

a Open the MassHunter Quantitative Analysis program.

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

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

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

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

f Apply the method to the batch.

g Save the batch and analyze it.

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

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

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

k Repeat these steps for every mix of standards that you still need to include in the Dynamic MRM method.

4. Create the final Dynamic MRM method.

Do this procedure only if you saw errors when you imported retention times directly from the data files, or if you have isomeric pesticides.

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

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

c Open the Dynamic MRM Update Options dialog box.

d Update the method using the Quant report folders you generated in "Use MassHunter Quant to extract remaining retention times."

e Save the method.

f Open the Dynamic MRM Viewer.

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

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

i Save the method.

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

Analyzing Samples

Prepare samples

•Follow the applicable QuEChERS method, or another sample preparation method you have chosen. If you need to select a QuEChERS method, see "Agilent SampliQ QuEChERS Kits" (Agilent publication 5990-3562EN).

Acquire data for standards and samples

a Make sure you have all the standards and samples ready to go in the appropriate containers for your LC autosampler.

b Put the standards and samples into the LC autosampler.

c Start the MassHunter Data Acquisition program.

d Set up a worklist.

e Run the worklist.

Perform quantitation and review results

a Quantitate the Dynamic MRM data files.

b Review the quantitation results.

Print a report

a Set up to print a quantitative analysis report.

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

c Create a custom report, if desired.

For More Information

For details about these procedures, see the *Agilent Triple Quadrupole LC/MS Quantitation of Pesticides Workflow Guide*.

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