Predicting drug penetration across the blood-brain barrier: comparison of different stationary phases for immobilized artificial membrane liquid chromatography

M. De Vrieze[1], F. Lynen[1], D. Verzele[2], R. Szucs[3], P. Sandra[4]

[1] Separation Science Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 S4-bis, B-9000 Ghent
[2] Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk
[3] Pfizer Global R&D, Sandwich CT13 9NJ, Kent, United Kingdom
[4] Research Institute for Chromatography, President Kennedypark 26, B-8500 Kortrijk

INTRODUCTION

The Blood-Brain Barrier (BBB) permeability evaluation is an essential task for developing effective drugs for the treatment of the Central Nervous System (CNS). Both for drugs already on the market or under development, it is essential to know to what extent a drug enters the BBB. A common measure of the degree of BBB permeation is the ratio of the steady-state concentration of the drug molecule in the brain to the concentration in the blood, usually expressed as log ($C_{brain/blood}$) or log BB [1].

In this study, the performance of three stationary phases for immobilized artificial membrane (IAM) liquid chromatographic approaches were compared on a set of 49 compounds. All data were correlated with actual log BB values and the relative performance of the approaches was studied.

IAMs mimic the lipid environment of a cell membrane by anchoring synthetic (phospho)-lipid analogues at monolayer density to silica particles. These particles are subsequently used as a column packing material for HPLC [2]. The drug interactions in IAM-LC are presented in Figure 1.

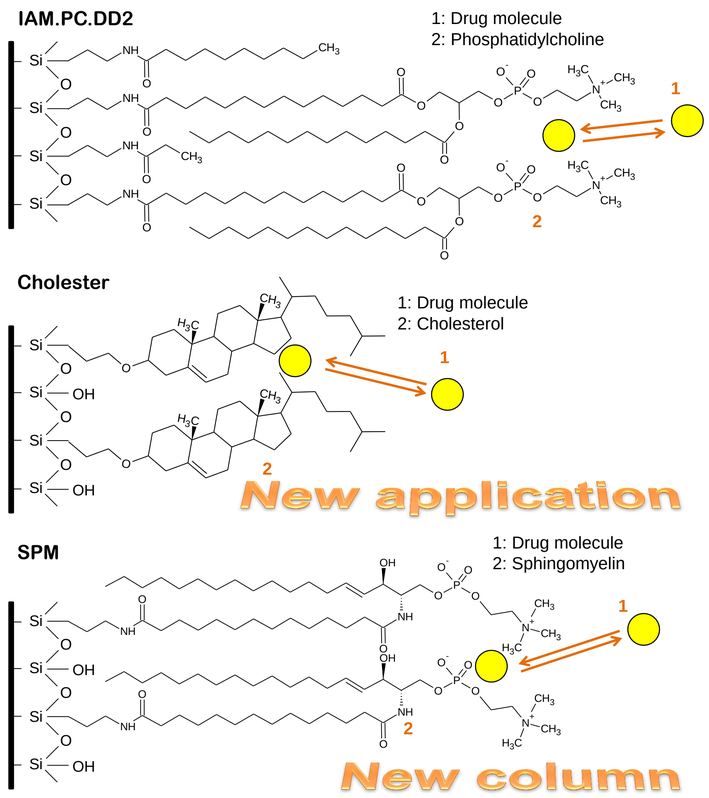

Figure 1: Schematic representation of drug interactions in Immobilized Artificial Membrane (IAM) liquid chromatography on an IAM.PC.DD2, a Cholester and a SPM column.

EXPERIMENTAL

IAM

Measurements were performed on three IAM-columns, namely an IAM.PC.DD2 column (10 µm, 150 x 4.6 mm), a Cholester column (5 µm, 250 x 4.6 mm) and an in-house synthesized Sphingomyelin column (150 x 3 mm) [3]. The mobile phase flow rate was 1 ml/min, except for the Sphingomyelin column, where a flow rate of 0.5 ml/min was used. The mobile phase was a mixture of methanol and Dulbecco's Phosphate-Buffered Saline (DPBS).

Log BB

The retention factors (k) of the compounds were measured. A Partial Least Squares (PLS) regression was performed in order to determine the correlation coefficient (R) between actual (in vivo) log BB values and log BB values predicted using log k values and several molecular descriptors. The most relevant descriptors were selected by systematic removal and/or reinsertion of all descriptors from the models while monitoring the effect on the Leave-One-Out Cross-Validation (LOOCV) regression coefficients.

RESULTS & DISCUSSION

To illustrate the difference in retention behavior (and thus also log BB prediction) of the columns, chromatograms obtained for three compounds are given in Figure 2.
There is no particular elution order for compounds on these columns, which is an indication of the difference in selectivity.

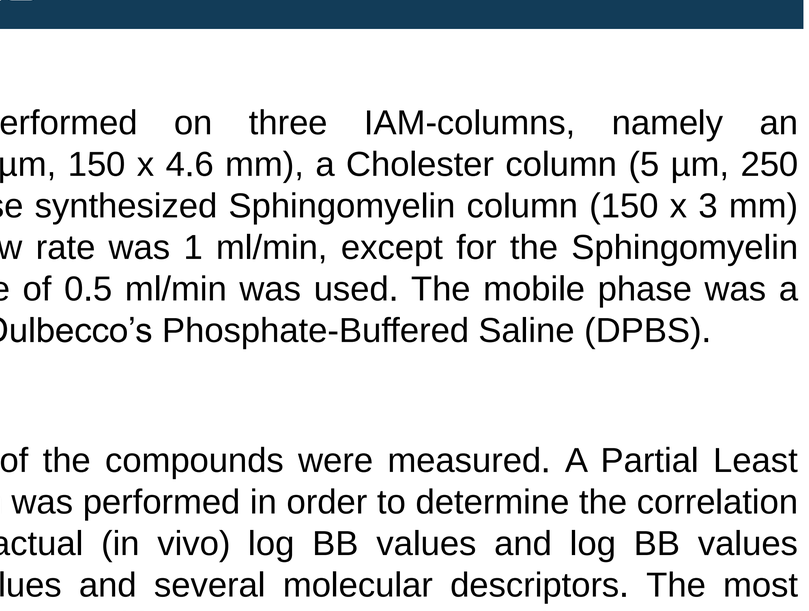

Figure 2: Chromatograms obtained by analyses of clonidine, halothane and ibuprofen on an IAM.PC.DD2, a Cholester and a SPM column.

The results from the PLS and LOOCV regressions before and after elimination of superfluous molecular descriptors are presented in Table 1. The large difference in correlation coefficient in Table 1A is an indication of overfitting in the model. By removing unnecessary descriptors, the overfitting was reduced a lot (Table 1B). For all three columns, a correlation coefficient of ± 0.80 was obtained, indicating a good log BB prediction.

Table 1: Correlation coefficients between actual and predicted log BB values using PLS and LOOCV (A) before and (B) after optimization of molecular descriptors.

(A)

	IAM.PC.DD2 30 % MeOH	Cholester 50 % MeOH	SPM 30 % MeOH
R (PLS)	0.8772	0.8604	0.8701
R (LOOCV)	0.6231	0.5620	0.6064

(B)

	IAM.PC.DD2 30 % MeOH	Cholester 50 % MeOH	SPM 30 % MeOH
R (PLS)	0.8542	0.8303	0.8429
R (LOOCV)	0.8129	0.7750	0.7994

The correlation between actual and predicted log BB values is illustrated in Figure 3 for all columns before and after optimization. Although there are a few outsiders, the predicted log BB values for most compounds are close to the actual (in vivo) determined values.

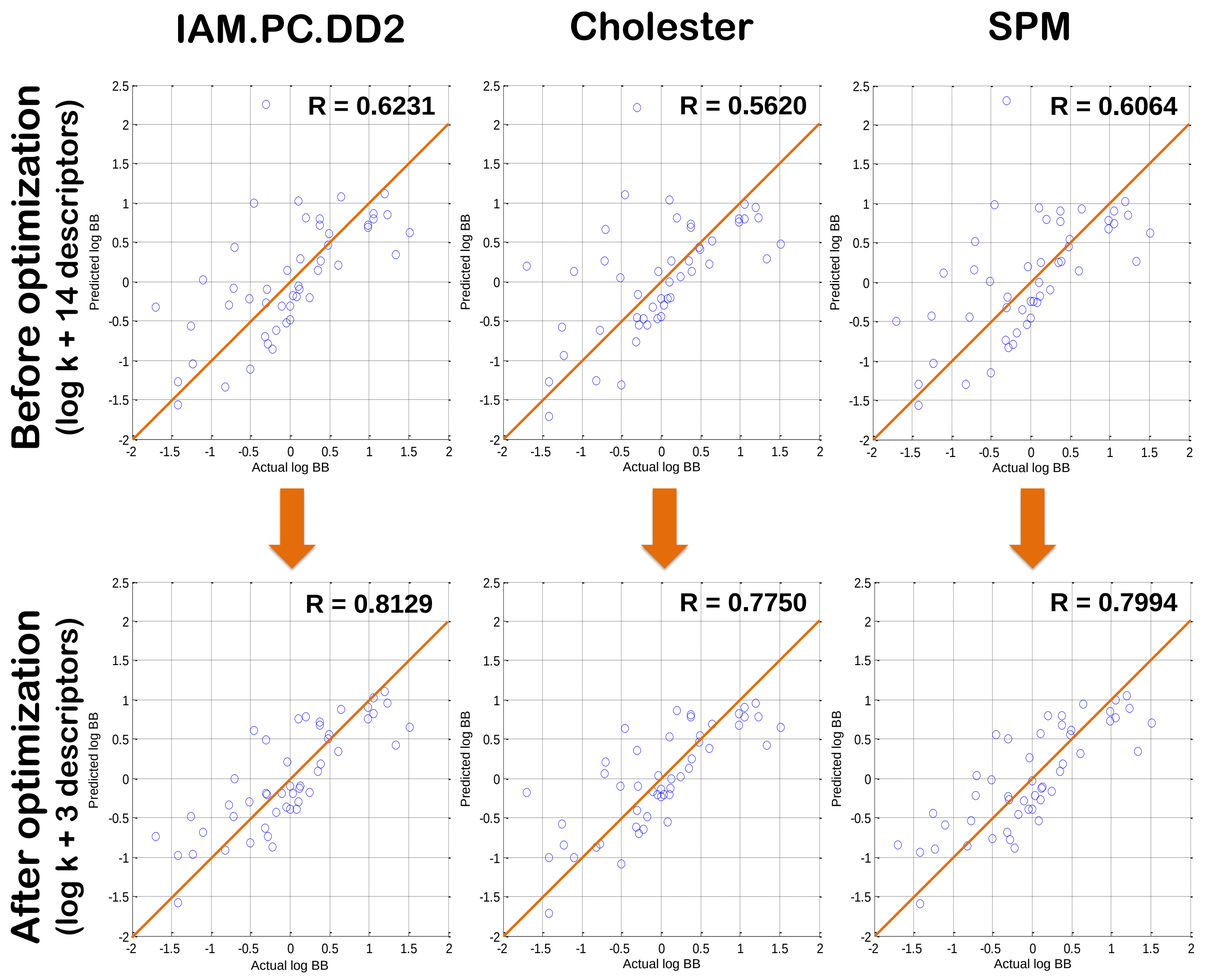

Figure 3: Visual representation of the correlation between Actual and Predicted log BB values using the LOOCV method before and after elimination of superfluous molecular descriptors

Prediction of log BB values

The coefficients of the equations obtained from PLS regressions that lead to the R values listed in Table 1B, are listed in Table 2. Except for the log k values, all descriptor values are available in literature or can be calculated.

Table 2: Coefficients generated by PLS regression after elimination of superfluous descriptors. The general equation for the predicted log BB values is:
log BB = a + b × α + c × Pr + d × HIA + e × log k

	IAM.PC.DD2 30 % MeOH	Cholester 50 % MeOH	SPM 30 % MeOH
a	-2.831	-3.374	-2.750
b	0.444	0.735	0.653
c	-0.003	-0.002	-0.003
d	0.042	0.044	0.039
e	0.703	0.629	0.706

CONCLUSION

- **The commercial IAM.PC.DD2 column was compared to a cholester and a new SPM column towards log BB prediction.**
- **All three models performed very good, illustrating that these three columns can be used for this kind of modeling.**
- **Other (phospho)lipid-like stationary phases should be developed and tested for prediction of log BB values.**

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