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A Partitioning Cleanup Technique Using Minimum Quantity of Hexane

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Insecticides are less soluble in hexane than in other organic solvents like acetone, acetonitrile, chloroform, dichloromethane etc., which are used in residue analysis. Plant pigments are more soluble in hexane and hence hexane is used sometimes in the cleanup of samples, and is discarded after partitioning. For estimating the residues of monocrotophos, which is highly soluble in water, a single step cleanup of partitioning the insecticide from hexane to water is sufficient (Urs and Rao 1980). For carbofuran, which is moderately soluble in water, the cleanup is done by partitioning acetonitrile extract with hexane (Cook 1973). In our laboratory, dissolving the dried dichloromethane extract of the samples in 50 ml water and transferring the pigments to 0.5 ml hexane was found sufficient to give good recovery of carbofuran, when an aliquot of the aqueous solution was analysed by colorimetry using cholinesterase inhibition technique. When the same cleanup was tried for the recovery of other insecticides having low solubility in water, the recovery was not consistent.

It was found, that when a plant extract was dissolved in minimum quantity of acetone: hexane solution, addition of a large quantity of water was sufficient to separate the pigments along with hexane. The reason is that hexane is less soluble in acetone than water. This hexane layer could be separated by centrifuging. The same procedure was adapted here also, and for this, the sample was first extracted with dichloromethane, evaporated to dryness in a tube, dissolved in 1 ml acetone, 0.1 ml hexane and 9 ml water. The tube was shaken vigorously, centrifuged and filtered to remove the pigments that got separated along with hexane. The filtrate was partitioned with a suitable organic solvent in a separating funnel before estimation. This cleanup procedure was evaluated for the recovery of some insecticides - endosulfan, phorate, quinalphos, carbofuran, aldicarb, phosalone, monocrotophos, fenitrothion and dichlorvos in some vegetables.

As dichloromethane cannot extract the bound insecticides, the samples were extracted by acid hydrolysis. The acid extract itself was partitioned with hexane after adding acetone and this method was tested for the recovery of the metabolites of carbofuran.

MATERIALS AND METHODS

Reagents : Acetone, hexane, dichloromethane and distil water.
Glassware : Glass centrifuge tubes (30 ml) with stopper.
Standards : Pesticide standards (Analytical grade) were obtained from the manufacturers. 1000 ppm stock solutions of the insecticides were prepared and diluted to 10 ppm serially with acetone for recovery studies.

Equipment : Centrifuge capable of running at 3000 rpm.

Extract 25 g of the chopped plant material with 150 ml dichloromethane in a waring blender and filter by suction into a 500 ml filtering flask. Add sufficient quantity of sodium sulphate to the flask to remove traces of water and filter to a 250 ml conical flask and evaporate gently over a water bath at 40°C. When the volume reaches 15-20 ml, remove the flask from water bath, transfer the extract into a volumetric flask and make up the volume to 25 ml by repeatedly rinsing the conical flask with small quantities of dichloromethane. Add 1 ml of 5% polyethlene glycol in dichloromethane and a few boiling chips to a glass centrifuge tube and concentrate over a water bath at 40°C. When the volume reaches 2 ml, remove the tube from water bath and by swirling action evaporate to dryness.

For extracting the bound metabolites of carbofuran, follow the acid hydrolysis method of Cook (1973). For this, reflux 25 g of the plant material for half an hour over a heating mantle with 125 ml of 0.25 N HCl in a round bottom flask fitted with Leibig condensor and filter into a 150 ml volumetric flask through glass wool. Repeatedly rinse the round bottom flask with small quantities of hot HCl, filter into the volumetric flask and make up the volume after cooling.

Before extraction, fortify vegetable samples with insecticides to study the recovery.

After evaporation, add 1 ml of acetone along the sides of the tube, followed by 0.1 ml hexane and by swirling dissolve all the extract. Add 9 ml water to the tube, stopper and shake vigorously for 30 secs. Remove the stopper and centrifuge at 3000 rpm (In this process, all the hexane gets evaporated leaving behind a film of pigments over aqueous layer). Filter the aqueous layer carefully through a funnel fitted with a wet plug of cotton into a 250 ml separating funnel. Repeat the process of partitioning with 0.1 ml hexane three more times and pool all the filtrate in the same separating funnel.

For the metabolites of carbofuran, instead of 9 ml of water, add 20 ml of acid extract, 1 ml of acetone and partition with 0.1 ml of hexane, centrifuge and filter as done for other insecticides. Similarly add 20 ml of the acid extract to the tube and repeat the partitioning procedure thrice. The fourth time add 1 ml acetone, 0.1 ml hexane and 9 ml water and follow the procedure as done for other insecticides. Rinse the tube two times with 10 ml of 10% aqueous acetone and filter. Pool the filtrate in the same separating funnel.

By partitioning, transfer the insecticide present in the pooled filtrate into 150 ml dichloromethane. However, to partition the insecticides which are readily soluble in hexane, i.e. endosulfan, use 150 ml hexane instead of dichloromethane. Ignore the milky aqueous layer encountered sometimes during partitioning, even after the separation of the organic phase. Filter the organic layer into a conical flask through a filter paper overlaid with sodium sulphate.

If the separating funnel partitioning is done with hexane no further cleanup is necessary. For estimating organophosphate and carbamate residues, concentrate the dichloromethane extract over a water bath at 45°C. When the volume reaches 25 ml, add 50 ml of hexane and evaporate at 55°C. When all the dichloromethane would have evaporated, i.e. when the extract turns slightly milky, stop the evaporation, if the volume is less than 15 ml. If the volume is more, concentrate further until the volume is about 15 ml. Remove the flask, add a pinch of sodium sulphate to the hexane and filter through a filter paper overlaid with a small quantity of sodium sulphate into a 25 ml volumetric flask. By rinsing the flask repeatedly with hexane and filtering, make up the volume to 25 ml. Use an aliquot of this volume for estimating residues colorimetrically either by the method of Getz and Watts (1964) or the enzyme inhibition technique of Urs and Rao (1980).

RESULTS AND DISCUSSION

This method employs both small tube and separating funnel partitioning. The affinity of pigments to hexane over aqueous acetone is fully exploited in the small tube partitioning, using very small quantities of hexane, acetone and water. The sample is extracted in dichloromethane, evaporated to dryness and dissolved in acetone. By adding water and hexane, plant pigments get separated along with hexane. To bring the insecticide to aqueous phase, the tube is shaken thoroughly. For complete separation and evaporation of the hexane, centrifuging is done. For maximum recovery of the insecticides the procedure is repeated three more times. In the small tube partitioning employed for the metabolites of carbofuran, the acid extract is used instead of water. The partitioning in the tube was repeated three more times to get maximum recovery. The small volume of solvents used facilitate centrifuging without any hazards. Most of the plant extractives are eliminated in the small tube partitioning and filtration. Consequently emulsions are not formed in the second partitioning done in separating funnel.

In the separating funnel cleanup, the aqueous layer may remain milky even after the organic layer is separated. Mistaking this for emulsion, sodium sulphate or sodium chloride should not be added to break it. Any such addition brings the pigments to the organic layer. The organic layer should be immediately separated, ignoring the milky aqueous phase.

In the partitioning cleanup employed by Luke et al. (1975) acetone extract is partitioned with petroleum ether and dichloromethane. The cleanup was sufficient for gas chromatographic estimation of residues. However, in the present method a gas chromatograph facility was not available, only colorimetric estimations were done and there were no interferences. This method requires very low quantities of solvents (only about 400 ml) and is hence economical.

Even though the dichloromethane extract was clear,the additional cleanup of transferring the extract to hexane removed some hexane insolube but dichloromethane soluble extractives. After the cleanup, the extract sometimes retained a faint yellow tinge. However, this did not interfere with the estimation done by cholinesterase inhibition. The yellow tinge was more pronounced in green leaf samples even after cleanup.

Table 1. Recovery of Insecticides in some vegetables

Insecticide	Solubility in water (ppm)	Crop	Insecticide Fortification level	Recovery Percent +	Method of estimation
Endosulfan	Insoluble	Cabbage	2.0	55 (+17.07)	Maitlen et al. (1963)
		Beans	2.0	60 (± 0)	"
Fenitrothion	Insoluble	Chow-chow	2.0	102.5(+ 6.75)	Urs and Rao (1980)
		Cabbage	0.8	67.5(±10.60)	"
Phosalone	10	Tomato	2.0	97.1(+ 0)	"
Quinalphos	22	Beans	0.8	80.0(± 0)	Getz and Watts(1964)
Phorate	50	Cabbage	0.1	90.0(± 0)	Urs and Rao (1980)
			0.2	99.0(± 1.41)	"
			1.0	86*	"
		Okra	0.5	83*	"
		Beans	1.0	75*	Getz and Watts (1964)
Carbofuran	700	Cabbage	1.0	110.0(+14.40)	Urs and Rao (1980)
		Beans	0.5	85.5(± 0.70)*	"
		Tomato	2.0	81.25(+1.25)**	"
3-hydroxy Carbofuran	-	Beans	2.0	84.05(±1.72)**	"
		Capsicum	2.0	88.45(±2.40)**	"
3-ketocarbofuran	-	Cabbage	2.0	88.75(±2.12)	"
		Cowpea	2.0	87.5 (± 0)	"
Aldicarb	6000	Beans	0.1	80.0 (± 0)	"
Dichlorvos	10,000	Beet root	0.5	85.0 (± 4.0)	"
Monocrotophos	Highly soluble	Tomato	1.0	98.75(±2.12)	"

\+ Average of 2 replications
* Values of single analysis
** Extraction was done by acid hydrolysis

This method of cleanup was evaluated by fortifying some vegetables with carbofuran and its two metabolites, aldicarb, endosulfan, phorate, quinalphos, phosalone, monocrotophos, fenitrothion and dichlorvos. As seen from table 1, the recovery of all the insecticides except endosulfan was above 60%. As endosulfan is readily soluble in hexane, some of it may get dissolved in hexane along with the pigments and lost in the tube and while filtering. Dichlorvos and aldicarb are volatile, but recovery is not affected as the final stage of evaporation in tubes is done by swirling only. This method requires low quantities of solvents and partitioning is done both in tubes and separating funnels. Also in the partitioning no emulsions are formed. Thus this method permits rapid and economic cleanup. Hence it can be used as a common cleanup for insecticides having varying solubility in water as evident from the recovery of fenitrothion (insoluble), phorate and quinalphos (less soluble), carbofuran and aldicarb (moderately soluble), and monocrotophos and dichlorvos (highly soluble). The possibility of using this method in multi residue analysis and gas chromatograph will also have to be worked out.

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REFERENCES

Cook RF (1973) Carbofuran In: Sherma J, Zweig G (eds) Analytical methods for pesticides and plant growth regulators, vol 7. Academic Press, New York, p 187.

Getz ME, Watts RE (1964) Application of 4 (P-nitrobenzyl) pyridine as a rapid quantitative reagent for organophosphate pesticides J Assoc Agri Chem 47: 1094-96.

Luke MA, Froberg JF, Masumoto HT (1975). Extraction and cleanup of organochlorine, organophosphate, organonitrogen and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. J. Assoc. Off Anal Chem 58: 1020-26.

Maitlen JC, Walker KC, Westlake WE (1963) An Improved colorimetric method for determining endosulfan (Thiodan) residues in vegetables and beef fat J Agr Food Chem 11: 416-18.

Urs KCD, Rao VLVP (1980). Residues of monocrotophos in cabbage, cauliflower and knol-knol In: Edwards CA, Veeresh GK, Krueger HR (eds) Pesticide residues in environment in India. UAS Tech. Ser. No. 32, University of Agricultural Sciences, Bangalore, India, p 219.

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