The GC/MS/MS Analyzer and the Pesticides and Environmental Pollutants MRM Database

Application Note

Food Safety and Environmental

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Abstract

Based on an Agilent 7890A GC System and an Agilent 7000 Series Triple Quadrupole GC/MS, these GC/MS/MS Multiresidue Analyzers were developed to simplify a lab's startup process. The database has an average of eight MRM transitions with relative intensities for each compound to provide alternative measurements for minimizing matrix interferences. Easy-to-use tools, as well as tutorial videos are included in the database to build an MRM acquisition method based on your list of compound CAS numbers in less than 5 minutes.

Introduction

Pesticide residue analysis is a complex task requiring the analyst to search for dozens, or even hundreds, of compounds in a wide variety of crop or environmental matrices. Triple Quadrupole GC/MS (GC/MS/MS) provides excellent sensitivity and selectivity in analyzing complex matrices. Agilent Technologies offers several pre-configured and pretested GC/MS/MS analyzers to simplify a lab's startup process. The analytical capability of a lab, however, is largely determined by the completeness of the MS/MS MRM transitions in an acquisition method. Agilent Technologies developed an MS/MS MRM Pesticides and Environmental Pollutants Database (G9250AA) of over 1,070 compounds to address many of the limitations most labs are facing. This database is in the form of a spreadsheet for ease-of-use. Significant efforts were invested to acquire multiple transitions (eight on average, with relative intensities) of each compound in the database to work around matrix interferences.

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Key features of the MS/MS MRM Database include:

- Allows users to build acquisition methods without buying all compound standards, thus saves time and money
- Includes retention times for either constant flow or constant pressure methods; corresponding retention index (RI) values are also included for easy migration to other GC oven programs
- Has multiple MS/MS transitions, eight on average, for each compound which provides alternatives to work around matrix interferences
- Shows relative intensity of each MS/MS transition within a compound that facilitates transition selection
- Allows a quick sort according to compound category (phthalates, PAHs, organophosphorus, fungicides, or semi-volatile pollutants and so forth, see Appendix A), CAS number, molecular formula, or molecular weight and so forth.

Using the tools in the database, an MRM acquisition method, based on a list of compound CAS numbers, can be easily created from a subset of MS/MS transitions in the database in less than 5 minutes.

Experimental Conditions

Sample Preparation

Without proper extraction and cleanup procedures, it is difficult to detect trace levels of analytes in complex matrices. The QuEChERS sample preparation technique was first introduced for pesticide analysis in foods by USDA scientists in 2003 [1]. It has been rapidly accepted worldwide for multi-residue pesticide analysis due to its special features known as Quick, Easy, Cheap, Effective, Rugged, and Safe. The QuEChERS extracts can be analyzed by LC and GC combined with MS to determine a wide range of pesticide residues.

Agilent's QuEChERS extraction kits and dispersive SPE cleanup kits have demonstrated excellent recoveries for the frequently used pesticides in different food matrices [2, 3]. The sample extracts used in this study were prepared using the QuEChERS technique as described in an application note by Zhao, L. *et al* [4].

GC/MS/MS Analyzer

The Multiresidue GC/MS/MS Analyzer is configured with Agilent's proprietary Capillary Flow Technology (CFT), enabling rugged, reliable GC column backflushing. Backflushing the GC column shortens run times, extends column life, reduces chemical background, provides consistent retention times and spectra, and keeps the MS ion source clean. A Multi-Mode Inlet (MMI) provides the flexibility to inject samples in cold, hot, or solvent-vent modes. Each analyzer system is tested with a 17-compound mixture and retention-time locked at the factory.

Two hardware configurations are available to meet different lab needs (see Figure 1):

- G3445A – option 411: This configuration is based upon constant pressure mode method with post-column backflushing. It provides the flexibility to add GC detectors and can be easily scaled for shorter run times.
- G3445A – option 412: This configuration is based upon constant flow mode method with mid-column backflushing. This method provides ultimate performance and shorter cycle times with reduced carrier gas consumption.

Both configurations (option 411 and option 412) are interchangeable by changing the column and adding or removing a capillary flow-restrictor. A Quick Start Guide for each analyzer discusses the retention time locking, checkout method results and report for your specific system, a list of supplies, and some troubleshooting tips.

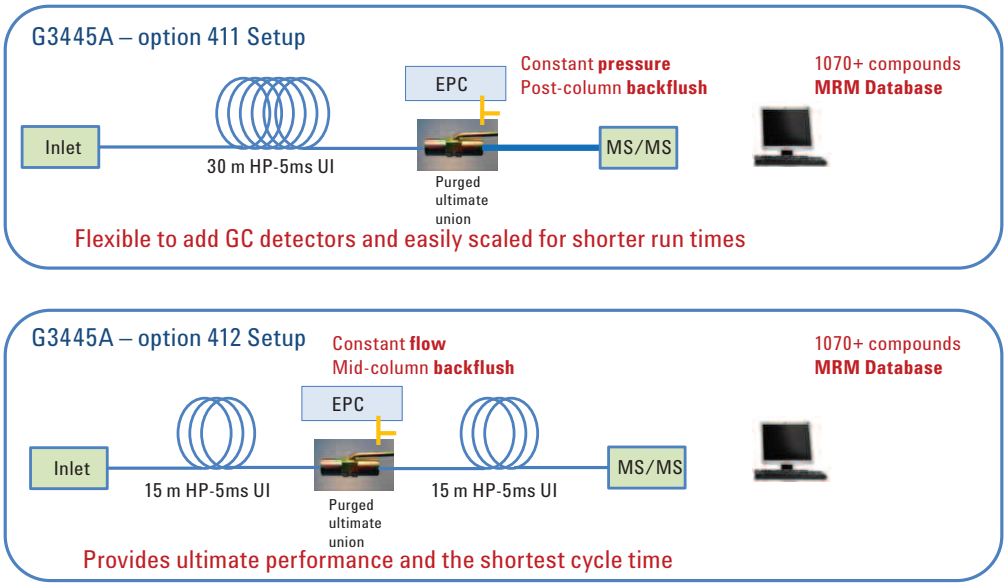

Figure 1. System configurations of Agilent GC/MS/MS Multiresidue Analyzers.

Methods

There are three sets of method parameters included with the database. Some of the method highlights are shown in Table 1.

Table 1. Method Parameters Included with the Databases

	Method 1	Method 2	Method 3
Run time	40.5 min	41.867 min	19.75 min
Column flow	Constant flow mode	Constant pressure mode	Constant flow mode
Feature	Allowing many more transitions in an analysis than Method 3	MS/MS Analyzer G3445A option # 411	MS/MS Analyzer G3445A option # 412
Column(s)	Agilent J&W HP-5ms UI 0.25 mm × 15 m, 0.25 µm (two each)	Agilent J&W HP-5ms UI 0.25 mm × 30 m, 0.25 µm (one each)	Agilent J&W HP-5ms UI 0.25 mm × 15 m, 0.25 µm (two each)
Oven program	Initial at 60 °C, hold for 1 min 40 °C/min to 120 °C, hold for 0 min 5 °C/min to 310 °C, hold for 0 min	Initial at 70 °C, hold for 2 min 25 °C/min to 150 °C, hold for 0 min 3 °C/min to 200 °C, hold for 0 min 8 °C/min to 280 °C, hold for 10 min	Initial at 60 °C, hold for 1 min 40 °C/min to 170 °C, hold for 0 min 10 °C/min to 310 °C, hold for 2 min
Locking compound and RT	Chlorpyrifos-methyl locked to 18.111 min	Chlorpyrifos-methyl locked to 16.593 min	Chlorpyrifos-methyl locked to 9.143 min
MS source temperature	300 °C	300 °C	300 °C
Quad temperature	Q1 = Q2 = 180 °C	Q1 = Q2 = 180 °C	Q1 = Q2 = 180 °C
Backflush	Mid-column, post-run	Post-column, post-run	Mid-column, post-run

Additional method details for each method are listed on individual pages (tabs) in the database.

The retention times (RTs) and retention indexes (RIs) corresponding to these three sets of methods are included for all compounds in the database. Therefore, you can either use one of the above prescribed methods (use RTs in the database) or your existing lab method (convert database RIs to expected RTs for your method). The database RIs were calculated using retention times of straight-chain hydrocarbons from C-8 to C-35. An RI_to_RT conversion tool is included with the database, so you can calculate expected retention times of your analytes based on the RIs in the database and RTs of hydrocarbon markers (C-8 to C-35) from your existing GC method. If your existing method uses HP-5ms UI column of the same phase ratio as a 0.25 mm, 0.25 µm column, you will see the smallest difference between the expected and actual retention times for your analytes.

Backflush

Food or environmental extracts after cleanup are usually still very complex containing various matrix residues such as high-boiling compounds. The extracts used in GC/MS analyses can cause contamination and deterioration of the analytical column and MS ion source, affecting data quality due to poor peak shape and loss of responses of active analytes. It also leads to a shorter lifetime of the analytical columns and frequent MS maintenance. Therefore, it is necessary to use best techniques and supplies to achieve reliable results and to protect the analytical column and MS ion source.

Column backflushing can be beneficial for the analysis of complex extracts because it significantly reduces analysis time and reduces both column head trimming and MSD ion source cleaning frequency [5]. Agilent CFT makes column backflushing routine [6, 7].

Database Overview

The G9250AA MRM Database is in Microsoft Excel format for easy searching and filtering. Compounds are separated by color bands for clarity. The following basic compound information is included for each compound:

- Common name
- Molecular formula
- Molecular weight (averaged)
- Molecular weight (mono-isotopic)
- CAS number, without dashes for easy sorting
- Classification 1 (see Appendix A)
- Classification 2 (see Appendix A)
- Retention times (RT) and retention indexes (RI) for constant flow and constant pressure methods (total three methods)
- Relative intensity of each transition within a compound
- Chinese name and Japanese name where available

In addition, information is included for building MassHunter MRM acquisition methods:

- CAS number, standard format with dashes
- Method RT
- Common name
- ISTD (true or false)
- Precursor ion
- MS1 resolution
- Product ion
- MS2 resolution
- Dwell time
- Collision energy (Voltage)
- Retention time window (used in the MassHunter Compound List Assistant tool)

Figures 2 and 3 give an overview of the database layout. Using the Excel filtering tool, it is easy to display the table array according to the criteria chosen in any column. Figure 4 shows the database after using Excel filtering in column AE to hide all transitions except the top two (Q0 and Q1). This flexibility allows the user to build methods according to compound categories, (for example, PAHs, Phthalates, or PCBs), or regulatory methods and so forth. Two groups of compound classifications in the Database are listed in Appendix A as a reference.

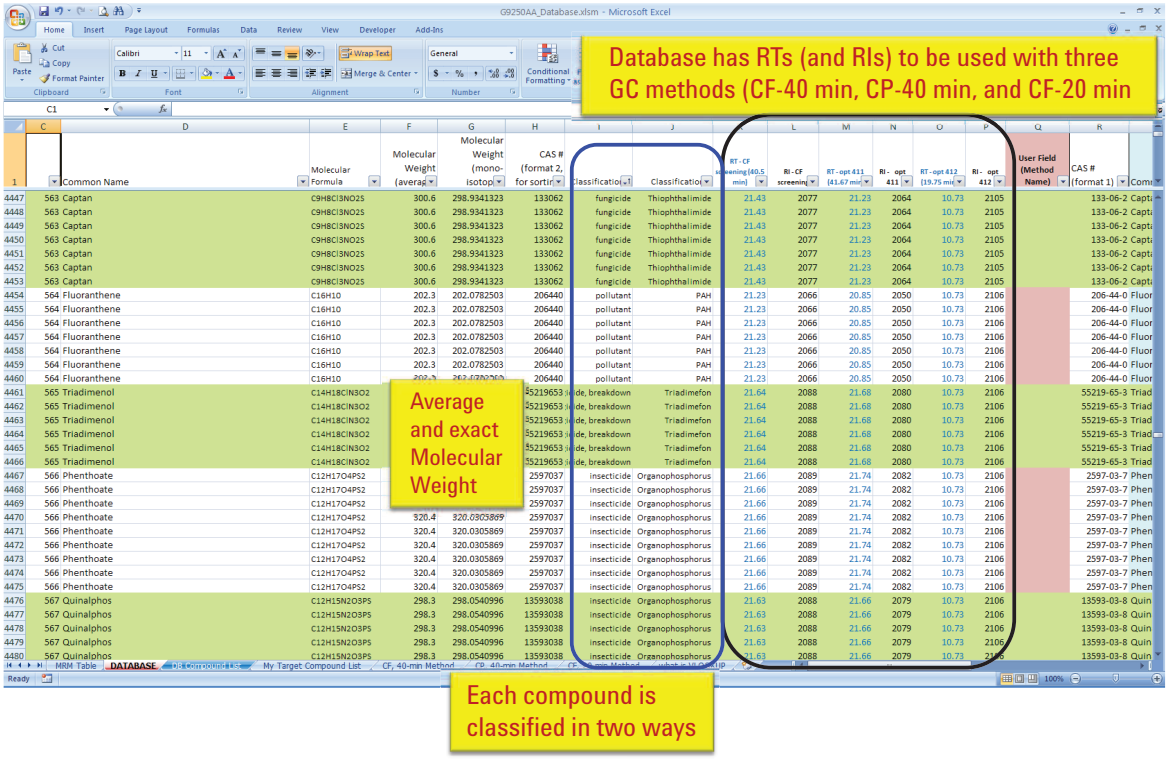

Figure 2. Layout of the Database 1: molecular weights, classifications, and three RTs and RIs.

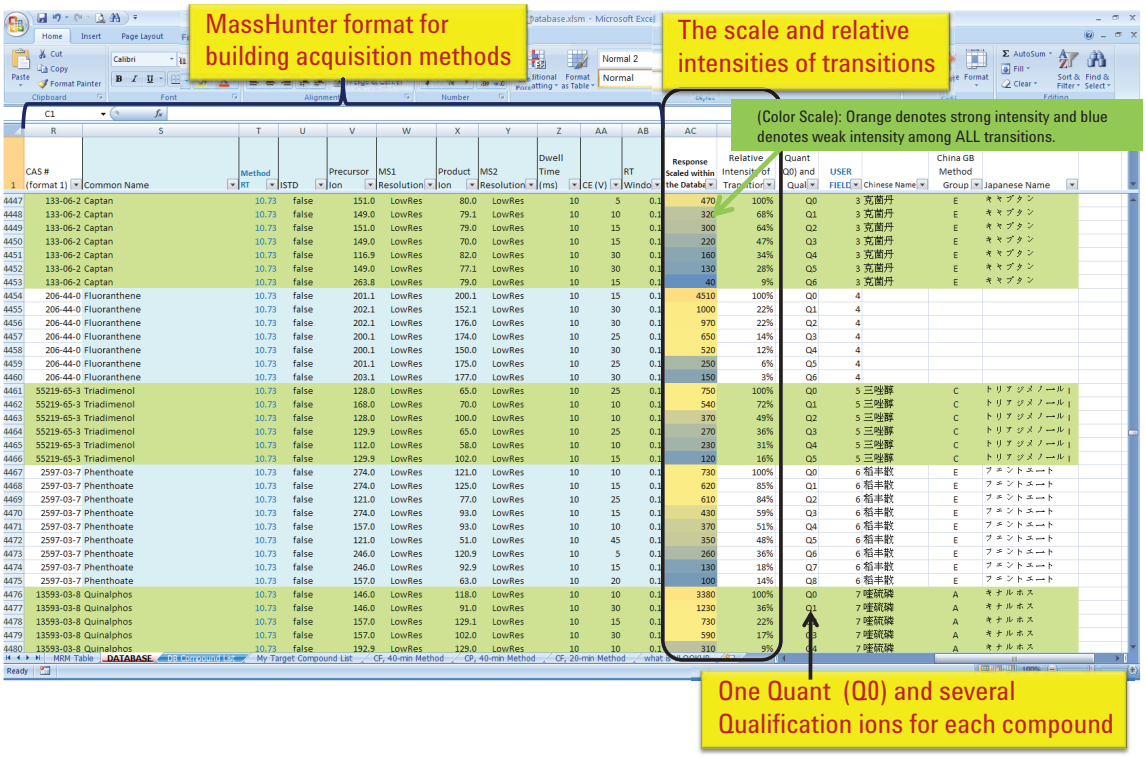

Figure 3. Layout of the Database 2: MassHunter format for building acquisition methods, multiple transitions, and relative intensities.

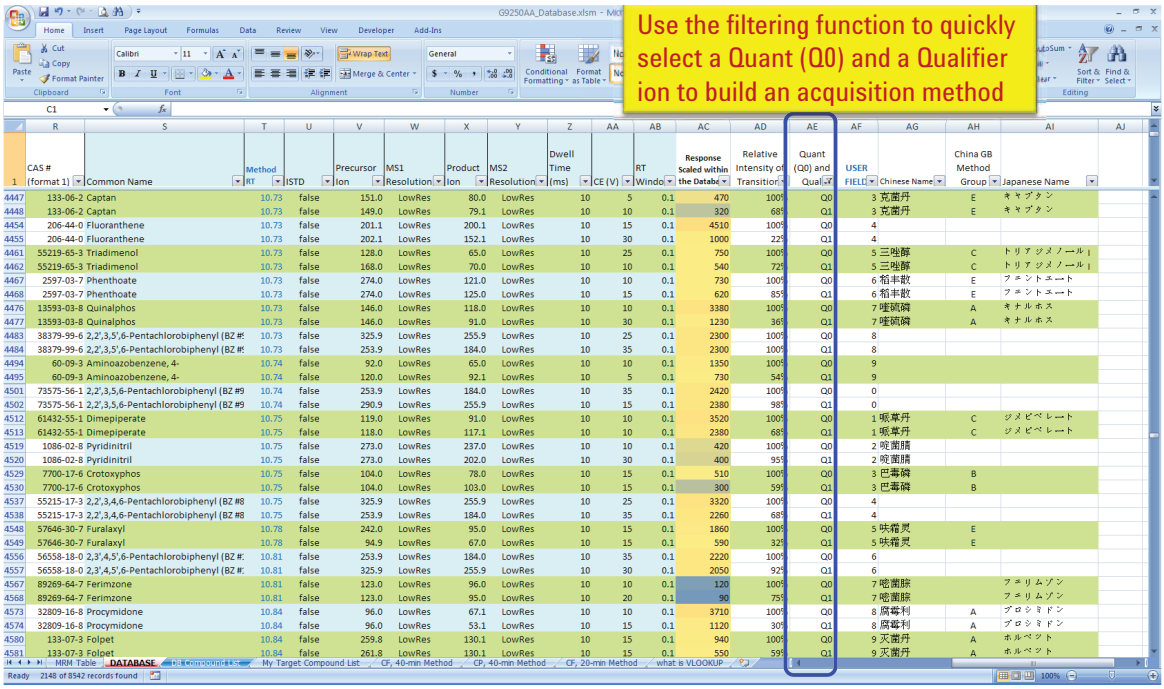

Figure 4. Using Excel filter to hide all transitions except the top two of each compound.

Table 2 gives a breakdown of the compounds included in the database.

Table 2. Compounds Included in the Database

	Total number
Pesticides (fungicides, herbicides, insecticides, rodenticides, and others)	675
Breakdown products	42
Deuterated compounds	6
Polybrominated Diphenyl Ether (PBDE)	4
Polybrominated Biphenyl (PBB)	1
Polychlorinated Biphenyl (PCB)	209
Polycyclic Aromatic Hydrocarbon (PAH)	26
Phthalates	17
Additional semivolatile pollutants	94

A complete list of compounds can be found on the **DB Compound List** tab in the database.

There are three videos included with the database to help the user learn the database:

- An overview of the content and layout of the database.
  → Each individual column and tab is explained in the video.
- A tutorial of building an MRM acquisition method based on your list of compound CAS numbers.
  → An MRM acquisition method, based on your list of CAS numbers, can be easily and quickly created from a subset of MS/MS transitions in the database.
- A tutorial showing how to add new compounds to the database.

The database ReadMe file shows a few Excel shortcuts to use with the database and a few additional ways of using the database. For example, it shows how to find all nitrogen containing compounds in the database, or how to select all PCB congeners, or the 14 most toxic PCB planar congeners in the database.

Results and Discussion

Chemical Background from the Matrix

Figure 5 shows four MRM total ion chromatograms (TICs) of pepper, spinach, orange, and pear extracts acquired using method 3 described on page 3. Thirty-five analytes at 10 ppb each were spiked in each matrix. Seven transitions of each analyte were used in the acquisition method. The TICs showed that the chemical background from the four matrices was quite different and sizeable. In this study, pear extract showed the highest background response in terms of number of peaks and intensity. The TIC from the orange extract was the cleanest among the four chromatograms. These different and high background responses all came from the matrix. To understand the matrix effect, we need to evaluate the chemical background in each individual transition.

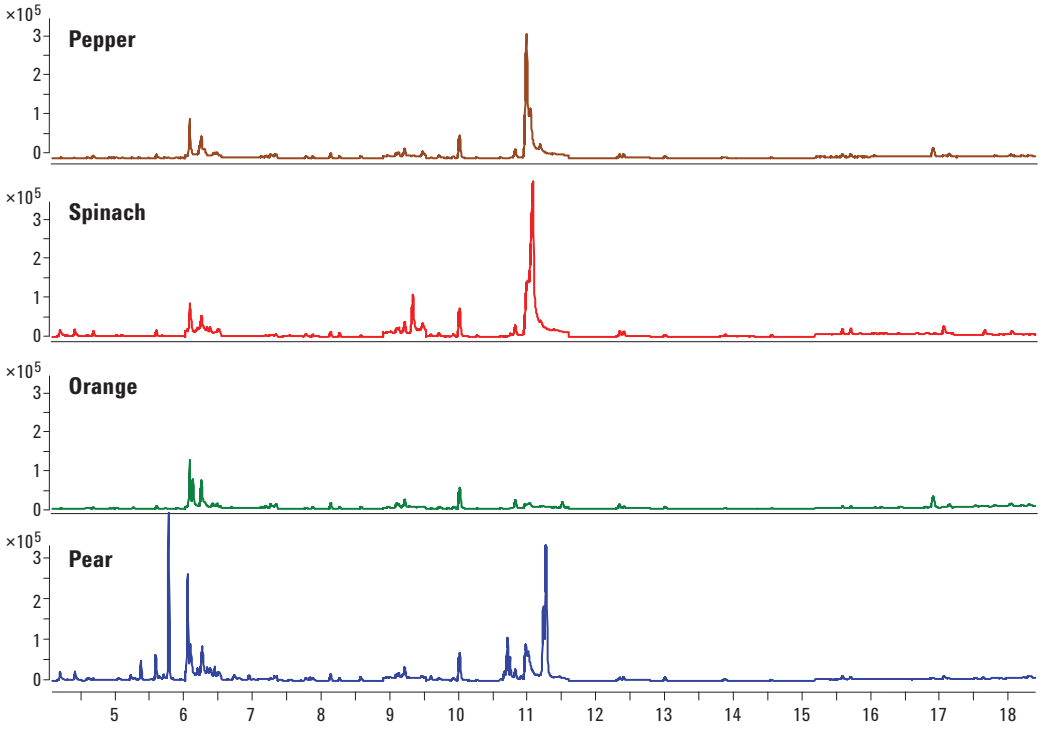

Figure 5. Total ion chromatograms (TICs) of pepper, spinach, orange, and pear extracts including 35 analytes spiked at 10 ppb each.

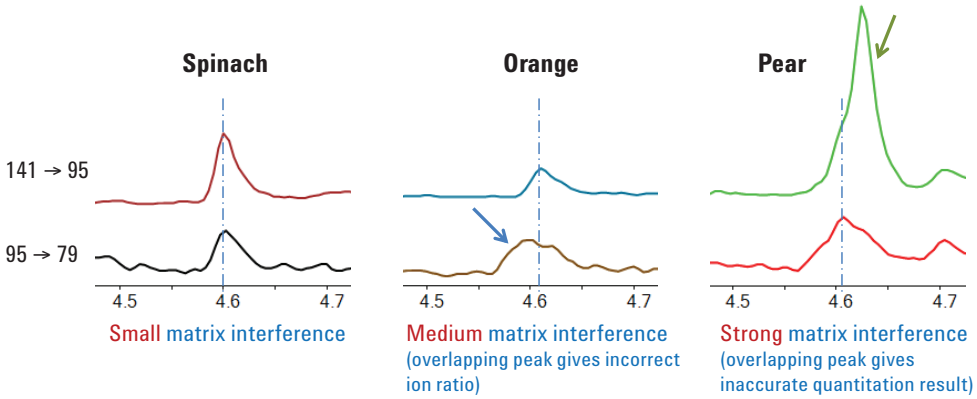

Figure 6. Top two transitions of methamidophos (at 10 pg) in three matrices.

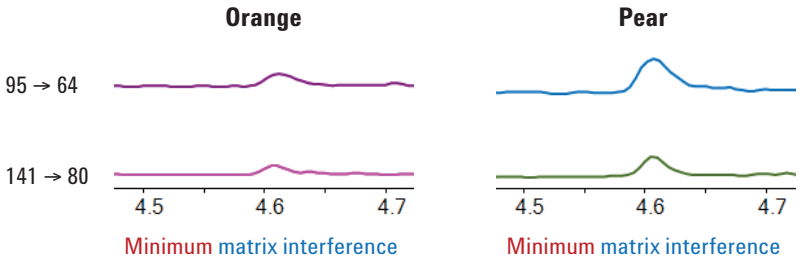

Figure 7. Two alternative methamidophos transitions with minimum matrix interference.

A typical MRM database or acquisition method has two MRM transitions for each analyte. Figure 6 shows extracted ion chromatograms (EICs) of the top two transitions of methamidophos (at 10 pg) in three matrices. The retention time of methamidophos is about 4.6 minutes. The transitions are arranged in the descending order of responses with the larger one on top. Figure 6 shows the obvious issues of getting inaccurate quantitation results due to medium or strong matrix interference. For orange matrix, an overlapping peak in the second transition marked by a blue arrow, affected integration results and the qualifier ion ratio. For pear matrix, an overlapping peak in the first transition, marked by a green arrow, which is typically used for quantitation, gave higher and inaccurate quantitation results.

If a user only has two MRM transitions available for each analyte, it is difficult to work around the matrix effect as seen in Figure 6. The G9250AA database has an average of eight transitions for each compound. This allows the user to choose alternative transitions easily when matrix interference affects peak shape and integration results.

Figure 7 shows EICs of two alternative methamidophos transitions in the database. Both transitions showed minimum matrix interferences in orange and pear matrices. In fact, the EICs of these two transitions showed minimum matrix interference in all four matrices. Although these two transitions do not provide the highest responses, they are suited for a universal or screening MRM method. It is always best to evaluate the chemical background of an analyte's multiple transitions in different matrices before selecting the most appropriate transitions in a particular matrix.

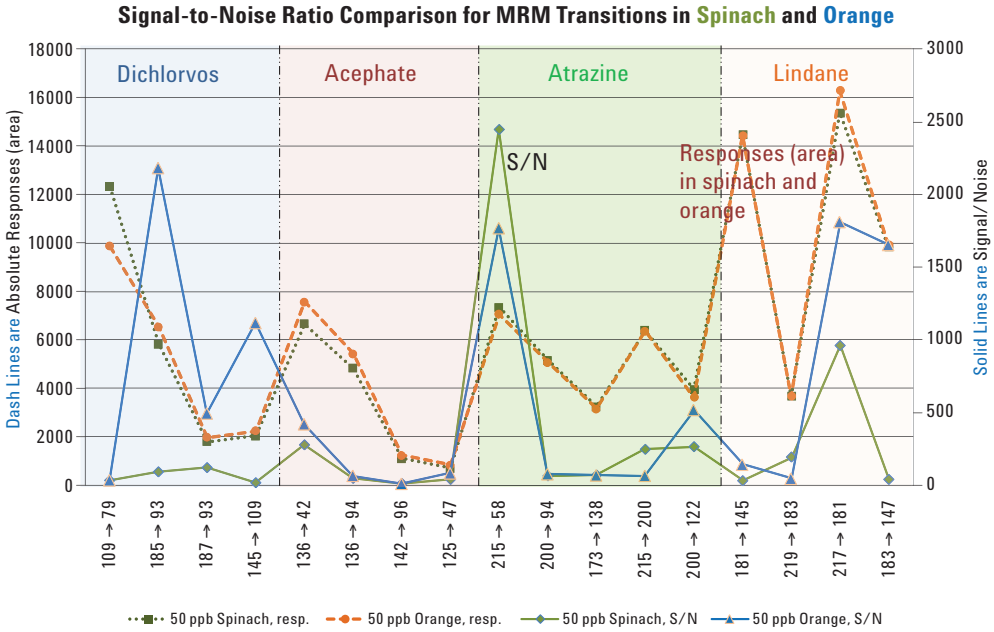

Figure 8. Comparison of area counts and signal-to-noise ratios of four analytes MRM transitions in spinach and orange matrices.

Signal-to-Noise Ratios

Evaluating the signal-to-noise ratios (S/N) of MRM transitions is another way to identify matrix effects. Some pesticides showed consistent MRM responses in different matrices, but many pesticides had different MRM responses in different matrices due to either matrix enhancement or matrix suppression.

Figure 8 shows responses, or area counts, and S/N's of several MRM transitions for four analytes in spinach and orange matrices. The orange dashed line and dark green dotted line represent area counts from four or five MRM transitions of each analyte in these two matrices. The solid blue and green lines represent the S/N from the same MRM transitions in these two matrices. The dashed and dotted lines, signifying area counts, superposed tightly. However, the solid lines, S/N, showed significant variations for some transitions within each analyte. Using atrazine as an example, the area count for transition 215→58 was about the same (approximately 7,000) for both matrices, but the S/N for this transition in spinach was about 40% higher than the S/N in orange matrix. In contrast, for transition 200→122, the S/N in orange matrix was almost double the S/N in spinach, even though the area counts in both matrices were about the same (approximately 4,000). This matrix effect was not unique to atrazine. The S/N variations from some of the MRM transitions of dichlorvos and lindane were more pronounced even though the area counts were comparable in both matrices. Again, if the number of MRM transitions available for each analyte is limited to two or three, it is difficult to select optimal MRM transitions suited for the matrix analyzed.

The multiple transitions available in the G9250AA database allow users to choose several selective transitions to achieve accurate confirmation and quantitation results. This study showed that MRM transitions should be chosen according to matrix to achieve optimal and reliable quantitation results. It is important to use matrix-matched calibrations and low background transitions to achieve accurate quantitation results.

Conclusion

Based on the Agilent 7890A GC and 7000 Series Triple Quadrupole GC/MS, the GC/MS/MS Multiresidue Analyzers were developed to simplify a lab's startup process. A special feature of the analyzer is the comprehensive and flexible MRM database of over 1,070 pesticides and environmental pollutants. The analyzer also includes CFT backflush for superior system robustness during routine operations.

Matrix can cause quantitation interference, lower responses, or poor peak shape. Each matrix has a different matrix effect. Therefore, it is critical to choose the most selective transitions for a particular matrix and use matrix-matched calibration curves to achieve accurate and reliable quantitation results. The G9250AA MRM Database has an average of eight MRM transitions with relative intensities for each compound to provide alternative measurements to minimize matrix interference. Easy-to-use tools as well as tutorial videos are also included in the database to build an MRM acquisition method based on your list of compound CAS numbers in less than 5 minutes.

References

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Appendix A

List of First Compound Classification in the Database

algicide
bird repellent
breakdown
defoliant, plant growth regulator
deuterated
fragrance
fumigant
fungicide
fungicide, insecticide
fungicide, microbiocide
fungicide, plant growth regulator
herbicide
herbicide safener
herbicide, algaecide
herbicide, microbiocide
herbicide, plant growth regulator
insect attractants
insect growth regulator
insect repellent, synergist
insecticide
insecticide, fungicide
insecticide, insect repellent
insecticide, molluscicide
insecticide, nematicide
insecticide, plant growth regulator
microbiocide
microbiocide, fungicide
molluscicide
nematicide
plant growth regulator
plant growth regulator, herbicide
pollutant
rodenticide
synergist
wood preservative, microbiocide

List of secondary compound classification in the Database

1,3-Indandione
2,6-Dinitroaniline
Amide
Anilide
Anilinopyrimidine
Aromatic
Aryl phenyl ketone
Arylalanine
Aryloxyphenoxy propionic acid
Auxins
Benzamide
Benzimidazole
benzofuranyl alkylsulfonate
Benzoic acid
Benzothiazole
Botanical
Bridged diphenyl
Carbamate
Carbanilate
Carbofuran
Carboxamide
Chitin synthesis inhibitor
Chlorinated phenol
Chloroacetanilide
Dinitrophenol derivative
Diphenyl ether
Dithiocarbamate
Folpet
Formamidine
Formamidine
Fumigant
Halogenated organic
Hydrobenzonitrile
Hydroxybenzonitrile
Imidazolinone
Juvenile hormone mimics
Keto-enol
Mercaptobenzothiazole
Morphactins
Morpholine
Naphthalene acetic acid derivative
Neonicotinoid
Nitrophenyl ether
N-Methyl carbamate
Organochlorine
Organophosphorus
Oxadiazolone
PAH
Phthalate
Phthalic acid
Picolinic acid
Pyrazole
Pyrethroid
Pyrethroid ester
Pyridazine
Pyridazinone
Pyridine
Pyridinecarboxylic acid
Pyrimidinamine
Pyrimidine
Pyrimidine organothiophosphate
Pyrimidinyloxybenzoic acid
Pyrrole
Quinoline
Quinone
Quinoxaline
SemiVOC
Strobin
Substituted benzene
Sulfite ester
Thiadiazole
Thiocarbamate

Continued

List of secondary compound classification in the Database

Chlorophenoxy acid or ester
Conazole
Coumarin
Cyclic dithiocarbamate
Cyclodiene
Cytokinins
Defoliant
Deuterated PAH
Deuterated semiVOC
Dicarboximide
PBB
PBDE
PCB
Phenol
Phenoxyacetic
Phenoxybutyric
Phenoxypropionic
Phenylsulfamide
Phosphoramidate
Phosphorodiamide
Thiophthalimide
Triadimefon
Triazine
Triazinone
Triazole
Triazolone
Uracil
Urea
Xylylalanine

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