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Analysis of Pesticide Residues in Fortified Water, Soil and Vegetable Samples

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Abstract

Water, soil and potato samples spiked with insecticides were analyzed for percent recoveries on Gas Chromatograph (GC) equipped with Electron Capture Detector (ECD), using capillary column. Percent recoveries of methyl parathion were calculated to be 100, 67 and 94 percent at spiking level of 0.17, 1.7 and 8.7 µg/liter and 310 and 138 percent for cypermethrin at spiking level of 0.146 and 1.46 µg/liter by liquid-liquid extraction. Through solid-phase extraction the percent recovery of deltamethrin was 37 and 78 percent at spiking level of 0.16 and 1.6 µg/liter. The percent recoveries of deltamethrin through solvent extraction technique were found to be 78 and 86 percent at spiking level of 2.56 and 25.20 µg/liter, while 81 and 147 percent for deltamethrin and cypermethrin through soxhlet extraction at 8.0 and 8.7 µg/liter concentration, respectively. The percent recoveries at spiking level of 0.1 ppm were calculated to be 0, 5.59, 35.52 and 0 percent and in 1.00 ppm recovery was calculated to be 91.98, 56.60, 44.56 and 58.93 percent in fortified samples of potato with dimethoate, methyl parathion, endosulfan and cypermethrin, followed by 0, 59.82, 111.20, 11.36 percent in blank spiked at 0.1 ppm, respectively. The data presented provides evidence that capillary column GC with ECU detection can be used reliably and advantageously for regulatory determination of pesticide residues in food, water and soil. The different methods described allow quantitative extraction of the pesticides. However, further experiments need to be conducted to ensure consistent results. The reproducibility of analytical methods require that the pesticide residue analysis be performed with the highest possible accuracy and so qualified that, the results obtain reflect the least deviation from the true value.

Introduction

The adverse effect of essential chemicals in agriculture are primarily residue in food and feed, occupational hazards, pollution of drinking water, contamination of environment both in aquatic and terrestrial habitat affecting non target beneficial organism and eventually narrowing down to bio diversity. In recent years substantial amount of chemicals and pesticides have been used by the agriculture industry (Au and Fung, 1988). In Pakistan the pesticide consumption has increased from 665 million tons a.i in 1980 to 44872 million tons a.i in 1997 (Government of Pakistan, 1997). The indiscriminate use of insecticides on fruits and vegetables causes direct or indirect problems (Mukherjee and Gopal, 1996; Sukul, 1995). The insecticides are carried in run-off and drainage from soil to water and also find their way into various food products. The widespread use of toxic insecticides has created a need for detecting them as spray residues (Jan and Shah, 1991). Modern analytical techniques are being used for analyzing low concentration down to 0.1 ppb in water, soil and food products. The fate of pesticide from the cradle to the grave needs to be closely monitored to ensure safety of the mankind and the environment. To ensure sustainable development with emphasis on a high degree of environmental and human health protection, Ecotoxicology Institute has been established in Pakistan. The aim of this project was to synchronize all parameters to perform pesticide residue analysis. In this study different methods of extraction were validated by calculating the recovery percentages of pesticides analysis on GC equipped with ECD using capillary column.

Materials and Methods

Reagents and Apparatus: All chemicals were analytical grade (BDH Chemicals). Insecticide standards of >95 percent pure were prepared in ethyl acetate. Perkin Elmer Autosystem Gas Chromatograph equipped with electron capture detector (ECD) using capillary column, Waring blender, Buchi rotary evaporator and Mikrolab Arrhus A/S Gel Permeation Chromatograph Biobeads SX3 (200-400 mesh).

Fortification of Samples: Distilled water was fortified by insecticides in 1 ml ethyl acetate to one liter of water. In case of solid-phase extraction 5 ml of methanol was added to each spiking water. Soil (50 g) were fortified with insecticides made in different concentrations. Potato samples were chopped small pieces. 25 g samples ware spiked with 0.1 ppm (2.5 µg/ml) and 1.0 ppm (25 µg/ml) of pesticide mixture containing bhc, dimethoate, methyl parathion, endosulfan ans cypermethr in at the blending stage. Two blanks i.e. solvent + 0.1 ppm and solvent + 1.0 ppm pesticide mixture were prepared. These were processed and spiked in a manner similar to potato samples. A third blank was prepared by directly mixing the 0.1 ppm mixture (final concentration 0.5 µg/ml). Two blinds (solvent + no pesticide), one before making all the other extracts in the blender and one after making the extracts and washing the blender jug were also processed in a similar manner.

Extraction of Samples

Water

Liquid-Liquid Extraction: The fortified samples were transferred to a 2 liter separator funnel and extracted vigorously for 1.5 minutes with 25 ml dichloro-methane (solvent-water ratio of 1:40 by volume). The process was repeated three times. The extracts were combined, dried with anhydrous sodium sulphate and concentrated at 40°C. The residue was quantitatively transferred to volumetric flask (5 ml) and stored at 4°C till analysis on GC.

Solid-Phase Extraction: Spiked water was extracted with C-18 extraction disk (pore size 60 A°). The disk was allowed to soak with 10 ml of methanol for 3 minutes and then methanol was eluted slowly with the help of suction pump. Spiked water was added to the disk before it dried and the vacuum was adjusted to a flow rate of 40-50 ml/min. 20 ml of

ethyl acetate was then filtered through the same disk to get all the remaining pesticide. The extract was concentrated to dryness on a rotary evaporator and quantitatively transferred in 1 ml volumetric flask.

Soil

Mechanical Shaker: Soil samples (50 g) were extracted by shaking with 150 ml of acetone: hexane in a mechanical shaker for one hour. The mixture was filtered through a glass wool plug into a separator funnel. This extract was washed with millipore distilled water (2* 100 ml). The lower aqueous layer plus any emulsified material were discarded. The remaining hexane was dried with anhydrous sodium sulphate (few gm) and 15 ml of the aliquot was transferred to a round bottom flask and evaporated to dryness on a rotary evaporator. The residue was quantitatively transferred to volumetric flask (5 ml).

Soxhiet Extraction: Fortified and untreated soil was samples (2 g) were extracted on Soxtec system HT2, Tecatar Sweden, with 40 ml of cyclohexane: ethyl acetate (1:1). Extraction knob was kept to boiling position for 45 minutes and then to rinsing position for 30 minutes. After rinsing the condenser valve was closed and all the solvent was collected in the condenser. The cup was air dried.

Vegetable: Fortified 25 g subsamples were blended separately in the blender, with 50 ml acetone for 3 minutes. To this mixture, 50 g sodium sulphate + 50 ml cyclohexane: ethyl acetate (1:1) were added and again blended for another 2 minutes. The whole mixture was allowed to stand for 20 minutes and then 30 ml of clear supernatant was taken out. Few drops of 10 percent propanediol were added and evaporated on rotary evaporator. The remaining liquid was air blasted to completely get rid of the solvent. This was quantitatively reconstituted in ethyl acetate in *a* volumetric flask (5 ml). Potato samples were filtered through Bio-beads SX-3 eluted with cyclohexane: ethyl acetate (1:1). The collected eluent was concentrated to dryness in 2 ml of ethyl acetate and analyzed by *gas* chromatography.

Analysis of samples: Quantitation of different pesticides in water, soil and potato extracts was accomplished by Perkin Elmer Autosystem Gas Chromatograph equipped with ECD under the parameters; Column: 17 m methyl 10& phenyl silicone 0.32 mm ID, 0.5 µm film thickness. Injector temp.:2200°C Detector temp.: 350°C, Detector makeup, Nitrogen: flow 33.3 ml/min, Oven :Temp. 80°C, initial time 0 min. Rate 20°C/min. Final temp. 280°C stay for 0 min, final time 10 min. injection: 1 µl splitless, Integration: Peak height was used for quantitation.

Results and Discussion

The recoveries of spiked sample of water with methyl parathion were calculated to be 100, 67.60 and 94.10 percent from spiking level of 0.17, 1.7 and 8.7 µg/liter respectively. Traces of insecticides were also observed in blank and blind samples (Table 1). A few unknown peaks appeared in the chromatogram due to coextractives but they did not interfere with the pesticide peaks. The retention time (RT) of methyl parathion was recorded to be 9.1 minutes, whereas 1013 was calculated to be > 01 µg/ml. The recoveries from spiked sample of water with cypermethrin were 310.34 and 138.62 percent at spiking levels of 0.146 and 1.46 µg/liter by liquid-liquid extraction (Table 1).

Table 1: Recovery percentages of methyl parathion and cypermethrin extracted by liquid-liquid extraction from water

Insecticide	Spiking level µg/l	µg in final volume (5 ml)	Calculated µg/ml	% Recovery
Methyl paration	Blank	0.00	<0.019	Traces
	Bling	0.00	<0.019	Traces
	0.17	0.03	0.03±49.8%	100
	1.7	0.34	0.23±11.3%	67.60
	8.7	1.7	1.60±11.3%	94.10
Cypermethrin	0.146	0.029	0.09±87.17%	310.34
	1.46	0.29	0.40±25.90%	138.65

Table 2: Recovery percentages of deltamethrin extracted by solid phase extraction from water

Insecticide	Spiking level µg/l	µg in final volume (5 ml)	Calculated µg/ml	% Recovery
Deltamethrin	Blank	0.00	0.00	Nil
	Blind	0.00	0.00	Nil
	0.16	0.16	0.03±99.8%	18.75
	0.16	0.16	0.06±55.2%	37.50
	1.60	1.60	0.11±28.9%	06.87
	1.60	1.60	0.80±11.7%	50.00
	1.60	0.32 (1:5)	0.25±14.0%	78.12

Table 3: Recovery percentages of deltamethrin extracted by solvent extraction from soil

Insecticide	Spiking level µg/l	µg in final volume (5 ml)	Calculated µg/ml	% Recovery
Deltamethrin	Blank	0.00	0.00	Nil
	Blind	0.00	0.00	Nil
	02.56	0.102	0.08±35.3%	78.43
	25.20	1.008	0.86±9.7%	86.00

Table 4: Recovery percentages of deltamethrin and cypermethrin extracted by Soxhlet extraction from soil

Insecticide	Spiking level µg/l	µg in final volume 25 ml	Calculated poiml	% Recovery
Blank	0.00	0.00	0.00	Nill
Blind	0.00	0.00	0.00	Nill
Deltamethrin	8.00	0.16	0.13±24.60%	81.25
Cypermathrin	8.70	0.17	0.25±25.27%	147.05

Table 5: Percent recovery of pesticides in potato

		Blank		Potato sample	
S.No.	Pesticides	0.1	1	0.1	1
1	Dimethoate	0.00	75.00	00.00	91.98
2	M. Parathion	59.82	60.00	05.59	56.60
3	Endosulfan	111.20	45.61	35.25	44.56
4	Cypermethrin	11.36	48.55	00.00	58.93

The presence of 0.05 µg/ml gird of cypermethrin in blank confirms the contamination of glassware. It should be pointed out that the recovery from fortified samples does not give the extractive efficiency of field samples. Some variation is due to the separation of liquid phases, especially in the case of emulsions being formed (Lantos *et al*., 1983). Solid phase extraction of deltamethrin resulted in poor recovery at spiking level of 0.16 µg/liter i.e 37 percent but fair at 1.6 µg/ml i.e 78 percent (Table 2). No contamination was recorded in blank and blind samples. in case of soil samples the recovery of deltamethrin was 78 and 86 percent at 2.56 and 25.20 µg/liter through mechanical shaker (Table 3), while, 147 percent at 8.0 *1.4* µg/liter through Soxhlet extraction (Table 4).

Thus Soxhlet extraction proved to he much better. Cypermethrin gave 81 percent recovery at 8.0 µg/liter through same extraction (Table 4). The results of recovery experiments of potato samples are summarized in Table 5.

The recovery of endosulfan was fairly good 111 percent (at 0.1 ppm level) in blank while in potato samples at 0.1 and 1.0 ppm it was only 35.25 and 44.56 percent respectively. On the other hand dimethoate gave 91.98 and 75.00 percent recovery in potato and blank samples at 1.0 ppm level but zero recovery at a level of 0.1 ppm. In case of methyl parathion variable recoveries were obtained from blank 59.82 and 60.00 percent (0.1 and 1.0 ppm). The addition of cypermethrin to blank resulted in 11.36 and 48.55 percent recoveries while 0.00 and 48.55 percent in potato samples at 0.1 and 1.0 ppm respectively. At ppm level satisfactory results of all insecticides were obtained except dimethoate at 0.1 ppm level. At this low level of fortification recoveries were 0 percent. Negative results in the blind sample prepared after making all the other extracts and washing the blender jug confirms the washing procedure. Variation in the recovery of different pesticides in vegetable experiment may be due to many factors. It is not always possible to predict accurately the behaviour of a pesticide crop combination based on prior information with other pesticides or other crops (Wheeler *et al.,* 1983). Although the Danish method of extraction for fruits and vegetables was adapted, the percent recoveries are not comparable. A collaborative study of a German working group with tomato homogenate showed that following one unique analytical procedure precisely step by step without deviation from the method description does not guarantee highly comparable results (Ebing, 1983). The probability of the pesticides being degraded due to complete evaporation of the solvents on rotary evaporator also exists. The pesticides may also have been oxidized due to solvent evaporation through direct air. Evaporation of solvents to dryness should be avoided especially in multi residue methods (Lantos *et al.,* 1983).

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