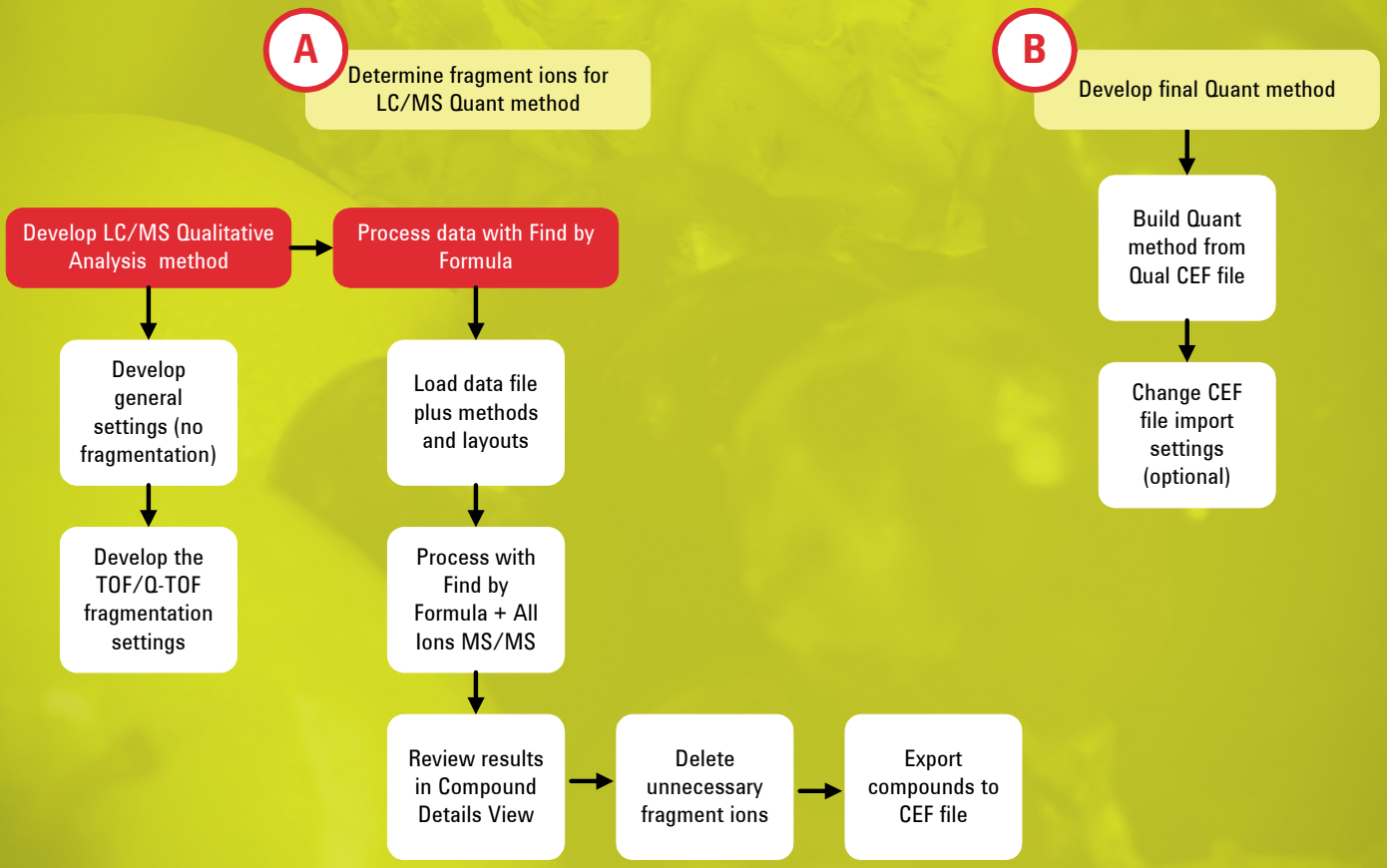
Agilent All Ions MS/MS

Workflow Overview



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Required Hardware and Software

This *Workflow Overview* describes use of the "All Ions MS/MS" technique for compound confirmation and setup of an LC/MS method for pesticide quantification. The method uses analysis by Agilent time-of-flight (TOF) or quadrupole time-of-flight (Q-TOF) instruments. You use the All Ions MS/MS solution from Agilent MassHunter Qualitative Analysis to create a file with masses and retention times. You later use that file to set up a method in Agilent MassHunter Quantitative Analysis.

For details about the steps in this *Workflow Overview*, see the *Agilent All Ions MS/MS Workflow Guide*.

Figure 1 *The workflow requires an Agilent LC and TOF or Q-TOF 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
- One of the following mass spectrometers:
  - Agilent 6200 Series Accurate-Mass TOF LC/MS System
  - Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System
- Agilent MassHunter software:
  - Agilent MassHunter Data Acquisition software for TOF/Q-TOF version B.05.01 or later
  - Agilent MassHunter Qualitative Analysis software version B.06.00 or later
  - Agilent MassHunter Quantitative Analysis software version B.05.02 or later (requirement for new PCDLs)
  - Agilent MassHunter Personal Compound Database and Library version B.04.00 or later
  - Agilent MassHunter Personal Compound Database and Library (PCDL) Manager software version B.04.00 or later
- Microsoft Windows 7 Professional 64 bit

Developing the LC/MS Qualitative Analysis Method

1. Develop the general LC/MS settings (no fragmentation).

2. Develop the fragmentation settings.

For the first step in this workflow, you run a qualitative analysis of a standard solution. In the next step, you use the data you acquire to find the masses and retention times of your analytes.

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

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

c Prepare and refrigerate the standard solution.

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a Start with an appropriate data acquisition method (such as the general method you just developed).

b Set the mode to MS.

c Set the acquisition speed to 3 spectra/second.

d If you have a Q-TOF, set up experiments with three collision energies.

e If you have a TOF, set up experiments with three fragmentor voltages.

f Save the method.

g Analyze the standard solution with the method.

Processing Data with Find by Formula

To quickly build a MassHunter Quantitative Analysis method, you follow these basic steps:

1. Use MassHunter Qualitative Analysis to process a data file with Find by Formula. In this step, you find all the target compounds in the file.

2. Use MassHunter Qualitative Analysis to select fragment ions for target compounds.

3. Build the MassHunter Quantitative Analysis method.

The following pages provide more information.

Process with Find by Formula

1. Load data file plus methods and layouts.

   a Make sure you are in the Navigator View.

   b Use the Target Compound Screening Workflow.

   c Load the data file.

   d Load the method.

   e Make sure your method points to the correct Personal Compound Database and Library (PCDL).

2. (Optional) Process a data file with Find by Formula *without* All Ions MS/MS

   a Set up the Find Compounds by Formula options and find the compounds.

   b Verify the results.

   This demonstration shows that you can successfully confirm compounds with Find by Formula using only the low-energy channel (low fragmentor voltage on a TOF or low collision energy on a Q-TOF). This is because the program assigns isomers by both mass and retention time, rather than by mass alone. However, the confirmation is more reliable when you use All Ions MS/MS, as described on page 5.

3. (Optional) Use Find by Formula with multiple structural isomers.

   If your analysis includes structural isomers:

   a Increase the number of isomers for Find by Formula. (In the Formula Source tab of Find by Formula, under **Matches per formula**, mark **Automatically increase for isomeric compounds**. Then set **Maximum number of matches** to **1**.)

   b Add retention times for isomers to the PCDL.

   c Find the compounds.

   d View the results.

4. Process a data file with Find by Formula *with* All Ions MS/MS confirmation.

These instructions describe how to find compounds using Find by Formula with spectra from the low-energy channel and then confirm them with fragment ions from the high-energy channels. You search a data file for target compounds using Find by Formula, and you confirm them with the coelution profile of fragment ions.

a Set up the Find Compounds by Formula options and find the compounds.
   - Be sure to click the **Fragment Confirmation** tab and mark the check box for **Confirm with fragment ions**.

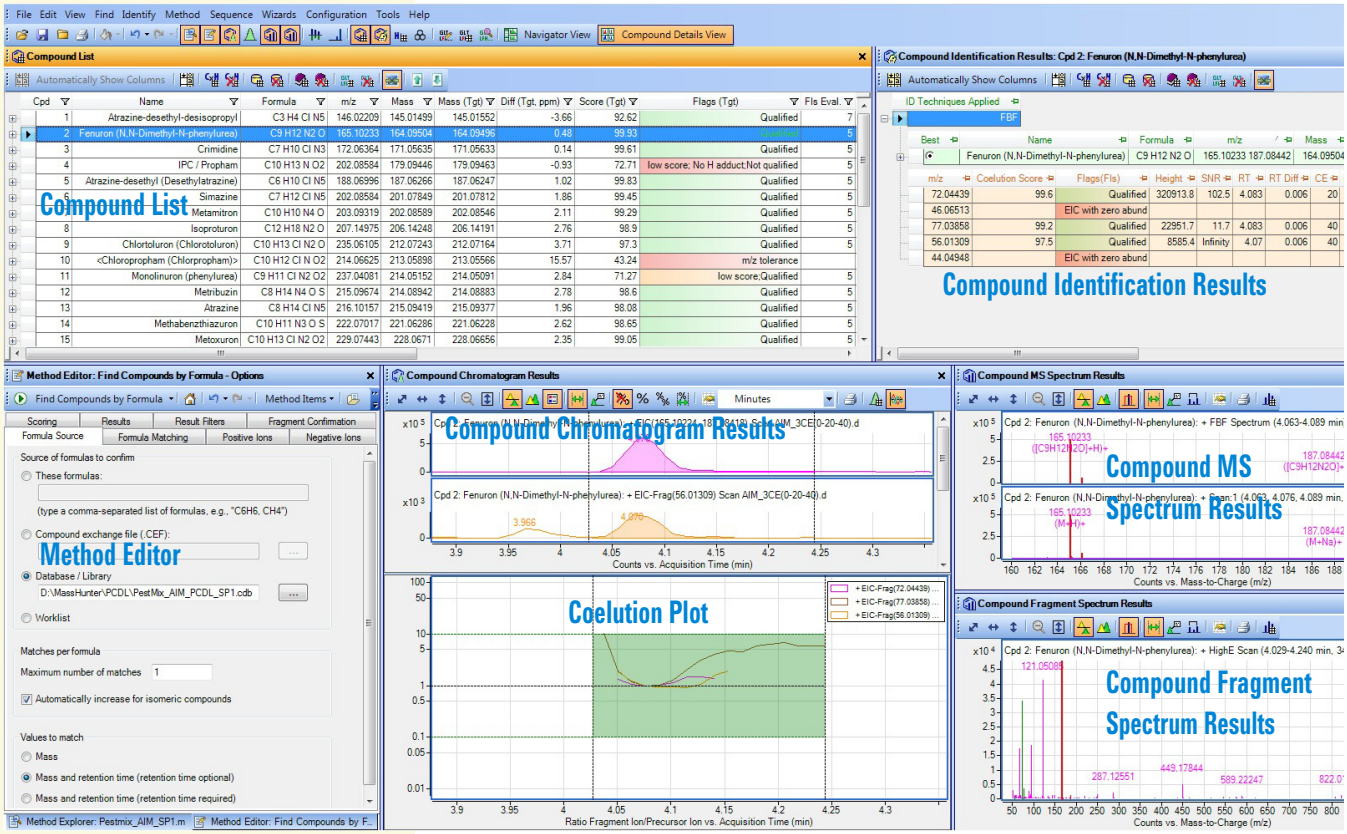
b Make sure you are in the Compound Details View.

c Verify the results.

5. Review results of Find by Formula in Compound Details View.

a Become familiar with the basic components in the Compound Details View.

b In the Compound Details View, review the:
   - Compound List (top left)
   - Compound Identification Results (top right)
   - Compound MS Spectrum Results (middle right)
   - Compound Fragment Spectrum Results (bottom right)
   - Compound Chromatogram Results (middle middle)
   - Coelution Plot (bottom middle).



6. (Optional) Select fragment ions without MS/MS spectra.

a Find compounds without the use of library spectra. (In the Method Editor for Find Compounds by Formula, click the **Fragment Confirmation** tab. Under Fragment Ion Source, click **Use average fragment spectrum**. Set **Number of most abundant ions from average fragment spectrum** to **10**.)

b Examine the results.

7. (Optional) Observe how the program selects fragment ions in case of multiple structural isomers.

a Observe the number of ions the program evaluated for structural isomers. It evaluated the combined list of most intense ions for each compound.

b If possible, find fragment ions that are unique for each structural isomer.

8. Delete unnecessary fragment ions.

- In the Compound Chromatogram Results, click a legend to select an ion. Then right-click the ion's colored bar and click **Disqualify Fragment**.

9. Export compounds to a CEF file.

- Click **File > Export > as CEF**.

Developing the LC/MS Quantitative Analysis Method

This section illustrates how to rapidly build a MassHunter Quantitative Analysis method using a CEF file that you exported from MassHunter Qualitative Analysis

1. Build a Quant method from the Qual CEF file.

   a In MassHunter *TOF* Quantitative Analysis, create a batch and add samples.

   b Start to build a new Quant method from the CEF file (**Method > New > New Method from File**).

   c Set up the compounds.

   d Set up the qualifier ions.

   e Set up the concentrations.

   f Add the origin to the calibration curve.

   g Validate the method.

   h Exit the Method Editor.

   i Define that the sample is a calibration sample and assign the level for the method.

   j Analyze the batch and examine the results.

   k If necessary, fix the problem with the qualifier ratios.

   l Exit and analyze the batch again.

   m To make it easier to review results, set all ion traces to 100% of full scale.

2. Change the CEF file import settings (optional).

   If you want to change the import settings for number of fragment ions and/or number of isotope ions to include as qualifiers:

   a Open the configuration file for editing. The file is **C:\Program Files\Agilent\MassHunter\Workstation\Quant\bin\CefImportConfig.xml**.

   b If desired, modify the All Ions MS/MS settings near the bottom of the file.

   c If you make changes, save the file.

   d Go back to Quant and recreate the method from the CEF file.

   e Verify that the method is properly set up.

For More Information

For details about these procedures, see the *Agilent All Ions MS/MS Workflow Guide*.

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