Bull. Environ. Contam. Toxicol. (1984) 32:651–656

Environmental Contamination and Toxicology

A Rapid Analysis for Pesticides in Milk and Oilseeds

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Extraction and cleanup of pesticide residues from food containing fats or oils has been a challenge to the residue analyst. Use of the recommended procedures in section 211 of the Pesticide Analytical Manual (1982) require that the fats first be isolated from the product followed by an extraction of the residues from the fats. Such attempts usually result in the final extracts containing enough fat to interfere with the gas chromatographic (GC) determination. Additional cleanups are required which are not always successful at recovering the full amount of the pesticide residue. In addition, the analytical time required for both the fat isolation and cleanups are extensive, limiting the number of samples that can be analyzed.

Several attempts have been made to improve this prevailing situation. Eidelman (1962) developed a cleanup using dimethyl sulfoxide after the fat was isolated which took 6 hr per sample and could be dangerous under certain conditions. McCulley & McKinney (1964) reported a lengthy low temperature fat precipitation method unsuited for routine use. Onley & Bertuzzi (1966) demonstrated the use of calcium stearate to coagulate fatty constituents. This method, however, involved the use of an unstable extraction mixture and a time consumming double extraction. Rogers (1972) investigated the use of Micro Cel-E, an adsorbant, to remove large quantities (30 g) of extracted fat from residues. Although the method exhibits a vast improvement in sensitivity over the recommended procedures, the final extract still contained unacceptable amounts of coextracted fats. Griffith & Craun (1974) employed gel permeation chromatography to remove fats from extracts. Involvement of this type of special equipment and expertise quickly eliminated this method from routine use because of economic reasons.

Since most of the above methods required a time consumming fat isolation and/or special equipment, other means of analysis were investigated in this laboratory. Aluminium oxide long used in the analysis of oils looked attractive as a promising candidate for absorbing oils. A single extraction using aluminium oxide

was attempted and proved successful at removing fats from residues. In this paper, a procedure for milk and oilseeds is presented that eliminates a fat isolation step and provides an extract acceptable for GC determination after a florisil cleanup.

MATERIALS AND METHODS

All solvents, reagents and equipment are as specified in section 211 of volume 1 of the Pesticide Analytical Manual (1982) except for the following: aluminium oxide (#A540, Fisher Scientific Co., Fair Lawn, NJ). All pesticide analytical standards were obtained from the Environmental Protection Agency. The gas chromatograph was a Tracor Model 560 equipped with either an electron capture detector, a Hall Model 700A electrolytic conductivity detector (halogen mode), or a flame photometric detector. Columns employed were 120 mm by 2 mm id glass coil columns packed with either 3% OV-17 on 80-100 mesh Chromosorb W HP (Supelco, Bellefonte, PA), 2% DEGS on 80-100 mesh Chromosorb W AW (Analabs Inc., New Haven, CT), or 2% OV-101 on 100-120 mesh Chromosorb W HP (Hewlett-Packard, Avondale, PA).

For milk samples containg 4% fats or less, weigh 50 g milk into a blender cup. Add 20 g aluminium oxide, 25 mL distilled water and 280 mL acetonitrile. [If significant GC interferences are present in the aluminum oxide, then wash with 200 mL ethanol (95%) followed by 200 mL hexane and dry on a steam bath]. Blend 2 min at high speed, wait for the solids to settle and filter the supernatant liquid with suction through sharkskin filter paper (previously washed with 100 mL acetonitrile). Measure 250 mL of the filtrate and transfer to a 1 L separatory funnel. Add 100 mL petroleum ether and shake for 30 sec. Add 10 mL saturated NaCl solution and 500 mL distilled water. Shake for 1 min and allow the layers to separate. Transfer the lower aqueous layer to a second 1 L separtory funnel containg 100 mL petroleum ether and shake for 1 min. Once again, allow sufficient time for the layers to separate and then discard the lower aqueous layer. Then combine the remaining layer with the layer in the first separtory funnel and wash the combined layers with 2 x 100 mL portions of distilled water. Dry the petroleum ether extract through 1" layer of anhydrous, granular $NaSO_4$ [if GC interferences are present in the $NaSO_4$, then heat to 600°C for 1 hr] (Luke et al. 1981) and collect in a Kuderna-Danish (K-D) concentrator with a Mills tube attached. Concentrate on a steam bath to 10 mL. Elute the extract on a Florisil column as stated in section 211 of Volume 1 of the Pesticide Analytical Manual (1982). Inject the eluate onto a GC. Use the factor 250/344 to calculate amount of sample in the final extract.

For oilseeds: grind product through a meat grinder equipped with a 2 mm screen at least 3 times. Weigh a portion of sample containing not greater than 2 g fat into a blender. Add 20 g of aluminium oxide and 350 mL 20% water in acetonitrile (add 200 mL distilled water to a graduated cyclinder and dilute to 1 L with acetontrile). Continue with the procedure for milk beginning with "Blend 2 min....". Use the factor 250/350 to calculate the amount of sample in the final extract.

RESULTS AND DISCUSSION

Successsful utilization of any fatty product procedure for residue analysis depends on its ability to extract the non-polar residues from the fat globule (Berosa & Bowman 1966) and its capacity to analyze many samples in a short period of time to properly evaluate residue use or potential misuse. Extraction with aluminium oxide appeared to satisfy both criteria for milk and oilseeds.

Recovery data for pesticide standards added to milk (Table 1) demonstrated the proposed procedure's ability to determine the same polarity range as the recommended procedure. Similar extraction efficiencies between the recommended method and the proposed procedure are illustrated by the comparative values of incurred residues found for various products compiled in Table 2. Procedure parameters, which can be considered a modification of the Bertuzzi method (1967) for low moisture products were based on the data listed in Table 3. Results illustrate that extraction of residues from the fat is dependent both on the amount of absorbant and content of water present in the solvent. Increasing the surface area of the absorbant increased the extraction efficiencies of residues from the fat. These experimental facts indicate that the fat must be coated in a thin layer around each absorbant particle and that the thinner the layer of fat, the more complete the residue extraction. Additionally, if the solvent contains too much water, the fat layer is not pentetrated to permit residue extraction. On the other hand, too little water in the solvent causes extensive coextraction of fat and a final extract unsuitable for GC determination. The compromise of 20% water in acetonitrile and 20 g of aluminium oxide, along with a maximum of 2 g fat are found to be optimal. Since milk containes water itself, the added water is adjusted so that the total amount of water is 20% of the total volume of solvent added. Oilseeds, on the other hand, contain so little moisture that adjustment is not required.

Elimination of the fat isolation step as well as a single step extraction reduces the time of analysis to about 30 min per sample from the usual 4-5 hr normally required if the recommended procedure is followed. Further reduction in analysis time is

Table 1. Percentage recoveries of pesticide standards from milk through the acetonitrile/aluminium oxide procedure.

Standard	Fortification Level (ppm)	Percentage Recovery
Dieldrin	0.16	85
p,p'-DDE	0.20	92
Heptachlor epoxide	0.21	92
Diazinon	0.25	81
Methyl parathion	0.10	80
Ethion	0.20	92
HCB	0.10	83
a-BHC	0.10	99
Aldrin	0.41	90
Endrin	0.55	94

Table 2. Comparative assay values of incurred residues with the PAM procedure.

Product	Residue	Acetonitrile Aluminium oxide Procedure (ppm)	PAM Procedure (ppm)
Sesame seeds	Endrin	0.06	0.05
	Endrin	0.08	0.09
	Endrin	0.08	0.10
	Endrin	0.04	0.06
Milk	p,p'-DDE	0.06	0.06
Peanuts	Endrin	0.11	0.11
	Primiphos-methyl	0.51	0.54

achieved by using air pressure on the Florisil column thereby decreasing the elution time. With this new analytical approach, it is possible to analyze about 20 samples per analyst per day.

Cleanup by Florisil elution produced extracts (6% and 15% diethyl ether/petroleum ether) that contained no coextracted fat. The more polar elutions of 50% diethyl ether/petroleum ether and 100% diethyl ether contained no more than about 5% of the total fat in the sample portion analyzed and caused no analytical problems

Table 3. Aluminium oxide/water/fat ratio affect on the amount of fat residue and pesticiude standard recovered.

Run No.	Water in CH_3CN	Al_2O_3 (%)	Fat Sample (g)	Fat Residue (g)	Pesticide Recovery (%)	
					p,p'-DDE	Dieldrin
1	35	20	3	<.1	50	80
2	10	20	3	0.6	*	*
3	35	20	2	<.1	69	83
4	35	10	2	<.1	61	74
5	20	10	2	0.1	84	95
6	20	20	2	<.1	93	96
7	20	30	2	<.1	89	93
8	20	40	2	<.1	93	85

* Not analyzed due to interferences from fat.

unless low levels of incurred residues were encountered (< 0.1 ppm) in the case of oilseeds.

In conclusion, the limited data presented for milk and oilseeds clearly demonstrates an alternate and rapid procedure for fatty products which produces a final extract that requires no further cleanup. Efforts to expand this procedure to other fatty products as well as to expand the polarity range of pesticides to include the highly polar residues such as methamidophos are currently under investigation.

Acknowledgements. The authors wish to express their thanks to J. Tsao for her recovery data and Dr. T. Cairns for his constant encouragement and assistance in preparing this manuscript.

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Received October 16, 1983; accepted November 16, 1983