Accurately Identify and Quantify A Hundred Pesticides in a Single GC Run

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Introduction

The global agricultural industry uses over a thousand pesticides for the production of food and foodstuffs. More and more methods are being created to analyze the extensive list of target pesticides. Analytical laboratories are then strained to evaluate and quantitate hundreds of pesticides in a single run. Currently GC-MS/MS MRM analyses use time segments (TS's) with predefined sets of MRM transitions for each segment. As sample complexity increases (i.e. quantifying low levels of hundreds of pesticide residues in a wide diversity of food matrices) the ability to utilize dynamic MRM (dMRM) provides laboratories with the capability to better tackle the large multi-analyte analysis and to accurately quantify trace quantities of pesticides from high-throughput methods. An evaluation was conducted to look at the set up of an MRM acquisition method in the traditional TS structure and the analogous dMRM paradigm. Three matrix optimized MRM transitions (Q0, Q1, and Q2) for a Target Compound List of 195 various pesticides were chosen for the analysis.

MS Acquisition Method Development

The MassHunter Pesticide & Environmental Pollutant MRM Database and Matrix Optimized Transitions were utilized to develop MRM methods for the evaluation off 195 target pesticides in a variety of matrices. Both the 40 min and 20 min constant flow methods referenced in the MRM Database were followed. The top 3 (highest responding) MRMs for each compound were selected for analysis.

Experimental

GC Methodology

The analysis was conducted on an Agilent 7890B GC and 7010 Series Triple Quadrupole GC/MS. See Tables 1 & 2 for the GC method parameters. The system was configured with a Multimode Inlet (MMI), equipped with an ultra-inert liner (p/n: 5190-2293). Two HP-5ms UI columns (15 m × 0.25 mm × 0.25 µm; p/n: 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) for the use of backflushing (see Figure 1). Both a 40 min resolution method and a 20 min fast analysis method were examined.

Table 1. 7890B GC Method Conditions

Injection port liner	4-mm Ultra Inert liner with wool		
Injection mode	Hot-splitless		
Injection volume	1 µL		
Inlet temperature	280 °C		
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)		
Oven program		60 °C	1 min
	40 °C/min	120 °C	0 min
	5 °C/min	310 °C	0 min
MS transfer line temperature	280 °C		

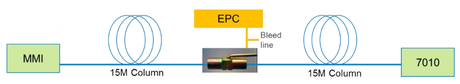

Figure 1. Column Configuration for Optimal MRM Application.

Table 2. PUU Backflush Settings*

Timing	1.5 min duration during post-run
Oven temperature	310 °C
Aux EPC pressure	~50 psi
Inlet pressure	~2 psi

*Backflush conditions optimized for application method in Agilent Laboratory. A 1.5 min backflush duration may be too short for other methods; recommendations can be made for a 5 min backflush duration.

Mass Spec Parameters

Tables 3 & 4 show the MS parameters for Time Segment (TS) MRM and dynamic MRM (dMRM) respectively.

Table 3. 7010 Time Segment (TS) MRM Parameters

Electron Energy	70 eV
Tune	atunes.eihs.tune.xml
EM gain	10
MS1 & MS2 resolution	Wide
Collision Cell	1.5 mL/min N_2 & 2.25 mL/min He
Quant/Qual transitions	Matrix Optimized
Dwell times	Time Segment (TS) specific*
Source temperature	300 °C
Quad temperatures	150 °C

*All dwells in each TS were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS

Figure 2. Image of 7010 MS/MS source

Table 4. 7010 dynamic MRM (*d*MRM) Parameters

Electron Energy	70 eV
Tune	atunes.eihs.tune.xml
EM gain	10
MS1 & MS2 resolution	*d*MRM unit
Collision Cell	1.5 mL/min N_2 & 2.25 mL/min He
Quant/Qual transitions	Matrix Optimized
Dwell times	Optimized by *d*MRM
Source temperature	300 °C
Quad temperatures	150 °C

*All dwells were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec. This was utilized to compare directly with the TS parameters.

Time Segment Method Development

Time Segment development was completed utilizing the Graphical User Interface (GUI) in the MRM Database and the MassHunter Compound List Assistant (CLA). Figures 3 – 8 show a quick representation of the development for analysis in Organic Honey.

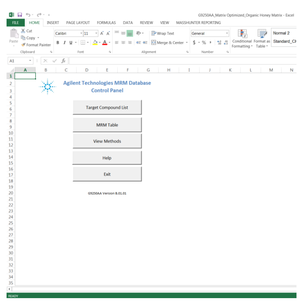

Figure 3. The Organic Honey Matrix Optimized MRM Database was utilized for the TS Method Development.

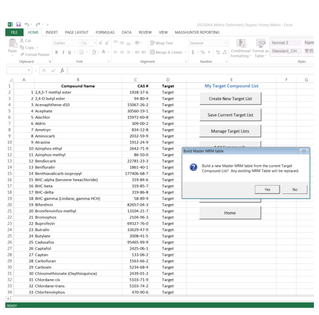

Figure 4. After the Target List was created, the MRM Table could be generated.

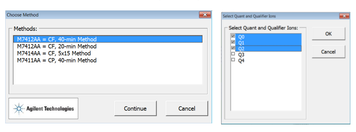

Figure 5. There are two selections that the Database calls for in order to develop the MRM Table for the correct method. 1) Method Selection (40 min method selected); 2) Quant and Qualifier Ion Selection

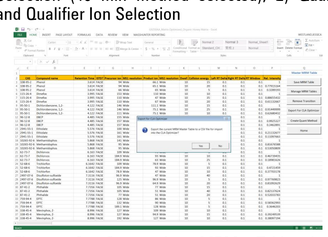

Figure 6. Once the MRM Table is completed, the Database exports the data to the CLA for MRM optimization.

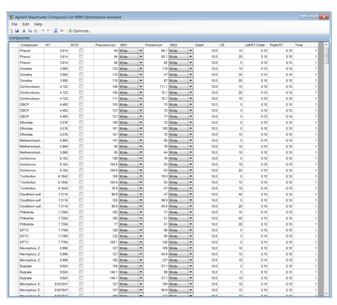

Figure 7. The CLA allows for the user to optimize the RT delta's and the dwell times based on the user defined cycle times.

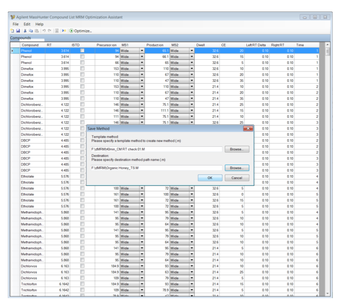

Figure 8. The method is then saved by the CLA and can be loaded into MassHunter GC/MS Data Acquisition

Elements of TS Method Development

Typical method development time: ~ 5 min

Adding target compounds: one-by-one selection or import CAS# list

Removing target compounds: one-by-one selection

Adding MRM transitions: recreation of the MRM Table from the Target List

Removing MRM transitions: one-by-one selection; must rerun CLA to re-optimize

Quant and Qualifier selection: same selection and amount for each target compound

Use of CLA for method optimization: RT deltas can be set one-by-one or "filled down" within columns; dwell optimization by algorithm or constant cycles/sec

dMRM Method Development

*d*MRM development was completed utilizing the MS Method Editor within MassHunter Workstation GC/MS Acquisition Software. Figures 9 – 14 show a quick representation of the development for analysis in Organic Honey.

Figure 9. From within the MS Parameters of MassHunter GC/MS Data Acquisition the Organic Honey Matrix Optimized MRM Database was imported and the Method of choice was selected.

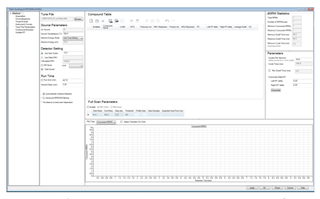

Figure 10. The MRM Method will be filled with the Target compounds and their chosen MRMs.

Figure 11. The compound browser allows the user to select their target compound list and the quant and qualifier ions. Once chosen, the MRMs are applied to the Import List.

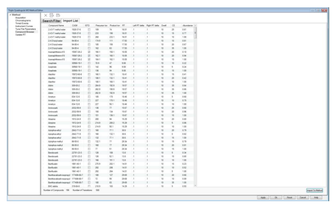

Figure 12. The Import List will maintain all of the target compounds and their respective MRMs that are to be utilized in the method. Once finalized they are then imported to the Method.

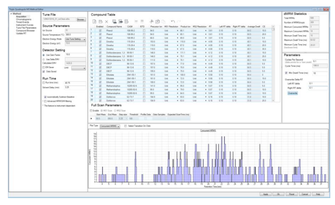

Figure 13. The Method Acquisition page is where the user will define the RT deltas and the define the cycles/sec and/or the dwells. Shown is the Target List and respective MRMs for the 40 min method.

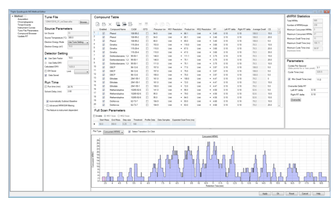

Figure 14. View of the 20 min method of the same Target List and the respective MRMs as from the 40 min method (Figure 13).

Elements of *d*MRM Method Development

Typical method development time: ~5-10 min depending on how complicated the MS method is

Adding target compounds: one-by-one selection, group selection, or import CAS# list

Removing target compounds: one-by-one or multiple selection

Adding MRM transitions: one-by-one or multiple selection

Removing MRM transitions: one-by-one or multiple selection removal

Quant and Qualifier selection: same selection for all or choice for each target compound

Use of MassHunter DA for method optimization: RT deltas can be set one-by-one or "filled down" within columns; dwell optimization by algorithm or user defined settings

Evaluation

The use of *d*MRM provides users with another way to set up their MS Acquisition Method Parameters. Whether the user chooses to utilize TSs or the *d*MRM functionalities they will be able to run their optimal analysis.

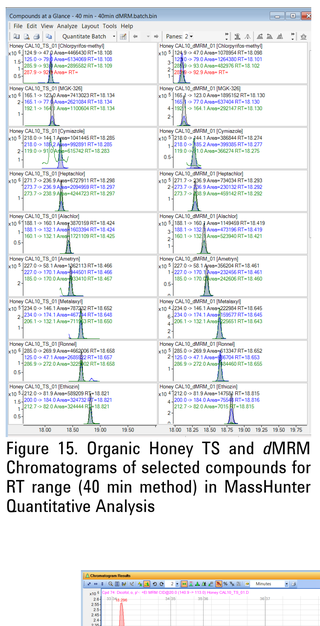

Figure 15. Organic Honey TS and *d*MRM Chromatograms of selected compounds for RT range (40 min method) in MassHunter Quantitative Analysis

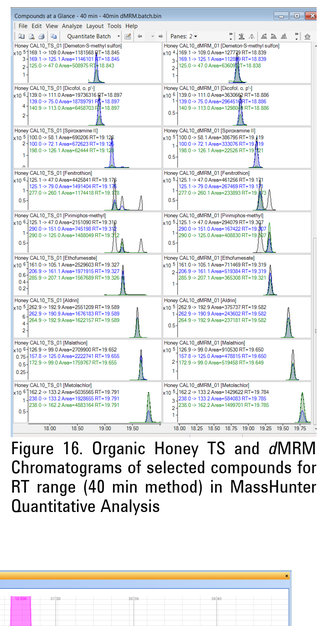

Figure 16. Organic Honey TS and *d*MRM Chromatograms of selected compounds for RT range (40 min method) in MassHunter Quantitative Analysis

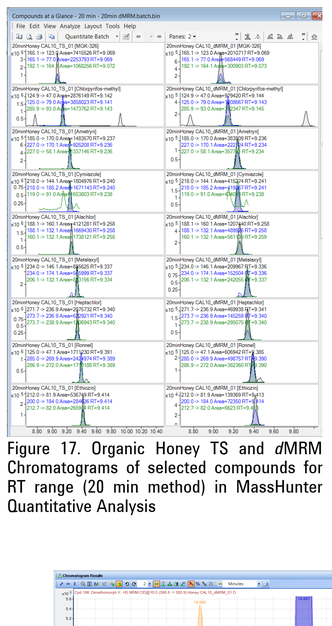

Figure 17. Organic Honey TS and *d*MRM Chromatograms of selected compounds for RT range (20 min method) in MassHunter Quantitative Analysis

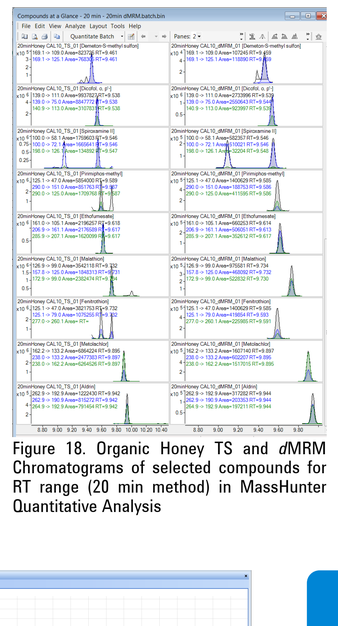

Figure 18. Organic Honey TS and *d*MRM Chromatograms of selected compounds for RT range (20 min method) in MassHunter Quantitative Analysis

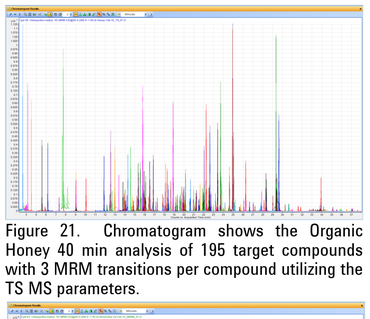

Figure 21. Chromatogram shows the Organic Honey 40 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the TS MS parameters.

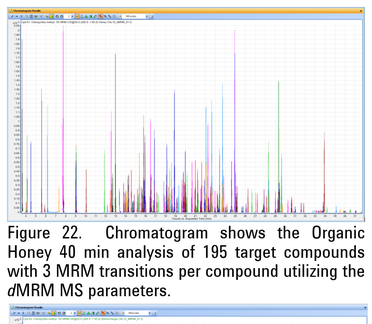

Figure 22. Chromatogram shows the Organic Honey 40 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the *d*MRM MS parameters.

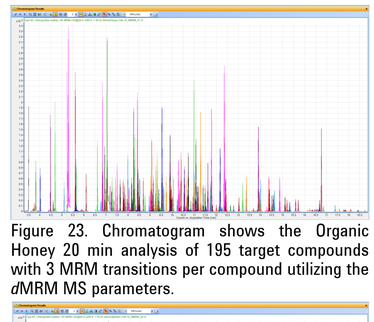

Figure 23. Chromatogram shows the Organic Honey 20 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the *d*MRM MS parameters.

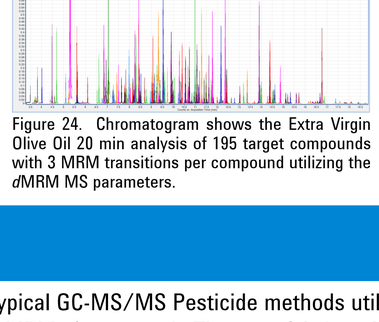

Figure 24. Chromatogram shows the Extra Virgin Olive Oil 20 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the *d*MRM MS parameters.

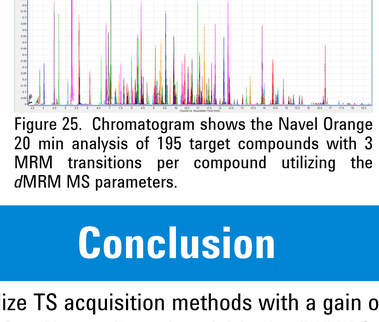

Figure 25. Chromatogram shows the Navel Orange 20 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the *d*MRM MS parameters.

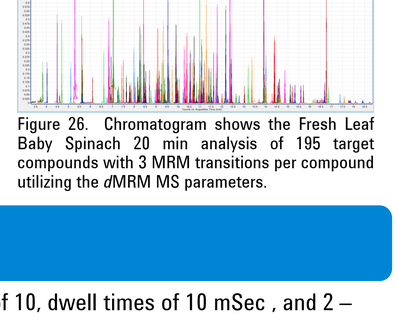

Figure 26. Chromatogram shows the Fresh Leaf Baby Spinach 20 min analysis of 195 target compounds with 3 MRM transitions per compound utilizing the *d*MRM MS parameters.

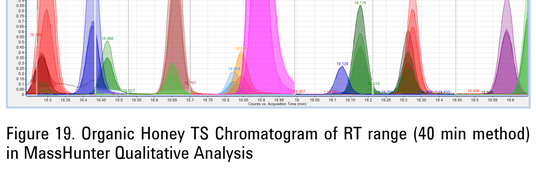

Figure 19. Organic Honey TS Chromatogram of RT range (40 min method) in MassHunter Qualitative Analysis

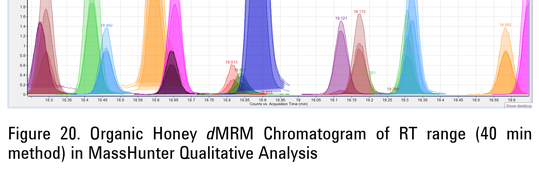

Figure 20. Organic Honey *d*MRM Chromatogram of RT range (40 min method) in MassHunter Qualitative Analysis

Conclusion

Typical GC-MS/MS Pesticide methods utilize TS acquisition methods with a gain of 10, dwell times of 10 mSec , and 2 – 3 MRMs/compound. The use of Agilent MassHunter Data Acquisition's *d*MRM functionality for MS acquisition method development provides users to achieve equivalent or better quality data and results by:

- Monitoring the MRM transitions based on the compounds' retention times as they elute from GC
- Reducing the number of MRM transitions active at any given time allowing for longer dwell times in many cases
- Optimizing the dwell times to maintain a constant MS cycle time and constant sampling rate across all peaks

As sample complexity increases the ability to utilize *d*MRM will provide laboratories with the capability to better tackle their large multi-analyte analysis and to accurately quantify trace quantities of pesticides from high-throughput methods.