Semi-automated method for in-line removal of matrix components from food for the analysis of residual pesticides by LC-MS/MS

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Abstract & Introduction

In-line sample prep (ILSP) provides a semi-automated cleanup procedure for the analysis of pesticide residues in food by LC-MS/MS. ILSP selectively retains matrix components from the sample extract and can be utilized as a standalone workflow or integrated into an existing QuEChERS workflow. In these experiments, ILSP was applied to multiple challenging commodities representing a wide range of compositions including spinach, soybean meal, avocado, whole orange, black tea, and hibiscus tea for the analysis of 61 pesticides. This solution provides a novel, semi-automated approach to reduce the abundance of matrix components entering the analytical column and MS source resulting in a decrease in instrument contamination and an improvement in data quality.

What is In-Line Sample Prep (ILSP)?

- Semi-automated workflow for the extraction, clean-up & analysis of pesticide residues in food commodities by LC-MS/MS
- Matrix components are removed from the sample extract concomitant with analysis
- Elute target analytes while flushing matrix components to waste with the aid of a 6-port high pressure switching valve in a single injection
- Can be used as a standalone workflow or in conjunction with an existing QuEChERS extraction
- Automation introduces less human error and increases throughput over traditional extraction and clean-up techniques

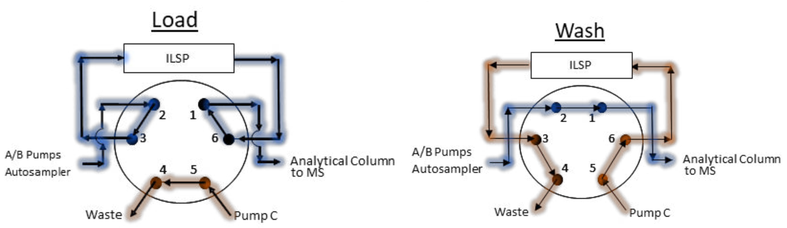


Figure 1: 6-port high pressure valve configuration for loading ILSP and washing ILSP.

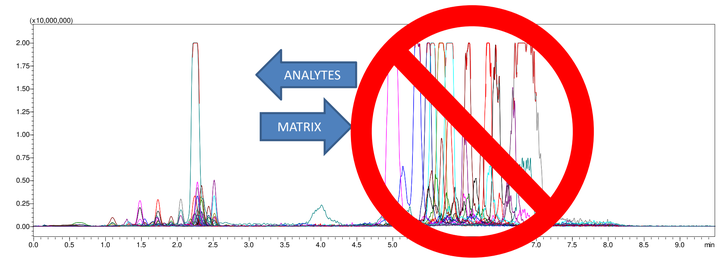


Figure 2: Visual representation of ILSP clean-up. Pesticides are minimally retained on the cartridge, while the matrix is strongly retained so that it may be flushed to waste.

System requirements:

- Binary HPLC system equipped with an auxiliary pump
- Column oven with 6 port high pressure switching valve
- Autosampler capable of internal needle rinsing
- Dual-directional ILSP cartridge (100% aqueous and high pressure stable to 12,000 psi)
- ILSP cartridge holder

Method Development

ILSP Method Setup – Step 1

Set up LC-MS/MS using previously optimized mobile phase and gradient from an existing pesticide residue instrument method

-For best results the mobile phase buffer should not exceed 5 mM

Configure 6-port switching valve

-Install the ILSP cartridge and holder as indicated below (load position)

-Remove the analytical column and replace with a union

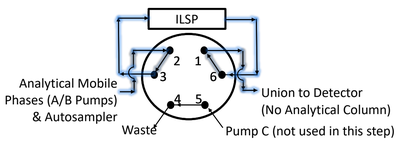


Figure 3 (left): 6-port valve configuration for step 1. In this step only mobile phases A & B are used with the ILSP cartridge (no analytical column).

Determine Valve Switch Time – Step 2

- Inject a solvent standard onto the ILSP cartridge using previously optimized conditions
- Locate the last eluting analyte in the resulting chromatogram
- This will be the time it takes all compounds to elute and the valve can be switched to wash pigments to waste after this time

MPA: 2 mM AF + 0.2% FA water
MPB: 2 mM AF + 0.2% FA Methanol
Pesticide in solvent
Oven temp: 50 C
Flow: 0.4 mL/min

Time	%B
0	5
2	60
4	75
6	100
7.5	100
7.51	5
9.5	STOP

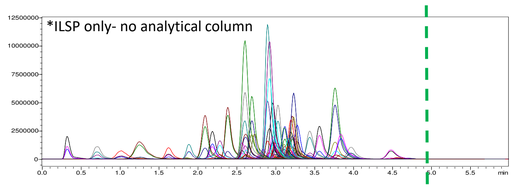


Figure 4: Chromatogram obtained for pesticide mixture using the outlined mobile phases and gradient on ILSP cartridge.

Determine ILSP Wash Time – Step 3

- Substitute a wash solvent for mobile phase B and program 100 %B for 1 mL/min
- With valve in the load position inject a matrix blank on the ILSP cartridge (no analytical column)
- Monitor the presence of matrix in SIM or SCAN modes and note the time it takes the TIC to return to baseline

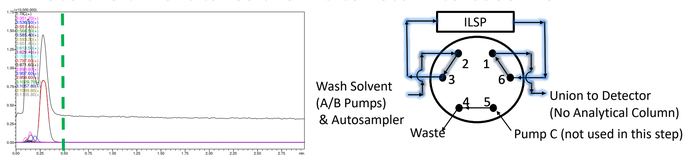


Figure 5: Chromatogram obtained from blank matrix sample injected onto ILSP cartridge with the valve in load position.

Application to Food Commodities

Spinach- High Water Content

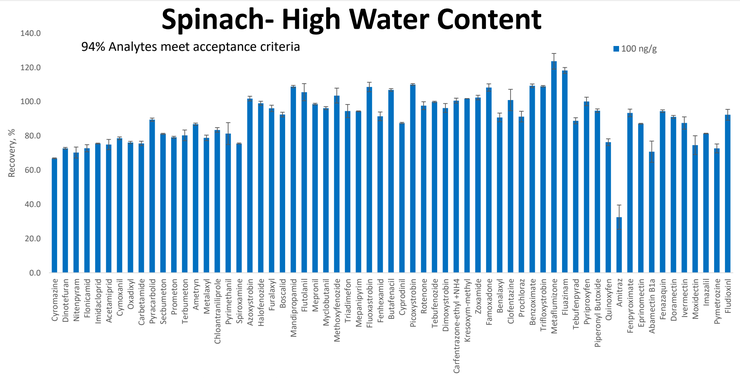


Figure 6: Spinach was homogenized and 15 g weighed into a 50 mL centrifuge tube. 15 mL of ACN + 0.1% AA added, vortexed, shaken for 5 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 100 ng/g.[1]

Soybean Meal- High Oil & Low Water

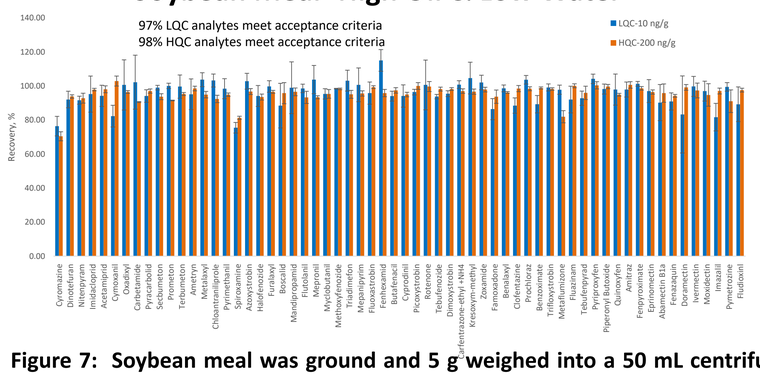


Figure 7: Soybean meal was ground and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 15 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

Avocado- High Oil and Intermediate Water

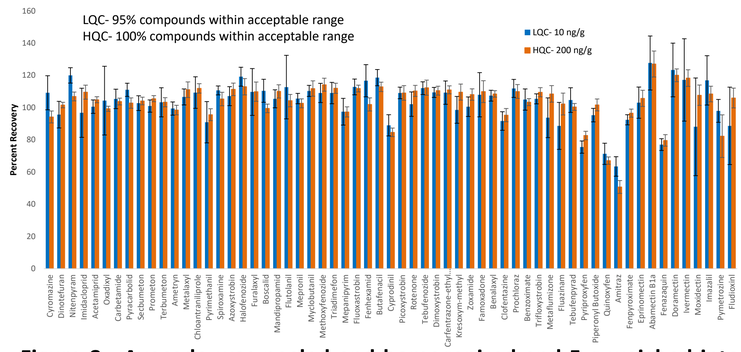


Figure 8: Avocado was peeled and homogenized and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 3 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

Whole Orange- High Acid and High Water

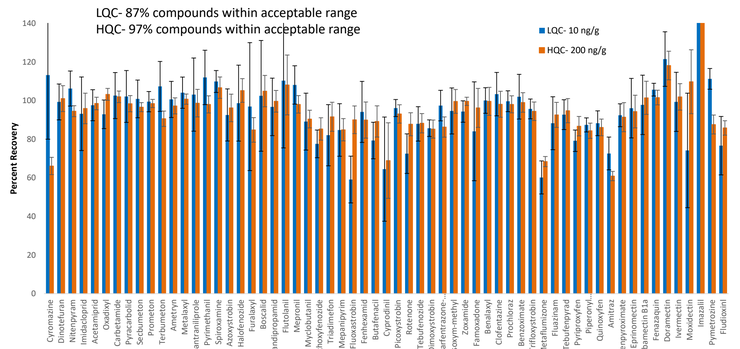
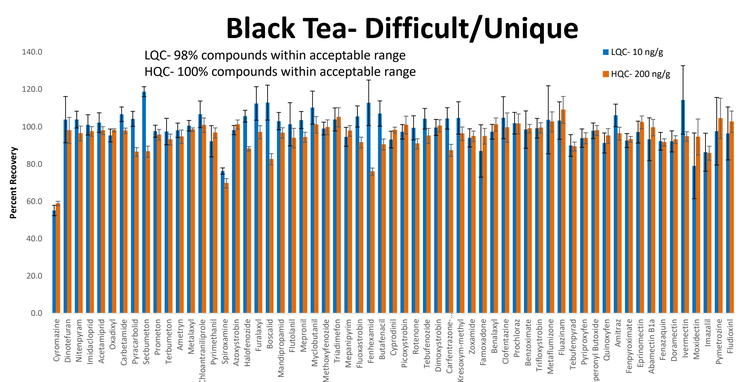


Figure 9: Whole orange was homogenized and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

Black Tea- Difficult/Unique



Hibiscus Tea- Difficult/Unique

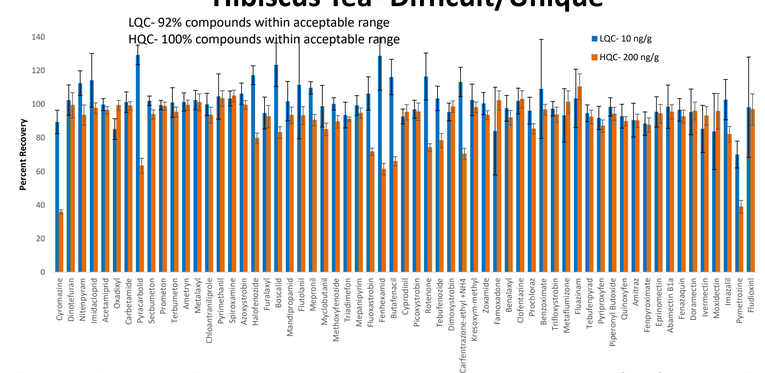


Figure 10 & 11: Black tea and hibiscus tea were weighed (1 g) into a 50 mL centrifuge tube. 5 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to filter vial and 10 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

Conclusions

The semi-automation of the ILSP methodology reduces error, simplifies sample preparation, and increases throughput compared to traditional extraction and cleanup techniques for a streamlined approach for the analysis of pesticide residues in food commodities.

References

1. S.A. Lupo, R.L. Romesberg, X. Lu, Automated inline pigment removal for the analysis of pesticide residues in spinach by liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1629 (2020) 461477.