Lipophilicity determination of *N*-(benzothiazol-2-yl)-α-amino alkyl phosphonic diesters by RP-HPLC and RP-HPTLC

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Using methanol-water mixtures as the mobile phase, the chromatographic retention parameters k' and R_f were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) and reversed-phase high-performance thin-layer chromatography (RP-HPTLC) for *N*-(benzothiazol-2-yl)-α-amino alkyl phosphonic diesters and the correlation with lipophilicity parameter (Clog*P*) was established. Logk_w values obtained from RP-HPLC and R_{m0} values obtained from RP-HPTLC can be used to evaluate the lipophilicity of this kind of compounds. Chromatographic method is a good alternative for lipophilicity measurement.

Keywords Reversed-phase HPLC, reversed-phase HPTLC, *N*-(benzothiazol-2-yl)-α-amino alkyl phosphonic diesters, lipophilicity determination

Introduction

Lipophilicity is an important physico-chemical parameter of substance[1] and it plays a vital role in QSAR (Quantitative Structure-Activity Relationships) studies.[2,3] Traditionally, lipophilicity of a compound is determined by "flask-shaking" method, but the traditional method has some disadvantages:[4] it is tedious, time consuming and can be applied only in a limited range on the lipophilicity scale.

These dificulties can be overcomed by using chromatographic method, such as reversed-phase high-performance liquid chromatography[5] and reversed-phase thin-layer chromatography:[6] it is rapid and relatively simple, very small amounts of substances are required and the compounds need not be very pure.

Logk' values obtained using RP-HPLC and R_m values obtained using RP-TLC have been used as a reliable alternative to the classical log*P* values in order to express the lipophilicity character of a compound.[7,8]

N-(Benzothiazol-2-yl)-α-amino alkyl phosphonic diesters show anti tobacco mosaic virus (TMV) activity and their lipophilicity is related with their activities. The objectives of this work were to determine the retention behavior of this kind of compounds on RP-HPLC and RP-HPTLC using methanol-water mixtures as the mobile phase, to find the relationship between retention characteristics and the calculated lipophilicity parameters (Clog*P*) of these compounds.

Experimental

Materials

The structures of nineteen *N*-(benzothiazol-2-yl)-α-amino alkyl phosphonic diesters are listed in Table 1. This series of compounds were synthesized in our organic synthesis laboratory, and their structures were demonstrated by many methods: IR, NMR, MS and elemental analysis. Approximately 1 mg/mL concentration in methanol was used for spotting.

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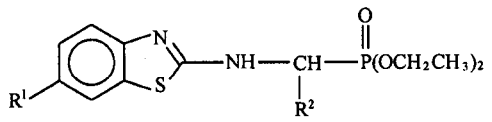
Apparatus

The HPLC instrument was a Backman Gold System II liquid chromatography (2500N, Harbor Boulevard, Fullerton, CA, U.S.A.) with a data system. The ODS column (Backman, Part No.: 235329, 250 × 4.6 mm id.) was packed with ODS packing of particle size 5 μm.

TLC was performed on precoated C_{18} reversed-phase HPTLC plates (10 × 10 cm, F_{254}) (Merck, Darmstadt, Germany). A Nanomat applicator (Camag, Muttenz, Switzerland) was used with a Pt-Ir pointed glass capillary. Plates were developed in a closed chamber (Camag).

SGI Indy Workstation (U.S.A.) with Sybyl 6.22 (Tripos Company) was used for data collecting, and a PC computer (Pentium II) was used for data processing.

Table 1 Structures of *N*-(benzothiazol-2-yl)-α-amino alkyl phosphonic diesters



No.	R^1	R^2
1	CH_3	4-CH_3OPh
2	CH_3	4-ClPh
3	CH_3	Ph
4	CH_3	3-ClPh
5	CH_3	4-NO_2Ph
6	CH_3	2-ClPh
7	CH_3	2,4-Cl_2Ph
8	CH_3	3-CH_3Ph
9	CH_3	4-OHPh
10	CH_3	(3,4-OCH_2O)Ph
11	CH_3	4-BrPh
12	CH_3	2-CH_3OPh
13	CH_3	2,4-(CH_3O)Ph
14	CH_3	4-$(CH_3)_2N$Ph
15	CH_3	4-CH_3Ph
16	CH_3	3-NO_2Ph
17	H	2,4-Cl_2Ph
18	CH_3	3-BrPh
19	H	Ph

Chromatography

HPLC Methanol-water mixtures were used as eluents, the concentration of methanol ranging from 70% to 90% in steps of 5%. The flow rate was 1.0 mL/min and 20 μL samples were injected. The column was thermostatic at 25℃ and a UV detector at 230 nm was used. The retention time of 100% methanol was employed as the unretained time t_0.

TLC Methanol-water mixtures were used as mobile phase, the concentration of methanol were 75%, 80%, 85%, 90%, respectively. Developments were carried out in a closed chamber at room temperature, the distance of development being about 5 cm. After development, the plates were dried in air and the spots were revealed under an UV lamp.

Results and discussion

The k' and R_f values of each compound obtained in all experimental conditions were listed in Table 2 and Table 3, respectively. The lipophilitic parameters (Clog$P$), which were obtained from Indy workstation, were also listed in Table 3.

Table 2 k' values of 19 compounds in RP-HPLC

No.	k'				
	0.70	0.75	0.80	0.85	0.90
1	5.428	3.022	1.995	1.195	0.6604
2	10.94	5.838	3.152	1.519	0.8274
3	5.435	3.293	1.853	1.024	0.6308
4	12.00	6.436	3.065	1.381	0.8170
5	5.958	3.417	1.883	1.160	0.6839
6	10.26	4.866	2.565	1.346	0.7206
7	24.55	13.18	5.284	2.447	1.1960
8	8.995	4.834	2.441	1.179	0.6477
9	3.100	1.293	0.8317	0.5662	0.3719
10	4.180	2.850	1.472	0.7907	0.4982
11	10.47	5.556	2.591	1.446	0.8385
12	5.238	3.162	1.585	0.8820	0.4964
13	5.662	3.319	1.624	0.9190	0.4998
14	6.100	4.149	2.004	1.137	0.6912
15	8.995	4.595	2.275	1.110	0.6560
16	5.746	3.324	1.585	0.8763	0.4757
17	14.96	7.193	3.556	1.661	0.9438
18	11.14	5.517	2.667	1.418	0.7805
19	3.580	1.924	1.102	0.6674	0.4426

The R_m values of each compound were obtained by Eq. (1):

$$R_m = \log(1/R_f - 1) \quad (1)$$

Table 3 R_f values of 19 compounds in RP-HPTLC and their Clog$P$ values

No.	R_f				log$P$
	0.75	0.80	0.85	0.90	
1	0.186	0.276	0.403	0.554	4.41
2	0.122	0.224	0.322	0.459	5.21
3	0.192	0.277	0.411	0.544	4.49
4	0.110	0.229	0.335	0.475	5.21
5	0.188	0.274	0.422	0.546	4.24
6	0.123	0.228	0.332	0.468	5.21
7	0.051	0.131	0.211	0.347	5.92
8	0.137	0.221	0.356	0.509	4.99
9	0.356	0.466	0.581	0.699	3.83
10	0.205	0.320	0.431	0.587	3.85
11	0.108	0.193	0.314	0.472	5.36
12	0.204	0.303	0.460	0.577	4.41
13	0.195	0.289	0.451	0.571	4.50
14	0.132	0.232	0.341	0.481	4.69
15	0.126	0.220	0.334	0.476	4.99
16	0.166	0.280	0.388	0.542	4.24
17	0.077	0.145	0.296	0.406	5.42
18	0.103	0.180	0.303	0.454	5.36
19	0.264	0.381	0.496	0.624	4.00

Linear correlation between R_m values and the concentration of organic modifier in the eluents were calculated separately for each compound according to Eq. (2):

$$R_m = R_{m0} + bc \quad (2)$$

where c is the concentration of methanol in the eluent, R_{m0} is the R_m value extrapolated to 0% organic modifier concentration, and b is the change of R_m value caused by unit change of organic modifier concentration in the mobile phase. The R_{m0} and b values were listed in Table 4.

There is also a linear relationship between the logk' values and the concentration of organic modifier in the mobile phase as shown in Eq. (3):

$$\log k' = \log k_w + S\varphi \quad (3)$$

where φ is the concentration of methanol in the eluent, logk_w is the logk' value extrapolated to 0% organic modifier concentration, and S is the change of logk' value caused by unit change of organic modifier concentration in the mobile phase. The logk_w and S values were also listed in Table 4.

Table 4 logk_w, S, R_{m0}, b values of each compound and the regression coefficients

No.	logk_w	S	r	R_{m0}	b	r
1	3.854	-4.465	0.9984	4.333	-4.908	0.9992
2	5.004	-5.654	0.9996	4.705	-5.159	0.9968
3	4.071	-4.756	0.9994	4.178	-4.725	0.9991
4	5.288	-6.004	0.9982	5.102	-5.646	0.9931
5	4.056	-4.690	0.9994	4.294	-4.866	0.9982
6	5.002	-5.730	0.9994	4.756	-5.237	0.9969
7	6.109	-6.712	0.9986	6.071	-6.468	0.9918
8	5.018	-5.796	0.9995	4.909	-5.469	0.9996
9	3.490	-4.401	0.9830	3.370	-4.142	0.9996
10	4.015	-4.809	0.9966	4.232	-4.861	0.9987
11	4.896	-5.555	0.9982	5.244	-5.773	1.000
12	4.374	-5.203	0.9992	4.298	-4.941	0.9980
13	4.495	-5.332	0.9992	4.409	-5.051	0.9975
14	4.246	-4.907	0.9965	4.685	-5.177	0.9987
15	4.993	-5.782	0.9988	4.802	-5.296	0.9994
16	4.609	-5.486	0.9992	4.491	-5.070	0.9984
17	5.414	-6.073	0.9990	5.770	-6.269	0.9940
18	5.090	-5.798	0.9992	5.256	-5.752	0.9999
19	3.711	-4.551	0.9970	3.740	-4.399	0.9997

All the compounds showed normal retention behavior, that is their R_m and $\log k'$ values decreasing linearly with increasing concentration of methanol in the mobile phase, as it can be seen clearly from Fig. 1.

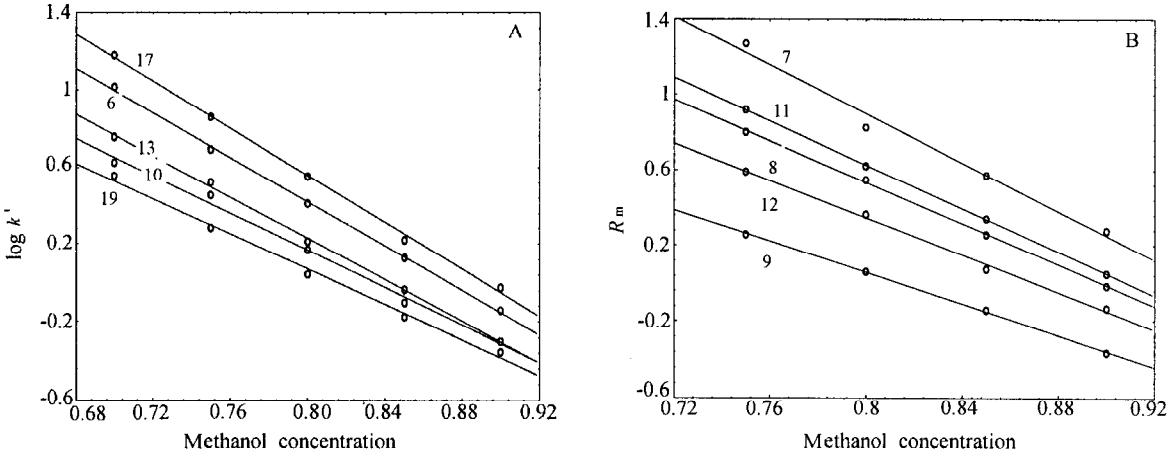


Fig. 1 Relationship between the retention values and methanol concentration in the mobile phase for some selected compounds: A: RP-HPLC B: RP-HPTLC.

The relationship between $\log k'$ values and Clog$P$ in HPLC and the relationship between R_m values and Clog$P$ in RP-HPTLC were listed in Table 5.

Table 5 Relationships between the retention parameters and Clog$P$ in all experimental conditions

Chromatography	Mobile phase composition	Regression equations
RP-HPLC	0.70	$\log k' = -0.8840 + 0.3697\text{Clog}P$
		$n = 19$ $r = 0.9711$ $F = 281.6$ $s = 0.05580$ $p < 0.00001$
	0.75	$\log k' = -1.024 + 0.3441\text{Clog}P$
		$n = 19$ $r = 0.9332$ $F = 114.6$ $s = 0.08142$ $p < 0.00001$
	0.80	$\log k' = -1.055 + 0.2899\text{Clog}P$
		$n = 19$ $r = 0.9358$ $F = 119.7$ $s = 0.06711$ $p < 0.00001$
	0.85	$\log k' = -1.033 + 0.2285\text{Clog}P$
		$n = 19$ $r = 0.9236$ $F = 98.65$ $s = 0.05828$ $p < 0.00001$
	0.90	$\log k' = -1.096 + 0.1913\text{Clog}P$
		$n = 19$ $r = 0.9088$ $F = 80.53$ $s = 0.05399$ $p < 0.00001$
RP-HPTLC	0.75	$R_m = -0.957 + 0.360\text{Clog}P$
		$n = 19$ $r = 0.9359$ $F = 120.1$ $s = 0.08334$ $p < 0.00001$
	0.80	$R_m = -0.842 + 0.278\text{Clog}P$
		$n = 19$ $r = 0.9220$ $F = 96.33$ $s = 0.07172$ $p < 0.00001$
	0.85	$R_m = -0.946 + 0.246\text{Clog}P$
		$n = 19$ $r = 0.9178$ $F = 90.89$ $s = 0.06529$ $p < 0.00001$
	0.90	$R_m = -1.096 + 0.225\text{Clog}P$
		$n = 19$ $r = 0.9282$ $F = 105.8$ $s = 0.05533$ $p < 0.00001$

* n is the number of compounds being studied, r is the regression coefficient, F is the overall F-test for the regression, s is the standard deviation, p is the level of significance.

Note that in Table 5 the R_m and $\log k'$ values are related with lipophilicity of these compounds. Basically,

the regression coefficient in the relationship between R_m, $\log k'$ and Clog$P$ increased with decreasing concentration of methanol in the mobile phase. So the extrapolated retention parameters R_{m0} and $\log k_w$ were considered, and there is a good correlation between the extrapolated retention parameters and the Clog$P$ values, as shown in the following equations:

$$\log k_w = -0.3278 + 1.040\text{Clog}P$$
$$n = 19 \quad r = 0.9273 \quad F = 104.3 \quad s = 0.2580 \quad p < 0.00001 \tag{4}$$

$$R_{m0} = -0.100 + 1.002\text{Clog}P$$
$$n = 19 \quad r = 0.9212 \quad F = 95.32 \quad s = 0.2601 \quad p < 0.00001 \tag{5}$$

Factor analysis was carried out for the five parameters: Clog$P$, $\log k_w$, R_{m0}, S and b, the result was listed in Table 6, which show that all the five parameters have high factor loadings in factor 1, which means that they are highly correlated and have much in common. That is to say that $\log k_w$, R_{m0}, S and b can reflect the lipophilicity of these compounds. S and b were considered:

$$S = -0.8584 - 0.9454\text{Clog}P$$
$$n = 19 \quad r = 0.8856 \quad F = 61.82 \quad s = 0.3045 \quad p < 0.00001 \tag{6}$$

$$b = -1.042 - 0.879\text{Clog}P$$
$$n = 19 \quad r = 0.9044 \quad F = 76.34 \quad s = 0.2549 \quad p < 0.00001 \tag{7}$$

Table 6 Factor analysis result

Parameters	Factor 1
R_{m0}	-0.9779
b	0.9730
$\log k_w$	-0.9849
S	0.9607
Clog$P$	-0.9557
Eigenvalue	4.709
Total variance (%)	94.19

It can be seen that the relationship between $\log k_w$, R_{m0} and Clog$P$ is better than that of S, b and Clog$P$, which means although S and b can reflect the lipophilicity of these compounds, they are of secondary importance. So $\log k_w$ and R_{m0} values were preferred to evaluate the lipophilicity of these compounds.

In this paper it was shown that $\log k_w$ and R_{m0} values obtained from RP-HPLC and RP-HPTLC respectively are a good alternative for lipophilicity determination.

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