RAPID AND EFFECTIVE PASS-THROUGH CLEANUP OF HIGH CHLOROPHYLL QUECHERS EXTRACTS PRIOR TO GC-MS/MS AND LC-MS/MS ANALYSIS

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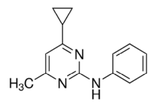
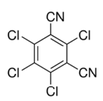
INTRODUCTION

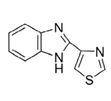
In recent years, food safety laboratories have adopted new and simplified sample preparation methods, such as QuEChERS, to reduce analysis time and to increase throughput. When spinach or other leafy vegetables are subjected to QuEChERS extraction, significant amounts of chlorophyll and other pigments are co-extracted along with the target pesticides. The presence of these co-extracted substances can lead to chromatographic interference, contamination of GC or LC systems, and contamination of the mass spectrometer. To avoid these complications, a cleanup step is recommended prior to the instrumental analysis. For spinach, this is often performed using dispersive SPE (dSPE) with graphitized carbon black (GCB) or other sorbents designed to remove chlorophyll. In this study, chlorophyll and other natural pigments in a raw spinach sample, such as lutein and carotene, were measured using UHPLC with photodiode array detection (PDA). Then various types of dispersive and pass-through SPE cleanup options were evaluated for pigment removal (monitored using PDA) and pesticide recovery (monitored using GC-MS/MS and LC-MS/MS). Particular attention was given to recoveries of three pesticides with planar geometry commonly used on spinach, cyprodinil, chlorothalonil, and thiabendazole (see below). These three compounds were spiked into a fresh spinach sample at a concentration of 50 µg/kg (ppb).



cyprodinil



chlorothalonil



thiabendazole

What is Oasis PRiME HLB Sorbent?

- A **reversed-phase** solid phase extraction device
  - PATENT PENDING
- **Simpler**
  - Streamlined protocols, no condition and equilibration steps are required
- **Faster**
  - Faster and more even flows through devices with less plugging and faster overall processing
- **Cleaner**
  - Reduce matrix effect by removing matrix interferences such as fats, pigments, and phospholipids

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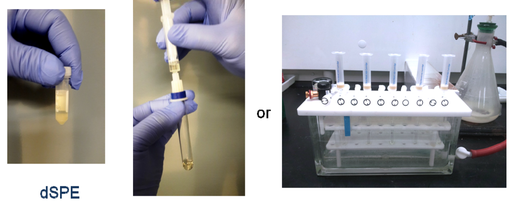
SAMPLE PREPARATION

QuEChERS Extraction. 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, pn 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 technique: by dSPE and by pass-thru cleanup with Oasis PRiME HLB cartridges.

Cleanup (dSPE). Into a 2 mL centrifuge tube was weighed 150 mg anhydrous sodium sulfate, 50 mg C18 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, 150mg, pn 186008717) was mounted on a pre-cleaned vacuum manifold set to minimal vacuum (approx 2 psi). *No cartridge conditioning is required or was performed.* A 0.8 mL aliquot of the supernatant was passed-through the Oasis PRiME cartridge and discarded. Then a 1.5 mL portion of the supernatant was passed through the cartridge and collected. Samples were then taken for APGC-MS and UPLC-MS analysis in the same manner as the dSPE samples.

dSPE or pass-through SPE can provide effective sample cleanup in only minutes



RESULTS

UPLC with PDA provides effective method for monitoring spinach pigment cleanup

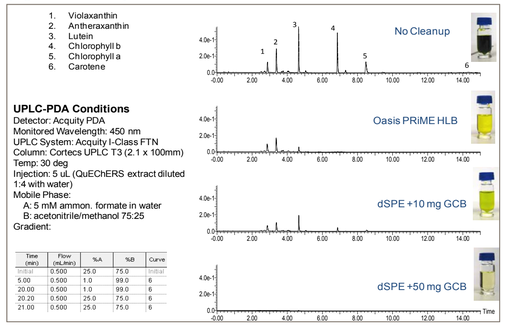


Figure 1. UPLC-PDA chromatograms showing removal of pigments from spinach extracts using the three cleanup protocols

APGC-MS/MS: sensitive and selective for GC pesticides

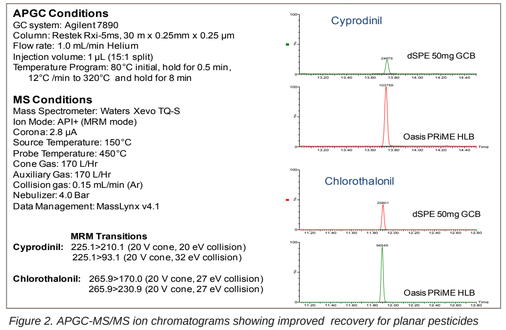


Figure 2. APGC-MS/MS ion chromatograms showing improved recovery for planar pesticides cyprodinil and chlorothalonil after cleanup with the Oasis PRiME HLB cartridge compared with dSPE cleanup with graphitized carbon

UPLC-MS/MS: sensitive and selective for LC pesticides

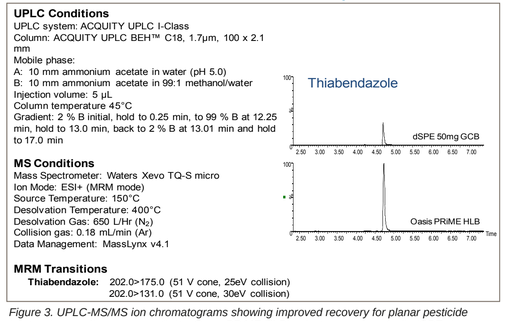


Figure 3. UPLC-MS/MS ion chromatograms showing improved recovery for planar pesticide thiabendazole after cleanup with the Oasis PRiME HLB cartridge compared with dSPE cleanup with graphitized carbon

DISCUSSION

Evaluation of the cleanup options. 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.

"Plus" cartridge formats for syringe sample processing. 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 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.

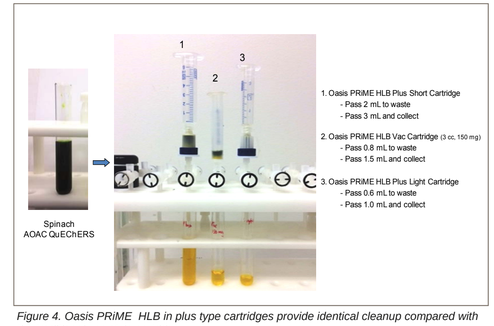


Figure 4. Oasis PRiME HLB in plus type cartridges provide identical cleanup compared with the traditional vac style cartridge

CONCLUSIONS

- **For the QuEChERS spinach extraction, significant amounts of chlorophyll and other pigments are co-extracted along with the target pesticides**
- **Pass-thru cleanup with an Oasis PRiME HLB cartridge effectively removed greater than 99% of chlorophyll and greater than 95% of lutein from the QuEChERS extract**
- **dSPE cleanup 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**

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