Using a QuEChERS Approach for the Determination of Pesticide Residues in Soil

UCT Part Numbers:
ECQUEU750CT-MP – 4000 mg MgSO4, 1000 mg NaCl, 500 mg Sodium Citrate dibasic sesquihydrate, 1000 mg Sodium Citrate tribasic dehydrate
CUMPSC18CT – 150 mg MgSO4, 50 mg PSA, 50 mg endcapped C18
SLC-18100ID21-3UM – Selectra® C18 HPLC Column 100 x 2.1mm, 3 µm
SLC-18GDC20-3UM - Selectra® C18 Guard Cartridge, 10 x 2.0mm, 3 µm

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Introduction

The use of pesticides in agriculture and households is widespread. To ensure food safety and prevent the unnecessary exposure of consumers to pesticides it is important to test for these residues in surveillance plans. While the greatest source of pesticide exposure comes from residues that remain in final food products, they can also be found in environmental samples such as water and soil. As a consequence, any pesticides that are present in soil can potentially be incorporated into growing crops. Contaminated soil also represents a serious environmental problem as the pesticides can be transported to other environmental systems such as ground water and air.

Due to the wide range of pesticides used in agriculture, the development of fast multi-residue methods that simultaneously determine a wide range of pesticides is essential. One of the most widely used multi-residue methodologies is the QuEChERS approach. This offers many advantages including speed, cost, ease of use, good performance characteristics and wide applicability range (matrices and analytes).

Soil is a complex matrix consisting of organic and inorganic material. It possesses many active sites (polar, non-polar and ionic) that are capable of retaining pesticides and other residues. Compared to other matrices commonly encountered in pesticide residue analysis (e.g. fruits and vegetable), soil samples are more difficult to extract and require longer extraction times due to the stronger interactions that may occur between the soil and the pesticides.

The aim of this study was to evaluate the effectiveness of the QuEChERS extraction and cleanup approach for the analysis of pesticides in soil. 21 pesticides, comprising various chemical properties, were used for the study. LC-MS/MS was used for detection and quantitation.

NOTE: It is possible for certain compounds to be covalently bound to the soil. These bound residues can only be removed using an acid or base hydrolysis step prior to extraction. However, if a hydrolysis step is employed, this may have a detrimental effect on pH sensitive analytes. Investigating this issue was outside the scope of this study and it was not evaluated.

QuEChERS procedure

Sample Extraction

1. Weigh 10g soil sample (≥70% H_2O content) into a 50mL centrifuge tube. Alternatively, weigh 3g air-dried soil sample into a 50mL tube and add 7mL H_2O, vortex briefly, and allow to hydrate for 30 min.
2. Add 10 mL of acetonitrile to each sample.
3. Shake (manually or mechanically) or vortex samples for 5 min to extract pesticides. (In this study a Spex SamplePrep Geno/Grinder 2010 operated at 1500 rpm was used).
4. Add the contents of an **ECQUEU750CT-MP** Mylar pouch (citrate buffered salts) to each centrifuge tube.
5. Immediately shake samples for at least 2 min.
6. Centrifuge for 5 min at ≥3000 rcf.

Sample Cleanup

1. Transfer a 1 mL aliquot of supernatant to a 2mL **CUMPSC18CT** dSPE tube ($MgSO_4$, PSA & C18).
2. Vortex samples for 0.5 - 1 min.
3. Centrifuge for 2 min at high rcf (e.g. ≥ 5000).
4. Filter purified supernatant through a 0.2 µm syringe filter directly into a sample vial.
5. Analyze samples by LC-MS/MS.

Analytical Procedure

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® C18, 100 × 2.1 mm, 3 µm (p/n: **SLC-18100ID21-3UM**)
Guard column	UCT Selectra® C18, 10 × 2.0 mm, 3 µm, (p/n: **SLC-18GDC20-3UM**)
Column temp.	40°C
Injection volume	3 µL
Autosampler	10°C
Wash solvent	MeOH:ultrapure water (1:1, v/v)
Mobile phase A	0.1% ammonium formate + 0.3% formic acid
Mobile phase B	methanol + 0.1% formic acid
Flow rate	300 µL/min
Run time	25 min (including 5 min re-equilibration)
Divert valve	Mobile phase was sent to waste for the initial 3 min and during re-equilibration to reduce ion source contamination.

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+
Spray voltage	4500 V
Vaporizer temperature	450°C
Capillary temperature	225°C
Sheath gas pressure	55 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.5 mTorr
Acquisition method	EZ method (SRM)
Cycle time	1 sec

MRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Carbendazim	4.9	192.09	132.08	29	160.08	17.0	81
Dicrotophos	5.6	238.01	108.60	33	126.58	17.0	73
Thiabendazole	8.6	202.06	131.06	31	175.07	24.0	103
DIMP	8.6	180.96	96.90	12	98.86	14.0	38
Simazine	8.6	202.01	67.97	32	131.97	17.0	104
Tebuthiuron	8.8	228.95	115.59	26	171.63	17.0	72
Carbaryl	9.0	201.96	126.97	29	144.96	6.00	40
Atrazine	9.9	215.96	67.65	35	173.60	16.0	79
DEET	10.1	191.95	90.66	28	118.63	15.0	92
Pyrimethanil	11.0	199.99	106.97	23	183.00	22.0	97
Malathion	12.3	331.01	98.57	23	126.86	12.0	60
Acetochlor	13.3	269.96	148.02	15	223.98	10.0	64
Cyprodinil	13.6	226.12	77.03	40	93.05	33.0	88
Tebuconazole	14.2	308.01	69.66	29	124.56	35.0	97
Diazinon	14.3	304.99	153.04	16	169.02	16.0	100
TPP	14.4	327.09	77.02	37	152.07	33.0	98
Zoxamide	14.4	335.92	158.91	36	186.91	19.0	89
Pyrazophos	14.7	374.10	194.06	20	222.13	20.0	104
Profenofos	15.7	372.89	127.92	41	302.79	17.0	99
Chlorpyrifos	16.4	349.70	96.81	29	197.76	20.0	81
Abamectin	17.6	889.98	304.92	25	751.21	35.0	112
Bifenthrin	18.2	440.04	165.21	39	180.42	11.0	66

Accuracy & Precision Data				
Analyte	**20 ng/g (n=6)**		**100 ng/g (n=6)**	
	Mean (%)	**RSD (%)**	**Mean (%)**	**RSD (%)**
Abamectin	74.9	11.17	71.8	6.28
Acetochlor	93.9	7.32	97.5	3.19
Atrazine	95.3	5.16	98.1	1.30
Bifenthrin	94.9	12.90	90.9	10.32
Carbaryl	95.2	7.13	93.9	3.53
Carbendazim	69.6	8.55	81.6	5.06
Chlorpyrifos	89.5	6.36	93.1	3.96
Cyprodinil	93.2	9.12	94.1	1.78
DEET	107.3	6.75	101.1	0.67
Diazinon	94.4	7.53	98.2	1.36
Dicrotophos	91.0	6.61	99.1	3.35
DIMP	82.5	6.74	88.1	1.47

Accuracy & Precision Data (cont)				
Analyte	**20 ng/g (n=6)**		**100 ng/g (n=6)**	
	Mean (%)	**RSD (%)**	**Mean (%)**	**RSD (%)**
Malathion	52.3	9.29	78.1	1.78
Profenofos	79.5	8.76	88.6	2.75
Pyrazophos	80.5	8.01	93.9	2.63
Pyrimethanil	90.2	4.88	92.2	2.36
Simazine	92.4	7.74	98.9	2.77
Tebuconazole	88.5	6.69	93.1	3.08
Tebuthiuron	100.7	7.39	101.1	2.14
Thiabendazole	52.8	5.61	63.1	6.80
Zoxamide	92.4	7.92	99.4	2.11
Note: TPP was used as an internal standard. Matrix-matched calibration curves were used for quantification.				

Figure 1. LC-MS/MS chromatogram of 21 pesticides and internal standard (TPP):

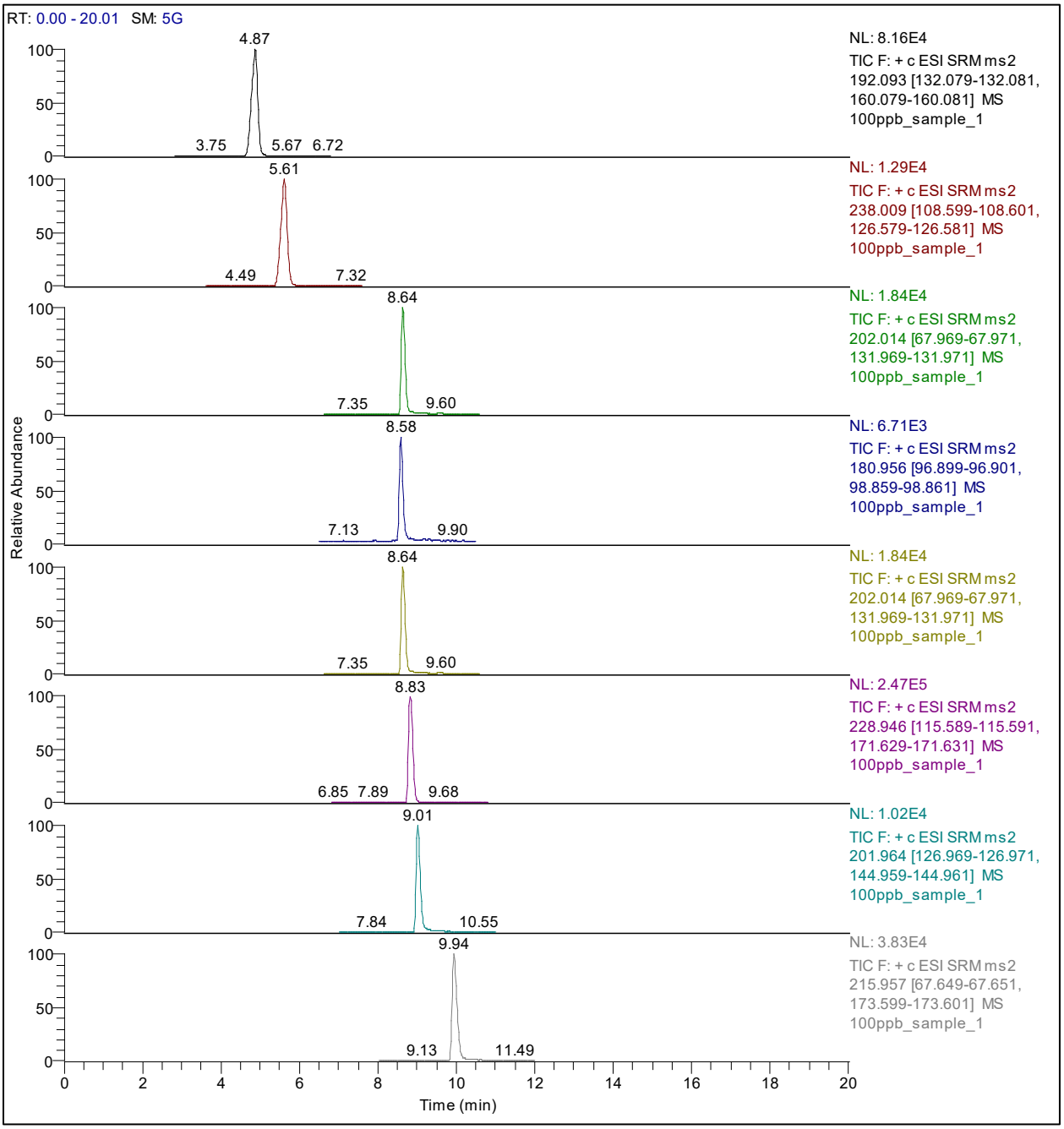


Figure 1 continued.

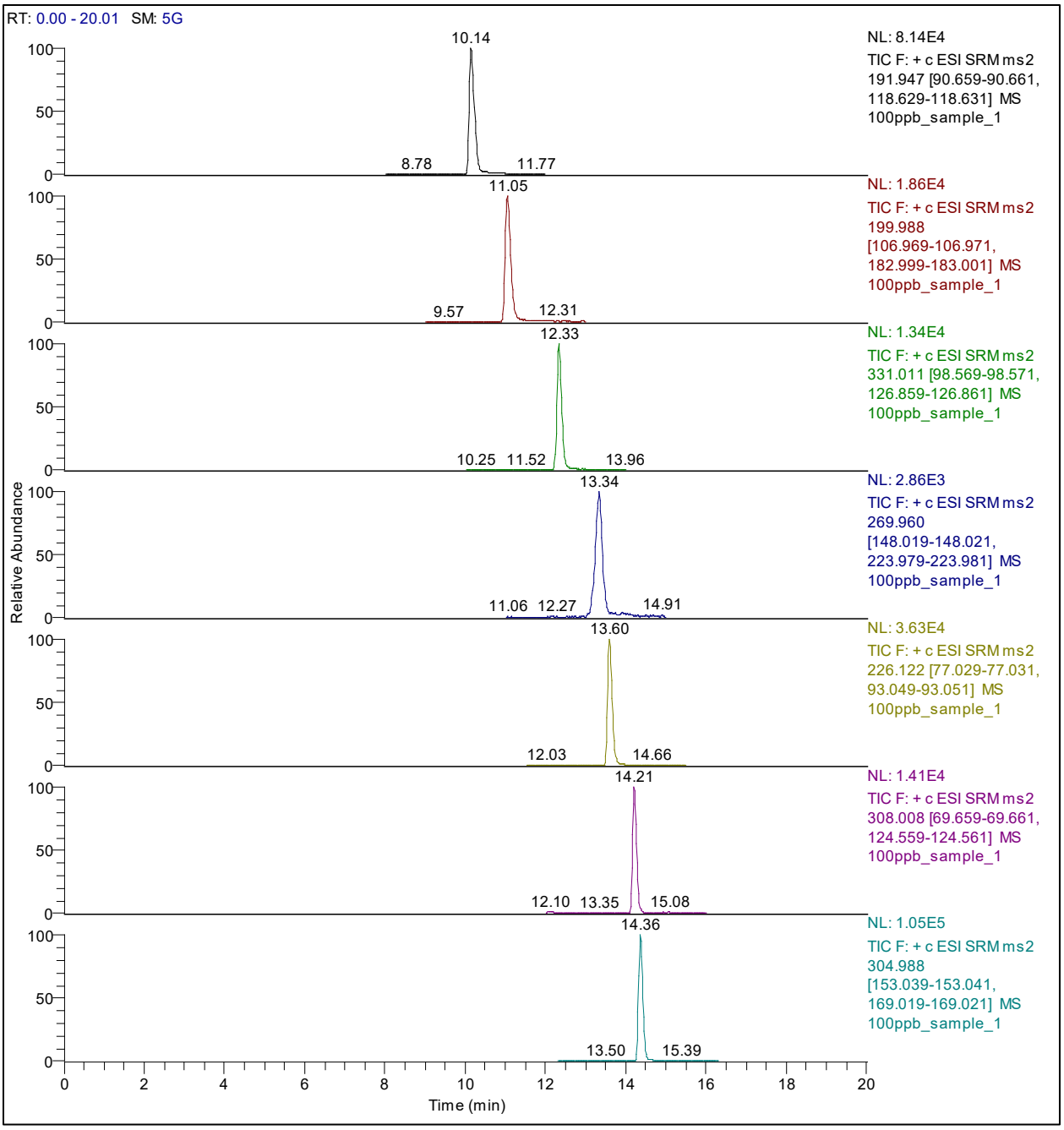
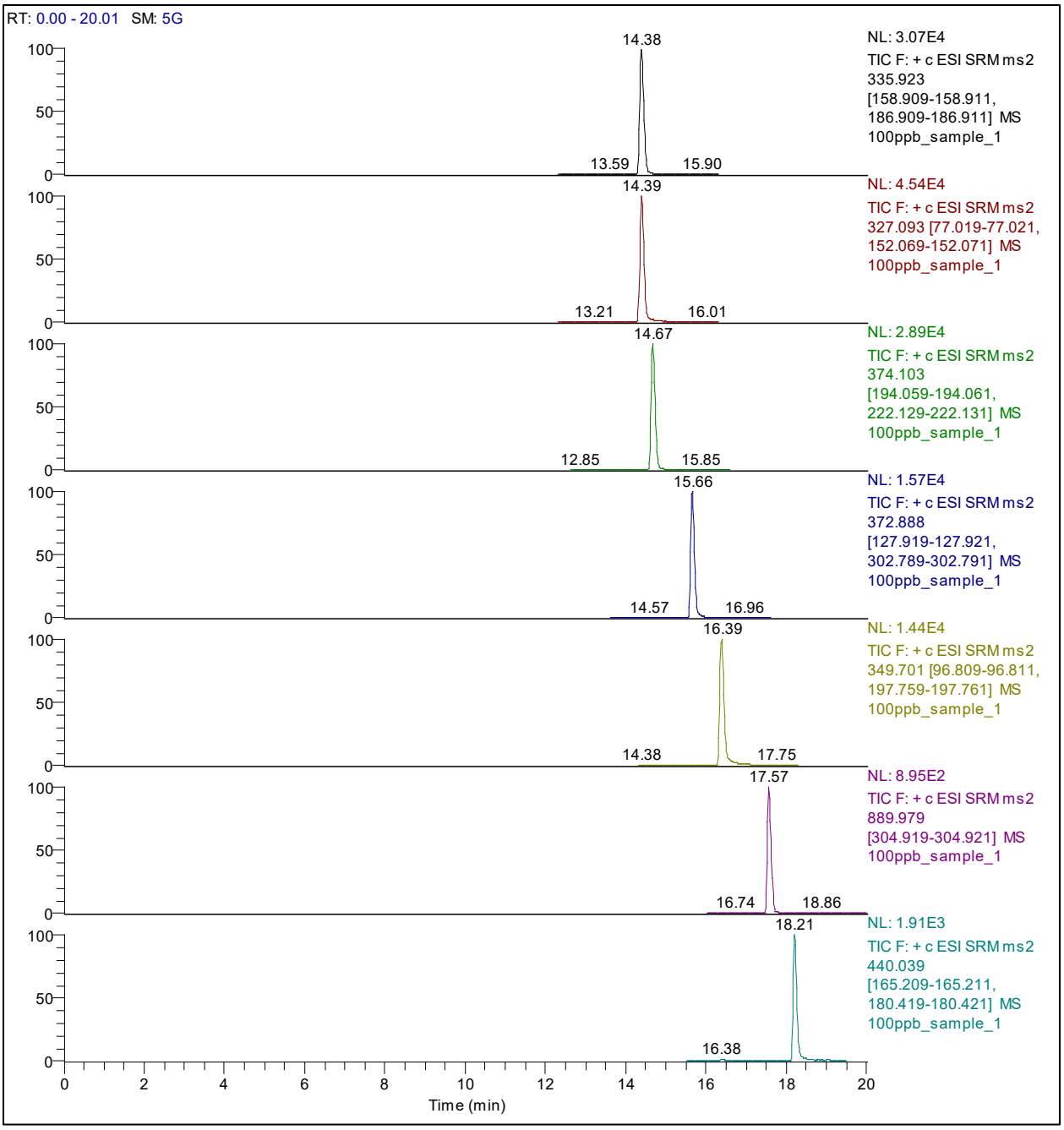


Figure 1 continued.



Results & Discussion

The vast majority of pesticides included in the study could be efficiently extracted from soil using the QuEChERS approach. Neutral pesticides, in particular, could be readily extracted using acetonitrile in combination with the citrate buffered QuEChERS salts. Thiabendazole on the other hand gave low, though reproducible, recovery throughout the study. Thiabendazole is a basic compound that is positively charged at low pH and is capable of being retained on the soil through ionic interactions, particularly by humic/fulvic acids. In addition, it is a planar pesticide and could potentially be retained by strong hydrophobic interactions on the soil (e.g. similar to analyte retention on graphitized carbon black (GCB)).

In the dispersive-SPE cleanup step, using a combination of PSA/C18 yields cleaner extracts than using PSA alone and should be used whenever possible. In this study, no major variation in results was observed between PSA and PSA/C18. In fact the PSA/C18 gave slightly better results, possibly due to reduced matrix effects.

Using UCT's Selectra® C18 HPLC column resulted in good retention and separation of the 21 pesticides and internal standard in less than 20 min. 6-point matrix-matched calibration curves (10, 20, 100, 200, 500 and 1000 ng/mL) were used to obtain the most accurate results possible. Linearity in detector response was observed over the concentration ranges investigated with correlation coefficients (R^2 values) greater than 0.99 for all 21 analytes. As outlined in the Accuracy and Precision Data table, the majority of results were found be within an acceptable recovery range of 70-110 % and have RSD values <10 %, demonstrating that the method meets acceptable performance criteria.

In conclusion, the QuEChERS sample preparation method provides a fast and simple approach for extracting and analyzing 21 pesticides in soil while achieving acceptable recovery and reproducibility. The use of UCT's Selectra® C18 HPLC column provided good chromatographic separation for all analytes included in the study.

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