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

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APPLICATION BENEFITS

- Rapid QuEChERS extraction of chili powder
- Simple, fast dSPE cleanup prior to UPLC-MS/MS analysis; cleaner extracts mean less time spent on routine instrument maintenance
- Straightforward SPE cleanup for GC-MS/MS analysis for longer column life and less injection port maintenance
- Atmospheric pressure ionization for GC-MS/MS (APGC)
- Excellent performance for both UPLC-MS and APGC-MS using the Xevo® TQ-S Mass Spectrometer – only 30 minutes to convert from LC to GC operation

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

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

LCMS Certified Vials

MassLynx® v4.1 data system with Quanpedia™ data base

KEY WORDS

QuEChERS, pesticides, SPE, chili powder, UPLC-MS/MS, GC-MS/MS, APGC

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 dried commodities. This is the third in a series of application notes that illustrate sample preparation for dried commodities. The first application note discussed the pesticide analysis of dried tea, a highly resinous leaf material.[1] The second application note discussed the strategies for pesticide analysis of ginseng powder, a highly resinous root material.[2] This application note presents QuEChERS extraction and SPE-based cleanup strategies for multi-residue pesticide analysis of chili powder, a highly resinous and oily material obtained from dried chili pepper fruits. For the analysis of chili powder, the sample is first equilibrated with water before extraction using the DisQuE pouch for CEN QuEChERS. Aliquots are then taken for cleanup and chromatographic 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/MS analysis. Recovery data for target pesticides extracted from chili powder will be presented using these cleanup protocols.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
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

GC conditions

Instrument:	Agilent 7890
Column:	J&W DB5 MS 30 mm x 0.25 mm x 0.25 µm
Injection vol.:	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 TQS
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

UPLC-MS/MS cone and collision parameters, as well as MRM transitions used for this study are presented in Table 1. APGC-MS/MS cone and collision parameters and MRM transitions used for this study are presented in Table 2.

Sample preparation

QuEChERS extraction

Place 2 g chili powder and 10 mL reagent water into a 50 mL centrifuge tube. Shake for 10 seconds then 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 pesticides analysis by UPLC-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 (p/n 600000671CV) and dilute to 1.0 mL with mobile phase A for UPLC-MS/MS analysis.

SPE Cleanup for pesticides analysis by APGC-MS

Dilute 1 mL of QuEChERS extract with 10 mL 3:1 acetone/toluene. Install a Sep-Pak PSA/carbon SPE cartridge (p/n 186004590) 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 to just below 0.5 mL and adjust final volume to 0.5 mL with toluene. Transfer to a certified vial (p/n 186000272C) for APGC-MS/MS analysis.

RESULTS AND DISCUSSION

Figure 1 shows a typical APGC-MS/MS chromatogram obtained from a 10 ppb spiked sample of chili powder; the selected compound is pendimethalin.

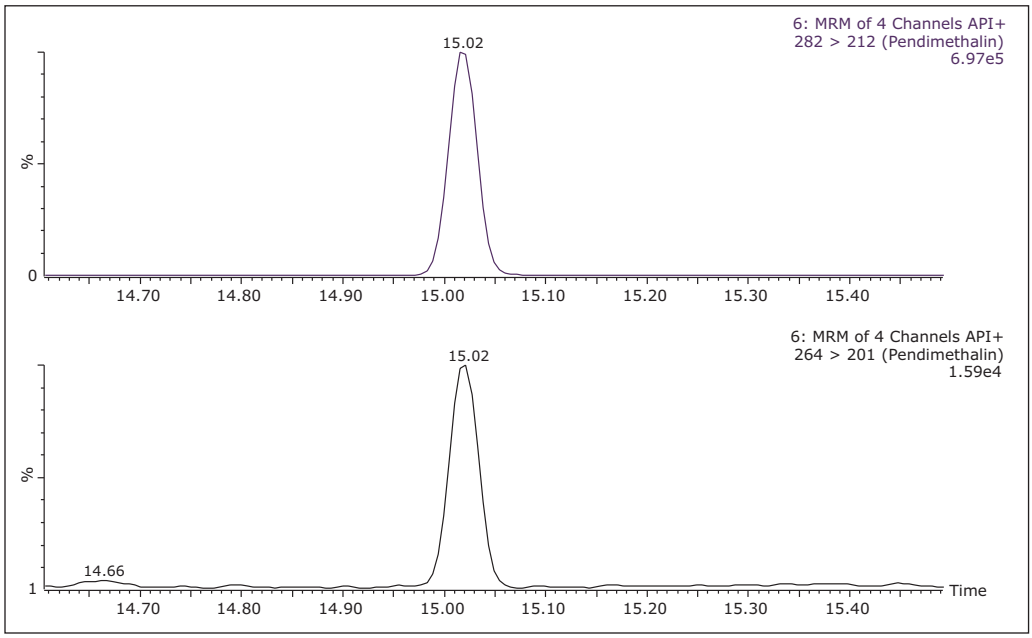

Figure 1. APGC-MS/MS analysis of chili powder, 10 ppb pendimethalin (transition for quantitation on top).

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. The primary MRM transition for quantitation is presented first (column 3) while the confirmatory MRM transition is presented in column 4.

Pesticide	MRL ppb(EU)	RT min	MRM *m/z*(Cone V, Collision eV)		%Recovery(n=6) @10, 100 ppb(% RSD)	
Abamectin	50	11.76	890.6>305.2(30,25)	890.6>567.4(30,13)	65(22)	70(16)
Acetamiprid	100	4.11	223.0>126.0(30,20)	223.0>56.1(30, 15)	90(4)	85(4)
Azoxystrobin	50	8.08	404.1>372.0(30,15)	404.1>329.0(30,35)	91(9)	88(10)
Buprofenzin	2000	10.65	306.1>201.0(30,12)	306.1>57.4(30,20)	85(5)	80(4)
Carbaryl	50	6.48	202.0>145.0(30,10)	202.0>127.0(30,26)	89(11)	102(4)
Carfentrazone-ethyl	20	9.34	412.0>346.0(30,24)	412.0>266.0(30,18)	82(9)	86(9)
Chlorfenapyr	10	10.06	406.2>251.0(34,22)	406.2>152.0(34,60)	81(11)	83(9)
Chlorpyriphos-methyl	50	9.96	321.8>125.0(30,20)	321.8>289.9(30,16)	89(12)	79(9)
Clothianidin	70	3.57	250.0>169.0(30,12)	250.0>132.0(30,17)	82(16)	82(6)
Cyprodinil	1000	9.42	226.0>93.0(40,33)	226.0>108.0(40,30)	89(11)	74(10)
Cymoxanil	50	4.32	199.0>128.0(30,8)	199.0>111.0(30,18)	92(10)	97(6)
Diazinon	50	9.56	305.1>169.0(30,22)	305.1>96.9(30,35)	92(16)	82(6)
Difenoconazole	500	10.06	406.0>251.1(37,25)	406.1>111.1(37,60)	85(9)	84(6)
Diflubenzuron	1000	9.18	311.1>158.1(30,18)	311.1>141.0(30,35)	86(18)	78(4)
Dimethoate	20	3.92	230.1>125.0(30,20)	230.1>199.0(30,10)	92(4)	89(2)
Emamectin	20	11.11	886.6>158.0(50,35)	886.6>126.0(50,38)	61(6)	65(10)
Fludioxonil	2000	8.33	247.0>180.0(42,28)	247.0>126(42,35)	113(4)	88(7)
Fenamidone	50	8.23	312.1>92.0(30,25)	312.1>236.1(30,16)	81(7)	84(3)
Fenpyroximat	300	11.22	422.2>366.1(30,18)	422.2>138.1(30,32)	82(5)	72(6)
Hexythiazox	500	10.89	353.0>228.1(30,14)	35.0 >168.1(30,26)	79(8)	80(6)
Imidacloprid	50	3.56	256.1>209.1(30,16)	256.1>175.1(30,19)	85(7)	85(3)
Indoxacarb	300	10.18	528.0>203.0(30,40)	528.0>150.0(30,30)	82(10)	89(7)
Lambda Cyhalothrin	100	11.33	467.2>225.0(30,20)	467.2>141.1(30,46)	87(31)	79(17)
Malathion	20	8.42	331.0>127.0(30,12)	331.0>99.0(30,24)	86(4)	87(4)
Mandipropamid	1000	8.41	412.3>328.2(30,16)	412.3>356(30,10)	91(13)	92(6)
Metalaxyl	100	7.36	280.1>220.1(30,13)	280.1>192.1(30,17)	90(3)	88(5)
Methamidophos	10	0.71	142.0>93.9(30,13)	142.0>124.90(30,13)	73(6)	73(2)
Methomyl	20	2.57	163.0>88.0(30,10)	163.0>106.0(30,30)	84(17)	86(7)
Methoxyfenozide	1000	8.52	369.1>149.1(30,20)	369.1>313.20(30,10)	112(13)	86(5)
Novaluron	10	10.34	493.0>158.0(30,19)	493.0>141.0(30,40)	91(3)	86(9)
Oxamyl	20	2.33	237.0>72.0(30,12)	237.0>90.0(30,10)	92(5)	86.3
Oxydemeton-methyl	10	2.62	247.0>168.8(30,14)	247.0>108.9(30,25)	88(8)	95(2)
Propetamphos	100(US)	8.62	282.0>138.0(30,20)	282.0>156(30,12)	113(15)	82(6)
Pendimethalin	50	10.88	282.2>212.2(30,10)	28.2>194.1(30,17)	81(11)	74(8)
Pyriproxifen	50	10.74	322.1>96.0(30,14)	322.1>227.1(30,14)	84(9)	78(7)
Pyraclostrobin	50	9.74	388.1>163.0(30,25)	388.1>193.9(30,12)	79(8)	89(6)
Spinosad A	50	11.04	732.6>142.0(40,35)	732.6>98.1(40,50)	67(12)	69(12)
Spinosad D	50	11.43	746.5>142.0(40,38)	746.5>98.1(40,48)	62(14)	66(16)
Spiromesifen	500	11.03	371.1>273.1(30,10)	371.1>255.1(30,24)	94(20)	81(6)
Tebuconzole	500	9.50	308.0>70.1(31,22)	308.1>125.0(31,40)	87(5)	82(3)
Thiacloprid	1000	4.67	253.0>126.0(30,20)	253.0>90.1(30,37)	90(3)	84(2)
Thiamethoxam	20000(20US)	2.78	292.0>211.0(30,13)	292.0>132(30,23)	93(5)	86(3)
Trifloxystrobin	50	10.17	409.0>145.0(30,40)	409.0>186.0(30,16)	89(7)	86(5)

Table 1. UPLC-MS/MS recovery data and tuning parameters. Abbreviations: MRL = maximum residue limit, RT = retention time, MRM = transition chosen in multiple reaction monitoring with cone voltage (V) and collision cell energy (eV = electron volts).

Pesticide	MRL ppb(EU)	RT min	MRM *m/z*(Cone V, Collision eV)		%Recovery(n=6) @10, 100 ppb(% RSD)	
Acephate	50	8.82	143>95(10,20)	143>125(10,10)	66(6)	62(5)
Bifenthrin	100	15.97	242.8>122.9(20,10)	242.8>154.9(20,10)	86(20)	122(15)
Carfentrazone ethyl	20	14.85	411.7>276.8(20,30)	411.7>301.8(20,30)	85(8)	74(7)
Chlorpyrifos methyl	100	10.90	321.6>124.7(35,20)	321.6>289.6(35,10)	73(4)	77(7)
Chlorfenapyr	50000	14.03	408.7>270.8(20,20)	408.7>378.7(20,10)	74(6)	82(5)
Diazinon	50	9.98	304.9>168.9(20,20)	304.9>276.9(20,10)	88(6)	81(5)
Deltamethrin	5000	20.40	505.6>252.7(20,20)	505.6>280.7(20,10)	LOQ	72(9)
Fenvalerate	50	19.54	419.8>124.8(10,40)	419.8>166.8(10,10)	61(4)	75(9)
L-Cyhalothrin	1000	16.95	449.8>196.8(15,20)	449.8>224.8(15,10)	77(8)	77(16)
Propetamphos	100(US)	9.76	281.9>137.8(10,20)	281.9>194.8(10,10)	83(13)	79(9)
Pyriproxyfen	50	16.67	321.9>95.8(10,20)	321.9>184.8(10,20)	79(11)	76(8)
Phenothrin	50	16.45	350.9>182.8(20,40)	350.9>248.8(20,20)	77(8)	88(9)
Resmethrin	200	15.46	338.9>170.9(25,10)	338.9>292.9(25,10)	52(9)	56(10)
Trifluralin	50	8.76	335.9>235.8(30,10)	335.9>251.8(30,20)	81(6)	79(7)

Table 2. APGC-MS/MS recovery data and tuning parameters (abbreviations as in Table 1).

A modified QuEChERS method was used for this study. The chili powder sample is first mixed with water and allowed to equilibrate before the addition of acetonitrile and QuEChERS salts. This procedure was shown to be effective for extraction of a wide range of pesticide residues from chili powder. An aliquot of the QuEChERS extract was cleaned up using dSPE prior to LC-MS analysis. Fatty acids, sugars, polyphenolic resins and other potential interferences are removed in this step (see Figure 2). Another aliquot of the QuEChERS extract was cleaned up using a carbon/PSA SPE cartridge prior to APGC-MS analysis. Figure 3 shows the cleanup obtained with the carbon/PSA cartridge; most of the colored matrix compounds are removed. The highly colored matrix compounds in chili are not amenable to GC analysis and will build up in the injection port and on the head of the column if not removed by SPE. Consquently, without the SPE cleanup, only a few samples could be analyzed by APGC-MS before injection port and column maintenance was required; after SPE cleanup hundreds of samples were analyzed without any routine maintenance.

Figure 2. SPE cleanup for LC-MS; vial on left shows a chili powder sample prepared with no dSPE cleanup, vial on right shows a sample prepared with dSPE cleanup.

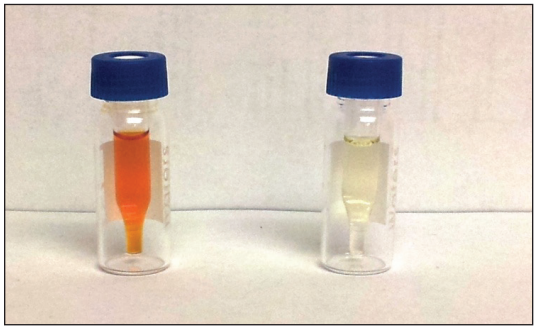

Figure 3. SPE cleanup for GC-MS; vial on left shows a chili powder sample with no cleanup, vial on right shows a sample after cleanup using carbon/PSA cartridge.

CONCLUSIONS

- The modified QuEChERS approach was effective for recovery of a wide range of pesticides from chili powder
- dSPE provided cleanup suitable for UPLC-MS/MS analysis
- A PSA/carbon cartridge based SPE cleanup was highly effective for GC-MS/MS analysis using APGC-MS
- One Xevo TQ-S Mass Spectrometer can be shared to provide excellent performance for both UPLC-MS and APGC-MS
  - Conversion from LC to GC interface takes only minutes
  - No venting of the mass spectrometer is required for the conversion

Reference

1. Multi-Residue Pesticide Analysis in Tea: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis, Waters Application Note 720004819EN.
2. Multi-Residue Pesticide Analysis in Ginseng Powder: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis. Waters Application Note 720005006EN.

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