Pražské analytické centrum inovací

Projekt CZ.04.3.07/4.2.01.1/0002 spolufinancovaný ESF a Státním rozpočtem ČR

Chromatografické metody v analyse lipidů

Eva Tvrzická

1. LF UK Praha

ZÁKLADNÍ STAVEBNÍ KAMENY ŽIVÉ HMOTY

proteiny

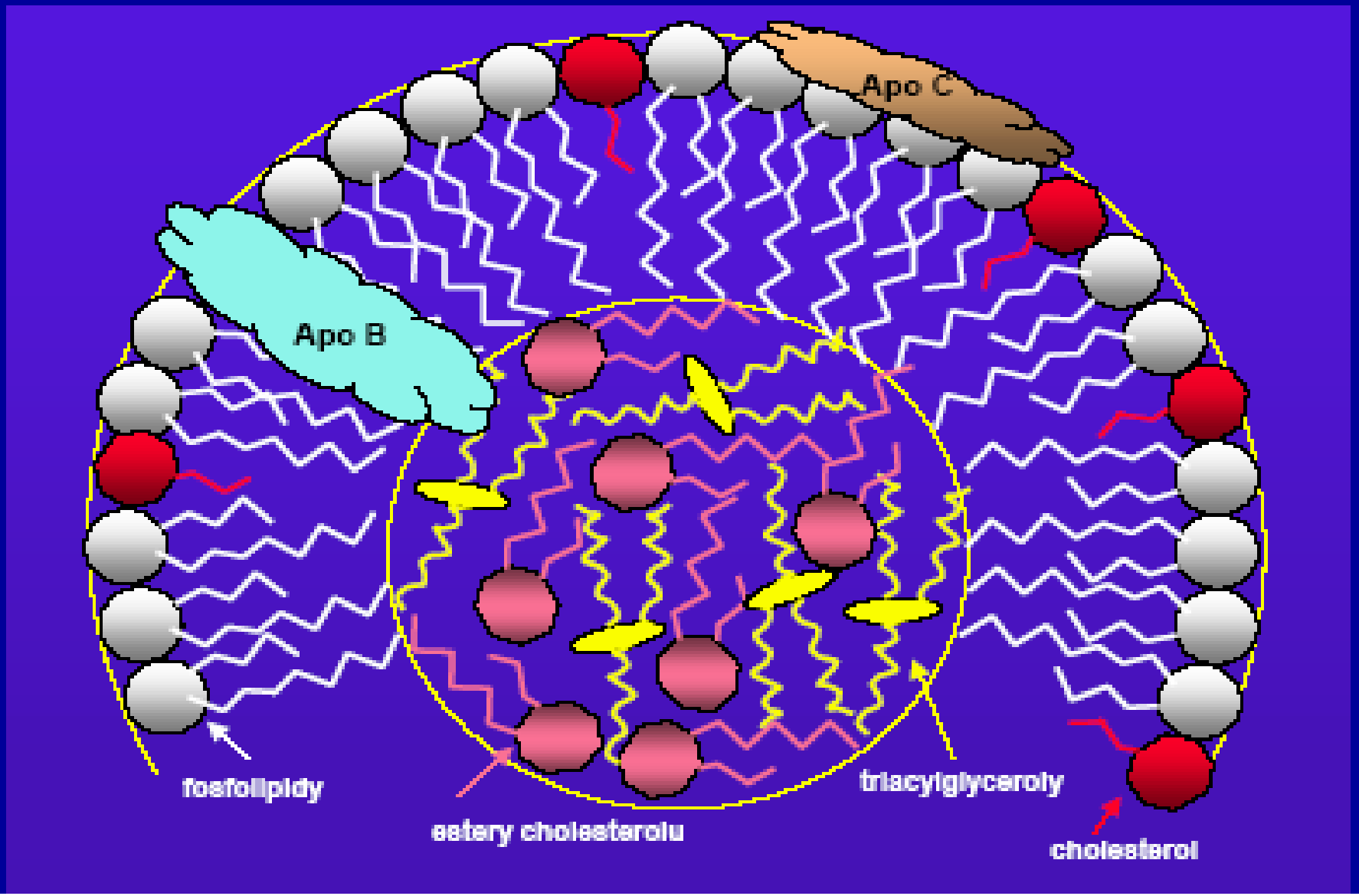
glycidy

lipidy

cirkulující **membránové**

LIPOPROTEINY

CIRKULUJÍCÍ FORMA LIPIDŮ



LIPOPROTEINY

FYSIKÁLNÍ VLASTNOSTI

LP	MW (Da)	Průměr (nm)	Hustota (g/ml)
CM	50 - 1,000,000,000	75 - 1200	< 0.930
VLDL	10 - 80,000,000	30 - 80	0.930 - 1.006
IDL	5 - 10,000,000	25 - 35	1.006 - 1.019
LDL	2 - 3,000,000	18 - 25	1.019 - 1.063
HDL	65 - 400,000	5 - 12	1.063 - 1.210

LIPOPROTEINY

ANALYTICKÉ METODY

PREPARACE – ULTRACENTRIFUGA

STANOVENÍ VELIKOSTI ČÁSTIC – HPLC (DIALYSA)

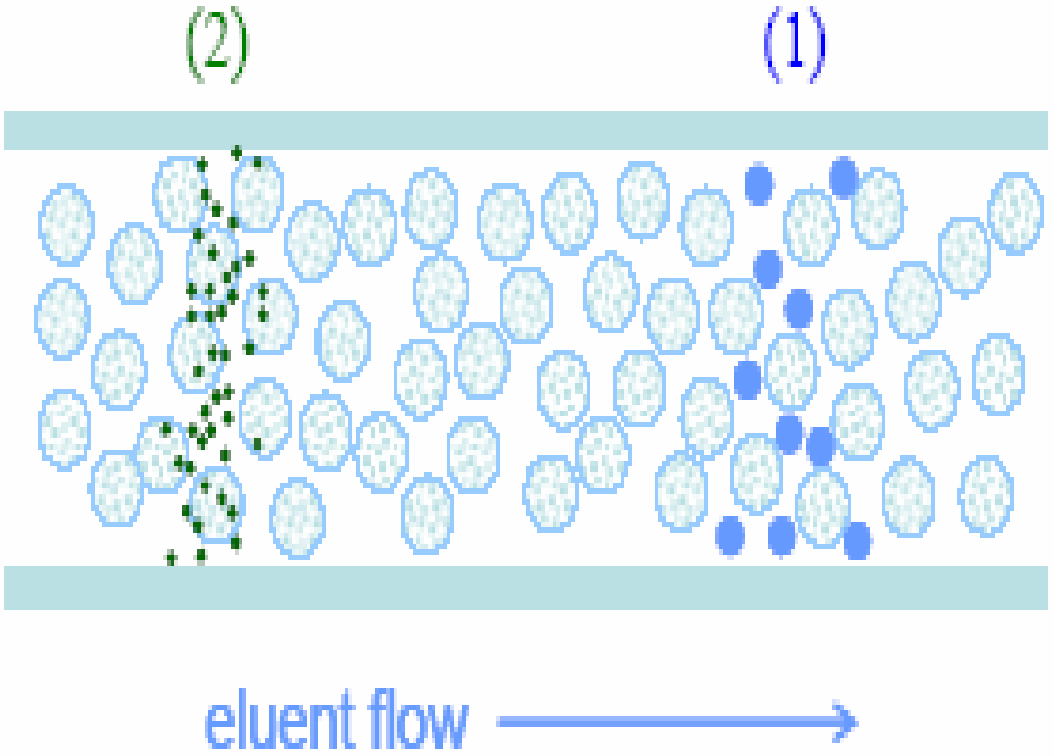
ANALYSA PROTEINOVÉ SLOŽKY – HPLC

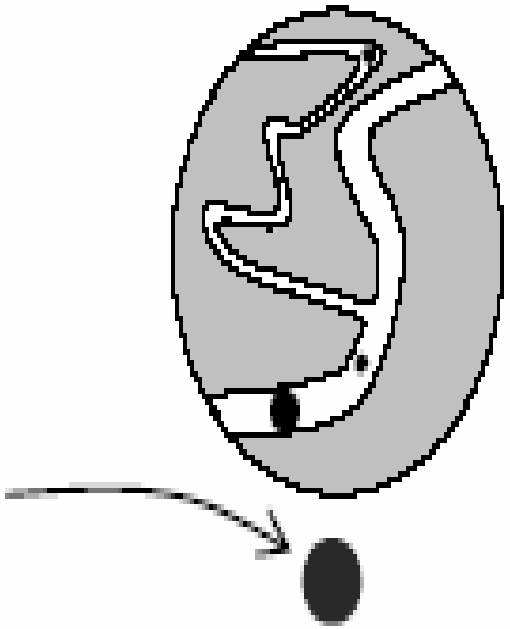
ANALYSA LIPIDOVÉ SLOŽKY – TLC, HPLC

ANALYSA MOLEKULÁRNÍCH DRUHŮ - GC, HPLC

ALTERNATIVNÍ METODY

SEC, GPC, MSC - PRINCIP METODY





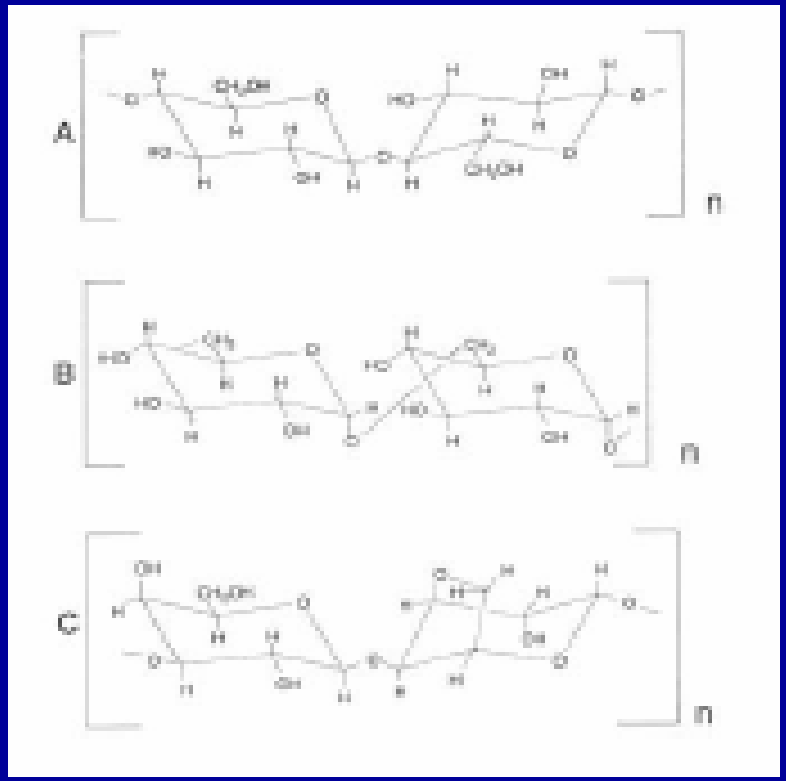
SEC, GPC, MSC - PODMÍNKY

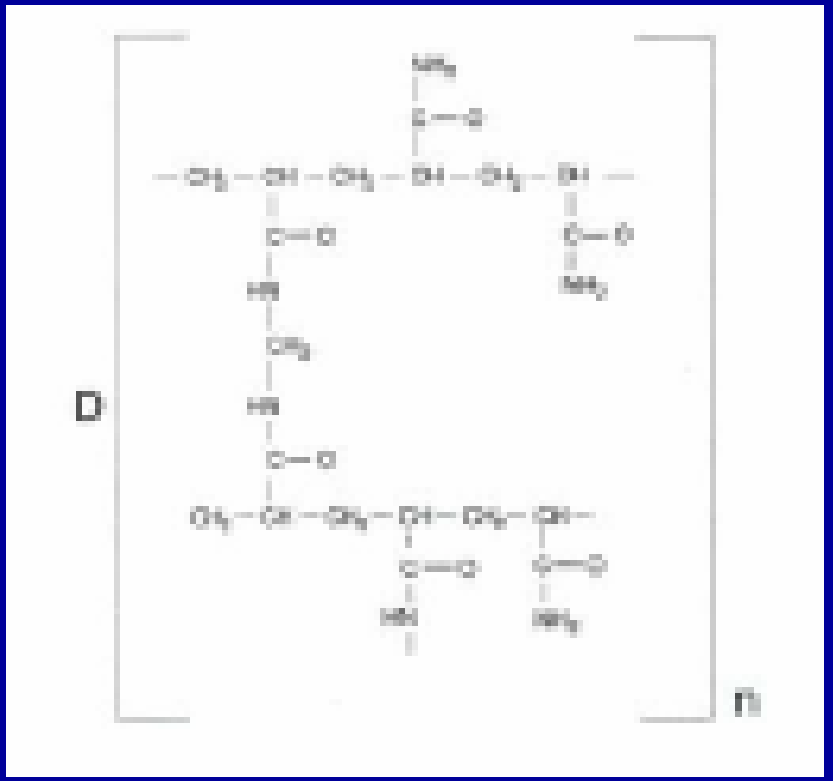
Separace molekul podle velikosti

SF - silikagel (SiOx)
polysacharidy (celulosa, dextran, agarosa)
polyakrylamid
MF - vodné roztoky pH 6 – 8
fosfát 7.2, tris(hydroxymethyl)aminomethan 8.1
NaCl 0.05 – 0.5M ↓ iontové interakce (protein-gel)
↓ neiontové interakce – detergent (ethylen glykol)
rozpouštědla (n-propanol, acetonitril)

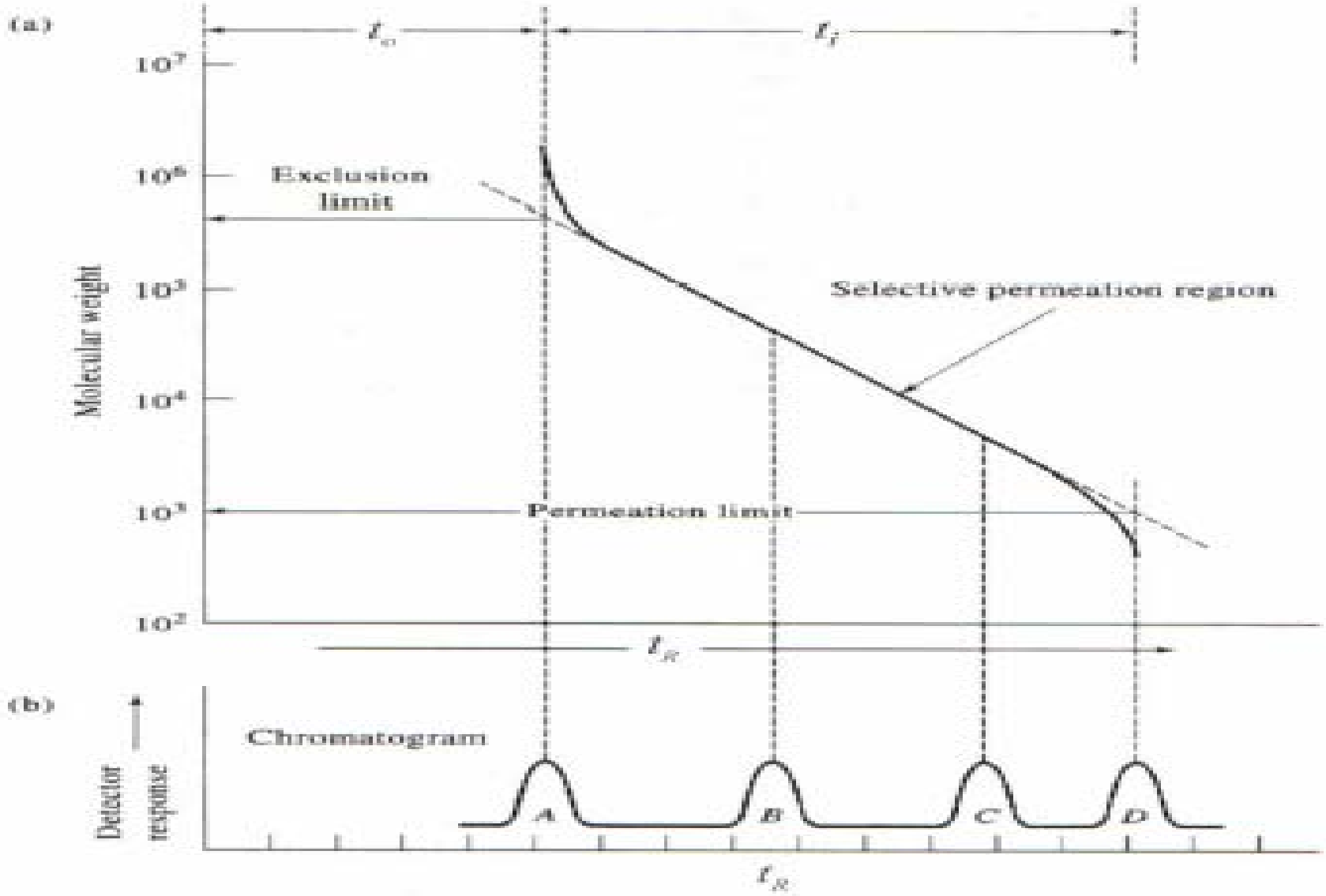
STRUKTURA SORBENTU

a) celulosa
b) dextran
c) Agarosa
d) polyakrylamid

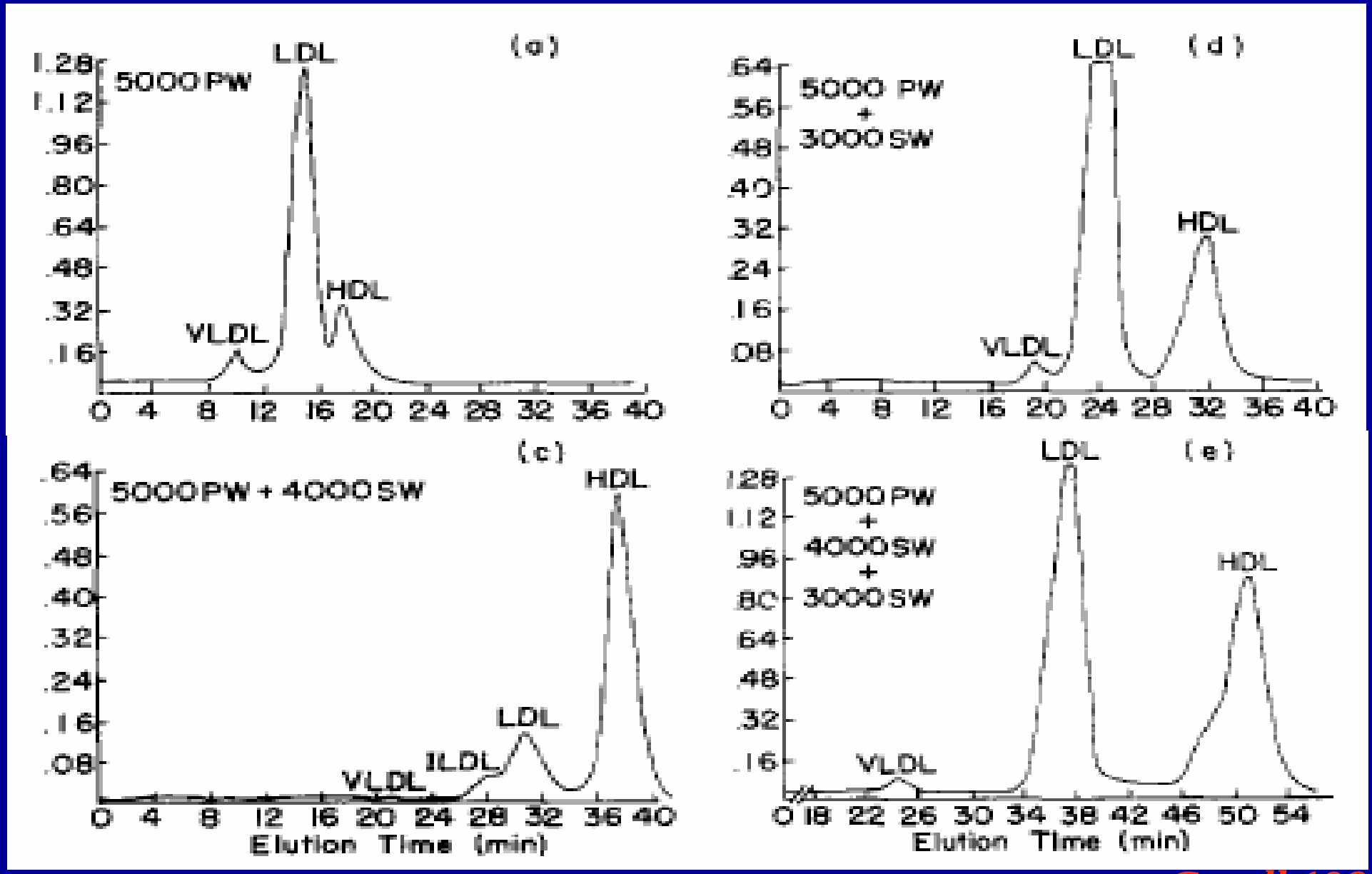




SEC - KALIBRACE



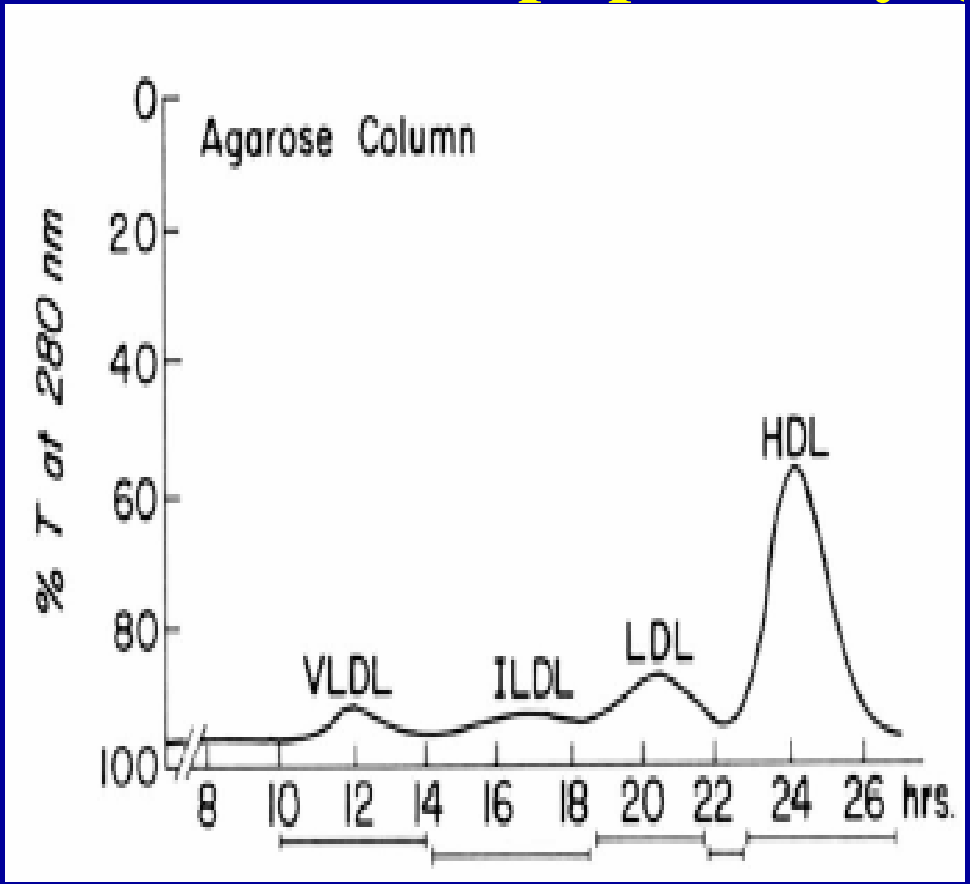
SEC - KOMBINACE KOLON

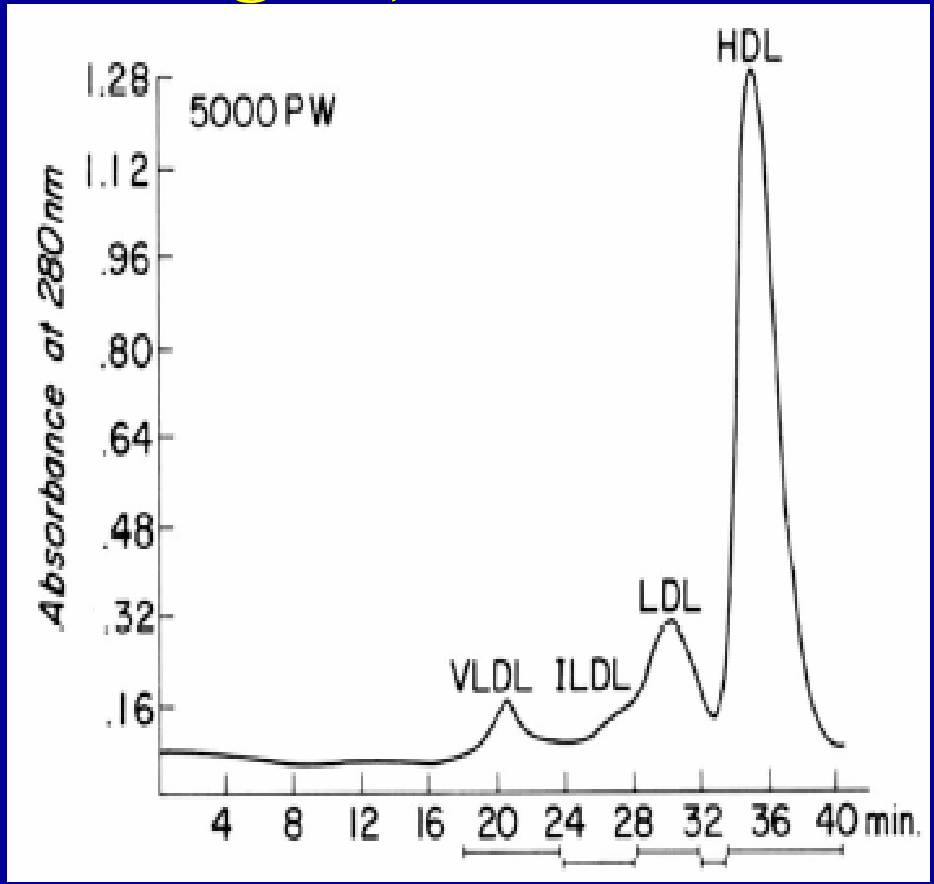


Caroll 1983

SEC - SROVNÁNÍ KOLON

lipoproteiny (d < 1.225 g/ml)





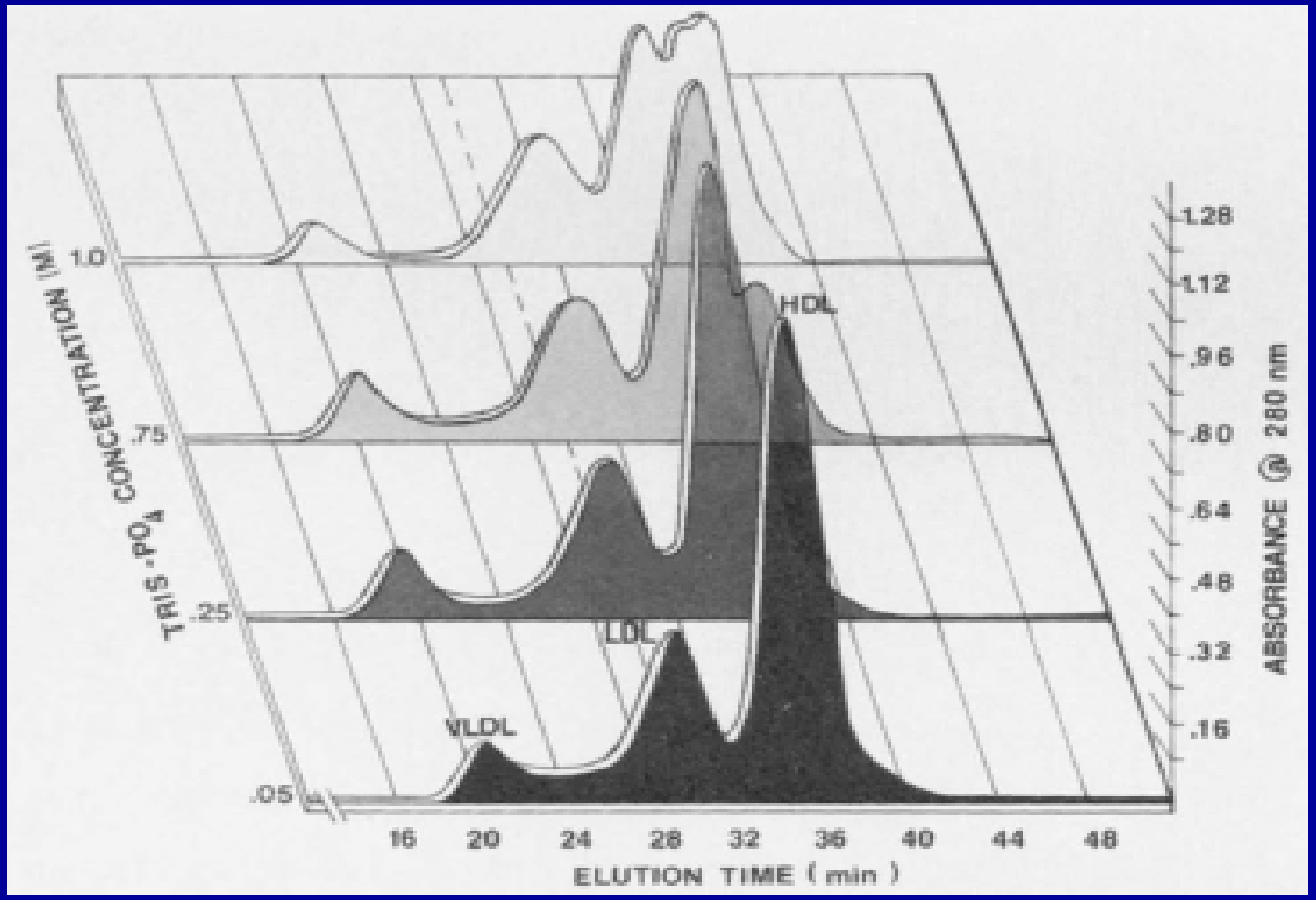
1.5x90 cm, 0.9% NaCl pH 7.4, 7 ml/h, 4°C

7.5x60 cm, 0.25M $TrisPO_4$, pH 7.6, 0.5 ml/mi

Caroll 1983

SEC -VLIV MOBILNÍ FÁZE

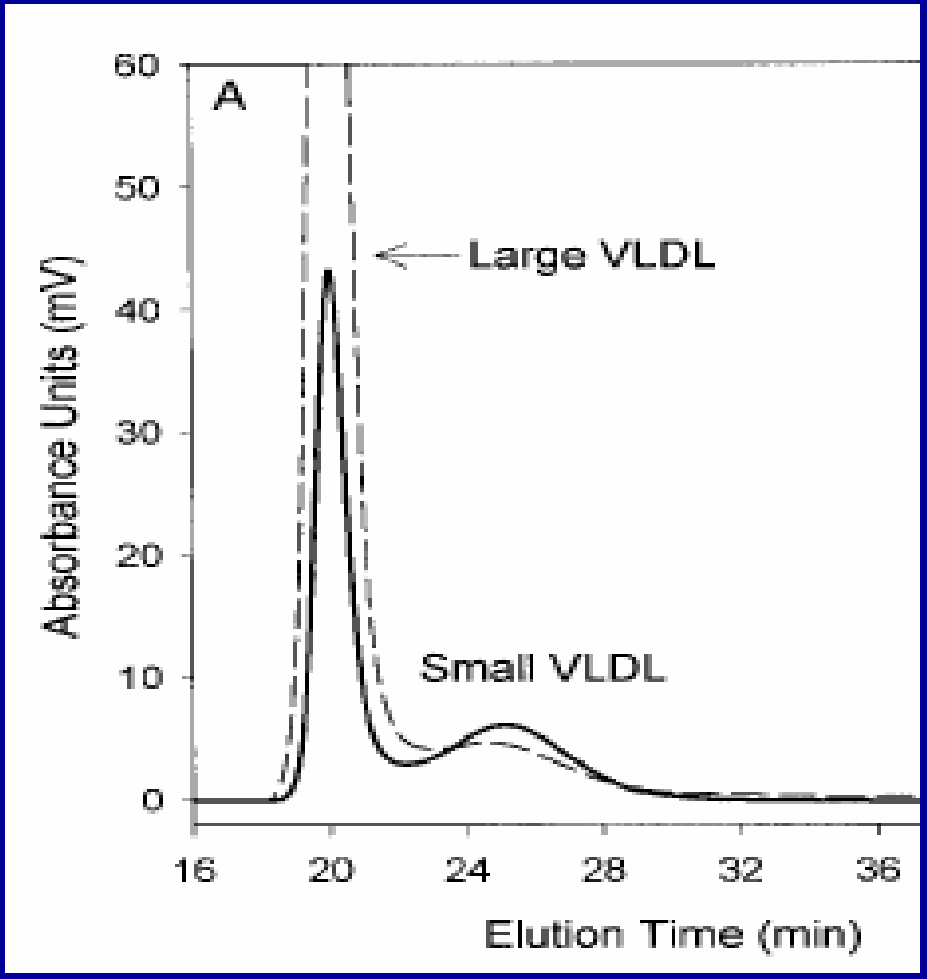
Lipoproteiny (d < 1.225 g/ml), TRIS-PO_4 pH 7.6

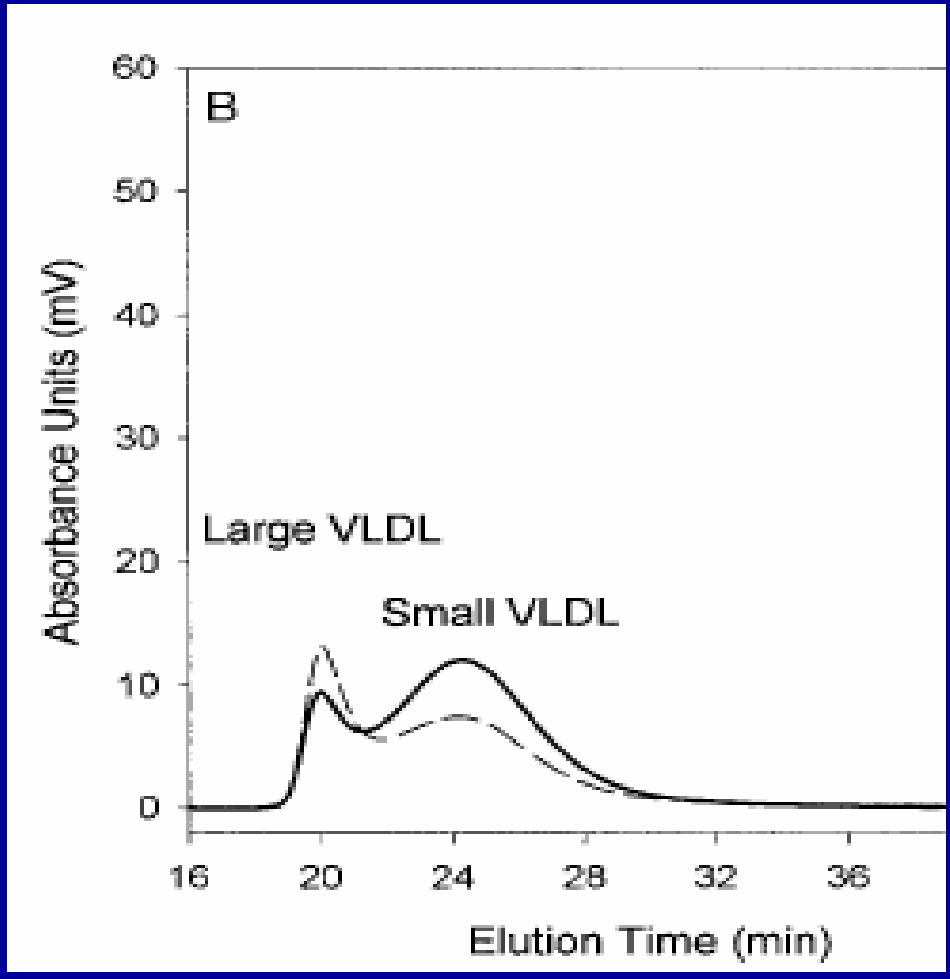


Caroll 1983

SEC – SROVNÁNÍ VZORKŮ

lipoproteiny (d < 1.006 g/ml)



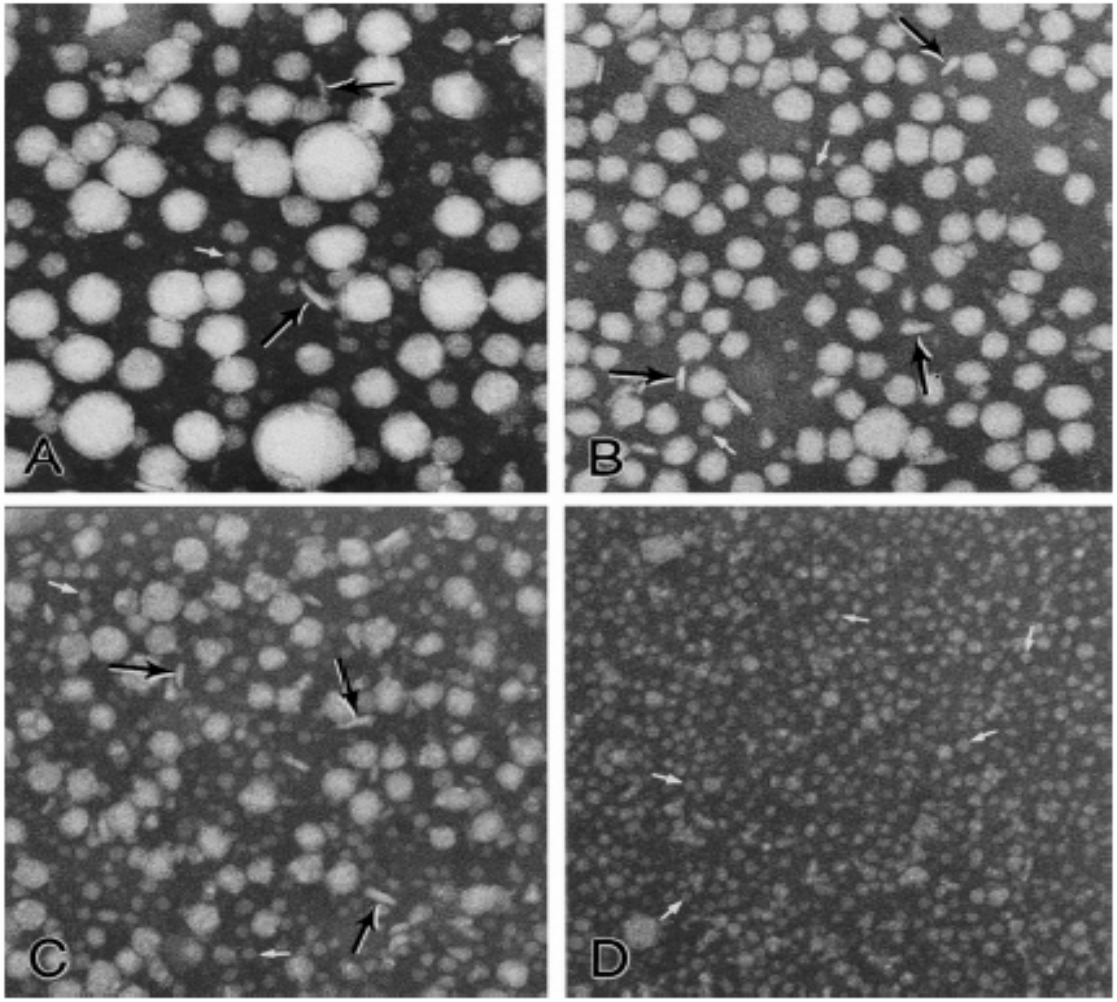


Superose 6HR, 1x30 cm, 0.05M PBS, pH 7.4, 0.5 ml/min

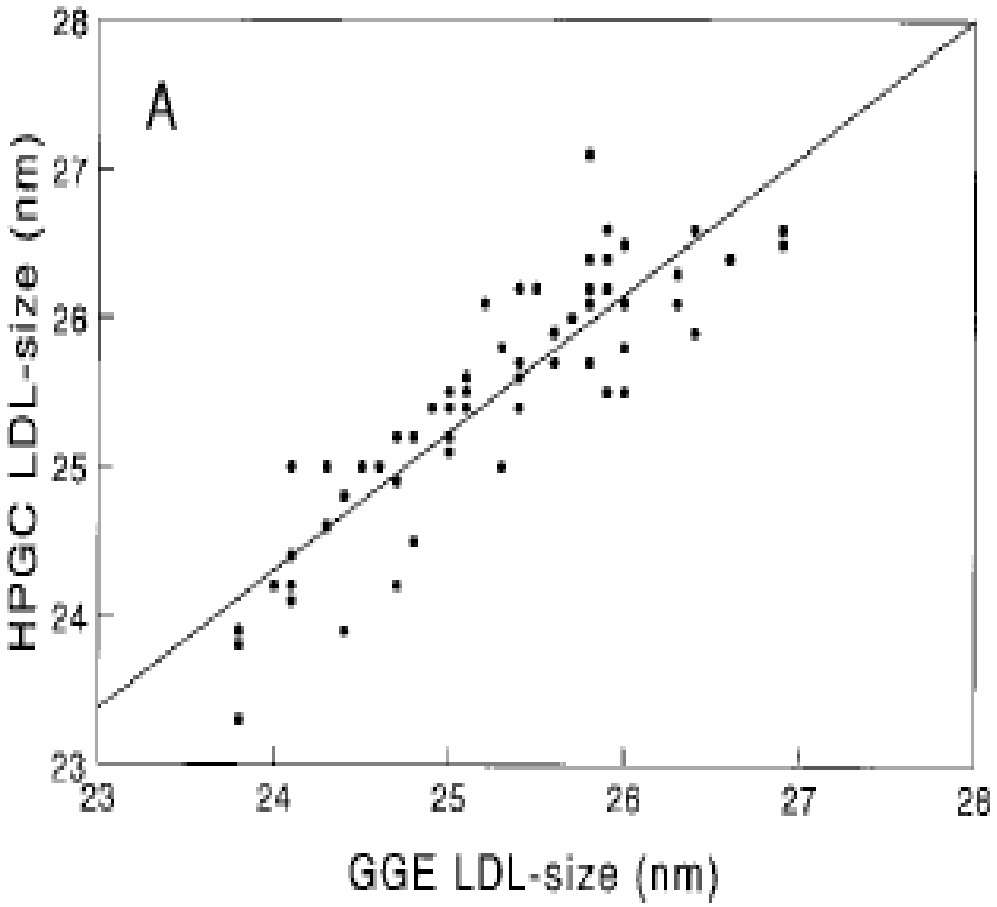
Usui 2002

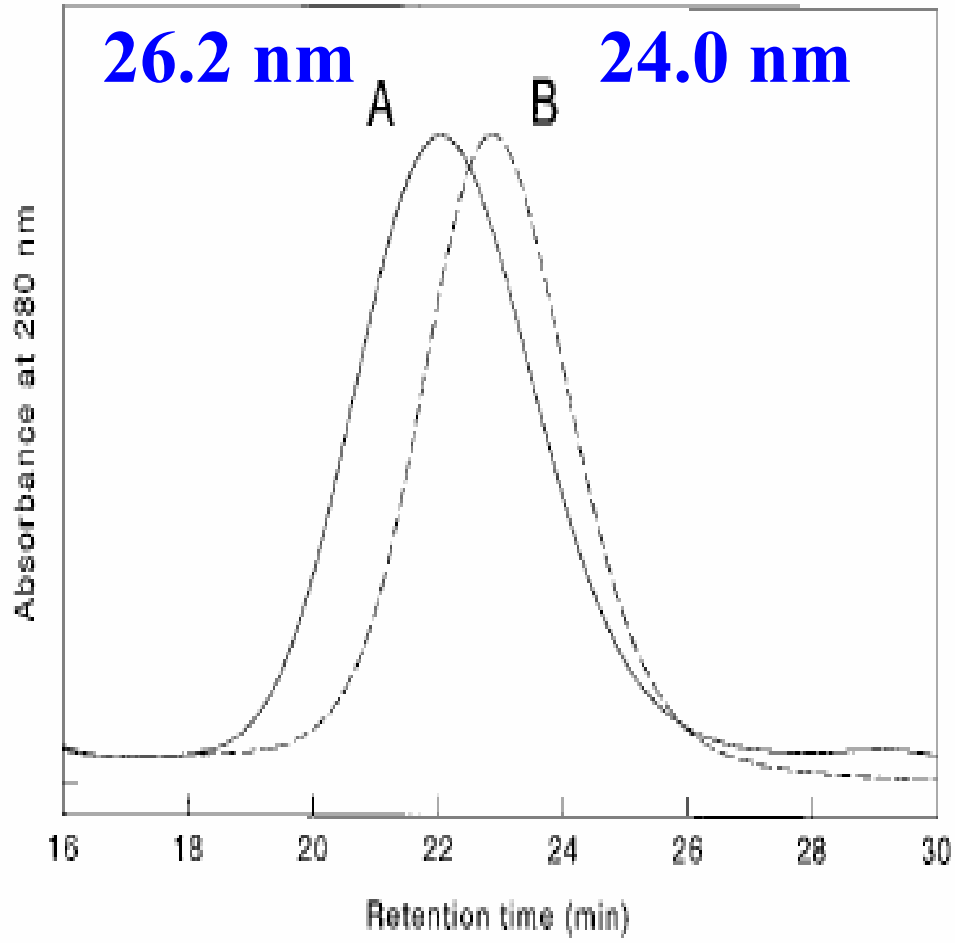
ELEKTRONOVÝ MIKROSKOP

a) 27 – 60 nm b) 22 – 33 nm
c) 17 – 33 nm d) 7 – 12 nm



SROVNÁNÍ SEC – PAGE VELIKOST LDL



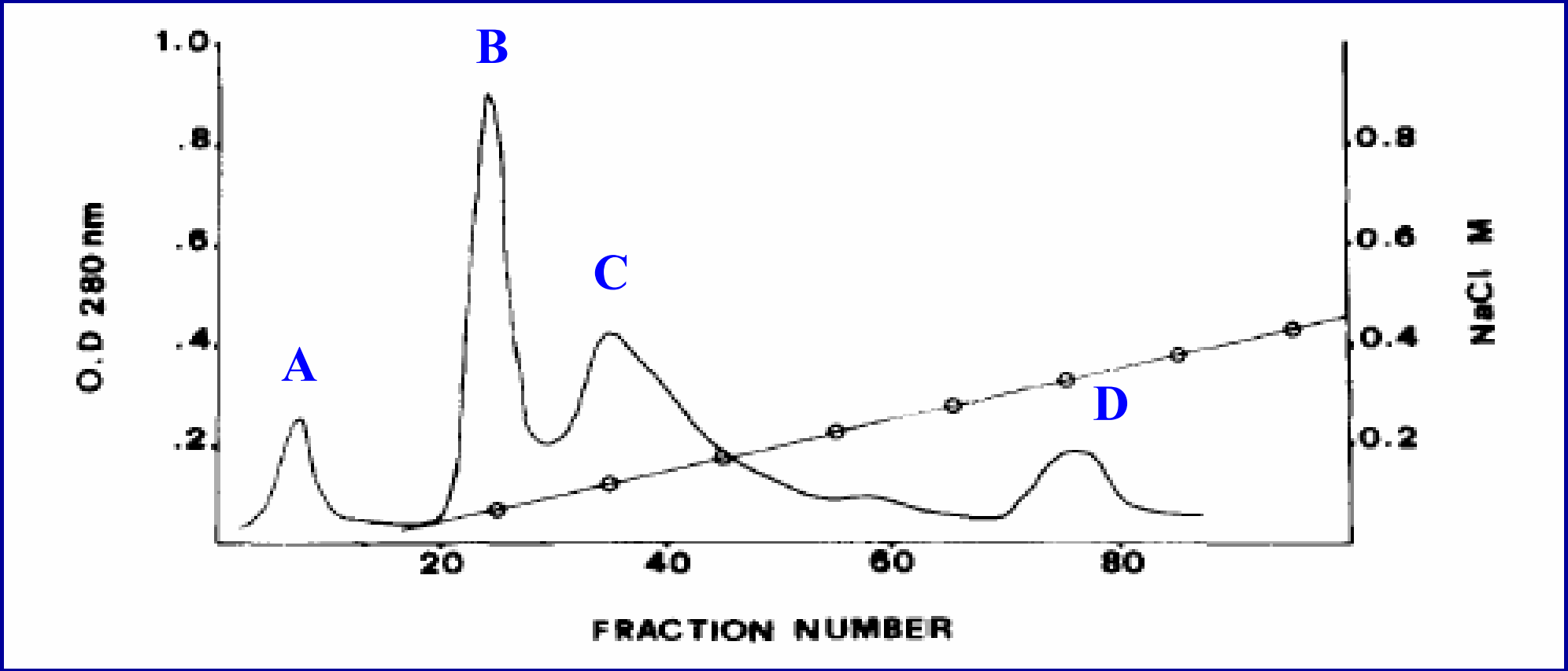


Superose 6, 1x30 cm, 0.1M PBS pH 7.4, 0.5 ml/min, PAGE 2-10%

Scheffer 1997

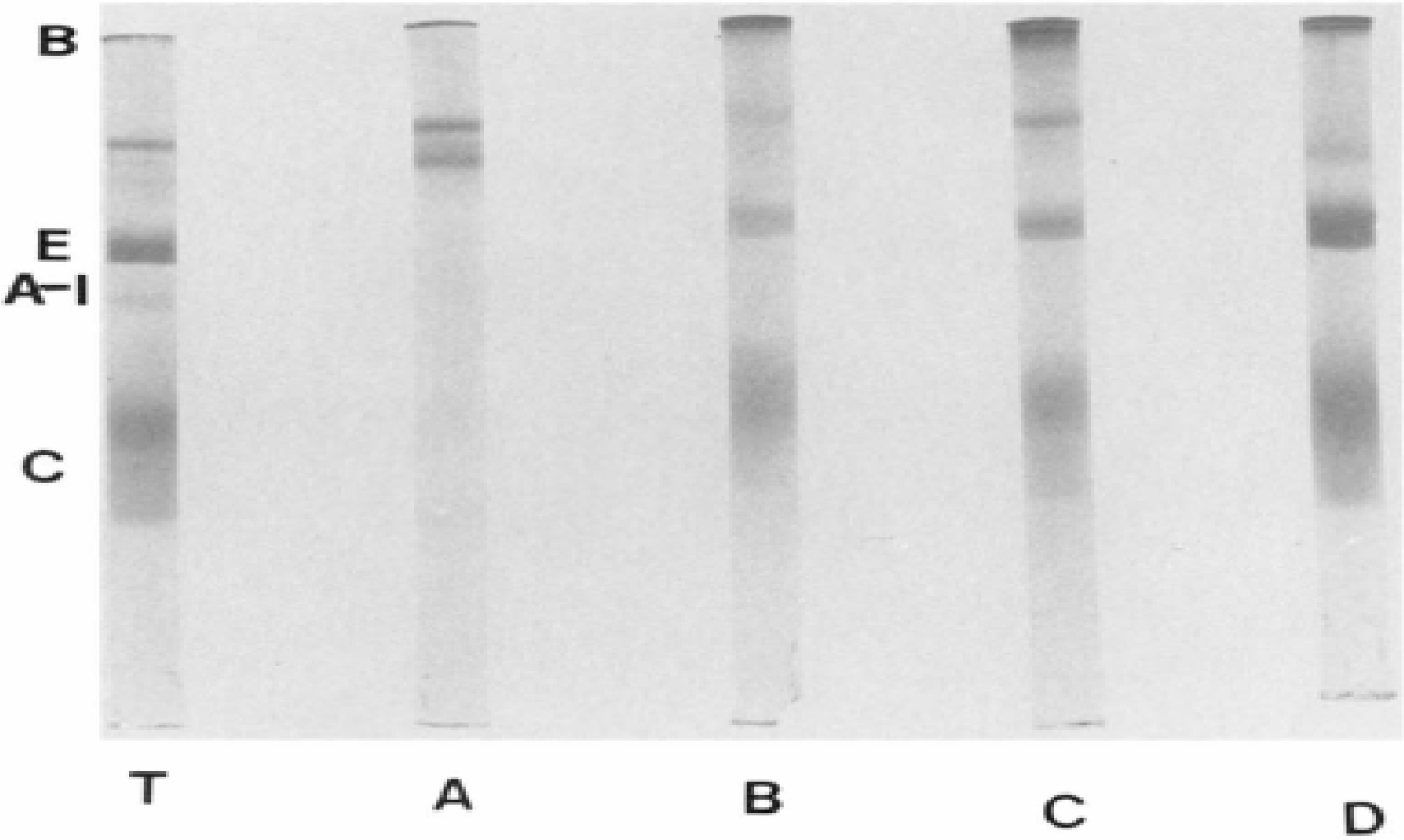
VLDL - AFINITNÍ CHROMATOGRAFIE

SF: heparin-Sepharosa 2.6 x 8 cm
MF: 0.005M Tris, pH 7.4, gradient NaCl 0.05–0.5M



Trezzi 1983

SDS-ELFO FRAKCÍ VLDL

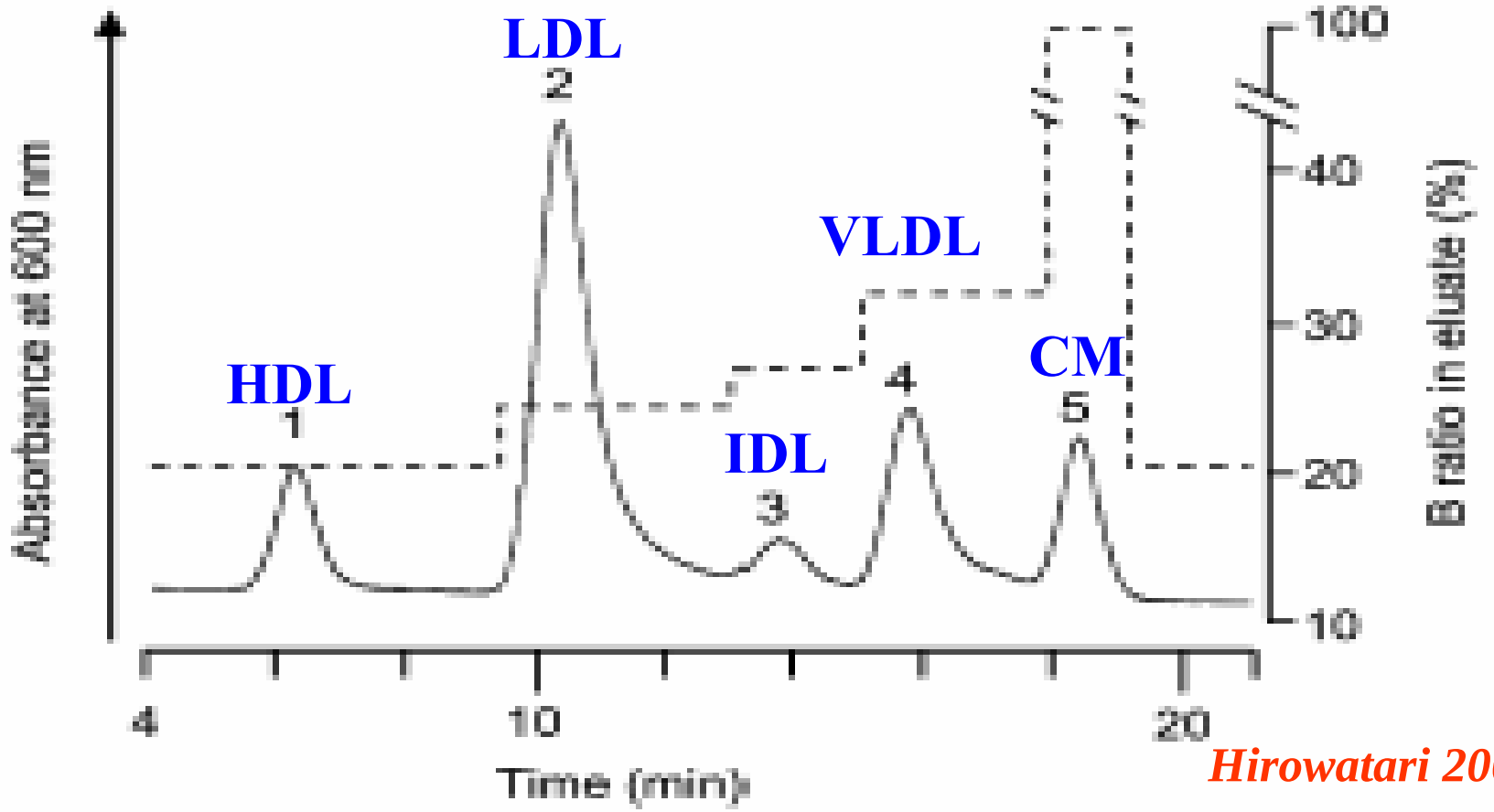


Trezzi 1983

LP - IONTOVÝMĚNNÁ CHROMATOGRAFIE

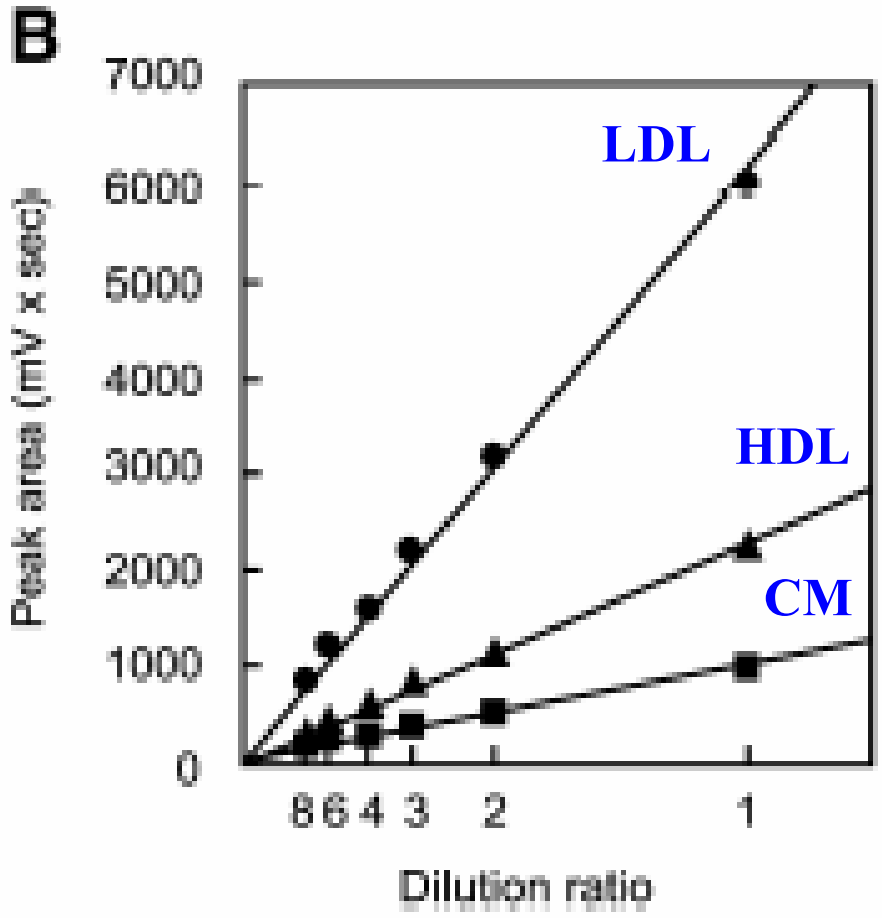
SF: DEAE-polyakrylamid 4.6 x 20 mm

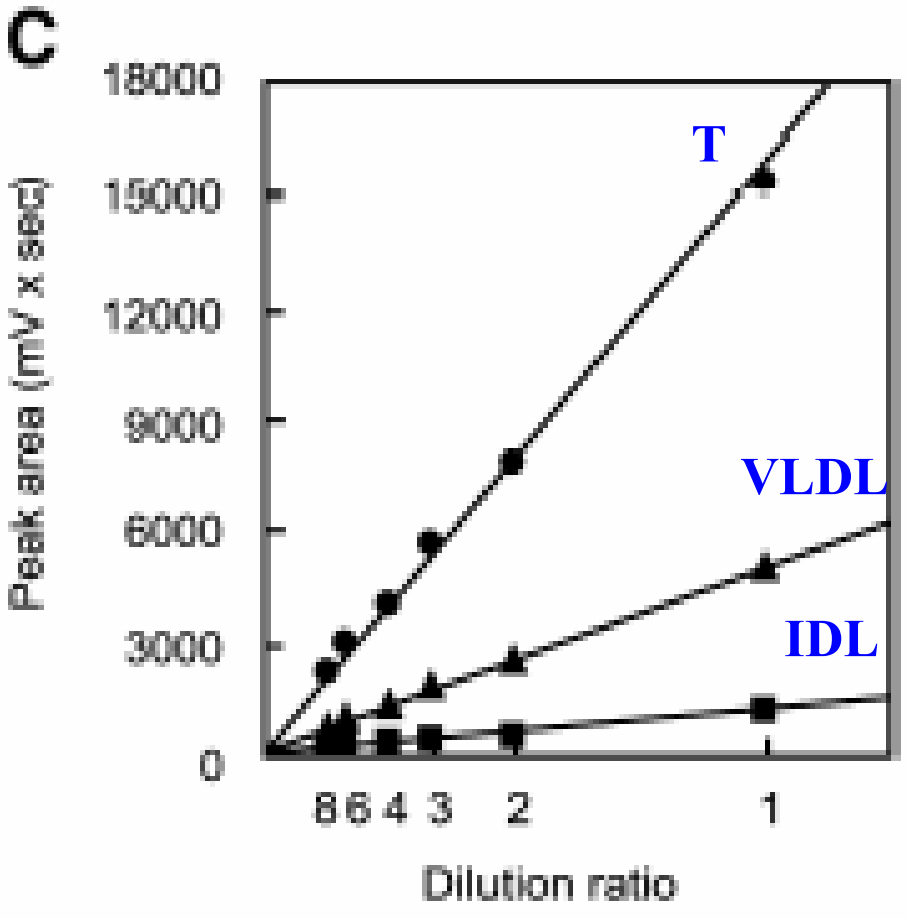
MF: 0.05M Tris-HCl, pH 7.5, gradient $NaClO_4$ 0.1–0.5M



Hirowatari 2003

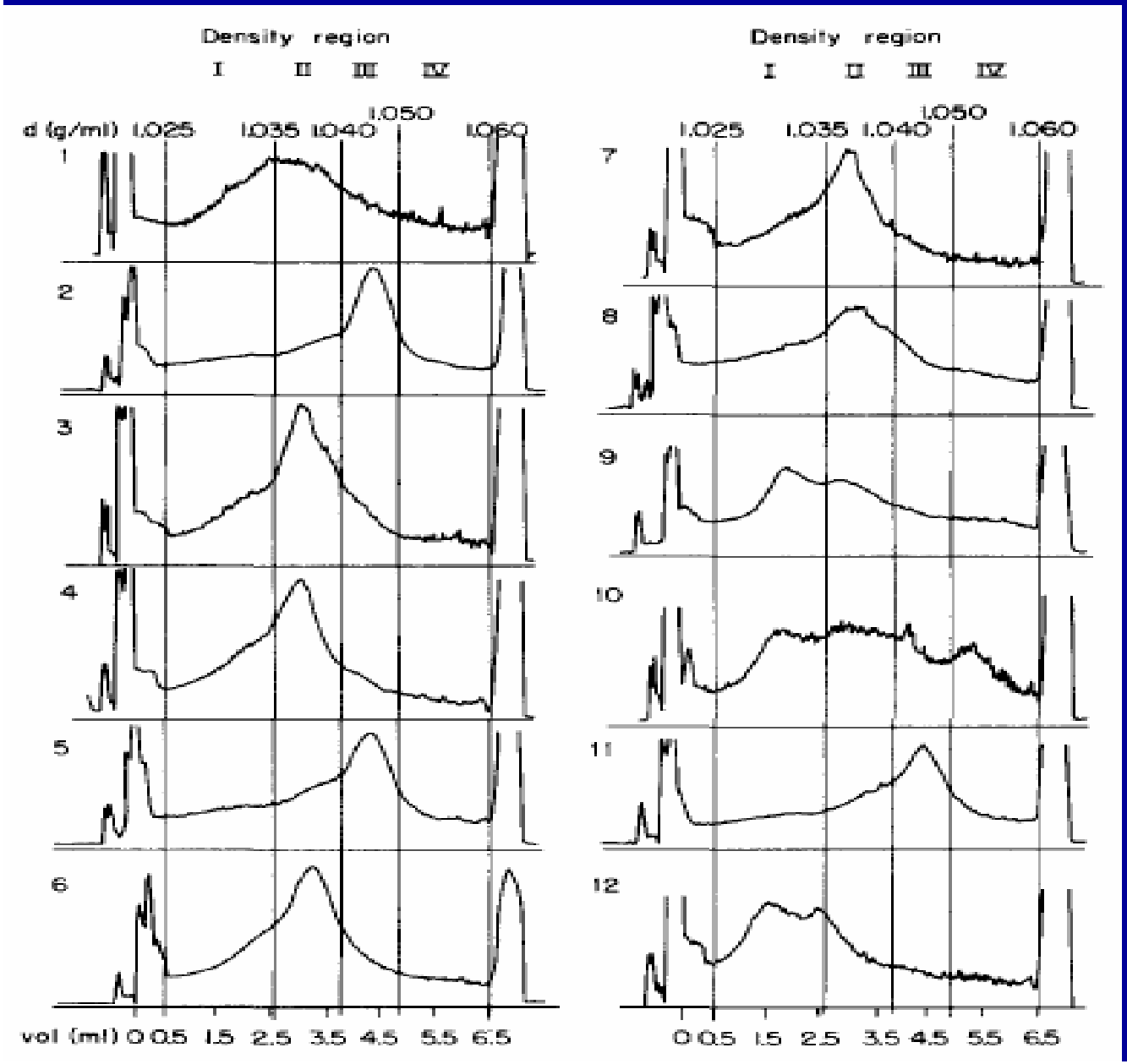
KALIBRACE - LP-AEC

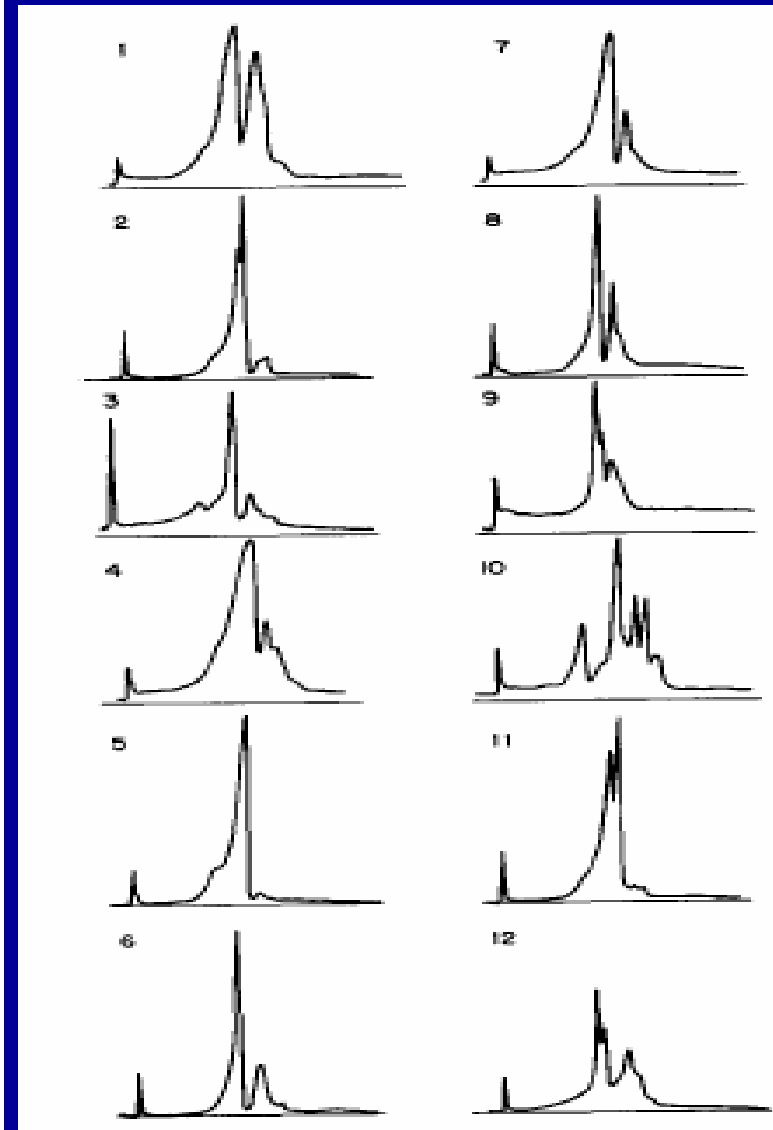




Hirowatari 2003

LDL - Analytická UC - PAGE





Kraus 1982

APOPROTEINY - MW

Apo	MW (Da)	Apo	MW (Da)
AI	28 000	D	30-33 000
AII	17 000	E	35 000
AIV	46 000	F	33 000
B48	264 000	G	72 000
B100	550 000	H	50-90-130 000
CI	5 800	J	2x40 000
CII	9 100	L	39-42 000
CIII	8 750	M	23-26 000

APOPROTEINY - METODY

RP-HPLC: SF – C4, C8, C18

MF – 0.1% TFA, 1% TEA-PO_4, gradient NaCl

SEC: SF – silikagel, methakrylát

MF – 0.1-0.2M pufr pH 7.0-8.2

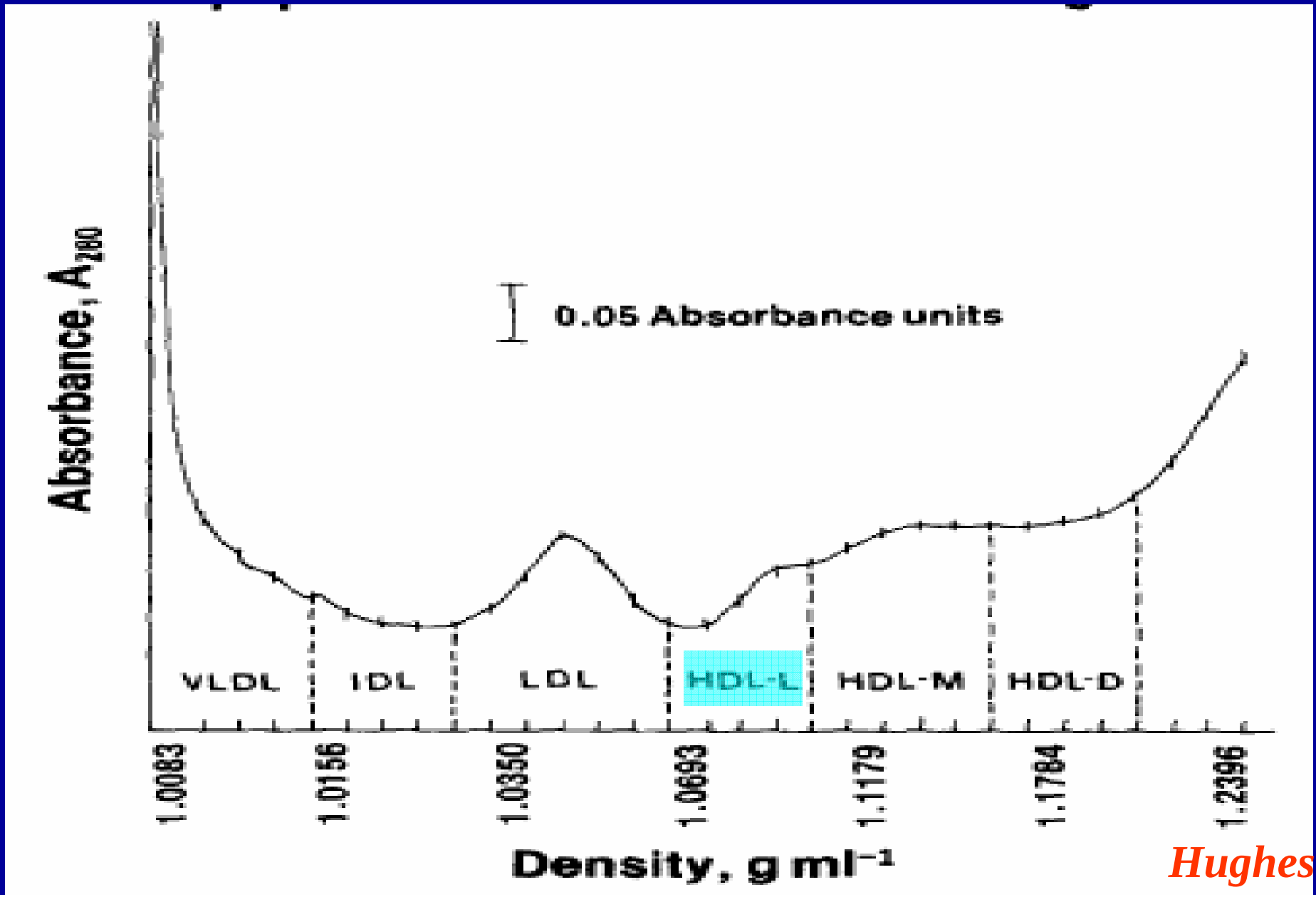
AC, IAC: SF – heparin-Sepharosa

MF – 0.002-0.005M pufr pH 7.4, gradient NaCl

IE-FPLC: SF – DEAE-trisakryl nebo celulosa, anex

MF – 0.01-0.1M tris-urea pH 7.6-8.2, gradient NaCl

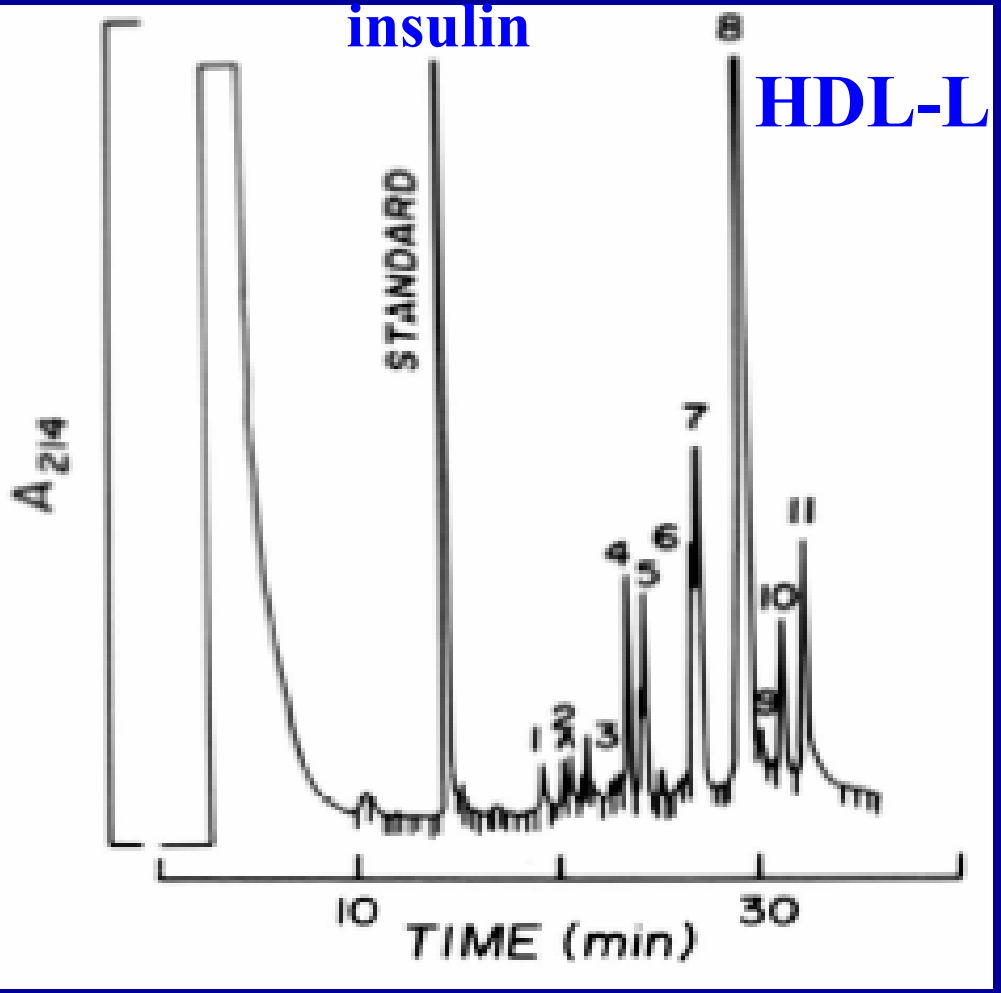
SEPARACE LP – GRADIENT UC

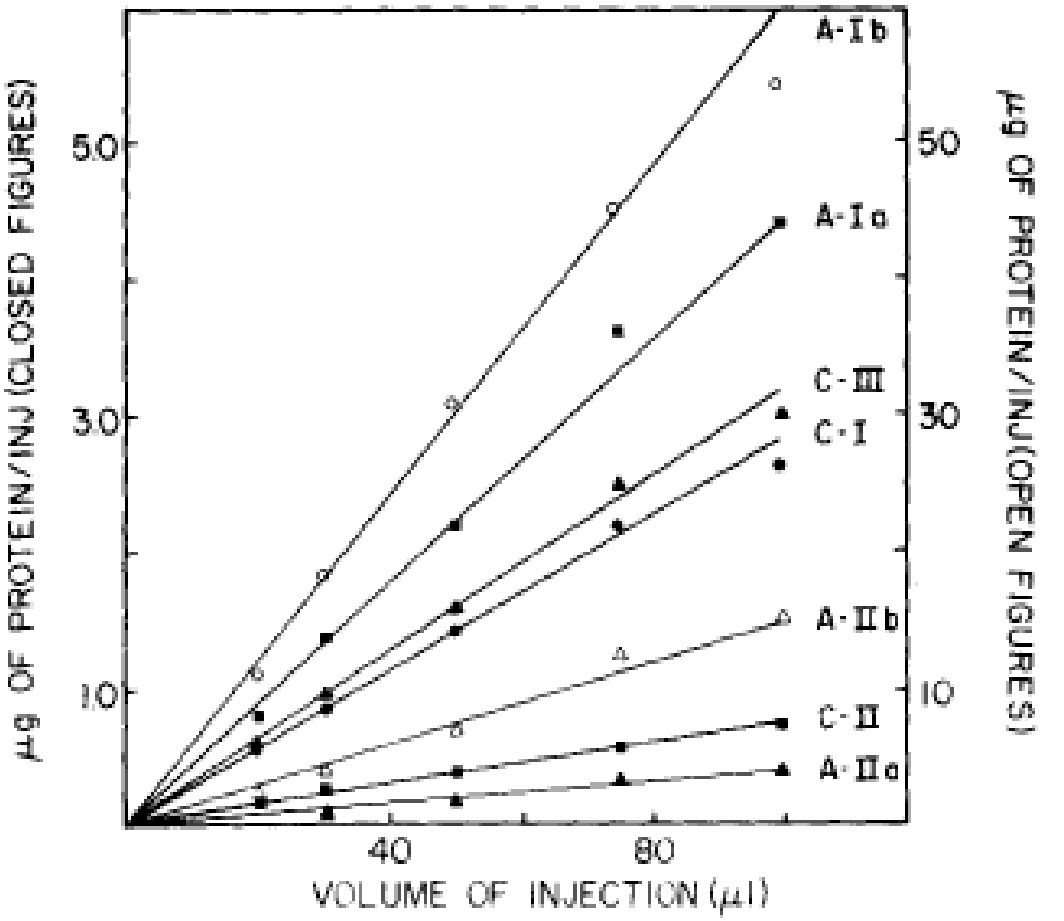


Hughes 1988

APO – RP-HPLC

1: C-IIIa, 2: C-IIIb, 3: C-IIa, 4: C-IIIc, 5: C-I, 6: C-IIb,
7: A-Ia, 8: A-Ib, 9: A-IIc, 10: A-IIa, 11: A-IIb

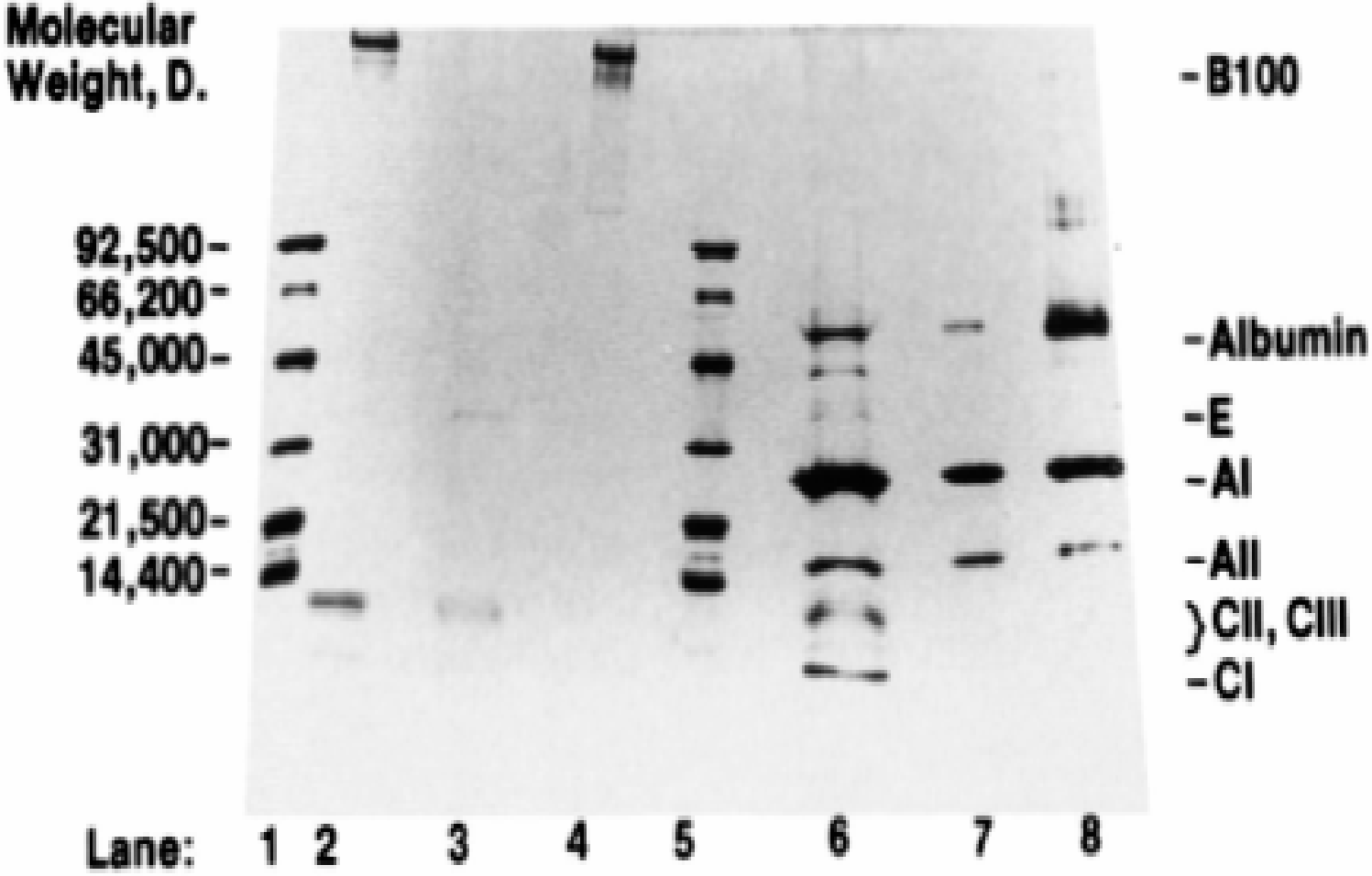




C18 4.6x250 mm, 0.1% TFA, CH_3CN 25-58%, 1.2 ml/min, 50°C *Hughes 1988*

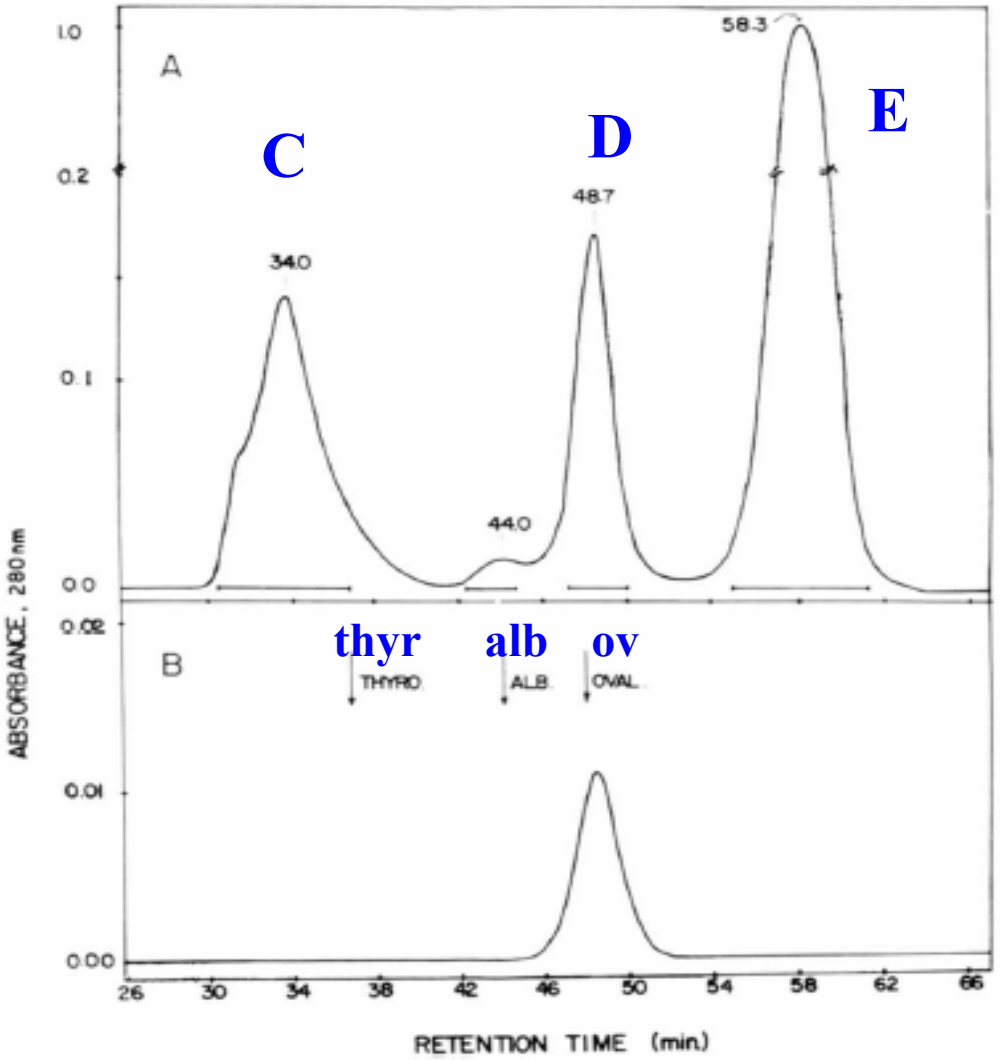
LP – GRADIENT SDS-PAGE

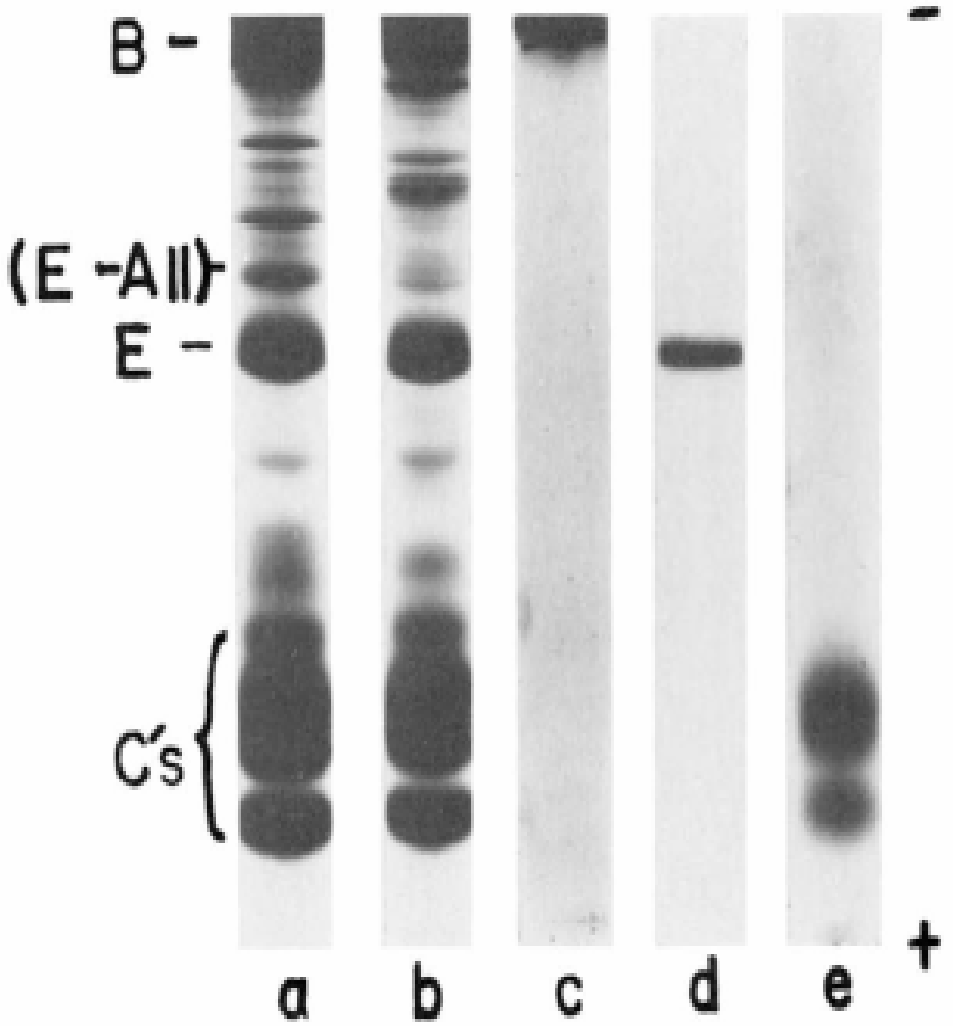
1: STD, 2: VLDL, 3: IDL, 4: LDL, 5: STD, 6: HDL-L, 7: HDL-M, 8: HDL-D



Hughes 1988

VLDL ApoE - SEC

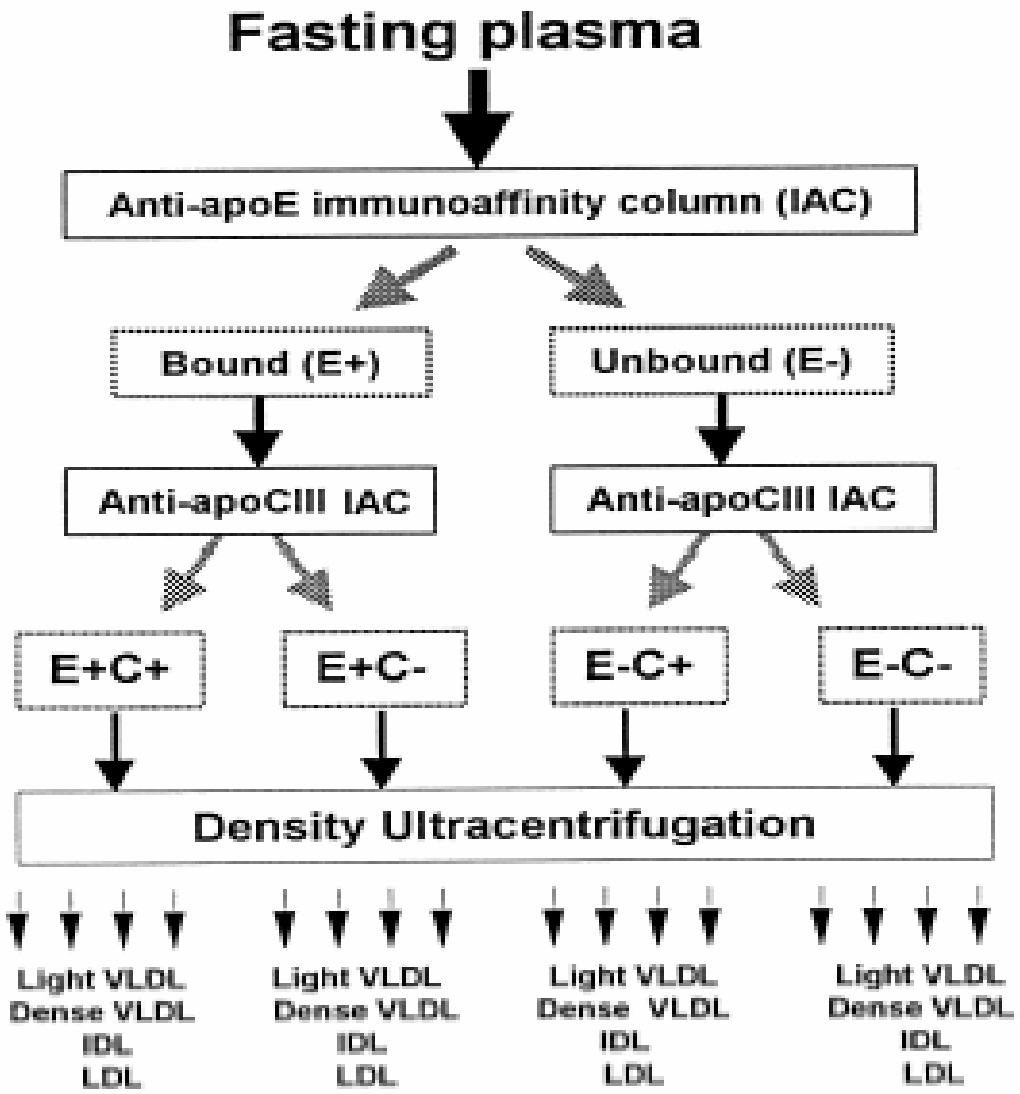


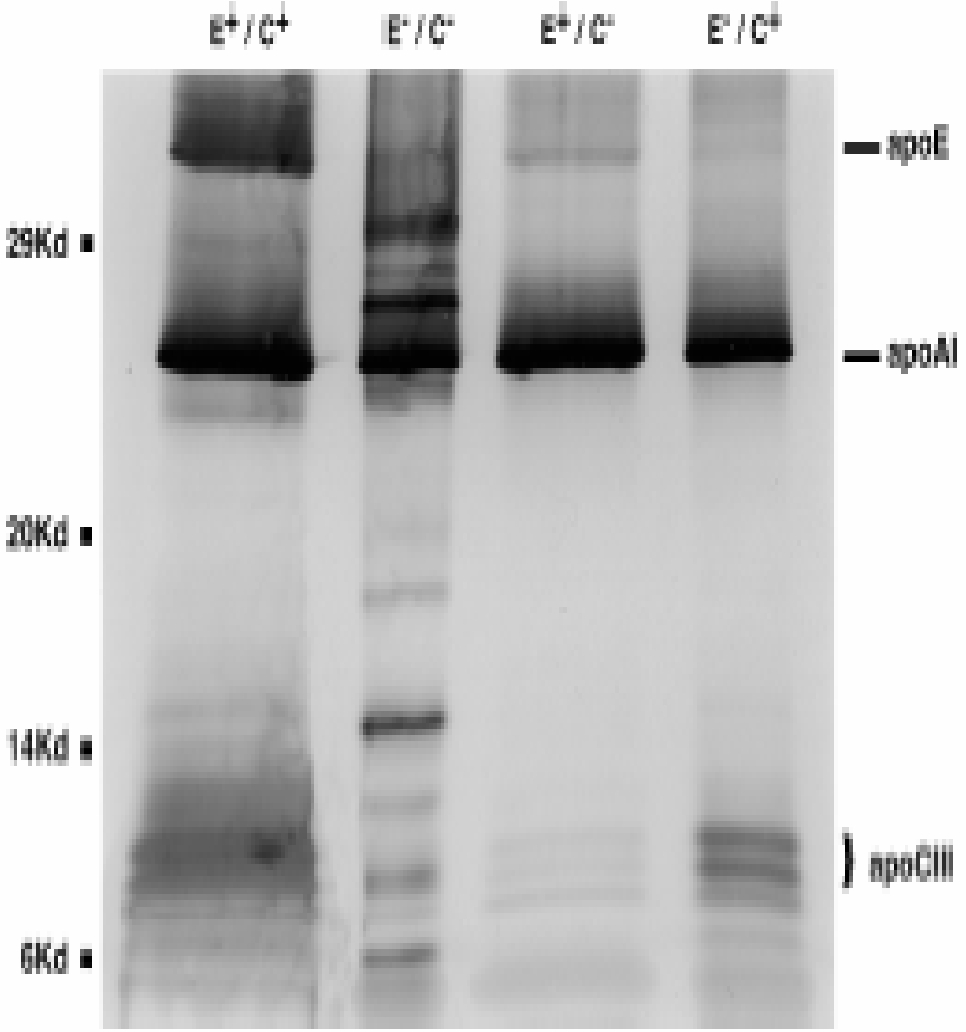


TSK50+TSK400+TSK3000, ā 7.5x300 mm, 0.01 Tris pH 7, 0.5 ml/min, SDS-PAGE 0.1-10%

Pfaffinger 1983

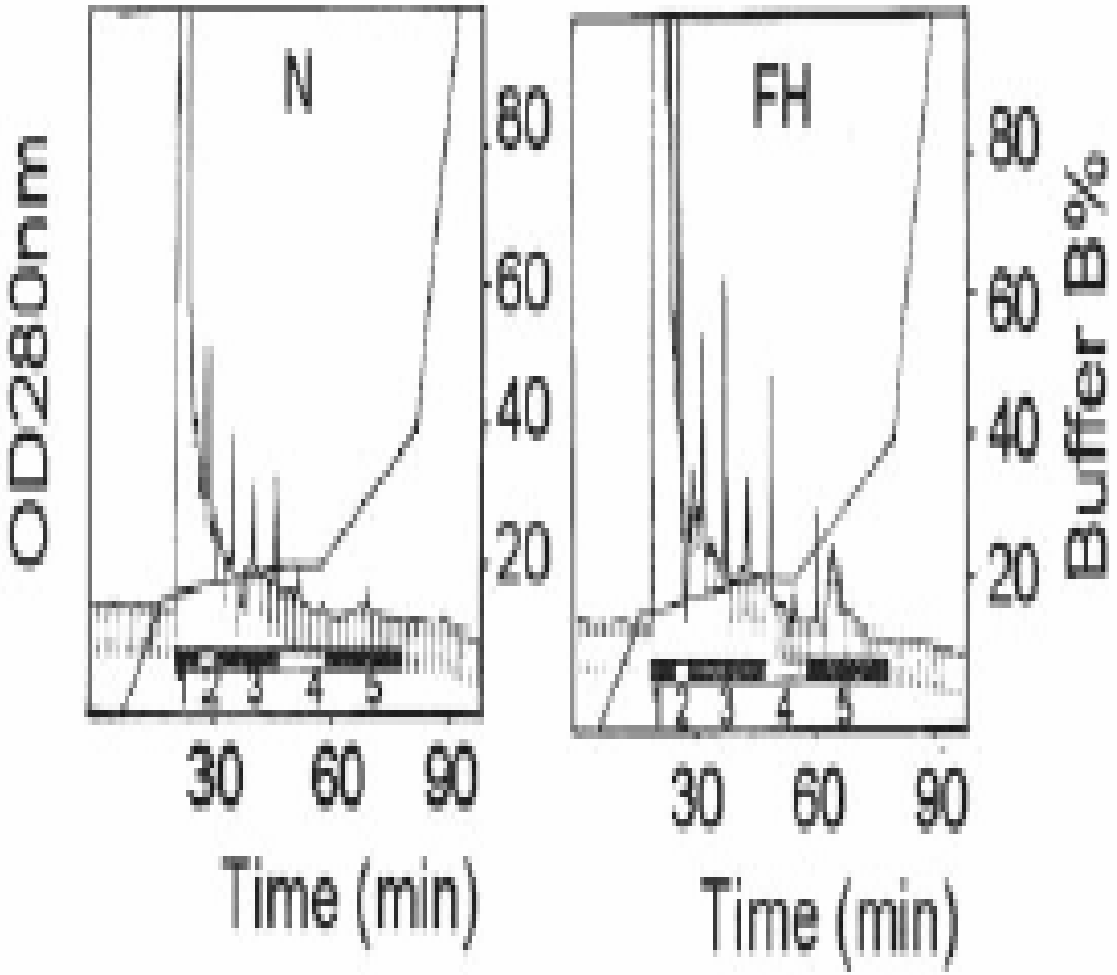
ApoLP - IAC

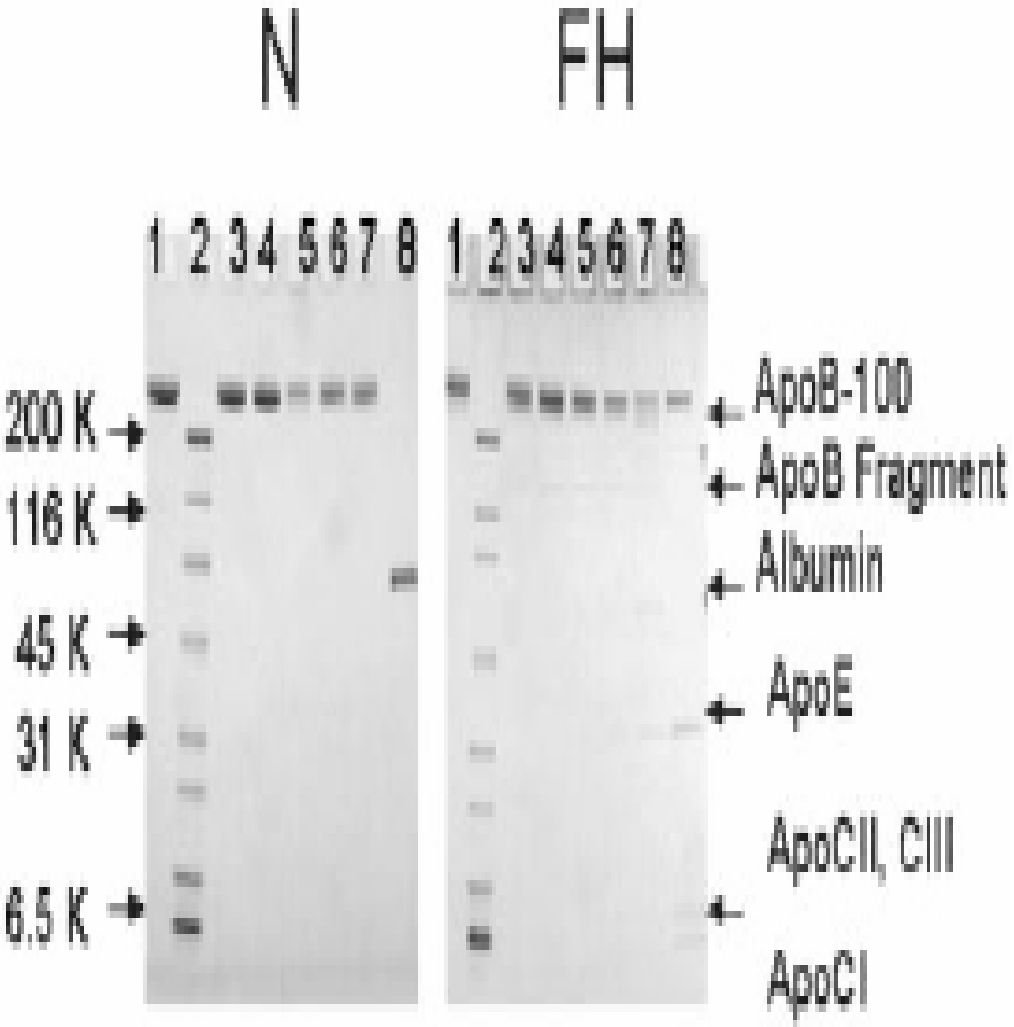




Campos 2001

LDL – iontovýměnná FPLC





UnoQ12, 0.02M Tris-HCl, pH 8, 2 ml/min, gradient 1M NaCl, 4°C *Yang 2003*

JEDNODUCHÉ LIPIDY

NEPOLÁRNÍ (NEUTRÁLNÍ)

WE, SE, FAME, GEDE, TG, FFA, AL, FS, DG, MG, (PL)

TLC (silikagel): heptan – diethylether – kyselina octová

POLÁRNÍ

(NL), CM, CL, PE, PI, LPE, PS, PC, SM, LPC

TLC (silikagel): chloroform – methanol – voda

JEDNODUCHÉ LIPIDY

ANALYTICKÉ METODY

PREPARACE – EXTRAKCE CELKOVÉHO LIPIDU

STANOVENÍ LIPIDOVÝCH TŘÍD – HPLC, TLC (prep.)

STANOVENÍ MOLEKULÁRNÍCH DRUHŮ – RP-HPLC, GLC

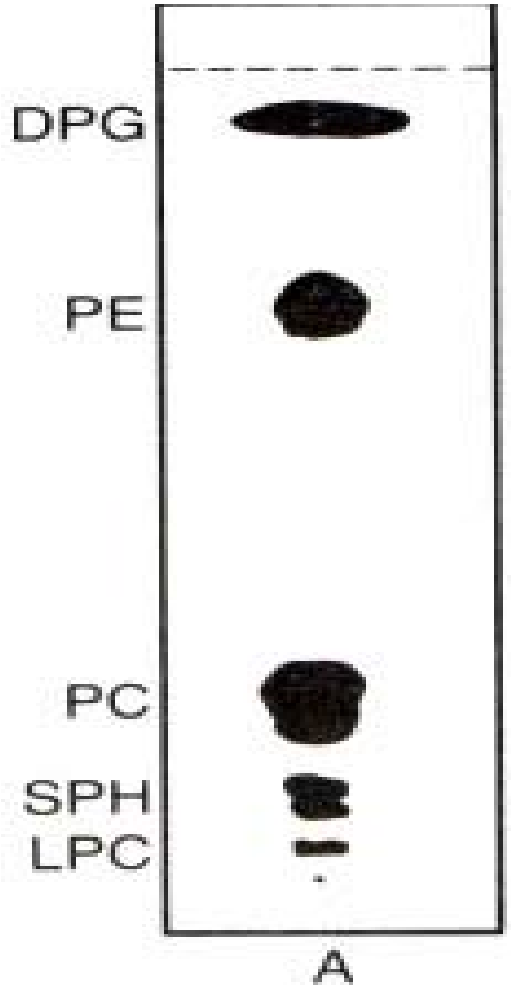
STANOVENÍ MASTNÝCH KYSELIN – GLC, HPLC

STANOVENÍ NEZMÝDELNITELNÉHO PODÍLU - GLC, HPLC

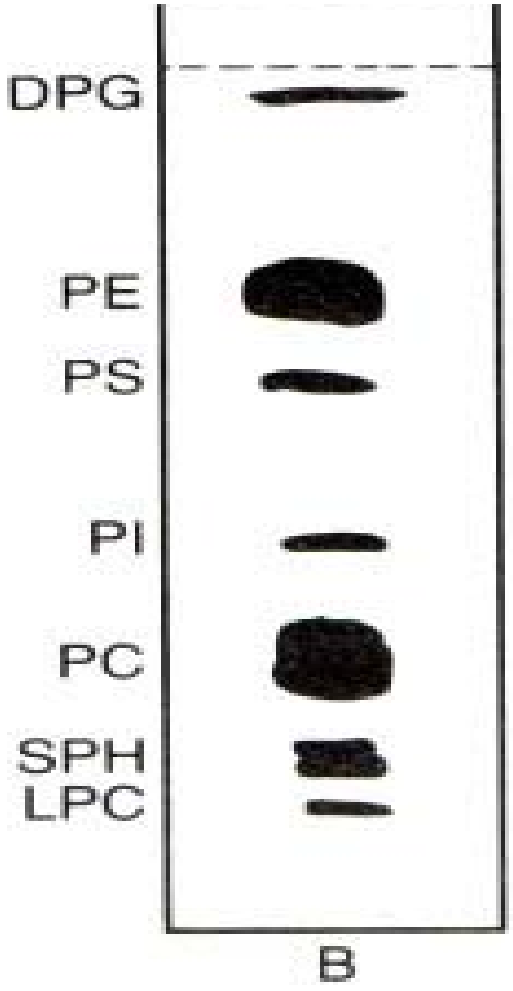
ALTERNATIVNÍ METODY

PREPARATIVNÍ TLC

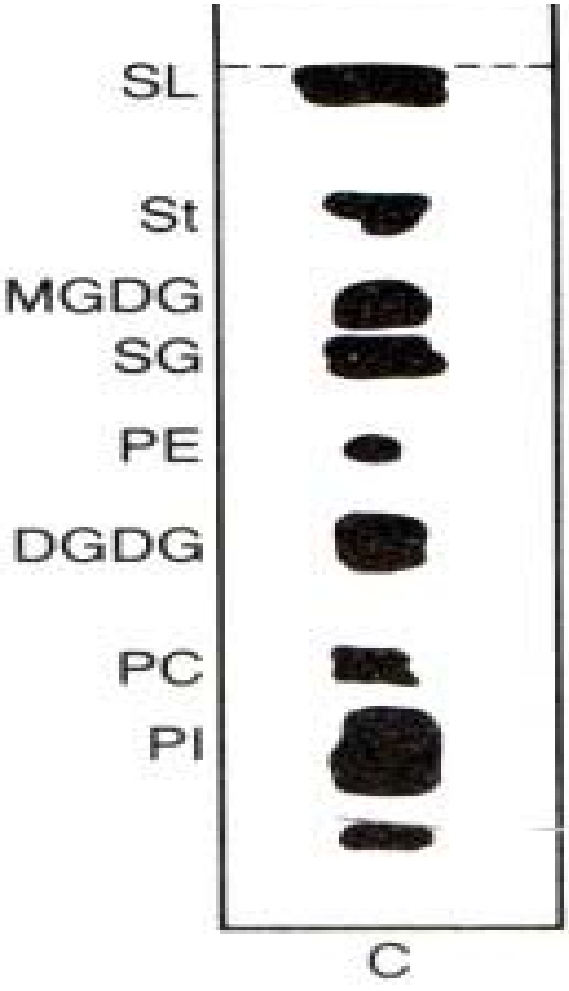
$CHCl_3$-MeOH-H_2O
25:10:1



$CHCl_3$-MeOH-HAc-H_2O
25:15:4:2



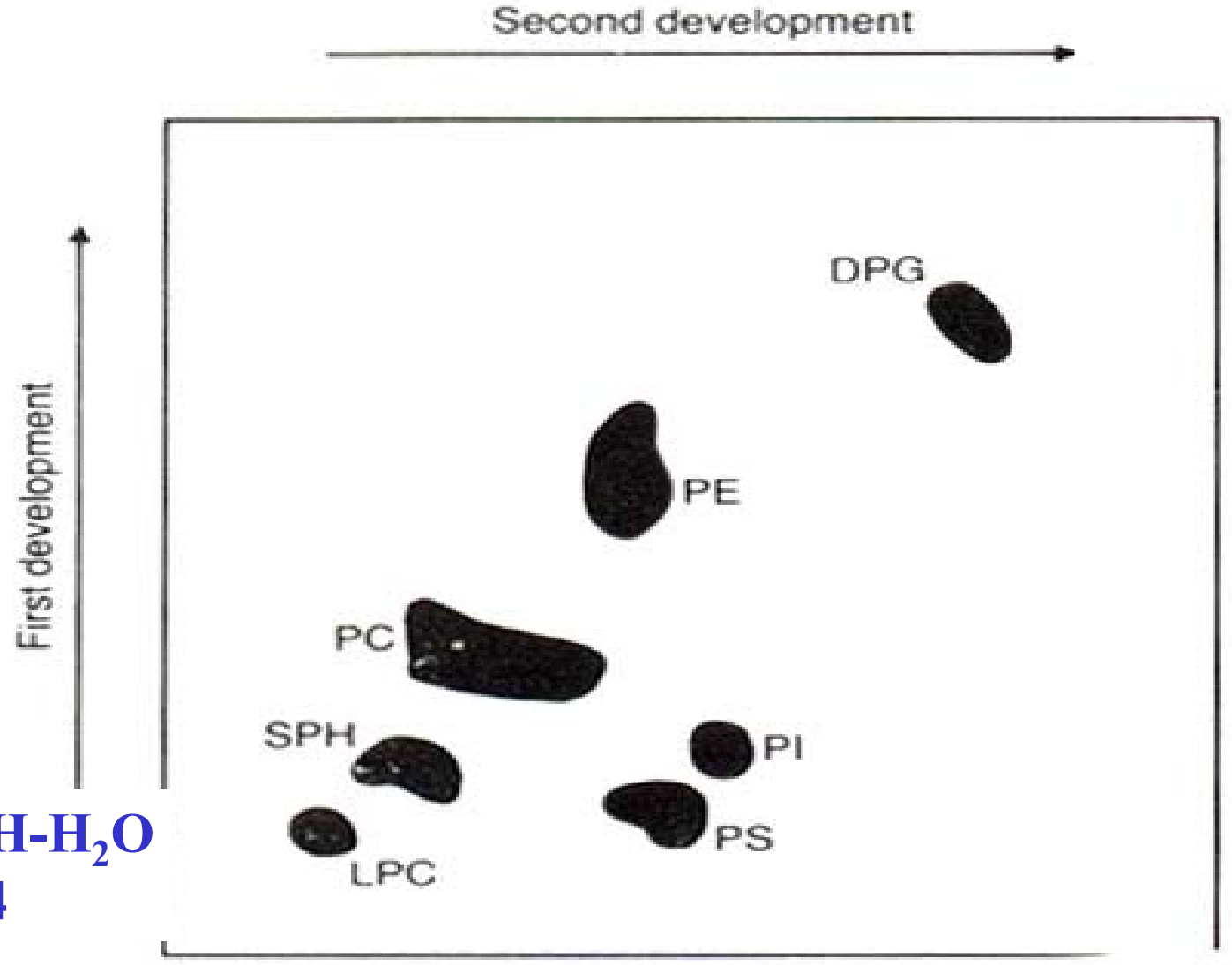
KET-HAc-H_2O
40:25:3,7



Skipski 1964

LIPIDOVÉ TŘÍDY 2D-TLC

n-BuOH-HAc-H_2O
3:1:1

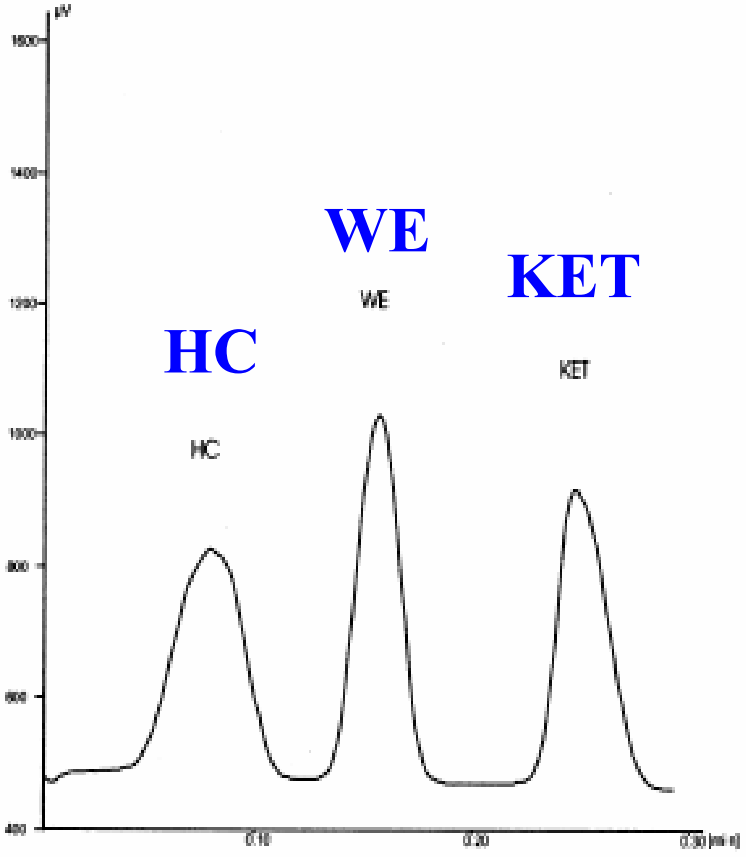


$CHCl_3$-MeOH-H_2O
65:25:4

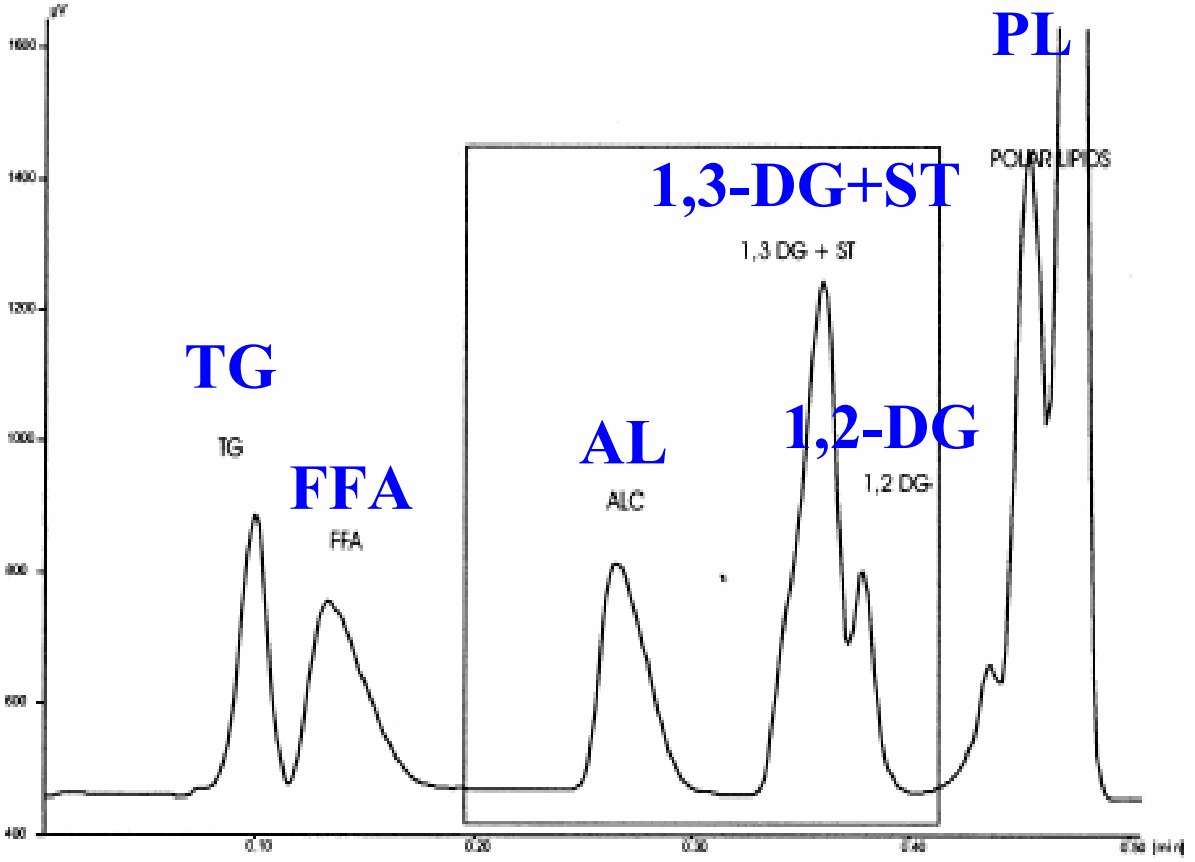
Rouser 1967

LIPIDOVÉ TŘÍDY – TLC-FID

Fytoplankton *Dunaliella viviridis*



HDF 97:3:0.2, 30 min

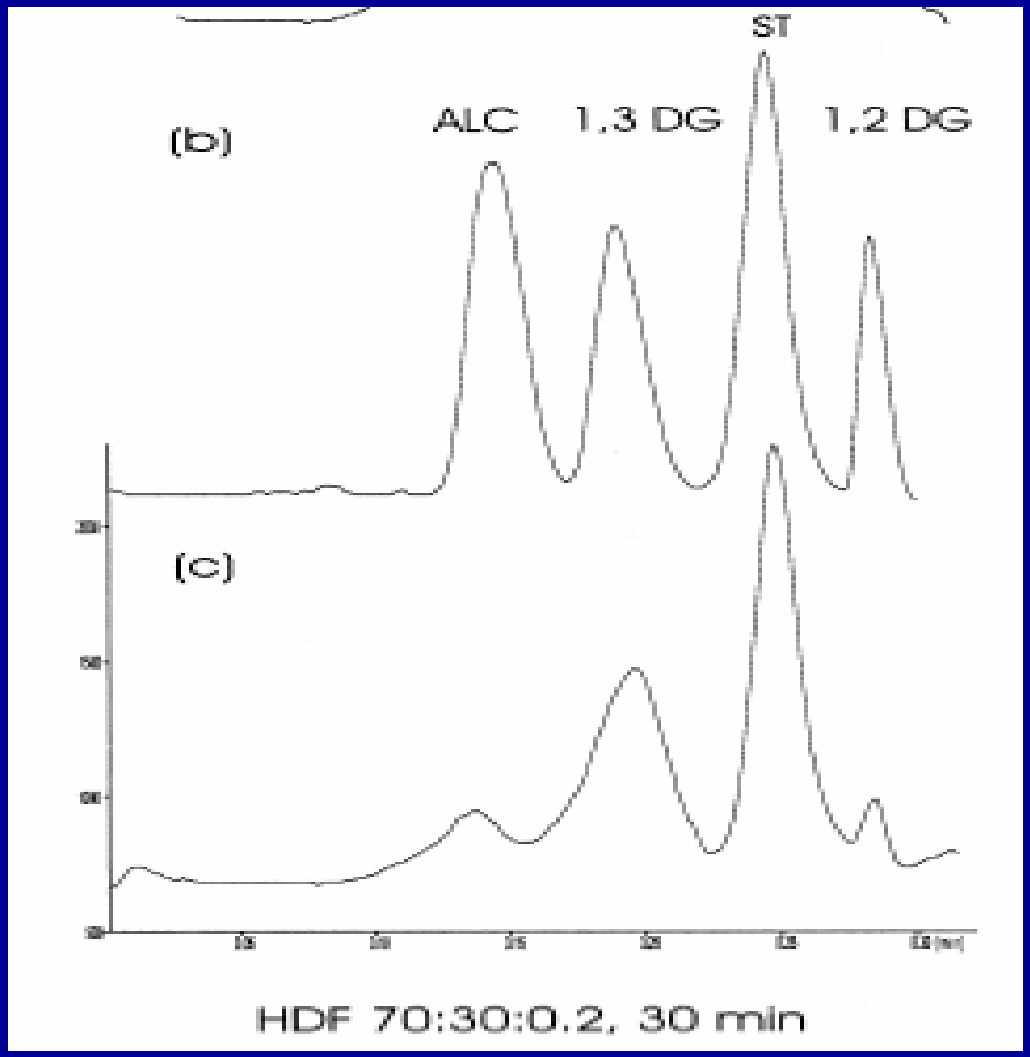


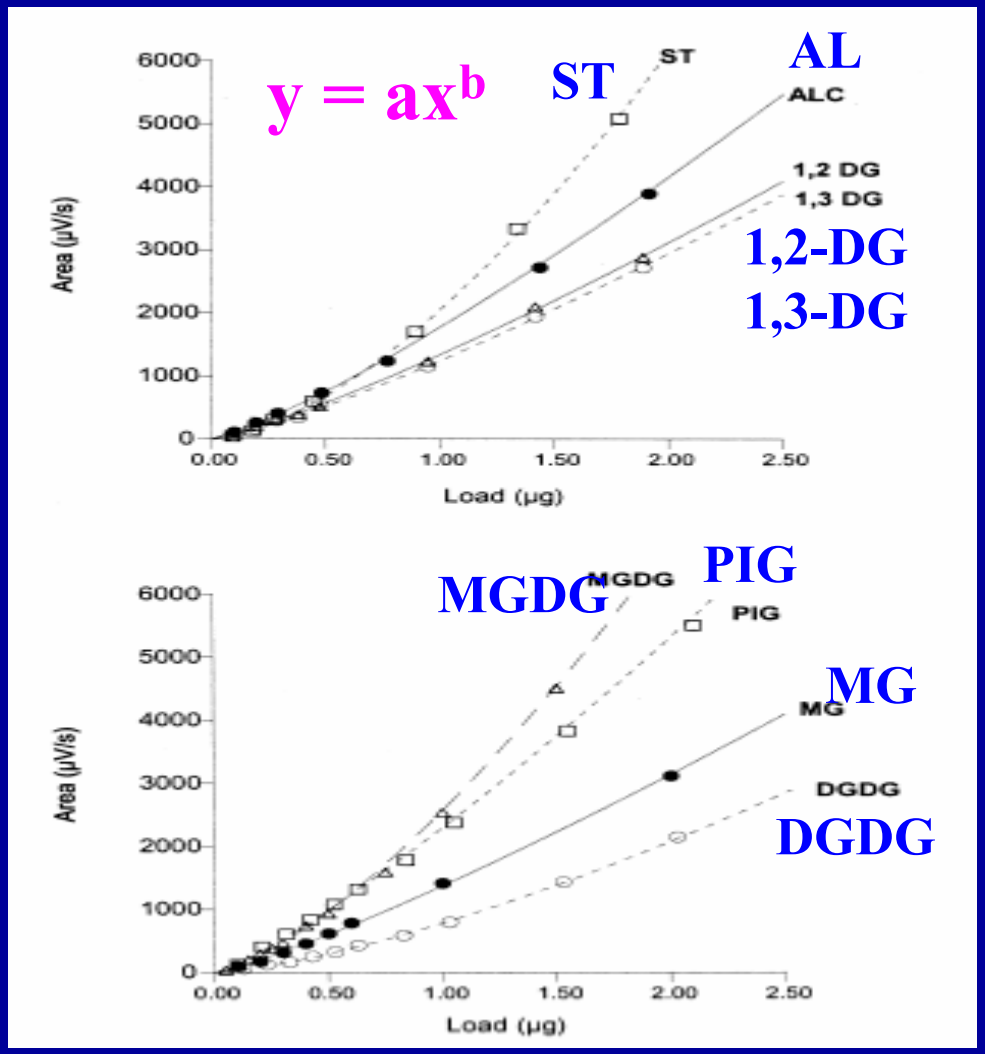
HDF 80:20:0.2, 30 min

Striby 1999

LIPIDOVÉ TŘÍDY – TLC-FID

Fytoplankton *Dunaliella viviridis*





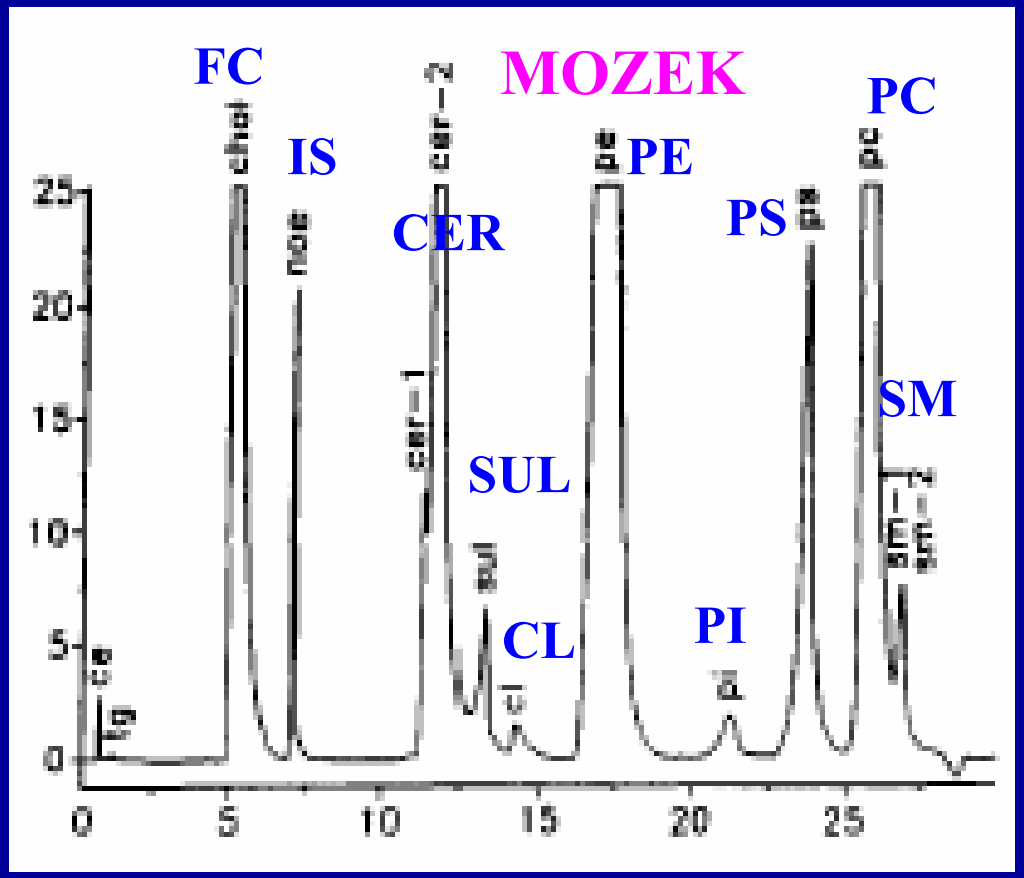
Striby 1999

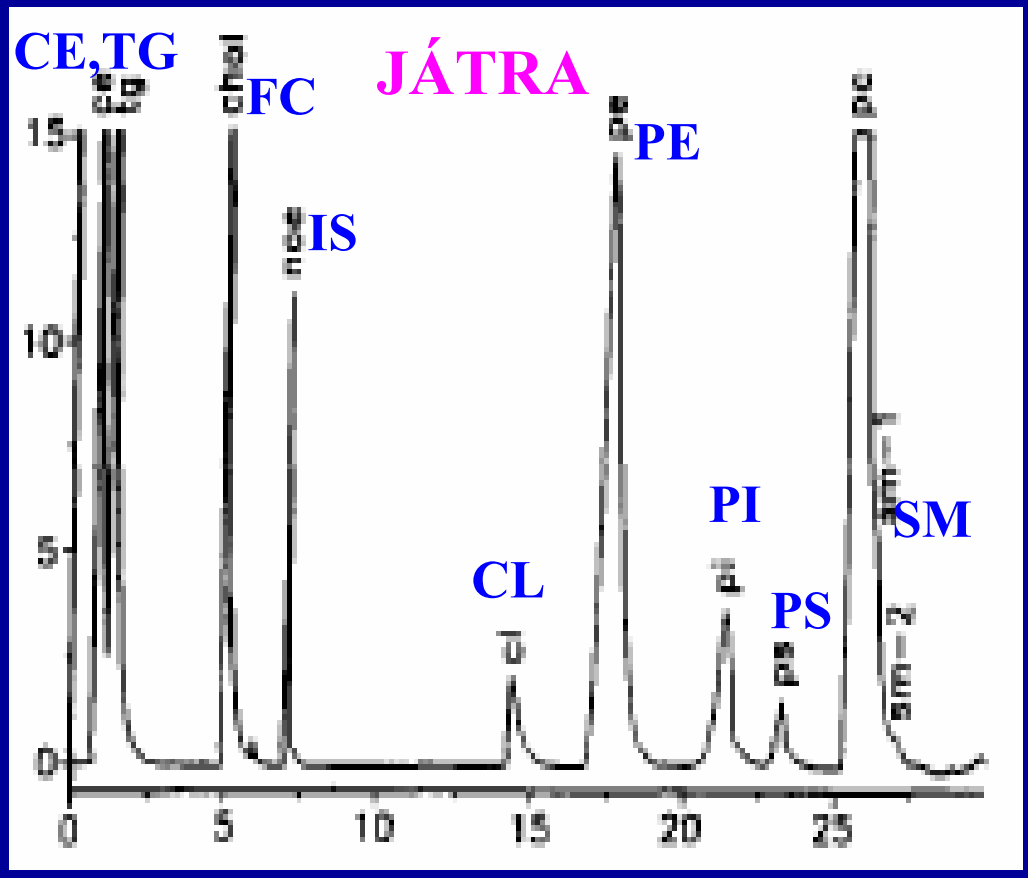
KALIBRAČNÍ ZÁVISLOST

rovnice	rozpětí r
$y = ae^{bx}$	0.86 – 0,89
$y = ax^{b}$	0,98 – 1,00
$y = a + b.\ln x$	0,72 – 0,91
$y = a + bx$	0,98 – 1,00
$y = a + bx + bx^{2}$	0,99 – 1,00
$y = a + b/x$	0,58 – 0,73
$y = a + bx^{½}$	0,97 – 0,99
$y = a + b.\log x$	0,89 – 0,95

Peuchant 1984, Parrish 1985

LIPIDOVÉ TŘÍDY – HPLC-ELSD



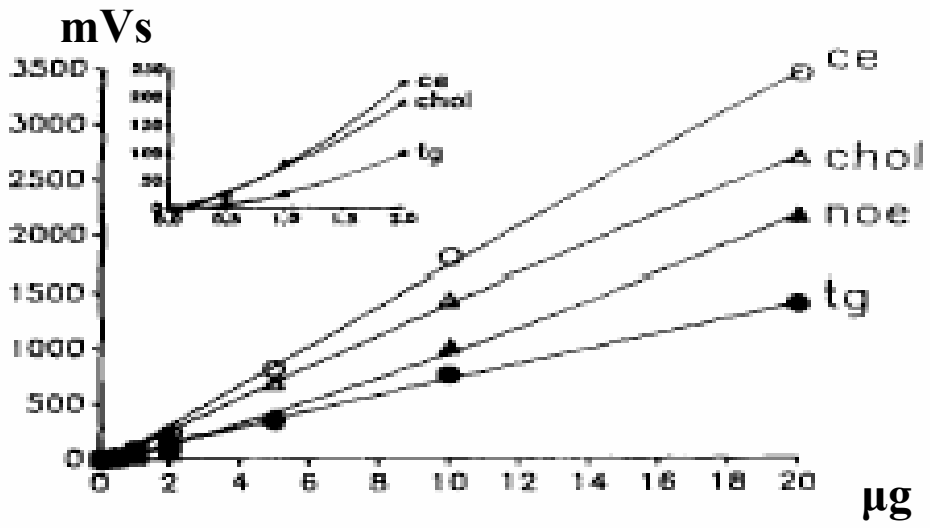


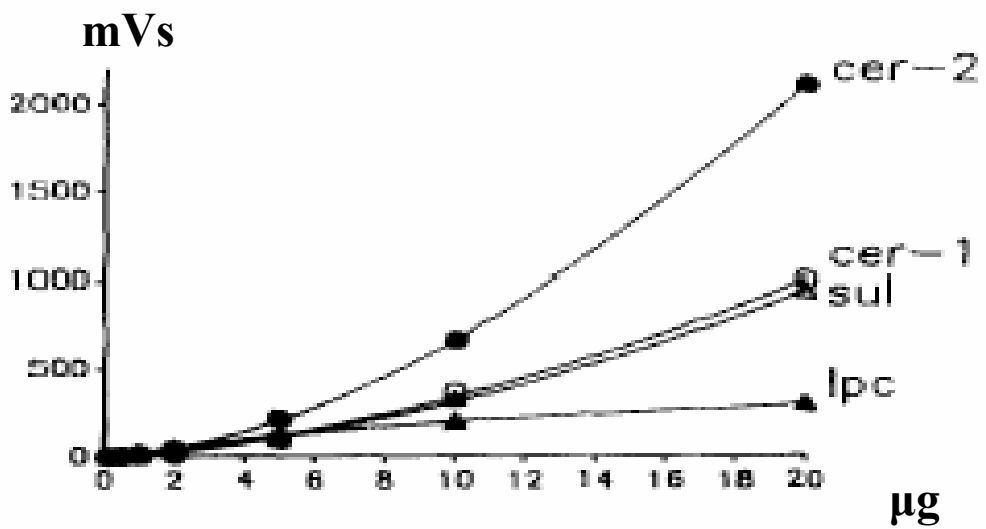
S3W Spherisorb (3µm silica), 4.6 x 100 mm, IS - n-oleoylethanolamin

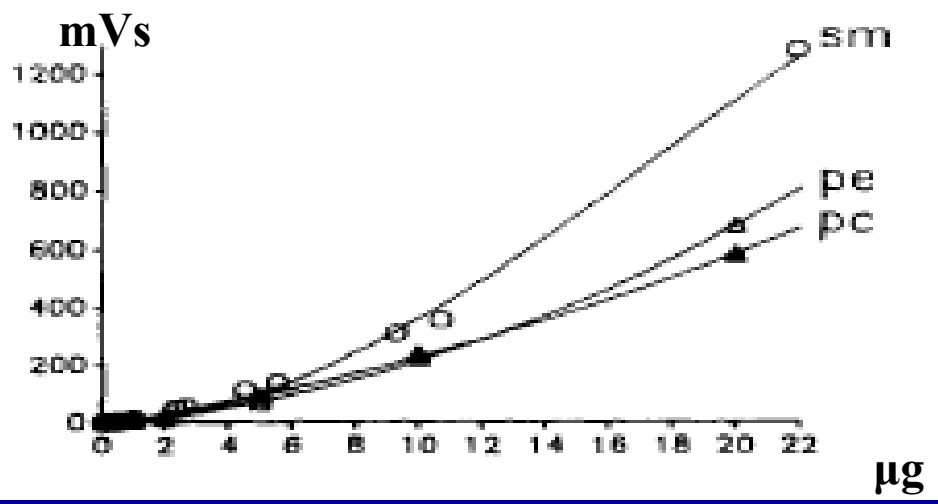
A: isooktan-tetrafydrofuan, B: chloroform-isopropanol, C: isopropanol-voda (0.5mM serin, pH 7.5 – ethylamin)

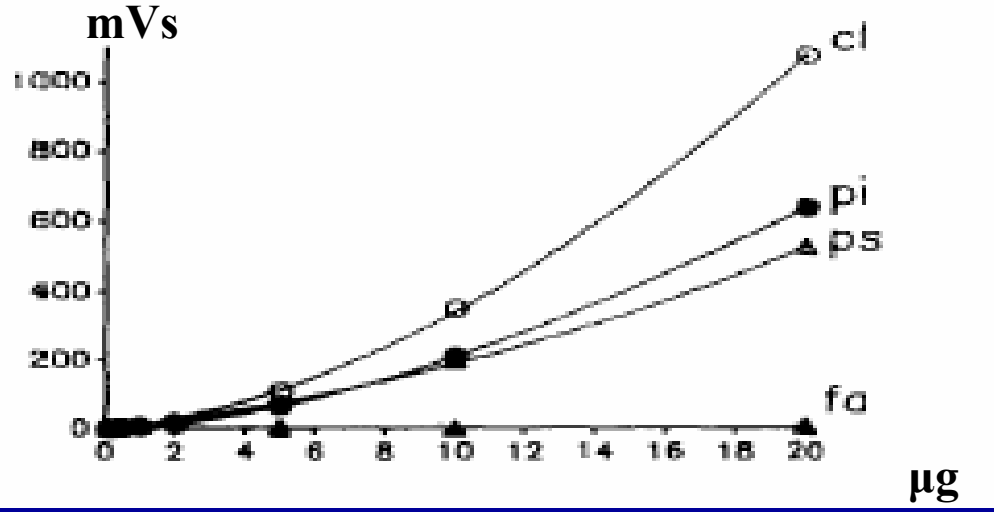
Lutzke 1990

HPLC-ELSD - KALIBRACE



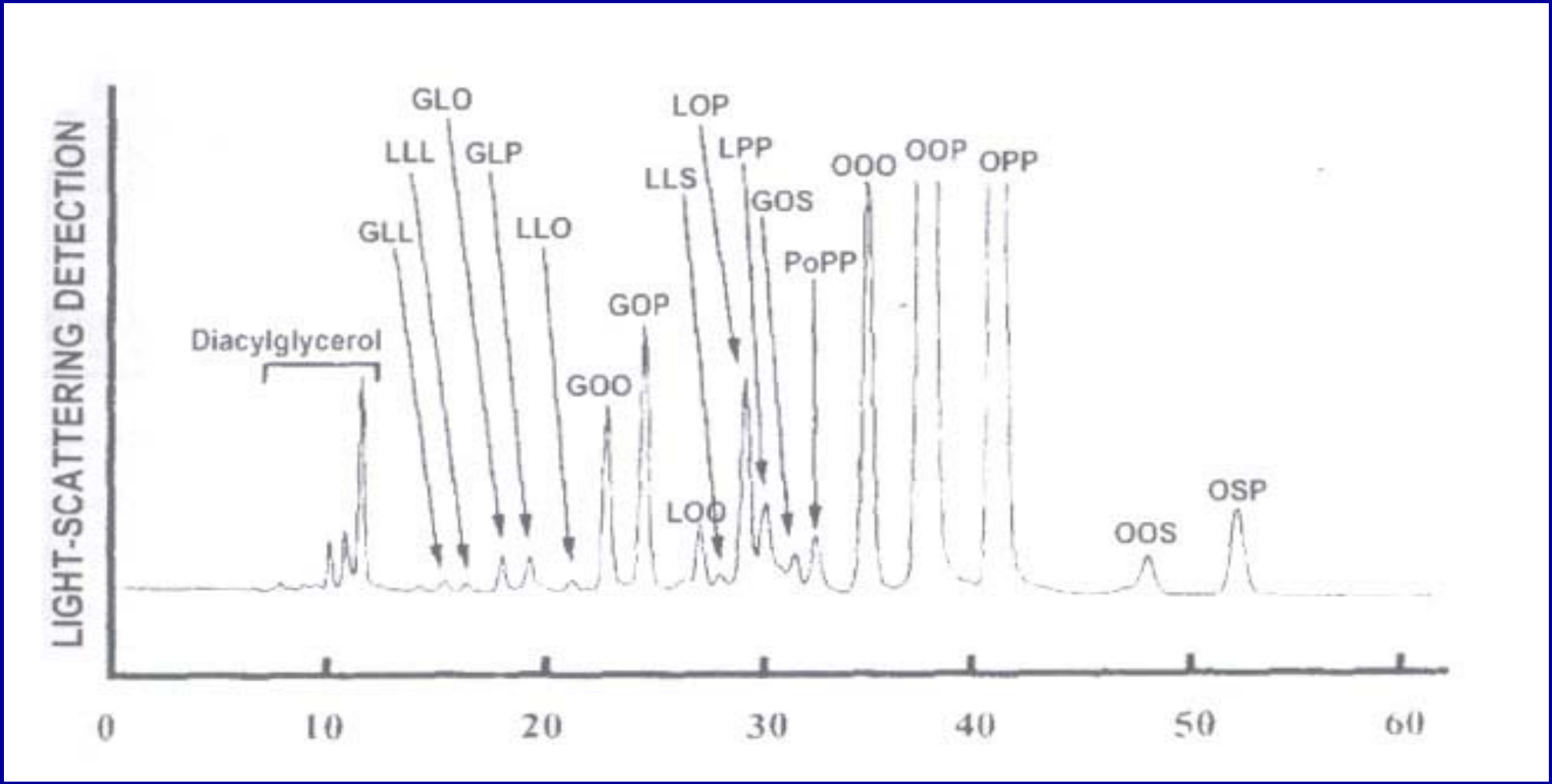






Lutzke 1990

MOLEKULÁRNÍ DRUHY TG – RP-HPLC



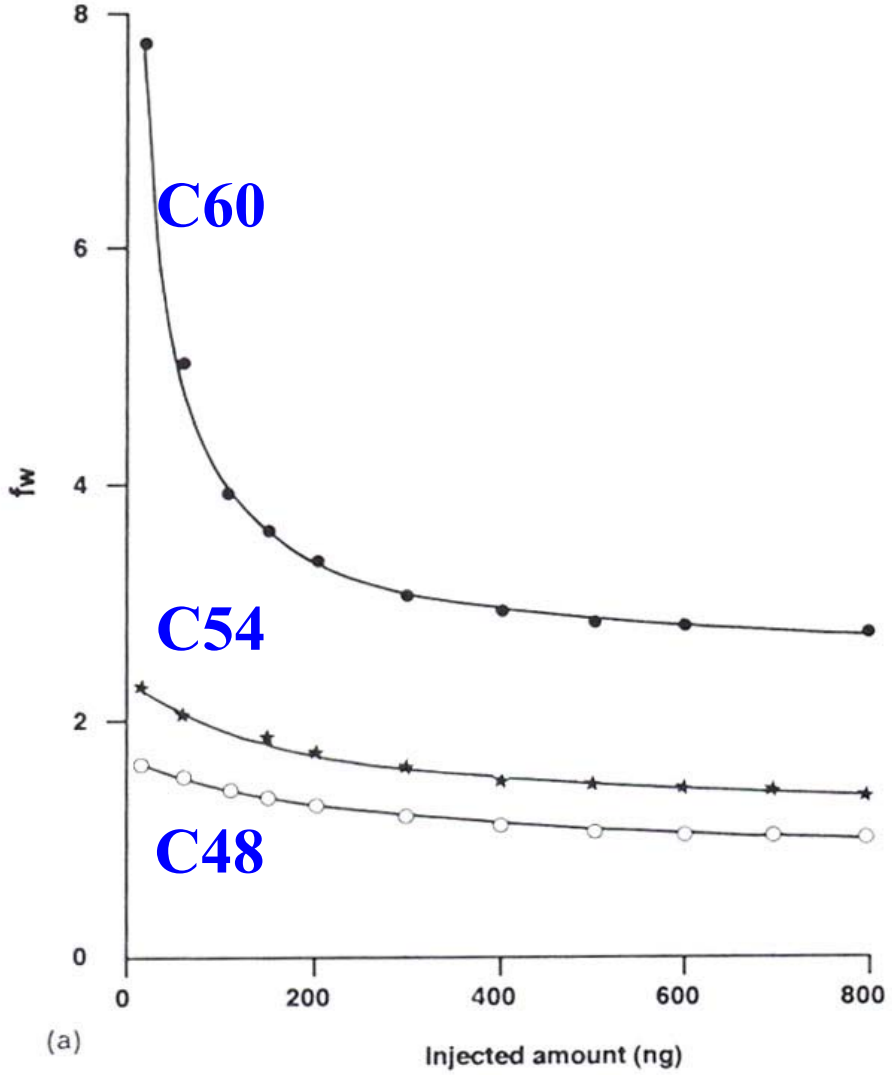
C18 6x250 mm, 5µm, ACN-2-propanol 60:40 iso, 1.5 ml/min

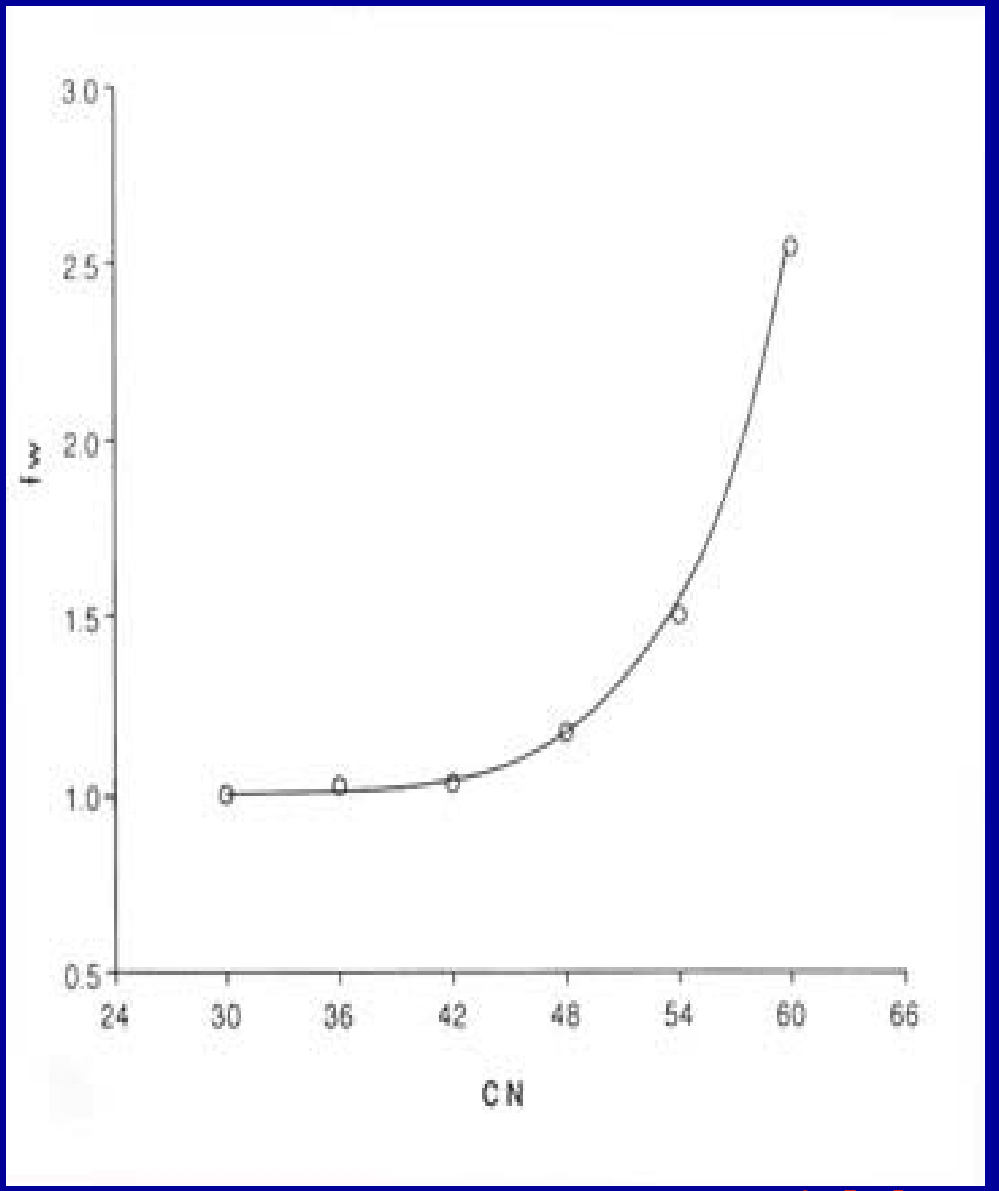
Pillai 2002

POŘADÍ ELUCE MOLEKULÁRNÍCH DRUHŮ TG

CN:P	HT-GLC	CN:P	RP-HPLC	CN:P	HT-GLC	CN:P	RP-HPLC
46:0	MPP	58:8-9	OOD/OAA	52:2	POO	52:4-48:2	PPA/MPL
46:1	MMO	56:6-54:7	LLA/PoLA	52:3	POL	50:3-48:2	PPLn/MPL
46:2	MML	54:8	PLD	52:4	PLL	44:0	MMP
48:0	PPP	52:5	PPoD	54:1	SSO	54:4	OOL
48:1	MPO/PPPo	48:4-46:3	MMA/MMLn	54:2	SOO	52:3	POL
	PoPoPo	46:7	OLA	54:3	OOO	50:2-50:1	PPL/MPO
48:2	MPL	54:6	LLL	54:3	SOL	46:0	MPP
48:3	MPoL	52:5	PoLL	54:4	OOL	54:3	OOO
50:0	MSS	56:6-7	POD	54:4	SLL	52:2	POO
50:0	PPS	54:6	PLA	54:5	OLL	50:1	PPO
50:1	PPO	52:5	PLLn/PPoA	54:6	LLL	48:0	PPP
50:2	PPL/PPoO	52:6-46:2	PPD/MML			54:2	SOO
50:3	MOL/PPoL	42:0	MMM			54:2	SSL
50:4	MLL	54:5	OLL			52:1	PSO
52:1	PSO	52:4-50:3	PLL/PPoL			50:0	MSS

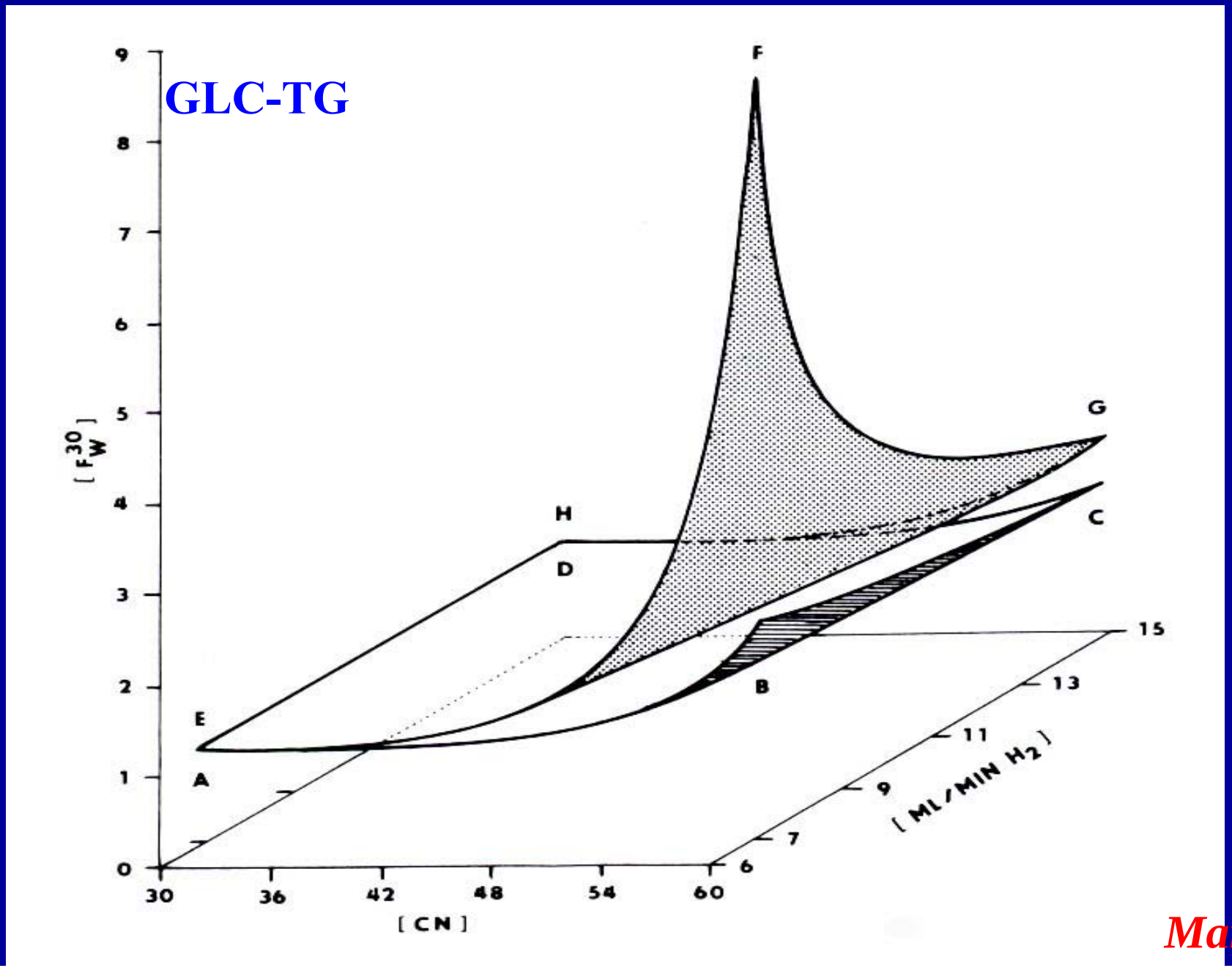
KALIBRACE – GLC-TG





Tvrzická 1994

SROVNÁNÍ NÁPLŇOVÉ A KAPILÁRNÍ KOLONY

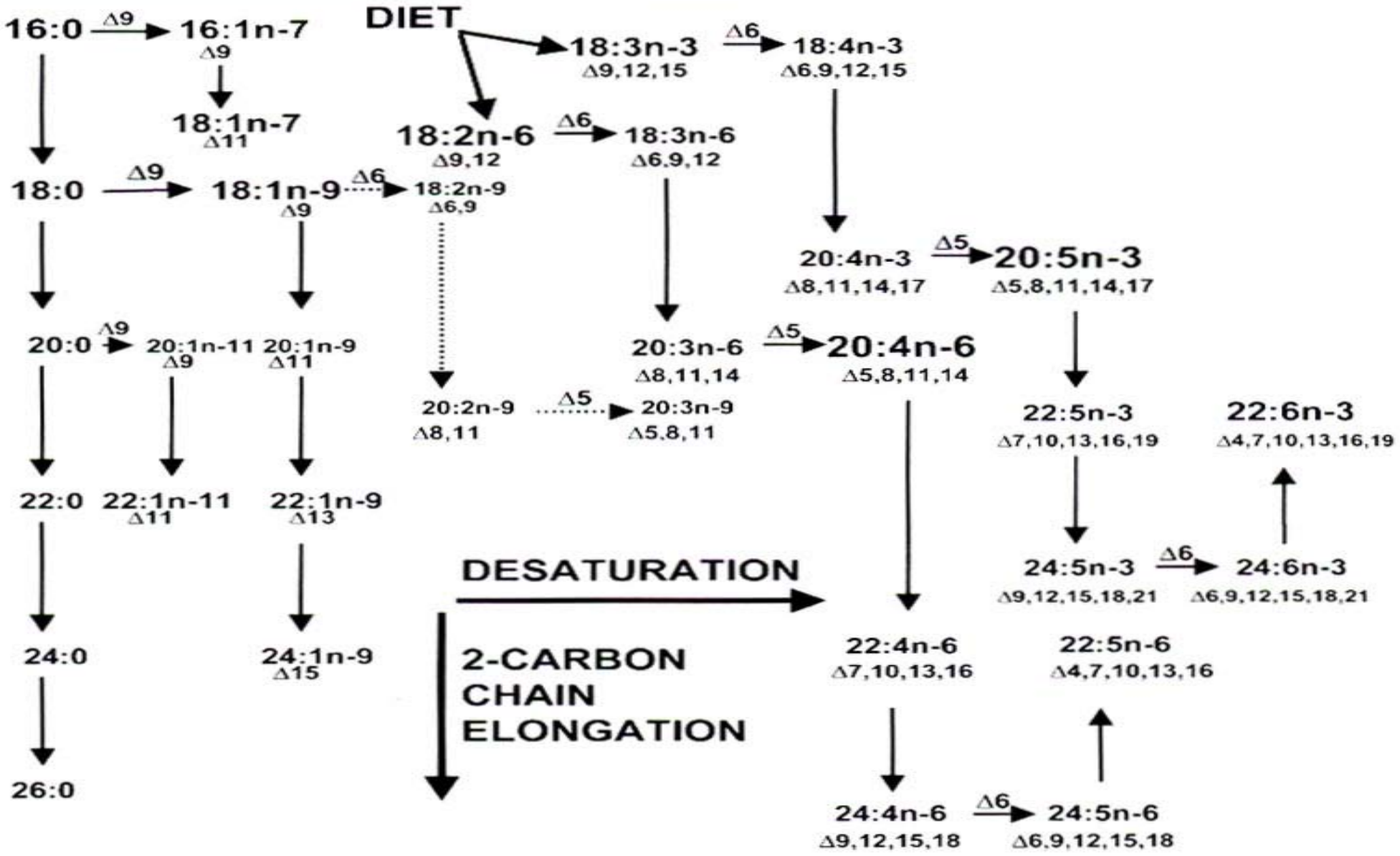


Mareš 1988

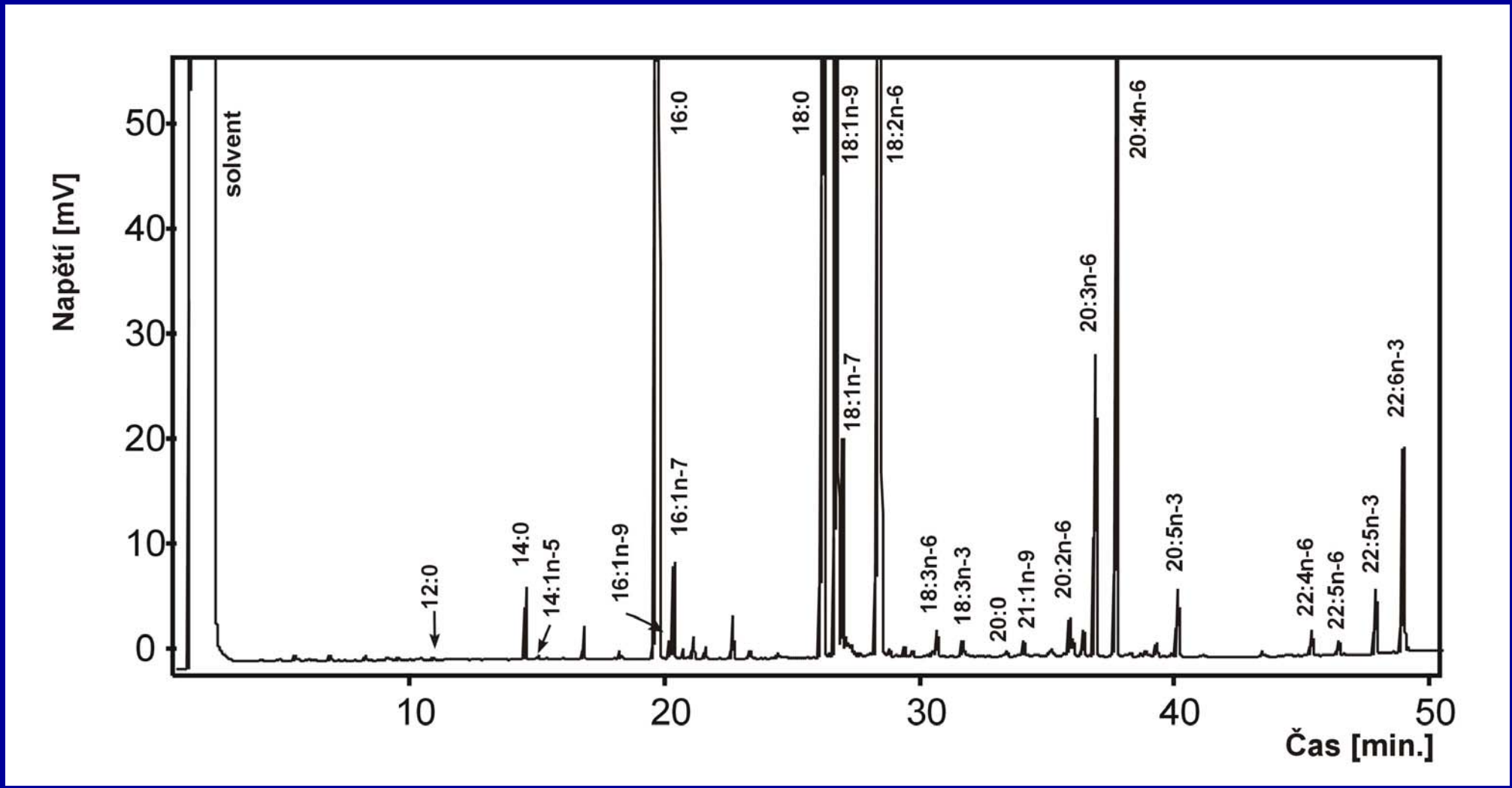
ROZDĚLENÍ MASTNÝCH KYSELIN

- SCFA - octová C2:0, propionová C3:0, máselná C4:0
- MCFA - kapronová C6:0, kaprylová C8:0, kaprinová C10:0
- LCFA - laurová C12:0, myristová 14:0, palmitová C16:0, stearová C18:0
- VLCFA - arachová C20:0, behenová C22:0, lignocerová C24:0, cerotová C26:0, montanová C30:0
- cis MFA - olejová C18:1n-9c, palmitolejová C16:1n-7c
- trans MFA - elaidová C18:1n-9t
- PUFAn-3 - α-linolenová C18:3n-3, eicosapentaenová C20:5n-3, docosahexaenová C22:6n-3
- PUFAn-6 - linolová C18:2n-6, γ-linolenová C18:3n-6, dihomo-γ-linolenová C20:3n-6, arachidonová C20:4n-6

METABOLICKÁ PŘEMĚNA MASTNÝCH KYSELIN



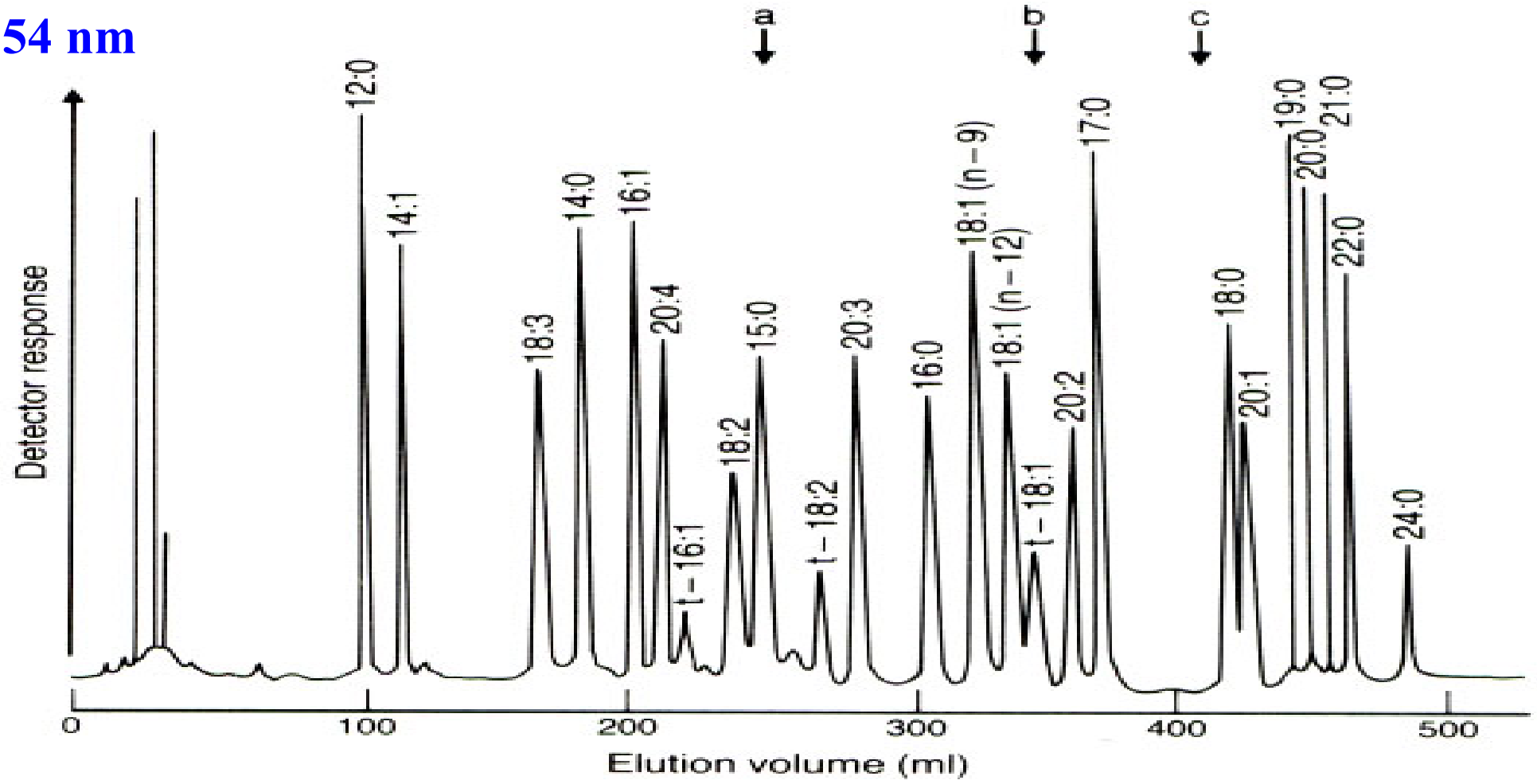
SEPARACE FAME - GLC



Tvrzická 2002

SEPARACE FA - RP-HPLC

254 nm

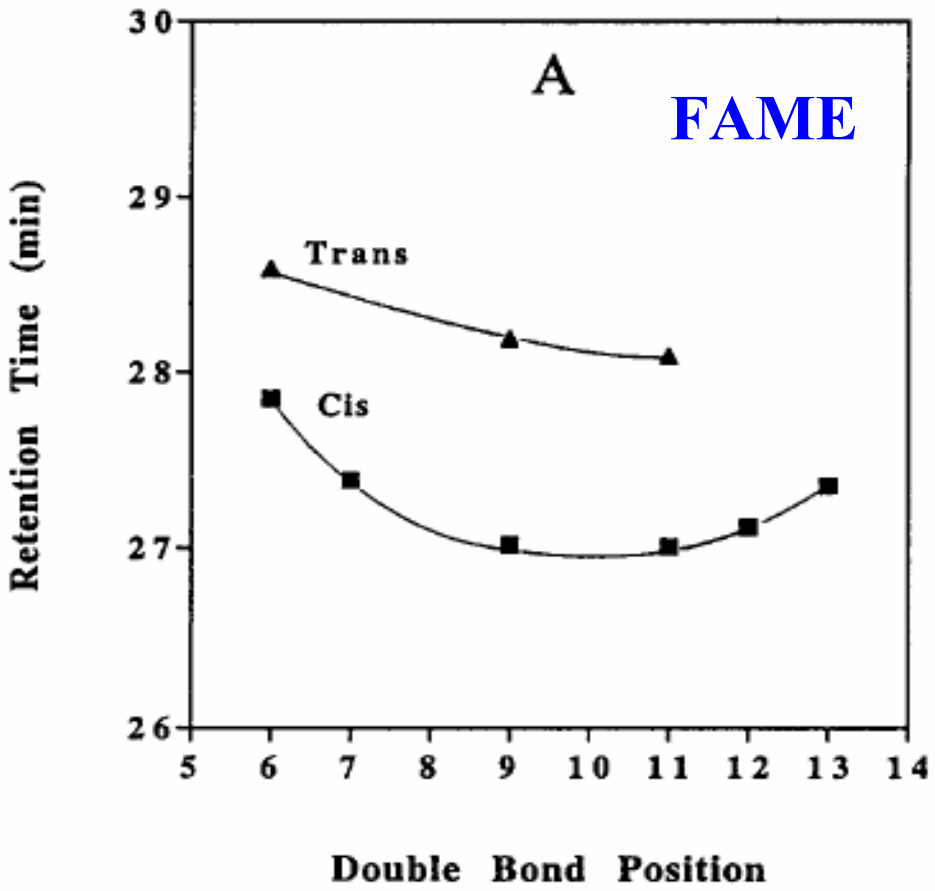


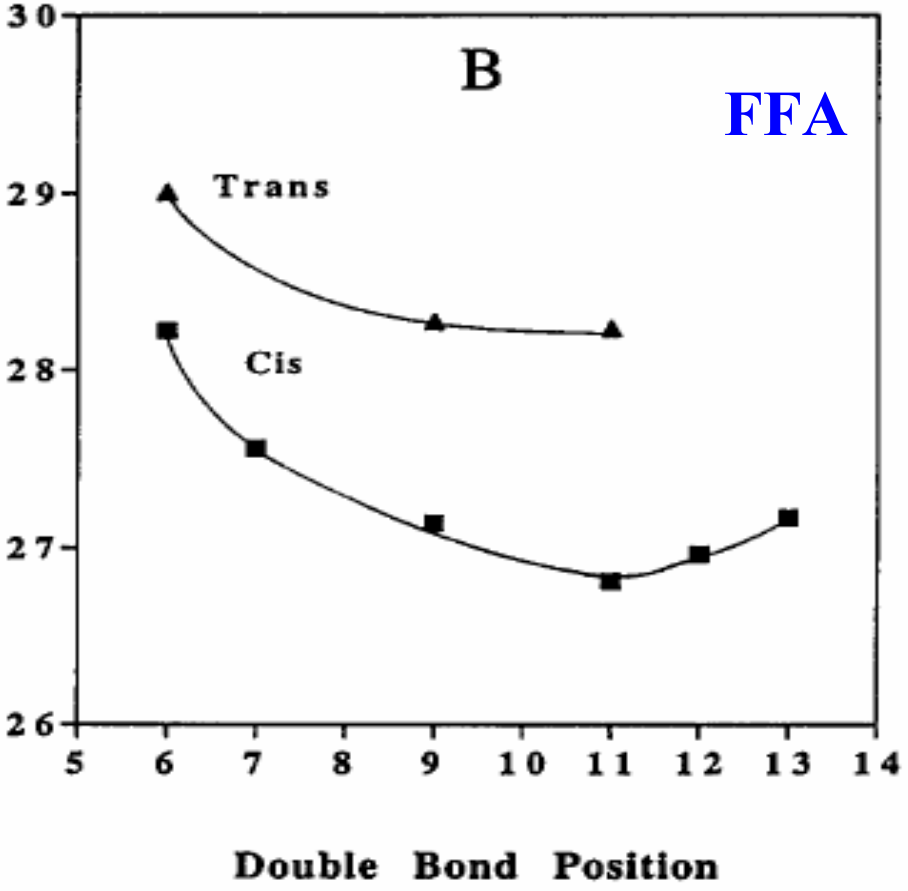
C18 6.4x900 mm, fenacyl der., ACN-H_2O gradient, 2 ml/min

Borch 1975

FAME – RP-HPLC-ELSD

Vliv polohy dvojné vazby na eluční čas





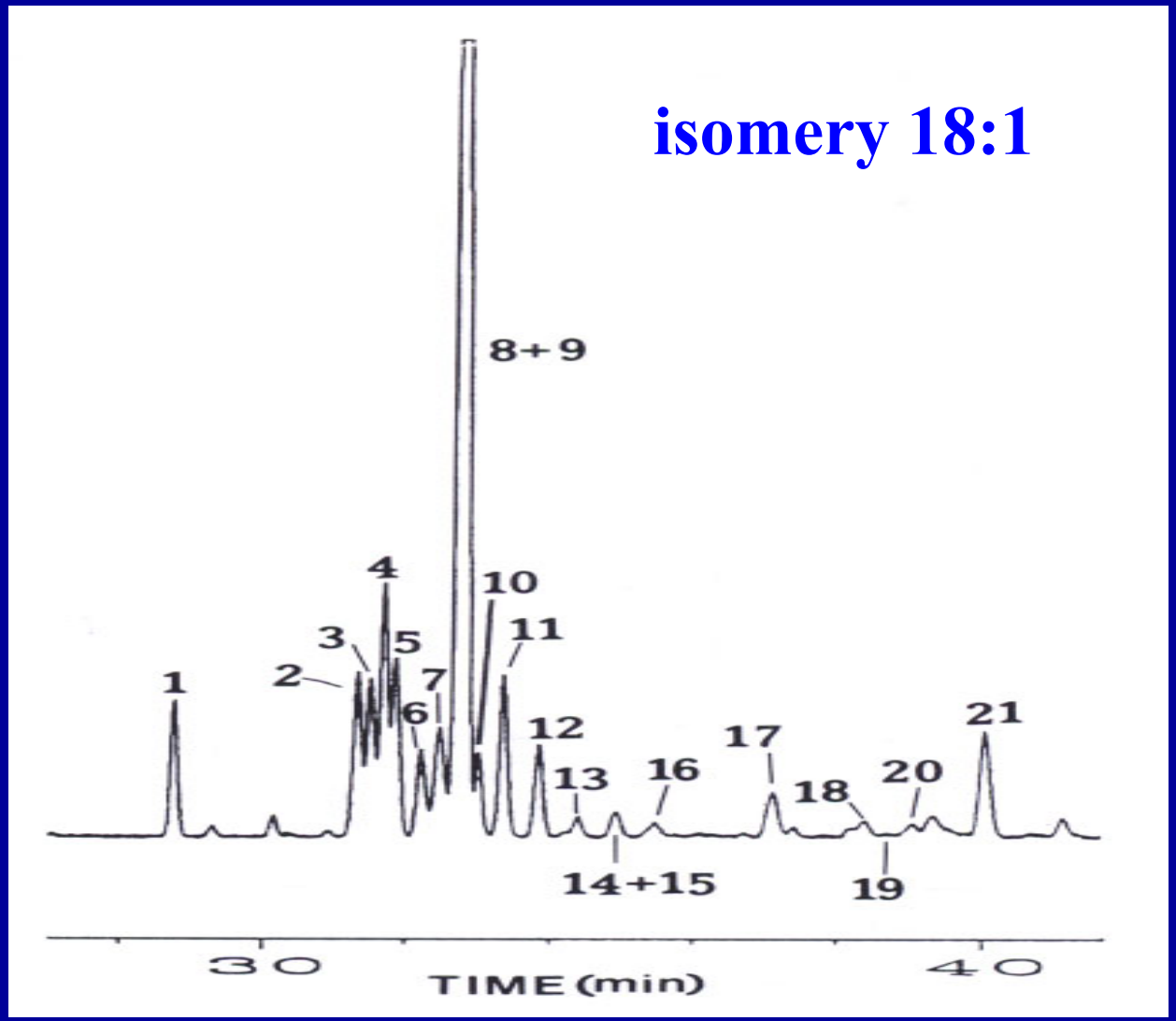
ODS 0.46 x 25 cm, 5 µm

MeOH/H_2O 90-100%, 1 ml/min

MeOH/H_20 85-100%, 0.05% HAc

Lin 1994

SEPARACE cis-trans FAME - GLC



1	18:0	9	15t
2	6t-8t	10	10c
3	9t	11	11c
4	10t	12	12c
5	11t	13	13c
6	12t	14	16t
7	13+14t	15	14c
8	6c-9c	16	15c

Christie 1998

PODĚKOVÁNÍ

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