

VALUATION OF SOLID PHASE EXTRACTION DISKS IN THE DETERMINATION OF PESTICIDE RESIDUES IN SURFACE AND GROUNDWATER IN PANAMA

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Abstract

In Panama large quantities of pesticides are used in agriculture and livestock farming and there is increasing concern about their impact on public health and the environment. Chiriqui is the Province that registers the largest number of producers whose activities have impact on the environment, especially on surface and groundwater. Systematic monitoring programmes are non-existent due, in part, to the high cost of laboratory determination of environmental residues of pesticides. Within the framework of the FAO/IAEA/SIDA Co-ordinated Research Programme, efforts were focused on evaluating and optimising the use of solid phase extraction C_{18} membrane disks in the analysis of surface and groundwater samples to determine pesticide residues. Factors studied were the effect of pre-washing and conditioning of the disks, flow rates, concentration level and matrix effects of field samples. Four pesticides, carbofuran, chlorothalonil, ametryn and chlorpyrifos were selected for these tests because preliminary analysis showed their presence in surface and groundwater. The technique significantly reduces the amount of organic solvents used as compared with the liquid-liquid extraction method. Quantifiable detection limits (Q_L) for the method were found to be 0.003 $\mu\text{g/L}$ carbofuran, 0.016 $\mu\text{g/L}$ chlorothalonil, 0.007 $\mu\text{g/L}$ ametryn and 0.003 $\mu\text{g/L}$ chlorpyrifos, when using standard spiked solutions. Recovery (%) was high when standard mixtures were used for the test runs but low when real surface water samples were tested, especially for chlorothalonil which was not recovered at all.

1. INTRODUCTION

Over 267 pesticides are distributed in Panama, of which 33.3% are insecticides, 27.9% are herbicides and 26.6% are fungicides. Imports are calculated to be in the order of 35 million US dollars [1]. Although an increasing number of farmers are using Integrated Pest Management techniques and more producers are resorting to organic farming, it is unlikely that the amount of agrochemicals will decrease significantly in the near future.

The Province of Chiriquí, located at the western region of the country, has the highest agricultural output and an important cattle-breeding tradition. A great percentage of the imported pesticides are used in this region, especially as crop protectors in banana plantations. Small and medium scale farming and cattle breeding account for many of the indiscriminate and improper use of pesticides. Due to heavy rainfalls and the relative fragility of tropical soils, many of these pesticides end up in surface and groundwater systems. An organochlorine pesticide residues study performed in 1989, on the waters of the Chiriquí Viejo River, which crosses highly fertile land, showed the presence of heptachlor epoxide, dieldrin, endrin and DDT with several samples showing residues above the maximum permissible limit [2]. These pesticides had already been banned from agricultural use.

In 1996 several water samples were taken from the Chiriquí Viejo River and the groundwater wells in the vicinity, and were extracted using solid phase membrane disks. The disks were analysed by the Food and Environmental Toxicology Laboratory of the University of Florida. Surface water samples had traces of chlorothalonil, alachlor, bromacil, chlorpyrifos and endosulfan, while groundwater samples had traces of chlorothalonil and chlorpyrifos. In both types of samples the levels were below 0.1 $\mu\text{g/L}$ [3].

Even though pollution by pesticide residues does not seem to be a problem in Panama at the present time there is a need for monitoring food and the environment for residues of pesticides and other chemical pollutants by using cost effective and affordable analytical methods. In most of the pesticide analytical methods pesticides are extracted by using large quantities of expensive organic solvents. For example in Method 3510A of the U.S. Environmental Protection Agency [4], which is based on liquid-liquid extraction (LLE), pesticides are extracted from water by using large volumes of methylene chloride followed by subsequent Kuderna-Danish concentration to a reduced volume and final solvent exchange to hexane. At low analyte concentrations, the LLE procedures can result in analyte losses due to incomplete extraction, adsorption on the glass surface, and losses during concentrations and solvent exchange. . There is, therefore, a need for alternative methods of extraction based on the use of reduced quantities of solvents.

Many solid-phase extraction (SPE) methods and specially disk solid-phase extraction (DSPE) of pesticides from water provides a useful alternative to the traditional liquid-liquid extraction-base methods, since it reduces the amount of solvent required and shortens the time needed for extraction [5,6]. In the present research Empore C₁₈ solid phase extraction disks was evaluated for extraction of four pesticides (carbofuran, chlorothalonil, ametryn and chlorpyrifos) from water prior to analysis by gas chromatography.

Because preliminary trials with ametryn, carbofuran, chlorothalonil, and chlorpyrifos had given poor recovery and low precision, modifications were made, which included disk-conditioning, change of extraction flow rate and solvent volume. The effect of spiking load and matrix effects was assessed.

2. METHODS AND MATERIALS

2.1. Solvents and reagents

All solvents used (acetone, toluene, methanol, iso-octane, ethyl acetate) and reagents (sodium sulphate anhydrous) were pesticide grade, and were purchased from J. T. Baker or Fisher.

2.2. Pesticides standards and solutions

Standards of ametryn, carbofuran, chlorpyrifos, and chlorothalonil were all 99% pure, and were obtained from Chem Service. Four standard mix solutions, containing 0.1, 0.2, 0.3 and 0.4 µg/mL of each of the four pesticides (ametryn, carbofuran, chlorothalonil and chlorpyrifos) in iso-octane was prepared for calibrations, and two standard mix solutions, containing 0.04 and 0.4 µg/mL of each of the four pesticides, in methanol was prepared for spiking. The solutions were kept at -10°C in amber screw cap vials with PTFE-silicone septa and open top phenolic closures.

2.3. Solid phase extraction disks

Empore™, 47-mm extraction disks with Bakerbond® octadecyl (C18) (J. T. Baker) were used in this study. A study protocol provided by Dr. Hugh Anson Moye [7] for the use of Empore™ SPE disks was used. The initial protocol established that the disks should be washed with 10 mL of ethyl acetate and conditioned with 15 mL of methanol, where the latter is allowed to saturate the membrane for 1 minute. Several researchers have used 10 mL methanol and a saturation time of 3 minutes to condition the membrane disks [8, 9, and 10]. It was, therefore, decided to do some tests using 10 and 15 mL methanol. The results showed that there was no

significant difference between the two volumes. Therefore, the disks were conditioned by using 10 mL of methanol and 3 minutes saturation time.

After several trials, pre-washing of the membranes with ethyl acetate was performed by placing the disks onto a petri dish, adding enough solvent to soak the membranes and leaving them covered for 6 hours. The disks were then placed on the filtration apparatus and dried by applying vacuum for 1 minute. Once dry, they were kept in aluminium foil until use.

2.4. Sample preparation

2.4.1. Extraction of pesticides from water

Glass distilled water, filtered through the Empore™ disks was used for sample preparation. One litre samples of water were spiked with 1 mL of standard mixture and filtered through the Empore™ disk, which had been previously conditioned with 10 mL of methanol. A Jr. Speedman system was used for extraction, which was kept at a flow rate of 30 mL/min. Once the sample was extracted, the disk was dried by applying vacuum for 20 minutes.

2.4.2. Elution of pesticides from the disks

Pesticides were eluted from the disks with ethyl acetate. Once the extraction of the disks was completed, they were placed on a 1 litre, glass filtering system (Wheaton TS 40/35), where a 200mm x 30mm glass tube, containing 1g of anhydrous sodium sulphate, was placed for collection of the extracts. The extracts were concentrated to approximately 4 mL and then transferred to a 10 mL Kuderna-Danish concentration tube where, with the aid of an adjustable Mini- Vap (Supelco 2-2970) concentrator/evaporator, the sample was evaporated to dryness, dissolved in iso-octane and then adjusted to a final volume of 0.2 mL for analysis.

2.5. Sample analysis

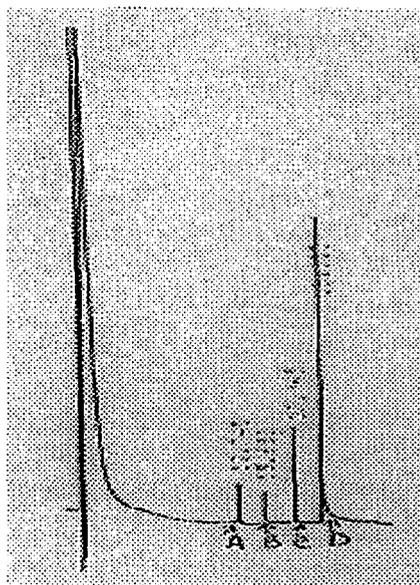
A Shimadzu model 14-B gas chromatograph equipped with direct injection system and a nitrogen-phosphorous thermionic detector (NPD) was used for the analysis. A fused silica, PTE-5®, wide bore capillary column, 30m x 0.53mm, with 0.53µm film thickness, (Supelco), was used for the separation of the pesticides. Direct manual injection, of 1µL, of both standards and samples were made with a 10µL (Hamilton #701) syringe. The operational conditions of the gas chromatograph were as follows:

Injector temperature (°C)	225
Column initial temperature (°C)	150
Column initial hold up time (min.)	3
Column temperature program (°C/min.)	4
Column final temperature (°C)	214
Column final hold up time (min.)	15
Detector temperature (°C)	300
Detector range	1
Detector current (nA)	6
Carrier gas flow (mL/min)	3 (Helium)
Backup gas flow (mL/min)	37 (Helium)
Hydrogen flow (mL/min)	3
Air flow (mL/min)	150
Attenuation	0

3. RESULTS AND DISCUSSIONS

3.1. Gas chromatography

The chromatogram of the analysis of the four pesticides is shown in Fig 1. The retention times for the pesticides were 13.7 min for carbofuran, 15.7 for chlorothalonil, 17.9 for ametryn and 19.9 for chlorpyrifos. The time span between injections was 45 minutes. Each sample was injected twice, and the standard pesticide mixture was run after every 6 injections to check the variability in the NPD response.



Std. mix: 0.2 μ g/mL (A: Carbofuran; B: Chlorothalonil; C: Ametryn; D: Chlorpyrifos)

FIG.1 Chromatogram of standard mixture of pesticides

The GC response (peak height) was plotted against concentration for each pesticide, and the best-fitted linear regression lines were estimated. Detector response of the calibration solutions was linear over a range of concentrations of 0.1 to 0.4 μ g/mL. The lowest response was given by carbofuran and the highest by chlorpyrifos (Fig. 2).

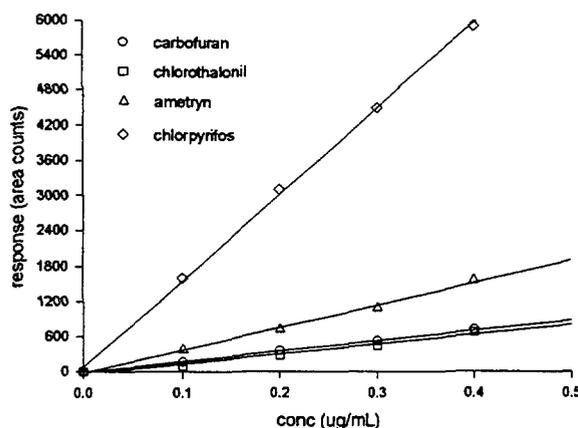


FIG. 2. Calibration curve of standard mixtures

The r^2 values for the linear regression model with non-zero intercept, of each of the four pesticides, ranged between 0.978 and 0.999. The limit of detection of the method calculated from the calibration curves, ($Q_L = 3s_{y/x} / slope$) was 0.003 $\mu\text{g/L}$ for carbofuran, 0.016 $\mu\text{g/L}$ for chlorothalonil, 0.007 $\mu\text{g/L}$ for ametryn and 0.003 $\mu\text{g/L}$ for chlorpyrifos.

3.2. The effect of extraction speed on recovery of pesticides

Extraction speed is one of the main advantages of solid phase extraction, since it is reduced significantly when a comparison is made with other extraction techniques. Flow rates reported by H.A. Moye for water extraction averaged 234 mL/min, giving an extraction time of 4 to 5 min/L. Tests with this high flow rates gave poor results and a literature search revealed that several authors recommend a flow rate of 30 mL/min when using Empore™ C₁₈ SPE disks [11]. This lower speed increased the extraction time to 33 minutes per liter.

Flow rate effects were tested by spiking six samples of distilled water with 1-mL standard mixture containing 0.04 $\mu\text{g/mL}$ each of ametryn, carbofuran, chlorothalonil and chlorpyrifos. The results (Table I and Fig. 3) show an increase in recovery rates and reduced variation (<6%) when the extraction of the water sample is carried out at 30 mL/min.

TABLE I. RECOVERY (%) OF PESTICIDES FROM DISTILLED WATER BY USING TWO DIFFERENT FLOW RATES FOR EXTRACTION

Pesticide	200 mL/min					30 mL/min				
	1	2	3	mean	cv	1	2	3	mean	cv
Carbofuran	89.3	97.4	123.6	103.4	17.3	109.8	104.5	99.8	104.7	4.9
Chlorothalonil	56.1	26.7	50.5	44.4	35.1	58.3	64.5	64.0	62.3	5.6
Ametryn	21.6	24.0	14.8	20.1	23.7	67.0	63.8	65.3	65.3	2.5
Chlorpyrifos	44.1	40.6	55.6	46.8	16.8	95.3	85.0	87.8	89.3	5.9

3.3. The effect of fortification level on the recovery of pesticides

The rate of degradation of pesticides in water is affected by their concentration because of kinetic effects over certain reactions due to hydrolysis and photolysis [12]. This effect was, therefore, studied in the laboratory by spiking distilled water with standard solutions of different at two fortification levels of 0.04 $\mu\text{g/L}$ and 0.4 $\mu\text{g/L}$ of each pesticide. These levels were chosen so that they would fall below and above the maximum permissible limit in water of 0.1 $\mu\text{g/L}$.

For each trial run 1L distilled water was spiked with either a 0.04 $\mu\text{g/L}$ standard pesticide mixture or with a 0.4 $\mu\text{g/L}$ spiking solution. Every run was done in triplicate and all fortified samples were extracted with an Empore C18 membrane at a 30 mL/min flow rate. The results (Fig. 4) show a significant reduction in the recovery percentages of the different pesticides when the fortification level is increased from 0.04 $\mu\text{g/L}$ to 0.4 $\mu\text{g/L}$. Ametryn and Chlorpyrifos are the pesticides most affected, from 71.5% to 16.1% in the first case and from 95.8% to 48.9% in the latter. It was also observed that the coefficient of variation were lower than 10% for both concentration levels except for ametryn at the higher fortification level; recoveries were quantitative, with the notable exception of ametryn.

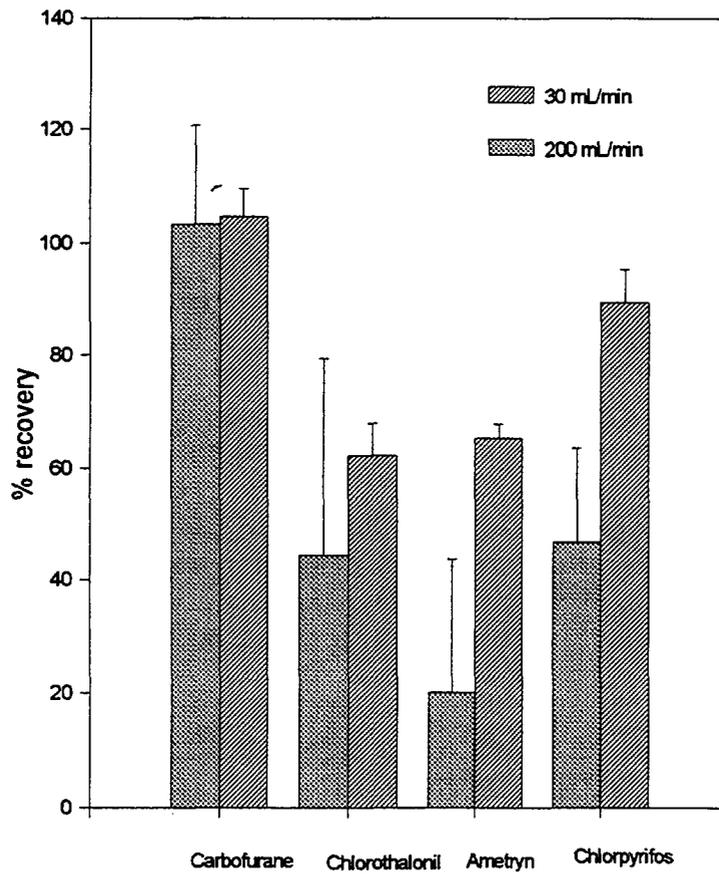


FIG. 3. Percent recovery of pesticides at two extraction flow rates (30 mL/min. and 200 mL/min.)

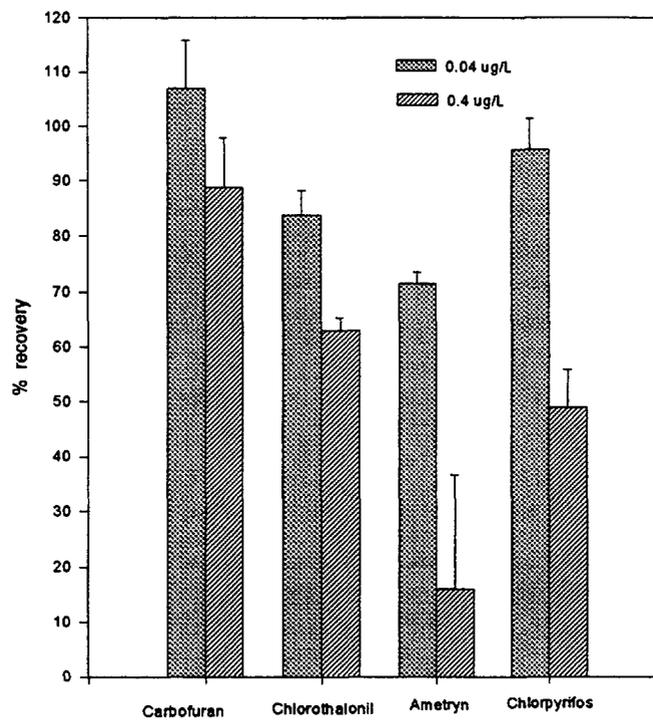


FIG. 4. Percent recovery of pesticides from distilled water at Two concentration levels (0.04µg/L and 0.4µg/L)

3.4. The effect of matrix on the recovery of pesticides

The effect of suspended particulate matter in the water on the extraction of the pesticides was studied by spiking 1L water samples from Platanares river with the four pesticides. The samples were spiked with a 1-mL aliquot of the standard pesticide mixture of 0.04 µg/L. A glass fibre pre-filter (Whatman GF/D) was placed above the Empore™ C₁₈ membrane prior to filtration. Some difficulty was encountered during filtration due to the amount of suspended particles but a flow rate of 30 mL/min was achieved.

Results in Fig. 5 show an increase in the recovery of carbofuran from 106.9% in distilled water to 126.5% in river water. Similarly, the recovery of ametryn increased from 71.5% in distilled water to 90.7% in river water. Chlorpyrifos, on the other hand, shows a reduction from 95.8% in distilled water to 77.3% in river water. No recovery, at all, was observed for chlorothalonil when river water was used. This could be due to sorption of chlorothalonil on organic matter suspended in the river water, a characteristic that has been reported for non-polar compounds (13,14).

An increase was also observed in the coefficient of variation (cv) when river water was used. However, they are lower than 20%, which means that the recoveries are quantitative, with the exception of chlorothalonil.

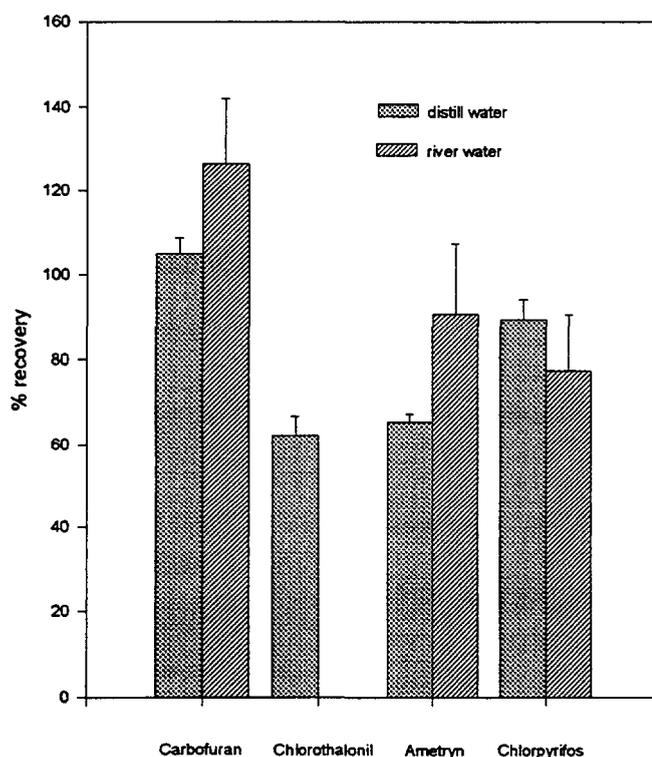


FIG. 5 Percent recovery of pesticides from surface water

4. CONCLUSIONS

The analytical method described in this work provided a rapid, quantitative, and convenient procedure for determining 4 pesticides at the low part per billion level. The extraction with the Empore™ C₁₈ filter disks, permitted an average pesticide recovery, from distilled water, of

71.5% (ametryn), 83.7% (chlorothalonil), 95.8% (chlorpyrifos) and 106% (carbofuran), at a concentration level of 0.04 µg/L. But for real samples, recoveries vary significantly, especially in the case of chlorothalonil, whose recovery depends strongly on the amount of suspended organic matter. Finally, the disks proved to withstand several extractions, which makes it attractive for laboratories of low budget.

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