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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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## Abstract

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.


### 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

## 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 × 100 mm, 1.7 µm |
| Injection volume: | 5 µL |
| Temp.: | 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 min, linear gradient to 99% B at 12.25 min, hold to 13.0 min, back to 10% B at 13.1 min, hold and re-equilibrate until 17 min |

### MS conditions for UPLC

| | |
|---|---|
| Instrument: | Xevo TQ-S |
| Mode: | Electrospray + and - |
| 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 × 0.25 mm × 0.25 µm |
| Injection vol.: | 2 µL splitless |
| Flow rate: | 2.0 mL/min helium (constant flow) |
| Temp. 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: | Xevo TQ-S |
| Mode: | API+ |
| 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.

| 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. Abbreviations: LOQ = below limit of quantification, NA = no allowed residue, (others as in Table 1).*

## Sample preparation

QuEChERS extraction

Place 2 g chili powder and 10 mL reagent water into a 50 mL centrifuge tube. Shake for 10 sec then let soak and equilibrate for 30 min. Add 10 mL acetonitrile, cap and vortex for 10 sec and then shake well for a 1 min. Add contents of DisQuE pouch for CEN QuEChERS and shake well for 1 min. Centrifuge the sample at 4000 RPM (rcf 3250 × g) for 5 min 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 MgSO4/25 mg PSA/25 mg C18/7 mg GCB, p/n: 186008071). Vortex for 10 sec and shake for 1 min. Centrifuge the sample at 12000 RPM (rcf 13400 × g) for 4 min 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 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.

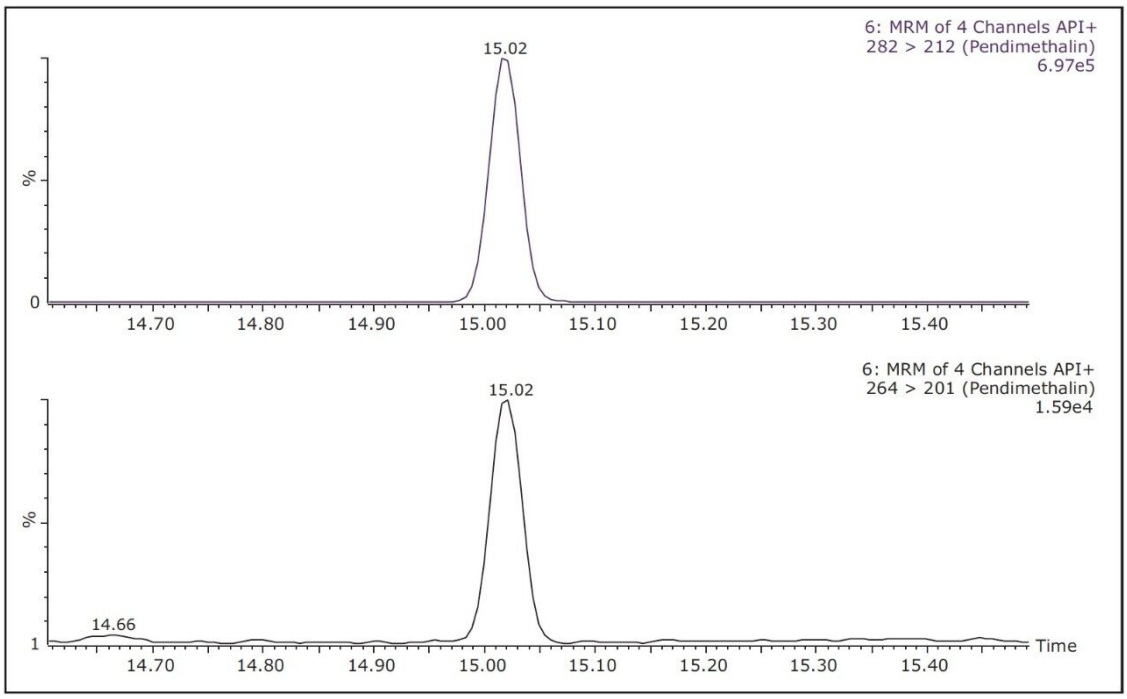


*Figure 1. APGC-MS/MS analysis of chili powder, 10 ppb pendimethalin (transition for quantitation on top).*

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.

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.*

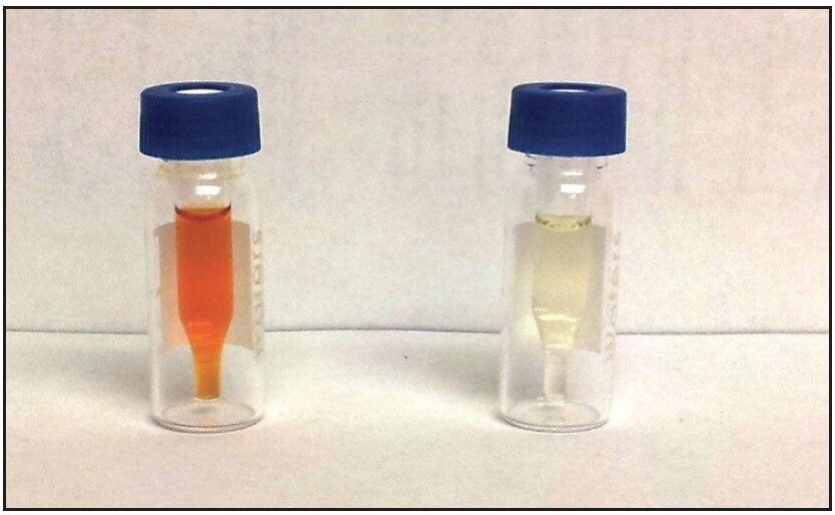

*Figure 3. SPE cleanup for GC-MS; vial on left shows a chili powder sample with no cleanup, vial on right shows a sample after cleanup using carbon/PSA cartridge.*

## Conclusion

- 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.

## References

1. Multi-Residue Pesticide Analysis in Tea: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis, Waters application note no. 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 no. 720005006EN.

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720005142, November 2015