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Analytical investigation of selected pesticide residues from fruits and vegetables by an improved extraction method using reverse phase high performance liquid chromatography

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Abstract: Pesticide residues like chlorpyrifos, cypermethrin, deltamethrin and dichlorovos were monitored by an improved extraction method from apple, peach and potato using reverse phase high performance liquid chromatography. The selected pesticides were extracted from fruits and vegetable samples using smaller volume of ethyl-acetate i.e. 60ml, anhydrous sodium sulphate (10gm) and sodium chloride (2.5gm). The cleanup of extract was done with 6 gm of activated charcoal. The final extract of fruits and vegetable samples were analysed using reverse phase high performance liquid chromatography with ultra-violet detector in an isocratic mode. The mean residual values of chlorpyrifos, cypermethrin, deltamethrin and dichlorovos found in apple, peach and potato extracts were 0.2866, 0.714, 0.08, 0.38, 1.46, 0.54, 0.151, 0.568, 0.216, 0.338, 0.064 and 0.0µg/g respectively. All the results were compared with Codex MRLs. It was found that all pesticides treated fruits and vegetables were safe for human consumption. Only chlorpyrifos residual amount was greater than MRLs, so its spray on crops should be limited as it is resistant to degradation.

Keywords: Pesticide residues, reverse phase high performance liquid chromatography, fruits, vegetables, chlorpyrifos, dichlorovos.
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INTRODUCTION

Pests are disease causing organisms that use plants as their source of food. The pests attack result in decreased yield of fruits and vegetables. Pesticides are agrochemicals designed to combat the attack of pests on agricultural crops. In modern agricultural practices, pesticides are widely used on crops for pre and post harvest applications. Pesticides are classified based on target organisms, chemical structure and physical state. Various classes of pesticides include herbicides, insecticides, rodenticides, biopesticides and weedicides[1,2]. A systematic pesticide follows absorption mechanism in plants. Many pesticides move upward (through the xylem) and outward[3].

Use of pesticides in Pakistan was started in 1952[4]. The pesticide companies motivated the farmers to use more than the recommended dose of pesticides through the media campaign. At present, 108 types of insecticides, 30 types of fungicides, 39 types of weedicides, 5 types of acaricides are being used in Pakistan[5]. 27% of the insecticides are being used on fruits and vegetables[6]. Pesticides have different target organisms and are available in concentrated liquid or powder form.

Pesticides cause potential toxicity to human beings and animals. Human health hazards vary with extent of exposure. Moderate health hazards include flu, skin diseases and headache, blurred vision where severe human health hazards include paralysis, blindness and even death[7]. According to Stockholm convention on persistent organic pollutants, 10 of the 12 most dangerous and persistent organic chemicals are pesticides[4]. These synthetic pesticides are mostly persistent to degradation, may remain in fruits and vegetables and cause health hazards to consumers.

Pesticides are usually analysed by gas chromatography and gas chromatography equipped with mass spectrometry[19]. Liquid chromatography hyphenated with tandem mass spectrometry proves to be a powerful technique for pesticide analysis in food samples[7]. Analytical chemists' target is to develop cost-effective, robust and sensitive analytical technique that can identify and quantify the residual amounts of pesticides in food samples.

In general, the main criteria for sample preparation for residue analysis of pesticides includes; multiresidue analysis, maximum recoveries, least matrix effects, good precision, easy and safe method. High performance liquid chromatography, HPLC, has found its applications for pesticide analysis with ultraviolet and fluorescent properties. HPLC is used for a broad spectrum analysis because it can determine all ionic, polar, non-polar, acidic, basic, neutral and thermally unstable pesticides[8].

Reverse phase high performance liquid chromatography is good for separating and identification of organophosphorus and pyrethroids group of pesticides. The objective of this research was to investigate pesticides residues in fruits and vegetable matrices with the improved extraction

method by using smaller volumes of extracting solvents to make the analysis economical.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of highest purity and HPLC grade purchased from MERCK and FLUKA. Anhydrous sodium sulfate, magnesium sulfate, activated charcoal and Florisil were of analytical grade. Stock and standard solutions of pesticides standards were prepared in acetonitrile and their retention time was recorded.

Sampling

Samples for fruits and vegetables were purchased from the local market of Lahore city. One kg of each apple, peach and potato was collected for the laboratory analysis.

Method

Each sample of fruit and vegetable was chopped in an electrical chopper and 50gm of chopped sample was taken in an Erlenmeyer flask. Sodium chloride, anhydrous sodium sulfate and ethyl acetate were added, shaked well on a horizontal shaker for an hour and filtered by an ordinary filter paper. Filtered extract was cleaned using activated charcoal. The cleaned extract was concentrated on a rotary evaporator and was then dried by bubbling nitrogen gas through it. The dried extract was then redissolved in acetonitrile and final extract of 1.00 ml was ready for injection to liquid chromatograph.

Analytical technique

The pesticide residue analysis was done on reverse phase high performance liquid chromatography, LC-10, Shimadzu, Japan with provided computer software.

RESULTS AND DISCUSSION

The methods for the pesticide residue analysis with conventional techniques are being improved day by day. The major steps involved in pesticide residue analysis are; extraction, cleanup and analysis. Therefore, reliable, cost-effective multi-residue methods are required to monitor pesticide residues in food. Extraction of pesticide residues from fruits produces complex mixtures that have often required sample purification and preparation procedures to isolate the targeted pesticides for analysis.

The reverse phase high performance liquid chromatography was used as an analytical technique to get chromatograms showing the residual effects of each pesticide.

Standard chromatograms for four pesticides i.e., chlorpyrifos, cypermethrin, deltamethrin and dichlorovos are shown in figure 1. The maximum pesticide residue levels analysed in apple, peach, and potato are given in table 1.

Table 1: Integration parameters and maximum residue levels (MRL) for fruit and vegetable samples using RP- HPLC equipped with UV-detector.

Name of the Pesticide	Fruit and vegetable	Retention Time	Maximum Residue Levels (µg/g)
Chlorpyrifos	Apple	2.784	0.860
	Peach	3.082	0.924
	Potato	3.162	0.137
Cypermethrin	Apple	2.785	0.875
	Peach	2.749	1.63
	Potato	2.881	0.192
	Tomato	ND	ND
Deltamethrin	Apple	2.787	0.241
	Peach	2.756	0.454
	Potato	2.700	0.065
Dichlorovos	Apple	6.418	0.283
	Peach	6.490	0.021
	Potato	6.076	0.014

The retention time of the pesticides was considered in the ±10 variations and the maximum residue levels (MRLs) in the selected fruits and vegetable were calculated. The results of these pesticides were then compared with the extraneous maximum residue limits (MRLs) of these pesticides in fruits and vegetables as specified in food standards by Codex Alimentarius issued by food and agriculture organization (FAO) and world health organization (WHO). The residual amount of chlorpyrifos in peach was higher than MRLs specified by Codex Alimentarius. Pesticides are applied to fruits, vegetables and various crops at various stages of cultivation and during post harvest storage to provide protection against a range of pests, before they become available to the consumer. The formulations used have been developed with specific pesticidal properties that are dependent upon the inherent chemical and physical characteristics of constituent chemicals. Consequently, pesticides comprise a large number of substances that belong to many different chemical classes. To ensure the safety of food for consumers and international trade, legislations such as European Union directives has established maximum residue limits for pesticides in food stuff. Thorough monitoring of pesticide residues is crucial for proper risk assessment of human exposure through food.

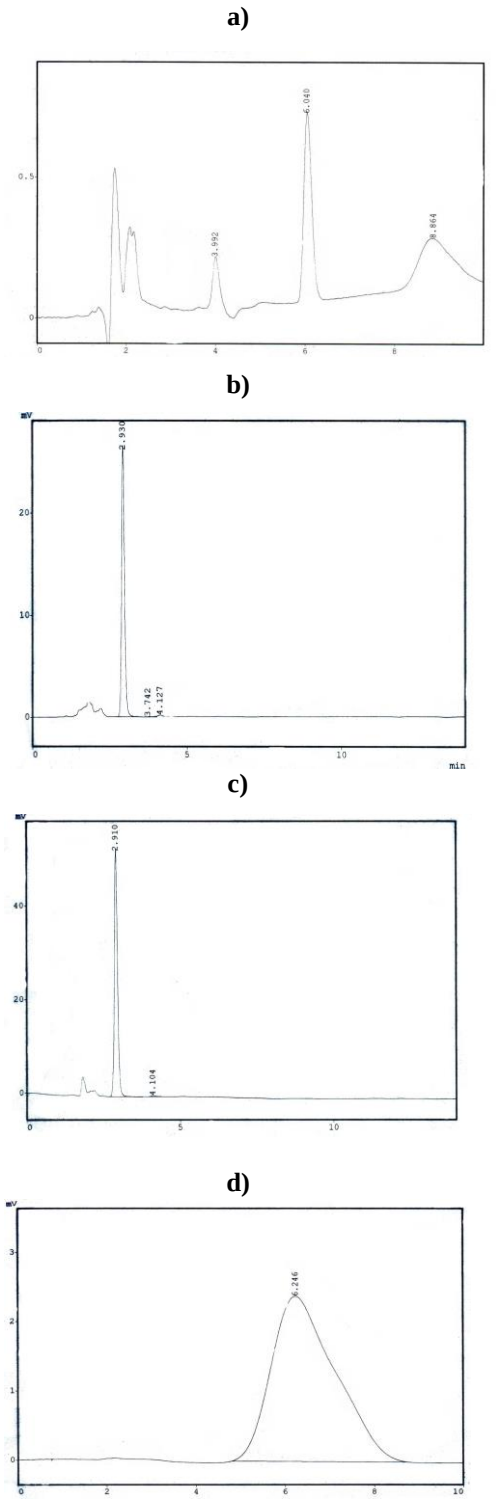

Figure 1: Chromatograms of standard pesticides; **a)** Chlorpyrifos, **b)** Cypermethrin, **c)** Deltamethrin, **d)** Dichlorovos

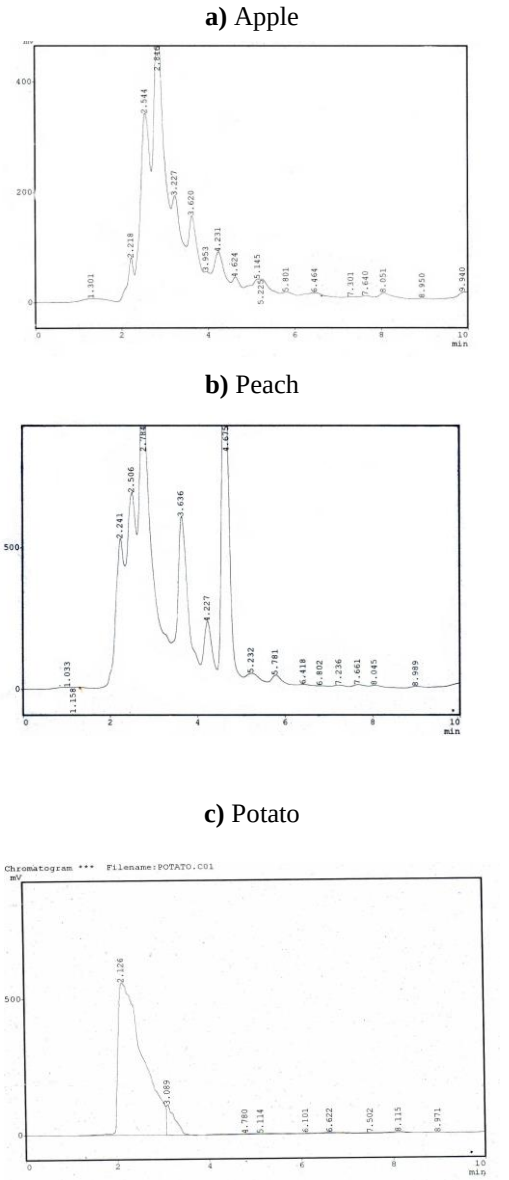

Figure 2. Chromatograms of pesticide residues from different fruit and vegetables; **a)** apple, **b)** peach, **c)** potato.

CONCLUSION

Fruits and vegetables production is substantially reduced by infestation of various insects and pests. The pest infestation causes 30-40% loss to the yields and sometimes the loss is high. Pesticides, indispensable components of IPM, can reduce these losses. However, indiscriminate applications of these pesticides to the crops results in residues in food and food commodities with consequential hazards. The health hazards can be minimized considerably or

eliminated if these residues are kept below their prescribed safe levels.

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