$ElogP_{oct}$: A Tool for Lipophilicity Determination in Drug Discovery†

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We present an RP-HPLC method, for the determination of $logP_{oct}$ values for neutral drugs, which combines ease of operation with high accuracy and which has been shown to work for a set of 36 molecules comprised largely of drugs. The general features of the method are as follows: (i) compound sparing (≤1 mL of a 30−50 μg/mL solution needed), (ii) rapid determinations (20 min on average), (iii) low sensitivity to impurities, (iv) wide lipophilicity range (6 $logP_{oct}$ units), (v) good accuracy, (vi) excellent reproducibility. A linear free energy relationship (LFER) analysis, based on solvation parameters, shows that the method encodes the same information obtained from a shake-flask $logP_{oct}$ determination. 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 $ElogP_{oct}$.

Introduction

The importance of $logP_{oct}$ is underscored, for example, by the generally observed correlation between high lipophilicity ($logP_{oct}$ > 4.5) and poor solubility.[1] Furthermore, lipophilicity is of paramount importance in several other ADME aspects, that is, absorption, distribution, metabolism, and excretion.[2] 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. A large volume of distribution, probably due to a high fraction of the compound bound to tissues, is often observed for lipophilic compounds. Thus, a method that can accurately and rapidly yield $logP_{oct}$ values would be a welcome addition to the experimental tools available for physicochemical properties screening in the discovery setting.

We note here that, for drug molecules, computed values are often inaccurate and, depending on the software used, they may differ by as much as two $logP_{oct}$ units, among different software packages and from the experimental values, for an entire class of compounds. 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 by using minuscule amounts of compound, the computed values should be replaced by measured values, especially in the case of intramolecular H-bonding and/or conformationally flexible compounds. SAR analyses and even alerts such as the Lipinski "rule of 5"[3] would greatly benefit by the introduction of accurate experimental values.

The classical shake-flask method, or variations of this method which have been described,[4] are neither rugged nor rapid enough for medium- to high-throughput applications, they are generally more sensitive to impurities, less amenable to automation than are RP-HPLC methods, and they do not usually offer a wide dynamic range.

RP-HPLC retention data have been shown to correlate well with absolute and relative lipophilicity values[5] but, perhaps not surprisingly, they have also been criticized as not being a true "replacement" for shake-flask values.[4] Part of the criticism might be thought as stemming from the fact that many reports were limited in their scope, focusing either on fairly simple monofunctional solutes[6,7] or on classes of analogues[8] with a limited $logP_{oct}$ range. When these correlations were extended beyond classes of drug-like analogues, less than encouraging results were obtained.[5] 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 $logP_{oct}$. In particular, log k' values respond to solute hydrogen-bond acidity, but $logP_{oct}$ values do not.[9] 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. These observations pointed us toward using the extrapolated value, rather than, as reported by Yamagami and co-workers,[10] 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. We shall return to the issue of the slope later but, as we note here and despite the criticism discussed, we are of the opinion that, with a judicious choice of conditions, RP-HPLC methods might be defined, following Taylor,[11] as "being in a class of their own".

Another factor of great importance, and perhaps of

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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, that is, the reproducibility of the packing chemistry and manufacturing. It might be argued that a well-kept column would last for a considerably long period of time, but it is essential to know that pooling data from different columns will not be difficult, although "scaling standards" might be used across columns. However, tests of reproducibility of the method from column to column should be performed, independently of the above arguments.

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 fairly modest resources, a fairly 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, albeit neutral ones in the present work. We present and discuss our results in the following sections.

Results and Discussion

Minick at al.[7,12] had used a MC-8 column, coupled with the addition of 1-octanol to both components of the eluent, together with the addition of small amounts of *n*-decylamine in the aqueous phase. We reasoned that an electrostatically coated silica phase[13] (such as the LC-ABZ column) might not require a modifier such as *n*-decylamine. At the same time octanol might be beneficial to reproduce the intermolecular interactions experienced by a solute in a classical "shake-vial" octanol/buffer partition determination, and it might reduce (however slightly) the retention times. Thus we "combined" the column used by Pagliara et al.[6] with the conditions used by Minick et al.,[7] with the exception of the use of *n*-decylamine, and explored its application to a set of diverse drug-like compounds. To maximize the speed of analysis, while still retaining a good accuracy, an appropriate flow rate was chosen for each range of lipophilicity (see Experimental Section), and this choice was combined with the use of a fairly short column to further enhance the throughput of the method.

The data we obtained for an initial set of 27 compounds, in the absence or presence of octanol, pointed to a clear difference in performance, as shown by Figures 1 and 2, and by their respective equations. The compounds used are the first 27 compounds reported in Table 1. The data used in Figure 1 were generated using only methanol and MOPS buffer as the mobile phase, as opposed to Figure 2, where octanol was added to methanol, and octanol-saturated water was used to prepare the buffer. In both cases the flow rate was 1 mL/min. Different columns were used for these runs, but the intercolumn variability was checked (via the protocol used in Figure 2, data not shown), and an excellent correlation was obtained for the three different columns used. In one case, the same column was tested

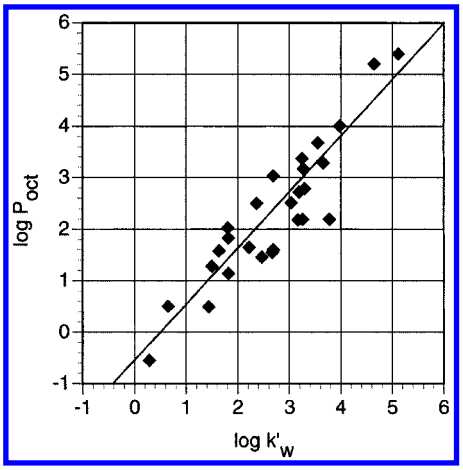

Figure 1. Correlation between $\log P_{oct}$ and k'_w for 27 solutes, in the absence of octanol.

initially and after 4 months of intensive use, and no significant difference was found. Therefore, we concluded that the difference in performance was brought about by the addition of octanol to both components of the mobile phase and not by potential differences in the column packing among individual columns.

Figures 1 and 2 clearly bring out the significant difference between the addition of 1-octanol to the mobile phase (both components), with respect to the data generated in its absence. In both cases the slope is close to unity, but the error is more than doubled in the absence of 1-octanol as expressed by eq 1 (no added octanol) and eq 2 (added octanol). In both cases a range of 6 $\log P_{oct}$ units is encompassed by these experiments.

$$\log P_{oct} = 1.0890(\pm 0.0969) \log k'_w - 0.5435(\pm 0.2768) \quad (1)$$

$$N = 27,\ R^2 = 0.835,\ R = 0.914,\ s = 0.556,\ F = 126,\ q^2 = 0.808$$

$$\log P_{oct} = 1.1014(\pm 0.0389) \log k'_w - 0.0045(\pm 0.0941) \quad (2)$$

$$N = 27,\ R^2 = 0.970,\ R = 0.985,\ s = 0.238,\ F = 803,\ q^2 = 0.965$$

The question of the diagnostic importance of the slope has been stressed by Minick et al.[12] Pointing to the work of Melander et al.,[14] these authors state that "...equations correlating log k'_w and $\log P_{oct}$ 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. In our case a slope very close to unity is obtained, with or without 1-octanol. Furthermore a larger slope would result in the magnification of any error in the determination of log k'_w if the estimation of $\log P_{oct}$ was the final goal. The log k'_w data could be used as a self-consistent scale of lipophilicity and, in this case, the second consideration would not be very important. An example might be represented by the work of Valkó et al.,[15] which describes a chromatographic hydrophobicity index (CHI), obtained via a gradient run. In this case a correlation with a

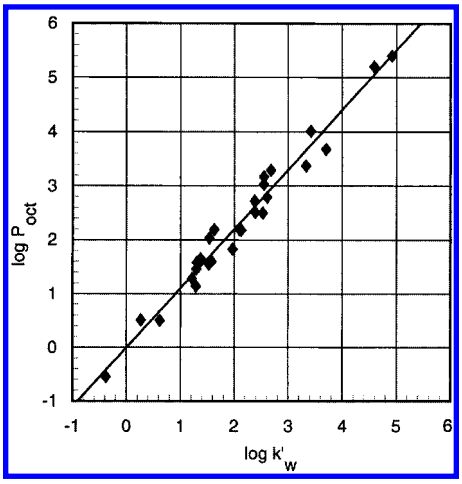

Figure 2. Correlation between $\log P_{oct}$ and k'_w for 27 solutes, in the presence of octanol.

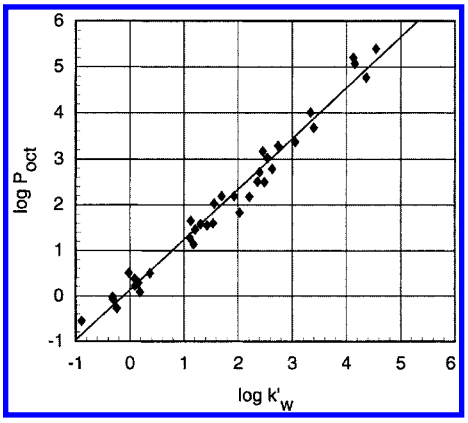

Figure 3. Correlation between $\log P_{oct}$ and k'_w for 36 solutes.

"classical" shake-flask $\log P_{oct}$ was not necessarily sought, and a self-consistent CHI scale was established. However, $\log P_{oct}$ (or $\log D_{oct}$) data are so widely used in many correlations by the medicinal chemistry community that a "classical" $\log P_{oct}$ 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 has been reported in the literature to date.

Armed with these very good results, we sought to expand the correlation by adding more drug compounds, aiming at extending the physicochemical diversity of the set. We added nine more compounds, as reported in Table 1, and sought, at the same time, an analysis of the data via the well-known solvation parameters of Abraham.[16,17] The solvation analysis was performed to answer the question of closeness between the classical shake flask $\log P_{oct}$ values,and our RP-HPLC derived values, beyond what could be surmised by a slope close to unity.

Figure 3 shows, graphically, the correlation between the shake-flask and the corresponding RP-HPLC data. Also, the slope in eq 3 is very similar to the slope in eq 2, further demonstrating the accuracy of the method and yielding an indirect assessment of its robustness in

Table 1. Retention Time and $\log P_{oct}$ Data for the 36 Solutes Used

compound	CAS no.	log k'_w[a]	Elog P_{oct}[b]	log P_{oct}[c]	residuals	refs[d]
3,5-dichlorophenol	591-35-5	3.40	3.88	3.68	−0.20	11
3-bromoquinoline	5332-24-1	2.54	2.93	3.03	0.10	11
3-chlorophenol	108-43-0	2.49	2.88	2.50	−0.38	20
acetaminophen	103-90-2	−0.02	0.11	0.51	0.40	21
acetophenone	98-86-2	1.31	1.58	1.58	0.00	20
allopurinol	315-30-0	−0.89	−0.85	−0.55	0.30	22
bromazepam	1812-30-2	1.13	1.38	1.65	0.27	23
carbamazepine	298-46-4	1.70	2.01	2.19	0.18	24
chloramphenicol	56-75-7	1.18	1.43	1.14	−0.29	25
clotrimazole	23593-75-1	4.13	4.69	5.20	0.51	*e*
dexamethasone	50-02-2	2.03	2.37	1.83	−0.54	26
diazepam	439-14-5	2.63	3.03	2.79	−0.24	27
estradiol	50-28-2	3.34	3.82	4.01	0.19	25
fluconazole	86386-73-4	0.37	0.54	0.50	−0.04	19
griseofulvin	126-07-8	2.21	2.57	2.18	−0.39	25
hydrocortisone	50-23-7	1.43	1.71	1.55	−0.16	26
hydrocortisone-21-acetate	50-03-3	1.93	2.26	2.19	−0.07	26
lorazepam	846-49-1	2.36	2.74	2.51	−0.23	28
lormetazepam	848-75-9	2.40	2.78	2.72	−0.06	29
naphthalene	91-20-3	3.06	3.51	3.37	−0.14	11
nifedipine	21829-25-4	2.46	2.85	3.17	0.32	this work
nifuroxime	6236-05-1	1.11	1.36	1.28	−0.08	this work
prednisolone	50-24-8	1.54	1.83	1.60	−0.23	this work
prednisone	53-03-2	1.21	1.47	1.46	−0.01	25
quinoline	91-22-5	1.56	1.85	2.03	0.18	30
testosterone	58-22-0	2.74	3.15	3.29	0.14	26
tolnaftate	2398-96-1	4.55	5.15	5.40	0.25	*e*
antipyrine	60-80-0	0.09	0.23	0.38	0.15	31
bifonazole	60628-96-8	4.37	4.95	4.77	−0.18	25
caffeine	58-08-2	−0.31	−0.21	−0.07	0.14	25
diethylstilbestrol	56-53-1	4.16	4.72	5.07	0.35	25
methylthioinosine	342-69-8	0.19	0.34	0.09	−0.25	this work
metronidazole	443-48-1	−0.32	−0.22	−0.02	0.20	32
nitrofurazone	59-87-0	0.10	0.24	0.23	−0.01	33
pentoxifylline	6493-05-6	0.16	0.31	0.29	−0.02	34
thiamphenicol	15318-45-3	−0.24	−0.13	−0.27	−0.14	35

[a] Average of at least three determinations on different LC-ABZ columns (average s.d. = 0.119). [b] Data from eq 3. [c] Shake-flask data. [d] References for shake-flask data. [e] Computed values using ClogP 3.55 v.210.

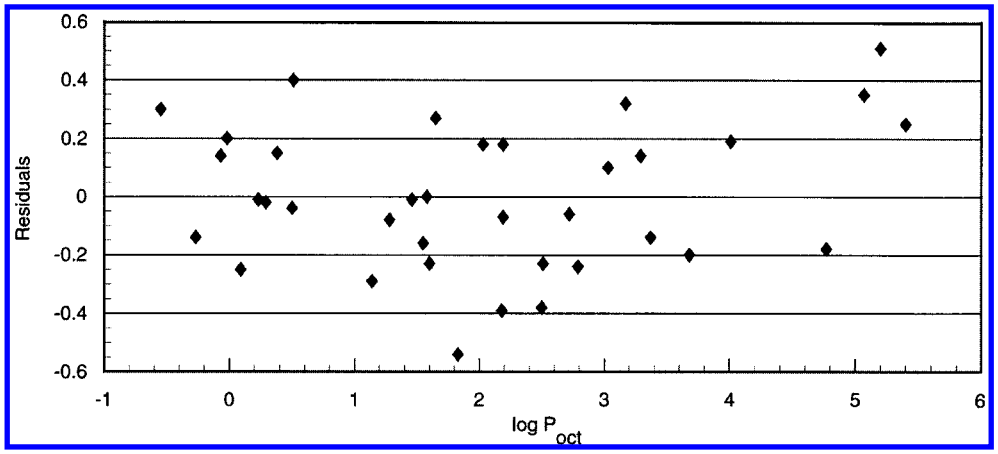

Figure 4. Plot of residuals vs $\log P_{oct}$.

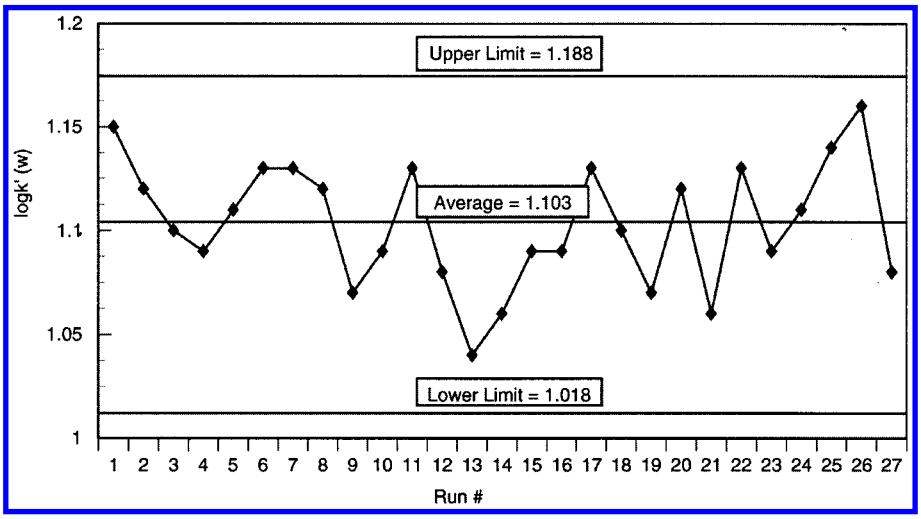

Figure 5. Control plot for nifuroxime.

predicting unknown compounds. The cross-validated coefficient of determination (leave-one-out), or q^2 value, shows the excellent predictive power of this method. The correlation was obtained by averaging the values determined, for each compound, using a minimum of three different columns, with an average standard deviation of 0.1187.

$$\log P_{oct} = 1.1021(\pm 0.0291) \log k'_w + 0.1344(\pm 0.0653) \quad (3)$$

$$N = 36,\ R^2 = 0.977,\ R = 0.988,\ s = 0.251,\ F = 1434,\ q^2 = 0.974$$

Furthermore it is worth noting that a plot of residuals vs the $\log P_{oct}$ values, as in Figure 4, 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 6 $\log P_{oct}$ units.

As a further measure for a day-to-day system suitability check, we used control charts, constructed for 10 compounds suitably chosen across the entire range, as shown in Figure 5 for nifuroxime (see Statistical Analysis section). An unexpected variation in these plots would immediately "flag" questionable results.

We termed the values obtained via eq 3 as $\text{Elog}P_{oct}$, and we will refer to them as such for the rest of the discussion.

We then turned our attention to an analysis of the balance of forces underlying these phenomena, using a linear free energy relationship, based on solvation parameters, as described by Abraham.[16,17] In these equations R_2 is the excess molar refraction, π_2^H is the dipolarity/polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ are the (summation) hydrogen bond acidity and basicity, respectively, and V_X is the McGowan's volume. In particular, the superscript "0", used for the hydrogen bond basicity parameter, refers to a particular scale of values, useful for certain types of solutes, when the organic portion of the binary system is a partially water miscible solvent, as in the case of water-saturated octanol. The other subscripts and superscripts have the usual meaning, i.e., they refer to hydrogen-bonding scales and to the solute.[16a] The calculated parameters are reported in Table 2, and the coefficients for the respective equations (eq 4 and 5), whether $\log k'_w$ or $\text{Elog}P_{oct}$ values are used, are very close to the coefficients of the equation based on shake-flask $\log P_{oct}$ values (eq 6), as reported for 613 solutes,[17] as well as to the equation based on shake-flask values for the 35 solutes examined (eq 7). We note that (i) the correlation matrix (Table 3) is satisfactory and (ii) the comparison between eqs 4 and 5 shows that the correlation between $\log k'_w$ and the shake-flask values relies on the *same* balance of forces

Table 2. Solvation Parameters for 35 Solutes

compound	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$	V_X
3,5-dichlorophenol	1.02	1.00	0.91	0.00	1.0199
3-bromoquinoline	1.64	1.23	0.00	0.42	1.2193
3-chlorophenol	0.91	1.06	0.69	0.15	0.8975
acetominophen	1.06	1.63	1.04	0.86	1.1724
acetophenone	0.82	1.01	0.00	0.48	1.0139
allopurinol	1.41	1.55	0.70	0.92	0.8818
bromazepam	2.31	1.38	0.33	1.62	1.9445
carbamazepine	2.15	2.07	0.52	1.13	1.8106
chloramphenicol	1.85	0.72	0.34	2.09	2.0728
clotrimazole	2.55	2.60	0.00	1.08	2.6230
dexamethasone	2.04	3.51	0.71	1.92	2.9132
diazepam	2.08	1.57	0.00	1.25	2.0739
estradiol	1.80	1.77	0.86	1.10	2.1988
fluconazole	2.34	2.80	0.47	1.65	2.0064
griseofulvin	1.75	2.69	0.00	1.50	2.3947
hydrocortisone	2.03	3.49	0.71	1.90	2.7976
hydrocortisone-21-acetate	1.89	2.88	0.46	2.16	3.0951
lorazepam	2.51	1.28	0.45	1.63	2.1141
lormetazepam	2.44	1.65	0.12	1.61	2.2550
naphthalene	1.34	0.92	0.00	0.20	1.0854
nifedipine	1.50	2.45	0.23	1.45	2.4945
nifuroxime	1.13	0.98	0.69	0.60	0.9669
prednisolone	2.21	3.10	0.71	1.92	2.7546
prednisone	2.14	3.58	0.36	1.89	2.7116
quinoline	1.27	0.97	0.00	0.54	1.0443
testosterone	1.54	2.59	0.32	1.19	2.3827
tolnaftate	2.97	2.20	0.00	0.93	2.3949
antipyrine	1.32	1.50	0.00	1.48	1.5502
bifonazole	2.41	2.25	0.00	1.12	2.5006
caffeine	1.50	1.60	0.00	1.33	1.3632
diethylstilbestrol	1.60	1.75	1.26	0.77	2.2440
metronidazole	1.05	1.60	0.18	1.03	1.1919
nitrofurazone	1.65	1.79	0.40	1.08	1.2644
pentoxifylline	1.64	2.28	0.00	1.84	2.0834
thiamphenicol	2.26	3.30	0.90	2.03	2.3204

Table 3. Correlation Matrix for the 35 Solutes in Table 2[a]

	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$	V_X
R_2	0.234	0.017	0.325	0.519
π_2^H		0.024	0.434	0.630
$\Sigma\alpha_2^H$			0.001	0.001
$\Sigma\beta_2^0$				0.576

[a] R^2 values.

encoded by the two parameters, beyond a slope of unity. Furthermore any cross-correlation of the parameters, for the data reported in this paper, does not affect the coefficients of eq 7 as compared to eq 6. Also, the ratios of coefficients, normalized to the V_X coefficient, are essentially identical for all four equations (data not shown). We conclude that the ElogP$_{oct}$ values, obtained using the method presented here, and expressed by eq 3, are identical to shake-flask values, but they can be generated at a much higher throughput, and combine ease of operation with a wide dynamic range. Due to lack of partition data in other solvent pairs, we were not able to obtain accurate parameters for methylthioinosine, and this solute was excluded from the correlation.

$$\log k'_w = 0.066(\pm0.144) + 0.409(\pm0.106)R_2 - 0.955(\pm0.078)\pi_2^H - 0.038(\pm0.109)\sum\alpha_2^H - 3.092(\pm0.101)\sum\beta_2^0 + 3.484(\pm0.127)V_X \quad (4)$$

$$N = 35,\ R^2 = 0.980,\ R = 0.990,\ s = 0.221,\ F = 288,\ q^2 = 0.970$$

$$E\log P_{oct} = 0.204(\pm0.015) + 0.452(\pm0.117)R_2 - 1.053(\pm0.086)\pi_2^H - 0.041(\pm0.120)\sum\alpha_2^H - 3.410(\pm0.111)\sum\beta_2^0 + 3.842(\pm0.104)V_X \quad (5)$$

$$N = 35,\ R^2 = 0.980,\ R = 0.990,\ s = 0.244,\ F = 288,\ q^2 = 0.968$$

$$\log P_{oct} = 0.088 + 0.562R_2 - 1.054\sum\pi_2^H - 0.034\sum\alpha_2^H - 3.460\sum\beta_2^0 + 3.814V_X \quad (6)$$

$$N = 613,\ R^2 = 0.995,\ R = 0.997,\ s = 0.116,\ F = 23162$$

$$\log P_{oct} = 0.273(\pm0.103) + 0.525(\pm0.076)R_2 - 1.034(\pm0.056)\pi_2^H - 0.049(\pm0.078)\sum\alpha_2^H - 3.510(\pm0.072)\sum\beta_2^0 + 3.787(\pm0.091)V_X \quad (7)$$

$$N = 35,\ R^2 = 0.992,\ R = 0.996,\ s = 0.16,\ F = 705$$

As a further improvement we have also automated the calculation procedure, through in-house software, to obtain the final ElogP$_{oct}$ value, without any manual intervention, directly from the chromatographic data file. This modification allows for an enhanced throughput, starting with an already rapid procedure. ElogP$_{oct}$ data for any compound are obtained, on average, in 20 min or less, on a single instrument.

The current method is, at the moment, limited to neutral (and weakly acidic or basic) compounds, but its development covers the practical limitation involved in the determination of compounds which are devoid of any significant ionization, and thus are not amenable to a logP$_{oct}$ determination via well-known potentiometric techniques.[18]

Conclusion

We have demonstrated that, by a judicious choice of mobile phase and RP-HPLC column, a very accurate ElogP$_{oct}$ determination method could be developed which responds to the criteria of rapid throughput, ruggedness, and minimal manual intervention set forth in the Introduction, for drug-like compounds. Since logP$_{oct}$ has been shown to be an important parameter for the ADME profiling of newly synthesized compounds,[3] such as estimation of solubility, intestinal permeability, and clearance, we believe this method will find useful applications in pharmaceutical discovery and development settings. Work is in progress to find a similarly rugged and efficient method for the determination of logD$_{oct}$ values, at pH 7.4. These findings will be reported in due course.

Experimental Section

Materials and Methods. All the solutes were purchased directly from commercial sources (Aldrich, Sigma, and Fluka) and used as received, in all cases. Fluconazole was obtained internally. Deionized water, 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 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, with the exception of the correlation obtained without octanol in either component of the mobile phase (vide

infra). 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 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.

All the chromatographic runs were performed on a HP-1100 HPLC ChemStation at the ambient temperature. The HPLC columns used were Supelcosil LC-ABZ, 5 μm, 4.6 × 50 mm. A diode array detector was used to monitor signals at 235, 255, 265, and 275 nm. We also tested columns having different silica bond lots to ensure reproducibility. Samples were dissolved in 1:1 methanol/water in a concentration range of 10−60 μg/mL. The flow rate was 0.5, 1, or 2 mL/min, depending on the lipophilicity range. Three lipophilicity ranges were established using, in all cases, three points for the extrapolation to k'_w, as described in the table below.

$logP_{oct}$ range	flow rate (mL/min)	% MeOH
−0.5 to 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. In each case the entire group of samples is run before the column is equilibrated to the next condition, in an automated fashion. The data analysis is automated via in-house software yielding the $ElogP_{oct}$ values (see Results and Discussion section), directly from the chromatographic data files.

The shake-flask $logP_{oct}$ data were taken from the literature, after careful evaluation of the experimental method reported in the original references, or they were determined in-house, except when data were not available and they 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 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.

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 36 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 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. A control chart for nifuroxime is shown in Figure 5.

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