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Sorbent Effect on Retention of Benzotriazoles in RP HPLC

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Abstract—The effect of the nature of the sorbent, molecular structure, and composition of the mobile phase on retention of benzotriazole derivatives was studied. Variation of the acetonitrile concentration in the eluent led to a considerable increase in the retention index of these substances, especially for Hypercarb and hypercrosslinked polystyrene. This is explained by the contribution of specific π–π interactions between the aromatic fragments of sorbates and hypercrosslinked polystyrene to sorption or by the polar retention effect for Hypercarb.

Keywords: benzotriazole, reversed-phase high-performance liquid chromatography, octadecyl silica gel, hypercrosslinked polystyrene, porous graphitized carbon, acetonitrile.

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INTRODUCTION

Interest of researchers in the chemistry of heterocyclic compounds primarily arises from their high biological and pharmacological activity and hence the possibility of developing drugs with a wide range of activity on their basis [1, 2]. Benzotriazole itself is a substrate for the synthesis of new aromatic heterocycles with different structures because of its ability to be involved in various reactions used in organic synthesis (arylalkylation, heterocyclization, heteroalkylation, etc.) [3, 4]. A term "benzotriazole methodology" has even appeared in the literature due to the useful properties of this compound; it encompasses currently known procedures for the synthesis of biologically active compounds from benzotrialole [5]. Moreover, many derivatives of aromatic heterocycles are used to create materials with nonlinear optical properties and develop photon technologies and biosensors; and they have other applications in electronics, optics, and various fields of technology [6]. Many publications were devoted to the use of azoles as ionic liquids with a unique set of properties [7, 8].

The widespread occurrence of aromatic heterocycles requires the development of methods for their qualitative and quantitative determination in complex mixtures of synthetic and natural origin and of procedures for analyzing the intermediates in the synthesis of various biologically active compounds on their basis. Liquid chromatography is one of the effective methods for investigating these compounds; it allows simultaneous separation and determination of compounds that widely differ in their structure and chemical properties due to the difference in their ability to be sorbed from aqueous organic eluents [9, 10].

The development of adsorption technologies now follows the trend of creating new sorption materials with preset properties or modifying the known and long-used materials. The active use of carbon and polymer materials with well-known properties remains a steady tendency; however, new publications appear, which indicate that the potential of sorbents of this type has not yet been exhausted [11]. The most widespread polymer sorbents are hypercrosslinked polystyrenes (HCPSs). Their main advantages are high chemical stability and hydrophobicity, high sorption capacity, and compatibility with many solvents [12, 13]. Porous graphitized carbon (PGC) has recently become widely used as a carbon sorbent possessing an important property of structural sensitivity in separation of complex synthetic and natural mixtures of substances with close molecular structures (geometrical and positional isomers, diastereomers, including highly polar phenols, amino acids, peptides, carbohydrates, and nucleosides) [14].

Therefore, the goal of our study was to investigate the peculiarities of chromatographic retention of benzotriazoles on various sorbents and the effect of the structure of analyte molecules and the composition of the eluent on their sorption under the conditions of reversed-phase (RP) HPLC.

EXPERIMENTAL

The chromatographic experiment was performed on a Varian ProStar liquid chromatograph with a ProStar UV spectrophotometric detector at a wavelength of 254 nm. The following sorbents were used: octadecyl silica gel (Waters, column size 150 × 4 mm, sorbent

Table 1. Selected characteristics of the compounds under study

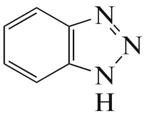
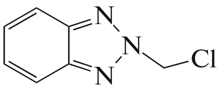
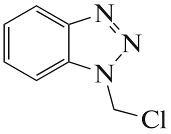
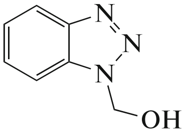
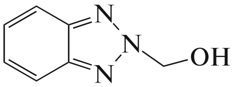
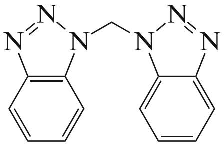
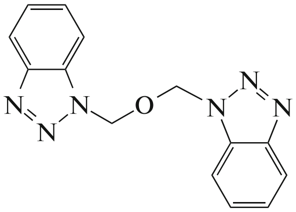
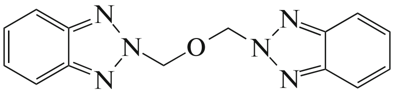
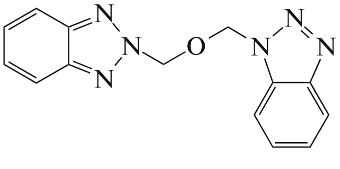
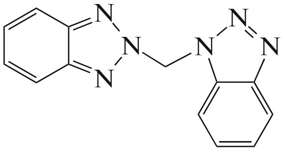
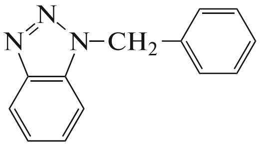
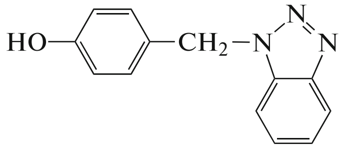
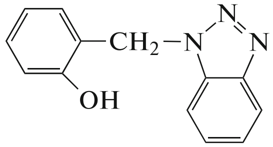
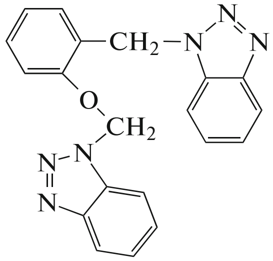
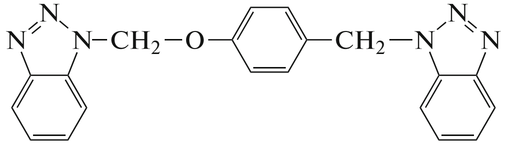
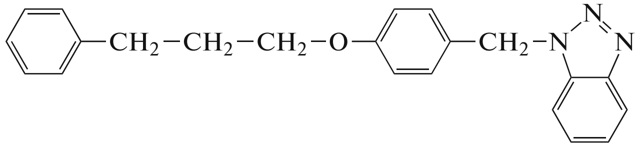
No.	Formula	Name	V, Å^3	log$P$*	α, Å^3	S, Å^2	μ, D
1		Benzotriazole	117.34	1.34	49.62	134.62	4.00
2		2-(Chloromethyl)-2*H*-benzotriazole	152.17	1.92	52.63	174.02	2.09
3		1-(Chloromethyl)-1*H*-benzotriazole	152.09	1.41	52.47	173.32	3.74
4		1*H*-Benzotriazol-1-ylmethanol	145.40	0.54	51.91	166.4	2.51
5		2*H*-Benzotriazol-1-ylmethanol	145.54	1.04	52.03	167.23	1.43
6		1,1'-Methylene-bis (1*H*-benzotriazole)	242.69	2.65	59.84	262.03	0.23
7		1,1'-(Oxydimethyl-diyl) bis(1*H*-benzo-triazole)	270.19	3.23	62.06	293.77	6.47
8		2,2'-(Oxydimethyl-diyl) bis(2*H*-benzo-triazole)	270.76	3.66	62.21	293.61	1.24
9		1-[(2*H*-Benzo-tri-azol-2-yl-methoxy)methyl]-1*H*-benzotriazole	270.55	3.74	62.19	290.15	5.13
10		1-(2*H*-Benzotriazol-2-ylmethyl)-1*H*-ben-zotriazole	242.87	3.15	59.95	262.83	4.04
11		1-Benzyl-1*H*-ben-zotriazole	222.32	2.92	58.16	239.56	4.12

Table 1. (Contd.)

No.	Formula	Name	V, Å^3	$\log P$*	α, Å^3	S, Å^2	μ, D
12		4-(1*H*-Benzotriazol-1-ylmethyl)phenol	229.40	2.18	58.76	248.12	3.46
13		2-(1*H*-Benzotriazol-1-ylmethyl)phenol	229.40	2.18	58.74	247.2	5.64
14		1-[2-(1*H*-Benzotriazol-1-ylmethoxy)benzyl]-1*H*-benzotriazole	354.01	3.55	68.87	370.45	3.02
15		1-[4-(1*H*-Benzotriazol-1-ylmethoxy)benzyl]-1*H*-benzotriazole	354.00	3.55	68.90	373.91	3.79
16		1-[4-(3-Phenylpropoxy) benzyl)]-1*H*-benzotriazole	370.53	5.40	70.24	391.42	4.48

In the tables and figures below, the numbering of substances is given in accordance with this table.

* Calculated with the ACDLabs'12 free software.

grain size 5 μm); hydrophobic (neutral) microporous hypercrosslinked polystyrene (column size 150 × 4.6 mm, sorbent grain size 5 μm); and porous graphitized carbon Hypercarb (column size 50 × 3 mm, sorbent grain size 5 μm). The sorbent based on hypercrosslinked polystyrene was synthesized and packed into a column at the Sorption Processes Stereochemistry Laboratory, Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences.

As objects of our research, we used the derivatives of benzotriazole, whose formulas are shown in Table 1.

Elution was performed in an isocratic mode. As a mobile phase, we used an acetonitrile–water mixture in different volume ratios (1 : 1, 1 : 3, 2 : 3, and 3 : 1). The eluent flow rate was 500 μL/min. To prepare the mobile phases, we used thrice distilled water and acetonitrile of HPLC-gradient grade (Panreac, Spain); degassing was performed by treating the prepared aqueous acetonitrile mixtures ultrasonically using a UZDN-2T unit. The chromatograms were recorded and processed with the Galaxie program for chromatographic analysis.

The sorbate solutions were prepared by dissolving dry individual substances in suitable solvents. The volume of the introduced sample was 20 μL.

The retention of substances was characterized in terms of the retention index (k) calculated by the equation

$$k = t'_R/t_M = (t_R - t_M)/t_M.$$

Sodium nitrite was used as a nonsorbed substance. The analysis included five parallel measurements.

The polarizability (α), molar volume (V), surface area (S), and dipole moment (μ) were calculated by the density functional theory method using the Spartan 10 (version 1.1.0) program. The lipophilicity (distribution coefficient in the n-octanol–water system, $\log P$) of the compounds was calculated with the ACDLabs'12 free program (Table 1).

Table 2. Retention indices of benzotriazoles on different sorbents

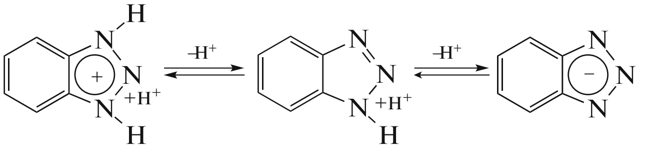
No.	Retention index										
	ODS				HCPS			Hypercarb			
	3 : 1*	1 : 1	2 : 3	1 : 3	3 : 1	1 : 1	2 : 3	3 : 1	1 : 1	2 : 3	1 : 3
1	0.80	0.88	0.96	1.98	1.61	2.96	3.53	0.82	1.09	1.90	4.86
2	0.58	1.41	2.55	7.64	4.41	14.41	32.29	1.60	4.40	8.55	24.28
3	0.53	1.52	2.58	8.36	4.56	16.15	34.57	1.56	4.55	8.43	25.24
4	0.61	0.67	0.86	1.69	1.63	3.04	4.73	0.82	1.33	1.94	5.43
5	0.44	0.74	0.86	1.66	1.62	3.04	4.60	0.85	1.27	2.04	5.41
6	1.04	1.96	3.01	13.57	4.29	19.31	43.69	3.22	9.13	22.42	100.15
7	0.98	2.00	3.08	14.09	5.07	23.89	55.37	3.32	9.65	21.24	111.13
8	0.97	2.00	3.04	14.44	3.68	21.37	56.48	3.30	9.59	21.76	87.19
9	0.99	2.24	3.05	14.85	5.14	20.64	65.18	3.01	9.31	21.49	81.19
10	0.99	2.12	3.03	14.84	5.09	21.91	66.83	3.26	9.73	19.44	–
11	0.96	1.62	1.91	17.03	2.19	16.57	–	0.75	11.15	–	–
12	0.70	1.42	2.65	9.67	3.75	12.18	25.02	2.69	5.87	16.32	82.48
13	0.81	1.82	3.46	16.26	4.83	13.74	34.53	4.33	10.87	31.91	–
14	0.94	5.79	6.61	55.06	19.83	80.23	–	23.56	12.08	25.16	–
15	0.94	4.02	8.62	–	27.69	67.89	–	29.54	10.41	–	–
16	0.70	1.05	1.97	11.27	4.00	9.57	–	2.84	8.06	16.95	–

* Composition of the eluent: acetonitrile–water (v/v).

RESULTS AND DISCUSSION

A peculiarity of the molecular structure of nitrogen heterocycles is the interaction of the lone electron pairs of the heteroatom with the electrons of the cyclic system; another peculiarity is the high degree of delocalization of π-electrons. For *N*-unsubstituted diazoles, triazoles, and tetrazoles, tautomeric forms can exist in the form of associates due to the intermolecular proton transfer. The *N*-substituted benzotriazoles exist as 1*H* and 2*H* isomers; it was assumed that the 1*H* isomers dominate in the solid state and solution, while the 2*H* isomers dominate in the gas phase [5].

Benzotriazole is an ampholyte because it can be protonated at the 3-position, forming salts with acids, and deprotonated at the 1-position. The following equilibrium was assumed to exist for it:



The changes in the electronic structure of triazole molecules lead to changes in their physicochemical parameters and hence in the sorption characteristics determined by the competing effect of the dispersion interaction with the sorbent and specific interactions of the nitrogen atoms of the benzotriazole fragment and the functional groups with the components of the polar eluent under the conditions of RP HPLC.

Table 2 presents the retention indices of the substances during chromatography on octadecyl silica gel (ODS), hypercrosslinked polystyrene (HCPS), and porous graphitized carbon (Hypercarb) at different quantitative compositions of the aqueous acetonitrile eluent. It follows from the obtained data that the retention characteristics of benzotriazoles in general on all the sorbents used change symbatically with the physicochemical parameters responsible for the intermolecular interactions (IMIs) in RP HPLC (volume, lipophilicity, and polarizability). The smallest retention indices are characteristic for compounds **1–5** with the minimum van der Waals volume, lipophilicity, and polarizability of molecules for almost all the compositions of the eluent and sorbents used. At the same time, the *k* index of these compounds changes depending on the nature and position of substituent in the benzotriazole fragment.

Thus, the structures of the pairs of substances **2, 3** and **4, 5** differ in the presence of a chlorine atom or hydroxyl group and their position, respectively; this primarily leads to a change in the N–C–Cl or N–C–OH bond angle (Fig. 1) and hence to a change in the electronic and physicochemical parameters of these compounds. Due to the presence of chlorine atoms liable to specific interactions with both the components of the mobile phase and the sorbents, the retention of

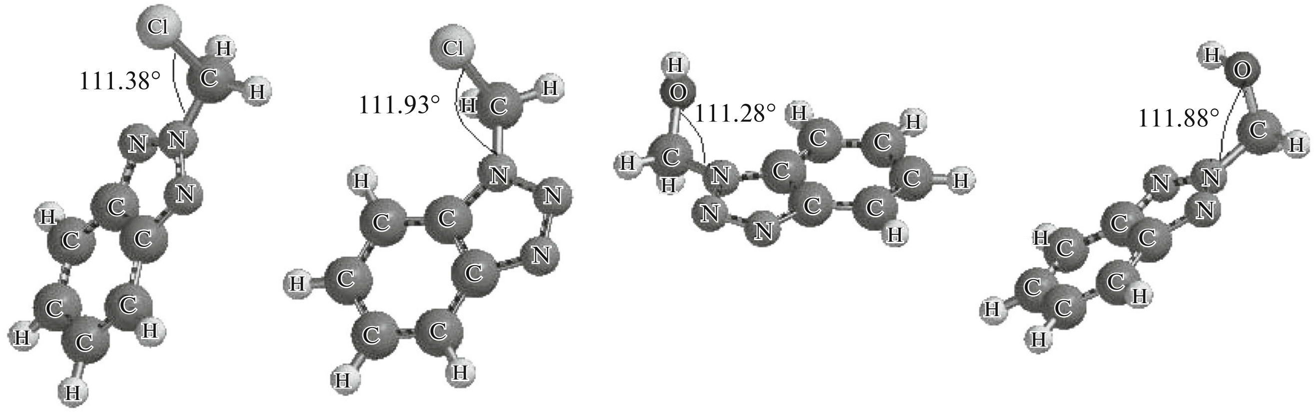


Fig. 1. Optimized molecules **2, 3** and **4, 5**.

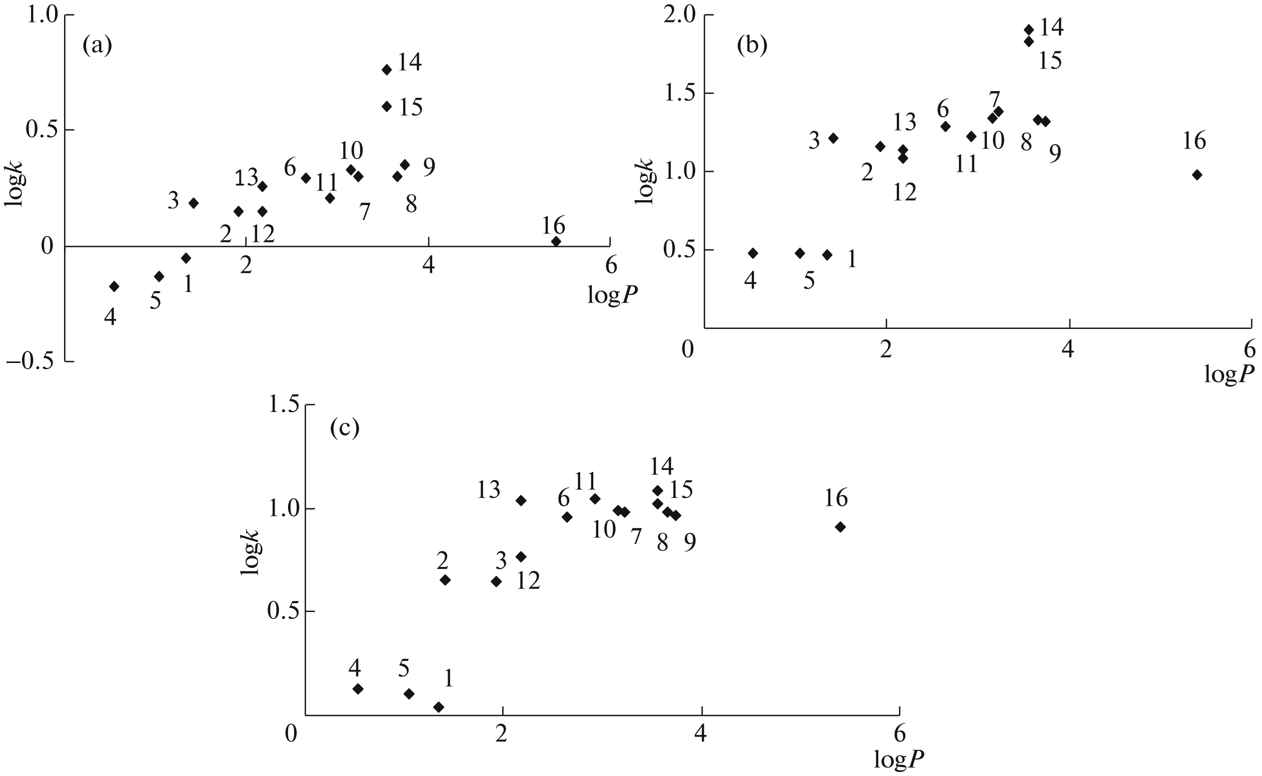


Fig. 2. Dependences of the logarithm of the retention index on the lipophilicity of benzotriazoles on the sorbents (a) ODS, (b) HCPS, and (c) Hypercarb; eluent composition: acetonitrile–water 1 : 1.

substances **2** and **3** changes more significantly than that of hydroxyl-containing compounds **4** and **5** with a similar structure, especially on HCPS and PGC. Sorbates **4** and **5** probably interact with the components of the mobile phase more strongly, as indicated by their high solvation energy and insignificant dependence of the retention index on the nature of the sorbent. The decrease in the acetonitrile content in the eluent also changes the retention of **2** and **3** more significantly than of **4** and **5**.

For compounds **6–16**, the van der Waals volume, polarizability, and lipophilicity increase, leading in general to an increase in the retention index due to the strengthening of dispersion interactions between the sorbate and the stationary phase. However, in this case, the increase is again ambiguous because it depends on the eluent composition, the nature of the sorbent, and the nature and position of substituents in the sorbate molecule.

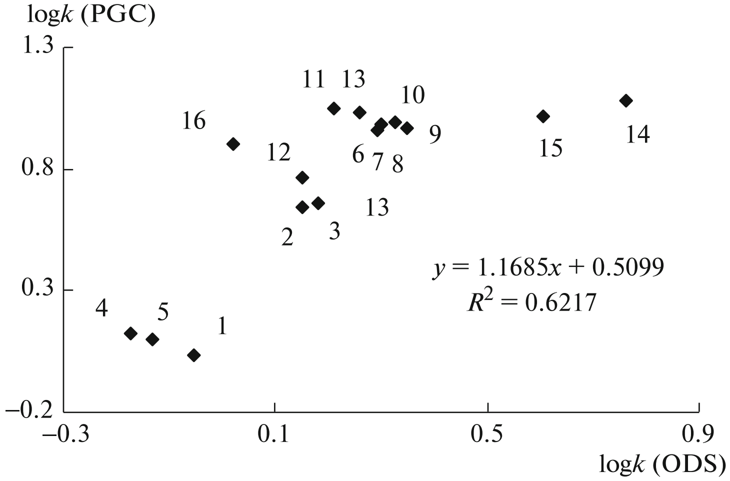


Fig. 3. Correlation between the retention indices of benzotriazoles on ODS and PGC.

According to the obtained data, on ODS with 75% acetonitrile, the retention index was smaller than unity for almost all the sorbates under study due to their dominant interaction with the polar eluent. As the acetonitrile concentration decreased, the *k* index of these substances on ODS increased only insignificantly; on Hypercarb and HCPS, it increased for almost all sorbates and eluent compositions. A decrease in the acetonitrile concentration to 25% led to an irreversible sorption of these substances on HCPS (for this reason, the corresponding retention indices are absent in Table 2). The significant retention of benzotriazoles in the case of HCPS is evidently primarily due to the higher specific surface area of nanoporous hypercrosslinked polystyrene compared with that of the silica sorbent and to the contribution of the specific π–π interactions between the aromatic fragments of sorbates and hypercrosslinked polystyrene to sorption in addition to dispersion.

The increased retention on PGC may be explained by the specific adsorbate–adsorbent intermolecular interactions. These interactions occur in addition to the dispersion interactions and are expressed as the so-called polar retention effect, which is known to be due to π-stacking between the aromatic fragments of adsorbate molecules and the delocalized electron density of the graphite-like surface of PGC and also to electrostatic interactions between the polar adsorbate molecules and induced charges on the surface of PGC [11]. These interactions are illustrated by the sorption of isomers **7**–**9**, whose retention indices on ODS differ by dozens of times from those for HCPS and PGC for several eluent compositions.

The effect of different types of IMIs on retention by different sorbents is illustrated by the plots presented in Fig. 2. The relationship between the retention on ODS and lipophilicity is almost linear mainly due to the dispersion interactions of benzotriazoles with this sorbent. The specific interactions additional to the dispersion interactions during sorption on HCPS and PGC lead to deviation from linearity and scatter of points on the plots of the corresponding dependences.

The degree of the thermodynamic relationship between processes in chromatographic columns can be judged from the level of correlation between the retention indices of sorbates on two different columns. These dependences are generally used for comparing the retention indices on two sorbents [13]; this leads to an equation of the form $\log k_A = a + b\log k_B$, where k_A and k_B are the retention indices on sorbents A and B, respectively. Considerable deviations from linearity on the plots of the retention indices of benzotriazoles on ODS and PGC (Fig. 3) point to considerable differences in the mechanisms of retention of these compounds by these sorbents despite the reversed-phase variant of HPLC in both cases.

CONCLUSIONS

Thus, the obtained data indicate that the HCPS and PGC sorbents are rather sensitive to the structure of benzotriazoles when the composition of the mobile phase is varied, due to the specific interactions additional to the dispersion interaction, which generally determines the retention under the conditions of RP HPLC. The sorption of these compounds by these sorbents is considerably affected not only by the electronic and physicochemical parameters (volume, polarizability, lipophilicity, etc.), but also by the number of heterocyclic and other aromatic fragments and heteroatoms in the molecule, the nature of substituents and functional groups, and molecular stereochemistry. It can be stated with confidence that inclusion of all these parameters in consideration will allow one to predict the retention and the possible order of elution of benzotriazoles from the chromatographic column with a high degree of accuracy.

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