Comprehensivity and hyphenation in analysis of fats

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INTERNATIONAL WORKSHOP

"CHALLENGES IN FOOD CHEMISTRY"

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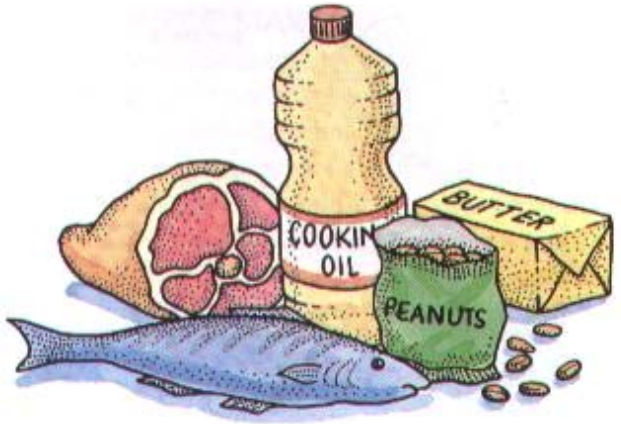
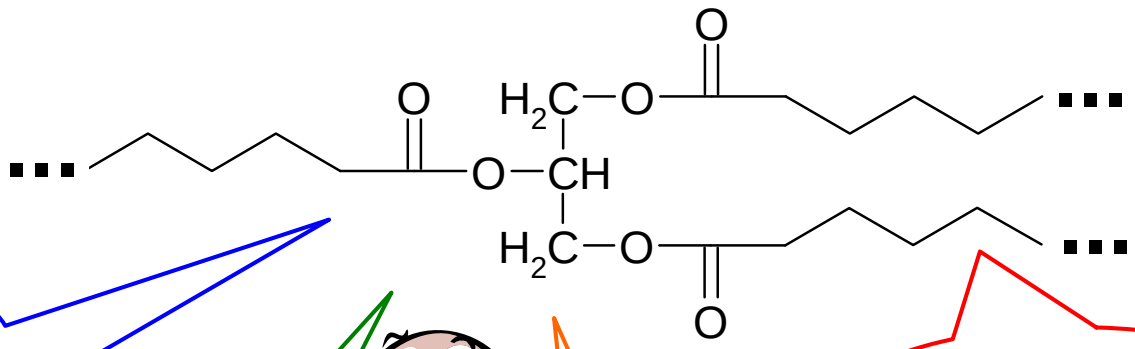
Constanta, Romania

Fat is good?!



Murphy Law: Everything nice during life is immoral, illegal or gets FAT!

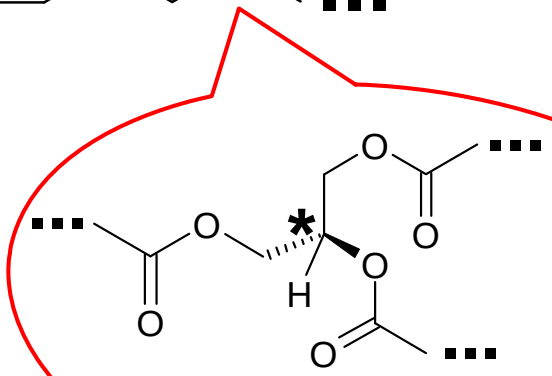
Triglycerides: **TGs**



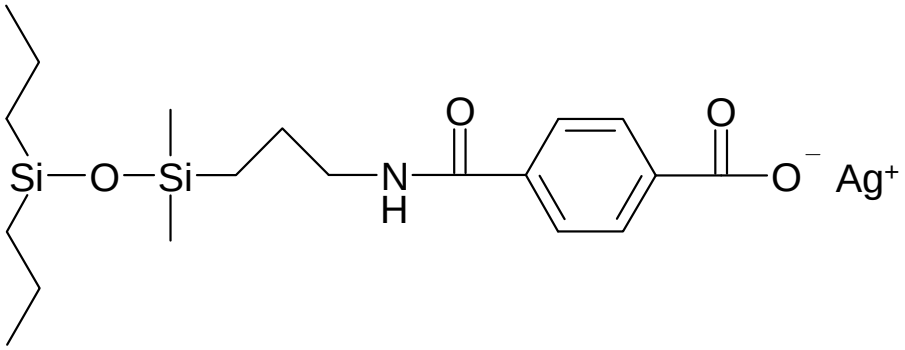
TGs
DGs
MGs
FFA

Position:
SOL ≠ OLS ≠ OSL

-saturated;
-unsaturated
-polyunsaturated
-place of the =



Chiral!

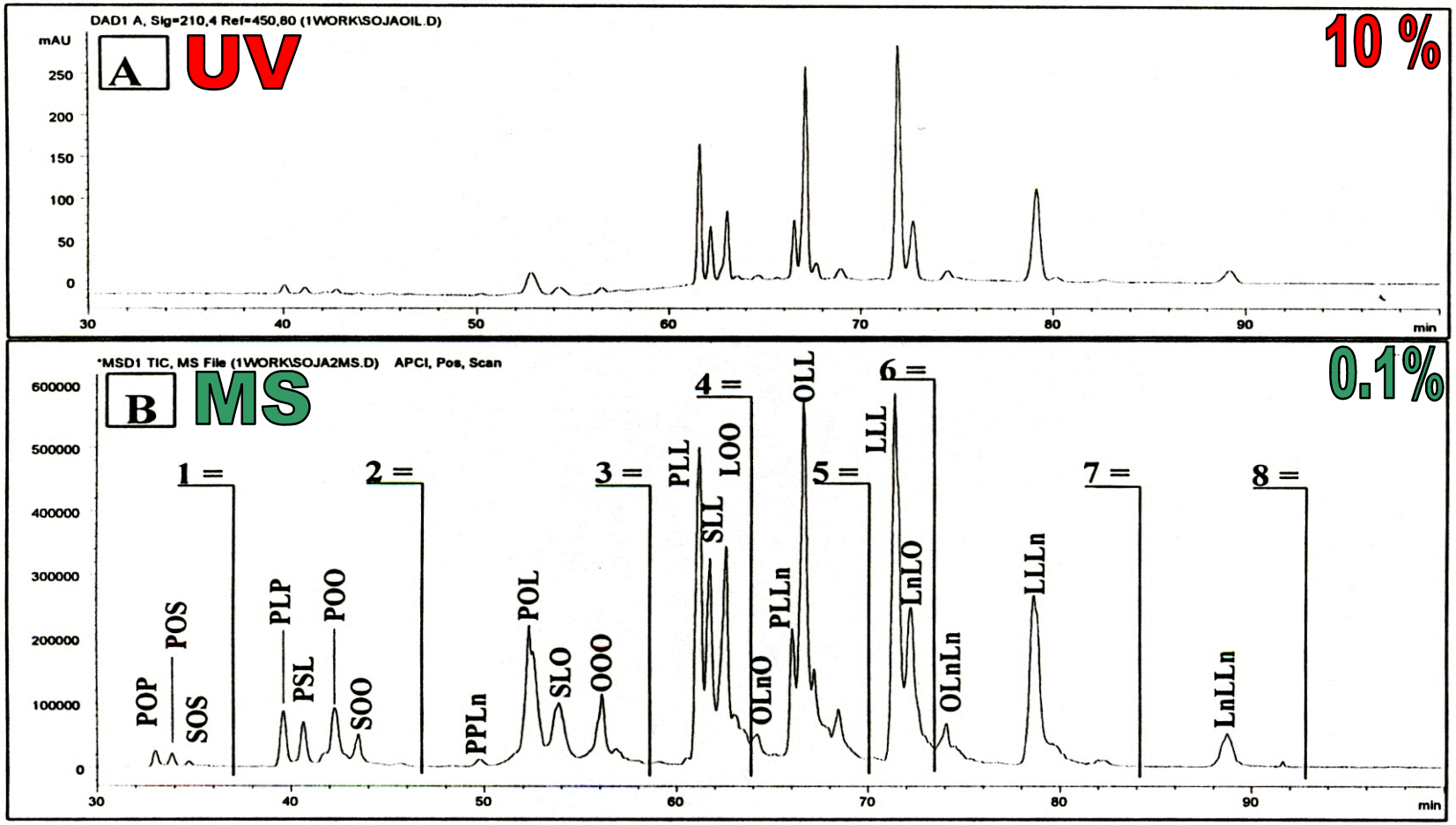


SFC

Column: Nucleosil 100-5 SA (Ag^+) form, 25 cm L x 4.6 mm i.d. x 5 um d.p.
Column temperature: 65 ^oC;
Pressure: 150 bar (2 min) ⇗ 300 bar: [1.5 bar/min]
Modifier: ACN/2-PrOH : 6/4
Modifier programming: 1.2% (2 min) ⇗ 7.2% (28 min): [0.3%/min] ⇗ 12.2%: [0.54%/min]
Flow rate: 1 mL/min

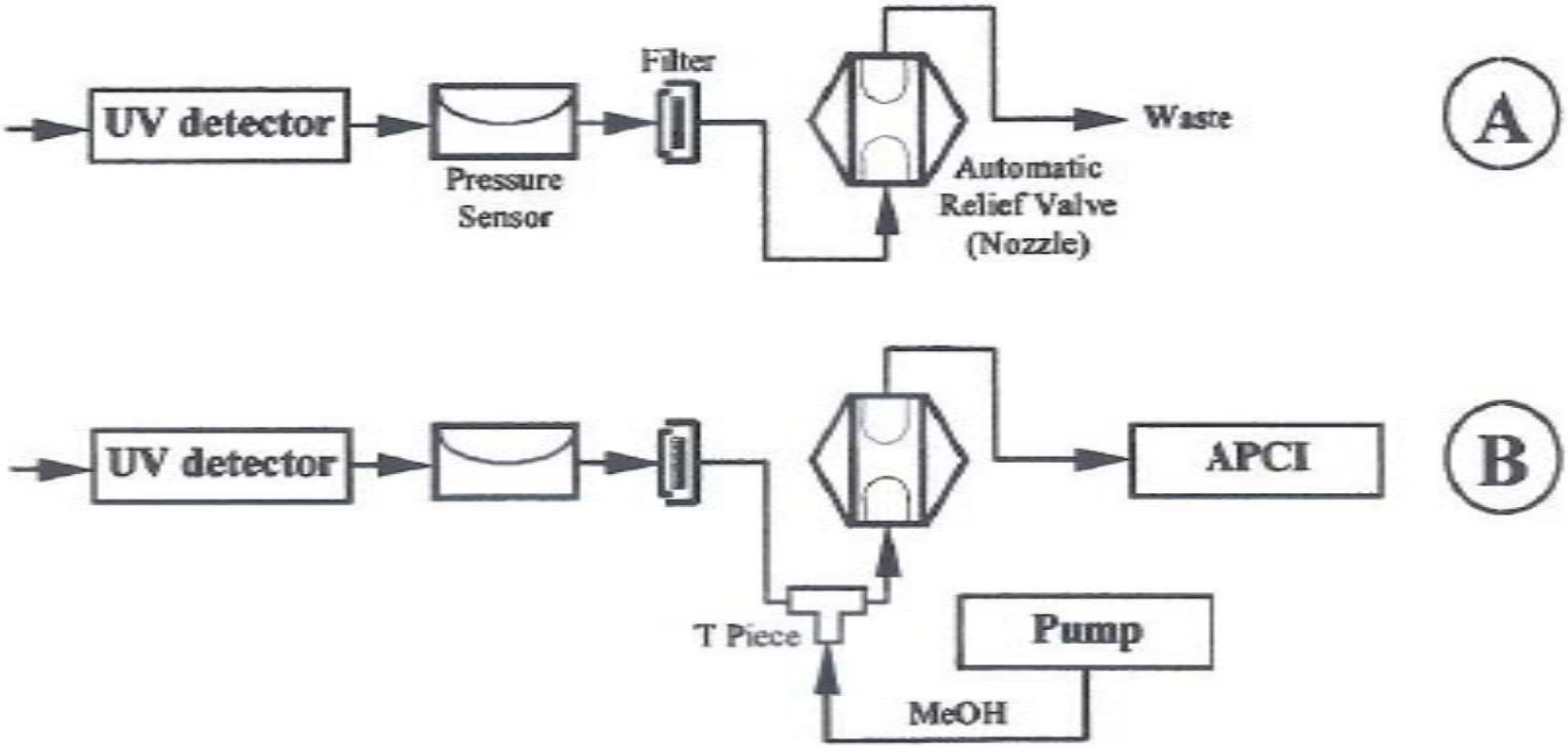
Structural identification / confirmation ability

pSFC/MS of TGs in fats

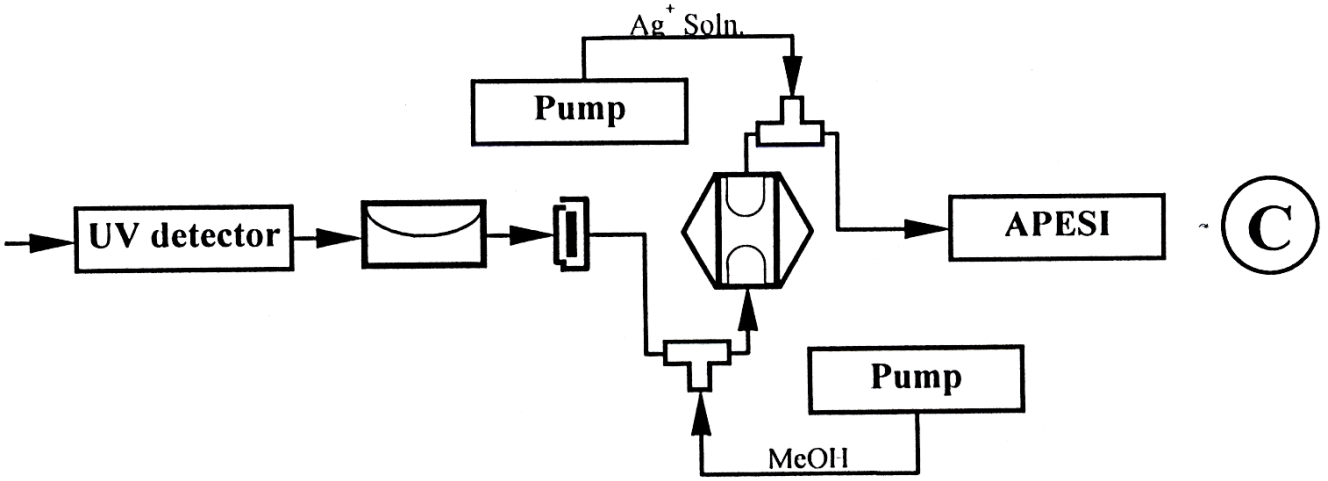


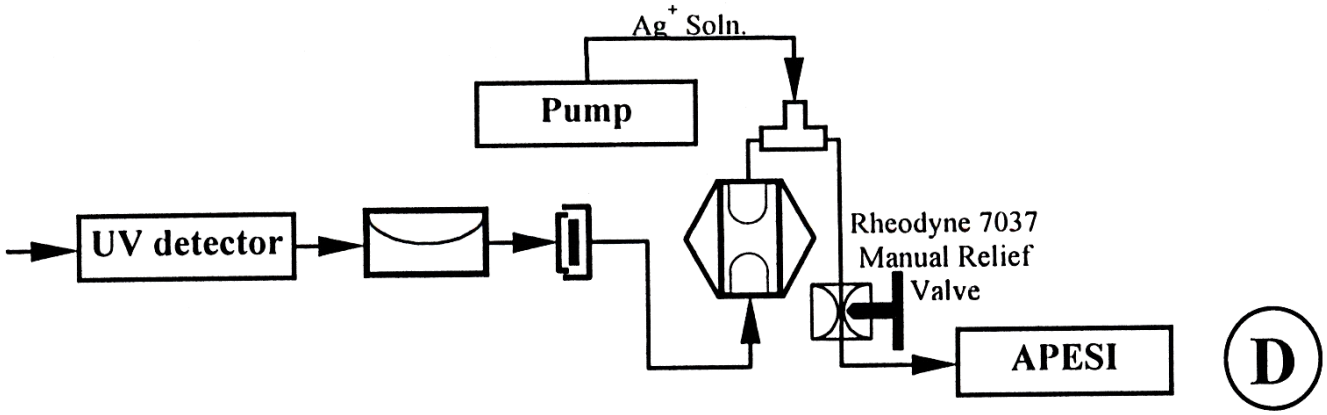
NDB first, CN within groups

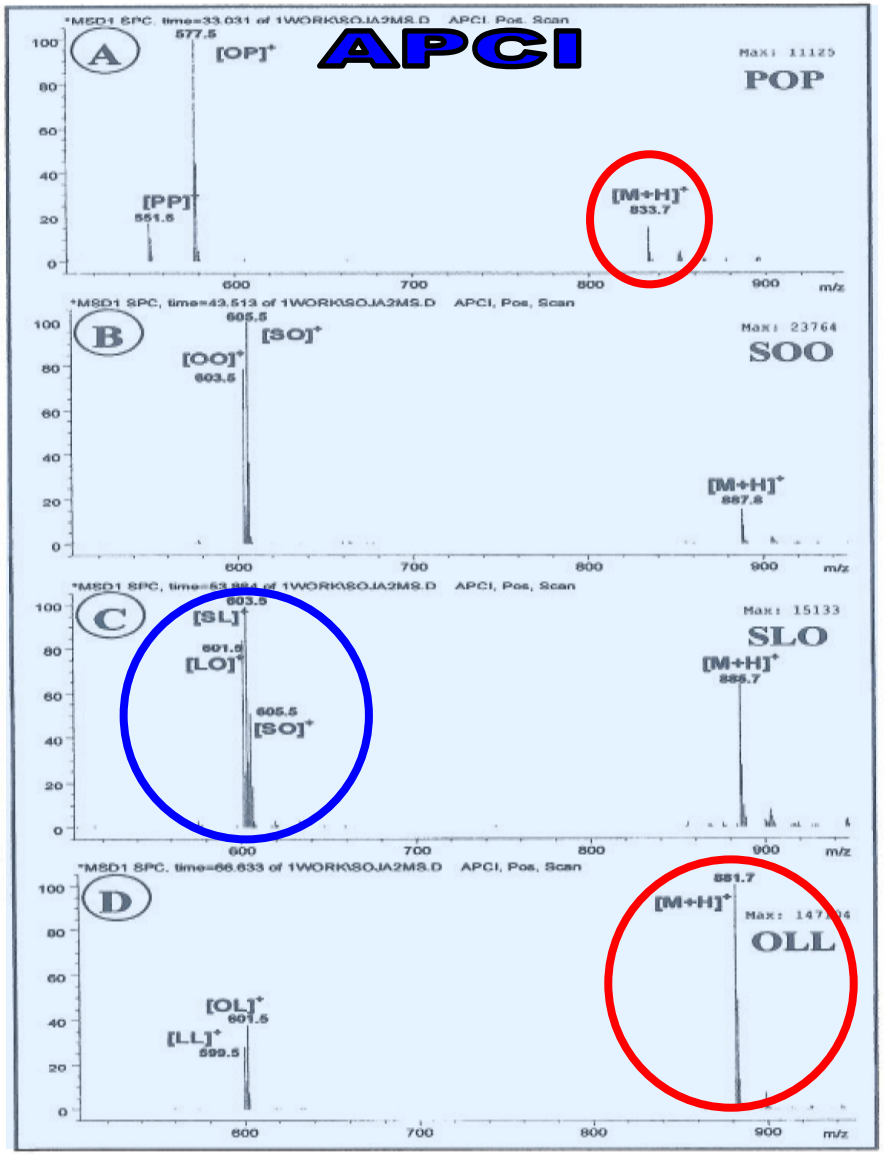
PSFC / MS interfacing !

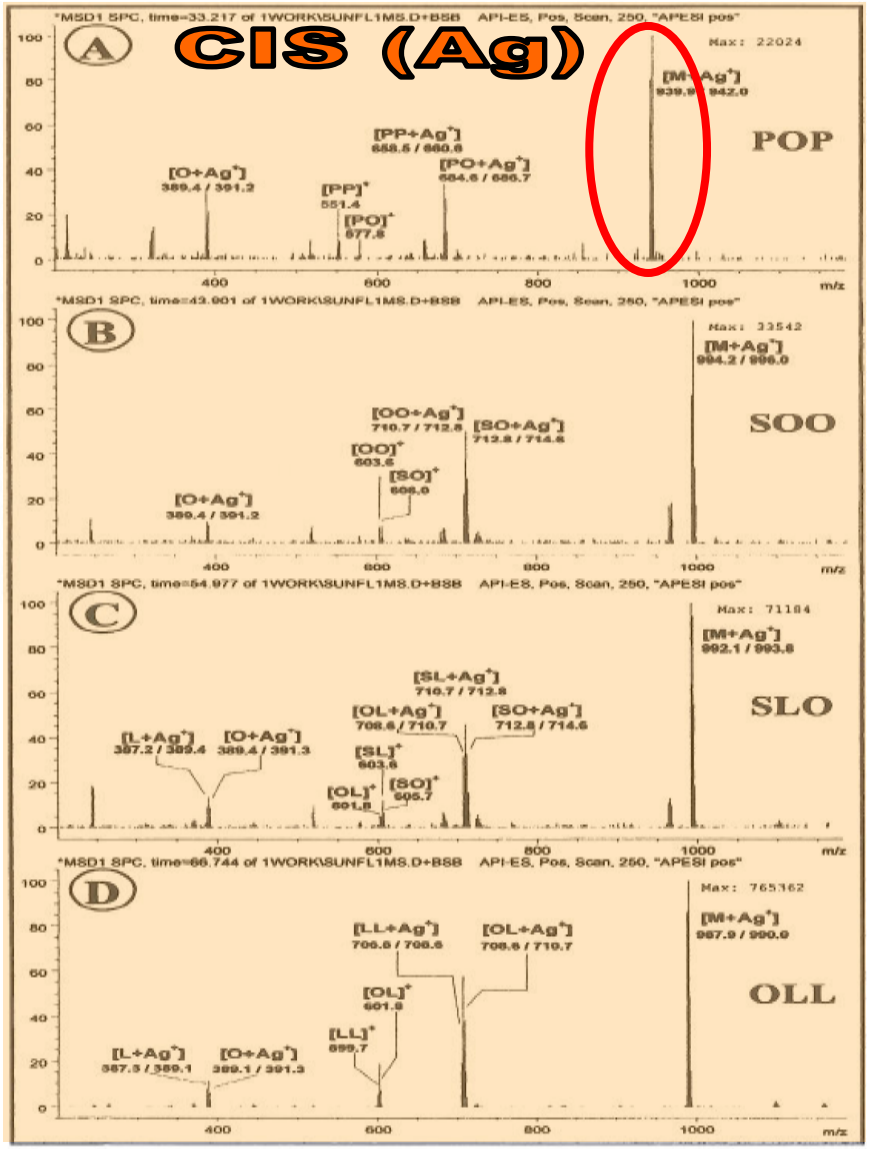


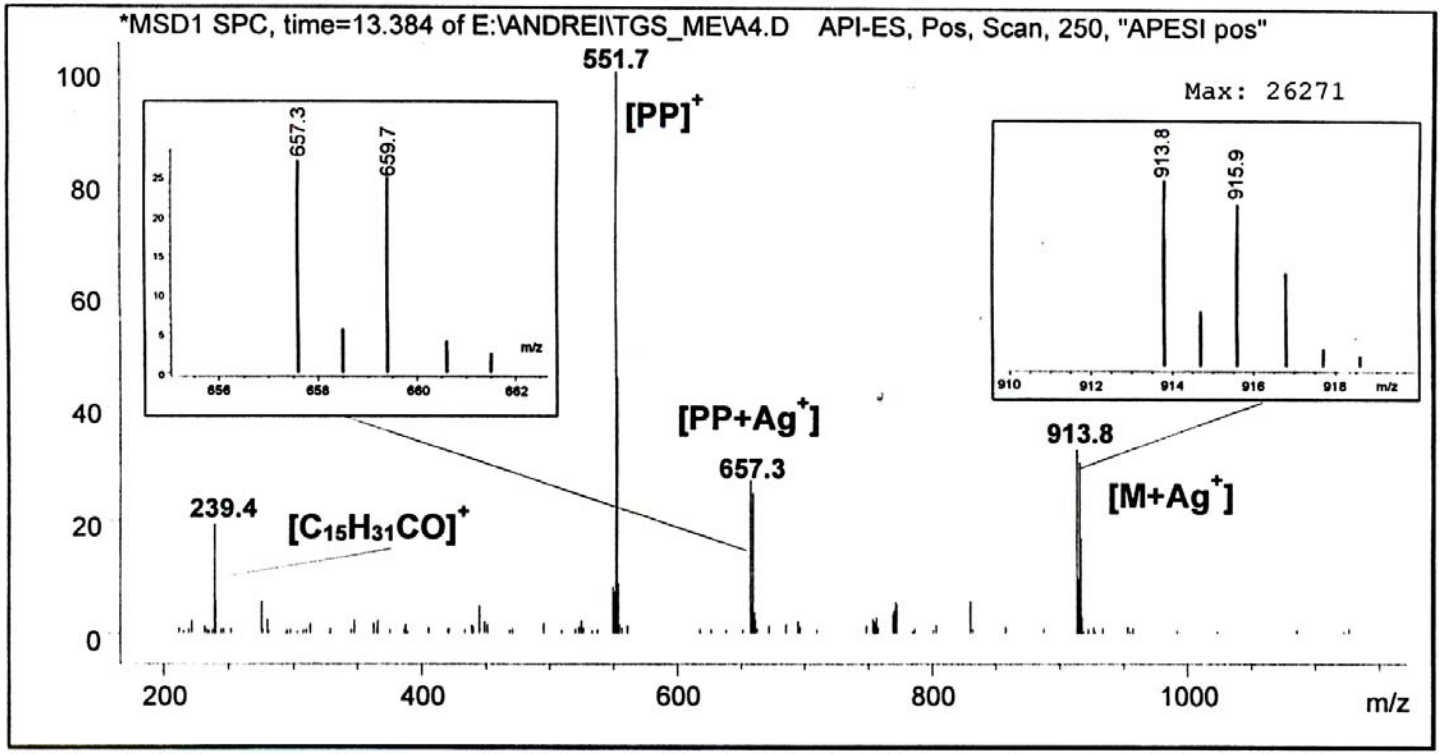
MS Ionization: AP-CI
Alternative: CIS-AP-CI (Ag+)






APCI
A
POP
[OP]+
577.5
[PP]+
551.5
[M+H]+
833.7
Max: 11125
B
SOO
[OO]+
603.5
[SO]+
605.5
[M+H]+
887.5
Max: 23764
C
SLO
[SL]+
603.5
[LO]+
601.5
[SO]+
605.5
[M+H]+
885.7
Max: 15133
D
OLL
[M+H]+
881.7
[OL]+
601.5
[LL]+
599.5


CIS (Ag)
A
POP
[O+Ag]+
389.4 / 391.2
[PP]+
551.4
[PO]+
577.5
[PP+Ag]+
658.5 / 660.6
[PO+Ag]+
684.5 / 686.7
[M+Ag]+
939.9 / 942.0
Max: 22024
B
SOO
[O+Ag]+
389.4 / 391.2
[OO]+
603.5
[SO]+
605.5
[OO+Ag]+
710.7 / 712.8
[SO+Ag]+
712.8 / 714.8
[M+Ag]+
994.2 / 996.0
Max: 33542
C
SLO
[L+Ag]+
387.2 / 389.4
[O+Ag]+
389.4 / 391.3
[SL]+
603.6
[OL]+
601.5
[SO]+
605.7
[SL+Ag]+
710.7 / 712.8
[OL+Ag]+
708.6 / 710.7
[SO+Ag]+
712.8 / 714.6
[M+Ag]+
992.1 / 993.8
Max: 71184
D
OLL
[L+Ag]+
387.3 / 389.1
[O+Ag]+
389.1 / 391.3
[LL]+
599.7
[OL]+
601.8
[LL+Ag]+
706.6 / 708.6
[OL+Ag]+
708.6 / 710.7
[M+Ag]+
987.9 / 990.0
Max: 765362



☞ **Positional isomers can be identified based on diglyceride ion ratios. (Equipment dependent)!**

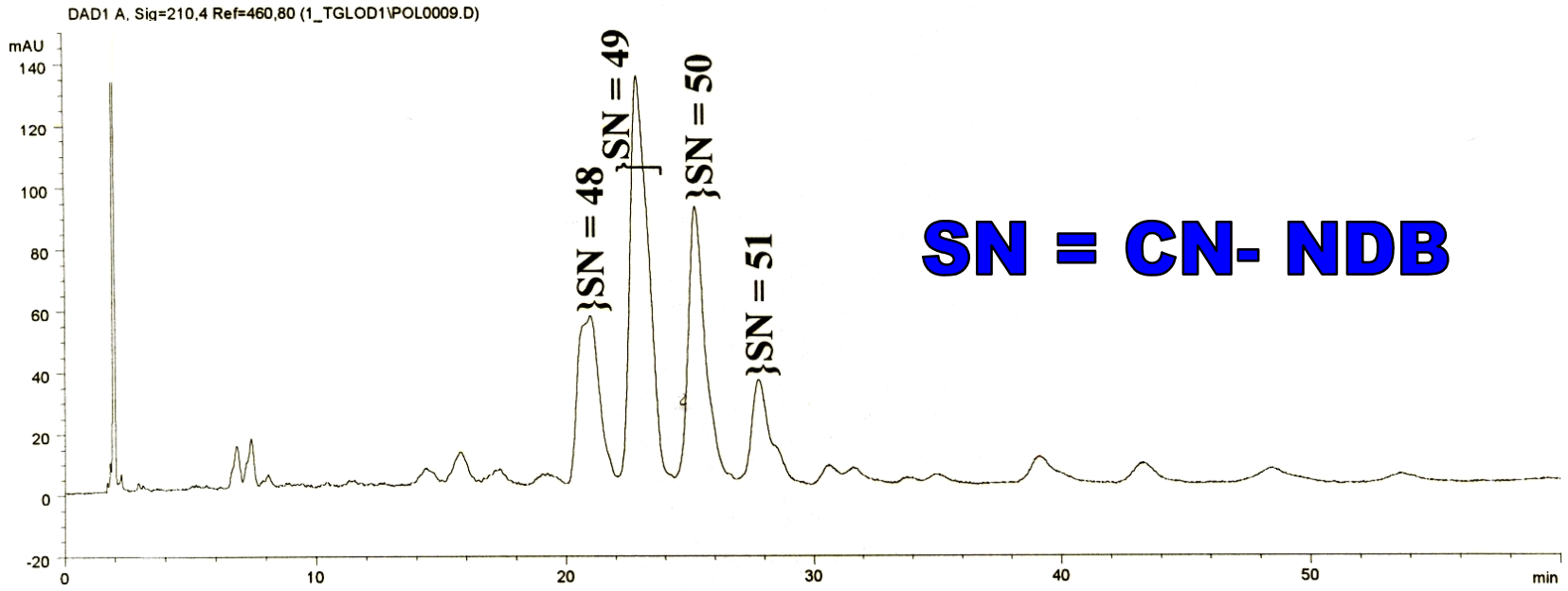
☞ **In CIS the [M+Ag$^+$] ion shows the highest intensity irrespective to the degree of unsaturation. (Equipment independent).**

☞ **Fully saturated TGs can be characterized through CIS. (Equipment independent).**

Structural identification / confirmation ability should still be considered equipment dependent!

A second dimension needed?

Comprehensivity: a deeper insight!

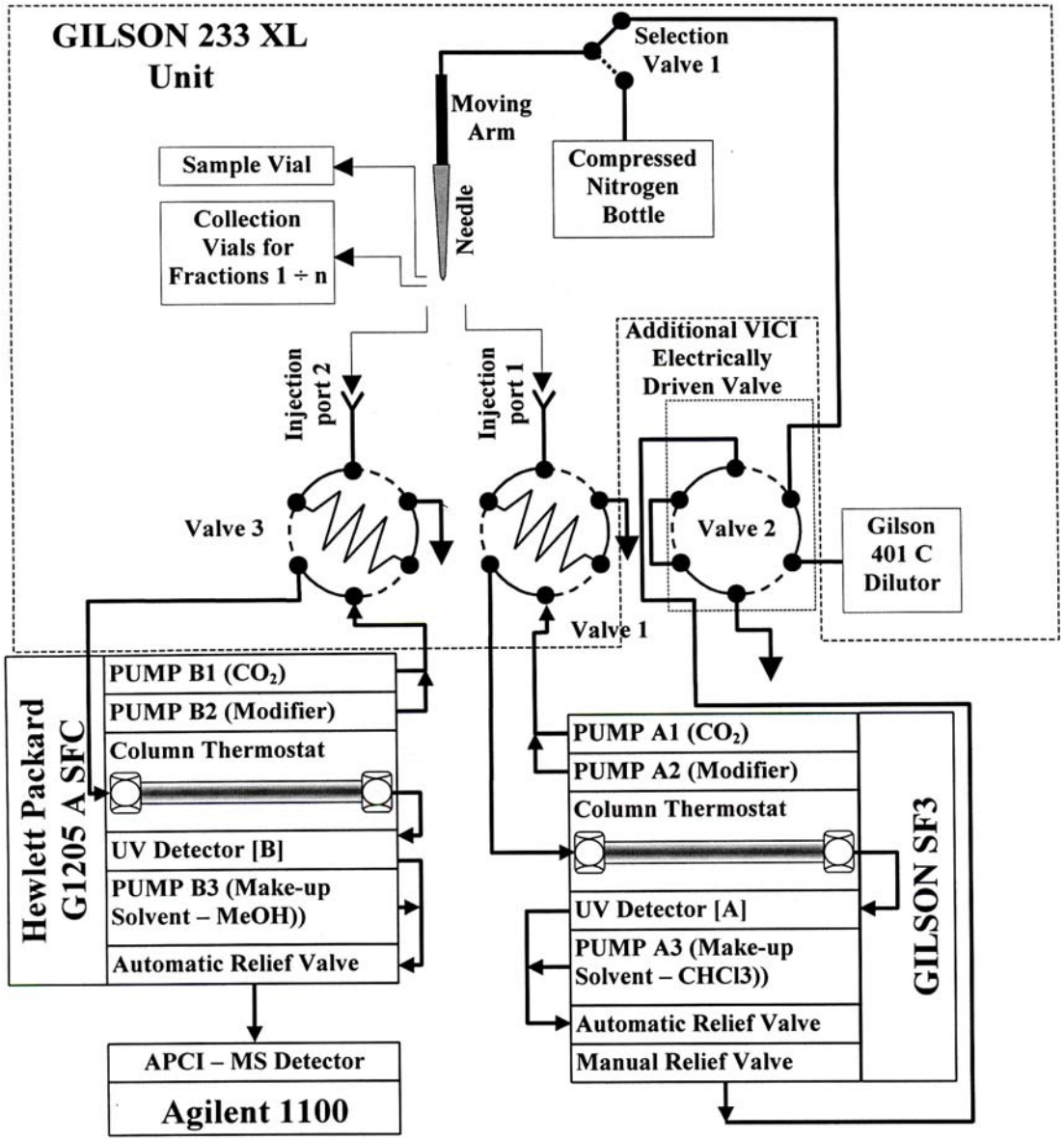


SN = CN- NDB

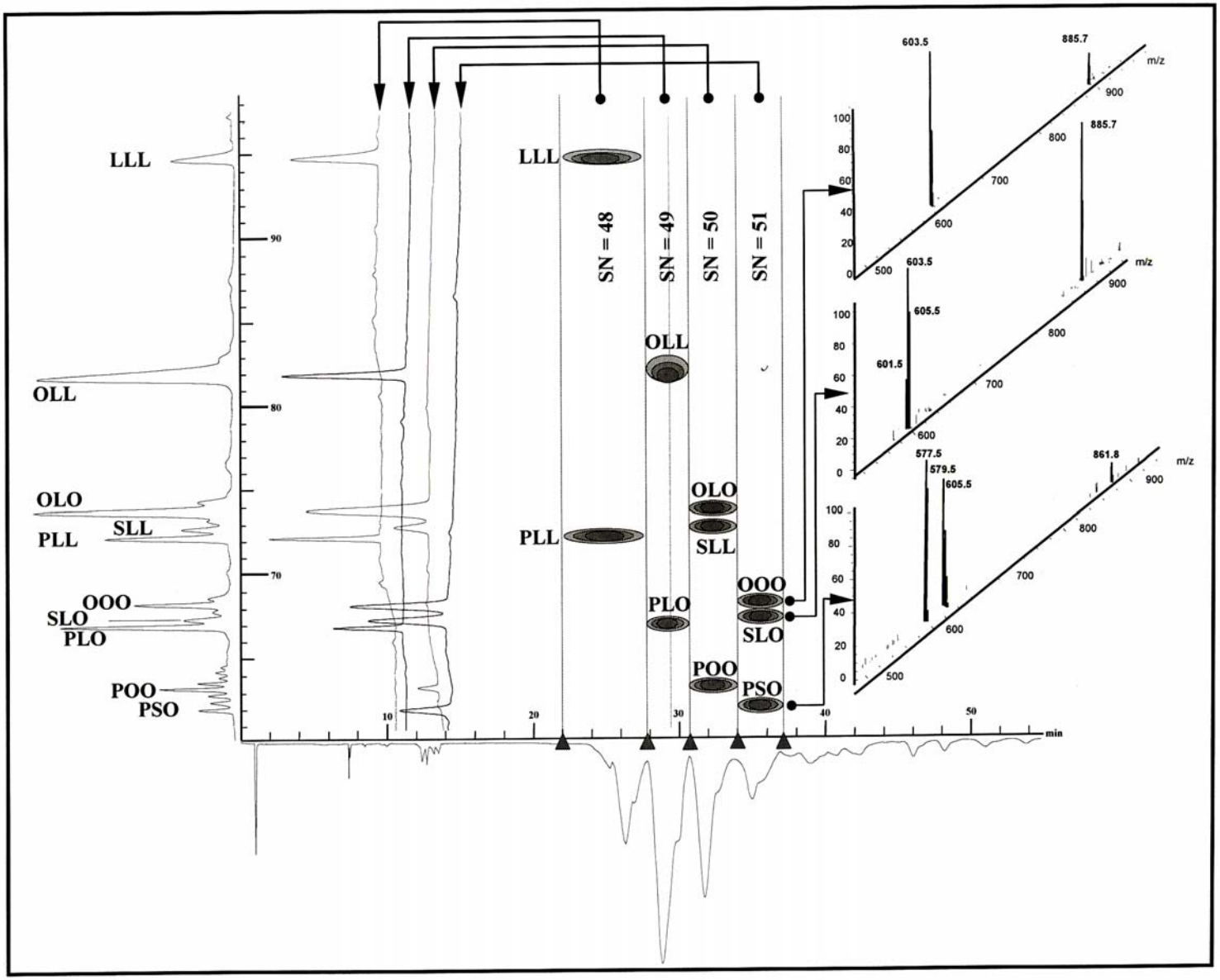
First dimension: PSFC on C18

Column: 2 x Adsorbosphere C18, 25 cm L x 4.6 mm i.d. x 5 um d.p.
Column temperature: 25 ^{o}C;
Pressure: 150 bar
Modifier: ACN/2-PrOH : 6/4; 2.5% in CO_2 isocratic
Flow rate: 2 mL/min

Experimental setup: PSFC x PSFC



Comprehensive pSFC x PSFC / MS: Results!



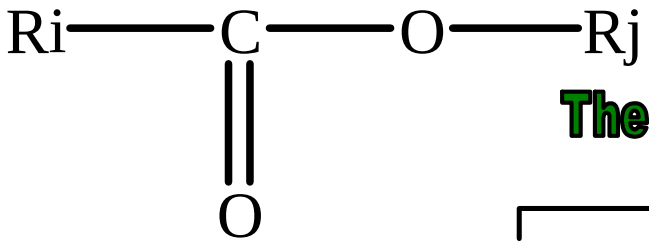
Sometimes NO APCI ionization occurs?

As an example:

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

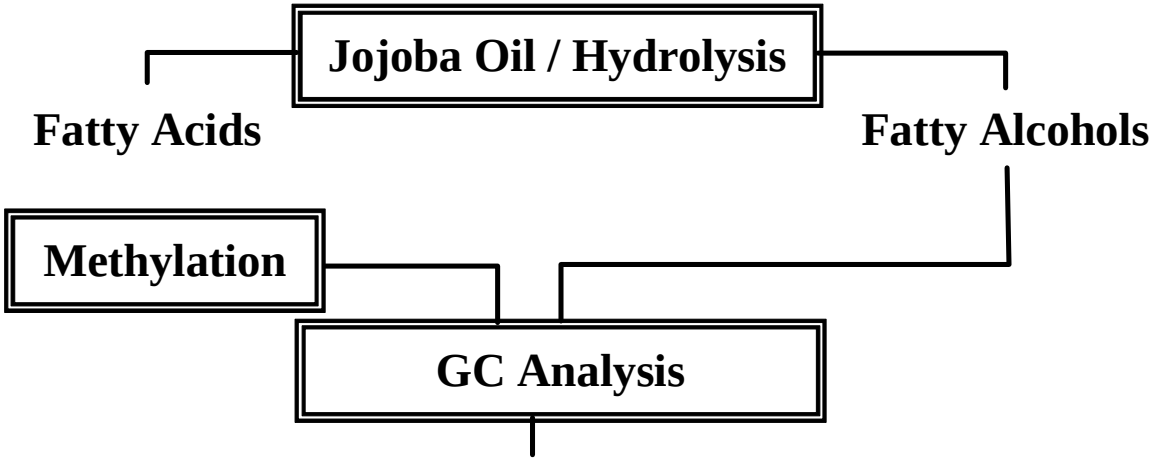
Jojoba Oil, light yellow, odorless

Composition: Mainly Wax Esters !



$C_{i:n} / C_{j:m}$

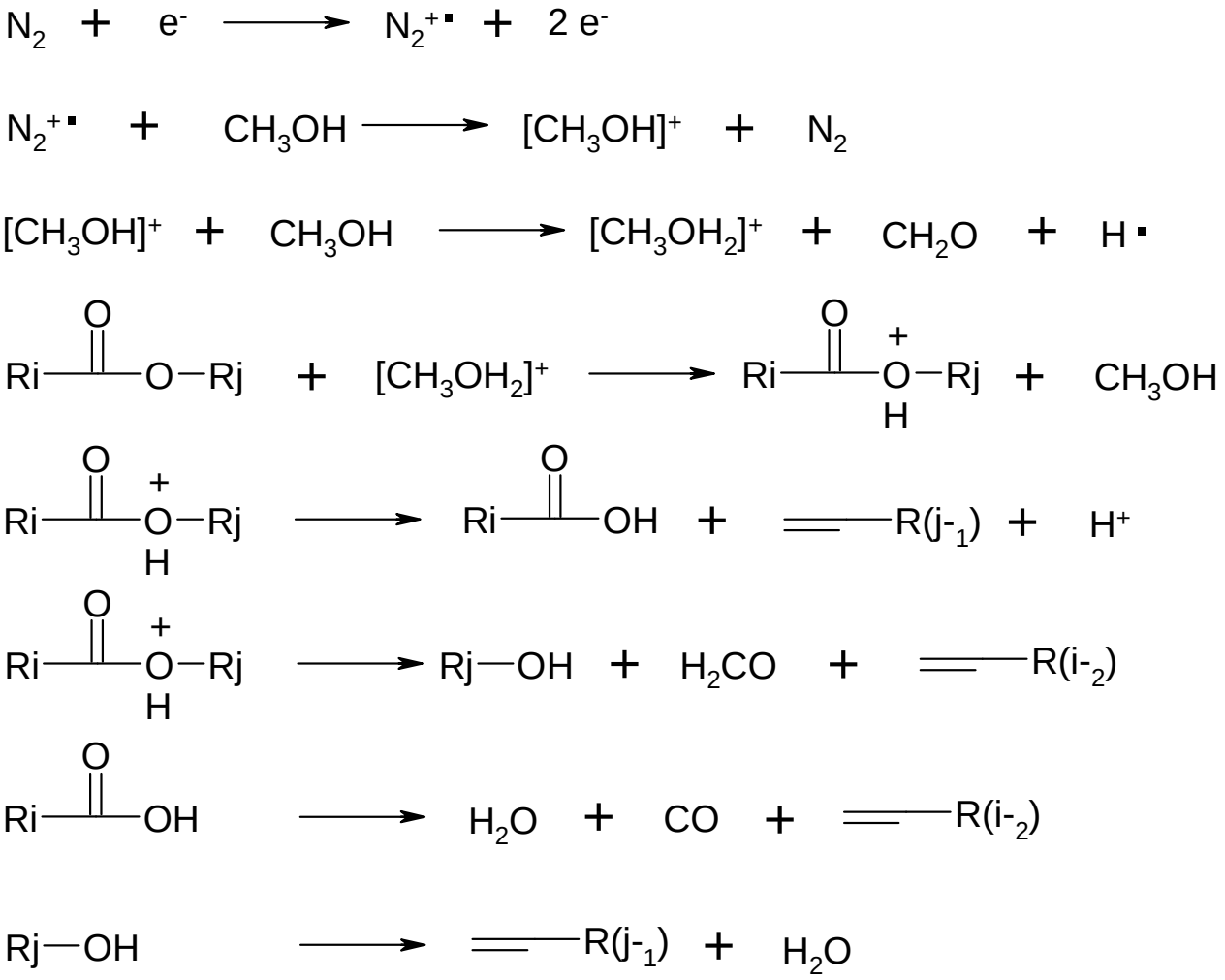
The classical analytical solution:



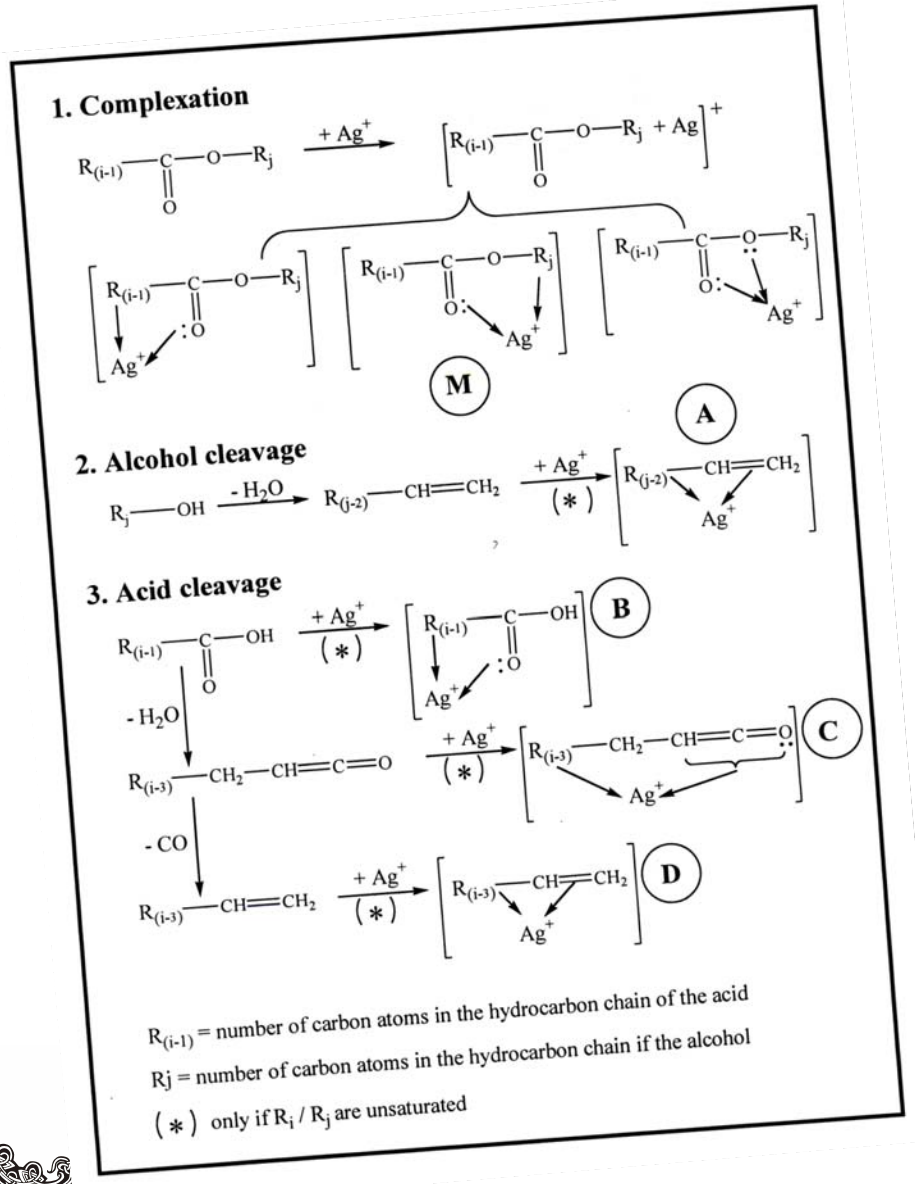
OV-1 - *FAMEs mass distribution;*
Carbowax - *FAs mass distribution*
CN – *unsaturation*

NO ionization!

Why?



Solution: CIS-APCI



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)

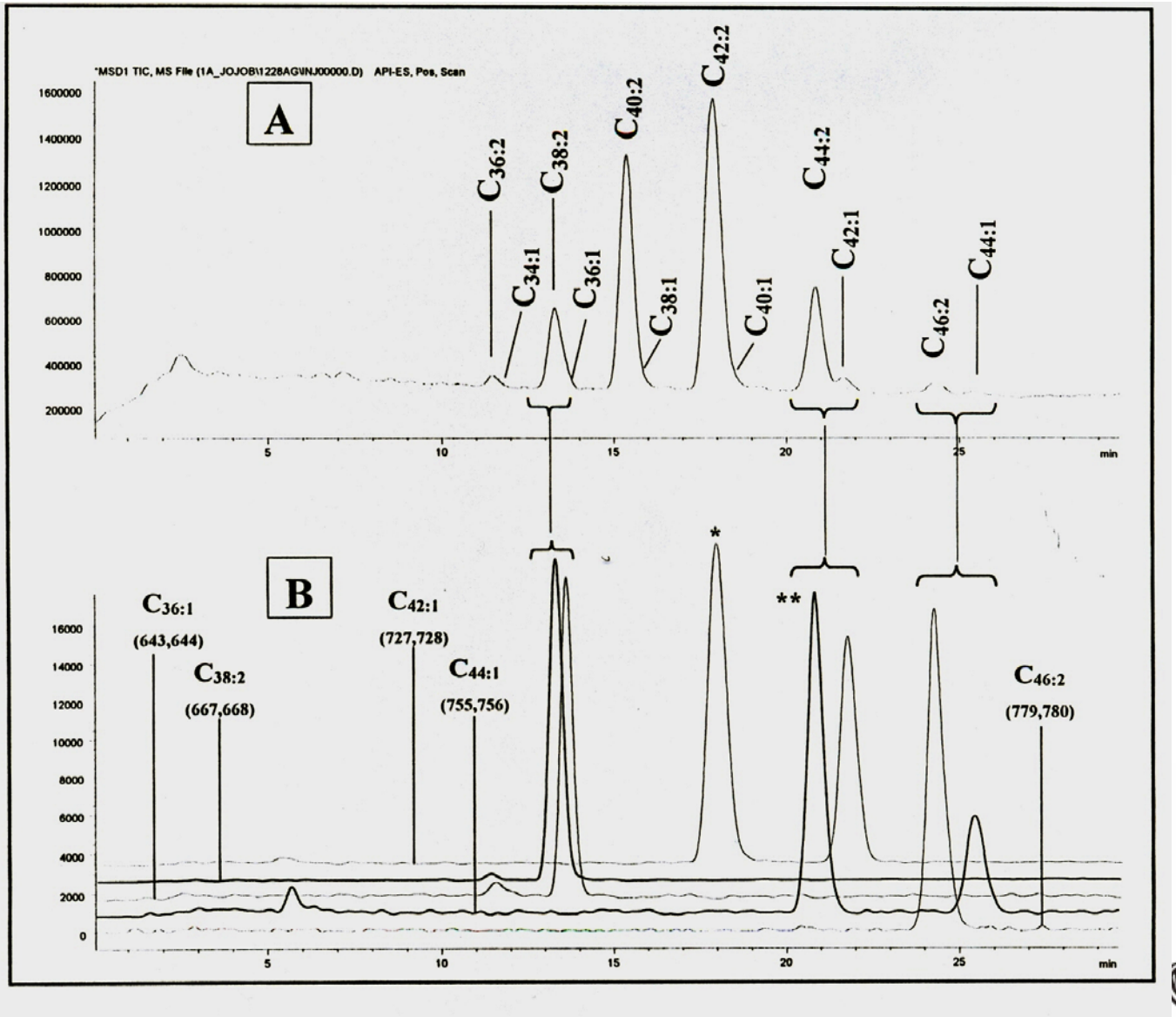
ECN = CN – 2 NDB

Ex.

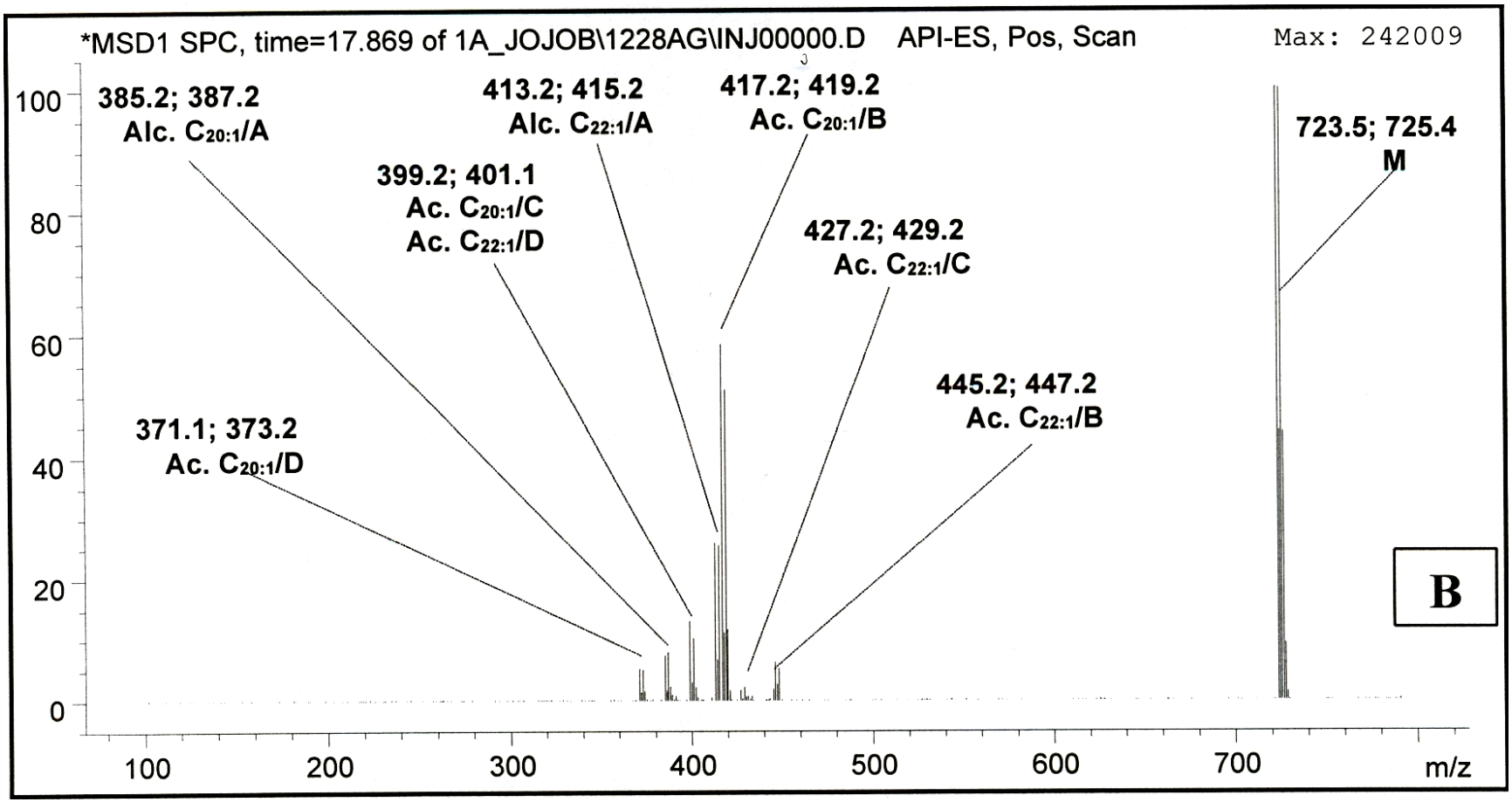
C_xH_{2x} ⇒ CN = x; NDB = 1; ECN = x-1

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

Results:

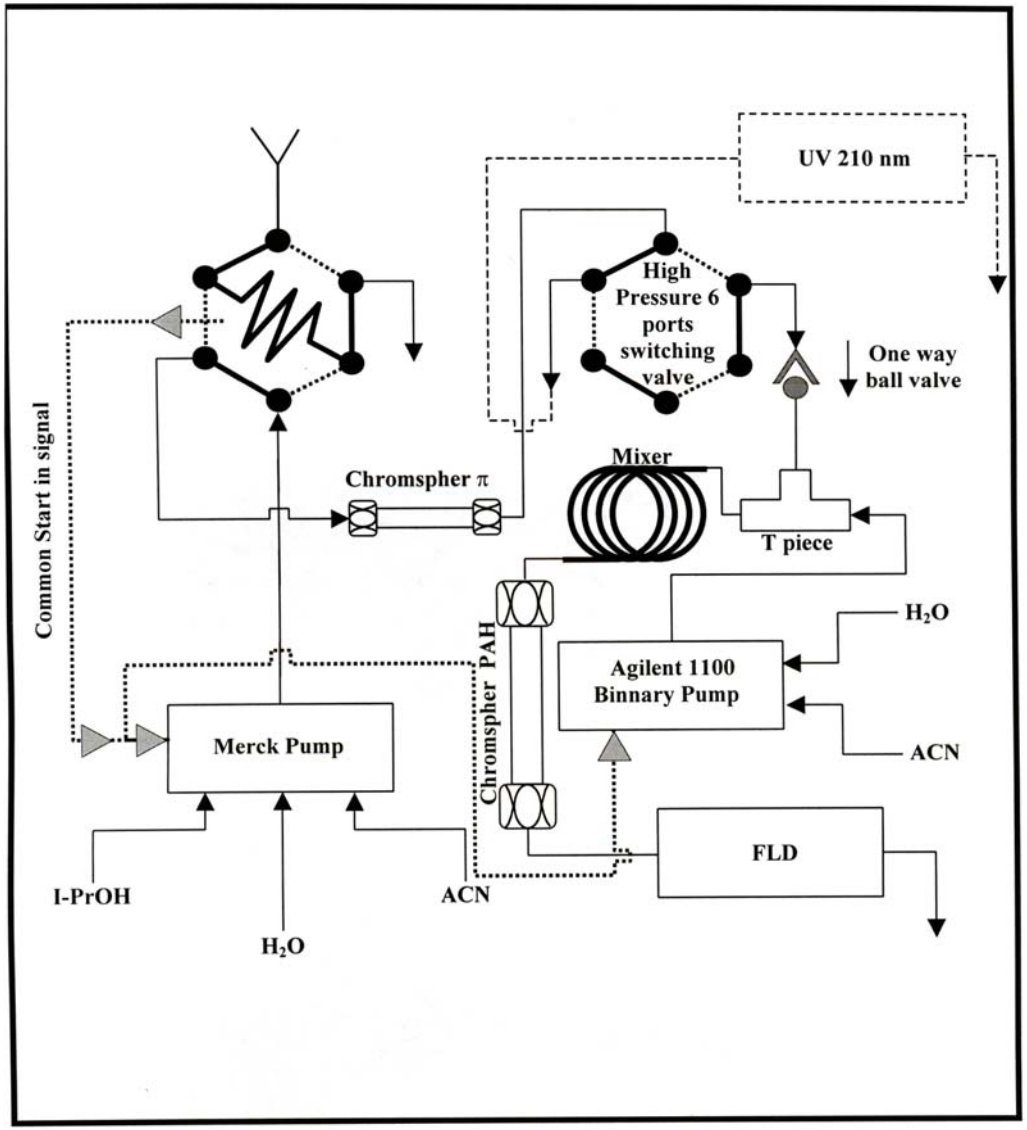

*MSD1 TIC, MS File (1A_JOJOB\1228AG\NJ00000.D) API-ES, Pos, Scan
A
C36:2
C34:1
C38:2
C36:1
C40:2
C38:1
C42:2
C40:1
C44:2
C42:1
C46:2
C44:1
1600000
1400000
1200000
1000000
800000
600000
400000
200000
5
10
15
20
25
min
B
C36:1
(643,644)
C38:2
(667,668)
C42:1
(727,728)
C44:1
(755,756)
*
**
C46:2
(779,780)
16000
14000
12000
10000
8000
6000
4000
2000
0
5
10
15
20
25
min

MS spectral information:

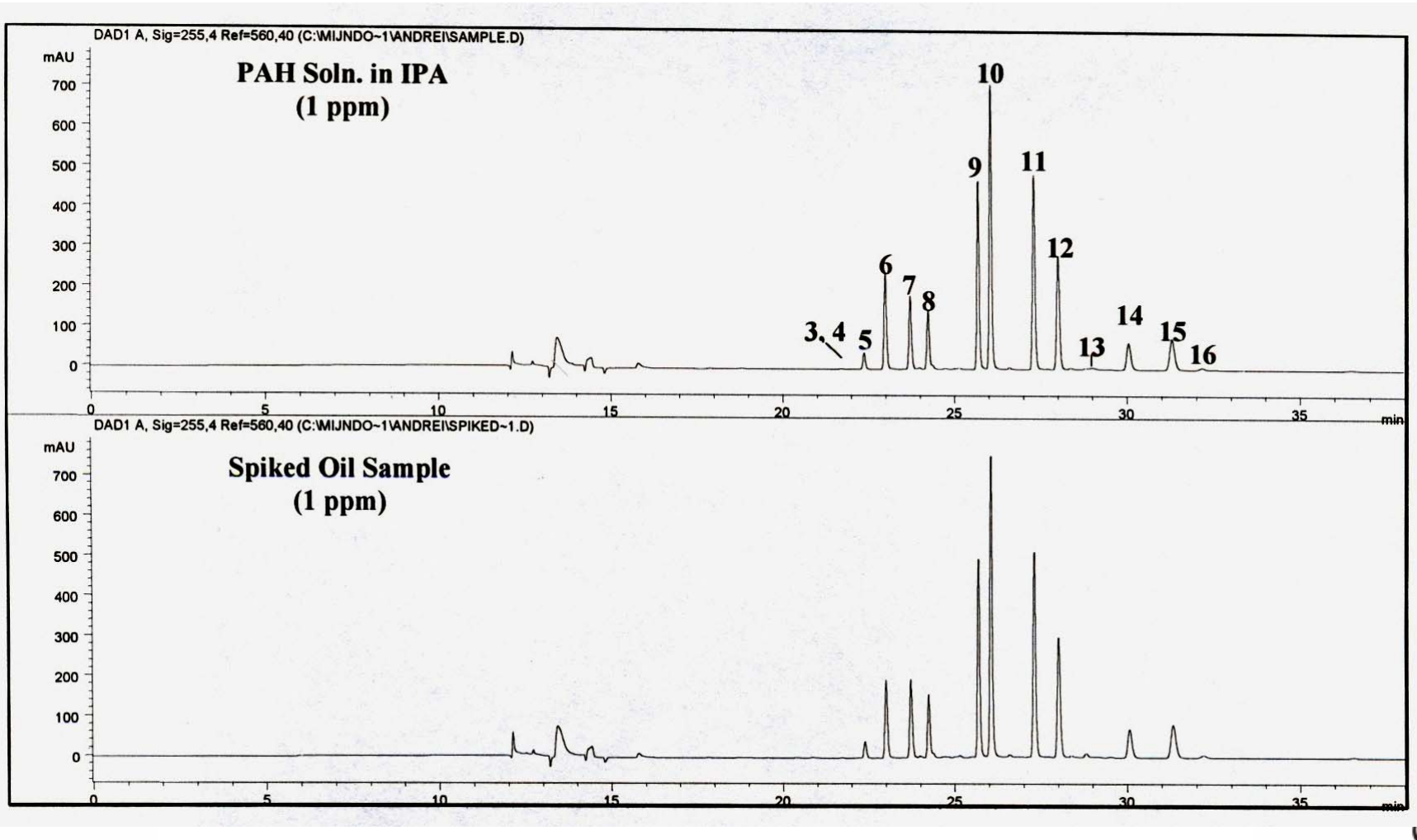


MS selectivity resolves non-ideal LC separation!

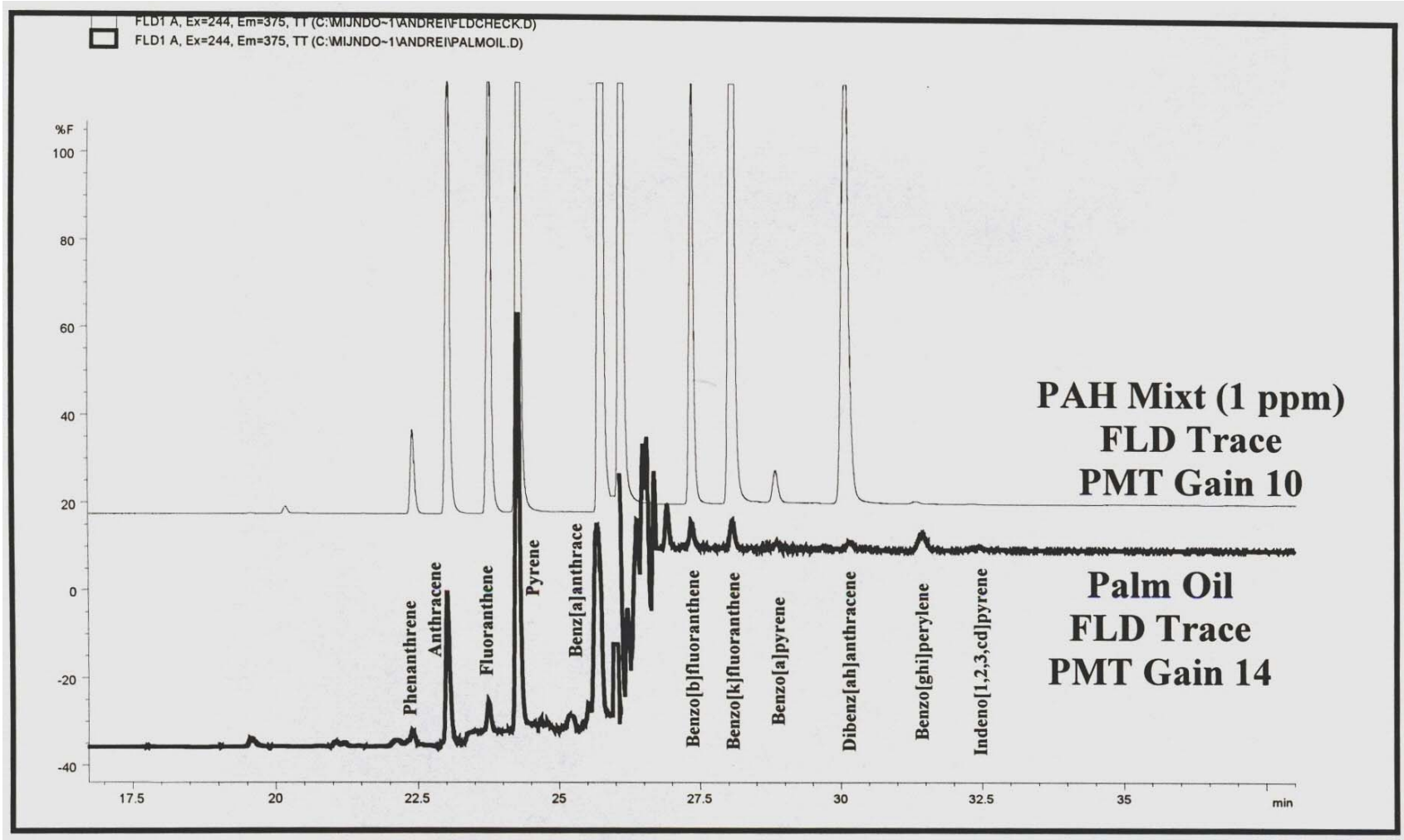
A real nightmare:
Residual PAHs in Palm Oil!



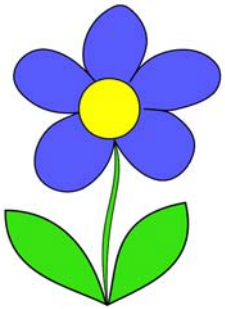
Results:


DAD1 A, Sig=255,4 Ref=560,40 (C:\MIJNDO~1\ANDRE\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\ANDRE\SPIKED~1.D)
mAU
Spiked Oil Sample
(1 ppm)
min

Unfortunately, reality can be hardly accepted sometimes!



Thank you for your attention!



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