

The Revolution of Automated Sample Preparation in Food

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Application Specialist

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Principle of QuEChERS

Quick Easy Cheap Efficient Rugged and Safe

What is QuEChERS?

Sample Preparation for pesticides and more

- The acronym stands for **Q**uick, **E**asy, **C**heap, **E**fficient, **R**ugged and **S**afe
- First published in 2003 by M. Anastassiades, S.J. Lehotay and team
- Originally used for pesticides analysis
- More than 1800 non-pesticides such as:
 - Mycotoxins
 - Antibiotics
 - Vet Drugs

412 ANASTASSIADES ET AL. JOURNAL OF AOAC INTERNATIONAL VOL. 86, NO. 2, 2003

RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

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FRANK J. SCHENCK

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A simple, fast, and inexpensive method for the determination of pesticide residues in fruits and vegetables is introduced. The procedure involves initial single-phase extraction of 10 g sample with 10 mL acetonitrile, followed by liquid-liquid partitioning formed by addition of 4 g anhydrous MgSO₄ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid-phase extraction (dispersive-SPE), in which 150 mg anhydrous MgSO₄ and 25 mg primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile extract. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, and sugars, to some extent from the food extracts. Gas chromatography/mass spectrometry (GC/MS) is then used for quantitative and confirmatory analysis of GC-amenable pesticides. Recoveries between 85 and 101% (mostly >95%) and repeatabilities typically <5% have been achieved for a wide range of fortified pesticides, including very polar and basic compounds such as methamidophos, acephate, omethoate, imazalil, and thiabendazole. Using this method, a single chemist can prepare a batch of 6 previously chopped samples in <30 min with approximately \$1 (U.S.) of materials per sample.

Pesticide residue analysis of food and environmental samples has been performed in numerous government and private laboratories throughout the world for approximately 40 years. However, the methods used for analysis of common pesticides are far from ideal. Some residue monitoring laboratories still use methods developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, extended analysis time and manual labor were the norm, and technology was less capable than today. Modern residue monitoring programs, however, are expected to be responsive to the latest developments in agriculture and new legislation. The introduction of new, more rapid, and effective analytical approaches, therefore, is essential for laboratories to improve overall analytical quality and laboratory efficiency.

Without question, the most efficient approach to pesticide analysis involves the use of multiresidue methods (MRMs). The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills (1). At that time, nonpolar organochlorine insecticides (OCs) were the main focus for analysis. With the Mills method, OCs and other nonpolar pesticides were extracted from nonfatty foods with acetonitrile (MeCN), which was then diluted with water, and the pesticides were partitioned into a nonpolar solvent (petroleum ether). As a consequence, relatively polar pesticides, such as certain organophosphorus insecticides (OPs), were partially lost during this step. The need to analyze more polar OPs and other pesticides in agriculture initiated the development of alternative procedures to determine compounds not extracted by the Mills method. These methods often simply modified the Mills procedure by using the initial MeCN extract but with different partitioning, cleanup, and determinative steps (2-4).

In the 1970s, new methods were developed to extend the analytical polarity range to cover OCs, OPs, and organonitrogen pesticides (ONs) in a single procedure (5, 6). These multiresidue MRMs differed from the Mills approach in that acetone, rather than MeCN, was used for the initial extraction. However, the new methods still used nonpolar sol-

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² Author to whom correspondence should be addressed, e-mail: slehotay@arserrc.gov.

Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

Anastassiades, M.; Lehotay, S. J.; Štajnbacher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* 2003, 86, 412-431.

Qu

Quick

E

Easy

Ch

Cheap

E

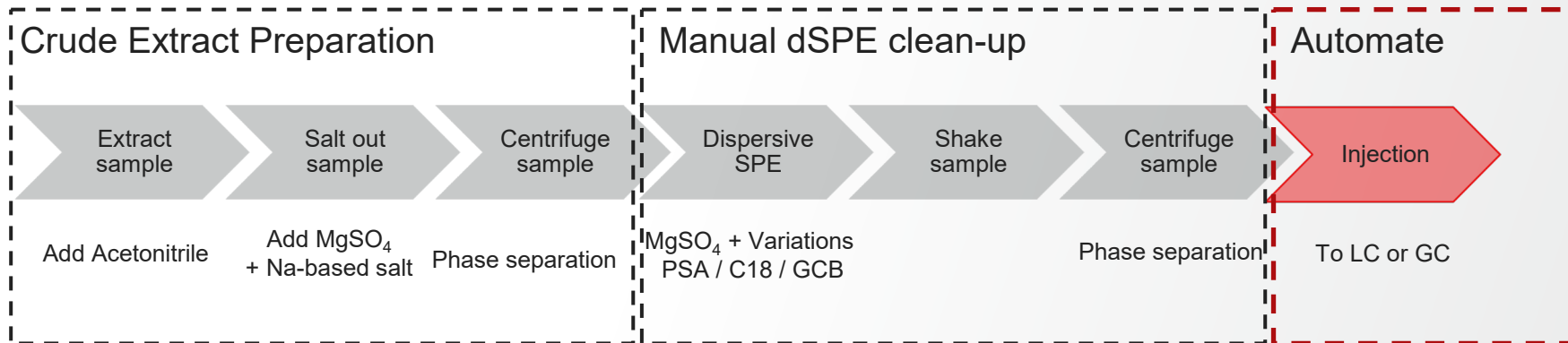
Effective

R

Rugged

S

Safe



Step 1: Crude Extract Preparation

- Principle
 - Using Liquid/Liquid portioning with Acetonitrile (ACN)
 - Water and Acetonitrile is miscible
 - Introduce high salt, change the affinity of water to lead to two phases separated
 - Require shaking and centrifugation for phase separation
- Methods and Regulations
 - Lehotay et. al. (AOAC Official Method 2007.01) modified the method using acetate buffer salt
 - Anastassiades et. al. (CEN Standard Method EN 15662) modified the method using citrate buffer salt



[3] S.J. Lehotay, K. Maštovská, A.R. Lightfield, J. AOAC Int. 88 (2005), 615–629 & 60A.

[4] M. Anastassiades, E. Scherbaum, B. Tas, delen, D. Stajnbaher, in: H. Ohkawa, H. Miyagawa, P.W. Lee (Eds.), Crop Protection, Public Health, Environmental Safety, Wiley-VCH, Weinheim, Germany, 2007, p. 439.

QuEChERS Methods

Step 2: Clean-up of the extract – The most suitable sorbent mix?

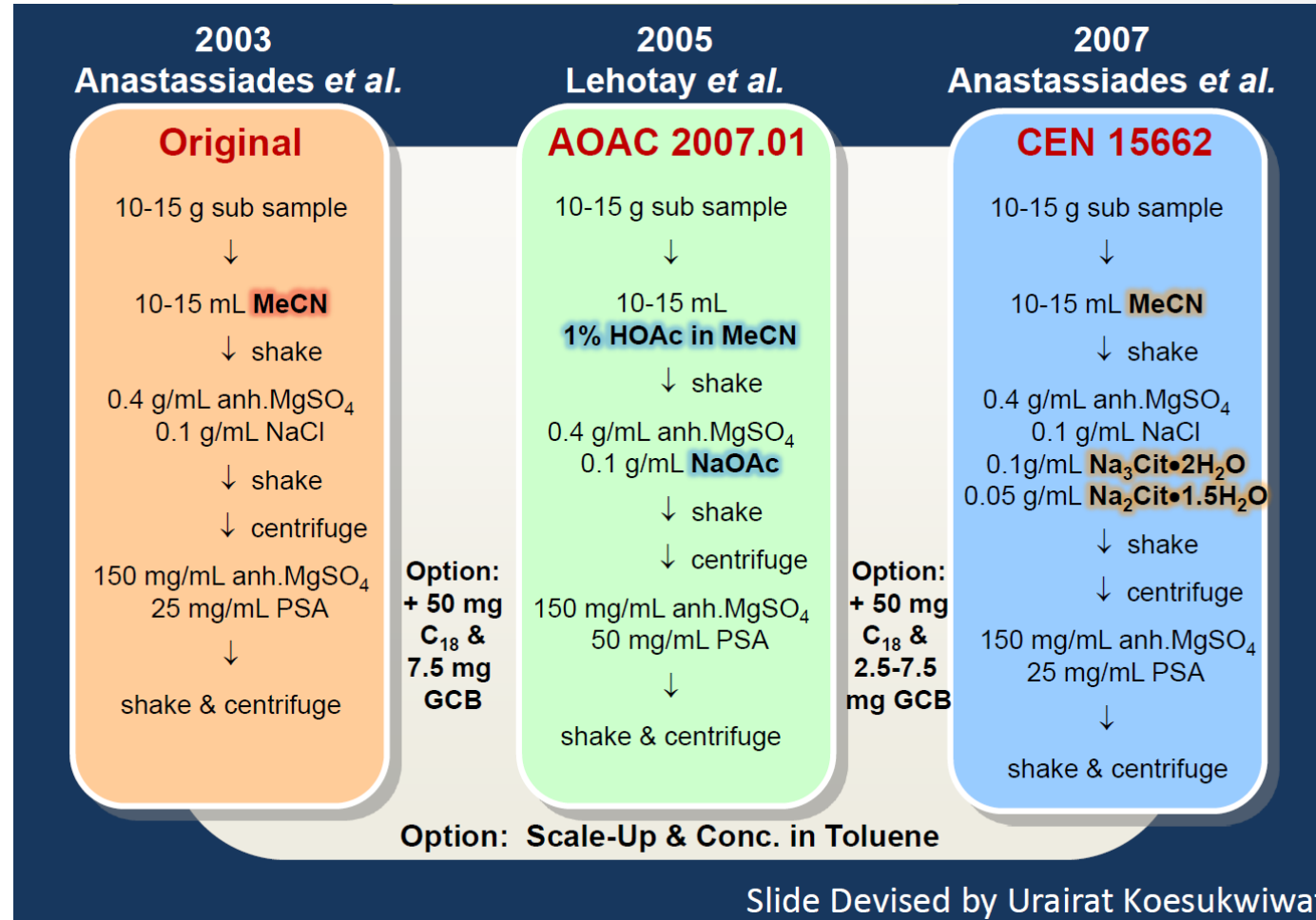
- dSPE is a manual step (= dispersive SPE)
 - Mixing the extract with various sorbent materials
 - Matrix specific mixes used
- Challenges and Limitations
 - Low recovery for fatty samples
 - Matrix effects with complicated matrices
 - e.g. spices, tea, and oils
 - or a high chlorophyll contents
- “Modified QuEChERS“ search in Google Scholar:
 - 5700 publications since 2003 deal with modified clean-up of the QuEChERS extract⁶
 - Customized dSPE clean-ups are used matrix dependent
 - with mixes of many different sorbent materials:
 - Like PSA, C18, GCB, CarbonX®, MgSO₄, Clorofiltr®, Z-Sep®, Z-Sep+®, ZrO₂, EMR®, ...
 - or freezing the extract



From: Lehotay, S.J. 2013. Revisiting the Advantages of the QuEChERS Approach to Sample Preparation. Separation Science Webinar.
Google Scholar search result: “modified QuEChERS”, 2003 to today.

QuEChERS Methods in Pesticide Analysis

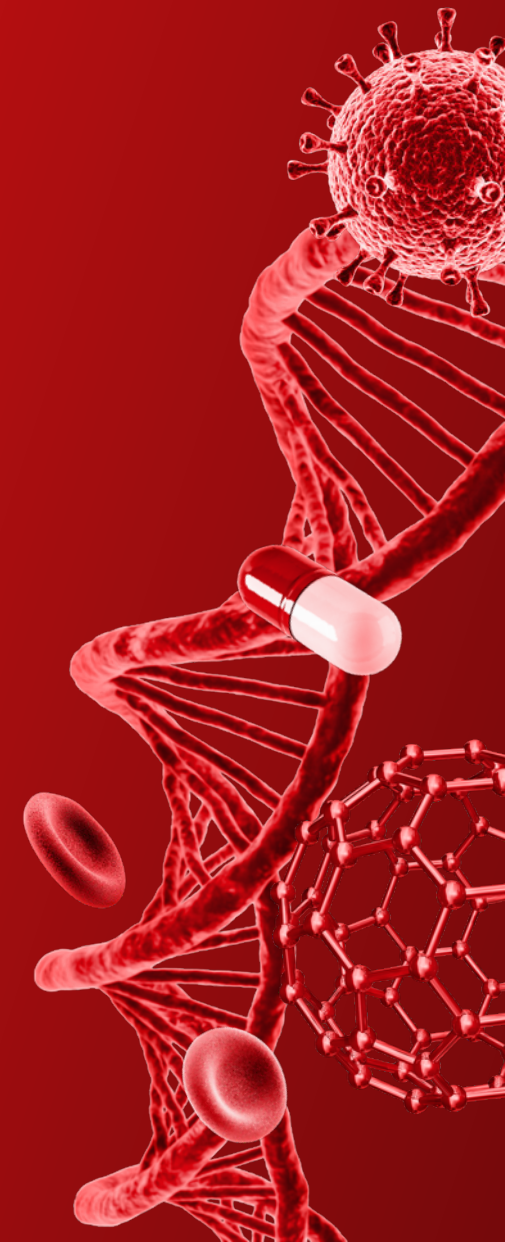
AOAC vs. EN



From: Lehotay, S.J. 2013. Revisiting the Advantages of the QuEChERS Approach to Sample Preparation. Separation Science Webinar.

QuEChERS-er

A better QuEChERS



Automated

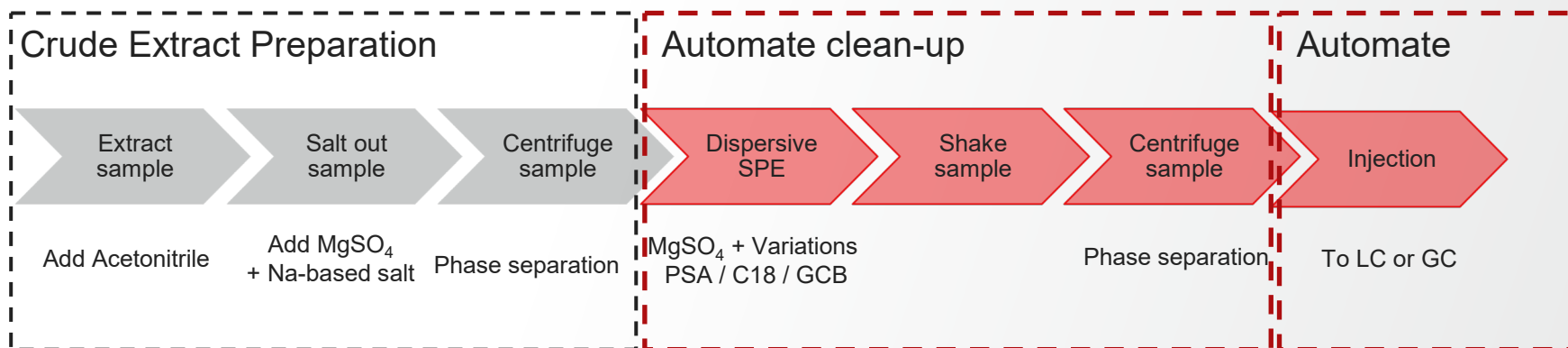
Q u E C h E R S

Quick

Easy

Cheap

Effective Rugged Safe



Automated

Qu

Quick

E

Easy

Ch

Cheap

E

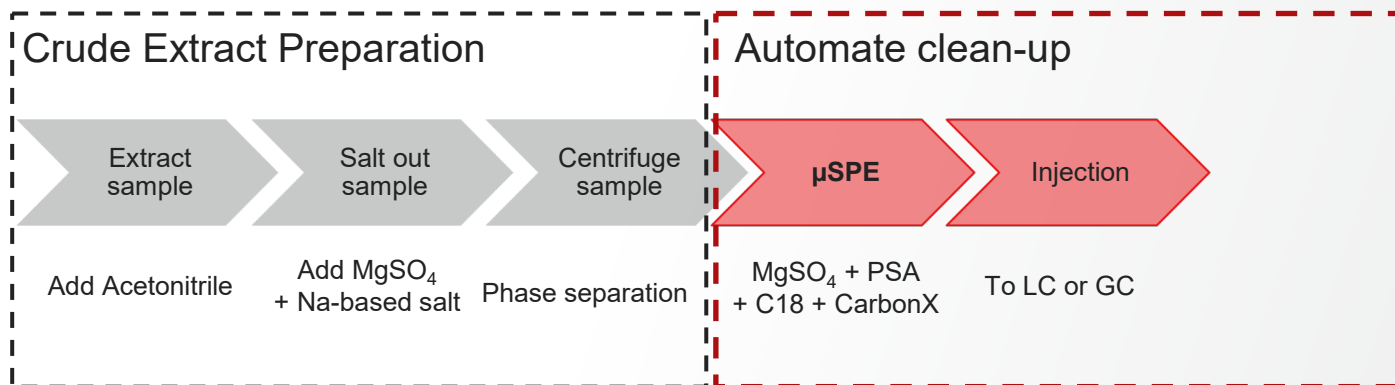
Effective

R

Rugged

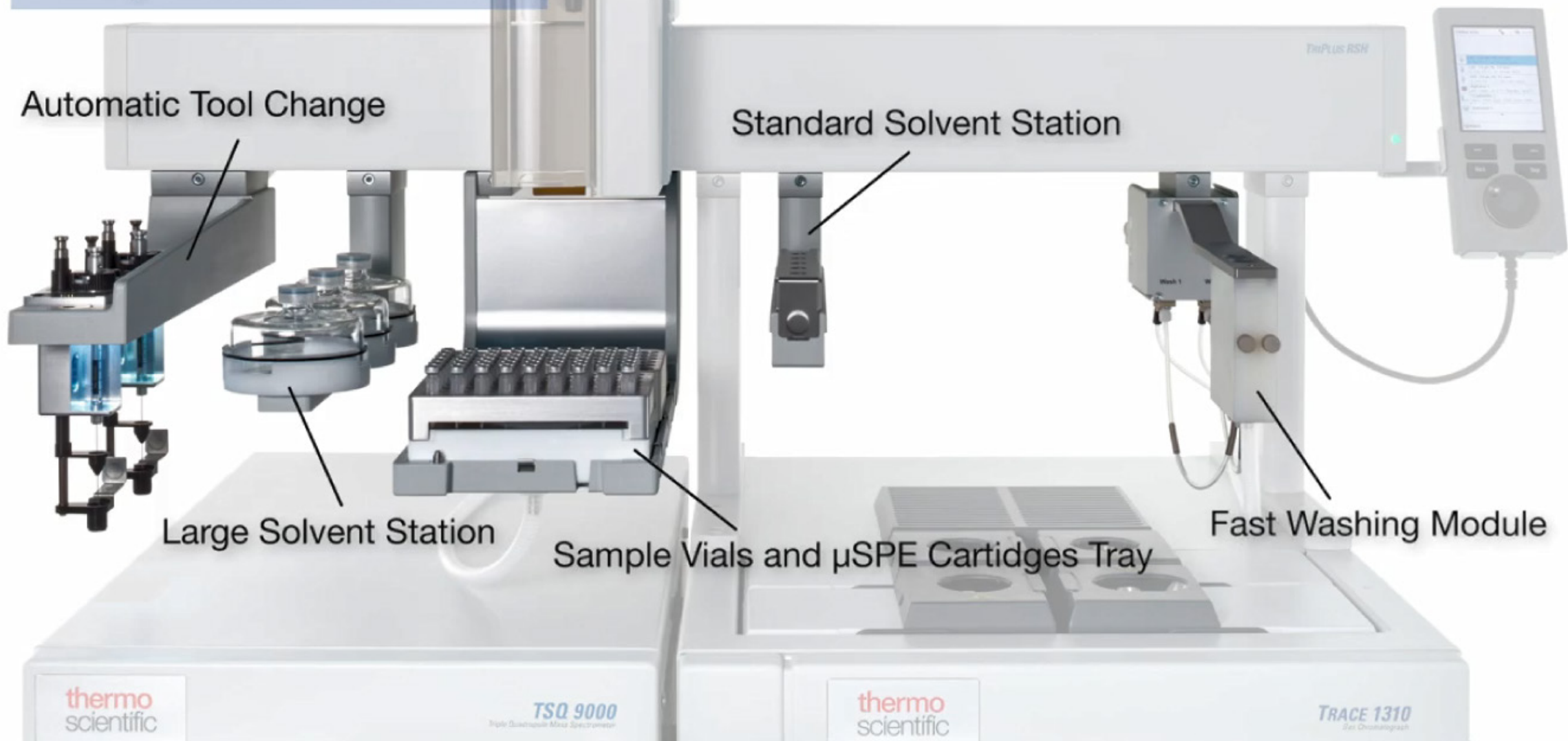
S

Safe



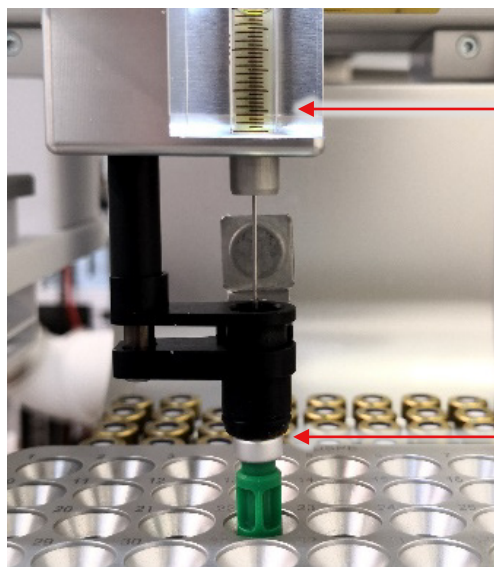
thermo scientific

Required Modules



μSPE: SPE designed for PAL Automation

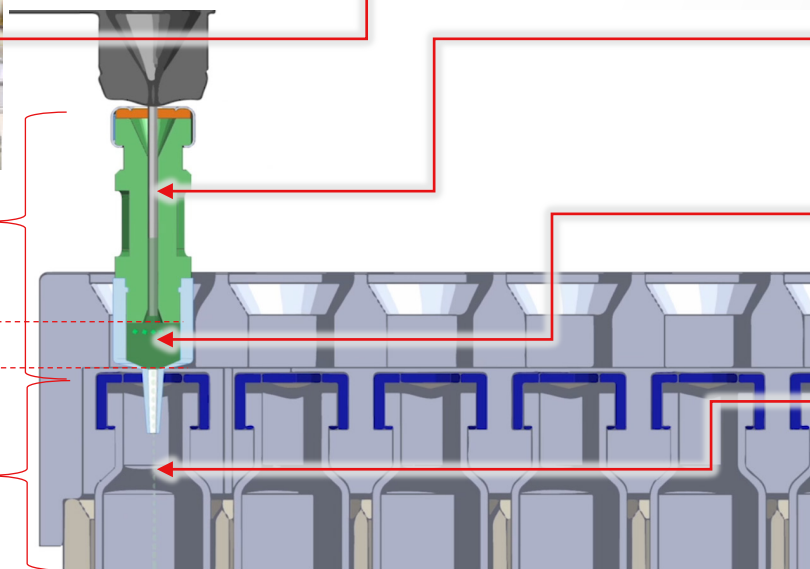
Controlled elution from low particle size sorbent bed



μSPE Cartridge

Sorbent Bed

Sample Vial



The PAL3 syringe replaces the SPE column sample reservoir above the sorbent bed as found in manual SPE cartridge formats.

The needle penetrates the septum and creates a seal so that the sample or solvent is forced through the sorbent bed by **positive pressure** when the plunger is depressed.

Solvent and sample flow rates are precisely controlled with the syringe plunger.

Solvents and sample are pushed through the SPE sorbent bed by the autosampler syringe plunger at a controlled rate.

The sample is eluted into a collection plate or vial and then injected into the LC injection valve or GC inlet.

Why use μ SPE?

Compare to the classical cartridge SPE

Classical SPE

- Limited selectivity
 - High sample and solvent volumes
 - Requires evaporation with N_2
 - End volume $\gg 100 \mu\text{L}$ in vial
- Vacuum operated
- Drying before elution
- Manual operation
 - Time consuming
 - Low sample throughput
 - Batch processing
- No QA/QC
 - As of manual operation



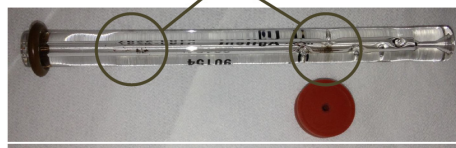
μ SPE

- High selectivity
 - compares to LC separation
 - Sharp elution peak profile, no concentration
 - Final volume $< 100 \mu\text{L}$ (or online)
- Positive pressure w liquid syringe
- No drying step
- Walk away automation
 - Fast with < 10 min
 - High productivity
 - Prep on chromatographic timescale
- Traceable
 - Processing well documented
 - 21CFR11 compatible



Steven Lehotay application for critical food stuff fish, meat, spices

- Shorter clean-up time
- Automated, compared to original QuEChERS
- Wide range of lipid content, e.g. salmon
- Critical range of matrices, e.g. dried spices
- Solid reference as clean-up for Quechers extracts
- Longer uptime for increased sample throughput
- Less maintenance – higher productivity
- Call the automated as QuEChERS-er (2020, EPRW)



“Injector liner after 230 matrix injections, only little dirt found”

Lehotay, S et al. *Chromatographia* (2016). doi:10.1007/s10337-016-3116-y

Chromatographia
DOI 10.1007/s10337-016-3116-y

CrossMark

ORIGINAL

Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography—Tandem Mass Spectrometry

Steven J. Lehotay¹ · Lijun Han^{1,2} · Yelena Sapozhnikova¹

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Abstract This study demonstrated the application of an automated high-throughput mini-cartridge solid-phase extraction (mini-SPE) cleanup for the rapid low-pressure gas chromatography—tandem mass spectrometry (LPGC-MS/MS) analysis of pesticides and environmental contaminants in QuEChERS extracts of foods. Cleanup efficiencies and breakthrough volumes using different mini-SPE sorbents were compared using avocado, salmon, pork loin, and kale as representative matrices. Optimum extract load volume was 300 µL for the 45 mg mini-cartridges containing 20/12/12/1 (w/w/w/w) anhydrous MgSO₄/PSA (primary secondary amine)/C₁₈/CarbonX sorbents used in the final method. In method validation to demonstrate high-throughput capabilities and performance results, 230 spiked extracts of 10 different foods (apple, kiwi, carrot, kale, orange, black olive, wheat grain, dried basil, pork, and salmon) underwent automated mini-SPE cleanup and analysis over the course of 5 days. In all, 325 analyses for 54 pesticides and 43 environmental contaminants (3 analyzed together) were conducted using the 10 min LPGC-MS/MS method without changing the liner or returning the instrument. Merely, 1 mg equivalent sample injected achieved <5 ng g⁻¹ limits of quantification. With the use of internal standards, method validation results showed that 91 of the 94 analytes including pairs achieved satisfactory results (70–120 % recovery and RSD ≤ 25 %) in the 10 tested food matrices (n = 160). Matrix effects were typically less than ±20 %, mainly due to the use of analyte protectants, and minimal human review of software data processing was needed due to summation function integration of analyte peaks. This study demonstrated that the automated mini-SPE + LPGC-MS/MS method yielded accurate results in rugged, high-throughput operations with minimal labor and data review.

Keywords High-throughput automation · Solid-phase extraction cleanup · Pesticide residue analysis · QuEChERS sample preparation · Fast GC-MS/MS · Analyte protectants · Environmental contaminants · Foods

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Electronic supplementary material The online version of this article (doi:10.1007/s10337-016-3116-y) contains supplementary material, which is available to authorized users.

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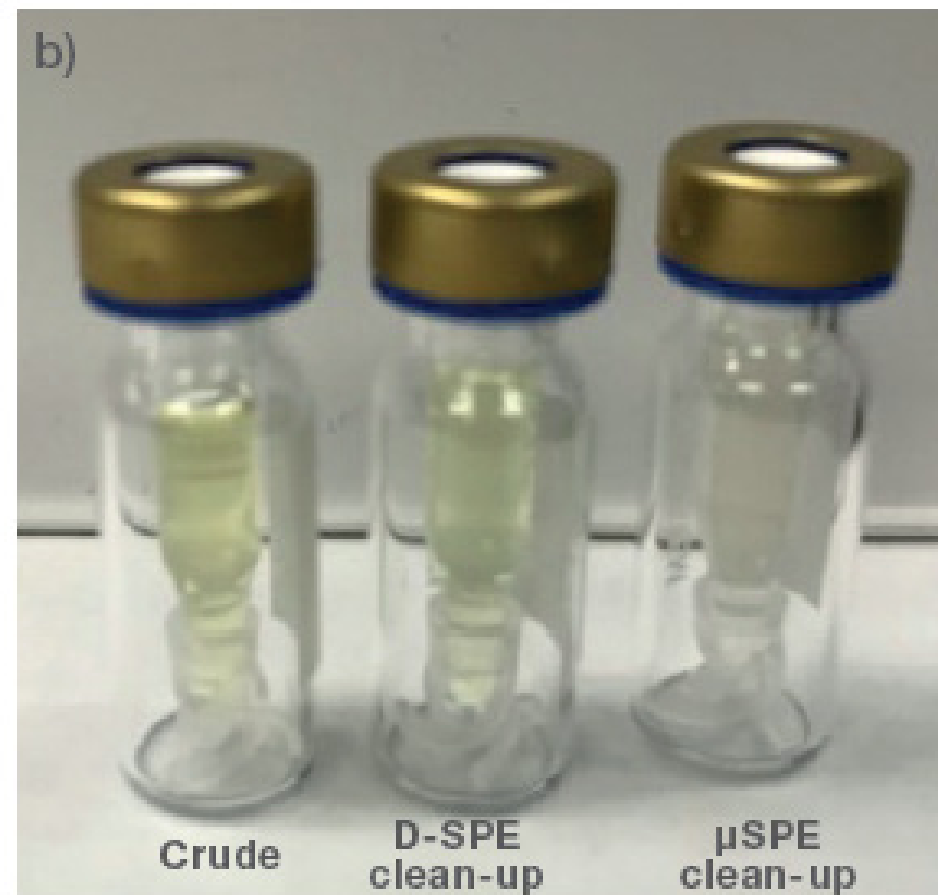
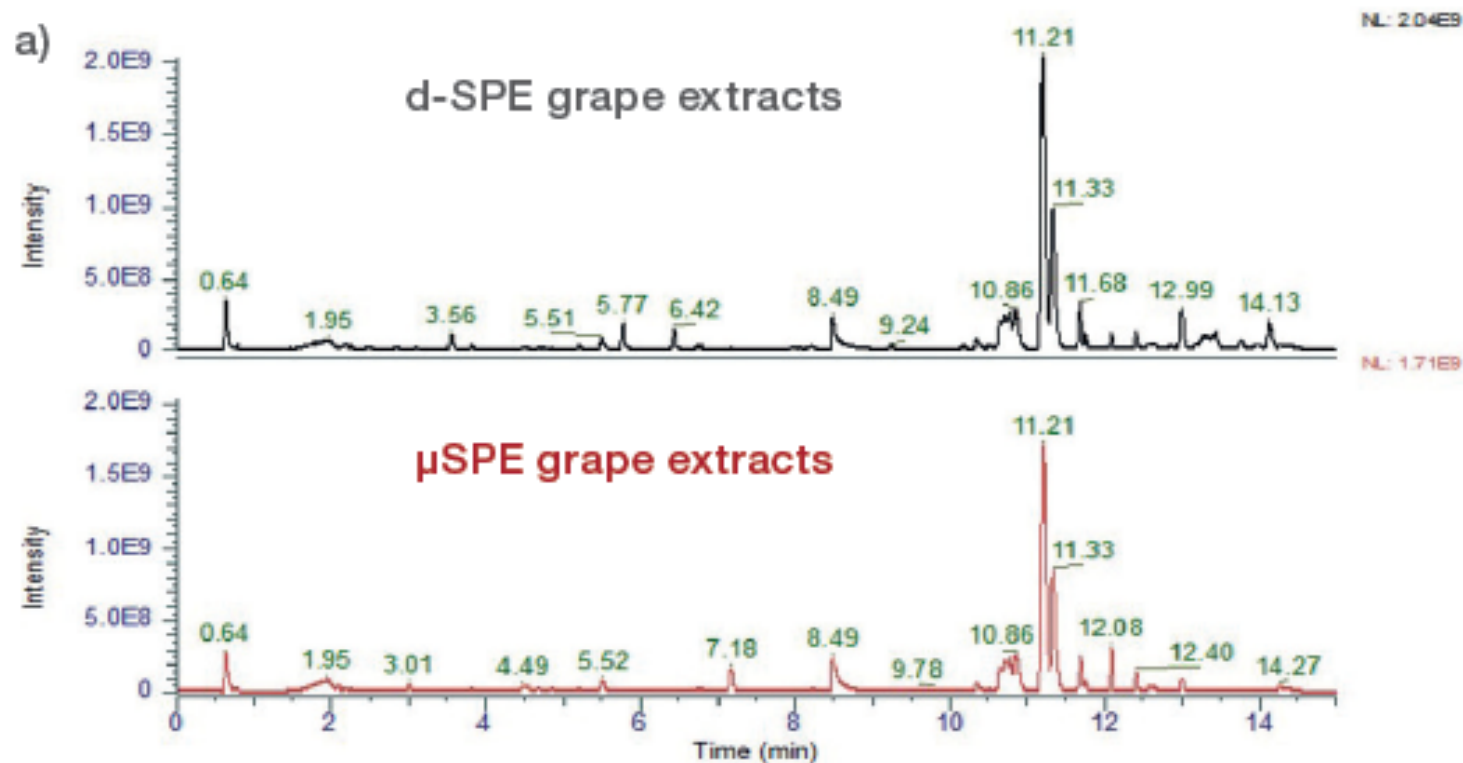
Clean-up Workflow

Fully automated by the TriPlus RSH

Procedure step	LCMS	GCMS
Clean syringe with elution solvent		
Condition μ SPE cartridges in the conditioning rack	150 μ L	200 μ L
Transfer cartridge to the elution rack		
Load QuEChERS extract from the sample vial onto the cartridge	150 μ L	300 μ L
Clean syringe with elution solvent		
Elute the cartridge with elution solvent	150 μ L	150 μ L
Collected eluents in 2 mL vial, total volume:	300 μ L	250 μ L
Discard cartridge to waste baker		
LCMS: Dilute combined extract and mix with syringe	1200 μ L	
GCMS: Add analyte protectant solution		30 μ L
Dilute combined extract with EtOAc and mix with syringe		250 μ L
Inject to GCMS or LCMS	10 μ L	3 μ L

Automated QuEChERS Clean Up

Clean up performance in Grape



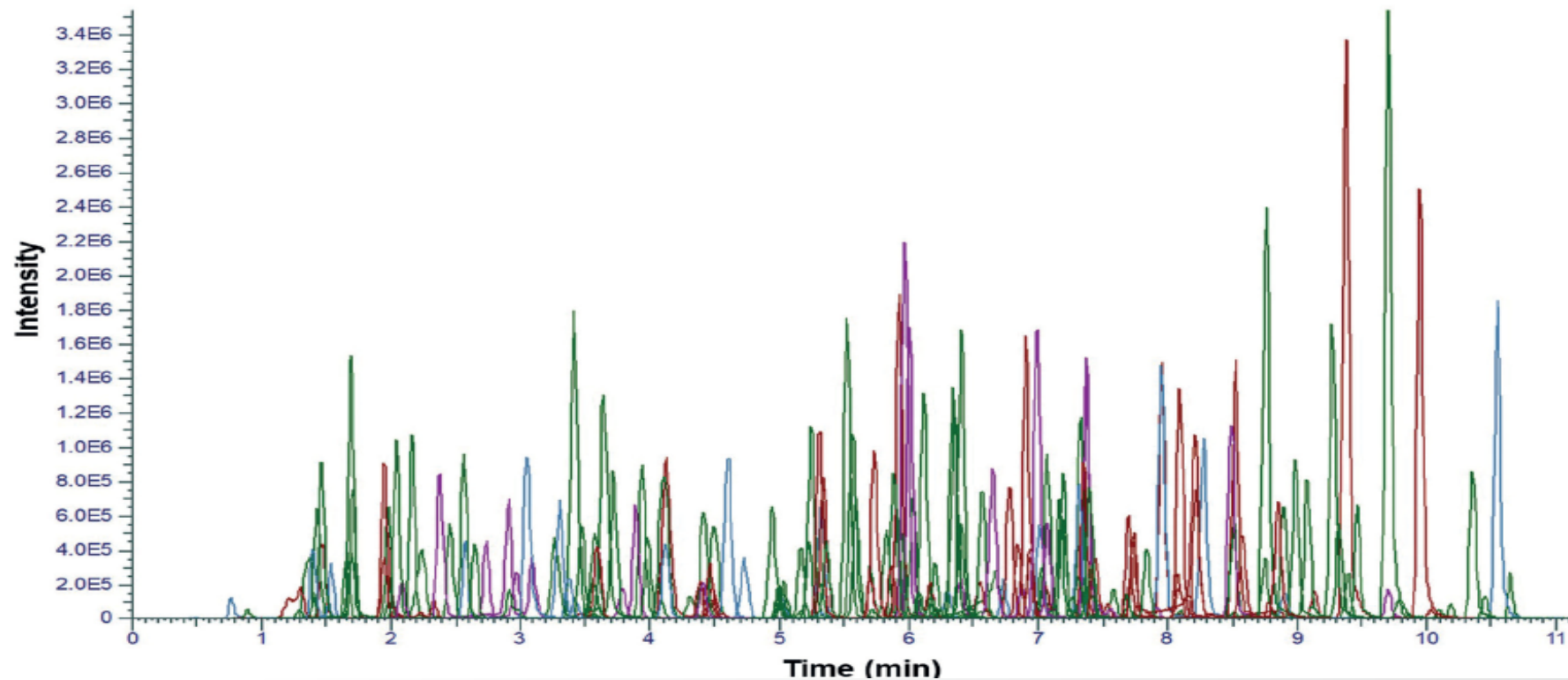
Fully Automated μ SPE in Rice

By TriPlus RSH with GC-MS/MS and LC-MS/MS

Automated QuEChERS Clean-up

Rice by μ SPE-GC-MS/MS and μ SPE-LC-MS/MS

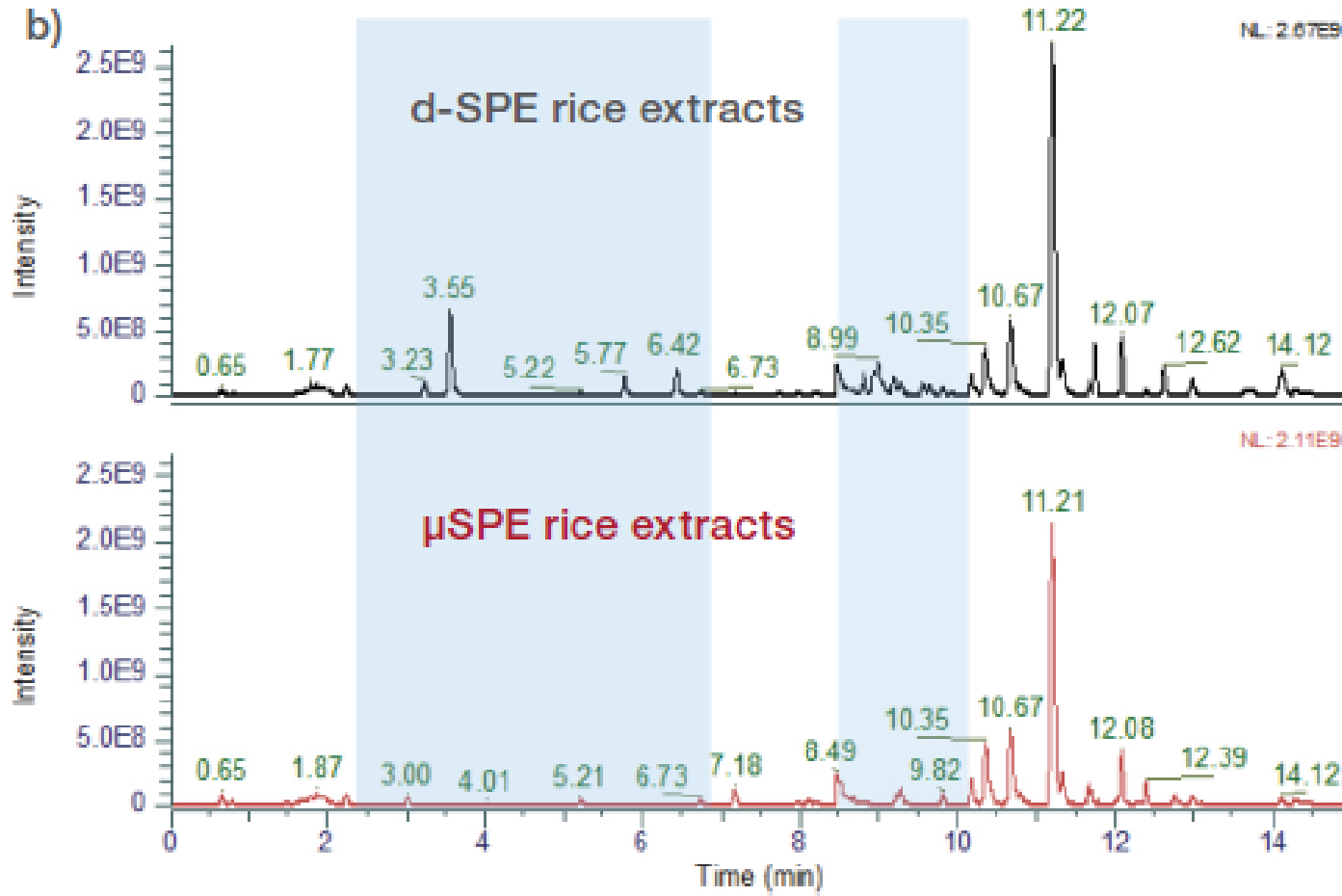
- Total 209 target Pesticides by GC-MS/MS
- Total 195 target Pesticides by LC-MS/MS



Overlaid chromatograms of 195 pesticides by the LC-MS/MS

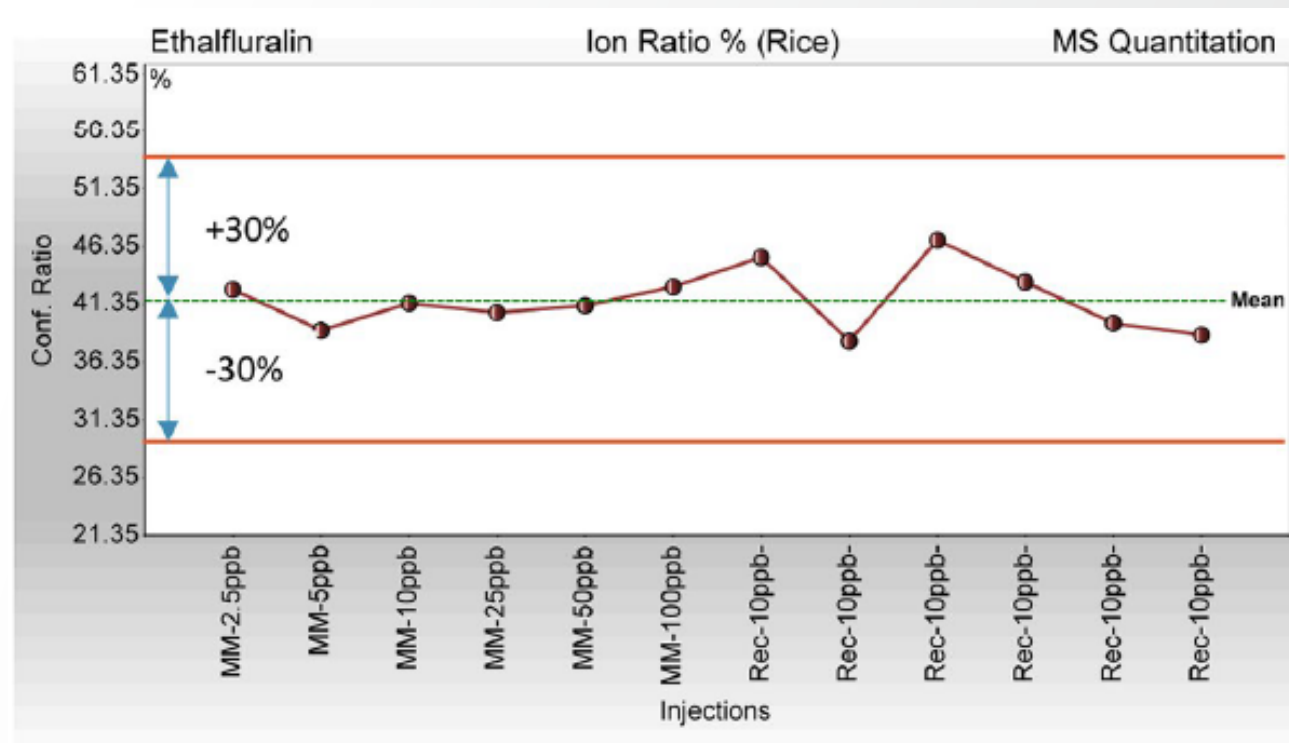
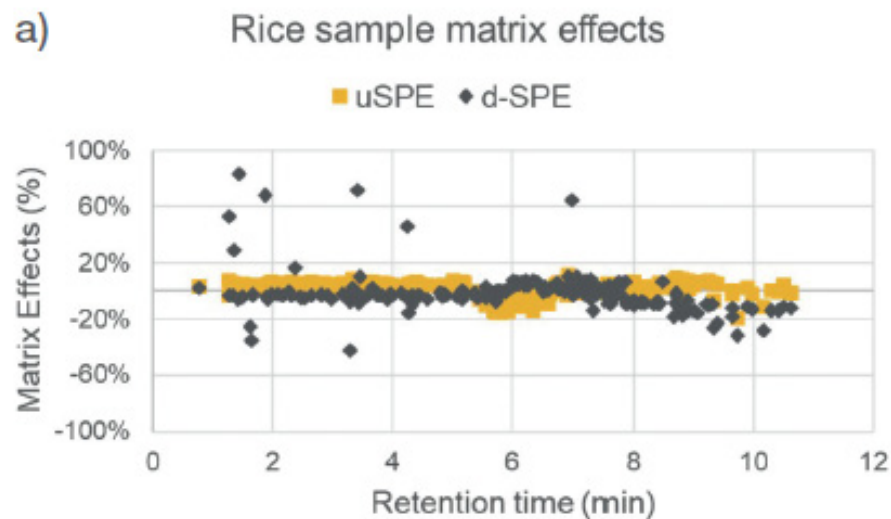
Automated QuEChERS Clean-up

Background interference



Automated QuEChERS Clean-up

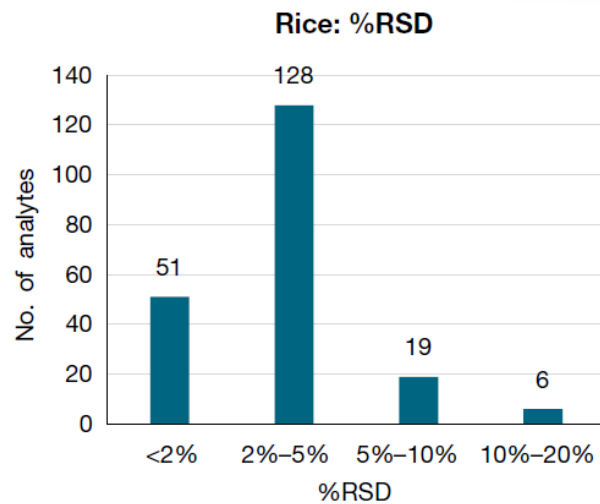
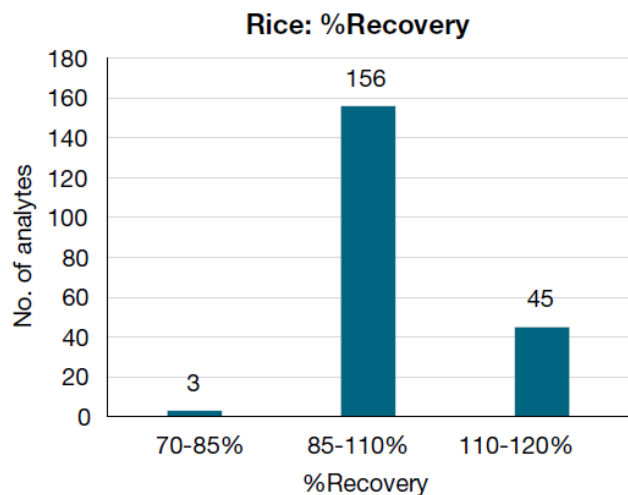
Matrix effects and system stability



Ion ratio % for ethalfluralin in rice from matrix-matched standards and recovery samples (n=6) prespiked at 10 μ g/kg and subjected to μ SPE clean-up

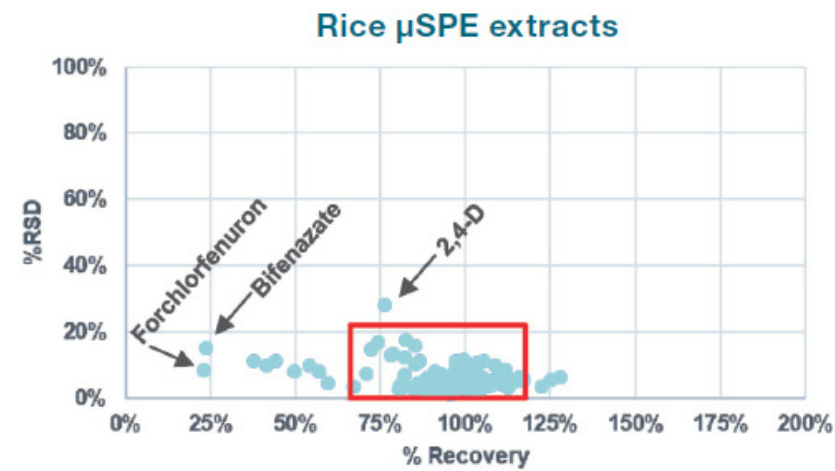
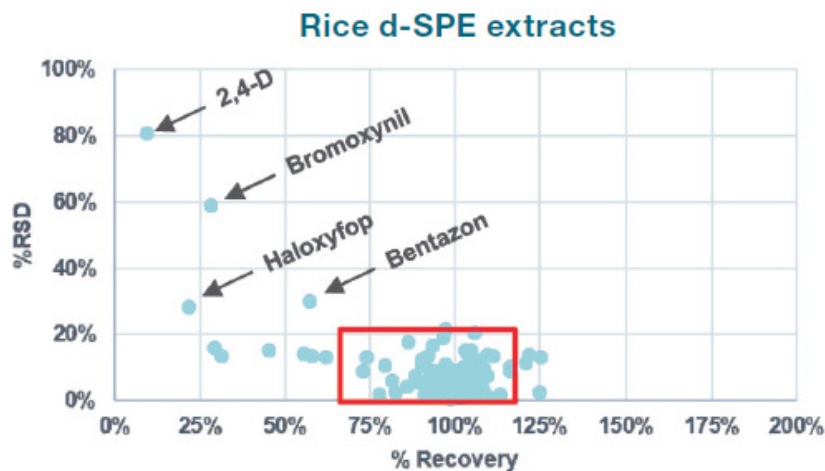
Automated QuEChERS Clean-up

Precision and Accuracy



In GC-MS/MS

In LC-MS/MS



Fully Automated Vet Drug Clean-up

By TriPlus RSH μ SPE

VetDrugs Residues Analysis



Automated

Qu

Quick

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Easy

Ch

Cheap

E

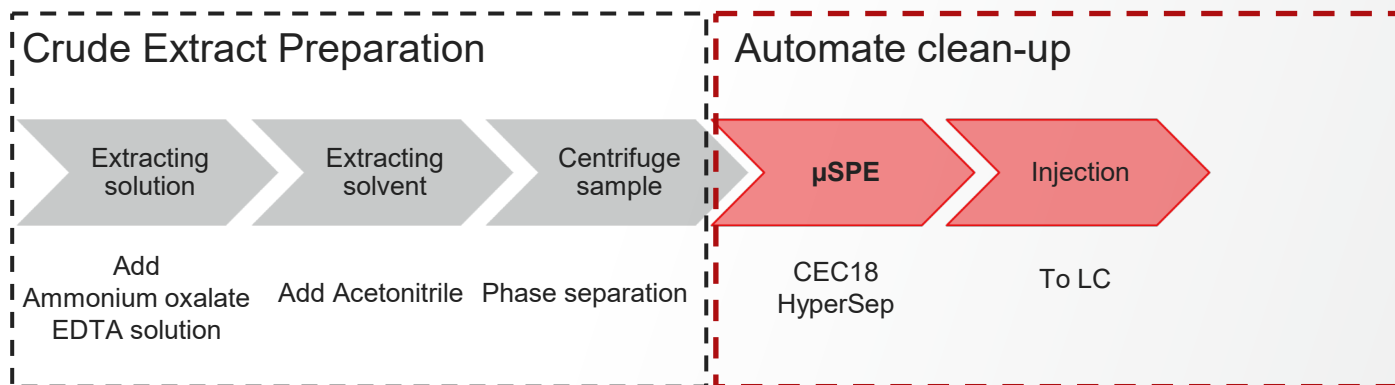
Effective

R

Rugged

S

Safe



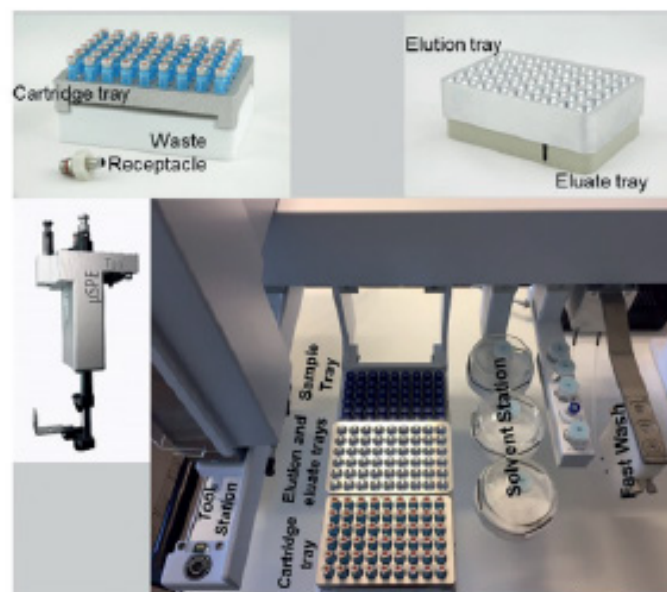
Automated Clean-up Workflow

With online analysis

Table 1. Steps for automated online μ SPE cleanup method with LC injection using the CEC18 cartridge

Step	Action
1	Aspirate 300 μ L of QuEChERS extract into the syringe
2	Move μ SPE cartridge to elution tray
3	Load 300 μ L QuEChERS extract onto μ SPE cartridge
4	Perform μ SPE -push extract through cartridge
5	Move-dispose of cartridge to waste bucket
6	Change to LC/MS injection tool
7	Perform sandwich injection
8	Change to prep syringe for next sample
9	Proceed with prep-ahead for next extract sample upon ready Signal

A



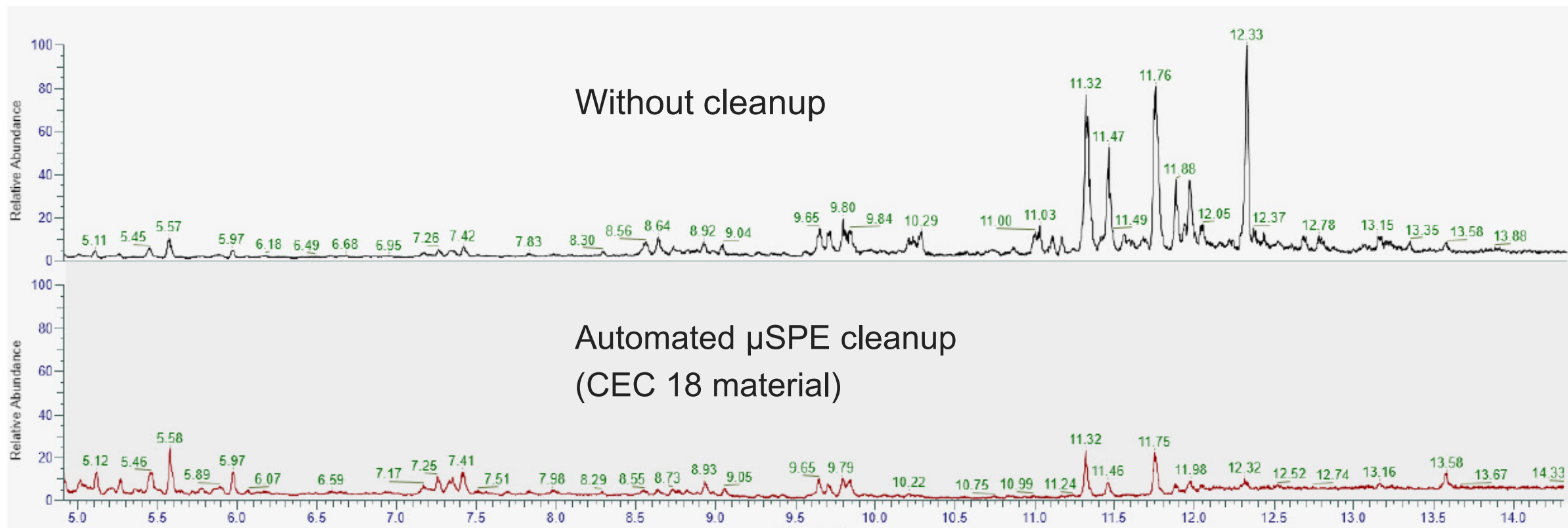
B



Sample 1	μ SPE clean-up	Analysis	✓		
Sample 2		μ SPE clean-up	Analysis	✓	
Sample 3			μ SPE clean-up	Analysis	✓

Comparing the Cleanup effects

Bovine muscle



Chromatogram of preSpiked Vet Drug

In Bovine Kidney

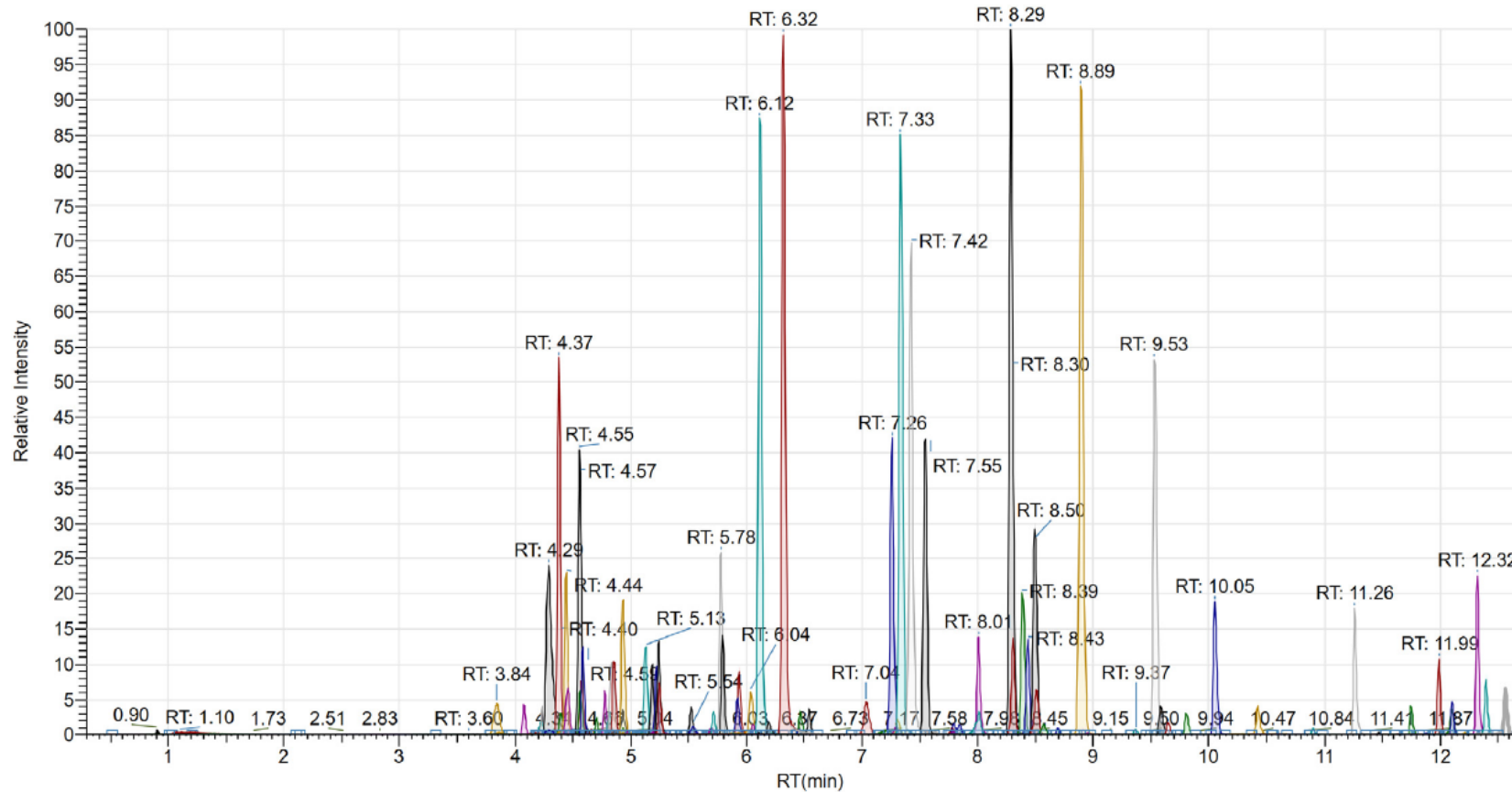
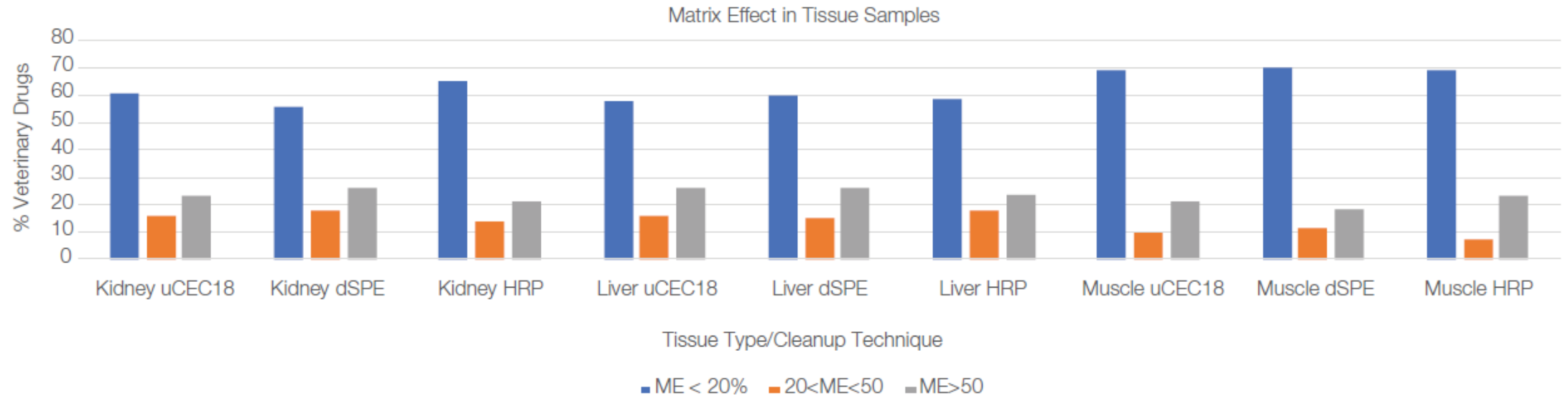
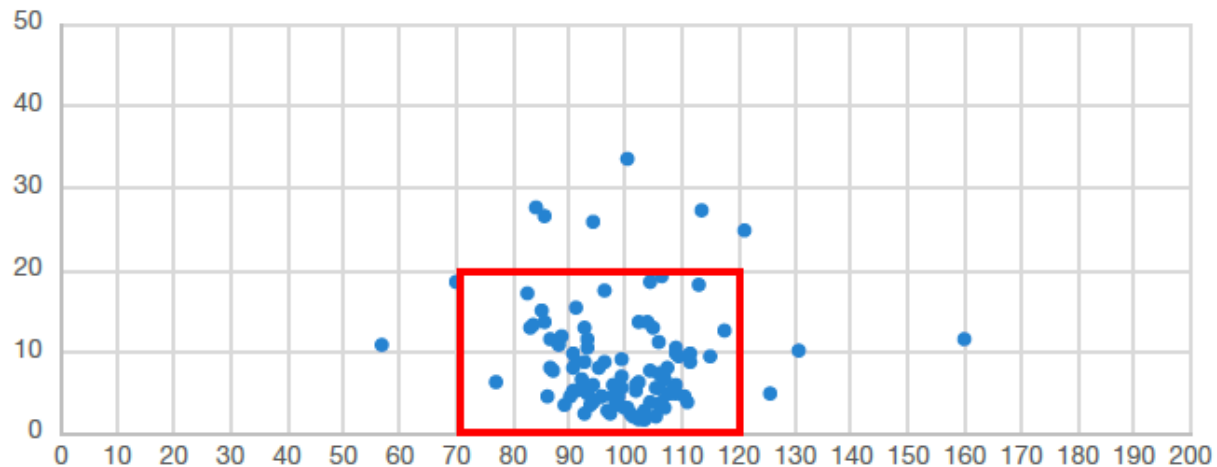


Figure 4. Overlaid chromatograms of all 103 veterinary drugs included in the method, 50 ng/g in bovine kidney extract.

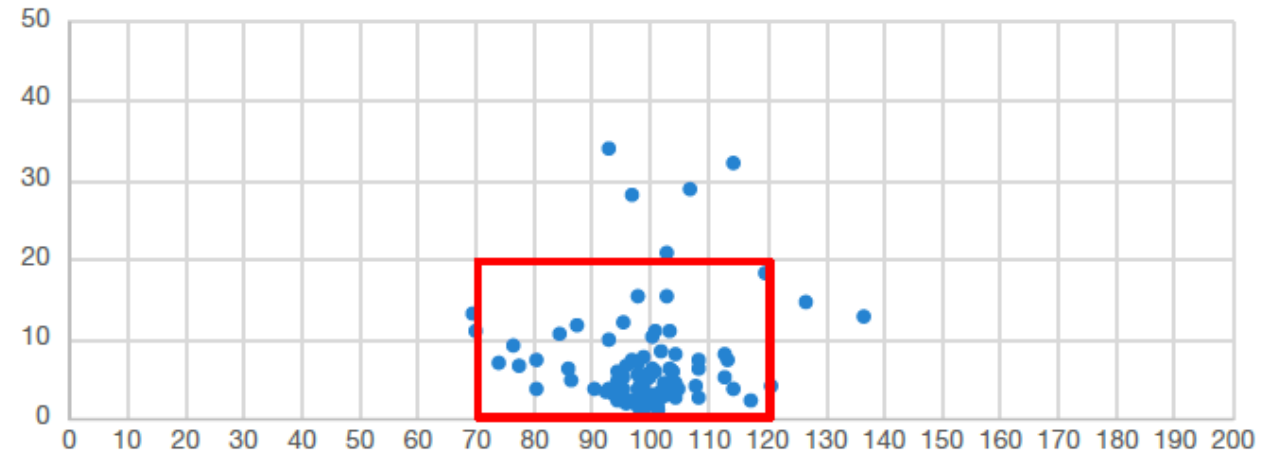
Automated μ SPE Cleanup performance



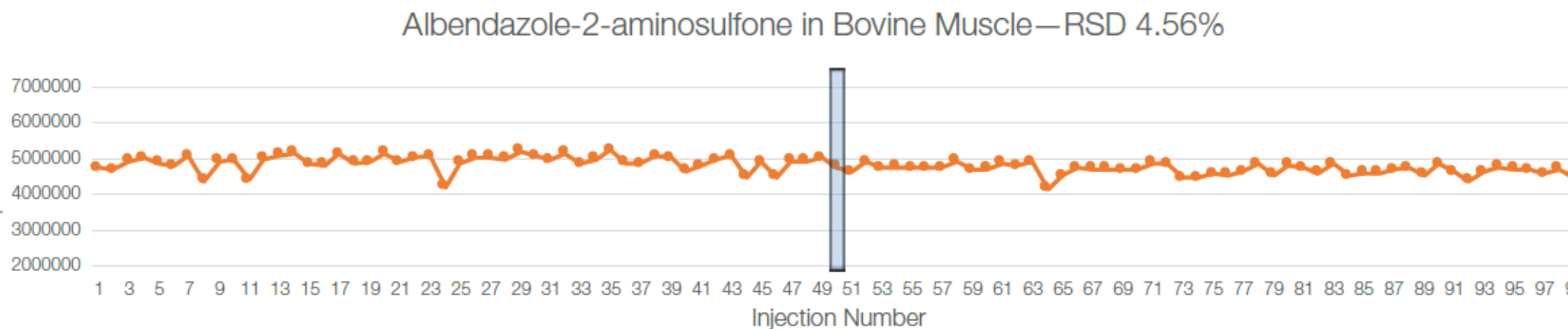
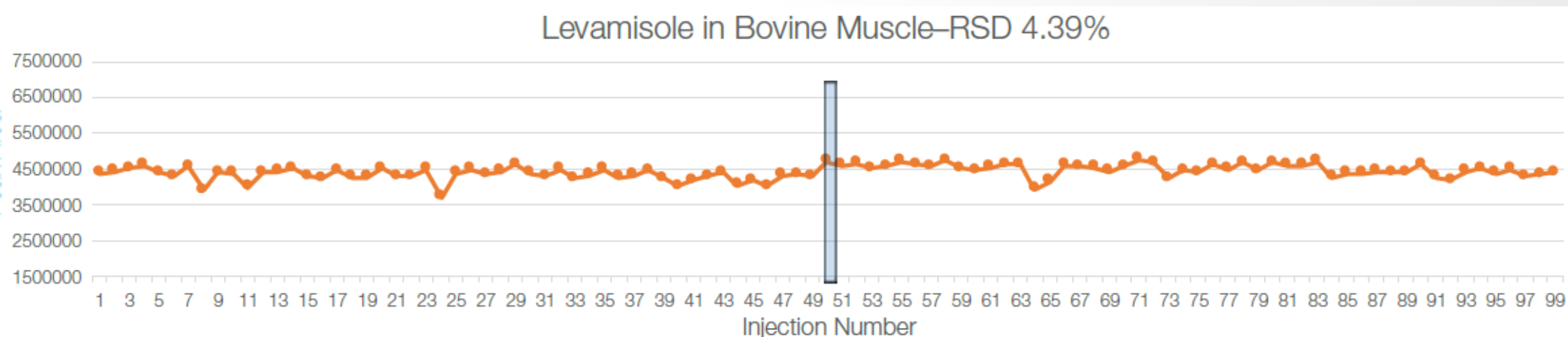
Bovine Kidney 5 ng/g CEC18- μ SPE



Bovine Kidney 50 ng/g CEC18- μ SPE



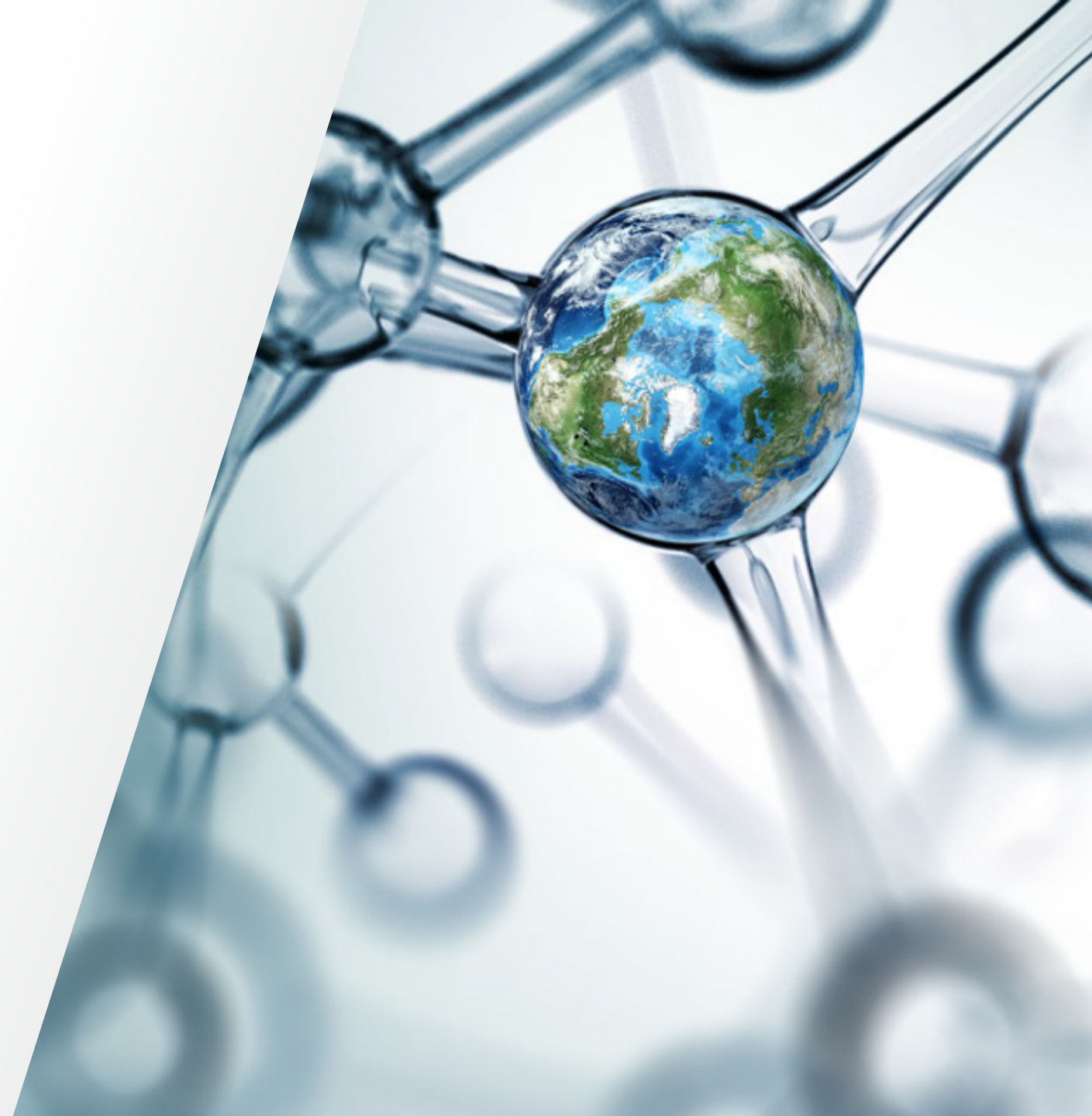
Long Term Performance



Fully Automated QuEChERS

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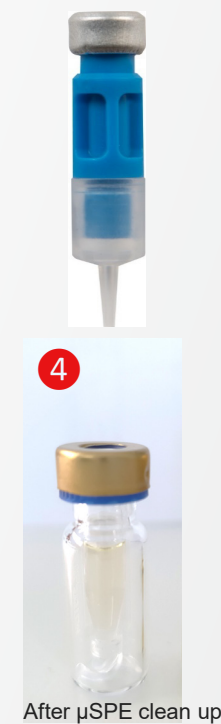
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Easily create and test sample preparation methods with the Thermo Scientific™ TriPlus™ RSH Sampling Workflow Editor Software. When connected with the Thermo Scientific™ TriPlus™ RSH and TriPlus RSH SMART, this software enables easy programming of the autosampler to fully automate common sample handling operations as well as more complex sample preparation procedures. The visual programming approach of the software makes this operation very easy and intuitive via its drag and drop functionality, not requiring any programming skills.

Fully QuEChERS Automation

Analyzing Organophosphates in Orange Juice

Liquid-Liquid Extraction	> Use Tool	✗	<p>1 Manually prepare 500μL of Orange Juice into 2 mL vial</p> <p>2 Add 3 x 200μL of Acetonitrile into Orange Juice sample</p> <p>3 Add 250μL of saturated NaCl into Orange Juice sample</p> <p>4 Wait for phase separation from the liquid-liquid extraction</p>
	> 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	✗	
μ SPE Clean-Up	> Use Tool	✗	<p>4 Load 250μL of the orange extract for μSPE clean up</p>
	> μ SPE Elute	✗	
	> Clean Syringe	✗	
Inject	> Liquid Injection	✗	
	> Wait	✗	



Application Notes

thermoscientific

APPLICATION NOTE

65906

Automated micro-SPE clean-up for GC-MS/MS analysis of pesticide residues in cereals

Authors: Sarvendra Pratap Singh, Subodh Kumar Budakoti, and Dasharath Oulkar
Customer Solution Center, Thermo Fisher Scientific, Ghaziabad, India

Keywords: Pesticide residues, cereals, QuEChERS, micro-solid phase extraction (μ SPE), GC-MS/MS, advanced electron ionization (AEI), targeted quantitation, TSQ 9000, Chromeleon Chromatography Data System



Goal

To assess the suitability of an automated micro-solid phase extraction (μ SPE) clean-up of QuEChERS extracts for the determination of pesticide residues in cereal samples by gas chromatography coupled to triple quadrupole mass spectrometry.

Introduction

Worldwide food demand is set to increase substantially in the next few decades¹, and consequently, food safety concerns are also growing quickly^{2,3}. To meet the demand for food, pesticides are used to control pests and ensure high crop yields, but there are some concerns that banned pesticides are still used illegally. If used incorrectly, pesticides can affect consumer's health, hence the importance regulatory bodies place on screening food samples for the presence of pesticide residues.

Given the large number and types of food samples that need to be tested, any delays in the analysis could ultimately impact the timely import/export of food products, which is crucial for perishable products. The extraction of pesticides from food matrices is typically carried out using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) acetonitrile method. Many versions of QuEChERS have been published but one of the most widely used versions is AOAC 2007.01⁴. This method includes a manual dispersive solid-phase extraction clean-up step (dSPE) of the initial non-cleaned extract. This clean-up procedure can be time-consuming and can result in limited removal of matrix co-extractives. By replacing this manual clean-up step with an automated μ SPE clean-up approach, laboratories can save time, achieve more effective removal of co-extractives, and thus improve the consistency of the results. A miniaturized SPE method, consisting of sorbents

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APPLICATION NOTE 65684

Multi-pesticide residues analyses of QuEChERS extracts using an automated online μ SPE clean-up coupled to LC-MS/MS

Authors: Long Sun¹, Qilei Guo¹, Cristina C. Jacob², Claudia P.B. Martins², Richard Fussell³; ¹Thermo Fisher Scientific, Customer Solution Center, Beijing, China; ²Thermo Fisher Scientific, San Jose, CA, USA; ³Thermo Fisher Scientific, Hemel Hempstead, UK

Keywords: Pesticide residues analysis, LC-MS/MS, QuEChERS, online μ SPE, clean-up, automation



Goal

To demonstrate the feasibility of an automated online sample clean-up solution coupled to LC-MS/MS for rapid and robust quantitation of multi-pesticide residues in food matrices.

Introduction

Pesticides are widely used to control pests worldwide, so crops, feed, and food products are routinely tested for the presence of pesticide residues and to check for compliance with permitted Maximum Residue Levels (MRLs)^{1,2}. Given the globalization of the food supply, the large number of different pesticides used, and the many samples to be analyzed, robust, accurate, reproducible,

and cost-effective multiresidue methods allowing the reliable analysis of hundreds of pesticides in a single experiment and in many different sample types are required.

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach is commonly used for the analysis of multi-pesticide residues, because of the substantial productivity gains that can be achieved.^{3,4} The QuEChERS approach usually involves extraction with acetonitrile in the presence of a mixture of salts followed by centrifugation. An aliquot of the supernatant is cleaned up by manual dispersive-solid phase extraction (d-SPE) in an attempt to remove unwanted matrix compounds, such as pigments, sugars, organic acids, excess water, and other components.

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APPLICATION NOTE

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Multi-class veterinary drugs analyses of QuEChERS extracts using an automated online μ SPE cleanup coupled to LC-MS/MS

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Keywords: Veterinary drug residue analysis, QuEChERS, Online μ SPE, LC-MS/MS, TSQ Altis, Automation, Solvent Sandwich Injection Technique, Auto Calibration Standard Preparation



Goal

To demonstrate an automated online sample cleanup solution coupled to LC-MS/MS for rapid and robust screening and quantitation of veterinary drug residues in animal tissues.

Introduction

Veterinary drugs are administered to animals to ensure animal welfare. It is necessary to screen food products for veterinary drug residues at the maximum residue limits (MRL) set by global regulatory agencies. This screening typically involves both identification and quantification of veterinary drugs using LC-MS/MS.

A sample preparation approach often applied to veterinary drug screening in animal tissues is QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction.¹ This process involves a liquid-solid extraction of the sample with acetonitrile and salts. After the extraction, sample cleanup is often preferred. One common cleanup approach is dispersive solid phase extraction (dSPE), which involves adding a fixed amount of a powdered reagent (such as C18 or PSA) to the extract, vortexing for several minutes, then centrifugation and transfer into an autosampler vial. A second approach is solid phase extraction (SPE), in which the extract is passed through a sorbent material contained in a cartridge using a vacuum manifold. The goal of both cleanup approaches is to avoid the loss of target analytes whilst removing as many matrix co-extractives as possible, since they can cause ionization suppression and faster contamination of the LC-MS/MS detection system.

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Thank you

Q&A

