In silico drug-protein binding studies using TLC retention data

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Introduction

One of the most important factors influencing the transport and distribution of a drug in a living organism is protein binding [1]. The thin-layer chromatography (TLC) experiments, with albumin-modified plates, were designed as an indicator of the protein binding affinity of the group of 129 drugs. Retention data, and molecular descriptors (MDs), were later used in multiple linear regression (MLR) analyses to create a model for predicting protein binding.

Materials and methods

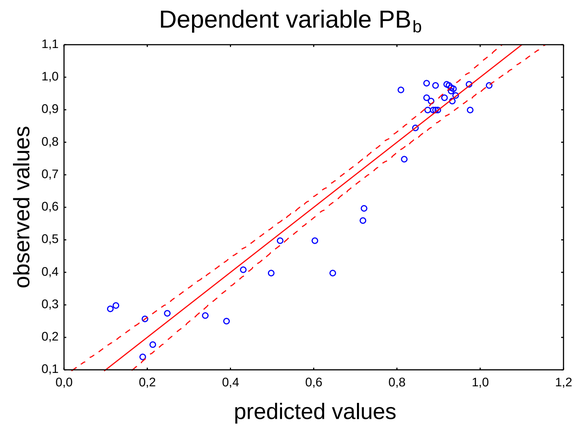
TLC chromatography: experiments were performed in normal and reverse phase (NP and RP-2 plates respectively). Plates were covered with a 2 mg/ml solution of bovine serum albumin (BSA) which is used as a substitute for human albumin. The plates, with the applied solutions of 129 drugs, were then developed in the mobile phase with acetonitrile: methanol: acetate buffer pH 7.4, 60:20:20 (v/v/v). Plates were scanned in a Desaga Densitometer CD 60 and densitograms with retardation factor (R_f) values were obtained. The reference plates, without BSA, were developed and scanned in the same way.

***Statistical modelling*:** MLR analyses were performed in STATISTICA 13.1 (TIBCO Software Inc.). Protein binding values were taken from DrugBank database, and they were used as dependent variable (PB). Molecular descriptors were taken from online databases: DrugBank [2] and CHEMBL [3] or calculated in HyperChem (HyperCube Inc, 2002). Compounds were later divided into groups of acidic(a), basic(b) and neutral(n) ones, and MLRs were performed for each group individually (PB_a, PB_b, PB_n) or for the combined sets (PB_{ab}, PB_{an}, etc.).

molecular descriptors (MDs)	description
NP; RP2	The R_f, obtained in TLC chromatography on the BSA impregnated plates, in normal and reversed mode, respectively
NP/C; RP2/C	The R_f from BSA impregnated NP or RP-2 plate/the R_f factor from a clear plate
NP/PB; RP2/PB	The R_f from BSA impregnated NP or RP-2 plate/protein binding value
NP/PSA; RP2/PSA	The R_f from BSA impregnated NP or RP-2 plate/polar surface area
NP/B2; RP2/B2	The R_f from BSA impregnated NP or RP-2 plate/ computational parameter B2, describes the bioavailability in the central nervous system

Results and disscussion

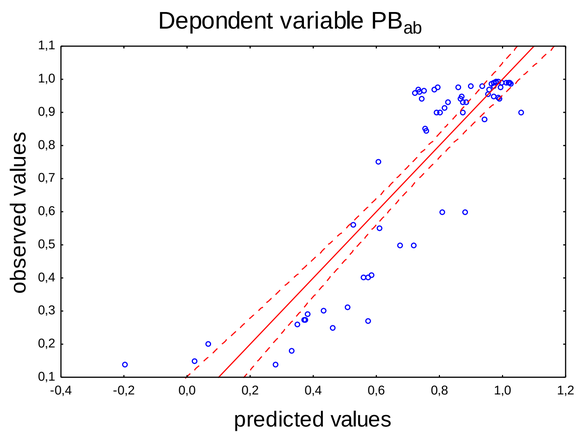
The best correlation were obtained for basic (PB_b), acidic (PB_a) drug sets and for combination of acids and bases (Pb_{ab}) .



PB_b = 0.76(±0.054) + 0.88(±0.11) NP – 0.54(±0.03) NP/PB

R= 0.96; R^2 = 0.91 F(2,31) = 160.74; p <0.0000; s = 0.09437; n_b = 34

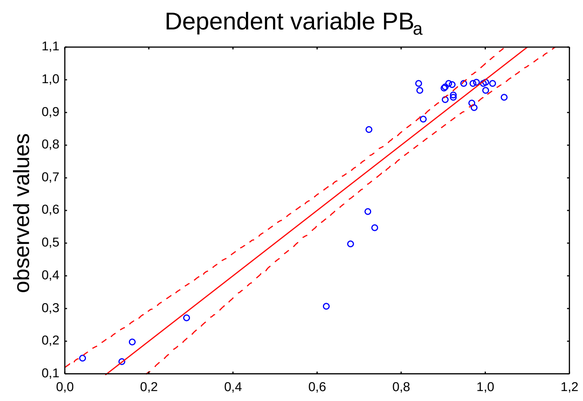
Q^2_{LOO} = 0.88, SDEP= 0.1063, PRESS =0.3636, S_{PRESS} = 0.1034, Q^2_{LMO} =0.86



PB_{ab} = 1.39(±0.41) + 0.80(±0.09) NP – 0.30(±0.02) NP/PB + 0.01(±0.00) NP/B2 + 0.03(±0.01) NP/PSA – 0.89(±0.43) NP/C

R= 0.89; R^2 = 0.80 F(5,57) = 44.235; p <0.0000; s = 0.14432; n_{ab} = 63

Q^2_{LOO} = 0.63, SDEP= 0.1883, PRESS =2.6723, S_{PRESS} = 0.2060, Q^2_{LMO} =0.63



PB_a = 0.68(±0.09) + 0.43(±0.12) RP2 – 0.23(±0.02)RP2/PB+0.01(±0.00) RP2/B2 + 0.10(±0.04) RP2/PSA

R= 0.93; R^2 = 0.86, F(4,24) = 36.4743; p <0.0000; s = 0.1213;

n_a = 29

Q^2_{LOO} = 0.74, SDEP= 0.1541, PRESS =0.8425, S_{PRESS} = 0.1704, Q^2_{LMO} =0.74

Regardless of the significant differentiation of the structure, acid-base character, the chromatographic parameters describe the ability of drugs to bind to proteins at a very similar level. The correlation coefficient in the presented models ranges from 0.89 to 0.96. Mathematical models with the participation of Rf variables explain 80-91% of the variability of PB in groups. Bovine serum albumin (BSA) modified stationary phase TLC analysis appears to provide data (Rf and derivatives) on protein binding for any drug class.

Bibliography

[1] C. Bertucci and E. Domenici, *Reversible and covalent binding of drugs to human serum albumin: Methodological approaches and physiological relevance,* Curr. Med. Chem. 9 (2012), pp.1463–1481.

[2] Drugbank. Available at https://www.drugbank.ca/drugs/DB01174.

[3] Chembl database. Available at https://www.ebi.ac.uk/chembl/.

Acknowledgement

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