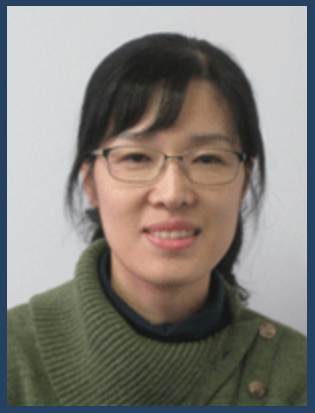
Sample Preparation and Matrix Effects in the Detection of Chemical Residues in Foods

Steven J. Lehotay, HyeYoung Kwon, and Lucía Geis-Asteggiante

USDA UNITED STATES DEPARTMENT OF AGRICULTURE

ARS Agricultural Research Service



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Presentation Outline

I. QuEChERS & Update

II. Matrix Effects (GC and LC-API-MS)

III. Pesticide Experiments & Results

IV. Veterinary Drug Residue Results

V. Conclusions

Multiclass, Multiresidue Analytical Approach:

- extraction
- clean-up

} QuEChERS method

Quick
Easy
Cheap
Effective
Rugged
Safe

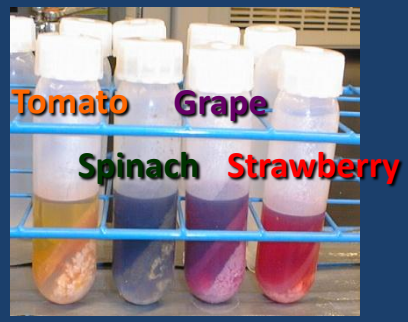
- quantification
- identification

} GC-MS
LC-MS

Slide Devised by Katerina Mastovska

QuEChERS Approach

1) Shake sample with solvent and salts



2) Centrifuge for 1 min

3) Mix a portion with a sorbent

4) Centrifuge for 1 min

5) Analyze Pesticides

Different QuEChERS Methods

2003 Anastassiades *et al.*

Original

10-15 g sub sample

↓

10-15 mL **MeCN**

↓ shake

0.4 g/mL anh.$MgSO_4$
0.1 g/mL NaCl

↓ shake

↓ centrifuge

150 mg/mL anh.$MgSO_4$
25 mg/mL PSA

↓

shake & centrifuge

Option:
+ 50 mg C_{18} & 7.5 mg GCB

2005 Lehotay *et al.*

AOAC 2007.01

10-15 g sub sample

↓

10-15 mL
1% HOAc in MeCN

↓ shake

0.4 g/mL anh.$MgSO_4$
0.1 g/mL **NaOAc**

↓ shake

↓ centrifuge

150 mg/mL anh.$MgSO_4$
50 mg/mL PSA

↓

shake & centrifuge

Option:
+ 50 mg C_{18} & 2.5-7.5 mg GCB

2007 Anastassiades *et al.*

CEN 15662

10-15 g sub sample

↓

10-15 mL **MeCN**

↓ shake

0.4 g/mL anh.$MgSO_4$
0.1 g/mL NaCl
0.1g/mL **$Na_3Cit•2H_2O$**
0.05 g/mL **$Na_2Cit•1.5H_2O$**

↓ shake

↓ centrifuge

150 mg/mL anh.$MgSO_4$
25 mg/mL PSA

↓

shake & centrifuge

Option: Scale-Up & Conc. in Toluene

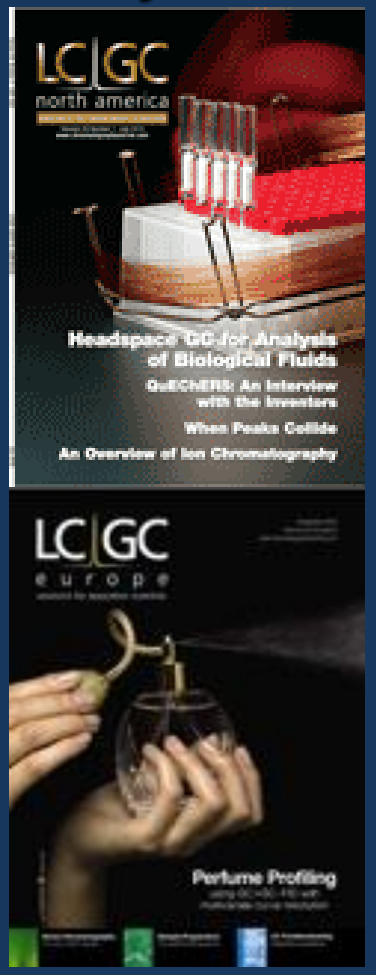
Slide Devised by Urairat Koesukwiwat

QuEChERS Update

Steve and Angelo “Interviewed” by Ron Majors

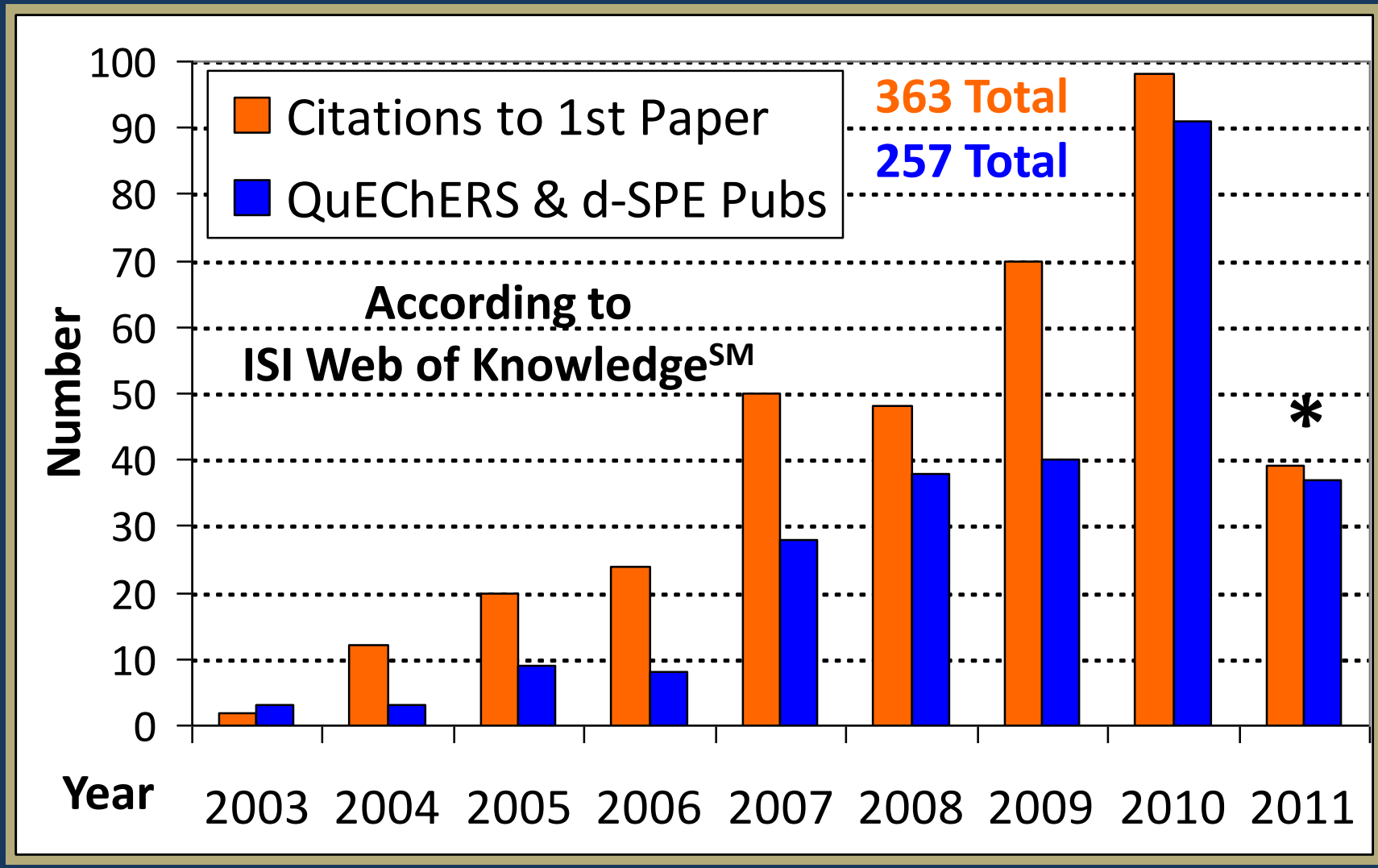
QuEChERS a Sample Preparation Technique that is “Catching On”: An Up-to-Date Interview with the Inventors, *LC GC North America*, **July, 2010**

The QuEChERS Revolution, *LC GC Europe*, **Sept., 2010**



Available from ChromatographyOnline.com

QuEChERS in the Literature

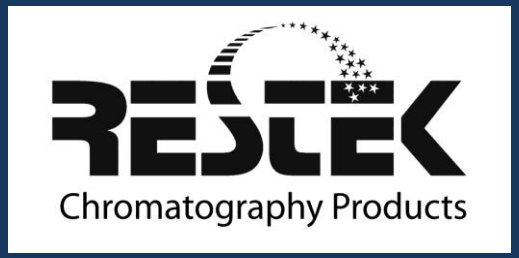


*search conducted on May 3, 2011

What's New with QuEChERS?

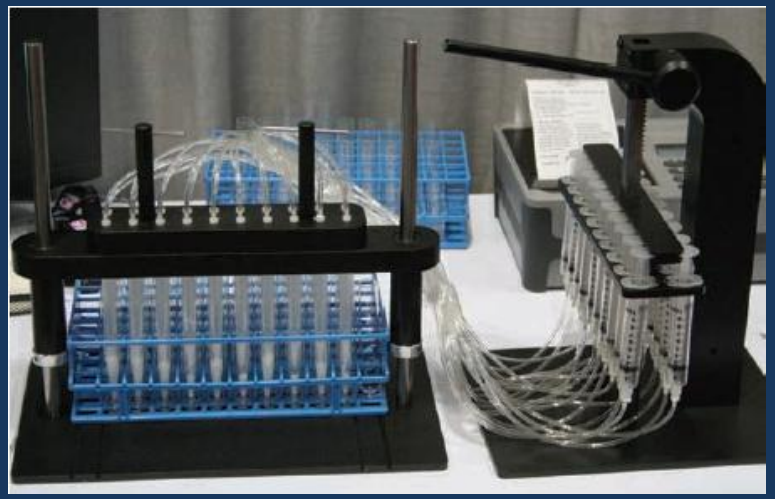
- More vendors and formats (e.g. DPX)
- GCB or ChloroFiltr for chlorophyll reduction
- Type of $MgSO_4$: can use 97% purity
- Shakers – *e.g.* Spex and Glas-Col
- Automation with robotic autosampler
- "Unified" method to undergo AOAC update
- d-SPE has a life of its own
- More applications (*e.g.* PAHs)
- Veterinary drug residue methods

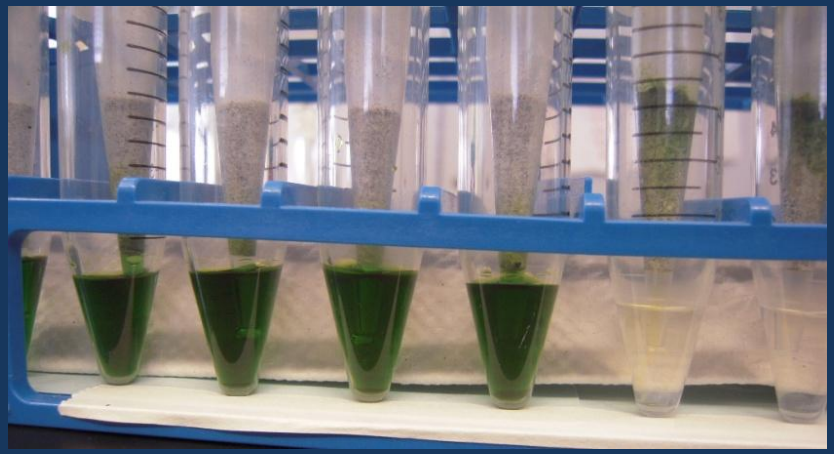
Vendors of QuEChERS Products



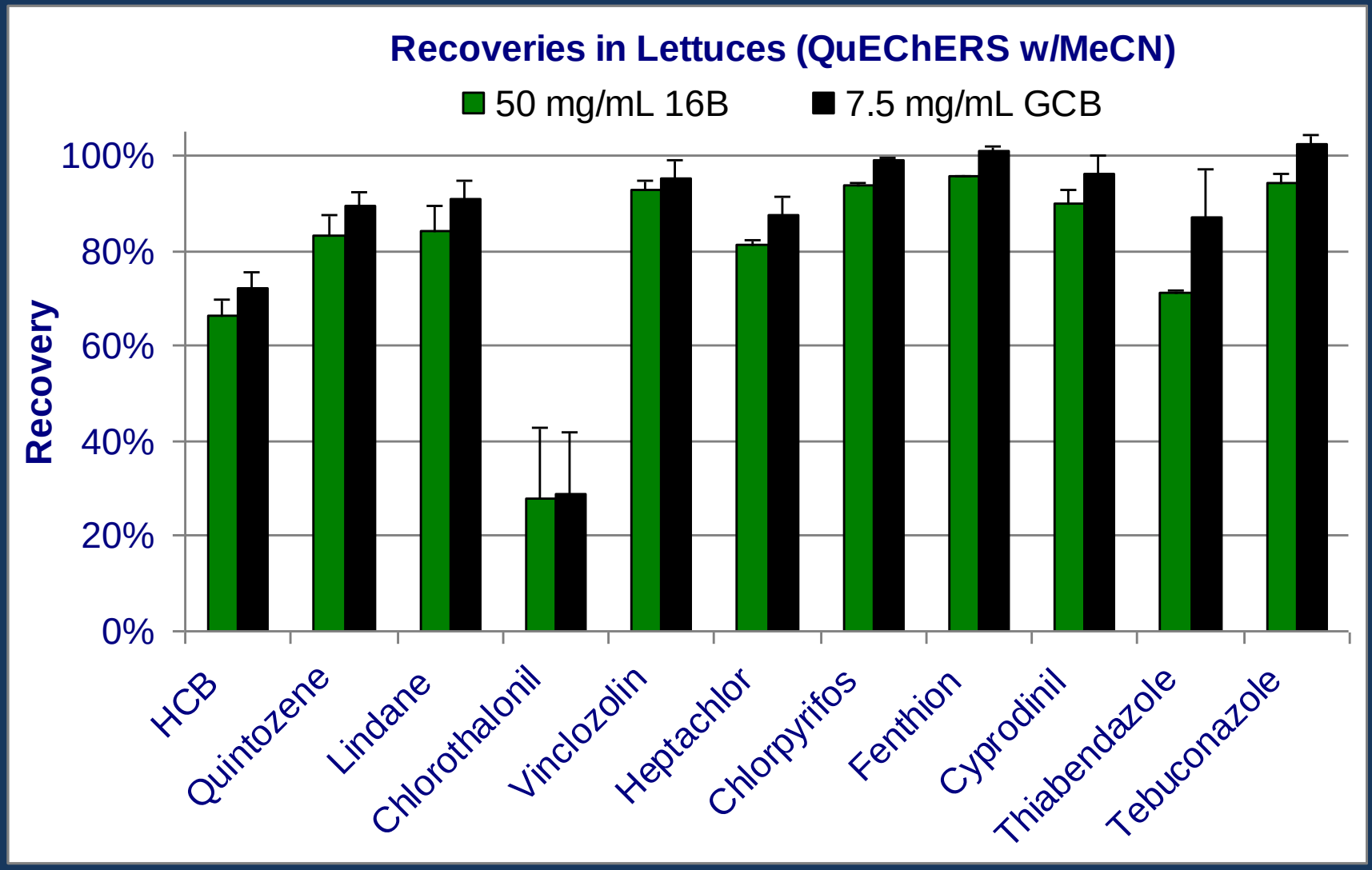
Disposable Pipette Extraction (DPX)

Patented in 2003 by William Brewer, University of South Carolina





Comparison of ChloroFiltr (16B) and GCB



Unified QuEChERS Method

extraction

1 g sample per 1 mL of MeCN w/ 1% HOAc
for fruits and vegetables

add internal standard

per g sample, add 0.4 g anh. $MgSO_4$
+ 0.1 g anh. NaOAc
shake or blend

centrifuge

(dispersive) SPE clean-up

per mL of the upper layer:
150 mg $MgSO_4$ + 50 mg PSA
+ 50 mg C18 + 7.5 mg GCB
mix and centrifuge

QuEChERS for Grains, Nuts, Doughs

extraction

2.5 - 5 g sample + 10 mL water*
+ 10 mL MeCN + internal standards

shake for 1 h

add 4 g $MgSO_4$ + 1 g NaCl
shake vigorously for 1 min

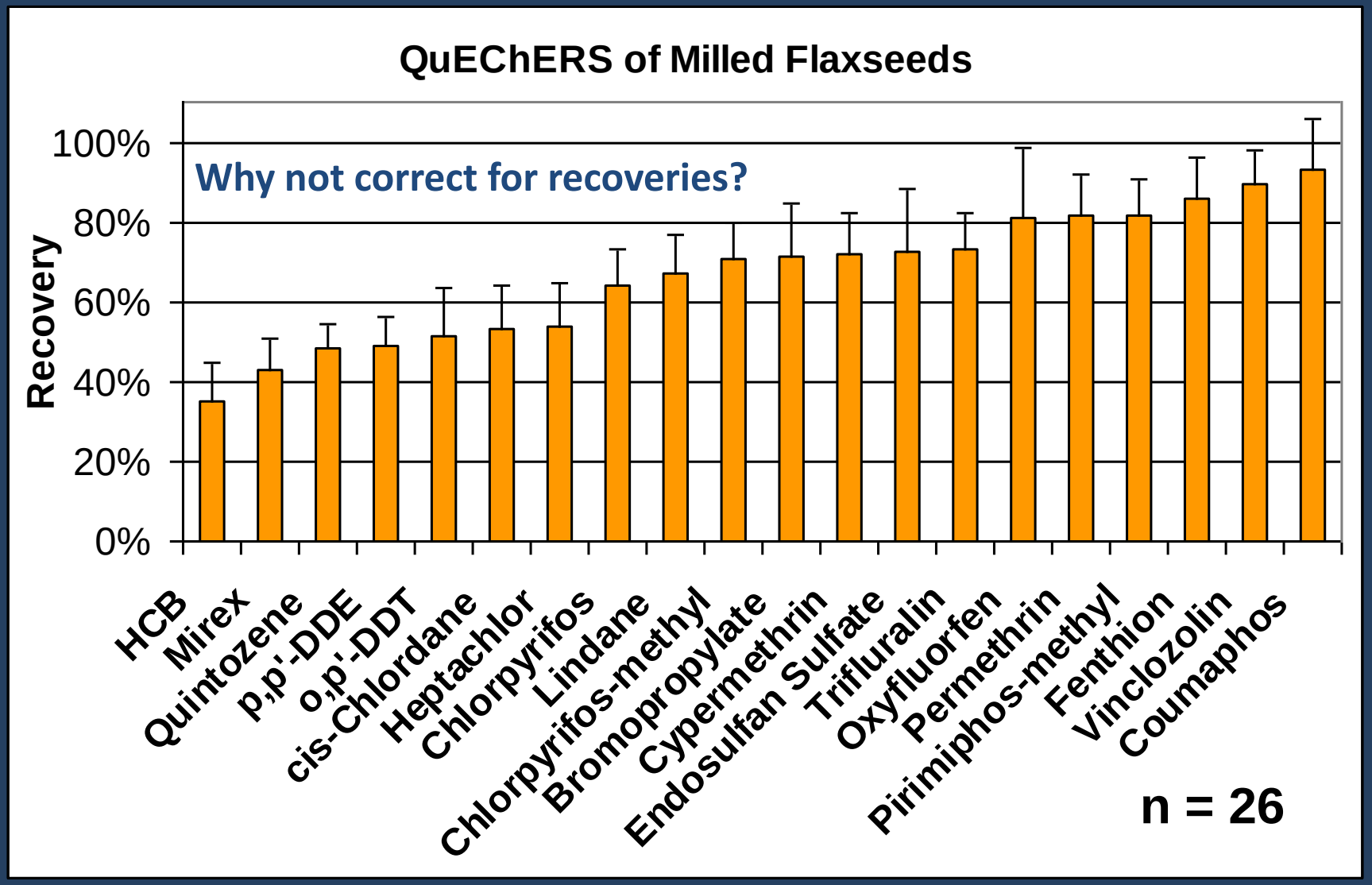
centrifuge for 1 min

*15 mL water for 5 g of rice

dispersive SPE clean-up

1 mL of the upper layer
+ 150 mg PSA + 50 mg C18 + 150 mg $MgSO_4$
mix for 30 s
centrifuge for 1 min

6 Data Sets from 3 Types of Flaxseeds



Biggest Problem with LC- and GC- MS(/MS)

Matrix Effects

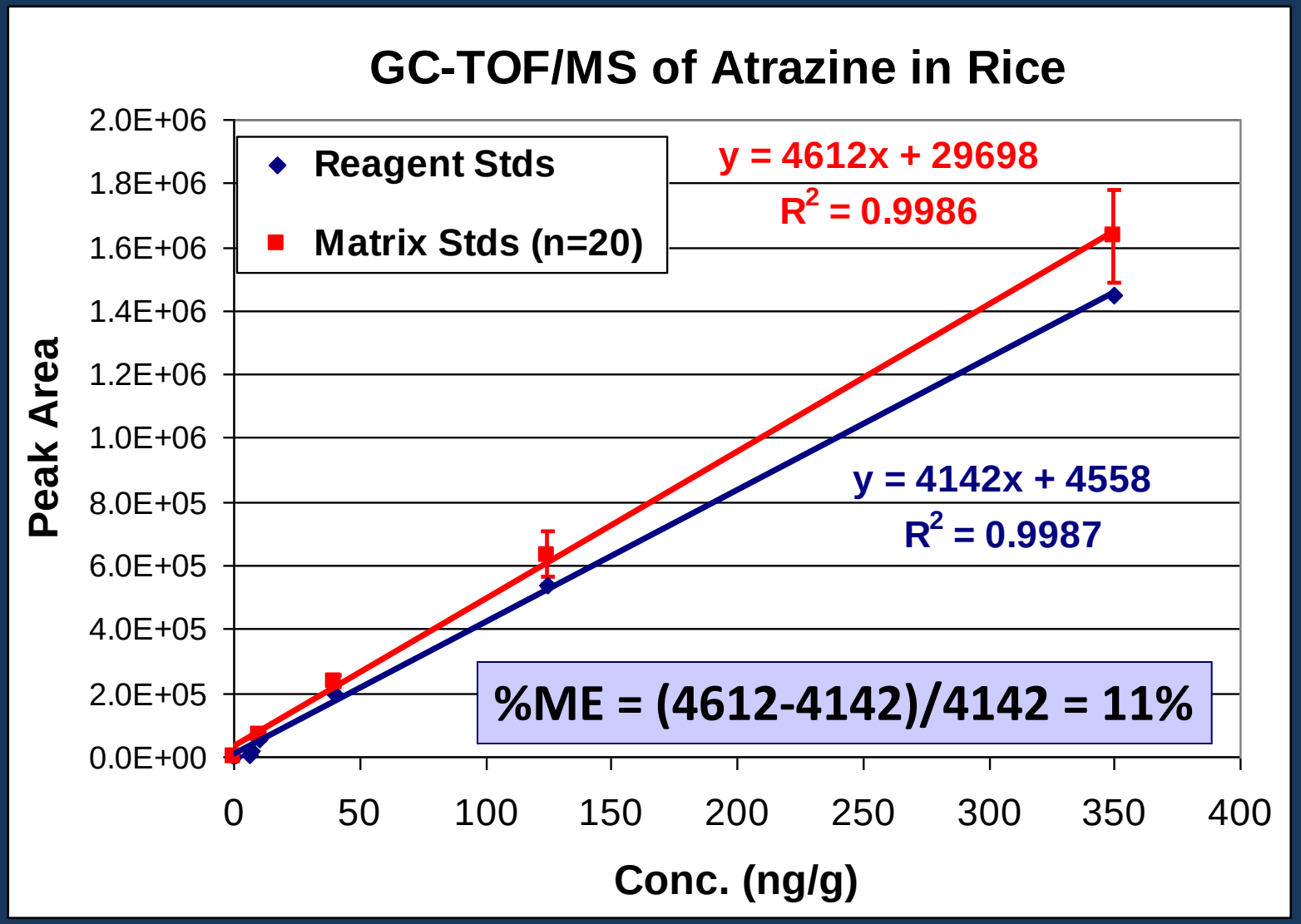
plus costs to purchase and maintain,
and facility requirements, and downtime,
and need for more expertise due to
greater complexity

It is still a pain!

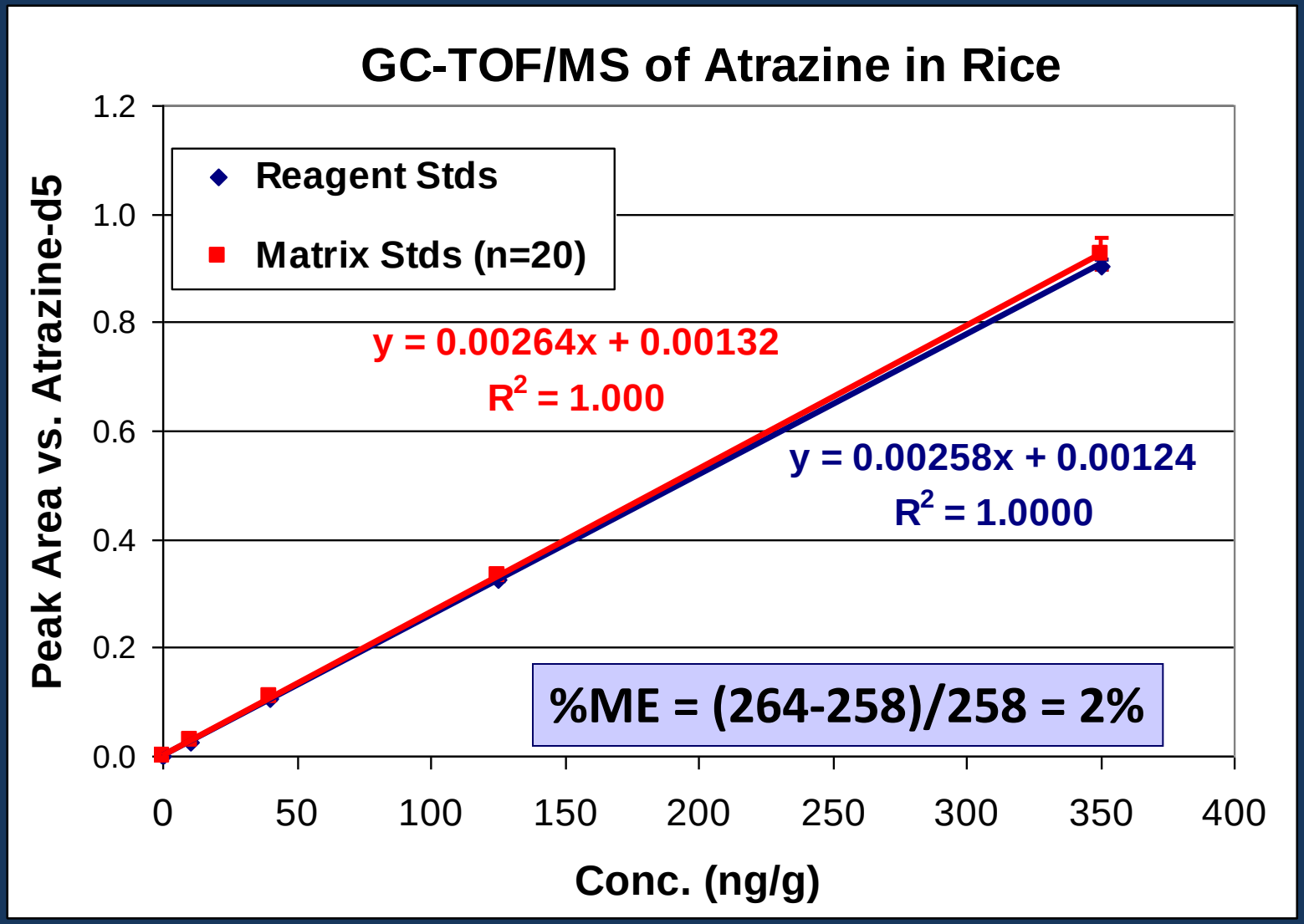
Experiment to Assess Matrix Effects

- 33 LC- and/or GC- amenable pesticides
- 4 matrices (apple, orange, spinach, and rices)
- 20 different sources of each commodity
- Calibration standards from 10-350 ng/g in each commodity/source and reagent-only
- Analyte protectants added to GC standards
- Analytical sequences conducted on API-3000 LC-(ESI$^+$)-MS/MS and LP-GC/ToF-MS (10 µL PTV)
- Matrix effects calculated (vs. I.S. and not)

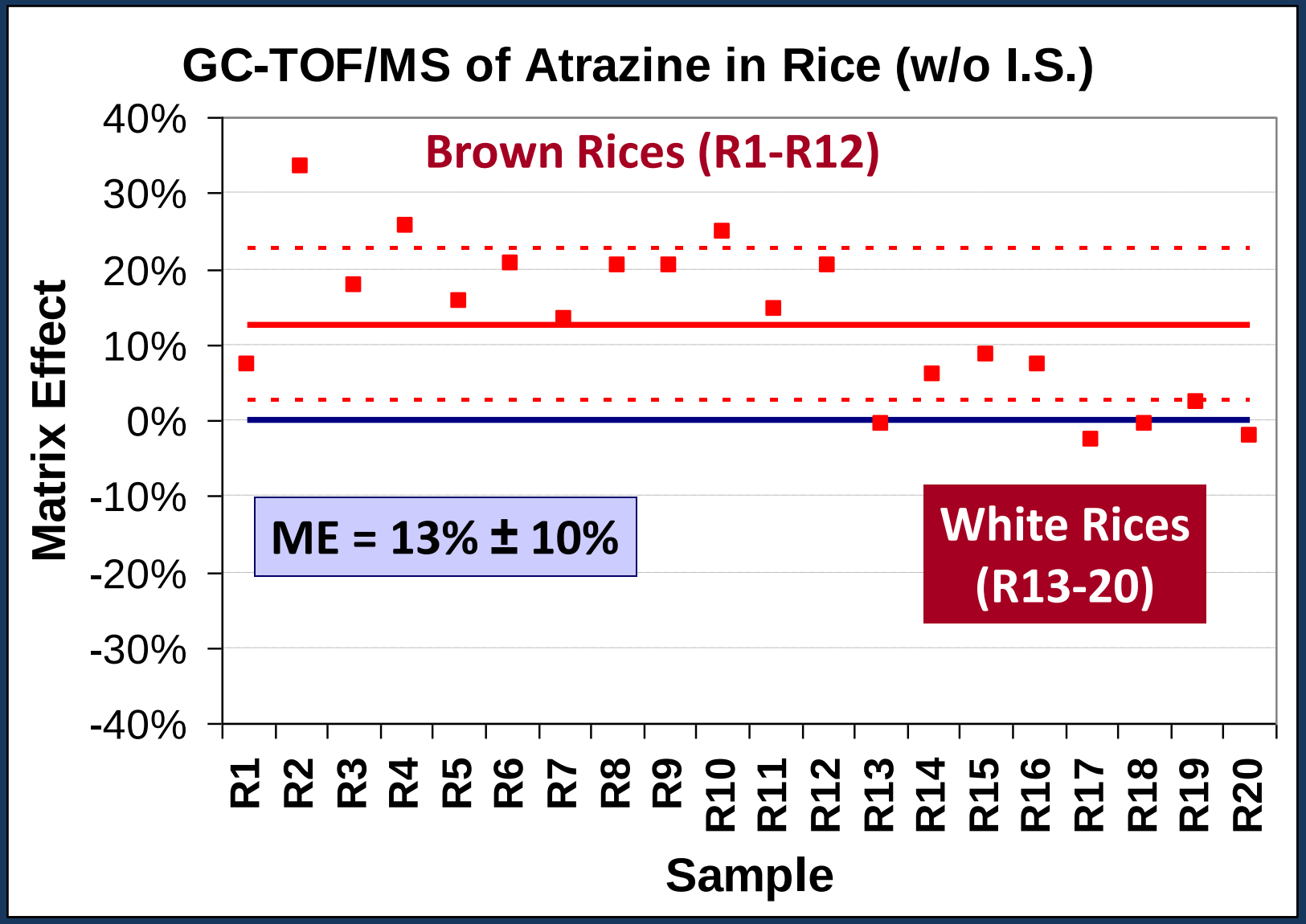
How to Calculate (Estimate) Matrix Effects



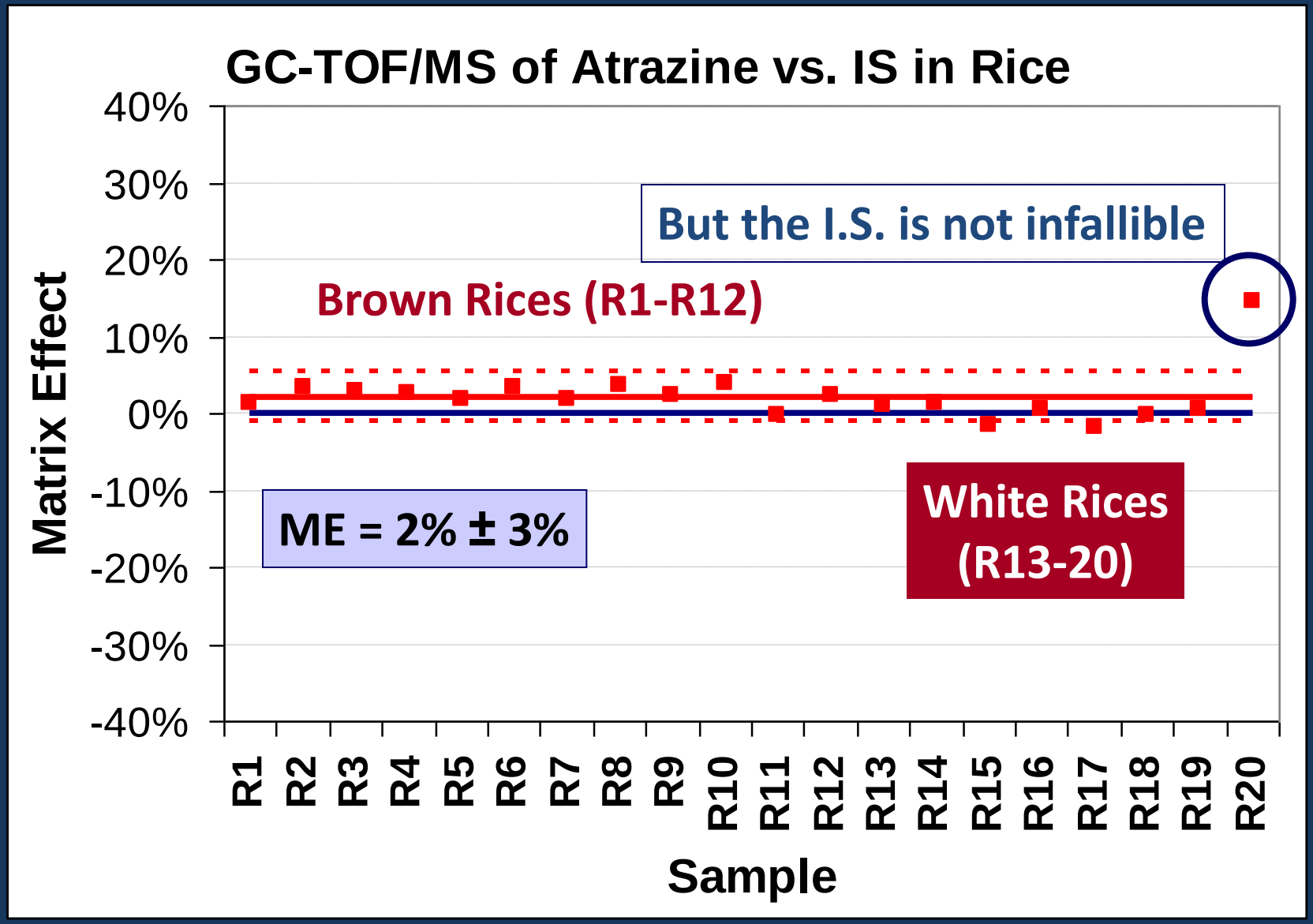
Isotopically-Labeled Internal Standard is Ideal



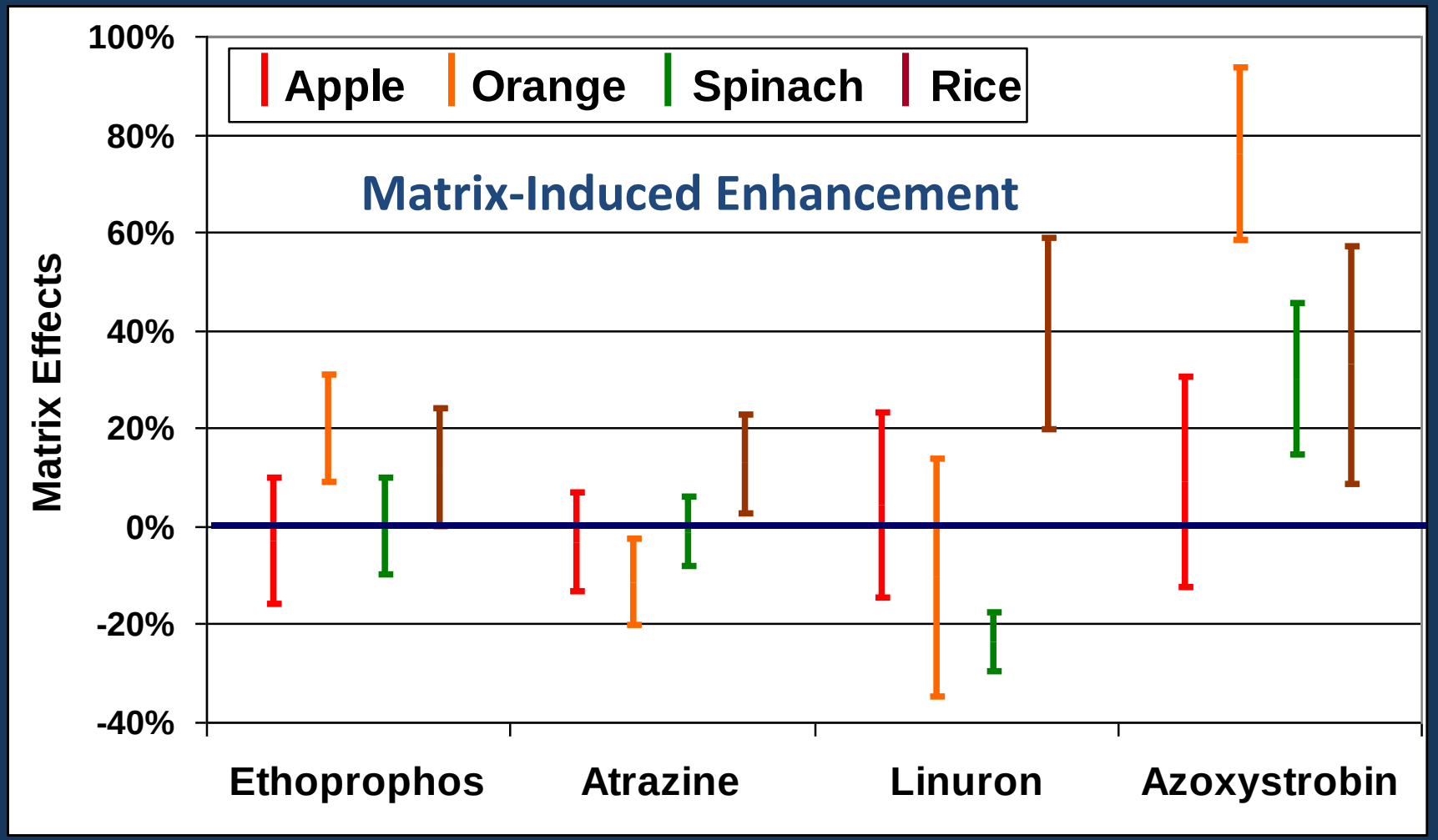
Alternative Calculation Method



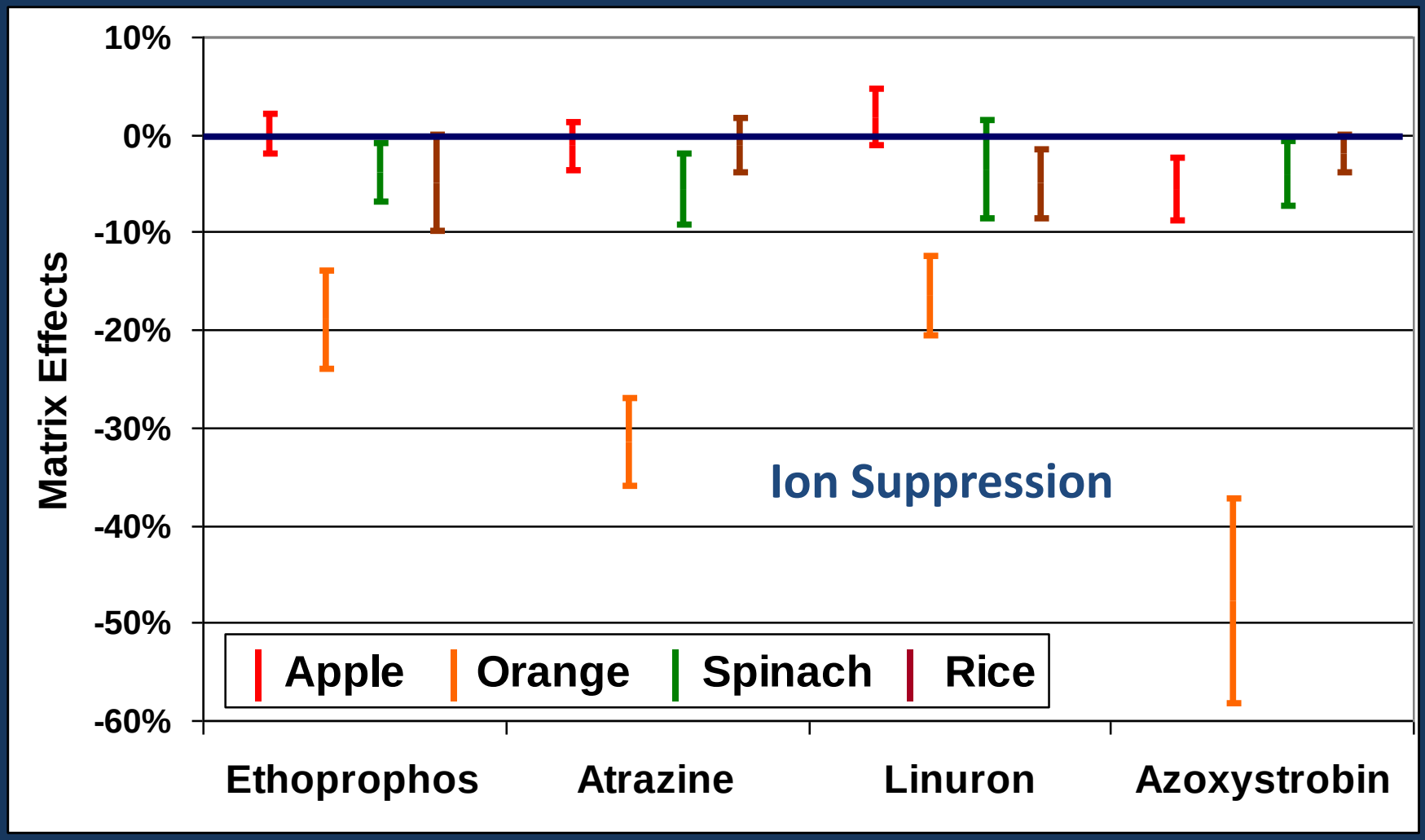
Effect of Isotopically-Labeled IS



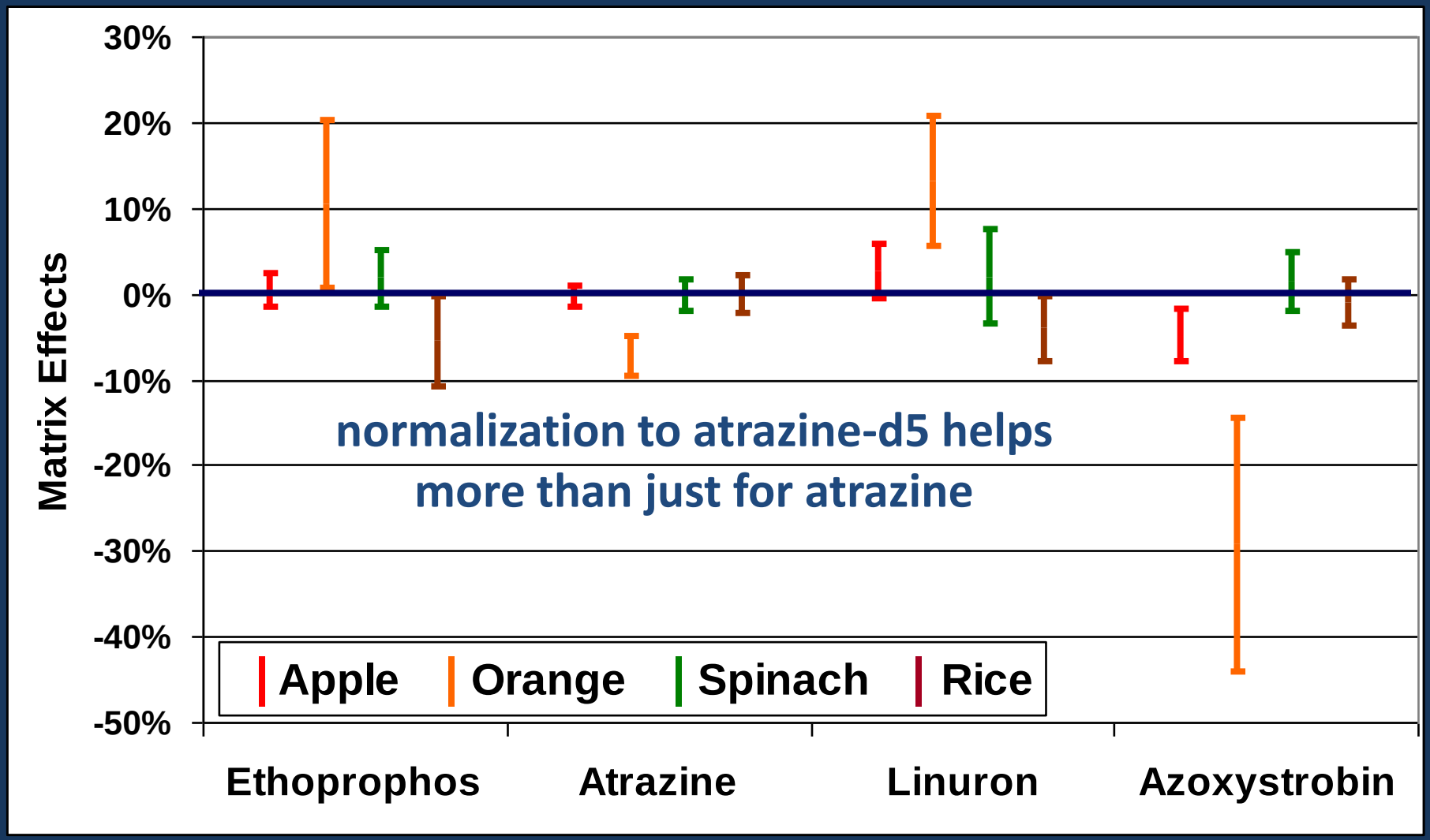
Results for LP-GC/ToF (w/o I.S.)



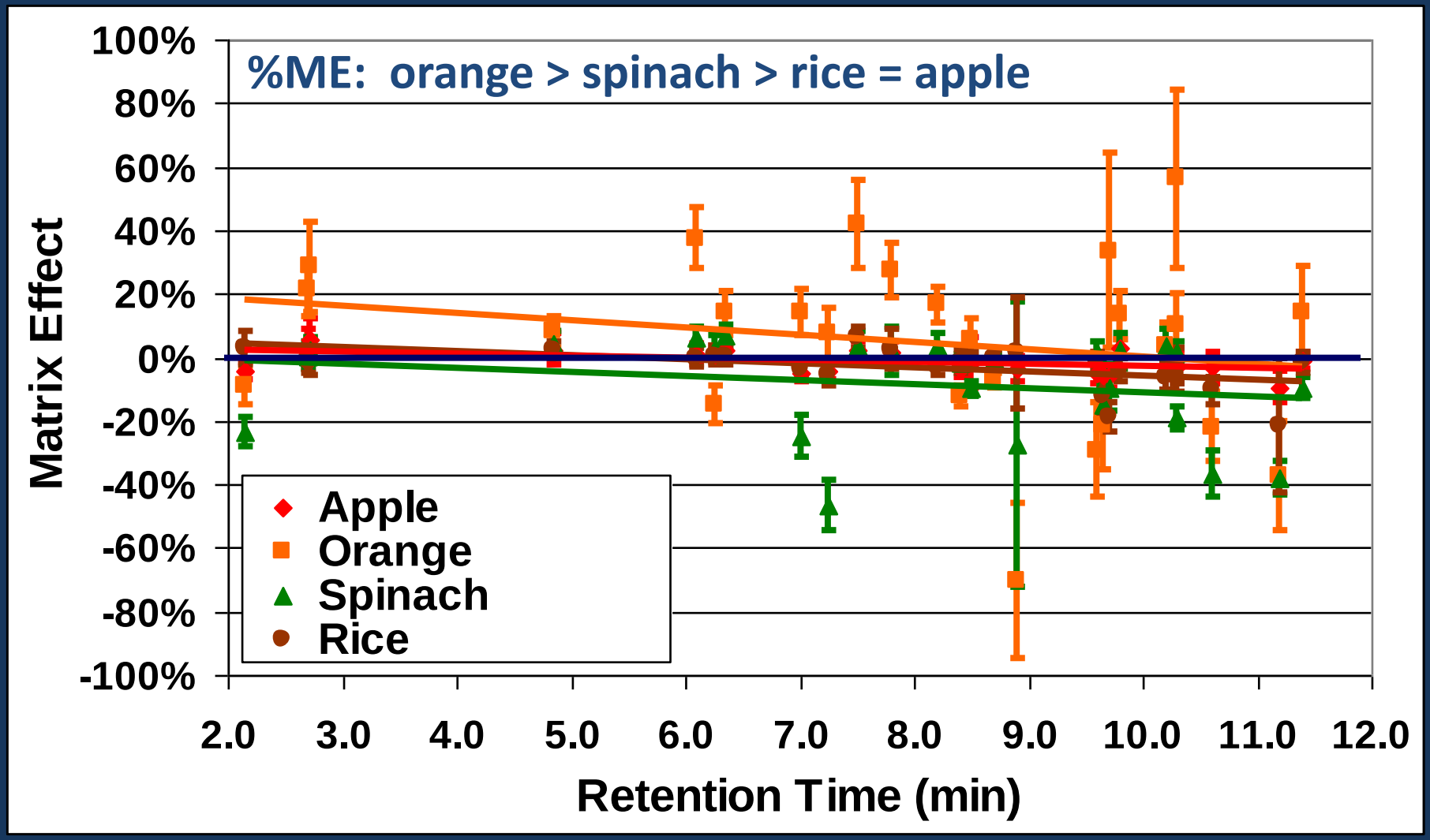
Results for LC-MS/MS (w/o I.S.)



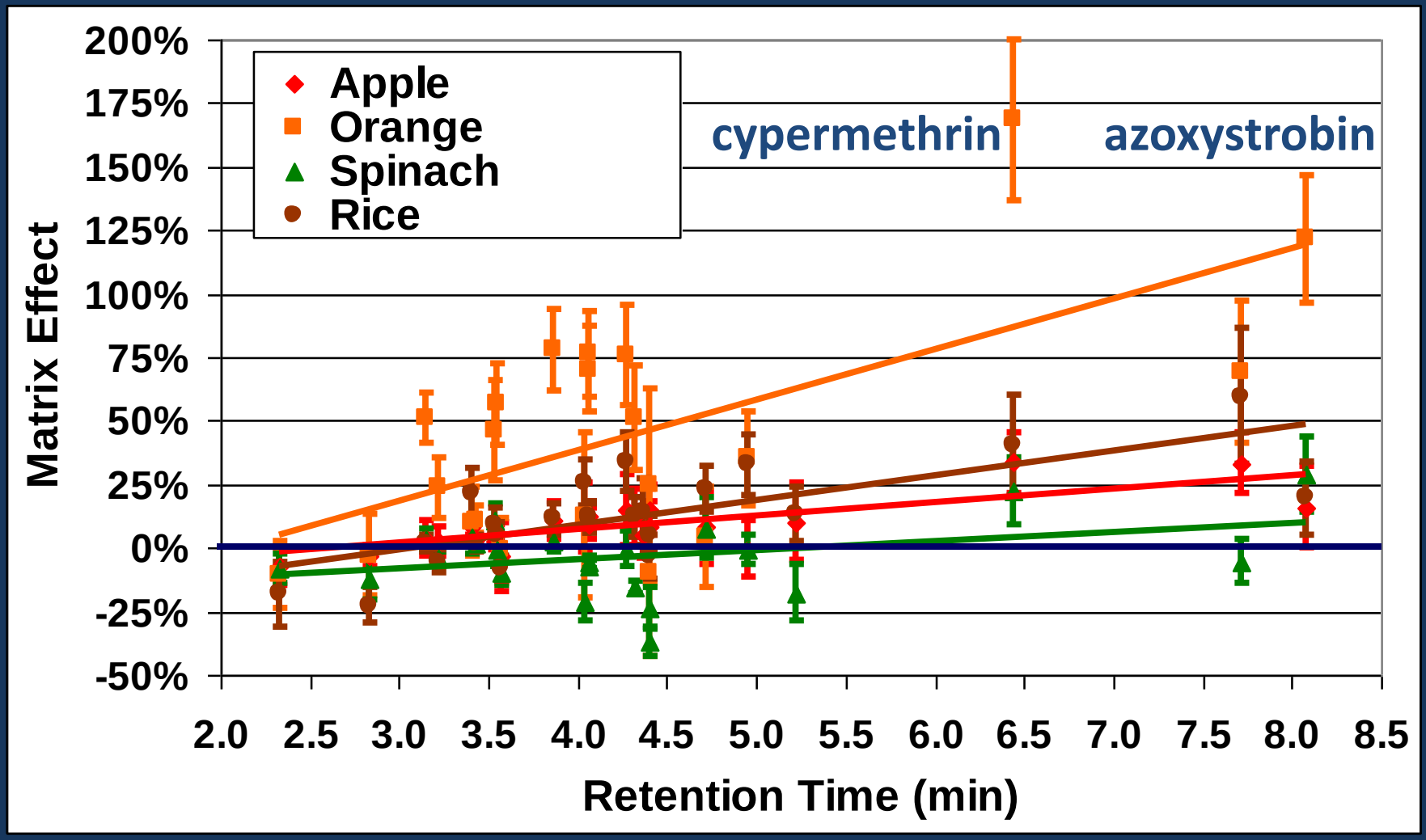
Results for LC-MS/MS (w/ I.S.)



Results for LC-MS/MS (w/ I.S.)



Results for LP-GC/ToF (w/ I.S.)

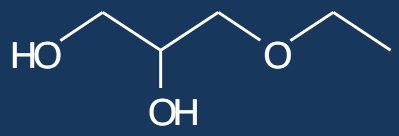


%ME: orange >> rice > spinach = apple

Analyte Protectants

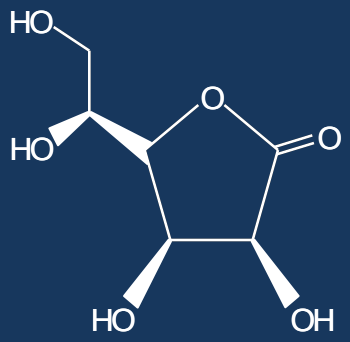
Strongly interact with active sites in GC system (inlet, column and ion source) to decrease degradation and adsorption of co-injected analytes.

Sharper peaks, less tailing, more ruggedness, lower LOD



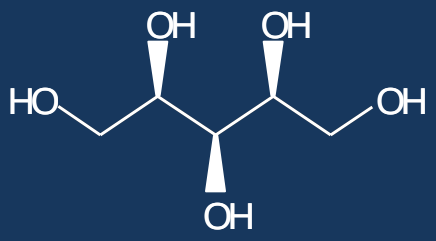
ethylglycerol

1 mg/mL



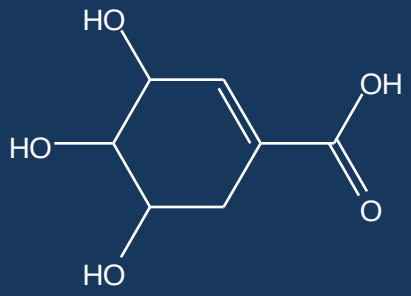
gulonolactone

0.1 mg/mL



sorbitol

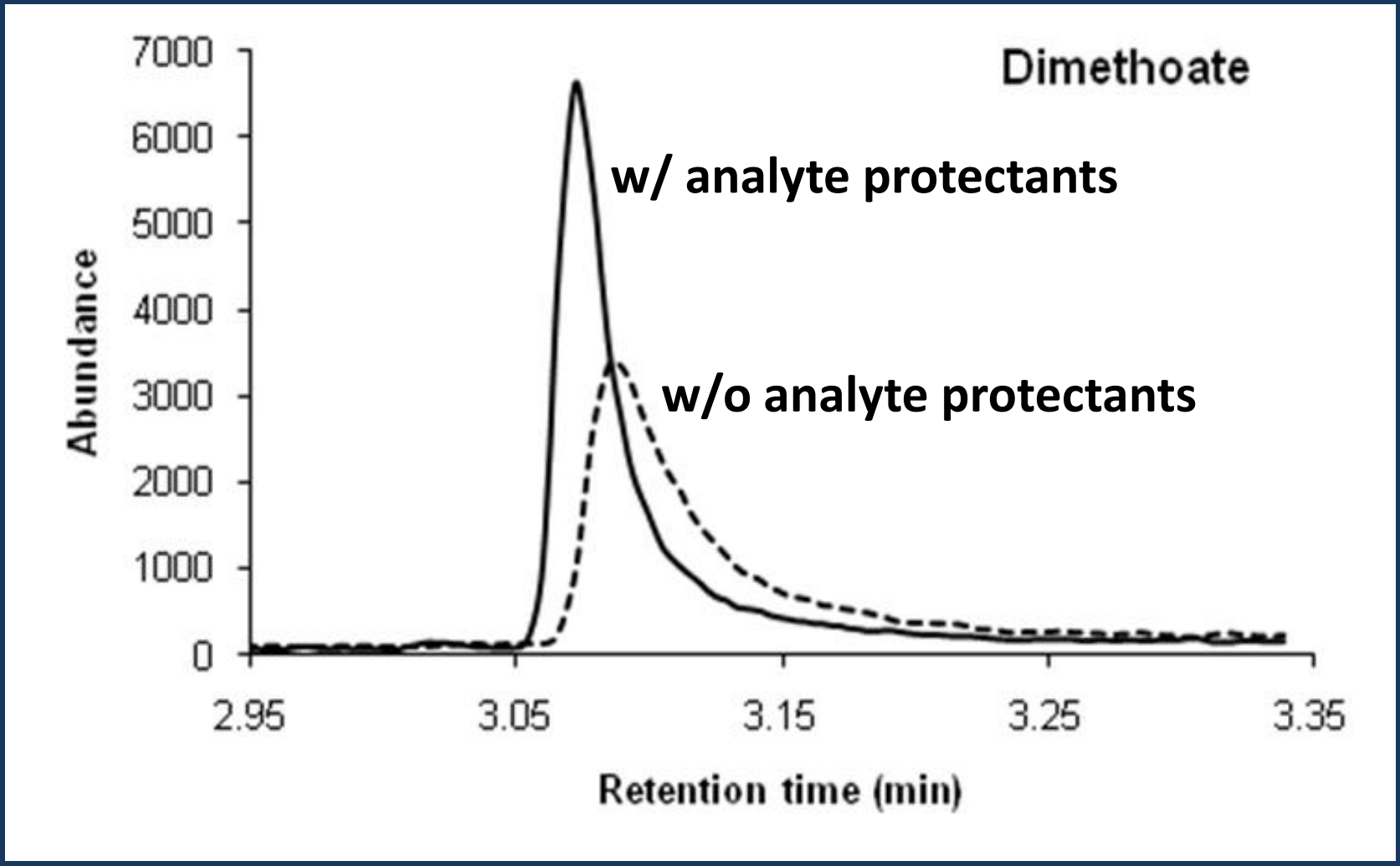
0.1 mg/mL



shikimic acid

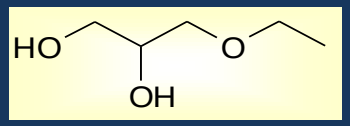
0.05 mg/mL

Effect of Analyte Protectants



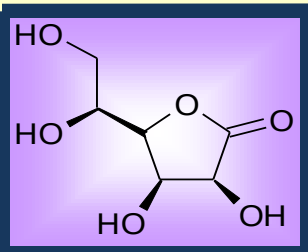
Anastassiades, Maštovská, Lehotay, *J. Chromatogr. A*, 1015, 163-184 (2003)

Combination of Analyte Protectants for GC Pesticide Residue Analysis

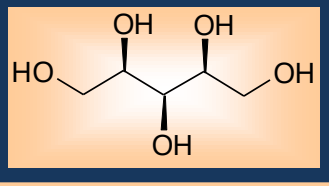


ethylglycerol (10 μg)

Signal enhancement:
- moderate
- strong



gulonolactone (1 μg)



sorbitol (1 μg)

dichlorvos
o-phenylphenol
dimethoate
heptachlor
cyprodinil
imazalil
endosulfan-SO4
coumaphos
deltamethrin

← retention time →

K. Mastovska, S.J. Lehotay, M. Anastassiades, *Anal. Chem.*, 77, 8129-8137 (2005)

Conclusions of Pesticides Study

- Matrix effects aren't so bad in QuEChERS with LC- and GC- MS(/MS) analyses, but worse in citrus
- In terms of matrix effects, one apple is much like another, and oranges are alike, too, but apples aren't like oranges, they're like plums, *etc.*
- Analyte protectants in GC improve results, but matrix-matching still needed for late-eluters, especially in citrus.
- Isotopically-labeled internal standards work best to overcome matrix effects, but not perfectly, and they even help reduce effects for other analytes.

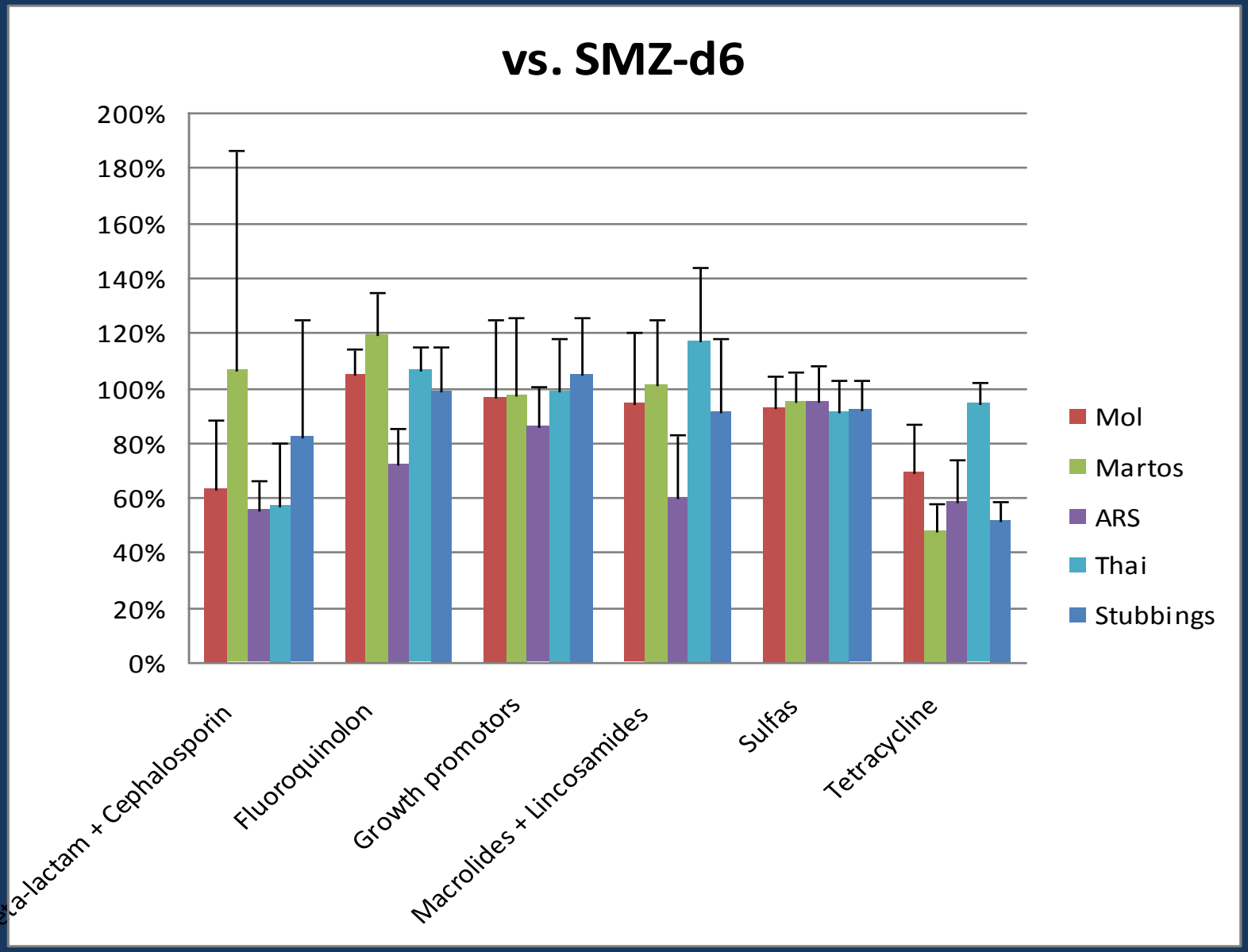
Comparison of 6 Vet. Drug Methods

1) Mol *et al.* (Rikilt – The Netherlands)
2) Martos *et al.* (U. Guelph – ON, Canada)
3) Lehotay *et al.* (USDA-ARS – Wyndmoor, PA)
4) Leepipatpiboon *et al.* (Chulalongkorn U., Thailand)
5) Stubbings *et al.* (FERA – York, UK)
6) Kaufmann *et al.* (Switzerland)

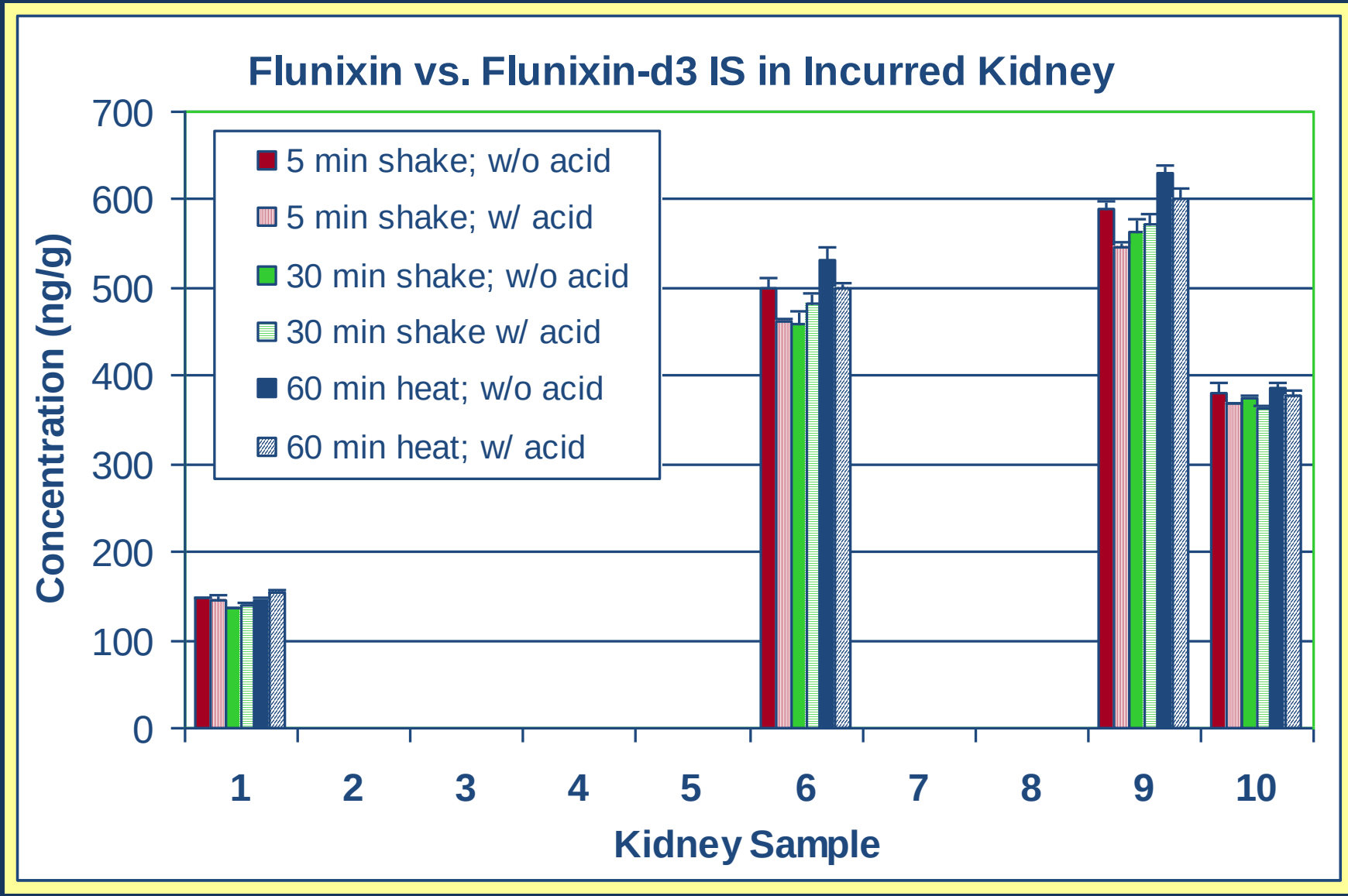
All methods gave similar qualitative MS/MS screening capabilities with nearly all 60 of the analytes meeting identification criteria at ½ "tolerance" level in kidney.

Speed, cost, ease of use and ruggedness become the differentiating aspects.

60 Vet. Drugs in Beef Kidney



Analysis of Incurred Kidney (2 g)



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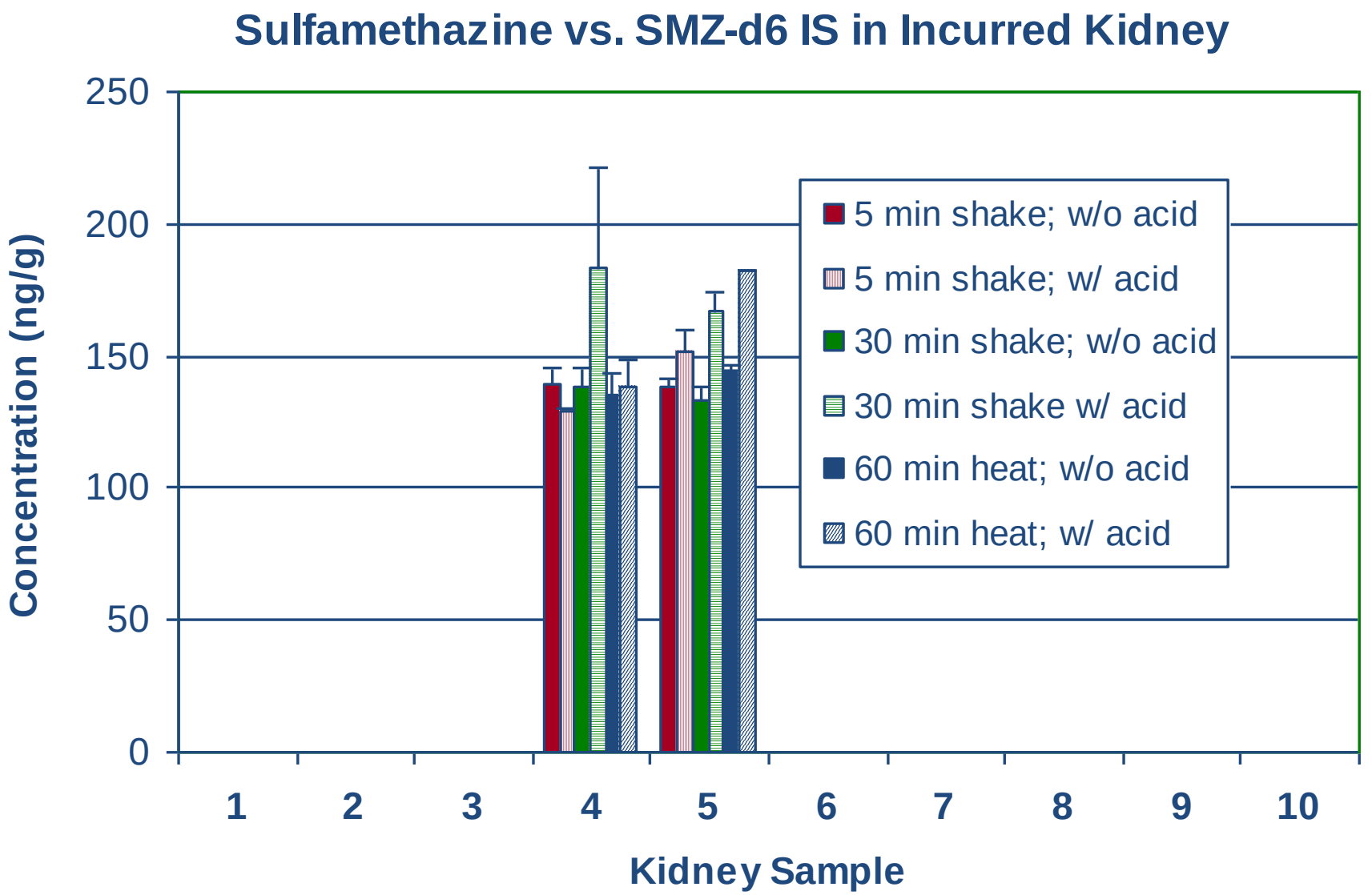

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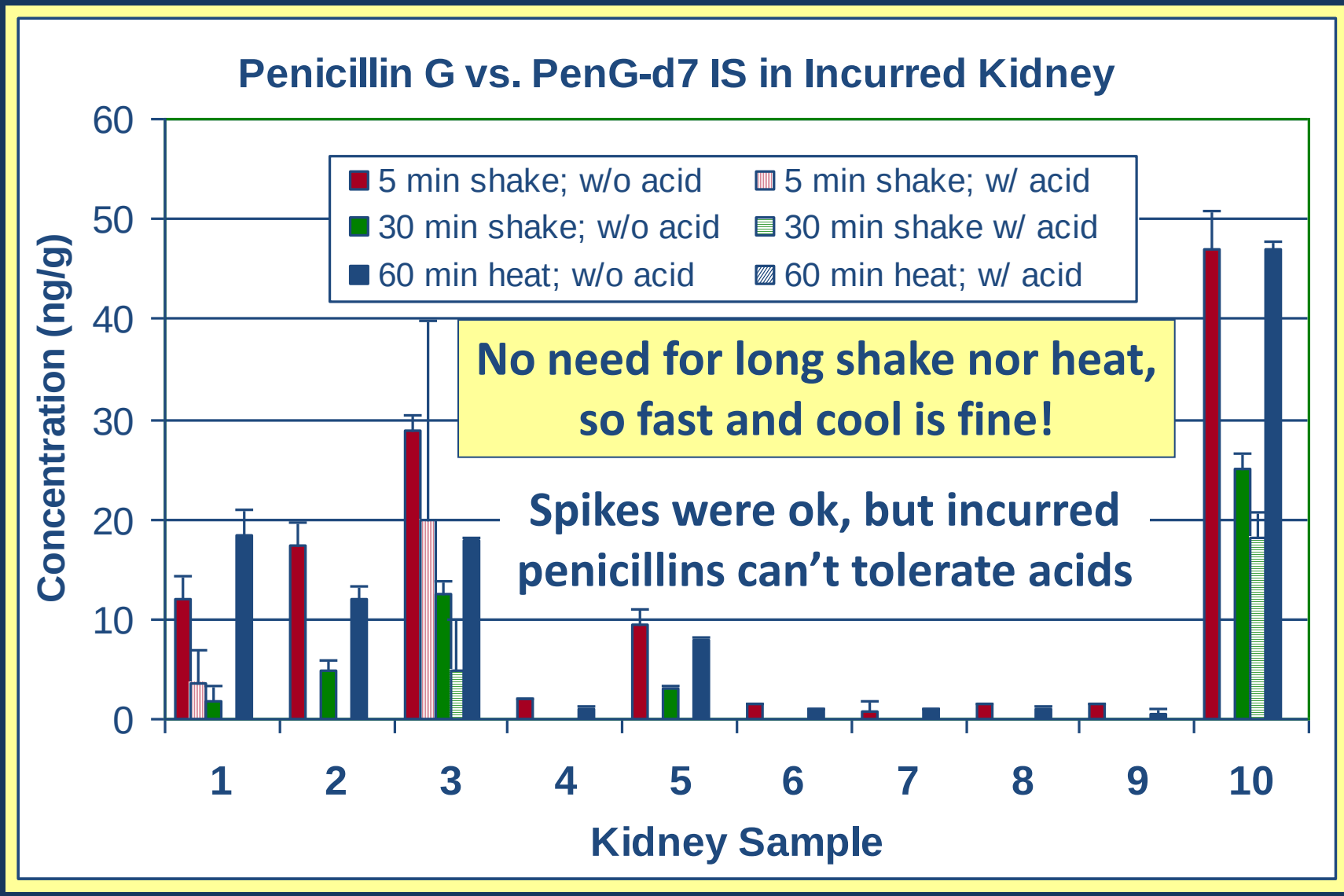


Fast Method for Vet. Drug Residues

clean-up

2 g tissue in a 50 mL tube
add IS mix (SMZ-d6; flunixin-d3; PenG-d7)

add 10 mL of 4/1 (*v/v*) MeCN/water
vortex briefly, shake for 5 min
centrifuge for 5 min >3500 rcf

supernatant + 500 mg C18 + 10 mL hexane sat'd w/MeCN; mix for 30 s, centrifuge for 5 min > 3500 rcf; aspirate hexane to waste

evaporate 5 mL extract to 1 mL final vol.

filter extract with the Mini-UniPrep™

UHPLC-MS/MS analysis

Streamlined Method Validation

Needs:

- Trueness (Recoveries at ≥3 Levels, n > 5)
- Precision (Repeatability & Reproducibility)
- Ruggedness (Multi-day, Multi-Analyst, etc.)
- Selectivity (Interferences in Blanks?)
- Range (calibration and matrix effects)
- Detection limits (MDL, LOD, LOQ, LOI)
- Qualitative (False Negatives/Positives)

Can We Meet All Needs in 3 Days?

3-Day Validation Experiment

Day 1:

- **Analyst 1 in hot Lab, Reagents A, 10 matrix blanks from different sources, 6 spikes at 3 levels each in 6 matrices + 4 spikes each at same levels in mixed matrices (1 in glass tubes); 5-point calibration each in mixed matrix and reagent-only stds; reagent blk = 0-Std inj'd after high std to check for carry-over**

Days 2 and 3:

- **Analysts 2 & 3 in cooler labs repeat using Reagents B & C with different sources of matrices**

Veterinary Drug Residues Conclusions

- The streamlined method has met validation criteria for most drugs in a 3-day validation for qualitative identification screening purposes.
- Sample throughput is 60 samples/day by 1 chemist for UHPLC-MS/MS analysis.
- The method is being implemented for routine monitoring of cattle (so far) by the USDA labs.
- Quantitation is acceptable for ≈75% of the drugs, but enforcement requires 2nd analysis anyway.
- The new streamlined method still needs a cool name.

Acknowledgments

Michelangelo Anastassiades

Katerina Mastovska

Alan Lightfield

Urairat (Oil) Koesukwiwat

Terry Dutko and others at USDA FSIS

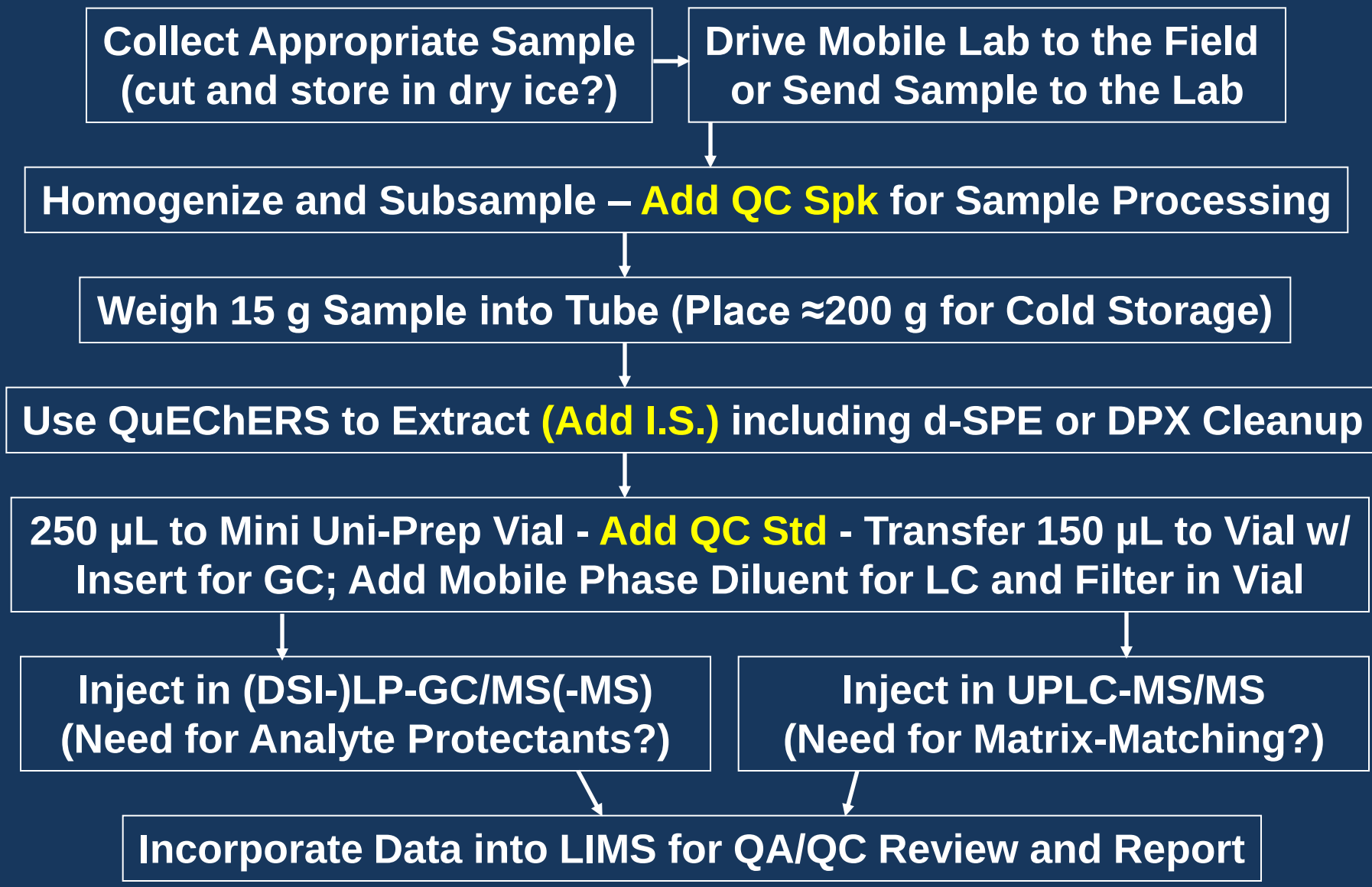
US-Israel Binational Agricultural Research and Development Grant US-4273-09

QuEChERS Baby Picture

This is what happens when two fathers who hate to do dishes have a baby together

Muchas Gracias!

Efficient Pesticide Residue Analysis



Conclusions

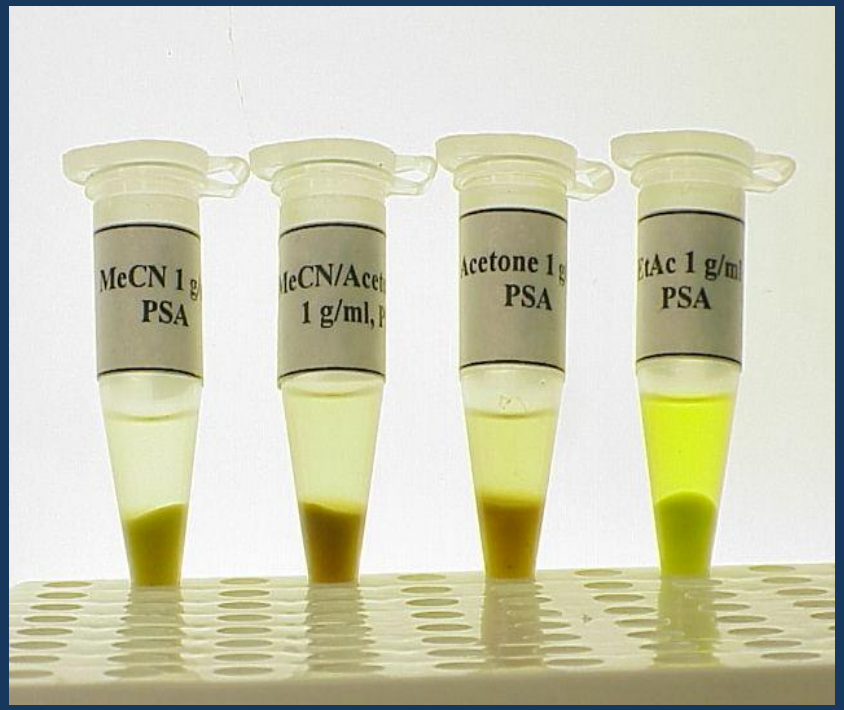
- QuEChERS is a well-proven, fast sample preparation method for hundreds of pesticide residues in different types of food matrices.
- UPLC-MS/MS can provide 10 min analysis of hundreds of LC-amenable pesticides.
- LP-GC/MS can also provide 10 min analysis of hundreds of GC-amenable pesticides.
- Currently, the **HUGE** sample throughput limitation is data processing and review!

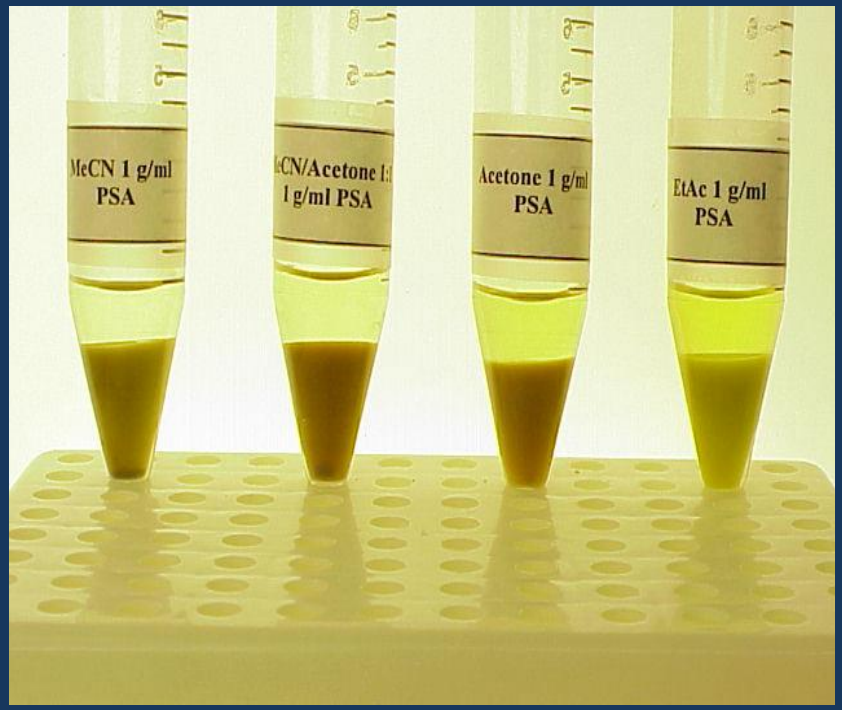
QuEChERS as a Teenager

- QuEChERS is no longer a baby, born of two fathers, it is a teenager influenced by friends, some you can trust and others you can't.
- The QuEChERS approach is still learning its potential and limitations in the big world.
- QuEChERS concepts are easy and fast to try in your application(s) – no big loss if it fails.
- Recovery experiments alone are not enough to validate methods – use incurred samples, proficiency testing, and/or interlab trials.

Dispersive-SPE

- **Why use an SPE apparatus for "chemical filtration?"**
- **Dispersive-SPE involves the mixing of the sorbent with the extract in a mini-centrifuge tube to retain matrix interferants, but not analytes.**





QuEChERS Features and Impact

- A single extract can be prepared in 10 min or a batch of 20 in an hr by a single analyst with ≈$1-3 of disposable materials per sample and generate <12 mL nonchlorinated solvent waste.
- Consistently high recoveries (mostly 90-110% with RSDs < 10%) of a wide range of GC- and LC-amenable pesticides are achieved from many food matrices.
- Countless labs have implemented QuEChERS successfully for up to 500 pesticides in food and increased efficiency (faster, less labor, lower cost, less waste, saves space, less labware, higher throughput).
- QuEChERS concepts have spread to other applications.

What is QuEChERS?

www.quechers.com

http://en.wikipedia.org/wiki/Quechers

The Quechers method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticide residues in food. The name is a **portmanteau word** formed from "**Quick, Easy, Cheap, Effective, Rugged, and Safe**."

QuEChERS History

2000-2002

2002 EPRW-Rome

2003 Publication

MgSO4

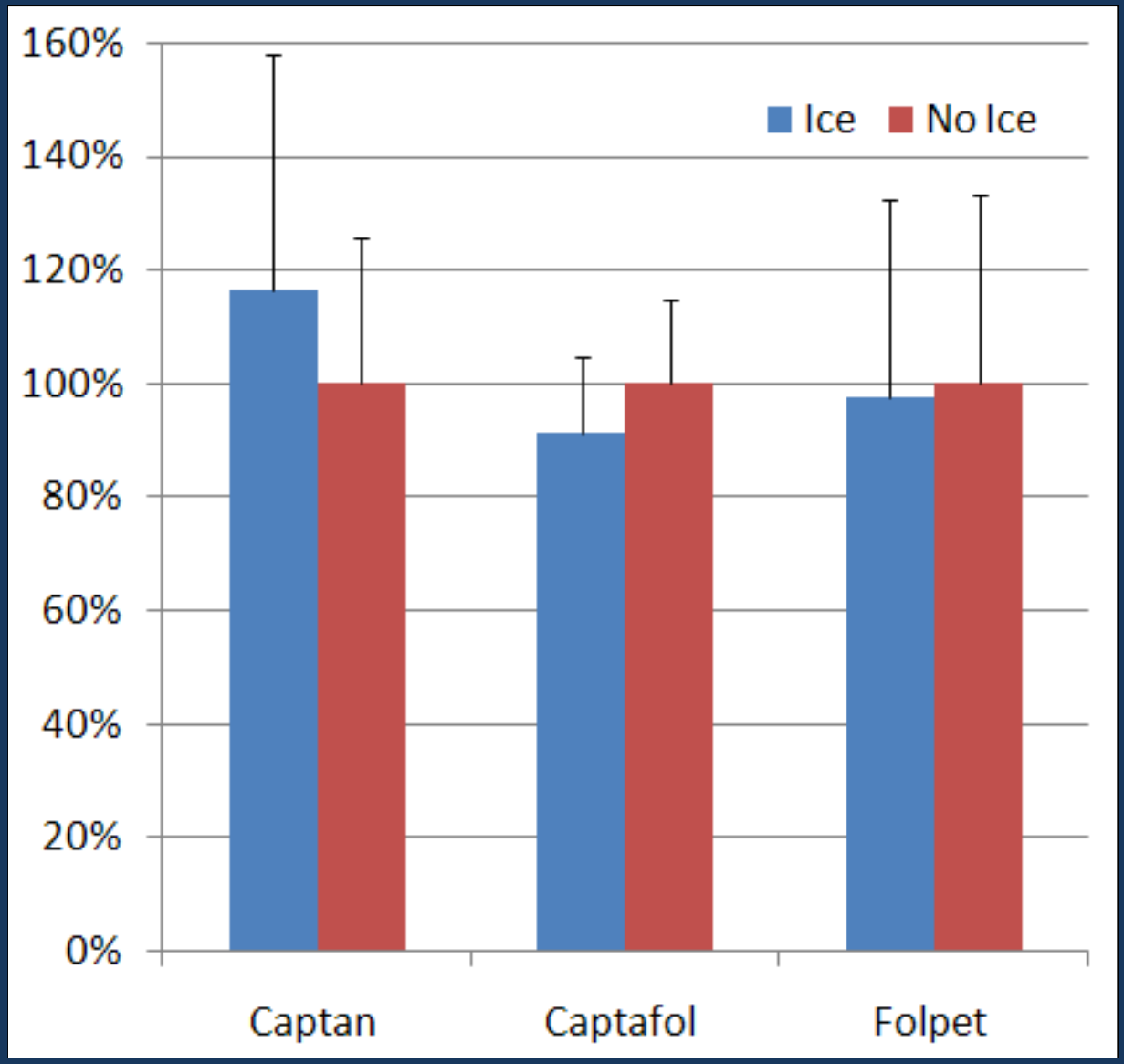
Dispersive-SPE

Analyte Protectants

Limitations of QuEChERS?

- Too Many Modified Versions
- Cereals require a separate protocol
- Still Problems with captan, folpet, captafol
- Spices and oils give problems
- Works best with modern MS systems
- Need PTV or solvent exchange for low LOD
- Matrix effects in complicated matrices
- Even simpler sample prep possible

QuEChERS Update



Syringeless Filters

Mini-UniPrep™ (Whatman)

1) Place unfiltered sample (max. 0.5 mL) in chamber.

2) Compress filter plunger into sample chamber. Clean filtrate fills reservoir bottom up.

3) Place the Mini-UniPrep™ vial in an autosampler.

aqueous samples: PVDF (polyvinylidenefluoride) filter

QuEChERS Sample Prep

(1) weigh 15 g homogenized sample into a 50 mL tube
(2) add spiking and I.S. solutions, and vortex for 1 min;
(3) add 15 mL of MeCN with 1% HOAc; shake for 30 s;
(4) add 6 g of anh. $MgSO_4$ and 1.5 g of anh. NaOAc;
(5) shake the tube immediately for 1 min;
(6) centrifuge the tube at 3,250 rcf for 2 min;
(7) transfer 1 mL extract to d-SPE tube containing 150 mg anh. $MgSO_4$ + 50 mg PSA + 50 mg C-18 + 7.5 mg GCB;
(8) mix for 30 s and centrifuge at 3,250 rcf for 2 min;
(9) transfer 0.5 mL into an autosampler vial;
(10) add 50 μL of the QC and analyte protectants mixture and 50 μL MeCN (to make sample volumes equal those of the calibration standards), and
(11) conduct LP-GC/MS-MS analysis.