Anaylsis of Pesticide Residues in Rice Using Agilent Bond Elut QuEChERS AOAC Kit by LC-MS/MS Detection

Application Note

Food Safety

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Abstract

This application note describes 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 pesticides classed in rice. 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 the target pesticides in the rice extracts were then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. The method was validated in terms of recovery and reproducibility for all of the analytes of interest. The 5 ng/g limit of quantitation (LOQ) for pesticides in rice shown in this application was well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments were 10, 50, and 250 ng/g. The mean recoveries ranged between 76% and 108% (average of 97.8%), with RSD below 10% (average of 4.7%).

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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, a dispersive solid phase extraction (dispersive SPE) is employed using a combination of primary secondary amine (PSA) to remove organic acids from the sample matrix, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. According to different food matrices, other ingredients may be added in this step, such as graphitized carbon black (GCB) to remove pigments and sterol, or C18 to remove lipids and waxes.

The AOAC dispersive SPE kits for products with fats and waxes was selected for this application. These kits for a 1 mL sample volume, contain 50 mg of PSA, 150 mg of $MgSO_4$, 50 mg of C18 is added per mL of ACN extracts. In this study, 12 pesticides were used for evaluating the performance of the Agilent Bond Elut AOAC Buffered Extraction kit (p/n 5982-5755) and Bond Elut QuEChERS AOAC Dispersive SPE kits for Fruits and Vegetables with fats and waxes (p/n 5982-5158). The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in rice.

Experimental

Reagents and Chemicals

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 (St Louis, MO, USA). The internal standard (tripenyl phosphate, TPP) was from Agilent Technologies Inc. (Wilmington, DE, USA).

Standard Solutions

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. Three QC spiking solutions of 0.2, 1, and 10 µg/mL, were made fresh daily in 1:1 ACN/water with 0.1% FA. A 10 µg/mL of TPP in 1:1 ACN/water with 0.1% FA was made as an IS spiking solution.

Equipment and Materials

Agilent 1200 series HPLC (Agilent Technologies Inc., CA, USA)

Agilent 6460 triple quadrupole LC/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA)

Agilent Bond Elut QuEChERS AOAC Buffered Extraction kits (p/n 5982-5755) and Bond Elut QuEChERS AOAC dispersive SPE kits for fruits and vegetables with fats and waxes (p/n 5982-5158) (Agilent Technologies Inc., DE, USA)

Agilent Ceramic Homogenizers, 50 mL tubes (p/n 5982-9313) (Agilent Technologies Inc., DE, USA)

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)	
Flow rate	0.4 mL/min	
Column temperature	30 °C	
Injection volume	5 µL	
Mobile phase	A: 0.1% FA in water	
	B: 0.1% FA in ACN	
Gradient	Time (min)	%B
	0	5
	1	5
	3	50
	7	90
	8	90
	8.2	5
	9	5
Post run	2 min	
Total cycle time	11 min	
MS conditions		
Positive mode		
Gas temperature	350 °C	
Gas flow	10 L/min	
Nebulizer	40 psi	
Capillary	3500 V	

Other conditions relating to the analytes are listed in Table 2.

Table 1. Pesticides Chemical and Regulatory Information [3-5]

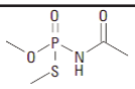
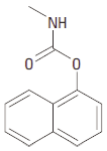
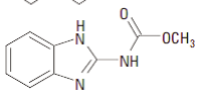
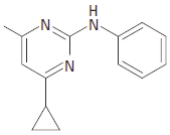
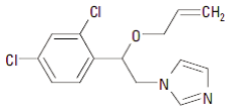
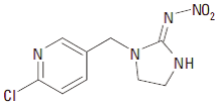
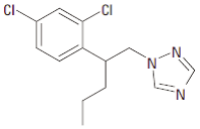
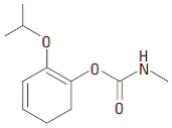
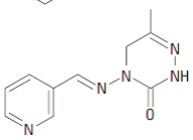
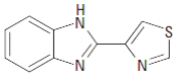
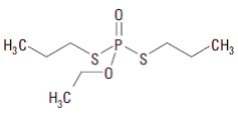
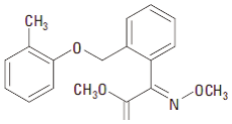
Name	Class	Log P	pKa	MRLs (ng/g)	Structure
Acephate	Organophosphate	–0.89	8.35	20	
Carbaryl	Carbamate	2.36	10.4	50	
Carbendazim	Benzimidazole	1.48	4.2	100	
Cyprodinil	Anilinopyrimidine	4	4.44	500	
Imazalil	Imidazole	3.82	6.53	20	
Imidacloprid	Neonicotinoid	0.57	NA	1000	
Penconazole	Triazole	3.72	1.51	50	
Propoxur	Carbamate	0.14	NA	2000	
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	

Table 2. Instrument Acquisition Data Used for the Analysis of 12 Pesticides by LC-MS/MS

Analyte	Channel	MRM (m/z)	Fragmentor (V)	CE (V)	RT (min)
Pymetrozine	1)	218.1>105	130	20	1.44
	2)	218.1>78.1		50	
Acephate	1)	184.0>143	65	3	1.59
	2)	184.0>95		20	
Carbendazim	1)	192.1>160.1	110	15	3.19
	2)	192.1>132.1		30	
Thiabendazole	1)	202.0>175.1	160	25	3.32
	2)	202.0>131.1		35	
Imidacloprid	1)	256.1>209	140	10	4.01
	2)	256.1>175		15	
Imazalil	1)	297.1>158.9	150	20	4.43
	2)	297.1>200.9		15	
Propoxur	1)	210.2>111	70	10	4.81
	2)	210.2>93		25	
Carbaryl	1)	202.0>145	70	15	4.99
	2)	202.0>127		40	
Cyprodinil	1)	226.1>93	150	37	5.55
	2)	226.1>77		52	
Ethoprophos	1)	243.1>130.9	115	15	6.00
	2)	243.1>96.9		35	
Penconazole	1)	284.0>70	125	10	6.22
	2)	284.0>158.9		30	
Kresoxim-methyl	1)	314.1>222	70	3	6.66
	2)	314.1>116		5	
TPP (IS)	1)	327.1>77	170	40	6.85
	2)	327.1>152		45	

1) Quantifier transition channel

2) Qualifier transition channel

Sample Preparation

Sample comminution

Organically grown, pesticide free rice was purchased from local market. The rice was placed into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the tea remained separate. The following day, only the required amount of frozen rice was removed and thoroughly blended. Dry ice was added while comminuting, when possible. Samples were comminuted thoroughly, offering sample homogeneity. It was verified that no pieces of rice 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. 50 µL of IS spiking solution (10 µg/mL of TPP) was added to all the samples except the control blank to yield a 100 ng/g concentration in the samples. Tubes were capped and vortexed for 1 min. Ten mL of water were added to each tube using the dispenser. Tubes were caped and vortexed for 1 min. Two ceramic homogenizers for 50 mL tubes (p/n 5982-9313) were added to each tube. A 15 mL aliquot of ACN (0.1% AA) was added to each tube using the dispenser. Tubes were capped and shaken by hand for 1 min. An Agilent Bond Elut QuEChERS AOAC extraction salt packet, containing 6 g anhydrous $MgSO_4$, 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. Sample tubes were centrifuged at 4,000 rpm for 5 min.

Dispersive SPE Cleanup

An 8 mL aliquot of upper ACN layer was transferred into Agilent Bond Elut QuEChERS AOAC dispersive SPE 15 mL tube (p/n 5982-5158). The 15 mL tube contained 400 mg of PSA, 1,200 mg of anhydrous $MgSO_4$ and 400 mg of C18. The tubes were capped tightly and vortexed for 1 min. The tubes were centrifuged with a standard centrifuge at 4,000 rpm for 5 min. A 1 mL portion of the extract was transferred into a 10 mL tube and dried under nitrogen below 40 °C. The resulting residue was dissolved and made to a constant volume of 1 mL using the ACN/water (1/9). Then the residue was filtered through a 0.45-µm filter membrane (p/n 5185-5836) and analyzed with LC-MS/MS.

Results and Discussion

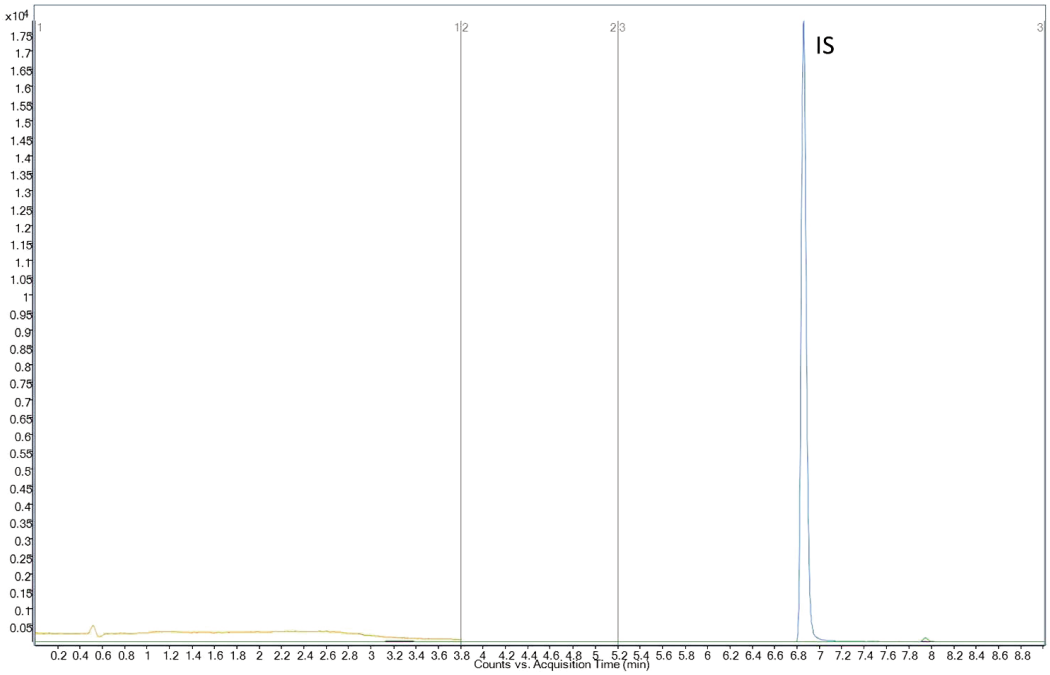


Figure 1. MRM chromatogram of rice matrix blank.

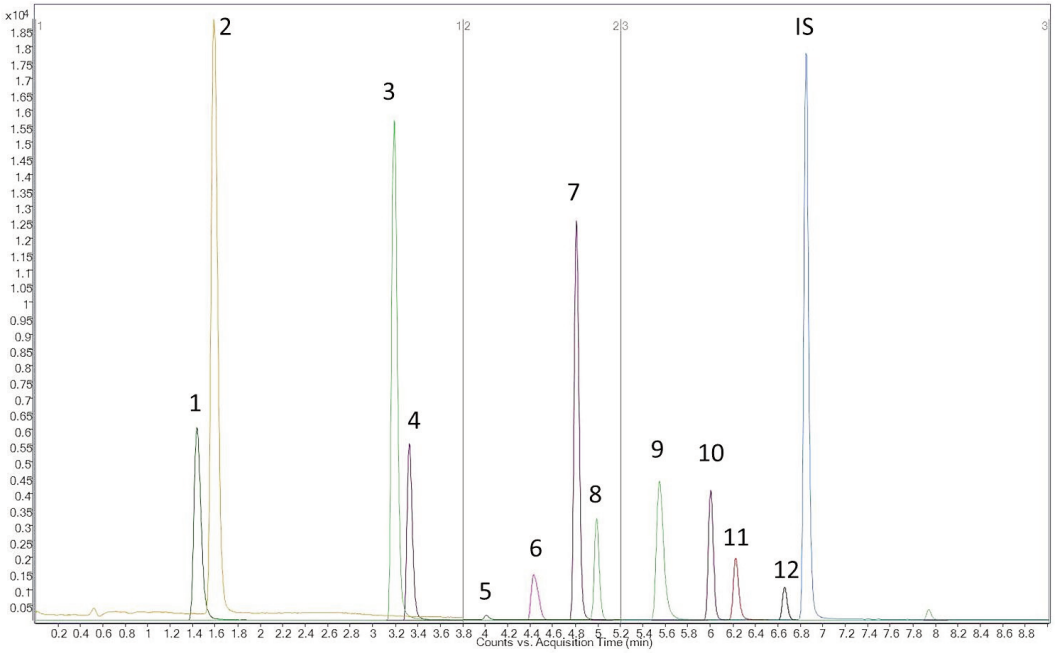


Figure 2. MRM chromatograms of 10 ng/g fortified sample processed by AOAC method. Peak identification: 1. Pymetrozine, 2. Acephate, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP.

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

Table 3. Linearity of Pesticides in Rice Extract

Name	Regression equation	R^2
Pymetrozine	Y = 1.0525x + 0.3331	0.997
Acephate	Y = 1.2109x + 0.0897	0.998
Carbendazim	Y = 1.9011x + 0.2080	0.998
Thiabendazole	Y = 0.8764x + 0.1622	0.999
Imidacloprid	Y = 0.0778x + 0.0135	0.991
Imazalil	Y = 0.3765x + 0.0552	0.997
Propoxur	Y = 1.8122x + 0.6237	0.993
Carbaryl	Y = 0.5832x + 0.0042	0.999
Cyprodinil	Y = 1.0002x + 0.3903	0.998
Ethoprophos	Y = 0.4793x + 0.0783	0.992
Penconazole	Y = 1.3872x + 0.0117	0.996
Kresoxim-methyl	Y = 0.3921x + 0.0058	0.996

Linearity and limit of quantification (LOQ)

The linearity calibration range for all of the pesticides tested was 5-500 ng/g. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 250, and 500 ng/g, the 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 LOQ (5ng/g) established for all pesticides is lower than the MRLs of these pesticides in fruits and vegetables. Table 3 shows the linear regression equation and correlation coefficient (R^2).

Table 4. Recovery and Reproducibility of Pesticides in Fortified Rice with QuEChERS

	10 ng/g fortified QC		50 ng/g fortified QC		250 ng/g fortified QC	
Analyte	**Recovery**	**RSD***	**Recovery**	**RSD***	**Recovery**	**RSD***
Pymetrozine	80.3%	4.5%	76.3%	3.8%	90.1%	4.2%
Acephate	85.0%	2.3%	92.8%	3.2%	96.3%	1.9%
Carbendazim	102.3%	8.3%	99.0%	2.0%	108.2%	5.8%
Thiabendazole	94.6%	5.4%	89.4%	4.7%	83.9%	6.1%
Imidacloprid	100.5%	10.2%	105.4%	2.8%	91.7%	8.2%
Imazalil	99.2%	4.4%	92.6%	5.5%	93.8%	5.3%
Propoxur	96.7%	3.7%	103.6%	1.1%	108.2%	2.7%
Carbaryl	88.0%	5.6%	100.7%	3.0%	108.1%	3.9%
Cyprodinil	90.3%	1.9%	92.5%	8.9%	92.4%	5.1%
Ethoprophos	104.1%	3.4%	105.8%	4.8%	110.5%	6.1%
Penconazole	103.9%	2.2%	93.9%	6.9%	90.5%	3.0%
Kresim-methyl	107.5%	10.3%	94.7%	2.5%	100.6%	2.7%

*RSD (n=6)

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted sample at levels of 10, 50, and 250 ng/g. 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 (shown as RSD) data are shown in Table 4 and Figure 3. It can be seen from the results that 12 pesticides give excellent recoveries and precision.

Conclusions

Agilent Bond Elut QuEChERS AOAC buffered extraction kits and dispersive SPE kits for fruits and vegetables with fats and waxes provide a simple, fast and effective method for the purification of representative pesticides in rice. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiclass, multi-residue pesticide determination in rice. The impurities and matrix effects from rice did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than regulated MRLs in rice. As the selected pesticides represented a broad variety of different classes and properties, the Agilent Bond Elut QuEChERS AOAC Extraction and Dispersive SPE kits is an excellent choice for other pesticides in similar food matricies.

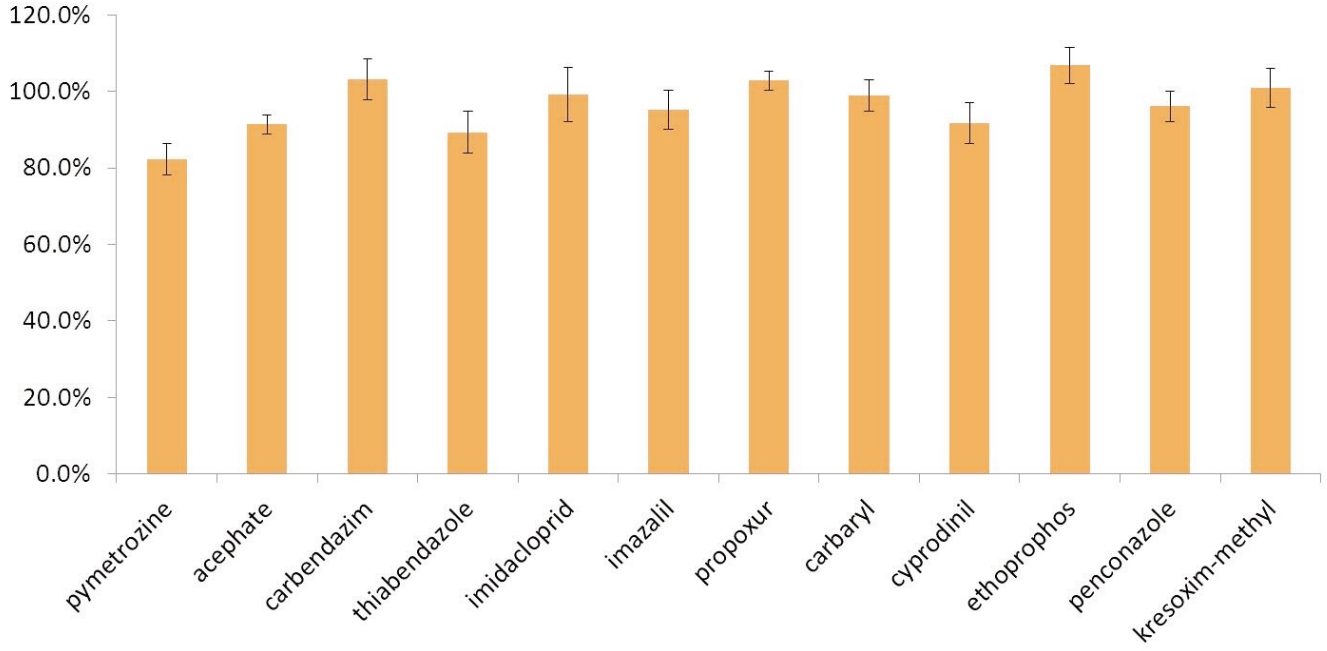


Figure 3. The recovery and precision results of 12 pesticides in rice.

References

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