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Influence of organic modifier and separation modes for lipophilicity assessment of drugs using thin layer chromatography indices

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ABSTRACT

Lipophilicity constitutes one of the most important physicochemical properties in the design and development of drug molecules. In the present work thin layer chromatography (TLC) has been utilized to evaluate lipophilicity of 11 representative drugs, which included six proton pump inhibitors (omeprazole, pantoprazole, rabeprazole, lansoprazole, ilaprazole, and tenatoprazole), an anti-vertigo drug, betahistine, nonsteroidal anti-inflammatory drug, ibuprofen, anti-malarial drug, atovaquone, an anti-HIV agent, atazanavir and a hormonal drug, calcitriol. Normal as well as reversed-phase separation modes were evaluated to study the effect of different organic modifiers for the estimation of lipophilicity. The quantitative descriptor of lipophilicity, the partition coefficient (logP) was estimated by suitably optimizing the solvent systems for both the modes. The best mobile phase pairs for NPTLC and RPTLC were toluene-acetonitrile and water-methanol respectively. Principal component analysis, hierarchical cluster analysis, as well as non-parametric methods like sum of ranking differences and generalized pair wise correlation revealed the dominant pattern in the data. The results obtained from both the separation modes were comparable and were in good agreement with the computational data for all the drugs.

1. Introduction

Lipophilicity of a drug molecule is of paramount importance because of its impact on metabolism, pharmacokinetics, pharmacodynamics and toxicological profile of the molecule. This is attributed to its contribution to absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of a drug like solubility, permeability through membranes, distribution, potency, promiscuity etc. [1]. As per the IUPAC definition of lipophilicity, it represents the affinity of a molecule or a moiety for a lipophilic environment [2].

Rutkowska et al. [3] have reviewed techniques for direct and indirect measurement of lipophilicity. Direct measurement methods include shake flask method and potentiometric titration method which provides encouraging results. However, they have some accompanying drawbacks like lengthy and tedious process, reproducibility issues, and requirement of compound with high purity and in relatively large amounts. On the contrary indirect measurement methods based on liquid chromatography and other techniques provide inherent advantages like shorter anal-

ysis time and analysis of compounds with extended logP values (also called $logK_{OW}$), ranging from 0 to 6. Furthermore, diversified thin layer chromatographic reports dominate the literature when it comes to the lipophilicity assessment of organic compounds, owing to its cost-effectiveness, ability to analyse multiple compounds in a single run and easier experimental manipulations. Literature reveals many reports on the determination of lipophilicity of drug molecules using reversed-phase thin layer chromatography (RPTLC) [4–6], while few reports are available utilizing the normal phase (NP) approach for lipophilicity assessment [7–9]. Keeping in mind the comparative cost of the plates, normal phase mode in TLC is more preferred than their RP counterpart. Even the degree of impregnation has huge impact on the lipophilicity parameters when the RPTLC plates are prepared through impregnation process [10]. Odovic et al. [8] studied the hydrophobic behaviour of five angiotensin converting enzyme (ACE) inhibitors and their four metabolites using both modes of TLC. They showed no significant difference in the hydrophobicity parameters (R_{M0} and C_0) obtained using both the modes and supported applicability of NPTLC for lipophilicity assessment. However, the study was done by analyzing two series of structurally analogous analytes using R_{M0} instead of C_0. Further, the results were not correlated using any advanced statistical test other than the classical regression. Two novel methods have been reported for such statistical ranking or grouping of

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2 N.M. Bhatt et al. / J. Chromatogr. A xxx (2018) xxx–xxx

lipophilicity assessment tools [11]. They have mainly used non-parametric methods like Sum of Ranking Differences (SRD) and Generalized Pair Correlation Method (GPCM) in addition to the more conventional Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) approaches. Though the report discusses a more rational approach towards the grouping or ranking methods for the lipophilicity assessment; it only encompasses the reversed-phase mode of TLC.

Generally, lipophilicity measurements have been carried out using RPTLC with a non-polar stationary phase [12]. Komsta et al. [13] have stressed upon development of standardized approach for selection of organic modifier and recommended use of more "water-like" modifier for measuring lipophilicity using RPTLC. Nevertheless, there are no methods that highlight the impact of modifier and its influence on the measurement of lipophilicity using NPTLC. This motivated us to design a study to evaluate the modifier-effect and provide a comparative evaluation of the results obtained from NPTLC as well as RPTLC. Moreover the present work reviews applicability of NPTLC as a cost-effective alternative to RPTLC for lipophilicity assessment of organic molecules using selected classes of drugs as model solute set. The experimental results were correlated with the theoretically computed lipophilicity descriptors using different computational programs. In addition to classification using PCA and HCA, different approaches to determine the lipophilicity were also ranked using novel non-parametric ranking methods namely SRD and GPCM.

2. Experimental

2.1. Materials and chemicals

All the chemicals used in the present study were of analytical grade unless otherwise stated. Organic solvents like toluene, methanol, ethanol, iso-propanol, n-butanol, and acetonitrile were of analytical grade from E. Merck (Mumbai, India). Reference standards of the selected drugs, omeprazole (OME, 98.71%), pantoprazole sodium sesquihydrate (PAN, 99.55%), rabeprazole sodium (RAB, 98.84%), and lansoprazole (LAN, 98.78%), were obtained from Ashutosh Pellets Ltd. (Gujarat, India), while ilaprazole (ILA, 99.47%), tenatoprazole (TEN, 98.86%), betahistine hydrochloride (BET, 99.48%), and ibuprofen (IBU, 99.45%) were procured from Clearsynth Labs (Mumbai, India). Atovaquone (ATO, 99.11%), atazanavir (ATA, 99.24%), and calcitriol (CAL, 98.78%) were from Titan Pharmaceuticals Ltd. (Mumbai, India).

2.2. Experimental descriptors of lipophilicity

The experimental lipophilicity descriptors for the selected class of drugs were determined using both NPTLC and RPTLC (Table S1). NPTLC measurements were performed using aluminium backed silica gel GF_{254} plates purchased from E. Merck (Darmstadt, Germany), while a RP stationary phase was obtained by impregnating silica gel GF_{254} layer with silicone oil. Plates were impregnated in a chromatographic chamber, with 200 mL of the 5% silicone solution in ether. Once the silicon solution reached the top of the plates, they were left for another 30 min and subsequently dried on a hot plate at 50 °C.

For sample spotting, reference standards of the investigated drugs were separately dissolved in methanol (1.0 mg/mL) and 1.0 μL aliquot was used for sample application on the plates. Chromatograms were developed using linear ascending mode at room temperature. Typical chromatographic chambers (CAMAG, Muttenz, Switzerland) were filled with 35 mL of mobile phase and saturated with the vapours for 20 min prior to plate development. For NPTLC experiments toluene was used as organic solvent with different polar mobile phase component, viz. methanol, ethanol, iso-propanol, n-butanol, and acetonitrile, at varying volume fractions. In the case of RPTLC, water was used as a mobile phase component with different organic modifiers namely methanol, ethanol and acetonitrile. For NPTLC and RPTLC the percent organic modifier volumes were varied within the range of 10–80 % and 40–80 %, respectively, in 10% increments. The plates were developed up to a distance of 8.0 cm, dried on a hot plate and spots were visualized under UV illumination at 254 nm. Mean retention factor (R_f) values of the visualized spots obtained from three replicate measurements were used for subsequent calculations. Statistical differences among the R_f values obtained from three chromatographic runs were not significant at $p < 0.005$ (t-test). MS Excel software packages were used to perform all calculations and graphics were prepared using OriginPro 8 software package.

The retardation factors of the visualized spots on a TLC plate were converted to R_M values which are defined using Equation 1,

$$R_M = log\left(\frac{1}{R_f} - 1\right) \tag{1}$$

The calculated R_M values were plotted against the volume percentage of organic modifier and extrapolated to zero concentration. The chromatographic parameter of lipophilicity (R_{M0RP}), which corresponds to the intercept of the linear dependence of the retention on the volume of the organic component of the mobile phase for RPTLC was determined by extrapolation of the said plot to zero concentration of organic modifier. This can be described using the Soczewiński–Wachtmeister's Equation 2 [3,14];

$$R_M = R_{M0RP} - S \cdot \varphi \tag{2}$$

where, R_M of respective compound was calculated from Equation 1, R_{M0RP} is the R_M value extrapolated to zero concentration of organic modifier in mobile phase, S is the slope of the regression plot and φ is the volume percentage of organic modifier in the mobile phase.

For NPTLC, the calculated R_M values obtained using Equation 1, were plotted against the logarithm of the volume percentage of organic modifier. The R_M values at zero modifier concentration (R_{M0NP}) were acquired by extrapolation of the linear response [15].

$$R_M = R_{M0NP} - b \cdot log\varphi \tag{3}$$

Where, R_{M0NP} is the R_M value extrapolated to zero modifier concentration; b is the slope of the regression plot; and φ is the percentage of organic modifier in the mobile phase. However, another lipophilicity descriptor (C_0) for NPTLC was calculated from the values obtained from the said plot using the following Equation 4 [15],

$$C_0 = -R_{M0NP}/b \tag{4}$$

2.3. Theoretical descriptors of lipophilicity

ChemDraw Ultra 12 program was applied to generate SMILES codes for all the studied drugs, which were used for calculation of lipophilicity using different programs that computes values of partition coefficient according to algorithms based on chemical structure of investigated compounds. Theoretical values of lipophilicity descriptors were computed on different scales using various online tools and available programs (Table S2). Some of the computed values namely ALOGPs, AC_logP, milogP, ALogP, MLogP, XLOGP2, and XLOGP3 were obtained using ALOGPS online program version 2.1 from Virtual Computational Chemistry Laboratory available online at http://www.vcclab.org. In silico molecular property prediction provided by Molsoft LLC (available at http://www.molsoft.com) was used to avail MolLogP. Evaluation version of BioLoom ver. 5 (BioByte Corp., CA) was used for the prediction of ClogP values. ACD/LogP, and LogKow values were obtained from https://