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Lipophilicity indices derived from the liquid chromatographic behavior observed under bimodal retention conditions (reversed phase/hydrophilic interaction): Application to a representative set of pyridinium oximes

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ABSTRACT

The liquid chromatographic behavior observed under bimodal retention conditions (reversed phase and hydrophilic interaction) offers a new basis for the determination of some derived lipophilicity indices. The experiments were carried out on a representative group (30 compounds) of pyridinium oximes, therapeutically tested in acetylcholinesterase reactivation, covering a large range of lipophilic character. The chromatographic behavior was observed on a mixed mode acting stationary phase, resulting from covalent functionalization of high purity spherical silica with long chain alkyl groups terminated by a polar environment created through the vicinal diol substitution at the lasting carbon atoms (Acclaim® Mixed Mode HILIC 1 column). Elution was achieved by combining different proportions of 5 mM ammonium formiate solutions in water and acetonitrile. The derived lipophilicity indices were compared with log *P* values resulting from different computational algorithms. The correlations between experimental and computed data sets are significant. To obtain a better insight on the transition from reversed phase to hydrophilic interaction retention mechanisms, the variation of the thermodynamic parameters determined through the van't Hoff approach was also discussed.

Abbreviations: AChE, acetylcholinesterase; AClogP, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches); ALOGP, log *P* estimation according to the Ghose–Crippen algorithm; ALOGPs, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches); CHI, lipophilicity index obtained in fast gradient elution conditions; d.p., diameter of the packed particles (mm); ΔG^0, standard Gibbs free energy; ΔH^0, standard enthalpy; ΔS^0, standard entropy; *F*, flow rate; ϕ, volume phase ratio ($V_{S.Ph.}/V_{M.Ph.}$); HILIC, hydrophilic interaction liquid chromatography; Hy, hydrophobicity estimation; HYL, linear extrapolation of the retention factor to 100% acetonitrile as mobile phase; i.d., column's internal diameter (mm); ISOELUT, isoelution composition (% of acetonitrile in the mobile phase corresponding to the minimum retention determined through a binomial regression approach); ISOELUT1, isoelution composition (% of acetonitrile in the mobile phase corresponding to the minimum retention determined through a linear regression approach); ISOELUT2, isoelution composition (% of acetonitrile in the mobile phase corresponding to the minimum retention determined through a power regression approach); *k*, retention factor; k_{min}, lipophilicity index corresponding to the minimum retention factor experimentally determined; $K_{o/w}$, distribution coefficient between *n*-octanol and water; KOWWIN, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches); k_w^{bin}, extrapolated retention factor to 100% aqueous mobile phase through a binomial approach; k_w^{lin}, extrapolated retention factor to 100% aqueous mobile phase through a linear approach; *L*, column length (cm); LC–MS, liquid chromatography coupled to mass spectrometric detection; log, mathematical symbol of the logarithm function; log *D*, logarithm of the distribution ratio between n-octanol and water brought to a given pH value; log k_w, lipophilicity index resulting from extrapolation of the retention factor to 100% aqueous mobile phase; log *P*, logarithm of $K_{o/w}$; M. Ph., mobile phase; miLogP, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches); MLOGP, log *P* estimation according to Moriguchi; OP, organophosphorus; PC, principal component; PCA, principal component analysis; QSAR, quantitative structure–activity relationship; QSPR, quantitative structure–properties relationships; *R*, universal gas constant (8.31 J K^{-1} mol^{-1}); RPLC, reversed phase liquid chromatography; RSD, relative standard deviation; S.Ph., stationary phase; SlogD, log *D* estimation according to ADMET Predictor software, version 5.0.0012; SlogP, log *P* estimation according to ADMET Predictor software, version 5.0.0012; *T*, temperature (°C or K); t_0, column's void time; TOC, total residual organic carbon content (ng mL^{-1}); XLOGP2, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches); XLOGP3, log *P* estimation according to the ALOGPS 2.1. software (different computational approaches)

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1. Introduction

Lipophilicity, accepted as an extension of the hydrophobic character, includes all favorable interactions that contribute to the distribution of a chemical entity between water and other solubilizing media, and represents a manifestation of the characteristics of the system in which the solute is placed [1].

The octanol–water distribution coefficient ($K_{o/w}$) got widely acceptance as the physicochemical property measuring the lipophilicity of chemicals [2]. It stands at the basis of quantitative structure–properties/activity relationships (QSPR/QSAR), having crucial roles in various domains, such as fundamental, industrial and food chemistry, pharmaceutical sciences (drug design, prediction of absorption/metabolization/distribution/excretion/toxicological features), environmental risk assessment (bioaccumulation in aquatic biota) and agriculture (soil sorption).

Experimental determination of $K_{o/w}$ is possible through the use of different direct or indirect approaches. Both alternatives were extensively reviewed in the literature [1,3–5]. As the separation methods (and more specifically, the chromatographic ones) are based on distribution of solutes between non-miscible phases, their use in estimation of $K_{o/w}$ is frequent.

No general consensus exists about the ability of reversed phase (RP) chromatography driven on chemically bonded stationary phases to estimate $K_{o/w}$ of chemical compounds. Many efforts have been paid to optimize/standardize the experimental conditions [6,7]. Immobilization of n-octanol and biomimetic moieties (artificial membranes, liposomes) as well as different chemistries of the modification of the silicagel surface was especially tailored to obtain the closest estimation [8–10]. Retention behavior in micellar and microemulsion electrokinetic chromatographic approaches was found highly correlated with $K_{o/w}$ [11,12]. The solvation parameter model acts as a tool frequently used for identification and evaluation of the suitability of a chromatographic system to closely estimate $K_{o/w}$ [13,14]. Undoubtedly, high performance liquid chromatography (HPLC) provides a generous platform for identification and measurement of various types of lipophilicity, resulting in numerous classes of indices, more or less correlated to $K_{o/w}$. Lipophilicity scales, based on different theoretical approaches and determined by different procedures, may somehow overcome the difficulties of standardization. The most used lipophilicity index is the retention factor (k) [15]. It was completed by log k_w index, representing the functional extrapolation to a hypothetical situation when the mobile phase is 100% water [16]. The introduction of the chromatographic retention index (CHI) by Valkó et al. leaded to an experimental approach based on fast gradient elution [17]. Sârbu et al. proposed an alternative scale, based on the modification of k on replicate increasing injection volumes of samples obtained in diluents non-miscible with the mobile phase [18]. The image may be completed by the scores obtained through application of the Principal Component Analysis (PCA) to the matrix of the specific retention factors obtained under different elution conditions [19,20]. Based on the previous mentioned approaches, many applications have been published in literature with respect to various classes of compounds, i.e. drugs, natural products, products for food industry, pesticides.

Oxime type reactivators are used as cleavage reagents of the bond between a former serine hydroxyl group situated on the esteratic active site of acetylcholinesterase (AChE) and the phosphorus atom of an inhibitor ((thio)phosphonyl/phosphoryl moiety belonging to a nerve agent or pesticide) [21]. The oximate anion acts as a nucleophilic agent while the quaternary nitrogen favorably positions the reactivator towards an anionic center representing a secondary binding site at the entrance of a narrow gorge of the enzyme. The reactivating potency of an oxime depends on the inhibitory characteristics of the organophosphorus compound (OP), the inter-correlation between spontaneous reactivation and aging processes, as well as on its ability of penetration through biological barriers (mainly the brain–blood one). The existence of quaternary pyridinium nitrogen atoms in the structures of most oximes used as AChE reactivators makes their ability to penetrate through biological barriers difficult [22]. A lot of effort has been made to control the hydrophilic character of a pyridinium oxime through the nature of the substituent of the quaternary nitrogen atom [23]. Thus, it appears important to find practical and fast experimental approaches for establishing the relative change in the hydrophilic character of newly synthesized pyridinium oximes according to the substitution of the quaternary nitrogen atoms. Retention behavior of quaternary oximes on various stationary phases (involving different retention mechanisms), as lipophilicity descriptors, has been discussed in recent publications [24–26].

The aim of the present approach is to evaluate the possibility of measuring lipophilicity indices from the chromatographic behavior obtained under bimodal retention conditions (reversed phase and hydrophilic interaction) on a dodecyl diol chemically modified silicagel as a stationary phase for a set 30 congeners belonging to the mono-pyridinium oxime class. As the retention profiles are "U" shaped, interest was focused on the correlation between the minimum retention conditions and the lipophilic characteristics of the investigated compounds. The experimental values were correlated with log P values deriving from different computational algorithms. Some thermodynamic aspects related to transition from RP to HILIC elution modes are also discussed.

2. Experimental

2.1. Reagents

Acetonitrile (HPLC gradient grade), and ammonium formate (eluent additive for LC–MS grade) from Merck (Darmstadt, Germany) were used during experiments. Water for chromatography (resistivity of minimum 18.2 MΩ and total residual organic carbon content—TOC—of maximum 30 ng mL^{-1}) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument (TKA Instruments as part of Thermo Fischer Scientific, Niederelbert, Germany). The chemical structures of the studied analytes are presented in Fig. 1. All compounds were bromide salts, exception being made by PAE-iodide (all position isomers). The studied oximes were synthesized at the National Research Institute of Police Science, Chiba, Japan. The analytes considered in the present approach were specifically selected for an illustrative structure–lipophilicity relationship study and not necessarily for their therapeutic reactivation capacity.

2.2. Equipments

Experiments were performed with an Agilent 1260 Infinity series LC/MWD (Agilent Technologies) system consisting of the following modules: quaternary pump (G1311B), automated injector (ALS-G1329B), column thermostat (TCC-G1316C), and a multichannel UV–vis detector (DVL-G1365D). System control and data acquisition were made with the Agilent Chemstation for LC 3D, version 04.03(16).

2.3. Chromatographic experiments

An Acclaim™ Mixed-Mode HILIC-1 column (150 mm L × 4.6 mm i.d. × 5 μm d.p.) from Thermo Scientific (P.N. 066843, S.N. 001261) was used and thermostated at 25 °C. The stationary phase consists in a high purity, 120 Å pore size, spherical silicagel