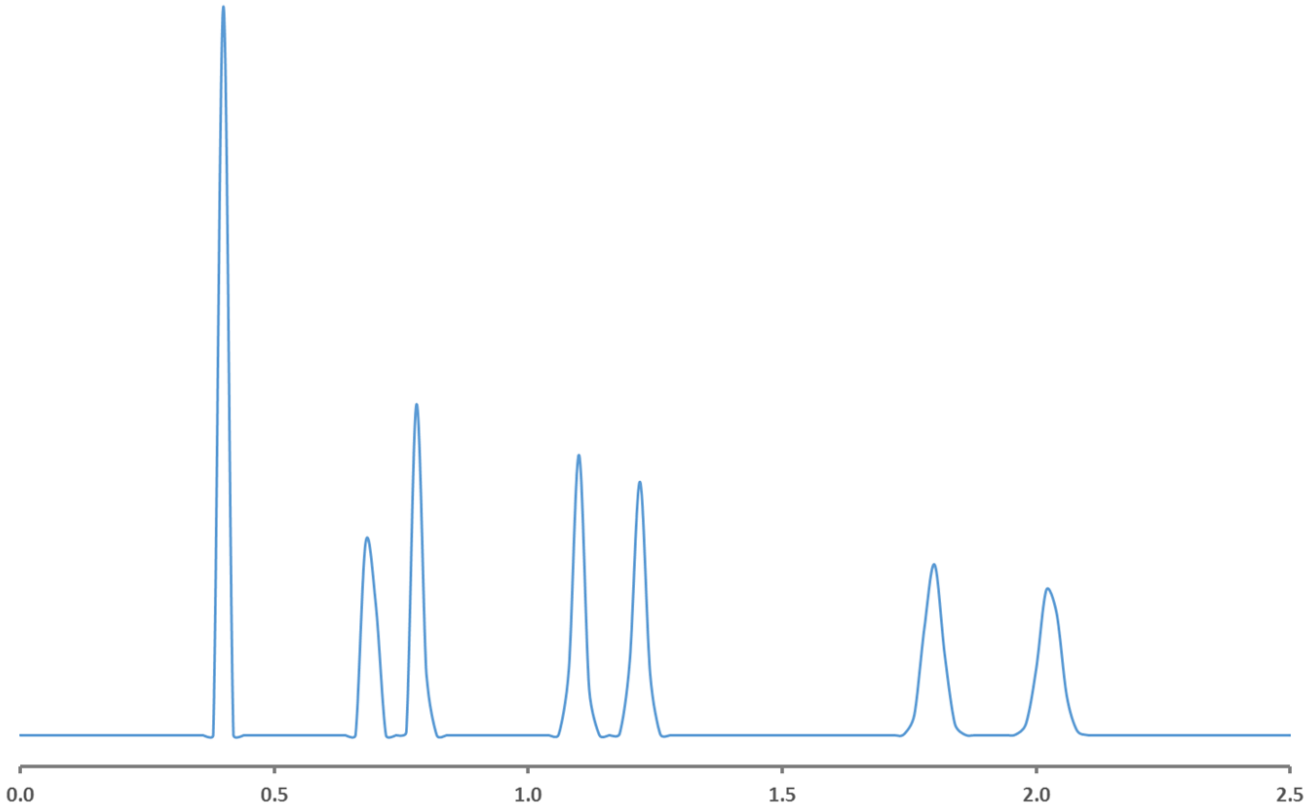


ULTRAVYSOKOTLAKÁ CHROMATOGRAFIE

Pokročilá kapalinová chromatografie

Jiří Urban, Ústav chemie, Přírodovědecká fakulta, Masarykova univerzita, Brno, urban@chemi.muni.cz

VELIKOST ČÁSTIC A ÚČINNOST

$$H = A + \frac{B}{u} + (C_s + C_m) \cdot u$$

$$H = 2\lambda d_p + \frac{2\lambda D_m}{u} + \frac{d_p^2(a + b \cdot k + c \cdot k^2)}{24(1+k)^2 \cdot D_m} u$$

VELIKOST ČÁSTIC A ÚČINNOST

$$H = A + \frac{B}{u} + (C_s + C_m) \cdot u$$

$$H = 2\lambda d_p + \frac{2\lambda D_m}{u} + \frac{d_p^2(a + b \cdot k + c \cdot k^2)}{24(1+k)^2 \cdot D_m} u$$

$$N = \frac{L}{2 \cdot d_p}$$

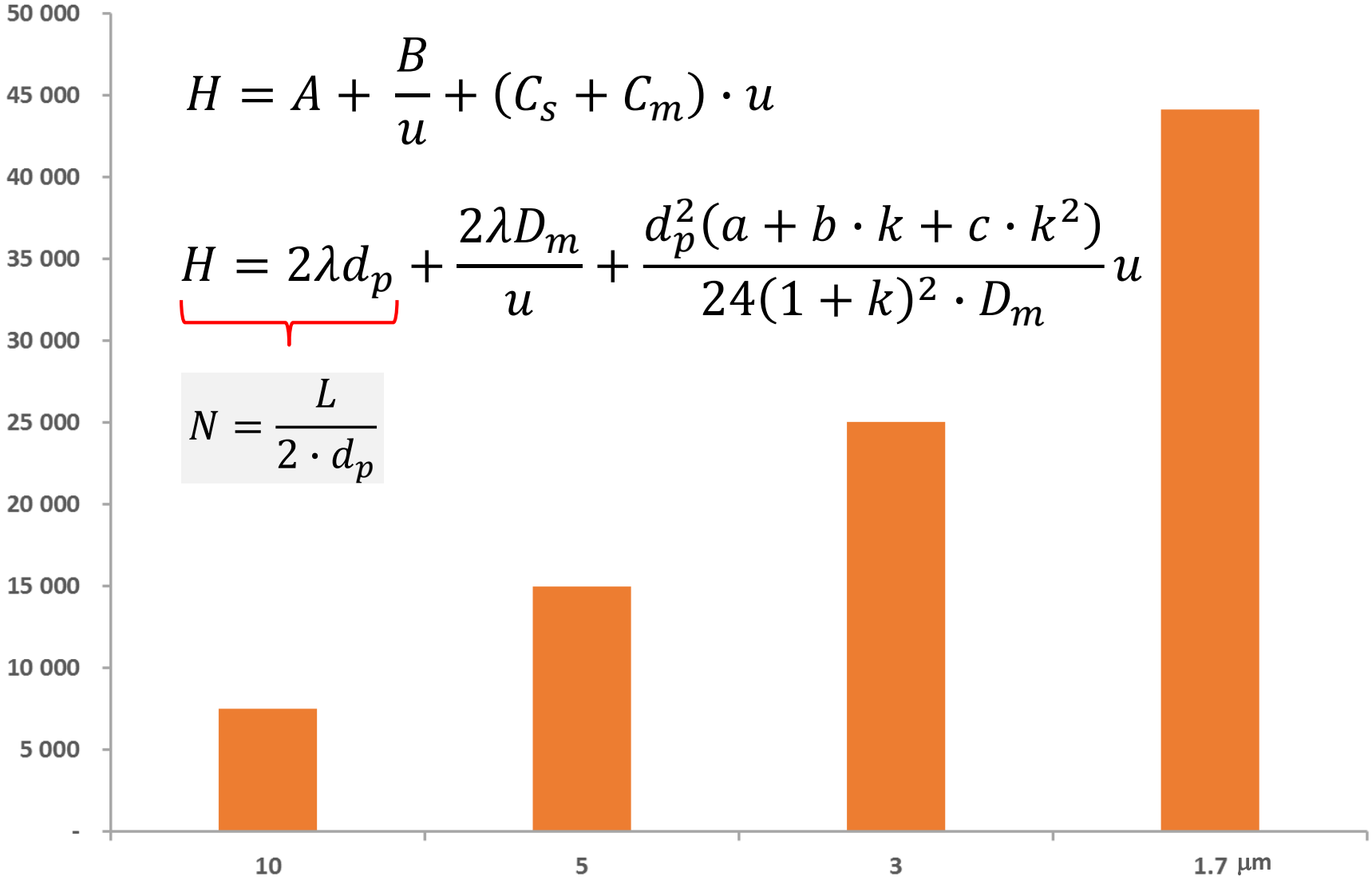
VELIKOST ČÁSTIC A ÚČINNOST

$$\downarrow d_p \approx \uparrow N$$

$$H = A + \frac{B}{u} + (C_s + C_m) \cdot u$$

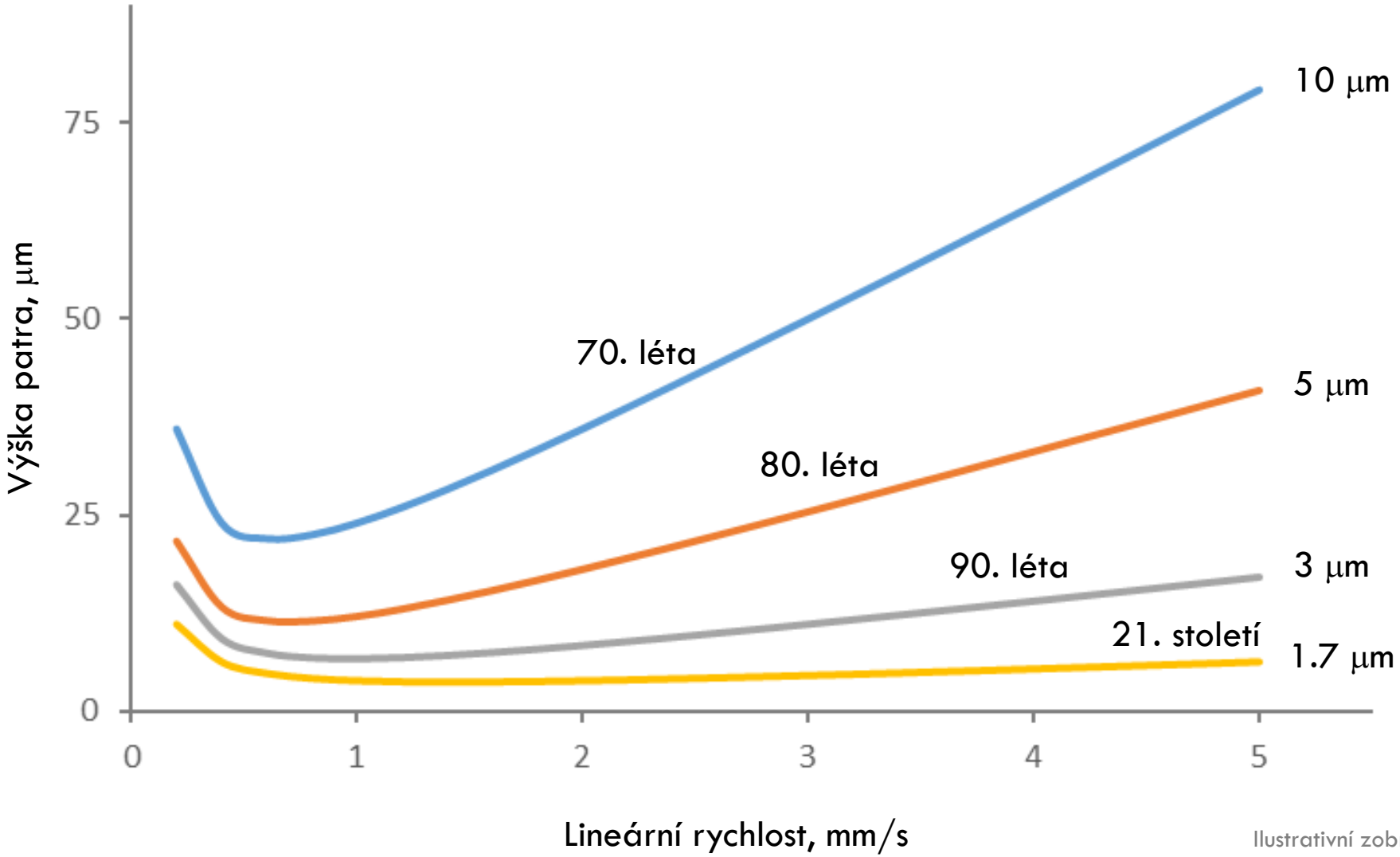
$$H = 2\lambda d_p + \frac{2\lambda D_m}{u} + \frac{d_p^2(a + b \cdot k + c \cdot k^2)}{24(1+k)^2 \cdot D_m} u$$

$$N = \frac{L}{2 \cdot d_p}$$



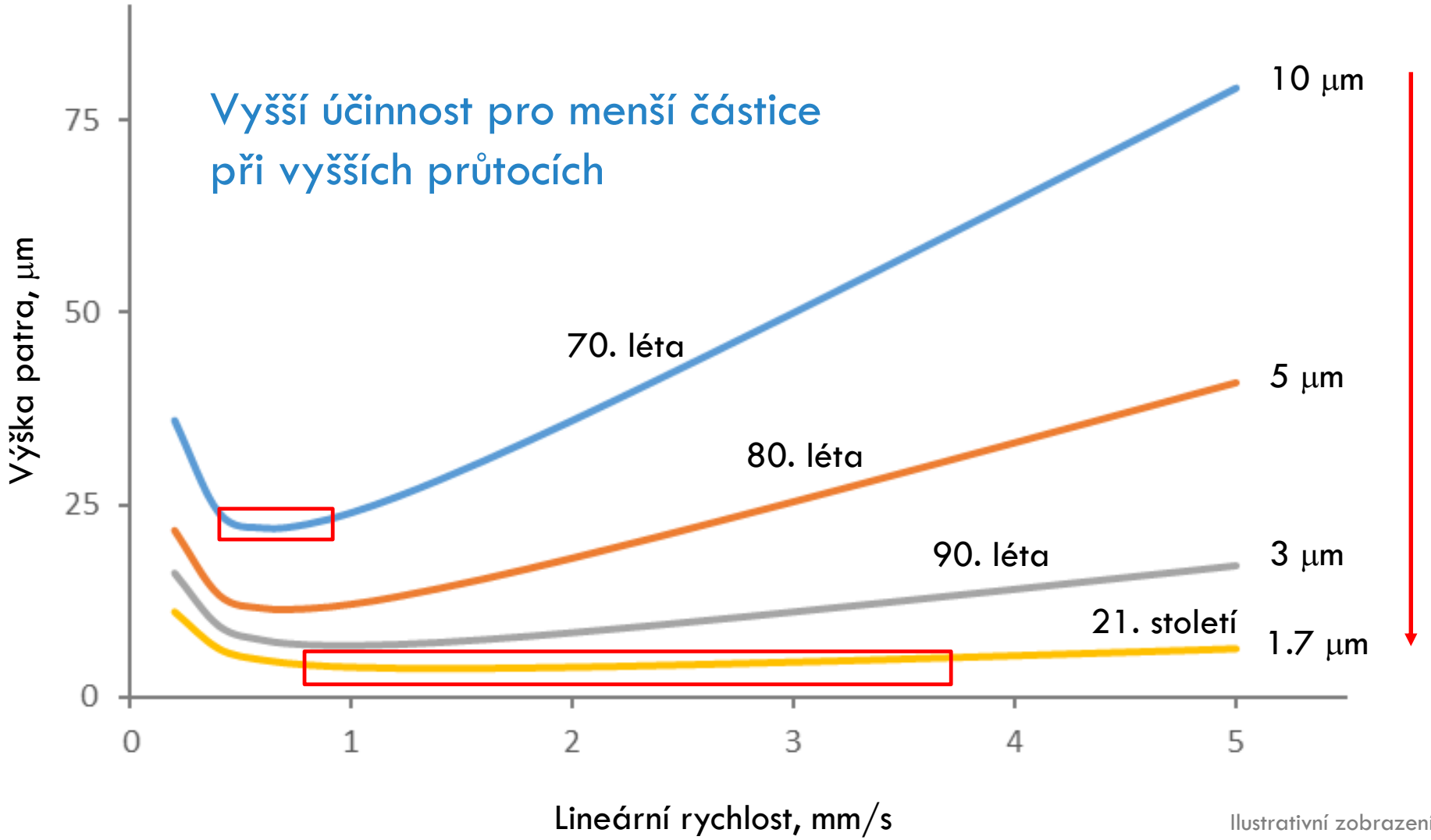
L = 150 mm

VELIKOST ČÁSTIC A ÚČINNOST



Ilustrativní zobrazení

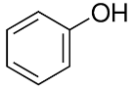
VELIKOST ČÁSTIC A ÚČINNOST



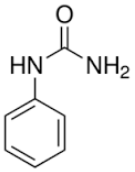
Ilustrativní zobrazení

VLIV VELIKOSTI ČÁSTIC NA SEPARACI

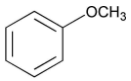
Testovací směs



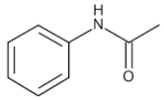
fenol



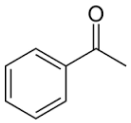
fenylurea



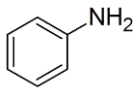
metoxybenzen



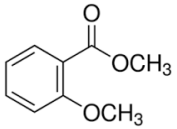
acetanilid



metylphenylketon



aniline



methylmethoxybenzoát

Sloučenina	k
Fenol	1.35
Fenylmočovina	3.06
Metoxybenzen	3.63
Acetanilid	5.48
Methylphenylketon	6.19
Anilin	9.60
Methylmetoxybenzoát	10.95

Kolona

150 x 4.6 mm, C18

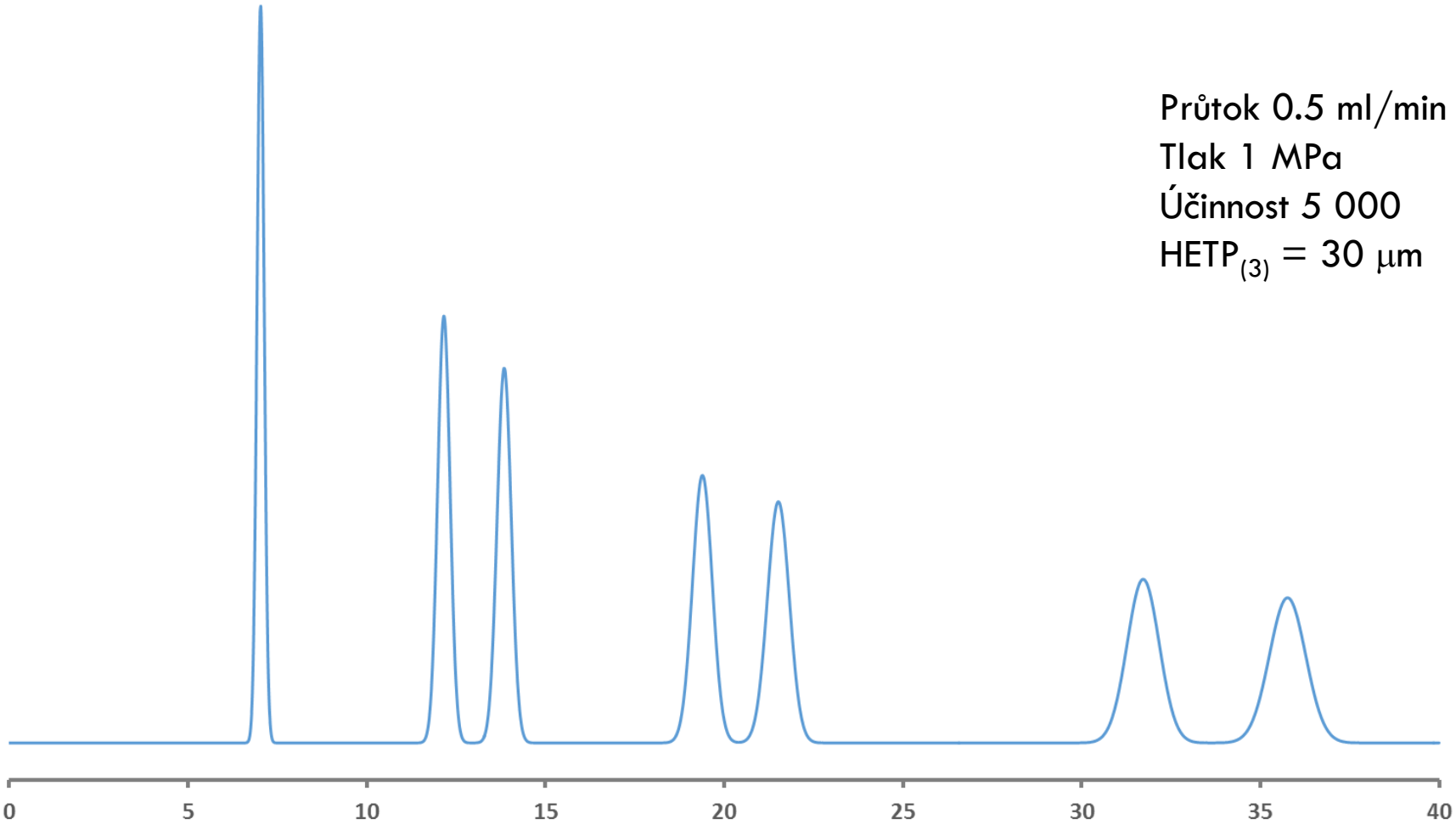
Velikost částic

10 µm 5 µm 3 µm 1.7 µm

Mobilní fáze

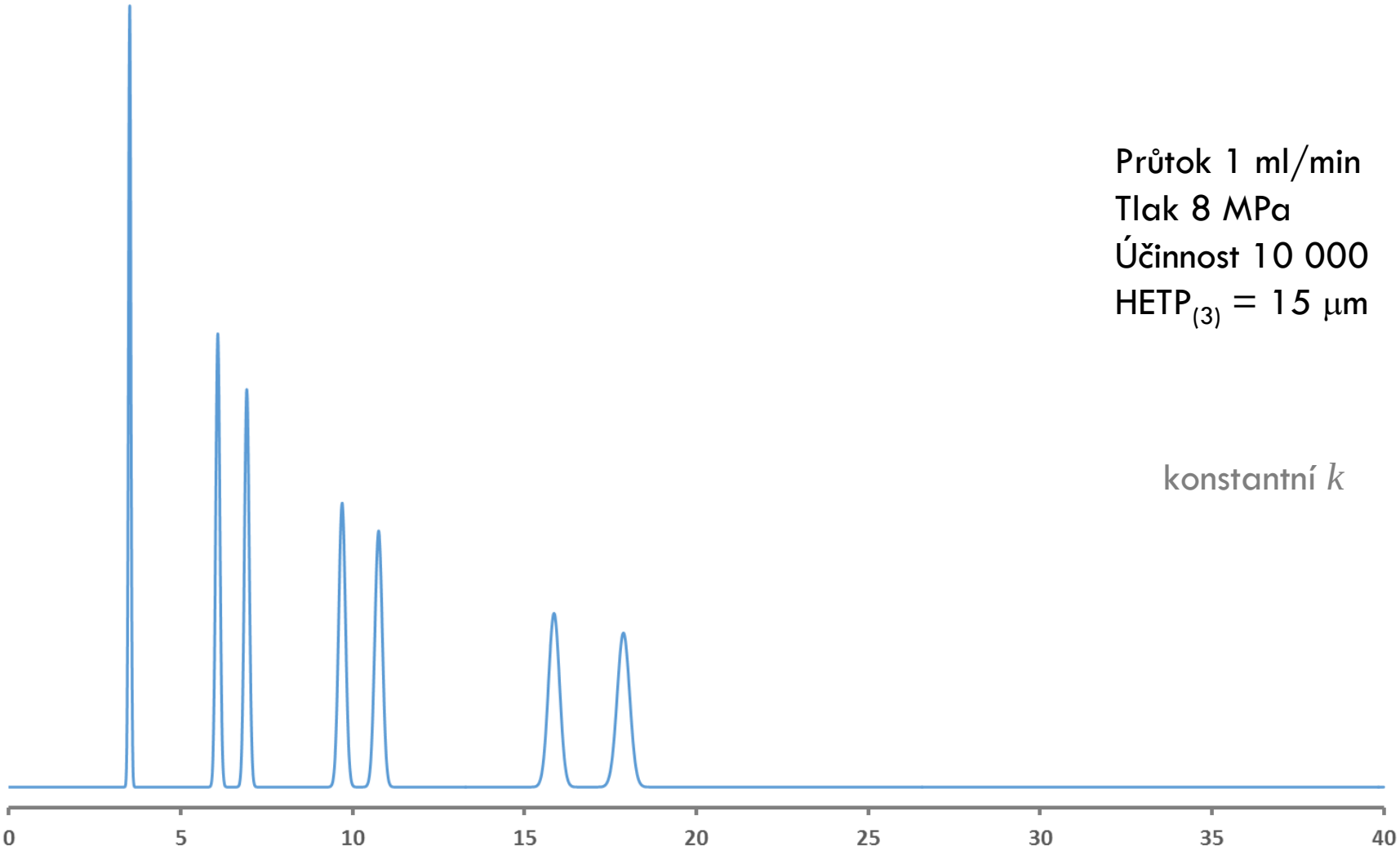
55% Acetonitril

150 X 4.6, 10 μm



Průtok 0.5 ml/min
Tlak 1 MPa
Účinnost 5 000
$HETP_{(3)}$ = 30 μm

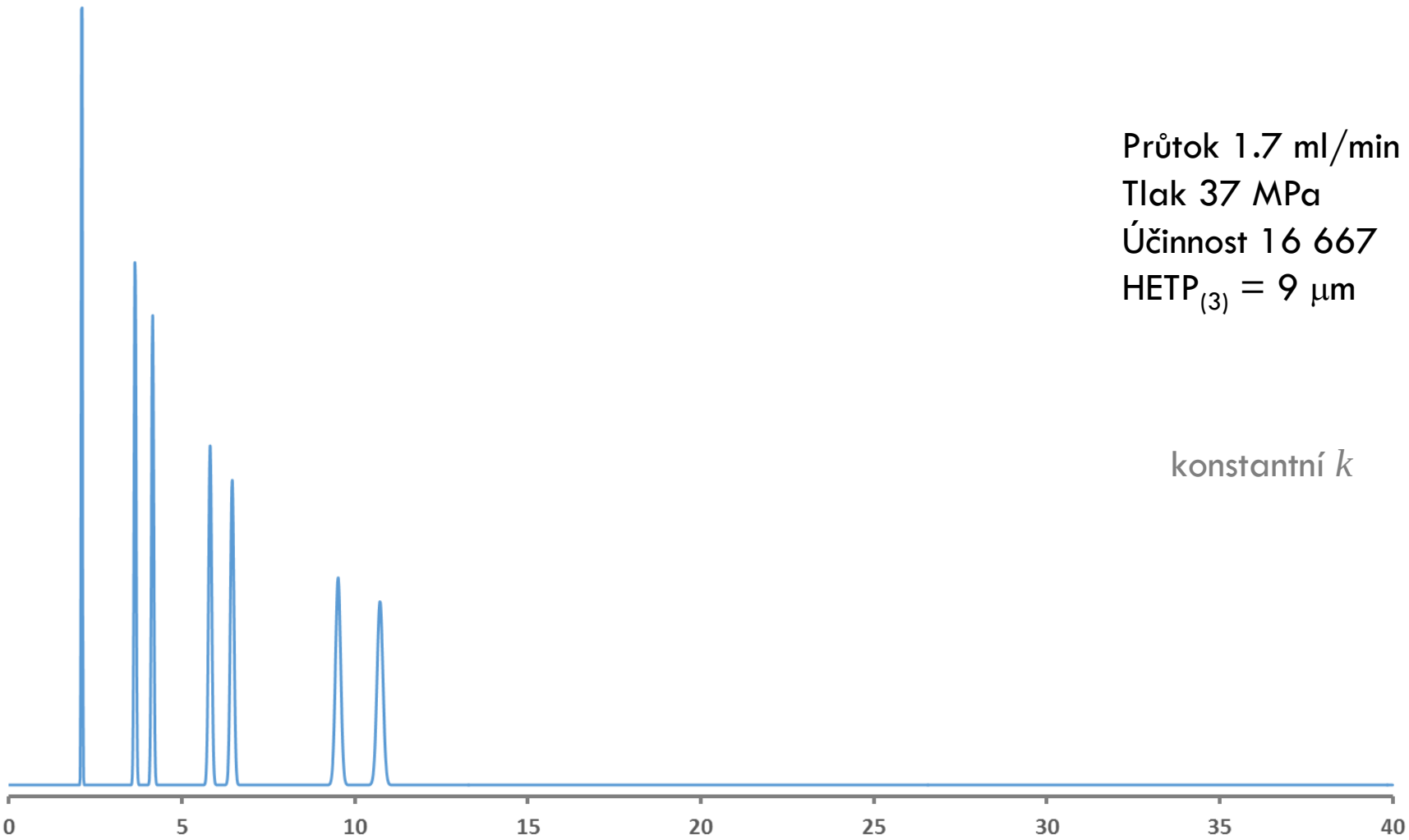
150 X 4.6, 5 μm



Průtok 1 ml/min
Tlak 8 MPa
Účinnost 10 000
$HETP_{(3)}$ = 15 μm

konstantní k

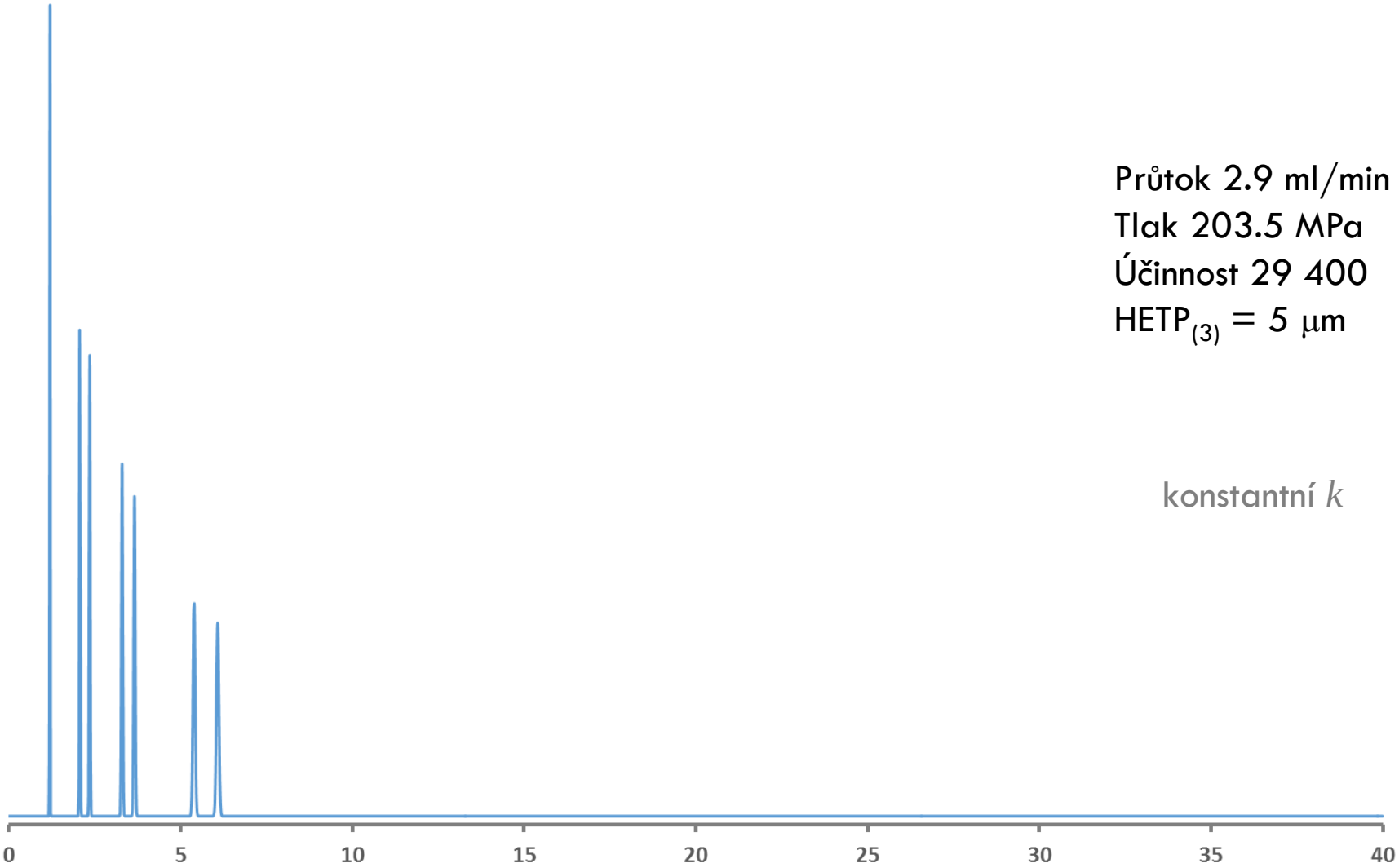
150 X 4.6, 3 µm



Průtok 1.7 ml/min
Tlak 37 MPa
Účinnost 16 667
$HETP_{(3)}$ = 9 µm

konstantní k

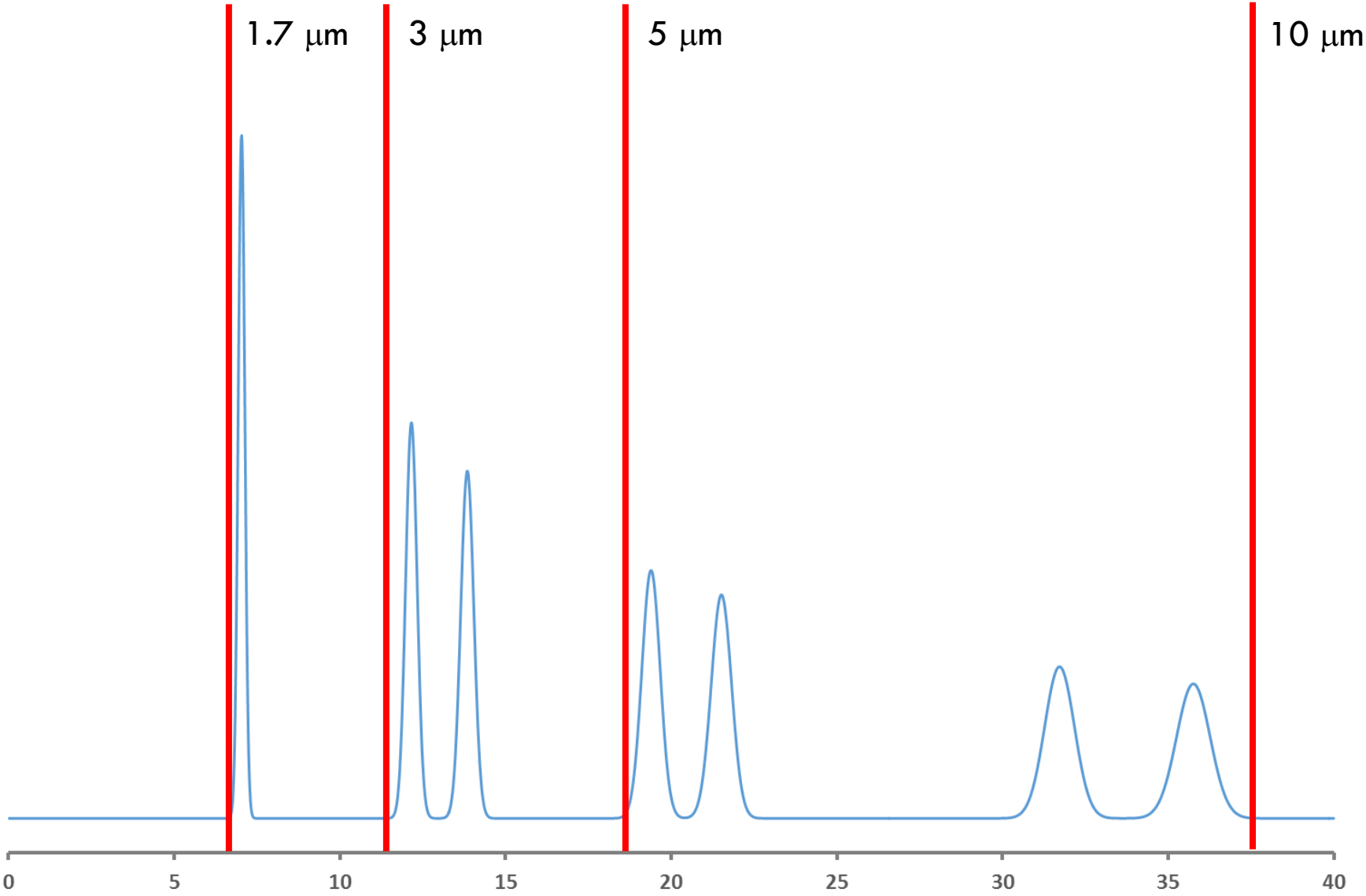
150 X 4.6, 1.7 µm



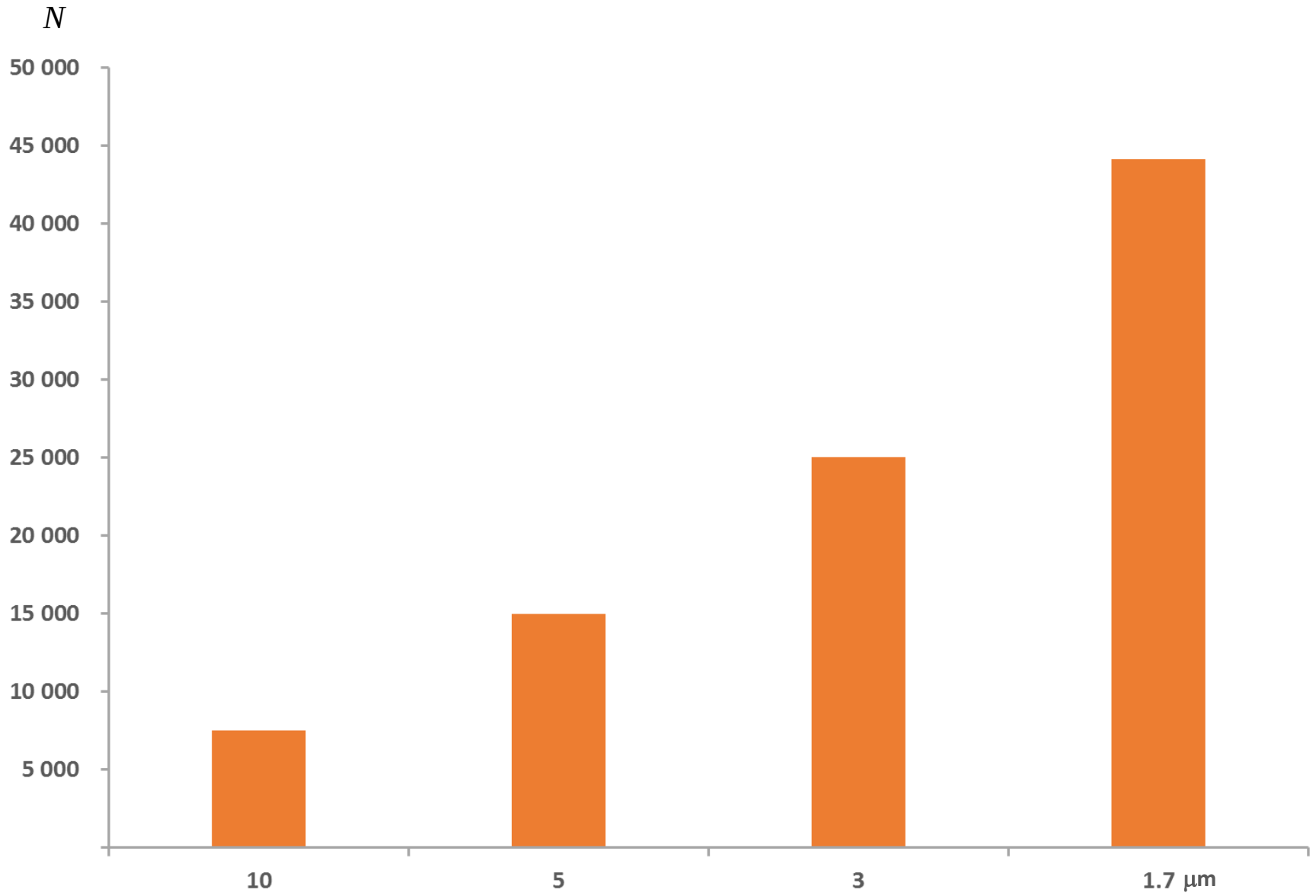
Průtok 2.9 ml/min
Tlak 203.5 MPa
Účinnost 29 400
$HETP_{(3)} = 5$ µm

konstantní k

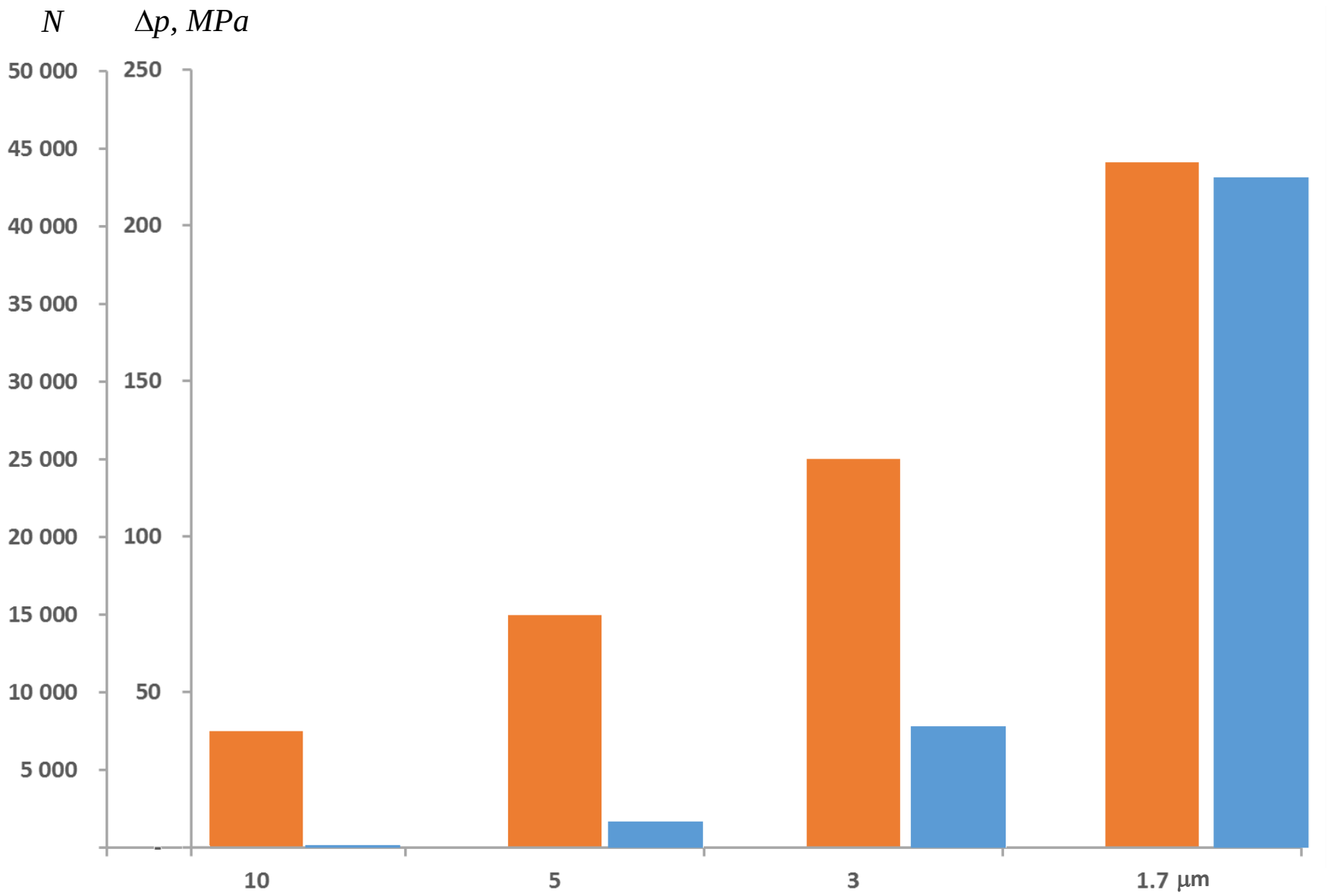
VELIKOST ČÁSTIC A RYCHLOST ANALÝZY



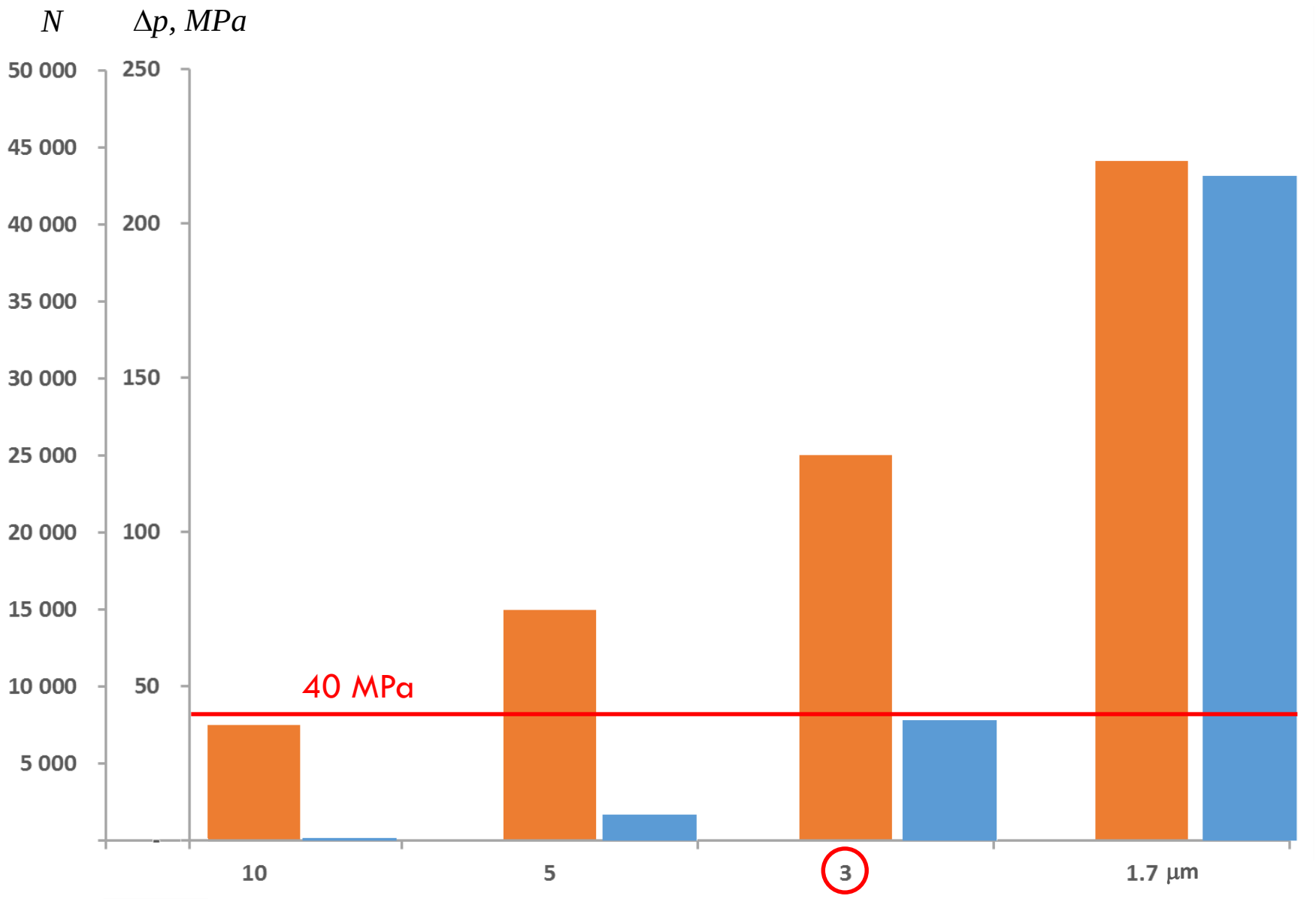
VELIKOST ČÁSTIC, ÚČINNOST A TLAK



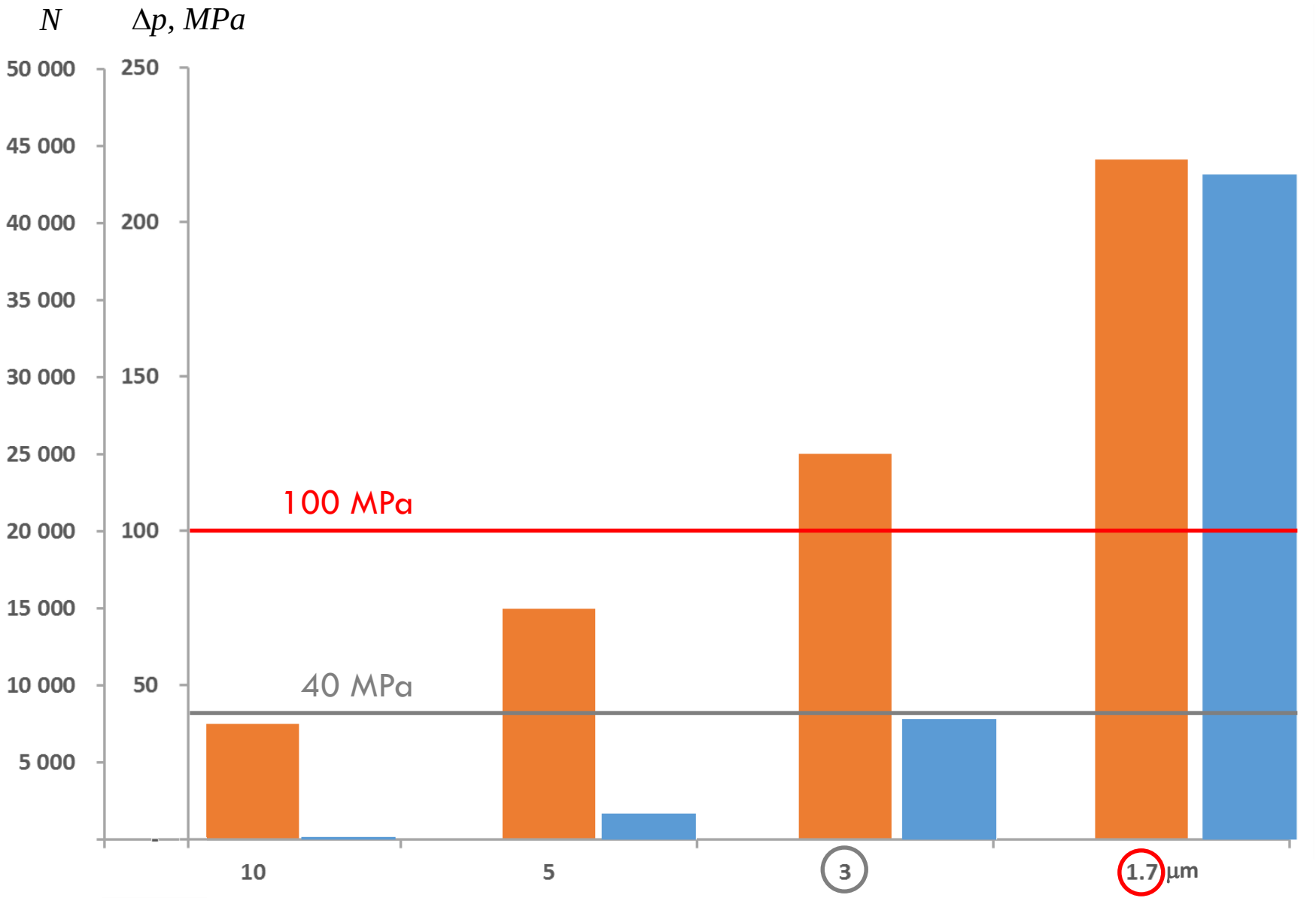
VELIKOST ČÁSTIC, ÚČINNOST A TLAK



VELIKOST ČÁSTIC, ÚČINNOST A TLAK



VELIKOST ČÁSTIC, ÚČINNOST A TLAK



VLIV VELIKOSTI ČÁSTIC

d_p, µm	F_m, ml/min	N	p, MPa	t_{ana}, min	$R_{3,2}$
10	0.5	5 000	1	36	2.29
5	1.0	10 000	8	18	3.25
3	1.7	17 000	37	11	4.20
1.7	2.9	30 000	204	6	5.57

150 x 4.6 mm
C18
55% acetonitril

konstantní k

$$\Delta p_{opt} \approx \frac{1}{d_p^3}$$

Při použití 1 µm částic bude pracovní tlak
125x větší než v případě 5 µm částic

VLIV VELIKOSTI ČÁSTIC

d_p, µm	F_m, ml/min	N	p, MPa	t_{ana}, min	$R_{3,2}$
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150 x 4.6 mm
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konstantní k

$$\Delta p_{opt} \approx \frac{1}{d_p^3}$$

Při použití 1 µm částic bude pracovní tlak 125x větší než v případě 5 µm částic

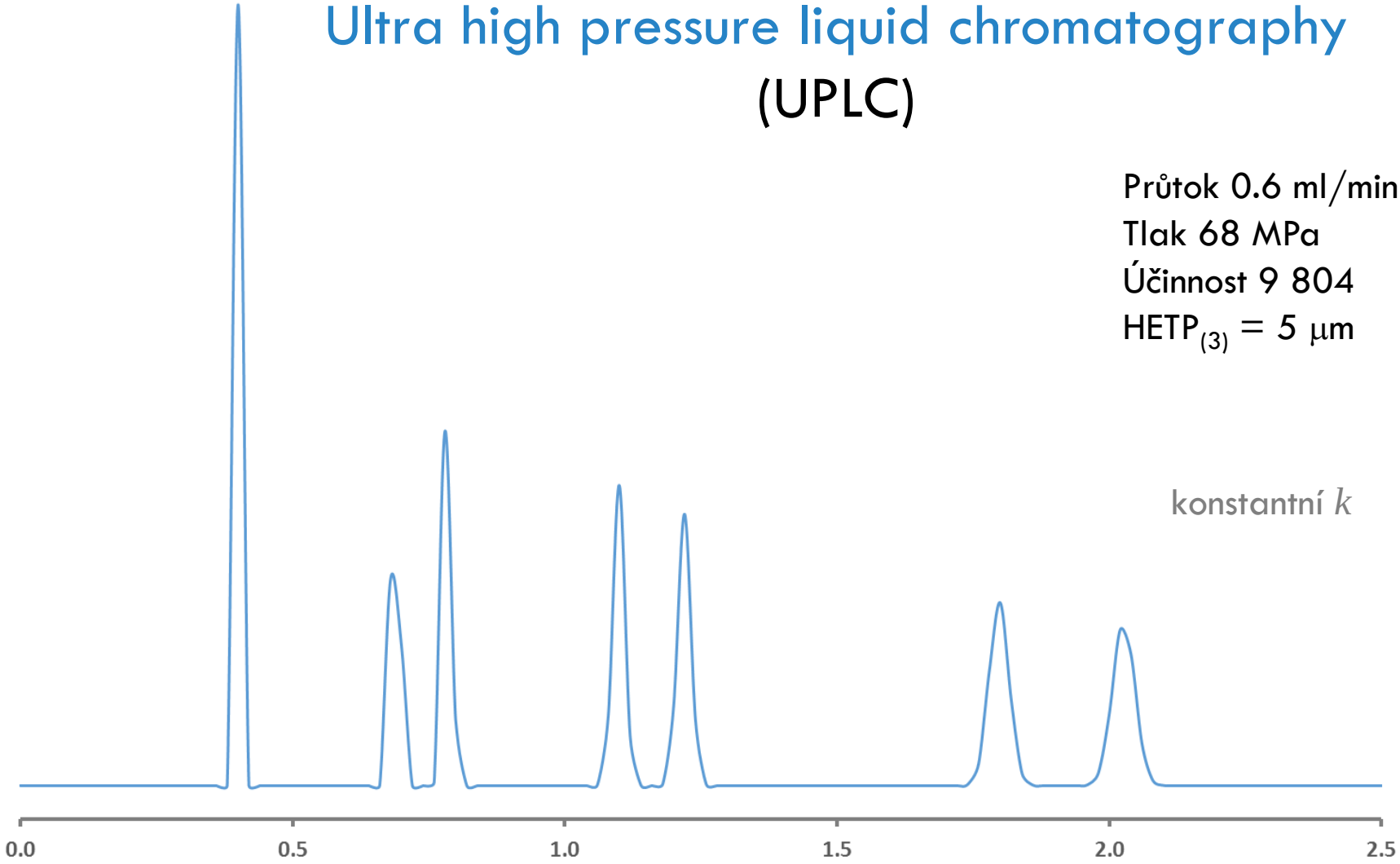
Nutnost použití kolon s menšími rozměry

50 X 2.1, 1.7 µm.

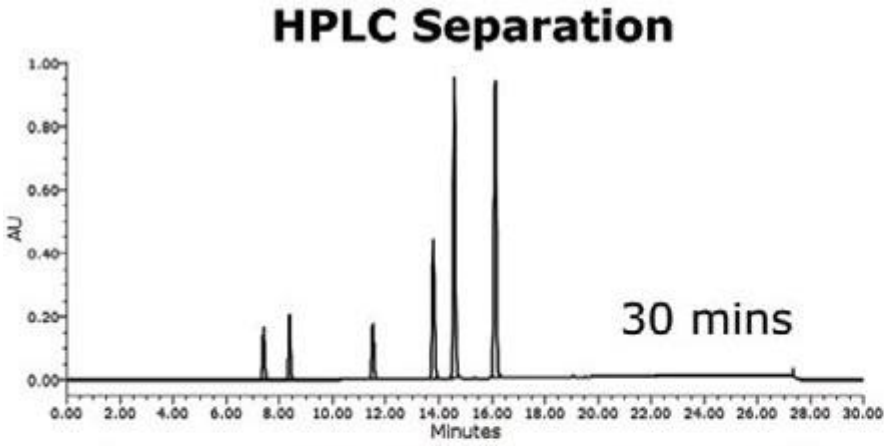
Ultra high pressure liquid chromatography (UPLC)

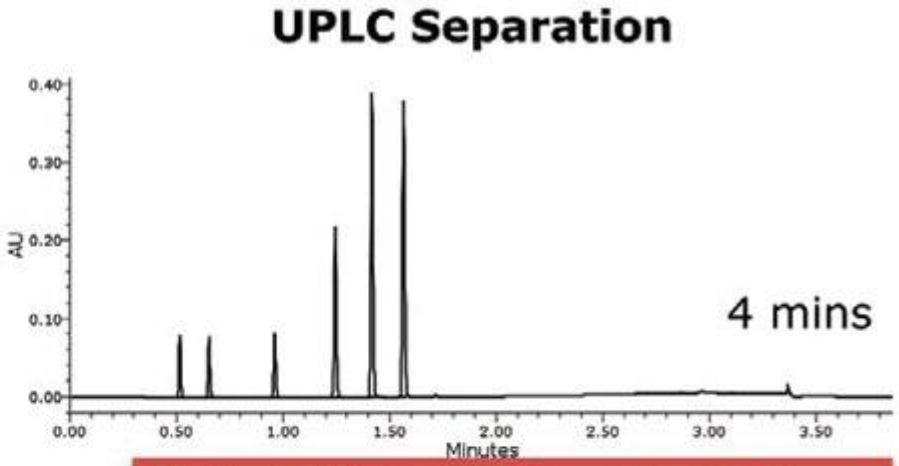
Průtok 0.6 ml/min
Tlak 68 MPa
Účinnost 9 804
$HETP_{(3)} = 5$ µm

konstantní k



HPLC vs. UPLC



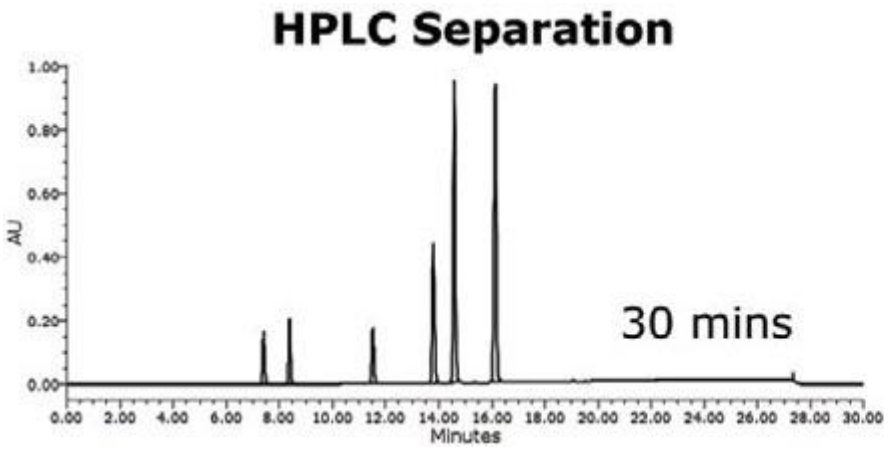


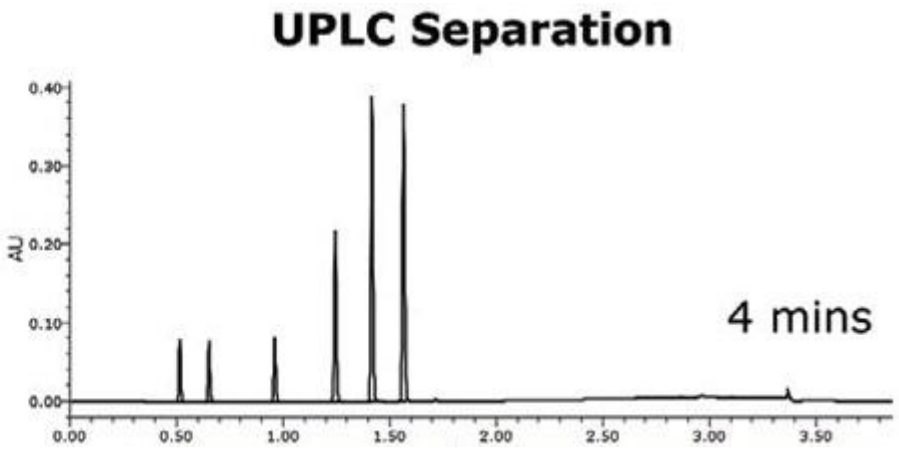
Typical Column Volume = 2.49 mL

Typical Column Volume = 0.17 mL

HPLC		UPLC Reduction	UPLC	
Run Time	30 min.	**87%**	Run Time	3.86 min.
Solvent Consumed	30 mL	**83%**	Solvent Consumed	4.97 mL
Sample Consumed	20 µL	**93%**	Sample Consumed	1.0 µL

HPLC vs. UPLC





Nevýhody UPLC

- Instrumentální nároky
- Vyšší pracovní tlak
- Teplota tření
- Nižší životnost kolon
- Lze řešit vyšší teplotou

Výhody UPLC

- Větší selektivita a citlivost
- Rychlé analýzy
- Nižší provozní náklady
- Vyšší produktivita
- Rychlejší optimalizace

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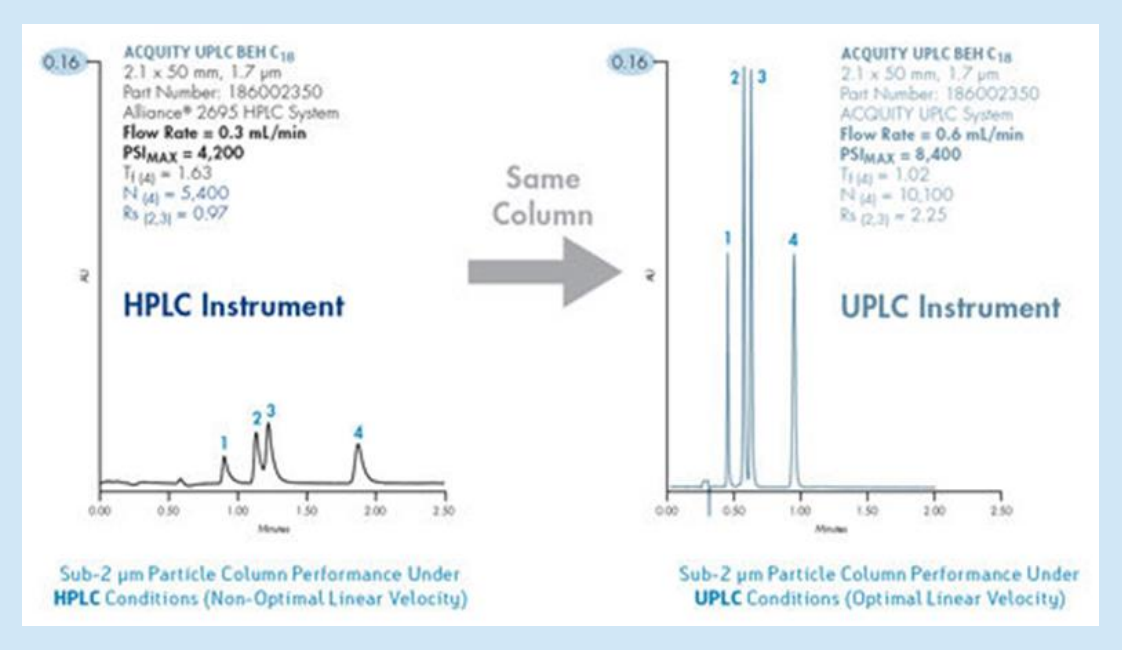
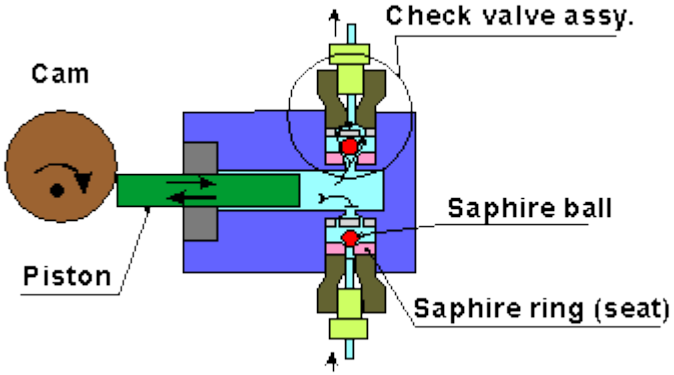
NUTNÁ OPTIMALIZACE SYSTÉMU!

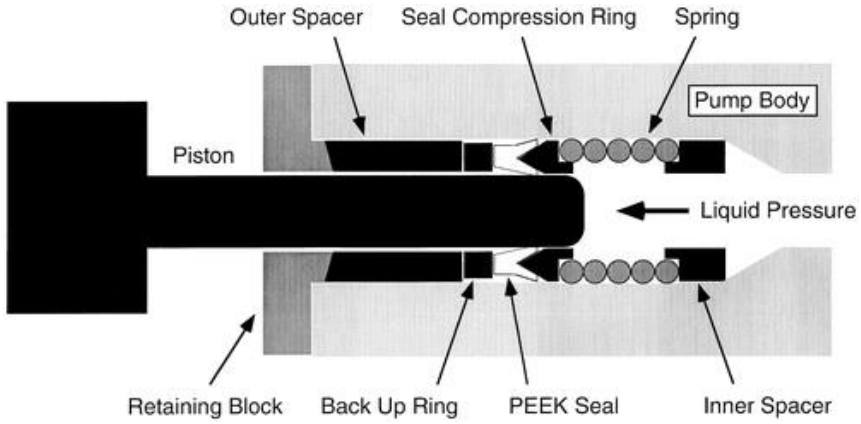


Nelze pouze „koupit přístroj“ nebo „UPLC“ kolonu

ČERPADLA

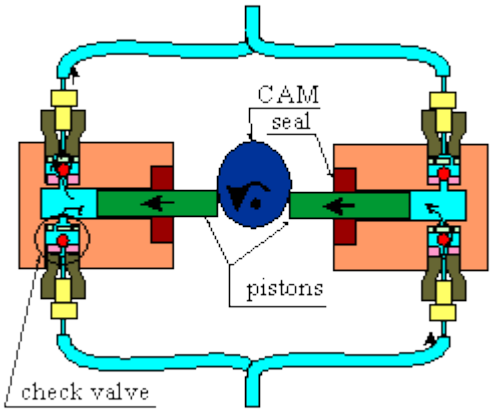
Jednopístový systém

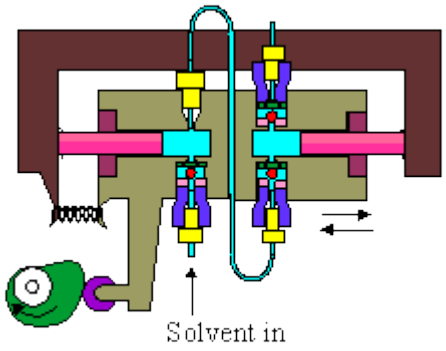


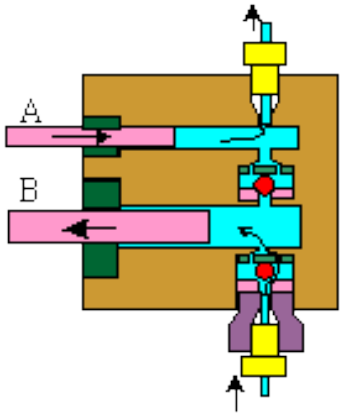


450 MPa (Anal. Chem. 69 (1997) 983–989.)

Dvoupístový systém

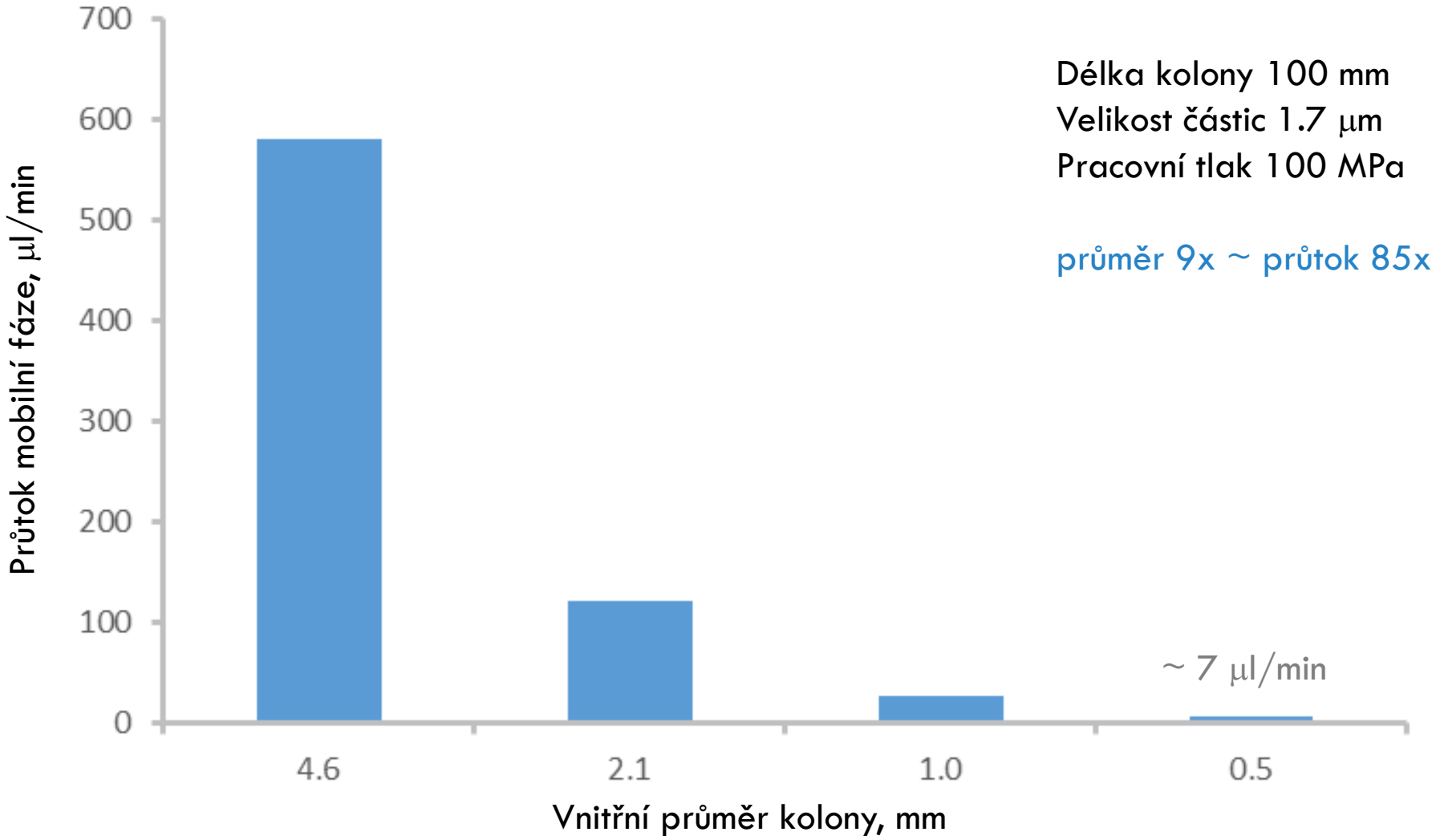






International Journal of Pharmaceutical Research & Analysis 2 (2012) 24-31.

PRŮMĚR KOLONY A PRŮTOK MOBILNÍ FÁZE



Délka kolony 100 mm
Velikost částic 1.7 µm
Pracovní tlak 100 MPa

průměr 9x ~ průtok 85x

OBJEM KOLONY

150 x 4.6 mm, V = 2.49 ml

100 x 4.6 mm, V = 1.66 ml

75 x 4.6 mm, V = 1.25 ml

75 x 2.1 mm, V = 0.26 ml

50 x 2.1 mm, V = 0.17 ml

DÁVKOVANÝ OBJEM

20 µl

150 x 4.6 mm, V = 2.49 ml

0.8 %

100 x 4.6 mm, V = 1.66 ml

1.2 %

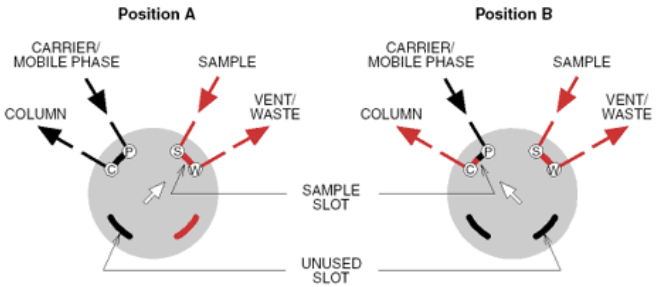
75 x 4.6 mm, V = 1.25 ml

1.6 %

max 135 MPa

75 x 2.1 mm, V = 0.26 ml

7.7 %



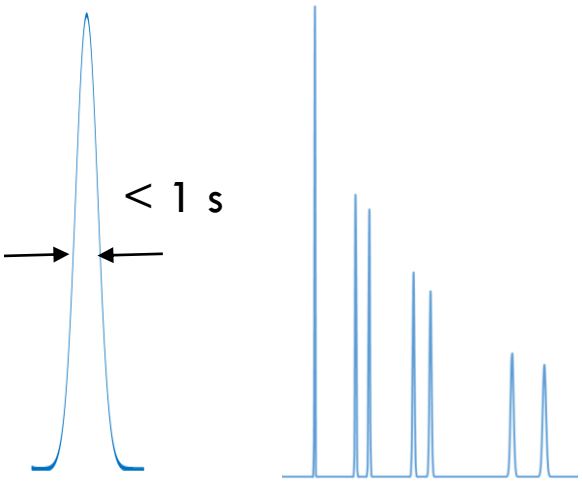
50 x 2.1 mm, V = 0.17 ml

11.5 %

DETEKCE V UPLC

Velmi úzké píky

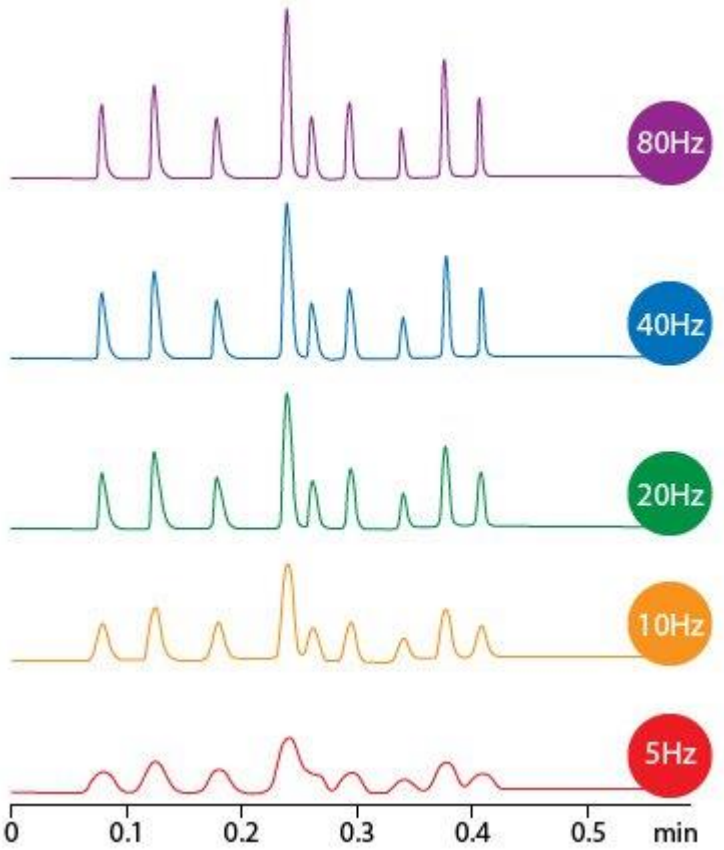
(malá disperze)



2 – 3x větší citlivost

(ve srovnání s HPLC)

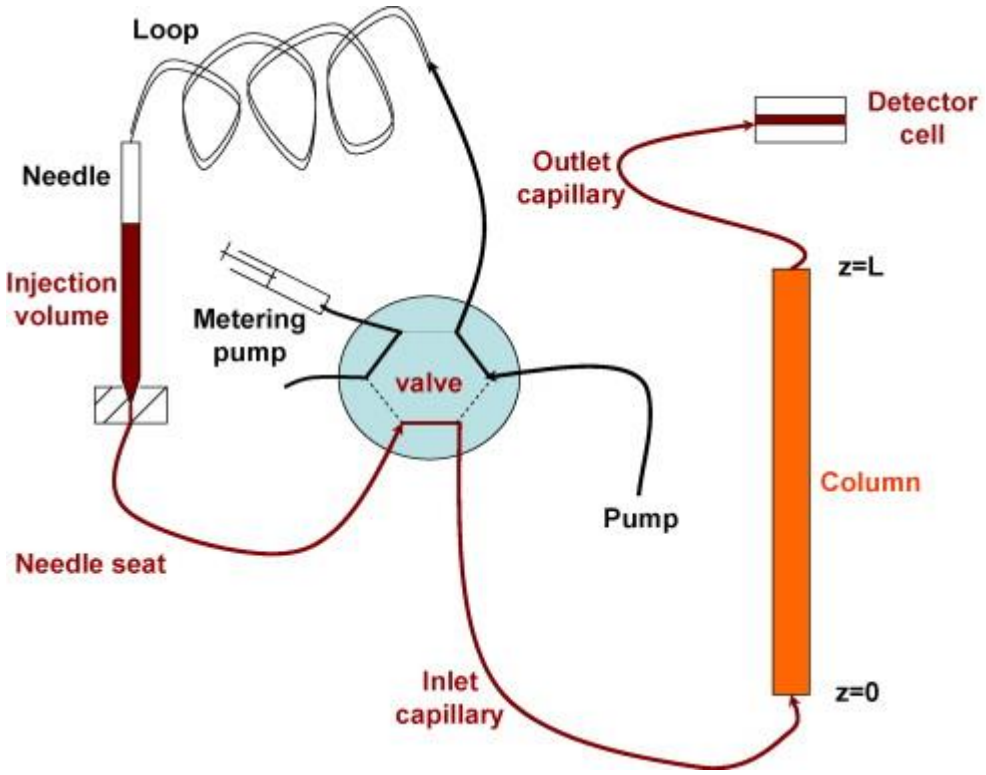
Nutné rychlé vzorkování



www.chromatographyonline.com/system-and-column-volumes-hplc-we-still-haven-t-got-message

MS, UV, DAD, EC, FL

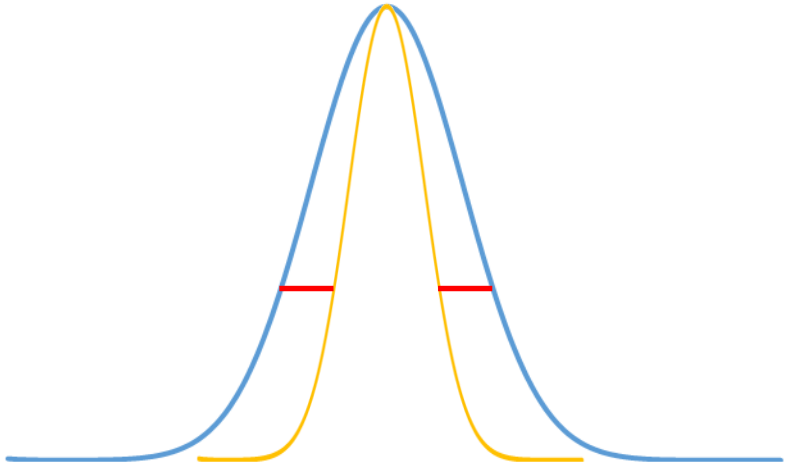
MIMOKOLONOVÉ OBJEMY



Rozmytí v systému

Rozmytí na koloně

Mimokolonové objemy



$$\sigma^2_{celkem} = \sigma^2_{kolona} + \sigma^2_{ext}$$

J. Chromatogr. A 1217 (2010) 3000–3012.

MIMOKOLONOVÉ OBJEMY

Agilent 1100

Agilent 1200

Agilent 1290

Waters Acquity

Každý systém je jiný!

J. Chromatogr. A 1217 (2010) 3000–3012.

MIMOKOLONOVÉ OBJEMY

Systém	Dávkovač, µl	Kapiláry, µl	Detekční cela, µl	
Agilent 1100	2.3	15.2	13	= 30.5 µl
Agilent 1100 opt.	1.2	3.8	1.7	= 6.7 µl
Agilent 1200 opt.	1.1	3.1	1.7	= 5.9 µl
Agilent 1290	1.7	6.2	2.4	= 10.3 µl
Waters Acquity		8.2	0.5	= 8.7 µl

J. Chromatogr. A 1217 (2010) 3000–3012.

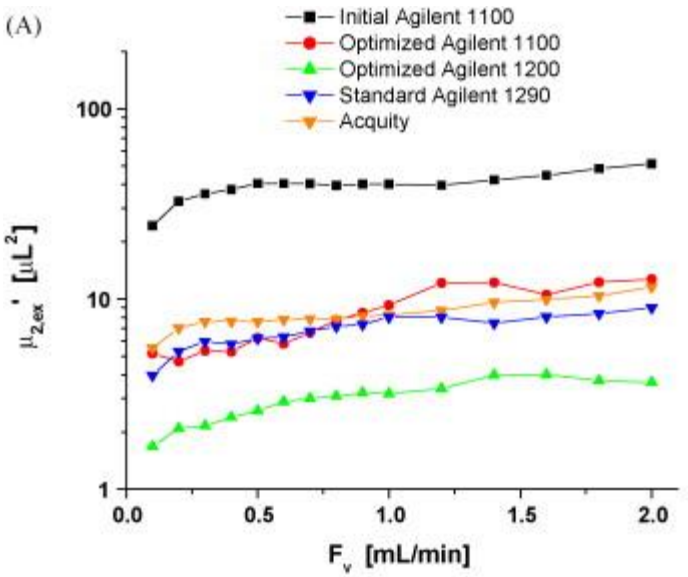
MIMOKOLONOVÉ OBJEMY

Systém	Dávkovač, µl	Kapiláry, µl	Detekční cela, µl	100% ACN, µl²	65% MeOH, µl²	Příspěvek V_{ex}, %
Agilent 1100	2.3	15.2	13	41.5	73.2	49
Agilent 1100 opt.	1.2	3.8	1.7	13.7	10.7	17
Agilent 1200 opt.	1.1	3.1	1.7	3.5	6.1	8
Agilent 1290	1.7	6.2	2.4	9.4	17.3	18
Waters Acquity		8.2	0.5	8.5	8.0	12

J. Chromatogr. A 1217 (2010) 3000–3012.

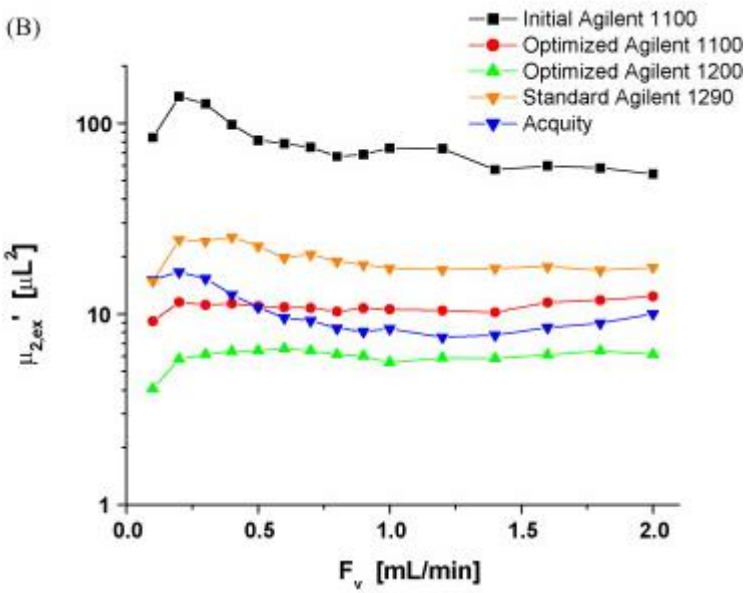
VLIV MOBILNÍ FÁZE

100% acetonitril



naphtho[2,3-α]pyren

65% metanol



4-tert-butylphenol

Větší viskozita, větší rozmytí píků (ale konstantní při vyšších průtocích).

J. Chromatogr. A 1217 (2010) 3000–3012.

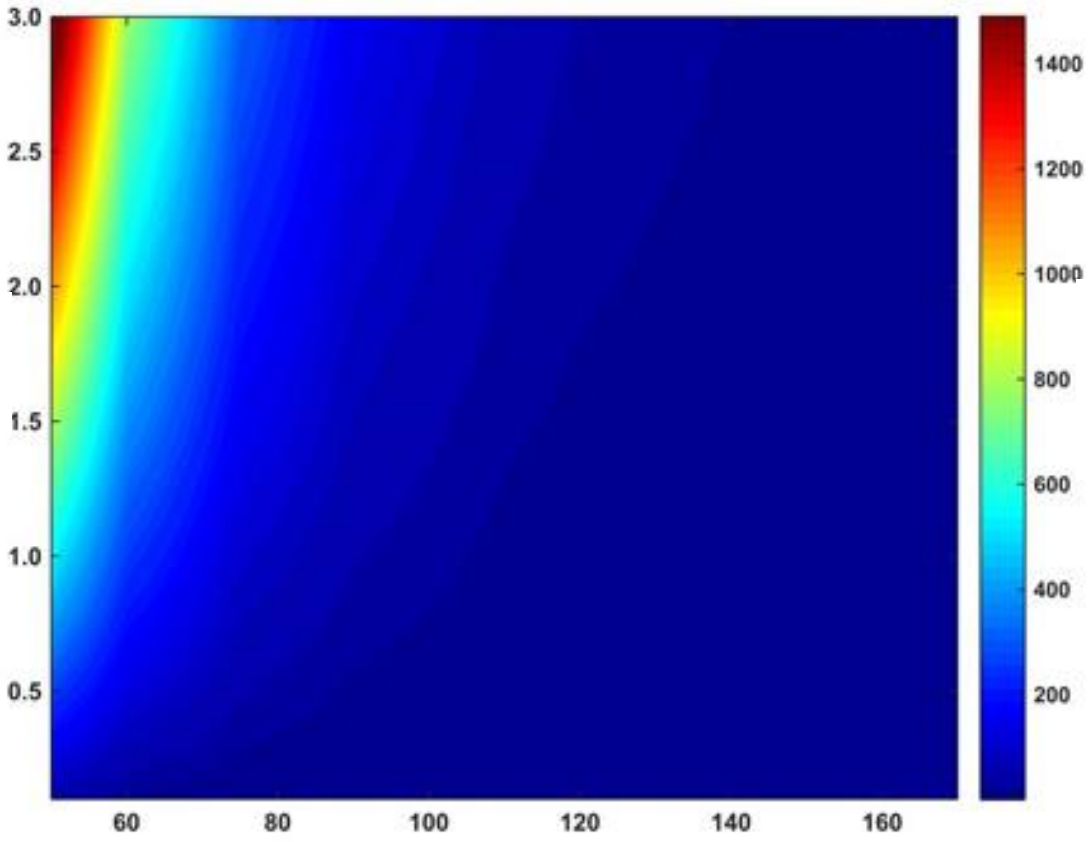
MIMOKOLONOVÉ OBJEMY

Rozměr kolony, mm	Maximální mimokolonové objemy, µl
100 x 4.6	35
50 x 4.6	25
100 x 3.0	15
50 x 3.0	10
100 x 2.1	7
50 x 2.1	5
30 x 2.1	4

www.chromatographyonline.com

Maximálně 2 – 3 % z objemu kolony

TLAK vs. PRŮMĚR KAPILÁRY



Průtok
ml/min

Pracovní tlak
bar

Průměr kapiláry
μm

$$\Delta P = \frac{8\eta L}{r^2}\upsilon$$

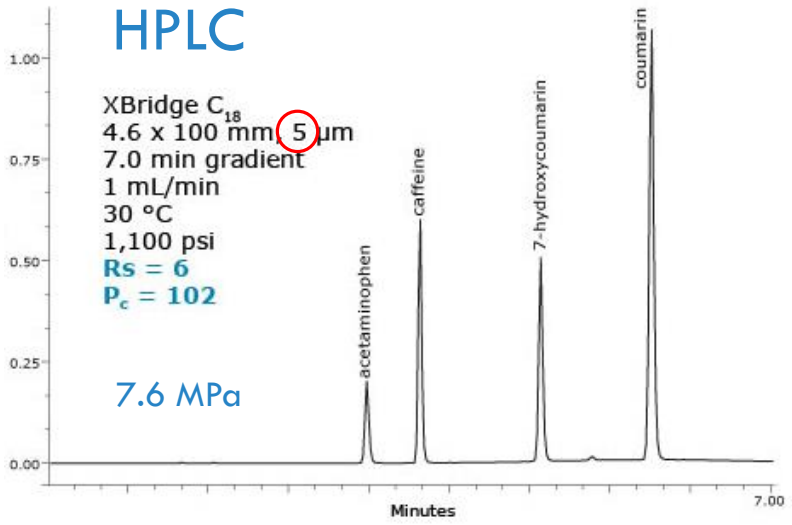
Viskozita vody při pokojové teplotě, délka kapiláry 50 cm, www.chromacademy.com

PŘEVOD METODY

HPLC

XBridge C_{18}
4.6 x 100 mm, 5 µm
7.0 min gradient
1 mL/min
30 °C
1,100 psi
Rs = 6
P_c = 102

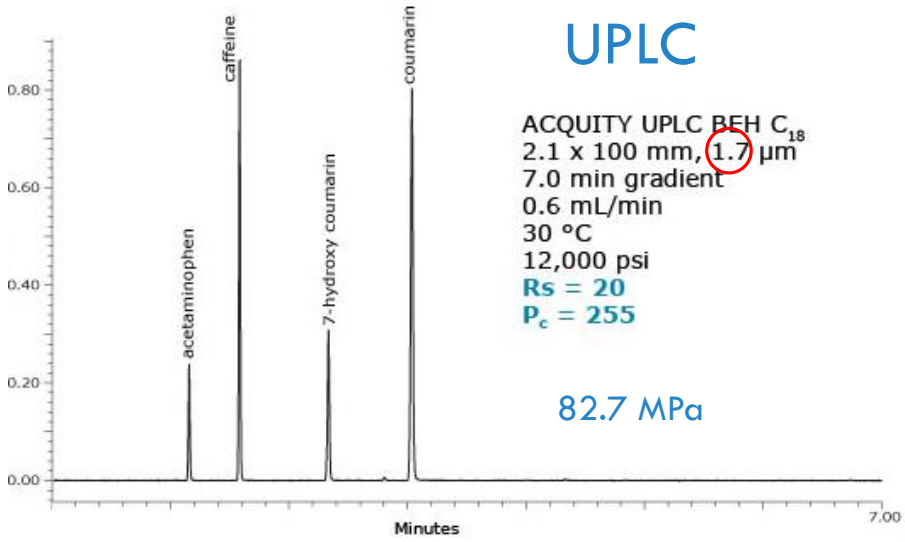
7.6 MPa



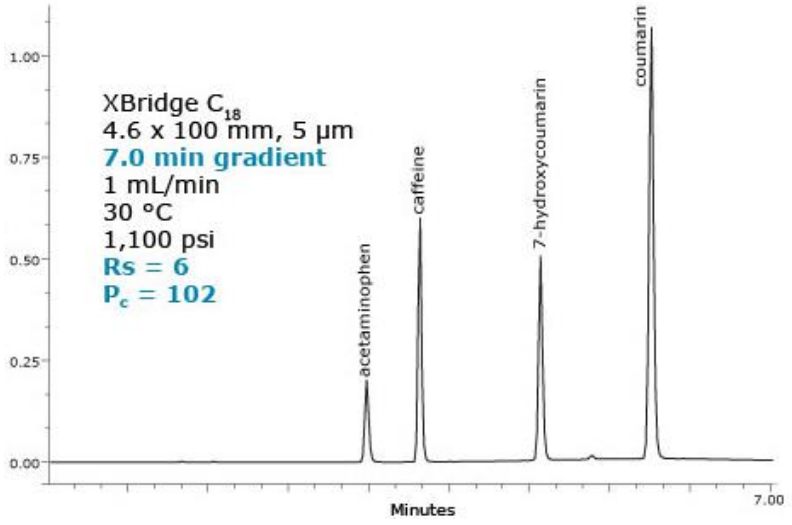
UPLC

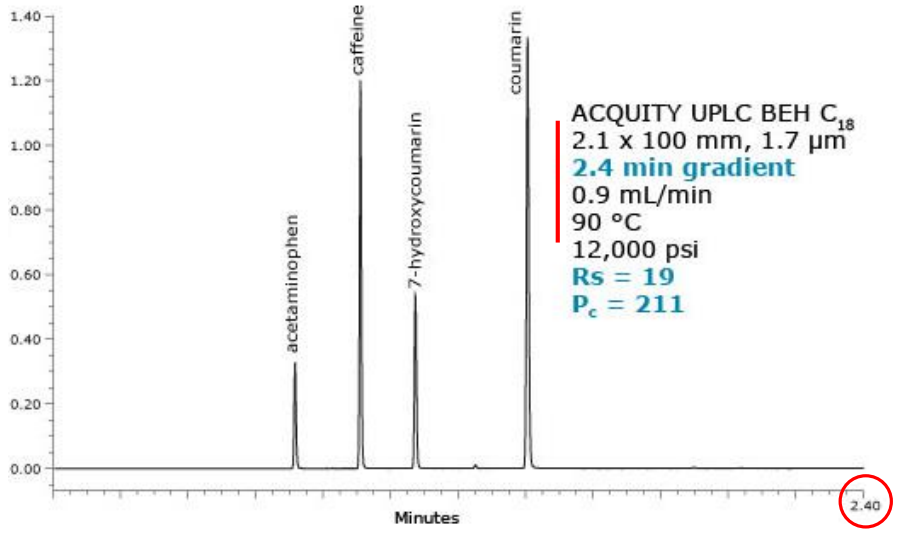
ACQUITY UPLC BEH C_{18}
2.1 x 100 mm, 1.7 µm
7.0 min gradient
0.6 mL/min
30 °C
12,000 psi
Rs = 20
P_c = 255

82.7 MPa



XBridge C_{18}
4.6 x 100 mm, 5 µm
7.0 min gradient
1 mL/min
30 °C
1,100 psi
Rs = 6
P_c = 102



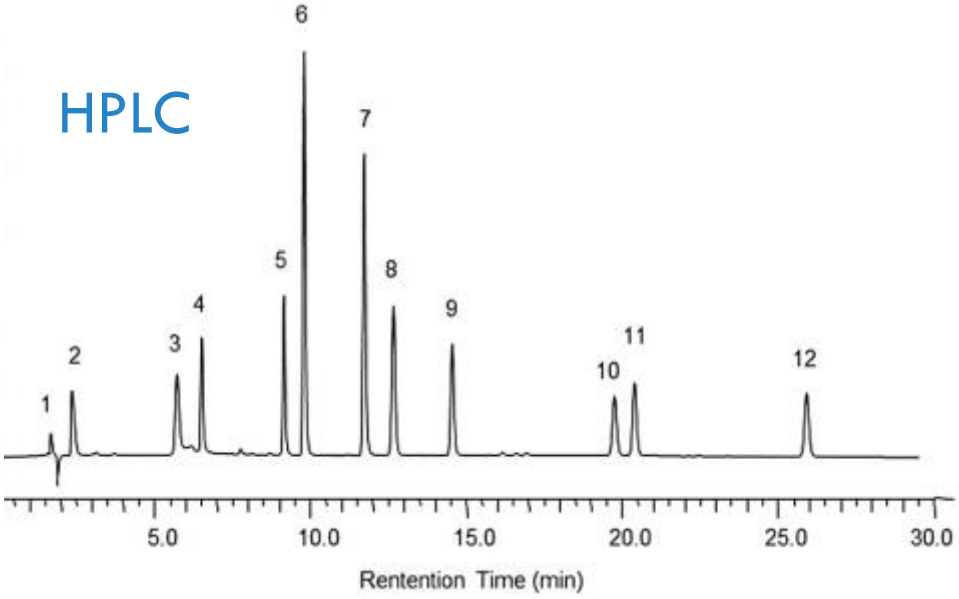


ACQUITY UPLC BEH C_{18}
2.1 x 100 mm, 1.7 µm
2.4 min gradient
0.9 mL/min
90 °C
12,000 psi
Rs = 19
P_c = 211

PŘEVOD METODY

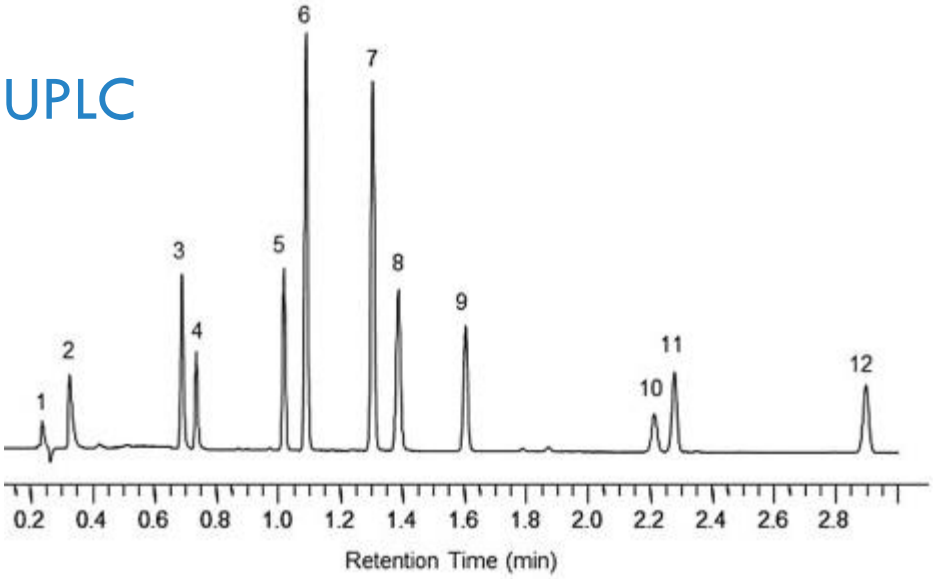
Farmaceutika

HPLC



C18, 150 × 4.6 mm, 5 µm, 1 mL/min,
V_{inj} = 20 µL, celkový čas analýzy 45 min

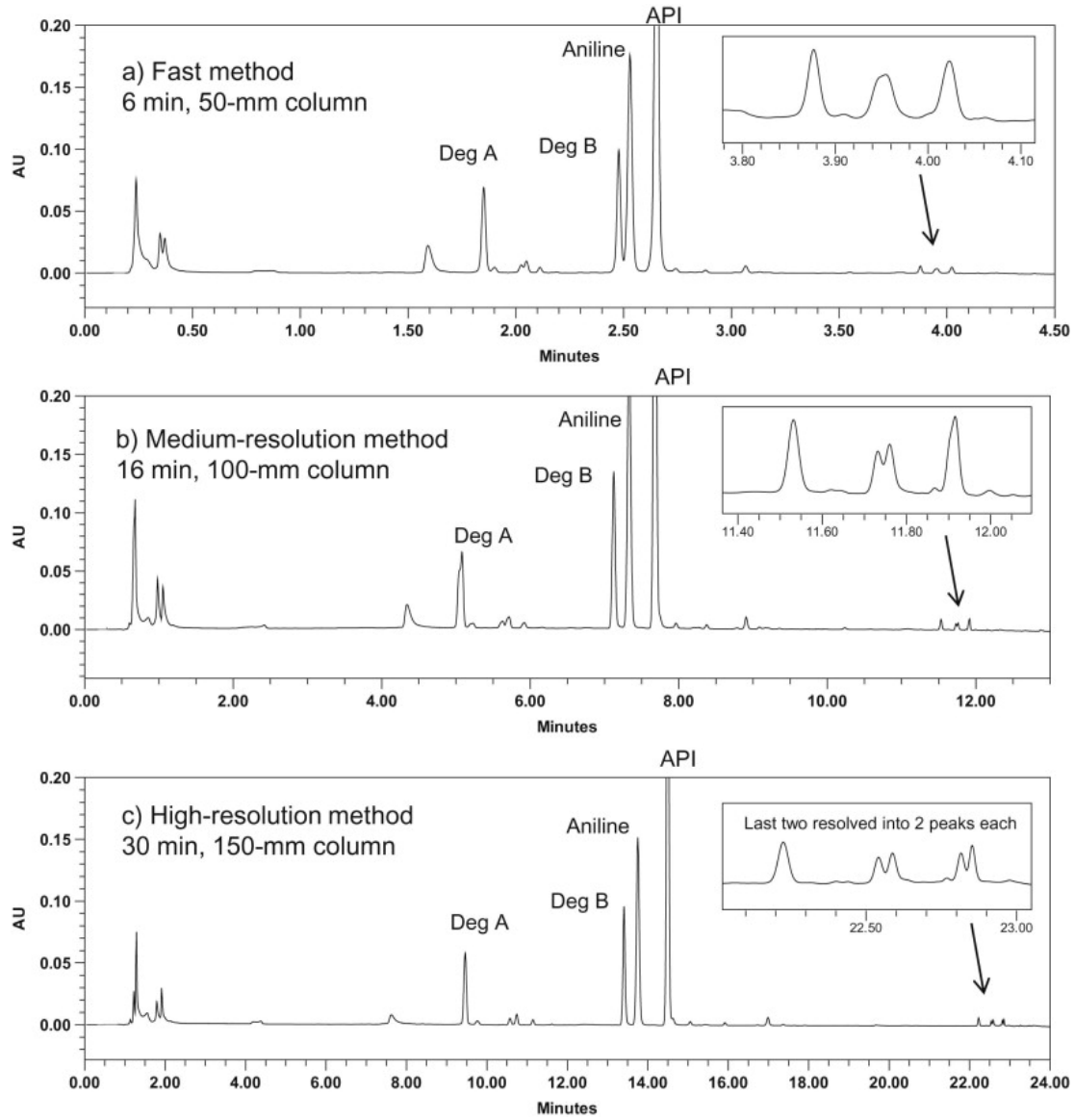
UPLC



C18 50 × 2.1 mm, 1.7 µm, 0.61 mL/min,
Vinj = 1.4 µL, celkový čas analýzy 5.1 min

Eu. J. Pharm. Biopharm 68 (2008) 430–440

RYCHLOST vs. ROZLIŠENÍ



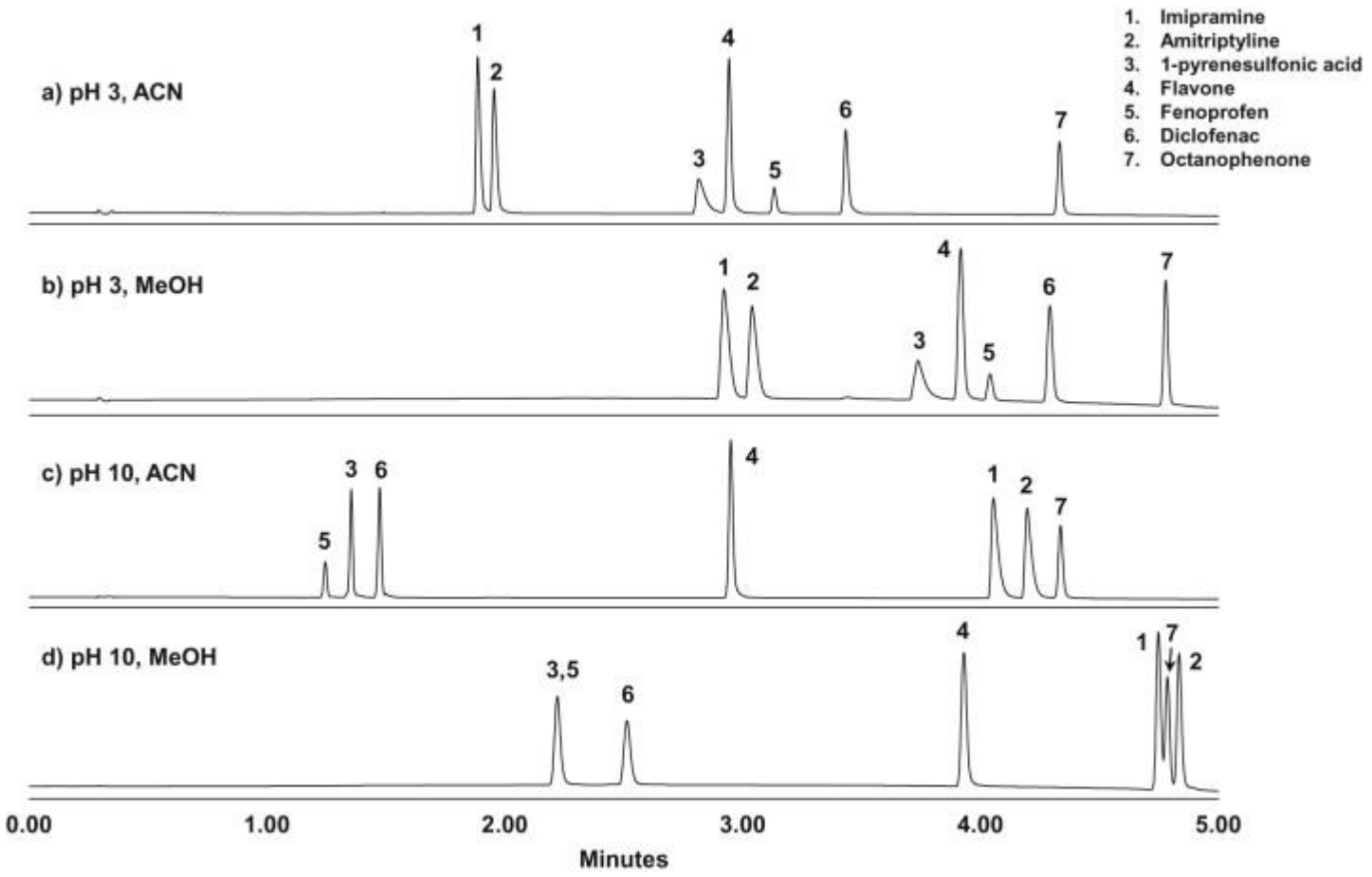
rychlost

rozlišení

Eu. J. Pharm. Biopharm 68 (2008) 430–440

(RYCHLÉ) TESTOVÁNÍ VLIVU MOBILNÍ FÁZE

Gradient 5 – 95%, kolona C18, 50 × 2.1 mm, 1.7 µm



TrAC Trends in Analytical Chemistry 63 (2014) 21–30. & www.waters.com

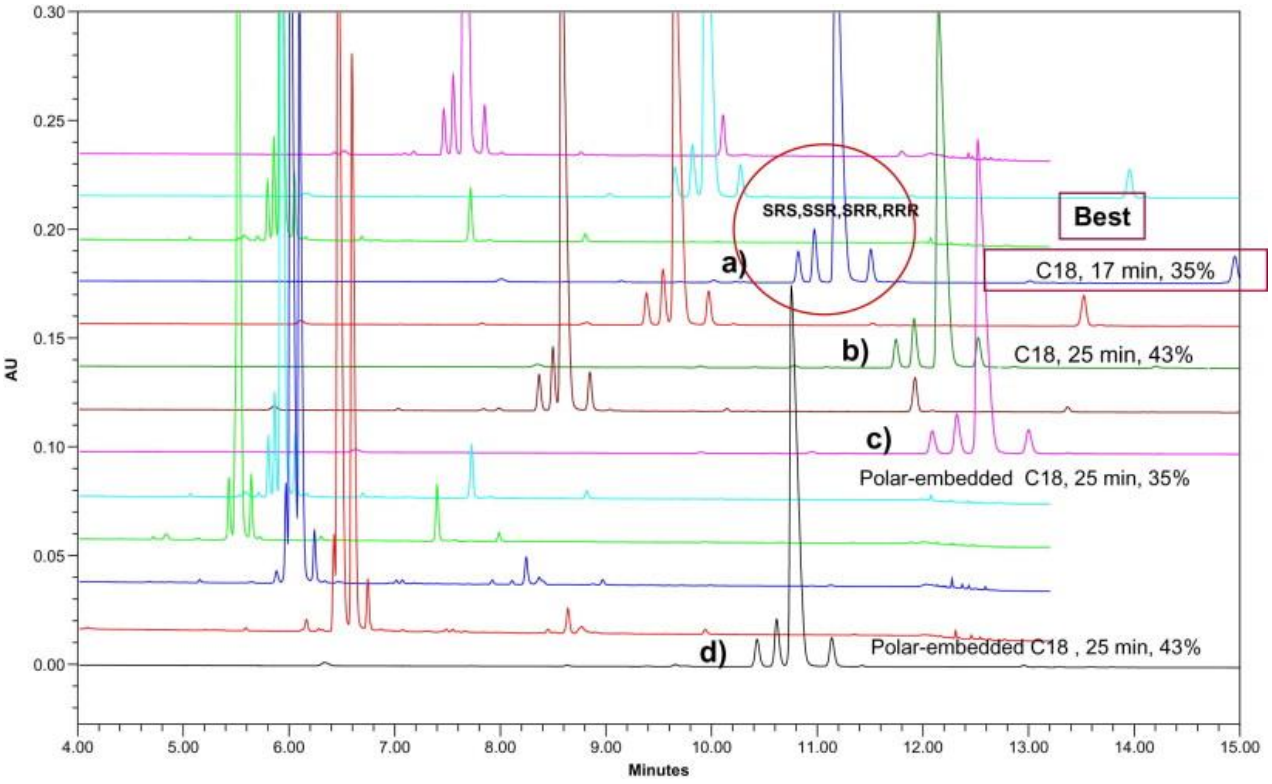
„AUTOMATICKÝ“ VÝVOJ METODY

Matematické plánování (DoE)

Chirální sloučenina
s třemi centry (SRR)

30 experimentů

- Čas gradientu
- Finální koncentrace
- Kolona
  - C18
  - C18 s polárními skupinami



Redukce času analýzy ze 42 na 17 min

TrAC Trends in Analytical Chemistry 63 (2014) 21–30.

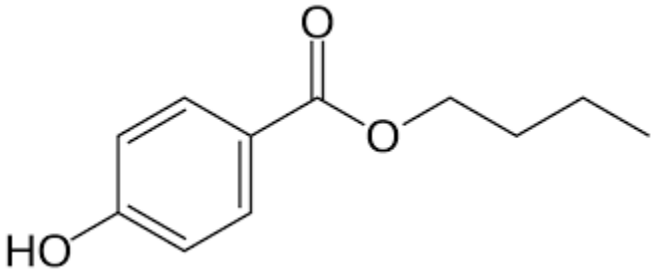
CO JE NEJLEPŠÍ?

Strategie	Kolona	Velikost částic, µm	Teplota, °C	Δp max, MPa
HPLC	Waters Xbridge C_{18}, 50 × 2.1 mm	5	30	40
HPLC, 1000 bar	Waters Xbridge C_{18}, 50 × 2.1 mm	5	30	100
HTLC	Waters Xbridge C_{18}, 50 × 2.1 mm	5	90	40
Sub-2 µm	Waters Acquity BEH C_{18}, 50 × 2.1 mm	1.7	30	40
UHPLC	Waters Acquity BEH C_{18}, 50 × 2.1 mm	1.7	30	100
HT-UHPLC	Waters Acquity BEH C_{18}, 50 × 2.1 mm	1.7	90	100
Fused-core	Supelco Ascentis C_{18}, 50 × 2.1 mm	2.7 Core-shell	30	60
Monolith	Phenomenex Onyx C_{18}, 50 × 2.1 mm	–	30	20

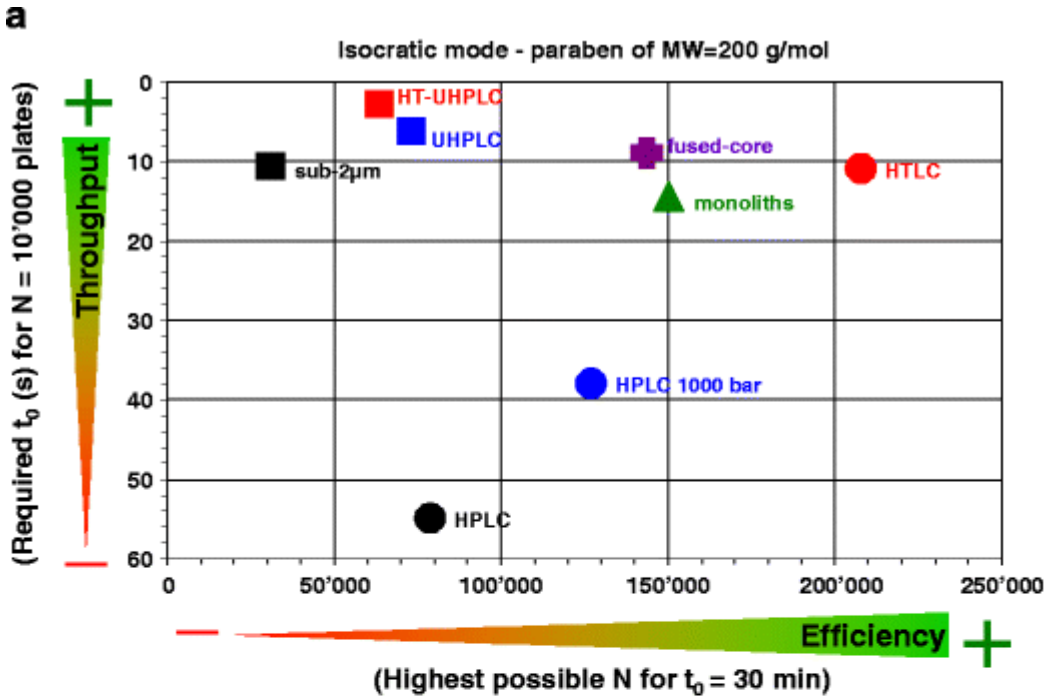
Analytical and Bioanalytical Chemistry 397 (2010) 1069–1082.

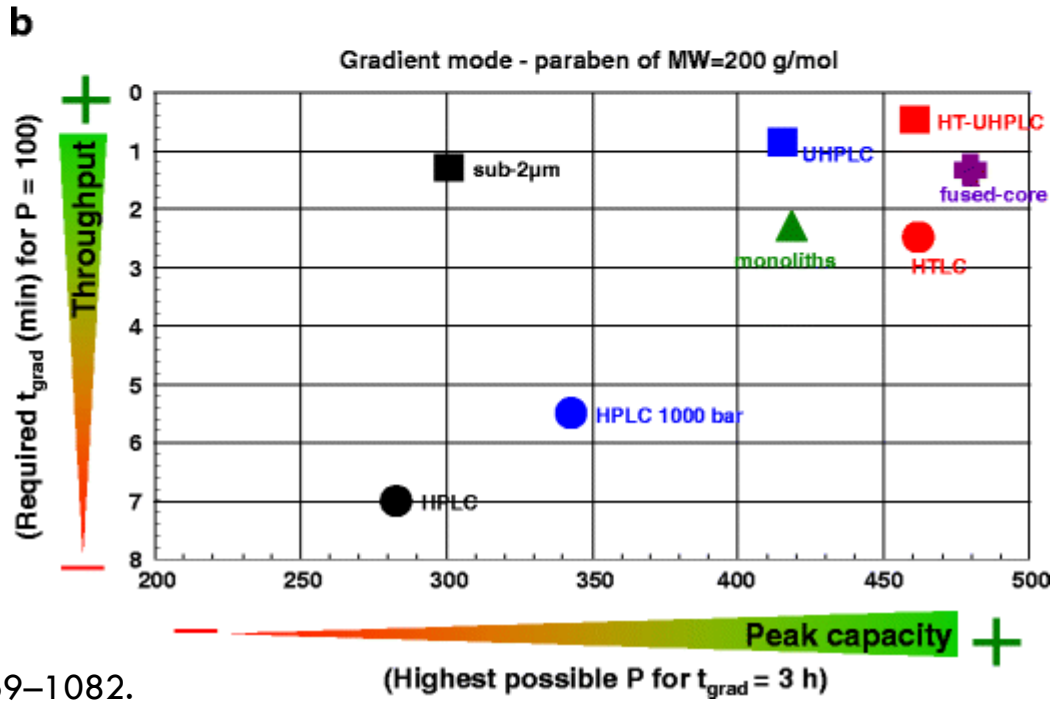
CO JE NEJLEPŠÍ?

butylparaben



200 g/mol

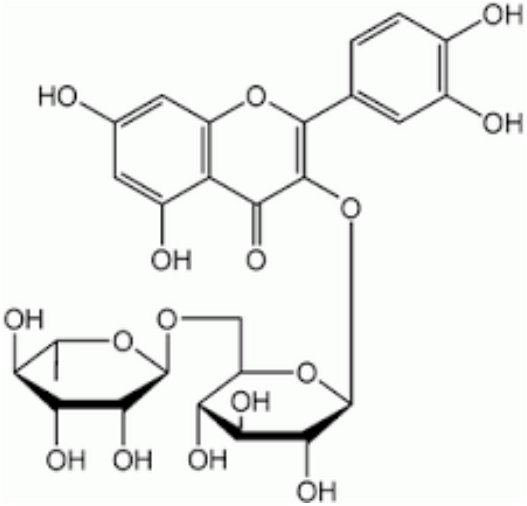




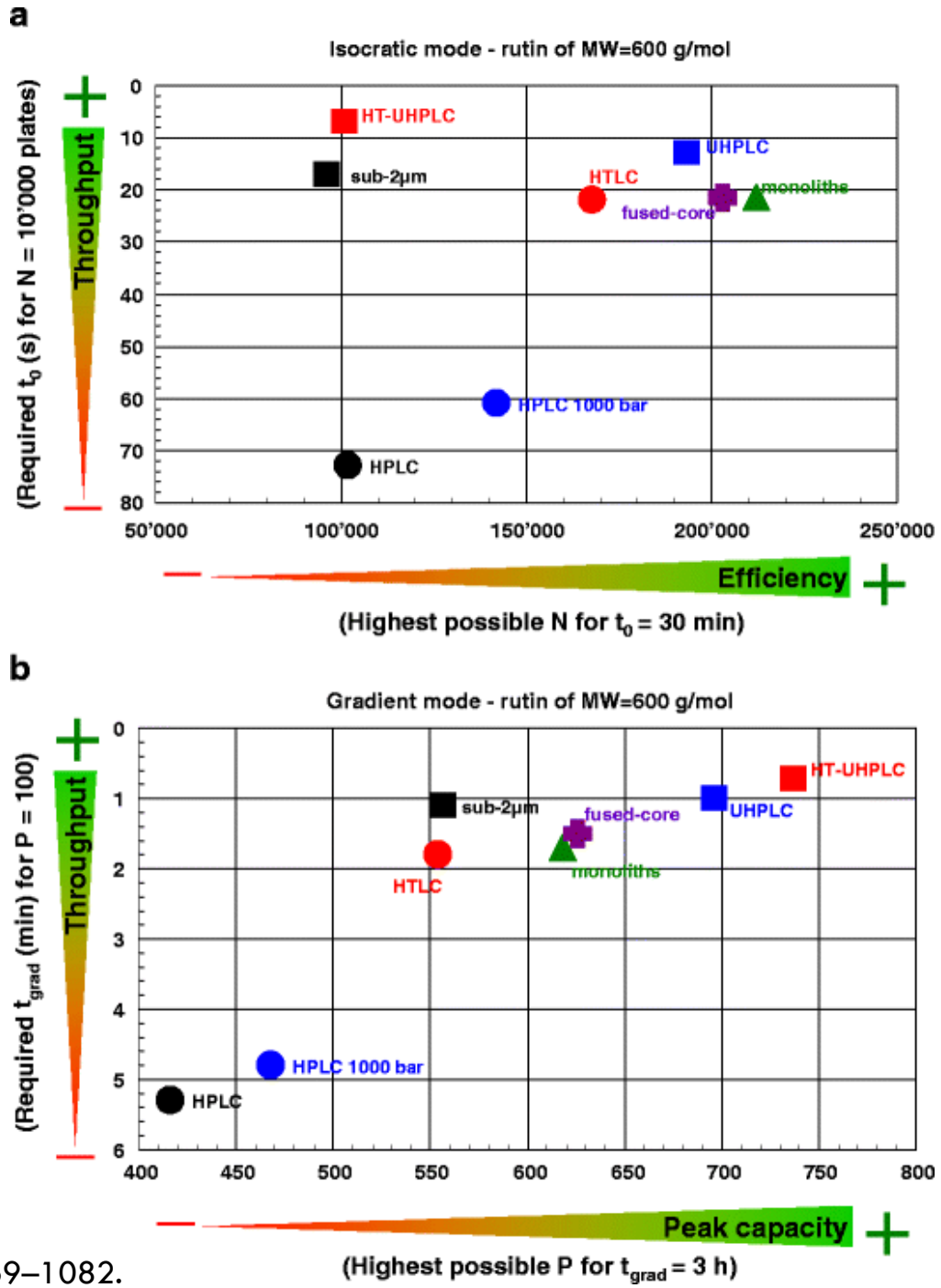
Analytical and Bioanalytical Chemistry 397 (2010) 1069–1082.

CO JE NEJLEPŠÍ?

rutin



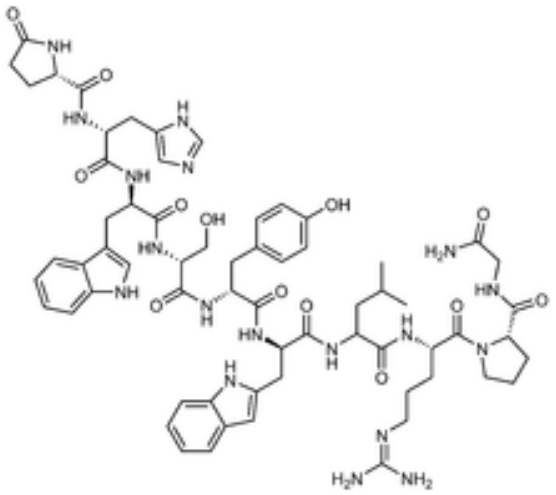
600 g/mol



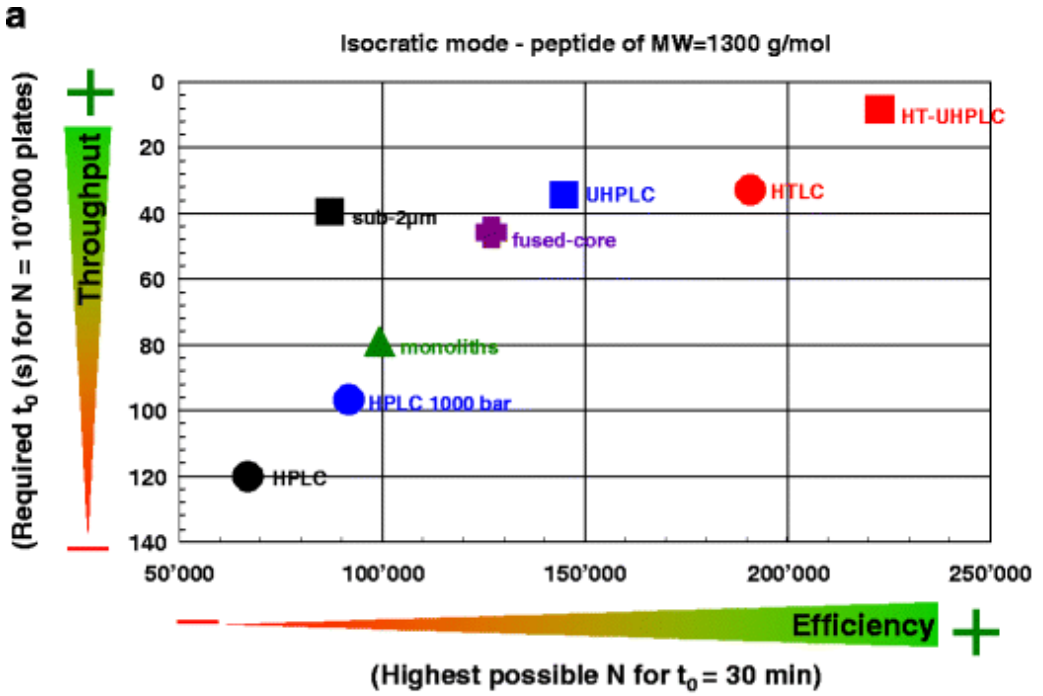
Analytical and Bioanalytical Chemistry 397 (2010) 1069–1082.

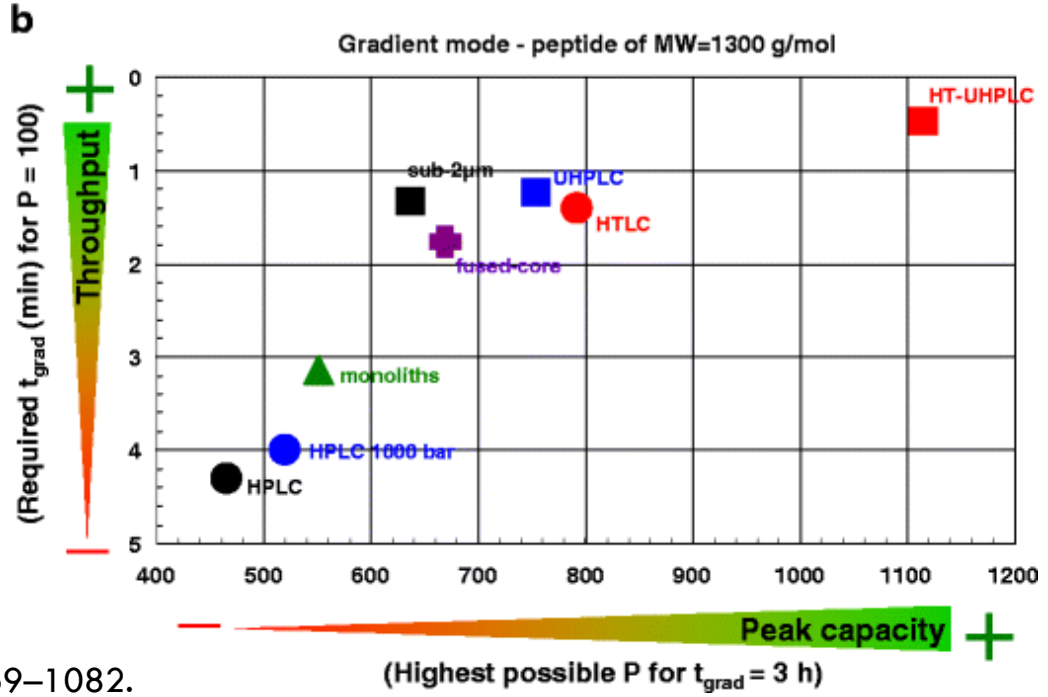
CO JE NEJLEPŠÍ?

triptorelin



1300 g/mol





Analytical and Bioanalytical Chemistry 397 (2010) 1069–1082.

“No pressure, no diamonds.“

Thomas Carlyle