APPLICATION NOTE #20149

Pesticide Detection in Food Products through Bead Mill Homogenization and QuEChERS Extraction

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The QuEChERS method was introduced in 2003 for "Quick, Easy, Cheap, Effective, Rugged and Safe" extraction of multiple pesticides and has since been modified and optimized to support a wide range of analytes (1). The QuEChERS method is widely used and is formalized in two documented methods, AOAC 2007.01 and EN 156223. Prior to the QuEChERS extraction the sample must first be comminuted. The comminuting process can be performed in a number of ways including, manual chopping, blending and milling. A more recent approach has been to homogenize the plant material through bead beating, a process in which the plant material is placed in a sealed tube with beads and vigorously shaken to produce a final homogenate of sub micron particle sizes (2). The advantage of this approach is that the homogenization can be performed in a 50 mL centrifuge tube containing 15 g of plant material and 5 to 15 mL of acetonitrile which is the starting solvent for common QuEChERS extractions.

Herein, we describe the application of bead mill homogenization and QuEChERS extraction for the detection of pesticides in food products. The 50 mL tube carriage for the Bead Ruptor 24 supports simultaneous homogenization of up to three samples in a standard 50 mL polypropylene centrifuge tube and provides sufficient force to homogenize even extremely hard samples such as seeds and roots.

Materials & Methods

Materials

Apples, grapes, carrots and whole soybeans were obtained from a local supermarket.

Equipment

- **Bead Ruptor 24 Bead Mill Homogenizer** (Cat # 19-040)
- **Bead Ruptor 24 50 mL Tube Carriage** (Cat #19-345-050)
- **4000 Q-Trap mass spectrometer** (AB Sciex) interfaced to a LC-20AD HPLC (Shimadzu) with a 100 x 2.1 mm ultra aqueous C18 column (Restek).

Sample Preparation

The produce was manually diced and ~15 grams of material was placed in a 50 mL polypropylene centrifuge tube containing 15 g of yttria-stabilized zirconium oxide ceramic beads (Cat #19-6508).

The produce samples were diluted with 15 mL of acetonitrile with 0.1 ppm d10-chlorpyrifos (as an internal standard) and homogenized on the Bead Ruptor 24 at 6 m/s for 30 seconds. 6 g $MgSO_4$ and 1.5 g NaCl was then added to the sample post homogenization. The tubes were returned to the Bead Ruptor 24 and shaken for 10 seconds at 6 m/s then centrifuged for 10 minutes at 3500 rpm. 0.5 mL of the top layer containing acetonitrile and extracted pesticides was removed and mixed with 0.5 mL of methanol.

Mass Spectrometry

1 µL of the mixture was analyzed by LC-MS/MS on a 4000 Q-Trap tandem mass spectrometer. Pesticides were separated by reverse phase chromatography on a Restek Ultra Aqueous C18 column (100 x 2.1 mm) during a 4 minute linear gradient from 5 to 60% buffer B followed by a 7.5 minute linear gradient from 60 to 95% Buffer B. Buffer A was 4 mM ammonium formate, 0.1% formic acid and Buffer B was methanol, 4 mM ammonium formate and 0.1% formic acid. Samples were electrosprayed at a voltage of 4500 V and source temp of 350°C. Twenty six transitions were monitored for detection of the thirteen pesticides as shown in Table 1.

continued

Table 1

Peak #	Q1	Q2	RT	Pesticides
1	192.2	160.2	4	Carbendazim.1
	192.2	132.1	4	Carbendazim.2
2	202.1	145	6	Carbaryl.1
	202.1	127	6	Carbaryl.2
3	318	160	6.8	Phosmet.1
	318	133	6.8	Phosmet.2
4	412.1	328.1	7	Mandipropamide 1
	412.1	356.1	7	Mandipropamide 2
5	331	99.1	7.2	Malathion.1
	331	127	7.2	Malathion.2
6	249.1	160	7.2	Linuron.1
	249.1	182.1	7.2	Linuron.2
7	343	307	7.3	Boscalid.1
	343	140	7.3	Boscalid.2
8	200	107	7.3	Pyrimethanil.1
	200	82	7.3	Pyrimethanil.2
9	325	108	8.1	Cyazofamid.1
	325	261.1	8.1	Cyazofamid.2
10	388	194	9.8	Pyraclostrobin.1
	388	163	9.8	Pyraclostrobin.2
11	406.1	251.1	11.2	Difenoconazole.1
	408.2	253.1	11.2	Difenoconazole.2
13	748.5	142.2	12	SpinetoramA.1
	748.5	98.1	12	SpinetoramA.2
12	308.1	197.1	12.6	Quinoxyfen.1
	308.1	162.1	12.6	Quinoxyfen.2
IS	324	260	11.6	d10-chlorpyrifos

Results

Pesticide extraction efficiency is in some part dependent on the ability to effectively homogenize the sample. As pesticides are present on both the fruit and vegetable skin and the interior flesh, it is advantageous to reduce the sample to a homogeneous mixture prior to pesticide extraction. However, this process can be challenging due to the vast diversity of sizes and densities of target plant and food products that need to be analyzed. Furthermore, many pesticides are volatile or semi-volatile and are not amendable to homogenization methods which generate large amounts of heat.

In this study we evaluated the use of bead milling for sample comminution on four common fruits and vegetables prior to pesticide extraction using the QuEChERS. Samples were homogenized in the presence of acetonitrile on the Bead Ruptor 24 outfitted with a 50 mL tube carriage. Full homogenization was achieved in 30 seconds for all sample types. Figure one is an image of the grape sample pre and post homogenization (Figure 1). Pesticides were then extracted and analyzed by LC-MS/MS operated in MRM mode (Figure 2).

Of the twelve pesticide transitions monitored two were detected in the apple, eight in the grape, one in the soybean, and four in the carrot samples (Figure 1). The internal standard d10-chlorpyrifos was also detected in each sample.

Figure 2 Pesticides were extracted and analyzed by LC-MS/MS operated in MRM mode

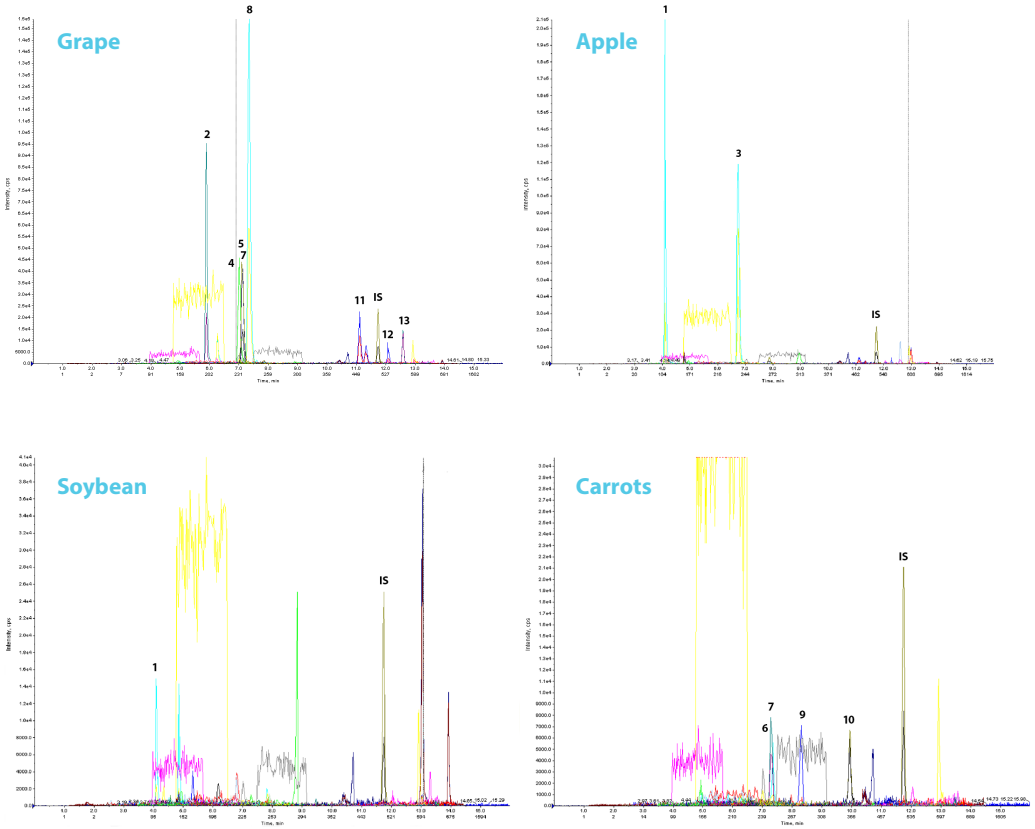

Figure 1
Grape sample pre and post homogenization

Omni Bead Ruptor 24

References

1. M. Anastassiades, S. J. Lehotay, Fast and easy multiresidue method employment acetonitrile extraction/ partitioning and "dispersive solidphase extraction" for determination of pesticide residues in produce. J. AOAC Int. 2003, 86, 412-431.
2. J. Wong et al, Development and interlaboratory validation of QuEChERS-based liquid chromatography-tandem mass spectrometry method for multiresidue pesticide analysis. J. Agric. Food. Chem. 2010, 58, 5897-5903.

Part Numbers Referenced

Bead Ruptor 24 Motor Unit: 19-040

Bead Ruptor 24 – 50 mL Tube Carriage Kit: 19-345-050

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