Chromatographic retention factor obtained on immobilized keratin stationary phase - what molecular properties does it encode?

Anna W. Sobańska*, Elżbieta Brzezińska
Department of Analytical Chemistry, Medical University of Lodz, ul. Muszyńskiego 1, 90-151 Łódź, Poland
*anna.sobanska@umed.lodz.pl

INTRODUCTION

Chromatographic retention factors (log k_{KER}) of 33 molecules (2-cresole, 2-naphtol, 3-cresole, 3-nitrophenol, 4-bromophenol, 4-chlorophenol, 4-cresole, 4-ethylphenol, 4-nitrophenol, baclofen, chlorocresole, methyl hydroxybenzoate, phenol, phenylalanine, resorcinol, salcylic acid, thymol, 1,2,3-tris(1-methylethyl)benzene, 1,4-dinitrobenzene, 3-(trifluoromethyl)phenol, 4-cyanophenol, 4-iodophenol, 4-nitrobenzoic acid, anizole, benzamide, benzene, benzoic acid, benzonitrile, caffeine, chlorobenzene, indazole, phenol and toluene) were obtained on an immobilized keratin stationary phase by Turowski and Kaliszan [1]. Their objective was to develop a novel stationary phase that could be used to investigate the skin permeability coefficient of solutes (log K_p) *in vitro*. However, log k_{KER} is not a sufficiently good predictor of skin permeability coefficient to be used as a sole descriptor in log K_p models. Turowski and Kaliszan reported that this descriptor can be used combined with the chromatographic retention factor obtained by Immobilized Artificial Membrane chromatography (log k_{IAM}) and the results of log K_p predictions using multiple linear regression (MLR) models are satisfying (**Equation 1**):

$$\log k_p = -6.558\ (\pm 0.130) + 1.920\ (0.242)\ \log k_{IAM} - 1.039\ \log k_{KER}\ (n = 17, R = 0.932) \qquad \textbf{Equation 1}$$

They noticed that skin permeability increases with the lipophilicity of solutes (encoded primarily by log k_{IAM}) and decreases with their affinity for keratin (expressed as log k_{KER}). Their conclusion is obviously logical, but the model they proposed (**Equation 1**) requires two sets of chromatographic data, obtained on different stationary phases, this being the likely reason why the immobilized keratin stationary phase they proposed has never gained much popularity and, to the best of our knowledge, is not commercially available.

The objective of this research was to investigate the possibility of using log k_{KER} as the only chromatographic parameter in studies of compounds' skin permeability – in combination with calculated descriptors that, for whatever reason, were not considered (or not available) in the original study by Turowski and Kaliszan.

MATERIAL AND METHOD

Molecular weight (M_w), heavy atom count (***#HvAt***), aromatic heavy atom count (***#ArHvAt***), fraction of sp^3 carbons (F_{Csp3}), rotable bond count (***FRB***), hydrogen donor count (***HD***), hydrogen acceptor count (***HA***), molecular refractivity (***MR***), octanol-water partition coefficient (***XLOGP3***) and topological polar surface area (***TPSA***) were calculated using SwissADME software available freely on-line [2]. Reference log K_p values were obtained using EPI Suite software [3]. Equations 2 and 3 were generated by stepwise multiple linear regression, MLR (forward mode) using Statistica v. 13.

RESULTS AND DISCUSSION

In this study the values of log k_{KER} obtained by Turowski and Kaliszan were correlated with a set of descriptors calculated using SwissADME software. It was discovered that log k_{KER} encodes primarily lipophilicity (***XLOGP3***), aqueous solubility (log ***S***) and molecular size descriptors (M_w), which are important factors governing the ability of compounds to cross the skin barrier, but the correlations are moderate (Table 1). On the other hand, log k_{KER} does not correlate with polar surface area (***TPSA***) and the molecule's ability to form hydrogen bonds (***HD***, ***HA***) - which are important properties in the context of solutes' skin permeability.

Table 1. Correlation Matrix (R), n=33

	log k_{KER}	M_w	#HvAt	#ArHvAt	F_{Csp3}	FRB	HA	HD	MR	TPSA	XLOGP3	log S
log k_{KER}	1.00	0.50	0.29	0.33	0.16	-0.05	-0.13	-0.25	0.47	-0.14	0.58	-0.68
M_w	**0.50**	1.00	0.78	0.13	0.45	0.58	0.44	0.16	0.81	0.40	-0.03	-0.10
#HvAt	0.29	0.78	1.00	0.26	0.63	0.75	0.57	0.12	0.90	0.54	-0.14	0.09
#ArHvAt	0.33	0.13	0.26	1.00	0.03	-0.23	-0.02	-0.09	0.25	0.03	-0.05	-0.05
F_{Csp3}	0.16	0.45	0.63	0.03	1.00	0.47	-0.08	-0.12	0.76	-0.14	0.13	-0.14
FRB	-0.05	0.58	0.75	-0.23	0.47	1.00	0.49	0.25	0.67	0.48	-0.26	0.28
HA	-0.13	0.44	0.57	-0.02	-0.08	0.49	1.00	0.37	0.19	0.87	-0.45	0.43
HD	-0.25	0.16	0.12	-0.09	-0.12	0.25	0.37	1.00	-0.01	0.36	-0.41	0.40
MR	0.47	0.81	0.90	0.25	0.76	0.67	0.19	-0.01	1.00	0.25	0.08	-0.15
TPSA	-0.14	0.40	0.54	0.03	-0.14	0.48	0.87	0.36	0.25	1.00	-0.57	0.56
XLOGP3	**0.58**	-0.03	-0.14	-0.05	0.13	-0.26	-0.45	-0.41	0.08	-0.57	1.00	-0.98
log S	**-0.68**	-0.10	0.09	-0.05	-0.14	0.28	0.43	0.40	-0.15	0.56	-0.98	1.00

$$\log K_p = -2.57\ (\pm 0.80) + 1.82\ (\pm 0.37)\ \log k_{KER} - 0.016\ (\pm 0.005)\ M_w + 0.12\ (\pm 0.08)\ \textbf{\#HvAt} - 0.27\ (\pm 0.11)\ \textbf{\#ArHvAt} - 0.017\ (\pm 0.005)\ \textbf{TPSA}$$

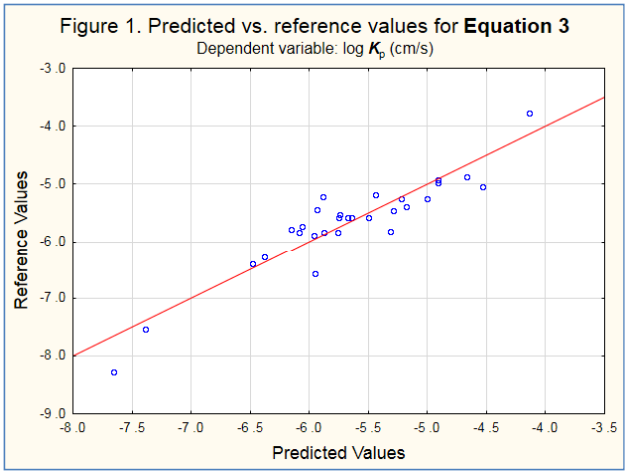
$(n = 33, R^2 = 0.70, R^2_{Adj.} = 0.65, F = 12.8, p < 0.01, s_e = 0.54)$ **Equation 2**

Equation 2 improved significantly when acidic/strongly ionizable compounds (phenylalanine, salicylic acid, benzoic acid, 4-nitrobenzoic acid) were removed and the resulting **Equation 3** accounts for 85% of total variability (Figure 1).

$$\log K_p = -2.73\ (\pm 0.53) + 1.80\ (\pm 0.25)\ \log k_{KER} - 0.015\ (\pm 0.003)\ M_w + 0.13\ (\pm 0.05)\ \textbf{\#HvAt} - 0.27\ (\pm 0.07)\ \textbf{\#ArHvAt} - 0.020\ (\pm 0.003)\ \textbf{TPSA}$$

$(n = 29, R^2 = 0.85, R^2_{Adj.} = 0.82, F = 25.9, p < 0.01, s_e = 0.35)$ **Equation 3**

Log K_p is, surprisingly, positively correlated with log k_{KER} and (which is less surprising) it is inversely correlated with M_w, ***#ArHvAt*** and ***PSA*** – larger and more polar molecules are not absorbed transdermally as easily as smaller and less polar ones.



CONCLUSIONS

It was concluded that log k_{KER} could be used as a descriptor in MLR models of log K_p in combination with other parameters , such as polar surface area, molecular weight and the count of heavy/aromatic heavy atoms. However, the model proposed in this study requires further development, as it seems to fail while applied to acidic compounds – it is likely that the interations between keratin and acidic compounds differ from those between keratin and neutral or basic solutes. Another explanation of the discrepancy between the reference and predicted log K_p values may be related to the reference data – EpiSuite log K_p calculations work best for compounds of "moderate" properties and tend to give erroneous results when applied to hydrophilic, ionizable, extremally lipophilic or very strongly H-bonding molecules [4,5].

REFERENCES

1. Turowski, M. and Kaliszan, R. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1325-1333
2. Daina, A.; Michielin, O.; Zoete, V. *Sci. Rep.* **2017**, *7*, 42717
3. EPI SuiteTM-Estimation Program Interface | US EPA Available online: https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface.
4. Fu, X.C.; Wang, G.P.; Wang, Y.F.; Liang, W.Q.; Yu, Q.S.; Chow, M.S.S. *Pharmazie* **2004**, *59*, 282-285
5. Cronin, M.T.D., Dearden, J.C., Moss, G.P., Murray-Dickson, G. *Eur. J. Pharm. Sci.* **1999**, *170*, 129-133

Funding: This research was supported by an internal grant of the Medical University of Łódź no. 503/3-016-03/503-31-001.