

## Oasis PRiME HLB Cartridges for Rapid and Effective Removal of Chlorophyll from QuEChERS Spinach Extracts

Michael S. Young and Jeremy C. Shia  
Waters Corporation, Milford, MA, USA



### GOALS

A simple, quick, and effective simple cleanup strategy to remove chlorophyll from QuEChERS extracts prior to GC-MS/MS analysis.

Demonstrating the flexible use of Oasis® PRiME HLB Plus Short and Plus Light Cartridge formats suitable for processing samples with or without the need for a vacuum or positive pressure manifold.

### BACKGROUND

The QuEChERS method is highly effective for extraction of a wide range of pesticides from fruits and vegetables. After initial QuEChERS extraction, dispersive SPE (dSPE) is typically used for cleanup prior to chromatographic analysis. A combination of various sorbents is dispersed into an aliquot of the acetonitrile based extract for selective removal of potential interferences. Among the interfering substances found in many vegetables, chlorophyll is particularly bad for gas chromatography; only a few injections of a high chlorophyll extract can result in severe contamination of the injection port and column head.

Oasis PRiME HLB Cartridges provide rapid removal of chlorophyll from spinach samples after QuEChERS extraction. This technology brief demonstrates Oasis PRiME HLB Sorbent in both Vac and Plus Formats.

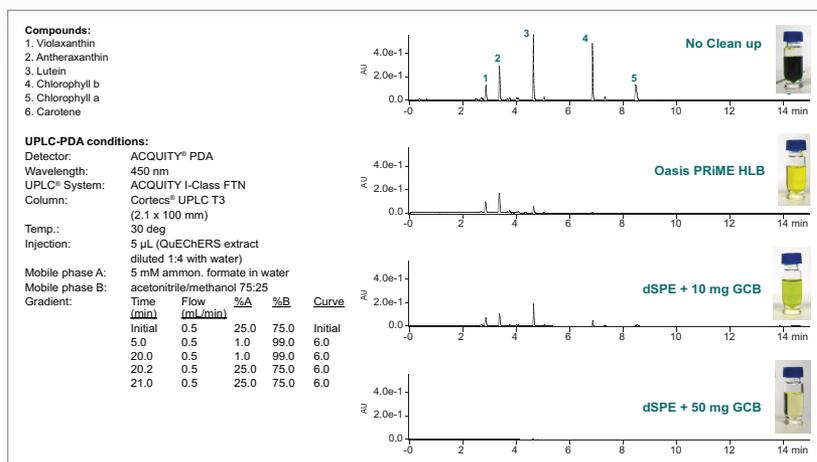


Figure 1. UPLC-PDA chromatograms showing removal of pigments from spinach extracts using the three cleanup protocols.

The most common sorbent used for removal of chlorophyll is graphitized carbon black (GCB). Although GCB is effective for removal of chlorophyll, recovery losses can occur for some pesticides, particularly those that have a planar geometry. Therefore, an alternative to GCB is desirable.

### THE SOLUTION

Pass-through cleanup using Oasis PRiME HLB Cartridges is an effective alternative to dSPE with GCB with no loss of planar pesticides.

## EXPERIMENTAL

**Test Compounds.** In a previous study,<sup>1</sup> good recoveries were shown for a wide variety of pesticides after cleanup using Oasis PRiME HLB Cartridges. Among those pesticides, there are three planar pesticides commonly used on high chlorophyll commodities, cyprodinil, chlorothalonil, and thiabendazole. These three compounds were chosen for this study and were spiked into the spinach sample at a concentration of 50 µg/kg (ppb).

**QuEChERS Extraction.** Raw spinach purchased at a local grocery store. A 15 g sample of homogenized sample was weighed into a 50 mL centrifuge tube and spiked with the test compounds. 15 mL 1:99 acetic acid/acetonitrile were added and the sample was manually shaken for 1 minute. Then, QuEChERS salts (contents of DisQuE™ pouch for AOAC QuEChERS, p/n 186006812) were added and the tube was shaken vigorously by hand for 1 minute. After centrifugation (3200 rcf for 5 minutes), portions of the supernatant were taken for cleanup using these two techniques: by dSPE and by pass-through cleanup with Oasis PRiME HLB Cartridges.

**Cleanup (dSPE).** Into a 2 mL centrifuge tube was weighed 150 mg anhydrous sodium sulfate, 50 mg C<sub>18</sub> silica, 50 mg PSA (primary/secondary amine silica) and 50 mg GCB. A second 2 mL tube was prepared with the same sorbents except with 10 mg GCB. A 1 mL portion of supernatant was transferred to each tube and the tubes were shaken by hand for 1 minute. After centrifugation (1 minute at 13500 rcf), a portion of sample was transferred to an auto-sampler vial for analysis by APGC-MS. Another portion of the sample (100 µL) was transferred to a separate vial and diluted with 400 µL water for UPLC-MS analysis.

**Cleanup (Using Oasis PRiME HLB Vac Style Cartridges).** An Oasis PRiME HLB Cartridge (3 cc, 150 mg) was mounted on a pre-cleaned vacuum manifold set to minimal vacuum (approximately 2 psi). **No cartridge conditioning is required or was performed.** A 0.8 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB Cartridge and discarded. Then a 1.5 mL portion of the supernatant was passed through the cartridge and collected.

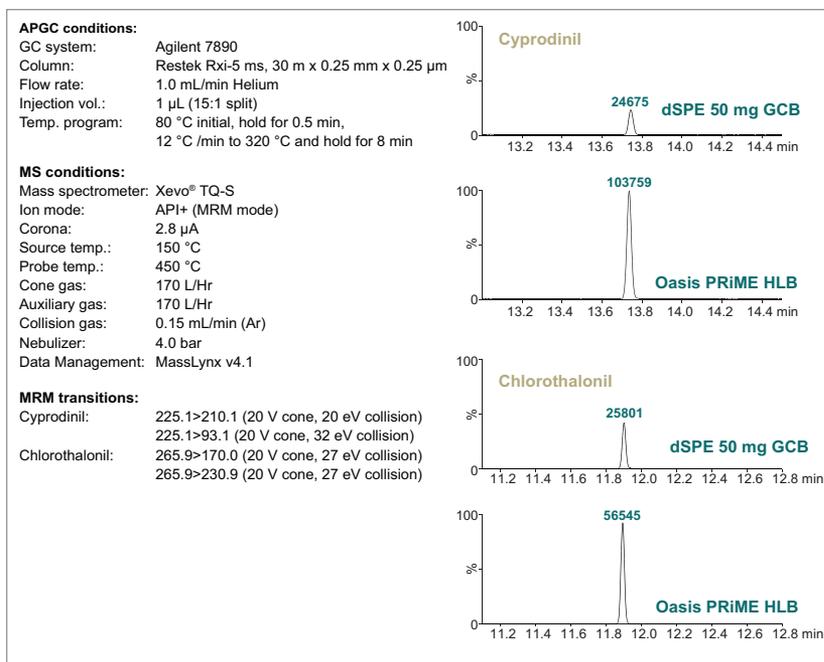


Figure 2. APGC-MS/MS ion chromatograms showing improved recovery for planar pesticides cyprodinil and chlorothalonil after cleanup with the Oasis PRiME HLB Cartridge compared with dSPE cleanup with graphitized carbon.

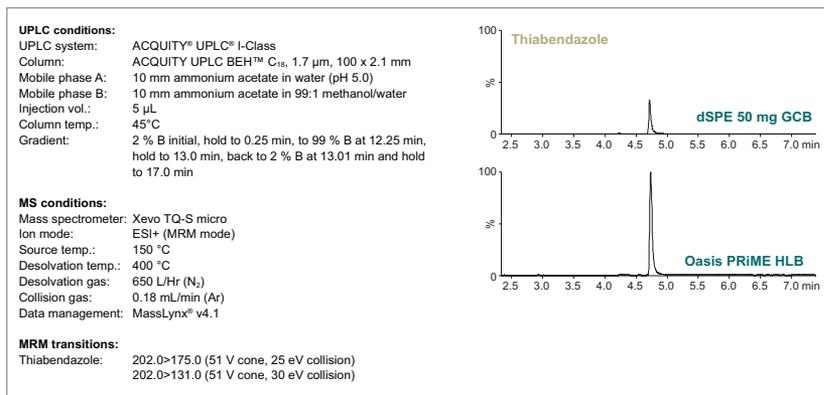


Figure 3. UPLC-MS/MS ion chromatograms showing improved recovery for planar pesticide thiabendazole after cleanup with the Oasis PRiME HLB Cartridge compared with dSPE cleanup with graphitized carbon.

Samples were then taken for APGS-MS and UPLC-MS analysis in the same manner as the dSPE samples.

**Cleanup (Using Oasis PRiME HLB Plus Style Cartridges). No cartridge conditioning was performed.** A 3 mL syringe was attached to the Oasis PRiME HLB Plus Light Cartridge used for cleanup. A 0.7 mL aliquot of the supernatant was passed through the Oasis PRiME HLB Cartridge and discarded. Then a 0.8 mL portion of the supernatant was passed through the cartridge and collected. A similar procedure was performed with the Plus Short format using a 6 mL syringe. A 2 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB cartridge and discarded. Then a 3 mL portion of the supernatant was passed through the cartridge and collected. Samples were then prepared for APGS-MS and UPLC-MS analysis in the same manner as the dSPE samples.

## INSTRUMENTAL ANALYSIS

Chlorophyll removal from the extracts was monitored using UPLC coupled to a photo-diode array detector (PDA). Pesticide concentrations were measured using APGC-MS/MS for cyprodinil and chlorothalonil, and using UPLC-MS/MS for thiabendazole.

## RESULTS

All three cleanup methods were effective for removal of the majority of chlorophyll and carotenes from the QuEChERS extract of spinach. Pass-through cleanup with the Oasis PRiME HLB Cartridge was slightly better than dSPE with 10 mg GCB for removal of chlorophyll. dSPE with 50 mg GCB per mL extract was the only cleanup that effectively remove all pigments from the QuEChERS extract. However, significant losses of planar pesticides were observed using dSPE with 50 mg GCB. In contrast, little or no recovery losses were observed for the three planar pesticides with Oasis PRiME HLB cleanup or using dSPE cleanup with 10 mg GCB per mL extract. Figure 1 shows UPLC-PDA chromatograms illustrating removal of the pigments using the three cleanup protocols. Figure 2 shows APGC-MS/MS ion chromatograms illustrating the recovery losses for cyprodinil and chlorothalonil. Figure 3 shows a UPLC-MS/MS ion chromatogram illustrating recovery loss for thiabendazole.

The Oasis PRiME HLB Cartridge is available in various sizes and formats. The “vac” type cartridges are most convenient for use with vacuum/positive pressure manifold while the “plus” type cartridges are suitable for use with

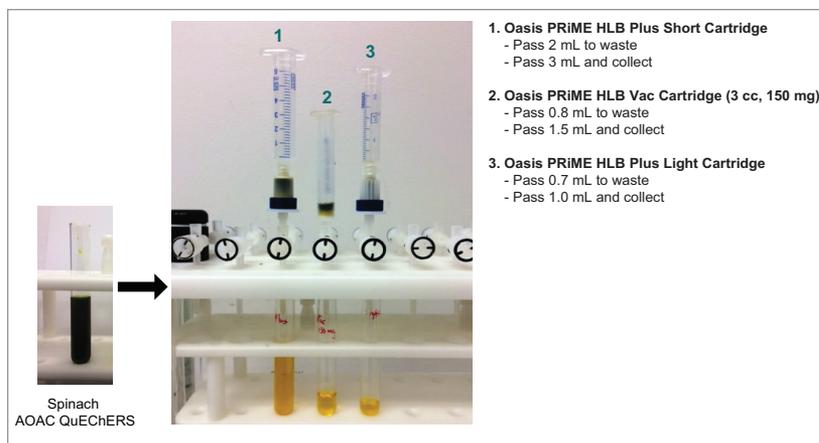


Figure 4. Oasis PRiME HLB in plus type cartridges provide identical cleanup compared with the traditional vac style cartridge.

a syringe (similar to a syringe filter) or with a vacuum/positive pressure manifold. The choice of cartridge size is made based on the volume of extract required by the analyst. Figure 4 illustrates this cartridge choice; no difference was seen in total pigment removal or pesticide recovery among the three cartridge choices.

## CONCLUSIONS

- For the QuEChERS spinach extraction, significant amounts of chlorophyll and other pigments are co-extracted along with the target pesticides.
- Pass-through clean-up with an Oasis PRiME HLB Cartridge effectively removed greater than 99% of chlorophyll and greater than 95% of lutein from the QuEChERS extract.
- dSPE clean-up with 10 mg GCB (per mL extract) was less effective compared with the Oasis PRiME HLB Cartridge for removal of chlorophyll and lutein from the QuEChERS extract.
- dSPE cleanup with 50 mg GCB (per mL extract) removed all pigments from the QuEChERS extract, but significant loss of planar pesticides was observed.

## References

1. Oasis PRiME HLB Cartridge for Rapid and Effective Cleanup of Avocado, A High Fat Matrix, Prior to APGC-MS/MS Analysis, Waters Application Note [720005816EN](#), 2016.

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