Bull. Environm. Contam. Toxicol. 30, 110–116 (1983)

A Modification of the Luke Multiresidue Procedure for Low Moisture, Nonfatty Products

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The analysis of dry products (<10% water) for pesticide residues has always been more difficult than in high moisture products. Removal of pesticides from this sample matrix is harder due to the concentration of plant material and/or lack of moisture. Low assay values for incurred residues were obtained when the Mills procedure (MILLS et al. 1963) was employed. Additionally, MUMMA et al.(1966) reported efficient extractions only with a chlorform/methanol Soxhlet extraction. On the other hand, BERTUZZI et al.(1967) investigated several solvent mixtures and found similar extraction efficiencies with a single blending of a 35% water/acetonitrile solvent. This procedure became the recommended procedure for dry products (PESTICIDE ANALYTICAL MANUAL VOL.1 1982).

During the early 1970's, a new highly polar pesticide, methamidophos (Monitor), was introduced and found not to be detectable by the recommended procedures. LUKE et al.(1975) published an acetone extraction procedure that recovered both the highly polar residues and the non-polar ones as well. Therefore it was reasoned, that for dry products, a corresponding water/acetone solvent could be used to detect the same wide polarity range of residues. An extraction modification using 350 mL of 35% water/acetone was used to successfully recover residues ranging in polarity from methamidophos to p,p'-DDE, and comparative results agreed well with the previous work by BERTUZZI et al.(1967).

MATERIALS AND METHODS

Materials. The reagents and apparatus used were as described in LUKE et al.(1975) except for the following: activated carbon (Darco G60, MCB, E. Rutherford NJ), magnesium oxide (200 mesh adsorptive grade, MCB, E. Rutherford NJ), Celite 545 (Johns-Manville, Lompoc, CA). The gas chromatograph(GC) was a Tracor model 560 equipped with a flame photometric detector(FPD-P) and a Hall model 700A electrolytic conductivity detector(HECD) in both the halogen and

0007-4861/83/0030-0110 $01.40

nitrogen modes. Columns used were a 75 cm x 2 mm id and 120 cm x 2 mm id glass coiled columns packed with either 2% stablized DEGS on 80-100 mesh Chromosorb W AW (Analabs, Inc. New Haven, CT) or 3% OV-17 on 80-100 mesh Chromosorb W HP (Supelco, Bellefonte, PA). Conditions used were in accordance with those published by LUKE et al. (1981). Analytical standards of acephate and methamidophos were obtained from the Chevron Chemical Company; dimethoate was obtained from American Cyanamid; chlorpyrifos was obtained from Dow Chemical USA; carbaryl, chlorthiophos, p,p'-DDE, dieldrin, endrin, methyl parathion, and primiphos methyl were obtained from the Enviromental Protection Agency.

Procedure. Grind sample containing less than 10% fat or oil to pass 20 mesh. Weigh 15.0 g into a 1-qt blender cup, and add 350 mL of 35% water/acetone [350 mL distilled water diluted to 1 L with acetone]. Blend at high speed for two min, and filter with pre-washed filter paper (LUKE et al. 1981). Transfer 80 mL of the filtrate to a 1-L separatory funnel containing 100 mL of dichloromethane. Add 100 mL petroleum ether [or 100 mL acetone plus ca. 7 g NaCl if methamidophos is suspected], and shake vigorously for 1 min. Let the layers separate, and transfer the aqueous layer (lower layer) to a second 1-L separatory funnel. Dry the organic layer (upper layer) through sodium sulfate [prepared in accordance with LUKE et al. (1981)], and collect in a Kuderna-Danish(K-D) concentrator with a Mills tube attached. Add ca. 7 g NaCl to the second separatory funnel [no salt added if added to first separatory funnel], and shake vigorously with 2 x 100 mL portions of dichloromethane for 1 min; dry each organic layer (lower layer) by passing it through sodium sulfate, and combining the layers in the K-D. Discard the aqueous layer. Rinse the sodium sulfate with 50 mL dichloromethane. Mix the combined layers, and concentrate on a steam bath to 2 mL. Remove the dichloromethane as per LUKE et al. (1981), and inject onto a GC or cleanup as follows.

Carbon Cleanup. For highly polar residues such as methamidophos, prepare a 25 x 200 mm glass column with a coarse porosity sintered-glass frit with ca. 2.5 cm Celite followed by 6 g carbon mixture [1 part carbon, 4 parts Celite, and 2 parts magnesium oxide thoroughly mixed] and topped with large plug of glass wool. Tamp down column firmly, and add ca. 25 mL dichloromethane to the column, and force the solvent with air pressure until the top of the solvent reaches the top of the packed column bed. Discard this wash. Place a K-D concentrator with a Mills tube attached below the column. Transfer the sample quantitatively to the column with small portions of dichloromethane, elute the solvent as before. Elute 200 mL of a 2:1 acetone/dichloromethane mixture through the column with air pressure. Mix the contents of the K-D concentrator, and concentrate as per the previous section.

Florisil Cleanup. For the non-polar or moderately polar residues such as carbaryl and p,p'-DDE, remove the acetone by concentrating with 2 x 100 mL portions of petroleum ether to 2 mL. Dilute the extract to 100 mL petroleum ether and elute through florisil in accordance with sec. 211.14d of the PESTICIDE ANALYICAL MANUAL VOL.1 (1982). Include a 200 mL 100% diethyl ether elution.

RESULTS AND DISCUSSION

Evaluation of residues for regulatory purposes can normally only be performed by analyzing large numbers of crops with methodology that detects a wide polarity range of residues. LUKE et al.(1981) succeeded in this goal by utilizing an acetone extracting solvent with minimal cleanup and GC detectors, such as the FPD-P and the HECD which were not greatly affected by coextractives. Application of this principle to low moisture products required an extraction modification to overcome two problems: lack of moisture, and chromatographic problems due to coextractives. BERTUZZI et al.(1967) reported similar extraction efficiencies when a 35% water/acetonitrile extraction was compared to the Mumma Soxhlet extraction (MUMMA et al. 1966). This improved efficiency was thought to be due to the deactivation of the cellulose active sites by the water (LUKE & MASUMOTO 1982). The choice of the 35% water/acetone extracting solvent was based on the experimental results of BERTUZZI et al.(1967). Comparison values of incurred residues (illustrated in Table 1) with those found by the Bertuzzi procedure (BERTUZZI et al. 1967) indicated similar extraction efficiences. Recovery values of pesticide standards (listed in Table 2) and assay values of residue extracted from various crops (reported in Table 3) obtained by this water/acetone method illustrated the method's ability to determine a wide polarity range of residues. The Mills procedure (MILLS et al. 1963) from which the Bertuzzi procedure (BERTUZZI et al. 1967) is derived failed to detect the highly polar residues (LUKE et al. 1975).

Table 1. Comparison of Assay Values of Incurred Residues with the Bertuzzi Procedure.

Product	Residue	Water/acetone Procedure (ppm)	Bertuzzi Procedure (ppm)
Mung Beans	Endrin	0.14	0.14
Mung Beans	Endrin	0.12	0.12
Mung Beans	Endrin	0.12	0.13
Mung Beans	Endrin	0.076	0.086
Mung Beans	Endrin	0.12	0.15
Mung Beans	Primiphos-methyl	0.28	0.32

Table 2. Pecentage Recoveries of Pesticide Standards Through the Water/acetone Method.

Product	Standard	Fortification Level(ppm)	% Recovery	
			Before Cleanup	After Cleanup
Mung Beans	Acephate	0.60	116	109^{*}
Mung Beans	Methamidophos	0.39	101	101^{*}
Mung Beans	Dimethoate	0.996	109	100^{*}
Dried Corn	Carbaryl	9.99	95	100^{+}
Dried Corn	Chlorpyifos	0.48	103	87^{+}
Dried Corn	Chlorthiophos	0.48	106	85^{+}
Dried Corn	Methyl-parathion	0.68	112	93^{+}
Dried Corn	p,p'-DDE	0.142	---	94^{+}
Dried Corn	Dieldrin	0.146	92	106^{+}
Dried Corn	Endrin	0.141	85	85^{+}
Mung Beans	Primiphos-methyl	0.489	---	104^{+}

* Carbon cleanup
\+ Florisil cleanup

Chromatographic difficulties such as an unstable baseline and extraneous peaks caused by coextractives from the large amounts of dehydrated plant material were alleviated in part by reducing the sample size down to 15 g from the 25 g reported by BERTUZZI et al. (1967). This modification coupled with the use of the FPD-P and HECD permitted most products to be analyzed without cleanup. To obtain adequate sensitivity (0.01 ppm endrin) however, the extract had to be concentrated to a volume of 2 mL. Cleanup was employed only in the cases where the lower levels (<0.1 ppm) were measured.

Table 3. Residues Obtained from Analysis of Various Products by the Water/acetone Method.

Product	Residue	Level Found(ppm)
Dried Bell Peppers	p,p'-DDE	0.05
Dried Bell Peppers	Methamidophos	2.9
	Acephate	1.1
Dried Bell Peppers	Methamidophos	0.4
	Parathion	0.8
	Endosulfan	0.2
Dried Bell Peppers	Methamidophos	2.7
	BHC	0.3
Dried Celery	Diazinon	0.1
	Methamidophos	0.4

Petroleum ether used in the first partitioning reduced the amount of polar coextractives (LUKE et al. 1975). However, the recovery of methamidophos was reduced to ca. 75% and emulsions sometimes occurred in products such as dried peppers. Substitution of acetone for the petroleum ether eliminated these problems, but the increased coextractives caused an unstable baseline on the chromatogram at lower levels of residues.

Recoveries of pesticide standards through the cleanup procedures are presented in Table 1. Although the data are limited for the carbon cleanup, most polar residues have successfully been eluted. Adsorption properties of carbon were generalized in the following manner (SNYDER 1968):

1. Relative sample adsorption was governed by molecular weight. The larger the molecular weight the more it was adsorbed.

2. Aromatics were preferentially adsorbed over aliphatics.

3. Nonionic compounds were preferentially adsorbed over the ionic and charged compounds.

STORHERR et al.(1971) had developed the carbon mixture as a cleanup prior to the determination of organophosphorous residues. Considerable time was required to evaporate the acetonitrile/benzene elution solvent during which residues could be lost (LUKE et al. 1975). Acetone/dichloromethane elution solvent had the distinct advantage of being able to be concentrated down to 2 mL in ca. 30 min at a low temperature. Aromatic compounds have been recovered through the Storherr carbon cleanup (STORHERR et al. 1971), but they were either totally or partially adsorbed with the acetone/dichloromethane procedure. However, the aromatics were eluted through florisil. Thus, this disadvantage was more than offset by the acetone/dichloromethane system's ability to elute the highly polar residues. One pesticide, acephate, did not elute from the carbon mixture. Removal of the magnesium oxide from the carbon mixture eliminated the problem, but produced a white residue upon concentration below 2 mL which had no effect upon the GC. Acidification of the commerial charcoal used to prepare the carbon mixture was found to be unnecessary. Norite S.G. Extra and Darco G60 were used and produced no differences.

After the acetone was removed from the sample extract, no problems were encounted when florisil was used in accordance with sec. 211.14d of the PESTICIDE ANALYTICAL MANUAL VOL 1 (1982). Inclusion of a 100% diethyl ether eluate allowed the cleanup of such pesticides as carbaryl and azinphos methyl. Acetone did affect the residue elution pattern by causing the residues to elute earlier. This observation was employed to decrease analysis time when the electron capture detector was used (LUKE et al. 1975).

Acknowledgments. We thank M. Bennett and A. Oken for their recovery work and Dr. Thomas Cairns for assistance in preparing this manuscript.

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Accepted November 3, 1982