Pesticide Residue Analyses of QuEChERS Extracts of Different Food Matrices Using an Online Robotic SPE Clean-up Procedure Coupled to LC-MS/MS

F. Michael Hudson; Cristina C. Jacob; and Claudia P.B. Martins, Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134, USA

ABSTRACT

Purpose: A simple and fast method for the clean-up of QuEChERS extracts, coupled with LC-MS/MS for multi-residue pesticide analysis in food matrices.

Methods: A robotic solid-phase extraction (SPE) using miniaturized cartridges on a CTC autosampler (TriPlus RSH with RTC) was coupled to Thermo Fisher Vanquish™ Flex Binary UHPLC System interfaced with a Thermo Fisher TSQ Fortis™ Triple Quadrupole Mass Spectrometer with a Thermo Scientific™ Heated Electrospray Ionization (HESI-II) probe operating in both positive and negative ionization modes.

Results: Improvements in analytical performance are here reported for an LC-MS/MS method for the multi-residue analysis of pesticides from food matrices after implementing a fully automated and online µSPE clean-up method for processing QuEChERS extracts.

INTRODUCTION

Pesticides have been widely used to control pests and their residue analyses in crops and food products are routinely performed in regulatory and industrial laboratories. QuEChERS method is commonly used for the extraction of pesticide residues from food samples[1]. Despite its high pesticide extraction efficiency, fatty and pigmented matrix is often coextracted along with the compounds of interest when QuEChERS method is employed. This propensity for matrix coextraction can cause problems in the LC-MS analysis-such as ion suppression-and therefore clean-up is beneficial. We report herein a fully automated and online µSPE clean-up method of QuEChERS extracts with direct LC-MS/MS analysis.

MATERIALS AND METHODS

TriPlus RSH System Configuration

- Triplus RSH configuration enables the use of dedicated syringes for µSPE extracts clean-up steps and LC/MS injection, and is also equipped with reservoirs for conditioning, washing and eluting solvents (Fig.1)
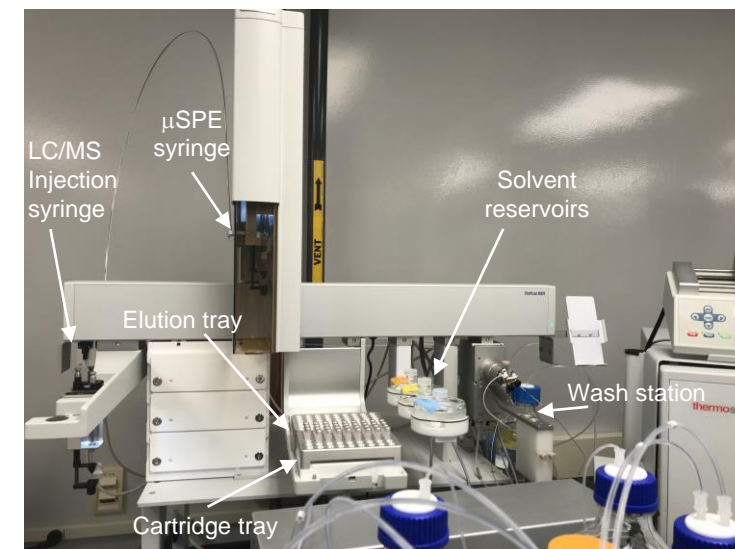

Figure 1. TriPlus RSH µSPE clean-up workflow setup

- µSPE Cartridges are sealed by a septum above the sorbent bed which allows the syringe to push sample extracts or solvents through the sorbent bed. Therefore, the syringe replaces the vacuum system of the classical SPE methodology with the added benefit of working at defined flow rates (Fig. 2)

Figure 2. Cartridge needle transport and ITSP µSPE cartridges

- The small dimensions allows the scale-down of the µSPE clean-up workflow (low microliter level)

µSPE workflow

- **Robotic Solid-Phase Extraction** (SPE) using miniaturized cartridges on a CTC autosampler (TriPlus RSH) with integrated injection to LC-MS/MS analysis (Table 1)

- **ITSP SPE Cartridges** QuEChERS Blend for LC; where C18/zirconia-coated silica and Carbon-X are used as base sorbent

- **Elution solvent** 1:1 Acetonitrile/ Methanol + 100 mM ammonium formate (pH 5.8)

- Blank matrix samples (Leek, avocado, and walnut) were purchased in local retail stores. The homogenized samples were then extracted with QuEChERS protocols and spiked with a 270 pesticide standard mixture.

- The clean-up cycle is completed in less than 10 min. It is scheduled as a prep-ahead task which allows continuous sample analysis within the cycle of the LC-MS system.

Table 1. QuEChERS extract automated clean-up workflow

Step	Action	Description
1. Clean	Prep Syringe with elution solvent
2. Condition	µSPE with 100 µL elution solvent
3. Move	µSPE cartridge to elution tray
4. Load	100 µL QuEChERS extract onto µSPE cartridge
5. Clean	The prep syringe
6. Elute	µSPE cartridge with 100 µL elution solvent
7. Move	Used µSPE cartridge to cartridge tray
8. Change	To LC/MS injection syringe
9. Inject	To LC-MS/MS
10. Change	To prep syringe for next sample
11. Proceed	With prep-ahead for next extract sample upon Ready Signal

LC-MS/MS system

- Vanquis Flex Binary pump interfaced with a Fortis Triple Quadrupole Mass Spectrometer equipped with a HESI ionization probe.

LC Conditions		Gradient:	Time (min)	% Solvent B
Analytical Column Thermo Accucore AQ, 2.1 x 100 mm, 2.6 µm			0	0
Flow rate 300 µL/min			0	0
Column temperature 25 °C			0.5	0
Solvent A Water containing 2mM ammonium formate, 2% methanol and 0.1% FA			7	70
Solvent B Methanol containing 2mM ammonium formate, 2% water and 0.1%FA			9	100
Injection volume 1 µL			12	100
			12.1	0
			15	0

MS Source Parameters	
HESI Source: both positive and negative modes	Vaporizer temperature : 350 °C
Spray Voltage: Positive 3.7 kV and Negative 2.5 kV	Cycle time :0.4 s
Sheath Gas (Arb): 30	Q1 Resolution (FWHM): 0.7 Da
Auxiliary Gas (Arb): 6	Q3 Resolution (FWHM): 1.2 Da
Sweep Gas (Arb): 1	CID gas: 1.5 mTorr
Ion transfer tube temp.: 300 °C	

Data Analysis

The acquired data were processed using Thermo Scientific™ TraceFinder™ 4.1 software.

RESULTS

Leek QuEChERS extracts

- µSPE procedure resulted in clear extracts (efficient decolorization, Fig. 3)

- µSPE clean-up provided recoveries in the range 60-120% for most of the compounds (Fig.3)

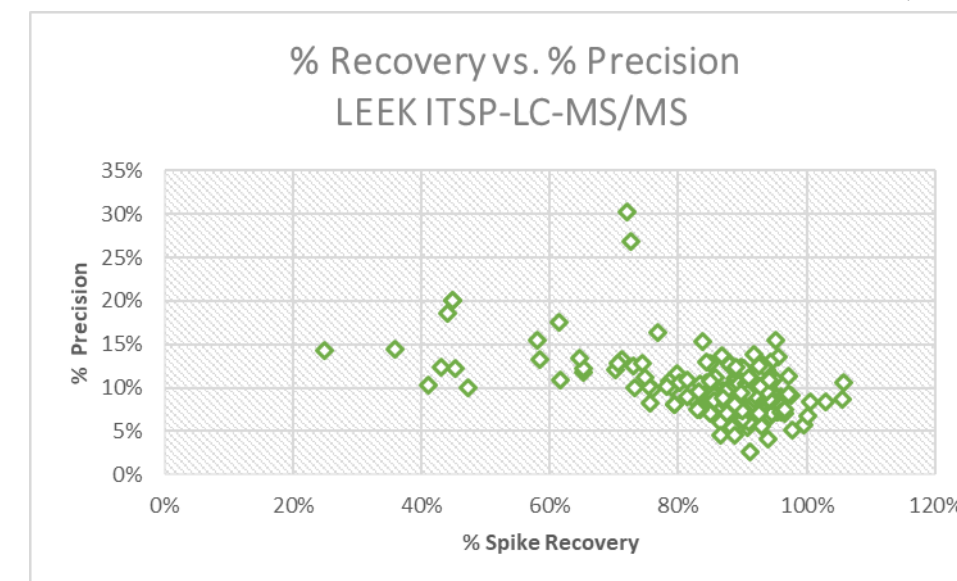- Precision <20% was achieve for most of the monitored compounds (Fig. 3)

Figure 3. Leek extract without clean-up and after µSPE clean-up

Figure 4. %Spike Recovery vs. % precision of Leek QuEChERS extracts
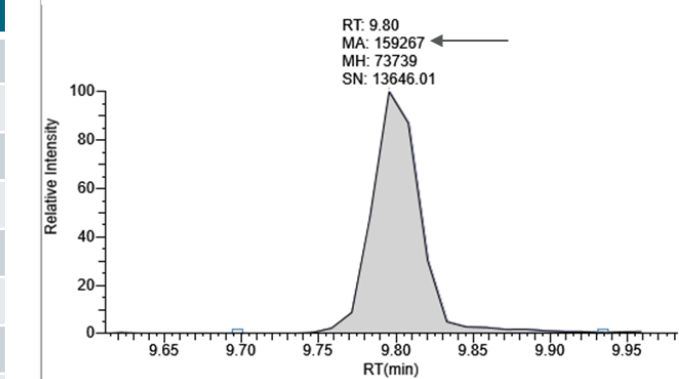
Avocado QuEChERS extracts

✓ High oil content and color which are removed by µSPE clean-up

Table 2. Reduction od matrix effects for certain compounds

| Selected Compounds	Matrix effects before mSPE	Matrix effects after mSPE
Brodifacoum	90%	78%
Etofenprox	81%	74%
Resmethrin	79%	73%
Fenpyroximat	65%	59%
Fenazaquin	65%	59%
Flufenoxuron	58%	55%
Bifenazate	94%	53%
chlorfluazuron	56%	48%
Clethodim	54%	47%
Profenofos	46%	44%
Tetramethrin	40%	44%
Tebufenpyrad	44%	44%
Penconazole	46%	40%
Tolfenpyrad	44%	39%
Hexythiazox	44%	39%
Furathiocarb	40%	37%
Propiconazole	48%	36%
Hexaconazole	52%	36%
Imibenconazole	48%	33%
Tebuconazole	49%	33%
Mexacarbate	48%	32%
Sulprofos	46%	29%
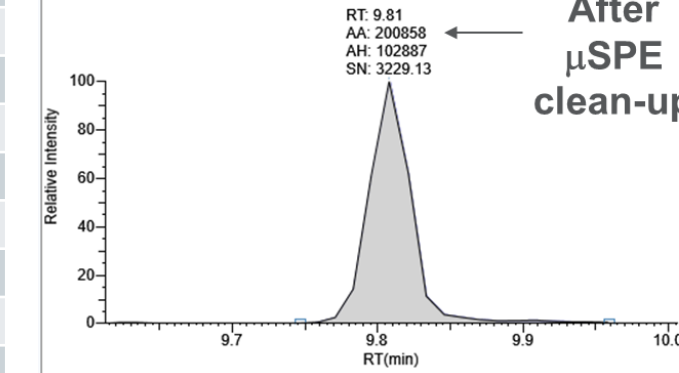
Figure 5. Sulprofos Extracted Ion Chromatograms showing better signal after µSPE clean-up.

Walnut QuEChERS extracts

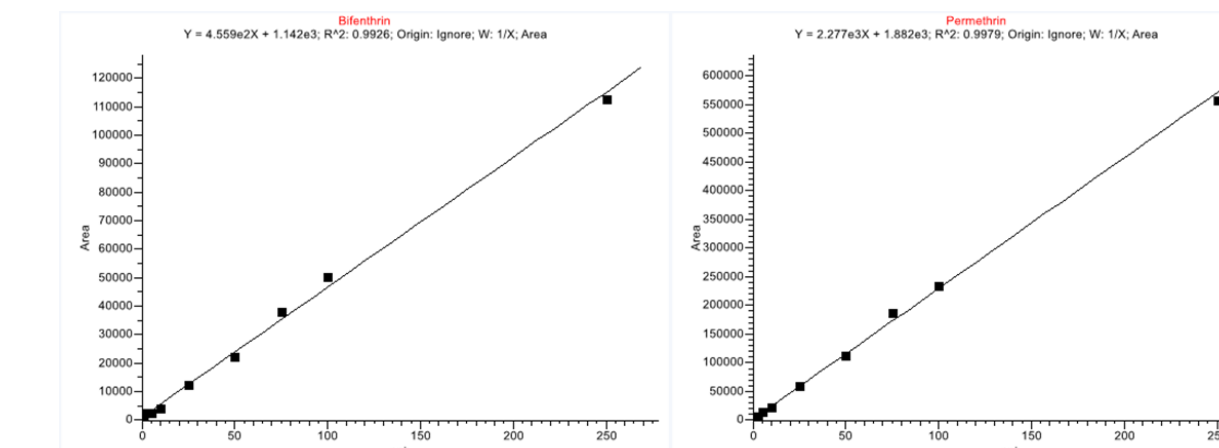✓ High oil content which is removed by µSPE clean-up

Figure 6. Calibration curves examples showing the good linearity over the range 1 – 250 ppb

- Calibrators were prepared in walnut matrix followed by µSPE clean-up
- Good linearity over the range 1 – 250 ppb for most of the compounds
- R² > 0.98 for most of compounds
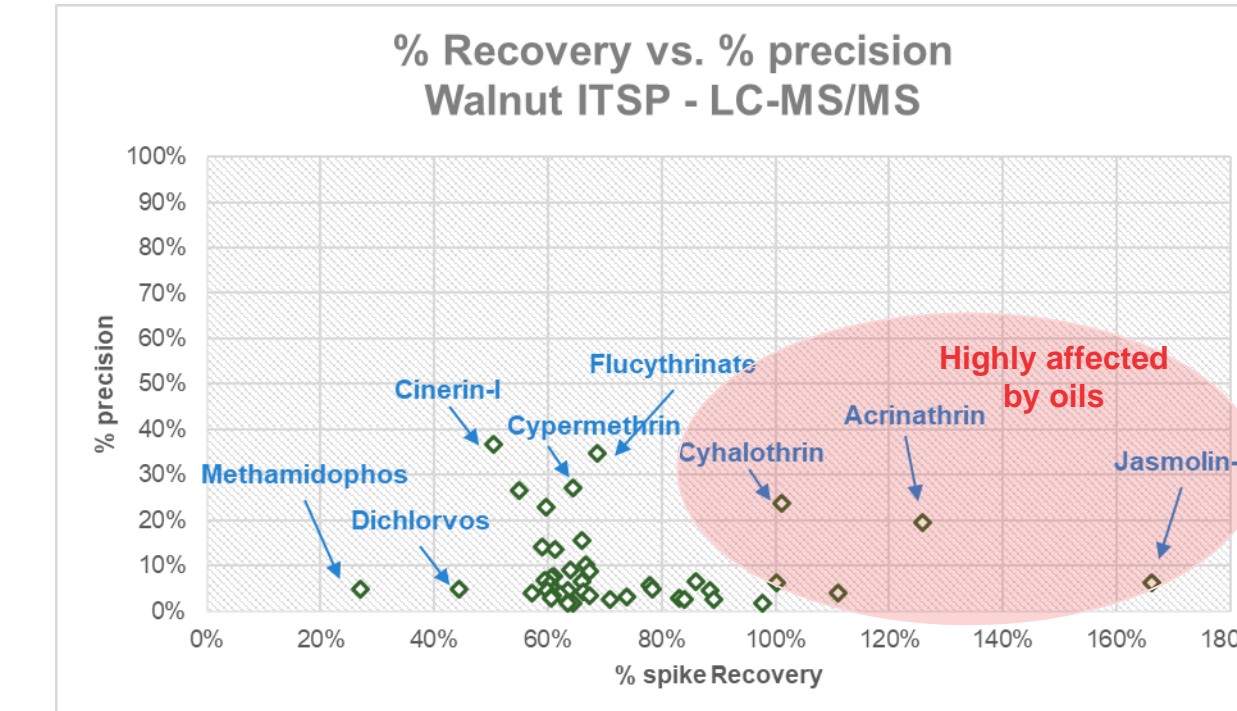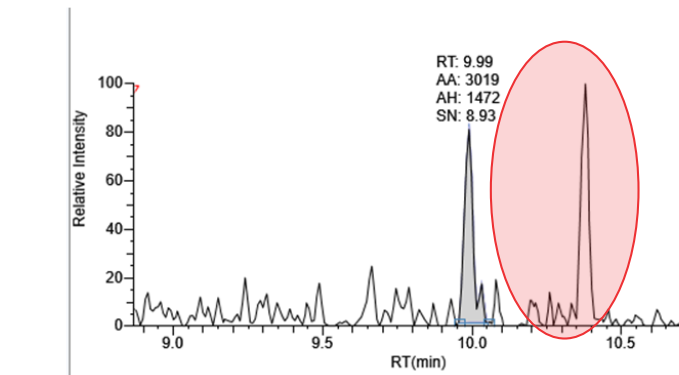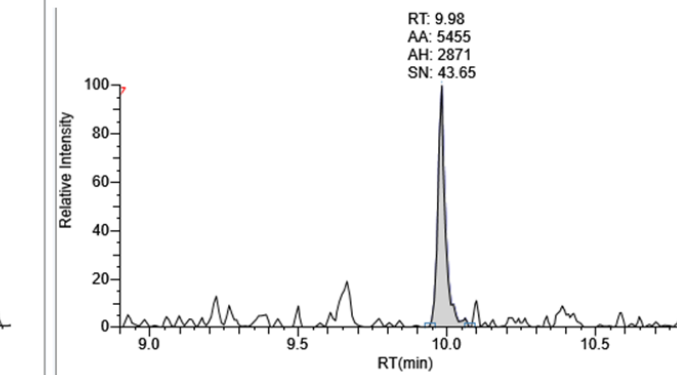- Accuracy < 20% for most of the compounds
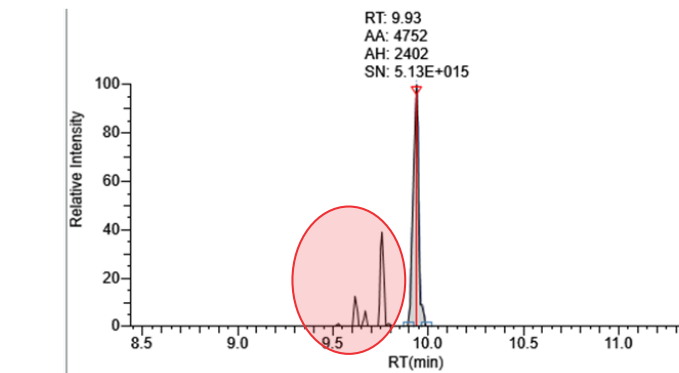

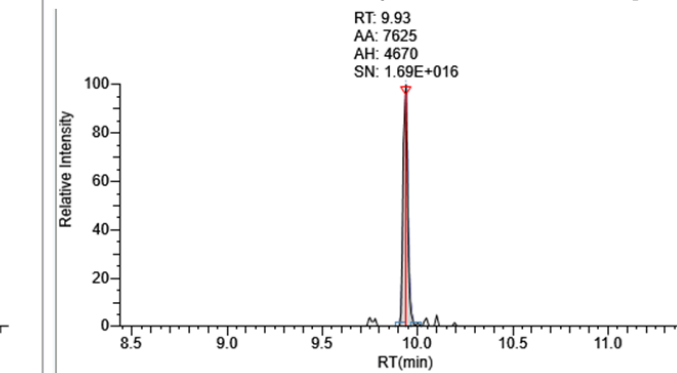Figure 7. %Recovery vs. % precision of Walnut QuEChERS extracts

Figure 8. Extracted ion chromatograms showing the removal of non-polar matrix components

CONCLUSIONS

Fully automated and online µSPE clean-up procedure with LC-MS/MS for the pesticide multi-residue analysis in food matrices was developed and evaluated.

- Average pesticides recoveries were in the range of 60 to 120% with RSDs <30% in different food matrices (Leek, avocado and walnut)
- µSPE procedure improved removal of matrix components and analytical performance
- The developed µSPE workflow is reliable, robust and suitable for LC-MS/MS analyses
- The automated µSPE procedure uses the idle time between injections, therefore LC-MS/MS run times are not affected
- Lab efficiency is improved by removing the need for manual clean-up procedure

REFERENCES

1. Anastassiades et al. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. Chromatogr. A.* 2003. 1015, 163-184.

ACKNOWLEDGEMENTS

TRADEMARKS/LICENSING

PO65484-EN0419S