High-Throughput log *P* Determination by Ultraperformance Liquid Chromatography: A Convenient Tool for Medicinal Chemists

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Abstract: Accurate determinations of lipophilicity indices benefit from recent advances in chromatographic sciences such as the launch of ultraperformance liquid chromatography (UPLC). The fast strategy presented here emerges as a powerful method suitable for high-throughput log *P* measurements of therapeutic compounds in isocratic and gradient modes. Because UPLC columns are highly stable in basic pH conditions, this approach allows a direct lipophilicity estimation of basic compounds in their neutral forms.

Precise knowledge of the physicochemical properties of new chemical entities (NCEs[a]) in early steps of drug design and discovery is of prime importance.[1,2] Among these properties, lipophilicity is a key parameter involved mainly in pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) and in ligand−target interactions.[3] Moreover, lipophilicity is the molecular parameter of choice in numerous (quantitative) structure–activity relationships ((Q)SAR) of different classes of compounds.[4] Thus, methods were developed to rapidly predict lipophilicity of new chemical entities from their chemical structures.[5,6] These valuable in silico approaches are unfortunately characterized by some well-known limitations such as uncertain predictions for innovative molecular fragments or a low accuracy due to the neglect of molecular 3D structure effect in the lipophilicity prediction.[7] In this context, accurate and fast experimental methods are mandatory to optimize in silico methods and build chemical libraries of experimental lipophilicity data.

The advantages of RPLC (reversed phase liquid chromatography) techniques for fast lipophilicity indices measurements are recognized.[8–14] In particular, they are characterized by low sample consumption, insensitivity to impurities, and automation possibilities. However, the benefits of this technique are partially reduced by the restricted application field of RPLC methods (i.e., not appropriate for determining partition coefficients of highly lipophilic compounds because of very long analysis time). In addition, the interest of RPLC for determining partition coefficients of highly basic compounds remains limited because of the silica-based stationary phases instability in high pH conditions.[15,16]

Liquid chromatography (LC) has recently evolved, particularly with the development of short columns packed with small particles (<2 μm) used in very high-pressure conditions (>5800 psi). This technology, called ultraperformance liquid chromatography (UPLC) and initially commercialized by Waters, enables a significant analysis time reduction without compromising chromatographic performance.[17] Two interesting features of UPLC, namely, the short analysis time and high chemical stability of stationary phases, make it attractive to medicinal chemists for high-throughput lipophilicity determination of NCEs. In this paper UPLC was used for the first time to determine partition coefficients in *n*-octanol/water system with a generic strategy.

As described elsewhere,[18] cluster analysis was used to build a set of well-balanced compounds in the space of molecular chemical properties quantified by the van der Waals volume (V_w), polarity/polarizability (π^*), H-bond donor acidity (α), H-bond acceptor basicity (β), and log P_{oct} values. Thirty-eight neutral, acidic, and weak basic (pK_a < 5.5) model compounds were selected. Their log P_{oct} range (from 0 to 5) is well adapted to medicinal chemistry. The proposed methodology was also used to determine 18 basic compounds: 10 β-blockers and 8 local anesthetics with log P_{oct} ranging from 1.8 to 4.3 and with pK_a between 7.5 and 9.9.

Analyses were performed using a mixture of aqueous buffer and organic solvent as mobile phases. Three different buffers (pH 2, 5, 9) were used to analyze all compounds in their neutral forms. Two organic modifiers were tested, i.e., methanol and acetonitrile. Four columns were tested, namely, Acquity BEH Shield RP18, Acquity BEH C18, Acquity BEH C8, and Acquity BEH phenyl (30 mm × 2.1 mm i.d., 1.7 μm). For basic compounds log *P* determination was performed using an ammonium acetate buffer at pH 10.5.

Among the four tested columns, the Acquity BEH Shield RP18 (which contains an embedded polar group) was chosen for further investigations, as it presented the best linear correlations of log k_w versus log P_{oct} values (data not shown). This support is a hybrid stationary phase with ethylene bridges inside the silica matrix. These chemically stable structures ensure high pH stability (up to 11).

In isocratic mode, retention factors (k) were measured with four or five different mobile phase compositions. Retention factors in pure water (log k_w) were obtained by extrapolation using linear or quadratic relationships between log k values and methanol or acetonitrile percentages, respectively.

In isocratic mode, appropriate mobile phase compositions had to be determined for each compound. For example, *N*,*N*-diethylacetamide, a rather polar compound (log P_{oct} = 0.34) was analyzed at low methanol concentrations (20−50%) with retention times lower than 0.5 min (Figure 1A). For pentamethylbenzene, a more lipophilic compound (log P_{oct} = 4.56) and higher methanol concentrations (60−80%) were necessary to elute the analyte from the column (Figure 1B). At 60% methanol, the retention time was about 3.3 min, which is acceptable for a compound with a retention factor higher than 25. However, a loss in sensitivity due to peak broadening was observed. In summary, over the 38 tested compounds, the average log P_{oct} determination time in isocratic mode was about 25 min per compound.

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[a]Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; k, retention factor; LC, liquid chromatography; log k_w, logarithm of the retention factor in pure water; log P_{oct}, partition coefficient in the *n*-octanol/water system; NCEs,new chemical entities; (Q)SAR, (quantitative) structure–activity relationships; RPLC, reversed phase liquid chromatography; UPLC, ultraperformance liquid chromatography.

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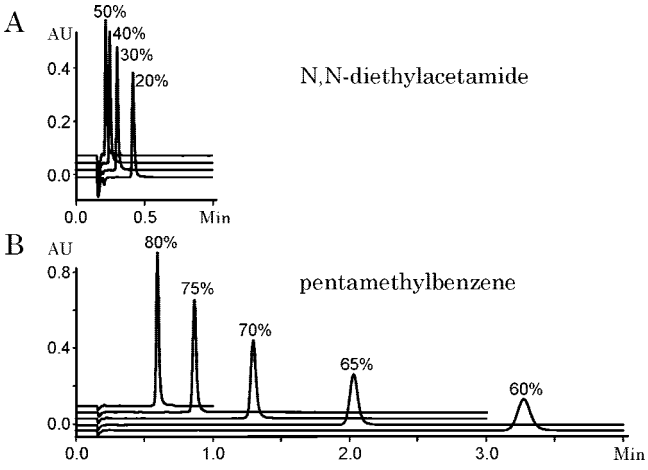


Figure 1. Chromatograms of *N*,*N*-diethylacetamide (A) and pentamethylbenzene (B) in isocratic mode: mobile phase, ammonium acetate, pH 5.0/MeOH; flow rate, 0.5 mL/min; detection, UV 215, 220 nm; column, AcquityShield BEH RP18, 2.1 mm × 30 mm, 1.7μm.

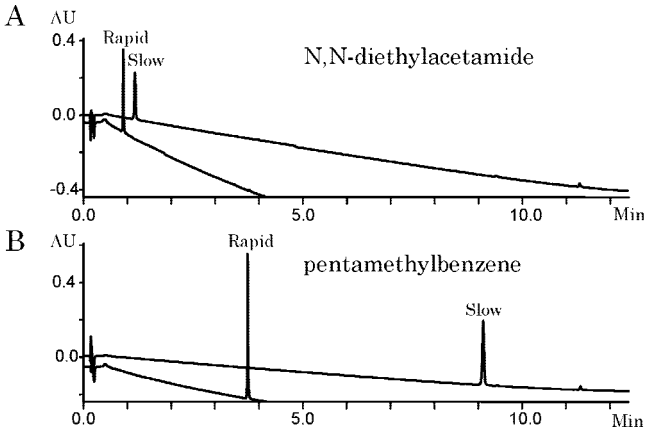


Figure 2. Chromatograms of *N*,*N*-diethylacetamide (A) and pentamethylbenzene (B) in gradient mode: mobile phase, ammonium acetate, pH 5.0/MeOH; flow rate, 0.5 mL/min; detection, UV 215, 220 nm; column, AcquityShield BEH RP18, 2.1 mm × 30 mm, 1.7μm.

In gradient mode, a generic procedure including two runs was applied to model the behavior of each compound in the whole organic modifier composition range using HPLC modeling software Osiris (Datalys, Grenoble, France). Initial and final organic modifier compositions were fixed to 2% and 95% organic modifier, respectively. By computation of the retention times obtained from the two gradient runs differing only in time, the software could calculate an exact value of log k_w by solving two equations.

The two gradient runs from 2% to 95% methanol lasted 12.4 (slow) and 4.13 (rapid) min, whatever the compound (parts A and B of Figure 2). In this mode, the total log P_{oct} determination time was about 20 min per compound, including column reequilibration. Moreover, sensitivity was constant because peak width in gradient mode is similar for early and later eluting compounds.

The plots of log k_w versus log P_{oct} values using methanol or acetonitrile as organic modifier in isocratic and gradient modes are shown in Figure 3.

In isocratic conditions, good linear correlations were obtained using methanol (Figure 3A) or acetonitrile (Figure 3B). The best correlation was obtained with methanol (determination coefficient $r^2 = 0.98$), and therefore, this solvent represents the best choice for measuring log P_{oct} between 0 and 5 in isocratic mode. In gradient mode, the linear correlation obtained using methanol as organic modifier (Figure 3C, $r^2 = 0.95$) was better than using acetonitrile (Figure 3D, $r^2 = 0.92$). Moreover, with methanol, the slope remained similar to the one obtained in isocratic mode (not the case with acetonitrile). Since the model is linear with methanol and quadratic with acetonitrile, the calculated log k_w values using only two retention time measurements are more accurate with methanol. Therefore, methanol is the solvent of choice for measuring log P_{oct} in gradient mode.

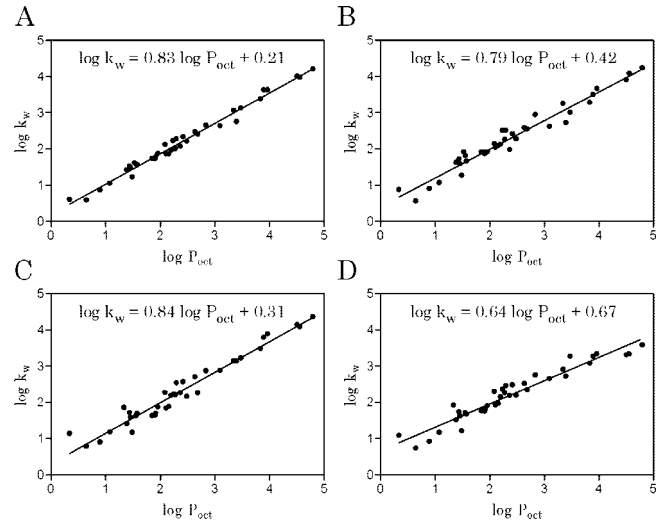


Figure 3. Relationship between log k_w and log P_{oct} for the 38 model compounds in isocratic mode using MeOH, $r^2 = 0.98$ (A), or ACN, $r^2 = 0.95$ (B), and in gradient mode using MeOH, $r^2 = 0.95$ (C), or ACN, $r^2 = 0.92$ (D).

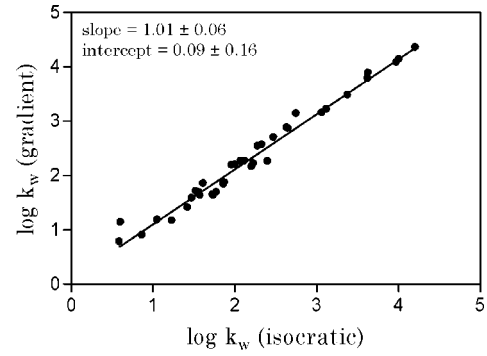


Figure 4. Relationship between log k_w obtained in isocratic versus gradient mode for the 38 model compounds, $r^2 = 0.98$.

Table 1. Isocratic versus Gradient Mode

	isocratic mode	gradient mode
correlation log k_w − log P_{oct}	MeOH, $r^2 = 0.98$	MeOH, $r^2 = 0.95$
log P_{oct} range, lower limit	>0	>0
log P_{oct} range, higher limit	dependent on sensitivity	sensitivity not an issue
log P_{oct} determination time	25 min/compound	20 min/compound
approach	compound-dependent	generic

The log k_w values obtained in isocratic and gradient modes were linearly correlated using methanol as an organic modifier (Figure 4). Considering the 95% confidence interval, the slope of the linear regression was 1 and the *y* intercept 0, confirming that the two log k_w values were not statistically different.

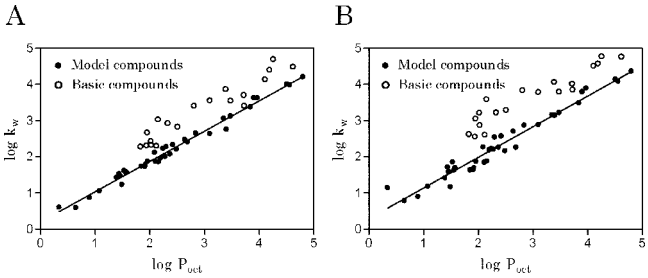


Figure 5. Relationship between log k_w and log P_{oct} for the 38 model compounds and 18 basic compounds in isocratic (A) and gradient mode (B).

Several additional parameters had to be taken into account to decide between isocratic and gradient modes. These parameters are listed in Table 1. In conclusion, the best way to achieve high-throughput log P_{oct} determination between 0 and 5 by UPLC is to work in gradient mode (generic method) with an Acquity BEH Shield RP18 column and methanol as an organic modifier.

In the final experimental conditions, the following model between log k_w and log P_{oct} values was obtained with the 38 model compounds:

$$\log k_w = 0.84(\pm 0.07)\log P_{oct} + 0.31(\pm 0.22) \quad (1)$$

$$n = 38, \quad q^2 = 0.95, \quad r^2 = 0.95, \quad s = 0.21, \quad F = 742$$

In order to fully characterize the Acquity BEH Shield RP18 stationary phase, linear solvation free-energy relationships (LSERs) were applied to the set of log k_w values for the 38 model compounds:

$$\log k_w = 2.90 \times 10^{-2}(\pm 0.19 \times 10^{-2})V_w - 0.34(\pm 0.25)\pi^* - 0.07(\pm 0.18)\alpha - 3.01(\pm 0.45)\beta - 0.10(\pm 0.28) \quad (2)$$

$$n = 38, \quad q^2 = 0.97, \quad r^2 = 0.98, \quad s = 0.13, \quad F = 451$$

After elimination of non significant variables, eq 3 confirms that only two main structural parameters govern the UPLC retention in the investigated experimental conditions, namely, the van der Waals volume V_w and hydrogen-bond acceptor basicity β.

$$\log k_w = 2.82 \times 10^{-2}(\pm 0.20 \times 10^{-2})V_w - 3.27(\pm 0.40)\beta - 0.24(\pm 0.26) \quad (3)$$

$$n = 38, \quad q^2 = 0.97, \quad r^2 = 0.98, \quad s = 0.15, \quad F = 703$$

The relative contributions of each variable obtained after Mager's standardization,[19] namely, 56% for the volume term and 44% for β, are similar to those characterizing the partitioning in *n*-octanol–water system (49% and 51% for V_w and β, respectively). The comparable parameters calculated for the two data sets show that both phenomena are governed by these two main structural properties.

Since the large majority of drugs are basic, the described UPLC procedure was used to measure log k_w values for 10 β-blockers and 8 local anesthetics at pH 10.5. The comparison between extrapolated retention factors and log P_{oct} values (model and basic compounds) are reported in Figure 5.

In both modes, basic compounds have a different behavior from the neutral ones. This behavior can be attributed to the effect of organic modifier on the mobile phase pH, the ionization state of solutes, and electrical characteristics of stationary phase. The balance of electrostatic intermolecular forces influences in a nonregular way isocratic log *k* measurements at different organic modifier percentages leading to highly uncertain log k_w values. Because it remains difficult to take into account all these effects, the relationships between log *k* values at a given organic modifier percentage (isocratic log *k*) and log P_{oct} values were explored as a pragmatic approach to reduce observed variations. Plots of isocratic log *k* values at different methanol percentages (40%, 50%, 60%, and 70%) versus log P_{oct} are shown in Figure 6.

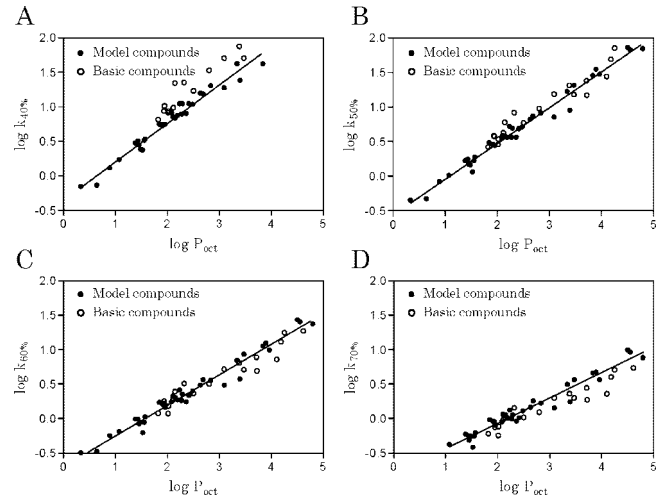


Figure 6. Relationship between isocratic log *k* values and log P_{oct} for the 38 model compounds and the 18 basic compounds. Methanol percentages are 40% (A), 50% (B), 60% (C), and 70% (D).

Table 2. Data Obtained for the 18 Basic Compounds

compd	log P_{oct} [20]	log $k_{50\%}$	log P_{UPLC}
pindolol	1.83	0.42	1.9
mepivacaine	1.94	0.58	2.2
metoprolol	1.95	0.57	2.2
acebutolol	2.02	0.45	2.0
procaine	2.03	0.53	2.1
prilocaine	2.12	0.62	2.3
bisoprolol	2.15	0.77	2.6
lidocaine	2.33	0.91	2.9
oxprenolol	2.51	0.77	2.6
metipranolol	2.81	0.97	3.0
alprenolol	3.10	1.18	3.4
tetracaine	3.39	1.31	3.7
propranolol	3.48	1.18	3.4
carazolol	3.73	1.17	3.4
bupivacaine	3.72	1.37	3.8
carvedilol	4.11	1.44	3.9
dibucaine	4.19	1.69	4.4
butacaine	4.26	1.85	4.7

The relationship between isocratic log *k* values at 50% methanol (log k_{50}) versus log P_{oct} was the best compromise between the highest discrimination power (i.e., highest slope) and the lowest difference between neutral and basic compounds behavior (Figure 6B). Moreover, the quality of the linear correlation obtained with the 38 model compounds in such conditions remained acceptable for model compounds, allowing the derivation of eq 4:

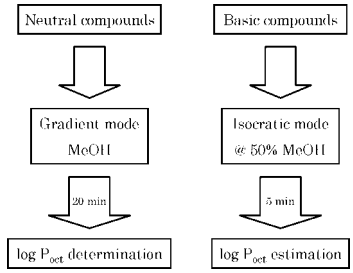
$$\log k_{50\%} = 0.52(\pm 0.02)\log P_{oct} - 0.56(\pm 0.05) \quad (4)$$

$$n = 38, \quad q^2 = 0.98, \quad r^2 = 0.98, \quad s = 0.08, \quad F = 2048$$

Equation 4 enabled estimation of log P_{oct} for the 18 tested basic compounds. The obtained values are reported in Table 2 and demonstrate that isocratic retention factors at pH 10.5 gave a suitable estimation for basic compounds ($7.5 < pK_a < 9.9$).

Moreover, measuring only log k_{50} saves time because the average analysis time was 5 min for an isocratic log *k* measurement versus 20 min for a log k_w measurement in gradient mode.

Scheme 1



In summary, high-throughput measurements of log *P* is very attractive for medicinal chemists. Depending on the nature of compounds, the following strategy should be used, using Acquity BEH Shield RP18 support (Scheme 1): gradient mode with methanol for log P_{oct} determination of neutral compounds (20 min per compound) and only one analysis in isocratic mode at 50% methanol for log P_{oct} estimation of basic compounds (around 5 min per compound).

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Supporting Information Available: Complete equations corresponding to Figure 3 and complete solvatochromic equations corresponding to partitioning in *n*-octanol–water system. This material is available free of charge via the Internet at http://pubs.acs.org.

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