# Automated In-Line Pigment Removal for Multi-Residue Pesticide Analysis in Spinach by LC-MS/MS

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## Introduction

- The presence of pigments in sample extracts can compromise the LC-MS/MS analysis of pesticides in green vegetables through ionization suppression or enhancement.
- A faster and simpler workflow has been developed which effectively removes pigments from sample extracts without the loss of pesticides inherent to dispersive SPE (dSPE) which contain graphitized carbon black (GCB).

## Methods

**Table 1: LC instrument conditions for ILSP workflow**

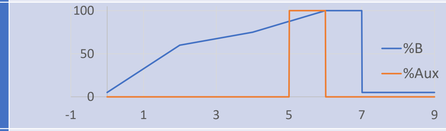
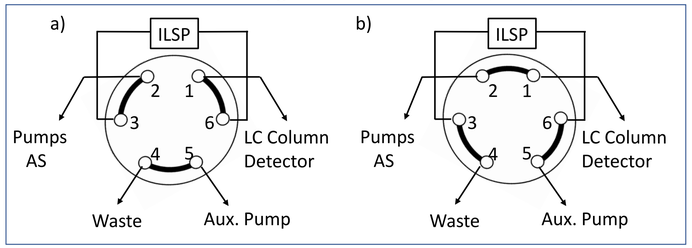
| | |
|---|---|
| **Instrument:** | Shimadzu Nexera UHPLC and 8045 MS/MS |
| **ILSP-PR Cartridge:** | 5 x 2.1 mm |
| **LC Column:** | Raptor ARC-18 2.7 µm, 100 x 2.1 mm (Cat# 9314A12) |
| **Pump A:** | Water, 2mM $NH_4HCO_2$ + 0.2% HCOOH |
| **Pump B/Aux.:** | Methanol, 2mM $NH_4HCO_2$ + 0.2% HCOOH |
| **Flow A/B, Aux.:** | 0.5 mL/min, 1.0 mL/min |
| **Time Program:** |  |
| **Oven Temp.:** | 50 °C |
| **Injection Volume:** | 4 µL of spinach extracts fortified with LC Multiresidue Pesticide Standard #4 (Cat# 31975) |



**Figure 1: ILSP valve configuration for: a) load, and b) wash**




## Results & Discussion

- Matrix constituents were removed using an UHPLC system equipped with an auxiliary pump, 6-port switching valve, and dual-directional ILSP cartridge (Table 1 and Figure 1).
- The ILSP procedure was compared to an AOAC QuEChERS extraction (Figure 2) and evaluated for recovery (Figure 3) and matrix effects (Figures 4-5) for 63 pesticides in spinach.

**Figure 2: Comparison of the number of steps and the time required to prepare 14 samples: ILSP vs. QuEChERS**

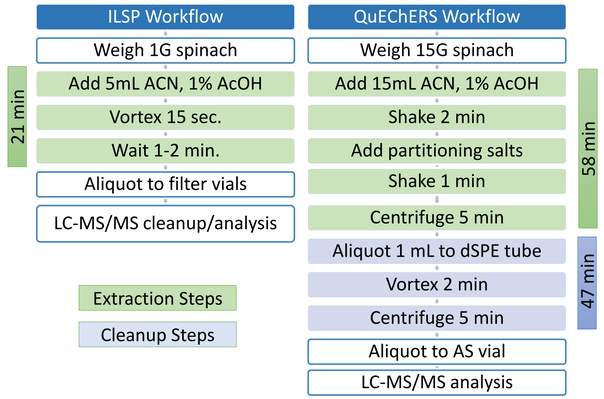



**Figure 3: Analytes with recoveries of 70-120% and RSD <20% for fortified spinach samples extracted by ILSP vs. QuEChERS**

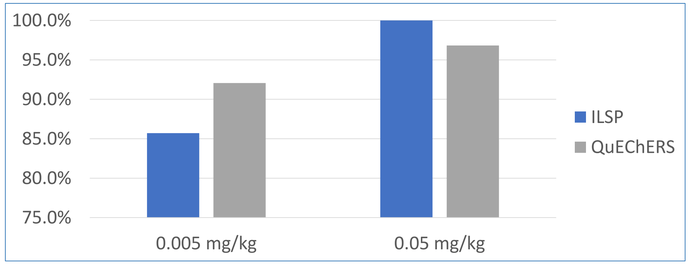



**Figure 4: Impact of cleanup procedure (dSPE vs. ILSP) on calculated matrix effects for QuEChERS extracted spinach samples: (a) overall distribution, (b) planar pesticides**

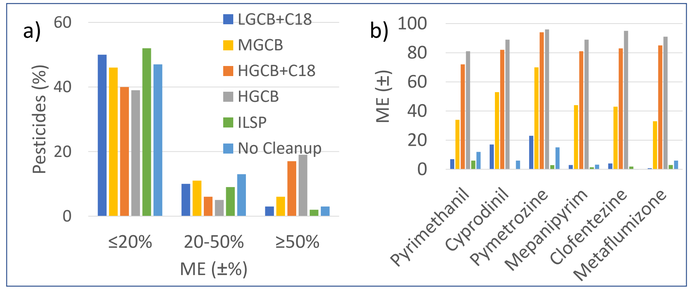
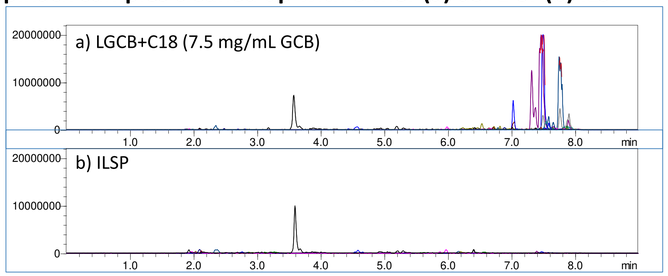



**Figure 5: XIC of pigments remaining in QuEChERS extracted spinach samples cleaned up with either (a) dSPE or (b) ILSP**




## Conclusion

- The ILSP workflow has fewer steps, takes 1/5 the time, and is more amenable to automation than QuEChERS.
- ILSP removes more pigments than dSPE with GCB, reducing MS contamination without loss of planar pesticides.
- Of the 63 pesticides tested, 86% met the acceptance criteria for samples fortified at 0.005 mg/kg and 100% met the criteria at 0.05 mg/kg.