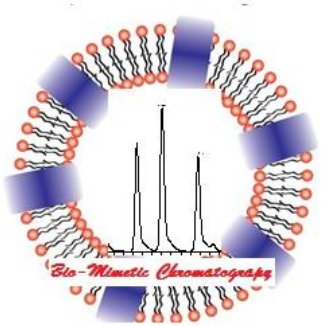
Lipophilicity in drug discovery



Klara Valko

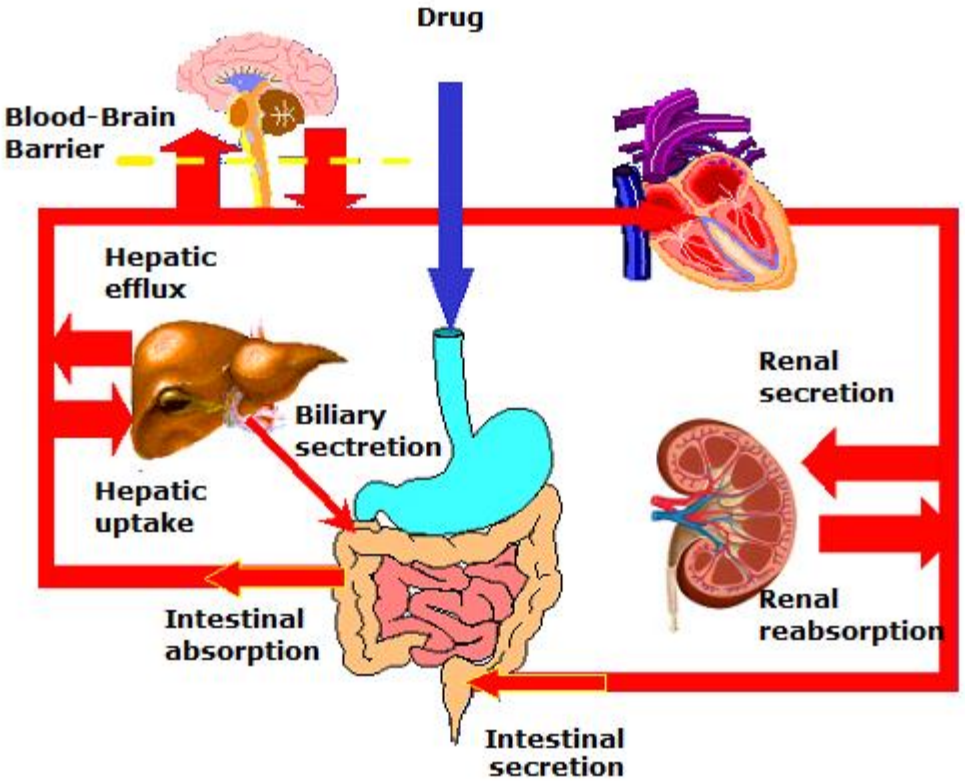
Bio-Mimetic Chromatography

Consultancy for Successful Drug Discovery

What is lipophilicity? IUPAC definition

- Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment.
- Hydrophobicity measures the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non-polar molecules.

Why do we need to measure lipophilicity?



Compound partitions between two immiscible solvent

A compound partitioning between aqueous and organic phase can model compound partitioning *in vivo*

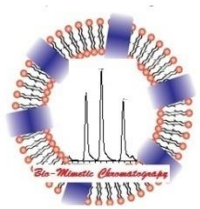
Lipophilicity is measured by partition coefficient

- Partition coefficient is the quotient of the compound concentration in an aqueous and a non-miscible solvent under equilibrium condition.

- It is expressed by the quotient of the compound concentrations in the two phases under equilibrium condition.

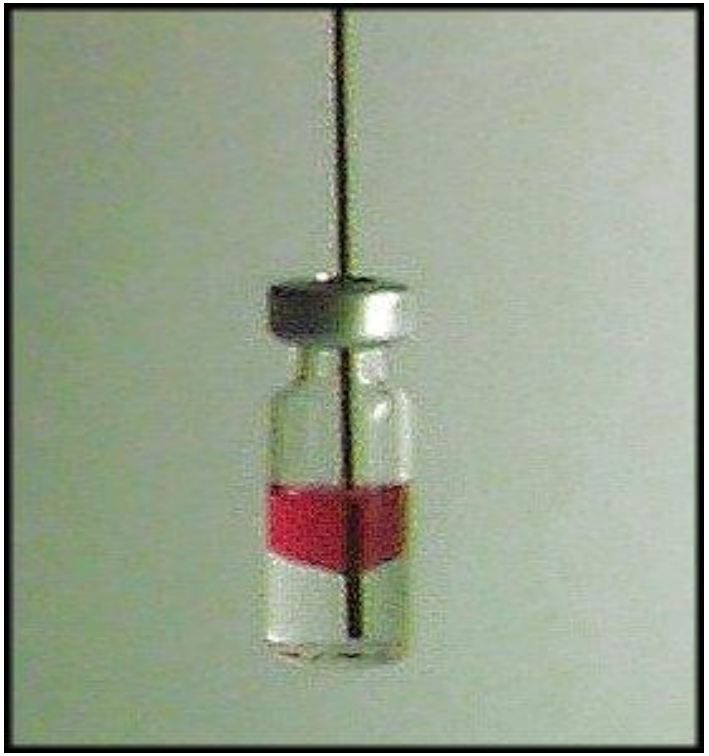
- It depends on the nature of the two phases, the compound properties, pH, and the temperature.

Procedure of measuring octanol/water partition coefficients



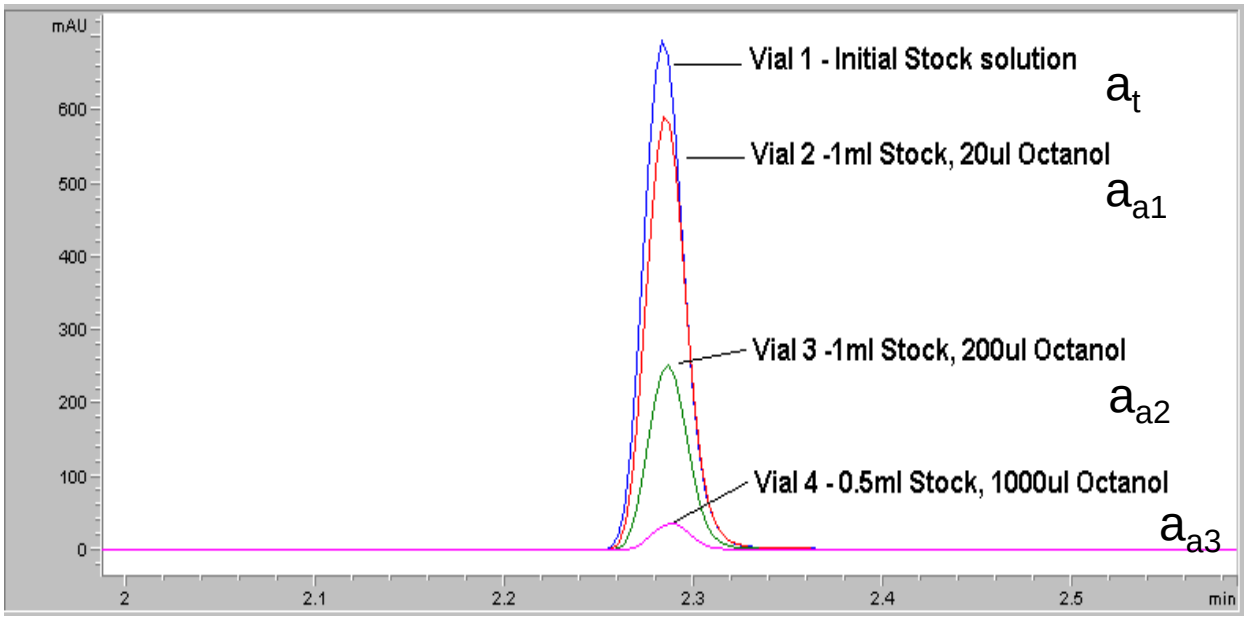
- Add the compound into the octanol saturated aqueous buffer
- Add various amounts of water saturated octanol to the constant volumes of buffer solution containing the compound
- Equilibrate with shaking/rolling
- Measure the peak area of the compound in the buffer phase by injecting the same amounts from each vial
- Calculate the partition coefficient

Roller-Vial method for the measurement of octanol/water partition coefficients.



Inject from the bottom layer (aqueous phase) to the HPLC for the concentration determination of the compound.

Concentration determination by measuring HPLC peak areas from the water phase



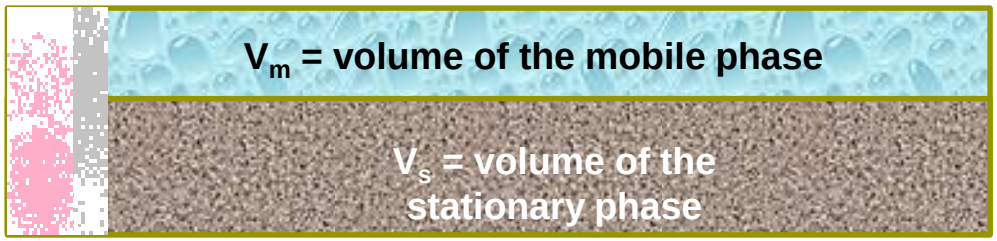
$$P = \frac{a_t - a_a}{a_a} * \frac{V_{water}}{V_{octanol}}$$

a_t is the peak area obtained from the aqueous phase without octanol
a_a is the peak area obtained from the aqueous phase after equilibration with octanol
$V_{octanol}$ volume of the octanol phase; V_{water} volume of the aqueous phase

Measuring lipophilicity by HPLC

Measuring bio-relevant association constants has great impact on drug discovery!

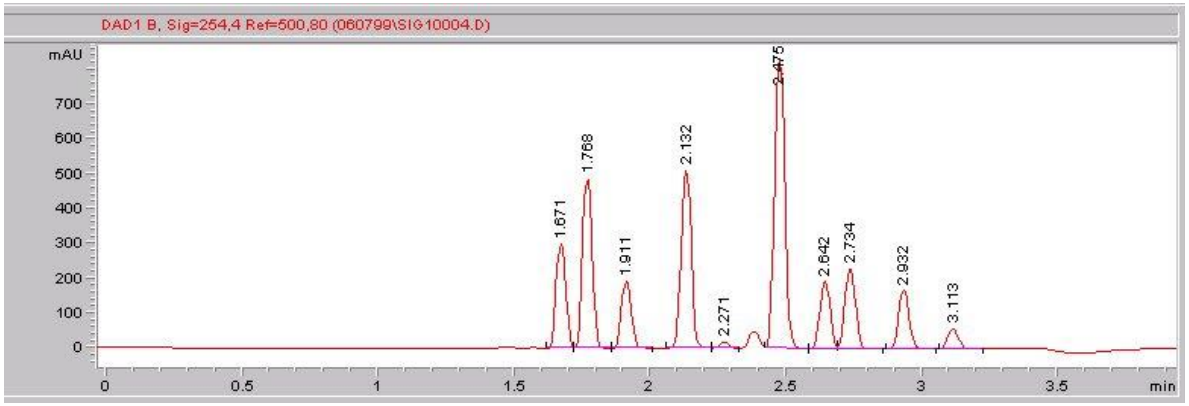
Based on the solvophobic theory the interaction between the solute and the stationary phase is considered as a reversible association of the solute molecules with the stationary phase moiety (hydrocarboneous, membrane, or protein). Accordingly solute retention is governed by the dynamic equilibrium constant.



Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.

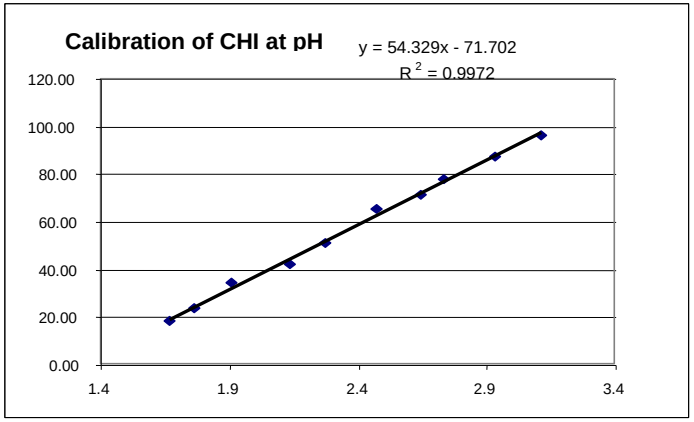
Chromatographic Hydrophobicity Index (CHI)

- Approximates the organic phase concentration (%) when the compound elutes from a reversed phase column using linear gradient. CHI gives a straight line with the gradient retention time.

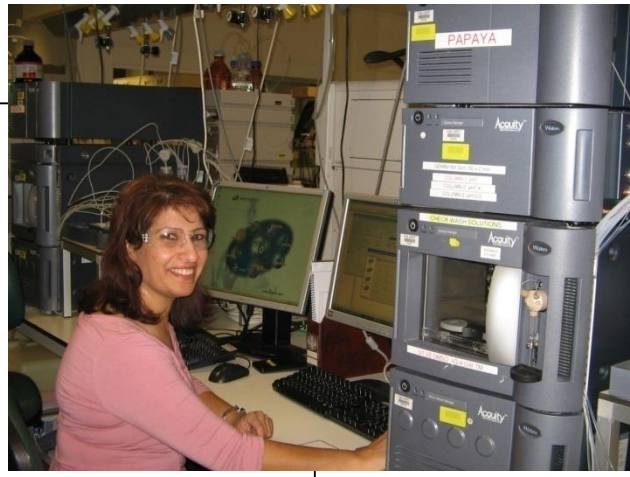


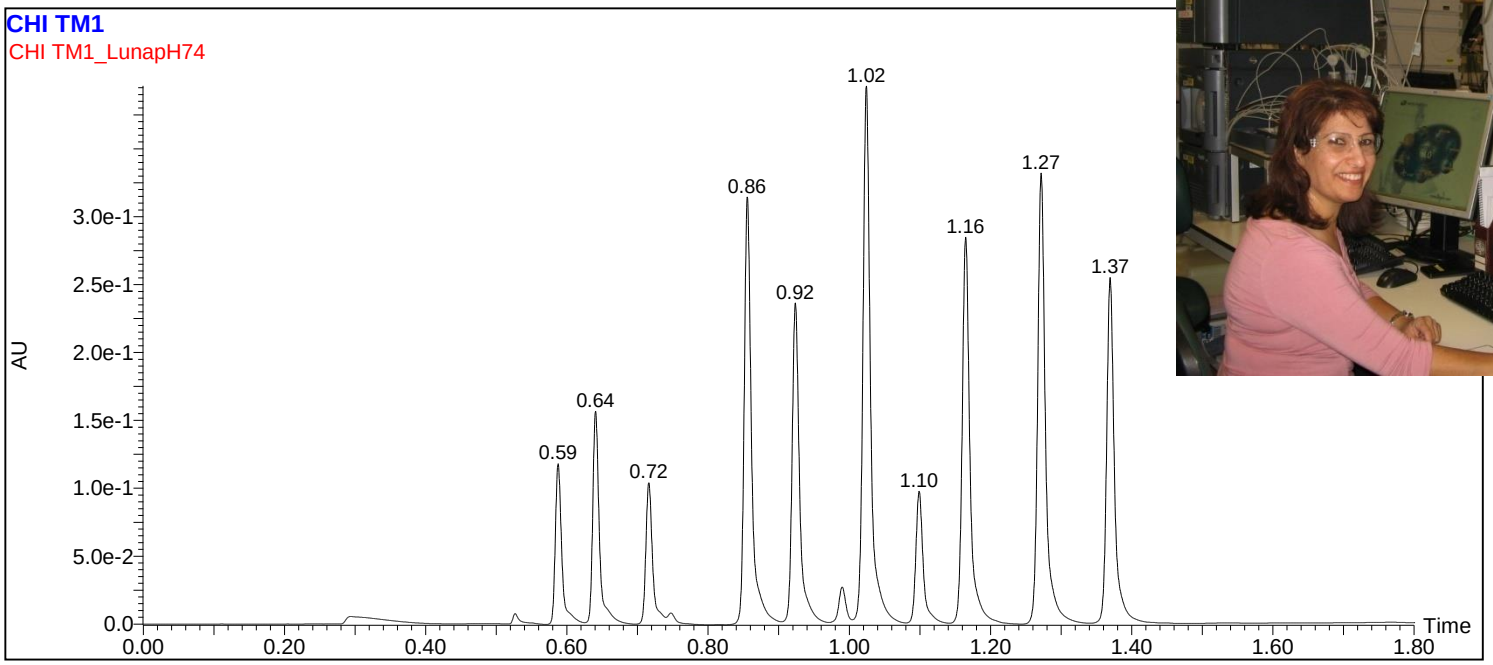
LunaC18(2) 50 x 3 mm; 1.00 ml/min; Mobile phase A 50 mM ammonium acetate pH 7.4 and B is 100% acetonitrile. Gradient: 0 - 2.5 min 0 - to 100% B; 2.5 - 2.7 min 100% B.

Compound	$CHI_{7.4}$ at pH 7.4	CHI_{2} at pH 2	$CHI_{10.5}$ at pH 10.5
Theophylline	18.4	17.9	5.0
Phenyltetrazole	23.6	42.2	16.0
Benzimidazole	34.3	6.3	30.6
Colchicine	43.9	43.9	43.9
Phenyltheophylline	51.7	51.7	51.7
Acetophenone	64.1	64.1	64.1
Indole	72.1	72.1	72.1
Propiophenone	77.4	77.4	77.4
Butyrophenone	87.3	87.3	87.3
Valerophenone	96.4	96.4	96.4



Ultrahigh Performance Liquid Chromatography (uPLC) for CHI





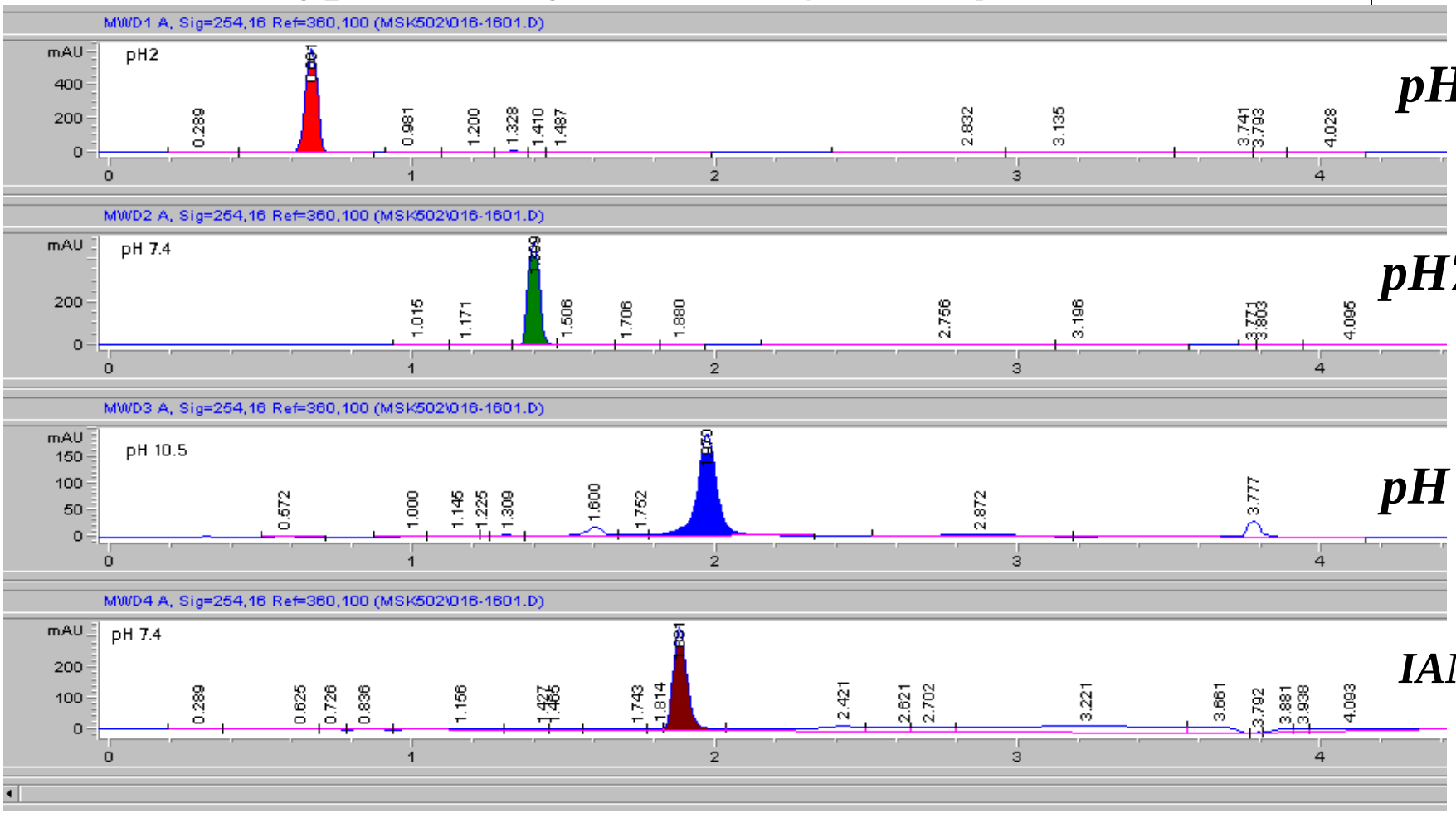
The CHI test mix is separated in less than 90 sec
Now we can determine a compound's lipophilicity in 90 sec using various starting mobile phase pH

www.bio-mimetic-chromatography.com

Courtesy of Shenaz Bunally at GSK

Parallel measurement of a compound's retention at various pH to reveal acid/base character

Typical 4-way chromatograms of a base



pH2

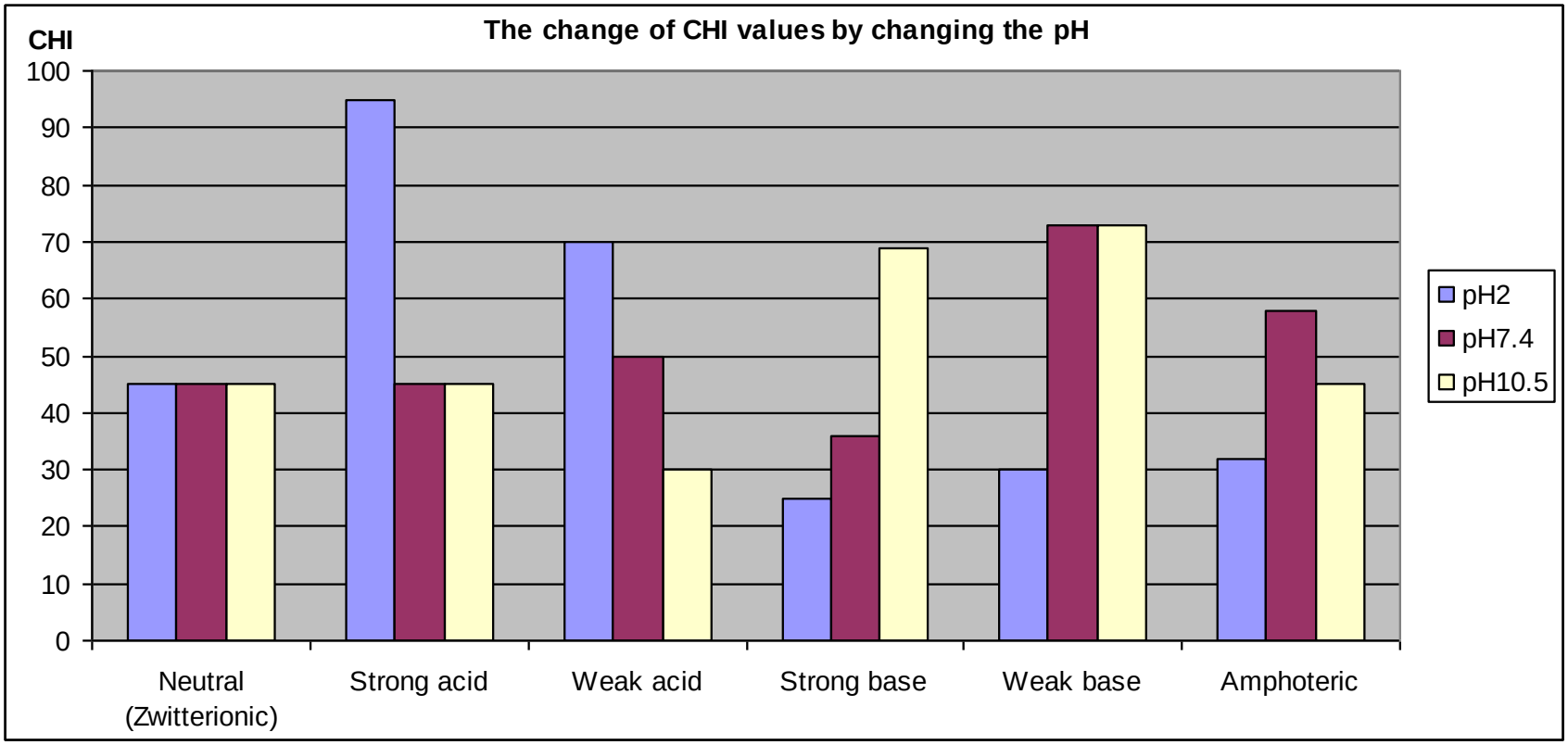
pH7.4

pH 10.5

IAM 7.4

www.bio-mimetic-chromatography.com

CHI values at pH 2, pH 7.4 and pH 10.5 reveal acid/base character of compounds

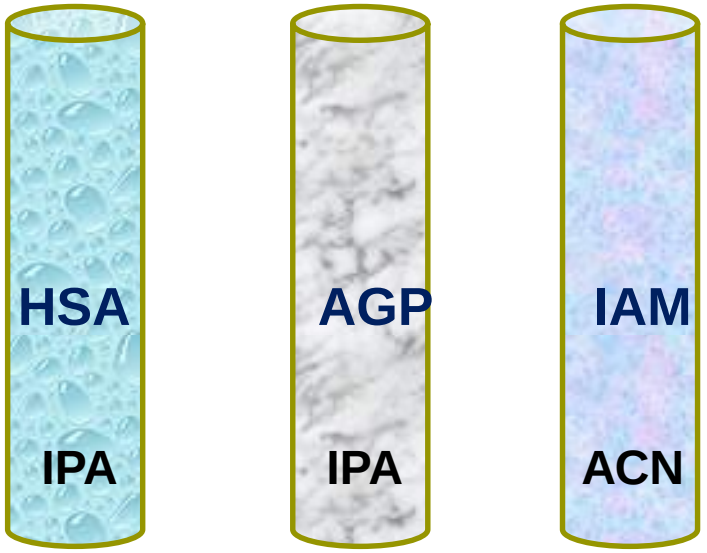


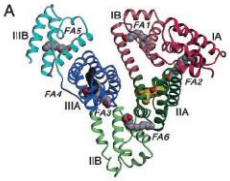
CHIs measured at 3 pHs provide an automatic way of grouping molecules according to acid/base character without structural information.

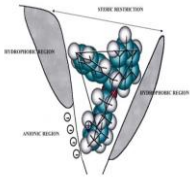
www.bio-mimetic-chromatography.com

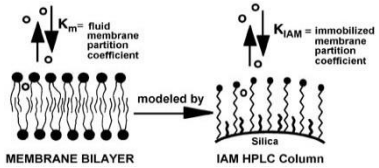
Bio-mimetic HPLC measurement of Human Serum Albumin (HSA), α-1acidglycoprotein (AGP) and Immobilized Artificial Membrane (IAM) partition

pH 7.4 aqueous mobile phases







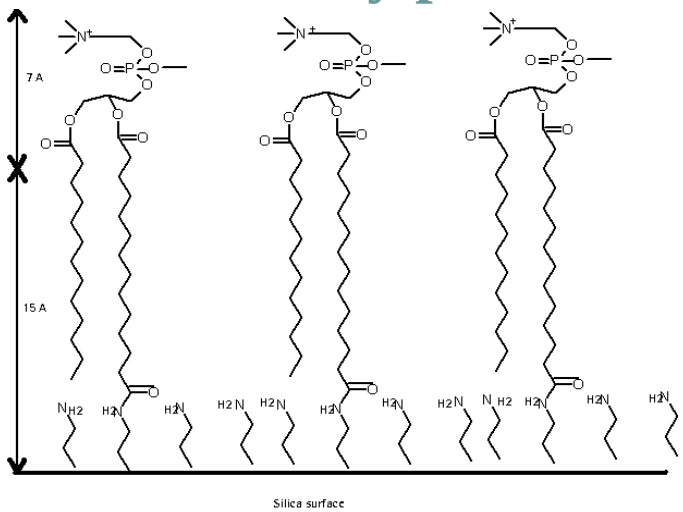


Bio-Mimetic Stationary phases

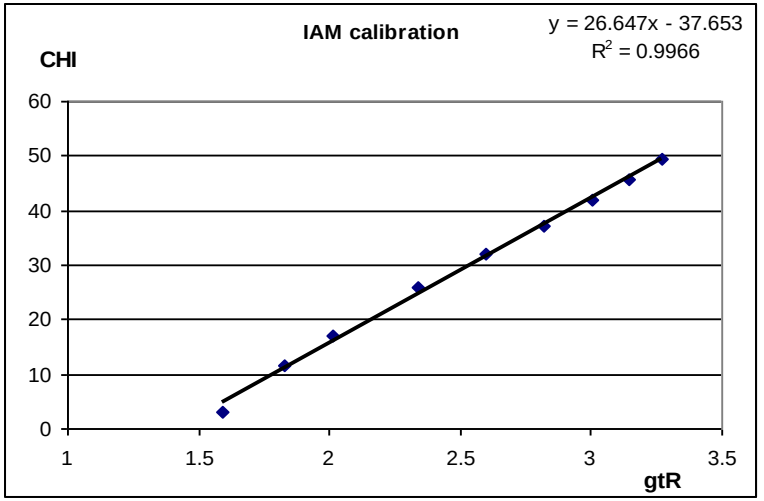
www.bio-mimetic-chromatography.com

Biomimetic lipophilicity measurements (Membrane partition) using Immobilised Artificial Membrane stationary phase

Stationary phase



Typical calibration



Compound	gtR	CHI IAM
Octanophenone	3.269	49.4
Heptanophenone	3.145	45.7
Hexanophenone	3.001	41.8
Valerophenone	2.822	37.3
Butyrophenone	2.601	32
Propiophenone	2.341	25.9
Acetophenone	2.013	17.2
Acetanilide	1.83	11.5
Paracetamol	1.591	2.9

Column: IAM PC2 (CH2)12 150 x 4.6

Mobile Phase flow rate: 2 ml/min

Gradient: 0 to 3 min 0 to 80% acetonitrile

3 to 3.5 min 80% acetonitrile

3.5 to 3.7 min 0% acetonitrile

Cycle time: 5 min

www.bio-mimetic-chromatography.com

K. Valko et al. J. Pharm. Sci.89 (2000) 1085-1096

Serum albumin binding measurement using chemically bonded serum albumin stationary phases

Column: HSA 50 x 3 mm (Chrom Tech, Chiral Technologies)

Flow rate: 1.8 ml/min at 30^{0}C

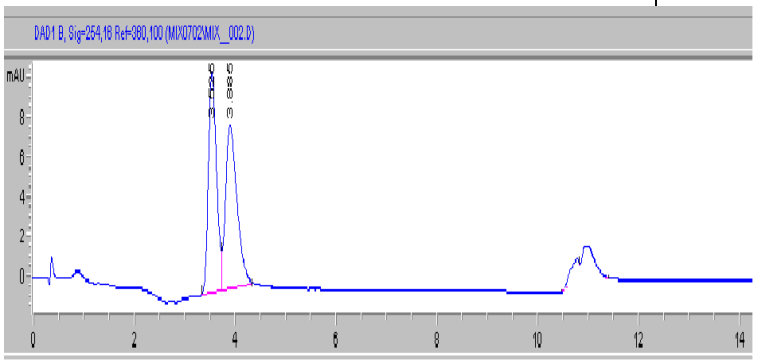
Mobile phase: 50 mM ammonium acetate pH7.4

Gradient: 0 - 3 min 0 to 30% 2-propanol;

3 to 10 min 30% 2-propanol;

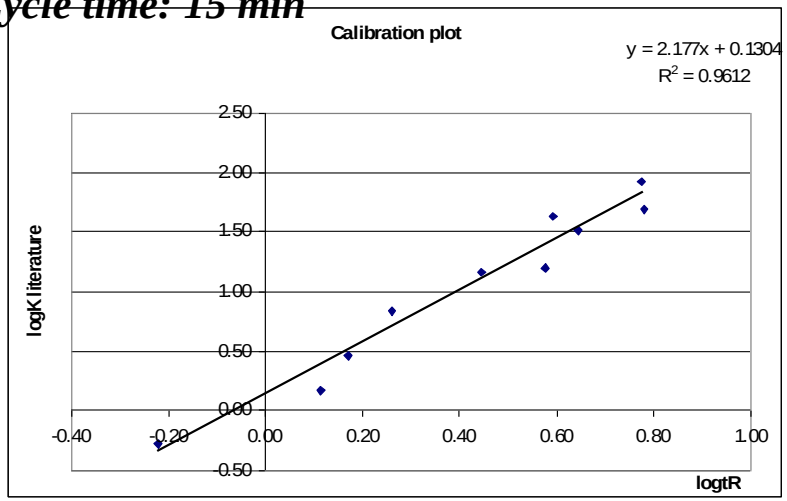
10 to 10.5 min 0% 2-propanol

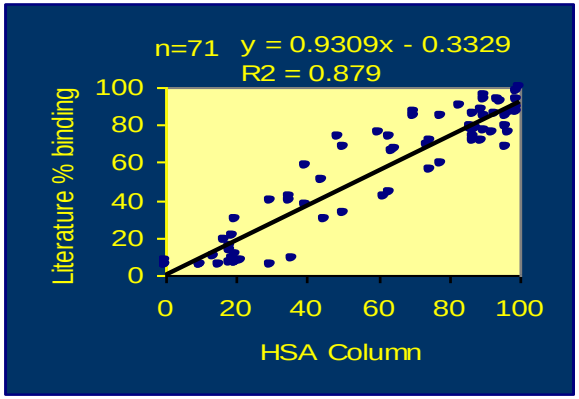
Cycle time: 15 min



Calculate %Binding

logK = slope * log(tR) + int
K = %B / (101-%B)

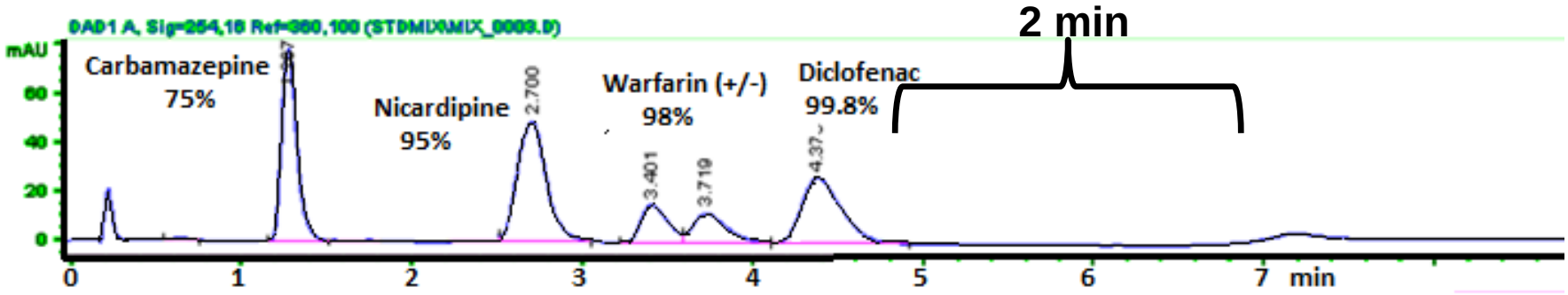


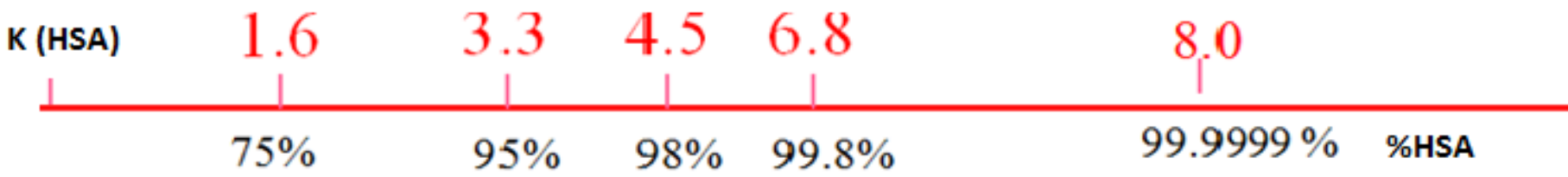


www.bio-mimetic-chromatography.com

K. Valko et al. J. Pharm. Sci. 92 (2003) 2236

Retention time of compounds can be converted to % binding or K association constant





$$e\log k(HSA) = \exp(\log k(HSA)) = K(HSA)$$

AGP binding measurement by HPLC

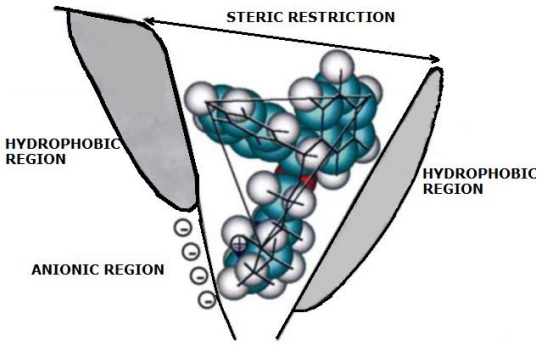
Same principle as HSA binding measurements:

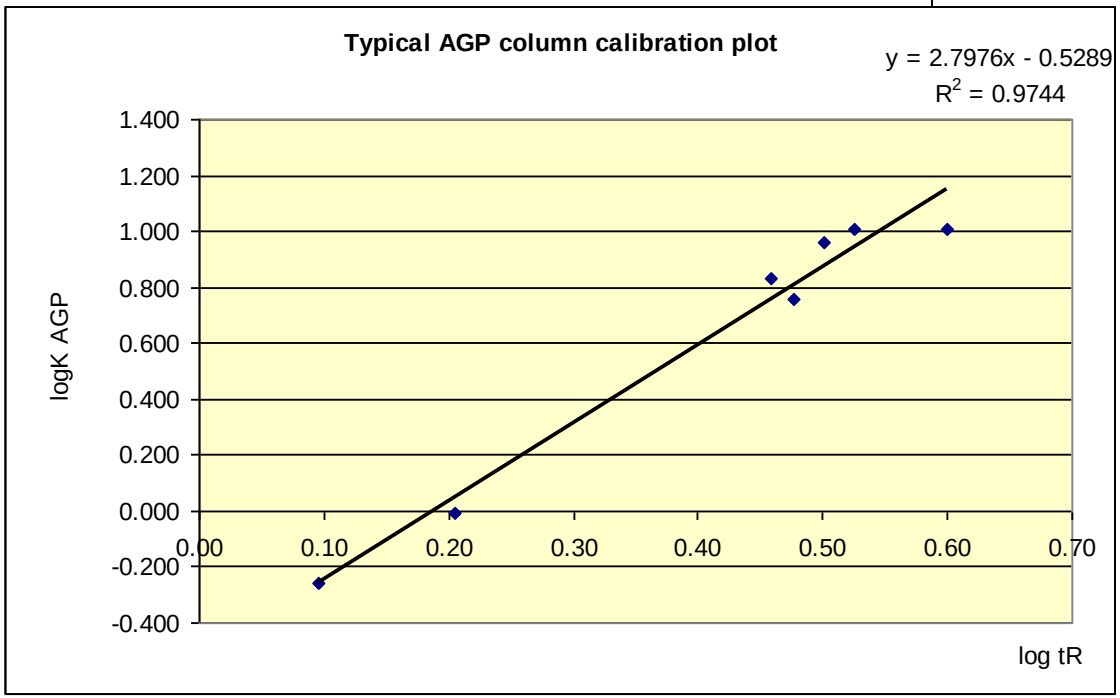
AGP column

2-propanol gradient

pH 7.4 ammonium acetate

Calibration with AGP binding data derived from published % AGP bound values





Calibration set of compounds: Nizatidine, Bromazepam, Warfarin, Propranolol, Imipramin, Nicardipine, Chlorpromazine

References

C. Hansch, A. Leo, ρ-σ-π analysis. A method for correlation of biological activity and chemical structure. J. Amer. Chem.Soc. 86(1964) 1616-1624

Horvath, Cs., Melander, W., Molnar, I. Solvophobic interactions in liquid chromatography with non-polar stationary phases, Journal of Chromatography, 125 (1976) 129-156

Valko, K.; Snyder, L.R.; Glajch, G.L. Retention in reversed-phase liquid chromatography as a function of mobile-phase composition. J. Chromatogr. A 656, (1993) 501–520.

Harnisch, M., Mockel, H. J., Shulze, G. J., Relationship between log P_{ow}, shake flask values and capacity factors derived from reversed-phase high performance liquid chromatography for n-alkylbenzenes and some OECD reference substances. *Journal of Chromatography* **282** (1983) 315-332

Tomlinson, E., Chromatographic hydrophobicity parameters in correlation analysis of structure – activity relationships. *Journal of Chromatography* **113** (1975) 1-45

Giaginis, A. Tsantili-Kakoulidou, Current state of the art in HPLC Methodology for lipophilicity assessment of basic drugs (Review) *Journal of Liquid Chromatography & Related Technologies, 31: (2008)* 79–96.

Lombardo, F., Shalaeva, M. Y., Tupper, K. A., Gao, F., $ElogD_{oct}$: A tool for Lipophilicity Determination in Drug Discovery. 2. Basic and neutral compounds. (2001) 2490-2497

Gocan, S., Cimpan, G., Comer, J., Lipophilicity measurements by liquid chromatography in Advances in Chromatography, Eds: E. Grushka, N. Grinberg, 44 (2005) 79-176, Taylor & Francis Group, 1574447343

Valko, K., Bevan, C., Reynolds, D., Chromatographic hydrophobicity index by fast-gradient RP-HPLC: A high throughput alternative to log P/log D. *Analytical Chemistry* **69** (1997) 2022-2029

Valko, K. Measurements of lipophilicity and acid/base character using HPLC methods. In “Pharmaceutical profiling in drug discovery for lead selection” Eds. Borchardt, R., Kerns, E., AAPS (2004)Arlington, VA 127-182