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An Improved Multiresidue Procedure for Determination of Pesticides in Vegetables

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A cost effective, less time consuming and effective analytical technique for estimation of multiresidues of 30 insecticides have been reported by introducing modifications at extraction and clean up stages, by using GC equipped with SPB-5 and HP-1 capillary columns and appropriate oven temperature programming.

KEY WORDS : Multireside analysis, organochlorine, organophosphorus, synthetic pyrethroid, carbamate.

Insecticides belonging to organochlorine (OC), organophosphate (OP), carbamate and synthetic pyrethroid (SP) are widely used on vegetable crops for controlling a variety of insect-pest. Generally, farmers do not observe the recommended waiting period after the final spray, therefore the likelihood of residue of applied insecticides on vegetables is very high. As the vegetables are consumed raw also, the presence of residues may pose health hazards to the consumer. In order to know the extent of residual contamination and the magnitude of exposure to human being, there is a need of multiresidue analytical technique which should be efficient, precise, sensitive and capable of detecting and estimating low concentration of pesticide residues. Recently many multiresidue chromatographic techniques have been reported[1-5]. Among these, the one technique[5] is considered to be most efficient. While adopting it for our studies, we faced difficulties in getting quantitative recoveries. Therefore, some modifications were introduced at the level of extraction, clean up and quantitation by GLC. The modified technique was validated by processing different vegetables spiked at different levels with insecticides of the four chemical groups.

MATERIALS AND METHODS

Reference standards of the insecticides in study were procured from Environmental Protection Agency (EPA), North Carolina, USA. Standard solutions (100 μg ml^{-1}) were prepared in *n*-hexane and further dilutions were made with *n*-hexane in the case of organochlorine and synthetic pyrethroid and with ethyl acetate for organophosphate and carbamate insecticides. Samples were analysed by Gas Chromatograph, Model Hewlett Packard HP-5890A, equipped with electron capture detector (ECD) and nitrogen phosphorous detector (NPD). Organochlorines and synthetic pyrethroids were estimated by GC equipped with ECD fitted with capillary column SPB-5 (30 m x 0.32 mm i.d. x 0.25 μm film thickness poly 5% diphenyl/95% *dimethyl* silicone). Nitrogen is used as carrier gas at a linear gas velocity of 2 ml through column and make up gas 60 ml min^{-1}. The injector port was maintained at 280°C and the oven temperature was 150°C for 5 min, then @ 8°C min^{-1} to

190°C for 2 min and finally @ 15°C min^{-1} to 280°C for 10 minutes. The detector temperature was 300°C. Organophosphates and carbamates were detected on NPD using HP-1 (10 m x 0.53 mm i.d. x 2.65 µm film of dimethyl polysiloxane). The flow rates were N_2 : 18 ml min^{-1}, H_2 : 1.5 ml min^{-1} and O_2 : 135 ml min^{-1}. The injector port was maintained at 250°C. The oven temperature programme was 100°C for 1 min, then @ 10°C min^{-1} to 200°C and finally 20°C min^{-1} to 260°C for 3 minute.

For the pesticidal estimation first individual reference standards and then standard cumulative solutions of OC and SP on ECD, and OP and carbamate on NPD were injected.

Recoveries : Recoveries of some pesticides of each group were carried out by processing the spiked vegetables i.e. cauliflower, brinjal and cucumber representing rough surface, smooth surface and vegetable with high water content, respectively. Chopped and macerated 20 g sample of each vegetable was spiked (0.01 µg g^{-1}) depending upon the sensitivity of each insecticide.

Extraction : The residues from the spiked samples were extracted with 100 ml acetone by mechanical shaking for 1 hour. The extract was filtered through 3-4 cm bed of anhyd. sodium sulphate and transferred into separatory funnel diluted with 10% NaCl and extracted with ethyl acetate (50, 25, 25 ml). Organic phase was combined and concentrated to about 5 ml under reduced pressure and analysed by GLC.

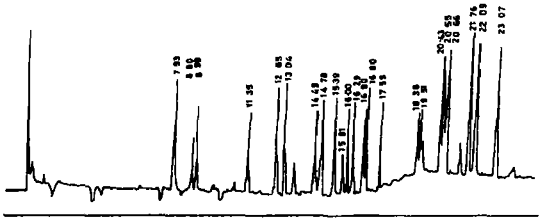
OC and SP : A glass column (60 cm x 22 mm) was packed with 5 g mixture of Florisil (60-100 mesh) and charcoal (5:1, w/w) in between two layers of anhyd. sodium sulphate. Column was prewashed with 50 ml hexane, loaded with the first part of concentrated extract and eluted with 125 ml mixture of ethyl acetate : hexane (3:7, v/v). The eluate was concentrated and final volume was made to 2 ml with ethyl acetate : *n*-hexane (3:7, v/v).

OP and carbamates : Chromatographic column was prepared by packing 5 g adsorbent mixture of silica gel (60-230 mesh) and activated charcoal (5:1, w/w). Column was pre-washed and loaded with the second part of the concentrated extract and eluted with 125 ml mixture of acetone : hexane (3:7, v/v). Eluate was concentrate and final volume was adjusted to 2 ml with acetone : *n*-hexane (3:7, v/v) and analysed.

RESULTS AND DISCUSSION

Chromatograms of organophosphorus and carbamate pesticides analysed by GC-NPD are shown in Figure 1 and 2. Retention times (Rts) and detection limit for each analyte are presented in Table 1. Recoveries of each pesticides in brinjal, cauliflower and cucumber are given in Table 2. The % recoveries were in the range of 80-111, 73-95, 83-125 and 82-104 for organochlorines, synthetic pyrethroids, organophosphates and carbamates, respectively. Thus, the recoveries were all adequate except for heptachlor (40-46%) and acephate (19-26%), which were relatively low.

By improved analytical technique, all the peaks of the standard pesticides could be resolved upto a desirable extent. The use of



Time →

Fig. 1. Gas Chromatogram showing separation of organochlorine and synthetic pyrethroids on SPB-5 column

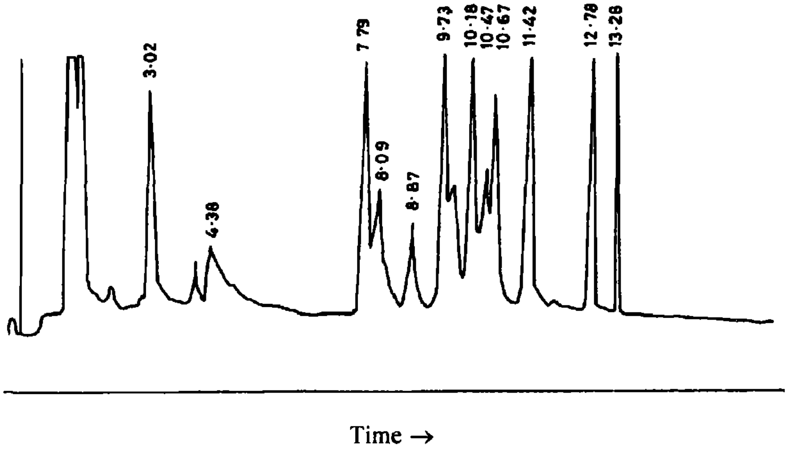


Fig. 2. Gas Chromatogram showing separation of organophosphorus and carbamate pesticides on HP-1 column

Table 1. Retention times and limits of detection of pesticides investigated

SPB-5			HP-1 column		
Pesticides	Rt (min)	Detection limit (pg)	Pesticides	Rt (min)	Detection limit (ng)
Organochlorines and Synthetic pyrethroids			Organophosphorus and carbamates		
α-HCH	7.93	10	DDVP	3.02	15
β-HCH	8.80	10	Acephate	4.38	40
γ-HCH	8.98	5	Phorate	7.79	25
Heptachlor	11.35	5	Monocrotophos	8.09	50
Aldrin	12.65	5	Carbofuran	8.87	50
Chlorpyrifos	13.04	10	Phosphamidon	9.73	25
o, p'-DDE	14.49	5	Carbaryl	10.18	50
Endosulfan-A	14.78	5	Fenitrothion	10.47	25
p, p'DDE	15.39	5	Malathion	10.67	12
Endosulfan-B	16.00	5	Chlorpyrifos	11.42	20
o, p' DDT	16.29	5	Quinalphos	12.78	12
Endosulfan sulfate	16.80	20	Triazophos	13.28	20
p, p'-DDT	16.90	10			
Endrin	17.59	12			
Cis-permethrin	19.38	100			
Trans-permethrin	19.51				
	20.43				
Cypermethrin	20.55	75			
	20.66				
Fenvalerate	21.76	50			
	22.09				
Deltamethrin	23.07	50			

activated charcoal ensured better removal of colour pigments without affecting recoveries from spiked vegetable samples. In five cases of cauliflower (rough surface), four in brinjal (smooth surface) and one in cucumber (high water content) recoveries exceeded 100%. This could possibly be due to adsorption of injected standards in the injection port or column, while co-extractives in the sample prevented adsorption. Thus, the modified technique can be considered economical, less time consuming, authentic and sensitive which involved less volumes of solvents at extraction and gave satisfactory recoveries by efficient clean-up. The technique is being followed successfully in screening vegetable samples for monitoring pesticide residues in our laboratory.

Table 2. Recoveries of pesticides recoveries in vegetables with improved technique

	* Recovery (%)		
Pesticide	Cauliflower	Brinjal	Cucumber
α-HCH	85	92	89
β-HCH	103	111	91
γ-HCH	95	91	79
Heptachlor	41	46	40
p, p'-DDE	84	87	80
o, p'-DDE	93	95	80
p, p'-DDT	90	92	89
Endrin	92	89	84
Aldrin	108	105	92
Endosulfan-A	91	89	85
Endosulfan-B	93	90	87
Endosulfan sulphate	94	92	96
Fenvalerate	95	89	85
Deltamethrin	92	93	82
Cypermethrin	92	93	82
DDVP	90	85	83
Acephate	19	26	22
Chlorpyrifos	91	90	85
Malathion	92	90	95
Monocrotophos	125	107	103
Quinalphos	113	101	96
Carbofuran	104	98	95
Carbaryl	93	87	82
Phorate	89	85	83
Fenitrothion	85	90	91
Phosphamidon	90	93	87

*average of three replicates.

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