Actual aspects in Separation Sciences

Micro-symposium dedicated to commemoration of one century from the birth of Prof. Dr. Candin Liteanu

September 11th 2014
Cluj Napoca, Romania

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Chromatography: How old is she?

Michail Semjonowitsch Tswet
19.05.1872 – 26.06.1919

Physikalisch-chemische Studien über das Chlorophyll. Die Adsorptionen. **Ber. Dtsch. Botan. Ges.** 24 (1906) S. 316–323.

Adsorptionsanalyse und chroma tographische Methode. Anwendung auf die Chemie des Chlorophylls, **Ber. Dtsch. Botan. Ges.** 24 (1906) S. 384–393.

History is sometimes unfaithful!

Friedlieb Ferdinand Runge
08.02.1795 – 25.03.1867

Farbenchemie I, (1834).

H. H. Bussemas, G. Harsch, L. S. Ettre. *Friedlieb Ferdinand Runge (1794–1867): "Self-grown pictures" as precursors of paper chromatography*, **Chromatographia**, 38 (1994), ss. 243–254.

Christoph Friedrich Goppelsroeder
(1837-1919)

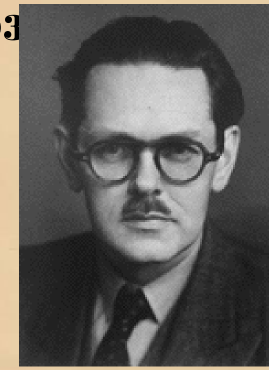
Zeit. Anal. Chem., 7 (1868) 195.

Godfathers of chromatographic sciences:

Arne W. Kaurin Tiselius
10.10.1902 – 29.10.1971

Science, 94 (1941) 145-146.

Archer John Porter Martin
01.03 .2002

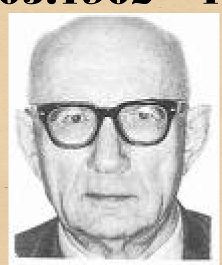


Biochem. J. (Lond.), 35 (1941)1358.

Richard L. Millington Synge 28.10.1914 –

Biochem. J. (Lond.), 35 (1941)1358.

Marcel J.E. Golay
03.05.1902 – 1989



van Deemter JJ, Zuiderweg FJ and Klinkenberg A. *Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography,* **Chem. Eng. Sc.**, 5 (1956) 271–289.

Internationally recognized Romanian contributors in the chromatography world

Candin Liteanu
06.07.1914 – 31.05.1990

C. Liteanu, S. Gocan, A. Bold, Separatologie Analitica, Ed. Dacia (1971).

C. Liteanu, I. Rîca, **Statistical Theory & Methodology of Trace Analysis,** Ellis Horwood, Chicester (1980).

George Emil Baiulescu
04.08.1931 – 09.06.2009

G.E. Baiulescu, V.A. Ilie, **Stationary Phases in Gas Chromatography,** Pergamon Press, Oxford (1975).

G.E. Baiulescu, P. Dumitrescu, P. Gh. Zugravescu, **Sampling,** Ellis Horwood, Chicester (1991).

Ionel Ciucanu, Cromatografia de gaze cu coloane capilare, Ed. Academiei Romane (1990).

Serban C. Moldoveanu, Victor David, Sample Preparation in Chromatography, Elsevier Science B.V. (2002).

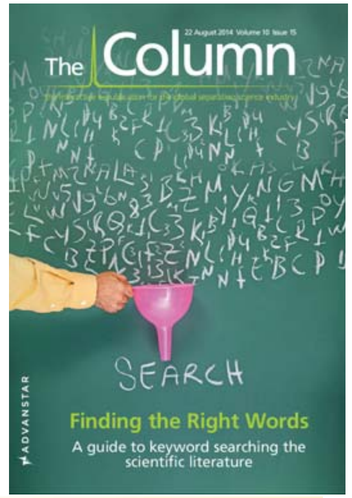
Serban C. Moldoveanu, Victor David, Essentials in Modern HPLC Separation, Elsevier (2013).

The "Jourdain complex" about Analytical Chemistry!

Monsieur Jourdain: Par ma fois, il y a plus de quarante ans que je dis de la prose, sans que j'en susse rien; et je suis le plus obligé du monde de m'avoir appris cela!

Everyone needs analytical chemistry (especially separation techniques), everyone makes analytical chemistry! What analytical chemists are still doing? Are they still useful?

Perception on Analytical Chemistry!



My Sushi Moment

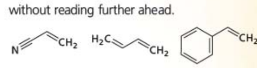
Incognito attends a sushi class and ponders the plight of the analytical scientist.

Recently while on summer vacation I found myself taking a class on sushi making — something I'd wanted to do for several years. The class was excellent and the chef/instructor was a very amiable young man who was keen on talking about the "chemistry" of the sushi-making process — the "pickling and fermentation" of the rice, the release of starch as you patted the rice down on the Nori paper that helped to bind the rolls etc.

As we were working away, I couldn't help but notice that among some very ornate and brightly coloured tattoos which the chef sported on his forearms, there was a tattoo of what I thought I recognized as styrene. My first thought was that my new chef acquaintance had been misled and that an internet trawl for interesting sushi-based chemicals had gone badly wrong, and he would be forever doomed to be tagged with a popular monomer rather than something much more pertinent, romantic, or even illegal...one never knows!

My curiosity eventually got the better of me and in a quiet moment at the end of the class I asked the chef the relevance of his tattoo. He went on to reveal further molecules on his arms, which I've drawn out in the following figure.

There's a prize for anyone who can guess the relevance of these molecules at this stage, without reading further ahead.



Well, from left to right in the figure the molecules are acrylonitrile, 1,3-butadiene, and styrene. When polymerized they form the very common thermoplastic terpolymer, acrylonitrile butadiene styrene, more popularly known as ABS. So chef, it turns out, knew more than just a smattering of chemistry and thankfully was not labouring under a misapprehension about his indelible markings.

But why ABS? Well, it turns out that brightly coloured tattoo inks are made, at least in part, from ABS, which makes the colours extremely vivid and certainly explained the very brightly coloured fish and other tattoos that the chef was also sporting. He had though it pertinent to pay homage to the chemistry behind his skin art.

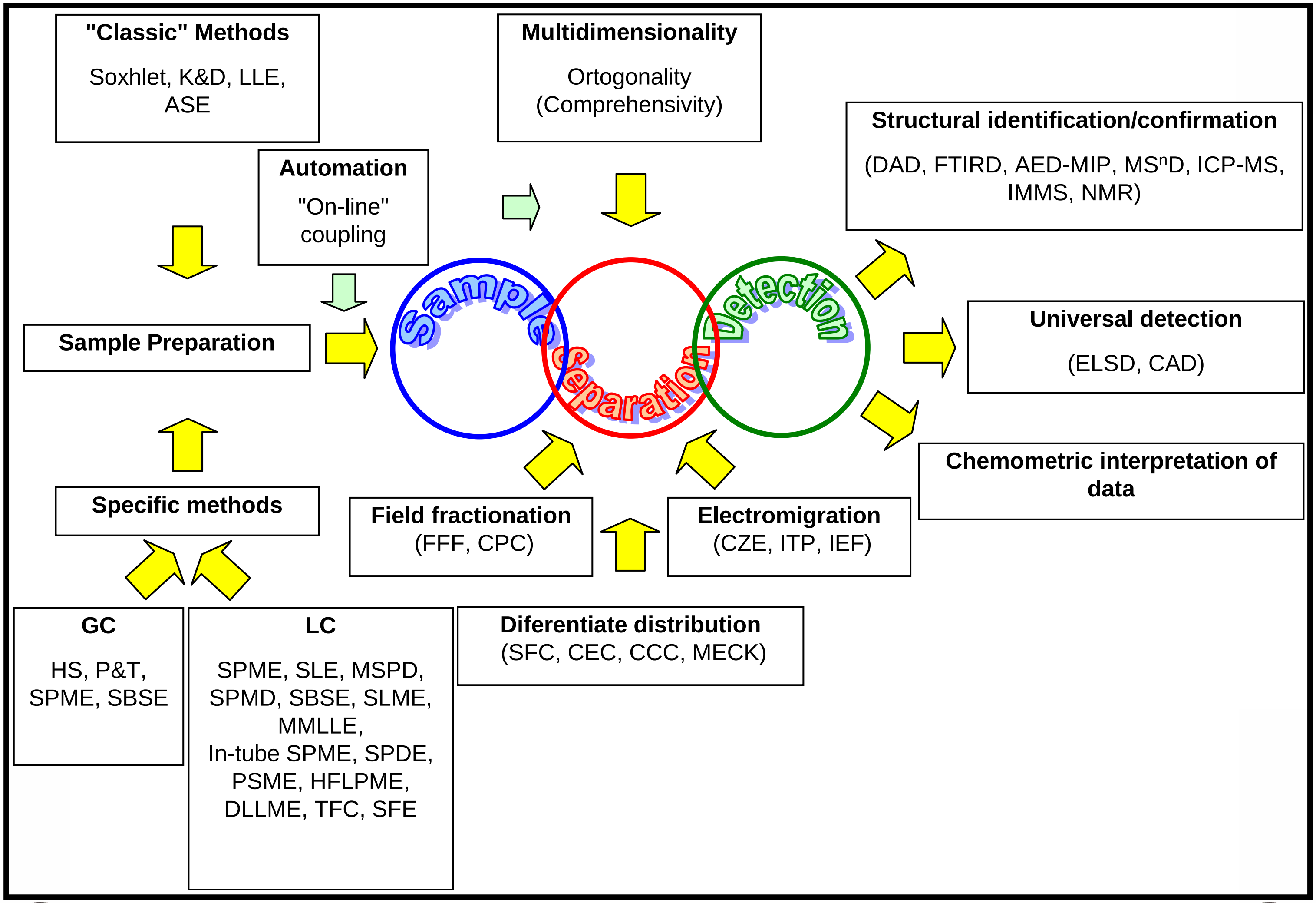
But let's get to the point of this instalment.

It turns out that the chef was a highly enthusiastic analytical chemist, who had spent some considerable time working in laboratories during his undergraduate programme and had initially taken a job as a trainee sushi chef to help fund himself through college. Indeed, he had graduated, undertaken a Master's degree, and had begun to work in a laboratory (a subsidiary of a multi-national corporation) that I had visited several years ago, and which was undertaking, at that time, some good research using reasonably advanced techniques. That's where the good news ended.

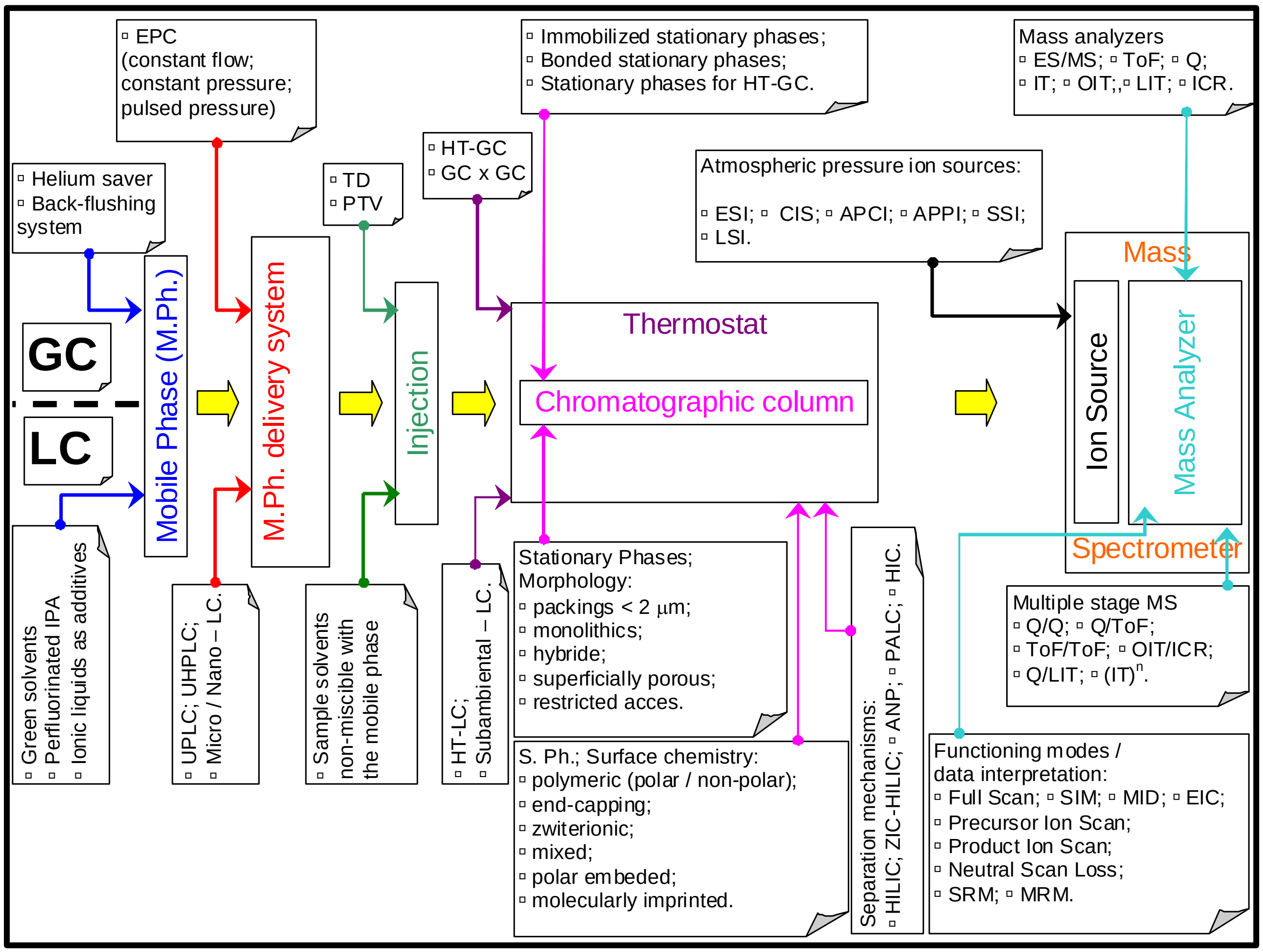
It turns out that my chef friend and many of his classmates were highly disillusioned with analytical science and while he obviously had a love and passion for the subject, he earned about 30% more as a sushi chef. His benefits package was also much better working for the sushi chain.

Furthermore, he went on to describe how his cohort, with whom he spoke regularly, all felt like second- or third-class citizens behind the chemical engineers, organic chemists, and biochemists with whom they worked. There was a common attitude that the analytical guys were there to simply turn the handle and generate data, which the "higher ups" would then interpret and contextualize to produce "information" useful to the business.

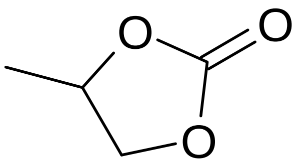
"Coagulation" role of chromatography



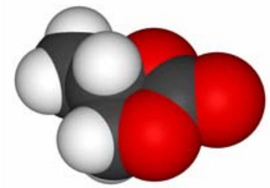
Trends in chromatographic techniques



Green solvents as alternative to ACN in RPLC



Propylene carbonate

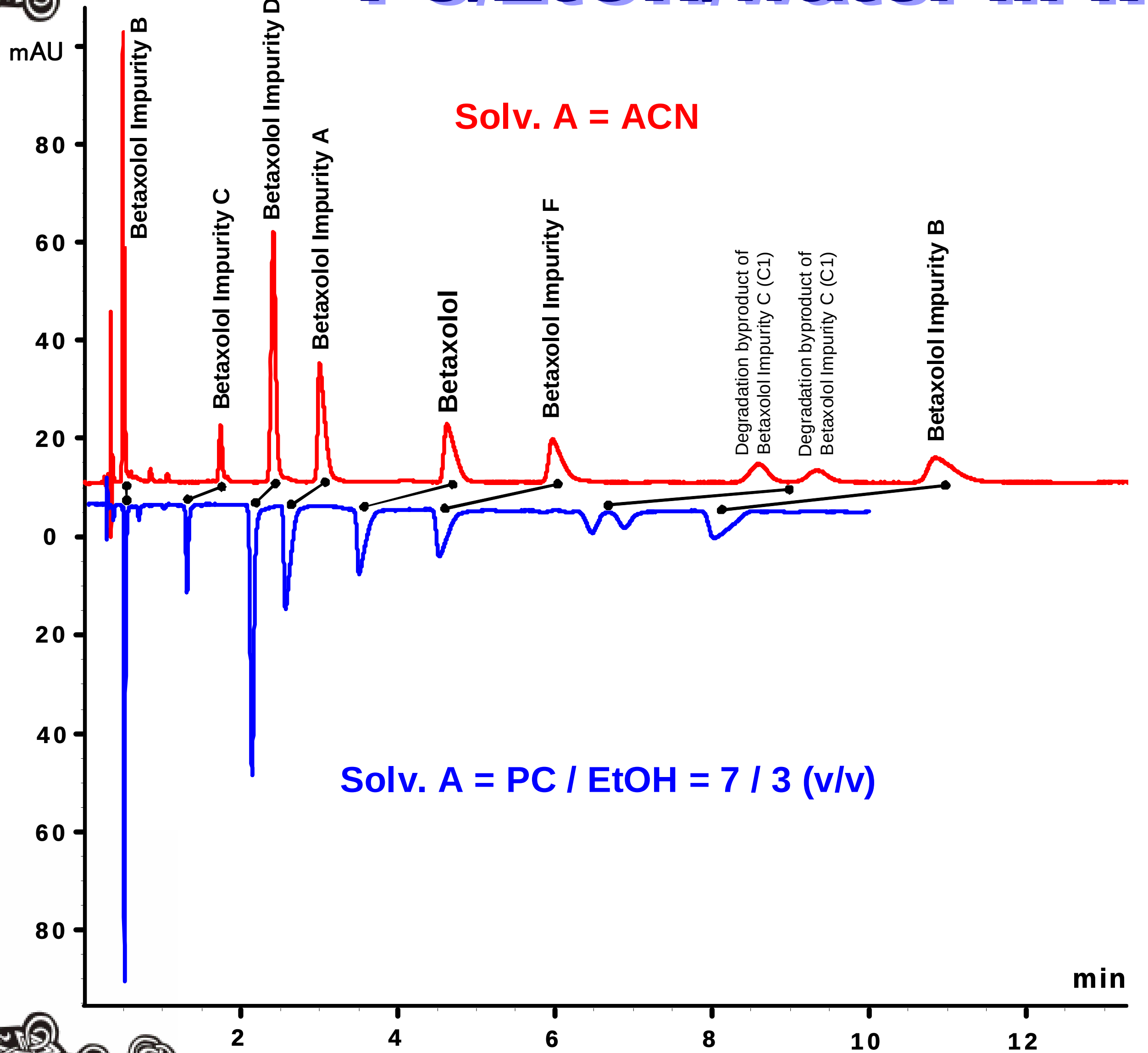


- ☞**Not listed as VOC**
- ☞**Readily biodegradable**
- ☞**Not flammable**
- ☞**Available as highly purified solvent**

☞**Only mixtures PC/EtOH are fully miscible with water**

Tache F, Udrescu S, Albu F, Micăle F, Medvedovici A. Greening pharmaceutical applications of liquid chromatography through using propylene carbonate-ethanol mixtures instead of acetonitrile as organic modifier in the mobile phases. J Pharm Biomed Anal. 2013;75:230-8.

PC/EtOH/water in IPLC



Chromatographic column: Zorbax SB-C18, 5 cm L x 4.6 mm i.d. x 1.8 μm d.p.
Column temperature: 70 ºC

Solvent A: **Organic modifier (ACN or PC/EtOH 7/3)**

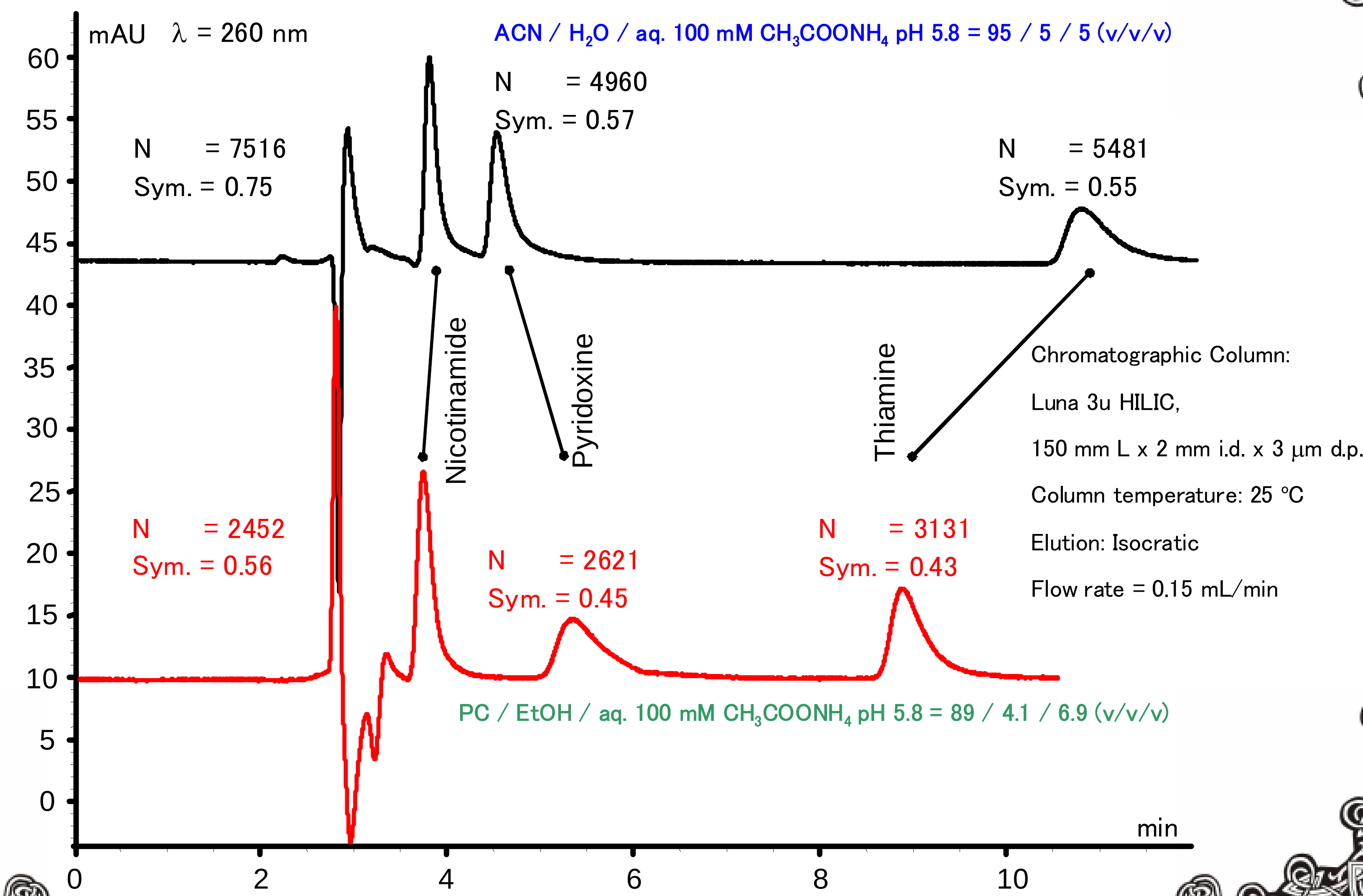
Solvent B: aqueous Sodium heptane sulphonate 15 mM and 0.1% H_3PO_4

Elution: Isocratic; Solvent A / Solvent B = 25 / 75 (v/v)

Flow rate: 2 mL/min;

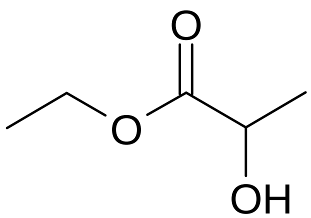
Detection: UV, 273 nm

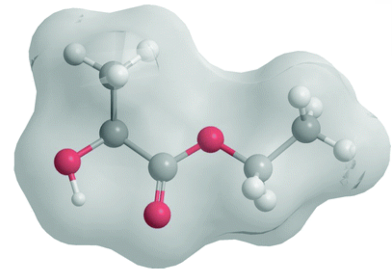
PC/EtOH/water in HILIC



Green solvents as alternative to ACN in RPLC

Ethyl Lactate



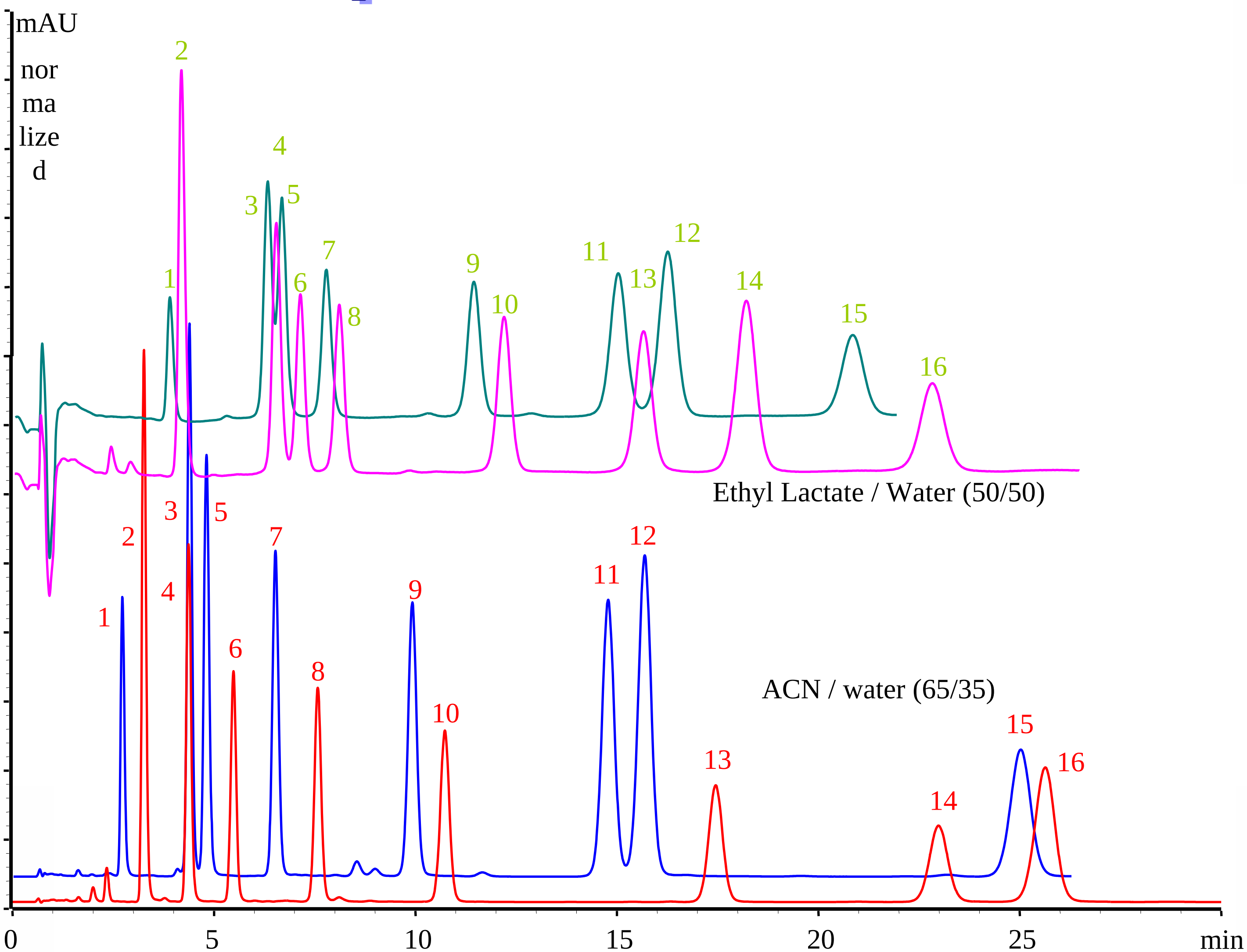


- ☞Not listed as VOC
- ☞Readily biodegradable
- ☞Not flammable
- ☞Fully miscible with water

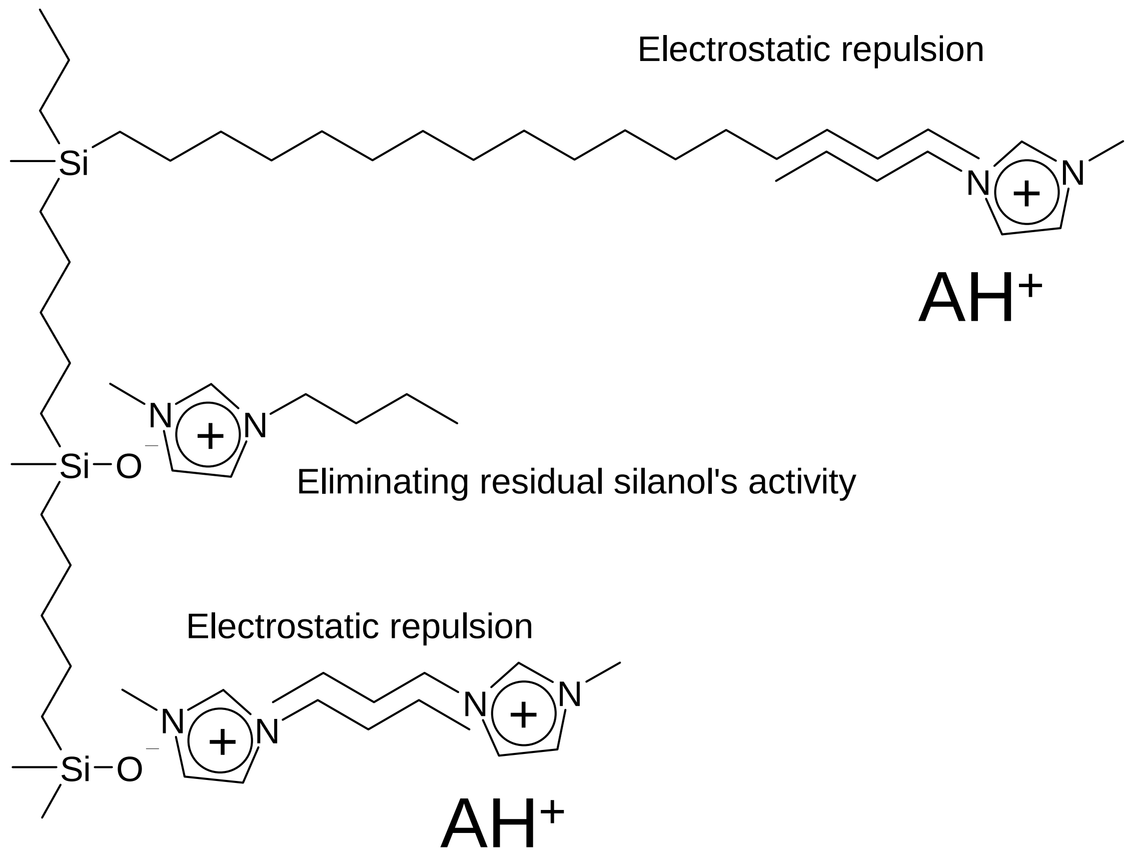
☞Not commercially available as LC grade solvent!

Micăle F., Albu F., Iorgulescu E.E., Medvedovici A., Tache F. Ethyl lactate as a greener alternative in RPLC: a realistic appraisal. J Chromatogr. Sci. submitted.

PAHs separation on a C18 column

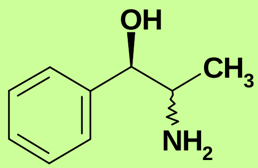


Ionic liquids as additives in the mobile phases for LC

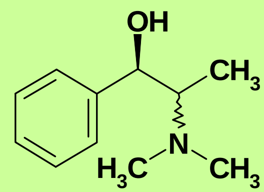


Applications: Forensic, Ephedrines in urine.

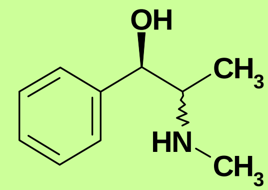
Target compounds (threshold limit ~ 5 ppm)



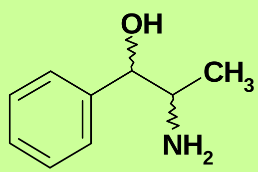
Norephedrine
[(1R,2S)-2-amino-1-phenylpropane-1-ol]
(NORE)



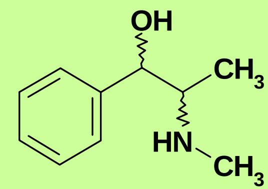
N-methylephedrine
[(1R,2S)-2-dimethylamino-1-phenylpropane-1-ol]
(NEFE)



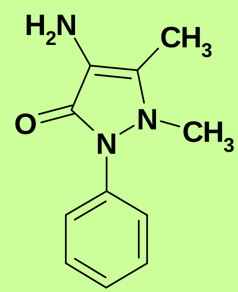
Ephedrine
[(1R,2S)-2-methylamino-1-phenylpropane-1-ol]
(EFE)



Cathine (Norpseudoephedrine)
[(1S,2S)-2-amino-1-phenylpropane-1-ol]
(CAT)

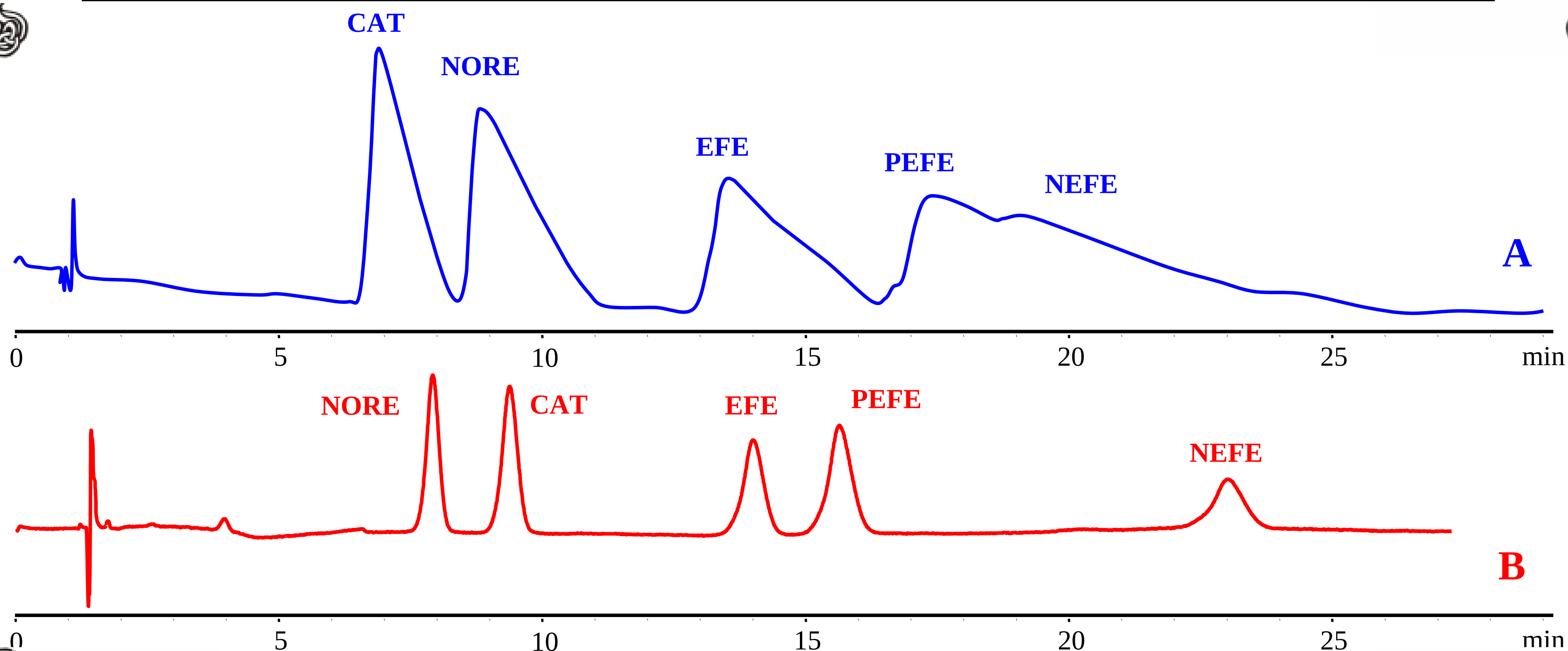


Pseudoephedrine
[(1S,2S)-2-methylamino-1-phenylpropane-1-ol]
(PEFE)



IS - 4-aminoantipyrine
4-amino-2,3-dimethyl-1-phenyl-3-pirazoline-5-onea

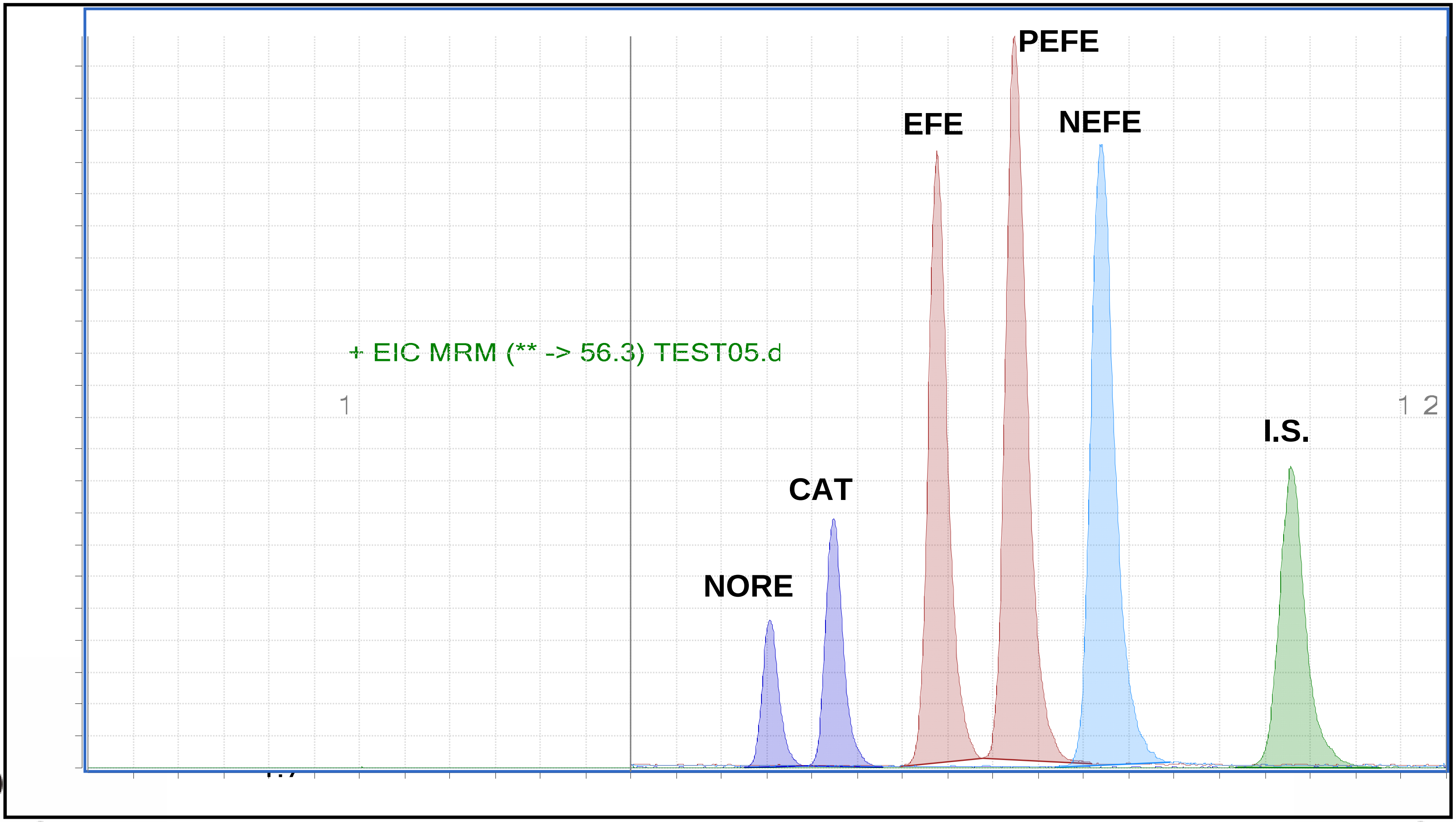
Separation on C18 stationary phase (strong end capping) with 100% aqueous mobile phase (0.1% H_3PO_4)



Separation on C18 stationary phase (strong end capping) with 90% aqueous (15 mM BMIm – $N(Tf)_2$ at pH 3) and 10% MeOH mobile phase

Need for additional interactions (i.e. π-π + ion pairing)

Separation on Phenyl stationary phase with 72% aqueous (15 mM HFBA) and 28% MeOH mobile phase; MS/MS detection.



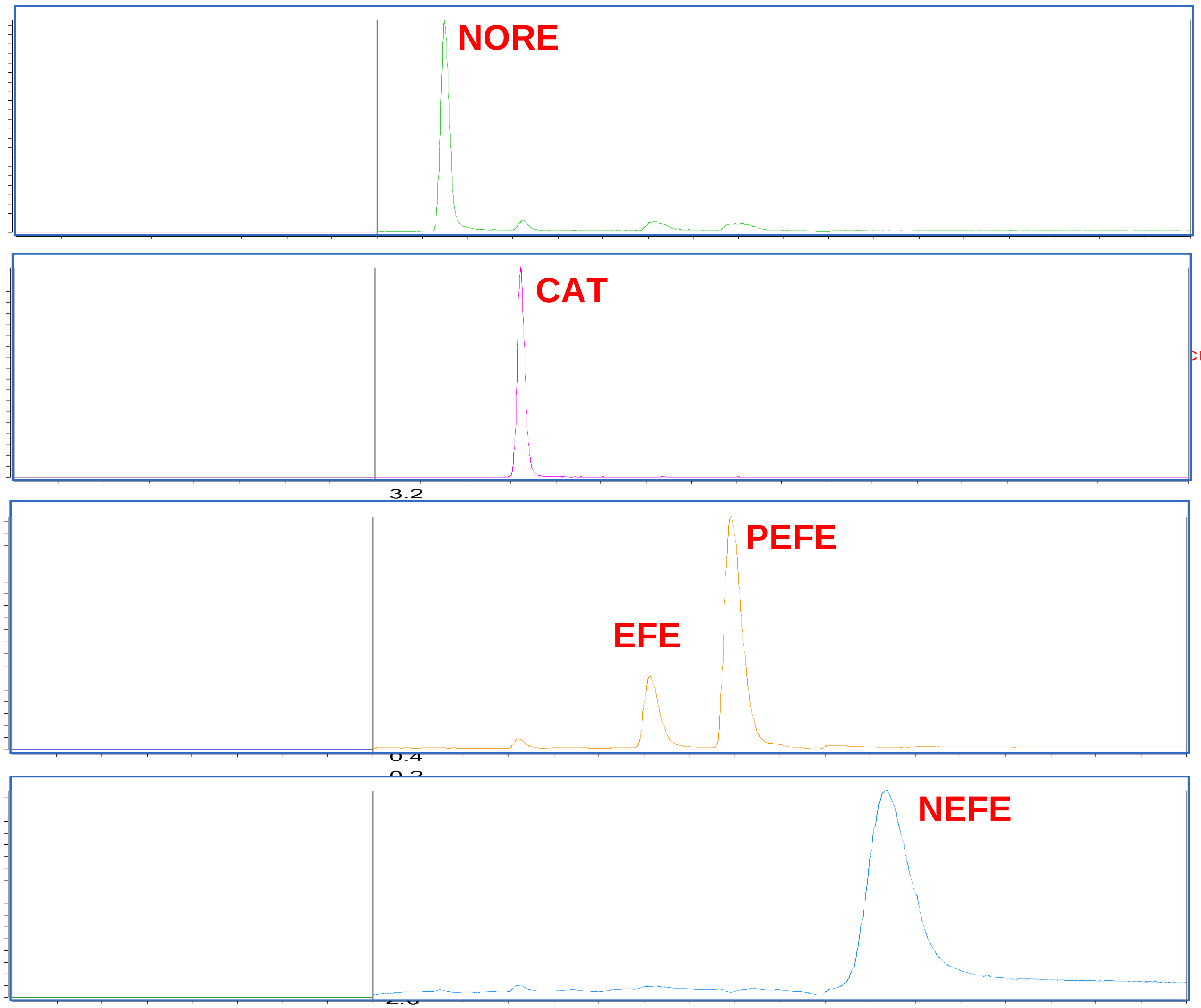
1.6

1.5

1.4

1.3

RP on C18 with strongly alkaline mobile phase (pH = 10.5)



I.S. stock solution
4-aminoantipyrin
conc.I.S. = 100 µg/mL
vol = 50 µL
solvent = methanol

10% TEA
vol = 50 µL
solvent = methanol

↓

Urine
vol = 1000 µL

↓

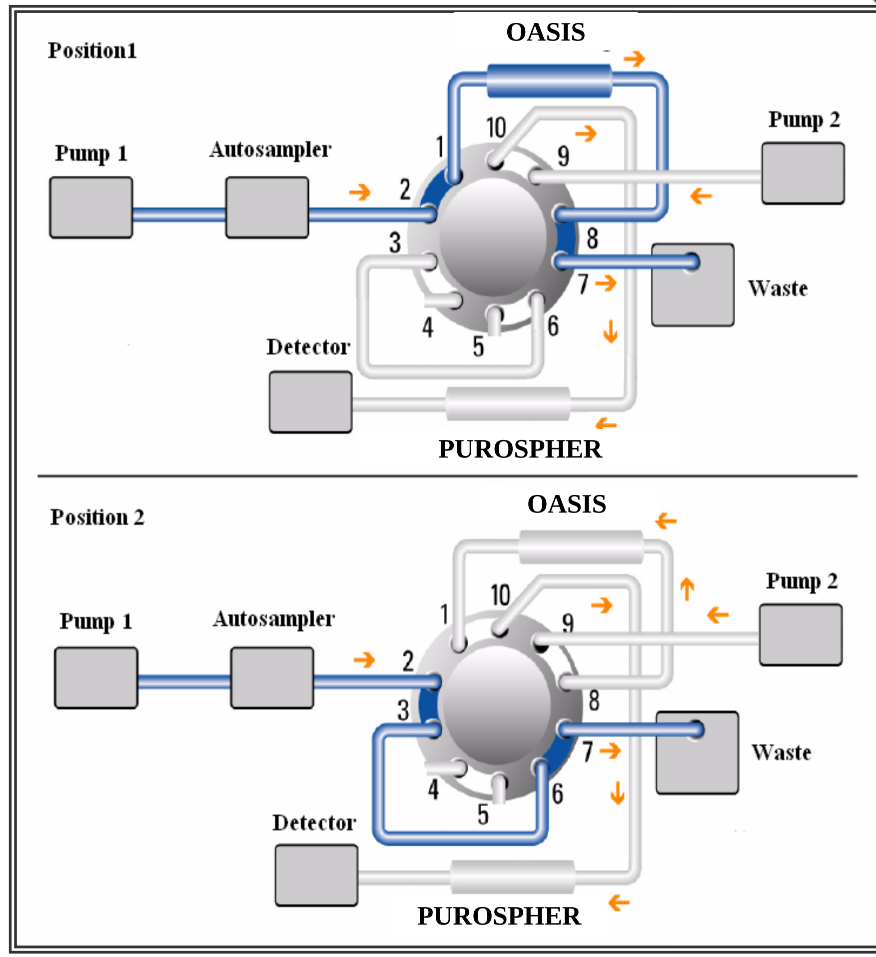
Vortex
t = 5 min.
speed = 2000 rpm

↓

Centrifuge
Temp.= 25°C
Time = 5 min.
speed = 12000 rpm

↓

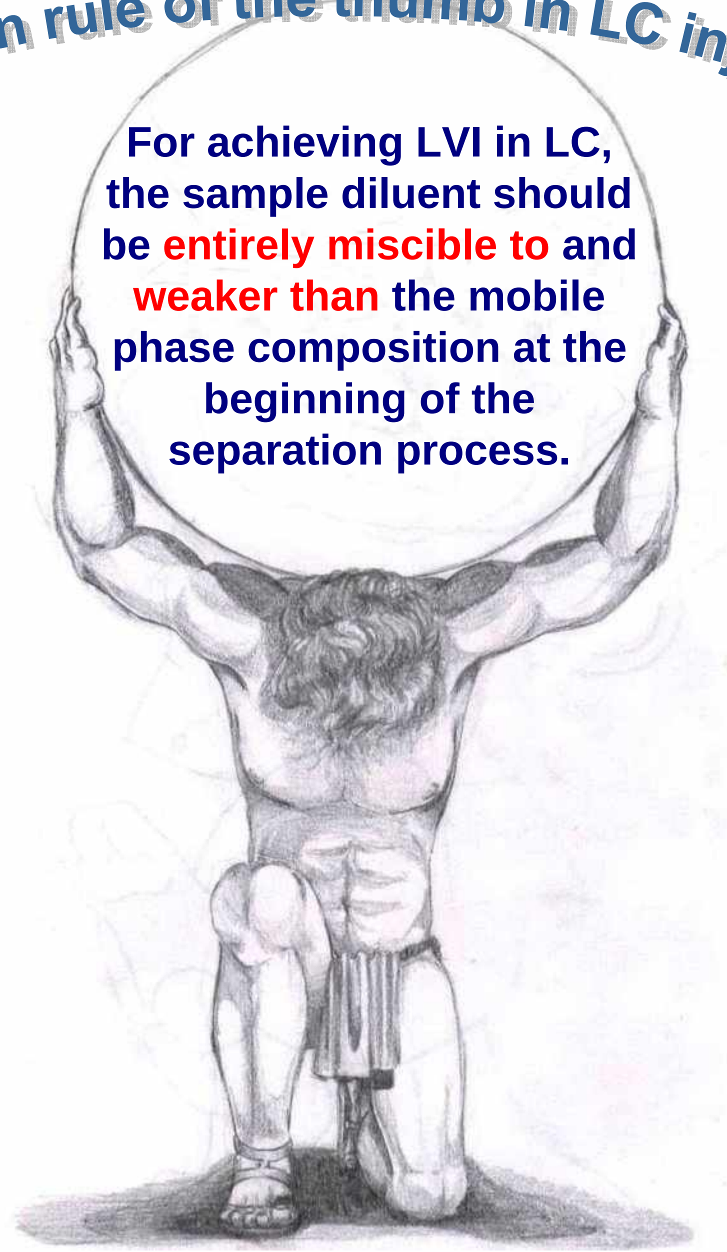
Transfer supernatant to vial
Inject 100 µL



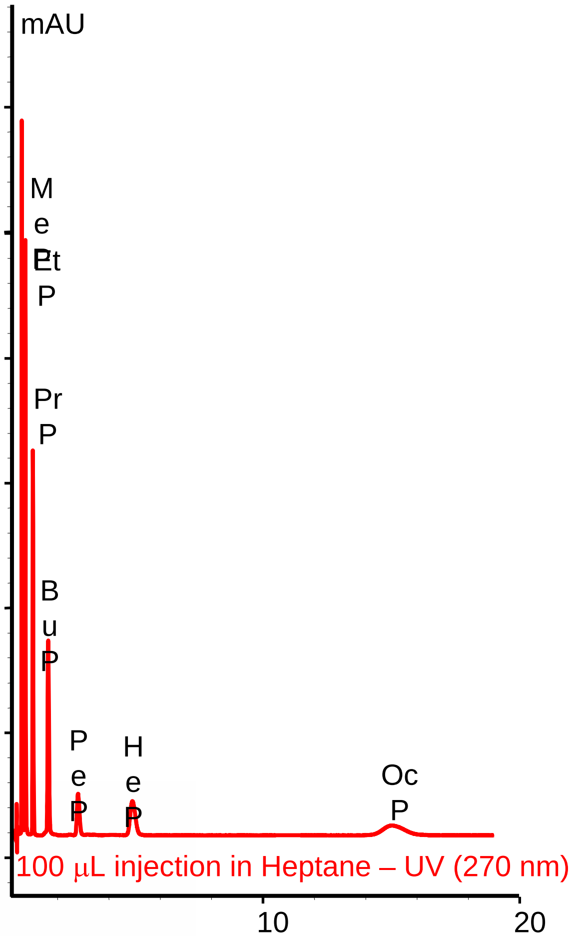
OASIS - vinyl pyrrolidone / divinyl benzene copolymer

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.



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

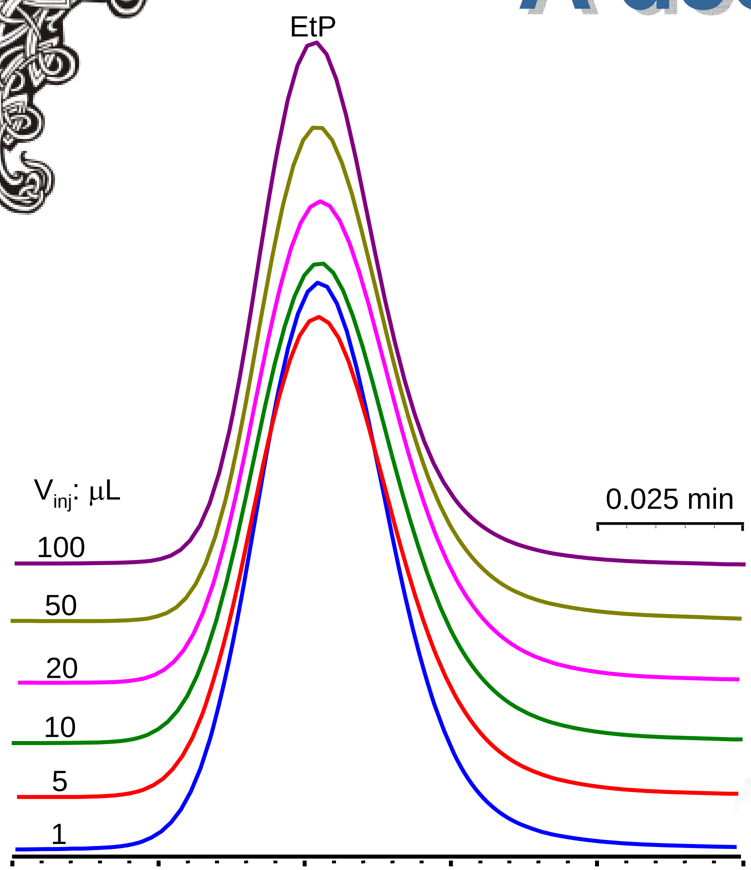
Detection: UV 270 nm

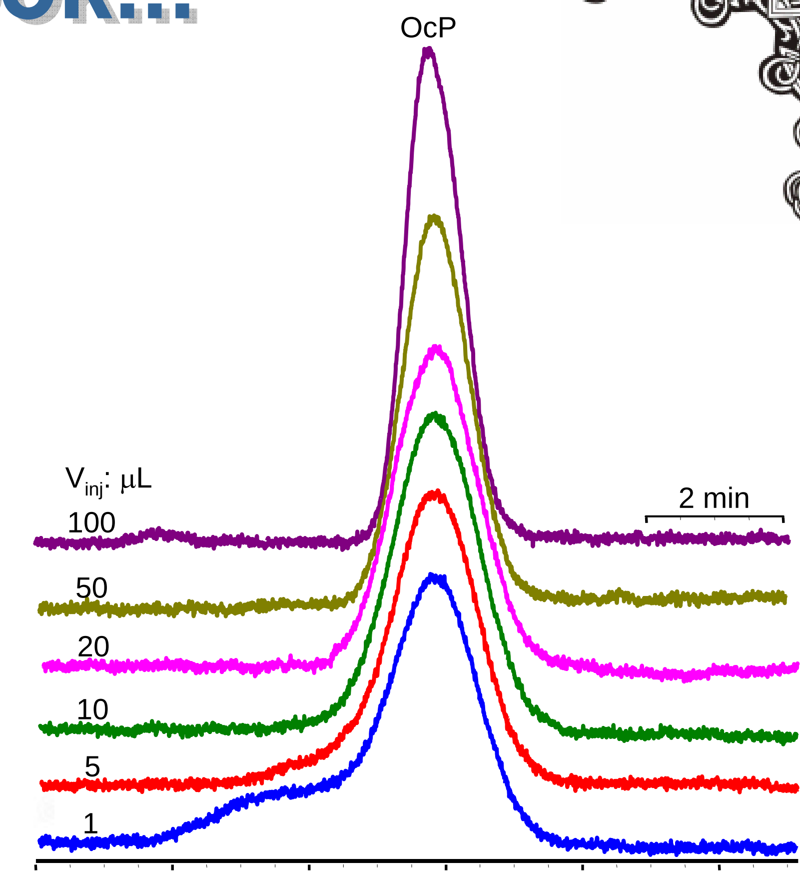
Injection volumes: 100 µL

Analytes: Methyl, Ethyl, Propyl, Butyl, Pentyl, Hexyl, Octyl Parabens

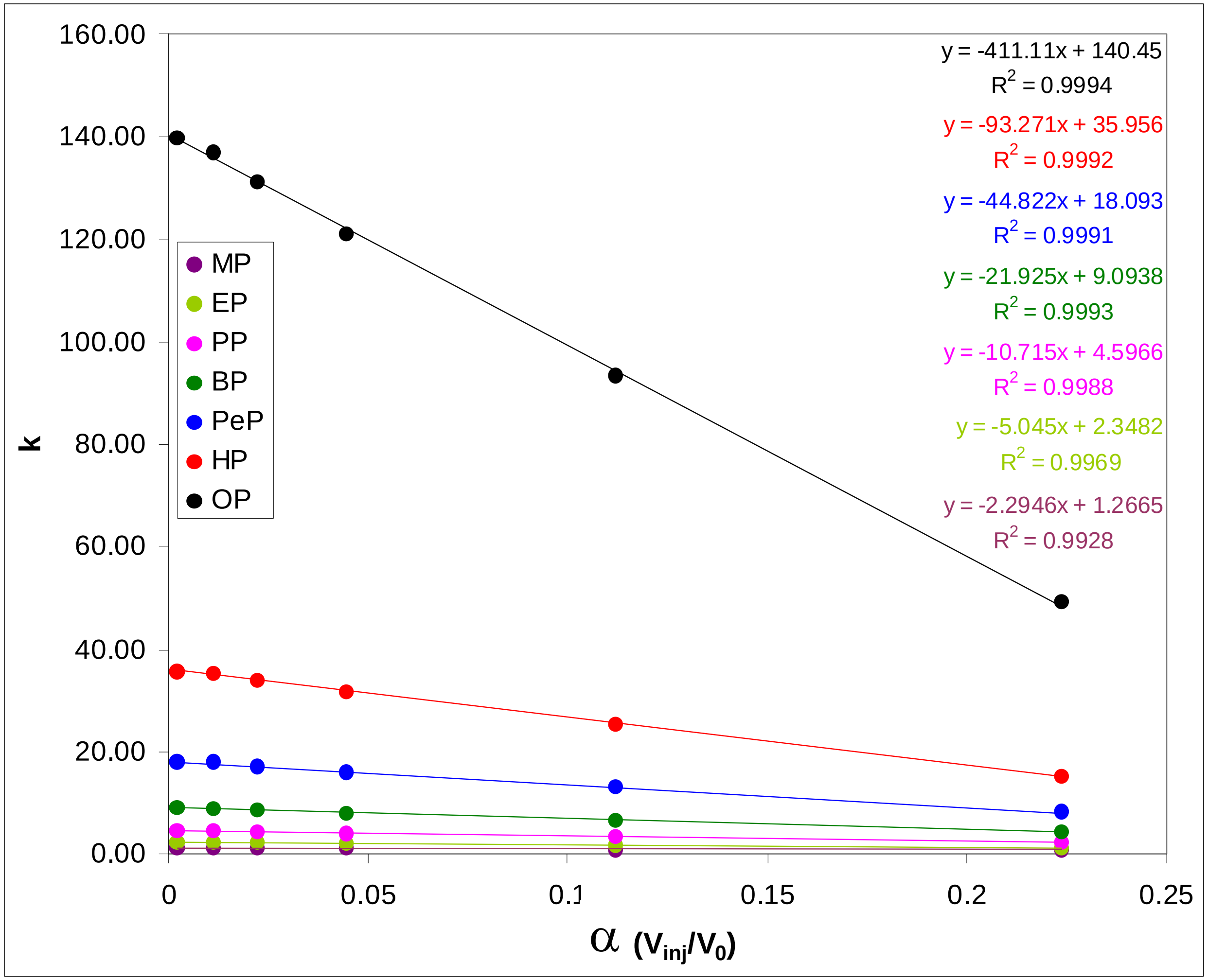
Diluents: Hexane, Heptane, iso-Octane, Decane, Dodecane

A deeper look...





and some calculations, it results that …



retention linearly decreases with the injected volume!

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] $\overset{\longrightarrow}{\leftarrow}$; [2] $\underset{\longleftarrow}{\rightarrow}$; [3] $\overset{\longrightarrow}{\leftarrow}$;

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

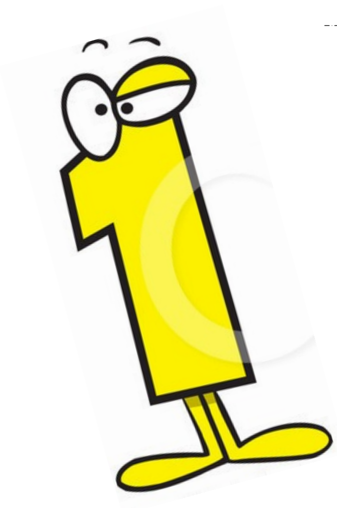
the $V'_{S.Ph.}$ available for A is a fraction of $V_{S.Ph.}$,

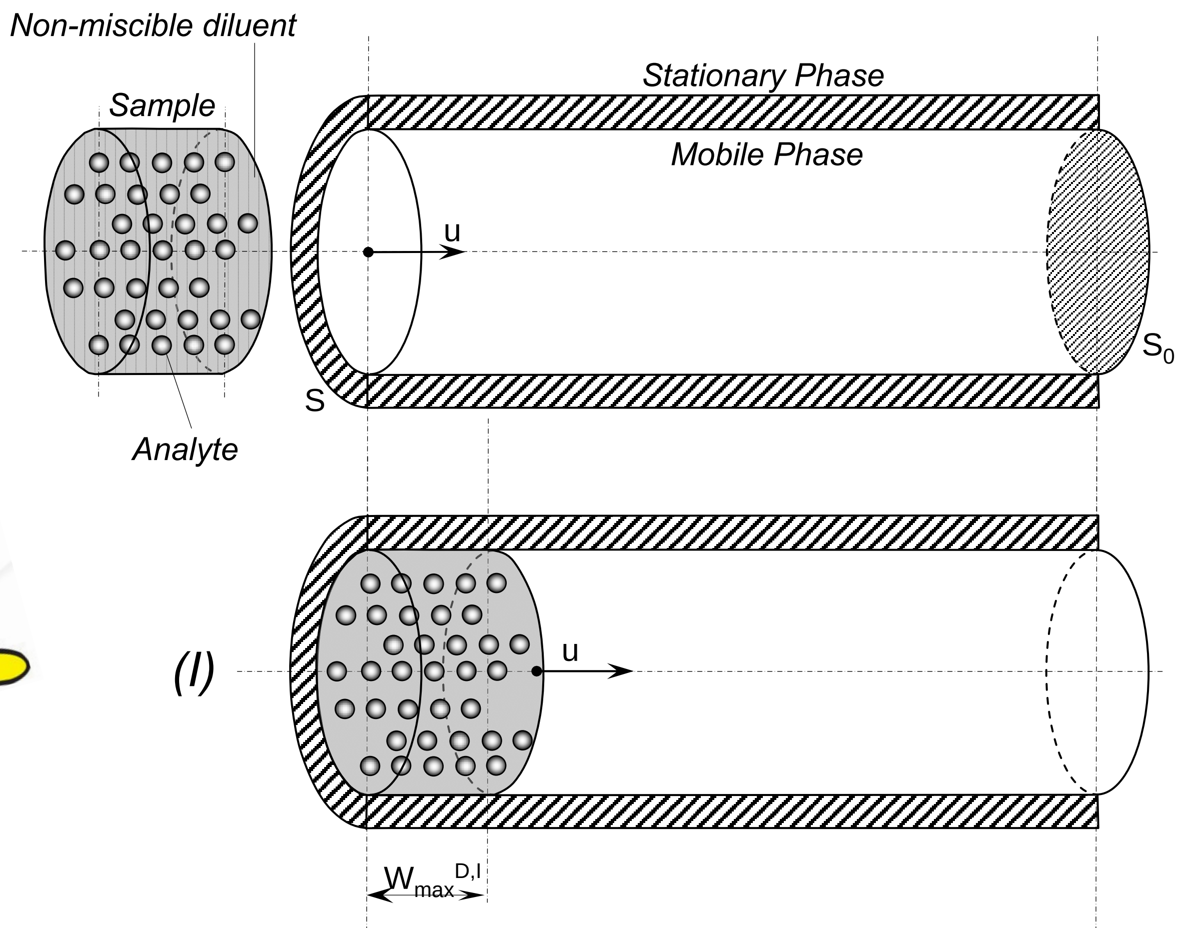
more precisely ($V_{S.Ph.} - \Delta V$), where $\Delta V = \zeta \times V_{inj}^D$, where ζ is a constant

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

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).

An alternative model: on-line SLE / RPLC



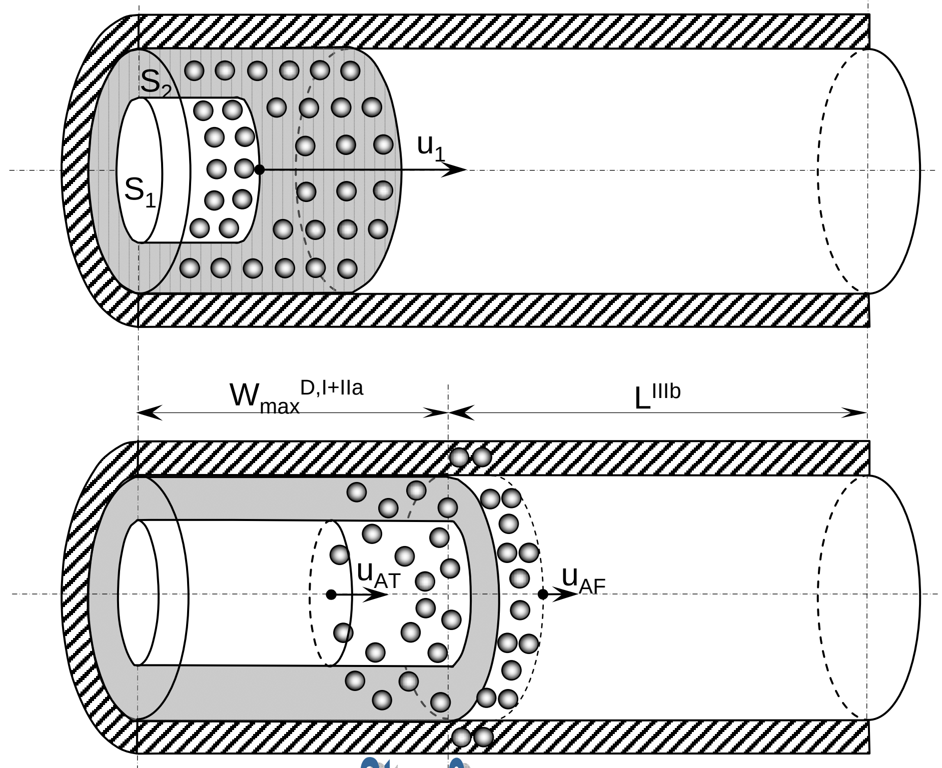


Step 1: Transport of the diluent in the column's head

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.

It can be demonstrated that:

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

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

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

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

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;

ι = inflation factor;

S_0 = M.Ph. cross section;

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

To conclude:

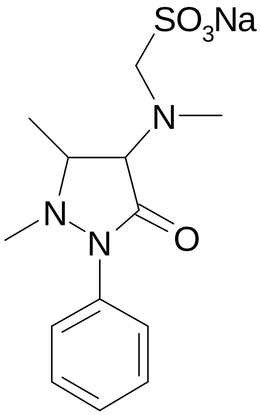
1. The on-line RP-SLE model fits better to experimental observations compared to the competitive adsorption model.

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.

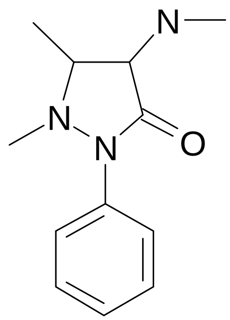
3. The kinetic of the LLE process is less important for analytes having an increased hydrophobic character, as long as the “free” stationary phase will refocus them.

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

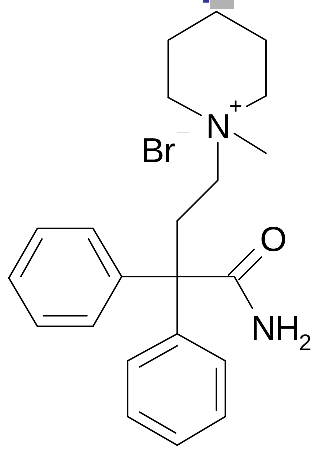
An application: solving "difficult" pharmaceutical formulations!



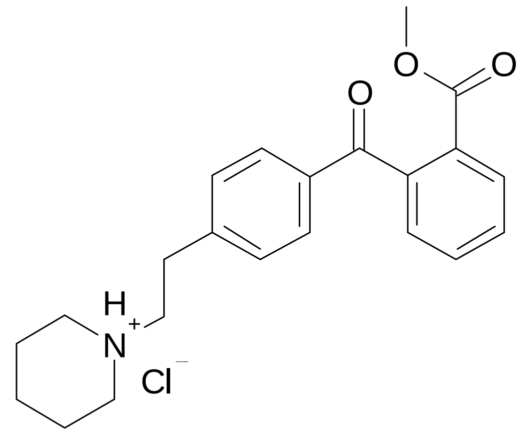
Metamizole sodium
(MTZ)
log Dow (pH=3)
-2.24
500 mg/mL
500 X dilution



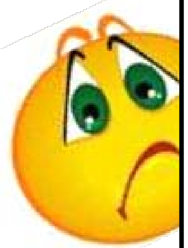
Metamizole Imp. C
(MTC)
Log Dow (pH=3)
0.76
(max. 3.5% from MTZ)
(17.5 mg/mL)
25 X dilution



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

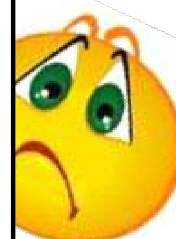


Polar Compounds!

Opposite ion pairing characteristics!

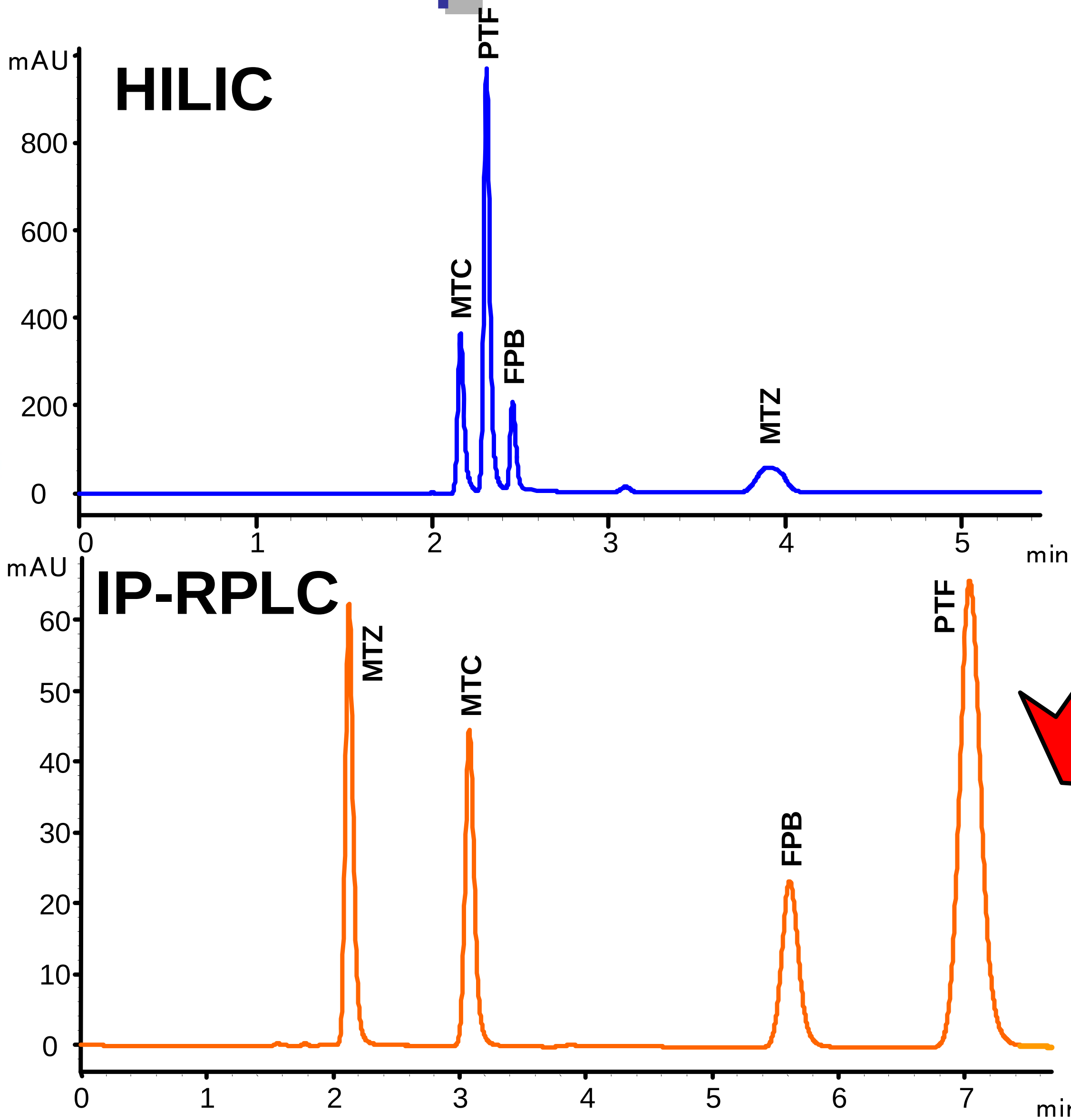
Tailing favored by increased interaction to residual silanols!

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:

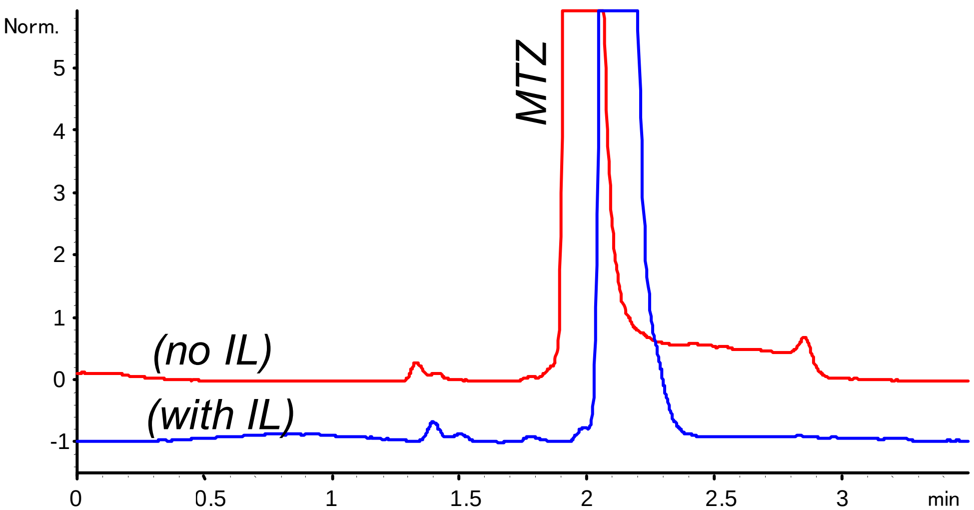
UV 290 nm (MTZ, MTC, PTF); UV 220 nm (FPB)

$\mathbf{V_{inj}}$ = 20 µL (for FPB);

Diluent : 1-Octanol

SHS = sodium hexane sulfonate

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



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

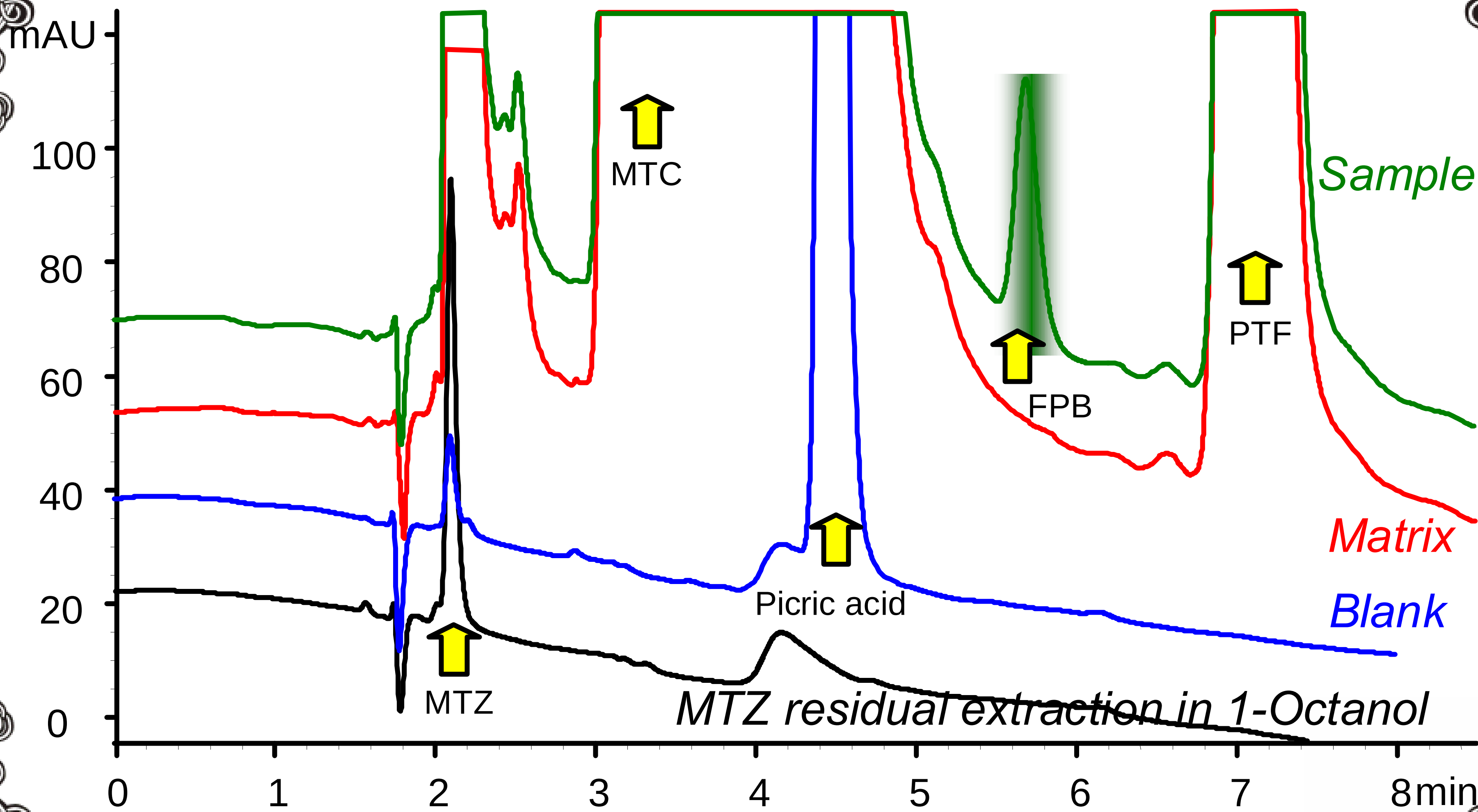
Vortex
t = 10 min.
Speed = 2000 rpm

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

Transfer organic layer to vial
Vol = 500 µL

Inject
V_{inj} = 20 µL

An incurred sample!



Validation was achieved!

Applications in bioanalysis:

#	Analyte/s	Matrix	Non-miscible diluent/extractant	Purpose
1	Fenspiride	Human Plasma	1-octanol	bioequivalence
2	Enalapril & Enalaprilat	Human Plasma	1-octanol	bioequivalence, greening approach (PC)
3	Simvastatin & simvastatic acid	Human Plasma	limonene	bioequivalence
4	Indapamide	Whole blood	1-octanol	bioequivalence



Cheregi M, Albu F, Udrescu Ş, R?ducanu N, Medvedovici A. Greener bioanalytical approach for LC/MS-MS assay of enalapril and enalaprilat in human plasma with total replacement of acetonitrile throughout all analytical stages. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2013;927:124-32.

Medvedovici A, Udrescu S, David V. Use of a green (bio) solvent - limonene - as extractant and immiscible diluent for large volume injection in the RPLC-tandem MS assay of statins and related metabolites in human plasma. Biomedical Chromatography. 2013;27(1):48-57.

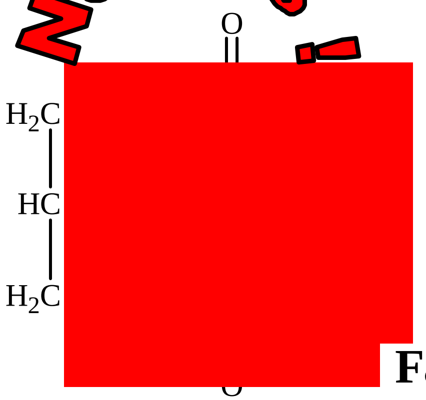
Medvedovici A, Udrescu S, Albu F, Tache F, David V. Large-volume injection of sample diluents not miscible with the mobile phase as an alternative approach in sample preparation for bioanalysis: An application for fenspiride bioequivalence. Bioanalysis. 2011;3(17):1935-47.

Udrescu S, Sora ID, Albu F, David V, Medvedovici A. Large volume injection of 1-octanol as sample diluent in reversed phase liquid chromatography: Application in bioanalysis for assaying of indapamide in whole blood. J Pharm Biomed Anal. 2011;54(5):1163-72.

MS detection troubles in LC!
i.e. no ionization!

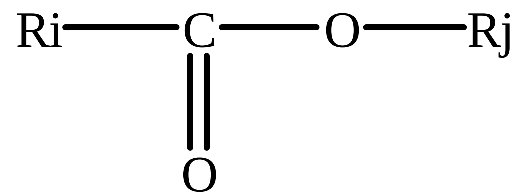
JOJOBA Seeds (*Simmondsia Chinesis*), orig. Sonora Desert – U.S.

No TGs !



Mainly Wax Esters !

$C_{i:n}$-COO-$C_{j:m}$



Jojoba Oil / Hydrolysis

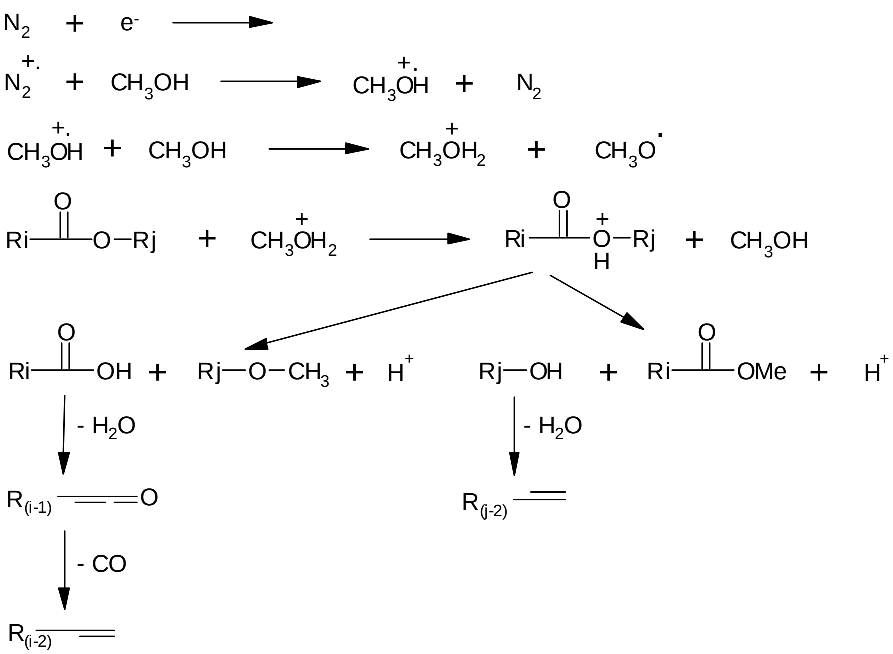
Fatty Acids **Fatty Alcohols**

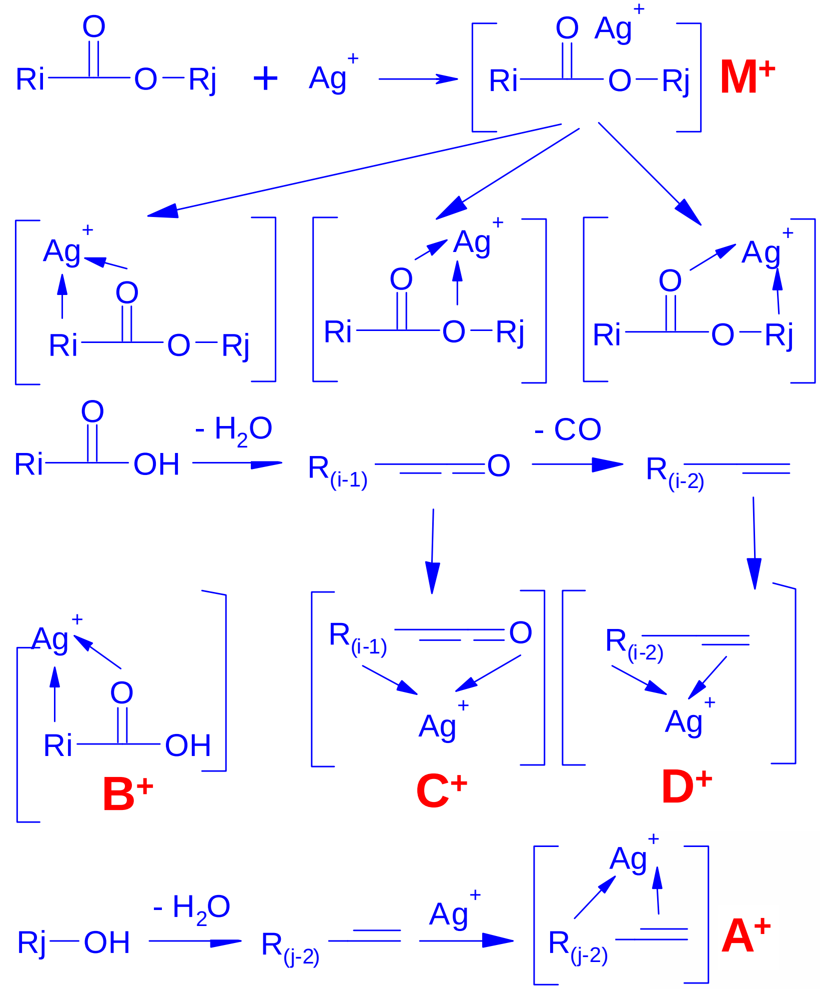
Methylation

GC Analysis

OV-1; Carbowax; CN

Ionization processes:





Coordination Ion Spray (CIS)

Separation conditions:

Column	Spherisorb BDS 25 cm x 2.0 mm x 5 µm
M. Phase	MeOH / Acetone / Hexane : 2 / 1 / 1
Elution	Isocratic
Flow	0.2 mL/min
$AgNO_3$ flow	10 µL/min (post column)
Vol. Inj.	10 µL
MS	(+) mode
Gas Temp.	200 °C
Gas Flow	12 L/min
Nebulizing P.	45 psi
Cap. Voltage	5 kV
Mass Scan	100-800 m/e
CID Voltage	290 V

RETENTION RULE

Acc. to **ECN** (Equivalent Chain Number)

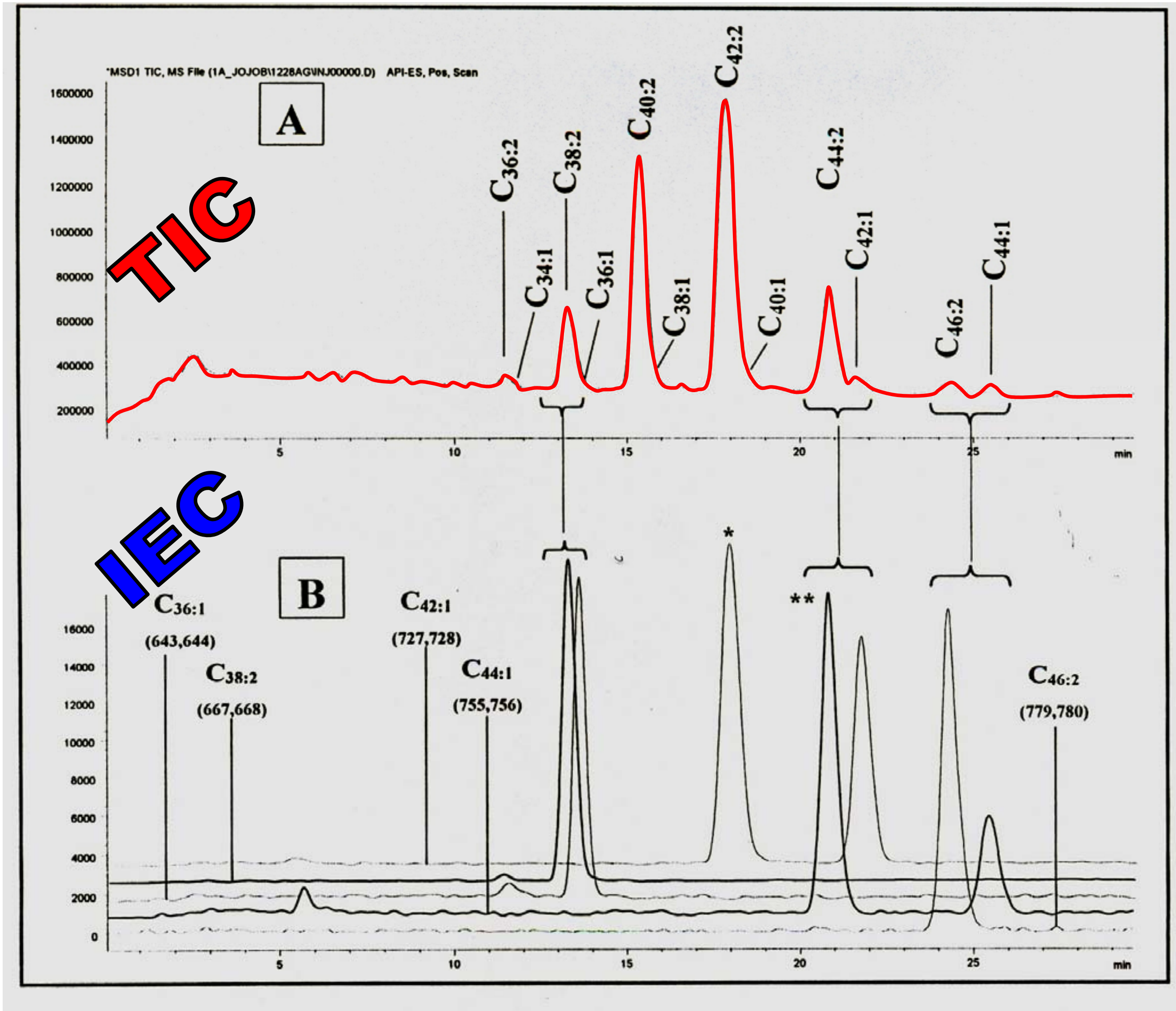
$$\mathbf{ECN = CN - 2\ NDB}$$

Ex.

$C_xH_{2x} \Rightarrow CN = x;\ NDB = 1;\ ECN = x-1$

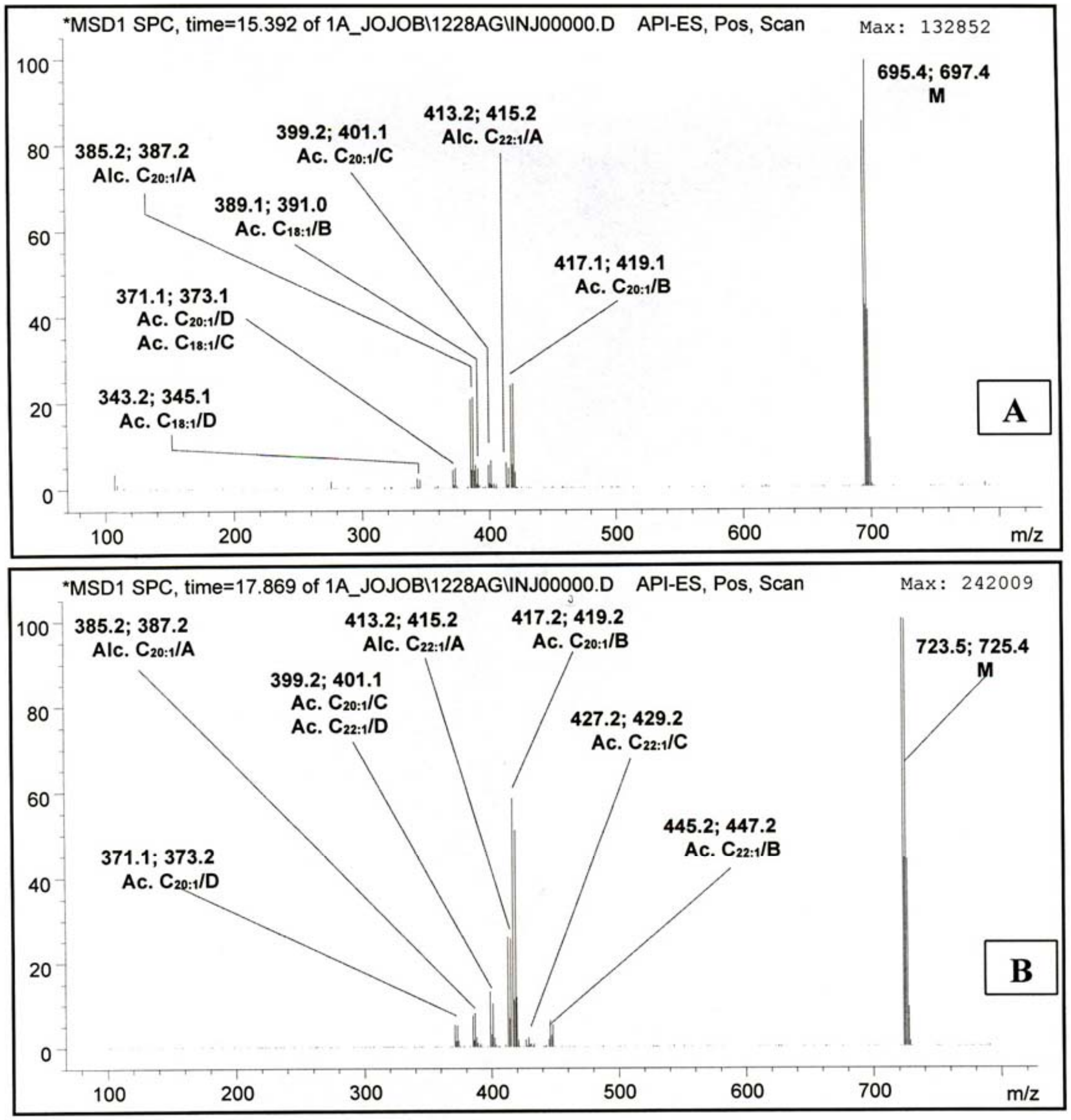
$C_{(i+2):2}$ ***elutes before*** $C_{i:1}$

Results:



*MSD1 TIC, MS File (1A_JOJOB\1228AG\INJ00000.D) API-ES, Pos, Scan
A
TIC
C36:2
C34:1
C38:2
C36:1
C40:2
C38:1
C42:2
C40:1
C44:2
C42:1
C46:2
C44:1
1600000
1400000
1200000
1000000
800000
600000
400000
200000
5
10
15
20
25
min
IEC
B
C36:1
(643,644)
C38:2
(667,668)
C42:1
(727,728)
C44:1
(755,756)
*
**
C46:2
(779,780)
16000
14000
12000
10000
8000
6000
4000
2000
0
5
10
15
20
25
min

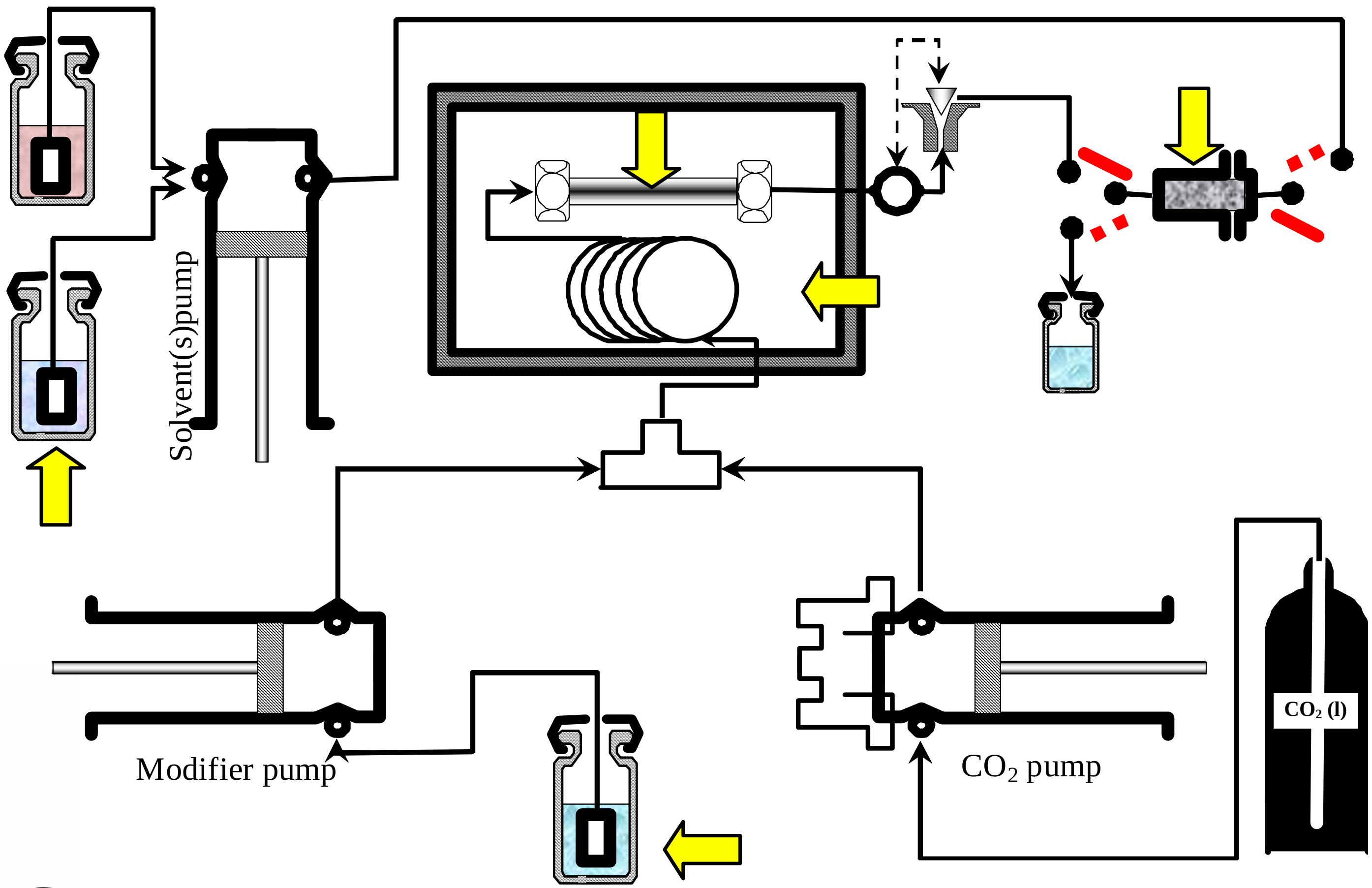
MS spectral information:



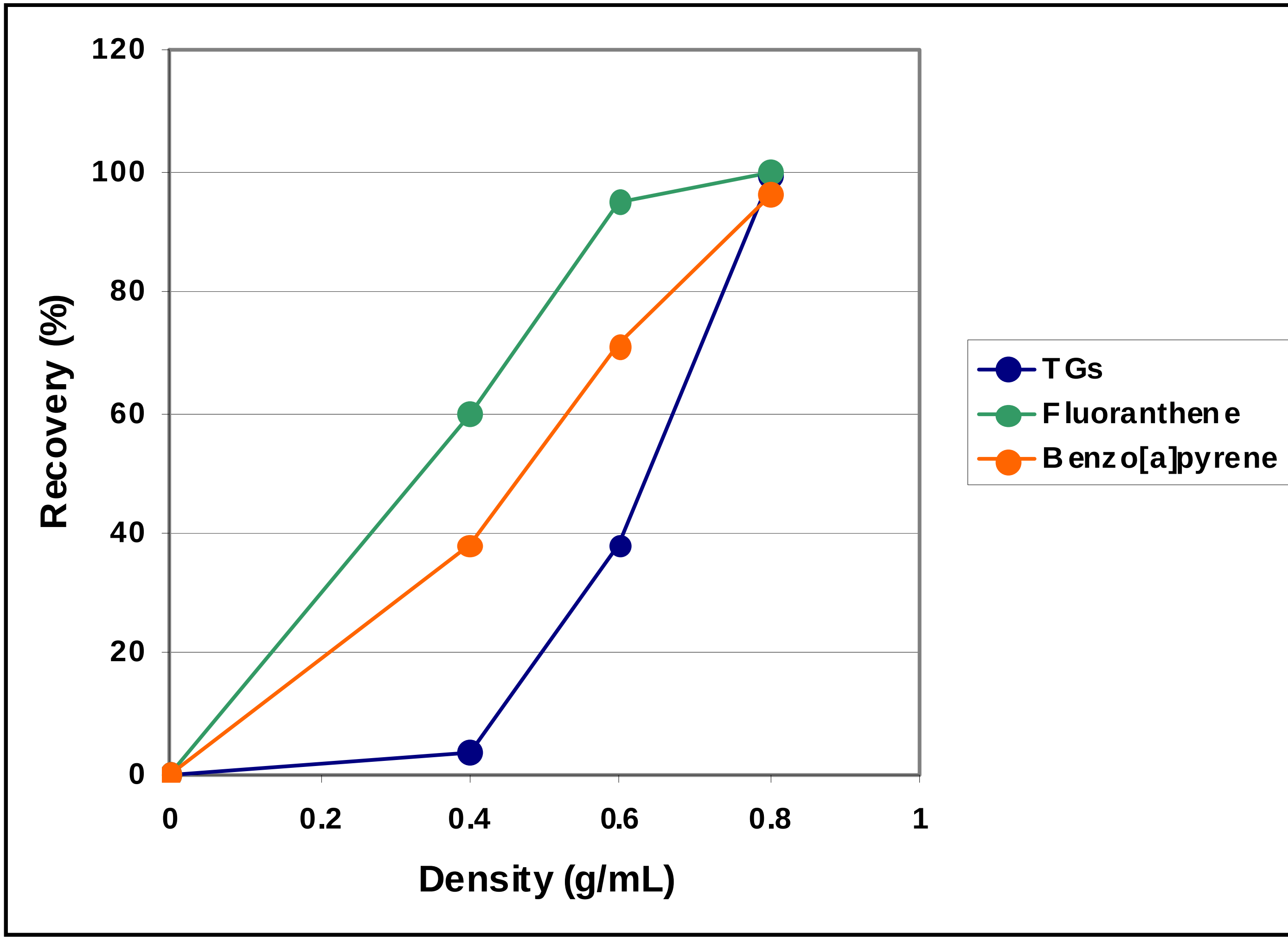
Analysis of jojoba oil by LC-coordination ion spray-MS (LC-CIS-MS) Medvedovici A, Lazou K, d'Oosterlinck A, Zhao Y, Sandra P, J. Sep. Sci., *25 (2002) 173-178.*

Sample preparation for "difficult" samples:

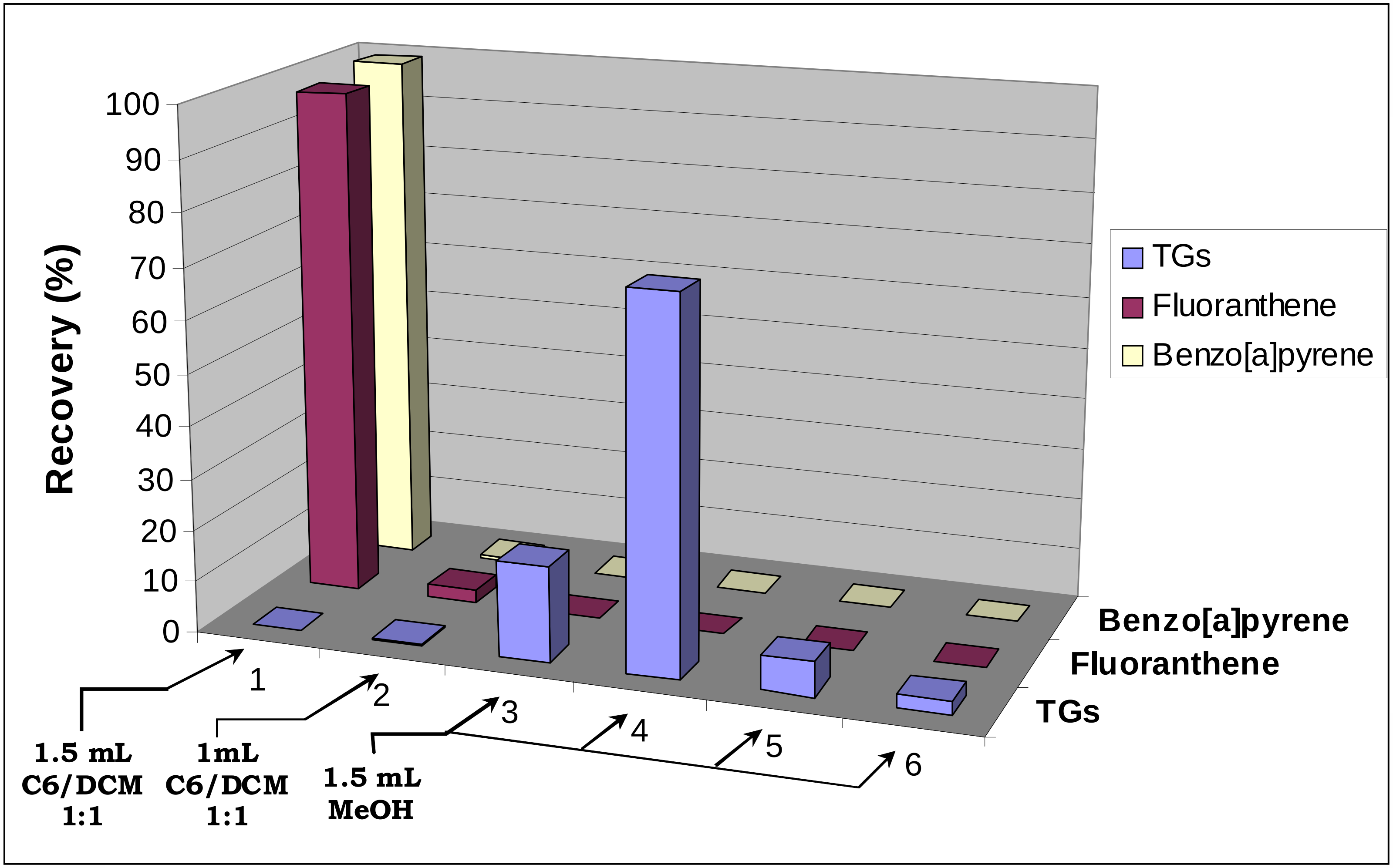
SFE



PAHs in barbecued steak?

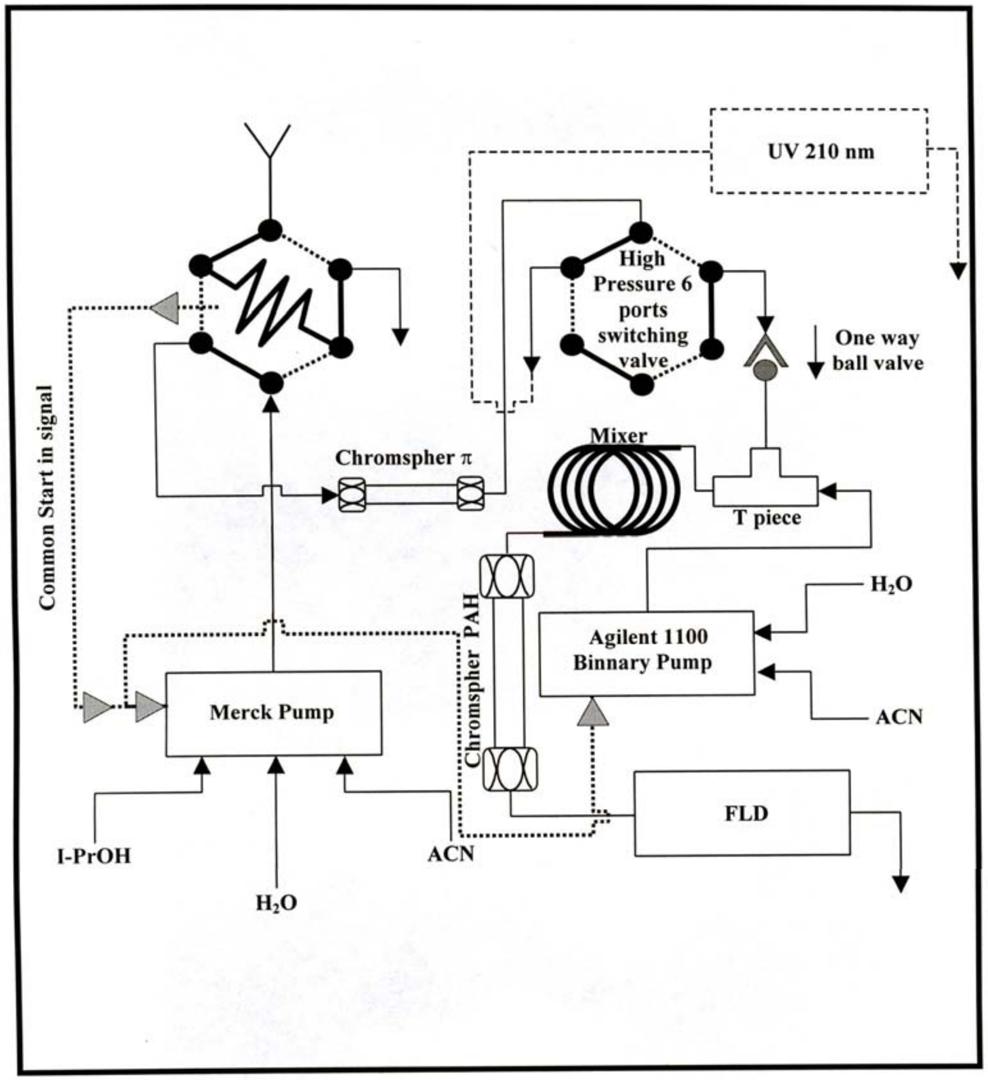


Selectivity through controlling desorption during the SPE step.



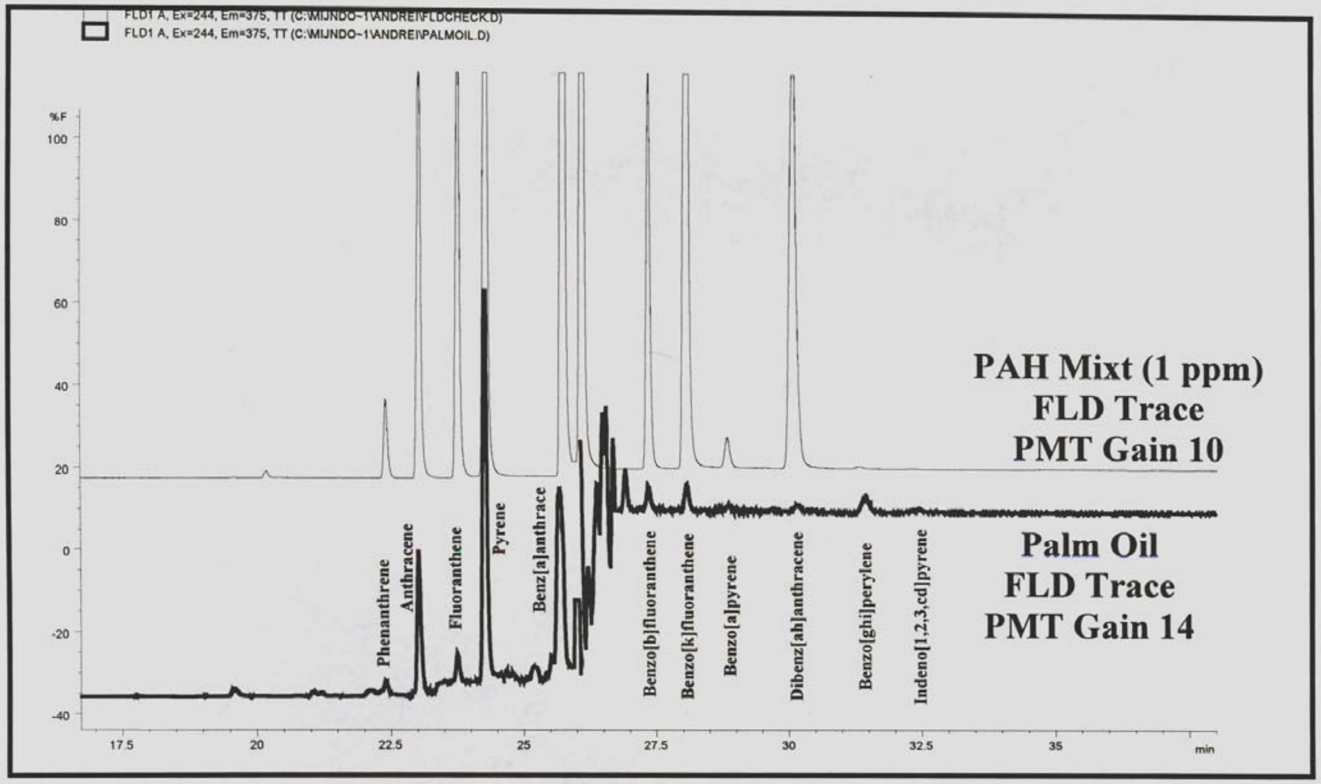
Sandra P, Medvedovici A, Kot A, David F, in Packed Column Supercritical Fluid Chromatography, (C. Berger; K. Anton Eds.) Marcel Dekker Publishing Inc., pg. 369 – 401 (1997).

On-line SPE: PAHs in Palm Oil



Chromsphere pi: Ag loaded adsorbent.

Analytical results:



Sandra P, Medvedovici A, unpublished results.

A final question:

Is chemistry an "orphan" domain in Romania?

Probably Yes

Consequently, we have to learn about "group solidarity"!

... and to not forget that the few jobs on

the market exist mostly in ... the analytical field!

Thank you for the invitation, patience and kind attention!

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PNII_ID_PCE_2011_3_0152/C. no. 310/2011.

I acknowledge the support of former and present co-workers (the list is quite long...)