On-line webinar: Microscale FAIMS-MS and Pharmaceutical Analysis

Date/time: Thursday 9th May, 4pm BST

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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)

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:

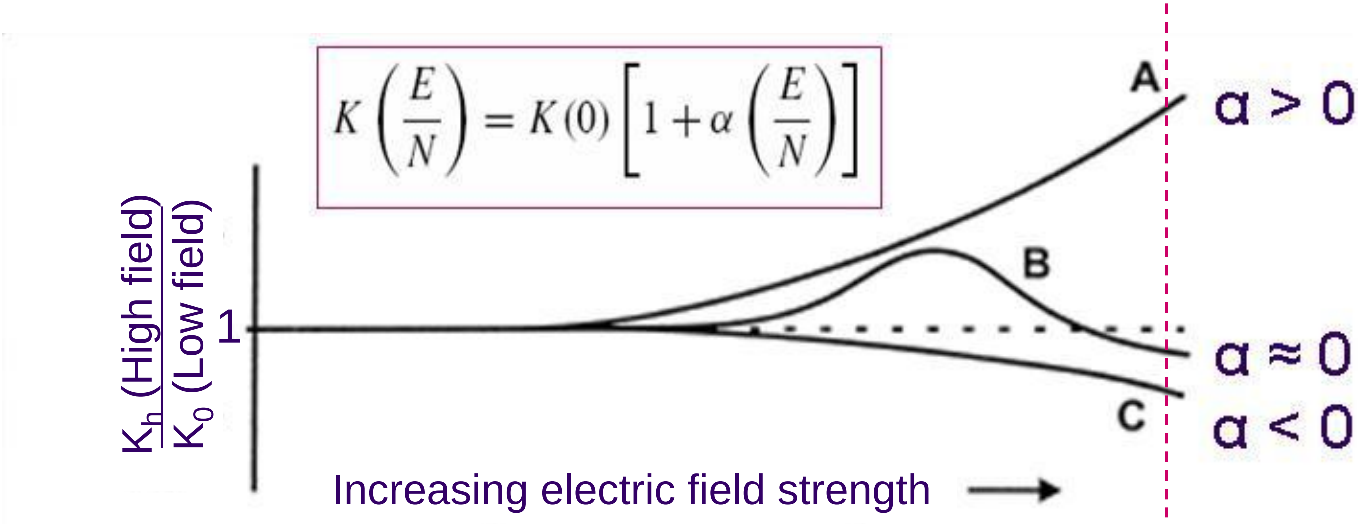
$$v = K . E$$

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

- This relationship only holds under low field conditions ($E \rightarrow 0$)

Ion mobility in high and low electric fields

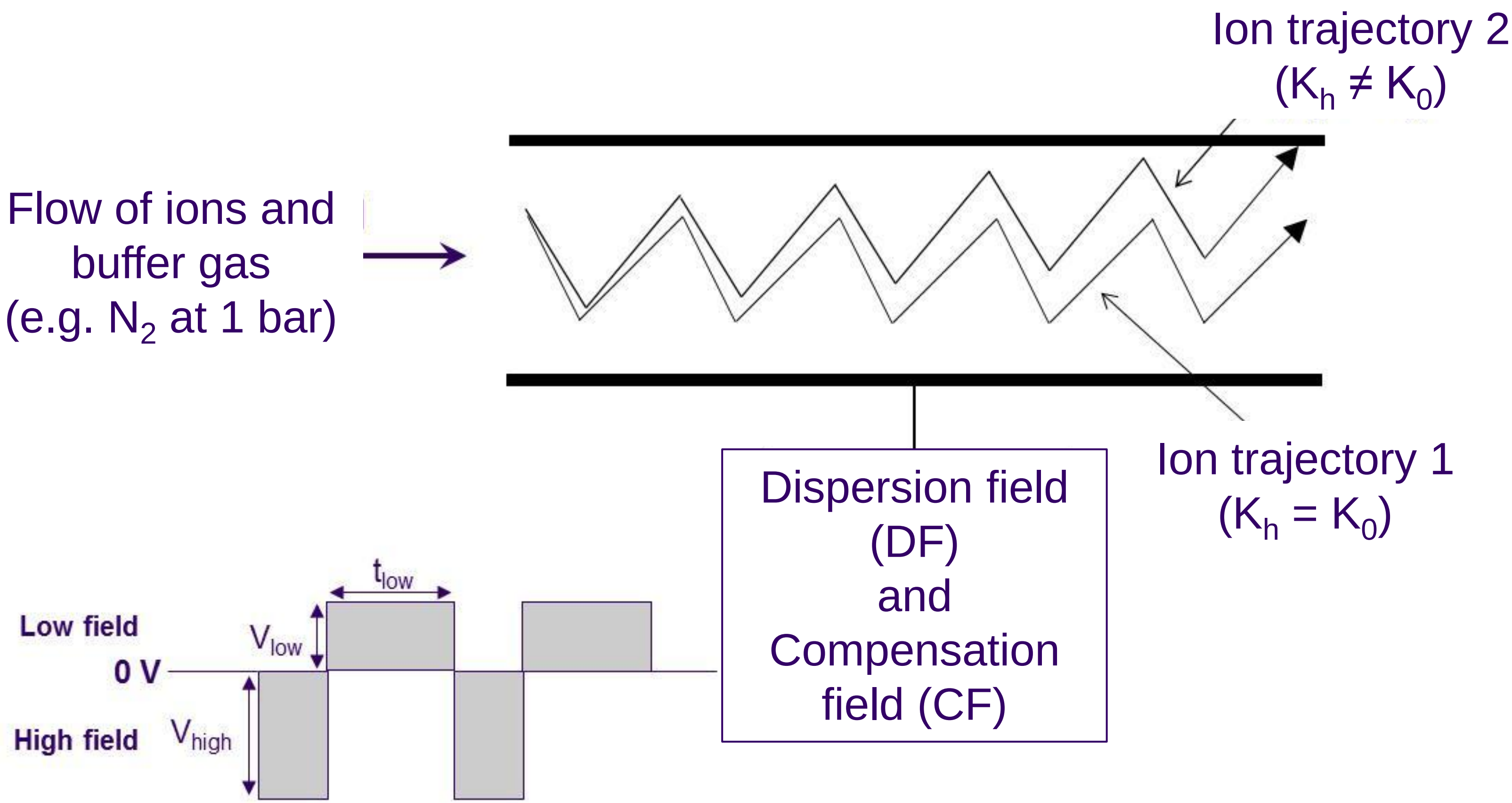
- Mobility is dependent on electric field strength



- Alpha coefficient – compound dependent

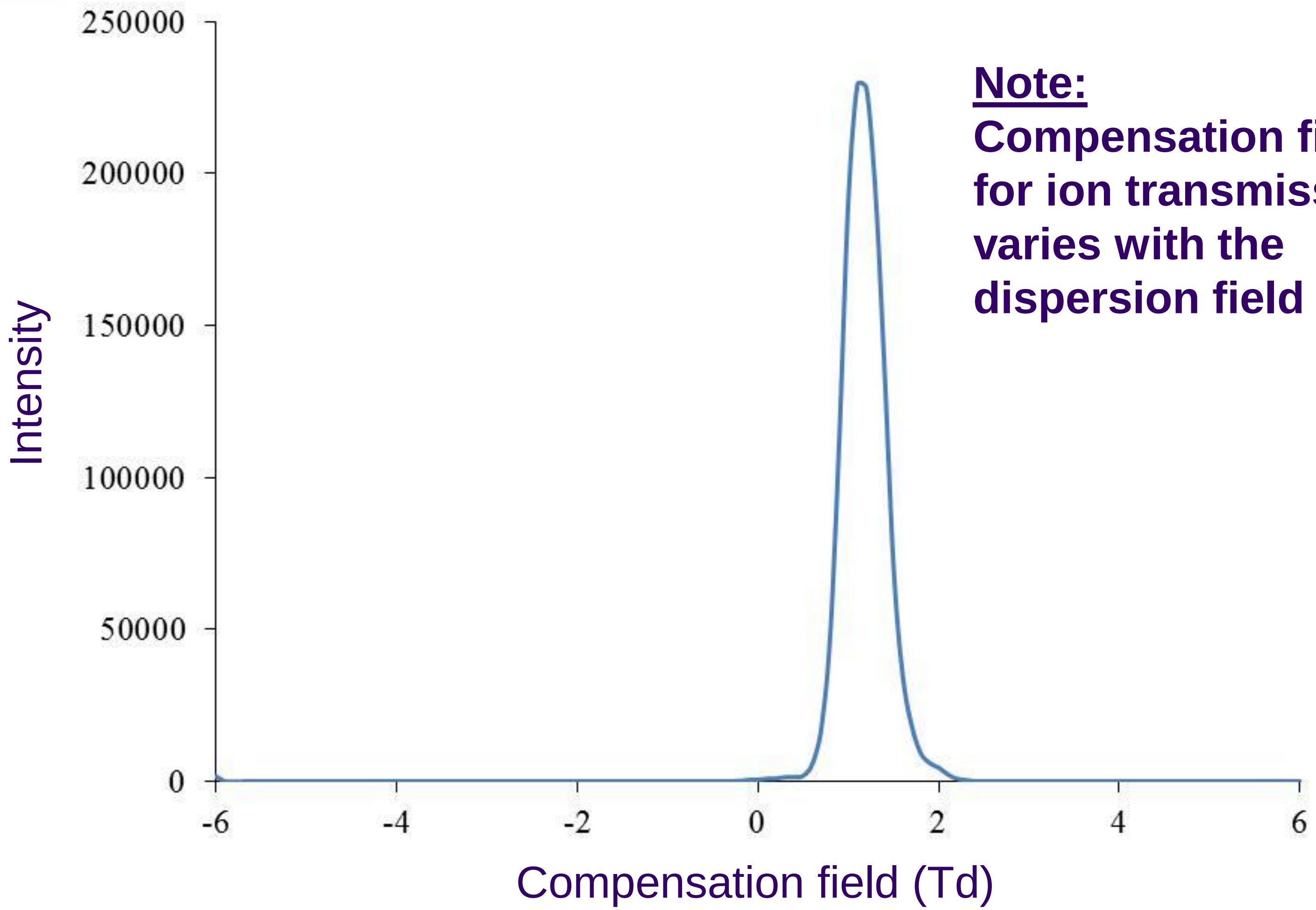
[Purves R W, Guevremont R, Anal. Chem. 1999, 71, 2346-2357]

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



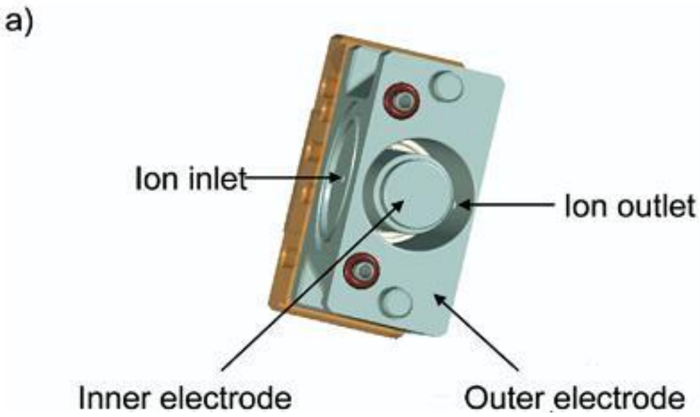
- Compensation field (CF), or voltage (CV, set to transmit ions of selected differential mobility
- Continuous ion beam (equivalent to quadrupole mass filter)

ESI-FAIMS-MS spectrum of protonated reserpine
(Owlstone miniaturised FAIMS-Agilent TOF, ESI)

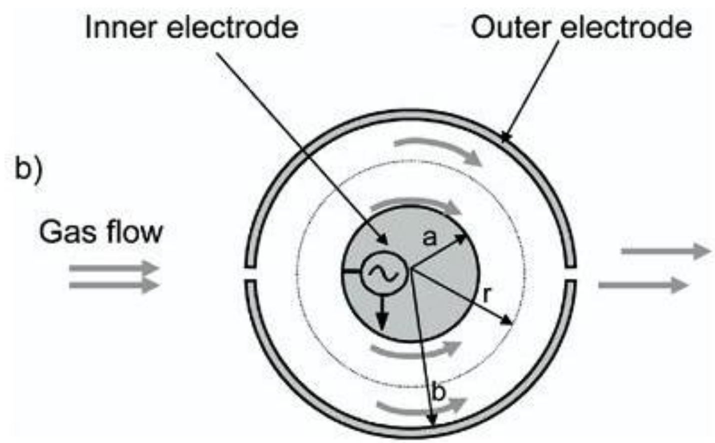


<u>Note:</u>
Compensation field for ion transmission varies with the dispersion field

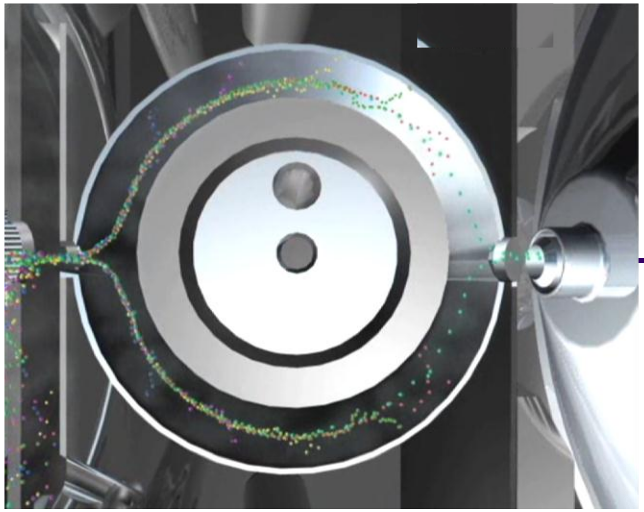
Combining FAIMS with mass spectrometry: Thermo Fisher Scientific cylindrical electrode schematic



(electrode gap ~ 2-3 mm)



Electrospray ion source →

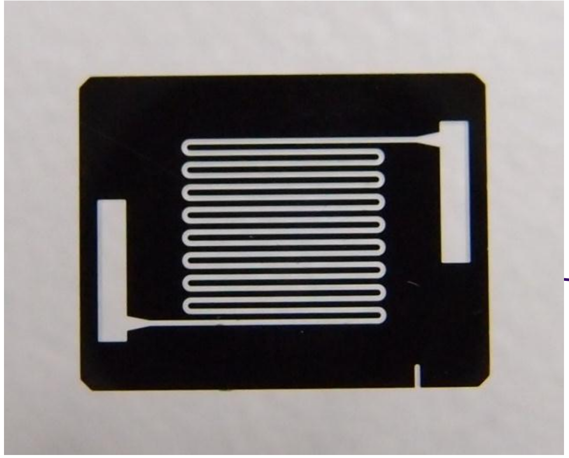


→ Mass spectrometer

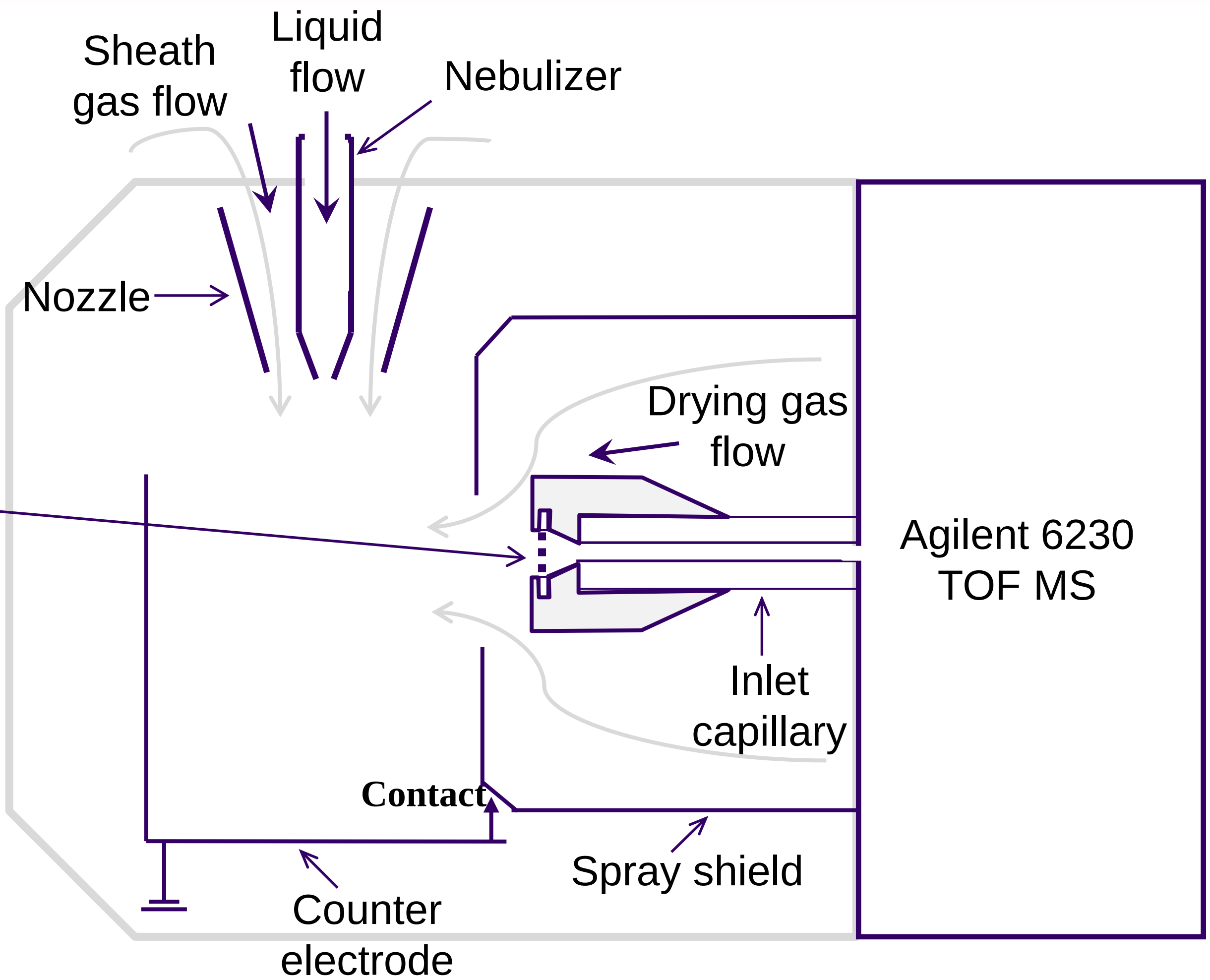
[Barnett et al., *J Am Soc Mass Spectrom,* 2007, 18, 1653–1663]

Prototype Owlstone miniaturised chip-based 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)



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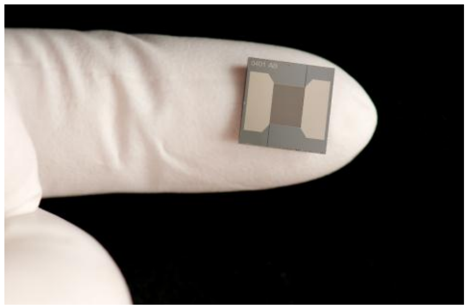
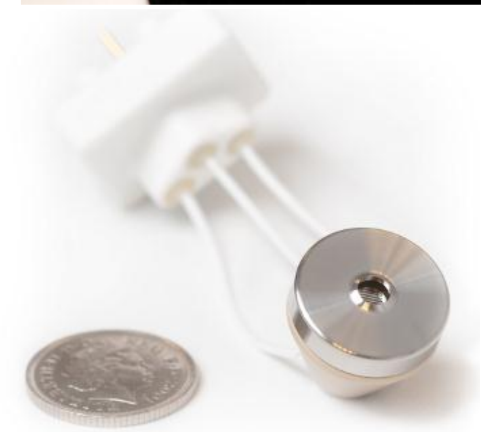
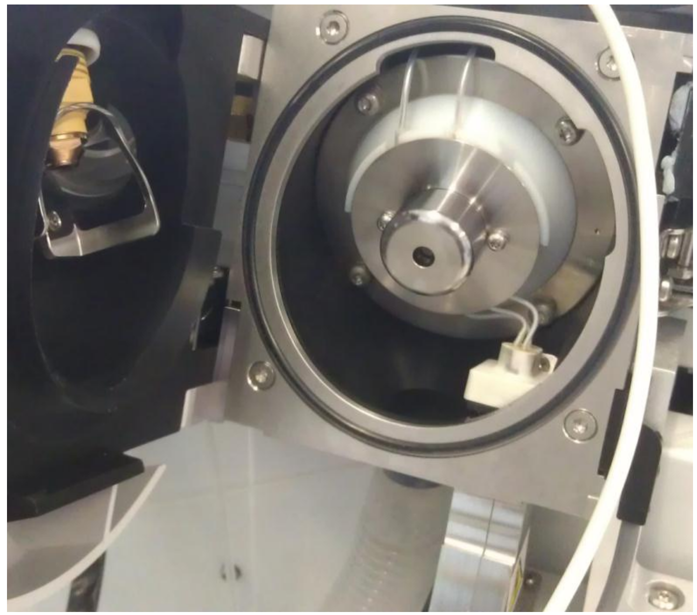
Prototype Owlstone miniaturised chip-based FAIMS cartridge located behind spray shield of the Jet Stream ESI source of an Agilent 6230 TOF MS







http://prezi.com/uqsyffbbdsmf/introduction-to-owlstone-ultrafaims/

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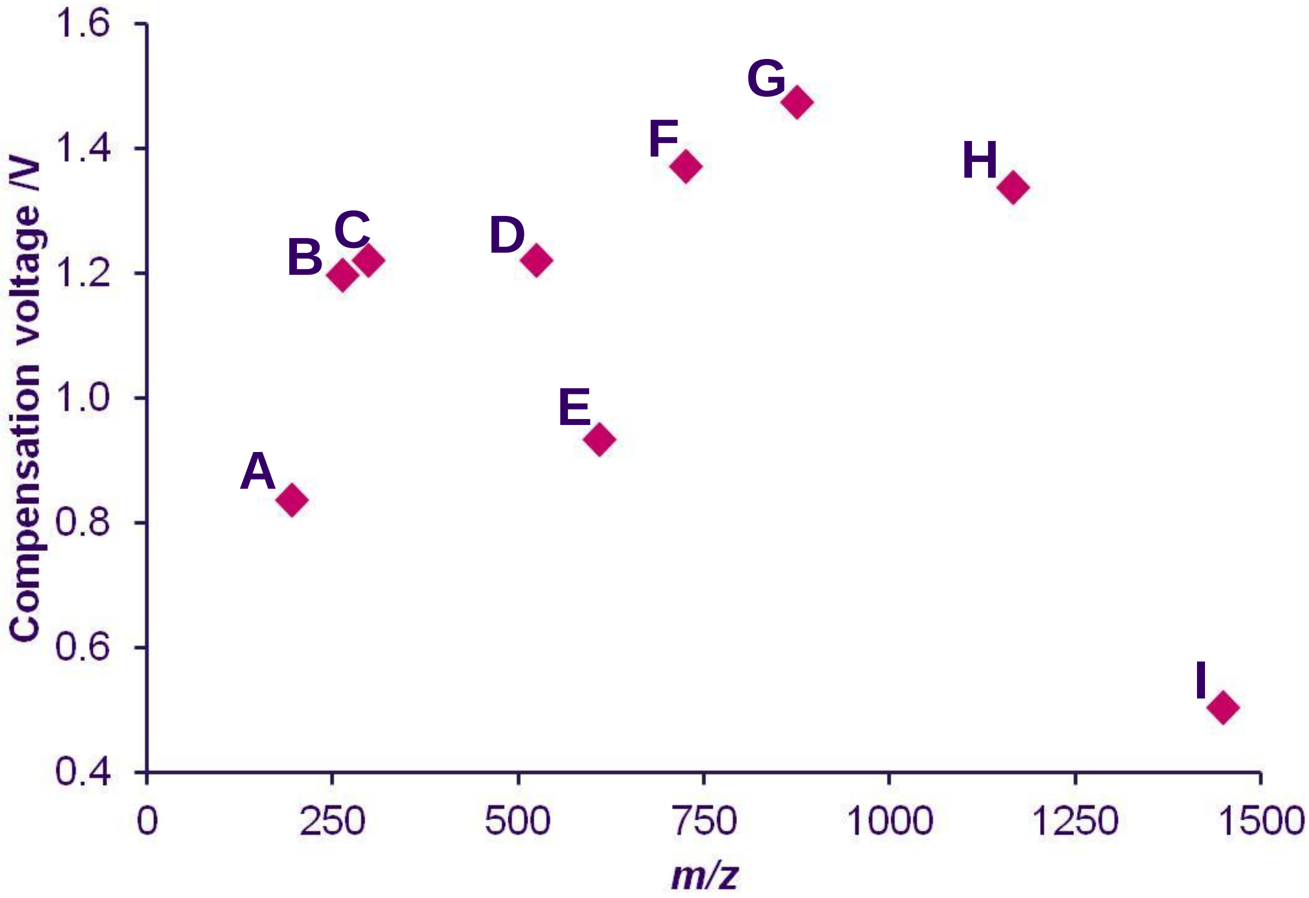
FAIMS combined with mass spectrometry: orthogonal separation enhances analytical space



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

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

C. $[\text{TPAB} - \text{Br}]^{+}$

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

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

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

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

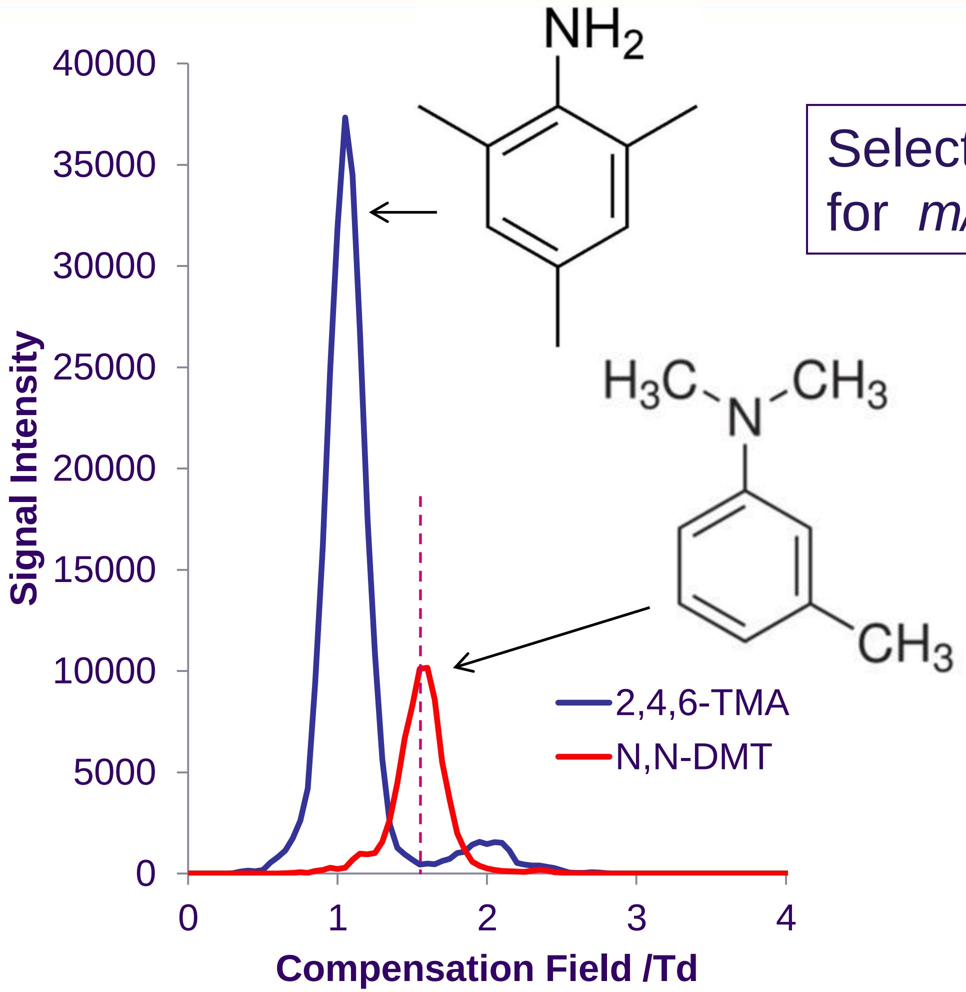
H. $[\text{Insulin B Chain} + 3H]^{3+}$

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

- Isobaric and isomeric compounds

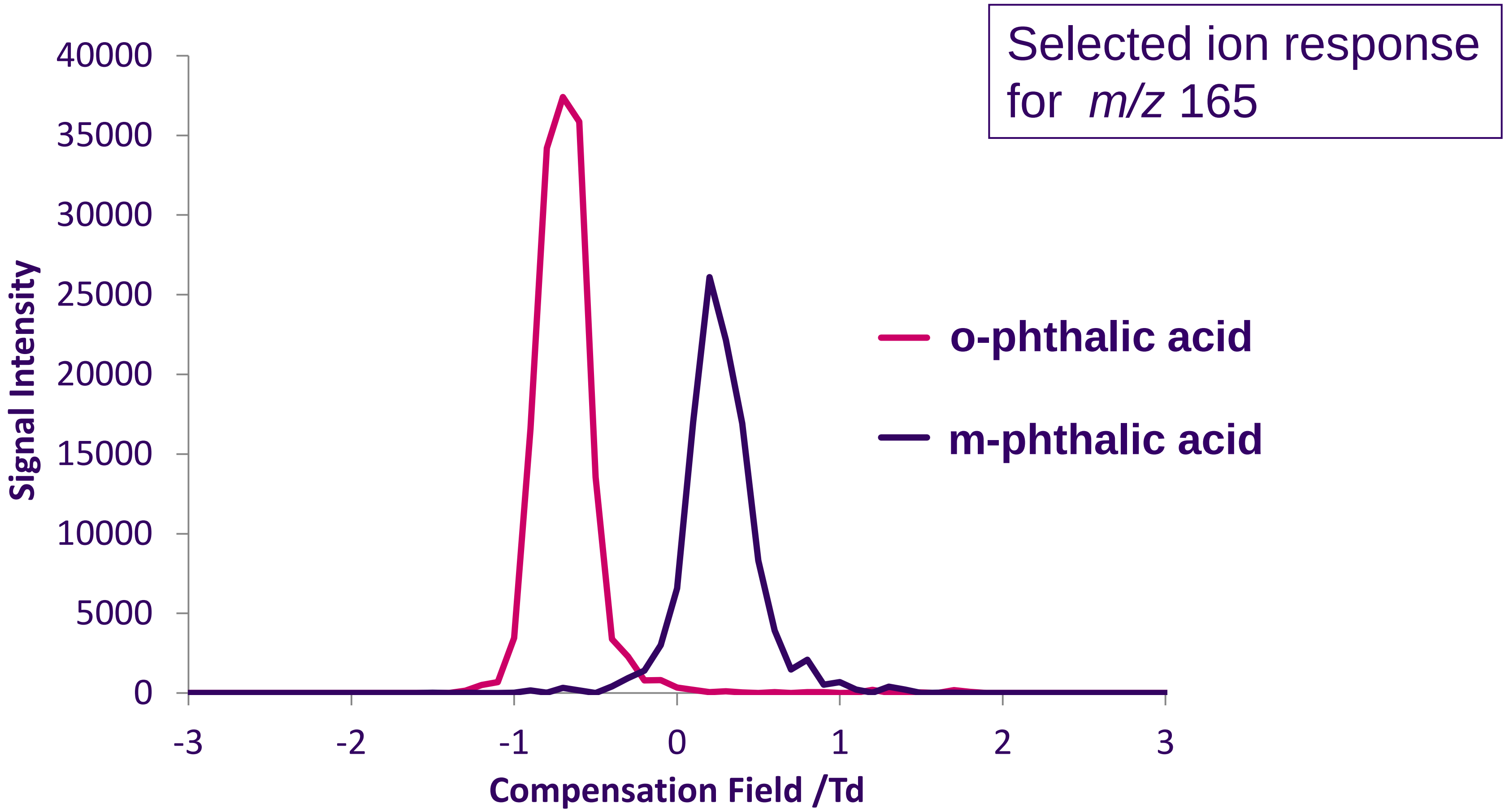
ESI-FAIMS-MS of 2,4,6-trimethylaniline and N,N-dimethyl-*m*-toluidine

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



Selected ion response for *m/z* 136

ESI-FAIMS-MS of isomeric o- and m-phthalic acids (Dispersion field = 200 Td; electrode gap = 100 μm) – 0.5% 2-Butanol



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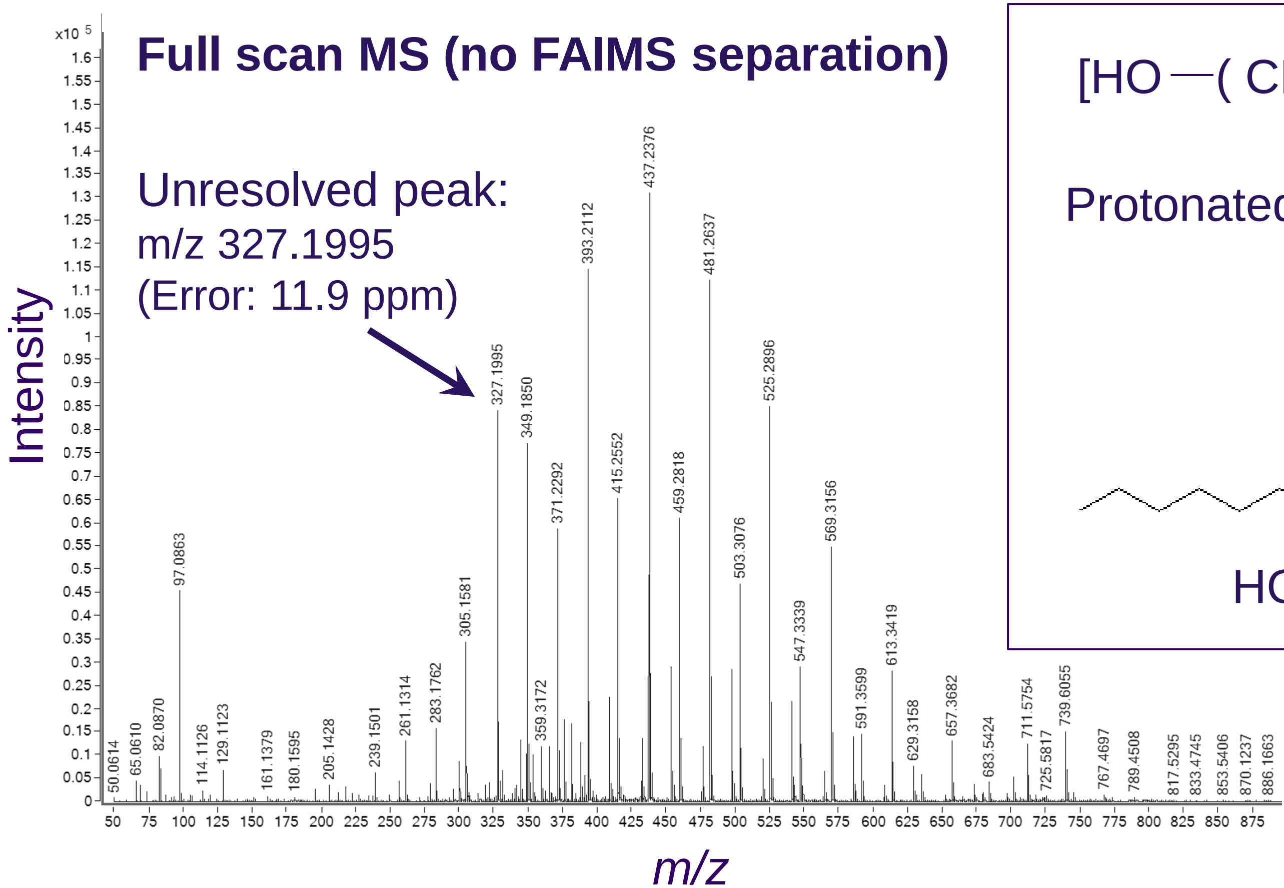
Applications of FAIMS-MS

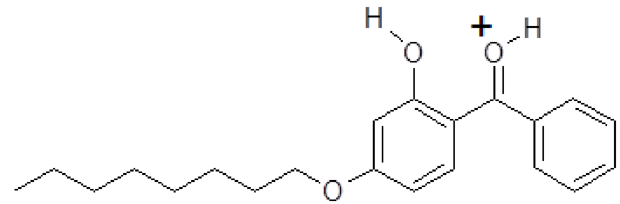
- Reducing sample complexity/chemical noise

ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture



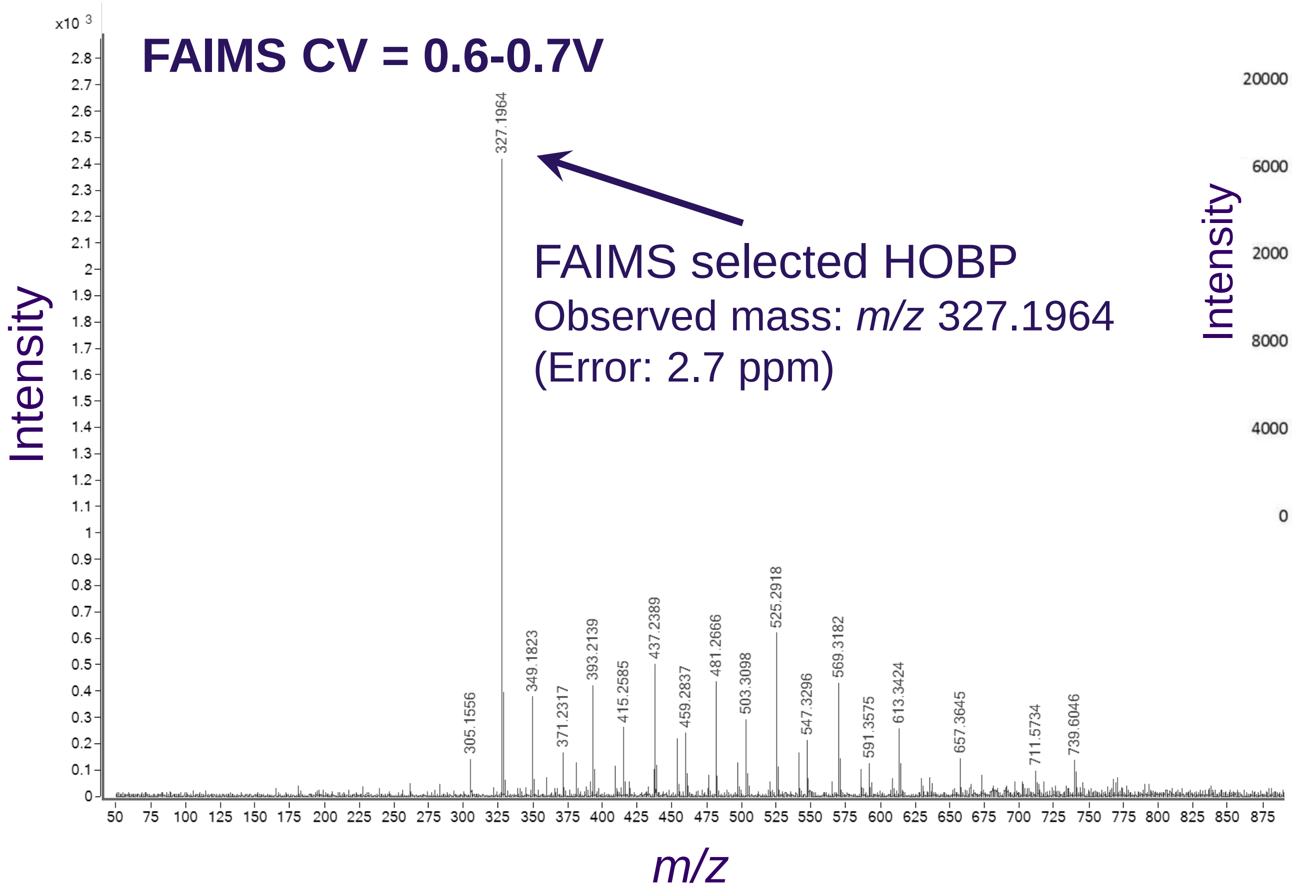
$[HO—(CH_2CH_2O)_n—H + H]^+$
(n = 7)

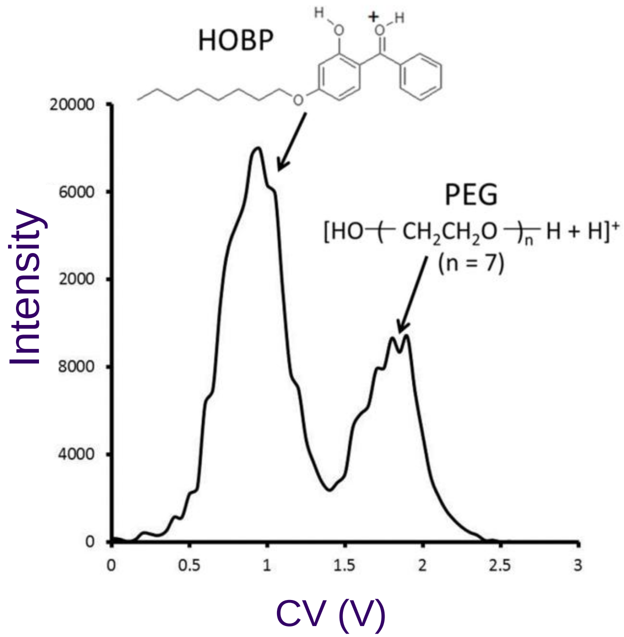
Protonated PEG (*m/z* 327.2013)



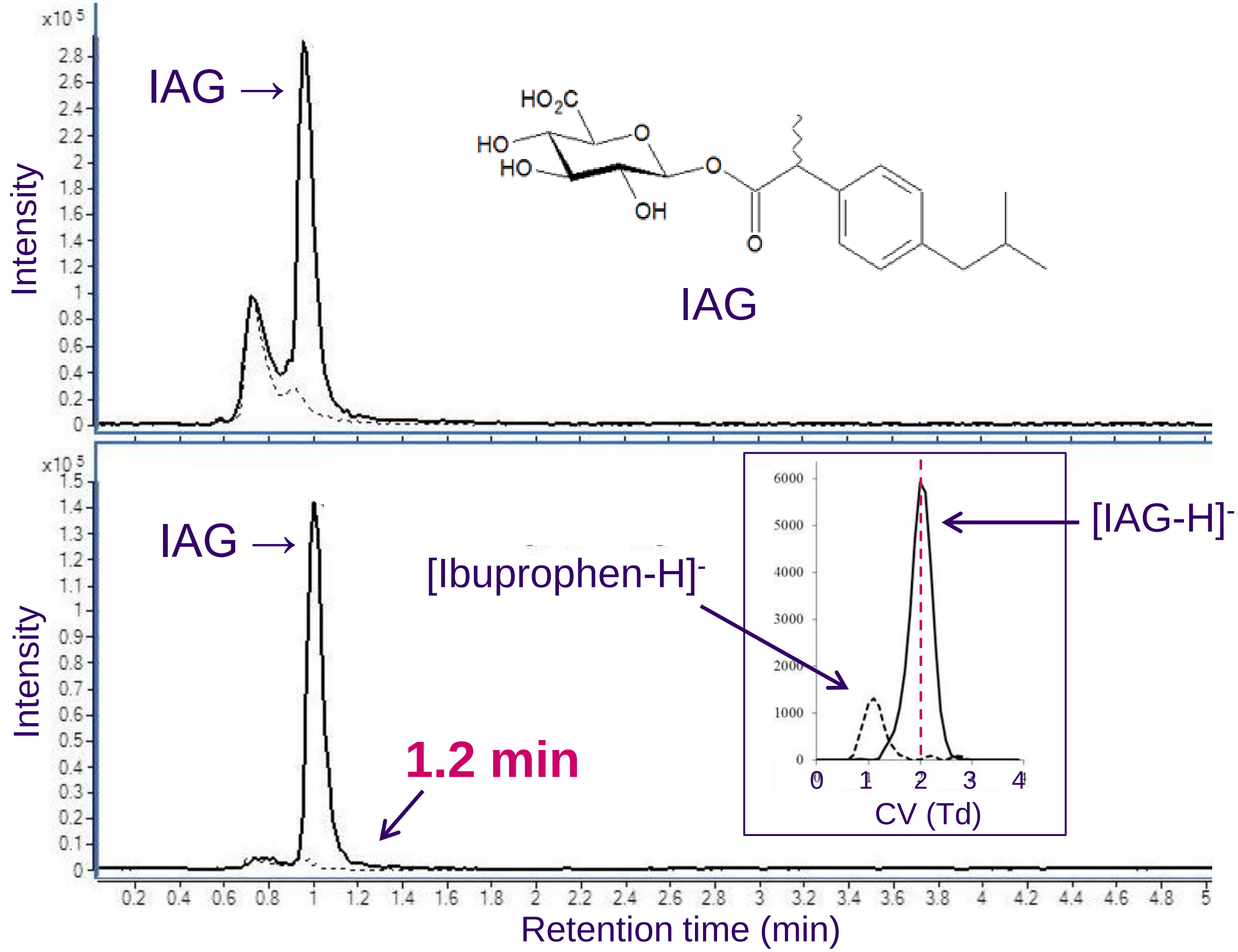
HOBP (*m/z* 327.1955)

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

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

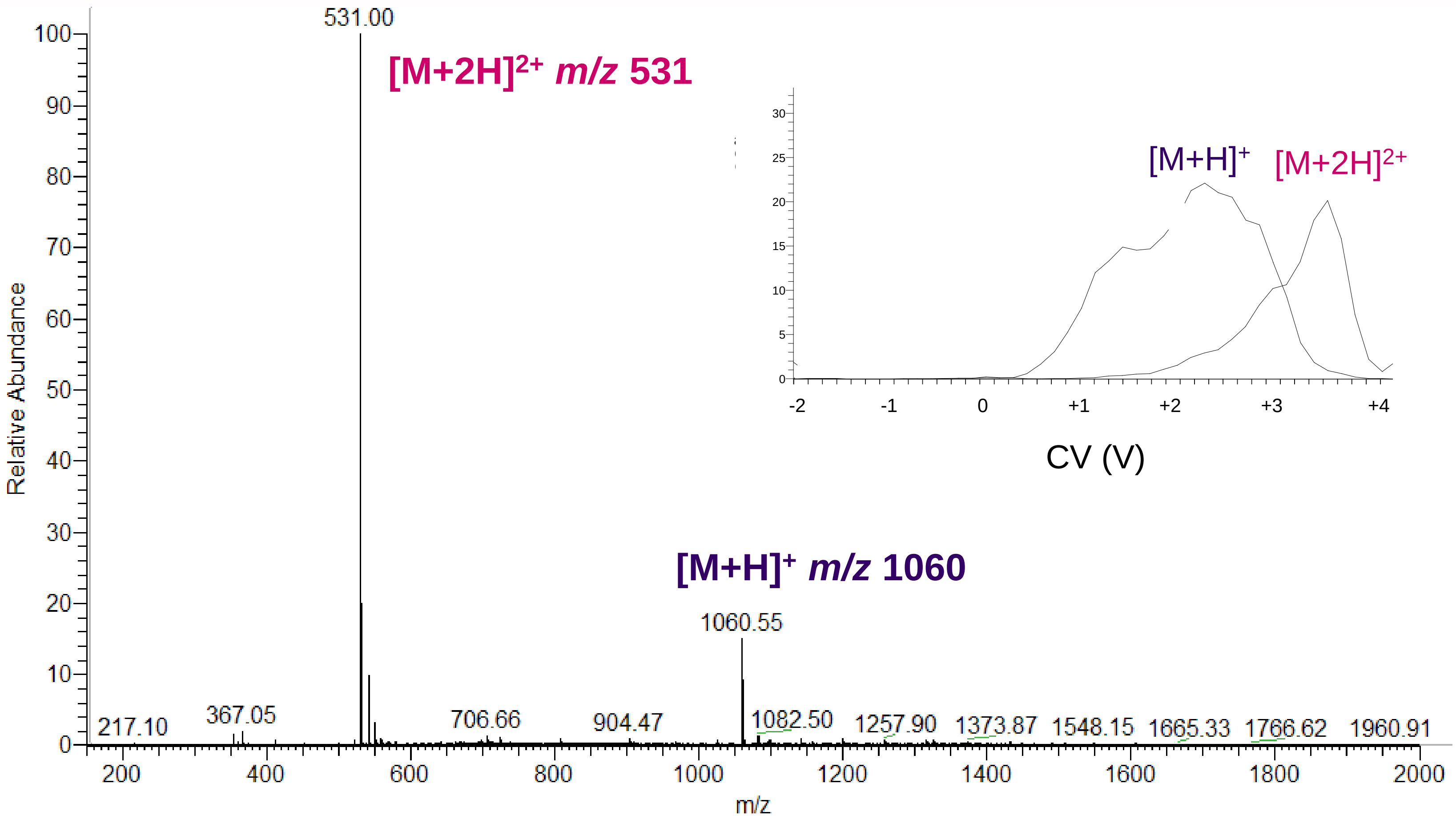
Comparison of LOQ; LDR (R^2) and intra-day reproducibility for the determination of IAG spiked into urine (15.5 *μ*g/ml, n=5)

	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 et al, *J. Chromatogr. A*, 127, 76-81, 2013]

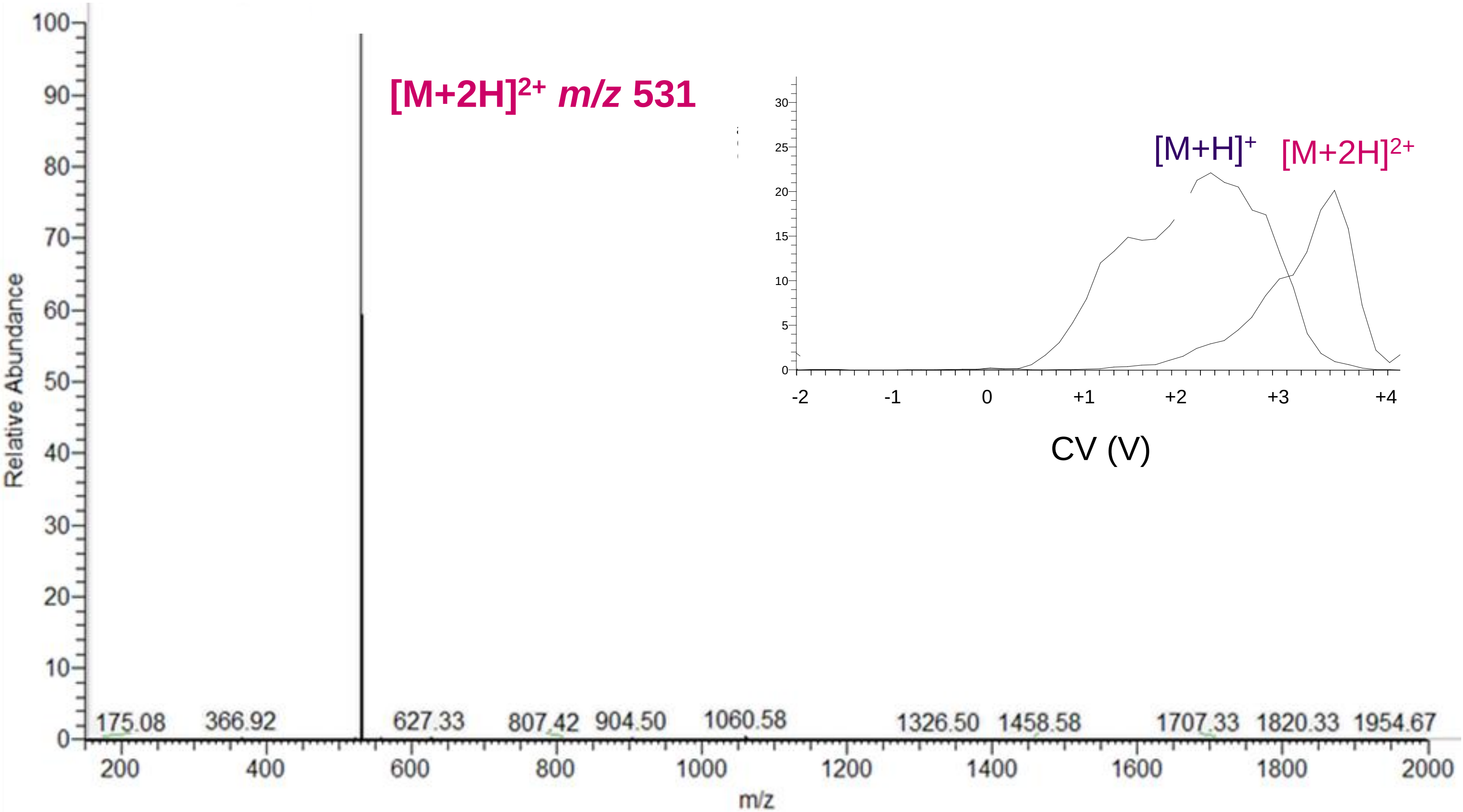
- Charge state and conformers

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})



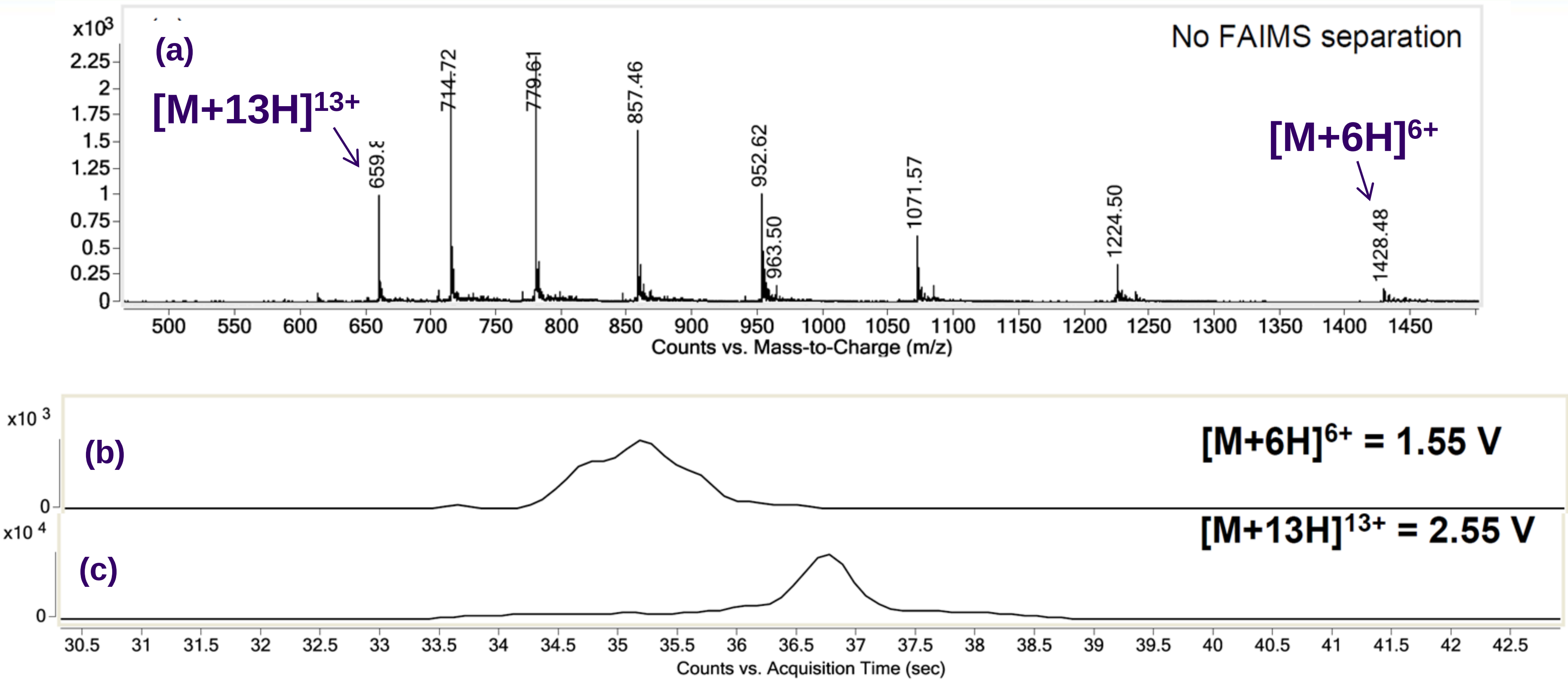
[L Brown et al., *Anal. Chem.*, 2010, 82, 9827-9834]

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]

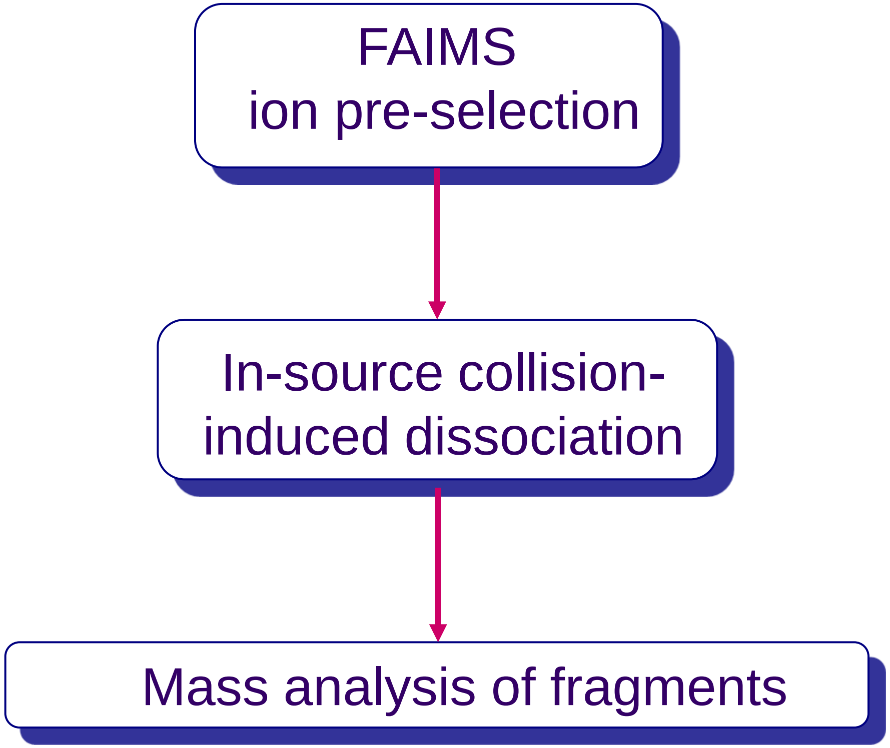
MS and FAIMS-MS of Ubiquitin



(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]

- Enhancing FAIMS-MS selectivity

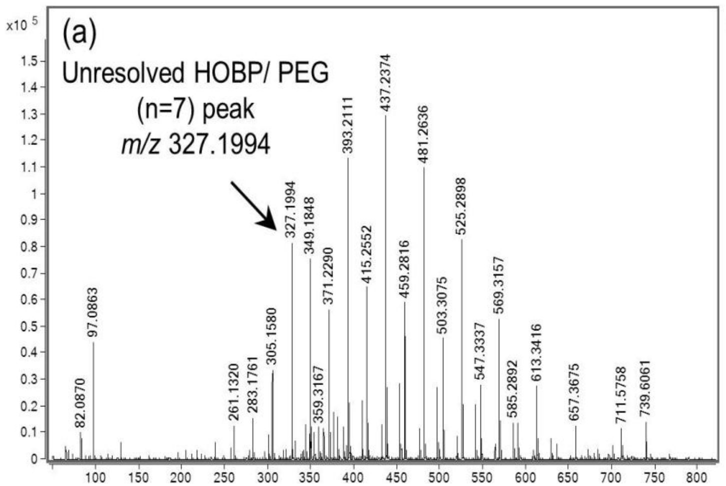
FAIMS-selected in-source CID-MS (FISCID-MS)



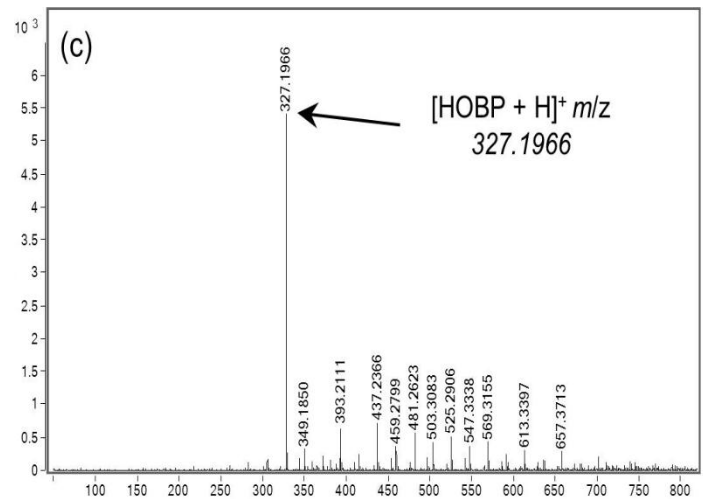
[Brown, L; Smith, R et al., *Anal Chem*, 2012, 84, 4095]

ESI-FAIMS-TOFMS analysis of PEG/HOPB mixture

No FAIMS



FAIMS-selected HOBP

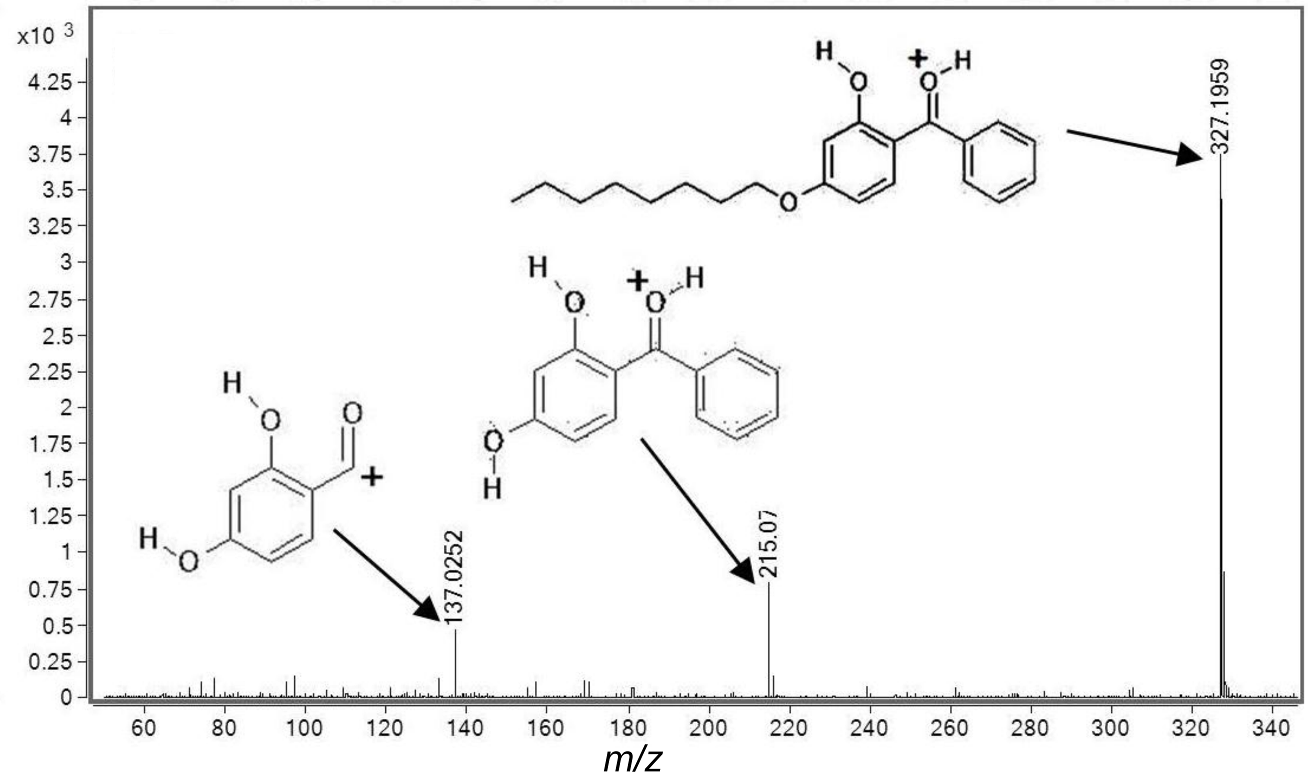


Protonated PEG (*m/z* 327.2013) and HOBP (*m/z* 327.1955) ions

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

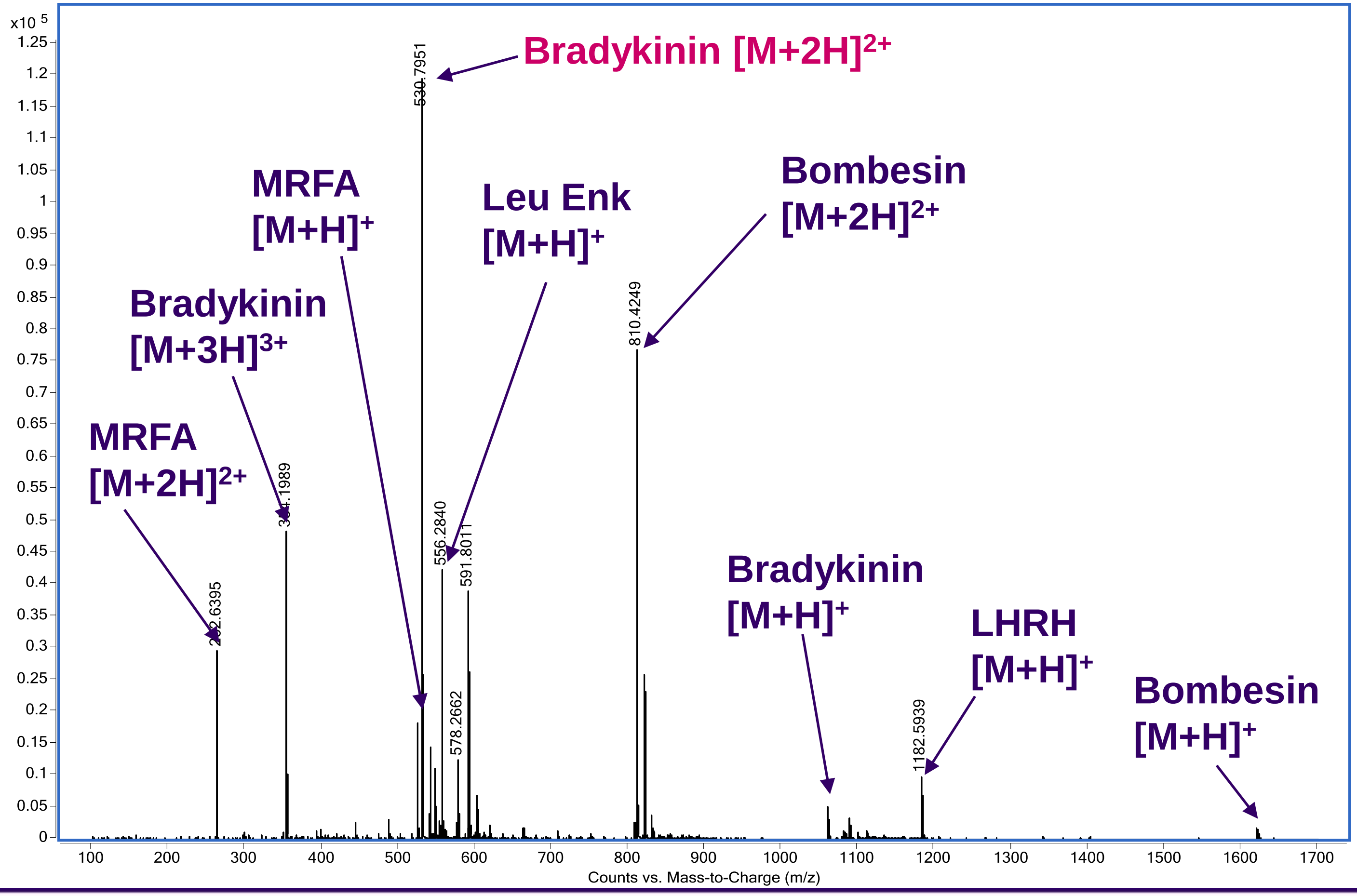
ESI-FISCID-TOFMS analysis of PEG/HOPB mixture

FAIMS-in source CID-MS: CV = 0.6 - 0.7V



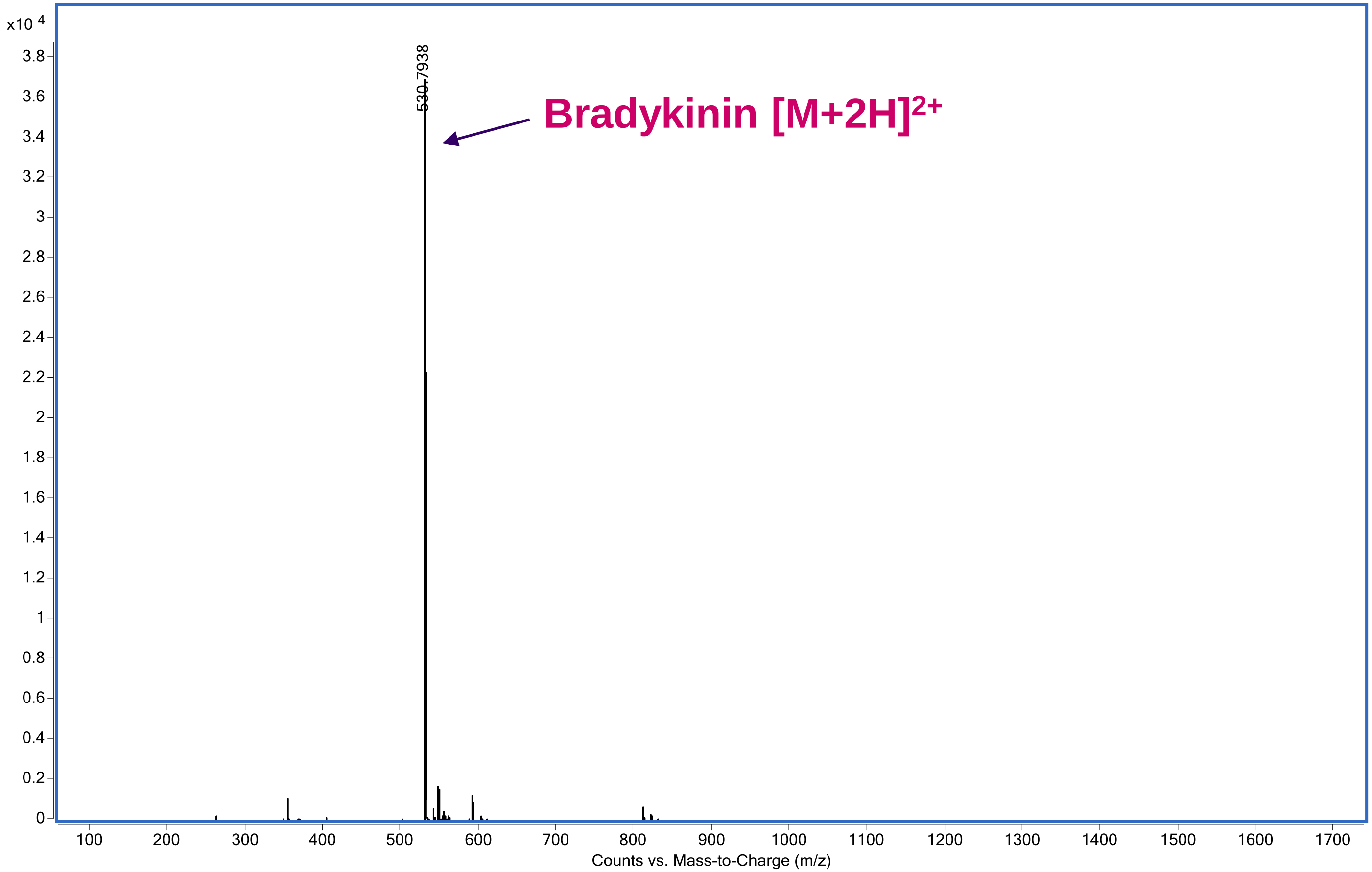
MS of peptide mixture without FAIMS separation

- Owlstone FAIMS-Agilent TOF MS: no FAIMS selection



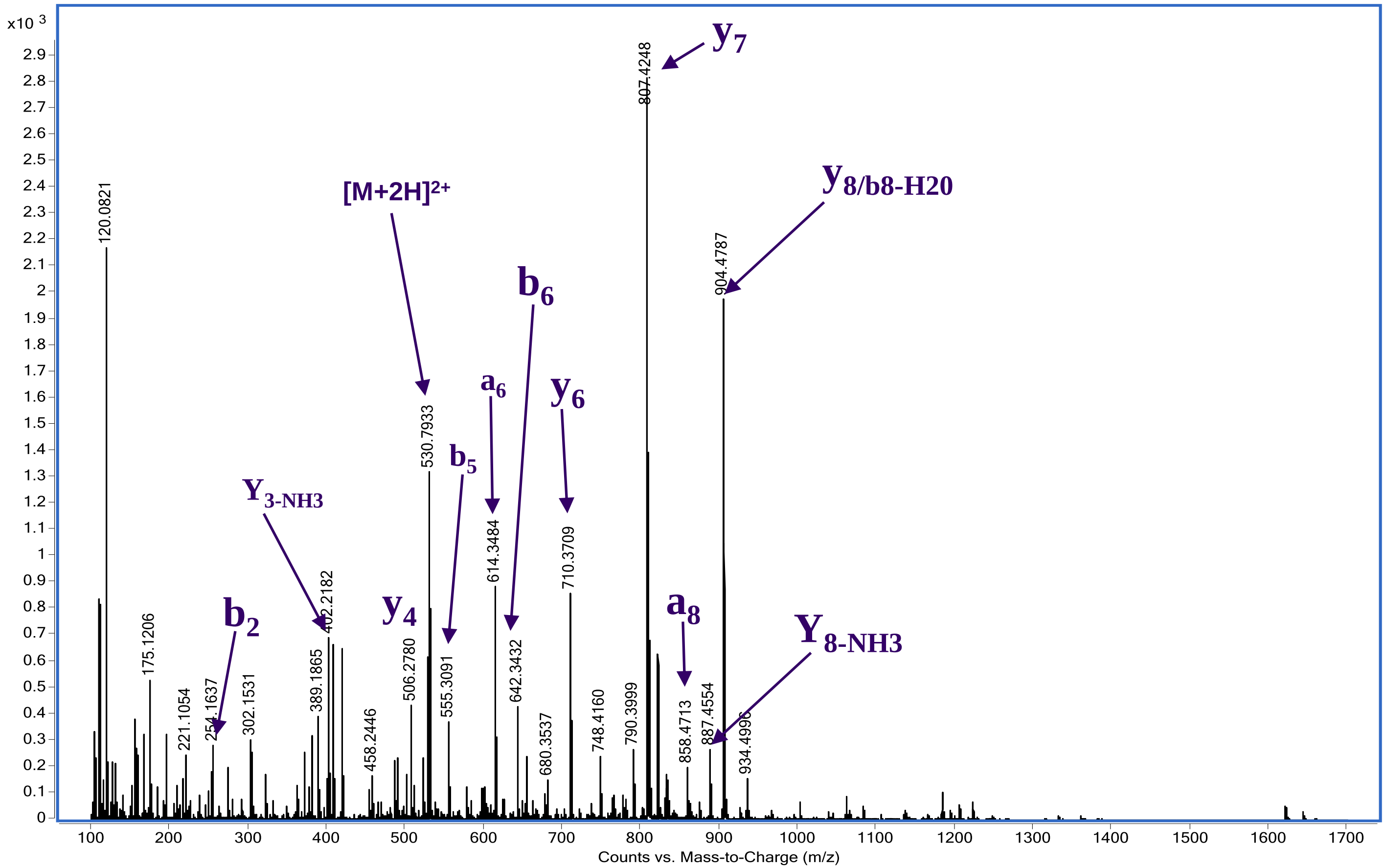
FAIMS-MS of peptide mixture with FAIMS pre-selection of Bradykinin (*m/z* 531, $[M+2H]^{2+}$)

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



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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

Further reading and FAIMS publications

Creaser group miniaturised FAIMS publications:

R. W. Smith, D. E. Toutoungi, J C Reynolds, A. W.T. Bristow, A. Ray, A. Sage, I. D. Wilson, D. J. Weston, B. Boyle, C. S. Creaser, *J. Chromatogr. A*, 2013, 127, 76.

L. J. Brown, C. S. Creaser, *Curr. Anal. Chem.*, 2013, 9, 192.

L. J. Brown, R. W. Smith, D. E. Toutoungi, J C Reynolds, A. W.T. Bristow, A. Ray, A. Sage, I. D. Wilson, D. J. Weston, B. Boyle, C. S. Creaser, *Anal Chem*, 2012, 84, 4095.

L. J. Brown, D. E. Toutoungi, N. A. Devenport, J. C. Reynolds, G. Kaur-Atwal, B. Boyle, C. S. Creaser, *Anal. Chem.*, 2010, 82, 9827.

Further reading:

R. Guevremont, High-field asymmetric waveform ion mobility spectrometry: a new tool for mass spectrometry, *J Chromatogr A,* 2004, 1058, 3.

B. M. Kolakowski, Z. Mester, Review of applications of high-field asymmetric waveform ion mobility spectrometry and differential mobility spectrometry. *Analyst,* 2007, 132, 842.

A. A. Shvartsburg, Differential ion mobility spectrometry: nonlinear ion transport and fundamentals of FAIMS, CRC Press, Boca Raton, 2009.

Acknowledgements

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Owlstone Limited: Danielle Toutoungi, Billy Boyle, Lauren Brown

Agilent Technologies: Michael Ugarov, George Stafford, Ashley Sage (Sciex)

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