

# Development and Inter-laboratory Validation of a Liquid Chromatography/Tandem Mass Spectrometry Method for Multi-residue Pesticides Analysis

## Florida Pesticides Residue Workshop

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# Outline

- Background Information
- Where and what could go wrong?
- Sample preparation and analysis
- Results
  - Method validation
  - Principal component analysis



# A Pesticide Multi-residue Method is Needed

- For initial screening and follow up determination of currently used and emerging pesticides by the United States Food and Drug Administration (FDA) and Ontario Ministry of the Environment (MOE)
- To complement existing multi-residue methods for the determination of polar and/or thermal labile pesticides
- To maximize analytical efficiency as more compounds can be analyzed with the same preparation effort and analytical time



# The Envisioned Multi-residue Method

- Use liquid chromatography / tandem mass spectrometry (LC/MS-MS) instrumentation
- Have the provision for the identification and follow up quantitation of pesticides with high confidence
- Be validated for various sample matrices and can be used by multiple laboratory
- Have consistent data quality for compounds monitored

# Details of the FDA/MOE Method (I)

- Uses “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) for sample preparation
- Based on work done using Waters Ultra LC and Premier MS-MS system (J. Wong), the method analyzes 191 target pesticides encompassing eleven categories of pesticides
- Uses LC/MS-MS instrumentation with the additional ability for information dependent data acquisition (IDA) for library collection and follow up library search
- All analytes determined by using two multiple reaction monitoring (MRM) transitions to achieve an identification point of four
- Validated for orange, peach, spinach, and water sample matrices

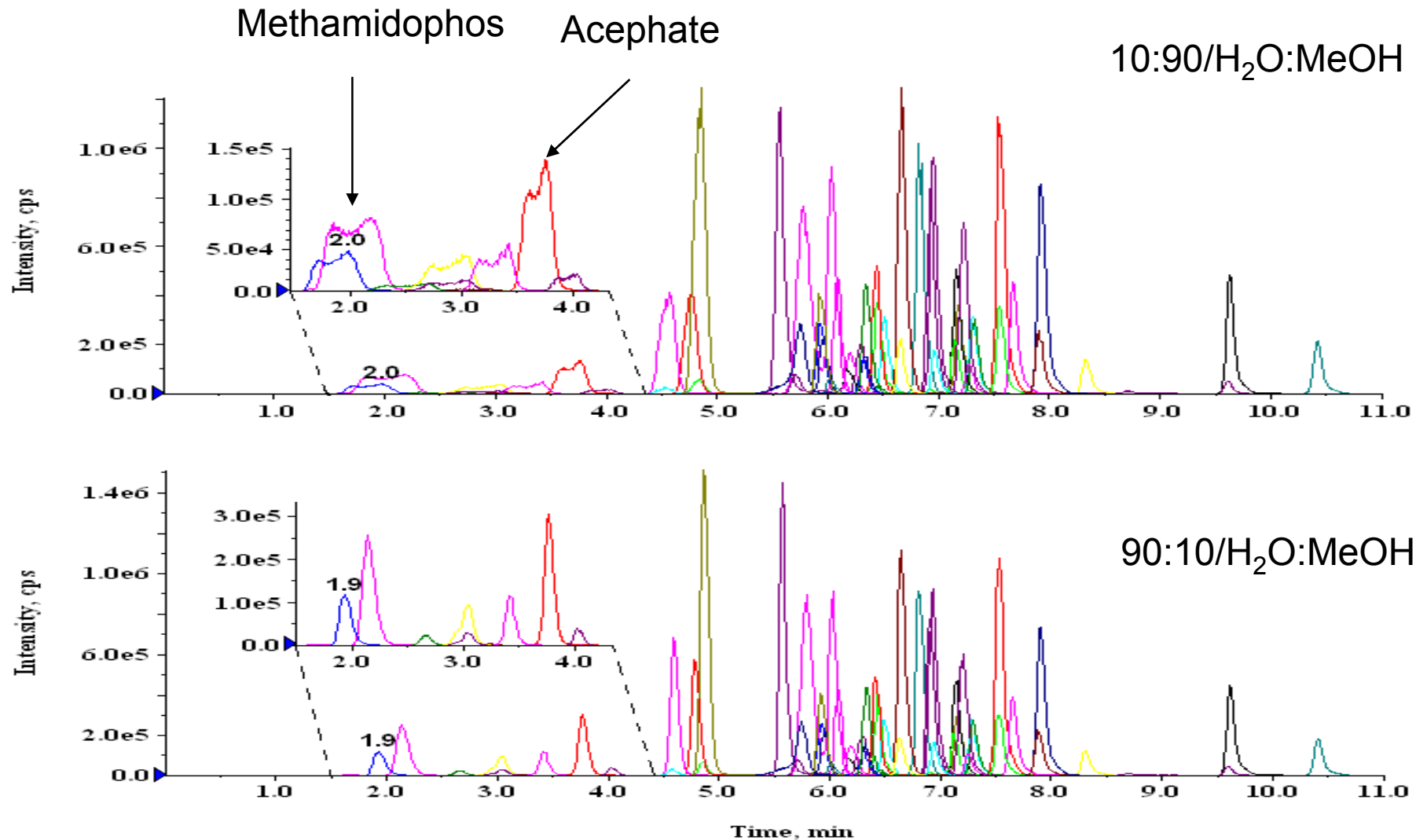


# Details of the FDA/MOE Method (II)

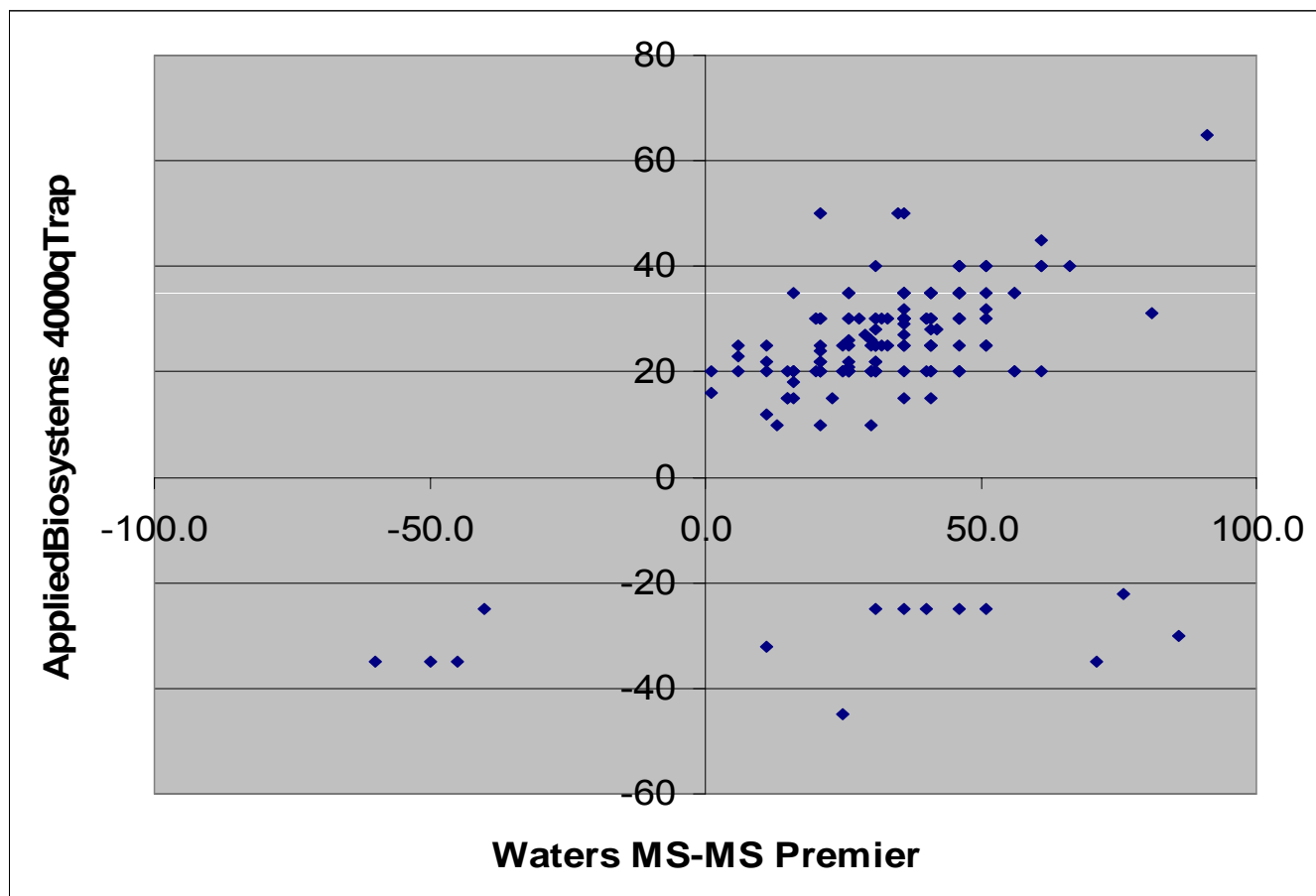
- Uses seven deuterium labelled pesticides as surrogates and internal standards
- LC/MS-MS parameters used were the same with modifications to meet local requirements
- QuEChERS samples were prepared @ FDA College Park
  - Extracts reconstituted in  $\text{CH}_3\text{CN}$  and 4mM  $\text{NH}_4\text{OAc}+0.1\%$   $\text{HCOOH}$  (FDA) for immediate analysis
  - Extracts shipped overnight, stored in freezer ( $< -20^\circ\text{C}$ ), reconstituted in  $\text{MeOH}$  and 10mM  $\text{NH}_4\text{OAc}$  (MOE) on the 3<sup>rd</sup> and 10<sup>th</sup> day and analyzed on LC/MS-MS
- Data merged and principal component analysis carried out to interpret the results



# Challenges in Method Development – Optimization of Sample Extract Solvent



# Challenges in Method Development – Electrospray Ionization Source Declustering Potential



# Challenges in Method Development — H<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, or Na<sup>+</sup> Adducts or Matrix Effect?

- 191 compounds optimized via infusion that resulted in various positive ion adducts
- A 36 hour instrument within-run (N=7) of low level standard showed 7 and 14 compounds, respectively, had a RSD of >50% and >20%
- Eight of the 191 pesticides had 0% recovery in the QuEChERS extracts

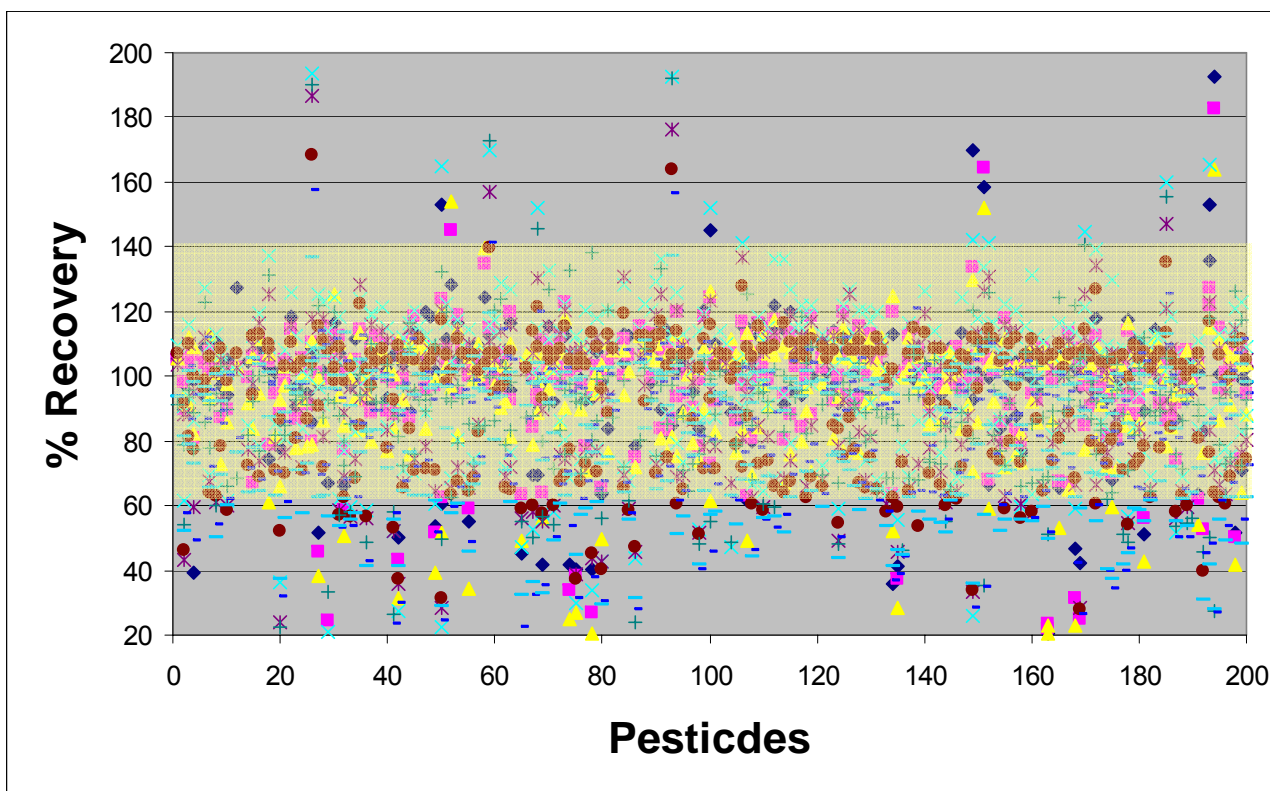
# LC Parameters

	FDA	MOE
Mobile phase	0.1% HCOOH/4 mM NH <sub>4</sub> OAc in H <sub>2</sub> O (A) and MeOH (B)	10 mM NH <sub>4</sub> OAc in H <sub>2</sub> O (A) and MeOH (B)
Flow rate	0.5 mL/min	0.35 mL/min
Total run time	11.5 min	16 min
Gradient	5% B to 95% B, 11.5 min	20% B to 100% B, 16 min
Injection Volume	8.4	6.8

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# Typical Recoveries of 191 Pesticides in Three Matrices

Analyzed by Two Laboratories



**88.5%**

3 matrices, 3 spiking levels, 72 samples and 191 pesticides

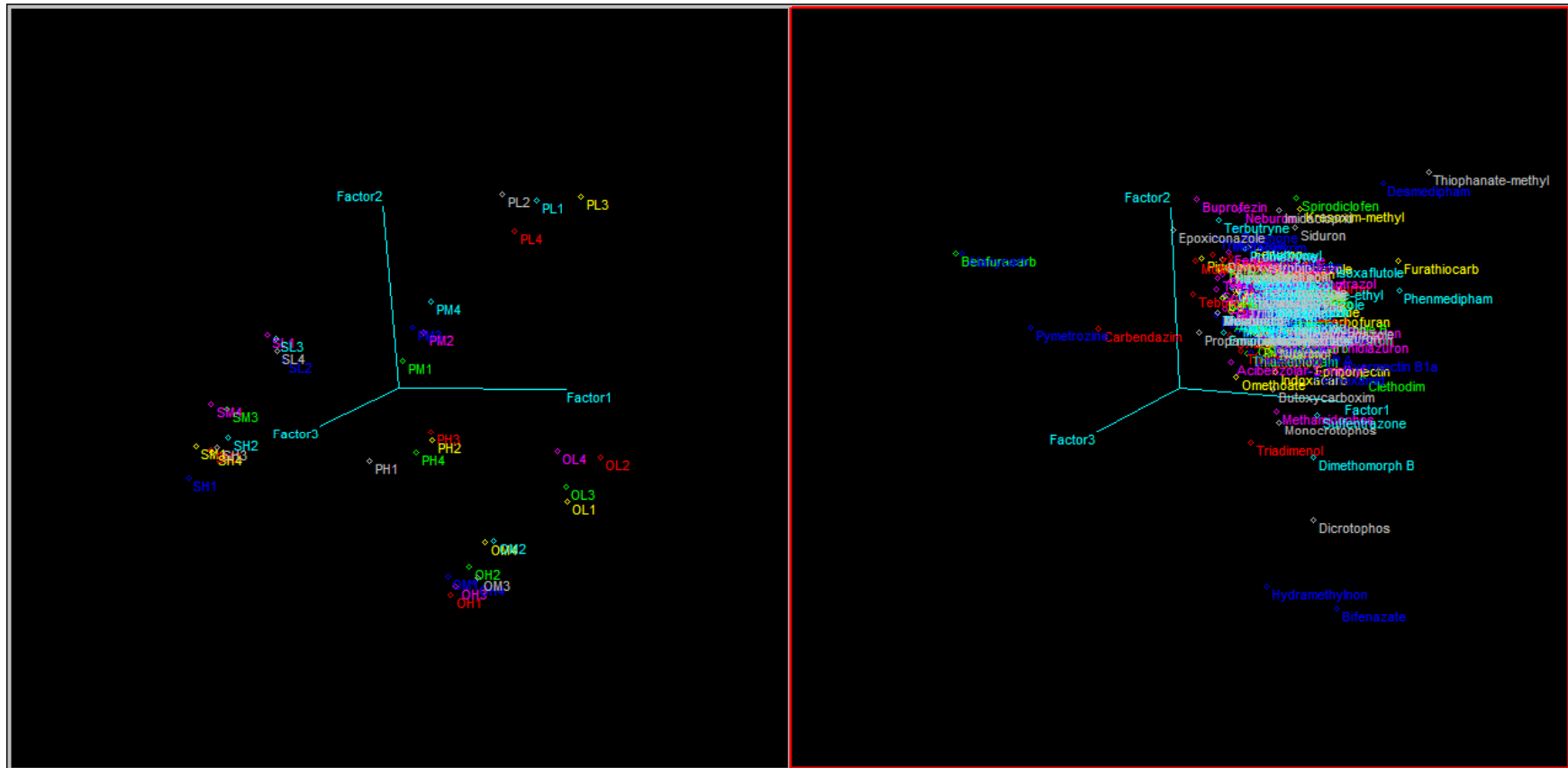
Not Detected: 8.4%

# Challenges & A Possible Solution

- The Challenge: simply too many data points to interpret to carry out trend analysis
- Use principal component analysis (PCA) to compare the results in mathematic domain
  - sample matrices
  - spiking levels (matrix effect, signal-to-noise of the experiment, analytical system loading)
  - recoveries
- Pesticide recovery used as variables. Eigen-values of 72 samples for 191 variables were used
- Qualitative analysis based on quantitative variables



# Principal Component Analysis of Pesticide Recoveries

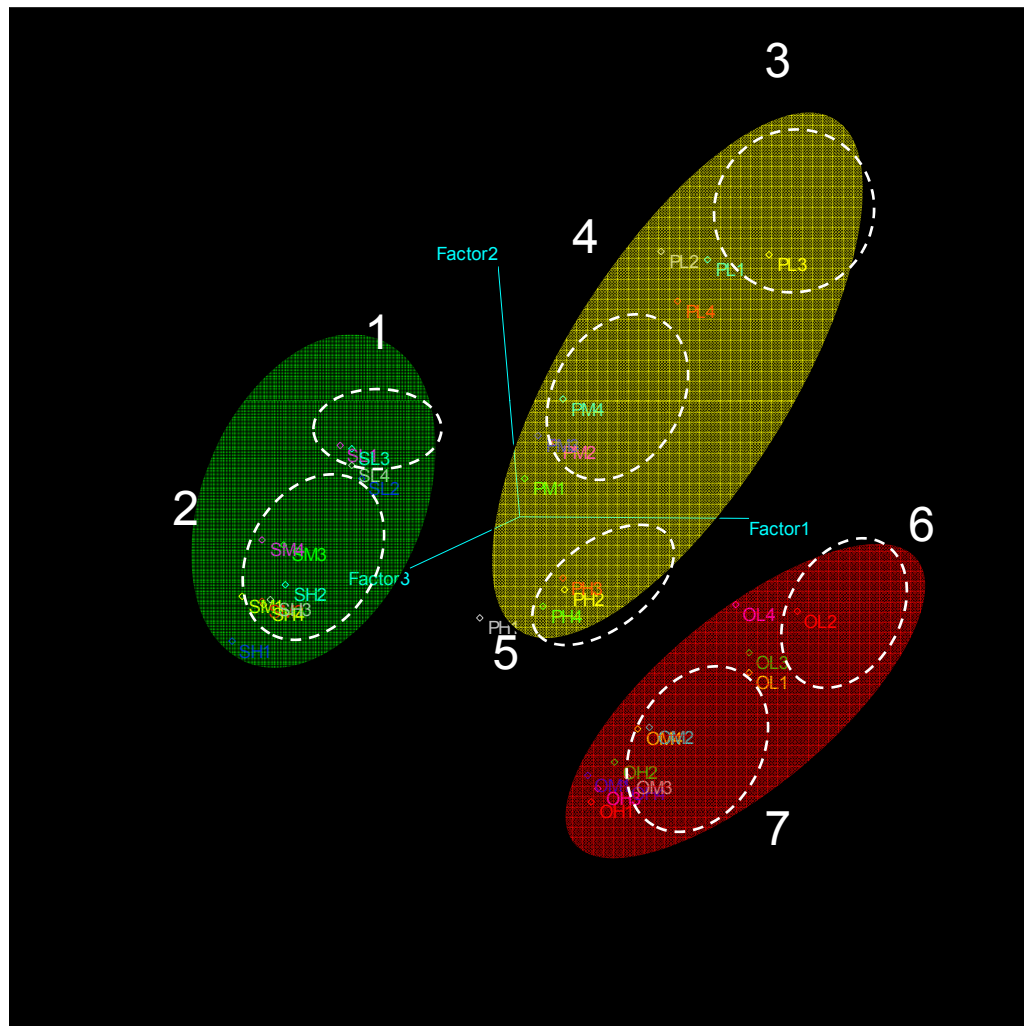


Scores (spiked samples )

Loadings ( pesticides)

S: spinach; O: orange; P: peach; L: 25ng/g spike; M: 100 ng/g; spike; H: 250 ng/g spike  
 1,2,3,4: replicates 1 to 4 at each spiking level  
 SL1 stands for spinach replicate sample #1 spiked at 25ng/g

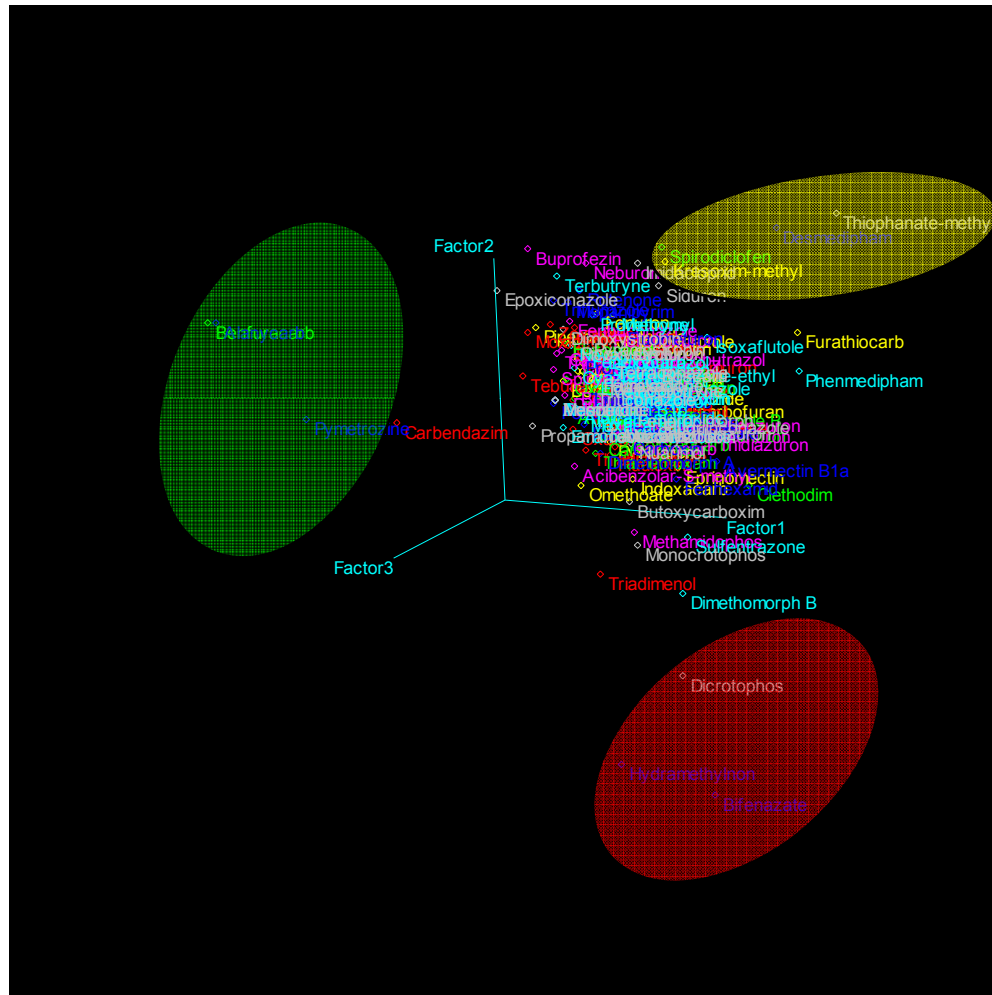
# Scores — Significant Components and Grouping






## Major groups      Subgroups

- Spinach
  - 1. SL 1–4
  - 2. SM 1–4 & SH 1–4
  
- Peach
  - 3. PL 1–4
  - 4. PM 1–4
  - 5. PH 1–4
  
- Orange
  - 6. OL 1–4
  - 7. OM 1–4 & OH 1–4

# Pesticide Loadings



In terms of the three major groups, the following pesticides contribute to the difference among them.

-  Alanycarb, Benfuracarb, Pymetrozine
-  Thiophanate-methyl, Desmelopham, Phenemdipham, Furathiocarb
-  Dimethomorph B, Dicrotophos, Hydramethylinon, Bifenazate

# An Explanation

	<b>Spinach</b>	<b>Peach</b>	<b>Orange</b>	<b>RSD, 18-hr Within Run</b>
Furathiocarb		<b>x</b>		<b>6.2</b>
Alanycarb	<b>x</b>			<b>9</b>
Thiophanate-methyl		<b>x</b>		<b>14.1</b>
Dicrotophos			<b>x</b>	<b>14.5</b>
Benfuracarb	<b>x</b>			<b>14.7</b>
Hydramethyion			<b>x</b>	<b>16.2</b>
Pymetrozine	<b>x</b>			<b>17.4</b>
Dimethomorph B			<b>x</b>	<b>19.9</b>
Phenemdipham		<b>x</b>		<b>20</b>
Bifenazate			<b>x</b>	<b>23.1</b>
Desmelopham		<b>x</b>		<b>25.3</b>

# Principal Component Analysis

- The PCA model demonstrates there are three major groups and two to three subgroups within each major group
- The model and LC/MS-MS within-run data show that matrix effects instead of the analytical system affected these groups
- Within each matrix/data set, samples analyzed at the lowest level (5 ng/mL, 20 uL injection) formed a unique group due to system sensitivity
- Samples analyzed at the other two levels (20 and 100 ng/mL) are in a “comfortable” zone of the LC/MS-MS, did not show significant difference, and might only be affected by sample matrices
- Method performance; hence, variation in the recovery of each pesticide was the major driving force that defines the trend