

CHROM. 12,314

## COMPARISON OF THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC BEHAVIOUR OF *s*-TRIAZINE DERIVATIVES ON VARIOUS STATIONARY PHASES

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(First received July 13th, 1979; revised manuscript received August 14th, 1979)

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### SUMMARY

The high-performance liquid chromatographic behaviour of 23 *s*-triazine derivatives was studied using LiChrosorb SI-5 as the stationary phase and 2-4% isopropanol in *n*-pentane as the mobile phase. The chromatographic data obtained were correlated with both the structures of the stationary phases and the polarities of the mobile phases that have been used so far for the analysis of *s*-triazines. Some of these relationships can be useful for the identification of *s*-triazines.

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### INTRODUCTION

The extensive use of *s*-triazine herbicides has stimulated a great interest in rapid and sensitive methods for their analysis. In addition to non-specific spectrophotometric methods<sup>1</sup>, paper chromatographic<sup>2</sup>, thin-layer chromatographic<sup>3,4</sup> and especially gas chromatographic (GC)<sup>5-12</sup> methods have been successfully applied in this field. GC plays a predominant role in the analysis of *s*-triazine residues. Using specific detectors<sup>9,10</sup>, subnanogram amounts of *s*-triazines can be determined in water, soil and grain by GC without prior purification of the extracts. The procedure has been fully automated<sup>11,12</sup>.

However, GC fails in some instances: some substances, *e.g.*, cyanatryne, decompose under the conditions used in GC and others, *e.g.*, 2-hydroxy derivatives, are so polar and therefore non-volatile that they cannot be analyzed by GC without prior derivatization. High-performance liquid chromatography (HPLC) seems an attractive alternative to GC as *s*-triazines absorb strongly in the UV region (the molar absorp-

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tivity is  $\epsilon = 3 \cdot 10^4 - 4 \cdot 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$  at  $\lambda_{\text{max}} = 220-240 \text{ nm}$ ) and the detection is sufficiently sensitive for the determination of residues over the required concentration range (0.005–0.1 ppm).

HPLC has not been used extensively for the analysis of *s*-triazine derivatives. Published work<sup>13–18</sup> deals chiefly with practical applications, always using the reversed-phase technique. Permaphase ETH (siloxane) has been used as the stationary phase for the analysis of terbutryne<sup>13</sup> and cyanatryne<sup>14,15</sup> with water–methanol as the mobile phase. Vitali *et al.*<sup>16</sup> separated 13 *s*-triazines on chemically bonded Zipax-ODS stationary phase with 5% aqueous methanol as the mobile phase. A reversed-phase system was used by Roth<sup>17</sup> and Jork and Roth<sup>18</sup>, who studied the effect of the mobile phase composition (methanol–water) on the separation of *s*-triazines on  $\mu$ Bondapak C<sub>18</sub> stationary phase. They compared various chromatographic methods and critically evaluated their advantages and disadvantages from the point of view of the practical determination of *s*-triazines in plant extracts.

In view of the importance of *s*-triazines in the environment, *s*-triazine herbicides have been studied systematically in our laboratories by various methods, namely spectrophotometry, electrochemistry, GC and GC combined with mass spectrometry<sup>5–8</sup>. The acid–base behaviour and the dissociation constants of a series of *s*-triazines were also studied<sup>10</sup>. The determination of *s*-triazine residues in soil has also been investigated<sup>6</sup>.

As some *s*-triazines could not be analysed by GC, HPLC was also used<sup>19,20</sup>. A number of *s*-triazines were not separated satisfactorily using the reversed-phase technique and therefore chemically bonded CN<sup>19</sup> and NH<sub>2</sub><sup>20</sup> stationary phases were employed in our earlier work. In this paper, the data obtained on LiChrosorb SI-5 stationary phase are given and the relationship between the structure of *s*-triazines, the structure of the stationary phase and the mobile phase composition is discussed.

## EXPERIMENTAL

### Materials

The *s*-triazine derivatives were obtained from Ciba-Geigy (Basle, Switzerland). Isopropanol (UV grade) was purchased from Lachema (Brno, Czechoslovakia) and *n*-pentane (p.a. grade) from Merck (Darmstadt, G.F.R.).

### Method

The HPLC measurements were carried out on a Varian Model 4100 liquid chromatograph equipped with a Variscan 635 UV detector. The wavelength selected for all measurements was 235 nm. A stainless-steel column (25 cm  $\times$  2.2 mm I.D.) was packed with LiChrosorb SI-5, particle size 5  $\mu\text{m}$ . *n*-Pentane containing 2–4% of isopropanol was used as the mobile phase, at a flow-rate of 30 ml/h for a 2% mobile phase and 18 ml/h for 3 and 4% mobile phases. The column was pre-tested by the manufacturer, showing 7800 theoretical plates for *m*-nitraniline at a flow-rate of 60 ml/h with *n*-hexane–dichloromethane–isopropanol (90:10:0.5) as the mobile phase.

The dead retention volume was determined in the same manner as in our previous paper<sup>19</sup> and a value of  $V_M = 0.72 \text{ ml}$  was obtained.

## RESULTS AND DISCUSSION

*s*-Triazines have similar chemical structures and offer wide possibilities for correlation of their structural characteristics with chromatographic data. A list of *s*-triazines studied and some of their physico-chemical characteristics are given in Table I.

The adjusted retention volumes,  $V'_R$ , and the capacity factors,  $k'$ , are given in Table II for the three compositions of the mobile phase. Optimal separation was achieved using 2% isopropanol in *n*-pentane. An example of the separation of a mixture of *s*-triazines with this mobile phase is shown in Fig. 1.

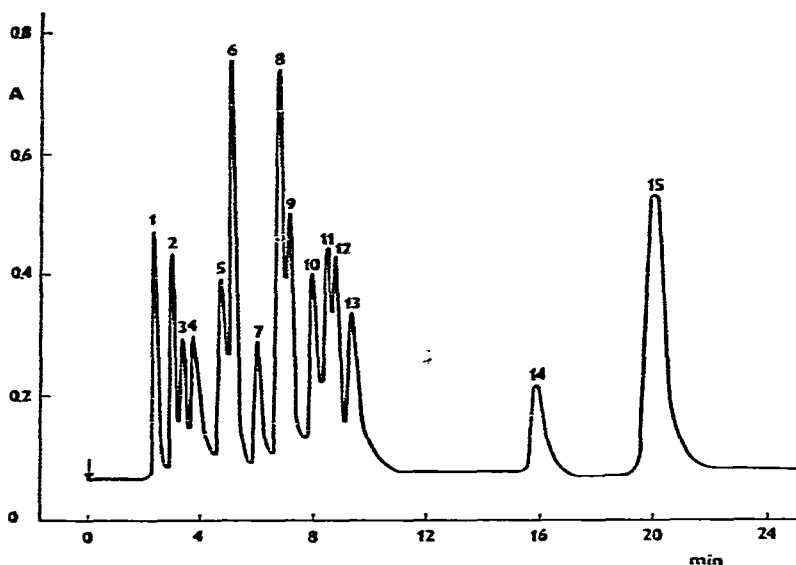


Fig. 1. Separation of a mixture of *s*-triazines on LiChrosorb SI-5 with 2% isopropanol in *n*-pentane as the mobile phase. Flow-rate, 30 ml/h; UV detection at 235 nm; 0–1 a.u.f.s.; 1 cm/min; 1050 p.s.i. Peaks: 1 = chlorazine; 2 = ipazine; 3 = aziprotryne; 4 = prometryne; 5 = terbutylazine; 6 = ametryne; 7 = atrazine; 8 = prometone; 9 = simetryne; 10 = metoprotryne; 11 = simazine; 12 = norazine; 13 = atratone; 14 = neretone; 15 = cyanazine.

To compare the retention behaviour of *s*-triazines on various stationary phases, ratios of the capacity factors (*i.e.*, the relative retentions) were calculated for substances that differ in only a single substituent, either in the alkyl group bound to the amino groups in positions 4 and 6, or in the substituent in position 2. These values are analogous to the functional group retention indices used in GC for identification purposes. The average  $k'_1/k'_2$  values corresponding to the replacement of one substituent in the molecule are given in Table III for all stationary phases so far studied, together with the standard deviations.

On the  $\mu$ Bondapak  $C_{18}$  reversed phase<sup>17,18</sup> with methanol–water (60:40) as the mobile phase, thiomethyl derivatives are the most strongly retained. Methoxy derivatives cannot be separated from chloro derivatives on this stationary phase (see Table III). *s*-Triazines containing the same number of carbon atoms in the alkyl groups

TABLE I  
STRUCTURE AND PROPERTIES OF *s*-TRIAZINES

$\epsilon_1$ ,  $\epsilon_2$  = molar absorptivities at absorption maxima (l/mol · cm);  $E_{1/2}$  = anodic half-wave potentials (V vs. S.C.E.);  $I_A$  = retention index on XE-60 at 195°;  $I_B$  = retention index on SE-30 + Reoplex 400 at 195°.

Common name	Substituent		Mol. wt.	$\epsilon_1^b$	$\epsilon_2^b$	$E_{1/2}^a$	$pK_a^{21-23}$	$I_A^{24}$	$I_B^{24}$
	2-	4-							
Chlorazine	Cl	N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	257.8	—	—	—	1.74	2286	1791
Ipazine	Cl	NHCH(CH <sub>3</sub> ) <sub>2</sub>	243.6	43,100	4300	1.77	1.99	2378	1907
—	Cl	NHC(CH <sub>3</sub> ) <sub>3</sub>	257.8	36,000	3300	2.07	—	2418	1938
Trietazine	Cl	NHC <sub>2</sub> H <sub>5</sub>	229.7	44,300	4300	1.77	1.88	2415	1932
Propazine	Cl	NHCH(CH <sub>3</sub> ) <sub>2</sub>	229.7	32,000	3100	1.95	1.85	2462	1973
Terbutylazine	Cl	NHC <sub>2</sub> H <sub>5</sub>	229.7	19,500	1800	2.01	1.94	2504	1999
Atrazine	Cl	NHC <sub>2</sub> H <sub>5</sub>	215.7	41,000	3900	2.02	1.68	2509	2023
Simazine	Cl	NHC <sub>2</sub> H <sub>5</sub>	201.5	36,000	3100	1.97	1.65	2553	2078
Norazine	Cl	NHCH(CH <sub>3</sub> ) <sub>2</sub>	201.5	—	—	—	1.88	2518	2029
Cyanazine	Cl	NHC <sub>2</sub> H <sub>5</sub>	240.7	—	—	—	1.30	—	—
Aziprotryne	SCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	224.3	—	—	—	—	—	—
Prometryne	SCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	241.3	42,000	—	1.83	4.05	2558	2099
Terbutryne	SCH <sub>3</sub>	NHC <sub>2</sub> H <sub>5</sub>	241.3	21,200	—	1.78	4.38	2608	2122
Ametryne	SCH <sub>3</sub>	NHC <sub>2</sub> H <sub>5</sub>	227.3	40,000	—	1.77	4.00	2610	2139
Simetryne	SCH <sub>3</sub>	NHC <sub>2</sub> H <sub>5</sub>	213.3	44,400	—	1.79	4.00	2656	2185
Desmetryne	SCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	213.3	33,700	—	1.78	3.93	2622	2141
Metoprotryne	SCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	271.4	—	—	—	3.98	2983	2457
Prometone	OCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	225.3	40,200	—	1.77	4.28	2350	1916
Terbutone	OCH <sub>3</sub>	NHC <sub>2</sub> H <sub>5</sub>	225.3	33,600	—	1.79	4.46	2396	1938
sec.-Bumetone	OCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	225.3	—	—	—	4.23	2470	2015
Atraton	OCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	211.3	—	—	—	4.20	2418	1972
Simetone	OCH <sub>3</sub>	NHC <sub>2</sub> H <sub>5</sub>	197.2	38,300	—	1.79	4.17	2435	1990
Noretone	OCH <sub>3</sub>	NHCH(CH <sub>3</sub> ) <sub>2</sub>	197.2	—	—	—	4.15	2411	1966

TABLE II  
RETENTION DATA FOR *s*-TRIAZINES ON LICHROSORB SI-5 STATIONARY PHASE  
Mobile phase: *n*-pentane with 2, 3 or 4% of isopropanol (IPA).

<i>s</i> -Triazine	2% IPA		3% IPA		4% IPA	
	$V_R$	$k'$	$V_R$	$k'$	$V_R$	$k'$
Chlorazine	0.06	0.08	0.02	0.04	0.02	0.04
Ipazine	0.26	0.36	0.20	0.28	0.15	0.20
2-Chloro-4,6-di- <i>tert.</i> -butyl- <i>s</i> -triazine	0.37	0.50	0.27	0.38	0.24	0.32
Trietazine	0.38	0.50	0.29	0.41	0.26	0.36
Aziprotryne	0.41	0.57	0.32	0.46	0.26	0.35
Prometryne	0.57	0.79	0.47	0.65	0.37	0.50
Terbutryne	0.61	0.85	0.55	0.76	0.40	0.54
Propazine	0.68	0.95	0.67	0.80	0.49	0.68
Terbutylazine	0.86	1.19	0.72	1.00	0.57	0.78
Ametryne	1.02	1.42	0.86	1.21	0.64	0.87
Atrazine	1.38	1.91	1.12	1.57	0.83	1.14
Prometone	1.75	2.43	1.36	1.92	0.98	1.34
Terbutone	1.86	2.58	1.53	2.15	1.07	1.47
Simetryne	1.86	2.58	1.47	2.07	1.08	1.48
Desmetryne	1.95	2.71	1.51	2.13	1.19	1.65
Metoprotryne	2.30	3.19	1.70	2.40	1.27	1.75
Simazine	2.40	3.33	1.86	2.62	1.37	1.88
<i>sec.</i> -Bumetone	2.53	3.51	1.92	2.70	1.49	2.04
Norazine	2.62	3.64	1.95	2.75	1.47	2.01
Atratone	2.88	4.00	2.15	3.02	1.61	2.21
Simetone	4.81	6.68	4.06	5.72	2.69	3.68
Noretone	5.43	7.54	4.09	5.76	3.02	4.14
Cyanazine	7.58	10.53	5.36	7.56	3.91	5.35

bound to the amino groups in positions 4 and 6 are also poorly separated. For example, simetone–simazine–norazine, prometone–propazine and atratone–atrazine–desmetryne–simetryne systems are not separated.

*s*-Triazines were better separated using a chemically bonded CN phase<sup>19</sup> in combination with both a non-polar and a polar mobile phase. Methoxy derivatives were well separated from chloro and thiomethyl derivatives using *n*-heptane–isopropanol. Whereas at a low isopropanol concentration the retention order is  $\text{OCH}_3 \gg \text{Cl} > \text{SCH}_3$ , an increase in the retention times of thiomethyl derivatives occurs with increasing polarity of the mobile phase and the retention order changes at a 15% isopropanol concentration ( $k'_{\text{Cl}}/k'_{\text{SCH}_3} < 1$ ; see Table III). The retention order in the *s*-triazine series with the same substituent in position 2 is unaffected by an increase in the polarity of the mobile phase over the range of isopropanol concentrations studied and depends solely on the spacial shielding of the amino groups by alkyl groups, analogous to the situation in GC<sup>5</sup>.

*s*-Triazines differing in the substituent in position 2 were best separated on a chemically bonded  $\text{NH}_2$  phase<sup>20</sup>. *s*-Triazines can form hydrogen bonds of the phase–N–H···N–triazine or phase–N···H–N–triazine type with the  $\text{NH}_2$  phase, which are responsible for an increased selectivity of the stationary phase. The strength of the hydrogen bonds depends on the mutual steric accessibility of the sites.

TABLE III

DEPENDENCE OF  $k_1/k_2$  RATIOS ON VARIOUS STATIONARY PHASES ON THE MOBILE PHASE POLARITYMobile phases: A = 60% methanol in water; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> = 2, 3, 4% isopropanol in *n*-pentane; C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> = 1.5, 5, 15% isopropanol in *n*-heptane; D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> = 0.25 M methanol, 0.25 M ethanol, 0.25 M isopropanol, 0.25 M *tert*-butanol in *n*-heptane; E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, E<sub>4</sub>, E<sub>5</sub> = 0.131, 0.196, 0.25, 0.261, 0.323 M isopropanol in *n*-pentane.

$R_1/R_2$	Parameter * C <sub>18</sub> <sup>18</sup>					SI-5					CN <sup>19</sup>					NH <sub>2</sub> <sup>20</sup>				
	A	B1	B2	B3	B4	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5	E1	E2	E3	E4	E5
Et/H	$\bar{x}$	3.96	0.17	0.14	0.16	0.19	0.22	0.26	0.21	0.20	0.21	0.20	0.18	0.17	0.15	0.15	0.17	0.18	0.19	0.21
	$s$	0.39	0.02	0.04	0.04	0.01	0.02	0.02	0.01	0.01	0.01	0.01	—	—	—	0.02	0.01	0.01	0.01	0.01
Et/Me	$\bar{x}$	1.57	0.53	0.55	0.54	0.62	0.74	0.80	0.61	0.58	0.61	0.58	0.56	0.58	0.53	0.55	0.55	0.56	0.56	0.56
	$s$	0.01	0.01	0.03	0.02	—	—	—	—	—	—	—	—	—	—	0.01	0.01	0.01	0.01	0.03
Et/iPr	$\bar{x}$	0.64	1.73	1.73	1.70	1.60	1.46	1.51	1.52	1.56	1.52	1.56	1.61	1.62	1.68	1.66	1.66	1.65	1.65	1.64
	$s$	0.01	0.19	0.18	0.05	0.09	0.26	0.09	0.12	0.10	0.12	0.10	0.07	0.07	0.16	0.12	0.12	0.10	0.10	0.11
Et/tBu	$\bar{x}$	—	2.70	2.69	2.52	2.39	1.90	1.98	2.16	2.27	2.16	2.36	2.42	2.61	2.51	2.51	2.41	2.39	2.34	
	$s$	—	0.28	0.16	0.15	0.07	0.42	0.10	0.17	0.13	0.13	0.04	0.01	0.01	0.11	0.01	0.06	0.11	0.11	
iPr/Me	$\bar{x}$	2.46	0.29	0.31	0.32	0.41	0.59	0.70	0.41	0.38	0.41	0.36	0.35	0.35	0.32	0.33	0.34	0.34	0.36	
	$s$	0.04	0.03	0.02	0.03	—	—	—	—	0.06	0.04	0.04	0.02	0.03	0.02	0.02	0.03	0.03	0.02	
OCH <sub>3</sub> /Cl	$\bar{x}$	0.98	2.18	2.14	1.96	2.84	2.73	2.61	1.29	1.32	1.29	1.32	1.39	1.42	1.40	1.39	1.38	1.37	1.38	
	$s$	0.02	0.22	0.17	0.06	0.16	0.09	0.12	0.05	0.05	0.05	0.05	0.05	0.14	0.05	0.05	0.05	0.06	0.05	
OCH <sub>3</sub> /SCH <sub>3</sub>	$\bar{x}$	0.58	2.86	2.75	2.57	3.57	2.86	2.32	1.91	1.99	1.91	2.06	2.06	2.14	2.18	2.15	2.08	2.05	2.00	
	$s$	0.01	0.20	0.17	0.09	0.12	0.06	0.02	0.17	0.16	0.16	0.08	0.08	0.11	0.18	0.18	0.15	0.15	0.06	
Cl/SCH <sub>3</sub>	$\bar{x}$	0.59	1.32	1.33	1.32	1.29	1.29	0.89	1.42	1.46	1.46	1.46	1.46	1.47	1.56	1.53	1.49	1.48	1.44	
	$s$	0.01	0.08	0.09	0.08	0.10	0.22	0.04	0.13	0.09	0.09	0.06	0.06	0.08	0.08	0.08	0.08	0.06	0.08	

\*  $\bar{x}$  = mean  $k_1/k_2$  value;  $s$  = standard deviation.

Compared with the  $\text{NH}_2$  phase, methoxy derivatives are more strongly retained on silica gel. The bonds of the  $-\text{Si}-\text{OH}$  groups to the  $\pi$ -electrons of the *s*-triazine ring or to the electron pairs on the nitrogen atoms in the substituent groups can play a role with silica gel. As the dependence on the ring electron density is not obeyed (the retention volume order is  $\text{OCH}_3 > \text{Cl} > \text{SCH}_3$ , whereas the total group electronegativity order, expressed in terms of  $\text{p}K_a$  or  $\sigma_m$ , is  $\text{Cl} > \text{SCH}_3 \geq \text{OCH}_3$ ), a predominant effect of silica gel on the ring  $\pi$ -electrons can be excluded, otherwise the ring would not be sterically affected by substitution and would exhibit only an induction effect. As far as the bonds of the silica gel OH groups to the electron pairs on the nitrogen atoms are concerned, the opposite effects of the substituent donor ability (*tert.*-butyl > isopropyl > ethyl > methyl) and steric accessibility (an increase in the entropic factor in the equilibrium) play a role, the latter predominating. Hence it can be assumed for the  $\text{Si}-\text{OH} \cdots \text{N}$ -triazine bond that the equilibrium constant for the complex formation will decrease with increased branching of the alkyl group, which has been confirmed experimentally.

From the point of view of the effect of substituents of types other than amine (with the possibility of hydrogen bonding), an increase in the retention volumes in the order  $\text{OCH}_3 \gg \text{SCH}_3 \approx \text{Cl}$  can be expected. However, these groups affect the electron density in the whole conjugated system in the order  $\text{Cl} > \text{SCH}_3 \gg \text{OCH}_3$ . The resultant retention order for substances differing only in the substituent in position 2 is  $\text{OCH}_3 \gg \text{Cl} > \text{SCH}_3$ .

*s*-Triazines with the same number of carbon atoms in the amino alkyl groups in positions 4 and 6, e.g., the pairs norazine-simazine and noretone-simetone, are best

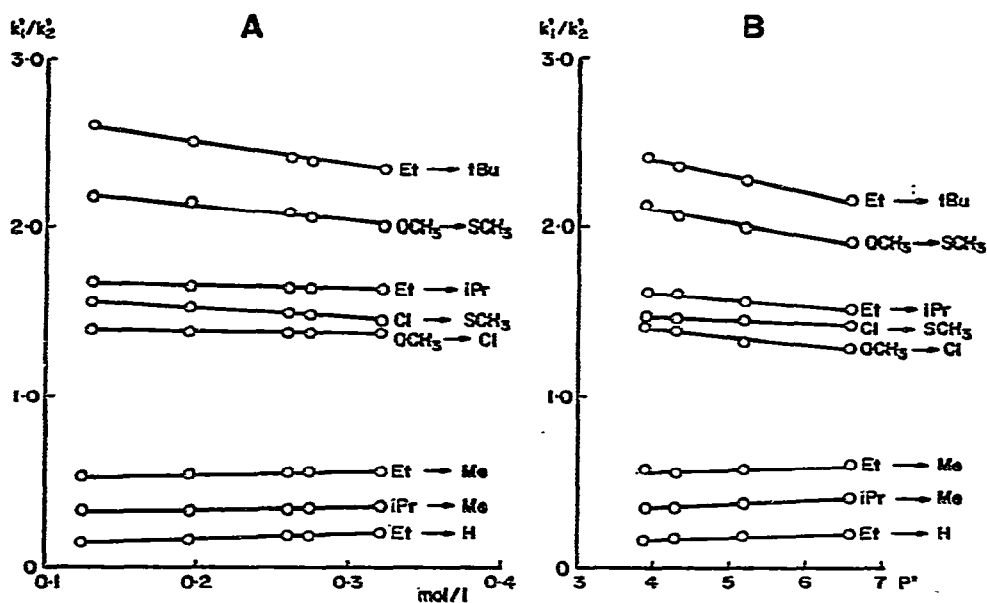


Fig. 2. Dependence of the capacity factors,  $k_1/k_2$ , on the isopropanol concentration (A) and the alcohol polarity,  $P'$ , (B) for LiChrosorb  $\text{NH}_2$  stationary phase.  $P' = 6.6$  for methanol, 5.2 for ethanol, 4.3 for isopropanol and 3.9 for *tert.*-butanol).

separated on silica gel, whereas their separation on the  $\text{NH}_2$  phase is difficult and on  $\mu\text{Bondapak C}_{18}$  impossible.

As can be seen from Table III, the  $k'_1/k'_2$  values depend both on the stationary phase and on the polarity of the mobile phase. With increasing isopropanol concentration (Fig. 2A) or with increasing alcohol polarity (expressed in terms of the polarity index,  $P'$ , see Fig. 2B)<sup>25</sup>, the  $k'_1/k'_2$  ratios decrease (on substitution of ethyl for isopropyl, ethyl for *tert.*-butyl,  $\text{OCH}_3$  for Cl, Cl for  $\text{SCH}_3$  and  $\text{OCH}_3$  for  $\text{SCH}_3$ ), or increase (on substitution of ethyl for methyl, ethyl for hydrogen and isopropyl for ethyl) and approach unity, which means that separation is not achieved at a certain isopropanol concentration. The dependence shown in Fig. 2 for the LiChrosorb  $\text{NH}_2$  phase also hold for the CN phase and silica gel (see Table III).

The  $k'_1/k'_2$  ratios given in Table III can be used to predict the retention behaviour of *s*-triazines that were not available. For example, the capacity ratios were calculated for ipatone (0.78), ipatryne (0.25), trietatone (1.09) and trietatryne (0.35) on LiChrosorb SI-5 and with 2% isopropanol in *n*-pentane as the mobile phase.

The constancy of the  $k'_1/k'_2$  ratio can be utilized in correlations of the retention data with the number of carbon atoms in the alkyl groups bound to the amino groups in positions 4 and 6. In the series of *s*-triazines obtained by substitution of a single kind of substituent, linear dependences of  $\log k'$  on the number of carbon atoms in the alkyl groups were obtained, and some of them are depicted in Fig. 3. The  $\log k'$  dependences on the number of carbon atoms are non-linear in series of *s*-triazines that differ only in the substituent in position 6, because of different spacial shielding of the alkyl groups. Dependence similar to those given in Fig. 3 could also be employed for the identification of *s*-triazines.

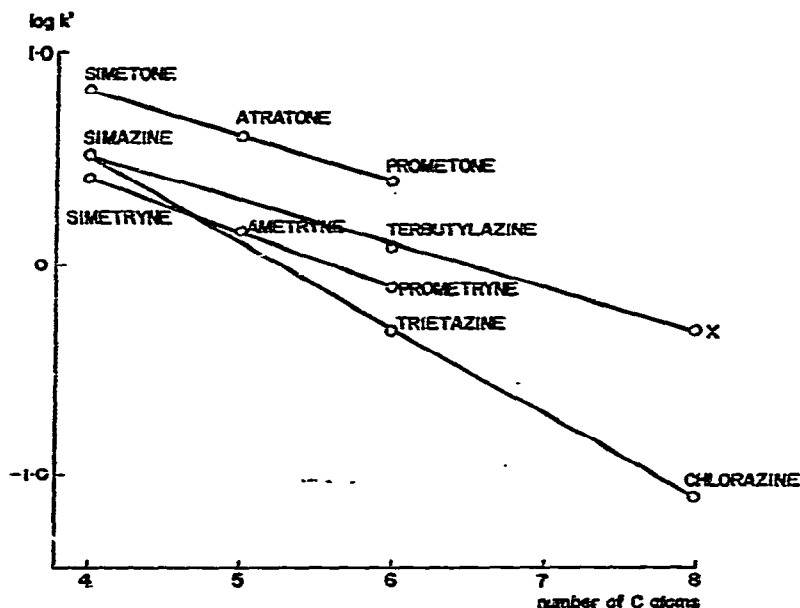


Fig. 3. Dependence of  $\log k'$  on the number of carbon atoms in the alkyl groups bound to *s*-triazine amino groups in positions 4 and 6. X = 2-chloro-4,6-di-*tert.*-butyl-*s*-triazine.



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