

# 使用安捷伦 SampliQ QuEChERS EN 试剂盒通过 LC/MS/MS 检测分析绿茶中的农药残留

## Application Note

Food Safety

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### Abstract

本应用摘要描述了如何使用快速、简便、经济、高效、耐用和安全 (QuEChERS) 的 EN 样品制备方法萃取和净化 12 种农药残留，这些农药代表了绿茶中可能存在的多种农药类型。最初的 EN 方法包括在水/乙腈缓冲系统中进行的首萃取，加入盐分后的萃取/分离步骤，以及使用分散固相萃取 (dispersive SPE) 的净化步骤。然后通过液相色谱与在阳离子多反应监测 (MRM) 模式下操作的电喷雾离子化串联质谱仪 (LC-ESI-MS/MS) 进行联用，来确定绿茶提取物中是否存在目标农药。对该方法检测所有感兴趣分析物的回收率和重现性进行了验证。如该应用中所示，它对绿茶中农药的定量限 (LOQ) 为 5 ng/g，远低于最大残留限量 (MRL)。回收率实验的加标水平为 10、50 和 250 ng/g。平均回收率大都在 87% 到 108% (平均值为 93.5%) 范围内，而相对标准偏差 (RSD) 低于 10% (平均值为 5.2%)。



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## Introduction

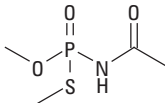
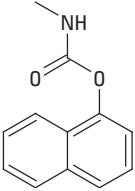
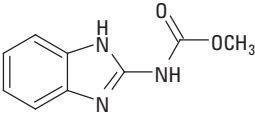
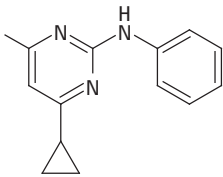
The EN QuEChERS method has been widely used in the analysis of pesticides in food, especially in Europe [1-2]. The method uses acetonitrile extraction, followed by salting out of the water from the sample using anhydrous magnesium sulfate ( $MgSO_4$ ), NaCl, and buffering citrate salts to induce partitioning. 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. Depending on the food matrix, 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.

Green tea is considered to be a highly pigmented sample since it contains high levels of chlorophyll. Therefore, the EN dispersive SPE kits for highly pigmented commodities was selected for this application. These kits for a 1-mL sample volume, contain 25 mg of PSA, 150 mg of  $MgSO_4$ . GCB in the amount of 7.5 mg was added per mL of ACN extracts. GCB absorbs planar molecules like pigments and sterols; therefore

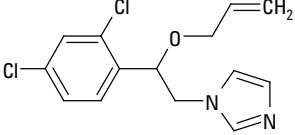
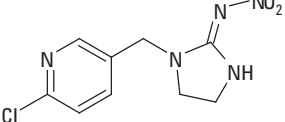
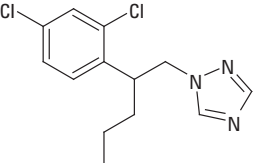
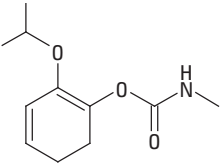
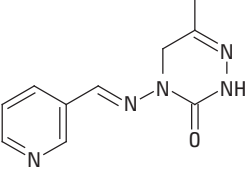
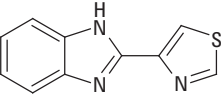
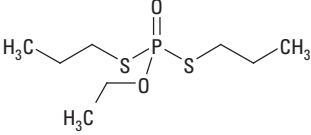
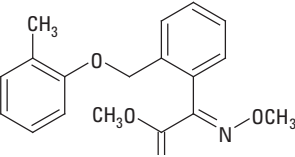
it is very helpful in cleaning up pigmented matrices such as green tea. The efficiency of cleanup is dependent upon the amount of GCB used. The more GCB used, the more planar molecules are absorbed, and therefore, a cleaner sample matrix is obtained. Instead of the relatively high amount of GCB used in the AOAC method (50 mg of GCB per mL of ACN extract), a much lower amount of GCB is used in the EN method (2.5 mg of GCB per mL of ACN extracts for "pigmented" produce, or 7.5 mg of GCB per mL of ACN extracts for "highly pigmented" produce). The GCB has an impact on the extraction of planar pesticides. The AOAC method yields a cleaner final sample matrix but caused significant loss of planar pesticides. The EN method, on the contrary, caused little to no loss of planar pesticides but generated a more complicated sample matrix.

In this study, 12 pesticides were used for evaluating the performance of the Agilent SampliQ EN Buffered Extraction kit (p/n 5982-5650) and SampliQ QuEChERS EN Dispersive SPE kits for Highly Pigmented Fruits and Vegetables (p/n 5982-5356). The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in green tea.

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

Name	Class	Log P	pKa	Structure	MRLs in apple (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

(Continued)

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
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

## 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 (triphenyl 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, and 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 2 µg/mL solution of TPP in 1:1 ACN/water with 0.1% FA was made as an IS spiking solution.

### Equipments and Materials

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

Agilent 6410 Triple Quadrupole MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA)

Agilent SampliQ QuEChERS EN Buffered Extraction kits, p/n 5982-5650 and SampliQ QuEChERS EN Dispersive SPE kits for highly pigmented fruits and vegetables, p/n 5982-5356 (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)

### Sample Preparation

#### Sample comminution

Organically grown, pesticide free green tea was purchased from Teavana Corp. The green tea 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 tea was

### Instrument Conditions

#### 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	10 µL	
Mobile Phase	A: 5 mM FA in water B: 5 mM 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.

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 tea were visible in the final sample.

### Extraction/Partitioning

A 2 g (±0.1g) amount of homogenized sample was placed into a 50-mL centrifuge tube. QC samples were fortified with 100 µL of appropriate QC spiking solution. One hundred microliters of IS spiking solution (10 µg/mL of TPP) were 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 min. An 8-mL amount of water was added to each tube using the dispenser. Tubes were capped and vortexed for 1 min. Two ceramic homogenizers for 50 mL tubes (p/n 5982-9313) were added to each tube. A 10 mL aliquot of ACN was added to each tube using the dispenser. Tubes were capped and shaken by hand for 1 min. An Agilent SampliQ QuEChERS EN extraction salt packet, containing 4 g anhydrous MgSO<sub>4</sub>,

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)	RT (min)
Acephate	1) 184.0 > 143.0	60	3	1.66
	2) 184.0 > 95.1		21	
Pymetrozine	1) 218.1 > 105.1	125	21	1.95
	2) 218.1 > 78.1		50	
Carbendazim	1) 192.1 > 160.1	110	16	3.25
	2) 192.1 > 132.1		32	
Thiabendazole	1) 202.0 > 175.1	145	26	3.37
	2) 202.0 > 131.1		38	
Imidacloprid	1) 256.1 > 209.1	125	11	4.02
	2) 256.1 > 175.1		17	
Imazalil	1) 297.1 > 159.0	110	23	4.40
	2) 297.1 > 69.1		17	
Propoxur	1) 210.2 > 111.1	70	11	4.81
	2) 210.2 > 93.1		23	
Carbaryl	1) 202.0 > 145.0	50	5	4.98
	2) 202.0 > 127.1		30	
Cyprodinil	1) 226.1 > 93.1	125	40	5.84
	2) 226.1 > 108.1		33	
Ethoprophos	1) 243.1 > 130.9	125	19	5.98
	2) 243.1 > 173.0		11	
Penconazole	1) 284.0 > 70.1	120	15	6.20
	2) 284.0 > 159.0		37	
Kresoxim-methyl	1) 314.1 > 222.1	90	11	6.62
	2) 314.1 > 235.1		11	
TPP (IS)	1) 327.1 > 77.1	160	49	6.80
	2) 327.1 > 152.1		49	

1) Quantifier transition channel

2) Qualifier transition channel

1 g NaCl, 1 g Na<sub>3</sub>Citrate, and 0.5 g Na<sub>2</sub>H Citrate sesquihydrate, 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 4000 rpm for 5 min.

#### Dispersive SPE Cleanup

A 6-mL aliquot of upper ACN layer was transferred into Agilent SampliQ QuEChERS EN dispersive SPE 15 mL tubes (p/n 5982-5356). The 15 mL tube contained 150 mg of PSA, 900 mg of anhydrous MgSO<sub>4</sub> and 45 mg of GCB. The tubes were capped tightly and vortexed for 1 min. The tubes were centrifuged with a standard centrifuge at 4000 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

According to the recommendation, the EN dispersive SPE kit for highly pigmented products was used for green tea in our study. The final sample processed by EN method still appeared green in color, however, 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 50 ng/g fortified green tea extract processed by the EN dispersive SPE method.

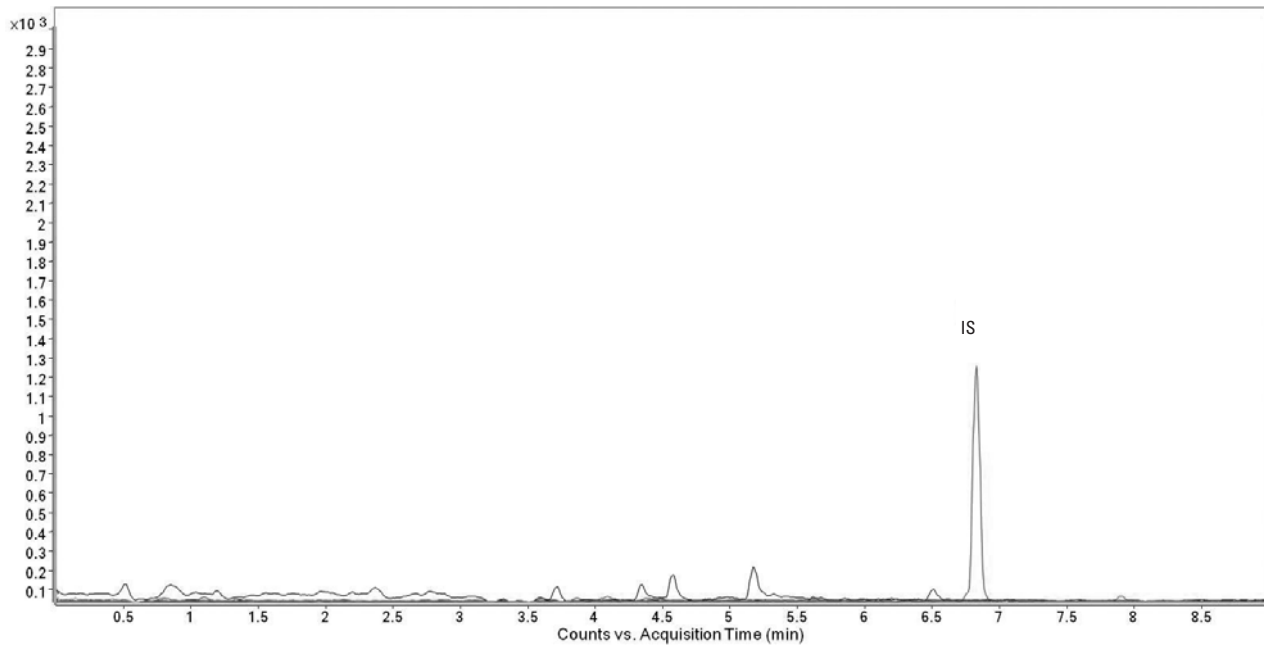


Figure 1. MRM chromatogram of green tea matrix blank. Peak identification: IS: TPP.

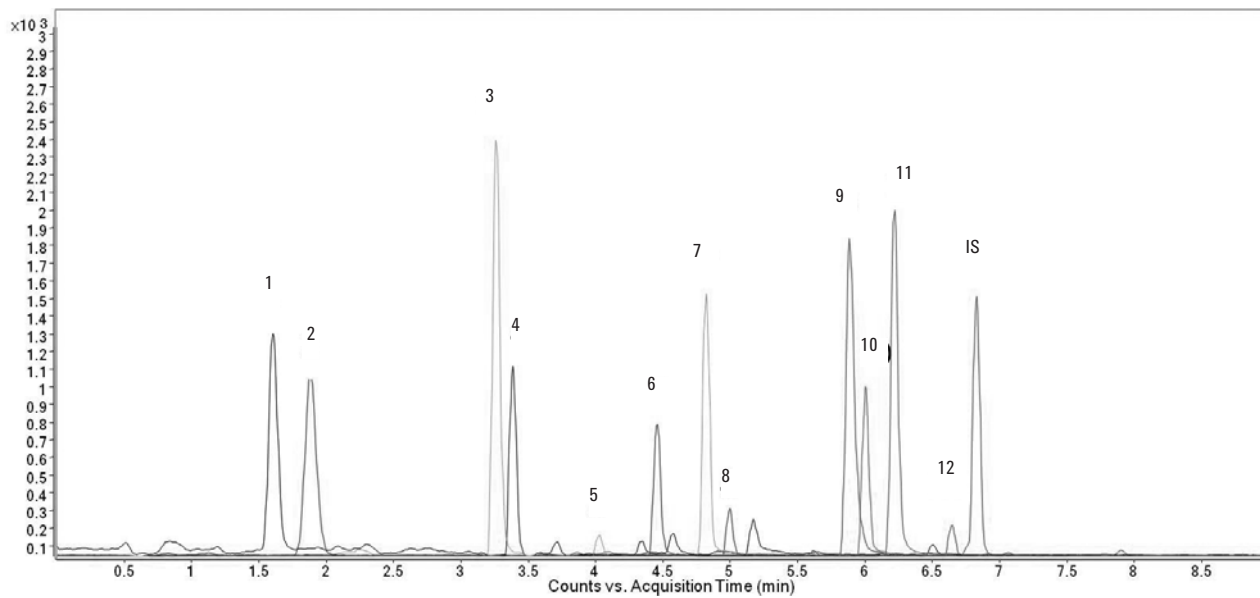


Figure 2. MRM chromatograms of 50 ng/g fortified sample processed by EN method. Peak identification: 1. Acephate, 2. Pymetrozine, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP.

### 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, and 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/con-

centration of IS). The 5 ng/g quantification limits LOQ (5 ng/g) established for all pesticides is lower than the MRLs of these pesticides in fruits and vegetables. Table 3 shows linear regression equations and correlation coefficients ( $R^2$ ).

### Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticide 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 11 pesticides give excellent recoveries and precision. The pymetrozine gave lower recovery but good precision. Pymetrozine is a pH sensitive pesticide that can be affected by the pH of the extraction procedure, at pH 5-5.5. Carbendazim, thiabendazole, and cyprodinil, which are planar pesticides like pymetrozine, show loss in recovery in the presence of the low amounts of GCB used in the d-SPE step.

Table 3. Linearity of Pesticides in Green Tea Extract

Name	Regression equation	R <sup>2</sup>
Acephate	$Y = 2.0260x + 0.0347$	0.9995
Pymetrozine	$Y = 2.3741x - 0.0141$	0.9999
Carbendazim	$Y = 2.9630x + 0.1685$	0.9991
Thiabendazole	$Y = 1.5021x - 0.0034$	0.9998
Imidacloprid	$Y = 0.0625x - 0.0002$	0.9998
Imazalil	$Y = 0.9445x + 0.0190$	0.9997
Propoxur	$Y = 1.8301x + 0.0362$	0.9998
Carbaryl	$Y = 0.3677x - 0.0014$	0.9999
Cyprodinil	$Y = 3.0000x + 0.0809$	0.9991
Ethoprophos	$Y = 1.2764x + 0.0425$	0.9986
Penconazole	$Y = 2.7559x + 0.0259$	0.9999
Kresoxim-methyl	$Y = 0.1976x + 0.0119$	0.9969

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

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		250 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Acephate	80.5%	5.4%	91.7%	2.9%	88.9%	8.2%
Pymetrozine	43.1%	3.0%	42.2%	3.4%	43.4%	9.8%
Carbendazim	114.6%	11.6%	97.6%	2.0%	105.0%	6.2%
Thiabendazole	98.1%	6.9%	90.4%	2.4%	81.7%	5.8%
Imidacloprid	104.3%	11.7%	108.6%	2.5%	93.9%	7.9%
Imazalil	97.5%	4.4%	87.8%	5.6%	92.4%	4.6%
Propoxur	98.1%	2.4%	110.2%	1.7%	107.8%	3.9%
Carbaryl	89.7%	11.4%	104.9%	3.3%	108.1%	5.2%
Cyprodinil	84.9%	2.1%	92.5%	3.7%	93.9%	5.5%
Ethoprophos	103.4%	3.1%	111.2%	3.2%	104.9%	5.7%
Penconazole	108.7%	2.9%	94.3%	4.5%	89.8%	3.3%
Kresim-methyl	105.7%	12.4%	96.4%	2.5%	99.2%	5.5%

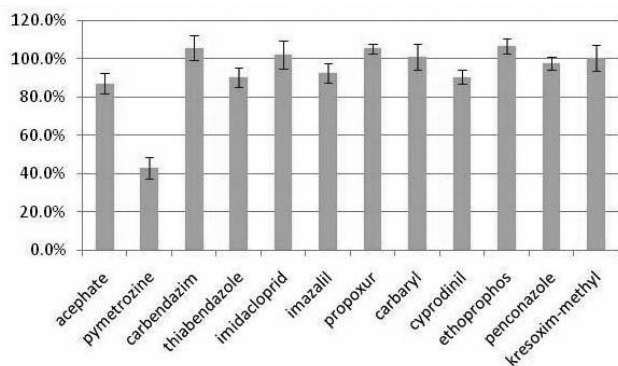


Figure 3. The recovery and precision results of 12 pesticides in green tea.

## Conclusions

Agilent SampliQ QuEChERS EN buffered extraction kits and dispersive SPE kits for highly pigmented fruits and vegetables provide a simple, fast and effective method for the purification of representative pesticides in green tea. The small amount of GCB used in dispersive SPE does not impact the extraction of planar pesticides. The recovery and reproducibility, based on matrix spiked standards, are acceptable for multiclass, multi-residue pesticide determination in green tea.

## References

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