A Method for the Trace Analysis of 175 Pesticides Using the Agilent Triple Quadrupole GC/MS/MS

Application Note

Food Safety

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Abstract

A GC/MS/MS multiple reaction monitoring (MRM) method has been developed on the Agilent 7890A/7000A GC triple quadrupole mass spectrometer system (GC/QQQ) for 175 commonly analyzed pesticides. Numerous fruit and vegetable extracts were analyzed by this method and by a GC single quadrupole mass spectrometer (GC/Q) for comparison. The GC/Q was operated in the selected ion monitoring (SIM) mode and in the scan mode. Scan results were evaluated using Agilent's Deconvolution Reporting Software (DRS) with the RTL Pesticide and Endocrine Disruptor Database. The GC/Q instrument was equipped with a Multimode inlet and injections of 5 µL were made in the cold splitless mode. These were compared to 1 µL injections of the same extracts on the GC/QQQ. The GC/QQQ was found to be far more sensitive and selective than either GC/Q approach, primarily because there is much less interference from co-extracted matrix. There is, however, still a need for GC/Q methods that use DRS to screen for more than 900 pesticides and other contaminants since the GC/QQQ in the MRM mode is only for target compound analysis.

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Introduction

Pesticide residue analysis is a complex task requiring the analyst to search for dozens, or even hundreds, of compounds in a wide variety of crop matrices. Extraction techniques, such as the QuEChERS method [1–3] leave large amounts of indigenous materials in the extract. The use of more extensive cleanup steps risks removing pesticide residues in addition to the matrix. As required detection limits for many pesticides fall to 10 µg/Kg (10 ppb) or lower, more sophisticated analytical tools are needed.

For GC-amenable pesticides, many laboratories are using two complementary techniques for screening and confirmation purposes. For broad screening at the 5 to 100 ppb level, GC/single quadrupole (GC/Q) is employed with Deconvolution Reporting Software (DRS) and the RTL Pesticide and Endocrine Disruptor library from Agilent Technologies [4–6]. This is a scan method to screen for 927 GC-amenable pesticides and endocrine disruptors in a single GC/MS run. Detection limits for most pesticides vary from approximately 5 to 100 ppb, depending upon the matrix and the injection volume [4]. For target pesticide analysis in the most complex matrices, the Agilent 7890A/7000A GC/triple quadrupole (GC/QQQ) is unmatched.

This paper compares three mass spectral techniques for the analysis of pesticide residues in a variety of crop matrices. Spiked and unspiked samples were analyzed by GC/Q in the selected ion monitoring (SIM) mode and in the scan mode with DRS analysis. The same samples were also analyzed by GC/QQQ using a multiple reaction monitoring (MRM) method for 175 pesticides. The objective was to compare the ability of these GC/Q and GC/QQQ methods to detect low levels of pesticides in several different crop matrices.

Experimental

Samples

Spiked and unspiked extracts of fresh produce were provided by the U.S. Food and Drug Administration (U.S. FDA, CFSAN, College Park, MD) and the U.S. Department of Agriculture (USDA ARS, ERRC, Wyndmoor, PA). Samples from the FDA were prepared using the QuEChERS [1–3] method modified to include the use of activated carbon as an additional sorbent. The resultant toluene solution contained 4.5 grams of produce per milliliter of extract. Samples from the USDA were extracted using the published QuEChERS method and contained 1 gram of produce per milliliter of acetonitrile solvent.

Instrumentation

The GC/Q and GC/QQQ systems used for these experiments are described in Tables 1 and 2.

Table 1.   Instrumentation and Analytical Conditions for the GC/Q System

GC	Agilent 7890A Series
Autosampler	Agilent 7693A Injector and sample tray
Inlet	Multimode inlet
Carrier gas	Helium
Inlet pressure	18.420 psi (constant pressure mode) during run 2.0 psi (during backflush)
Splitless Mode Inlet Parameters	
Temperature	250 °C
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Injection volume	1 µL
Purge flow to split vent	30 mL/min at 0.75 min
Cold Splitless Mode Inlet Parameters	
Temperature program	60 °C (0.01 min), 700 °C/min to 280 °C (hold)
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Injection volume	5 µL
Purge flow to split vent	30 mL/min at 1.25 min
Oven temperature program	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (5 min)
Capillary flow technology	2-way splitter with one port capped – used for backflushing the analytical column and retention gap
Pneumatic Control Module (PCM)	Helium plumbed to 2-way splitter
PCM pressure	4.0 psi during run, 60.0 psi during backflush
Analytical column	Agilent J&W HP-5ms UI 15 m × 0.25 mm × 0.25 µm (P/N 19091S-431UI)
Connections	Between retention gap and 2-way splitter
Retention gap	2.0 m × 0.25 mm Siltek deactivated fused silica tubing (Restek, Bellefonte, PA)
Connections	Between inlet and analytical column using an Ultimate Union (P/N G3182-61580) to couple the retention gap to the column
Restrictor	80 cm × 0.15 mm deactivated fused silica tubing (Agilent)
Connections	Between the 2-way splitter and the MSD
Initial column flow rate	2.705 mL/min (nominal)
Retention time locking	Chlorpyrifos-methyl locked to 8.298 min
Mass selective detector	Agilent 5795C Series with performance turbo pump
Mode	Electron impact
Transfer line temperature	280 °C
Source temperature	230 °C
Quadrupole temperature	150 °C
Threshold	100
Sampling rate	A/D = 4
Gain factor	1
SIM dwell times	Variable from 4 to 25 ms
Trace ion detection	On
Solvent delay	2.5 min
Backflushing Conditions	
Timing	5 min duration during post-run
Oven temperature	280 °C

(Continued)

Table 1. Instrumentation and Analytical Conditions for the GC/Q System (continued)

Aux EPC pressure	60 psi
Inlet pressure	2 psi
Software	
GC/MSD	Agilent GC/MS ChemStation control and data analysis software (P/N G1701EA E.02.00 SP1)
Deconvolution Reporting Software	Agilent P/N G1716AA (Ver. A.04.00)
Library Searching Software	NIST MS Search (Ver 2.0d) (comes with NIST mass spectral library – Agilent P/N G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.62 or greater; comes with NIST mass spectral library – Agilent P/N G1033A)
MS Libraries	NIST 08 mass spectral library (Agilent P/N G1033A) Pesticide and Endocrine Disruptor Database (Agilent P/N G1672AA)

Table 2. Instrumentation and Analytical Conditions for the GC/QQQ system

GC	Agilent 7890A Series
Autosampler	Agilent 7683A Injector and sample tray
Inlet	Split/splitless
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Carrier gas	Helium
Inlet pressure	18.350 psi (constant pressure mode) during run 1.0 psi (during backflush)
Inlet temperature	250 °C
Injection volume	1 µL
Purge flow to split vent	30 mL/min at 0.75 min
Gas saver	On (20 mL/min at 2.0 min)
Oven temperature program	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (5.5 min)
Capillary flow technology	Purged Ultimate Union (P/N G3186B) – used for backflushing the analytical column and retention gap
Aux EPC gas	Helium plumbed to Purged Ultimate Union
Aux EPC pressure	4.0 psi during run, 80.0 psi during backflush
Analytical column	Agilent J&W HP-5ms UI 15 m × 0.25 mm × 0.25 µm (P/N 19091S-431UI)
Connections	Between retention gap and Purged Ultimate Union (P/N G3186B)
Retention gap	2.0 m × 0.25 mm Siltek deactivated fused silica tubing (Restek, Bellefonte, PA)
Connections	Between inlet and analytical column using ultimate union (P/N G3182-61580) to couple the retention gap to the column
Restrictor	65 cm × 0.15 mm deactivated fused silica tubing (Agilent)
Connections	Between the Purged Ultimate Union (P/N G3186B) and the MSD
Initial column flow rate	2.688 mL/min (nominal)
Retention time locking	Chlorpyrifos-methyl locked to 8.298 min

Triple Quadrupole Mass Spectrometer	Agilent 7000A Series
Mode	Electron impact
Transfer line temperature	280 °C
Solvent delay	2.3 min
Source temperature	300 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
MRM Mode Conditions	
MS1 resolution	1.2 u
MS2 resolution	1.2 u
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Backflushing Conditions	
Timing	3 min duration during post-run
Oven temperature	280 °C
Aux EPC pressure	80 psi
Inlet pressure	1 psi
Software	
Data acquisition	Agilent MassHunter Data Acquisition Software (Ver. B.04.00)
Qualitative analysis	MassHunter Workstation Software for Qualitative Analysis (Ver. B.03.01)
Quantitative analysis	MassHunter Workstation Software for Quantitative Analysis (Ver. B.03.01)

Results and Discussion

GC Configuration

Both GC systems used a 15-m × 0.25 mm × 0.25 µm Agilent J&W HP-5ms UI column and were running the standard Agilent pesticide method [7] at 2X speed. This method uses an initial oven temperature of 70 °C, which works for most GC solvents without using a retention gap. However, 1-µL injections of samples in toluene lead to poor peak shape, so a 2-m deactivated retention gap was coupled to the front of the column. This improved the peak shapes.

Column backflushing is essential for the analysis of food extracts [4, 8–9] because they usually contain high-boiling indigenous compounds. In just a few runs, these materials can collect on the head of the column (or retention gap), causing peak tailing and retention time shifts. Over time, they can migrate from the column to the mass spec source, which would eventually have to be cleaned.

Agilent's capillary flow technology makes column backflushing routine (4, 8–9) and several different capillary flow devices can be used for the purpose. The GC/QQQ system used a Purged Ultimate Union, while the GC/Q system used a two-way splitter (with one port capped). In both cases, the analytical column was connected to the capillary flow device. A short restrictor was used to couple the capillary flow device to the mass spectrometer. Figure 1 shows the configuration of each instrument.
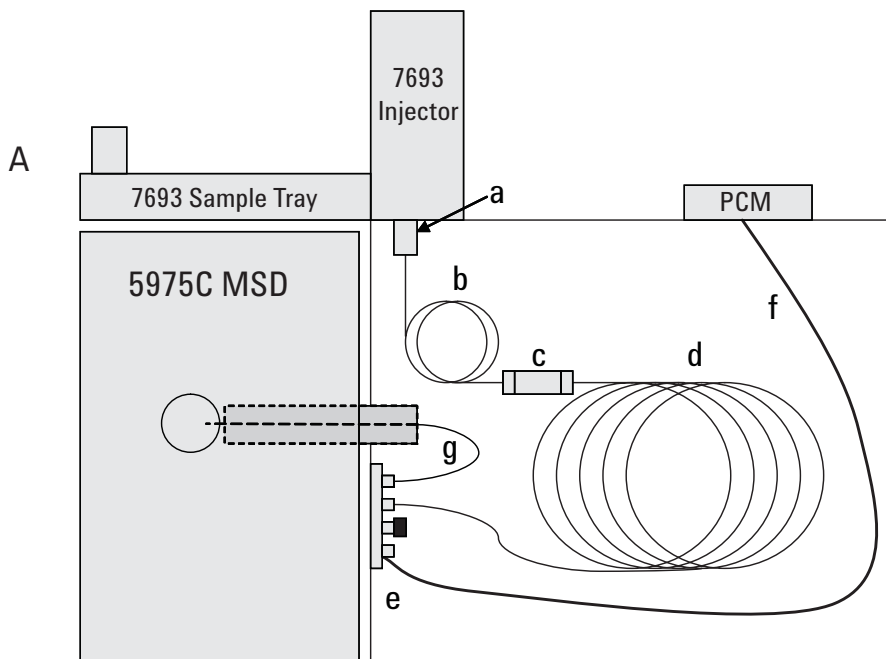
Figure 1A. *The GC/MSD used for scan and SIM analyses was configured with a) Multimode inlet, b) 2 m × 0.25 mm deactivated retention gap, c) Ultimate Union, d) 15 m × 0.25 X 0.25 μm Agilent J&W HP-5ms UI column, e) two-way purged splitter with one port capped, f) helium purge flow controlled by a pneumatic control module (PCM), and g) 80 cm × 0.15 mm deactivated restrictor.*

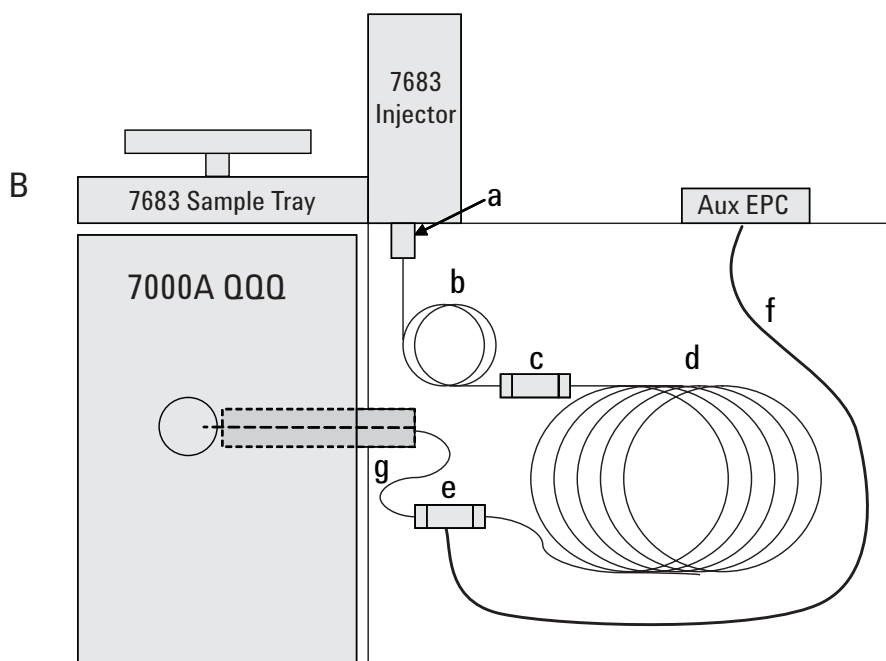*Figure 1B.* *The GC/QQQ used for MRM analyses was configured with a) split/splitless inlet, b) 2 m × 0.25 mm deactivated retention gap, c) Ultimate Union d) 15 m × 0.25 × 0.25 μm Agilent J&W HP-5ms UI column, e) Purged Ultimate Union, f) helium purge flow, and g) 65 cm × 0.15 mm deactivated restrictor.*

MRM Method

A method was developed for the analysis of 175 commonly analyzed pesticides. Two transitions were determined for each compound and the collision energy was optimized for each. Since the method was locked to the Agilent Pesticide method (running at twice the original speed), the retention times correspond to those recorded in Agilent's RTL Pesticide and Endocrine Disruptor Database (P/N G1672AA) divided by two. There are small differences in RT between the database and values shown here because this method used a retention gap, capillary flow device, and a restrictor. Table 3 lists the pesticides in alphabetical order with their retention times, quant and qual transitions, and the collision energies for each.

Table 3.   Target and Qualifier Transitions for 175 Pesticides

Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Acrinathrin	15.371	181.1	152.1	25	181.1	127.1	30
Akton	11.403	282.9	219.0	10	282.9	184.0	25
Alachlor	8.507	188.1	130.1	40	188.1	160.1	10
Aldrin	9.247	262.9	192.9	40	262.9	190.9	40
Allethrin	10.908	123.1	81.1	10	123.1	79.1	20
Atrazine	6.581	200.1	122.1	10	200.1	104.0	20
Azamethidaphos (Azamethiphos)	13.248	215.0	171.0	15	215.0	128.0	30
Azinphos-methyl	14.835	160.1	77.1	20	160.1	132.1	0
Benfluralin	5.842	292.1	264.0	10	292.1	160.1	20
BHC, α-	6.025	181.0	145.0	15	181.0	109.0	30
BHC, ß-	6.595	181.0	145.0	15	181.0	109.0	30
BHC, δ-	7.266	181.0	145.0	15	181.0	109.0	30
Bifenthrin	14.428	181.1	165.1	30	181.1	166.1	15
Bromacil	9.186	205.0	132.0	30	205.0	187.9	20
Bromophos	10.020	330.9	315.9	20	330.9	285.9	35
Bromophos-ethyl	11.261	358.9	302.9	15	358.9	284.8	35
Bromopropylate	14.320	183.0	155.0	15	183.0	76.0	35
Captan	10.617	79.1	77.1	10	79.1	51.1	25
Carbophenothion	13.316	157.0	121.0	25	157.0	75.1	40
Chlordane, *cis-*	11.410	372.9	265.9	40	372.9	263.9	30
Chlordane, *trans-*	11.010	372.9	265.9	20	372.9	263.9	25
Chlordene, α-	8.562	230.0	160.0	40	230.0	195.0	25
Chlordene, ß-	9.376	230.0	160.0	35	230.0	195.0	25
Chlordene, γ-	9.314	230.0	160.0	40	230.0	195.0	25
Chlorfenvinphos, ß-	10.779	267.0	159.0	20	267.0	81.0	40
Chlorobenzilate	12.706	139.0	111.0	15	139.0	75.0	30
Chloroneb	4.323	191.0	113.0	15	191.0	141.0	10
Chlorothalonil	7.395	265.9	133.0	40	265.9	230.9	20
Chlorpyrifos	9.606	196.9	168.9	15	196.9	107.0	40
Chlorpyrifos-methyl	8.284	286.0	93.0	25	286.0	270.9	20
Chlorthiophos	13.051	268.9	205.0	15	268.9	177.0	25
Coumaphos	15.859	362.0	109.0	15	362.0	81.0	40
Cyanazine	9.694	212.1	123.1	20	212.1	151.1	10
Cyanophos	6.887	243.0	109.0	10	243.0	79.0	30
Cyfluthrin 1	16.144	163.0	127.1	5	163.0	91.1	15
Cyfluthrin 2	16.212	163.0	91.1	15	163.0	127.1	5

(Continued)

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Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Cyfluthrin 3	16.273	163.0	127.1	5	163.0	91.1	15
Cyfluthrin 4	16.307	163.0	127.1	5	163.0	91.1	15
Cyhalothrin, λ-	15.208	181.1	152.1	30	181.1	127.1	35
Cypermethrin 1	16.381	181.1	152.1	25	181.1	127.1	35
Cypermethrin 2	16.463	181.1	152.1	30	181.1	127.1	35
Cypermethrin 3	16.531	181.1	152.1	25	181.1	127.1	30
Cypermethrin 4	16.558	181.1	152.1	25	181.1	127.1	30
Dacthal (DCPA) (Chlorthal-Dimethyl)	9.721	300.9	222.9	25	300.9	166.9	40
DDD, o,p'-	12.170	235.0	165.1	30	235.0	199.1	15
DDD, p,p'-	12.841	235.0	165.1	25	235.0	199.1	20
DDE, o,p'-	11.241	246.0	176.1	40	246.0	211.0	20
DDE, p,p'-	12.007	246.0	176.1	40	246.0	175.1	40
DDT, o,p'-	12.882	235.0	165.1	30	235.0	199.1	20
DDT, p,p'-	13.492	235.0	165.1	30	235.0	199.1	20
DEF (Tribufos)	12.054	169.0	57.1	5	169.0	112.9	5
Deltamethrin	18.016	181.1	152.1	25	181.1	127.1	25
Demeton-S	6.303	88.1	60.0	5	88.1	59.0	20
Demeton-S-methyl	5.230	88.1	60.0	5	88.1	59.0	15
Dialifos	15.432	208.0	102.1	40	208.0	89.0	40
Diallate 1	5.957	234.1	150.0	20	234.1	192.0	10
Diallate 2	6.127	234.1	150.0	20	234.1	192.0	10
Diazinon	7.226	179.1	121.1	40	179.1	137.2	20
Dicapthon	9.694	262.0	216.0	15	262.0	123.0	40
Dichlofenthion	8.067	279.0	223.0	15	279.0	205.0	30
Dichlofluanid	9.199	123.0	77.1	20	123.0	51.1	40
Dichlorobenzophenone, 4,4'-	9.593	139.0	111.0	15	139.0	75.1	30
Dichlorvos	2.905	109.0	79.0	5	109.0	47.0	15
Diclobenil	3.367	171.0	100.0	25	171.0	136.0	15
Dicloran	6.269	206.0	176.0	10	206.0	124.0	30
Dieldrin	11.926	262.9	192.9	40	262.9	190.9	35
Dimethachlor	8.080	134.1	105.1	15	134.1	77.1	30
Dioxathion	15.934	125.0	97.0	5	125.0	65.0	25
Disulfoton	7.260	88.1	60.0	5	88.1	59.0	25
Ditalimfos	11.586	130.0	102.1	15	130.0	75.0	30
Edifenphos	13.377	173.0	109.0	15	173.0	65.1	40
Endosulfan ether	7.660	240.9	205.9	20	240.9	203.9	20
Endosulfan I	11.308	240.9	205.9	15	240.9	136.0	40
Endosulfan II	12.570	195.0	125.0	25	195.0	159.0	10
Endosulfan sulfate	13.377	271.9	236.9	20	271.9	116.9	40
Endrin	12.366	262.9	193.0	35	262.9	190.9	35
Endrin aldehyde	12.956	249.9	214.9	35	249.9	141.9	40
Endrin ketone	14.116	316.9	101.0	20	316.9	245.0	20
EPN	14.333	157.0	77.1	25	157.0	110.0	15
Ethalfluralin	5.632	276.1	105.1	35	276.1	202.0	20
Ethion	12.997	231.0	128.9	25	231.0	174.9	10
Ethoprop (Ethoprophos)	5.357	158.0	97.0	15	158.0	114.0	5
Etridazole	3.963	183.0	139.9	20	183.0	108.0	40

(Continued)

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Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Famphur	13.329	218.0	109.0	15	218.0	79.0	30
Fenamiphos (Phenamiphos)	11.803	303.1	80.0	40	303.1	154.0	20
Fenarimol	15.222	139.0	111.0	15	139.0	75.0	35
Fenchlorphos (Ronnel)	8.650	284.9	269.9	15	284.9	239.9	35
Fenitrothion	9.030	277.0	109.0	20	277.0	260.0	5
Fenpropathrin	14.503	181.1	152.1	30	181.1	127.1	35
Fensulfothion	12.780	292.0	156.0	25	292.0	109.0	20
Fenthion	9.552	278.0	109.0	20	278.0	125.0	20
Fenvalerate 1	17.202	167.1	125.0	15	167.1	89.1	40
Fenvalerate 2	17.412	167.1	125.0	10	167.1	89.1	35
Fluchloralin	7.321	306.1	264.1	5	306.1	206.0	15
Flucythrinate 1	16.571	199.1	107.1	30	199.1	157.1	10
Flucythrinate 2	16.741	199.1	107.1	25	199.1	157.1	5
Fluridone	16.944	328.1	259.0	30	328.1	189.1	40
Fluvalinate τ- 1	17.412	250.1	55.1	15	250.1	200.1	20
Fluvalinate τ- 2	17.480	250.1	55.1	15	250.1	200.1	25
Folpet	10.807	147.1	103.1	5	147.1	76.0	30
Fonophos	6.934	246.1	109.0	15	246.1	137.0	5
Heptachlor	8.379	271.9	236.8	25	271.9	116.9	40
Heptachlor exo-epoxide isomer A	10.474	183.0	118.9	30	183.0	154.9	15
Heptachlor exo-epoxide isomer B	10.352	352.9	262.8	25	352.9	281.9	20
Hexachlorobenzene	6.168	283.9	213.9	35	283.9	248.8	25
Hexazinone	13.702	171.1	71.1	15	171.1	85.1	15
Iprobenfos (IBP)	7.660	204.0	91.1	10	204.0	121.0	40
Iprodione	14.211	187.0	124.0	25	187.0	159.0	15
Isazophos	7.517	161.1	119.0	10	161.1	146.0	5
Isofenfos	10.813	213.1	121.0	20	213.1	185.0	5
Jodfenphos (Iodofenphos)	11.776	376.9	361.9	20	376.9	93.0	35
Leptophos	14.876	171.0	77.1	25	171.0	124.1	10
Lindane (γ-BHC)	6.710	181.0	145.0	15	181.0	109.0	30
Malathion	9.396	173.1	99.0	15	173.1	117.0	10
Methidathion	11.146	145.0	85.1	5	145.0	58.1	15
Methoxychlor, o,p'-	13.730	227.1	121.1	15	227.1	91.1	35
Methoxychlor, p,p'-	14.442	227.1	141.1	40	227.1	169.1	30
Metolachlor	9.450	162.1	133.1	15	162.1	132.1	25
Mevinphos	3.782	127.0	109.0	10	127.0	95.0	15
Mirex	14.923	271.9	236.9	15	271.9	116.9	40
Nonachlor, cis-	12.848	408.8	109.0	20	408.8	299.9	25
Nonachlor, trans-	11.539	408.8	299.8	25	408.8	301.8	30
Oxadiazon	12.210	175.0	112.0	15	175.0	76.1	40
Parathion	9.633	291.1	109.0	10	291.1	81.0	40
Parathion methyl	8.284	263.0	109.0	10	263.0	79.0	35
Pentachloroaniline	7.761	264.9	193.9	30	264.9	155.9	30
Pentachlorobenzene	4.459	249.9	214.9	25	249.9	142.0	40
Pentachlorobenzonitrile	6.866	274.9	239.9	20	274.9	204.9	35
Pentachlorophenyl methyl ester	6.283	264.9	236.9	10	264.9	142.9	40
Pentachlorothioanisole	9.016	295.9	245.8	40	295.9	262.9	15

Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Permethrin, *cis-*	15.703	183.1	153.1	15	183.1	168.1	15
Permethrin, *trans-*	15.798	183.1	155.1	10	183.1	165.1	10
Phenanthrene-d10	6.863	188	160	10	188	186	10
Phenothrin	14.713	183.1	153.1	15	183.1	168.1	15
Phenthoate	10.861	274.0	121.0	10	274.0	125.0	20
Phorate	5.961	231.0	128.9	25	231.0	174.9	10
Phosalone	14.855	182.0	111.0	15	182.0	75.1	40
Phosmet	14.259	160.0	77.1	30	160.0	133.0	15
Pirimiphos ethyl	10.332	318.1	166.1	15	318.1	182.1	15
Pirimiphos methyl	9.138	290.1	125.0	25	290.1	233.0	10
Procymidone	10.983	283.0	96.1	10	283.0	67.1	40
Profenofos	11.953	207.9	63.1	40	207.9	99.0	25
Propachlor	5.164	120.1	77.1	20	120.1	92.1	5
Propargite	13.858	135.1	107.1	15	135.1	77.1	30
Propazine	6.676	214.1	172.0	10	214.1	104.0	20
Propetamphos	6.948	138.0	110.0	5	138.0	64.0	15
Propyzamide	6.975	173.0	145.0	15	173.0	109.0	35
Prothiophos	11.878	162.0	63.1	40	162.0	98.0	20
Pyraclofos	15.439	360.0	96.9	35	360.0	194.0	15
Pyrazophos	15.351	221.1	193.1	10	221.1	149.1	15
Pyridaphenthion	14.272	340.1	199.1	5	340.1	97.0	40
Quinalphos	10.827	146.1	118.1	10	146.1	91.1	30
Quintozene	6.832	236.9	118.9	25	236.9	142.9	30
Resmethrin	13.994	123.1	81.1	5	123.1	95.1	5
Simazine	6.473	201.1	173.1	5	201.1	138.1	10
Sulfotep-ethyl	5.902	322.0	146.0	25	322.0	65.0	40
Sulprofos	13.180	322.0	97.0	30	322.0	156.0	5
Tebupirimfos	7.687	261.1	137.1	15	261.1	153.1	20
Tecnazene (TCNB)	5.110	202.9	83.0	25	202.9	142.9	20
Tefluthrin	7.524	177.1	127.1	20	177.1	137.0	20
Temephos	20.525	125.0	47.0	20	125.0	79.0	10
Terbufos	6.890	231.0	128.9	25	231.0	174.9	10
Terbuthylazine	6.907	214.1	104.0	20	214.1	132.0	10
Tetrachloroaniline, 2,3,5,6-	5.293	230.9	158.0	25	230.9	122.0	40
Tetrachlorvinphos	11.478	329.0	109.0	25	329.0	79.0	35
Tetramethrin I	14.299	164.1	107.1	15	164.1	135.1	10
Tetramethrin II	14.421	164.1	107.1	10	164.1	135.1	5
Thiometon	6.161	125.0	47.0	20	125.0	79.0	10
Tolclofos methyl	8.392	265.0	250.0	15	265.0	93.0	25
Tolyfluanid	10.623	137.0	91.1	20	137.0	65.1	35
Triallate	7.470	268.0	183.9	25	268.0	226.0	15
Triazophos	13.241	161.0	134.1	10	161.0	91.1	20
Trifluralin	5.808	306.1	264.0	5	306.1	160.0	30
Triphenyl phosphate	13.865	326.1	169.1	35	326.1	233.0	10
Vinclozolin	8.311	212.0	145.0	25	212.0	109.0	40

Carrot Extract

A carrot extract with incurred pesticide residues was analyzed in the scan and SIM modes with the GC/Q. In each case, 5-µL injections were made using Agilent's new Multimode inlet operated in the cold splitless mode. Three SIM methods were used to monitor > 170 compuonds with about 60 pesticides in each method. Four ions were monitored for each compound. The scan data were analyzed automatically using Agilent's Deconvolution Reporting Software, together with the 927-compound RTL Pesticide and Endocrine Disruptor Database.

The same carrot sample was also analyzed on the 7890A/7000A GC/QQQ system using the MRM transitions listed in Table 3. An 11-point calibration curve was prepared in carrot matrix for 170 pesticides from 3.33 µg/kg (ppb) to 6670 µg/kg. Table 4 shows the results of these analyses.

Table 4. Results from the Analysis of a Carrot Extract with Incurred Pesticides by GC/MS in the Scan Mode with DRS Analysis, by GC/MS in the SIM Mode, and by GC/MS/MS in the MRM Mode (An X implies that the compound was found by that method.)

Pesticide	GC/Q 5 µL (Multimode inlet) Cold SL scan + DRS	Cold SL SIM	GC/QQQ[a] 1 µL Hot SL (ppb)
Diclobenil			0.38[b]
Pentachlorobenzene			0.75[b]
Trifluralin			2.3[b]
Tefluthrin			0.53[b]
4,4'-Dichlorobenzophenone			1.2[b]
Chlorpyrifos			24.7
o,p'-DDE			3.7
p,p'-DDE	X	X	240
o,p'-DDD			9
p,p'-DDD	X		Sum = 45
o,p'-DDT	X		
p,p'-DDT	X	X	130
Fenazaquin	X	Not in method	Not in method

a. The actual concentration of these compounds was lower in the original carrot sample by a factor of 4.5 since the extraction method results in 4.5 g of produce per mL of extract.

b. The reported values fall below the lowest point on the calibration curve.

The single quad methods were not quantitative, so Table 4 only indicates (with an X) if a pesticide was found, either by DRS or by manual examination of the SIM data. Since the triple quad method was calibrated, the amount of each pesticide could be determined. The amounts reported are those found in the extract. Because the extraction method concentrated this sample by a factor of 4.5:1 (4.5 g of carrot to 1.0 mL of final extract), the pesticide concentrations in the original carrot samples were actually lower by this factor.

The scan method with DRS analysis has the capability to find any of the 927 compounds in the database, while the SIM and MRM methods are limited to the 175 target compounds listed in Table 3. DRS found fenazaquin, a pesticide that was not in the SIM or MRM methods. This demonstrates the advantage of using GC/MS with DRS for screening purposes in combination with GC/MS/MS for target compound analysis.

In spite of the concentrated carrot matrix, the GC/QQQ was able to detect three pesticides below 1 ppb (1 µg/kg) and three more below 5 ppb. The lowest level calibration standard was prepared at 3.33 ppb, so numbers reported below that level are extrapolated values. The optimal MRM transitions for p,p'-DDD and o,p'-DDT are the same and, since these two compounds were only partially resolved chromatographically, they are reported together.

Figure 2A shows the extracted quant ion (*m/z* 246) for p,p'-DDE from the scan analysis of the carrot sample. Interferences in these chromatograms make it harder to do an accurate quantitative analysis without first deconvoluting the spectrum. After deconvolution (Figure 2B), ChemStation integration is trivial. Figure 2C shows the EIC (*m/z* 246) from the GC/MS SIM analysis of the same sample. Although the signal/noise ratio (S/N) is 10-fold better, there appear to be more interferences.

It is easy to see the advantage of the GC/QQQ for target compound analysis. A 1-µL injection of the carrot extract on this instrument gave a clean MRM chromatographic peak (Figure 2D) with better S/N (434) than was obtained for the 5-µL GC/Q SIM analysis (S/N = 375)(Figure 2C).
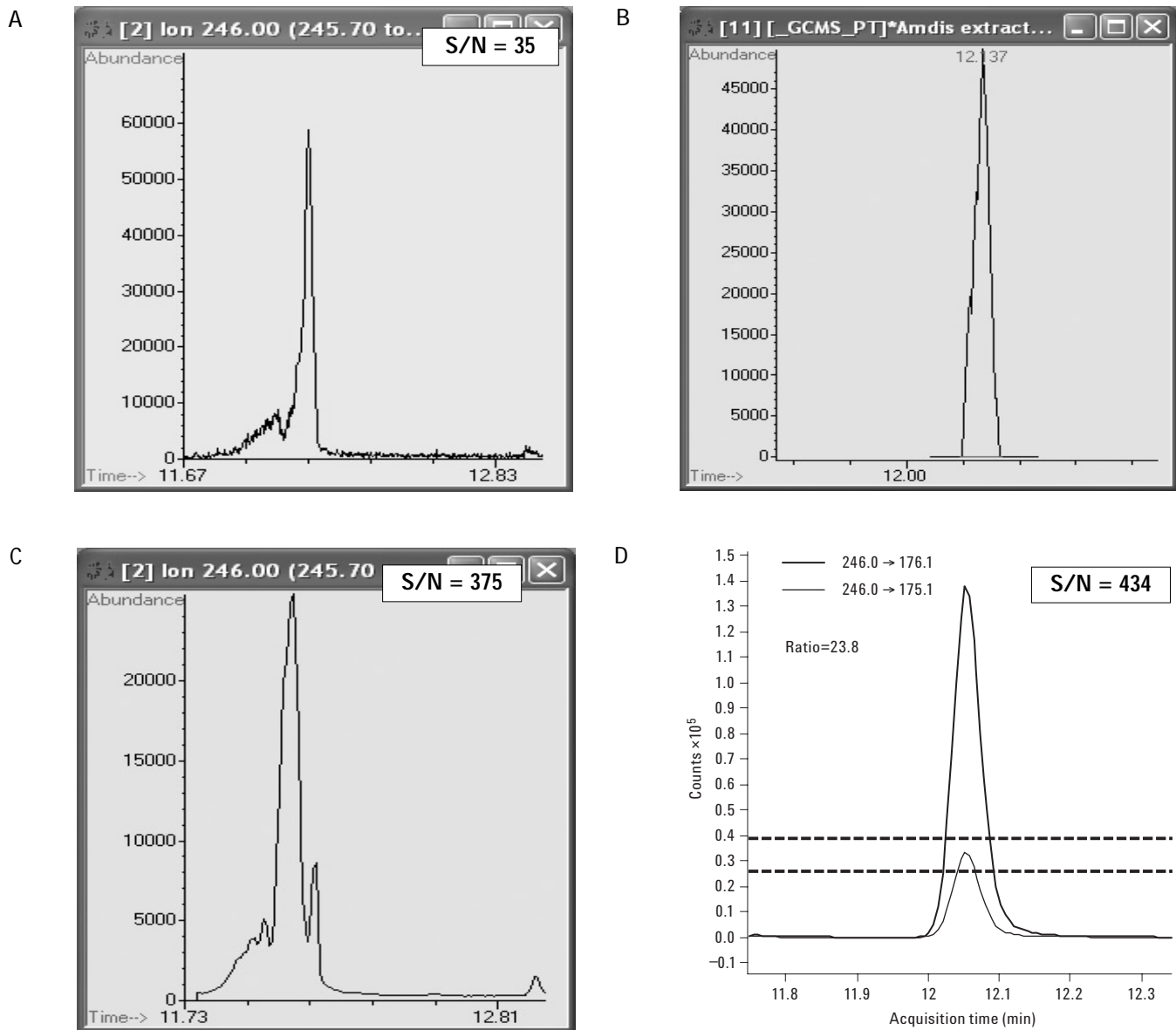
Figure 2. *A) p,p'-DDE quant ion (m/z 246) extracted from the scan chromatogram obtained from a 5-µL cold splitless injection of a carrot extract with incurred pesticides. B) Same as in (A) but after deconvolution. C) p,p'-DDE quant ion (m/z 246) extracted from a SIM chromatogram obtained from a 5-µL cold splitless injection of the same sample. D) Quant and qualifier transitions (246.0 → 176.1 and 246.0 → 175.1, respectively) for the GC/MS/MS analysis of a 1-µL hot splitless injection of the same carrot extract. Peak-to-peak signal/noise ratios for the extracted ions and the quant transition are shown. The ratio of the two transition ions (D) is 23.8, confirming the presence of p,p'-DDE.*

Comparing GC/MS SIM to GC/MS/MS MRM – Various Matrices

Figure 3 compares GC/MS SIM results to GC/MS/MS MRM results for p,p'-DDE spiked into various commodities at 10 ppb. On the left, the SIM EICs for the quant ion (*m/z* 246) show increasing amounts of matrix interference from the apple, cabbage, ginseng, orange, and spinach samples. In contrast, the p,p'-DDE GC/MS/MS transitions shown on the right have no interferences from any of the extracts. The large S/N values shown for the quant transition (246.0 → 176.1) suggest that one should be able to detect p,p'-DDE at the sub-ppb level.

EIC (246)
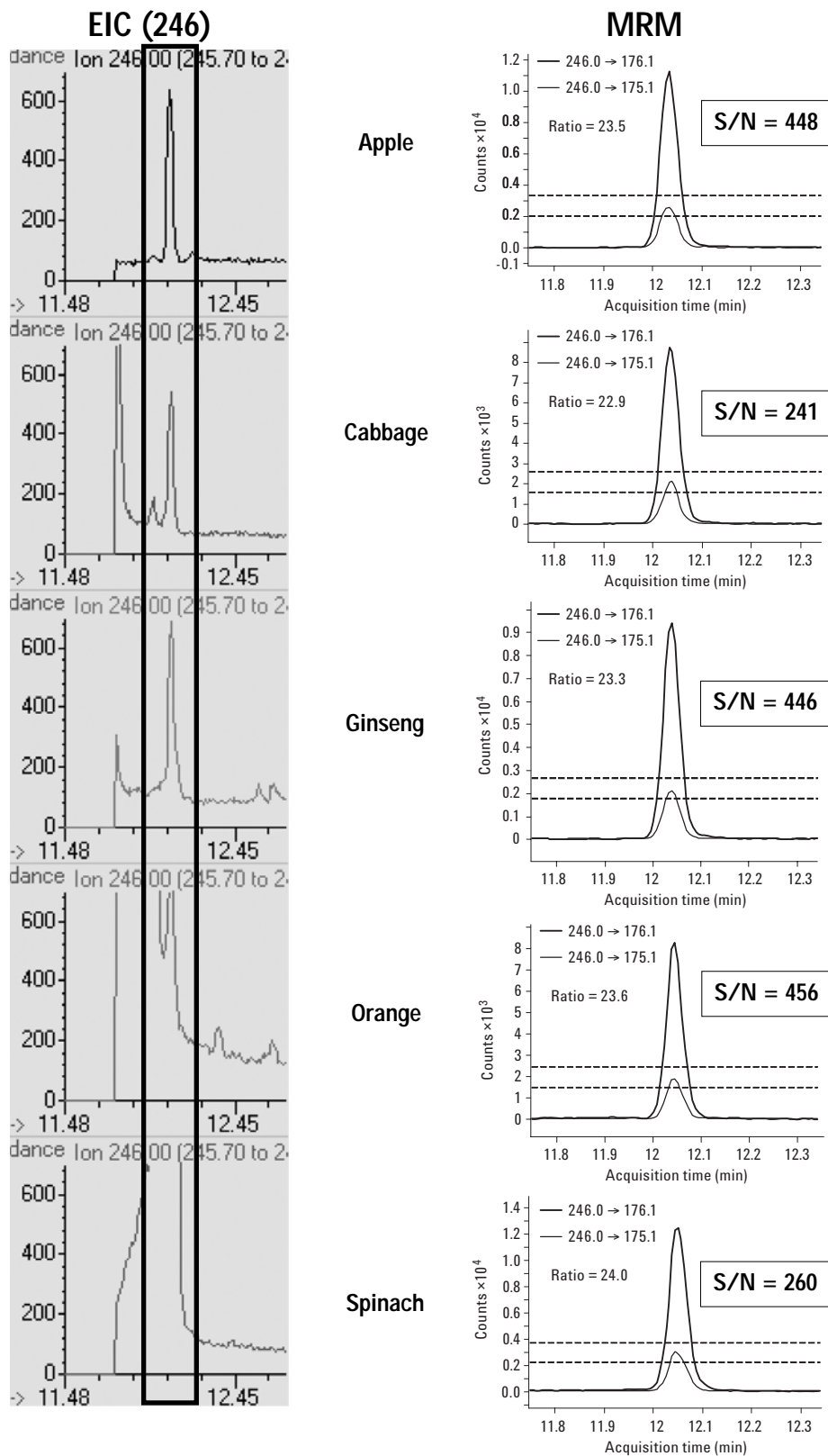
MRM

Apple

— 246.0 → 176.1
— 246.0 → 175.1

Ratio = 23.5

S/N = 448

Cabbage

— 246.0 → 176.1
— 246.0 → 175.1

Ratio = 22.9

S/N = 241

Ginseng

— 246.0 → 176.1
— 246.0 → 175.1

Ratio = 23.3

S/N = 446

Orange

— 246.0 → 176.1
— 246.0 → 175.1

Ratio = 23.6

S/N = 456

Spinach

— 246.0 → 176.1
— 246.0 → 175.1

Ratio = 24.0

S/N = 260

Figure 3.   A comparison of GC/MS SIM and GC/MS/MS MRM analysis for p,p'-DDE spiked at 10 ppb (10 µg/kg) into five different produce extracts. On the left, the EICs for the p,p'-DDE quant ion (m/z 246) show increasing amounts of interference from the matrix. The transitions on the right (246.0 → 176.1 and 246.0 → 175.1) for p,p'-DDE are clean, with peak-to-peak S/N values ranging from 241 to 448. All injections were 1 µL.

Tomato Extract

All three techniques being discussed were able to identify incurred chlorothalonil in a tomato extract, which was present at 1 ppm. However, only the GC/QQQ was able to identify pentachlorobenzonitrile, a chlorothalonil metabolite, which it measured at 9.3 ppb. Figure 4 shows the MRM transitions for pentachlorobenzonitrile and a calibration curve for the compound ranging from 3.33 ppb to 6670 ppb.

Backflushing the Column

The norm when analyzing dirty samples by GC/MS is to replace the inlet liner and clip the column frequently. Many labs do this daily. Otherwise, matrix accumulates in the liner and column, degrading the chromatography. Over time, these materials migrate through the GC column and contaminate the source, which then needs to be cleaned. This problem may be compounded with a GC/QQQ instrument because one does not see much evidence of the matrix and the temptation is to ignore maintenance until the source (and sometimes the first quadrupole) needs to be cleaned.

The Agilent 7000A Series triple quad MS uses the same inert source and gold-plated quartz quadrupole that are found in the 5975C MSD. These can be heated up to 350 °C and 200 °C, respectively, which greatly minimizes the need for cleaning, even when high-boiling matrix compounds do reach the detector.

The best way to prevent chromatographic degradation and reduce the need for source cleaning is to backflush the GC column during or after each run. With the configurations shown in Figure 1, backflushing is done for 3 to 5 minutes after the run by raising the pressure at the capillary flow device (two-way splitter or the Purged Ultimare Union) and lowering the inlet pressure. This reverses the flow through the column and purges high-boiling matrix components from the head of the column and out through the inlet's split vent.

During the course of this work, approximately 100 1-µL injections of concentrated food extracts were made into the GC/QQQ system with no evidence of column or MS performance problems. Nearly 300 µL of these same extracts were injected into the GC/Q system before column and inlet maintenance was required. With the capillary flow device installed, you can do this maintenance without venting the mass spectrometer.

Conclusions

Agilent's 7890A/7000A triple quadrupole MS system is a sensitive and rugged tool for target pesticide analysis. There is far less interference from matrix than one sees in single quadrupole methods, making it much easier to quantify pesticides at the low ppb levels required by today's legislation. In many cases a 1-µL injection into the GC/QQQ produced far better results than a 5-µL injection into the GC/Q. Nevertheless, there is still a need for screening methods that look for hundreds of pesticides. For this, we recommend using large-volume injection with Agilent's new Multimode inlet, GC/Q analysis in the scan mode, and data analysis using Deconvolution Reporting Software with Agilent's Pesticide and Endocrine Disruptor Database. The combination of these two approaches is the best way to screen for more than 900 contaminants (by GC/Q with DRS) while performing ultra-trace analysis for a smaller list of target compounds (using GC/QQQ). Both approaches benefit from column backflushing, which is highly recommended when analyzing dirty samples, such as food extracts.
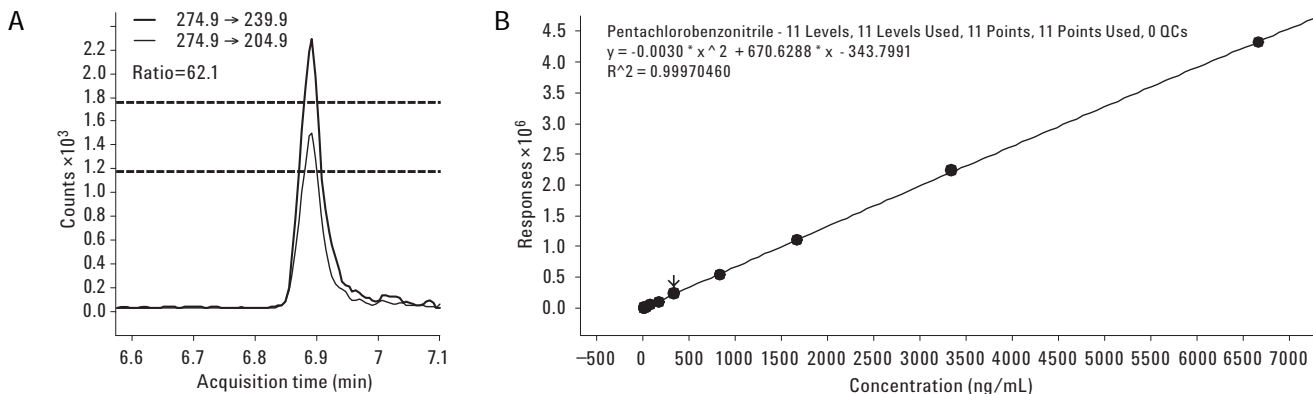

Figure 4.   A) MRM transitions identifying pentachlorobenzonitrile at 9.3 ppb in a tomato extract. B) A calibration curve for pentachlorobenzonitrile from 3.33 to 6,670 ppb with a quadratic curve fit > 0.999.

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