The New PAL Micro-SPE Cartridge for Automated Pesticides Extraction and Clean-up

Micro-SPE for Green Analytical Chemistry

PAL SYSTEM
Ingenious sample handling

Solid Phase Extraction

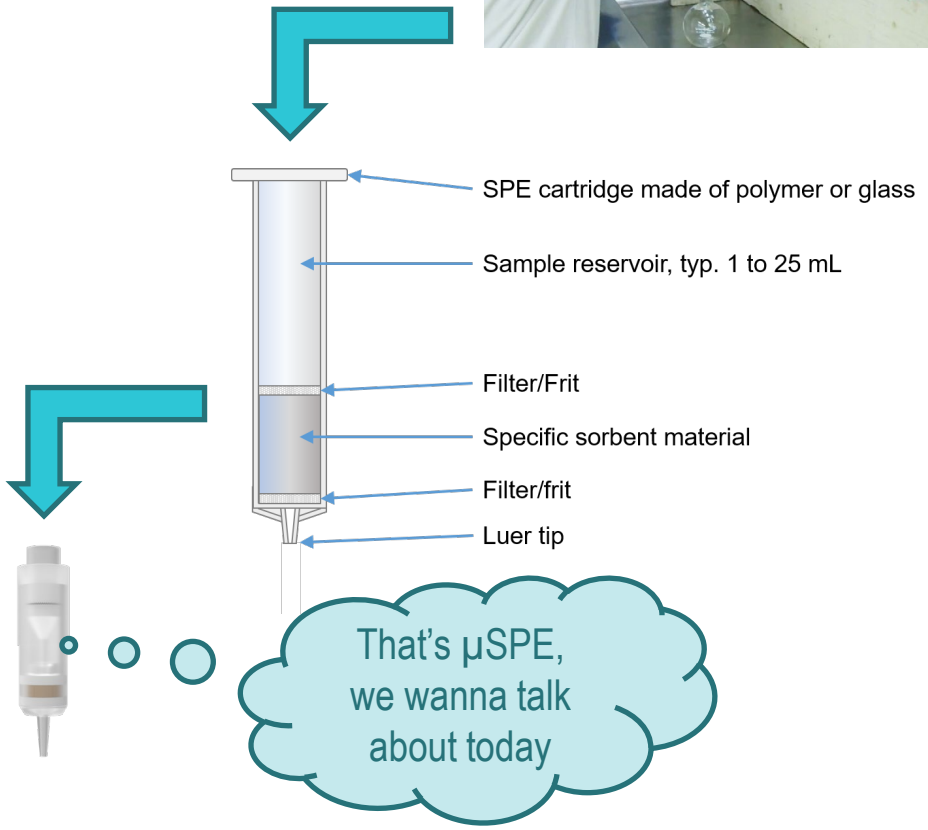
A quick look back and where do we go today

- ‘The separating funnel is a museum piece’

- Modern SPE originated in 1974
  - by Reginald Adams, Thomas Good, and Michael Telepchak
  - First dispersive (dSPE)
  - Later cartridge formats

- Much simpler for the lab
  - Less sorbent material
  - Less solvent
  - Faster
  - More concentrated analytes
  - More selective
  - Compatible with GC-MS and LC-MS

Exactly the same is true again for µSPE

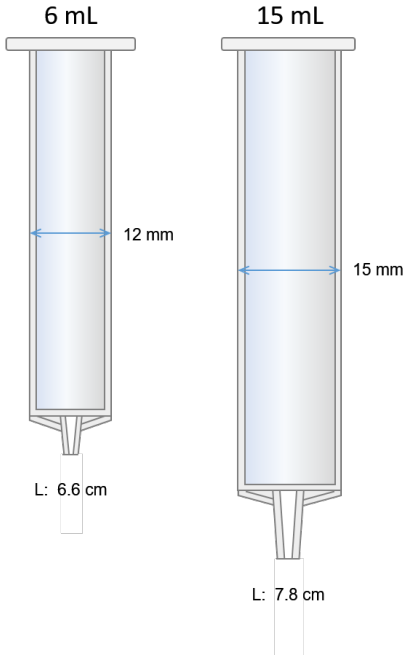


What is Micro-SPE (µSPE)

Compare to the classical cartridge SPE

Classical SPE

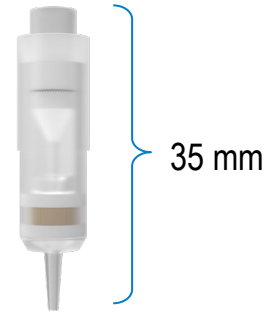
- Limited selectivity
  - High sample and solvent volumes
  - Requires evaporation with N_2
  - End volume >>100 µL in vial
- Vacuum operated
- Drying before elution
- Manual operation
  - Time consuming
  - Low sample throughput
  - Batch processing
- No QA/QC
  - As of manual operation



µSPE

- High selectivity
  - Sharp elution peak profile,
  - Compares to LC separation
  - No dilution, no concentration
  - Final eluates < 100 µL (or online)
- Positive pressure w liquid syringe
  - Very low solvent use
- No drying step
- Walk away automation
  - Fast
  - Works on chromatographic timescale
  - High productivity
- Traceable
  - Processing well documented

Works like a mini LC column !



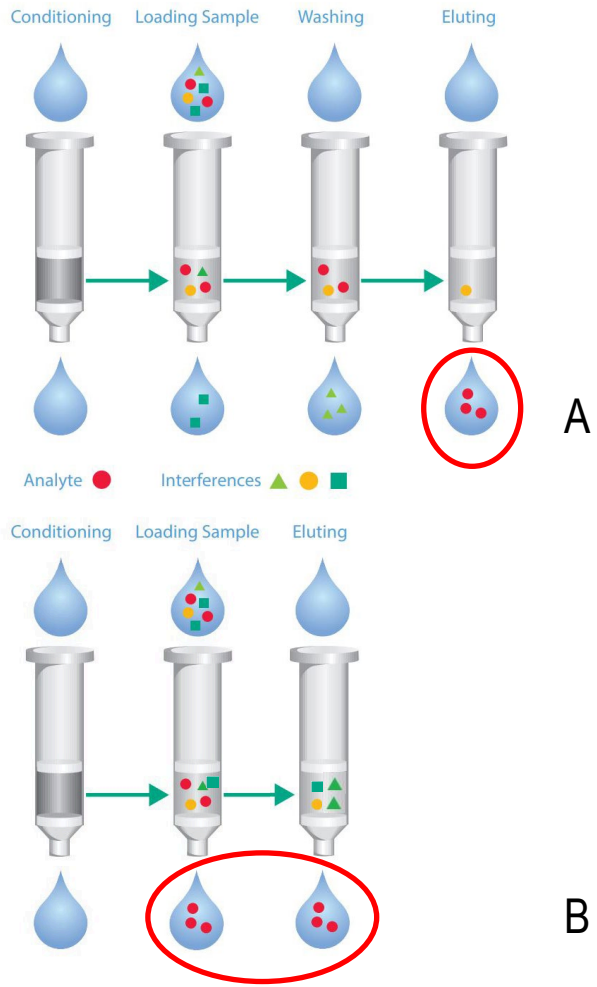
OD 8.5 mm

SPE Workflows

Both available for µSPE

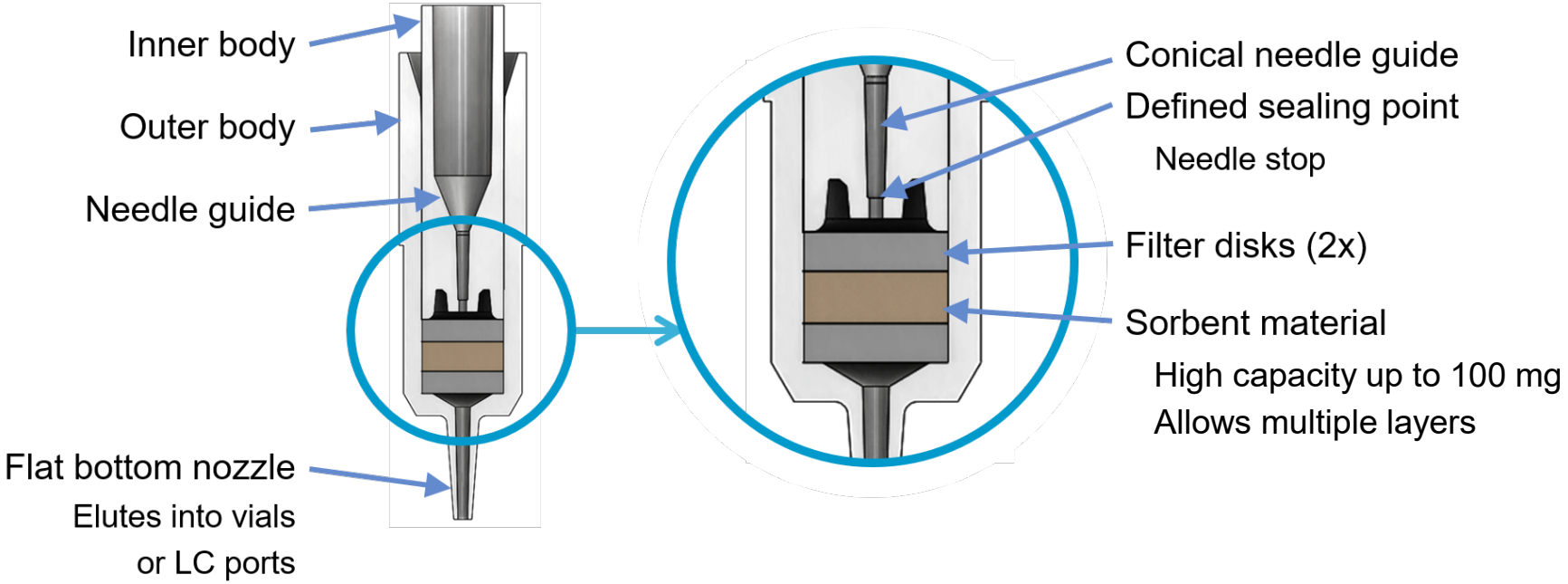
- **“Enrichment” mode** (the “classical” procedure)
  - aka Load-Wash-Elute mode
  - Analytes ● retained
    - Matrix ▲■ washed away
  - Analytes ● eluted by solvent change
    - e.g. with C18, or SAX material for Glyphosate, AMPA in EURL Almeria

- **“Scavenging” mode**
  - Matrix ▲■ retained
    - kept on cartridge
  - Analytes ● elute with extract
    - e.g. for ‘QuEChERS’ with acetonitrile, or ‘SweEt’ with ethyl acetate
      - Pesticides in Hill Labs, USDA Lehotay, Zurich Kanton Lab, EURL Almeria …)
      - C18 material for veterinary drugs analysis



Inside the µSPE Cartridge

How does the µSPE Cartridge work

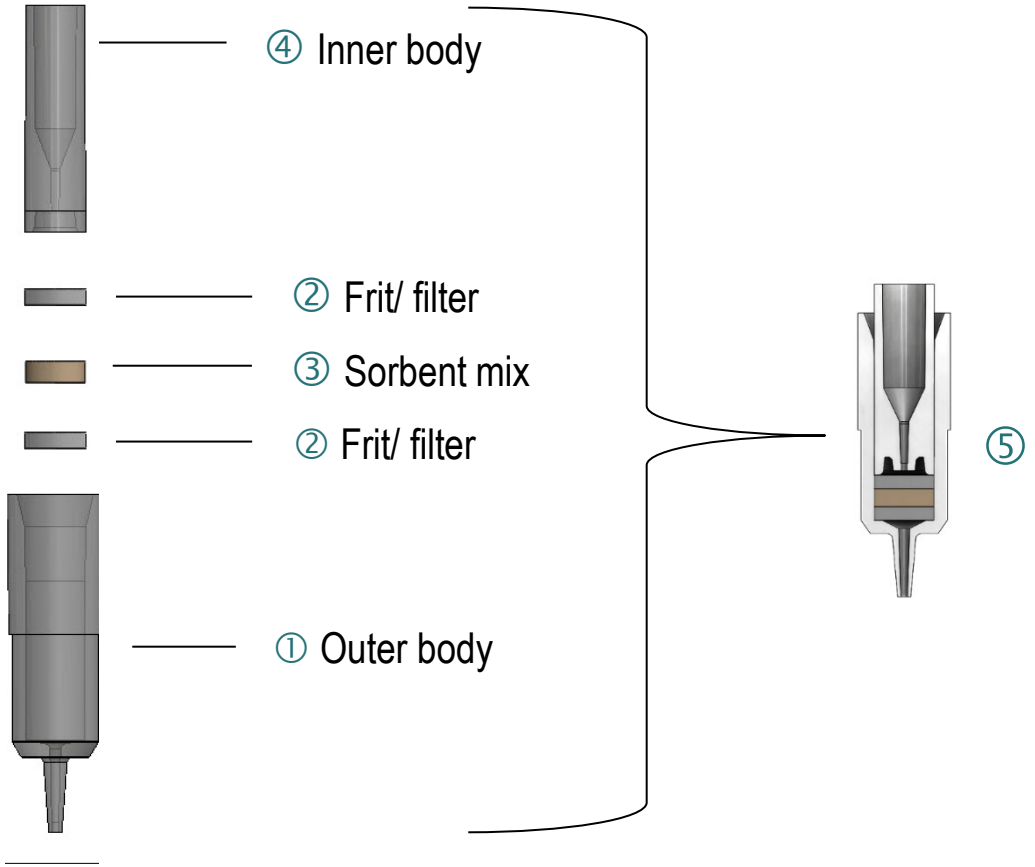


- The µSPE cartridges offer
  - combinations of sorbent materials - as used for the QuEChERS clean-up,
  - customized and proprietary sorbents are available,
  - just filter materials of different pore sizes, e.g. for LC and IC applications.

PAL System µSPE – Automated Cartridge Assembly

Automated cartridge filling and assembly with built in QC

- PAL System µSPE cartridges components



PAL System µSPE - QC and Reporting

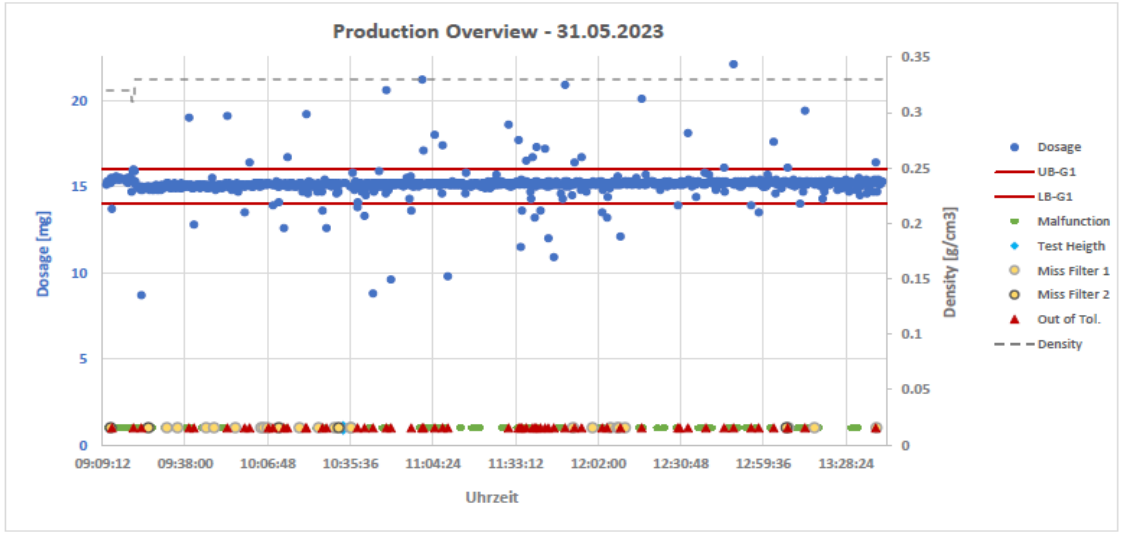
Automated QC reporting and checks

- Production of sorbent mixture (dry storage)
- Testing on the dosed amount of powder (weight)
- Check for correct packing density of the powder (height)
- Lot number assignment
- Packing and labelling of cartridges from a produced lot
- Dry storage of packed cartridges

- Lot tracking of production
  - Sorbent amount (weight)
  - Sorbent packing (height)

- Certificate of Conformity for each lot
  - Filling Amount: Target weight ± 10%
  - Overall cartridge height Target height ± 1.0mm

Production Control



		Statistics	
S	Mean Value and Deviation	Average CF	15.13 mg
		Std. Dev.	1.00 mg
G1	Percentile Quality level limits	Perc. 0.05	14.70 mg
		Perc. 0.95	15.47 mg
E	Limits- / Extreme values	Min. Value	-0.10 mg
		Max. Value	25.00 mg
F	Out of Quality Reasons	Out of Tolerance	58 pcs
		Missing Filter	22 pcs
		IBS Height Out of Tol.	1 pcs

PAL System µSPE - QC and Reporting

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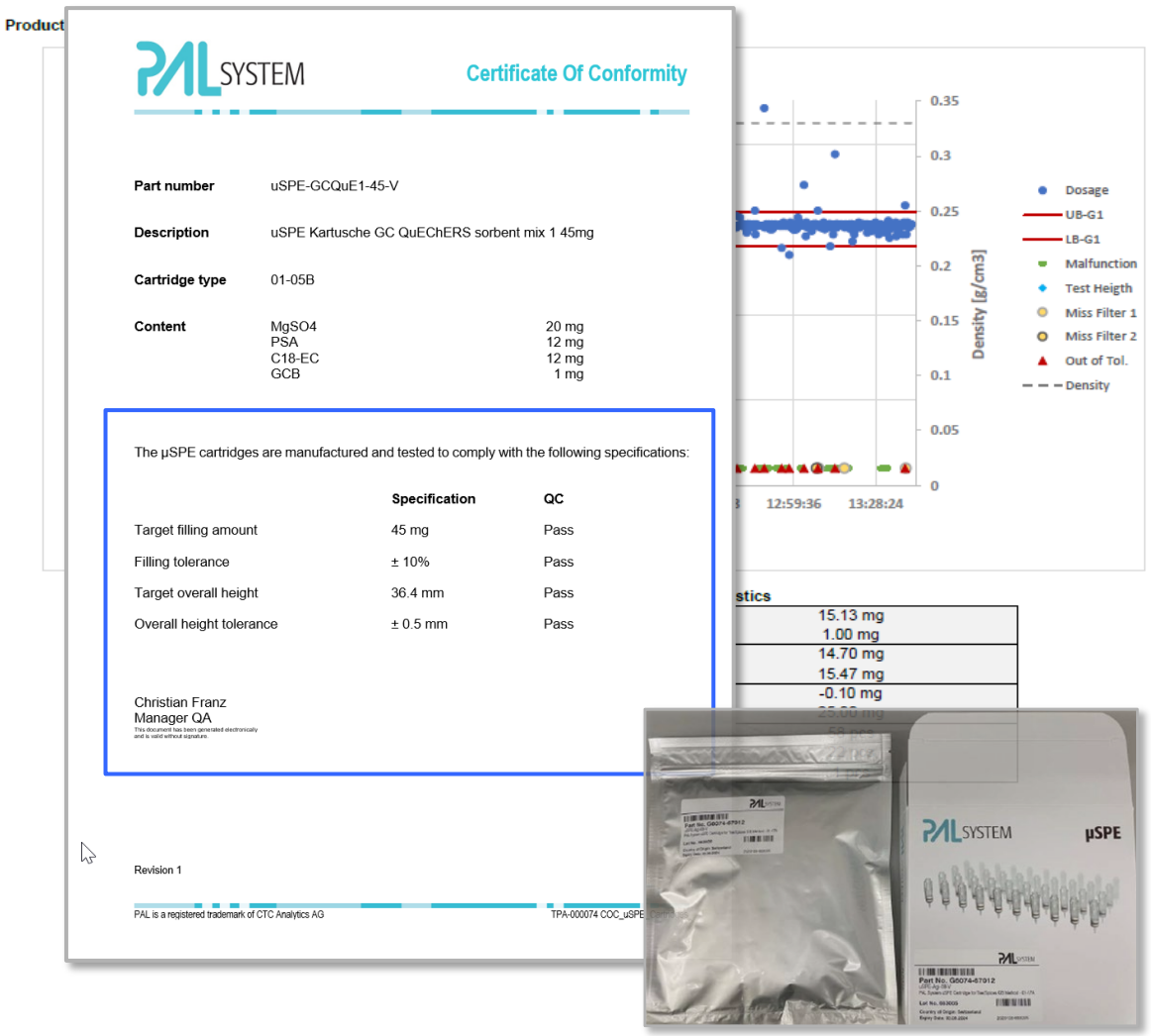
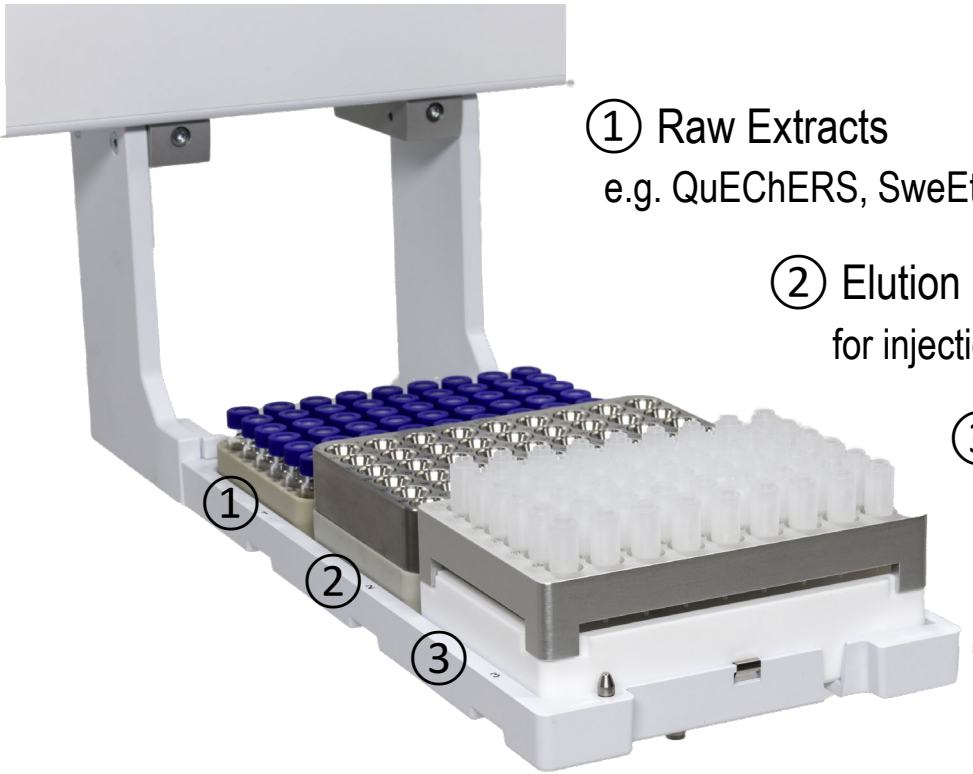


How Does µSPE Work on a PAL System

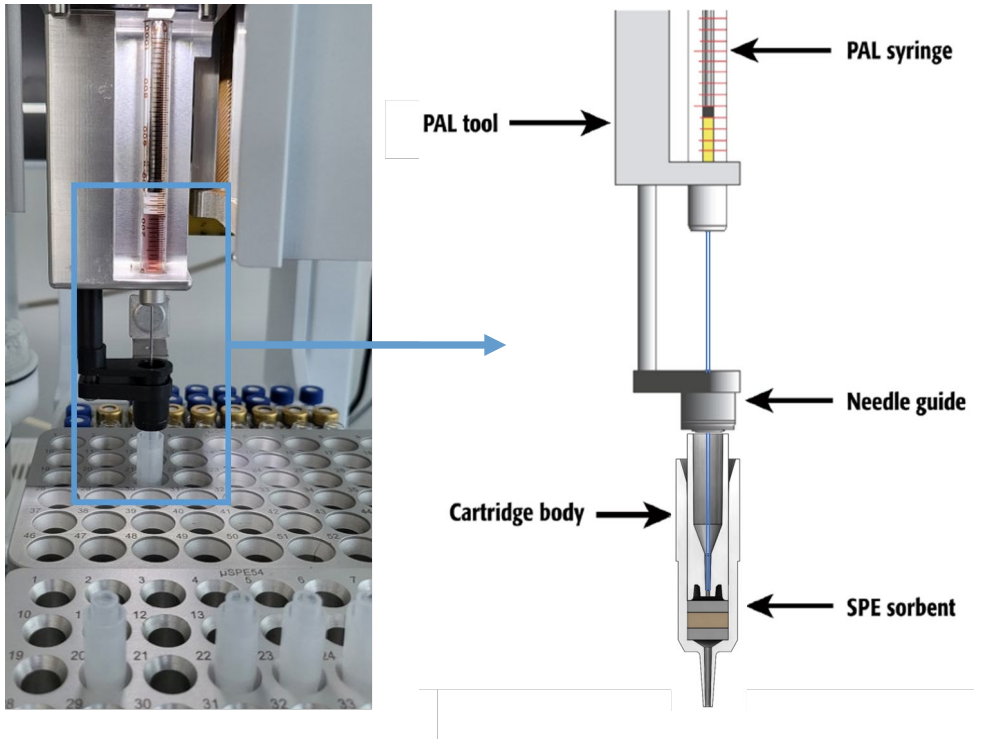
One Trayholder is installed on a PAL System (upgradeable)



① Raw Extracts
e.g. QuEChERS, SweEt, or other ...

② Elution - Cleaned Extracts
for injection GC-MS, LC-MS

③ µSPE cartridges
for opt. conditioning



Cartridge transport on syringe needle
for elution and return/waste bin

Standard QuEChERS Protocol*

10 g of sample

Shake 1 min

Salt out

Shake 1 min

Centrifuge

Dispersive SPE

Shake 1 min

Centrifuge

Analysis

Use 50 mL tubes
Add 10 mL acidified acetonitrile
Add ISTD (Triphenylphosphate)

4 g $MgSO_4$ anh.,
1 g NaCl,
1 g Citrate buffer (CEN 15662)
1 g Acetate buffer (AOAC 2007.01)

- Freeze step for fatty samples, or GLP clean-up
- Direct LCMS analysis of polar pesticides

1 mL aliquot transferred into 10 mL tubes,
add PSA, $MgSO_4$, C18, GCB, Chlorofiltr, ...
as required by the food commodity

LLE Extraction

dSPE Clean-up

* *QuEChERS - Mini-Multiresidue Method for the Analysis of Pesticides, M. Anastassiades, 2003*

QuEChERS Protocol* with µSPE Clean-up

10 g of sample

Shake 1 min

Salt out

Shake 1 min

Centrifuge

Dispersive SPE

Shake 1 min

Centrifuge

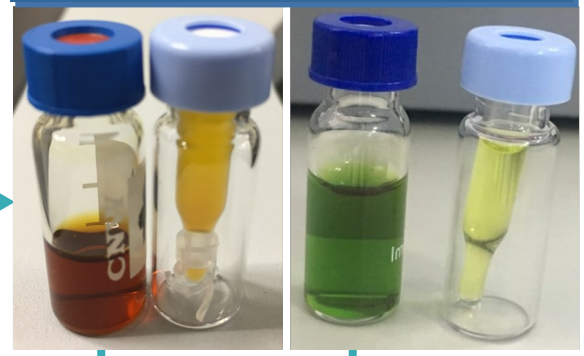
Use 50 mL tubes
Add 10 mL acidified acetonitrile
Add ISTD (Triphenylphosphate)

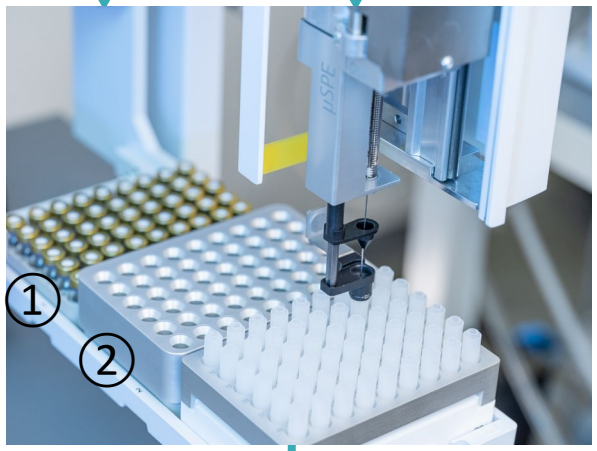
➡ Add buffer kit, AOAC 2007.1/EN15662

➡ No freeze - No GPC

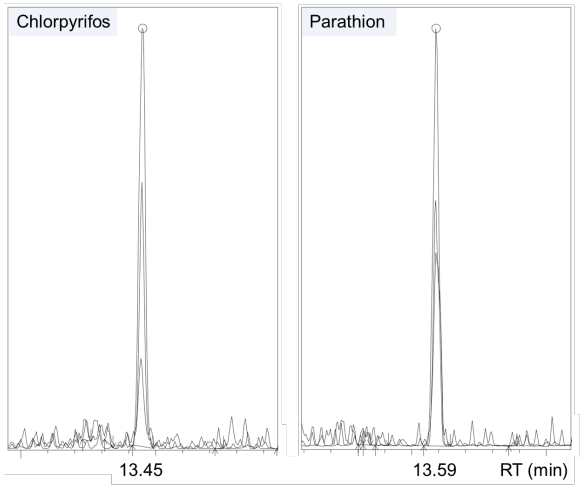
➡ **Take 1 mL for µSPE clean-up**

Black and green tea samples





GC-MS and LC-MS Injection



Chlorpyriphos 1 ppb
RT 13.45 min
m/z 313.90 > 285.90
m/z 313.90 > 193.90

Parathion 1 ppb
RT 13.59 min
m/z 291.10 > 137.00
m/z 291.10 > 81.00

Pesticides Clean-up by Hill Laboratories, Hamilton, NZ

PAL SYSTEM
Ingenious sample handling

More than 10 Years Experience for > 900 Matrices, >1000 Samples/wk

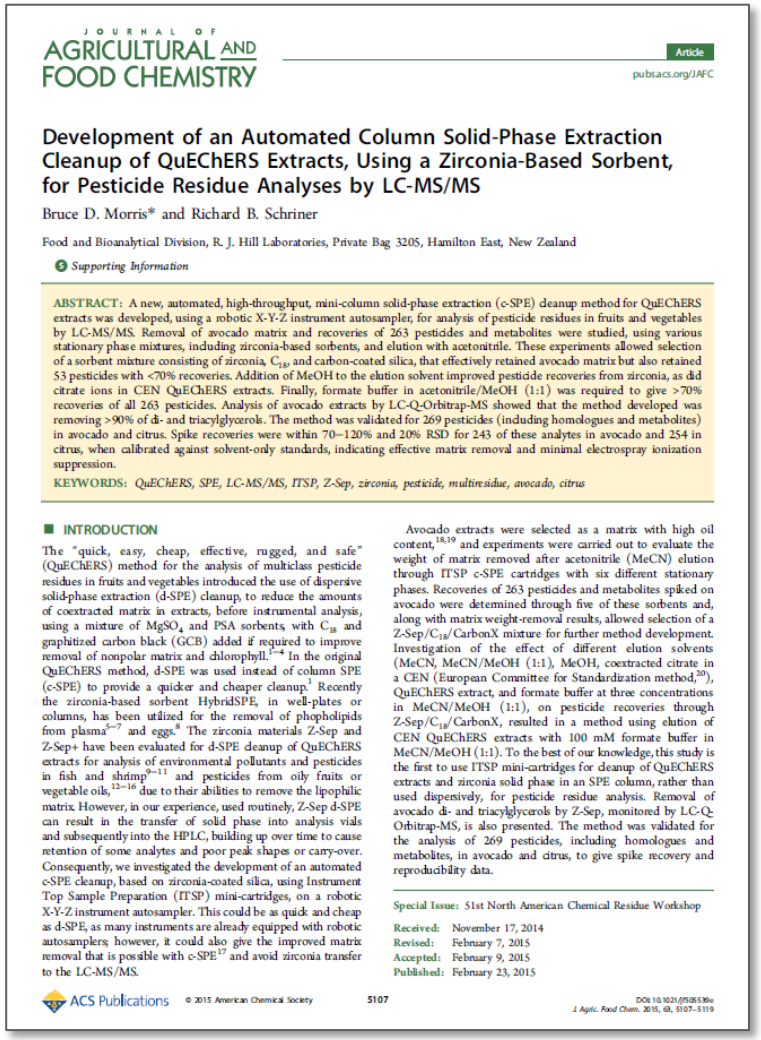
- Automated, compared to original QuEChERS
- Reduced manual labor in sample prep
- Wider range of samples

  high lipid content, incl. Avocados, with difficult matrices like dried herbs and spices
- Off-line clean-up on PAL Systems

  only 1/5 of analysis times, serves several GC-MS and LC-MS systems.

µSPE GC-MS clean-up		
Sorbent	Amount	Percentage
PSA	12 mg	27%
C18	12 mg	27%
GCB	1 mg	2%
MgSO4	20 mg	44%
Total	45 mg	100%

µSPE LC-MS clean-up		
Sorbent	Amount	Percentage
Z-Sep	8 mg	27%
C18	21 mg	70%
GCB	1 mg	3%
Total	30 mg	100%



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Development of an Automated Column Solid-Phase Extraction Cleanup of QuEChERS Extracts, Using a Zirconia-Based Sorbent, for Pesticide Residue Analyses by LC-MS/MS

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Food and Bioanalytical Division, R. J. Hill Laboratories, Private Bag 3205, Hamilton East, New Zealand

Supporting Information

ABSTRACT: A new, automated, high-throughput, mini-column solid-phase extraction (c-SPE) cleanup method for QuEChERS extracts was developed, using a robotic X-Y-Z instrument autosampler, for analysis of pesticide residues in fruits and vegetables by LC-MS/MS. Removal of avocado matrix and recoveries of 263 pesticides and metabolites were studied, using various stationary phase mixtures, including zirconia-based sorbents, and elution with acetonitrile. These experiments allowed selection of a sorbent mixture consisting of zirconia, C_{18}, and carbon-coated silica, that effectively retained avocado matrix but also retained 53 pesticides with <70% recoveries. Addition of MeOH to the elution solvent improved pesticide recoveries from zirconia, as did citrate ions in CEN QuEChERS extracts. Finally, formate buffer in acetonitrile/MeOH (1:1) was required to give >70% recoveries of all 263 pesticides. Analysis of avocado extracts by LC-Q-Orbitrap-MS showed that the method developed was removing >90% of di- and triacylglycerols. The method was validated for 269 pesticides (including homologues and metabolites) in avocado and citrus. Spike recoveries were within 70−120% and 20% RSD for 243 of these analytes in avocado and 254 in citrus, when calibrated against solvent-only standards, indicating effective matrix removal and minimal electrospray ionization suppression.

KEYWORDS: *QuEChERS, SPE, LC-MS/MS, ITSP, Z-Sep, zirconia, pesticide, multiresidue, avocado, citrus*

■ INTRODUCTION

The "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method for the analysis of multiclass pesticide residues in fruits and vegetables introduced the use of dispersive solid-phase extraction (d-SPE) cleanup, to reduce the amounts of coextracted matrix in extracts, before instrumental analysis, using a mixture of $MgSO_4$ and PSA sorbents, with C_{18} and graphitized carbon black (GCB) added if required to improve removal of nonpolar matrix and chlorophyll.[1−4] In the original QuEChERS method, d-SPE was used instead of column SPE (c-SPE) to provide a quicker and cheaper cleanup.[1] Recently the zirconia-based sorbent HybridSPE, in well-plates or columns, has been utilized for the removal of phospholipids from plasma[5−7] and eggs.[8] The zirconia materials Z-Sep and Z-Sep+ have been evaluated for d-SPE cleanup of QuEChERS extracts for analysis of environmental pollutants and pesticides in fish and shrimp[9−11] and pesticides from oily fruits or vegetable oils,[12−16] due to their abilities to remove the lipophilic matrix. However, in our experience, used routinely, Z-Sep d-SPE can result in the transfer of solid phase into analysis vials and subsequently into the HPLC, building up over time to cause retention of some analytes and poor peak shapes or carry-over. Consequently, we investigated the development of an automated c-SPE cleanup, based on zirconia-coated silica, using Instrument Top Sample Preparation (ITSP) mini-cartridges, on a robotic X-Y-Z instrument autosampler. This could be as quick and cheap as d-SPE, as many instruments are already equipped with robotic autosamplers; however, it could also give the improved matrix removal that is possible with c-SPE[17] and avoid zirconia transfer to the LC-MS/MS.

Avocado extracts were selected as a matrix with high oil content,[18,19] and experiments were carried out to evaluate the weight of matrix removed after acetonitrile (MeCN) elution through ITSP c-SPE cartridges with six different stationary phases. Recoveries of 263 pesticides and metabolites spiked on avocado were determined through five of these sorbents and, along with matrix weight-removal results, allowed selection of a Z-Sep/C_{18}/CarbonX mixture for further method development. Investigation of the effect of different elution solvents (MeCN, MeCN/MeOH (1:1), MeOH, coextracted citrate in a CEN (European Committee for Standardization method,[20]), QuEChERS extract, and formate buffer at three concentrations in MeCN/MeOH (1:1), on pesticide recoveries through Z-Sep/C_{18}/CarbonX, resulted in a method using elution of CEN QuEChERS extracts with 100 mM formate buffer in MeCN/MeOH (1:1). To the best of our knowledge, this study is the first to use ITSP mini-cartridges for cleanup of QuEChERS extracts and zirconia solid phase in an SPE column, rather than used dispersively, for pesticide residue analysis. Removal of avocado di- and triacylglycerols by Z-Sep, monitored by LC-Q-Orbitrap-MS, is also presented. The method was validated for the analysis of 269 pesticides, including homologues and metabolites, in avocado and citrus, to give spike recovery and reproducibility data.

Special Issue: 51st North American Chemical Residue Workshop

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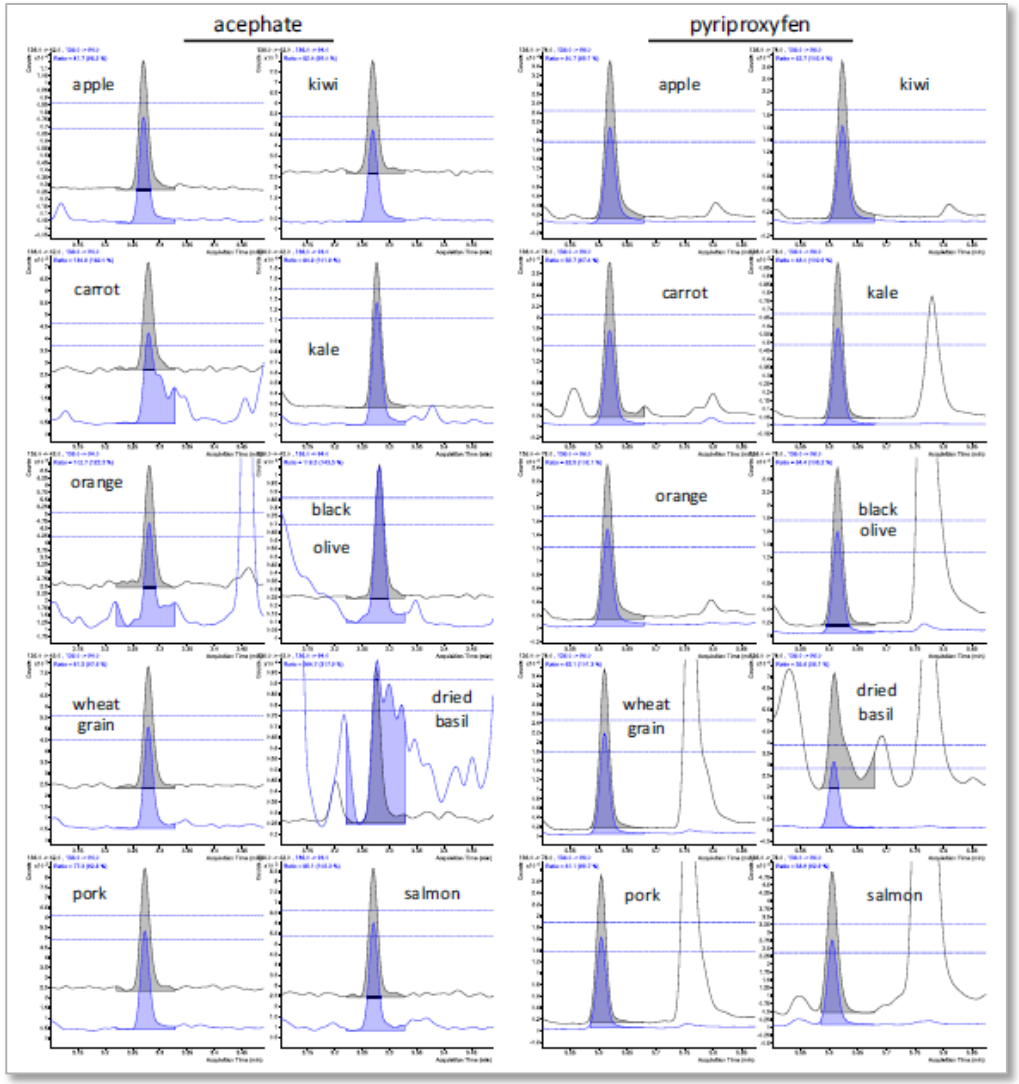
DOI: 10.1021/jf505630e
J. Agric. Food Chem. 2015, 63, 5107−5119

B.Morris, R.Schriner (2015) J. Agi. Food. Chem. 63, 5107-19.

Automated µSPE Clean-up for GC-MS - Only 8 min *

PAL SYSTEM
Ingenious sample handling

1. Take a 1 mL syringe
2. Wash the 1 mL syringe with MeCN
3. **Take 500 µL raw extract** from rack 1 into 1 mL syringe
4. Get the µSPE cartridge from rack 3 with the syringe needle
5. Move the cartridge to the elution rack 2
6. **Push the raw extract through the µSPE cartridge** at 5 µL/s
7. Discard µSPE cartridge into waste beaker
8. Wash the 1 mL syringe with MeCN/MeOH/water (vol 1/1/1)
9. Wash the 1 mL syringe with MeCN
10. Switch to 100 µL syringe and wash with MeCN
11. **Add 25 µL AP + QC solutions** to the collection vial in rack 2
12. Wash the 100 µL syringe with MeCN/MeOH/water (vol 1/1/1)
13. Wash the 100 µL syringe with MeCN
14. Switch to 10 µL GC injection syringe
15. Wash the 10 µL syringe with MeCN
16. Aspirate the cleaned extract from the elution vial in rack 2
17. **Inject** 1 µL of extract to GC-MS/MS
18. Wash the 10 µL syringe with MeCN



* as of Steven J. Lehotay, Lijun Han, Yelena Sapozhnikova (2016) and Nicolas Michlig, Steven J. Lehotay (2022)

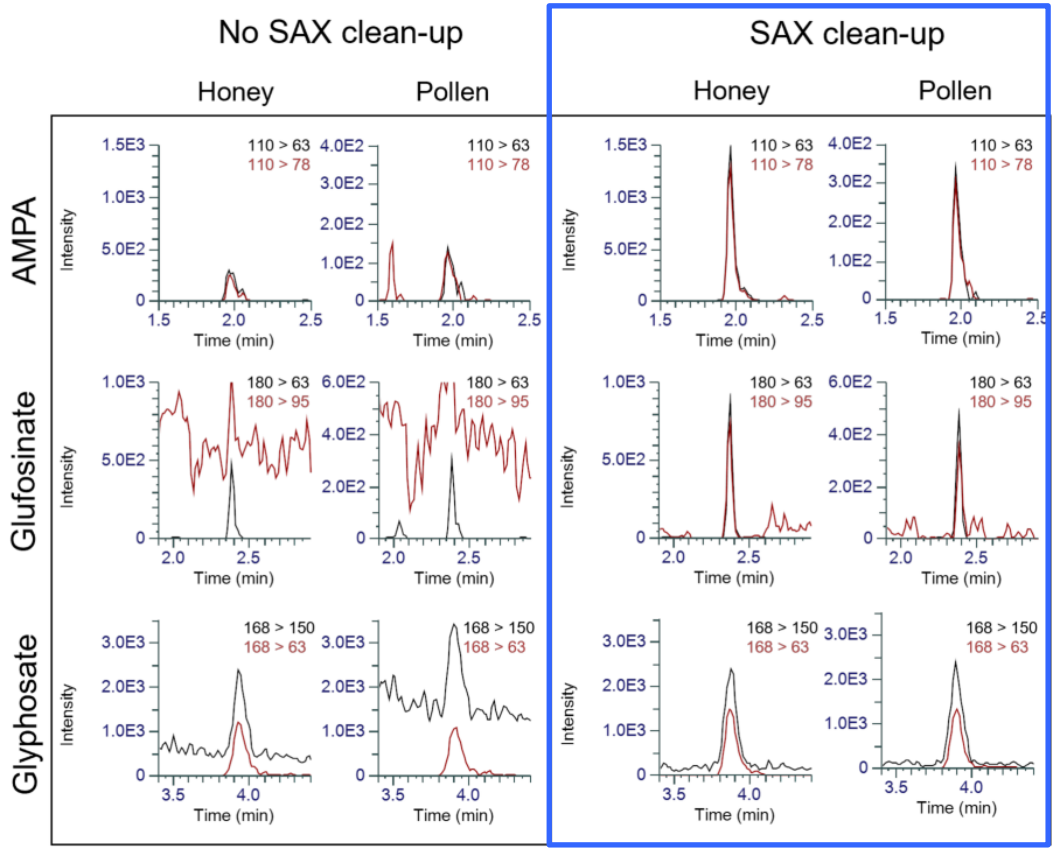
Highly Polar Pesticides in Complex Matrices (QuPPe)

Glyphosate, AMPA, Glufosinate, … EURL for Pesticides in Fruit and Vegetables, Almeria, Spain

- Matrix honey, pollen, coffee beans
- Acidified methanol extraction
- Automated µSPE clean-up
  50 mg SAX (strong ion exchange)

- Clean-up procedure
  1000 µL methanolic raw extract
  Load at 5 µL/s
  Matrix washed with 600 µL methanol
  Analytes elute with 400 µL methanol/HCl (9:1)
- Inject 10 µL to LC-MS/MS

- Cost saving: 500 mg → 50 mg SAX material
- Time saving: manual → automated 10:1
- Analytical: Improved recoveries up from avg. 70 → 86%



Extracted LC-MS/MS ion chromatograms of standards of AMPA, glufosinate, and glyphosate at 0.010 mg/kg spiked to honey and pollen matrix without (left) and after SAX clean-up (right)

Jesus, F., A. R. García, et al. 2023. "Determination of Highly Polar Anionic Pesticides in Beehive Products by Hydrophilic Interaction Liquid Chromatography Coupled to Mass Spectrometry." Anal. Bioanal. Chem. https://doi.org/10.1007/s00216-023-04946-7.

Ethylacetate Extraction of Pesticides from Foods (*aka* SweEt)

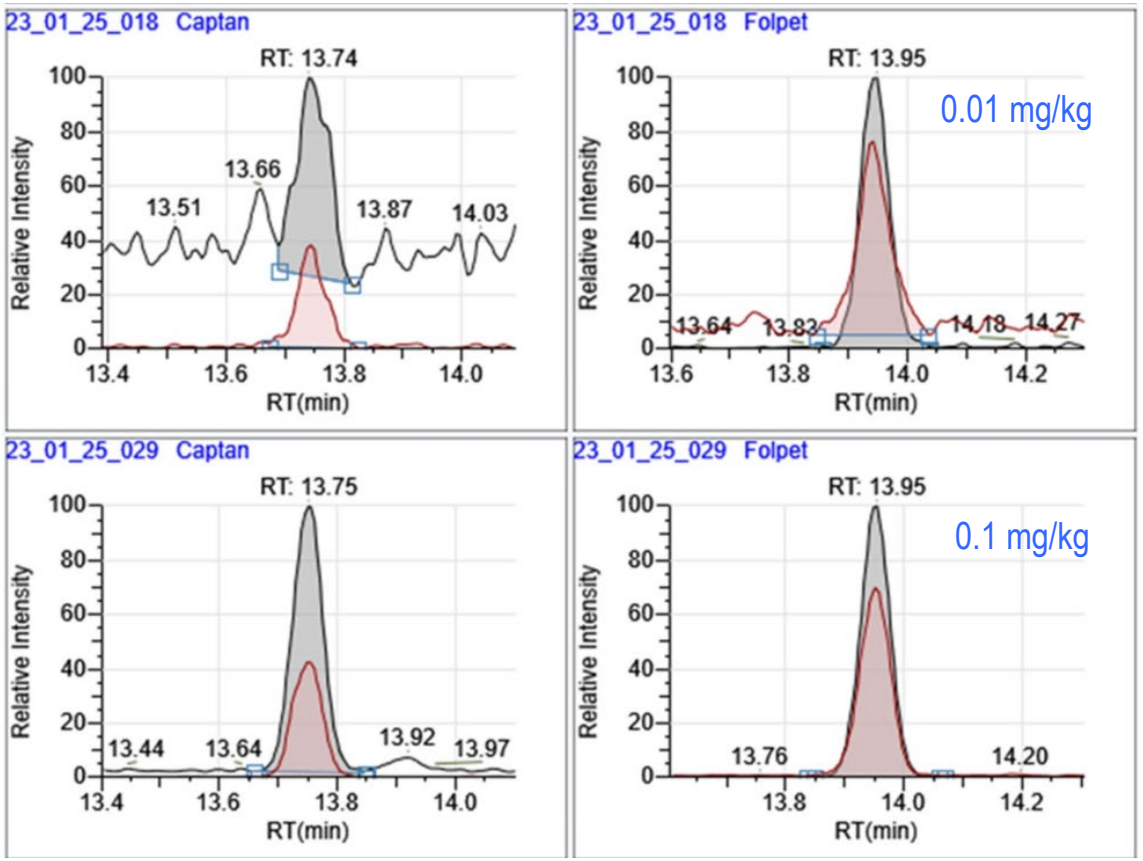
PAL SYSTEM Ingenious sample handling

Big time savings and reduced manual effort for high fat matrices – Cantonal Lab. Zurich, CH

- EtOAc extracts wider range of polar pesticides
- But, also extract high amounts of matrix
- GPC or extract freezing was used as clean-up

- Clean-up using µSPE
  - 45 mg of PSA, C18, GCB, $MgSO_4$
  - Load 200 µL raw EtOAc extract, 2 µL/s
  - Blow-out 1 mL air
- Injection 3 µL cleaned extract to GC-MS/MS

- Significant improvements:
  - Difficult and fatty samples e.g. dried spices, egg, avocado, or liver are successfully processed,
  - Captan and Folpet with good recoveries.
  - One µSPE cartridge for all food matrices.
  - No time-consuming freeze-out or GPC required anymore.



Captan and folpet in raspberry samples after µSPE clean-up.

Schürmann, A., C. Crüzer, et al. 2023 "Automated Micro-Solid-Phase Extraction Clean-up and Gas Chromatography-Tandem Mass Spectrometry Analysis of Pesticides in Foods Extracted with Ethyl Acetate." Anal. Bioanal. Chem. 416 (3): 689–700. https://doi.org/10.1007/s00216-023-05027-5.

Veterinary Drug Screening by online µSPE – LC-MS/MS

PAL SYSTEM
Ingenious sample handling

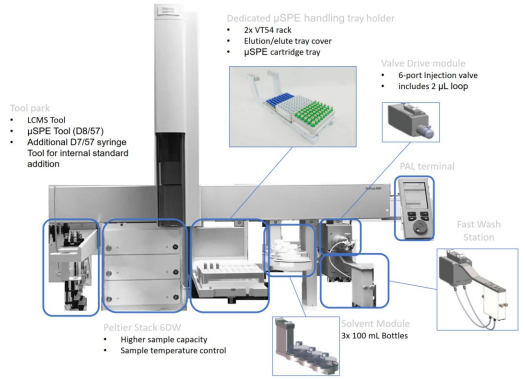
From the Veterinary Diagnostic Laboratory, Iowa State University, USA.

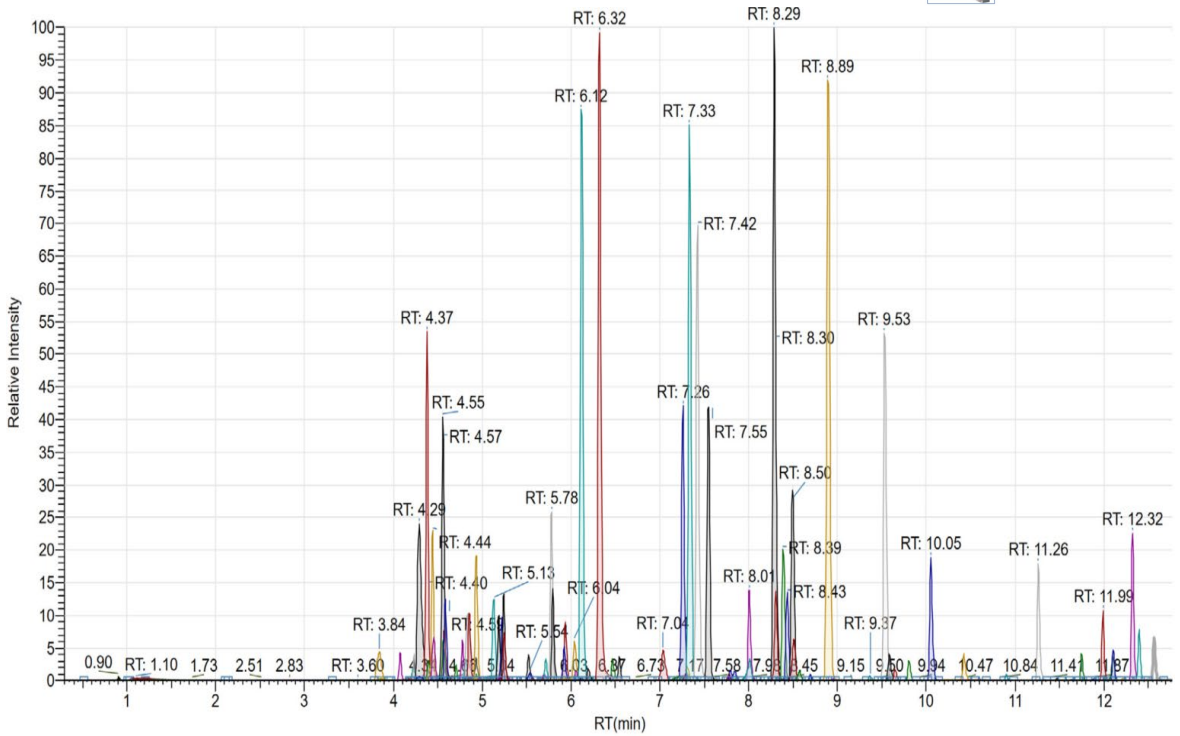
- Veterinary drugs are legally controlled in large number of samples in difficult matrix.
- QuEChERS extraction (LLE with MeCN)
  from 5 g muscle, or kidney

- Automated µSPE clean-up
  on Thermo Scientific™ TriPlus™ RSH sampler,
  µSPE cartridge with 15 mg of endcapped C18.
  300 µL of supernatant at 2 µL/s,
  the eluate diluted 3+1 with mobile phase,
  injected into a 2 µL loop on the injection valve.
  Clean-up takes only 8.5 min to complete

- Cost saving on C18: 500 mg → 15 mg (30x less)
- Time saving: 80 min/15 samples → Zero, online prep
- No additional consumables

103 veterinary drugs at 50 ng/g
in bovine kidney extract.
Total cycle time 15 min.





Schrunk, D., L.E. Burns, et al. 2021. “Multi-Class Veterinary Drugs Analyses of QuEChERS Extracts Using an Automated Online MSPE Cleanup Coupled to LC-MS MS”. Application Note, CTC Analytics AG.

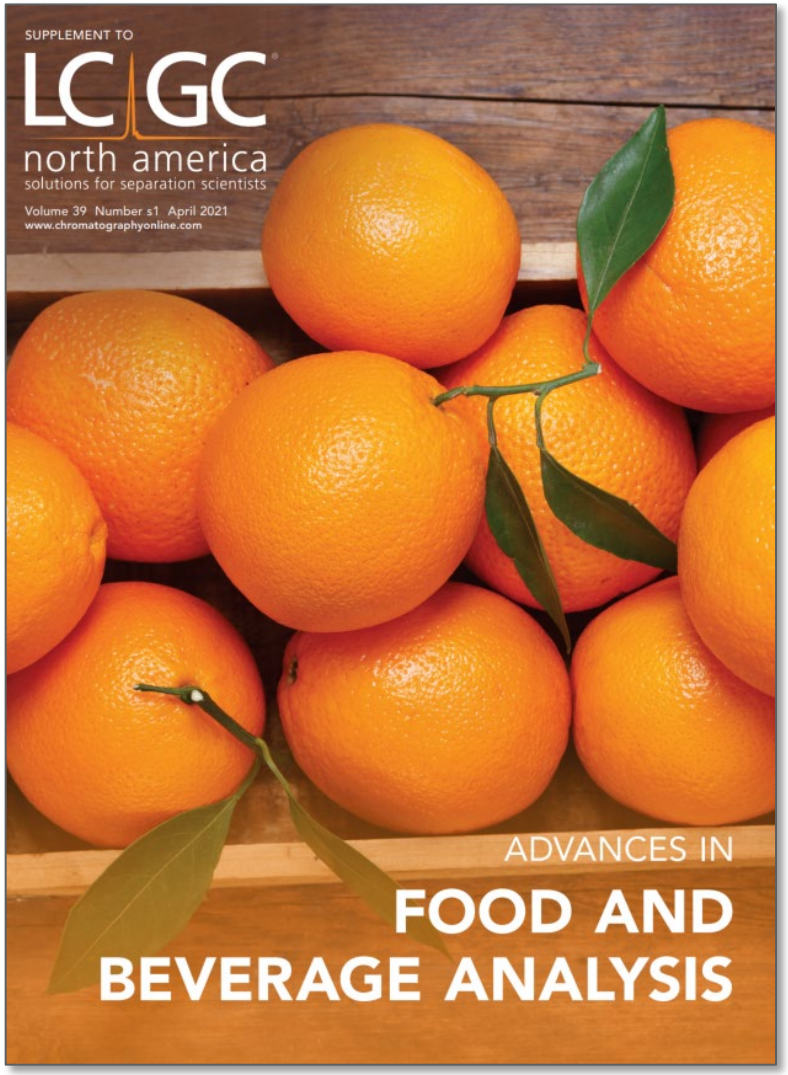
Automated QuEChERS Extraction and µSPE Clean-up

Application for homogeneous samples like beverages

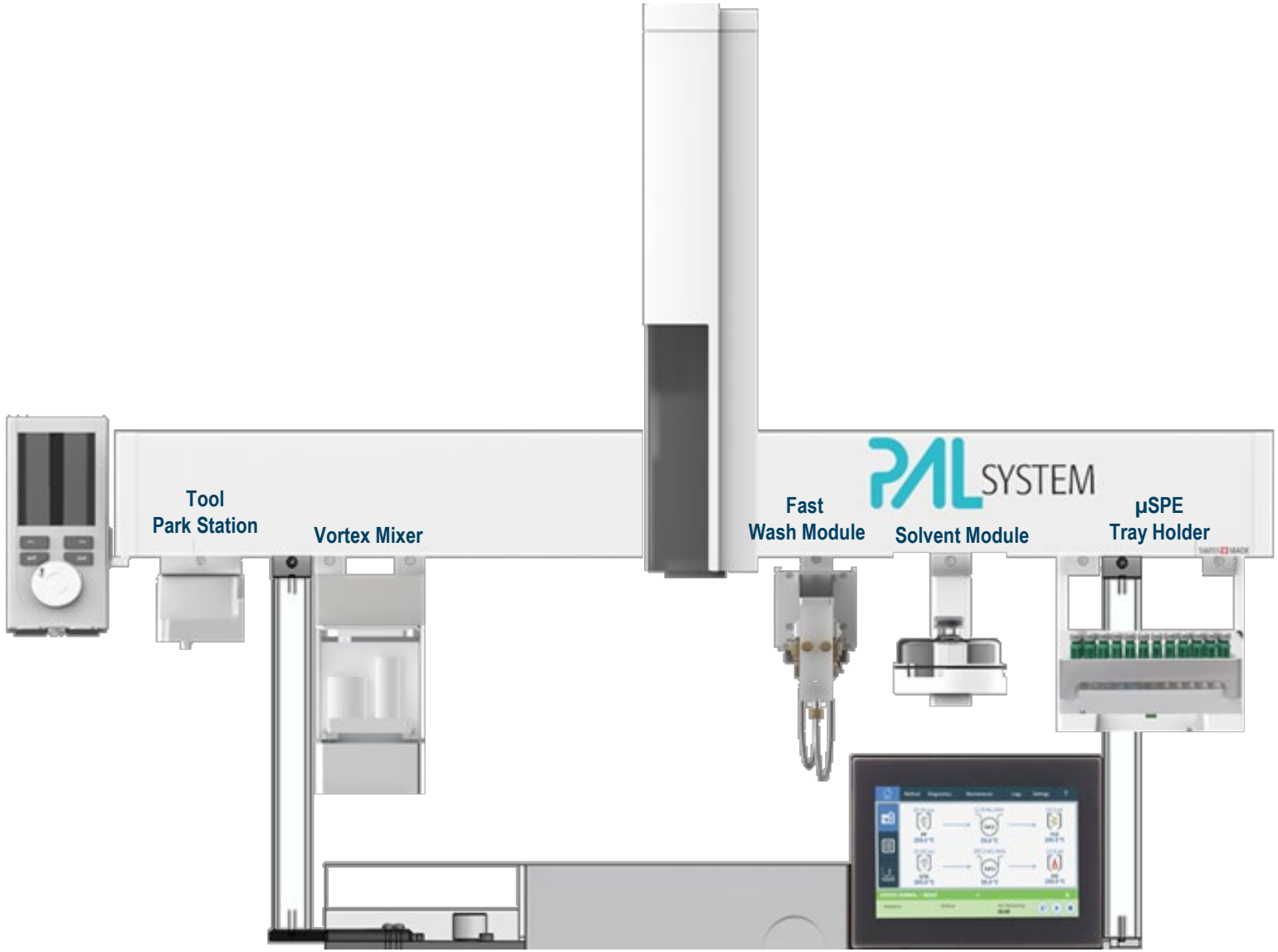
- Why not automate all steps? The QuEChERSs extraction with SPE clean-up?

- Homogenous samples
  - Do not need much manual treatment
  - Can be pipetted into 2 mL vials (also automatically!)
- QuEChERS extraction is automated in 2 mL vials
  - with buffer salts previously added.
  - Then added acidified MeCN,
  - sat. NaCl solution.
- µSPE clean-up
  - 45 mg of PSA, C18, GCB, $MgSO_4$
  - Load 250 µL raw extract, 2 µL/s
- Injection 3 µL cleaned extract to GC-MS/MS

- Combines all benefits
  - from QuEChERS, µSPE clean-up, and prep-ahead automation



PAL RTC System for QuEChERS, µSPE, and online GC-MS



Tool Park Station

- Pos.1 GC Injection syringe (10 µL)
- Pos.2 µSPE Tool (1000 µL)
- Pos.3 APs/ISTD syringe (25 µL)

Solvent Module

- Pos.1 Acetonitrile
- Pos.2 NaCl, sat.
- Pos.3 not used

Fast Wash Module

- Pos.1 Water
- Pos.2 Acetonitrile

QuEChERS Extraction and µSPE Clean-up Workflow

PAL SYSTEM
Ingenious sample handling

Liquid-Liquid Extraction

- Use Tool
- Repeat
  - Get Liquid From Vial
  - Dispense Liq. Into Vial
- Repeat End
- Vortex Vial
- Clean Syringe
- Get Liquid From Vial
- Dispense Liq. Into Vial
- Clean Syringe
- Vortex Vial
- Wait

µSPE

- Use Tool
- µSPE Elute
- Clean Syringe

Inject

- Liquid Injection
- Wait

1. Prepare 500 µL of Orange Juice into 2mL vial
2. Add 600 µL MeCN into Orange Juice sample
3. Shake
4. Add 250 µL sat. NaCl into sample vial, shake
5. Wait for phase separation from the liquid-liquid extraction
6. Load 250 µL of the orange extract to the µSPE clean-up
7. Inject 2 µL of the cleaned extract to GC-MS/MS

Orange juice

Orange juice + acetonitrile

+ NaCl for phase separation

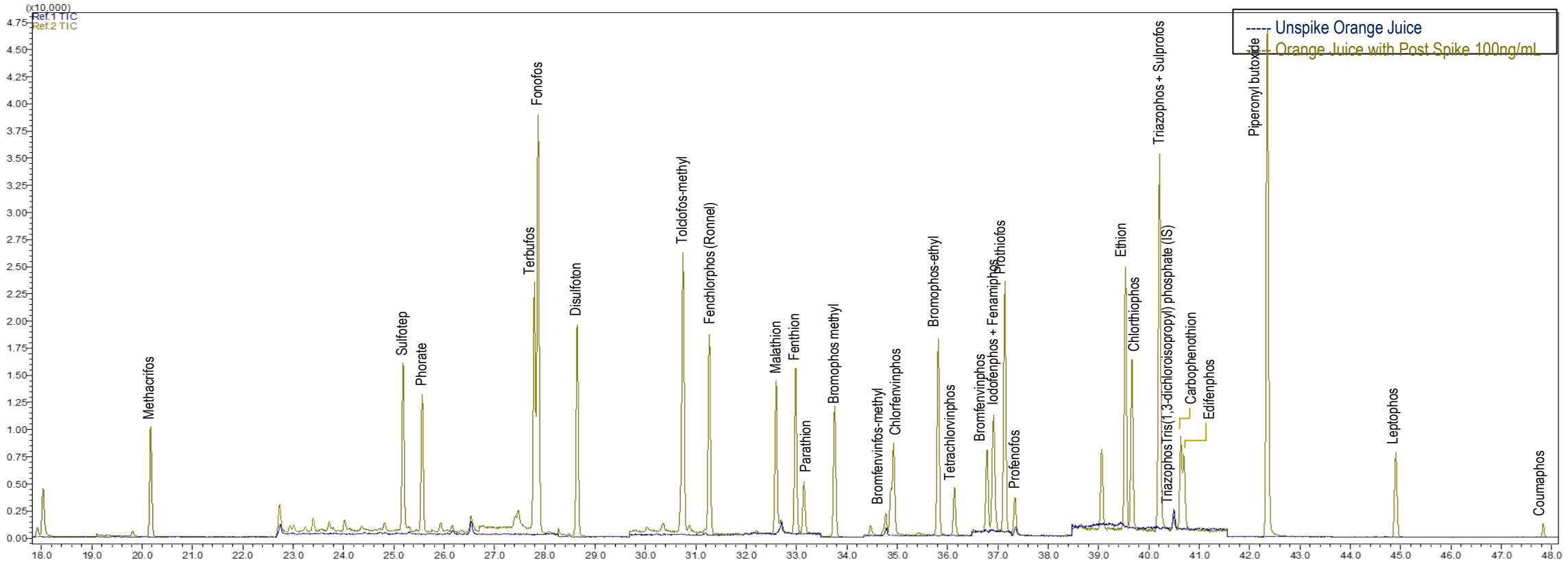
After µSPE clean up, for injection

OCP Pesticides PAL Extracted from Orange Juice

Total ion chromatogram from GC-MS/MS in MRM mode

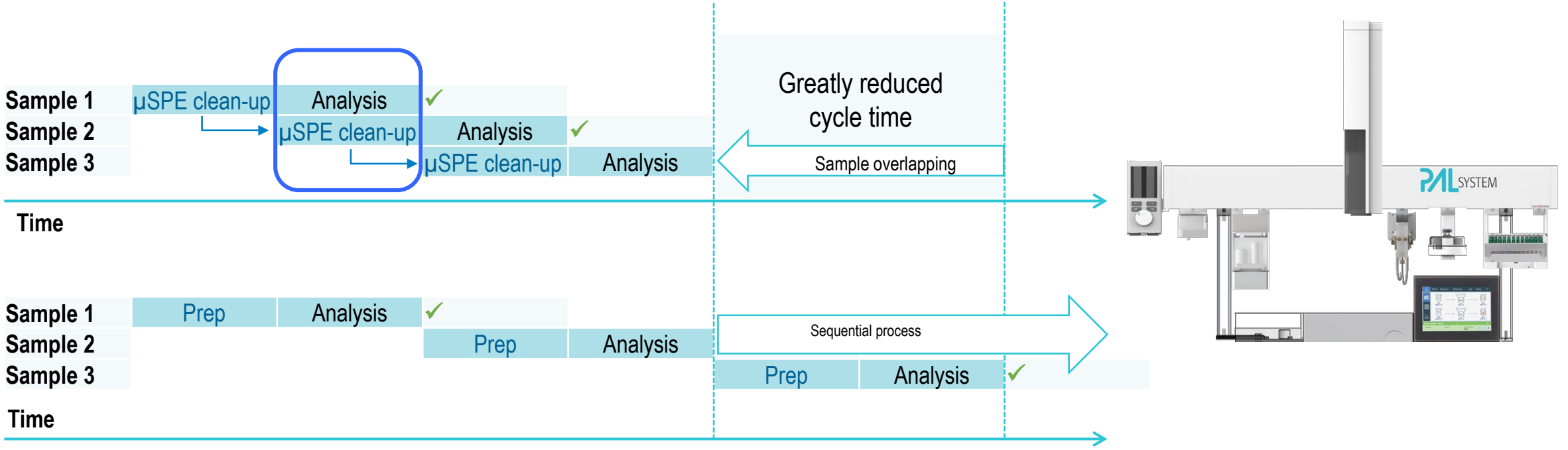
- At MRL level the pre-spike RSDs were mostly below 10%. Recoveries achieved 70% to 115%, calibration linearity > 0.995.
- LODs range from 1.8 ng/mL to 4.1 ng/mL (n = 8) well below the general MRL at 10 ng/g level.

  In the original orange juice from a local supermarket about 1.8 ng/mL Malathion was detected.



Parallel Extraction and µSPE Clean-up by PAL Prep-ahead

PAL serves sample prep and GC-MS/LC-MS analysis in parallel



- Strong benefits:
- High reproducibility: All samples are treated on the same timeline.
- Saves time: Continuous analysis of samples, improved sample throughput, overnight processing.
- Highly efficient: Increased use of the GC-MS and LC-MS unit, reduces cost/sample.

Summary

µSPE replaces the traditional SPE concentration and clean-up procedures

- µSPE is the next step available towards a Greener Analytical Chemistry
  - Less solvents
  - Less consumables
  - Less waste
  - Less energy consumption

- µSPE delivers strong analytical advantages
  - One clean-up cartridge for all type of samples
  - Improved recoveries
  - Improved clean-up
  - Improved precision

- µSPE reduces cost/sample
  - Efficient use of GC-MS and LC-MS by online prep-ahead
  - Increased sample throughput
  - Walk-away automation
  - Less manual workload
  - Less repeat measurements
  - Faster report out

µSPE at the EPRW 2024

PAL SYSTEM
Ingenious sample handling

In Vendor Seminars, Presentations and Posters

Today is Theme Day: Green Analytical Chemistry, Miniaturization and Automation

- **V-5 Gerstel Vendor Seminar**, Wednesday, 18 September 2024 I 10:50 – 11:15
- Steven J. Lehotay, Nicolas Michlig: Comparison of Automated vs. Centrifugal µ-SPE for the Analysis of Pesticides and Other Contaminants in a Variety of Foods.
- **O-16 Steven J. Lehotay**: High Throughput, Miniaturized, Automated, and "Green" Analysis of a Wide Scope of Pesticides and Other Residues in Fatty and Non-fatty Foods.
- **O-17 Amadeo Fernandez-Alba**: Automation and Miniaturization for Enhancing Analytical Methods in Pesticide Residue Evaluation in Food.
- **O-27 Mette Erecius Poulsen**: Evaluation, Optimization, and Validation of Different Micro-SPE Clean-up Cartridges for Pesticide Residuals in Cereals.

And a number of interesting posters:

- **PD-01 Jose Antonio Martinez**, et al. Pesticide Residue Analysis in Spice Samples by Automated Micro-SPE Clean-up
- **PD-18 Ivan Aloisi** et al. High Throughput Automated Off-line Micro-SPE for Clean-up of Complex QuEChERS Extracts
- **PD-49 Ederina Ninga**, et al. Optimization of Customized Micor-SPE Cartridges for Pesticides in Cereals
- **PD-50 Ederina Ninga**, et al. Validation of Pesticides in Insects Using "QuEChERSER"-Micro-SPE Clean-up
- **PD-51 Ederina Ninga**, et al. QuEChERS Micro-SPE Clean-up Method for Pesticides in Feed Samples by GC-Orbitrap

- Visit the new CTC µ-SPE cartridges at:
- https://www.palsystem.com
- https://www.bgb-info.com/home.php?cat=38964

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