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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF s-TRIAZINES AND THEIR DEGRADATION PRODUCTS USING ULTRAVIOLET PHOTOMETRIC AND AMPEROMETRIC DETECTION

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SUMMARY

The chromatographic behaviour of eighteen s-triazine derivatives has been studied in a reversed-phase system with a Separon SIC 18 stationary phase and a mobile phase of aqueous sodium dihydrogenphosphate and methanol. The dependences of the capacity factors on the methanol content, the pH and the ionic strength have been obtained. Ultraviolet photometric detection can generally be used whereas amperometric detection with a carbon-fibre array electrode is useful for selective detection of s-triazine hydroxy derivatives, but with a sensitivity poorer than that of photometric detection. The method has been applied to a study of the photolysis of s-triazines and the effect of the substituents and the pH on the photolysis rate.

INTRODUCTION

s-Triazine derivatives are among the most important selective herbicides. They are degraded in soil through hydrolysis and the effects of UV radiation, oxidation, soil microorganisms, etc., leading primarily to dealkylation in positions 4 and 6 and hydrolysis of the substituent in position 2[1–4]. s-Triazines and their degradation products are highly resistant, survive many years in soil and thus analysis for them is important. Gas chromatography (GC), *e.g.*, refs. 3, 5–12 and high-performance liquid chromatography (HPLC), *e.g.*, refs. 12–22 have been routinely used for this purpose. GC has an high sensitivity and a good separation efficiency; down to tens of pg of s-triazines can be determined using a thermoionization detector[10,12]. Both packed[5–7] and capillary[8–10] columns can be used. A disadvantage of GC is its limitation to volatile s-triazines; the hydroxy derivatives cannot be analyzed directly and must be suitably derivatized[11]. HPLC is directly applicable both to s-triazines and their degradation products, on stationary phases such as silica gel[15,19] and chemically bonded C_{18}[12,15,20,22], CN[14] and NH_2[13] phases. Hydroxy derivatives of s-triazines have been separated in reversed-phase systems employing acidic and weakly alkaline mobile phases[22]. The sensitivities of various chromatographic methods, GC, HPLC, thin-layer chromatography (TLC) and HPTLC, for the analysis of s-triazine herbicide residues have been compared[12].

TABLE I

PHYSICO-CHEMICAL PROPERTIES OF THE s-TRIAZINES

No.	s-Triazine	Substituent in position			$pK_a^{5,12,24}$	Absorp. maximum (nm)*	Anodic half-wave potential $(V)^5$
		2	4	6			
1	Hydroxysimazine	OH	NHC₂H₅	NHC₂H₅	–	218	0.9*
2	Hydroxyatrazine		NHC₂H₅	NHCH(CH₃)₂	5.15	218	0.96*
4	Hydroxypropazine		NHCH(CH₃)₂	NHCH(CH₃)₂	5.20	217	0.96*
4	Desethylhydroxyatrazine		NH₂	NHCH(CH₃)₂	4.75	212	1.0*
5	Deisopropylhydroxyatrazine		NHC₂H₅	NH₂	4.65	213	1.0*
6	Simetone	OCH₃	NHC₂H₅	NHC₂H₅	4.15	222	1.79
7	Atratone		NHC₂H₅	NHCH(CH₃)₂	4.2	217	–
8	Desmetryne	SCH₃	NHCH₃	NHCH(CH₃)₂	3.93	221	1.78
9	Simetryne		NHC₂H₅	NHC₂H₅	4.0	222	1.79
10	Ametryne		NHC₂H₅	NHC₂H₅	4.0	222	1.77
11	Prometryne		NHCH(CH₃)₂	NHCH(CH₃)₂	4.05	223	1.83
12	Terbutryne		NHC₂H₅	NHC(CH₃)₃	4.40	223	1.78
13	Simazine	Cl	NHC₂H₅	NHC₂H₅	1.65	222	1.97
14	Atrazine		NHC₂H₅	NHCH(CH₃)₂	1.68	222	2.02
15	Propazine		NHCH(CH₃)₂	NHCH(CH₃)₂	1.85	221	1.95
16	Terbutylazine		NHC₂H₅	NHC(CH₃)₃	1.94	223	2.01
17	Trietazine		NHC₂H₅	N(C₂H₅)₂	1.88	227	1.77
18	Ipazine		N(C₂H₅)₂	NHCH(CH₃)₂	1.85	228	1.77

* In the mobile phase of 0.01 M sodium dihydrogenphosphate + 70% (v/v) methanol, pH 6.8.

In view of the importance of solar radiation in the degradation of *s*-triazines it is desirable to study the photolysis of these substances under defined conditions and HPLC seems to be most suitable for this purpose. So far, very little work has been done in this field. The selectivity of amperometric detection might be useful in such a study but, to our knowledge, has not yet been employed. For these reasons we have undertaken the present work. First it was necessary to optimize the separation conditions from the point of view of amperometric detection (a sufficiently conductive mobile phase). Then photometric and amperometric detection were studied in detail and compared. Finally, the method was applied to monitor the photolysis of *s*-triazines, under different experimental conditions.

EXPERIMENTAL

Reagents

The *s*-triazine derivatives (Ciba-Geigy, Switzerland) are listed in Table I. The other chemicals of p.a. purity were obtained from Lachema (Czechoslovakia) and were not further purified.

Apparatus

The liquid chromatograph consisted of a 2150 pump, a 2151 variable-wavelength spectrophotometric detector (both from LKB, Sweden), a Rheodyne 7125 sampling valve, a carbon-fibre array amperometric detection cell[23] with an EDLC electrometer and a dual TZ 4620 recorder (all from Laboratorní Přístroje, Czechoslovakia). A glass column (150 mm × 4.5 mm I.D.) was packed with Separon SIC 18, 5 μm (Laboratorní Přístroje). A mobile phase flow-rate of 0.3 ml min^{-1} was used and 20-μl samples with a concentration of around $5 \cdot 10^{-5}$ mol l^{-1} were injected, *i.e., ca.* 200 ng of the test substance in the injected volume.

Aqueous solutions of *s*-triazines were photolyzed by irradiation with an RVL-X 250 UV lamp (Tesla, Czechoslovakia; maximum emission at 250 nm) in quartz test-tubes placed in parallel with the lamp at a distance of 10 cm. The exposure times ranged from 1 to 120 min. The samples were then diluted five-fold in methanol and analyzed by HPLC. The effect of the pH on the photolysis was studied using *s*-triazine solutions in 0.01 *M* solutions of hydrochloric acid and sodium hydroxide and in distilled water.

The absorption spectra were obtained on an SP-800 instrument (Unicam, U.K.) and the pH was measured using a PHM 64 instrument (Radiometer, Denmark).

The mobile phase was deaerated by passage of helium. All the measurements were carried out at laboratory temperature (22 ± 2°C) and the electrode potentials are referred to the saturated silver–silver chloride electrode.

RESULTS AND DISCUSSION

s-Triazine separation

The capacity factors have been obtained for the *s*-triazines at various methanol contents, pH and the ionic strength of the mobile phase. The results are given in Table II and the typical dependences of the capacity factors are shown in Figs. 1
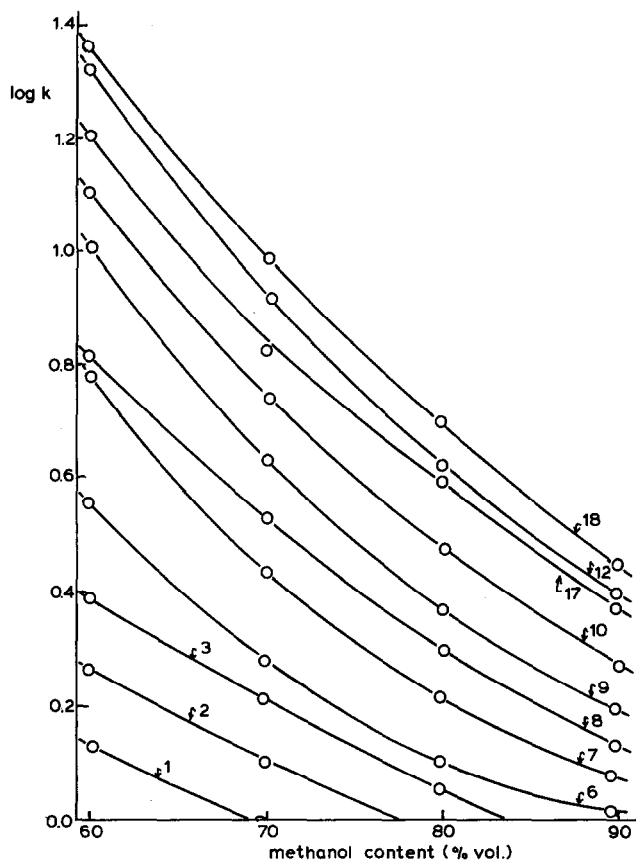
Fig. 1. Dependence of log k on the methanol content in the mobile phase of 0.01 M sodium dihydrogen-phosphate, pH 6.8. Compounds numbered as in Table I.

and 2. The substances with the same substituents in positions 4 and 6 and different substituents in position 2 exhibit retention times increasing in the order of decreasing polarity of the substituent. In the series of s-triazines with the same substituent in position 2, the retention increases with increasing number of carbon atoms in the substituents in positions 4 and 6, *i.e.*, with increasing substitution of the amino groups by alkyls. The optimum methanol content is 70% (v/v), except for degradation products of atrazine, with which the value is 40% (v/v)[22].

 s-Triazine derivatives are weak bases whose pK_a values range from *ca.* 1.6 for chloro derivatives to about 5 for hydroxy derivatives, through *ca.* 4 for methoxy and thiomethyl derivatives (see Table I). The dependence of the capacity factors on the mobile phase pH was measured from pH 3 to 7, with 70% (v/v) methanol in the mobile phase. The chloro derivatives are not protonated at these pH values and thus their retention is pH-independent. The retention times of thiomethyl, methoxy and hydroxy derivatives increase with increasing pH, the change being largest within the pK_a region (Fig. 2). A pH of 6.8 has been selected as optimal, as the differences in
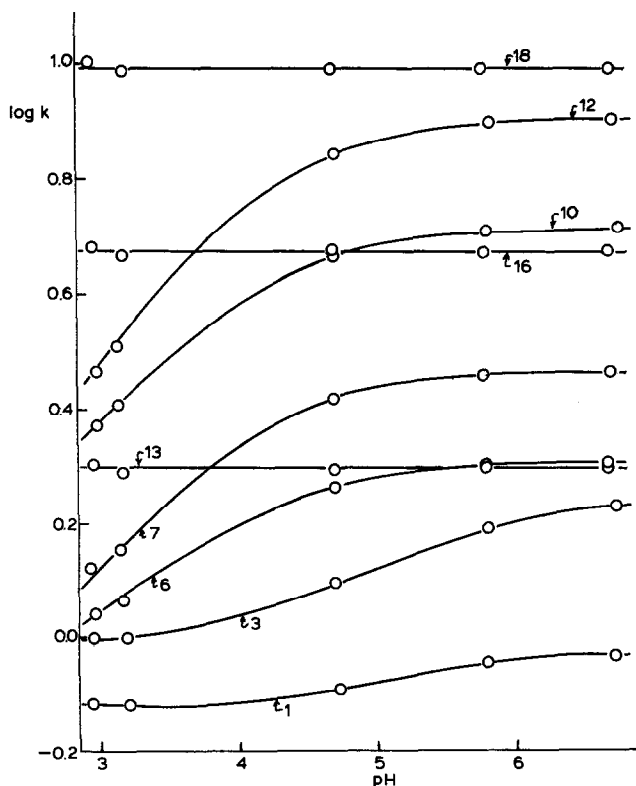
Fig. 2. Dependence of log *k* on the mobile phase pH. Mobile phase: 0.01 *M* sodium dihydrogenphosphate + 70% (v/v) methanol. Compounds numbered as in Table I.

the capacity factors are greatest. Another reason for the use of a neutral mobile phase was an improvement in the peak symmetry; in acidic mobile phases the peaks exhibit tailing, owing to *s*-triazine protonation. A neutral mobile phase was used analogously in ref. 22.

The dependence of the capacity factor on the ionic strength was measured in the mobile phases containing 70% (v/v) methanol and 0.001–0.05 *M* aqueous sodium dihydrogenphosphate. The retention of the substances with log *k* > 0.4 is unaffected by changes in the ionic strength, whereas the retention times of more rapidly eluted derivatives decrease with increasing ionic strength.

On the above basis, the mobile phase consisting of 0.01 *M* aqueous sodium dihydrogenphosphate and 70% (v/v) methanol, pH 6.8, has been chosen as optimal. An example of the separation of a mixture of *s*-triazines under these conditions is given in Fig. 3. As the photolysis products of *s*-triazines are better separated in the mobile phases containing only 40% (v/v) methanol, this lower methanol content was used to analyze them (see Fig. 4).

Detection

The UV absorption maxima of the test substances were determined in the

TABLE II

CAPACITY FACTORS OF s-TRIAZINE DERIVATIVES AT VARIOUS METHANOL CONTENTS

| s-Triazine	Methanol content (%, v/v)			
	60	70	80	90
Hydroxysimazine	1.334	0.998	0.759	0.671
Hydroxyatrazine	1.848	1.263	0.933	0.753
Hydroxypropazine	2.427	1.660	1.139	0.851
Desethylhydroxyatrazine	2.039*	1.287**		
Desisopropylhydroxyatrazine	1.141*	0.883**		
Simetone	3.602	1.935	1.243	1.047
Atratone	6.026	2.754	1.634	1.202
Desmetryne	6.526	3.362	1.980	1.349
Simetryne	10.154	4.331	2.317	1.556
Ametryne	12.618	5.435	3.027	1.871
Prometryne	17.906	7.244	3.828	2.296
Terbutryne	20.941	8.190	4.285	2.492
Simazine	3.261	2.011	1.259	1.003
Atrazine	5.346	2.754	1.633	1.131
Propazine	8.851	3.951	2.105	1.408
Terbutylazine	10.116	4.501	2.399	1.634
Trietazine	15.849	6.714	3.920	2.380
Ipazine	23.60	9.506	5.011	2.511

* 30% (v/v) methanol.
** 40% (v/v) methanol.
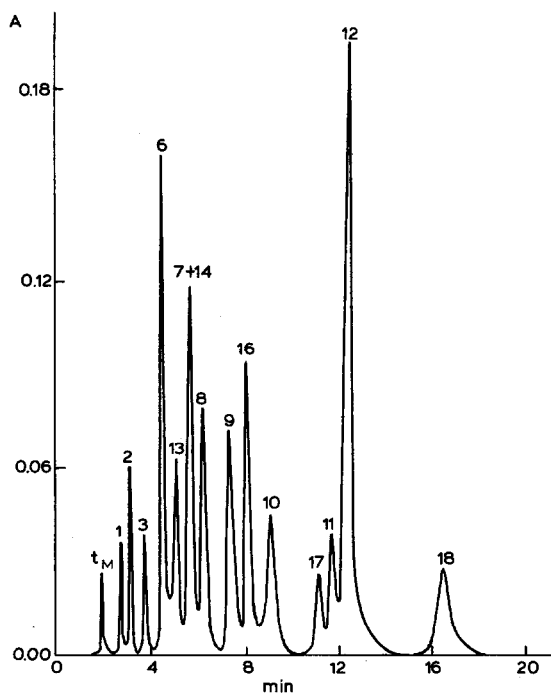
Fig. 3. Separation of s-triazine derivatives. Mobile phase: 0.01 M sodium dihydrogenphosphate, 70% (v/v) methanol, pH 6.8. Compounds numbered as in Table I.
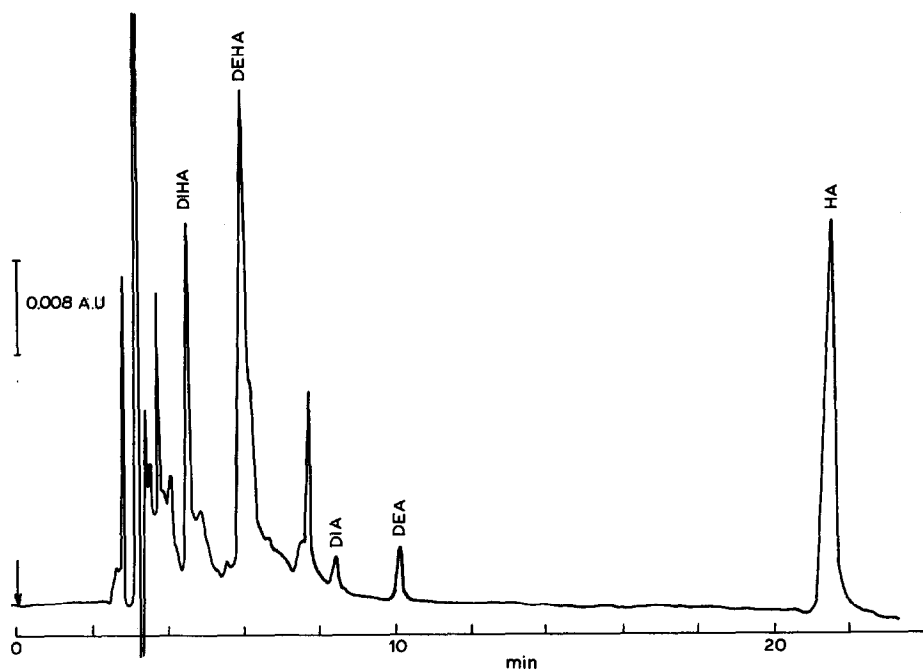
Fig. 4. Separation of photolysis products of atrazine. Mobile phase: 0.01 M sodium dihydrogenphosphate, 40% (v/v) methanol, pH 6.8. DIHA = Deisopropylhydroxyatrazine; DEHA = desethylhydroxyatrazine; HA = hydroxyatrazine; DIA = deisopropylatrazine; DEA = desethylatrazine.

optimum mobile phase and are given in Table I. The position and intensity of the absorption bands depend on the solution pH and the nature of the substituents in positions 2, 4 and 6 (refs. 5, 24). The main absorption maximum is located between 217 and 230 nm. Another, broader and lower maximum occurs at 235–271 nm.

The UV photometric detection employed a wavelength of 225 nm. The limits of detection, determined as the intercepts of the regression calibration plots with three times the standard deviation of the noise, are about 1–2 ng of the test substance in the injected volume, *i.e.*, 0.05–0.1 ppm. The dependence of the measurement precision on the amount injected can be seen from Table III.

TABLE III

STATISTICAL PARAMETERS FOR THE PHOTOMETRIC DETERMINATION

s-Triazine	Detection limit (ng)	Correl. coeff.	R.S.D. for		
			4 ng	40 ng	400 ng
Hydroxysimazine	2	0.998	35.6	1.9	3.0
Atratone	1	0.996	28.5	2.3	3.8
Atrazine	1	0.994	21.0	2.2	2.9
Prometryne	2	0.999	34.1	2.0	3.6

s-Triazine derivatives can be reduced at a dropping mercury electrode[25], but their electrochemical oxidation (except for hydroxy derivatives) is difficult. A single anodic wave with an half-wave potential of around + 1.7 to + 2.0 V is obtained in anhydrous acetonitrile (Table I), but the wave disappears in mixed media and acidic solutions[5]. Only hydroxy derivatives of s-triazines are electrochemically oxidized in the given mobile phase. The hydrodynamic voltammograms were measured in the optimum mobile phase and yielded the potential of + 1.1 V at which the highest signal-to-noise ratio was obtained. The limits of detection were ca. 50–100 ng. The amperometric detection does not attain the sensitivity of the UV photometric detection, but its selectivity may be advantageous in the analysis of the degradation products of s-triazine herbicides in complex matrices.

Photolysis

s-Triazines are degraded by solar radiation; dealkylation predominates in soils in the presence of fulvic acids, whereas hydrolysis to hydroxy derivatives is predominant in aqueous solutions in the absence of fulvic acids[26].

We have studied the photodegradation of atrazine, atratone, ametryne, desmetryne and prometryne, *i.e.*, s-triazines with various substituents in positions 2 and 4, by irradiating their aqueous solutions and analyzing them by HPLC. The dependences of the degree of degradation on the exposure time and the solution pH were followed.

It is seen from Fig. 5 that the photolysis rate depends strongly on the substituent in position 2 and increases in order methoxy < chloro ≪ thiomethyl derivatives.
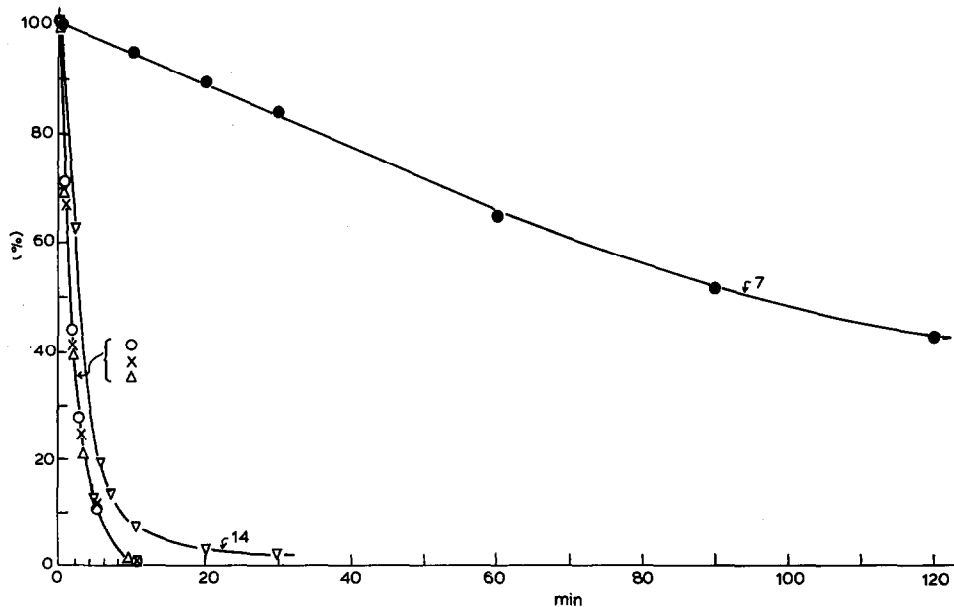

Fig. 5. Dependence of the degraded fraction of selected s-triazine derivatives on the photolysis time. Compounds numbered as in Table I.

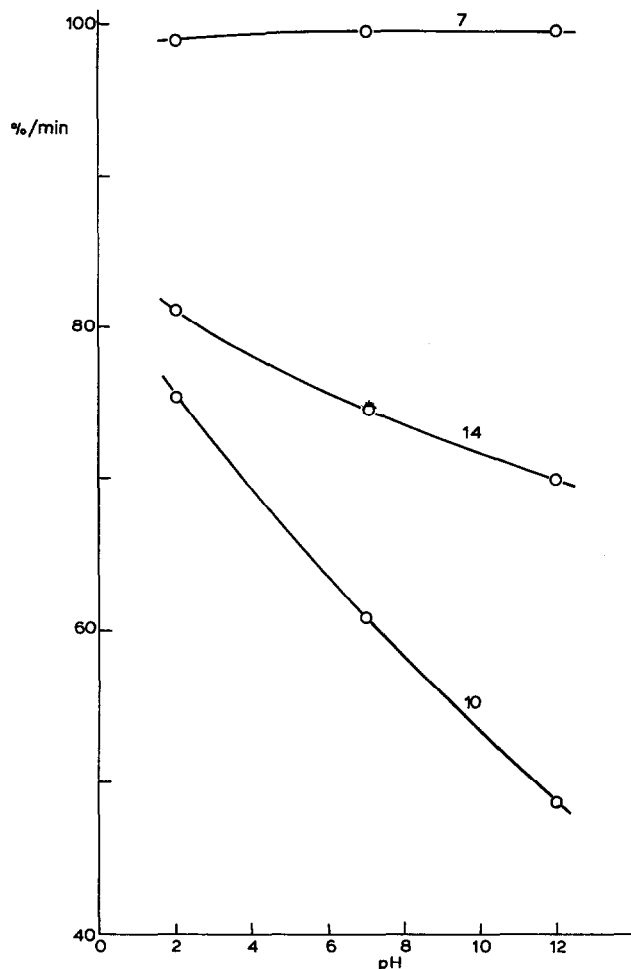Fig. 6. Dependence of the rate of photolysis for selected s-triazine derivatives on the solution pH. Photolysis times: atrazine (14), 4; ametryne (10), 2; atratone (7), 60 min.

A comparison of the photolysis of desmetryne, ametryne and prometryne, which differ only in the substituent in position 4, indicates that the photolysis rate is independent of the alkyl bound to nitrogen.

The dependence of the photolysis rate on the pH was studied for atrazine, atratone and ametryne. The rate decreases with decreasing pH for atrazine and ametryne and increases for atratone (see Fig. 6).

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