

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

Jamie L. York, Sharon Lupo, Connor Flannery; Restek Corporation

## 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

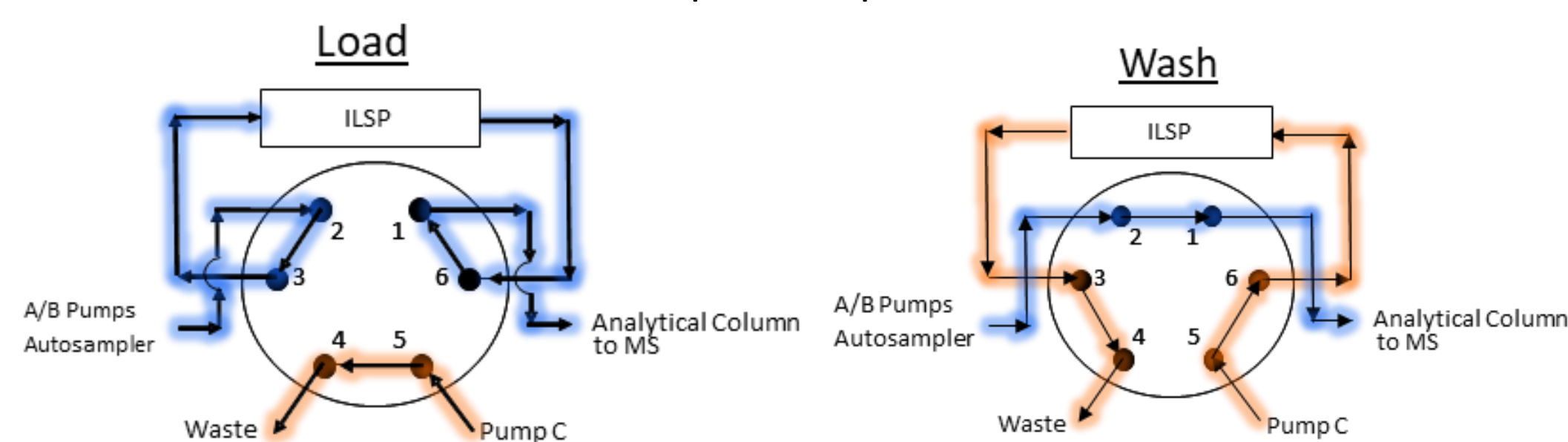


Figure 1: 6-port high pressure valve configuration for loading ILSP and washing ILSP.

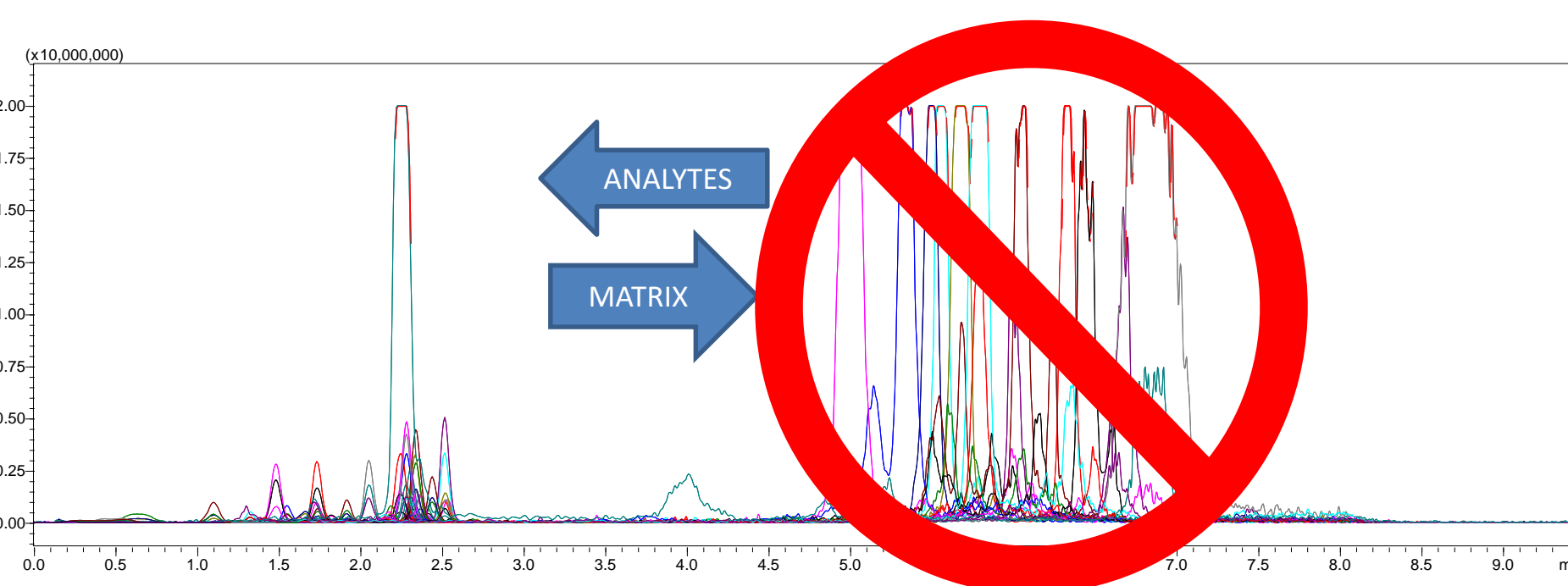


Figure 2: Visual representation of ILSP clean-up. Pesticides are minimally retained on the cartridge, while the matrix is strongly retained so that it may be flushed to waste.

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

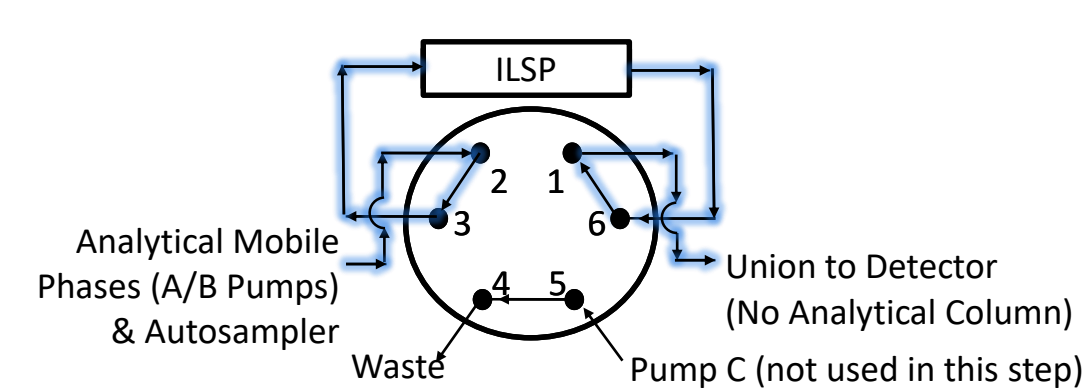


Figure 3 (left): 6-port valve configuration for step 1. In this step only mobile phases A & B are used with the ILSP cartridge (no analytical column).

### 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

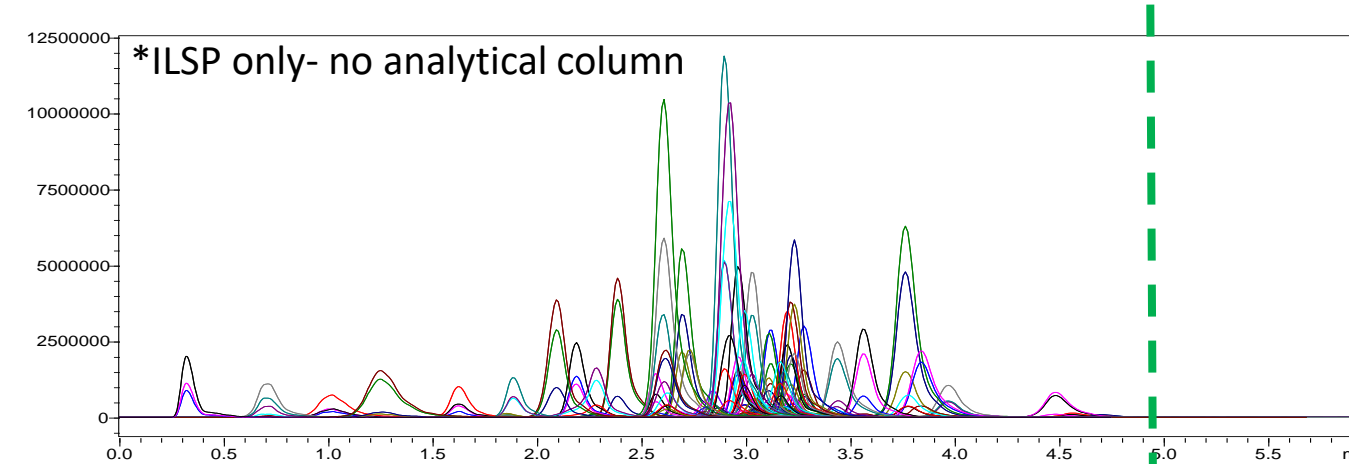


Figure 4: Chromatogram obtained for pesticide mixture using the outlined mobile phases and gradient on ILSP cartridge.

### 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

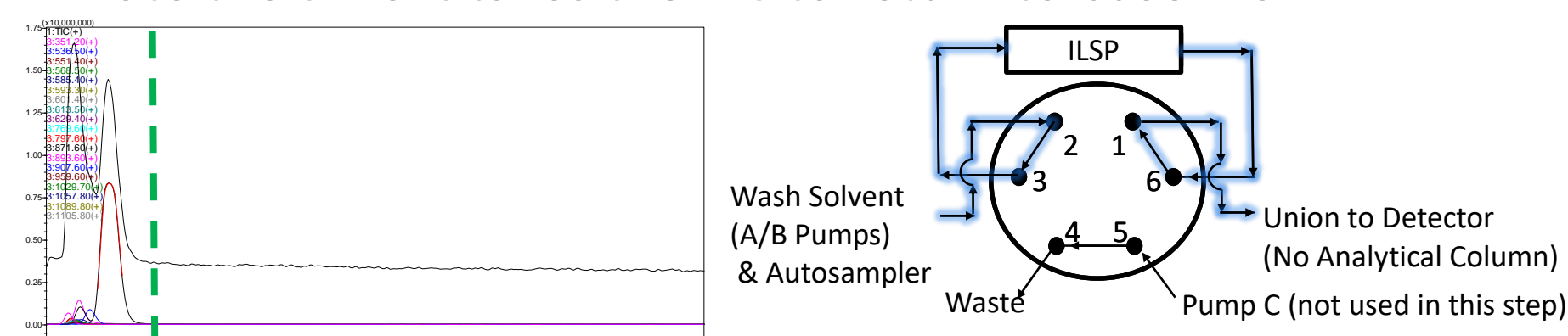


Figure 5: Chromatogram obtained from blank matrix sample injected onto ILSP cartridge with the valve in load position.

## Application to Food Commodities

### Spinach- High Water Content

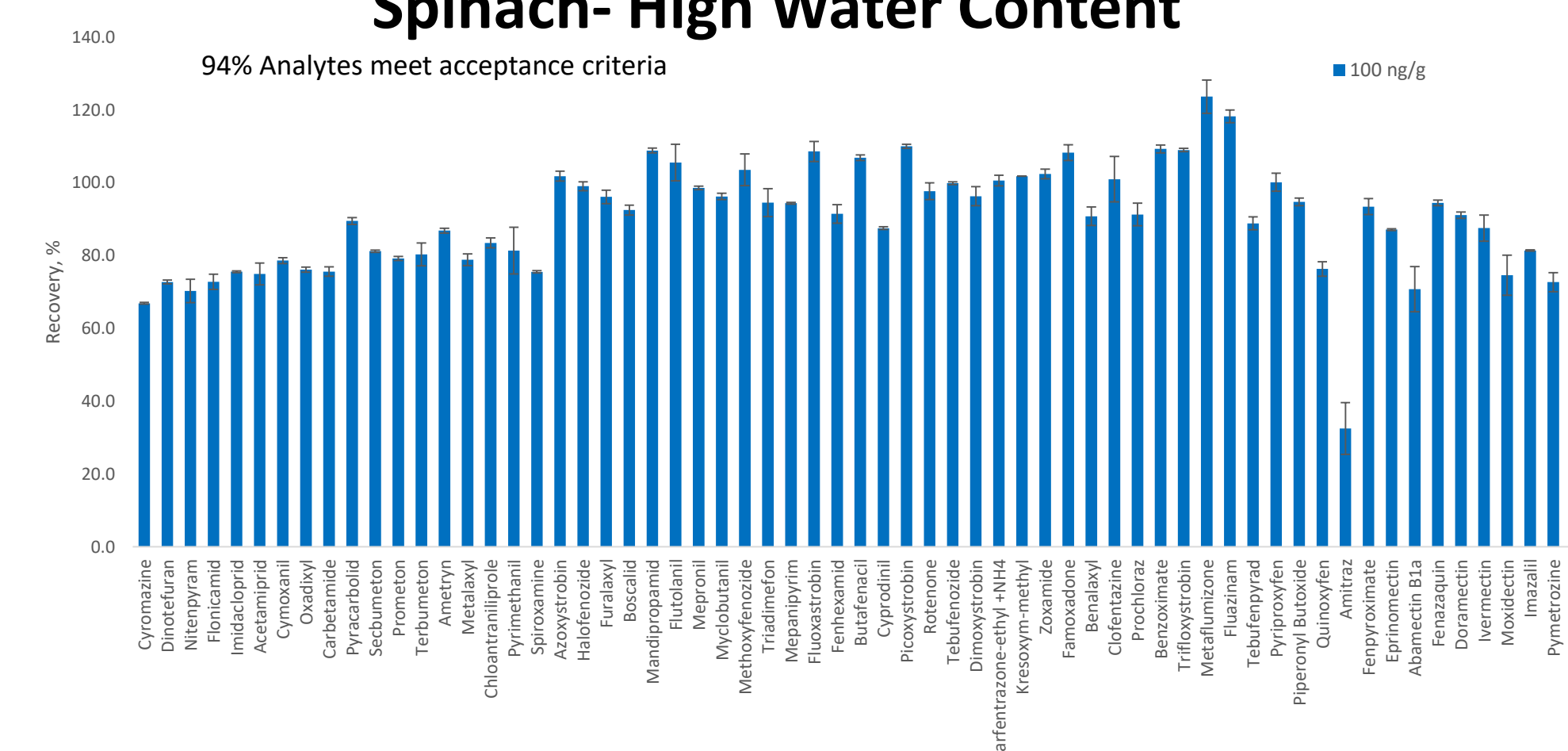


Figure 6: Spinach was homogenized and 15 g weighed into a 50 mL centrifuge tube. 15 mL of ACN + 0.1% AA added, vortexed, shaken for 5 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 100 ng/g.<sup>1</sup>

### Soybean Meal- High Oil & Low Water

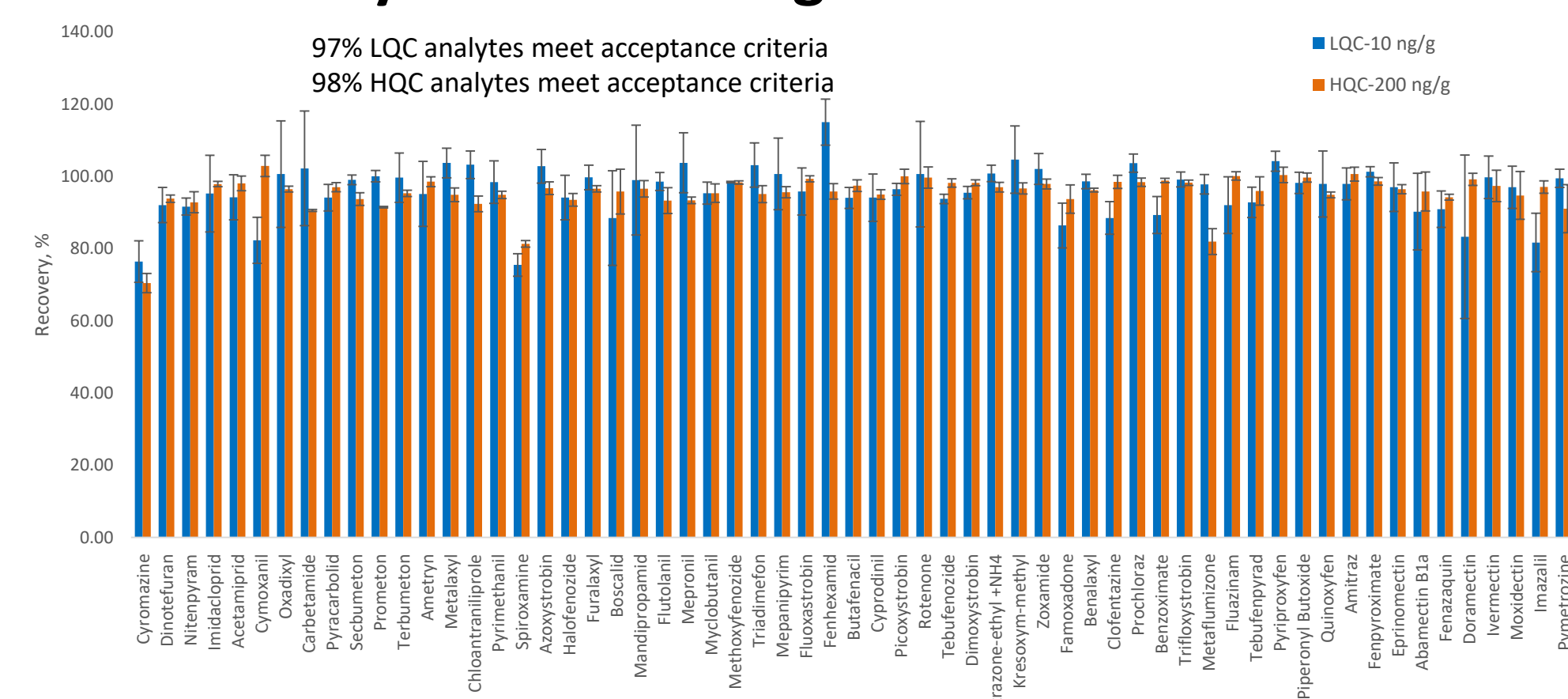


Figure 7: Soybean meal was ground and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 15 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

## References

- 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.

### Avocado- High Oil and Intermediate Water

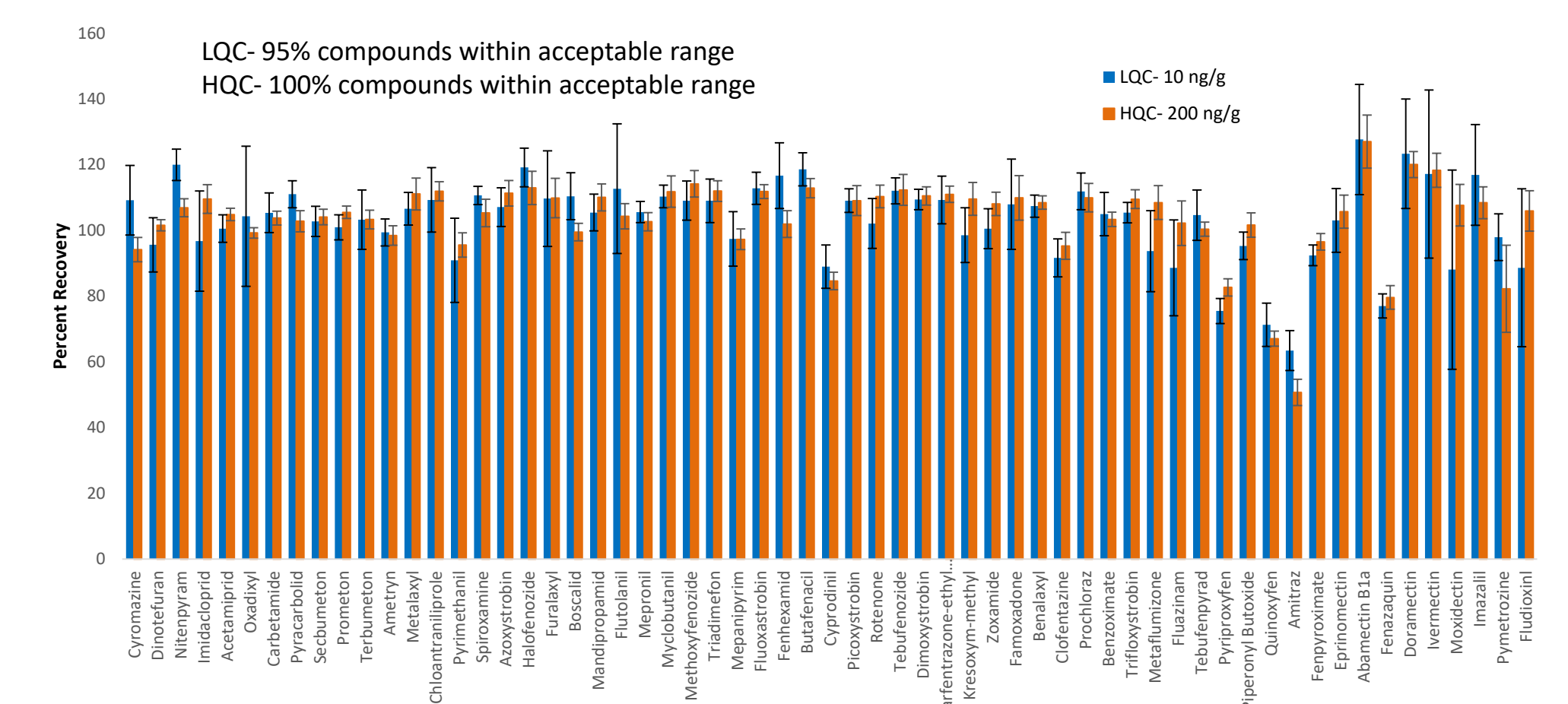


Figure 8: Avocado was peeled and homogenized and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 3 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

### Whole Orange- High Acid and High Water

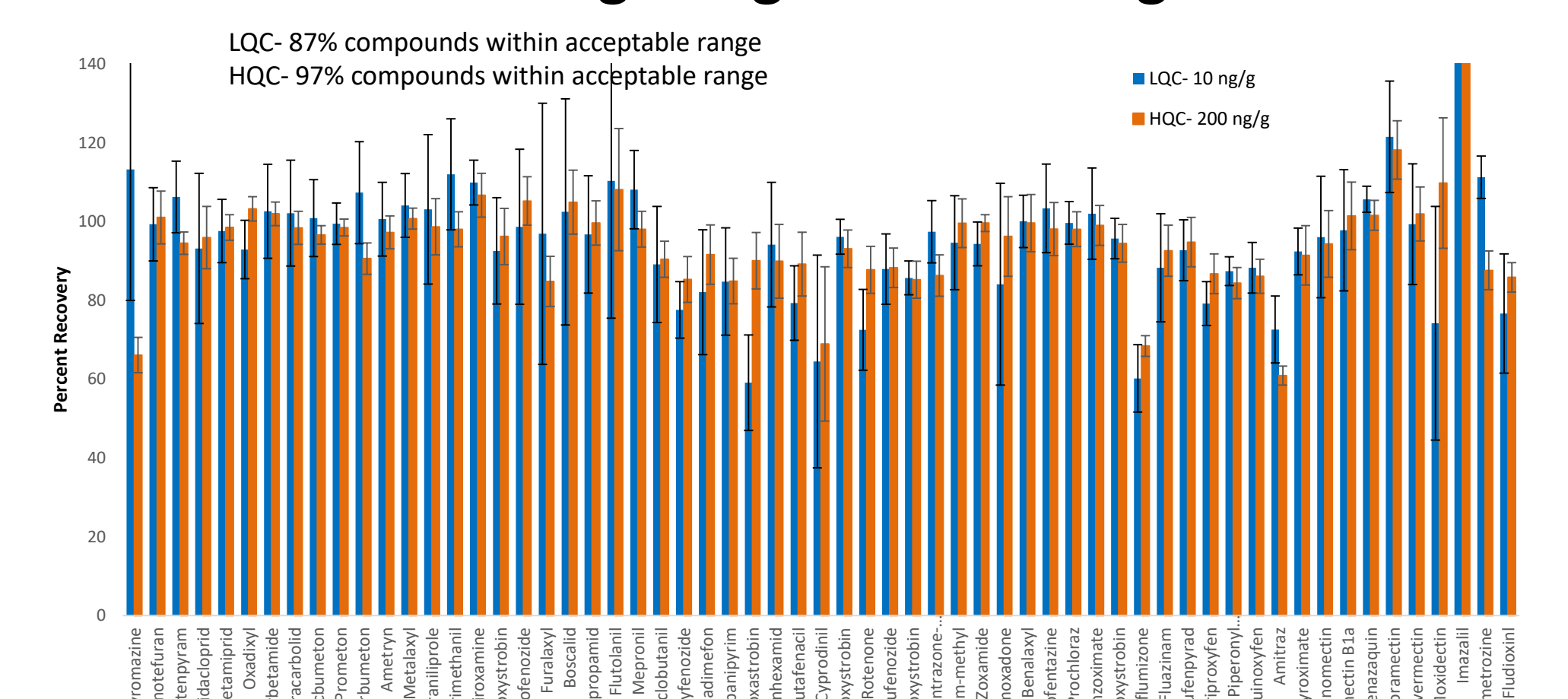
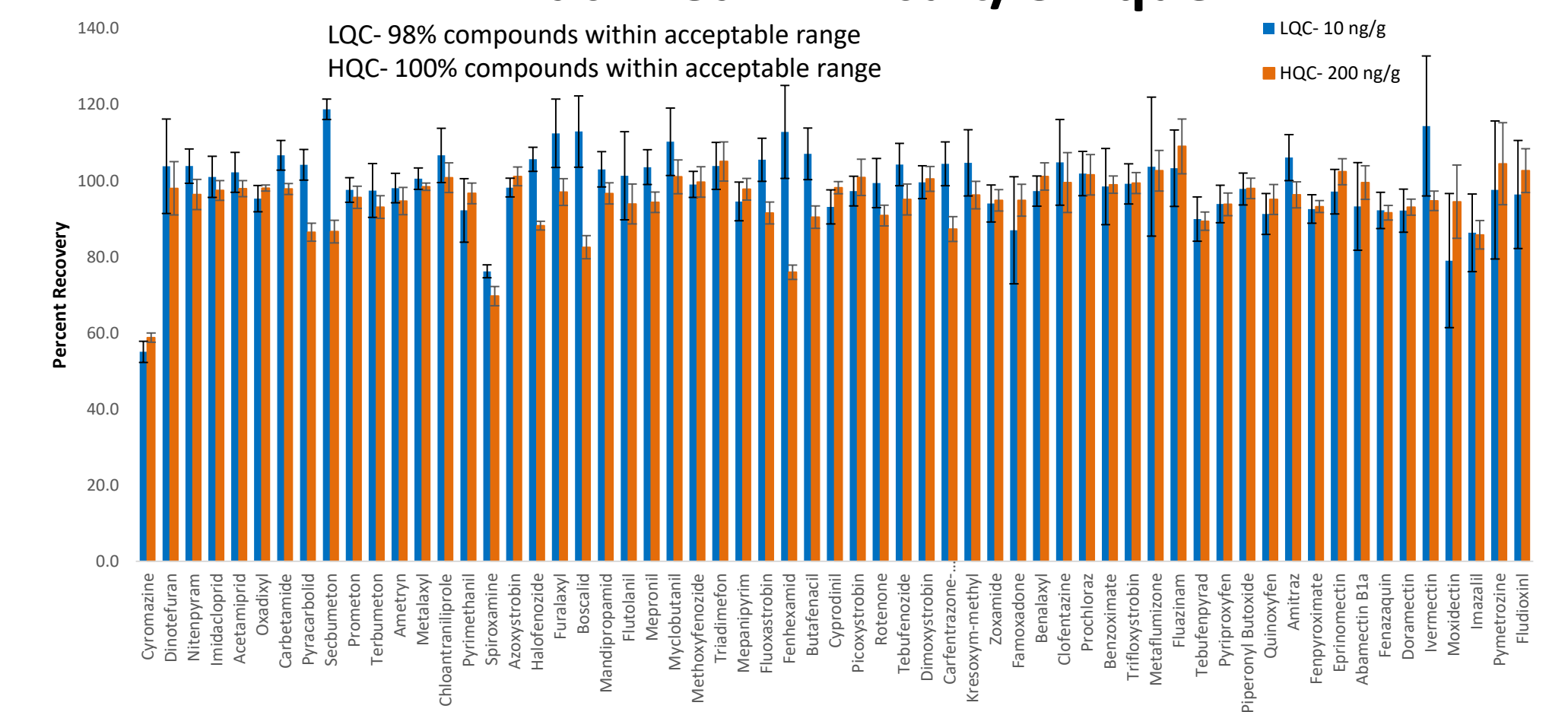


Figure 9: Whole orange was homogenized and 5 g weighed into a 50 mL centrifuge tube. 10 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to vial and 4 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

### Black Tea- Difficult/Unique



### Hibiscus Tea- Difficult/Unique

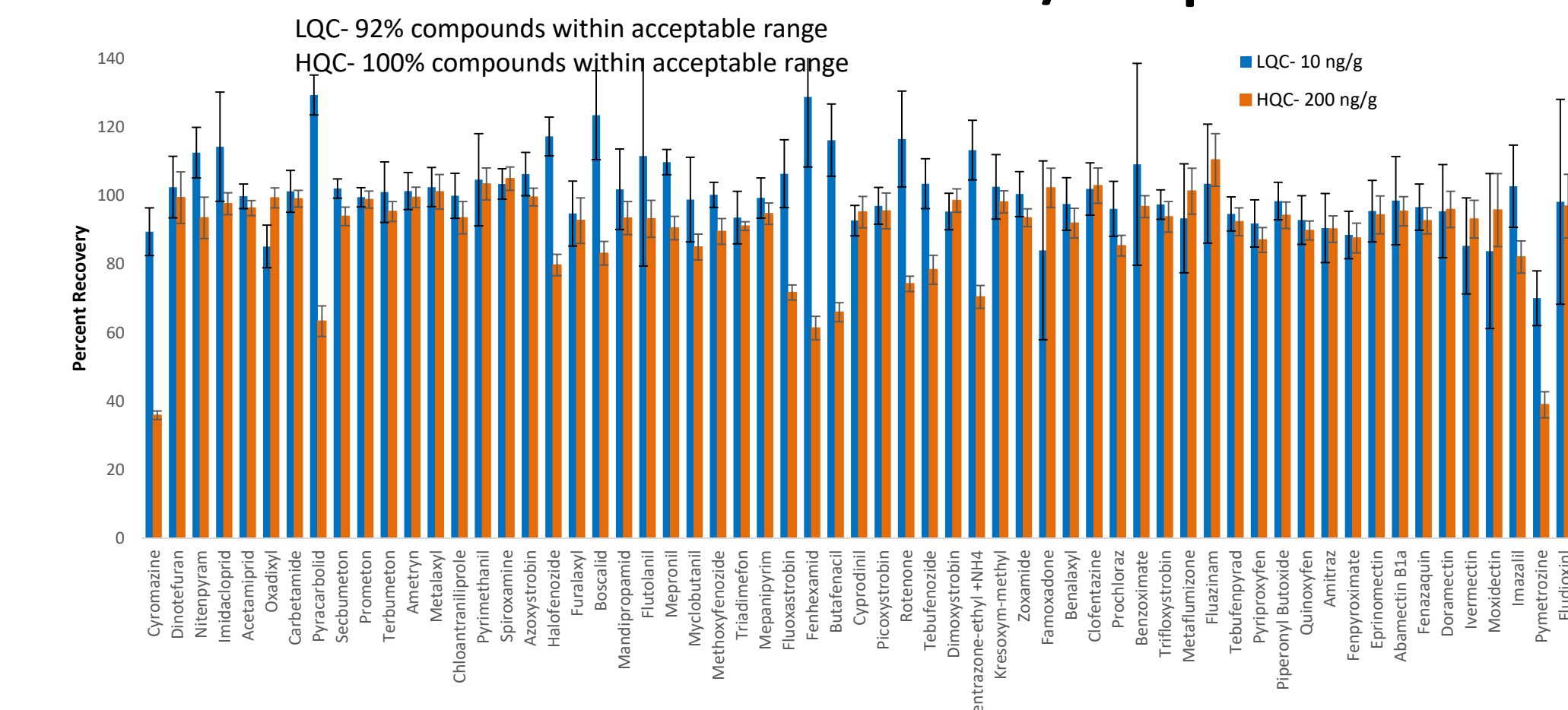


Figure 10 & 11: Black tea and hibiscus tea were weighed (1 g) into a 50 mL centrifuge tube. 5 mL of ACN + 0.1% AA added, vortexed, shaken for 10 min, and centrifuged 10 mins @ 4000 rpms. Sample was aliquoted to filter vial and 10 µL injected. Graph represents recovery data at 10 ng/g and 200 ng/g.

## 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.