Extraction of Pesticides from Spices

Abstract

The QuEChERS method has been shown to be practical for pesticide clean up and extraction on a number of different sample types and is increasingly being used on more difficult matrices. Unfortunately, this process is a manual, multi-step process that can be time consuming and yield variable results. With so many different types of food matrices and pesticide residues to be analyzed, there is a need for a faster, simpler extraction method. Furthermore, some matrices, either by their nature or their economic value, can be difficult to extract with just the QuEChERS method alone. Spices are among these difficult matrices, because of the dehydrated state in which they are received. The EDGE® provides a valuable alternative to the QuEChERS method for the extraction of pesticides from spices, with its ability to extract pesticide residues in less than seven minutes, in one automated step.

Introduction

In recent years, consumers have had a growing concern for additives and contaminants in their foods. Of most concern are those additives that can cause long term hazardous health effects, such as pesticides. Consumer demand, coupled with an ever increasing list of regulated pesticides, has created a need for rapid and accurate pesticide analysis. The QuEChERS method has been a widely accepted method to meet some of these challenges; however, because of the large number of pesticides being monitored and the decrease in the maximum residue limits, sample preparation and analysis can be a big challenge.

The manual, multi-step process of the QuEChERS method requires multiple sample transfers and generates a lot of consumable waste. Alternative methods can help streamline this workflow, yielding improved recoveries for difficult matrices, such as spices, with faster and simpler methods. The EDGE automated extraction system performs extraction and clean-up, when necessary, in a single automated step. The collected extract is automatically filtered, cooled, and ready for analysis. Both the rinsing and washing of the sample, ensuring no carryover, are included in the run time. The EDGE offers the fastest automated pesticide extraction possible in one simple method.

Materials and Methods

Reagents

Black pepper, cinnamon, oregano, and paprika were obtained from a local grocery store. All samples were spiked with 10 μL of 100 μg/mL stock solution of Restek LC multi-residue pesticide standards 1, 6, 7, and 9. Samples were set aside for 30 minutes after spiking, prior to analysis. Sodium acetate, magnesium sulfate, and primary secondary amine were purchased in bulk from Silicycle. Spiked samples were extracted via the EDGE or QuEChERS method. Acetonitrile with 1% acetic acid was used as the extraction, rinse, and wash solvent.

Sample Preparation

A 2 g portion of each spice was weighed into an assembled Q-Cup containing a S1 Q-Disc® stack (C9+G1+C9 sandwich). A Q-Screen® was placed on top of each sample using a Q-Screen tool. The Q-Cups were placed in the EDGE removable rack, along with 50 mL polypropylene conical tubes. The rack was slid into position on the EDGE. The CEM-approved EDGE method for Pesticide Residues was run. The extract was transferred to a Q-Dry™ evaporator for evaporation to <5 mL. Extracts were then diluted to 5 mL with acetonitrile with 1% acetic acid. All samples and blanks were prepared in triplicate. The extracts were transferred to vials for analysis.

EDGE Method for Pesticide Residues

Q-Disc: S1 Q-Disc stack (C9+G1+C9 sandwich)

Cycle 1
Extraction Solvent: 1% Acetic Acid in Acetonitrile
Top Add: 25 mL
Bottom Add: 0 mL
Rinse: 5 mL
Temperature: 40 °C
Hold Time: 03:00 (mm:ss)

Cycle 2 (Rinse Only)
Extraction Solvent: 1% Acetic Acid in Acetonitrile
Top Add: 0 mL
Bottom Add: 0 mL
Rinse: 5 mL
Temperature: - - -
Hold Time: - -:- -

Wash 1
Wash Solvent: 1% Acetic Acid in Acetonitrile
Wash Volume: 10 mL
Temperature: 40 °C
Hold Time: 00:03 (mm:ss)

QuEChERS Method

A 2 g portion of each spice was weighed into a 50 mL centrifuge tube. 10 mL of DI water was added to the tubes and then the tubes were manually shaken for 30 seconds and left to stand for 30 minutes, per modified QuEChERS methodology for dry samples. A volume of 10 mL of acetonitrile with 1% acetic acid was added to the tubes and vortexed for 1 minute on a VWR Analog Vortex Mixer. 1.5 g of sodium acetate and 6 g of magnesium sulfate were added to each tube and the resealed tubes were shaken for 1 minute and centrifuged at 6000 rpm for 5 minutes in a Thermo CL2 Centrifuge. A volume of 1 mL of the acetonitrile layer was added to a 50 mL centrifuge tube containing 150 mg of magnesium sulfate and 50 mg of primary secondary amine. The tubes were shaken for 1 minute and centrifuged at 9000 rpm for 10 minutes. The supernatant was transferred to a vial for analysis. All samples and blanks were prepared in triplicate.

Analysis

A volume of 10 µL of each extract was injected into a Waters Acquity UPLC with a Xevo TQD triple quad mass spectrometer and a Restek ARC-18, 2.7 µm, 100 x 2.1 mm column with a flow of 0.4 mL/min. An elution program with a 7-minute ramp from 95% A (water containing 2 mM ammonium formate with 0.2% formic acid) and 5% B (methanol containing 2 mM ammonium formate with 0.2% formic acid) to 100% B and one MSD transition was used for quantification for each pesticide. Each sample was analyzed in triplicate.

Results

The EDGE efficiently extracted the pesticides from spices in under seven minutes, including filtration, cooling, and system washing. **Table 1** (page 3) shows the EDGE recovery data of multiple pesticides from cinnamon, oregano, black pepper, and paprika via UPLC MS/MS analysis. **Table 2** (page 4) shows the QuEChERS recovery data of multiple pesticides from cinnamon, oregano, black pepper, and paprika via UPLC MS/MS analysis. Overall, better recoveries and RSD values were obtained via the EDGE extraction in comparison to QuEChERS. Furthermore, the modifications needed to make the QuEChERS method applicable to the dry nature of the spices made the process long and tedious. With QuEChERS already being a tedious manual process, the EDGE offers the benefit of a simplified automated method, along with improved recoveries.

Conclusion

The extraction process used on the EDGE automated extraction system allowed for the difficult matrix of spices to be extracted efficiently. One CEM-approved extraction method was utilized for all spices that greatly simplified the process, compared to QuEChERS. With one automated method, pesticides were more efficiently extracted than with the traditional QuEChERS process. In this study, spices were the focus; however, the same EDGE method would be applicable to all food samples and a wide range of pesticides. Some pesticides are known to be heat labile. For samples where temperature is a concern, a room temperature extraction can be programmed on the EDGE. The EDGE, with its efficient pesticide extraction method, is ideal for testing labs that want repeatable results for all samples with just one automated method.

Table 1. EDGE Recovery of Multiple Pesticides from Spices

	Cinnamon		Oregano		Black Pepper		Paprika	
Compound	**Average**	**STDEV**	**Average**	**STDEV**	**Average**	**STDEV**	**Average**	**STDEV**
Acephate	84.72%	4.26%	91.35%	7.22%	91.04%	2.60%	82.25%	4.01%
Fuberidazole	78.44%	4.13%	86.11%	8.32%	94.44%	0.61%	86.54%	2.41%
Omethoate	94.08%	9.95%	95.73%	11.25%	87.61%	4.01%	95.09%	1.17%
Monocrotophos	104.36%	18.67%	92.35%	8.35%	87.44%	1.65%	86.50%	4.53%
Dimethoate	100.60%	2.41%	86.46%	8.19%	99.31%	5.35%	95.10%	1.47%
Dicrotophos	85.50%	5.89%	90.75%	8.32%	95.19%	1.83%	97.87%	4.14%
Trichlorfon	91.67%	5.14%	105.09%	11.05%	86.39%	3.71%	89.92%	11.11%
Penconazole	95.01%	2.88%	88.55%	9.06%	84.43%	10.70%	91.79%	1.16%
Vamidothon	86.76%	5.49%	103.66%	9.46%	92.33%	3.83%	110.78%	3.85%
Cyproconazole Isomers	90.22%	9.71%	83.68%	11.35%	77.50%	13.45%	94.35%	1.19%
Paclobutrazol	81.70%	3.25%	89.88%	3.35%	72.91%	4.44%	89.04%	1.33%
Flutriafol	95.07%	3.03%	88.68%	8.44%	82.41%	10.26%	93.55%	0.50%
Tebuconazole	95.39%	3.07%	82.62%	9.22%	102.21%	2.47%	95.36%	2.42%
Hexaconazole	92.42%	3.51%	85.36%	9.58%	80.71%	2.73%	90.35%	2.02%
Flusilazole	95.01%	3.00%	85.84%	9.60%	91.01%	2.48%	92.69%	2.80%
Desmedipham	100.60%	2.50%	78.61%	0.42%	93.37%	3.71%	96.15%	1.03%
Metconazole	94.16%	1.68%	87.39%	8.88%	80.56%	3.75%	89.49%	5.55%
Diniconazole	93.24%	0.62%	90.20%	9.20%	92.54%	4.98%	99.08%	7.16%
Etaconazole	95.57%	4.49%	83.72%	8.19%	77.00%	13.32%	88.62%	7.69%
Pencycuron	90.21%	1.13%	78.60%	3.31%	93.17%	7.80%	95.83%	6.67%
Epoxiconazole	97.40%	2.95%	84.60%	8.70%	96.25%	18.69%	87.85%	8.81%
Fenarimol	96.35%	4.89%	84.28%	8.07%	95.35%	15.98%	91.11%	6.32%
Ipconazole	93.17%	3.39%	84.65%	11.38%	95.66%	2.95%	86.64%	8.96%
Fenbuconazole	95.34%	3.57%	85.35%	9.60%	98.21%	18.14%	89.09%	6.32%
Bitertanol	100.38%	2.82%	84.08%	7.74%	107.76%	24.04%	89.18%	5.81%
Triflumizole	91.61%	5.92%	88.40%	11.70%	85.86%	4.56%	98.02%	0.25%
Etoxazole	91.55%	2.74%	80.78%	9.46%	99.28%	4.13%	83.67%	1.45%
Spirotetramat	97.21%	3.59%	66.52%	13.86%	110.81%	5.49%	89.56%	9.22%
Dimethomorph	92.18%	2.39%	86.63%	7.22%	88.89%	9.58%	87.09%	10.41%
Difenoconazole	76.53%	0.35%	84.50%	9.94%	103.31%	1.20%	90.39%	7.73%
Spirodiclofen	91.96%	1.97%	79.72%	8.37%	100.64%	7.70%	97.18%	4.97%
Spinosyn A	37.81%	5.79%	65.99%	11.58%	85.75%	12.91%	94.45%	0.06%

Table 2. QuEChERS Recovery of Multiple Pesticides from Spices

	Cinnamon		Oregano		Black Pepper		Paprika	
Compound	**Average**	**STDEV**	**Average**	**STDEV**	**Average**	**STDEV**	**Average**	**STDEV**
Acephate	41.03%	5.69%	34.37%	3.76%	60.37%	14.35%	63.47%	10.03%
Fuberidazole	32.18%	7.50%	35.02%	5.05%	55.43%	14.21%	44.89%	6.87%
Omethoate	44.14%	6.39%	33.80%	3.77%	61.83%	14.84%	54.09%	9.42%
Monocrotophos	46.38%	7.09%	39.30%	3.00%	62.78%	15.95%	50.20%	10.09%
Dimethoate	46.29%	6.65%	37.31%	3.72%	66.40%	15.68%	72.41%	15.89%
Dicrotophos	40.27%	5.72%	40.15%	8.04%	64.60%	16.38%	64.35%	11.88%
Trichlorfon	48.40%	7.42%	40.69%	3.76%	64.16%	16.61%	85.83%	6.76%
Penconazole	46.23%	7.64%	43.47%	5.08%	73.62%	13.18%	62.49%	14.94%
Vamidothon	46.91%	5.89%	49.49%	14.82%	63.00%	16.45%	61.80%	11.91%
Cyproconazole Isomers	48.91%	5.94%	44.67%	4.91%	58.74%	10.65%	64.40%	23.31%
Paclobutrazol	67.31%	10.41%	63.46%	26.63%	91.60%	28.59%	65.17%	17.44%
Flutriafol	49.11%	7.36%	46.34%	5.98%	62.54%	16.31%	65.82%	16.58%
Tebuconazole	42.98%	7.26%	44.82%	2.06%	32.21%	8.12%	63.29%	15.71%
Hexaconazole	49.65%	8.04%	46.91%	6.38%	60.41%	17.70%	65.28%	14.21%
Flusilazole	48.47%	7.87%	79.82%	6.42%	53.40%	13.24%	65.96%	14.00%
Desmedipham	46.35%	8.19%	39.58%	1.83%	55.06%	14.74%	59.42%	14.59%
Metconazole	42.44%	6.29%	41.88%	1.53%	51.96%	16.46%	60.00%	17.65%
Diniconazole	34.67%	6.89%	35.34%	3.90%	100.95%	2.15%	55.66%	11.24%
Etaconazole	45.34%	8.88%	45.76%	4.12%	0.00%	0.00%	63.22%	16.31%
Pencycuron	48.55%	7.08%	42.83%	4.94%	64.29%	14.55%	62.59%	15.71%
Epoxiconazole	46.96%	7.29%	44.93%	2.76%	54.25%	18.33%	61.03%	13.97%
Fenarimol	48.90%	7.19%	31.04%	26.17%	74.14%	16.39%	65.97%	17.11%
Ipconazole	42.00%	7.04%	42.62%	4.06%	56.87%	15.33%	60.08%	14.11%
Fenbuconazole	47.38%	6.95%	45.35%	2.62%	62.10%	15.75%	63.10%	15.32%
Bitertanol	46.32%	5.72%	43.67%	4.82%	69.58%	44.82%	66.25%	17.46%
Triflumizole	43.67%	7.73%	44.22%	4.75%	60.27%	15.65%	63.98%	16.18%
Etoxazole	43.37%	5.30%	41.48%	4.54%	56.78%	14.12%	64.12%	19.35%
Spirotetramat	60.77%	12.71%	54.61%	5.03%	52.93%	13.78%	64.65%	15.96%
Dimethomorph	43.67%	8.68%	39.25%	20.25%	57.71%	16.30%	80.20%	32.04%
Difenoconazole	43.02%	6.65%	42.76%	2.93%	59.50%	13.79%	61.36%	15.05%
Spirodiclofen	55.05%	10.94%	52.77%	3.31%	52.60%	12.58%	63.48%	17.80%
Spinosyn A	48.81%	36.19%	34.28%	3.93%	32.76%	25.95%	29.16%	8.62%

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