Analysis of Pesticide Residues in Chicken Muscle with QuEChERS and Triple Quadrupole GC

Application Note

Food Testing & Agriculture

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Introduction

A GC/MS/MS multiple reaction monitoring method was developed on the Agilent 7890A GC and an Agilent 7000B GC Triple Quadrupole GC/MS System for 54 pesticide residues in chicken. Sample preparation followed the QuEChERS AOAC extraction method with two different dispersive cleanup techniques. Chicken samples were analyzed to verify calibration coefficient and reproducibility. The quantification limit of most compounds can be down to 5 ng/g (5 ppb), even with matrix interference. The method recoveries for all of the compounds were 70 to 120%, with RSD below 15%.

Experimental

All reagents and solvents were MS or HPLC grade. Acetonitrile (ACN) and methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) and acetic acid (AA) were from Fluka (Sigma-Aldrich, Steinheim, Germany). The pesticide standards and internal standard triphenyl phosphate (TPP) were provided by internal customers.

Agilent Technologies

Equipment and materials

Agilent 7890A GC

Agilent 7000B Triple Quadrupole GC/MS System

Agilent Bond Elut QuEChERS AOAC Extraction Kit (p/n 5982-5755CH)

Agilent Bond Elut QuEChERS Dispersive Kit, Other Food Methods (p/n 5982-4956CH)

Agilent Bond Elut QuEChERS Dispersive Kit, All Food Types (p/n 5982-0029CH)

Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)

Conditions

GC

Column:	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm
Carrier:	Constant flow, 1.0 mL/min
Oven temperature:	Initial 60 °C for 1 min, then 40 °C/min to 170 °C for 0 min, then 10 °C/min to 310 °C hold for 5 min 310 °C 5 min
Injector temperature:	Initial 60°C for 0 min, then 500 °C/min to 280 °C for 10 min, then 500 °C/min to 60 °C hold for 0 min
Injection:	Splitless, 1.0 µL

MS

Ion source:	EI
Source temperature:	300 °C
Collision gas:	Nitrogen, 1.5 mL/min
Transfer line temperature:	280 °C
Quad temperature:	180 °C
Solvent delay:	3.7 min
MRMs:	see Table 1

Table 1. CAS number, MRMs and retention time for 54 pesticides and internal standard.

Name	CAS No.	Ion for quantitation	Ion for qualification	Retention time (min)
Methamidophos	10265-92-6	141.0 → 95.0	95.0 → 79.0	4.65
Dichlorvos	62-73-7	109.0 → 79.0	184.9 → 93.0	4.75
Acephate	30560-19-1	136.0 → 94.0	142.0 → 96.0	5.77
Omethoate	1113-02-6	109.9 → 79.0	155.9 → 110.0	6.86
Monocrotophos	6923-22-4	127.1 → 109.0	127.1 → 95.0	7.42
BHC-*alpha*	319-84-6	216.9 → 181.0	218.9 → 183.0	7.76
Hexachlorobenzene	118-74-1	283.8 → 213.9	283.8 → 248.8	7.90
Dimethoate	60-51-5	86.9 → 46.0	92.9 → 63.0	7.91
BHC-*beta*	319-85-7	216.9 → 181.1	181.0 → 145.0	8.15
BHC-*gamma*	58-89-9	216.9 → 181.0	181.0 → 145.0	8.28
Diazinon	333-41-5	137.1 → 84.0	137.1 → 54.0	8.41
BHC-*delta*	319-86-8	181.1 → 145.1	217.0 → 181.1	8.63
Vinclozolin	50471-44-8	187.0 → 124.0	197.9 → 145.0	9.24
Parathion-methyl	298-00-0	125.0 → 47.0	262.9 → 109.0	9.27
Chlorpyrifos-methyl	5598-13-0	124.9 → 78.9	285.9 → 92.9	9.27
Metalaxyl	57837-19-1	234.0 → 146.1	220.0 → 192.1	9.46
Heptachlor	76-44-8	271.7 → 236.9	273.7 → 238.9	9.47
Pirimiphos-methyl	29232-93-7	290.0 → 125.0	232.9 → 151.0	9.72
Fenitrothion	122-14-5	125.1 → 47.0	125.1 → 79.0	9.72
Malathion	121-75-5	126.9 → 99.0	172.9 → 99.0	9.86
Fenthion	55-38-9	124.9 → 47.0	278.0 → 109.0	10.05
Aldrin	309-00-2	262.9 → 192.9	254.9 → 220.0	10.08
Chlorpyrifos	2921-88-2	196.9 → 169.0	198.9 → 171.0	10.09
Parathion	56-38-2	138.9 → 109.0	290.9 → 109.0	10.10
Triadimefon	43121-43-3	208.0 → 181.1	208.0 → 111.0	10.14
Kelthane	115-32-2	139.0 → 111.0	251.0 → 139.0	10.17
Pendimethalin	40487-42-1	251.8 → 162.2	251.8 → 161.1	10.66
Heptachlor exo-epoxide	1024-57-3	352.8 → 262.9	354.8 → 264.9	10.76
Chlordane-oxy	27304-13-8	114.9 → 51.1	114.9 → 87.0	10.78
Isofenphos	25311-71-1	212.9 → 121.1	212.9 → 185.1	10.79

Table 1. CAS number, MRMs, and retention time for 54 pesticides and internal standard (continued).

Name	CAS No.	Ion for quantitation	Ion for qualification	Retention time (min)
Triadimenol	55219-65-3	168.0 → 70.0	128.0 → 65.0	10.87
Quinalphos	13593-03-8	146.0 → 118.0	146.0 → 91.0	10.87
Methidathion	950-37-8	144.9 → 85.0	144.9 → 58.1	11.13
DDE-p,p'	72-55-9	246.1 → 176.2	315.8 → 246.0	11.76
Myclobutanil	88671-89-0	179.0 → 125.1	179.0 → 90.0	11.86
Dieldrin	60-57-1	262.9 → 193.0	277.0 → 241.0	11.86
Endrin	72-80-8	262.8 → 193.0	244.8 → 173.0	11.86
Chlorfenapyr	122453-73-0	136.9 → 102.0	246.9 → 227.0	12.17
DDD-p,p'	72-54-8	234.9 → 165.1	236.9 → 165.2	12.50
Ethion	563-12-2	230.9 → 129.0	230.9 → 175.0	12.56
DDT-o,p'	789-02-6	235.0 → 165.2	237.0 → 165.2	12.58
DDT-p,p'	50-29-3	235.0 → 165.2	237.0 → 165.2	13.17
Iprodione	36743-19-7	313.8 → 55.9	313.8 → 244.9	13.83
Acetamiprid	135410-20-7	152.0 → 116.1	126.0 → 90.0	13.97
Phosmet	732-11-6	160.0 → 77.1	160.0 → 133.1	14.03
Bifenthrin	82657-04-3	181.2 → 165.2	181.2 → 166.2	14.04
Tetradifon	116-29-0	158.9 → 111.0	226.9 → 199.0	14.54
Phosalone	2310-17-0	182.0 → 111.0	182.0 → 102.1	14.71
Cyhalothrin	91465-08-6	208.0 → 181.0	197.0 → 141.0	15.00
Permethrin I	51877-74-8	183.1 → 168.1	183.1 → 165.1	15.73
Permethrin II	52341-32-9	182.9 → 168.1	182.9 → 155.1	15.85
Fenvalerate I	51630-58-1	167.0 → 125.1	208.9 → 141.1	17.51
Difenoconazole I	119446-68-3	322.8 → 264.8	264.9 → 202.0	17.92
Deltamethrin	52918-63-5	250.7 → 172.0	252.9 → 174.0	18.23
Triphenyl phosphate (internal standard)	115-86-6	214.9 → 168.1	326.0 → 325.0	13.49

Sample preparation

Organic farm chicken was purchased from a local food market. The chicken muscle was removed and chopped into small pieces. The muscle sample was then homogenized thoroughly with a food grinder and stored at -20 °C. The QuEChERS extraction procedure is shown below.

1. Weigh 7.5 g chicken muscle (± 0.1 g) in 50 mL centrifuge tube and add 7.5 mL of water.
2. Add internal standard, and QC spike solution if necessary, vortex for 1 minute.
3. Add 15 mL ACN containing 1% AA.
4. Add Bond Elut QuEChERS extraction salt packet.
5. Cap and shake vigorously for 30 seconds.
6. Centrifuge at 4,000 rpm and 4 °C for 5 minutes.
7. Transfer 8 mL of upper ACN layer to Bond Elut Dispersive-SPE 15 mL tube*.
8. Vortex 1 minute, centrifuge at 4,000 rpm and 4 °C for 5 minutes.
9. Transfer 4 mL extract to tube and dry under nitrogen.
10. Reconstitute with 1 mL ACN and analyze.

*Bond Elut QuEChERS Dispersive Kit, Other Food Methods and Bond Elut QuEChERs Dispersive Kit, All Food Types were used.

Results and Discussion

The AOAC extraction method was used for pesticide extraction. Two different dispersive-SPE kits were used for sample cleanup; one for other food methods and one for all food types. Figure 1 shows the sample solutions in vials from the QuEChERS cleanup procedures. The color of the sample with the dispersive-SPE kit for other food methods is slightly yellow, the one with the dispersive-SPE kit for all food types is clear. The pesticides included organochlorine, organophosphate, and pyrethroids. All target pesticides were separated and well detected by the HP-5ms Ultra Inert GC column. With the powerful selectivity provided by GC/MS/MS, the MRM chromatograms of the matrix blank did not show any interference peaks with the target analytes. Figures 2 to 5 show the GC/MS/MS chromatograms of the matrix blank and 10 ng/g-fortified chicken muscle extract processed by the AOAC QuEChERS extraction method and the two different cleanup methods.

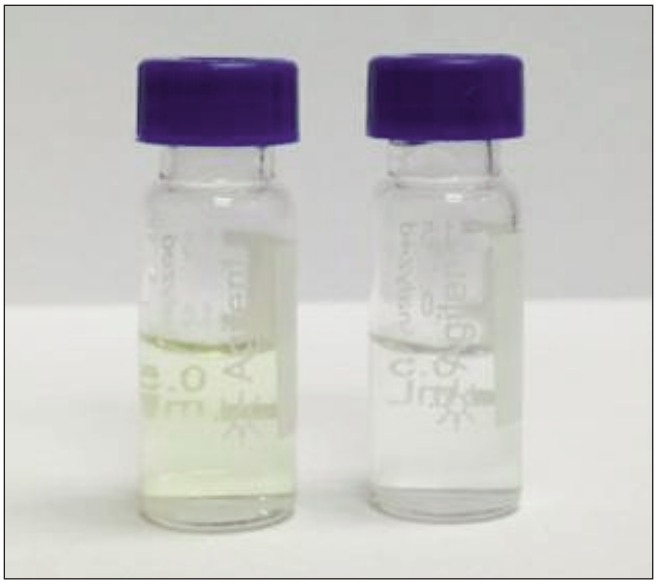

Figure 1. Sample solutions of chicken muscle with QuEChERS; cleanup with dispersive-SPE kit for other food methods (left) and cleanup with dispersive-SPE kit for all food types (right).

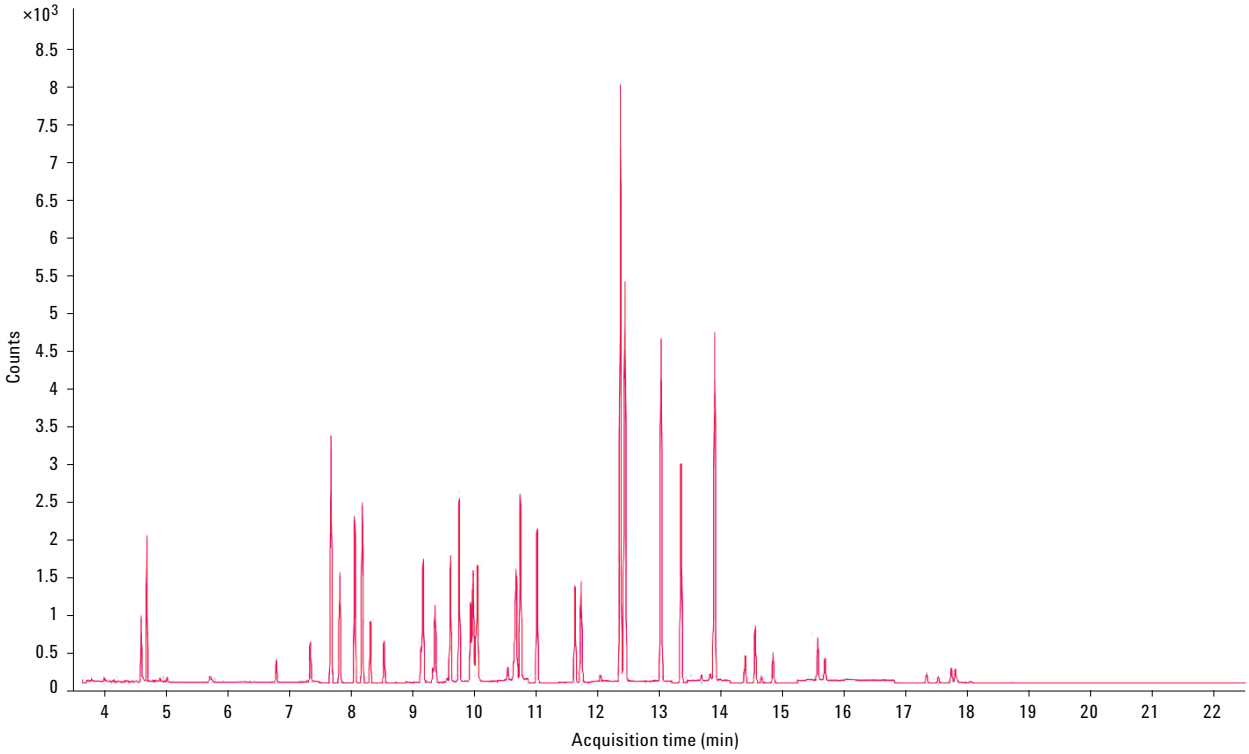

Figure 2. TIC of spiked 10 ng/g pesticides in chicken muscle after extraction with Agilent Bond Elut QuEChERS AOAC Extraction Kit and cleanup with Agilent QuEChERS Dispersive Kit, Other Food Methods.

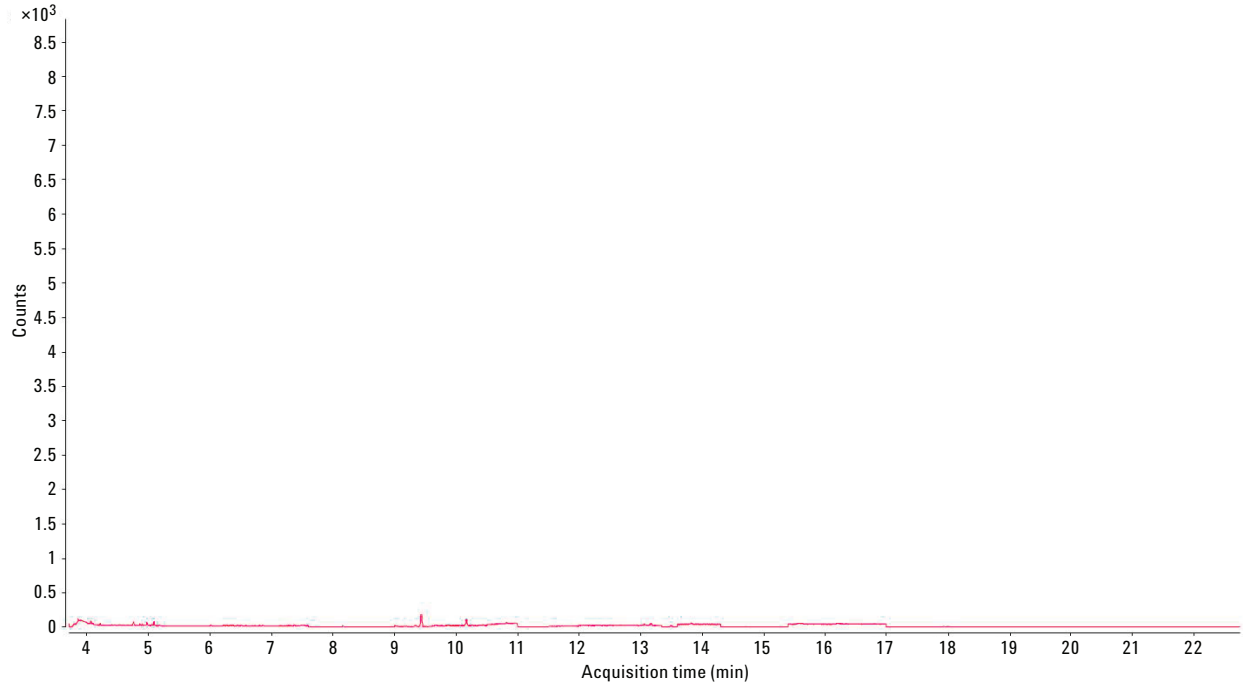

Figure 3. TIC of chicken muscle sample blank after extraction with Agilent Bond Elut QuEChERS AOAC Extraction Kit and cleanup with Agilent Bond Elut QuEChERS Dispersive Kit, Other Food Methods.

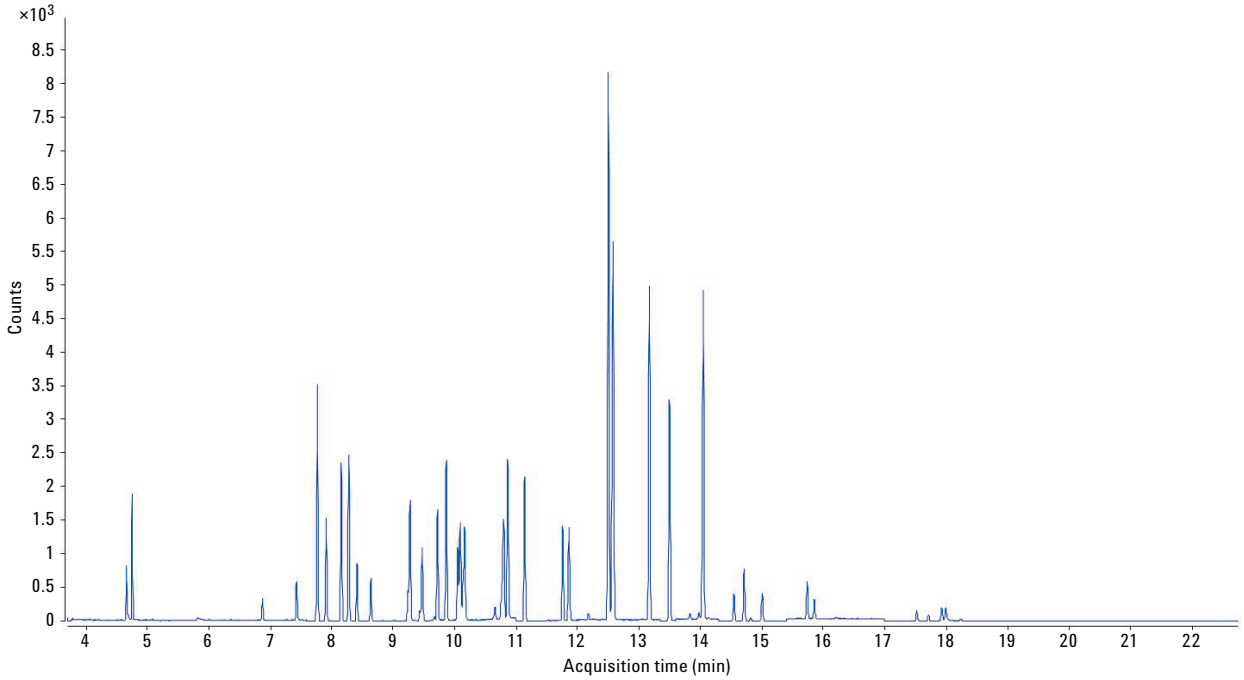

Figure 4. TIC of spike 10 µg/kg pesticides in chicken muscle after extraction with Agilent Bond Elut QuEChERS AOAC Extraction Kit and cleanup with Agilent Bond Elut QuEChERS Dispersive Kit, All Food Types.

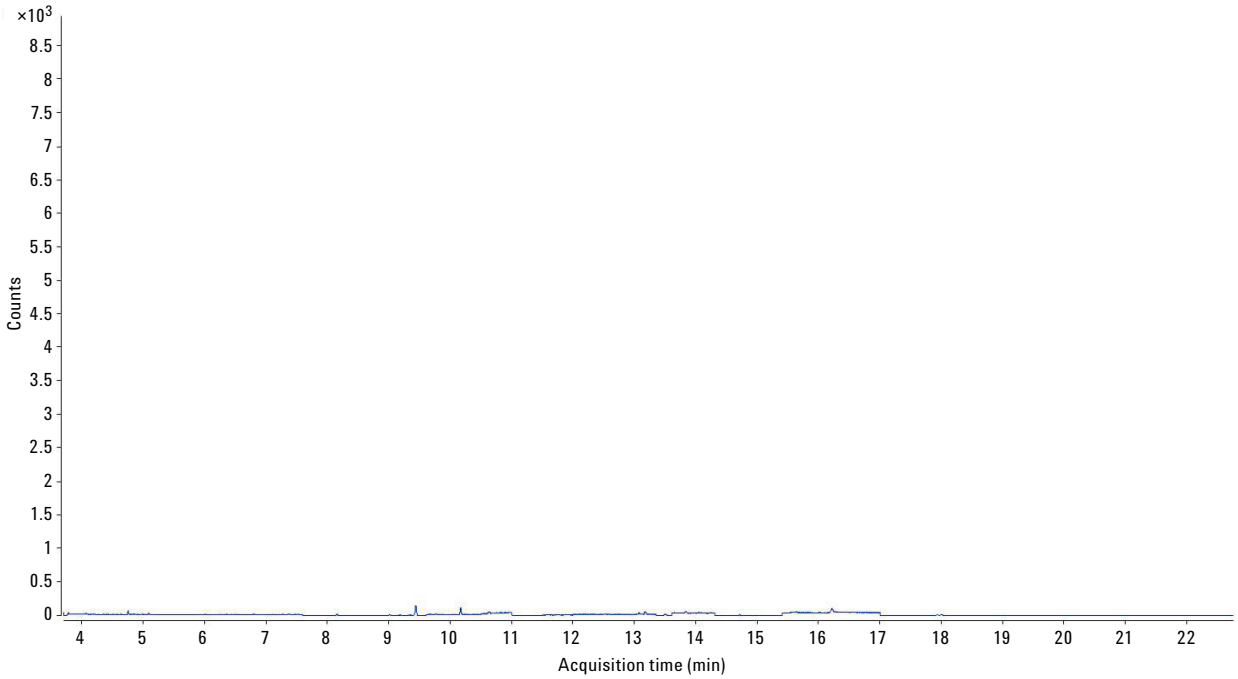

Figure 5. TIC of chicken muscle sample blank after extraction with Agilent Bond Elut QuEChERS AOAC Extraction Kit and cleanup with Agilent Bond Elut QuEChERS Dispersive Kit, All Food Types.

Linearity and limit of quantification (LOQ)

The linearity calibration range for all of the pesticides tested was 5 to 100 ng/g. Calibration curves , spiked in matrix blanks, were made at 5, 10, 20, 50, and 100 ng/g. TPP was used as an internal standard at 50 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). The 5 ng/g quantification limits established for all pesticides were lower than the MRLs of these pesticides in fruits and vegetables. The correlation coefficients (R^2) for all of the compounds were between 0.996 and 0.999.

Recovery and reproducibility

The recovery and reproducibility were evaluated by spiking pesticide standards in comminuted sample at levels of 10 and 50 ng/g and prepared with the AOAC QuEChERS extraction method and the two different cleanup methods. These QC samples were quantitated against the matrix spike calibration curve. The analysis was performed in replicates of six at each level. The recovery and reproducibility RSD data are shown in Tables 2 and 3. It can be seen that 54 pesticides had excellent recoveries and precision with the two different cleanup kits.

Table 2. Recovery and reproducibility of pesticides in chicken muscle with QuEChERS and cleanup with dispersive SPE kit for other food methods (n = 6).

	10 ng/g Spiked		50 ng/g Spiked	
Name	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Methamidophos	88.4	6.0	93.3	5.8
Dichlorvos	100.3	3.9	82.4	2.8
Acephate	84.1	14.0	79.5	7.6
Omethoate	84.3	8.9	84.9	8.6
Monocrotophos	90.7	6.7	75.6	8.2
BHC-*alpha*	95.3	4.3	94.7	6.9
Hexachlorobenzene	81.5	3.3	95.6	5.4
Dimethoate	93.1	4.5	94.5	6.3
BHC-*beta*	97.3	3.1	85.6	3.1
BHC-*gamma*	96.6	3.9	85.0	7.1
Diazinon	97.6	3.2	96.8	6.3
BHC-*delta*	95.0	4.7	94.1	4.8
Vinclozolin	99.8	2.0	93.8	4.5
Parathion-methyl	97.0	3.3	92.3	5.5
Chlorpyrifos-methyl	97.2	4.7	94.2	2.4
Metalaxyl	102.5	3.6	93.7	4.5
Heptachlor	93.9	3.9	97.9	5.0
Pirimiphos-methyl	98.9	3.6	89.7	5.5
Fenitrothion	96.9	2.4	92.7	3.8
Malathion	95.8	2.2	95.3	3.7
Fenthion	99.8	1.0	93.9	3.2
Aldrin	93.0	1.8	93.6	3.5
Chlorpyrifos	100.5	2.2	92.7	2.9
Parathion	93.4	6.8	97.1	6.1
Triadimefon	102.1	1.9	92.1	3.9
Kelthane	90.4	2.1	92.1	2.0
Pendimethalin	99.7	2.3	92.3	5.8
Heptachlor exo-epoxide	92.5	2.5	94.4	4.4
Chlordane-oxy	95.1	2.2	89.7	2.7
Isofenphos	98.8	2.7	91.1	6.1
Triadimenol	94.9	3.3	92.6	2.1
Quinalphos	97.2	2.1	73.9	5.9
Methidathion	97.4	2.6	89.3	2.9
DDE-p,p'	94.8	1.8	90.3	3.9
Myclobutanil	98.3	1.2	92.7	5.0
Dieldrin	95.7	2.8	92.6	5.3
Endrin	96.0	3.8	91.3	2.1
Chlorfenapyr	101.1	2.2	95.4	2.5
DDD-p,p'	96.9	1.5	90.7	3.5
Ethion	95.6	2.1	90.6	3.2
DDT-o,p'	92.7	1.8	93.2	3.5
DDT-p,p'	95.9	1.2	89.0	4.1

Table 2. Recovery and reproducibility of pesticides in chicken muscle with QuEChERS and cleanup with dispersive SPE kit for other food methods (n = 6) (continued).

	10 ng/g Spiked		50 ng/g Spiked	
Name	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Iprodione	72.7	5.6	89.3	6.6
Acetamiprid	97.4	2.8	89.9	1.2
Phosmet	92.6	3.3	98.2	3.1
Bifenthrin	96.1	2.0	90.0	3.3
Tetradifon	98.4	1.1	91.6	10.3
Phosalone	103.5	7.1	93.3	1.6
Cyhalothrin	99.7	5.4	90.8	6.0
Permethrin I	91.4	10.3	89.3	4.9
Permethrin II	95.5	9.2	91.1	5.2
Fenvalerate I	107.9	8.8	92.3	14.3
Difenoconazole I	93.0	5.5	88.2	7.5
Deltamethrin	94.4	5.1	103.0	16.1

Table 3. Recovery and reproducibility of pesticides in chicken muscle with QuEChERS and cleanup with dispersive-SPE kit for all food types (n = 6).

	10 ng/g Spiked		50 ng/g Spiked	
Name	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Methamidophos	95.1	5.4	82.4	2.8
Dichlorvos	115.4	2.8	93.3	5.8
Acephate	81.0	12.0	79.5	7.6
Omethoate	93.3	10.6	75.6	8.2
Monocrotophos	89.0	8.2	84.9	8.6
BHC-*alpha*	110.9	2.7	94.7	6.9
Hexachlorobenzene	73.6	3.7	85.0	7.1
Dimethoate	109.8	3.6	85.6	3.1
BHC-*beta*	112.6	2.6	95.6	5.4
BHC-*gamma*	108.4	7.5	94.5	6.3
Diazinon	113.8	2.4	94.1	4.8
BHC-*delta*	107.1	2.6	96.8	6.3
Vinclozolin	113.1	1.3	97.9	5.0
Parathion-methyl	109.5	2.2	93.7	4.5
Chlorpyrifos-methyl	108.6	3.4	93.8	4.5
Metalaxyl	109.7	3.0	94.2	2.4
Heptachlor	105.1	1.7	92.3	5.5
Pirimiphos-methyl	108.2	1.5	92.1	3.9
Fenitrothion	109.4	0.5	95.3	3.7
Malathion	111.3	2.5	92.7	2.9
Fenthion	103.2	1.6	93.9	3.2
Aldrin	98.6	0.3	89.7	5.5
Chlorpyrifos	107.4	2.8	92.7	3.8
Parathion	104.2	3.8	97.1	6.1

Table 3. Recovery and reproducibility of pesticides in chicken muscle with QuEChERS and cleanup with dispersive-SPE kit for all food types (n = 6) (continued).

	10 ng/g Spiked		50 ng/g Spiked	
Name	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Triadimefon	110.3	2.1	92.1	2.0
Kelthane	98.1	1.6	93.6	3.5
Pendimethalin	104.7	1.1	91.1	6.1
Heptachlor exo-epoxide	110.0	2.4	94.4	4.4
Chlordane-oxy	106.2	0.8	92.3	5.8
Isofenphos	108.9	1.5	89.7	2.7
Triadimenol	102.1	1.5	73.9	5.9
Quinalphos	107.3	1.7	92.6	2.1
Methidathion	104.4	0.8	89.3	2.9
DDE-p,p'	101.1	0.2	90.3	3.9
Myclobutanil	104.9	0.3	91.3	2.1
Dieldrin	101.7	4.4	92.7	5.0
Endrin	105.5	1.6	92.6	5.3
Chlorfenapyr	101.7	3.1	95.4	2.5
DDD-p,p'	106.1	1.5	90.7	3.5
Ethion	102.9	1.8	89.0	4.1
DDT-o,p'	98.3	1.4	90.6	3.2
DDT-p,p'	98.9	1.1	93.2	3.5
Iprodione	66.6	5.3	98.2	3.1
Acetamiprid	94.8	3.4	89.3	6.6
Phosmet	92.0	3.2	90.0	3.3
Bifenthrin	98.5	1.4	89.9	1.2
Tetradifon	102.4	1.5	93.3	1.6
Phosalone	92.5	2.3	91.6	10.3
Cyhalothrin	97.8	2.6	90.8	6.0
Permethrin I	89.0	7.9	89.3	4.9
Permethrin II	94.7	6.6	91.1	5.2
Fenvalerate I	88.9	4.1	92.3	14.3
Difenoconazole I	81.1	4.1	99.6	9.1
Deltamethrin	75.8	5.3	88.2	7.5

Conclusions

Agilent Bond Elut QuEChERS AOAC buffered extraction kits, dispersive-SPE kits for other food methods, and dispersive-SPE kits for all food types, provided a simple, fast and effective method for the purification of representative pesticides in chicken muscle. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiclass, multiresidue pesticide determination in chicken muscle. The impurities and matrix effects from chicken muscle did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than regulated MRLs in food. As the selected pesticides represented a broad variety of different classes and properties, BondElut QuEChERS AOAC Extraction and Dispersive-SPE kits are excellent choices for other pesticides in similar food matrixes.

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