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Simple Determination of 40 Organophosphate Pesticides in Raw Wool Using Microwave-Assisted Extraction and GC-FPD Analysis

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ABSTRACT: A validated analytical method for the multiresidue analysis of 40 organophosphate pesticides (OPs) and conversion products in raw wool has been developed. The method is based on the selective microwave-assisted extraction (MAE) of raw wool with acetonitrile and analysis of extracts by gas chromatography—flame photometric detector. The optimum MAE conditions were 20 min duration at 80 °C with 30 mL of acetonitrile per gram of wool. A validation study was performed according to the European SANCO guidelines 10684/2009. Limits of detection and quantification for all pesticides tested were from 0.01 to 0.2 mg/kg and from 0.2 to 1.0 mg/kg, respectively. The average recoveries of pesticides spiked at different levels were in the range of 70—120% with relative standard deviations of ≤20%. The extraction performance was compared to the one obtained with a reference Soxhlet extraction. The method was also applied in the analysis of real wool (after field application) samples.

KEYWORDS: MAE, organophosphate pesticides, wool, GC-FPD

INTRODUCTION

The improvement of both quantity and quality of wool production is a major objective for sheep breeders. Ectoparasites, such as lice, scabies, and flies, are common pests for sheep and can deteriorate the quality of the fleece. Organophosphate pesticides (OPs) are currently employed to control them. Moreover, some pesticides that are not directly applied to sheep could be found on wool due to indirect transference from the environment. For example, in some cases sheep are included in mixed production systems where they are in direct contact with agriculture. Consequently, they could be in contact with insecticides and acaricides used in agricultural production. The lipophilic pesticides are generally found in wool lipids and, consequently, wool byproducts such as wool grease and lanolin show relatively high levels of pesticides.[1] The International Wool Textile Organization (IWTO) rules the different wool trading aspects, particularly those focused on pesticide residues in wool. Despite the fact there is no official analytical protocol to analyze these residues, IWTO suggests some procedures. The most frequently applied methodology is a laborious and time-consuming process wherein pesticides are extracted by the use of petroleum ether in a Soxhlet apparatus. The resulting extract is fractionated through gel permeation chromatography, and the pesticide residues are determined by chromatographic analysis.[2] Some pesticide residue extraction methods with different principles such as supercritical fluid extraction and accelerated solvent extraction have been reported. These methods either need a cleanup step prior to GC analysis[3−6] or do not accomplish all of the necessary analytical quality control standards (i.e., high relative standard deviations (RSDs)).[7] Recently, microwave-assisted extraction (MAE) has been applied in pesticide residue analysis.[8−12] Stability problems under MAE conditions for some thermally labile OPs have been also reported.[13] MAE has only been used for the analysis of dieldrin in processed wool products.[8] To our knowledge, analysis of OP residues in raw wool by MAE has never been undertaken. The aim of this study was to develop and validate a fast and environmentally friendly multiresidue method for the analysis of OPs in raw wool samples. The proposed method allows the simultaneous determination of 40 pesticides in raw wool by a gas chromatography—flame photometric detection (GC-FPD) system, avoiding a laborious cleanup step. The broad range of OPs that can be analyzed with this method demonstrates its robustness. Moreover, the method was applied in the analysis of real samples and the results compared with the validated, routinely used method in our laboratory.

MATERIALS AND METHODS

Apparatus and Reagents. The MSP 1000 laboratory microwave system (CEM, Matthews, NC) equipped with 12 vessel carousel operated in the closed mode was used for the microwave-assisted solvent extraction (MAE) of raw wool. PTFE-lined extraction vessels were used. During operation, both temperature and pressure were monitored in a single vessel, and a sensor monitoring the solvent leaks in the interior of the microwave oven was also used. The operational parameters of the microwave-assisted extraction apparatus are shown in Table 1.

For separation and quantification of analytes a Thermo Fisher Scientific, model Finnigan Trace GC (Rodano, Milan, Italy), gas chromatograph equipped with a flame photometric detector (FPD) was used. The injection was made in a programmed temperature

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vaporizer (PTV): initial temperature, 60 °C; hold for 1.5 min; increase to 220 °C at the rate of 5 °C/s; and hold at 220 °C for 35 min. Gas chromatography (GC) was carried out with two columns (HP-1, 10 m, 0.53 mm, 2.65 μm film thickness) in tandem obtained from Agilent Technologies (Avondale, PA) and Hewlett-Packard (Palo Alto, CA), respectively. The detector temperature was 300 °C. Helium was used as carrier gas at a constant flow rate of 7 mL/min. For operation of the FPD the hydrogen flow was set at 90 mL/min and the air at 115 mL/min. Helium was used as the detector makeup gas at 30 mL/min. The oven was operated under a temperature gradient with initial temperature set at 50 °C (hold for 1 min), increase to 170 °C at the rate of 16 °C/min, increase to 220 °C at the rate of 6 °C/min (hold 1 min), increase to 240 °C at the rate of 4 °C/min, increase to 280 °C at the rate of 5 °C/min (hold 10 min), and return to initial conditions in 5 min. Total run time was 40.8 min. Two microliter injections were made by use of an autosampler (model AS 3000). The control of the GC-FPD system and data processing were performed by ChromCard, ThermoFinnigan (Rodano, Milan, Italy) software.

Pesticide-free wool was obtained from sheep that were not treated with pesticides for the past 3 years. Absence of pesticides in wool was confirmed through chemical analysis. The wool was stored at 21 °C and 60% realtive humidity prior to use, weighed, and used as such. Acetonitrile, toluene, *n*-hexane, and ethyl acetate of pro-analysis grade were purchased from Merck (Darmstadt, Germany). Analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Stock solutions of individual analytes at 1 mg/mL were made in ethyl acetate; mixed standard stock solutions made were serially diluted with ethyl acetate to produce a series of working standard solutions of 0.1−100 μg/mL. The latter solutions were used for the construction of calibration curves and the preparation of the fortified wool samples. Stock solutions were stored in a deep freezer (−23 °C), whereas the working standard solutions were stored refrigerated and renewed at bimonthly intervals. Matrix-matched calibration solutions were prepared as follows: 0.2 mL of blank raw wool extract (prepared by extracting pesticide-free raw wool samples, as described above) was dried under a N_2 stream and fortified with 0.2 mL of working standard solutions of pesticides in ethyl acetate at various concentrations. These matrix-matched solutions were used to prepare calibration curves, to evaluate the linear range, and to calculate recoveries in fortified samples.

Table 1. Optimal Parameters for Microwave-Assisted Extraction

magnetron power	100% (1000 W)
maximum temperature	80 °C
maximum pressure cutoff	100 psi
extraction duration	20 min
solvent	acetonitrile
solvent volume	30 mL
sample weight	1 ± 0.01 g

For confirmation of positive results in real wool samples a GC-MS/MS system consisting of a Trace 2000 gas chromatograph equipped with a split/splitless injector and connected with the GCQ plus ion-trap mass spectrometer (Thermoquest, Austin, TS) was used. Gas chromatographic analysis was carried out on a 30 m, 0.25 mm i.d., 0.25 μm film thickness CP-SIL 8 CB (5% phenyl, 95% dimethylpolysiloxane) low bleed/MS column (Varian Analytical Instruments, The Netherlands) with a 1 m, 0.25 mm i.d. guard column of deactivated fused silica (Alltech). The GC-MS/MS operational conditions were as described elsewhere.[10]

Microwave-Assisted Solvent Extraction. Wool samples (1 g) were extracted with 30 mL of acetonitrile in microwave-assisted

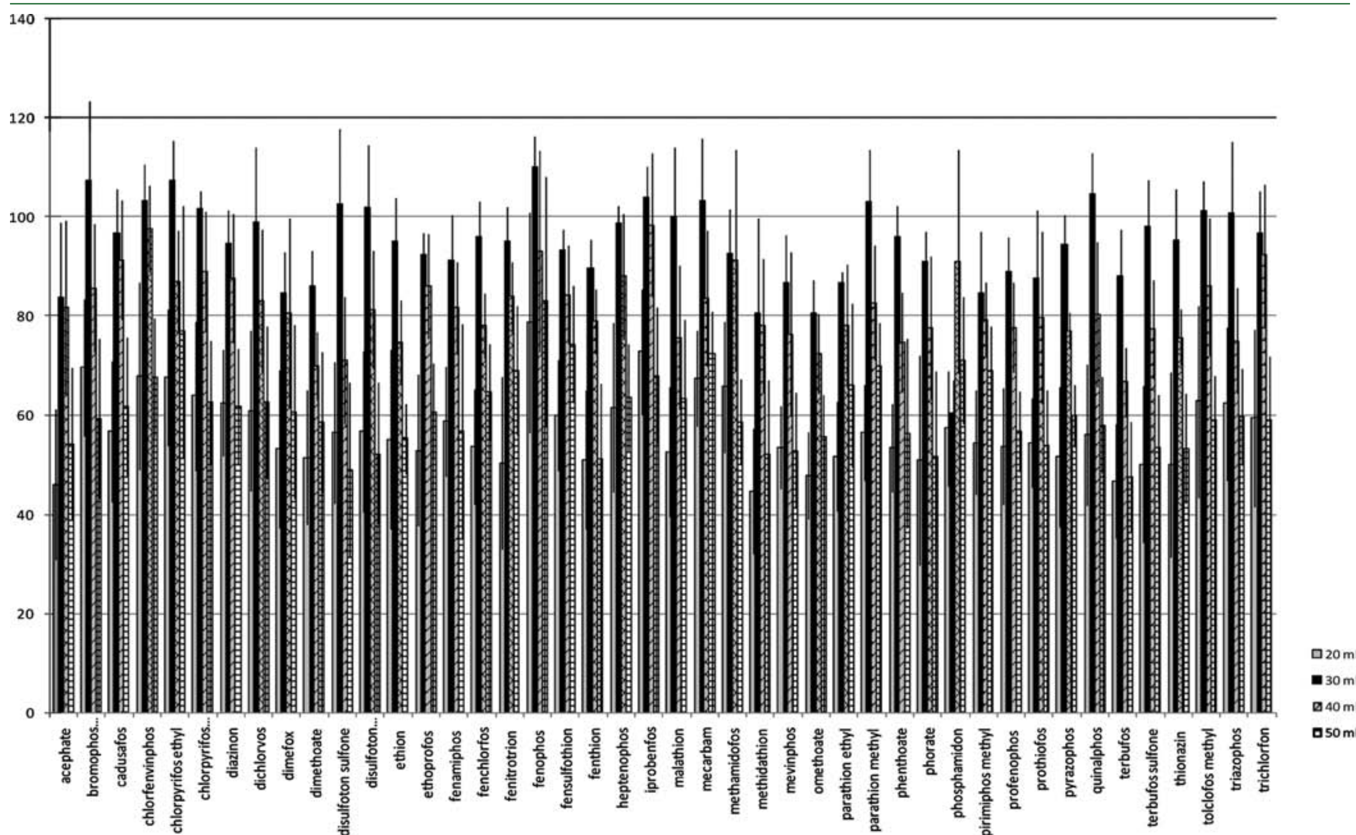

Figure 1. Optimization of extraction volume: recoveries versus different volumes for each pesticide.

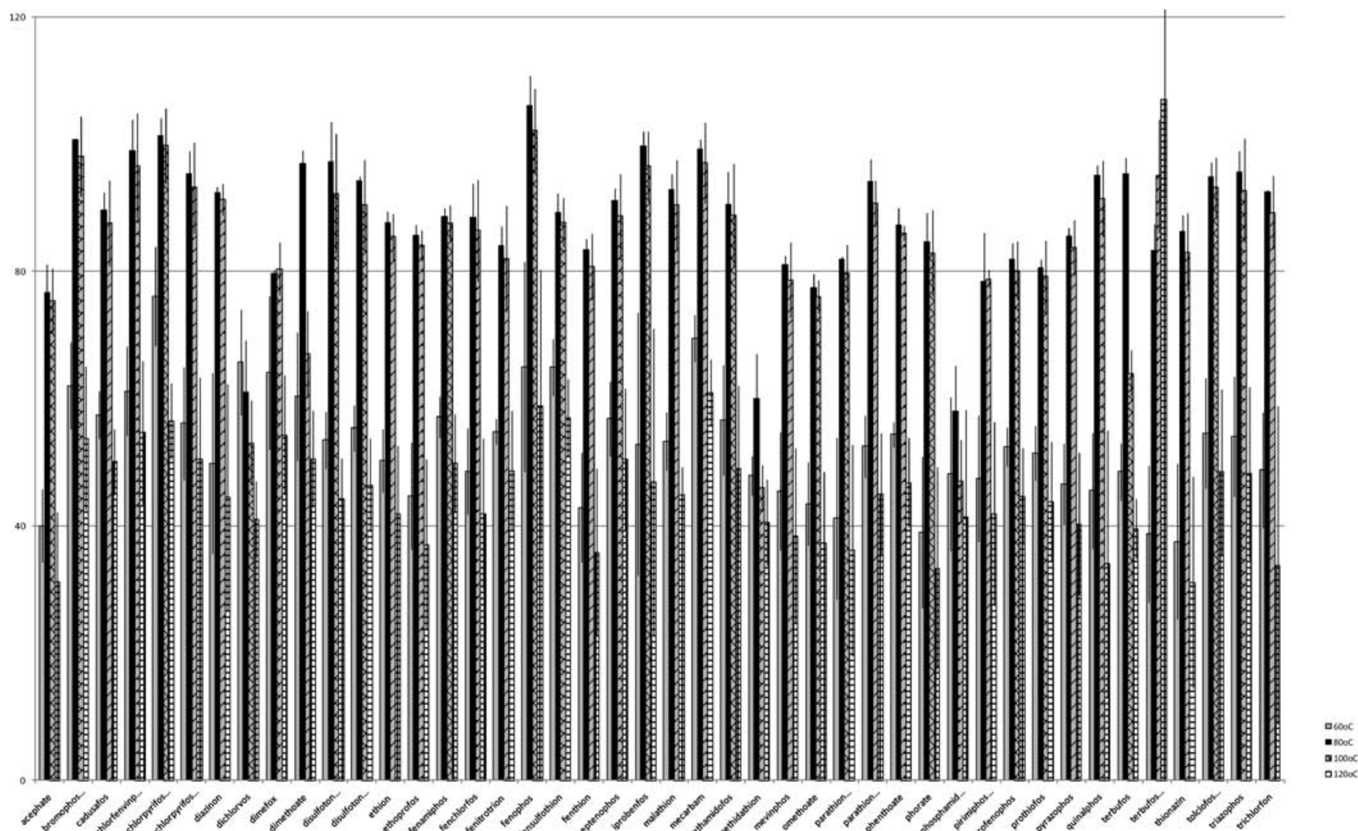

Figure 2. Optimization of microwave-assisted extraction temperature: recoveries versus different temperatures for each pesticide.

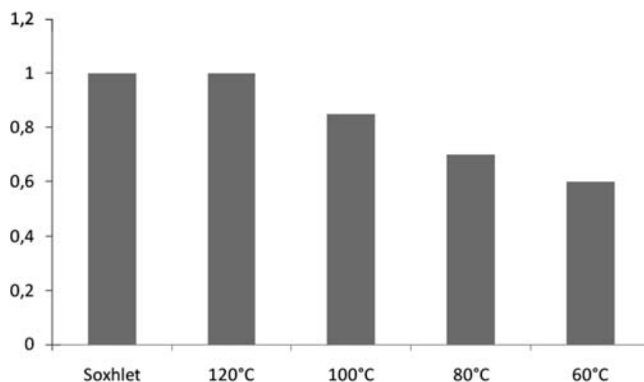

Figure 3. Comparison of wool wax coextractives using Soxhlet extraction and MAE at different temperatures: grams of MAE extract per grams of Soxhlet extract.

extraction equipment in sets of 12 vessels according to the operational program shown in Table 1. The vessels, before being removed from the microwave oven, were allowed to stand for about 10 min to cool to 38−40 °C. Then the samples were filtered using a Büchner vacuum filtration device with a 0.45 μm PTFE membrane disk and rinsed with 10 mL of acetonitrile. The combined extract was transferred to a round-bottom flask and concentrated to 5 mL under reduced pressure. The flask was additionally rinsed with 2 mL of acetonitrile, transferred to a 10 mL tube, and placed for 10 min in a freezer (−20 °C). The supernatant was transferred to another tube, and 0.2 mL of toluene was added prior to evaporation until dryness under a N_2 stream. The residues were redissolved in 0.2 mL of ethyl acetate and GC analyzed.

Soxhlet Extraction. Wool samples (1 g) were extracted with 100 mL of *n*-hexanes in a Soxhlet apparatus during 3 h. Then the samples were filtered using a vacuum filtration device and rinsed with 10 mL of *n*-hexanes. A 0.2 mL portion of toluene was added prior to evaporation until dryness under a N_2 stream. The residues were redissolved in 0.2 mL of ethyl acetate containing 1.0 mg/kg bromophos methyl as internal standard and GC analyzed.

Reference Method. The method employed for the determination of pesticide residue levels in raw wool is a validated version of the method from Jones.[1,2] In short, 10 g of wool was Soxhlet extracted with 300 mL of hexanes for 3 h, and the extract was driven to dryness under reduced pressure and redissolved in 25.0 mL of a 1:1 CH_2Cl_2/hexane mixture. Five milliliters of this solution was injected in a homemade MPLC equipped with a column containing 10 g of Biobeds X3 (Bio-Rad Inc.) and chromatographed at 2 mL/min isocratically with a 1:1 CH_2Cl_2/hexane mixture. The elution of wool wax esters was followed spectrophotometrically at 220 nm. After the elution of lipids was completed (approximately 7 min), the eluates were collected to a final volume of 100 mL and the solvent was eliminated under reduced pressure. The residue was redissolved in 1 mL of ethyl acetate containing 1.0 mg/L bromophos methyl as internal standard.

RESULTS AND DISCUSSION

Extraction Optimization. A preliminary study was conducted to select the experimental variables that should be optimized. The central composite design has been adopted for the optimization of the extraction process.[9,14,15] However, in our case the extraction of pesticides by MAE in different substrates was in routine analysis for many years, and thus it was only necessary to adjust and/or modify the previously selected

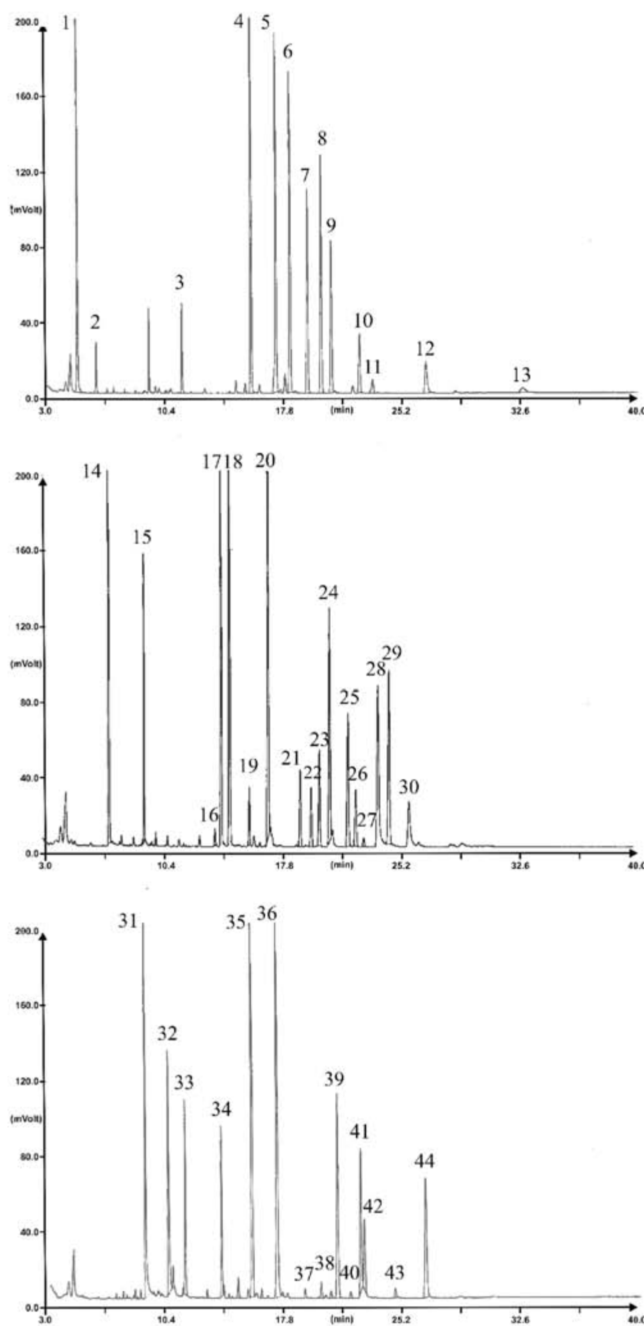

Figure 4. Chromatograms of fortified raw wool samples at 1.0 mg/kg with the proposed method by GC-FPD. Peaks: 1, trichlorfon; 2, phosphamidon; 3, acephate; 4, phorate; 5, diazinon; 6, iprobenfos; 7, tolclofos methyl; 8, pirimiphos methyl; 9, fenthion; 10, chlorfenvinphos; 11, disulfoton sulfone; 12, triazophos; 13, pyrazophos; 14, dimefox; 15, dichlorvos; 16, omethoate; 17, thionazin; 18, ethoprofos; 19, dimethoate; 20, terbufos; 21, chlorpyrifos methyl; 22, fenchlorfos; 23, malathion; 24, parathion ethyl; 25, terbufos sulfone; 26, phenthoate; 27, methidathion; 28, fenamiphos sulfone; 29, prothiofos; 30, fensulfothion; 31, methamidofos; 32, disulfoton sulfoxide; 33, mevinphos; 34, heptenophos; 35, cadusafos; 36, fenophos; 37, parathion methyl; 38, fenitrotrion; 39, chlorpyrifos ethyl; 40, bromophos methyl; 41, mecarbam; 42, quinalphos; 43, profenophos; 44, ethion.

conditions to the extraction of organophosphate pesticides from wool matrix.[11,12,16,17] Among the parameters used in MAE, the magnetron power was set to 100% (1000 W) for a 12 vessel tray because no degradation of the target solutes was observed during the rapid heating of the extraction vessels. In cases when fewer than 12 vessels are used, the application of less magnetron power should be considered. A sample weight of 1 ± 0.01 g was selected because this is the amount required for residue analytical methods based on the instrumental analytical systems applied in this study to obtain low limit of quantification (LOQ) values.[16,17] The analytical method was developed for the analysis of OP residues in raw wool containing high amounts of wool grease, a waxy material containing not only high amounts of esters, diesters, and hydroxyl-esters, formed by condensation of high molecular weight lanolin alcohols with lanolin fatty acids, but also cholesterol, triterpenoids, and free fatty acids. Preliminary experiments with 5 g sample portions showed that recovery was reduced due to the high proportion of wool grease coextracted with pesticides, which partition between the extraction solvent and the wool lipids. Thus, the selection of the solvent was a crucial factor for selective OP extraction. Among the different solvents (methanol, acetone, acetone/hexane, and acetonitrile) tested, acetonitrile was finally selected. Due to its polarity, acetonitrile can absorb the magnetron power to solubilize the OPs under study while dissolving small amounts of wool grease. The acetonitrile volume was optimized to simultaneously achieve the highest recoveries for all analytes and minimize wool grease extraction. Figure 1 shows the pesticide recoveries at 2 mg/kg using different MeCN volumes for pesticide residue extraction from wool. Mean recoveries obtained with a 30 mL solvent extraction volume were statistically significantly higher from the recoveries obtained when 20 or 50 mL of acetonitrile was used (Duncan's test, $\alpha = 0.05$). For most of the tested compounds, a volume of 20 mL yielded the lowest recoveries of the three extraction volumes assayed and the highest standard deviation (RSD > 20%). Similar results on the influence of the ratio extractant volume to solutes recovery were also previously observed in MAE-based methods.[12,19] Taking into consideration the above observations, 30 mL of acetonitrile was employed as extracting medium. The reported recoveries were obtained after the equipment had been further rinsed with 10 mL of acetonitrile.

The influence of the extraction temperature on the efficiency of the MAE was also evaluated. A series of extractions were performed at 60, 80, 100, and 120 °C. OP recovery data derived from temperature optimization experiments are shown in Figure 2. At 60 and 120 °C recoveries were in many cases unacceptably low, in the range of 38−76 and 31−107%, respectively, with RSD values higher than those obtained when the extraction was performed at either 80 or 100 °C. The temperature of 60 °C was quite low to extract OPs from raw wool. On the other hand, extraction at 120 °C was also inadequate because at this temperature extracts contained high amounts of wool grease substances. It has been reported in previous studies that elevated temperatures improve extraction efficiency as long as the solutes are not thermally labile,[12,18] but in our case at 120 °C degradation of terbufos and formation of its respective degradation product (terbufos sulfone) was observed.

No significant differences were observed for the recovery values of all analytes at either 80 or 100 °C, except for dimethoate and terbufos. Both pesticides showed mean recovery values significantly higher when the extraction was carried out at 80 °C. Figure 3 shows the comparison of the lipids extracted using Soxhlet and MAE at the temperatures assayed as discussed above, expressed as the relationship grams of MAE extract per

Table 2. Linear Range of Matrix-Matched Calibration Curves and the Respective Correlation Coefficients (r^2) as well as Limits of Detections (LOD) and Quantitation (LOQ)

pesticide	r^2	linear range (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)
acephate	0.9978	0.01−1.6		
bromophos methyl	0.9946	0.1−1.6	0.5	0.1
cadusafos	0.9973	0.01−0.8	0.2	0.01
chlorfenvinphos	0.9969	0.01−1.6	0.2	0.01
chlorpyrifos ethyl	0.9907	0.01−1.6	0.2	0.01
chlorpyrifos methyl	0.9954	0.01−1.6	0.2	0.01
diazinon	0.9925	0.01−1.6	0.2	0.01
dichlorvos	0.9979	0.01−1.6		
dimefox	0.9965	0.01−0.2	1.0	0.01
dimethoate	0.9895	0.01−1.6	0.2	0.01
disulfoton sulfone	0.9948	0.02−1.6	0.2	0.02
disulfoton sulfoxide	0.9908	0.01−1.6	0.2	0.01
ethion	0.995	0.02−1.6	0.2	0.02
ethoprofos	0.9981	0.01−0.8	0.2	0.01
fenamiphos sulfone	0.9997	0.01−1.6	1.0	0.01
fenchlorfos	0.9932	0.01−1.6	0.2	0.01
fenitrotrion	0.9832	0.2−0.8	0.5	0.2
fenophos	0.9978	0.01−0.8	0.2	0.01
fensulfothion	0.9971	0.01−1.6	0.5	0.01
fenthion	0.9971	0.01−1.6	0.2	0.01
heptenophos	0.9944	0.01−1.6	0.2	0.01
iprobenfos	0.9935	0.01−1.6	0.2	0.01
malathion	0.9967	0.01−1.6	0.2	0.01
mecarbam	0.9901	0.01−1.6	0.2	0.01
methamidofos	0.9976	0.01−0.8	0.2	0.01
methidathion	0.9891	0.1−1.6		
mevinphos	0.995	0.01−1.6	0.2	0.01
omethoate	0.9804	0.01−1.6	1.0	0.01
parathion ethyl	0.9987	0.01−1.6	0.2	0.01
parathion methyl	0.9927	0.2−0.8	0.5	0.2
phenthoate	0.9966	0.01−1.6	0.5	0.01
phorate	0.9985	0.01−1.6	0.2	0.01
phosphamidon	0.9939	0.01−1.6		
pirimiphos methyl	0.9957	0.01−1.6	0.2	0.01
profenophos	0.9932	0.1−1.6	0.5	0.1
prothiofos	0.998	0.01−1.6	0.2	0.01
pyrazophos	0.9812	0.1−1.6	1.0	0.1
quinalphos	0.9916	0.01−1.6	0.2	0.01
terbufos	0.9923	0.01−1.6	0.5	0.01
terbufos sulfone	0.9983	0.01−1.6	0.2	0.01
thionazin	0.9977	0.01−0.8	0.2	0.01
tolclofos methyl	0.9969	0.01−1.6	0.2	0.01
triazophos	0.9931	0.01−1.6	0.2	0.01
trichlorfon	0.9918	0.01−1.6	0.2	0.01

grams of Soxhlet extract. The best compromise between the minimal lipid coextraction and maximum pesticide recoveries was 80 °C, and all of the experiments were carried out at this temperature.

Chromatographic Analysis. The 44 OPs included in the analytical method were separated in three stock solutions on the basis of the retention time of each solute. Separation of the targeted solutes was done to avoid coelution of some pesticides. When positive results were found in real samples, they were confirmed through mass spectrometry. Sample chromatographic data derived from the analysis of fortified raw wool samples at 1.0 mg/kg using the proposed method by GC-FPD is shown in Figure 4. The chromatograms were clean of interferences mainly due to the selective extraction by the use of microwave energy, the removal of the coextracted wool grease by low-temperature precipitation (placing the extract at −20 °C for 10 min), and the use of a phosphorus-specific detector (FPD). Reduction of coextracted substances by low-temperature cleanup, during pesticide residue

Table 3. Mean Recoveries ($n = 5$) and Respective RSDs of Target Compounds from Fortified (at Three Levels) Raw Wool Samples

	fortification level					
	0.2 mg/kg		0.5 mg/kg		1.0 mg/kg	
pesticide	% recovery	%RSD	% recovery	%RSD	% recovery	%RSD
acephate[a]	*37*	*1*	*79*	*27*	*82*	*24*
bromophos methyl	211	11	102	4	110	12
cadusafos	85	4	88	4	97	13
chlorfenvinphos	113	3	100	5	104	8
chlorpyrifos ethyl	91	6	98	6	110	18
chlorpyrifos methyl	111	12	83	1	100	10
diazinon	108	14	93	3	96	3
dichlorvos	*43*	*9*	*59*	*6*	*66*	*13*
dimefox	46	9	42	9	71	10
dimethoate	120	15	83	9	96	9
disulfoton sulfone	115	0	104	31	111	10
disulfoton sulfoxide	80	5	105	8	99	7
ethion	115	7	99	6	101	8
ethoprophos	87	9	93	1	104	12
fenamiphos	54	8	65	19	92	5
fenchlorfos	118	14	70	15	95	3
fenitrothion	158	11	103	4	89	0
fenofos	76	5	76	4	96	6
fensulfothion	103	21	91	4	94	5
fenthion	82	6	77	3	85	6
heptenophos	71	7	86	0	95	13
iprobenfos	104	2	98	3	99	3
malathion	106	12	74	13	88	12
mecarban	82	5	98	5	103	11
methamidofos	72	5	90	6	89	11
methidathion	*195*	*38*	*73*	*21*	*71*	*24*
mevinphos	73	5	91	6	94	17
omethoate	38	17	55	6	76	18
parathion ethyl	95	7	92	3	116	15
parathion methyl	176	9	104	4	87	1
phenthoate	125	15	84	3	96	12
phorate	83	2	68	4	83	4
phosphamidon	*26*	*6*	*36*	*4*	*38*	*11*
pirimiphos methyl	104	2	100	0	101	5
profenophos	189	15	99	1	98	4
prothiofos	106	8	92	0	115	14
pyrazophos	128	27	121	36	101	8
quinalphos	85	5	99	6	99	10
terbufos	63	8	97	10	89	5
terbufos sulfone	102	10	90	2	95	1
thionazin	96	8	86	11	93	4
tolclofos methyl	103	3	95	4	101	6
triazophos	85	6	100	7	105	8
trichlorfon	73	4	94	6	99	2

[a] Compounds in italics show unacceptable recoveries and/or %RSD values with the proposed method at all three fortification levels.

analysis in different matrices, has also been previously reported in MAE[20] or other extraction techniques.[21,22]

Among the pesticides initially included in the analytical method, acephate, phosphamidon, methidathion, and dichlorvos exhibit unacceptable extraction and/or chromatographic performance. Dichlorvos was lost during the N_2 evaporation step, whereas acephate, phosphamidon, and methidathion were degraded in the injection port even though a PTV inlet was used to minimize degradation of the thermally labile compounds.

Although chromatograms were clean of interferences, pesticide quantification was conducted by matrix-matched calibration curves because after the repetitive injection of raw wool extract a signal enhancement was observed. The matrix enhancement depended on the concentration and the solute polarity. At higher fortification levels, no matrix effect was observed even after 100 sample injections with the same injector liner and precolumn. On the other hand, at the lowest fortification level a signal enhancement was observed after 10 injections. The matrix effect was more evident in the polar pesticides (dimefox, omethoate, thionazin, ethoprophos, dimethoate, methamidophos, terbufos sulfone, disulfoton sulfone, and disulfoton sulfoxide). As mentioned elsewhere,[23,24] matrix-induced signal enhancement is usually attributed to the matrix blocking of active sites in the injector liner that subsequently protect solute from thermal degradation.

Method Validation and Performance. Matrix-matched calibration curves, which were prepared with blank wool extracts, were linear in the 0.01−1.6 mg/kg range in most cases (Table 2). Pesticides that were more influenced by the matrix showed a narrower range of linearity. Another option would be to quantify a standardized matrix such as 0.1% peanut oil. Although this could eliminate the problems due to the variability of the matrices over time and between laboratories, the use of blank wool extracts is the most realistic situation. The mean recovery values of solutes from raw wool spiked at 0.2, 0.5, and 1.0 mg/kg and processed by the proposed method are shown in Table 3. The accuracy and precision of the method, as depicted by the percent mean recovery values and the respective RSDs, were acceptable according to the quality standard established by the SANCO guidelines 10684/2009[25] because recovery values were between 70 and 120% and their respective RSDs were <20% for all solutes, at least at one fortification level, except for acephate, dichlorvos, methidathion, and phosphamidon. Their respective mean recovery values were <40% with RSDs of >20%. LOD values, determined as the minimum concentration of each analyte in the wool matrix providing a signal-to-noise ratio of at least 3, were determined to range from 0.01 to 0.2 mg/kg. LOQ values, determined as the lowest concentration of a given compound with recoveries of >70% and a RSD of <20%, were in the range of 0.2−1 mg/kg (Table 2).

Compared to Soxhlet extraction the MAE is faster. It allows the simultaneous processing of 12 samples in 20 min overall time, whereas Soxhlet extraction takes 3 h. It has less solvent consumption: only 30 mL is needed against 300 mL employed in Soxhlet extraction. MAE showed recoveries as good as those obtained with Soxhlet extraction and the traditional GPC method at the different levels compared for the representative OPs shown in Table 4. If sample homogeneity is a concern, the analysis can be carried out by triplicate averaging the results and evaluating the RSD. In this situation, still four different samples can be extracted in a single MAE run. Nevertheless, the results of pesticide residues in real wool samples obtained using 1 g sample were similar to those obtained using 10 g. Both extract residues were weighed, finding that MAE presents less coextractives

Table 4. Comparison of Soxhlet and Microwave-Assisted Extraction for Some Representative Pesticides at Different Levels

	1.0 mg/kg				0.5 mg/kg				0.25 mg/kg			
	MAE		Soxhlet		MAE		Soxhlet		MAE		Soxhlet	
pesticide	% recovery	%RSD	% recovery	%RSD	% recovery	%RSD	% recovery	%RSD	% recovery	%RSD	% recovery	%RSD
dichlorvos	57	8	33	45	67	8	62	24	54	7	42	30
diazinon	105	2	99	4	104	1	111	6	94	3	91	5
pirimiphos methyl	100	1	100	4	102	1	109	4	96	1	91	3
chlorpyrifos ethyl	99	0.4	101	4	102	0.3	110	3	97	2	95	4
chlorfenvinphos	95	9	100	8	96	2	105	3	105	8	92	5
ethion	86	8	101	4	93	1	114	2	108	3	116	5

Table 5. Pesticides Found in Real Wool Samples

	ethion (mg/kg)		diazinon (mg/kg)	
sample	MAE	Soxhlet	MAE	Soxhlet
A	118	91	1.0	1.1
B	0.35	0.45	<LOQ	<LOQ
C	<LOQ	<LOQ	29	24
D	<LOQ	<LOQ	nd	nd

(Figure 3). This was also visualized in the respective chromatograms.

Analysis of Real Raw Wool Samples. Real raw wool samples that were analyzed using the standard Soxhlet/GPC procedure during the routine surveillance program for the detection of pesticide residue levels in raw wool in Uruguay were also analyzed using the MAE procedure. Diazinon and ethion positives were confirmed, and the concentration values found for both methods were similar (Table 5). Most of the samples contained both of them. Although both methods extracted quantitatively the pesticide residues from real wool samples, the main advantage of the MAE method is that no further sample handling after the extraction solution had been frozen was needed to obtain results that compare well with the routine method. Confirmation of positive results was made according to SANCO guidelines 10684/2009 through GC-MS/MS.

The proposed method combining MAE, low-temperature cleanup, and gas chromatographic analysis is a simple, efficient, and rapid approach for the accurate determination of commonly found OPs in raw wool samples.

SAFETY

Due to the handling of a MAE system, particular attention should be given to the purity of the wool samples (the samples should not contain metallic contaminants that could interact with magnetron power).

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