Determination of Pesticide Recovery Rates from Fruit and Vegetables Using QuEChERS Extraction and Bead Mill Homogenization on the Biotage® Lysera

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This application note was developed in a collaboration between Biotage and Omni International application laboratories.

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 a standard QuEChERs extraction.

Herein, we evaluate the effectiveness of bead mill homogenization on the Biotage® Lysera for multiple pesticide extraction using the QuEChERS AOAC 2007.01 method. The newly designed 50 mL tube carriage for the Lysera 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

Reagents

17 component pesticide standard was obtained from Restek (Cat#31155). Q-Sep QuEChERs salts and dispersive solid phase extraction (dSPE) materials were purchased from Restek (Cat#26238 and 26222). HPLC grade acetonitrile was purchased from Fisher.

Standards

The 17 component pesticide standards were obtained at 300 µg/mL concentration for each component. A 7.5 µg/mL spiking solution was created by diluting 5 µL of the stock solution in 195 µL of acetonitrile (0.1% acetic acid).

Sample Preparation

Certified organic strawberries, soybeans (shells removed) and apples were purchased from a local grocery store.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 (P/N 19-6508). 200 µL of pesticide spiking solution was gently pipetted over each produce sample to create a final pesticide concentration of 100 ppb and the tubes were rocked for one minute to disperse the pesticides. The spiked samples were incubated at 4 °C overnight.

Comminution

The spiked produce samples were diluted with 5 mL of acetonitrile (0.1% acetic acid) and homogenized on the Lysera using the settings outlined in Table 1. It was determined in a previous study that the optimum amount of buffer for 15 g of starting material was between 4–6 mL in a 50 mL centrifuge tube. Increased amounts of buffer in excess of 6 mL resulted in poor homogenization efficiency and increased processing time requirements.

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Table 1. Product Type and Biotage® Lysera Settings.

Produce	Speed (m/s)	Processing Time (Sec.)
Strawberry	4.2	30
Soybean	5.0	30
Apple	5.0	30

Post processing, the samples were visually inspected and determined that complete homogenization was achieved (Figure 1).

Figure 1. Produce Samples Pre and Post Homogenization. 15 g samples of strawberry, soybeans, and apples were homogenized on the Biotage® Lysera with 15 g of ceramic beads in 30 seconds.

Pesticide Extraction

10 mL of acetonitrile was added to each homogenate followed by the addition of one Q-Sep AOAC Method packet containing 6.0 g magnesium sulfate and 1.5 g sodium acetate. The samples were returned to the Biotage® Lysera and gently shaken for 1 minute at a speed of 0.8 m/s. The samples were centrifuged at 3000 rpm for 2 min in a Hettich Rotanta HP centrifuge. The supernatant was removed and placed in a 15 mL centrifuge tube containing 1.2 g magnesium sulfate, 400 mg PSA, 400 mg C18 and 400 mg graphitized carbon (Restek Cat#26222), shaken for 1 minute then centrifuged to pellet solid material. 500 µL of the supernatant was extracted and placed in an autosampler vial for GC/MS.

Mass Spectrometry

Extracted pesticides were analyzed on a HP 5890 Series II GC fitted with a Rtx-5 30 m x 0.25 mm x 0.25 um column, hydrogen carrier gas and injection temperature at 250 °C. Injection volume was 1 µL with column heating increasing from 100 to 330 °C and the MS scan range set from 50 – 600 m/z.

Results

The sensitivity of any QuEChERs based pesticide analysis is in some part dependent on the ability to effectively homogenize the sample. As pesticides are present on both the sample surface and interior, the sample must first be reduced to a single homogeneous mixture prior to 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. Bead milling is an attractive method for front end pesticide sample prep due to its ability to rapidly homogenize multiple hard or soft samples in a variety of sizes while maintaining a relatively low level of temperature rise. In this study, three produce samples with varying degrees of density were spiked with 17 standard pesticides at a concentration of 100 ppb (100 ng/g) (Table 2).

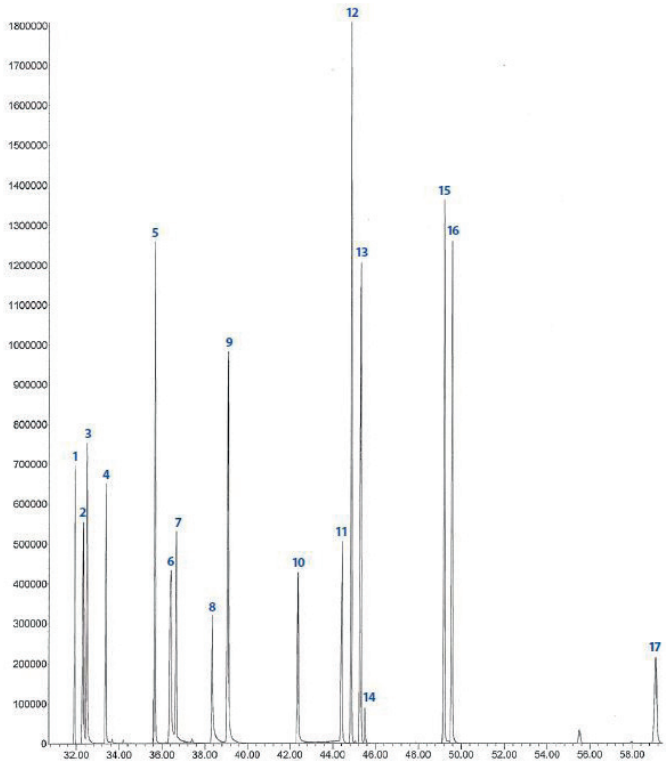

Figure 2. GC-MS Total Ion Chromatogram – 100 ppb Spiked Strawberry Sample. TIC displays the elution profile of the 17 pesticide standards extraction from the 100 ppb spiked strawberry sample post QuEChERs extraction.

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Table 2. 17 Pesticide Compounds in Spiked Mixture.

#	Pesticide	Strawberry	Soybean	Apple
1	Vinclozolin	69.5	75.4	68.9
2	Carbaryl	71.4	80.7	69.6
3	Metalaxyl	82.4	88.1	78.9
4	Methiocarb	90.9	83.4	74.1
5	Cyprodinil	78.1	84.8	75.1
6	Thiabendazole	87.9	81	83.2
7	Captan	65.4	60	72.5
8	Folpet	58.7	59.6	69.9
9	Imazalil	86.9	85	83.3
10	Myclobutanil	91.5	80.4	75.8
11	Fenhexamid	75	85.3	69.9
12	Iprodione	82.5	77.5	58.4
13	Bifenthrin	89.4	86.7	96.4
14	Fenpropathrin	91.7	76.4	83.7
15	cis-Permethrin	104.3	94.8	88.8
16	trans-Permethrin	95.7	80.9	89.5
17	Deltamethrin	76.4	88.9	91.7

Pesticides were extracted from three produce samples and analyzed by GC-MS to determine percent recoveries.

Homogenization of the three produce samples was accomplished in 30 secs with no detectable heat increase (Figure 1). While the strawberry sample had intact seeds visible post homogenization, both the apple flesh and peel were completely homogenized with no visible peel present after processing.

Following extraction, each pesticide mixture was analyzed by GC-MS to quantify the pesticide yields generated from the combined bead milling and QuEChERs process (Figure 2). Individual peak areas were calculated and compared to the stock mixture of known concentration to determine recovery values. Pesticide recoveries ranged from 58.4 to 104.3% based on the analyte and produce material with the highest recovery being seen for strawberry and apple samples. This may be due to differences in the sample composition between soybeans and the fruits analyzed in this study.

Conclusion

The Biotage® Lysera supports bead milling in volumes up to 50 mL and has been demonstrated to effectively disrupt both soft and hard samples. When combined with the QuEChERs pesticide extraction method, high recoveries can be achieved from a variety of produce types. Future studies will aim at expanding to a wider range of sample matrices and target analytes to further evaluate the effectiveness of the bead milling approach as a comminution solution.

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.

Ordering Information

Part Number	Description	Quantity
119-060	Biotage® Lysera	1
19-345-50	50 mL Tube Carriage Kit, Biotage® Lysera	1
19-6508	Hard Tissue Homogenizing Mix (50 mL Tubes)	1

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