

ULTRAVYSOKOTLAKÁ CHROMATOGRRAFIE

Pokročilá kapalinová
chromatografie



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

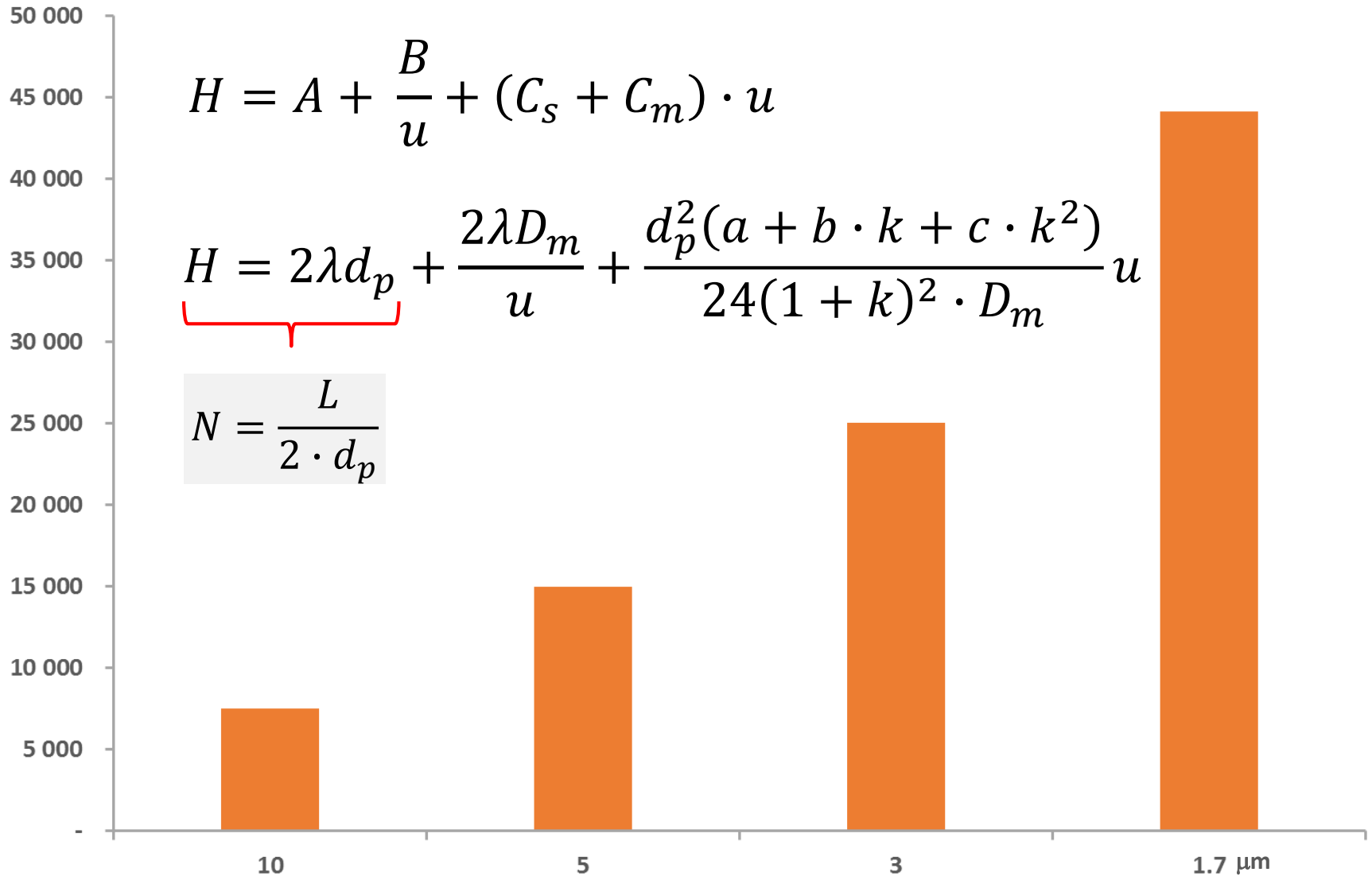
$$H = A + \frac{B}{u} + (C_s + C_m) \cdot u$$

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

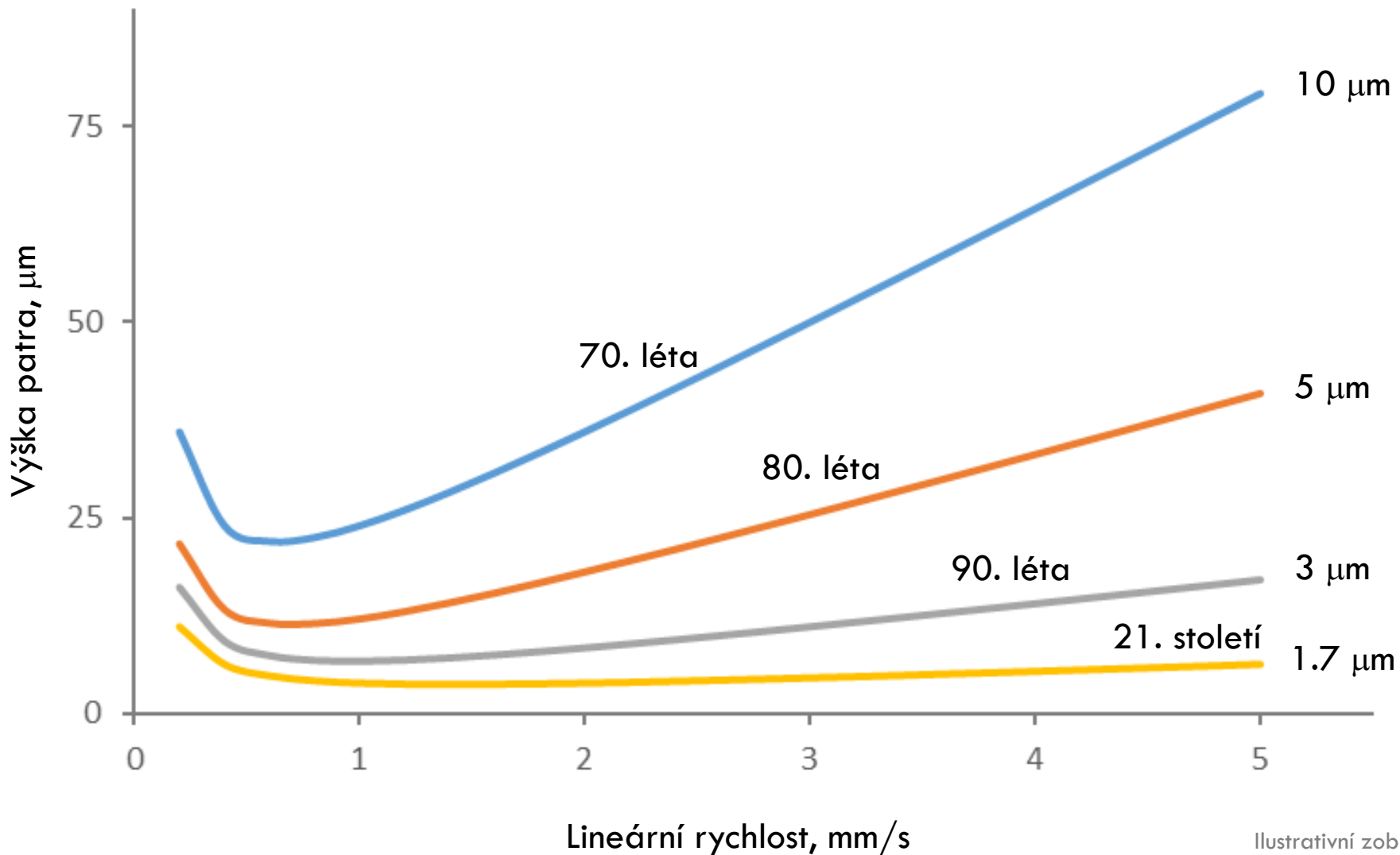
$$N = \frac{L}{2 \cdot d_p}$$

VELIKOST ČÁSTIC A ÚČINNOST

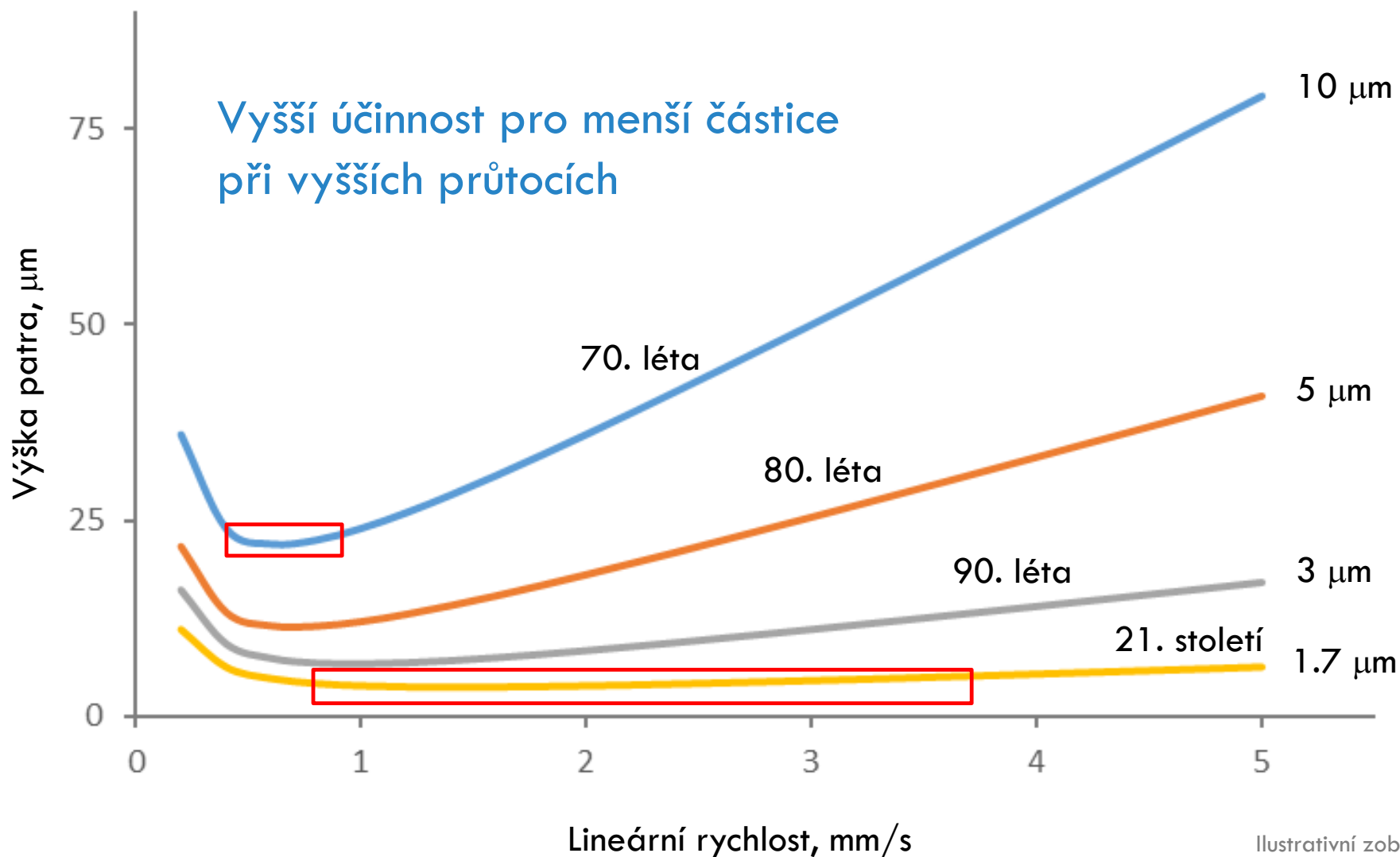
↓ $d_p \approx \uparrow N$



VELIKOST ČÁSTIC A ÚČINNOST

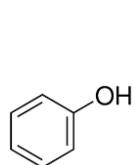


VELIKOST ČÁSTIC A ÚČINNOST

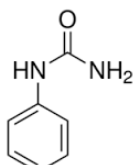


VLIV VELIKOSTI ČÁSTIC NA SEPARACI

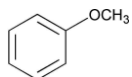
Testovací směs



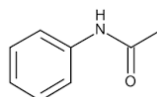
fenol



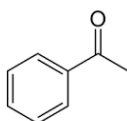
fenylurea



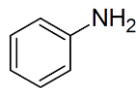
metoxybenzen



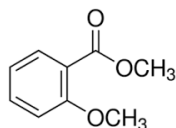
acetanilid



methylphenylketon



aniline



methylmethoxybenzoát

| Sloučenina | k |
|----------------------|-------|
| Fenol | 1.35 |
| Fenylmočovina | 3.06 |
| Metoxybenzen | 3.63 |
| Acetanilid | 5.48 |
| Methylphenylketon | 6.19 |
| Anilin | 9.60 |
| Methylmethoxybenzoát | 10.95 |

Kolona

150 x 4.6 mm, C18

Velikost částic

10 μm



5 μm



3 μm




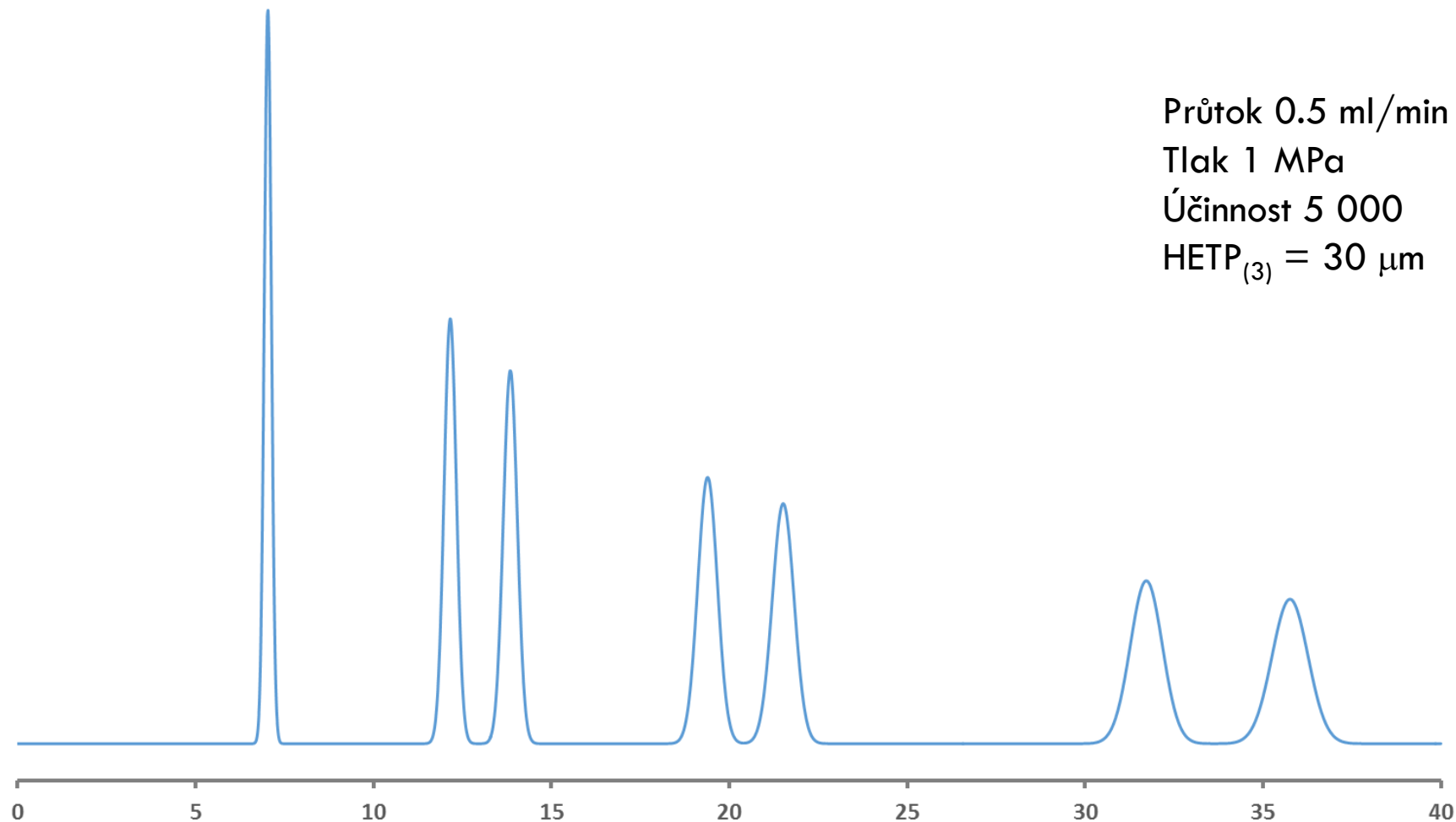
1.7 μm



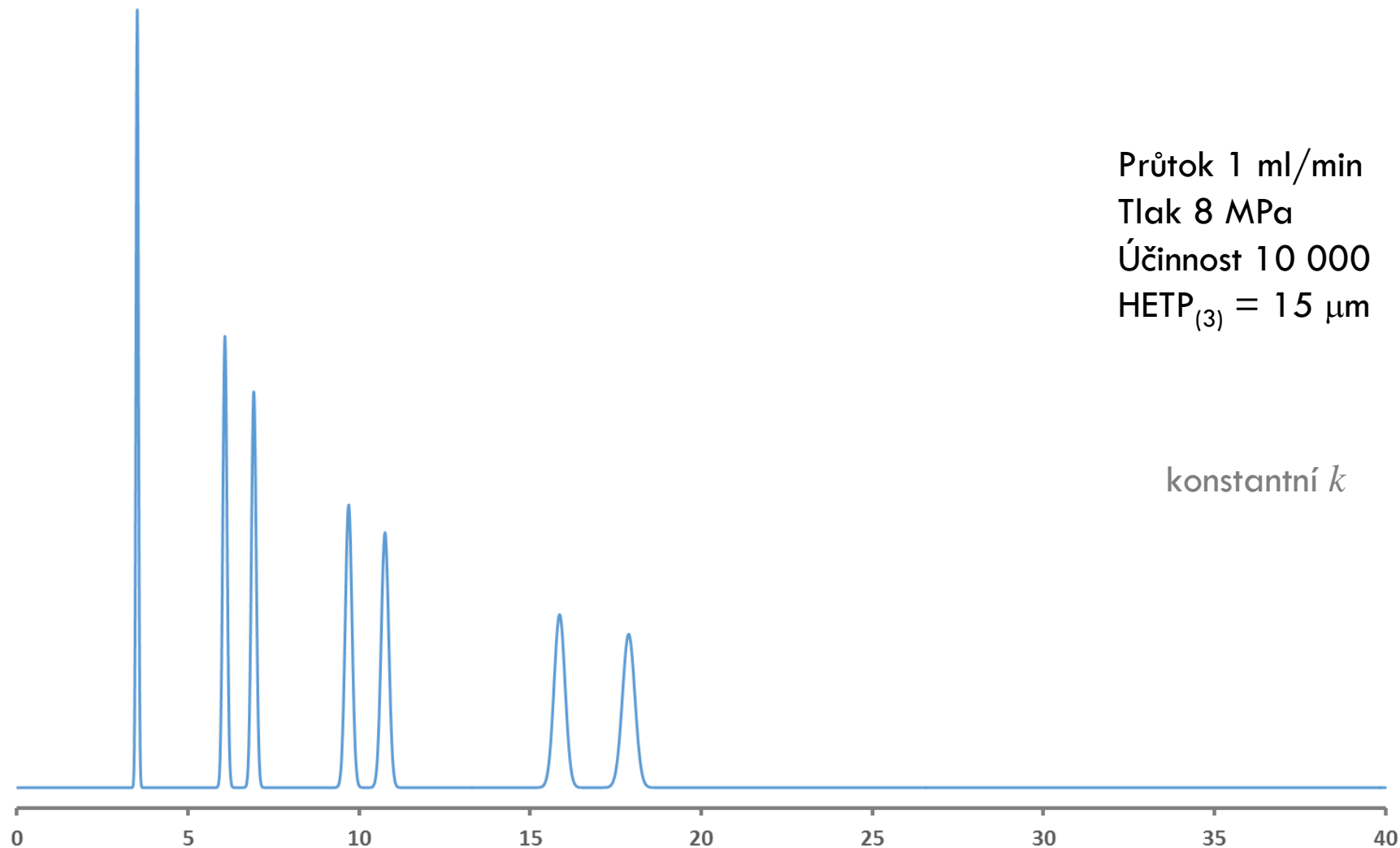
Mobilní fáze

55% Acetonitril

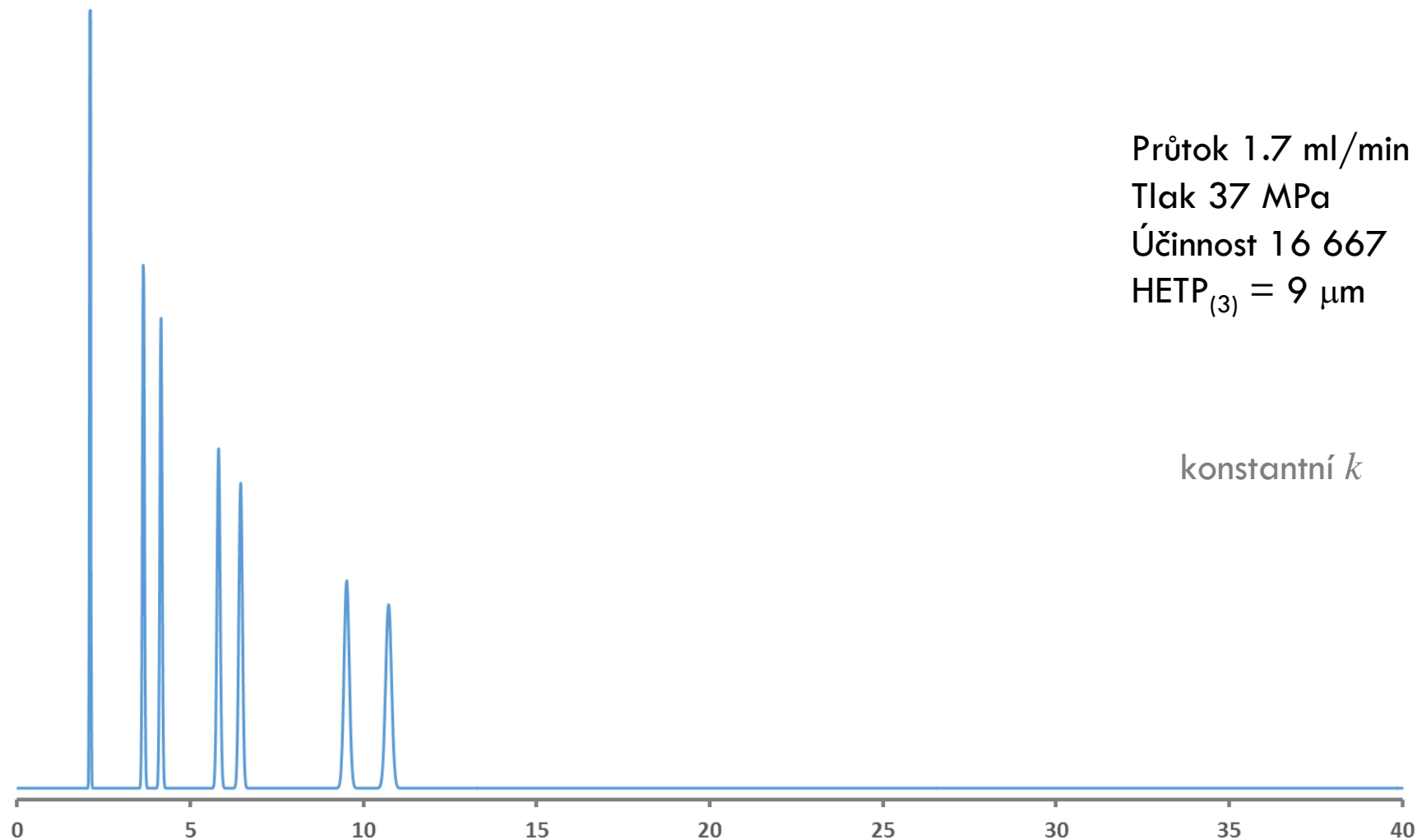
150 X 4.6, 10 μm 



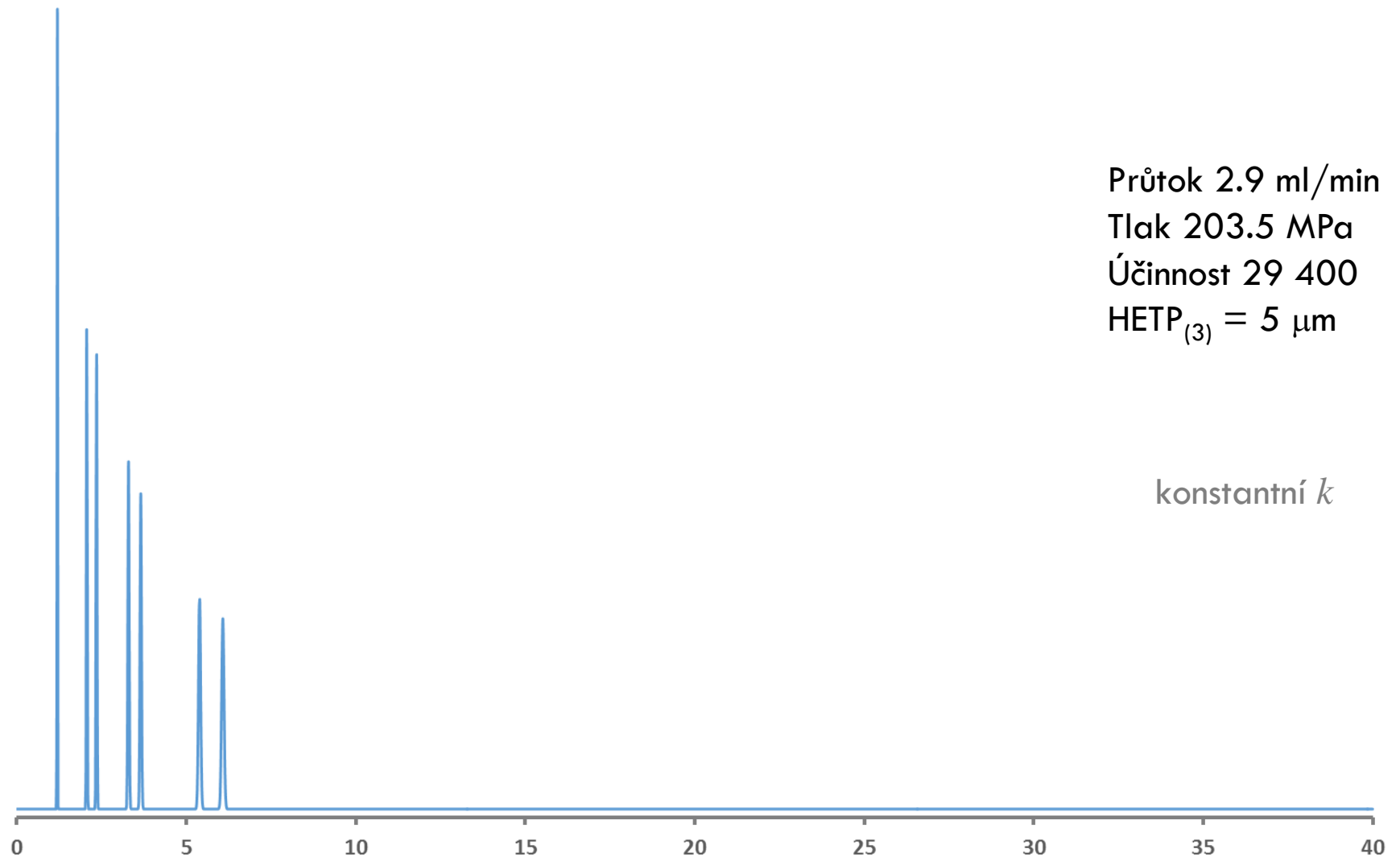
150 X 4.6, 5 μm ○



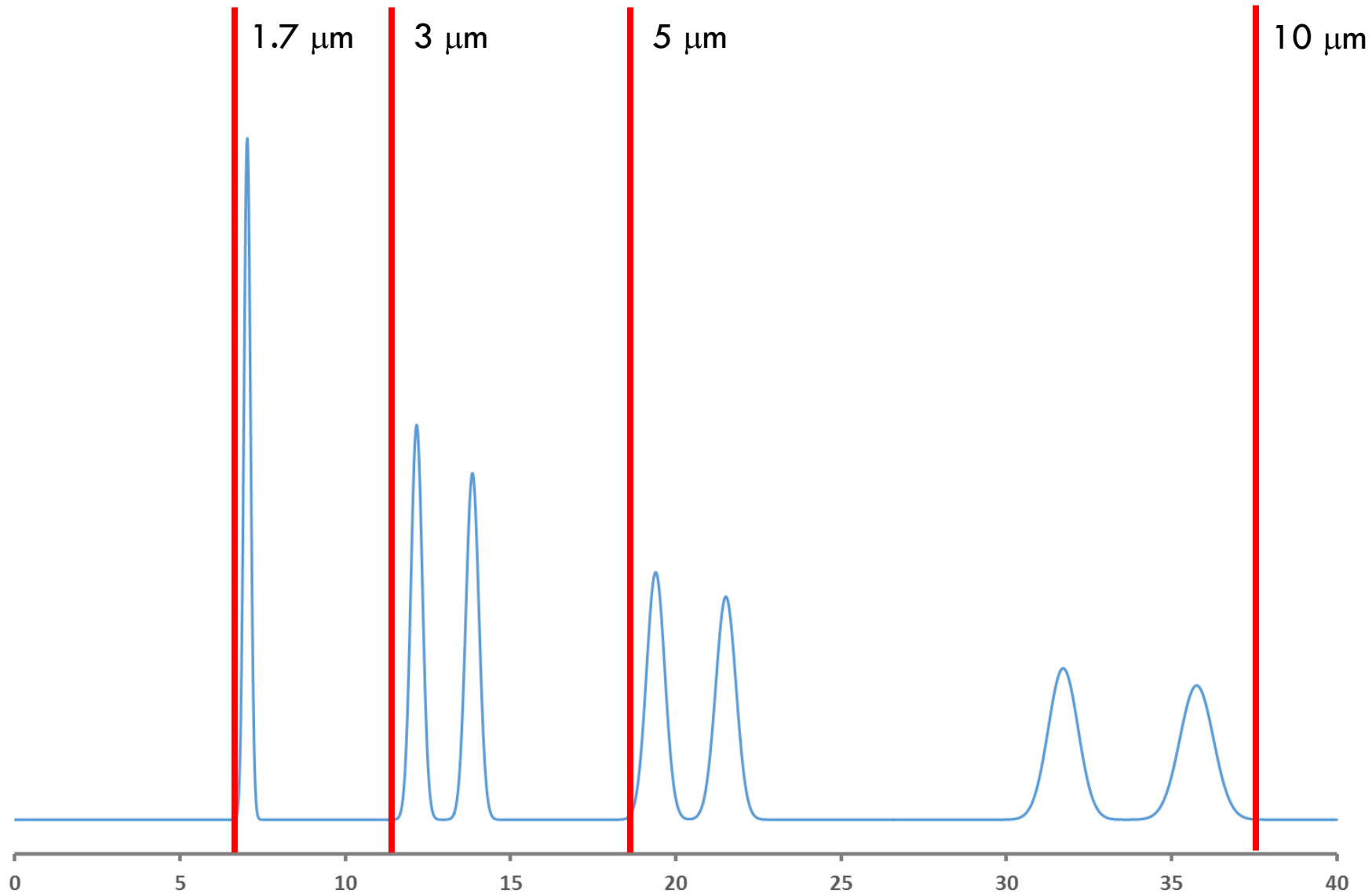
150 X 4.6, 3 μm \circ



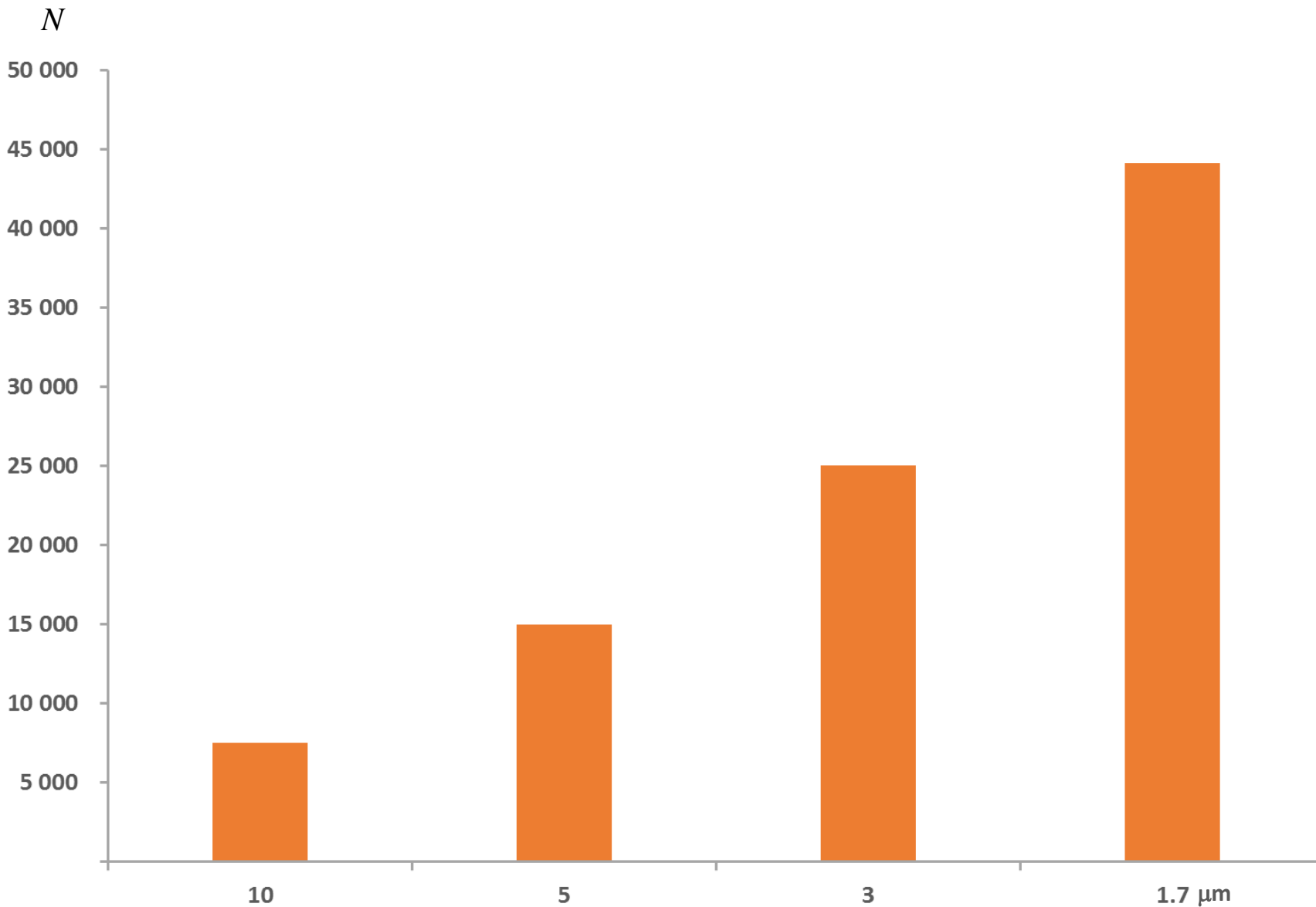
150 X 4.6, 1.7 μm



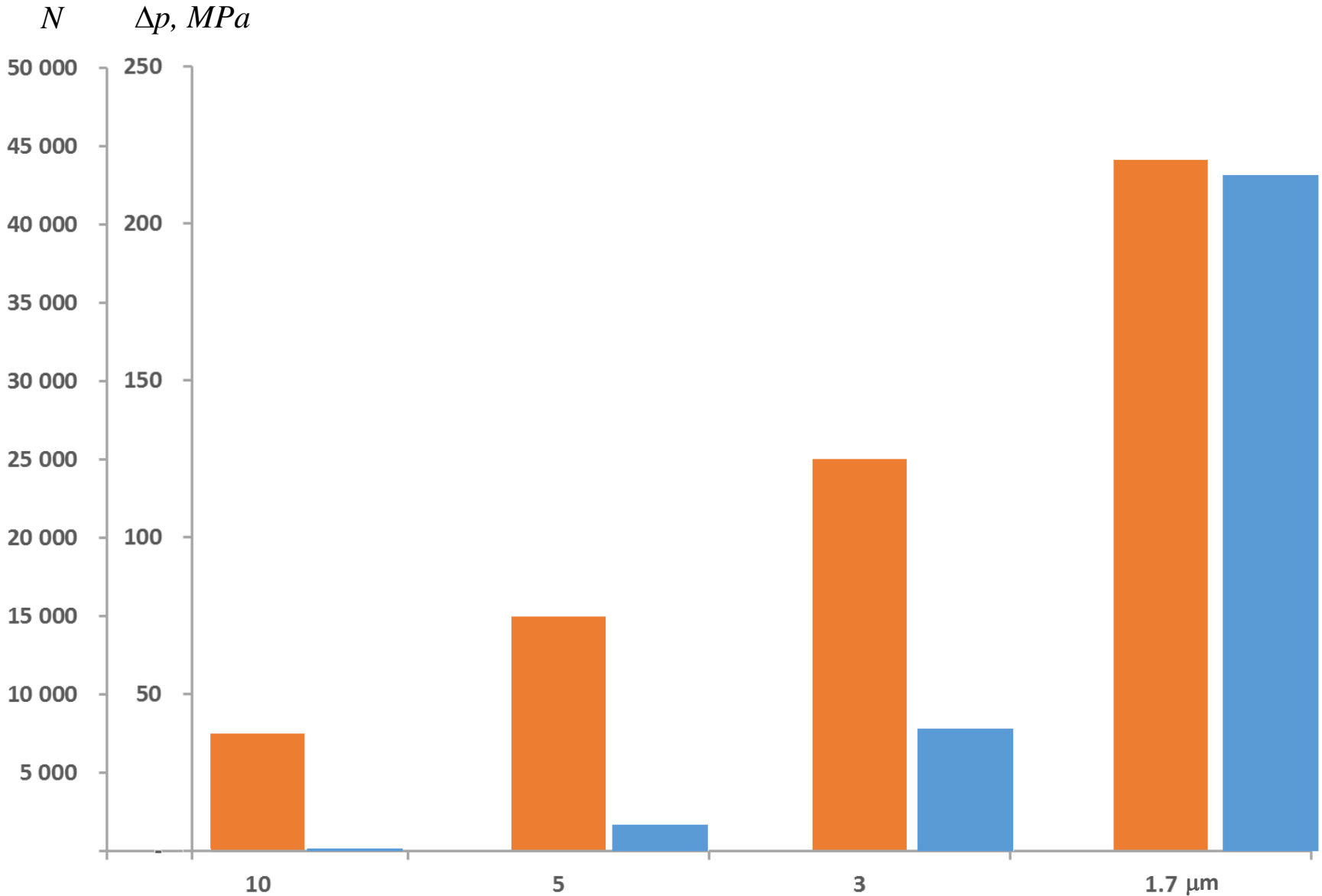
VELIKOST ČÁSTIC A RYCHLOST ANALÝZY



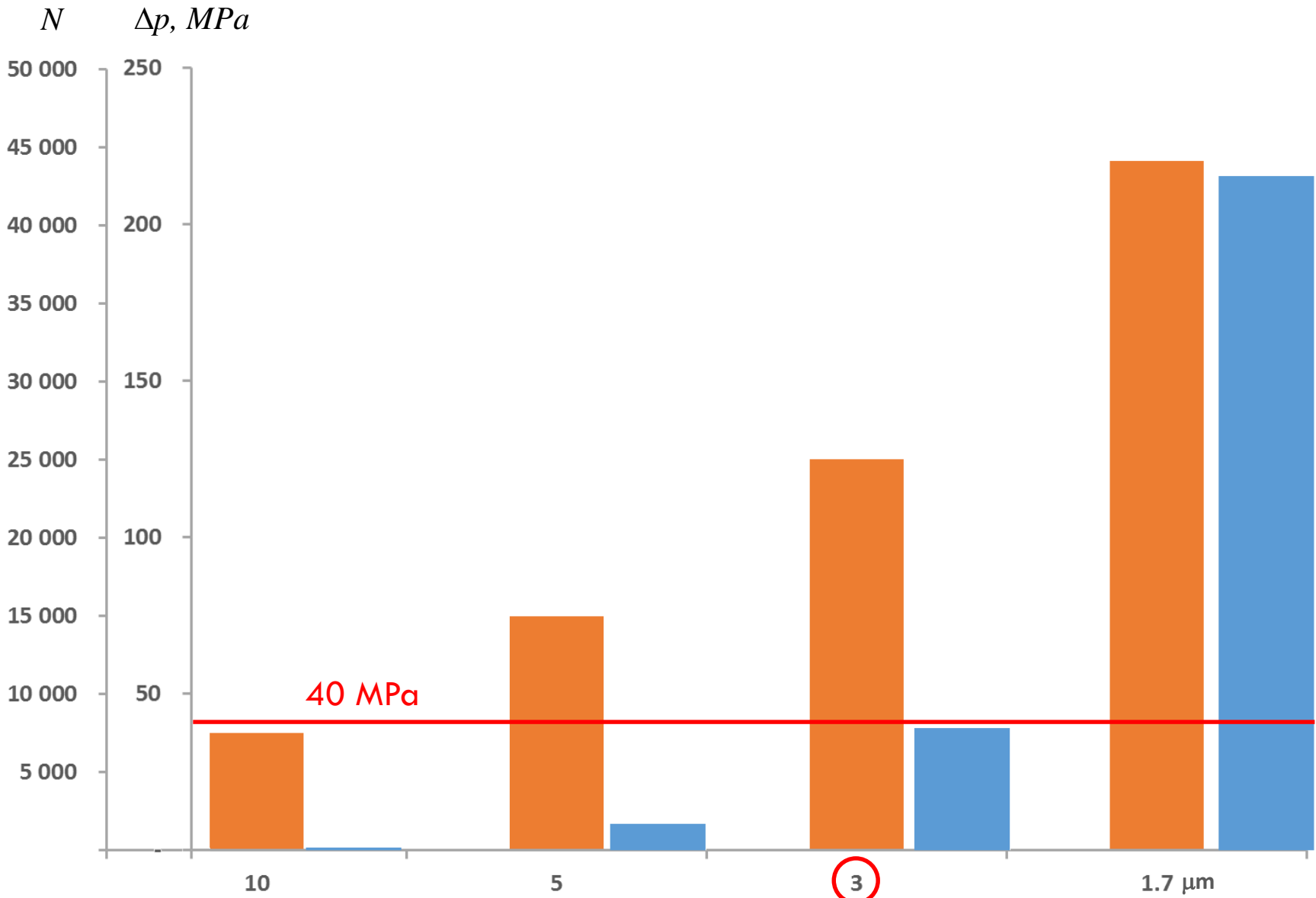
VELIKOST ČÁSTIC, ÚČINNOST A TLAK



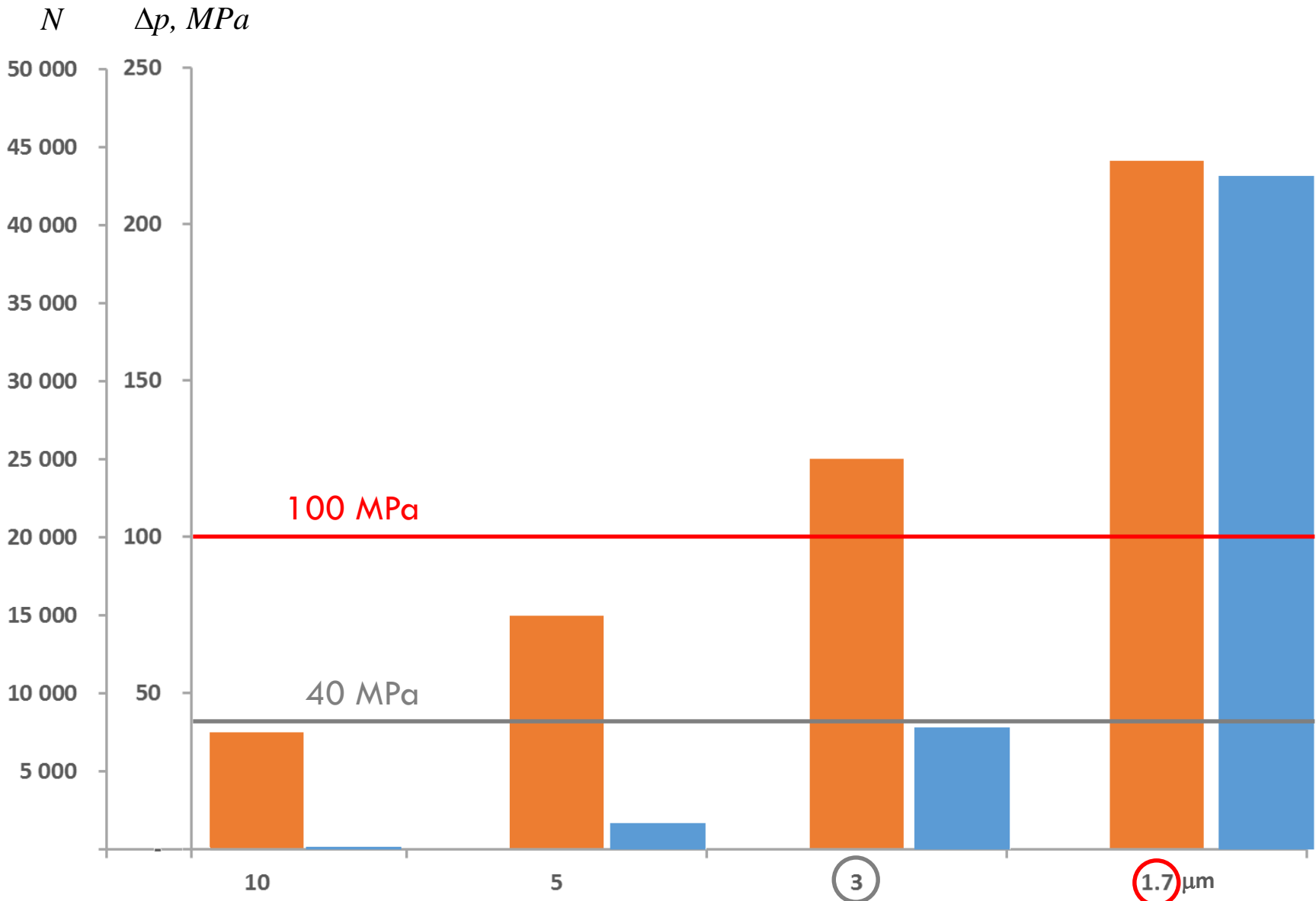
VELIKOST ČÁSTIC, ÚČINNOST A TLAK




VELIKOST ČÁSTIC, ÚČINNOST A TLAK



VELIKOST ČÁSTIC, ÚČINNOST A TLAK



VLIV VELIKOSTI ČÁSTIC



| $d_p, \mu\text{m}$ | $F_{m'}, \text{ml/min}$ | N | p, MPa | $t_{\text{ana}'}, \text{min}$ | $R_{3,2}$ |
|--------------------|-------------------------|--------|-----------------|-------------------------------|-----------|
| 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

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

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

VLIV VELIKOSTI ČÁSTIC



| $d_p, \mu\text{m}$ | $F_{m'}, \text{ml/min}$ | N | p, MPa | $t_{\text{ana}'}, \text{min}$ | $R_{3,2}$ |
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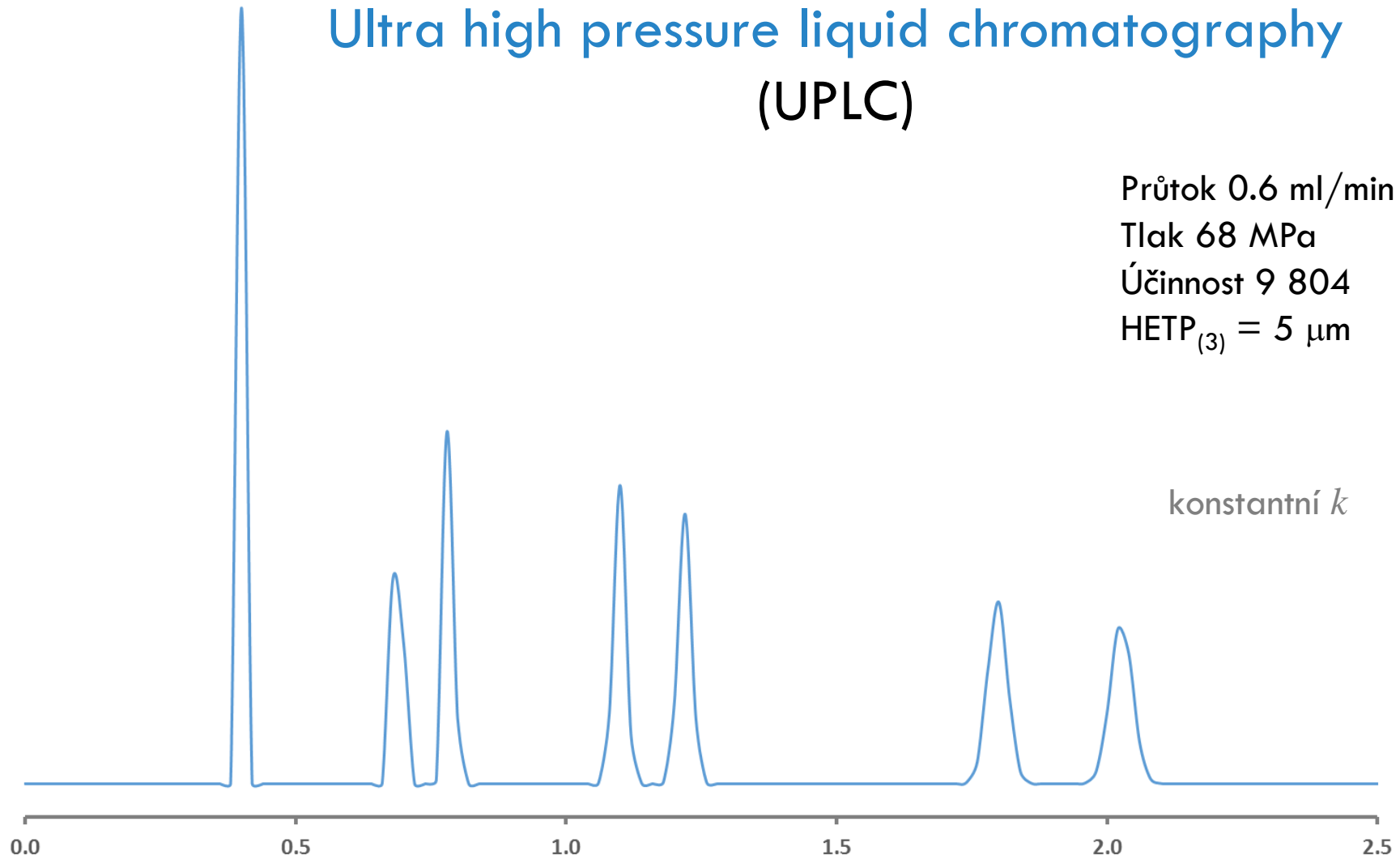
Při použití 1 μm částic bude pracovní tlak
125x větší než v případě 5 μm částic

Nutnost použití kolon s menšími rozměry

50 X 2.1, 1.7 μm

Ultra high pressure liquid chromatography (UPLC)

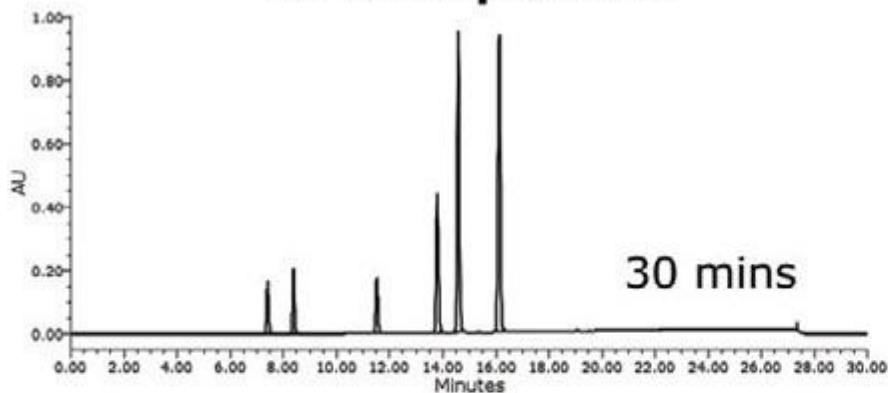
Průtok 0.6 ml/min
Tlak 68 MPa
Účinnost 9 804
HETP₍₃₎ = 5 μm



konstantní *k*

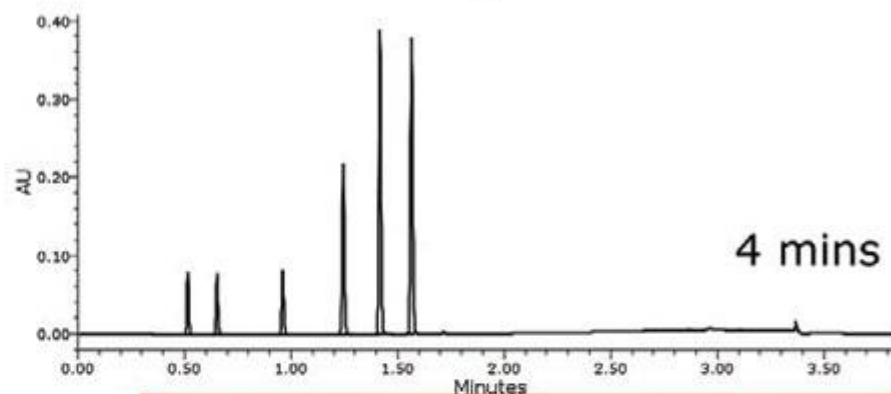
HPLC vs. UPLC

HPLC Separation



Typical Column Volume = 2.49 mL

UPLC Separation

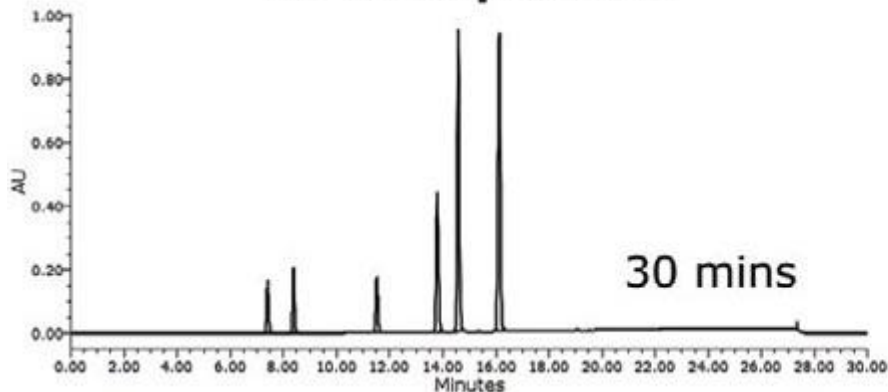


Typical Column Volume = 0.17 mL

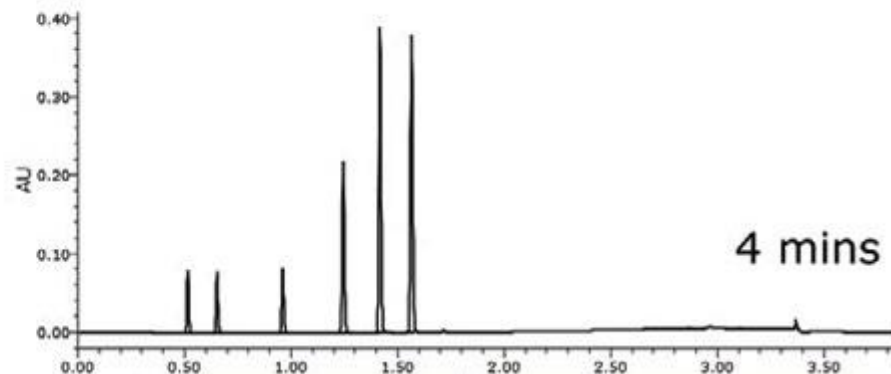
| HPLC | | UPLC Reduction | UPLC | |
|------------------|------------|----------------|------------------|-------------|
| Run Time | 30 min. | 87% | Run Time | 3.86 min. |
| Solvent Consumed | 30 mL | 83% | Solvent Consumed | 4.97 mL |
| Sample Consumed | 20 μ L | 93% | Sample Consumed | 1.0 μ L |

HPLC vs. UPLC

HPLC Separation



UPLC Separation



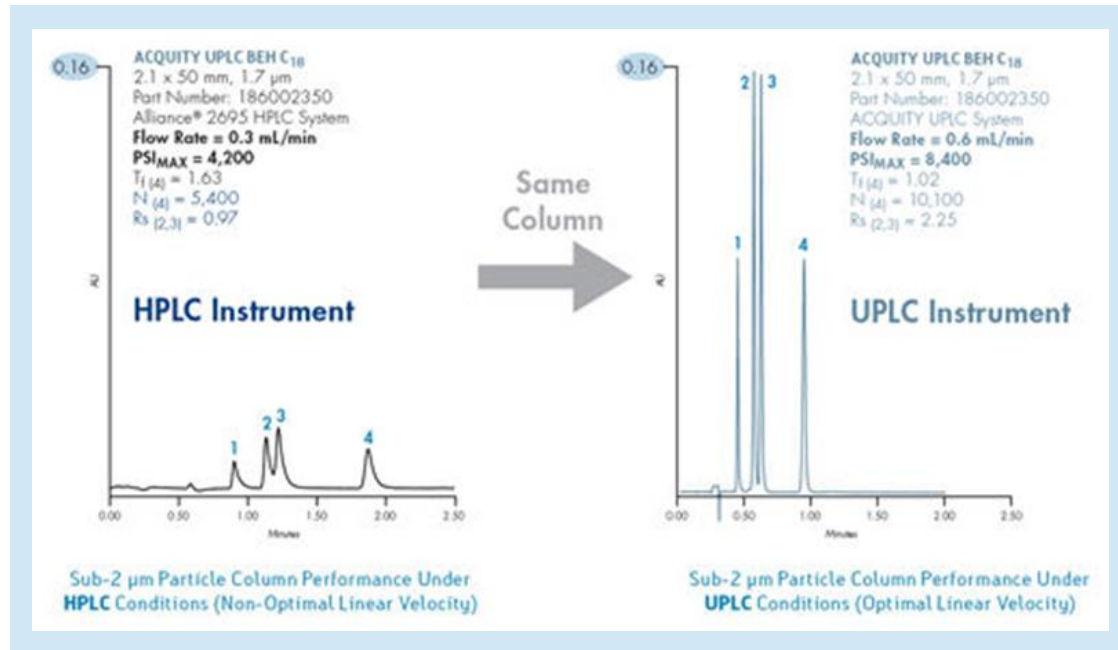
Nevýhody UPLC

- 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

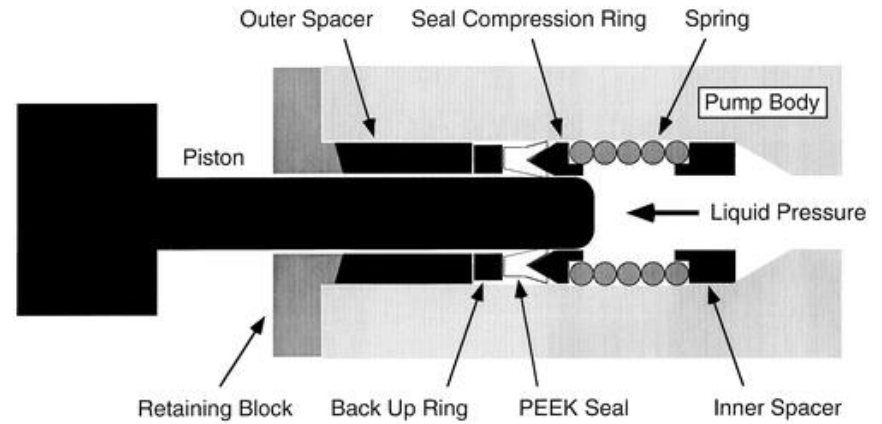
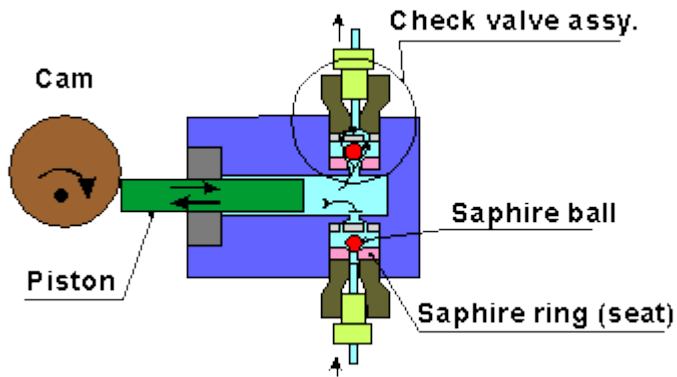
NUTNÁ OPTIMALIZACE SYSTÉMU!



Nelze pouze „koupit přístroj“ nebo „UPLC“ kolonu

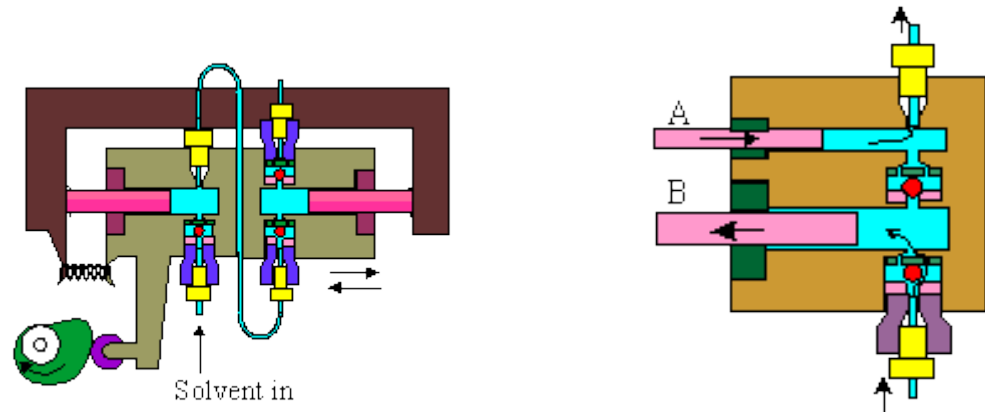
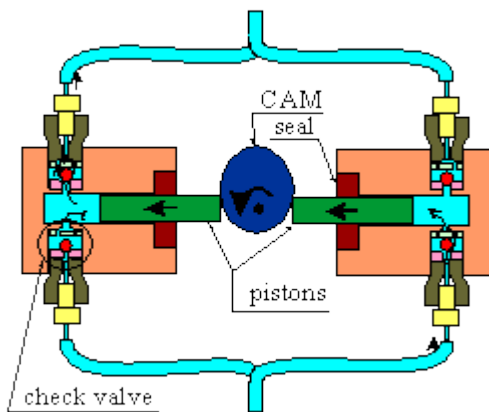
ČERPADLA

Jednopístový systém

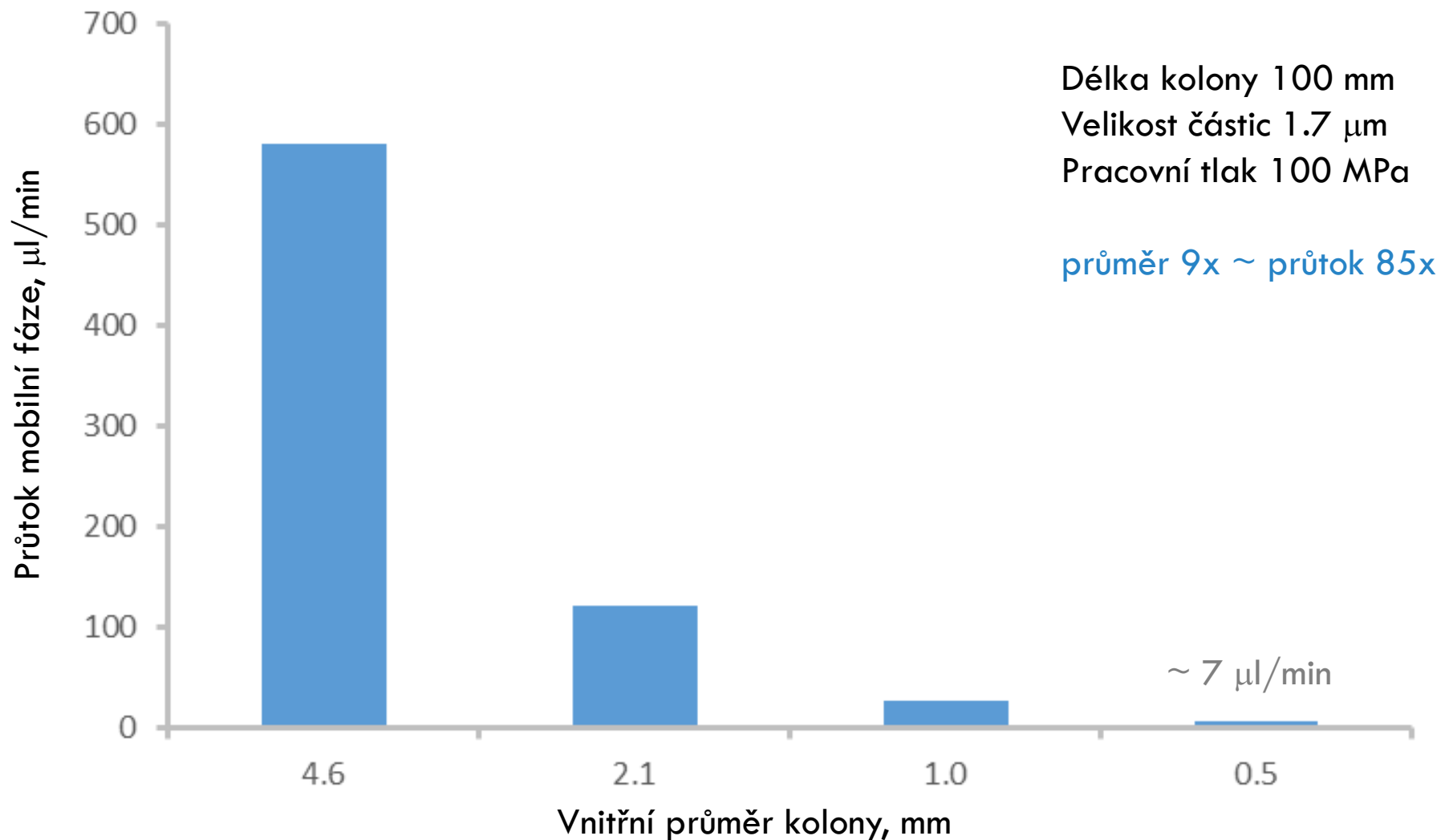


450 MPa (Anal. Chem. 69 (1997) 983–989.)

Dvoupístový systém




PRŮMĚR KOLONY A PRŮTOK MOBILNÍ FÁZE




OBJEM KOLONI


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 μ l

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 %

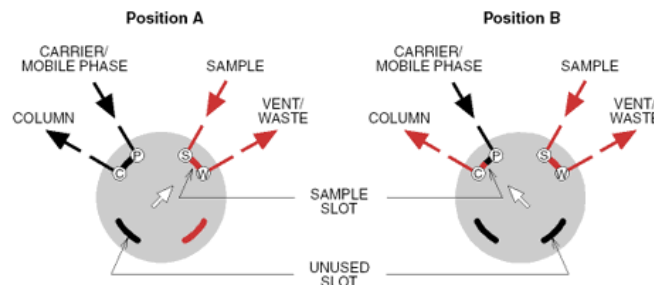
75 x 2.1 mm, $V = 0.26$ ml



50 x 2.1 mm, $V = 0.17$ ml



max 135 MPa

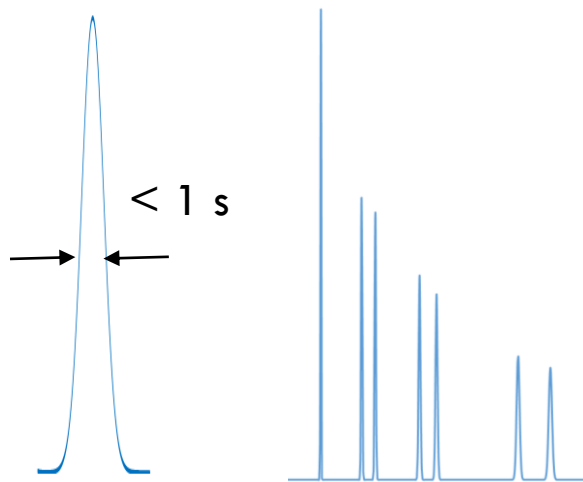


7.7 %

11.5 %

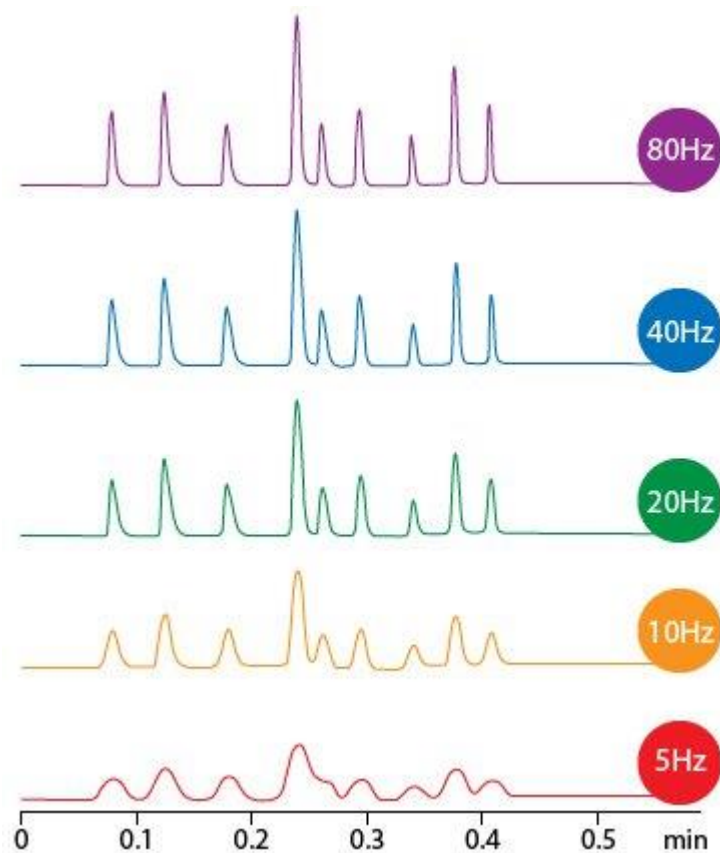
DETEKCE V UPLC

Velmi úzké píky
(malá disperze)



2 – 3x větší citlivost
(ve srovnání s HPLC)

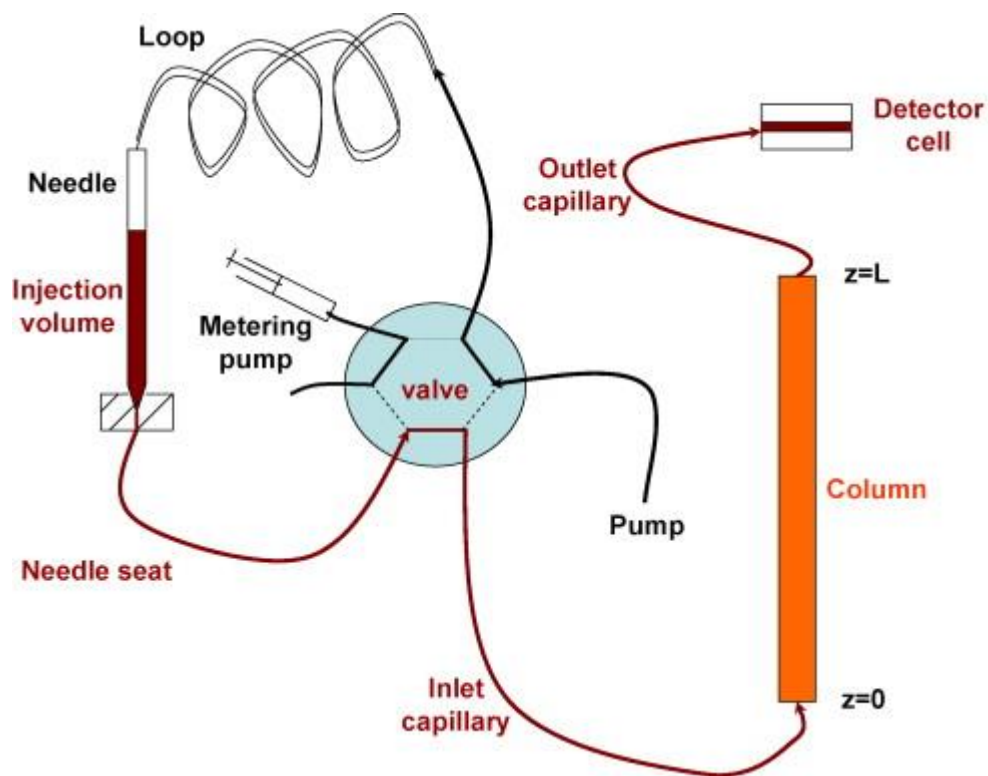
Nutné rychlé vzorkování



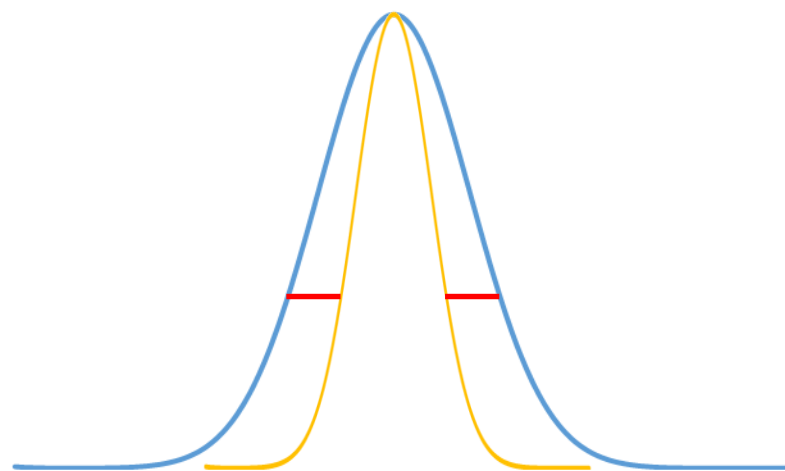
www.chromatographyonline.com/system-and-column-volumes-hplc-we-still-haven-t-got-message

MS, UV, DAD, EC, FL

MIMOKOLONOVÉ OBJEMY



Rozmytí v systému
Rozmytí na koloně
Mimokolonové objemy



$$\sigma_{celkem}^2 = \sigma_{kolona}^2 + \sigma_{ext}^2$$

MIMOKOLONOVÉ OBJEMY

Agilent 1100



Agilent 1200



Agilent 1290



Waters Acquity



Každý systém je jiný!



MIMOKOLONOVÉ OBJEMY

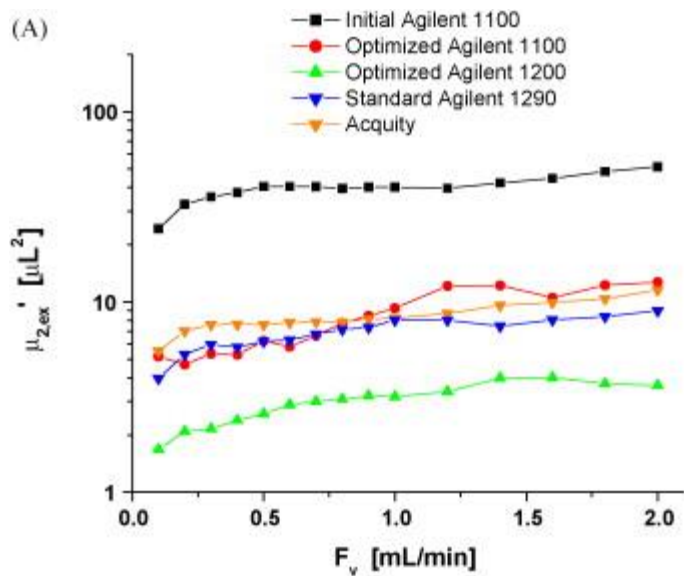
| System | Dávkoř, μl | Kapiláry, μl | Detekční cela, μl | |
|-------------------|-----------------------|-------------------------|------------------------------|----------------------|
| Agilent 1100 | 2.3 | 15.2 | 13 | = 30.5 μl |
| Agilent 1100 opt. | 1.2 | 3.8 | 1.7 | = 6.7 μl |
| Agilent 1200 opt. | 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 |

MIMOKOLONOVÉ OBJEMY

| System | Dávkoř, μl | Kapiláry, μl | Detekční cela, μl | 100% ACN, μl^2 | 65% MeOH, μl^2 | Přispěvek 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 |

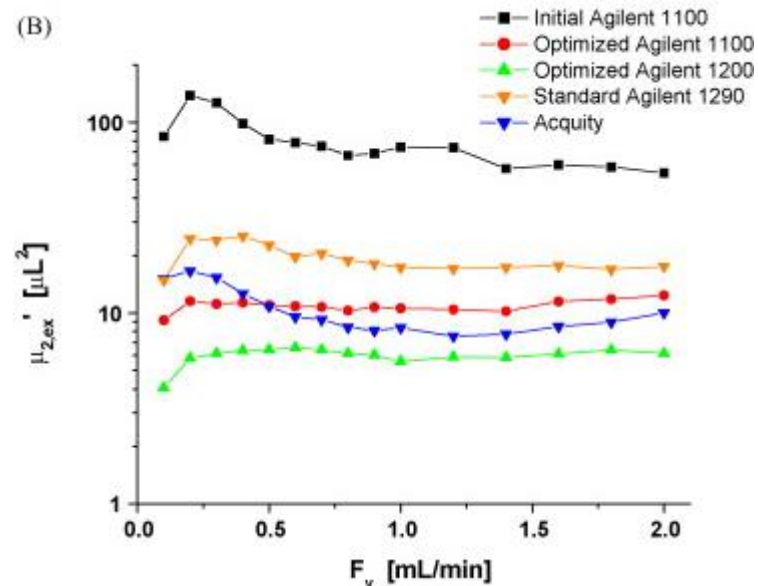
VLIV MOBILNÍ FÁZE

100% acetonitril



naphtho[2,3- α]pyren

65% metanol



4-*tert*-butylphenol

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

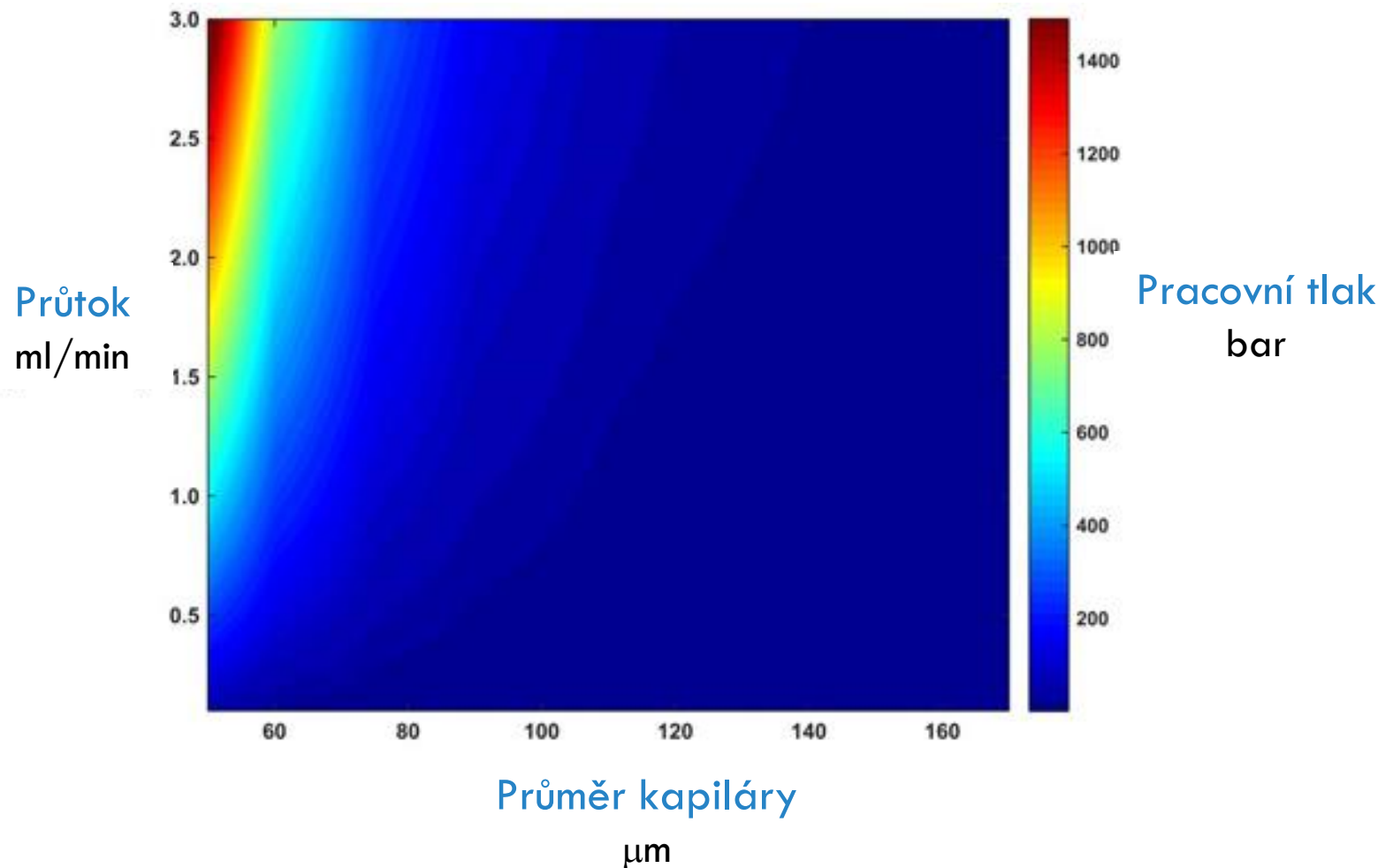
MIMOKOLONOVÉ OBJEMY

| Rozměr kolony, mm | Maximální mimokolonové objemy, μ l |
|-------------------|--|
| 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

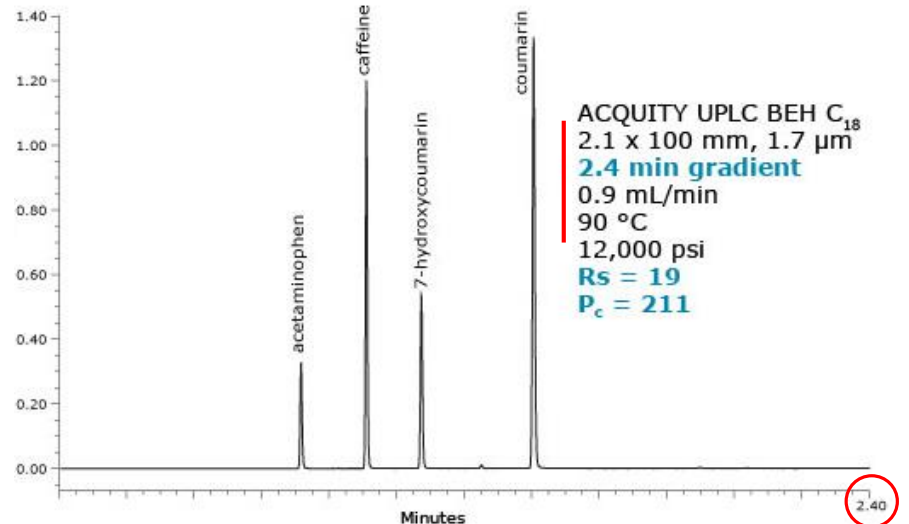
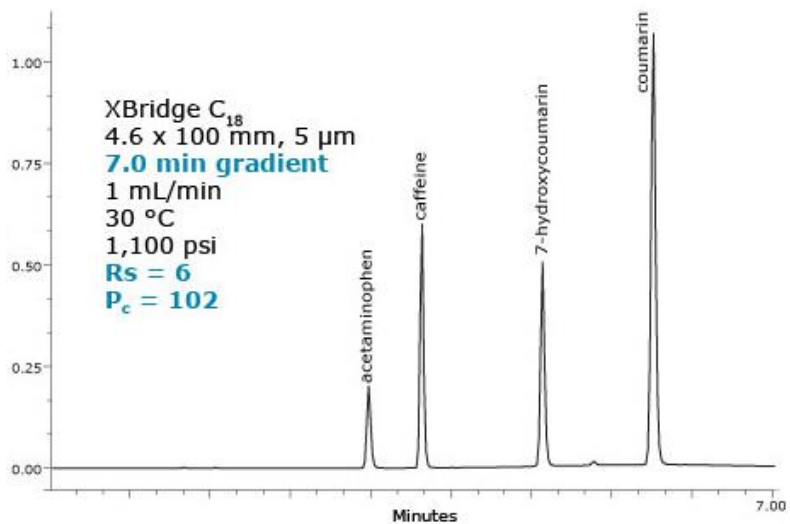
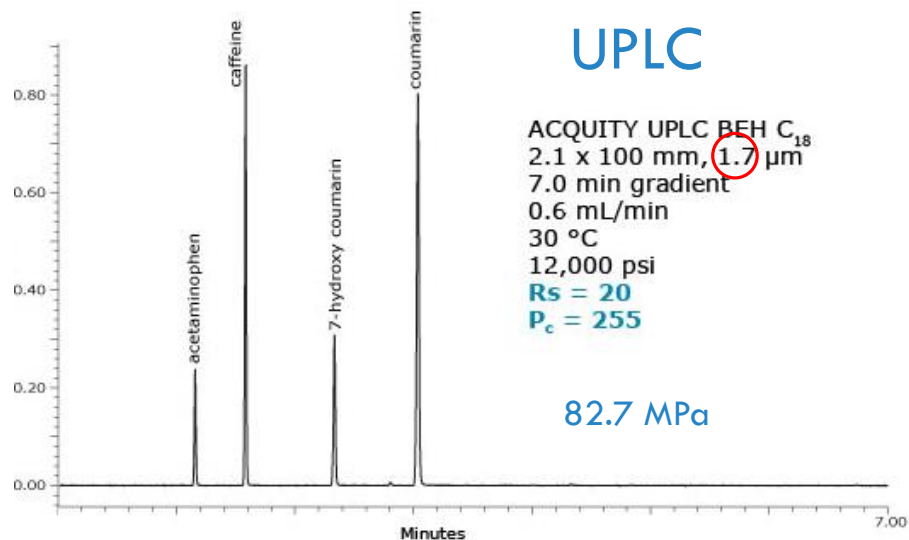
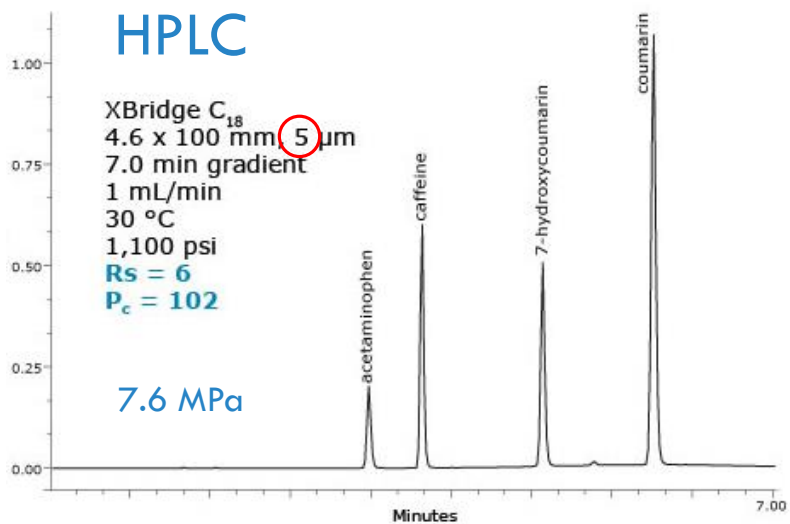
TLAK vs. PRŮMĚR KAPILÁRY



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

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

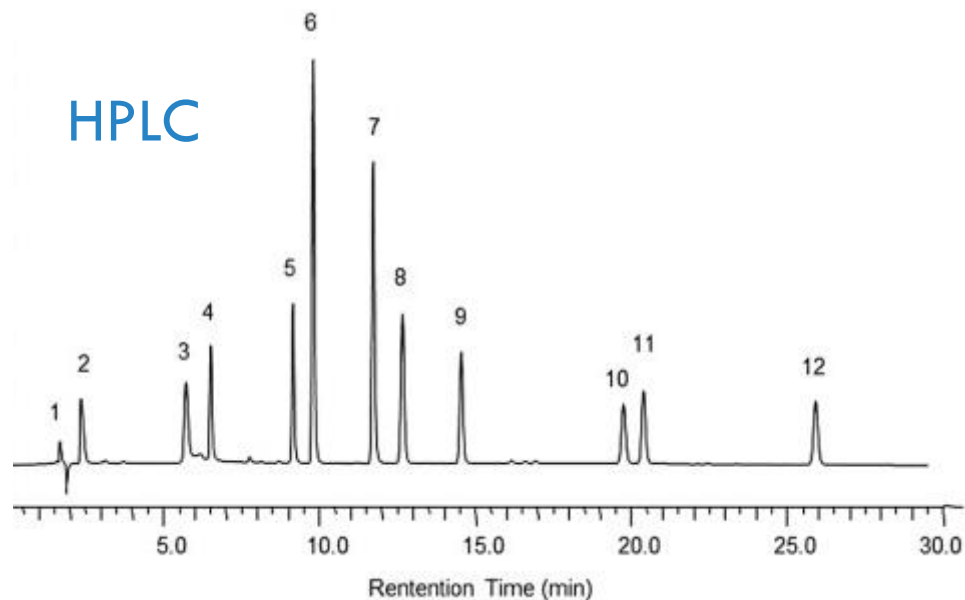
PŘEVOD METODY



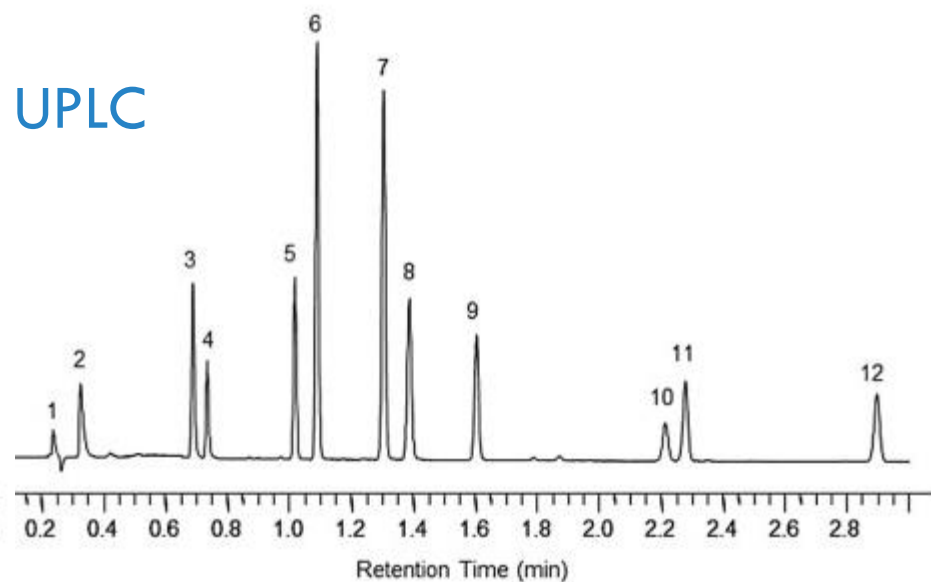
PŘEVOD METODY

Farmaceutika

HPLC



UPLC

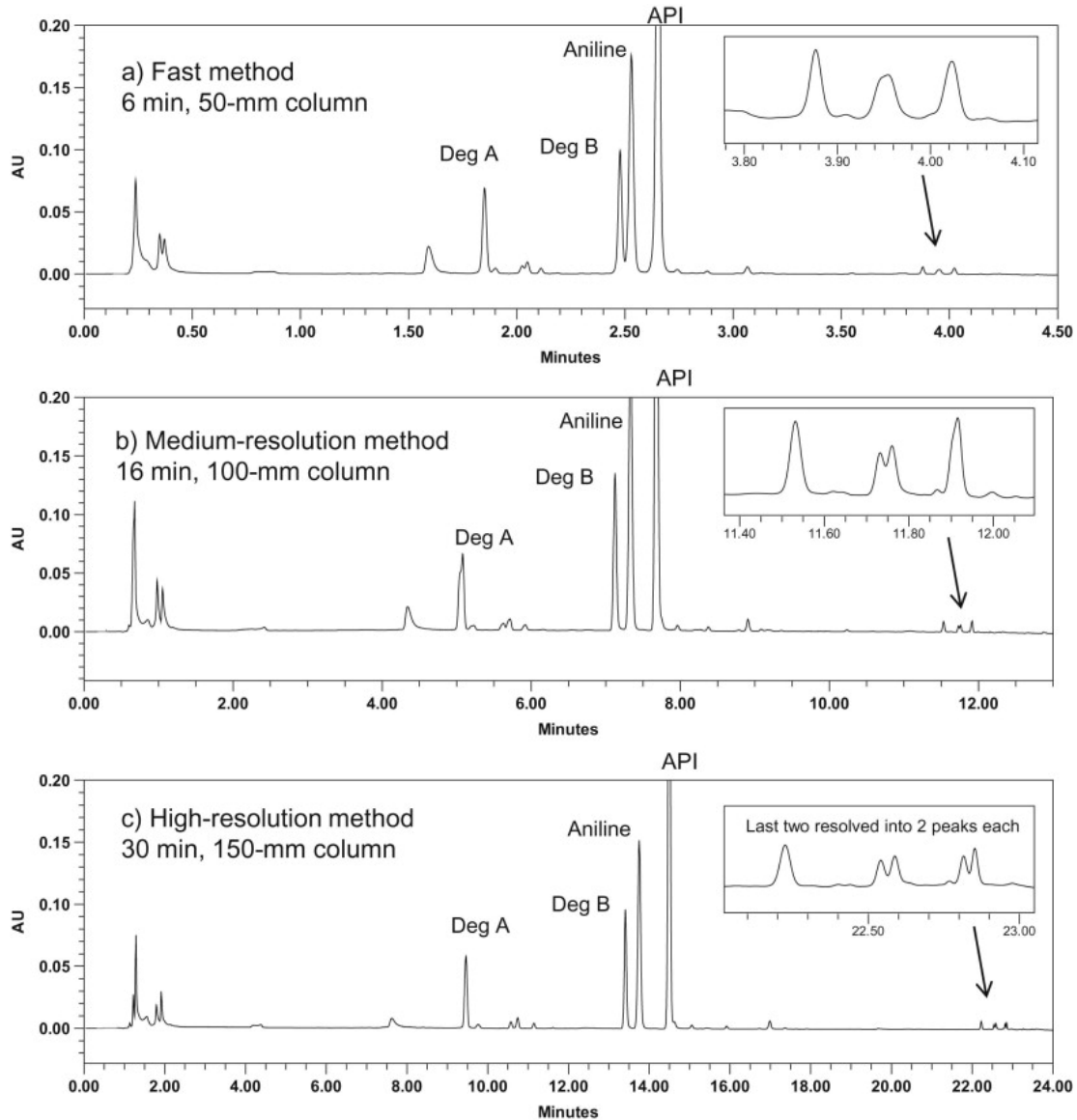


C18, 150×4.6 mm, $5 \mu\text{m}$, 1 mL/min,
 $V_{inj} = 20 \mu\text{L}$, celkový čas analýzy 45 min

C18 50×2.1 mm, $1.7 \mu\text{m}$, 0.61 mL/min,
 $V_{inj} = 1.4 \mu\text{L}$, celkový čas analýzy 5.1 min

RYCHLOST vs. ROZLIŠENÍ

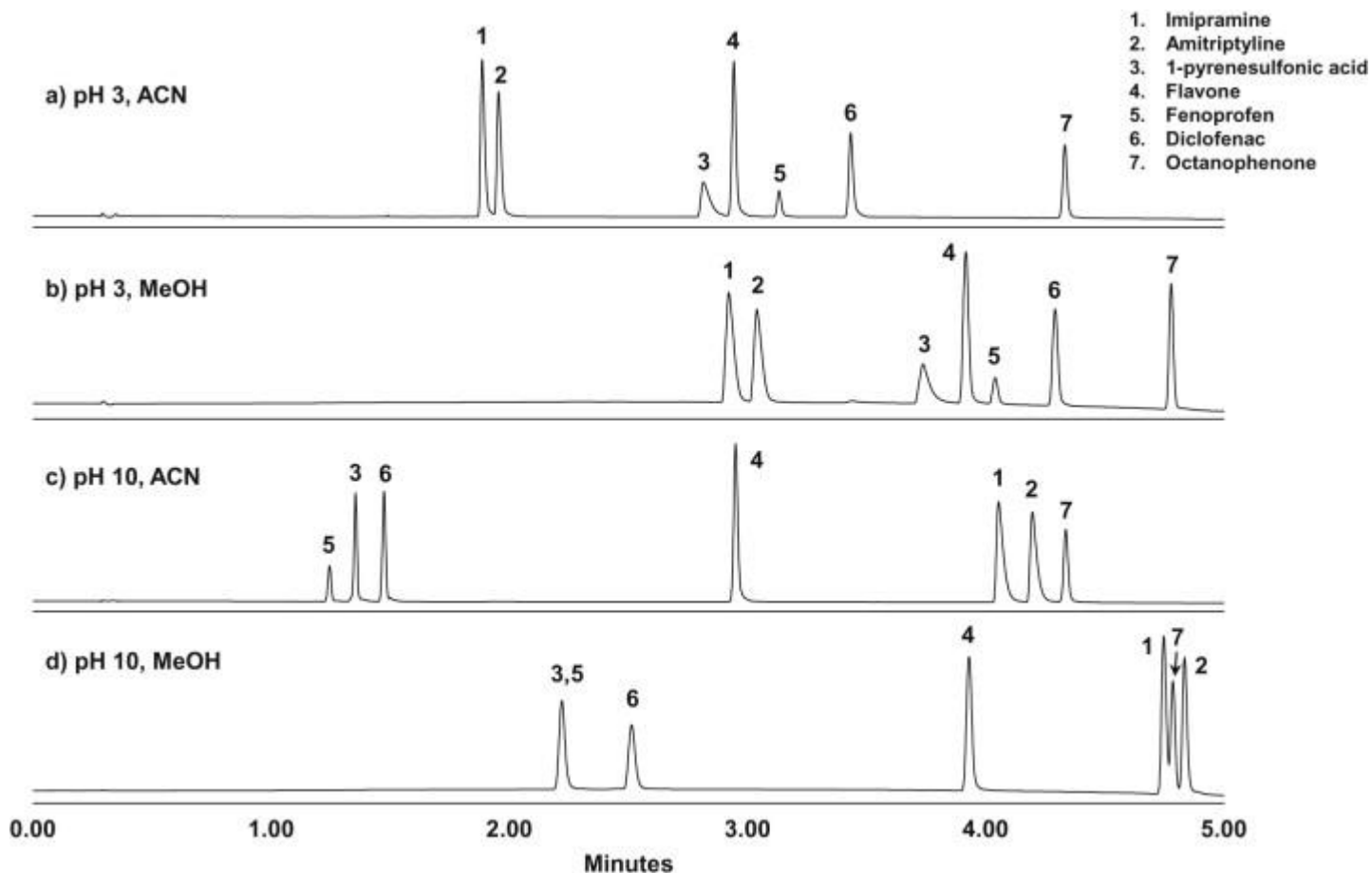
rychlost



rozlišení

(RYCHLÉ) TESTOVÁNÍ VLIVU MOBILNÍ FÁZE

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



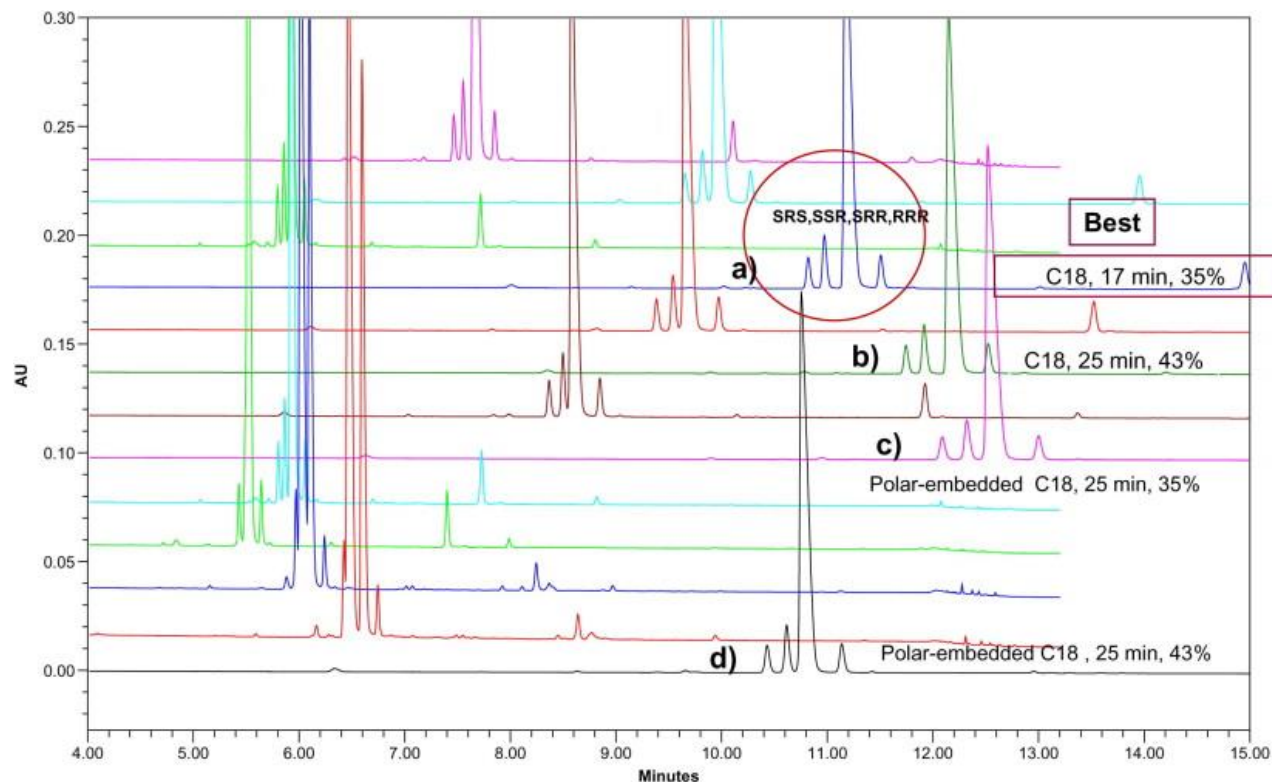
„AUTOMATICKÝ“ VÝVOJ METODY

Matematické plánování (DoE)

Chirální sloučenina
s třemi centry (SRR)

30 experimentů

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



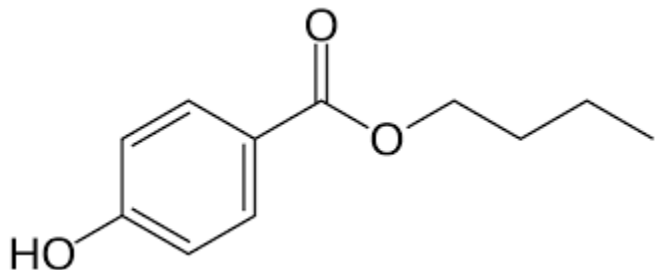
Redukce času analýzy ze 42 na 17 min

CO JE NEJLEPŠÍ?

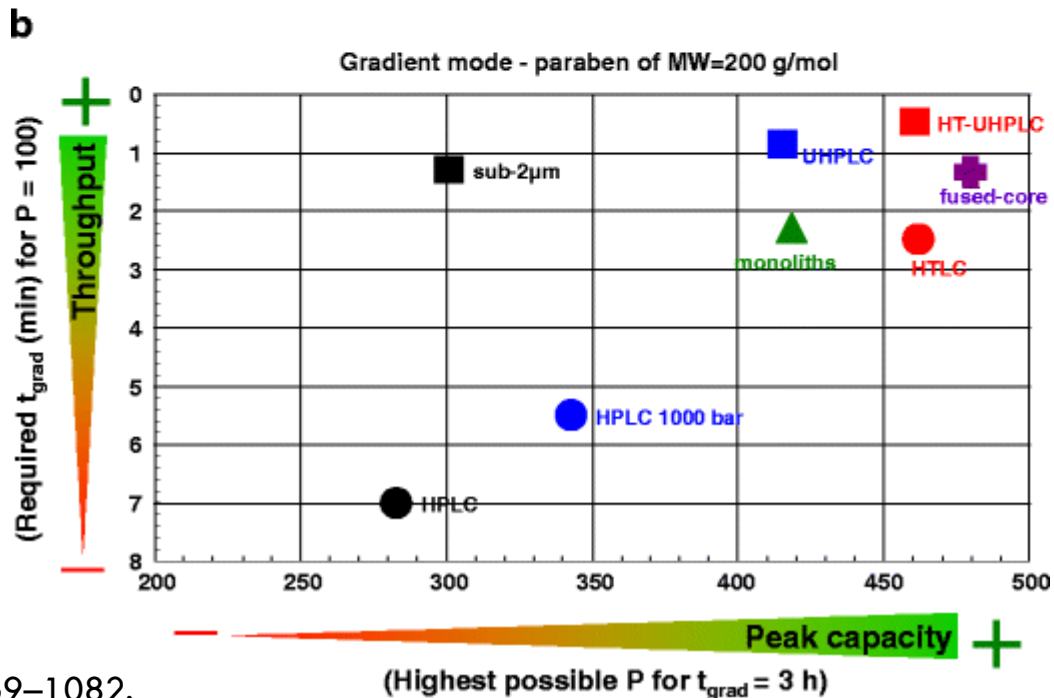
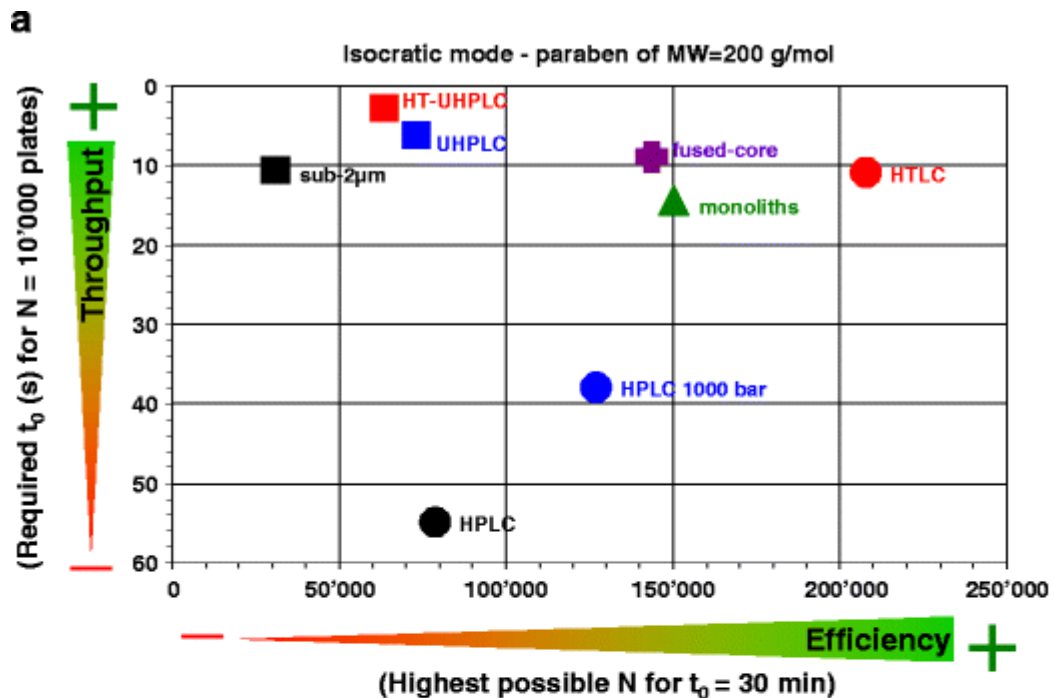
| Strategie | Kolona | Velikost částic, μm | Teplota, $^{\circ}\text{C}$ | Δp max, MPa |
|---------------------|---|--------------------------------|-----------------------------|---------------------|
| HPLC | Waters Xbridge C ₁₈ , 50 × 2.1 mm | 5 | 30 | 40 |
| HPLC, 1000 bar | Waters Xbridge C ₁₈ , 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 |

CO JE NEJLEPŠÍ?

butylparaben

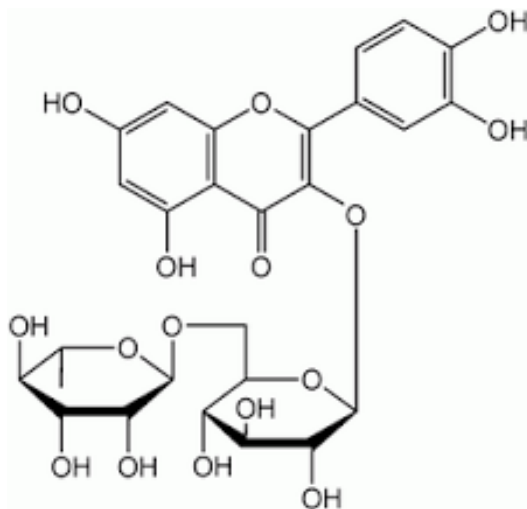


200 g/mol



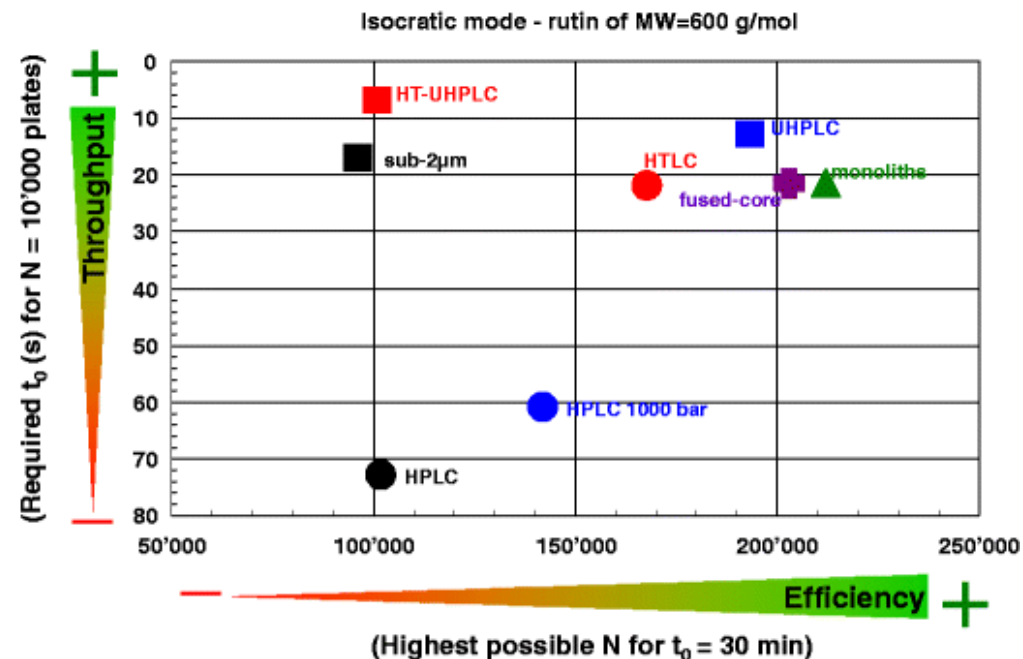
CO JE NEJLEPŠÍ?

rutin

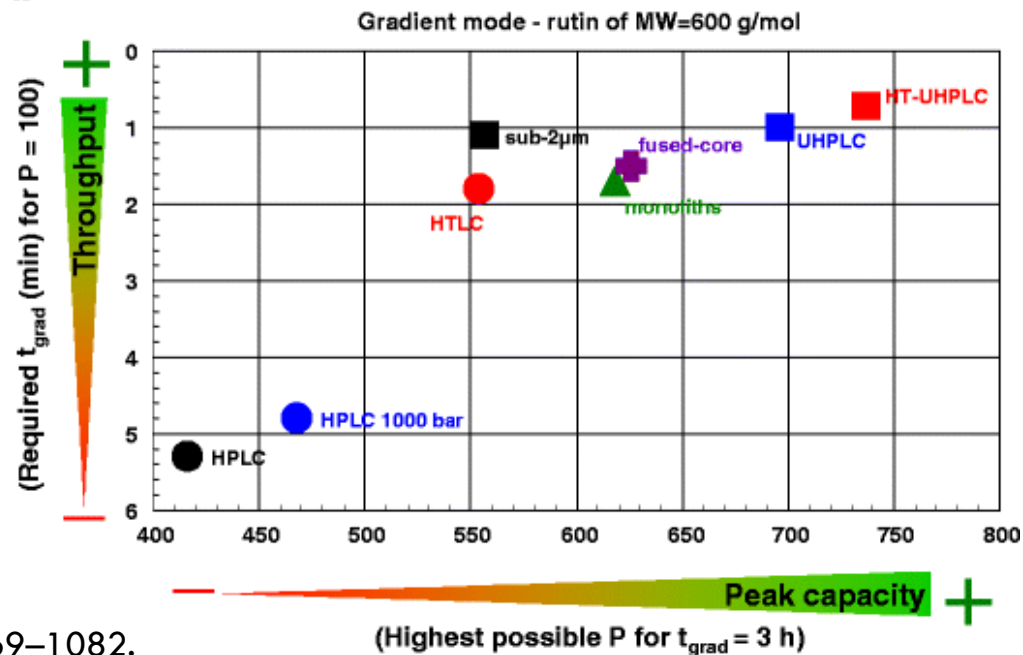


600 g/mol

a

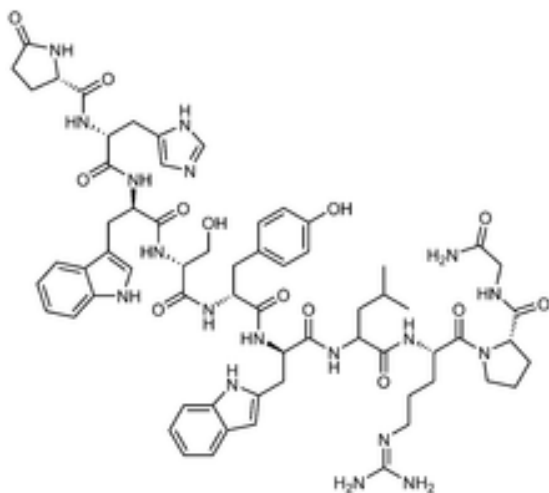


b

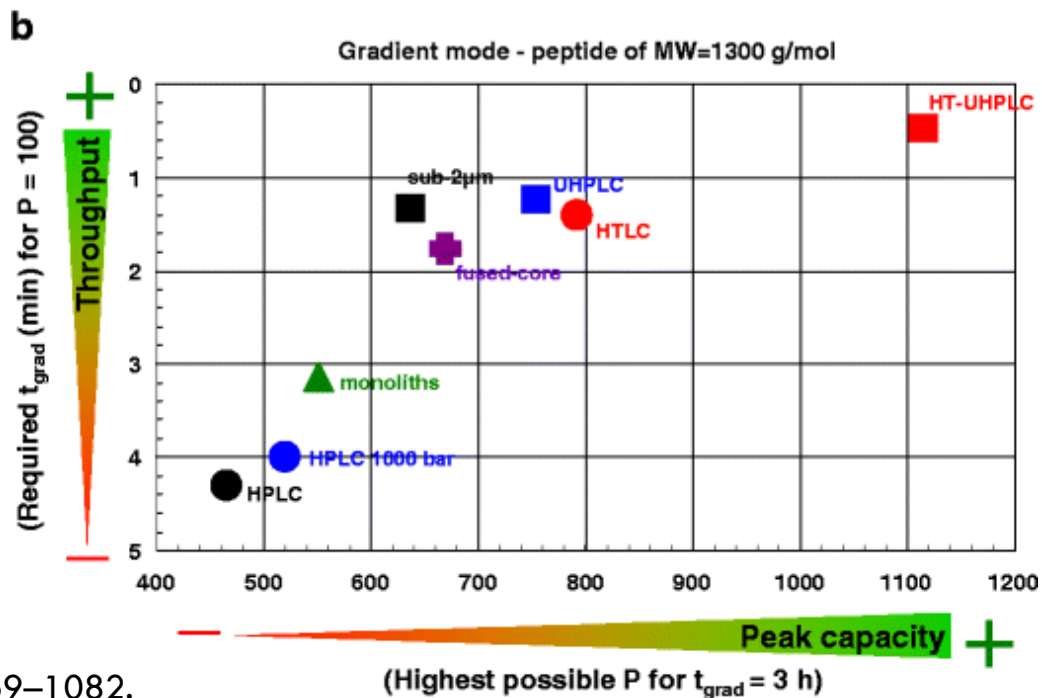
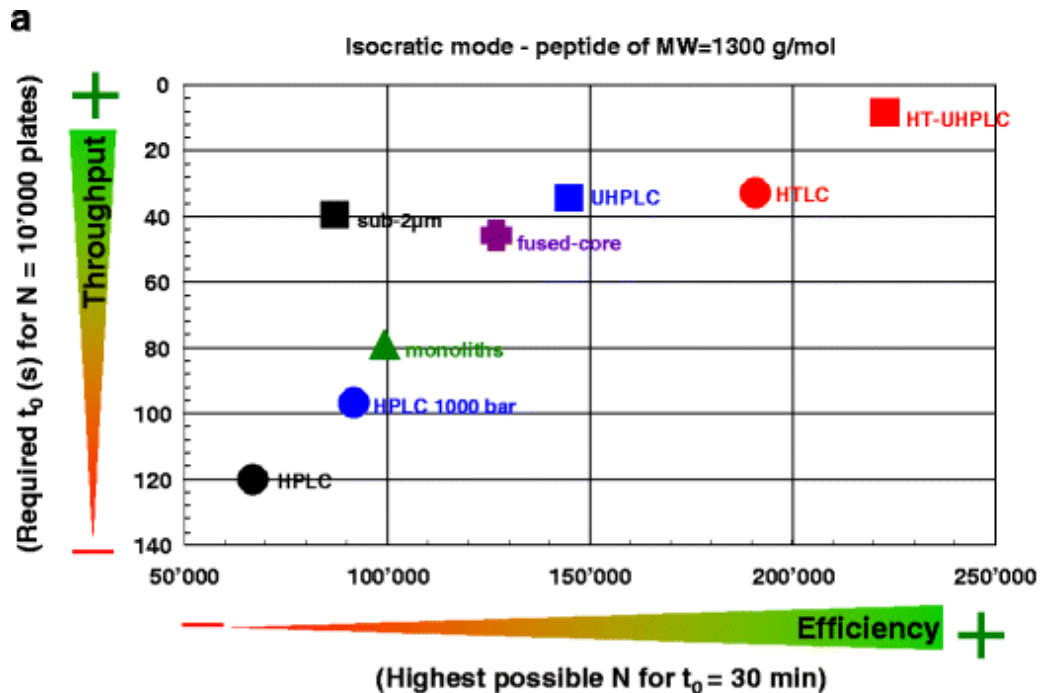


CO JE NEJLEPŠÍ?

triptorelin



1300 g/mol



“No pressure, no diamonds.”

Thomas Carlyle

