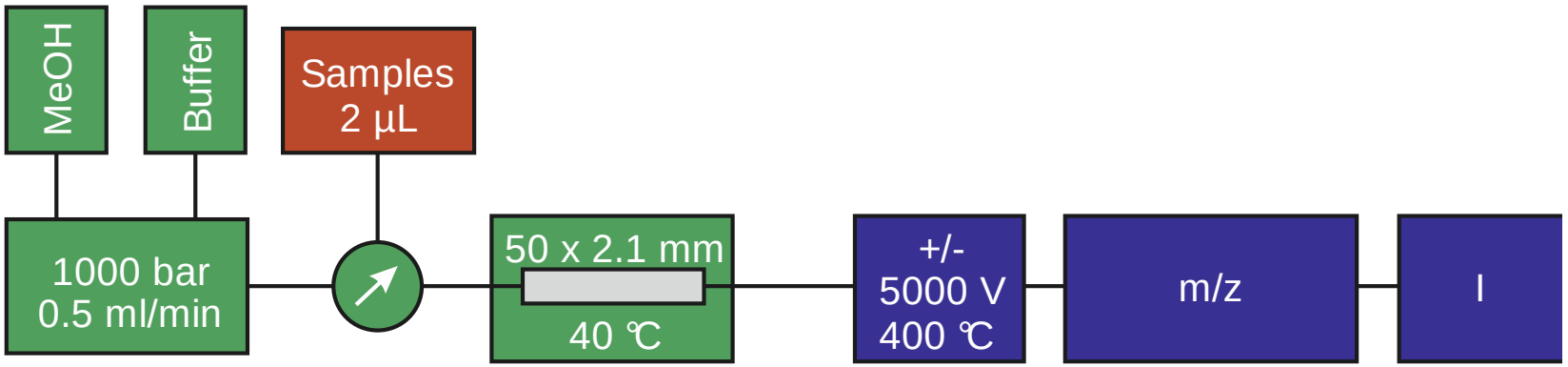
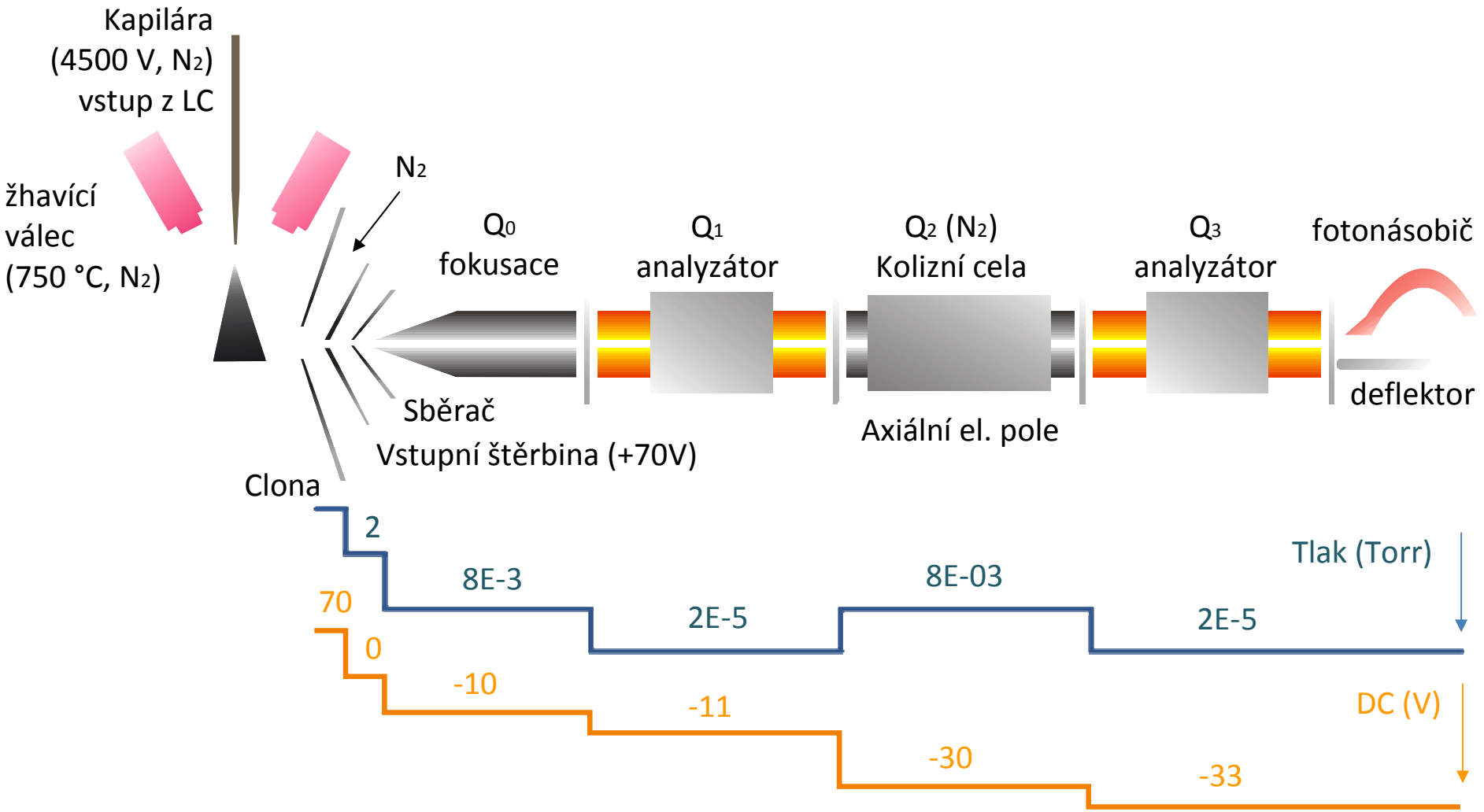
# Limity využití tandemové hmotnostní spektrometrie v klinické biochemii

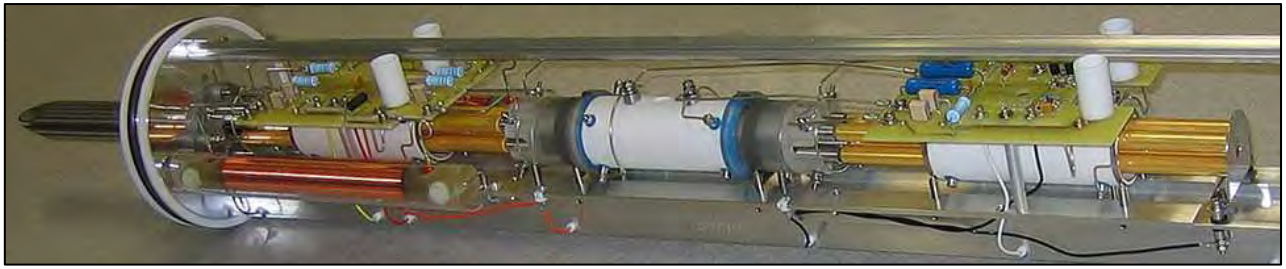
**D. Friedecký**

*Laboratoř dědičných metabolických poruch*

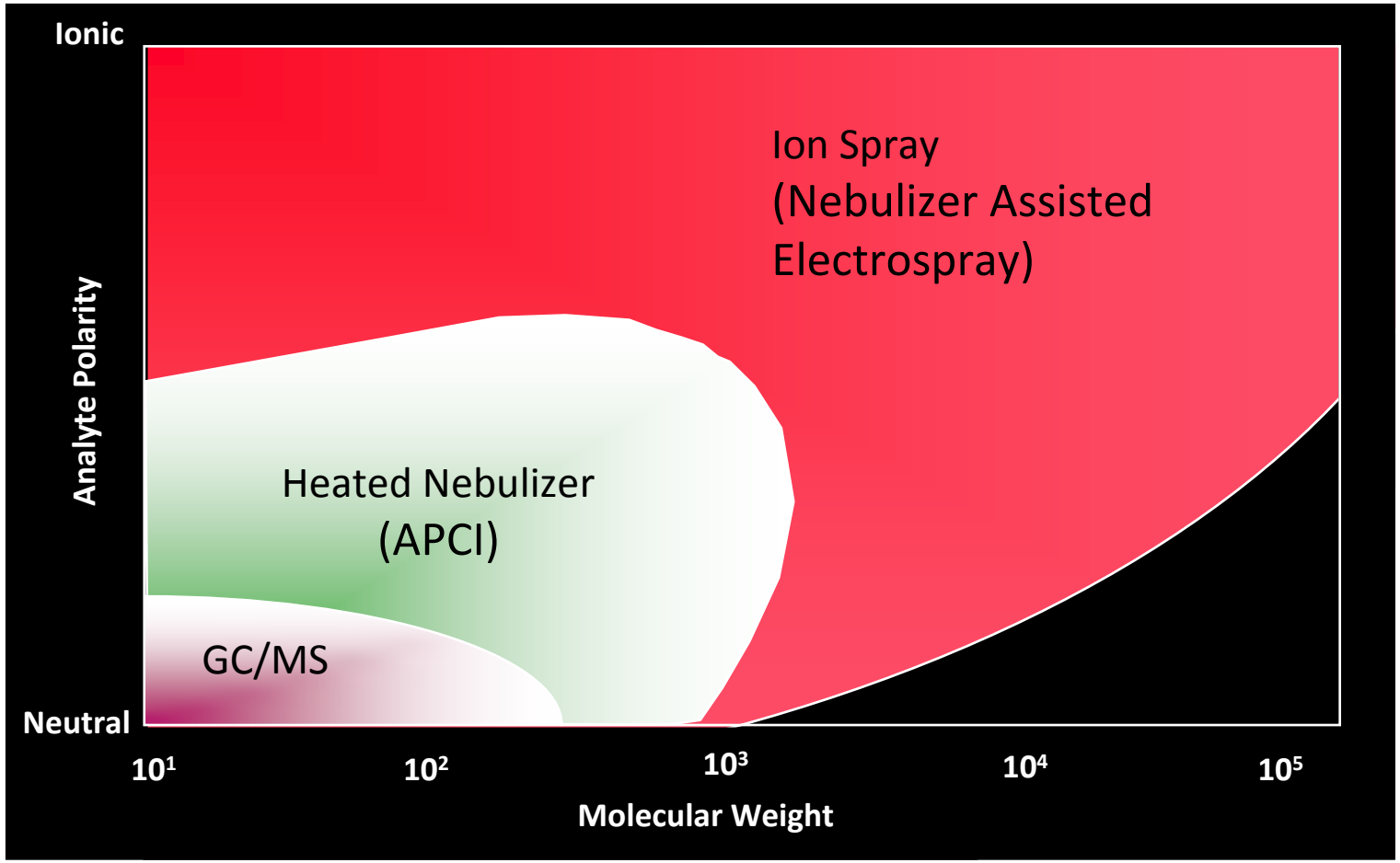
*FN Olomouc*



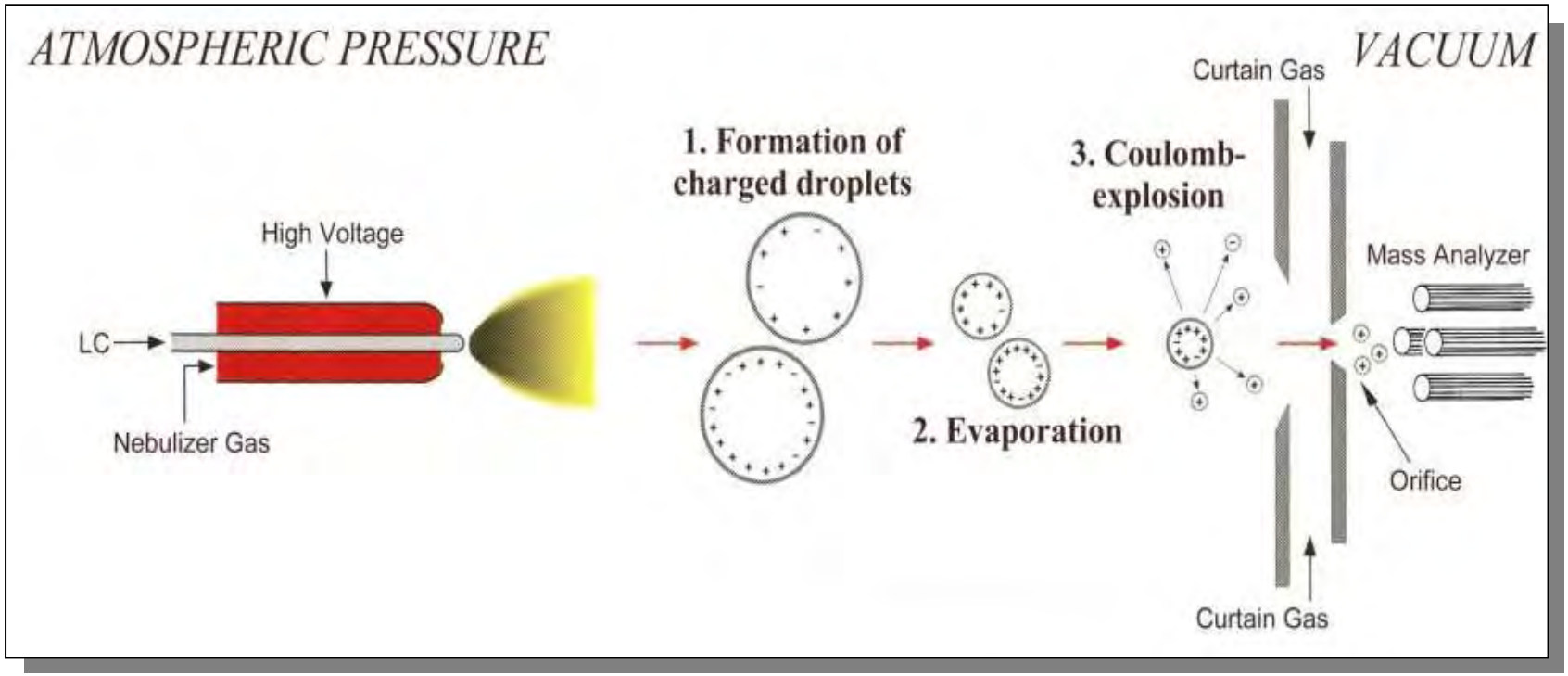

Kapilára
(4500 V, N2)
vstup z LC
žhavící
válec
(750 °C, N2)
N2
Q0
fokusace
Q1
analyzátor
Q2 (N2)
Kolizní cela
Q3
analyzátor
fotonásobič
deflektor
Sběrač
Vstupní štěrbina (+70V)
Axiální el. pole
Clona
2
8E-3
2E-5
8E-03
2E-5
Tlak (Torr)
70
0
-10
-11
-30
-33
DC (V)



# Ionization Methods

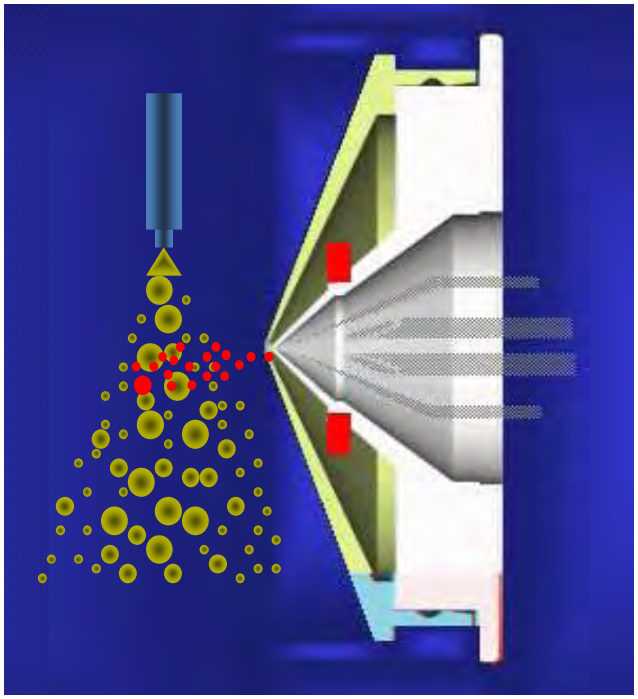


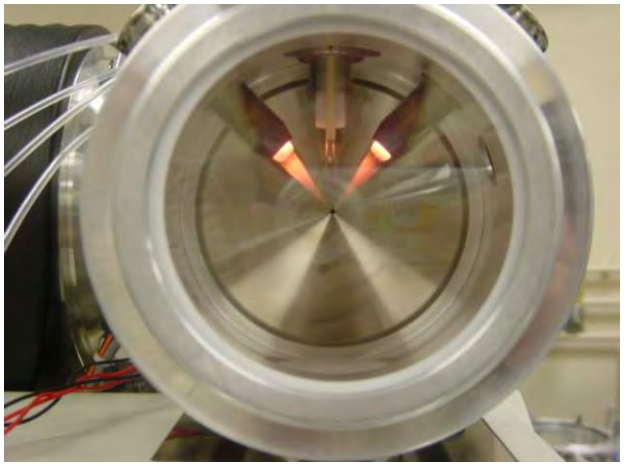
# Ionization Methods - ESI



# Ionization Methods - ESI

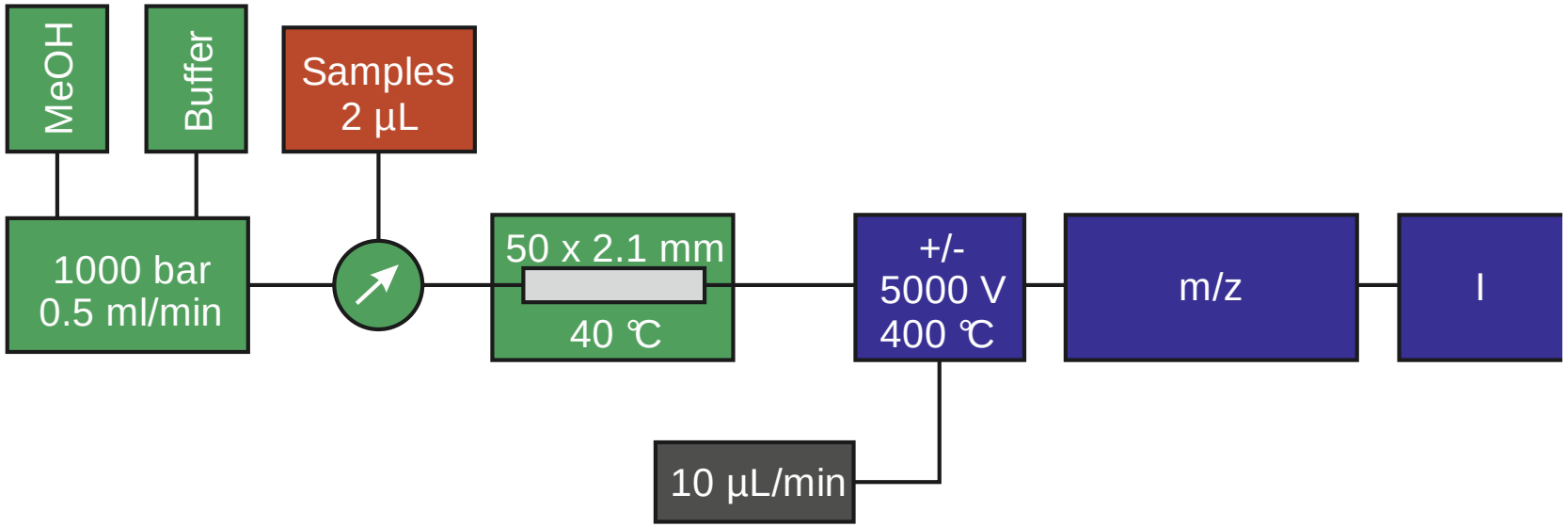
- Low transfer of ions
- Soft ionisation technique
- Adducts with mobile phase
- Ion suppression
- Sensitivity dependent on structure





# Ion suppression study

- Online postcolumn injection STD




- Ratio Sample/STD

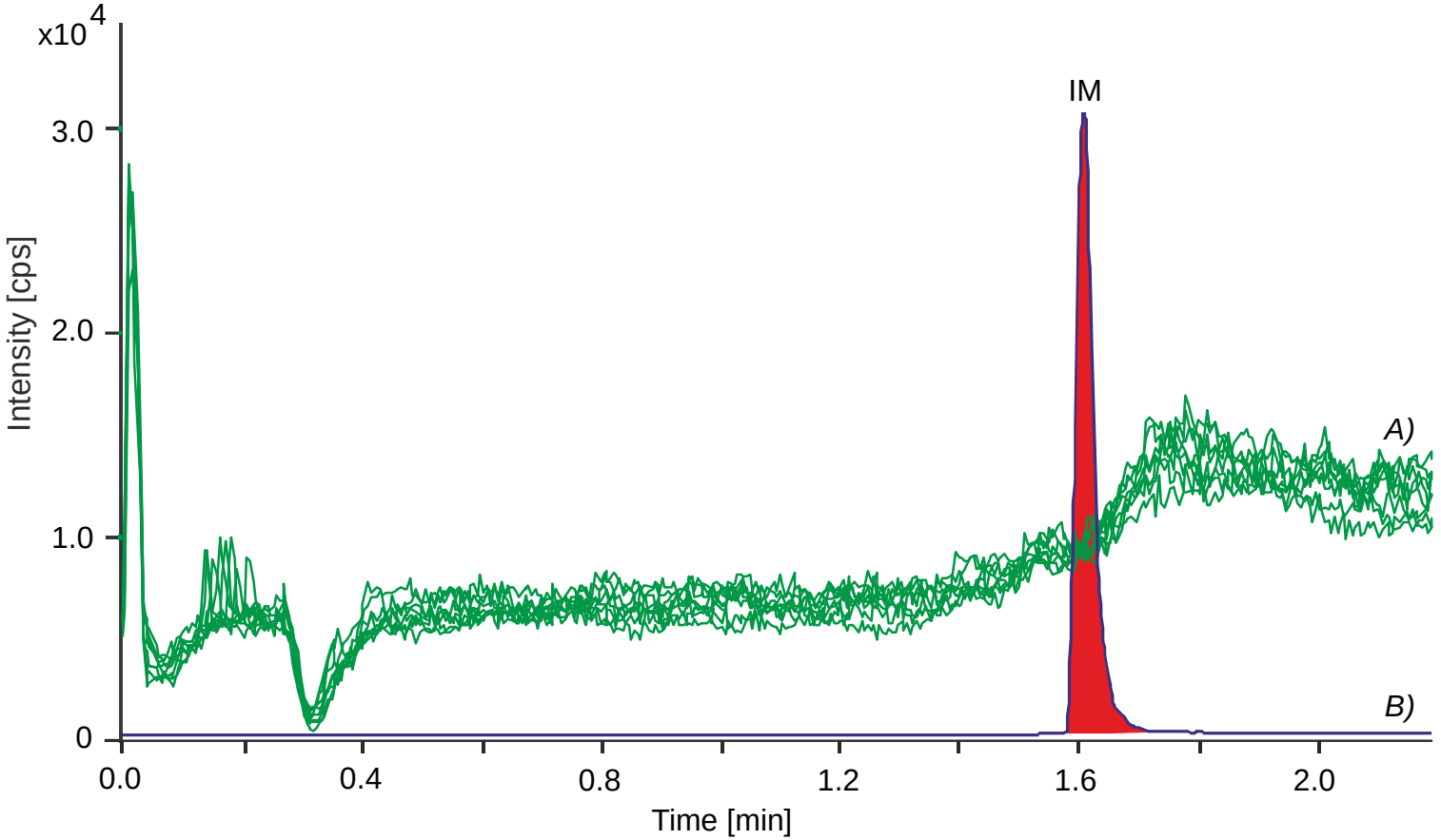
$$\text{Ion suppression \%} = \left[1 - \frac{I_{std\ in\ blank} - I_{blank}}{I_{std\ in\ MeOH}}\right] \times 100$$

# Stanovení TKI LC-MS/MS

Plasma - deproteinace MeOH 1:10, ACQUITY UPLC®BEH C18 (Waters) 1.7 µm, 2.1 x 50 mm

MF: *A)* $NH_4COOH$, *B)* MeOH; 83 % *A* (1.0 min) => 50 % *A* (2.1 min); 40 °C, 0.5 ml/min,

350 => 450 bar, MS: ESI+, LOD: 1.2 ng/ml, celkový čas: 3,2 min

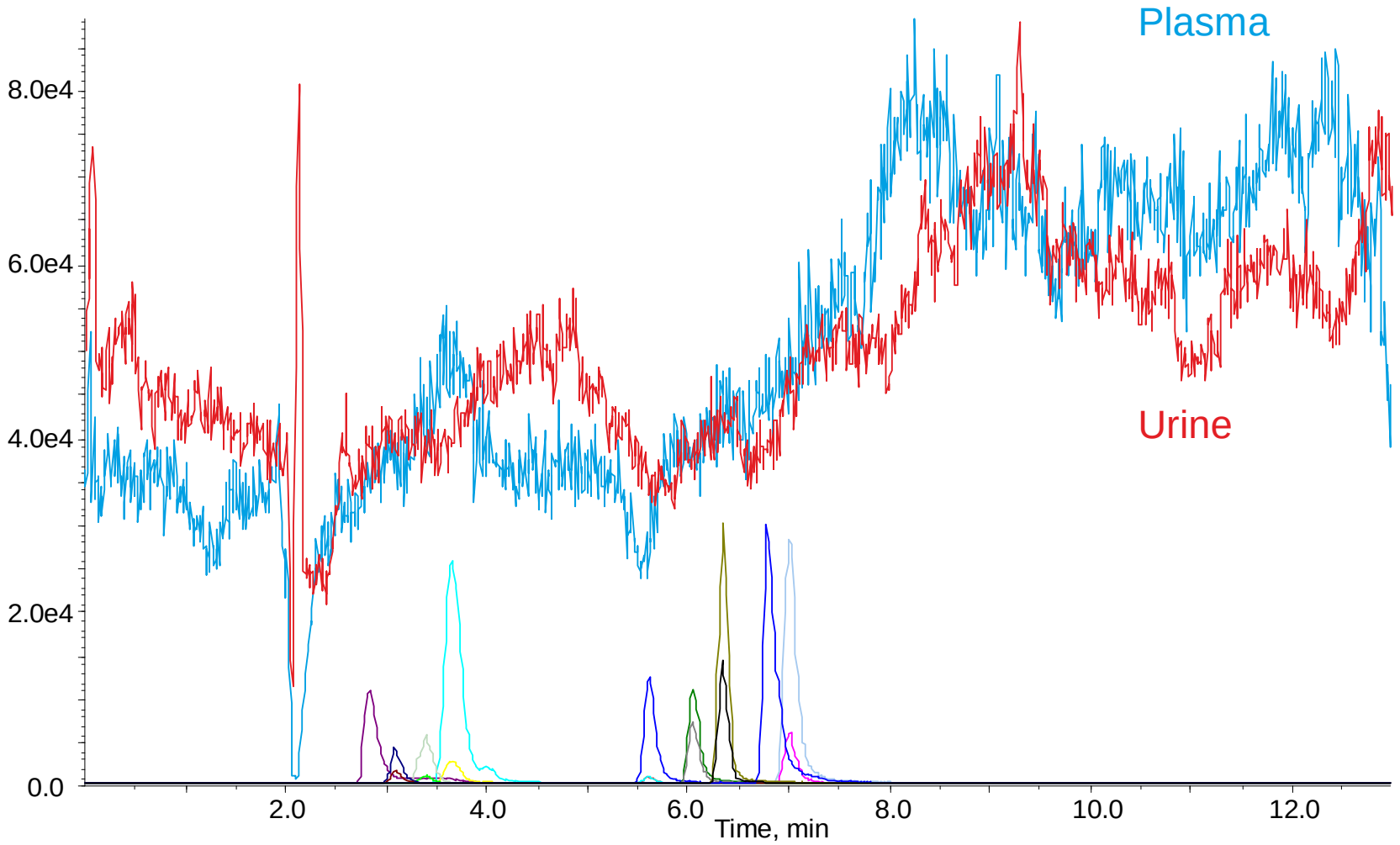


# Stanovení ribosidů PDNS LC-MS/MS

Moč naředěna MF na 0,5 mM kreatinin; Kinetex C18 (Phenomenex); 2.6 µm, 2.1 x 150 mm

MF: *A)* NH4FA (20mM, pH 3.5), *B)* MeOH; 100 % *A* (1.0 min) => 50 % *A* (5.0 min)

40 °C, 0.15 ml/min, 150 => 200 bar; MS: ESI+, 4500 V, 500 °C, 50 ms



# Stanovení TKI FIA-MS/MS

Plasma - deproteinace (MeOH+D8IMA), sonifikace, vortex, zamražení, centrifugace

MF: MeOH + FA, 300/30 µl/min, 80/10 bar; celkový čas analýzy: 45 s

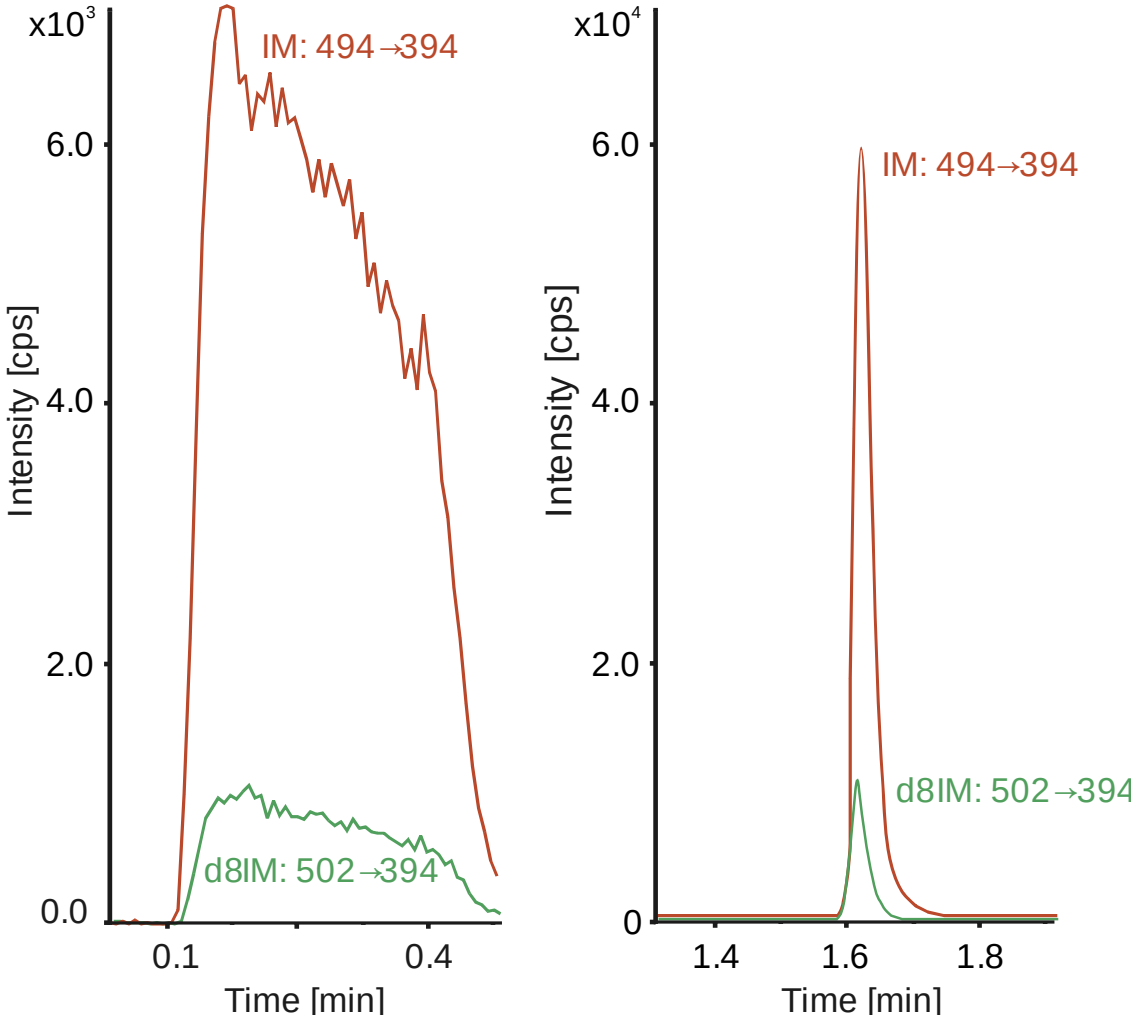
MS: ESI+, 4500 V, 300 °C, 200 ms; LOD: 9.2 ng/ml

**Ion suppressions:**

**FIA: 73.61 - 88.42%**

**LC: 7.21 - 18.86%**

The matrix effects were fully eliminated by the use of d8IM.

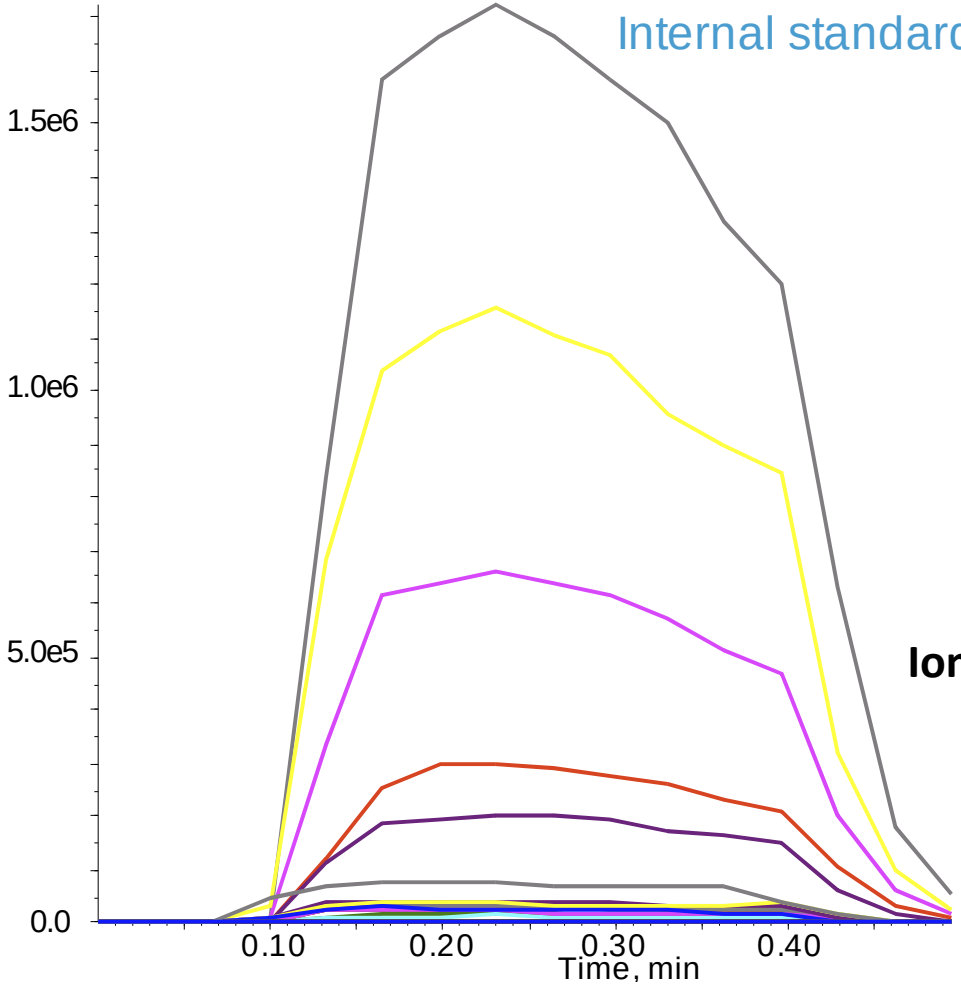


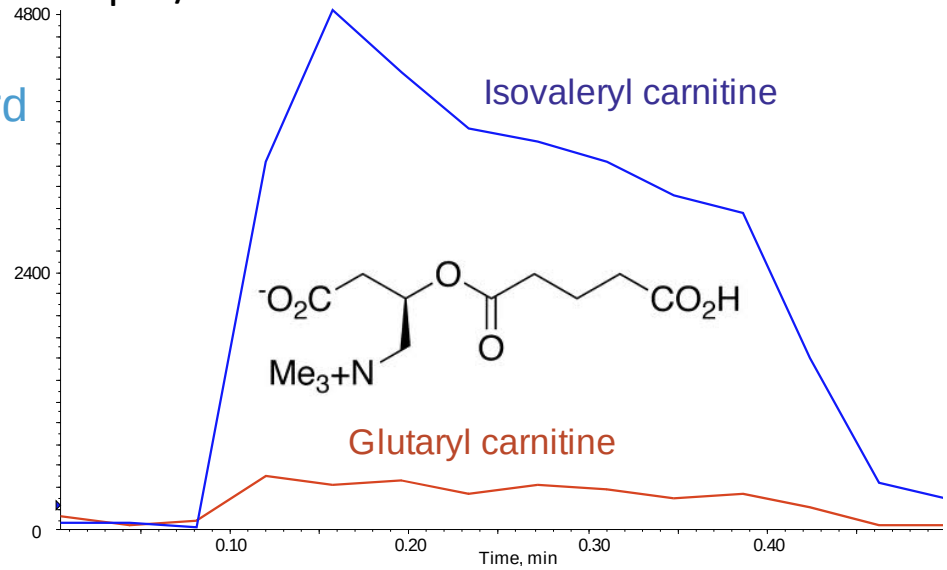
# Stanovení AMK a AK FIA-MS/MS

DBS - deproteinace (MeOH+IS), vortex, centrifugace

MF: MeOH + FA, 300/30 µl/min, 80/10 bar; celkový čas analýzy: 55 s

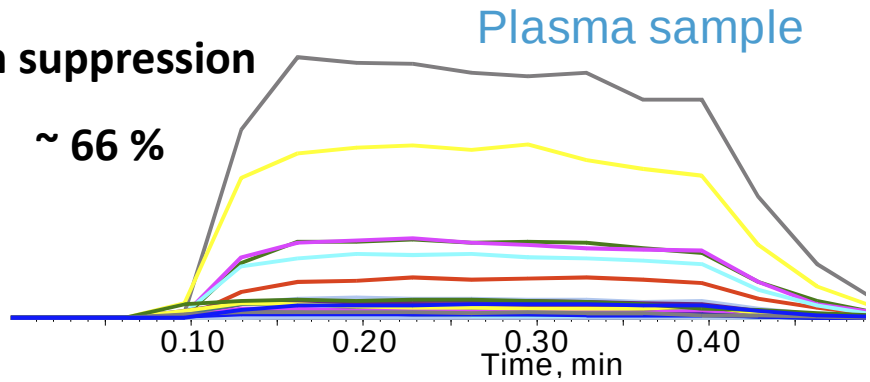
MS: ESI+, 4500 V, 300 °C, 70 ms; LOD: 5 nM/L – 10 µM/L

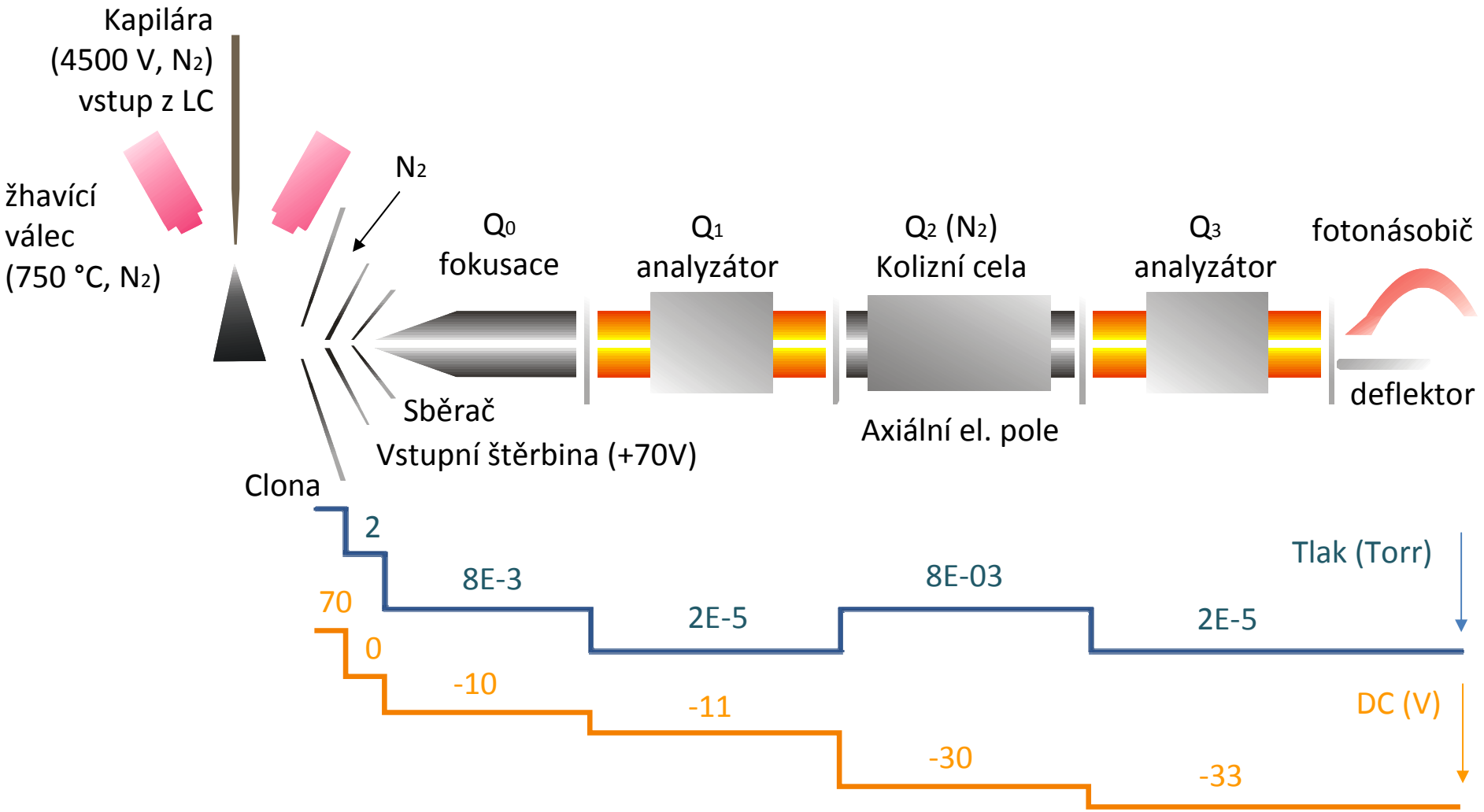



**Ion suppression**

**~ 66 %**

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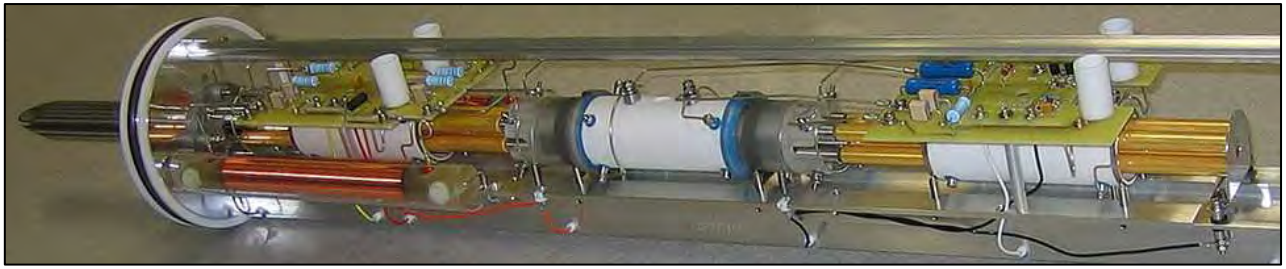
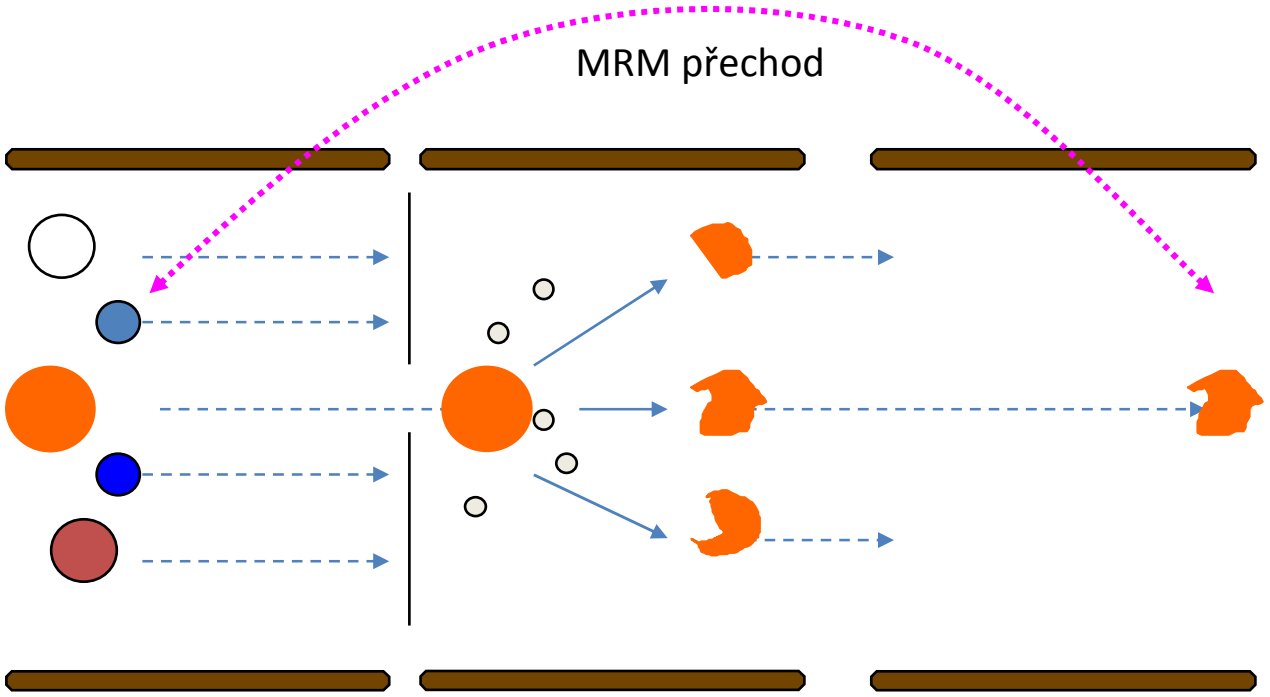


# MRM = multiple-reaction monitoring

- Rozlišení

- Dwell time = čas měření přechodu // počet přechodů (analytů) // počet bodů na peak

- Lower sensitivity for small Mw

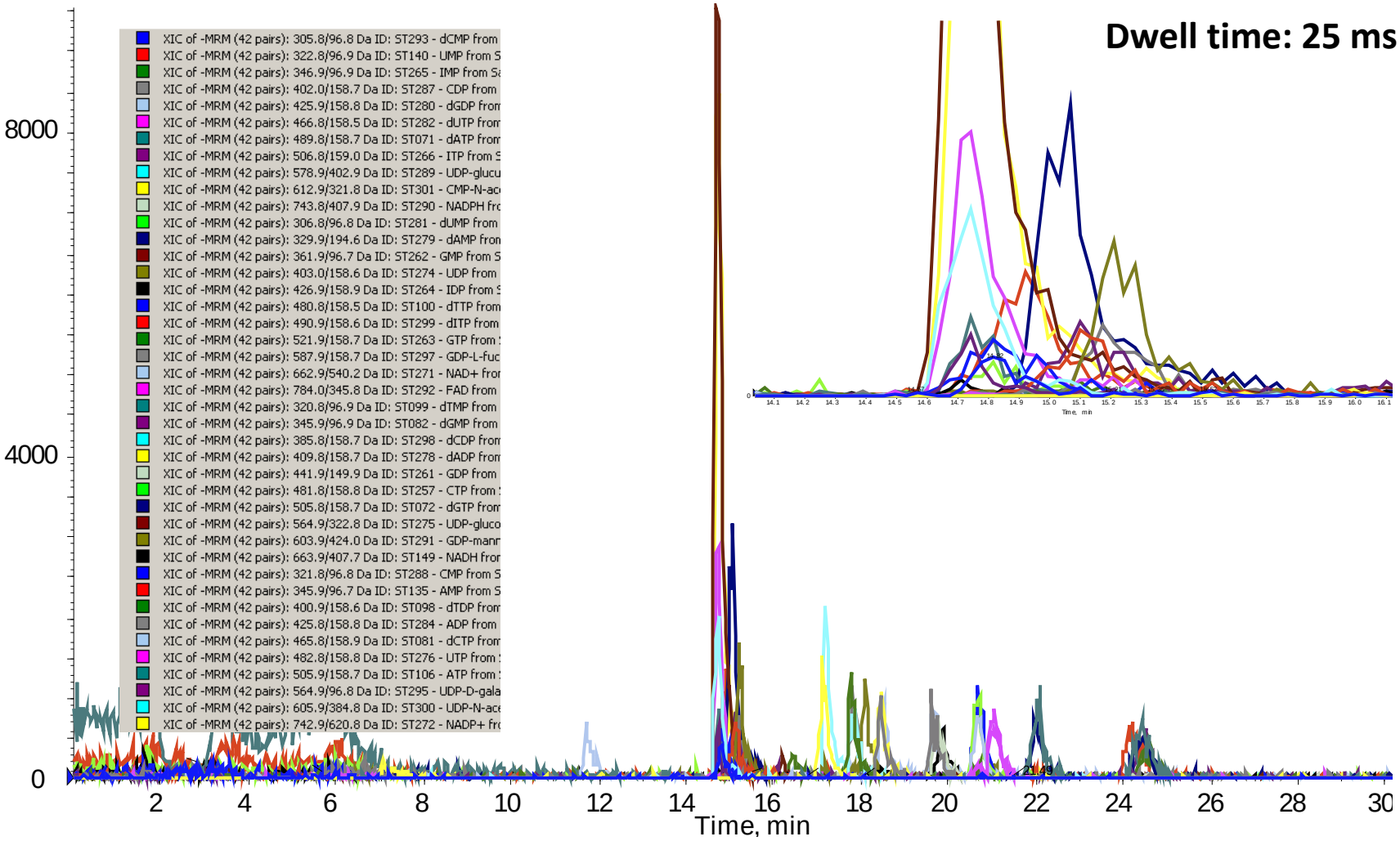


# Stanovení nukleotidů (LC-MS/MS)

Fibroblasty - deproteinace (MeOH), Luna NH2 - ANP, (Phenomenex) 3.0 µm, 1.0 x 150 mm

MF: *A)* $NH_4Ac$, *B)* MeOH; 0 % *A* (1 min) => 95 % *A* (30 min); 30 °C, 50 µl/min, 200 bar

MS: ESI-, -4500 V, 500 °C, LOD: < 0.1 µM, celkový čas analýzy: 35 min



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Plasma - deproteinace MeOH 1:10, ACQUITY UPLC®BEH C18 (Waters) 1.7 µm, 2.1 x 50 mm

MF: *A)* $NH_4COOH$, *B)* MeOH; 83 % *A* (1.0 min) => 50 % *A* (2.1 min); 40 °C, 0.5 ml/min, **1µM**