Hill Laboratories
BETTER TESTING BETTER RESULTS

www.hill-laboratories.com

Use of a Robotic Solid Phase Extraction Clean-up of QuEChERS Extracts to Give Improved Matrix Removal for Pesticide Residue Analyses by GC-MS/MS and LC-MS/MS.

Bruce Morris, Richard Schriner*, Rick Youngblood† and Kim Gamble†*

**RJ Hill Laboratories, Hamilton, New Zealand.*
†ITSP Solutions Inc., Hartwell, GA, U.S.A.

www.itspsolutions.com

ITSP SOLUTIONS, INC.

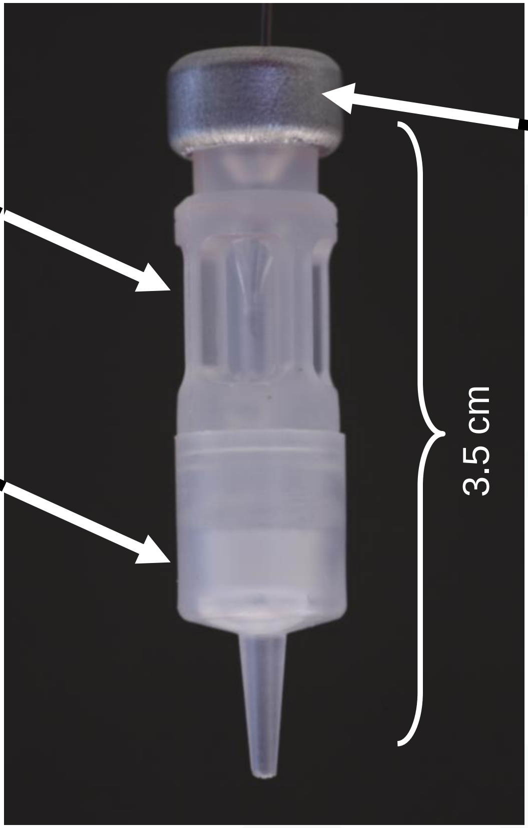

Bay of Islands
Auckland
Tauranga
Napier
Picton
Wellington
NEW ZEALAND
Christchurch
(Akaroa)
National Park
ic cruising)
Dunedin (Port Chalmers)

Introduction

- Why did we look at a robotic column SPE alternative to commonly used dispersive SPE?
- In our lab, QuEChERS for LC-MS/MS was successful, but less so for GC.
  - **dSPE gave extracts that were poor for GC, with the samples we wanted to analyse.**
- Bench cartridge SPE gave better clean-up, but seemed impractical for 100s of samples per day.
- With some experience in the use of CTC autosamplers, it seemed a robotic method could be the answer.
- ITSP SPE cartridges became available for CTCs, and we started a collaboration developing these for QuEChERS.

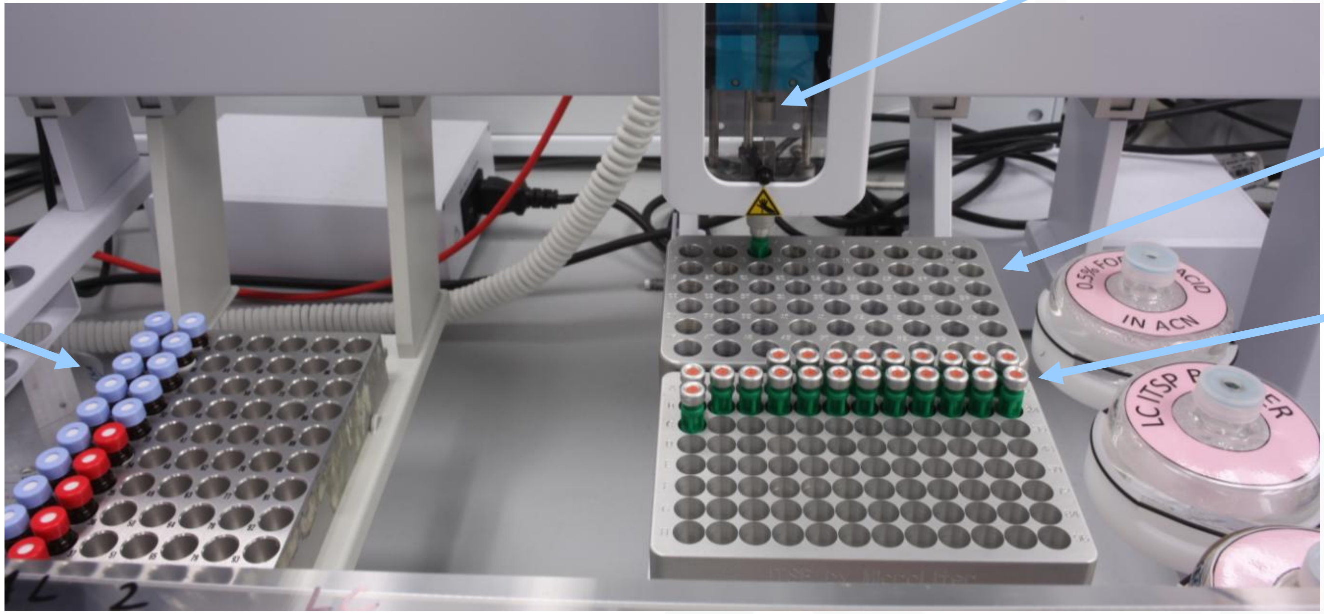
Robotic SPE clean-up Cartridges.

- A miniaturised SPE cartridge invented by ITSP Solutions.
- Stationary phase mixtures co-developed and trialled for QuEChERS at Hill Labs.
  - **30 – 45mg of stationary phase, depending on mixture.**



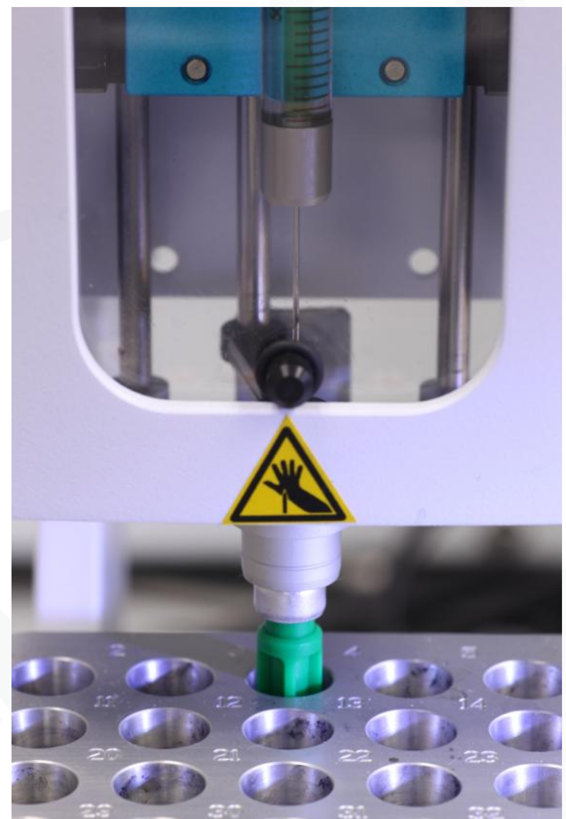
Robotic SPE clean-up
- CTC autosampler

- Robotized on a CTC autosampler
  - **Hence ITSP "Instrument Top Sample Prep", although it can also be done stand-alone on a bench top.**
  - **Well-plate dimensions, although 2-mL vials used rather than wells.**



Robotic SPE clean-up
- Load and Elute tray

- Developed specifically for QuEChERS-ITSP.
- Samples eluted into 2-mL vials in a 54-well tray, allowing volume for LC-buffer addition.
- Tray cover aligns cartridges and allows them to be removed from vials after elution, for instrument injection



ITSP-QuEChERS Procedure

QuEChERS extraction (20g for fresh fruit)

LC-MS/MS

Aliquot taken off into 2-mL vial, ISTD added

Extract loaded

LC-ITSP cartridge

Analytes eluted

Diluted in aqueous buffer for LC-MS/MS analysis.

GC-MS/MS

Aliquot taken off into 2-mL vial, ISTD added

Extract loaded

Analytes eluted

GC-ITSP cartridge

Analyte protectants added for GC-MS/MS injection.

Takes place on Load and Elute tray

Cartridge Development

- **LC-MS/MS cartridges.**

  The aim was to remove non-polar extract matrix (oils/waxes).

  - **Drop out of solution when diluted in aqueous buffer for injection.**
  - **Are retained on the LC column which becomes fouled.**
  - **Cause matrix suppression in high organic end of chromatogram, or in the next injection.**

  Polar sugars/acids washed out at the start of the LC gradient, are not such a big problem.

- **GC-MS/MS cartridges**.

  Wanted to remove extracted sugars, fatty acids, sterols and pigments.

  - **Foul the inlet liner/pre-column, creating active sites.**
  - **Cause interferences or suppression in the chromatogram.**

  GC can cope with some higher MW oils/waxes, especially with backflushing.

LC-MS/MS Cartridge Sorbent Trials.

- Various cartridge stationary phase mixtures were trialled with Avocado extract (oily).
  - **5g fruit/20mL acetonitrile, 150µL of extract loaded onto cartridges (slightly overloaded), eluted with 150µL of acetonitrile.**

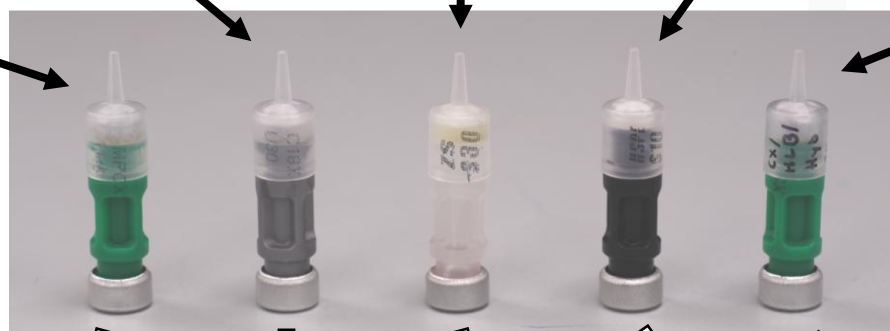
$MgSO_4$/PSA/C-18/ CarbonX 45mg

Z-Sep/C-18/ CarbonX 30mg

Z-Sep+ 30mg

Z-Sep 10mg

Z-Sep/HLB/ CarbonX 20mg



Dry weight Recovered (mg)

Raw extract	PSA/C-18 /CarbonX	Z-Sep/C-18 /CarbonX	Z-Sep+	Z-Sep	Z-Sep/HLB/ CarbonX
0.41	0.23	0.15	0.07	0.18	0.20

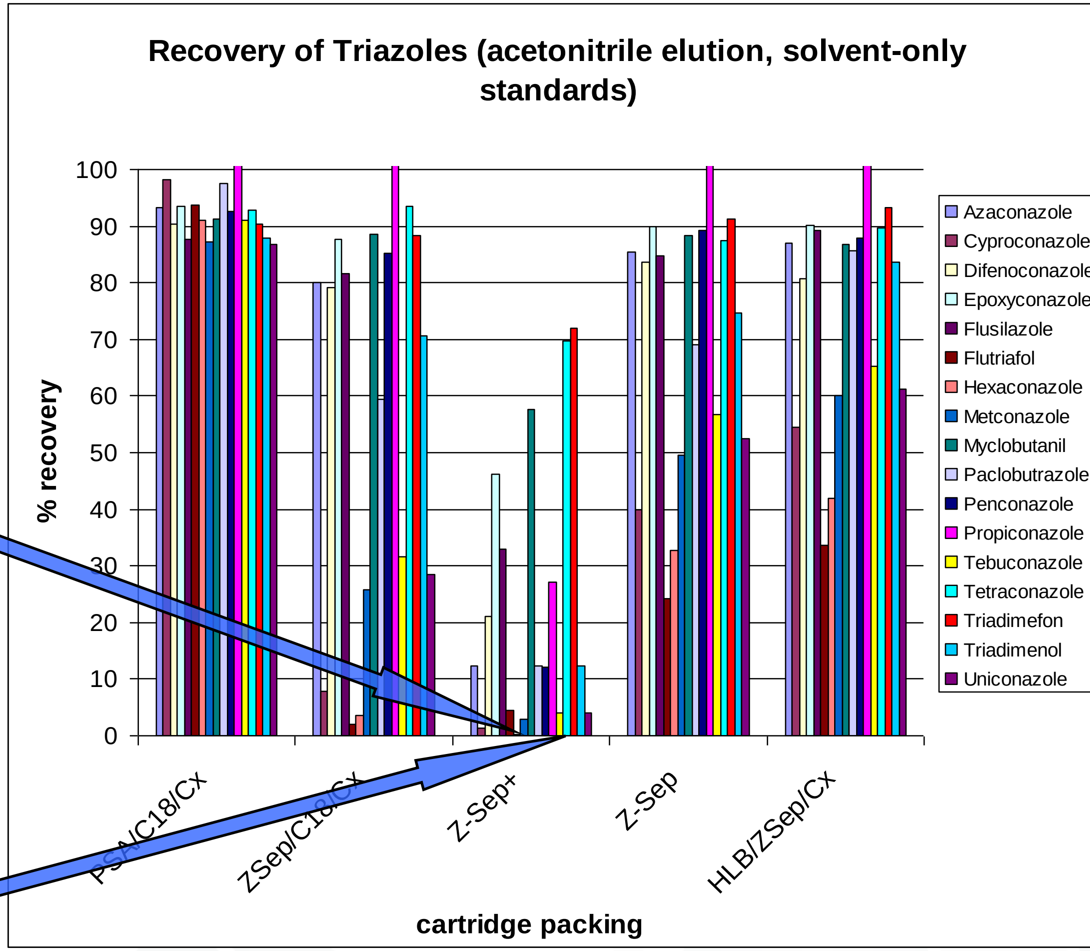
2nd best oil removal, but better pesticide recoveries

Best oil removal, but lose many pesticides

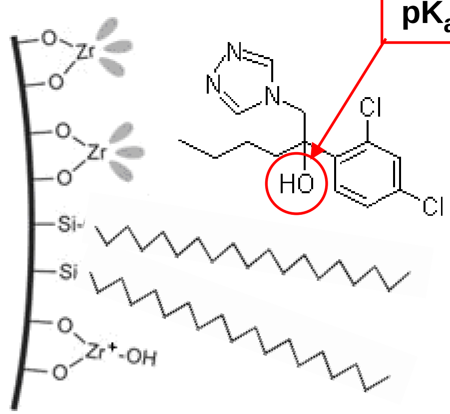
Recoveries off Trial Cartridges

Pesticide spike on Avocado extract.

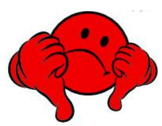
- **For Z-Sep+, 68 out of 266 pesticides lost (< 70% recovery).**
- **Z-Sep/C-18: 40 analytes < 70% recovery.**
- **As show by 17 triazole fungicide recoveries.**



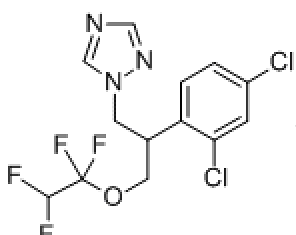
Z-Sep+



Hexaconazole
-highly retained

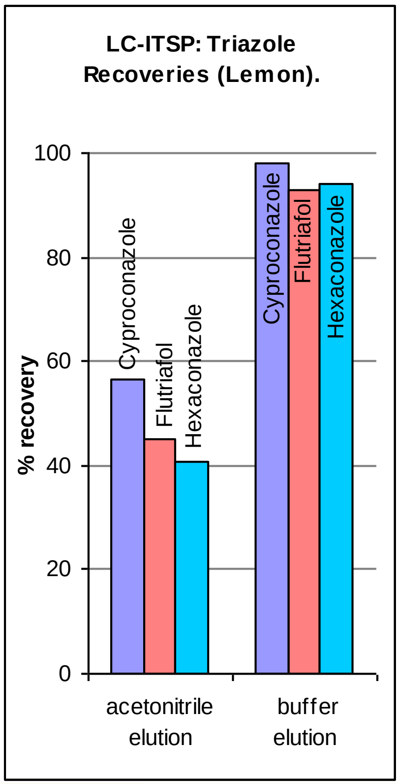


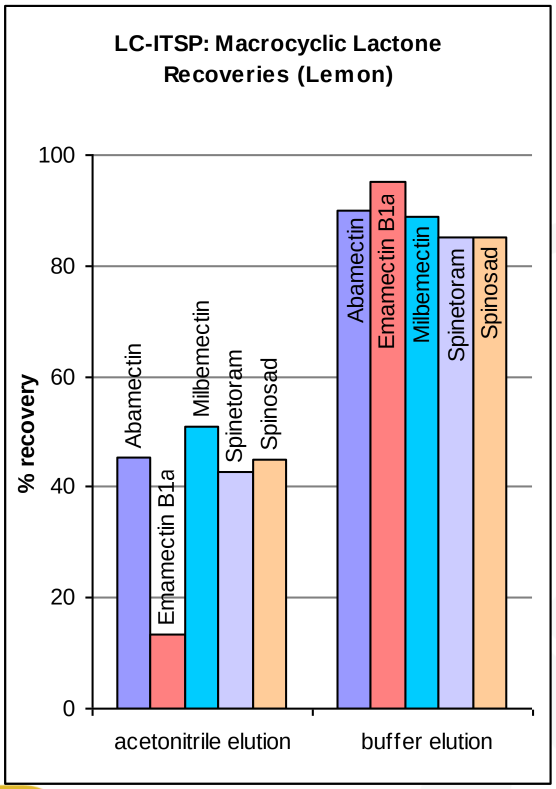
Tetraconazole
-lesser retention

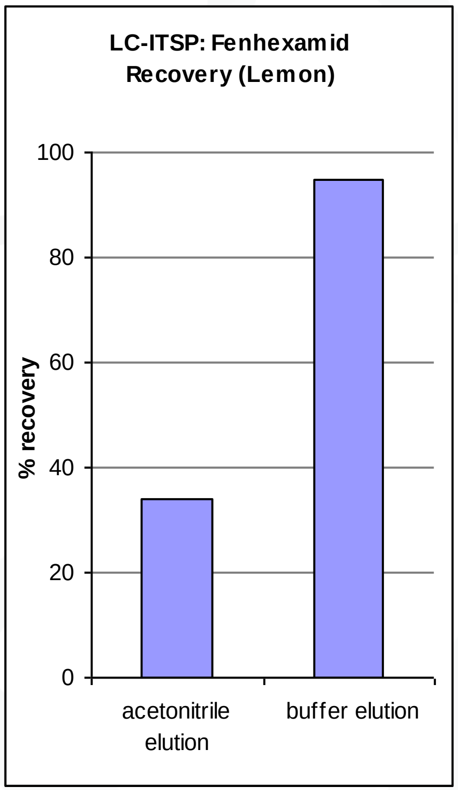


Z-Sep/C-18 ITSP : Effect of Buffer Elution.

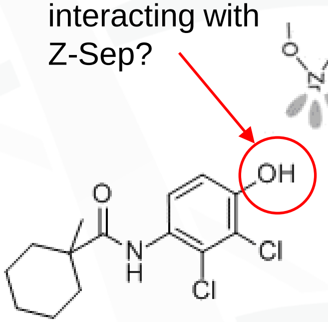
- Z-Sep/C-18/Cx was chosen as the best compromise between retention of oils and recovery of analytes.
- However 40 compounds were still “lost”, so we tried elution with formate buffer.
- This improved recoveries of e.g. triazole fungicides, macrocyclic lactones, fenhexamid.
- However, buffer can push some matrix oils off, so have to be careful with elution volumes.





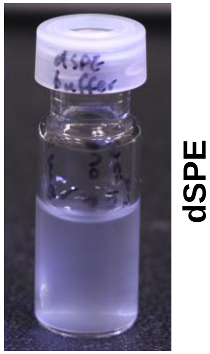


Phenolic –OH interacting with Z-Sep?

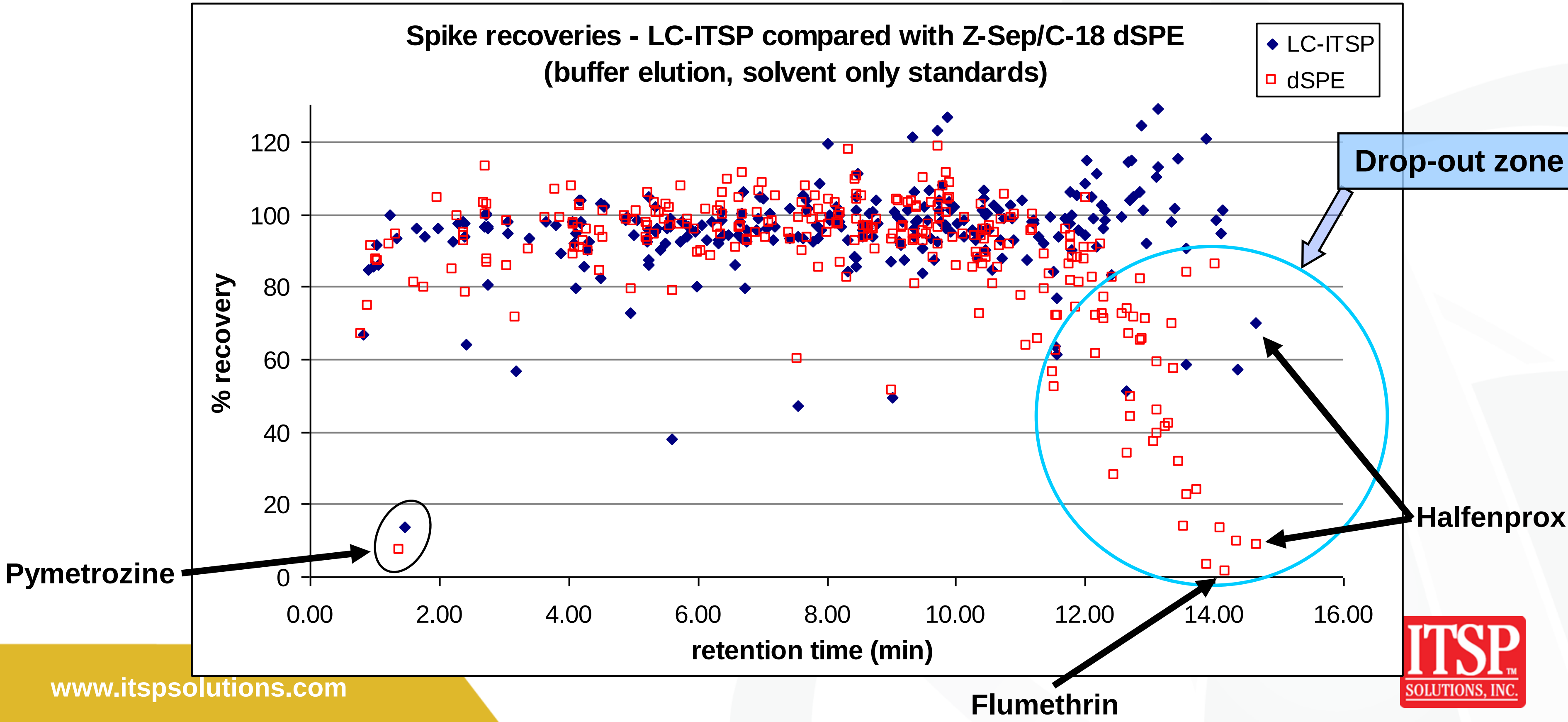


Fenhexamid
(anilide fungicide)

Z-Sep/C-18 : dSPE vs. LC-ITSP - Avocado



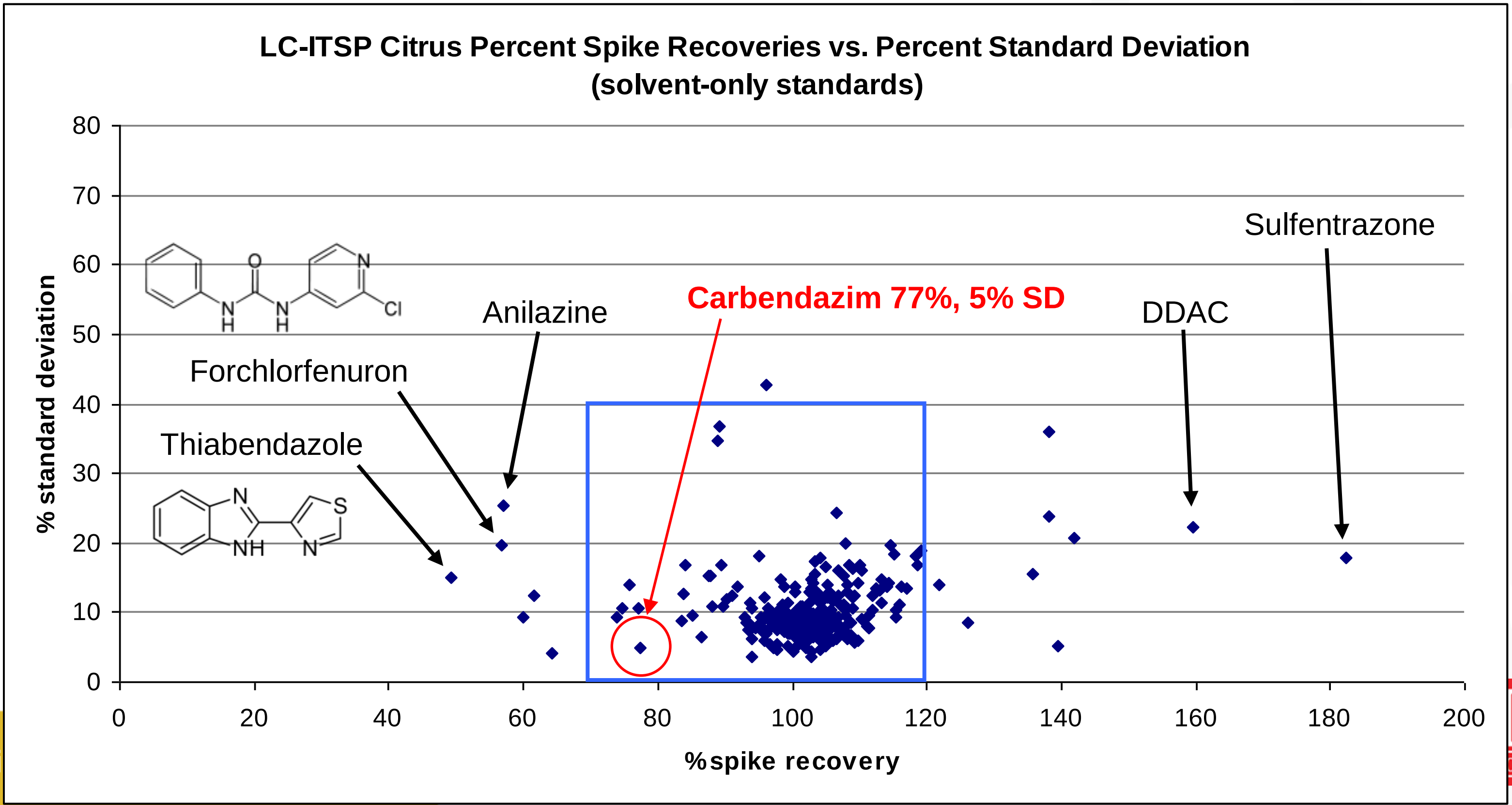
- **dSPE**, with buffer added, significant amounts of matrix oils are pushed off, giving cloudy solutions, resulting in drop-out of non-polar analytes.
  - **Out of 272 analytes, 19 with retention times > 12 minutes (logP_{ow}≥ 5) have < 50% recovery.**
- **LC-ITSP** with buffer elution, only 4 out of 272 analytes have < 50% recovery.
  - **e.g. Pymetrozine with poor extraction recovery. _Solutions are clear._**



ITSP SOLUTIONS, INC.

LC-ITSP spike recoveries for Citrus.

- 282 Pesticides (incl. metabolites/homologues) analysed by LC-MS/MS, shown in the plot below.
- 16 falling outside 70-120% recovery and 40% standard deviation (*N* = 10).
  - **Low recoveries - during extraction, and/or some retention on Z-Sep/C-18/CarbonX, however highly reproducible with ITSP (e.g. Anilazine, Forchlorfenuron, Thiabendazole).**
  - **High recoveries (e.g. DDAC, Sulfentrazone), due to losses in solvent-only calibration standards.**



Cartridge Development

- **LC-MS/MS cartridges.**

  The aim was to remove non-polar extract matrix (oils/waxes).

  - **Drop out of solution when diluted in aqueous buffer for injection.**
  - **Are retained on the LC column which becomes fouled.**
  - **Cause matrix suppression in high organic end of chromatogram, or in the next injection.**

  Polar sugars/acids washed out at the start of the LC gradient, are not such a big problem.

- **GC-MS/MS cartridges**.

  Wanted to remove extracted chlorophyll/pigments, sugars, fatty acids, sterols, HCs.

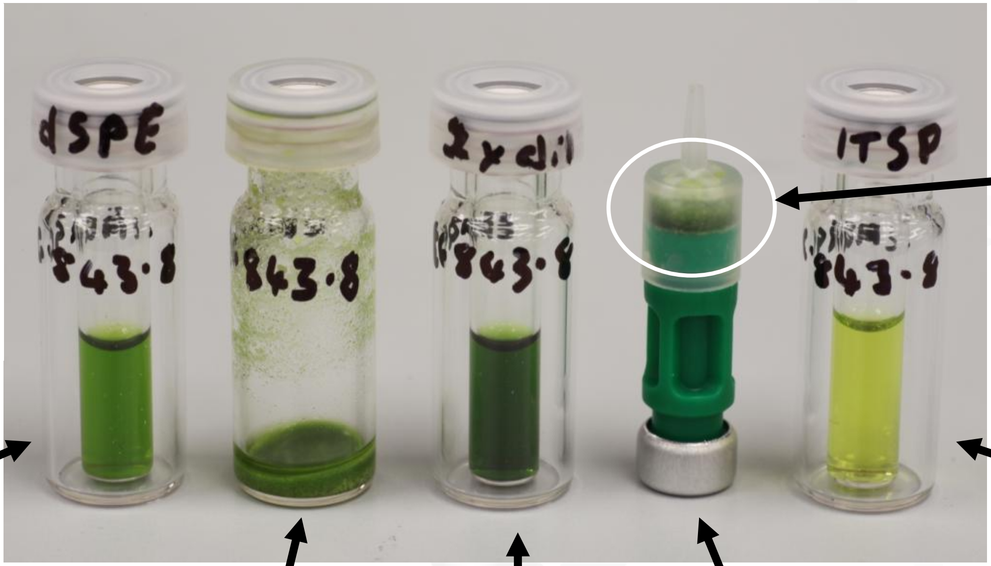
  - **Foul the inlet liner/pre-column, creating active sites.**
  - **Cause interferences or suppression in the chromatogram.**

  GC can cope with some higher MW oils/waxes, especially with backflushing.

GC-ITSP - Chorophyll removal

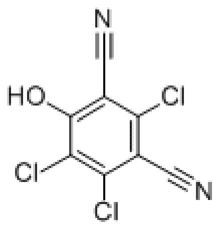
- GC-ITSP stationary phase mixture uses the same sorbents as standard QuEChERS dSPE.
  - **PSA/C-18/$MgSO_4$ with CarbonX for chorophyll removal.**
- CarbonX appears to be more effective in ITSP format than in dSPE.

Spinach Extract

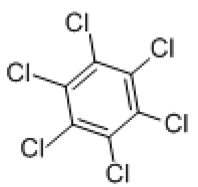


Recovery of Planar Aromatics off ITSP

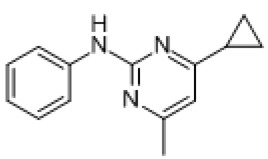
- Using CarbonX, recovery of planar aromatic pesticides has been acceptable, without toluene eluent.



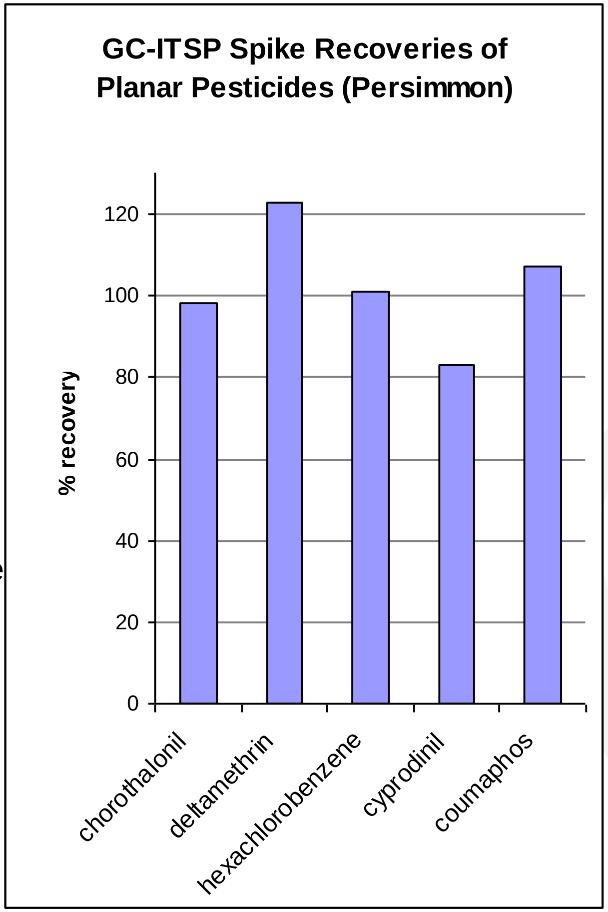
Chlorothalonil

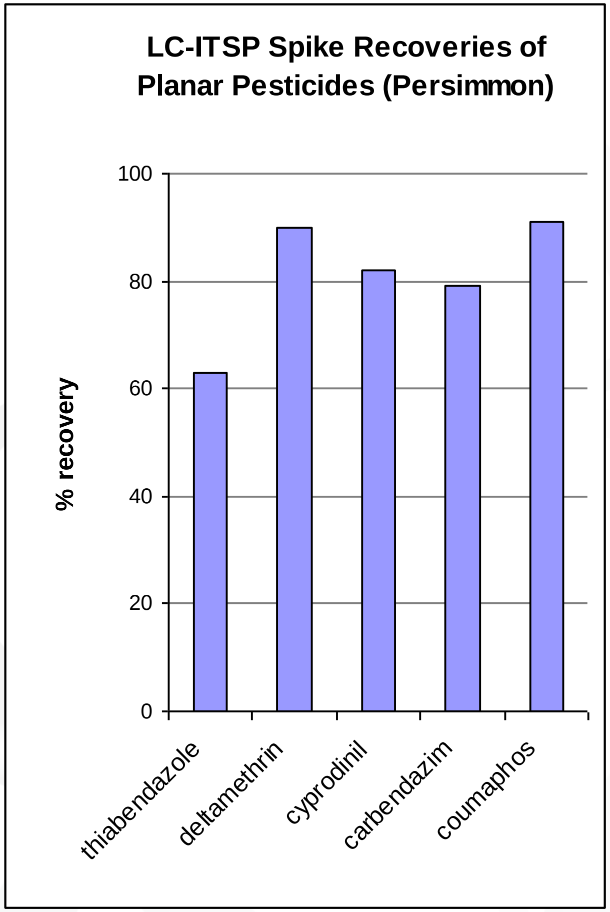


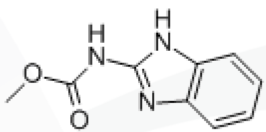
Hexachlorobenzene



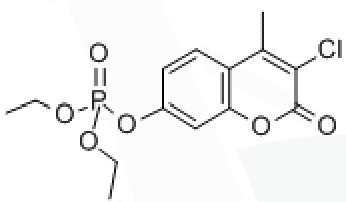
Cyprodinil







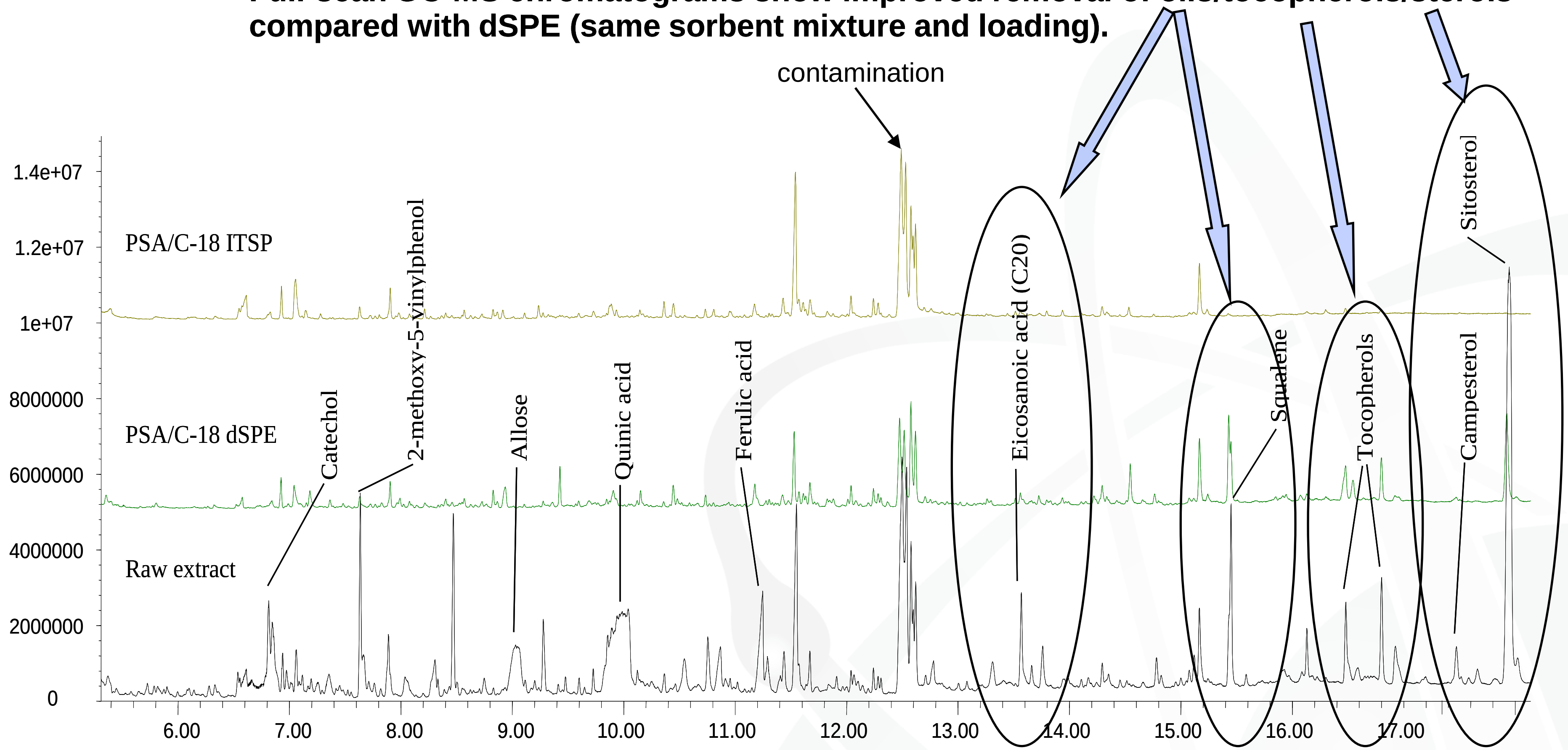
Carbendazim



Coumaphos

GC-ITSP clean-up of Blueberry extract.

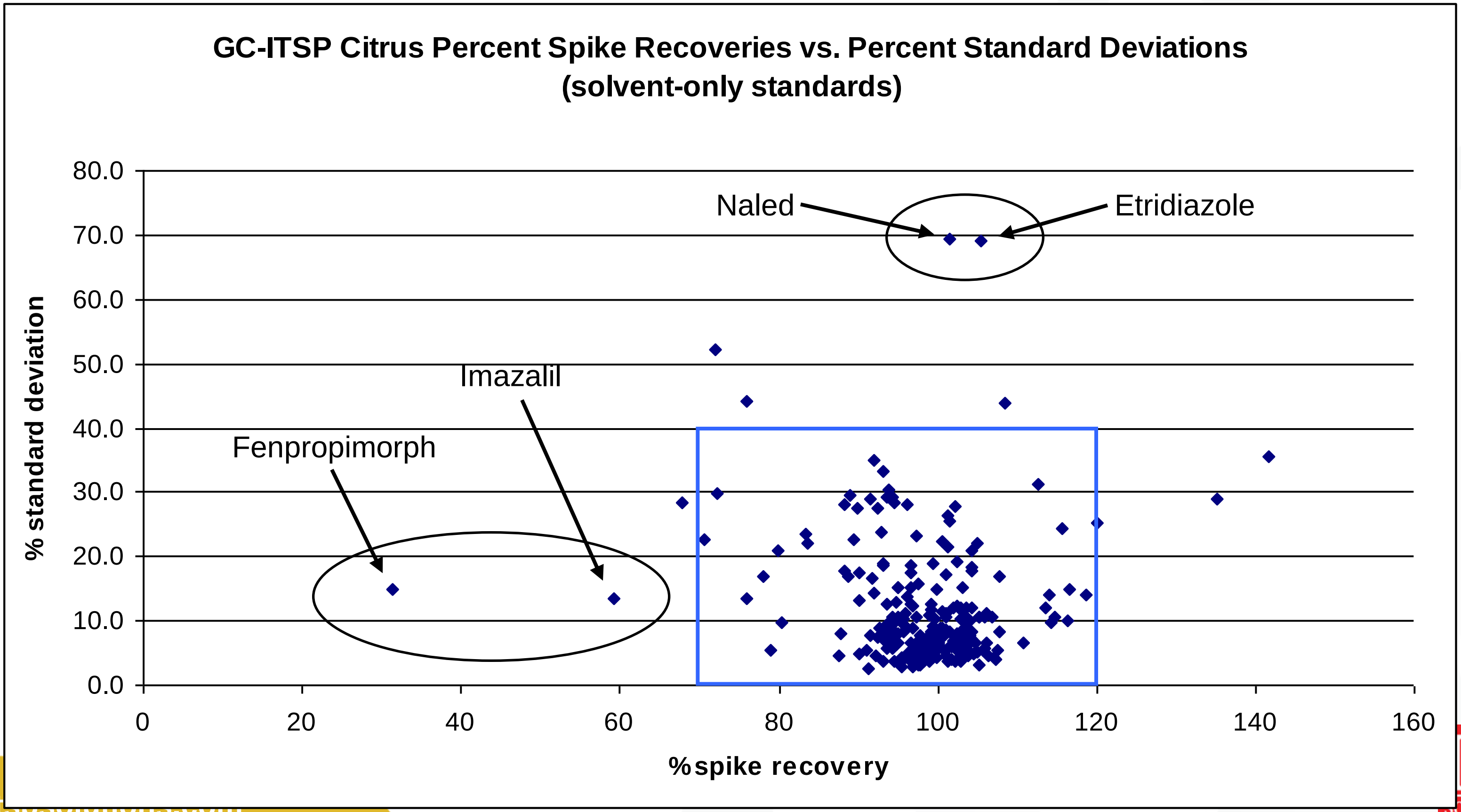
- 10g fruit/20mL acetonitrile, GC-ITSP **compared with PSA/C-18 dSPE.**
  - **Full-scan GC-MS chromatograms show improved removal of oils/tocopherols/sterols compared with dSPE (same sorbent mixture and loading).**



ITSP SOLUTIONS, INC.

GC-ITSP spike recoveries for Citrus.

- 228 Pesticides analysed by GC-MS/MS, in plot below.
- 10 falling outside 70-120% recovery and 40% standard deviation (*N* = 10).
  - **Those with some retention on the cartridge (e.g. imazalil), show highly reproducible recoveries.**
  - **Poor reproducibility is due to variable breakdown or losses (e.g. naled, etridiazole).**



Summary

- Robotic clean-up means high-throughput, low labor input, and high reproducibility.
- Column SPE format means improved clean-up compared with dispersive SPE.
  - **Desirable for more difficult samples, reducing instrument fouling (esp. for GC).**
  - **Can generally use solvent-only calibration standards, due to fewer matrix effects.**
  - **Reduces MS/MS interferences and oil drop-out in-vial (for LC).**
  - **Avoids dSPE stationary phase transfer into analysis vials (ends up in LC injector or in-line filter).**
- Careful choice of stationary phase mixtures and elution solvents allows an extended suite of pesticides to be recovered, while retaining effective matrix removal.
  - **Chlorophyll, pigments, sugars, sterols, oils/fatty acid removal, keeps columns and inlets clean!**
- SPE integrated into the instrument cycle and takes place between injections.
  - **Uses idle autosampler time (takes ~7 minutes per clean-up).**
  - **Gives freshly cleaned up extracts, with reduced potential for analyte breakdown, especially for LC with the addition of aqueous buffer.**
- Has proven to be reliable and robust in a commercial laboratory environment.

References

- AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.
- EN15662:2008, Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS-method.
- Increased Removal of Fat and Pigment from Avocado Extracts Prior to GC-MS Analysis of Pesticide and Metabolite Residues. Katherine K. Stenerson and Jennifer Claus, *Reporter US Volume 31.2.*
- EURL-FV(2013-M11). Determination of pesticide residues in avocado and almond by liquid and gas chromatography tandem mass spectrometry (http://www.eurl-pesticides.eu/userfiles/file/EURL-FV%20(2013-M11)Determination%20of%20pesticide%20residues%20in%20high%20oil%20vegetal%20commodities.pdf).
- Novel Porous Carbon Sorbent Materials for Use in Sample Preparation. Dwight Stoll, David C. Harmes, Jon Thompson, Doug Fryer, Conor Smith, and Bill Barber. EAS poster presentation, 2012. (http://uniscicorp.com/wp-content/uploads/2012/12/2012-Quechers-EAS-Presentation-v61.pdf)

Thankyou for your Attention