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Multi-Residue Pesticide Analysis in Tea: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis

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APPLICATION BENEFITS

Rapid QuEChERS extraction of dried tea

Simple, fast dSPE cleanup prior to UPLC-MS/MS analysis; less time spent on instrument maintenance

Straightforward SPE cleanup for an acidic pesticide prior to UPLC-MS/MS analysis

Straightforward SPE cleanup for GC-MS/MS analysis for longer column life and less injection port maintenance

Atmospheric pressure ionization for GC-MS/MS

WATERS SOLUTIONS

ACQUITY UPLC® I-Class

Xevo® TQ-S Mass Spectrometer

APGC

ACQUITY UPLC BEH C_{18} Column

DisQuE™ products for QuEChERS extraction and dSPE cleanup

Sep-Pak® PSA/Carbon cartridge cleanup for GC-MS

Oasis® MAX cartridge cleanup for acidic pesticides

LCMS Certified Vials

MassLynx® V4.1 data system with Quanpedia™ data base

KEY WORDS

QuEChERS, pesticides, SPE, tea, LC-MS/MS, GC-MS/MS

INTRODUCTION

The QuEChERS methods have simplified and streamlined sample preparation for pesticide analysis. Although effective for fruits, vegetables and many other types of samples there are challenges when this technique is applied to certain dried commodities such as teas. Not only must the proper amount of water be added and equilibrated prior to QuEChERS extraction, but highly resinous leafy materials such as tea may require significant cleanup prior to LC-MS and GC-MS analysis.

This application note presents QuEChERS extraction and SPE-based cleanup strategies for multi-residue pesticide analysis of dried teas. The tea sample is first equilibrated with water before extraction using the DisQuE pouch for CEN QuEChERS. Then, three aliquots are taken for further analysis. One aliquot is subjected to a dSPE cleanup specific for determination of base/neutral pesticides by UPLC-MS/MS. A second aliquot is taken and subjected to an SPE cartridge cleanup optimized for determination of base/neutral pesticides by GC-MS analysis. The third aliquot is subjected to an SPE cleanup for LC-MS/MS determination of acidic pesticides not amenable to cleanup on PSA (primary secondary amine silica). Recovery data are presented for target pesticides extracted from dried black tea using these three cleanup protocols.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Injection volume:	5 µL
Temperature:	45 °C
Mobile phase A:	10 mM ammonium acetate in water (pH 5.0)
Mobile phase B:	10 mM ammonium acetate in methanol
Flow rate:	0.45 mL/min
Gradient:	10 %B initial and hold to 0.25 minutes, linear gradient to 99 %B at 12.25 minutes, hold to 13.0 minutes, back to 10 %B at 13.1 minutes, hold and re-equilibrate until 17 minutes

MS conditions for UPLC

Instrument:	Waters Xevo TQ-S
Mode:	Electrospray positive and negative (ES+, ES-)
Capillary:	3.0 kV
Extractor:	3.0 V
Source temp.:	150 °C
Cone gas:	150 L/hr
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/hr
Collison gas (Argon):	0.18 mL/min

LC-MS/MS cone and collision parameters, as well as MRM transitions used for this study are presented in Table 1.

GC conditions

Instrument:	Agilent 7890
Column:	J&W DB% MS 30 m x 0.25 mm x 0.25 µm
Injection volume:	2 µL splitless
Flow rate:	2.0 mL/min helium (constant flow)
Temperature program:	80 °C initial, hold for 0.5 min, 12 °C/min to 300 °C and hold for 10 min

MS conditions for APGC

Instrument:	Waters Xevo TQ-S
Mode:	API positive
Corona:	2.2 µA
Source temp.:	150 °C
Probe temp.:	450 °C
Cone gas:	170 L/hr
Aux gas:	250 L/hr
Nebulizer gas:	4.0 Bar
Collison gas (Argon):	0.18 mL/min

GC-MS/MS cone and collision parameters and MRM transitions used for this study are presented in Table 2.

LC-MS/MS (dSPE Cleanup)

Pesticide	MRL ppb (EU)	RT min	MRM *m/z*	(Cone V, Collision eV)	% Recovery (n=6) @ 10, 100 ppb (% RSD)	
Acephate	50	1.35	184.1 > 125.1 (8,18)	184.1 > 143.0 (8,8)	LOQ	101 (11)
Acetamiprid	100	4.03	223.0 > 126.0 (30,20)	223.0 > 56.1 (30,15)	96 (7)	101 (2)
Bitertinol	100	9.81	338.1 > 70.1 (30,8)	338.1 > 99.1 (30,16)	107 (47)	87 (16)
Carbaryl	50	6.40	202.0 > 145.0 (30,10)	202.0 >127.0 (30,26)	84 (23)	112 (11)
Carfentrazone ethyl	20	9.27	412.0 > 346.0 (30,24)	412.0 > 266.0 (30,18)	108 (11)	114 (3)
Clofentazine	50	9.72	303.0 > 138.0 (30,18)	303.0 > 102.0 (30,33)	94 (10)	97 (6)
Clothianidin	70	10.97	250.0 > 169.0 (30,12)	250.0 > 132.0 (30,17)	89 (40)	111 (9)
Diazinon	50	9.50	305.1 > 169.0 (30,22)	305.1 > 96.9 (30,35)	108 (6)	103 (2)
Dichlorvos	20	5.82	221.0 > 109.0 (30,22)	221.0 > 79.0 (30,34)	101 (8)	107 (5)
Diflubenzuron	100	9.10	311.1 > 158.1 (30,18)	311.1 > 141.0 (30,35)	115 (20)	107 (3)
Dimethoate	50	3.82	230.1 > 125.0 (30,20)	230.1 > 199.0 (30,10)	105 (18)	109 (3)
Diuron	100	9.82	233.0. > 72.1 (30,30)	233.0 > 46.3 (30,14)	83 (15)	110 (6)
Fenpropathrin	2000	11.14	350.1 > 125.0 (30,14)	350.1 > 97.0 (30,34)	74 (6)	80 (5)
Fenpyroximate	100	11.18	422.2 > 138.1 (30,32)	422.2 > 366.1 (30,18)	90 (10)	92 (5)
Imidacloprid	50	3.50	256.1 > 209.1 (30,16)	256.1 > 175.1 (30,19)	97 (10)	105 (11)
Malathion	500	8.34	331.0 > 127.0 (30,12)	331.0 > 99.0 (30,24)	98 (10)	107 (5)
Monocrotophos	50	2.95	224.1 > 127.1 (30,16)	224.1 > 98.0 (30,12)	98 (7)	105 (5)
Novaluron	10	10.31	493.0 > 158.0 (30,19)	493.0 > 141.0 (30,40)	100 (21)	92 (6)
Phosalone	50	9.74	367.9 > 181.9 (30,14)	367.9 > 110.9 (30,42)	90 (9)	95 (12)
Pyraclostrobin	50	9.68	388.1 > 163.0 (30,25)	388.1 > 193.9 (30,12)	91 (11)	105 (2)
Pyriproxyfen	50	10.67	322.1 > 227.1 (30,14)	322.1 > 96.0 (30,14)	95 (8)	90 (3)
Simazine	50	5.84	202.0 > 96.0 (30,26)	202.0 > 124.0 (32,22)	92 (30)	107 (4)
Spinosad A	50	10.97	732.6 > 142.0 (40,35)	732.6 > 98.1 (40,50)	102 (7)	92 (3)
Spinosad D	50	1.36	746.5 > 142.0 (40,38)	746.5 > 98.1 (40,48)	101 (13)	85 (5)
Spiromesifen	50000	10.97	371.1 > 273.1 (30,10)	371.1 > 255.1 (30,24)	111 (19)	70 (8)
Thiacloprid	10000	4.60	253.0 > 126.0 (30,20)	253.0 > 90.1 (30,27)	99 (14)	101 (5)
Thiamethoxam	20000 (20 US)	2.71	292.0 > 211.0 (30,13)	292.0 > 181.0 (30,22)	110 (23)	108 (4)
Triazophos	20	8.62	314.1 > 161.9 (30,18)	314.1 > 118.9 (30,35)	101 (5)	112 (3)
(Oasis MAX Cartridge SPE Cleanup)						
2,4-D	100	2.09	219.0 > 124.8 (15,25)	219.0 > 160.8 (15,20)	73 (5)	73 (4)

Table 1. LC-MS/MS recovery data.

GC-MS/MS (PSA/Carbon Cartridge Cleanup)

Pesticide	MRL ppb (EU)	RT min	MRM *m/z*	(Cone V, Collision eV)	% Recovery (n=6) @ 10, 100 ppb (% RSD)	
Acephate	50	5.18	183.8 > 94.8 (10,20)	183.8 > 142.8 (10,10)	66 (9)	59 (9)
Bifenthrin	100	15.97	242.8 > 122.9 (20,10)	242.8 > 154.9 (20,10)	91 (8)	71 (10)
Bitertanol	100	17.63	337.9 > 98.8 (20,10)	337.9 > 268.9 (20,10)	86 (9)	78 (13)
Carfentrazone	20	14.86	411.7 > 276 (20,30)	411.7 > 301.8 (20,30)	101 (17)	93 (11)
Chlorpyrifos methyl	100	10.96	321.6 > 124.7 (35, 20)	321.6 > 289.6 (35,10)	63 (14)	76 (11)
Chlorfenapyr	50000	14.05	408.7 > 270.8 (20,20)	408.7 > 378.7 (20,10)	98 (10)	93 (12)
Cyfluthrin	100	18.81	433.7 > 126.8 (15,30)	433.7 > 190.8 (15,10)	101 (6)	91 (20)
Cypermethrin	500	18.67	415.8 > 126.8 (25,25)	415.8 > 190.8 (25,10)	78 (16)	89 (18)
Dicofol	20000	16.06	352.6 > 281.7 (20,20)	352.6 > 316.6 (20,10)	65 (67)	88 (2)
Diazinon	50	9.99	304.9 > 168.9 (20,20)	304.9 > 276.9 (20,10)	98 (16)	79 (9)
Dichlorvos	20	5.17	220.8 > 108.9 (20, 10)	220.8 > 144.8 (20, 10)	87 (9)	77 (17)
Deltamethrin	5000	20.42	505.6 > 252.7 (20,20)	505.6 > 280.7 (20,10)	63 (47)	88 (23)
Ethion	3000	14.44	384.6 > 142.7 (10,20)	384.6 > 170.8 (10,10)	90 (10)	90 (13)
Etoxazole	15000	16.13	359.9 > 140.8 (35,30)	359.9 > 303.8 (35,20)	77 (14)	71 (11)
Endosulfan	30000	13.17	406.5 > 252.6 (10,20)	406.5 > 288.6 (10,10)	145 (7)	101 (18)
Fenpropathrin	2000	16.11	349.9 > 96.8 (25,30)	349.9 > 124.8 (25, 10)	94 (7)	81 (10)
Fenvalerate	50	19.54	419.8 > 124.8 (10,40)	419.8 > 166.8 (10,10)	72 (14)	86 (16)
l-Cyhalothrin	1000	16.76	449.8 > 196.8 (15,20)	449.8 > 224.8 (15,10)	61 (20)	40 (11)
Malathion	500	11.67	330.8 > 126.8 (15, 10)	330.8 > 210.8 (15,20)	57 (33)	97 (30)
Monocrotophos	5	8.88	223.8 > 97.8 (25, 10)	223.8 > 126.8 (25, 10)	85 (6)	74 (17)
Propargite	5000	15.17	230.8 > 80.8 (20, 20)	230.8 > 162.8 (20,10)	94 (21)	107 (28)
Propetamphos	100 (US)	9.78	281.9 > 137.8 (10,20)	281.9 > 194.8 (10,10)	101 (16)	88 (5)
Pyriproxyfen	50	16.67	321.9 > 95.8 (10,20)	321.9 > 184.8 (10,20)	89 (10)	80 (10)
Phenothrin	50	16.45	350.9 > 182.8 (20,40)	350.9 > 248.8 (20,20)	69 (22)	72 (7)
Phosalone	50	16.62	367.7 > 124.8 (15,20)	367.7 > 181.8 (15,20)	80 (8)	71 (11)
Resmethrin	200	15.46	338.9 > 170.9 (25,10)	338.9 > 292.9 (25,10)	36 (22)	53 (16)
Trifluralin	50	8.76	335.9 > 235.8 (30,10)	335.9 > 251.8 (30, 20)	96 (14)	124 (24)

Table 2. GC-MS recovery data.

Sample preparation

QuEChERS extraction

Place 2 g dried tea and 10 mL reagent water into a 50 mL centrifuge tube. Let soak and equilibrate for 30 minutes. Add 10 mL acetonitrile, cap and vortex for 10 seconds and then shake well for a 1 minute. Add contents of DisQuE pouch for CEN QuEChERS and shake well for 1 minute. Centrifuge the sample at 4000 RPM (rcf 3250 x g) for 5 minutes and collect the supernatant. Aliquots of the supernatant are used for SPE cleanups.

dSPE cleanup for base/neutral pesticides by LC-MS

Place 1 mL of QuEChERS extract into 2 mL DisQuE dSPE tube (150 mg $MgSO_4$/25 mg PSA/25 mg C_{18}/7 mg GCB, p/n 186008071). Vortex for 10 seconds and shake for 1 minute. Centrifuge the sample at 12000 RPM (rcf 13400 x g) for 4 minutes and collect the supernatant. Transfer 200 µL of supernatant to a LC-MS certified vial and dilute to 1.0 mL with mobile phase A for LC-MS.

SPE cleanup for base/neutral pesticides by GC-MS

Dilute 1 mL of QuEChERS extract with 10 mL 3:1 acetone/toluene. Install a Sep-Pak PSA/carbon SPE cartridge on vacuum manifold with collection vessel in place. Place 200 mg anhydrous $MgSO_4$ atop the cartridge frit. Pass all of the diluted extract through cartridge and collect. Rinse the cartridge with 2 mL 3:1 acetone/toluene and collect (combine with pass-through fraction above). Evaporate to just below 0.5 mL, add 2 mL toluene and evaporate to 0.5 mL.

SPE cleanup for acidic pesticides by LC-MS

Dilute 1 mL of QuEChERS extract with 2 mL water and adjust to pH 7.5-8.5 with 2 % ammonium hydroxide solution (a few drops). Condition Oasis MAX Cartridge (3 cc, 60 mg) with 1 mL methanol and 1 mL water. Load diluted QuEChERS extract at a flowrate of about 3 mL/minute. Wash with 1 mL 1 % aqueous ammonium hydroxide followed by 2 mL methanol. Install collection vessel and elute cartridge with 3 mL 93:5:2 MTBE/methanol/formic acid. Evaporate and reconstitute in mobile phase (80:20 mobile phase A/B).

RESULTS AND DISCUSSION

Recovery data for this study are presented in Tables 1 and 2 and were determined by comparison of the peak areas for samples spiked into the sample matrix prior to sample preparation with the peak areas for samples spiked after all sample preparation steps.

LC-MS matrix effects (measured at 100 ppb) were under 35% for all compounds except imidacloprid (45% suppression).

The QuEChERS procedure was shown to be effective for extraction of a wide range of pesticide residues from tea. Appropriate cleanup steps (dSPE, SPE) were chosen based on the subsequent chromatographic analysis and the chemical properties of the pesticides. The dSPE cleanup is suitable for neutral and basic pesticides but not for acidic pesticides that would be retained on the PSA sorbent. Therefore, a cleanup strategy optimized for acidic pesticides was employed for determination of 2,4-D. The dSPE cleanup proved to be insufficient for GC-MS analysis so the more rigorous pass-through cleanup was performed using the carbon/PSA cartridge. The QuEChERS procedure and the cleanup strategies utilized for this study are discussed below.

QuEChERS extraction

A modified QuEChERS method was used for this study. The dried tea sample is first mixed with water and allowed to equilibrate before the addition of acetonitrile and QuEChERS salts. The subsequent cleanups discussed below were more effective for the citrate buffered extraction (CEN QuEChERS) compared with acetate buffered extraction (AOAC QuEChERS); therefore the CEN approach was chosen. Unfortunately, QuEChERS is also very effective at extracting many highly colored natural tea components that can act as interferences in the subsequent chromatographic analyses. Therefore, further cleanups are essential. Figure 1 shows a typical QuEChERS extraction of black tea.

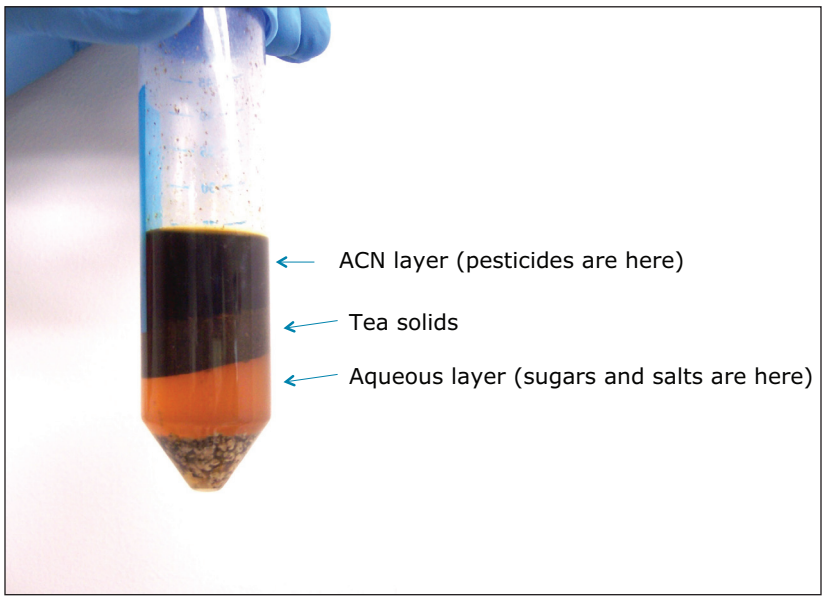

Figure 1. Tea sample extracted with DisQuE products for CEN QuEChERS after centrifugation.

dSPE cleanup

Dispersive solid-phase extraction (dSPE) is commonly performed on QuEChERS extracts prior to GC or LC analysis. For black tea, this cleanup approach was insufficient for reproducible GC-MS analysis. For LC-MS analysis, the chosen dSPE cleanup was acceptable. The amounts of C_{18}, PSA (primary-secondary amine silica) and GCB (graphitized carbon black) were chosen for the best cleanup with high recovery. For LC-MS analysis without dSPE cleanup, approximately 20 samples could be analyzed before routine maintenance (cone cleaning) was required. With dSPE cleanup, over 100 samples could be analyzed before routine maintenance was required.

SPE cleanup for 2,4-D (Oasis MAX)

This cleanup was highly effective, removing most tea component interferences. To optimize the cleanup MTBE (methyl t-butyl ether) was used as the principal elution solvent, modified with formic acid and methanol. Although formic acid/methanol (without MTBE) was effective for elution of 2,4-D, retained tea component acids co-eluted with the analyte. With the MTBE based eluent the retained tea acids remained on the cartridge and did not co-elute with the analyte. Figure 2 shows a typical QuEChERS tea sample extract cleaned up using an Oasis MAX cartridge compared with a sample with no cleanup.

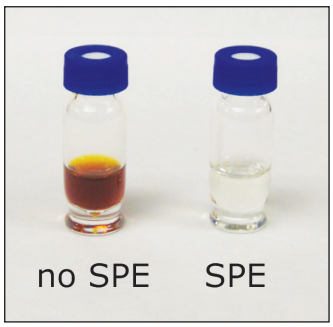

Figure 2. QuEChERS tea extract cleaned up with Oasis MAX cartridge for acids analysis by LC-MS (right) compared with no cleanup (left).

SPE cleanup for GC-MS (Sep-Pak PSA/Carbon)

This cleanup was highly effective, removing all colored components from the QuEChERS extract prior to GC-MS analysis. Initial experiments were performed by loading 1 mL of the QuEChERS extract onto the SPE cartridge followed by elution with 10 mL of acetone/toluene. However, much better cleanup was obtained if 1 mL QuEChERS extract was diluted with 10 mL of acetone/toluene before applying to the cartridge. For GC-MS analysis without the SPE cleanup, only a few sampes could be analyzed before injection port and column maintenance was required. With SPE cleanup, hundreds of samples could be analyzed before maintenance was required. Figure 3 shows Sep-Pak PSA/carbon cleanup of the QuEChERS extract.

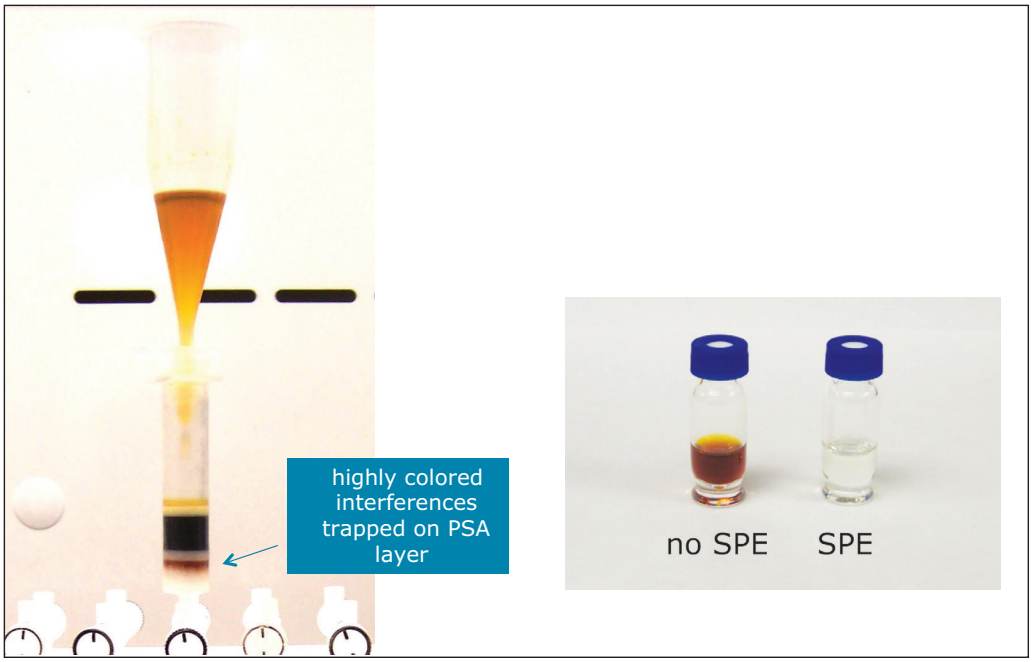

Figure 3. QuEChERS tea extract cleaned up Sep-Pak PSA/carbon cartridge for GC-MS analysis; pass thru cartridge cleanup is shown on left and resulting sample vials are shown on right.

CONCLUSIONS

- The modified QuEChERS approach was effective for recovery of a wide range of pesticides from dried tea
- dSPE provided acceptable cleanup for LC-MS/MS analysis
- A PSA/carbon cartridge based SPE cleanup was highly effective for GC-MS/MS analysis using APGC
- Oasis MAX cartridge cleanup was highly effective for LC-MS determination of 2,4-D

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