ON-LINE REVERSED PHASE SUPPORTED LIQUID EXTRACTION (RP-SLE) AS A BASIC SCENARIO FOR EXPLAINING LARGE VOLUME INJECTION (LVI) OF IMMISCIBLE DILUENTS IN RPLC

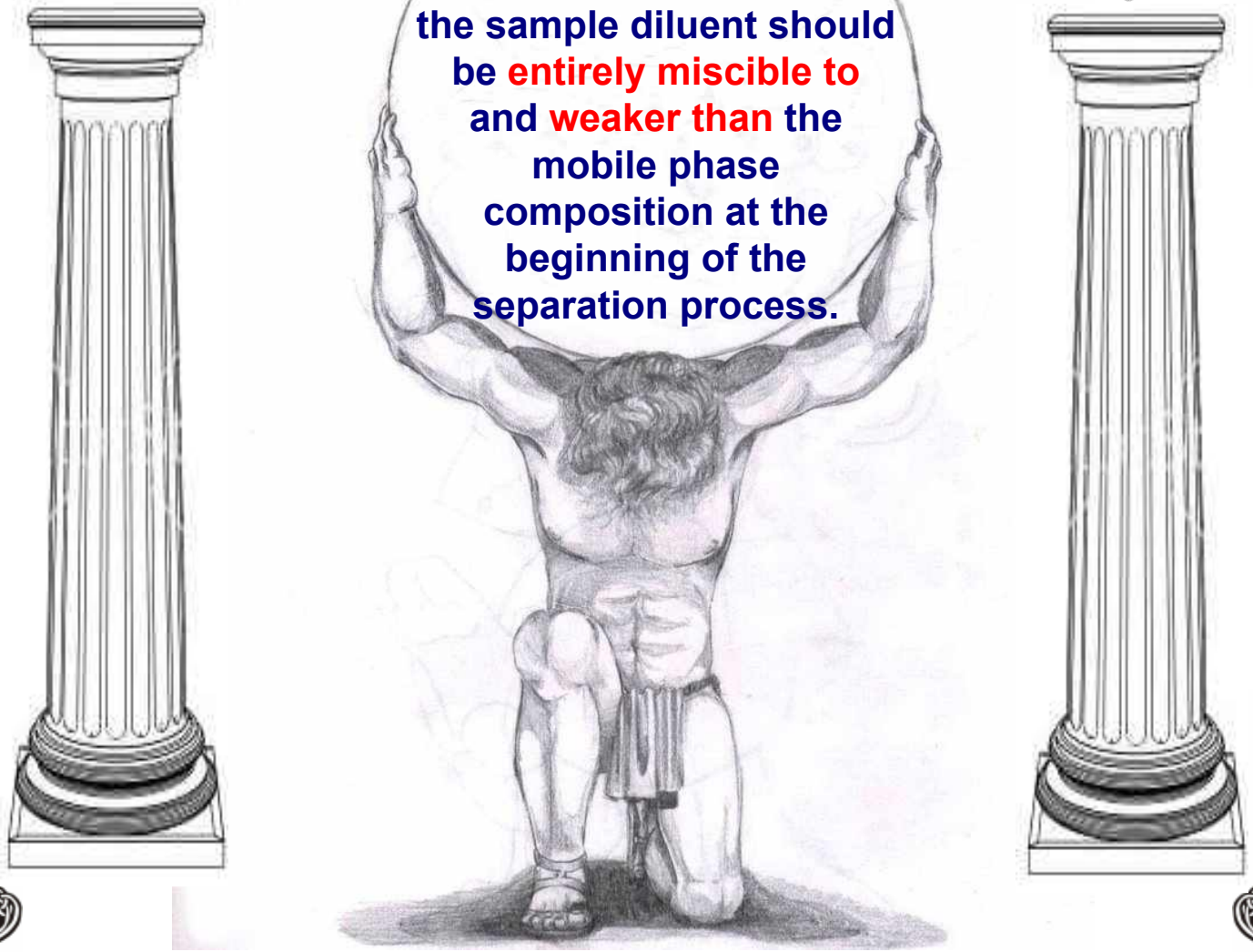
Andrei Medvedovici, Paul Lazăr
Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, # 90-92 Panduri Ave., Bucharest-050663, Romania
Fax no. + 40214102279;
E-mail: avmedved@yahoo.com; paul_lazar@yahoo.com

CHIMIA 2014

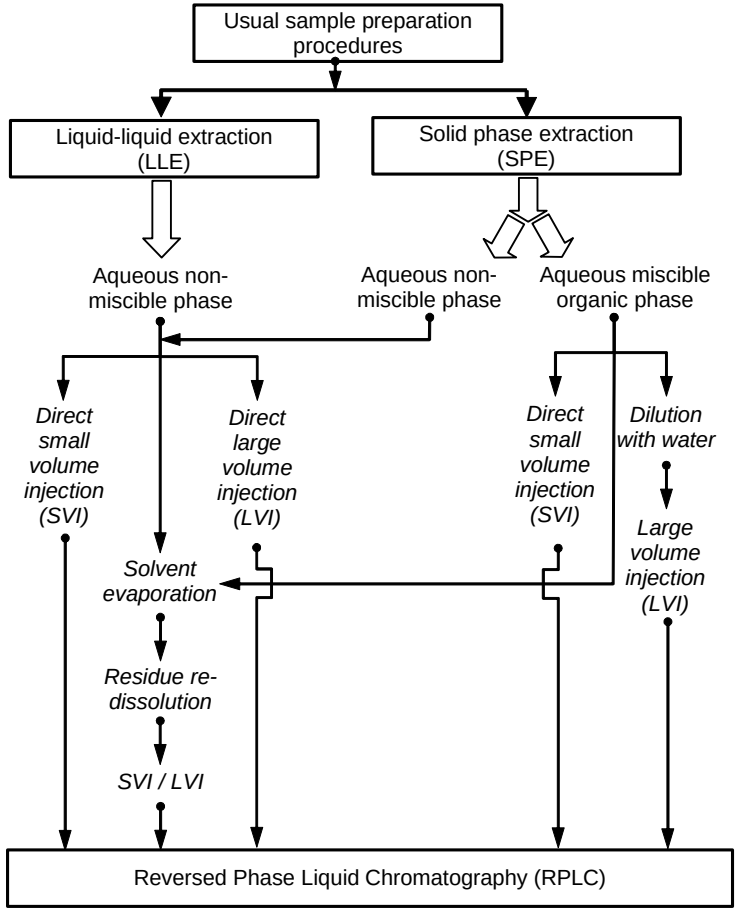
New Trends in Applied Chemistry
May 23-24th 2014
Constanţa, Romania

The golden rule of the thumb in LC injections

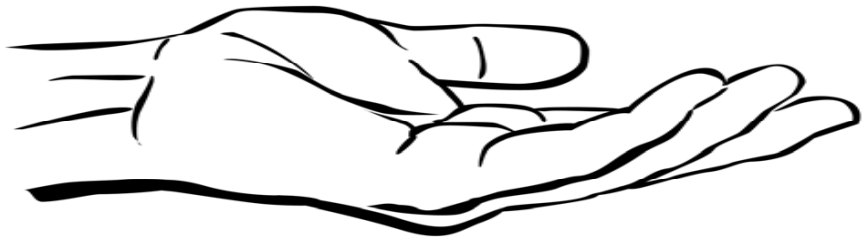
For achieving LVI in LC, the sample diluent should be entirely miscible to and weaker than the mobile phase composition at the beginning of the separation process.



Most of the sample preparation techniques provide



target compounds in water non-miscible organic solvents !



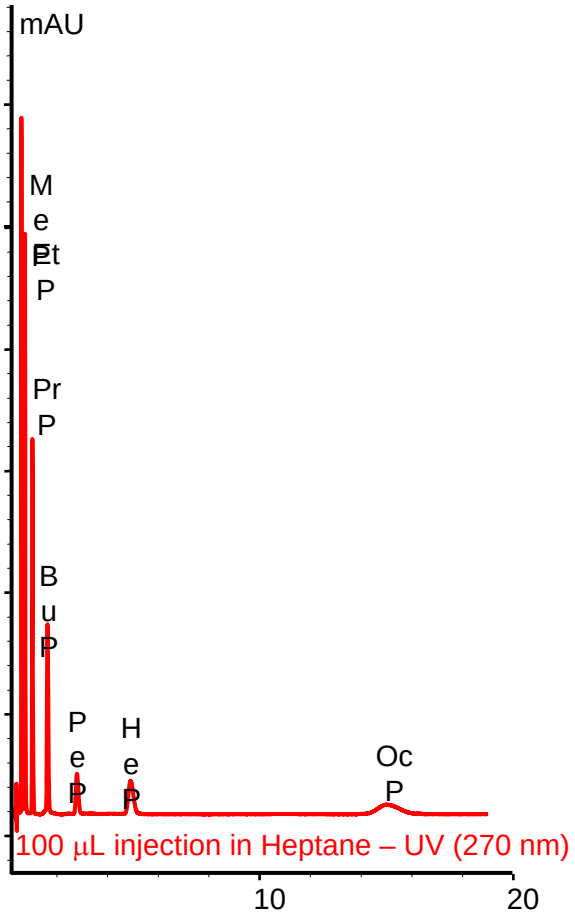
Injecting Organic Solvents in Reversed-Phase

A reader wrote me recently asking what would happen if he injected his sample, which came dissolved in hexane, into a reversed-phase mobile phase of methanol-buffer. The first answer is that this is not a good habit, but is it possible? We'll see.

Is the injection solvent much stronger than the mobile phase, but it is not miscible either. This sounds **like a disaster waiting to happen** – and it may be. But not so fast! If the injection volume is small enough, it may be possible to disperse the injection solvent in the mobile phase sufficiently that acceptable peak shape results. This will be a matter of trial and error. I would start by making a solution of my sample in hexane at a high enough concentration that I get a good response, even at very small volumes. First, inject the sample dissolved in the mobile phase as reference. Then inject 1, 2, 5, 10 and 20 μL of the hexane solution and see what happens. I expect that 1 μL will be acceptable, but at some volume, the peaks will start coming out too early and will be distorted. No, it's no ideal, but it may work. I remember doing exactly this with a sample we received that was dissolved in toluene. We already had a method for the same compound as a reversed phase method. If I recall correctly, we were able to run with 5 μL injections and obtain acceptable results.

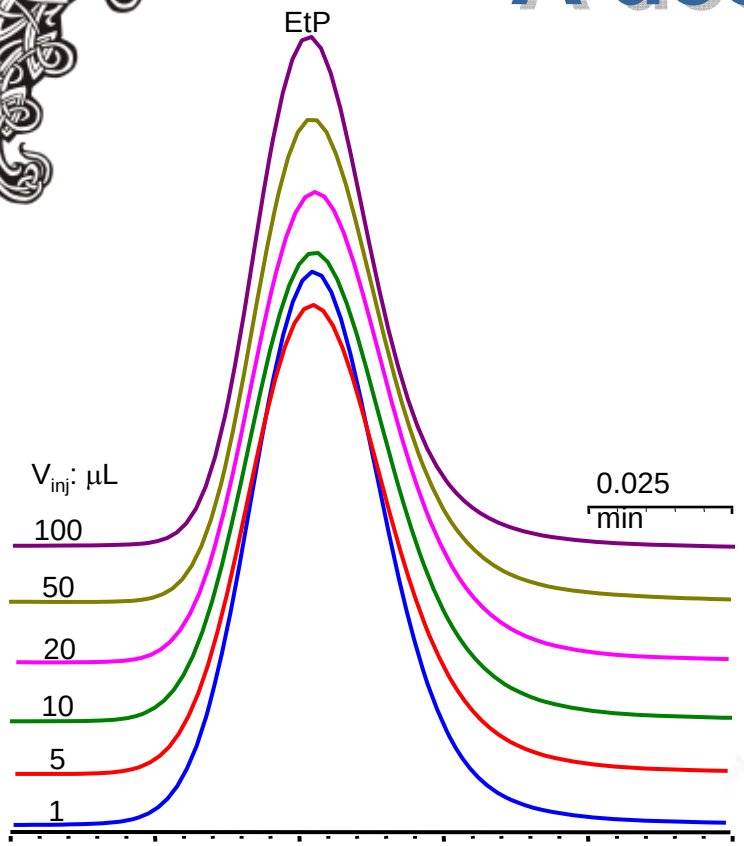
My favorite chemistry quote is from Izaak Kolthoff, considered by many to be the father of analytical chemistry: "**Theory guides, experiment decides**". Here is a good example of that – no, hexane is not compatible with a methanol-water mobile phase, but under the right conditions, you just might get away with it.

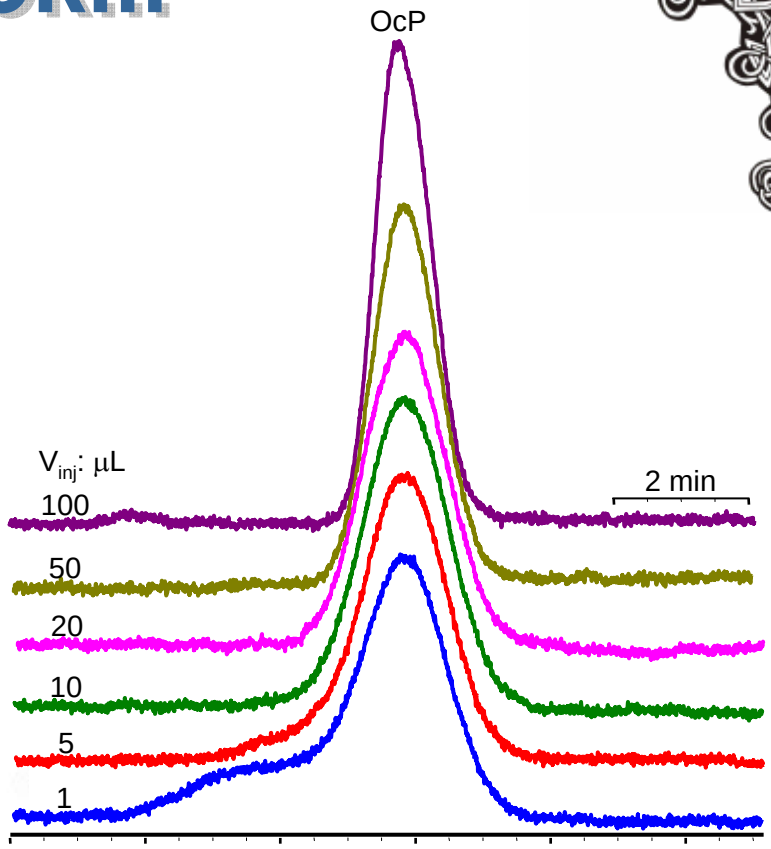
Do you find this disastrous?



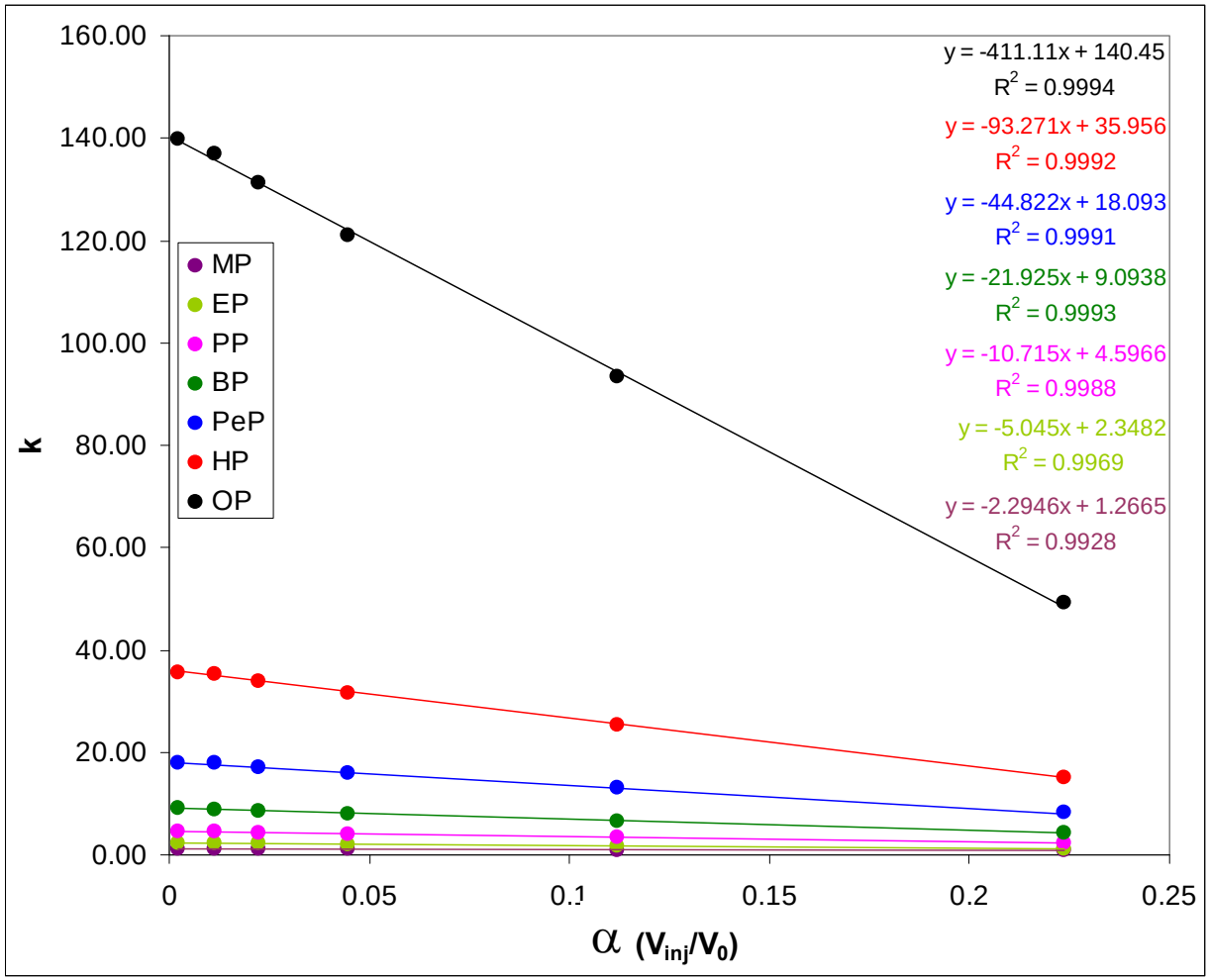
Chromatographic Column: Zorbax SB-C18 column
(50 mm L x 4.6 mm i.d. x 1.8 µm d.p.)
Column temperature: 25 °C
Mobile phase: ACN / H2O 40 / 60 (v/v)
Flow rate: 1.5 mL/min
Elution: isocratic
Detection: UV 270 nm
Injection volumes: 1 to 100 µL
Analytes: Methyl, Ethyl, Propyl, Butyl, Pentyl, Hexyl, Octyl Parabens
Diluents: Hexane, Heptane, iso-Octane, Decane, Dodecane
Absolute amounts loaded to column kept constant.

A deeper look...





and some calculations, it results that ...



retention linearly decreases with the injected volume!

and it is not only a pure hazard...

Diluent	Characteristics of the linear regression $k_{app}=f(\alpha)$	Model compounds						
		MeP	EtP	PrP	BuP	PeP	HeP	OcP
Hexane	Slope	-2.387	-5.144	-10.912	-22.374	-45.845	-95.813	-392.974
	Intercept	1.26	2.33	4.57	9.06	18.00	35.88	131.34
	Correlation Coefficient	**0.9976**	**0.9990**	**0.9995**	**0.9997**	**0.9997**	**0.9996**	**0.9991**
Heptane	Slope	-2.295	-5.045	-10.715	-21.925	-44.822	-93.271	-411.107
	Intercept	1.27	2.35	4.60	9.09	18.09	35.96	140.45
	Correlation Coefficient	**0.9964**	**0.9984**	**0.9994**	**0.9996**	**0.9995**	**0.9996**	**0.9997**
Iso-Octane	Slope	-2.114	-4.796	-10.366	-21.387	-43.902	-92.047	-409.310
	Intercept	1.26	2.34	4.59	9.10	18.08	36.08	142.22
	Correlation Coefficient	**0.9960**	**0.9987**	**0.9996**	**0.9999**	**0.9998**	**0.9998**	**0.9997**
Decane	Slope	-2.025	-4.970	-11.103	-23.005	-47.240	-99.732	-444.952
	Intercept	1.51	2.72	5.23	10.26	20.32	40.54	160.11
	Correlation Coefficient	**0.9938**	**0.9984**	**0.9996**	**0.9999**	**0.9998**	**0.9997**	**0.9996**
Dodecane	Slope	-2.137	-5.102	-10.971	-22.113	-46.101	-97.501	-445.343
	Intercept	1.528	2.735	5.250	10.297	20.402	40.694	161.327
	Correlation Coefficient	**0.9979**	**0.9997**	**0.9996**	**0.9998**	**0.9999**	**0.9999**	**0.9997**

First model: a competitional adsorption!

$A_{(D)} \leftrightarrows A_{(M.Ph.)}$ [1]

$A_{(M.Ph.)} + L_{(S.Ph.)} \leftrightarrows A^*L_{(S.Ph.)}$ [2]

if assuming [D] >> [A] and log P^D > log P^A

$i\ D_{(M.Ph.)} + L_{(S.Ph.)} \leftrightarrows D_i^*L_{(S.Ph.)}$ [3]

[1] ⇄ ; [2] ⇄ ; [3] ⇄ ;

$$k_A = K_A \times V'_{S.Ph.}/V_{M.Ph.}$$

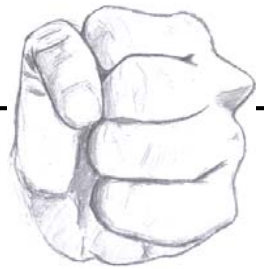
the $V'_{S.Ph.}$ available for A is a fraction of $V_{S.Ph.}$,
more precisely ($V_{S.Ph.} - \Delta V$), where $\Delta V = \zeta \times V_{inj}^D$, where ζ is a constant

$$k_A = K_A \times V_{S.Ph.}/V_{M.Ph.} - (K_A \times \zeta/V_{M.Ph.}) \times V_{inj}^D$$

A. Medvedovici, Victor David, Vasile David, C. Georgita, Retention phenomena induced by LVI of solvents non-miscible with the mobile phase in RPLC, *J. Liq. Chromatogr. Relat. Technol.*, 30, 199-213 (2007).

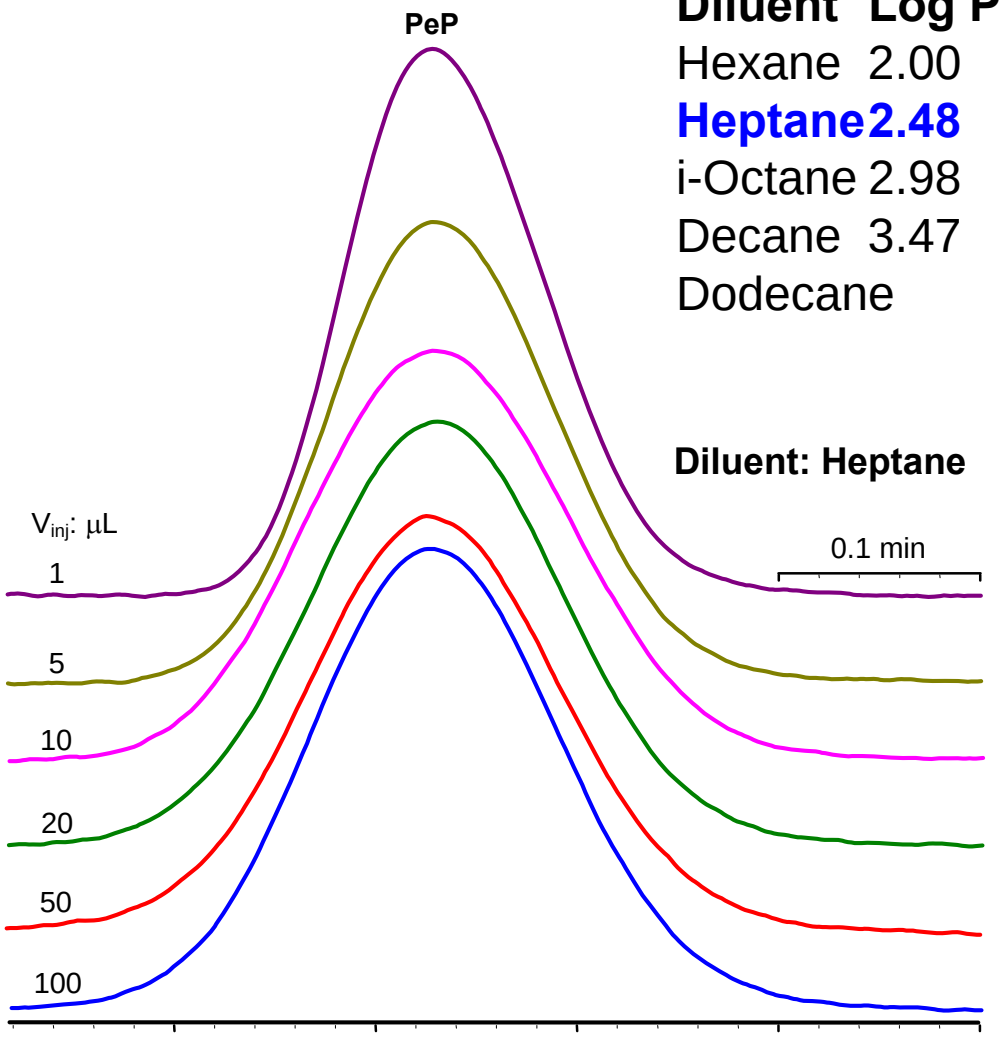
As resulting consequences: rules governing LVI of immiscible diluents.

1. D exhibits an increased chromatographic retention compared to target compounds ($k_D^{front} > k_A$);
2. Solubility of D in the M.Ph. should be as low as possible;
3. The initial chromatographic resolution supports the “apparent” reduction of the column length (affecting selectivity).
4. D plug from a previous injection is already eliminated from the column before starting a new separation process;
5. Fingering effects due to different viscosities (D vs. M.Ph.) need attention and should be controlled;



Are all these rules really applying?

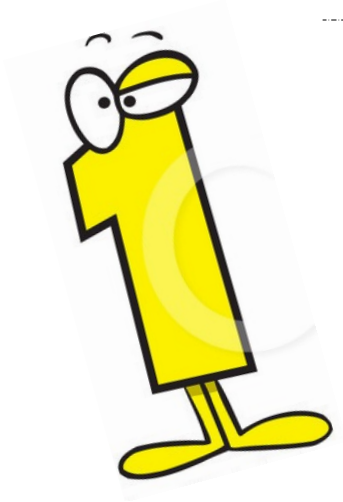
Analyte	Log P
MeP	2.00
EtP	2.48
PrP	2.98
BuP	3.47
PeP	**3.96**
HeP	4.45
OcP	5.43

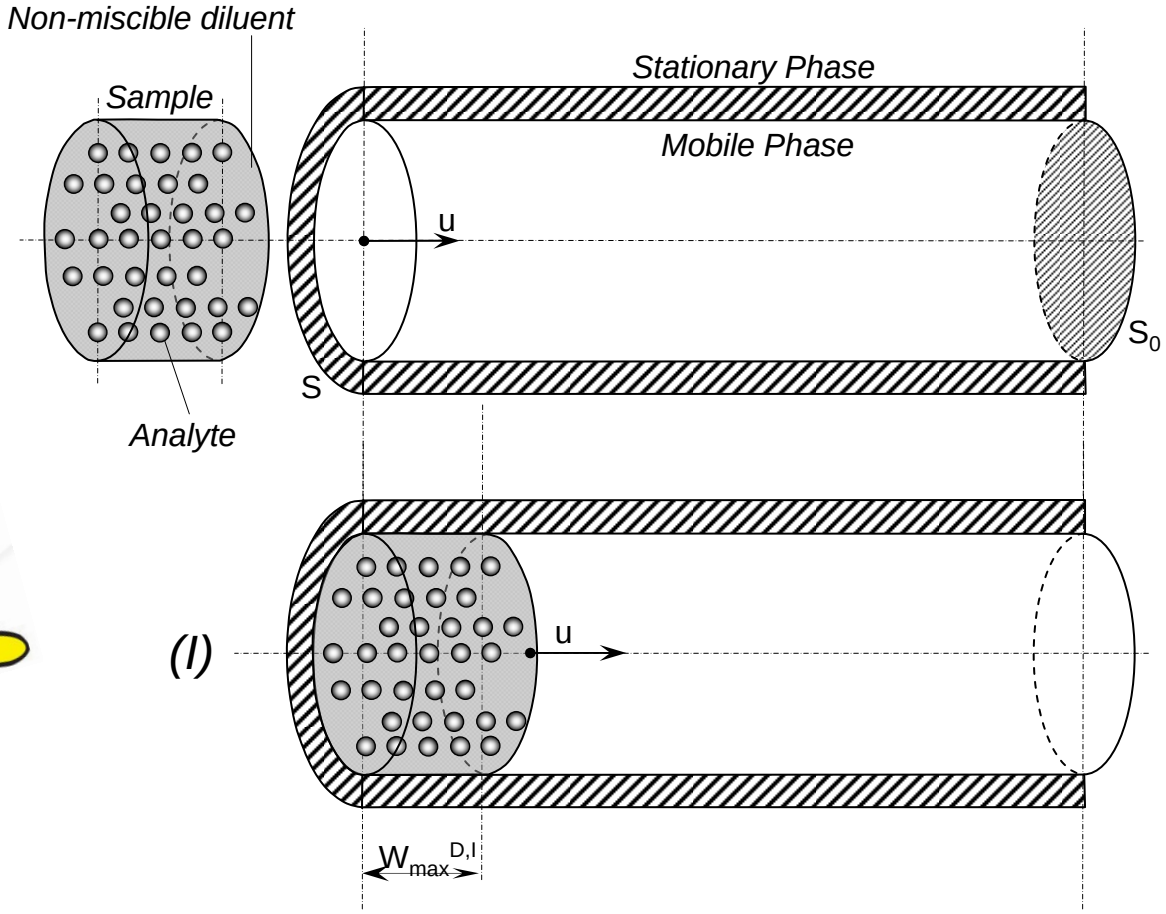


Diluent	Log P
Hexane	2.00
Heptane	**2.48**
i-Octane	2.98
Decane	3.47
Dodecane	3.96

Diluent: Heptane

An alternative model: on-line SLE / RPLC

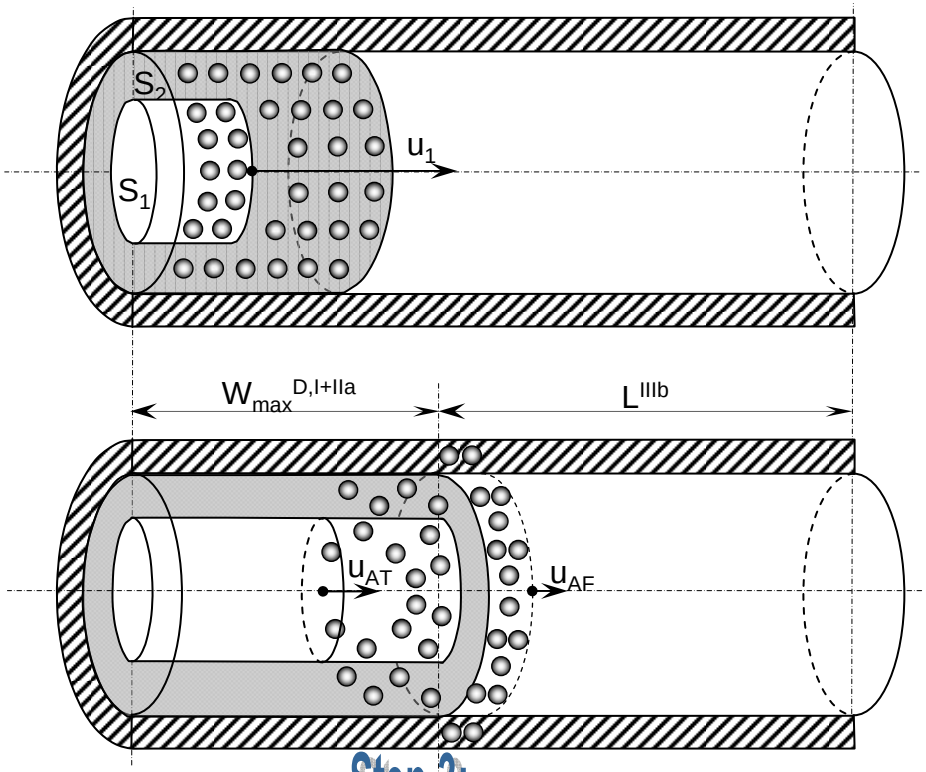




Step 1: Transport of the diluent in the column's head

Step 2:

a) running channels of the M.Ph. through D plug;
b) inflation of the D plug; c) LLE of the analyte from D to the M.Ph.



Step 3:

a) re-injection of the analyte in the head of the remaining column through D plug;
b) chromatographic separation in the remaining column.

If the following general assumptions are fulfilled ...

1. the diluent completely replaces the mobile phase during loading (no entrapping of the mobile phase between the solid phase particles arises);

2. the diluent plug remains immobile after its transport in the column's head and its inflation;

3. the reciprocal solubility of the diluent and the mobile phase should be considered as negligible;

4. the hydrophobic character of the diluent is significantly similar to the stationary phase character;

5. the model ignores the effect of the longitudinal/axial and radial/transversal mass transfer widening the analyte zone; thus, the short range (at the order of magnitude of particle size dimensions) mass transfer via radial/transversal diffusion is considered instantaneous but axial/longitudinal diffusion is considered 0;

6. the number of the mobile phase penetrating channels through the diluent is significantly similar to the number of chromatographic elution channels through the packing material;

7. the stationary phase contribution to the analyte partition in the diluent plug is negligible (the retention activity of the stationary phase is quenched by the presence of the diluent existing in a much larger amount); the contribution of the stationary phase to the dilution effect of the analyte in the diluent zone is negligible;

8. no fingering effects at the interfaces between the immiscible liquid zones were considered.

It can be demonstrated that:

$$k_{app} = \alpha\left(\frac{K}{2} - \imath k\right) + k;$$

$$\alpha = \frac{V_{inj}}{V_0}; K = \frac{[A]_D}{[A]_{M.Ph.}};$$

$$\imath = \frac{S_0}{S_2}$$

$K < 2 \times S/S_0 \times K_0$

S = S.Ph. cross section;

K_0 = chromatographic equilibrium constant

K = LLE distribution constant of A between M.Ph. and D;

α = reduced injection volume;

$\imath$ = inflation factor;

S_0 = M.Ph. cross section;

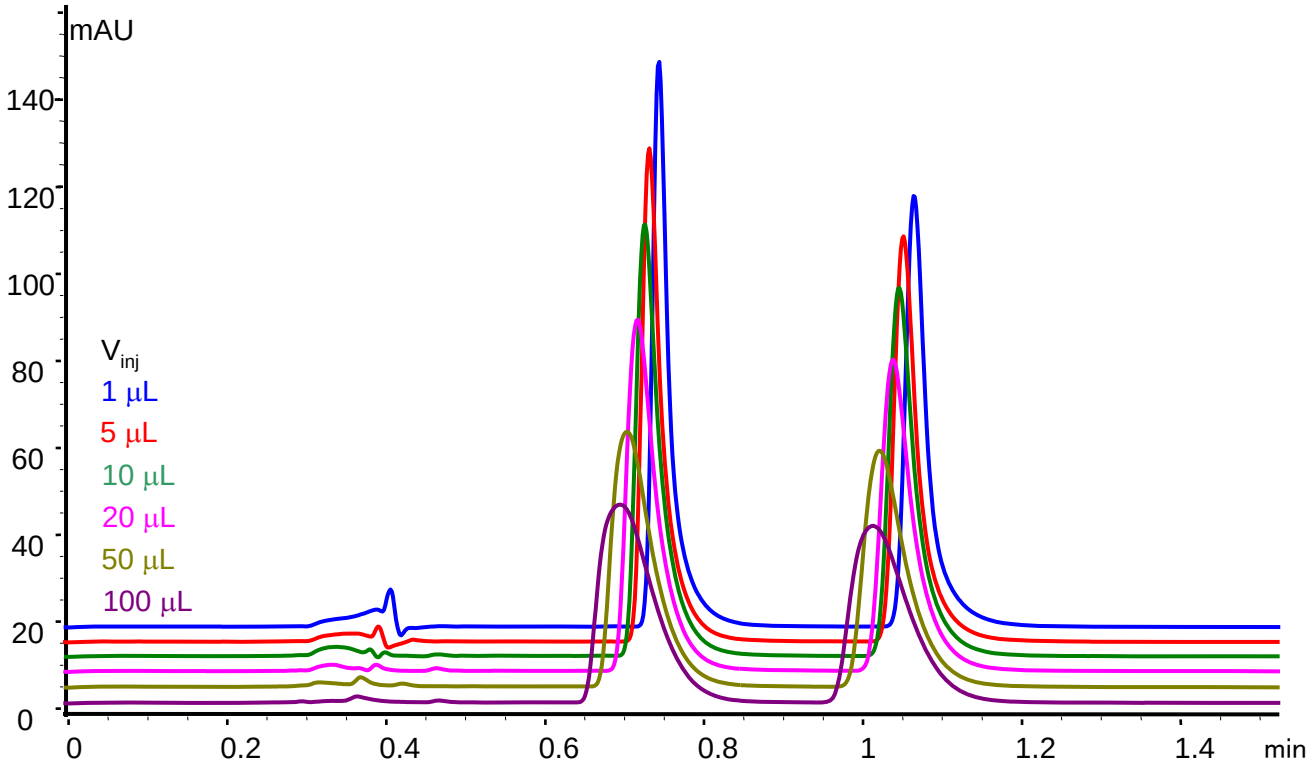
S_2 = D cross section after M.Ph. penetration through the plug;

Some numeric examples ...

	V_{inj} (µL)	k	k_{app}	K	K_0	K_0/K	ι	Mean ι	L of D plug	L'	N_{app}	N
MeP	1	1.27	1.27	0.011	28.5	2537.6	-	2.4; s = 0.33; RSD% = 13.8	0.27	49.73	**7128**	**6988**
	5		1.25				-		1.35	48.65	5757	5602
	10		1.21				2.1		2.70	47.30	5550	5087
	20		1.16				2.0		5.39	44.61	5096	3860
	50		0.98				2.1		13.48	36.52	4830	1541
	100		0.77				1.8		26.96	23.04	**4577**	**485**
BuP	1	9.04	9.06	0.144	202.3	1407.6	-		0.27	49.73	**9705**	**8596**
	5		8.92				-		1.35	48.65	9435	8181
	10		8.57				2.3		2.70	47.30	8581	8050
	20		8.06				2.4		5.39	44.61	7351	7744
	50		6.59				2.4		13.48	36.52	6717	6627
	100		4.22				2.4		26.96	23.04	**3832**	**4289**
OcP	1	141.34	139.80	2.938	3161.9	1076.3	-		0.27	49.73	**4353**	**5001**
	5		136.89				-		1.35	48.65	4542	5115
	10		131.38				3.2		2.70	47.30	4326	5015
	20		120.98				3.2		5.39	44.61	3647	4972
	50		93.47				3.0		13.48	36.52	3458	5044
	100		49.12				2.9		26.96	23.04	**1413**	**5212**

with heptane as diluent.

LVI of fasting eluting compounds dissolved in the M.Ph.



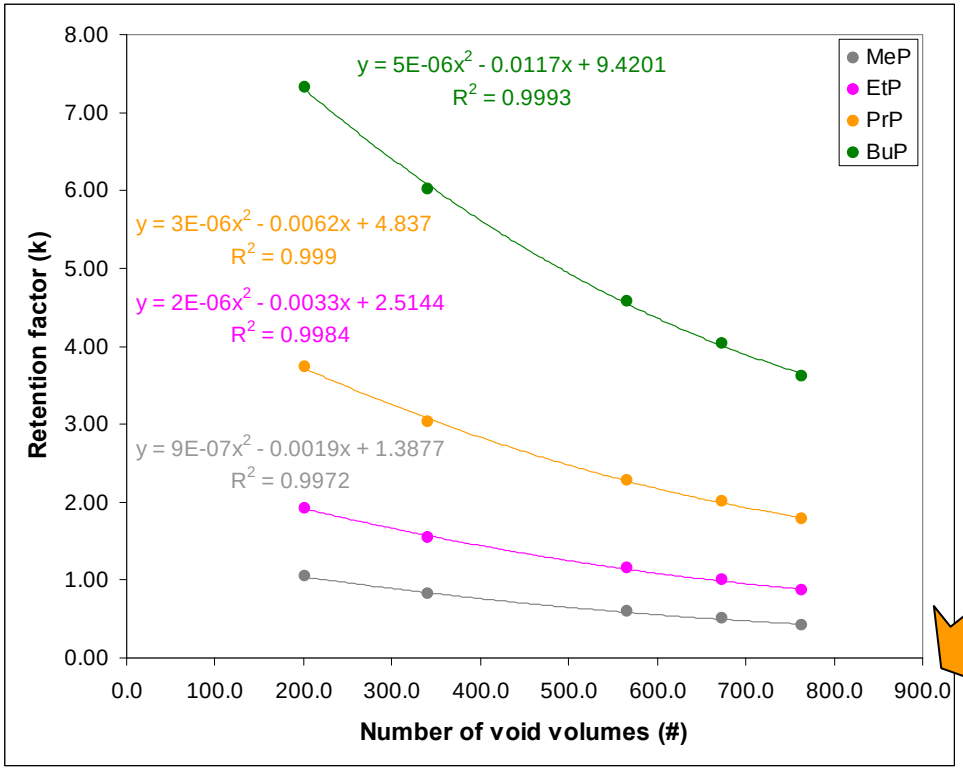
Innability of the S.Ph. to refocus analytes.

Some suspicions may however arise...

if reduction of retention is due to M.Ph. saturation with D? (diluent plug acts as a M.Ph. saturator)

a) Saturate M.Ph. with heptane;

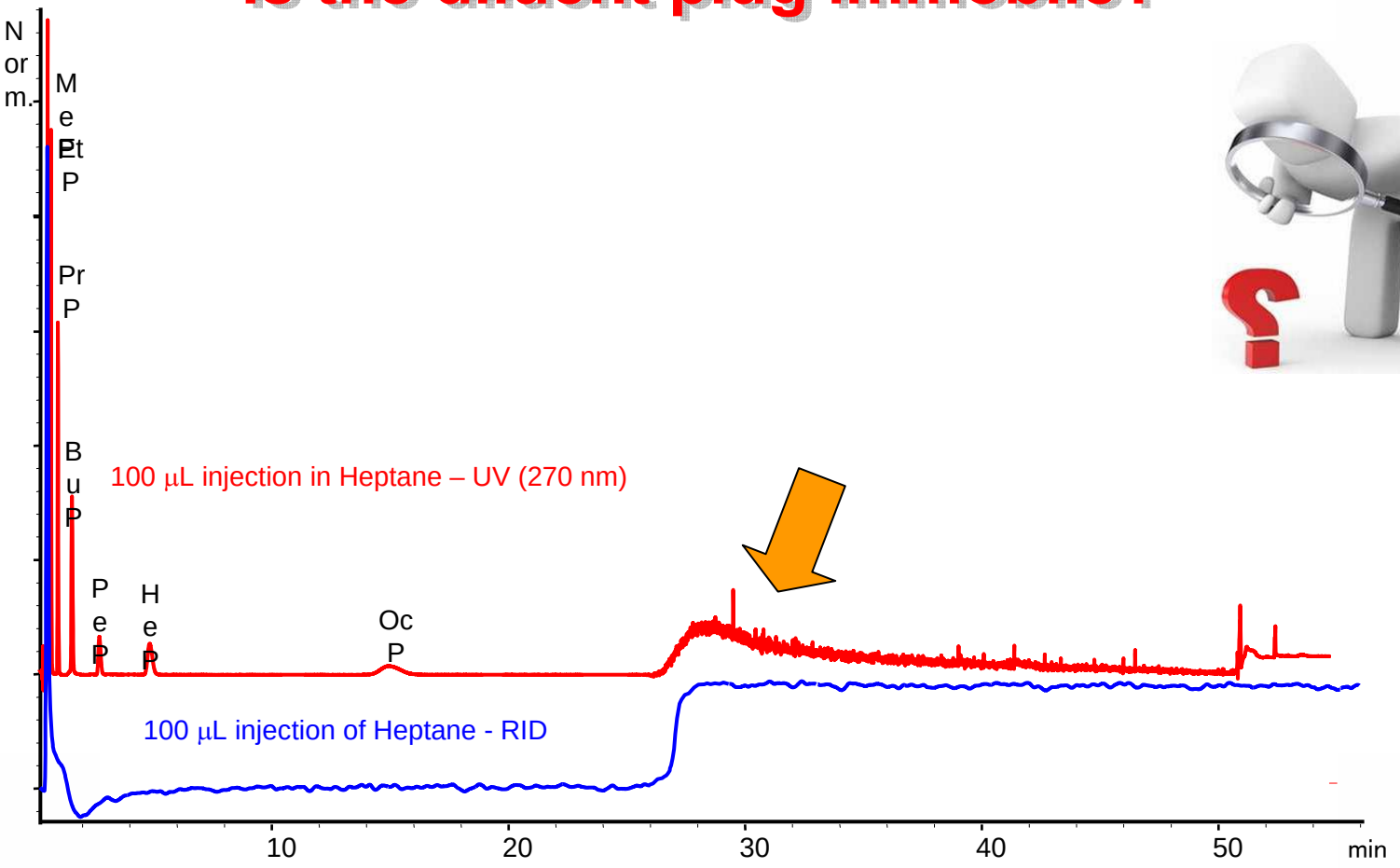
b) Observe retention on consecutive injections of 1 uL in MeOH



For the assumed on-line RP-SLE/RPLC scenario, mobile phase saturation in the diluent should not produce any measurable effects!

Some suspicions may however arise...

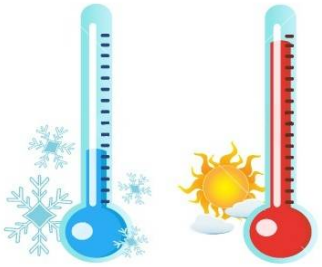
Is the diluent plug immobile?

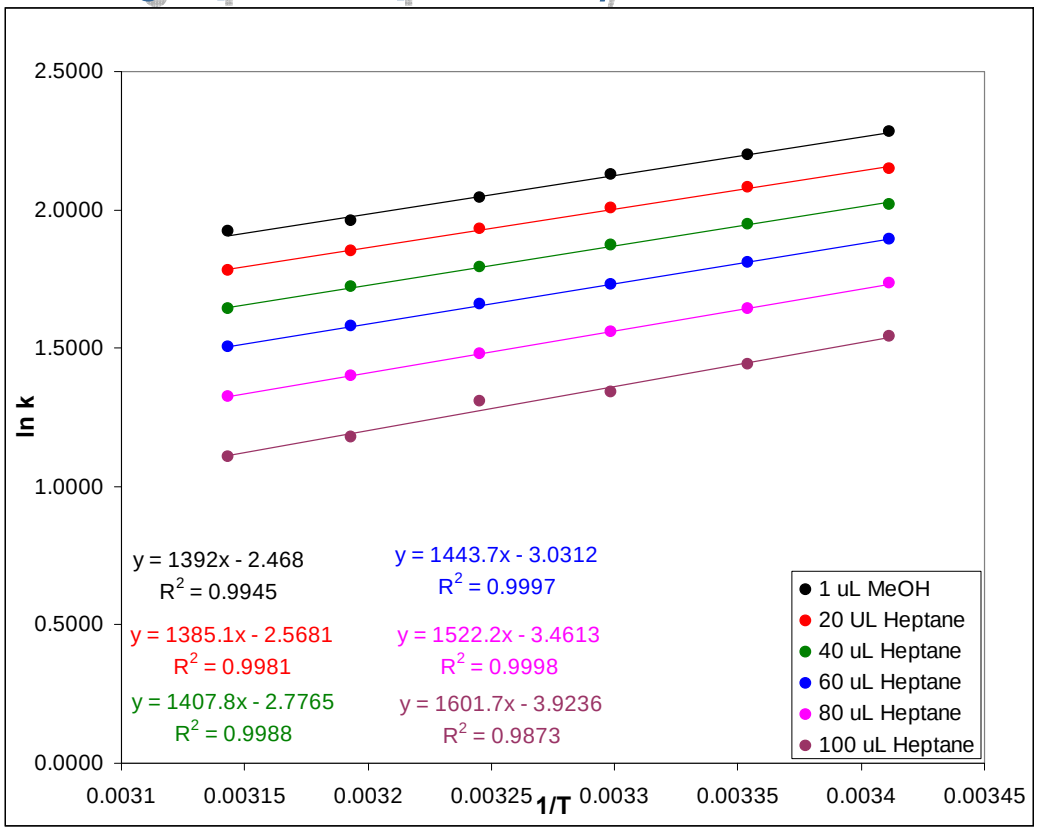


NO, it moves!

But its movement is not chromatographically based!

May the two processes (LLE & chromatographic separation) thermodinamically prooved?





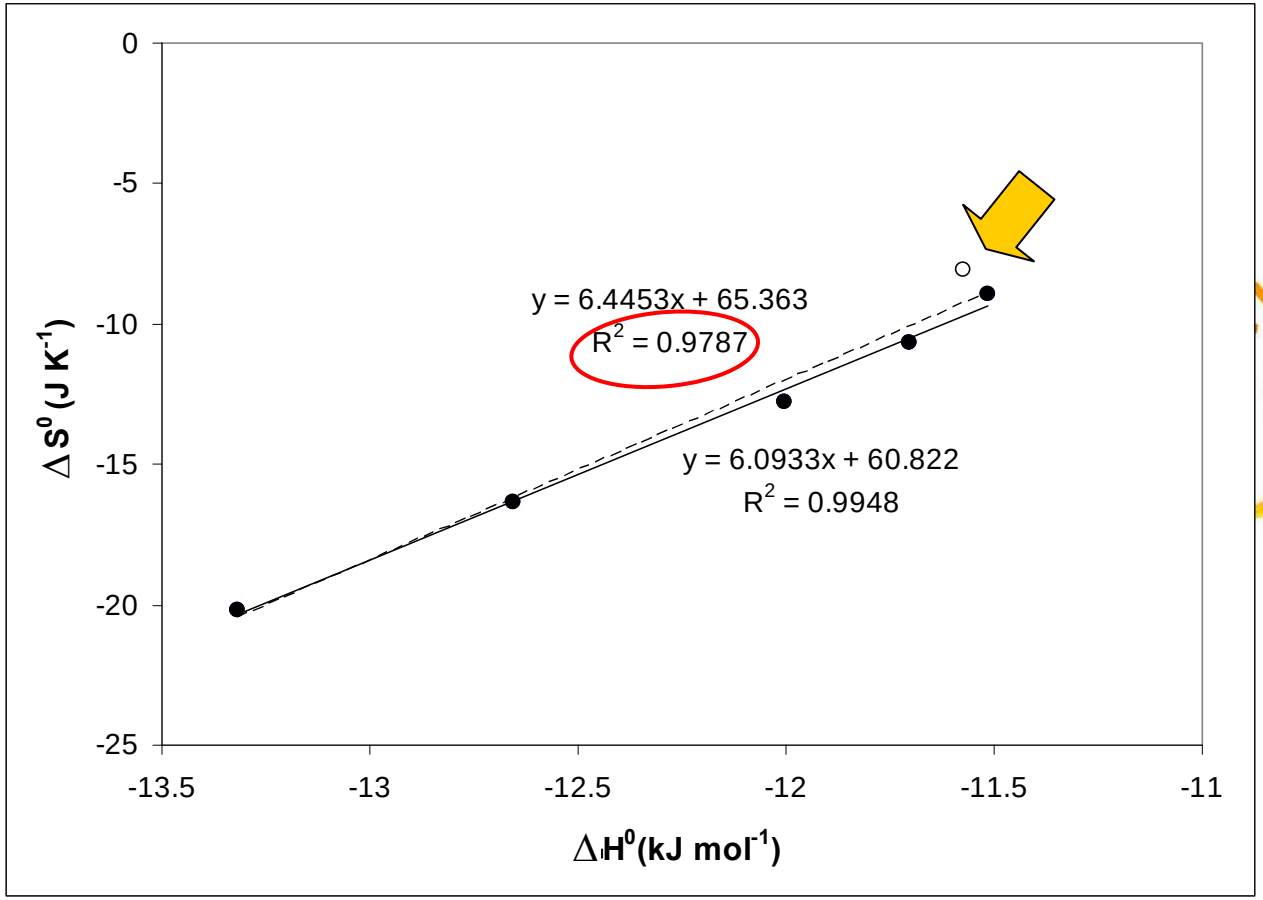
The use of the van't Hoff approach:

Analyte: BuP; Diluent: Heptane
Injected volumes: 20, 40, 60, 80 and 100 uL; additionally 1 uL in MeOH;
Temperature: 20 to 45 oC with increments of 5 oC.

van't Hoff plots are linear.

The standard enthalpies appeared at a first sight to be constant (similar slopes).

However ...



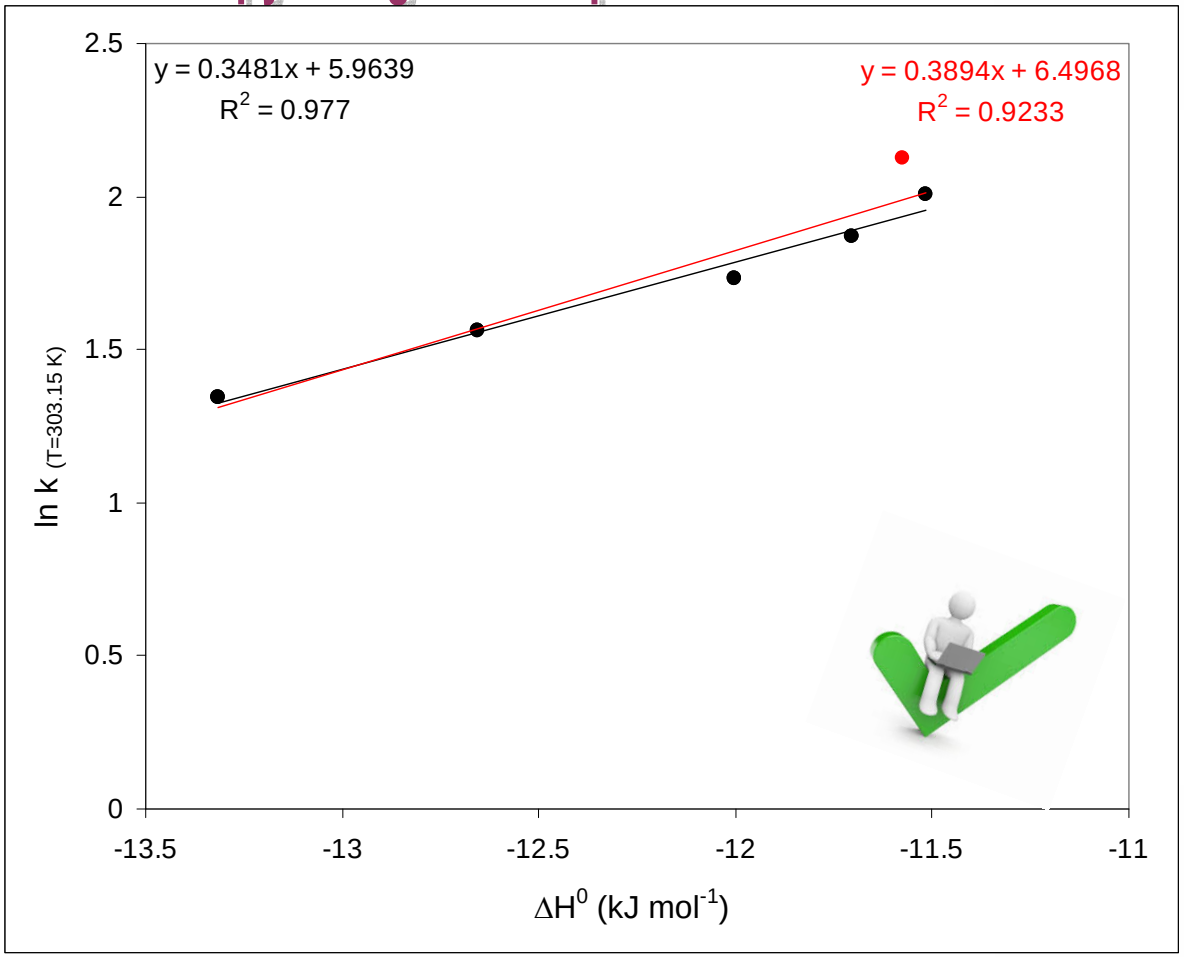
Enthalpy - entropy compensation occurs!

When considering only the chromatographic process, linearity is reduced!

When enthalpy - entropy compensation occurs, the plot ln(k) as function of the standard enthalpy at a given temperature should be linear.

The thermodynamic study sustains the existence of the LLE and the RP chromatographic distinctive steps.

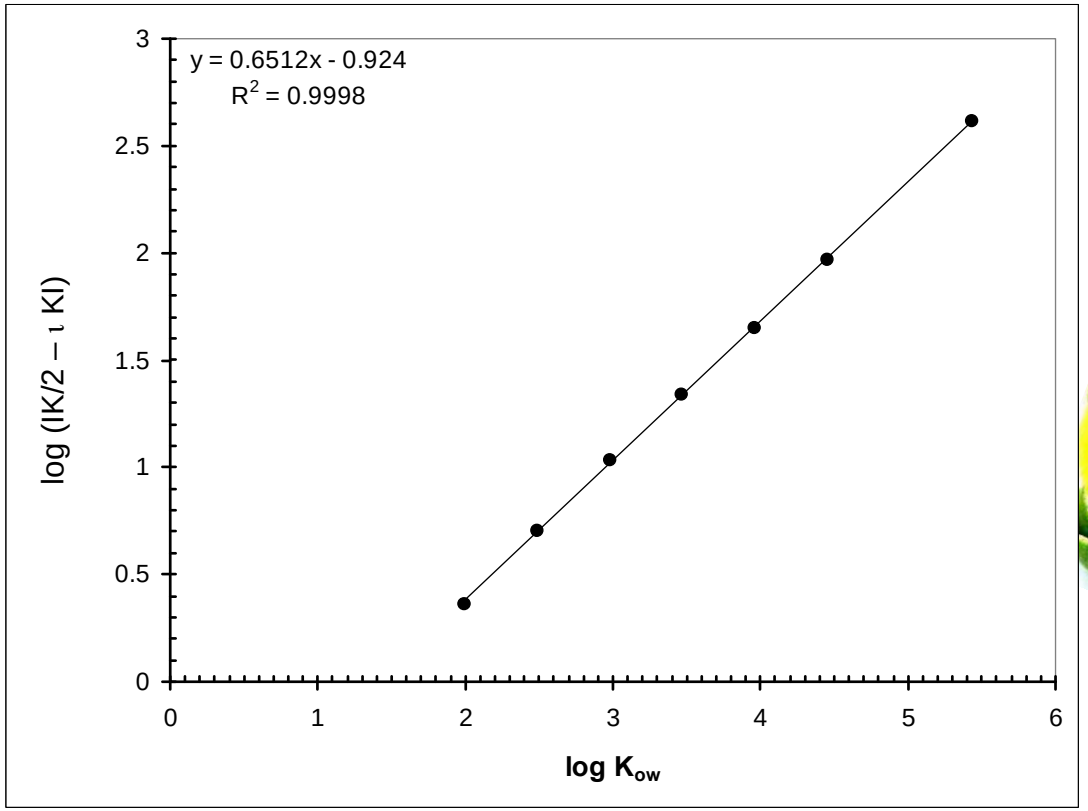
As both steps are based on similar interactions of the analyte distributed between a hydrophobic phase (D and SP) and the MP, van't Hoff plots remain linear.



Again, when considering only the chromatographic process, linearity is reduced!

Is the following statement true?

$$Log\left[abs\left(\frac{\partial k_{app}}{\partial \alpha}\right)\right] = f\left(LogP\right)$$



A new hydrophobicity descriptor is born!

To conclude:

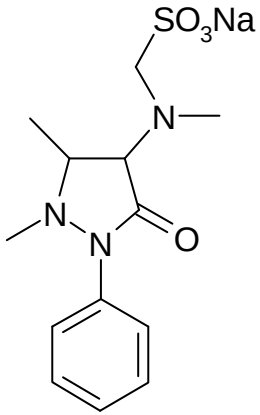
1. The on-line RP-SLE model fits better to experimental observations compared to the competitive adsorption model.

2. The non-miscibility of the diluent with the mobile phase seems to play the most important role compared to the relationship between the hydrophobic characteristics of the diluent and analytes.

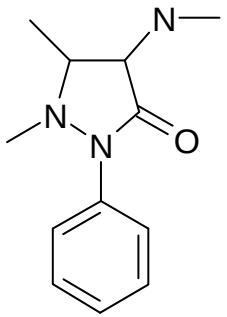
3. The kinetic of the LLE process is less important for analytes having an increased hydrophobic character, as long as the “free” stationary phase will refocus them.

4. For analytes having hydrophilic character, band compression is achieved during running M.Ph. channel formation through the diluent plug.

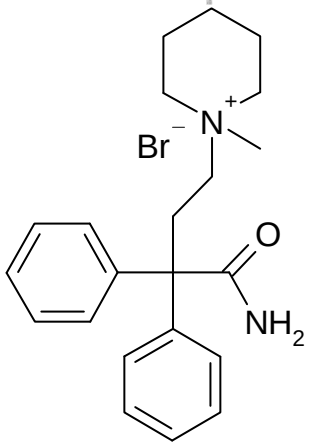
An application: solving "difficult" pharmaceutical formulations!



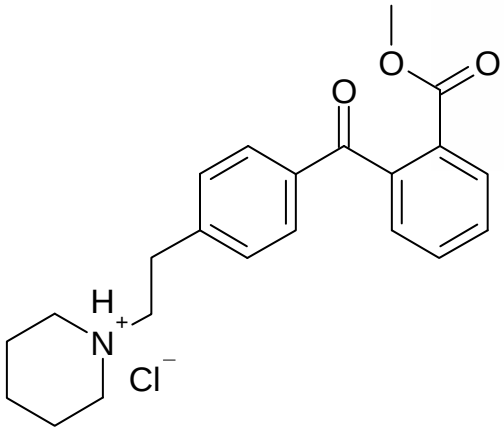
Metamizole sodium
(MTZ)
log Dow (pH=3)
-2.24
500 mg/mL
500 X dilution



Metamizole Imp. C
(MTC)
Log Dow (pH=3)
0.76
(max. 3.5% from MTZ)
(17.5 mg/mL)
25 X dilution



Fenpiverine Bromide
(FPB)
Log Dow (pH=3)
-0.56
20 ug/mL
IP-LLE+RP-SLE



Pitofenone Hydrochloride
(PTF)
Log Dow (pH=3)
0.66
2 mg/mL
25 X dilution

Polar Compounds!

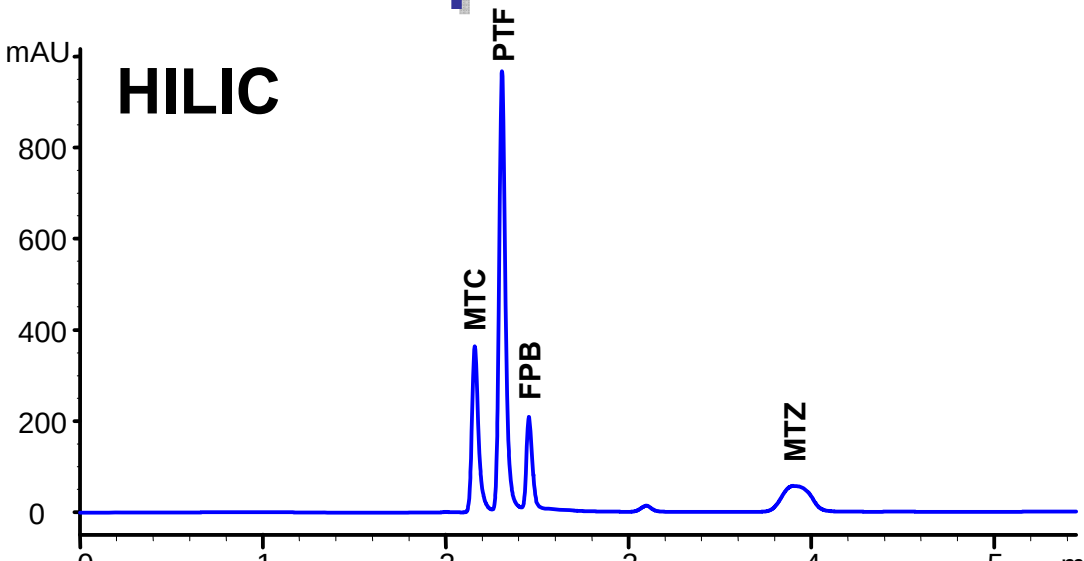
Opposite ion pairing characteristics!

Tailing favored by increased interaction to residual silanols!

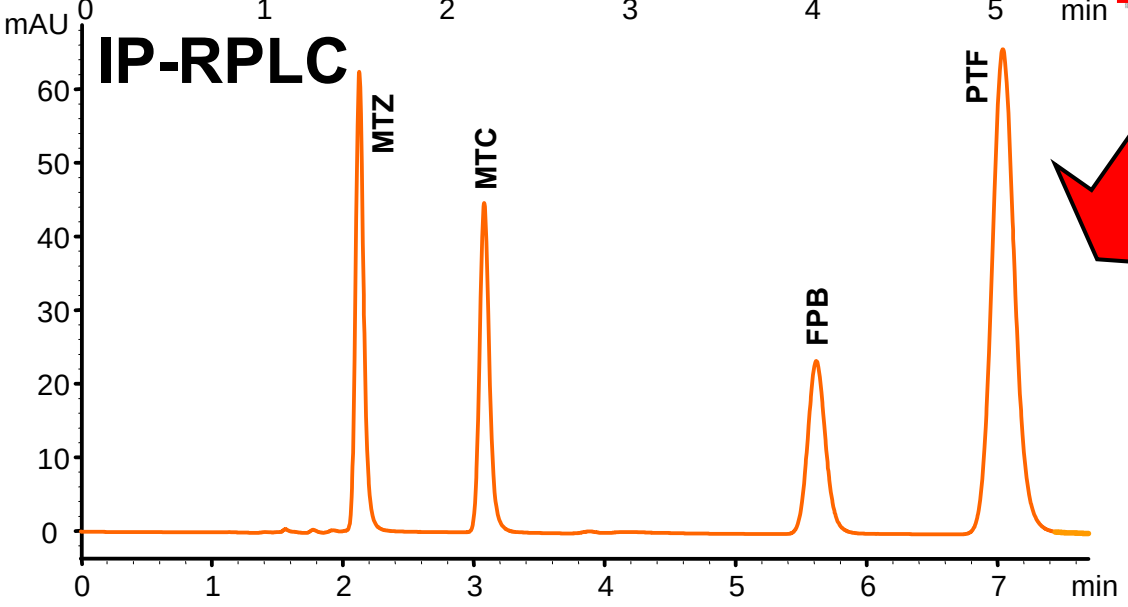
Quantitatively uncompensated mixture:
(MTZ/FPB = 1/25,000; PTF/FPB = 1/100; MTZ/PTF = 1/250)

T. Galaon, M. Radulescu, V. David, A. Medvedovici, use of an immiscible diluent in ionic-liquid / ion-pair LC for the assay of an injectable analgesic, *Cent.Eur. J. Chem.*, 10(4), 1360-1368 (2012).

Choice of a separation mechanism!



Better selectivity!



Experimental conditions:

Column: Luna C8(2): 150 mm x 4.6 mm x 5 μm;

T °C = 25 °C;

Organic modifier: MeOH;

Aqueous component:

aq. 10 mM SHS + 10 mM BMP-TFB at pH=3 with H_3PO_4 ;

Elution mode: Isocratic, Org./Aq. 48/52 (v/v)

Detection:

UV 290 nm (MTZ, MTC, PTF); UV 220 nm (FPB)

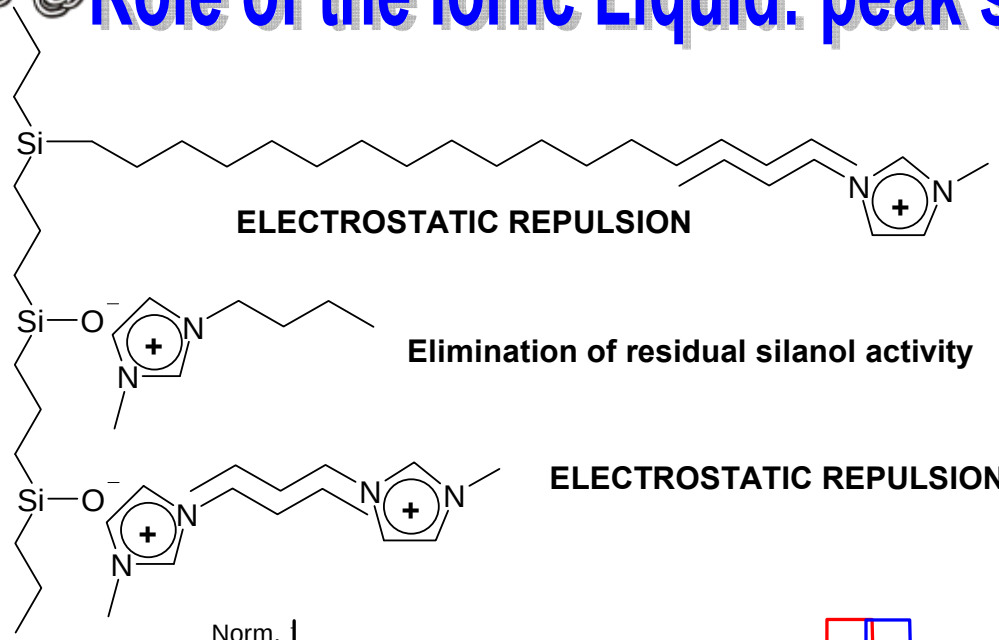
$\mathbf{V_{inj}}$ = 20 μL (for FPB);

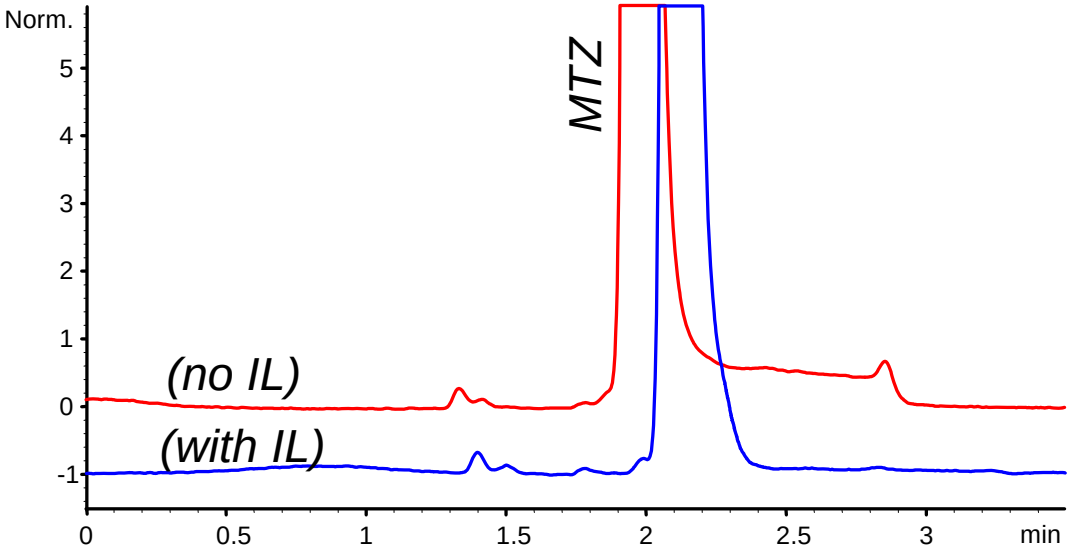
Diluent : 1-Octanol

SHS = sodium hexane sulfonate

BMP-TFB = 1-butyl 1-methyl pyrrolidinium tetrafluoroborate

Role of the Ionic Liquid: peak shape control!





Sample preparation procedure for fenpiverine bromide

Injectable Solution
Vol = 500 µL

Britton Robinson Buffer pH=10.4
Vol = 250 µL

30 mM aq. Picric Acid Solution
Vol = 250 µL

Vortex
t = 10 sec.
Speed = 2000 rpm

1-Octanol
Vol = 1000 µL

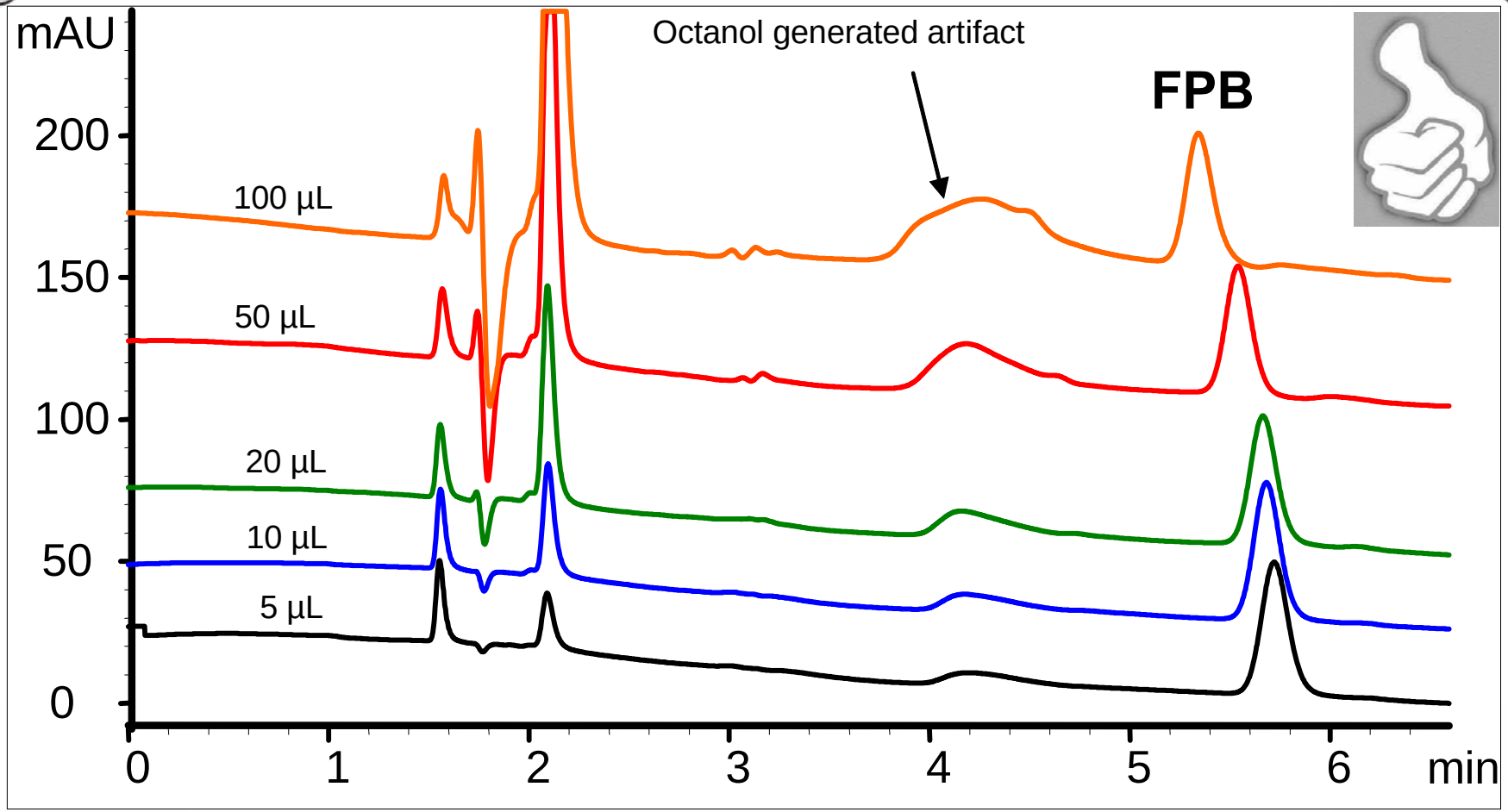
Vortex
t = 10 min.
Speed = 2000 rpm

Centrifuge
Temp.= 25°C
Time = 5 min.
Speed = 14000 rpm

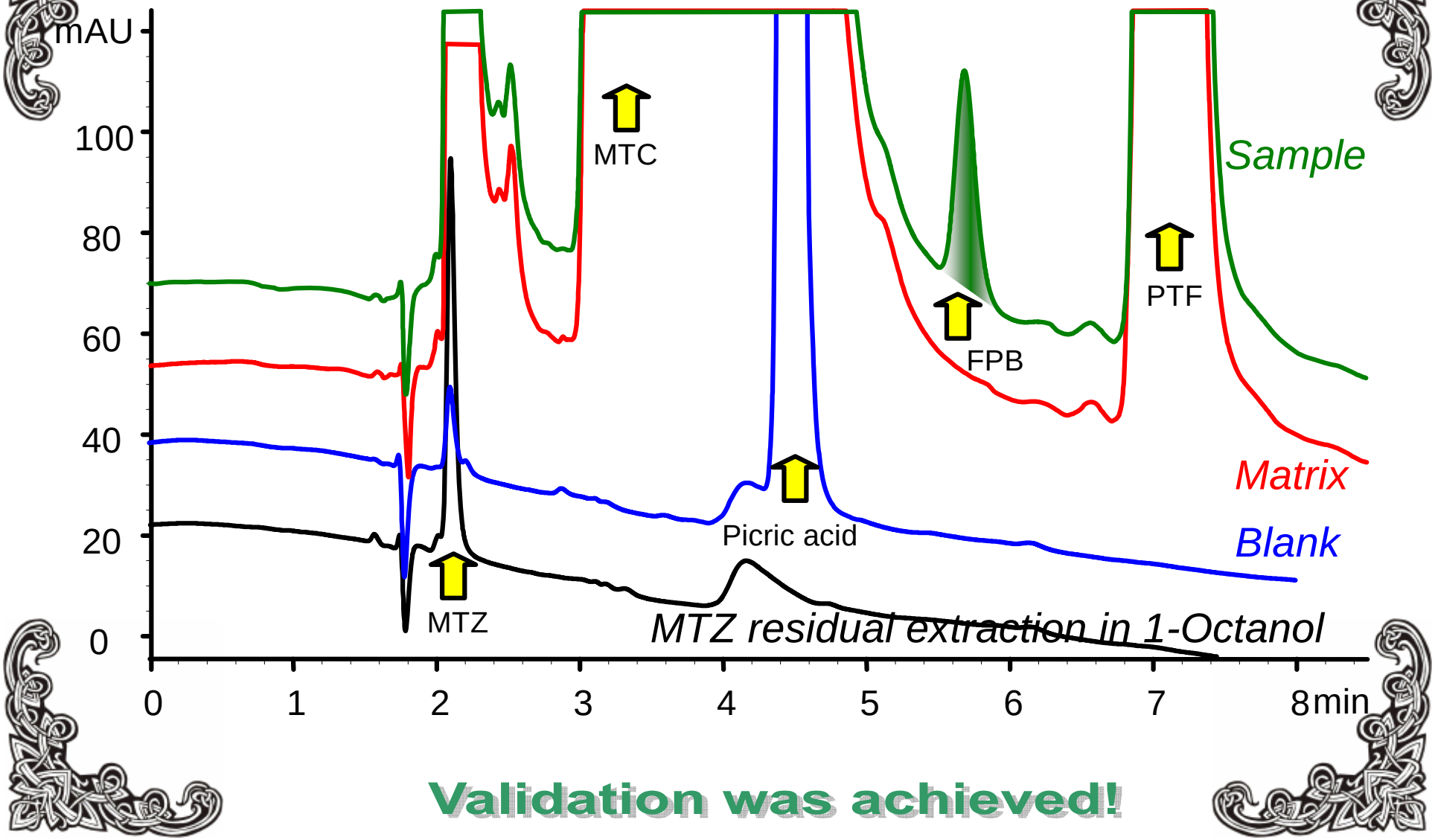
Transfer organic layer to vial
Vol = 500 µL

Inject
V_{inj} = 20 µL

On-line RP-SLE / RPLC for FPB does work?



An incurred sample!



Validation was achieved!

Final comments:

LVI of M.Ph. non-miscible diluents in RPLC is in fact an on-line RP-SLE. Although complex and difficult to be brought at a parametrization stage, the process may be successfully controlled and used as a valuable tool for enhancing on sensitivity/selectivity.

The process logically continues sample preparation „classical" procedures, offering interesting opportunities for high throughput and/or automated approaches.

V. David, M. Cheregi, A. Medvedovici, Alternative sample diluents in bioanalytical LC-MS, *Bioanalysis*, 5(24), 3051-3061 (2013).

Acknowledgements:

(≈ 50%) The financial support given by the Romanian project PNII_ID_PCE_2011_3_0152/C. no. 310/2011.

To my past & present co-workers Corina (Barcutean / Endes), Cristina (Georgita), Iulia (Sora), Florin (Albu), Stefan (Udrescu), Mihaela (Cheregi), Mona (Iorgulescu) and Florentin (Tache) for their contributions (and hard work) to the topic.