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APPLICATION NOTE

Analysis of Pesticides in Fruit and Vegetable Products using a Standard QuEChERS Method and a Modified Method Involving the Geno/Grinder.

With kind permission of:
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- APPLICATION NOTE SP024: Pesticide Anaylsis: Standard QuEChERS vs Modified Method
- APPARATUS: **Geno/Grinder®**
- APPLICATIONS: QuEChERS / Pesticide Extraction

Introduction

Pesticide residues in agricultural food sources are widely considered to cause adverse health effects when consumed by humans. In particular, much of the produce sold in the U.S. is imported and concern over pesticide levels in these fruits and vegetables in comparison to those grown domestically has resulted in increased testing for pesticide residues.

In 2003, the QuEChERS method for pesticide analysis was introduced by Anastassiades and Lehotay et al. QuEChERS is an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe and allows for analysis of multiple pesticides, while offering faster and easier handling over previous methods. The QuEChERS method is now widely used and has been adopted by the AOAC as method 2007.01 "Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate." In Europe, the official method is EN 15662.

For a typical analysis, 10-15 g samples of chopped and homogenized agricultural produce are placed in 50 ml centrifuge tubes and an extracting solvent, such as acetonitrile, and anhydrous magnesium sulfate and sodium acetate or sodium chloride salts are added. The tubes are capped and shaken by hand for one minute to mix the contents and extract the pesticide into the solvent. The samples are then treated with clean up materials, concentrated, and analyzed by GC/MS or LC/MS

In this study, the Geno/Grinder was employed to homogenize the fruit/vegetable samples and to mix the produce rapidly and thoroughly with the salts and solvent in an effort to improve the extraction step. The goal of the study was to determine whether the use of the Geno/Grinder during the extraction step would increase pesticide recovery over the traditional, manual QuEChERS method.

Experimental

Three fruit and vegetable matrices were chosen for the purpose of evaluating materials with differing density and toughness: 1) strawberries – soft; 2) apples – dense and tough; and 3) celery – fibrous. Strawberries are quite soft and preliminary tests verified that they could easily be ground to a mushy, liquefied substance using the Geno/Grinder (grinding method described below). Apples are tougher and denser and, not surprisingly, a longer grinding time was required to effectively mash the fruit to an applesauce-like consistency. While celery is not as dense as apple, it is fibrous and tough and therefore difficult to grind effectively.

Fresh strawberries, apples (Granny Smith), and celery were purchased from a local supermarket and cut into 1/4-1/2 inch-sized chunks, weighed out in 15.1 g quantities, and placed into 50 ml round-bottomed LDPE centrifuge tubes. Each sample was spiked with 250 µl of a 40 µg/

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ml solution of CAL-CARB-13 (a mix of 13 common pesticides available from SPEX CertiPrep) in dichloromethane (10 ppm per sample). The pesticide mix was introduced into each tube by syringe, taking care to control the flow of pesticide solution onto the fruit/vegetable sample. The tubes were capped and gently shaken by hand for 15 sec. to ensure an even distribution of the pesticide solution over the entire sample. The samples were placed in a refrigerator at 4°C and stored overnight.

Traditional QuEChERS Method

The spiked strawberry samples from four centrifuge tubes (each containing 15.1 g) were collectively transferred into a single-serve blender and homogenized to a smooth consistency. Samples of 15.1 g were re-measured from this mixture and transferred back into the same centrifuge tubes. Care was taken to transfer out liquid as well as solid material. Apple and celery samples were spiked and homogenized in the same manner as the strawberry.

To each tube of homogenized produce was added 6.0 g anhydrous magnesium sulfate, 1.5 g anhydrous sodium acetate, and 15 ml acetonitrile containing 1% glacial acetic acid. The tubes were capped and shaken by hand for 1 min. The liquid in the strawberry tubes was observed to be pink in color, the apple extract was pale yellow, and the celery extract a very saturated green color.

All tubes were then centrifuged at 3500 rpm for 3 min. The supernatant liquid was removed, measured, and divided into two equal portions (5 ppm maximum pesticide per sample after this step) and transferred into 15 ml centrifuge tubes.

Primary secondary amine (PSA) (25 mg x Vol (ml) supernatant) and graphitized carbon black (GCB) (5 mg x Vol (ml) supernatant) were added to each tube. The sample tubes were capped and shaken by hand for 30 sec., then centrifuged at 3200 rpm for 1 min.

Modified QuEChERS Method using the Geno/Grinder

To centrifuge tubes containing spiked produce samples were added 3 ceramic grinding cylinders (3/8" x 7/8" angle cut part # 2183) and 5 ml acetonitrile containing 1% glacial acetic acid. The tubes were capped and placed into a tube holder, clamped in place on the Geno/Grinder, and ground at 1500 rpm. After grinding for 2 min., the strawberry samples were well ground to a pulpy, liquid consistency. Since apple and celery are tougher materials, 6 min. grind times at 1500 rpm were required to achieve a well ground, mushy consistency.

During preliminary test runs, it was found that addition of a small amount (5 ml) of solvent improved the grinding process by aiding the movement of the produce in the tube. Without solvent, the harder fruits and vegetables tended to become packed down in the bottom of the tube and effective grinding was difficult to achieve. Alternatively, addition of the full 15 ml of solvent prior to grinding provided too much fluid and the grinding media were not able to make sufficient contact with the produce to achieve effective grinding. Through experimentation, it was determined that addition of 5 ml of solvent was optimal for effective grinding of a 15 g sample.

To each tube was then added 6 g anhydrous magnesium sulfate, 1.5 g anhydrous sodium acetate, and the remaining 10 ml acetonitrile (1% glacial acetic acid). The tubes were recapped, placed back on the Geno/Grinder and shaken for 1 min. at 1500 rpm.The liquid in the strawberry tubes The liquid was observed to be pink in color, the apple extract was pale yellow, and the celery extract a very saturated green color. All material was well mixed and free-flowing.

The samples were then centrifuged at 3500 rpm for 3 min. The supernatant liquid was removed from each tube, measured, and divided into two equal samples (5 ppm max. pesticide after this step) and transferred into 15 centrifuge tubes.

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PSA (25 mg x Vol (ml) supernatant) and GCB (5 mg x Vol (ml) supernatant) were added to each tube. The sample tubes were capped, shaken on the Geno/Grinder for 30 sec. at 1500 rpm, then centrifuged at 3200 rpm for 1 min.

Modified QuEChERS Method using the Geno/Grinder – All-In-One

Additional samples of spiked strawberry were handled in a second manner. In an effort to determine whether the pre-homogenization step can be eliminated for soft fruits, 6 g anhydrous magnesium sulfate, 1.5 g anhydrous sodium acetate, and 15 ml acetonitrile (1% glacial acetic acid) were added to tubes containing 15.1 g of chunked strawberry. The tubes were capped and clamped on the Geno/Grinder.

After 2 min. of grinding at 1500 rpm the strawberry was fairly well ground, but some visible chunks remained. The samples were ground an additional 2 min. (4 min. total), at which point the strawberry was very well ground and thoroughly mixed with the salts and solvent. The samples were centrifuged, the supernatant removed, and clean up was conducted as above, using the Geno/Grinder.

Attempts to combine the homogenization and extraction steps for apple and celery were less successful. When the salts were combined with small chunks of produce, a tube was filled to capacity and the produce and grinding media could not move freely. Thus, impact of the grinding media on the produce was impeded and only partial grinding was achieved.

Preparation of Samples

Following clean up and centrifugation, the supernatant was removed from each sample, transferred to a clean 15 ml tube (all methods), and concentrated down to near dryness (approximately 100 µL) by gently heating the tubes while passing a slow stream of nitrogen over the sample. Toluene was added to each sample to bring the total sample volume to 1 ml. In many cases a viscous drop of material settled to the bottom of the centrifuge tube after addition of toluene. The material appeared to be soluble in aqueous solvents, but not in non-polar solvents and had a syrupy consistency.

The toluene solution was removed by syringe and injected into GC sample vials, while the residue material was left in the tube. For strawberry the residue was red for the samples that had been prepared using the Geno/Grinder and yellow for samples prepared using the standard QuEChERS method. In the case of apple, the residue was golden for Geno/Grinder samples and brown for standard QuEChERS samples. For celery, a yellow residue remained for Geno/Grinder samples, while for standard QuEChERS samples a pale yellow drop was observed.

Sample Analysis

Samples were analyzed using an HP 5890-GC with a CV-5 capillary column and a 5972-MSD detector. Scan range was 35-450 m/z with signal-to-noise of 3:1. Injected sample size was 1 µl.

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Results and Discussion

At the beginning of the study, each sample was spiked with 250 µl of a solution of 13 pesticides, each present in a concentration of 40 ppm/ml. Thus, 10 ppm of each pesticide was introduced into each produce sample. After extraction and centrifugation, the supernatant was removed and divided into two equal replicates to continue the study. Therefore, each sample was divided into two and the maximum concentration of each pesticide in a sample at this point was 5 ppm.

Recovery results for all three produce matrices are shown in Table 1 as total concentration recovered in ppm. Results for strawberry are shown graphically in Figure 1. The blue bars represent the samples that were prepared by manually shaking homogenized samples with the salts and acetonitrile. The red bars represent the samples that were homogenized using the Geno/Grinder prior to the addition of the salts. Finally, the green bars (All in one) show the results for the samples that were ground and mixed with the salts and acetonitrile simultaneously (no pre-homogenization).

For all pesticides detected, significantly higher recoveries were obtained for the samples prepared using the Geno/Grinder than for those prepared by hand. Interestingly, the samples ground simultaneously with the salts and solvent (All in one)performed as well, or in some cases better, than the samples that were ground on the Geno/Grinder before addition of the salts. This indicates that this method is viable for soft produce matrices.

The poorest recoveries were obtained for Dichloran and Chorothalonil. This was the case for all matrices evaluated. In fact, for celery Chlorothalonil was not detected, even when the Geno/Grinder was used. Because the method used in this study was not optimized for a particular type of pesticide, it is not surprising that some pesticides were not stable under the conditions used.

Table 1 – Recovery of Pesticides in Produce Samples (ppm)

Pesticide	Strawberry			Apple		Celery	
	Geno/ Grinder	All in one	QuEChERS	Geno/ Grinder	QuEChERS	Geno/ Grinder	QuEChERS
Carbofuran	2.33	2.2	0.8	2.4	1.2	1.0	ND*
Carbaryl	1.8	1.6	ND	1.8	0.4	ND	ND
Diphenylamine	1.8	2.2	ND	6.2	3.8	0.9	ND
Chlorpropham	2.7	3.3	0.3	3.0	1.1	1.5	0.1
Dichloran	0.7	0.4	ND	0.4	ND	0.2	ND
Chlorothalonil	0.7	ND	ND	1.2	ND	ND	ND
Pirimicarb	1.8	1.4	ND	1.4	ND	0.1	ND
Vinclozolin	2.5	2.9	0.1	2.8	1.1	2.3	ND
Metalaxyl	3.1	3.2	1.3	3.0	1.8	2.3	1.5
Parathion	2.9	3.4	1.3	3.0	1.9	3.2	1.4
Systhane	2.8	3.1	1.0	3.0	1.6	2.3	1.1
Azinphos-Methyl	2.5	2.7	1.9	3.1	2.9	3.2	2.4

*ND=Not Detected

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Figure 1 – Recovery of Pesticides in Strawberry Samples

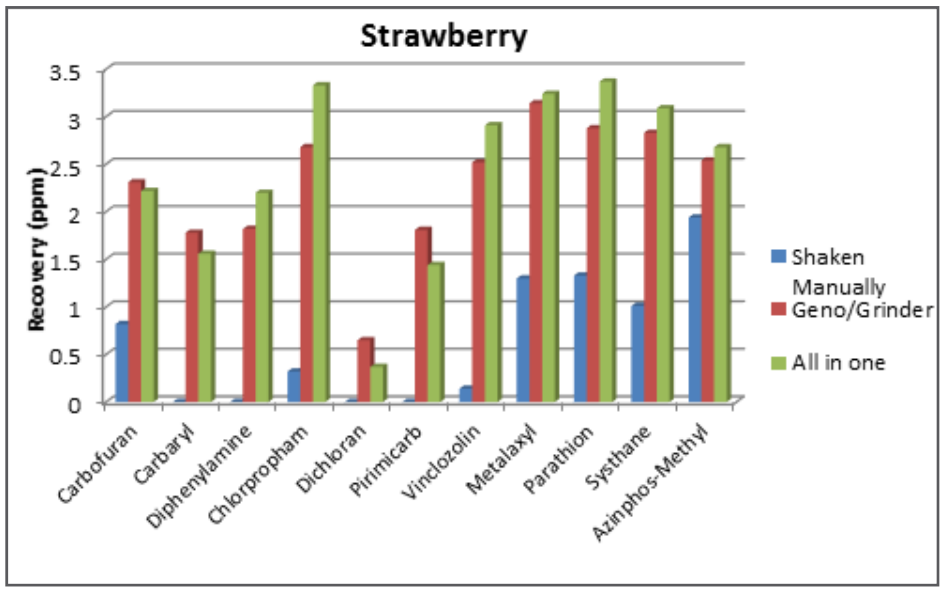

Figure 2 shows the recovery results for apple. Again, the Geno/Grinder method outperformed the standard manual method. Greater recoveries were obtained for all detected pesticides when the Geno/Grinder was used to grind the apple and mix the resulting pulpy mass with the salts and acetonitrile, with the exception of Azinphos-Methyl where the recovery for the two methods was similar. For most pesticides, the recoveries were similar to those obtained from the strawberry samples. However, the Diphenlyamine results from the Geno/Grinder samples gave a recovery of 6.2 ppm, which is greater than the 5 ppm that was introduced to the sample. The recovery for this pesticide using the manual method (3.8 ppm) was also higher than for the other pesticides for these same samples. Diphenylamine is commonly used in crop protection for apples and the high concentration for this pesticide is likely a result of its presence on the apple before the addition of the pesticide spike. Nevertheless, the results for the Geno/Grinder samples were significantly higher than for the standard QuEChERS sample.

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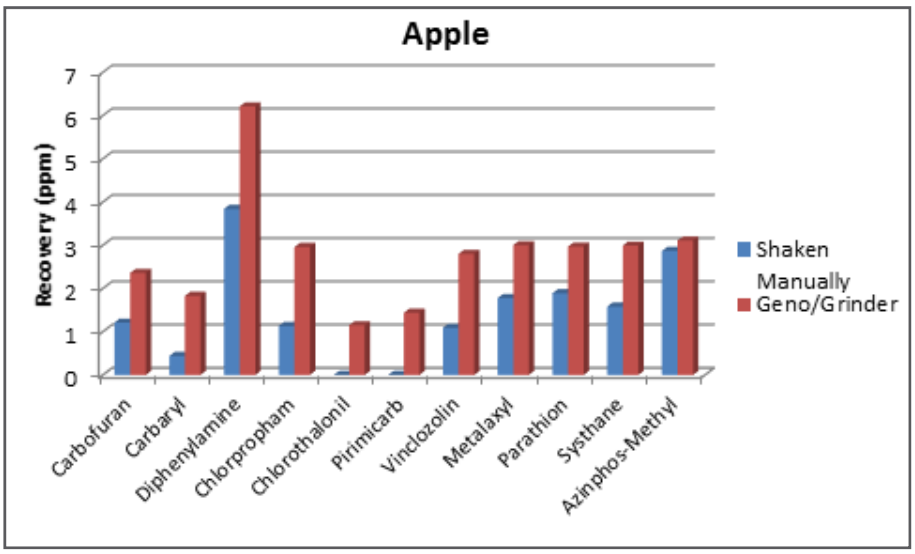
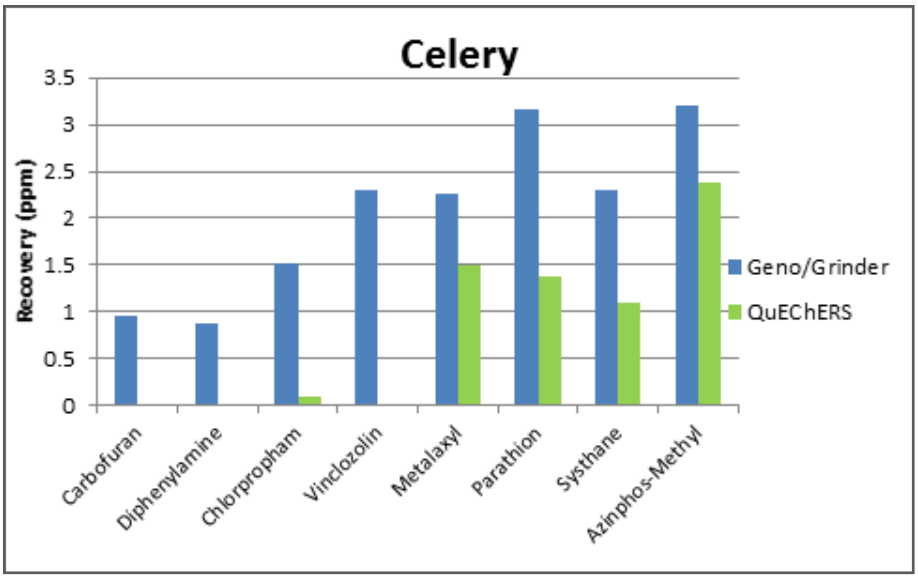
Figure 2 - Recovery of Pesticides in Apple Samples



Results for celery are shown in Figure 3. As with strawberry and apple, the recoveries for the Geno/Grinder samples are much higher than for the manually prepared samples. For all three matrices evaluated, the Geno/Grinder extracted some pesticides that were not detected in the samples prepared by hand. Particularly noteworthy in the case of celery is Vinclozolin; the recovery is on par with Metalxyl and Systhane for samples prepared using the Geno/Grinder, but was completely undetected in the standard QuCEhERS samples.

Figure 3 - Recovery of Pesticides in Celery Samples



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It is clear from the preceding graphs that use of the Geno/Grinder significantly increased pesticide recovery in this study. Since the Geno/Grinder shakes the samples so much more vigorously and rapidly than can be accomplished by hand, it is not surprising that extraction was more effective. During a 1 minute run at a setting of 1500 rpm, the Geno/Grinder shakes a tube in a complete up and down cycle 1500 times, whereas a human can shake a tube about 200 times in 1 minute. In addition, the sample tubes run on the Geno/Grinder each contained 3 ceramic, angle-cut grinding cylinders. These aid in mixing the sample thoroughly with the solvent and salts, but also grind the produce, ensuring a thorough extraction of the pesticide.

Thus, for soft produce matrices, it may be possible to eliminate the pre-homogenization step, thereby further reducing sample preparation time.

In addition to improved pesticide recovery, the use of the Geno/Grinder increases throughput since a greater number of samples can be homogenized and extracted at one time. In this study, twelve 50 ml tubes were shaken simultaneously on the Geno/Grinder, whereas a maximum of four tubes were shaken by hand at one time. During the clean-up step, the Geno/Grinder accommodated twenty-four 15 ml tubes, while a maximum of six were shaken manually.

Conclusion

Produce samples of varying density and toughness were evaluated and in all cases significantly greater pesticide recovery was obtained for samples prepared using the Geno/Grinder than for samples shaken manually.

In addition, since run time and operating rate are automatically controlled by the Geno/Grinder, all samples within a run and from run to run are shaken in a consistent manner, eliminating variability. The Geno/Grinder can also shake up to sixteen 50 ml centrifuge tubes in one run, thus increasing sample throughput. This not only reduces time spent on sample preparation, but also reduces fatigue for laboratory workers.

Finally, produce samples can be ground in 50 ml centrifuge tubes using the Geno/Grinder, possibly eliminating the need to pre-homogenize the produce. Soft fruits, such as strawberries, can be ground in the presence of the salts and extracting solvent used in the QuEChERS method with no negative impact on pesticide recovery.

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