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Oasis PRiME HLB Cartridge for Clean-up of QuEChERS Extracts of Soybean Pods Prior to UPLC-MS/MS Determination of Free Acidic Herbicides

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APPLICATION BENEFITS

- Efficient, time-saving multi-class/ multi-residue methodology
- Simple, rapid, and effective sample clean-up suitable for determination of acidic herbicides
- Simultaneous extraction and clean-up of neutral and basic pesticides
- Fast, sensitive UPLC™-MS/MS analysis

WATERS SOLUTIONS

ACQUITY™ UPLC I-Class System

Xevo™ TQ-XS Tandem Mass Spectrometer

Oasis™ PRiME HLB Cartridge for SPE clean-up

DisQuE™ Pouch for CEN QuEChERS

MassLynx™ MS Software

KEYWORDS

UPLC-MS/MS, acidic herbicides, multi-residues, pesticides, edamame, QuEChERS

INTRODUCTION

Acidic herbicides are commonly used for agricultural weed control. To help insure public health and safety, reliable analytical methods are necessary to determine residues of these herbicides in fruits and vegetables grown for human or animal consumption. For the analytical chemist, it is desirable to screen for multiple acidic herbicides with a single analytical method in order to maximize throughput and minimize costs. It is even more cost effective if the same single analytical extraction and clean-up method can be used to screen for acidic, neutral and basic pesticides. In this application note, a QuEChERS extraction and UPLC-MS/MS analysis method is demonstrated for multiresidue analysis of free (unbound) acidic herbicides in soybean pods. This vegetable (known as edamame) is a popular and nutritious foodstuff. However, this commodity is challenging for pesticide analysis; typically edamame is about 5–6% total fat and 0.3% phospholipid (lecithin) with significant amounts of pigments such as chlorophyll and carotenes. The presence of these co-extracted substances in the QuEChERS extract can lead to interference in the UPLC-MS analysis, contamination of the analytical column, and other components of the UPLC system, and contamination of the mass spectrometer itself. The Oasis PRiME HLB Cartridge is highly effective for removing fats, phospholipids, and chlorophyll from QuEChERS extracts of edamame. A QuEChERS extraction method has been successfully applied to the analysis of free acidic herbicides, but dSPE clean-up was not employed.[1] Common dispersive SPE methods (dSPE) using PSA sorbents for clean-up cannot be used for acidic herbicides because the acidic compounds are retained on the sorbent.[2] However, pass-through clean-up with the Oasis PRiME HLB Cartridge provides good recovery for acidic herbicides. Therefore, the same QuEChERS extract can be used to screen for acidic and non-acidic herbicides and other pesticides after a single pass-through clean-up using the Oasis PRiME HLB Cartridge.

This application note highlights a clean-up protocol, part of a multiresidue analytical method, suitable for unbound (free) acidic herbicides and also suitable for base/neutral herbicides. This is not a class specific method optimized for bound and unbound acidic herbicides; such an optimized method is currently under development. An application note or other publication will soon be presented for a class specific method for acidic herbicides and metabolites after basic hydrolysis.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS T3 1.8 µm, 2.1 × 100 mm
Mobile phase:	A: 0.02% Formic in water
	B: Acetonitrile:MeOH (50:50)
Injection volume:	10 µL
Injection mode:	Partial loop injection
Column temp.:	25 °C
Weak needle wash:	10:90 Acetonitrile:water (600 µL)
Strong needle wash:	50:30:40 Water:acetonitrile:IPA (200 µL)
Seal wash:	10:90 acetonitrile:water

Gradient:

Time	Flow (mL/min)	%A	%B
0.00	0.400	95.0	5.0
5.00	0.400	5.0	95.0
6.00	0.400	5.0	95.0
6.10	0.400	50.0	50.0
6.50	0.500	50.0	50.0
6.80	0.500	95.0	5.0
7.00	0.400	95.0	5.0
8.00	0.400	95.0	5.0

MS conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI+
Source temp:	120 °C
Desolvation temp.:	300 °C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	30 L/hr
Collision gas flow:	0.15 mL/min
Data management:	MassLynx v4.2
Monitored transitions:	see Table 1

Table 1. MRM transitions and instrument parameters used for this study; also presented in Table 1 are observed retention times (RT).

Name	MRM	Cone (v)	Collision (eV)	Retention time (min)
Compounds Analyzed in ES-				
2, 4-DP	233.0>161.0 233 .0>125.0	28	10 30	4.80
2,4-D	218.9>161.0 218.9>125.0	26	15 40	4.45
2,4-DB	246.9>160.9 246.9>125.0	12	10 10	4.97
2,4,5-T	252.8>194.9 252.8>158.9	19	14 36	4.86
2,4,5-TP (Silvex)	268.9>196.9 268.9>161.0	28	15 30	5.16
3,6-Dichloro-2-hydroxy benzoic acid (Dicamba metabolite)	204.9>160.9 204.9>124.9	14	11 11	3.77
4-CPA	185.0>127.0	28	16	3.93
Bentazone	239.0>132.0	30	30	4.06
Bromoxynil	275.8>80.8 275.8>78.8	48	30	4.34
Dicamba	218.8>174.8 218.8>145.0	9	9 9	3.69
Fenoxaprop-P	332>151.9 332>115.9	70	50 32	5.24
Fluazafop-P (butyl)	384.1>282.1 384.1>328.1	38	22 16	5.78
Fluroxypyr	254.9>208.8 254.9>180.8	28	16 12	3.82
Fomesafen	437.1>195.0 437.1>222.0	59	30 30	5.14
Imazaquin	310.0>266.0 310.0>233.0	20	16 25	4.01
Ioxynil	369.7>126.8 369.7>215.0	40	30 30	4.65
MCPA	199.2>140.9 201.0>143.0	20	10 8	4.48
MCPB	227.0>140.9	15	20	4.99
MCPP	213.0>141.0 213.0>118.8	21	14 14	4.81
Triclopyr	255.9>220.1 255.9>197.9	20	5 10	4.68
Compounds Analyzed in ES+				
Cycloxydim	326.0>280.0 326.0>180.0	34	16 22	5.82
Imazapyr	262.2>86.1 262.2>69.2	38	26 26	2.76
Imazethapyr	290.2>245.2 290.2>177.1	45	20 25	3.67
Haloxyfop	362.0>288.0 362.0>272.0	28	26 32	5.26
Imazosulfuron	413.0>152.8 413.0>155.9	7	12 18	4.77
Metosulam	418.0>175.0 418.0>140.0v	41	28 52	4.19
Metsulfuron methyl	382.0>167.0 382.0>198.9	28	16 22	4.03
Picloram	241.0>168.0 241.0>195.0	26	30 21	2.56
Quinmerac	222.2>204.2 222.2>141.1	17	15 30	3.24
Thifensulfuron methyl	388.0>167.0 388.0>56.0	25	15 15	3.93

Sample preparation

Initial extraction/precipitation

Place a 5 g homogenized sample into a 50-mL centrifuge tube. Add fortification standards if required and allow 30 min to equilibrate. Add 10 mL of water, vortex for 10 seconds, and add 10 mL of acetonitrile. Vortex for 30 seconds, and then add contents of DisQuE QuEChERS Pouch for CEN, p/n: 186006813. Vortex for 10 seconds and then place on mechanical shaker for 10 minutes. Centrifuge at 4000 rpm for 5 min. Portions of the supernatant (top layer) are taken for clean-up.

Note: The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fat and phospholipids.

Oasis PRiME HLB clean-up (left) and no clean-up (right).

SPE clean-up

Mount an Oasis PRiME HLB 3 cc Vac Cartridge, 150 mg , p/n 186008717 on a pre-cleaned vacuum manifold. The vacuum is set to 1–2 psi. Approximately 0.7 mL of the QuEChERS supernatant is passed through the Oasis PRiME HLB Cartridge and discarded. After collection vessels are installed in the manifold, approximately 1.2 mL of the supernatant is passed through the cartridge and collected. Exactly 0.20 mL of the collected fraction is diluted with 0.40 of reagent water for UPLC-MS/MS analysis.

RESULTS AND DISCUSSION

Figure 1 shows the total method recovery data obtained from six replicate analyses of edamame samples spiked at 1, 10, and 100 ng/g. The chromatograms shown in Figure 2 demonstrate the effectiveness of the Oasis PRiME HLB Cartridge for removal of ≥95% of the phopholipids from the edamame extracts. The overall method recoveries are generally above 70% although lower recovery was observed for a few of the more polar acidic herbicides such as picloram and the dicamba metabolite. It is important to distinguish any recovery losses resulting from the SPE clean-up from losses resulting from the initial QuEChERS extraction. The graph presented in Figure 3 compares the total recovery (red bars) with the SPE recovery (blue bars) measured at the 100 ng/g spike level. The red data were obtained by spiking the sample before QuEChERS extraction and SPE clean-up; the blue data were obtained by spiking the extract after QuEChERS extraction and before SPE clean-up. For picloram, this shows that most of the recovery loss occurred during the QuEChERS extraction step and not from the Oasis PRiME HLB pass-through clean-up step.

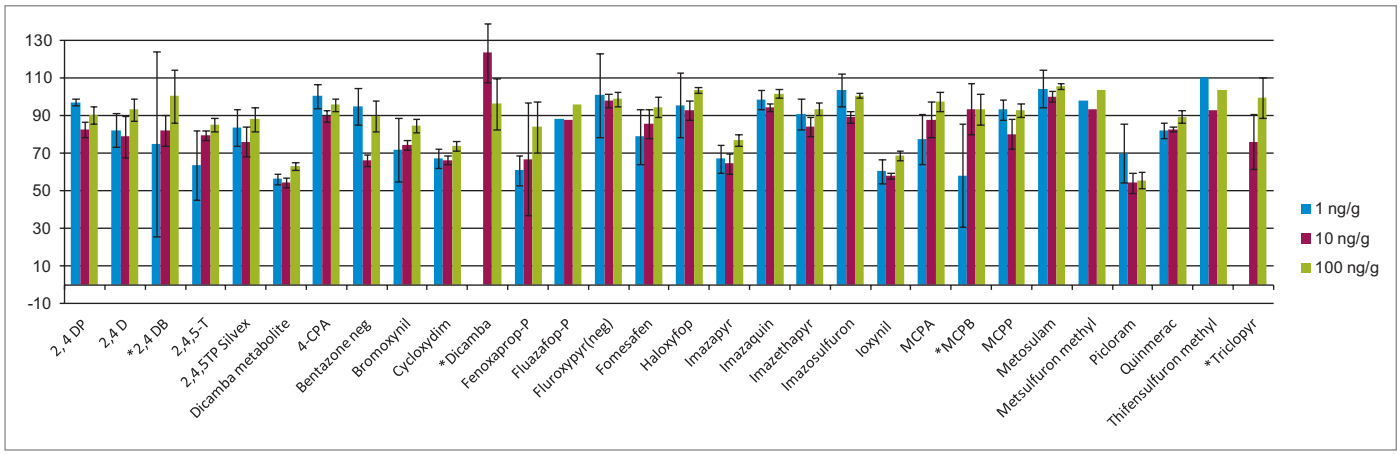

*Figure 1. Recovery of acidic herbicides from edamame after QuEChERS extraction and Oasis PRiME HLB pass-through clean-up (error bars indicate standard deviation for six replicate analyses, * indicates LOQ above 1 ng/g).*

QuEChERS methods have been widely accepted for pesticide analysis in many commodities. Among the hundreds of pesticides amenable to extraction using QuEChERS are many acidic herbicides. Traditional dispersive clean-up (dSPE) using PSA (primary/secondary amine silica) cannot be used for acidic herbicides because the compounds will be retained on the sorbent. Therefore, the analyst must prepare two separate aliquots of the QuEChERS extract for analysis with separate clean-up strategies for the acids and for base/neutrals. However, unlike PSA, Oasis PRiME HLB sorbent does not rely on ion-exchange mechanisms for removal of phospholipids and related contaminants and does not retain acidic pesticides. Therefore, a single aliquot of QuEChERS extract can be subjected to a rapid pass-through clean-up prior to analysis for both acids and base/neutrals.

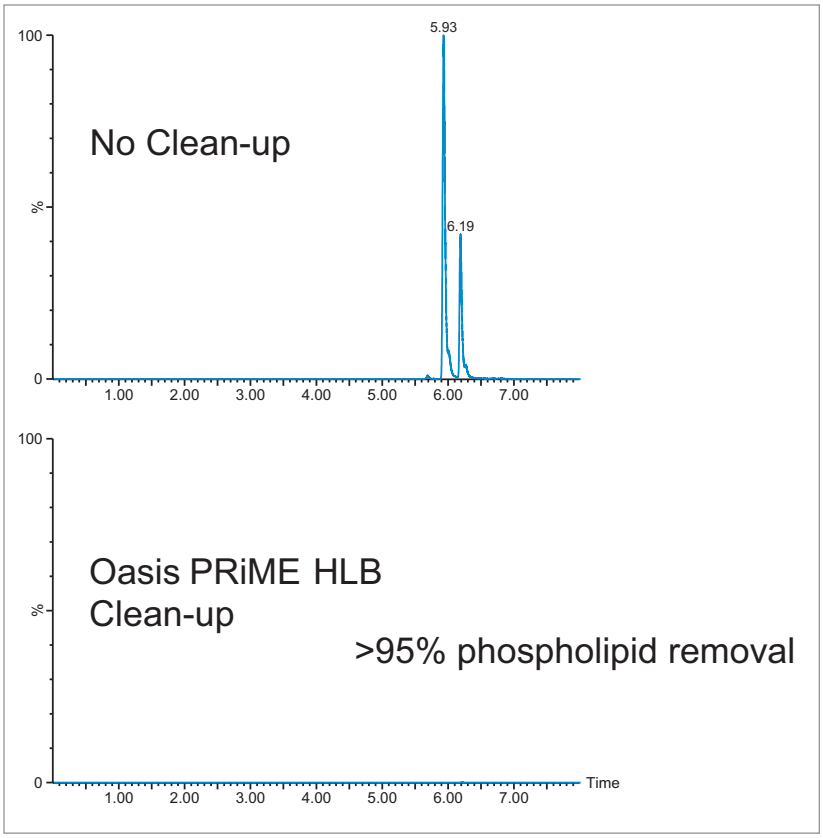

Figure 2. UPLC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from edamame QuEChERS extract (transitions monitored: 496.4, 520.0, 522.0, and 524.0 m/z, all to 184.4 m/z).

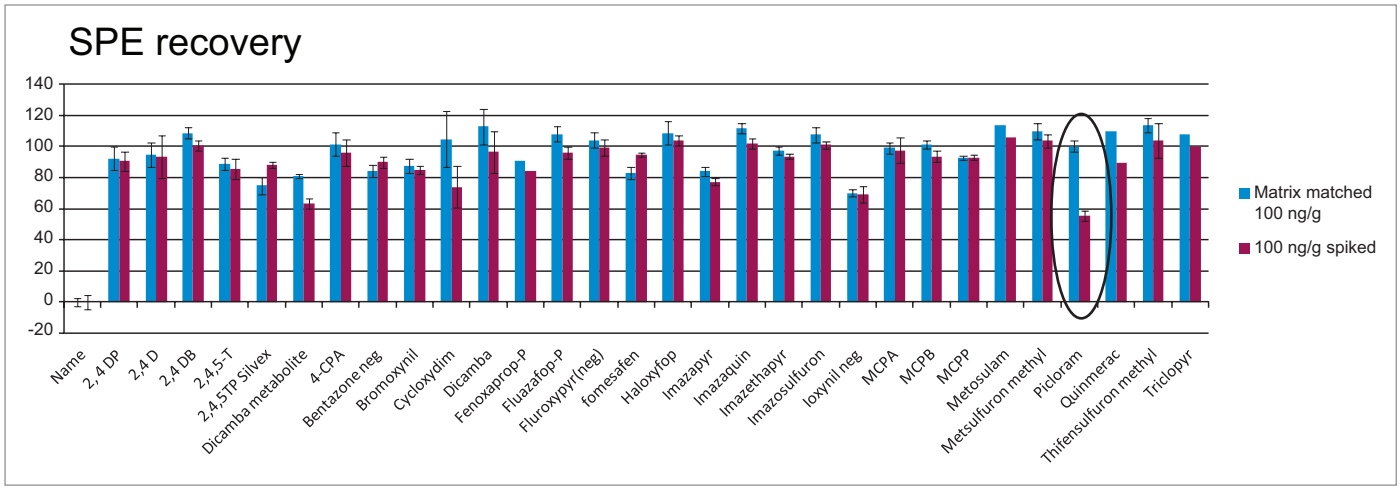

Figure 3. Comparison of total method recovery (red bars) with SPE recovery (blue bars) of acidic herbicides.

CONCLUSIONS

- Pass-through clean-up with the Oasis PRiME HLB Cartridge is effective for the removal of fats, phospholipids, and pigments from QuEChERS extracts of edamame.
- Good recoveries of acidic herbicides were obtained after pass-through clean-up with the Oasis PRiME HLB Cartridge; this is not possible using dSPE with PSA.
- Oasis PRiME HLB provides good cleanup for acid, base, and neutral pesticides in one step; dSPE with PSA cannot.

References

1. Carneiro RP, et. al. *Food Control.* (2013) 33: 413–423.
2. Lehotay S, et.al. *J AOAC Int.* (2005) 88(2): 595–614.

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