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Comparison of Pesticide Extraction in Agricultural Products Using a Manual Shaking Method and Mechanical Mixing with the HG-600 Geno/Grinder® 2010

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SUBJECT (SP023): Tissue Homogenization and Cell Lysis
APPARATUS: Geno/Grinder®
APPLICATION: QuEChERS/Pesticide Extraction

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Abstract

Since its introduction in 2003 by Anastassiades and Lehotay et al, the QuEChERS method[1] (quick, easy, cheap, effective, rugged, safe) has proven to be effective and convenient for analysis of multiple pesticides in agricultural products. Increasing concern over the health effects of residual pesticides on fruits and vegetables has led to increased testing of these products to determine the levels of pesticides on produce when it goes to market. The QuEChERS method has allowed analysts to process a greater number of samples in a shorter period of time than with previous methods.

In this study, samples of strawberry, apple, and green pepper were prepared for analysis using both the standard QuEChERS method and a modified method involving mechanical mixing using the HG-600 Geno/Grinder. The Geno/Grinder is a mechanical disrupter that grinds and mixes materials using a rapid vertical motion. GC/MS results of samples prepared using both methods were compared.

Experimental

Sample Preparation

Mason jars of frozen, homogenized strawberry, apple, and green pepper (a blend of bell and non-bell) were obtained from *UC Davis (IR-4 Project). Each fruit or vegetable contained a known pesticide applied to the crop in the field; strawberry and apple contained flutianil, and green pepper contained etoxazole. The jars were thawed at room temperature prior to measuring out 15 g samples into 50 mL centrifuge tubes.

To each tube was then added 15 mL acetonitrile containing 1% acetic acid. To the strawberry and apple samples, 6.0 g anhydrous magnesium sulfate and 1.5 g sodium acetate were added. To the green pepper samples, 6.0 g anhydrous magnesium sulfate and 1.0 g sodium chloride were added. All sample tubes were capped and the samples of each fruit or vegetable were divided into two sets, A and B. Set A was shaken manually, while Set B was shaken mechanically using the Geno/Grinder. Each sample set consisted of 5 to 7 samples.

Method 1 – Manual Mixing

Sample Set A (from each material: strawberry, apple, and green pepper) was shaken by hand for 1 minute.

Method 2 – Mechanical Mixing

Two angle cut ceramic cylinders (⅜” x ⅞”) were added to each tube in Sample Set B. The tubes were capped and shaken in the Geno/Grinder at 1500 strokes/minute (spm) for 1 minute.

Clean Up

After mixing, all samples from Sets A and B were centrifuged at 3500 rpm for 3 minutes. An aliquot (12-13 mL) of the supernatant liquid was taken from each sample and transferred to a clean 15 mL centrifuge tube. To each sample was added primary secondary amine (PSA, 25 mg/mL sample), graphitized carbon black (GCB, 5 mg/mL sample), and anhydrous magnesium sulfate (75 mg/mL sample) and the tubes were capped.

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Sample Set A was shaken by hand for 30 seconds. Sample Set B was shaken in the Geno/Grinder for 30 seconds at 1500 strokes/minute. Following this treatment, both sample sets were visibly lighter in color. All samples were centrifuged at 3200 rpm for 30 seconds. The supernatant liquid was again removed from each sample and transferred to a clean 15 mL centrifuge tube. The total volume of each sample was brought to 1 mL in toluene and an internal standard mix was added. The samples were analyzed by GC/MS using a SIM method.

GC/MS Conditions

An HP 5890 gas chromatograph coupled to a 5972 mass selective detector was used for the analysis of the samples. The GC/MS was equipped with an HP 7673 GC/SFC injector. The analytes were separated on a HP-5 capillary column (3.0 mm x 0.25 mm x 0.25 µm). The GC oven temperature program was set to an initial temperature of 70 °C for one minute, and raised to 230 °C at 20° C/minute with a total run time of 15 minutes. The MS was operated in electron impact ionization in the SIM mode scan range of 35 to 450 m/z. The GC/MS interface and MS source were both 250 °C. The injected volume of sample extract was 2 µL with splitless injection. Chemstation B.02.05 and Enviroquant G1701 BA Ver. B.01.00 were used for the data collection and analysis of samples.

Results and Discussion

The results are shown in Table 1. For all three matrices, the measured pesticide concentration was greater when the Geno/Grinder was used to shake the samples. The increase in recovery was similar for strawberry and apple, 20% and 22 % respectively. Both of these fruits contained the pesticide flutianil. For pepper, recovery of the pesticide etoxazole increased 35%. This indicates that use of the Geno/Grinder improves pesticide extraction from the fruit or vegetable matrix. At a setting of 1500 strokes/minute, samples are shaken in a complete up and down motion 1500 times during a one minute run. In contrast, a lab worker can shake a sample approximately 200 times in one minute. Due to the more rapid and vigorous agitation using the Geno/Grinder, mixing should be more thorough than can be achieved manually and it is not surprising that extraction is improved over the manual method.

The addition of two ceramic grinding cylinders to each tube shaken on the Geno/Grinder also may have aided the extraction process. The ceramic cylinders mash and grind the fruit or vegetable matrix further during the shaking process, thereby increasing surface area and allowing a more complete extraction of pesticide from the matrix. In fact, the matrices in the samples shaken on Geno/Grinder visibly appeared more thoroughly ground than samples shaken by hand.

Table 1. Comparison of GC/MS Results for Samples Prepared Manually and Using the Geno/Grinder

	Manual			Geno/Grinder			
Matrix	Conc. (ppb)	Std. Dev.	RSD (%)	Conc. (ppb)	Std. Dev.	RSD (%)	Increase Using Geno/Grinder (%)
Strawberry	20	1.0	5	24	1.7	7	20
Apple	9	0.8	9	11	1.4	12	22
Pepper	17	2.5	15	23	2.4	10	35

An additional benefit of using the Geno/Grinder for sample preparation is elimination of variation in shaking technique. Samples are held vertically in a foam support and every sample within a run is subjected to the same shaking conditions. Also, since the operating rate and run time can be set on the Geno/Grinder, identical conditions from run to run are ensured.

However, when samples are shaken manually, shaking conditions can vary and this can affect how well the samples are mixed. As a worker becomes tired from shaking multiple samples, he/she will likely slow down and later samples may not be as well shaken as earlier ones. If two or more people are preparing samples, there is also the possibility that the workers will not shake the samples in the same manner. Again, this can affect the effectiveness of the extraction.

Finally, use of the Geno/Grinder allows for greater sample throughput. While a lab worker can shake 2 to 4 samples (50 mL tubes) simultaneously by hand, the Geno/Grinder can accommodate 16 samples. Thus, more samples can be prepared in a given amount of time.

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