An Optimal Method for the Analysis of Pesticides in a Variety of Matrices

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NACRW 2016
P-070

Agilent Technologies

Introduction

The global agriculture industry uses over a thousand pesticides for the production of food and foodstuffs. Producers require pesticides to meet the increasing demand for reasonably priced food both in and out of season. This growing demand has increased the use of pesticides and expanded poor agricultural practices elevating risks in the food supply and environment. Analytical laboratories are then strained to evaluate and quantitate hundreds of pesticides in a wide range of matrices. Not only are laboratories faced with time constraints, but they also face matrix interferences that degrade their ability to accurately identify and quantitate the multitude of target pesticides.

The MassHunter Pesticide & Environmental Pollutant MRM Database (Rev. A.01.01) is the most comprehensive GC MRM Database on the market. With over 1000 compounds with at least 8 MRMs/compound, analysts have the ability to optimize their acquisition methods for their target compounds in a variety of matrices. The availability of multiple MRM transitions not only helps to address matrix interferences, but it also aids in accurately identifying compounds that may have several MRMs in common.

Matrix interferences have been a common complaint for MRM acquisitions in pesticides analysis. It has been seen that the usefulness of a given compound's MRMs can change depending on the matrix being measured, due to factors such as increased/decreased response (which changes the quant and qual ions). The ability to have multiple MRMs from which to choose aids in lab productivity, improved quant method generation, and achieving optimal analysis.

Experimental

Methodology

The analysis was conducted on an Agilent 7890B GC and 7010 Series Triple Quadrupole GC/MS system. See Tables 1 – 3 for method parameters. The system was configured with a Multimode Inlet equipped with an ultra-inert liner (p/n: 5190-2293). The inlet was then connected to two HP-5ms UI columns (15 m × 0.25 mm × 0.25 µm; p/n: 19091S-431 UI) coupled to each other through a purged ultimate union (PUU) for the use of backflushing (see Figure 1).

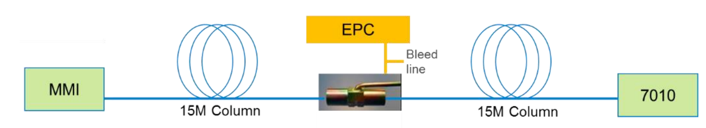


Figure 1. Column Configuration for Optimal MRM Application.

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.

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		

Table 3. 7010 MS/MS 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

Sample Prep

A selection of matrices were chosen over a variety of categories. Table 4 provides the chosen matrix per category and a quick look at the QuEChERS sample prep procedure that was followed.

Table 4. 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)

Matrix Optimized MRMs

Identification of Matrix Optimized MRM Transitions

Agilent Technologies offers the most comprehensive GC MRM Database for Pesticides and Environmental Pollutants (PN: G9250-60006). The MRM Database contains 1000+ compounds and up to 10 MRMs/compound. The all-inclusive database provides a surplus of MRMs to aid in accurate identification, utilize MRMs that fall within the ion ratio confidence limits, and avoid matrix interferences.

Across the globe there are a multitude of different applications and regulations that are followed. The P&EP MRM Database provides all of the material for users to identify the optimal MRMs for their specific analysis. In order to provide guidance on the optimal use of these MRMs, Agilent has begun to look at target compounds in a variety of matrices.

A total of 195 compounds were selected for the analysis. Each compound was analyzed in each of the 8 matrices and in acetonitrile (ACN). The top 5 MRM transitions for each target compound were selected based on response, ion ratio, and selectivity. From these, the top 3/4 MRMs were transferred to a matrix specific method for further optimal analysis.

Please note that due to the large amount of data collected, not all observations will be detailed in this poster. Contact the authors for further information.

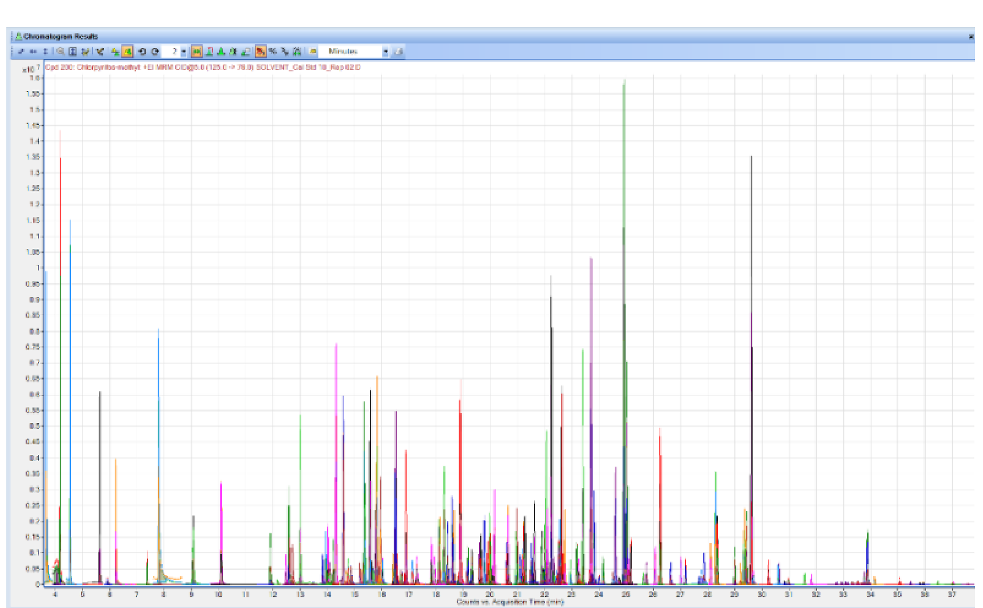


Figure 2. Chromatogram of all Target Compounds in Acetonitrile (~200 – 400 ppb; compound dependent)

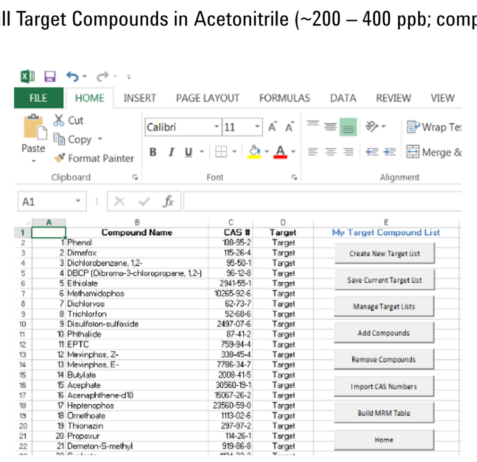
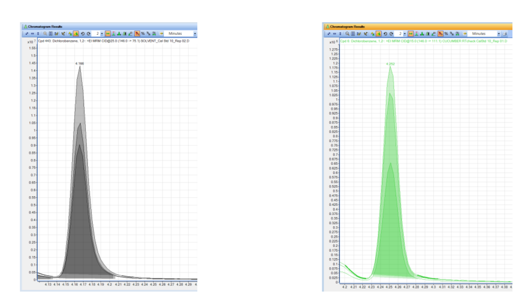


Figure 3. Screen capture of top portion of Target Compound List from the P&EP MRM Database (A.01.01)

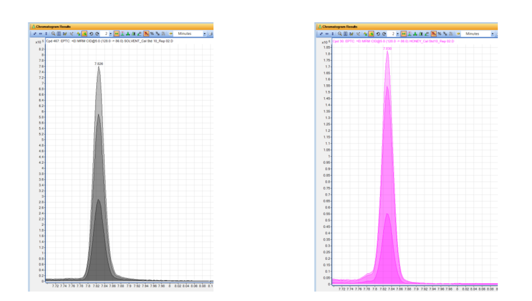
Changes in Quant (Q0) and Qualifier Ions (Q1, Q2, ...)

The majority of pesticides analyzed indicated that the responses of the optimal MRM transitions often change.



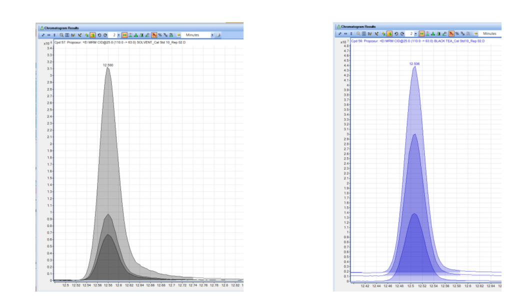
1,2-Dichlorobenzene in ACN — 1,2-Dichlorobenzene in Cucumber

Solvent MRMs			Cucumber MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
148	75.1	25	146	75.1	25
111	75.1	10	146	111.1	15
146	75.1	25	111	75.1	10



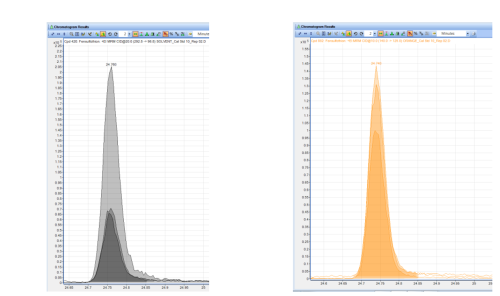
EPTC in ACN — EPTC in Organic Honey

Solvent MRMs			Organic Honey MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
128	86	5	128	86	5
132	90	5	132	90	5
132	62	10	189.1	128	5



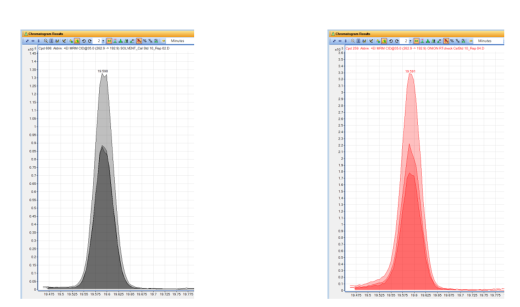
Propoxur in ACN — Propoxur in Black Tea

Solvent MRMs			Black Tea MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
110	63	25	110	63	25
152	110	10	110	64	15
110	92	10	152	110	10



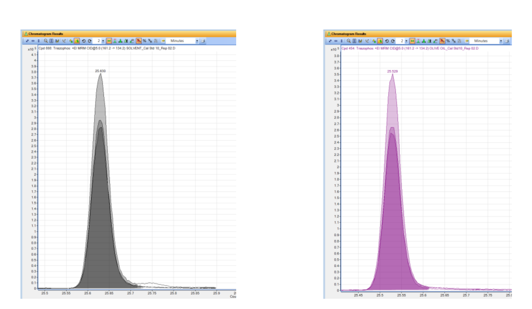
Fensulfothion in ACN — Fensulfothion in Navel Orange

Solvent MRMs			Navel Orange MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
292.8	96.8	20	140	125	10
140	125	10	156	141	10
156	141	10	291.8	156	15



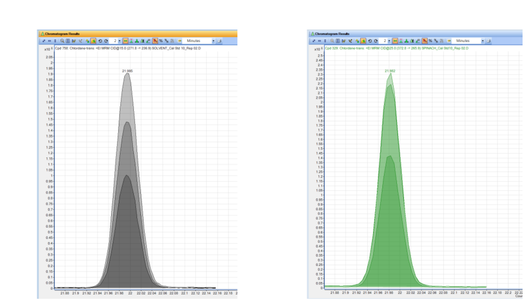
Aldrin in ACN — Aldrin in Yellow Onion

Solvent MRMs			Yellow Onion MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
262.9	192.9	35	262.9	192.9	35
254.9	220	20	262.9	190.9	35
262.9	190.9	35	254.9	220	20



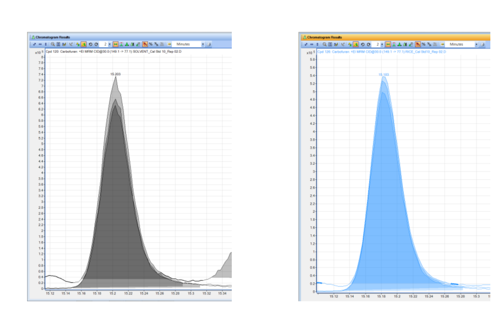
Triazophos in ACN — Triazophos in Olive Oil

Solvent MRMs			Olive Oil MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
161.2	134.2	5	161.2	134.2	5
161.2	106.1	10	161.2	106.1	10
257	162.1	5	161.2	91	15



trans-Chlordane in ACN — trans-Chlordane in Spinach

Solvent MRMs			Spinach MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
271.8	236.9	15	372.8	265.9	25
372.8	265.9	25	271.7	236.9	15
374.8	265.8	15	374.8	265.8	15

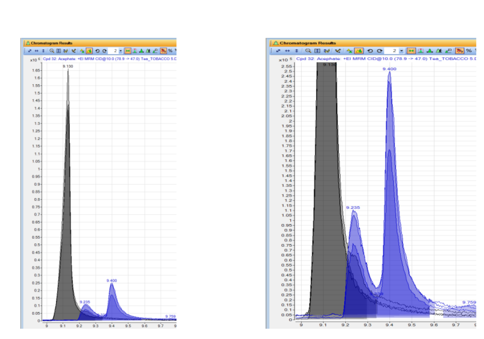


Carbofuran in ACN — Carbofuran in Jasmine Rice

Solvent MRMs			Jasmine Rice MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
149.1	77.1	30	151	136.1	15
164.2	149.1	10	150	134	20
164.2	103.1	25	136	77	25

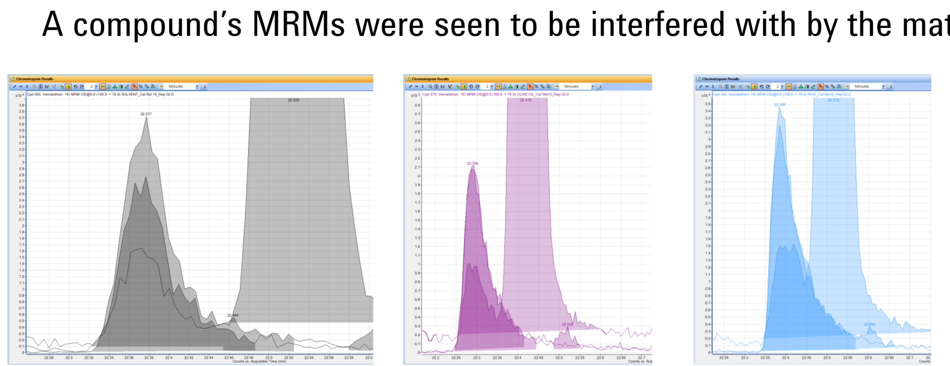
Matrix Interferences Are Real

A compound's MRMs were seen to be interfered with by the matrix.



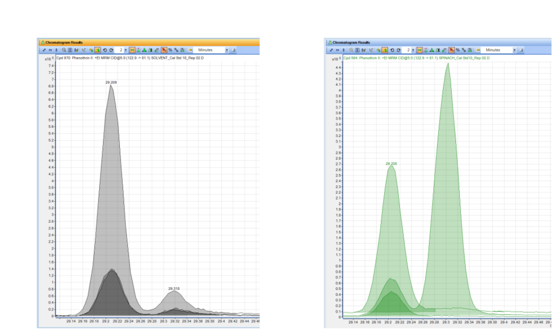
Acephate in ACN — Acephate in Black Tea

Solvent MRMs			Black Tea MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
136	94	15	142	96	5
78.9	47	10	78.9	47	10
142	96	5	124.9	47	15



Vamidothion in ACN — Vamidothion in Olive Oil — Vamidothion in Jasmine Rice

Solvent MRMs			Olive Oil MRMs			Jasmine Rice MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
141.9	78.9	10	145	87	5	145	87	5
145	87	5	141.9	78.9	10	141.9	78.9	10
108.9	78.9	5	108.9	78.9	5	108.9	78.9	5



Phenothrin II in ACN — Phenothrin II in Spinach

Solvent MRMs			Spinach MRMs		
m/z	m/z (prod.)	CE	m/z	m/z (prod.)	CE
122.9	81.1	5	122.9	81.1	5
182.9	168.1	10	122.9	79.1	20
182.9	153.1	15	182.9	168.1	10

Results

The 7010 Series Triple Quadrupole GC/MS system can confirm pesticide residues at the low ppb level even in the most complex extracts. The calibration standards were prepared at concentrations ranging from 0.12 pg/µL to 50 pg/µL; for 90% of compounds a calibration curve with a R2 ≥ 0.990 was produced. All analyzed pesticides obtained a %RSD of repeated measurements of ≤ 30%, and 90% have a LOQ ≤ 1.5 pg/µL.

A representative selection of compounds and their calculated values are shown for Organic Honey and Baby Spinach compared to ACN solvent.

CMPD	SOLVENT					ORGANIC HONEY					BABY SPINACH				
	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error
Ethoprophos	11.13	0.386	0.408	1.477	5.72	8.72	0.303	0.293	1.061	3.11	8.25	0.286	0.295	1.068	3.18
Phorate	12.40	0.429	0.453	1.640	5.78	29.01	1.002	1.023	3.699	2.01	16.11	0.557	0.549	1.987	1.33
BHC-alpha	9.38	0.325	0.343	1.241	5.51	7.83	0.271	0.261	0.943	4.01	7.94	0.275	0.276	0.997	0.16
Dazomet	11.15	0.386	0.412	1.492	6.81	4.38	0.152	0.152	0.552	0.45	9.10	0.315	0.322	1.165	2.22
BHC-beta	9.27	0.321	0.340	1.229	5.80	17.19	0.596	0.541	1.959	9.10	8.76	0.304	0.303	1.096	0.24
Aminocarb	19.89	0.690	0.737	2.665	6.75	8.40	0.291	0.285	1.032	2.09	9.76	0.339	0.362	1.310	6.91
Phenanthrene-D10	7.68	0.266	0.280	1.014	5.19	6.59	0.229	0.217	0.786	4.92	8.95	0.311	0.312	1.129	0.49
Diazinon	9.63	0.333	0.352	1.274	5.69	7.33	0.254	0.238	0.862	6.15	5.78	0.200	0.201	0.728	0.46
Iprobenfos	21.38	0.740	0.784	2.837	6.04	4.69	0.162	0.157	0.569	2.94	17.14	0.593	0.624	2.257	5.26
2,4-D butyl ester	15.67	0.541	0.576	2.083	6.35	8.09	0.280	0.260	0.940	7.08	19.66	0.679	0.687	2.485	1.13
Chlorpyrifos-methyl	9.96	0.345	0.364	1.316	5.37	7.76	0.269	0.252	0.910	6.42	7.04	0.244	0.244	0.884	0.20
Triadimefon	12.71	0.440	0.464	1.680	5.41	4.26	0.148	0.137	0.497	6.97	10.17	0.352	0.363	1.313	2.95
Terbufos sulfone	12.14	0.419	0.444	1.605	5.78	3.46	0.119	0.112	0.404	6.54	14.01	0.484	0.482	1.745	0.33
Heptachlor endo-epoxide	9.57	0.662	0.698	2.526	5.41	7.75	0.536	0.493	1.783	8.13	7.17	0.496	0.490	1.774	1.25
Flurenol-butyl	9.09	0.311	0.328	1.185	5.47	6.85	0.234	0.219	0.793	6.32	18.80	0.642	0.650	2.350	1.13
Haloxyfop-r-methyl	12.54	0.436	0.460	1.664	5.60	6.74	0.234	0.217	0.786	7.20	17.48	0.607	0.614	2.222	1.18
Chlordane-cis	8.35	0.290	0.305	1.103	5.26	13.08	0.453	0.411	1.486	9.42	21.67	0.751	0.750	2.713	0.14
DDT-o,p'	4.42	0.153	0.162	0.585	5.31	8.78	0.305	0.270	0.977	11.35	23.04	0.799	0.785	2.839	1.84
Hexazinone	11.71	0.407	0.431	1.558	5.67	4.91	0.171	0.157	0.569	7.85	7.40	0.257	0.263	0.951	2.10
Benthiavalicarb-isopropyl	16.32	0.561	0.596	2.157	6.24	14.22	0.489	0.437	1.583	10.49	19.13	0.658	0.668	2.415	1.51
Azinphos-ethyl	9.01	0.312	0.329	1.191	5.45	13.77	0.477	0.436	1.579	8.53	16.08	0.557	0.564	2.042	1.25
Permethrin, (1R)-trans-	10.89	0.377	0.396	1.431	5.05	10.25	0.354	0.335	1.211	5.57	22.36	0.773	0.793	2.870	2.56
Ethofenprox	9.95	0.349	0.368	1.331	5.35	16.50	0.579	0.539	1.951	6.88	28.66	1.006	1.026	3.714	2.03
Difenoconazole II	28.94	1.001	1.062	3.843	6.14	19.99	0.691	0.663	2.399	4.12	27.37	0.947	0.973	3.522	2.82

Conclusions

The growing demand on the global agriculture industry has increased the number of targeted pesticides and expanded to include a multitude of matrices. Not only are analytical laboratories faced with time constraints, but they also face matrix interferences that degrade their ability to accurately identify and quantitate the multitude of target pesticides.

Eight various matrices that span over multiple varieties the following observations were identified:

- Changes in Quant (Q0) and Qualifier ion (Q1, Q2, ...) responses are the most common. These changes merely affect the relative abundances of the MRMs which plays a part in method development for optimum quantitative data analysis.
- The availability of multiple MRMs per compound allows a user to discriminate among compounds with similar transitions, and to select MRMs that fulfill desired ion ratio confidence limits.
- The main challenges come from extremely large matrix interferences, which are encountered more often in complex matrices such as Loose Leaf Black Tea or Spinach. The number of usable MRMs for a given target compound can be reduced, and the shift in RT can push a target out of a Time Segment. In these cases great care must be exercised to produce accurate results for all analytes.

Matrix interferences are a common complaint for MRM acquisitions in pesticides analysis and can alter a target compound's MRMs. Matrix Optimized MRM Transitions aid in lab productivity, improved quant method generation, and optimal analysis!

To help provide customers with their optimal pesticides analysis! Agilent's MassHunter P&EP MRM Database (Rev. A.01.01) is the most comprehensive GC MRM Database on the market. With the evolving market and demand for matrix optimized transitions, Agilent will add to the value of their MRM Database with the addition of 7800 matrix optimized MRM transitions!