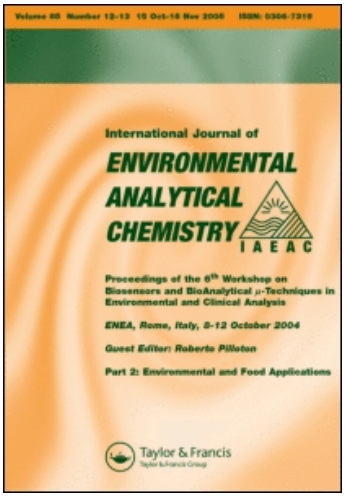

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LEACHING OF TRIAZINE PESTICIDES IN LOAMY SOIL AND THEIR DETERMINATION BY REVERSED PHASE HPLC

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The separation and identification of triazine pesticides (ametryn, atrazine, cyanazine and simazine) was carried out on Nova Pak C_{18} column (150 × 3.9 mm). The mobile phase used was acetonitrile-water (65:35, v/v) adjusted to pH 4.5 with acetic acid. The flow rate of the mobile phase used was 1.0 mL/min. The detection of the pesticides was carried out at 250 nm. The values of the separation factor (α) were in the range of 1.49–5.32 and the values of the resolution factors (R_s) were ranged from 1.18 to 2.99 for the separated pesticides. The developed HPLC method was used to determine the concentrations of the reported pesticides in the loamy soil samples. The recovery of the pesticides from soil samples was found to be about 50%. The relative standard deviation and limit of detection were in the range of 0.01–0.02 and 0.5–1.0 μg/mL respectively.

Keywords: Triazine pesticides; Amytrin; Atrazine; Cyanazine; Simazine; Soil samples; HPLC

INTRODUCTION

Pesticides are dangerous and harmful substances because of their tissue degradation when introduced into the body[1]. Pesticides are bioaccumulative and relatively stable, as well as toxic or carcinogenic, and therefore require close monitoring. Thus, European countries and the US EPA

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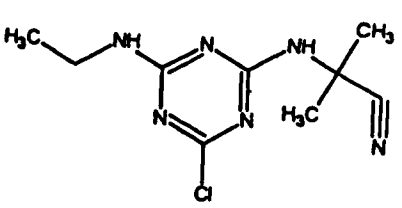
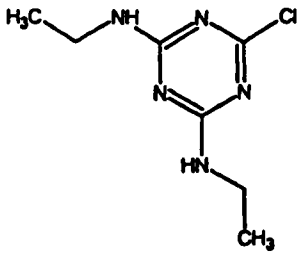
(Environmental Protection Agency) have established legal limits of 0.1 μg/L for individual pesticides and 0.5 μg/L for the sum of the pesticides present in water[2–4]. Ametryn, atrazine, cyanazine and simazine belong to triazine group of pesticides (Fig. 1). These pesticides are weak bases and they are most widely used in agricultural activities and are also most persistent in the environment[5–7].

In spite of the hazardous effects of pesticides, their use has increased in the recent years, particularly in agriculture and forestry activities[4,8,9]. It has been reported that increasing amounts of pesticide residues may be present in the soil and these can ultimately be leached to aquifer levels and hence contaminate the ground water, or they may be carried away by run-off water and soil erosion[10,11] resulting in the contamination of the surface water. Therefore, pollution of ground and surface water due to pesticides



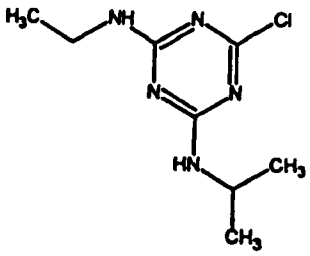
cyanazine

I



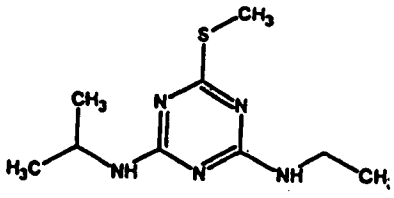
simazine

II



atrazine

III



ametryn

IV

FIGURE 1 The chemical structures of the triazine pesticides used in this study.

has become a worldwide problem. Due to the toxic and carcinogenic nature of pesticides, their detection at trace level in water, soil and other biological samples is of great importance. Chromatographic methods[12,13] have been used widely for the determination of pesticides in different samples. These methods include high performance liquid chromatography (HPLC) and gas chromatography (GC). HPLC has many advantages over GC method because HPLC permits the simultaneous analysis of acidic, basic, neutral, ionic and thermally unstable pesticides. Additionally, derivatization is also not required in HPLC. Keeping all the above facts in consideration, attempts have been made to develop a suitable HPLC method for the determination of ametryn, atrazine, cyanazine and simazine pesticides. The developed method was used for the detection of the reported pesticides in the soil samples.

EXPERIMENTAL

Chemicals and reagents

Ametryn, atrazine and simazine were obtained from Supelco Chem. Co., Bellefonte, USA and cyanazine was purchased from Ciba-Geigy Co., Greensboro, USA. The solutions (0.01 mg/mL) of the individual and the mixture of the pesticides were prepared in double distilled water. Acetonitrile of HPLC grade was purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Acetic acid was purchased from Sigma Chem. Co., USA.

Instruments used

The HPLC system consisting of Waters solvent delivery pump (model 510, Milford, Massachusetts, USA), Waters injector (model WISP 710B), Waters tunable absorbence detector (model 484) and Waters integrator (model 740) was used for the analysis of pesticides. The column used was Nova Pak C_{18} (150 × 3.9 mm) and was obtained from Waters (Milford, USA). The pH was adjusted with the pH meter (model 611, Orion Research Inc., USA).

Chromatographic conditions

20 μL of the mixture of these pesticides was injected on to an HPLC system as described above. The mobile phase used in this study was acetonitrile–water (65:35, v/v) adjusted to 4.5 pH with acetic acid. The effect of the pH

on the separation was carried out by adjusting the pH of the mobile phase by acetic acid and triethylamine. The mobile phase was filtered and degassed before use. The flow rates of the mobile phase was 1.0 mL/min. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at $23 \pm 1°C$. The detection was carried out at 250 nm. The chromatographic parameters such as retention factor (k), separation factor (α) and resolution factor (R_s) were calculated. The identification of the separated pesticides of the mixture was carried out by running the chromatograms of the individual pesticides under identical conditions. The retention times of the individual pesticides were compared with the retention times of the pesticides in the mixture. Further, the identification was confirmed by the internal addition method. The quantitative analysis of these pesticides in the soil samples was carried out by comparing the peak areas of the individual pesticides with the peak areas of the pesticides in the mixture.

Extraction of the pesticides from soil samples

The developed chromatographic system was used to separate and identify the pesticides in the soil samples. A field of loamy soil (60×60 cm) was selected and the spraying of the mixture (100 mL) of these pesticides (100 mg/L each) was done. The field was irrigated three times at an interval of 30 days each. The soil samples were collected from the surface at 15, 30, 45 and 60 cm depths respectively. Water from the collected soil sample was removed by pressing the soil samples in a pressure machine. The soil samples were dried at room temperature and the pesticides were extracted from these collected soil samples with distilled water. The soil samples (10 g) were shaken with distilled water (100 mL) three times and filtered through Whatman 24 filter paper separately. The three filtrates for each sample were combined. These filtrates were concentrated under vacuum. The concentrations of the pesticides in the concentrated filtrates were determined by HPLC.

RESULTS AND DISCUSSION

The retention factor (k) for the separated triazine pesticides are given in Table I while the values of the separation factor (α) and the resolution factor (R_s) for all these separated pesticides are reported in Table II. The typical chromatograms of the separated pesticides are shown in Fig. 2.

TABLE I The retention factors (k) for the separated cyanazine, simazine, atrazine and ametryn pesticides on Nova Pak C_{18} column (150 × 3.9 mm) using mobile phase acetonitrile–water (65:35, v/v), adjusted to pH 4.5 with acetic acid, at 1.0 mL/min flow rate

Pesticides	*Retention factor (k)*
Cyanazine	2.60
Simazine	6.08
Atrazine	9.03
Ametryn	13.83

For details see Experimental section.

TABLE II The separation (α) and resolution factors (R_s) for the separated cyanazine, simazine, atrazine and ametryn pesticides on Nova-Pak C_{18} column (150 × 3.9 mm) using mobile phase acetonitrile–water (65:35, v/v), adjusted to pH 4.5 with acetic acid, at 1.0 mL/min flow rate

Pesticides	*Separation Factor (α)*	*Resolution Factor (R_s)*
Cyanazine + Ametryn	5.32	2.99
Cyanazine + Atrazine	3.47	2.57
Cyanazine + Simazine	3.04	1.39
Simazine + Ametryn	2.28	2.07
Simazine + Atrazine	1.49	1.18
Atrazine + Ametryn	1.53	1.28

For details see Experimental section.

It is clear from Tables I and II that all the studied pesticides were successfully base lined separated. The order of the elution of these pesticides was confirmed by running the chromatograms of the individual pesticides under the identical chromatographic conditions. The chromatograms of the pesticides of the mixture were identified by comparing their retention times with the retention times of the individual pesticides. The confirmation of the pesticides in the mixture was also carried out by the internal addition method. The order of the elution was cyanazine > simazine > atrazine > amytrin (Table I).

To optimize the chromatographic conditions, mixtures of various alcohols, acetonitrile, hexane, diethylamine, acids etc. were tested. As a result of extensive experiments, the optimized chromatographic conditions were developed and reported herein. The effect of pH of the mobile phase on the separation of the reported pesticides was also carried out between 3 and 8 pH. The pH was adjusted with triethylamine and acetic acid. It has been observed that the best separation of these pesticides was observed at 4.5 pH.

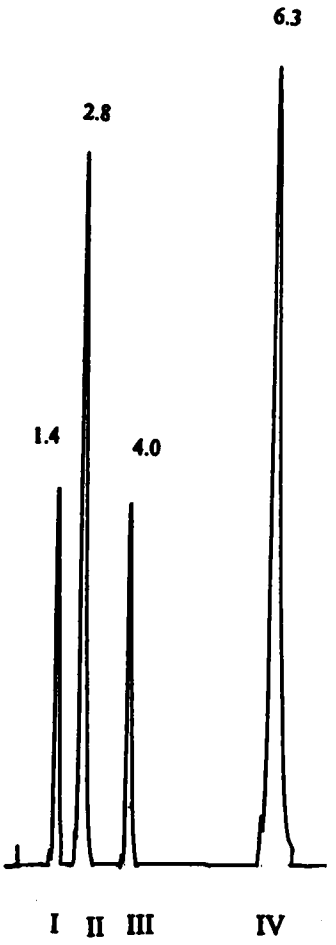


FIGURE 2 The chromatograms of the separated triazine pesticides (Fig. 1) on Nova Pak C_{18} column (150 × 3.9 mm) using mobile phase acetonitrile–water (65 : 35, v/v), adjusted to 4.5 pH with acetic acid, at 1.0 mL/min flow rate. I to IV: The separated chromatograms of the triazine pesticides (Fig. 1).

The stationary phase in Nova Pak C_{18} column is silica gel bonded to octadecyl carbon chain. Therefore, the separation of these pesticides on this column may be explained simply as the adsorption and the partition of the pesticides between the mobile and stationary phases. The pesticides, having different physical and chemical properties, also have different adsorption and partition properties which resulted in their separation under the reported chromatographic conditions. Figure 1 indicates that these pesticides contain amino, halogen and sulfur groups which may

TABLE III The concentrations of the pesticides (mg/kg) in the soil samples at different depths

Sl. No.	*Soil Depths* (cm)	*Pesticides*			
		Ametryn	*Atrazine*	*Cyanazine*	*Simazine*
1.	0	0.12	0.11	0.10	0.09
2.	15	0.10	0.09	0.08	0.06
3.	30	0.07	0.07	0.06	0.05
4.	45	0.05	0.05	0.04	0.03
5.	60	0.02	0.02	0.01	0.01

For details see Experimental section.

form hydrogen bonding with the silica oxygen of the stationery phase. Besides, dipole-induced dipole interactions and van der Waals forces also control the separation of the pesticides under the reported chromatographic conditions.

The concentrations of the four pesticides in the soil samples at different depths are reported in Table III. The concentrations of all the pesticides in the soil samples at different depths are in the range of 0.09–0.12, 0.06–0.10, 0.05–0.07, 0.03–0.05 and 0.01–0.02 mg/kg of soil at 15, 30, 45 and 60 cm depths respectively (Table III). The total concentrations of the recovered pesticides in $60 \times 60 \times 60$ cm cube of soil was calculated. It was found that about 50% of the pesticides were recovered (by extraction with distilled water) from the soil samples. Therefore, it may be concluded that about 50% of the pesticides has been adsorbed in the soil. Besides, the degradation of the pesticides in the environment may be responsible for this finding. It is also interesting to note that the presence of the pesticides at different depths of the soil indicates the leaching and distribution of these pesticides. The relative standard deviation (RSD) was also calculated for these pesticides and it was in the range of 0.01–0.02. The lower limit of detection of these pesticides was also determined and it varied from 0.5 to 1.0 μg/mL. The efficiency of the developed HPLC system was tested by running the chromatograms of the pesticide samples from soils three times. The recovery (%) of the pesticides with the corresponding replicates was between 99.5 and 99.8%.

CONCLUSION

It is clear from this study that the developed HPLC system is suitable for the separation and identification of the reported pesticides. The developed

HPLC method has been used to separate and identify these pesticides in the loamy soil samples. The reported HPLC system is fast, reproducible and simple. The presence of the pesticides at different soil depths indicates the distribution and the leaching of the pesticides. About 50% recovery of the pesticides in the soil samples shows the adsorption and degradation nature of these pesticides.

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