Hill Laboratories
BETTER TESTING BETTER RESULTS

Eliminating the need for Matrix-matched Calibration Standards for GC and LC Pesticide Residue Analyses of QuEChERS Extracts using a Robotic Solid Phase Extraction Clean-up Procedure.

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1. OVERVIEW

Standard EN QuEChERS method[1]:

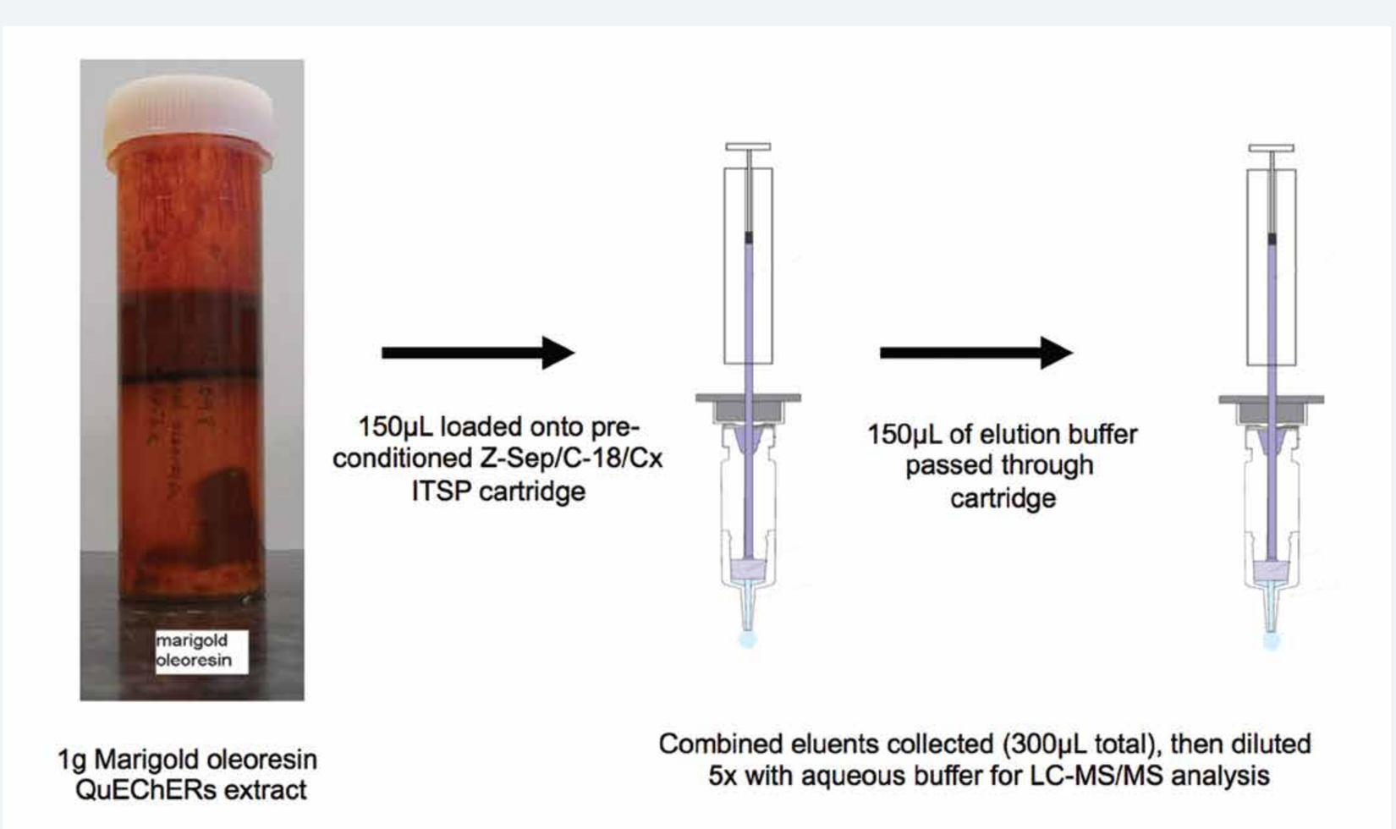
- Acetonitrile/water extraction with "salting-out" and citrate buffering.
- Dispersive SPE (dSPE) clean-up with $MgSO_4$ / primary-secondary amine (PSA) alone, or in combination with reversed phase (C-18) silica and graphitized carbon black (GCB).
- Dilution in aqueous formate or acetate buffer, followed by LC-MS/MS analysis.
- Addition of analyte protectants and GC-MS/MS analysis.
- Matrix-matched calibration standards generally used to compensate for matrix effects, especially for complex spice/herb or concentrated food ingredient samples.

ITSP (Instrument Top Sample Preparation):

- Robotic solid-phase extraction (SPE) clean-up, using miniaturized cartridges on a CTC autosampler (**Figure 1**).
- Replaces dSPE step, giving improved clean-up and matrix removal.
- Stationary phases: LC-MS/MS - Z-Sep/C-18/CarbonX. GC-MS/MS - MgSO4/PSA/C-18/CarbonX.
- Elution solvents: LC-MS/MS - 1:1 MeCN/MeOH + 100mM NH_4formate (pH 5.8). GC-MS/MS - acetonitrile/0.5% formic acid.
- Enables the use of solvent-only calibration standards.

Figure 1: ITSP cartridges.

Figure 2: ITSP clean-up of QuEChERS extracts for LC-MS/MS, using Z-Sep/C-18/ Carbon-X stationary phase.

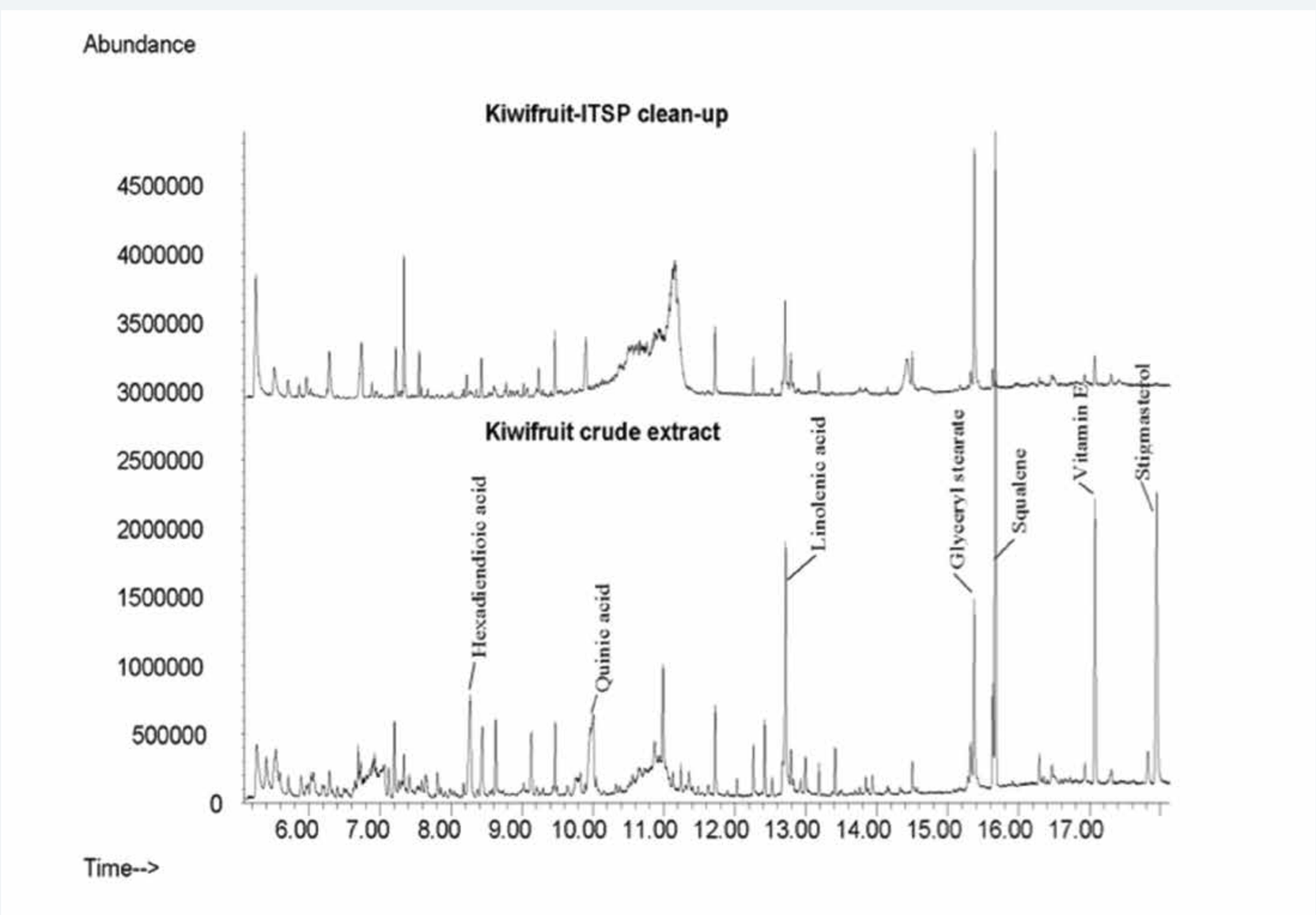


150μL loaded onto pre-conditioned Z-Sep/C-18/Cx ITSP cartridge
150μL of elution buffer passed through cartridge
1g Marigold oleoresin QuEChERs extract
Combined eluents collected (300μL total), then diluted 5x with aqueous buffer for LC-MS/MS analysis

2. KIWIFRUIT

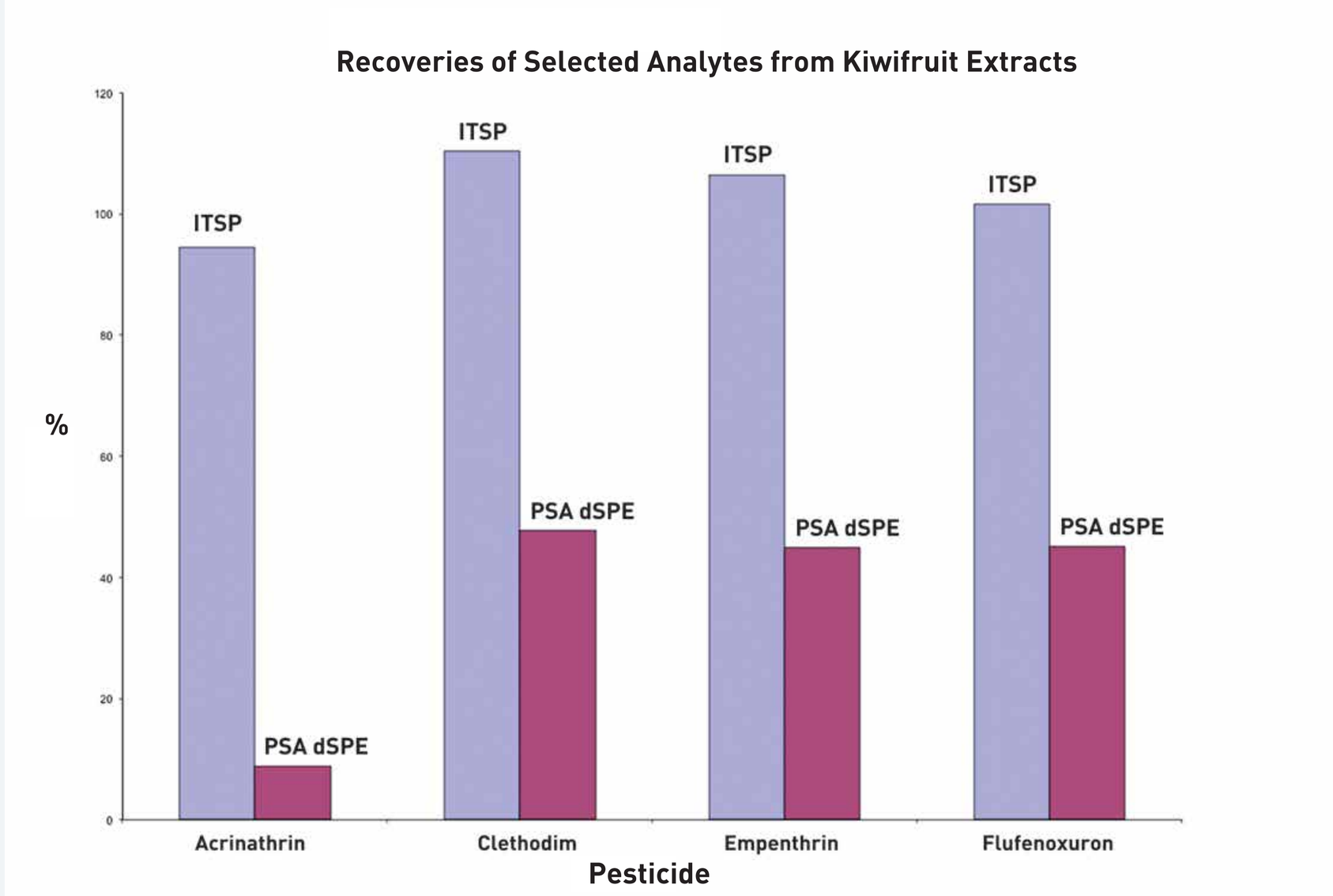
- Extracts contain oils
  - These interferences are removed by GC-ITSP clean-up (**Figure 3**).

Figure 3: GC-MS full-scan chromatograms showing removal of non-polar matrix by GC-ITSP.



- Kiwifruit matrix is poorly removed by PSA dSPE giving suppression of some analytes (**Figure 4**):
  - Acrinathrin, empenthrin and flufenoxuron (non-polar, effected by oils).
  - Clethodim appears to be retained by PSA.
- ITSP (Z-Sep/C-18/Cx)clean-up showed lack of suppression or losses (**Figure 4**), with improved oil removal, allowing use of solvent-only standards.

Figure 4: Kiwifruit extract LC-MS/MS spike recoveries, calculated against solvent standards, showing suppression/loss with PSA dSPE, avoided using Z-Sep/C-18/Cx ITSP.

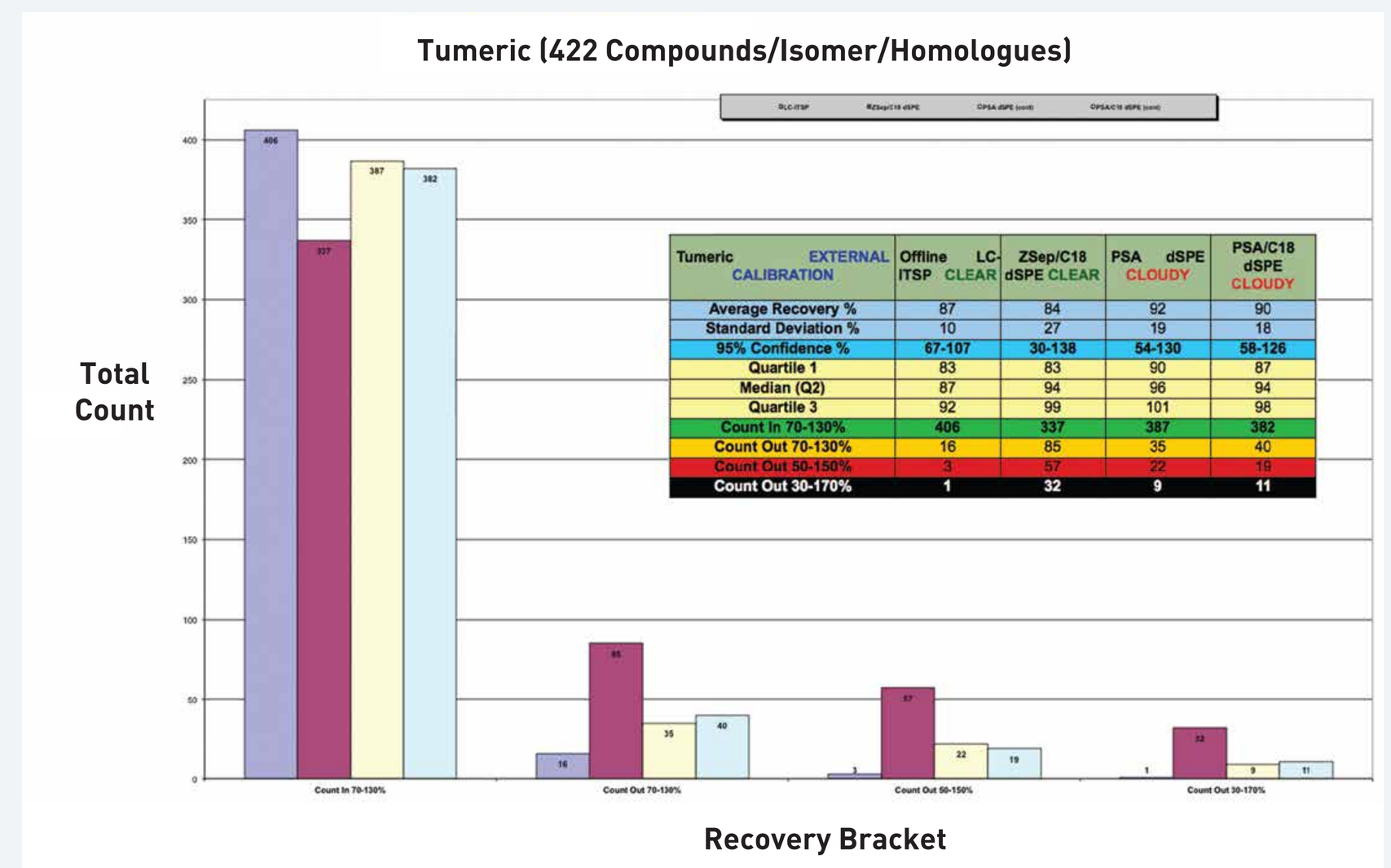


3. SPICES BY LC-MS/MS

- Spices commonly have high oil content and color (e.g. carotenoids), or non-polar flavor compounds (e.g. curcumin in turmeric, capsaicin in chili pepper), which may interfere with LC-MS/MS.
- Turmeric and chili pepper powder extracts were cleaned up by LC-ITSP (Z-Sep/C-18/Cx), and compared with three different dSPE phase-mixtures (**Figures 5 and 6**).
- ITSP clean-up performed best, resulted in fewer pesticides falling outside 70 – 130% recovery than dSPE, and fewer "failures" (outside 30 – 170%), due to loss or suppression.
- One pesticide (trinexapac-ethyl) failed to be recovered from all clean-ups.

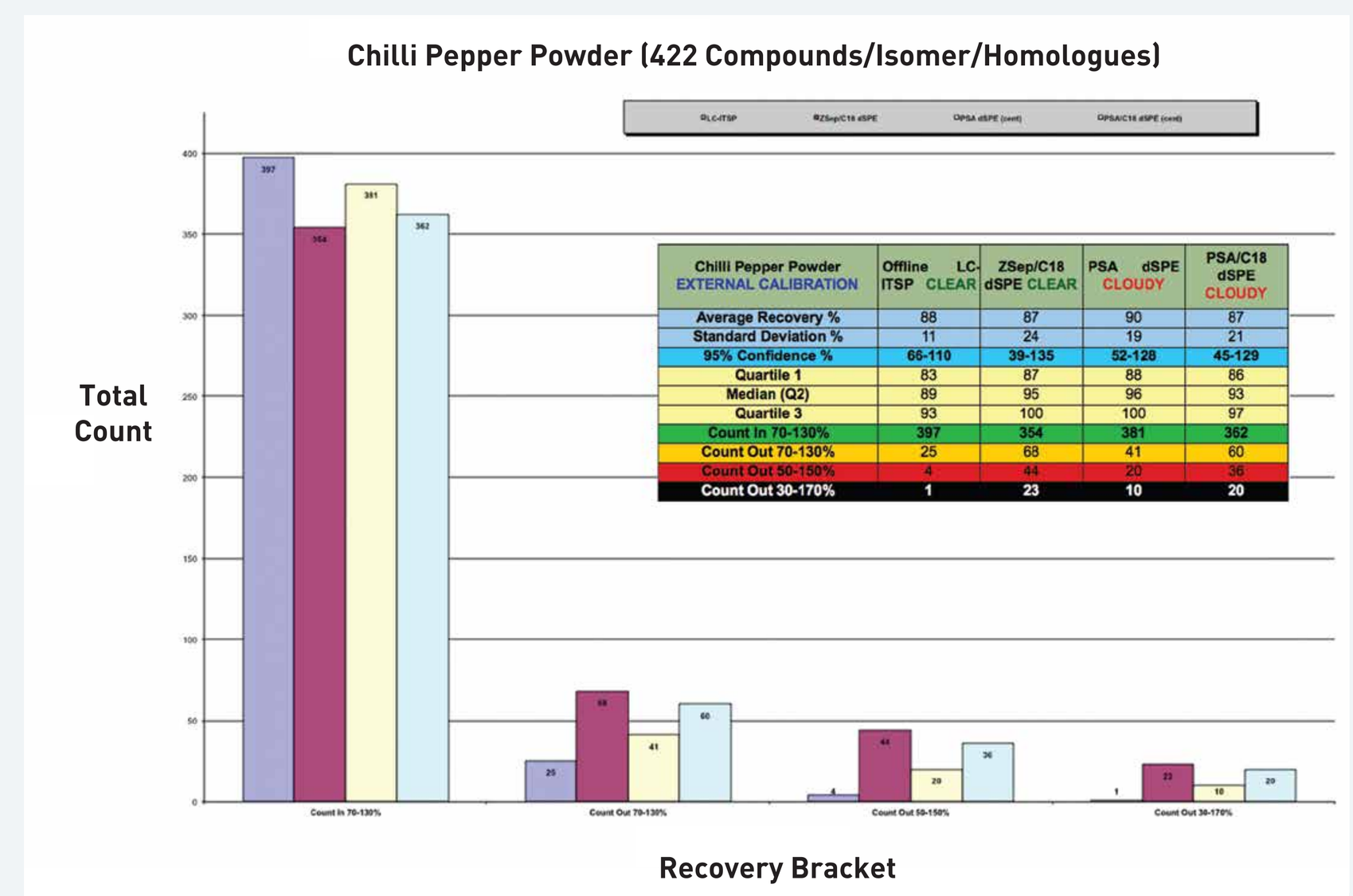
TURMERIC

Figure 5: Comparison of LC-MS/MS pesticide recoveries from Turmeric extract, cleaned up by ITSP or dSPE, calculated against solvent-only calibration standards.



CHILI POWDER

Figure 6: Comparison of LC-MS/MS pesticide recoveries from Chili powder extract,cleaned up by ITSP or dSPE, calculated against solvent-only calibration standards.



4. CONCENTRATED FOOD INGREDIENTS BY LC-MS/MS

- These are used as food colorings or flavorings, such as paprika color and marigold oleoresin, and are often highly colored and oily (**Figure 2**).
- Effective removal of non-polar matrix oils is needed, otherwise significant suppression/loss of non-polar pesticides is observed, as seen with PSA dSPE clean-up of Marigold oleoresin (**Figure 7**).
- Use of Z-Sep/C-18/Cx packed ITSP cartridges for clean-up of Marigold oleoresin resulted in acceptable recoveries of non-polar pesticides, due to superior matrix oil removal (**Figure 8**).

Figure 7: Pesticide recoveries versus LC-MS/MS retention time (aqueous to organic gradient, C-18 column), for PSA dSPE. Suppression or loss of non-polar pesticides is evident.

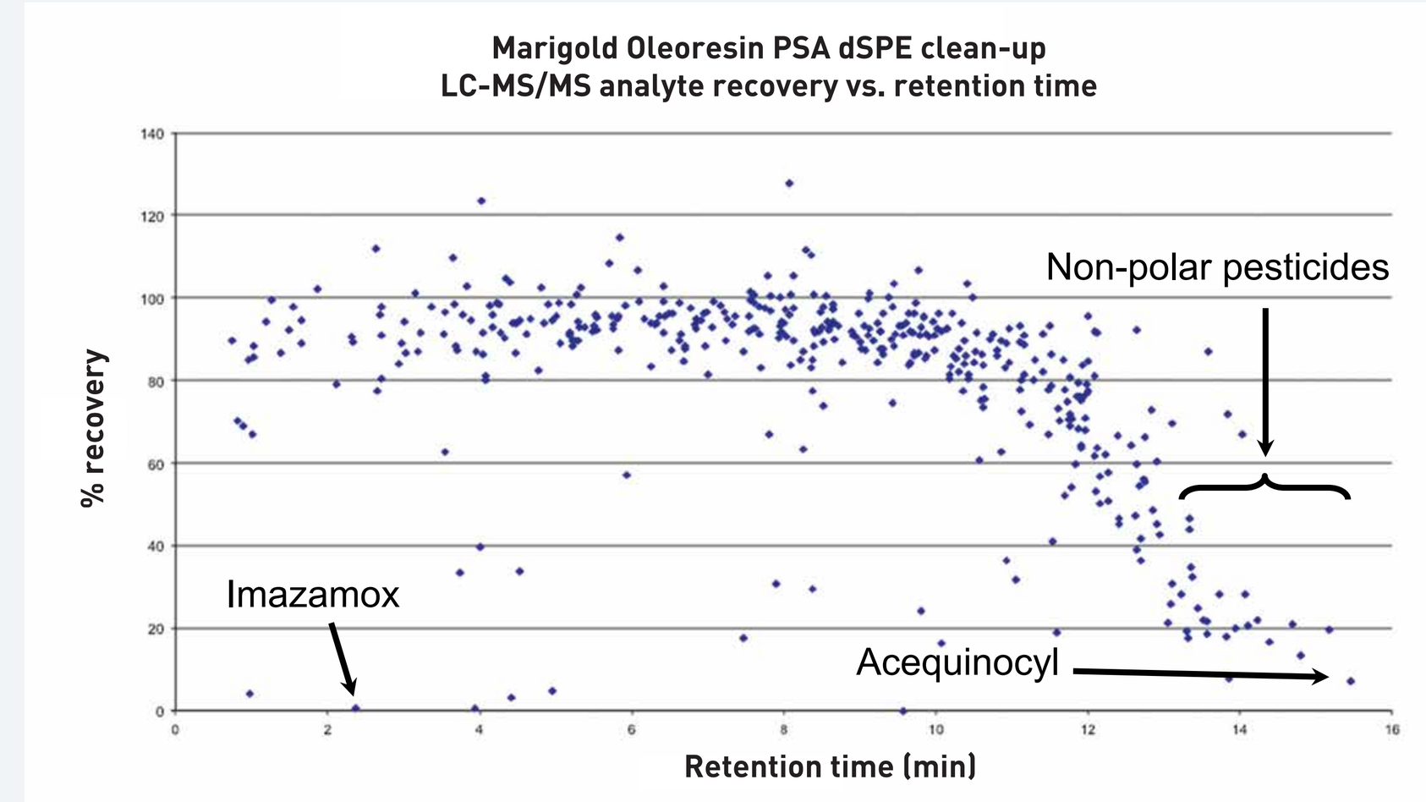
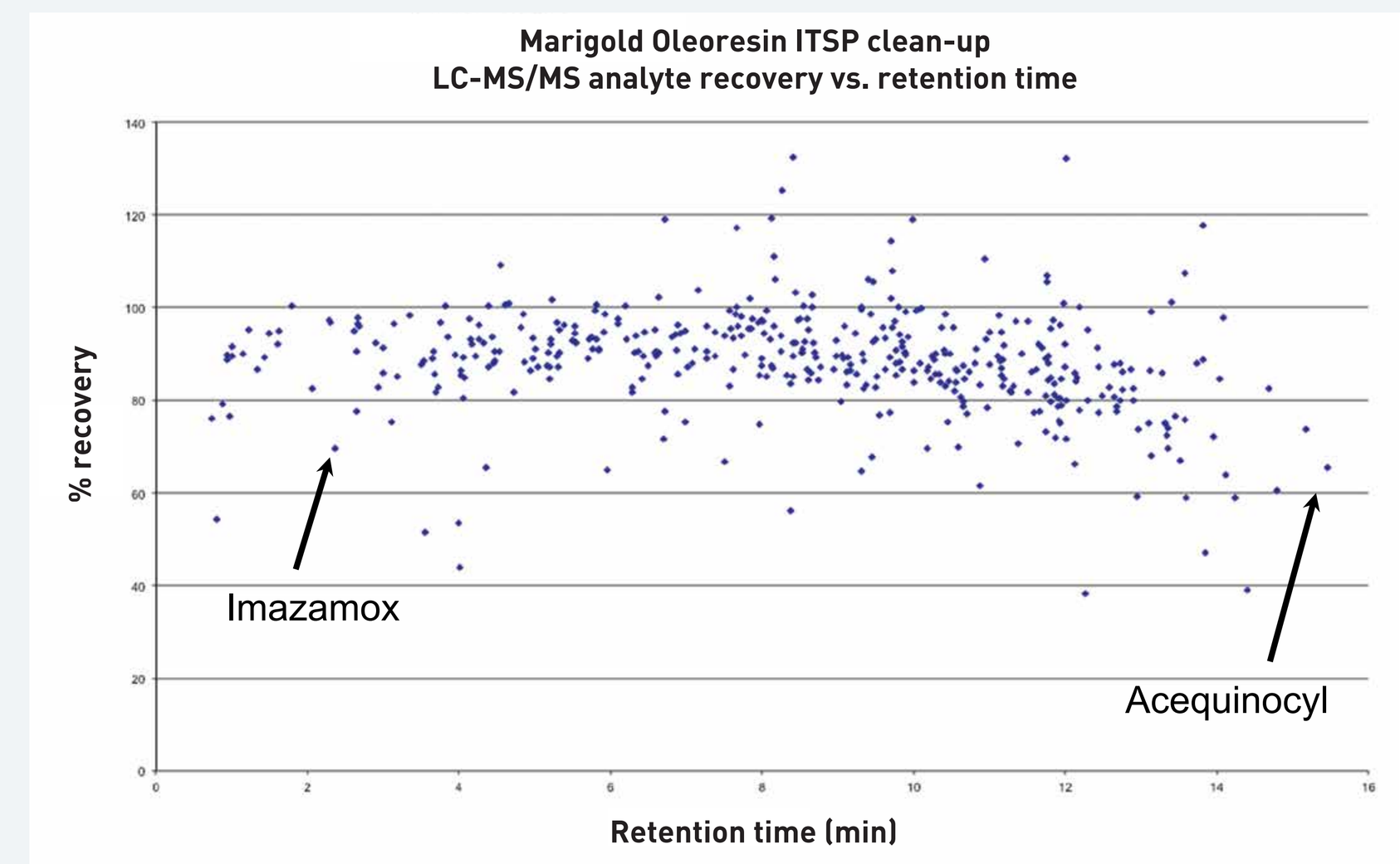


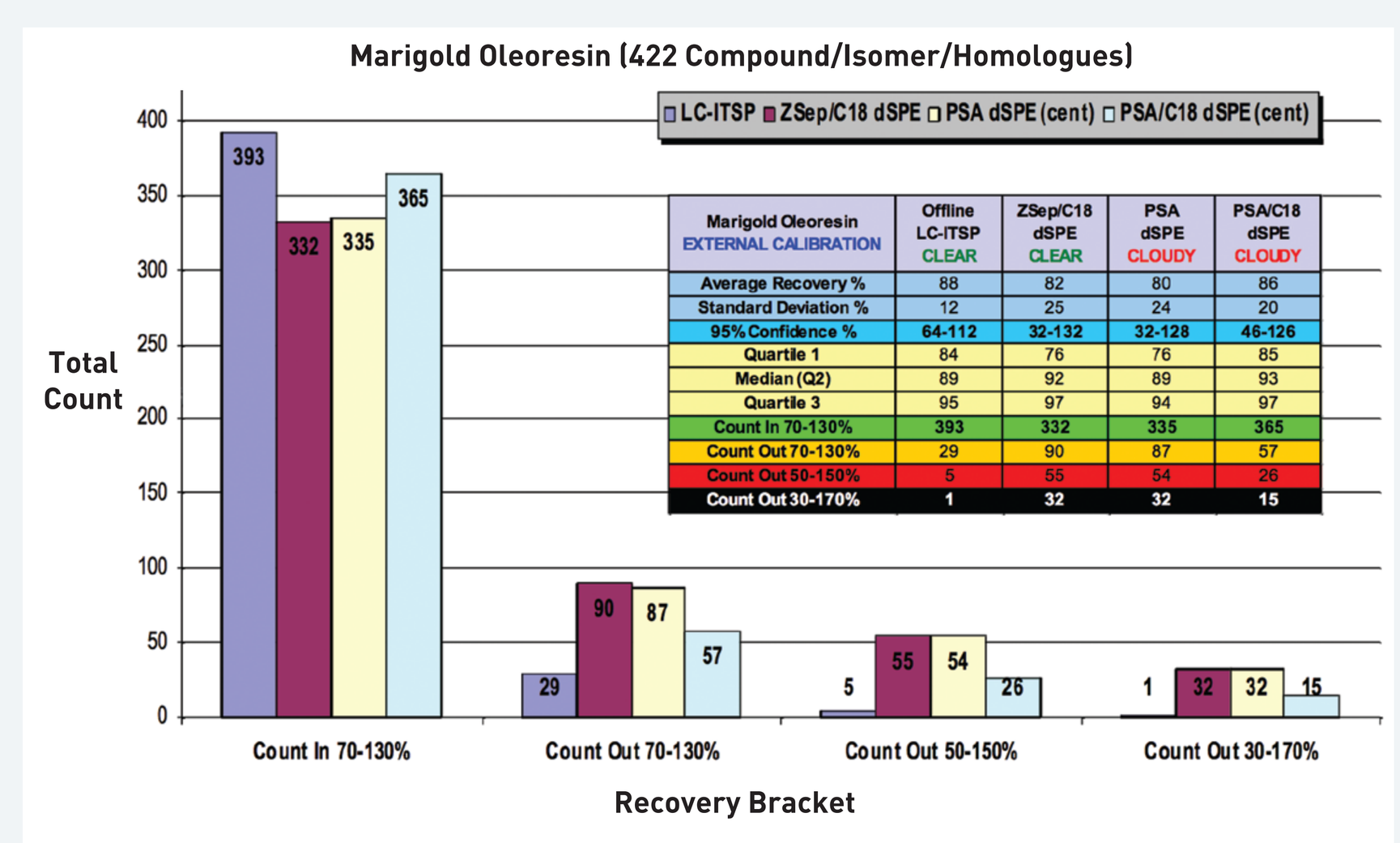
Figure 8: Pesticide recoveries versus LC-MS/MS retention time (aqueous to organic gradient, C-18 column), for ITSP (Z-Sep/C-18/Cx), showing little suppression or loss of non-polar pesticides.



- Comparisons of Marigold oleoresin and paprika color extracts, cleaned up by LC-ITSP (Z-Sep/C-18/Cx), and three different dSPE phase mixtures are presented in **Figures 9 and 10**.
- The greater removal of matrix (predominantly oils), by ITSP resulted in fewer pesticides falling outside the 70 – 130% recovery bracket.
- Especially evident is the lower counts in the "outside 50 – 150%" and "outside 30 – 170%" brackets for ITSP compared with all three dSPE methods. These brackets can be classified as "recovery failures".
- Acequinocyl is an example of a non-polar compound, exhibiting low (7%) recovery with PSA dSPE, and acceptable (66%) recovery using ITSP clean-up of Marigold oleoresin (**Figures 7 and 8**).
- Acidic compounds such as Imazamox can be lost on PSA by ion exchange (**Figure 7**), or on Z-Sep by lone-pair intaction with vacant Zr orbitals. Use of formate buffer allows 70% recovery from ITSP (**Figure 8**).
- Azoles (e.g. Uniconazole) are also retained on Z-Sep (29% recovery off Z-Sep/C-18 dSPE), however are recovered off ITSP (87%) with formate buffer elution.

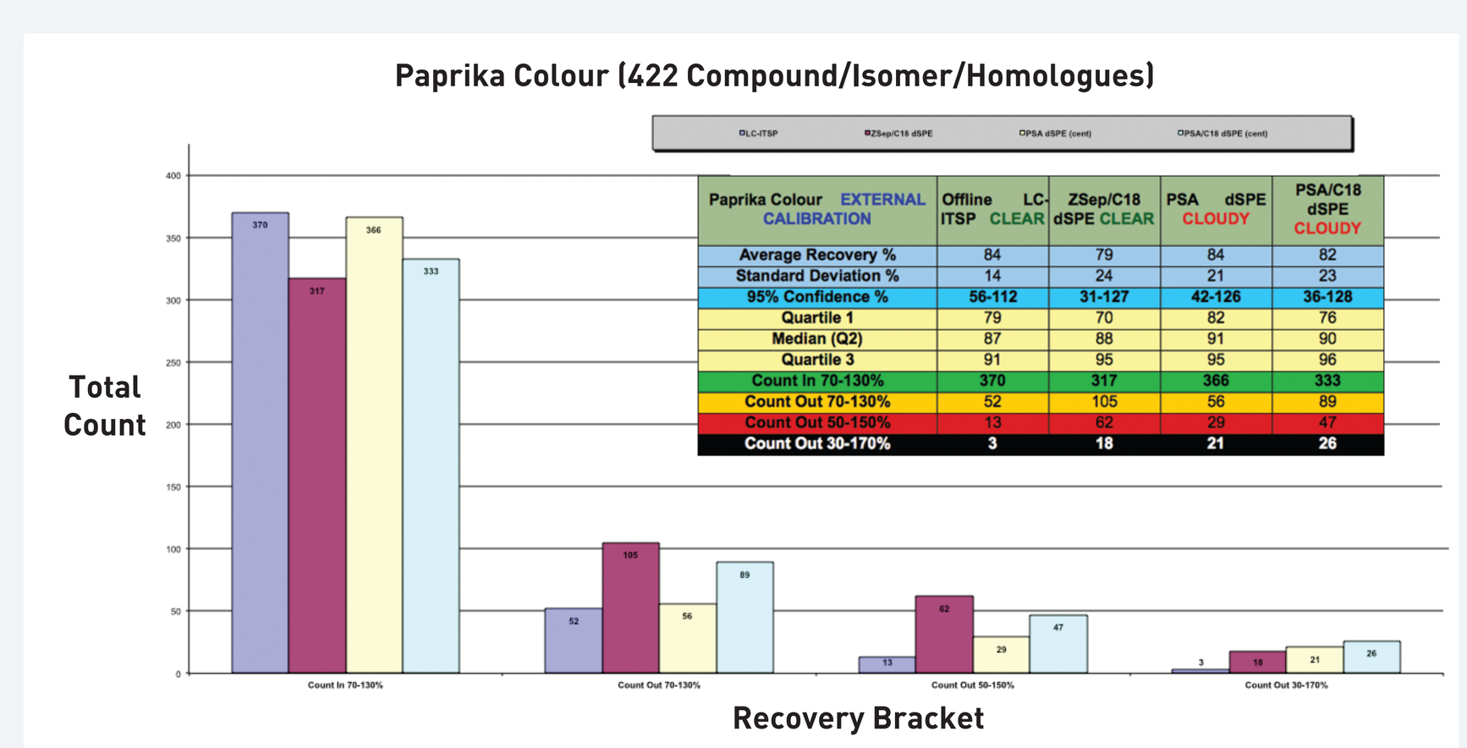
MARIGOLD OLEORESIN

Figure 9: Comparison of LC-MS/MS pesticide recoveries from Marigold oleoresin extract,cleaned up by ITSP or dSPE, calculated against solvent-only calibration standards.



PAPRIKA COLOR

Figure 10: Comparison of LC-MS/MS pesticide recoveries from Paprika color extract,cleaned up by ITSP or dSPE, calculated against solvent-only calibration standards.



5. DISCUSSION

QuEChERS methods commonly use matrix-matched calibration standards, to overcome suppression or enhancement of instrument signals by sample matrix. Non-polar matrix (oils) can be problematic for LC-MS/MS analyses of more difficult sample types, such as spices/food ingredients. Z-Sep/C-18 dSPE has been shown to be more effective than PSA/C-18 at removing oils.[2] Data presented here shows that Z-Sep/C-18, packed into an ITSP cartridge, removes more non-polar matrix than PSA dSPE and also gives improved pesticide recoveries compared with dSPE using Z-Sep/C-18, or PSA/C-18. Effective matrix removal by ITSP allows use of solvent-only LC-MS/MS calibration standards with acceptable recoveries of most pesticides in the large suite trialled (422 LC-MS/MS analyte peaks).

6. REFERENCES AND ACKNOWLEDGEMENTS

1. EN 15662: Determination of Pesticide Residues Using GC-MS and/or LC-MS (/MS) following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE - QuEChERS method. In European Committee for Standardization, Technical Committee CEN/TC 275; "Food analysis - Horizontal Methods"; Brussels, Belgium, 2008 ().
2. Increase 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 - http://www.sigmaaldrich.com/technical-documents/articles/analytical/food-beverage/avocado-extracts-zsep.html#sthash.ZMJzHRdC.dpuf.

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