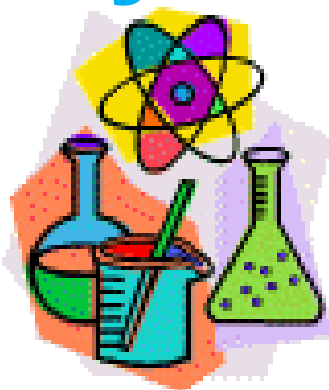


Citlivé elektroforetické stanovení farmaceutik a metabolitů v klinických vzorcích s acetonitrilovým zaostřením



Petr Tůma

Ústav hygieny

3. lékařská fakulta Univerzity Karlovy

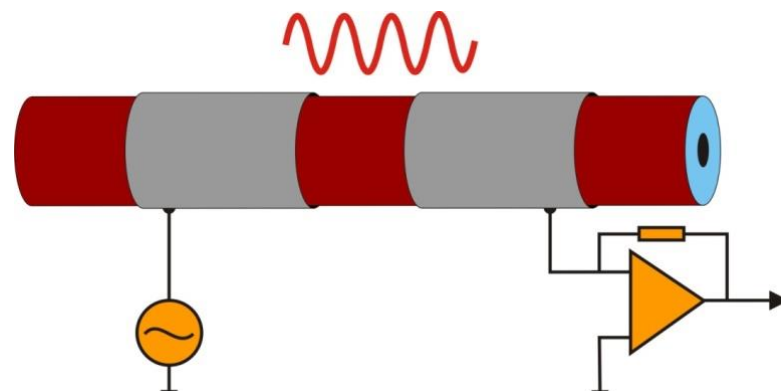
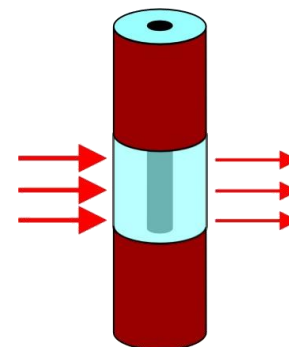
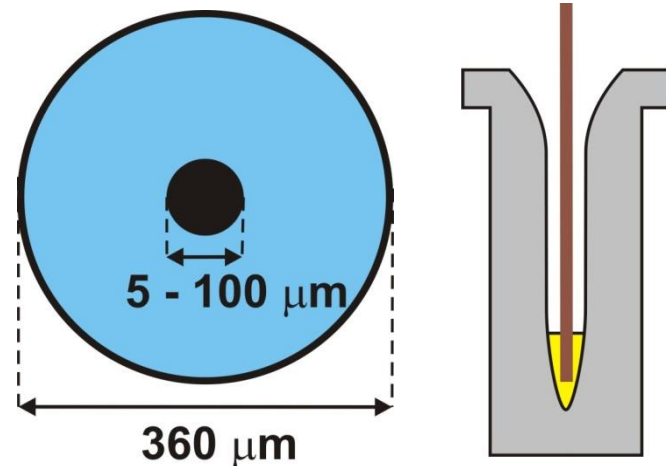
Proč kapilární elektroforéza v klinické analýze

Přednosti

- vysoká separační účinnost: cca 10^5 pater/m
- krátká doba separace: 5-15 min
 - velké soubory jedinců
- malé množství vzorku k analýze: $10 \mu\text{L}$
 - mikrodialyzáty, zvířecí modely
- snadná úprava biologického materiálu
 - přímé dávkování
- malá spotřeba separačního pufu
- nízká cena kapiláry

Nevýhody

- malá citlivost
 - UV detekce $10^{-5} - 10^{-6}$ M



Přídavek organického rozpouštědla

deproteinizace

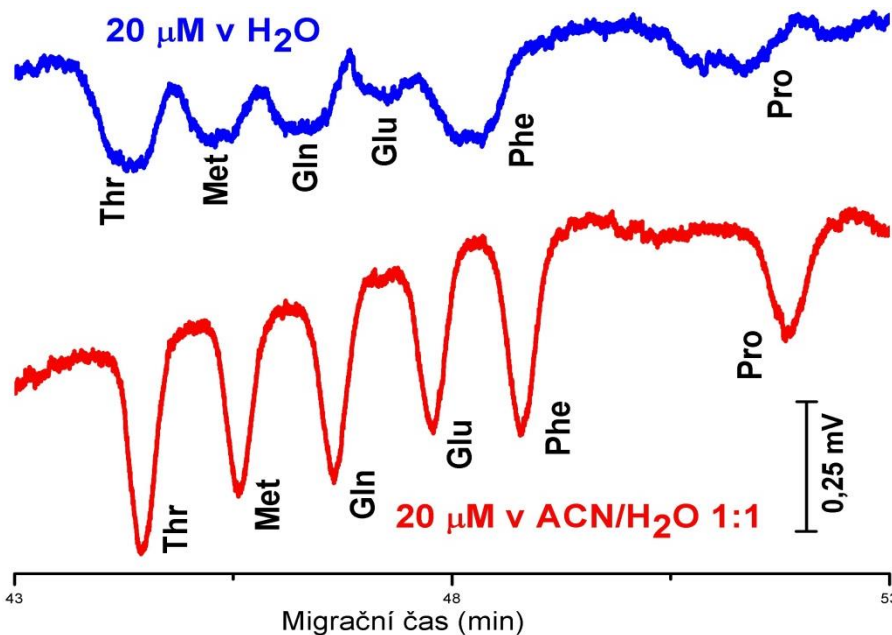
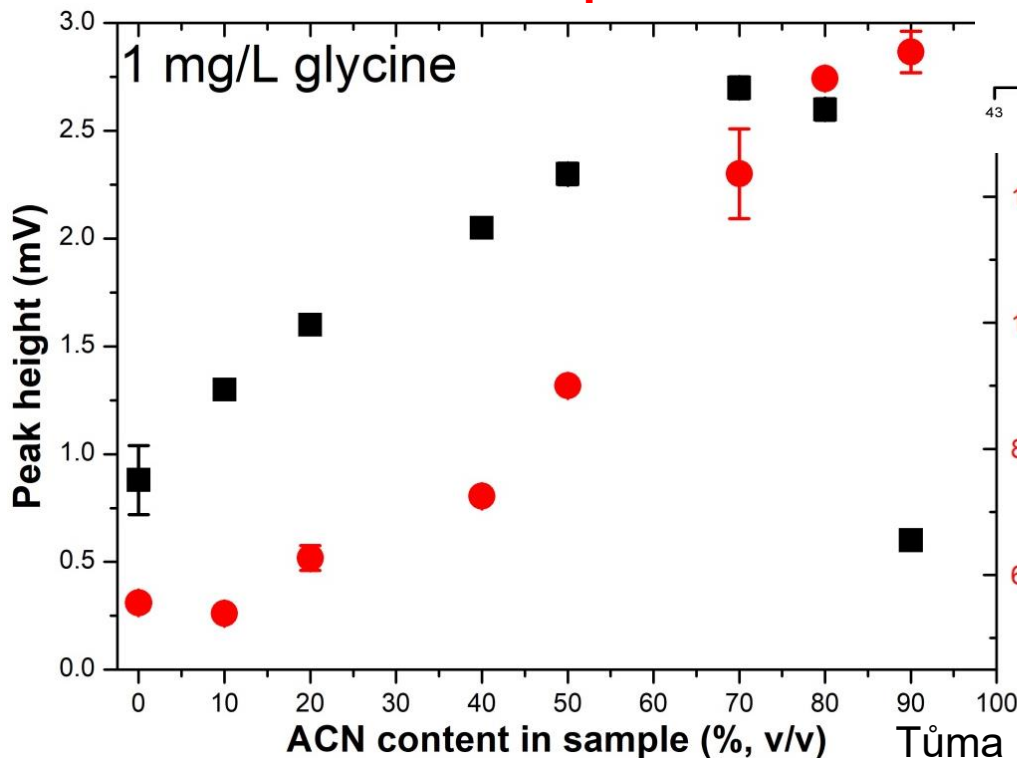
- silná kyselina: sulfosalicylová, HClO_4

$G(\text{vzorek}) > G(\text{BGE})$

- organické rozpouštědlo: acetonitril, aceton, isopropanol

$G(\text{vzorek}) < G(\text{BGE})$

zaostření píků



Number of theoretical pla

1.2×10^5

1.0×10^5

8.0×10^4

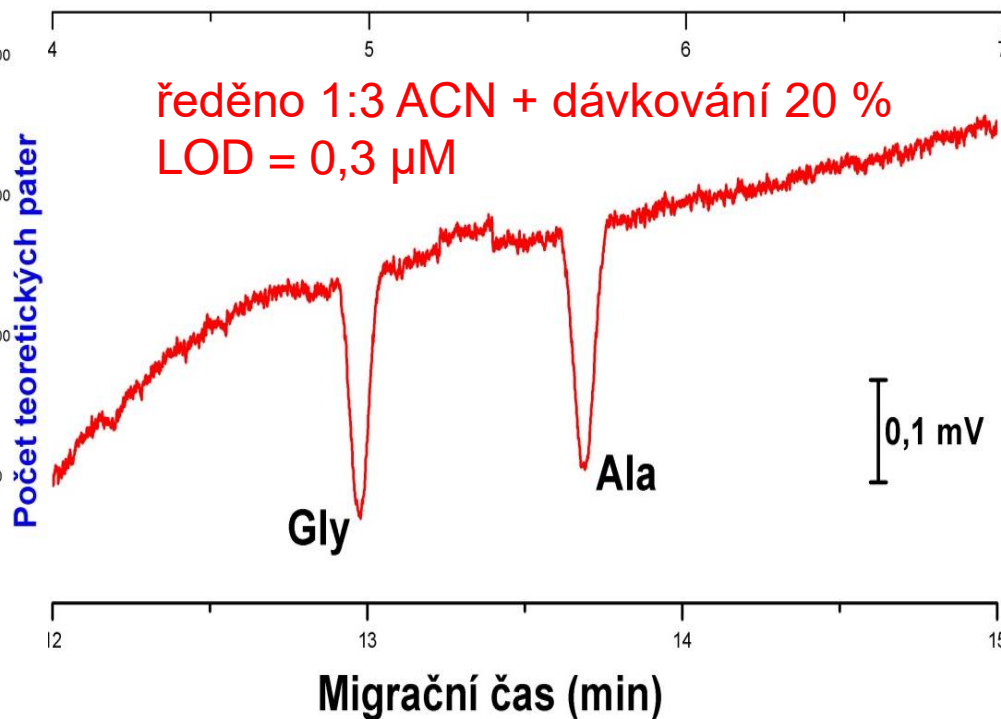
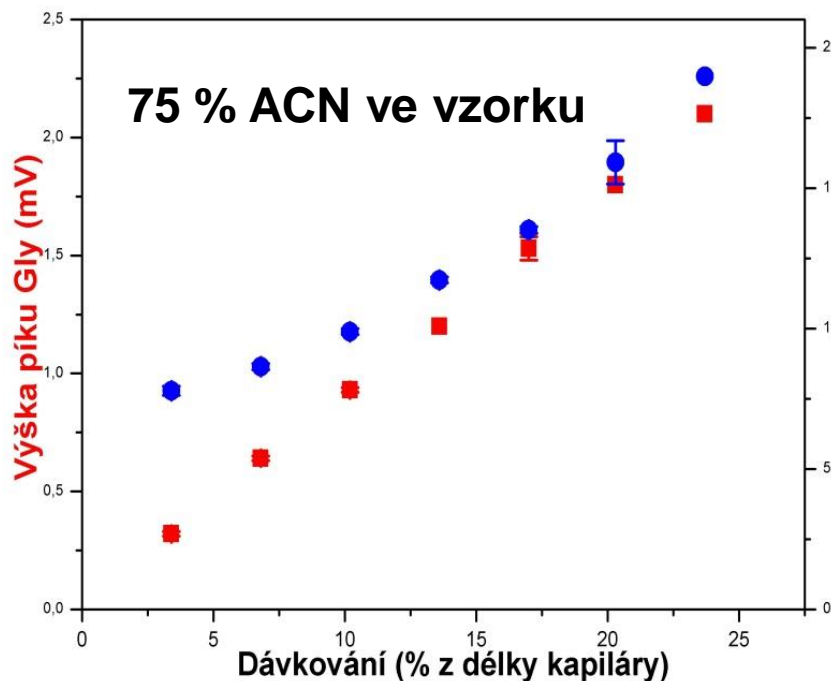
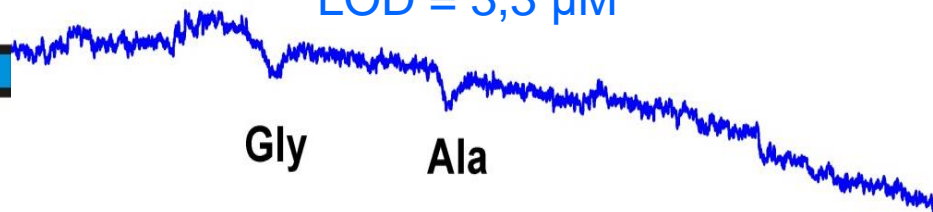
6.0×10^4

Zvýšené dávkování vzorku

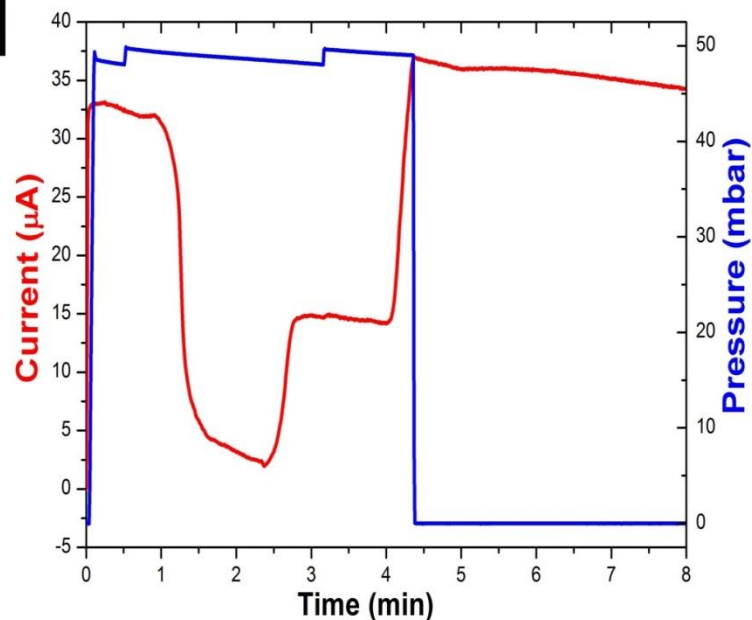
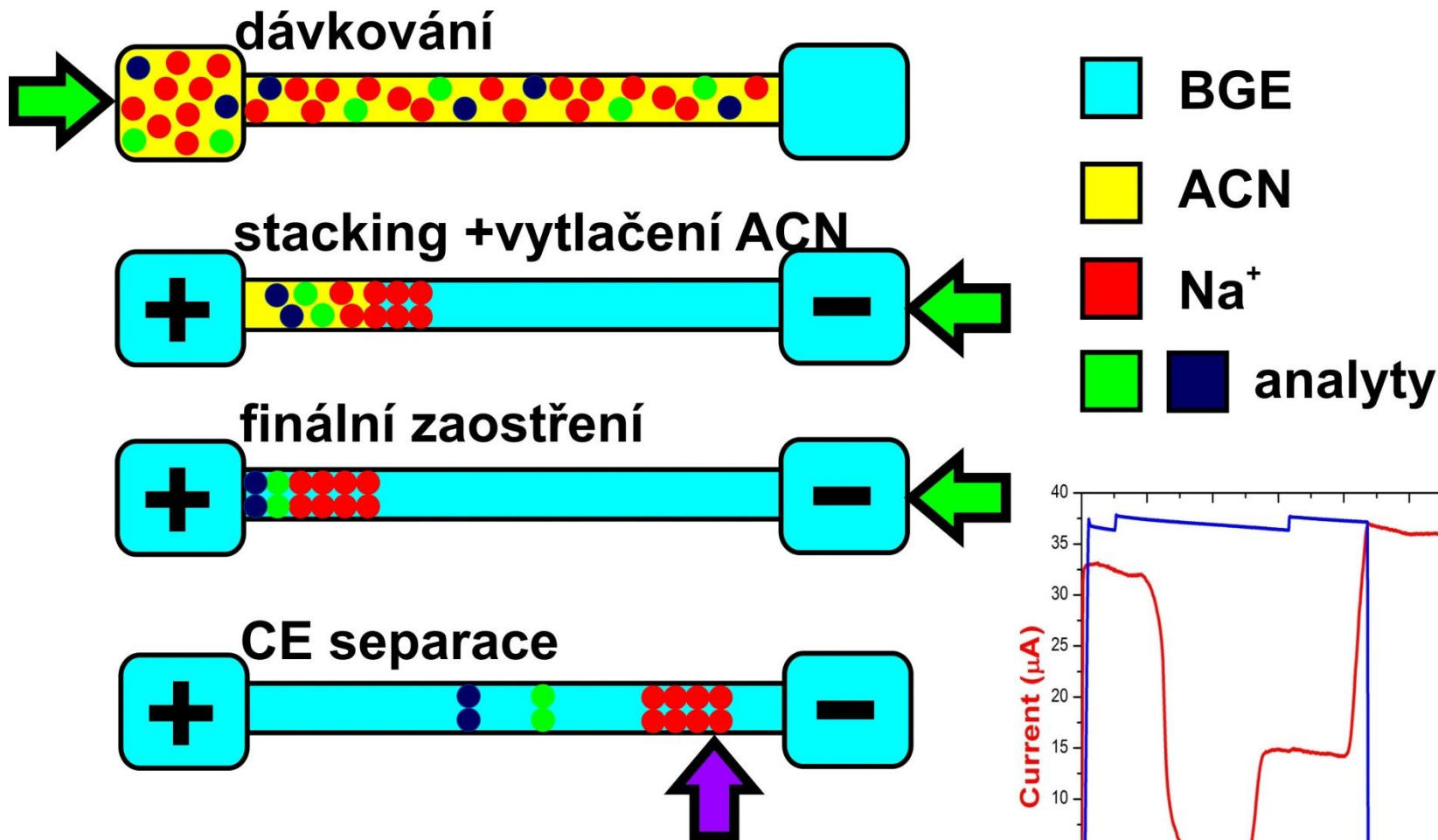


Separace 4 μM směsi Gly + Ala

bez ředění + dávkování 1 %
LOD = 3,3 μM



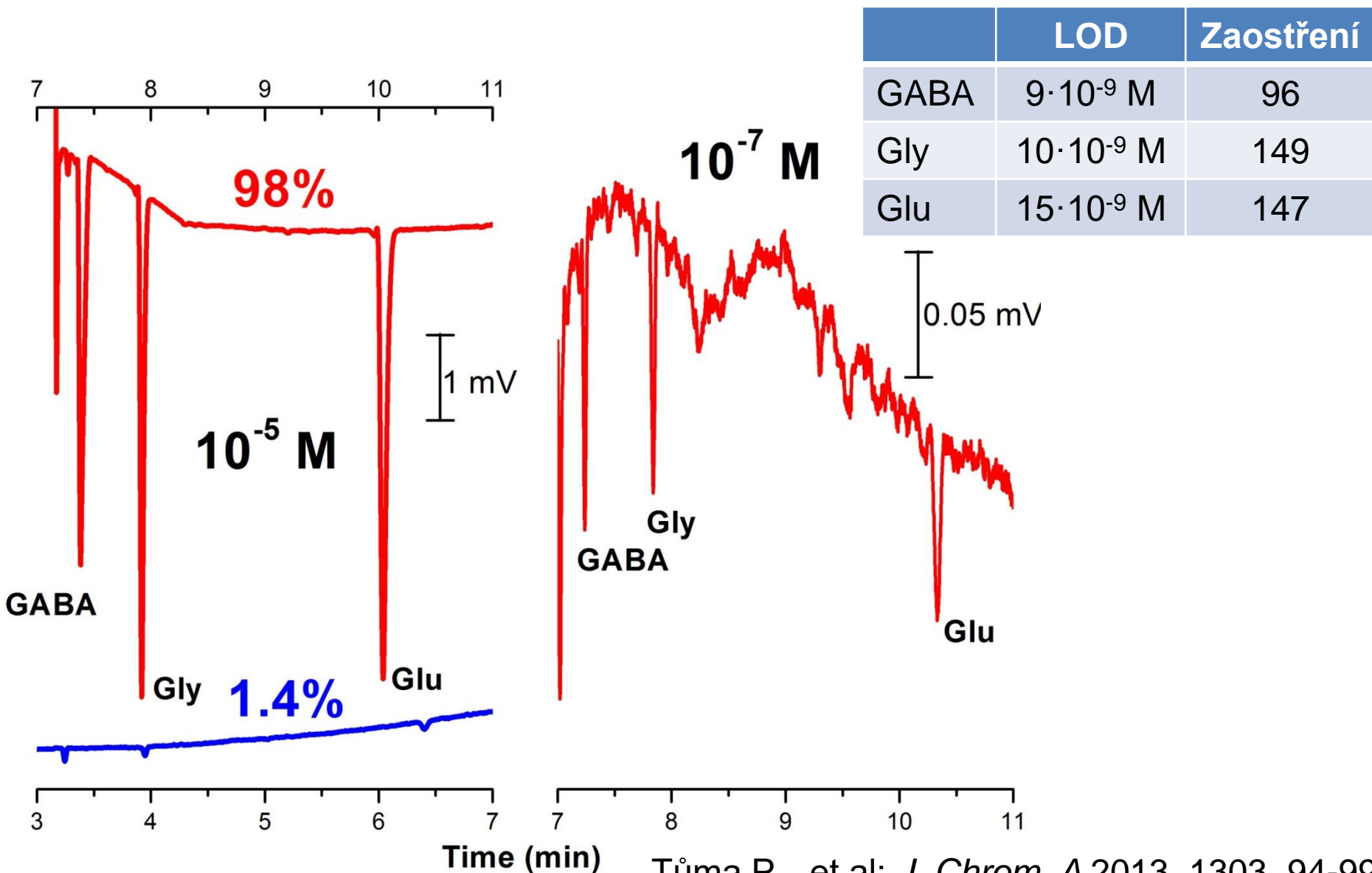
On-line prekoncentrace vzorku s aplikací hydrodynamického tlaku



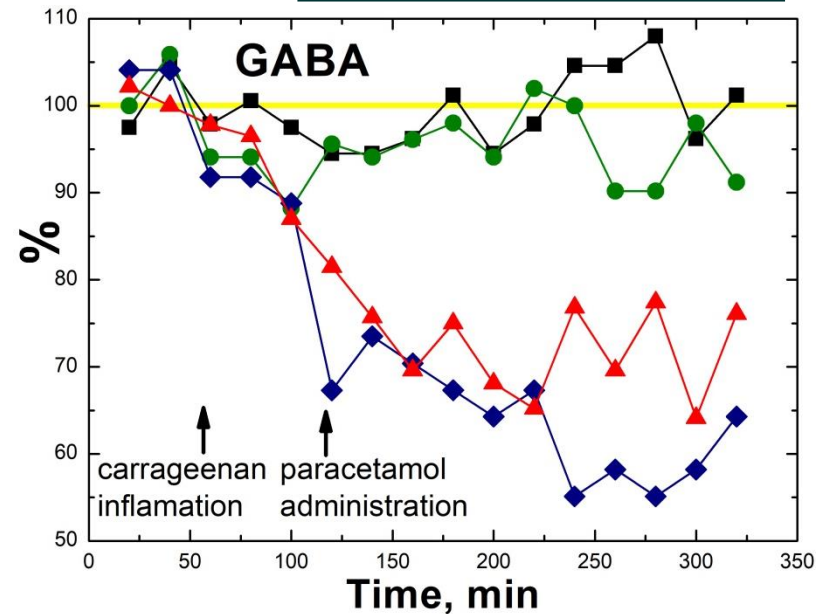
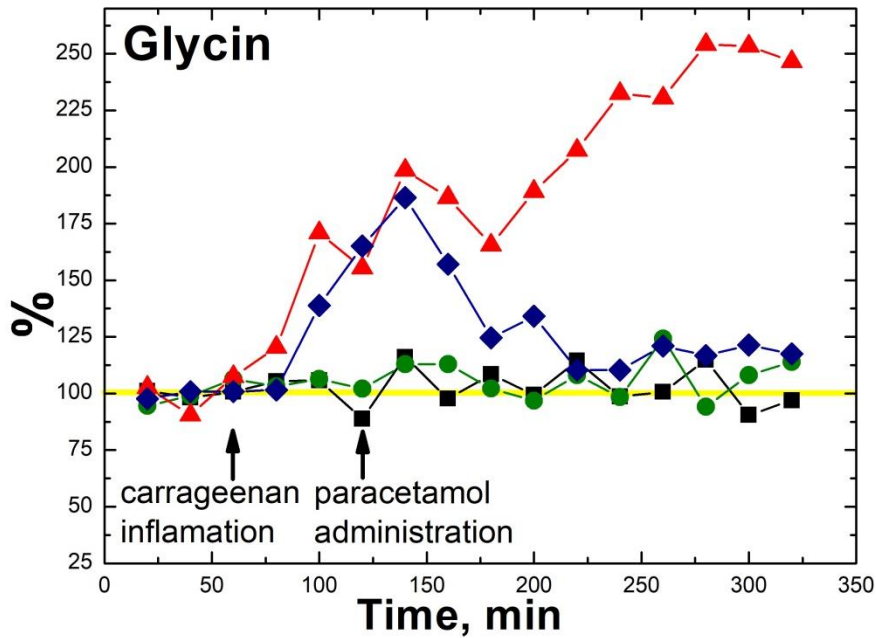
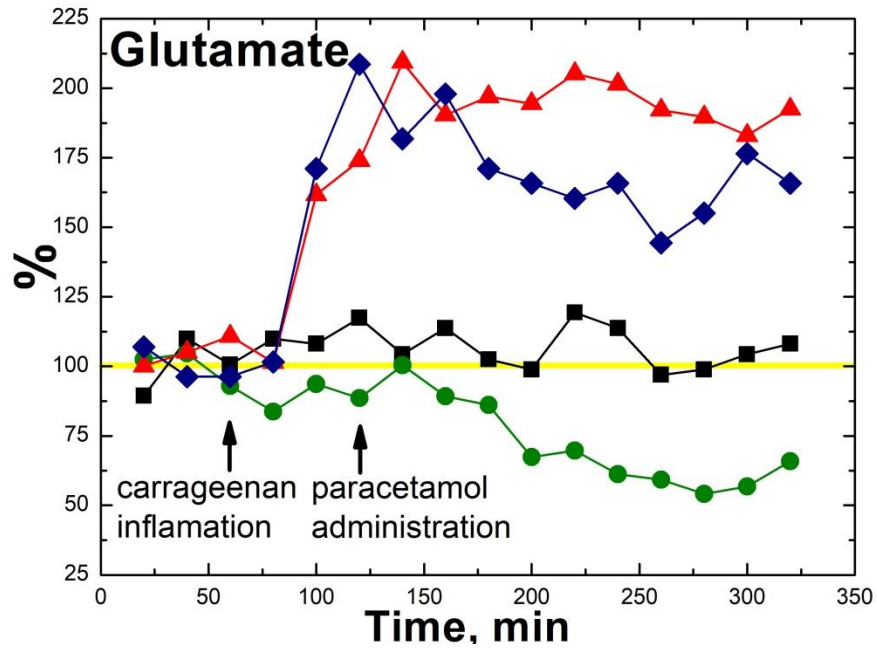
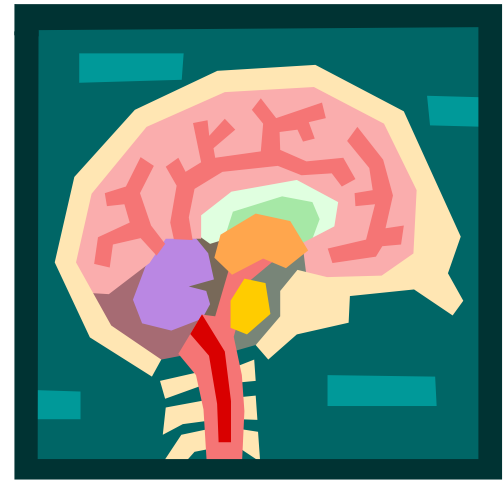
Tůma P. et al: *J. Pharm. Biomed. Anal.* 2018, 160, 368-373.

Tůma P. et al: *Electrophoresis* 2018, 39, 2605-2611.

Stanovení neurotransmitterů v mikrodialyzátech



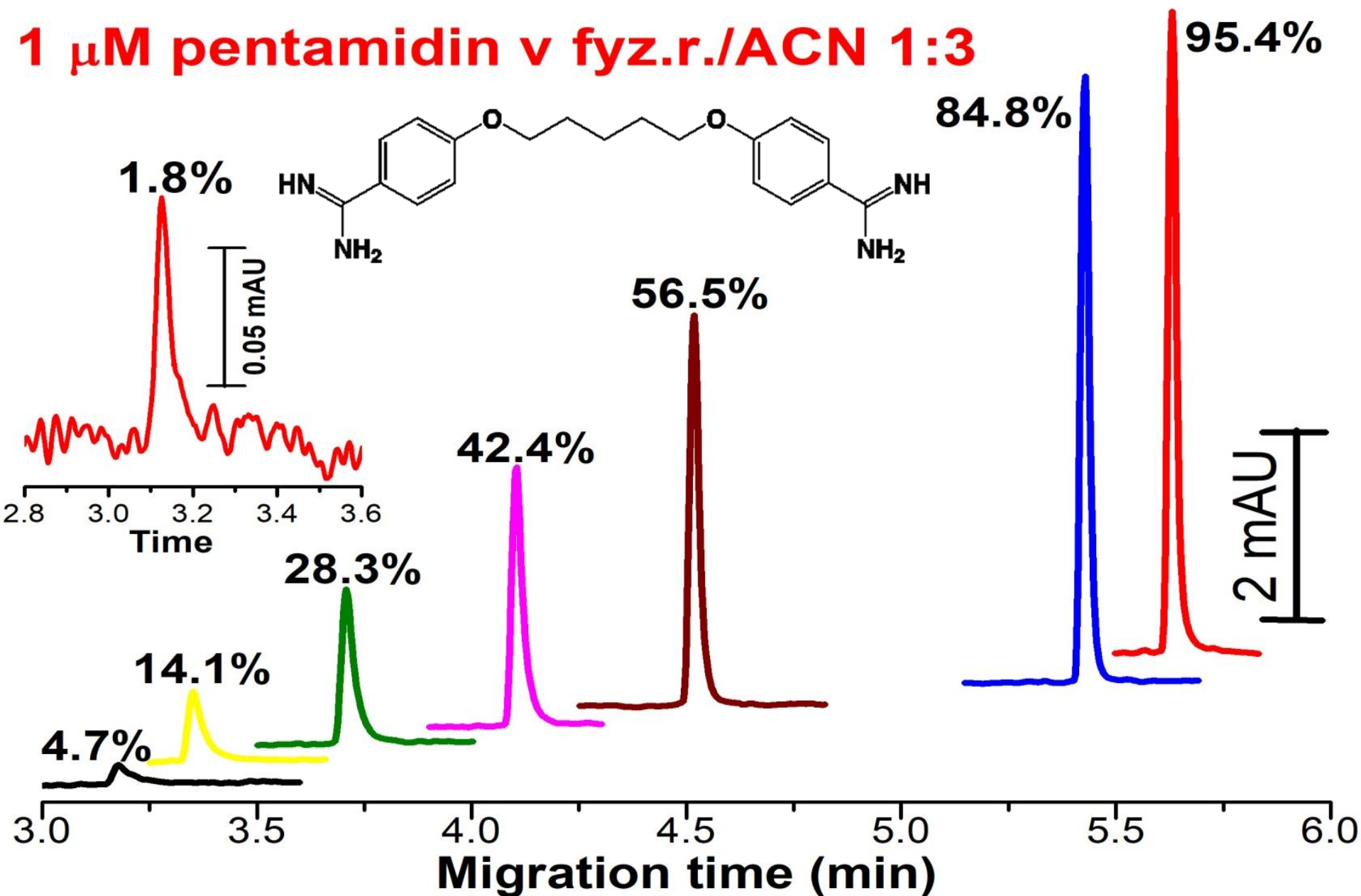
Studium bolesti v PAG



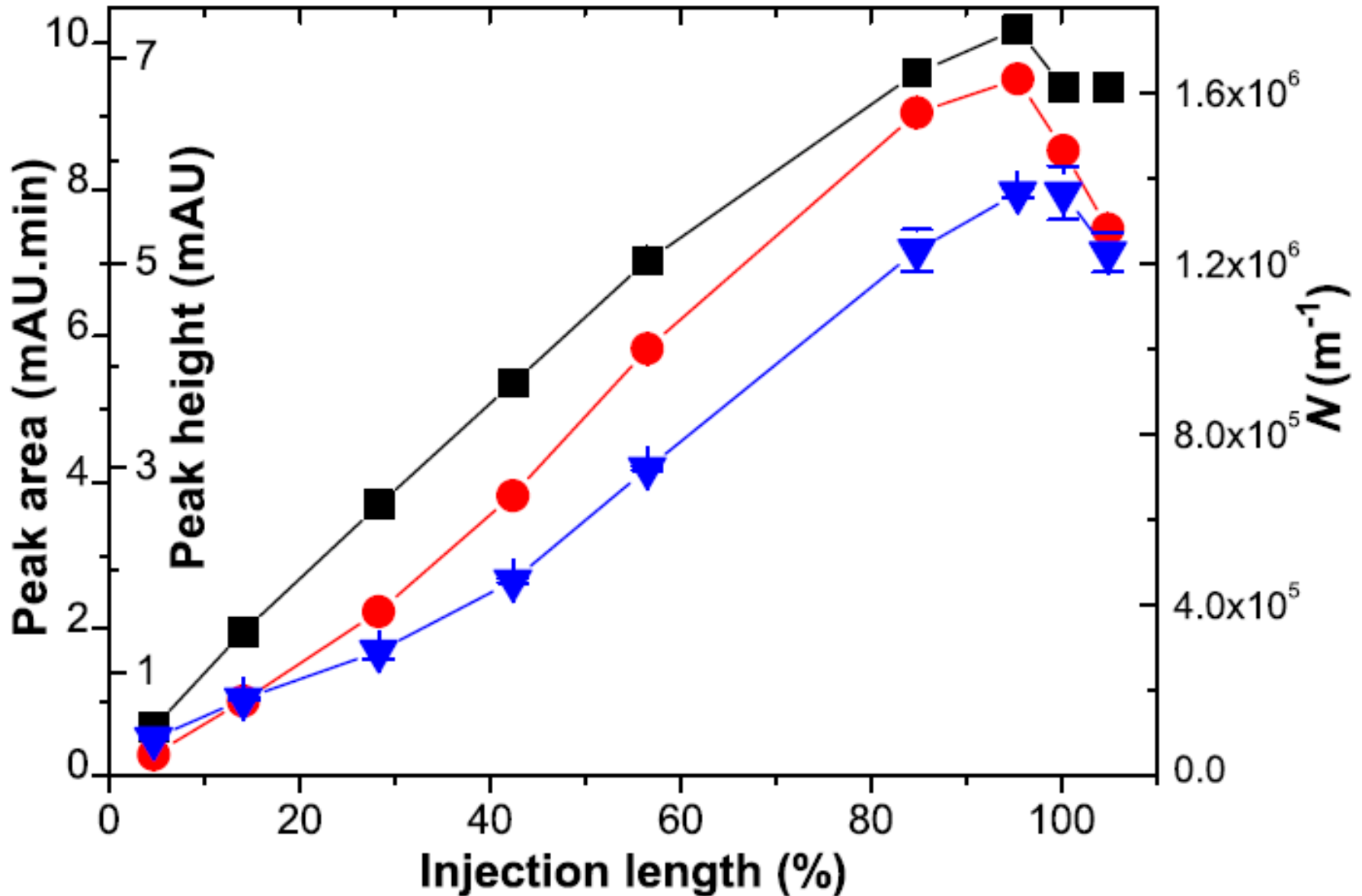
1. fyziologický roztok + voda
2. fyziologický roztok + paracetamol
3. karagenan + voda
4. karagenan + paracetamol

Stacking při stanovení léčiv v krvi

1 μ M pentamidin v fyz.r./ACN 1:3



Separáčnı parametry ACN-stacking





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Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

ELSEVIER



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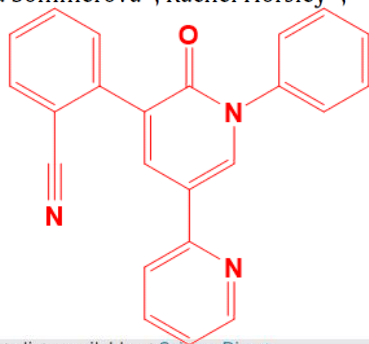
Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

ELSEVIER

Novel electrophoretic acetonitrile-based stacking for sensitive monitoring of the antiepileptic drug perampanel in human serum

Petr Tůma^a, Miroslava Bursová^{b,c}, Blanka Sommerová^a, Rachel Horsley^d, Radomír Čabala^{b,c}, Tomáš Hložek^{b,c,*}



Contents lists available at ScienceDirect

Journal of Chromatography A

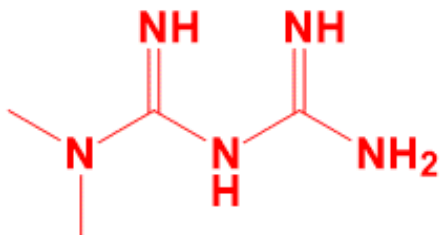
journal homepage: www.elsevier.com/locate/chroma



ELSEVIER

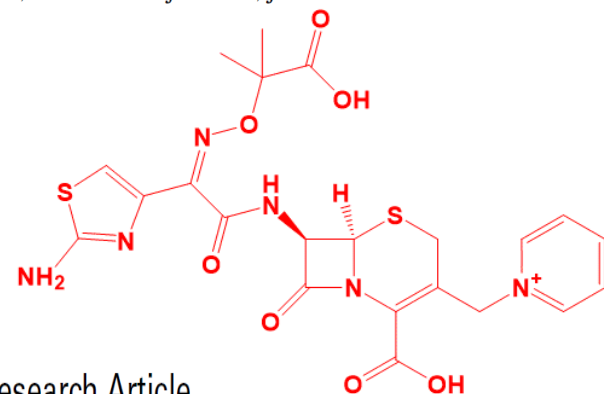
Large volume sample stacking for rapid and sensitive determination of antidiabetic drug metformin in human urine and serum by capillary electrophoresis with contactless conductivity detection

Petr Tůma*



Electrophoretic stacking for sensitive determination of antibiotic ceftazidime in human blood and microdialysates from diabetic foot

Petr Tůma^{a,*}, Martin Jaček^a, Vladimíra Fejfarová^b, Jan Polák^{c,d}



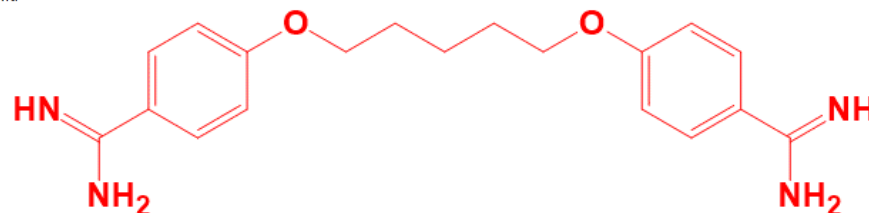
Electrophoresis 2018, 39, 2605–2611

Petr Tůma¹
Petr Heneberg²
Šimon Vaculín³
Dušan Koval⁴

¹Department of Hygiene, Third Faculty of Medicine, Charles University, Prague, Czechia
²Third Faculty of Medicine, Charles University, Prague, Czechia

Research Article

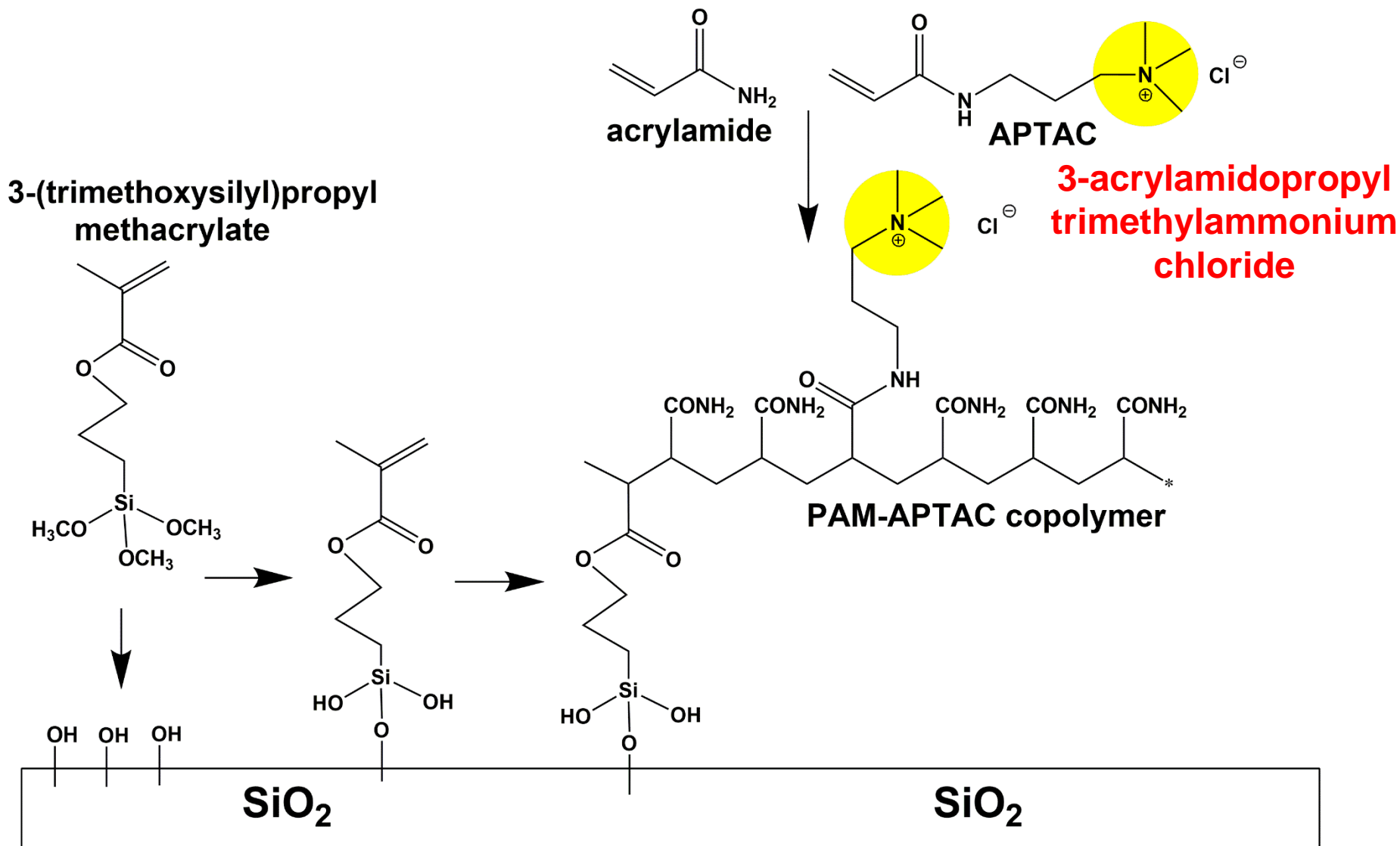
Electrophoretic large volume sample stacking for sensitive determination of the anti-microbial agent pentamidine in rat plasma for pharmacological studies



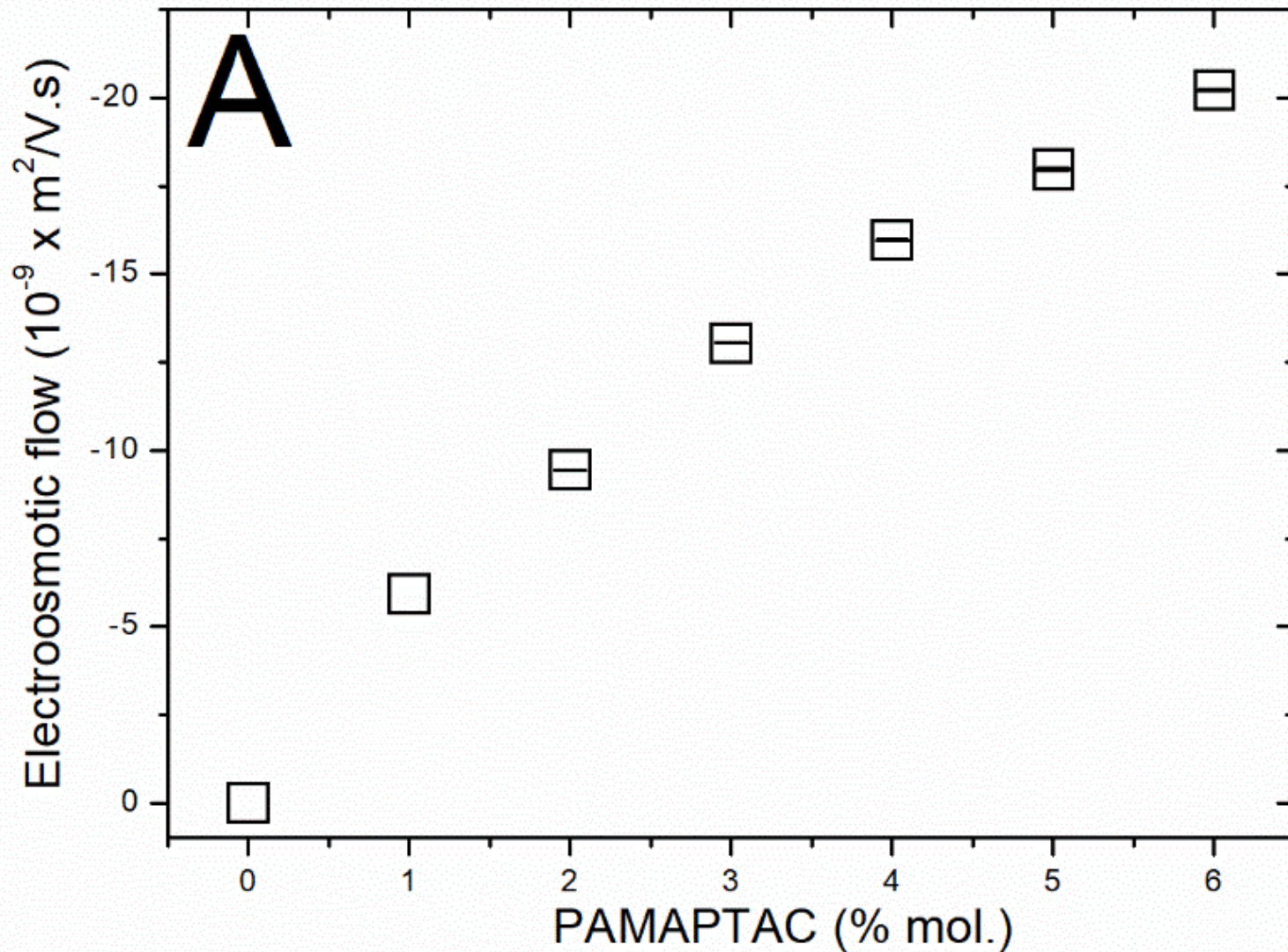
Stacking při stanovení léčiv v krvi

Léčivo	BGE	Kapilára - délka (cm)/id (μm)	Dávkování - % z délky	Detekce	LOD, μM
Pentamidine - antimalarikum	100 mM HAc/Tris, pH 4.7	31.5/50	28	UV	0.03
Ceftazimide - antibiotikum	50 mM Cl-HAc v 20% MeOH, pH 2.32	31.5/25	27	UV	0.8
Metformin - antidiabetikum	2.0 M HAc, pH 2.15	31.5/50	7,3	C ⁴ D	0.03
Perampanel - antiepileptikum	50 mM Cl-HAc, pH 2.15	35/50	37	FD	0.008

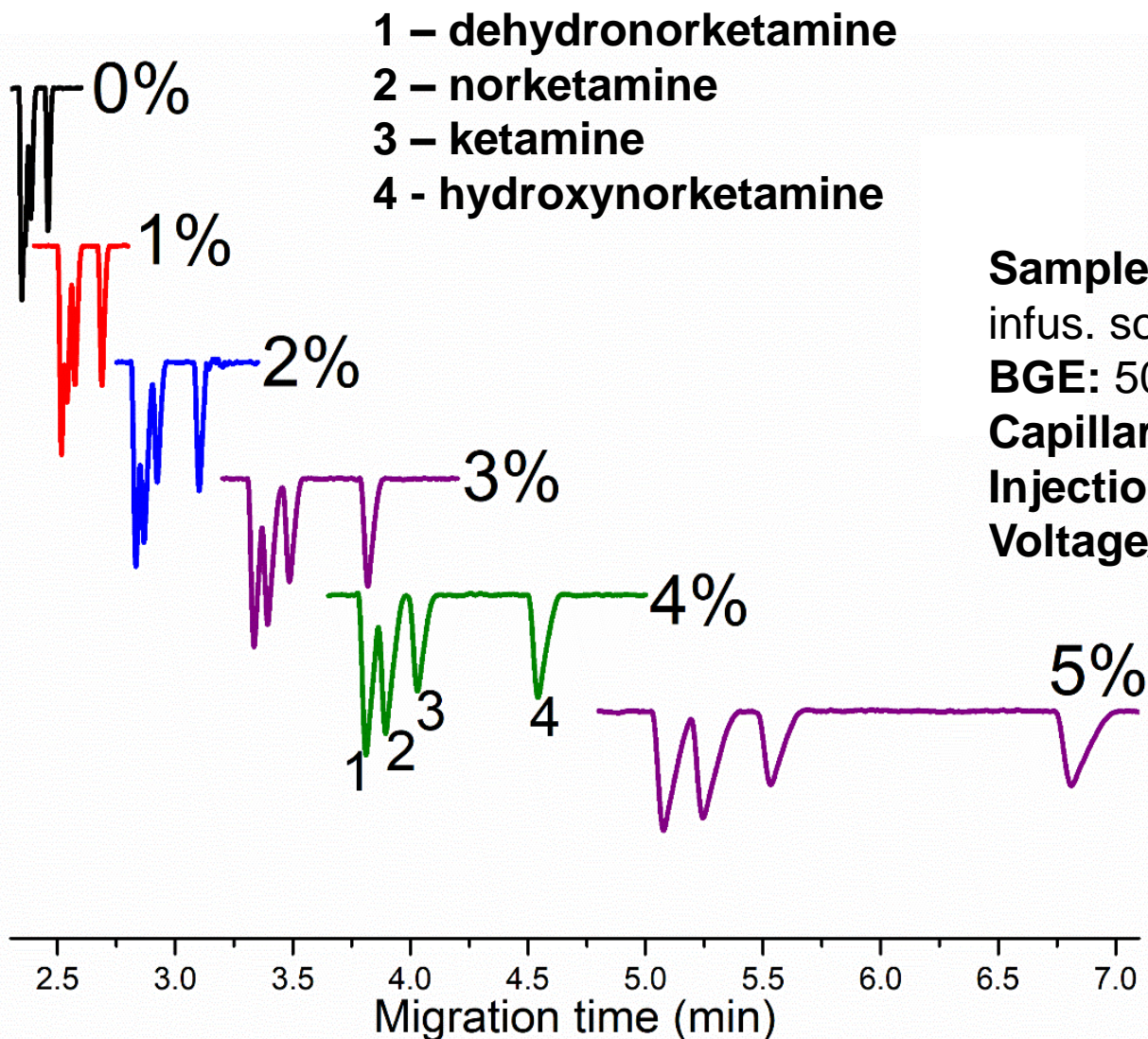
Kovalentní coating kapiláry



Protisměrná elektroforéza



Protisměrná separace ketaminů



Sample: 1.0 µg/mL in 75% ACN/ 25% infus. sol.

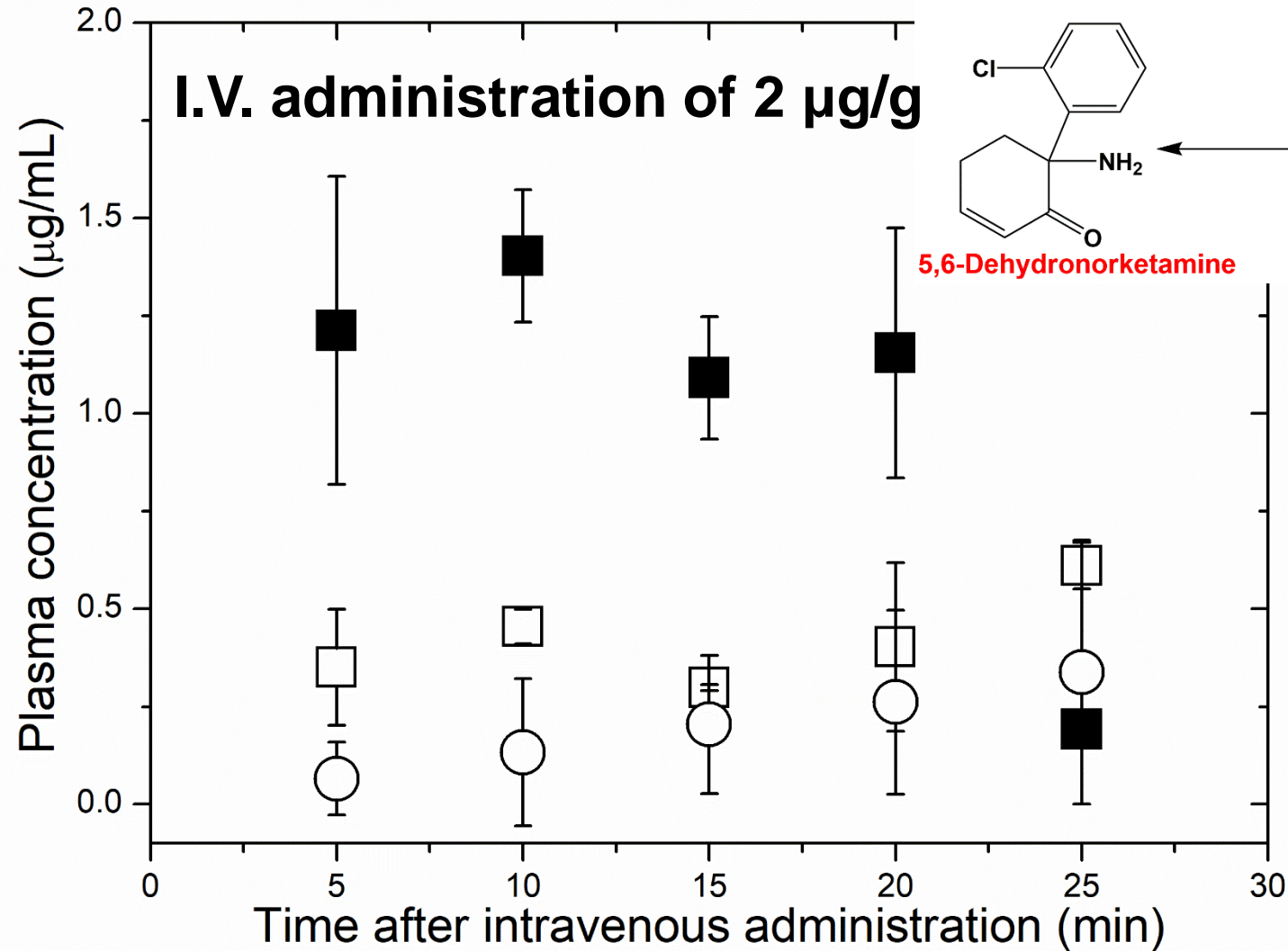
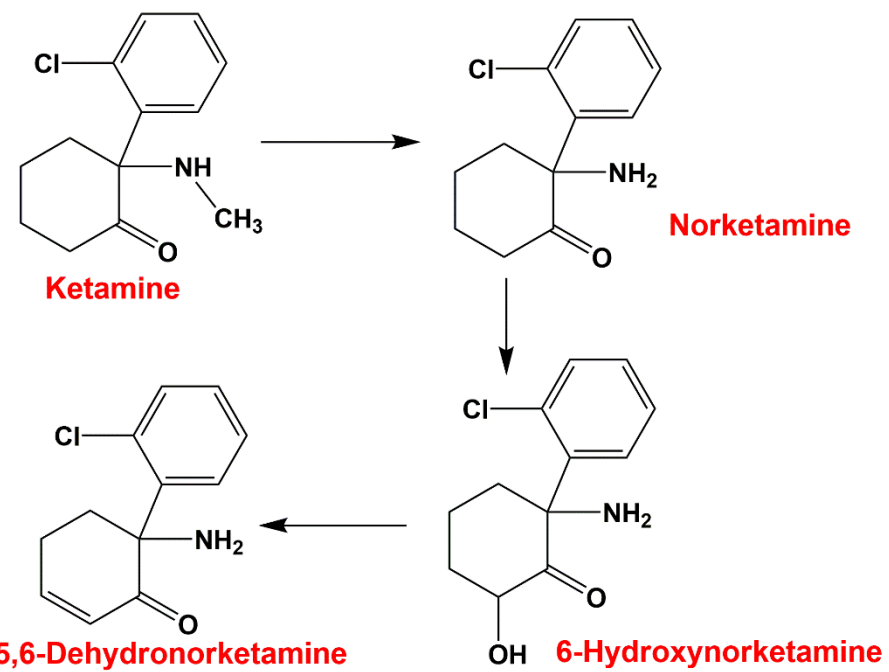
BGE: 500 mM AcOH, pH 2.3

Capillary: 31.4/16.6 cm

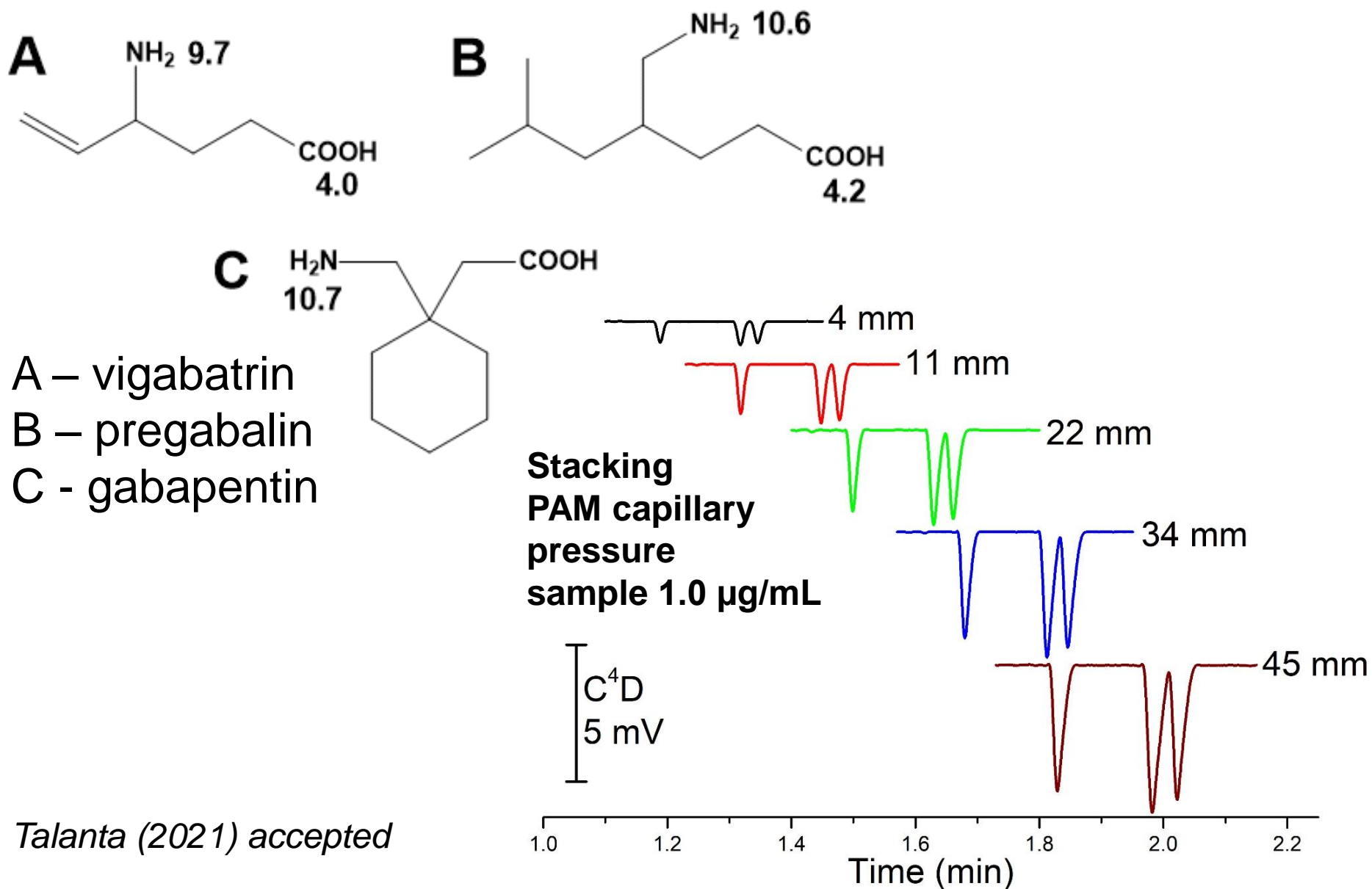
Injection: 50 mbar for 120 s

Voltage/current: +30 kV/5.4 µA

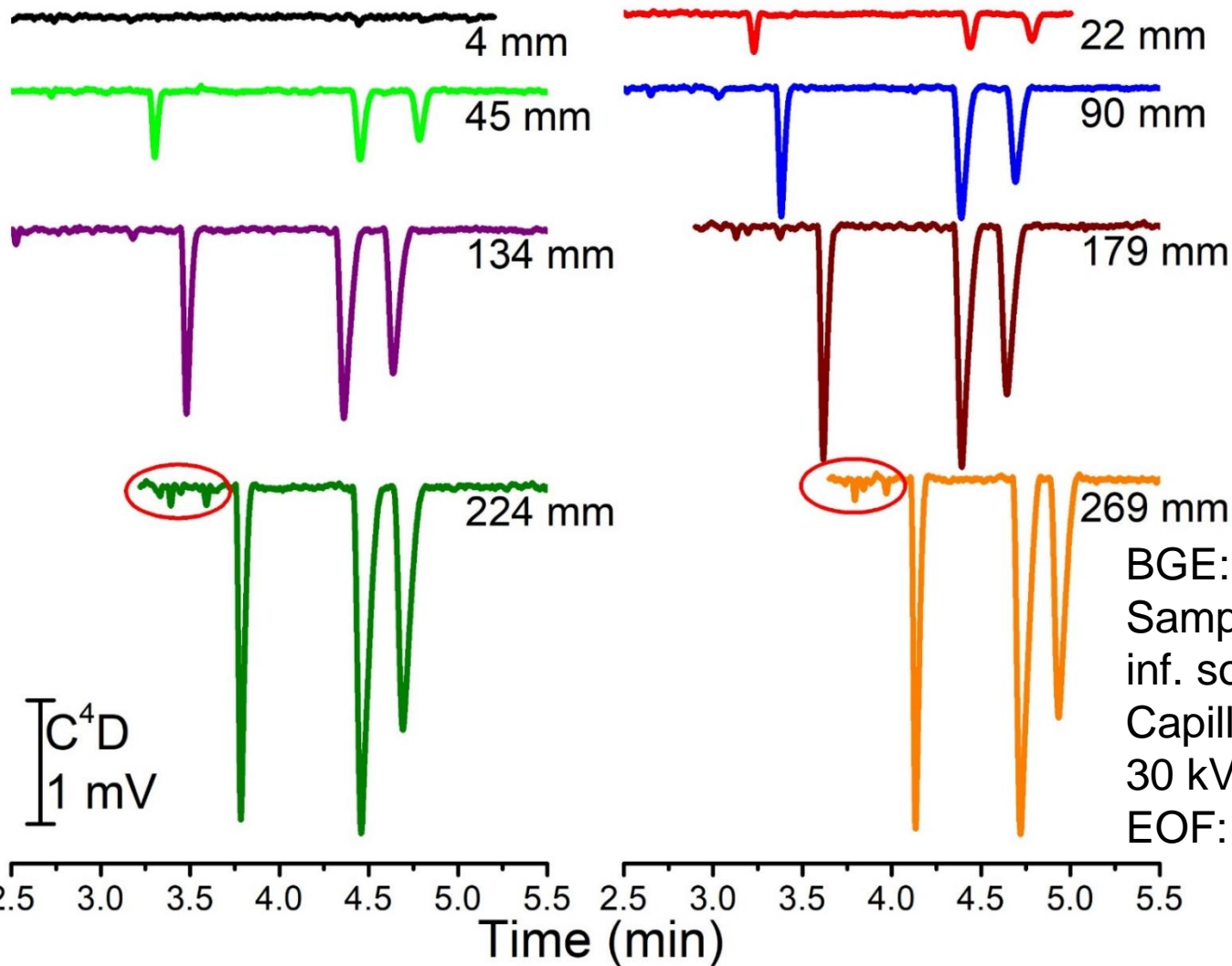
Metabolism in rats



ACN stacking antiepileptics in PAMAPTAC



Stacking in 5% PAMAPTAC



BGE: 500 mM AcOH, pH 2.3
Sample, 0.1 $\mu\text{g}/\text{mL}$ in 25%
inf. sol.+ 75% ACN
Capillary: 25 μm , 31.5/17 cm
30 kV/5 μA
EOF: $-18.6 \cdot 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$

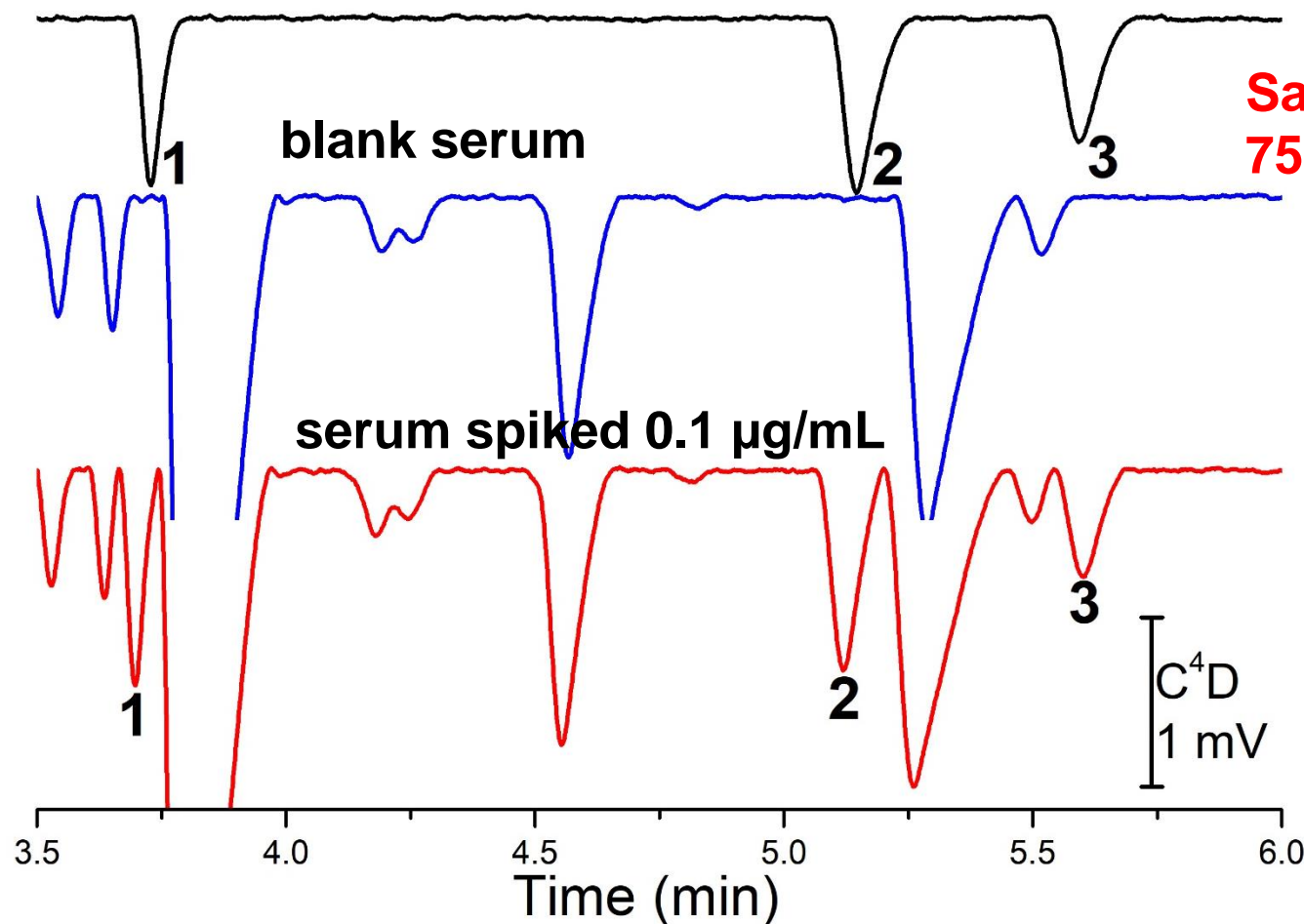
Antiepileptika v lidském séru

model sample - 0.1 $\mu\text{g/mL}$

LOD: 0.8 – 1.2 ng/mL

LOD: 5 -7 nmol/L

Sample: 25 μL serum +
75 μL ACN



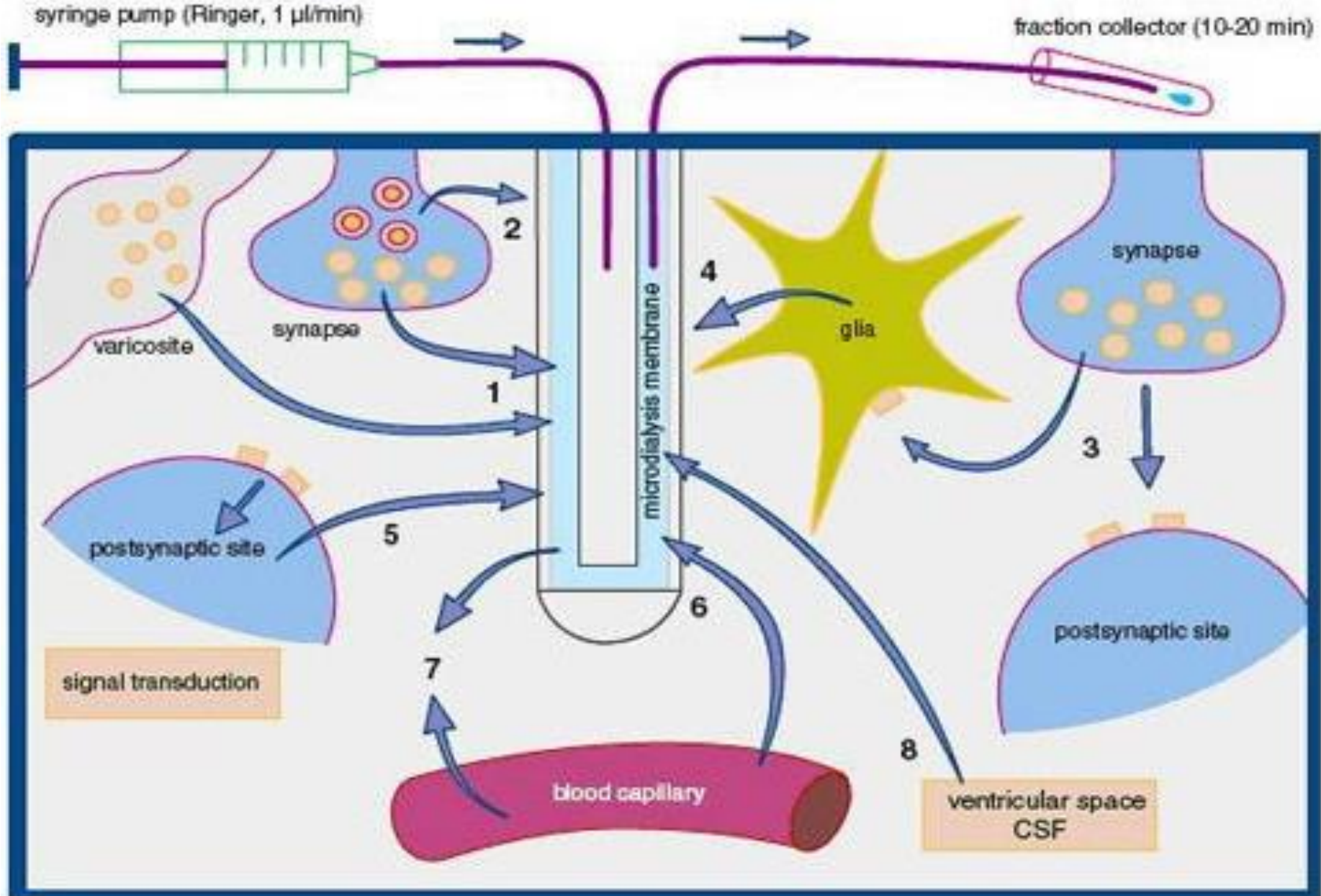
- 1 – vigabatrin
- 2 – pregabalin
- 3 - gabapentin

Poděkování

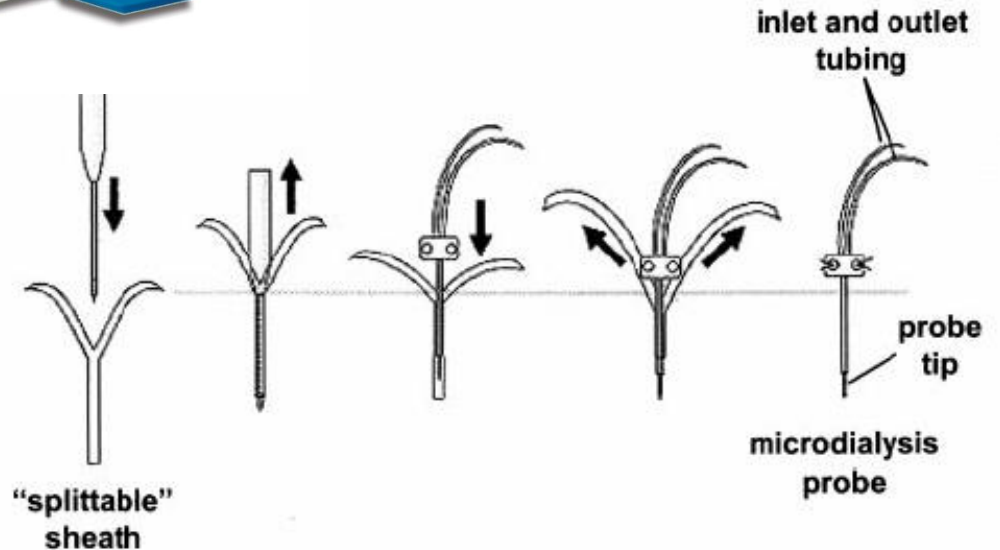
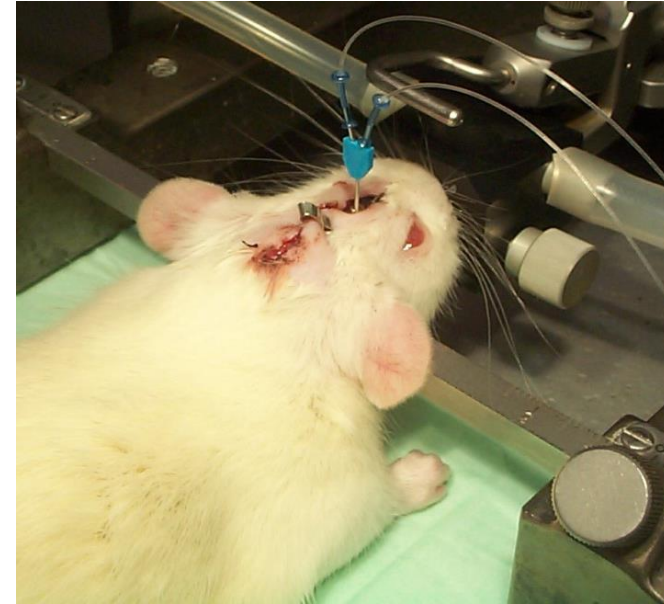
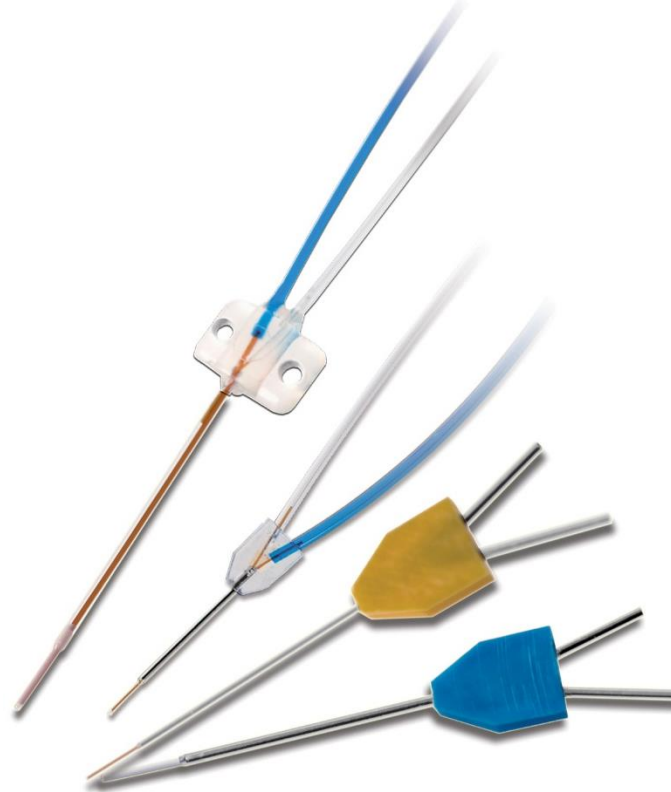
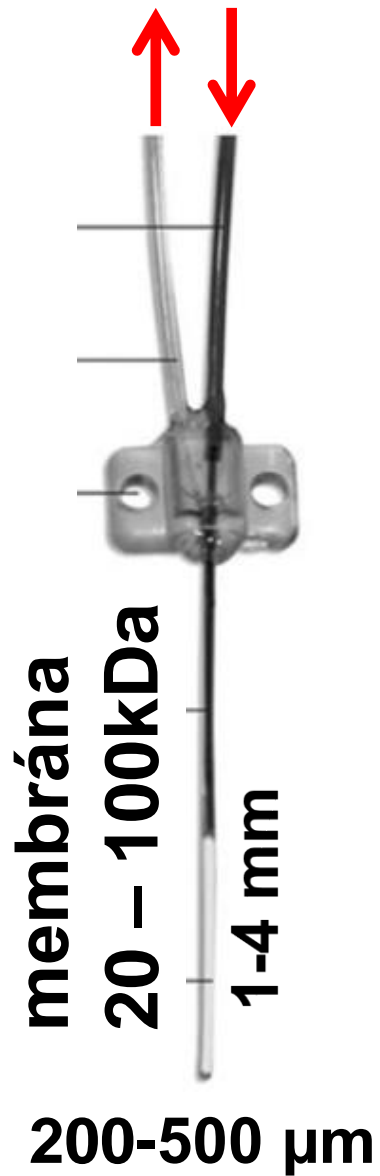
Autoři děkují GAČR, grant 18-04902S a 17-12648S
za poskytnutou finanční podporu a Vám všem
děkuji za pozornost.



Princip mikrodialýzy

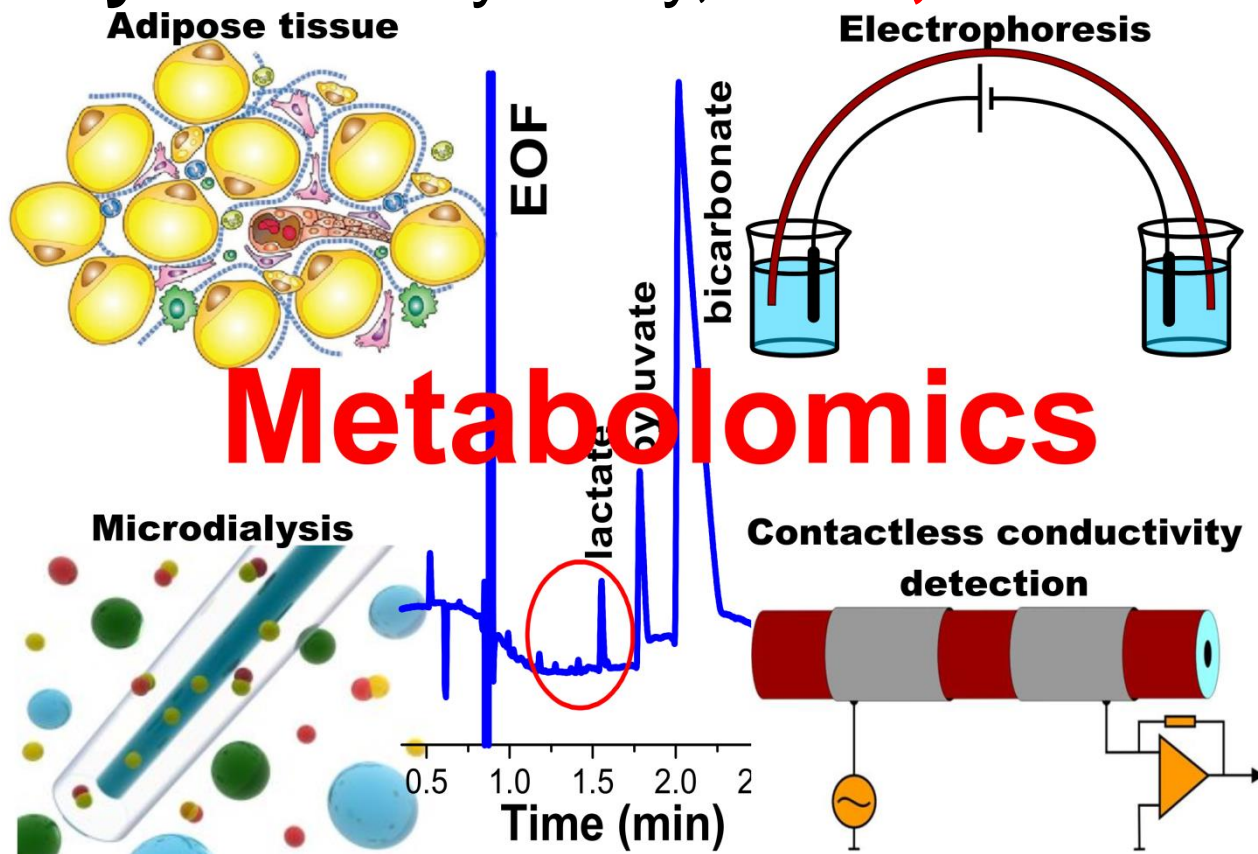


Mikrodialyzační sondy

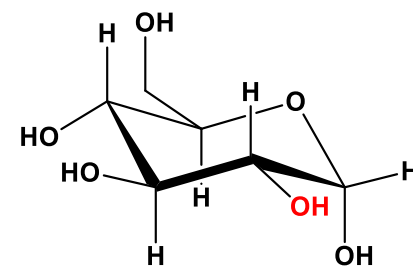
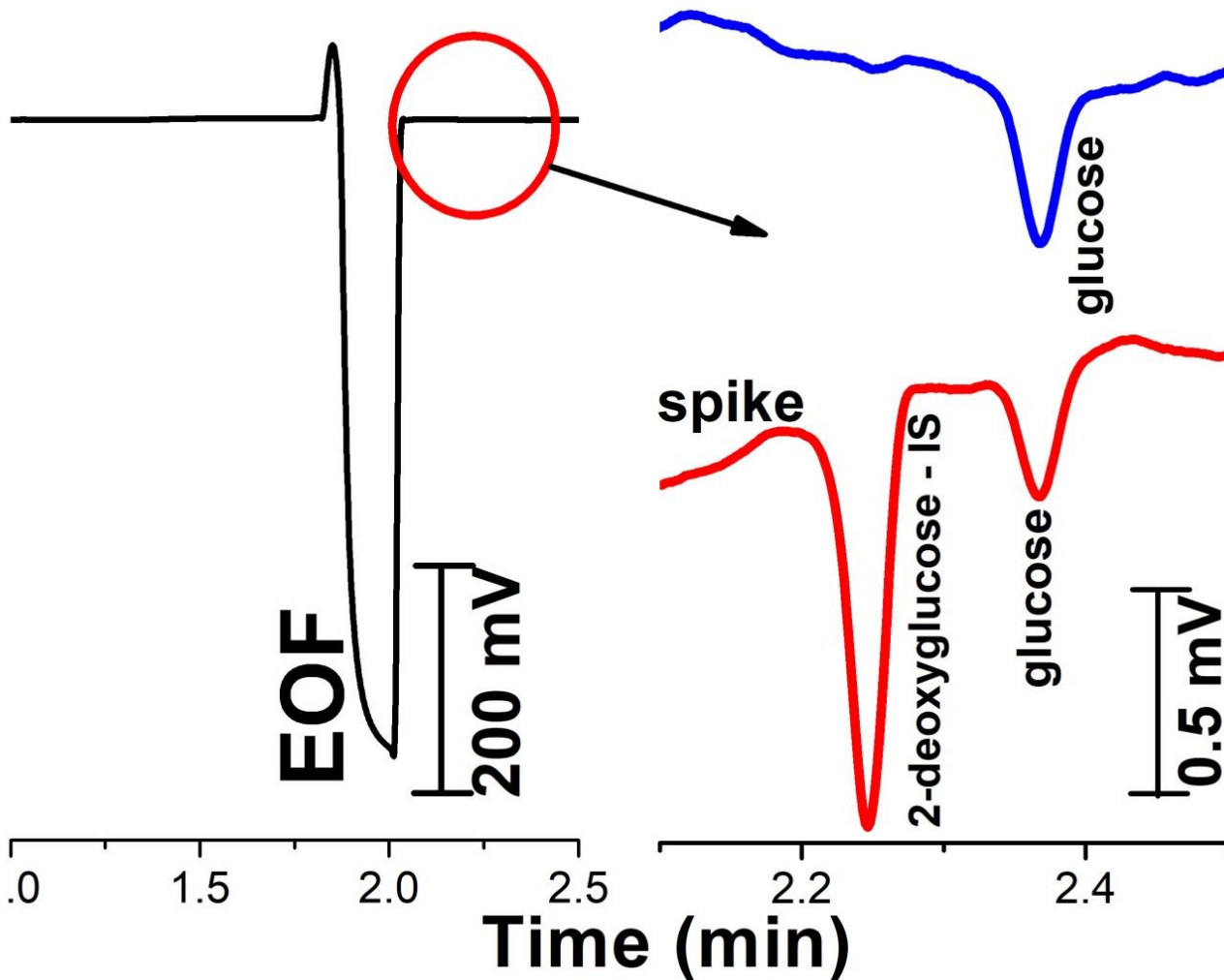
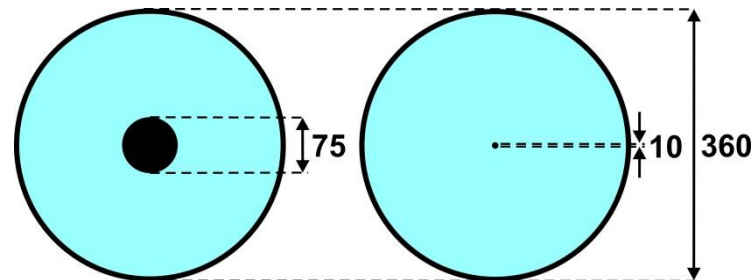


Metabolismus tukové tkáně

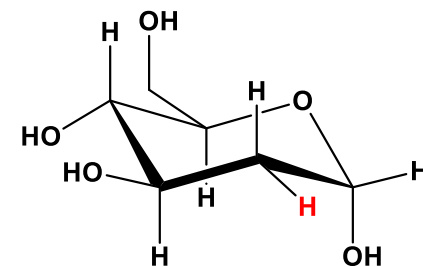
- lipidy – **glycerol** + mastné kyseliny
- sacharidy – **glukóza** + **laktát**
- proteiny – aminokyseliny, **Valin, Leucin a Isoleucin**



CE/C⁴D - glukóza



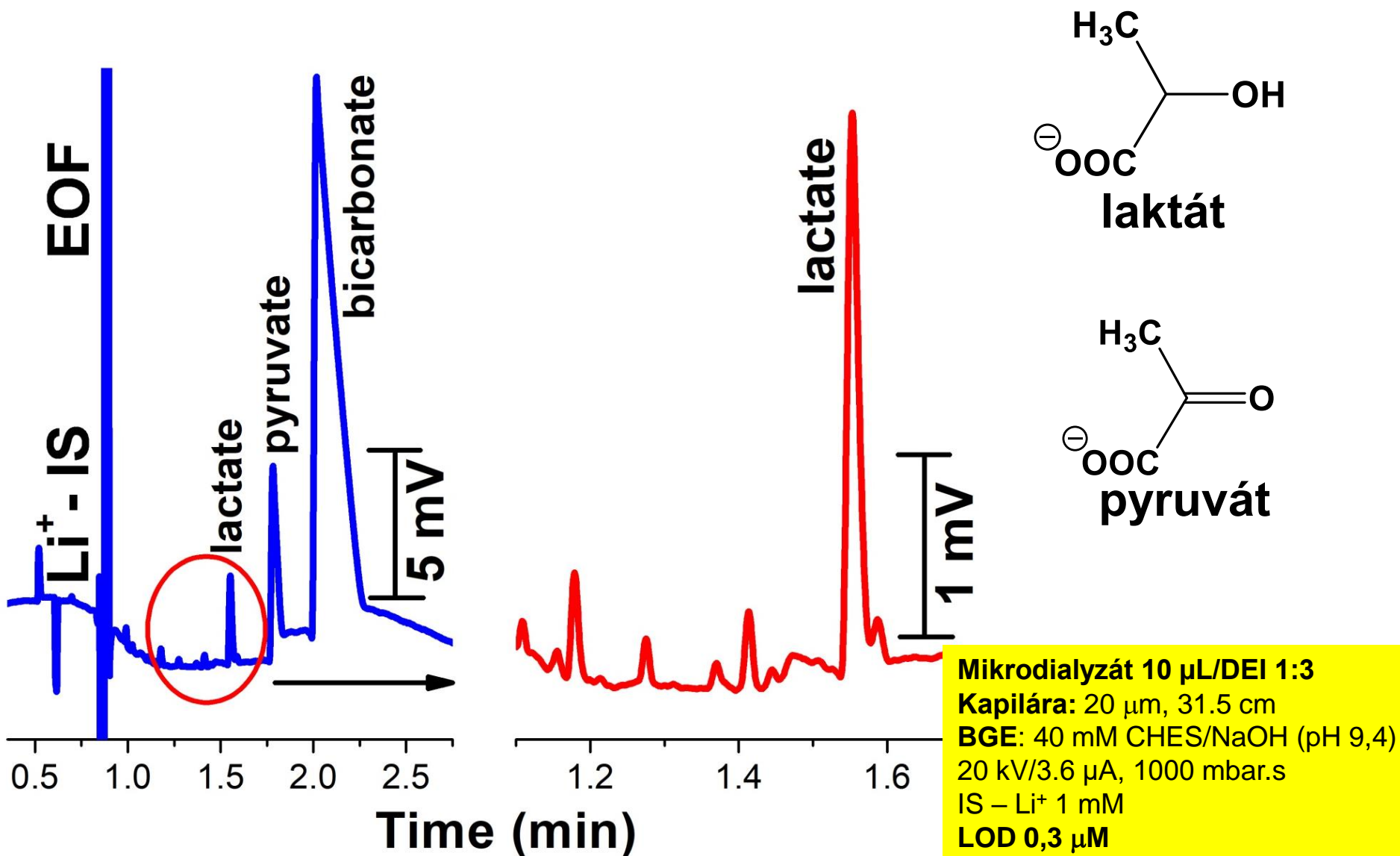
glukóza



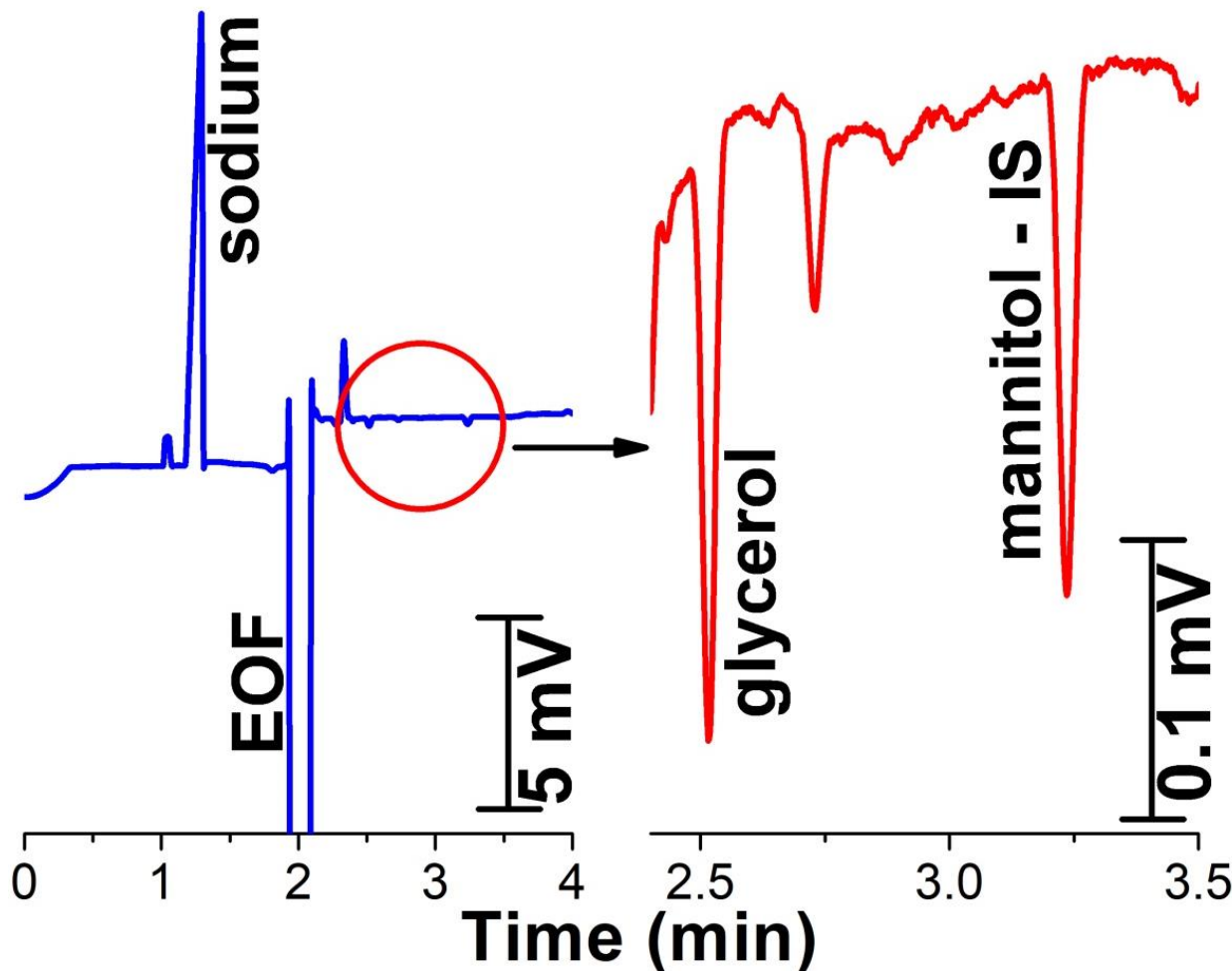
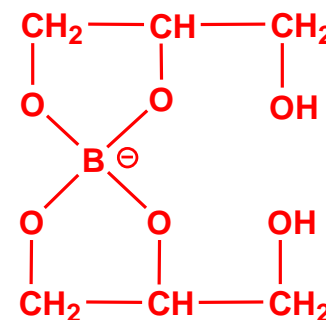
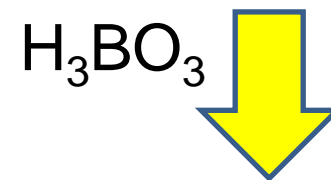
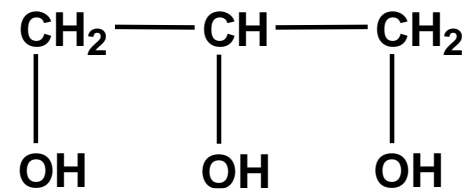
2-deoxyglukóza

Mikrodialyzát 10 μ L/ACN 1:3
Kapilára: 10 μ m, 31.5 cm
BGE: 50 mM NaOH (pH 12,6)
10 kV/2 μ A, 1000 mbar.s
IS – 2-deoxyglukóza 100 mg/L
LOD 3,0 μ M

CE/C⁴D - laktát



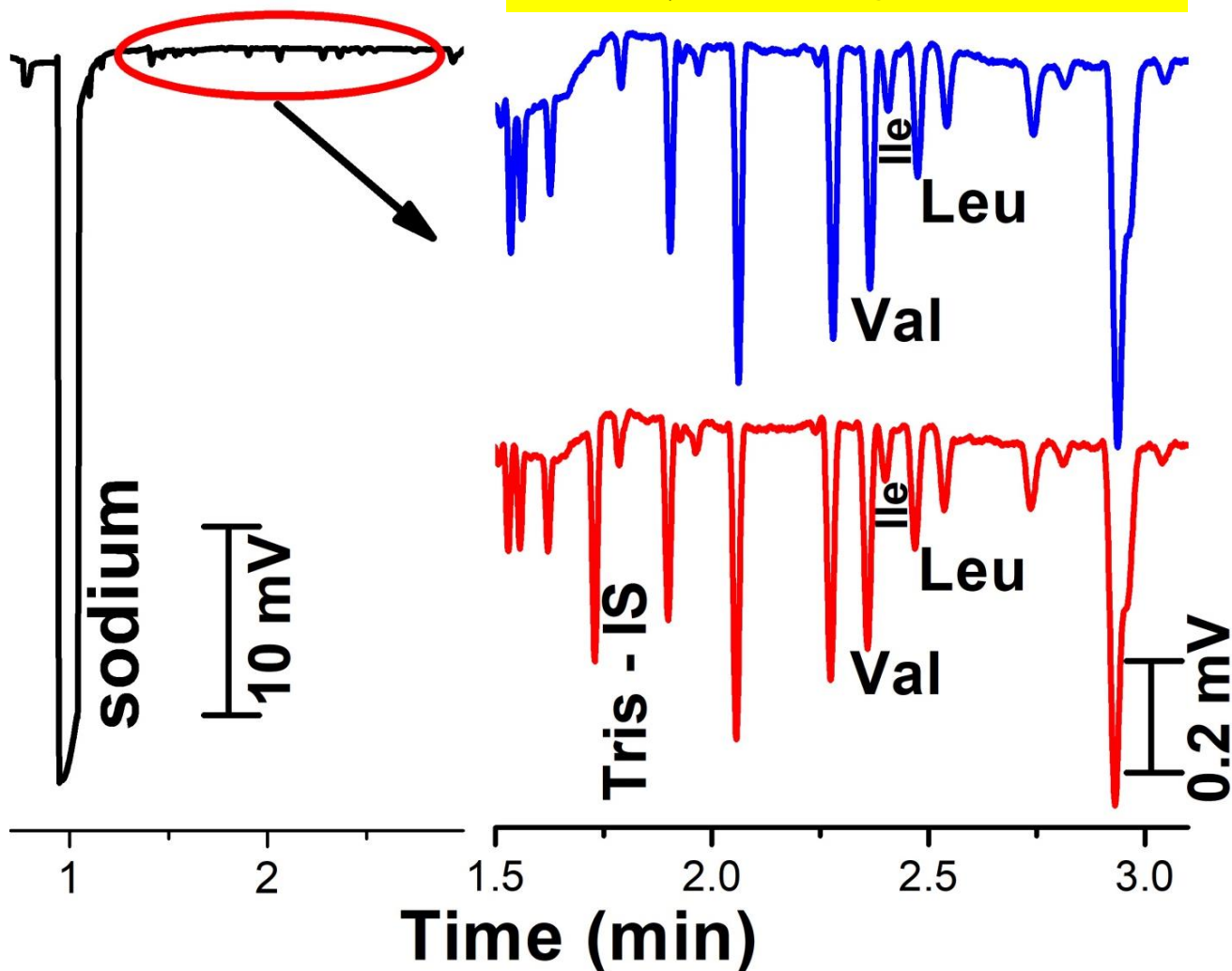
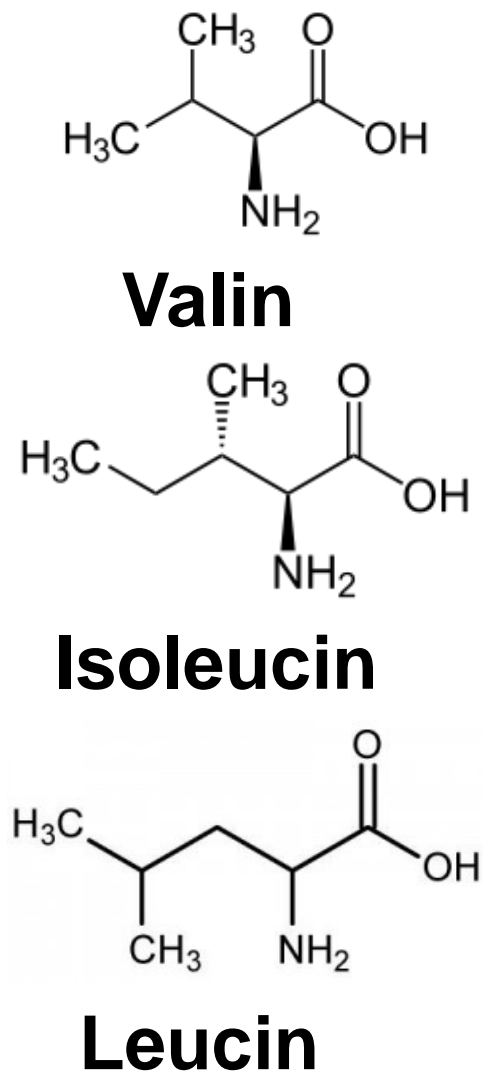
CE/C⁴D - glycerol



Mikrodialyzát 10 µL/ACN 1:3
Kapilára: 20 µm, 31.5 cm
BGE: 100 mM H₃BO₃/LiOH (pH 9,5)
10 kV/3.5 µA, 2000 mbar.s
IS – manitol 100 µM
LOD 2.1 µM

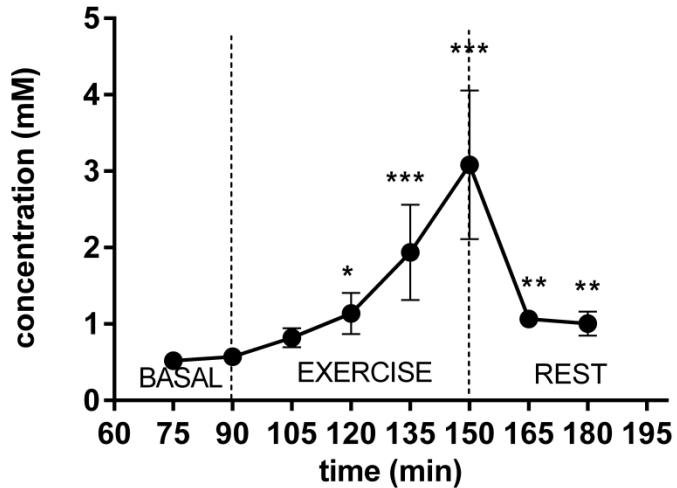
CE/C⁴D - BCAA

Mikrodialyzát 10 µL/ACN 1:3
Kapilára: 25 µm, 31.5 cm, INST-coated
BGE: 2,1 M Hac + 20% MeOH (pH 2,1)
30 kV/4 µA, 800 mbar.s
IS – Tris 10 µM
LOD 0,1 µM - stacking

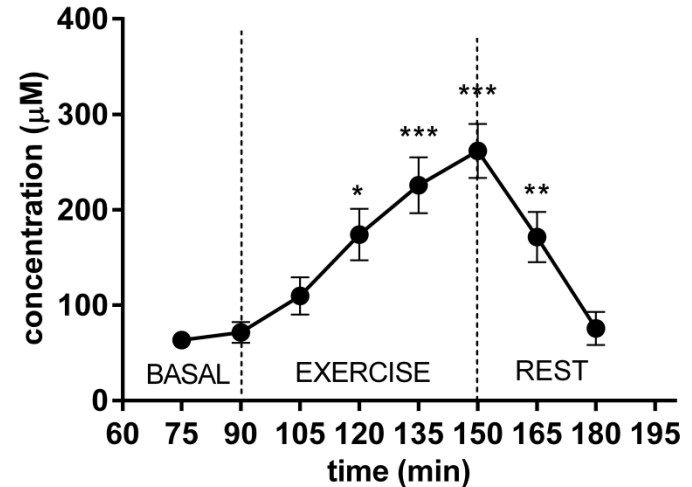


Sekvenční monitoring metabolitů

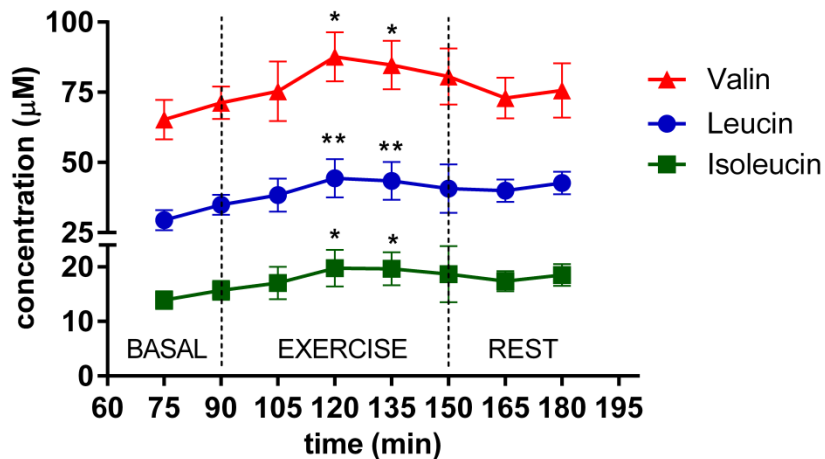
Lactate in dialysate



Glycerol in dialysate



BCAA in dialysate



Consent to Publish

Series Title:
MiMB



Title of Book/Volume/Conference: Clinical Applications of Capillary Electrophoresis: Methods and Protocols, Second Edition

Editor(s) name(s): Terry Phillips, PhD., D.Sc.

Title of Contribution: The Control of Glucose and Lactate Levels in Nutrient Medium after Cell Incubation and in Microdialysates of Human Adipose Tissue by Capillary Electrophoresis with Contactless Conductivity Detection

Author(s) full name(s): Petr Tůma

Consent to Publish

Series Title:
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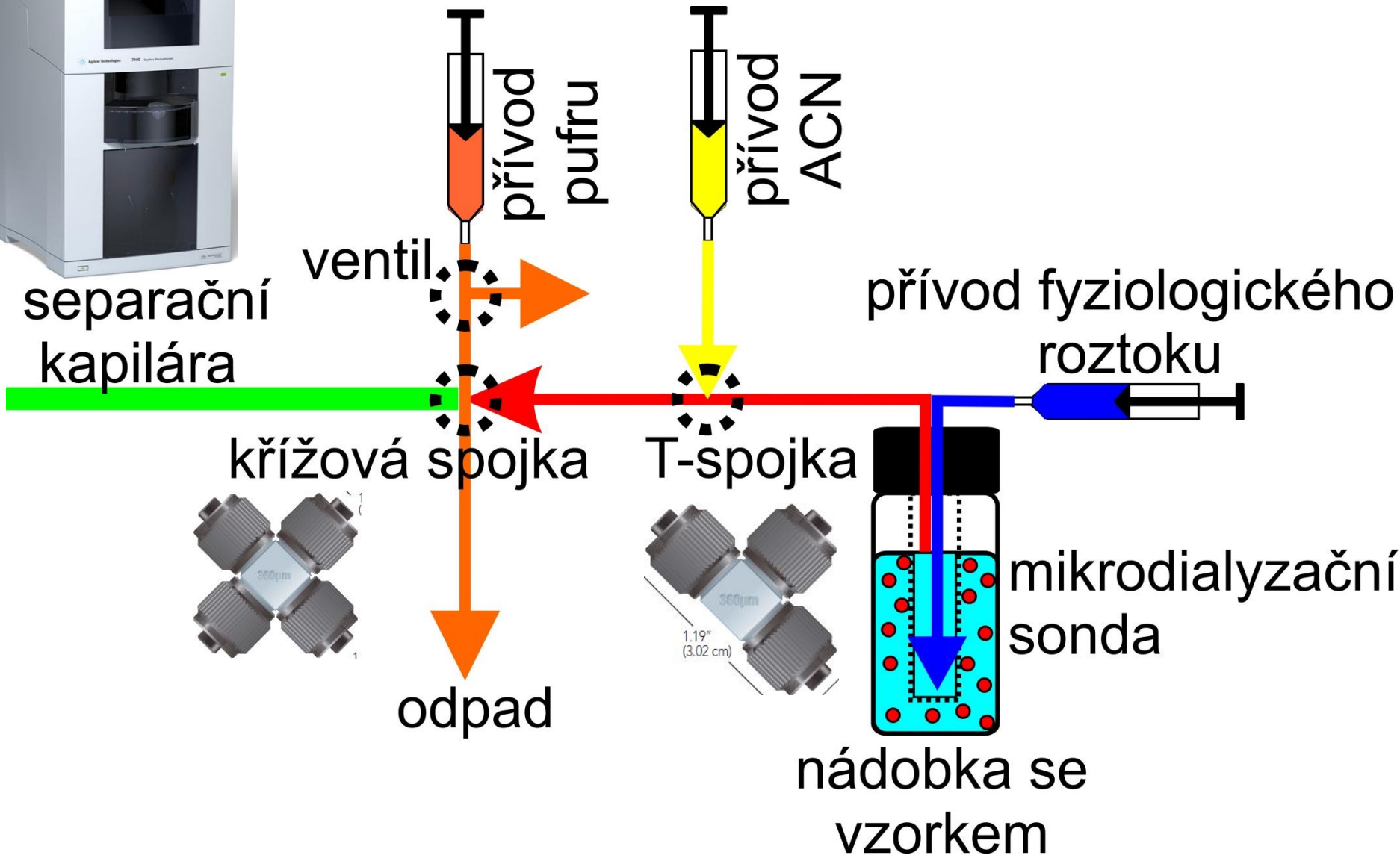
Title of Book/Volume/Conference: Clinical Applications of Capillary Electrophoresis: Methods and Protocols, Second Edition

Editor(s) name(s): Terry Phillips, PhD., D.Sc.

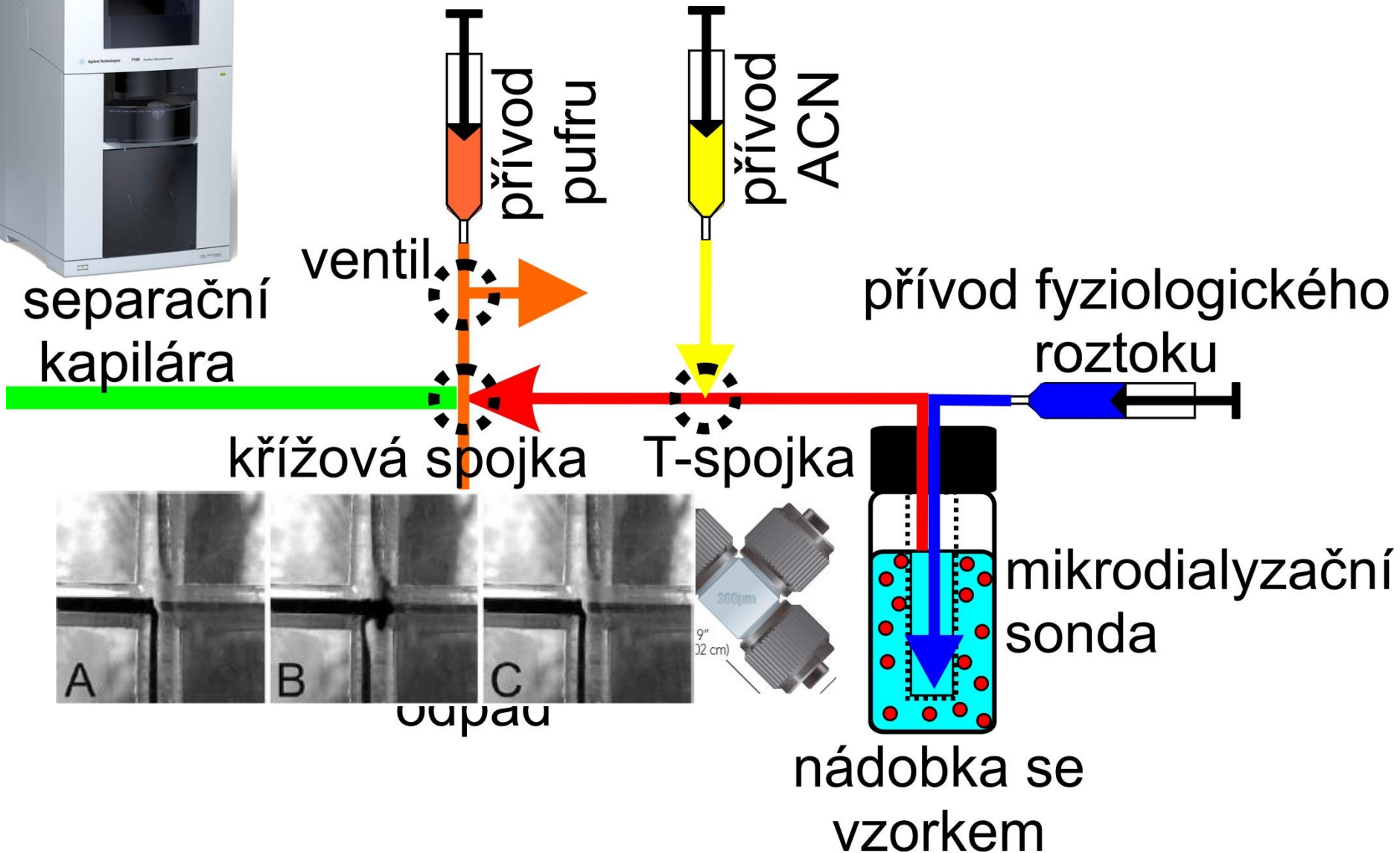
Title of Contribution: Rapid and Sensitive Determination of Branched-Chain Amino Acids in Human Plasma by Capillary Electrophoresis with Contactless Conductivity Detection for Physiological Studies

Author(s) full name(s): Petr Tůma

On-line spojení mikrodialýzy, úpravy vzorku a CE analýzy



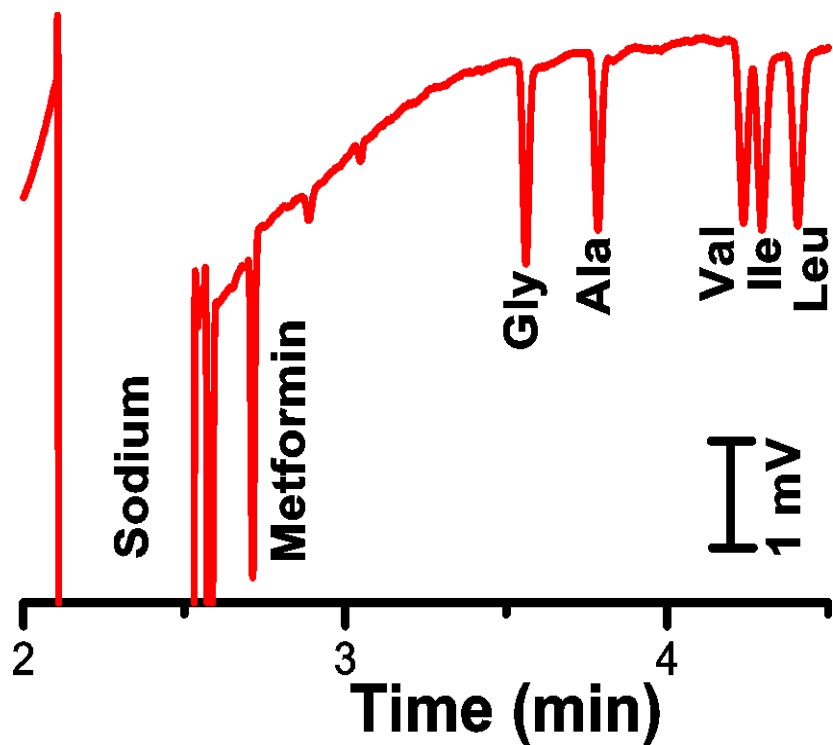
On-line spojení mikrodialýzy, úpravy vzorku a CE analýzy



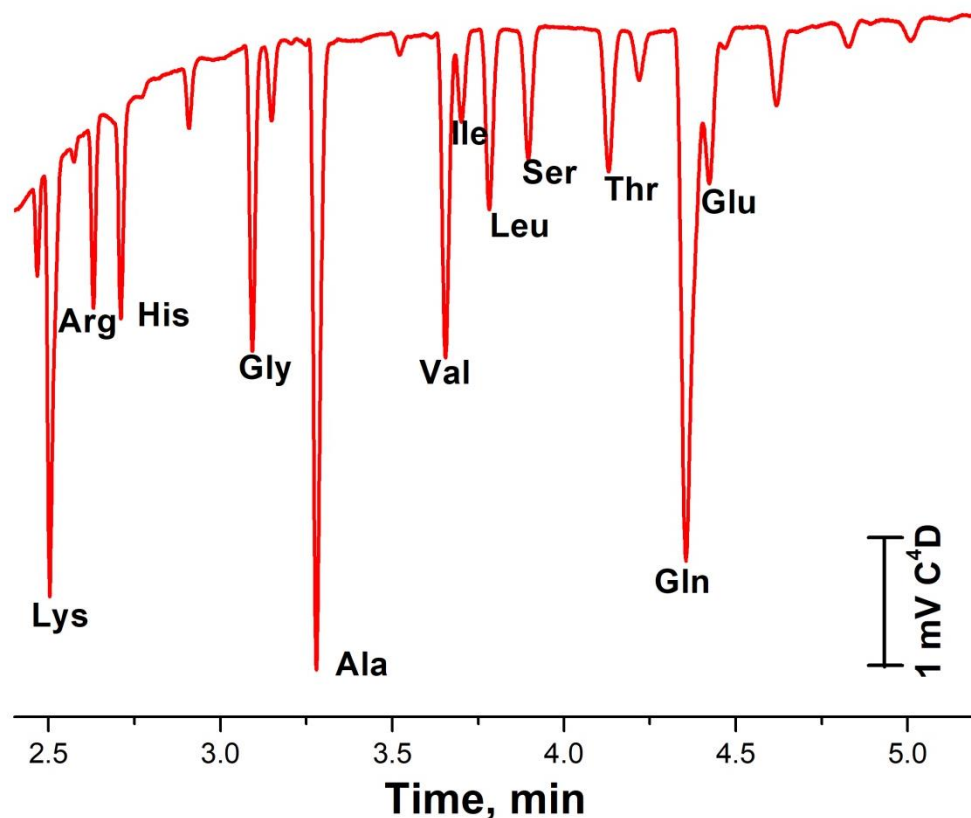


On-line analýza aminokyselin

100 μM směs aminokyselin
ve 150 mM NaCl



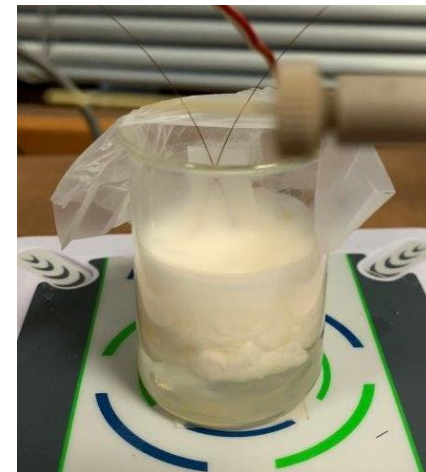
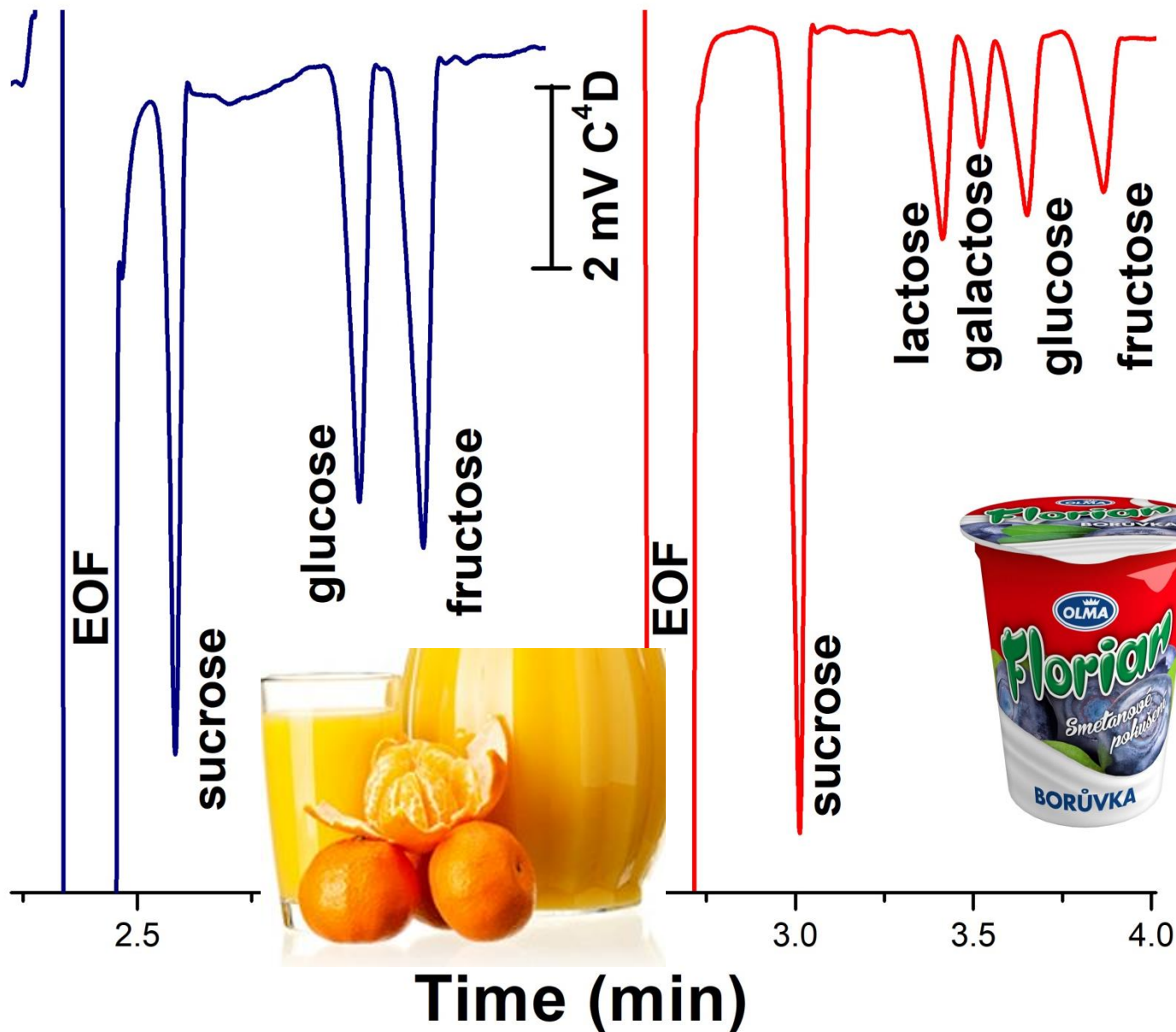
Lidská krevní plasma



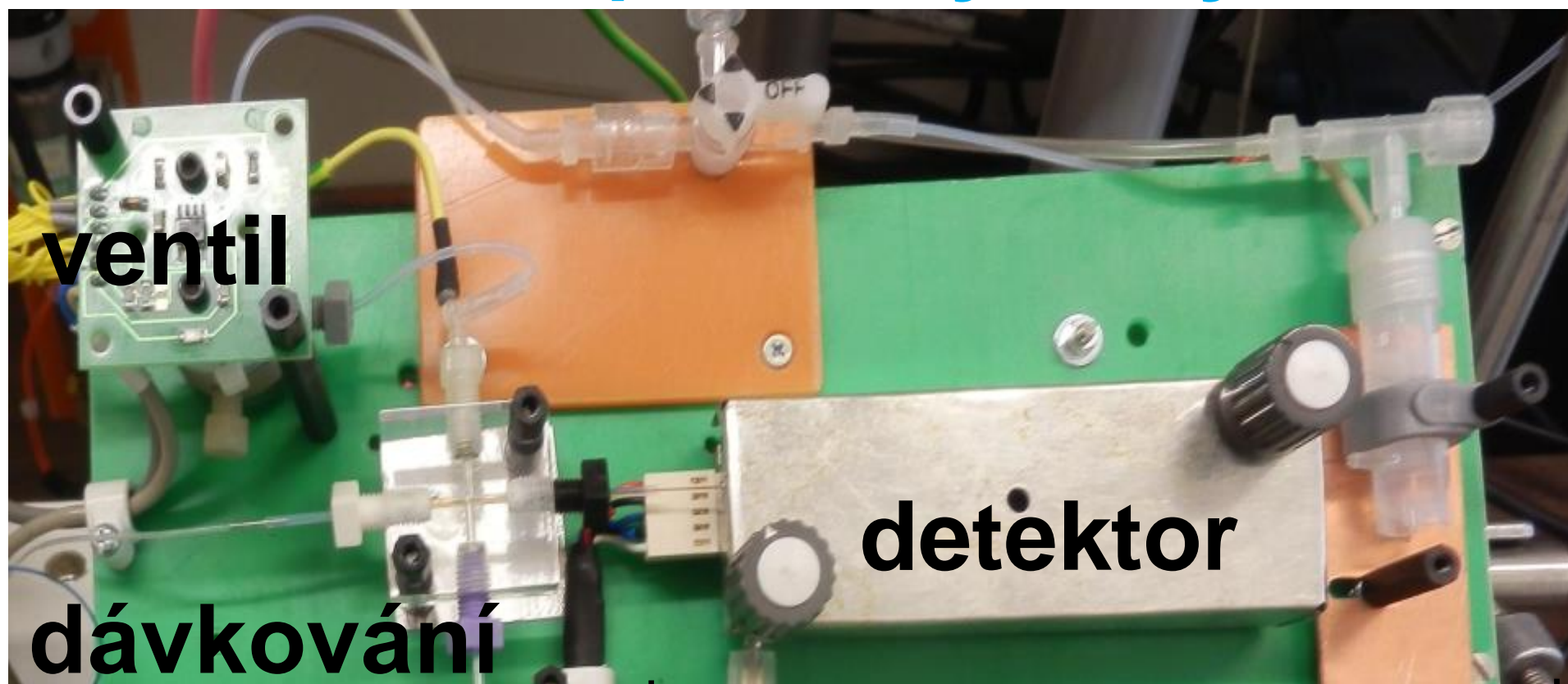
mikrodialyzační sonda: cut-off 20 kDa
promývání fyz. roztokem: 2,5 $\mu\text{L}/\text{min}$
úprava vzorku: ACN s 0,01M HCl 7,5 $\mu\text{L}/\text{min}$
flow gating solution: BGE 50 $\mu\text{L}/\text{min}$

kapilára: INST-coated 25 μm , 40 cm, 25 cm C⁴D,
BGE: 3,2 M Hac + 20% MeOH + 1% INST (pH 2,1)
separace: 30 kV/4 μA
hydrodynamické dávkování: 800 mbar.s

On-line analyza sacharidů

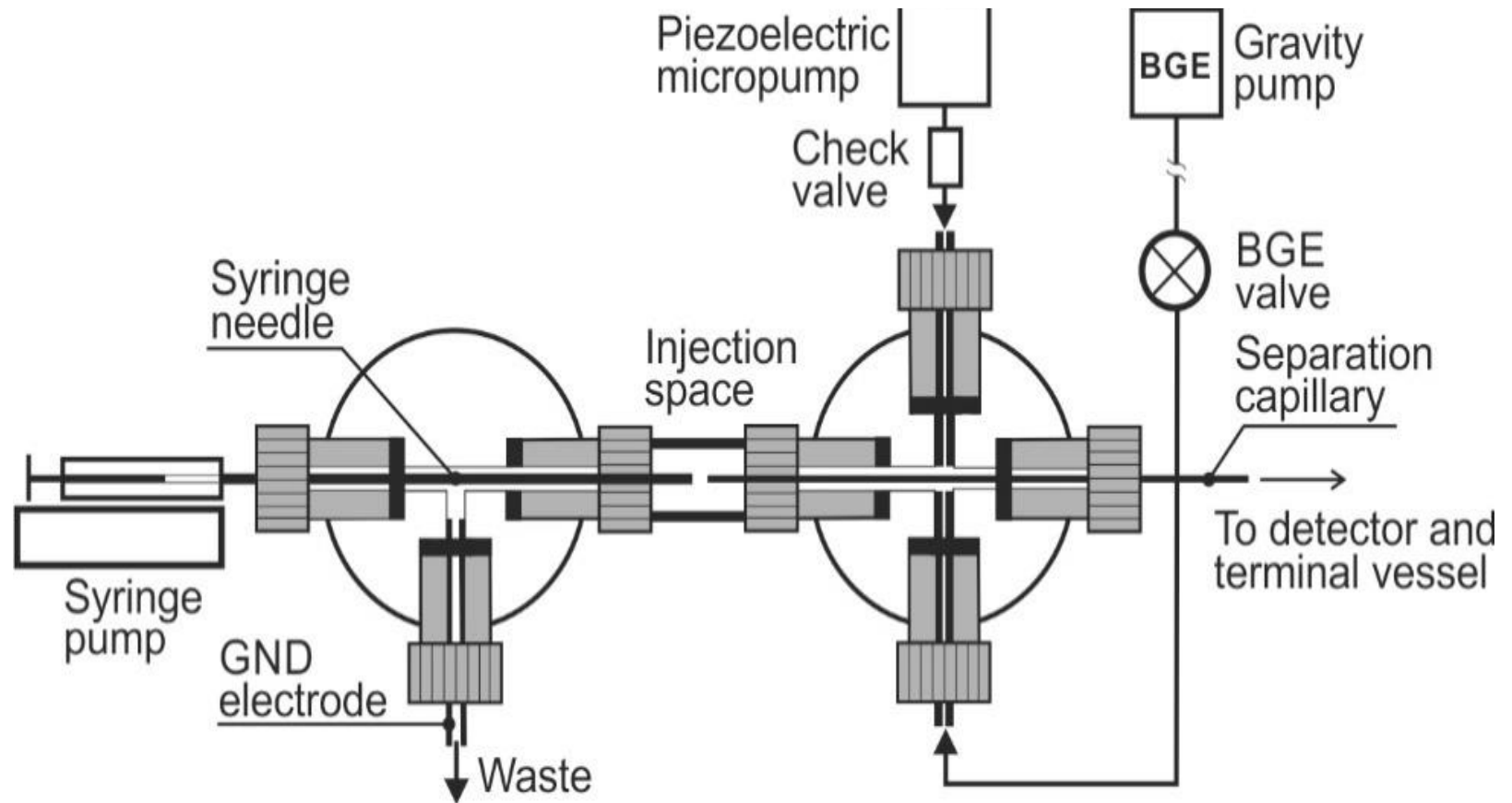


Modulární a přenosný analyzátor

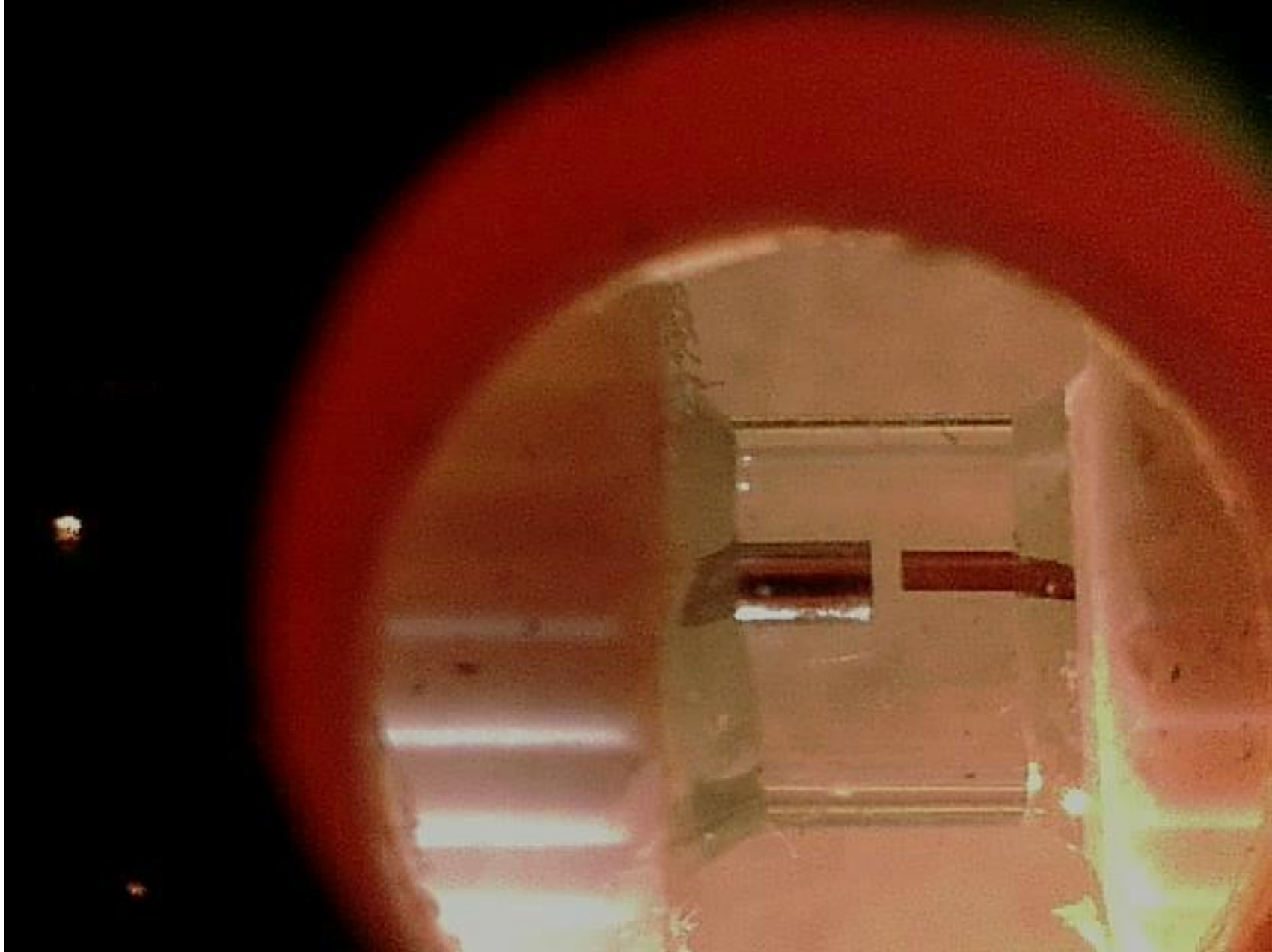


10 cm

Miinjector pro 0,2 μL vzorku

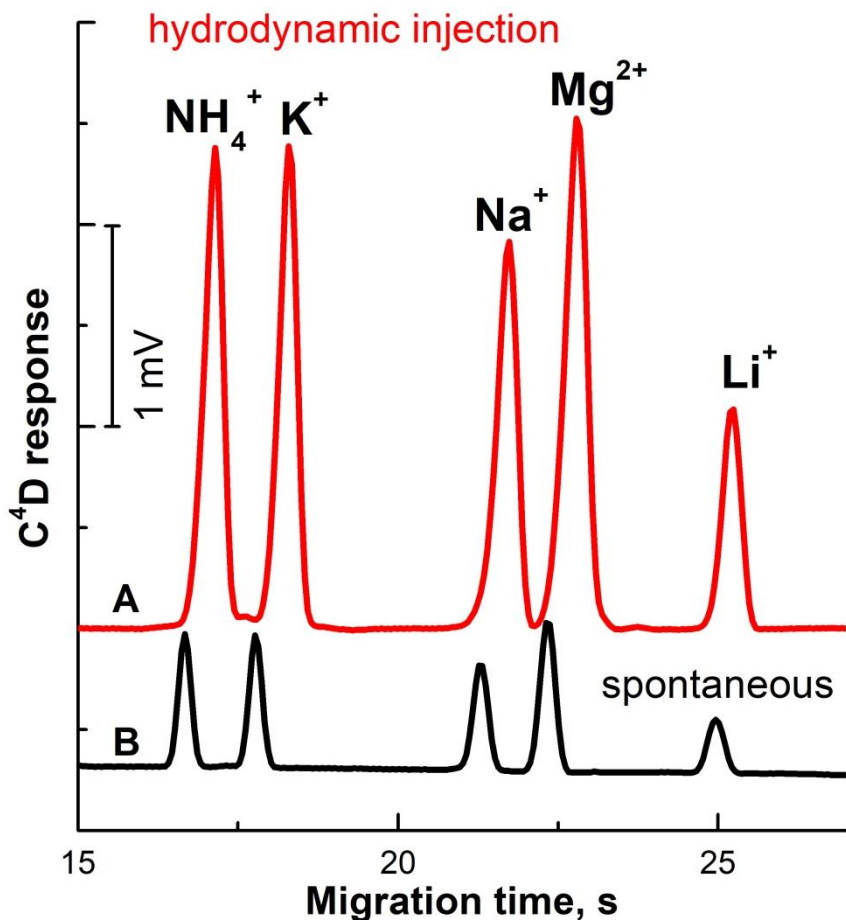


Opekar, Tůma: *Anal. Chim. Acta* 2018, 1042, 133-140.



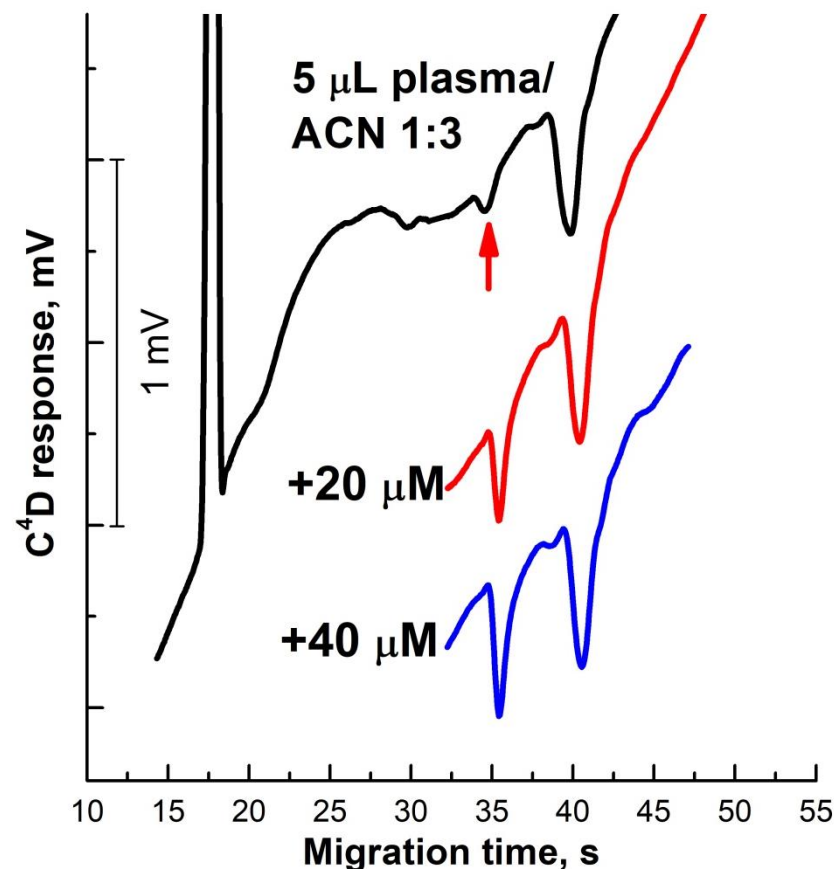
Aplikace – analýza 0,2 μL vzorku

Minerály ve vodách

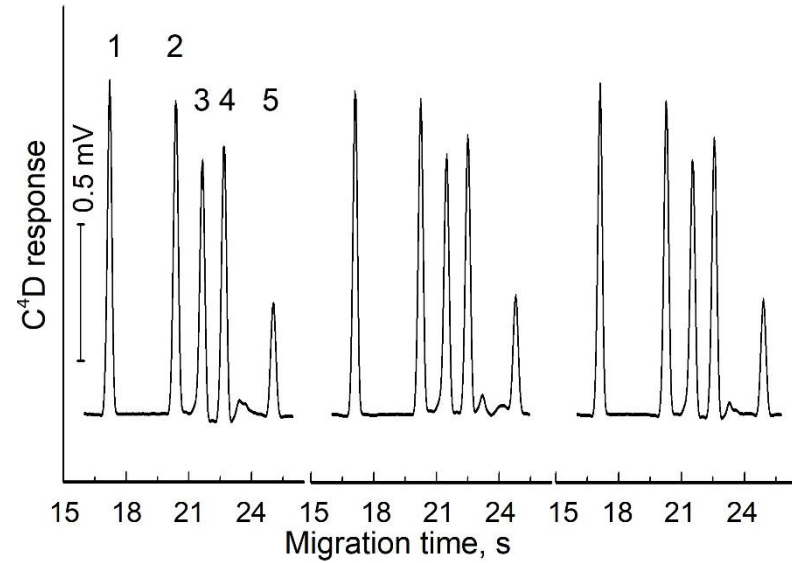
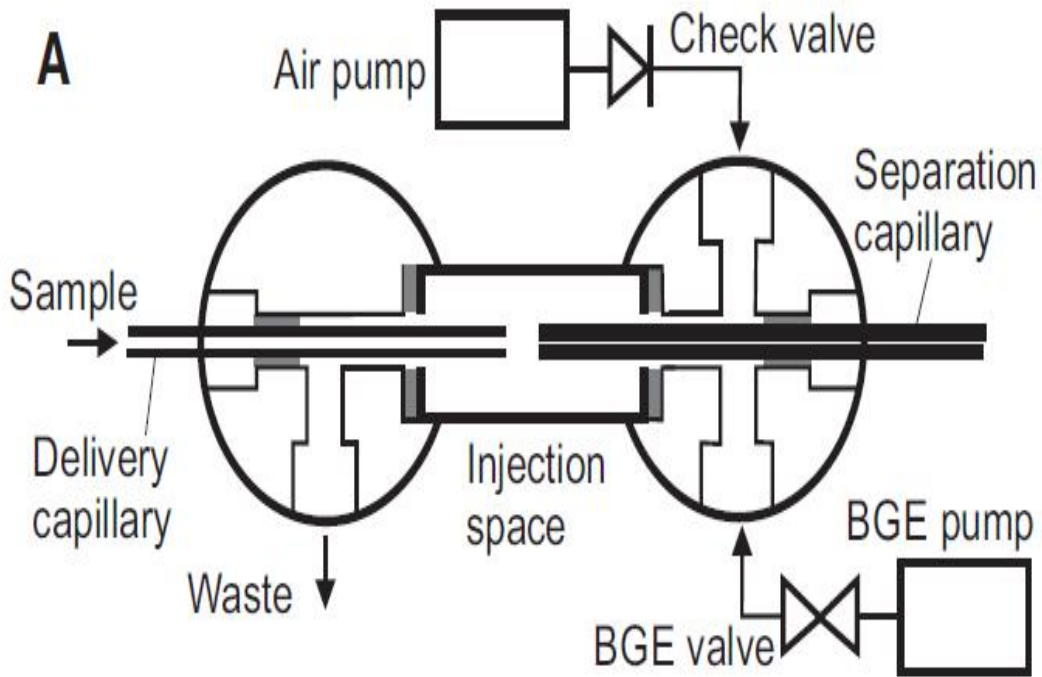


kapilára: 25 μm, 16,5 cm, 10,5 cm C⁴D
BGE: 50 mM HAc + 20 mM Tris + 2 mM 18-crown-6
separace: 10 kV/6 μA, 250 mbar.s
vzorek: 100 μM modelová směs

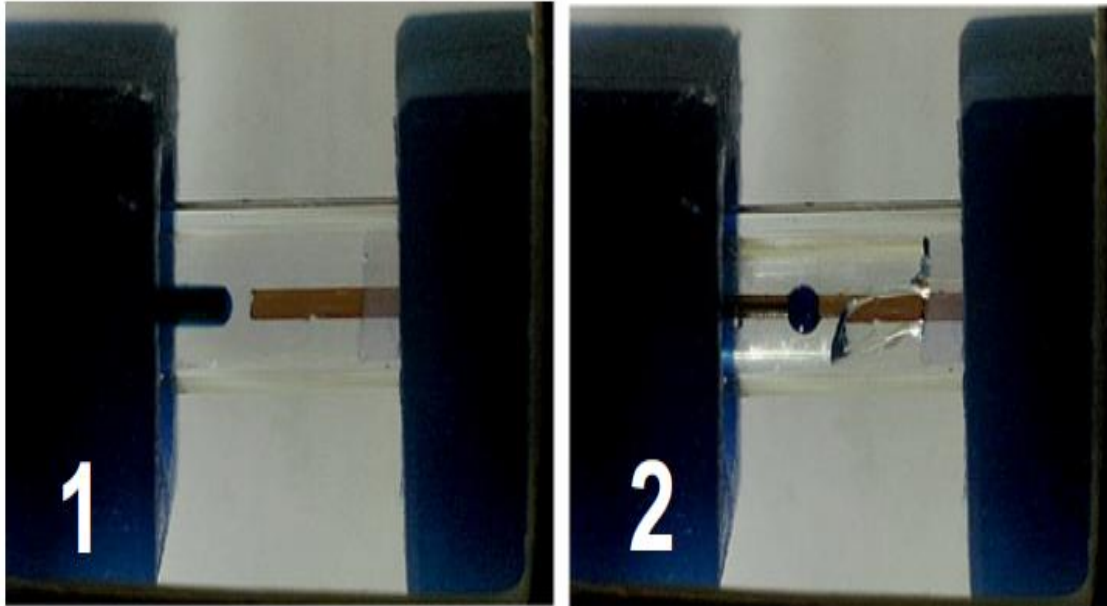
Pentamidin v krvi



kapilára: 25 μm, 16,5 cm, 10,5 cm C⁴D
BGE: 100 mM HAc + 50 mM NaOH
separace: 10 kV/18 μA, 150 mbar.s
vzorek: 5 μL plasmy pacienta + 15 μL ACN



B



Opekar F., Tůma P.: **An air-assisted flow-gating interface for capillary electrophoresis**, *Electrophoresis* 2019, 40, 1-5.