

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

Application Note

Author

Jessica Westland Agilent Technologies, Inc.

Abstract

A selected target compound list of 195 various pesticides was chosen for the evaluation of both the traditional time segment (TS) acquisition and the dMRM acquisition structures. Not only were the MRM acquisition setup procedures examined, but the acquired data were also evaluated. As sample complexity increases, the ability to use dMRM 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. The use of dynamic MRM (dMRM) acquisition method development provides users the ability 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
- Optimizing the dwell times to maintain a constant MS cycle time and constant sampling rate across all peaks



Agilent Technologies

Introduction

The global agriculture industry uses over a thousand different pesticides for the production of food and foodstuffs. Producers require pesticides to meet the increasing demand for reasonably priced food. Analytical laboratories are then strained to evaluate and quantitate hundreds of pesticides in a single run. Currently, GC/MS/MS MRM analyses use time segment (TS) acquisition methods. TS methods focus on specified MRM transitions within a fixed retention time (RT) window. The more transitions in a time segment, the lower the dwell time and thus the sensitivity of the data acquired. Adding new compounds to the method usually results in redoing the time segments manually, and can be very time-consuming. Using the automated process of dynamic MRM (dMRM) acquisition saves a large amount of method development time. dMRM uses retention time locking (RLT) of the GC/MS system to set the RT of concurrent MRM transitions in a RT window. This automated procedure determines the number of these transitions to group in a RT window based on dwell criteria entered by the user to determine optimal sensitivity for the instrument.

Experimental

Sample preparation

Many laboratories focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [1,2]. This straightforward sample preparation allows for the analysis of hundreds of pesticides at low concentrations with a single extraction. A selection of eight different matrices were analyzed. These commodities included yellow onion, navel orange, organic honey, basic cucumber, jasmine rice, fresh leaf baby spinach, black loose leaf tea, and extra virgin olive oil [3]. Each matrix was extracted with a specified QuEChERS methodology, in which various dispersive SPE (dSPE) were used for matrix cleanup (Table 1).

Instrumentation

All analyses were run on an Agilent 7890B GC equipped with an Agilent 7693B Autosampler and an Agilent 7010A Triple Quadrupole GC/MS. Table 2 displays the GC and backflush parameters, and Tables 3 and 4 show the MS/MS method parameters for TS and dMRM, respectively. The GC was configured with a multimode inlet (MMI) equipped with an 4 mm ultra inert, splitless, single taper, glass wool liner (p/n 5190-2293). From the inlet, two Agilent J&W HP-5ms Ultra Inert 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 midcolumn/post run backflushing (Figure 1).



Figure 1. Column configuration for an optimal MRM application.

Table 1. Matrix Selection and Sample Preparation Used for Optimal MRM Application.

Category	Matrix	Sample Prep
High oil	Extra virgin olive oil	3 g oil/7 mL water, EN salts (5982-5650), EMR—L (5982-1010), Polish Pouch (5982-0102), Dry step
Difficult	Black loose leaf tea	3 g tea/7 mL water, EN salts, EN dSPE pigment (5982-5256)
High pigment	Fresh leaf baby spinach	10 g, EN salts, EN dSPE pigment (5982-5356)
High starch	Jasmine rice	3 g rice/7 mL water, EN salts, EN dSPE Fatty (5982-5156)
High water	Basic cucumber	10 g, EN salts, EN dSPE General (5982-5056)
High sugar	Organic honey	5 g honey/5 mL water, EN salts, EN dSPE General (5982-5056)
High acid	Navel orange	10 g, EN salts, EN dSPE Fatty (5982-5156)
Clean 15	Yellow onion (not sweet)	10 g, EN salts, EN dSPE Fatty (5982-5156)

MS Acquisition Method Development

The Agilent MassHunter Pesticide & Environmental Pollutant MRM Database (Rev. A.04.00) and Matrix Optimized Transitions [3] were used to develop the MRM acquisition methods for the evaluation of 195 target pesticides in each matrix (Figure 2). Both the 40 minute and 20 minute constant flow methods referenced in the MRM Database were followed. The top three (highest responding) MRMs for each compound were selected for analysis.

	A	В	C	D	E	F	(
1		Compound Name	CAS #	Target	My Target Compound List		
2	1 Phenol		108-95-2	Target			
3	2 Dimefox		115-26-4	Target	Create New Target List		
4	3 Dichlorob	enzene, 1,2-	95-50-1	Target			
5	4 DBCP (Dib	romo-3-chloropropane, 1,2-)	96-12-8	Target			
6	5 Ethiolate		2941-55-1	Target	Save Current Target List		
7	6 Methamic	lophos	10265-92-6	Target			
8	7 Dichlorvo	3	62-73-7	Target	Manage Target Lists		
9	8 Trichlorfo	n	52-68-6	Target			
10	9 Disulfotor	-sulfoxide	2497-07-6	Target			
11	10 Phthalide		87-41-2	Target	Add Compounds		
12	11 EPTC		759-94-4	Target			
13	12 Mevinpho	s, Z-	338-45-4	Target	Remove Compounds		
4	13 Mevinpho	s, E-	7786-34-7	Target	Nemove compounds		
15	14 Butylate		2008-41-5	Target			
16	15 Acephate		30560-19-1	Target	Import CAS Numbers		
17	16 Acenapht	nene-d10	15067-26-2	Target			
8	17 Heptenop	hos	23560-59-0	Target			
19	18 Omethoat	e	1113-02-6	Target	Build MRM Table		
20	19 Thionazin		297-97-2	Target			
21	20 Propoxur		114-26-1	Target	Home		
22	21 Demeton-	S-methyl	919-86-8	Target	Home		
23	22 Cycloate		1134-23-2	Target			
4	23 Ethopropi	105	13194-48-4	Target			
25	24 Naled		300-76-5	Target			
26	25 Bendiocar	b	22781-23-3	Target			
27	26 Trifluralin		1582-09-8	Target			
28	27 Benflurali	n	1861-40-1	Target			
9	28 Monocrot	ophos	6923-22-4	Target			
0	29 Cadusafo		95465-99-9	Target			
81	30 Phorate		298-02-2	Target			
32	31 BHC-alpha	(benzene hexachloride)	319-84-6	Target			
13	32 Hexachlor	obenzene	118-74-1	Target			

Figure 2. Screen capture of the top portion of the Target Compound List from the P&EP MRM Database (A.04.00).

Table 2. Agilent 7890B GC method conditions.

Parameter	Value
MMI 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)
MS transfer line temperature	280 °C
Oven program (40 minute method)	60 °C for 1 min 40 °C/min to 120 °C, 0 min 5 °C/min to 310 °C, 0 min
Oven program (20 minute method)	60 °C for 1 min 40 °C/min to 170 °C, 0 min 10 °C/min to 310 °C, 3 min
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 are optimized for an application method in an Agilent Laboratory. A 1.5 minute backflush duration may be too short for other methods; recommendations can be made for a 5 minute backflush duration.

Table 3. Agilent 7010A Triple Quadrupole GC/MS time segment (TS) MRM parameters.

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	Wide
Collision cell	1.5 mL/min N_2 and 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.

** The recommended source temperature is 280 °C. The source temperature here was run hotter due to internal lab settings.

Table 4. Agilent 7010A Triple Quadrupole GC/MS dynamic MRM (dMRM) parameters.

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	dMRM unit
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix optimized
Dwell times	Optimized by dMRM*
Source temperature	300 °C
Quad temperatures	150 °C

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

Time Segment Method Development

Time segment acquisition development was completed using the graphical user interface (GUI) in the MRM Database and the MassHunter Compound List Assistant (CLA). The Organic Honey Matrix Optimized MRM Database was used as an example for the TS method development (Figure 3). After the Target List was created, the **Build MRM Table** option was selected (Figure 4). Two selections are needed for the development of the MRM Table:

- Method selection (the 40 minute constant flow method was selected in this example)
- Quantifier and qualifier ion selections (Figure 5)

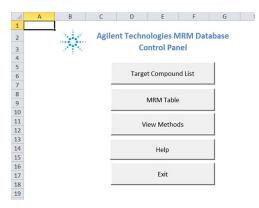


Figure 3. The GUI Homepage of the Organic Honey Matrix Optimized MRM Database, used for TS method development.

E	
My Target Compound List	
Create New Target List	
Save Current Target List	
Manage Target Lists	
Add Compounds	
Remove Compounds	
Import CAS Numbers	Build Master MRM table
Build MRM Table	 Build a new Master MRM table from the current Target Compound List? Any existing MRM Table will be replaced.
Home	Yes No

Figure 4. Selecting **Build MRM Table** from the generated Target Compound List.

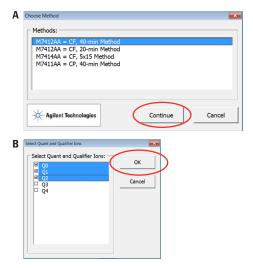


Figure 5. Two selections for MRM Table development: A) Method Selection (the 40 minute M7412AA method option was selected here); B) Quant and Qual Ion selections.

Once the MRM Table was completed, the **Export for CLA Optimizer** option was selected, and the CLA program was launched. The Database saved this export file as a .csv file, and was then imported into the CLA (Figure 6). The optimization parameters were set to use a constant cycle time of 5 msec throughout each TS (Figure 7). The RT deltas can also be edited within the CLA. The method was saved and loaded into MassHunter GC/MS Data Acquisition (DA) B.07.05 (Figure 8).

Q		
Master MRM Table		
Save MRM Table		
Manage MRM Tables	Export for CLA Optimizer	83
Remove Transition	Export the current MRM Master Table to a CSV file for import	
Export For CLA Optimizer	into the CLA Optimizer?	
Create Quant Method	Yes No	
Home		



Edit Help		Ontimize	5									
ounds		Optimize	2									
Compound	RT	ISTD	Precursor ion	MS1	P	Production	MS2	D	well	CE Let RT D	eta Right	RT Time
Phenol	3.614	1	94	Wide	-	66.1	Wide	-	10.0	15	0.10	0.10
Phenol	3.614	1	94	Wide		65.1	Wide	-	10.0	20	0.10	0.10
Phenol	3.614	1	66	Wide	Ŧ	65	Wide	*	Paramet	200		
Dimefox	3.995	1	153	Wide	•	110	Wide	-				
Dimefox	3.995	1	110	Wide	Ŧ	47	Wide	*	V Use	constant cycle time		
Dimefox	3.995	1	110	Wide	-	67	Wide	-		Cycles per second		
Dichlorobenz	4.122	1	146	Wide	-	111.1	Wide	-		S S		
Dichlorobenz	4.122		146	Wide	-	75.1	Wide	-				
Dichlorobenz	4.122	1	111	Wide	-		Wide	*		Use equal dwell time		
DBCP	4.485		155	Wide	-	75	Wide	-	Eleve	Deliver Terry		
DBCP	4.485		157	Wide	-	75	Wide		Rer	nove Duplicate Transitions		
DBCP	4.485		157	Wide	*	77	Wide	*		Default	Optimize	Cancel
Ethiolate	5.576		100	Wide		72	Wide	-		Delaur	Obaurte	Canver
Ethiolate	5.576		161	Wide	*	100	Wide	-	10.0	0	0.10	0.10
Ethiolate	5.576		161	Wide		72	Wide	*	10.0	15	0.10	0.10
Methamidoph	5.868	1	141	Wide	*	95	Wide	-	10.0	5	0.10	0.10
Methamidoph	5.868		95	Wide	*	79	Wide	-	10.0	10	0.10	0.10
Methamidoph	5.868		95	Wide	•	64	Wide	-	10.0	10	0.10	0.10
Dichlorvos	6.163		109	Wide	-	79	Wide	*	10.0	5	0.10	0.10
Dichlorvos	6.163		184.9	Wide	-	93	Wide	-	10.0	10	0.10	0.10
Dichlorvos	6.163		184.9	Wide	-	63	Wide	*	10.0	25	0.10	0.10
Trichlorfon	6.1642		109	Wide	-	78.9	Wide	-	10.0	5	0.10	0.10
Trichlorfon	6.1642		184.9	Wide	-		Wide	-	10.0	15	0.10	0.10
Trichlorfon	6,1642		78.9	Wide	-		Wide	-	10.0	10	0.10	0.10
Disulfoton-sulf	7.3116		96.9	Wide		47	Wide	-	10.0	40	0.10	0.10
Disulfoton-sulf	7.3116		125	Wide		96.9	Wide	-	10.0	5	0.10	0.10
Disulfoton-sulf	7.3116		96.9	Wide	•		Wide	-	10.0	20	0.10	0.10
Phthalide	7.7356		105		*		Wide	-	10.0	15	0.10	0.10
Phthalide	7.7356		105		•		Wide		10.0	40	0.10	0.10
Phthalide	7.7356		77		•		Wide	*	10.0	20	0.10	0.10
EPTC	7.7788		128		*		Wide	-	10.0	5	0.10	0.10
EPTC	7.7788		132		-		Wide	-	10.0	5	0.10	0.10
EPTC	7.7788		189.1		-		Wide	-	10.0	5	0.10	0.10
Mevinphos, Z-	8.996		127		-		Wide	-	10.0	10	0.10	0.10
Mevinphos, Z-	8.996		127		-		Wide	-	10.0	15	0.10	0.10
Mevinphos, Z-	8.996		192		-		Wide	-	10.0	10	0.10	0.10
Butylate	9.024		156		-		Wide	-	10.0	5	0.10	0.10
Butylate	9.024		146.1		-		Wide	-	10.0	5	0.10	0.10
Butylate	9.024		146.1		•		Wide	-	10.0	10	0.10	0.10
Mevinphos, E-	9.031847			Wide	*		Wide	-	10.0	10	0.10	0.10
Mevinphos, E-	9.031847		127	Wide	-	94.9	Wide		10.0	15	0.10	0.10

Figure 7. The acquisition optimization parameters were set to use a constant cycle time, of 5 cycles/sec, throughout each TS.

Edit Help			Optimization Assista										
	0 🕫 💽 🕨 🛛	Optimi:	ze										
ounds													
Compound	RT	ISTD	Precursor ion	MS1		Production	MS2		Dwell	CE	Left RT Delta	RightRT	Time
Phenol	3.614			Wide	•		Wide	•	32.6	20	0.10	0.10	
Phenol	3.614			Wide	•		Wide		32.6	15	0.10	0.10	
Phenol	3.614			Wide	•		Wide	•	32.6	5	0.10	0.10	
Dimefox	3.995			Wide	•		Wide	-	32.6	10	0.10	0.10	
Dimefox	3.995		110	Wide		67	Wide	•	32.6	20	0.10	0.10	
Dimefox	3.995		110	Wide		47	Wide	•	32.6	35	0.10	0.10	
Dimefox	3.995		153	Wide		110	Wide		21.4	10	0.10	0.10	
Dimefox	3.995		110	Wide		67	Wide		21.4	20	0.10	0.10	
Dimefox	3.995		110	Wide		47	Wide	-	21.4	35	0.10	0.10	1
Dichlorobenz	4.122		146	Wide		75.1	Wide		21.4	25	0.10	0.10	
Dichlorobenz	4.122		146	Wide		111.1	Wide	-	21.4	15	0.10	0.10	
Dichlorobenz	4.122		111	Wide	-	75.1	Wide		21.4	10	0.10	0.10	
Dichlorobenz	4.122		146	Wide	-	75.1	Wide		32.6	25	0.10	0.10	
Dichlorobenz	4.122		Save Method								× 0.10	0.10	
Dichlorobenz	4.122		Template method								0.10	0.10	
DBCP	4.485		Please specify a te	mplate me	thed to	create new met	hod (m)				0.10	0.10	
DBCP	4.485										0.10	0.10	
DBCP	4.485		F:\dMRMj20 min Or	ganicHone	w_151	и				Browse	0.10	0.10	
DBCP	4.485	1	Destination: Please specify des	tentine me	Rodo	ath came (m)					0.10	0.10	
DBCP	4.485	17									0.10	0.10	
DBCP	4.485	171	F:\dMRMj20 min Or	ganicHon	W_TS_	Optimized[M				Browse	0.10	0.10	
Ethiolate	5.576	11							ОК	Cancel	0.10	0.10	
Ethiolate	5.576	1							UK	Cancer	0.10	0.10	
Ethiolate	5.576	1	100	Wide		72	Wide		32.6	5	0.10	0.10	
Ethiolate	5.576	1		Wide			Wide		32.6	0	0.10	0.10	
Ethiolate	5.576	1		Wide	-		Wide		32.6	15	0.10	0.10	
Ethiolate	5.576	1		Wide			Wide		32.6	5	0.10	0.10	
Methamidoph	5.868	10		Wide			Wide		32.6	5	0.10	0.10	
Methamidoph	5.868	17		Wide			Wide		32.6	10	0.10	0.10	
Methamidoph	5.868	17		Wide	-		Wide		32.6	10	0.10	0.10	
Methamidoph	5.868	10		Wide	-		Wide	-	32.6	5	0.10	0.10	-
Methamidoph.	5.868			Wide	-		Wide	-	32.6	10	0.10	0.10	
Methamidoph	5.868	1		Wide	•		Wide	-	32.6	10	0.10	0.10	
Methamidoph	5.868	1		Wide			Wide	-	21.4	5	0.10	0.10	
Methamidoph.	5.868	1		Wide			Wide		21.4	10	0.10	0.10	
Methamidoph.	5.868	-		Wide			Wide		21.4	10	0.10	0.10	
	6 163		95		•				21.4	10	0.10	0.10	
Dichlorvos							Wide	_					
Dichlorvos	6.163	1	184.9				Wide	•	21.4	25	0.10	0.10	
Dichlorvos	6.163	1		Wide			Wide		21.4	5	0.10	0.10	
Trichlorfon	6.1642		184.9		•		Wide	-	21.4	15	0.10	0.10	
Trichlorfon	6.1642			Wide	•		Wide		21.4	5	0.10	0.10	

Figure 8. The TS MRM method was saved by the CLA, and made ready for Agilent MassHunter GC/MS Data Acquisition.

Key Elements of TS method development

- Typical method development time: ~ 5 minutes
- 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

dMRM acquisition development was completed using the MS Method Editor within MassHunter Workstation GC/MS Acquisition Software. From within the MS Parameters of MassHunter GC/MS Data Acquisition (B.07.05), the Organic Honey Matrix Optimized MRM Database was imported, and the 40 minute M7412AA constant flow method was selected (Figure 9). The MRM Acquisition Method page is where all of the target compounds for the method are shown (Figure 10). The Compound Browser was used to locate target compounds and their respective MRMs (the same target list and ions were used as the TS method development). Once chosen, the MRMs are applied to the Import List (Figure 11). The Import List maintains all of the target compounds that are to be used in the method, and their respective MRMs. Once the target list is finalized, they are imported to the Method (Figure 12). The Method Acquisition page is where the RT deltas can be edited, the cycles/sec can be defined, and the dwell times are optimized (Figure 13). Figure 14 displays a view of the 20 minute dMRM acquisition method for the same Target List and respective MRMs.

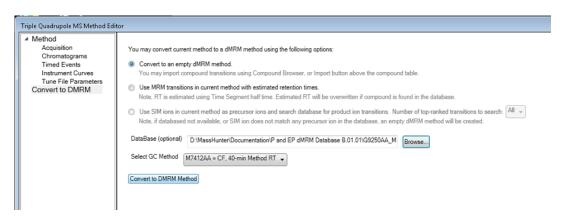


Figure 9. The Organic Honey Matrix Optimized MRM Database was imported into the MS Parameters of Agilent MassHunter GC/MS Data Acquisition B.07.05.

Method	Tune File	O manufacture in the second se	dMRM Statistics
Acquisition		Compound Table	Total MRMs 1
Chromatograms Timed Events	12MAY2016_01_noClean eihs Browse	国際× 県島県 米国 米国的 わべ	Number of MRMGroups 1
Instrument Curves Tune File Parameters	Source Parameters	Enabled Compound CAS# ISTD Precursor Ion MST Resolution Product Ion MS2 Resolution RT Left RT delta Right RT delta Average Dwell CE	Minimum Concurrent MRMs 1
Compound Browser	Ion Source El	Name CHOW INTO PROJECT IN HIST REDUCTION HIST REDUCTION HIST REDUCTION INTO DEL NOT DELL'ENTRE CEL	Maximum Concurrent MRMs 1
Update RT	Source Temperature ("C) 300.0		Ninimum Dwell Time (ms) 99.3
	Electron Energy Mode Use Tune Setting		Maximum Dwell Time (ms) 99.3
	Electron Energy (eV) 70.0		
	Detector Setting		Minimum Cycle Time (ms) 1.2 (hardware limit)
	Use Gain Factor 10.0		Parameters
	Use Delta EMV		Custon Res Second
	Calculated EMV 1426.8		(data points for a 1sec peak) 10.0
	EM Saver Limit		Cycle Time (ms) 100.0
	Data Saved		Min Dwell Time (ms) 4.0
	Run Time		
	Run time (min) 40.75		
	Solvent Delay (min) 3.25		
	and the second second second second		Overwrite
	Automatically Subtract Baseline Marca MRM/SIM filtering		
		Both W 1015 Sam Miss Sam	Overwrite Delta RT
		Plot Type Concurrent MRMS . Elect Transition Cin Click	
		10-1 Concurrent MEMS	
		99-1- 8 8 8 7-7- 7-7- 5-5- 5-	
		02 04 06 08 1 12 14 16 18 2 22 24 26 28 3 32 34 36 38 4 42 44 46 48 5 52 54 56 58 6 62 64 66 68 7 72 74 76	78 8 82 84 86 88 9 92 94 96
		¹⁰ 02 04 06 08 1 12 14 15 18 2 22 24 26 28 3 32 34 36 38 4 42 44 46 48 5 52 54 56 58 5 62 64 65 68 7 72 74 76 Reterior Time (min)	7.8 8 8.2 8.4 8.6 8.8 9 9.2 9.4 9.6

Figure 10. A blank Agilent MRM Acquisition Method page.

	Sea	rch/Fil	ter Import	List																
s sters		Select GC	Method	M7412AA =	CF, 40-min Method RT	•	🗐 Sh	ow All Records												
	Filter						Compo	und List					Sea	rch Text				Columns		
- 1	E	Target List	Name	Molecular Fo	ormula ~		2.4.5- 2.4-D	F methyl ester hutyl ester					*					Compound	I Name	
		Group Nar	THE	Algaecide: F	ungicide; Nematicide 🔍		Aceph Alachi Aldrin Ametr Amino Atrazii Azinol	or carb te tos-ethyl tos-methyl coarb										Formula Formu	ne Ion	
							Select	All Select					Sea	arch Compou	nds					
	N	umber of to	op-ranked transition	s to select: 3 •	1							**						Ranked By	Abundance	Re
- 1		-	Formula	Molecular Weight	Compound Name	CAS#	ISTD	Precursor Ion	Product Ion	RT	Left RT delta	Right RT delta	RT Window	v Dwell	CE	Abundance	Response /			
- 1		V		94.1112	Phenol	108-95-2	1	94	66.1	3.61	.1	.1	0.2	10	15	1.00	8.001464E+07	1		
- 1		1	CEHEO	94.1112	Phenol	108-95-2	1	94	65.1	3.61	.1	.1	0.2	10	20	0.78	62343536			
- 1		1	CEHEO	94.1112	Phenol	108-95-2	13	66	65	3.61	.1	.1	0.2	10	5	0.33	26318358			
- 1		1	CEHEO	94.1112	Phenol	108-95-2		95	67.1	3.61	.1	.1	0.2	10	15	0.08	6266749			
- 1		E3	C6H6O	94.1112	Phenol	108-95-2	1	66	51	3.61	.1	.1	0.2	10	20	0.06	4997724			
- 1		1	C4H12FN2OP	154.1	Dimefox	115-26-4		153	110	4	.1	.1	0.2	10	10	1.00	5431075			
- 1		4	C4H12FN2OP	154.1	Dimefox	115-26-4		110	47	4	.1	.1	0.2	10	35	0.89	4814943			
- 1		1	C4H12FN2OP	154.1	Dimefox	115-26-4		110	67	4	.1	.1	0.2	10	20	0.61	3320161			
- 1			C4H12FN2OP		Dimefox	115-26-4		111	109.9	4	.1	.1	0.2	10	0	0.58	3177175			
- 1			C4H12FN2OP		Dimefox	115-26-4		154	58	4	.1	.1	0.2	10	-	0.39	2120272			
- 1		1		147	Dichlorobenzene, 1,2-			146	111.1	4.12	.1	.1	0.2	10	15	1.00	7.490563E+07			
- 1		1		147	Dichlorobenzene, 1,2-			146	75.1	4.12	.1	.1	0.2	10		0.91	6.85012E+07			
1		1		147	Dichlorobenzene, 1,2-			111	75.1	4.12	.1	.1	0.2	10		0.63	46951176			
1				147	Dichlorobenzene, 1,2-			148	75.1	4.12	.1	.1	0.2	10		0.62	46592972			
1		1		147	Dichlorobenzene, 1,2-			148		4.12	.1	.1	0.2	10		0.38	2.834693E+07			
1				236.3	DBCP	96-12-8		155	75	4.49	.1	.1	0.2	10	5	1.00	5.02397E+07			
-1		4		236.3 236.3	DBCP	96-12-8 96-12-8		157	75 77	4.49	.1	.1	0.2	10	~	0.99	49864544 1.739746E+07			
1				236.3 236.3	DBCP	96-12-8 96-12-8		157	77	4.49	.1		0.2	10	0	0.35	1.739746E+07 17152336			
1		13		236.3	DBCP	96-12-8 96-12-8	0	155	49	4.49	1	1	0.2	10	30	0.34	1,298091E+07			
1				230.5	Ethiolate	2941-55-1		100	43 72	5.58	.1	.1	0.2	10	5	1.00	7.155342E+07			
1		2		161.3	Ethiolate	2941-55-1	0	161	100	5.58	1	1	0.2	10		0.21	15121155			
1		V		161.3	Ethiolate	2941-55-1	E. 1	161	72	5.58	.1	1	0.2	10		0.11	7940758			
1				161.3	Ethiolate	2941-55-1	-	118	90	5.58	1	.1	0.2	10		0.02	1435439			
1		1		161.3	Ethiolate	2941-55-1	0	118	58	5.58	.1	.1	0.2	10		0.01	690654			
1		1		141	Methamidophos	10265-92-6		141		5.87	.1	.1	0.2	10	5	1.00	11401272			
1		1	C2H8NO2PS		Methamidophos	10265-92-6		95	79	5.87	.1	.1	0.2	10	10	0.82	9312170			
1		-			14	*****		05	~	5.07			0.0	+0	*0	0.00				

Figure 11. The compound browser displays all of the compounds and respective MRMs that are within the loaded Database.

X IR IP													
Search/Filter Impo	Search/Filter Import List												
Compound Name	CAS#	ISTD	Precursor Ion	Product Ion	RT	Left RT delta	Right RT delta	Dwell	CE	Abundance			
rs 2.4.5-T methyl ester	1928-37-6		109	74	16.01	.1	.1	10	25	0.61			
2,4,5-T methyl ester	1928-37-6		233	190	16.01	.1	.1	10	15	0.77	=		
2.4.5-T methyl ester	1928-37-6		268	233.1	16.01	.1	.1	10	10	1.00			
2,4-D butyl ester	94-80-4		174.9	111	17.93	.1	.1	10	10	0.61			
2.4-D butyl ester	94-80-4		185	155	17.93	.1	.1	10	20	0.67			
2,4-D butyl ester	94-80-4		162	63	17.93	.1	.1	10	35	1.00			
Acenaphthene-d10	15067-26-2	1	160.1	158.1	10.05	.1	.1	10	20	0.18			
Acenaphthene-d10	15067-26-2	4	162.1	160.1	10.05	.1	.1	10	20	0.94			
Acenaphthene-d10	15067-26-2	4	164.1	162.1	10.05	.1	.1	10	15	1.00			
Acephate	30560-19-1		78.9	47	9.08	.1	.1	10	10	0.22			
Acephate	30560-19-1		142	96	9.08	.1	.1	10	5	0.24			
Acephate	30560-19-1		136	94	9.08	.1	.1	10	15	1.00			
Alachior	15972-60-8		160.1	132.1	18.41	.1	.1	10	15	0.41			
Alachior	15972-60-8		188.1	132.1	18.41	.1	.1	10	20	0.42			
Alachior	15972-60-8		188.1	160.1	18.41	.1	.1	10	10	1.00			
Aldrin	309-00-2		264.9	192.9	19.57	.1	.1	10	35	0.64			
Aldrin	309-00-2		262.9	190.9	19.57	.1	.1	10	35	0.66			
Aldrin	309-00-2		262.9	192.9	19.57	.1	.1	10	35	1.00			
Ametryn	834-12-8		185	170	18.46	.1	.1	10	5	0.68			
Ametryn	834-12-8		227	170.1	18.46	.1	.1	10	10	0.70			
Ametryn	834-12-8		227	58.1	18.46	.1	.1	10	10	1.00			
Aminocarb	2032-59-9		136	77	15.67	.1	.1	10	25	0.68			
Aminocarb	2032-59-9		150	134	15.67	.1	.1	10	20	0.96			
Aminocarb	2032-59-9		151	136.1	15.67	.1	.1	10	15	1.00			
Atrazine	1912-24-9		200	94	15.29	.1	.1	10	20	0.80			
Atrazine	1912-24-9		214.9	200.2	15.29	.1	.1	10	5	0.86			
Atrazine	1912-24-9		214.9	58.1	15.29	.1	.1	10	10	1.00			
Azinphos-ethyl	2642-71-9		160	77.1	30.6	.1	.1	10	20	0.79			
Azinphos-ethyl	2642-71-9		160	132.1	30.6	.1	.1	10	0	0.82			
Azinphos-ethyl	2642-71-9		132	77.1	30.6	.1	.1	10	15	1.00			
Azinphos-methyl	86-50-0		132.1	77	29.34	.1	.1	10	15	0.79			
Azinphos-methyl	86-50-0		160	77	29.34	.1	.1	10	20	0.81			
Azinphos-methyl	86-50-0		77	51	29.34	.1	.1	10	15	1.00			
Bendiocarb	22781-23-3		126	108	13.8	.1	.1	10	5	0.34			
Bendiocarb	22781-23-3		126	52.1	13.8	.1	.1	10	15	0.65			
Bendiocarb	22781-23-3		166	151.1	13.8	.1	.1	10	10	1.00			
Benfluralin	1861-40-1		275.9	202.1	14.01	.1	.1	10	15	0.23			
Benfluralin	1861-40-1		292	206	14.01	.1	.1	10	10	0.52			
Benfluralin	1861-40-1		292	264	14.01	.1	.1	10	5	1.00			
Benthiavalicarb-isopropyl	177406-68-7		180	127	29.69	.1	.1	10	20	0.68			
Benthiavalicarb-isopropyl	177406-68-7		72	55	29.69	.1	.1	10	10	0.89			
Benthiavalicarb-isopropyl	177406-68-7		180	83	29.69	.1	.1	10	35	1.00			
BHC-alpha	319-84-6		218.9	183	14.29	.1	.1	10	5	0.93	*		

Figure 12. The Import List maintains all of the target compounds and their respective MRMs that are intended for the acquisition method.

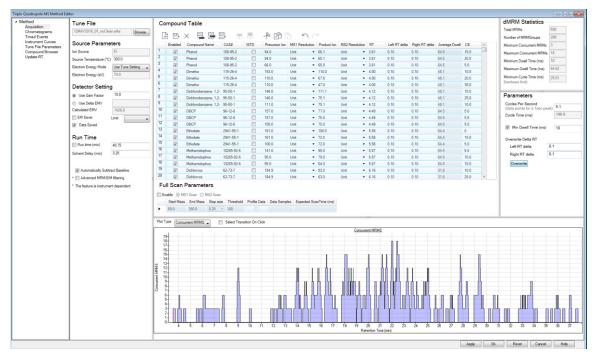


Figure 13. The Method Acquisition page shows the Target List and respective MRMs for the 40 minute method.

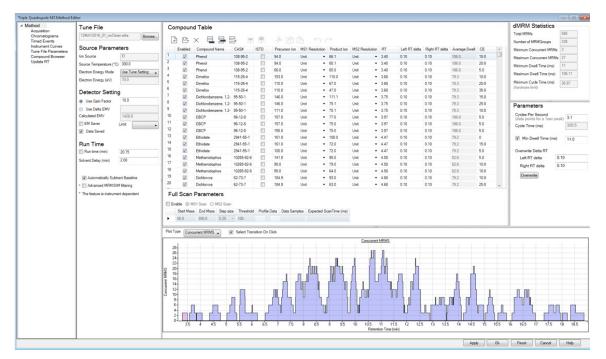


Figure 14. The Method Acquisition page shows the same Target List and respective MRMs for the 20 minute method.

Key elements of dMRM method development

- Typical method development time: ~5-10 minutes depending on how detailed the MS method is
- Adding target compounds: One-by-one selection, group selection, or searching a 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 dMRM acquisition method provides users with another way to set up their MS acquisition method parameters. Whether the user chooses to use TSs or the dMRM functionalities, they both aid in achieving optimal analysis. Figures 15-20 are various selected chromatograms that were observed and analyzed in both TS and dMRM acquisition methods.

Results and Discussion

There are two ways to view the difference in the chromatographic displays:

- MassHunter Qualitative Analysis Software (B.07.00 SP1, or later)
- MassHunter Quantitative Analysis Software (B.07.01, or later)

Figures 19-26 show a selected representation of the 195 target compounds in various matrices. The concentration shown for the various target analytes ranged between 180-380 ppb. A higher concentration was used for viewing ability; further analysis was done showing that 90% of all target compounds achieved a calibration curve with $R^2 \ge 0.990$. All analyzed pesticides obtained a %RSD of repeated measurements of $\le 30\%$, and 90% of the analyzed pesticides were found to have a limit of quantitation (LOQ) $\le 1.5 \text{ pg/}\mu\text{L}$ [3].

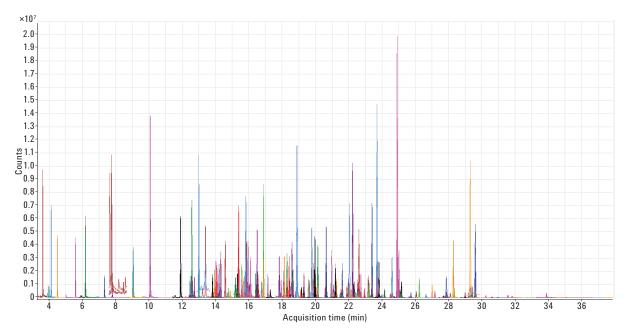


Figure 15. Organic honey 40 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the TS MS parameters.

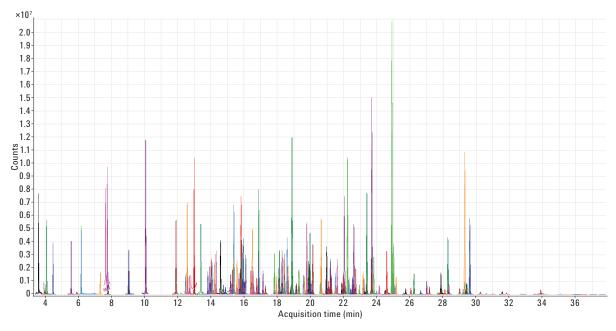


Figure 16. Organic honey 40 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

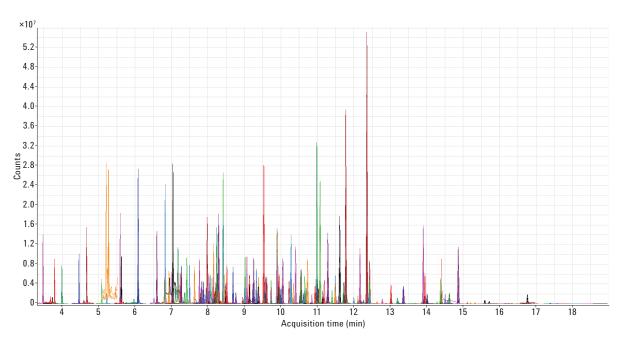


Figure 17. Organic honey 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

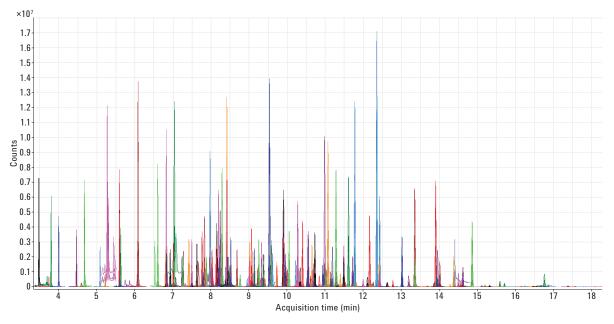


Figure 18. Extra virgin olive oil 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

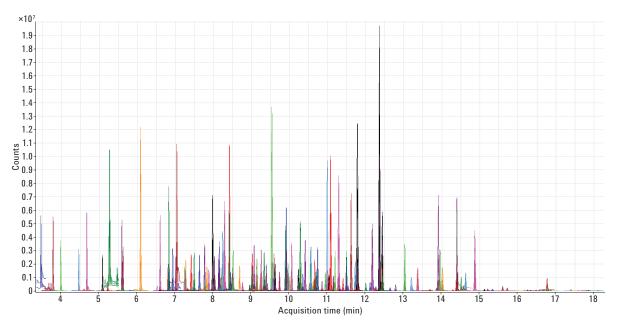


Figure 19. Navel orange 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

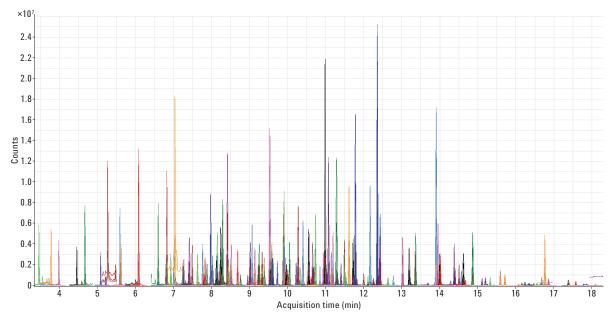


Figure 20. Fresh leaf baby spinach 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

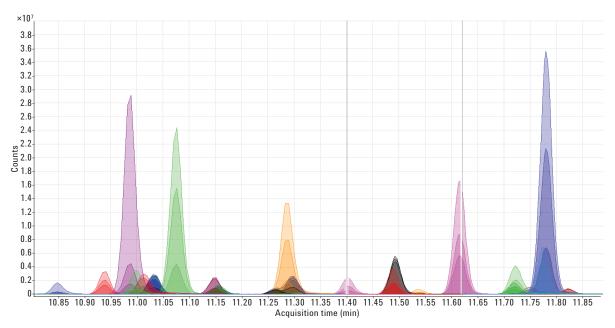


Figure 21. Organic honey TS chromatogram of RT range (40 minute method) in Agilent MassHunter Qualitative Analysis.

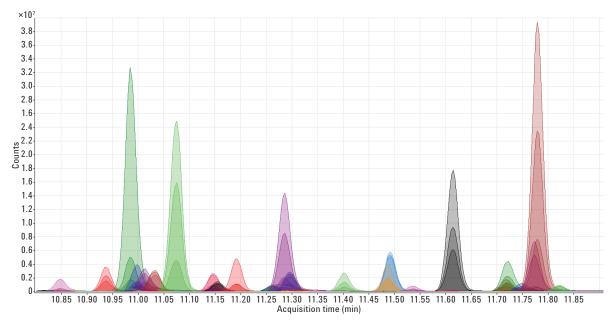


Figure 22. Organic honey dMRM chromatogram of RT range (40 minute method) in Agilent MassHunter Qualitative Analysis.

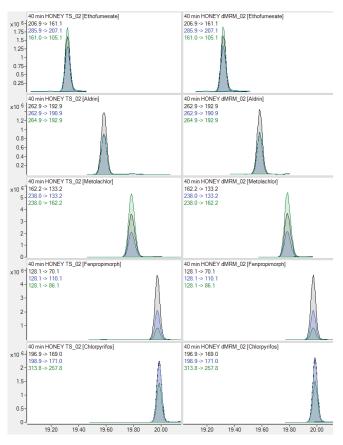


Figure 23. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (40 minute method) in Agilent MassHunter Quantitative Analysis.

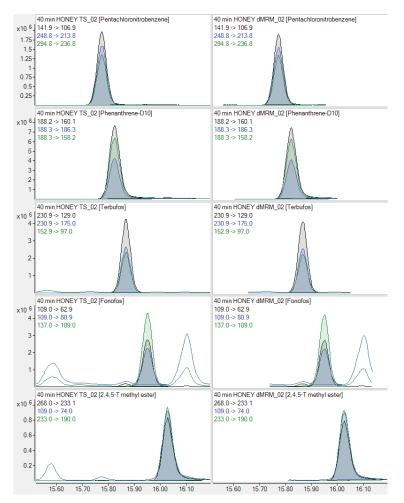


Figure 24. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (40 minute method) in Agilent MassHunter Quantitative Analysis.

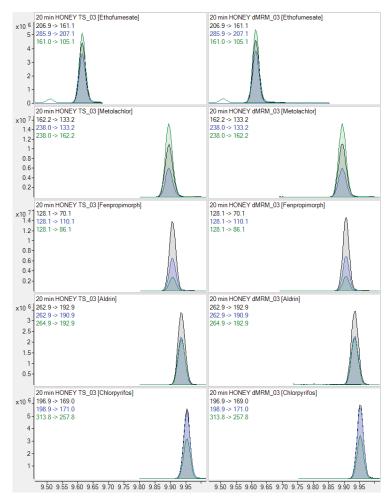


Figure 25. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (20 minute method) in Agilent MassHunter Quantitative Analysis

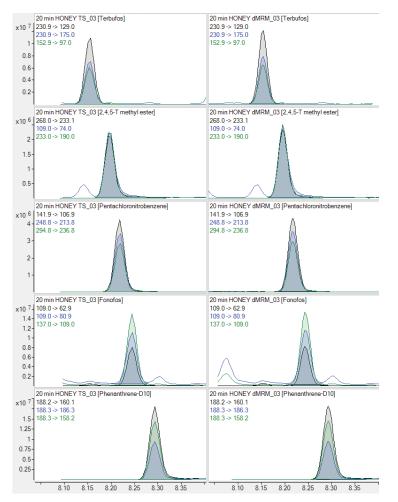


Figure 26. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (20 minute method) in Agilent MassHunter Quantitative Analysis.

Conclusions

Standard GC/MS/MS Pesticide methods use TS acquisition methods with a gain of 10, dwell times of 10 msec, and 2-3 MRMs/compound. The Agilent MassHunter Data Acquisition's dMRM functionality for MS acquisition method development provides users the ability to achieve equivalent or better quality data and results by:

- Monitoring the MRM transitions based on the compounds' retention times as they elute from the GC
- Reducing the number of MRM transitions active at any given time allowing for longer dwell times
- 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 use dMRM 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.

References

- Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. S. J. AOAC Int. 2003, 86, 412-431.
- Lehotay, S. J.; Mastovská, K.; Lightfield, A. R. J. AOAC Int. 2005, 88, 615-629.
- Westland, J.; Stevens, J. An Optimal Method for the Analysis of Pesticides in a Variety of Matrices; Application note, Agilent Technologies, Inc. Publication number 5991-7303EN, 2016.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 2016, 2017 Printed in the USA April 12, 2017 5991-7302EN

