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Note

Separation of atrazine and some of its degradation products by high-performance liquid chromatography

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Environmental studies on the fate of herbicides in soils have raised much interest in recent years[1–3]. Atrazine is one of the *s*-triazine group of herbicides which are currently being used world-wide[4]. Uncontrolled factors such as rainfall and temperature affect the atrazine degradation rate in certain soils[1], necessitating the measurement of residual levels before rotational crops can be planted. High-performance liquid chromatography (HPLC) is eminently suitable for monitoring the fate of small amounts of such herbicides and their degradation products, especially in complex mixtures such as soil extracts[5,6]. Often no elaborate pretreatment[7] or derivatization is necessary as for gas chromatography (GC)[1,8]. The HPLC behaviour of atrazine has been studied on amino[9], cyano[10] and reversed-phase (C_{18})[11] columns. Atrazine and its derivatized degradation products have also been studied by GC[1]. This paper describes the determination of atrazine and its underivatized degradation products by reversed-phase (C_8) HPLC.

MATERIALS AND METHODS

Atrazine and its degradation products were kindly donated by Ciba Geigy (Johannesburg, South Africa). Methanol, acetic acid and ammonium acetate of the highest quality were obtained from Merck (South Africa), while water was obtained from a Millipore Milli-Q system. The compounds investigated are listed in Table I and were injected individually as 100 ppm solutions, or as a mixture containing 10 ppm of each, with a 20-μl sample loop.

An HPLC system comprising a Beckman Model 322 Gradient Liquid Chromatograph with a fixed (254 nm) wavelength detector was used. Ultrasphere octyl (25 × 0.4 cm) columns were employed at ambient temperature and a constant flow-rate of 1 ml/min. Investigations at 220 nm were performed on a Spectra Physics SP 8000 B liquid chromatograph equipped with a Model SP 8400 UV–visible variable-wavelength detector using the same operating conditions as above. The water and metha-

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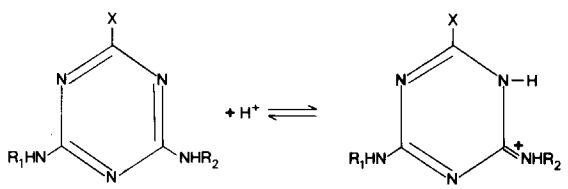
nol solvents were either neat or fortified with 1 % acetic acid or 50 m*M* of ammonium acetate. When ammonium acetate was used the pH was adjusted to 7.4; for the methanol phase, acetic acid, and for the water phase, ammonium hydroxide, was used to adjust the pH.

The pK_a values of the *s*-triazine compounds were determined spectrophotometrically[12] on a Beckman Spectrophotometer Acta Model MVI and are listed in Table I. The absorption spectra of the atrazine derivatives were determined in methanol at a concentration of 6.25 μg/ml and are shown in Fig. 5.

A sandy loamy soil obtained from the University of Pretoria's experimental farm was spiked with 5 ppm of atrazine and each of its degradation products. The spiked soil samples were extracted with a 10 % water–acetonitrile solution[5,7] and the extract filtered through a Whatman No. 1 filter-paper. Three ml of the filtrate were evaporated in a stream of nitrogen. The residue was dissolved in 3 ml methanol, filtered through a 0.45-μm Millipore filter and injected (20 μl) as such without further purification.

RESULTS AND DISCUSSION

Initial studies on the separation of atrazine and its degradation products by reversed-phase HPLC indicated that a column with a C_8 bonded stationary phase should be used in preference to a C_{18} bonded stationary phase, since some of the derivatives were too polar to be retained on the latter column. Furthermore, these initial studies also revealed that tailing of the hydroxyatrazines could be improved by addition of 1 % acetic acid to both solvents. This problem could also be completely eliminated by addition of 50 mmol ammonium acetate per litre of each solvent and adjusting the pH to 7.4. This could be expected since *s*-triazines are weakly basic polar compounds which dissociate in aqueous solutions according to eqn. 1:



(1)

if X = Cl pKa ≃ 2 $R_1 = -CH_2-CH_3$

if X = OH $pKa_1 \simeq 5$ $R_2 = -CH(CH_3)_2$

Thus the separation of atrazine and its derivatives should be influenced by a change in pH. The principle of suppressing or enhancing ionization to improve tailing of peaks is well established[13,14]. From the dissociation constant (pK_a) values in Table I it can be seen that these compounds can be divided into two groups, namely the chloro- and hydroxyatrazines, with pK_a about 2 and 5, respectively.

The dissociation constant for the hydroxy group on the hydroxyatrazines is about 11. This group would therefore remain undissociated in the pH range of 2–8, wherein silica-based columns can be used. The dependence of k' of these derivatives on solvent strength of the mobile phase, containing water–methanol, both of which

TABLE I

STRUCTURES OF THE DEGRADATION PRODUCTS OF ATRAZINE USED

Name	*Abbreviation*	pK_a	R_1	R_2	*X*
Atrazine	ATRZ	1.71*	C_2H_5	$CH(CH_3)_2$	Cl
Deethylatrazine	DEA	1.65	H	$CH(CH_3)_2$	Cl
Deisopropylatrazine	DIA	1.58	C_2H_5	H	Cl
Hydroxyatrazine	HA	5.15**	C_2H_5	$CH(CH_3)_2$	OH
Deethylhydroxyatrazine	DEHA	4.57	H	$CH(CH_3)_2$	OH
Deisopropylhydroxyatrazine	DIHA	4.65	C_2H_5	H	OH

* Reported as 1.85 and 1.68 in ref. 12 and 16, respectively.

** A pK_a of 5.20 has been reported for 2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine in ref. 12.

contain 1% acetic acid, is shown in Fig. 1. The pH of these mixtures varies from 3.0 to 3.6 increasing with methanol concentration, as expected from the dependence of pK_a of weak acids on the dielectric constant of solvents. At these pH values the chloroatrazines should be neutral while the hydroxyatrazines should be protonated. The retention times of the neutral chloroatrazines decrease with increasing solvent strength. However, the retention times of the hydroxyatrazines increased with increasing solvent strength. These anomalous results can possibly be explained in terms of solubility effects. It is known that hydroxy-*s*-triazines are relatively more soluble in aqueous solutions of pH 3 rather than neutral pH as they

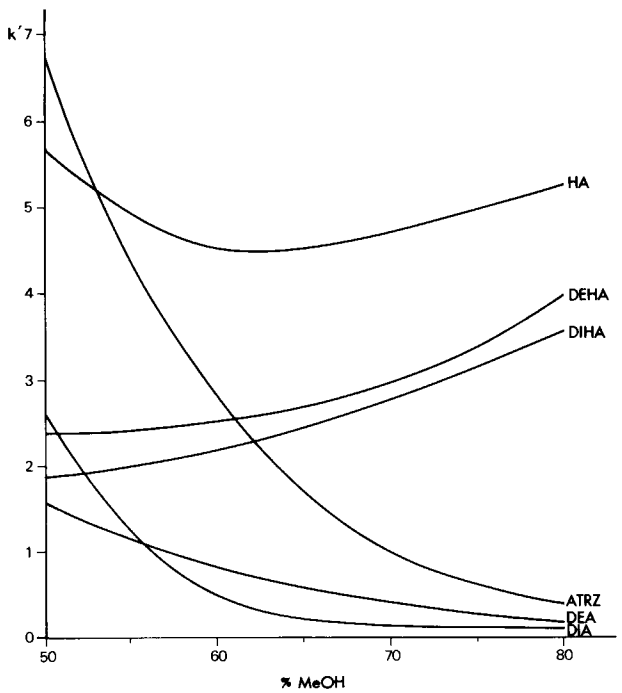


Fig. 1. Dependence of k' on the % methanol (MeOH) in the mobile phase. Both the methanol and water reservoirs contained 1% acetic acid, and the pH was 3.0–3.6.

are protonated at pH 3 (ref. 15). Reduction of the polarity of the solvent (increasing methanol concentration) should decrease their solubility.

Notwithstanding the fact that these molecules are positively charged, they are still retained on the column, as shown in Fig. 1. The differences in retention times between the hydroxyatrazine derivatives indicate that typical reversed-phase forces are probably operative, through the difference in their alkyl groups. That is, the retention times increase in the order of DIHA < DEHA < HA with two, three and five alkyl carbons respectively.

It was then decided to develop a solvent system where all the atrazine derivatives were present in the unprotonated form. This was achieved by adding 50 mmol of ammonium acetate to each litre of the two solvents and adjusting the pH to 7.4. The results of these investigations are shown in Fig. 2. Satisfactory separation of all these derivatives can be obtained with methanol–water (40:60), with ammonium acetate at pH 7.4, as shown in Fig. 3. The separation can be increased if necessary by decreasing the percentage of methanol.

The sensitivity of this method depends on the molar absorptivity of the compounds at a particular wavelength. The detection limits for atrazine at 220 and 254 nm were about 0.1 and 1 ppm respectively. From the spectra shown in Fig. 5, it can be seen that the detection limit of the other compounds will be of the same order. We were able to extract and determine atrazine and its degradation products from a soil sample which had been spiked with 5 ppm of each of these compounds, as shown in Fig. 4.

The linearity of the detector had been confirmed in the 1–10 ppm range for all

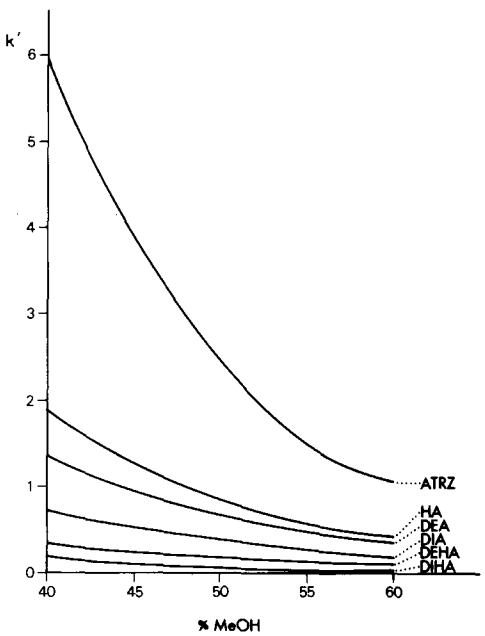
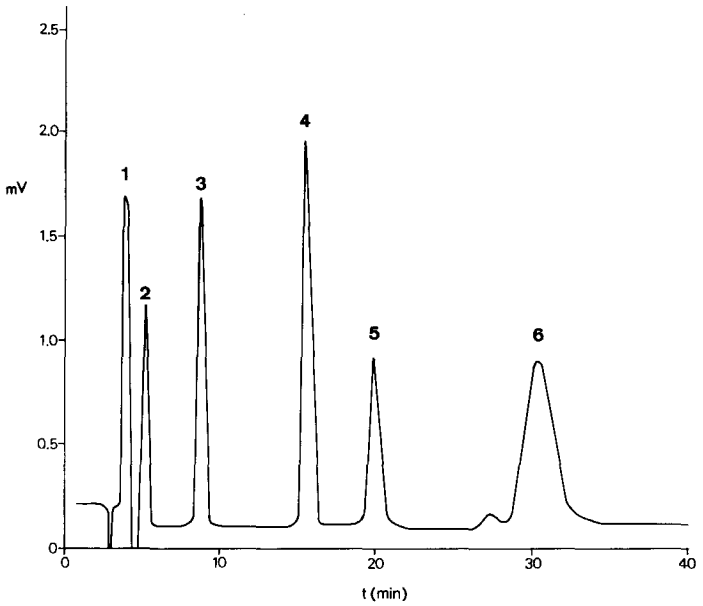


Fig. 2. Dependence of k' on the % methanol in the mobile phase. Both the methanol and water reservoirs contained 50 mM ammonium acetate and the pH was adjusted to 7.4.



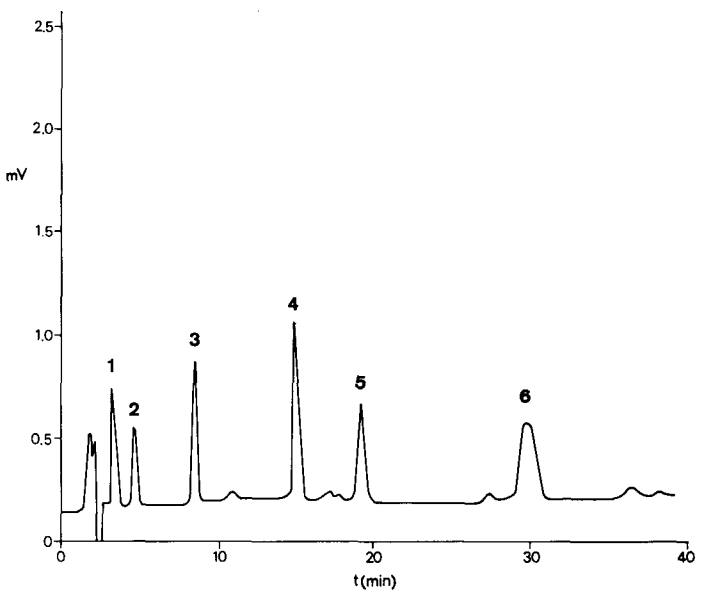


Fig. 3. Separation of atrazine and its degradation products on Ultrasphere octyl (C_8) stationary phase. Mobile phase: methanol–water (40:60) both solvents with 50 m*M* ammonium acetate at pH 7.4. Flow-rate: 1.0 ml/min. Pressure: 2000 p.s.i. Detection at 220 nm, each compound 10 ppm. Peaks: 1 = DIHA; 2 = DEHA; 3 = DIA; 4 = DEA; 5 = DHA; 6 = ATRZ.

Fig. 4. Separation of atrazine and its degradation products extracted from a soil sample which had been spiked with 5 ppm of each. Chromatographic conditions as in Fig. 3.

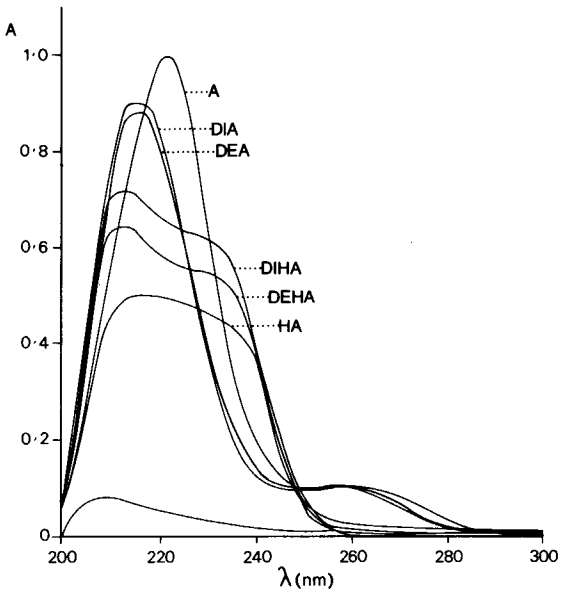


Fig. 5. Absorption spectra of atrazine (A) and its degradation products, 62.5 μg derivative per 10 ml methanol.

the derivatives. The recoveries of the spiked soil samples were 78.4 ± 4.2% for the atrazines and 72.5 ± 4.8% for the hydroxyatrazines. However, we have found that these recoveries vary with soil type.

CONCLUSION

This method can be used to measure residual amounts of atrazine and its degradation products in soil samples. It should be possible to measure most *s*-triazines and their degradation products in soil samples, as well as in plant and animal tissues, with slight modifications.

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