Enhancing Biological Analyses using UltraFAIMS-MS

Lauren Brown, Owlstone Medical Ltd.

New Advances in Biological Mass Spectrometry,
University of Birmingham, December 13th 2016.

Introduction

- Key to biological analyses using mass spectrometry is being able separate a particular target component from other species that are also present in the sample.

- Achieving more separation:

  - Improve mass spectrometer resolution so that ever increasingly smaller differences in *m/z* can be resolved.

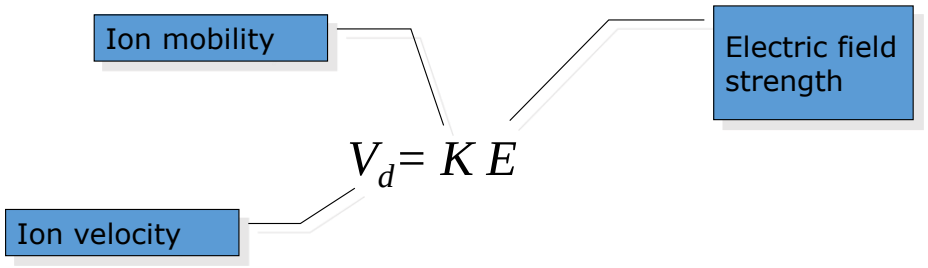
  - Apply another separation technique upstream of the mass spectrometer.

- Research into alternative separation techniques continues, as analysts look for ways of tackling separation problems that existing techniques cannot solve.

Ion mobility background

- FAIMS = Field Asymmetric Ion Mobility Spectrometry (or DMS)

- Variant of Ion mobility spectrometry (IMS)
  - Distinguishing ions according to differences in the speed that they move through a buffer gas under the influence of an electric field.

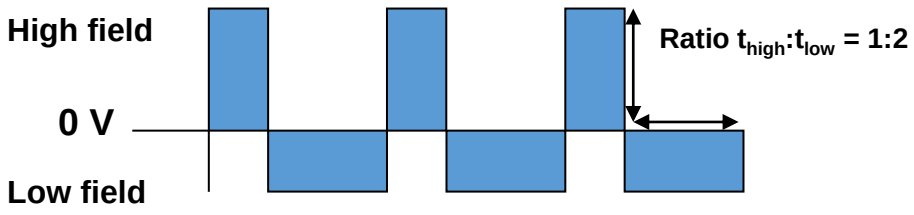


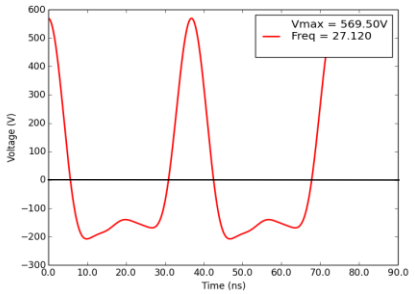
- At low fields, an ion's mobility (K) is constant and is a function of charge (z) and collision cross-section (Ω)

- Ions with a larger cross-section are more likely to collide with gas molecules, travelling more slowly than smaller ions.

FAIMS basics

- FAIMS uses an asymmetric alternating electric field, perpendicular to the direction of travel.





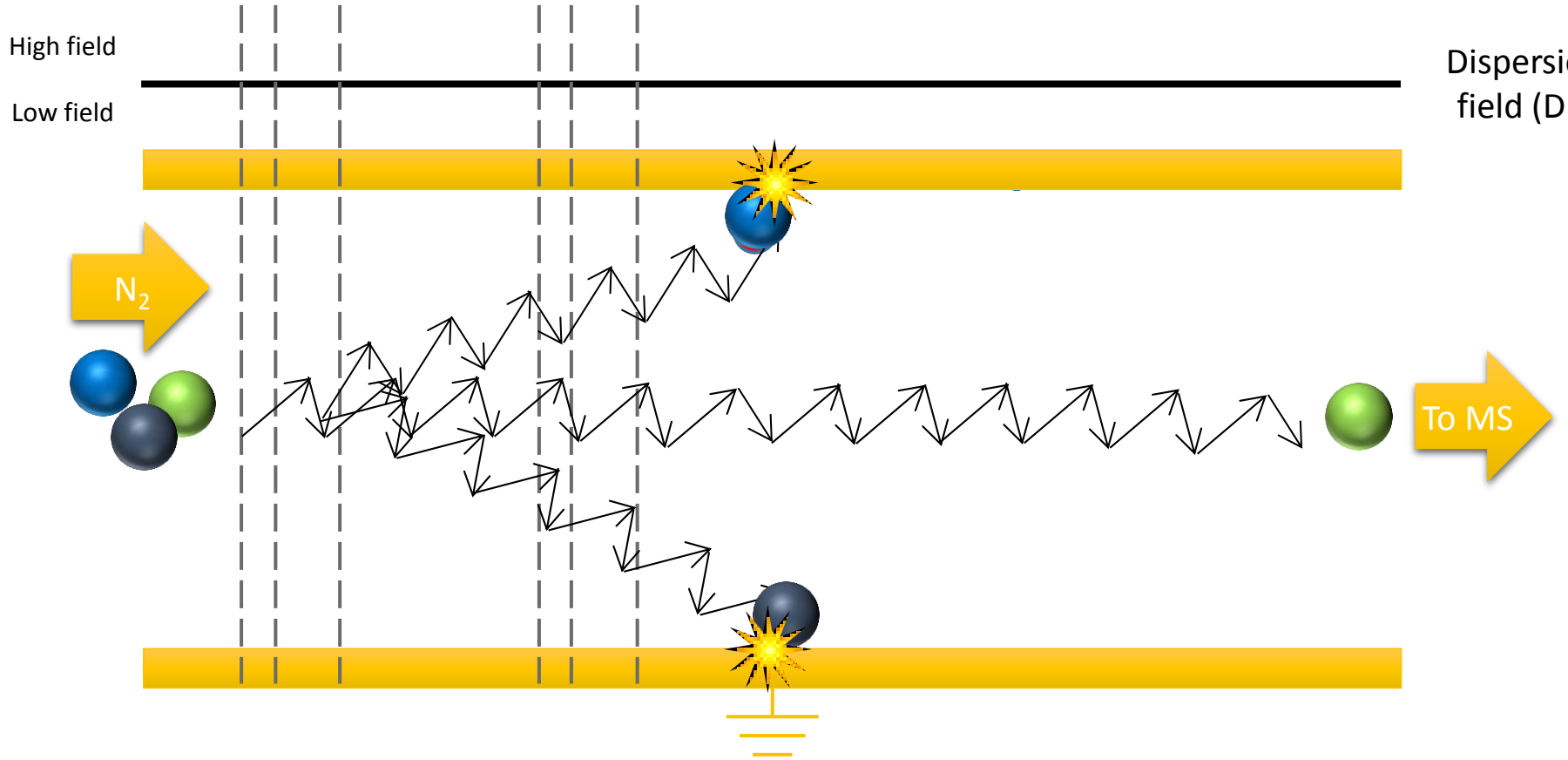
- Ions will be subjected to an electric field condition which causes them to drift in one direction at a velocity based on its ion mobility.

- As the field is reversed in direction and magnitude, the ion changes direction and speed based on its new mobility at the new electric field conditions.

- This is repeated at a rate based on the operating frequency of the device and usually results in a net drift towards an electrode.

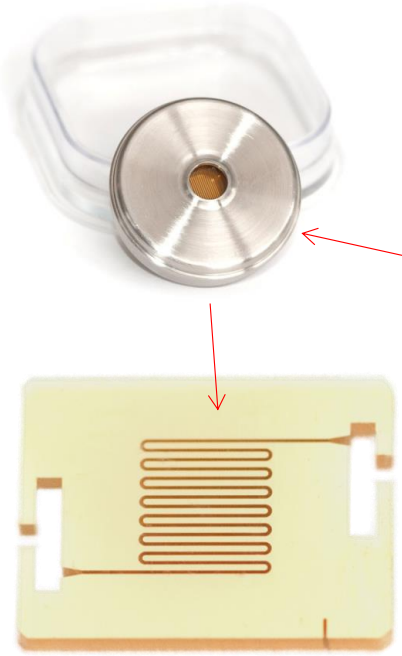
- By applying an additional DC compensation field (CF), this sideways drift can be cancelled out, correcting for the drift and focusses ions through the device
  - Analogy to an atmospheric pressure quadrupole

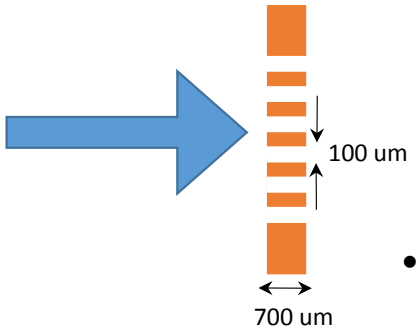
The FAIMS device is a tunable filter



- As the field reverses direction and magnitude, the ion changes direction and speed
- Each ion has a specific net sideways drift velocity
- The sideways drift can be cancelled out by applying the CF

What is UltraFAIMS?





- Miniaturised version of FAIMS, in which the electrodes are formed from a micro-manufactured “chip”
- Each device consists of a set of parallel gaps in a metal substrate that forms the electrodes
- The key dimensions are the electrode gap (100 um) and the channel length (700 um)
- The small scale enables very fast separation, due to:
  - Short ion residence times <200µs (MS dependent)
  - low-voltage drive electronics
- Provides an additional separation stage prior to mass spectrometry

Why use ultraFAIMS with mass spectrometry?

- To improve mass spectral detection in the absence of other pre-separation techniques
  - Pre-separation of ions formed by ambient ionisation techniques

- Separation of isobaric/isomeric interferences, eliminating the need for or speeding up LC for higher throughput analyses
  - Online SPE

- Extra dimension of fast separation in combination with LC-MS
  - Enhanced detection of low abundance analytes for untargeted omics applications

UltraFAIMS combined with ambient ionisation sources

- Ambient ionisation advantages:
  - Analysis of samples i*n situ*
  - Determination of spatial distribution of species
  - Minimal sample preparation

- Disadvantages
  - Complex biological samples have inherent matrix effects
  - No pre-separation means analysis cannot be selective

- Some preliminary **DART** work:
  http://www.owlstonenanotech.com/sites/default/files/ultrafaims/UMC0042-DART-preliminary-test-results.pdf

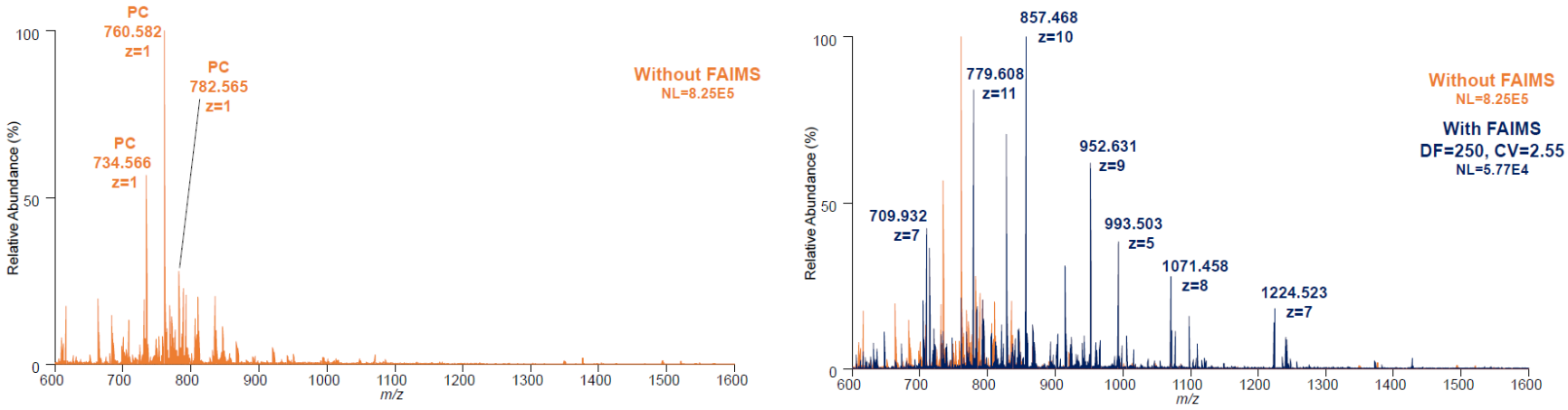
- Liquid Extraction Surface Analysis (**LESA**)

- Desorption Electrospray Ionisation(**DESI**)

  Ambient Ionization and FAIMS Mass Spectrometry for Enhanced Imaging of Multiply Charged Molecular Ions in Biological Tissues
  Clara L. Feider, Natalia Elizondo, and Livia S. Eberlin; *Analytical Chemistry* **2016** *88* (23), 11533-11541 DOI: 10.1021/acs.analchem.6b02798

DESI-UltraFAIMS-MS

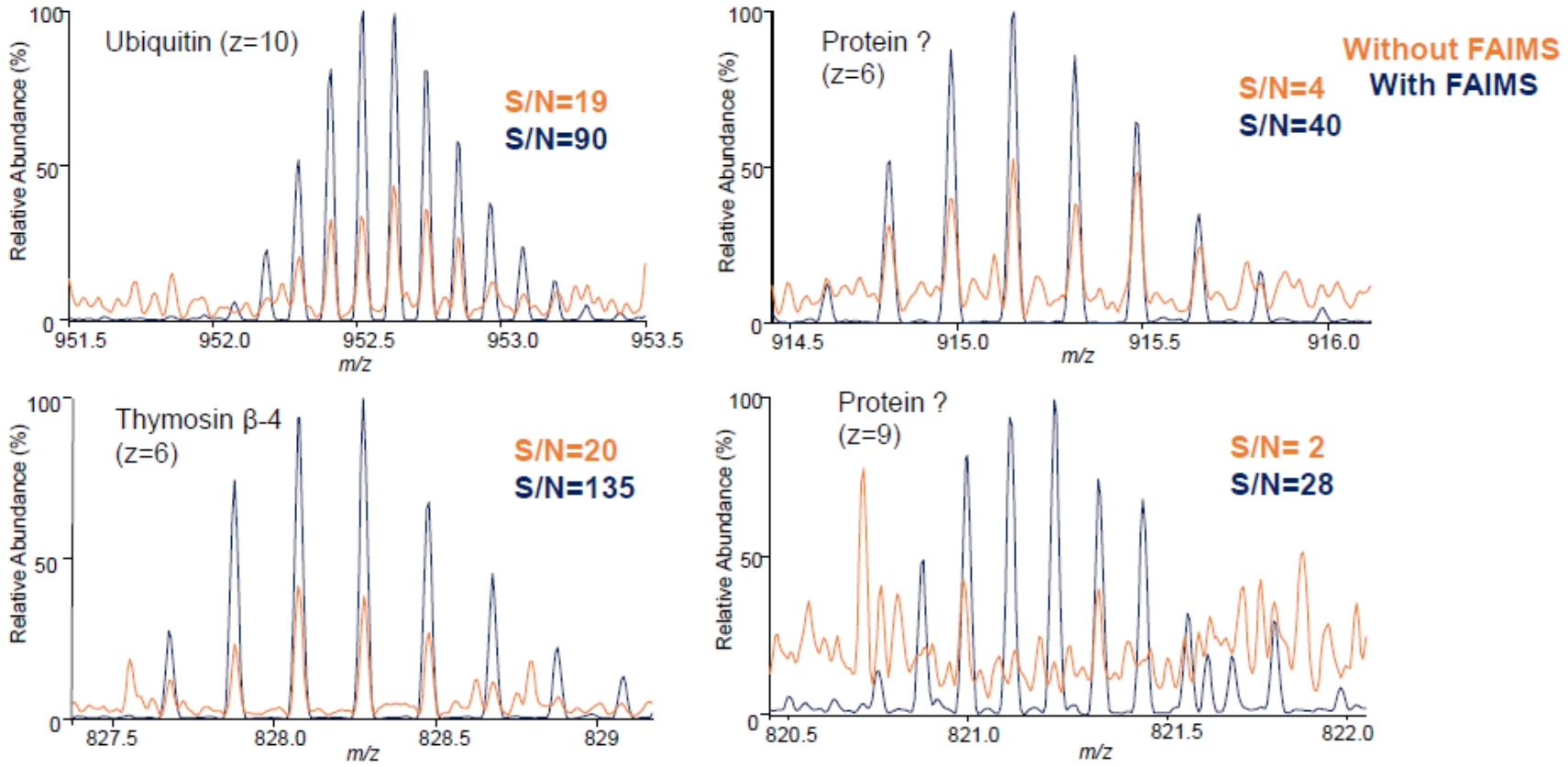
- FAIMS enabled semi targeted detection of multiply charged molecular species at enhanced S/N.
- Improved visualization of spatial distributions.
- Allowed detection of species which were unseen by ambient ionization MSI alone.



- This mass spectral enhancement was two-fold:
  - Increased S/N due to suppressed background.
  - Increased absolute signal in some cases as the trap was preferentially filled with FAIMS-transmitted low abundance ions, rather than naturally high abundance species.

Feider, C.L.; Elizondo, N.; Eberlin, L.S.; *Anal. Chem.* **2016** *88* (23)

DESI-UltraFAIMS-MS

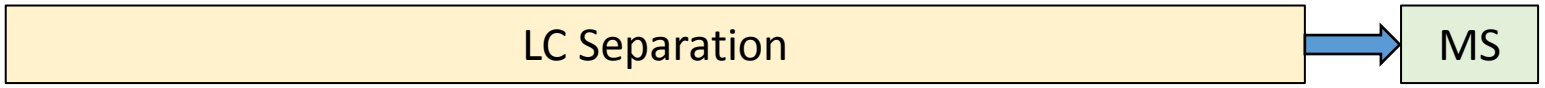


- 84 proteins were detected using FAIMS, 66 of which were not otherwise detected
- Used to distinguish high grade serous ovarian cancer, necrotic ovarian tissue, and normal ovarian tissue under optimized LMJ-SSP-FAIMS conditions.

Feider, C.L.; Elizondo, N.; Eberlin, L.S.; *Anal. Chem.* **2016** *88* (23)

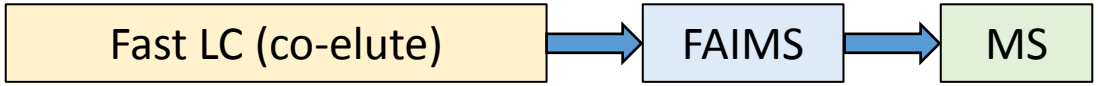
High throughput analysis

- Traditional biological mass spectrometry:



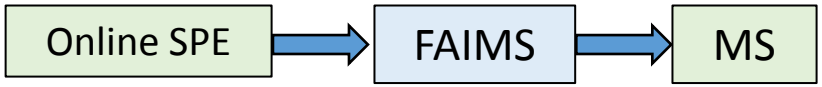
Up to ~30 mins

- Shorten LC separation using a quick gradient to remove salts prior to mass spectral detection and use FAIMS to separate the co-eluting isomers:



1-2 mins

- Lose LC completely, remove salts via SPE and use FAIMS to separate isomers prior to mass spectral analysis:



~20 s

Vitamin D3 metabolite isomers

- Vitamin D, along with calcium, promotes bone growth in children and aids in the prevention of osteoporosis in older adults.

- Vitamin D_3 is metabolized in the liver to form 25-hydroxyvitamin D_3 (25-OH D_3) and levels are routinely measured for diagnostic assessment of vitamin D related diseases

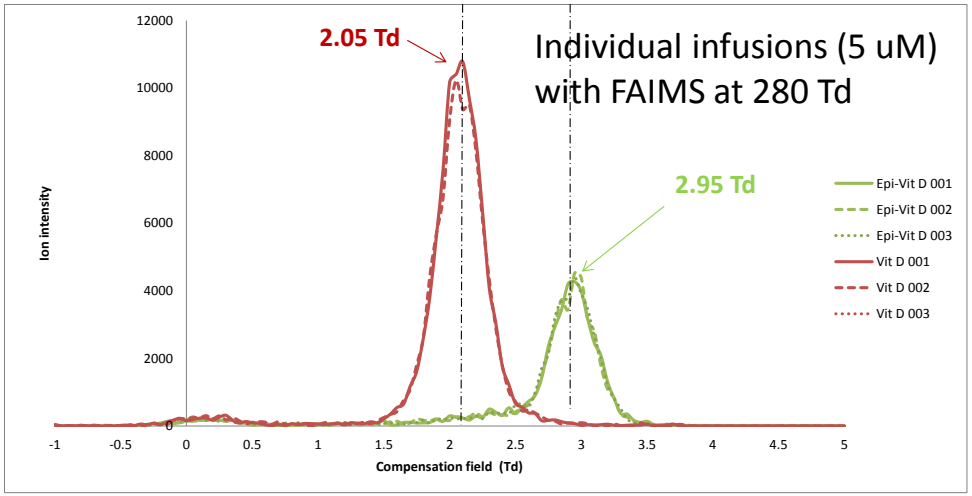
- Biologically inactive 3-epi analog of 25-OH D_3 (3-epi-25OH-D_3) has been reported
  - Interference from the inactive 3-epi analog may lead to inaccurate information for treatment and prevention.

- LC-MS/MS is currently used to quantify 25-OH D_3 and diagnose vitamin D disorders, however, lengthy analysis times limit its utility for high-throughput analyses.

- Used UltraFAIMS-MS to separate the hydroxyvitamin D_3 epimers using in timescales suitable for high-throughput clinical analysis.

Vitamin D3 metabolite isomers

- The 2 isomers can be identified due to differences in their CF position



	25-OH D_3 (CF = 2.05 Td)	3-epi-25OH-D_3 (CF = 2.95 Td)
Average signal	10283	4393
SD	110.03	125.48
% RSD	1.07	2.86
Interference %	2.38	2.19
Variance (+/- %)	0.32	0.77

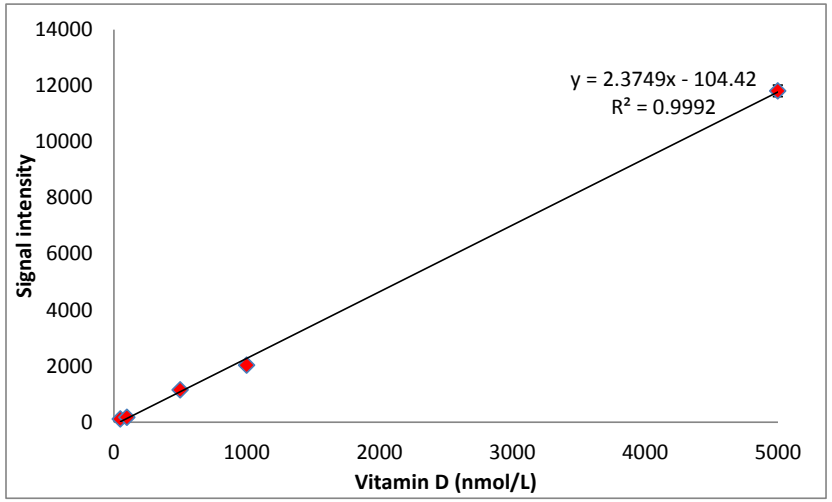
- In equimolar solutions, both 25-hydroxy vitamin D3 and 3-epi-25-hydroxy vitamin D3 can be monitored with just 2% interference from the other epimer.

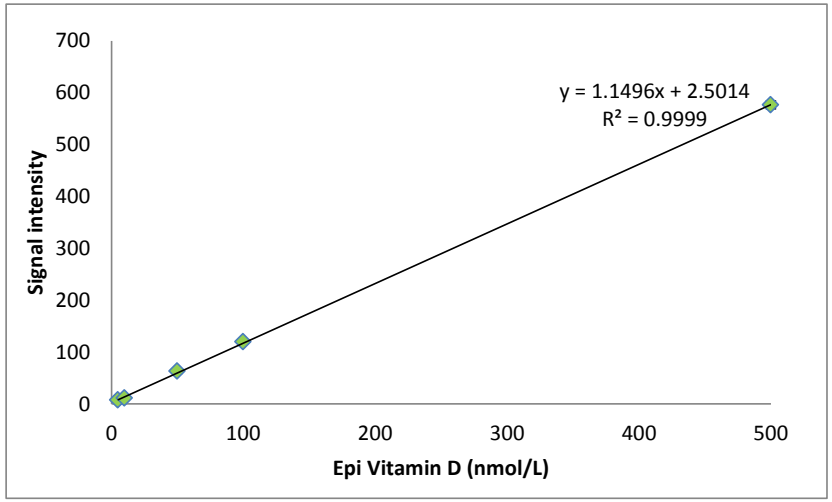
- Each epimer could be identified from a mixture over ratios of 2:1 (25-OH vitamin D3: epi 25-OH vitamin D3) to at least 20:1, covering the whole biologically relevant range

Applications TOC

Vitamin D3 metabolite isomers

- Samples containing 25-OH vitamin D3 and epi 25-OH vitamin D3 were analysed at increasing concentrations at a fixed10:1 ratio of 25-OH D_3 to 3-epi 25-OH D_3.
- Linear calibration curves were generated for both epimers, showing peak height to be related to concentration.



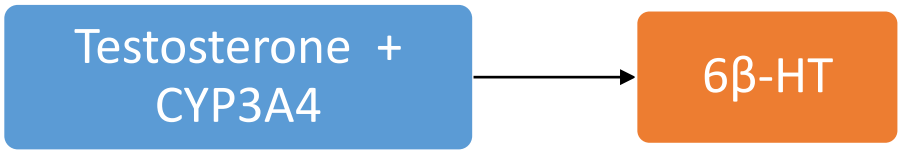


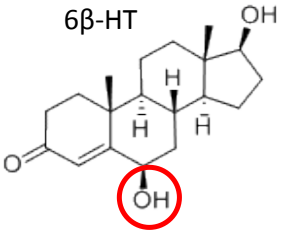
- A test 'unknown' sample, containing 500 n Mol L^{-1} 25-OH vitamin D3 and 50 n Mol L^{-1} epi 25-OH vitamin D3 was analysed:

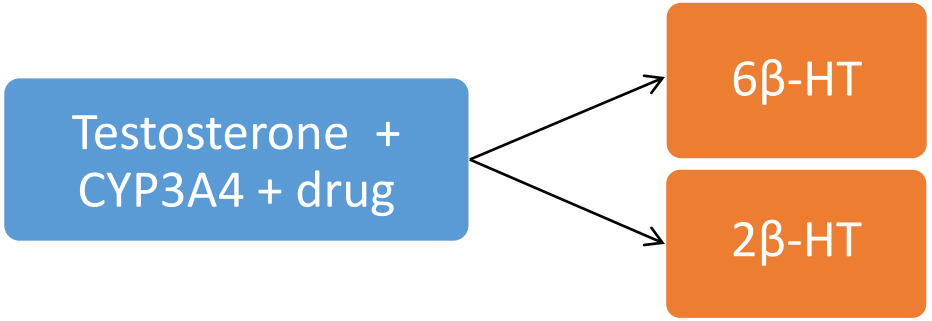
True concentration (n Mol L^{-1})	Calculated concentration (n Mol L^{-1})	Error
50	53	+ 6%

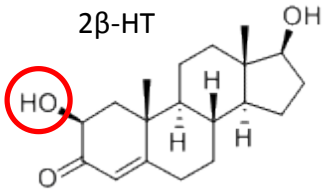
2β and 6β-hydroxytestosterone

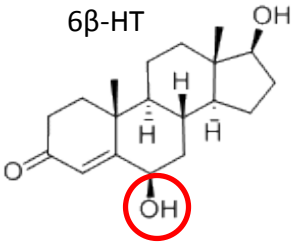
- 6β-hydroxytestosterone (6β-HT) is the major metabolite of testosterone
- However some drug candidates promote formation of 2β-HT while inhibiting 6β-HT







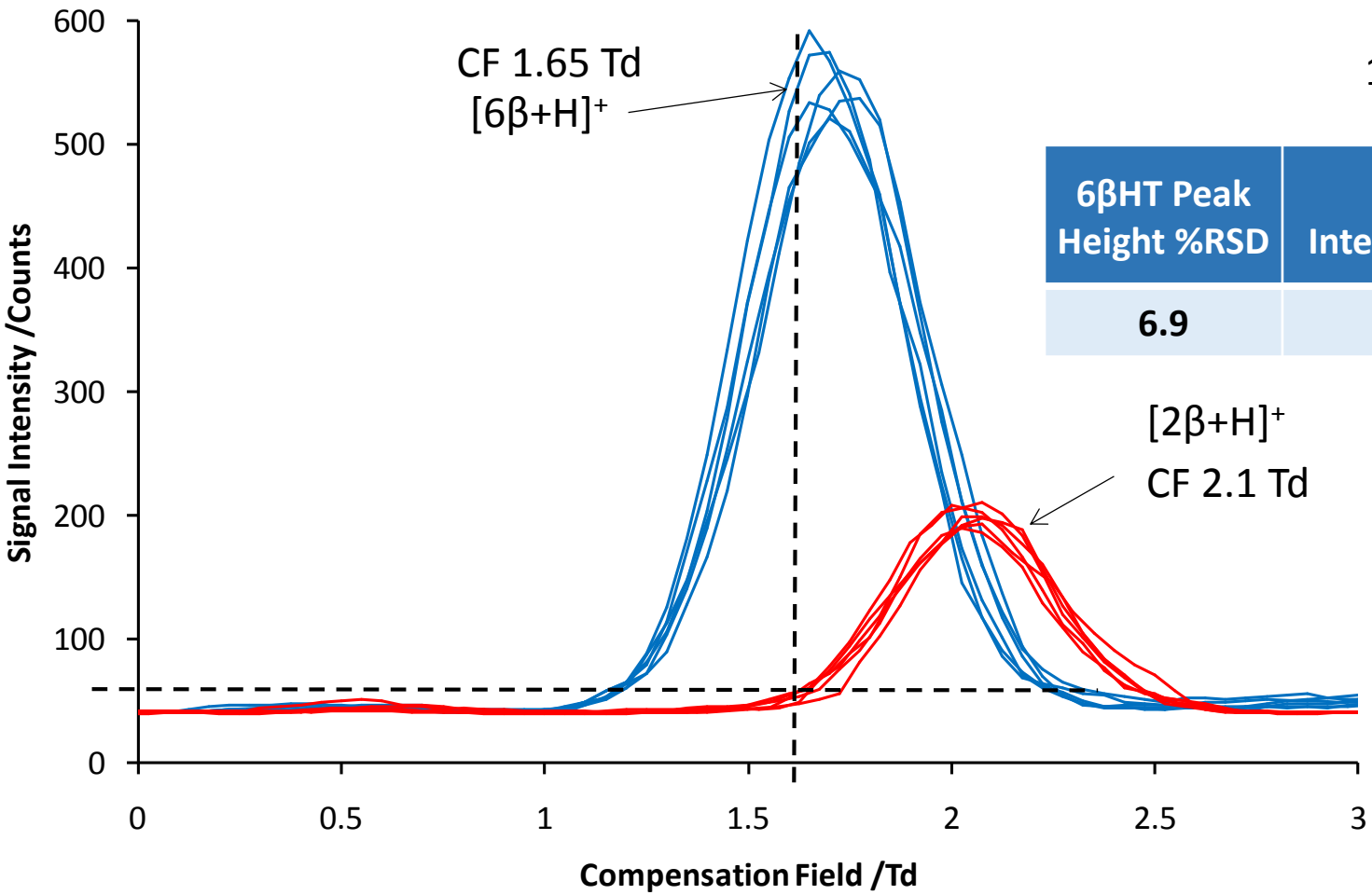




- 2β-HT and 6β-HT are isobaric with no unique MS/MS fragmentations
- LC separation is currently used in these cases
- A faster method for testing potential drug candidates is highly desirable
  - E.g. Online-SPE methods

2β and 6β-hydroxytestosterone separation

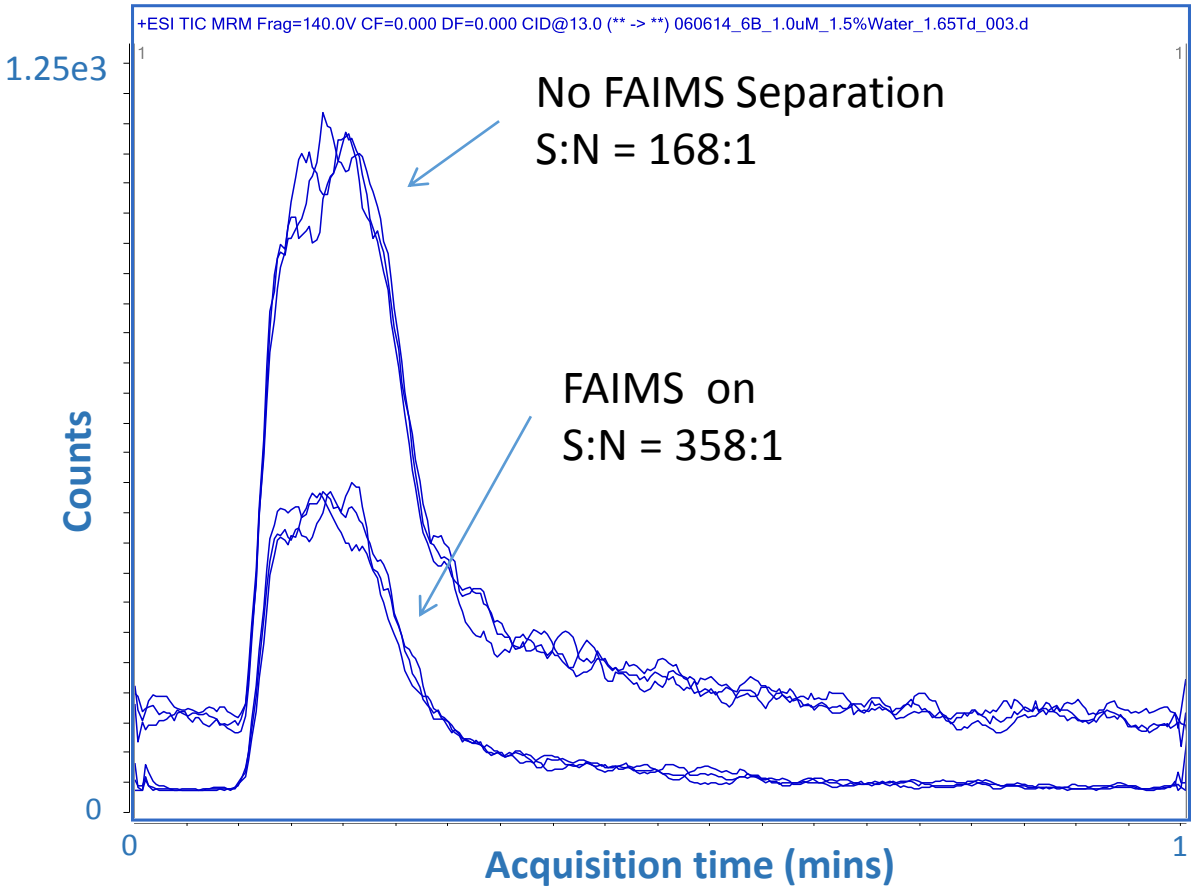
MRM: *m/z* 305.2>269.4
DF 305 Td
1.5% water.



6βHT Peak Height %RSD	2βHT Interference (%)
6.9	**3.6**

Signal to noise improvements

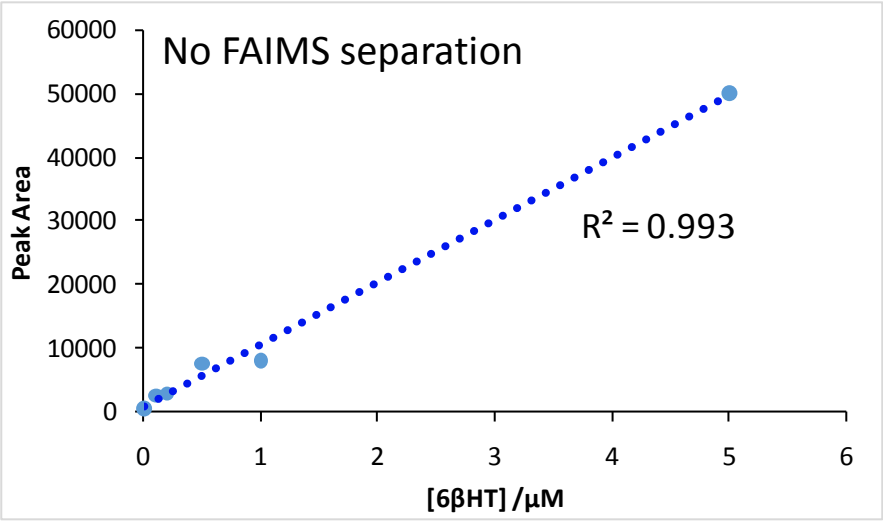
Loop injections of 6β-HT (no 2β-HT in sample) at 1µM, with and without FAIMS separation.



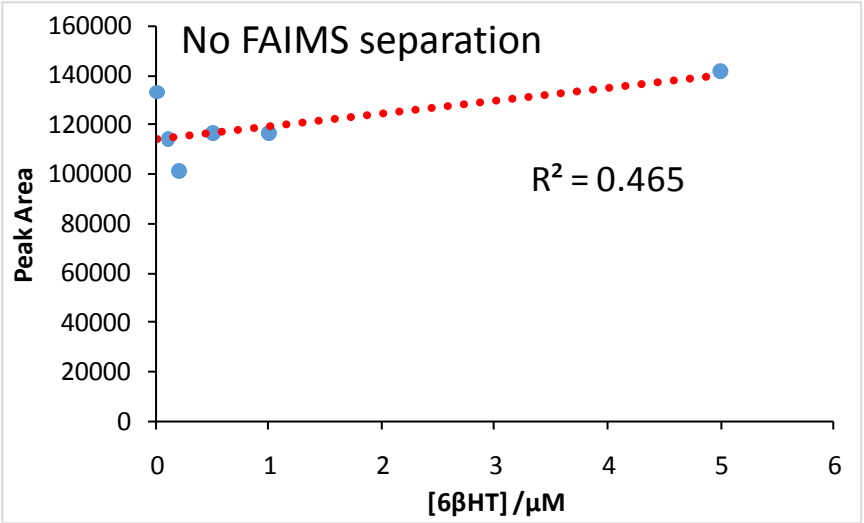
As well as removing interference from the isomer, FAIMS is also improving signal to noise.

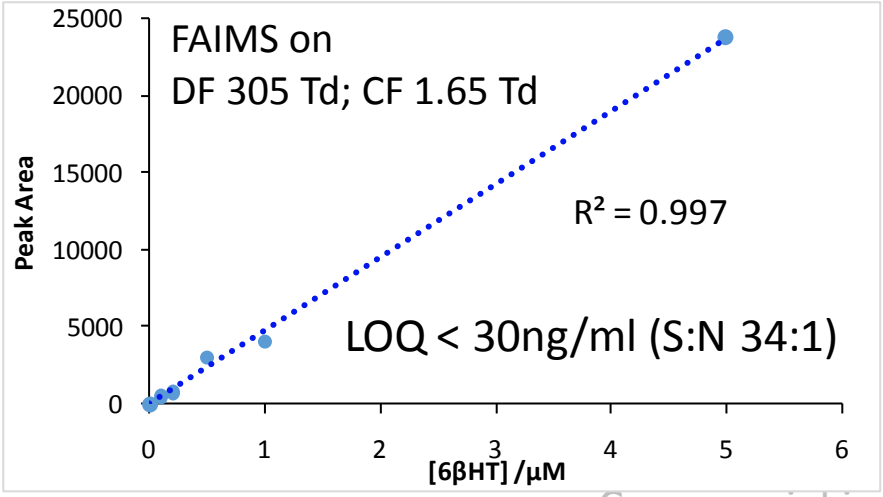
Quantitative Performance - 6β-HT Calibration curve

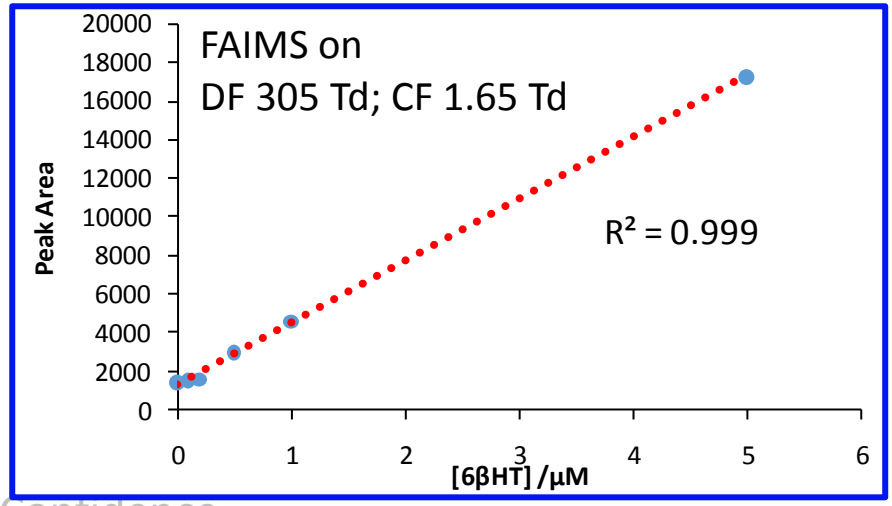
6β-HT only



6β-HT with 2β-HT interference (1µM)







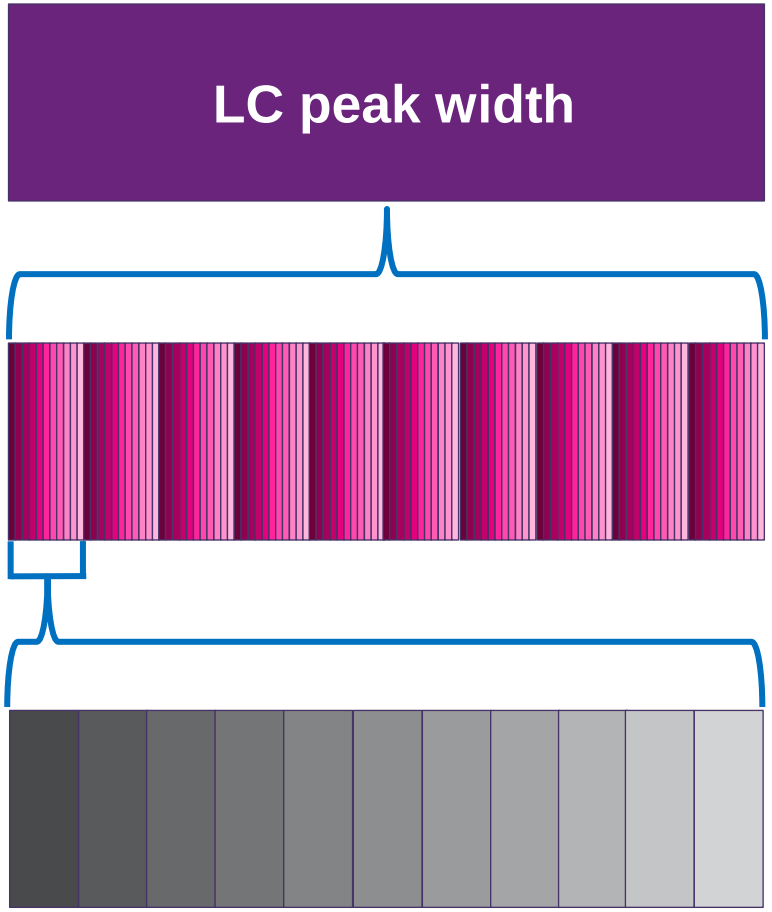
LC-UltraFAIMS-MS for untargeted “-omics” applications

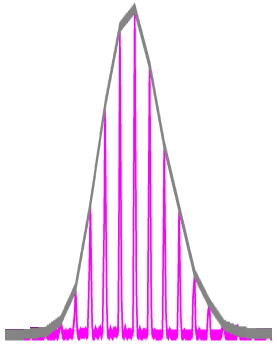
- In non-targeted ‘omics’ experiments such as metabolomics and proteomics, typically liquid or gas chromatography (LC or GC) combined with mass spectrometry (MS) is used to separate and analyse complex biological matrices

- Molecular features can however be missed or remain hidden within the dataset using these techniques due to:
  - Trace level features unresolved from the noise
  - Unresolved isomeric or isobaric species

- UltraFAIMS operating in a scanning mode provides an extra dimension of separation for LC-MS to improve peak capacity and reduce chemical noise

LC-UltraFAIMS-MS data acquisition





All CFs scanning

- FAIMS hops between 11 CF settings/s
- ToF set to scan 11 spectra/second
  - 1 mass spectra per CF
- Nested data sets within the chromatographic peak
- Synchronised using contact closure interface

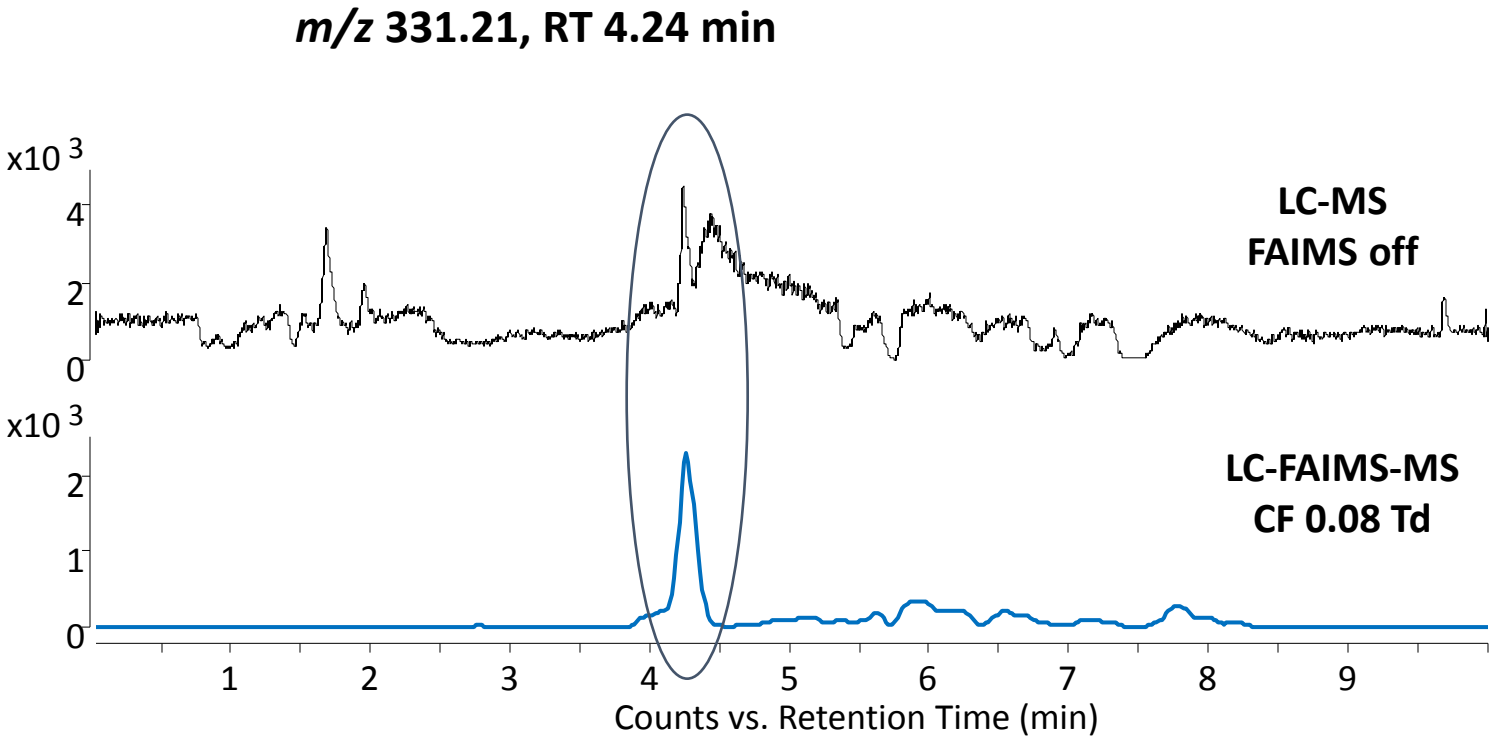
Detection of isobaric compounds

FAIMS off S:N

= 2.7

FAIMS on S:N =

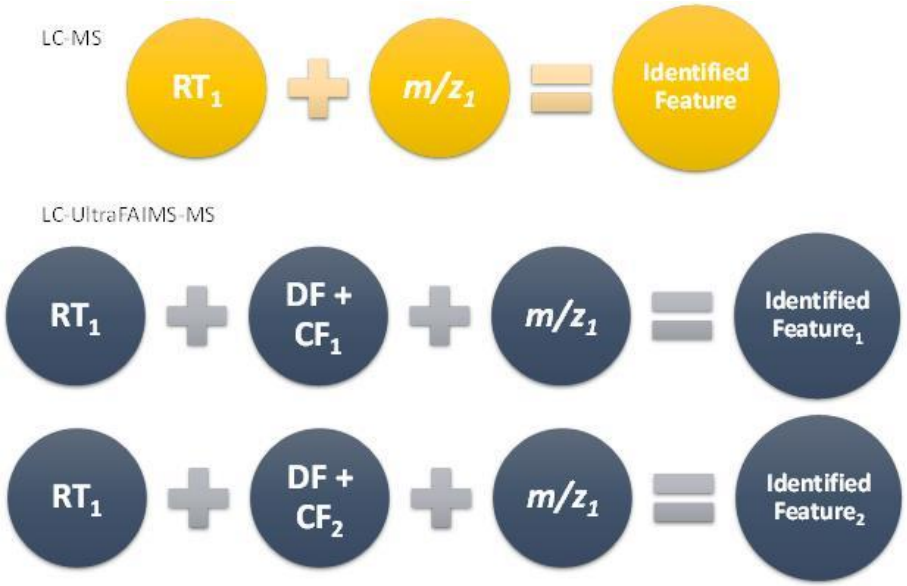
124.1



- FAIMS off = 0 features due to high baseline
- FAIMS on = Reduction in noise, resulting in additional feature detected
  - (45x improvement in S/N)

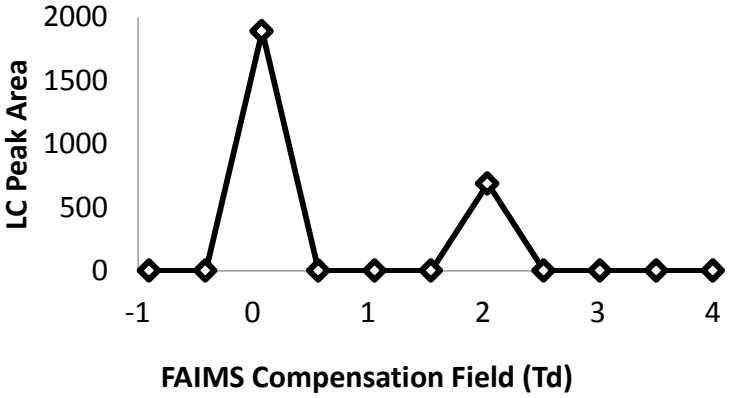
Untargeted feature determination

- UltraFAIMS is capable of separating things undistinguishable by LC-MS alone
- UltraFAIMS reduces chemical background, making low abundance ions easier to detect

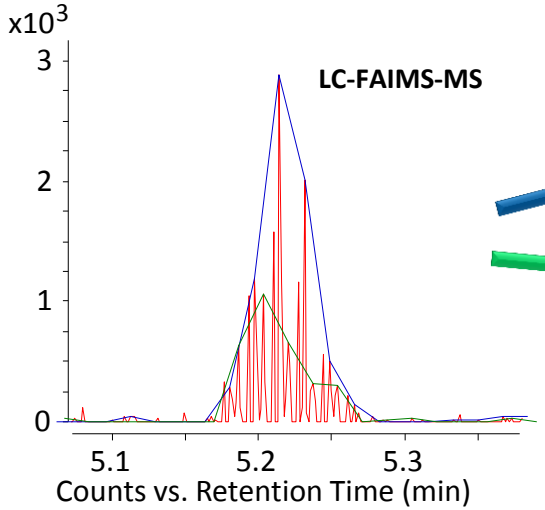


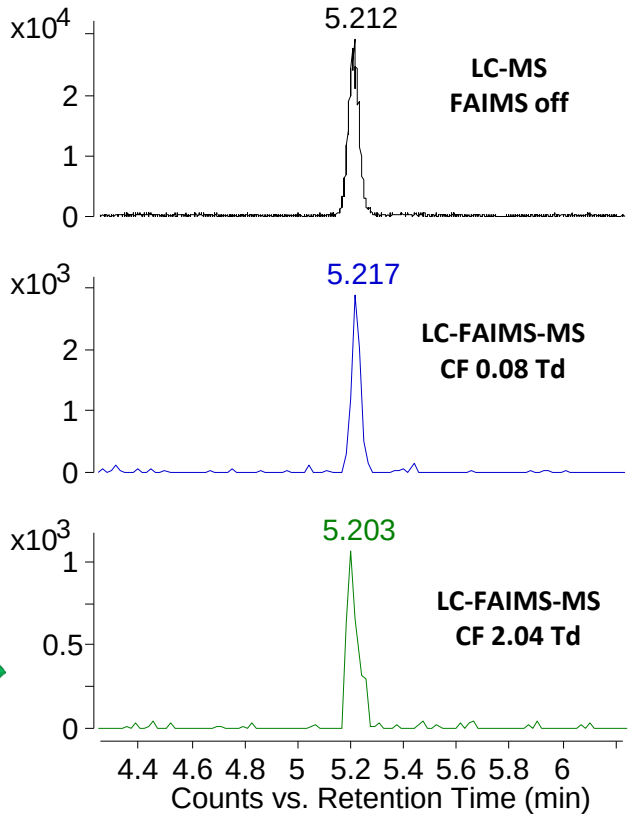
- Both effects contribute to an increased number of detected features and therefore an increased likelihood of detecting metabolites.

Detection of isobaric compounds



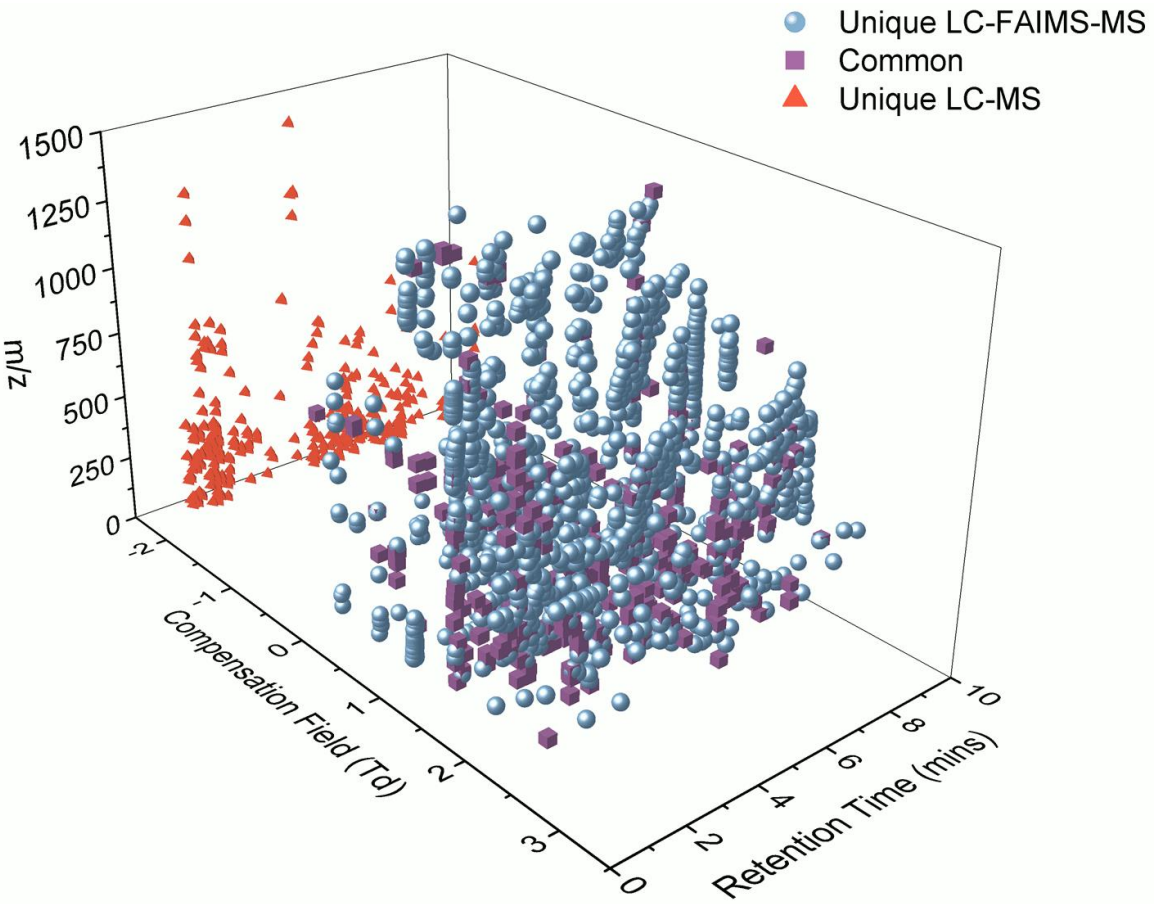
***m/z* 207.11**
RT 5.21 min

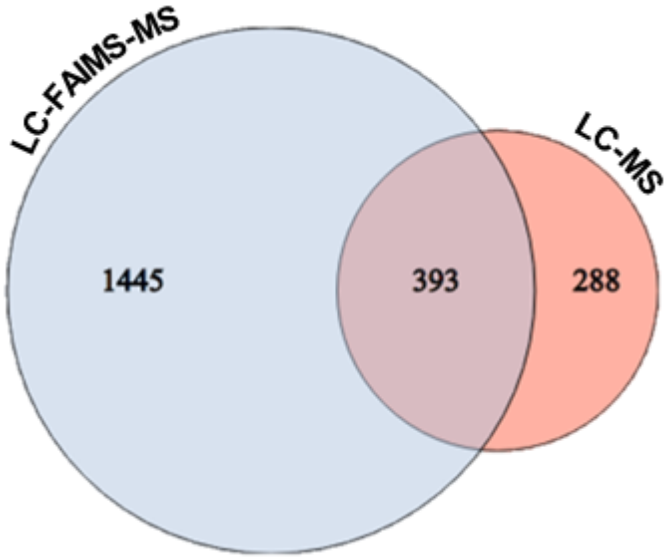




- FAIMS off = 1 feature detected
- FAIMS on = 2 features due to additional orthogonal separation

Identified feature comparison





Identified features based upon RT, *m/z* and CF

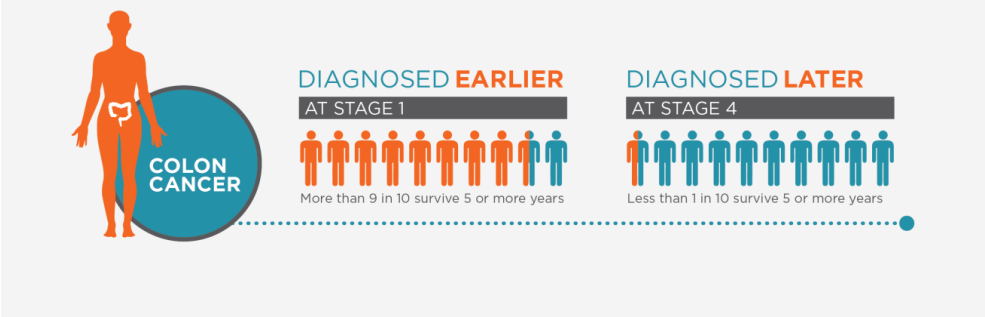
- 1838 spectral features were identified using LC-ultraFAIMS-MS, 1445 of which were unique to UltraFAIMS, compared with 681 identified by LC-MS alone.

Application to metabolomics

- Metabolomics looks to characterise a set of metabolites present within an organism.
    - Metabolite changes due to cellular processes
        - e.g. detection of particular disease states due to the presence or absence of certain metabolites.
- Historically been difficult due to the complexity of biological samples.
- We believe the reduction of background and subsequent enhanced feature detection due to the addition of FAIMS to LC-MS opens up new opportunities for LC-MS based metabolomics.

Utility to disease diagnostics: Colorectal cancer

- Patients identified at Stage I have around a 97% five-year relative survival rate, while those identified at Stage IV have around a 7.5% five-year relative survival rate.



- Existing non-invasive colorectal cancer (CRC) screening methods show relatively low sensitivities (66 - 96%, method dependent).

- To increase the number of early diagnoses, CRC screening must be improved.

- In collaboration with UHCW and University of Warwick we are undergoing a 2000 patient study into the detection of biomarkers of CRC.

- Can we apply the LC-UltraFAIMS-MS method to biological samples from CRC patients and use the additional feature detection ability to diagnoses early stage disease?

Summary

- UltraFAIMS is a fast gas-phase separation stage for MS and LC-MS
- Available for Thermo, Agilent and Bruker mass spectrometers
- Used in combination with ambient ionisation sources to enhance analyte detection in the absence of traditional pre-separation methods
- Can replace LC separations of isomeric species, speeding up analysis times
- An UltraFAIMS step can be added to LC-MS to improve peak capacity and aid non-targeted analysis
  - Resolving isobaric species
  - Separating trace level components from chromatographic noise
- Further detail/tech specs etc on our website www.ultrafaims.com
- For further info on IMS in general....

"Ion Mobility Mass Spectrometry: The Next Five Years"
(88-page e-book)

Download free from:
http://bit.ly/owlstone-jsb

Are you a member of the LinkedIn IMS-MS group yet? Join to find out what the IMS-MS community is discussing.

http://bit.ly/IMMSgroup

Acknowledgements

- University of Texas
  - ***Livia Eberlin***
  - ***Clara Feider***
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  - ***Noah Weiss***
  - ***Roland Thiele***
  - ***Uwe Kobold***
- Owlstone
  - ***Robert Smith***
  - ***Kayleigh Arthur***
- Loughborough University
  - ***Colin Creaser***
  - ***James Reynolds***
  - ***Matthew Turner***

THANK YOU

FOR YOUR ATTENTION!

Any questions?

To find out more about UltraFAIMS, visit the product page at: www.ultrafaims.com