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Miscellaneous

Solid phase microextraction–high pressure liquid chromatographic determination of Nabam, Thiram and Azamethiphos in water samples with UV detection: preliminary data

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Abstract

Nabam, Thiram and Azamethiphos are important agrochemicals and were analysed in agricultural water samples by extraction with solid phase microextraction coupled to HPLC with UV detection. The role of solid phase microextraction technique for the determination of these pesticides in water samples was investigated. Water samples spiked with these pesticides were preconcentrated using a polydimethylsiloxane (PDMS) fiber. Both dynamic and the static modes were tried and the static mode was found to give better recoveries and peak areas. The method enabled the determination of the pesticides at low concentrations with a limit of detection ($S/N = 3$) is 1–10 ng/ml.

Keywords: Solid phase microextraction (SPME); HPLC; Nabam; Thiram; Azamethiphos; Water samples

1. Introduction

Residue analysis of the pesticides [1–5] is important as pesticide pollution is a cause of great concern. Thiram and Nabam are dithiocarbamate [6] fungicide used to protect seed, fruit, vegetable, ornamental and turf crops from a variety of fungal diseases. They are also used as accelerator and vulcanization agents in the rubber industry. Thiram has been used in the treatment of human scabies, as a sunscreen, and as a bactericide applied directly to the skin or incorporated into soap. Azamethiphos (Fig. 1) is an organophophorus insecticide, which acts by the inhibition of cholinesterase activity. It is used as a pesticidal spray for control of flies [7] and cockroaches in warehouses and and in veterinary medicine. Azamethiphos has been found to be effective against psocids belonging to the genus lipocelis, which are serious pests of the stored commodities [8].

Solid phase microextraction technique (SPME) was first developed in 1989 at the University of Waterloo (Ont., Canada) by Pawliszyn and co-workers (Belardi and Pawliszyn) [9,10]. It is a preconcentration technique that has been applied to determine several environmental pollutants in the aqueous samples and first used with gas chromatography. Combined to HPLC, SPME was first used for the determination of polycyclic aromatic hydrocarbons (PAHs) [11]. Desorption of the analyte from the fiber is done in the specially built desorption chamber (Fig. 2) of the SPME–HPLC interface. There are two ways for desorption of the analyte from the fiber. When the analyte is not strongly adsorbed on the fiber, dynamic mode of desorption is sufficient, here analyte can be removed by moving stream of mobile phase. But when the analytes are more strongly adsorbed on the fiber, the fiber is dipped in the mobile phase or other strong solvent for specified time, and desorption performed in this way is known as static [12,13]. The aim of the present work was to elucidate

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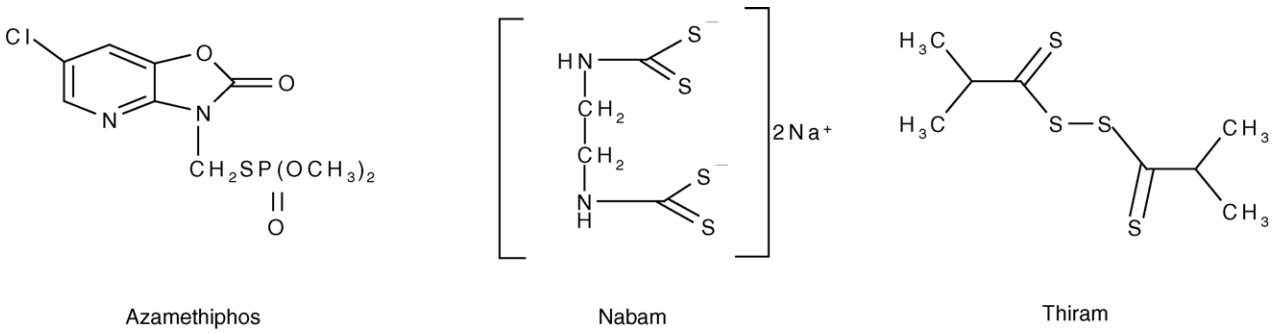

Fig. 1. Structures of the Azamethiphos, Nabam and Thiram.

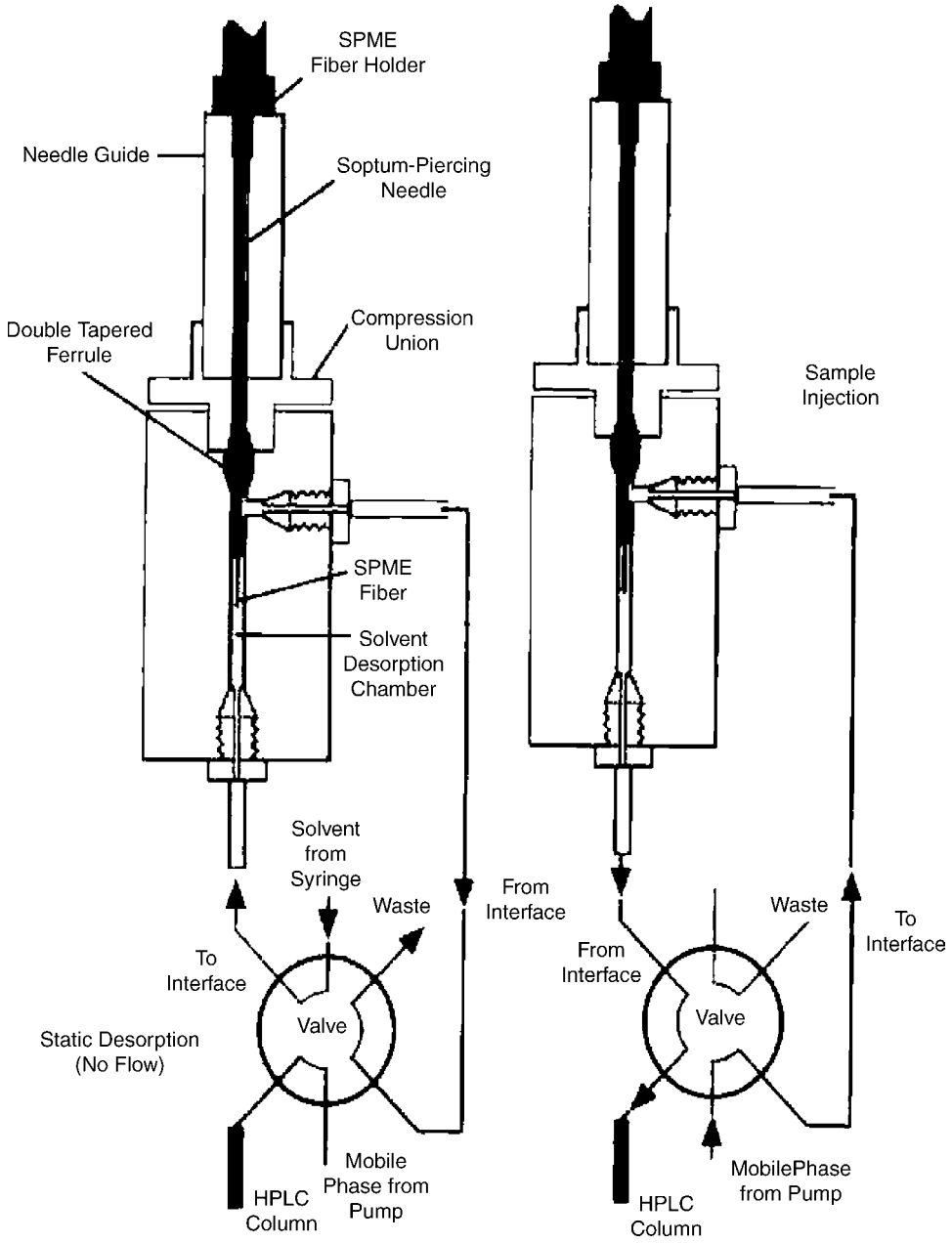

Fig. 2. Desorption chamber of SPME–HPLC interface (reproduced with permission from Supelco).

the behavior of polydimethylsiloxane (PDMS) fibers and to develop methods for the rapid analysis of Nabam, Thiram and Azamethiphos in water samples.

2. Experimental

Instrumentation: The HPLC system consists of high-pressure pumps (Waters 501, 1985) for pushing the mobile phase at high pressure controlled by the gradient controller. A Rheodyne valve was used as a interface for sample injection, C_{18} bond pack column (5 μm, 250 mm, 4.6 mm i.d.) with C_{18} guard column, a flow rate of 0.7 ml/min with water:acetonitrile (30:70) in isocratic mode were used for the separation. Chromatographic data were collected using Waters 484 UV absorbance detector and recorded with a Winchrom data station (INDTECH instruments). The injection volume was 60 μl.

2.1. Reagents

Thiram was obtained from CDH Laboratories, New Delhi (laboratory reagent), and its purity was checked by melting point (155.5 °C). Nabam and Azamethiphos were obtained from Reidel de Haën (Seelze, Germany). Acetonitrile (Thomas Baker, Mumbai; HPLC grade) and water (Spectrochem, Mumbai; HPLC grade) were used. All the solvents and solutions were filtered through filter papers (Millipore, USA) 0.45 μm (for aqueous solvents) and 0.5 μm (for organic solvents). Stock solution of the Thiram and Azamethiphos (0.4 mg/ml) were prepared in acetonitrile and Nabam (0.4 mg/ml) in water. The solutions were further diluted using acetonitrile:water (70:30). These solutions were filtered through the filters (Millipore; 0.45 μm pore size) and stored in an amber colored bottle. Further dilutions were done with acetonitrile:water (70:30) as desired.

2.2. Optimizations

The separation behavior of these pesticides was studied by direct injection of the samples, and the different parameters like selection of a suitable wavelength, effect of flow rate and composition of the mobile phase were optimized. The mobile phase acetonitrile:water (70:30) was selected for the best separation of the these pesticides. It was observed that high concentration of acetonitrile in the mobile phase caused overlapping of the peaks but lower concentration of acetonitrile in the mobile phase caused peak broadning. A wavelength of 254 nm was selected for the measurements. A PDMS fiber of 100 μm coating was used for the SPME extraction. An extraction time of 30 min and desorption time of 5 min was selected.

2.3. SPME procedure

Fibers were conditioned before the use; this conditioning was performed by keeping the fiber in the mobile phase for 30 min at 50 °C. Fiber blank was run after the conditioning so as to confirm that there were no peak that could be assigned to the compounds introduced during the manufacture of the fiber. The conditioned fiber was put into the spiked water sample and kept under stirring for a period of 30 min at room temperature. NaCl was added to increase the extraction of the sample. Desorption chamber of the SPME was filled with the desorption solvent (here it was mobile phase). Then the fiber was placed into the desorption chamber of the SPME in the load position of the injector. The fiber was kept for 5 min in desorption chamber before changing the knob to inject mode in case of static desorption and no time lapse was made in case of the dynamic mode.

3. Results and discussion

3.1. Characteristics of the calibration curve

Under the optimum conditions developed above the calibration curves were constructed for the determination of Nabam, Thiram and Azamethiphos using SPME-HPLC with UV detection by spiking the water samples in the range of 20–1 ppm of these pesticides. Characteristics of the calibration curves are summarized in Table 1. A characteristic chromatogram is shown in Fig. 3 and an overlay of the direct injection and SPME–HPLC with UV detection at 254 nm is shown in Fig. 4.

3.2. Effect of temperature

Effect of temperature was studied on the extraction of pesticides from the water samples, which were spiked at

Table 1
Characteristics of the calibration curve for Nabam, Thiram and Azamethiphos

Parameter	Nabam	Azamethiphos	Thiram
R^2	0.942	0.9384	0.996
Y	11.72x + 4.6359	4.38x + 2.0452	36.4x − 5.0162
m	11.72	4.38	36.4
Linear range (ng/ml)	20–600	10–600	5–600
LOD (ng/ml, $S/N = 3$)	10	10	1

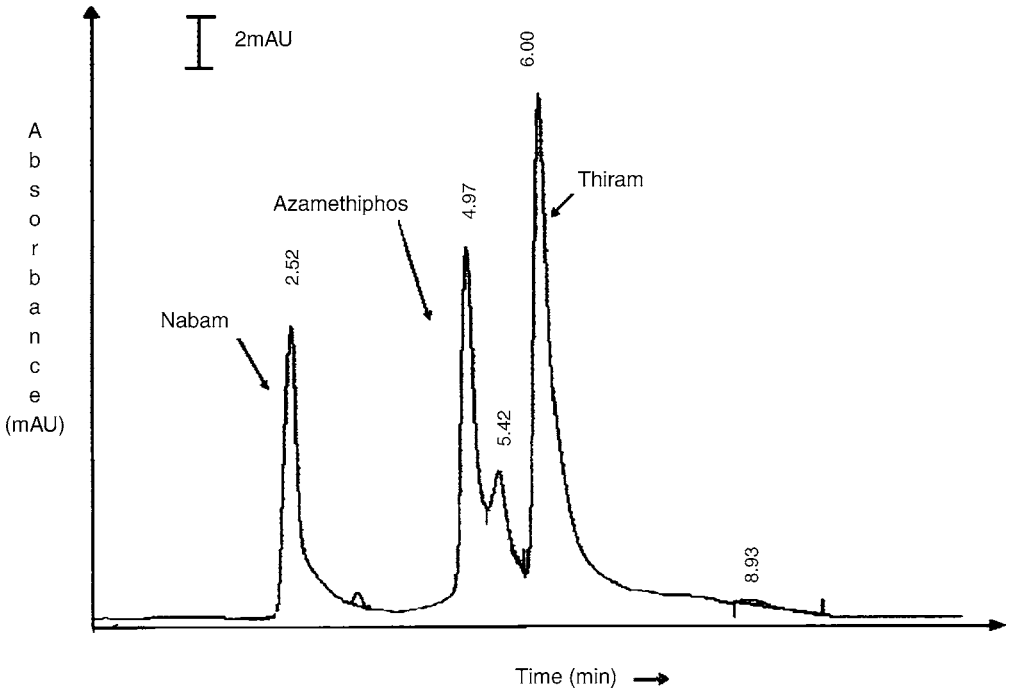

Fig. 3. HPLC–UV chromatogram for the separation of Nabam, Azamethiphos and Thiram (0.5 ppm each) at the flow rate of 0.7 ml/min using mobile phase in ratio of acetonitrile:water 70:30 detection at 254 nm.

0.1 μg/l level. The extractions were performed at 30, 45 and 60 °C. There was a slight increase in the extraction of the pesticides till 45 °C and then a decrease was observed. This is because the adsorption of the analyte onto a fiber is an exothermic process and also solubility of the Nabam, Thiram and Azamethiphos in the sample solution increases with the temperature. Rest of the studies were performed at room temperature.

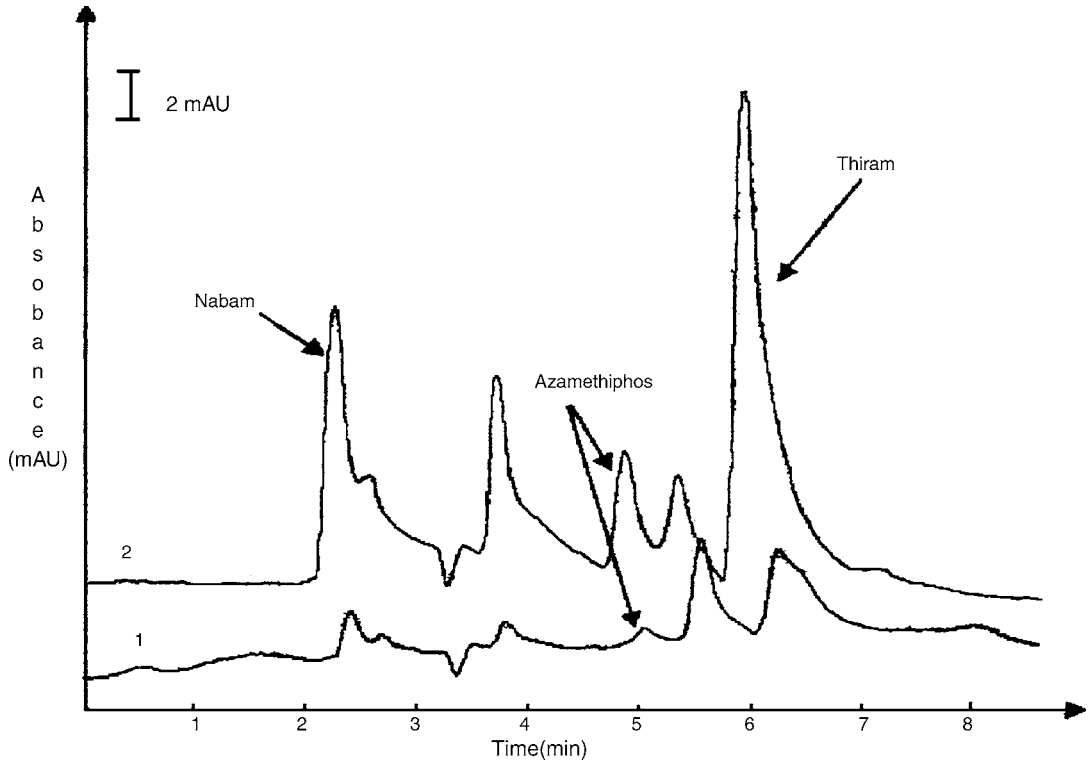

Fig. 4. Overlay of the HPLC–UV spiked tap water sample 0.08 ppm at wavelength 254 nm (1) direct injection (2) SPME–HPLC–UV. Rest of the conditions are same as in Fig. 3.

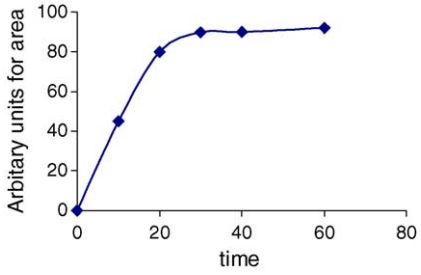

Fig. 5. Effect of time for the extraction of the Thiram using the PDMS fiber from the spiked water samples (Thiram, 0.1 mg/l). Rest of the conditions are same as in Fig. 3.

3.3. Effect of time on the extraction and desorption behavior

The water samples spiked at 0.1 ppm were examined for the study of extraction time. The effect was studied by recording the peak area versus the extraction time. As shown in Fig. 5, the adsorption reaches its maximum in 30 min. Desorption time is also very important parameter to insure the complete desorption and hence increased sensitivity. Desorption increases with time in static mode. The results shows that 5 min time was sufficient for the complete desorption (Fig. 6), hence was used through out.

3.4. Effect of salt addition

Effect of the addition of the salt sodium chloride (1–10 g) in the water samples, which were spiked with Nabam, Thiram and Azamethiphos (0.1 ppm), was studied. Increase in the extraction was observed as the salt concentration was increased. This is because the increase in the salt concentration reduces the analyte solubility [14]. Therefore, the experiments were performed by the addition of 5 g of NaCl.

3.5. Effect of nature of the fiber

Polydimethylsiloxane was used for the extraction. It was observed that 100 μm PDMS fiber was satisfactory for extraction and hence was used for the studies.

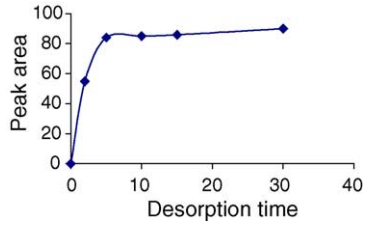

Fig. 6. Effect of time for desorption of Thiram from the PDMS fiber after dipping in the spiked water samples (Thiram, 0.1 mg/l). Rest of the conditions are same as in Fig. 3.

Table 2
Determination of Nabam, Thiram and Azamethiphos in spiked tap water samples

Pesticide	Added (ng/ml)	Found (ng/ml)	R.S.D. (%)
Nabam	19.4	19.3	3.3
	40.0	38.4	3.1
Azamethiphos	20.0	19.1	3.5
	40.0	38.2	3.2
Thiram	20.0	19.5	2.8
	40.0	38.6	2.4

Each reading is the mean of three experiments.

3.6. Application to tap water samples

We tested the performance of the method using the tap and tube-well water samples obtained from the Guru Nanak Dev University, Amritsar, Punjab, India. Absence of various peaks in the chromatogram at the retention time of compounds studied verified the absence of these compounds. No interfering peaks appeared at the retention time of the compounds studied. These water samples were spiked at 10 and 20 ng/ml with Nabam, Azamethiphos and Thiram. The general procedure was applied for the analysis these pesticides. The results of the determination are given in Table 2.

4. Summary

SPME combined with HPLC–UV was found to be suitable for the determination of the Nabam, Thiram and Azamethiphos in the water samples. The various parameters of adsorption and desorption of these pesticides for adsorption and desorption in SPME–HPLC were optimized. The static desorption mode gave better results than the dynamic mode for all the compounds. SPME allows the detection of concentration down to 1 μg/l ($S/N = 3$). Matrix effects do not interfere with the quantitation process.

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