Relationship Between Log K' Values of Benzodiazepines and Composition of the Mobile Phase

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PLEASE SCROLL DOWN FOR ARTICLE

RELATIONSHIP BETWEEN LOG K' VALUES OF BENZODIAZEPINES AND COMPOSITION OF THE MOBILE PHASE

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ABSTRACT

Log k' values were determined, at various compositions of mobile phase, for a series of benzodiazepines. The analysis of the relationship between the log k' values and the mobile phase composition yielded extrapolated log k' values as a measure of the partitioning between the hydrophobic stationary phase and water. The log k' values extrapolated from the linear part of the curve were shown to be very well correlated with both R_M and log P values. As a further step of our study the extrapolated log k' values were calculated by means of the equations describing the linear or the quadratic relationship between retention times and methanol concentration over the full range of the mobile phase composition. However the results did not seem to be satisfactory. Similarly the intro-

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duction of a square root term into the quadratic equations
did not improve the relationship between the extrapolated
log k' values and the R_M or log P values.

INTRODUCTION

The study of the quantitative structure-activity relationship
demonstrated the importance of physico-chemical parameters
in determining the biological activity of drugs. In particular
the lipophilic character of molecules seems to play a very
important role (1,2,3). The direct partitioning between octanol
and water is the classic procedure for the determination of
the partition coefficient as a measure of the lipophilic char-
acter. However in order to avoid some disadvantages of the
classic procedure the chromatographic R_M values had been
proposed as an alternative to the direct partitioning (4,5,6).
In reversed-phase TLC system, when the mobile phase is repre-
sented by only water, the R_M values can be considered as a
measure of the partitioning of the compounds between the
hydrophobic stationary phase and the water itself. For more
lipophilic compounds the partitioning between the hydrophobic
stationary phase and the aqueous mobile phase affects their
chromatographic behaviour in such a way that they do not move
from the starting line, unless a certain amount of an organic
solvent, i. e. methanol or acetone, is added to the mobile
phase. The plots of the R_M values versus the composition of
the mobile phase show that for each compound there is a range
of linear relationship. The theoretical R_M values at 0% organic
solvent in the mobile phase can be calculated by extrapolation
from the linear part of the curve by means of the equations
describing such linear relationship. The extrapolated R_M

values at 0% were considered as a measure of the partitioning
of the compounds between the hydrophobic stationary phase
and water. For several series of compounds it was possible
to show a very good correlation between the experimental or
extrapolated R_M values at 0% and the log P values. In more
recent years there has been an increasing interest in comparing
different procedures for measuring the lipophilicity of drugs.
In particular reversed-phase high-performance liquid chroma-
tography (HPLC) has been presented as an alternative to both
reversed-phase TLC and direct partitioning (7,8,9,10).The range
of linear relationship observed in reversed-phase TLC was also
demonstrated between the log k' values and the composition
of the mobile phase in reversed-phase HPLC (11,12,13,14).
The extrapolated log k' values from HPLC were shown to be
very well correlated with both R_M and log P values (7,15,16).
However several authors (17,18,19) in studying the relationship
between log k' values and composition of the mobile phase
have turned their attention to the deviations from linearity.
Tijssen et al. (20) have developed a theoretical model relating
solubility parameter and retention behaviour. On this basis
it has been predicted that log k' values may change quadrat-
ically with the composition of the mobile phase. Schoenmakers
et al. (21,22) confirmed this model by means of experimental
data. More recently Schoenmakers et al. (23) showed that in
some cases the relationship between log k' values and mobile
phase composition can be better described with an extension
of the quadratic equation by means of a term proportional
to the square root of the organic solvent concentration in

the mobile phase. The aim of the present study was to investiga-
te the relationship between the chromatographic retention
of a series of benzodiazepines (24,25) and the composition
of the mobile phase in reversed-phase HPLC system. Since for
that series of benzodiazepines the log P and R_M values had
been previously obtained (24), this has allowed to study the
relationship between log k' values and both R_M and log P values.

MATERIALS AND METHODS

HPLC was performed on a Spectra Physics Cromatograph consisting
of a SP 87000 Solvent Delivery System and a SP 8750 Organizer
Module. A Varian Aerograph U. V. detector operated at 254
nm. A 30 cm. x 3.9 mm. i. d. Bondapak C_{18} Column from Waters
Assoc. was used. The mobile phase was methanol in various
mixtures (v/v) with phosphate buffer (pH = 7.4, ionic strenght
= 0.1 M) at a flow rate of 1 ml/min. The benzodiazepines,
obtained from commercial sources and reported in Table 1,
were dissolved in methanol or methanol-water mixtures in the
concentration range between 100 and 400 µg/ml. The benzodia-
zepines solutions were injected into the column by a 10 µl
loop. All solutions were first filtered to reduce contamination.
The elution time (t_0) of a non-retained component was regarded
as being equal to the elution time of potassium nitrate. The
log k' values were calculated as log $(t_r-t_0)/t_0$. The experiments
were performed at room temperature. For each compound retention
data were measured at a minimum of five different methanol
concentrations in the mobile phase. Each measurement was rep-
licated at least three times. The R_m and log P values previously
measured (24) are reported in Table 1. The data for the plots

TABLE I

Structures, log P and R_M values of Benzodiazepines.
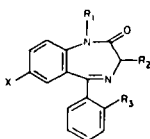
no.	compound	X	R_1	R_2	R_3	log P	R_M
1	N-demethyldiazepam	Cl	H	H	H	2.14	1.77
2	diazepam	Cl	CH_3	H	H	2.60	1.95
3		Cl	CH_3	H	Cl	3.25	2.15
4		Cl	H	H	F	2.15	1.73
5		Cl	CH_3	H	F	2.67	1.91
6		Cl	CH_2CF_3	H	H	3.73	2.54
7	prazepam	Cl	$CH_2\text{-}c\text{-}C_3H_5$	H	H	3.72	2.41
8	flurazepam	Cl	$(CH_2)_2N(C_2H_5)_2$	H	F	2.35	1.68
9	nitrazepam	NO_2	H	H	H	2.12	1.47
10		NO_2	$CONHCH_3$	H	H	1.95	1.46
11		Cl	$(CH_2)_2Cl$	OC_2H_5	H	3.95	2.32
12	oxazepam	Cl	H	OH	H	2.17	1.51
13	temazepam	Cl	CH_3	OH	H	1.99	1.58
14		Cl	$(CH_2)_2OH$	OH	H	1.35	1.35
15	lorazepam	Cl	H	OH	Cl	2.38	1.65
16	methyllorazepam	Cl	CH_3	OH	Cl	2.58	1.82
17		Cl	H	OH	F	1.48	1.48
18		NO_2	$(CH_2)_2OH$	OH	H	0.72	0.87
19		Cl	H		H	3.54	2.43
20		Cl	H	$OCOCH_2CH_2COOH$	H	1.41	1.33
21		Cl	H	$OCOCH(CH_2CH_2CH_3)_2$	H	5.25	3.01
22	medazepam					4.05	2.65
23						0.44	1.00
24						0.19	0.78
25	chlordiazepoxide					2.50	1.81

of Fig. 2 were taken from previous papers, with the exception of a few R_m values which were determined at the time of the present work. The details of the basic reversed-phase TLC technique had been already reported (4).

RESULTS

The reversed-phase HPLC system provided reliable log k' values for all the test compounds. In fact t_r must be not too close to t_o because in this case $t_r - t_o$ cannot be measured accurately, likewise, if t_r is too long, excessive peak broadening will limit accuracy. The plots of Fig. 1 show the relationship between the experimental log k' values and the methanol concentration in the mobile phase. At lower methanol concentrations for each compound there was a range of linear relationship between log k' values and composition of the mobile phase. At higher methanol concentrations deviations from linearity were observed. In fact the log k' values tend to a minimum value indicating that the compounds move with the solvent. The plots of Fig. 1 can be usefully compared with the plots of Fig. 2, where are reported some examples describing the relationship between the R_M values for several classes of compounds and the composition of the mobile phase in a reversed-phase TLC system. For the more lipophilic compounds such as prazepam, testosterone propionate, and 1-(pyridin-2-azo)-2-naphtol over the full range of acetone concentrations the relationship might be described as an S-shaped curve. In fact at the lower acetone concentrations the compounds do not move from the starting line, while at the higher concentrations they tend to move with the solvent front. The extrapolation
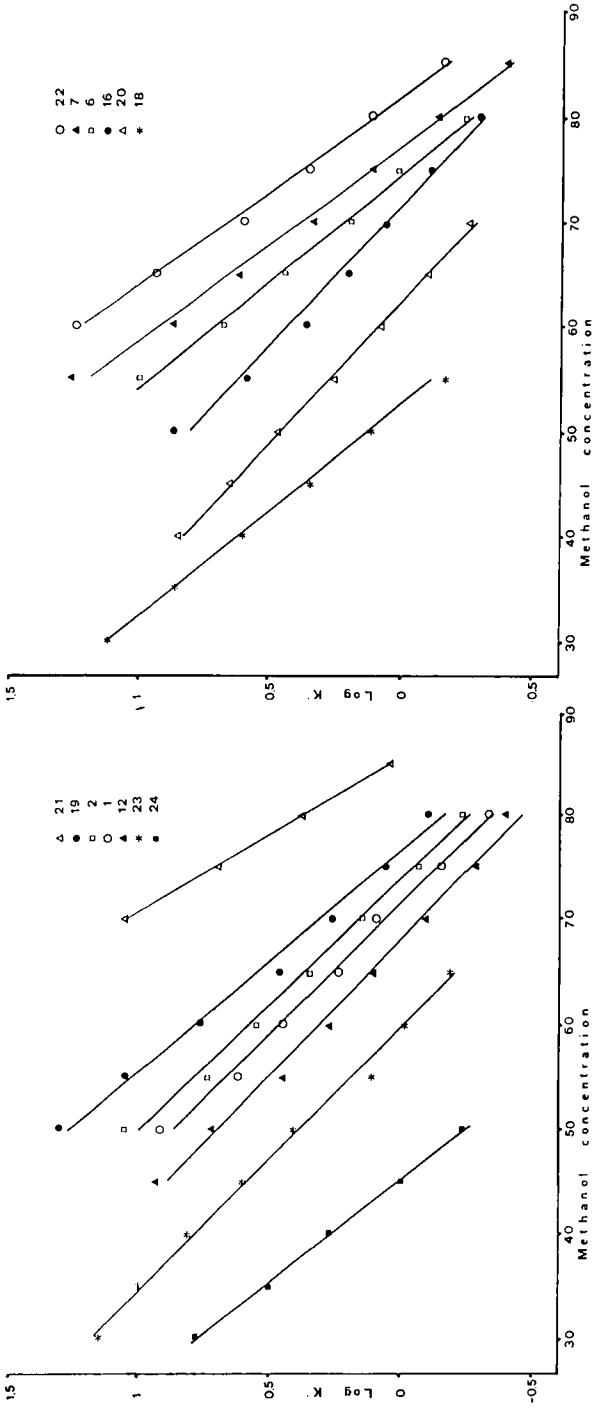
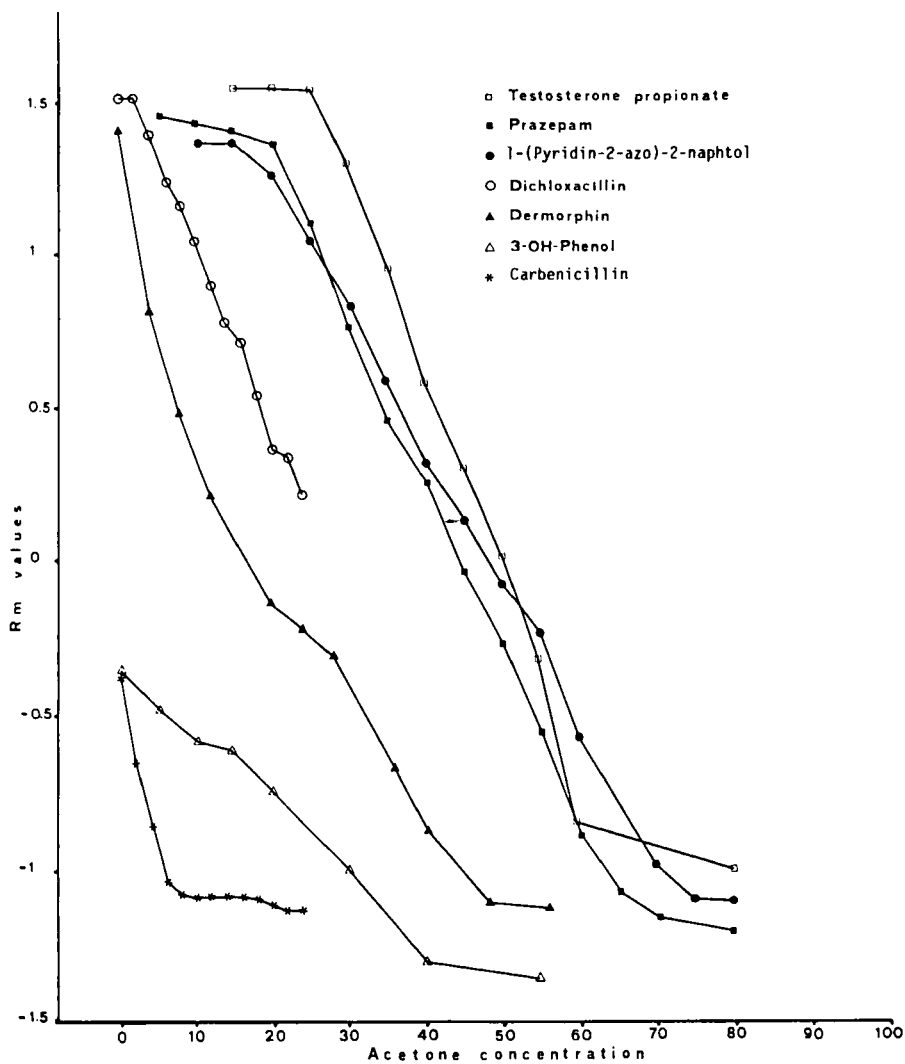FIGURE 1. Relationship between log k' values and methanol concentration in the mobile phase.

FIGURE 2. Relationship between R_M values and acetone concentration in the mobile phase.

from the linear part of the curve yields the theoretical R_M values at 0% acetone in the mobile phase. Werkhoven-Goewle et al. (26) showed very similar S-shaped curves describing the relationship between log k' values and composition of the mobile phase in a HPLC system. In our present series of benzodiazepines the very long t_r at the lower methanol concentrations did not allow to calculate reliable log k' values. As a consequence the plots of Fig. 1 do not show the upper part of the S-shaped curve. In Fig. 2 the more hydrophilic compounds such as carbenicillin, 3-OH- phenol and the dermorphin derivative show deviations from linearity only at the higher acetone concentrations, since even at 0% organic solvent in the mobile phase their chromatographic behaviour yields experimental R_M values. The first part of the curve can be fitted by a straight line and a theoretical R_M value at 0% can be obtained by interpolation. Werkhoven-Goewle et al. (26) showed in HPLC a similar behaviour for monochlorophenols. The chromatographic study of a series of 10 penicillins and a series of 21 5-nitroimidazoles (4, 27) provided the R_M values for calculating eqns. 1 and 2 which point out a very good correlation between the interpolated and the experimental R_M values at 0% acetone or methanol in the mobile phase. Eqns. 1 and 2 seem to support the validity of the linear extrapolation at 0% for hydrophilic compounds.

Penicillins

$$R_M \text{ (exptl.)} = 0.065 + 0.990 \, R_M \text{ (calcd.)} \tag{1}$$
$$(n = 10; \quad r = 0.999; \quad s = 0.024; \quad F = 6357.35; \quad P < 0.005)$$

5-nitroimidazoles

$$R_M \text{ (exptl.)} = -0.024 + 1.018 \ R_M \text{ (calcd.)} \tag{2}$$

$(n = 21; \quad r = 0.999; \quad s = 0.026 \quad F = 12396.30; P < 0.005)$

In conclusion the plots of Fig. 1 and 2 seem to show very si-
milar chromatographic behaviours in TLC and HPLC. Therefore
according to the empirical procedure we have been using in
reversed-phase TLC, the equations describing the relationship
between log k' values and composition of the mobile phase
in the linear part of the curve were calculated (Table 2).
The extrapolated log k' values at 0% of methanol in the mobile
phase were considered as a measure of the partitioning between
stationary phase and water. The relationship between the
extrapolated log k' values of Table 2 and both the R_M or log
P values reported in Table 1 is described by eqns. 3 and 4.

$$\log k' = 0.870 \ (\pm 0.185) + 1.455 \ (\pm 0.099) \ R_M \tag{3}$$

$(n = 25; \quad r = 0.951: \quad s = 0.269; \quad F = 215.91; \ P < 0.005)$

$$\log k' = 1.805(\pm 0.111) + 0.686 \ (\pm 0.041) \ \log P \tag{4}$$

$(n = 25; \quad r = 0.961, \quad s = 0.240; \quad F = 278.00; \ P < 0.005)$

The very good correlation coefficients of eq 3 and 4 seem
to suggest that the extrapolation technique can be satisfac-
torily used also in HPLC. In order to extend our analysis
of the relationship between log k' values and mobile phase
composition, in Table 3 are reported the linear equations
calculated from the full range of methanol concentrations.
All the equations of Table 3 were highly significant and their
intercepts yielded eqns. 5 and 6 describing the relationship
with the R_M and log P values of Table 1. However the correlation
coefficients of eqns. 5 and 6 are rather low. Obviously this

TABLE 2

Linear relationship between log K' values and composition of the mobile phase at the lower organic solvent concentrations according to the equation log K' = a + b C where C is the methanol concentration.

Compound no.	a	b	r*	Methanol % range
1	3.25	-0.0470	0.988	50-60
2	3.59	-0.0510	0.989	50-60
3	4.17	-0.0610	1.000	50-60
4	3.26	-0.0470	0.998	45-60
5	3.68	-0.0540	1.000	50-60
6	4.02	-0.0550	0.996	55-65
7	4.77	-0.0640	0.992	55-65
8	3.37	-0.0480	1.000	50-60
9	3.20	-0.0500	1.000	45-55
10	3.02	-0.0470	1.000	45-55
11	4.12	-0.0530	0.993	55-65
12	3.16	-0.0490	0.997	45-55
13	3.17	-0.0470	1.000	50-60
14	2.64	-0.0480	1.000	35-45
15	3.26	-0.0500	0.999	45-55
16	3.42	-0.0510	0.998	50-60
17	2.90	-0.0440	0.999	45-55
18	2.68	-0.0518	1.000	30-45
19	4.08	-0.0554	0.999	50-65
20	2.47	-0.0402	0.999	40-55
21	5.74	-0.0670	1.000	70-80
22	5.04	-0.0630	1.000	60-70
23	2.18	-0.0340	0.998	30-40
24	2.27	-0.0500	0.998	30-40
25	3.29	-0.0490	0.999	45-55

*: Correlation coefficient

TABLE 3

Linear relationship between log K' values and composition of the mobile phase over the full range of methanol concentrations according to the equation log K' = a + b C where C is the methanol concentration.

Compound no.	a	b	r*	Methanol % range
1	2.88	-0.0403	0.997	50-80
2	3.09	-0.0419	0.997	50-80
3	3.21	-0.0435	0.989	50-80
4	3.13	-0.0444	0.999	45-70
5	3.03	-0.0422	0.995	50-80
6	3.61	-0.0482	0.997	55-80
7	4.13	-0.0534	0.997	55-85
8	2.71	-0.0360	0.991	50-80
9	2.88	-0.0435	0.999	45-70
10	2.69	-0.0404	0.998	45-70
11	3.86	-0.0487	0.997	55-80
12	2.62	-0.0385	0.996	45-80
13	2.69	-0.0383	0.997	50-80
14	2.30	-0.0395	0.997	35-65
15	2.56	-0.0359	0.992	45-75
16	2.66	-0.0370	0.993	50-80
17	2.69	-0.0398	0.999	45-70
18	2.65	-0.0510	1.000	30-55
19	3.65	-0.0477	0.996	50-80
20	2.33	-0.0373	0.999	40-70
21	5.69	-0.0664	1.000	70-85
22	4.55	-0.0555	0.999	60-85
23	2.37	-0.0396	0.998	30-65
24	2.28	-0.0504	0.999	30-50
25	2.65	-0.0365	0.991	45-80

*: Correlation coefficient

is due to the deviations from linearity observed at the higher
methanol concentrations in the mobile phase.

$$\log K'_{Linear} = 0.809 \ (\underline{+} \ 0.265) + 1.269 \ (\underline{+} \ 0.142) \ R_M \qquad (5)$$
$$(n = 25; \quad r = 0.882; \quad s = 0.385; \quad F = 80.24; \ P<0.005)$$

$$\log K'_{Linear} = 1.638 \ (\underline{+} \ 0.177) + 0.693 \ (\underline{+} \ 0.066) \ \log P \qquad (6)$$
$$(n = 25; \quad r = 0.803; \quad s = 0.383; \quad F = 81.33; \ P<0.005)$$

In fact in Table 3 the differences between the observed and
calculated log k' values might suggest a parabolic relationship
with the composition of the mobile phase. Therefore the equa-
tions describing the quadratic relationship between the log
k' values and the full range of methanol concentrations were
calculated (Table 4).

The intercepts were used in calculating eqns. 7 and 8 which
are much better than eqns. 5 and 6 and even better than eqns.
3 and 4.

$$\log k'_{quadratic} = 0.501 \ (\underline{+} \ 0.218) + 2.097 \ (\underline{+} \ 0.117) \ R_M \qquad (7)$$
$$(n = 25; \quad r = 0.966; \quad s = 0.317; \quad F = 323.48; \ P<0.005)$$

$$\log k'_{quadratic} = 1.840 \ (\underline{+} \ 0.113) + 0.992 \ (\underline{+} \ 0.042) \ \log P \qquad (8)$$
$$(n = 25; \quad r = 0.980; \quad s = 0.244; \quad F = 561.08; \ P<0.005)$$

However the analysis of variance of the equations of Table
4 showed that the introduction of the quadratic term into
equations of Table 3 did yield a significant improvement in
only 7 of the equations of Table 4. The lack of any significance
for the squared term in most of the equations of Table 4 does
not allow much confidence in the quadratic relationship between
log k' values and methanol concentration at least in the
present series of compounds. Finally according to the model
suggested by Schoenmakers. et al. (23) in Table 5 are reported

TABLE 4

Quadratic relationship between log K' values and composition
of the mobile phase over the full range of methanol concentra-
tions according to the equation log K' = a + b_1 (Me OH%) +
b_2 (Me OH%)^2.

Compound no.	a	b_1	b_2	r*	$F_{(1,\alpha)}$
1	3.55	-0.0613	0.00016	0.997	0.92
2	4.37	-0.0822	0.00031	0.997	6.00
3	5.31	-0.1097	0.00051	0.989	3.61
4	3.89	-0.0715	0.00024	0.999	3.28
5	4.81	-0.0985	0.00043	0.995	23.81**
6	5.21	-0.0963	0.00036	0.997	3.31
7	5.78	-0.1014	0.00034	0.997	4.62
8	4.44	-0.0905	0.00042	0.991	4.41
9	3.80	-0.0763	0.00029	0.999	8.58
10	3.62	-0.0733	0.00029	0.998	8.07
11	5.71	-0.1046	0.00041	0.997	6.50
12	3.76	-0.0762	0.00030	0.996	15.13**
13	3.79	-0.0729	0.00027	0.997	7.13
14	3.00	-0.0686	0.00029	0.997	11.56**
15	4.26	-0.0942	0.00049	0.992	25.30**
16	4.30	-0.0890	0.00040	0.993	7.45
17	3.38	-0.0644	0.00021	0.999	6.35
18	2.69	-0.0528	0.00002	1.000	0.06
19	5.61	-0.1096	0.00048	0.996	18.23**
20	2.89	-0.0582	0.00019	0.999	14.29**
21	6.89	-0.0975	0.00020	1.000	1.32
22	6.25	-0.1030	0.00033	0.999	5.15
23	2.34	-0.0382	0.00001	0.998	0.01
24	2.46	-0.0595	0.00011	0.999	0.30
25	4.08	-0.0838	0.00038	0.991	8.01**

*:Correlation coefficient
**:The quadratic regression is significant at the 5% level

the equations describing the relationship between log k' values
and methanol concentrations in the mobile phase where a third
independent variable is represented by the square root of
the methanol concentration. Eqns. 9 and 10 describing the
relationship between the log k' values from Table 5 and the
R_M and log P values respectively, show very low correlation
coefficients.

$$\log k' = 21.919 \ (\pm 10.216) - 16.339 \ (\pm 19.077) \ R_M \qquad (9)$$

(n = 25;   r = 0.408;   s = 27.786;   F = 4.603 ; n.s.

$$\log k' = 9.491 \ (\pm 4.828) - 0.220 \ (\pm 28.165) \ \log P \qquad (10)$$

(n = 25;   r = 0.379;   s = 28.165;   F = 3.864 ; n.s.

On the other hand the validity of the equations of Table 5
is limited by the fact that the square root term is not signifi-
cant. One could add that there is a strict limit to the number
of independent variables which may be included in the regression
equation. In other words the number of observations in Table
5 seems to be too small to justify a third independent variable.
This seems to rule out any general validity also for the use
of the square root term. Finally Schoenmakers et al. (23)
themselves had pointed out that their model should hold only
when the square root term is smaller than 1.

CONCLUSIONS

The present data show that by means of the HPLC technique
it is possible to obtain log k' values of benzodiazepines
which are highly correlated with their R_M or log P values
as an expression of the lipophilic character molecules. The
empirical technique based upon the extrapolation from the
linear part of the curve describing the relationship between

TABLE 5

Relationship between log K' values and composition of the mobile phase when considering the introduction of the square root term into the equations of Table 4 according to the equation:
$\log K' = a + b_1 \, (\text{Me OH\%}) + b_2 \, (\text{Me OH\%})^2 + b_3 \sqrt{\text{Me OH\%}}$

Compound no.	a	b_1	b_2	b_3	r*
1	46.82	1.3043	-0.0034	-14.5218	0.995
2	38.44	0.9931	-0.0025	-11.4348	0.995
3	78.25	2.1922	-0.0055	-24.4783	0.995
4	-7.59	-0.4781	0.0014	4.0813	0.995
5	24.38	0.5188	-0.0012	- 6.5652	0.995
6	93.04	2.5599	-0.0063	-28.8351	0.997
7	79.72	2.0643	-0.0049	-23.8984	0.996
8	- 2.03	-0.2949	0.0010	2.1739	0.995
9	21.79	0.5608	-0.0016	- 6.3958	0.995
10	15.79	0.3571	-0.0010	- 4.3243	0.995
11	13.70	0.1368	-0.0002	- 2.6214	0.997
12	3.13	-0.0971	0.0004	0.2166	0.993
13	38.64	1.0269	-0.0026	-11.6957	0.995
14	9.43	0.1992	-0.0006	- 2.4833	0.991
15	27.47	0.7017	-0.0017	- 8.1216	0.994
16	18.33	0.3039	-0.0005	- 4.4507	0.996
17	- 0.99	-0.2193	0.0007	1.5548	0.995
18	9.65	0.2874	-0.0013	- 2.9117	0.992
19	-31.57	-1.2830	0.0035	12.4783	0.995
20	- 3.04	-0.2811	0.0009	2.1739	0.993
21	7.86	-0.0724	0.0001	- 0.2949	0.999
22	60.99	1.4347	-0.0032	-17.3164	0.997
23	2.47	-0.0383	0.0000	- 0.0258	0.939
24	7.74	0.2115	-0.0010	- 2.2625	0.994
25	18.02	0.3782	-0.0008	- 4.7982	0.993

*: Correlation coefficient

log k' values and mobile phase composition was shown to be reliable also in HPLC, at least for the present series of benzodiazepines. Since the relationship between log k' or R_M values and mobile phase composition can be generally described by an S-shaped curve, the extrapolation from the linear part of the curve seems to avoid the physical limitations of the chromatographic system. These are represented by the upper and lower part of the S-curve. Therefore the extrapolated log k' or R_M values should be a real measure of the partitioning between stationary phase and water. The usefulness of log k' or R_M values linearly or quadratically extrapolated from the full range of mobile phase composition should only depend upon the particular range of organic solvent concentration which has been used. For example, a quadratic extrapolation could be useful in the presence of a range of organic solvent yielding only the lower part of an S-shaped curve. The critical point seems rather to be the choice of the range of linear relationship from which to calculate the extrapolated log k' or R_M values. With lipophilic compounds a small difference in the slope of the straight line would make a great difference in the extrapolated log k' or R_M values. A 0-100% range of organic solvent in the mobile phase would help in avoiding large errors. In fact in this way one should be able to describe most of the S-shaped curve, at least in reversed-phase TLC. In a HPLC system because of the very long retention times at the lower organic solvent concentrations, it may be difficult to describe the upper part of the S-curve. However if the aim is to calculate from the linear part of the curve the

extrapolated log k' values at 0% organic solvent in the mobile phase this should not limit the usefulness of the extrapolation procedure.

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