

NORMAL AND REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF SOME NEW 1,2,4-TRIAZOLE DERIVATIVES

Marijana M. Ačanski, Nada U. Perišić-Janjić and Vesna Dimova

The retention behaviour and separation ability of normal and reversed phase HPLC with one non-polar and two polar mobile phases, have been studied by measuring the retention constants of a series of newly synthesized 1,2,4-triazole derivatives. The results are discussed in terms of the nature of the solute, eluent and stationary phase.

KEY WORDS: High performance liquid chromatography (HPLC), normal phase, silica gel column, reversed phase, C-8 bonded silica gel column, 1,2,4-triazole derivatives

INTRODUCTION

1,2,4-triazoles are a well known group of physiologically active compounds. They have found application as herbicides, fungicides (1), plasticizers, adhesives and chemicals in the photographic industry. Preliminary investigations have shown that newly synthesized 1,2,4-triazole derivatives are biologically active against *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritidis* and *Aspergillus niger*. Antimicrobial investigation were performed by the filter paper disk methods (2). The compounds had the highest activity against *Salmonella enteritidis*.

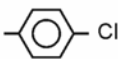

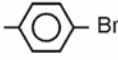
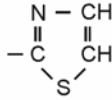
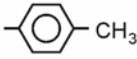
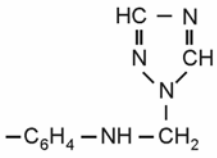
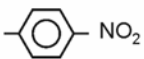
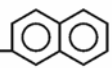
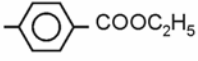
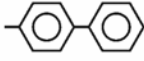
The primary intention in chromatography is to achieve satisfactory resolution of the sample components. The retention, and consequently resolution in liquid chromatography (LC), is determined by the interactions of the solute with both mobile and stationary phases of the system. Thus, the molecular structure of a solute, the polar and non-polar function of the stationary phase and the mobile phase composition influence retention in

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LC. It is well known that the same position of different substituents in a molecule can have significantly pronounced effects on retention in chromatography. Because of that, in this work we studied the retention behaviour of two series of 1,2,4-triazole derivatives by normal- and reversed-phase HPLC on silica gel and C-8 modified silica gel columns, respectively, using non-aqueous and aqueous mobile phases.

The structures of the investigated compounds are presented in Table 1.

Table 1. Structural formulas of the 1,2,4-triazoles studied

$ \begin{array}{c} \text{N} - \text{CH} \\ \parallel \quad \parallel \\ \text{HC} \quad \text{N} \\ \diagdown \quad / \\ \text{N} \\ \\ \text{CH}_2 - \text{NH} - \text{X} \end{array} $			
Compound	Series I	Compound	Series II
I ₁		II ₁	
I ₂		II ₂	
I ₃		II ₃	
I ₄		II ₄	
I ₅		II ₅	

EXPERIMENTAL

New 1-(arylaminoethyl)-1,2,4-triazoles (Table 1) were synthesized by the reaction of 1-hydroxymethyl-1H-1,2,4-triazole with the corresponding aromatic amine. They were characterized by determining melting points, IR and NMR spectra and elemental analysis.

Separations were performed with a Milton Roy liquid chromatograph (Riviera Baech, FL, USA) consisting of a model Consta Metric 3000 pump and a Milton Roy Spectro Monitor 3160 variable-wavelength UV-Vis detector set at 254 nm. Samples were injected using a Rheodyne 7125 valve (Cotati, CA, USA) fitted with a 20 μL loop. The columns

used were commercially available Spherisorb SI (250×4 mm i.d.; 5 μm) and LiChrosorb RP-8 (150×4 mm i.d.; 5 μm (both, E. Merck, Darmstadt, Germany).

1,2,4-triazole derivatives were dissolved (0.02 %) in methanol and the solutions filtered through a 0.2 μm Chromafil filter (Macherey-Nagel, Düren, Germany).

A binary solvent system (hexane - propan-1-ol) was used as a mobile phase on the silica gel column. Binary mixtures methanol - water, and acetonitrile - water, containing a volume fraction (φ) of organic solvent between 0.4 and 0.9 were used as mobile phases on C-8 modified silica gel column. The eluents used to prepare mobile phases were of analytical grade and were filtered through a 0.45 μm filter and degassed before use. The flow rate was 1 mL min⁻¹ at room temperature.

The retention factor, k , was calculated as $k = \frac{t_r - t_0}{t_0}$, where t_r is the retention time of the solute and t_0 the column void time of methanol. Each t_r value was measured in triplicate and averaged.

RESULTS AND DISCUSSION

Two series of 1,2,4-triazole derivatives were studied. In series I there were derivatives which have different substituent at the *p*- position of nitrogen atom (position 7), while in series II consisted of derivatives which have different substituent bound to the nitrogen atom at position 7 (Table 1). All derivatives were examined by normal and reversed phase HPLC on the silica gel and C-8 bonded silica gel columns.

Normal phase chromatography

The retention data for the 1,2,4-triazole derivatives obtained on silica gel column with hexane - propan-1-ol (8:2; v/v) are as eluent given in Table 2.

Table 2. Retention data for the 1,2,4-triazole derivatives on a Spherisorb SI column, using hexane-1-propan-ol as the eluent; designation of the compounds is as in Table 1

Compound	$\log k$
I ₁	-0,623
I ₂	-0,608
I ₃	-0,240
I ₄	-0,067
I ₅	-0,177
II ₁	0,351
II ₂	0,247
II ₃	0,038
II ₄	-0,646
II ₅	-0,666

The retention orders of the compounds of the particular groups were as follows:

Series I: $I_1 \leq I_2 < I_3 < I_5 < I_4$

Series II : $II_5 \leq II_4 < II_3 < II_2 < II_1$

In series I the least retention had compounds I_1 and I_2 . These two compounds were not resolved. Compound I_1 at *p*- position has a Cl atom, and compound I_2 a Br atom. These data did not confirm our former observations that the retention of derivatives possessing a halogen atom at the same position decreased from fluorine to iodine (3, 4). On the other hand, in these investigations, like in previous ones (3, 4), retention of halogen derivatives was low.

Compound I_3 which has a methyl group at the *p*- position was stronger retained than compounds I_1 and I_2 , but less than compound I_5 which has a polar ester group at the same position.

In series I the most retained was compound I_4 having a polar nitro group at the *p*- position.

In series II the least retention had derivatives II_4 and II_5 , which were not mutually resolved.

The most retained was compound II_1 because the molecule of pyridine at position 7 is more polar compared to the molecule of thiazole at the same position.

The sequence of separation on the silica gel column of all compounds was:

$II_5 \leq II_4 \leq I_1 \leq I_2 < I_3 < I_5 < I_4 < II_3 < II_2 < II_1$

On the basis of the separation sequence, it is shown that the compounds having in the molecule different nonpolar groups at the same position were not resolved because the nonpolar substituents did not affect the retention. Compounds from series I were generally less retained than compounds from series II. The most polar solute in series I, compound I_4 , was less retained than compound II_3 which is in series II of middle polarity.

The compounds that have a nitrogen atom as the substituent in their molecule, capable to form hydrogen bonding with silica gel surface, were the most retained (compounds II_3 , II_2 , II_1).

In these investigations, like in previous ones (4, 5) we showed that silica gel did not always indicate the real polarity of the compound, but it can be said that the retention behaviour of new 1,2,4-triazole derivatives on silica gel were in accordance with general principles in normal-phase liquid chromatography.

Reversed phase chromatography

The change in compounds retention of the 1,2,4-triazole derivatives with increasing volume fraction of the modifier in aqueous mobile phases was in accordance with the well known equation, generally accepted in partition chromatography:

$$\log k = \log k_0 - m\varphi \quad [1]$$

where φ is the volume fraction of the organic component of the binary aqueous mobile phase, $\log k_0$, is the value of $\log k$ extrapolated to $\varphi = 0$, and m is a constant. The relationship between the retention factor, $\log k$, of the investigated compounds and volume

fraction, φ , of the modifier in the aqueous mixture was linear (Table 3). The compounds were more mobile in the eluent with acetonitrile as the eluent modifier than with methanol owing to the lower polarity of acetonitrile. It is empirically known that a change from methanol to acetonitrile generally decreases the selectivity (Figs. 1 and 2).

Table 3. Constants m and $\log k_0$ of Equation [1] for the relationship between retention and mobile phases composition; designation of the compounds is as in Table 1

Compound	Methanol-water			Acetonitrile-water		
	$\log k_0$	m	r	$\log k_0$	m	r
I ₁	1.6527	2.403	0.9663	1.5836	2.327	0.9983
I ₂	1.7890	2.478	0.9550	1.6910	2.430	0.9990
I ₃	1.3890	2.034	0.9942	1.2759	1.953	0.9936
I ₄	1.2340	2.255	0.9983	1.3138	2.166	0.9929
I ₅	1.9430	2.999	0.9995	1.5142	2.344	0.9986
II ₁	0.9220	2.020	0.9765	0.9120	0.899	0.9986
II ₂	0.5785	1.790	0.9921	0.3239	1.258	0.9835
II ₃	0.6197	1.620	0.9630	0.2828	0.786	0.9998
II ₄	4.3450	4.795	0.9991	3.5405	4.265	0.9886
II ₅	2.6741	3.431	0.9872	2.4070	3.308	0.9913

The numerical values of the absolute value of constants m and $\log k_0$ for each examined compound and used mobile phases, containing water and methanol or acetonitrile as modifier, are given in Table 3. Correlation coefficients from linear regression analysis of experimental $\log k$ values varied from 0.9550 to 0.9998.

The retention data obtained for separation on the C-8 bonded silica gel column are generally typical of reversed phase chromatographic behaviour: less polar solutes are more strongly retained. The retention order of the compounds with both mobile phases were mainly as follows:

$$\text{Series I: } I_4 < I_5 \leq I_3 < I_1 < I_2$$

$$\text{Series II: } II_2 \leq II_1 < II_3 < II_5 < II_4$$

Compounds II₄ and II₅, which were not resolved on silica gel column, were clearly resolved on C-8 bonded silica gel (Figs. 1b and 2b), because the molecule of naphthalene at the position 7 is more hydrophobic than the molecule of diphenyl at the same position. Besides, the halogen atom contributed to the increase of hydrophobicity 1,2,4-triazole derivatives. Because of that, compounds I₁ and I₂ were the most retained in series I in both aqueous mobile phases; in all cases these two compounds were clearly resolved on reversed phases showing a higher retention of compound I₂ (Figs. 1a and 2a).

It is apparent from data in Table 3 that on C-8 bonded silica gel column and with both mobile phases the constant $\log k_0$ and the absolute value of the constant m increase with increasing compound retention (Figs. 3 and 4). There are, therefore, linear relationships between these two constants, with high correlation coefficients.

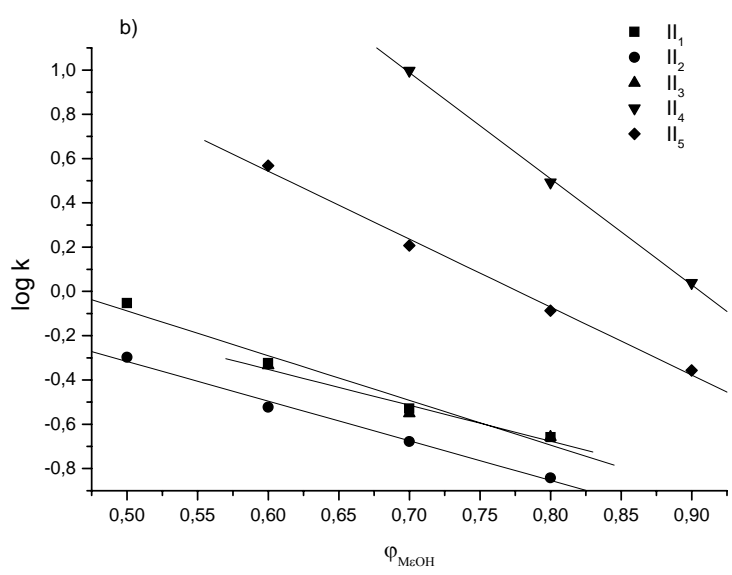
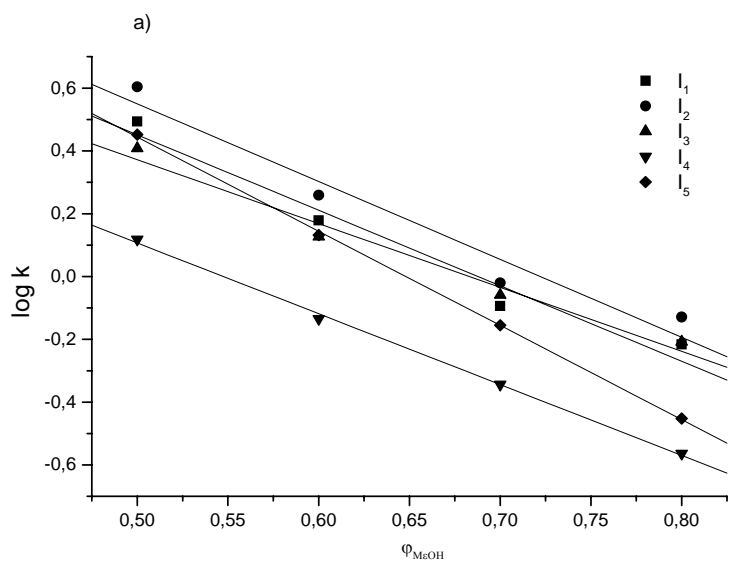


Fig. 1. Relationship between the retention constant ($\log k$) and the methanol concentration (φ_{MeOH}) in the mobile phase methanol-water; designation of the compounds is as in Table 1

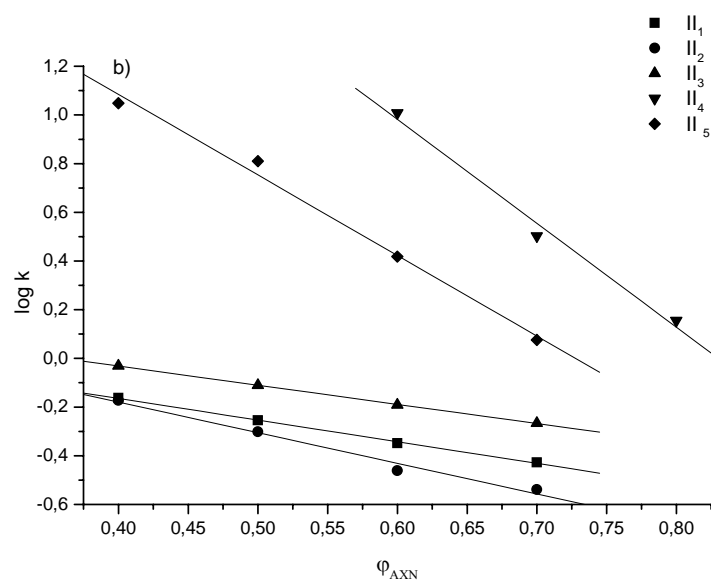
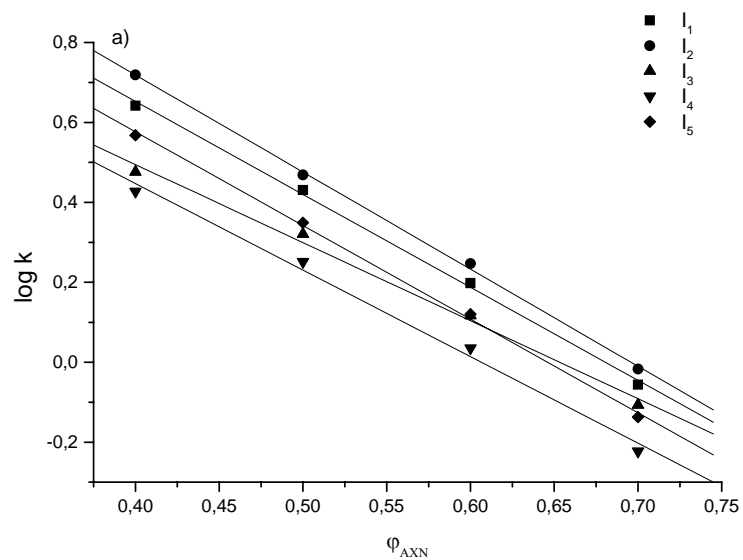


Fig. 2. Relationship between the retention constant ($\log k$) and the acetonitrile concentration (φ_{ACN}) in the mobile phase acetonitrile-water; designation of the compounds is as in Table 1

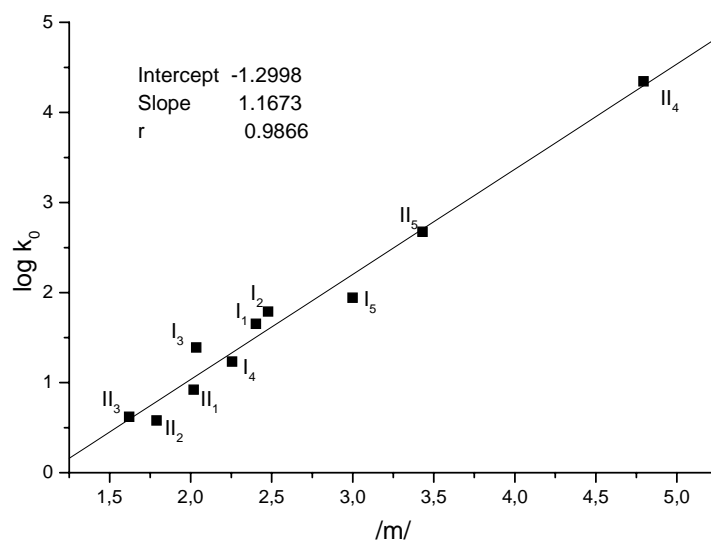


Fig. 3. Plot of $\log k_0$ against $/m/$ for the mobile phase methanol-water; designation of the compounds is as in Table 1

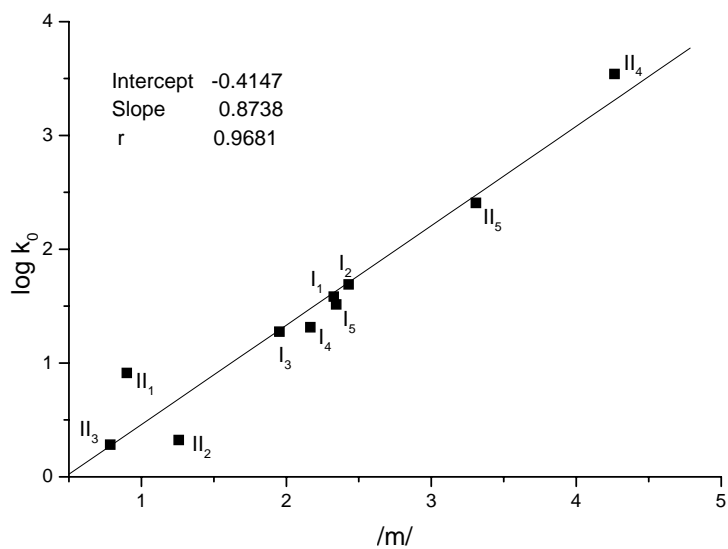


Fig. 4. Plot of $\log k_0$ against $/m/$ for the mobile phase acetonitrile-water; designation of the compounds is as in Table 1

CONCLUSION

- On silica gel, the retention sequence of 1,2,4-triazole derivatives obtained with non-polar eluent is that predicted on the basis of polarity of the compounds. However, some retention data show that the retention on silica gel does not always indicate the real polarity of the compound.
- On C-8 bonded silica gel, the retention sequence of the compounds obtained by applying aqueous-organic mobile phases, is basically a consequence of the compound hydrophobicity.
- Compounds that are not resolved in normal phases are resolved in reversed phases and vice versa.
- For resolving 1,2,4-triazole derivatives it is necessary to have one column of silica gel and one column of C-8 silica gel. This combination guarantees resolution of the 1,2,4-triazole derivatives.

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**ТЕЧНА ХРОМАТОГРАФИЈА ПОД ВИСОКИМ ПРИТИСКОМ НА
НОРМАЛНОЈ И ОБРНУТИМ ФАЗАМА НЕКИХ НОВИХ
1,2,4-ДЕРИВАТА ТРИАЗОЛА**

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У раду је испитано ретенционо понашање и раздвајање новосинтетизованих деривата 1,2,4-триазола, течном хроматографијом под високим притиском на нормалној и обрнутим фазама. Уз извесне ограде, резултати показују да је раздвајање на силика гелу (нормална фаза) у складу са поларношћу једињења, док је раздвајање на октил силика гелу (обрнута фаза) у складу са хидрофобношћу једињења. Деривати 1,2,4-триазола који нису раздвојени на силика гелу раздвојени су на октил силика гелу и обрнуто. У хроматографији на обрнутим фазама испитана је и промена ретенције деривата 1,2,4-триазола са променом запреминског удела органске компоненте у двокомпонентној воденој покретној фази.

Received 14 August 2003
Accepted 29 September 2003