Comprehensive Pesticide Analysis in Juice Using a Combination of GC/MS and LC/MS Methods

Application Note

Food Safety

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Abstract

Comprehensive detection of pesticides in food matrices requires a combination of both GC/MS and LC/MS techniques. Methods have been developed on Agilent GC/MS and LC/MS instrument platforms that reliably detect and quantitate 39 pesticides in a vegetable juice matrix. Most of the limits of quantitation (LOQ) values for GC/MS SIM ranged from 5 to 50 ng/mL, compared with 0.25 ng/mL to 10 ng/mL for GC/MS/MS, and 0.1 ng/mL to 10 ng/mL for LC/MS/MS. Both sensitivity and selectivity are improved using MS/MS and recovery of most spiked pesticides ranged from 70 to 120%.

Introduction

With the increasing globalization of the food industry, there is greater scrutiny on food safety, resulting in major changes in the number of pesticides that are being regulated and monitored, as well as the allowable levels of those pesticides in food. There are more than 1,000 registered pesticides in the US, and approximately 400 with tolerances established by the Environmental Protection Agency (EPA) and enforced by the Food and Drug Administration (FDA). The European Union (EU) and Japan also strictly regulate pesticide residues in food, setting maximum residue levels (MRLs) for food and animal feed. While these levels vary, the default tolerance is 10 parts per billion (ppb).

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Food testing laboratories, therefore, require the ability to detect and quantify hundreds of pesticides, in a myriad of foodstuffs, at very low levels of contamination. No single analytical approach can provide the flexibility required to meet this need. Wide variations in the chemical properties of pesticide contaminants and the necessity to detect a very large number of compounds require a range of chromatography and mass spectrometry systems. For pesticides that can be easily vaporized without degradation, GC/MS (gas chromatography-single quadrupole mass spectrometry) is an ideal analytical tool, due to the availability of large libraries of pesticide spectra and deconvolution software. Complex foodstuffs can require GC/MS/MS (GC-tandem mass spectrometry) analysis to provide the required selectivity and sensitivity in a matrix containing a very large number of background compounds. Pesticides such as carbamates and organophosphates that are quite polar, not easily vaporized, thermally labile, or not easily derivatized, are best analyzed using liquid chromatography (LC) methods. LC/MS/MS is particularly useful for analyzing sets of known target pesticides, due to its sensitivity and specificity in complex food matrices.

This application note illustrates the effective use of these three mass spectral techniques for the comprehensive analysis of 39 pesticide residues in vegetable juice. Detection limits and recoveries were determined for all 39 pesticides on all three instrument systems, when the pesticide was detectable. Spiked samples were analyzed by GC/MS in scan mode using an Agilent mass selective detector (MSD) with Agilent Deconvolution Reporting Software (DRS) and Retention Time Locked (RTL) Pesticide and Endocrine Disruptor Library. GC/MS confirmation and quantification were done in the SIM mode. Increased sensitivity and specificity were obtained with GC/MS/MS and multiple reaction monitoring (MRM) performed on an Agilent Triple Quadrupole GC/MS. The more polar pesticides were best analyzed using LC/MS/MS, on an Agilent Triple Quadrupole LC/MS. Sample preparation for all three instrument systems was performed using the AOAC QuEChERS method [1]. Using GC/MS in the SIM mode, the linear range was typically 25 ng/mL (ppb) to 1 µg/mL (ppm), while most of the pesticides had a linear range from 2.5 ng/mL to 1 µg/mL using GC/MS/MS. A wider linear range (0.5 ng/mL to 1 µg/mL) was achieved on the LC/MS/MS for some pesticides. The use of these three analysis platforms thus assures screening and confirmation capability for all 39 pesticides to determine whether the vegetable juices meet the requirements of international regulations.

Experimental

Reagents and Standards

Most of the pesticide standards were obtained from the U.S. EPA Pesticide Repository (Ft. Meade, MD), and others were obtained from Fluka/Sigma Aldrich. Pesticide standards were prepared in unspiked, blank vegetable juice samples provided by the Grocery Manufacturers Association-Food Industry Analytical Chemists Committee. Separate stock solutions were prepared (1 mg/mL) by weighing 10 mg each and dissolving in 10 mL of methanol. Intermediate stock solutions of a mixture of all 39 pesticides were prepared in a 100 mL volumetric flask in acetonitrile at a concentration of 50 µg/mL by mixing the stock solutions. Matrix-matched calibration standards (0.5 ng/mL–1 µg/mL) were prepared by spiking the intermediate standards into a vegetable juice blend.

Pesticide grade acetonitrile and methanol, and optima or LC/MS-grade water, and formic acid were purchased from Fisher Scientific.

Instruments

The GC/MS experiments were performed on an Agilent 7890A Series GC coupled to an Agilent 5975C Series GC/MS inert XL MSD with Triple Axis Detector and operated in electron ionization (EI) mode. Retention time locking and Deconvolution Reporting Software were used to screen for the spiked pesticides in the vegetable juice matrix in scan mode. The Automated Mass spectral Deconvolution and Identification System (AMDIS) developed by the National Institute of Standards and Technology (NIST) and incorporated into DRS was used to separate spectra of interest from dirty matrix spectra present in vegetable juice. Samples were then analyzed in Single Ion Monitoring (SIM) mode using target ions and qualifier ions determined from the scan mode. The instrument conditions are given in Table 1.

Table 1. GC/MS Run Conditions

GC Run Conditions		
Columns	Deactivated Restrictor: Agilent 0.7 m × 0.15 mm (p/n 160-7625-5) Analytical Column: Agilent J&W HP-5ms column, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431)	
Injection volume	1 µL	
Injection mode	Cold splitless using a multimode inlet	
Inlet temperature program	60 °C (0.35 min hold); 900 °C/min to 280 °C (15 min hold); 900 °C/min at 300 °C.	
Oven program	Scan mode 70 °C for 1 min 70 °C to 150 °C at 50 °C/min 150 °C to 200 °C at 6 °C/min 200 °C to 280 °C at 16 °C/min, 5 min hold 4 min added on to the run at 290 °C for column backflush	SIM mode 60 °C for 1.5 min 60 °C to 150 °C at 50 °C/min 150 °C to 240 °C at 8 °C/min 240 °C to 280 °C at 50 °C/min, 2.5 min hold 280 °C to 290 °C at 100 °C/min, 2.05 min hold 4 min added on to the run at 290 °C for column backflush
Flow rate for SIM	1 mL/min constant flow mode	
Initial flow rate for scan	2.7 mL/min (nominal, constant pressure mode)	
Retention time locking	Chlorpyrifos-methyl locked to 8.298 min for scan runs	
Transfer line temperature	280 °C	
Backflush configuration	The analytical column was connected between the multimode inlet and a purged ultimate union. The 0.7 m restrictor was connected between the purged ultimate union and the MSD. Pressure at the purged ultimate union was set to 4 psig using an auxiliary EPC module.	
MSD conditions		
Scan mode	Scan and SIM run separately	
Mode	Electron ionization (EI)	
Source temperature	300 °C	
Quadrupole temperature	200 °C	

The GC/MS/MS experiments were performed on an Agilent 7890A Series GC coupled to an Agilent 7000B Triple Quadrupole GC/MS operated in EI mode. Samples were analyzed using Multiple Reaction Monitoring (MRM). The instrument conditions are given in Table 2, and the retention times, quantifier and qualifier transitions, and collision energies for each pesticide are given in Table 4.

The LC/MS/MS experiments were performed on an Agilent 1200 Series HPLC system coupled to an Agilent 6460 Series Triple Quadrupole LC/MS System with Jet Stream technology. The instrument conditions are listed in Table 3 and the retention times, quantifier and qualifier transitions, and collision energies for each pesticide are given in Table 4.

Table 2. GC/MS/MS Run Conditions

GC Run Conditions	
Columns	Two Agilent J&W HP-5ms, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI) columns joined by a purged ultimate union
Injection volume	1 µL
Injection mode	Cold splitless using a multimode inlet
Inlet temperature program	60 °C (0.35 min hold); 600 °C/min to 270 °C
Oven program	60 °C for 1 min 60 °C to 170 °C at 40 °C/min 170 °C to 310 °C at 10 °C/min, 1.25 min hold
Flow rate	1.224 mL/min (constant flow)
Solvent delay	2.3 min
Flow mode	Constant flow, chlorpyrifos methyl retention time locked to 9.143 min
Transfer line temperature	300 °C
Run time	19 min
Backflush configuration	A purged ultimate union (PUU) was connected between the two 15 m analytical columns. Column 1 was backflushed for 4 min at the end of the run with the GC oven at 310 °C, the inlet pressure at 1 psi, and the pressure at the PUU held at 60 psi.
Triple Quadrupole MS conditions	
Mode	Electron ionization (EI), MRM
Source temperature	300 °C
Quadrupole temperatures	Both at 180 °C

Table 3. LC/MS/MS Run Conditions

LC Run Conditions	
Column	Agilent ZORBAX Eclipse-plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Column temperature	40 °C
Injection volume	5 µL
Mobile phase	A = 0.1% formic acid in ddH_2O B = 0.1% formic acid in acetonitrile
Run time	15 min
Flow rate	0.3 mL/min
Gradient	Initial 5% B; 10 min gradient to 95% B, then step to 100% B for 5 min
Triple Quadrupole MS conditions	
Mode	ESI, positive, MRM
Sheath gas	350 °C, 11 L/min
Drying gas flow	11 L/min
Nebulizer pressure	40 psi
Capillary voltage	4,000 V
Nozzle voltage	1,000 V

Table 4. Target and Qualifier Transitions for 39 Pesticides Identified by GC/MS/MS and LC/MS/MS

	GC/MS/MS							LC/MS/MS						
		Quantitative transition			Qualitative transition				Quantitative transition			Qualitative transition		
Compound name	**RT (min)**	**Precursor ion**	**Product ion**	**CE (V)**	**Precursor ion**	**Product ion**	**CE (V)**	**RT (min)**	**Precursor ion**	**Product ion**	**CE (V)**	**Precursor ion**	**Product ion**	**CE (V)**
acephate	5.567	136	94	13	136	42	8	2.458	184	142.9	4	184	95	20
acetamiprid								5.216	223.1	125.9	20	223.1	99	44
azoxystrobin	18.189	344.1	172	46	344.1	156	46	8.518	404.1	372.1	8	404.1	344.1	24
bifenthrin	13.721	181	166	15	181	165	35	12.447	440.2	181.2	5	440.2	166.2	40
boscalid	16.384	140	112	15	140	76	30	8.698	343	307	16	343	271.1	32
carbaryl	9.054	144	115	30	144	89	50	7.112	202.1	145	4	202.1	127	28
carbendazim								3.272	192.1	160.1	20	192.1	132.1	25
chlorothalonil	8.412	265.9	169.9	28	265.9	133	53							
chlorpropham	6.952	213	171.7	5	127	92	20	8.856	214.1	172	5	214.1	154	15
chlorpyrifos	9.768	196.9	168.9	17	196.9	107	40	11.025	350	293.9	15	350	197.9	30
cyfluthrin	15.533	163	127	5	163	91	14							
cyhalothrin, λ	14.687	181	152	30	181	127	30							
cypermethrin	16.293	181.1	152.1	25	181.1	127.1	35							
cyprodinil	10.215	224.1	208.1	27	224.1	118.1	45	7.951	226.1	108	30	226.1	93	40
dicloran	7.638	206	176	13	206	124	30							
dieldrin	11.513	262.9	192.9	40	262.9	190.9	35							
dimethoate	7.63	125	93	15	125	79	5	5.098	230	199	5	230	171	10
fenvalerate 1	17.202	225	119	15	167.1	125	12							
fenvalerate 2	17.396	225	119	15	167.1	125	12							
fenpropathrin	13.846	181.1	152	26	181.1	127.1	26	11.025	350.2	197.9	16	350.2	125.1	5
fludioxonil	11.358	248	154	25	248	127	30	8.268	266.1	158	15	266.1	229	20
folpet	10.982	259.9	130	20	259.9	102	40							
imidacloprid								4.952	256.1	209	10	256.1	175.1	10
malathion	9.549	173.1	117.1	10	173.1	99	15	9.049	331.1	127	5	331.1	99	15
methomyl								3.886	163.1	106	4	163.1	88	4
methoxyfenozide								8.927	369.2	149.1	16	369.2	313.1	0
myclobutanil	11.538	179	152	6	179	125	14	8.47	289.1	125	20	289.1	89	50
omethoate	6.755	156	110	7	156	79	27	2.832	214	124.9	20	214	109	28
oxamyl								3.675	220.1	90.1	0	220.1	72.1	4
cis-permethrin	15.413	183.1	168.1	15	183.1	153	18							
trans-permethrin	15.534	183.1	165.1	10	183.1	115.1	30							
o-phenylphenol	6.113	170	141	30	170	115	45							
phosmet	13.705	160	133	15	160	77	30	8.438	318	160	5	318	133	40
piperonyl butoxide	13.181	176	131	16	176	103	30	10.762	356.2	177.1	10	356.2	119.1	35
propiconazole	12.738	259.1	191	5	259.1	173	20	9.223	342.1	159	20	342.1	69	20
spinosad A								7.847	732.5	142.1	35	732.5	98	55
spinosad D								8.137	746.5	142.1	35	746.5	98	55
tebufenozide								9.394	353.2	297.2	5	353.2	133.1	15
thiabendazole	10.487	201	174	20	201	65	54	3.475	202	175	24	202	131.1	36
thiamethoxam	10.112	246.9	212	10	246.9	182	20	4.277	292	211	4	292	181	20
thiobencarb	9.599	257	100	5	257	72	22	10.045	258.1	125.1	25	258.1	100.1	5
trifloxystrobin	12.758	222	130	14	116	63	30	10.322	409.2	186.1	10	409.2	145	40

CE = Collision energy

Sample Preparation

Extraction and cleanup of thirty-nine pesticides spiked in vegetable juice were achieved with an Agilent Bond Elut QuEChERS Extraction kit for pesticides (p/n 5982-5755) and an Agilent Bond Elut QuEChERS AOAC Dispersive SPE kit for fruits and vegetables (p/n 5982-5022) used per kit instructions. The method used involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and then a cleanup step using dispersive solid phase extraction (dispersive SPE) [2]. Following extraction and cleanup, 200 µL of each sample was transferred to an autosampler vial, ready for GC/MS, GC/MS/MS and LC/MS/MS analysis.

Data Analysis

Spiked samples were analyzed by GC/MS in scan mode using an Agilent mass selective detector (MSD) with Agilent Deconvolution Reporting Software (DRS, p/n G1716AA) and Retention Time Locked (RTL) Pesticide and Endocrine Disruptor Library (p/n G1672AA).

Matrix-matched calibration curves (0.5 ng/mL to 1,000 ng/mL) for each pesticide were constructed for 39 pesticides on all three instruments. These were obtained by plotting the pesticide concentration versus the signal intensity (area) and determining the R^2 using weighted linear regression (1/x) with the Agilent MassHunter Quantitative Analysis software for GC/MS/MS and LC/MS/MS, and Agilent Chemstation data analysis software for GC/MS data. Limits of detection (LODs) and LOQs were calculated based on signal-to-noise ratios (S/N) of 3:1 and 10:1 respectively, using the same software.

Results and Discussion

Comprehensive Pesticide Coverage

Three methods were developed for the analysis of 39 pesticides commonly found in fruits and vegetables. While no single method could detect all 39 pesticides, the combination of GC/MS, GC/MS/MS, and LC/MS/MS provided the required sensitivity of detection and quantification for all 39. Retention time locking and Deconvolution Reporting Software were used to screen for the spiked pesticides in the vegetable juice matrix in the GC/MS scan mode. Samples were then analyzed by GC/MS SIM using target ions and qualifier ions determined from the scan mode. The GC/MS SIM method used one target and three qualifier ions for pesticide identification, and the GC/MS/MS MRM method used two transitions. The Optimization tool in Mass Hunter Acquisition software was used to determine LC/MS/MS MRM transitions, fragmentor voltages and collision energies for the 39 pesticides. Each MRM analysis subsequently used up to three transitions. Table 4 lists the pesticides that were identified by GC/MS/MS or LC-MS/MS or both methods. The pesticides are listed in alphabetical order with their retention times, precursor, and product ions for both the quantitative and qualitative transitions, and the collision energies for each.

Linearity, LOD, and LOQ

Calibration curves were determined using matrix-matched standards (0.5 ng/mL to 1 µg/mL) for all 39 pesticides on all three instruments. Table 5 summarizes the linear range, curve fit using the weighted linear regression (1/x), and the LODs and LOQs for each pesticide using all three methods. Most of the pesticides could be determined in the 2.5 ng/mL (ppb) to 1 µg/mL (ppm) range with $R^2 > 0.99$ when analyzed by GC/MS/MS and 25 ng/mL to 1 µg/mL range with $R^2 > 0.99$ with GC/MS SIM. A wider linear range (0.5 ng/mL to 1 µg/mL) was achieved on the LC-MS/MS for some pesticides, generally with $R^2 > 0.98$.

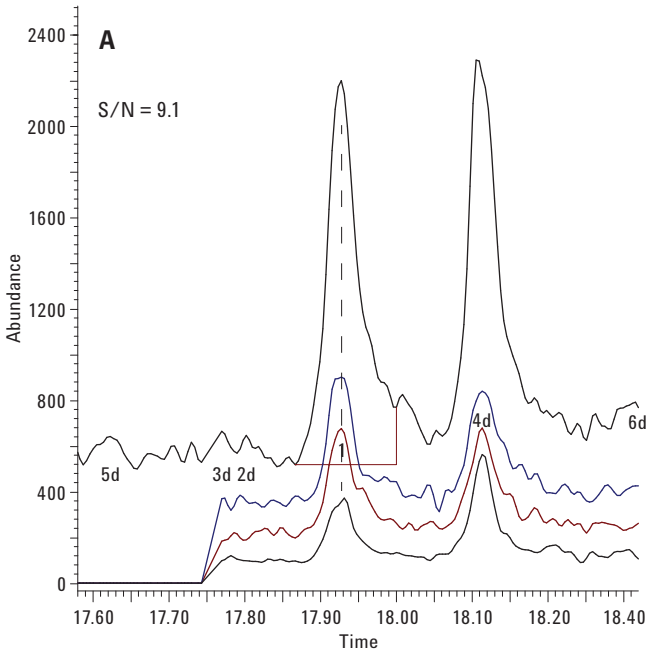
The LOD for each pesticide analyzed by GC/MS/SIM, GC/MS/MS and LC/MS/MS was determined to be the amount of pesticide that would produce at least 3/1 signal-to-noise in the matrix-matched standards for the target ion (SIM) or quantifying MS/MS transition. Most of the LOD values for GC/MS/MS ranged from 0.1 ng/mL to 10 ng/mL, compared with 1 ng/mL to 25 ng/mL for GC/MS SIM and 0.05 ng/mL to 10 ng/mL for LC/MS/MS.

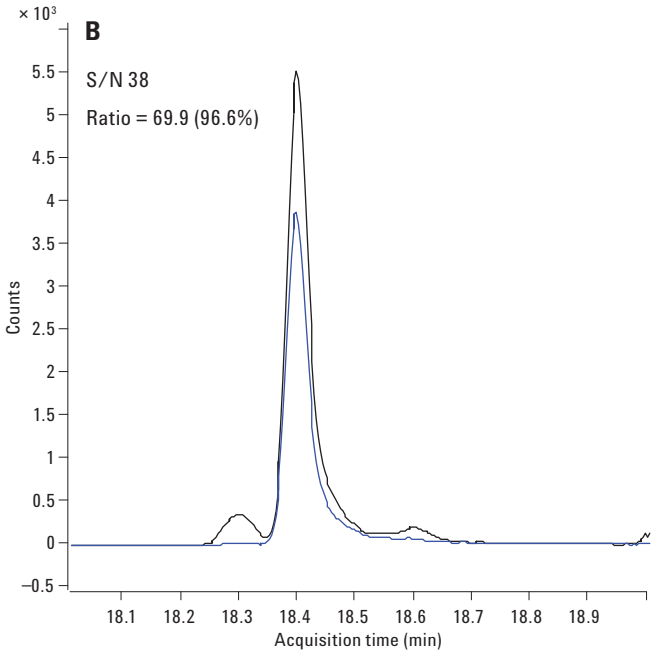
The LOQ for each pesticide analyzed by GC/MS SIM, GC/MS/MS and LC/MS/MS was determined to be the amount of pesticide that would produce at least 10/1 signal-to-noise in the matrix-matched standards for the target ion (SIM) or quantifying MS/MS transition. Most of the LOQ values for GC/MS SIM ranged from 5 to 50 ng/mL, compared with 0.25 ng/mL to 10 ng/mL for GC/MS/MS, and 0.1 ng/mL to 10 ng/mL for LC/MS/MS.

Table 5. Linearity, LOD, and LOQ

	GC/MS				GC/MS/MS				LC/MS/MS			
Compound name	**Linearity (ng/mL)**	**R^2**	**LOD (S/N=3) (ng/mL)**	**LOQ (S/N=10) (ng/mL)**	**Linearity (ng/mL)**	**R^2**	**LOD (S/N=3) (ng/mL)**	**LOQ (S/N=10) (ng/mL)**	**Linearity (ng/mL)**	**R^2**	**LOD (S/N=3) (ng/mL)**	**LOQ (S/N=10) (ng/mL)**
acephate					10-1000	0.9983	5	10	5-1000	0.9996	2.5	5
acetamiprid									50-500	0.9951	0.1	0.25
azoxystrobin	25-1000	0.9947	10	25	10-1000	0.9935	1	2.5	25-1000	0.9929	<0.01	<0.05
bifenthrin	25-1000	0.9989	1	5	2.5-1000	0.9975	0.1	0.25	50-500	0.9981	25	50
boscalid	25-1000	0.9985	10	25	2.5-1000	0.9963	0.5	1	0.5-1000	0.9967	0.25	0.5
carbaryl	25-1000	0.9976	10	25	2.5-500	0.9998	0.5	1	50-500	0.9952	0.05	0.1
carbendazim									25-500	0.9926	0.25	0.5
chlorpropham	50-1000	0.9966	25	50	1-1000	0.9971	0.1	0.25	10-1000	0.9986	5	10
chlorothalonil	25-1000	0.9971	1	5	2.5-1000	0.9945	0.1	0.25				
chlorpyrifos	25-1000	0.9992	10	25	10-1000	0.9999	0.5	1	5-1000	0.9962	0.05	0.1
cyfluthrin	50-1000	0.9950	25	50	10-500	0.9885	5	10				
cyhalothrin, λ	50-1000	0.9907	25	50	2.5-500	0.9993	1	5				
cypermethrin	50-1000	0.9957	25	50	2.5-500	0.9941	1	2.5				
cyprodinil	25-1000	0.9994	5	10	10-1000	0.9996	1	5	50-500	0.9979	0.25	0.5
dicloran	25-1000	0.9999	10	25	1-500	0.9797	0.5	1				
dieldrin	50-1000	0.9991	25	50	10-1000	0.9996	2.5	5				
dimethoate	25-1000	0.9989	10	25	10-1000	0.9999	0.5	1	50-500	0.9861	0.1	0.25
fenpropathrin	5-1000	0.9999	25	50	2.5-1000	0.9952	1	2.5	5-1000	0.9965	0.25	0.5
fenvalerate 1	50-1000	0.9959	25	50	2.5-500	0.9930	1	2.5				
fenvalerate 2	50-1000	0.9928	25	50	2.5-500	0.9919	1	2.5				
fludioxonil	25-1000	0.9985	10	25	10-1000	0.9979	1	2.5	25-500	0.9963	10	25
folpet					2.5-1000	0.9939	10	25				
imidacloprid									50-1000	0.9967	0.1	0.25
malathion	50-1000	0.9993	25	50	10-1000	0.9992	1	2.5	50-1000	0.9917	0.05	0.1
methomyl									25- 500	0.9956	10	25
methoxyfenozide									50-1000	0.9943	0.05	0.1
myclobutanil	25-1000	0.9990	10	25	1-1000	0.9975	0.25	1	0.5-1000	0.9981	0.25	0.5
omethoate					10-500	0.9966	5	10	10-1000	0.9968	2.5	5
oxamyl									25-500	0.9876	10	25
o-phenyl phenol	25-1000	0.9995	10	25	2.5-1000	0.9984	<0.5	1				
phosmet					25-1000	0.9945	2.5	10	0.5-1000	0.9979	0.1	0.25
piperonyl butoxide	25-1000	0.9979	10	25	10-1000	0.9995	0.5	2.5	50-500	0.9966	0.05	0.1
propiconazole					10-1000	0.9997	0.5	2.5	0.5-1000	0.9976	0.25	0.5
spinosad A									50-500	0.9895	0.25	0.5
spinosad D									25-1000	0.9954	0.25	0.5
tebufenozide									50-500	0.9919	0.25	0.5
thiabendazole					10-500	0.9982	2.5	10	25-1000	0.9972	0.25	0.5
thiamethoxam					2.5-500	0.9926	0.25	2.5	50-500	0.9921	0.25	0.5
trifloxystrobin					2.5-1000	0.9932	0.5	2.5	50-1000	0.9922	0.05	0.1
thiobencarb					10-1000	0.9996	1	2.5	0.5-1000	0.9979	0.25	0.5

All three methods were able to identify azoxystrobin, bifenthrin, boscalid, carbaryl, chlorpyrifos, cyprodinil, dimethoate, fenpropathrin, fludioxonil, malathion, and myclobutanil. Figure 1A shows the Extracted Ion Current (EIC) (*m/z* 344, 388, 345, and 372) from the GC/MS SIM analysis of azoxystrobin in the 25 ng/mL spiked vegetable juice blend extract. Figure 1B illustrates the advantage of GC/MS/MS over GC/MS SIM for target compound analysis. A 1-µL injection of the 25 ng/mL spiked vegetable juice blend extract on GC/MS/MS gave a clean MRM chromatographic azoxystrobin peak with better S/N (38:1) than was obtained for 1 µL GC/MS SIM analysis (S/N = 9.1:1, Figure 1A). Analysis of the same sample extract gave an even better S/N (327:1) using LC/MS/MS analysis for azoxystrobin rather than GC/MS/MS. This is due to the polar nature of azoxystrobin, making it more suitable for analysis by LC/MS/MS.





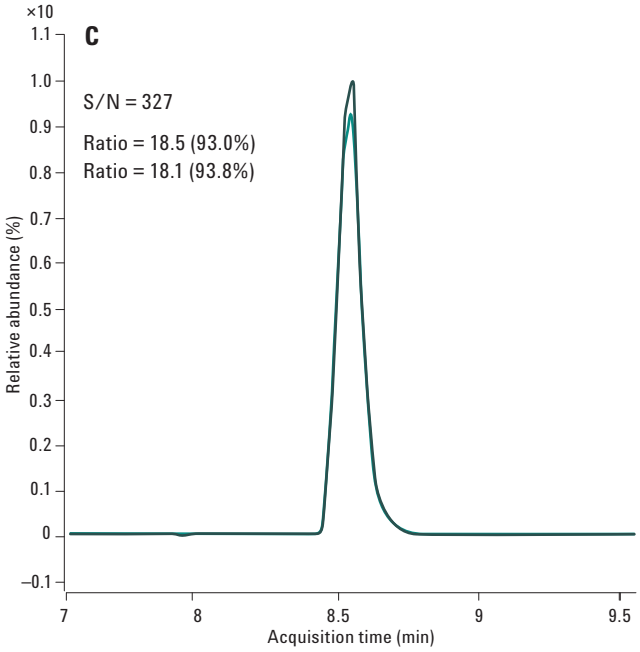


Figure 1. Analysis of a 25 ng/ml spiked vegetable juice blend extract using all three methods. A) Azoxystrobin quantifier ion (m/z 344) and three qualifier ions (m/z 388, 345, 372) extracted from a GC/MS SIM chromatogram B) Quantifier and qualifier transitions (344.1→172.0, 344.1→156.0) for the GC/MS/MS analysis, C) Quantifier and qualifier transitions (404.1→372.1, 404.1→344.1, and 401.1→329.1) for the LC/MS/MS analysis. The ratios of the qualifier to quantifier ions are shown for B and C, confirming the presence of azoxystrobin in each case.

Conversely, the nonpolar pyrethroid bifenthrin was better detected by either GC method (LOD 1 ng/mL by GC/MS and LOD 0.1 ng/mL by GC/MS/MS), than by LC/MS/MS (LOD 25 ng/mL). Carbaryl, a carbamate, can be detected with higher sensitivity (LOD 0.05 ng/mL) when analyzed using LC/MS/MS, but better linearity (2.5 ng/mL to 500 ng/mL) was achieved with GC/MS/MS. Chlorpropham, also a carbamate, has better sensitivity (LOD 0.1 ng/mL) and linear range (1 ng/mL to 1000 ng/mL) with GC/MS/MS, compared to LC/MS/MS. Fenpropathrin and chlorpyrifos elute very closely to each other when analyzed on the LC/MS/MS, making identification difficult due to similar retention times and product ions. Both compounds have the same transition (350→198). Fenpropathrin can be detected and confirmed with similar sensitivity on the GC/MS/MS (2.5 ng/mL–1,000 ng/mL). Chlorpyrifos is a polar organophosphate and thus has a wider linear range (5 ng/mL to 1,000 ng/mL) and better sensitivity (LOD 0.05 ng/mL) with LC/MS/MS. Chlorpyrifos can be analyzed by LC/MS/MS using the $C_9H_{11}{}^{35}Cl_2{}^{37}ClNO_3PS$ isotope as the precursor ion (*m/z* 349.9), and the product ions resulting from this precursor will not interfere with fenpropathrin, which is separated by a 3 minute difference in retention time by GC/MS/MS. Thus, analyzing chlropyrifos using these transitions and LC/MS/MS and analyzing fenpropathrin by GC/MS/MS provides good sensitivity for both compounds and no interference from either. Malathion has better sensitivity (LOD 0.05 ng/mL) when analyzed with LC/MS/MS, but a wider linear range (10 ng/mL to 1,000 ng/mL) with GC/MS/MS. Thus, using all three methods assures the highest quality data across the 39 pesticides, as an analyte that is poorly detected by one method will be detected better by one or more of the other methods.

Figure 2 shows the MRM transitions (in overlay) of the pesticides spiked at 10 ppb that can be detected in the vegetable juice blend matrix with GC/MS/MS (Figure 2A) and LC/MS/MS (Figure 2B).

Recovery Studies

The 39 pesticides were spiked into vegetable juice extract at two different fortification levels (50 and 250 ng/mL) and then extracted with the QuEChERS kit. The analysis was performed in four replicates at each level (n = 4). Table 6 shows the recoveries and standard deviations for each pesticide on each of the three instruments.

Most, but not all, spike recoveries fell between 70–120%. The compounds that could only be analyzed by GC/MS: chlorothalonil, cyfluthrin, cyhalothrin, λ cypermethrin, dicloran, dieldrin, and o-phenyl phenol, gave good recoveries. Folpet was one exception with recovery for the high spike at 53.6%, and this compound had a poor response by GC/MS/MS. Likewise, compounds that only responded by LC/MS/MS: acetamiprid, carbendazim, imidacloprid, methomyl, oxamyl, spinosad A, spinosad D, and tebufenozide, also showed acceptable recoveries. It should be noted that GC/MS SIM would be more susceptible to matrix interferences than MS/MS. Thiabendazole gave high recovery in the low spike by GC/MS but acceptable recovery by GC/MS/MS. For this compound, the consistently high recovery by LC/MS/MS may indicate a calibration bias.

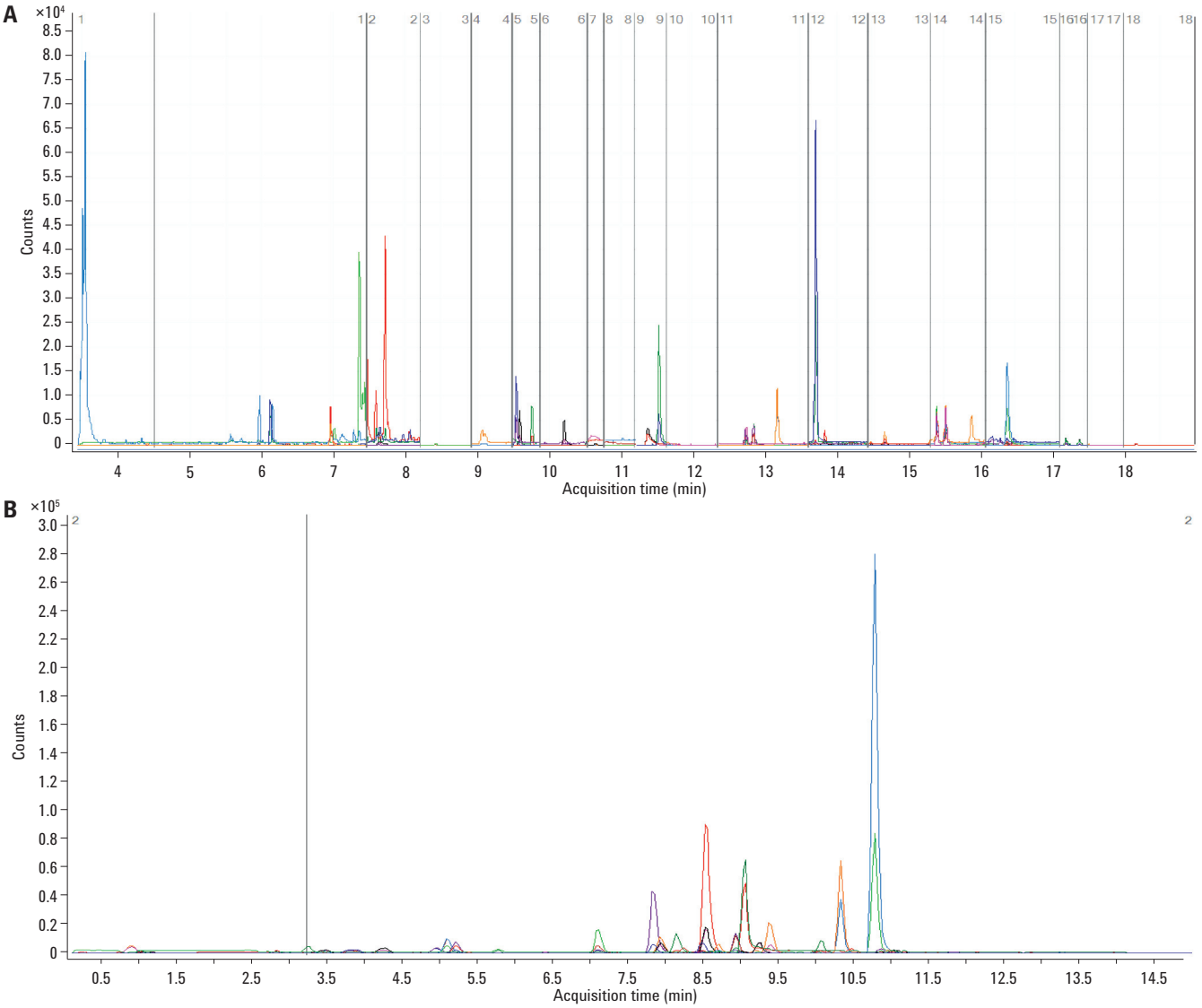


Figure 2. The MRM transitions (in overlay) of the pesticides spiked at 10 ppb (ng/mL) and detected in the vegetable juice blend matrix with GC/MS/MS (Figure 2A) and LC/MS/MS (Figure 2B).

Table 6. Recoveries for 39 Pesticides in Spiked Vegetable Juice Samples (n-4)

	GC-MS		GC-MS/MS		LC-MS/MS	
Compound name	**50 ng/mL Average % recovery ± stdev**	**250 ng/mL Average % recovery ± stdev**	**50 ng/mL Average % recovery ± stdev**	**250 ng/mL Average % recovery ± stdev**	**50 ng/mL Average % recovery ± stdev**	**250 ng/mL Average % recovery ± stdev**
acephate			90.8 ± 8.1	81.8 ± 6.6	106.4 ± 1.7	102.9 ± 4.6
acetamiprid					114.8 ± 11.7	121.4 ± 3.5
azoxystrobin	116.3 ± 3.6	87.9 ± 2.2	85.5 ± 1.7	81.12 ± 4.0	128.4 ± 4.8	115.1 ± 4.3
bifenthrin	114.9 ± 2.7	93.7 ± 0.8	100.7 ± 2.6	90.7 ± 2.5	110.2 ± 5.4	94.8 ± 5.9
boscalid	117.5 ± 3.3	93.6 ± 1.3	90.8 ± 3.3	87.3 ± 3.0	128.4 ± 9.7	120.6 ± 3.9
carbaryl	118.6 ± 4.3	80.3 ± 3.5	78.8 ± 5.8	64.1 ± 5.7	111.7 ± 6.1	115.6 ± 2.9
carbendazim					115.6 ± 7.2	112.2 ± 1.6
chlorpropham	96.9 ± 12.0	95.61 ± 4.0	100.3 ± 6.4	99.7 ± 2.5	107.0 ± 6.9	106.2 ± 2.7
chlorothalonil	115.08 ± 5.0	73.8 ± 2.4	53.4 ± 3.8	50.1 ± 4.3		
chlorpyrifos*	121.49 ± 4.2	98.0 ± 0.8	99.1 ± 5.2	95.1 ± 1.9	125.5 ± 7.5	102.4 ± 10.0
cyfluthrin	120.4 ± 5.5	81.8 ± 1.8	93.5 ± 2.7	90.4 ± 2.5		
cyhalothrin, λ	121.2 ± 5.9	83.7 ± 12.7	93.3 ± 2.5	94.6 ± 3.6		
cypermethrin	118.8 ± 3.4	80.2 ± 5.1	84.9 ± 5.2	80.2 ± 3.6		
cyprodinil	114.4 ± 4.49	98.7 ± 1.4	103.3 ± 5.6	98.9 ± 1.2	98.6 ± 6.0	115.6 ± 4.1
dicloran	113.7 ± 10.1	92.3 ± 2.8	166.1 ± 4.0	98.6 ± 2.6		
dieldrin	101 ± 2.8	113.1 ± 1.8	103.6 ± 4.9	95.9 ± 4.0		
dimethoate	103.1 ± 10.2	84.02 ± 7.0	91.1 ± 5.8	82.2 ± 4.3	89.4 ± 8.1	115.2 ± 9.8
fenpropathrin*	98.5 ± 8.3	88.7 ± 5.9	87.2 ± 2.8	90.1 ± 2.5	139.4 ± 7.9	105.9 ± 8.8
fenvalerate 1	136.5 ± 5.7	90.8 ± 3.9	80.6 ± 5.0	71.9 ± 5.2		
fenvalerate 2	148.8 ± 3.1	87.9 ± 2.1	78.5 ± 5.3	70.2 ± 4.7		
fludioxonil	124.9 ± 3.2	97.6 ± 1.3	109.5 ± 6.4	100.3 ± 1.8	145.8 ± 12.7	120.6 ± 8.3
folpet			72.4 ± 11.1	53.6 ± 9.4		
imidacloprid					103.7 ± 6.1	117.1 ± 5.1
malathion	112.0 ± 12.4	96.7 ± 11.7	92.4 ± 3.3	88.4 ± 1.6	95.6 ± 11.0	114.4 ± 8.0
methomyl					121.2 ± 10.0	112.5 ± 6.1
methoxyfenozide					95.10 ± 0.40	123.4 ± 6.8
myclobutanil	118.2 ± 5.5	102.3 ± 1.7	108.1 ± 5.4	101.2 ± 1.5	120.0 ± 8.3	109.3 ± 2.7
omethoate			144.10 ± 27.5	73.8 ± 10.0	114.3 ± 4.6	110.3 ± 2.2
oxamyl					110.5 ± 12.3	119.9 ± 4.8
o-phenyl phenol	106.3 ± 3.6	104.49 ± 0.7	103.8 ± 5.5	102.96 ± 2.0		
phosmet	136.4 ± 6.0	70.0 ± 3.5	64.4 ± 4.8	50.3 ± 6.3	121.7 ± 5.0	107.3 ± 3.2
piperonyl butoxide	123.7 ± 6.0	93.9 ± 2.2	103.1 ± 5.0	96.9 ± 0.7	94.7 ± 7.1	119.2 ± 1.5
propiconazole			104.0 ± 4.3	99.0 ± 2.4	124.3 ± 4.5	114.4 ± 1.7
spinosad A					122.1 ± 5.8	114.6 ± 4.0
spinosad D					114.7 ± 4.9	113.6 ± 1.5
tebufenozide					109.6 ± 9.5	102.2 ± 18.3
thiabendazole	126.8 ± 7.9	81.6 ± 5.3	85.0 ± 6.5	84.5 ± 3.6	120.7 ± 4.6	123.5 ± 6.1
thiamethoxam			76.0 ± 7.5	69.3 ± 5.3	110.3 ± 7.1	122.2 ± 5.6
trifloxystrobin	123.9 ± 2.0	95.0 ± 1.0	96.5 ± 2.5	94.7 ± 1.8	133.7 ± 8.2	144.3 ± 4.0
thiobencarb	109.8 ± 6.4	96.0 ± 2.9	104.2 ± 5.4	99.0 ± 1.3	123.1 ± 3.5	108.8 ± 3.3

*Fenpropathrin and chlorpyrifos were spiked separately to obtain recoveries by LC/MS/MS using the quantitative transitions provided in Table 4.

Conclusions

Food safety laboratories require methods that can analyze all pesticides of interest in a particular matrix, with levels of detection and quantification that meet regulatory requirements. Some pesticide compounds are best analyzed using GC/MS, while others are best measured using LC/MS. The best approach to assure comprehensive detection of a large number of pesticides is to use both GC and LC/MS techniques. Methods have thus been developed on both Agilent GC/MS and LC/MS instrument platforms using the most common pesticide analytical techniques for the analysis of pesticides in vegetable juice. Used together, they can meet any laboratory's need for screening and confirmation of 39 pesticides in vegetable juice at levels well below the established MRL's. Used separately, they provide screening or confirmation of most of these pesticides, without requiring acquisition of new equipment.

Table 5 shows why most labs are moving their GC single quadrupole pesticide methods to GC/MS/MS. The Agilent 7000B Triple Quadrupole GC/MS has much better selectivity and offers much better LOD and LOQ values. However, the Agilent 5975C Series GC/MS single quadrupole instrument can be used to screen for more than 900 pesticides using DRS with the Agilent Pesticide and Endocrine Disruptor Library. For screening purposes, this approach still has great value [3].

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