Unraveling the molecular interactions driving retention and selectivity of a sphingomyelin-based stationary phase by QSPR interpreted through block relevance analysis.

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INTRODUCTION

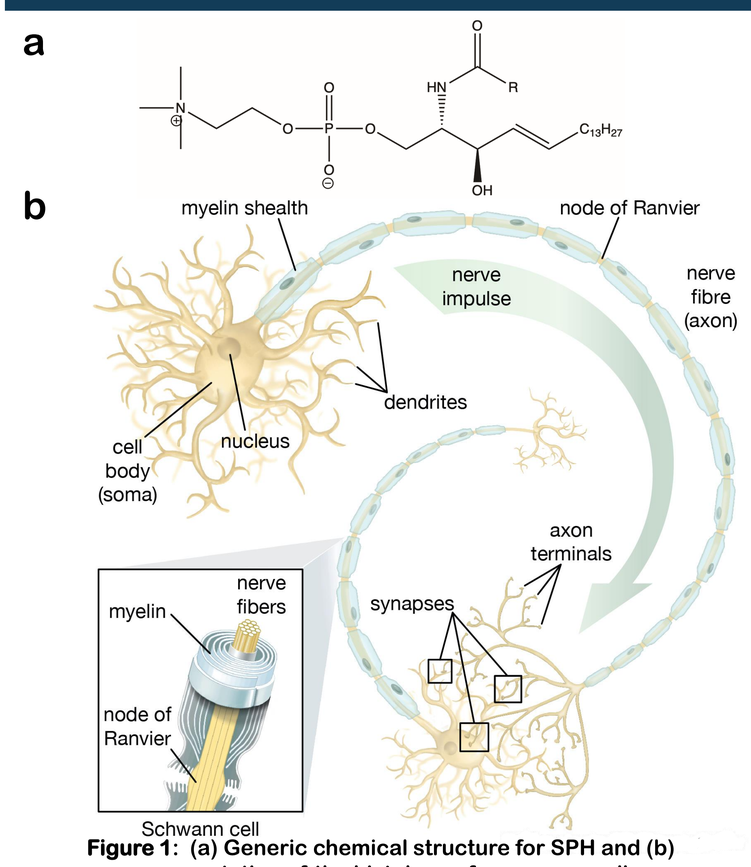


Figure 1: (a) Generic chemical structure for SPH and (b) representation of the histology of a nervous cell.

Sphingomyelin (SPH, Fig. 1a) is a type of sphingolipid found in animal cell membranes in a range from 2 to 15% mol/mol in most tissues. However, SPH features higher concentrations in red blood cells, the ocular lenses, nerve tissues and especially in the membranous myelin sheath that surrounds some nerve cell axons. (Fig. 1b). Because of its characteristics, SPH stationary phases represents an ideal additional tool to mimic the interactions taking place between active pharmaceutical ingredients and neurons.

EXPERIMENTAL

Column packing

The SPH stationary phase (0.821 mg), synthesized by the Separation Science Group in 2012 [1] (Figure 2), was suspended in methanol (7.0 mL) and the resulting slurry was packed (600 bar) in an HPLC column (10 cm x 2.1 mm).

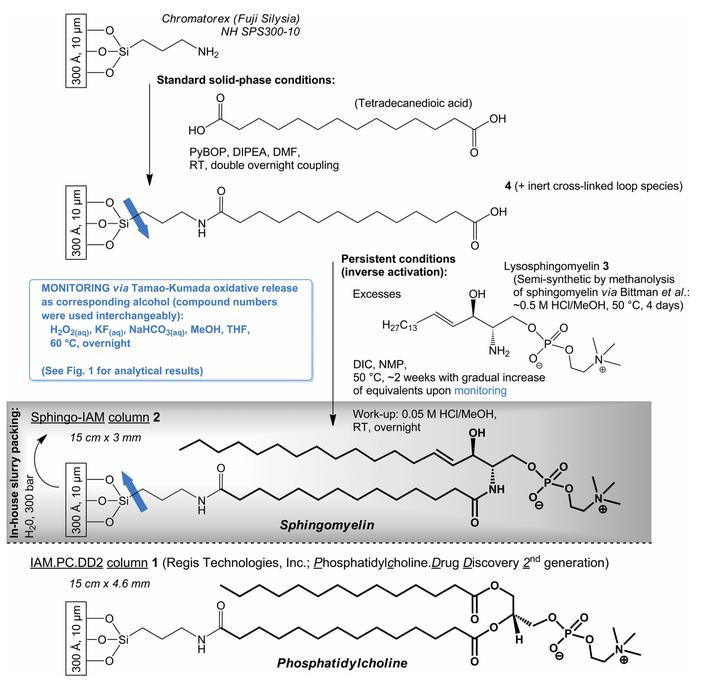


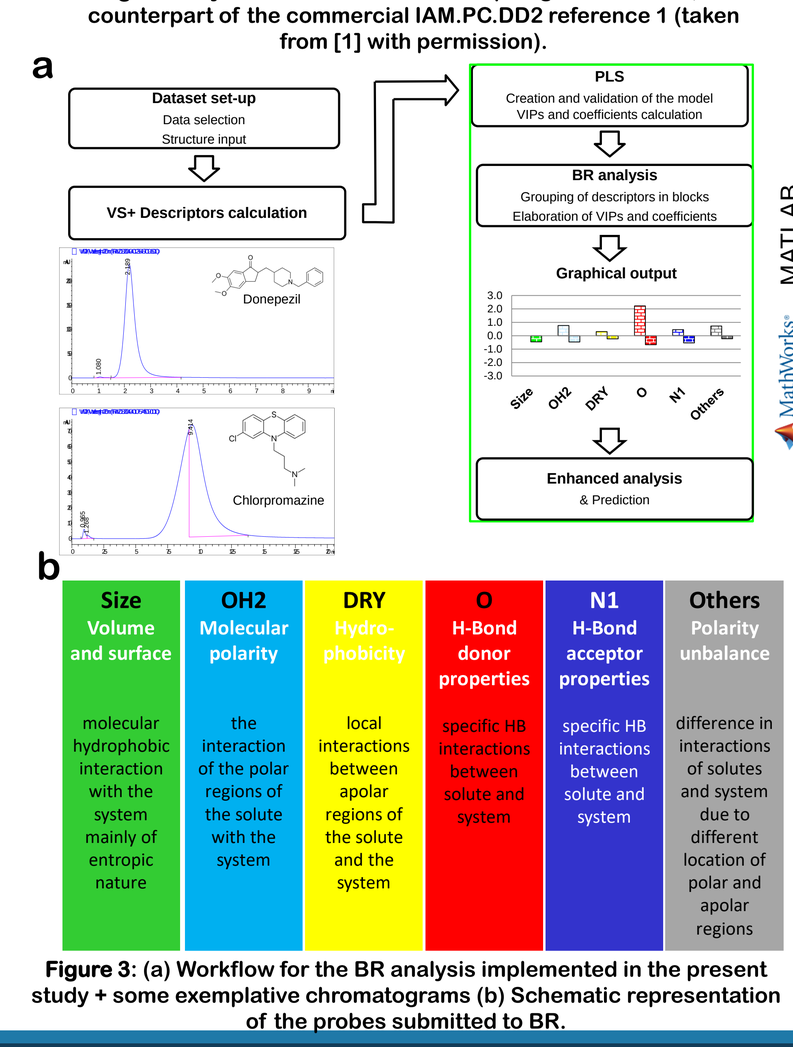
Figure 2: Synthesis and structure of Sphingo-IAM silica 2, as counterpart of the commercial IAM.PC.DD2 reference 1 (taken from [1] with permission).

Analytical method

The column was operated on an Agilent 1100 HPLC quaternary pump at 300 µL min^{-1} at 25 ° C using a mobile phase consisting of 60/25/15 Dulbecco's phosphate buffer saline pH 7.4/ methanol/ acetonitrile. The injection volume was 10 µL. The elution was achieved isocratically and monitored by UV detection at 220 nm. The databased assayed consisted of 36 neutral compounds, 26 basic molecules and 26 acids. All the analyses were performed in triplicate.

Block relevance (BR) analysis

VolSurf+ (VS+) models were built by submitting the SMILES codes of the compounds to VS+ (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). The BR was accomplished by an in house implemented Matlab script run on a Windows 10 multicore machine.



b

Size	OH2	DRY	O	N1	Others
Volume and surface	Molecular polarity	Hydro-phobicity	H-Bond donor properties	H-Bond acceptor properties	Polarity unbalance
molecular hydrophobic interaction with the system mainly of entropic nature	the interaction of the polar regions of the solute with the system	local interactions between apolar regions of the solute and the system	specific HB interactions between solute and system	specific HB interactions between solute and system	difference in interactions of solutes and system due to different location of polar and apolar regions

Figure 3: (a) Workflow for the BR analysis implemented in the present study + some exemplative chromatograms (b) Schematic representation of the probes submitted to BR.

RESULTS & DISCUSSION

Neutral molecules

We screened the Lombardo's [2] 36 neutral solutes having wide lipophilicity range (-0.55 (allopurinol) < log P > + 5.50 (tolnaftate)).

Model	LV	R^2	Q^2	RMSE_CV	N
log k SPH IAM	3	0.80	0.60	0.53	36
	3	0.85	0.66	0.48	35 (antipyrine)

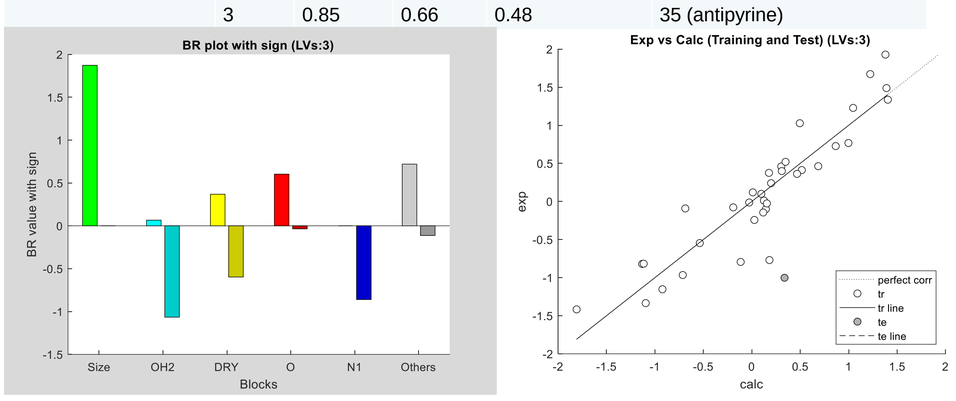


Figure 4: Statistical validation, BR analysis and plot experimental vs predicted retention coefficient for the 36 neutral compounds assayed.

Highly significant relationships (Fig. 4) were observed, with only one data point (antipyrine) behaving as weak outlier.

The retention seem to be largely dependent on molecular size and exhibit a pattern similar to *n*-octanol/water lipophilicity

Acidic molecules

Model	LV2	R^2	Q^2	RMSE_CV	N
log k SPH IAM	2	0.76	0.57	0.46	26

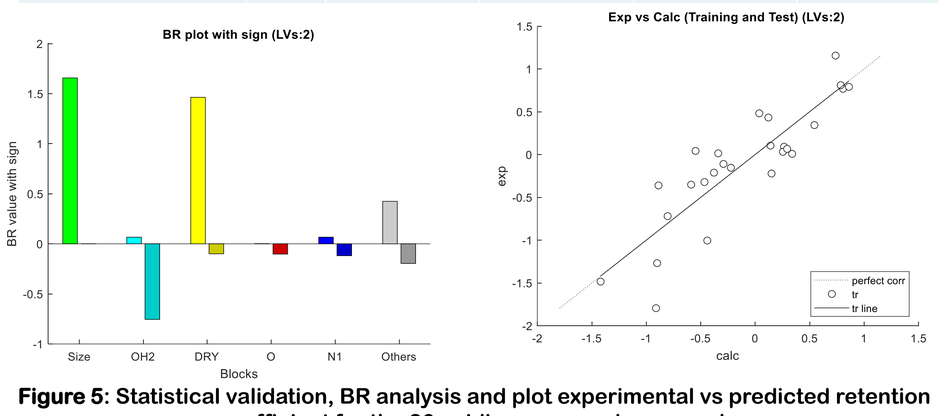


Figure 5: Statistical validation, BR analysis and plot experimental vs predicted retention coefficient for the 26 acidic compounds assayed.

The retention of acidic compounds (Fig. 5) seems to be mostly hydrophobicity-driven. The regression models are reasonably good.

Basic molecules

Model	LV2	R^2	Q^2	RMSE_CV	N
log k SPH IAM	2	0.72	0.11		26
	2	0.81	0.29	0.55	25 (paroxetine)

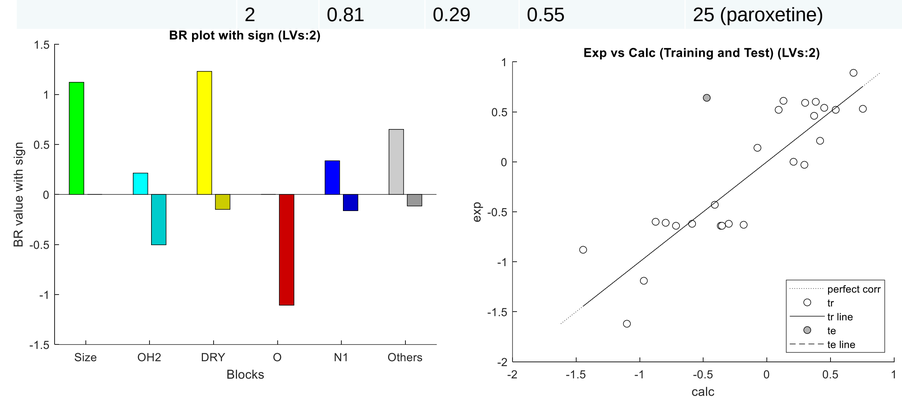
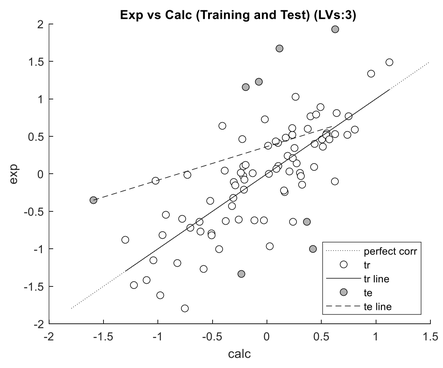


Figure 6: Statistical validation, BR analysis and plot experimental vs predicted retention coefficient for the 26 basic compounds assayed.

The prediction of retentive behavior of basic solutes was more problematic (Fig. 6), with paroxetine behaving as strong outlier, and poor statistical validation for the considered subgroup.

Whole dataset

Model	LV	R^2	Q^2	RMSE_CV	N
log k SPH IAM	3	0.54	0.35		80
	3	0.64	0.52	0.47	72



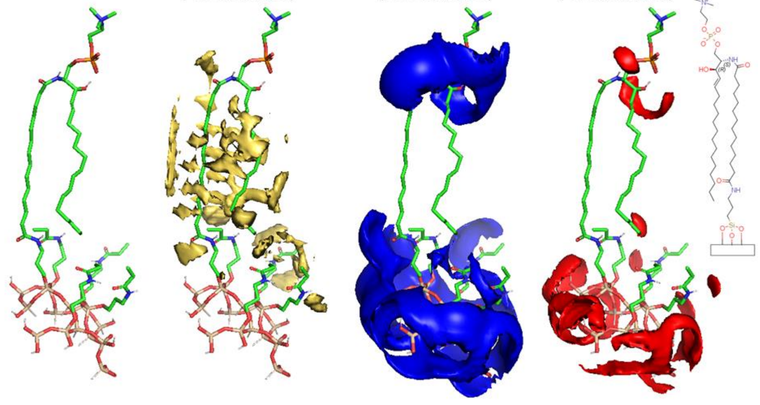


Figure 7: Statistical validation, MIFs representation and plot experimental vs predicted retention coefficient for the whole dataset.

As can be seen, excluding bases benefit the statistics of the global model.

CONCLUSION

- The mechanism of retention of pharmaceutically relevant compounds was studied, unraveled and graphically represented (Fig. 7) by BR analysis.
- The BR analysis was found to be significant for neutral and acidic molecules, but more critical for basic compounds.

REFERENCES

[1] Verzele D., et al. Development of the first sphingomyelin biomimetic stationary phase for immobilized artificial membrane (IAM) chromatography. Chem Commun (Camb). 48 (8) 1162-1164.
[2] Lombardo F. et al., ElogPoct: a tool for lipophilicity determination in drug discovery. J Med Chem. 2000 Jul 27;43(15):2922-8.