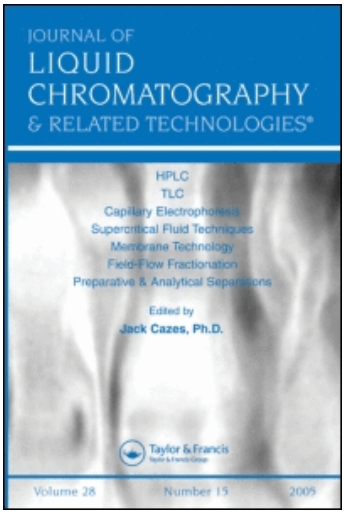


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Method for Determination of Partition Coefficients by High-Performance Liquid Chromatography: Application to O-Hydroxylbenzenesulfonanilides

X. Sun[a]; M. Xin[a]; J. Zhao[a]
[a] Department of Chemistry, Inner Mongolia University, Huhehot, Inner Mongolia

To cite this Article Sun, X. , Xin, M. and Zhao, J.(1994) 'Method for Determination of Partition Coefficients by High-Performance Liquid Chromatography: Application to O-Hydroxylbenzenesulfonanilides', Journal of Liquid Chromatography & Related Technologies, 17: 5, 1183 — 1194
To link to this Article: DOI: 10.1080/10826079408013394
URL: http://dx.doi.org/10.1080/10826079408013394

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(5), 1183-1194 (1994)

METHOD FOR DETERMINATION OF PARTITION COEFFICIENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: APPLICATION TO O-HYDROXYLBENZENESULFONANILIDES

XIAOLIN SUN, MIN XIN, AND JUN ZHAO
Department of Chemistry
Inner Mongolia University
Huhehot, Inner Mongolia 010021
The People's Republic of China

ABSTRACT

The capacity factors (log k′) for 26 compounds of widely varying structural types were determined by high-performance liquid chromatography method using methanol—water as mobile phase and ODS column as stationary phase in which free silanol groups were fully suppressed. These determined log k′ values are correlated with known partition coefficient (log P_{OCT}) values of 26 compounds and the regression parameters show that accordingly there are two sets of correlation lines when 26 compounds are divided into two data sets depending on their hydrogen-bonding ability. The factors which caused different partitioning mechanisms of the compounds are discussed.

As an application, the partition coefficients of a series of o-hydroxylbenzenesulfonanilides (HBSA) were determined by using obtained relative correlation line and then correlated with their anthelmintic activities.

INTRODUCTION

A variety of theoretical and experimental works [1—3] have shown that the properties of drugs such as absorbability and transportation are closely correlated with their hydrophobicity, which is a critical factor affecting activities of drugs in some cases. As a result, the partition coefficients (log P_{OCT}) which present hydrophobicity of drugs are commonly used in studies on QSAR (quantitative structure—activity relationships). Through studing the anthelmintic activities, we discovered that o-hydroxyl benzenesulfonanilides (HBSA) were some very potent fasciolicides. Our initial studies[4,5] revealed that one of the most important conditions for HBSA to possess potent activities was their stability in lipophilic phase of biomembranes. For this reason, analysis of the partition coefficients of HBSA is necessary for us to thoroughly investigate anthelmintic mechanism of these compounds and search for more potent drugs.

We had previously tried to determine partition coefficients of HBSA in an n-octanol—water partitioning system by the shake-flask technique and failed because of emulsions forming between n-octanol and water. It is well known that high-performance liquid chromatography has been successfully used as a convenient method for determination of the partition coefficient, so we used the octadecylsilane (ODS) column as a stationary phase in reversed-phase HPLC, on the basis of similarities in the hydrophobic partitioning processes occurring in an octanol—water system and in a reversed-phase HPLC system with an aqueous mobile phase, to determine partition coefficients of a series of HBSA compounds. In this studing, we tried to correlate the determined partition coefficients

of HBSA to their anthelmintic activities, and we also tried to clarify the factors which govern the value of k′ (capacity factor) in terms of chemical structure and partition behaviour in chromatography on an ODS column.

EXPERIMENTAL

Materials:

All the chemicals used were analytical or chemical grade. Water for solvents were glass redistilled and methanol was spectral grade. HBSA compounds were synthesized in our laboratory and purified by recrystallization method. All samples were dissolved in methanol at 1 mg/ml, adequate amount of samples was injected on to the column according to their UV detector responses.

Apparatus:

HPLC was carried out at 30℃ with a Shimadzu LC-6A system equipped with a SPD-6AV ultraviolet detector (wavelength range 195－700nm) and LC-6A high pressure pump. Chromatographic data were recorded and processed on C-R3A data system.

The stationary phase was an ODS column (Shim-Pack CLC-ODS, 5 μ particle size, 150mm×6mm i. d.). These silica packings are fully end-capped to suppress residual silanol group influences.

The mobile phase was a mixture of water and methanol. In order to obtain acceptable retention time, the volume fraction of methanol in mobile phase was set at 0. 7, the flow rate of mobile phase was constantly 1. 4 ml/min.

Procedures:

The correlation between the partition coefficient of samples and their chromatographic capacity factor k′ (log k′) can be expressed as follows :

$$\log P_{OCT} = a + b \log k'$$

capacity factor, $k' = \frac{t_R - t_0}{t_0}$

where t_R is the sample's retention time and t_0 is the retention time of an unretained substance, determined by formamide.

We had chosen 26 compounds of widely varying functionality and structure types for the determination of k′ values and established correlation described above. All values of log P_{OCT} of these compounds were obtained from the reference [6]. The pH of the mobile phase was adjusted to 3.0 with acetic acid to avoid the effect of acid dissociation of the samples and HBSA compounds.

In another experiment, ammonium chloride (0.035M in mobile phase) was added to mobile phase as a masking agent [7] to examine the extent to which the free silanol groups existed in the stationary phase. Experimental values of log k′ of the 26 compounds is listed in Table 1.

RESULTS AND DISCUSSION

At first, we determined the effect of free silanol groups in the CLC-ODS column used in our experiments. By comparing the log k′ and log k'_N (log k′ of the compounds when NH_4Cl was added into mobile phase) values listed in Table 1, it is clear that almost all the values of log k'_N of the compounds are little less than that of log k′ of the compounds, including the compounds such as benzene, toluene etc. which have minimal interactions with

Table 1

Experimental Capacity Factors and Literature Octanol—Water Partition Coefficients

No.	Compound	log P_{OCT}	log k′	log k_N' *
1	Benzyl alcohol	1.10	−0.580	−0.587
2	o-Amino benzoic acid	1.21	−0.559	−0.581
3	Cyclohexanol	1.23	−0.557	−0.545
4	Phenylacetic acid	1.41	−0.376	−0.379
5	Phenol	1.46	−0.451	−0.465
6	Benzoic acid	1.87	−0.301	−0.324
7	m-Nitrophenol	2.00	−0.267	−0.276
8	m-Methoxylbenzoic acid	2.02	−0.237	−0.260
9	Salicylic acid	2.26	0.017	−0.002
10	p-Chlorophenol	2.39	−0.036	−0.055
11	1-Naphthol	2.98	0.128	0.110
12	p-Iodobenzoic acid	3.02	0.258	0.234
13	2,4,6-Trichlorophenol	3.72	0.588	0.587
14	Anisole	2.11	0.173	0.170
15	Benzene	2.13	0.174	0.171
16	p-Nitrotoluene	2.37	0.202	0.180
17	m-Nitrochlorobenzene	2.41	0.310	0.300
18	p-Nitrochlorobenzene	2.41	0.230	0.220
19	m-Nitrobromobenzene	2.64	0.369	0.340
20	Toluene	2.69	0.452	0.441
21	Chlorobenzene	2.84	0.452	0.445
22	p-Dichlorobenzene	3.39	0.662	0.652
23	Azobenzene	3.82	0.978	0.978
24	Diphenyl	4.04	0.883	0.859
25	Phenyl ether	4.21	1.002	0.998
26	Anthracene	4.45	1.123	1.125

* log $k_N' =$ log k′ of the compounds when NH_4Cl was added into mobile phase

silanol groups. This indicates that the diminished values of log k'_N may not be caused by the effect of silanol group but by changes in mobile phase ionic strength, for mobile phase ionic strength is also a factor that can affect partitioning processes for compounds in an HPLC system[8]. We believe that residual silanol groups in the ODS column have fully been suppressed.

Figure 1 shows a plot of the log P_{OCT} values of these 26 compounds versus their values of log k′ determined from the experiment. Linear regression parameters indicate that a linear relationship between these log P_{OCT} and log k′, and further, after comparing the chemical structure types of 26 compounds, it is found that these compounds can be divided into two classes with respect to hydrogen-bonding ability[6,9]: (i) HB(hydrogen-bonding), such as phenolic and carboxyl acid compounds etc. and (ii) NHB (non-hydrogen bonding), such as alkylbenzene, halogenbenzene and nitrobenzene compounds etc.. As shown in Figure 1, the HB (compound **1**～**13**, see Table 1)and NHB (compound **14**～**26**)have good correlations between log P_{OCT} and log k′, respectively. This again verified the view that partitioning of a compound between water and octanol is governed not only by its hydrophobicity, but also by the extent to which the compound can hydrogen-bond to octanol.

Table 2 lists the log P_{OCT} vs log k′ linear regression parameters obtained for this HPLC system when the 26 compounds are considered altogether as well as are divided into one set containing the HB and one containing the NHB. Except that correlation coefficients and variances of the fit for the two divided data sets are much better than that for the overall data set, the regression parame-

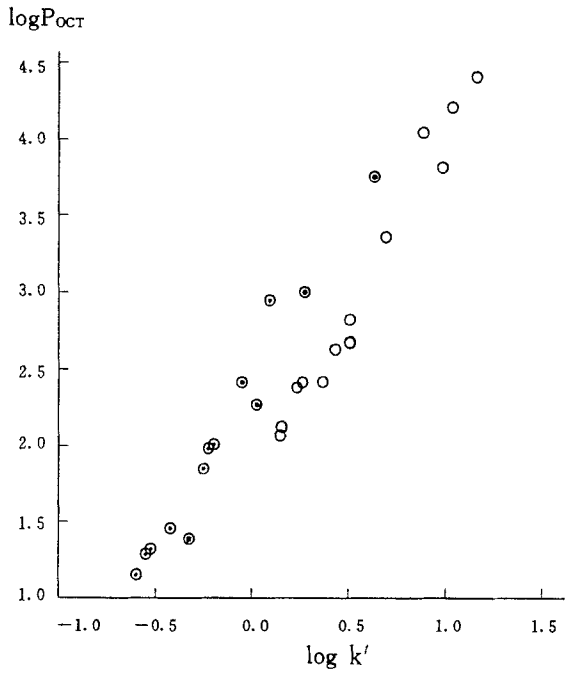


FIGURE 1. Plot of the log P_{OCT} versus the experimental log k′ for 26 compounds discussed

◉ HB compound

○ NHB compound

ters of the correlation lines for HB and NHB are significantly different from each other, especially the intercepts of the correlation lines. As demonstrated in previous studies [9—11] the differences in the intercepts of the correlation lines appears to be directly related to the differences in hydrogen-bonding ability between HB and NHB.

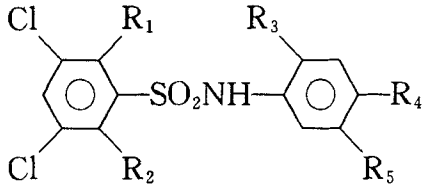
Some papers[12,13], which reported the similar experimental phenomena, attributed the causes of the phe-

Table 2
Log P_{OCT} vs. Log k′ Linear Regression Parameters for 26 Compounds Data Set

Parameter	All Data	NHB	HB
slope	1.806	2.371	2.241
intercept	2.223	1.761	2.461
correlation coefficient	0.960	0.990	0.987
variance of fit	0.074	0.016	0.017

nomena to hydrogen bonding of HB to residual silanol sites on the ODS column. However, as described above, silanol groups on the ODS column used in our experiments were fully suppressed to minimize interactions between HB and silanol groups here. Haky and Vemulapalli [14] had used ODA (Octadecyl-Bonded Alumina) as a stationary phase in HPLC system to determine the log k′ for compounds of various chemical classes. In contrast to results obtained with other columns, HB did not need to be treated as a separate data set on the ODA column to obtain good correlations between log k′ and log P_{OCT}. This clearly indicates that the HPLC system which used ODA as a stationary phase is more similar to n-octanol—water system than the ODS—HPLC system. Therefore, these data suggest that different partitioning behavior between HB and NHB in the ODS—HPLC system may be caused by differences other than those caused by analyte interactions with silanol groups.

As an application of the HPLC method, we employed correlation lines deduced above to estimate the log P_{OCT} of some HBSA compounds with different substituents. The general structural formula of these compounds can be represented as follows:



compound:

Ⅰ. $R_1=OH$, $R_2=R_3=R_4=R_5=Cl$
Ⅱ. $R_1=OH$, $R_2=H$, $R_3=R_4=R_5=Cl$
Ⅲ. $R_1=OH$, $R_2=Cl$, $R_3=CH_3$ $R_4=R_5=H$
Ⅳ. $R_1=OH$, $R_2=R_3=Cl$, $R_4=R_5=H$
Ⅴ. $R_1=OH$, $R_2=Cl$, $R_3=R_4=R_5=H$
Ⅵ. $R_1=OH$, $R_2=R_3=R_5=H$, $R_4=Cl$
Ⅶ. $R_1=H$, $R_2=Cl$, $R_4=OH$, $R_3=R_5=H$

Acorrding to result from our previous studies[5,15], the HBSA compounds shown above (except Ⅶ) are considered non-hydrogen bonding molecules due to intra-molecular hydrogen bonding, estimated log P_{OCT} values of the HBSA compounds are shown in Table 3.

In concordance with positive value of π_x (hydrophobic constant for halogen atoms), the values of determined log P_{OCT} of HBSA compounds increased with the total number of halogen atoms substituted in the benzene rings of the compounds, but obviously they did not follow the additive-constitutive property of Hansch's hydrophobic constants. This will call our attention to the special conformations of these compounds in solution.

Table 3
The Determined Log P_{OCT} and Anthelmintic Activities of HBSA Compounds

Compound	log k′	log P_{OCT}	Activity*
Ⅰ	1.096	4.360	++++
Ⅱ	1.078	4.317	+++
Ⅲ	0.951	4.016	
Ⅳ	0.930	3.966	
Ⅴ	0.834	3.738	++
Ⅵ	0.802	3.663	+
Ⅶ	0.766	4.178	−

* The activities of HBSA compounds were examined in sheep which were experimentally infected. Activities of compound Ⅲ and Ⅳ were not yet obtained.

It was revealed in our earlier paper[5]that the phenolic-OH group in the HBSA molecule was the most important active-group and the anthelmintic activity of these compounds is strengthened with increasing acidity of the phenolic-OH group in their molecules. By correlating log P_{OCT} of HBSA compounds with their anthelmintic activities, we are informed that the greater log P_{OCT} (or more hydrophobic) of HBSA compounds correlate with the higher anthelmintic activities of the compounds.

In addition, although the log P_{OCT} determined for HBSA compound Ⅶ demonstrates that the compound is hydrophobic, the acid dissociation constant PKa determined [16]for this compound told us that phenolic-OH group in molecule of HBSA compound Ⅶ had extremely weak acidity. For this reason, it is not surprising that HBSA com-

pound Ⅶ possesses no anthelmintic activity. In summary, the mechanism of biological activity for HBSA compounds is influenced by a variety of factors such as acidity, hydrophobicity and even molecular conformations of HBSA compounds in solution.

ACKNOWLEDGEMENT

This work was supported by National and Inner Mongolia's Natural Science Foundation of China.

REFERENCES

1. Ariens, E. J., Drug Design, Vol. 5, P81, Academic Press, New York (1975).
2. Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, wiley, New York (1979).
3. Hansch, C., in "Correlation Analysis in Chemistry and Biology", plenum, New York, N. Y. (1978).
4. Hiroshi Terada, Biochim Biophys Acta, 639: 225 (1981).
5. Sun Xiaolin, Xin Min and Lee Zhe, Proceedings of the Third National Symposium on Molecular Mechanics and Design of Drugs, Beijing, P2—34 (1991).
6. Leo. A., Hansch, C. and Elkins, D., Chem. Rev., 71:525 (1971).
7. Tsukinaka, Y., Akira Tsuji, Etsuko Miyamoto and Osamu Kubo, J. Pharm. Sci., 66:747 (1977).
8. Braumann, Th., J. Chromatogr., 373:191 (1986).
9. Keishiro Miyake, Nobuyasu Mizuno and Hiroshi Terada, Chem. Pharm. Bull., 34:4787 (1986).
10. Haky, J. E. and Yong, A. M., J. Liquid Chromatogr., 7:675 (1984).
11. Seiler, P., Eur. J. Med. Chem, 9:473 (1974).

12. Unger, S. H., Cook, J. R. and Hollenberg, J. S., J. Pharm. Sci., 67:1364 (1978).

13. Unger, S. H., and Chiang, G. H., J. Med. Chem., 24:262 (1981).

14. Haky, J. E. and Vemulapalli, S., J. Liquid Chromatogr, 13:3111 (1990).

15. (a)Xin Min, Sun Xiaolin, Lee Zhe and Lian Shouren, Chinese J. Appl. Chem, 8:87 (1991).
(b)Xin Min, Sun Xiaolin and Lee Zhe, Acta Scientiarum Naturalium Universitatis Intra mongolicac, 22:141 (1991).

16. Sun Xiaolin and Xin Min ,to be published.

Received: June 1, 1993
Accepted: September 28, 1993