ELSEVIER

Journal of Chromatography A, 722 (1996) 69–79

JOURNAL OF CHROMATOGRAPHY A

Retention behavior of quinolones in reversed-phase liquid chromatography

Ho Seob Kim, Dai Woon Lee*

Department of Chemistry, Yonsei University, Seoul, 120-749, South Korea

Abstract

Quantitative structure–retention relationships (QSRRs) have been used successfully to predict and explain retention behavior in reversed-phase liquid chromatography (RPLC). The informative structural descriptors for quinolones on a PRP-1 column and aqueous organic solvent system were proposed. Since quinolones are ionizable, the retention behavior of quinolones was investigated at both pH 3 and pH 11. Cluster analysis was applied to assign compounds to specific categories according to k'-value. Based on this analysis quinolones could be divided into two groups regardless of the organic modifier and pH. At pH 3, the solvent-accessible surface area was an informative descriptor which could explain the retention behavior of quinolones. In contrast, the y-component of the dipole moment was a useful descriptor at pH 11. Calculations for finding descriptors were performed by MM+ and AM1 methods.

1. Introduction

Reversed-phase liquid chromatography (RPLC) is the most popular method of analytical LC. This implies continuing research interest in this separation method. Extensive studies have been carried out over several decades to improve understanding of the solute retention mechanism, but much remains to be elucidated. There have been several approaches to solute retention theory, involving classical thermodynamics, molecular interaction kinetics [1], or the empirical method.

Although the thermodynamic approach can explain the different mechanisms that are effective after the data are obtained and identify distribution systems that are energy driven or entropically driven, it cannot predict a specific change in excess free energy for a particular solute distribution system [2–4]. The molecular interactive kinetic approach can explain and predict changes of retention behavior that occur by modifying the composition of either or both phases [5–7]. However, to predict retention, the distribution coefficients of each solute between the pure phases are required. With the empirical approach, the statistical correlation of known or measurable characteristics of the compounds with their observed retention data are used to determine the retention model. These studies are known as quantitative structure–retention relationships (QSRRs). Recently, individual applications of QSRRs were reviewed by Kaliszan [8]. QSRRs have three main goals: prediction of chromatographic retention, elucidation of the chromatographic separation mechanism, and

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00687-7

identification of the informative structural descriptors. There are many descriptors available that are commonly used in QSRRs studies [8]: topological, geometrical, electronic, physicochemical, and intermolecular ones [9].

Since the chromatographic retention is determined by intermolecular interactions such as the solute–stationary phase, solute–mobile phase, and mobile phase–stationary phase and all conditions can be kept constant in a chromatographic separation, the solute structure is the single independent variable in a chromatography system [8].

The goal of this study is to find which of the available descriptors describe the retention behavior of quinolones in a given chromatography system.

2. Experimental

2.1. Materials

High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile and dioxane (Burdick and Jackson, Muskegon, MI, USA) were used without further purification. Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) fitted with a 0.45-μm filter. Pure solutes (Table 1) were used as received, and stock solutions made in HPLC-grade acetonitrile. Among the 19 compounds, some (no. 1–7) were from Sigma (St. Louis, MO, USA), and the remainder were from the Korean Research Institute of Chemistry and Technology (Dae Jeon, South Korea).

2.2. HPLC conditions

All retention measurements were carried out using a Model 45 pump equipped with a U6K universal sample injector and a Model 440 fixed-wavelength (254 nm) absorbance detector (Waters Associates, Milford, MA, USA). Detector output was recorded on a C-R6A Chromatopac (Shimadzu, Kyoto, Japan) integrator.

A PRP-1 column (250 × 4.1 mm I.D., 10 μm, polystyrene–divinylbenzene, Hamilton, Reno, NV, USA) was used. Mixtures of phosphate buffer (0.05 *M* NaH_2PO_4) and organic modifier (v/v) were used as the mobile phase: pH 3 was adjusted using 99.9% H_3PO_4 to reduce dissociation of the acidic group, while pH 11 was adjusted using 10 *M* NaOH for its dissociation.

The column was thermostatted at 30°C using a home-made water jacket. Isocratic elution was carried out, and the flow-rate was 1.0 ml/min. The hold-up time (t_0) used to calculate capacity factors was determined for each composition from the elution of sample solvent.

2.3. Cluster analysis

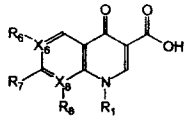
Statistical calculations were performed with a Macintosh Classic II using SYSTAT software (SYSTAT, Evanston, IL, USA). For cluster analysis the Euclidian distance was used, and the data were standardized before computing distances. Two common clustering methods, hierarchical and non-hierarchical clustering, were performed to identify groups, both in number and in content. For quantification of the clustering in the *K*-means, a popular non-hierarchical clustering technique, the variable means and the *F*-values for each cluster were considered.

2.4. Descriptor generation

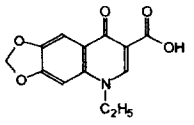
Calculations for obtaining descriptors were carried out with a IBM compatible 486 PC using HyperChem (Hypercube, Waterloo, Ont., Canada). For each solute geometry optimization calculation was carried out by MM+[1] in a periodic box. The dimension, in angstrom, of the periodic box is a cube 17.0 Å on a side. The total energy and the energies of the individual bonding component (bonds, angles, dihedrals, Van der Waals interactions, hydrogen bondings, electrostatic energy) arising from optimization calculation were used as descriptors. Single-point calculation was done with a semi-empirical method, AM1 [11], using optimized geometry. Then, the total energy in semi-empirical calculation, each

[1] The MM+ force field is an extension of MM2 which was developed by Allinger [10].

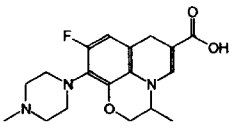
Table 1
Structures of quinolones



[I]



4



19

Compound no.	Substituents in [I]					
	R_1	R_6	R_7	R_8	X_6	X_8
1	C_2H_5	–		–	N	N
2	C_2H_5	F		–	C	CH
3	c-C_3H_5	F		–	C	CH
5	C_2H_5	–	CH_3	–	CH	N
6	C_2H_5	–		–	N	N
7		F		–	C	CH
8		F	Cl	–	C	CH
9		F	Cl	–	C	CH
10	C_2H_5	F	Cl	–	C	CH
11		F	Cl	–	C	CH
12	c-C_3H_5	F	F	–	C	CH
13		F		–	C	CH
14	c-C_3H_5	F		–	C	CH
15	C_2H_5	F		Cl	C	C
16	c-C_3H_5	NO_2	Cl	–	C	CH
17	C_2H_5	F		–	C	N
18	C_2H_5	F		F	C	C

energy component, heat of formation, dipole moment, and dipole moment components were used as descriptors. Also, solvent-accessible surface area, solvent-accessible molecular volume, log P, molar refractivity, and polarizability were calculated with the ChemPlus module (Hypercube).

3. Results and discussion

Table 2 gives the capacity factors (k') of quinolones in three mobile phases of each pH. Since the compounds showing similar retention behavior might generally have the same retention mechanism under a given chromatographic condition, the question was which of the compounds would have a similar retention behavior.

Cluster analysis is a technique for classifying individuals into unknown groups [12]. Therefore, this analysis was applied to classify compounds that showed similar retention behavior on the basis of their k'-values. Two ways of clustering, the hierarchical and non-hierarchical methods, were carried out to verify classification. Both clustering methods showed the same result in this study.

It is clear from Tables 3 and 4 that quinolones were divided into two groups based on k'-value regardless of the organic modifier and pH. The variable means for each group aid in understanding the characteristics. On the basis of group mean, each group was well separated; Group I had relatively less retained compounds, while Group II had relatively more retained compounds. Also, a large F-value is an indication that the corresponding variable is useful in separating the groups. At pH 3, the largest F-value indicating that the groups differ the most from each other in terms of organic modifier was observed in 80% methanol. At pH 11, the largest F-value was observed in 10% acetonitrile.

Based on the cluster analysis it was suggested that there were two retention behaviors for quinolones on the PRP-1 column. Therefore, in order to find the structural descriptors explaining the retention behavior, QSRRs study was performed for each group.

Table 2
Capacity factors (k') of quinolones in dioxane (D), acetonitrile (A), and methanol (M) as the organic modifiers at pH 3 and 11

Compound number	pH 3			pH 11		
	D 30%	A 40%	M 80%	D 10%	A 10%	M 40%
1	0.194	0.001	0.022	0.153	0.450	0.500
2	0.192	0.011	0.024	0.147	0.470	0.498
3	0.235	0.022	0.028	0.574	0.381	0.643
4	2.339	1.639	2.590	0.907	0.455	0.604
5	6.551	3.443	4.611	1.947	3.124	1.780
6	11.088	5.758	11.977	8.543	16.851	7.238
7	0.279	0.029	0.035	0.632	0.403	0.543
8	21.830	8.310	8.140	10.075	16.430	7.158
9	29.955	11.116	8.816	15.066	25.992	9.453
10	10.058	4.930	4.411	3.792	6.752	2.728
11	3.633	1.300	1.040	1.242	2.262	0.591
12	7.403	4.015	3.513	2.708	4.928	2.105
13	0.257	0.060	0.050	2.486	6.013	4.032
14	3.749	1.365	1.376	0.138	0.017	0.019
15	0.153	0.027	0.019	1.286	3.403	1.858
16	17.512	8.065	6.929	10.169	18.973	5.774
17	0.247	0.008	0.005	2.837	1.762	0.643
18	0.204	0.053	0.014	2.879	4.906	2.961
19	0.195	0.029	0.001	2.916	4.592	2.858

Table 3
Groups of quinolones as the result of the K-means clustering based on the k'-values at pH 3 (D: dioxane, A: acetonitrile, M: methanol)

Variable	F-ratio			
k' at D 30%	50.28			
k' at A 40%	51.43			
k' at M 80%	58.43			
Compounds	Variable	Minimum	Mean	Maximum
Group I				
1,2,3,4,5,7,	k'(D 30%)	−0.69	−0.43	0.46
10,11,12,13,14,	k'(A 40%)	−0.76	−0.44	0.66
15,17,18,19	k'(M 80%)	−0.76	−0.44	0.48
Group II				
6,8,9,16	k'(D 30%)	0.58	1.63	2.78
	k'(A 40%)	0.90	1.63	2.44
	k'(M 80%)	1.11	1.66	2.47

At pH 3, k' was plotted against solvent-accessible surface area in Fig. 1. Similar retention patterns were observed for Group I. Retention curves appear to plateau at a surface area greater than 500 Å^2, and the k'-value increases as the surface area decreases for values smaller than 450 Å^2.

Since the stationary phase is basically non-polar in RPLC, it is impossible to expect any strong interaction with a solute such as ionic interaction, hydrogen bonding, and dipole–dipole interaction. The only attractive force between the stationary phase and the solute would be Van der Waals force. However, since polystyrene-divinylbenzenes (Fig. 2) have phenyl groups, additional interactions, such as $\pi-\pi$

Table 4
Groups of quinolones as the result of the K-means clustering based on the k'-values at pH 11 (D: dioxane, A: acetonitrile, M: methanol)

Variable	F-ratio			
k' at D 10%	103.7			
k' at A 10%	114.2			
k' at M 40%	67.99			
Compounds	Variable	Minimum	Mean	Maximum
Group I				
1,2,3,4,5,7,	k'(D 10%)	−0.82	−0.47	0.04
10,11,12,13,14,	k'(A 10%)	−0.82	−0.47	0.07
15,17,18,19	k'(M 40%)	−0.98	−0.45	0.47
Group II				
6,8,9,16	k'(D 10%)	1.17	1.75	2.72
	k'(A 10%)	1.35	1.76	2.61
	k'(M 40%)	1.10	1.69	2.42

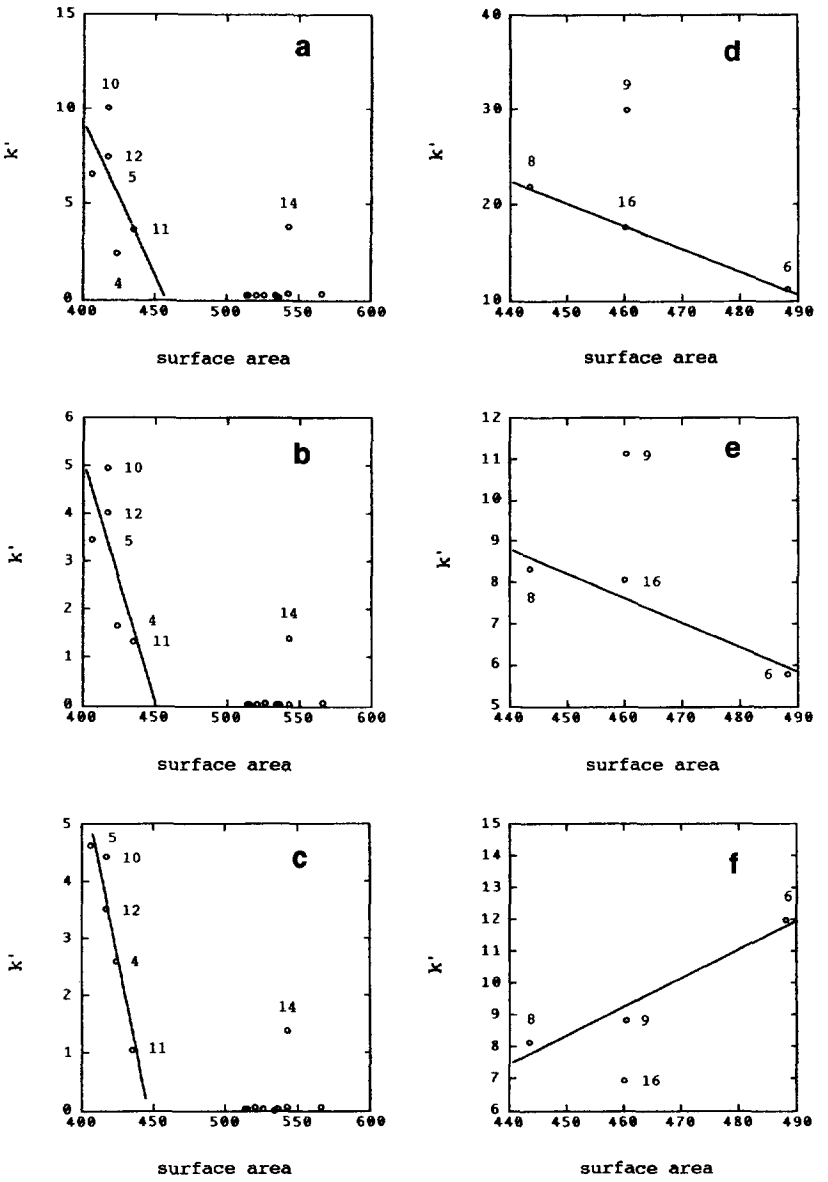


Fig. 1. Solvent-accessible surface area (Å^2) versus k' on PRP-1 column using each organic modifier (vol.%) at pH 3. (a) Group I in dioxane 30%; (b) Group I in acetonitrile 40%; (c) Group I in methanol 80%; (d) Group II in dioxane 30%; (e) Group II in acetonitrile 40%; (f) Group II in methanol 80%.

interaction and dipole–induced dipole interaction between solute and stationary phase might be possible, and face-to-face stacking conformation between solute and phenyl group would be considered.

Several compounds (Nos. 1, 2, 3, 7, 13, 14, 15, 17, 18, and 19) with a surface area greater than 500 Å^2 have the piperazinyl group in R_7 (see Table 1). Based on optimized geometry this substituent is positioned perpendicularly to the quinolone ring. Since the phenyl groups in the stationary phase are facing each other, there are

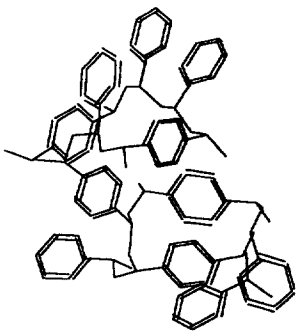


Fig. 2. Optimized structure of polystyrene–divinylbenzene used as stationary phase by MM+.

steric hindrances between stationary phase and piperazinyl group. Thus, these solutes are hardly retained at all. No. 14 was retained rather more than the others because the Van der Waals interaction between stationary phase and cyclopropyl group in R_1 might be greater than the interaction between stationary phase and ethyl group in R_1. Although no. 3 has the cyclopropyl group in R_1, no. 3 was less retained than no. 14 because the piperazinyl group was ionized at pH 3.

To verify this explanation, geometry optimization was performed between solute and stationary phase. It can be seen from Fig. 3 that no. 14 is facing the phenyl group while no. 15 (eluted early by 30% dioxane) faces away from the phenyl group.

In general, both the mobile phase and the solute in LC interact with the stationary phase. If the mobile phase consists of a mixture of solvents, the surface is partly covered by one solvent and partly by the other [1]. The distribution of a solute with respect to the aqueous phase is linearly related to the concentration of methanol unassociated with water in the mixture, and benzene does not interact with the methanol associated with water or water itself but with the methanol unassociated with water [13]. Therefore, it was suggested that phenyl groups in the stationary phase might interact only with an organic modifier such as dioxane, acetonitrile, and methanol. Interactions of the phenyl group with dioxane or acetonitrile might be stronger than its interactions with methanol because there are electrostatic potential stabilization between the dioxane molecule and phenyl group (Fig. 4) and $\pi-\pi$ charge transfer interaction between the acetonitrile molecule and phenyl group.

There are basically two types of interaction between solute and stationary-phase surface [1]. First, the solute can interact with the adsorbed solvent layer and get placed on the layer. This interaction takes place when the interactions between the solute and the stationary phase are relatively weak compared with those between the solvent and the stationary phase, and is known as sorption interaction. Second, the solute can displace the solvent molecules from the stationary-phase surface and interact directly with the surface. This occurs when the interactions between the solute and the stationary phase are much stronger than those between the solvent and the stationary phase, and is known as displacement interaction.

If there are multi-layers of solvent on the stationary-phase surface and solute interacts with

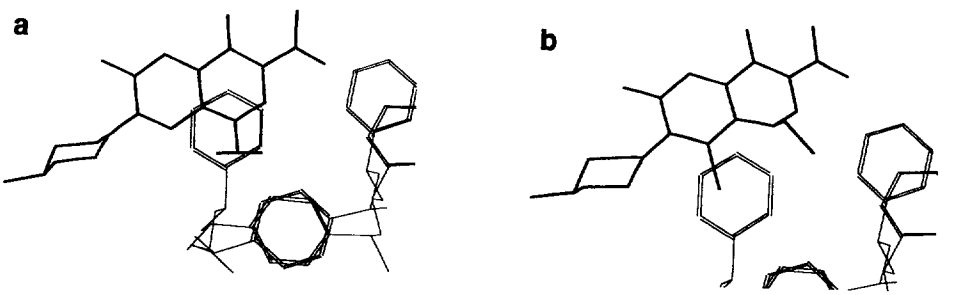


Fig. 3. Optimized interacting structure between the solute and the stationary phase at pH 3 by MM+: (a) compound no. 14, (b) compound no. 15.

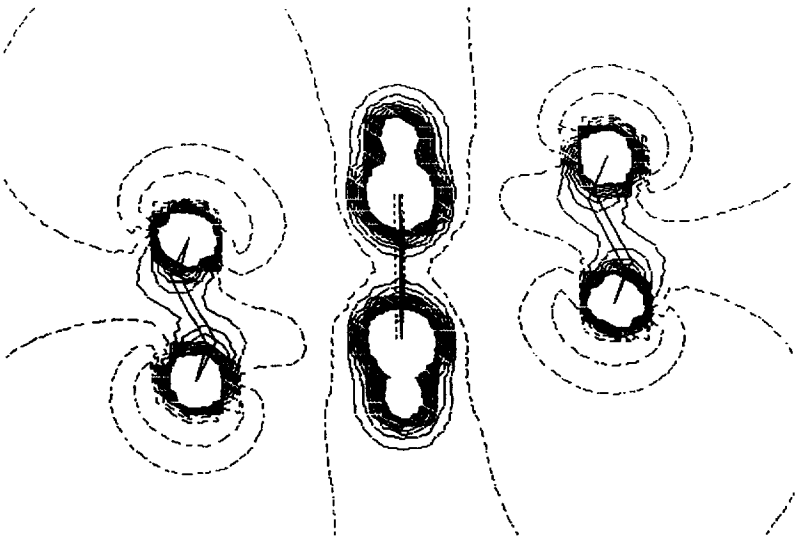


Fig. 4. Electrostatic-potential contour map calculated by AM1 for benzene–dioxanes complex (solid line: positive potential, dotted line: negative potential).

the lower layer of solvent by displacing the solvent from the upper layer, the cavity-formation energy in the solvent layer is proportional to the increase in surface area of the solute, and hence retention of the solute decreases as the surface area increases. It can be seen from Figs. 1a to 1c that the retention of compounds (no. 4, 5, 10, 11, and 12) increases as the surface area decreases[2].

In the case of Group II, there are two trends (Figs. 1d to 1f). When dioxane or acetonitrile were used as the mobile phase, a similar trend in retention between Group I and Group II was found. Thus, it was suggested that displacement interaction from the upper layer was dominant to retention of quinolones using dioxane or acetonitrile at pH 3. But, retention of no. 9 was unusually longer than for other members. The optimized conformation between no. 9 and stationary phase is shown in Fig. 5. The cyclopropylmethyl group in R_1 is located in the middle of the cross-linked divinylbenzene ring. Therefore, no. 9 showed an unusually longer retention.

When methanol was used as the mobile phase (Fig. 1f), solute could be adsorbed directly with stationary phase because there was only dipole–induced dipole interaction between them, which is relatively weak compared with hydrogen bonding between methanol molecules themselves. If retention occurs due to direct adsorption on the

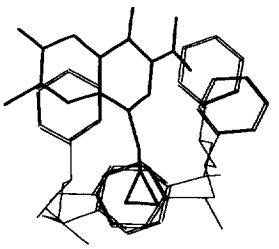


Fig. 5. Optimized interacting structure between compound no. 9 and the stationary phase at pH 3 by MM+.

[2] The hydrophobic effect plays a dominant role in determining the overall energetics of the equilibrium distribution in a reversed-phase chromatographic system. Of course, at pH 3, the k'-values of several solutes (no. 4, 5, 10, 11, 12) increased with the $\log P$, which serves as a measure of hydrophobicity. The retention order of solutes should be in order of size if the other components of the interaction are the same in the RP system. However, the retention behavior of solutes which have a piperazinyl group in position R_7 was not explained only by hydrophobicity. In that case, a steric effect between solute and polystyrene-divinylbenzene should be considered, and this effect could be explained by the solute surface area and its conformation. In order to understand other specific effects on retention, the following study is in process.

surface of the stationary phase, the significant factor would be contact area in the solute–stationary phase complex. Thus, the retention is proportional to the surface area. Since the nitro group in R_6 can form hydrogen bonds with the methanol molecule, no. 16 was less retained than the others.

At pH 11, k' was plotted against the y-component of the dipole moment in Fig. 6. Similar retention patterns were observed for each group (Table 4) regardless of organic modifier. In contrast to the situation at pH 3, all compounds were dissociated at pH 11, and hence it would be reasonable to presume that the electronic effect

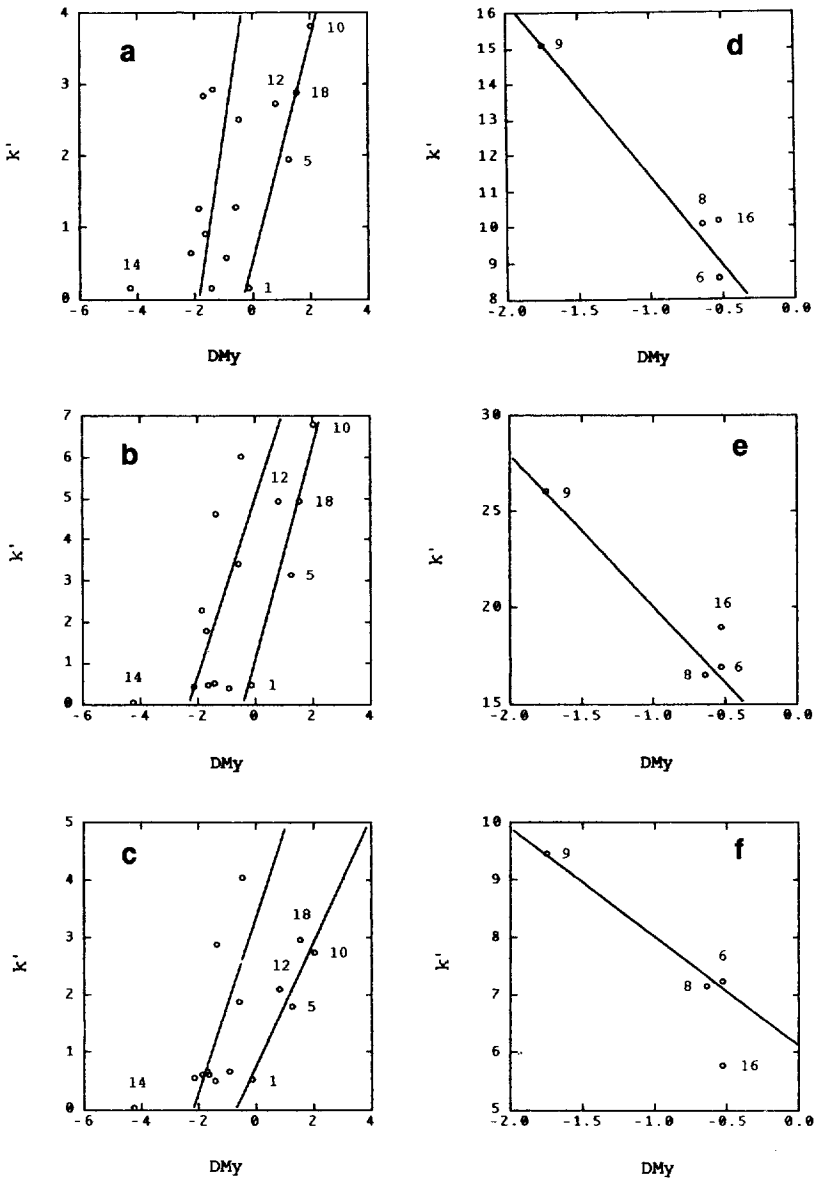


Fig. 6. The y-component of dipole moment (DMy) versus k' on PRP-1 column using each organic modifier (vol.%) at pH 11. (a) Group I in dioxane 10%; (b) Group I in acetonitrile 10%; (c) Group I in methanol 40%; (d) Group II in dioxane 10%; (e) Group II in acetonitrile 10%; (f) Group II in methanol 40%.

on retention is relatively more dominant than the dispersive effect. Also, considering the structure of polystyrene-divinylbenzene (Fig. 2), the solute should approach the stationary phase in the direction of the y-axis. Thus, the y-component of the dipole moment might have an important effect on retention.

For Group I (Figs. 6a to 6c) retention increases as the y-component of the dipole moment increases positively. Also, it can be seen from Fig. 7 that $\log P$ is proportional to the y-component of the dipole moment. An empirical hydrophobicity parameter, 1-octanol/water partition coefficient ($\log P$), is a good measure of the ease with which solutes bind to hydrophobic surfaces. According to the interrelation between the y-component of the dipole moment and the partition coefficient, the hydrophobicity of the solute increases as the former increases positively. There are two proportionality relations between $\log P$ and the y-component of the dipole moment according to the direction of the latter (the sign of dipole moment), one for positive and the other for negative sign. In Group I, hydrophobicity might be affected not only by the magnitude of the y-component of the dipole moment but also by the direction. Therefore, it was assumed that solute was retained by partitioning into the solvent layer on the stationary phase surface, and hydrophobicity was the driving force of partition.

For Group II (Figs. 6d to 6f) retention increases as the y-component of the dipole moment increases negatively. A solute might induce a dipole in the dioxane molecules associated on the phenyl group, and the orientation of the induced dipole depends on the orientation of the solute molecule. Both acetonitrile and methanol molecules have a permanent dipole. Therefore, it was suggested that the induced dipole or permanent dipole of solvent on the stationary-phase surface could interact with the dipole of the solute, and then the negatively directed y-component of the dipole moment would affect the dipole interaction. In this case the dipole interaction was the driving force of partitioning into the solvent layer on the stationary-phase surface. Using methanol as the mobile phase (Fig. 6f), no. 16 was less retained than other compounds due to hydrogen bonding between the nitro group and the methanol molecule. In contrast, using dioxane or acetonitrile (Figs. 6d and 6e), the additional Van der Waals force between solute and solvated stationary phase could be greater than that of no. 6 and 8 due to the cyclopropyl group in R_1 of no. 16, and hence no. 16 would be better retained.

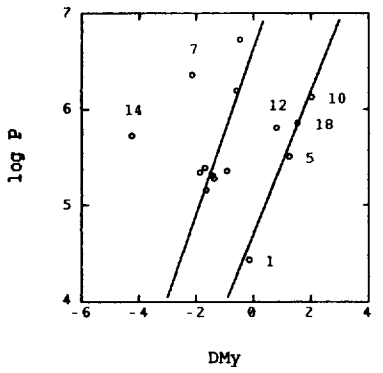


Fig. 7. Log P versus y-component of dipole moment (DMy) for compounds of Group I at pH 11.

In conclusion, two kinds of retention behavior for quinolones at pH 3 and pH 11 have been suggested. At pH 3, the solvent-accessible surface area was an informative descriptor which could explain the retention behavior of quinolones. At pH 11, the y-component of the dipole moment was a useful descriptor. Although Van der Waals interaction is known as the dominant force between the solute and the stationary phase in RPLC, the molecular shape of solute is important to understand the retention in a chromatography system.

Acknowledgements

This research was supported (in part) by the Center for Molecular Catalysis and the Korean Science and Engineering Foundation. We thank Dr. Chang-Ju Yoon for many helpful discussions. Also, Hoon Joo Kim of Lucky Research Park is acknowledged for his assistance in the application of the SYSTAT software.

References

[1] R.P.W. Scott, Silica Gel and Bonded Phases, Wiley, New York, 1993.
[2] L.R. Snyder, J. Chromatogr., 36 (1968) 455.
[3] D. Martire and R. Boehm, J. Phys. Chem., 91 (1987) 2433.
[4] A. Alverez-Zepeda and D.E. Martire, J. Chromatogr., 550 (1991) 285.
[5] R.J. Laub and J.H. Purnell, J. Chromatogr., 112 (1975) 71.
[6] E.D. Katz, K. Ogan and R.P.W. Scott, J. Chromatogr., 352 (1986) 67.
[7] E.D. Katz, C.H. Lochmüller and R.P.W. Scott, Anal. Chem., 61 (1981) 344.
[8] R. Kaliszan, J. Chromatogr. A, 656 (1993) 417.
[9] H.S. Kim, T.K. Kim and D.W. Lee, J. Liq. Chromatogr., 17 (1994) 2615.
[10] N.L. Allinger, J. Am. Chem. Soc., 99 (1977) 8127.
[11] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy and J.J.P. Stewart, J. Am. Chem. Soc., 107 (1985) 3902.
[12] A.A. Afifi and V. Clark, Computer-Aided Multivariate Analysis, Van Nostrand Reinhold, New York, 1990.
[13] E.D. Katz, C.H. Lochmüller and R.P.W. Scott, Anal. Chem., 61 (1989) 349.