Column Extraction of Pesticides from Fish, Fish Food and Mud

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Introduction

Many methods or variations of methods are used for the extraction of pesticides from nonliquid samples for residue analyses. Probably the three most common methods are extraction by Soxhlet (1,2,7), blending techniques (1,4,5,6), and shaking the ground sample with the extracting solvent (3). Each of these methods has significant disadvantages.

Soxhlet extraction is time consuming and requires relatively expensive, fragile equipment. Blending also requires expensive equipment and is hindered by formation of emulsion which necessitates separate filtering and drying steps. In addition, the use of organic solvents near an electric motor is a fire hazard. Two or more extractions are needed for good recovery by the shaking method, and the extract must be subsequently filtered and dried.

This paper reports a new column extraction procedure that is efficient, fast and inexpensive for separating pesticides from fish, fish food, and mud samples. Extraction, filtration, and drying can be combined in one step. We have also used this column method on soil and whole blood with success, however, sufficient data is not available to report these applications.

Materials and Equipment

1. Sodium sulfate, anhydrous granular.

2. Florisil, 60-80 mesh, activated for 2 hours at 650° C and deactivated by the addition of deionized water 5% H_2O (v)/95% Florisil (w).

3. Chromatography columns (20 x 400 mm), with integral reservoir (Figure 1).

4. Acetonitrile, pesticide grade, saturated with redistilled hexane.

5. Redistilled hexane saturated with acetonitrile.

6. Redistilled cyclohexane, diethyl ether, toluene, petroleum ether and iso-octane.

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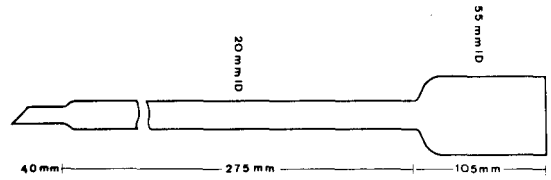

Figure 1. Extraction Column.

7. Five and ten-percent diethyl ether in petroleum ether.

8. One-percent solution of reagent grade H_3PO_4 in redistilled methanol.

9. ^{14}C-labeled pesticides; 2,4-D, p,p'-DDT, dieldrin, endrin, methoxychlor and simazine.

10. Scintillation fluor; 8.5 g Beckman Fluorally dry mix dissolved in 1 liter toluene (A.R. grade).

11. Liquid scintillation counter, Beckman model 200 L.

12. Model 803 Packard gas chromatograph with ^{63}Ni electron capture detector. Column: 2 mm I.D. x 180 cm borosilicate glass column packed with 80-100 mesh Corning GLC-110 glass beads coated with 0.3-percent (w/w) OV-7. The column was operated at 150° C, the detector at 235° C, and the nitrogen flow rate was maintained at 30 ml/min.

Procedure

Preparation of the sample: Each fish sample was prepared according to the procedure reported by Benville and Tindle (8). By this method, frozen fish were cut in small pieces, placed in a blender and ground with an equal weight of dry ice until a homogeneous mixture was obtained. The mixture was then poured into a beaker or loosely sealed plastic bag and placed in a freezer at -15° C for 8-12 hours. A 20-g sample of the free flowing, frozen sample was placed in a 250-ml beaker and spiked with a ^{14}C-labeled or a non-labeled pesticide. Eighty grams of anhydrous granular Na_2SO_4 was added and the mixture was stirred occasionally with a glass rod until the sample was dry.

With mud or fish food, a 20-g sample was blended with 80-g of anhydrous Na_2SO_4 until a free flowing dry mixture was obtained. Even with mud, which contains 30-40 percent moisture, a sample can be dried in a short time with anhydrous Na_2SO_4.

Extraction: The extraction column was prepared by inserting a small amount of glass wool in the bottom of a 20 x 400 mm chromatography column. Two grams of anhydrous Na_2SO_4 were poured into the column and then the sample was added. The sample was lightly

TABLE 1

Pesticide recovery data from spiked fish, fish food and mud

Pesticide	Sample type[1]	Pesticide added (ug)	Extracting solvent	Recovery[2] (percent)	Range (percent)
2,4-D	fish	2.00	1% H_3PO_4 in CH_3OH	99	± 1
2,4-D	fish	4.00	same	100	± 0
p,p'-DDT	fish	0.20	cyclohexane	100	± 0
dieldrin	fish	0.20	cyclohexane	96	± 1
endrin	fish	0.94	cyclohexane	96	± 2
methoxychlor	fish	0.9	cyclohexane	97	± 3
dieldrin	fish food	0.20	cyclohexane	96	± 2
p,p'-DDT	fish food	0.30	cyclohexane	100	± 0
simazine	fish	0.1	diethyl ether	96	± 3
simazine	fish	0.4	diethyl ether	95	± 2
simazine	fish	2.0	diethyl ether	98	± 5
parathion[3]	fish	16.0	10% ether in petroleum ether	100	± 0
parathion[3]	fish	160.0	same	100	± 0
simazine	mud	6.0	diethyl ether	83	± 7

[1] Sample weight was 20 g in all cases.
[2] Each value represents the mean of at least three observations.
[3] Analyzed by GLC.

TABLE 2

Comparative extraction efficiency of gizzard shad tissue (Tennessee River) by column extraction and the blending method.

	Column extraction		Blender extraction		
Pesticide	Mean conc.[1] (µg/g)	S.E.	Mean conc.[1] (µg/g)	S.E.	t[2]
p,p'-DDE	0.28	0.003	0.15	0.009	13.8
p,p-DDD	0.65	0.012	0.33	0.046	6.8
p,p'-DDT	0.22	0.011	0.17	0.010	3.4
Aroclor 1254	1.75	0.029	1.09	0.092	6.9
Aroclor 1260	3.97	0.053	1.93	0.288	7.0

[1] Means were calculated from the results of three independent analysis by each extraction method.
[2] Fisher t (P = 0.05) = 3.18. For a 95% confidence level.

packed by tamping with a glass rod. Tightness of packing and sample texture determine the solvent flow rate during extraction. The sample beaker and wall of the column reservoir were rinsed with approximately 10 ml of the extracting solvent (Table 1) and allowed to flow into the sample. An additional 190 ml of extracting solvent was then added and the effluent collected in a 400 ml beaker. The flow rate was usually 3-6 ml/min. The extract was concentrated by evaporation to ca. 3 ml on a warm hot plate in a fume hood and transferred to a culture tube by rinsing with iso-octane and made up to an appropriate volume when ^{14}C-labeled pesticides were used. When nonradioactive samples were used the samples were transferred directly to the appropriate container used in the clean-up operation.

The chromatography columns were cleaned after extraction by first soaking in warm water for 15 minutes and then pushing the sample out with a metal rod.

Sample clean-up and analysis: Nonradioactive samples were cleaned up by diluting the sample extract to 30-ml with hexane saturated with acetonitrile and partitioning from the hexane with two-30 ml portions of acetonitrile saturated with hexane. The acetonitrile was evaporated to ca. 5 ml on a hot plate in a fume hood. Ten milliliters of toluene were added, and the sample was evaporated to ca. 2 ml. The sample was transferred with hexane to a pre-rinsed 20 x 100 mm column of Florisil and eluted with 125 ml of 10-percent diethyl ether in petroleum ether. Each sample was collected in a 250-ml beaker and concentrated to 10 ml before injecting it into the gas chromatograph.

No cleanup was used with ^{14}C-labeled pesticides. The extract was concentrated to 10 ml, and a 1-ml aliquot was placed in a liquid scintillation counting vial. Ten milliliters of counting fluor were added, and the mixture was counted in a Beckman scintillation unit. Counts were corrected for background and quench. Observed counts were compared with the expected value to determine the recovery from the column extraction.

Comparative method: Two fish of approximately 450 g each, a gizzard shad and a northern pike were ground separately by the dry ice procedure. Six 20 g samples were weighed out from each ground fish and 80 g of anhydrous Na_2SO_4 was added to each sample. With each fish, three samples were extracted with 200 ml 5% diethyl ether in petroleum ether by column extraction and three with Sorvall (R) blender. Three extractions with 67 ml of 5% diethyl ether in petroleum ether were done on each of the blender samples. The three extracts were then combined and filtered.

Gizzard shad extracts from both the column extraction and the blender method were cleaned up by gel permeation (9) to prevent high PCB losses during cleanup. PCBs were then separated from DDT and metabolites by column chromatograph (10). The northern pike samples were cleaned up by the partition-Florisil method described earlier. Pesticide concentrations from both samples were determined by gas chromatography (Tables 2 and 3).

TABLE 3

Comparative extraction efficiency of northern pike tissue (South Dakota) by column extraction and the blending method.

Pesticide	Column extraction Mean conc.[1] (µg/g)	S.E.	Blender extraction Mean conc.[1] (µg/g)	S.E.	t[2]
p,p'-DDE	0.0076	0.00021	0.0064	0.00039	2.8
p,p'-DDD	0.0042	0.00002	0.0033	0.00012	7.6
p,p'-DDT	0.0086	0.00040	0.0078	0.00021	1.8

[1] Means were calculated from the results of three independent analysis by each extraction method.

[2] Fisher t (P = 0.05) = 3.18. For a 95% confidence level.

Results and Discussion

Recoveries of organochlorine insecticides from spiked fish and fish food ranged from 96-100 percent (Table 1). Recovery precision was good, generally with less than 3-percent variation between successive samples.

Parathion, an organophosphorus insecticide, was quantitatively recovered from fish samples when a mixture of ten-percent diethyl ether in petroleum ether was used as the extracting solvent.

Simazine and 2,4-D (dimethylamine salt) were extracted from fish using the solvents listed in Table 1. Recoveries for these compounds ranged from 95 to 99 percent with this method. These compounds are often difficult to extract efficiently from fish by blending or Soxhlet method. Extraction of simazine-spiked mud yielded 83-percent recovery.

With nonspiked native fish, column extraction was superior to the blending method in recovering DDT metabolites and PCBs in two species of fish. From the gizzard shad the column method extracted twice as much DDE and DDD (Table 2 and 3). The standard error (S.E.) between replicate samples for each method was less for the column extraction procedure except for two calculations in which the S.E. was very low for both methods. The calculated Fisher's t-test (Tables 2 and 3) was greater than 3.18 (P = 0.05) for the mean difference in all but two cases, indicating a significant difference in the two methods.

We believe that column extraction is an efficient method for extracting pesticide from several types of samples. Few modifications are required in processing the various types of samples. The similarities in procedures are highly advantageous in the operation of routine analytical programs. This method should be usable at low temperatures if desired, to minimize possible changes or losses of extracted compounds.

The high extraction efficiency of this method coupled with small expense suggests that this method should find wide application in pesticide residue analysis.

References

1. ARCHER, T. E., and CROSBY, D. G., Bull. Environ. Contam. Toxicol., 2, 191 (1967).

2. COLE, H. D., BARRY, D., FREAR, D. E. H., and BRADFORD, A., Bull. Environ. Contam. Toxicol., 2, 127 (1967).

3. KIRKLAND, J., and PEASE, H. L., J. Agr. Food Chem., 15, 187 (1967).

4. ANDERSON, R. J., THORNTON, J. S., ANDERSON, C. A., and KATAGUE, D. B., J. Agr. Food Chem., 14, 619 (1966).

5. MILLS, P. P., ONLEY, J. H., and GAITHER, R. R., J. Ass. Offic. Agr. Chem., 46, 186 (1963).

6. ONLEY, J. H., and BERTUZZI, P. F., J. Ass. Offic. Agr. Chem., 49, 370 (1966).

7. TINDLE, R. C., GEHRKE, C. W., and AUE, W. A., J. Ass. Offic. Anal. Chem., 51, 682 (1968).

8. BENVILLE, P. E., and TINDLE, R. C. J. Agr. Food Chem., 18, 5 (1970).

9. STALLING, D. L., TINDLE, R. T., and JOHNSON, J. L. "Pesticide Cleanup by Gel Permeation Chromatography." Presented 161st National ACS Meeting, Los Angeles, California, March 28-April 2, 1971.

10. ARMOUR, J., and BURKE, J., J. Ass. Offic. Anal. Chem., 53, 761 (1970).