Chromatographic approaches for evaluation of the hydrophobic characteristics of chemicals with emphasis on newly developed separation mechanisms

Andrei Medvedovici (1), Victor Voicu (2)

(1) University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Panduri Ave. # 90-92, Bucharest-060553, Romania.

(2) University of Medicine and Pharmacy "Carol Davila", Department of Pharmacology, Toxicology and Clinical Psychopharmacology, Floreasca St. # 8, Bucharest-014461, Romania.

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"From Science to Guidance and Practice"
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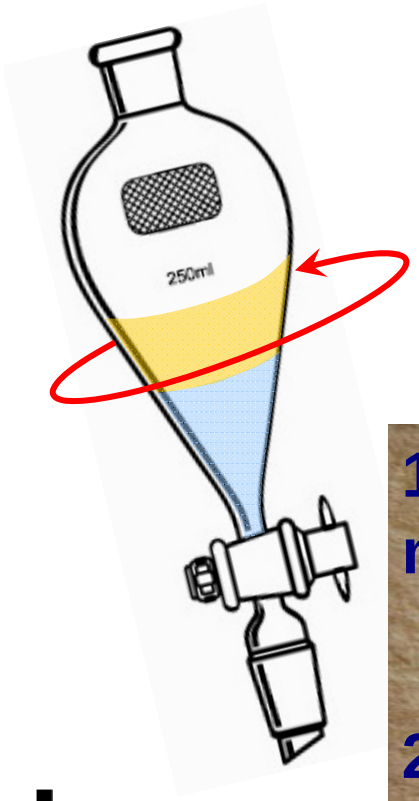
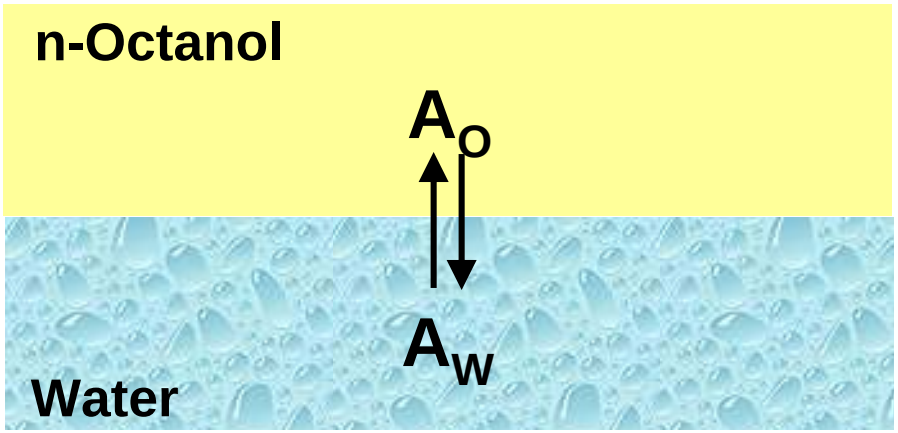
Hydrophobicity: the physical property of a molecule that is seemengly repelled from a mass of water.

Lipophilicity (as an extension of the hydrophobic character): includes favorable interactions that contribute to the distribution of a chemical entity between water and other solubilizing media, representing a manifestation of the characteristics of the system in which the solute is placed.

The measure of the hydrophobic character:
log $K_{o/w}$ or simply, log P.

Experimental determination of log P: the shake flask method





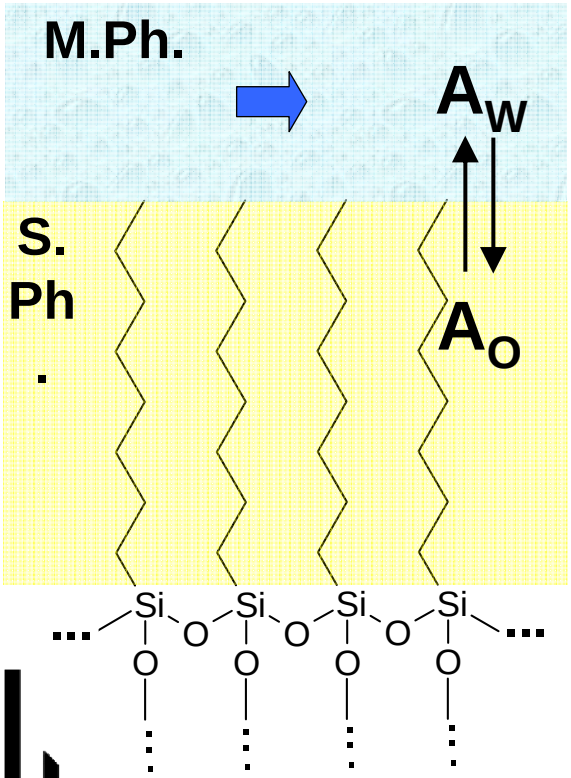
$$K_{O/W} = \frac{[A]_O}{[A]_W}$$

1. If $[A]_o$ or $[A]_W$ are ⇘⇘, very sensitive methods are necessary for assaying A.

2. Mutual relative solubility of the two media (*n*-octanol in water and water in *n*-octanol, respectively). i.e. - solubility of *n*-octanol in water at 25 °C is 0.56 g/L.

log P simulation from chromatographic retention data

$$K_A = \frac{[A]_{S.Ph.}}{[A]_{M.Ph}} = k \times \frac{V_{M.Ph.}}{V_{S.Ph.}} = k \times \beta$$



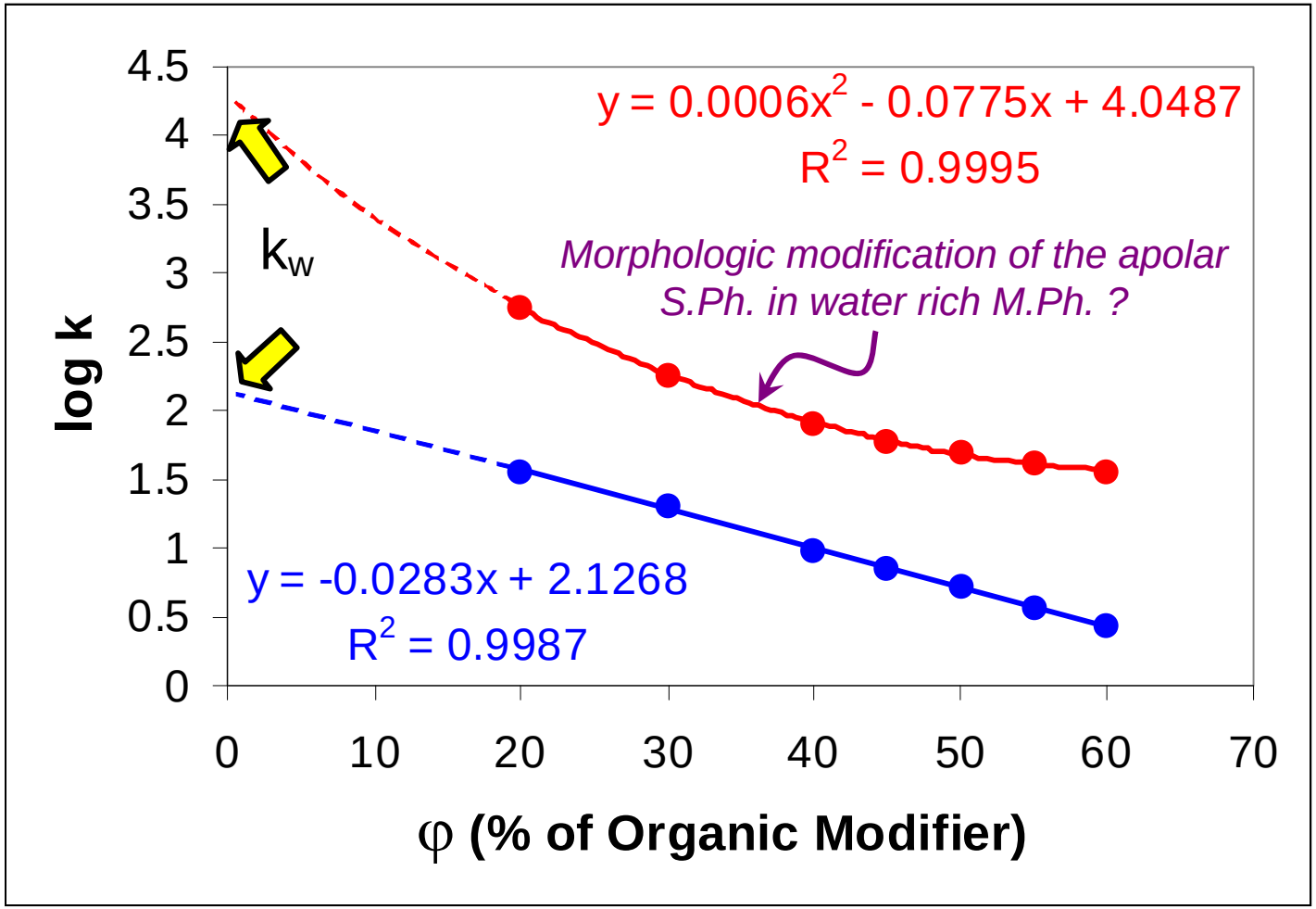
C8 bulk assimilated to *n*-octanol layer

If k_w is the retention factor for a hypothetical M.Ph. composition containing 0% Organic Modifier:

$$K_{O/W}^{A} = k_W \times \beta;$$

$$\log\left(K_{O/W}^{A}\right) = \log P^{A} = \log\left(k_W\right) + Log\left(\beta\right)$$

Limitations of the model:

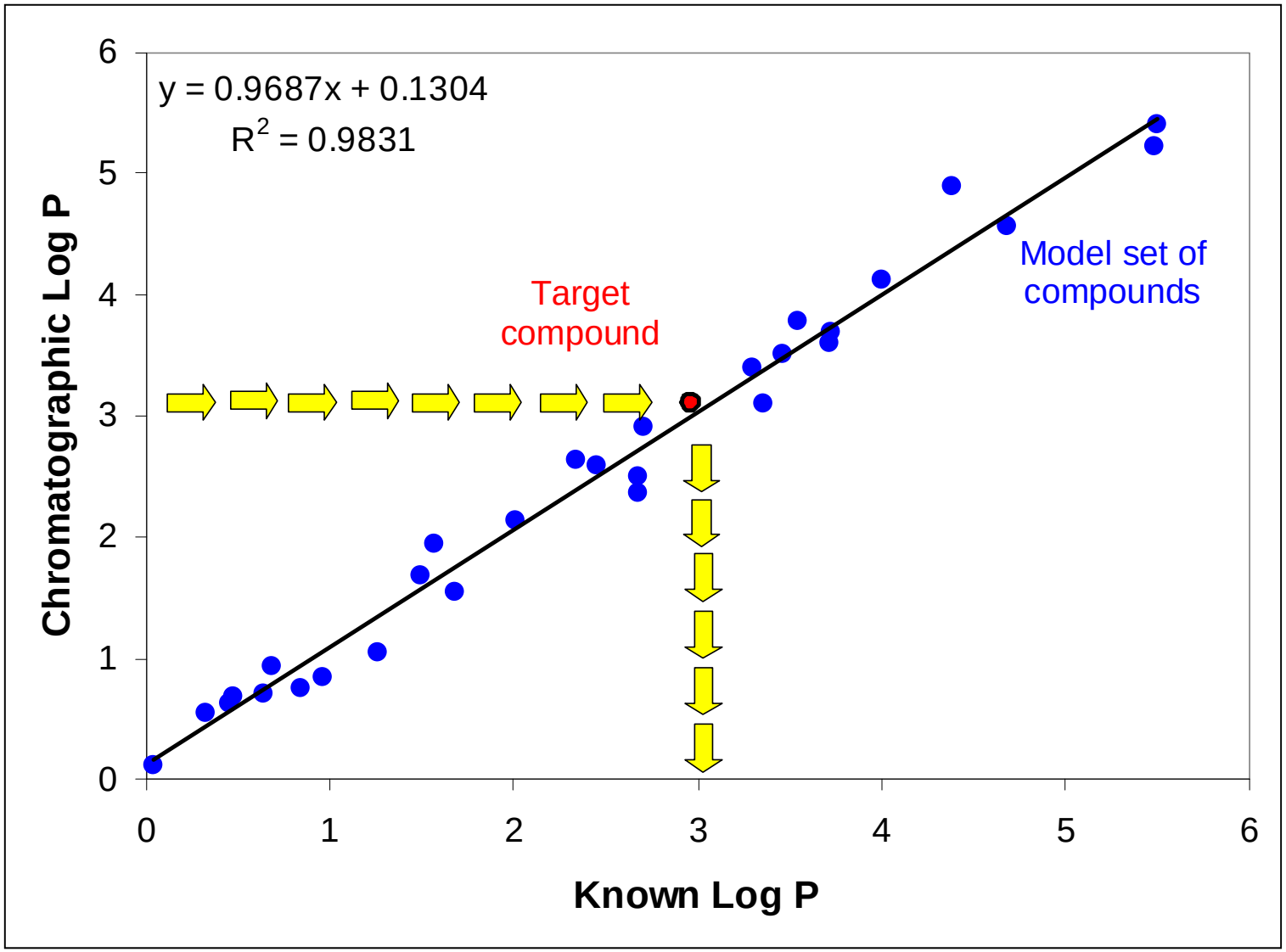


$$\log P = a \log k_W + bS + c$$

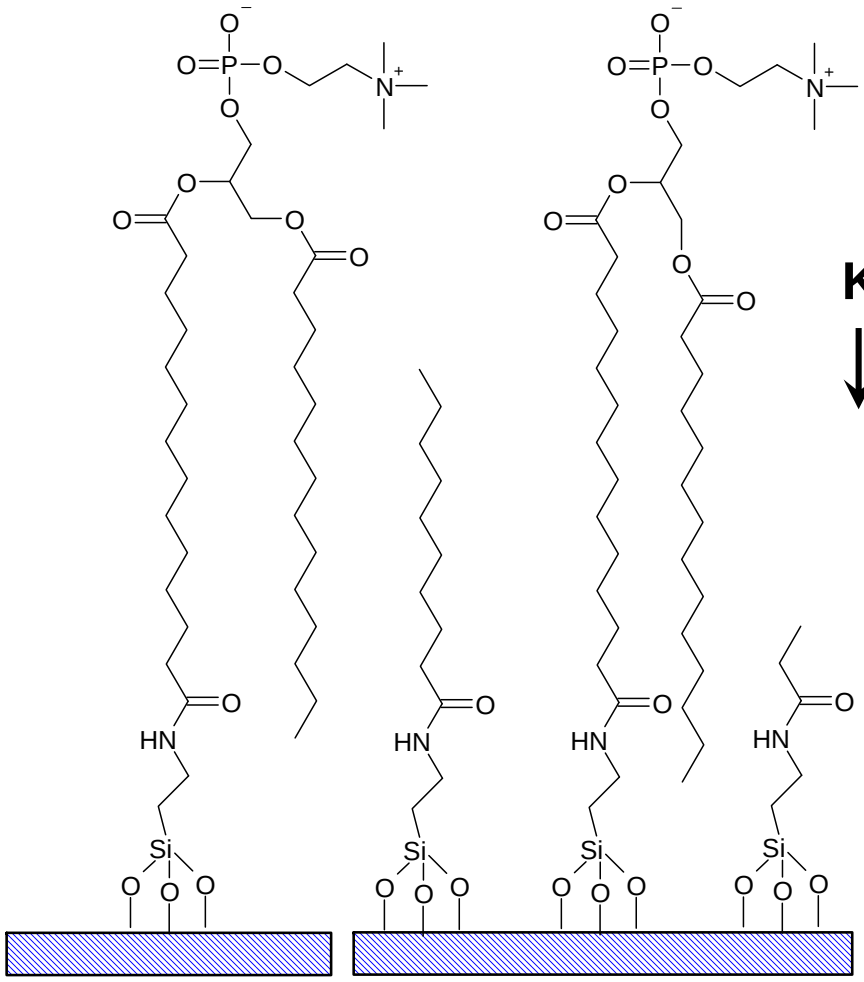
S is the slope of the linear regression,
a, b, c are characteristics of the S.Ph.

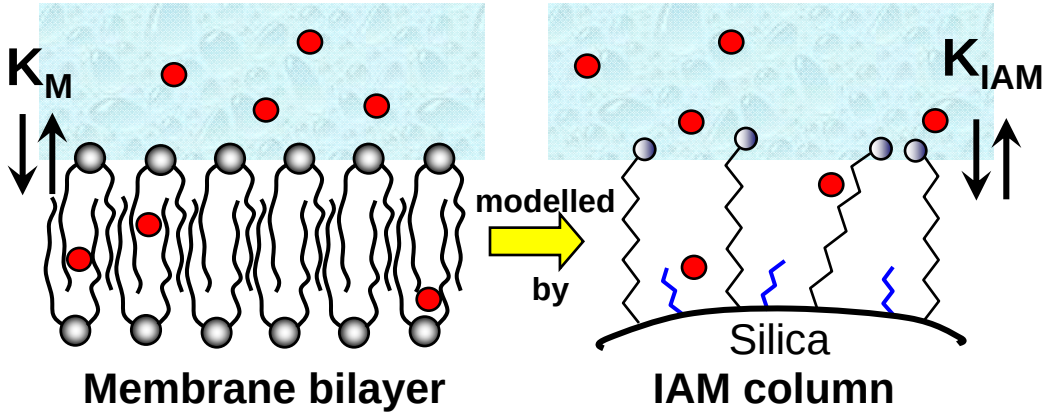
K. Valko, V. Slegeli, J. Chromatogr. A, 631 (1993) 49-61.

A time consuming compromise:



Special Stationary Phases
tailored for chromatographic log P determination
Biomimetic S. Ph. (Immobilised Artificial Membranes, liposomes)





Predicting Intestinal Drug Permeability

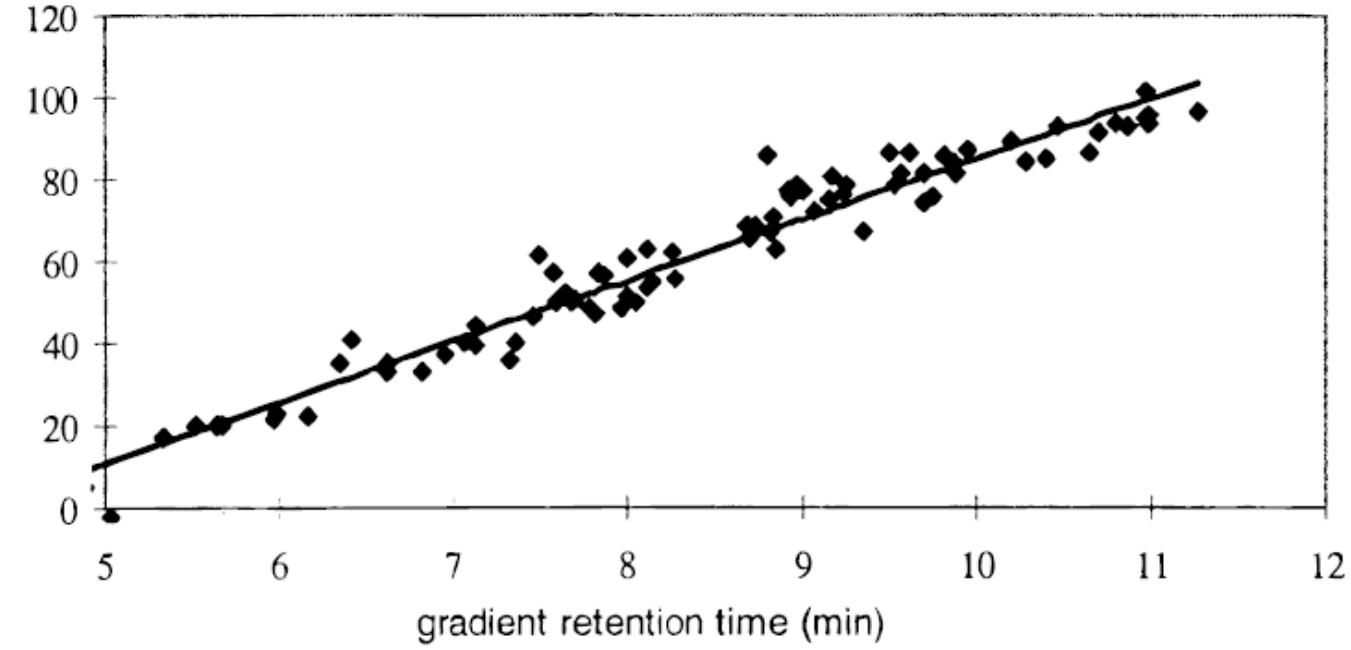
C. Pidgeon, et al., J. Med. Chem., 38 (1995) 590-594.

The CHI hydrophobicity scale:

The CHI scale: % (v) of ACN required to achieve an equal distribution of the compound between M.Ph. and S. Ph.

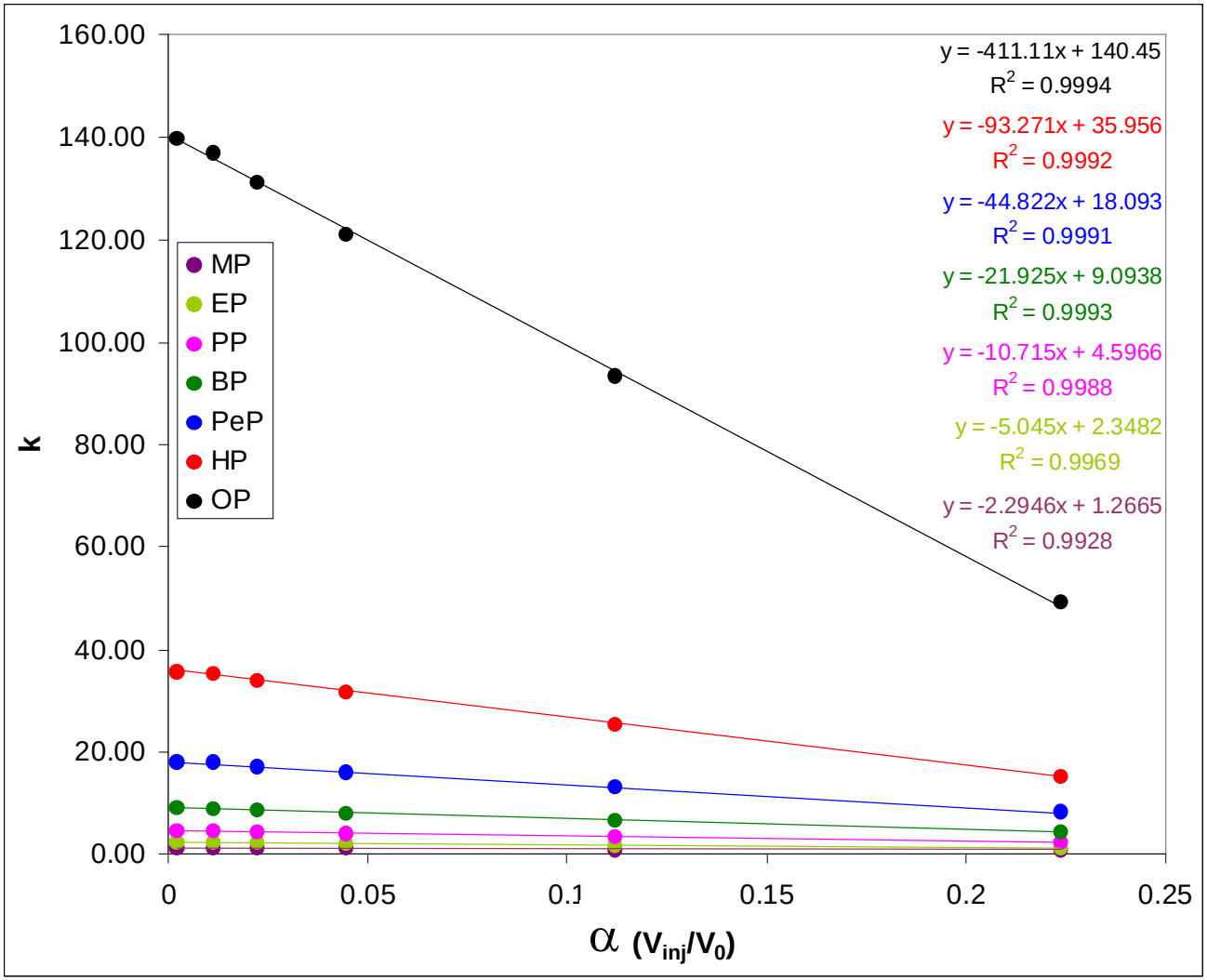
Gradient elution conditions!

$$t_R = f(-\frac{\log k_W}{S})$$



K. Valko, C. Bevan, D. Reynolds, Anal. Chem., 69 (1997) 2022-2029.

Hydrophobicity scale from Large Volume Injection (LVI) study in non-miscible LVI diluents:



C. Sarbu, R.D. Nascu-Briciu, D. Casoni, A. Kot-Wasik, J. Namiesnik, J. Chromatogr. A, 1266 (2012) 536-544.

Why chosing oximes as model compounds?
Their poor penetrability through biological barriers!

$$Log\,(BB) = \left[\frac{[A]_{Brain}}{[A]_{Blood}}\right]_{SS}$$

Log(BB) ∈ [-2 ÷ 1] [1]

Favorable distribution: Log(BB) > 0.3;
Poor Distribution: Log(BB) < -1;

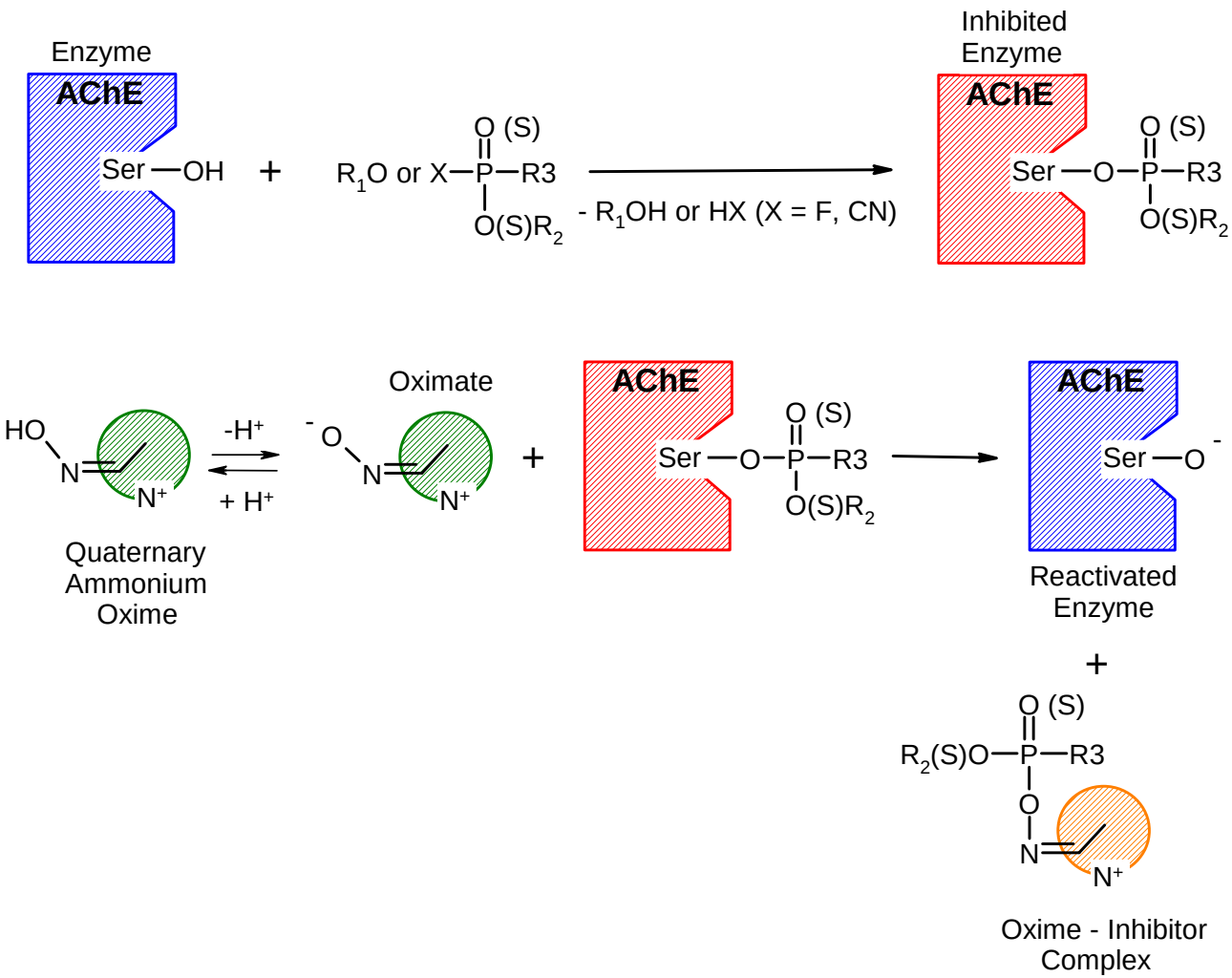
Log(BB) = f (*molecular mass – Mw; hydrophobic character – log P; polar surface area – PSA; nº. of rotatable bonds; nº. of H-bond donors; nº. of H-bond acceptors; 3D-molecular field descriptors; electropological state indices; critical micelle concentration – CMC_D; cross sectional area – A_D; permeability coefficient – PC; polarizability – CMR … and others*)

Successful CNS Drug [2]

Descriptor	*Condition*	*Descriptor*	*Condition*
Molecular weight	Mw < 450	Water solubility	S > 60 μg/mL
Hydrophobic character	Log P < 5	Metabolic stability	> 80% after 1 h
No. of H-bond donor	≤ 3	P450 enzyme CIP inhibition	< 50% at 30 μM
No. of H-bond acceptor	< 7	Metabolization by CYP2D6	Not significant
No. of rotatable bonds	< 8	CYP3A4 inducer	No potent
Polar surface area	PSA < 60÷70 $Å^2$	P-glycoprotein substrate	No
H-bonds	<8	Affinity to serum albumin	K_D < 10 μM
Acid character	pK_a ∈ [7.5÷10.5]	Effective permeability	> 1 × 10^{-6} cm/sec

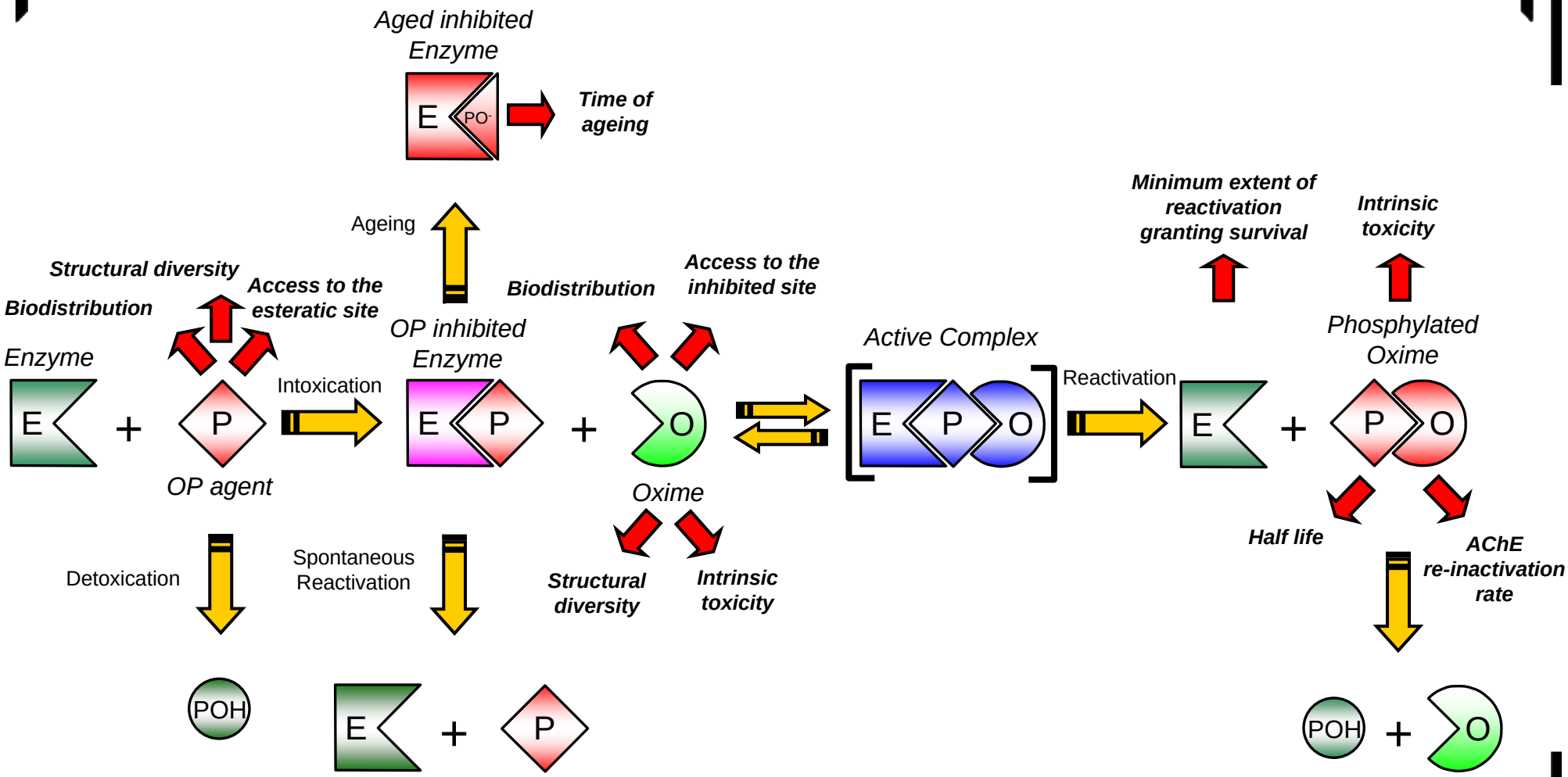
(1) M.H. Abraham et al., J. Pharm.Sci., 86 (1992) 310-315.
(2) H. Pajouhesh, G.R. Lenz, NeuroRx®, 2 (2005) 541-553.

Quaternary ammonium oximes: reactivators of the phosphylated AChE



V. Voicu, F.S. Radulescu, A. Medvedovici, EOMT, 9 (2013) 31-50.

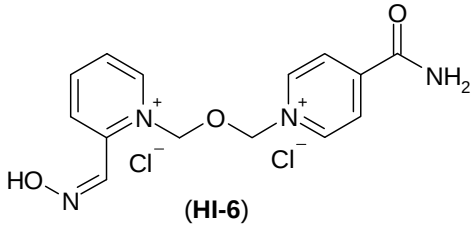
Complex scenario of OP intoxication / reactivation of AChE by oximes



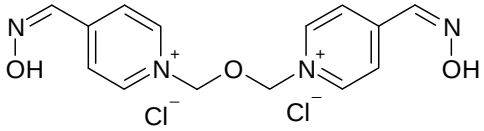
Resulting from such complexity: very large number of synthesized oximic congeners.

Attempts to determine the hydrophobic character of oxime from chromatographic retention data on different S. Ph.

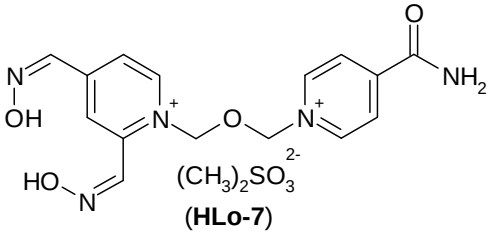
Pralidoxime (**PAM**)
2-[(hydroxyimino)methyl]-1-methylpyridin-1-ium



(**HI-6**)
2,1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane



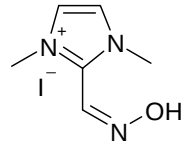
Obidoxime (**LuH-6**)
1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane



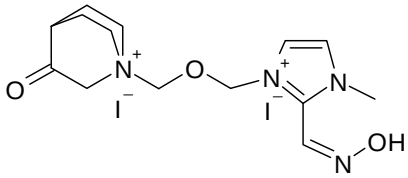
(**HLo-7**)
1-(4-aminocarbonylpyridinio)methoxymethyl-2,4-bis(hydroxyiminomethyl)pyridinium

RP (C8)
IP (C8)
RP (PFP)
HILIC
ZIC-HILIC
AGP

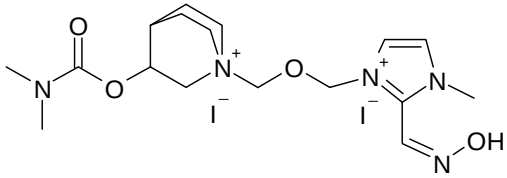
Elution at 37 ºC, with 0.9% NaCl in the aqueous component of the M.Ph.



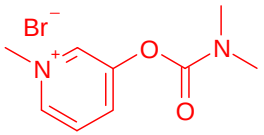
2-(Hydroxyimino-methyl)-1,3-dimethyl-3H-imidazol-1-ium **(1)**



1-Methyl-3-(3-oxo-1-aza-bicyclo[2.2.2]octane-1-ylmethoxymethyl)-1H-imidazole-2-carbaldehyde oxime **(2)**



Dimethyl-carbamic acid 1-[2-(hydroxyimino-methyl)-3-methyl-3H-imidazol-1-ylmethoxymethyl]-1-aza-bicyclo[2.2.2]octane-3-yl ester **(3)**



Pyridostigmine (**PRDS**)
Dimethyl-carbamic acid 1-methyl-pyridin-3-yl ester

V. Voicu, I. Sora, C. Sarbu, V. David, A. Medvedovici, J. Pharm. Biomed. Anal., 52 (2010) 508-516.

Some "strange" experimental findings:

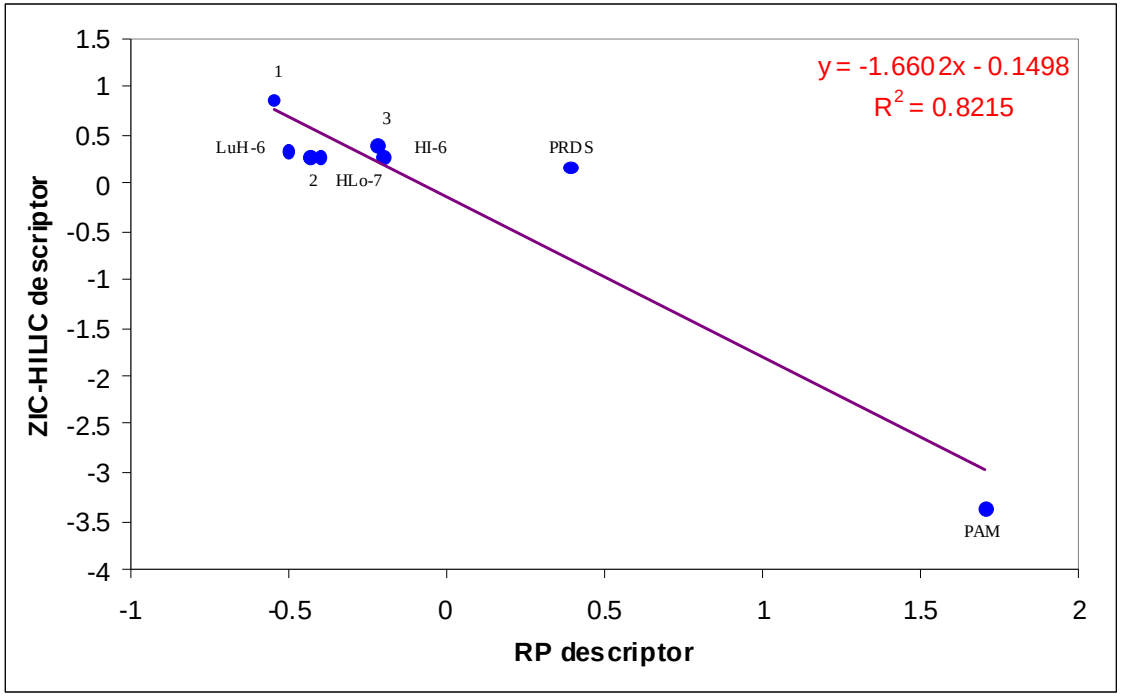
r_{xy}		ALOGPs	AClog P	AB/Log P	miLogP	ALOGP	MLOGP	XLOGP2	XLOGP3	K_{OW}^{WIN}
log k_w	**C8**	-0.3203	0.4506	0.0480	0.5710	0.6181	0.2848	0.4872	0.5613	0.0980
	IP/C8	-0.4376	0.0837	-0.3339	0.2506	0.5477	0.5174	0.5099	0.1954	0.3173
	PFP	-0.4263	0.2218	0.1311	***0.9014***	0.6207	0.2632	0.5730	0.4450	0.5120
	ZIC- HILIC	0.0245	***-0.8452***	-0.6368	-0.7299	-0.5416	0.1208	-0.3697	***-0.9185***	-0.0949
	HILIC	0.2229	-0.6872	-0.3437	-0.7154	-0.7032	-0.1652	-0.5616	***-0.8085***	-0.2121
	AGP	-0.1513	***-0.8519***	-0.6918	-0.5747	-0.4001	0.2369	-0.2458	***-0.8630***	0.1005

● Extrapolation to φ= 0% Organic Modifier; ● Extrapolation to φ= 100% Organic Modifier

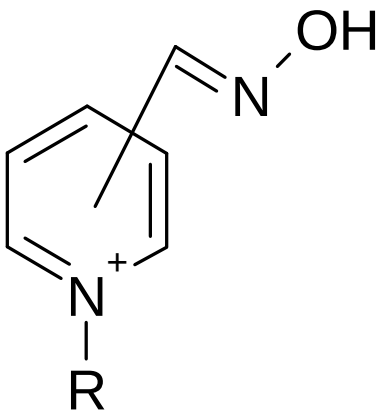
Hydrophobicity descriptors resulting from the chromatographic retention behaviour are poorly correlated to log P values resulting from different computational algorithms.

Correlations between descriptors determined through extrapolation of experimental retention data:

r_{xy}	C8	IP/C8	PFP	HILIC	ZIC-HILIC	AGP
C8	*1.0000*	0.7311	0.6180	-0.6758	**-0.9064**	-0.6010
IP/C8		*1.0000*	0.5259	-0.1822	-0.5995	-0.0424
PFP			*1.0000*	-0.5340	-0.6967	-0.3789
HILIC				*1.0000*	**0.8819**	**0.9487**
ZIC-HILIC					*1.0000*	0.8086
AGP						*1.0000*



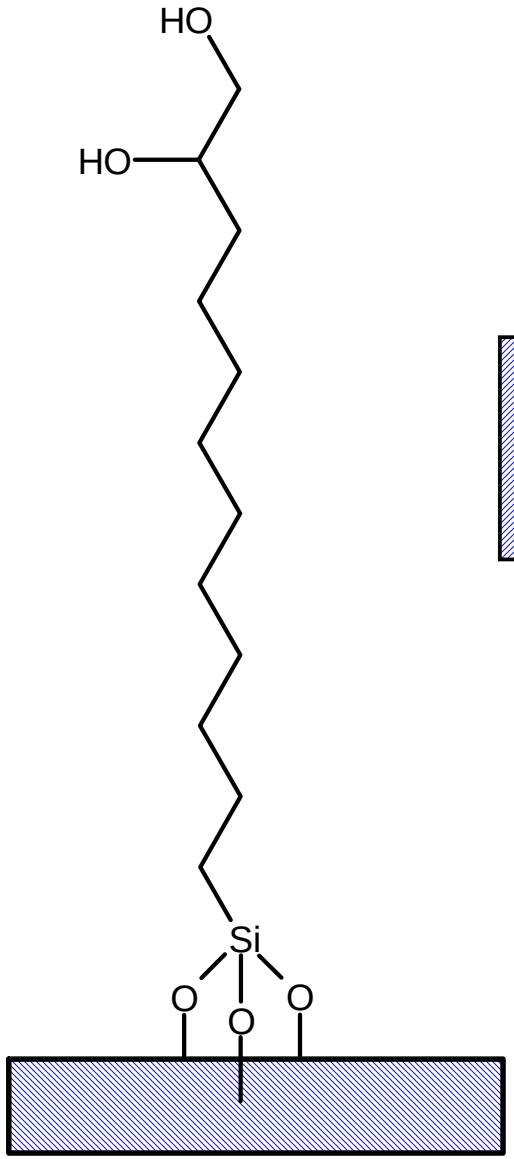
Newly synthesized oximes.
Target: Increasing log P to increase log BB

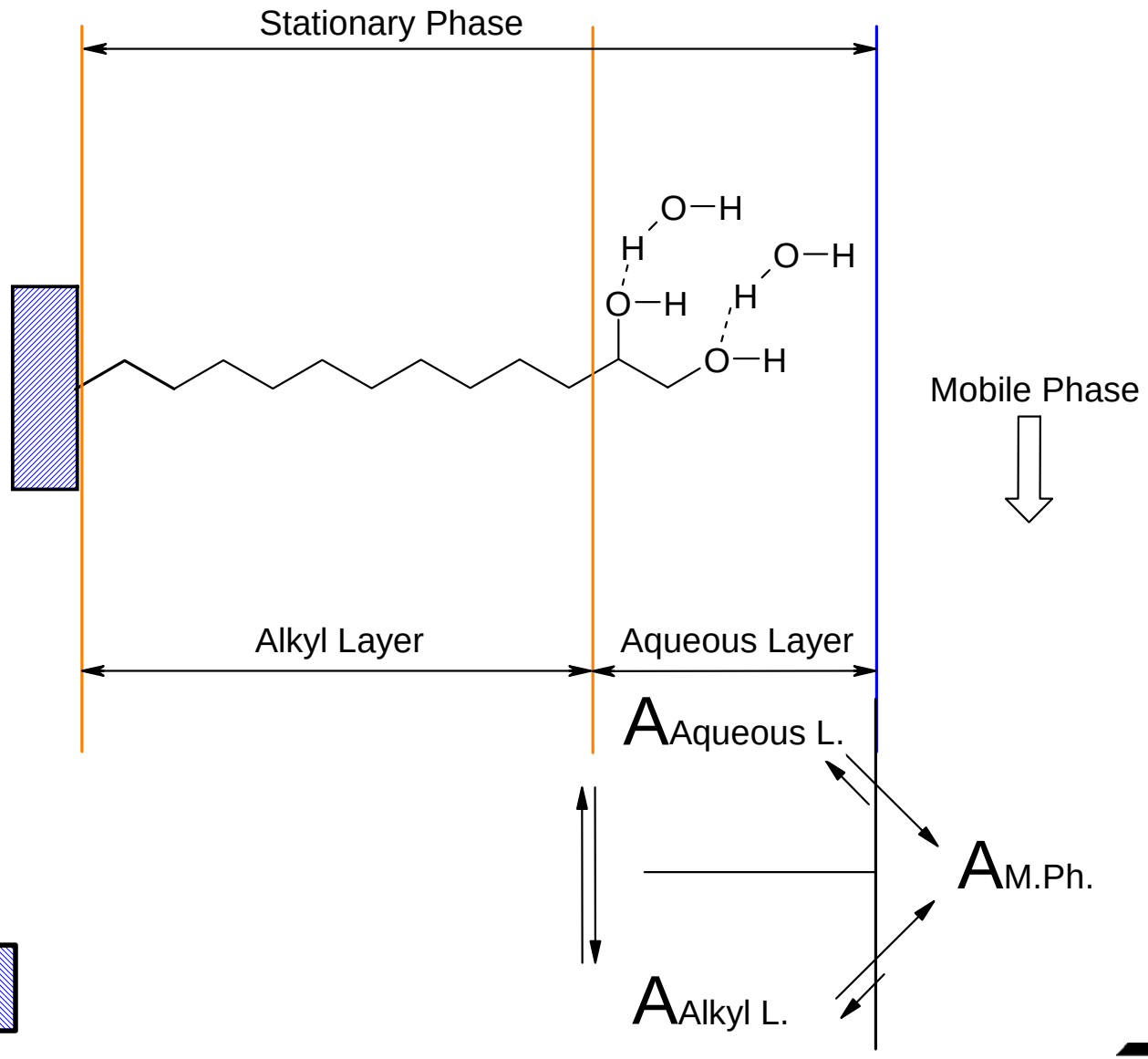


#	Substituent (R) Name	Substituent (R) Formula	Position of the oxime moiety	Acronym(s)
1	Ethyl	$-C_2H_5$	2, 3, 4	2-PAE, 3-PAE, 4-PAE
2	Butyl	$-C_4H_9$	2, 3	2-PAB, 3-PAB
3	Hexyl	$-C_6H_{13}$	2, 3, 4	2-PAH, 3-PAH, 4-PAH
4	Octyl	$-C_8H_{17}$	2, 3, 4	2-PAO, 3-PAO, 4-PAO
5	Decyl	$-C_{10}H_{21}$	2, 3	2-PAD, 3-PAD
6	Dodecyl (Lauryl)	$-C_{12}H_{25}$	2, 3, 4	2-PAL, 3-PAL, 4-PAL
7	Benzyl	$-CH_2-C_6H_5$	2, 3, 4	2-PABn, 3-PABn, 4-PABn
8	Ethyl-phenyl	$-(CH_2)_2-C_6H_5$	2, 3, 4	2-PAPE, 3-PAPE, 4-PAPE
9	Propyl-phenyl	$-(CH_2)_3-C_6H_5$	3	3-PAPP
10	Butyl-phenyl	$-(CH_2)_4-C_6H_5$	3, 4	3-PAPB, 4-PAPB
11	4-Methylbenzyl	$-CH_2-C_6H_4-CH_3$	2, 3, 4	2-PAMB, 3-PAMB, 4-PAMB
12	4-*t*-Butylbenzyl	$-CH_2-C_6H_4-C(CH_3)_3$	3, 4	3-PATB, 4-PATB

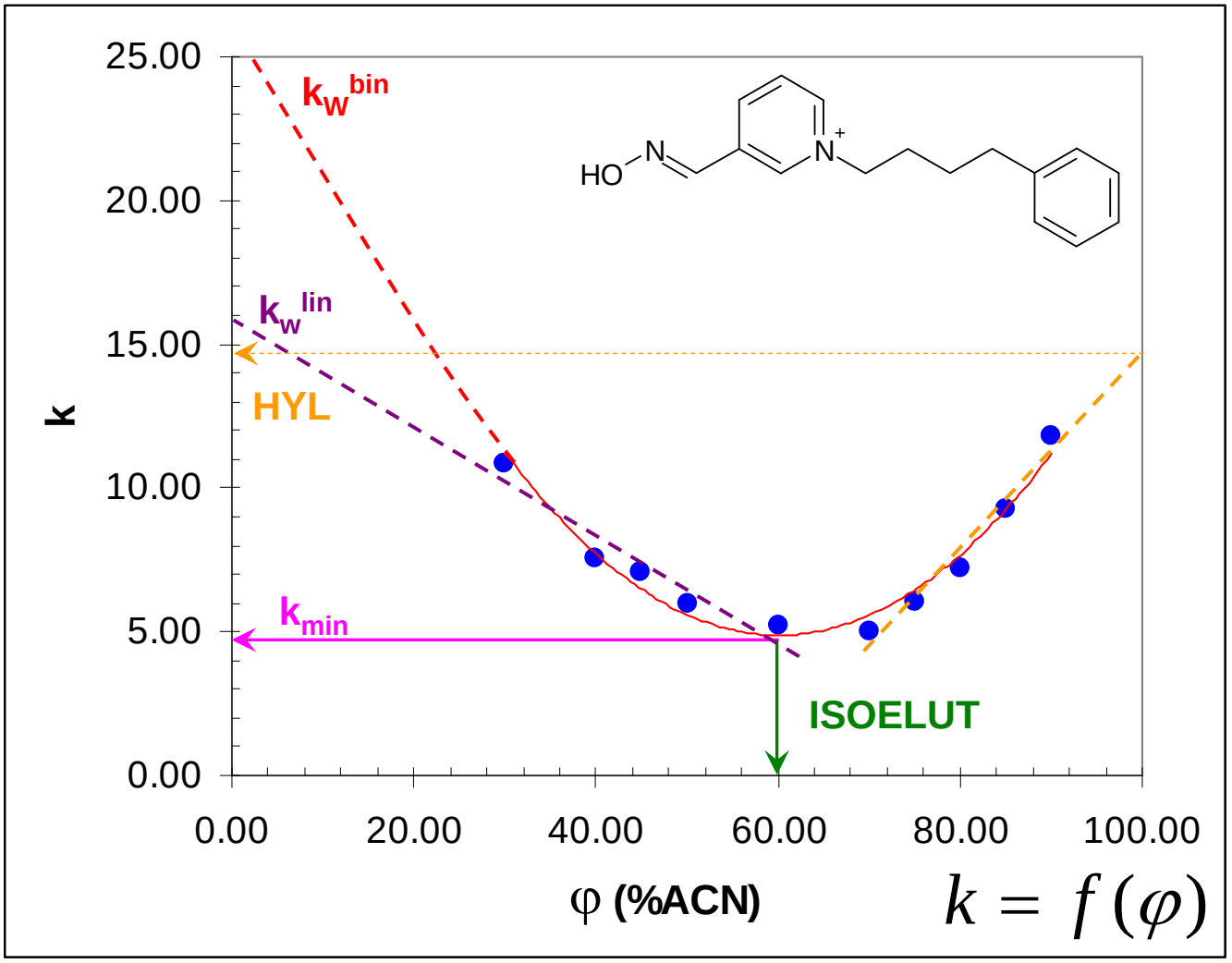
H. Ohta, T. Ohmori, S. Suzuki, H. Ykegaya, K. Sakurada, T. Takatori, Pharm. Res., 23 (2006) 2827-2833.

The Stationary Phase: A bimodal action (RP + HILIC)



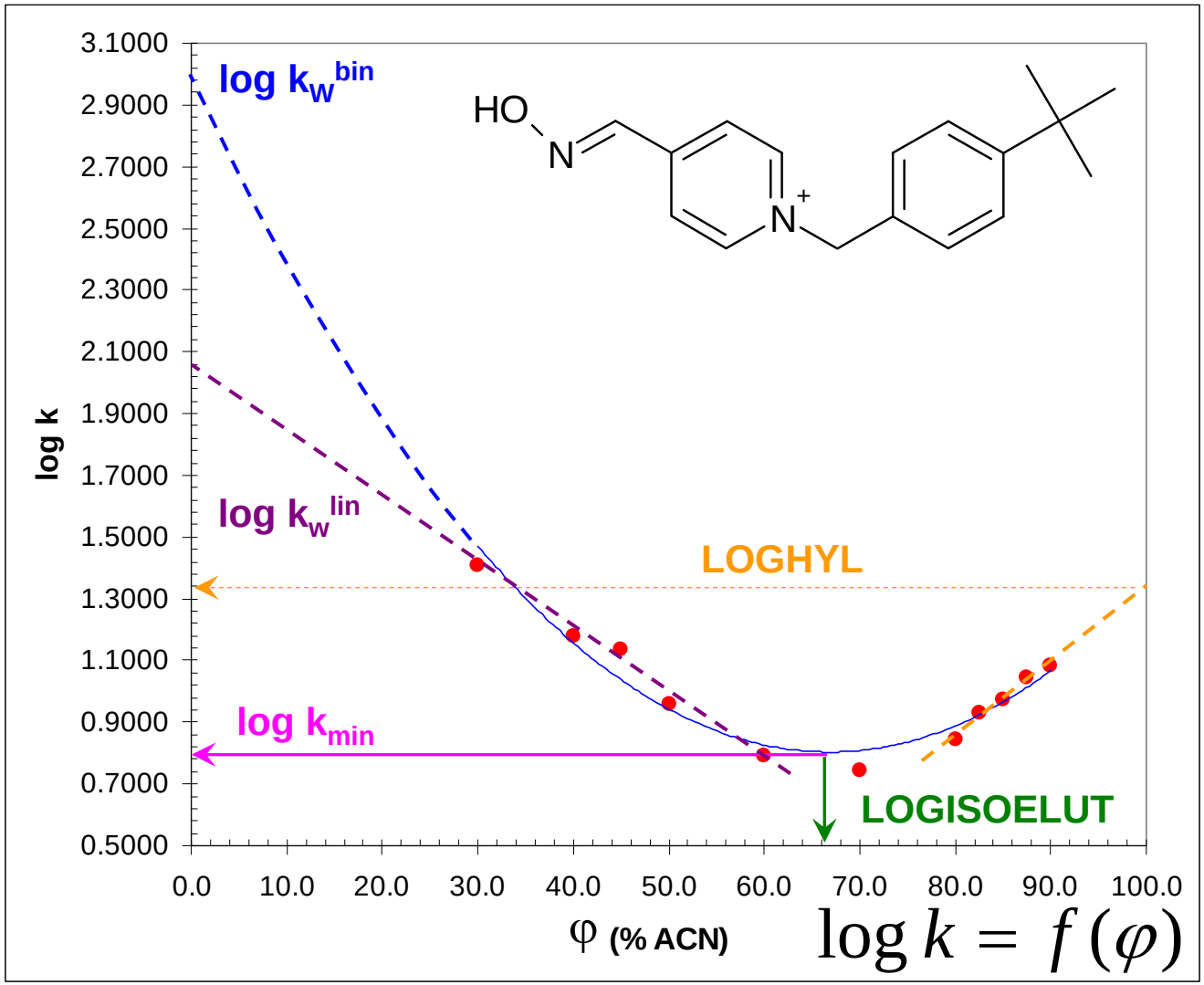


Results: "U" shaped retention profiles (I).



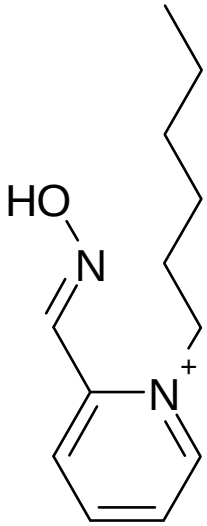
Potential hydrophobicity indicating descriptors!

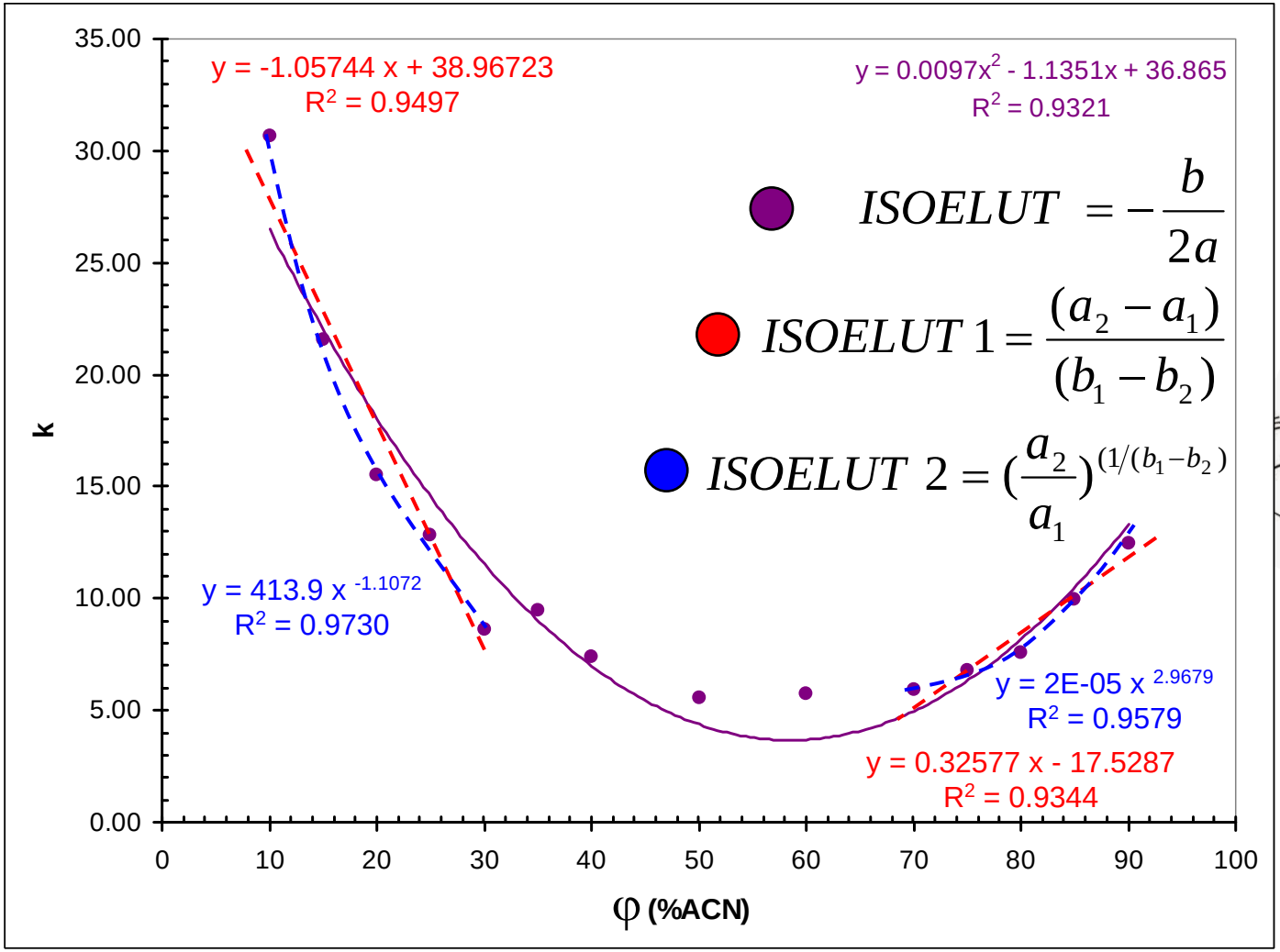
Results: "U" shaped retention profiles (II).



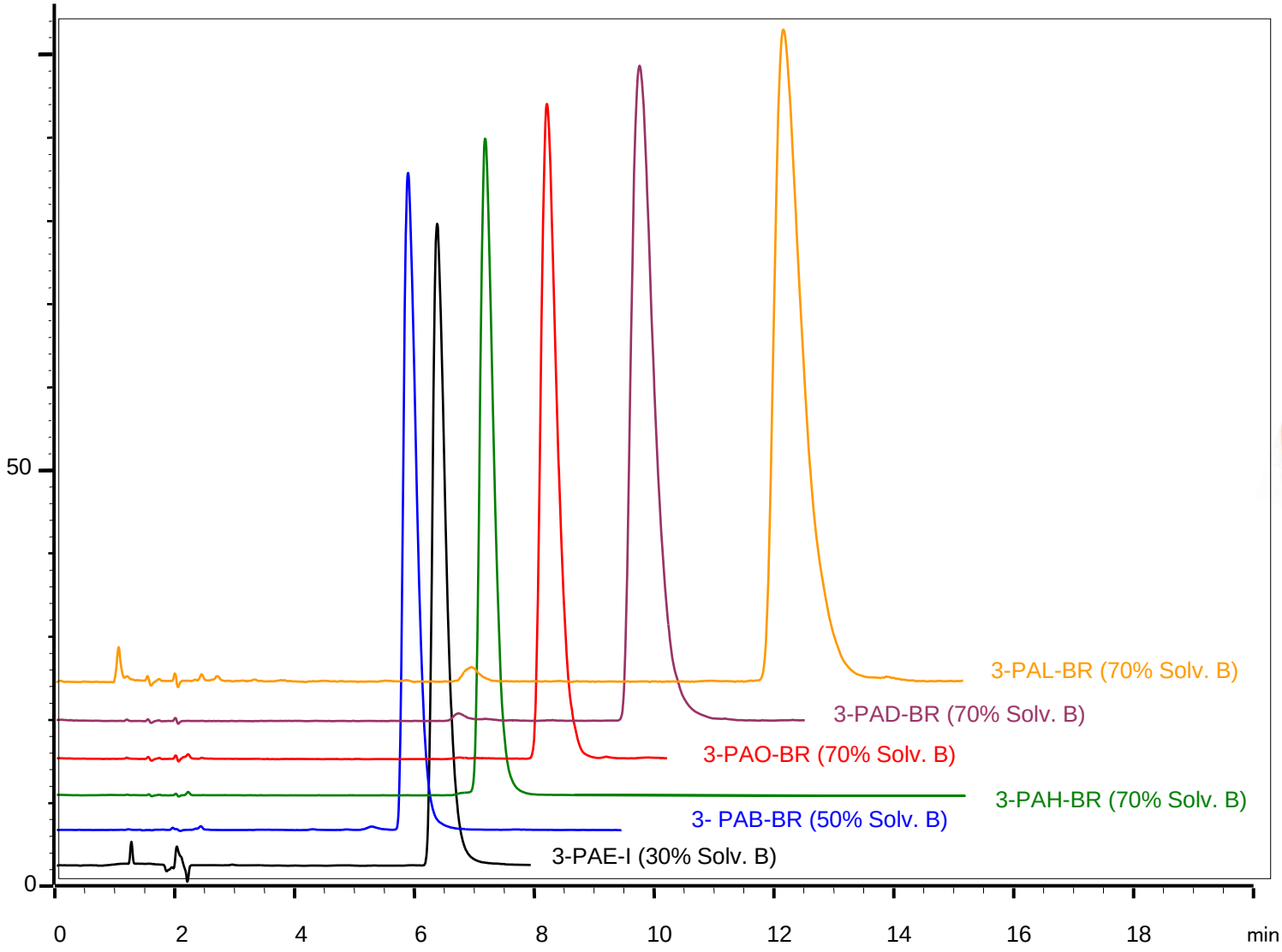
and the corresponding logarithms ...

(log)ISOELUT determination alternatives:





No concerns about peak shapes !



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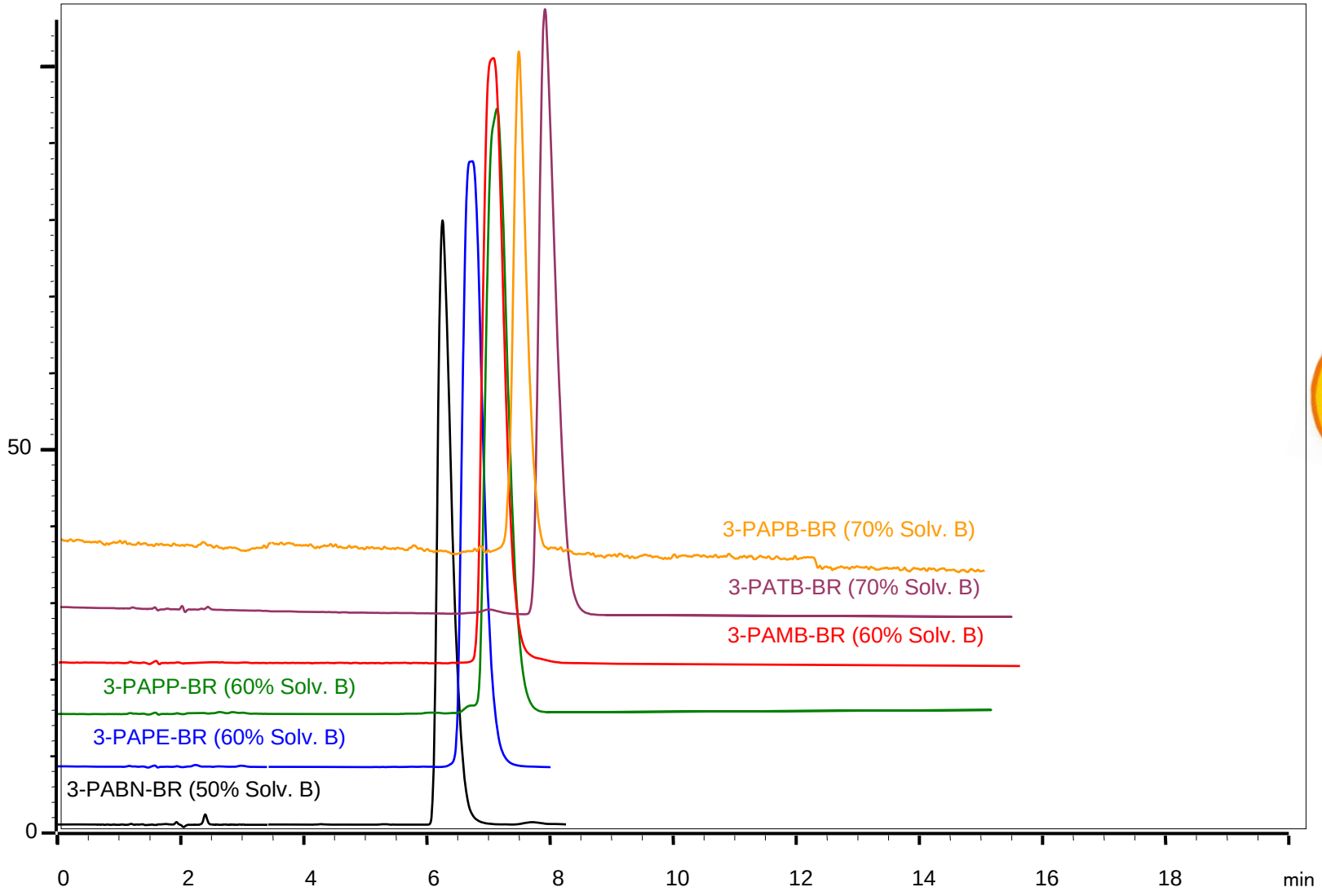


Correlations between determined indices and computed log P values:

Correlated sets	Correlation Coefficients										
	ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	Hy	MLOGP	ALOGP	LogD7	SlogD7.4
k_{min}	0.958	0.977	0.934	0.963	0.977	0.983	-0.758	0.935	0.943	0.884	0.969
log k_{min}	0.938	0.977	0.927	0.973	0.964	0.978	-0.849	0.954	0.944	0.918	0.969
ISOELUT	0.842	0.915	0.835	0.942	0.895	0.899	-0.891	0.914	0.904	0.931	0.902
LOG ISOELUT	0.809	0.881	0.810	0.904	0.865	0.873	-0.874	0.880	0.855	0.894	0.864
ISOELUT 1	0.479	0.512	0.460	0.508	0.484	0.529	-0.518	0.577	0.393	0.537	0.503
LOG ISOELUT 1	0.678	0.740	0.661	0.752	0.700	0.750	-0.817	0.806	0.640	0.788	0.738
ISOELUT 2	0.552	0.598	0.526	0.602	0.558	0.621	-0.730	0.668	0.493	0.642	0.609
LOG ISOELUT2	0.193	0.209	0.117	0.214	0.201	0.226	-0.267	0.240	0.135	0.232	0.227
k_w^{lin}	0.788	0.784	0.767	0.753	0.793	0.787	-0.546	0.803	0.770	0.652	0.770
log k_w^{lin}	0.847	0.862	0.836	0.843	0.862	0.863	-0.672	0.878	0.840	0.766	0.845
k_w^{bin}	0.754	0.812	0.756	0.787	0.817	0.800	-0.570	0.814	0.778	0.714	0.771
log k_w^{bin}	0.139	0.278	0.202	0.304	0.246	0.239	-0.377	0.257	0.259	0.374	0.230
HYL	0.488	0.472	0.479	0.413	0.458	0.502	-0.327	0.520	0.363	0.363	0.464
LOG HYL	0.430	0.405	0.420	0.346	0.400	0.440	-0.245	0.464	0.298	0.291	0.396

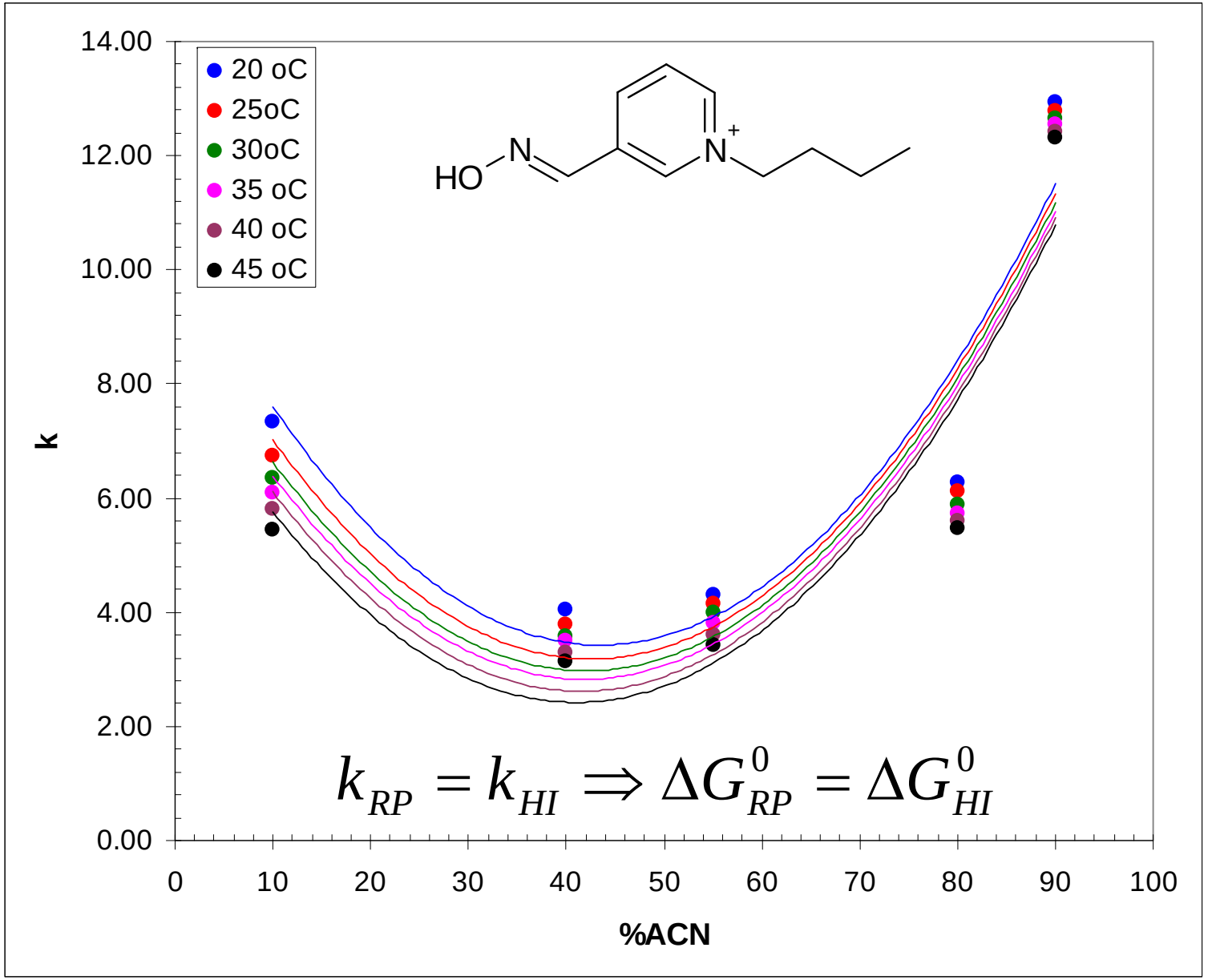
V. Voicu, C. Sarbu, F. Tache, F. Micale, S.F. Radulescu, K. Sakurada, H. Ohta, A. Medvedovici, Talanta, 122 (2014) 172-179.

Correlations to other descriptors:

Descriptors	Mw	Mol.Vol.	Diff. Coef.	P_{eff}	Log (MDCK COS)	P_{comea}	Log (Sw)	Log (FaSSGF)	Log (FaSSIF)	Log (FeSSIF)	LogBBB	PrUnbnd	Vd
k_{min}	0.861	0.946	-0.887	0.711	-0.764	0.304	-0.899	-0.969	-0.859	-0.894	0.852	-0.416	0.889
log k_{min}	0.909	0.967	-0.953	0.726	-0.728	0.401	-0.939	-0.966	-0.924	-0.932	0.792	-0.539	0.884
ISOELUT	0.929	0.950	-0.971	0.776	-0.707	0.512	-0.941	-0.923	-0.931	-0.949	0.648	-0.629	0.849
LOG ISOELUT	0.894	0.914	-0.947	0.729	-0.633	0.486	-0.900	-0.884	-0.905	-0.905	0.647	-0.622	0.836
k_w^{lin}	0.600	0.720	-0.717	0.438	-0.687	0.105	-0.661	-0.736	-0.673	-0.708	0.754	-0.205	0.629
log k_w^{lin}	0.724	0.820	-0.822	0.544	-0.709	0.217	-0.774	-0.827	-0.777	-0.809	0.768	-0.339	0.732

M_w - molecular mass; **Mol. Vol.** - Molecular Volume: **Diff. Coeff.** – molecular diffusion coefficient in water; **P_{eff}** – human jejunal effective permeability; **log MDCK** – apparent MDCK COS permeability; **P_{cornea}** – permeability through rabbit cornea; **log(FaSSGF)** – solubility in fasted state simulated gastric fluid, **log(FaSSIF)** – solubility in fasted state simulated intestinal fluid, **log(FeSSIF)** – solubility in fed state simulated intestinal fluid; **Log BBB** - the logarithm of the brain/blood barrier; **PrUnbnd** – the percent unbound to blood plasma proteins; **V_d** – the pharmacokinetic volume of distribution in humans; by ADMET Predictor, vers. 5.0.0012, Simulation Plus Inc. (U.S.A)

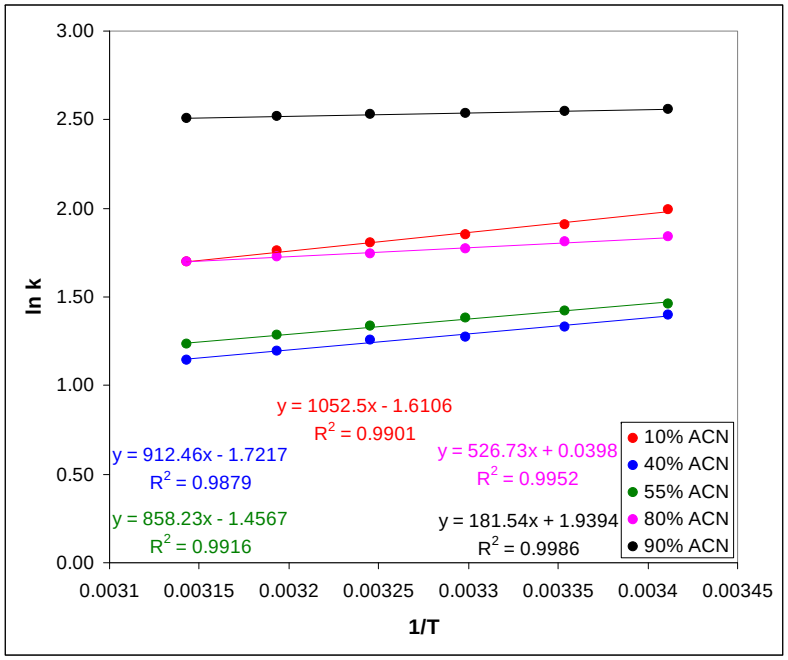
Thermodynamic aspects related to the bimodal retention mechanism:



The van't Hoff plots.

$$\Delta H^0_{RP} = -8.75 KJ/mol^0K$$

$$\Delta H^0_{HILIC} = -4.38 KJ/mol^0K$$



$$\Delta S^0_{RP} = 6.38 J/mol^0K$$

$$\Delta S^0_{HILIC} = 19.93 J/mol^0K$$

$$\Delta G^0_{RP} = \Delta H^0_{RP} - T \times \Delta S^0_{RP}; \Delta G^0_{RP} = \Delta H^0_{HILIC} - T \times \Delta S^0_{HILIC}; \ln k_{RP} = -B_{RP} \times \frac{1}{T} + A_{RP}; \ln k_{HILIC} = -B_{HILIC} \times \frac{1}{T} + A_{HILIC}$$

$$\Delta H^0_{RP} = -R \times B_{RP}; \Delta H^0_{HILIC} = -R \times B_{HILIC}; \frac{\Delta S^0}{R} + \ln \varphi = A; \frac{\Delta S^0}{R} = A - \ln \varphi; \Delta S^0 = R \times (A - \ln \frac{V_{FS}}{V_{FM}})$$

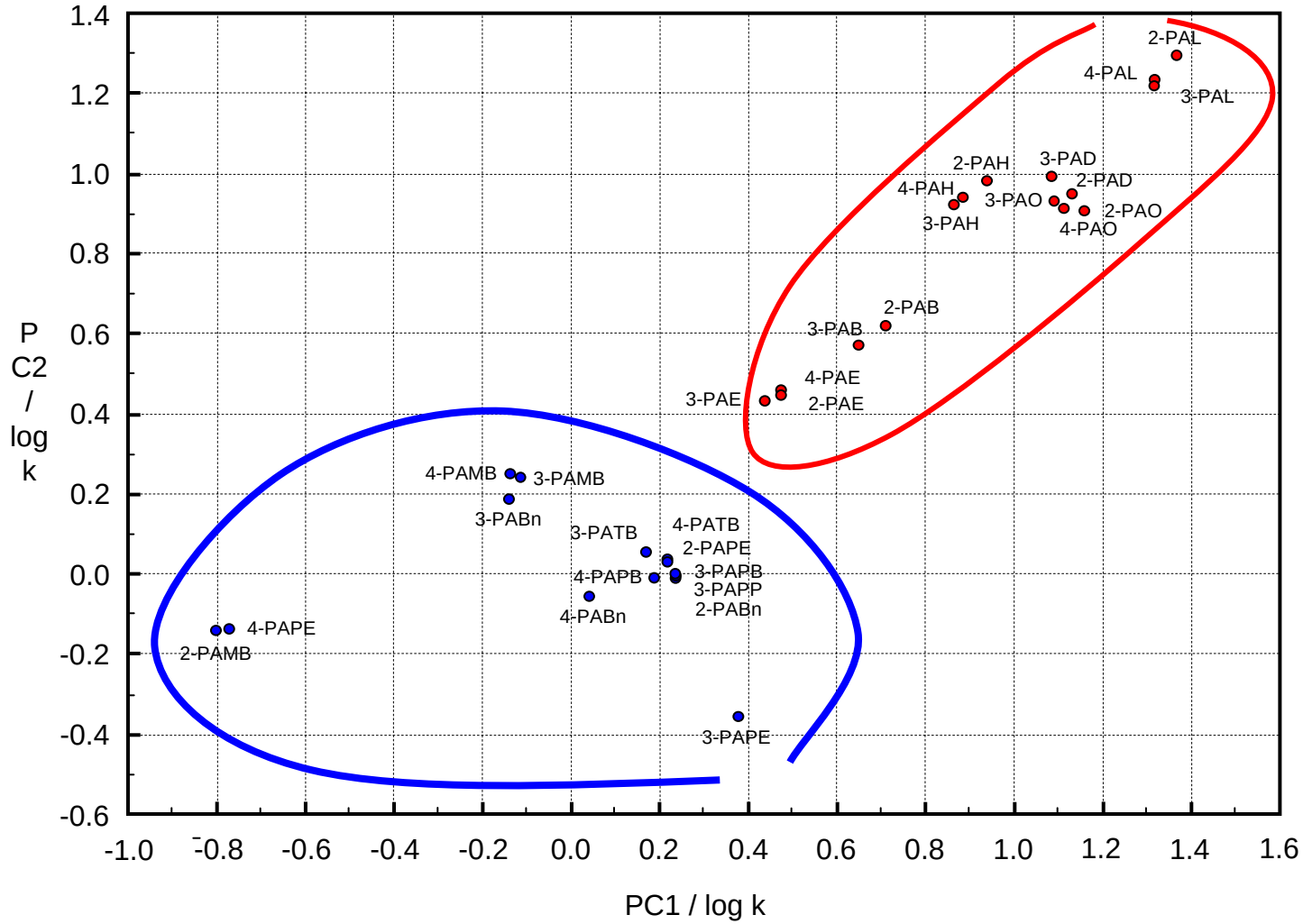
$$\Delta S^0_{RP} = R \times (A_{RP} - \ln \frac{V^{RP}_{FS}}{V_{FM}}); \Delta S^0_{HILIC} = R \times (A_{HILIC} - \ln \frac{V^{HILIC}_{FS}}{V_{FM}}); \Delta H^0_{RP} - \Delta H^0_{HILIC} = T \times (\Delta S^0_{RP} - \Delta S^0_{HILIC})$$

$$\frac{R \times (B_{HILIC} - B_{RP})}{T} = \Delta S^0_{RP} - \Delta S^0_{HILIC}$$

$$V^{HILIC}_{FS} = V^{RP}_{FS} \times 2.71828^{(e \frac{\Delta B^{HILIC/RP} + T\Delta A^{HILIC/RP}}{T})}$$

$$V^{RP}_{S.Ph.} \approx V^{HILIC}_{S.Ph.}$$

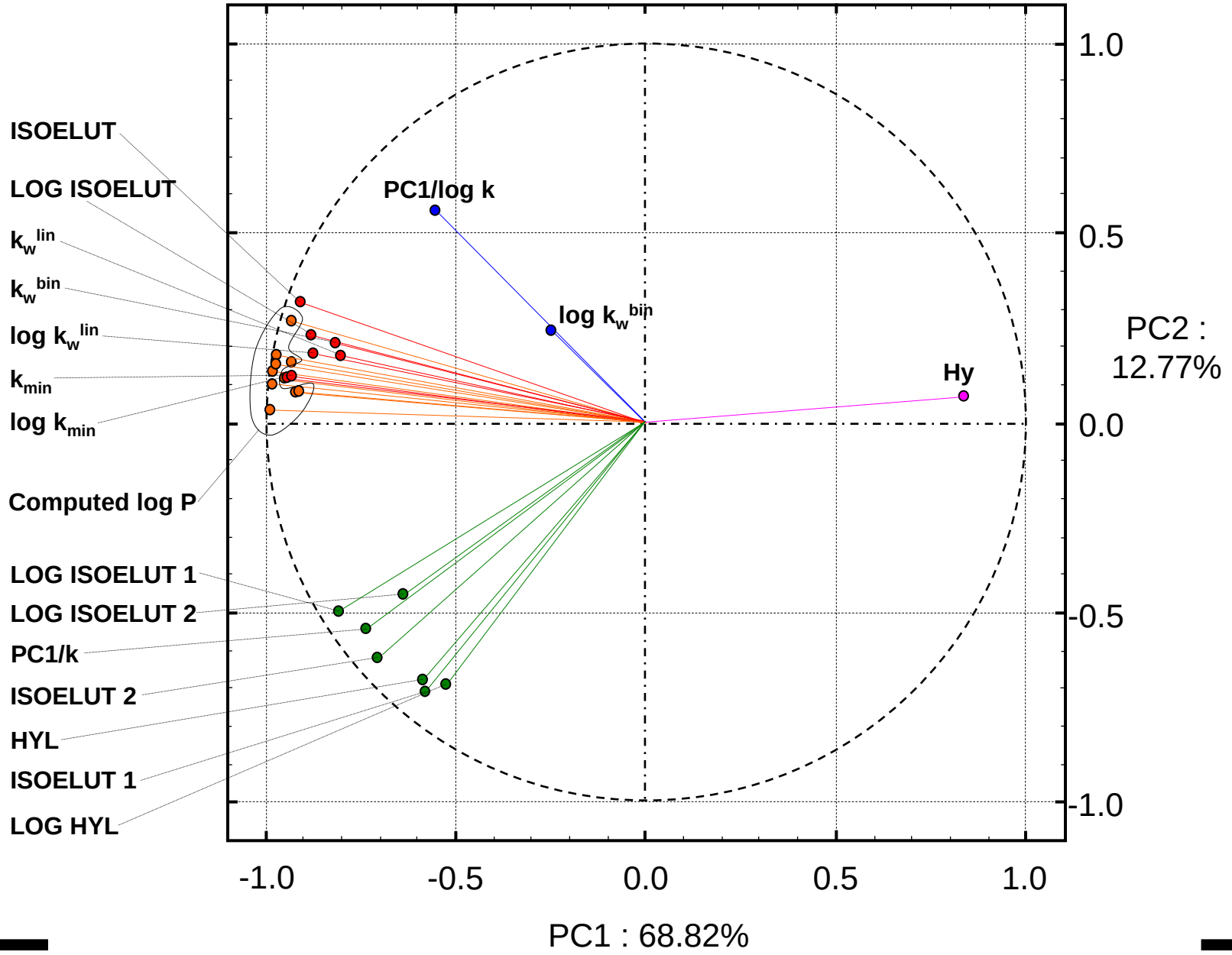
Some chemometric approaches:



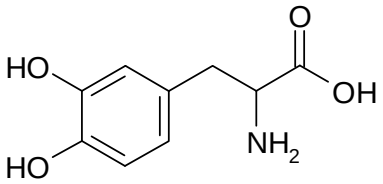
PCA on covariance matrices k/log k and computed descriptors

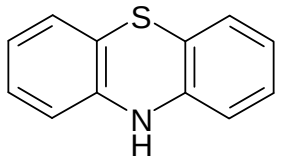
(Dis)Similarity Charts:

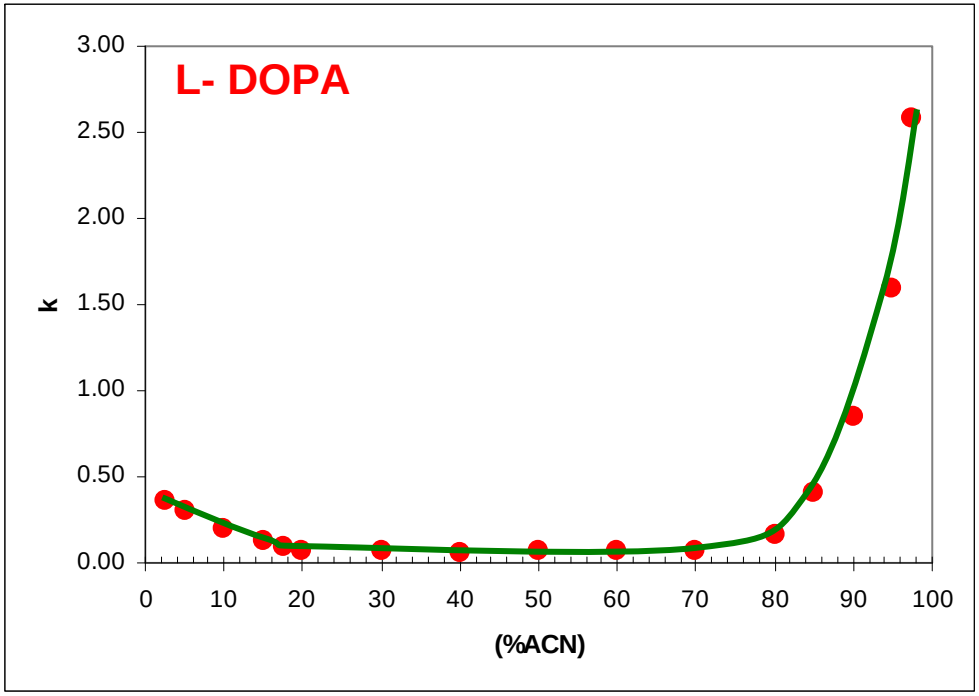
Loadings representation after applying PCA on matrices formed by calculated log P and hydrophobicity indices.

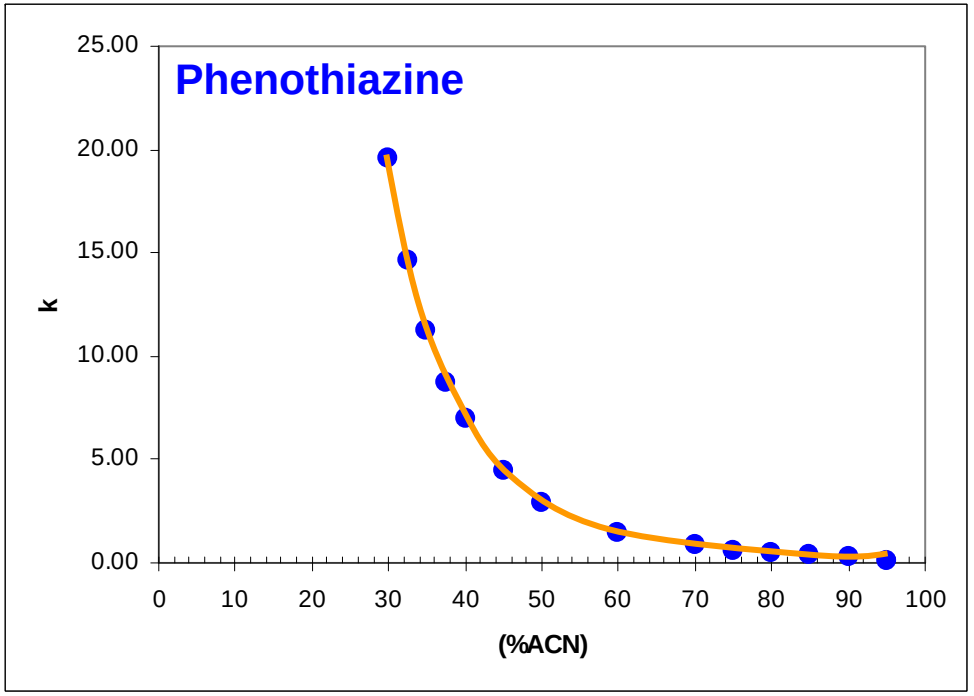


Does it always work ????









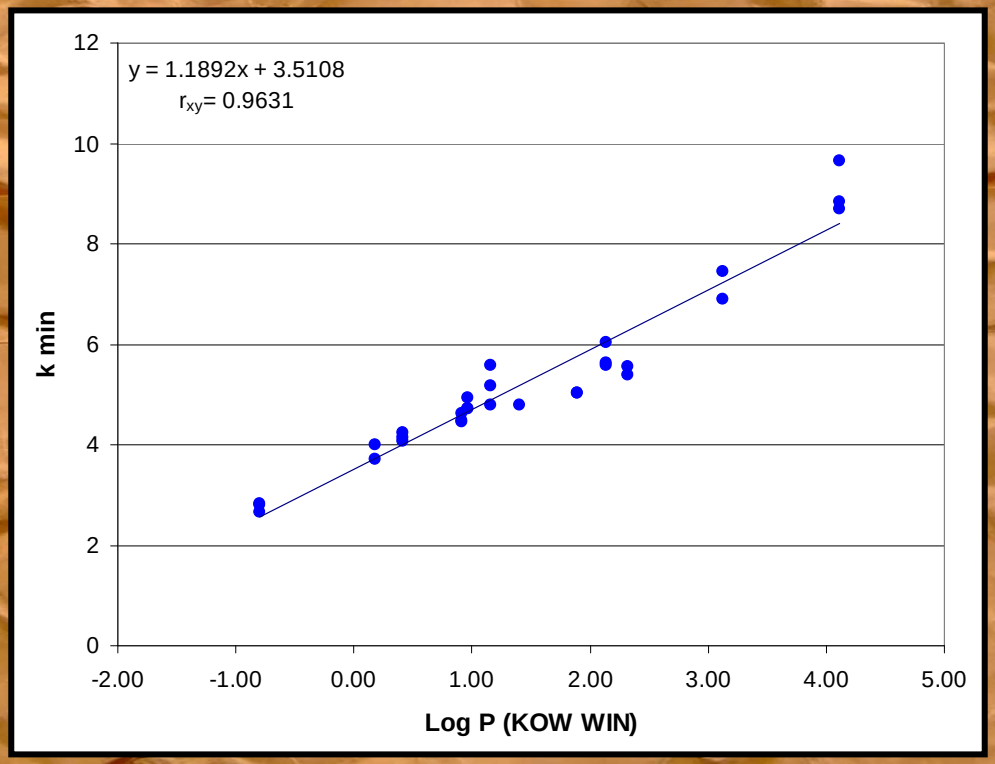
log P (s/f method) = -2.39

log P (s/f method) = 4.15

Conclusions:

1. Other chromatographic retention mechanisms than RP may successfully produce indices for lipophilicity scales.

2. For the bimodal RP+HILIC retention behaviour, k_{min}, ISOELUT, log k_{min}, LOGISOLEUT closely correlate with computed log P.



3. Such approaches work better within compound classes.

Thank you for your attention and patience !

Things you'll meet, of many a kind,
Sights and sounds, and tales, no end,
But to keep them all in mind,
Who would bother to attend?
Very little does it matter
If you can yourself fulfil,
That, with idle empty chatter,
Days go past and days come still.

M. Eminescu, Glossa
Translation: Corneliu M. Popescu

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