ElogD$_{oct}$: A Tool for Lipophilicity Determination in Drug Discovery. 2. Basic and Neutral Compounds

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We present an RP-HPLC method for the determination of the octanol–water distribution coefficients at pH 7.4, as log $D_{oct}^{7.4}$ values, for neutral and basic drugs, which combines ease of operation with high accuracy. The method is shown to work for a training set of 90 molecules comprised largely of drugs, and it was also applied to a test set of 10 proprietary compounds. This work expands the applicability of the method presented in our earlier report, for the determination of logP$_{oct}$ for neutral compounds (*J. Med. Chem.* **2000**, *43*, 2922–2928), and it offers the same general features but widens the scope. Generally, the method (i) is compound sparing ($\leq$1 mL of a 50–100 μg/mL solution needed), (ii) is insensitive to concentration and phase ratio effects observed in some shake-flask determinations, (iii) is amenable to rapid determinations ($\leq$20 min on average), (iv) is insensitive to impurities, (v) possesses a wide lipophilicity range (>7 log $D_{oct}^{7.4}$ units), and (vi) offers a good accuracy, (vii) an excellent reproducibility, (viii) and an excellent potential for automation. To the best of our knowledge, a similar performance, on a set of noncongeneric drugs, has not been previously reported. We refer to the value generated via this method as ElogD$_{oct}$.

Introduction

The importance of lipophilicity can be understood, for example, by considering the correlation between high lipophilicity and poor solubility, which has generally been explored with neutral solutes.[1] Furthermore, lipophilicity has been shown to be of paramount importance in several other ADME aspects, that is, absorption, distribution, metabolism, and excretion. It is generally held that very lipophilic compounds are "preferred" targets for metabolism, often leading to high clearance values and, quite often, lipophilicity positively correlates with a high plasma protein binding.[2–5] A large volume of distribution, probably due to a high fraction of the compound bound to tissues, is often observed for lipophilic compounds.[4] At physiological pH many basic or acidic drugs are ionized, and the partition coefficient is indeed a distribution coefficient, D, which is generally taken to be the distribution between an aqueous buffer at pH 7.4 and n-octanol, and it is indicated by the notation $D_{oct}^{7.4}$.

Scherrer[3] defines the distribution coefficient (in the form of its logarithm) for monoprotic bases as

$$\log D_{oct} = \log P_{oct} + \log [1/(1 + 10^{pK_a - pH})]$$

For monoprotic acids the equation has the same form, except that the exponent is written as pH-pK_a. For polyprotic compounds the equations become more complicated, and these aspects have been described in detail by Avdeef.[6] These equations assume that only unionized species partition in the organic phase (octanol). In fact, this may or may not be always true. Depending on the nature and concentration of the species present (including counterions), there may be other phenomena at work, such as ion partitioning.

Given the widespread use and application of distribution coefficients, a method that can accurately and rapidly yield log $D_{oct}^{7.4}$ values would be a welcome addition to the experimental tools available for physicochemical properties screening in the discovery setting. We will use, in the rest of this work, the general notation logD$_{oct}$, to mean the values determined at pH 7.4.

Although computational packages for the estimation of logD$_{oct}$ are available,[7] and this value could also be calculated from estimated logP$_{oct}$ and pK_a values, we find that, for drug molecules, computed values are often inaccurate. Depending on the software used, they may differ by as much as two to three logD$_{oct}$ units among different software packages and/or from experimental values, since the accuracy of pK_a as well as logP$_{oct}$ has to be factored in. These methods are, of course, valuable when virtual libraries (or individual virtual molecules) are being designed and, with proper training, more accurate values might be obtained. However, as early as possible and especially if a compound-sparing method is available, the computed values should be replaced by measured values, with particular regard to cases where intramolecular H-bonding is possible, and/or in the presence of conformational flexibility, and/or with molecules which can tautomerize. These occurrences typically offer an even greater challenge to fragment-based software packages. SAR and SPR analyses and alerts such as the Lipinski "rule of 5"[8] would greatly benefit by the introduction of accurate experimental values.

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The classical shake-flask method, or variations of this method which have been described,[9] are neither rugged nor rapid enough for medium to high-throughput applications, they are generally more sensitive to impurities and less amenable to automation than are RP-HPLC methods, and they do not usually offer a wide dynamic range.

RP-HPLC retention data, expressed either as log k' or k'_w, have been shown to correlate well with absolute and relative lipophilicity values,[10] but they have also been criticized as not being a true "replacement" for shake-flask values.[9] Part of the criticism may stem from the limited scope of some reports, focusing either on fairly simple monofunctional solutes[11,12] or classes of analogues[13] with a limited $\log P_{oct}$ range, and in many cases there was only mention of neutral solutes, under the pH conditions of the method. When these correlations were extended beyond classes of analogues, less encouraging results were obtained.[10] Furthermore, in several cases, the slope of these correlations was quite different from unity, casting doubts about the different balance of forces responsible for the two values. Indeed, LFER analyses have shown that log k' on typical RP-HPLC systems do not encode the same blend of factors as does $\log P_{oct}$.[14] In particular, log k' values respond to solute hydrogen-bond acidity, but $\log P_{oct}$ values do not.

k' represents the capacity factor of the solute at a given organic solvent concentration, and k'_w is the capacity factor extrapolated to a 0% concentration of the organic solvent. The observations reported above pointed us toward using the extrapolated value rather than, as reported by Yamagami and co-workers,[15] a log k' value, which is likely to be limited in its applicability to a wide variety of drug-like compounds (amphiprotic) and, therefore, of limited use for the goals we set. As we reported previously,[16] a judicious choice of columns and mobile phase, as well as flow rates, would greatly enhance the performance of RP-HPLC methods.

Another factor of great importance and concern, if the data were to be used for software training purposes or for the creation of a large database, is the reproducibility from column to column, which depends on the quality of the packing chemistry and manufacturing. We have shown, in the case of the ElogP data,[16] that the latter aspect does not appear to be a problem. However, it is advisable to monitor the column performance for possible deterioration, especially if high-throughput screening is the goal.

The speed of the determination and the ability to handle diverse structures and lipophilicity values are, of course, of paramount importance in an industrial research setting. These aspects translate into the capability of screening, with modest resources, a large number of compounds, with a good degree of accuracy across a wide range of lipophilicity values and hydrogen-bonding properties.

Considering the points discussed above, we set out to develop a method that would be accurate, rapid, and possess a good dynamic range, together with being applicable to a variety of drug-like molecules, and which would be robust with respect to ion-pairing and concentration-related variability, often observed across different protocols and laboratories. We present and discuss our results in the following section.

Results and Discussion

Minick et al.[12,17] had used an MC-8 column, coupled with the addition of 1-octanol to both components of the eluent, together with the addition of small amounts (0.5% v/v) of *n*-decylamine in the aqueous phase, and they obtained a very good correlation but on a limited data set and using monofunctional solutes. Previously, Unger[18] had obtained seemingly good results with the addition of a tertiary amine but the data set, albeit mostly comprised of drugs, was not very extensive, in terms of structural diversity and $\log D_{oct}$ values, with the latter spanning approximately two units. Furthermore, the optimized conditions reported (RP-18) yielded better results with a stationary phase slurry packed in the presence of octanol, and this procedure still yielded a fairly high (1.24) intercept, although a slope very close to unity was obtained. Obviously, there are more advanced choices today, in terms of pre-packed RP phases. An embedded polar amide column, such as the LC-ABZ,[19] had raised our interest from the work of Pagliara et al.[11] and had performed very well for neutral drug-like solutes in the absence of decylamine.[16] Thus, we started our work with an attempt at extending identical conditions to ionized solutes. However, despite use of several different masking agents, some of which were zwitterionic or anionic, we could not achieve high accuracy in reproducing the $\log D_{oct}$ values for sets including acidic, neutral, and basic compounds. It has been reported that anions seem prone to specific interactions with this stationary phase,[11] and our observations would seem to confirm that, especially after a fairly extensive screen of potential masking agents. We have tried other stationary phases but without any significant improvement, and we are of the opinion that it might be very difficult, by a single method, to determine the lipophilicity of all three classes of compounds across a wide range of structures and lipophilicity with the high accuracy we had set as a goal. However, neutral and basic compounds seem to comprise most of the drug-like compounds, and a survey on 2500 compounds submitted to our laboratory for various physicochemical determinations showed the (strongly) acidic compounds to be a fairly small fraction of about 5%. Furthermore, for therapeutic areas such as CNS, basic and neutral compounds generally represent an even higher percentage of compounds.

We soon found that for a subset of the compounds reported here, including basic and neutral compounds, *n*-decylamine would yield improved correlations, although it was obviously not necessary for the neutral solutes.[16] To maximize the speed of analysis, while still retaining a good accuracy, the same flow rate previously reported[16] was chosen for each range of lipophilicity (see Experimental Section). We also studied the effect of a further increase of flow rate on accuracy and performed statistical analysis in order to determine experimental lipophilicity "thresholds" for each range of conditions, which would yield accurate results. The current "thresholds" for lipophilicity ranges are reported in the Experimental Section.

Extensive work was conducted using the 90 solutes reported in Table 1, and each log k'_w value is the average of at least three determinations, on different columns, with an average standard deviation of 0.07.

Table 1. Extrapolated Capacity Factors and $\log D_{oct}$ Data for the 90 Solutes Used

compd[a]	CAS no.	$\log k'_w$[b]	sd[c]	$\mathrm{E}\log D_{oct}$[d]	$\log D_{oct}$[e]	res.[f]	refs
acebutolol	37517-30-9	−0.53	0.04	−0.39	−0.29	0.10	25, 26
acetaminophen*	103-90-2	0.15	0.01	0.38	0.51	0.13	27
acetophenone	98-86-2	1.21	0.03	1.57	1.58	0.01	28
allopurinol*	315-30-0	−0.27	0.01	−0.10	−0.44	−0.34	*g*, 29
alprazolam	28981-97-7	1.73	0.04	2.16	2.12	−0.04	30
alprenolol	13655-52-2	0.37	0.04	0.62	0.97	0.35	*j*, 25
amiodarone*	1951-25-3	5.10	0.21	5.95	6.10	0.15	31
amlodipine	88150-42-9	1.72	0.05	2.15	1.66	−0.49	*g*, *h*, 2, 32
antipyrine	60-80-0	0.12	0.03	0.34	0.38	0.04	33
atropine	51-55-8	−0.33	0.19	−0.16	−0.55	−0.39	*j*, 34
bifonazole	60628-96-8	4.33	0.07	5.09	4.77	−0.32	35
bromazepam	1812-30-2	1.04	0.04	1.38	1.65	0.27	36, 37
3-bromoquinoline*	5332-24-1	2.53	0.06	3.06	3.03	−0.03	38
caffeine	58-08-2	−0.19	0.02	−0.01	−0.07	−0.06	35
carbamazepine	298-46-4	1.40	0.03	1.78	2.19	0.41	39
chloramphenicol*	56-75-7	1.19	0.07	1.55	1.14	−0.41	35
3-chlorophenol	108-43-0	2.58	0.01	3.11	2.50	−0.61	28
chlorpheniramine	132-22-9	1.20	0.16	1.56	1.41	−0.15	*j*, 18, 40
chlorpromazine	50-53-3	2.66	0.08	3.20	3.38	0.18	18, 26, 41
chlorthalidone	77-36-1	0.76	0.10	1.06	1.11	0.05	*g*, *j*, 42
cimetidine	51481-61-9	0.17	0.03	0.40	0.35	−0.05	*j*
clonidine	4205-90-7	0.07	0.03	0.29	0.62	0.33	43
clotrimazole*	23593-75-1	4.03	0.06	4.75	5.20	0.45	16
clozapine	5786-21-0	2.82	0.03	3.38	3.13	−0.25	*j*, 18
cocaine	50-36-2	0.24	0.05	0.48	1.05	0.57	44
codeine	76-57-3	0.16	0.13	0.39	0.23	−0.16	41, 45
cyclothiazide	2259-96-3	2.15	0.09	2.63	2.09	−0.54	*j*
deprenyl	2323-36-6	2.19	0.11	2.67	2.70	0.03	31
desipramine	50-47-5	0.97	0.06	1.30	1.28	−0.02	*g*, *j*, 46
dexamethasone	50-02-2	1.62	0.04	2.03	1.83	−0.20	47
diazepam	439-14-5	2.46	0.03	2.98	2.79	−0.19	48
3,5-dichlorophenol	591-35-5	3.44	0.08	4.08	3.68	−0.40	38
diethylstilbestrol	56-53-1	4.12	0.10	4.85	5.07	0.22	35
diltiazem	33286-22-5	1.59	0.22	2.00	2.06	0.06	*j*
diphenhydramine	58-73-1	1.04	0.06	1.38	1.29	−0.09	*g*, *j*, 40
disopyramide	3737-09-5	−1.21	0.10	−1.16	−0.66	0.50	*j*
estradiol	50-28-2	3.28	0.08	3.90	4.01	0.11	35
fentanyl citrate	990-73-8	1.94	0.09	2.39	2.91	0.52	49
flecainide	54143-55-4	0.25	0.06	0.49	0.97	0.48	*g*, 50
fluconazole*	86386-73-4	0.40	0.15	0.66	0.50	−0.16	51
griseofulvin	126-07-8	1.73	0.06	2.16	2.18	0.02	35
haloperidol	52-86-8	2.00	0.05	2.46	2.98	0.52	*g*, *j*
hydrocortisone	50-23-7	1.14	0.06	1.49	1.55	0.06	49
hydrocortisone-21 acetate	50-03-3	1.64	0.01	2.06	2.19	0.13	49
imipramine	50-49-7	1.56	0.19	1.97	2.40	0.43	*g*, *j*, 18, 40
lidocaine	137-58-6	0.96	0.13	1.29	1.71	0.42	*g*, 18, 41
loratadine	79794-75-5	4.02	0.07	4.74	4.40	−0.34	40
lorazepam	846-49-1	2.30	0.03	2.80	2.51	−0.29	52
lormetazepam	848-75-9	2.27	0.04	2.77	2.72	−0.05	53
methotrimeprazine	60-99-1	2.10	0.27	2.57	2.77	0.20	*j*
methylthioinosine	342-69-8	0.25	0.02	0.49	0.09	−0.40	16
metoclopramide	364-62-5	0.46	0.16	0.73	0.64	−0.09	*j*
metoprolol	56392-17-7	−0.73	0.08	−0.62	−0.16	0.46	25, 31, 34, 46
metronidazole	443-48-1	−0.08	0.02	0.12	−0.02	−0.14	54
mexiletine	31828-71-4	0.02	0.03	0.23	0.47	0.24	*j*
morphine sulfate	64-31-3	0.10	0.10	0.32	0.03	−0.29	41, 44, 45
naphthalene	91-20-3	3.03	0.06	3.62	3.37	−0.25	38
nicotine	54-11-5	0.02	0.06	0.23	0.40	0.17	*g*, *j*
nifedipine	21829-25-4	2.34	0.06	2.84	3.17	0.33	16
nifuroxime*	6236-05-1	1.14	0.07	1.49	1.28	−0.21	16
nitrofurazone	59-87-0	0.29	0.01	0.53	0.23	−0.30	55
nizatidine	76963-41-2	−0.13	0.04	0.06	−0.52	−0.58	56
omeprazole	73590-58-6	1.59	0.04	2.00	2.30	0.30	*g*, 57
pentoxifylline	6493-05-6	0.03	0.02	0.24	0.29	0.05	58
pirenzepine	28797-61-7	−0.15	0.06	0.04	−0.61	−0.65	*g*
prednisolone	50-24-8	1.24	0.05	1.60	1.60	0.00	16
prednisone	53-03-2	0.90	0.06	1.22	1.46	0.24	35
procainamide	51-06-9	−0.69	0.24	−0.57	−0.91	−0.34	*g*, 59
propafenone	54063-53-5	1.14	0.10	1.49	1.81	0.32	*g*, *j*
propranolol	525-66-6	0.64	0.01	0.93	1.26	0.33	*g*, 25, 26, 31, 34, 46, 60
quinidine	56-54-2	1.16	0.12	1.51	2.04	0.53	18, 26, 61
quinoline	91-22-5	1.52	0.04	1.92	2.03	0.11	62
ranitidine	66357-35-5	−0.63	0.01	−0.50	−0.29	0.21	*j*
risperidone	106266-06-2	1.23	0.13	1.59	2.04	0.45	*g*
sotalol	3930-20-9	−1.47	0.10	−1.45	−1.35	0.10	25, 31
sumatriptan	103628-46-2	−0.54	0.02	−0.40	−1.00	−0.60	*g*, *h*, 63

Table 1. (Continued)

compd[a]	CAS no.	log k'_w [b]	sd[c]	$ElogD_{oct}$ [d]	$logD_{oct}$ [e]	res.[f]	refs
terbutaline sulfate	23031-32-5	−1.51	0.06	−1.49	−1.35	0.14	31, 46, 64
testosterone	58-22-0	2.63	0.04	3.17	3.29	0.12	47
tetracaine	94-24-6	1.70	0.07	2.12	2.29	0.17	31
thiamphenicol	15318-45-3	−0.05	0.01	0.15	−0.27	−0.42	65
thioridazine	50-52-2	2.88	0.10	3.45	3.34	−0.11	*j*, 66
tiapride	51012-32-9	−0.58	0.05	−0.45	−0.90	−0.45	*j*
tiotidine	69014-14-8	0.57	0.01	0.85	0.57	−0.28	*g*
tolnaftate*	2398-96-1	4.53	0.10	5.31	5.40	0.09	16
trazodone	19794-93-5	2.45	0.06	2.97	2.54	−0.43	j
triamterene	396-01-0	0.71	0.05	1.01	1.21	0.20	*g*, *j*, 42, 67
trichlormethiazide	133-67-5	0.26	0.02	0.50	0.43	−0.07	*g*, *j*
triflupromazine*	146-54-3	3.05	0.13	3.64	3.61	−0.03	*j*, 66
trimethoprim	738-70-5	0.36	0.02	0.61	0.63	0.02	*j*
zaltidine	85604-00-8	0.53	0.02	0.80	0.74	−0.06	*g*

[a] Asterisk denotes standard compound. [b] Average of three to five determinations [c] Standard deviation of 3 to 5 log k'_w determinations. [d] Equation 1. [e] Average $logD_{oct}$ from all the references or methods indicated. [f] $logD_{oct}$ - $ElogD_{oct}$. [g] Shake-vial procedure A. [h] Shake-vial procedure B. [j] Potentiometric determination.

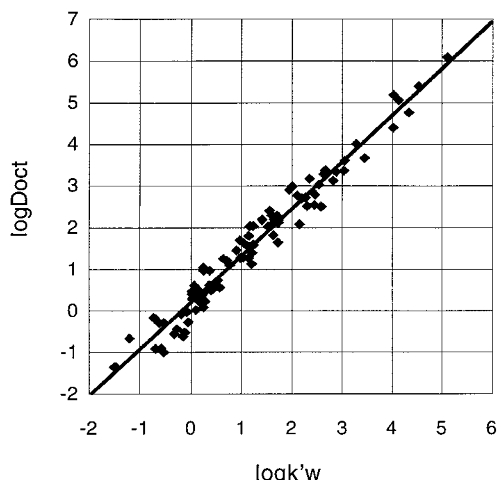

Figure 1. Correlation between $logD_{oct}$ and log k'_w for 90 solutes.

The table also reports the standard deviation of log k'_w for each compound ($N = 3$ to 5), and no large deviations were observed, regardless of structure and range of lipophilicity, with methotrimeprazine yielding the largest standard deviation (sd) with a value of 0.27. It is worth noting that routine potentiometric lipophilicity determinations have a typical sd of 0.4 $logD_{oct}$ units, for replicate determinations, and often they have to be extrapolated to 0% organic solvent from mixed solvents, due to the poor aqueous solubility of many compounds. The fit of the log k'_w to the averaged $logD_{oct}$ values is reported below (eq 1), and shown graphically in Figure 1.

$$logD_{oct} = 1.1267\ (\pm 0.0233) \log k'_w + 0.2075\ (\pm 0.0430) \quad (1)$$

$$N = 90,\ R^2 = 0.964,\ R = 0.982,\ s = 0.309,\ F = 2339,\ q^2 = 0.962$$

The slope obtained is very close to unity, with a small intercept, and these parameters offer a good comparison of the balance of forces which play a role in the shake-flask vs RP-HPLC distribution coefficient. The question of the diagnostic importance of the slope has been stressed by Minick et al.[17] Pointing to the work of Melander et al.,[20] these authors state that "...equations correlating log k'_w and $logP_{oct}$ data represent linear free energy relationships in which the slope is an estimate of how closely the free energies of the processes compare." A slope close to unity implies that the two processes are homoenergetic, i.e., the free energy changes are the same. Furthermore, if the goal is the determination of the "classical" $logD_{oct}$, then a slope significantly different from 1 would enhance any error in the determination of $logD_{oct}$. A slope significantly different from unity is an indication of a fairly large over- or underestimation of lipophilicity by the method. Obviously, if a different scale of lipophilicity is the goal, log k'_w values could be used as such, or different indices could be developed. Valkó et al.[21] described a chromatographic hydrophobicity index (CHI), obtained via a gradient run. In this case a correlation with a "classical" shake flask $logP_{oct}$ was not necessarily sought, and a self-consistent CHI scale was established. However, $logD_{oct}$ data are so widely used in many correlations by the medicinal chemistry community that a "classical" $logD_{oct}$ value is likely to be desired. To the best of our knowledge no other method capable of encompassing all the accuracy and ruggedness requirements we set as goals for this work, including a very practical set of conditions and speed, has been reported in the literature to date. By analogy with our previous work[16] we termed the values obtained via eq 1 as $ElogD_{oct}$, and we will refer to them as such for the rest of the discussion.

A plot of residuals vs $logD_{oct}$ values, as in Figure 2, shows that the error distribution is very consistent across the entire range, and no curvature (larger error) is observed at extreme values. This is important because it shows that similarly accurate determinations can be obtained across a dynamic range of 7 $logD_{oct}$ units, and the standard error is fairly small, considering also the variability of some of the $logD_{oct}$ data reported in the literature.

The question might be asked, about whether decylamine acts as a modifier other than a masking agent for potentially ionized silanols, since its absence is detrimental to the performance of the method. A comparison between log k'_w values obtained under the conditions reported in our previous work in 36 neutral solutes,[16] termed here log k'_w(P), and the values obtained under the present condition for the same solutes, i.e., log k'_w(D), shows that the balance of forces is the same, as demonstrated by a very small intercept (nonsignificant) and a slope very close to unity, in eq 2. Since

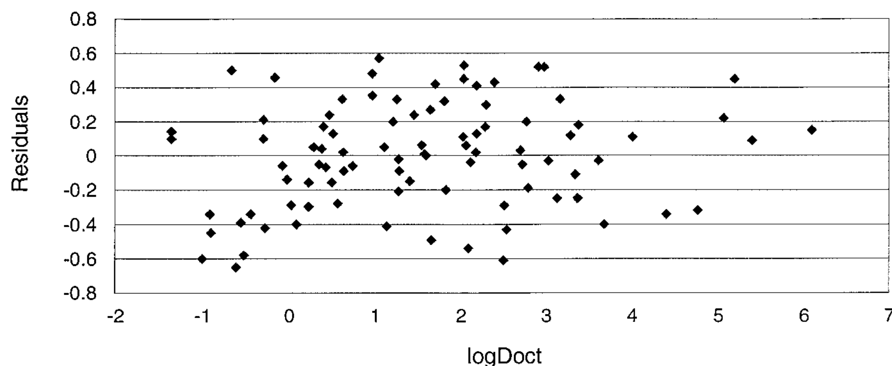

Figure 2. Plot of residuals vs $logD_{oct}$.

Table 2. Extrapolated Capacity Factors and $logD_{oct}$ Data for 10 Proprietary Compounds

compd	log K'_w[a]	sd[b]	$ElogD_{oct}$[c]	$logD_{oct}$[d]	residuals[e]
1	0.15	0.07	0.38	0.31	−0.07
2	0.79	0.03	1.10	1.16	0.06
3	2.75	0.25	3.31	3.20	−0.11
4	0.30	0.06	0.55	0.68	0.13
5	1.14	0.12	1.49	1.66	0.17
6	2.17	0.10	2.65	2.19	−0.46
7	3.46	0.02	4.11	3.37	−0.74
8	2.70	0.01	3.25	2.58	−0.67
9	3.68	0.16	4.35	3.85	−0.50
10	2.14	0.06	2.62	2.10	−0.52

[a] Average of three to five determinations. [b] Standard deviation for log K'_w determinations. [c] Predicted $ElogD_{oct}$ from eq 1. [d] Data from shake-vial and/or potentiometric determinations. The average was taken if more than one value was available. [e] Average residual, − 0.27. $ElogD_{oct}$ vs $logD_{oct}$: $R^2 = 0.969$.

we have demonstrated that log K'_w under our previous conditions encodes the same balance of forces as in a biphasic octanol−water system,[16] we conclude that the values obtained by this method are "true" $logD_{oct}$ values.

$$\log K'_w(\mathrm{P}) = 1.0429\ (\pm\ 0.0241) \log K'_w(\mathrm{D}) - 0.0219\ (\pm\ 0.0522) \quad (2)$$

$$N = 36,\ R^2 = 0.982,\ R = 0.991,\ s = 0.198,\ F = 1866,\ q^2 = 0.980$$

We have also tested a set of 10 proprietary compounds, basic and neutral, with molecular weights between 209 and 532 Da. These compounds are structurally dissimilar from the compounds in the training set and possess a wide range of functional groups. The results of the ElogD determinations are compared, in Table 2, with shake-vial and/or potentiometric determinations, and they show a good performance of the method for compounds **1**−**10**.

As in our previous method,[16] we used control charts as measure for a day-to-day system suitability check, which were constructed for 10 compounds chosen across the entire range and which are indicated in Table 1 (see Statistical Analysis section). An unexpected variation in these plots would immediately "flag" questionable results. Furthermore, we find that triflupromazine (CAS no. 146-54-3) offers a very sensitive "probe" for the column performance monitoring, as well as its analogue chlorpromazine (CAS no. 50-53-3). A decline in the log K'_w value of either of these two compounds is a good indication of column deterioration. A log K'_w value for triflupromazine below 2.7 (2.6 for chlorpromazine) would indicate that the column should be replaced.

Currently, the ElogD determinations are run with the aqueous portion of the mobile phase prepared by a commercial laboratory, according to our specifications, and in fairly large batches (>20 gallons), and we have noticed no significant difference in performance, after an initial adjustment of pH as needed. This practical enhancement helps with the speed of analysis, and it may also be taken as an indication of the ruggedness of the method.

As a further improvement, in terms of speed of analysis, we have also attempted to increase the flow rate, and we have further automated the calculation procedure through in-house software to obtain the final $ElogD_{oct}$ value, with very limited manual intervention, directly from the chromatographic data file. These modifications allow for an enhanced throughput, starting with an already rapid procedure. $ElogD_{oct}$ data for any compound are obtained, on average, in ≤20 min, on a single instrument. Equation 3 shows a high correlation between the log K'_w values obtained with the "standard" flow rate (sf, 1.0 mL/min) and the faster flow rates (ff, 1.5 mL/min), used for a "mixed" set of data comprising 56 proprietary and commercial compounds, using the same compounds under each condition, and encompassing roughly 3 log K'_w units, largely across the medium range defined in the Experimental Section. We have not yet implemented this flow rate in our routine work, while we have run over 2000 compounds with the "standard" flow rates.

$$\log K'_w(\mathrm{sf}) = 0.8823\ (\pm\ 0.0378) \log K'_w(\mathrm{ff}) + 0.1474\ (\pm\ 0.0654) \quad (3)$$

$$N = 56,\ R^2 = 0.910,\ R = 0.954,\ s = 0.175,\ F = 544,\ q^2 = 0.9$$

Similarly, we have obtained good results by increasing the flow rate of the high lipophilicity range, from 2 to 3 mL/min (data not shown).

As a further caveat with the use of shake methods we report the widely different results we obtained with guanoxan (a guanidine derivative, CAS no. 5714-04-5) for which a value of −0.83 was reported as $logD_{oct}$.[22] Using the shake-vial procedure B (see Experimental Section), values of −0.1 and −0.3 were obtained, in duplicate determinations. In a fairly extensive $logD_{oct}$ vs concentration study, using the shake-vial procedure A, and we found that the values ranged from −1.6 to −1.0 upon decreasing the concentration, from 1.5 to 0.1 mg, in a 50:2 octanol:buffer system. Indeed Perlman[23]

has reported a large variation in the $\log D_{oct}$ values of diarylguanidines, up to 2 $\log D_{oct}$ units depending upon the counterion present, and that might be the case here. Under our conditions we found an $E\log D_{oct}$ value of −0.3, which is in very close agreement with the data from shake-flask procedure B. However, it deviated significantly from the values from procedure A, even at the lowest concentration we have reached, and might be borderline acceptable for estimation, against the literature data.

The present chromatographic method is not usable, at the moment, for the determination of the $E\log D_{oct}$ of acidic compounds (significantly ionized at pH 7.4). However, the method offers high accuracy for compounds which are devoid of any significant ionization and thus are not amenable to a $\log D_{oct}$ determination via well-known potentiometric techniques.[24] It also offers high accuracy, together with the other characteristics of the RP-HPLC method, for compounds having low solubility in water, without recourse to mixed solvents and extrapolation, as in shake-flask and potentiometric techniques.

Conclusion

We have demonstrated that, by a judicious choice of mobile phase and RP-HPLC column, a very accurate $E\log D_{oct}$ determination method, for basic and neutral compounds, could be developed. This method responds to the criteria of rapid throughput, ruggedness, and minimal manual intervention set forth in the Introduction, for drug-like compounds. We have also offered some practical considerations to help its application to high-throughput screening. Since $\log D_{oct}$ has been shown to be an important parameter for the ADME, QSAR, and QSPR profiling of newly synthesized compounds,[3,5] we believe this method will find useful applications in pharmaceutical discovery and development settings. As a final comment we note that, with a minor adjustment of pH, the method should be amenable of use for the determination of $E\log D_{oct}$ at pH 6.5, thus finding application in correlations specifically involving intestinal absorption.[5]

Experimental Section

Materials and Methods. Most the solutes were purchased directly from commercial sources (Aldrich, Fluka, ICN, RBI, Sigma, Tocris) and used as received, in all cases. In several cases they were available through our Materials Management group as either proprietary compounds or samples extracted from commercial formulations. Deionized water and HPLC grade methanol (J. P. Baker) and 1-octanol (Fisher Scientific) were used throughout.

The mobile phase consisted, in all cases, of 20 mM MOPS buffer at pH 7.4, with the addition of 0.15% of *n*-decylamine[12,13] and methanol in varying proportions from 70 to 15% v/v. A 0.25% (v/v) amount of octanol was added to methanol, and octanol-saturated water was used to prepare the buffer. The mobile phase is now routinely prepared, according to our specifications, in larger batches, by Brand-Nu Labs, Meriden, CT.

The capacity factors data ($k' = (t_R - t_0)/t_0$), obtained at various amounts of methanol, were then extrapolated to 0% methanol and reported as $\log k'_w$, using a linear procedure. In all cases, except for allopurinol ($R^2 = 0.96$), the square of the correlation coefficient was 0.99. Injections of pure methanol were used to determine t_0, i.e., the dead time, while t_R has the usual meaning of the retention time for the analyte. For very low $\log D_{oct}$ compounds, atenolol (CAS no. 29122-68-7) was used to determine t_0 and is now used routinely for the low range.

All the chromatographic runs were performed on an Agilent 1100 HPLC ChemStation at the ambient temperature. The HPLC column used was Supelcosil LC-ABZ, 5 μm, 4.6 × 50 mm. A diode array detector was used to monitor signals at 235, 255, 265, 275, and 310 nm. We also tested columns manufactured from different silica bond lots to ensure reproducibility. Samples were dissolved in 1:1 methanol/water in a concentration range of 50−100 μg/mL. The flow rate was 0.5, 1, or 2 mL/min, depending on the lipophilicity range. Three experimental lipophilicity ranges were established using, in all cases, three points for the extrapolation to k'_w, as described in the table below.

$E\log D_{oct}$ range	flow rate (mL/min)	% MeOH
<1	0.5	15, 20, 25
1−3	1	40, 45, 50
>3	2	60, 65, 70

The samples are placed in the appropriate range by estimating their lipophilicity via computed values or by prior experience with a given class. Experimental values obtained from runs outside the appropriate range are typically run de novo. However, a "screen" using a single injection at 75% methanol can be performed to "weed out" high lipophilicity compounds. If the retention time is >1.1 min, at a flow rate of 2 mL/min, the compound would likely yield an $E\log D_{oct} > 5$. The user may then decide to adjust the conditions for that compound, such as the duration of each run, or to use such an estimated value, thus increasing the throughput and guarding against potential carry-overs.

In each case the entire group of samples is run before the column is equilibrated to the next condition, in an automated fashion. We typically start from the high range (highest methanol content) and run, sequentially, all the compounds. For the low range it was found that a period of equilibration between 1.5 and 2 h is needed. At the end of a complete run the column is flushed with acetonitrile, at 2.0 mL/min, for 10−20 min.

The data analysis is then performed via an automated procedure relying on in-house software, which yields the $E\log D_{oct}$ values (see Results and Discussion section), directly from the chromatographic data files.

The shake-flask $\log D_{oct}$ data, and in some cases data from countercurrent chromatography, were taken from the literature, after careful evaluation of the experimental method and temperature reported (generally between 20 and 25 °C) in the original references or they were determined in-house. In some cases, data were not available or could not be determined experimentally due to the high lipophilicity of the compound. In such cases (clotrimazole and tolnaftate), a computed value was used. The shake-vial experimental measurements performed in-house (Procedure A) were all conducted at least in duplicate, in amber glass vials, and in some cases, with varying ratios of octanol and MOPS buffer, always mutually presaturated prior to the experiment. The vials were shaken at least overnight. HPLC analysis at different wavelengths, after centrifugation and separation of the phases, was used for the quantitative analysis, and both phases were analyzed. In some cases compounds were sent to PGRD, Sandwich Laboratories, Sandwich, U.K., for a semiautomated $\log D_{oct}$ shake-vial determination, using a phosphate buffer at pH 7.4 as the aqueous phase. In this case (Procedure B), a 1:1 ratio of *n*-octanol and buffer (both phases were mutually presaturated) was used, generally with an agitation time ≥30 min, followed by centrifugation and analysis of both phases.

In several instances, as indicated in Table 1, the data were obtained from pH-metric determinations performed by pIon Inc., Woburn, MA, after submission of commercial or proprietary samples.

Statistical Analysis. All regression analyses were performed via the JMP statistical software package (v. 3.2.1, SAS

Institute Inc.). Ten compounds were selected across the set of 90 compounds, covering the entire range of lipophilicity, to monitor the day-to-day performance of the method. Statistical calculations showed that the use of the 10 compounds would ensure that the estimated slope, in the final regression equation, would be within ±0.09 of true one. The JMP software was also used for the quality monitoring. Data accumulated for the standard set of compounds and regularly plotted on the control charts constitute a powerful method for the detection of trends and variations in performance. Variations in log k'_w values, for the selected compounds, should not exceed $\pm 3k_s$, where k_s is the standard deviation estimate based on data collected under well controlled experiments.[16] Triflupromazine log k'_w is recorded for each run to ensure that the column is performing suitably.

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