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Determination of lipophilicity by reversed-phase high-performance liquid chromatography
Influence of 1-octanol in the mobile phase

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Abstract

Lipophilicity was evaluated using a novel RP-HPLC stationary phase (Discovery-RP-Amide-C16) with and without 1-octanol added to the mobile phase. A set of 46 drugs and flavonoids characterized by a broad structural diversity and a wide $\log P_{oct}$ range (−0.69 to 5.70) was selected for this study. This set consists of neutral solutes and solutes with acidic or ampholytic functionalities which were maintained neutral at pH 2.5 or 4. In our conditions, the addition of 1-octanol in the mobile phase proved a key factor to derive a lipophilicity index $\log k_w$ highly correlated with $\log P_{oct}$ for all investigated solutes. 1-Octanol improved the correlation between $\log P_{oct}$ and $\log k_w$ mainly by influencing the retention behavior of the solutes with $\log P_{oct}$ values below +3. This study brings additional evidence that under proper experimental conditions of stationary and mobile phases, RP-HPLC is a very useful method to obtain $\log P_{oct}$ values.

Keywords: Lipophilicity; $\log P_{oct}$; $\log k_w$; Mobile phase; Stationary phase; 1-Octanol; LC

1. Introduction

Since many years, lipophilicity is recognized as a meaningful parameter in structure–activity and structure–ADME relationships. It is also the single most informative and successful physicochemical property in medicinal chemistry. Not only has lipophilicity found innumerable applications in quantitative structure–activity relationships (QSARs) and quantitative structure-pharmacokinetic relationships (QSPkRs), but its study has revealed a wealth of information on intermolecular forces, intramolecular interactions, and molecular structure in the broadest sense [1–4].

The most widely used measure of lipophilicity is the partition coefficient in the 1-octanol/water system (noted $\log P_{oct}$). The reference procedure to measure $\log P_{oct}$ is the shake-flask method, which however is time-consuming and limited in range (ca. $-3 < \log P < 4$). Beyond these limits, $\log P_{oct}$ values measured by the shake-flask method become unreliable.

The reversed-phase HPLC (RP-HPLC) method is a promising alternative to the shake-flask method, having such advantages as a higher throughput, an insensitivity to impurities or degradation products, and a broader lipophilicity range. In RP-HPLC, lipophilicity indices are derived from the capacity factor $\log k$, which is calculated by Eq. (1):

$$k = \frac{t_r - t_0}{t_0} \quad (1)$$

where t_r and t_0 are the retention times of the solute and of an unretained compound, respectively. Some workers have used isocratic $\log k$ values measured in an appropriate mobile phase as a lipophilicity parameter [5–7]. However, many more investigators have used capacity factors extrapolated

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to 100% water ($\log k_w$) to eliminate organic solvent effects [8–13], and they have indeed demonstrated the usefulness of the $\log k_w$ parameter when investigating series of solutes covering a broad lipophilicity range. Generally, the extrapolation to 100% water is based on a quadratic relationship between the isocratic capacity factor $\log k$ and the volume fraction of organic solvent in the mobile phase, φ [14]. When methanol is used as the organic modifier, a linear relationship (Eq. (2)) is often obtained for neutral solutes [15,16]:

$$\log k = -S\varphi + \log k_w \quad (2)$$

where S is the slope and $\log k_w$, the intercept of the regression curve.

Until recently, most lipophilicity studies were based on RP-HPLC octadecyl silica (ODS) stationary phases. The correlations between $\log P_{oct}$ and $\log k_w$ or $\log k$ values so obtained are usually good for structurally related solutes [15,17,18]. The decrease in correlation between capacity factors and $\log P_{oct}$ with increasing structural diversity of solutes is believed to result from specific interactions of the compounds with the residual silanol groups in such stationary phases [19]. Therefore it is a very big challenge in this method to find the optimal stationary and mobile phase conditions in order to obtain $\log k_w$ values highly correlated with $\log P_{oct}$ for a broad range of noncongeneric compounds.

Measures have been taken to decrease the effects of free silanol groups. Great progress has been achieved with silica-based stationary phases exhibiting a high level of silanol deactivation, of which LC-ABZ and Discovery-RP-Amide-C16 phases are good examples. In these stationary phases, the alkyl chains contain an amido group which electrostatically shields silanols from highly polar analytes. In addition, it is hypothesized that the combination of amido groups and a hydration layer at the silica surface leads to a high degree of orientation of the alkyl chains of the stationary phase [20], which facilitates their hydrophobic interaction with the solutes, in contrast to what happens in conventional ODS stationary phases where the alkyl chains are mostly folded. The advantage of LC-ABZ over the conventional ODS stationary phases in $\log P_{oct}$ measurement has been verified and discussed [18,21–23]. A highly significant correlation was found between $\log P_{oct}$ and $\log k_w$ values obtained with the Discovery-RP-Amide-C16 phase for a wide range of compounds including model solutes and drugs [24].

The influence of the mobile phase on the $\log k_w$ versus $\log P_{oct}$ correlation has also been investigated using different organic modifiers [25,26] and/or adding low levels of *n*-decylamine or 1-octanol [12,13,24,27,28]. Lombardo et al. [12] investigated the influence of 1-octanol in the mobile phase on the $\log P_{oct}$ measurement for a set of noncongeneric neutral drugs on LC-ABZ stationary phase. A highly significant correlation between $\log k_w$ and $\log P_{oct}$ was obtained in the presence of 1-octanol in the mobile phase. The authors also expanded the applicability of the method to the determination of the 1-octanol/water distribution coefficients ($\log D_{oct}$) at pH 7.4 for neutral and basic drugs [13].

In order to further investigate the optimal conditions and the applicable range for obtaining $\log P_{oct}$ values from RP-HPLC measurements, we selected here a set of 46 neutral solutes and solutes with acidic and ampholytic functionalities, which were maintained neutral at pH 2.5 or 4. We determined their $\log k_w$ values on the Discovery-RP-Amide-C16 stationary phase using a methanol/phosphate buffer eluent with and without 1-octanol. The compounds in this set are all biologically active and cover a broad structural diversity as well as a wide $\log P_{oct}$ range (−0.69 to 5.70). They are nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, 4-phenyldihydropyridine (DHPs) calcium-channel blockers, antibacterials and flavonoids, as shown in Fig. 1. The correlation between $\log k_w$ and $\log P_{oct}$ values, and the relation between $\log k_w$ and S (see Eq. (2)), were explored. In addition, the mechanism of the influence of 1-octanol in the mobile phase on the $\log k_w$ versus $\log P_{oct}$ correlation was investigated.

2. Experimental

2.1. Solutes and reagents

All compounds were obtained from commercial sources (Wako, Osaka, Japan; TCI, Tokyo, Japan; Sigma–Aldrich, Tokyo, Japan and Steinheim, Germany; ICN, Aurora, USA; Merck, Schuchardt, Germany; TRC, North York, Canada; LKT laboratories Inc. Tokyo, Japan) and in the highest available purity. Distilled water, HPLC grade methanol, and 1-octanol (Sigma–Aldrich, Steinheim, Germany) were used throughout.

2.2. Measurement of capacity factors

The capacity factors were measured with a liquid chromatograph equipped with a *880-PU*-HPLC pump, a *875*-UV–vis detector (both from Jasco, Tokyo, Japan), a *655A-40* autosampler and a *D-2000* chromato-integrator (both from Hitachi, Tokyo, Japan).

The column was a Supelcosil Discovery-RP-Amide-C16 (5 cm × 4.6 mm I.D., 5 μm) from Supelco (Bellefonte, PA, USA). The mobile phase consisted of 0.02 M phosphate buffer and methanol in proportions varying from 70 to 10% (v/v). The phosphate buffer was adjusted to pH 3 for all neutral drugs (steroids 16–23, DHPs calcium-channel blockers 24–29 in Table 2) and flavonoids (42–46 in Table 2), and for the ionizable drugs to a pH value where the neutral form was in large excess (pH 2.5 for NSAIDs 1–15 and pH 4 for antibacterials 30–41 in Table 2). Two sets of measurements were conducted for all compounds. In one set, a 0.25% (v/v) amount of 1-octanol was added to methanol [27,28], and 1-

octanol saturated water was used to prepare the buffer. In the other set, the mobile phase condition was the same as that used in the first set except for the absence of 1-octanol in the eluents. The phosphate buffer was filtered under vacuum through a 0.45 μm HA Millipore filter (Millipore, Milford, MA, USA) before being mixed with methanol. The retention times were measured at ambient temperature by the UV–vis detector at the λ_{max} of the analytes.

The solutions to be injected (10^{-4} M to 10^{-3} M) were prepared by dissolving the solutes in the mobile phase; the injection volume was 10 μL. Uracil was used as the unretained compound. The measurements were carried out at a

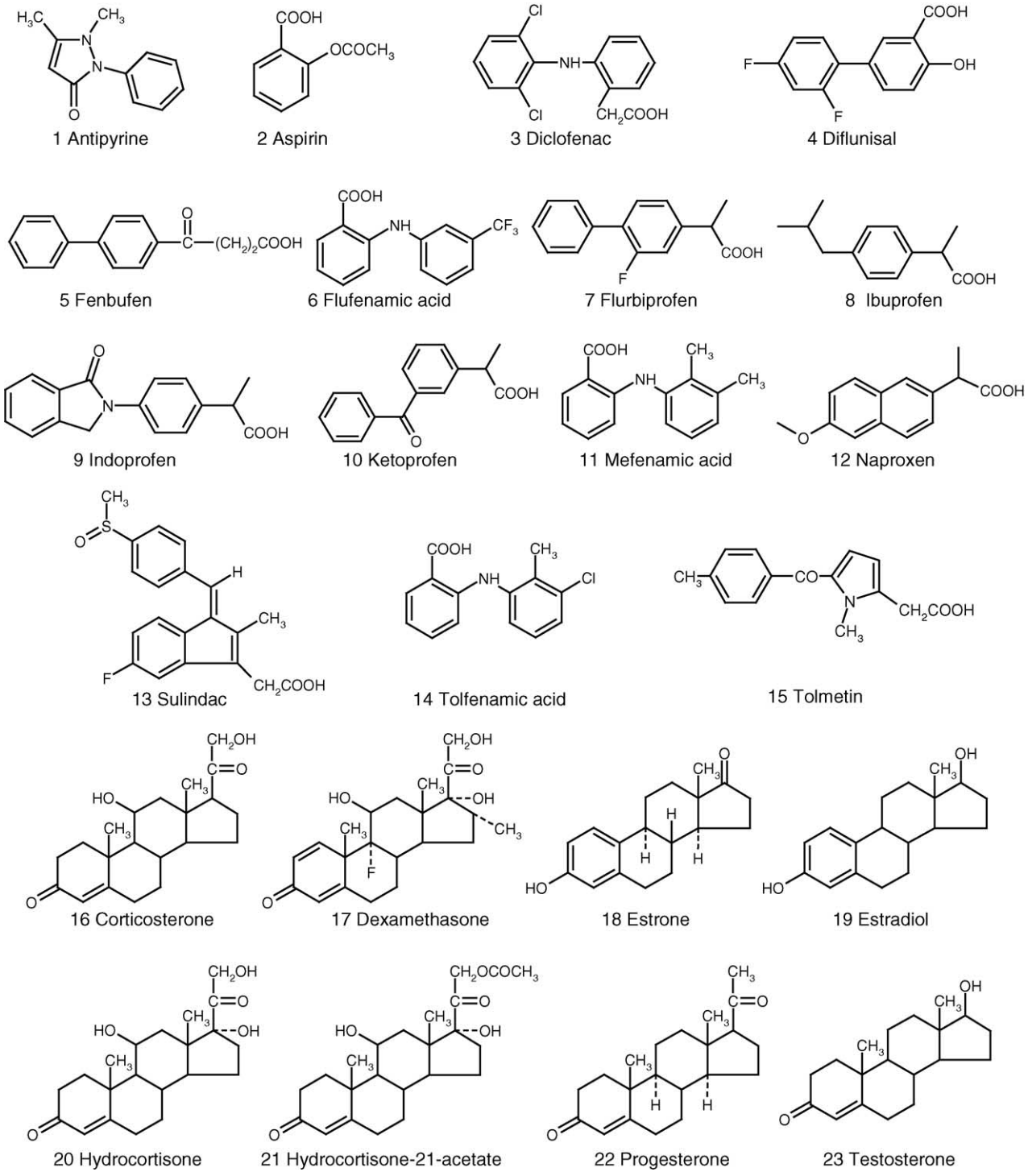

Fig. 1. Structures of the drugs and flavonoids under study.

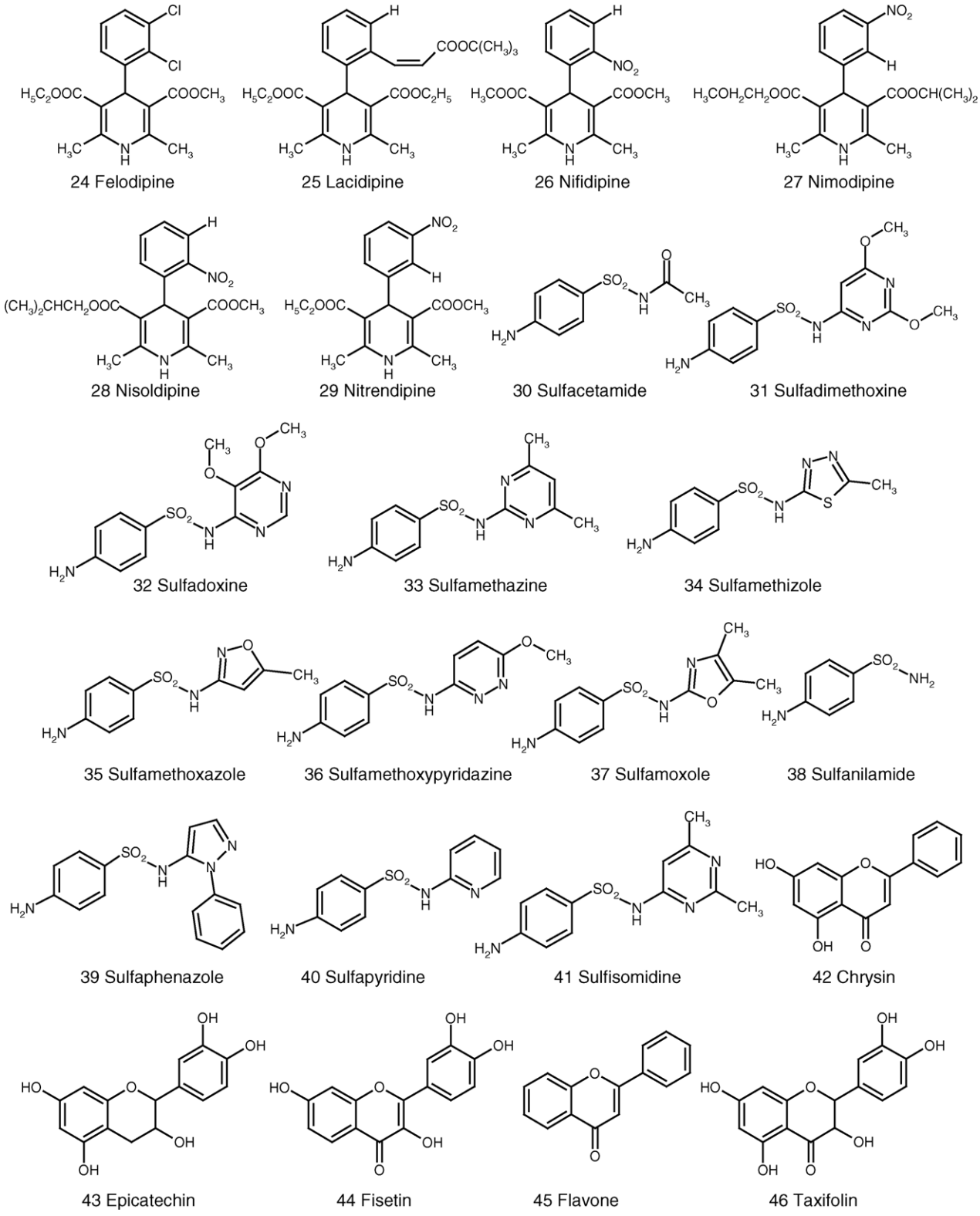

Fig. 1. (*Continued*).

flow rate 1.0 mL/min for compounds with a $\log P_{oct}$ value higher than +1, and 0.5 mL/min for compounds with $\log P_{oct}$ below +1. In all cases, three different methanol concentrations were used for extrapolation to $\log k_w$. Methanol concentrations were adapted to the $\log P_{oct}$ values of the solutes as described in Table 1.

The capacity factor $\log k$ was calculated by Eq. (1). All $\log k$ values were the average of three measurements. The

Table 1
Concentrations of organic modifier (methanol) used in the two sets of experiments

log P_{oct} of the solutes	%MeOH
>3	60, 65, 70
1–3	40, 45, 50
<1	10, 20, 25

log k values were then extrapolated to 100% water using Eq. (2).

2.3. Statistical analysis

All regression analyses were performed via the JMP statistical software package (Version 5.1.1, Japanese Edition, SAS Institute Inc.).

3. Results and discussion

3.1. Relationship between log k and φ

A linear relationship between log k and φ (the volume fraction of organic solvent in the eluent) was found for all compounds under both eluent conditions. In all cases, the squared correlation coefficient was higher than 0.99, excepting sulfamoxole and sulfapyridine ($r^2 = 0.98$) under the mobile phase with the presence of 1-octanol. The log k_w and S (slope) values of the 46 solutes were calculated by Eq. (2) and are presented in Table 2.

3.2. Correlation between log k_w and S

The correlation between log k_w and S was investigated under the two mobile phase conditions. Fig. 2 shows a large difference in statistical quality in the presence or absence of 1-octanol. The correlation was highly significant in the presence of 1-octanol (Eq. (3A) and Fig. 2A), while a poor correlation was established in the absence of 1-octanol (Eq. (3B) and Fig. 2B).

$$S = 0.86(\pm 0.04)\ \log k_w + 1.70(\pm 0.11)$$
$$n = 46;\ q^2 = 0.97;\ r^2 = 0.97;\ s = 0.24;\ F = 1549 \quad (3A)$$

$$S = 0.36(\pm 0.13)\ \log k_w + 3.54(\pm 0.36)$$
$$n = 46;\ q^2 = 0.41;\ r^2 = 0.41;\ s = 0.50;\ F = 31 \quad (3B)$$

In this and the following equations, 95% confidence limits are in parentheses; n, the number of the compounds; q^2, the cross-validated correlation coefficient; r^2, the squared correlation coefficient; s, the standard deviation and F the Fisher's test.

In spite of the significant correlation between log k_w and S for the complete set of solutes in Eq. (3A), there are three outliers as shown in Fig. 2A, namely sulfanilamide (38), sulfapyridine (40) and epicatechin (43). The reason for sulfapyridine being an outlier is probably the lower quality of the linear regression between log k versus φ. For the other two outliers, no reason is apparent and further investigation is needed. After omission of the three outliers, the correlation between log k_w and S becomes even better ($r^2 = 0.98$).

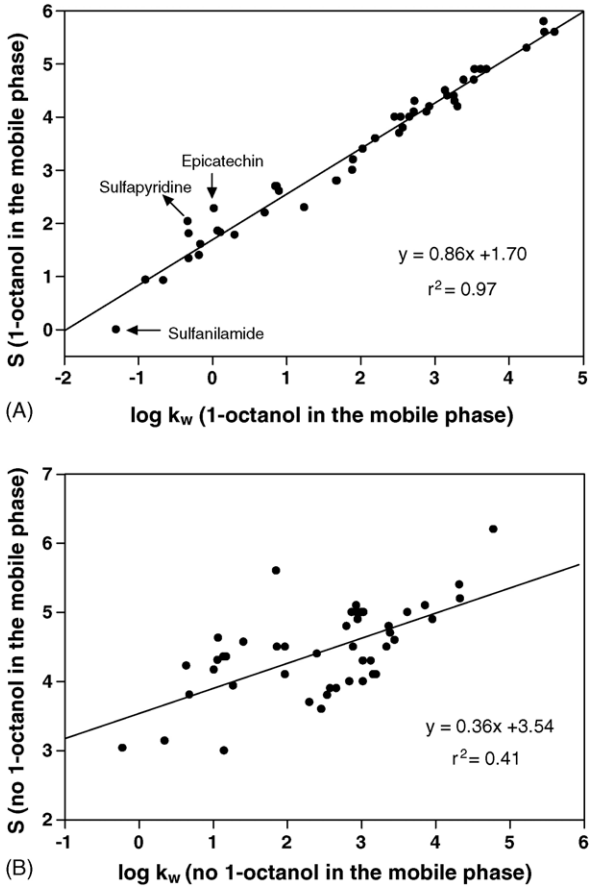

Fig. 2. Relationship between log k_w and slope S (A) in the presence of 1-octanol in the mobile phase and (B) in the absence of 1-octanol in the mobile phase.

Since the only difference between the two sets of experimental conditions was the presence or absence of 1-octanol in the mobile phase, the factor producing the much better correlation between log k_w and S is clearly the addition of 1-octanol. The significant correlation in Eq. (3A) implies that S is controlled by the same intermolecular forces as those in log k_w, which are van der Waals volume, H-bond acceptor basicity and dipolarity/polarizability as unraveled by a solvatochromic analysis in our previous study [24]. On the contrary, the non-significant correlation in the absence of 1-octanol (Eq. (3B) and Fig. 2B) means that these two parameters encode different structural information. A clear interpretation of S in this condition needs further quantitative structure-property (here S) relationship analysis.

In previous studies, good correlations between log k_w and S were obtained mostly for simple or closely related

Table 2
Investigated compounds and their physicochemical parameters

Number	Solutes	$\log P_{oct}$ [a]	1-Octanol in the mobile phase		No 1-octanol in the mobile phase	
			$\log k_w$ [b]	S [c]	$\log k_w$ [d]	S [e]
	NSAIDs					
1	Antipyrine	0.17	−0.16	1.61	1.06	4.31
2	Aspirin	1.13	0.71	2.20	1.15	3.00
3	Diclofenac	4.40	3.53	4.70	3.45	4.60
4	Diflunisal	4.44	3.31	4.20	3.20	4.10
5	Fenbufen	3.39	2.66	4.00	2.54	3.80
6	Flufenamic acid	5.25	4.48	5.60	4.32	5.40
7	Flurbiprofen	3.81	3.54	4.90	3.39	4.70
8	Ibuprofen	3.87	3.62	4.90	3.34	4.50
9	Indoprofen	2.77	1.90	3.20	2.93	5.10
10	Ketoprofen	2.77	2.54	4.00	3.03	5.00
11	Mefenamic acid	5.12	4.24	5.30	3.96	4.90
12	Naproxen	3.06	2.72	4.10	2.58	3.90
13	Sulindac	3.42	2.73	4.30	2.89	4.50
14	Tolfenamic acid	5.70	4.62	5.60	4.33	5.20
15	Tolmetin	2.79	2.46	4.00	2.95	4.90
	Steroids					
16	Corticosterone	2.20	1.68	2.80	2.80	4.80
17	Dexamethasone	1.83	1.67	2.80	2.87	5.00
18	Estrone	3.13	2.89	4.10	2.84	4.00
19	Estradiol	4.01	3.26	4.40	3.02	4.00
20	Hydrocortisone	1.55	1.24	2.30	2.40	4.40
21	Hydrocortisone-21-acetate	2.19	1.89	3.00	3.00	5.00
22	Progesterone	3.57	3.17	4.40	3.13	4.30
23	Testosterone	3.29	2.57	3.80	2.66	3.90
	DHPs caicium-channel blockers					
24	Felodipine	4.80	3.70	4.90	3.86	5.10
25	Lacidipine	5.56	4.47	5.80	4.78	6.20
26	Nifedipine	3.22	2.20	3.60	2.30	3.70
27	Nimodipine	4.18	3.14	4.50	3.37	4.80
28	Nisoldipine	4.53	3.39	4.70	3.62	5.00
29	Nitrendipine	4.15	2.93	4.20	3.02	4.30
	Antibacterials					
30	Sulfacetamide	−0.16	−0.66	0.93	0.35	3.14
31	Sulfadimethoxine	1.40	0.87	2.70	1.97	4.50
32	Sulfadoxine	0.56	0.11	1.83	1.41	4.57
33	Sulfamethazine	0.25	−0.32	1.34	1.01	4.17
34	Sulfamethizole	0.55	0.07	1.85	1.18	4.36
35	Sulfamethoxazole	0.72	0.30	1.78	1.27	3.94
36	Sulfamethoxypyridazine	0.35	−0.18	1.40	1.14	4.36
37	Sulfamoxole	−0.14	−0.32	1.81	1.07	4.63
38	Sulfanilamide	−0.69	−1.30	0.00	−0.22	3.04
39	Sulfaphenazole	1.27	0.85	2.70	1.86	4.50
40	Sulfapyridine	0.02	−0.33	2.04	0.68	3.81
41	Sulfisomidine	−0.37	−0.90	0.94	0.64	4.23
	Flavonoids					
42	Chrysin	3.52	3.27	4.30	3.16	4.10
43	Epicatechin	0.56	0.02	2.28	1.85	5.60
44	Fisetin	2.53	2.03	3.40	2.93	5.00
45	Flavone	3.56	2.52	3.70	2.46	3.60
46	Taxifolin	0.95	0.90	2.61	1.97	4.10

[a] The values of drugs 1–41 are taken from [12,29–32], those of flavonoids 42–46 are MlogP from the Bio-loom software [33].
[b] $0.01 \leq SD \leq 0.18$.
[c] $0.01 \leq SD \leq 0.30$.
[d] $0.01 \leq SD \leq 0.19$.
[e] $0.01 \leq SD \leq 0.30$.

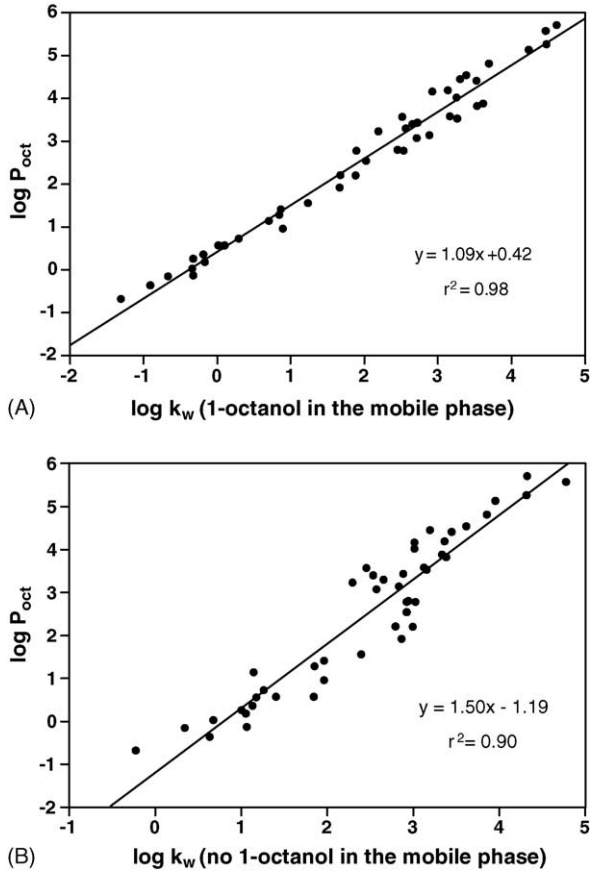

Fig. 3. Relationship between $\log P_{oct}$ and $\log k_w$ (A) in the presence of 1-octanol in the mobile phase and (B) in the absence of 1-octanol in the mobile phase.

compounds [10,14,15]. Here, significant correlations were obtained for a structurally diverse set of chemically complex drugs and flavonoids by using a 1-octanol enriched eluent. In agreement with previous results [18], the slope of the correlation between $\log k_w$ and S in Eq. (3A) is close to unity.

3.3. Correlation between $\log P_{oct}$ and $\log k_w$

The correlations between $\log P_{oct}$ and $\log k_w$ values obtained with the two sets of eluents are also markedly different, with 1-octanol producing a significant improvement. Eq. (4A) and Fig. 3A show that, in the presence of 1-octanol, the correlation between $\log P_{oct}$ and $\log k_w$ was highly significant for the whole set of solutes investigated. In contrast, the correlation was good but of lower quality when 1-octanol was absent (Eq. (4B) and Fig. 3B). This result is in agreement with the work of Lombardo et al. [12], who compared lipophilicity measurement in the presence or absence of 1-octanol using a set of 27 structurally diverse neutral solutes on a Supelcosil LC-ABZ column.

$$\log P_{oct} = 1.09(\pm 0.05)\log k_w + 0.42(\pm 0.13)$$
$$n = 46;\ q^2 = 0.97;\ r^2 = 0.98;\ s = 0.28;\ F = 1785 \quad (4A)$$

$$\log P_{oct} = 1.50(\pm 0.15)\log k_w - 1.19(\pm 0.42)$$
$$n = 46;\ q^2 = 0.89;\ r^2 = 0.90;\ s = 0.58;\ F = 387 \quad (4B)$$

In addition to the higher squared correlation coefficient and smaller standard deviation of Eq. (4A) compared to Eq. (4B), the slopes of these two equations are very different. As stated by Minick et al. [27], the slope of an equation correlating $\log k_w$ and $\log P_{oct}$ is an estimate of how closely the free energies of the processes compare. A unit slope in such a plot indicates that the two processes are homoenergetic. In Eq. (4A), the slope is very close to unity, meaning that the chromatographic retention process on the Discovery-RP-Amide-C16 stationary phase with 1-octanol in the eluent is very energetically similar to the partitioning process in 1-octanol/water.

On the contrary, the large deviation from unity in the slope in Eq. (4B) implies that RP-HPLC retention in the absence of 1-octanol and 1-octanol/water partitioning are governed by dissimilar processes.

The above results show that, with a set of highly diverse and functionally complex solutes (including neutral compounds and ionizable compounds which were maintained neutral at pH 2.5 or 4) and using the Discovery-RP-Amide-C16 stationary phase, 1-octanol in the eluent is a key factor to obtain a lipophilicity index $\log k_w$ highly correlated with $\log P_{oct}$. In other words, RP-HPLC with proper stationary and mobile phases is a very promising alternative to the traditional shake-flask method to derive $\log P_{oct}$ values not only for neutral drugs, as verified by Lombardo et al. [12], but also for drugs with acidic and ampholytic functionalities, although, neutral at the conditions studied.

As for the majority of basic drugs, $\log P_{oct}$ values cannot be determined with most silica-based stationary phases due to the pH limitation of these stationary phases. Instead, their distribution coefficient $\log D_{oct}$ at pH 7.4 was successfully determined by this method [13].

The wide applicable range of the Discovery-RP-Amide-C16 stationary phase in lipophilicity measurement demonstrated and confirmed the advantage of the amide embedded stationary phases over the conventional ODS stationary phases which could only be successful in $\log P_{oct}$ measurement of structurally related compounds. The reasons why this kind of stationary phases is a better model for the 1-octanol/water partition system are possibly (1) the high level of silanol deactivation on this stationary phase due to the electrostatic coating and (2) the selective solvation of the silica surface by water attracted into the bonded phase by the amide group as discussed by Dias et al. [22].

By comparing the $\log k_w$ values obtained with the two sets of experiments, Fig. 4 shows how the addition of 1-octanol to the eluent differently affects the chromatographic retention of solutes with $\log P_{oct}$ values below and above a value of 3. Indeed, all compounds with $\log P_{oct}$ values greater than 3 (open circles in Fig. 4) are close to the unity line, meaning that their $\log k_w$ values are not influenced by the addition of 1-octanol to the eluent. In contrast, the solutes with $\log P_{oct}$

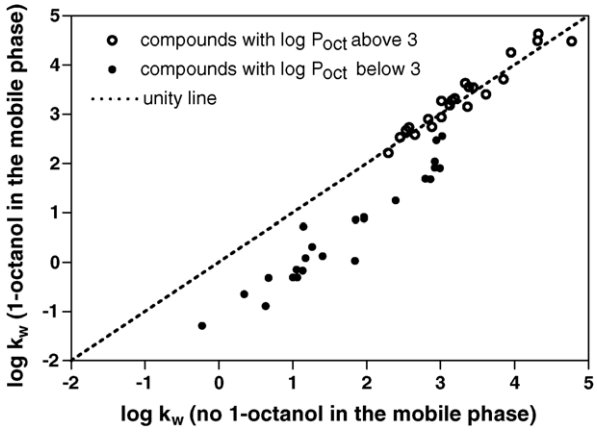

Fig. 4. Relationship between log k_w values derived from the two set of experiments.

values lower than 3 (closed circles in Fig. 4) deviate clearly from the unity line, implying that the addition of 1-octanol markedly decreases their log k_w values. This is interpreted to mean that the improved log P_{oct} versus log k_w correlation resulting from the addition of 1-octanol is due mainly to a modification of the retention behavior of the less lipophilic solutes.

As shown in Table 1, the log k_w values of the compounds with log P_{oct} values greater than 3 were extrapolated from higher methanol concentrations (60, 65, 70%) in the eluent. The negligible influence of 1-octanol on the retention behavior of these solutes may be related to their specific properties and/or to the higher methanol concentrations used.

4. Conclusion

Using the Discovery-RP-Amide-C16 stationary phase, linear relationships were found between isocratic log k values and the volume fraction of MeOH in the eluent in the presence and absence of 1-octanol. The correlation between the derived log k_w and S (Eq. (2)) is highly significant when a 1-octanol-enriched eluent was used, implying that under such conditions the two parameters encode the same intermolecular forces. In contrast, no significant correlation between these two parameters was seen in the absence of 1-octanol.

The addition of 1-octanol to the mobile phase is a key factor to obtain a lipophilicity index log k_w highly correlated with log P_{oct} values for a set of structurally complex and diverse solutes (log P_{oct} ranging from −0.69 to 5.70). This implies that the RP-HPLC method with proper stationary and mobile phases is of value to derive log P_{oct} values for neutral drugs, as found by Lombardo et al. [12], and for drugs with acidic and ampholytic functionalities which were maintained neutral at the experimental conditions.

Our study also unravels the mechanism by which 1-octanol improves the log k_w versus log P_{oct} correlation. By comparing the log k_w values obtained in the presence or absence of 1-octanol, it can be concluded that the influence of 1-octanol on the chromatographic retention is smaller for the more lipophilic compounds (log $P_{oct} > 3$) than for the less lipophilic ones (log $P_{oct} < 3$).

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