Electronic Supplementary Information (ESI)

Chem. Commun.

Development of the first sphingomyelin biomimetic stationary phase for immobilized artificial membrane (IAM) chromatography

Dieter Verzele,[a] Frédéric Lynen,[a] Mike De Vrieze,[a] Adrian G. Wright,[b] Melissa Hanna-Brown[b] and Pat Sandra[ac]*

[a] *Pfizer Analytical Research Centre, Department of Organic Chemistry, Ghent University, Krijgslaan 281-S4bis, B-9000 Ghent, Belgium.*
Fax: +32 9 264 4998; Tel: +32 9 264 9608; E-mail: pat.sandra@ugent.be

[b] *Pfizer Global R&D Laboratories, Analytical Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom.*
Tel: +44 1304 642125; E-mail: melissa.hanna-brown@pfizer.com

[c] *Research Institute for Chromatography, Kennedypark 26, B-8500 Kortrijk, Belgium.*
Fax: +32 56 204859; Tel: +32 56 204031; E-mail: pat.sandra@richrom.com

---

Details of the Partial Least Squares (PLS) regression analysis (Manuscript Fig. 2)

The Partial Least Squares (PLS) regression analysis, shown in Fig. 2 in the manuscript, was performed with Matlab software. For 23 test compounds, *in vivo* log BB values were used to construct the y-block (response variable), while calculated molecular descriptors and experimental log k_{IAM} values were used to construct the X-block (descriptor variables) in a log BB prediction model.[1] Both were normalized before PLS analysis.

The following molecular descriptors were used: total molar charge (α), molecular weight (MW), molar refractivity (MR), molar volume (MV), parachor (Pr), polarizability (Pol), the logarithm of the octanol-water partition coefficients (log P), the logarithm of the octanol-water distribution coefficients at pH 7.4 (log $D_{7.4}$), intrinsic aqueous solubility (log WSo), solubility profile at pH 7.4 ($WS_{7.4}$), plasma protein binding (PB), Ames test mutagenic index (MI and MIA) and human intestinal absorption (HIA).

The values of the acidity constants were used to calculate the α values. Structural parameters (MW, MR, MV, Pr, Pol) were calculated with ACD-Chemsketch software. Other parameters (log P, log $D_{7.4}$, log WSo, $WS_{7.4}$, PB, MI, MIA, HIA) were predicted with ChemSilico software.

The k_{IAM} (IAM retention factor) and corresponding log k_{IAM} values on both IAM.PC.DD2 column **1** and Sphingo-IAM column **2** were determined for the 23 test compounds from retention time measurements [$k_{IAM} = (t_{ret} - t_0) / t_0$], as detailed in Table S1.

Table S1 k_{IAM} values [= (t_{ret} - t_0) / t_0] of 23 test compounds measured on IAM.PC.DD2 column **1** and Sphingo-IAM column **2** (+ conditions)

Conditions:

Isocratic elution (25 °C):
NH_4OAc_{aq} buffer (10 mM, pH 7.4) + MeOH (40 % v/v),[2]
Waters 2690 Alliance HPLC chromatograph + Waters 2487 dual λ absorbance detector (210-300 nm).

Data acquisition/processing:
PeakSimple Chromatography Data System (model 202).

		20 µL, 50 µg/mL t_0 = 1.20 min	
		15 cm x 4.6 mm Flow 1 mL/min	15 cm x 3 mm Flow 0.5 mL/min
Entry	*Compound*	*IAM.PC.DD2 column **1***	*Sphingo-IAM column **2***
1	acetaminophen	0.51	0.72
2	acetylsalicylic acid	0.08	2.49
3	aminopyrine	0.63	0.81
4	amobarbital	1.81	2.78
5	antipyrine	0.51	0.63
6	benzene	1.89	2.87
7	carbamazepine	2.31	3.04
8	cimetidine	1.05	0.83
9	eserine	4.63	1.18
10	ethylbenzene	6.07	11.43
11	hexobarbital	1.27	1.89
12	ibuprofen	0.73	11.95
13	indomethacin	1.66	27.72
14	N-methyl-2-pyridineethanamine	2.85	0.51
15	omeprazole	2.34	3.31
16	oxazepam	5.93	7.77
17	pentobarbital	1.89	2.93
18	phenylbutazone	0.54	10.32
19	phenytoin	3.16	5.07
20	ranitidine	2.75	0.68
21	ropinirole	6.76	1.04
22	salicylic acid	0.06	2.18
23	toluene	3.45	5.66

Using IAM.PC.DD2 column **1**, the correlation between *in vivo* log BB values and predicted *in vitro* log BB values can be expressed with equation 1. This model explains 98 % of variance in the data.

Equation 1:
$\log BB = - 0.24 + 0.03\ \alpha - 0.53\ MW - 217.78\ MR - 1.31\ MV + 2.48\ Pr + 216.82\ Pol - 0.14 \log P - 0.21 \log D_{7.4} + 0.21 \log WSo - 0.39\ WS_{7.4} + 0.17\ PB - 0.15\ MI + 0.04\ MIA + 0.59\ HIA + 0.85 \log k_{IAM}$

Using Sphingo-IAM column **2**, the correlation between *in vivo* log BB values and predicted *in vitro* log BB values can be expressed with equation 2. This model explains 95 % of variance in the data.

Equation 2:

$$\log BB = -0.25 + 0.37\,\alpha - 0.29\,MW - 36.38\,MR + 0.57\,MV + 0.10\,Pr + 35.88\,Pol - 0.06\,\log P + 0.20\,\log D_{7.4} + 0.36\,\log WSo - 0.22\,WS_{7.4} + 0.05\,PB + 0.15\,MI + 0.15\,MIA + 0.28\,HIA + 0.33\,\log k_{IAM}$$

➲ Visual results: **Fig. 2** in the manuscript.

---

[1] Adapted from: Escuder-Gilabert, L.; Molero-Monfort, M.; Villanueva-Camañas, R. M.; Sagrado, S.; Medina-Hernández, M. J. *J. Chromatogr. B* **2004**, *807*, 193-201. Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood-brain barrier.

[2] Braddy, A. C.; Janáky, T.; Prokai, L. *J. Chromatogr. A* **2002**, *966*, 81-87. Immobilized artificial membrane chromatography coupled with atmospheric pressure ionization mass spectrometry.