

## Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the Determination of 148 Pesticides in Berries

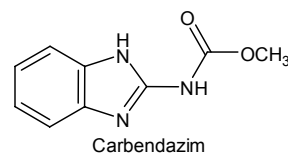
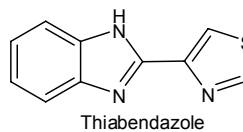
Jian Wang, PhD  
Calgary Laboratory  
Canadian Food Inspection Agency  
Calgary, Alberta, Canada  
Jian.Wang@inspection.gc.ca

The 46th Annual Florida Pesticide Residue Workshop (FPRW), July 19 - 22, 2009



## Objectives

- Method development:  
analysis of ~148 LC-  
amenable pesticides in  
berries including  
strawberries, Saskatoon  
berries, blackberries,  
raspberries, blueberries  
and cherries.





## Multi-residues and Challenges

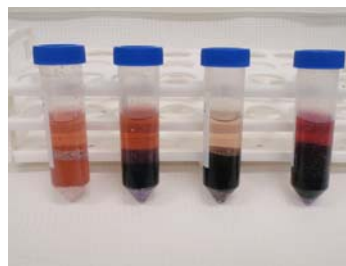
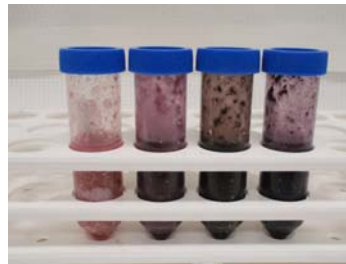
- Extraction: extract all in a single extraction
  - Generic vs QuEChERS (Buffered)
- Instrumentation: detect all in a single injection
  - LC/ESI-MS/MS (Pre-target analysis)
    - Fast data acquisition  $\leq 5$  ms dwell time
    - Scheduled MRM
  - UHPLC QqTOF (Post-target analysis)
    - Full-scan
- Method validation and data evaluation
  - Statistical experimental design
    - Nested data analysis



## Extraction: Buffered QuEChERS

### STEP 1: Extraction

- ☞ 15 g samples + 15 mL of acetonitrile/acetic acid (99:1, v/v) + 1.5 g of sodium acetate anhydrous + 6.0 g of magnesium sulfate anhydrous.
- ☞ Shake 45 s.
- ☞ Centrifuge  $\sim 2100 \times g$  for 3 min.



## STEP 2: Clean-up

☞ 9 mL of supernatants + 0.6 g PSA + 1.8 g of magnesium sulfate anhydrous.

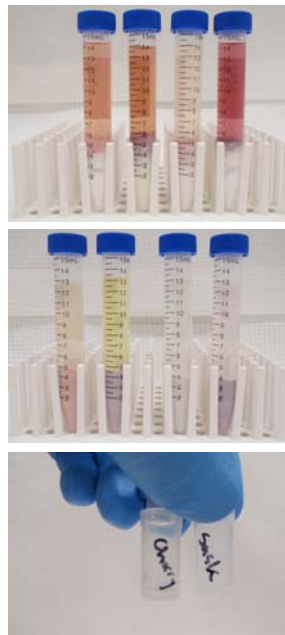
☞ Shake 45 s.

☞ Centrifuge  $\sim 2100 \times g$  for 3 min.

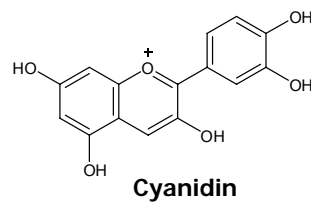
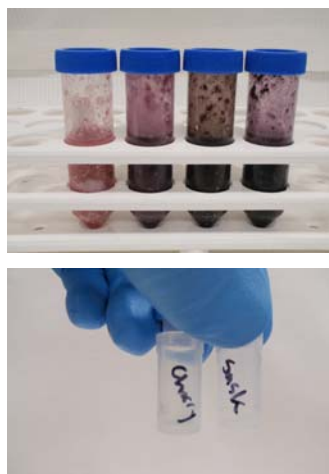
☞ Concentrate (1 mL) and reconstitute (1 mL) in 0.1 M ammonium acetate/methanol (50/50, v/v).

☞ Vial (Mini-UniPrep vial) 1/6 g matrix per mL for LC/ESI-MS/MS (6 times dilution).

☞ Vial 1g matrix per mL for UHPLC QqTOF MS.



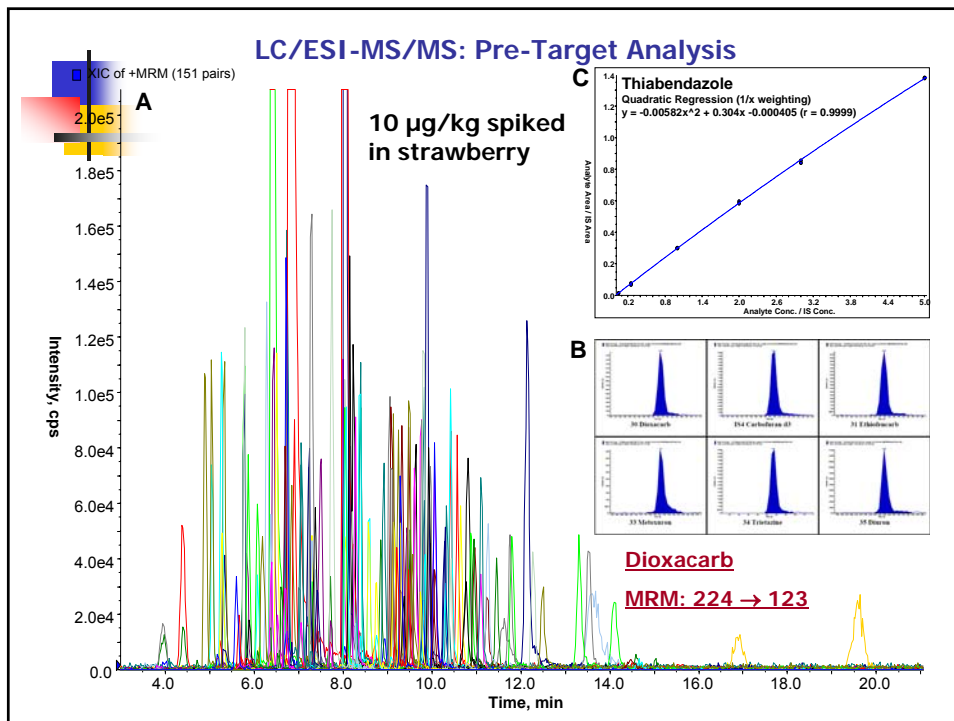
## QuEChERS: effective to remove anthocyanins in berries

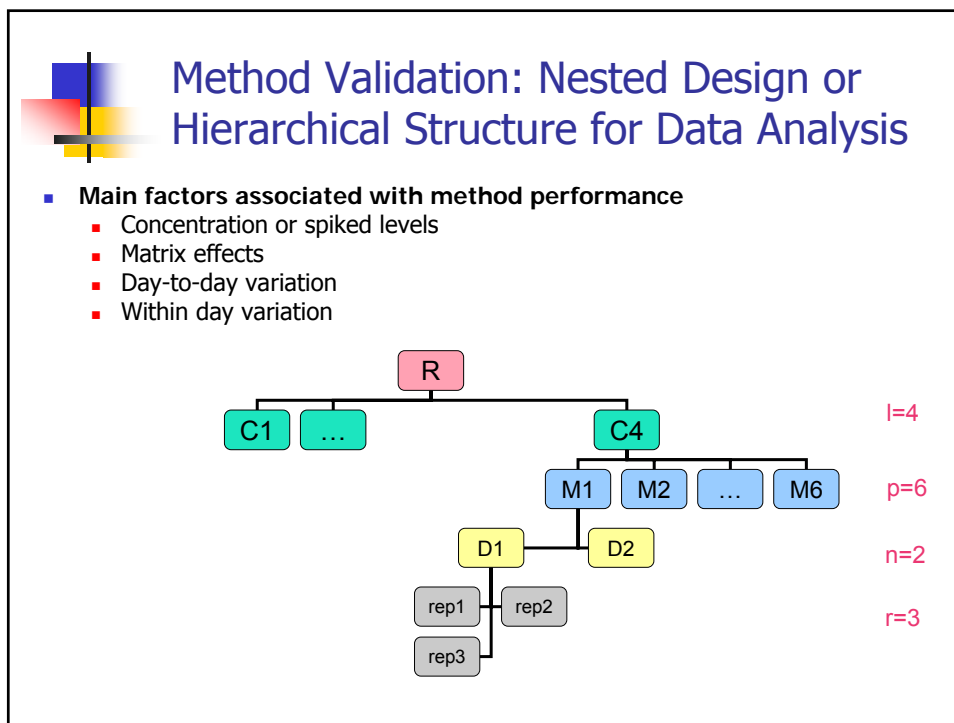
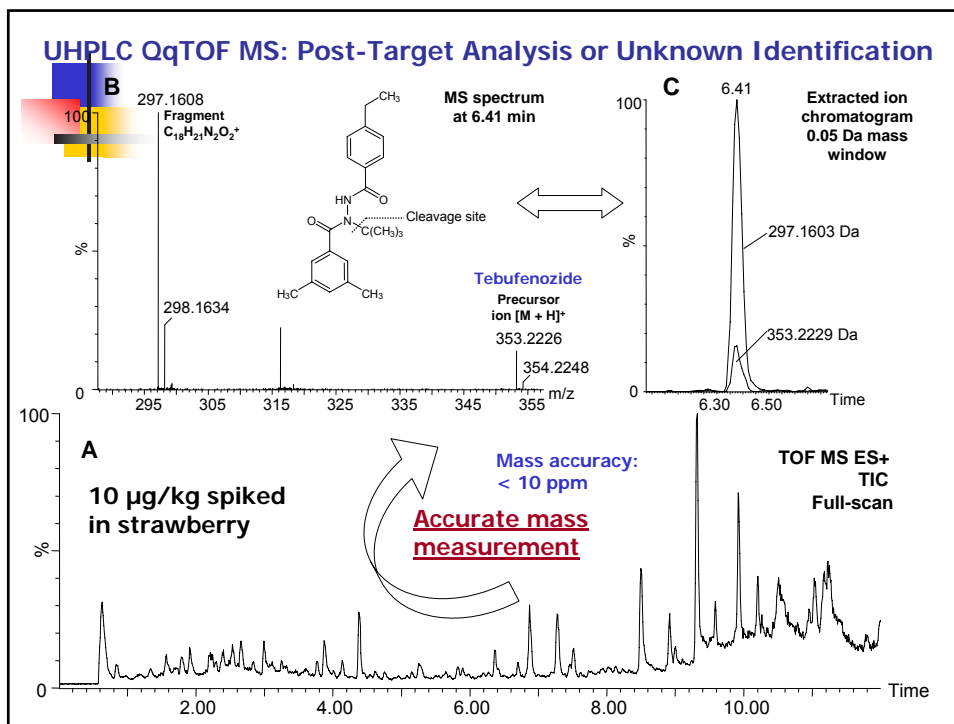




## Instrumentation

- **LC/ESI-MS/MS (MRM)**
  - Agilent 1200 SL and API 5000
    - Atlantis dC18 100 x 2.1 mm, 3µm column
    - Mobile phases: 10 mM ammonium acetate and acetonitrile
    - Injection volume: 5 µL
    - Run-time: 35 min
- **UHPLC QqTOF MS (Full-scan)**
  - Waters UPLC Q ToF Primer
    - Acquity UPLC BEH C18 100 x 2.1 mm, 1.7 µm column
    - Mobile phases: 10 mM ammonium acetate and acetonitrile
    - Injection volume: 10 µL
    - Run-time: 14 min







## Nested Experimental Design: Implementation

- **Matrices (6)**
  - 6 large samples of berries from local stores.  
Matrix-matched calibration with internal standards for quantification
- **Days (2)**
  - processing one matrix per day, spiked at 4 levels in triplicate, for 2 days
- **Number of experiments (12)**
  - $2 \times 6 = 12$  (up to 12 days)



## Nested Experimental Design: Implementation (cont.)

- **Number of extractions per experiment or batch**
  - 1 matrix blank split to 6 for six-point calibration
  - 1 reagent blank
  - 1 matrix blank
  - 12 spiked samples (4 levels in triplicate)
- **Total number of spikes per analyte**
  - $2 \times 6 \times 4 \times 3 = 144$



## Method Performance

- Accuracy: overall or method recovery

$$\bar{R}_m = \frac{\sum_{i=1}^l \bar{R}_i}{l}$$

- Precision: intermediate precision

$$u(R_I)^2 = u(r)^2 + u(D)^2 \quad u(P) = \frac{u(R_I)}{\bar{R}_m}$$



## Measurement Uncertainty

- Combined uncertainty based on **Law of Error Propagation**

$$u(X_{a.i}) = \frac{1}{\bar{R}_m} \sqrt{\frac{x_{a.i}^2 \times u(R_I)^2}{\bar{R}_m^2} + x_{a.i}^2 \times u(R)^2}$$

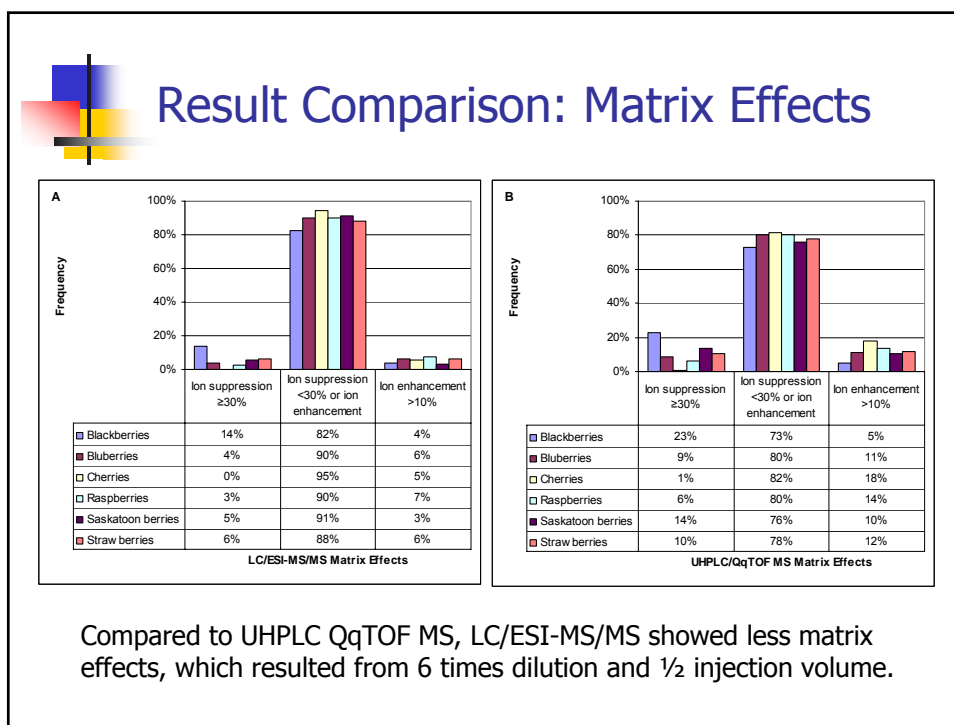
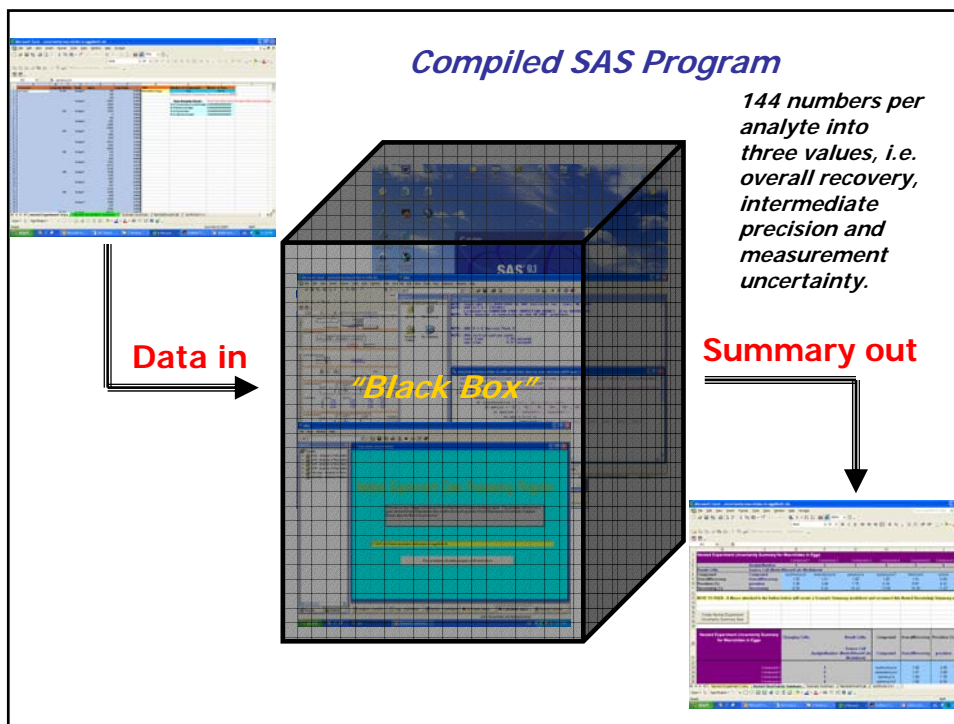
- Expanded uncertainty

$$U = k \times u(X_{a.i})$$

*k*: coverage factor

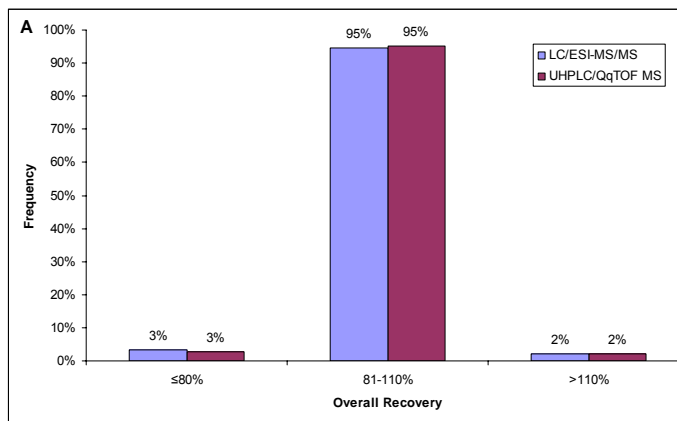
*k* = 2, for 95% confidence

*k* = 3, for 99% confidence





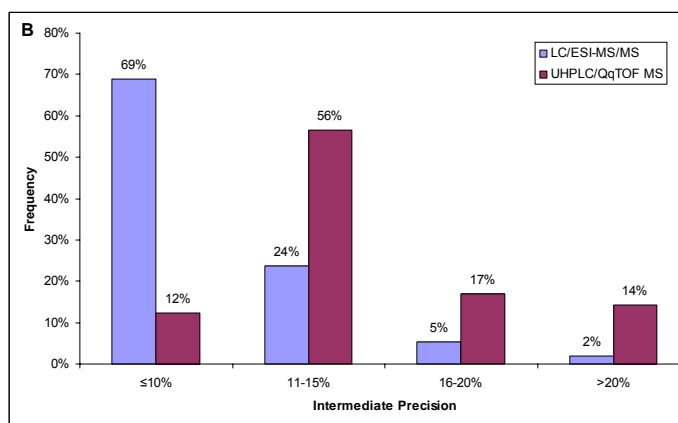
## Result Comparison: Overall Recovery



Matrix-matched standard calibration with internal standards improved the method accuracy.



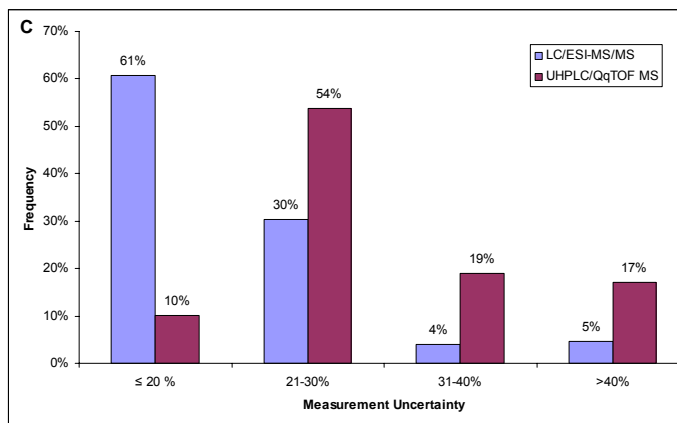
## Result Comparison: Intermediate Precision



LC/ESI-MS/MS demonstrated much better repeatability than UHPLC QqTOF MS.



## Result Comparison: Measurement Uncertainty



Compared to LC/ESI-MS/MS, UHPLC QqTOF MS showed larger measurement uncertainty, which resulted from its poor repeatability.



## Result Comparison: Sensitivity

- LC/ESI-MS/MS is much more sensitive than UHPLC QqTOF MS.
- LC/ESI-MS/MS
  - 2 out of 148 (1.4 %) not detected with S/N >3 at 5 µg/kg (0.8 µg/L in vial).
    - Aclonifen and chlorthiamid
- UHPLC QqTOF MS
  - 18 out of 147 (12.2 %) not detected with S/N >3 at 5 µg/kg (5.0 µg/L in vial).
    - Aclonifen, benoxacor, chlorbromuron, chlorthiamid, cyanofenphos, diclocymet, haloxyfop, isocarbamide, linuron, metolcarb, molinate, oxamyl-oxime, prodiamine, pyridalyl, quizalofop, thiofanox, tolylfluanid and zinophos



## Pros and Cons: Complementary

- **LC/ESI-MS/MS**
  - Pre-target analysis (MRM): parameter optimized for each individual
  - Ideal for quantification with good linearity, repeatability and sensitivity
  - Less matrix effects
- **UHPLC QqTOF MS**
  - Generic instrumental settings (full-scan): easy for method development.
  - Flexibility: post-target analysis
  - Unknown and fragments identification
  - Data storage: 20 to 30 MB for a 14 min run.



## Acknowledgements

Daniel Leung  
Willis Chow  
Calgary Laboratory  
Canadian Food Inspection Agency