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Learning how to use IAM chromatography for predicting permeability

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ABSTRACT

The interest for IAM (Immobilized Artificial Membranes) chromatography in the prediction of drug permeability is increasing. Here we firstly set-up a dataset of 253 molecules including neutral and ionized drugs and few organic compounds for which we either measured or retrieved from the literature IAM.PC.DD2 log K_w^{IAM} data. Then we applied block relevance (BR) analysis to extract from PLS models the relative contribution of intermolecular forces governing log K_w^{IAM} and $\Delta\log K_w^{IAM}$ (a combined descriptor calculated from log K_w^{IAM}). Finally, the relationship between log K_w^{IAM}, $\Delta\log K_w^{IAM}$ and passive permeability determined in both PAMPA and MDCK-LE systems was looked for. Models provided the basis for a rational application of IAM chromatography in permeability prediction.

1. Introduction

The interest for IAM (Immobilized Artificial Membranes) chromatography in drug discovery is known and increasing in recent years (Russo et al., 2017; Tsopelas et al., 2016; Valko, 2016). IAM columns are prepared by covalent binding of a monolayer of phospholipids to silica particles (Pidgeon et al., 1995) thus mimicking the lipid environment of a fluid cell membrane on a solid matrix (Taillardat-Bertschinger et al., 2002a, 2002b). Models based on IAM retention data were found for instance for permeability, volume of distribution, phospholipidosis risk and solubility enhancement in simulated intestinal fluid (Valko, 2014).

Permeability and solubility, determine the oral absorption of small molecules. Passive lipoidal diffusion is one of the most relevant permeation mechanisms by which drug molecules cross biological membranes. Passive diffusion involves extensive molecular interactions of drug candidates with physiological gastrointestinal (GI) barrier. In turn, these interactions mostly rely on the physicochemical properties of drug substances. For project teams handling with series experiencing permeability-limited issues, it is thus crucial to identify the most influencing physicochemical properties to guide the establishment of structure-property relationship for chemistry optimization (Wang and Skolnik, 2013). To this respect biomimetic properties measured by HPLC (e.g. IAM chromatography) could play a pivotal role in drug discovery strategies (Valko, 2016).

However, to clarify the potential application of IAM chromatography in drug discovery, it is crucial to know the balance of intermolecular forces governing IAM retention mechanisms. This topic has been investigated by some authors in the past using different strategies (mostly linear solvation energy relationships (LSER) (Abraham et al., 1999; Lázaro et al., 2006)). There is a general agreement about the fact that IAM retention is characterized by the two traditional components of lipophilicity (i.e. hydrophobicity and polarity) and additional recognition forces such as electrostatic interactions are also present when solutes are partially or fully ionized (Taillardat-Bertschinger et al., 2003; Van Balen et al., 2004). The paper by Li et al. (2006) introduced a molecular electronic factor in the standard LFER procedure to model IAM.PC.DD2. retention of a dataset of 53 structurally diverse compounds, including neutral, acidic and basic compounds.

One method to deconvolute the balance of intermolecular forces governing physico-chemical and biological events is the Block Relevance (BR) analysis which was successfully applied to model chromatographic indexes (Caron et al., 2016, 2015, 2013; Ermondi and Caron, 2012). Shortly BR analysis allows the analysis of the balance of intermolecular interactions governing the retention using common 3D-QSAR/QSPR models (here based on the VolSurf + (VS +) software) based on many descriptors and thus difficult to interpret. These descriptors are aggregated into property-related groups (blocks) which provide a convenient framework for their interpretation. Moreover, since VS + descriptors do take ionization into account (i.e. the same VS + descriptor could have different values for the neutral and the ionized species of a compound), BR analysis is expected to be particularly suited when ionized compounds are present in the datasets.

Abbreviations: BR Analysis, Block Relevance Analysis; IAM, Immobilized Artificial Membranes; LFER, Linear Free Energy Relationships; MDCK-LE, Low-Efflux Madin-Darby canine kidney cell line; PAMPA, Parallel Artificial Membrane Permeability Assay; PPL, phospholipids; PSA, Polar Surface Area; R, rugosity; SFC, Supercritical Fluid Chromatography

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In this study, we firstly set-up a dataset of 253 molecules including significantly populated subsets of neutral, acidic and basic drugs for which IAM.PC.DD2 log K_w^{IAM} values were either measured or extracted from the literature. For neutral molecules $\Delta\log K_w^{IAM}$ (a polarity descriptor recently introduced by Grumetto et al., 2012) was also determined. Then we applied BR analysis to unravel the balance of intermolecular forces governing both log K_w^{IAM} and $\Delta\log K_w^{IAM}$. Finally, the relationship between log K_w^{IAM} and $\Delta\log K_w^{IAM}$ and passive permeability (MDCK-LE log P_{app} and PAMPA log P_{eff} retrieved from the literature) was looked for and results mechanistically interpreted using information provided by BR analysis.

2. Methods

2.1. Chromatographic measurements

HPLC Varian ProStar instrument equipped with a 410 autosampler, a PDA 335 LC Detector and Galaxie Chromatography Data System Version 1.9.302.952 was used.

The analyses were performed at 30 °C with 20 mM ammonium/acetate at pH 7.0 in mixture with acetonitrile at various percentages. The stationary phase was IAM.PC.DD.2: its surface is formed by covalently bonding the membrane forming phospholipids to silica (Regis, 10 cm × 4.6 cm 10 µm packing 300 Å pore size). The flow rate was 1.0 ml/min. All samples were dissolved in the mobile phase.

Chromatographic retention data are reported as log k (the logarithm of the retention factor), calculated by the expression

$$log\ k_{IAM} = log[(t_r - t_0)/t_0] \quad (1)$$

where t_r and t_0 are the retention times of the drug and a non-retained compound (citric acid), respectively. Direct measurements of log k_{IAM} values in fully aqueous mobile phases (log K_w^{IAM}) were only possible for the compounds eluting within 30 min. For the solutes requiring the addition of acetonitrile in the eluent, the log K_w^{IAM} values were calculated by an extrapolation method. Log k values were determined at least three different acetonitrile percentages (ϕ) in the mobile phases (from 10 to 50%, v/v) and the intercept values of the linear relationships ($R^2 \geq 0.99$) between log k and ϕ values were assumed as log K_w^{IAM} values. All reported log k values are the average of at least three measurements. To avoid that the experimental measurements were affected by retention changes due to column ageing, the retention times of five gold standard compounds (caffeine, carbamazepine, ketoprofen, theobromine and toluene) were checked daily.

2.2. VS + models

VS + models for log K_W^{IAM} were built for the main dataset and the four subsets, whereas two models were built for $\Delta\log K_w^{IAM}$ data (all and ionized compounds). Firstly, compounds were split in a training and a test set and then the SMILES codes of the compounds were submitted to VS + (version 1.0.7, http://www.moldiscovery.com) using default settings and four probes (OH2, DRY, N1, and O probes that mimic, respectively, water, hydrophobic, HBA, and HBD properties of the environment). PCA and PLS tools implemented in VS + were used to obtain and validate models.

All datasets were randomly split in a training and in a test set (about 75% and 25% of the compounds, respectively).

2.3. BR Analysis

BR analysis was performed as described elsewhere (Ermondi and Caron, 2012; Caron et al., 2013). Briefly, BR analysis allows useful interpretation of PLS models by organizing the VS + descriptors into six blocks (Size, Water, DRY, N1, O, and Others) of straightforward significance detailed in Fig. 1, panel B. In a very simplistic way, BR analysis gives the relevance of each parameter (block) to the model.

Processing was done on two 8 cores Xeon E5 at 3.3 GHz. CPUs and 128GB of RAM.

2.4. Permeability models

Models and statistics reported in Table 3 were obtained with QSARINS (Gramatica et al., 2014; Gramatica et al., 2013) v.2.2.2 (www.qsar.it). The QSARINS free license was kindly provided by Prof. Paola Gramatica who is acknowledged by the authors. All models were obtained after data normalization.

2.5. PSA calculation

PSA were calculated using PyMol (version 1.8, https://sourceforge.net/projects/pymol/).

3. Results and discussion

3.1. Data collection

The dataset consists of 253 compounds (Table S1 in SI) for which we obtained log K_w^{IAM} values. 62 out of 253 data were acquired in our laboratory and 191 data retrieved from the literature and validated with experimental values. These latter were taken from different papers (Grumetto et al., 2016; Lázaro et al., 2006; Liu et al., 2008; Taillardat-Bertschinger et al., 2002a, 2002b). Details of the validation procedure adopted for integrating log K_w^{IAM} data extracted from the literature with values measured in our laboratory are reported in the (Fig. S1 in SI). Briefly, 8–10 compounds were chosen from the selected literature papers and analysed using our conditions. log K_w^{IAM} extracted from the paper were plotted vs experimental log K_w^{IAM} and a linear correlation was found and used to correct literature data.

Starting from the 253 compounds, four subsets were generated after calculating pK_a with MoKa v.2.6.5. The first included neutral compounds (n = 153), the second ionized compounds (n = 100), the third cations (n = 58) and the fourth anions (n = 42). Compounds were considered as ions when > 50% of ionized species was present.

$\Delta\log K_w^{IAM}$ was calculated for neutral compounds according to Grumetto et al. (2016). log K_W^{IAM} of structurally non related neutral compounds that have PSA = 0 was unambiguously correlated with the logarithm of *n*-octanol repartition coefficient log P^N_{oct} (Fig. S5 in SI):

$$log\ K_w^{IAM} = 0.92\, log\ P^N{}_{oct} - 1.03$$
$$n = 24\ R^2 = 0.95\ Q_{LOO}{}^2 = 0.94\ Q_{LMO}{}^2 = 0.94\ SDEP = 0.22 \quad (2)$$

where n is the number of compounds, R^2 is the determination coefficient, SDEP is the standard deviation of errors of prediction, $Q_{LOO}{}^2$ and $Q_{LMO}{}^2$ are the predicted determination coefficients calculated using the Leave One Out (LOO) and Leave More (or Many) Out (LMO) validation techniques respectively. LMO was performed using default parameters, a percentage of prediction elements equal to 30% and 2000 iterations.

$\Delta\log K_w^{IAM}$ is the difference between the logarithm of the experimental chromatographic retention factor (log K_W^{IAM}) and the value calculated using Eq. (2) using the log P^N_{oct} value of the analyte.

For compounds with few polar atoms, $\Delta\log K_w^{IAM}$ is low, e.g. benzyl benzoate (0.102), whereas compounds rich of polar moieties show high $\Delta\log K_w^{IAM}$ values, e.g. acyclovir (2.061).

Although obtained from different datasets, Eq. (2) does not appreciably differ from analogue equations reported in the literature (Grumetto et al., 2016, 2012).

3.2. log K_w^{IAM}: Vs + models and BR analysis

log K_w^{IAM} data were firstly imported into VS + as response variables (Y, the property) and a relation between Y and the 82 VS + molecular descriptors (X, the structure) was sought using the PLS algorithm