Analysis of Pesticide Residues in Chicken Using Agilent BondElut QuEChERS and LC/MS/MS

Application Note

Food Testing and Agriculture

Author

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Abstract

This application note described the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for the extraction and cleanup of 12 pesticide residues representing various pesticide classes in chicken. The original AOAC method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/partitioning step by the addition of salts, and a cleanup step using dispersive solid-phase extraction (dispersive SPE). The presence of target pesticides in the chicken extracts were then determined by liquid chromatography coupled to an electrospray-ionization tandem mass spectrometer (LC/ESI/MS/MS) operating in positive-ion multiple-reaction-monitoring (MRM) mode. The spiking levels for the recovery experiments were 10 and 50 ng/g. The mean recoveries ranged between 76% and 107%, with RSD below 10%.

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Introduction

The AOAC QuEChERS method has been widely employed in the analysis of pesticides in food [1,2]. The method uses acetonitrile extraction, followed by salting out of the water from the sample using anhydrous magnesium sulfate ($MgSO_4$), and buffering acetate salts to induce partitioning. For cleanup, the Agilent Dispersive SPE kit for other food methods was selected for this application. These kits, for an 8 mL sample volume, contain 900 mg $MgSO_4$, and 150 mg C18 is added per mL of ACN extract. In this study, 12 pesticides were used for evaluating the performance of the Agilent Bond Elut AOAC Buffered Extraction kit and Bond Elut QuEChERS Dispersive SPE kit for other food methods. The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for the pesticides in chicken.

Experimental

All reagents and solvents were HPLC or analytical grade. Methanol (MeOH) and acetonitrile (ACN) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards were purchased from Sigma-Aldrich Corp. (St Louis, MO, USA). The internal standard (triphenyl phosphate, TPP) was from Agilent Technologies, Inc. (Wilmington, DE, USA).

Standard and internal standard (IS) stock solutions (2.0 mg/mL for all except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at –20 °C. Two QC spiking solutions of 0.5 and 2.5 µg/mL were made fresh daily in 1:1 ACN:water with 0.1% FA. A 2.5 µg/mL aliquot of TPP in 1:1 ACN:water with 0.1% FA was made as an IS spiking solution.

Equipment and materials

- Agilent 1200 Infinity Series
- Agilent 6460 Triple Quadrupole LC/MS System with electrospray ionization
- Agilent Bond Elut QuEChERS AOAC Buffered Extraction kit (p/n 5982-5755CH)
- Agilent Bond Elut QuEChERS Dispersive SPE kit for other food methods (p/n 5982-4956CH)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Flying Pigeon Centrifuge (Anting Science Instrument, Shanghai, P.R.China)

HPLC conditions

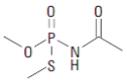
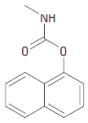
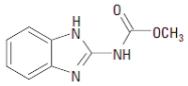
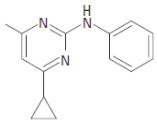
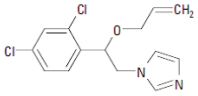
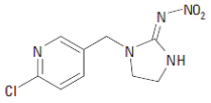
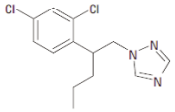
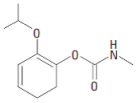
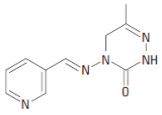
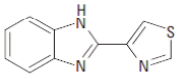
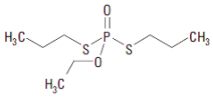
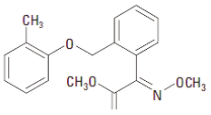
Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)	
Mobile phase:	A: 0.1% FA in water B: 0.1% FA in ACN	
Injection volume:	5 µL	
Flow rate:	0.4 mL/min	
Gradient:	Time (min)	%B
	0	5
	1	5
	3	50
	7	90
	8	90
	8.2	5
	9	5
Temperature:	30 °C	
Post run:	2 minutes	
Total cycle time:	11 minutes	

MS conditions

Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	3,500 V

The pesticides were monitored in positive mode. Other conditions relating to the analytes are listed in Table 2.

Table 1. Pesticide chemical and regulatory information [3-5].

Name	Class	Log P	pKa	Structure	MRLs (ng/g)
Acephate	Organophosphate	-0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		500
Imazalil	Imidazole	3.82	6.53		20
Imidacloprid	Neonicotinoid	0.57	NA		1,000
Penconazole	Triazole	3.72	1.51		50
Propoxur	Carbamate	0.14	NA		2,000
Pymetrozine	Pyridine	-0.19	4.06		600
Thiabendazole	Benzimidazole	2.39	4.73 12.00		50
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

Sample preparation

Chicken was purchased from local market. The meat was cut into small pieces and placed into a clean plastic bag and frozen at –20 °C overnight. The following day, only the required amount of frozen chicken was removed, and thoroughly blended. Samples were comminuted thoroughly for sample homogeneity. It was verified that no pieces of chicken were visible in the final sample.

Extraction/partitioning

A 5 g (± 0.1 g) amount of homogenized sample was placed into a 50 mL centrifuge tube. QC samples were fortified with 100 µL of appropriate QC spiking solution. IS spiking solution (100 µL) was added to all the samples except the control blank to yield a 50 ng/g concentration in the samples. Tubes were capped and vortexed for 1 minute. A 10 mL aliquot of water was added to each tube using a dispenser. Tubes were capped and vortexed for 1 minute. A 15 mL aliquot of ACN (0.1% AA) was then added to each tube using a dispenser. Tubes were capped and shaken by hand for 1 min. A Bond Elut QuEChERS AOAC extraction salt packet, containing 6 g anhydrous $MgSO_4$ and 1.5 g NaAcetate, was added directly to each tube. Tubes were sealed tightly and shaken vigorously for 20 seconds by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Finally, sample tubes were centrifuged at 4,000 rpm for 5 minutes.

Dispersive SPE cleanup

An 8 mL aliquot of the upper ACN layer was transferred to a Bond Elut QuEChERS AOAC dispersive SPE 15-mL tube. The tube contained 900 mg of anhydrous $MgSO_4$ and 150 mg C18. The tubes were capped tightly and vortexed for 1 minute. The tubes were then centrifuged with a standard centrifuge at 4,000 rpm for 5 minutes. A 2 mL portion of the extract was transferred into a tube and dried under nitrogen below 40 °C. The resulting residue was dissolved and made up to a constant volume of 1 mL using ACN:water (1:9). Then the residue was filtered through a 0.2-µm Agilent Premium syringe filter (p/n 5190-5106) and analyzed by LC/MS/MS.

Table 2. Instrument acquisition data used for the analysis of 12 pesticides by LC/MS/MS.

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)
Pymetrozine	1) 218.1 > 105 2) 218.1 > 78.1	130	20 50
Acephate	1) 184.0 > 143 2) 184.0 > 95	65	3 20
Carbendazim	1) 192.1 > 160.1 2) 192.1 > 132.1	110	15 30
Thiabendazole	1) 202.0 > 175.1 2) 202.0 > 131.1	160	25 35
Imidacloprid	1) 256.1 > 209 2) 256.1 > 175	140	10 15
Imazalil	1) 297.1 > 158.9 2) 297.1 > 200.9	150	20 15
Propoxur	1) 210.2 > 111 2) 210.2 > 93	70	10 25
Carbaryl	1) 202.0 > 145 2) 202.0 > 127	70	15 40
Cyprodinil	1) 226.1 > 93 2) 226.1 > 77	150	37 52
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 96.9	115	15 35
Penconazole	1) 284.0 > 70 2) 284.0 > 158.9	125	10 30
Kresoxim-methyl	1) 314.1 > 222 2) 314.1 > 116	70	3 5
TPP (IS)	1) 327.1 > 77 2) 327.1 > 152	170	40 45

1) Quantifier transition channel
2) Qualifier transition channel

Results and Discussion

According to the recommendation, the AOAC dispersive SPE kit for other food methods was used for chicken in our study. With the powerful selectivity provided by LC/MS/MS, the MRM chromatogram of the matrix blank did not show any peaks of interference to the target analytes. Figures 1 and 2 show the LC/MS/MS chromatograms of the matrix blank (IS spiked) and 10 ng/g fortified chicken extract processed by the dispersive SPE method.

Recovery and reproducibility

The recovery and reproducibility were evaluated by spiking pesticide standards in comminuted samples at levels of 10 and 50 ng/g. The analysis was performed in replicates of six at each level. The recovery and reproducibility (shown as RSD) data are given in Table 3. It can be seen that excellent recoveries and precision were obtained for the 12 pesticides.

Table 3. Recovery and Reproducibility of Pesticides in Fortified Chicken with QuEChERS.

	10 ng/g fortified QC		50 ng/g fortified QC	
Analytes	**Recovery (%)**	**RSD (n = 6)**	**Recovery (%)**	**RSD (n = 6)**
Pymetrozine	76.5	3.8%	80.2	6.9%
Acephate	82.1	10.2%	86.7	4.5%
Carbendazim	95.6	8.3%	93.7	2.5%
Thiabendazole	99.7	5.4%	98.2	3.2%
Imidacloprid	92.3	4.7%	106.7	2.8%
Imazalil	85.3	5.5%	82.5	1.1%
Propoxur	88.6	3.7%	90.7	4.4%
Carbaryl	79.8	5.6%	93.8	3.0%
Cyprodinil	88.5	1.9%	105.7	2.2%
Ethoprophos	101.2	3.4%	82.9	4.8%
Penconazole	87.9	10.3%	86.6	2.3%
Kresim-methyl	99.3	8.9%	89.4	2.0%

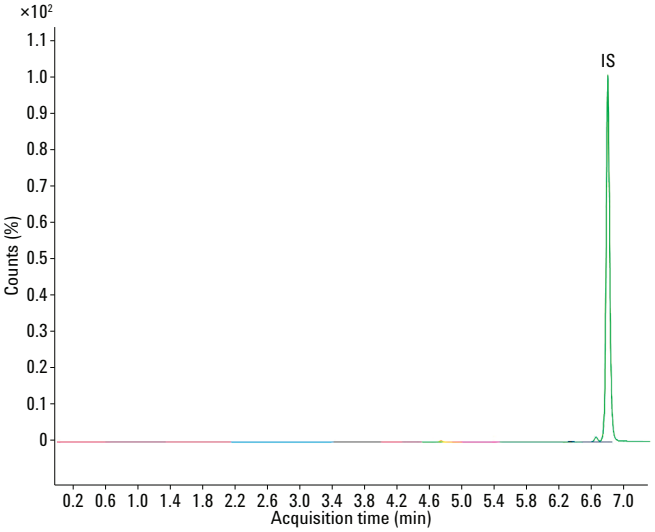


Figure 1. MRM chromatogram of chicken matrix blank. IS = TPP.

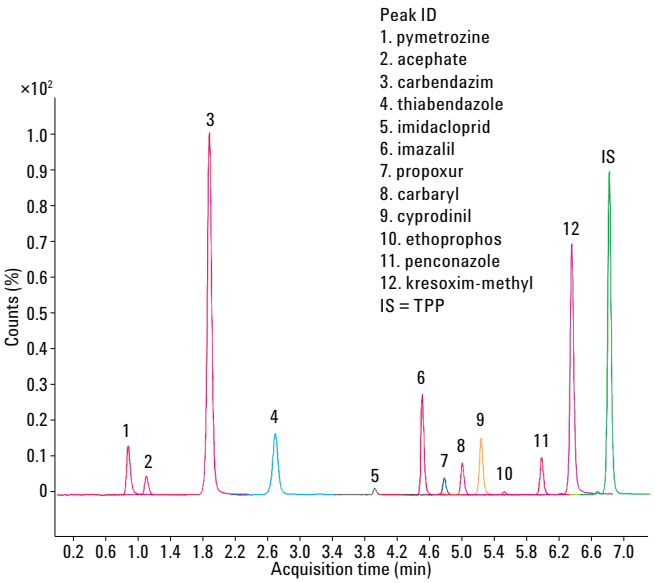


Figure 2. MRM chromatograms of 10 ng/g fortified chicken processed by QuEChERS.

Conclusions

Agilent Bond Elut QuEChERS AOAC Buffered Extraction kits and Dispersive SPE kits for other food methods provide a simple, fast, and effective method for the purification of representative pesticides in chicken. The recovery and reproducibility, based on matrix-spiked standards, were acceptable for multiclass, multiresidue pesticide determination in chicken. The impurities and matrix effects from chicken did not interfere with the quantitation of target compounds. As the selected pesticides represent a broad variety of different classes and properties, the Bond Elut QuEChERS AOAC Extraction and Dispersive SPE kit is an excellent choice for other pesticides in similar food matrixes.

References

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Printed in the USA
December 11, 2013
5991-3191EN

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