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ARTICLE

Extension of the QuEChERS Method for Pesticide Residues in Cereals to Flaxseeds, Peanuts, and Doughs†

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A simple method was evaluated for the determination of pesticide residues in flaxseeds, doughs, and peanuts using gas chromatography−time-of-flight mass spectrometry (GC-TOF) for analysis. A modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, which was previously optimized for cereal grain samples, was evaluated in these fatty matrices. This extraction method involves first mixing the sample with 1:1 water/acetonitrile for an hour to swell the matrix and permit the salt-out liquid−liquid partitioning step using anhydrous $MgSO_4$ and NaCl. After shaking and centrifugation, cleanup is done by dispersive solid-phase extraction (d-SPE) using 150 mg of anhydrous $MgSO_4$, 150 mg of PSA, and 50 mg of C-18 per milliliter of extract. This method gave efficient separation of pesticides from fat and removal of coextracted substances better than gel permeation chromatography or use of a freeze-out step, which involved excessive use of solvent and/or time. The optimized analytical conditions were evaluated in terms of recoveries, reproducibilities, limits of detection, and matrix effects for 34 representative pesticides using different types of flaxseeds, peanuts, and doughs. Use of matrix-matched standards provided acceptable results for most pesticides with overall average recoveries between 70 and 120% and consistent RSDs <20% for semipolar pesticides and <26% for lipophilic pesticides. The recoveries of these latter types of pesticides depended on the fat content in the matrices and partitioning factor between the lipids and acetonitrile. We believe that the consistency of the pesticide recoveries for different samples in multiple experiments and the physicochemical partitioning explanation for <70% recoveries of lipophilic pesticides justify compensation of results for the empirically determined recovery values. In any case, this method still meets 10 ng/g detection limit needs for lipophilic pesticides and may be used for qualitative screening applications, in which any identified pesticides can be quantified and confirmed by a more intensive method that achieves >70% recoveries for lipophilic pesticides.

KEYWORDS: Pesticide residues; analysis; QuEChERS; gas chromatography−time-of-flight mass spectrometry (GC-TOF); flaxseed; peanut; dough

INTRODUCTION

Flaxseeds are an excellent source of nutrients that promote good health. Specifically, flaxseed is rich in α-linoleic acid, which belongs to a group of essential omega-3 fatty acids that appear to be beneficial against heart disease. It is a good source of fiber, lignin, manganese, magnesium, and antioxidants. These nutrients have been shown to reduce the risk of diabetes, lower blood cholesterol levels, control sugar and insulin levels, and promote gastrointestinal health (*1*, *2*). For these possible reasons, consumer demand for flaxseed is increasing. Flaxseeds are commonly used as diet ingredient in breads, doughs, breakfast cereals, and other processed and bakery food products.

Pesticides are widely applied in a variety of different ways during the production of foods to control the growth of weeds and fungi or to prevent crop damage by insects, mites, rodents, and other pests. Pesticides are also frequently used on crops post-harvest to prolong storage life and improve quality. About half of American consumers consider pesticide residues in foods a strong or very strong concern (*3*), and due to food safety and environmental reasons, laws have been established in most countries worldwide to set maximum permissible levels of pesticide residues in foods. Analytical monitoring is often conducted to determine if residues are present for food safety, regulatory, product liability, quality, research, and/or food labeling purposes.

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Table 1. Compositions of the Sample Types of Interest According to the USDA Food Composition Database (*4*) (www.nal.usda.gov/fnic/foodcomp/search/)

	flaxseeds	peanuts, raw	biscuit dough	cinnamon roll dough	pie crust
water (%)	7.0 ± 1.6, *n* = 3	6.50 ± 0.09, *n* = 31	33.5	28	19.2
lipids (%)	**42.2 ± 3.2, *n* = 6**	**49.2 ± 0.3, *n* = 98**	**13.8**	**11.4**	**25.5**
carbohydrates (%)	28.9	16.1	41.4	52.3	51.1
protein (%)	18.3 ± 0.9, *n* = 7	25.8 ± 0.2, *n* = 78	6.9	4.5	3.0

In flaxseeds and other fatty types of foods, nonpolar pesticides may be of particular concern due to their greater solubility in lipids. **Table 1** gives the composition of the sample matrices of interest in the study according to the USDA Nutrient Data Laboratory (*4*). The monitoring of lipophilic pesticides at trace levels can be very challenging in the case of fatty matrices because the lipid coextractives can adversely affect the extraction efficiency and instrument performance for quantitative detection. For these reasons, stringent sample extraction and cleanup methods are usually conducted to remove most of the high molecular mass lipid from the sample extracts.

Traditionally, liquid−liquid extraction (*5*), gel permeation chromatography (GPC) (*6*), or low-temperature fat precipitation (*7*−*9*) has been used as a postextraction cleanup procedure for fatty matrices. However, these methods often require large solvent volumes, use a lot of glassware, and take much time and labor, which reduce the laboratory efficiency and sample throughput. Solid-phase extraction (SPE) (*10*), microwave-assisted extraction (MAE) (*11*), and matrix solid-phase dispersion (MSPD) (*12*, *13*) applied to pesticide residue analysis have also been used for fatty samples. SPE based on carbon nanotubes is a recent technique applied to oily matrices for extraction and cleanup (*14*).

Recently, the "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) approach has become the method of choice for the rapid extraction and cleanup of various sample types to determine pesticide residues (*13*, *15*−*20*). This approach has been shown in numerous laboratories to provide high-quality results, save time and labor, and lower solvent consumption. Even though QuEChERS has been employed in several kinds of vegetables, fruits, grains, and other foods, it has not been reported on the extraction of pesticides residue in flaxseeds and doughs. In particular, can the method previously optimized for the analysis of nearly 200 pesticides in cereal grains (*17*) be extended to foods with higher lipid content, such as flaxseeds, peanuts, and doughs?

The aim of this work was to evaluate the simple and rapid QuEChERS multiresidue method for the determination of 34 pesticides in high-fat (>20%) commodities (*13*) using gas chromatography (GC) coupled with time-of-flight (TOF) mass spectrometry. This study describes the extension of QuEChERS methodology for the determination of pesticides in flaxseeds, doughs, and peanuts. We also sought to demonstrate if compensation of recovery factors for different types of flaxseeds could be used to obtain consistent results for lipophilic pesticides.

MATERIALS AND METHODS

Chemicals and Materials. Pesticide standards (atrazine, azoxystrobin, bromopropylate, carbaryl, *cis*-chlordane, chlorothalonil, chlorpyrifos, chlorpyrifos-methyl, coumaphos, cypermethrin, deltamethrin, *p*,*p*′-DDE, *o*,*p*′-DDT, diazinon, dichlorvos, dimethoate, endosulfan sulfate, ethoprophos, fenthion, folpet, heptachlor, hexachlorobenzene (HCB), lindane, malathion, metolachlor, mirex, oxyfluorfen, permethrin, pirimiphos-methyl, procymidone, quintozene, tolylfluanid, trifluralin, and vinclozolin) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and the U.S. Environmental Protection Agency's National Pesticide Repository (Fort Meade, MD) with the highest available purity >95%.

Individual stock solutions of pesticides at concentrations ≥2000 ng/μL were prepared in toluene, acetonitrile (MeCN), or ethyl acetate (EtOAc) according to their solubility. A mixture of working standard solution containing 40 ng/μL of each pesticide was prepared by diluting the stock solutions with MeCN. Separate working standard solutions of the internal standard (IS), diazinon, and the quality control (QC) standard, procymidone, were also prepared in MeCN at 40 ng/μL. Milled flaxseeds (brown and golden varieties), ground peanut with shell, and dough (pie crust, Buttermilk Grand biscuits, and Flaky Supreme cinnamon rolls) samples were from General Mills (Golden Valley, MN).

Solvents used in the study were of analytical grade obtained from J. T. Baker (Phillipsburg, NJ). For the salting-out and dispersive solid-phase extraction (d-SPE) steps, anhydrous magnesium sulfate (anh $MgSO_4$) and 2 mL centrifuge tubes containing 150 mg of anh $MgSO_4$, 150 mg of primary−secondary amine (PSA), and 50 mg of octadecylsilane (C-18) were obtained from UCT, Inc. (Bristol, PA). ACS-grade sodium chloride (NaCl) was purchased from Mallinckrodt (Paris, KY).

Apparatus and Conditions. *Gel Permeation Chromatography*. For initial GPC experiments, we used a J2 Scientific (Accuprep MPS, Columbia, MO) GPC instrument and Express column (2.5 cm i.d. × 22.5 cm length containing Biobeads S-X3). The mobile phase was 1:1 (v/v) EtOAc/cyclohexane, flow rate was 5 mL/min, and injection volume was 5 mL. A UV detector was used to monitor the elution profile. Preliminarily, 100% flaxseed oil was used instead of the extracted flaxseed samples to verify the elution profile of oil and pesticides from the GPC system. The 2 g of flaxseed oil spiked with 34 representative pesticides at 2 μg/g concentration was dissolved in 10 mL of 1:1 (v/v) EtOAc/cyclohexane (equivalent to 25 mg of injected flaxseed oil). The GPC elution fraction from 10 to 16 min was automatically collected in test tubes and evaporated to dryness at 40 °C using a Zymark Turbovap evaporator (Hopkinton, MA) under a stream of nitrogen. The residues of each fraction were reconstituted in MeCN and transferred to autosampler vials with microvial inserts for GC-TOF analysis.

Gas Chromatography−*Time-of-Flight Mass Spectrometry*. GC-TOF analysis was performed using an Agilent Technologies (Palo Alto, CA) model 6890 gas chromatograph coupled to a Leco (St. Joseph, MI) Pegasus 4D TOF mass spectrometer. The analytical column setup was a Restek (Bellefonte, PA;) Rtx-5MS with Integra-Guard (20 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled to an Rtx-CPL Pesticides II (1.5 m × 0.10 mm × 0.10 μm thickness) column at the transfer line and detector. This corresponded to a 5.34 m × 0.18 mm i.d. "virtual" column configuration for the flow control calculation on the instrument.

The GC-TOF conditions for the analysis were similar to those in Mastovska et al. (*17*) as follows: ultrahigh-purity He carrier gas at constant flow of 1.5 mL/min, a 2 μL pulsed splitless injection volume with a pressure pulse of 75 psig for 1 min, 250 °C inlet temperature, a column oven program with initial temperature of 60 °C (held for 2 min), then a 20 °C/min ramp to 180 °C, then a 5 °C/min ramp to 230 °C, then a 20 °C/min ramp to 280 °C, followed by a 40 °C/min ramp to 300 °C (held for 12 min). The total analysis time was ∼25 min. The transfer line was set at 280 °C. The ion source temperature was 250 °C, and the electron energy was −70 eV. The mass range was *m*/*z* 70−600, the detector voltage was 1800 V, and the spectral data acquisition rate was 10 spectra/s. Data processing was performed using Leco ChromaTOF software. The NIST mass spectral library and Agilent's pesticide and endocrine disruptor database were used for mass spectral matching and peak identification.

The retention times (t_R) and ions used for each analyte in GC-TOF are shown in **Table 2**. The peak areas of each pesticide divided by peak areas of diazinon (IS) served as the signal for quantitative analysis using matrix-matched calibration standards prepared from blank matrix extracts. Diazinon was chosen as the IS in the ARS laboratory because it is known to yield consistently ≈100% recoveries with little chance of matrix interference. In the General Mills laboratory, d_5-atrazine was used for similar reasons as the IS and also because it would not occur in the samples.

Table 2. Optimized GC-TOF Acquisition Method Parameters for the 34 Representative Pesticides[a]

pesticide	t_R (s)	quant ion (*m*/*z*)	other ions (*m*/*z*)	linearity (linear regression)
dichlorvos	457.61	109	185, 220	0.998
ethoprophos	646.04	158	139, 200	0.999
triflulalin	666.83	264	306, 335	0.999
hexachlorobenzene	709.46	284	214, 249	0.998
dimethoate	715.72	125	229	0.998
atrazine	722.85	200	215	0.999
lindane	744.88	181	111, 219	0.995
quintozene	750.95	237	249, 295	0.999
diazinon (IS)	756.41	137	179, 304	n/a
chlorothalonil	787.81	266	229	0.992
vinclozolin	835.47	285	212, 198	0.995
chlorpyrifos-methyl	837.17	125	286	0.994
carbaryl	848.36	144	115	
heptachlor	852.16	272	237, 372	0.997
pirimiphos-methyl	881.00	290	276, 305	0.997
malathion	897.08	173	125, 158	0.999
metolachlor	910.81	162	238	0.998
fenthion	914.87	278	125, 169	0.999
chlorpyrifos	916.74	197	258, 314	0.999
tolylfluanid	987.26	137	238, 346	
folpet	1009.74	260	147, 297	
procymidone (QC)	1010.74	283	255	n/a
cis-chlordane	1051.29	373	237, 410	0.996
p,*p*′-DDE	1088.37	246	176, 318	0.994
oxyfluorfen	1103.97	252	302, 361	0.995
o,*p*′-DDT	1157.74	235	165, 354	0.999
endosulfan sulfate	1197.51	272	387, 422	0.996
bromopropylate	1245.82	341	155, 183	0.999
mirex	1283.91	272	237, 332	0.999
permethrin	1315.95	183	163	0.987
coumaphos	1331.57	226	210, 362	0.986
cypermethrin	1358.48	163	181, 209	0.997
deltamethrin	1454.51	253	172, 181	0.998
azoxystrobin	1480.67	344	388, 403	0.998

[a] Linearities (linear regression) were obtained from the matrix-matched calibration curves of flaxseed extract using a traditional GC column and a 10 μL PTV Optic-3 injection.

In some experiments, we investigated large-volume injection (LVI) to achieve lower detection limits. We used an Atas-GL International (Veldhoven, The Netherlands) Optic 3 programmable temperature vaporizer for LVI of 10 μL. The initial injector temperature was 75 °C for 18 s, ramped to 280 at 8 °C/s with a splitless period for 2 min. The inlet temperature was held at 250 °C and 50 mL/min split ratio until the end of the run.

Sample Preparation. The modified QuEChERS sample preparation method was the same as devised by Mastovska et al. (*17*): (1) milled/homogenized samples (2.5−5 g depending on the matrix and experiment) were weighed into 50 mL polypropylene centrifuge tubes; (2) known pesticide concentrations and the IS were spiked into the samples, which were allowed to stand for 1 h at room temperature; (3) 10 mL each of water and MeCN was added to the samples and mixed using an automatic vortexer for 1 h to swell the matrix and extract the samples; (4) 4 g of anh $MgSO_4$ and 1 g of NaCl were added to each tube, which was shaken vigorously by hand for 1 min, ensuring that the powders did not agglomerate; (5) the tubes were centrifuged at 3000 rcf for 3 min; (6) 1 mL aliquots of the MeCN extracts (upper layer) were transferred to 2 mL minicentrifuge tubes containing 150 mg of anh $MgSO_4$, 150 mg of PSA, and 50 mg of C-18, which were shaken for 30 s; (7) the d-SPE tubes were centrifuged at 3000 rcf for 3 min; and (8) 0.2 mL of the final extracts was transferred to autosampler vials with microvial inserts, and 20 μL of QC standard solution was added.

RESULTS AND DISCUSSION

Optimization of Sample Preparation Procedure. *GPC for Lipid Removal*. In the analysis of GC-amenable pesticide residues in high-fat matrices, sample cleanup is important because a small amount of lipids can reduce signal or cause column damage. This requires the removal of macromolecular mass lipids from the extracts before GC analysis. According to the USDA Nutrient Composition Database (*4*), raw flaxseeds and peanuts have a fat content of >40% (see **Table 1**). Traditionally, GPC is commonly applied as an effective cleanup step after a preliminary liquid−solid extraction for fat removal. In GPC, the separation on the column occurs by size, in which smaller molecules such as pesticides elute after the larger molecules such as fats. We initially felt that GPC was needed for sample cleanup of such fatty samples as flaxseeds, peanuts, and doughs, and we conducted experiments to evaluate GPC in this application.

Figure 1 shows the GPC elution profile of 1 g of flaxseed oil equivalent injection and some pesticides from the GPC fractions collected and analyzed by GC-TOF. The flaxseed oil was measured by weight in the 1 min fractions. In working with samples containing animal fats (e.g., cod liver oil, whale blubber, lard), we had found that separation of the lipids from the pesticide and environmental contaminant analytes could be easily achieved with the smaller size GPC column that we used (*21*). However, the separation of pesticides from the flaxseed oil was more difficult due to the different lipid profile of the vegetable oil, which overlapped with the early eluting pesticide analytes, such as trifluralin. Any pesticide that eluted at <13 min was not adequately separated from the flaxseed oil, as shown in **Table 3**.

Also, the pesticide recoveries of spiked flaxseed oil obtained after GPC cleanup were lower than desirable for trace analysis, especially for relatively polar analytes. Furthermore, the GPC method could not be used for analytes detected in liquid chromatography (LC), which would necessitate the extraction of the same sample by different methods to achieve wide analytical

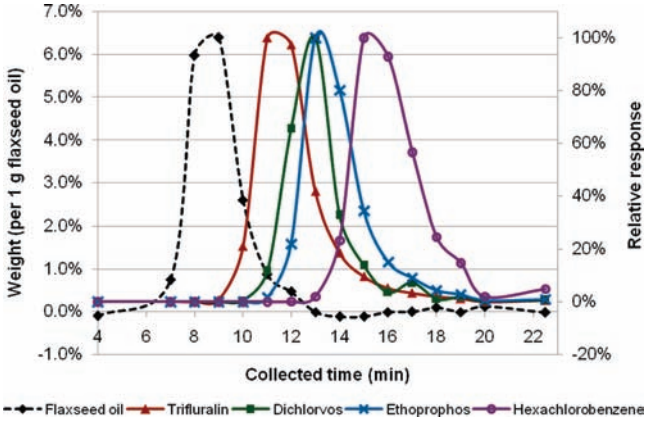

Figure 1. GPC elution profile for 1 g of injected equivalent of flaxseed oil and selected pesticides collected in 1 min fractions using the conditions given under Materials and Methods.

scope. Even for GC-amenable analytes, a larger GPC column was needed, but this produces even more solvent consumption and takes longer for cleanup. For these reasons, we decided to test the current QuEChERS method used by General Mills for analysis of pesticides in cereal grains (*17*) rather than continue the experiments using GPC.

Initial Extraction Step. The QuEChERS approach has already been shown to be effective in minimizing coextraction of lipids from fatty foods due to low solubility of the lipids in MeCN and still achieve high recoveries of a wide range of relatively polar LC- and semipolar GC-amenable pesticides (*13*, *18*). Lipophilic analytes are partially recovered depending on the partitioning ratios and volumes of the MeCN and lipid in the sample (*13*).

In an experiment, 2 g of milled flaxseeds was extracted by vortexing for 10 min at room temperature with 10 mL of three different types of solvents, 1:1 EtOAc/cyclohexane (GPC mobile phase), EtOAc, and MeCN. The amount of coextractives was measured after the extracts had been dried, which were 18.9 and 17.9% of original sample weight when using 1:1 EtOAc/cyclohexane and EtOAc, respectively, whereas coextractives were only 4.3% in the case of MeCN. Even after GPC as described above with a collection time of 10−20 min (see **Figure 1**), the amount of coextractives from the EtOAc and EtOAc/cyclohexane flaxseed extracts ranged from 3.5 to 4.7%. It was clearly advantageous in this application to use the QuEChERS approach if it is found to be fit-for-purpose.

As traditionally conducted (*22*), the swelling of dry samples (< 25% water content) with water is essential to allow the extraction solvent access into the sample and increase the extraction efficiency. Mastovska et al. (*17*) found that either 2.5 or 5 g of dry samples should be used with 10 mL of water plus 10 mL of MeCN (the solvents should be added together during the swelling step) depending on the amount of fatty acids in the samples. In this study, we also compared the use of 2.5 or 5 g of milled flaxseed or dough sample.

The 4 g of anh $MgSO_4$ and 1 g of NaCl were added to the mixture to induce the phase separation and force the pesticides to partition into the MeCN phase. This also increased the selectivity of the extraction process versus MeCN alone. No significant differences in the amount of coextractives were observed when the initial agitation time was changed.

Low-Temperature Lipid Precipitation and d-SPE Cleanup. Although the MeCN-based partitioning step minimized fat coextractives for GC analysis, an additional cleanup procedure was still necessary prior to injection. The low-temperature fat precipitation method (or freeze-out) is generally known as a practical way to help remove bulk coextractives from aqueous or other relatively polar solvents (*7*−*9*). The approach is simple and inexpensive, uses no extra solvent, and requires only additional time and the use of a freezer (or other means to expose the extracts to low temperature).

Table 3. Elution Times of the 34 Pesticides from the GPC System (1:1 EtOAc/Cyclohexane, Flow Rate = 5 mL/min, 5 mL Injection Volume)

elution time (min)	pesticides
11	trifluralin
12	atrazine, cypermethrin, deltamethrin, diazinon, endosulfan sulfate, malathion, oxyfluorfen, permethrin, procymidone, tolylfluanid, vinclozolin
13	carbaryl, *cis*-chlordane, chlorpyrifos, chlorpyrifos-methyl, coumaphos, *p,p′*-DDE, *o,p′*-DDT, dichlorvos, dimethoate, ethoprophos, heptachlor, lindane, metolachlor, pirimiphos-methyl
14	chlorothalonil, fenthion, folpet, mirex, quintozene
15	hexachlorobenzene

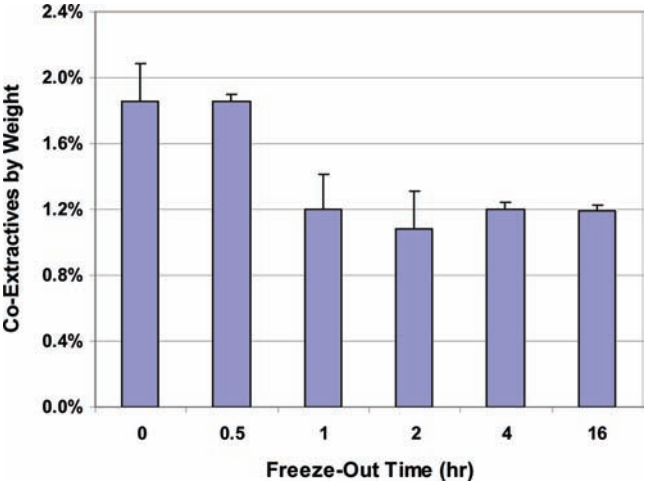

Figure 2. Effect of time on low-temperature fat precipitation from the initial QuEChERS flaxseed extracts (before d-SPE cleanup) at −20 °C.

By using the QuEChERS approach with 2.5 g of flaxseeds and 10 mL of water plus 10 mL of MeCN followed by the addition 4 g of anh $MgSO_4$ and 1 g of NaCl, the amount of coextracted matrix was reduced from ≈4% (using MeCN only) to 1.9% in the initial QuEChERS extract. As shown in **Figure 2**, this was further decreased to ≈1.2% when the extract was kept at −20 °C for 1 h or longer. A portion of the coextracted material (presumably lipids) precipitated and settled to the bottom of MeCN extracts in the vials. **Figure 3** also shows the effect of temperature for a 2 h freeze-out step, which shows that −20 °C is satisfactory. The use of dry ice at −85 °C provides no additional benefit and is too cold logistically because the MeCN must reach −45 °C to melt before it can be transferred.

Despite the visual observations and weight measurements, GC-TOF chromatograms showed no differences before or after the low-temperature precipitation step. However, when the simple and rapid d-SPE procedure was conducted for cleanup at room temperature using a mixture of 150 mg of anh $MgSO_4$, 150 mg of PSA, and 50 mg of C-18 per milliliter of extract, independent of low-temperature precipitation cleanup, both the gravimetric and chromatographic results show marked improvements. **Figure 3** provides the gravimetric results, which demonstrates how the flaxseed extracts after d-SPE gave the same values of ≈0.25% coextractives by weight whether the freeze-out step

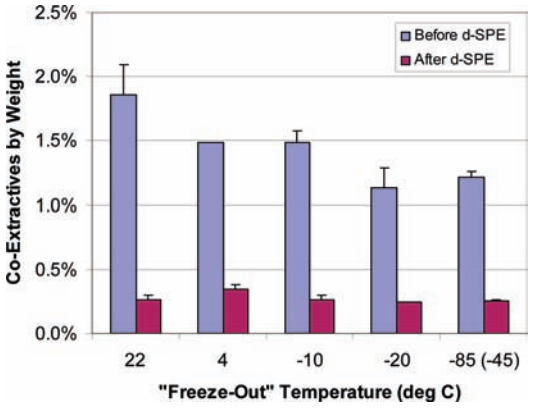

Figure 3. Effect of temperature on the amount of fat precipitation from QuEChERS flaxseed extracts kept at the given temperatures for 2 h (the −85 °C extracts reached −45 °C when the melted MeCN was taken for evaporation to determine weight differences).

was performed or not. Thus, the additional option of a freeze-out step was unnecessary.

In the d-SPE procedure, anh $MgSO_4$ was useful in removing the trace amount of water in the extract. PSA is a weak anion exchange sorbent that retains carboxylic acids, such as fatty acids, from the MeCN extracts, and the increase from 25 to 150 mg per milliliter of extract versus the original method (*15*) provides the greater capacity needed for reduction of fatty acids in these types of sample extracts (*17*). C-18 is a nonpolar sorbent that more effectively retains trace amounts of lipids from the extract, which was demonstrated to be particularly effective in milk and egg extracts (*13*). The use of C-18 was not included in the original method or interlaboratory trials, but subsequent trials have shown that it does not affect pesticide recoveries, and it can only help with cleanup. Thus, we recommend its use in d-SPE for our QuEChERS applications now. Furthermore, 7.5 mg of graphitized carbon black (GCB) per gram of sample equivalent (*19*) has also been found to be a good compromise for providing some degree of cleanup at the cost of ≈30% lower recovery of HCB in nonfatty matrices. However, we could not afford that degree of additional reduction in the fatty samples, and GCB was not used in this application.

In terms of chromatographic results, the QuEChERS extraction with d-SPE cleanup gave cleaner extracts than those obtained by GPC cleanup of flaxseeds extracted in the solvents tested, especially for volatile components at the front of the chromatogram. Our results led us to the conclusion that the d-SPE was a good cleanup procedure to remove coextracted material from the flaxseeds, even better than GPC or low-temperature precipitation.

Analytical Method Performance. Although we were prepared to use GPC or make alterations to the QuEChERS method as described above for the fattier matrices, we settled on the same approach as before (*17*) because it still provided the most effective cleanup in a highly streamlined method, and it eased implementation for routine analysis. We chose to sacrifice potentially higher recoveries for those lipophilic pesticides in the fatty matrices for practical factors of lower cost, higher sample throughput, greater ease of use, less labor, and reduced solvent consumption and waste. This approach was still found to meet fit-for-purpose detection limit needs, and we conducted further experiments to empirically justify the use of a method with lower recoveries, even if it is just used for screening purposes. The performance of the proposed method was evaluated to check the usefulness of quantitative and/or qualitative analysis in the high-fat matrices.

Table 4. Comparison of the Matrix-Induced Enhancement Effect in GC-TOF for Pesticides in QuEChERS Extracts from the Different Types of Doughs Using Pressure-Pulsed Splitless Injection (PSI) of 2 μL for 0.5 g/mL Final Extracts (5 g of Sample; 1 mg Equivalent Injected) or Large Volume Injection (LVI) of 10 μL for 0.25 g/mL Final Extracts (2.5 g of Sample; 2.5 mg Equivalent Injected)[a]

	biscuit		cinnamon roll		pie crust	
pesticide	PSI	LVI	PSI	LVI	PSI	LVI
atrazine	0	+++	++	+++	0	+
bromopropylate	++	++	+++	+++	++	+
cis-chlordane	0	0	+	+	0	0
chlorothalonil	0	++	++	+	++	+
chlorpyrifos	0	+	++	++	0	0
chlorpyrifos-methyl	+	+	+++	++	+	0
coumaphos	+	+++	+++	+++	++	+
cypermethrin	+	+++	+++	+++	++	++
p,p'-DDE	0	+	+	++	0	0
endosulfan sulfate	+	+	+	++	0	0
ethoprophos	++	++	+++	+++	+	+
fenthion	0	+	++	++	0	0
heptachlor	+	+	+++	++	++	0
hexachlorobenzene	0	+	+	++	0	+
lindane	0	+	++	++	+	0
malathion	++	++	+++	+++	++	+
metolachlor	+	+	++	++	0	0
mirex	0	0	++	++	0	0
oxyfluorfen	+	++	+	+++	+	+
permethrin	++	+	+++	+++	+	0
pirimiphos-methyl	0	+	++	++	+	0
quintozene	+	+	++	+++	+	+
trifluralin	0	+	++	++	+	+
vinclozolin	+	0	++	+++	0	+

[a] The matrix effect was calculated by taking the percent difference in the slopes of the matrix matched vs solvent-only linear calibration plots with "0" <20%; "+" = 20−50%; "++" = 50−100%, and "+++" >100% differences.

The method was evaluated in terms of linearity, matrix effects, recoveries, detectability, and precision.

Linearity and Matrix Effects. Matrix-matched calibration standards were prepared by adding known pesticide amounts from 0.25 to 1.5 μg/g equivalents to the six representative blank extracts (brown flaxseeds, golden flaxseeds, peanuts, butter biscuit dough, cinnamon roll dough, and pie crust dough). The results were calculated by plotting the peak area ratios of the analytes versus diazinon (IS) against the analyte concentrations. Good linearity was found with regression coefficients of the least linear squared calibration curves up to 0.999 for many pesticides.

In GC analysis, the matrix-induced chromatographic response effect has been widely observed in complex matrices (*23*−*25*). Peak enhancements occur for susceptible analytes when matrix components fill the active sites in the injection port, column, and MS source that would be filled by the analyte if matrix was not present. Therefore, a positive bias occurs when quantitative results are calculated using solvent-only calibration standards. To estimate this effect, the slopes of calibration curves achieved from solvent-based standards and from matrix-matched standards were compared. The calculated matrix enhancements ranged from −1 to 306% depending on the analyte, matrix, and GC conditions.

Matrix effects in GC cannot be measured precisely, thus **Table 4** gives only an overview for the pesticides deemed to be most reliable in estimating matrix effects for the different dough samples tested. Percent differences in sensitivity (slopes of the linear calibration plots) are given in different ranges rather than using the measured values, which give the impression of greater

Table 5. Averaged Percent Recoveries and %RSDs (within Sets) Obtained for 32 Pesticides Normalized to Diazinon (IS) Spiked at 1 μg/g into the Matrices[a]

	peanut (with shell)		flaxseeds (six sets)		doughs, 2.5 g (three sets)		doughs, 5 g (three sets)	
pesticide	mean	RSD	mean	av RSD	mean	av RSD	mean	av RSD
atrazine	101	5	114	6	102	6	115	4
azoxystrobin	103	7	145	22	107	5	122	13
bromopropylate	87	5	71	4	95	6	102	5
carbaryl	102	6	nd		nd		105	4
cis-chlordane	73	6	53	4	85	3	87	6
chlorothalonil	22	1	nd		39	5	42	2
chlorpyrifos	86	4	64	4	96	4	107	4
chlorpyrifos-methyl	96	5	71	4	99	3	104	4
coumaphos	111	2	93	10	111	8	111	5
cypermethrin	104	9	72	8	102	8	106	12
p,p′-DDE	59	1	49	3	72	3	73	2
o,p′-DDT	59	4	51	4	75	3	73	3
deltamethrin	nd		nd		105	7	71	5
dichlorvos	112	13	113	10	99	6	105	3
dimethoate	113	4	96	9	nd		nd	
endosulfan sulfate	98	4	73	6	101	5	112	6
ethoprophos	106	4	114	4	103	4	114	4
fenthion	96	5	86	4	99	4	113	3
folpet	nd		nd		nd		nd	
heptachlor	65	2	54	4	84	5	50	8
hexachlorobenzene	47	4	35	2	66	4	52	2
lindane	90	6	67	3	97	3	85	5
malathion	113	4	106	5	103	4	117	4
metolachlor	103	4	110	4	101	4	112	4
mirex	36	1	43	3	48	2	39	2
oxyfluorfen	94	8	81	5	103	5	120	5
permethrin	80	5	81	6	92	12	88	4
pirimiphos-methyl	102	5	82	3	101	4	118	4
quintozene	74	6	48	4	88	5	76	2
tolylfluanid	29	1	nd		36	1	9	0
trifluralin	95	5	73	3	102	3	111	8
vinclozolin	104	5	89	3	104	3	109	3

[a] A set of samples is $n = 3-8$ replicate spikes on a given day. nd, not detected.

precision. Two different types of injection and sample extraction sizes were compared as described in the table title. Diazinon (IS) was not used in these calculations because it also undergoes a degree of matrix enhancement, which complicates the assessment.

As previously described (*23*−*25*), the most nonpolar analytes were affected least by the matrix-induced enhancement, and the pesticides with higher polarity were more strongly affected. The cinnamon roll dough induced the greatest effects compared to the other dough due to the greater amount of simpler sugars present. The pie crust dough gave the least matrix effects in the experiment. Matrix-induced effects occurred for the same pesticides in both types of injection of the QuEChERS final extracts in MeCN (10 μL LVI equivalent to 2.5 mg of sample injected and 2 μL pressure-pulsed splitless equivalent to 1 mg of sample injected), but the effects were often more intense when a greater amount of matrix was injected. For these reasons, quantitative results were calculated using matrix-matched standards throughout the study. Fortunately, plenty of blank material of these matrices is available to allow this practice routinely.

Recoveries. Many experiments were conducted involving spiking the representative GC-amenable pesticides into the different matrices to determine recoveries with the final method. The samples were typically spiked with appropriate volumes to achieve 1 μg/g of spiked standards. **Table 5** shows the summarized recoveries for all pesticides analyzed in the four matrices (the results for the different types of flaxseeds and biscuit, cinnamon roll, and pie crust doughs were combined). The majority of pesticides gave 70−120% acceptable recoveries with associated

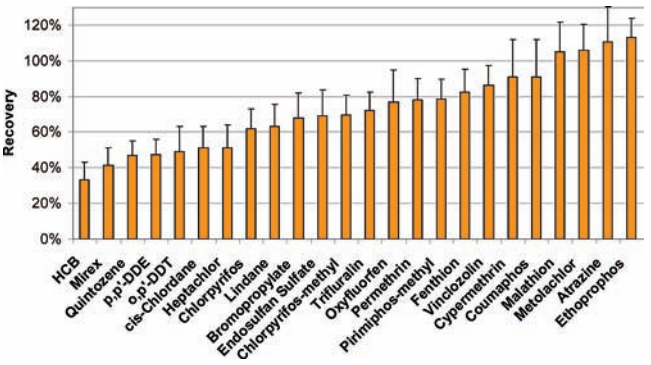

Figure 4. Recoveries and standard deviations (error bars) for overall averaged results from six data sets for pesticides listed in increasing order of recovery (generally decreasing lipophilicity) for three different sample types of flaxseeds analyzed by the final method ($n = 25-26$).

< 20% RSD over the course of multiple days. The more lipophilic pesticides (quintozene, chlorpyrifos, *cis*-chlordane, hexachlorbenzene, heptachlor, *p,p′*-DDE, *o,p′*-DDT, and mirex) gave recoveries of < 70% in flaxseeds and peanut samples, which have > 40% lipid content, but showed acceptable recoveries in the doughs. Thus, the recoveries of these types of pesticides depended on the lipophilicity of the pesticides and the lipid content in the matrices.

Missing and variable recoveries were also obtained for pesticides that are better analyzed by LC-MS/MS analysis, such as carbaryl, dimethoate, and azoxystrobin. As usual for the

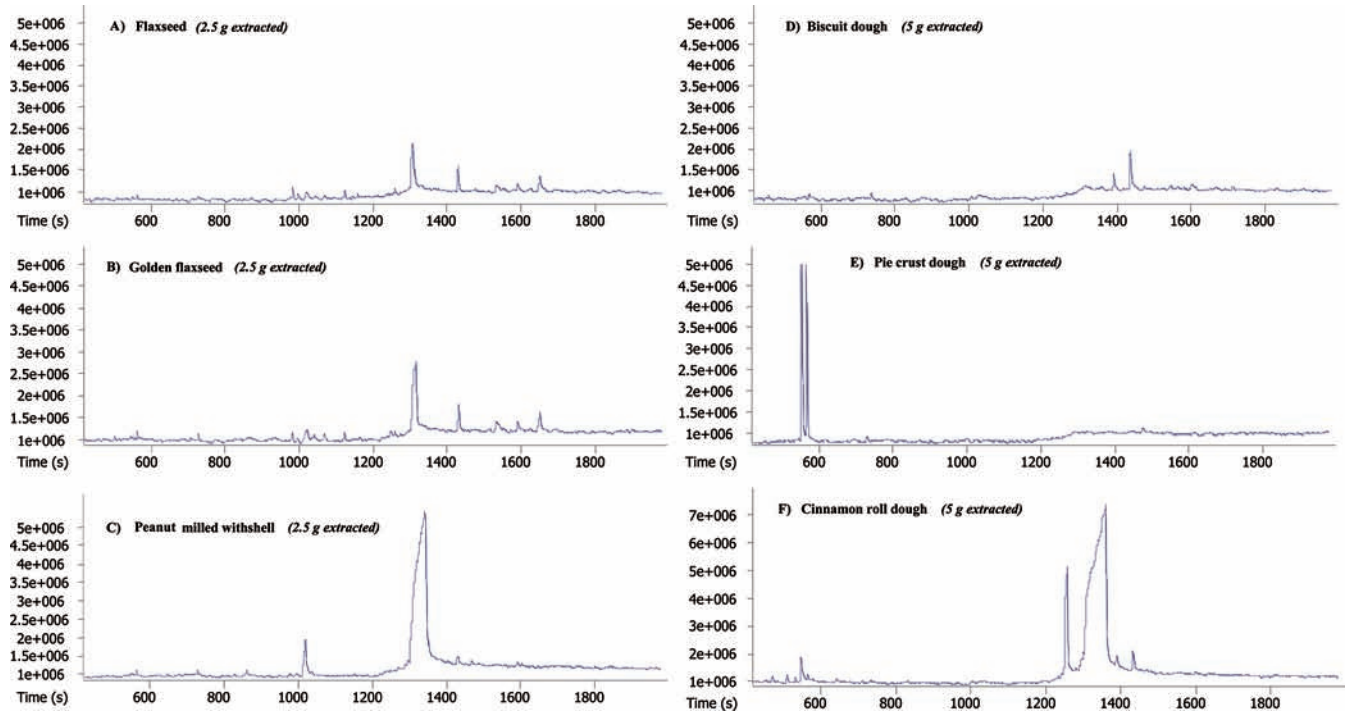

Figure 5. GC/TOF-MS total ion chromatograms for the (**A**, **B**) flaxseeds, (**C**) peanut, and (**D**) biscuit, (**E**) pie crust, and (**F**) cinnamon roll dough samples with different subsample extraction sizes.

base-sensitive analytes, folpet, chlorothalonil, and tolylfluanid degraded during sample preparation, storage time of the extracts, and/or GC injection using MeCN, the latter of which also affects deltamethrin (*26*).

For quantitation of the lipophilic pesticides, the critical issue relates to the reproducibility of the results from multiple days and different sample types, particularly for the flaxseeds, which were of greater interest to us and would be expected to have the greater variability (see **Table 1**). The interday RSDs were <26% RSD for lipophilic pesticides. **Figure 4** shows the average recoveries and standard deviations of 24 selected pesticides in three different types of flaxseeds over the course of 6 days of extractions and analyses (two sets of each different source of flaxseeds). The recoveries were reasonably consistent for our fit-for-purpose goals. We believe that because the reason for the low recoveries of the lipophilic pesticides relates to a well-understood physicochemical property, it is justifiable to correct the results for the recovery factor during quantitation. Alternately, the method can be used for semiquantitative screening and identification of these analytes, and if desired, a second method using a nonpolar solvent for extraction and GPC cleanup may be used (after validation) for quantitation and confirmation.

Figure 5 shows the total ion chromatograms of the different sample types tested, which demonstrate the greater fatty acid content (peak at 1300 s) of peanuts and cinnamon rolls compared to the flaxseeds and other doughs. The large peak did not interfere in the analysis of coeluting pesticides, nor did it lead to ghost peaks or reduced instrument ruggedness.

Second Laboratory Verification. A second laboratory verification study was performed on flaxseed samples by General Mills. This was done to evaluate the full suite of approximately 180 pesticides, including those analyzed by ultraperformance liquid chromatography−tandem mass spectrometry (UPLC-MS/MS), that were previously validated and implemented for cereal grains (*17*). To implement the method, we needed to ensure its ruggedness for use on flaxseeds and better determine the LODs by spiking the samples at lower concentrations. The materials used, sample preparation, and analysis conditions were the same as given previously, including quality control and data processing using d_5-atrazine as the internal standard (*17*). The validation consisted of ground flaxseed free of detected pesticide residues, spiked at three different levels performed in duplicate. Concentrations of the spiked samples ranged from 0.060 to 0.40 μg/g.

Recovery and LOD data are presented in **Table 6**. For the sake of brevity and simplicity, only the GC-TOF results for 32 chosen representative pesticides are listed. The independent General Mills results showed good agreement with those from the USDA validation experiments. Although several lipophilic pesticides showed low recoveries (~30−50%), the GC-TOF was able to detect many pesticides at 0.005 μg/g. Some of the poorer responding pesticides were amenable by UPLC-MS/MS, which has previously been demonstrated to provide better sensitivity and selectivity (*17*). As usual, problematic pesticides included folpet, tolylfluanid, some pyrethroids, and chlorothalonil. The folpet degradation product, phthalimide, was easily detected and was used as an indicator for the presence of folpet.

In conclusion, a rapid and efficient modified QuEChERS method was extended to high-fat matrices and evaluated for the determination of many GC-amenable pesticides by two different laboratories. The method also provides excellent results for relatively polar pesticides analyzed by UPLC-MS/MS. This method avoided GPC and still provided clean final extracts for rapid and rugged analysis. Lipophilic pesticides gave lower recoveries, but they were found to be reasonably consistent for our needs from different batches of matrices, and LODs were still acceptably low. In actual samples, the results can be corrected for the known recoveries, or the screened samples can be reanalyzed using a nonpolar extraction solvent to yield higher recoveries followed by GPC or other forms of cleanup. The proposed modified QuEChERS approach substantially minimized the time, labor, and cost and allowed simultaneous quantitation

Table 6. Averaged Percent Recoveries, %RSDs, and LODs obtained from the General Mills Laboratory for 32 Pesticides Normalized to d_5-Atrazine (IS) Spiked at Three Levels into Flaxseeds[a]

	0.40 μg/g		0.12 μg/g		0.06 μg/g		LOD (μg/g,
pesticide	mean	RSD	mean	RSD	mean	RSD	extracted)
atrazine[b]	96	3	109	3	102	0	0.005
azoxystrobin[b]	93	5	120	5	100	8	0.005
bromopropylate	61	2	54	10	57	3	0.005
carbaryl[b]	111	13	111	23	nd		0.04
cis-chlordane	54	8	55	17	49	4	0.01
chlorothalonil	nd		nd		nd		
chlorpyrifos[b]	61	3	54	11	55	4	0.01
chlorpyrifos-methyl	71	13	65	11	56	1	0.005
coumaphos[b]	79	31	nd		nd		0.35
cypermethrin	52	13	nd		nd		0.3
p,p′-DDE	52	15	43	22	52	12	0.005
o,p′-DDT	47	10	37	45	22	31	0.01
deltamethrin[b]	nd		nd		nd		
dichlorvos[b]	82	1	109	3	64	18	0.01
dimethoate[b]	106	2	132	15	nd		0.06
endosulfan sulfate	65	24	nd		nd		0.3
ethoprophos	94	5	107	6	95	4	0.005
fenthion	82	11	72	1	66	7	0.005
folpet[c]	nd		nd		nd		
heptachlor	48	5	54	4	53	2	0.005
hexachlorobenzene	34	9	32	8	25	21	0.005
lindane	65	16	58	2	70	23	0.03
malathion[b]	96	3	82	0	82	2	0.01
metolachlor[b]	92	7	92	0	95	1	0.005
mirex	41	10	35	35	24	10	0.005
oxyfluorfen	71	10	56	7	42	21	0.01
permethrin[b]	97	11	119	7	nd		0.1[d]
pirimiphos-methyl[b]	76	10	69	4	69	7	0.005
quintozene	56	8	42	7	38	8	0.005
tolylfluanid[b]	nd		nd		nd		
triflulalin	74	8	58	8	47	26	0.005
vinclozolin	80	8	80	5	76	4	0.005

[a]A set of samples consisted of duplicate spikes on a given day. nd, not detected. [b]Analyte is UPLC-MS/MS amenable. [c]Phthalimide is the primary degradation product of folpet and was qualitatively identified at all levels. [d]LOD based on the trans isomer only. The cis isomer was not resolved due to matrix interference.

and identification of the pesticide analytes. Good separation, linearity, and reproducibility of most target pesticides were achieved by this method. For those lipophilic pesticides with < 70% recoveries, the analyst has the option to justify compensation of the result for empirically determined recoveries or to use the method for qualitative screening identification purposes only and use a more intensive traditional method for quantitative and confirmation purposes.

LITERATURE CITED

(1) Oomah, B. D.; Sitter, L. Characteristics of flaxseed hull oil. *Food Chem.* **2009**, *114*, 623–628.

(2) Popova, I. E.; Hall, C.; Kubátová, A. Determination of lignans in flaxseed using liquid chromatography with time-of-flight mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 217–229.

(3) Brewer, M. S.; Rojas, M. Consumer attitudes toward issues in food safety. *J. Food Saf.* **2008**, *28*, 1–22.

(4) U.S. Department of Agriculture, Agricultural Research Service, USDA National Nutrient Database for Standard Reference, Release 21, 2008, Nutrient Data Laboratory Home Page,www.ars.usda.gov/ba/bhnrc/ndl.

(5) Pizzutti, I. R.; de Kok, A.; Zanella, R.; Adaime, M. B.; Hiemstra, M.; Wickert, C.; Prestes, O. D. Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid chromatography−tandem mass spectrometry using positive and negative electrospray ionization. *J. Chromatogr., A* **2007**, *1142*, 123–136.

(6) Fernandez Moreno, J. L.; Arrebola Lieanas, F. J.; Garrido Frenich, A.; Martinez Vidal, J. L. Evaluation of different sample treatments for determining pesticide residues in fat vegetable matrices like avocado by low-pressure gas chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1111*, 97–105.

(7) Li, L.; Xu, Y.; Pan, C.; Zhou, Z.; Jiang, S.; Liu, F. Simplified pesticide multiresidue analysis of soybean oil by low-temperature cleanup and dispersive solid-phase extraction coupled with gas chromatography/mass spectrometry. *J. AOAC Int.* **2007**, *90* (5), 1387–1393.

(8) Li, L.; Zhang, H.; Pan, C.; Zhou, Z.; Jiang, S.; Liu, F. Multiresidue analytical method of pesticides in peanut oil using low-temperature cleanup and dispersive solid phase extraction by GC-MS. *J. Sep. Sci.* **2007**, *30*, 2097–2104.

(9) Lentza-Rizos, C.; Avramides, E. J.; Visi, E. Determination of residues of endosulfan and five pyrethroid insecticides in virgin olive oil using gas chromatography with electron-capture detection. *J. Chromatogr., A* **2001**, *921*, 297–304.

(10) Amvrazi, E. G.; Albanis, T. A. Multiclass pesticide determination in olives and their processing factors in olive oil: comparison of different olive oil extraction systems. *J. Agric. Food Chem.* **2008**, *56*, 5700–5709.

(11) Papadakis, E. N.; Vryzas, Z.; Papadopoulou-Mourkidou, E. Rapid method for the determination of 16 organochlorine pesticides in sesame seeds by microwave-assisted extraction and analysis of extracts by gas chromatography−mass spectrometry. *J. Chromatogr., A* **2006**, *1127*, 6–11.

(12) Blasco, C.; Font, G.; Pico, Y. Determination of dithiocarbamates and metabolites in plants by liquid chromatography−mass spectrometry. *J. Chromatogr., A* **2004**, *1028*, 267–276.

(13) Lehotay, S. J.; Maštovská, K.; Yun, S. J. Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Int.* **2005**, *88* (2), 630–638.

(14) López-Feria, S.; Cárdenas, S.; Valcárcel, M. One step carbon nanotubes-based solid-phase extraction for the gas chromatographic−mass spectrometric multiclass pesticide control in virgin olive oils. *J. Chromatogr., A* **2009**, *1216*, 7346−7350.

(15) Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–231.

(16) Lehotay, S. J. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *J. AOAC Int.* **2007**, *90*, 485–520.

(17) Mastovska, K.; Dorweiler, K.; Lehotay, S. J.; Wegscheid, J.; Szpylka, K. Pesticide multiresidue analysis in cereal grains using modified QuEChERS method combined with automated direct sample introduction GC-TOFMS and UPLC-MS/MS techniques. *J. Agric. Food Chem.* **2010**, doi: 10.1021/jf9029892.

(18) Cunha, S. C.; Lehotay, S. J.; Mastovska, K.; Fernandes, J. O.; Oliveira, B.M.P.P Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives. *J. Sep. Sci.* **2007**, *30*, 620–632.

(19) European Committee for Standardization (CEN) standard method EN 15662, 2008 (www.cen.eu and www.quechers.com).

(20) Payá, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal. Bioanal. Chem.* **2007**, *389*, 1697–1714.

(21) Hoh, E.; Lehotay, S. J.; Pangallo, K. C.; Mastovska, K.; Ngo, H. L.; Reddy, C. M.; Vetter, W. Simultaneous quantitation of multiple classes of organohalogen compounds in fish oils with direct sample introduction-comprehensive two-dimensional gas chromatography and time of flight mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 2653–2660.

(22) Food and Drug Administration. *Pesticide Analytical Manual Vol. I: Multiresidue Methods*, 3rd ed.; U.S. Department of Health and Human Services: Washington, DC, 1999; www.cfsan.fda.gov/~frf/pami3.html.

(23) Erney, D. R.; Gillespie, A. M.; Gilvydis, D. M.; Poole, C. F. Explanation of the matrix-induced chromatographic enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection. *J. Chromatogr*. **1993**, *638*, 57–63.

(24) Hajslová, J.; Zrostlíková, J. Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices. *J. Chromatogr., A* **2003**, *1000*, 181–197.

(25) Schenck, F. J.; Lehotay, S. J. Does further clean-up reduce the matrix enhancement effect in GC analysis of pesticide residues in food? *J. Chromatogr., A* **2000**, *868*, 51–61.

(26) Mastovska, K.; Lehotay, S. J. Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. *J. Chromatogr., A* **2004**, *1040*, 259–272.

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