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Cleanup of QuEChERS Extracts using SBSE for LC/MS/MS Determination of Pesticides in Food Products

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Abstract

One of the most important aspects of reducing pesticide exposure is monitoring of pesticide residues in foods. A number of analytical methods have been developed, many of them based on traditional liquid-liquid extraction in combination with GC-MS or LC-MS. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples [1]. The dispersive Solid Phase Extraction (SPE) used to clean up these extracts can leave co-extractants, which can result in interferences such as ion suppression with the analytical results.

Stir bar sorptive extraction (SBSE) is a sorptive extraction technique based on polydimethylsiloxane (PDMS) coated stir bars. SBSE was developed to concentrate nonpolar analytes from aqueous solutions, and has recently been shown to effectively extract and concentrate PAHs from QuEChERS extracts while eliminating matrix interference for GC/MS analysis [2].

In this study we describe the potential benefits of using SBSE to concentrate pesticides from QuEChERS extracts and provide additional clean-up resulting in less matrix interference during LC-MS/MS.

Introduction

Spices and teas represent some of the most widely traded commodities in the global food market. Considering the geographical differences and variations of native and invasive pest populations, pesticide application approaches vary widely by region. Even properly managed pesticide use coupled with non-harmonized Maximum Residue Levels pose significant challenges for import and export. Recent concerns about economically motivated adulteration further complicate the safe and compliant marketing of spices and teas. Unapproved and heavy pesticide use jeopardizes the safety of consumers and the integrity of established brands of food products. Regular pesticide testing is the only means of providing the necessary data to help verify whether such commodities are safe for human consumption and comply with global pesticide regulations.

Unfortunately, spices and teas pose analytical challenges for successful extraction, isolation, and detection. QuEChERS based techniques often result in massive matrix interference that mask or inhibit the identification and quantification of analytes of interest. While excessive dilution may help improve identification by reducing matrix interference, it can result in exceedingly high limits of quantitation, thereby limiting the effectiveness of the technique.

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In this study we describe the potential benefits of using SBSE to concentrate pesticides from QuEChERS extracts. Recovery of the pesticides concentrated on the SBSE phase by liquid desorption provides better analytical sensitivity for the pesticides being monitored with reduced matrix interference. Manual steps such as evaporation, reconstitution, and dilution as well as the subsequent LC/MS/MS analysis of the final extracts can be automated to improve laboratory productivity for monitoring pesticide residues in foods.

Experimental

Materials

All pesticide analyte stock solutions were purchased from AccuStandard, Inc. Intermediate analyte stock solutions were prepared by combining the analyte stock solutions with methanol, at appropriate concentrations, to evaluate the different analytes. Final standards for calculating %Recoveries were prepared by combining the appropriate analyte stock with (90:10) water:acetonitrile.

A deuterated analogue, d_5-atrazine, was purchased from Restek. A working internal standard stock solution containing the d_5-atrazine internal standard was prepared at a concentration of 10 µg/mL in methanol.

Ground organic ginger and ground organic turmeric samples were purchased from a local market.

The Twister stir bars (10 mm length x 0.5 mm film thickness, Figure 1) used for extractions were from GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany. Stirbar sorptive extraction parameters were held constant throughout this work by mixing samples at 1200 rpm on a multiposition stir plate for 1 hour at room temperature.

Figure 1: The GERSTEL Twister® stirring and extracting a liquid sample.

The dispersive SPE blend used during the QuEChERS approach extractions contained 150 mg of magnesium sulfate and 50 mg of PSA and were from Agilent Technologies . All other reagents and solvents used were reagent grade.

Instrumentation

Samples were analyzed using an Agilent 1290 HPLC with an Agilent Eclipse Plus C18, RRHD, (2.1 x 50 mm, 1.8 µm) column and an Agilent 6460 Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. A GERSTEL MPS XL autosampler configured with an Active Washstation performed all injections as well as automated evaporation and reconstitution of samples during the extraction procedure using the mVAP Option. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 2 µL stainless steel sample loop.

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The final QuEChERS extraction and stir bar sorptive extraction procedure followed during the course of this work is shown below. Variations and modifications from this procedure and their effects on the resulting extracts are discussed within the Results and Discussion section.

QuEChERS/Twister Extraction Procedure:

- Weigh 0.25 gram of ground, organic, dry spice into a 50 mL polypropylene centrifuge tube.
- Add 10 mL of deionized water to the sample.
- Add the appropriate amount of pesticide spiking stock (if applicable) and internal standard solution (if applicable) to the sample.
- Add 10 mL of 100% acetonitrile to the sample.
- Vortex mix for 30 seconds.
- Add 6 grams of $MgSO_4$ to the sample.
- Add 1 gram of NaCl to the sample.
- Vortex mix for 30 seconds and then shake vigorously by hand for 5 minutes, making sure that the samples, solvents, and salts mix well.
- Centrifuge the sample at 3000 g for 5 minutes.
- Transfer 7.5 mL of the supernatant from the 1st QuEChERS extract sample into a 10 mL vial and cap with a magnetically transportable cap.
- Evaporate the extract to dryness using the GERSTEL mVAP Option at 55 °C under vacuum (100 mbar).
- Reconstitute the resulting residue using 5 mL of a saturated NaCl solution by vortex mixing and then sonication for 30 minutes.
- Add a Twister stir bar and stir for 1 hour at 1200 rpm.
- Remove the Twister from the sample, dip it into clean deionized water, and blot dry with a lint-free tissue.
- Place the Twister into a 2 mL vial and add 1 mL of 100% acetonitrile.
- Sonicate the vial for 30 minutes.
- Remove the Twister.
- Dilute 100 µL of the acetonitrile from the Twister back extraction with 900 µL of deionized water.
- Inject 2 µL into the LC/MS/MS system.

Analytical Method LC Method Parameters

Mobile Phase	A - 5 mM ammonium formate in water, with 0.01% formic acid	
	B - 0.01% formic acid in acetonitrile	
Gradient	Initial	6% B
	0.3 min	6% B
	14 min	95% B
	17 min	95% B
	17.1 min	6% B
Pressure	600 bar	
Flowrate	0.5 mL/min	
Run time	20 minutes	
Injection volume	2 µL (loop over-fill technique)	
Column temperature	55 °C	

Analysis Conditions MS

Operation	electrospray positive mode + Agilent Jetstream
Gas temperature	325 °C
Gas flow (N_2)	8 L/min
Nebulizer pressure	35 psi
Sheath Gas Temp	375 °C
Sheath Gas Flow	11 L/min
Capillary voltage	4500 V
Nozzle voltage	500 V

The mass spectrometer acquisition parameters for all compounds are shown in Table 1 along with the qualifier ion transitions.

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Table 1: Mass Spectrometer Acquisition Parameters for the Pesticides monitored.

Compound	Precursor Ion [m/z]	Product Ion [m/z]	Fragmentation [V]	CE [V]	Ret Time [min]
Deltamethrin	523	280.9	70	15	11.4
		181	70	50	
Permethrin	391.1	355	100	5	11.8
		183	100	5	
Malathion	331	211	80	10	7.46
		127	80	5	
Chlorpyrifos-methyl	321.9	289.9	80	15	8.79
		125	80	15	
Diazinon	305.1	169	160	20	8.54
		153	160	20	
Metolachlor	284.1	252	120	10	7.45
		176	120	15	
Atrazine-d5	221.1	179	120	20	4.96
		137	120	20	
Atrazine	216.1	174	120	15	5.00
		132	120	20	
Carbaryl	202.1	145.1	80	5	4.91
		117	80	10	
Carbendazim	192.1	160	90	20	2.14
		132.1	90	25	

Results and Discussion

Reduced Matrix Background

During routine LC-MS/MS determinations of pesticides in some matrices it was found that results for some pesticides extracted using the QuEChERS approach were unreliable, suggesting significant matrix interference even after optimizing the choice of dSPE sorbent. Since an earlier study [2] had shown that sample concentration using Stir Bar Sorptive Extraction (SBSE) also provided the benefit of eliminating some matrix interference in GC/MS analysis we decided to evaluate whether SBSE might also provide this benefit when analyzing by LC-MS/MS.

We first determined that performing an additional cleanup step following the dSPE of the typical QuEChERS strategy did provide some benefit for eliminating background matrix effects from QuEChERS extracts. In Figure 2 (organic ginger) and Figure 3 (organic turmeric) it can be seen that extracts that had undergone the typical QuEChERS cleanup plus an additional SBSE cleanup have much less matrix interference peaks compared with extracts that had undergone only the typical QuEChERS cleanup plus dSPE extraction.

We then tested whether SBSE alone provided significant cleanup of the QuEChERS extract. As shown in Figure 4, the resulting background from a QuEChERS extract of organic ginger resulted in a cleaner background when comparing with the results from the same extract that had undergone a typical QuEChERS plus dSPE extraction.

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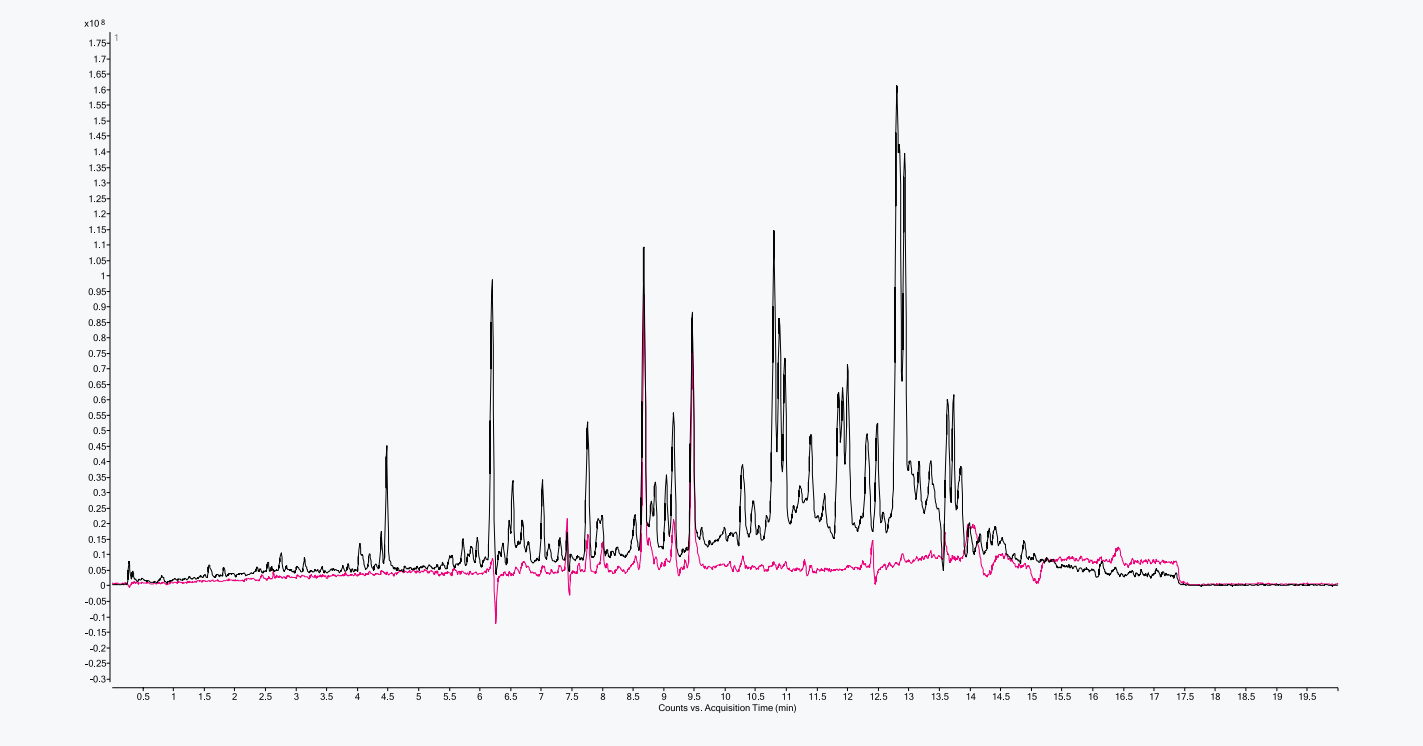

Figure 2: Comparison of background matrix interference between QuEChERS (black trace) and QuEChERS combined with SBSE (red trace) for ground organic ginger. Full scan (m/z 85-550) Frag 135; blank mobile phase subtracted from each TIC.

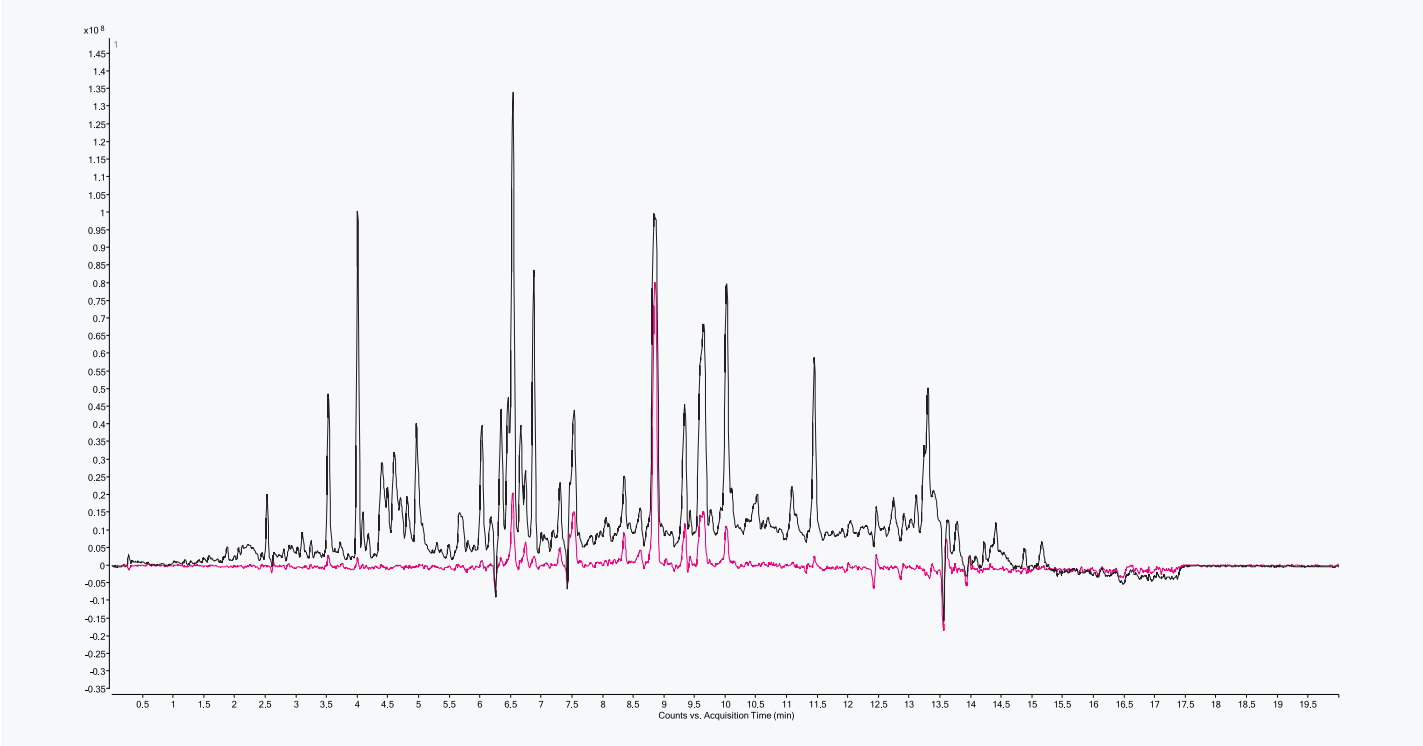

Figure 3: Comparison of background matrix interference between QuEChERS (black trace) and QuEChERS combined with SBSE (red trace) for ground organic turmeric. Full scan (m/z 85-550) Frag 135; blank mobile phase subtracted from each TIC.

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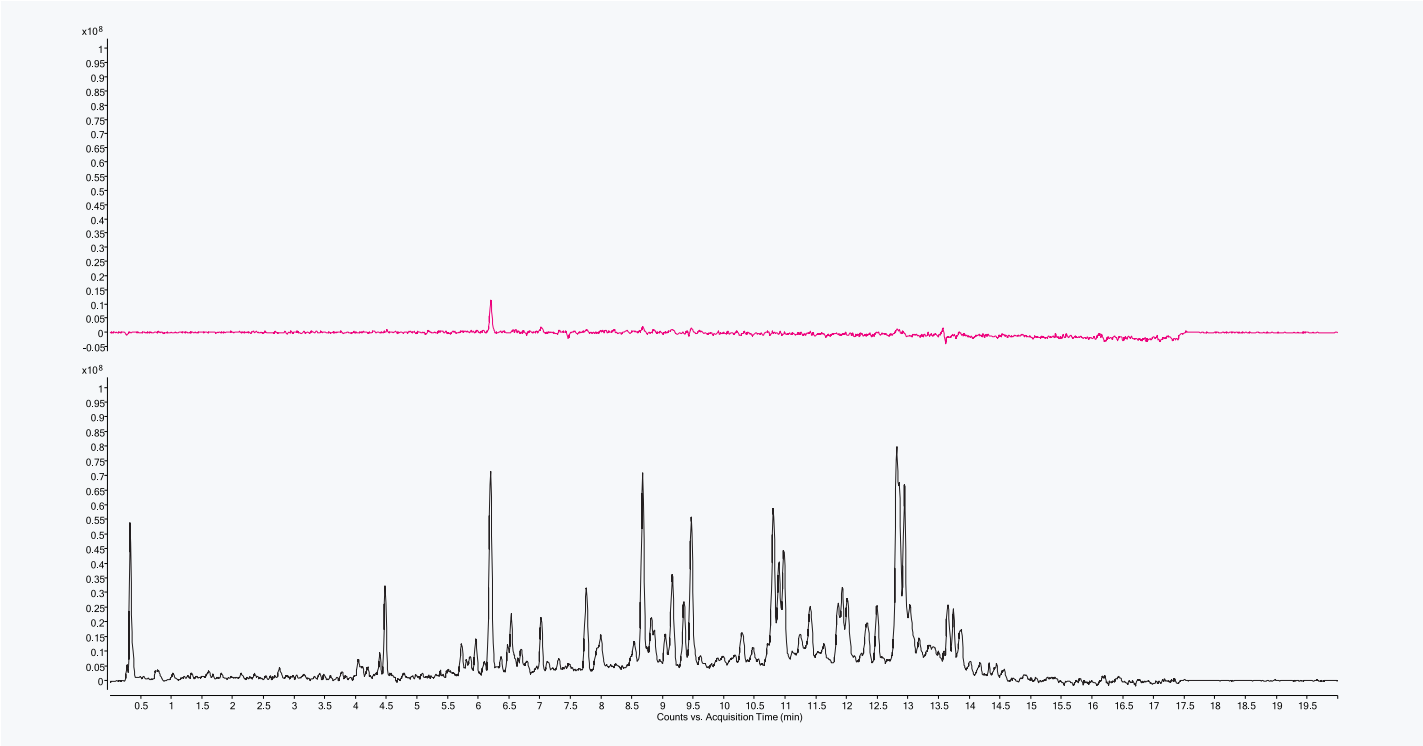

Figure 4: Comparison of background matrix interference between 1 mL of 1st QuEChERS extract combined with SBSE (A) and 1 mL of 1st QuEChERS extract combined with dSPE (B) for ground organic ginger.

Optimizing SBSE

QuEChERS acetonitrile extracts can be prepared for SBSE in several ways. We compared direct 1:10 dilution of the acetonitrile as performed in the previously mentioned study to evaporating and then redissolving the extracts since the analytes of interest in this study were primarily polar and nonvolatile. Eliminating the acetonitrile by evaporation followed by redissolving the extracts in water should improve the extraction efficiency for polar pesticides; redissolving the extracts in water saturated with NaCl should further improve recovery of polar pesticides by helping to drive the pesticides toward the PDMS phase during SBSE.

As shown in Table 2, the recovery of pesticides in organic ginger from acetonitrile/water using the SBSE is best for analytes with higher log $K_{o/w}$'s. In addition, pesticides with lower $K_{o/w}$'s showed relatively low recovery from acetonitrile/water. Evaporating the acetonitrile and reconstituting in water provided the best recovery for the highest $K_{o/w}$ compound (Diazanon) whereas reconstituting in a saturated NaCl solution provided the best recovery for most polar pesticides.

Table 2: Table of %Recoveries for SBSE extracts with various extraction conditions.

Pesticide	Log $K_{o/w}$	dSPE ONLY	Twister (1:9) in H_2O*	Twister (no ACN) H_2O	Twister (no ACN) sat. NaCl
Carbendazim	1.48	88.7	0.06	4.7	5.9
Malathion	2.29	115	9.27	45.1	43.6
Carbaryl	2.35	186	0.34	14.1	44.8
Atrazine-D5	2.82	90.3	0.36	5.6	21.6
Atrazine	2.82	91.9	0.44	6.6	22.3
Metolachlor	3.24	94	6.32	21.7	25.9
Diazinon	3.86	111	34.9	79.4	52.7

** Established using higher concentration*

Improving Detection Limits

Improvement of detection limits is possible for QuEChERS extracts when combined with SBSE because additional sample extract volume can be used without increasing the amount of matrix. Figure 5 shows a comparison of the matrix background for organic ginger when either 1 mL or 7.5 mL of extract was evaporated and reconstituted followed by either dSPE or SBSE cleanup. Drying down a larger volume of acetonitrile followed by dSPE resulted in significant increase in the matrix background whereas the background following SBSE remained very low. In addition, the pesticides recovered by evaporating, reconstituting and cleanup using SBSE showed minimal ion supression compared to those recovered using dSPE cleanup alone (Table 3).

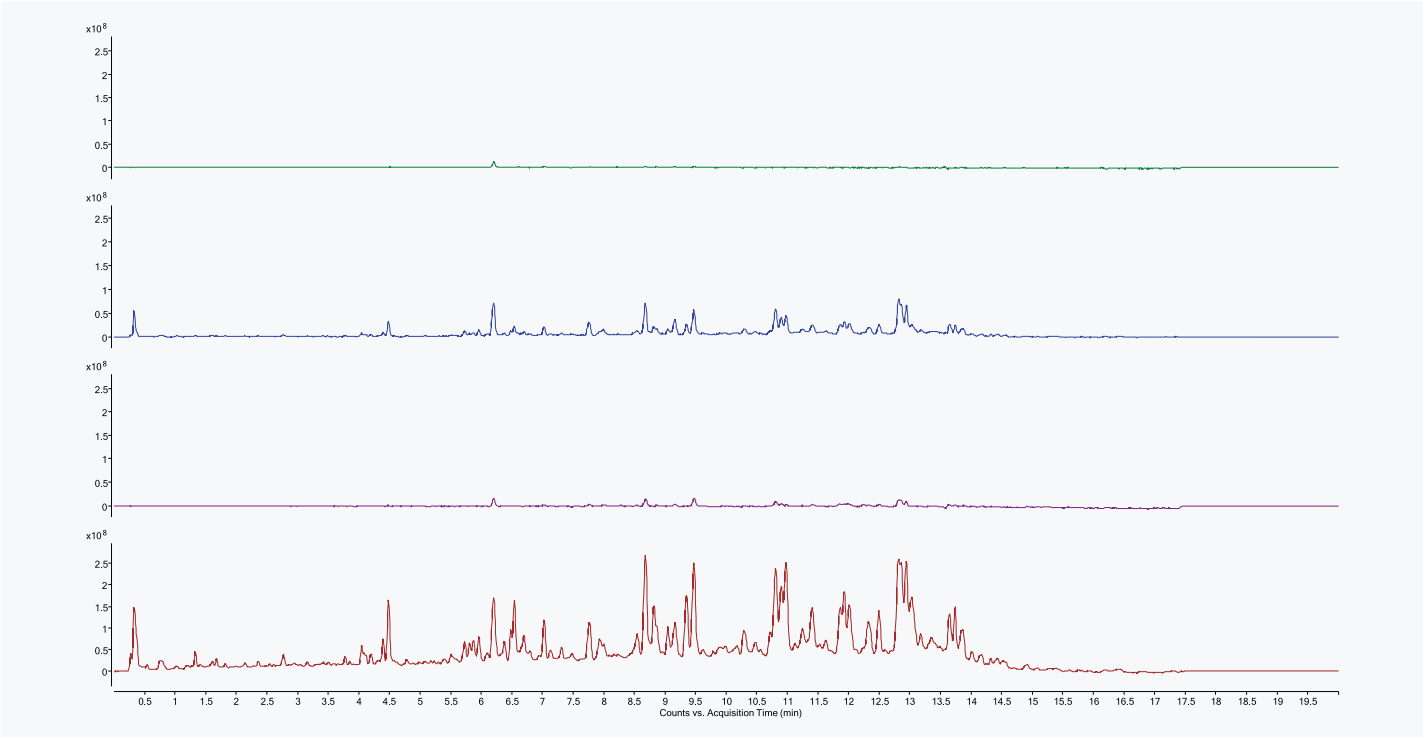

Figure 5: Comparison of background matrix interference between increased volumes of QuEChERS extracts for ground organic ginger, combined with dSPE and SBSE cleanup respectively. A: 1 mL with SBSE, B: 1 mL with dSPE, C: 7.5 mL with SBSE, D: 7.5 mL with dSPE.

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Table 3: Table of %Recoveries comparing dSPE extracts with SBSE extracts. Twister enables increase of sample amount without increasing matrix amount.

Analyte	Log $K_{o/w}$	0.25 g H_2O dSPE ONLY [1 mL]	0.25 g Ginger dSPE ONLY [1 mL]	0.25 g Ginger dSPE ONLY [7.5 mL]*	0.25 g H_2O Twister (no ACN) sat. NaCl	0.25 g Ginger Twister (no ACN) sat. NaCl
Carbendazim	1.48	90.9	101	97	3.7	5.9
Malathion	2.29	92.9	269	278	68.5	43.6
Carbaryl	2.35	85	729	746	48.8	44.8
Atrazine-D5	2.82	93.6	101	102	56.2	21.6
Atrazine	2.82	88.8	99	100	56.8	22.3
Metolachlor	3.24	92.8	105	100	72.7	25.9
Diazinon	3.86	88.2	175	163	32.3	52.7

** 7.5mL % Recoveries adjusted by dividing peak area by 7.5 mL first*

Representative calibration curves for Atrazine and Metolachlor in organic ginger are shown in Figure 6 and show that calibration curves can be successfully created using the QuEChERS-SBSE extraction and cleanup strategy.

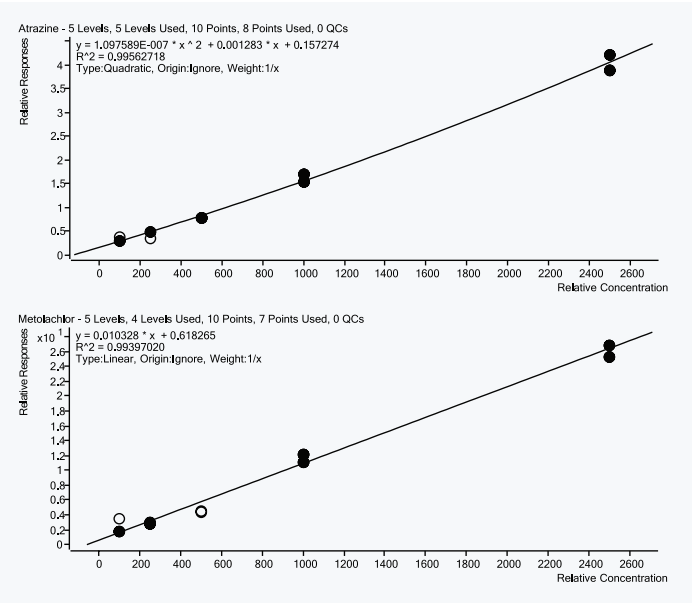

Figure 6: Representative calibration curves for Atrazine and Metolachlor in ground organic ginger.

Future work is planned in order to assess other QuEChERS and SBSE extraction parameters as well as to evaluate additional pesticides and commodities.

Conclusions

As a result of this study, we were able to show:

- Stir Bar Sorptive Extraction using the GERSTEL Twister® offers an alternative to dSPE for cleanup of QuEChERS extracts and has been shown to help decrease matrix interference.
- Improved detection limits are possible for QuEChERS extracts when combined with SBSE since additional sample volume can be used without simultaneously increasing the amount of potentially interfering matrix.
- Automation of sample injection, liquid handling and evaporation steps can be performed using the GERSTEL MultiPurpose Sampler (MPS).

References

[1] *Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate*; collaborative study. Lehotay, SJ., J. AOAC Int. 90, 485 (**2007**).

[2] *A High Throughput Method for Measuring Polycyclic Aromatic Hydrocarbons in Seafood Using QuEChERS Extraction and SBSE*. International Journal of Analytical Chemistry Volume 2015 (**2015**), Article ID 359629