# Large Volume Injection of Sample Diluents Non-Miscible with the Mobile Phase in RPLC: Principles & Applications

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# Why do we really need LVI?


1) ↗ amount of A loaded to column ⇒ ↗ D.R.
2) ↗ amount of A loaded to column (as $c_A$ is specific to the sample) ⇒ ↗ $V_{inj}$
3) ↗ $V_{inj}$ ⇒ ↗ D.R. ⇒ ↗ Sensitivity

The simplest way to enhance on Sensitivity is to increase $V_{inj}$ !

separation | science
new horizons in chromatography and detection techniques

John Dolan's
# HPLC Solutions

## 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.**

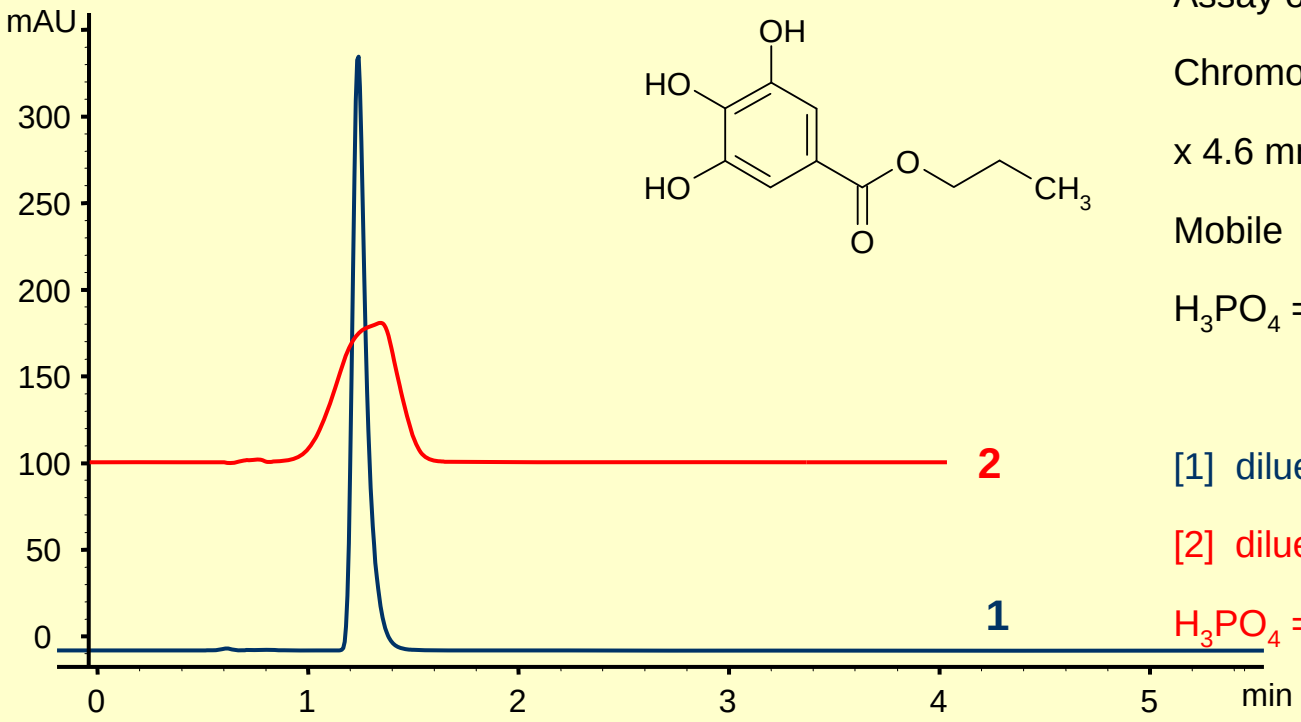
is the injection solvent much stronger than the mobile phase, but it is not miscible either. This sounds like a disaster waiting to happen – and it may be. But not so fast! If the injection volume is small enough, it may be possible to disperse the injection solvent in the mobile phase sufficiently that 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 mobile phase as a reference. Then inject 1 µL, 2 µL, 5 µL, 10 µL, 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, as in Figure 1(a). No, it's not 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.

# The Golden Rule of the Thumb:

For Large Volume Injection (LVI) in liquid chromatography the sample diluent should be **entirely miscible to** and **weaker than** the mobile phase composition at the beginning of the separation process.

# Poorly controlled LVI = Peak broadening + Peak symmetry distortions







**Diluent focusing on injection**

Assay of propylgalate:

Chromolith Performance RP-18-e, 10 cm x 4.6 mm ; 25 °C, 2 mL/min;

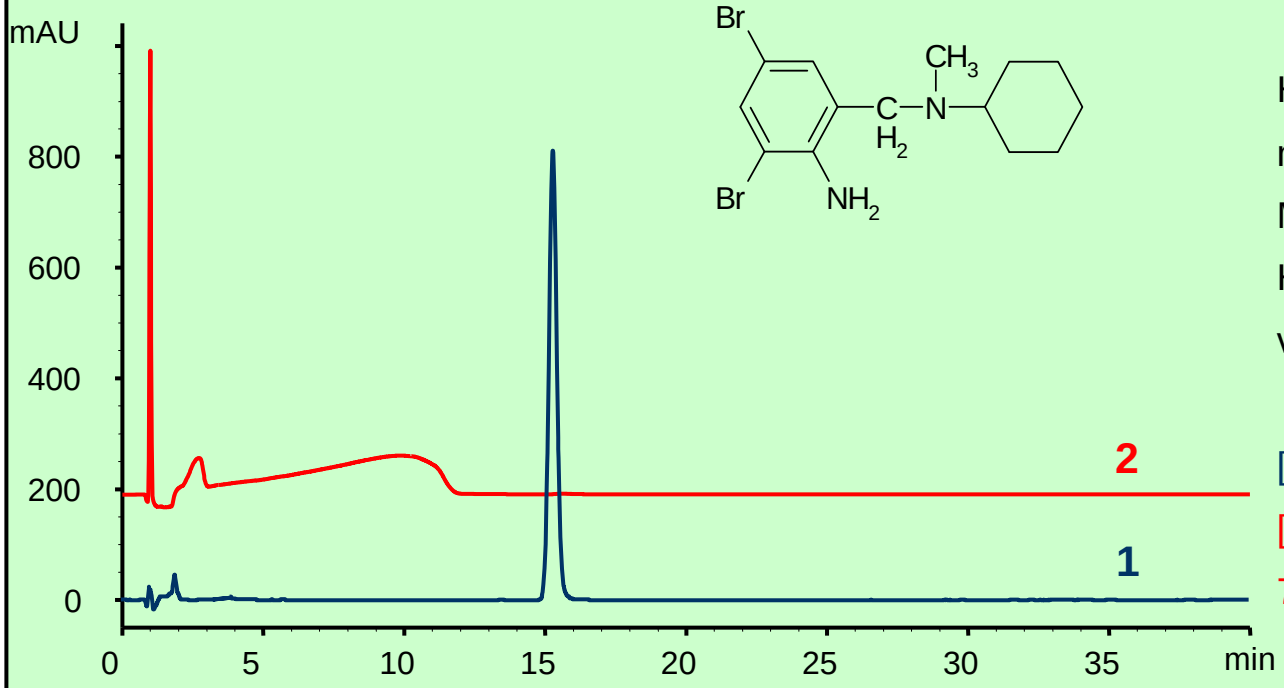
Mobile phase: MeOH / ACN / aq. 0,1% $H_3PO_4$ = 12 / 24 / 64 v/v/v;

$V_{inj}$ = 100 µL;

[1] diluent = Mobile phase;

[2] diluent = MeOH / ACN / aq. 0,1% $H_3PO_4$ = 20 / 40 / 40 v/v/v;

# Additional factors: pH focusing




**pH focusing on injection**

Assay of bromhexin:

Hypersil BDS 3-C18, 10 cm x 4.6 mm x 3 μm ; 25 °C, 1 mL/min;

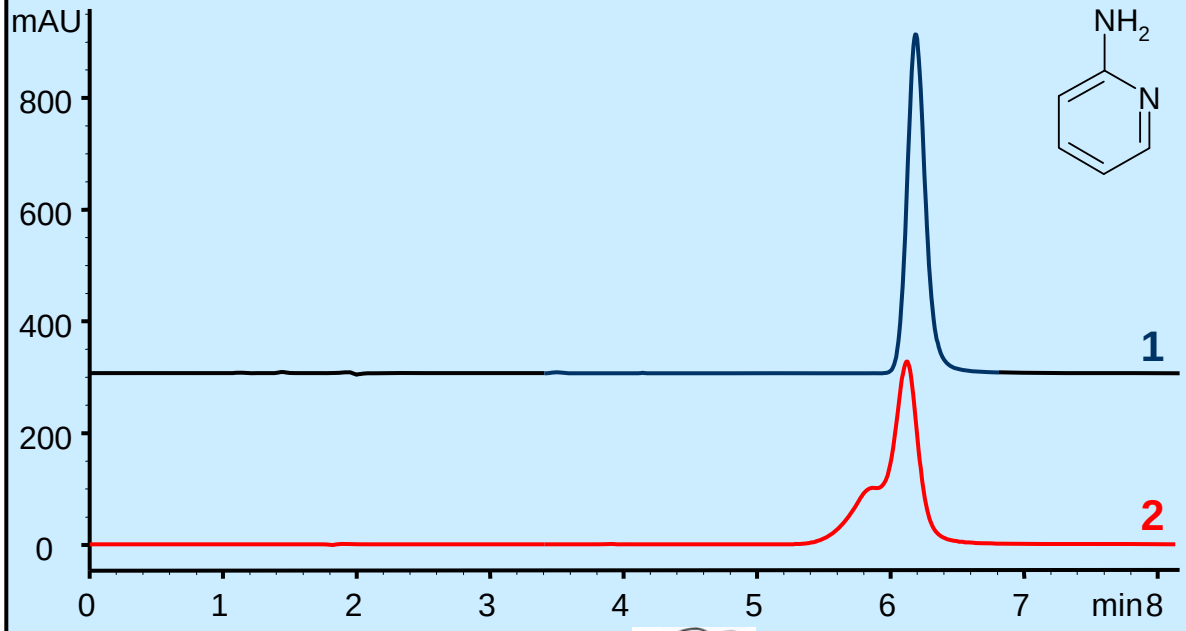
Mobile phase: ACN / aq. 0,1% $H_3PO_4$ at pH=7 with TEA : 75 / 25 v/v;

$V_{inj}$ = 5 μL;

[1] diluent = Mobile phase;

[2] diluent = ACN / aq. 3 % HCl = 75 / 25 v/v;

# Additional factors: differences with respect to the mobile phase additivation




**Focusing due to the lack of the I.P. agent**

Assay of 2-aminopyridine:

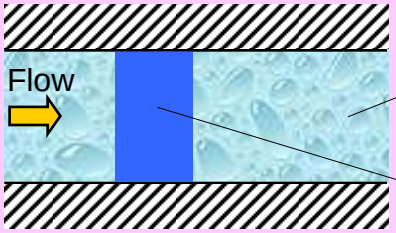
Purosphere Star-C18, 12.5 cm x 4.6 mm x 5 μm ; 25 °C, 0.7 mL/min;
Mobile phase: ACN / aq. 80 mM Sodium Octane Sulfonate at pH 2.8 with $H_3PO_4$ : 20 / 80 v/v;

$V_{inj}$ = 50 μL;

[1] diluent = Mobile phase;

[2] diluent = ACN / aq. $H_3PO_4$ at pH=2.8 : 20 / 80 v/v

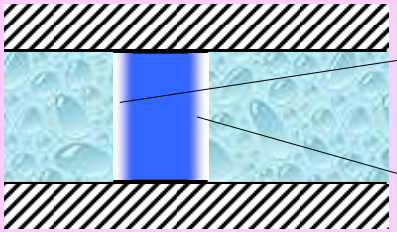
# Additional factors: Viscous fingering




Mobile phase

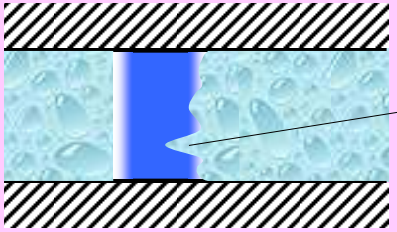
$\eta$ S > $\eta$ M.Ph.

Diluent (S) plunger



Boundary 1: ↗ $\eta$ gradient

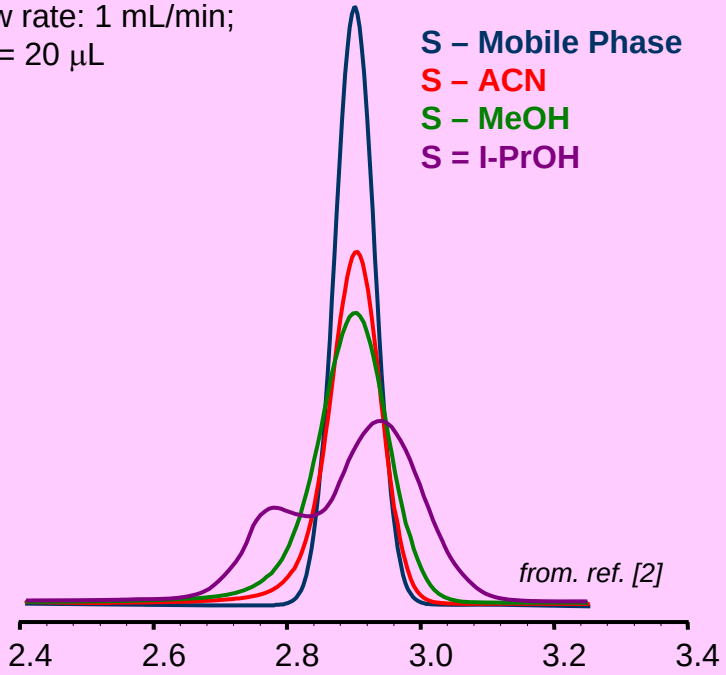
Boundary 2: ↘ $\eta$ gradient



Fingering effect

1-Phenyl-2-butanol (k=1.25)

Zorbax Eclipse XDB-C8 (150 mm x 4.6 mm x 5 μm);
T°C = 15 °C;
Mobile Phase: ACN / water : 60:40 (v/v);
Flow rate: 1 mL/min;
$V_{inj}$ = 20 μL

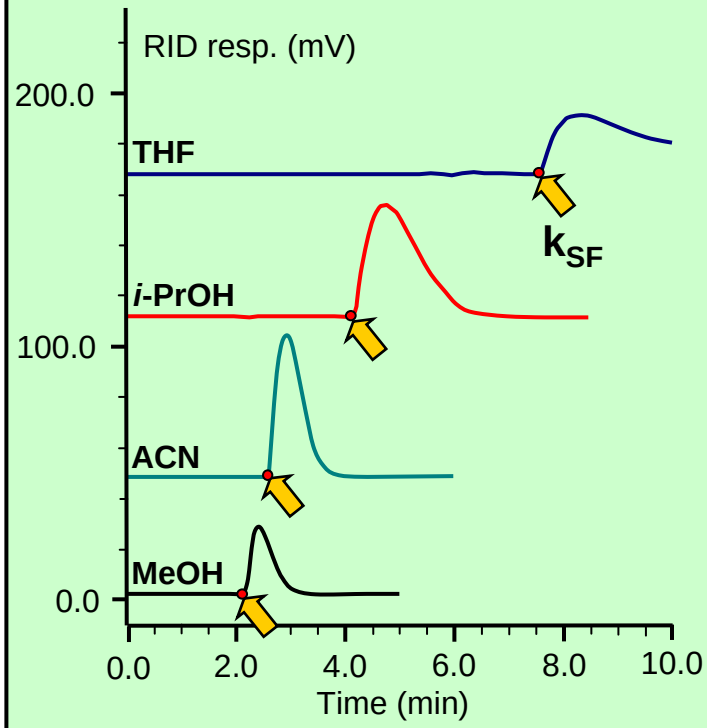


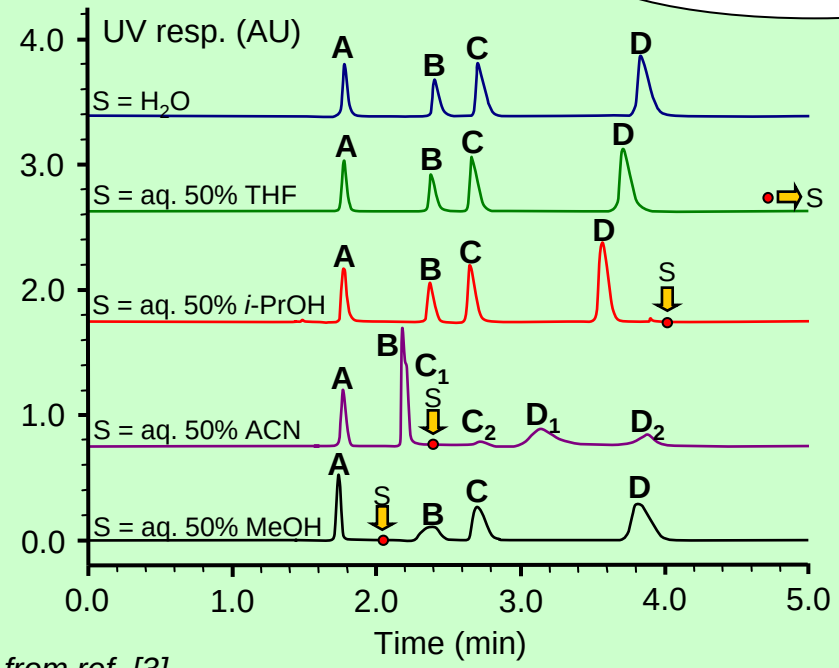

[1] D. Cherrak et al., Viscous fingering: A systematic study of viscosity effects in methanol-isopropanol systems, *Chromatographia*, 46(11-12), 647-654 (1997).

[2] S. Keunchkarian et al., Effect of sample solvent on the chromatographic peak shape of analytes eluted under RPLC conditions, *J. Chromatogr. A*, 1119, 20-28 (2006).

# First step forward: using strong injection solvents with 100% aqueous mobile phases

$$k_{SF} = (t_R^{SF} - t_0) / t_0$$



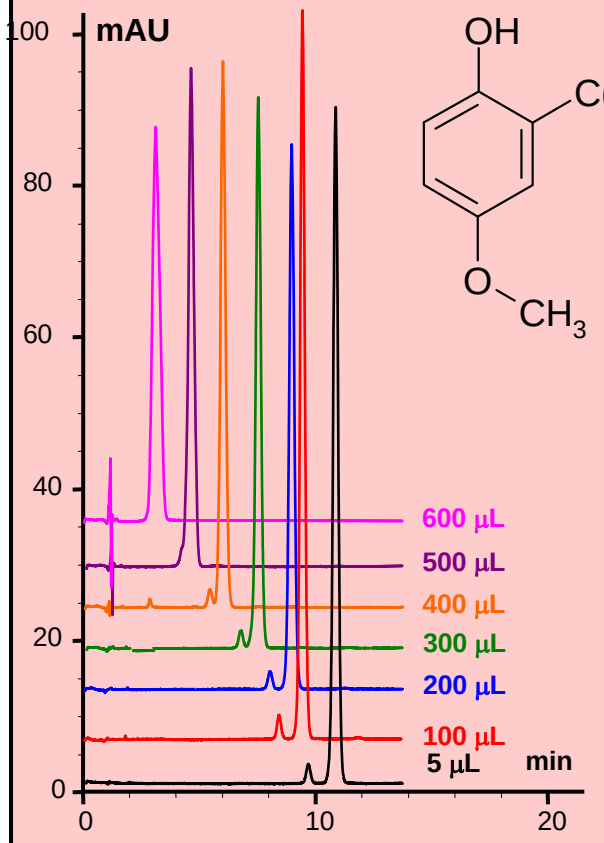

*from ref. [3]*

**A** – Tartaric Acid; **B** – Malonic Acid; **C** – Acetic Acid; **D** – Maleic Acid.

Column: YMC ODS-Aq C18 150 mm x 4.6 mm x 5 μm; T°C = 35 °C; Flow rate = 1.25 mL/min; UV – 205 nm


[3] E. Loesser et al., Using strong injection solvents with 100% aqueous mobile phase in RPLC, *J. Sep. Sci.*, 29, 2847-2852 (2006).

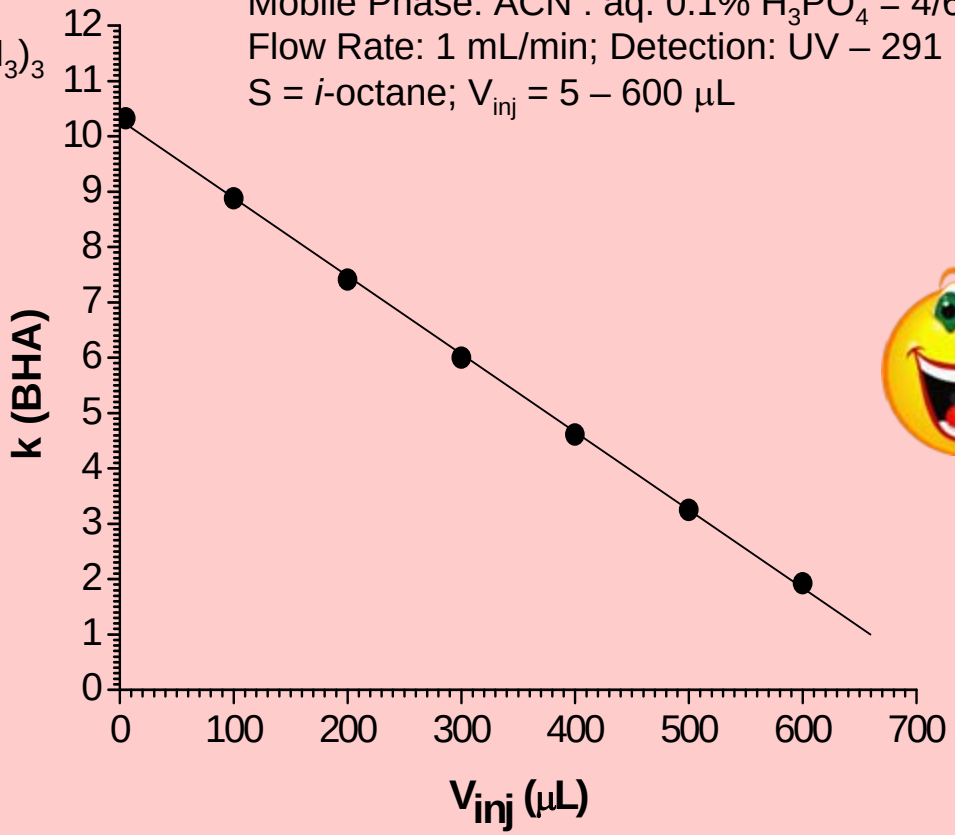
# Second step forward: an application for assaying BHA in pharmaceutical formulations of statines





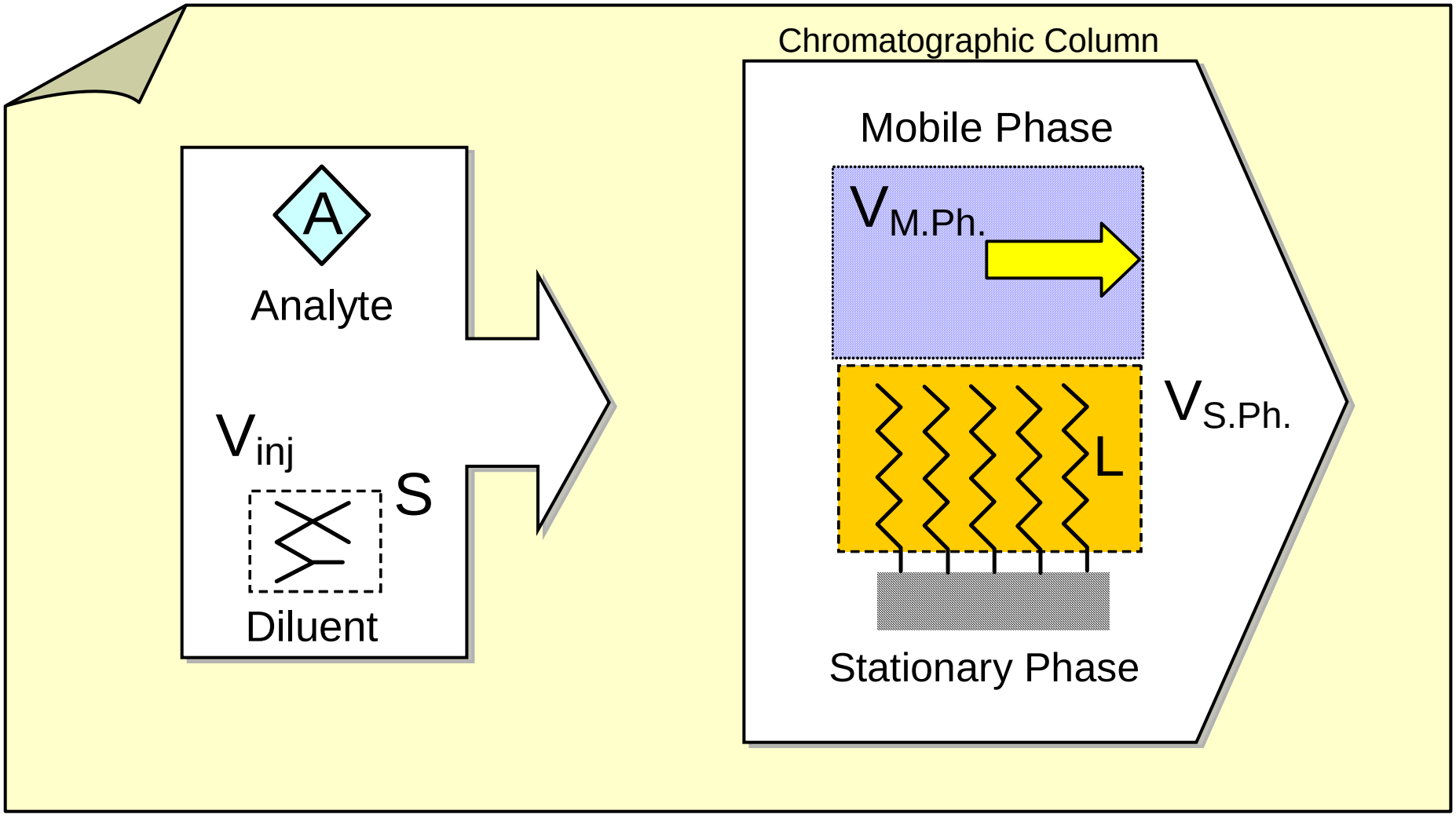


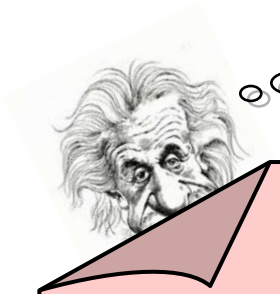
Column: Zorbax Eclipse C-8 150 mm x 4.6 mm x 5 µm;
Mobile Phase: ACN : aq. 0.1% $H_3PO_4$ = 4/6 (v/v);
Flow Rate: 1 mL/min; Detection: UV – 291 nm;
S = *i*-octane; $V_{inj}$ = 5 – 600 µL




[4] V. David, C. Barcutean, C. Georgita, A. Medvedovici, Non-miscible solvent LVI-HPLC/DAD method for determination of butylated hydroxyanisole in lovastatin and simvastatin pharmaceutical formulations, *Rev. Roum. Chim.*, 5, 445-451 (2006).

# To resume - the actors are:





Screenplay v.1:
Partition

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

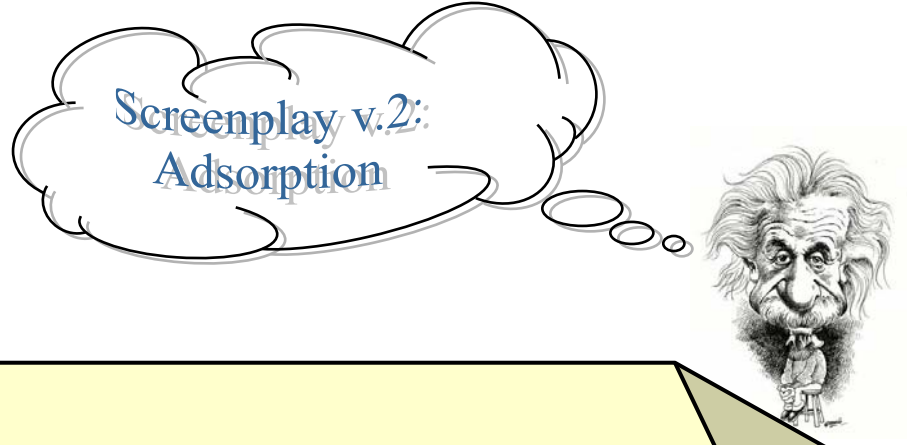
$$k_A = K_A \times V_{S.Ph.} / V_{M.Ph.}$$

*S is practically totally partitioned in the S.Ph. and exhibits similar properties; consequently:*

$$V_{S.Ph.}^{real} = V_{S.Ph.} + V_{inj}$$

$$k_A = K_A \times (V_{S.Ph.} + V_{inj}) / V_{M.Ph.}$$

*if* $V_{inj} \nearrow$ *than* $k_A \nearrow$




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

$A_{(M.Ph.)} + L_{(S.Ph.)} \leftrightarrows A^*L_{(S.Ph.)}$ [2] $K_A = [A^*L]_{S.Ph.} / ([A]_{M.Ph.} \times [L]_{S.Ph.})$

*if assuming* $[S] >> [A]$

$i\ S_{(M.Ph.)} + L_{(S.Ph.)} \leftrightarrows S_i^*L_{(S.Ph.)}$ [3] $K_S = [S_i^*L]_{S.Ph.} / ([S]^i_{M.Ph.} \times [L]_{S.Ph.})$

*if assuming* $K_{ow}^S > K_{ow}^A$ *and* $K_A = \gamma_1 \times K_{ow}^A$; $K_S = \gamma_2 \times K_{ow}^S$

*than*

[3] $\longrightarrow$ ; [2] $\rightleftharpoons$ ; [1] $\longrightarrow$ ;

$[A^*L]_{S.Ph.} / [A]_{M.Ph.} = k_A \times V_{M.Ph.} / V_{S.Ph.}$; $k_A = [(\gamma_1 \times K_{ow}^A \times [L]_{S.Ph.}) / V_{M.Ph.}] \times V_{S.Ph.}$;

*but a volume* $\Delta V_{S.Ph.}$ *is available only for* S; $\Delta V_{S.Ph.} = \gamma_3 \times V_{inj}$; *consequently*$\Rightarrow$

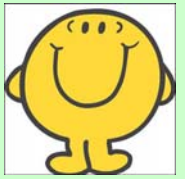
$k_A = \theta \times (V_{S.Ph.} - \Delta V_{S.Ph.}) = \theta \times V_{S.Ph.} - \theta \times \gamma_3 \times V_{inj} = \alpha - \beta \times V_{inj}$

$$k_A = \gamma_1 \times K_{ow}^{A} \times [L]_{S.Ph.} \times \frac{V_{S.Ph.}}{V_{M.Ph.}};$$

$$[L]_{S.Ph.}^{tot} = [L]_{S.Ph.} + [A * L]_{S.Ph.} + [S_i * L]_{S.Ph.};$$

*if assuming that* $[A * L]_{S.Ph.} << [L]_{S.Ph.} + [S_i * L]_{S.Ph.}$

$$[L]_{S.Ph.} = [L]_{S.Ph.}^{tot} - [S_i * L]_{S.Ph.}; \quad [L]_{S.Ph.}^{tot} = \frac{n_L^{tot}}{V_{S.Ph.}} = \frac{\rho_L}{M_w^L};$$



$$[S_i * L]_{S.Ph.} = \frac{[S]_{S.Ph.}}{i} = \frac{V_{inj} \times \rho_S}{i \times M_w^S \times V_{S.Ph.}};$$

$$k_A = \gamma_1 \times K_{ow}^{A} \times \frac{\rho_L \times V_{S.Ph.}}{M_w^L \times V_{M.Ph.}} - \gamma_1 \times K_{ow}^{A} \times \frac{\rho_S \times V_{inj}}{i \times M_w^S \times V_{M.Ph.}};$$

*for* $k_A = 0$ *(at* $V_{inj} = V_{inj}^{k_A=0}$*)* $\quad i = \frac{M_w^L \times \rho_S}{M_w^S \times \rho_L} \times \frac{V_{inj}^{k_A=0}}{V_{S.Ph.}}$; *q.e.d.!*

[5] 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).

Addendum

# The remaining Diluent plug ?!

# Rule of five for LVI in diluents non-miscible with the M.Ph.

1. D has increased chromatographic retention compared to target compounds ($k_{SF} > k_A$);

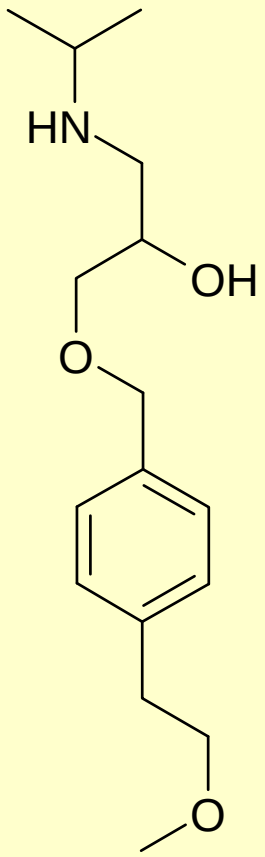
2. Solubility of D in the M.Ph. is low enough to force the saturation of the S.Ph. with D immediately after injection;

3. Fingering effects due to different viscosities (D vs. M.Ph.) are controlled;

4. D plug from a previous injection is already eliminated from the column before starting a new separation process;

5. The initial chromatographic resolution supports the "apparent" reduction of the column length (affecting selectivity).

# Verifying the theory:

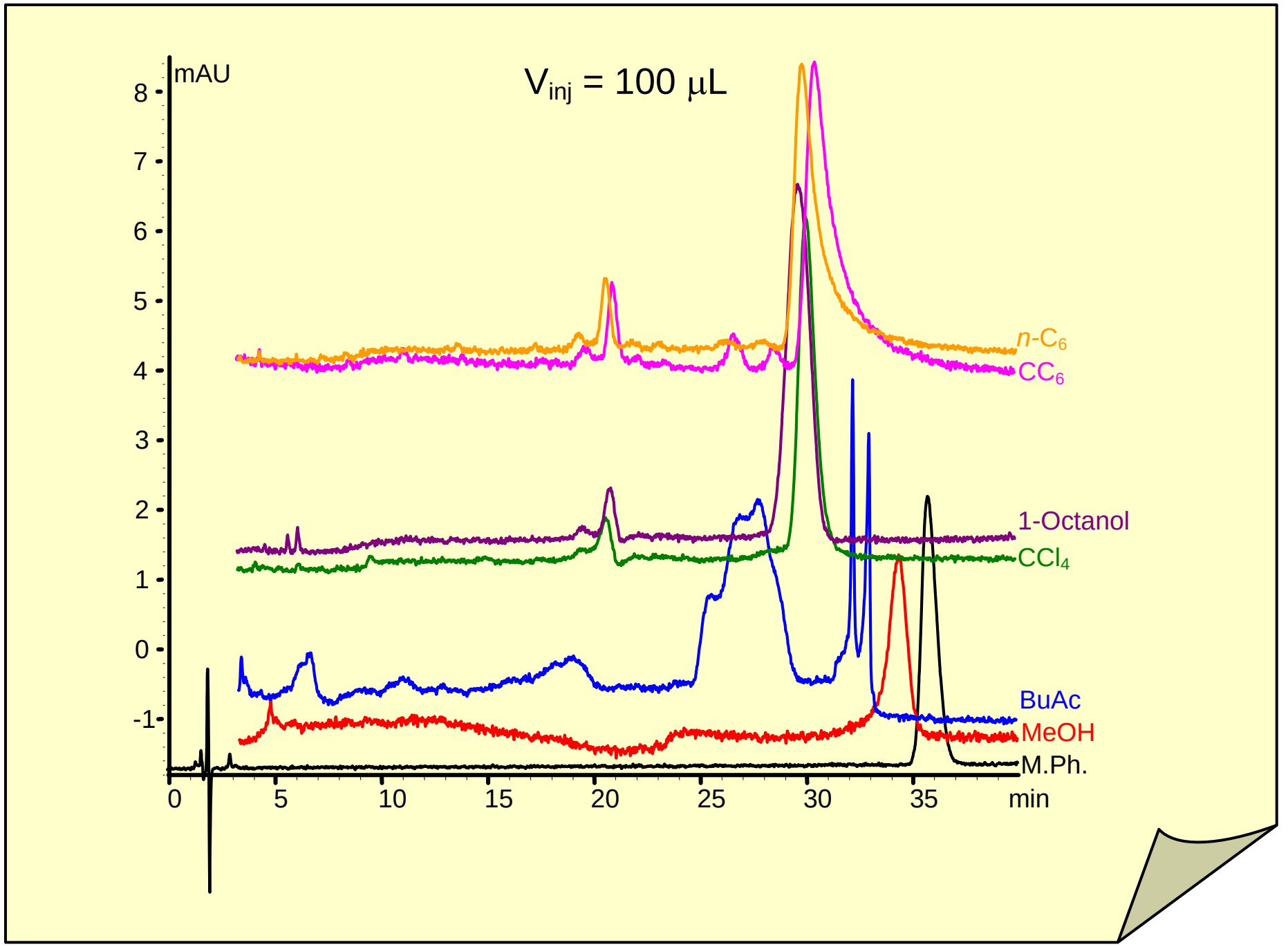


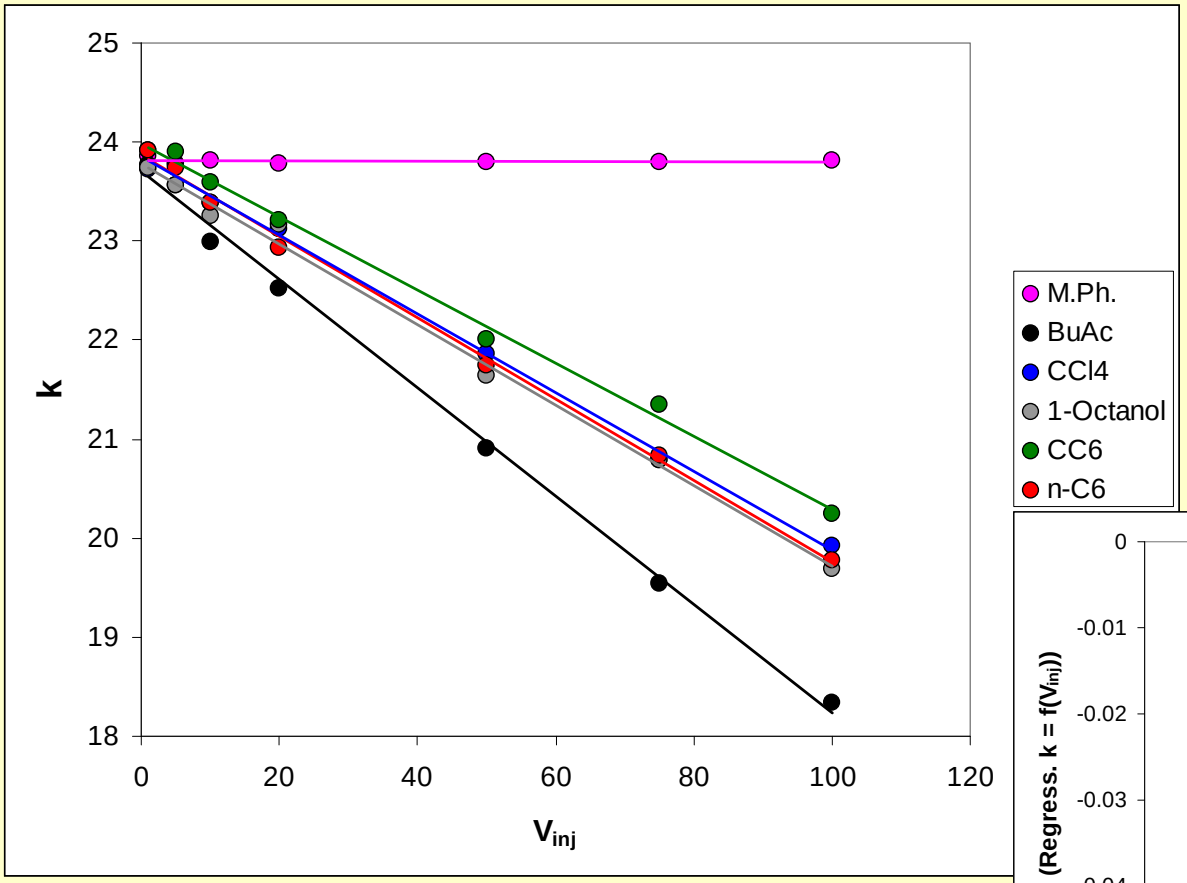

Solute: Metoprolol (log P = 1.88)

Diluents:
Methanol (log P = -0.77)
Butyl acetate (log P = 1.78)
Carbon tetrachloride (log P = 2.83)
1-Octanol (log P = 3.00)
Cyclohexane (log P = 3.44)
n-Hexane (log P = 3.9)
Injection Volumes: 1, 5, 10, 20, 50, 75, 100 μL

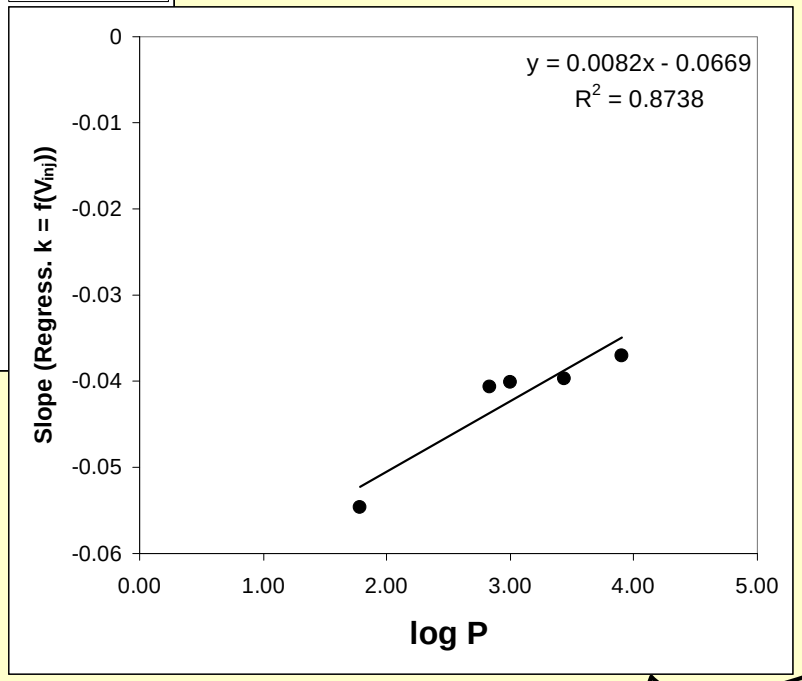
Chromatographic Columns:
Zorbax XDB C-18 (150 mm x 4.6 mm x 5 μm);
Chromolith Performance C-18 (100 mm x 4.6 mm);
C-18 Stable Bond AQ (150 mm x 4.6 mm x 5 μm);
Betasyl Phenyl (150 mm x 4.6 mm x 5 μm);

Mobile Phase:
Isocratic Elution
Organic Solvent: ACN
Aqueous Solvent: 50 mM HCOONa + 0.2% TEA at pH = 3.5 with HCOOH
Composition: Organic / Aqueous Solvents = 10/90 (v/v)

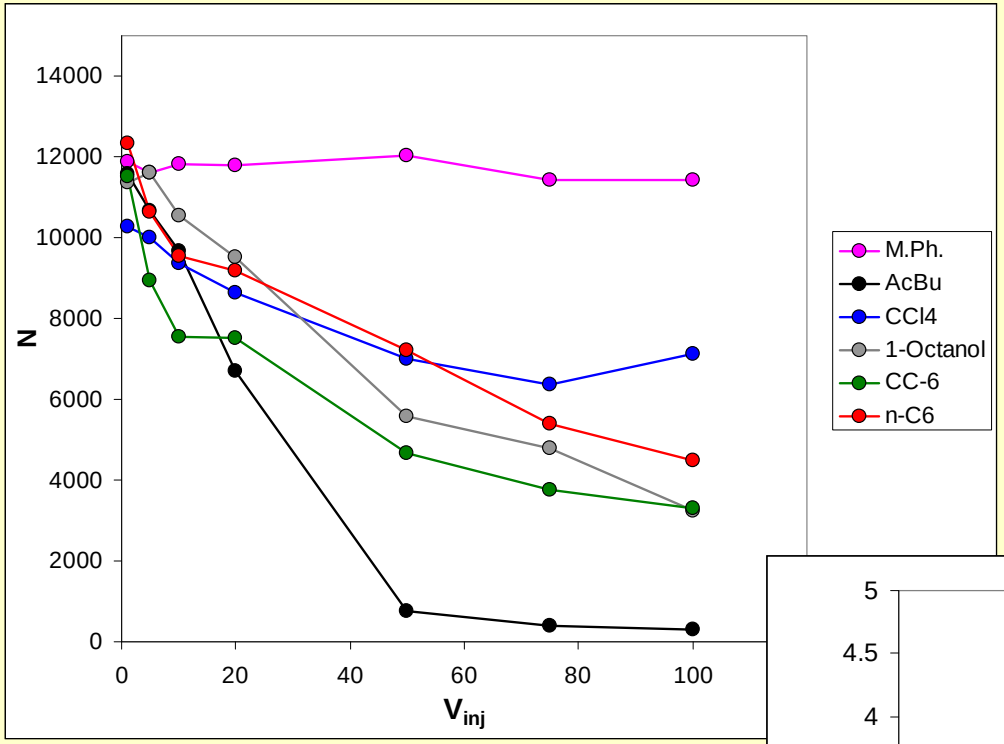

mAU
$V_{inj}$ = 100 µL
8
7
6
5
4
3
2
1
0
-1
0
5
10
15
20
25
30
35
min
*n*-$C_6$
$CC_6$
1-Octanol
$CCl_4$
BuAc
MeOH
M.Ph.



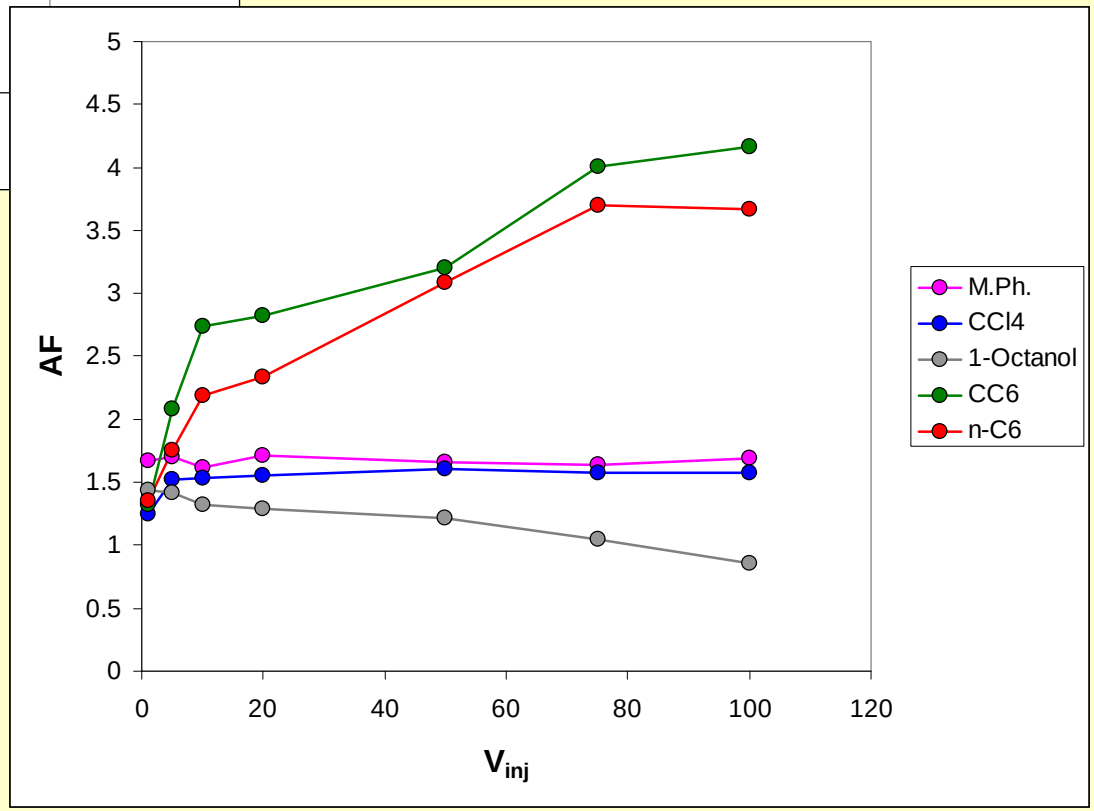

# Retention behavior:



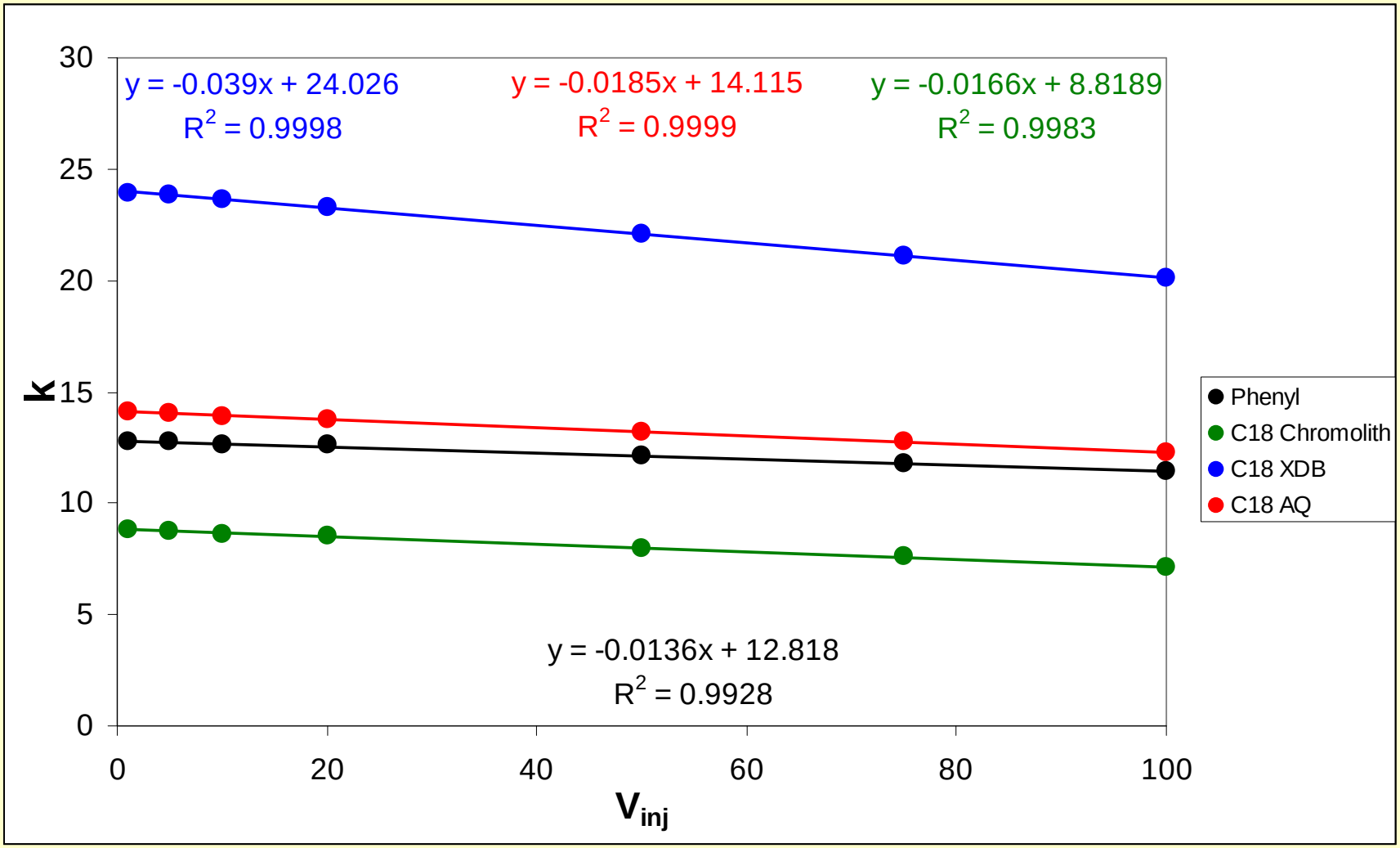
## Chromatographic efficiency variations:

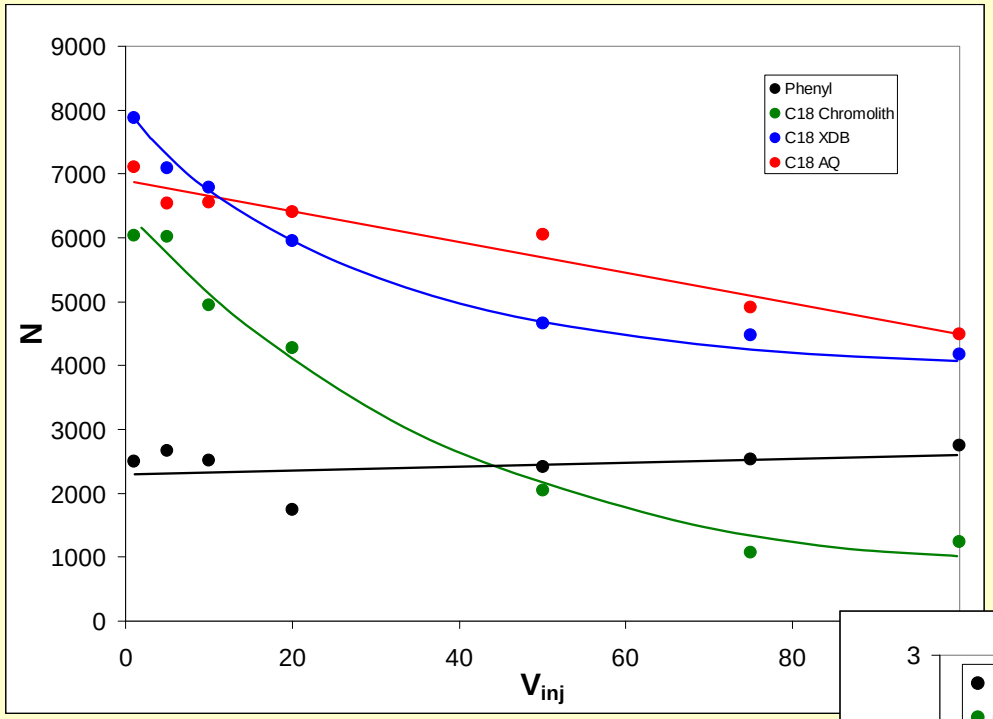



## Peak symmetry variations:



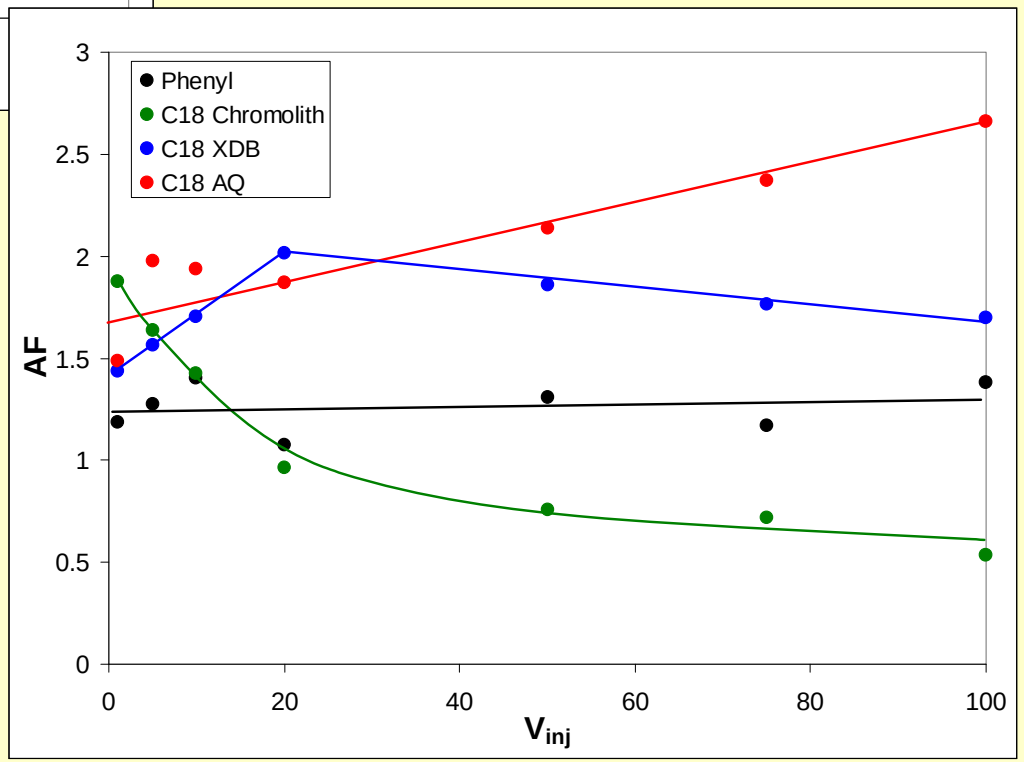
# Diluent: Carbon tetrachloride






## Chromatographic efficiency variations:

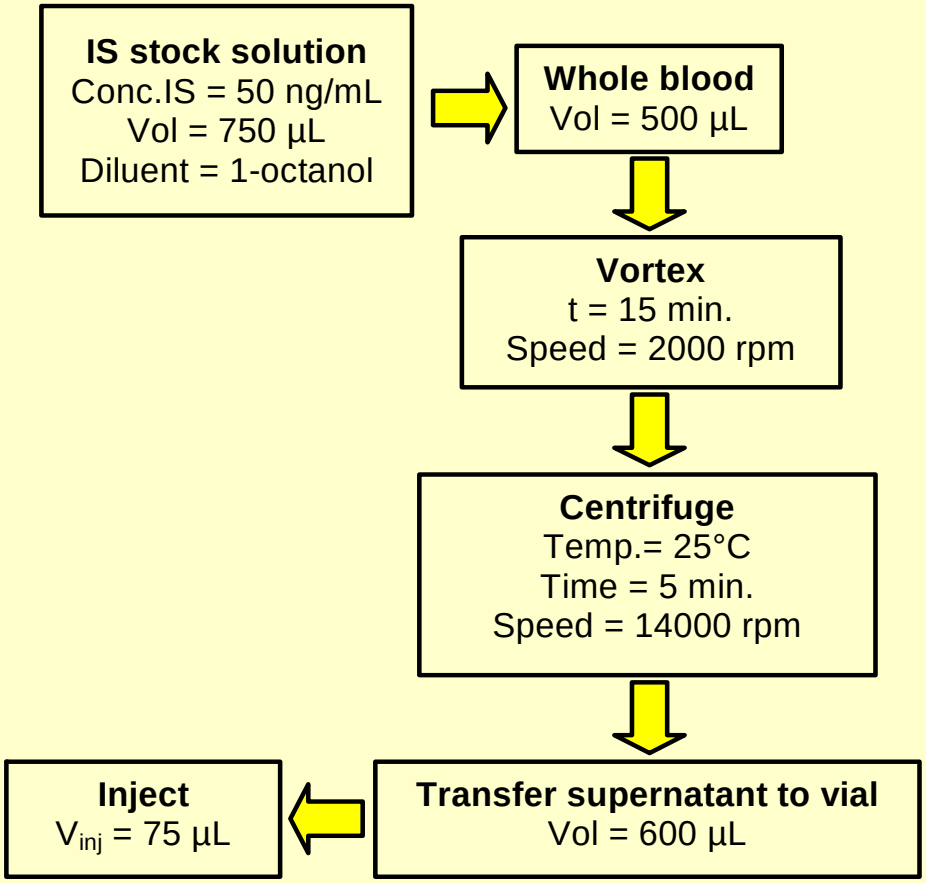
## Peak symmetry variations:



# Application 1: Indapamide in whole blood - LC/ESI/MS-MS

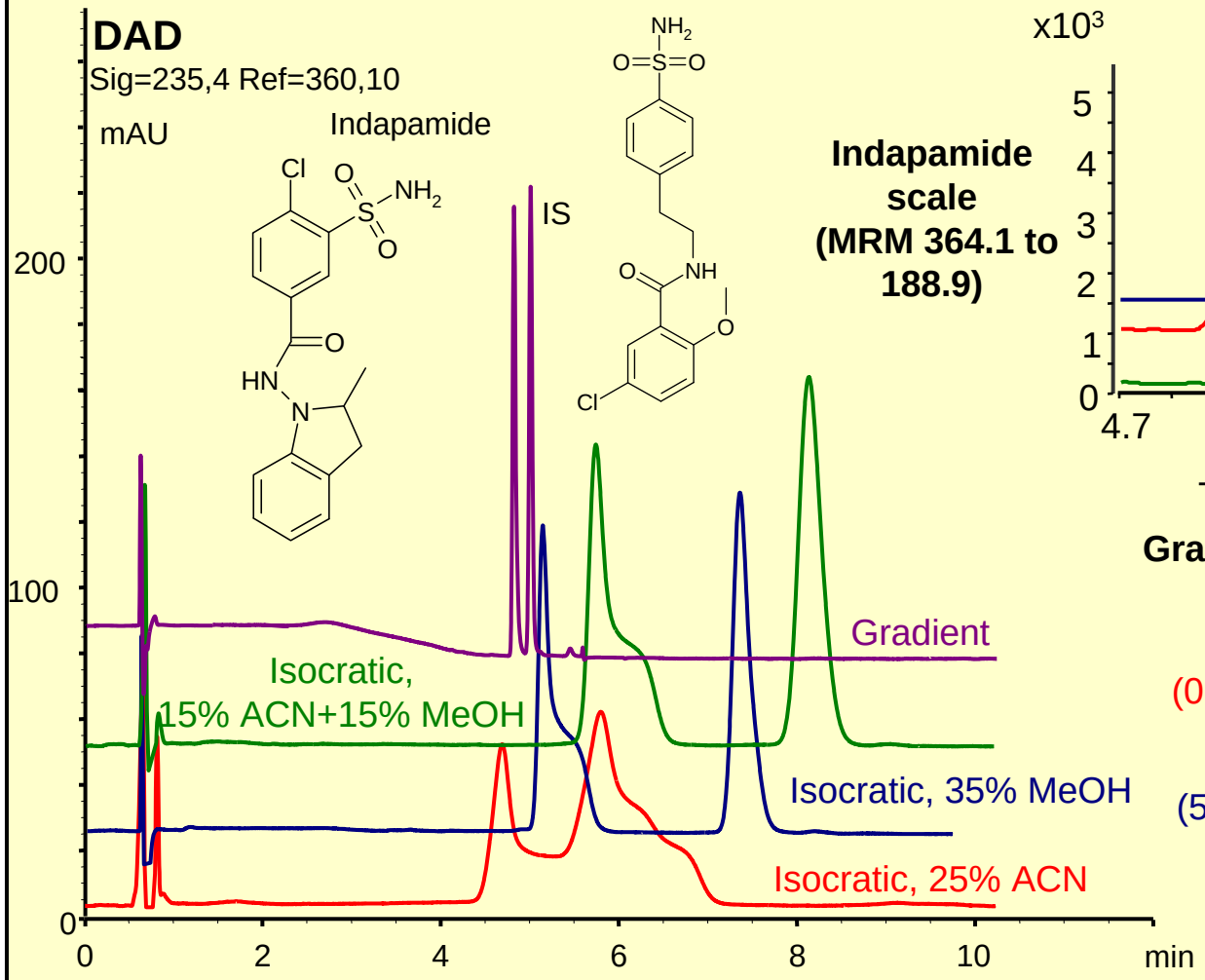
Column: Zorbax SB C18 RR
50 mm x 4.6 mm x 1.8 µm;
T °C = 40 °C;
Organic modifier: ACN/MeOH = 1/1 (v/v);
Aqueous component: aq. 0.1% HCOOH ;
Gradient profile :

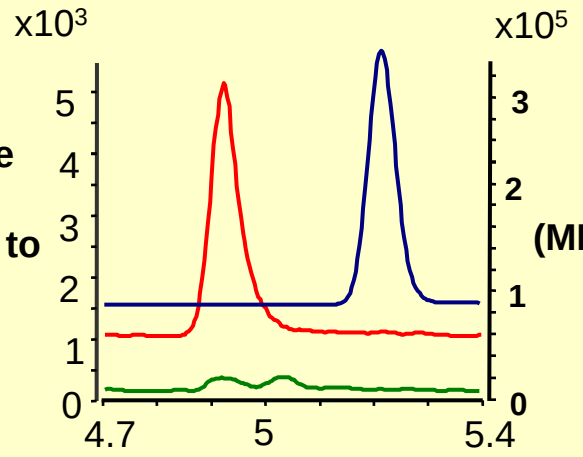
| Time (min.) | Organic modifier (%) | Flow rate (mL/min) |
|---|---|---|
| 0 | 5 | 0.8 |
| 2 | 45 | 0.8 |
| 5.5 | 45 | 0.8 |
| 5.51 | 100 | 0.8 |
| 6.0 | 100 | 0.8 |
| 6.50 | 100 | 1.2 |
| 6.51 | 5 | 1.2 |
| 7.5 | 5 | 1.2 |




$V_{inj}$ = 75 µL ;
Diluent : 1-Octanol

# Application 1: Indapamide in whole blood - LC/ESI/MS-MS




**Indapamide scale (MRM 364.1 to 188.9)**

**IS scale (MRM 367.1 to 170.1)**

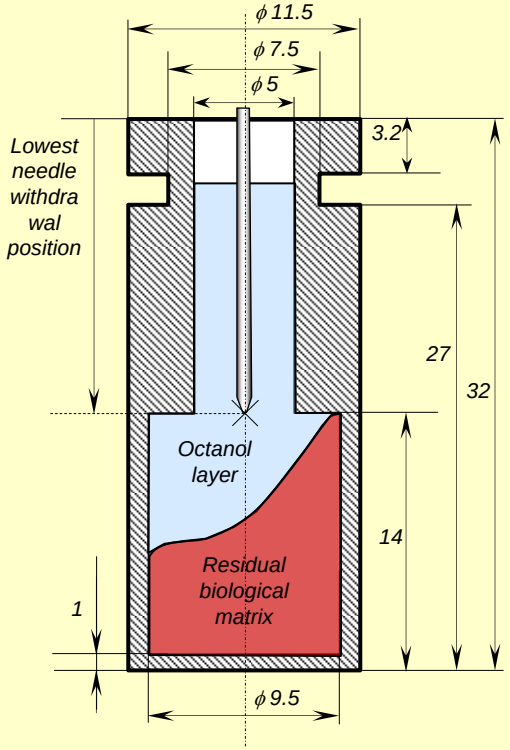
**Gradient -MS trace (detail)**

Indapamide
(0.5 ng/mL – MRM 364.1 to 188.9)

IS
(50 ng/mL – MRM 367.1 to 170.1)

Blank sample
(MRM 364.1 to 188.9)

# Application 1: Indapamide in whole blood - LC/ESI/MS-MS

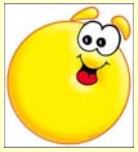



## Sample preparation & injection from a single vial!

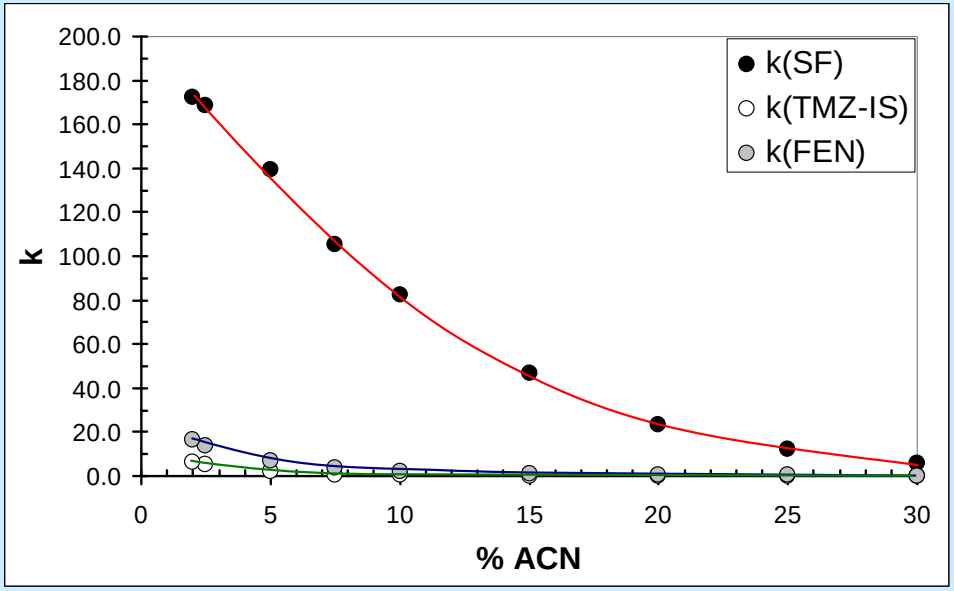
[6] S. Udrescu, I.D. Sora; F. Albu, V. David, A. Medvedovici, LVI of 1-octanol as sample diluent in RPLC: Application in bioanalysis for assaying of indapamide in whole blood, *J. Pharm. Biomed. Anal.*, 54, 1163-1172 (2011).

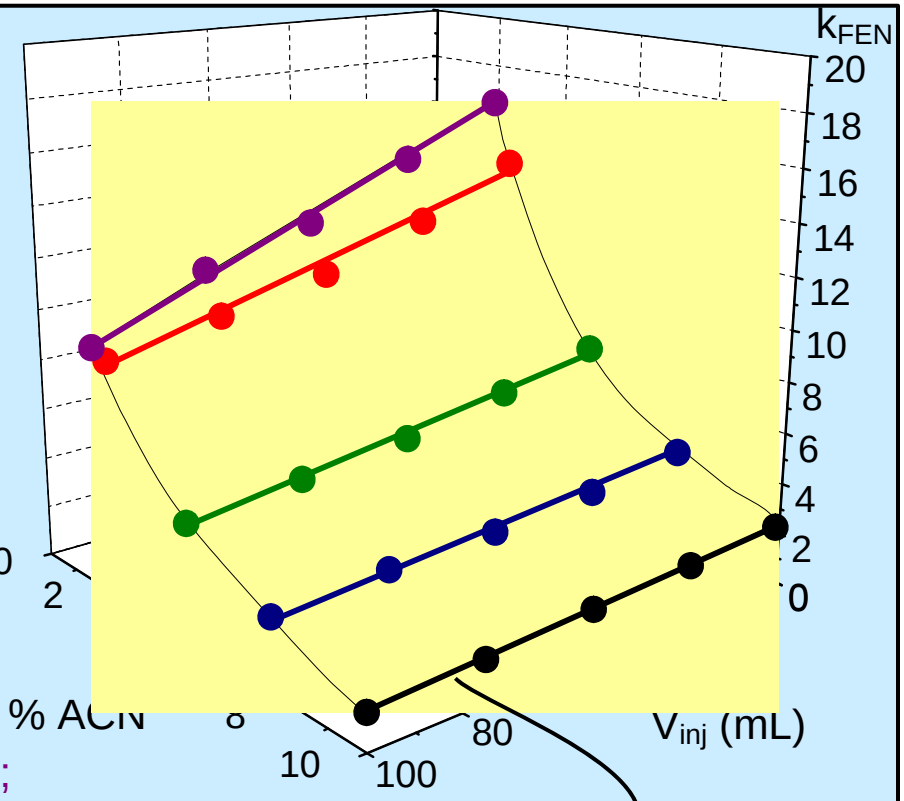
# Application 1: Indapamide in whole blood - LC/ESI/MS-MS

| Stage | Quality Characteristics |
|---|---|
| Linearity | LOD = 0.3 ng/mL (S/N = 3); LLOQ = 0.5 ng/mL (S/N≥5); ULOQ = 100 ng/mL; Conc. levels = 0.5/1/5/10/25/50/80 ng/mL; samples/level: n = 6 |
| | RSD% ∈ [4.4 ÷ 9.7]%; % Bias ∈ [-7.4 ÷ 8.0]%; Response function = linear, weighted $1/x^2$ |
| Precision | QC levels = 1.5/7.5/35/75 ng/mL; Repeatability: n = 10; Intermediate precision: n = 6 |
| | Repeatability: RSD% ∈ [0.8 ÷ 1.3]%; % Bias ∈ [-7.9 ÷ 8.1]% |
| | Intermediate precision: RSD% ∈ [7.3 ÷ 8.5]%; % Bias ∈ [-3.0 ÷ 3.3]% |
| Matrix Effects | MF(indapamide) = 0.77 (RSD% = 8.1, matrices = 6, n = 3/matrix, c = 3 x LLOQ) |
| | MF (IS) = 0.79 (RSD% = 7.1, n = 18, c = 50 ng/mL); Normalized MF = 0.98 (RSD% = 6.7). |
| Recovery | Indapamide: from 0.9% NaCl; Recovery = 97.8% (RSD% = 2); from whole blood; Recovery = 83.3% (RSD% = 5.8) |
| | IS: from 0.9% NaCl; Recovery = 103.8% (RSD% = 1.7); from whole blood; Recovery = 103.7% (RSD% = 2.1) |
| Ionization effects | Residual co-extracted matrix effect: Indapamide - Recovery = 98.4% (RSD% = 3.3); IS – Recovery 75.7% (RSD% = 2.1) |
| Dilution Integrity | Dilution ratios = 1/10; 1/5; 1/2; Dilution fluid: a) whole blood; b) aqueous 0.9% NaCl; samples per case: n = 3 |
| | 1/10: whole blood; mean RSD% = 5.4%; mean % bias = 13.9%; aq. 0.9% NaCl; mean RSD% = 0.8%; mean % bias = 7.9%; |
| | 1/5: whole blood; mean RSD% = 2.8%; mean % bias = 5.8%; aq. 0.9% NaCl; mean RSD% = 0.8%; mean % bias = 2.7%; |
| | 1/2: whole blood; mean RSD% = 0.1%; mean % bias = -2.9%; aq. 0.9% NaCl; mean RSD% = 2.6%; mean % bias = -5.9%; |



# Application 2: Fenspiride in human plasma by HPLC/ESI/MS-MS




Y = -0.0691 X + 16.387 (r^2 = 0.9929);

Y = -0.0482 X + 13.612 (r^2 = 0.9606);

Y = -0.0251 X + 4.9786 (r^2 = 0.9840);

Y = -0.0103 X + 3.7591 (r^2 = 0.9144);

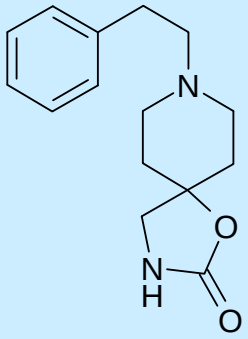
Y = -0.0095 X + 2.3267 (r^2 = 0.9981);

# Application 2: Fenspiride in human plasma by HPLC/ESI/MS-MS

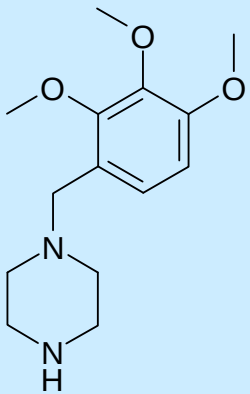
Column: Zorbax SB C18 RR
50 mm x 4.6 mm x 1.8 μm;
T °C = 50 °C;
Organic modifier: ACN;
Aqueous component: aq. 0.1% HCOOH ;
Gradient profile :

| Time (min.) | ACN (%) | Flow rate (mL/min) |
|---|---|---|
| 0 | 2 | 0.8 |
| 5 | 30 | 0.8 |
| 5.01 | 100 | 0.8 |
| 5.50 | 100 | 0.8 |
| 6.0 | 100 | 1.2 |
| 6.01 | 2 | 1.2 |
| 7.0 | 2 | 1.2 |

$V_{inj}$ = 75 μL ;
Diluent : 1-Octanol



**FEN**



**TMZ (IS)**

**5% $Na_2CO_3$ aq. solution**
Volume = 50 μL

**Plasma**
Volume = 500 μL

**Vortex**
Time = 2 min.
Speed = 2000 rpm

**IS working solution**
*Conc. Trimetazidine = 20 ng/mL*
Volume = 750 μL
Solvent = 1-octanol

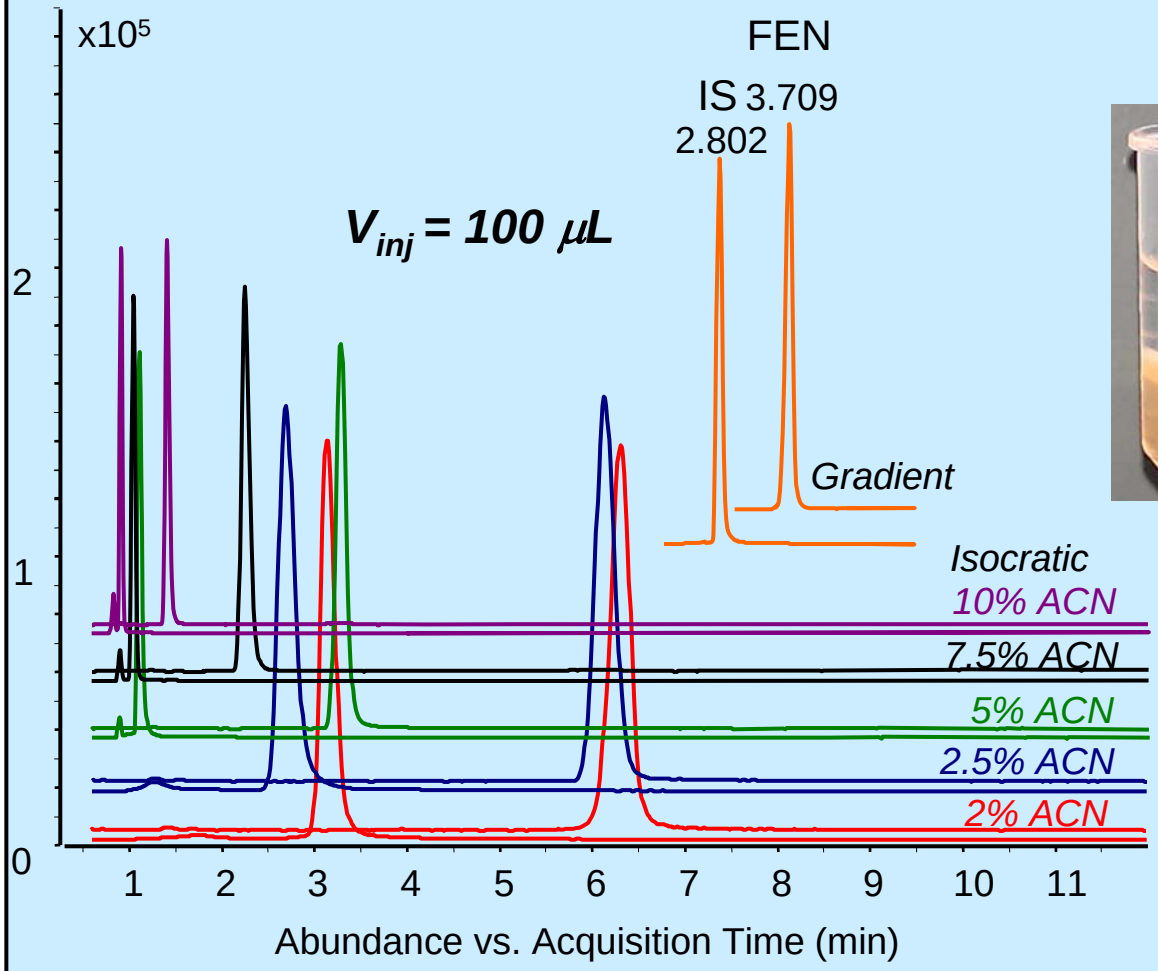
**Vortex**
Time = 10 min.
Speed = 2000 rpm

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

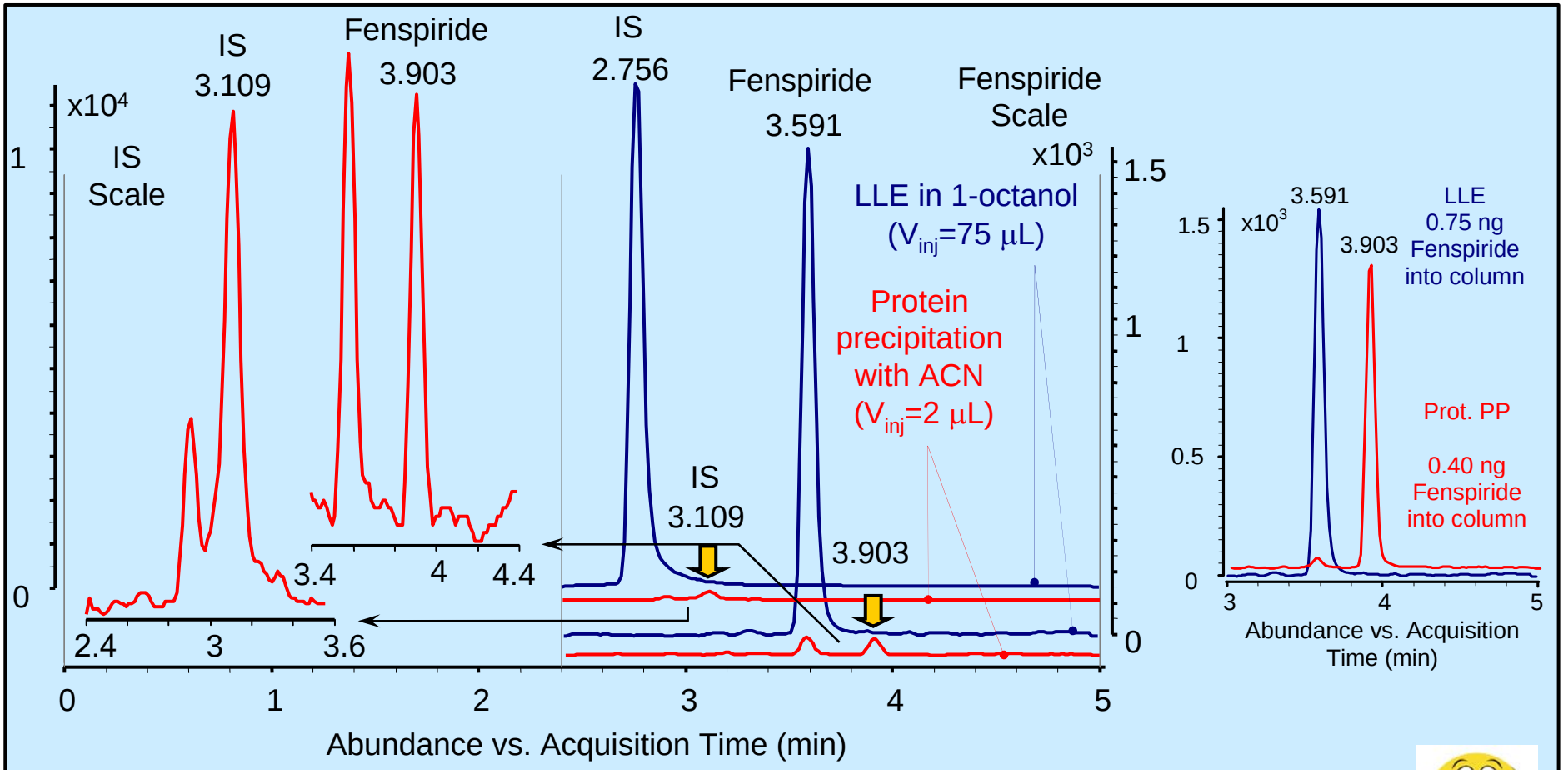
**Quantitatively transfer the supernatant to vial**

**Injection**
$V_{inj}$ = 75 μL

# Application 2: Fenspiride in human plasma by HPLC/ESI/MS-MS



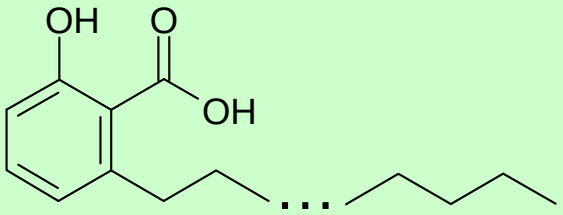

# Application 2: Fenspiride in human plasma by HPLC/ESI/MS-MS




[7] A. Medvedovici, S. Udrescu, F. Albu, F. Tache, V. David, LVI of sample diluents not miscible with the mobile phase as an alternative approach in sample preparation for bioanalysis: An application for fenspiride bioequivalence, *Bioanalysis*, 3(17), xxx-xxx (2011).

# Application 3: Assay of ginkgolic acids in Ginkgo dry extracts refined and quantified

Analytes: Ginkgolic Acids (C13; C15; C17)




Diluent: Hexane

Log P (Hexane) = 3.29;
Log P (C13:GA) = 8.69;
Log P (C15:GA) = 9.45;
Log P (C17:GA) = 10.4;

$k_{SF} < k_A$


0.5 g Dry Extract + 5 mL MeOH + 0.5 mL HCOOH (98%) up to 10 mL with $H_2O$

0.5 mL Hexane

**Vortex –mix**
1 min

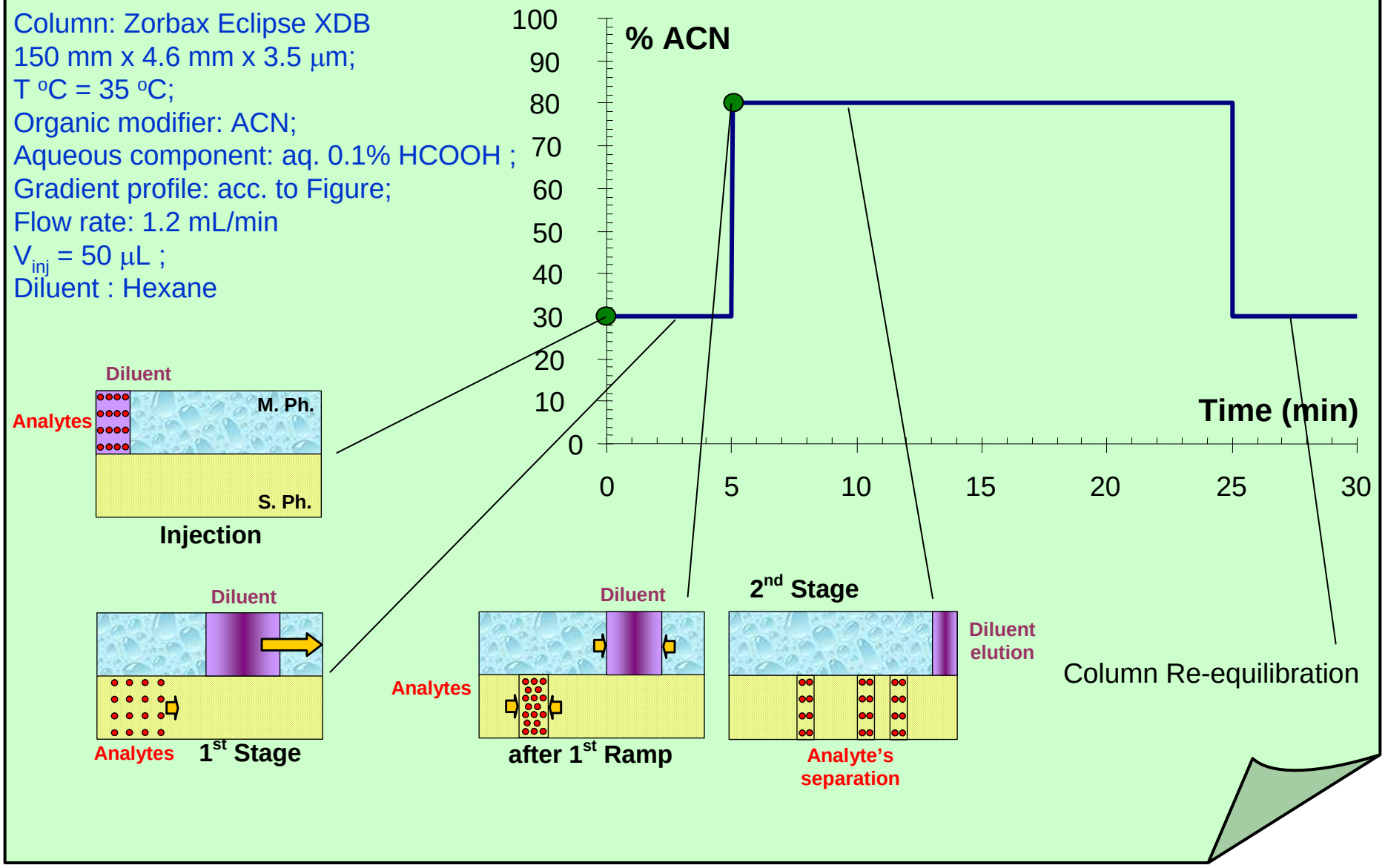
**Wait until phase separation**
5 min (no centrifugation needed)

**Aliquot from upper layer**
~ 0.2 mL

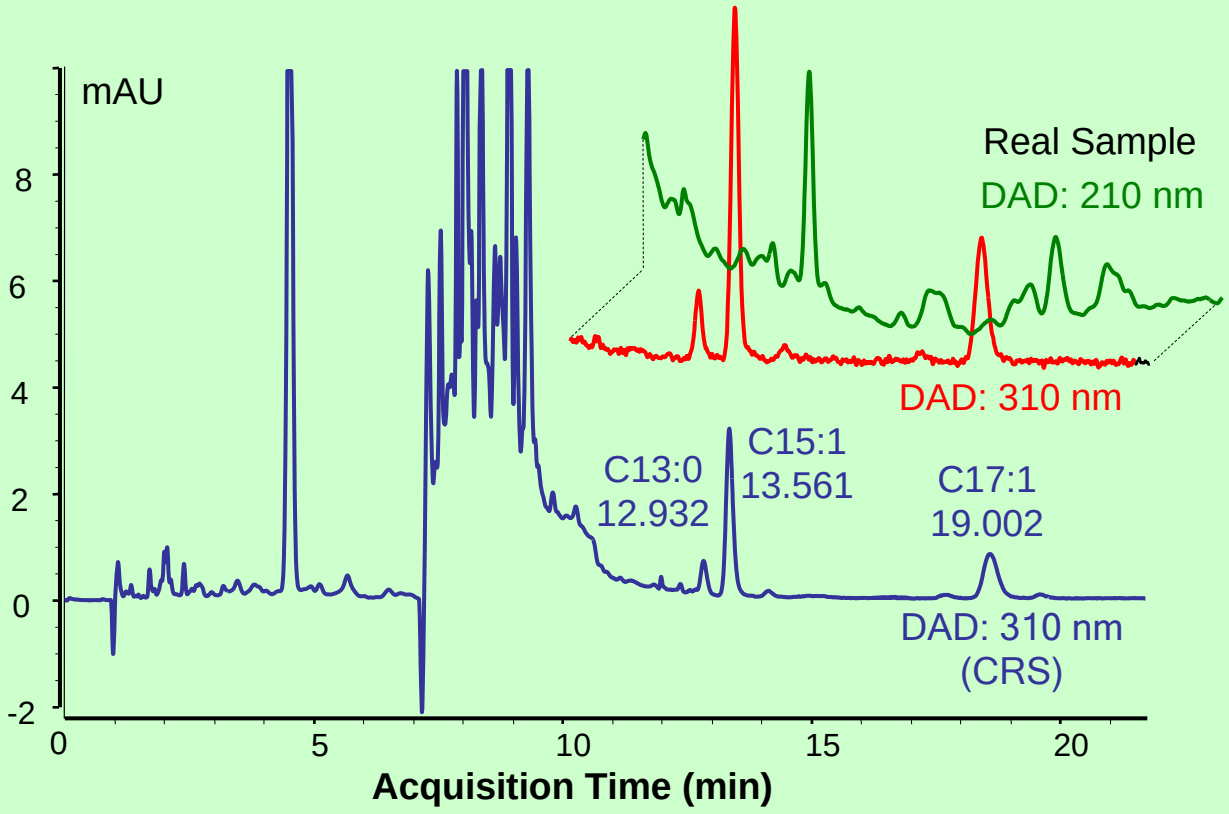
**Inject**
50 µL

# Application 3: Assay of ginkgolic acids in Ginkgo dry extracts refined and quantified

Column: Zorbax Eclipse XDB
150 mm x 4.6 mm x 3.5 μm;
T °C = 35 °C;
Organic modifier: ACN;
Aqueous component: aq. 0.1% HCOOH ;
Gradient profile: acc. to Figure;
Flow rate: 1.2 mL/min
$V_{inj}$ = 50 μL ;
Diluent : Hexane



# Application 3: Assay of ginkgolic acids in Ginkgo dry extracts refined and quantified

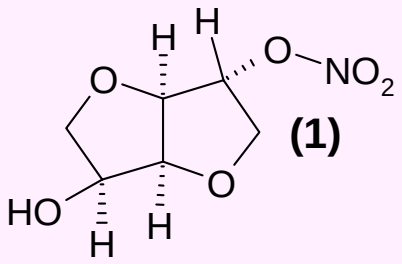



[8] S. Udrescu, I.D. Sora, V. David, A. Medvedovici, LVI of hexane solutions in RPLC/UV to enhance on sensitivity of the assay of Ginkgolic Acids in Ginkgo Biloba standardized extracts, *J. Liq. Chromatogr. Rel. Technol.*, 33, 133-149 (2010).

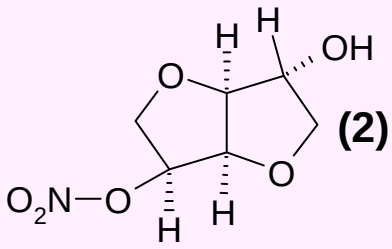
# Application 4: LVI of polar compounds of pharmaceutical interest dissolved in aqueous immiscible hydrophobic solvents (alkanes)

**Analytes:**

(1) Isosorbide 2-nitrate (log P = - 0.40;
(2) Isosorbide 5-nitrate (log P = -0.15);
(3) Pentoxifylline (log P = 0.56);
(4) Tropicamide (log P = 1.19);
(5) Methyl-*p*-hydroxybenzoate (log P = 1.96)



**(1)**



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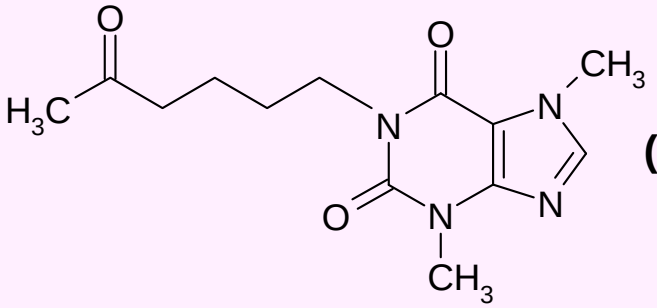

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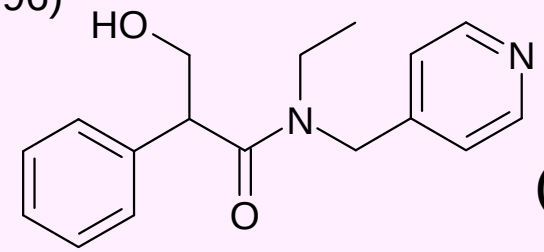

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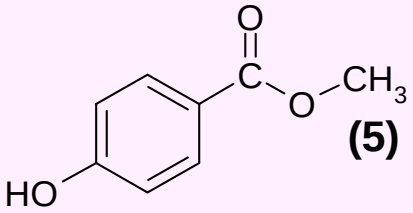


**(5)**

**Columns:**

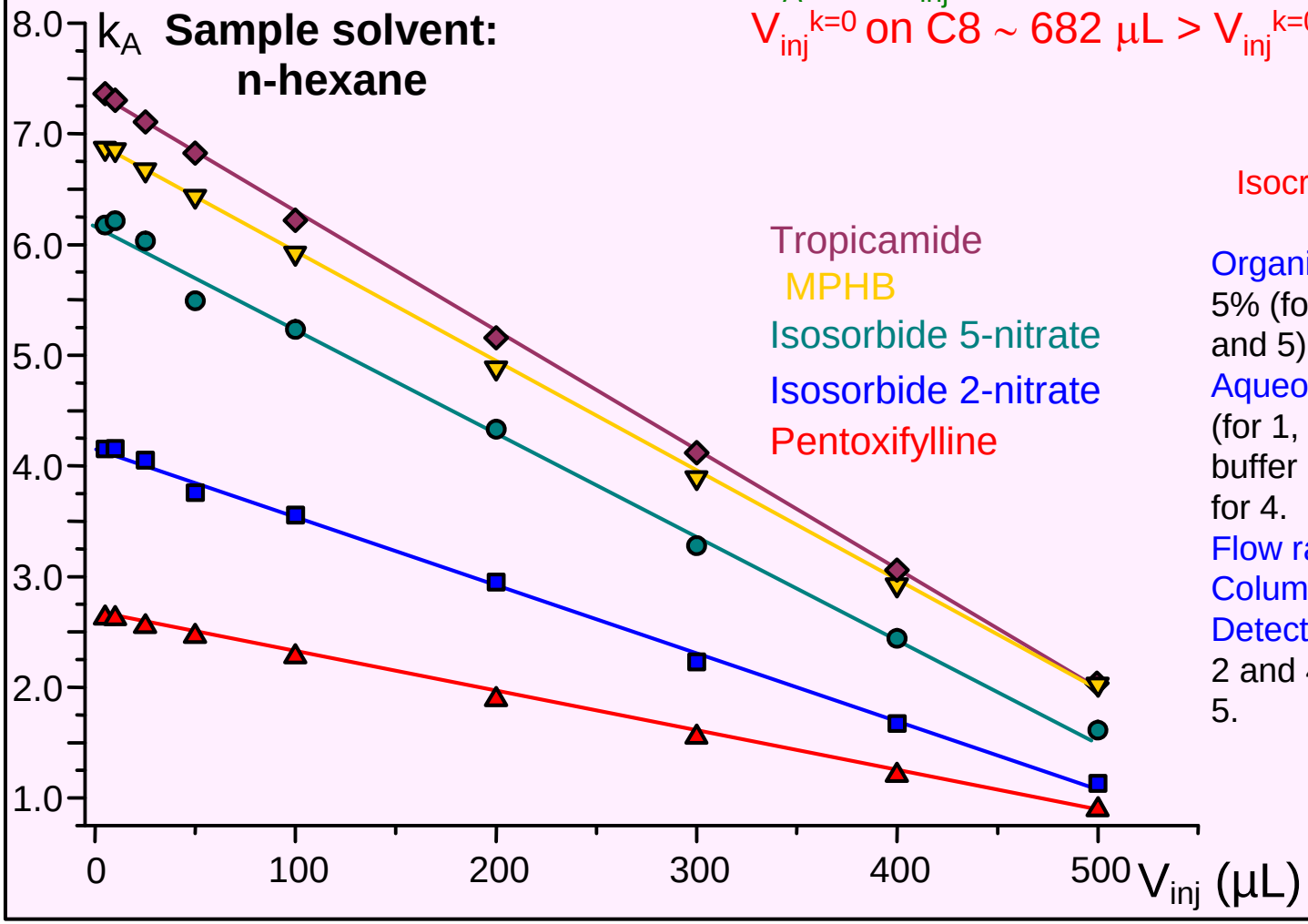
(A) Zorbax Eclipse XDB C-8; 150 mm x 4.6 mm x 3.5 μm;
(B) Zorbax Eclipse XDB C-18; 150 mm x 4.6 mm x 5 μm;

**Diluents:**

(I) *n* – hexane (log P = 3.28); (II) *n* – heptane (log P = 3.78); (III) *i* – octane (log P = 4.09)

# Application 4: LVI of polar compounds of pharmaceutical interest dissolved in aqueous immiscible hydrophobic solvents (alkanes)

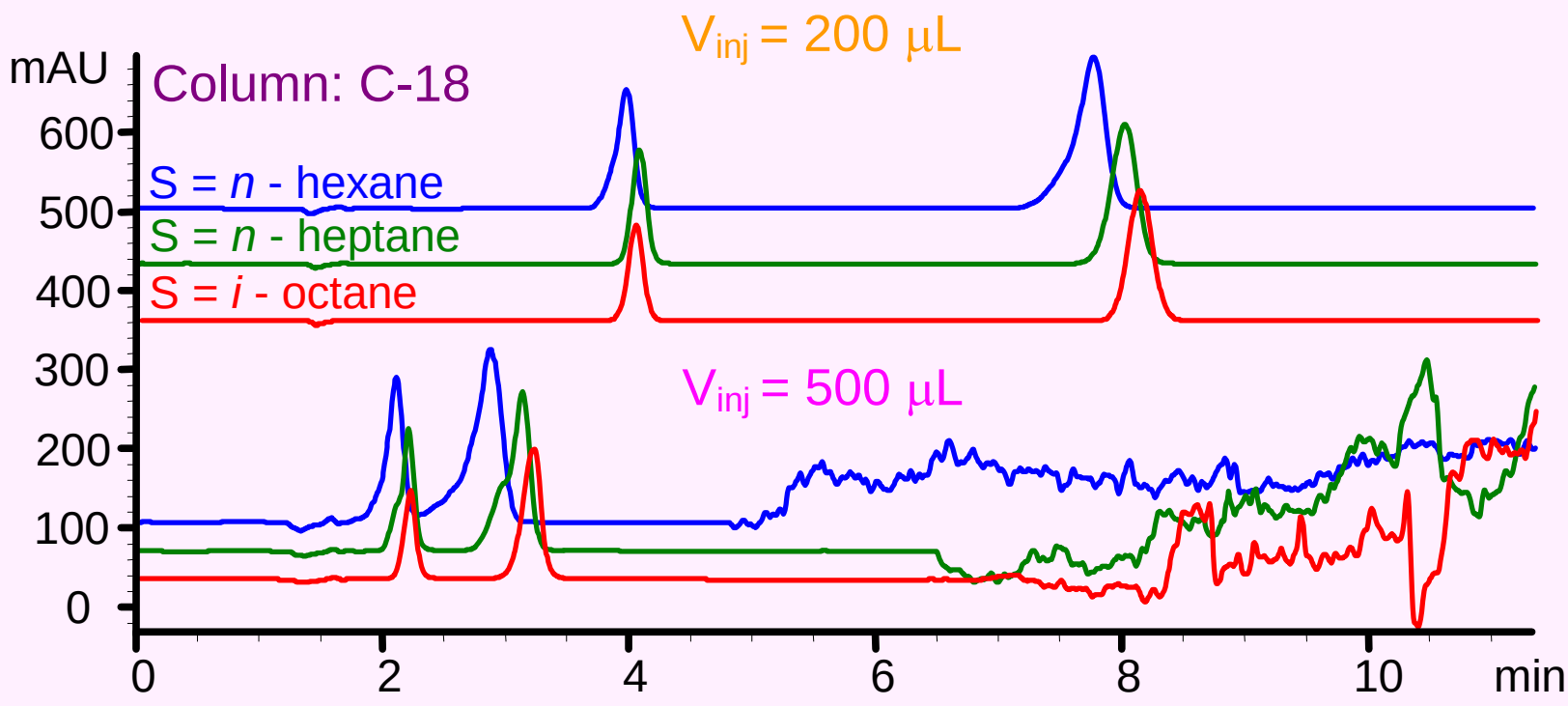
$k_A$ = f ($V_{inj}$); linear; negative slopes; r^2 > 0.99;
$N_A$ = f ($V_{inj}$) linear; negative slopes; r^2 > 0.99
$V_{inj}^{k=0}$ on C8 ~ 682 µL > $V_{inj}^{k=0}$ on C18 ~ 586 µL;




Isocratic elution conditions

Organic modifier: Acetonitrile 5% (for 1 and 2); 20 % (for 3 and 5) and 22% for 4.
Aqueous component: water (for 1, 2, 3, 5) or aqueous buffer $H_3PO_4$ / TEA at pH 7 for 4.
Flow rate: 1 mL/min;
Column temperature: 30 °C
Detection: UV – 210 nm for 1, 2 and 4 and 270 nm for 3 and 5.

# Application 4: LVI of polar compounds of pharmaceutical interest dissolved in aqueous immiscible hydrophobic solvents (alkanes)




[9] S. Udrescu, A. Medvedovici, V. David, LVI of polar pharmaceutical solutes in hydrophobic solvents: influence on retention under RPLC separation mechanism, *J. Sep. Sci.*, 31(16-17), 2939-2945 (2008).

# Conclusions:

LVI of M.Ph. non-miscible diluents in RPLC is feasible. Although complex, once understood, the process may be successfully controlled (mainly through gradient elution) and used as a valuable tool for enhancing on sensitivity.

LLE and LVI of non-miscible diluents are logically fitting together, offering interesting opportunities for high throughput approaches.

# Acknowledgements:

**To my colleague and friend, Prof. Dr. Victor David, for sharing the interest on the topic and continuing to develop it together.**

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

# Acknowledgements:

**To the unknown reviewer rejecting our first manuscript on LVI of immiscible diluents, for encouraging us to continue.**

**"The work carried out in this area is very limited and I do not think that it will acquire a broad practical significance in the future. The work presented here seems to be original, it is an interesting combination of experiment and theory and for this reason it is publishable, however …"**

Critics you, of sterile blossoms,
Driven out by pride and spell,
It is easy to write verses,
Having nothing all to tell.

**M. Eminescu**
**(To my critics)**