GREEN CHROMATOGRAPHIC ASSAY OF PAHs IN DIETARY SUPPLEMENTS AND FOODSTUFF

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INTERNATIONAL WORKSHOP
"Food Chemistry & Engineering"

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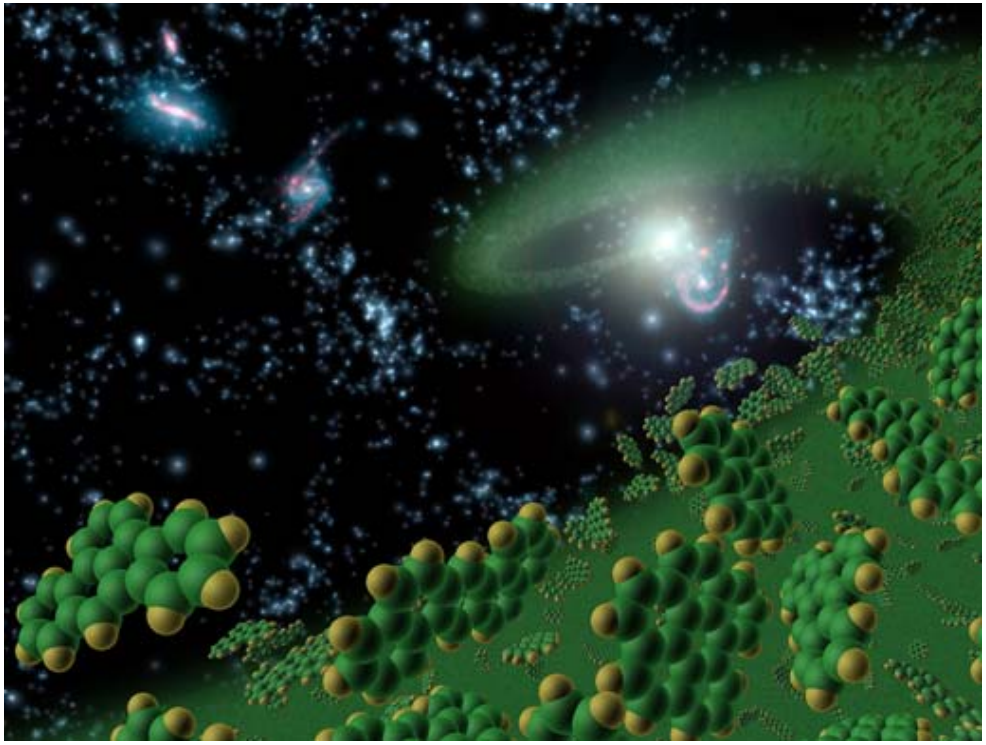
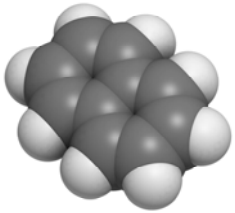
PAHs - everywhere in ... time and space!



" NASA's Spitzer Space Telescope is the first telescope to see polycyclic aromatic hydrocarbons so early -10 billion years further back in time than seen previously. Spitzer detected these molecules in galaxies when our universe was one-fourth of its current age of about 14 billion years. These large molecules, are among the building blocks of life."

PAHs



Definition - Hydrocarbons with fused aromatic rings.

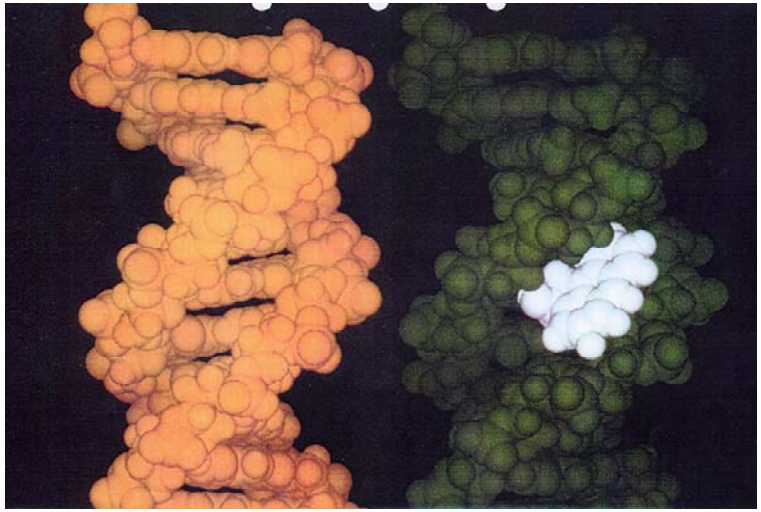
Sources - Biogenic, Petrogenic, Pyrogenic (natural & anthropogenic)

Generation - O_2 deficient combustion of organic matter.

Occurrence - Air, Water, Soil, Dry/Wet deposition on plants.

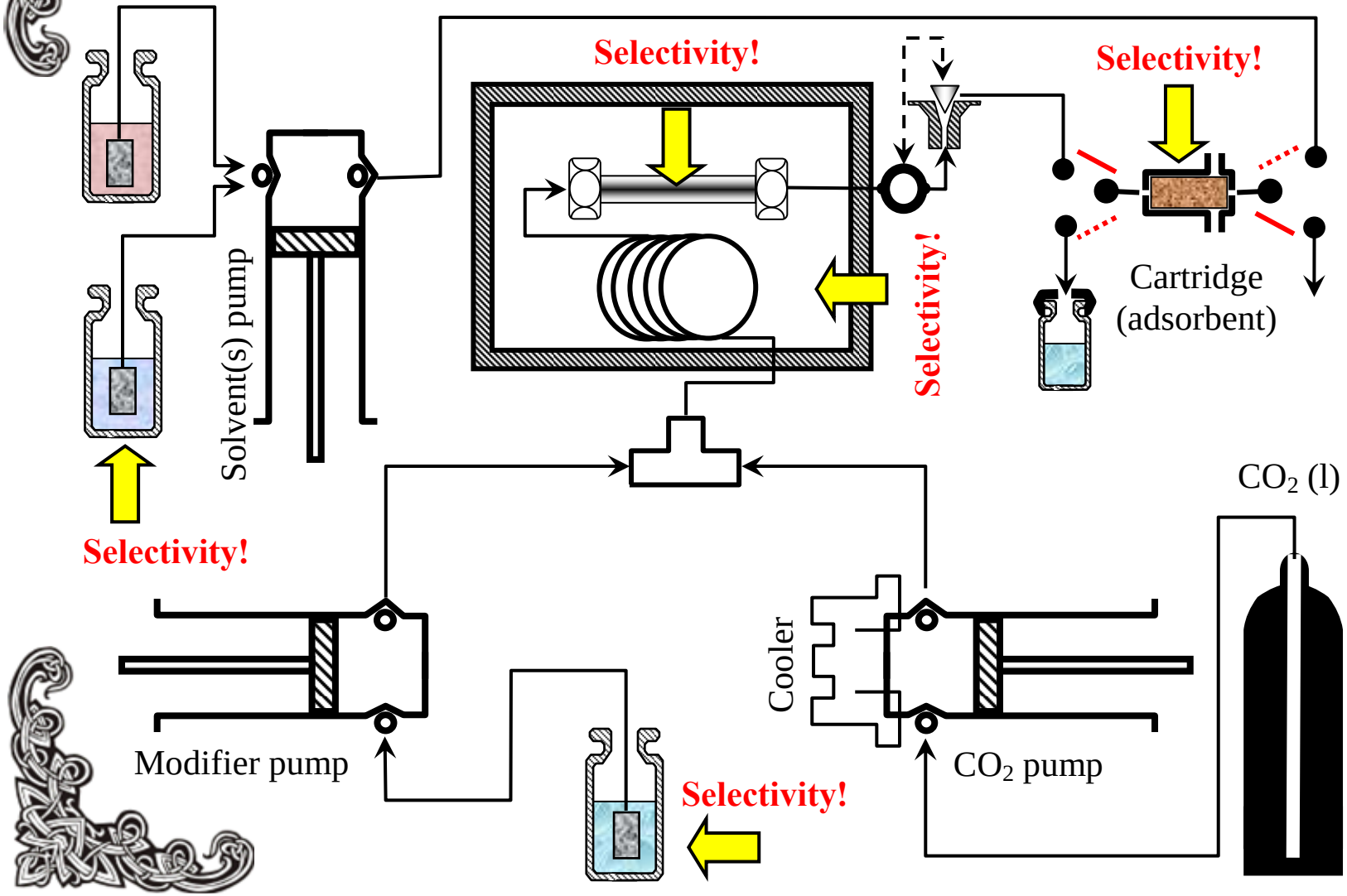
Exposure - Direct contact, breathing, eating, drinking.

Impact - Toxic, Carcinogenic, Mutagenic

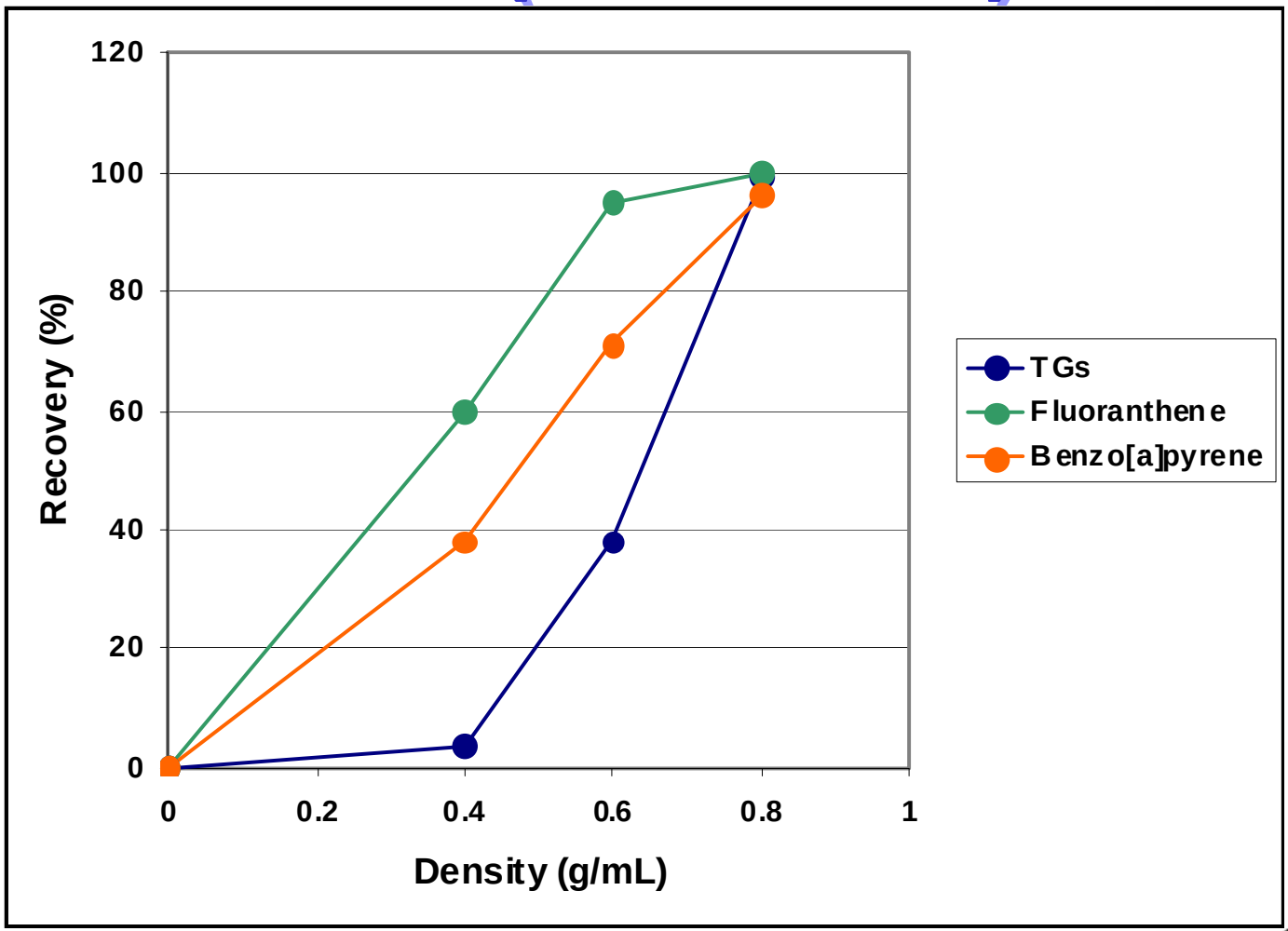


PAHs in fatty matrices

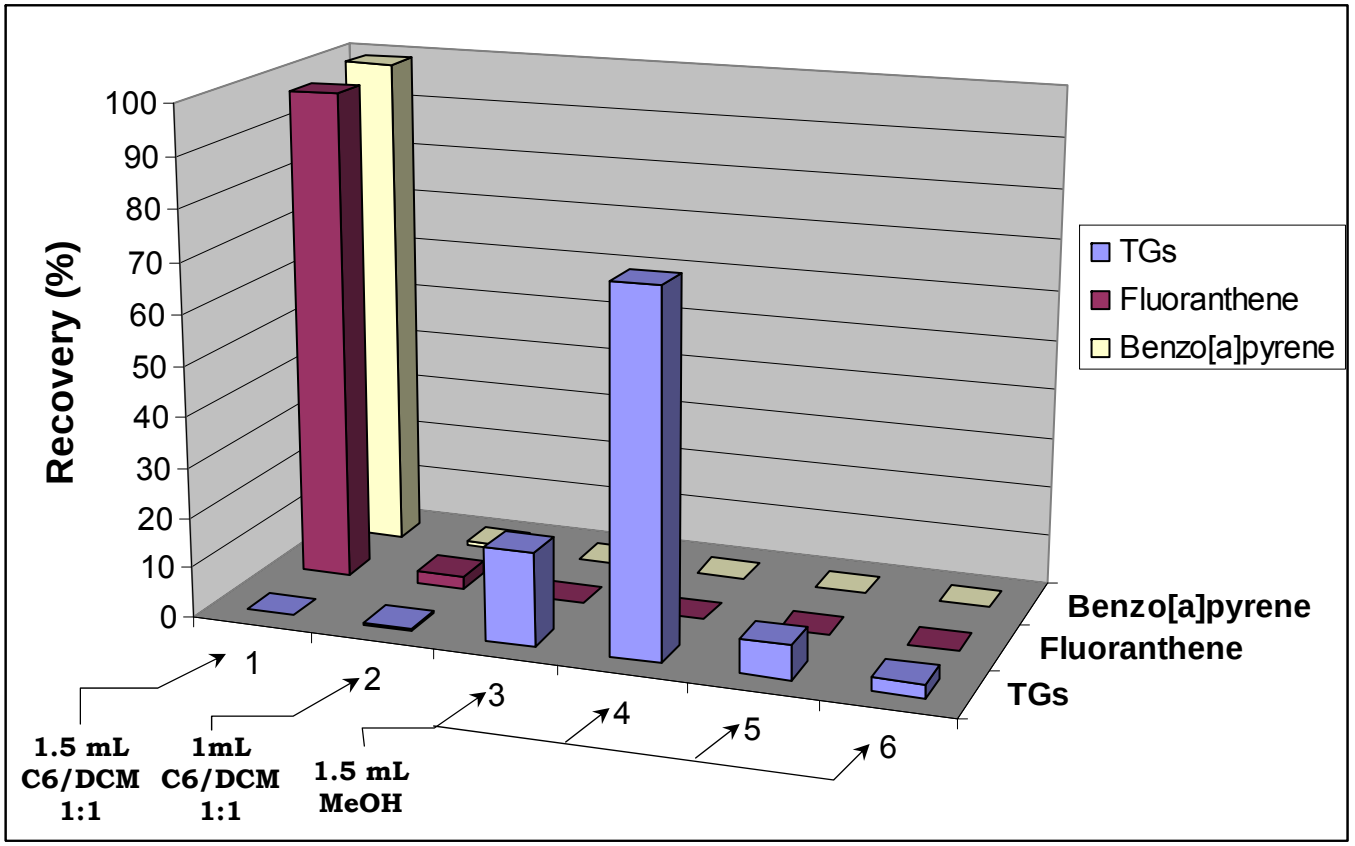
SFE



PAHs in smoked (barbecued) meat.

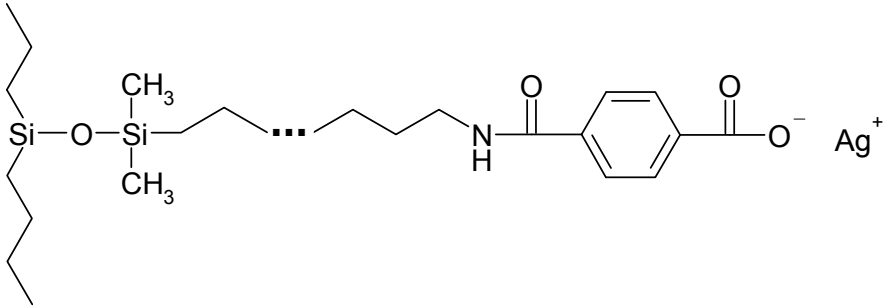


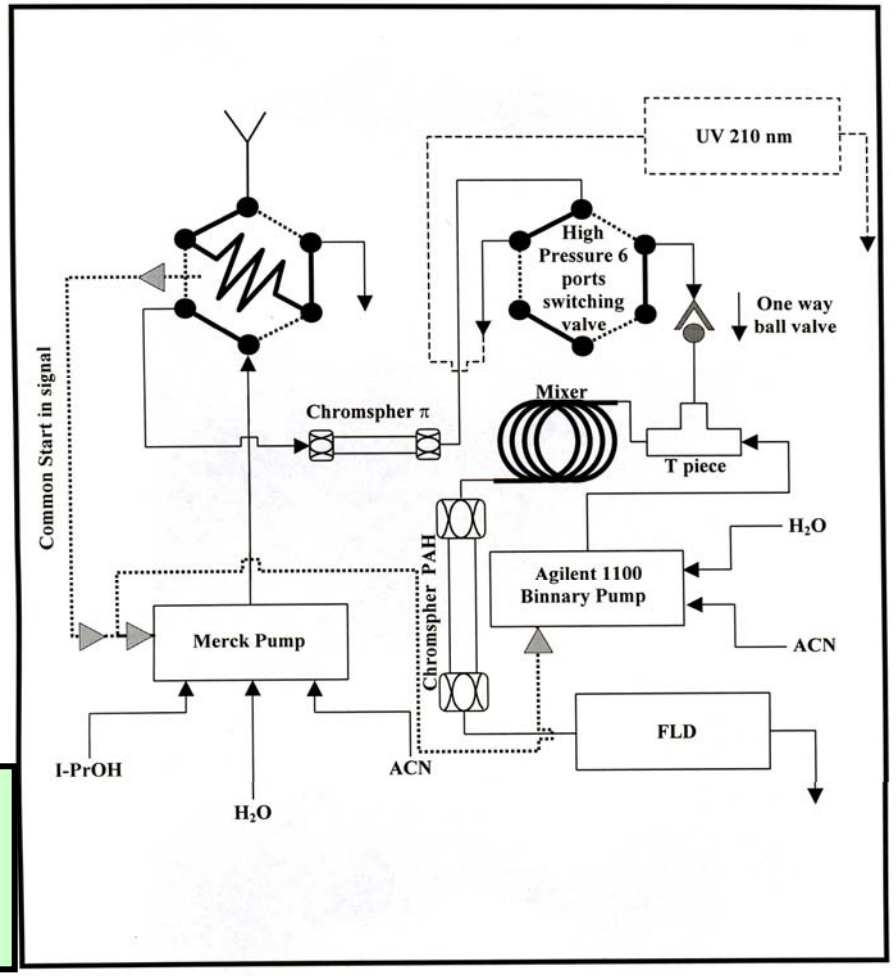
Selectivity through controlling desorption during the SPE step.



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).

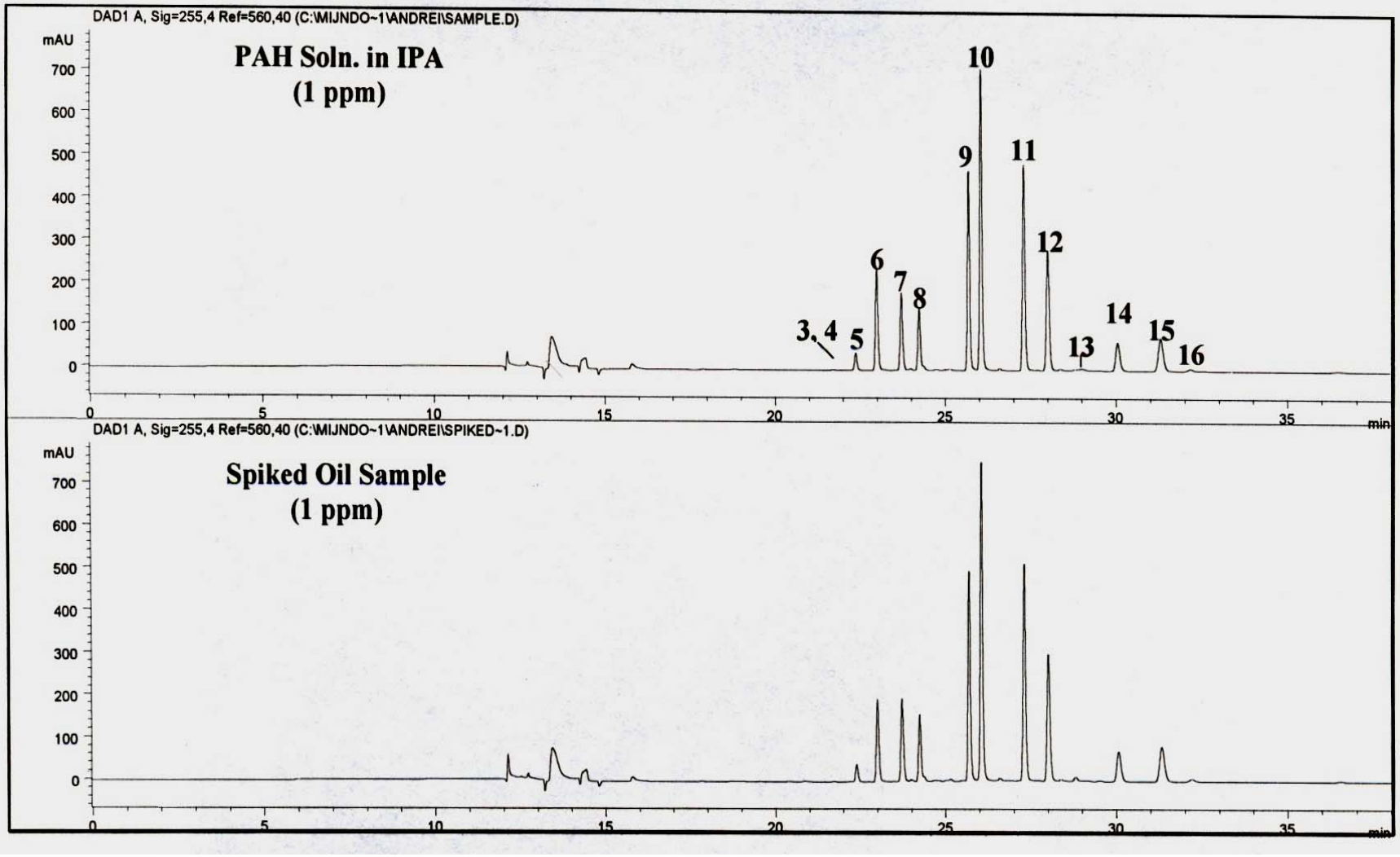
On-line SPE: PAHs in Vegetable Oils



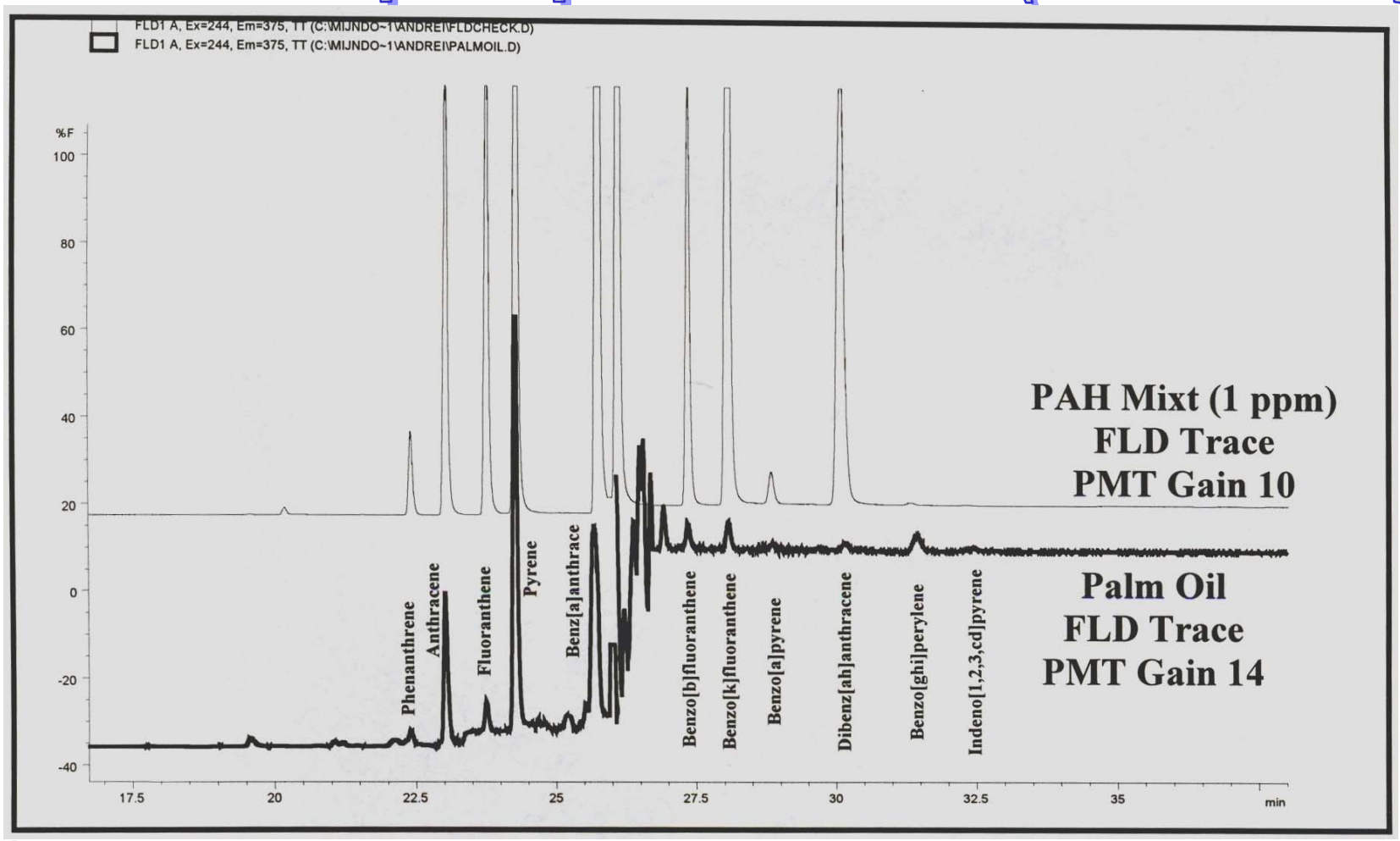


"Special" Adsorbents:
Metal Ion Embedded (for π-π interactions)

Results - (FLD)

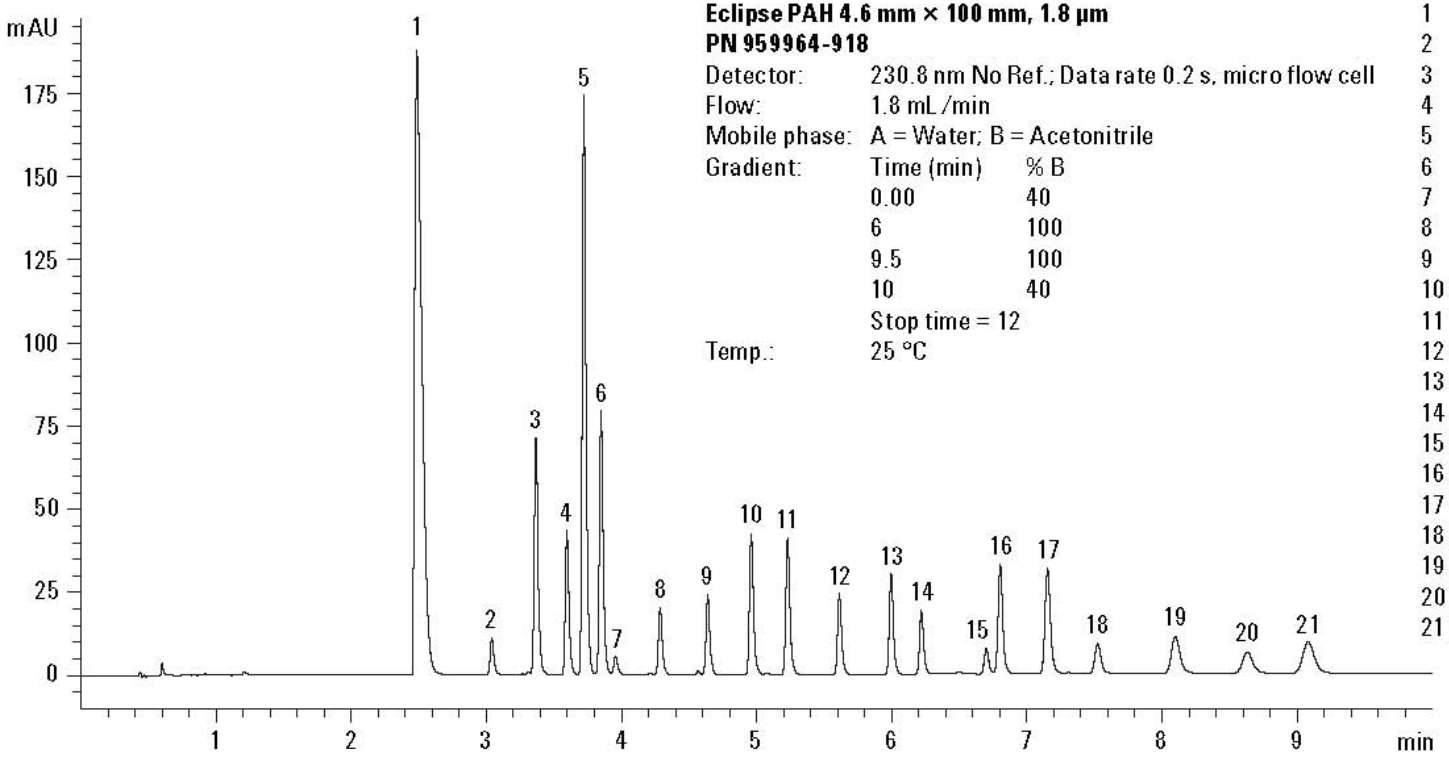

DAD1 A, Sig=255,4 Ref=560,40 (C:\MIJNDO~1\ANDREI\SAMPLE.D)
mAU
PAH Soln. in IPA
(1 ppm)
3, 4
5
6
7
8
9
10
11
12
13
14
15
16
min
DAD1 A, Sig=255,4 Ref=560,40 (C:\MIJNDO~1\ANDREI\SPIKED~1.D)
mAU
Spiked Oil Sample
(1 ppm)
min

Incurred samples - positive results (unfortunately)



Agilent Technologies: Application Note 5989-7968 EN (2008) .

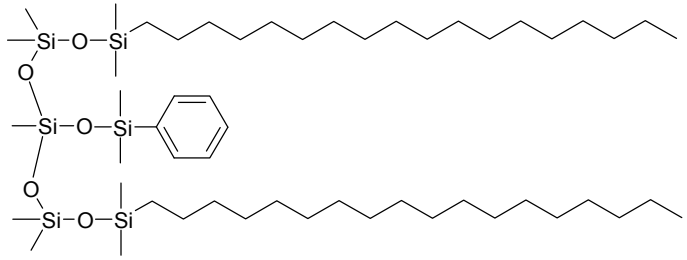
Straight ways for LC separation of PAHs



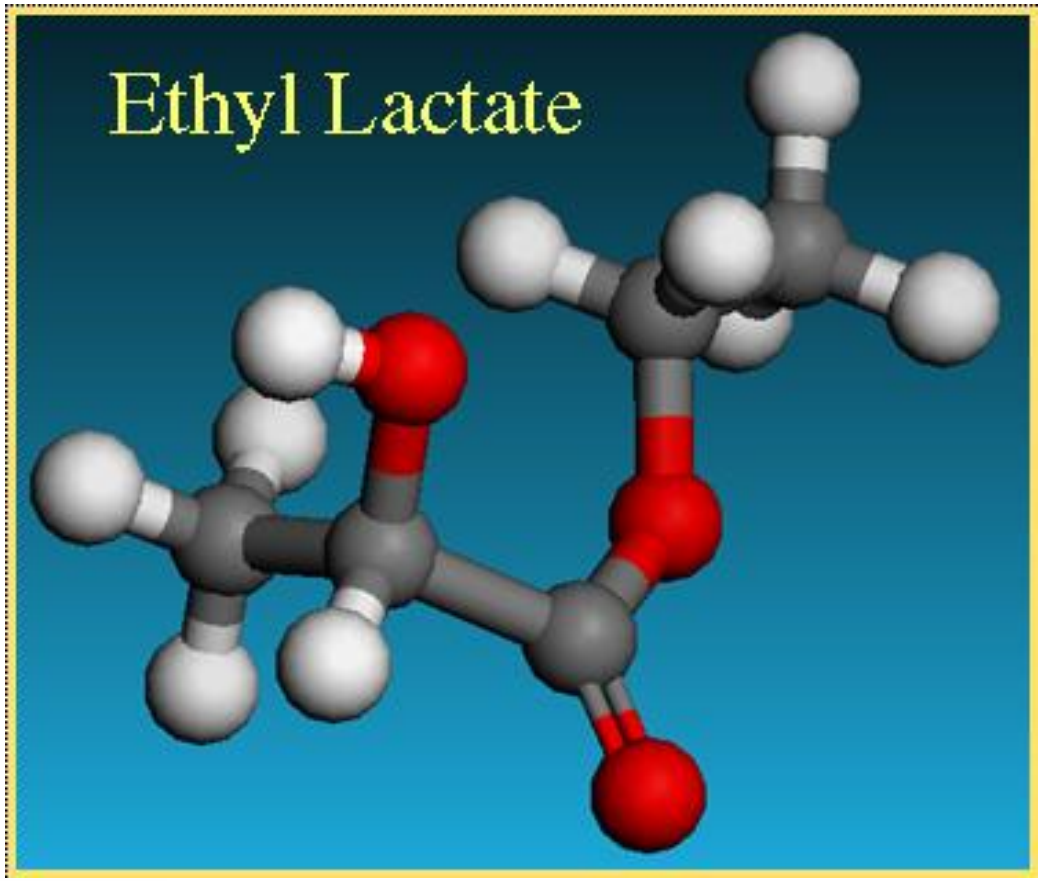
Eclipse PAH 4.6 mm × 100 mm, 1.8 µm
PN 959964-918

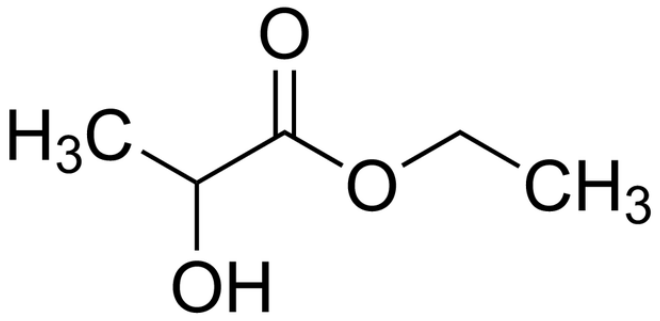
Detector:	230.8 nm No Ref.; Data rate 0.2 s, micro flow cell	
Flow:	1.8 mL /min	
Mobile phase:	A = Water; B = Acetonitrile	
Gradient:	Time (min)	% B
	0.00	40
	6	100
	9.5	100
	10	40
	Stop time = 12	
Temp.:	25 °C	

1 Toluene
2 Naphthalene
3 Acenaphthylene
4 1-Methyl naphthalene
5 2-Methyl naphthalene
6 Acenaphthene
7 Fluorene
8 Phenanthrene
9 Anthracene
10 Fluoranthene
11 Pyrene
12 Terphenyl-d14
13 Benzo(a)anthracene
14 Chrysene
15 Benzo(e)pyrene
16 Benzo(b)fluoranthene
17 Benzo(k)fluoranthene
18 Benzo(a)pyrene
19 Dibenzo(a,h)anthracene
20 Benzo(g,h,i)perylene
21 Indeno(1,2,3-c,d)pyrene



Rendering "green" LC separation of PAHs!





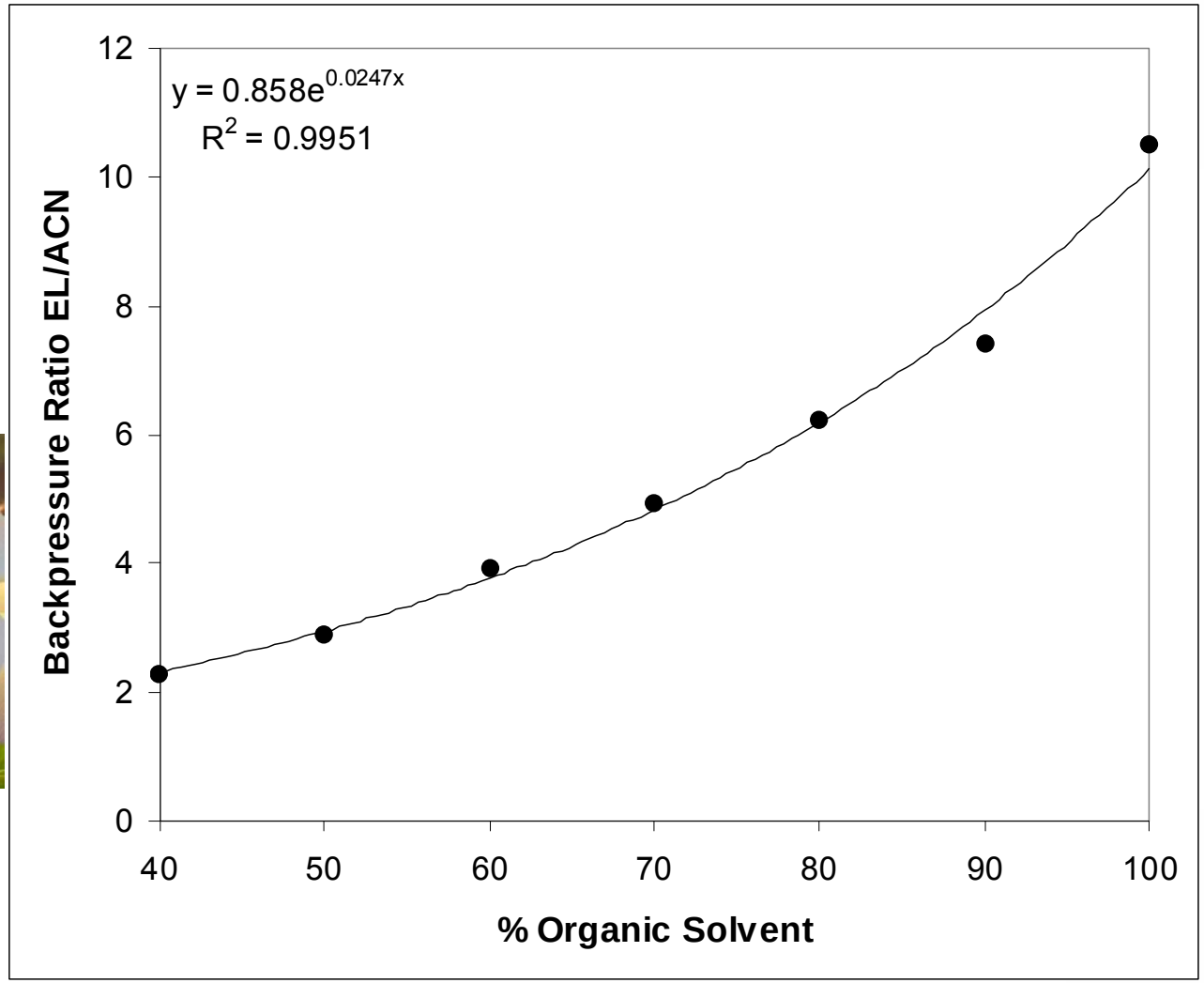
Replacement of ACN in the M.Ph.

Comparative overview:

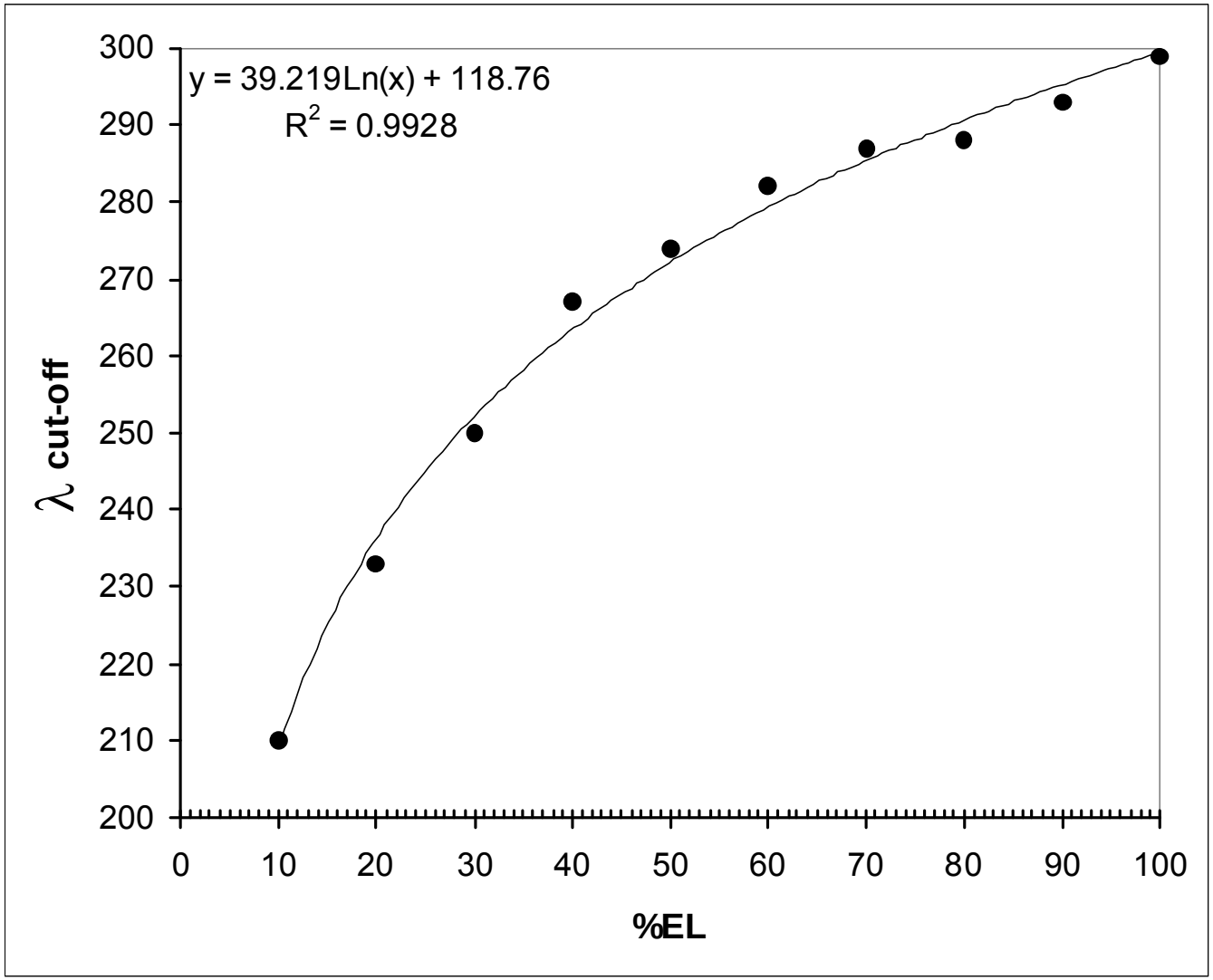
Characteristics	ACN	EL
CAS no.	75-05-8	97-64-3
Type of solvent	Polar aprotic	Polar protic
Molecular weight	41.04	118.13
Molecular dipole moment (D)	3.92	2.55
Dielectric constant	35.94	15.7
Hansen solubility parameters – Dispersive (δD)	15.3	16
Polar (δP)	18.0	7.6
Hydrogen bonding (δH)	6.1	12.5
Hildebrand solubility parameter (δT)	24.3	21.3
Boiling point (°C)	81.6	154
Melting point (°C)	-45.0	-25.0
Density (g/cm^3)	0.781	1.03
Log $K_{o/w}$	-0.34	-0.18
Water solubility	Miscible	Miscible
Viscosity/25 °C (cps)	0.36	2.53
Vapor pressure (kPa/20 °C)	9.7	0.22
Flash point (°C)	2	46.1
Auto ignition temperature (°C)	524	400
Lower flammable limit (LFL) (%)	4	1.5
Upper flammable limit (UFL) (%)	16	11.4
Oral LD50 rat (mg/kg)	2460	2500
Dermal LD50 rabbit (mg/kg)	980	>5000
Acute LC50 Daphnia 48 hrs (mg/mL)	3600	320000
HMIS Health	2	1
HMIS Flammability	3	2
HMIS Physical hazards	0	0
NFPA Rating Health	2	2
NFPA Rating Fire	3	2
NFPA Rating Safety	2	0
Exempt VOC (acc. 40 CFR 51.100)	No	Yes
Biodegradability	~ 40%/10 days	Readily biodegradable

First concern: viscosity!

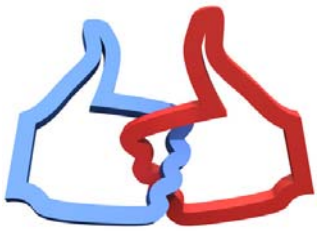
The UPLC reserve!

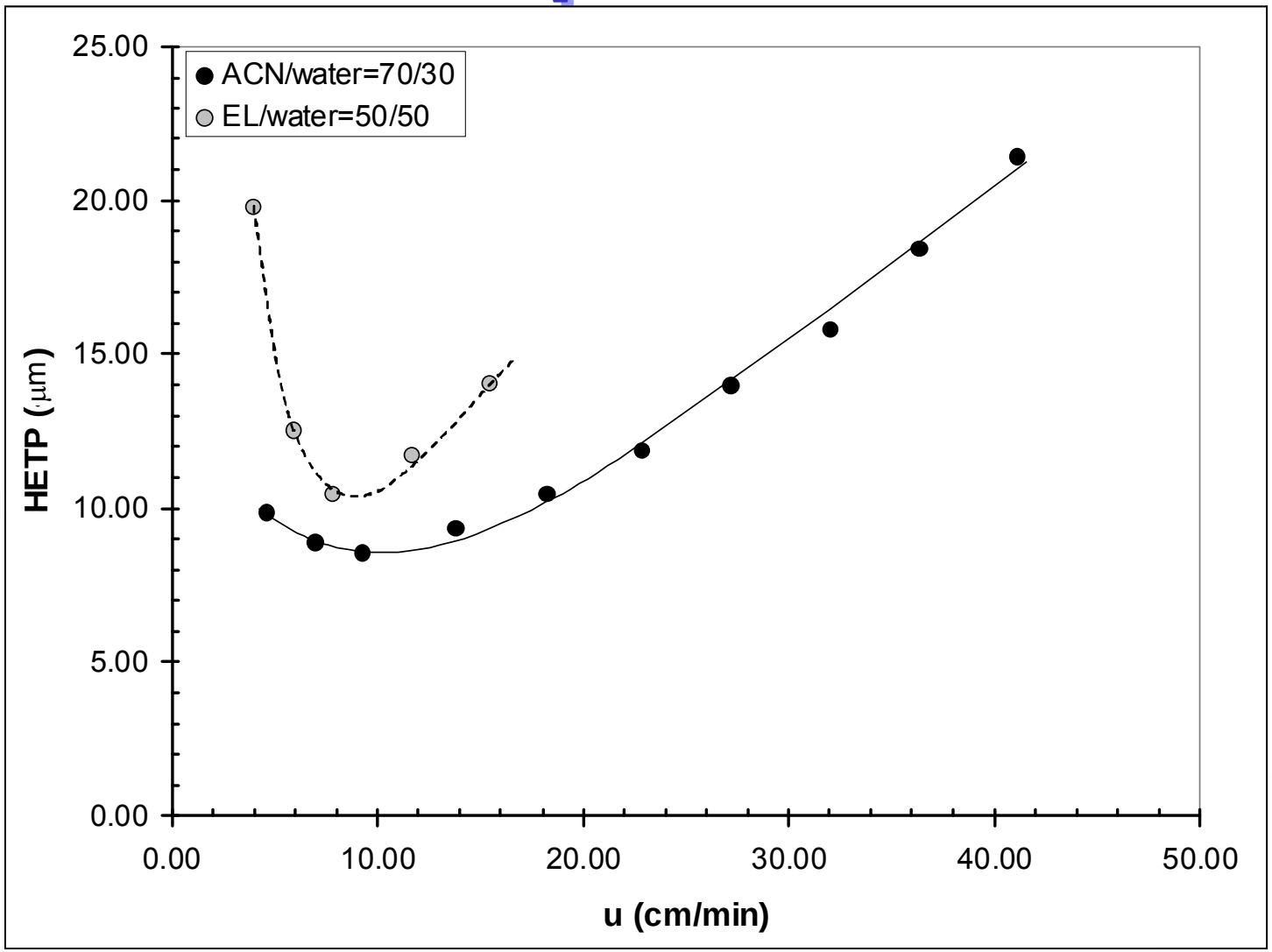


Second concern: spectral transparency!



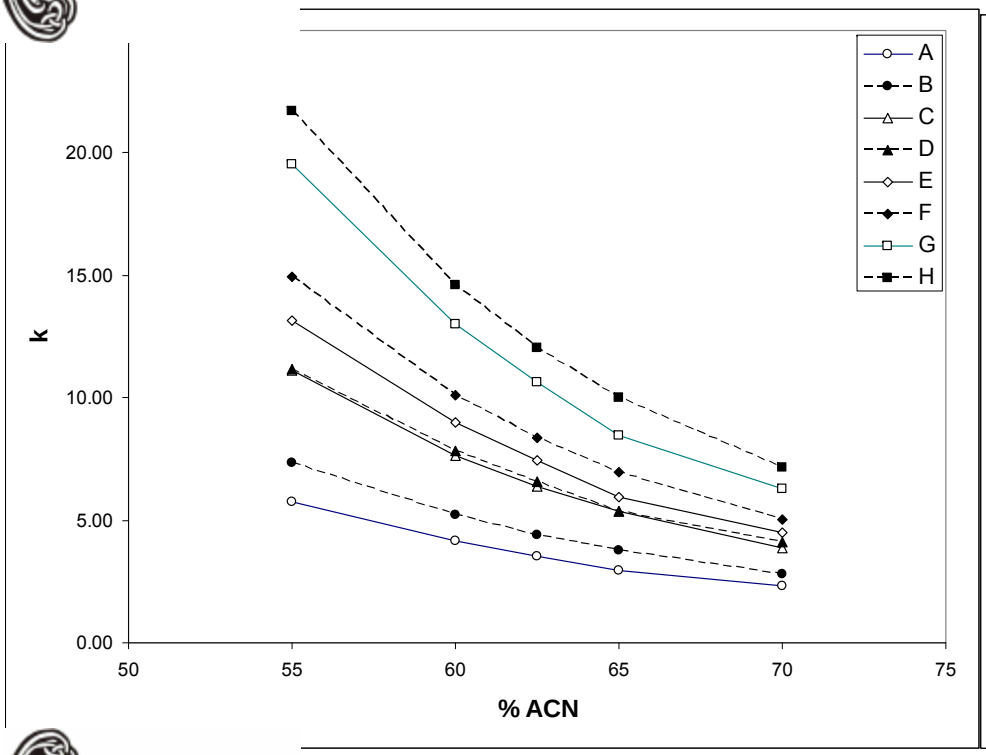
Third concern: optimal flow rates!

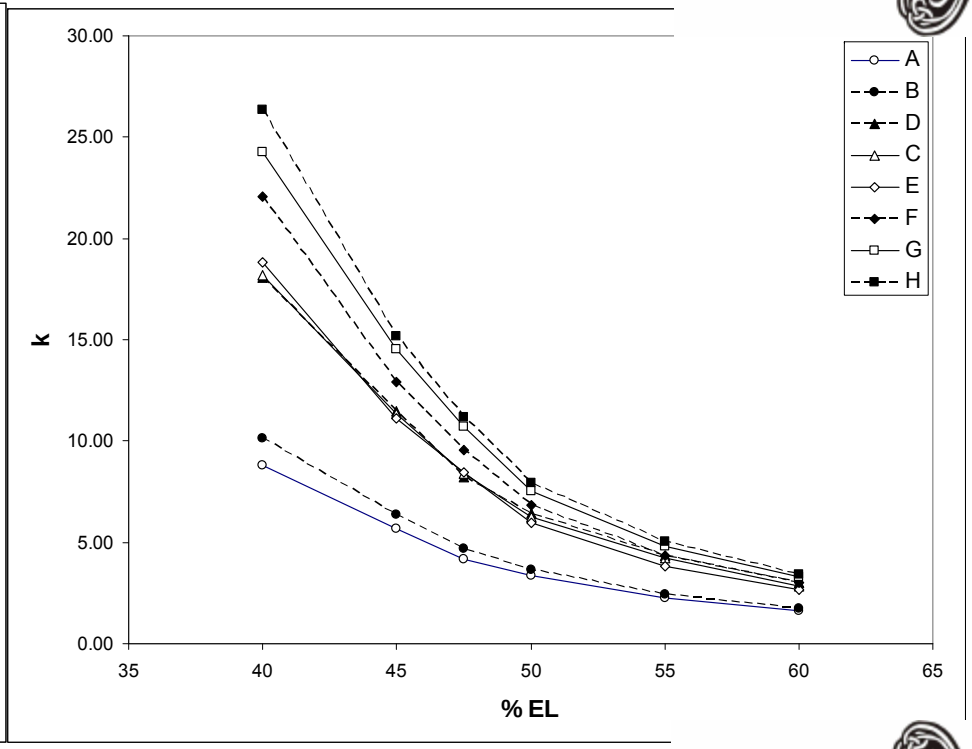




Retention profiles (under isocratic elution conditions)

Zorbax Eclipse XDB C18 (Agilent Technologies, PN 961753-902), 100 mm L × 2.1 mm i.d. × 3.5 µm d.p.
Flow rate of 0.3 mLmin^{-1}; Temperature: 25 °C





naphthalene – **A**; acenaphtylene – **B**; fluorene – **C**; acenaphtene –**D**; phenanthrene – **E**; anthracene – **F**; fluoranthene – **G**; pyrene – **H**; chrysene – **I**; benzo*(a)*anthracene – **J**; benzo*(b)*fluoranthene – **K**; benzo*(k)*fluoranthene – **L**; benzo*(a)*pyrene – **M**; dibenzo*(a,h)*anthracene – **N**; benzo*(g,h,i)*perylene – **O**; indeno*(1,2,3-c,d)*pyrene - **P**

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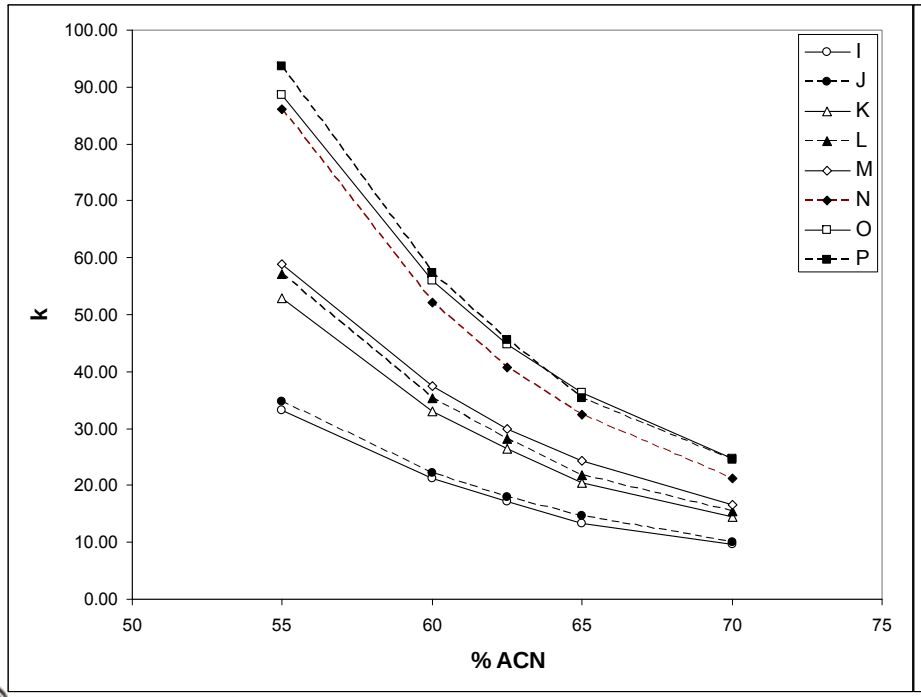
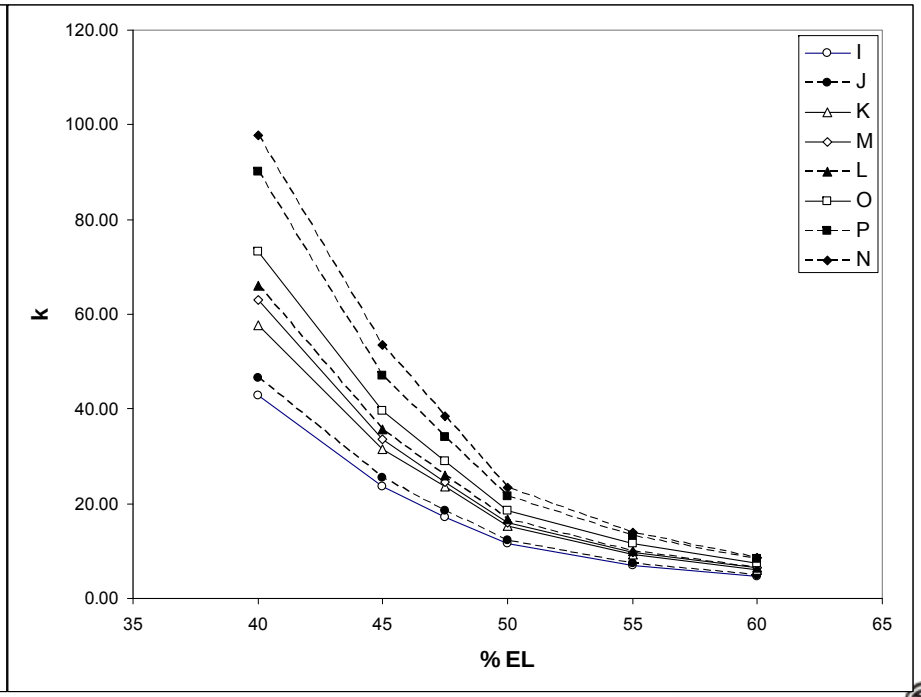


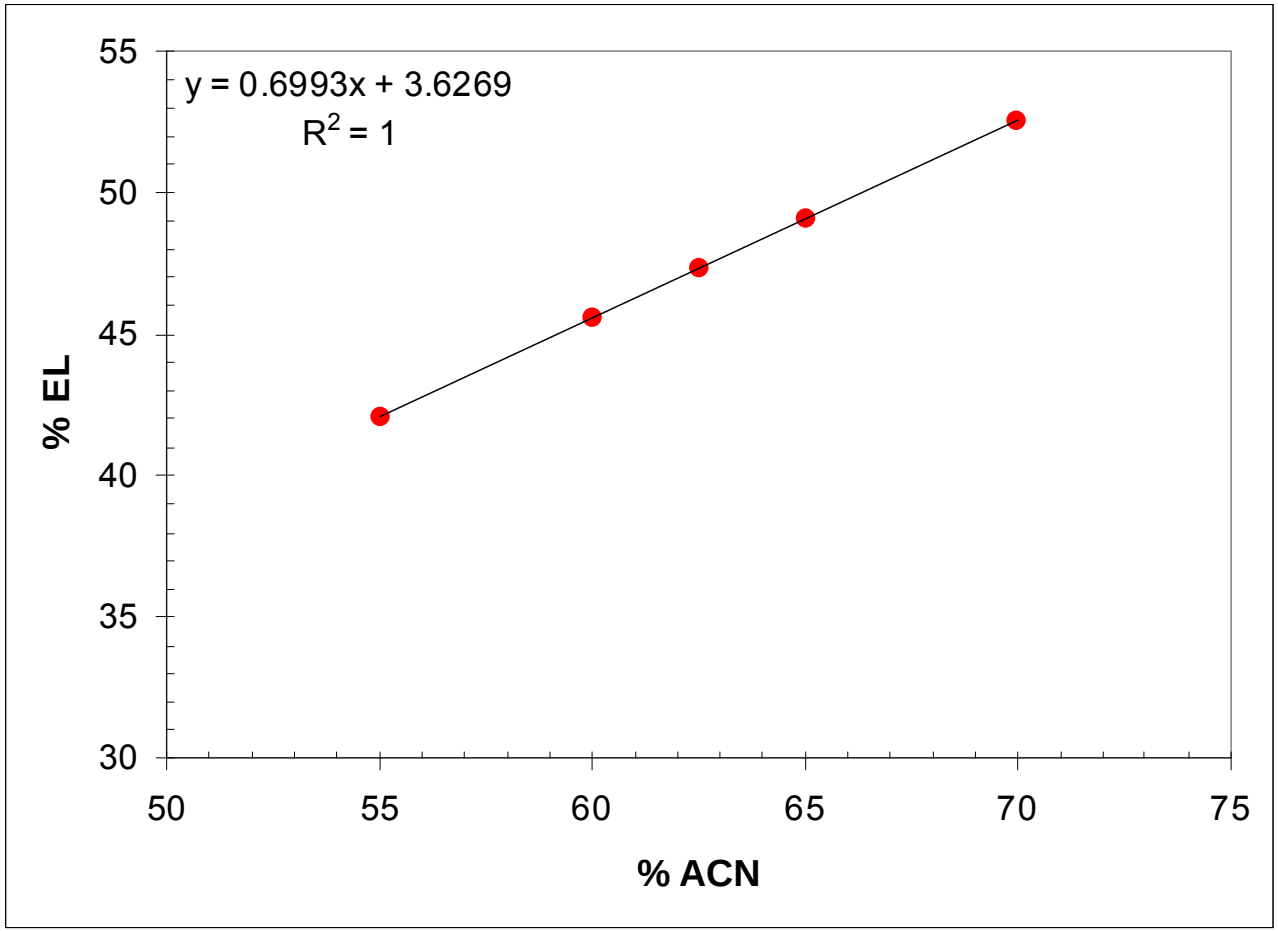


Equivalent retention:

$$\log k^{EL} = B^{EL} \times \log(\%EL) + A^{EL}$$

$$\log k^{ACN} = B^{ACN} \times \log(\%ACN) + A^{ACN}$$

$$(\%EL) = (\%ACN)^{\frac{B^{ACN}}{B^{EL}}} \times 10^{\frac{A^{ACN} - A^{EL}}{B^{EL}}}$$



Equivalent selectivity:

naphthalene – **A**;
acenaphtylene – **B**;
fluorene – **C**;
acenaphtene –**D**;
phenanthrene – **E**;
anthracene – **F**;
fluoranthene – **G**;
pyrene – **H**;
chrysene – **I**;
benzo*(a)*anthracene – **J**;
benzo*(b)*fluoranthene – **K**;
benzo*(k)*fluoranthene – **L**;
benzo*(a)*pyrene – **M**;
dibenzo*(a,h)*anthracene – **N**;
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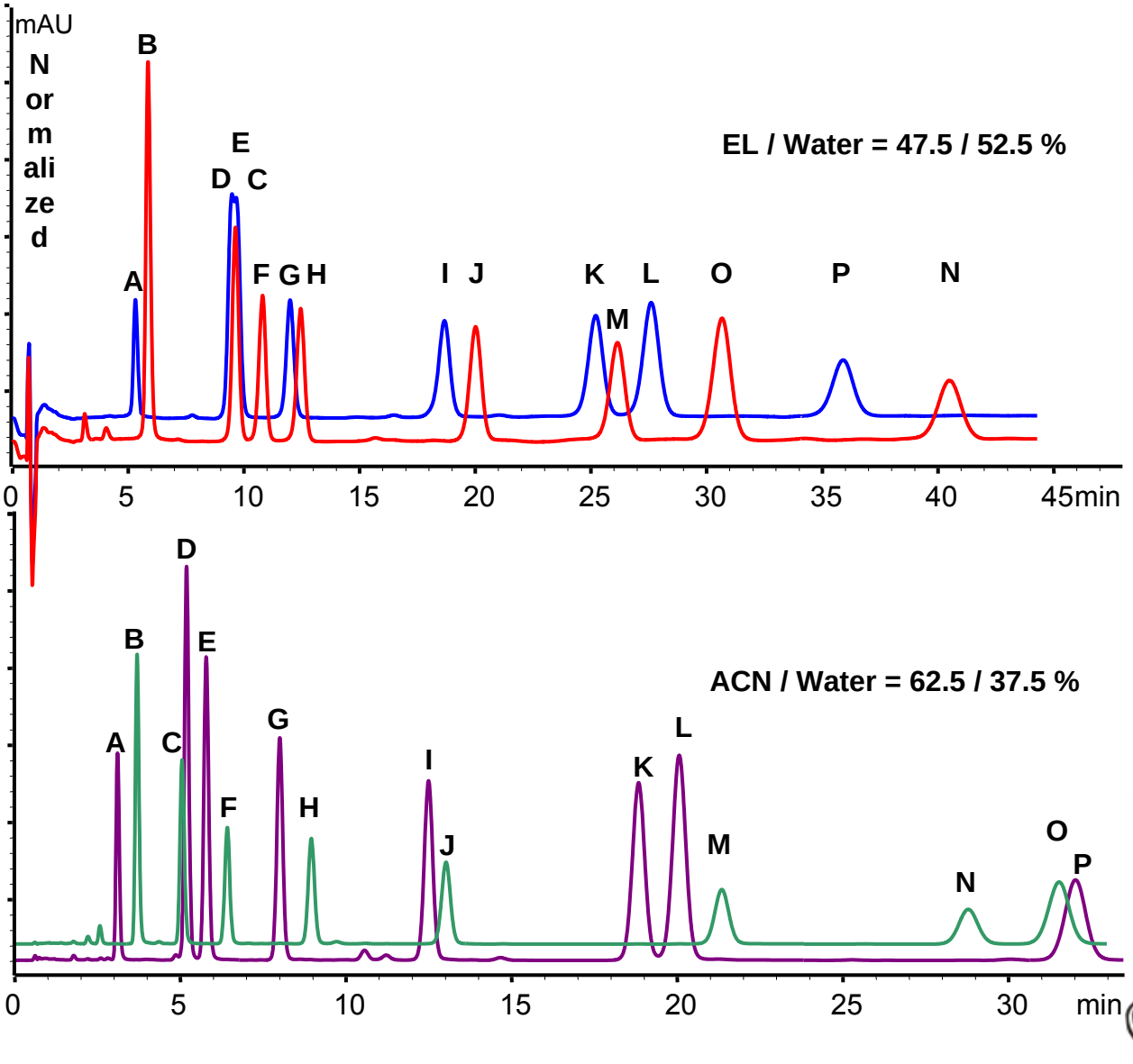
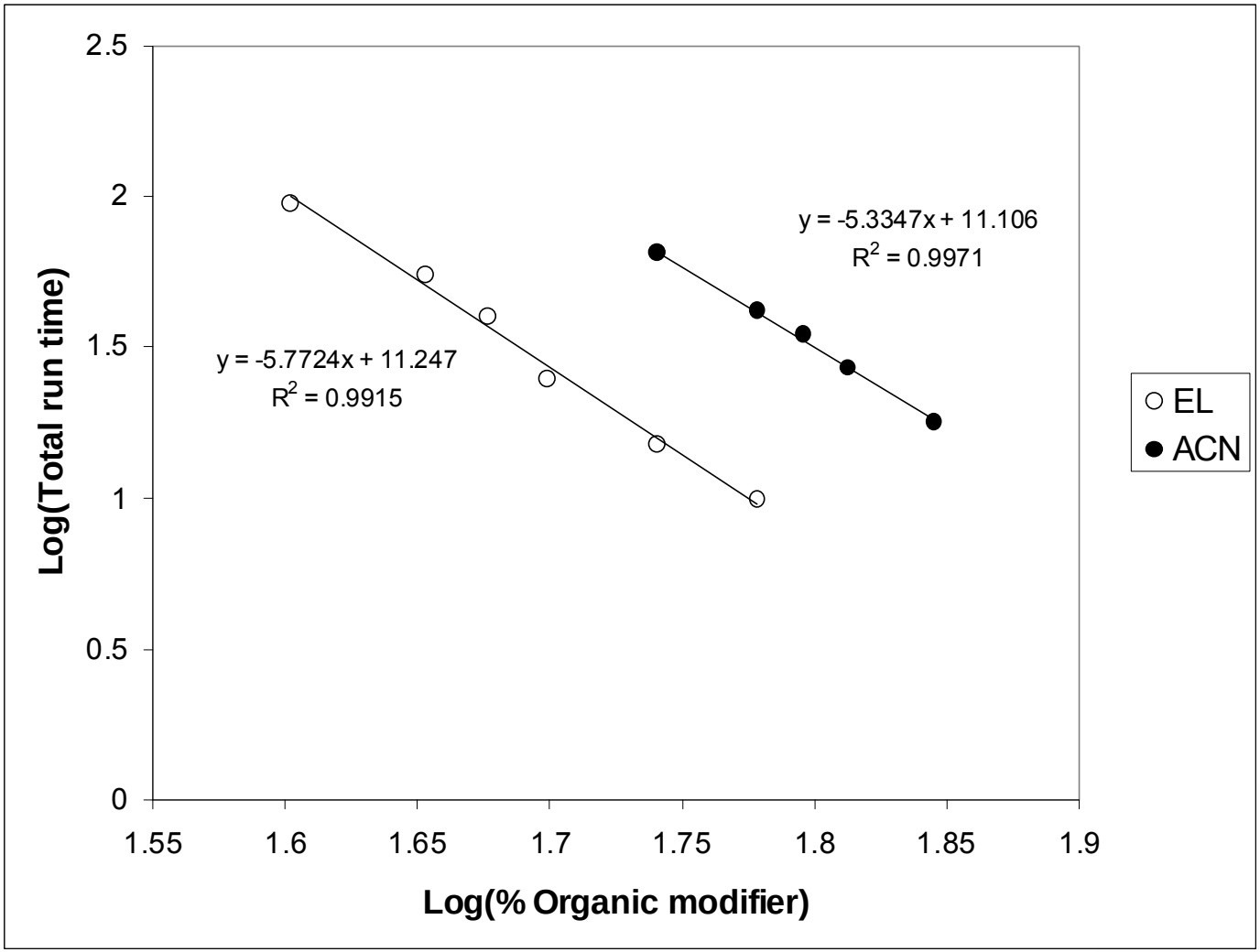


Equivalence of the run time duration:



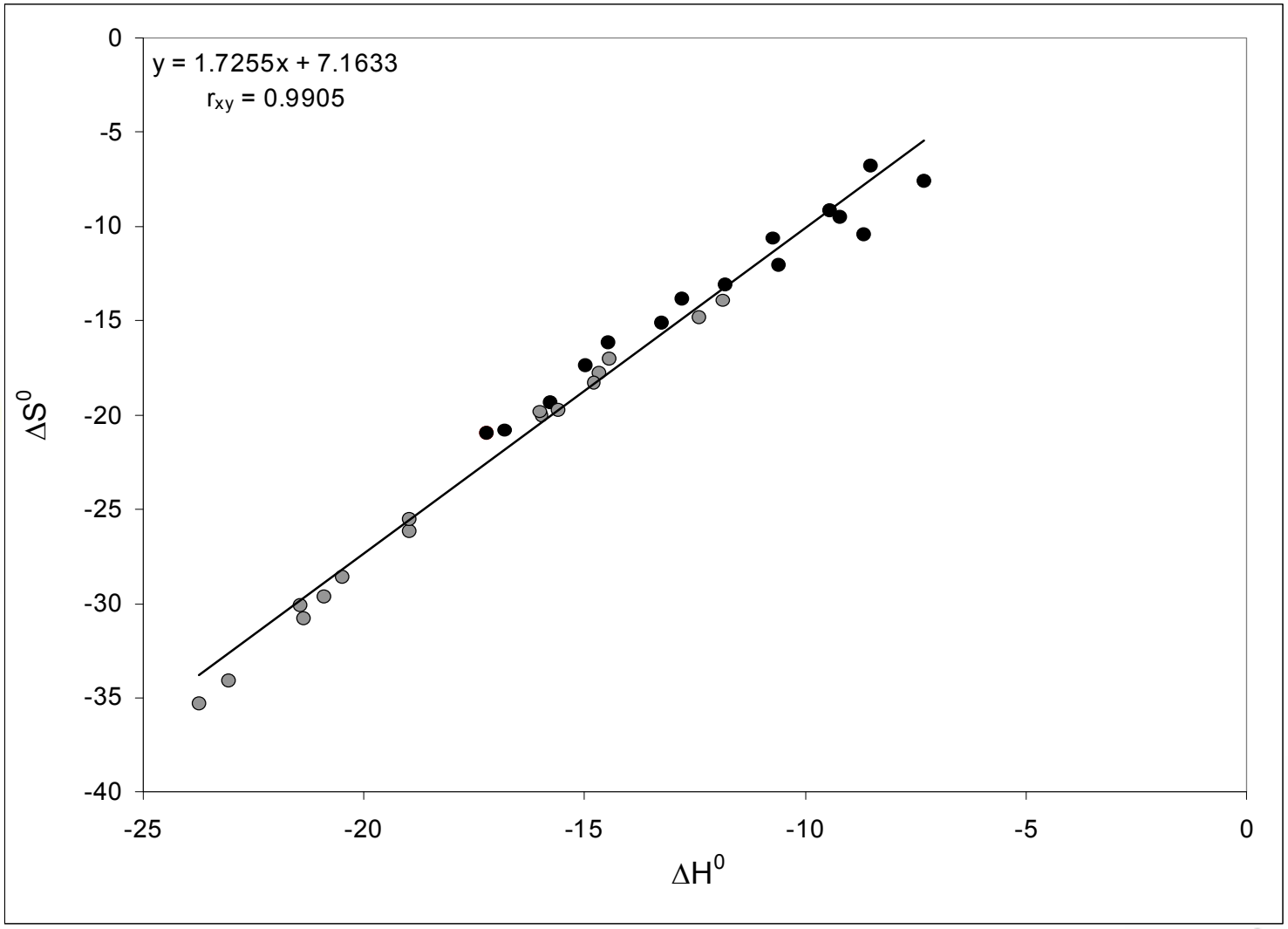
van't Hoff plots

(for increased selectivity elution conditions)

Compound	ACN/water = 70/30 (v/v)						EL/water = 45/45 (v/v)					
	B	A	r_{xy}	ΔH^0 $KJmol^{-1}K^{-1}$	ΔS^0 $Jmol^{-1}K^{-1}$	ΔG^0 (25 °C) $KJmol^{-1}K^{-1}$	B	A	r_{xy}	ΔH^0 $KJmol^{-1}K^{-1}$	ΔS^0 $Jmol^{-1}K^{-1}$	ΔG^0 (25 °C) $KJmol^{-1}K^{-1}$
A	878	-2.21	0.9847	**-7.30**	**-7.6**	-5.04	1427	-2.97	0.9889	-11.86	**-13.9**	-7.71
B	1043	-2.55	0.9923	**-8.67**	**-10.4**	-5.56	1495	-3.08	0.9913	-12.43	**-14.9**	-8.00
C	1108	-2.43	0.9922	**-9.21**	**-9.5**	-6.39	1765	-3.43	0.9927	-14.67	**-17.8**	-9.37
D	1025	-2.11	0.9860	**-8.52**	**-6.7**	-6.51	1739	-3.34	0.9922	-14.46	**-17.0**	-9.39
E	1138	-2.40	0.9876	**-9.46**	**-9.2**	-6.73	1780	-3.49	0.9924	-14.80	**-18.3**	-9.34
F	1276	-2.74	0.9926	**-10.61**	**-12.0**	-7.03	1876	-3.67	0.9919	-15.60	**-19.8**	-9.70
G	1290	-2.57	0.9886	**-10.73**	**-10.6**	-7.57	1921	-3.71	0.9913	-15.97	**-20.1**	-9.99
H	1422	-2.87	0.9922	**-11.83**	**-13.1**	-7.92	1925	-3.68	0.9913	-16.00	**-19.8**	-10.09
I	1538	-2.96	0.9906	**-12.79**	**-13.9**	-8.65	2283	-4.44	0.9897	-18.98	**-26.2**	-11.18
J	1594	-3.11	0.9929	**-13.25**	**-15.1**	-8.76	2281	-4.36	0.9900	-18.97	**-25.5**	-11.36
K	1741	-3.24	0.9915	**-14.48**	**-16.2**	-9.65	2466	-4.74	0.9894	-20.51	**-28.6**	-11.97
L	1801	-3.38	0.9919	**-14.98**	**-17.4**	-9.80	2573	-5.00	0.9892	-21.39	**-30.8**	-12.22
M	1899	-3.62	0.9931	**-15.79**	**-19.3**	-10.02	2514	-4.86	0.9893	-20.90	**-29.7**	-12.06
N	2073	-3.81	0.9930	**-17.24**	**-20.9**	-11.00	2857	-5.54	0.9887	-23.75	**-35.3**	-13.23
O	2024	-3.80	0.9937	**-16.83**	**-20.8**	-10.62	2578	-4.91	0.9891	-21.43	**-30.1**	-12.47
P	2072	-3.82	0.9925	**-17.23**	**-21.0**	-10.97	2774	-5.40	0.9890	-23.06	**-34.1**	-12.89

naphthalene – **A**; acenaphtylene – **B**; fluorene – **C**; acenaphtene –**D**; phenanthrene – **E**; anthracene – **F**; fluoranthene – **G**; pyrene – **H**; chrysene – **I**; benzo*(a)*anthracene – **J**; benzo*(b)*fluoranthene – **K**; benzo*(k)*fluoranthene – **L**; benzo*(a)*pyrene – **M**; dibenzo*(a,h)*anthracene – **N**; benzo*(g,h,i)*perylene – **O**; indeno*(1,2,3-c,d)*pyrene - **P**

Enthalpy / Entropy compensation!



Same interaction mechanisms!

Correlation of Molecular Descriptors with Retention:

Physico-chemical descriptors:

Molecular weight (M_w);
Solute's solubility in water (**S**);
Logarithm of the partition coefficient between *n*-octanol and water (log **P**).

Geometrical descriptors:

van der Waals volume (V_w);
van der Waals surface area (A_w).

Molecular shape descriptors:

Length to breadth ratio - **L/B**;

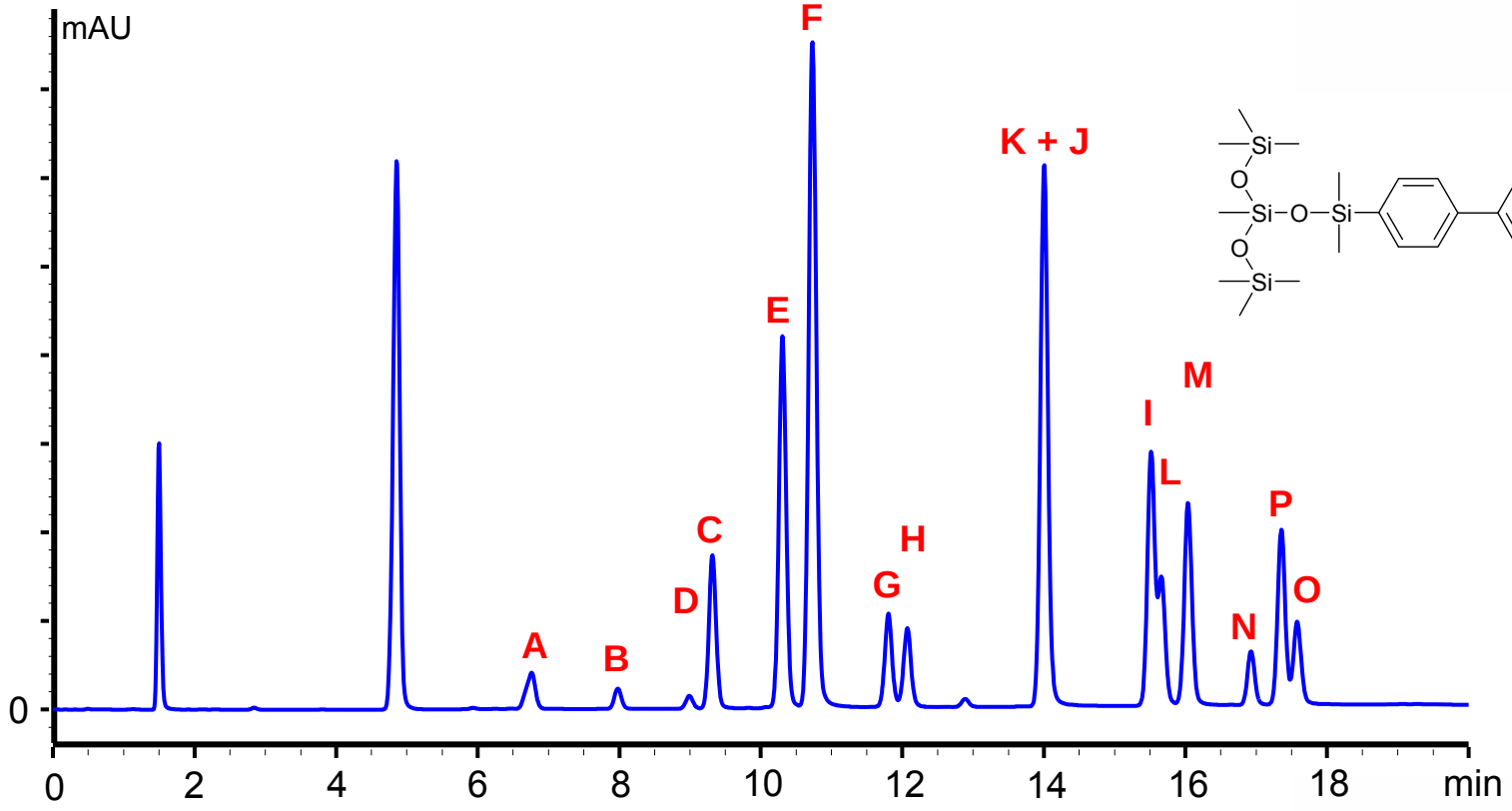
Molecular topology:

Connectivity index - χ;
Correlation factor - **F**.

Organic Modifier	Descriptor	M_w	V_w	log P	S	χ	F	L/B
	Function	M_w=f(log k)	V_w=f(log k)	Log P=f(log k)	Log S=f(log k)	χ=f(log k)	F=f(log k)	L/B=f(log k)
ACN	Mean r_{xy}	0.9941	0.9918	0.9937	-0.9701	0.9964	0.9974	0.3540
	s	0.0010	0.0017	0.0009	0.0007	0.0006	0.0010	0.0111
EL	Mean r_{xy}	0.9800	0.9852	0.9865	-0.9584	0.9832	0.9874	0.4367
	s	0.0040	0.0043	0.0024	0.0048	0.0021	0.0023	0.0156

Are π—π interactions responsible for the selectivity of the LC chromatographic separation of PAHs ?

Column:
Ultra Biphenyl,
150 mm L x
2.1 mm i.d. x
3 μm d.p.
H_2O/ACN:
50/50 to 20/80 in
15 min.;
0.3 mL/min;
25 °C;



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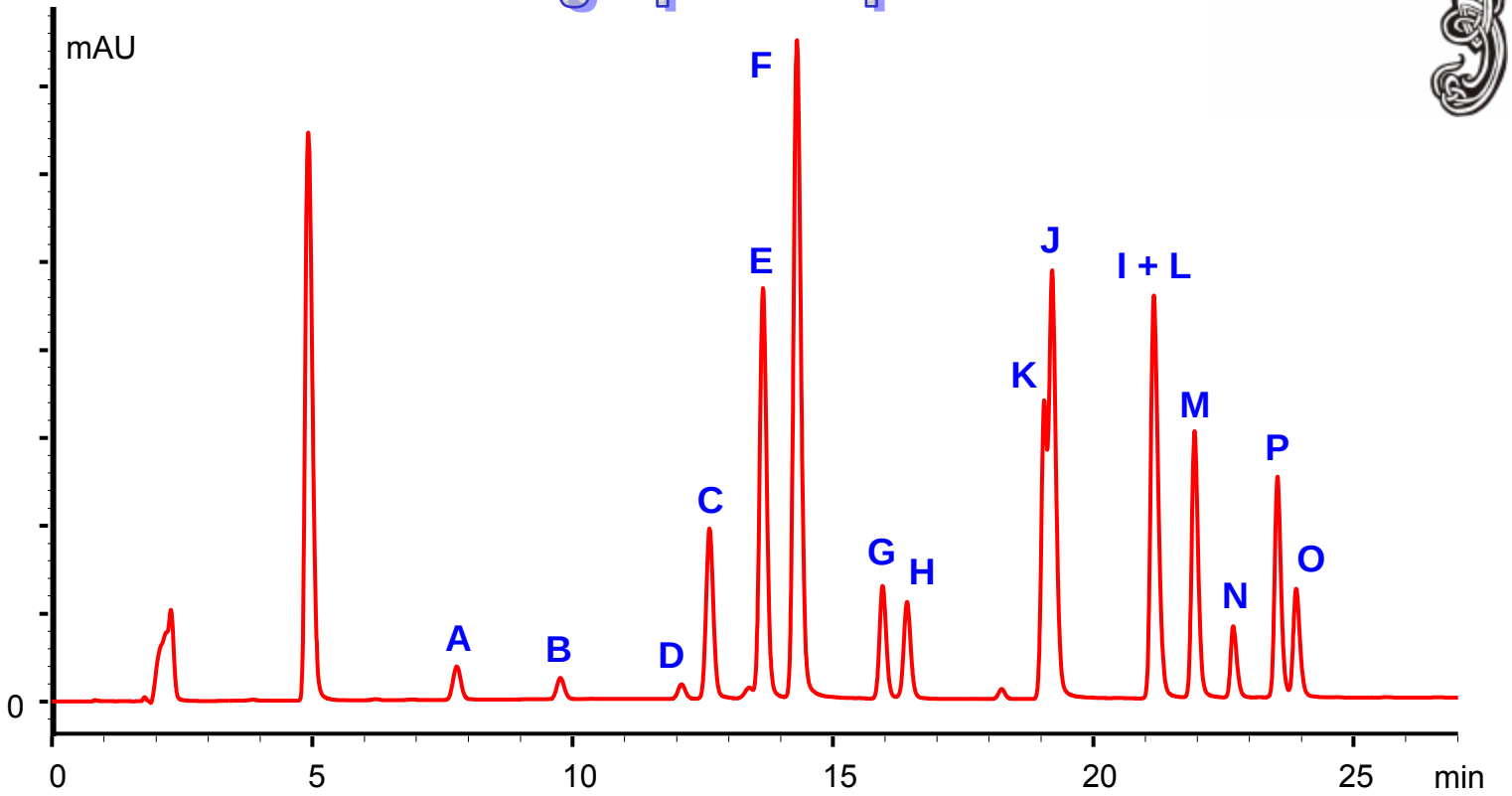
Column:
Ultra Biphenyl,
150 mm L x
2.1 mm i.d. x
3 μm d.p.
H_2O/MeOH:
30/70 to 0/100 in
20 min.;
0.2 mL/min;
25 °C;



naphthalene – **A**; acenaphtylene – **B**; fluorene – **C**; acenaphtene –**D**; phenanthrene – **E**; anthracene – **F**; fluoranthene – **G**; pyrene – **H**; chrysene – **I**; benzo*(a)*anthracene – **J**; benzo*(b)*fluoranthene – **K**; benzo*(k)*fluoranthene – **L**; benzo*(a)*pyrene – **M**; dibenzo*(a,h)*anthracene – **N**; benzo*(g,h,i)*perylene – **O**; indeno*(1,2,3-c,d)*pyrene - **P**

Conclusions:

1. The assay of PAHs in natural/artificial matrices is still of high importance and actuality.

2. Highly lipophilic matrices are extremely challenging for sample preparation procedures of PAHs for chromatographic analysis.

3. Replacement of ACN by EL in LC mobile phases does not essentially change retention, selectivity and interactions with the S. Ph. While the increased pressure drop may be sustained by means of UP/UHP-LC approaches, detectability remains a major concern.

4. Involvement of π–π interactions in the LC chromatographic mechanism of PAHs does not necessarily increase separation selectivity.

Thank you for your attention!

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