

LC/MS kvantitativní analýza s iontovou mobilitou

Jan Buček



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authorized distributor
thermoscientific

High-Field Asymmetric Waveform Ion Mobility Spectrometry System



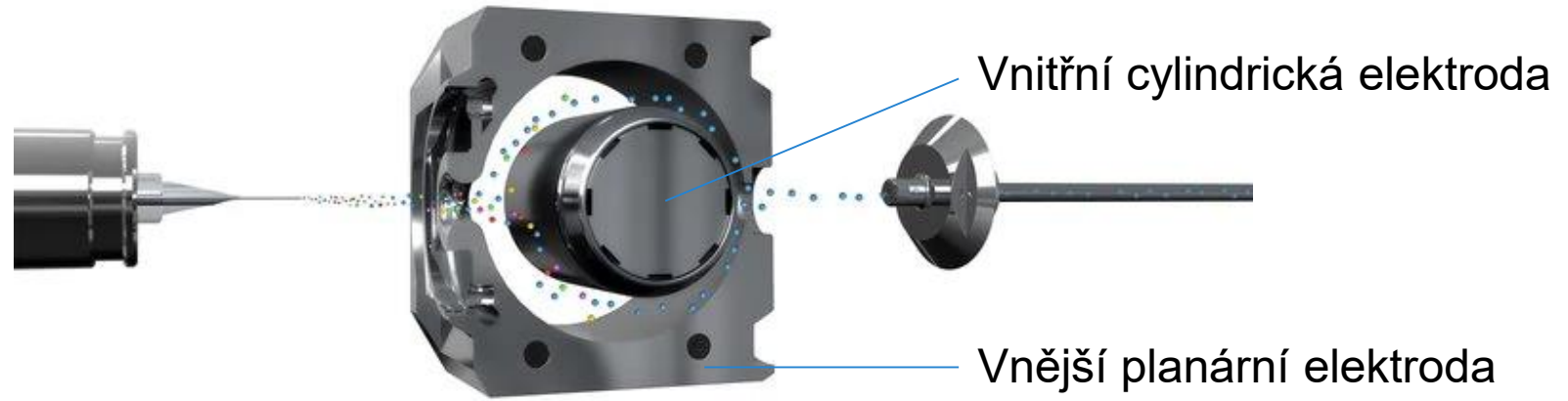
High-Field Asymmetric Waveform Ion Mobility Spectrometry System

TSQ II Series

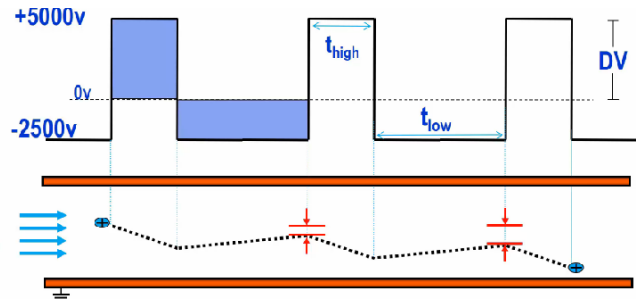
- TSQ Endura™
- TSQ Quantiva™
- TSQ Altis™
- TSQ Quantis™

HRMS Instruments

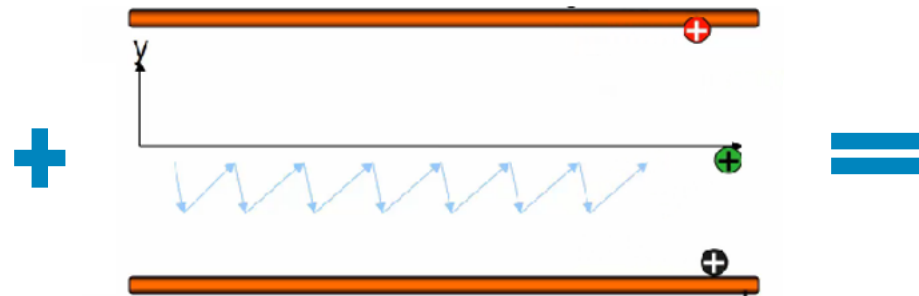
- Tribrid Orbitrap Fusion™
- Tribrid Orbitrap Fusion Lumos™
- Tribrid Orbitrap Eclipse™
- Orbitrap Exploris™
- NanoSpray Flex™
- Easy Spray™
- VeriSpray™



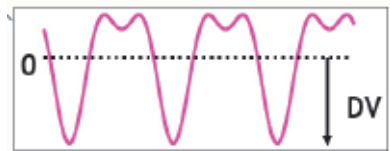
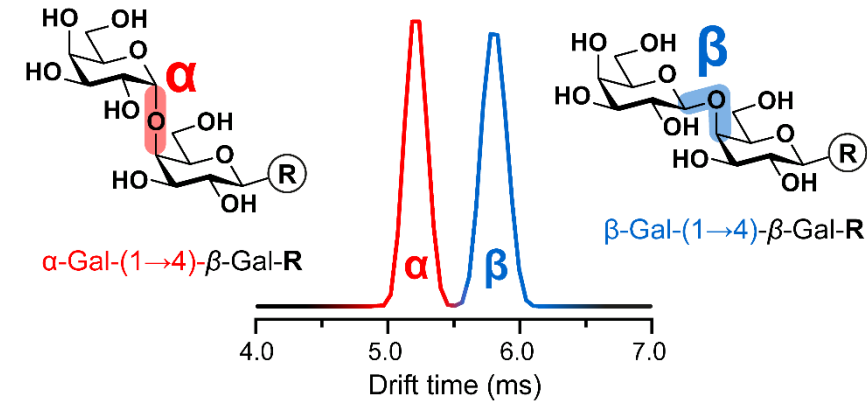
DV – Dispersion Voltage



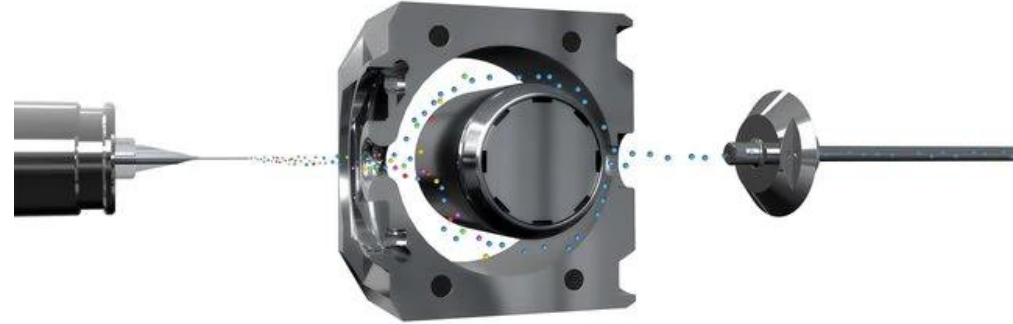
CV – Compensation Voltage



IM-MS Separation

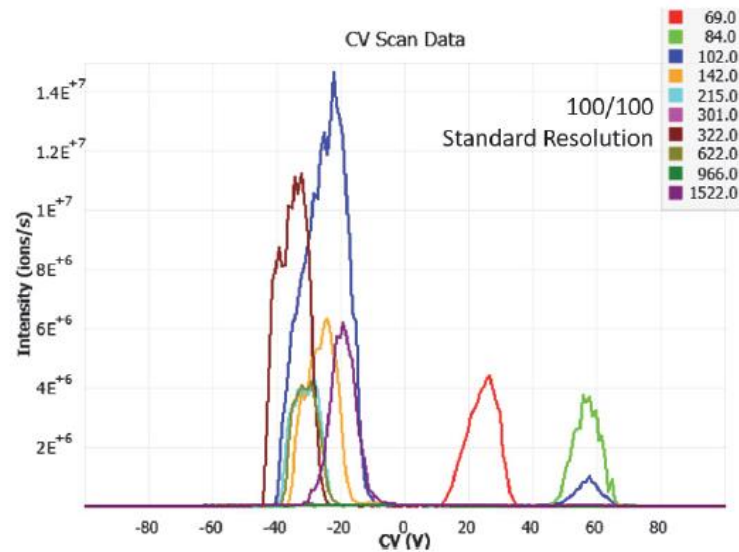


High-Field Asymmetric Waveform Ion Mobility Spectrometry System



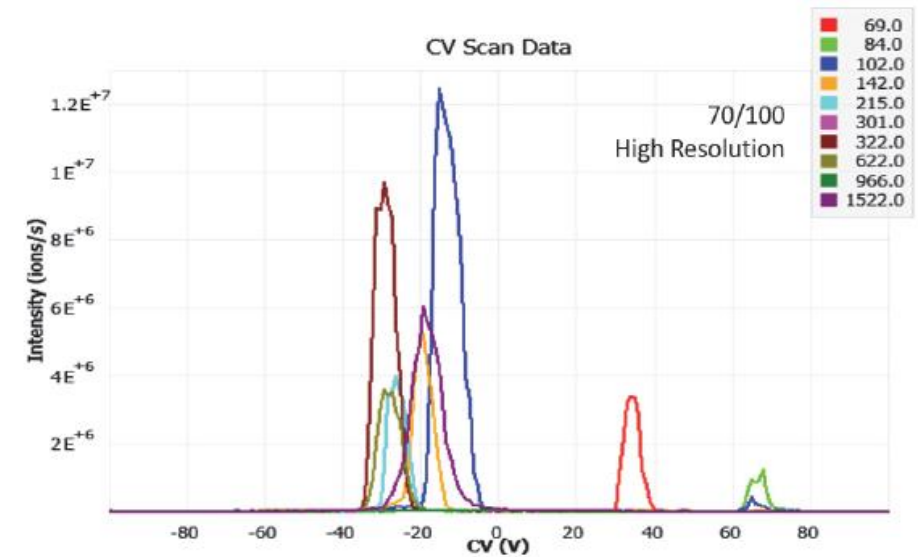
Standard Resolution Mode

zvýšená citlivost na úkor rozlišení



High Resolution Mode

vysoké rozlišení na úkor citlivosti

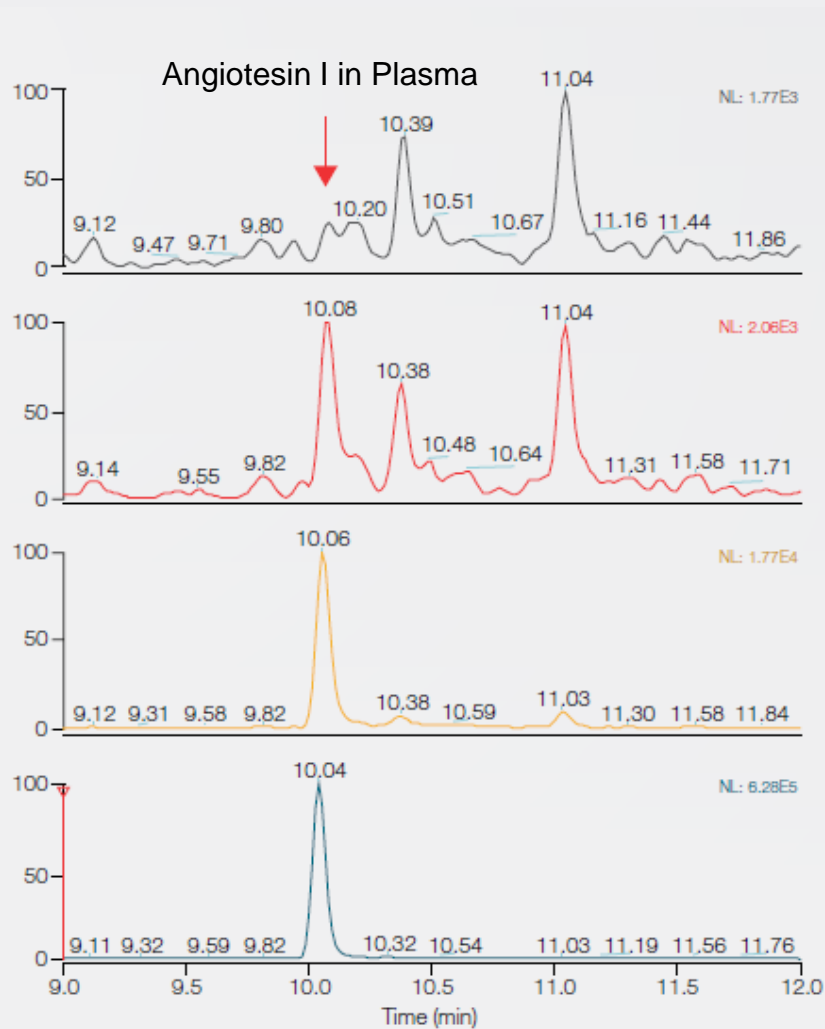




- Zlepšení S/N díky selekci molekul vstupujících do MS
- Zlepšení S/N propouštěním homogenní populace molekul
- Zlepšení limitů detekce a kvantifikace (LOD, LOQ)
- Separace a kvantifikace isobarických molekul
- Zkrácení / eliminace chromatografie
- Zlepšení kvality MS dat - snadnější identifikace peptidů
- Větší „Throughput“

FAIMS Pro Interface - S/N, LOD, LOQ

nLC-MS/MS



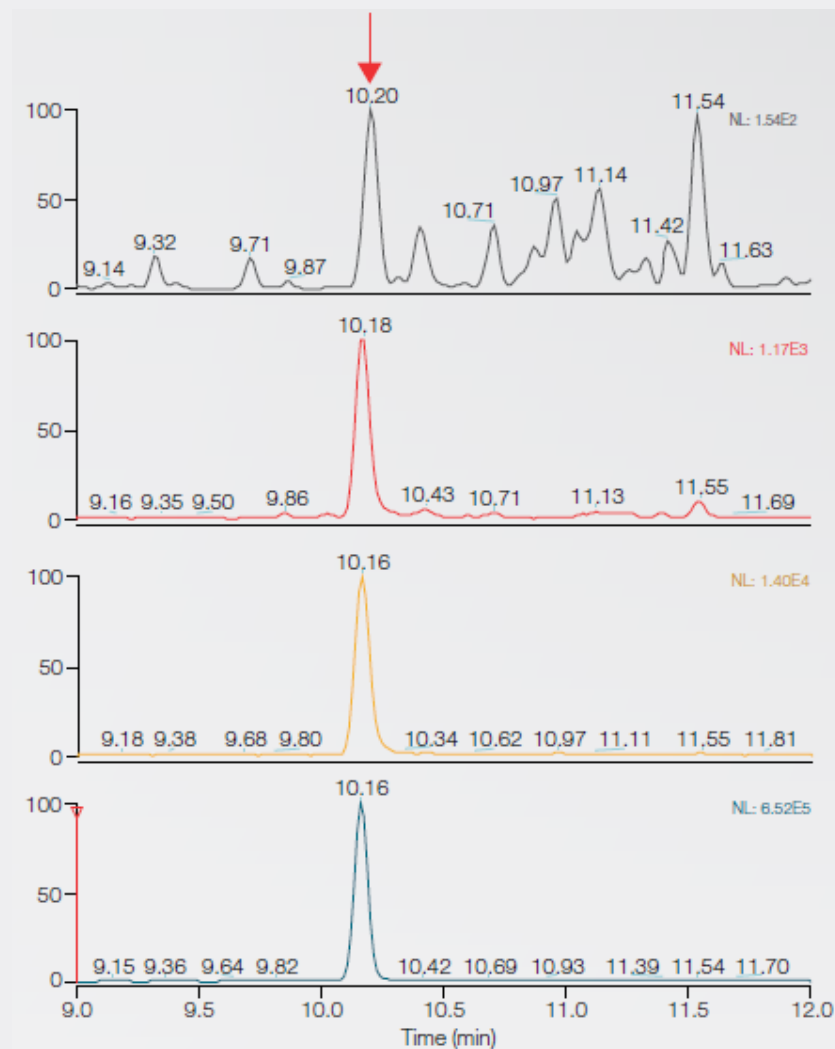
70 attomol

700 attomol

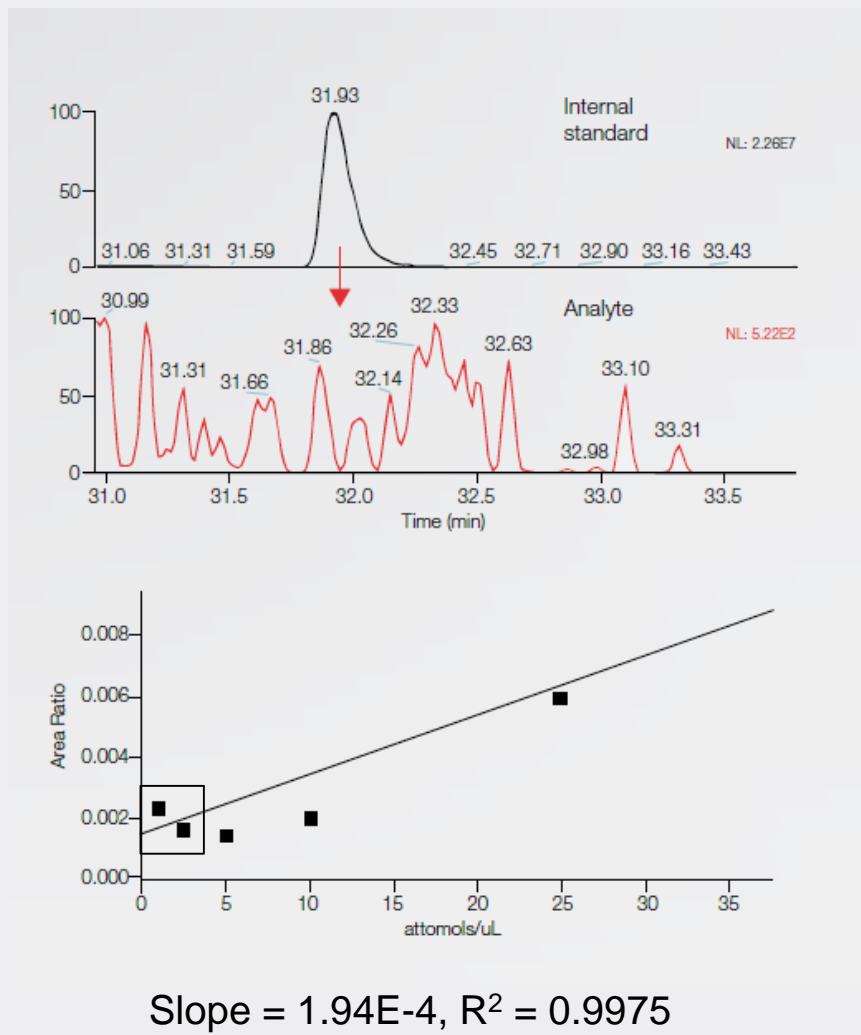
7 femtomol

700 femtomol

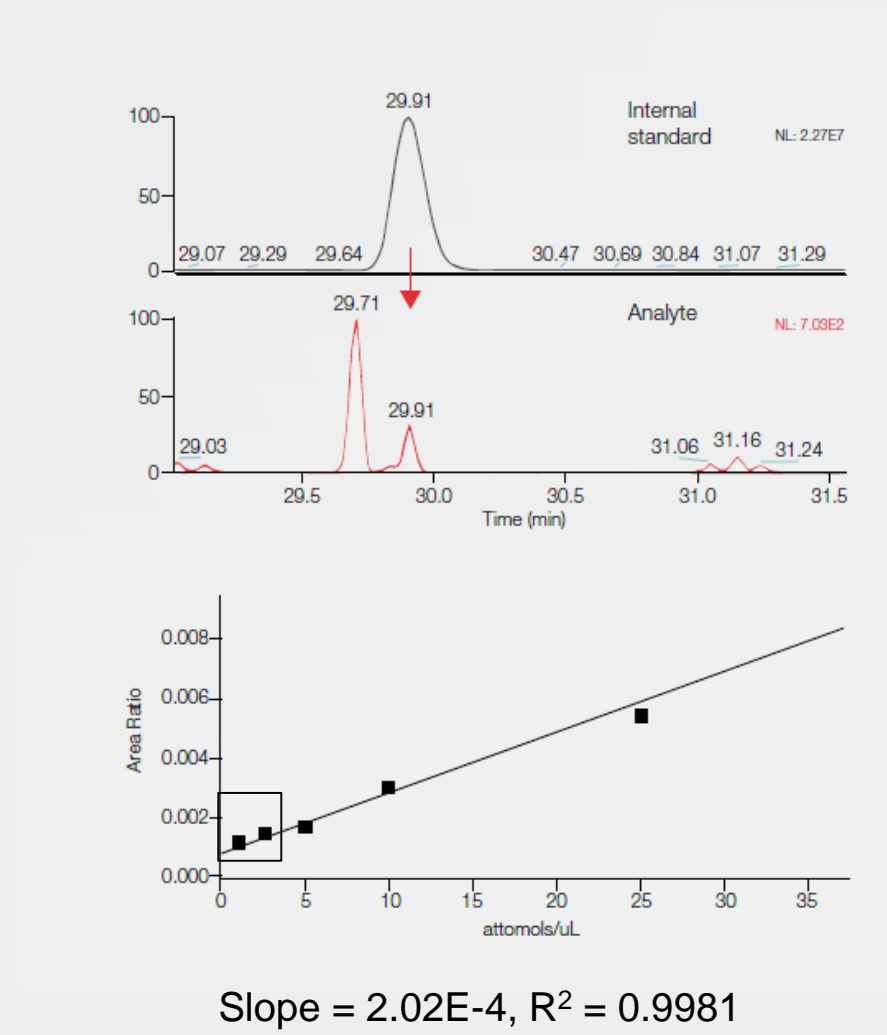
nLC-FAIMS-MS/MS



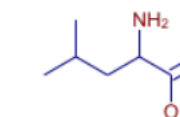
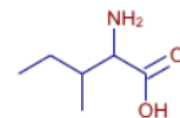
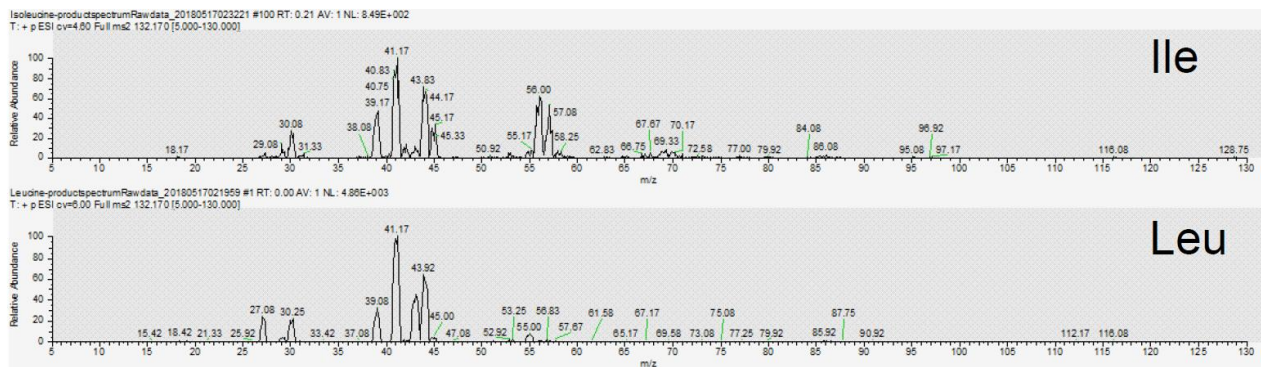
nLC-MS/MS



nLC-FAIMS-MS/MS



FAIMS Pro Interface – separace a kvantifikace isobarických molekul



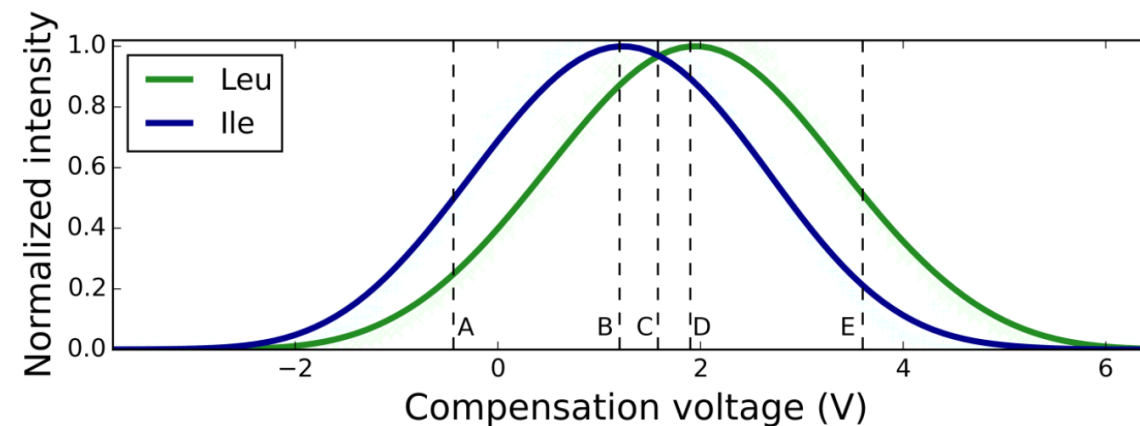
Monoisotopická hmotnost = 132.17

Produktové MS/MS spektra, 40eV CID, 2 m Torr

A
B
C
D
E

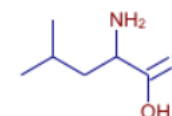
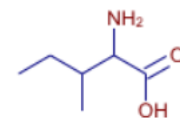
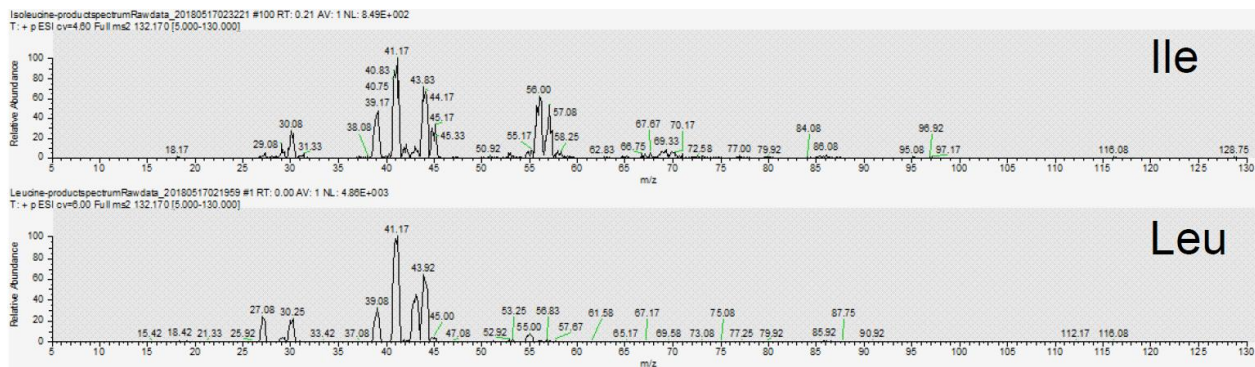
-0.44 V
1.2 V
1.58 V
1.9 V
3.6 V

Outer 50% Ile
Max Ile
Crossing
Max Leu
Outer 50% Leu



Zdroj: Kalafut B, Snyder AS, Quantitation of Isomers by Multi-VC FAIMS MS Scans, Thermo Scientific Application Note

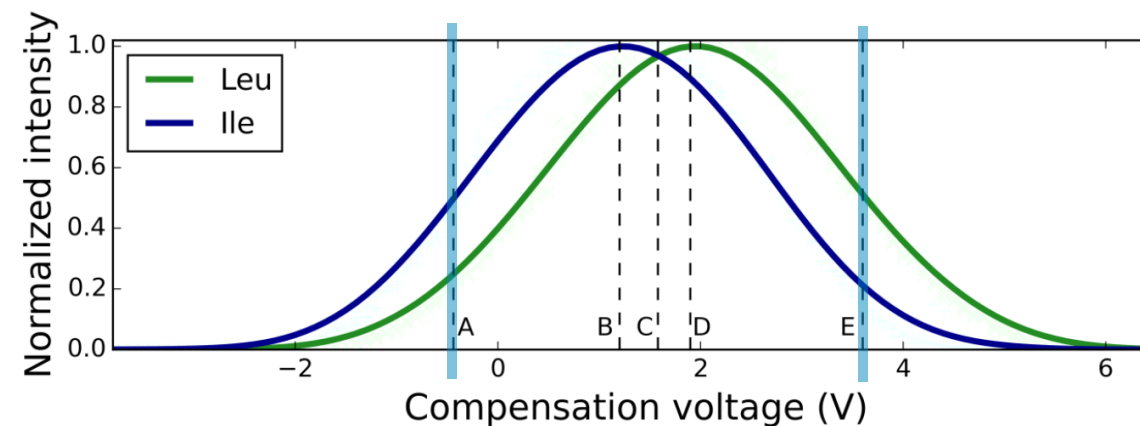
FAIMS Pro Interface – separace a kvantifikace isobarických molekul



Monoisotopická hmotnost = 132.17

Produktové MS/MS spektra, 40eV CID, 2 m Torr

A	-0.44 V	Outer 50% Ile
B	1.2 V	Max Ile
C	1.58 V	Crossing
D	1.9 V	Max Leu
E	3.6 V	Outer 50% Leu



Zdroj: Kalafut B, Snyder AS, Quantitation of Isomers by Multi-VC FAIMS MS Scans, Thermo Scientific Application Note

FAIMS Pro Interface – separace a kvantifikace isobarických molekul

7 směsí

Leu : Ile

0 : 100

10 : 90

30 : 70

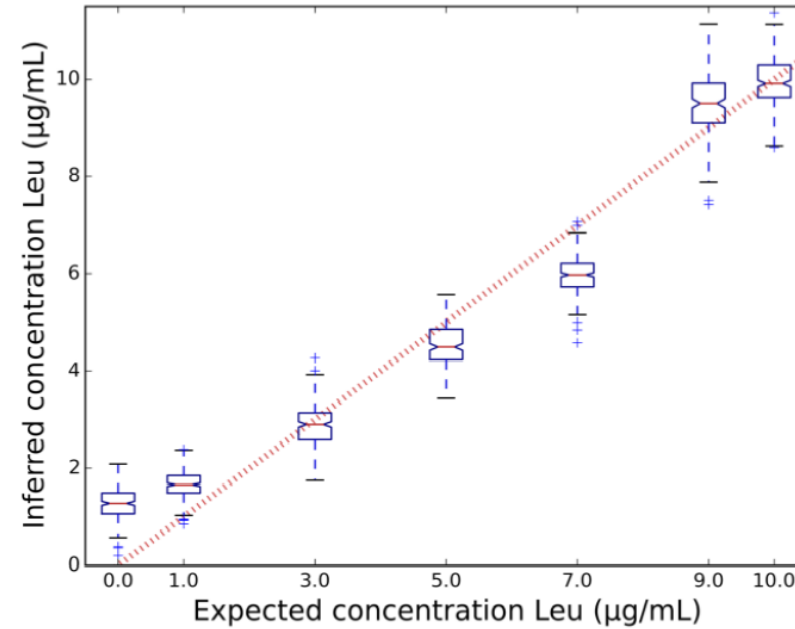
50 : 50

70 : 30

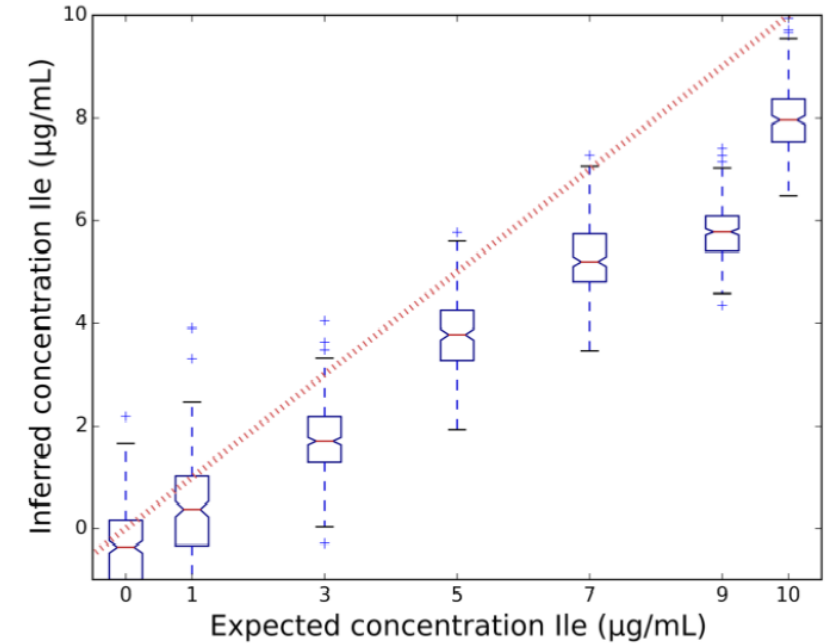
90 : 10

100 : 0

Leucin



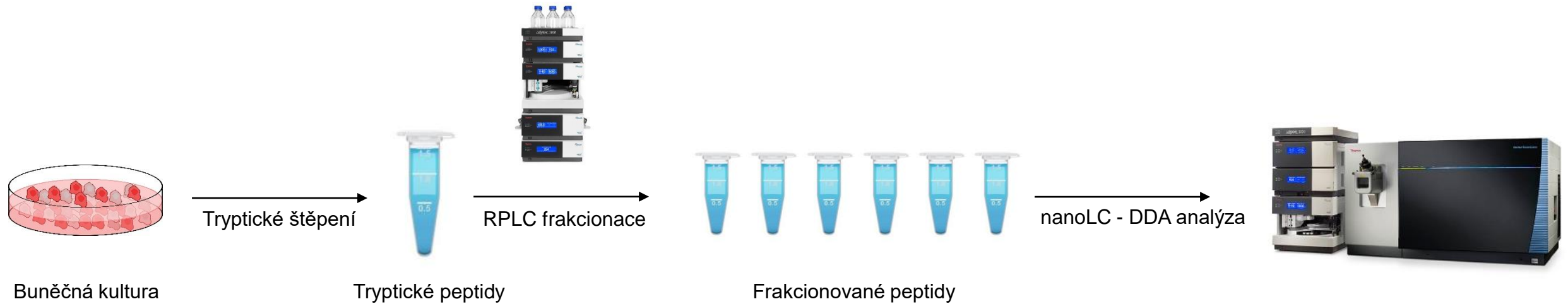
Isoleucin



Zdroj: Kalafut B, Snyder AS, Quantitation of Isomers by Multi-VC FAIMS MS Scans, Thermo Scientific Application Note

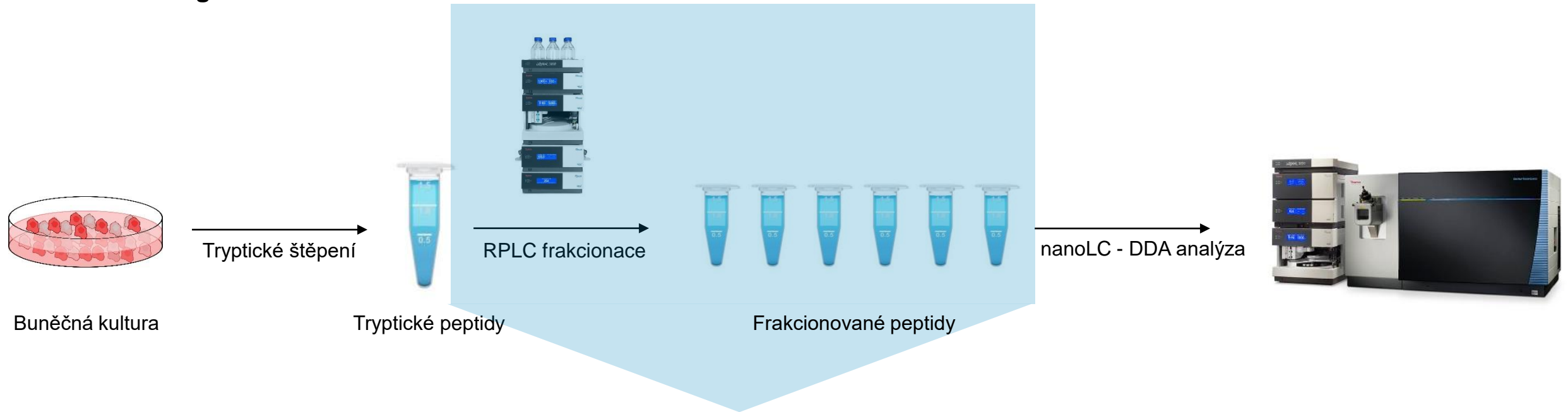
FAIMS Pro Interface – Zkrácení / Eliminace chromatografie

No FAIMS Single Shot Proteomics Workflow



FAIMS Pro Interface – Zkrácení / Eliminace chromatografie

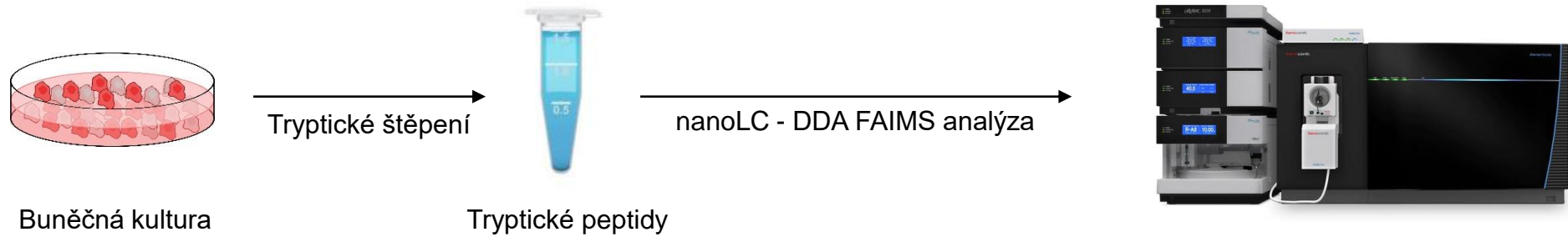
No FAIMS Single Shot Proteomics Workflow



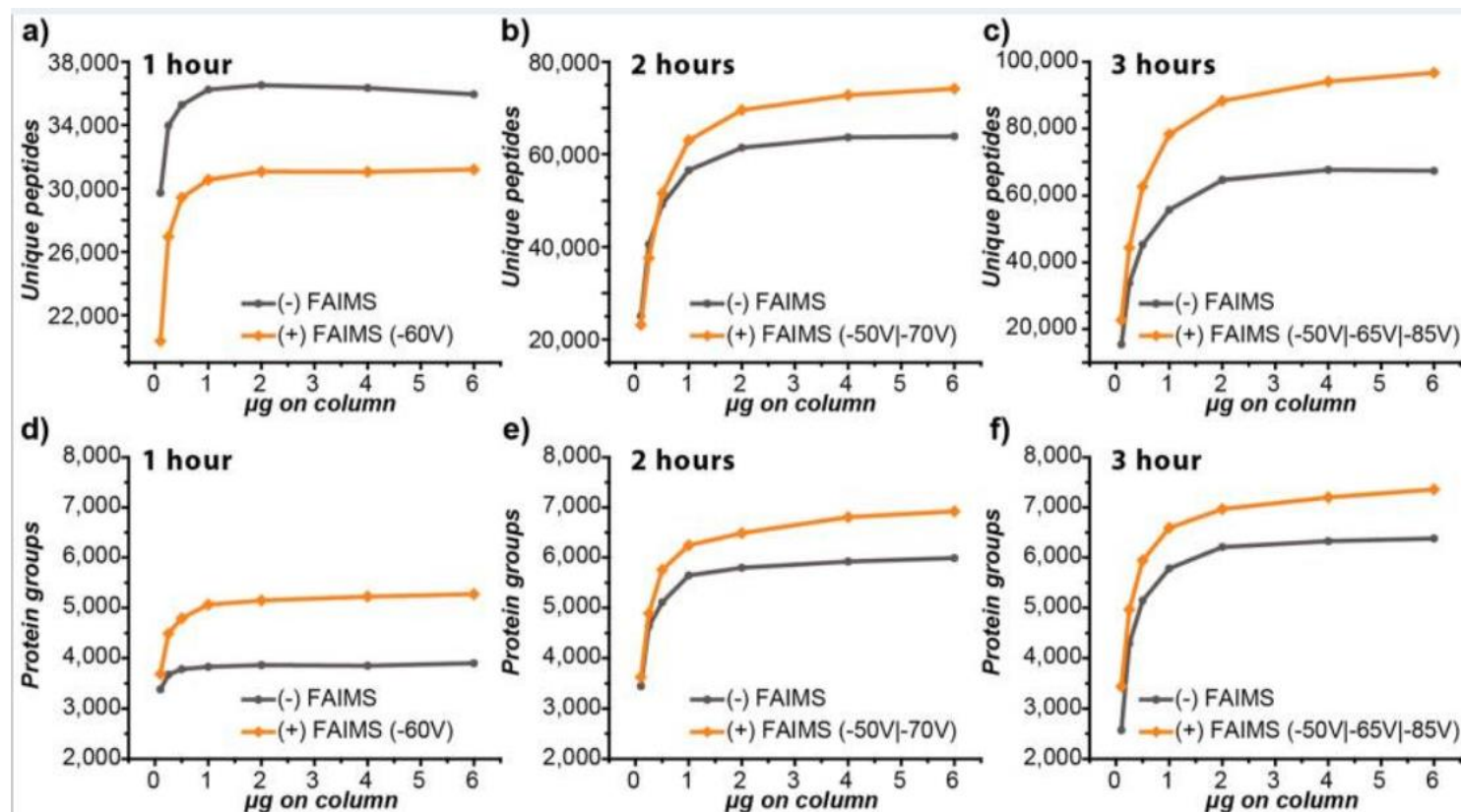
- + 1 den na přípravu vzorku
- + 1 LC systém
- větší objem vstupního vzorku
- náročnější manipulaci a větší prostor pro chybu

FAIMS Pro Interface – Zkrácení / Eliminace chromatografie

FAIMS Single Shot Proteomics Workflow



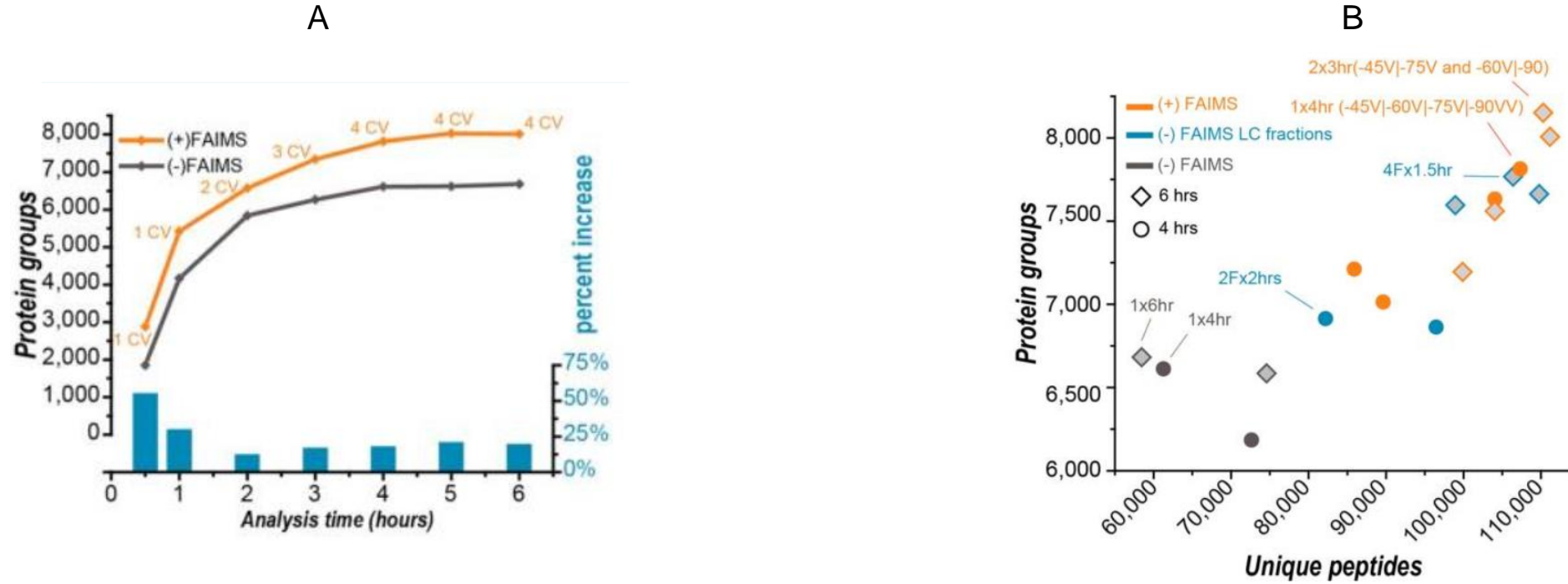
No FAIMS Workflow vs. FAIMS Workflow



Počet identifikovaných peptidů (a-c), respektive proteinů (d-f) v závislosti na délce analýzy a množství vzorku naneseného na kolonu

Zdroj: Hebert AS; Prasad S; Belford MW; Bailey DJ; McAlister GC; Abbatiello SE; Huguet R; Wouters ER; Dunyach JJ; Brademar DR; Westphall MS; Coon JJ *Anal. Chem* 2018, 90, 9529-9537.

No FAIMS Workflow vs. FAIMS Workflow

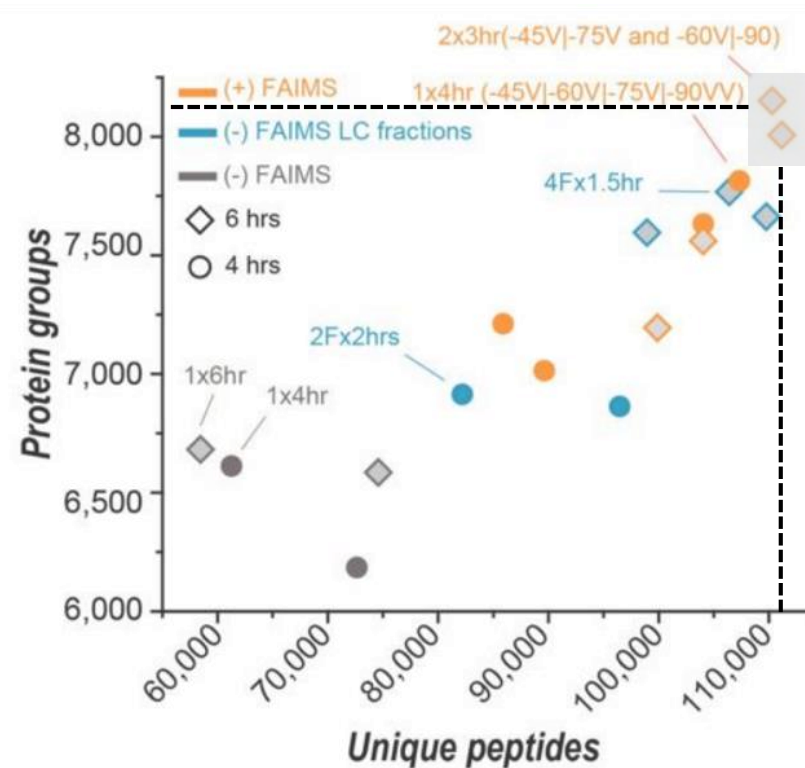


A - Počet identifikovaných peptidů v závislosti na době analýzy (0.5 – 6 h) včetně mezihodinového rozdílu

B – Počet identifikovaných peptidů / proteinů v závislosti na metodice

Zdroj: Hebert AS; Prasad S; Belford MW; Bailey DJ; McAlister GC; Abbatiello SE; Huguet R; Wouters ER; Dunyach JJ; Brademar DR; Westphall MS; Coon JJ *Anal. Chem* 2018, 90, 9529-9537.

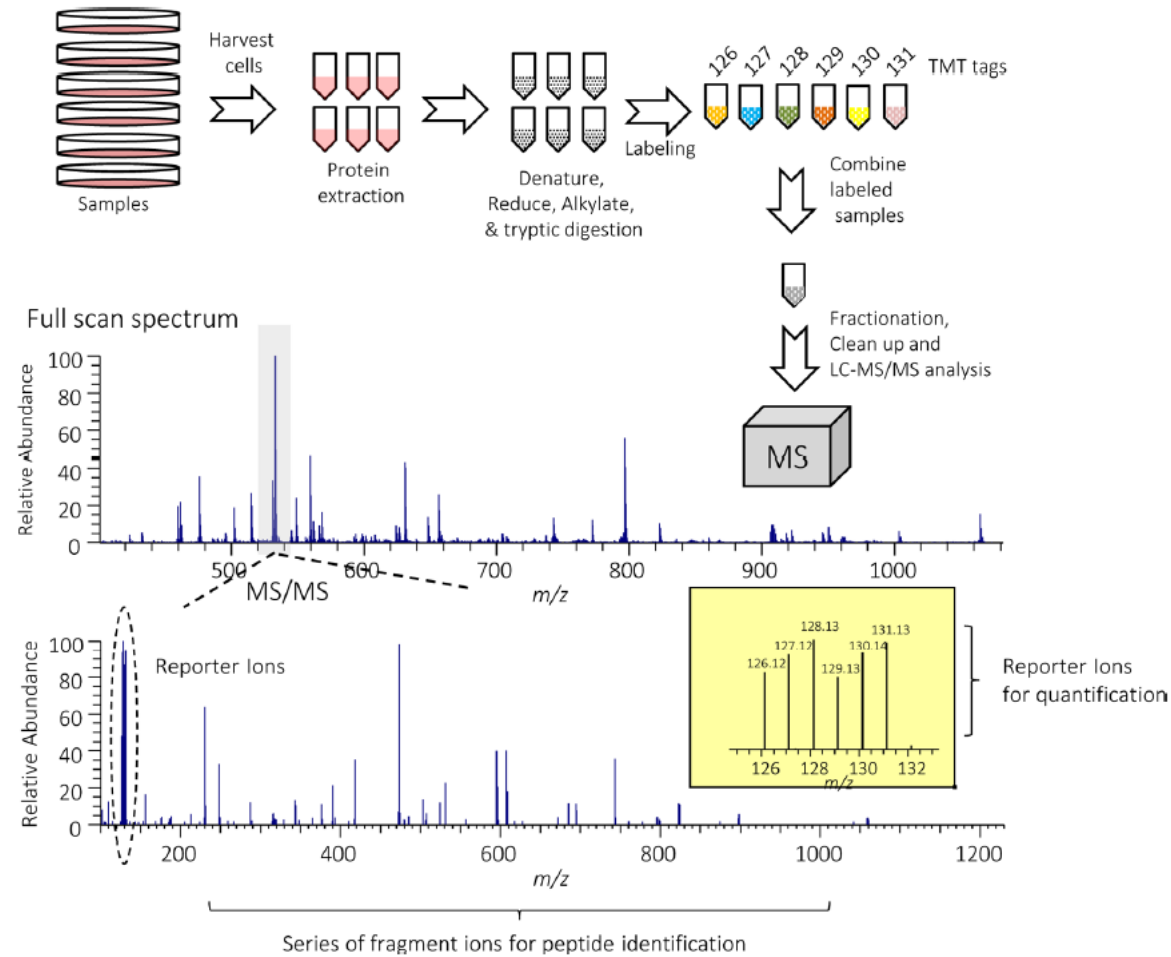
No FAIMS Workflow vs. FAIMS Workflow



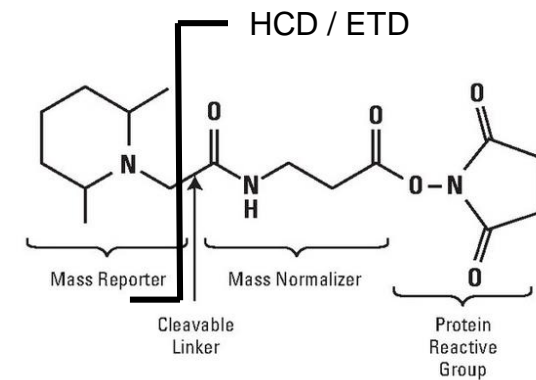
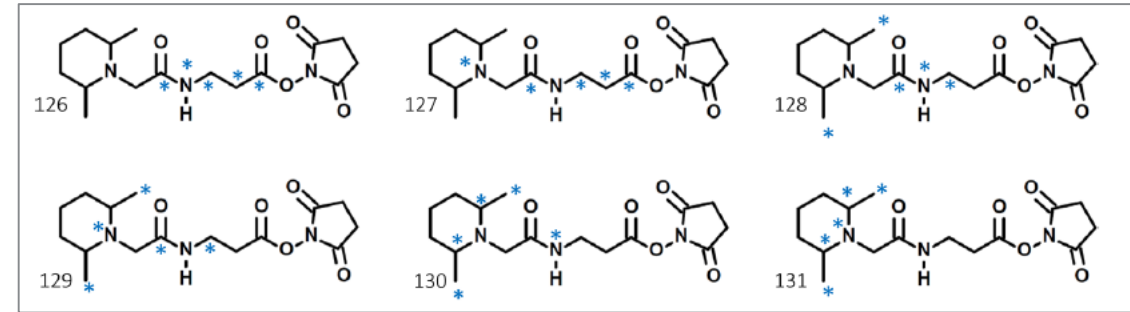
Počet identifikovaných peptidů / proteinů v závislosti na metodice

Zdroj: Hebert AS; Prasad S; Belford MW; Bailey DJ; McAlister GC; Abbatiello SE; Huguet R; Wouters ER; Dunyach JJ; Brademar DR; Westphall MS; Coon JJ *Anal. Chem* 2018, 90, 9529-9537.

FAIMS Pro Interface – Lepší relativní kvantifikace peptidů pomocí TMT*



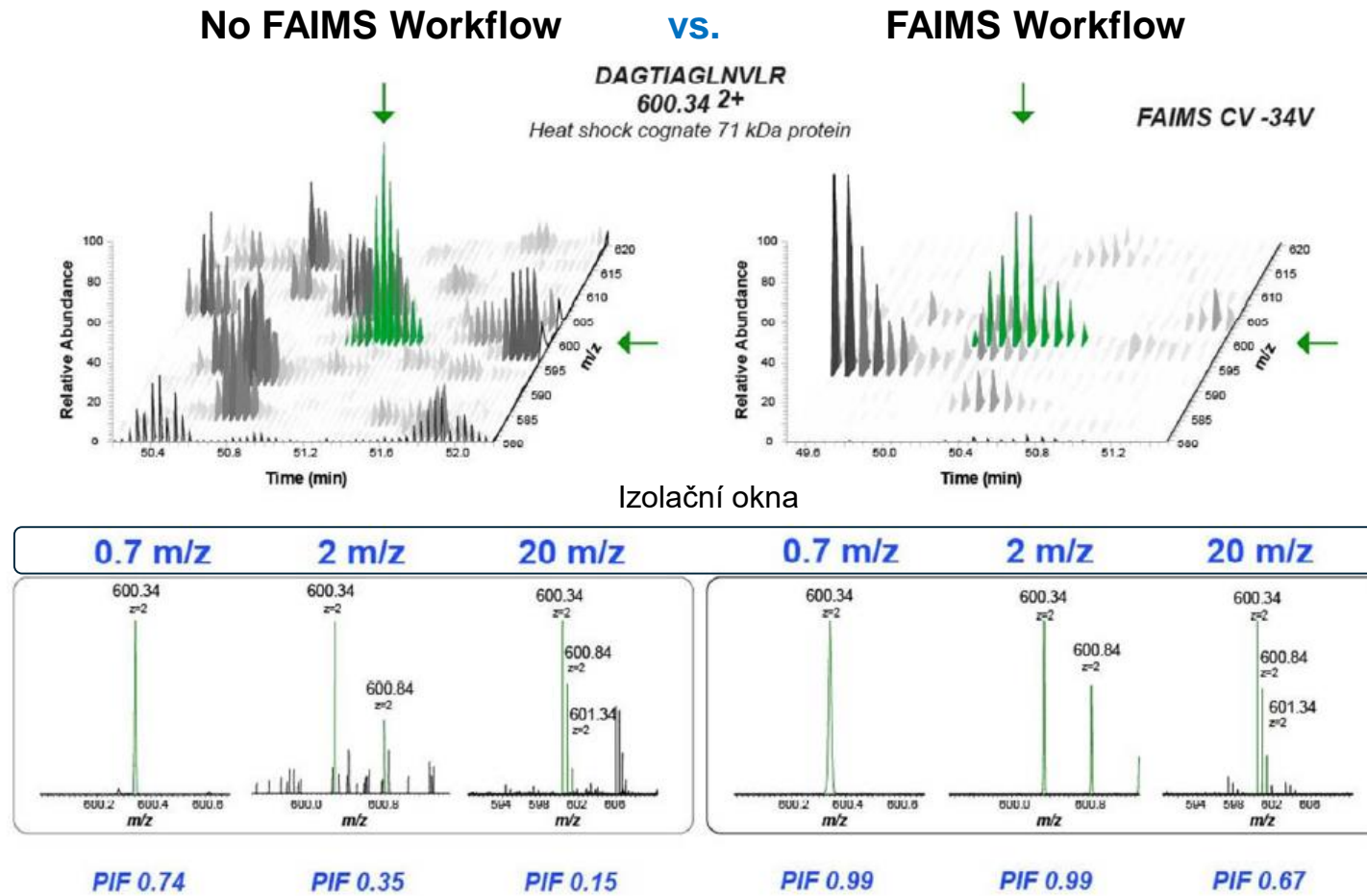
Thermo Scientific TMTsixplex™



Zdroj: Rauniyar N; Yates JR; *Journal of Proteome Research* 2014, 13, 5293-5309.

*TMT – Tandem Mass Tag

FAIMS Pro Interface – Lepší relativní kvantifikace peptidů pomocí TMT

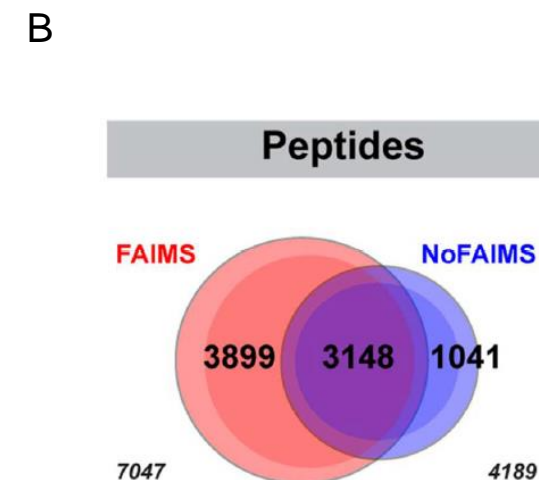
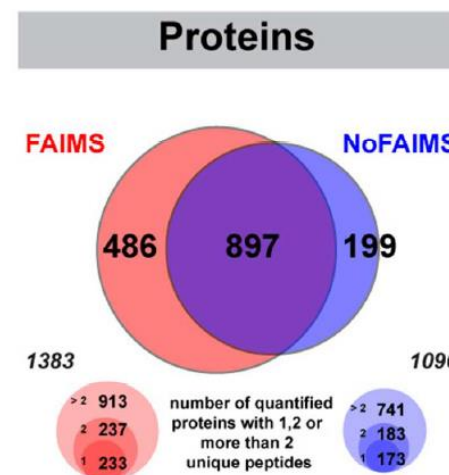
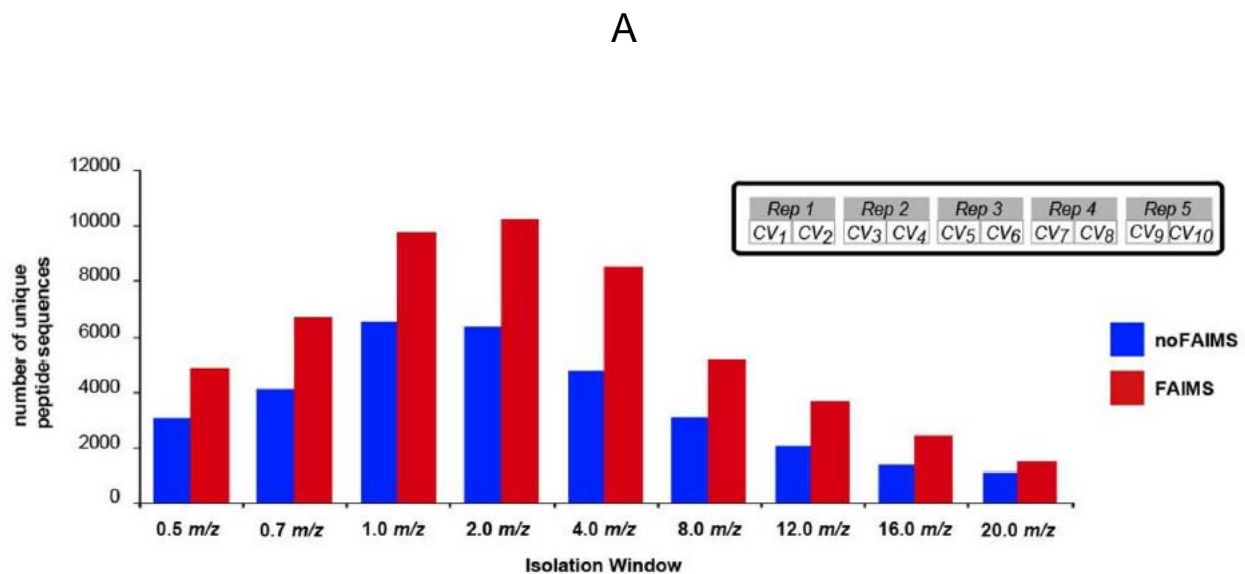


PIF – Precursor Ion Fraction - hodnota determinující kolik z celkového iontového fluxu připadá prekurzorovému iontu (0-1)

Zdroj: Phammatter S; Bobbeil E; Thibault P *Journal of Proteome Research* 2016, 12, 4653-4665.

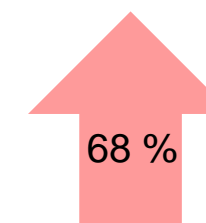
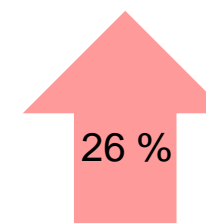
FAIMS Pro Interface – Lepší relativní kvantifikace peptidů pomocí TMT

No FAIMS Workflow vs. FAIMS Workflow



A - Počet identifikovaných peptidů v každém izolačním okně

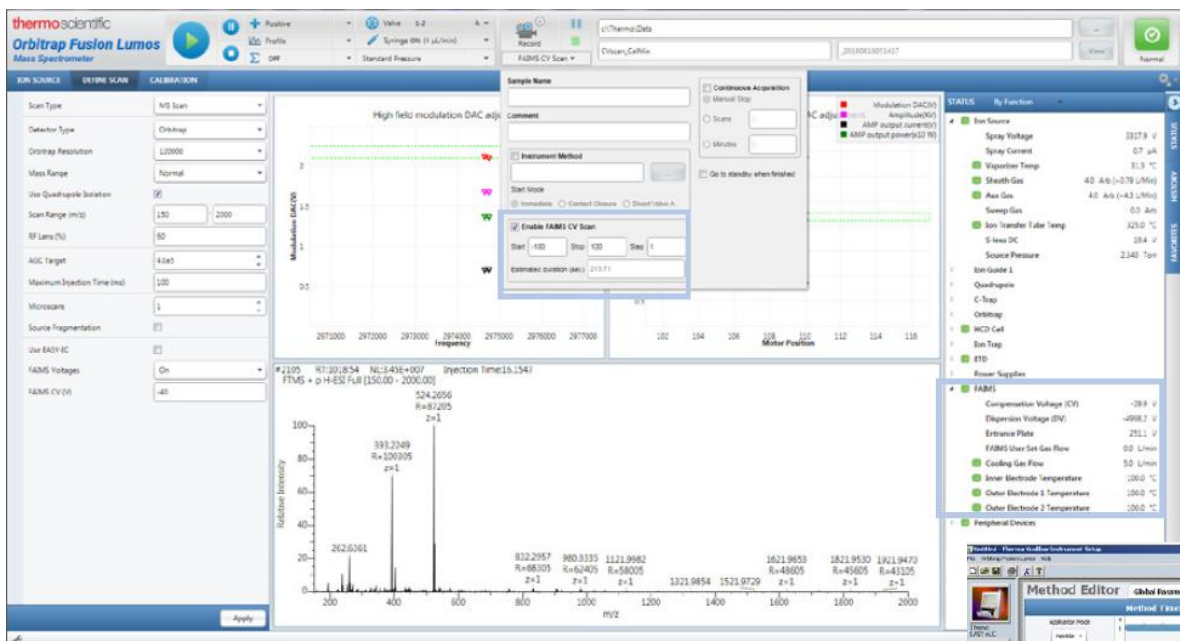
B – Vennovy diagramy počtu identifikovaných peptidů / proteinů



Zdroj: Phammatter S; Bobbeil E; Thibault P Journal of Proteome Research 2016, 12, 4653-4665.

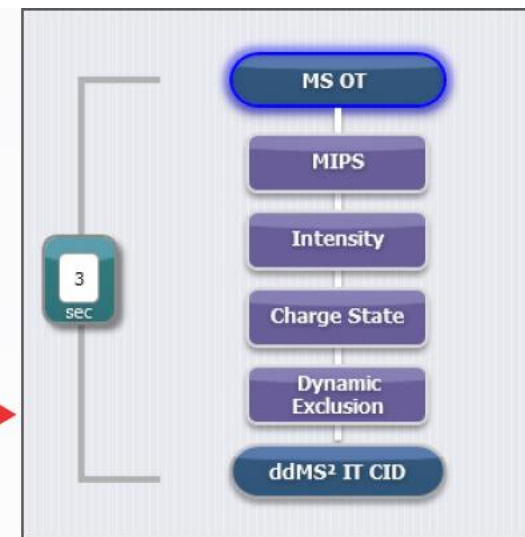
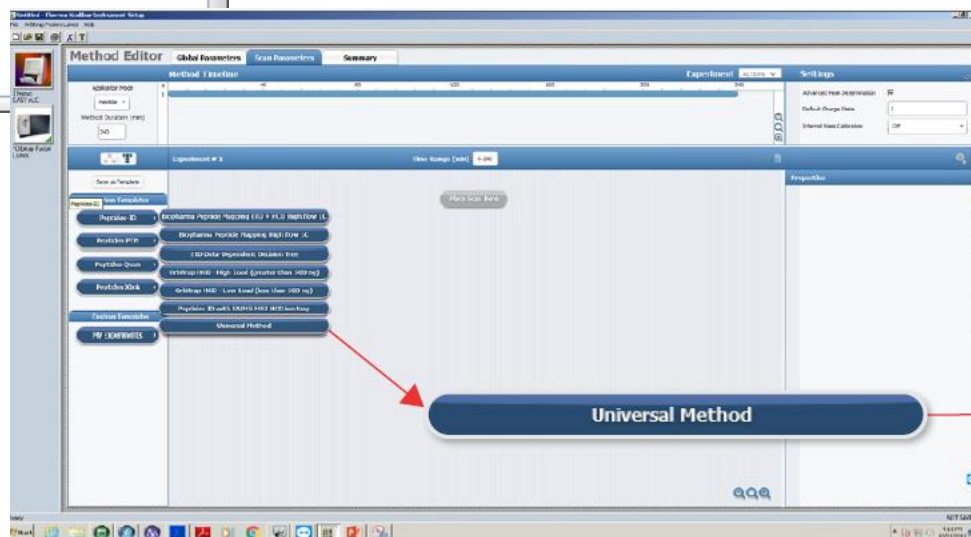
FAIMS Pro Interface – Automatická softwarová inkorporace

Automatické rozpoznání FAIMS v Tune UI



Optimalizace Compensation Voltage (CV) pro TIC, SIM, PRM

Editace přednastavených templátů instrumentových metod



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bucek@pragolab.cz