Applications of microscale FAIMS combined with mass spectrometry in pharmaceutical analysis

Colin Creaser

Centre for Analytical Science

Loughborough University, UK

(c.s.creaser@lboro.ac.uk)

Ion Mobility Spectrometry: Basics

- Rapid separation of gas phase ions as a result of differences in their mobility in an electric field and a buffer gas

- Two types of device:
  - Drift tube ion mobility spectrometry
  - Field asymmetric waveform ion mobility spectrometry (FAIMS) or differential mobility spectrometry (DMS)

Ion mobility spectrometry

Mobility of an ion in the presence of an electric field gradient and a buffer gas (e.g. He, N_2 or air; 1-10 mbar or 1 bar) is given by:

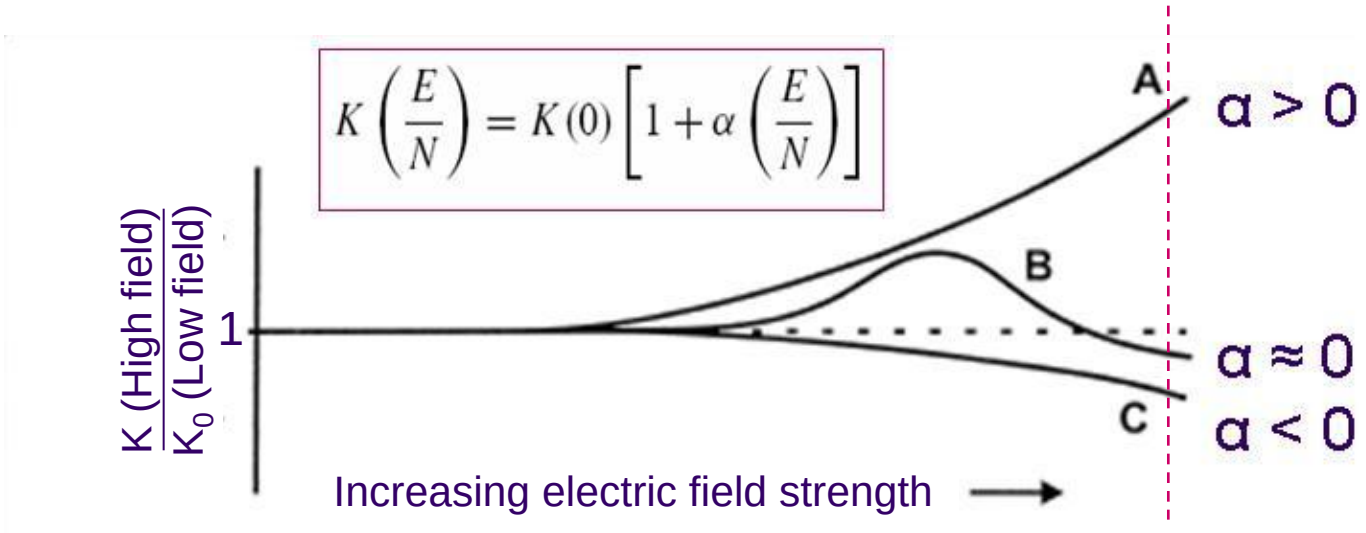
$$v_d = K.E$$

[v_d = ion velocity, E = electric field gradient, K = ion mobility]

- This relationship only holds under low field conditions (E → 0)

Ion mobility in high and low electric fields

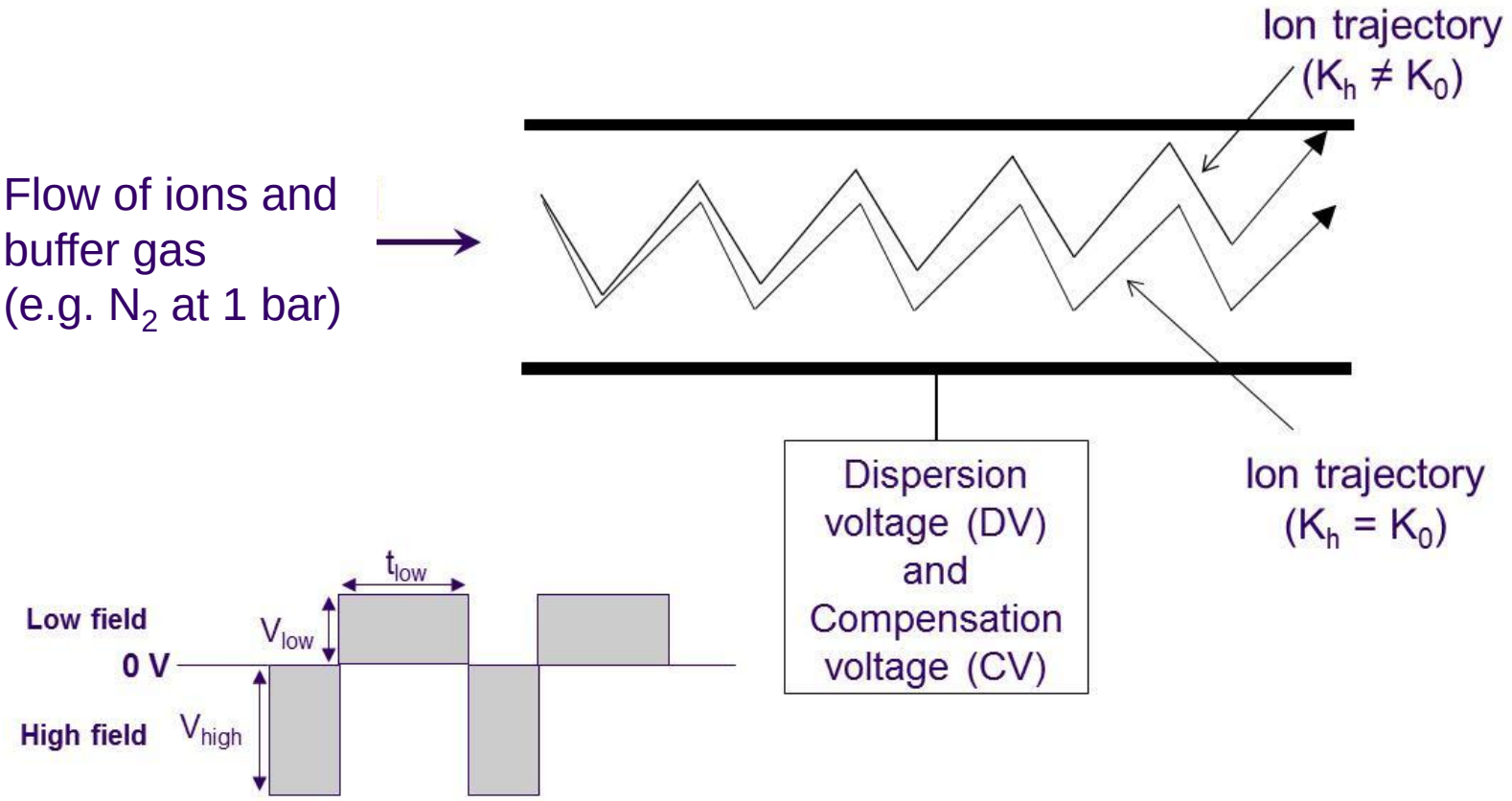
- Mobility is dependent on electric field strength



- Alpha coefficient – compound dependent

[Purves R W, Guevremont R, Anal. Chem. 1999, 71, 2346-2357]

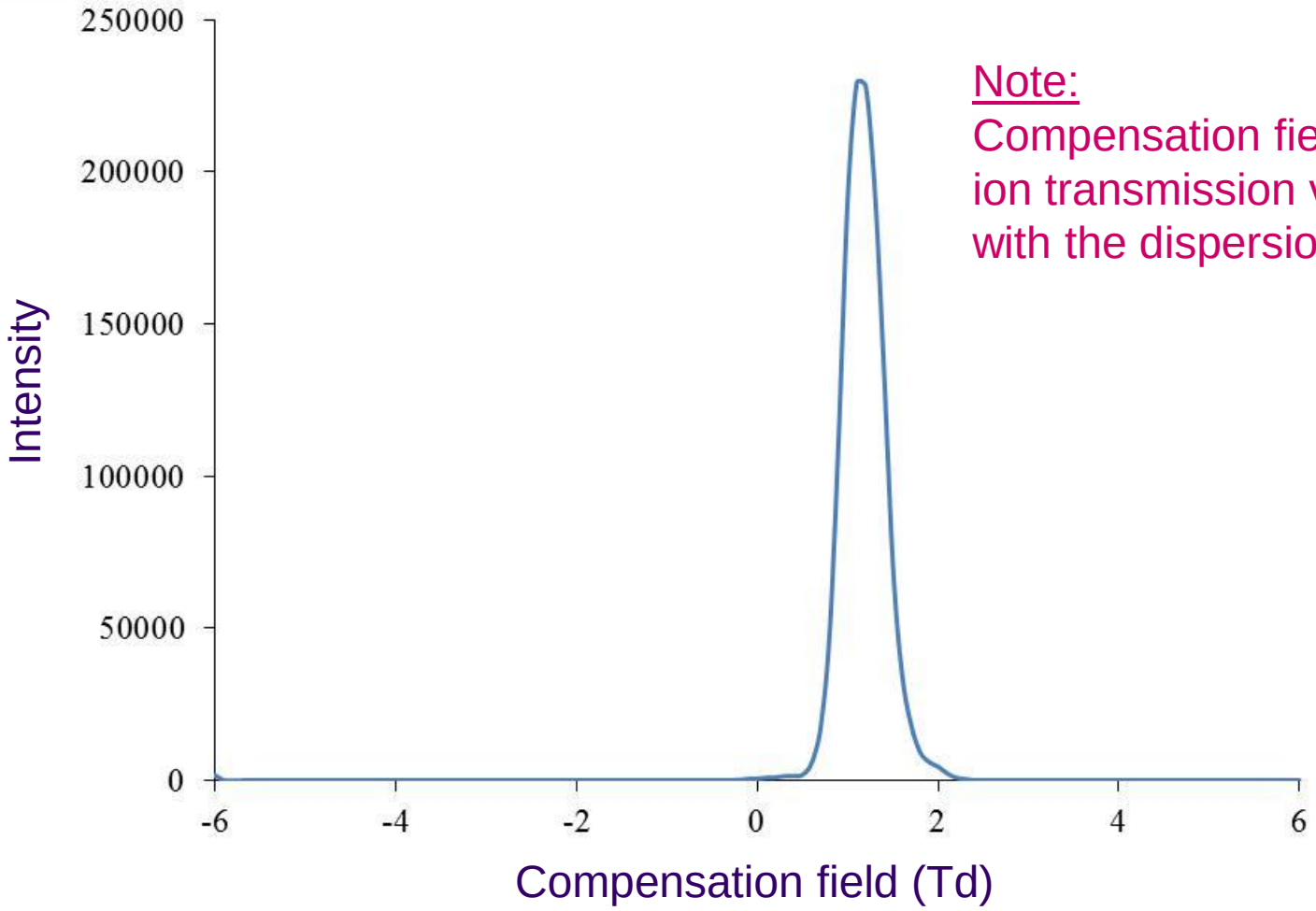
Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)/Differential mobility spectrometry (DMS)



- Compensation voltage (CV) or field (CF) set to transmit ions of selected differential mobility
- Continuous ion beam (equivalent to quadrupole mass filter)

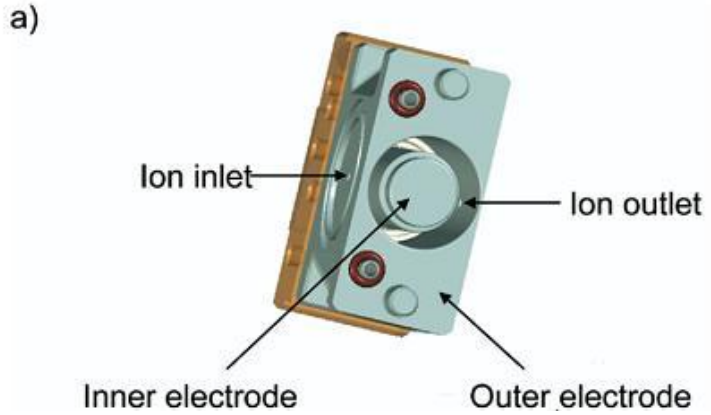
ESI-FAIMS-MS spectrum of protonated reserpine

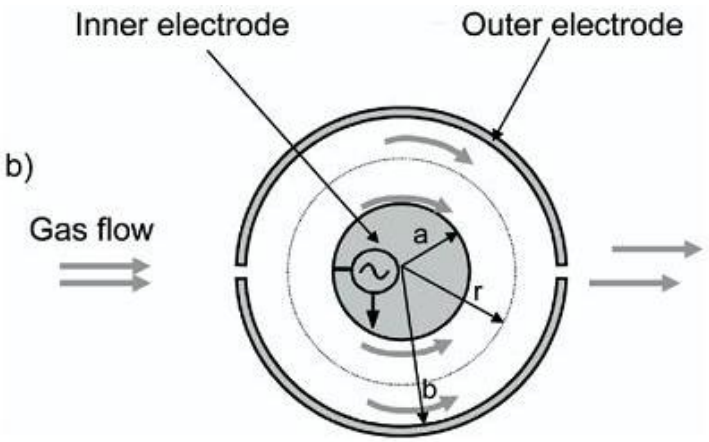
(Owlstone miniaturised FAIMS-Agilent TOF, ESI)



Note:
Compensation field for ion transmission varies with the dispersion field

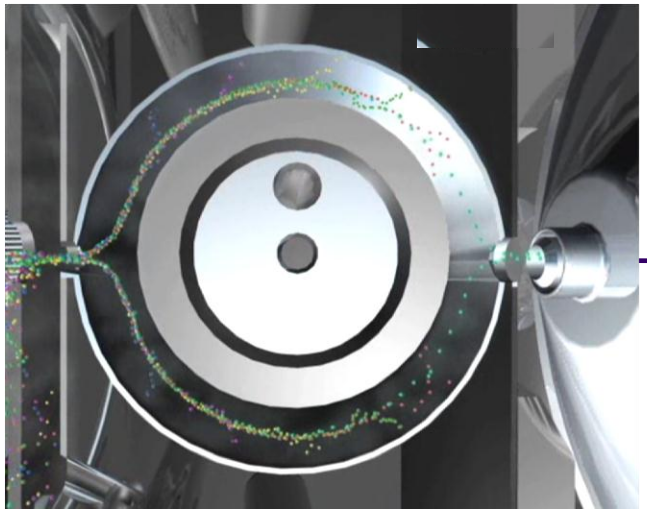
Combining FAIMS with mass spectrometry: Thermo Fisher Scientific cylindrical electrode schematic





(electrode gap ~ 2-3 mm)

Electrospray ion source →

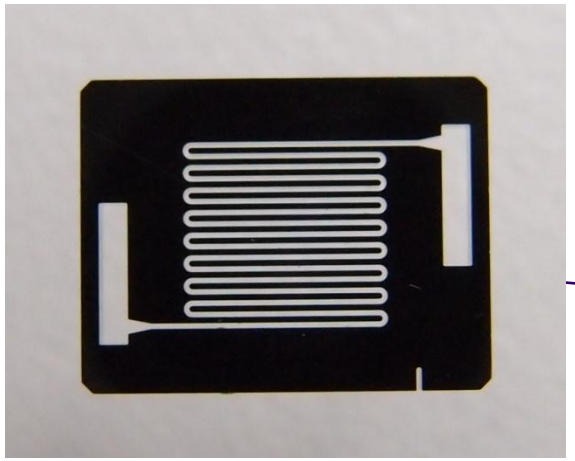


→ Mass spectrometer

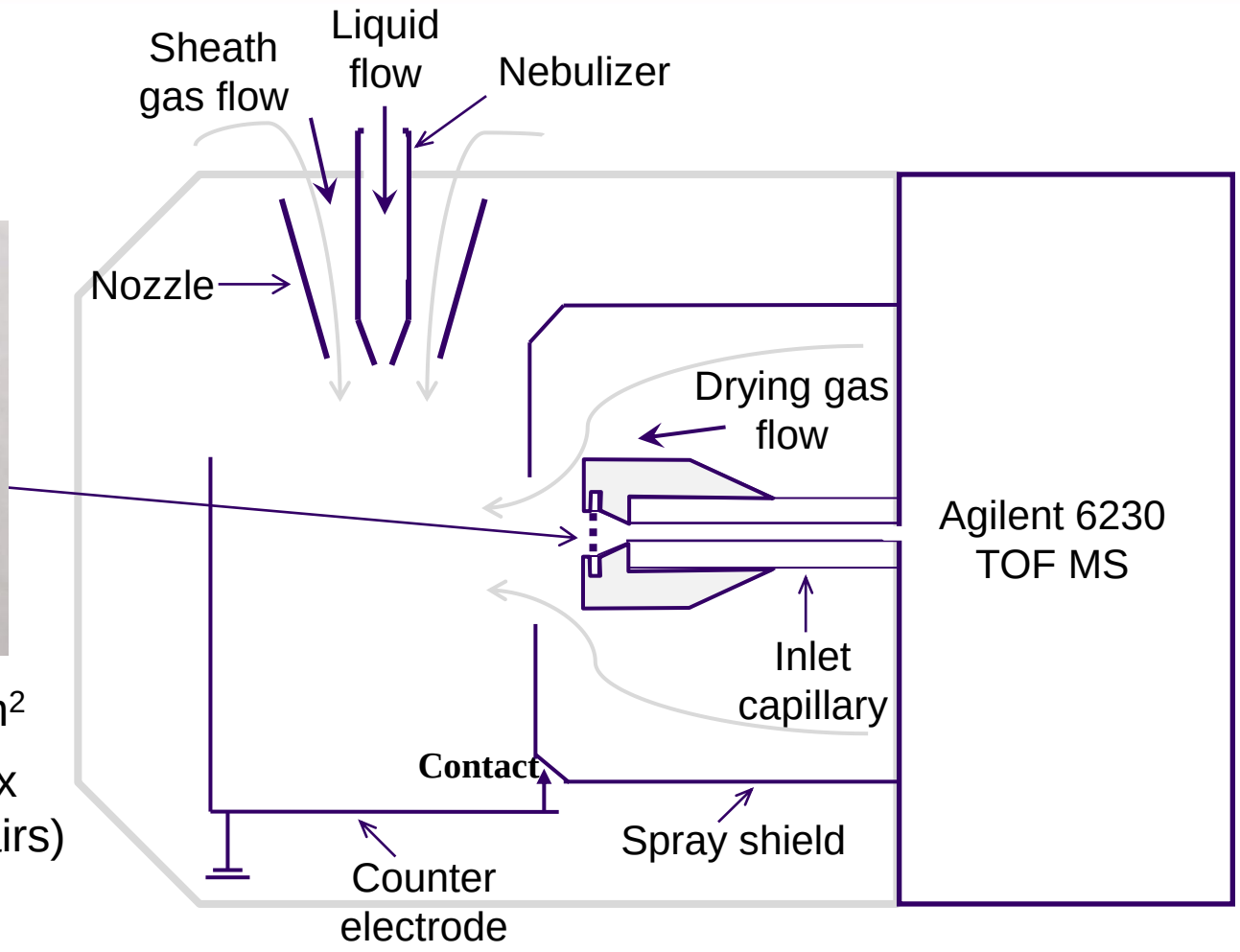
[Barnett et al., *J Am Soc Mass Spectrom,* 2007, 18, 1653–1663]

Prototype Owlstone ultra-FAIMS-Agilent 6230 TOF MS: modified JetStream ESI ion source region

Miniaturised chip-based FAIMS

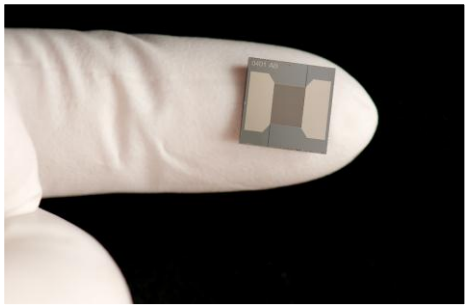


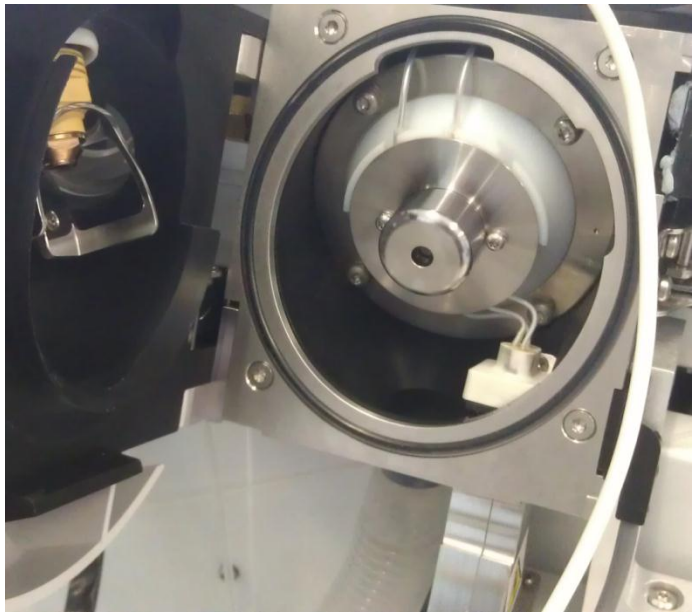
- Total area of chip: 4 mm^2
- Electrode gap: 100 µm x 700 µm (16 electode pairs)

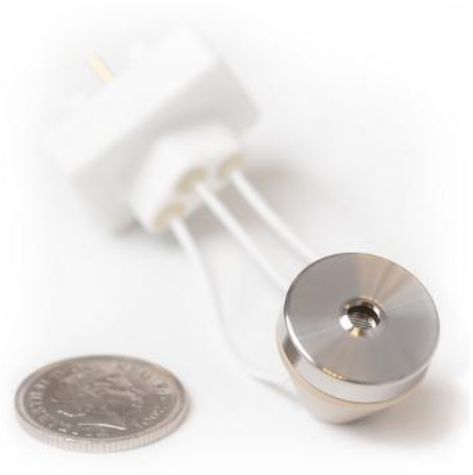


Prototype Owlstone ultra-FAIMS-Agilent 6230 TOF MS

Prototype Owlstone ultra-FAIMS chip mounted into chip cartridge located behind spray shield in Jet Stream ESI source in front of transfer capillary







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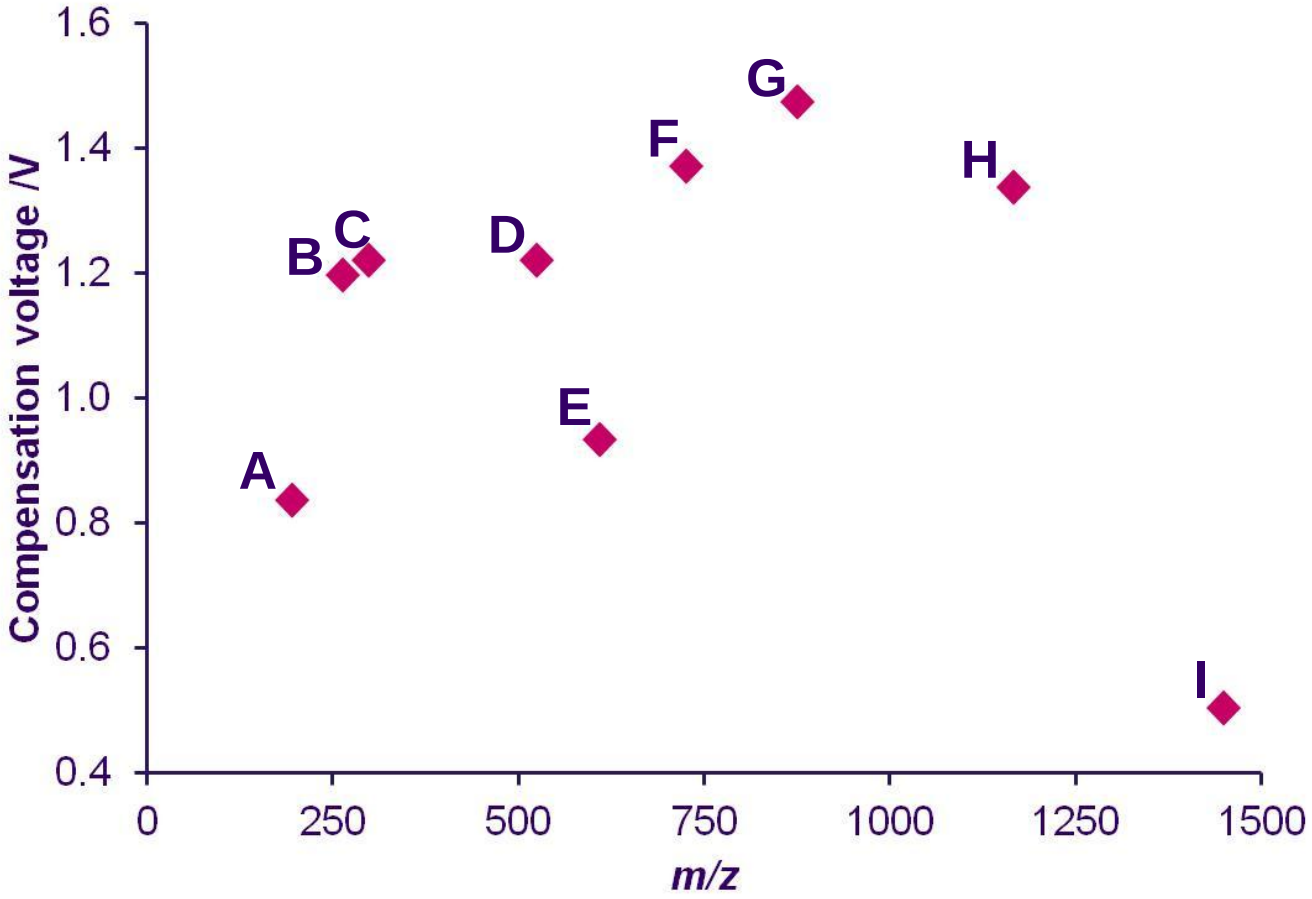
FAIMS combined with mass spectrometry: orthogonal separation enhances analytical space



A. [Caffeine + H]$^{+}$

B. [MRFA + 2H]$^{2+}$

C. [TPAB – Br]$^{+}$

D. [MRFA + H]$^{+}$

E. [Reserpine + H]$^{+}$

F. [Vancomycin + 2H]$^{2+}$

G. [Insulin B Chain + 4H]$^{4+}$

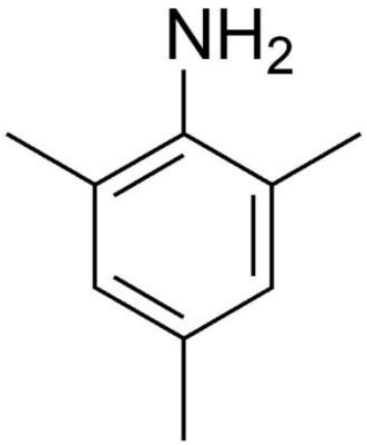
H. [Insulin B Chain + 3H]$^{3+}$

I. [Vancomycin + H]$^{+}$

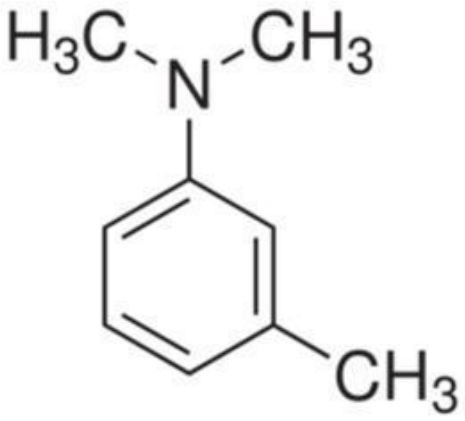
Applications of FAIMS-MS

- Isobaric and isomeric compounds

2,4,6-trimethylaniline and N,N-dimethyl-*m*-toluidine (*m/z* 136.1123)



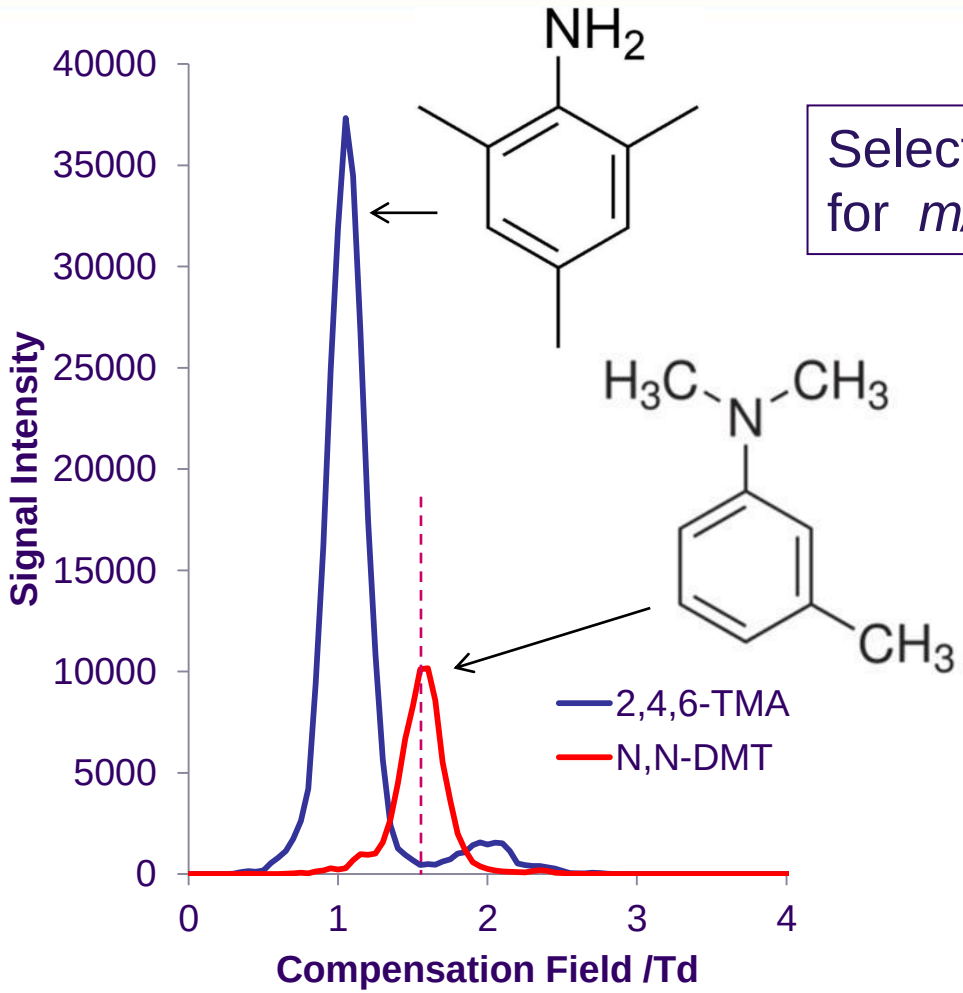
2,4,6-Trimethyl aniline



N,N-dimethy-*m*-toluidine

ESI-FAIMS-MS of 2,4,6-trimethylaniline and N,N-dimethyl-*m*-toluidine

(Dispersion field = 230 Td; electrode gap = 100 µm; 50 ng/ml)



Selected ion response for *m/z* 136

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TD- ESI-FAIMS-MS of N,N-dimethyl-*m*-toluidine (DF = 230 Td)
N,N-DMT concentration: 1 ppm wrt surrogate API

Thermal desorption of N,N-DMT from an API surrogate
(2-stage desorption of N,N-DMT)

↓

Extractive electrospray ionization

↓

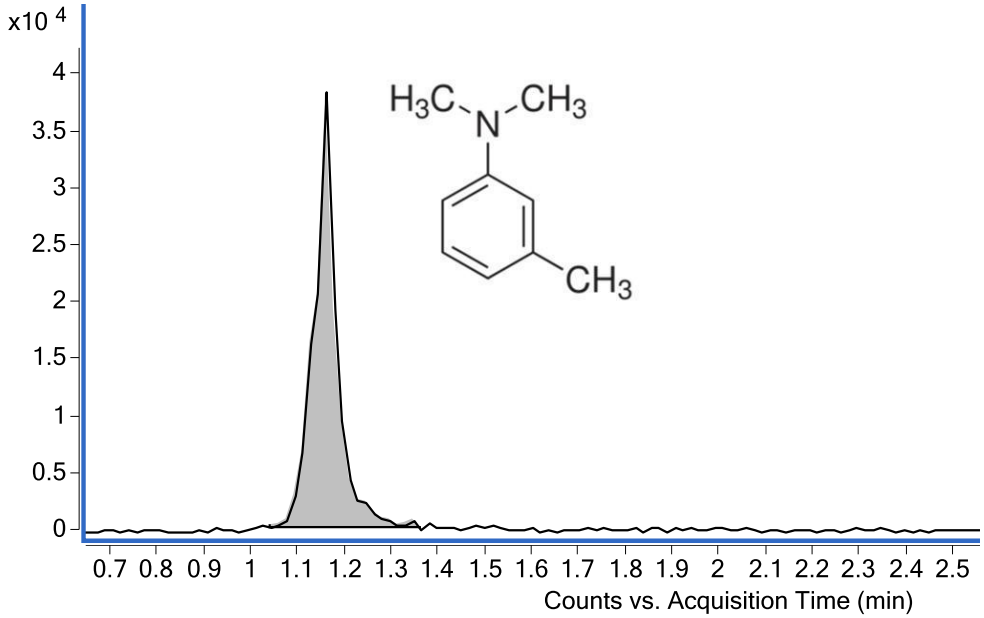
FAIMS selection
(DF = 230 Td; CF = 1.5 Td)

↓

Mass spectrometry detection
(SIR *m/z* 136)

Thermal Desorption-ESI-FAIMS-MS

N,N-DMT SIR (*m/z* 136)



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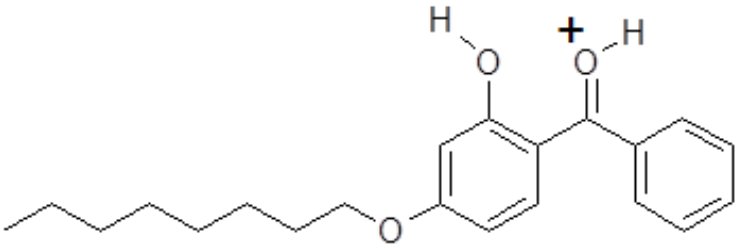
Applications of FAIMS-MS

- Reducing sample complexity/chemical noise

ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture

$$[HO—(CH_2CH_2O)_n—H + H]^+$$
(n = 7)

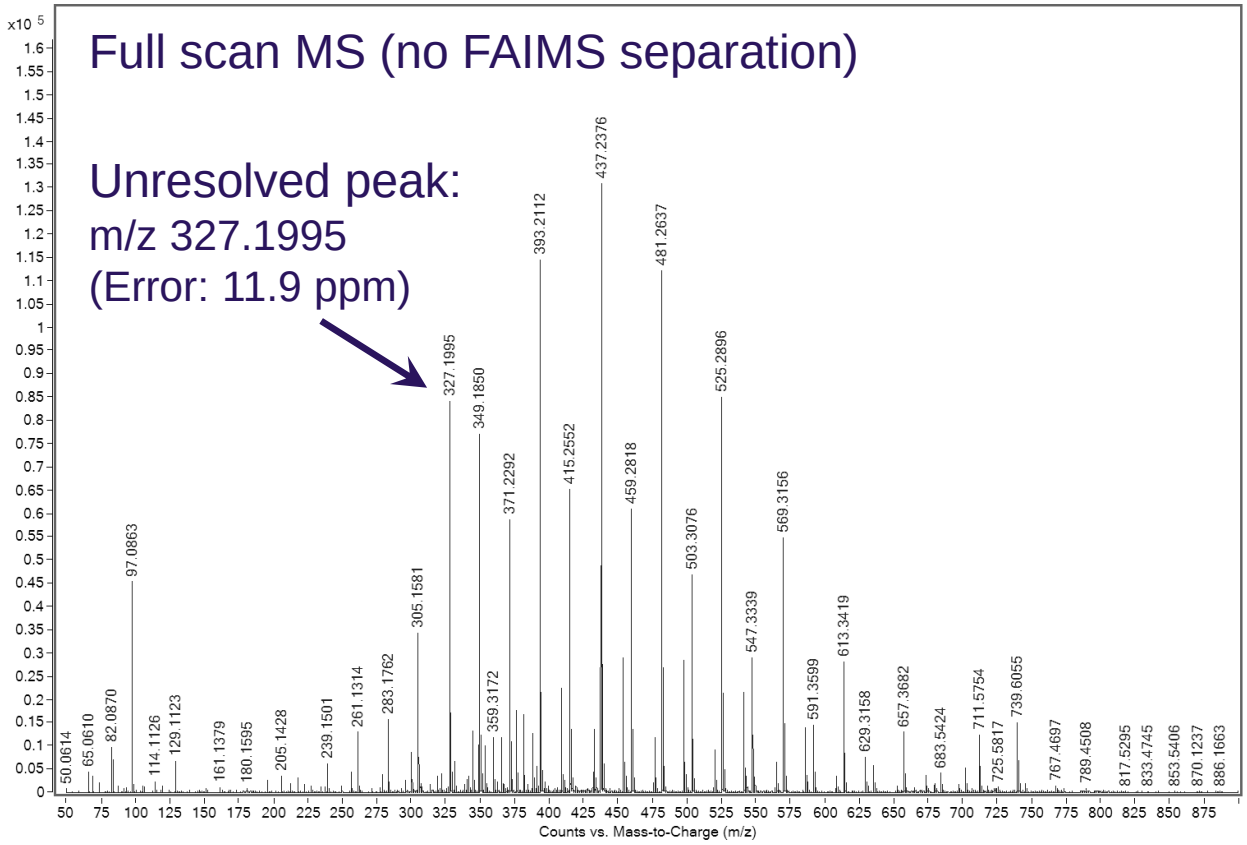


Protonated PEG (*m/z* 327.2013)

HOBP (*m/z* 327.1955)

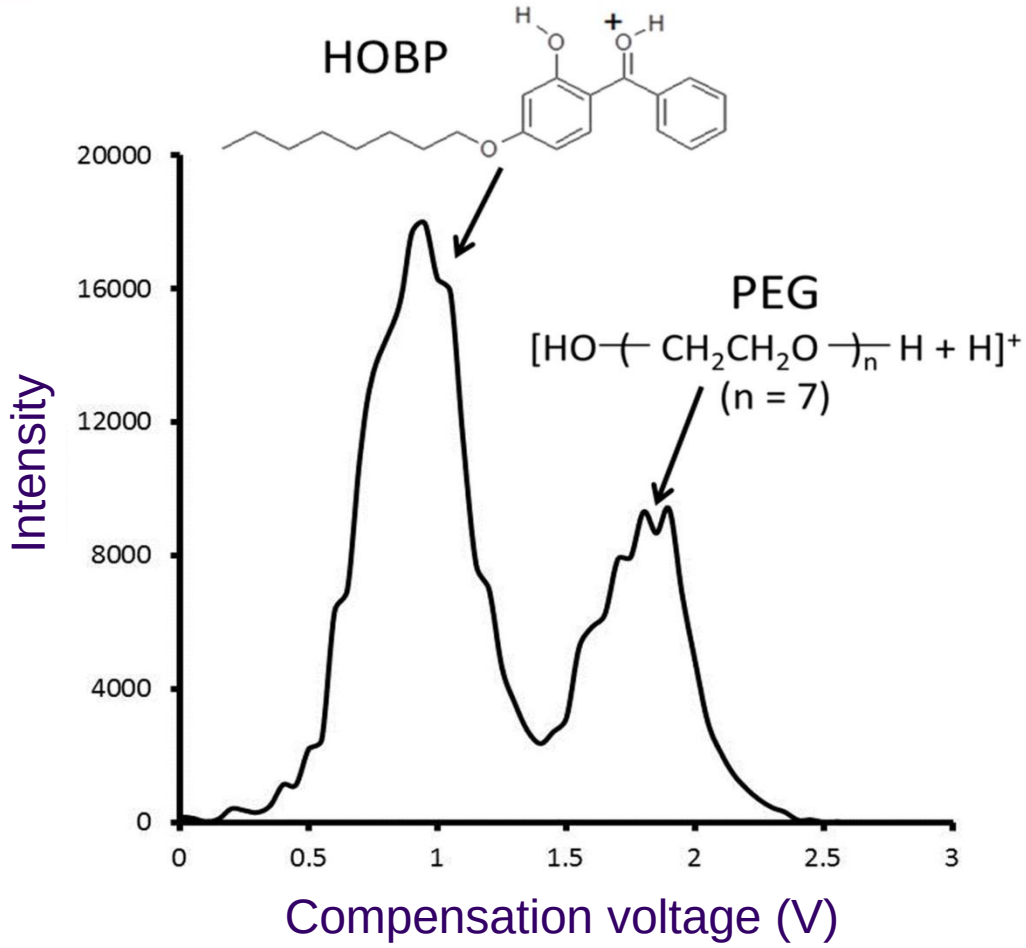
[L Brown, R Smith, D Toutoungi, J Reynolds, A Bristow, A Ray, A Sage, D Weston, I Wilson, B Boyle, C Creaser, *Anal. Chem.*, 2010, 82, 9827-9834]

ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture

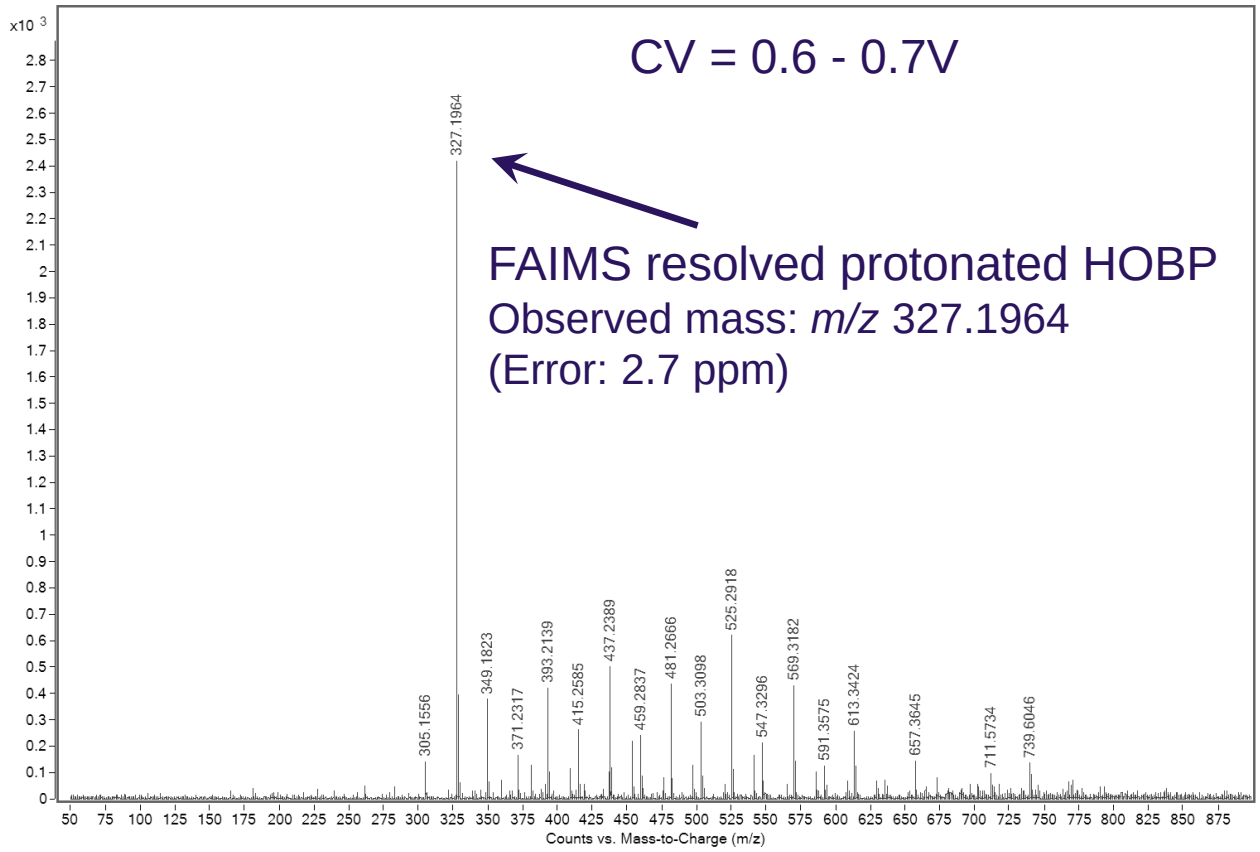


Protonated PEG (*m/z* 327.2013) and HOBP (*m/z* 327.1955) ions

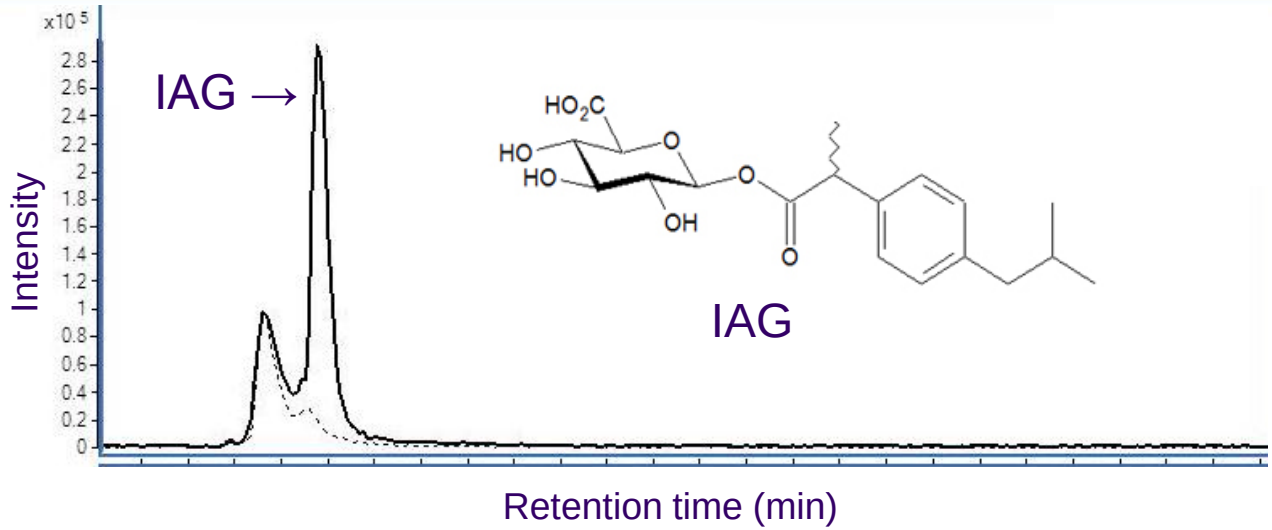
ESI-FAIMS-TOFMS separation (*m/z* 327.2) of HOBP:PEG mixture with resolved PEG 400 (n=7, *m/z* 327.2013) and HOBP (*m/z* 327.1955) ions



ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture



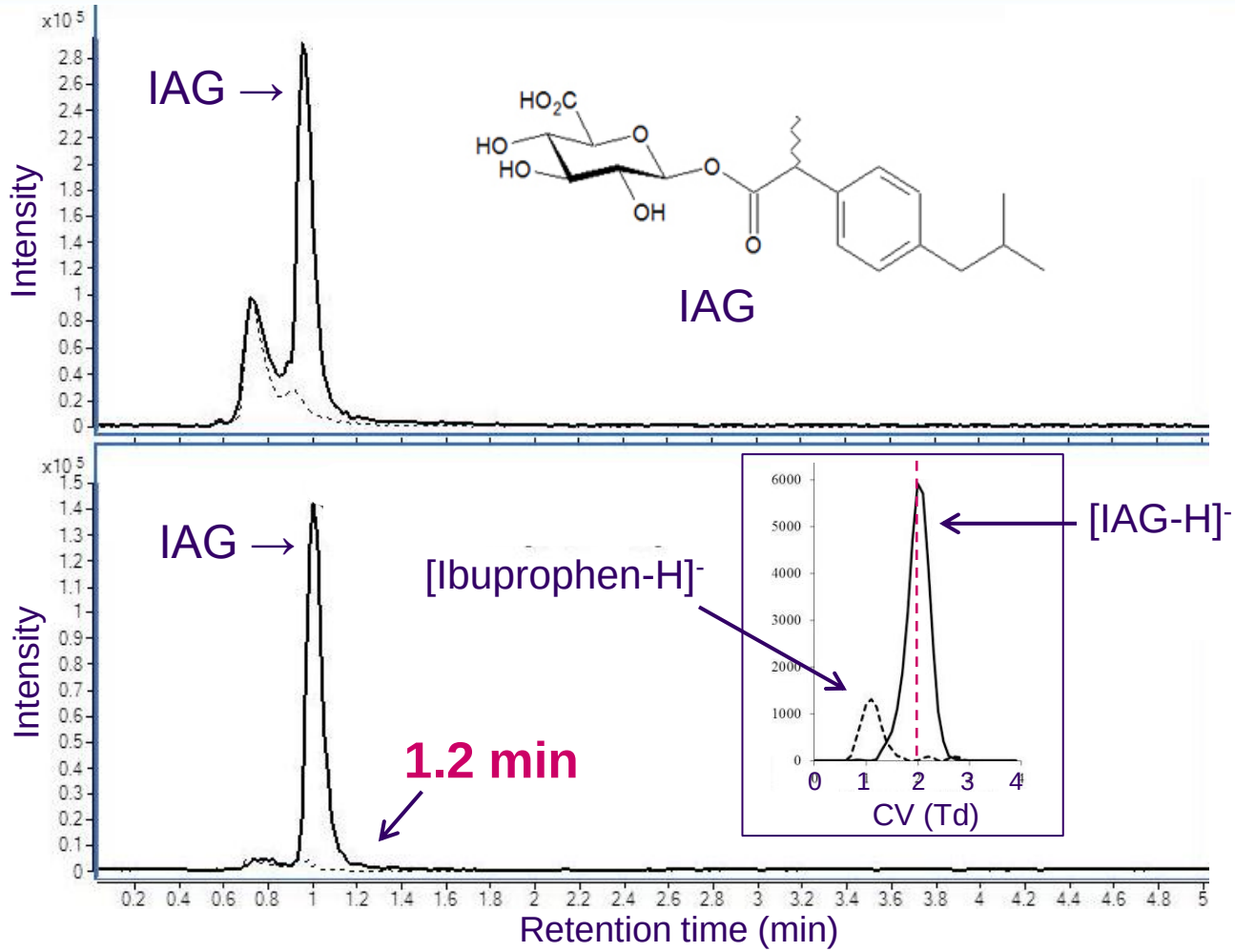
U(H)PLC-FAIMS-TOFMS analysis (-ve ion) of (R/S) ibuprofen 1-β-O acyl glucuronide (IAG) in human urine



FAIMS off

m/z 381±0.02

U(H)PLC-FAIMS-TOFMS analysis (-ve ion) of (R/S) ibuprofen 1-β-O acyl glucuronide (IAG) in human urine



FAIMS off
m/z 381±0.02

FAIMS on
m/z 381±0.02
DF 260 Td
CF 2.2 Td

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U(H)PLC-FAIMS-TOFMS analysis (-ve ion) of (R/S) ibuprofen 1-β-O acyl glucuronide (IAG) in human urine

Comparison of LOQ; LDR (R^2) and intra-day reproducibility for the determination of IAG spiked into urine (15.5 *μ*g/ml, n=5)

	LC-MS	LC-FAIMS-MS
LOQ (μg/ml)	0.018	0.010
LDR (μg/ml)	0.018-11	0.010-11
R^2	0.9991	0.9987
Intra-day (% RSD)	5.0	2.7

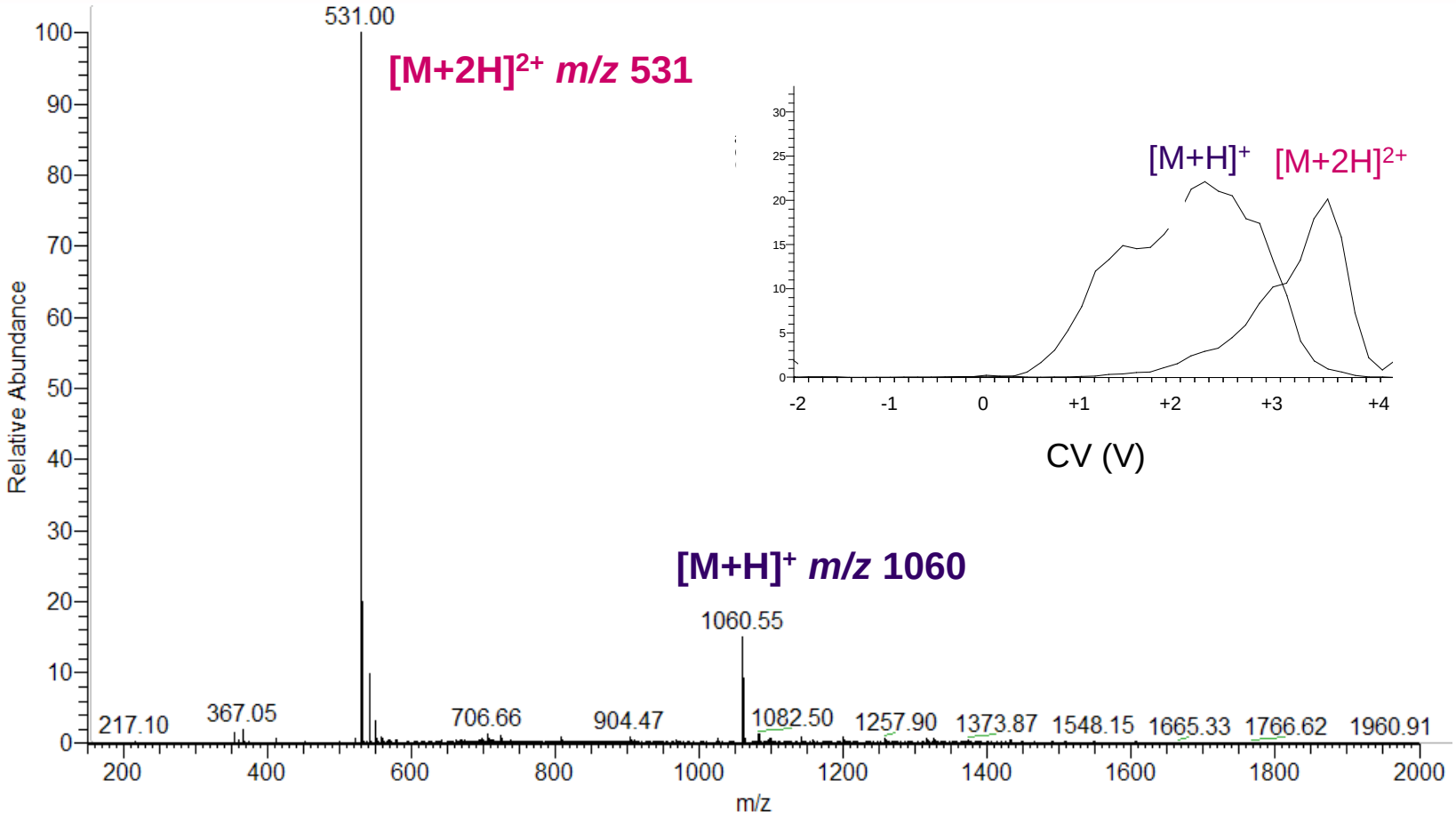
[Smith el al, *J. Chromatogr. A*, 127, 76-81, 2013]

Applications of FAIMS-MS

- Charge state and conformers

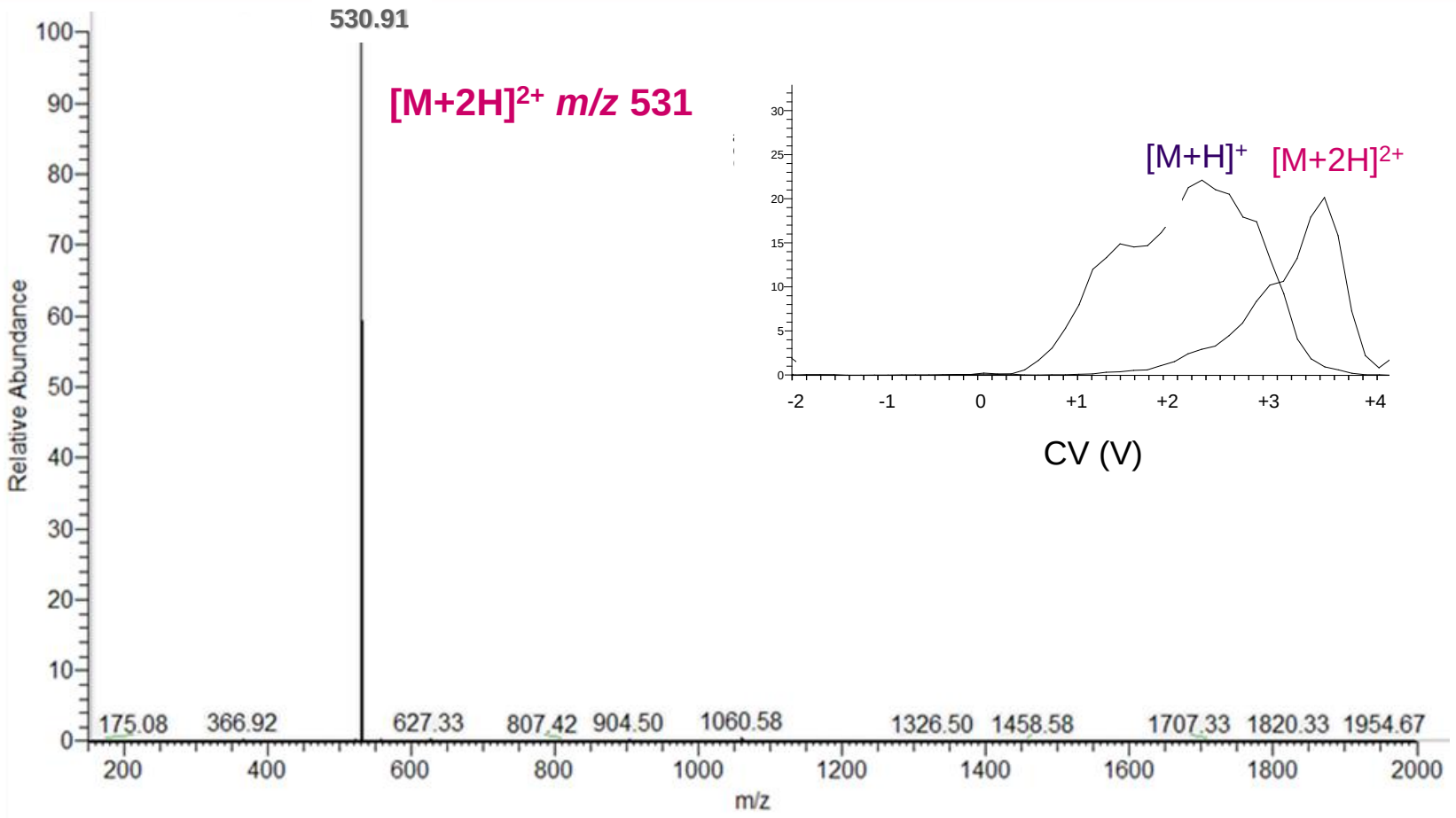
Bradykinin nano-ESI-LTQ mass spectrum without FAIMS separation

(DV 60 kV cm^{-1}; 22 MHz; N_2 drift gas; CV scan 0.5 V cm^{-1}; 10 pmol μl^{-1})



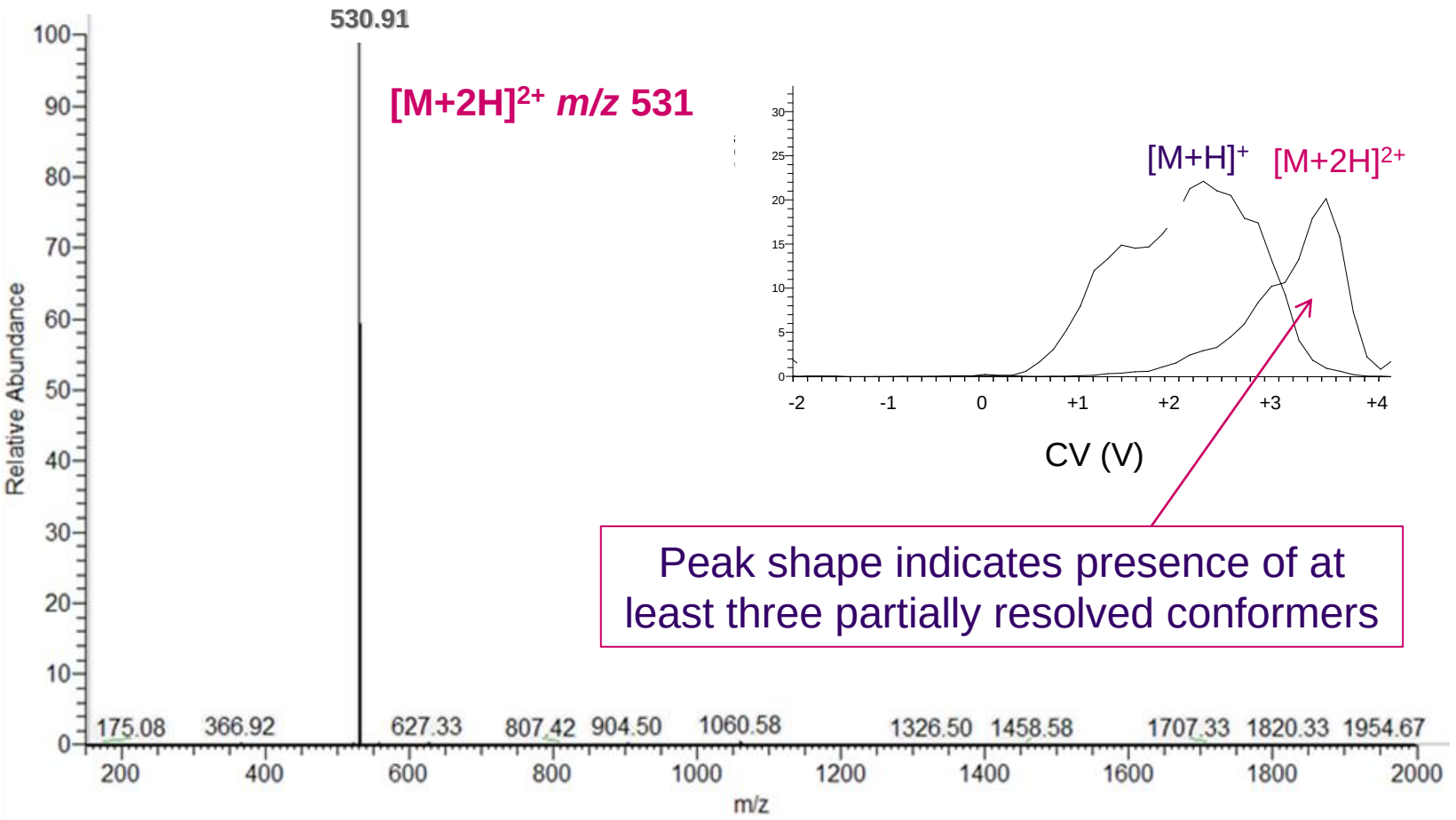
[L Brown et al., *Anal. Chem.*, 2010, 82, 9827-9834]

Bradykinin NSI-FAIMS-LTQ mass spectrum with FAIMS pre-selection of $[M+2H]^{2+}$ ion (CV +3.5 - +3.6 V)



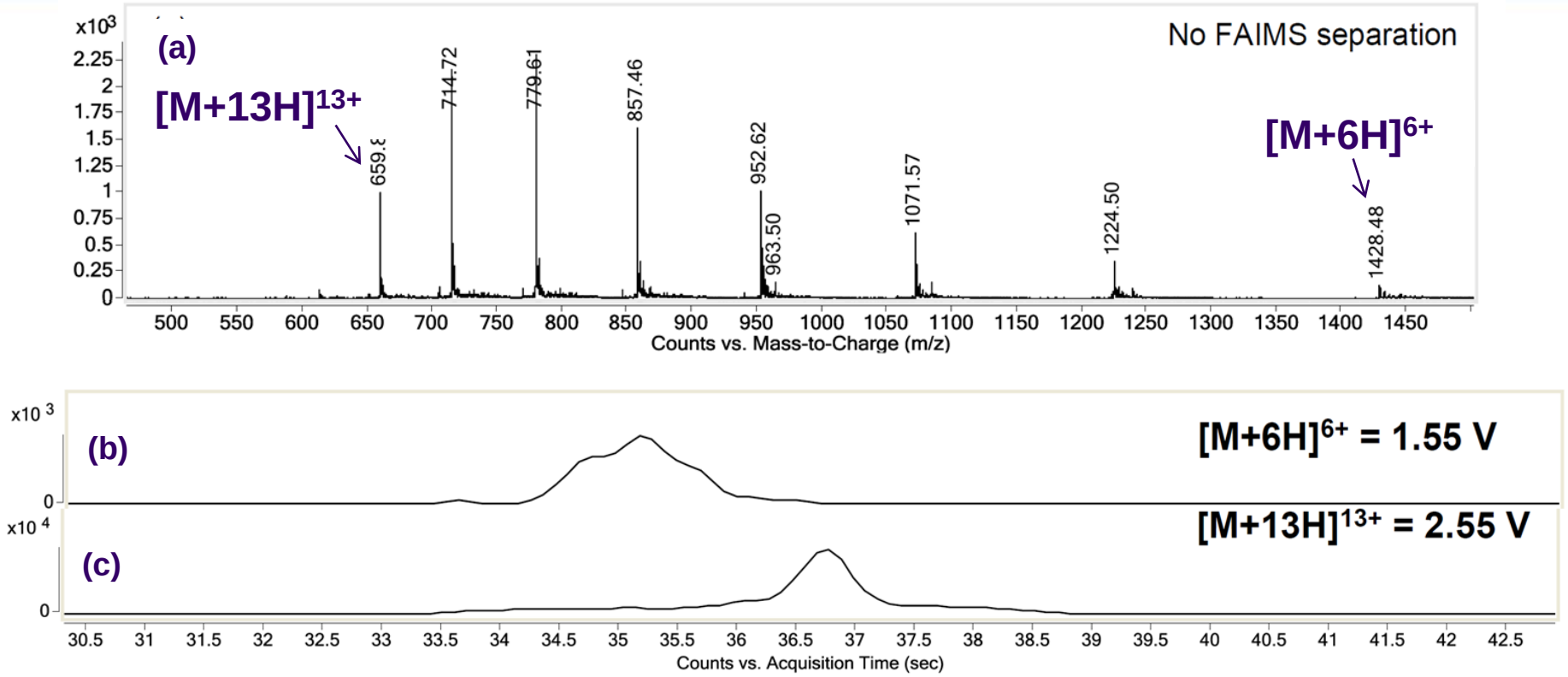
[L Brown et al., *Anal. Chem.*, 2010, 82, 9827; *Current Analytical Chemistry*, 9, 192, 2013]

Bradykinin NSI-FAIMS-LTQ mass spectrum with FAIMS pre-selection of $[M+2H]^{2+}$ ion (CV +3.5 - +3.6 V)



[L Brown et al., *Anal. Chem.*, 2010, 82, 9827; *Current Analytical Chemistry*, 9, 192, 2013]

MS and FAIMS-MS of Ubiquitin

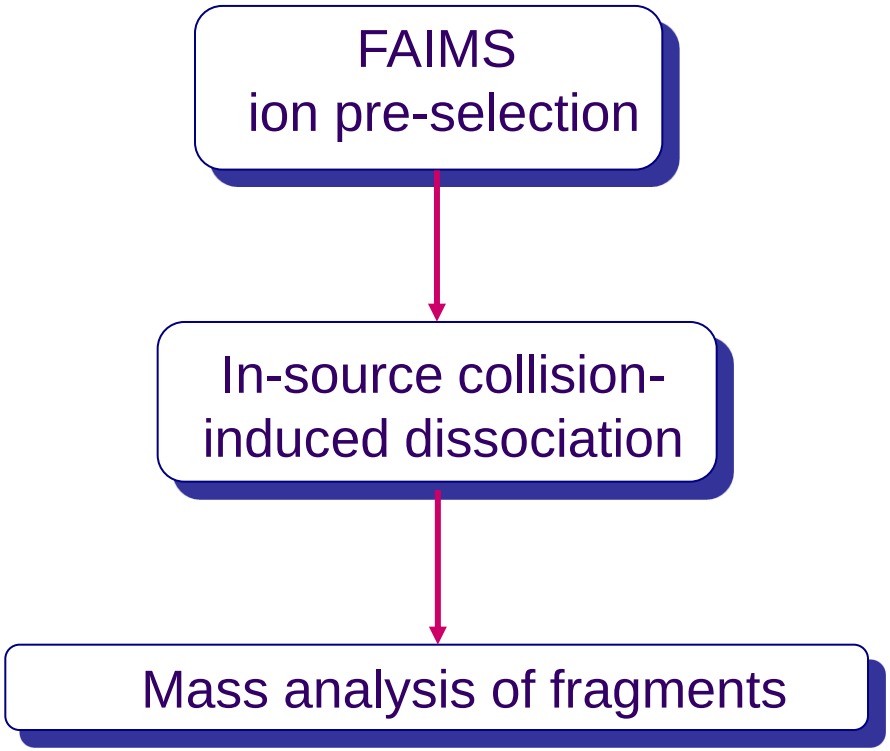


(a) Mass spectrum of Ubiquitin, (b) FAIMS spectrum of $[M+6H]^{6+}$ (*m/z* 1428), (c) FAIMS spectrum of $[M+13H]^{13+}$ (m/z 659) [FAIMS DF = 60 kV/cm]

Applications of FAIMS-MS

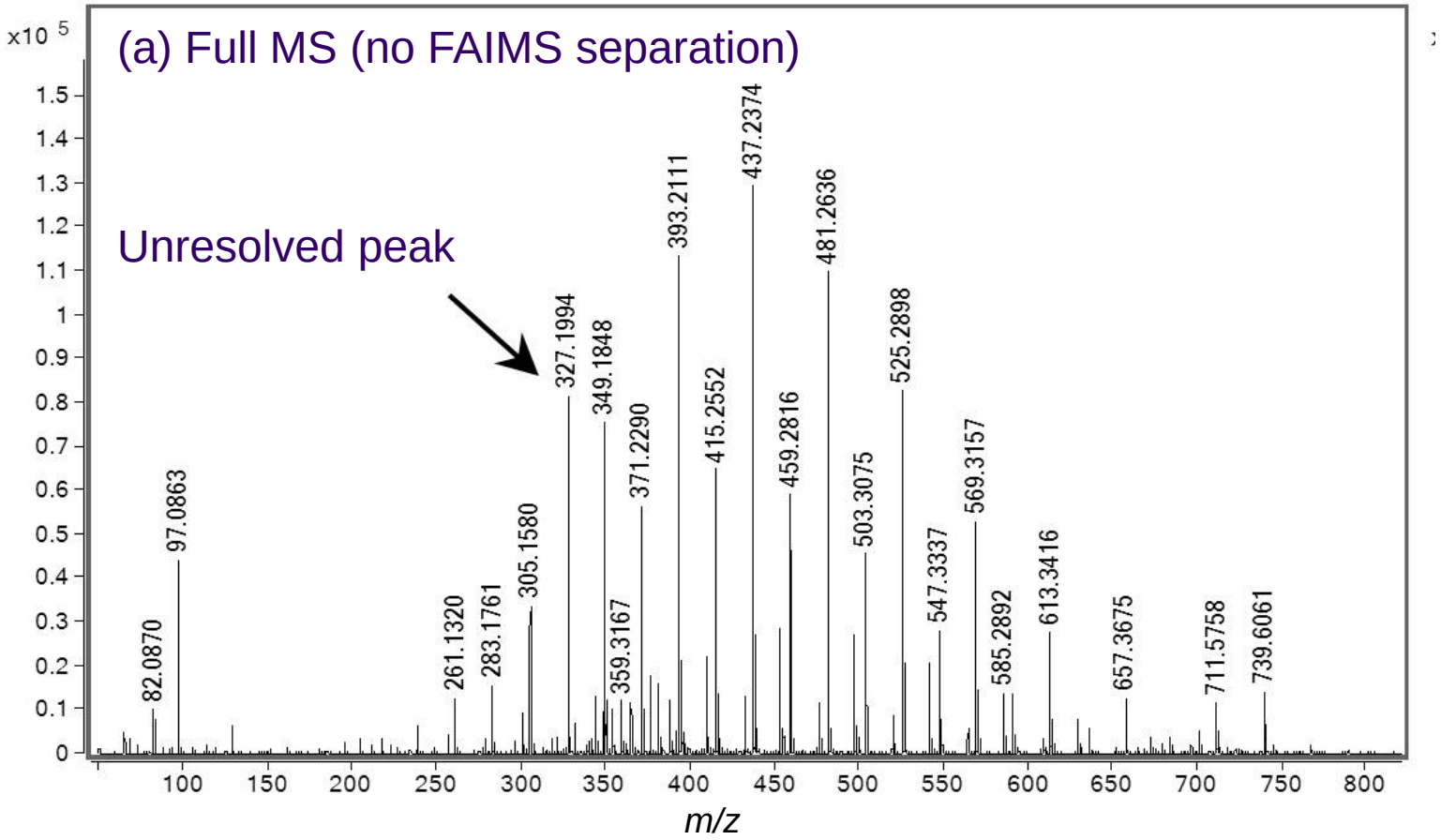
- Enhancing FAIMS-MS selectivity

FAIMS-selected in-source CID-MS (FISCID-MS)



[Brown, L; Smith, R et al., *Anal Chem*, 84, 4095, 2012]

ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture

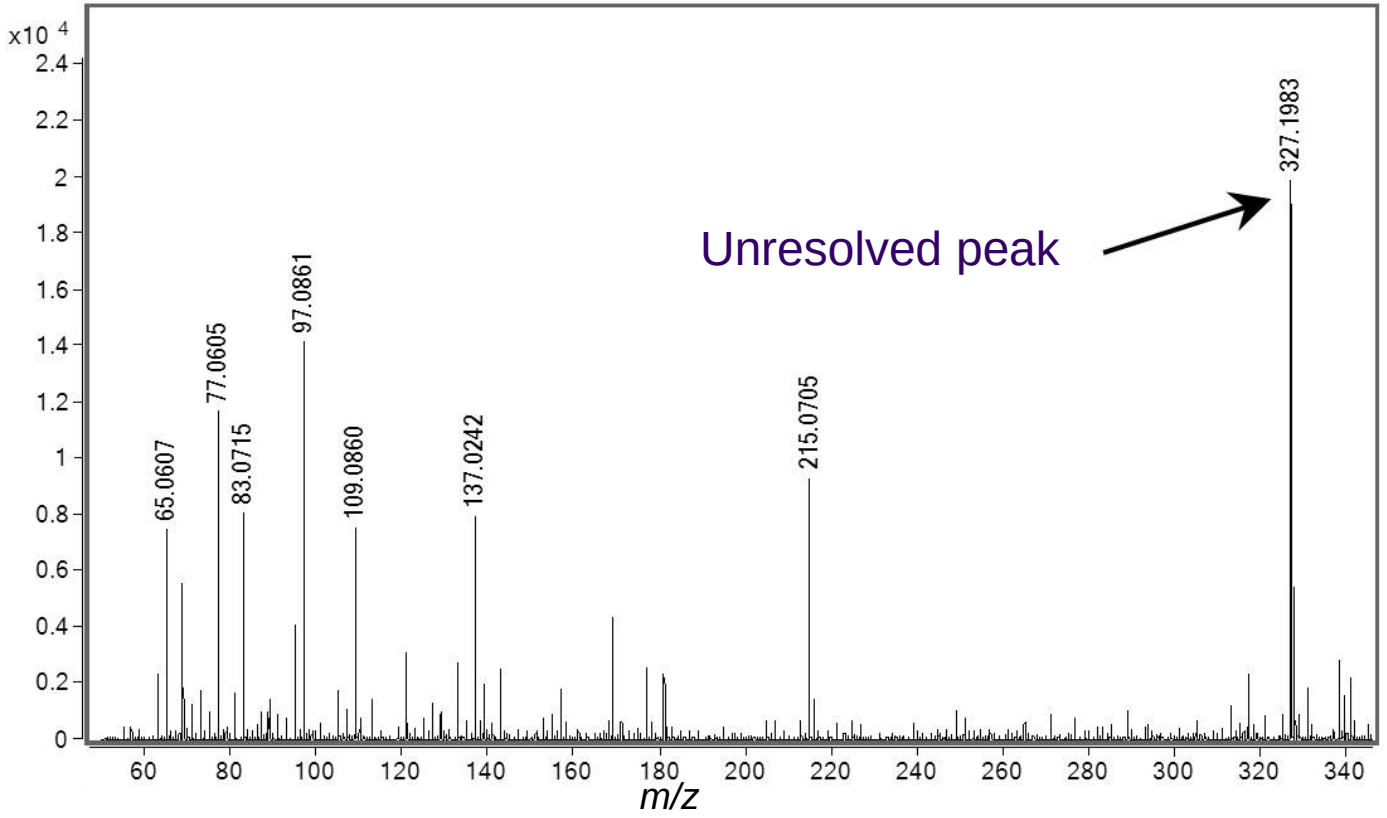


Protonated PEG (*m/z* 327.2013) and HOBP (*m/z* 327.1955) ions

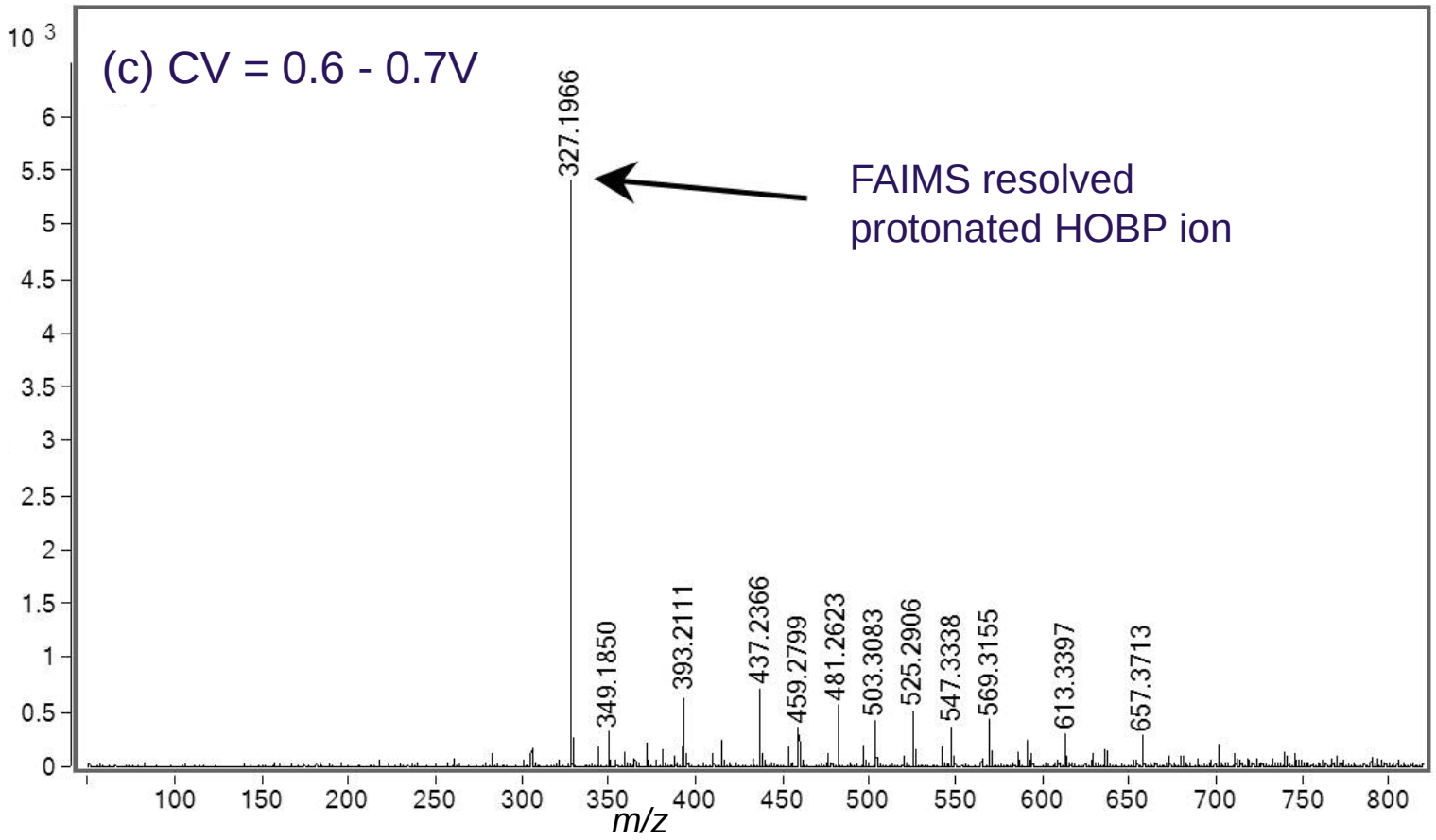
[Rob Smith]

ESI-CID-TOFMS analysis of PEG/HOPB mixture

(b) CID-MS without FAIMS separation

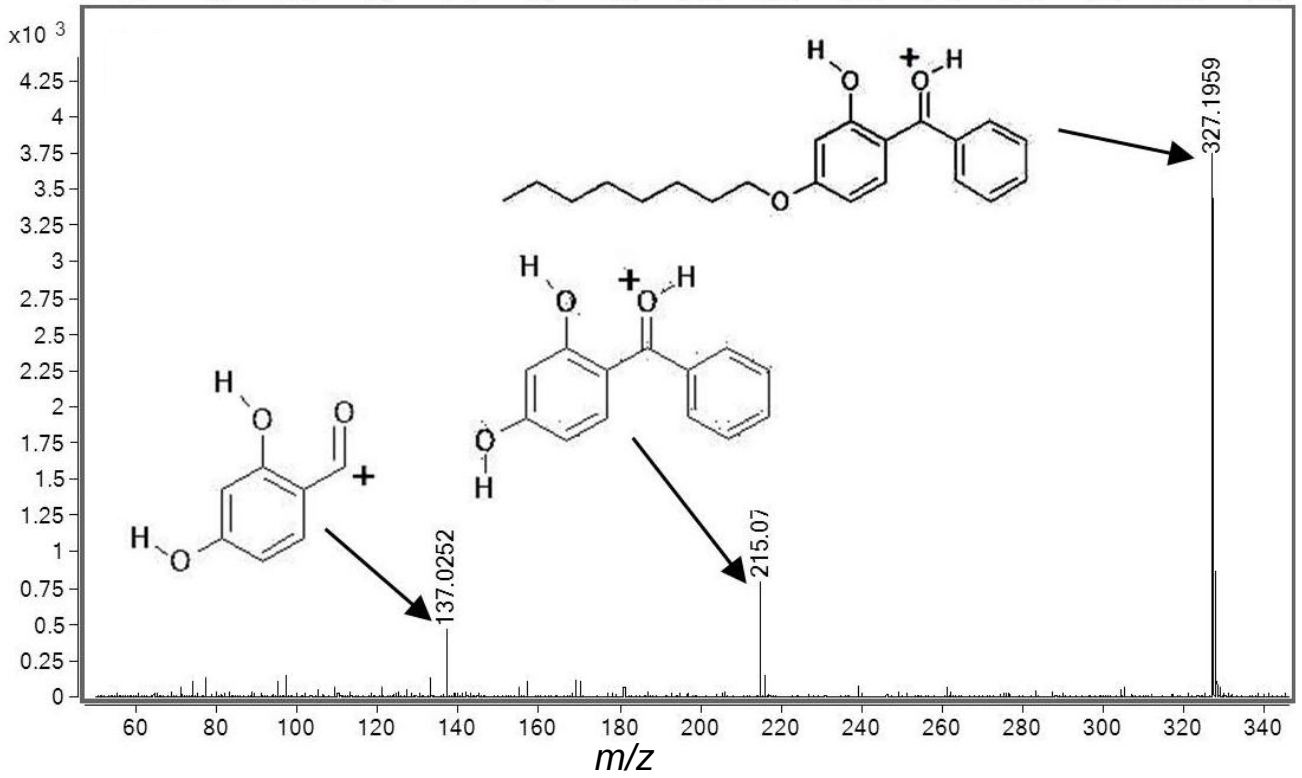


ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture



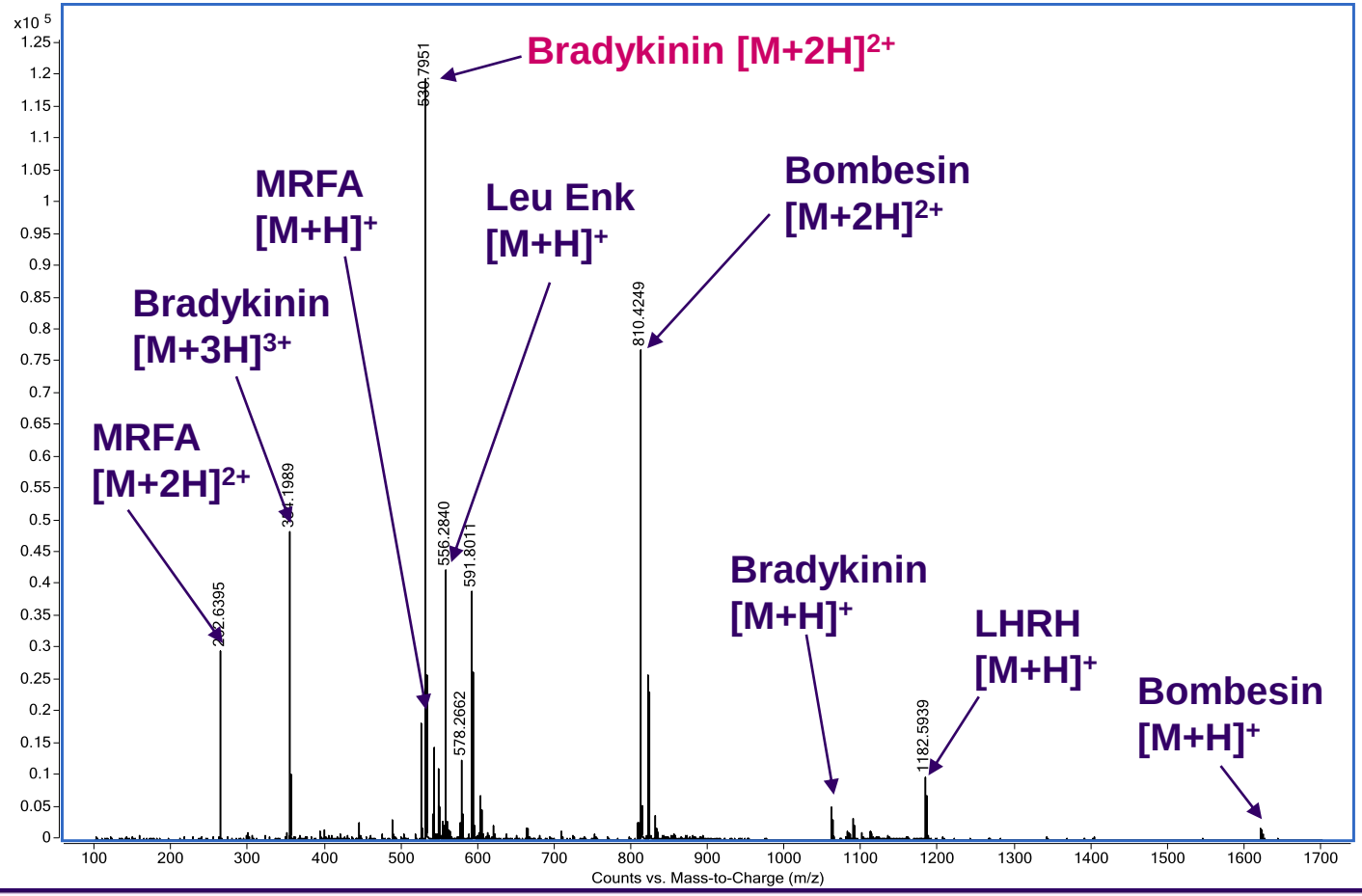
ESI-FISCID-TOFMS analysis of PEG/HOPB mixture

(d) ultra-FAIMS-CID-MS, CV = 0.6 - 0.7V



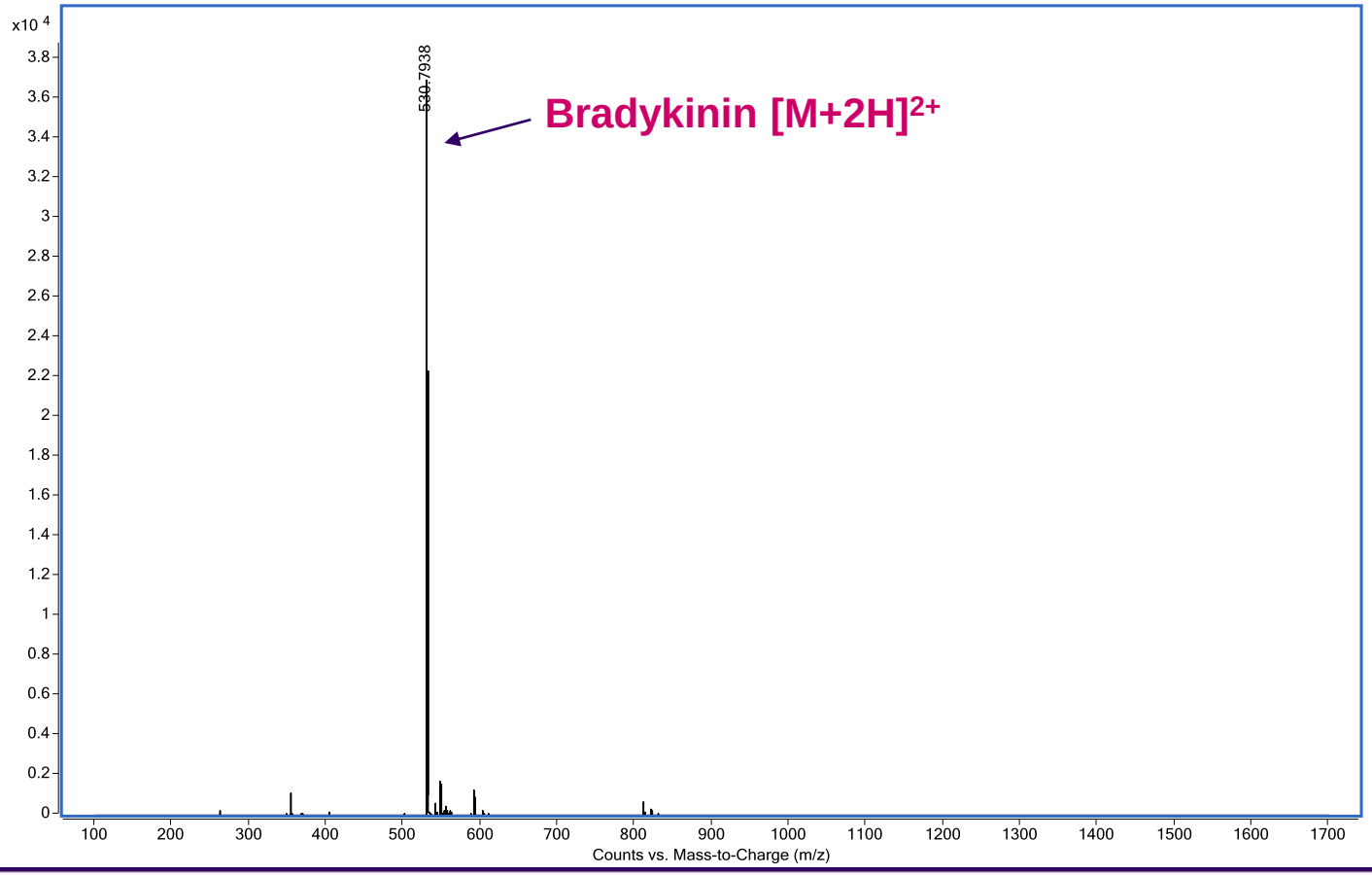
MS of peptide mixture without FAIMS separation

- Owlstone ultra-FAIMS-Agilent TOF MS: no FAIMS selection



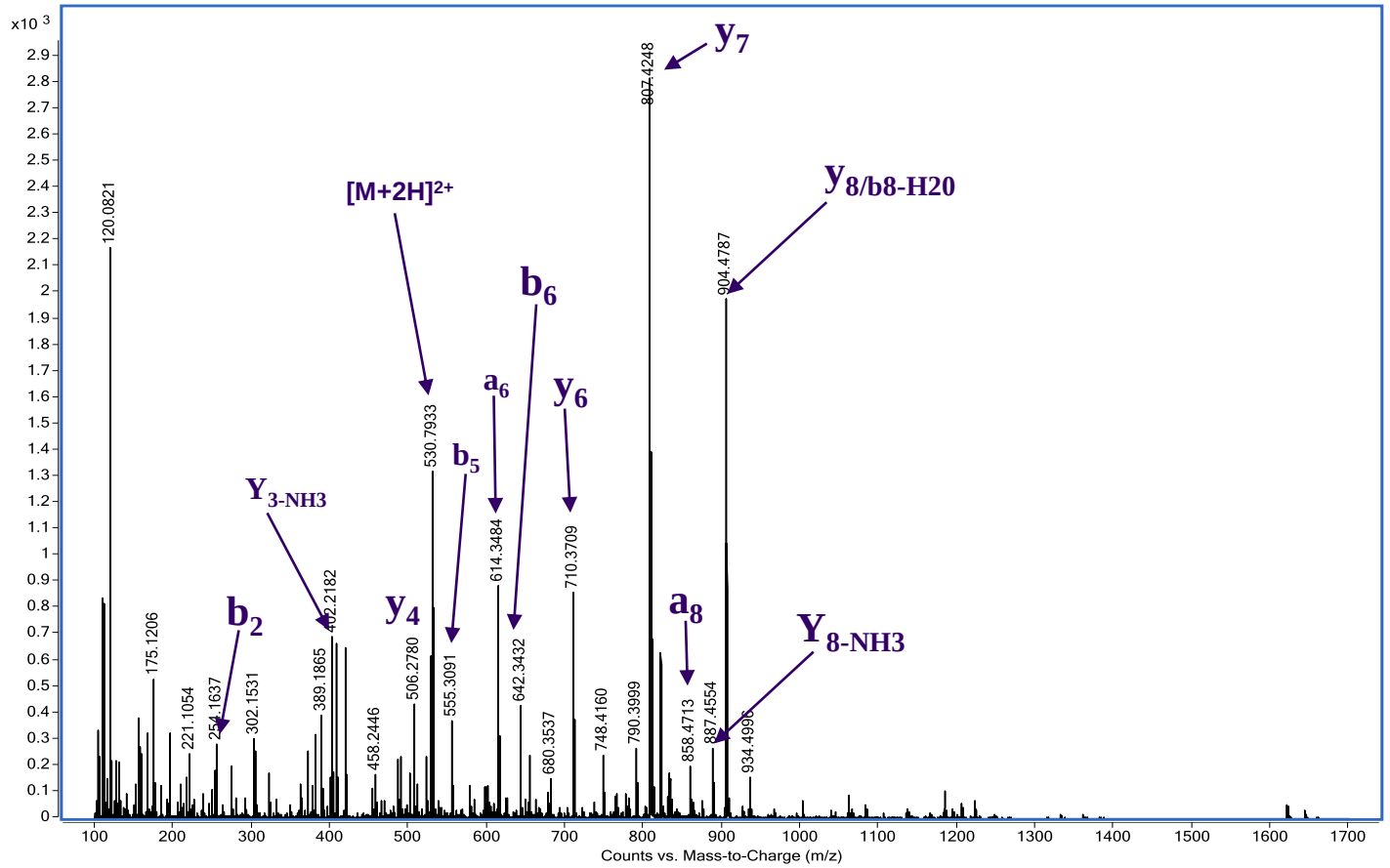
FAIMS-MS of peptide mixture with FAIMS pre-selection of Bradykinin (*m/z* 531, $[M+2H]^{2+}$)

- Owlstone ultra-FAIMS-Agilent TOF MS: CV = 2.6-2.7 selected

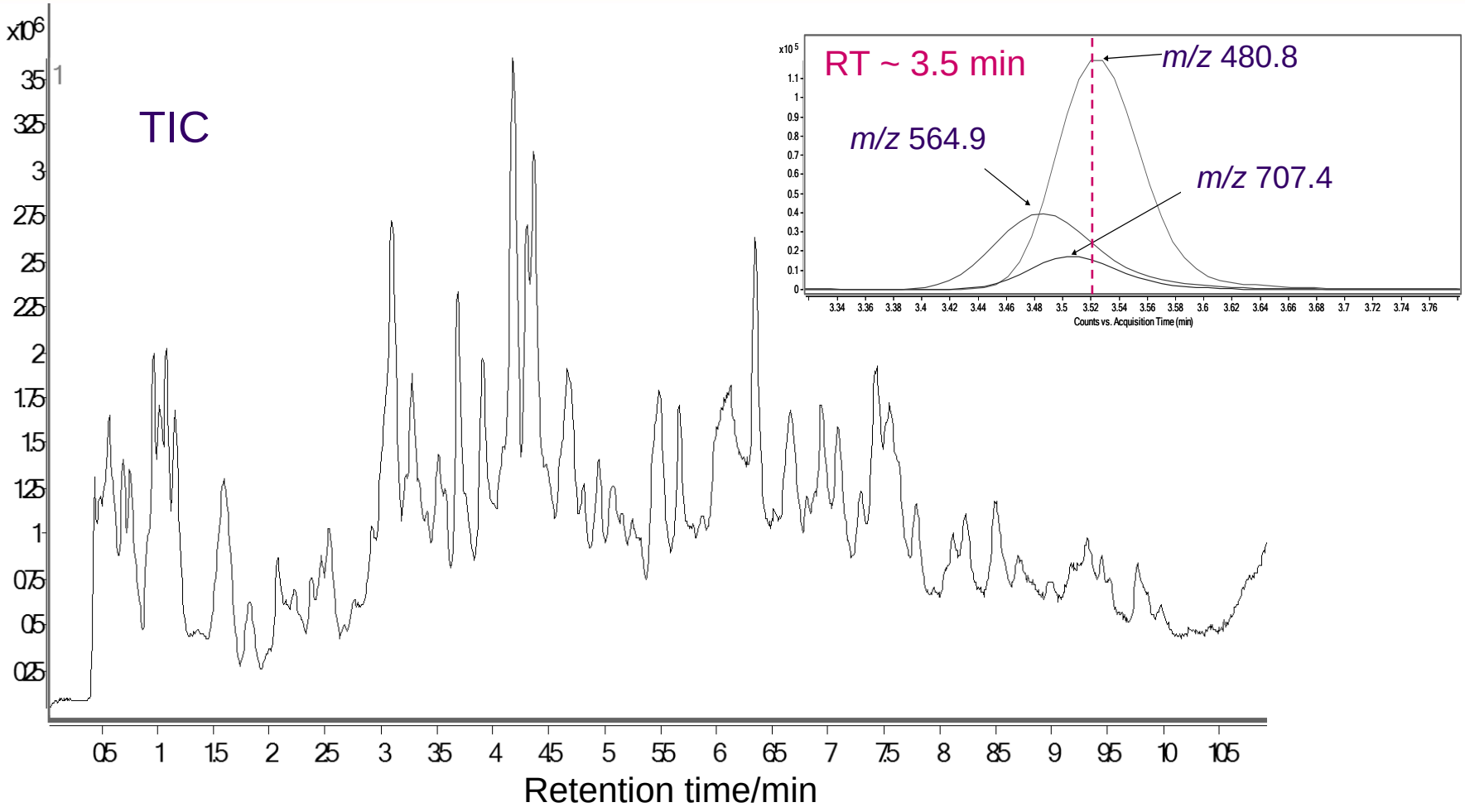


FISCID-MS of peptide mixture with FAIMS pre-selection of Bradykinin (*m/z* 531, $[M+2H]^{2+}$) and in-source CID

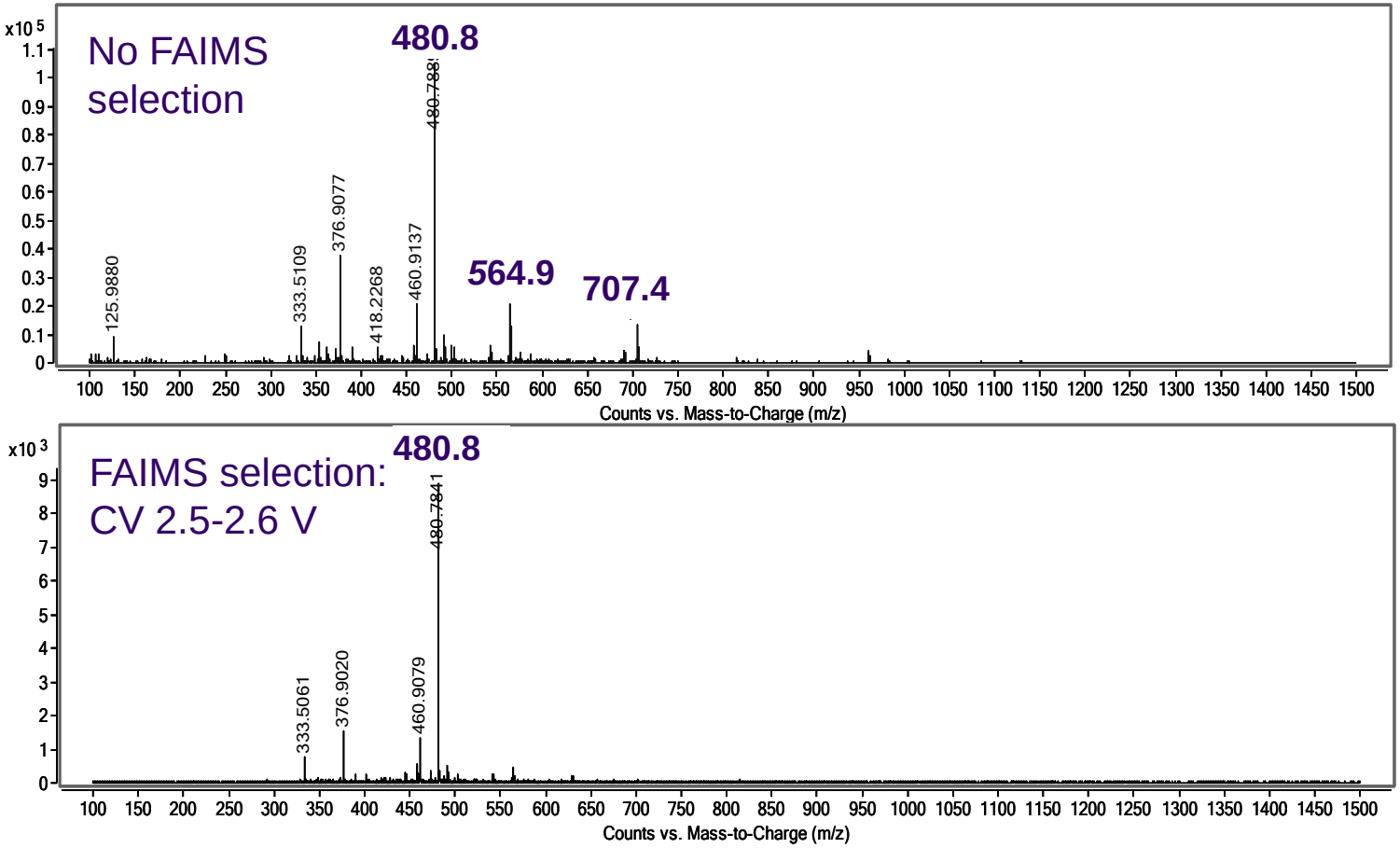
- Owlstone ultra-FAIMS-Agilent TOF MS; CV = 2.6-2.7; Fragmentor 350 V



LC-MS analysis of tryptic digest of depleted human plasma



LC-MS and LC-FAIMS-MS analysis of tryptic digest of depleted human plasma



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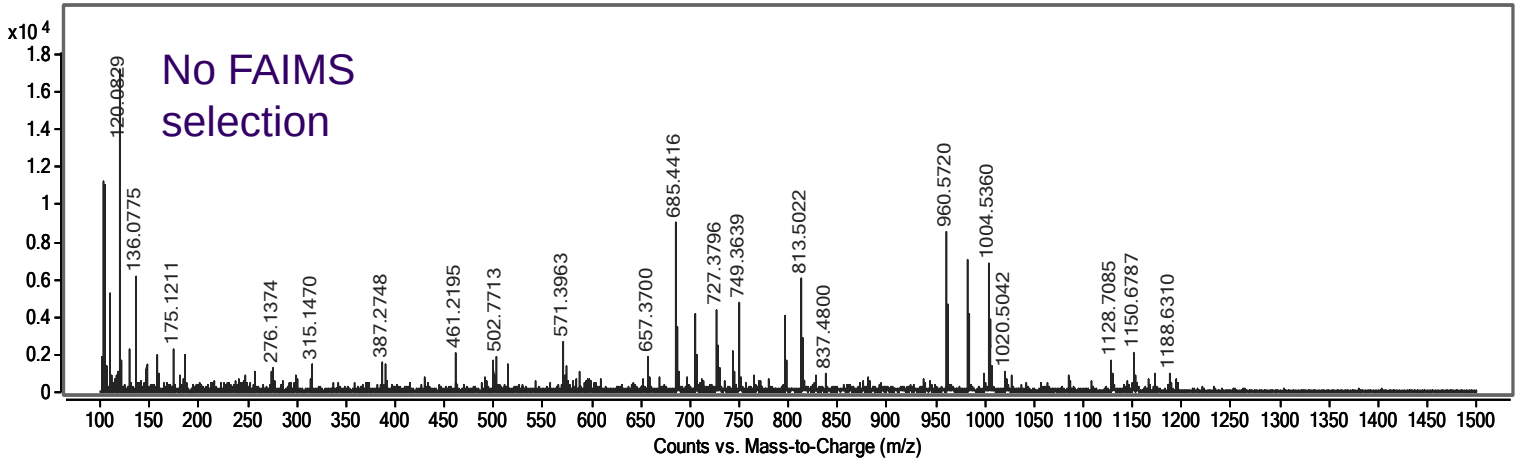
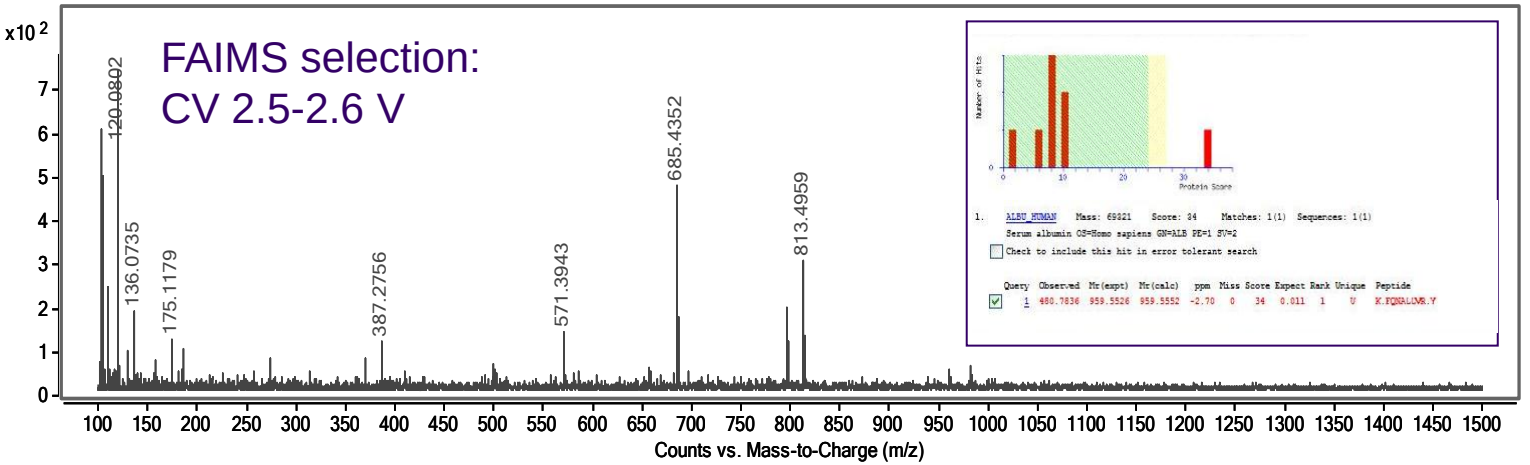
LC-FISCID-MS analysis of tryptic digest of depleted human plasma





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FAIMS-mass spectrometry: Conclusions

- FAIMS-MS configurations allow orthogonal separation of ions based on differential ion mobility and *m/z* (and retention time with LC or GC)
  - Enhanced analytical space (improved peak capacity)
- Separation of isobaric/isomeric ions (in some cases)
- Charge state/conformer separation
- Reduction in sample complexity:
  - Improved S:N and discrimination of target ions from background chemical noise
  - Enhanced quantitative performance
- Wide range of structural, qualitative and quantitative applications

Acknowledgements

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