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SEPARATION AND BEHAVIOUR OF *s*-TRIAZINE DERIVATIVES ON A NH_2-CHEMICALLY BONDED STATIONARY PHASE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The behaviour of *s*-triazine derivatives on a NH_2-chemically bonded stationary phase was studied by high-performance liquid chromatography and their sorption mechanism was explained. Pentane and heptane mobile phases were selected, the polarity of which was increased by the addition of aliphatic alcohols (methanol, ethanol, isopropanol and *tert.*-butanol).

The NH_2-phase provides a better separation of *s*-triazines differing in the substituent at position 2 (methoxy-, chloro- and thiomethyl-*s*-triazines) compared with the formerly used chemically bonded CN– and reversed (C_{18}) phases.

INTRODUCTION

s-Triazine derivatives are used mainly in agriculture as herbicides, but they also have fungicidal properties and some of them are utilized as pharmaceuticals. The methods employed for their determination have been surveyed by Gysin and Knüsli[1] and Stammbach *et al.*[2]. Chromatographic methods predominate at present[3] and permit both the determination of the preparation purity and the separation and identification of *s*-triazine residues in the environment.

Gas chromatography is the most important of these methods[4–11]. Polar stationary phases coated on deactivated supports are generally used for the separations, in combination with packed or capillary columns. Trace concentrations of *s*-triazines in soil, grain, etc., can be determined using specific detectors, especially the Coulson electrolytic conductance detector.

However, liquid chromatography is advantageous in some cases, chiefly with strongly polar substances (for example 2-hydroxyderivatives) which are insufficiently

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volatile or, on the other hand, very thermolabile. The first papers using high-performance liquid chromatography (HPLC) for analysis dealt with the practical determination of terbutryne and cyanatryne[12–14]. Permaphase ETH as stationary phase and a methanol–water mixture as mobile phase were used. Vitali *et al.*[15] employed the same mobile phase for separation of thirteen *s*-triazines. They also used a reversed-phase system with the Zipax-ODS chemically bonded stationary phase. The most extensive study has been published by Jork and Roth[16,17]. These authors studied the conditions for chromatographic separation of *s*-triazines on the μBondapak C_{18} chemically bonded phase with the aim of determining trace concentrations of these substances in plant extracts.

Because the separation of many *s*-triazines on non-polar stationary phases is unsatisfactory, a chemically bonded CN-phase of medium polarity was used in our previous paper[18], in combination with both non-polar and polar mobile phases. Although better results were obtained than with the C_{18} reversed phase, some triazines could not be separated even in this system. The LiChrosorb NH_2 stationary phase was selected in this work in view of the weakly basic properties of *s*-triazines, and the effect of the mobile phase composition on the chromatographic behaviour of twenty-two *s*-triazine derivatives was studied.

EXPERIMENTAL

Materials

The *s*-triazines tested were products of Ciba-Geigy (Basle, Switzerland). Methanol, ethanol and isopropanol, UV grade (Lachema, Brno, Czechoslovakia), pentane, p.a. (E. Merck, Darmstadt, G.F.R.), heptane, p.a. (Loba-Chemie, Wien, Austria), *tert.*-butanol, p.a. (Reanal, Budapest, Hungary), and octanol, pure (Lachema, Brno, Czechoslovakia) were purified by redistillation.

Method

The HPLC measurements were carried out on a Varian Model 4100 liquid chromatograph equipped with a Variscan 635 UV detector. The wavelengths selected for all measurements were 230 and 235 nm, respectively. The stainless-steel column used (25 cm × 2.2 mm I.D.) was packed with LiChrosorb NH_2 chemically bonded phase, particle size 10 μm, and was pretested by the manufacturer; 2837 theoretical plates were obtained for *o*-nitroaniline in hexane–methylene chloride–isopropanol (69.5:30:0.5) at a flow-rate of 1 ml/min. The mobile phases used are given in Table I. Flow-rates were 18 ml/h and 36 ml/h. The retention dead volume was determined as previously described[18], giving a value of $V_M = 0.68$.

RESULTS AND DISCUSSION

The retention data for twenty-two *s*-triazine derivatives were measured on the NH_2-chemically bonded phase using the mobile phases summarized in Table I. The value of the capacity factor, k', are given in Table II.

In gas–liquid chromatography (GLC) the retention behaviour is primarily determined by the volatility of the substances chromatographed, which in this case decreases in the series, 2-methoxy->2-chloro->2-thiomethyl-*s*-triazine (*s*-triazine

TABLE I

MOBILE PHASE USED

Phase	*Composition*
A	0.25 *M* methanol in heptane
B	0.25 *M* ethanol in heptane
C	0.25 *M* isopropanol in heptane
D	0.25 *M tert.*-butanol in heptane
E	0.131 *M* isopropanol in pentane
F	0.196 *M* isopropanol in pentane
G	0.25 *M* isopropanol in pentane
H	0.261 *M* isopropanol in pentane
I	0.323 *M* isopropanol in pentane

derivatives are also eluted from a GLC column in this order), whereas in liquid chromatography the retention order is chiefly affected by the polarity of the solutes relative to the polarities of the stationary and mobile phases.

The elution order in liquid chromatography on the NH_2 phase with a non-polar mobile phase (a mixture of an alkane with an aliphatic alcohol) is the same as that with the CN-phase[18], *i.e.*, thiomethyl derivatives are eluted first, then chloro derivatives and methoxy derivatives are retained most strongly. However, the differences in the retention of chloro- and thiomethyl derivatives are small on the CN-phase, whereas good separation is achieved on the NH_2-phase.

s-Triazines are weak bases, their dissociation constants varying from 1.65 for simazine to 4.46 for terbutone[19,20]. The basicity is most strongly affected by the substituent in position 2. The number of alkyl groups in positions 4 and 6 has a smaller, but also pronounced effect on the basicity of *s*-triazines[21,22]. The ability of *s*-triazines to form hydrogen bonds plays a role in their separation. With the NH_2 phase, the predominant effect is exerted by hydrogen bonds of the type phase–N–H···N-triazine, or phase–N···H–N-triazine. The hydrogen bond strenght depends on the steric accessibility of the centres. The steric hindrance is additive, because the bonds can be formed on several centres.

In the series of *s*-triazines differing only in the substituent on position 4, the elution order is *tert.*-butyl-, isopropyl-, ethyl- and finally methyl-derivatives. The effects of the donor ability of the amino-group substituent (*tert.*-butyl>isopropyl>ethyl>methyl) and of the steric accessibility are opposed, but the latter predominates. With identical alkylamines, the additional effect of the bond centres, OCH_3>Cl>SCH_3, plays a role.

Pentane and heptane were used as basic components of the mobile phase. The viscosity of pentane is approximately half that of heptane[23] (0.23 cP for pentane and 0.41 cP for heptane), which is reflected in a higher column efficiency. A disadvantage is the low boiling point of pentane which may be the cause of bubble formation in the detector. The two solvents further differ in their solubility parameters[23] (7.1 for pentane and 7.4 for heptane, *i.e.*, polar substances are somewhat less soluble in pentane than in heptane, leading to higher values of the capacity factor of *s*-triazines in pentane than in heptane. These conclusions have also been confirmed by our experiments with mobile phases C and G (see Table II).

Alcohols in a mobile phase are capable of forming hydrogen bonds, com-

TABLE II

RETENTION DATA FOR *s*-TRIAZINE DERIVATIVES ON LICHROSORB NH_2 STATIONARY PHASE IN VARIOUS MOBILE PHASES

Triazine	*k'*								
	Heptane				*Pentane*				
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
Ipazine	0.51	0.52	0.52	0.53	1.01	0.85	0.74	0.71	0.59
2-Chloro-4,6-bis(*tert.*-butyl)-*s*-triazine	0.65	0.65	0.66	0.66	1.13	1.00	0.90	0.88	0.75
Trietazine	0.82	0.84	0.83	0.84	1.54	1.38	1.21	1.18	1.05
Aziprotryne	0.94	0.93	0.97	0.97	1.79	1.51	1.42	1.40	1.18
Prometryne	1.15	1.16	1.18	1.22	2.34	1.85	1.58	1.50	1.25
Terbutryne	1.28	1.32	1.39	1.42	2.88	2.25	1.95	1.90	1.51
Propazine	1.85	1.86	1.84	1.87	3.91	3.06	2.50	2.41	1.87
Ametryne	1.90	1.90	1.93	1.98	4.17	3.26	2.70	2.56	1.98
Terbutylazine	1.93	1.97	2.00	2.04	4.37	3.41	2.83	2.67	2.07
Prometone	2.30	2.42	2.52	2.60	5.50	4.27	3.52	3.33	2.59
Atrazine	2.49	2.67	2.84	3.10	6.20	4.81	3.97	3.74	2.92
Terbutone	2.59	2.72	2.88	3.20	6.31	4.88	4.03	3.80	2.96
Metoprotryne	2.65	2.92	3.16	3.31	6.94	5.35	4.42	4.17	3.26
Simetryne	2.92	3.11	3.32	3.45	7.30	5.63	4.62	4.38	3.42
sec.-Bumetone	2.98	3.17	3.40	3.65	7.46	5.76	4.75	4.47	3.50
Desmetryne	3.10	3.30	3.52	3.70	7.82	6.02	4.92	4.66	3.65
Atratone	3.24	3.44	3.82	4.02	8.32	6.40	5.23	4.95	3.88
Simazine	3.94	4.28	4.66	4.92	11.75	8.60	6.93	6.60	4.99
Norazine	4.13	4.69	4.95	5.09	11.9	8.70	7.02	6.68	5.05
Simetone	5.20	7.08	9.39	11.6	19.1	15.5	12.85	12.4	10.1
Noretone	5.29	7.16	9.87	12.25	20.6	16.7	13.9	13.5	10.9
Cyanazine	12.8	13.2	13.6	14.8	24.2	18.6	15.9	14.4	11.3

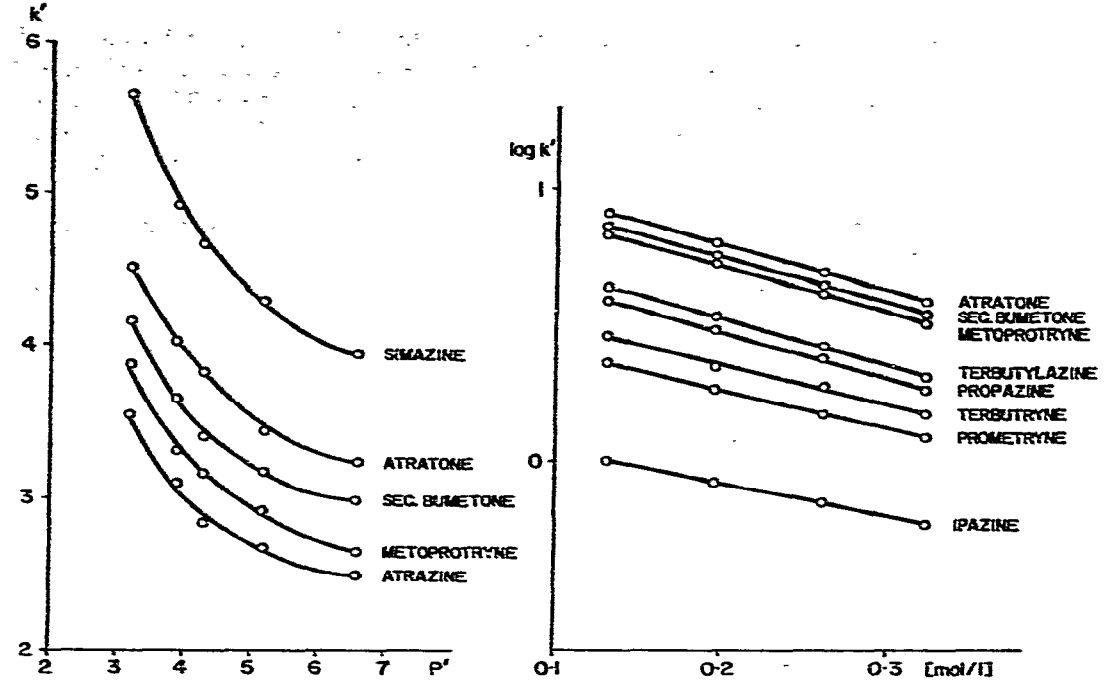

Fig. 1. Dependence of k' on the polarity of the mobile phase. P' = Polarity index[24] (methanol, 6.6; ethanol, 5.2; isopropanol, 4.3; *tert.*-butanol, 3.9; octanol, 3.2).

Fig. 2. Dependence of log k' on the concentration of isopropanol in pentane.

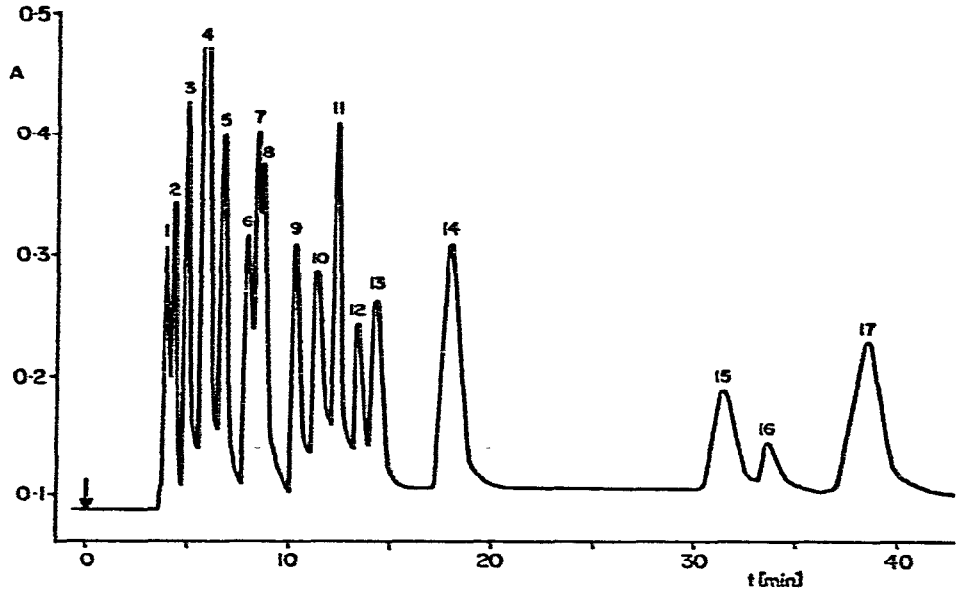

Fig. 3. Separation of *s*-triazines on LiChrosorb NH_2-10 stationary phase. Mobile phase: 0.25 *M* isopropanol in pentane; flow-rate, 18 ml/min. Pressure, 300 p.s.i. Detection: UV at 230 nm, 0–0.5 a.u.f.s. Peaks: 1 = ipazine; 2 = 2-chloro-4,6-bis(*tert.*-butyl)-*s*-triazine; 3 = trietazine; 4 = prometryne; 5 = terbutryne; 6 = propazine; 7 = ametryne; 8 = terbutylazine; 9 = prometone; 10 = atrazine; 11 = metoprotryne; 12 = desmetryne; 13 = atratone; 14 = simazine; 15 = simetone; 16 = noretone; 17 = cyanazine.

petitively displace *s*-triazines from the bonds with the NH_2 phase and thus also effect their elution. The effect of an alcohol on the retention behaviour of *s*-triazines was studied with heptane as the basic component of the mobile phase. The k' values generally increased with increasing molecular weight and decreasing polarity of the alcohols (see mobile phases A, B, C and D in Table II), *i.e.*, the solubility in the mobile phase decreased.

The k' values for *s*-triazines in mobile phases A, B, C and D were correlated with the polarity of the alcohol added. Snyder[24] recommends the polarity index, P', for characterization of solvents, which is partially corrected for the effects of dispersion interactions and molecular weight. The dependences of k' on P' for atrazine, metoprotryne, *sec.*-bumetone, atratone and simazine are given in Fig. 1, from which it can be seen that k' decreases with increasing polarity of the alcohol added. Isopropanol seems to be optimal from the point of view of the separation.

The dependence of the capacity factor on the alcohol concentration was studied with isopropanol (mobile phases E, F, G, H and I, see Fig. 2). The optimal separation was attained for 0.25 *M* isopropanol in heptane. An example of separation of a mixture of *s*-triazines is depicted in Fig. 3.

2-Hydroxypropazine and 2-hydroxysimazine, which cannot be directly analyzed by gas chromatography and must be converted into volatile derivatives can be separated on the NH_2 phase with pure methanol as mobile phase, both from one another and from the corresponding 2-chloro derivatives. This finding could be utilized in the study of degradation of *s*-triazines in the environment.

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