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Multivariate assessment of lipophilicity scales—computational and reversed phase thin-layer chromatographic indices

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ABSTRACT

Needs for fast, yet reliable means of assessing the lipophilicities of diverse compounds resulted in the development of various *in silico* and chromatographic approaches that are faster, cheaper, and greener compared to the traditional shake-flask method. However, at present no accepted "standard" approach exists for their comparison and selection of the most appropriate one(s). This is of utmost importance when it comes to the development of new lipophilicity indices, or the assessment of the lipophilicity of newly synthesized compounds. In this study, 50 well-known, diverse compounds of significant pharmaceutical and environmental importance have been selected and examined. Octanol-water partition coefficients have been measured with the shake-flask method for most of them. Their retentions have been studied in typical reversed thin-layer chromatographic systems, involving the most frequently employed stationary phases (octadecyl- and cyano-modified silica), and acetonitrile and methanol as mobile phase constituents. Twelve computationally estimated log*P*-s and twenty chromatographic indices together with the shake-flask octanol-water partition coefficient have been investigated with classical chemometric approaches—such as principal component analysis (PCA), hierarchical cluster analysis (HCA), Pearson's and Spearman's correlation matrices, as well as novel non-parametric methods: sum of ranking differences (SRD) and generalized pairwise correlation method (GPCM). Novel SRD and GPCM methods have been introduced based on the Comparisons with One VAriable (lipophilicity metric) at a Time (COVAT). For the visualization of COVAT results, a heatmap format was introduced. Analysis of variance (ANOVA) was applied to reveal the dominant factors between computational log*P*s and various chromatographic measures. In consensus-based comparisons, the shake-flask method performed the best, closely followed by computational estimates, while the chromatographic estimates often overlap with *in silico* assessments, mostly with methods involving octadecyl-modified silica stationary phases. The ones that employ cyano-modified silica perform generally worse. The introduction of alternative coloring schemes for the covariance matrices and SRD/GPCM heatmaps enables the discovery of intrinsic relationships among lipophilicity scales and the selection of best/worst measures. Closest to the recommended logK_{OW} values are Clog*P* and the first principal component scores obtained on octadecyl-silica stationary phase in combination with methanol-water mobile phase, while the usage of slopes derived from Soczewinski-Matyisik equation should be avoided.

Abbreviations: ANOVA, analysis of variance; C18, octadecyl silica; CEPW, conditional exact test with probability weighted ranking; CN, cyanopropyl-modified silica; COVAT, comparison with one variable at a time; CRRN, comparison of ranks with random numbers; GPCM, generalized pairwise correlation method; HCA, hierarchical cluster analysis; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; IAM, immobilized artificial membrane chromatography; LSER, linear solvation energy relationships; MEKC, micellar electrokinetic chromatography; MLC, micellar liquid chromatography; PC, principal component; PCA, principal component analysis; Rg, range scaling; Rk, rank transformation; SRD, sum of (absolute) ranking differences; St, standardized (autoscaled); TLC, thin-layer chromatography.

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1. Introduction

Throughout the last century lipophilicity evolved into an essential physicochemical parameter that is used in pharmaceutical and environmental sciences abundantly. It is related to the distribution of compounds in the environment and biota, to bioavailability and bioconcentration in the food chain, as well as to the transport in the soil-sediment-water compartments [1]. It is a crucial factor influencing passive transport through biological membranes such as the blood-brain or the gastrointestinal barriers [2,3]. Lipophilicity has a high impact on protein binding, drug-receptor interactions, which consequentially affect the desired physiological response, as well as drug-related toxicity and adverse effects [4,5].

Nevertheless, since the first works of Meyer and Overton [6,7], lipophilicity has been tailored to suit our practical needs, while its strict definition remains ambiguous. In that sense, according to the International Union for Pure and Applied Chemistry (IUPAC), lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment [8]. It is still not clear what a "lipophilic environment" actually is, and how it should be modelled. Such a vague definition of lipophilicity itself might be one of the reasons that create additional space for development of various lipophilicity measures and numerous experimental approaches for its measurement and estimation. In order to establish some constraints the IUPAC gives some recommendations how lipophilicity should be or could be measured [8]. The traditionally adopted shake-flask method – based on the distribution between *n*-octanol and water (commonly denoted as log*P*, but more frequently replaced with $\log K_{OW}$ in contemporary literature) – is time and reagent consuming, experimentally demanding, tedious, and mostly applicable to pure compounds that have partition coefficients in the range of −3 to 4.5 log units (some modifications of the shake flask method are applicable for compounds with $\log K_{OW} > 4.5$). In order to overcome these difficulties many chromatographic methods have been developed, and some of them have been adopted as standard methods, parts of OECD guidelines (Organization for Economic Cooperation and Development), such as Test No. 117, HPLC method [9]. Aside from very specific applications of chromatographic approaches that tend to mimic biosystems such as micellar liquid chromatography (MLC) [10–15], immobilized artificial membrane chromatography (IAM) [16,17], immobilized proteins [18] *etc*., the mainstream methods in the determination of lipophilicity are still based on typical reversed-phase chromatography involving a variety of chemically bonded stationary phases [19–22], where octyl-, octadecyl-, and cyanopropyl-modified silica beds are the most frequently used in combination with a polar mobile phase (usually binary mixtures of miscible organic solvents and water) [23–25].

Both high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) produce a high number of chromatographic lipophilicity indices. However, TLC has a significant advantage over HPLC because of its simplicity, significantly reduced costs, short analysis time, low consumption of solvents and reagents, and its ability to simultaneously handle dozens of samples.

Several lipophilicity measures stem from TLC experiments. The intercept ($R_M{}^0$) and the slope (b) of the linear dependence of the retention on the volume fraction of the organic component of the mobile phase (φ), proposed by Soczewinski and Matyisik [26] (Eq. (1)), have been introduced among them first. The R_M value is defined according to Eq. (2).

$$R_M = R_M^0 - b\varphi \tag{1}$$

$$R_M = \log\left(\frac{1}{R_F} - 1\right), \tag{2}$$

where R_F is the retardation factor, *i.e.* the ratio of the distance of a solute target zone and the solvent front.

The parameter b can be related to the specific hydrophobic surface area of the solute [27] and the surface tension of the mobile phase [28], while the intercept describes partitioning between pure water and the non-polar, hydrophobic stationary phase.

In addition, the concentration of the organic solvent in the mobile phase resulting in equal distribution of a solute among the stationary and mobile phases, C_0, was introduced by Bieganowska *et al.* [29], and is frequently used. It is defined as the ratio of the intercept ($R_M{}^0$) and the slope (b):

$$C_0 = -\frac{R_M^0}{b} \tag{3}$$

Alongside the extrapolated chromatographic lipophilicity measures, the ones based on primary retention data are also used as *e.g.* the first principal component scores ($PC1/R_M$) derived from principal component analysis (PCA) of multivariate retention data [30,31], and arithmetic means of R_M values, more frequently denoted as mR_M [23–25].Besides the experimental methods, computational approaches for the prediction of log*P* values are extensively used. Their main advantage is that they simply do not require experimental measurements. They can be classified in two large families: substructure-based and property-based methods. Substructure-based methods decompose the molecular structure into smaller fragments (or even down to the level of single atoms). Depending on the algorithm used, each fragment is then associated with a particular log*P* contribution. The final log*P* value of the unknown compound is obtained by a summation over all fragment contributions, and using correction factors, where necessary [32]. Examples of fragmentation/group contribution based methods are: ClogP, AClogP, ALOGP, miLogP, KOWWIN, XLOGP2, XLOGP3 [33–38]. Property-based methods, on the other hand, consider the molecule as an undivided entity [32]. Calculation of log*P* is based on quantitative structure – property relationship (QSPR) models using physicochemical parameters such as the case with the Linear Solvation Energy Relationships (LSER) approach [39], or from molecular descriptors obtained from 3D representations (*e.g.* COSMOFrag) [40], or simple 1D topological, and electrotopological indices (MLOGP, ALOGPs) [41,42]. Nevertheless, both property- and substructure-based methods are accompanied by estimation errors that reach orders of magnitude for the same molecule as compared to each other. Computational methods that are used in the present work are listed in Section 2.3.

When it comes to the selection of an appropriate approach to lipophilicity assessment there are several problems, errors, and misconceptions, especially in the case of newly synthesized compounds or novel lipophilicity indices. If there is no possibility to obtain octanol-water partitioning data, chromatographic and computational estimates are most frequently used to estimate lipophilicity. However, no systematic or widely accepted approach exists for the selection of appropriate lipophilicity measures. Many procedures use similarities among computationally estimated values and experimentally derived lipophilicity indices as a criterion to select the best one. Such similarities are most often obtained from hierarchical clustering (HCA) [43,44], principal component analysis [21,23,25,45], or simple correlations based on parametric statistics such as Pearson's correlation coefficient [24,25,44]. The last one is applicable only if the data is normally distributed, which is often not the case. PCA and HCA do not provide information about the statistical significance of such similarities, while the use of correlation measures most often lead to selection of the most correlated pairs, neglecting the rest of the statistically significant ones.

The aim of the present work was to rank and group lipophilicity measures from the typical reversed-phase thin-layer chromatographic data, to find the most similar and dissimilar ones, to suggest