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High performance liquid chromatographic capacity factor and partition coefficient relationship in a benzamide series

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Summary

High performance liquid chromatography was used to establish the relationship between capacity factor (log k') and partition coefficient (log P octanol/water) in a benzamides series.

The chromatographic parameter was correlated to log P values experimentally determined by the shake-flask method and to log P calculated from Rekker's molecular fragments approach.

In both cases, for the 10 molecules studied, capacity factor on partition coefficient fit very closely a regression line.

The linear model was tested for another molecule in the same series.

When comparing the computed values and the experimental ones, it can be concluded that HPLC is a good tool in predicting partition coefficient in this series.

Introduction

During this last decade, there is a considerable interest in determining octanol–aqueous partition coefficient for this parameter is frequently used to characterize hydrophobicity in quantitative structure–activity relationship (QSAR). Until now, the shake-flask method widely used for this determination certainly remains one of the most suitable procedures, but it has the disadvantage sometimes of being time consuming and limited in its applicability. Indeed it requires products of high purity

and presents practical problems when applied to unstable compounds, as pointed out by Yamana et al. (1977) who emphasized that the difficulties in determining partition coefficient of penicillins and cephalosporins.

Among the authors interested in quantifying hydrophobic properties in QSAR, Chen and Horvath (1979) found very good agreement between statistically predicted values obtained by reversed-phase thin-layer high-performance chromatography and those determined by Kuchar et al. (1979, 1980) for the same compounds: aromatic cinnamic acid and phenylacetic acid derivatives; these experiments were carried out on silicagel thin layers impregnated with a non-polar solvent like paraffin oil or octanol. This system was also used by Gasparic (1980) who studied a series of sulfonamides.

Bachrata et al. (1979) obtained good correlation between R_m values and partition coefficient in a series of local anaesthetics and they established a relationship between the chromatographic parameter and the logarithm of the surface anaesthetic activity. But thin-layer chromatography can be time-consuming in some particular applications because it can sometimes take many hours to get an equilibrium between the plate and the mobile phase as pointed out by Unger et al. (1978) who rather used high-performance liquid chromatography (HPLC). One of the systems suitable for partition coefficient octanol/water determination is an octadecyl chemically bonded stationary phase and an aqueous mobile phase modified by a certain amount of organic solvent, in order to obtain reasonable retention times for highly hydrophobic compounds.

The advantages of this method, namely, rapidity, versatility, precision and reliability, have been demonstrated by many authors who applied this method in various series such as, vasodilatators (McCall, 1975), 1,4-benzodiazepines (Hulshoff and Perrin, 1976), penicillins and cephalosporins (Yamana et al., 1977), phenolic derivatives (Miyake and Terada, 1978), chlorinated benzenes, toluenes and anilins (Konemann et al., 1979), and various simple aromatic molecules (Mirrlees et al., 1976) (Nahum and Horvath, 1980).

In the present work, high-performance liquid chromatography was successfully applied in a series of benzamides among which were procainamide, metoclopramide (Primperan) and tiapride (Tiapridal). The structure and some chemical characteristics of these molecules are given in Table 1. The technique proved to be a good tool in predicting partition coefficients, as capacity factors (log k') correlate well with log P values directly determined in the octanol/water system, considered as a reference system, and values calculated from literature (Rekker, 1977 and 1979).

Materials and Methods

Reagents

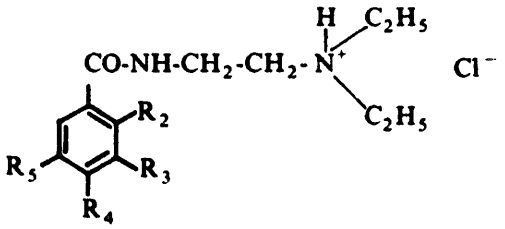
Benzamides were purified as hydrochlorides by recrystallization in ethanol and ether.

Pure octanol (Merck 991) was redistilled under reduced pressure.

All the reagents were of analytical grade (Merck).

TABLE I

BENZAMIDES STRUCTURE AND CHEMICAL CHARACTERISTICS



No.	R_2	R_3	R_4	R_5	%B at pH 6.08 [a]	Acidity constants	
						pK_{A_T} [b]	S.D.
1	H	H	NH_2	H	0.05	9.34	0.03
2	OCH_3	H	H	SO_2CH_3	0 11	9.05	0.02
3	OCH_3	H	NH_2	CN	0.06	9.32	0.02
4	OCH_3	H	NH_2	NO_2	0.07	9.25	0.04
5	OCH_3	H	NH_2	Cl	0.05	9.36	0.03
6	OCH_3	H	H	F	0.07	9.22	0.09
7	OCH_3	H	H	Cl	0.08	9.19	0.07
8	OCH_3	Cl	H	F	0.10	9.09	0.08
9	OCH_3	H	H	Br	0.08	9.19	0.02
10	H	Cl	H	Cl	0.09	9.13	0.02

[a] $\%B = \frac{100}{1+10\,pK_A - pH}$

[b] pK_{A_T} determined by Hanocq et al. (1973) and van Damme et al. (1976)

N.B.: 1 = procainamide; 2 = tiapride; 5 = metoclopramide.

For partition coefficient determination, the aqueous phase was a potassium dihydrogen phosphate–dipotassium hydrogen phosphate buffer, pH 7.40, ionic strength adjusted to 0.15 M with potassium chloride. These conditions have been chosen in agreement with the characteristics of the biological medium (van Damme et al., 1973 and 1979).

The mobile phase used for high-performance liquid chromatography determinations was a mixture of 18% acetonitrile and 82% phosphate buffer, pH 6.00, ionic strength adjusted to 0.40 M with sodium chloride. This solvent has been selected after systematic study of 3 parameters (ionic strength, pH and acetonitrile percentage) to get a good separation in a minimum of time (Verbiese et al., 1979 and 1980).

Apparatus and procedure

(a) Partition coefficient determination [1]

Octanol and phosphate buffer were first reciprocally saturated. Benzamides

[1] Computations have been made on a Tektronix 4051 calculator.

hydrochlorides were dissolved in the pH 7.40 and ionic strength 0.15 M phosphate buffer to obtain concentrations between 10^{-3} and 10^{-4} M. One to 20 ml of these samples were maintained under shaking with 1–10 ml of octanol for 8 h at constant controlled temperature of 20.00 ± 0.05°C. The aqueous phase was then centrifuged and the extinctions were read with a spectrophotometer (Beckman Acta V in a 1 cm quartz cell) at a wavelength corresponding to an isosbestic point in order to avoid any influence of small pH variations. The volumes of both phases were adjusted so that the final absorbance measured in the aqueous layer was reduced to about half of its initial value (van Damme, 1978). Partition coefficient was then statistically computed for 9 to 13 values by a least-squares analysis of the following equation:

$$\frac{[C_T]_0}{[B]_a} = P\frac{1}{[B]_a} + \frac{2P^2}{K_d}$$

where $[C_T]_0$ is the total molar concentration of all the species in organic phase; $[B]_a$ is the molar concentration of the basic form in aqueous phase; P is the partition coefficient; and K_d is the dimeride dissociation constant. The term $2P^2/K_d$ is a correction due to the formation of a dimeric form appearing for some molecules in the organic phase.

Capacity factor determination

The experiment was performed on a liquid chromatograph Perkin Elmer Model 1220. It was equipped with a syringe sample-loading injector, Rheodyne 7105, and fitted with an octadecyl Nucleosil column (Machery Nagel) of length 0.25 m, i.d. 4.0 mm, particle size 10 μm.

The mobile phase described above was pumped at a flow rate of 1.2 ml/min at ambient temperature. The detection was made at 215 nm. Capacity factors were classically calculated by determining the retention time of the derivative studied in comparison with the one of a non-retained product (in this study, the standard solvent, methanol, was chosen).

In the case of long retention time (very hydrophobic compounds), to balance an inevitable resolution decrease, the organic solvent in the aqueous phase was increased to get a reasonable retention time; capacity factors were then extrapolated from the diagrams obtained by plotting k′ against organic solvent concentration (see Fig. 1).

An example of such a procedure is given for compound no. 9 (see Fig. 1).

Results and Discussion

Capacity factors determined by HPLC and experimental partition coefficient together with the corresponding values calculated from Rekker (1977 and 1979) are listed in Table 2. In this table, K_N represents the key number which is Rekker's magic constant multiplier. The magic constant is a corrective factor which has been introduced as a modifier of the calculated log P from Rekker's fragmental incre-

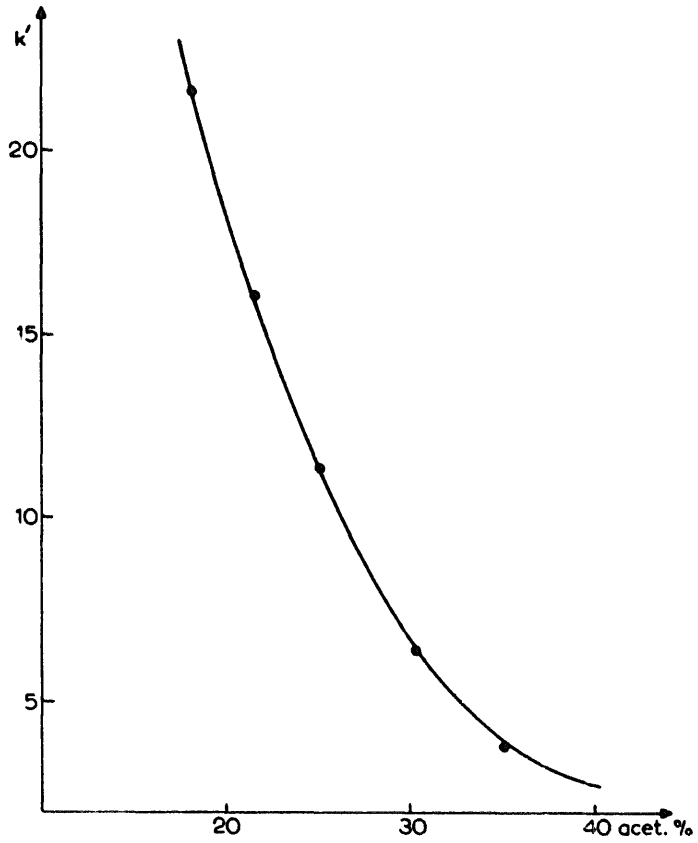


Fig. 1. k' as a function of acetonitrile percentage given as an example for compound number 9.

ments in order to consider some molecular interactions and some proximity effects. The second term 'UU' is the uncertainties unity. It corresponds to the sum of the standard deviations of each fragment included in log P calculation. For partition coefficient computation from experimental data, another correction term could be added because of 'ion pair' formation between monohydrogen phosphate, dihydrogen phosphate and chloride negative ions present in the aqueous phase and the positively charged benzamide at the working pH (van Damme, 1978 and 1979). Indeed as important pH variations had been observed during partition coefficient determination, the hypothesis was made that there was an 'ion pair' formation that could explain some abnormal values of partition coefficient. But if the pH 7.40 and the ionic strength 0.15 M were accurately maintained as it was the case in this experiment, the correcting term could be neglected and the above equation is good enough to calculate partition coefficient.

An 'ion pair' formation was confirmed by the HPLC technique. A detailed description of the chromatographic mechanism was given in a previous paper (Verbiese et al., 1979). At the operating pH (6.08) the molecules are completely dissociated (99% as can be seen from the pK_a values given in Table 1 and they can form an 'ion pair' with the $H_2PO_4^-$, HPO_4^{2-} and Cl^- ions present in the eluent; this phenomenum does not at all affect the correlation between capacity factor and log P.

The percentages of benzamides in basic form have been calculated following

TABLE 2

CAPACITY FACTOR AND PARTITION COEFFICIENT OF 12 MOLECULES IN THE BENZAMIDES SERIES

Chromatographic parameters				Experimental			Calculated from Rekker (1978)		
	k'	S	Log k'	P	CV%	Log P	K_N [a]	Log P	UU [b]
1	0.77	0.01	−0.113	6.19	2.5	0.792	−1	0.78	0.07
2	1.69	0.02	0.227	7.96	1.9	0.901	+1	1.11	0.15
3	3.88	0.02	0.588	88.0	0.6	1.944	+4	1.95	0.23
4	5.08	0.03	0.706	130	0.4	2.114	+5	2.36	0.22
5	7.66	0.08	0.884	416	1.0	2.619	+3	2.76	0.06
6	8.57	0.03	0.933	396	0.9	2.598	+1	2.67	0.15
7	17.1	0.07	1.233	1274	0.6	3.106	+1	3.21	0.12
8	19.2	0.08	1.283	1501	2.7	3.176	+1	3.41	0.16
9	22.3	0.08	1.348	1600	0.4	3.204	+1	3.40	0.12
10	29.2	0.08	1.465	5817	0.7	3.765	+1	3.87	0.13

[a] K_N = key number: Rekker's magic constant multiplier.

[b] UU = uncertainties unity.

S.D., standard deviation (number of assays: 9–13); CV%, coefficient of variation.

TABLE 3

COMPUTED LOG P VALUES FROM REGRESSION EQUATIONS FOR ANOTHER BENZAMIDE IN THE SERIES

Side chain	2	3	4	5	pK_A	Exp. log k'	Log P				
							Reg I [a]	Reg II [a]	$\epsilon f + K_N$ [b]	UU	Experimental
R	OCH_3	H	H	H	9.24	0.733	2.31	2.180	2.17	0.10	2.176

[a] See text (Reg I from literature; Reg II from experimental values).

[b] ϵf = sum of Rekker's hydrophobic fragmental constants.

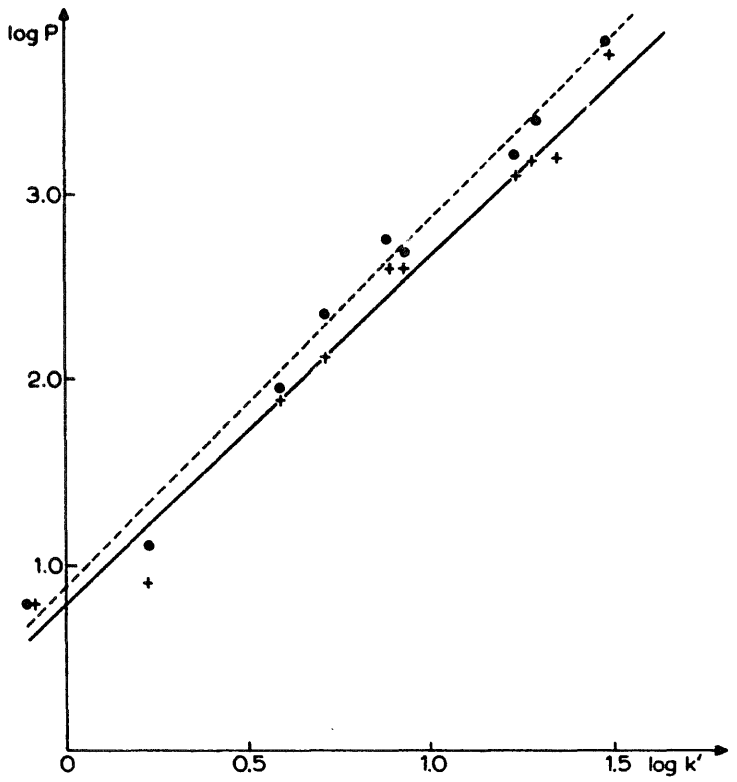


Fig. 2. ------, Reg. I (Rekker)—log P = 0.871 + 1.965 · log k'.
————, reg. II (Exp.)—log P = 0.795 + 1.901 · log k'.

Henderson-Hasselbalch's equation where the considered pK_a is only the thermodynamic pK_{a_T}. This has been done because the buffers' ionic strengths were different for the shake-flask method and HPLC. Capacity factors against calculated log P from literature or experimental log P very accurately fit a regression line. The statistical significances of the regression equations were tested by a Student's *t*-test on regression coefficient and on the slope of the two curves at the significance level $\alpha = 0.001$. From Rekker's hydrophobic fragmental constants we obtained:

$\log P = 0.871 + 1.965 \log k'$

with $r = 0.993$ and $t^{***}_{8} = 23$ and from experimental values:

$\log P = 0.795 + 1.901 \log k'$

with $r = 0.987$ and $t^{***}_{8} = 17$.

The validity of the linear model was tested for another benzamide. After a rapid measurement of retention time (capacity factor) by HPLC in the same system as the one used before, log P was calculated with the regression equations and these predicted computed values were checked by comparing on the one hand with partition coefficients experimentally determined, and on the other hand with the values calculated from Rekker's molecular fragments (K_N value was chosen by comparison of analog molecules in the series). As can be seen by examining the results given in Table 3, the shake-flask method and high-performance liquid chromatography are in good agreement with partition coefficient determination.

Conclusion

The data which have been presented in this study demonstrate that for this series of examined benzamides, the retention times (log k') are linearly related to octanol/water partition coefficient and to log P calculated from literature (Rekker, 1977, 1979).

High-performance liquid chromatography is a reliable, fast and reproducible method for any compound, even a strongly retained molecule, as the solvent lipophilicity can be rapidly adjusted.

Another advantage lies in the fact that the samples do not need to be very pure; indeed, once the solvent has been carefully chosen in order to get a good separation, the impurities do not interfere with the compound under study.

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References

Bachrata, M., Blesova, M., Schultzova, A., Grolichova, L., Bezakova, Z. and Lukas, A., Chromatographic study of local anesthetics—basic esters of substituted carbanilic acids. II The relationship between chromatographic values, other physico-chemical parameters and activity. J. Chromatogr., 171 (1978) 29–36.

Chen, B.K. and Horvath, C., Evaluation of substituent contribution to chromatographic retention: quantitative structure retention relationship. J. Chromatogr., 171 (1979) 15–28.

Gasparic, J., Use of reversed-phase thin-layer chromatography to assess hydrophobicity in studies of quantitative structure–activity relation for drugs. J. Chromatogr., 196 (1980) 391–396.

Hanocq, M., Topart, J., van Damme, M. and Molle, L., Détermination des constantes d'ionisation de quelques N-(diéthylamino-2 éthyl) benzamides substitués. J. Pharm. Belg., 28, 6 (1973) 649–662.

Hulshoff, A. and Perrin, J.H., A comparison of the determination of partition coefficient of 1,4-benzodiazepines by high performance liquid chromatography and thin layer chromatography. J. Chromatogr., 129 (1976) 263–276.

Konemann, H., Zelle, R. and Busser, F., Determination of log P oct. values of chloro-substituted benzenes and anilines by high performance liquid chromatography on ODS silica. J. Chromatogr., 178 (1979) 559–565.

Kuchar, M., Rejholec, V., Jelinkova, M. and Nemecek, O., Parametization of lipophilic properties of some aromatic aliphatic acids in paper chromatography. J. Chromatogr., 150 (1978) 419–428.

Kuchar, M., Rejholec, V., Brunova, B. and Jelinkova, M., R_M values from reversed-phase thin-layer chromatography as parameters of lipophilicity in quantitative structure–activity relationship in four series of arylaliphatic acids. J. Chromatogr., 195 (1980) 329–338.

McCall, J.M., Liquid–liquid partition coefficients by high pressure liquid chromatography. J. Med. Chem., 18 (1975) 549–555.

Mirrlees, N.S., Moulton, S.J., Murphy, C.T. and Taylor, P.J., Direct Measurement of octanol–water partition coefficients by high-pressure liquid chromatography. J. Med. Chem., 19, 5 (1976) 615–619.

Miyake, K. and Terada, H., Preparation of a column with octanol-like properties for high performance liquid chromatography. Direct measurements of partition coefficients in an octanol-water system. J. Chromatogr., 157 (1978) 386–390.

Nahum, A. and Horvath, C., Evaluation of octanol–water partition coefficient by using high-performance liquid chromatography. J. Chromatogr., 192 (1980) 315–322.

Rekker, R.F., The hydrophobic fragmental constant, Elsevier, Amsterdam, 1977.

Rekker, R.F., The hydrophobic fragmental constant; an extension to 1,000 data point set. Eur. J. Med. Chem. Chim. Ther., 14, 6 (1978) 479–488.

Topart, J., Hanocq, M., van Damme, M. and Molle, L., Etude spectroscopique de quelques N-(diéthylamino-2-éthyl) benzamides substitués. J. Pharm. Belg., 28 (1973) 633–648.

Unger, S.H., Cook, J.R. and Hollenberg, J.S., Simple procedure for determining octanol–aqueous partition, distribution and ionization coefficients by reversed phase high-pressure liquid chromatography. J. Pharm. Sci., 67 (1978) 1364–1367.

van Damme, M., Hanocq, M., Topart, J. and Molle, L., Détermination des coefficients de partage et des constantes de dissociation des dimères de quelques N-(diéthylamino-2-éthyl) benzamides substitués. Eur. J. Med. Chem.-Chim. Ther., 11 (1976) 209–216.

van Damme, M., Hanocq, M., Topart, J. and Molle, L., Détermination des constantes d'ionisation du N-(éthyl-1 pyrrolidyl-2 methyl) méthoxy-2-sulfomoyle-5 benzamide (sulpiride). Analysis, 4 (1976) 299–307.

van Damme, M., Hanocq, M., Auquier, H. and Molle, L., Méthode de calcul de coefficients de partage tenant compte de la formation de paires ioniques en présence d'ions phosphates: application à deux dérivés hétérocycliques. Anal. Lett., 12 (1979) 357–370.

van Damme, M., Contribution à la Détermination de l'Hydrophobie et des Constantes d'Acidité de Benzamides Substitués, Thèse d'agrégation de l'Enseignement Supérieur, Université Libre de Bruxelles (1978).

Verbiese-Génard, N., Hanocq, M., van Damme, M., Separation, identification and quantitative analysis of substituted benzamides in pharmaceutical dosage forms by HPLC. Int. J. Pharm., 2 (1979) 155–166.

Verbiese-Génard, N., Hanocq, M. and Molle, L., Proceedings of the 9th International Symposium on Chromatography and Electrophoresis. A. Frigerio and L. Renoz (Eds.), Recent Developments in Chromatography and Electrophoresis, Vol. 1, Elsevier, Amsterdam, 1979, pp. 79–86.

Verbiese-Génard, N., Hanocq, M. and Molle, L., High performance liquid chromatographic determination of sulpiride (Dogmatil) in urine. J. Pharm. Belg., 35 (1980) 24–30.

Yamana, T., Tsuji, A., Miyamoto, E. and Kubo, O., Novel method for determination of partition coefficients of penicillins and cephalosporins by high pressure liquid chromatography. J. Pharm. Sci., 66, 5 (1977) 747–748.