Retention Behavior of Benzodiazepines in Normal-Phase HPLC. Silica, Cyano, and Amino Phases Comparison

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PLEASE SCROLL DOWN FOR ARTICLE

RETENTION BEHAVIOR OF BENZODIAZEPINES IN NORMAL-PHASE HPLC. SILICA, CYANO, AND AMINO PHASES COMPARISON

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ABSTRACT

The retention data for 16 benzodiazepines was evaluated on different normal-phase HPLC columns using the Soczewinski-Snyder model. The slopes and intercepts of the linear relationships between log k´ and the mobile phase composition have been discussed in relation to solute and stationary phase characteristics. The good correlation between substituent chromatographic contribution and substituent electronic constant showed that the benzodiazepine molecule acts as a proton donor towards the adsorbent sites of the stationary phases investigated.

INTRODUCTION

Recent investigations on solute retention behaviour on polar bonded
phases (1-8) have shown that the Snyder adsorption model (9-11) is a
valuable tool in characterizing the adsorptive properties of cyano
and amino derivatized silica columns. Many studies (2, 3, 7, 12, 13)
cleared that the amino groups constitute the primary adsorption
sites on amino-silica packing. Therefore, this packing significantly
differs from silica in terms of differences in relative solvent
strength and relative retention of solute groups. The mechanism of
solute retention on the cyano phase is still controversial. Some
authors (1, 5, 7) assumed that cyano-silica behaves much like a
deactived silica towards non polar and moderately polar solutes.
Other studies (6, 14-16) showed that, as the polarity of solute and
solvents increases, cyano-silica possess unique   retention
characteristics.

Snyder developed a general   molecular   model   for   adsorption
chromatography (9-11, 17) where the retention of a solute molecule X
onto an adsorbent can be regarded as a displacement process where a
molecule of X in the non-sorbed phase displaced some number of
preadsorbed mobile phase molecules. The following relationship
between log k´ value and the composition of the mobile phase (as
X_s = mole fraction of the strong solvent in the binary mobile phase)
can be derived:

$$\log k_2' = \log k_1' - \frac{A_s}{n_b} \log X_s \qquad\qquad (1)$$

where A_s is the molecular area of the solute, n_b is the molecular

area of the strong solvent, k'_2 is the capacity factor for the solute eluted with the binary mobile phase, and k'_1 is the capacity factor of the solute eluted with just the strong solvent.

Soczewinski's approach (18-20) describes a special case of the Snyder's model, when more polar molecules of the solute and solvent are centred over adsorption sites with strong interactions between localized adsorbate and adsorption sites. In this case retention is related to X_s by the equation:

$$\log k' = \log \frac{K_{AX} \, X_{AS}}{K_{AS}} - n \log X_s \qquad (2)$$

where X_{AS} is the mole fraction of the strong solvent adsorbed on the stationary phase, K_{AX} and K_{AS} are the adsorption constants of the solute and solvent, respectively. In both eqs. 1 and 2 the slope values suggest the number of solute molecule active adsorption sites interacting with the surface sites of the stationary phase, while the intercept values are related to the strength of solute-stationary phase binding.

In this work the chromatographic behaviour of benzodiazepines (BDZ) on silica, cyano- and amino-silica stationary phases is investigated. BDZs are a class of clinically important drugs owing to their wide spectrum of anxiolitic, anticonvulsant, hypnotic and mio-relaxant properties (21-23). Recently (24-26) evidence has been given of the fact that the binding process of BDZ molecules to their specific receptors in the central nervous system is controlled by the hydrogen bonds (HB) they are able to accept. Retention data are examined on the basis of the Soczewinski-Snyder model for an understanding of the mechanism governing the adsorption of BDZs on

the different stationary phases. Analysis of relationship between retention characteristic and molecular structure makes it possible to obtain additional information about active adsorption sites in BDZ molecules and to predict analytical parameters determining optimum separation.

MATERIALS AND METHODS

Equipment

All chromatographic measurements were carried out with a Spectra Physics Chromatograph (SP87100 pump and SP87500 organizer module) equipped with a 10 μl sample loop, and a Varian UV detector operated at 254 nm.

Columns

The columns used were 30cm x 3.9mm I.D. obtained from Waters Assoc. (Millford, MA, U.S.A.): a μPorasil packed with 10-μm porous silica, a μBondapak CN and a μBondapak NH$_2$ with propylnitrile and propylamina groups chemically bonded to 10-μm porous silica, respectively.

Reagents

Solvent. HPLC grade n-hexane, 2-propanol, and ethyl acetate were obtained from Rudi Pont. These solvents were prefiltered though a Millipore type F-H 0.5μm filter and degassed by bubbling helium gas. For each column, a minimum of 7 different n-hexane-2-propanol and n-hexane-ethyl acetate mixture compositions were studied.

BDZ standards. The BDZs, obtained from commercial sources, were

dissolved in 2-propanol or 2-propanol-water mixtures in the concentration range between 100 and 400 μg/ml. Molecular structures of the studied BDZs are reported in Figure 1.

RESULTS AND DISCUSSION

Application of the Soczewinski-Snyder model to BDZs.

The linear relationship expressed by eqs. 1 and 2 was confirmed for the compounds investigated on all three columns. Slope, intercept and correlation coefficient values of log k´ vs. log X$_s$ plots with 2-propanol and ethyl acetate as strong solvents are reported in Tables I-III for silica, cyano- and amino-silica, respectively.

On each column, the slope n experiences relevant variations with solvent type: the ratios between average values of slopes in ethyl acetate and 2-propanol are 2.2 for amino and cyano and 1.8 for silica. These values agree with the value 2.0 found, with several classes of compounds, for the ratio between the strengths of these solvents on silica (10). Thus the number of ethyl acetate molecules displaced by one BDZ molecule in the adsorption process appears approximatively double of that of 2-propanol molecules. Assuming that the binding of a BDZ molecule needs, on the average, some 2.5 adsorption sites, these sites can be made available either by the release of same 2.5 molecules of ethyl acetate or 1.2 of 2-propanol.

Qualitative and quantitative studies of the structure-pharmacological activity correlation (27, 28) reported that BDZ-receptor interaction is determined by a HB mechanism

1) Medazepam

2)

3) Prazepam

4)

5) Oxazepam

6) Temazepam

7) Diazepam

8) N—demethyl

diazepam

9)

10)

11) Lorazepam

12) Methyl

lorazepam

13)

14)

15)

16)
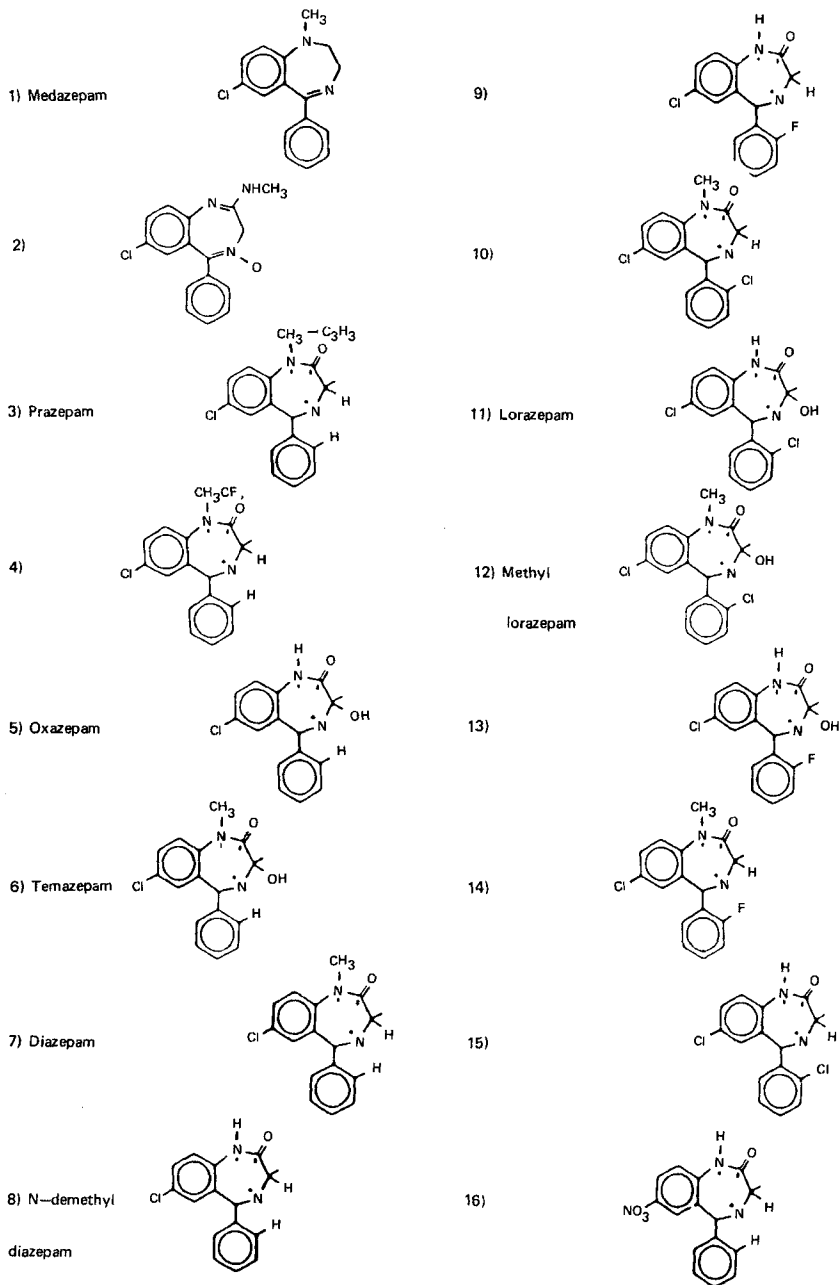
Figure 1: Molecular structures of the studied BDZs.

Table I

Slope, intercept, correlation coefficient values from the relationship between log k´ and the concentration of the strong solvent ($\log k' = a - n \log X_s$) on silica phase.

Compound	2-propanol			Ethyl acetate		
	a	n	r	a	n	r
1	-0.86	0.90	0.998	-0.87	1.80	0.999
2	-0.71	0.98	0.997	-0.68	1.78 `	0.998
3	-0.80	1.10	0.998	-0.79	1.77	0.997
4	-0.67	1.04	0.999	-0.67	1.91	1
5	-0.51	1.52	0.999	-0.46	2.51	0.998
6	-0.52	1.50	0.998	-0.46	2.43	0.999
7	-0.56	1.10	0.999	-0.52	2.29	0.999
8	-0.55	1.10	0.996	-0.51	1.92	0.998
9	-0.45	0.99	0.997	-0.40	1.79	0.998
10	-0.55	1.10	0.998	-0.53	2.09	0.997
11	-0.39	1.43	0.999	-0.33	2.49	0.998
12	-0.46	1.56	0.997	-0.38	2.47	0.996
13	-0.41	1.25	0.998	-0.34	2.28	0.998
14	-0.50	0.91	0.999	-0.46	1.79	0.999
15	-0.45	1.10	1	-0.40	1.91	0.997
16	-0.33	1.44	0.997	-0.26	2.49	0.997

Table II

Slope, intercept, correlation coefficient values from the relationship between log k´ and the concentration of the strong solvent (log k´ = a - n log X_s) on cyano-silica phase.

	2-propanol			Ethyl acetate		
Compound	a	n	r	a	n	r
1	-1.40	0.81	0.998	-1.42	2.09	0.999
2	-1.19	1.07	0.996	-1.20	2.32	0.996
3	-1.37	0.83	0.998	-1.27	1.90	0.999
4	-1.27	0.91	0.997	-1.36	1.93	0.999
5	-0.80	1.20	1	-0.76	2.82	0.999
6	-0.82	1.04	0.999	-0.79	2.56	1
7	-0.93	0.95	0.993	-0.82	1.67	0.991
8	-0.99	1.01	0.998	-1.03	2.33	0.998
9	-0.91	1.04	0.998	-0.85	2.20	0.998
10	-0.97	0.89	0.998	-0.90	2.10	0.998
11	-0.71	1.22	0.999	-0.64	2.38	1
12	-0.71	1.15	0.998	-0.69	2.12	0.999
13	-0.76	1.17	1	-0.71	2.51	0.999
14	-0.82	0.80	0.998	-0.84	1.88	0.996
15	-0.91	0.95	0.993	-0.86	2.28	0.997
16	-0.96	1.12	0.998	-0.95	2.35	0.997

Table III

Slope, intercept, correlation coefficient values from the relationship between log k´ and the concentration of the strong solvent ($\log k' = a - n \log X_s$) on amino-silica phase.

	2-propanol			Ethyl acetate		
Compound	a	n	r	a	n	r
1	-1.14	1.00	0.999	-1.08	2.69	0.999
2	-0.35	1.19	0.994	-0.56	3.32	0.996
3	-0.82	1.00	0.995	-0.79	2.65	0.996
4	-0.84	1.08	0.999	-0.74	3.17	0.993
5	0.10	1.32	0.994	0.16	2.88	0.998
6	0.11	1.34	0.995	0.14	2.60	0.997
7	-0.62	1.00	0.999	-0.57	2.55	0.997
8	-0.56	1.41	0.999	-0.55	2.28	0.999
9	-0.53	1.44	0.996	-0.51	2.78	0.998
10	-0.56	1.09	0.995	-0.49	2.82	0.997
11	0.62	1.30	0.996	0.86	4.96	0.999
12	0.57	1.41	0.998	0.84	5.50	0.995
13	0.58	1.41	0.999	0.77	5.60	0.996
14	-0.54	1.09	1	-0.49	2.19	0.996
15	-0.49	1.30	0.998	-0.44	2.42	0.998
16	-0.31	1.35	0.998	-0.27	3.17	0.997

mainly associated with the groups which are able to form HBs: 7-Cl,

4-N, 2-O, 3-OH and 2´-halogen. Slope and intercept values of Tables

I-III are thus checked in the following by assuming that such groups

are also the active sites in the BDZ adsorption on the studied

columns.

Binding properties of BDZs on normal-phase columns.

According to eqs. 1 and 2, on silica and cyano phases the value of

nearly 2 (1 for 2-propanol) for compounds 3, 4, 7, 8 would imply

that these molecules have two active functions for localizing on the

stationary phase surface. On the other hand, molecular structure

give evidence of three active groups: 4-N, 2-O and 7-Cl. Soczewinski

(20) indicated that, in a solute molecule with strongly polar groups

(aromatic, nitrogen, OH), substituents such as Cl and F do not cause

a marked variation in slope value since, even if they noteably

influence adsorption affinity, they have only a small effect on the

molecular mechanism. This behaviour is also confirmed by the fact

that compounds 9, 10, 14, 15 containing one more active group (2´-Cl

or 2´-F) have approximately the same slope value 2. Compounds 5, 6,

11-13 containing a 3-OH group as one more active group show slope

values in the range 2.1-2.8 (1.1-1.5 for 2-propanol). This behaviour

confirms the marked contribution of the OH group to the three-point

attachment (29).

On amino-silica the halogen group appears to contribute

significantly to multipoint adsorption, even if the retention

behaviour appears quite complex. Both compounds containing three or

four active groups have the same mean slope value of 2.7 (1.3 for

2-propanol)indicating the significant presence of a three-point attachment. Compounds 11-13 with five active groups have a slope of 5.4 which seems to bear witness to the activity of all five groups for a multipoint adsorption. The slopes of compounds 2 and 16 seem to indicate that even the polar groups 4-NO and 7-NO$_2$ significantly contribute to a multipoint adsorption on all three columns. The hypothesis that the BDZ molecule binds to the adsorbent surface by means of HB acceptor groups is also confirmed by the behaviour of intercept values reported in Tables I-III. The increasing number of such active sites causes ever increasing intercept values.

On each column, the intercept values are larger for ethyl acetate solvent compared to 2-propanol. Since ethyl acatate is a weaker solvent, K$_{AS}$ should be smaller and therefore the intercept value of eq. 2 is larger than that for 2-propanol. The linear relationship between the intercept values for both these solvents show good statistical parameters for each column (Table IV).

Eqs. 3-5 suggest no significant differences between the retention mechanism with the two solvents, even if ethyl acetate does not donate hydrogen in HB formation as 2-propanol does (3, 30).

From the data reported in Tables I-III for each solvent and each solute it was confirmed that the adsorption strength increases in the following order:

$$\text{silica} \simeq \text{cyano} < \text{amino.}$$

In order to investigate the specific contribution of solute-adsorbent interactions to solute retention on the different bonded phases, the relationships between intercept values on cyano

Table IV

Statistical parameters of the relationships between the intercepts with ethyl acetate and 2-propanol as strong solvents.

$$a_{EA} = 0.125(\pm0.013) + 1.153(\pm0.022) \; a_{2pr.} \qquad \text{silica} \qquad (3)$$

$$n = 16 \qquad r = 0.997 \qquad s = 0.013 \qquad F = 2617.12 \quad P < 0.001$$

$$a_{EA} = 0.129(\pm0.054) + 1.109(\pm0.054) \; a_{2pr.} \qquad \text{cyano} \qquad (4)$$

$$n = 16 \qquad r = 0.984 \qquad s = 0.045 \qquad F = 421.09 \quad P < 0.005$$

$$a_{EA} = 0.101(\pm0.027) + 1.109(\pm0.045) \; a_{2pr.} \qquad \text{amino} \qquad (5)$$

$$n = 16 \qquad r = 0.989 \qquad s = 0.093 \qquad F = 615.41 \quad P < 0.005$$

and amino vs. silica as reference were calculated. Since ethyl acetate and 2-propanol solvents gave parallel results, only the data with ethyl acetate are reported in Table V.

The good statistical parameters of eq. 6 indicate that the separation on cyano is parallel to that on silica. This behaviour seems to support the hypothesis that residual silanols are the primary adsorption sites for cyano-silica (1, 5, 7).

The poor correlation between retention data on amino vs. silica (eq. 7) evidences the change in the adsorptive properties of silica as a

Table V

Statistical parameters of the relationships between intercepts

on the different stationary phases (ethyl acetate as strong solvent).

$$a_{CN} = -0.319(\pm 0.102) + 1.238(\pm 0.193) \ a_{Si} \qquad (6)$$

$$n = 16 \qquad r = 0.864 \qquad s = 0.127 \qquad F = 41.29 \qquad P < 0.005$$

$$a_{NH_2} = 1.015(\pm 0.361) + 2.473(\pm 0.681) \ a_{Si} \qquad (7)$$

$$n = 16 \qquad r = 0.696 \qquad s = 0.449 \qquad F = 13.18 \qquad P < 0.05$$

$$a_{NH_2} = 2.746(\pm 0.430) + 5.564(\pm 1.081) \ a_{Si} \qquad (8)$$

$$n = 5(with \ OH) \qquad r = 0.948 \qquad s = 0.136 \qquad F = 26.47 \qquad P < 0.01$$

$$a_{NH_2} = 0.015(\pm 0.087) + 1.088(\pm 0.149) \ a_{Si} \qquad (9)$$

$$n = 11(without \ OH) \qquad r = 0.924 \qquad s = 0.086 \qquad F = 52.92 \qquad P < 0.005$$

result of the derivatization reaction with amino group (2, 3, 7, 12,

13). Figure 2 shows that compounds containing an hydroxyl group are

selectively retained on amino-silica. Therefore the linear

relationship between retention data on amino and silica is

significantly improved when the solutes are divided into two

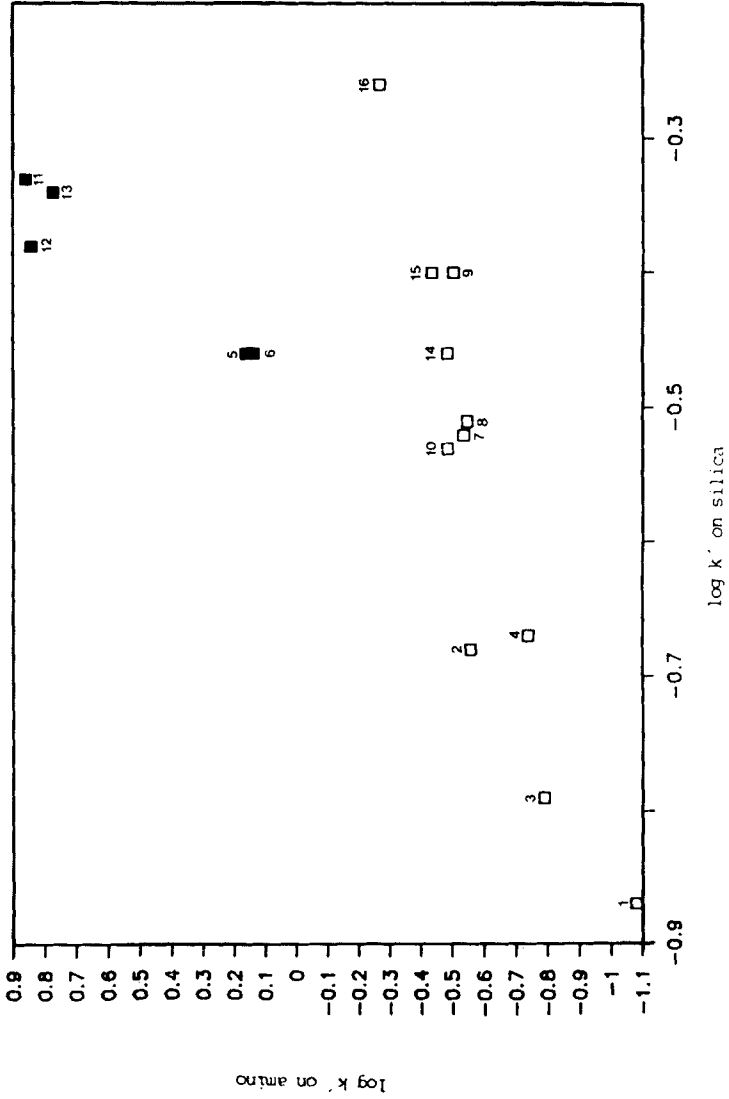■ Compounds with OH

□ Compounds without OH

Figure 2: Relationship between the retention data on amino vs. silica

(ethyl acetate as strong solvent: eqs.8 and 9)

classes: containing an 3-OH group or not (eqs. 8 and 9 in Table V).

This selective behaviour of the amino phase towards the OH group can

be explained by assuming that the amino group (a very strong Lewis

base) show some specific interactions with the acid group OH (12).

In order to quantitatively correlate solute retention to molecular

structure, the linear free energy relationship developed by Hammett

(31) was used to describe the substituent electronic effect on

adsorptive strength (32-34). The substituent chromatographic

contributions $\Delta \log k'$ were calculated from intercept values of

Tables I-III and reported in Table VI. $\Delta \log k'$ values have been

correlated with substituent constants σ using the following

equation:

$$\Delta \log k' = c + \varrho \sigma \qquad (10)$$

Significant correlations were found for all three columns with both

solvents (Table VII). As previously reported (12, 34) the results

with 2-propanol and ethyl acetate are very similar. The positive

value of the coefficient ϱ indicates that electronwithdrawing

substituents increase the adsorption strength of the solute

molecule. This can be explained by assuming that the BDZ molecule

acts as a proton donor towards the adsorbent sites (1, 12, 33). For

cyano-silica a statistically significant regression line was

obtained only when the strongly deviating NO_2 group was neglected.

For amino-silica the eqs. (c) and (c') were obtained without the

data related to the OH group. The presence of this substituent

determined retention values much higher than those predicted by eq.

10. As described above, this behaviour can be explained by assuming

Table VI

Substituent Hammett constants σ and substituent retention contributes $\Delta \log k'$ in both strong solvents on the investigated columns.

		2-propanol			Ethyl acetate		
Group	σ	Si	CN	NH_2	Si	CN	NH_2
OH	0.12	0.05	0.07	0.70	0.06	0.02	0.70
Cl	0.37	0.09	0.11	0.07	0.11	0.12	0.10
F	0.34	0.09	0.11	0.06	0.09	0.04	0.06
NO_2	0.59	0.22	0.03	0.25	0.25	0.08	0.28
CH_3	-0.07	-0.04	-0.06	-0.05	-0.03	-0.04	-0.03

Table VII

Statistical parameters of the relationship: $\log k' = c + \varrho\,\sigma$ (eq. 10)

with 2-propanol (eqs. a-c) and ethyl acetate (eqs. d-f)

as strong solvents.

c	ϱ	r	F	s	Column	
-0.015(±0.018)	0.359(±0.052)	0.970	47.50	0.026	Silica	(a)
-0.013(±0.024)	0.369(±0.091)	0.943	16.24	0.033	Cyano	(b)
-0.047(±0.044)	0.422(±0.114)	0.934	13.76	0.054	Amino	(c)
-0.008(±0.022)	0.386(±0.064)	0.961	36.39	0.032	Silica	(d)
-0.020(±0.026)	0.287(±0.101)	0.896	8.13	0.036	Cyano	(e)
-0.031(±0.052)	0.434(±0.133)	0.917	10.60	0.064	Amino	(f)

the existence of specific strong interactions between amino and OH groups.

CONCLUSIONS

The adsorption theory of Snyder appears to be a valuable means of describing adsorption behaviour of BDZs on silica, cyano- and amino-silica columns. Moreover study of the Hammett equation showed that BDZ molecules play the same role as HB donors both in the adsorption on the studied stationary phases and in BDZ-receptor binding.

The normal-phase chromatographic approach seems to be very promising for pharmacologically active compounds containing specific HB donor or acceptor groups implied in the drug-receptor interaction. Since chromatographic retention and receptor binding affinity are both controlled by HB forming capability, a chromatographic column could be a suitable reference system to discriminate whether a specific HB donor or acceptor group is primarily involved in the drug-receptor interaction. For the studied BDZs a strong correlation between the abilities to bind to the receptor and to the amino-silica column was found for all the groups involved in the BDZ-receptor interaction (35).

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