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Chromatographic behaviour of triazine compounds

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Abstract

A comparative study was made of the chromatographic behaviour of eight 1,3,5-triazine compounds, including Cl-triazines and S-triazines (Ametryne, Atrazine, Cyanazine, Prometryne, Propazine, Simazine, Terbutryne, Terbutylazine). The techniques investigated included liquid–liquid partitioning and ion interaction chromatography with UV detection. Capacity factors are discussed as a function of mobile phase parameters: pH, ionic strength and organic modifier and ion interaction reagent concentrations.

1. Introduction

The chromatographic determination of triazine herbicides is of great importance in environmental control. Generally, analytical methods deal with the simultaneous determination of different chemical families of pesticides [1]. The techniques usually used include gas chromatography (GC) [2] and liquid chromatography (LC) [3–11], the latter being preferable for polar or thermolabile analytes [12]. LC methods have been developed with particular attention to detection [3–7] and sample preconcentration [8,9]. In order to obtain high selectivity and lower detection limits, a method coupling LC with GC–MS has recently been developed [13]. A liquid–liquid partition study was reported by Günther and Kettrup [10] with reference to the retention time behaviour of these compounds, but the retention mechanism as a function of the eluent composition has received little attention.

The aim of this work was a comparison of the chromatographic behaviour and the separation mechanism for eight triazine species (Fig. 1), using liquid–liquid partitioning (LLC) and ion interaction (IIC) procedures.

2. Experimental

A Varian (Walnut Creek, CA, USA) LC 5000 liquid chromatograph equipped with a UV 100 spectrophotometric detector and a Vista 401 data system was used. Graphic representations were performed with an IBM PS/2 Model 57 SX desk-top PC and Sigma Plot version 5 software (Jandel Scientific). The analytical column was LiChrospher 100 RP-18 (5 μm) (250 × 4 mm I.D.), obtained from Merck (Darmstadt, Germany).

Acetonitrile (HPLC grade), phosphoric acid, sodium hydroxide, lithium perchlorate, tetrabutylammonium hydroxide (TBA-OH) and sodium dodecyl sulphate (SDS) were Merck analytical-reagent grade products.

Eluents were prepared with high-purity water obtained from a Milli-Q system (Millipore, Bedford, MA, USA), filtered and degassed under vacuum before use.

Reference standards for triazine compounds

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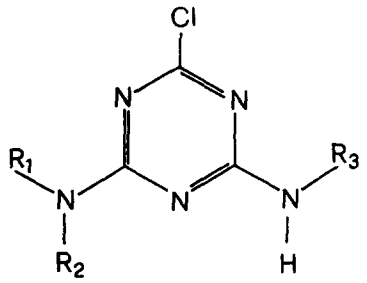
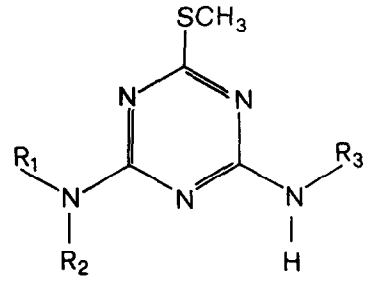


Names	R_1	R_2	R_3	pK_a
Simazine	H	Et	Et	1.7
Atrazine	H	Et	i-Pr	1.7
Propazine	H	i-Pr	i-Pr	1.7
Terbutylazine	H	Et	t-Bu	2.0
Cyanazine	H	Et	CH_3 C-C≡N CH_3	1.0



Names	R_1	R_2	R_3	pK_a
Ametryne	H	Et	i-Pr	4.1
Prometryne	H	i-Pr	i-Pr	4.1
Terbutryne	H	Et	t-Bu	4.3

Fig. 1. Structure and acid dissociation constants (pK_a) of the investigated triazine compounds. Et = Ethyl; i-Pr = isopropyl; t-Bu = *tert.*-butyl.

were obtained from Riedel-de Haën–Schering (Seelze, Germany) (98.0–99.9% purity). Stock standard solutions of the analytes (200 mg/l) were prepared in acetonitrile and stored in the dark at 4°C. Working standard solutions were obtained daily by diluting the stock standard solutions with acetonitrile. The amount of each analyte injected was 600 ng, unless stated otherwise.

The UV detector was set at 220 nm as a compromise between the maximum absorbance of the analytes and the reduced background of the eluents at this wavelength. Acetonitrile was chosen owing to its low absorbance background in the UV region.

Both reversed-phase LLC and IIC techniques were applied and the eluent flow-rate was 1.0 ml/min unless stated otherwise. Sample volumes injected were 100 μl. Chromatographic retention times (t_R) are the means of triplicate determinations and the dead time (t_0) was evaluated by injection of nitrate ion (1.0 m*M* $NaNO_3$), taken as the unretained peak in LLC, and by injection of water (water dip) in IIC. The capacity factors (k') were calculated from the retention times of the triazine compounds using the relationship $k' = (t_R - t_0)/t_0$.

The eluents were acetonitrile–water mixtures (see below) containing, depending on the chromatographic technique, the following components. For LLC studies, sodium phosphate buffer (15.0 m*M* H_3PO_4 + NaOH to adjust the pH) and, if stated, lithium perchlorate (0–1.2 *M*) or TBA-OH (0.25 m*M*) were used. After optimization, the system adopted was acetonitrile–water (45:55), 15 m*M* H_3PO_4 (pH 7.0) at a flow-rate of 1.5 ml/min. The pH values of all eluent mixtures were measured with reference to the actual medium (acetonitrile–water buffer) with the aid of a combined glass–calomel electrode. For IIC studies, SDS (0–1.0 m*M*) and sodium phosphate buffer (15.0 m*M* H_3PO_4 + NaOH to adjust the pH) were used. After optimization, the system adopted was acetonitrile–water (50:50), 1.0 m*M* SDS, 15 m*M* H_3PO_4 (pH 7.0) at a flow-rate of 1.0 ml/min.

3. Results and discussion

3.1. Liquid–liquid partition chromatography (LLC)

Experiments were performed, with respect to the mechanism acting in LLC, to evaluate the influence of organic modifier concentration, pH, ionic strength and modification of the stationary phase polarity. Fig. 2 shows, as an example, the

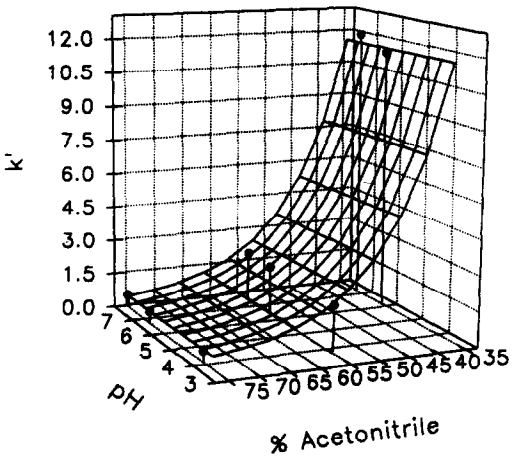


Fig. 2. Retention surface for ametryne in LLC. For experimental conditions, see text.

behaviour of k' for ametryne as a function of eluent composition (pH, acetonitrile concentration) in terms of the "retention surface". The dependence of the capacity factor on the concentration of the organic modifier is expressed by an exponential decrease corresponding to the increase in acetonitrile concentration. The retention time is not significantly affected by the eluent pH, which means that the variations in the partial charges on the nitrogen atoms do not change substantially the molecular lipophilicity. The same trend of the capacity factor was observed for all the triazines investigated, as a function of either pH or acetonitrile concentration. Nevertheless, the stronger inductive effect ($-I$) of the chloride atom in position 2 with respect to the $-SCH_2$ group generates an increase in the charge density on the triazine ring, in particular on the nitrogen atoms in positions 1 and 3, making such species more polar and with less affinity for the stationary phase.

Cl-triazines generally have lower retention times than S-triazines. This behaviour is observed with the exception of terbutylazine, for which the influence of the *tert.*-butyl group in position 4 prevails over the effect of chlorine in position 2, so that terbutylazine has a lower polarity, that is, a higher k', than the S-triazine ametryne. For a defined substituent in position 2, the k' value may be explained by considering the structure of the group in positions 4 and 6. The k' values for Cl-triazines show the sequence cyanazine < simazine < atrazine < propazine < terbutylazine, where cyanazine has the lowest k' value owing to the polar substituent group $-NHCH(CH_3)CN$.

Triazines are slightly basic compounds; the pK_a values (Fig. 1) show that significant protonation occurs at pH < 2 for Cl-triazines and at pH < 4 for S-triazines. As the dielectric constant of water–acetonitrile mobile phases is lower than that of water alone, the k' values are approximately constant at pH > 3. Anyway, the pH may influence other chromatographic parameters, such as the column resolution. Table 1 shows the number of theoretical plates [$N = 16(t_R/W)^2$, where W = peak width] evaluated for prometryne and propazine, which are representative of S- and Cl-triazines, respectively. According to the pK_a values, the pH effect is greater for S-triazines. This is also confirmed by the behaviour of the peak asymmetry factor (AF10), evaluated at 10% of the peak height; S-triazines show a tailed peak at acidic pH values of the mobile phase.

Fig. 3 shows an example of the separation obtained by LLC at neutral pH. Mobile phases containing perchlorate ion have been used [10]; this addition improves the resolution and chromatographic performance with a significant effect on the retention times. In our experiments, the lithium salt, which is more soluble, was preferred as the effect is evidenced only at very high concentrations. By increasing the perchlorate concentration the affinity between the eluent and the more polar species increases, resulting in lower k' values (pH 3.0). As expected, S-triazines show a greater deviation. The inversion of

Table 1
Effect of pH on the number of theoretical plates for propazine and prometryne (Cl- and S-triazine, respectively)

pH	Prometryne	Propazine
3	548	481
5	1118	510
7	1150	547

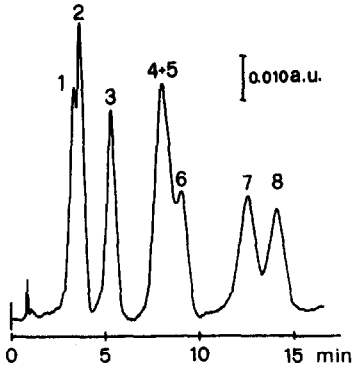


Fig. 3. Chromatogram of (1) cyanazine, (2) simazine, (3) atrazine, (4) propazine, (5) ametryne, (6) terbutylazine, (7) prometryne and (8) terbutryne obtained by LLC. Eluent, acetonitrile–water (45:55, v/v); buffer, pH 7.00; flow-rate, 1.5 ml/min; analytes, 1.0 μg each.

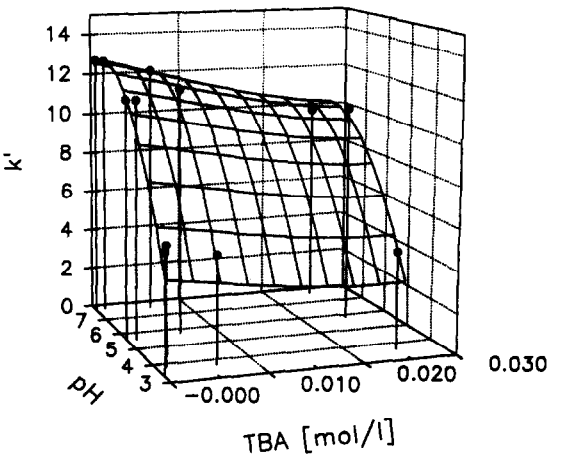


Fig. 4. Retention surface of ametryne (S-triazine) obtained using IIC with analyte and ion interaction reagent having the same positive charge. Eluent, acetonitrile–water (35:65, v/v); buffer pH and TBA concentration as shown.

the elution sequence due to the ionic strength effect is noteworthy. In contrast, for the neutral species the partitioning on the stationary phase is increased in addition to their k' values. For higher pH values (*e.g.*, pH 5) the increase in ionic strength results in greater k' values for all the species.

In addition of the organic modifier, pH and ionic strength effects on k' for triazines, we also investigated the effect of a compound able to modify the polarity of the stationary phase in order to improve the separation of the analytes. To avoid too short elution times and to evidence the effect of a positively charged lipophilic cation, the percentage of acetonitrile in the mobile phase was kept below 35%. Experiments were performed by adding tetrabutylammonium hydroxide (TBA-OH) to the mobile phase, the TBA cation being adsorbed on the surface of the stationary phase, which becomes positive. Fig. 4 shows the retention surface for ametryne as representative of S-triazines. The capacity factors decrease as the TBA concentration increases; this effect may be attributed to the repulsive effect between the positively charged TBA adsorbed on the stationary phase and the analytes in cationic form. Thus the pH of the mobile phase strongly affects the retention of S-triazines whereas Cl-triazines, having lower pK_a values, are not affected in the pH range investigated. On the other hand, the peak resolution does not improve in comparison with the previous LLC procedure when TBA is added to the eluent.

3.2. *Ion interaction chromatography (IIC)*

By working at a suitable pH, according to the triazine pK_a values, the analytes can be treated as cations and IIC can be applied for their separation. Experiments were carried out in order to optimize the IIC procedure and to evaluate the effectiveness of SDS as a counter ion.

When the eluent contains SDS, the stationary phase assumes a negative charge owing to SDS partitioning, and the strong affinity between the alkyl chain of SDS and the reversed phase modifies the stationary phase with the introduction of dynamic ion-exchange sites and a slight decrease in lipophilic character. The exchange reaction between the protonated triazine compounds ($T-H^+$) in the mobile phase (m) and the SDS adsorbed on the stationary phase (s) can be formally written as

$$T - H^{+}_{(m)} + Na^{+}\ {}^{-}O_3SOC_{12}H_{25(s)} \rightleftharpoons T - H^{+}\ {}^{-}O_3SOC_{12}H_{25(s)} + Na^{+}_{(m)}$$

Under these conditions, the retention of the triazine species increases as much as their partial positive charge is strong. Fig. 5 shows the behaviour of the k' values of the analytes as a function of SDS concentration. According to their pK_a values, S-triazines (ametryne, prometryne and terbutryne) show greater k' increases at higher SDS concentrations. It must be noted that the k' variations as a function of pH are opposite to those in LLC, where an increase in pH does not or only slightly affects k'. The decrease in k' at high pH values, in the presence of SDS, and the more consistent effect on S-triazines gives rise to an inversion in the sequence of elution between ametryne and terbutylazine.

The retention surface as a function of pH and SDS concentration in the eluent, for a generic triazine, changes its slope for either different pH or SDS concentration. Fig. 6 shows, as an example, the cyanazine retention surface. The decrease in k' at high pH indicates a decrease in the lipophilicity in the stationary phase when the SDS concentration increases. This represents an interesting chemometric case of interaction between the two factors (pH and SDS concentration) on the response surface (k').

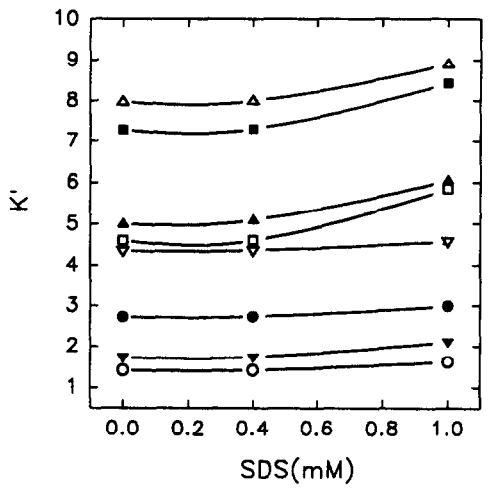


Fig. 5. Effect of SDS concentration on capacity factors in IIC: ○ = cyanazine; ▼ = simazine; ● = atrazine; ▽ = propazine; □ = ametryne; ▲ = terbutylazine; ■ = prometryne; △ = terbutryne. Eluent, acetonitrile–water (50:50, v/v); buffer pH 3.00; SDS concentration as shown.

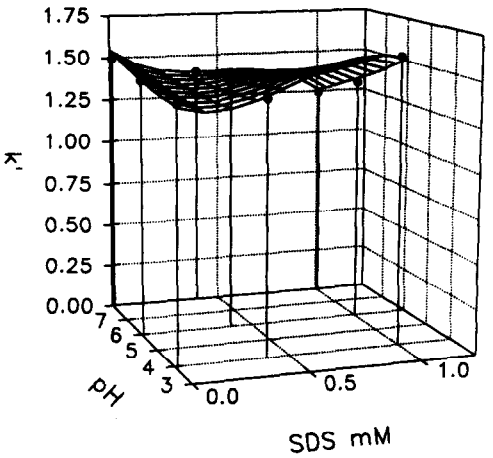


Fig. 6. Retention surface of cyanazine obtained by IIC in the presence of SDS. Eluent, acetonitrile–water (50:50, v/v); buffer pH and SDS concentration as shown.

Fig. 7 shows the separation of atrazines using the optimized IIC procedure, the resolution being better than that for the LLC method.

The ion interaction mechanism investigated in this work allows greater possibilities than LLC

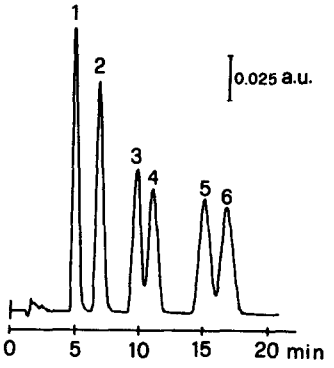


Fig. 7. Chromatogram of (1) simazine, (2) atrazine, (3) propazine, (4) terbutylazine, (5) prometryne and (6) terbutryne obtained by IIC in the presence of SDS. Eluent, acetonitrile–water (50:50, v/v); buffer, pH 7.00; SDS concentration, 1.00 m*M*.

and results in a competitive and more flexible technique for the determination of herbicides and pesticides.

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