

## Method Development for the Determination of Pesticide Residues in Vegetables and Fruits by using Solid-Phase Microextraction

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**Abstract :** A headspace solid phase microextraction (HS-SPME) method in combination with Gas Chromatography –Electron Capture Detector (GC-ECD) was used for the extraction and quantification of diazinon, chlorothalonil, malathion, chlorpyrifos, quinalphos, alpha endosulfan, profenofos and beta endosulfan. The method was developed using a 100  $\mu$ m poly dimethylsiloxane (PDMS) fiber. The results showed higher responses of the pesticides after addition of aliquots of water and an organic solvent to the vegetable and fruit samples. The extracting solvent consisting of a mixture of methanol/acetone (1:1) when added to the sample in the proportion of 2% vol/weight (v/w) showed the best recovery, resulting in more than 80% recovery of the pesticides for all the investigated vegetable and fruit samples. Relative standard deviations (RSD) for triplicate analyses of samples were less than 10%. Calibration curves that were constructed for the analytes spiked into vegetable and fruit samples followed linear relationships with good correlation coefficients ( $R^2 > 0.9900$ ). SPME has been shown to be a simple extraction technique, which has a number of advantages such as solvent free extraction, simplicity and compatibility with the chromatographic analytical system.

Keywords: method development, pesticides, solid phase microextraction, GC-ECD

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### Introduction

Organophosphorus (OP) and organochlorine (OC) pesticides are widely used in agriculture as insecticides and leave residues to varying extents in agricultural produce such as vegetables and fruits. Due to their toxic properties and potential risk to consumers, their residues in food commodities is an issue of public concern and controlled by legislation [1].

The current developments of analytical technologies to detect pesticide residues in vegetables and fruits have mostly focused on the simplification, miniaturization and improvement of the sample extraction and clean-up methods with universal microextraction procedures [2,3]. Solid phase microextraction (SPME) is a solvent free extraction technique [1-3] that represents a convenient alternate to conventional extraction methods. This technique has become increasingly popular in the extraction of organic compound [2-6]. It is an inexpensive solvent free and reliable technique with excellent sensitivity and good selectivity.

SPME has been successfully applied to the determination of pesticide residue analysis in water, soil, food and biological samples as reported in recent reviews published by Beltran et al. [2] and Kataoka et al. [3]. Water samples are by far the most widely analyzed by this technique [7-9].

The low number of references about pesticide determination in food samples by SPME derives from the complexity of these matrices, which makes an extraction of the sample prior to determination by direct immersion SPME necessary in most of cases. This problem can be overcome if headspace SPME is applied, as described in several studies dealing with pesticide residue determination in fruits [10-13] and in studies related to the determination of volatile compounds in food commodities [14-17]. Due to the success of HS-SPME as a method for quantitative determination of organophosphorus insecticides in fruit juices [1], the present study was performed as an attempt to couple HS-SPME sampling and GC-ECD analysis for the determination of five organophosphorous (diazinon, malathion, quinalphos, chlorpyrifos, profenofos) and three organochlorine (chlorothalonil, alpha endosulfan, beta endosulfan) pesticides in vegetables (cucumber, tomato, leafy) and fruit (star fruit, strawberry, guava) samples.

### METHODOLOGY

#### 1.0 Chemicals and Reagents

All solvents used were HPLC grade and all the pesticide standards (diazinon, chlorothalonil, malathion, chlorpyrifos, quinalphos, profenofos, alpha endosulfan and beta endosulfan) were > 95%

pure. Stock solutions of each pesticide at different concentration level 1.0 – 400.0 mg/kg were prepared in methanol and stored at 4 °C. Preparation of different concentration levels of stock solution is due to their sensitivity to the ECD detector. Working standard solutions of a mixture of pesticides were freshly prepared daily by volume dilution in distilled water. 1-chloro-4-fluorobenzene (2 mg/kg) was used as an internal standard and was added to the vial prior to GC analysis.

Fresh vegetables and fruits (100g) were weighed and chopped. 30 g of chopped vegetables and fruits were placed in a 150 mL beaker. 1.0 mL of stock solution was spiked into the vegetables and fruits drop by drop. After being kept at room temperature for 1 hour, the spiked vegetables and fruits were added with 30 g of distilled water, blended and homogenized in a food blender. Then, the samples were placed in separate vials and analyzed following the HS-SPME analysis procedure below.

## 2.0 HS-SPME Analysis

All determinations were performed using a 100 µm film thickness poly (dimethylsiloxane) – PDMS coated fiber mounted in a manual syringe holder (Supelco, Bellefonte, PA, USA). Initially, 5.0 g of the vegetable and fruit sample was placed in a 15 mL clear glass vial and capped with a PTFE-faced silicon septum. The samples were added with the internal standard. PDMS fiber was exposed to the headspace above the sample for 30 min at 60 °C. Each sample was stirred constantly during the sorption step using a stir bar and a stirring plate. Thermal desorption of the analytes was achieved by inserting the sorbent fiber into the injection port (held at 240 °C) for 15 min. Quantification of pesticides in vegetable and fruit samples was carried out by a five point-calibration

in the matrix using spiked samples and analyzing every sample in triplicate.

## 3.0 Gas Chromatography – Electron Capture Detector (GC-ECD)

A Shimadzu GC 17A version 2.21 gas chromatograph with an electron capture detector ECD was used. A SGE BPX5, 30m x 0.32 mm id capillary column with a 0.25 µm film was used in combination with the following oven temperature program: initial temperature 120 °C, then heated at 7 °C/min to a final temperature of 250 °C, and then held for 4.5 min. The total run time was 23.07 min. The splitless mode was used for the injection. The injector temperature was at 240 °C and the detector temperature was at 300 °C. Nitrogen gas (99.999%) was used as the carrier gas with a gas flow at 24.4 cm/sec linear velocity and the pressure at 94 kPa.

## Results and Discussion

The effects of adding water on the samples in order to favor the release of analyte from the matrix was established by using different amounts of water ranging from a dilution factor of 1 to 10. Figure 1 shows the graph of percentage recoveries versus the dilution factor (recoveries were calculated by comparing the peak ratio of the relevant chromatographic peak to the spiked sample and an aqueous solution at the same pesticides concentration level, that was progressively subjected to the same dilution). From the results, a dilution factor of 2 increased the recovery of all the investigated pesticides from the range 25% - 32% to the range 65% - 75%. So, the dilution factor of 2 was chosen for cucumber as the optimum dilution factor to increase extraction efficiency and was then adopted for further work on the cucumber samples. The detection response of all pesticides was enhanced with the addition of water and decreased when the amount of water added exceeded a certain level.

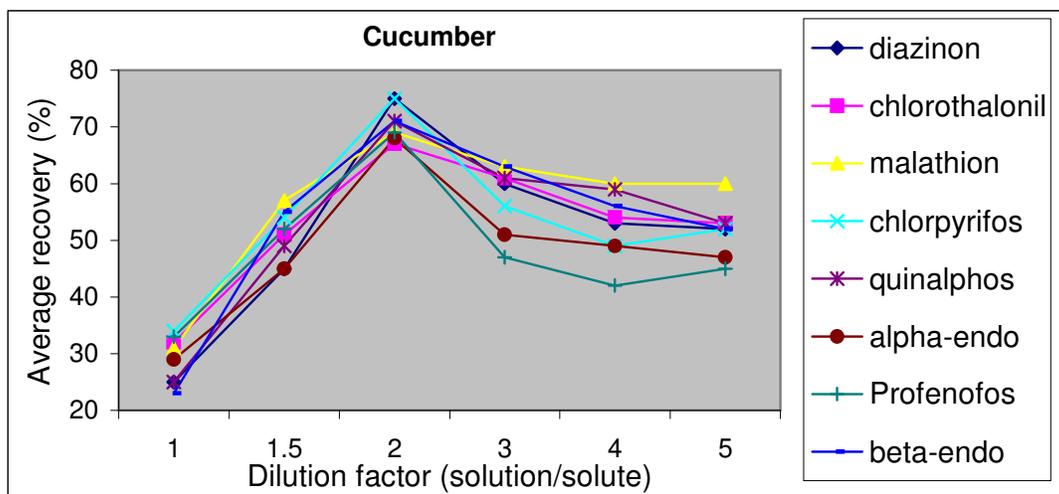


Figure 1 : Cucumber : percentage recoveries plotted versus the dilution factor.

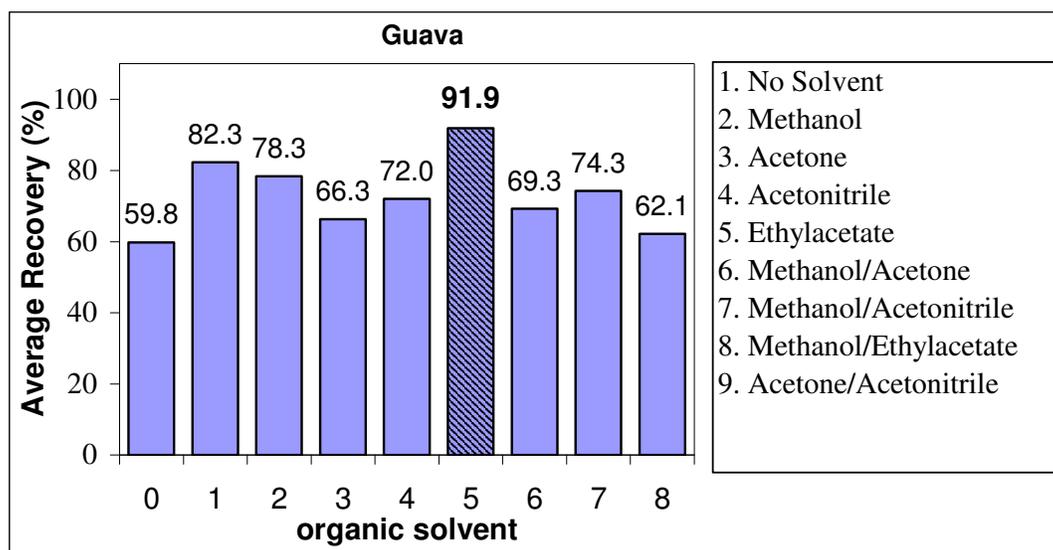
Table 1 shows the average recovery (%) of diazinon for all the investigated vegetables and fruits. The optimum dilution factor for cucumber and tomato samples were 2 and 3. For the other samples it was a factor of 5. This could be due to the water content of the vegetables and fruits. The HS-SPME process is affected by the suspended matter and dissolved compounds (sugar, pectins etc) contained in the vegetable and fruit samples which could adsorb the analytes, forming micelles and thus making it difficult for the analytes to reach the fiber (interfering with diffusion) [1]. Since the analytes were analyzed by HS-SPME, the addition of higher amounts of water would dilute the concentration of the analytes and increase the diffusion barrier of pesticides from aqueous phase to gaseous phase.

The addition of an organic solvent could also promote the release of organic compounds from the vegetable and fruit samples. However, the presence of a high concentration of an organic solvent would lead to a significant decrease in the extraction efficiency of the analytes [1]. Therefore, only a

small amount of solvent is recommended for use as the additive. Based on previous works related to the analysis of pesticides in complex matrices [1], the organic solvent tested were methanol, acetone, acetonitrile, ethylacetate, methanol/acetone (1:1), methanol/acetonitrile (1:1), methanol/ethylacetate (1:1) and acetone/acetonitrile (1:1). In this study, 2% (vol/weight) of organic solvent was added to the vegetable and fruit samples. The influence of water (dilution factor of 5) and the addition of organic solvent in guava samples are shown in figure 2. The average percentage shown is the average percentage recovery (%) of all the investigated pesticides. From the results, an average percentage recovery (%) obtained using a mixture of methanol/acetone (1:1) was much higher compared to that using the other organic solvents. The average recovery of all the investigated pesticides of guava sample under these conditions was 91.9 %. Besides the extraction efficiency, a mixture of methanol/acetone (1:1) was selected because it is relatively non-toxic, easy to volatilize and readily obtainable in the laboratory.

**Table 1:** Average recovery (%) of Diazinon for all the investigated vegetables and fruits

Compound	Average Recovery (%)							
	Dilution Factor	1	2	3	4	5	7	10
Cucumber	25	<b>75</b>	60	53	52	-	-	-
Tomato	38	68	<b>75</b>	73	73	-	-	-
Leafy	-	20	37	39	<b>50</b>	46	47	-
Star Fruit	-	49	48	53	<b>77</b>	73	69	-
Strawberry	-	31	43	45	<b>77</b>	74	71	-
Guava	-	28	45	55	<b>63</b>	58	55	-



**Figure 2:** Effect of organic solvents addition on extraction efficiency in guava samples with dilution factor of 5.

**Table 2:** Comparison of average recovery (%) of vegetable and fruit samples between Condition 1 (without dilution or organic solvent added) and Condition 2 (optimum dilution and 2 % (vol/weight) of methanol/acetone (1:1) added).

Recovery (%)	Condition 1		Condition 2		Average Increased (%)
	Range (%)	Average (%)	Range (%)	Average (%)	
Diazinon	20 - 40	29.7	83 - 95	88.7	59.0
Chlorothalonil	5 - 44	28.0	75 - 93	87.0	59.0
Malathion	13 - 33	26.2	81 - 97	90.5	64.3
Chlorpyrifos	7 - 34	18.0	74 - 94	82.0	64.0
Quinalphos	12 - 37	22.0	81 - 97	90.0	68.0
Alpha-Endo	5 - 29	14.3	74 - 92	85.7	71.4
Profenofos	8 - 33	20.2	82 - 99	90.5	70.3
Beta-Endo	7 - 24	15.7	70 - 99	88.0	72.3
Range (%)	<b>5 - 44</b>	14.3 – 29.7	<b>70 - 99</b>	82.0 – 90.5	<b>59.0 – 72.3</b>

Significant differences were found between results obtained from condition 1 (without dilution or organic solvent added) and condition 2 (optimum dilution and 2% (vol/weight) of methanol/acetone (1:1) added). The recoveries obtained from condition 1 were very low, ranging from 5 to 44%. This could be due to the fact that removing pesticides from a complex matrix is not very effective because the suspended matter interferes in the extraction process. In the attempt to reduce the matrix effect and to ameliorate the analyte recovery, the sample matrix was diluted with distilled water and the addition of different organic solvents. The addition of aliquots of water and organic solvent increased the extraction recoveries to between 70% and 99% for all the selected pesticides in all the vegetable and fruit samples studied. The increases in percentage of average recovery extracted from condition 1 to condition 2 were shown on Table 2. The average recovery increased is from 59.0% to 72.3%. The relative standard deviations for replicate experiments were less than 10% and the calibration curves were linear for the full range with correlation coefficient values greater than 0.9900.

### Conclusion

The complexity of the vegetable and fruit matrix makes it difficult to obtain a quantitative extraction of pesticides, but the decrease in concentration of the interfering components by a simple dilution and the addition of a small amount of organic solvent to the sample makes it possible to quantify pesticide residues. Optimized HS-SPME procedure can be proposed as a fast and accurate method for analyzing pesticides in vegetable and fruit samples and can be used instead of the conventional extraction technique, which involves large volumes of solvents, clean up procedures and time consuming steps.

Although many aspects of the application of SPME for analyzing pesticide residues in complex samples have to be resolved, this extraction technique may be considered as a viable alternative

in multiresidue techniques. The convenience, simplicity and reliability of the SPME technique should undoubtedly make it a valuable tool for the environmental screening of pesticides in the future.

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