Journal of Chromatography, 129 (1976) 263–276

CHROM. 9241

A COMPARISON OF THE DETERMINATION OF PARTITION COEFFICIENTS OF 1,4-BENZODIAZEPINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY

ABRAM HULSHOFF and JOHN H. PERRIN

Pharmaceutical Laboratories, University of Utrecht, Catharijnesingel 60, Utrecht (The Netherlands)

(Received March 24th, 1976)

SUMMARY

Relative partition coefficients (P) of 1,4-benzodiazepines were determined by a reversed-phase thin-layer chromatographic (RP-TLC) technique in the system oleyl alcohol–water, and by high-performance liquid chromatography (HPLC) using several column packing materials. R_{M_w} values, obtained by RP-TLC, correlated well with log P values determined directly in the system oleyl alcohol–water and with the literature values for the system 1-octanol–water. The log k'_w values from the HPLC experiments could be determined with greater precision, but the correlations of log k'_w with log $P_{\text{oleyl alcohol}}$ and log P_{octanol} were not as good as those found for the R_{M_w} values.

INTRODUCTION

Chromatographic methods have frequently been applied for the determination of relative partition coefficients (P) for use in quantitative structure–activity relationships (QSAR). Probably the most widely used technique is reversed-phase thin-layer chromatography (RP-TLC)[1–7], but recently, the use of high-performance liquid chromatography (HPLC) with a chemically bonded stationary phase for this purpose has been described [8–10]. It has been claimed[8] that HPLC with a chemically bonded apolar stationary phase yields retention data from which log P_{octanol} values can be calculated. In the work described here, the (relative) partition coefficients of 1,4-benzodiazepines were determined by a RP-TLC method that has been developed for this purpose[11], by HPLC using several packing materials, and by shaking the compounds in an oleyl alcohol–water system. Comparisons are made between the relative partition coefficients obtained by these techniques and with values from the literature[12].

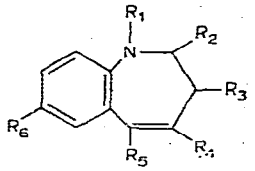
EXPERIMENTAL

Materials

The chemical formulae of the 1,4-benzodiazepines and their sources are summarized in Table I.

TABLE I

CHEMICAL FORMULAE AND SOURCES OF 1,4-BENZODIAZEPINES



The drugs were supplied by Hofman-La Roche (HR; Mijdrecht, The Netherlands), B.V. Substantia (S; Mijdrecht, The Netherlands) and Wyeth Laboratoria B.V. (W; Amsterdam, The Netherlands), or purchased from commercial sources (C).

Compound	R_1	R_2	R_3	R_4	R_5	R_6	*Source*
Medazepam	$-CH_3$	$-H$	$-H$	—	$-C_6H_5$	$-Cl$	HR
Prazepam	$-CH_2-CH(CH_2)_2$	$=O$	$-H$	—	$-C_6H_5$	$-Cl$	S
Flurazepam*	$-CH_2CH_2N(C_2H_5)_2$	$=O$	$-H$	—	$-C_6H_4F$	$-Cl$	HR
Diazepam	$-CH_3$	$=O$	$-H$	—	$-C_6H_5$	$-Cl$	C
Chlordiazepoxide*	—	$-NHCH_3$	$-H$	$\rightarrow O$	$-C_6H_5$	$-Cl$	HR
Lorazepam	$-H$	$=O$	$-OH$	—	$-C_6H_4Cl$	$-Cl$	W
Oxazepam	$-H$	$=O$	$-OH$	—	$-C_6H_5$	$-Cl$	W
Temazepam	$-CH_3$	$=O$	$-OH$	—	$-C_6H_5$	$-Cl$	W
Clonazepam	$-H$	$=O$	$-H$	—	$-C_6H_4Cl$	$-NO_2$	HR
Nitrazepam	$-H$	$=O$	$-H$	—	$-C_6H_5$	$-NO_2$	C
Flunitrazepam	$-CH_3$	$=O$	$-H$	—	$-C_6H_4F$	$-NO_2$	HR
Bromazepam	$-H$	$=O$	$-H$	—	$-C_5H_4N$	$-Br$	HR

* Supplied as the monohydrochloride.

Oleyl alcohol (Carl Roth OHG, Karlsruhe, G.F.R.), containing 98% of *cis*-9-octadecen-1-ol, was distilled (b.p. 135–140°, 0.05 mm Hg), passed through a column of basic aluminium oxide (Merck, Darmstadt, G.F.R.) and used for some of the RP-TLC experiments and all of the direct measurements of the partition coefficients.

Dioxan (Merck, "reinst"), Kieselguhr G (Merck), hexamethyldisilazane (HMDS) and trimethylsilyl chloride (TMSCl) (Merck, "für die Gaschromatographie") and dichlorodimethylsilane (DClDMS) (Merck, "zur Synthese") were used as supplied. Porasil C (37–75 μm) and C18/Corasil (37–50 μm) were purchased from Waters Ass. (Milford, Mass., U.S.A.) and were silylated before use. Chromosorb P-NAW (30–60 mesh) was obtained from Chrompack (Vlissingen, The Netherlands). Distilled water was used throughout. All other materials were of reagent grade.

Direct measurement of partition coefficients.

A 2- or 5-ml volume of oleyl alcohol, saturated with phosphate buffer, and 5 or 10 ml of phosphate buffer (pH = 7.4, ionic strength = 0.1 *M*), saturated with oleyl alcohol, were shaken together for 1½ h at 25° (weighed amounts of chlordiazepoxide hydrochloride, flurazepam hydrochloride and bromazepam were dissolved

in the buffer solution; the other compounds were dissolved in the oleyl alcohol). After centrifugation, the concentration of the drug in the aqueous layer was determined by measuring the ultraviolet absorbance, using a Perkin-Elmer Model 139 UV–visible spectrophotometer. An equal number of blanks was carried through the procedure.

The amount of drug and the portions of oleyl alcohol and buffer were such that the absorbance of the aqueous layer, measured at the wavelength of maximum absorption (λ_{max}) in a 0.5- or 1-cm cell, was between 0.2 and 0.7. The partition coefficient of chlordiazepoxide was determined by adding (after centrifugation) 2 ml 0.22 *N* hydrochloric acid to 2 ml of the aqueous layer and by measuring the absorbance of the resulting solution (pH = 1). The same procedure was followed in some of the diazepam experiments. In all of the other experiments, the absorbance was measured directly in the phosphate buffer. In the flurazepam experiments, the pH of the aqueous layer was also measured after centrifugation.

The partition coefficients, *P*, were calculated from the equation $P = C_{ol}/C_w$, where C_{ol} and C_w are the molar concentrations of the drug in the oleyl alcohol and aqueous layer, respectively.

Determination of the acid dissociation constant of flurazepam hydrochloride

The concentration-dependent acid dissociation constant of flurazepam hydrochloride was measured in water and in a 50% (w/w) methanol–water mixture by the titration method of Benet and Goyan[13], in the manner described by Hulshoff and Perrin[14]. Solutions of flurazepam hydrochloride ($5 \cdot 10^{-4}$ *M*; ionic strength = 0.1) were titrated at 25° against 0.1 *N* sodium hydroxide solution. Titrations in aqueous solutions could be executed, although near the end of the titration the concentration of flurazepam (free base) exceeded its solubility. The flurazepam was not precipitated until after the titration had been completed.

RP-TLC experiments

The method used was as described previously by Hulshoff and Perrin[11]. The stationary phase consisted of a Kieselguhr G layer that had been impregnated directly with oleyl alcohol, by coating the glass plates (20 × 20 cm) with a slurry of Kieselguhr G in a mixture of oleyl alcohol, acetone and dioxan. The polar mobile phases were represented by methanol–water buffer solutions (ammonia and ammonium salt; pH* = 9–9.5*; ionic strength = 0.1 *M*) saturated with oleyl alcohol. The methanol concentration in the mobile phase ranged from 7.5 to 55% (w/w). The developed plates were allowed to dry at room temperature and were then sprayed with acidified Dragendorff's reagent (to 50 ml of Dragendorff's reagent[15], 2 ml of 50% nitric acid was added).

HPLC measurements

The experiments were performed on a Waters ALC/GPC 202/6000 liquid chromatograph, with U6K injection system. Stainless-steel columns and pre-columns were used (2 ft. × 1/8 in. I.D. and 2 ft. × 3/8 in. I.D., respectively).

* pH meter readouts of measurements in methanol–water mixtures are denoted by the symbol pH*.

The dead volume, outside the column, between the injection system and the ultraviolet detector was measured and a correction for this dead volume was made when calculating the retention times. Samples were dissolved in methanol–water mixtures (0.05–0.1%) with the same methanol concentration as in the mobile phase; the sample solutions were saturated with oleyl alcohol when columns filled with oleyl alcohol-coated Porasil C as the stationary phase were used. Volumes of 2–25 μl of the sample solutions were injected. The elution time (t_0) of a non-retained component was regarded as being equal to the elution time of potassium nitrate, which was added to all of the sample solutions (0.5–1%).

The drugs were eluted with buffer solutions of ammonia and ammonium sulphate in methanol–water mixtures (pH* = 9; ionic strength = 0.1 *M*), at the rate of 0.9–1 ml/min. When using columns packed with oleyl alcohol-coated Porasil C, the mobile phase was saturated with oleyl alcohol by passing it through two pre-columns which were filled with oleyl alcohol-coated Chromosorb P-NAW, and placed between the pump and the injection system. All of the sample solutions and the mobile phases were filtered before use (pore size 0.2–1.2 μm). The temperature of the mobile phase and the temperature in the column and pre-columns were maintained at 25°.

Silylation with hexamethyldisilazane and trimethylsilyl chloride

The method as described by McCall[8] was used for the silylation of Porasil C and C18/Corasil.

Silylation with dichlorodimethylsilane

Porasil C (25 ml) was dissolved in 100 ml of a 5% solution of DClDMS in toluene. The solution was degassed by reducing the pressure over the solution. After standing for 30 min at room temperature, the silylated product was collected and thoroughly washed with methanol. The material was dried by gentle heating (40–50°) under a stream of nitrogen.

Impregnation of Porasil C and Chromosorb P-NAW with oleyl alcohol

Porasil C (silylated) and Chromosorb P-NAW were impregnated with oleyl alcohol by the solvent evaporation technique[16]. Weighed amounts of the support were immersed in a methanolic solution of oleyl alcohol (3 or 5% (w/w) of the amount of silylated Porasil C, and 30% (w/w) of the amount of Chromosorb P-NAW).

After gentle mixing of the slurry, the methanol was evaporated under reduced pressure and the powder was further dried under a stream of nitrogen. The columns were filled with silylated (HMDS + TMSCl) C18/Corasil, with silylated (DClDMS) Porasil C impregnated with 3 and 5% (w/w) oleyl alcohol and with silylated (HMDS + TMSCl) Porasil C impregnated with 5% (w/w) oleyl alcohol. The pre-columns were filled with Chromosorb P-NAW that had been impregnated with 30% (w/w) oleyl alcohol. The finest particles of all packing materials were blown off by passing a stream of nitrogen through a glass tube, in a vertical position, containing the packing material. The columns and the pre-columns were dry-packed by the tap–fill method. A column packed with non-silylated C18/Corasil by the manufacturer (Waters Assoc.) was also used.

RESULTS AND DISCUSSION

Reversed-phase chromatography of very lipophilic compounds cannot be performed with 1-octanol as the stationary phase[11], because the methanol concentration in the mobile phase, necessary for obtaining measurable retention times, would solubilize the 1-octanol. For this reason, oleyl alcohol was chosen as the stationary phase in all of the chromatographic experiments, and in the direct measurements for comparison purposes.

The ultraviolet characteristics of the benzodiazepines of which the partition coefficients were directly measured in the oleyl alcohol–phosphate buffer system are presented in Table II, together with the log $P_{\text{oleyl alcohol}}$ values. At pH = 7.4, all of the compounds except flurazepam are present in the neutral form[17]. Log P for flurazepam, mentioned in Table II, is the logarithm of the true partition coefficient[18] (P) calculated from the apparent partition coefficient (P_a) (measured at pH = 7.4) and the apparent dissociation constant (K_a^c; pK_a^c value determined to be 8.43). The partition coefficients in the system oleyl alcohol–phosphate buffer were correlated with the 1-octanol–phosphate buffer partition coefficients of Müller and Wollert[12], using a Collander-type equation[19]:

$$\log P_{\text{oleyl alcohol}} = a \log P_{\text{octanol}} + b$$

where a and b are constants.

A good linear correlation was found between log $P_{\text{oleyl alcohol}}$ and log P_{octanol} (Table VI, eqn. 1).

Before measuring the relative partition coefficients of the benzodiazepines by the RP-TLC method, the possible adsorption of the compounds on to the support phase during the migration was investigated. R_F values of eight benzodiazepines were measured using 30% (w/w) methanol–water buffers as the mobile phase and with

TABLE II

WAVELENGTHS OF MAXIMUM ABSORPTION (μ_{max}) AND MOLAR ABSORPTIVITIES (ε) OF BENZODIAZEPINES IN PHOSPHATE BUFFER (pH = 7.4) AND LOG $P_{\text{oleyl alcohol}}$ VALUES

s = standard deviation; n = number of determinations (of P)

Compound	λ_{max} *(nm)*	ε	*log P*	*s*	*n*
Prazepam	228	30,050	3.50	0.02	8
Flurazepam	231	33,390	3.11**	0.08	12
Diazepam	230	35,330			
			2.64	0.03	10
	285*	13,910*			
Chlordiazepoxide	245*	34,700*	2.25	0.02	10
Lorazepam	228	38,800	2.02	0.07	14
Temazepam	232	35,600	1.90	0.06	15
Nitrazepam	259	17,050	1.89	0.06	19
Bromazepam	235	33,490	1.18	0.02	11

* Values in 0.1 *N* HCl.

** The true partition coefficient is calculated from the values of the apparent partition coefficient (P_a) measured at pH 7.43.

TABLE III

VALUES OF $1/R_F$ OF BENZODIAZEPINES FROM RP-TLC EXPERIMENTS WITH THE SYSTEM OLEYL ALCOHOL–30% (w/w) METHANOL, AND THE VALUES OF THE INTERCEPTS (a) AND SLOPES (b) OF THE GRAPHS OF $1/R_F$ AGAINST C_{ol}

C_{ol} is the percentage (v/v) of oleyl alcohol in the impregnating mixture. n = number of determinations; s_a and s_b = standard deviations of a and b, respectively.

Compound	*Oleyl alcohol % (v/v)*					a	s_a	b	s_b	n
	1.25	*2*	*3*	*4*	*6*					
Diazepam	2.33	3.01	3.91	5.38	7.06	0.977	0.127	1.031	0.032	48
Chlordiazepoxide	1.77	2.11	2.63	3.32	4.38	0.966	0.065	0.572	0.016	48
Lorazepam	1.51	1.66	1.99	2.42	3.03	0.978	0.043	0.345	0.011	48
Temazepam	1.48	1.64	1.95	2.35	2.91	1.012	0.040	0.319	0 010	44
Clonazepam	1 47	1.61	1.93	2.30	2.86	0.994	0.035	0.314	0.009	48
Nitrazepam	1.41	1.53	1.80	2.16	2.60	1.007	0.031	0.271	0.008	48
Flunitrazepam	1.36	1.47	1.72	2.03	2.41	1.022	0.025	0.236	0.006	48
Bromazepam		1.19	1.30	1.41	1.57	1.013	0.012	0.095	0.003	47

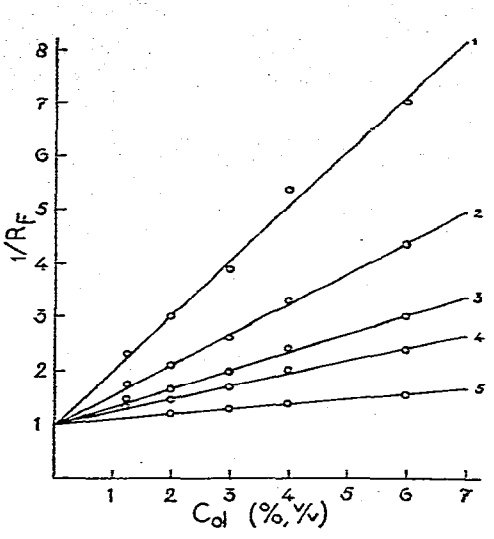
varying amounts of oleyl alcohol in the impregnating mixture. If the R_F values of compounds in reversed-phase chromatography are determined exclusively by partitioning between the stationary phase (oleyl alcohol) and the mobile phase (methanol–water mixtures), then a linear correlation exists between the R_F value and the oleyl alcohol concentration (C_{ol}) in the impregnating mixture:

$$\frac{1}{R_F} = 1 + {}_sP \cdot k \cdot C_{ol}$$

Where ${}_sP^*$ is the partition coefficient in the system oleyl alcohol/methanol–water mixture and k is a constant. Graphs of $1/R_F$ values against C_{ol} should then result in straight lines with intercepts of unity and slopes of ${}_sP \cdot k$. Values of $1/R_F$ for different oleyl alcohol concentrations are presented in Table III, with the calculated slopes and intercepts of the graphs. It is clear (Table III, Fig. 1) that the graphs are indeed straight lines and that the intercept on the $1/R_F$ axis does not differ significantly from unity for any compound. The 30% (w/w) methanol–water mixtures that were used as the mobile phase in these experiments usually contained ammonia and ammonium chloride (as in all further RP-TLC experiments). In some of these experiments other ammonium salts were used, and also the pH* of the buffer was changed ($7 < \text{pH}^* < 9.5$); these variations did not cause any change in the R_F values of the compounds. There was also no change in the chromatographic behaviour of the compounds when purified oleyl alcohol (Schuchardt, Munich, G.F.R.) was used instead of that obtained from Roth.

It can be concluded that, under the conditions used, partitioning of the benzodiazepine drugs is the sole process that governs the R_F values. The relative partition coefficients of the benzodiazepines in the oleyl alcohol–water system were then determined by measuring the R_M values of the compounds with various methanol concentrations in the mobile phase, using Kieselguhr G plates that had been impregnated

* The subscript indicates that a methanol–water mixture is involved.



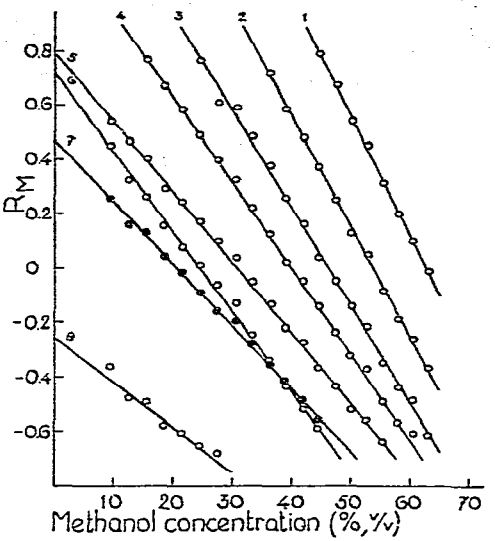


Fig. 1. $1/R_F$ as a function of the oleyl alcohol concentration (C_{ol}) in the impregnating mixture (RP-TLC) for five benzodiazepines. 1 = Diazepam; 2 = chlordiazepoxide; 3 = lorazepam; 4 = flunitrazepam; 5 = bromazepam.

Fig. 2. Effect of the methanol concentration of the mobile phase on the R_M values of benzodiazepines [RP-TLC; 1.25% (v/v) oleyl alcohol]. 1 = Medazepam; 2 = prazepam; 3 = flurazepam; 4 = diazepam; 5 = chlordiazepoxide; 6 = lorazepam; 7 = temazepam; 8 = bromazepam.

with 1.25 and 4% (v/v) oleyl alcohol in the impregnating mixture, and by extrapolating the graphs of R_M against methanol concentration to zero methanol concentration.

Flurazepam is a stronger base than the other benzodiazepines whose R_M values were determined. The $p({}_sK_a{}^c)$ of flurazepam in 50% (w/w) methanol–water was found to be 7.35, while in water $pK_a{}^c = 8.43$. The $p({}_sK_a{}^c)$ values of flurazepam at other methanol concentrations were estimated by assuming a linear decrease of $p({}_sK_a{}^c)$ with increasing methanol concentration*. The pH* of each mobile phase was at least 1.5 pH units higher than the estimated $p({}_sK_a{}^c)$ value of flurazepam at that methanol concentration (it was found that the R_F values of flurazepam remained constant at higher pH* values).

R_M** varies linearly with the volume fraction of the organic solvent in the mobile phase[20,21]. This can be represented by[11]

$$R_M = R_{M_w} + b'C$$

where R_{M_w} is the value of R_M at zero methanol concentration, b' is a constant and C is the methanol concentration (%, v/v). Graphs of R_M against C are shown in Fig. 2. The calculated slopes and intercepts of the graphs for both series [1.25 and 4% (v/v) oleyl alcohol] are shown in Table IV. The correlation between the R_{M_w} values of the

* Although the $p({}_sK_a{}^c)$ value does not change linearly with methanol concentration[14], the errors introduced by ignoring this non-linearity are small and of no consequence in the present investigations.

** $R_M = \log(1/R_F - 1) = \log P + \log r$ (where r is the phase volume ratio).

TABLE IV

SLOPES AND INTERCEPTS OF GRAPHS OF R_M AGAINST THE METHANOL CONCENTRATION OF THE MOBILE PHASE FROM RP-TLC EXPERIMENTS WITH 1.25 AND 4% (v/v) ALCOHOL IN THE IMPREGNATING MIXTURE

n = total number of R_M values; s = standard deviation; R_{M_w} and b' = intercept and slope, respectively, of the graphs $R_M = R_{M_w} + b'C$, where C is the methanol concentration (%, v/v).

Compound	*1.25% (v/v) oleyl alcohol*					*4% (v/v) oleyl alcohol*				
	n	R_{M_w}	s	b'	s	n	R_{M_w}	s	b'	s
Medazepam	96	2.75	0.027	−0.0438	0.0005					
Prazepam	148	2.19	0.019	−0.0407	0.0004					
Flurazepam	180	1.65	0.025	−0.0357	0.0005	92	2.18	0.027	−0.0368	0.0005
Diazepam	196	1.26	0.008	−0.0315	0.0002	132	1.76	0.021	−0.0317	0.0004
Chloriazepoxide	204	0.80	0.006	−0.0258	0.0002	204	1.31	0.010	−0.0268	0.0002
Lorazepam	156	0.71	0.008	−0.0291	0.0003	180	1.25	0.012	−0.0308	0.0003
Clonazepam	156	0.68	0.008	−0.0289	0.0003	201	1.13	0.011	−0.0284	0.0003
Temazepam	156	0.47	0.008	−0.0228	0.0003					
Flunitrazepam	144	0.44	0.009	−0.0241	0.0003	204	0.89	0.011	−0.0250	0.0003
Nitrazepam	156	0.43	0.009	−0.0235	0.0003	204	0.88	0.011	−0.0235	0.0006
Bromazepam	80	−0.25	0.013	−0.0165	0.0007	132	0.22	0.019	−0.0175	0.0005

two series proved to be very good, as well as the correlation between R_{M_w} (1.25% oleyl alcohol) and $\log P_{\text{oleyl alcohol}}$ (Table VI, eqns. 2 and 3). The value for the intercept on the R_{M_w} (4%) axis (Table VI, eqn. 2) is 0.46. As $R_{M_w}(4\%) = \log P + \log r_4$ and $R_{M_w}(1.25\%) = \Delta \log P + \log r_{1.25}$ (where r_4 and $r_{1.25}$ represent the phase volume ratios for 4% and 1.25% oleyl alcohol, respectively), this intercept is equal to $\log r_4/r_{1.25}$. The term r changes linearly with the oleyl alcohol concentration (C_{ol}) in the impregnating

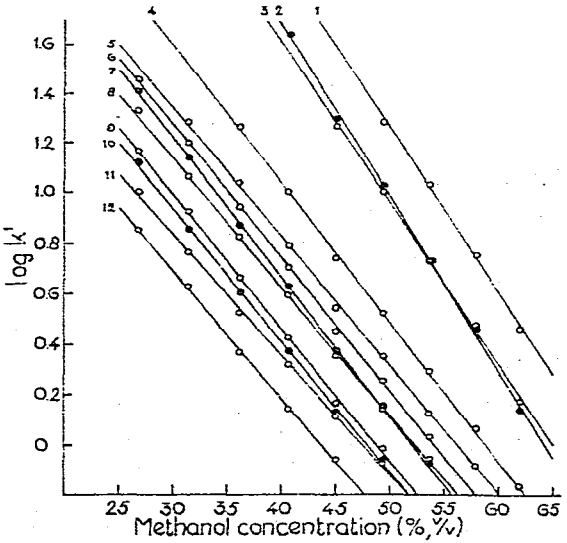


Fig. 3. Effect of the methanol concentration of the mobile phase on the log k' values of benzodiazepines (HPLC, C18/Corasil with HMDS + TMSCl). 1 = Medazepam; 2 = flurazepam; 3 = prazepam; 4 = diazepam; 5 = chlordiazepoxide; 6 = temazepam; 7 = lorazepam; 8 = oxazepam; 9 = flunitrazepam; 10 = clonazepam; 11 = nitrazepam; 12 = bromazepam.

mixture[11] and therefore $\log r_4/r_{1.25} = \log 4/1.25 = 0.51$. This value is in good agreement with the experimentally found value for the intercept. Apparently the RP-TLC technique is very suitable for the determination of the relative partition coefficients of the benzodiazepines. The technique had already been shown to give good results for the phenothiazines[11]. Differences in R_{M_w} values (ΔR_{M_w}) of the compounds are equal to $\Delta \log P_{\text{oleyl alcohol}}$, and as the $\Delta \log P_{\text{oleyl alcohol}}$ values are almost equal to the log P_{octanol}[22] (or π) values that are normally used in QSAR studies, this RP-TLC technique is a good substitute for the direct measurement of the 1-octanol–water partition coefficients.

A drawback of the RP-TLC method is the necessity for carrying out large numbers of determinations in order to obtain accurate R_{M_w} values with low standard deviations. This disadvantage is largely overcome by the use of HPLC, because log k' values, obtained with a thermostated column, are much more precise than R_M values from RP-TLC experiments*.

Retention times, t_R, of the benzodiazepines were measured by HPLC using 5 different column packing materials. Graphs of log k' against the methanol concentration were plotted, as in the RP-TLC experiments**. The resulting straight lines for a column packed with silylated C18/Corasil are shown in Fig. 3. The calculated slopes and intercepts of these graphs, with the five column packing materials, are presented in Table V, the general equation being

$$\log k' = \log k'_w + b''C$$

where $\log k'_w$ is the value of $\log k'$ at zero methanol concentration, b'' is a constant and C is the methanol concentration (%, v/v). The $\log k'_w$ values for the columns that were packed with Porasil C, which had been silylated with DClDMS and impregnated with 3 and 5% (w/w) oleyl alcohol, showed an excellent correlation (Table VI, eqn. 5). A very good correlation (Table VI, eqn. 6) was also found between the $\log k'_w$ values on Porasil C silylated with DClDMS and impregnated with 3% (w/w) oleyl alcohol and the $\log k'_w$ values on Porasil C silylated with HMDS + TMSCl and impregnated with 5% (w/w) oleyl alcohol. The absolute values of the slopes of the graphs of log k' against C, obtained with the latter column, where larger than those found for the two DClDMS silylated columns (Table V). With all three Porasil C columns, however, the absolute values of the slopes of these graphs were much larger than those found in the RP-TLC experiments. Also, the $\log k'_w$ values of the benzodiazepines obtained with the silylated and oleyl alcohol-coated Porasil C columns did not correlate very well with the log $P_{\text{oleyl alcohol}}$ values and the R_{M_w} (1.25% oleyl alcohol) values (Table VI, eqns. 7 and 8). These differences may be due to adsorption of the benzodiazepines on to the residual silanol groups of the Porasil C surface.

Log k'_w values, obtained with a column that is packed with an oleyl alcohol-

---

* Using an oleyl alcohol (5%)-coated Porasil C (silylated) column and 50% (w/w) methanol–water mixture as the mobile phase, the mean log k' for diazepam was found to be 0.055, with a standard deviation of 0.009; in an RP-TLC experiment [six plates, 1.25% oleyl alcohol, 30% (w/w) methanol–water] the mean R_M value for diazepam, which was spotted twice on each plate, was 0.124, with a standard deviation of 0.030.

** $\log k' = \log \dfrac{t_R - t_0}{t_0} = \log P + \log r.$

TABLE V

SLOPES (b'') AND INTERCEPTS (LOG k'_w) OF THE GRAPHS OF LOG k'_w AGAINST METHANOL CONCENTRATION IN THE MOBILE PHASE FROM HPLC EXPERIMENTS WITH FIVE DIFFERENT COLUMN PACKING MATERIALS

n = number of determinations; s = standard deviaton.

Compound	*Porasil C (3% oleyl alcohol)**					*Porasil C (5% oleyl alcohol)**					*Porasil C (5% oleyl alcohol)***				
	n	Log k'_w	s	b''	s	n	Log k'_w	s	b''	s	n	Log k'_w	s	b''	s
Medazepam	9	3.73	0.12	−0.0548	0.0022										
Prazepam	15	2.81	0.026	−0.0439	0.0005						7	3.33	0.080	−0.0530	0.0014
Flurazepam	14	3.03	0.055	−0.0487	0.0011	11	3.11	0.039	−0.0494	0.0009	7	3.40	0.12	−0.0500	0.0021
Diazepam	18	2.08	0.009	−0.0360	0.0002	22	2.17	0.013	−0.0367	0.0003	12	2.74	0.029	−0.0420	0.0006
Chlordiazepoxide	25	1.82	0.006	−0.0336	0.0001	24	1.91	0.017	−0.0343	0.0004	16	2.36	0.014	−0.0380	0.0003
Lorazepam	19	1.72	0.023	−0.0344	0.0006	21	1.82	0.023	−0.0368	0.0005	19	2.36	0.014	−0.0407	0.0003
Oxazepam	20	1.62	0.007	−0.0328	0.0002	25	1.73	0.019	−0.0349	0.0004	19	2.24	0.016	−0.0378	0.0003
Clonazepam	21	1.47	0.010	−0.0324	0.0003						20	2.05	0.014	−0.0375	0.0003
Temazepam	22	1.67	0.007	−0.0326	0.0002						17	2.30	0.019	−0.0381	0.0004
Flunitrazepam	20	1.41	0.010	−0.0308	0.0003						19	1.95	0.015	−0.0361	0.0003
Nitrazepam	17	1.26	0.008	−0.0288	0.0002	19	1.39	0.016	−0.0312	0.0004	18	1.86	0.011	−0.0341	0.0002
Bromazepam	14	1.04	0.008	−0.0273	0.0002						13	1.55	0.054	−0.0281	0.0011

Compound	*C18/Corasil (untreated)*					*C18/Corasil** (silylated)*				
	n	Log k'_w	s	b''	s	n	Log k'_w	s	b''	s
Medazepam	7	4.16	0.034	−0.0535	0.0006	11	4.58	0.069	−0.0663	0.0012
Prazepam	12	3.92	0.026	−0.0578	0.0005	15	4.20	0.045	−0.0647	0.0008
Flurazepam	9	4.56	0.068	−0.0633	0.0012	16	4.46	0.036	−0.0694	0.0007
Diazepam	12	3.14	0.035	−0.0603	0.0007	23	3.24	0.015	−0.0549	0.0003
Chlordiazepoxide	16	2.95	0.023	−0.0480	0.0005	23	2.89	0.019	−0.0515	0.0004
Lorazepam	18	2.82	0.014	−0.0505	0.0003	23	2.88	0.015	−0.0553	0.0003
Oxazepam	14	2.53	0.023	−0.0450	0.0005	23	2.69	0.013	−0.0514	0.0003
Clonazepam	16	2.54	0.017	−0.0501	0.0004	20	2.50	0.018	−0.0522	0.0005
Temazepam						21	2.86	0.017	−0.0528	0.0004
Flunitrazepam	19	2.72	0.017	−0.0521	0.0004	21	2.58	0.019	−0.0531	0.0005
Nitrazepam	19	2.35	0.011	−0.0464	0.0003	21	2.26	0.018	−0.0475	0.0004
Bromazepam	16	2.35	0.031	−0.0473	0.0008	17	2.20	0.017	−0.0504	0.0005

* Silylated with DCIDMS.

** Silylated with HMDS + TMSCl.

coated Kieselguhr G (of low surface area) will possibly give a better correlation with log $P_{\text{oleyl alcohol}}$ and R_{M_W} values from RP-TLC. The only advantage of the use of such a column over the PR-TLC technique would be the possibility of obtaining more precise values of the relative partition coefficients. The R_{M_W} values from RP-TLC measurements, however, can be determined with a precision that is sufficient for most QSAR studies, as the measurements of the biological activity of the compounds usually have much larger standard deviations than those of the physicochemical constants. A definite drawback of the use of column packing materials, which are coated with a solvent to serve as the stationary phase, is the need to use pre-columns, coated with that solvent. Without pre-saturation of the mobile phase, the stationary phase would soon be stripped off the column. The lifetime of an oleyl alcohol-coated support phase is limited even when rigorous precautions are taken to prevent "bleeding" of the column because of oxidation. The flow-rate must be low, otherwise some of the stationary phase might be removed. These disadvantages are overcome by the use of chemically bonded stationary phases.

C18/Corasil and C18/Porasil B, both consisting of silica gel particles to which octadecyl chains have been chemically bonded, were used as the stationary phase in reversed-phase HPLC[8–10]. In the present investigation, a commercially available C18/Corasil column was used (filled with non-silylated C18/Corasil), as well as a column packed with C18/Corasil that had been silylated with HMDS + TMSCl. According to McCall[8], it is necessary to silylate the C18/Corasil packing in order to block active silanol sites that might interfere with the desired liquid–liquid partition process. This seemed to be confirmed by the present investigations. The log k'_w value and the slopes of the graphs of log k' against methanol concentration in the mobile phase are presented in Table V. Graphs of log k' against C (silylated C18/Corasil) are shown in Fig. 3. The silylated C18/Corasil column yielded log k'_w values that correlated better with log $P_{\text{oleyl alcohol}}$ than the log k'_w values from the untreated C18/Corasil column (Table VI, eqns. 10 and 11). However, log k'_w values from the silylated C18/Corasil column did not correlate as well with log $P_{\text{oleyl alcohol}}$ and log P_{octanol} as did the R_{M_W} (1.25% oleyl alcohol) values from the RP-TLC measurements with log $P_{\text{oleyl alcohol}}$ and log P_{octanol} (Table VI, eqns. 11, 12 and 3, 4). The larger deviations, when comparing the graph of log k'_w (silylated C18/Corasil) against log $P_{\text{oleyl alcohol}}$ with the graph of R_{M_W} (1.25% oleyl alcohol) against log $P_{\text{oleyl alcohol}}$, are clearly visible in Fig. 4. Again, the presence of residual surface silanol groups may cause adsorption of the benzodiazepines on to the support phase, and also some adsorption may occur on the bonded phase itself[23]. It should also be noted that the silyl ether terminating the octadecyl chain where it is bonded to the Corasil surface is less polar than the hydroxyl group of an alcohol and (probably more important) has no hydrogen bond donating properties as do the alcohols. Correlations between log k'_w and log P_{octanol} can therefore be expected to be less good when compounds with hydrogen bond donating as well as compounds with hydrogen bond accepting properties are included[22]. This is the case for the benzodiazepines in the present investigation. McCall[8] calculated log P_{octanol} values of minoxidil analogues directly from the log k' values of these compounds using a silylated C18/Corasil column and 1% triethylamine (TEA) in water. The log P_{octanol} values were calculated by adding a term log K to the log k' values, log K being equal to the difference between the values for benzene of log P_{octanol} and log k' (1% TEA in water). However, when the slope (a) of the Collander

TABLE VI

REGRESSION EQUATIONS CORRELATING LOG $P_{\text{oleyl alcohol}}$, R_{M_W}, LOG k'_w AND LOG P_{octanol} VALUES OF BENZODIAZEPINES

The values in parentheses are the standard deviations. n = number of compounds; r = correlation coefficient; s = standard deviation of correlation.

*Regression equation**	n	r	s	*Eqn.*
Log $P_{\text{oleyl alcohol}}$ = 1.024 (± 0.082) log P_{octanol} − 0.338 (± 0.224)	7	0.984	0.151	1
R_{M_W} (4% oleyl alcohol) = 1.039 (± 0.022) R_{M_W} (1.25% oleyl alcohol) + 0.460 (± 0.019)	8	0.999	0.033	2
R_{M_W} (1.25% oleyl alcohol) = 1.029 (± 0.033) log $P_{\text{oleyl alcohol}}$ − 1.472 (± 0.079)	8	0.997	0.064	3
R_{M_W} (1.25% oleyl alcohol) = 1.127 (± 0.075) log P_{octanol} − 1.991 (± 0.217)	9	0.985	0.173	4
Log k'_w (Porasil, 3%)** = 1.025 (± 0.008) log k'_w (Porasil, 5%)** − 0.151 (± 0.018)	6	1.000	0.011	5
Log k'_w (Porasil, 3%)** = 1.056 (± 0.044) log k'_w (Porasil, 5%)*** − 0.698 (± 0.106)	11	0.992	0.080	6
Log k'_w (Porasil, 3%)** = 0.887 (± 0.117) log $P_{\text{oleyl alcohol}}$ − 0.122 (± 0.284)	8	0.951	0.231	7
Log k'_w (Porasil, 3%)** = 0.924 (± 0.083) R_{M_W} (1.25% oleyl alcohol) + 1.068 (± 0.109)	11	0.966	0.230	8
Log k'_w (C18/Corasil, untreated) = 0.865 (± 0.054) log k'_w (C18/Corasil, silylated) + 0.383 (± 0.176)	11	0.983	0.150	9
Log k'_w (C18/Corasil, untreated) = 0.920 (± 0.220) log $P_{\text{oleyl alcohol}}$ + 0.975 (± 0.546)	7	0.881	0.423	10
Log k'_w (C18/Corasil, silylated) = 1.029 (± 0.165) log $P_{\text{oleyl alcohol}}$ + 0.745 (± 0.399)	8	0.930	0.326	11
Log k'_w (C18/Corasil, silylated) = 1.119 (± 0.109) log P_{octanol} + 0.142 (± 3.14)	9	0.968	0.251	12
Log k'_w (C18/Corasil, silylated) = 0.929 (± 0.115) R_{M_W} (1.25% oleyl alcohol) + 2.210 (± 0.151)	11	0.938	0.318	13

* Log P_{octanol} values are taken from ref. 12.

** Silylated with DCIDMS.

*** Silylated with HMDS + TMSCl.

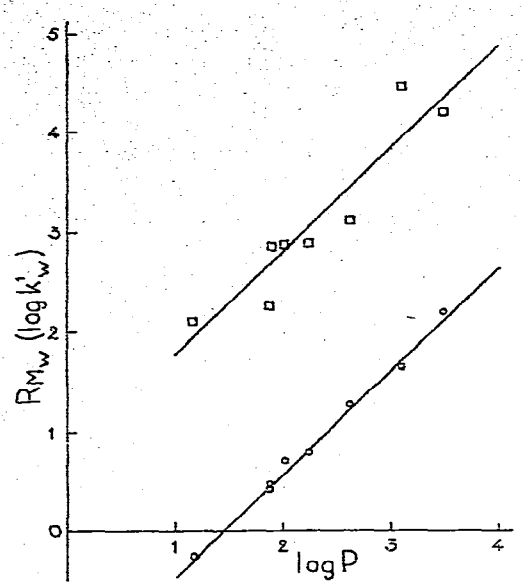


Fig. 4. Graphs of R_{M_w} and log k'_w against log $P_{\text{oleyl alcohol}}$ for eight benzodiazepines. The R_{M_w} values are those found by RP-TLC with 1.25% (v/v) oleyl alcohol in the impregnating mixture. The log k'_w values were obtained with silylated C18/Corasil.

equation correlating log k' with log P_{octanol} is not equal to unity, log K will not have be constant but will depend on the value of a and the log k' value:

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

and therefore

$$\log P_{\text{octanol}} - \log k' = \log k' \left(\frac{1}{a} - 1\right) - \frac{b}{a}$$

When an aqueous mobile phase is used with little or no organic solvent added to it, the value of a will be close to unity[22] (see also Table VI, eqns. 11 and1 2). Serious deviations can be expected when mixtures of water and an organic solvent are used as the mobile phase, because the differences in the log k' values of the compounds, and therefore the value of a, have a tendency to become smaller at higher concentrations of the organic solvent (Fig. 3). When dealing with the more lipophilic minoxidil analogues, McCall[8] measured their k' values using 1% TEA in 40% methanol–water. The k' values for the 1% TEA solution in water were calculated by measuring k' of one "calibrating" compound with both mobile phases, and by subsequently multiplying the k' values of the other compounds measured in the 40% methanol–water mixture by the ratio of the k' values of the calibrating compound. When applying this procedure, it was assumed by McCall[8] that this ratio would be constant for a class of closely related compounds. The graphs of log k' *versus* methanol concentration of the mobile phase are thus expected to be parallel. It is clear (Fig. 3) that this assumption is not valid for the (closely related) benzodiazepines. It would seem to be

safer, therefore, to determine log k' values at different methanol concentrations (C) and to extrapolate the graphs of log k' against C to zero methanol concentration. This procedure has also been recommended by Tomlinson[1] and Biagi *et al.*[3]. From the results of the present investigation, it is clear that the RP-TLC technique, with oleyl alcohol-coated Kieselguhr G, is the method of choice when one is interested in obtaining ΔR_M values, comparable with $\Delta \log P_{\text{octanol}}$ values, for use in QSAR studies. Chemically bonded stationary phases for HPLC experiments can possibly be developed with a hydroxyl group in the alkyl chain; such phases might yield log k'_w values that correlate better with log P_{octanol} than those presently obtained with octadecyl chains.

REFERENCES

1 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
2 E. Tomlinson and J. C. Dearden, *J. Chromatogr.*, 106 (1975) 481.
3 G. L. Biagi, O. Gandolfi, M. C. Guerra, A. M. Barbaro and G. Cantelli-Forti, *J. Med. Chem.*, 18 (1975) 868.
4 G. L. Biagi, A. M. Barbaro, O. Gandolfi, M. C. Guerra and G. Cantelli-Forti, *J. Med. Chem.*, 18 (1975) 873.
5 M. C. Bonjean, J. Alary and M. C. Luu Duc, *Chim. Ther.*, (1973) 93.
6 A. E. Bird and A. C. Marshall, *J. Chromatogr.*, 63 (1971) 313.
7 C. B. C. Boyce and B. V. Millborow, *Nature (London)*, 208 (1965) 537.
8 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
9 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
10 W. J. Haggerty and E. A. Murrill, *Res. Develop.*, 25 (1974) 30.
11 A. Hulshoff and J. H. Perrin, *J. Chromatogr.*, 120 (1976) 65.
12 W. Müller and U. Wollert, *Naunyn-Schmiedebergs Arch. Pharmakol.*, 278 (1973) 301.
13 L. Z. Benet and J. E. Goyan, *J. Pharm. Sci.*, 54 (1965) 1179.
14 A. Hulshoff and J. H. Perrin, *Pharm. Acta Helv.*, 51 (1976) 65.
15 R. Munier and M. Macheboeuf, *Bull. Soc. Chim. Biol.*, 31 (1949) 1144.
16 J. J. Kirkland, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971, Ch. 5, p. 176.
17 W. Müller and U. Wollert, *Naunyn-Schmiedebergs Arch. Pharmakol.*, 283 (1974) 67.
18 K. S. Murthy and G. Zografi, *J. Pharm. Sci.* 59 (1970) 1281.
19 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.
20 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
21 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.
22 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
23 R. E. Majors and M. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.