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Reversed-phase high-performance liquid chromatography for evaluating the distribution of pharmaceutical substances in suppository base–phosphate buffer system

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Abstract

The aim of this study was to assess the potency of the reversed-phase high-performance liquid chromatography (RP-HPLC) for in vitro evaluation of the distribution behavior of common drugs between one of the generally used suppository bases Witepsol H_{15} and the rectal liquid which is imitated by a phosphate buffer, pH 7.2. The distribution coefficients (log K) of nine compounds — paracetamol, caffeine, diclofenac, propyphenazone, indomethacin, codeine base, codeine phosphate, phenobarbital acid and phenobarbital sodium salt were determined by the classical 'shake-flask' method followed by RP-HPLC quantitative assay. The capacity factors log k' of the compounds were determined on reversed-phase C_{18} column at a number of methanol–5 mM phosphate buffer, pH 7.2 mobile phases containing different percentages of methanol (ϕ_{MeOH}). The apparent capacity factors log k_w^{app} were derived by extrapolation of the methanol concentration to zero and using the correction for ionization, the real capacity factors log k'_w were calculated. The lipophilicity of the compounds was assessed by the partition coefficients CLOGP and the distribution coefficients $CLOGD_{7.2}$, calculated for the n-octanol–water system. Correlations between log k'_w and CLOGP, log k_w^{app} and $CLOGD_{7.2}$, log k_w^{app} and log K were found. The last correlation indicated that the parameter log k_w^{app} was suitable for evaluating the distribution behavior of the studied drugs in the examined Witepsol H_{15}-rectal liquid system. The predictive power of this correlation was tested by a set of nine non-congeners. It was shown that the classical 'shake-flask' method for determination of the distribution behavior of the studied drugs between the suppository base Witepsol H_{15} and the phosphate buffer, pH 7.2 might be replaced by the RP-HPLC technique due to its priorities of rapid, stable and reproducible experiments.

Keywords: 'Shake-flask' method; Distribution coefficient log K for Witepsol H_{15}–phosphate buffer system; Capacity factors log k', log k'_w and log k_w^{app}; Partition coefficient CLOGP; Distribution coefficient $CLOGD_{7.2}$

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1. Introduction

Suppositories are useful dosage forms due to certain advantages in the local therapy and the systemic effect of drugs in the case of contraindicated and difficult oral administration. In order to release the formulation easily the drug should possess a low affinity to the suppository base. Therefore, the knowledge of the distribution coefficient of the drug in the suppository base–rectal liquid system is important for predicting the drug release. The determination of the distribution coefficients by the traditional 'shake-flask' method [1] is conventional but is laborious, time consuming and lacks purity, stability and mass balance. An effective alternative technique for rapid and reproducible assessment of the lipophilicity of organic solutes and their distribution behavior is the reversed-phase high-performance liquid chromatography (RP-HPLC). A good correlation between the chromatographic retention data ($\log k'$) and the octanol–water partition coefficients ($\log P$) were found [2–11] and the evaluated lipophilicity correlates well with the absorption, distribution and biological activity of the compounds. However, little is known about the utility of the RP-HPLC method in determining the drug distribution coefficients between suppository base and rectal liquid.

The aim of the present study was to assess the potency of RP-HPLC technique for in vitro evaluation of the distribution behavior of common drugs between the generally used Witepsol H_{15} suppository base and the rectal liquid imitated by a phosphate buffer, pH 7.2. The set of the studied compounds (Table 1) included paracetamol, caffeine, diclofenac, propyphenazone, indomethacin, codeine (as a base and as a phosphate) and phenobarbital (as an acid and as a sodium salt) which are widely used for suppository formulations either individually or in combination with different drugs. Further the relationships between the different lipophilicity parameters derived by 'shake-flask' method ($\log K$), by HPLC ($\log k'_w$ and $\log k_w^{app}$) or calculated (CLOGP and $CLOGD_{7.2}$) were investigated in order to prove the priority of the chromatographic technique for determination of the distribution behavior of drugs in suppository base–rectal liquid system.

2. Experimental

2.1. Materials

The drug substances were obtained from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). HPLC-grade methanol and analytical-reagent grade dipotassium hydrogenphosphate, sodium dihydrogenphosphate and orthophosphoric acid were provided by Merck (Darmstadt, Germany). The suppository base Witepsol H_{15} was purchased from Nobel Dynamite (Witen Werke, Germany).

2.2. Chromatographic apparatus and conditions

A chromatographic system (Varian, USA) consisting of tertiary pump Model 9012, Rheodyne injector with a 10 μl loop and UV-VIS detector Model 9050 set at 254 nm was used. The Varian Star Chromatography workstation and computer software (version 4.5) were utilized for controlling the HPLC system and collecting the data. A cartridge column (LiChroCART 250×4 mm, Merck) packed with endcapped material LiChrospher 100 RP-18, 5 μm was used and maintained at room temperature. The mobile phases were prepared from 5 mM phosphate buffer, pH 7.2 (adjusted with 1 M H_3PO_4) and modified with different percentages of methanol.

The eluents were filtered through a 0.45 μm filter (Millipore), degassed in ultrasonic bath before use and delivered isocratically at a flow rate of 1 ml/min. The retention time t_r of the compounds was determined with three replicate injections of each sample and the capacity factors k' were calculated for every composition of the mobile phase as $(t_r - t_0)/t_0$, where t_r is the retention time of the studied compound and t_0 is the retention time of an unretained compound, defined as the time from an injection to the first distortion on the baseline.

2.3. Determination of capacity factors (log k', log k_w^{app} and log k'_w)

Chromatographic retention data was obtained measuring the capacity factors k' for each com-

Table 1
Values of all experimental and calculated parameters used in the present study

Compound	log K	log k_w^{app}	S	log k'_w	pK_a	CLOGP	CLOGD$_{7.2}$	Density (g/cm^3)	Surface tension (dyne/cm)
Paracetamol	−0.9032	0.444	−0.017	0.444	10.82	0.34	0.34	1.249	52.8
Caffeine	−0.8127	0.952	−0.022	0.952	1.39	−0.07	−0.07	1.450	55.7
Diclofenac	0.0424	4.190	−0.063	7.210	4.18	3.28	0.26, 3.28[a]	1.431	57.9
Propyphenazone	0.5513	2.745	−0.039	2.745	2.37	1.74	1.74	1.081	38.5
Indomethacin	0.0600	4.371	−0.063	7.220	4.17	3.10	0.07, 3.10[a]	1.320	47.4
Codeine base	−0.8943	2.276	−0.032	3.308	8.19	1.83	0.80	1.340	60.5
Phenobarbital acid	−0.3004	1.961	−0.037	2.098	7.63	1.71	1.57	1.233	43.3
Phenobarbital sodium	−0.2892	1.942	−0.037	2.079	7.63	1.71	1.57	1.233	43.3
Codeine phosphate	−0.8927	2.400	−0.035	3.432	8.19	1.83	0.80	1.340	60.5

[a] Distributed as ion-pair.

pound at 0.5 increment of the methanol (ϕ_{MeOH}) in aqueous phosphate buffer, pH 7.2 mobile phases. In order to optimize the retention time of the solutes the volume fraction ϕ_{MeOH} was varied within different ranges: $0.25 \leq \phi_{MeOH} \leq 0.75$ for paracetamol, caffeine, phenobarbital acid and phenobarbital sodium, $0.35 \leq \phi_{MeOH} \leq 0.75$ for codeine base, codeine phosphate and propyphenazon and $0.45 \leq \phi_{MeOH} \leq 0.75$ for diclofenac and indomethacin. The data obtained was utilized for calculation of $\log k'$ and to derive values of $\log k_w^{app}$ (intercept) and S (slope) from the plot $\log k'$ versus ϕ_{MeOH}. The correlation coefficients, R, were > 0.97 for all compounds indicating that $\log k'$ varies linearly with ϕ_{MeOH} within the examined ranges of the eluent composition. Log k_w^{app} is the logarithm of the apparent capacity factor extrapolated for 100% aqueous phosphate buffer, pH 7.2 mobile phase. Using the correction for ionization $\log k'_w$ values were calculated, according to the following equations:

$$\log k'_w = \log k_w^{app} + \log (1 + 10^{pH - pK_a}) \quad \text{for acids}$$

$$\log k'_w = \log k_w^{app} + \log (1 + 10^{pK_a - pH})$$

for bases

The $\log k'_w$ value is the logarithm of the capacity factor of the non-ionized compound extrapolated for 100% aqueous mobile phase.

2.4. Determination of distribution coefficients (log K) in Witepsol H_{15}–phosphate buffer, pH 7.2 system

The distribution coefficients (K) of the studied pharmaceutical substances in Witepsol H_{15}–phosphate buffer, pH 7.2 system were determined by a traditional 'shake-flask' method according to Ibrahim et al. [1]. The buffered aqueous medium was prepared by mixing the appropriate volumes of 0.1 M NaH_2PO_4, 0.1 M K_2HPO_4 and 0.1 M H_3PO_4 to obtain the working 6.6 mM phosphate buffer, pH 7.2. Initially, buffered standard solutions of the drugs were prepared in a proper concentration range varying from 2.10^{-3} to 6.10^{-5} M in order to obtain linear response of UV absorption versus concentration for each compound. The measurments were performed using UV-VIS diode-array spectrophotometer (Hewlett Packard-HP8452) within 220–360 nm against 6.6 mM phosphate buffer, pH 7.2 as a blank solution. According to the preliminary assessment of the concentrations an appropriate amount of each compound was dissolved in 15 ml buffered aqueous solution. The solution was saturated with Witepsol H_{15} and added to 5 ml melted suppository base prior saturated with the phosphate buffer. The samples were placed in well-closed flasks and were shaken for about 12 h in a thermostated at 37 ± 1°C water bath (Vibrotherm L204, Hungary). The flasks were removed and left in a vertical position for 1 h at 37°C to allow the separation of the two immiscible phases, then cooled to room temperature, and frozen to solidify the suppository base. The two phases were separated using a filter paper and the aqueous buffered phase was additionally passed through 0.45 μm filter. The amount of the distributed drug in the aqueous phase was determined by RP-HPLC utilizing the method of the external standard. The applied mobile phase of methanol–aqueous phosphate buffer, pH 7.2 was modified either with 50 or 70% methanol in order to optimize the analysis time. The eluent with 50% methanol was used for determination of paracetamol, codeine base and codeine phosphate but for more lipophilic compounds like caffeine, diclofenac, propyphenazone, indomethacin, phenobarbital acid and phenobarbital sodium salt the proportion of methanol was increased to 70%. The unknown concentration of the drug in the aqueous phase C_u was calculated according to the equation:

$$C_u = C_s h_u / h_s$$

where C_s is the concentration of the external standard, h_s is the peak height of the external standard and h_u is the peak height of the compound assayed.

The distribution coefficient K was calculated according to the equation:

$$K = (C - C_u)/C_u$$

where C is the concentration of the drug in the aqueous phase before distribution. The determined distribution coefficients K were used in their logarithmic form, log K.

2.5. Calculation of pK_a, CLOGP and $CLOGD_{7.2}$

pK_a values and octanol–water partition coefficients CLOGP were calculated by the computer program ACD/Labs [12]. Using the correction for ionization the distribution coefficients $CLOGD_{7.2}$ were calculated.

2.6. Calculation of some physical properties

A set of physical properties including molecular mass M_m, molar refractivity MR, molar volume MV, parachor P, surface tension SURF, density DEN and polarizability POL was calculated using ACD/Labs [12]. These parameters were included in the regression equation after step-wise procedure by forward selection (adding further significant variables according to their contribution to the model) to improve the relationships between the operated lipophilicity parameters in the study. The correlations between the parameters were analyzed by the linear regression program in the SYSTAT statistical package [13].

3. Results and discussion

The values of all experimentally derived and calculated parameters used in this study are presented in Table 1. Low log K values indicate the rapid drug release from the suppository formulation. Thus, the Witepsol H_{15} suppository base is appropriate for phenobarbital, caffeine, codeine and paracetamol. Their log K values were below zero that is indicative for the low affinity of these drugs towards Witepsol H_{15}. The close values of log K for codeine base and codeine phosphate as well as for phenobarbital sodium salt and phenobarbital acid indicated that the chemical form and the accompanying ion was not of significance for the distribution behavior of these compounds.

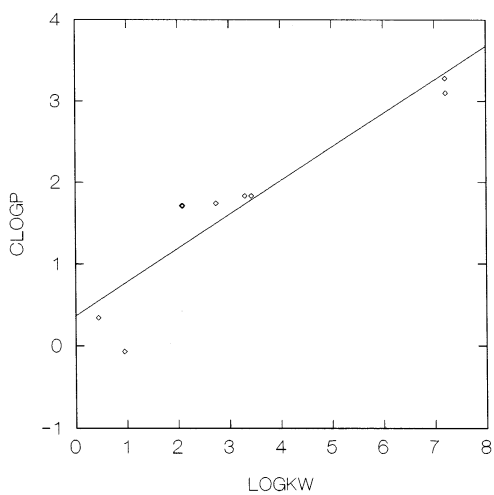

Fig. 1. Graph of CLOGP versus log k'_w ($n = 9$).

3.1. Correlation between log k_w^{app} and S

Although the compounds investigated were non-congeners and their slopes S differed, a correlation between log k_w^{app} and S was found (Eq. (1)):

$$\log k_w^{app} = 0.804(0.068)S - 0.718(0.279) \quad (1)$$

$$n = 9, \qquad R = 0.976,$$

$$s = 0.303 \quad \text{and} \quad F_{1,7} = 140.926.$$

where n is the number of data points, R is the correlation coefficient, s is the overall standard deviation of the regression, $F_{k,m}$ is Fisher's criterion of significance at k and m degrees of freedom. The standard deviations of the slope and intercept are shown in parentheses.

The good correlation of log k_w^{app} and the slope S allows the use of both the parameters for assessment of lipophilicity by the RP-HPLC method.

3.2. Correlation between log k'_w and CLOGP

The HPLC derived parameter log k'_w can be considered as a lipophilicity parameter independent of pH and analogous to the conventional octanol-water partition coefficient log P. Since both the parameters are related to the partition of the non-ionized molecules between polar and non-polar phases one can assume their correlation. For the studied set of drugs the following equation was found:

$$\text{CLOGP} = 0.414(0.063)\log k'_w + 0.364(0.253) \quad (2)$$

$$n = 9, \quad R = 0.963, \quad s = 0.436, \quad F_{1,7} = 42.851$$

The graph of CLOGP versus log k'_w is shown in Fig. 1. A good correlation exists between these

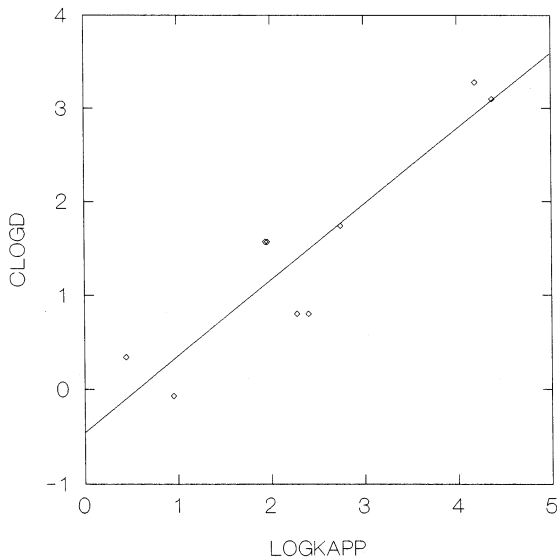

Fig. 2. Graph of $CLOGD_{7.2}$ versus $\log k_w^{app}$ ($n = 9$).

parameters. In recent literature there have been several studies on the relationship between log P and retention in a reversed-phase system for non-congeners [3–5].

3.3. Correlation between log k_w^{app} and $CLOGD_{7.2}$

The parameters $\log k_w^{app}$ and $CLOGD_{7.2}$ express the distribution of the non-ionized molecules between polar, pH 7.2 and non-polar phases. An attempt to correlate $\log k_w^{app}$ and $CLOGD_{7.2}$ for all nine compounds was unsuccessful. The analysis of the retention times indicated that indomethacin and diclofenac manifested higher retention times than expected for compounds with such a degree of ionization.

Inagi et al. [14] have demonstrated that indomethacin tends to form ion-pairs with monovalent cations (K^+, Na^+, NH_4^+) in pH-range higher than its pK_a value passing easily into the n-octanol phase as a neutral associate. In this case, the experimentally determined log P value in n-octanol–water system was higher than the calculated CLOGP. One can assume that the observed longer retention time for indomethacin in the study was due to the ion-pair formation with the potassium ions of the phosphate buffer eluent. Being a weak acid structurally related to indomethacin, the distribution behavior of diclofenac was presumed to be close to that of indomethacin. Therefore, in cases like this the experimentally determinated $\log k_w^{app}$ was more appropriate than the calculated distribution coefficient $CLOGD_{7.2}$ for the assessment of lipophilicity.

Assuming the distribution of diclofenac and indomethacin as ion-pairs their $CLOGD_{7.2}$ values were considered as equal to CLOGP. Thus, a good correlation between $CLOGD_{7.2}$ and $\log k_w^{app}$ was found:

$$CLOGD_{7.2} = 0.811(0.133)\log k_w^{app} - 0.458(0.354) \quad (3)$$

$$n = 9, \quad R = 0.958, \quad s = 0.489, \quad F_{1,7} = 37.257$$

The graph of $CLOGD_{7.2}$ versus $\log k_w^{app}$ is presented in Fig. 2. The exclusion of indomethacin and diclofenac from the examined set gave a linear regression with low R and F values (Eq. (4)):

$$CLOGD_{7.2} = 0.604(0.258)\log k_w^{app} - 0.132(0.509) \quad (4)$$

$$n = 7, \quad R = 0.850, \quad s = 0.522, \quad F_{1,5} = 5.458$$

In order to improve the regression a step-wise procedure by forward selection among of the physical properties was performed and thus Eq. (5) was generated:

$$CLOGD_{7.2} = 0.391(0.188)\log k_w^{app} - 3.634(1.338)DEN + 4.889(1.879) \quad (5)$$

$$n = 7, \quad R = 0.955, \quad s = 0.346, \quad F_{2,4} = 9.901$$

The density ($DEN = M_m/MV$) reflects the molecular mass and volume simultaneously. The negative value of the regression coefficient in front of DEN indicates that the compounds with lower DEN will possess higher $CLOGD_{7.2}$ and vise versa.

3.4. Correlation between log K and lipophilicity parameters and physical properties

The Pearson correlation matrix for log K, lipophilicity parameters ($\log k'_w$. $\log k_w^{app}$, CLOGP

and $CLOGD_{7.2}$) and physical properties (M_m, MR, MV, P, SURF, DEN and POL) is given in Table 2. Log K correlated with $\log k_w^{app}$ ($R = 0.650$) from the set of lipophilicity parameters and with SURF ($R = -0.755$) from the set of calculated physical properties. Both correlations were weak ($R \leq 0.8$) but the fact that $\log k_w^{app}$ and SURF did not intercorrelate ($R = -0.177$) permitted us to combine them in one regression equation (Eq. (6)):

$$\log K = 0.217(0.066)\log k_w^{app} - 0.040(0.010)\text{SURF} + 1.200(0.571) \quad (6)$$

$$n = 9, \quad R = 0.959, \quad s = 0.240, \quad F_{2,6} = 16.471$$

Eq. (6) showed that $\log k_w^{app}$ was an appropriate parameter for evaluating the affinity of compounds towards Witepsol H_{15} suppository base and higher $\log k_w^{app}$ values corresponded to higher distribution coefficients ($\log K$). Moreover, the last model indicated that the surface tension of the studied molecules was of importance for their distribution behavior in the investigated system of Witepsol H_{15}–phosphate buffer. The surface tension is a physical property reflecting the possibility of a molecule to take part in polar intermolecular interactions. The negative value of the regression coefficient in front of SURF indicated that molecules with high values of surface tension demonstrated lower affinity to the lipophilic Witepsol H_{15}. The correlation, expressed by Eq. (6), is presented in a three-dimensional plot (Fig. 3).

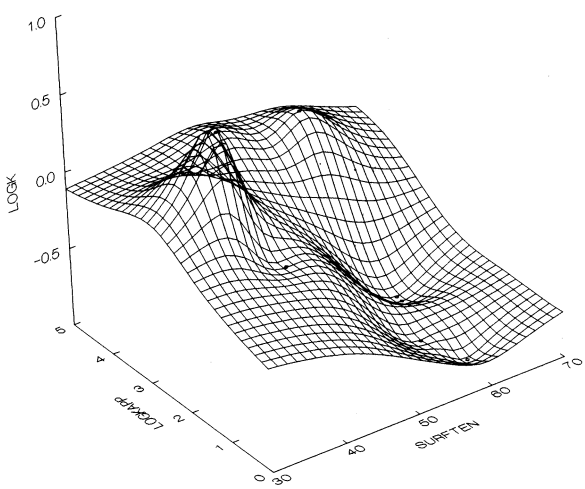

Fig. 3. Three-dimensional plot of the correlation between the distribution coefficient $\log K$ (LOGK), $\log k_w^{app}$ (LOGKAPP) and surface tension (SURFTEN).

3.5. Verification of the predictive power of the correlation between log K, log k_w^{app} and surface tension

In order to verify the predictive power of Eq. (6) a test set of nine non-congeners was chosen (Table 3). Reported data for their release rate from the suppository base Witepsol H_{15} was found in the literature but there was no data for the distribution coefficients in Witepsol H_{15}–phosphate buffer system with the exception of oxyphenbutazone. For the calculation of $\log k_w^{app}$ values Eq. (5) was preferred to Eq. (4) because the former equation was based on the actual values of $CLOGD_{7.2}$.

The distribution coefficients ($\log K$) were calculated according to Eq. (6). The obtained K values below 1.0 indicated a low affinity of the compound to the suppository base and rapid release from it but the values higher than 1.0 indicated high affinity of the drug to Witepsol H_{15} and slow release. The calculated K values were in a good agreement with the data obtained from the literature. Propranolol tends to form ion-pair like indomethacin and diclofenac and thus pass into non-polar phases [15–17]. Therefore, it might be concluded that the affinity to Witepsol H_{15} base of compounds possessing the tendency to form ion-pairs can not be predicted only by calculating $CLOGD_{7.2}$ values but experimentally derived $\log k_w^{app}$ values are needed as well.

In addition, the small difference between the calculated and the experimentally derived K values for oxyphenbutazone can be ascribed to the different methods of quantitative determination of the compound after the 'shake-flask' procedure. According to Ibrahim et al. [1] the amount of oxyphenbutazone was determined spectrophotometrically while in the present study the concentration of the compounds was assayed by the RP-HPLC method.

Table 2
Pearson correlation matrix for log K, lipophilicity parameters and physical properties

	log K	log k'_w	log k_w^{app}	CLOGP	$CLOGD_{7.2}$	M_m	MR	MV	PAR	SURF	DEN	POL
log K	1.000	–	–	–	–	–	–	–	–	–	–	–
log k'_w	0.493	1.000	–	–	–	–	–	–	–	–	–	–
log k_w^{app}	0.650	0.969	1.000	–	–	–	–	–	–	–	–	–
CLOGP	0.579	0.927	0.954	1.000	–	–	–	–	–	–	–	–
$CLOGD_{7.2}$	0.365	−0.296	−0.085	0.057	1.000	–	–	–	–	–	–	–
M_m	0.263	0.869	0.867	0.846	−0.155	1.000	–	–	–	–	–	–
MR	0.276	0.825	0.841	0.808	−0.105	0.980	1.000	–	–	–	–	–
MV	0.464	0.787	0.860	0.851	0.130	0.937	0.955	1.000	–	–	–	–
PAR	0.329	0.808	0.846	0.842	0.019	0.974	0.990	0.983	1.000	–	–	–
SURF	−0.755	0.033	−0.177	−0.158	−0.675	0.064	0.067	−0.215	−0.036	1.000	–	–
DEN	−0.480	0.304	0.115	0.040	−0.797	0.271	0.168	−0.076	0.069	0.722	1.000	–
POL	0.276	0.825	0.841	0.808	−0.105	0.980	1.000	0.955	0.990	0.067	0.167	1.000

Table 3
Verification of the predictive power of the correlation log K/log k_w^{app} and surface tension (Eq. (6))

Compound	pk_a	CLOGP	CLOGD$_{7.2}$	DEN	SURF	log k_w^{app} [a]	log K [b]	K	Release from Witepsol H$_{15}$ [c]
Morphine	8.14	1.06	0.073	1.440	72.8	1.066	−1.481	0.033	Rapid [18]
Propranolol	9.14	3.10	1.155, 3.100[d]	1.093	42.6	0.609, 5.583[d]	−0.372, 0.708[d]	0.425, 5.100[d]	Slow [19] (ion-pair formation)
Chlorpromazine	9.30	5.36	3.257	1.212	46.6	7.090	0.874	7.482	Slow [20]
Theophiline	9.93	0.06	0.059	1.465	67.6	1.263	−1.230	0.059	Rapid [21]
Oxyphen-butazone	11.18	3.03	3.030	1.241	52.9	6.780	0.555	3.589	3.81 [1]
Antipyrine	1.21	0.27	0.270	1.156	42.7	−1.069	−0.740	0.182	Rapid [22]
Chloroquine	10.48, 6.33	4.69	1.331	1.111	43.9	1.225	−0.290	0.513	Rapid [23]
Apornorphine	9.41, 6.50	3.09	3.008	1.299	58.2	7.263	0.448	2.805	Slow [24]
5-Flourouracil	7.95	−0.78	−0.851	1.530	46.1	−0.460	−0.744	0.180	Rapid [25]

[a] Calculated according to Eq. (5).
[b] Calculated according to Eq. (6).
[c] References are shown in parenthesis.
[d] Distributed as ion-pair.

4. Conclusions

The RP-HPLC derived $\log k_w^{app}$ reflects the ion-pair formation of the compounds and is therefore more appropriate as a parameter for the lipophilicity assessment than the calculated $CLOGD_{7.2}$ parameter is. Moreover, the combination of $\log k_w^{app}$ and the surface tension of the compounds is appropriate for evaluating the affinity to Witepsol H_{15} suppository base. The distribution coefficients of the compounds in Witepsol H_{15}–rectal liquid system ($\log K$) indicate that this suppository base is appropriate mainly for phenobarbital, caffeine, codeine and paracetamol from the studied drugs. The good correlations obtained between the lipophilicity parameters and the retention of the solutes in a reversed-phase system demonstrate the potency of HPLC technique for predicting the affinity of different drugs to any suppository base with many advantages over the traditional methods.

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