Brief Articles

Lipophilicity of Basic Drugs Measured by Hydrophilic Interaction Chromatography

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RPLC gains acceptance in pharmaceutical research for the rapid determination of lipophilicity but remains limited for the determination of partition coefficients of moderate to strong basic compounds under their neutral form because stationary phases are not compatible with high pH conditions. In this work, HILIC technique was used to accurately measure log P_{oct} of the neutral form of basic drugs by measuring the difference between 2 isocratic log k values ($\Delta\log k_{0-95}$) of their cationic form.

Introduction

Chromatographic methods and in particular reverse phase liquid chromatography (RPLC[a]) methods are currently largely used and recognized for the determination of log P_{oct}, as illustrated by the many well-documented reviews published on this subject.[1−7] The well-known advantages of these methods are their good throughput and easy automation, their low sample consumption, and their general insensitivity to impurities. However, RPLC techniques, in particular using conventional silica-based stationary phases, still remain limited for the determination of partition coefficients of moderate to strong basic compounds under their neutral form because of the silica-based stationary phases instability at high pH conditions[8−12] even if new strategies allow modern stationary phases to be resistant to a broader pH range.[9,13,14] Furthermore, in a recent ultra high pressure liquid chromatography (UHPLC) method applied to the determination of partition coefficients, deviant retention behavior has been observed for a series of β-blockers and local anesthetics compared to reference compounds.[15]

An approach to overcome the problem of basic drugs could be the determination of a retention factor of the cationic forms because relationships between the lipophilicity of the neutral and charged species were well-described.[16−18] Hydrophilic interaction chromatography (HILIC)[19] is a separation technique using eluents containing appreciable amounts of water and a relatively hydrophobic water-miscible solvent, generally acetonitrile (ACN), and is particularly suitable for the retention of polar and charged compounds. HILIC retention mechanism of charged compounds was reported to be a complex combination of different interactions[20] depending on the experimental conditions involved. Hydrophilic retention[19,21−24] dominates when high proportions of organic modifier are used in the mobile phase, and the retention increases by increasing organic modifier proportion. In contrast hydrophobic reverse-phase (RP) type retention[25,26] plays a major role when the mobile phase contains high proportions of water and the retention decreases by increasing organic modifier proportion. The third mechanism, the ion exchange mechanism, exists over the whole range of mobile phase composition.[27] Therefore, because lipophilicity expresses the balance of polarity and hydrophobicity, the particularity of HILIC retention has been exploited to obtain 1-octanol/water partition coefficients of basic compounds, including β-blockers and local anesthetics, using two diametrically opposite mobile phase compositions (i.e., 0% and 95% (v/v) of ACN in the mobile phase) involving different balance of intermolecular interactions.

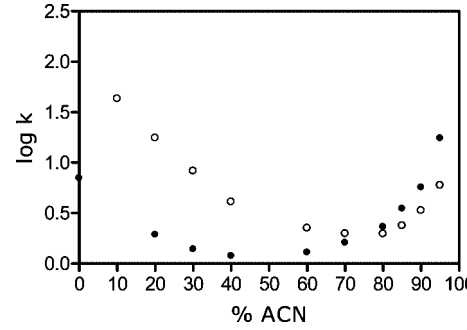

Figure 1. Relationship between the measured log k and the percentage of acetonitrile in the mobile phase (mixtures of ACN−ammonium acetate $^{w}_{w}$pH 4, I = 20 mM). ● atenolol; ○ pindolol.

Results and Discussion

The retention mechanism involved for cationic solutes in HILIC mode has shown to be a complex combination of ion exchange, hydrophilic, and "RP-like" mechanisms.[20] This HILIC particularity is generally expressed by opposite variations of log k at respectively high and low ACN percentages as illustrated in Figure 1 for atenolol and pindolol.

According to the work of Laurent et al.,[28] influence of organic modifier fraction on cation exchange chromatography using ammonium (having a positive free energy of transfer from water to ACN) as buffer cation is not expected to vary importantly by increasing ACN fraction until quite high modifier concentrations, where a weak decrease should be observed. Therefore,

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[a] Abbreviations: RPLC, reversed phase liquid chromatography; UHPLC, ultra high pressure liquid chromatography; HILIC, hydrophilic interaction chromatography; RP, reversed phase; ACN, acetonitrile; log P^{N}_{oct}, 1-octanol−water system partition coefficient of the neutral form; log P^{C}_{oct}, 1-octanol−water system partition coefficient of the cationic form; log k_0, retention factor measured with 100% water as mobile phase; log k_{95}, retention factor measured with 95% acetonitrile as mobile phase; $\Delta\log k_{0-95}$, difference between log k_0 and log k_{95} values; TFA, trifluoroacetic acid; LSER, linear solvation energy relationship.

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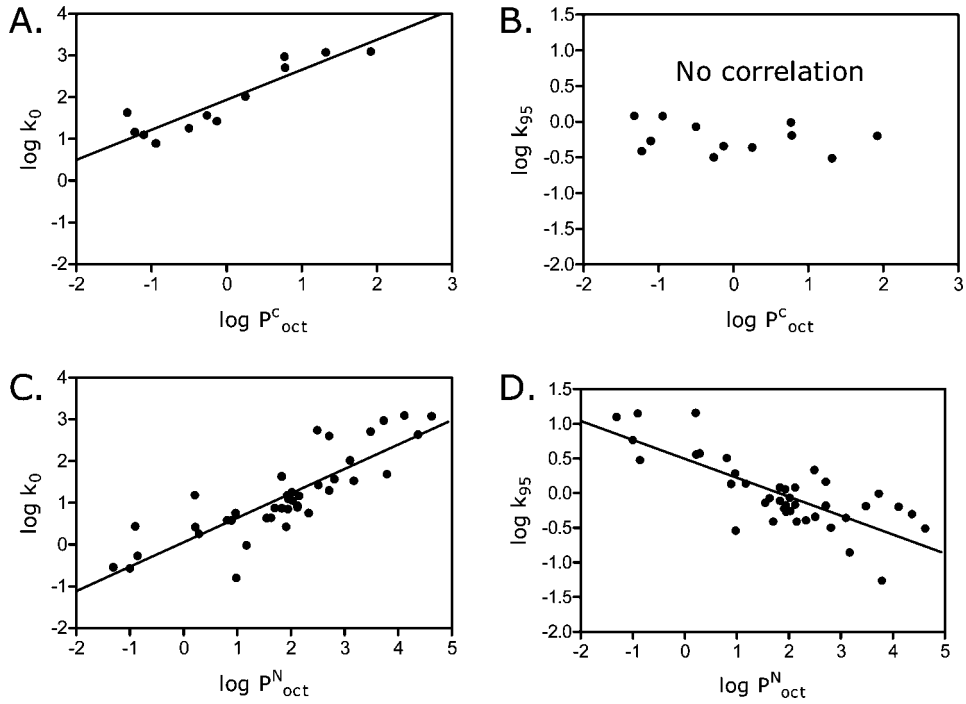

Figure 2. Relationship between (A) log k_0 and log P^c_{oct}; (B) log k_{95} and log P^c_{oct}; (C) log k_0 and log P^N_{oct}; (D) log k_{95} and log P^N_{oct}, where log k_0 was the isocratic log k obtained in a 100% buffer TFA−ammonium pH 2 (I = 100 mM) mobile phase and log k_{95} measured using a (95:5, v/v) ACN−buffer TFA−ammonium pH 2 (I = 100 mM) mobile phase.

the log k decrease registered at low ACN percentages can be attributed to RP-like hydrophobic retention, whereas at high ACN percentages, the log k increase can be ascribed to hydrophilic retention. The more lipophilic drug, pindolol, showed a more marked RP-like retention than atenolol, while hydrophilic retention was higher for atenolol (i.e., the more hydrophilic solute).

Zic-*p*HILIC stationary phase was tested with two diametrically opposite mobile phase compositions (i.e., 0% and 95% (v/v) of ACN) on a set of 39 moderate to strong basic model compounds ($7.10 < pK_a < 10.95$) including 14 β-blockers and four local anesthetics and having log P^N_{oct} values ranging from −1.31 to 4.62.

The reason for selecting 95% instead of 100% ACN is clearly related to the hypothetic mechanism of HILIC. The retention mechanism for a separation carried out in the HILIC mode is attributed to the thick layer of water adsorbed on silica, which induces liquid−liquid partition between the bulk mobile phase and the adsorbed aqueous liquid layer.

Furthermore, because the retention mechanism when low percentages of acetonitrile are used is a "RP-like" retention, the most lipophilic compounds were strongly retained on the stationary phase, which considerably increased the analysis time and diminished the accuracy. Therefore, for such compounds, the log k_0 was obtained by quadratic extrapolation from 4 or 5 isocratic log k measured at different percentages of acetronitrile. To be sure that extrapolated and nonextrapolated values were identical, several compounds with moderate lipophilicity were measured in the two conditions and no significant different were observed between log k_0 and extrapolated log k_0 (data not shown).

The corresponding retention factors (log k_0 and log k_{95}) were compared with experimental log P^C_{oct} and log P^N_{oct} values, representing respectively the logarithm of the partition coefficient of the cationic and the neutral form in 1-octanol/water system (numerical values in Supporting information).

Aqueous fraction was constituted of a trifluoroacetic acid (TFA)−ammonium (I = 100 mM) acidic buffer pH 2 to have basic compounds fully under their cationic forms. Figure 2 shows that no or low correlation was found between log P^C_{oct} or log P^N_{oct} and log k_{95}, while log k_0 was better correlated with log P^C_{oct} and log P^N_{oct} (r^2 = 0.84 and 0.71, respectively).

However, the use of an unique isocratic retention factor (log k_0 or log k_{95}) of charged compounds seems to be insufficient to accurately determine 1-octanol/water partition coefficients because notably of the cation exchange contribution involved over the whole range of ACN content in the mobile phase. Except cation exchange contribution, which is poorly influenced by changing ACN fraction in the mobile phase, log k_0 expresses almost hydrophobicity, which involves important contribution of the volume, whereas log k_{95} is an expression of compounds polarity (hydrogen bond donor and acceptor abilities). On the basis of linear solvation energy relationships (LSERs) analysis, compounds lipophilicity in 1-octanol/water partition coefficients is governed predominately by the volume and hydrogen bond acceptor basicity.[14] Therefore both log k_0 and log k_{95} give important information for lipophilicity.

The difference between log k_0 and log k_{95} (Δlog k_{0-95}) was then calculated and compared to log P^N_{oct} (Figure 3 and eq 1).

A good linear regression was obtained between these two parameters:

$$\log k_{0-95} = 0.87(\pm 0.04) \log P^N_{oct} - 0.45(\pm 0.09)$$
$$n = 39;\ r^2 = 0.93;\ s = 0.34;\ F = 480 \quad (1)$$

In these equations, 95% confidence limits are in brackets, n is the number of compounds, r^2 is the coefficient of determination (squared correlation coefficient), s is the standard deviation, and F is the Fisher value.

The Δlog k_{0-95} parameter of cationic forms is then an efficient descriptor to obtain log P^N_{oct} of basic compounds with partition coefficients ranging from −1.5 to 5.

However, the recent work of McCalley[20] on different silica stationary phases used in HILIC mode has shown that contrary to neutral compounds, HILIC retention of charged compounds

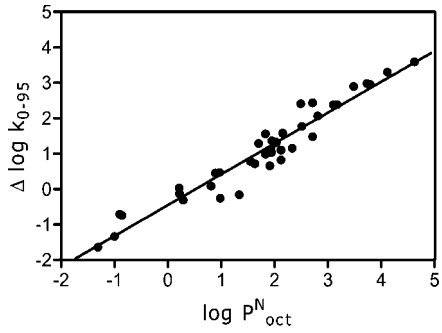

Figure 3. Relationship between $\Delta\log k_{0-95}$ (phase mobile = acetonitrile−buffer TFA−ammonium pH 2 ($I = 100$ mM) and $\log P^N_{oct}$.

could be highly influenced by pH, buffer nature, or buffer ionic strength of the mobile phase. These experimental parameters apparently acted on ion exchange retention mechanism by ionic competition and have to be carefully controlled as described in the guidelines proposed in Supporting Information.

Conclusion

Although the retention mechanism of charged compounds in HILIC mode is much more complicated than for neutral solutes, it was demonstrated that on Zic-pHILIC stationary phase, the difference between two isocratic log *k* values ($\Delta\log k_{0-95}$) was well correlated with $\log P^N_{oct}$ values, thus offering an innovative promising way to obtain 1-octanol/water partition coefficients of the neutral form of basic compounds. This technique is particularly interesting for strong basic compounds such as β-blockers, which are hardly measurable under un-ionized form by RPLC due to high pH limitations of many stationary phases.

Experimental Section

Chemicals. Acebutolol HCl, alprenolol HCl, apomorphine HCl, atropine, bisoprolol, carbamoylcholine, methylhomatropine, metoprolol tartrate, moxisylyte HCl, oxprenolol, pindolol, propranolol HCl, pyridostigmine, *S*-butyrylthiocholine, scopolamine bromide, strychnine, timolol maleate, and verapamil HCl were purchased from Sigma (Buchs, Switzerland).

Butylamine, dicyclohexylamine, ethanolamine, morpholine, MPTP HCl (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl), octopamine HCl, serotonine HCl, tetra-*N*-ethylammonium, tetra-*N*-methylammonium, and triethanolamine were obtained from Fluka (Buchs, Switzerland). Atenolol, tacrine HCl, and tripropylamine were purchased from Aldrich (Steinheim, Germany). Carazolol, carvedilol, and metipranolol were kindly offered by Boehringer-Mannheim (Mannheim, Germany). Penbutolol sulfate was offered by Hoechst Pharma (Zürich, Switzerland). Tyrosine methyl ester was obtained from Bachem (Bubendorf, Switzerland).

Acetonitrile of superpure quality for HPLC was purchased from VWR (Dietikon, Switzerland), water was obtained with the Milli-Q Water Purification System from Millipore (Bedford, MA), trifluoroacetic acid and acetic acid were provided by Fluka (Buchs, Switzerland), ammonium hydroxide solution (25% in H_2O) and formic acid were obtained from Merck (Darmstadt, Germany) and Reactolab (Servion, Switzerland) respectively.

Measurements of Retention Factors. All experiments were performed using a Merck Hitachi EliteLaChrom liquid chromatograph (Merck, Darmstadt, Germany, and Hitachi Instrument, Inc., San Jose, CA) equipped with a L-2200 auto sampler, a L-2130 pump, and a L-7614 degasser. Detection was performed using both a L-2400 UV detector operating at 230 nm for all compounds and a Sedex 85 LT-ELSD detector (Sedere, Alfortville, France).

ELSD conditions were as followed; for pure aqueous mobile phases, nebulization air pressure and temperature were set to 3.0 bar and 70 °C, respectively, while for ACN-rich mobile phases, these parameters were adjusted to 3.5 bar and 40 °C.

The chromatographic system was controlled by a EzChrom Elite System Manager software version 3.1.7 (Merck Hitachi). Retention measurements were performed at 23 °C on a ZIC-*p*HILIC column (sulfoalkylbetaine phase on a polymeric support, 10 cm × 4.6 mm, 5 μm) from SeQuant (Umeå, Sweden). Flow rate was set to 1 mL/min.

Phoebus software 1.0 (Sedere, Centre Analyze, Orleans, France) was used to prepare buffer (TFA (trifluoroacetic acid)/ammonium) at pH 2 and ionic strength (I) equal to 100 mM. The pH of the mobile phase were measured both before (^w_wpH) and after the adjunction of organic solvent (^s_wpH), with the pH-meter calibrated in aqueous buffer in both cases, even if ^s_wpH values have to be taken carefully.[29]

Supporting Information Available: pK_a, log P_{oct} of cationic and neutral forms, log k_0 and log k_{95} values of the 39 tested basic compounds and guidelines for partition coefficients measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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