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Applications of novel acquisition modes and instrument geometries in Time-of-Flight Mass Spectrometer for Targeted Quantitation

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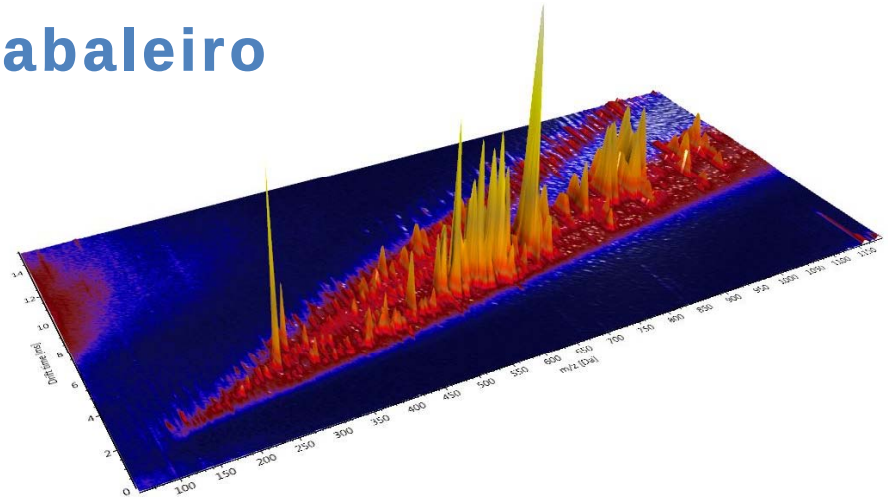
Diego Rodriguez Cabaleiro

EBF 2015



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Overview

- TQ vs HRMS systems
- TOF Acquisition modes
  - MS
  - MSMS
  - IMS-MSMS (Ion Mobility Spectrometry)
- The additional benefits of microfluidics

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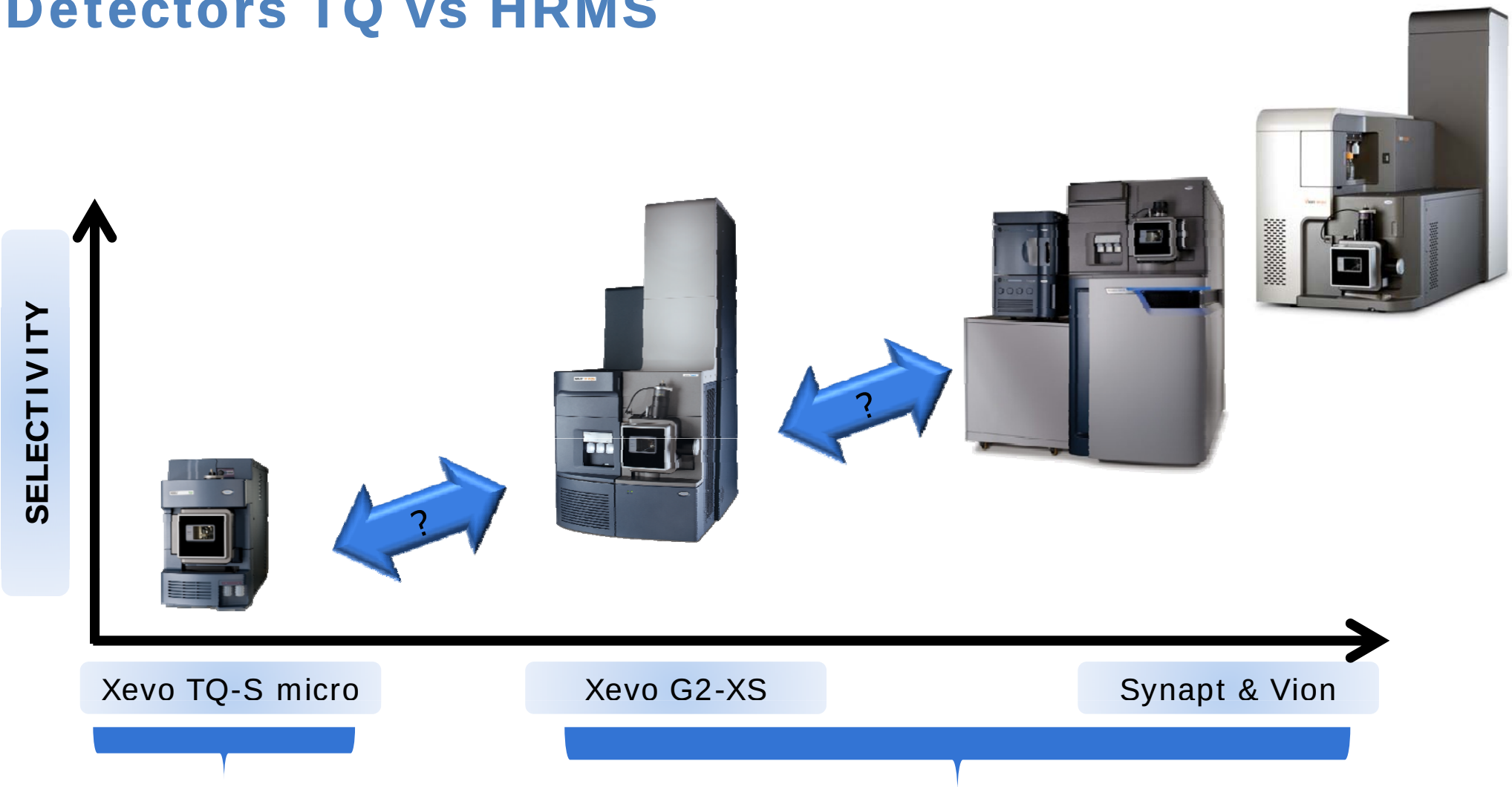
Detectors TQ vs HRMS



- Best sensitivity
- Best LDR (4-5 orders)
- Software for quick Met. Dev.
- Stablished accross departments
- **Selectivity based on fragmentation**

- Best sensitivity in full scan
- Good dynamic range (3-4 orders)
- Full scan or targetted quantitation
- **Selectivity**
  - **Exact Mass, IMS, Fragments**

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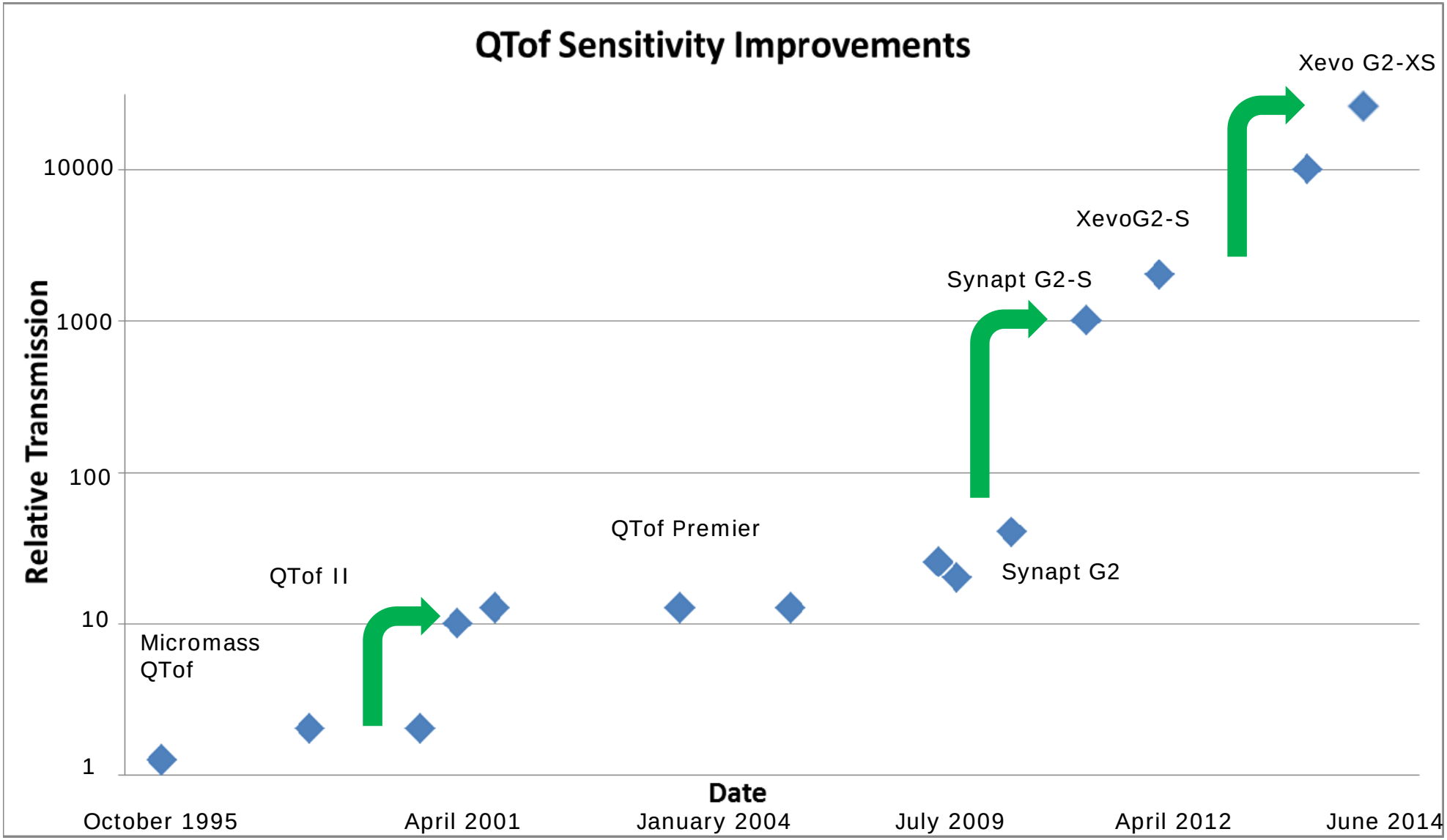
Evolution of TOF Sensitivity



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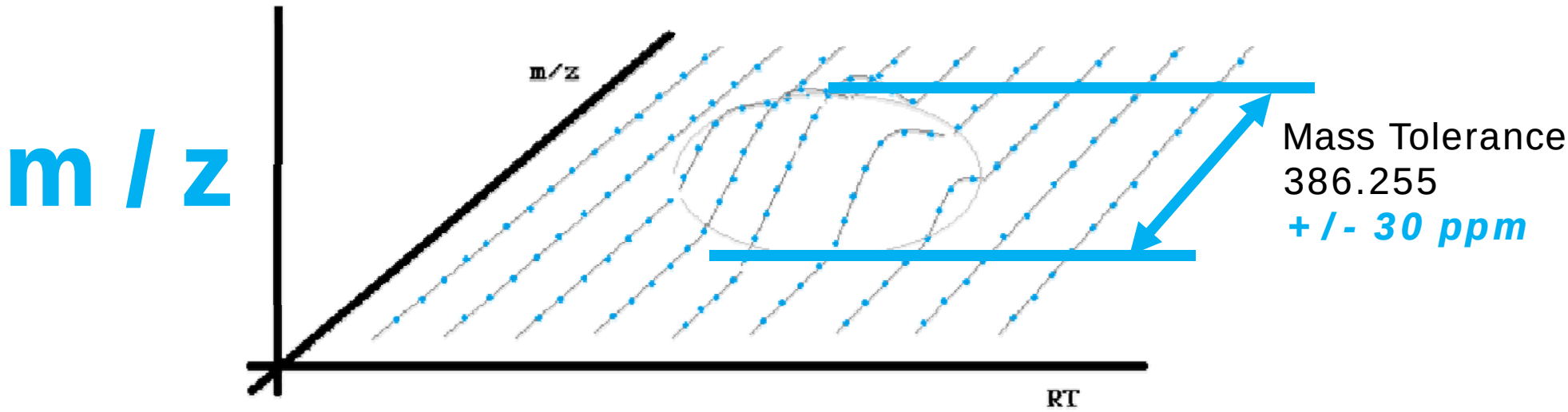
How do we select a peak using m/z?



We can choose how to sample a peak

What width we choose is related (inversely) to the resolution of the instrument and the noise around the peak of interest

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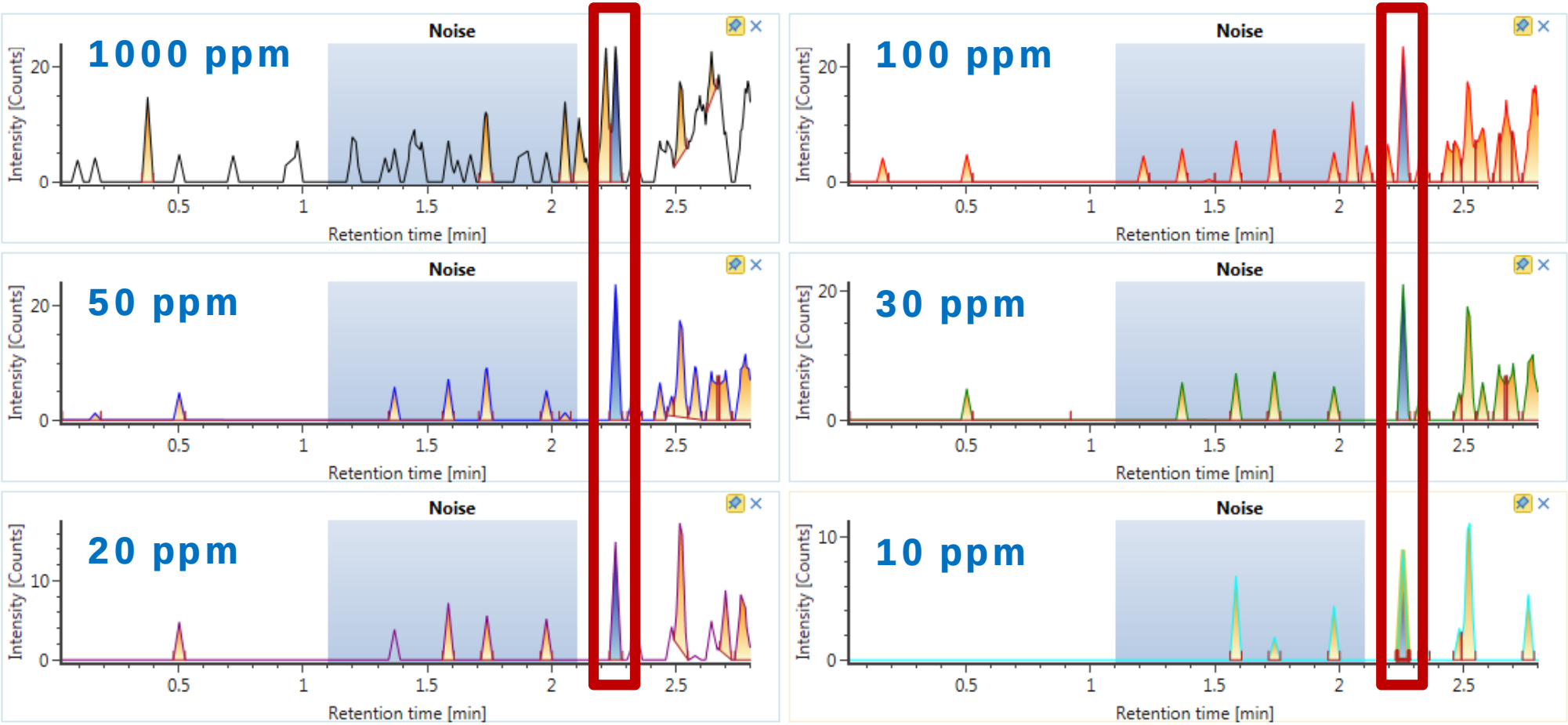
Buspirone in Plasma (10 pg/mL)
Adjusting Selectivity of PPM tolerance



For this assay, a 30 or 50 ppm window is optimal

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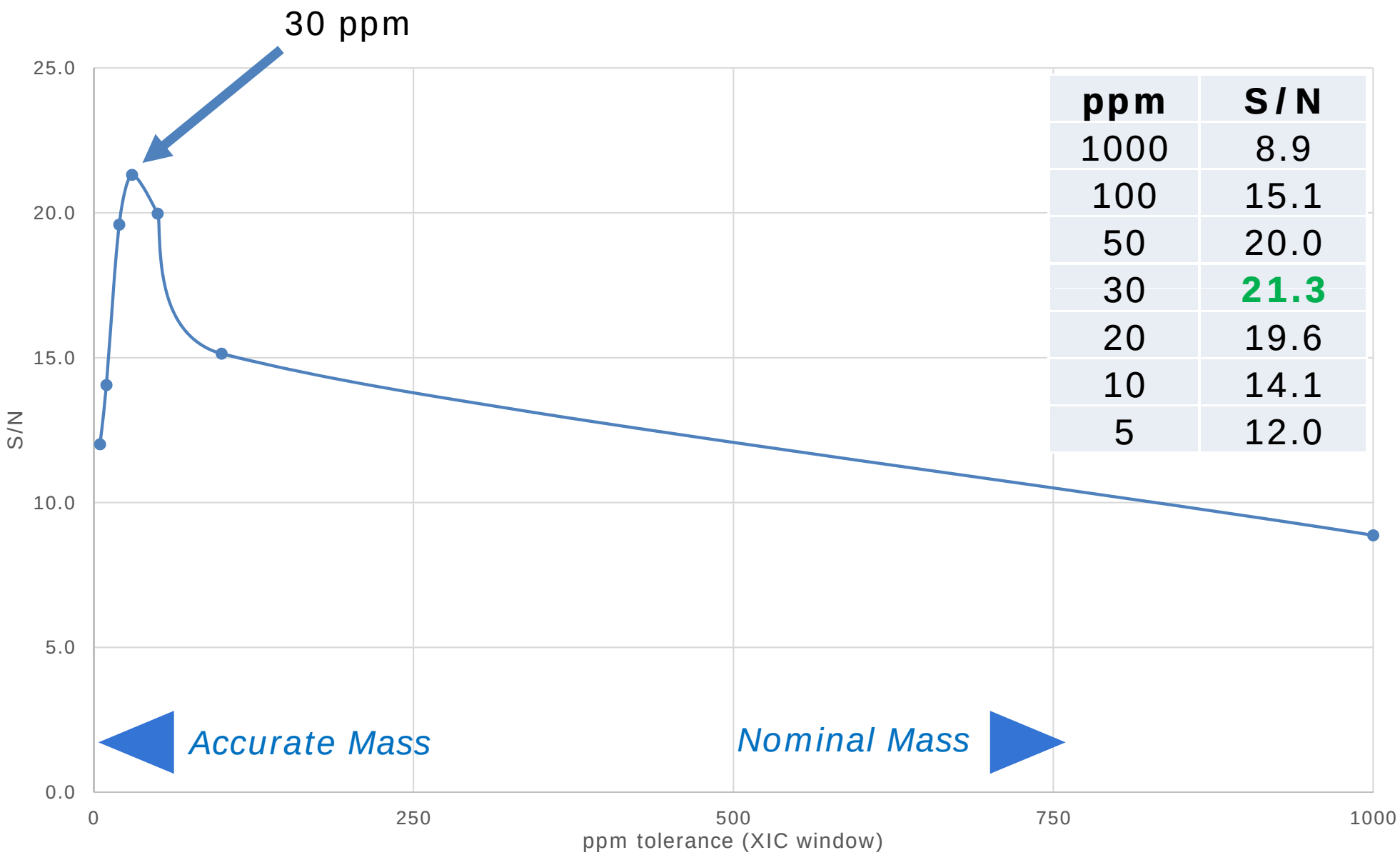
XIC tolerance to S/N Relationship Plot (Buspirone in Plasma)



ppm	S/N
1000	8.9
100	15.1
50	20.0
30	**21.3**
20	19.6
10	14.1
5	12.0

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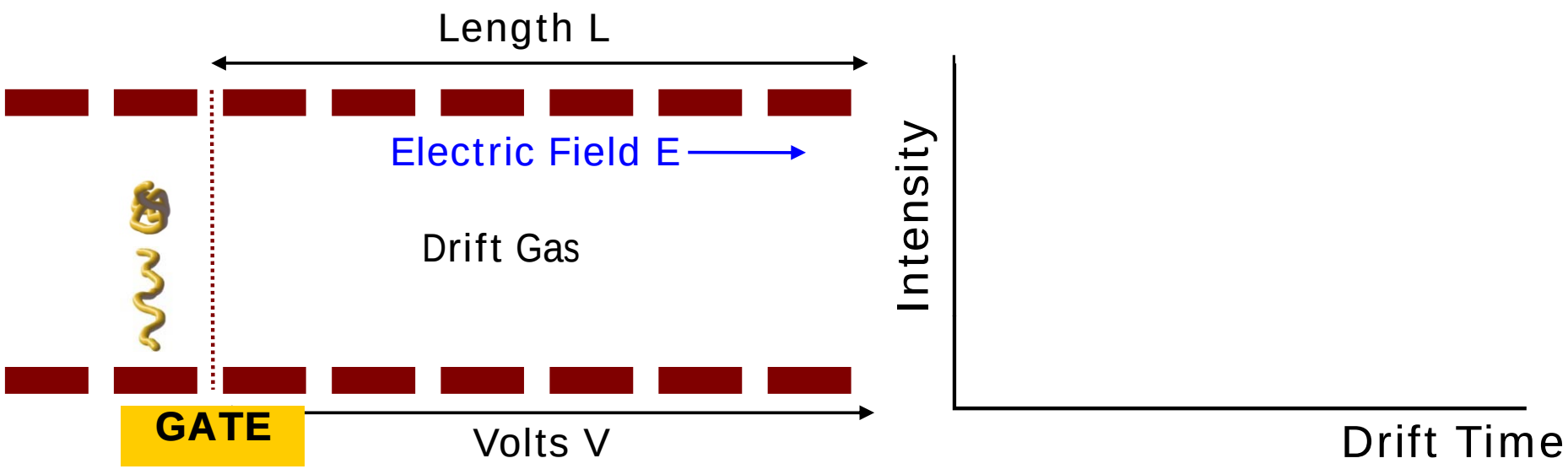
Ion Mobility Spectrometry



- Separation based on size and shape
- Orthogonal separation different from chromatographic and mass resolution

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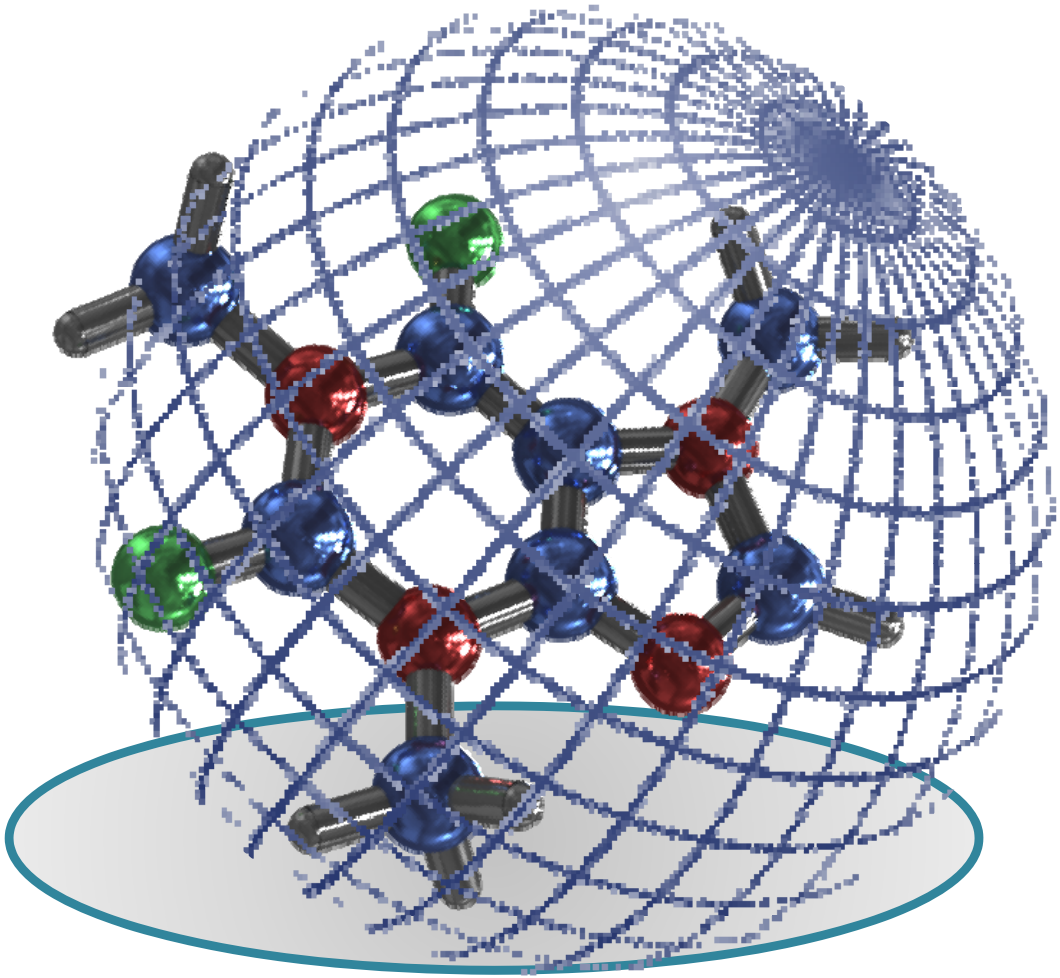
New distinctive parameter = CCS

- Unique physicochemical property of each ion
  - chemical structure
  - 3-dimensional conformation



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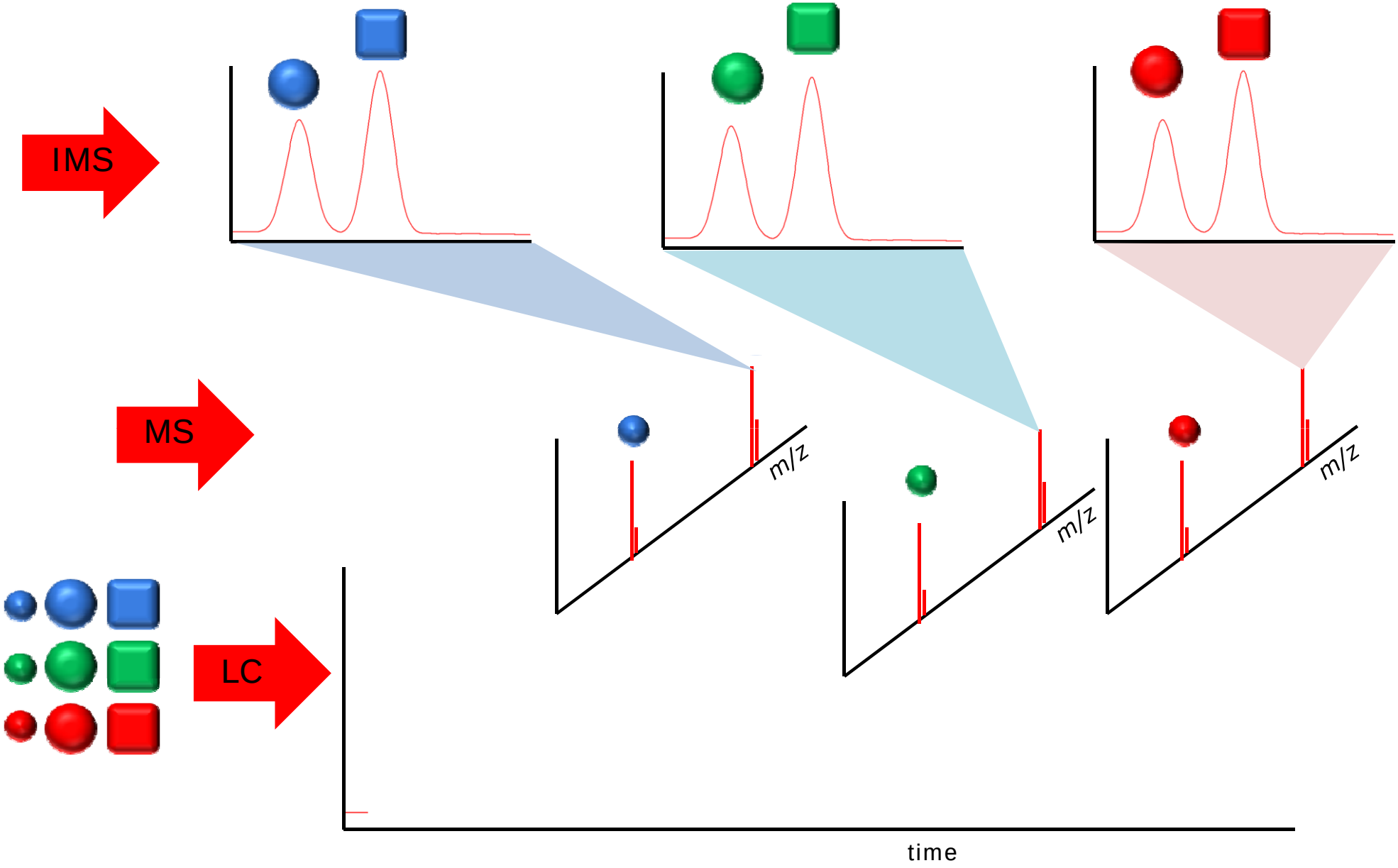
LC-IM-MS



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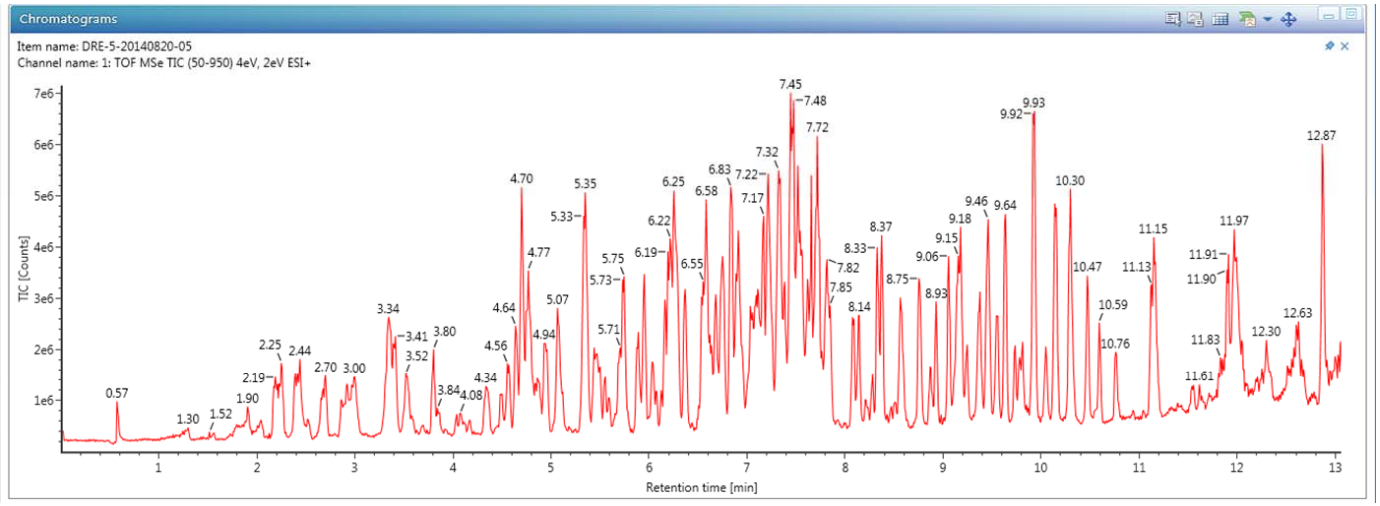
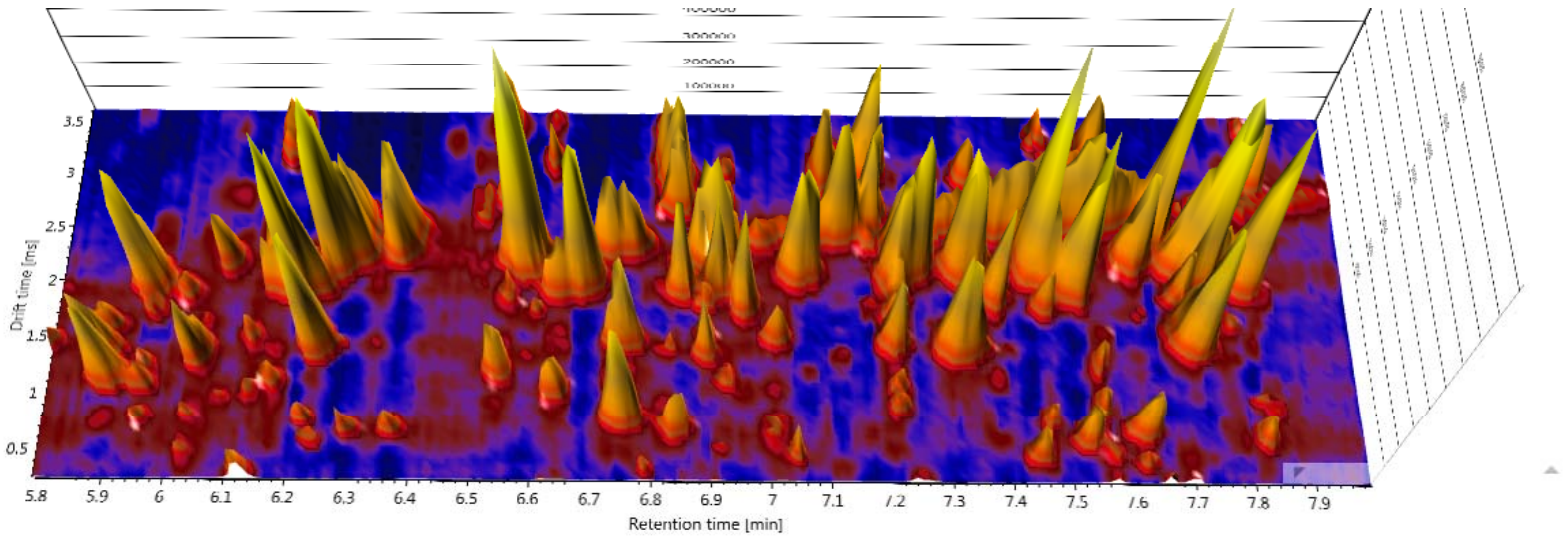
Greater peak capacity

Resolution in 2 dimensions = Retention X Mass



Resolution in 3 dimensions = Retention X Mobility X Mass



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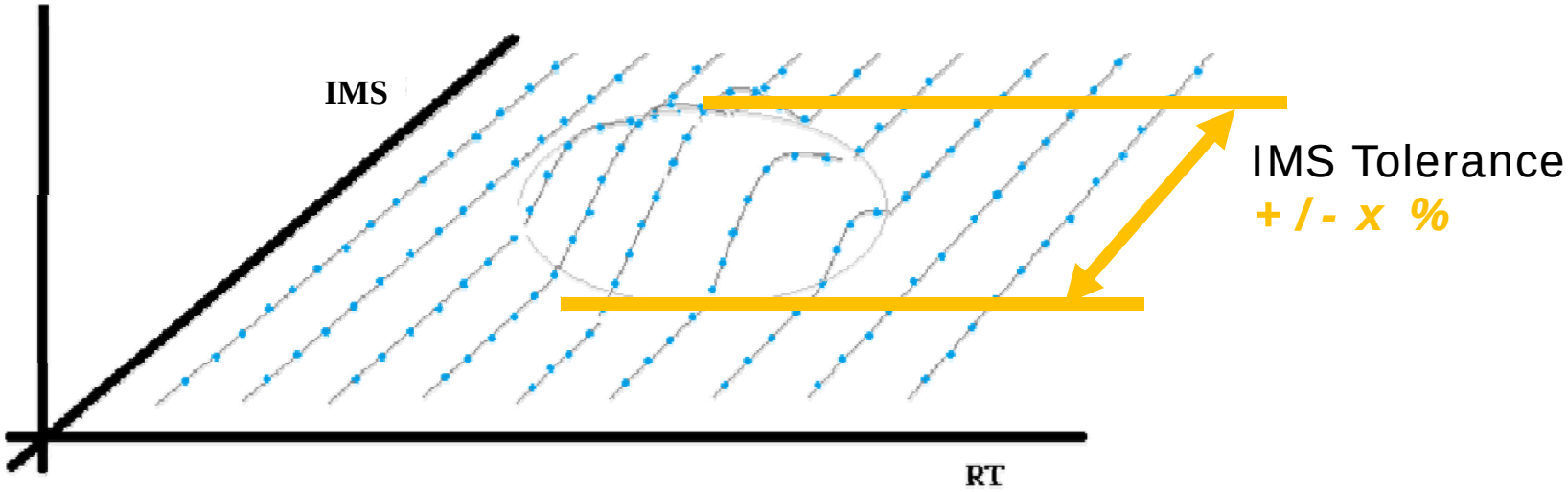
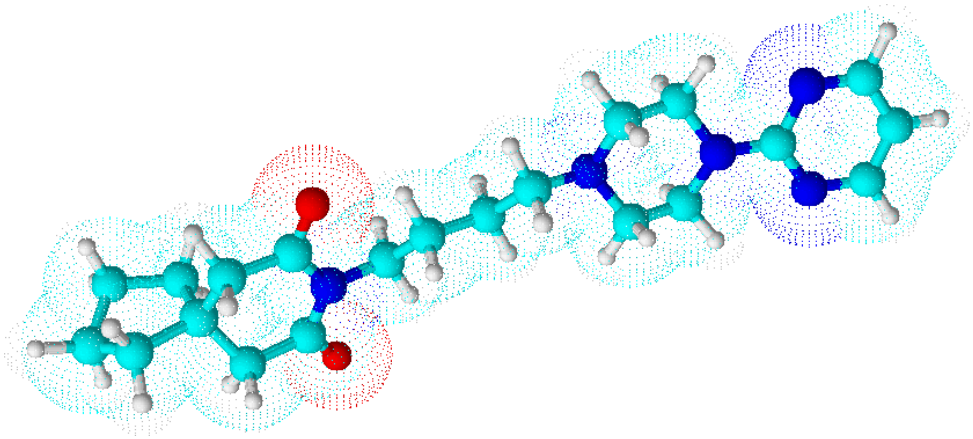
How do we select a peak using CCS?

CCS





Buspirone

We can set a tolerance around the CCS and choose to exclude isobaric interferences (NOISE)

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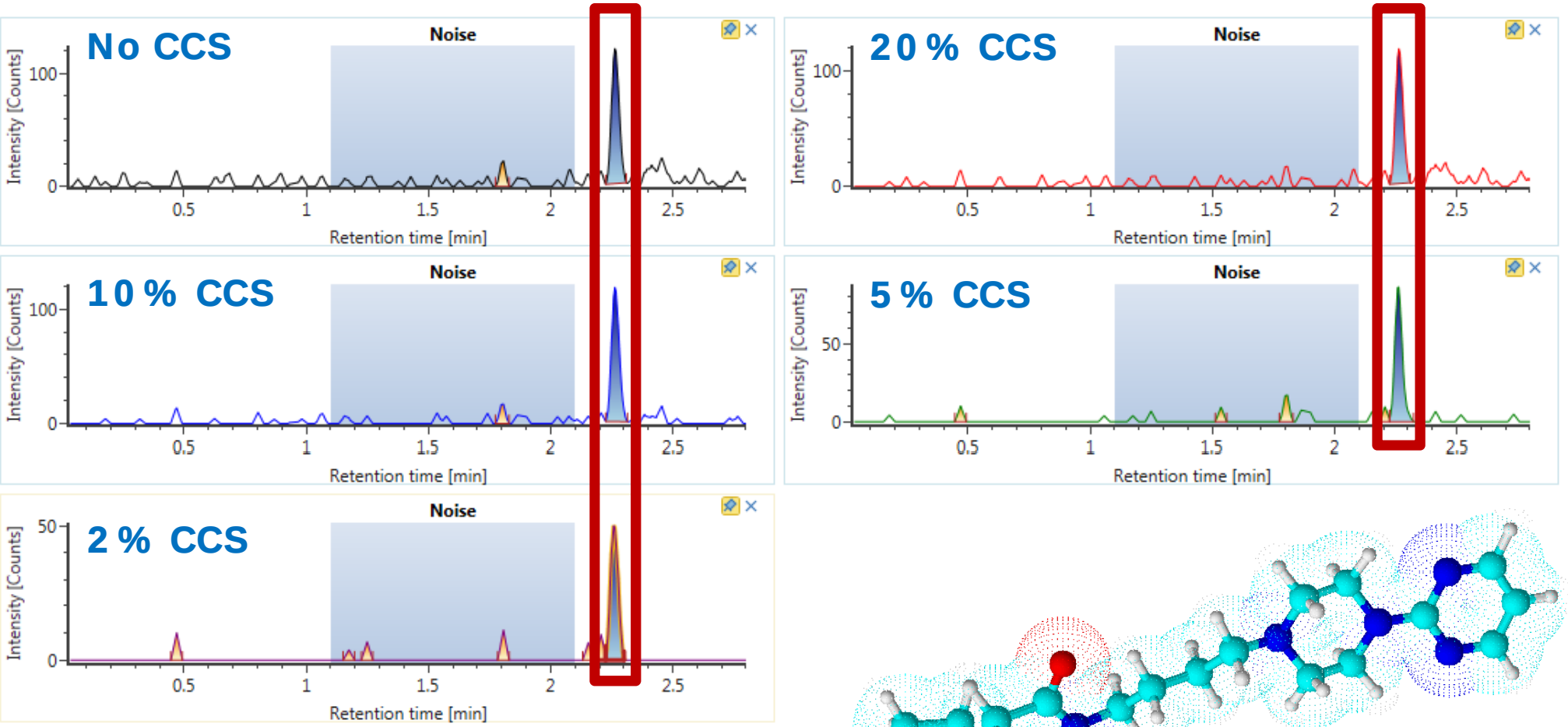
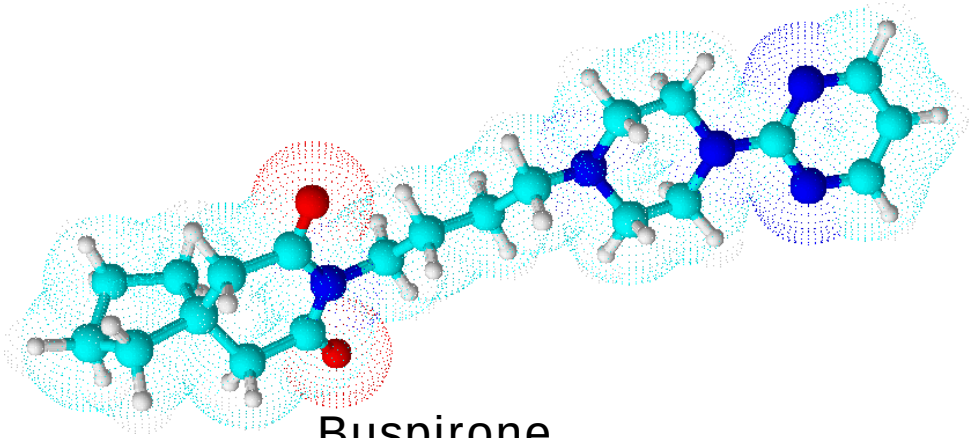
Buspirone in Plasma (100 pg/mL)
Adjusting Selectivity of CCS tolerance



For this assay 10% is optimal



Buspirone

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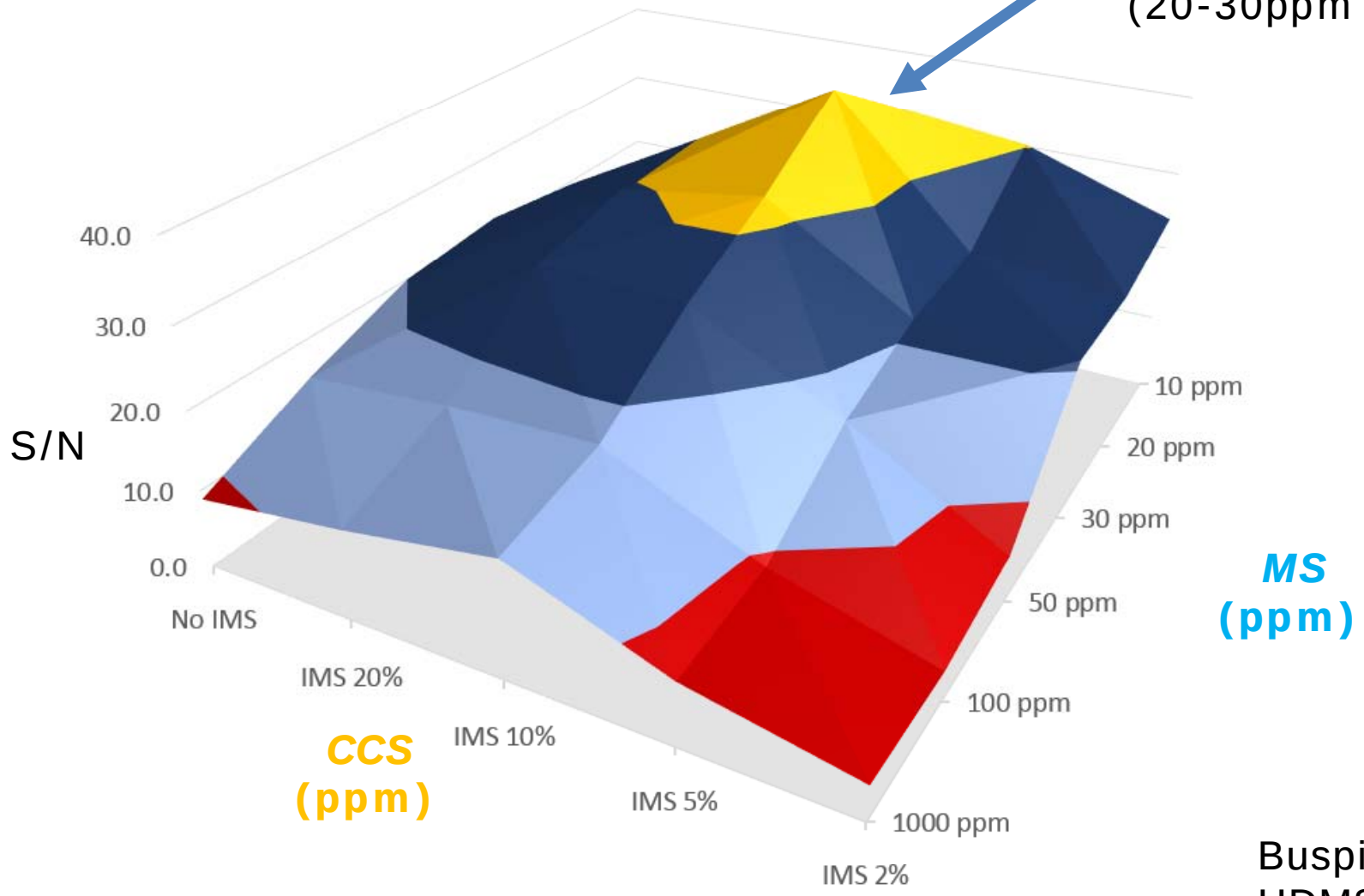
Combining Selectivity Factors CCS + m/z

Both ppm window and IMS window result in S/N hotspot (20-30ppm and 10% IMS)



Buspirone in Plasma
HDMSE, 100 pg/mL

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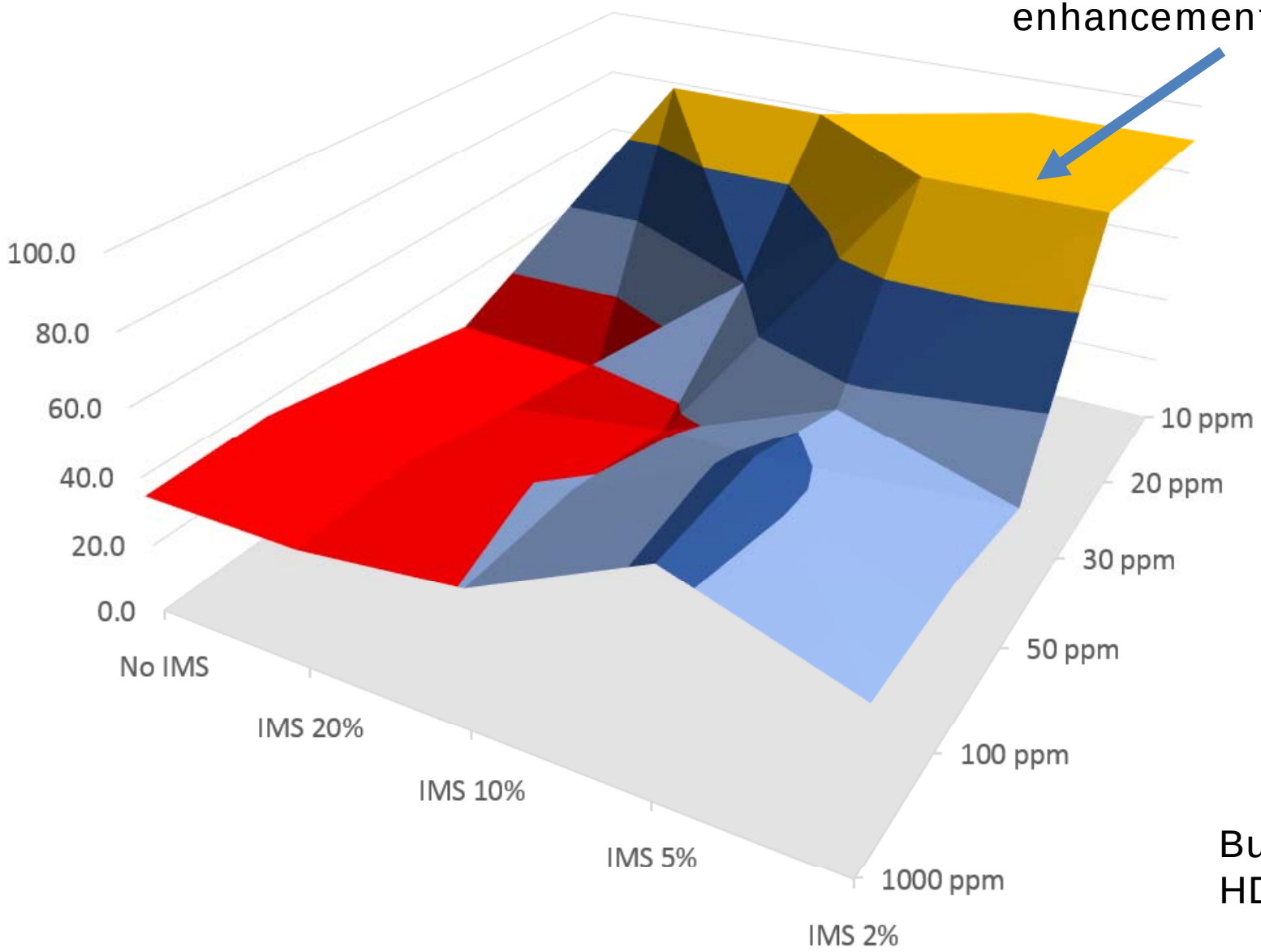
Combining Selectivity Factors
CCS + m/z + Quadrupole

Additional 2-3 fold further enhancement in S/N with Quad



Buspirone in Plasma
HDMSMS, 100 pg/mL

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Applying IMS principles to other compounds in Plasma

Clopidogrel (late eluter)

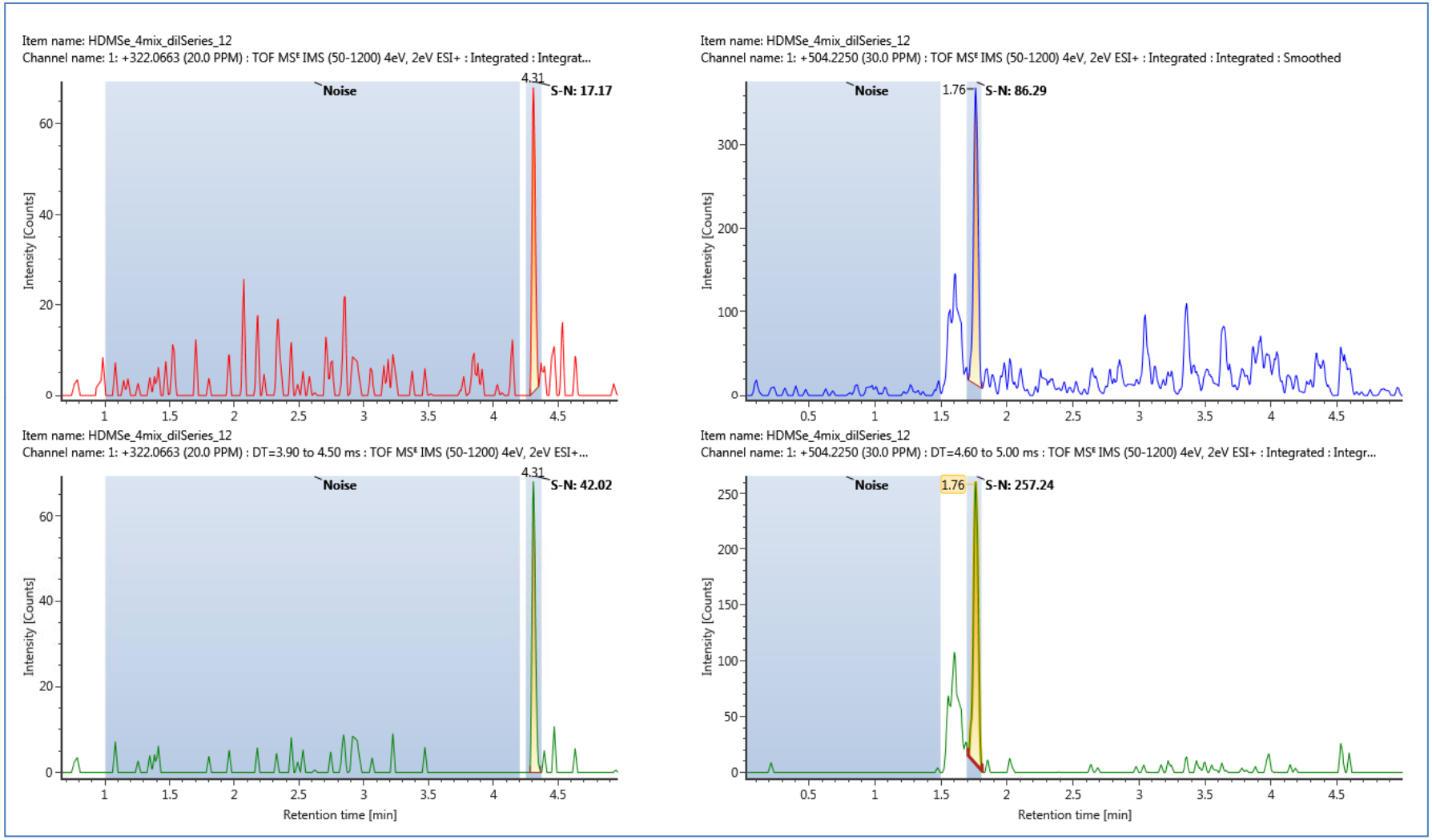
Oxytocin (+2 Peptide)

20 ppm

No IMS

20 ppm

+ *IMS*



IMS Cleanup is generically applicable

(RMS Noise Calculated)

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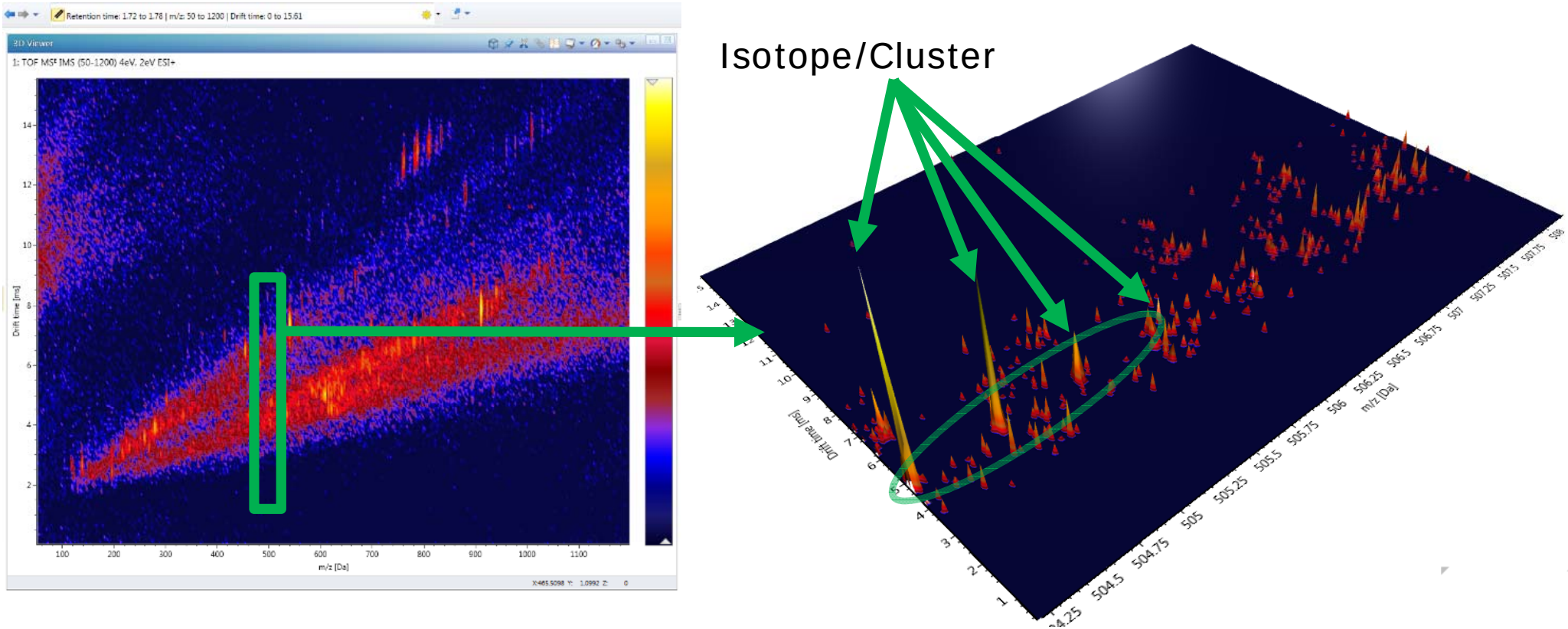
How do we do IMS/CCS Filtering and mass filtering together?

- Look at the RT where our compound elutes
- View the m/z vs CCS/IMS Plot



- Isolate the peak(s) of interest
  - smallest ppm and IMS window

UNIFI 1.8

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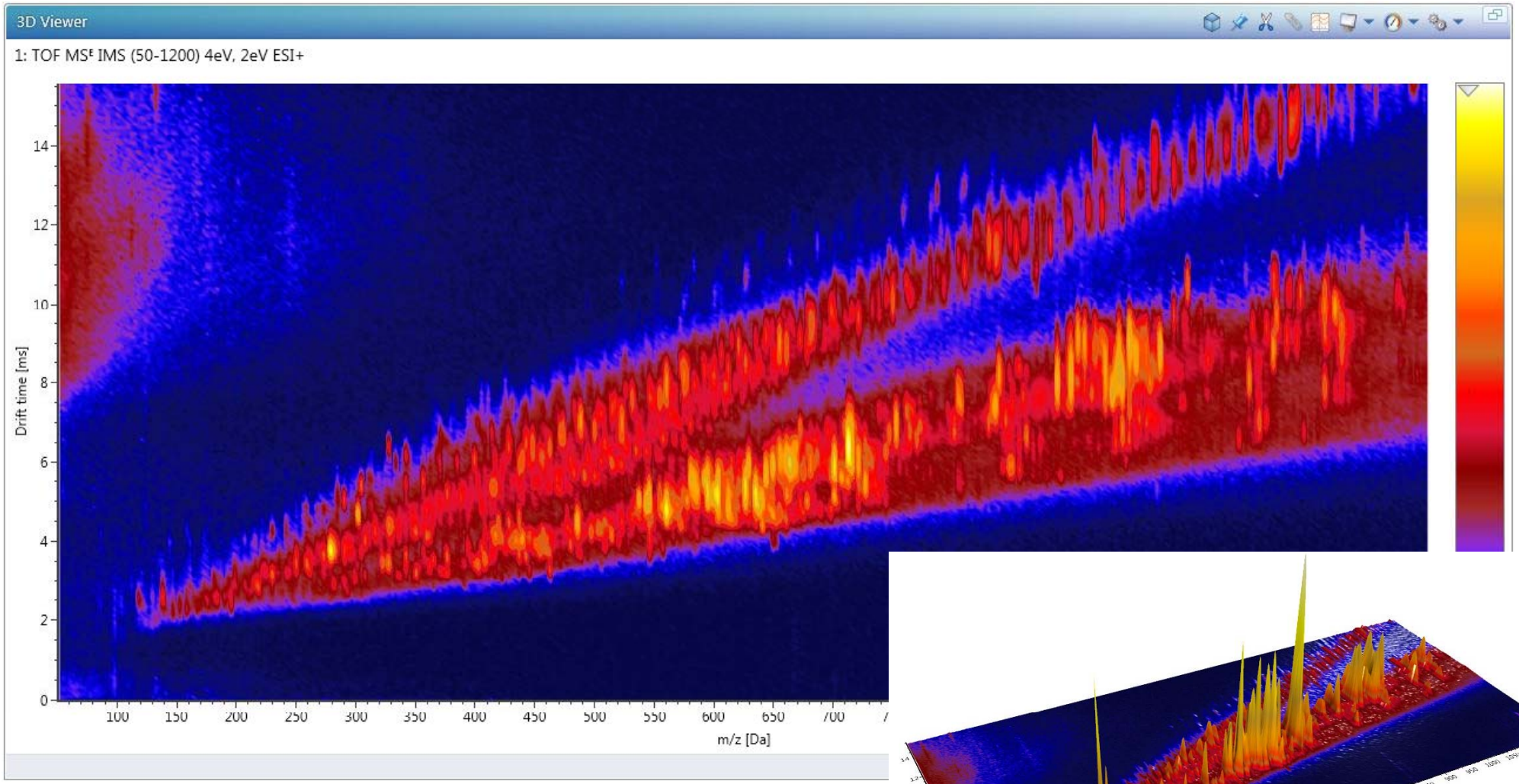
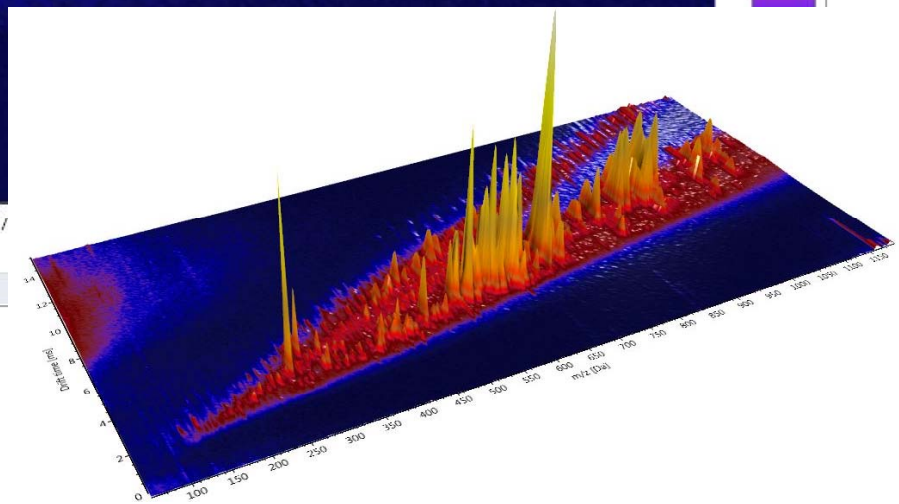
Peptide Quantitation
Dealing with extra complexity





Every peptide can be tracked by MASS (m/z) AND CCS

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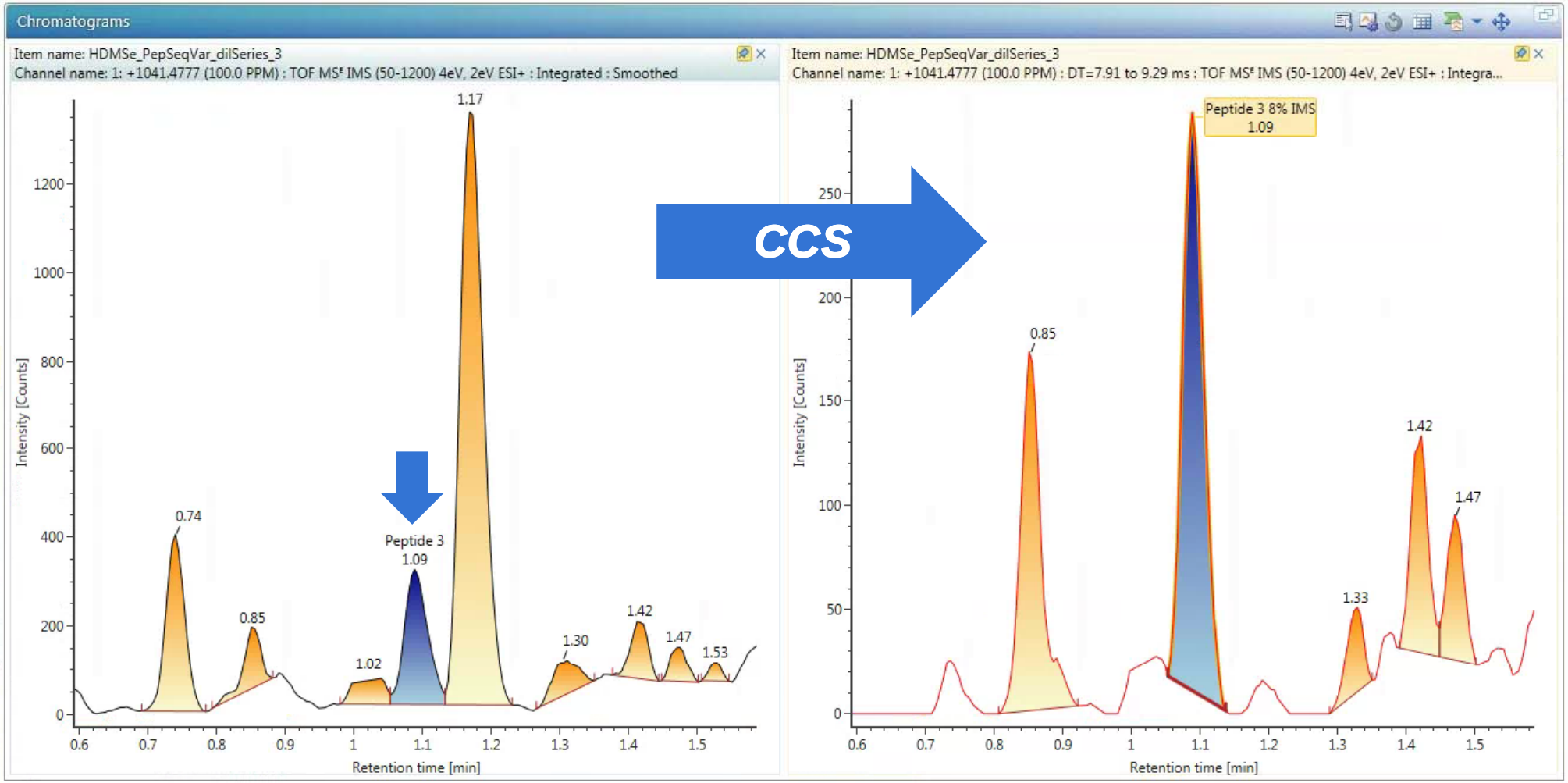
IMS Filtering of Peptide Quantitative Data



100 ppm

100 ppm + 8% IMS

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Advantages of Integrated Microfluidics and Micro-Flow ESI

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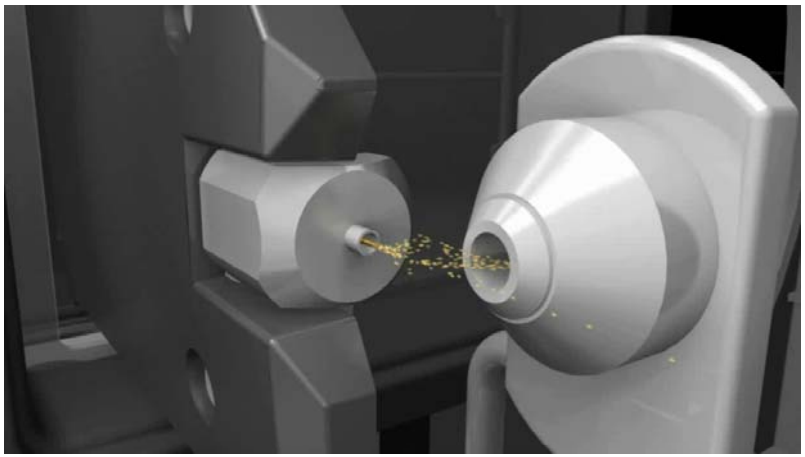
- Improved Sensitivity over 2.1 mm scale: **10-20X**
  - Improved Sampling Efficiency
  - Improved Ionization Efficiency
  - Reduced Ion Suppression



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Micro-Flow ESI vs Analytical-scale ESI Charge State Distribution Shift

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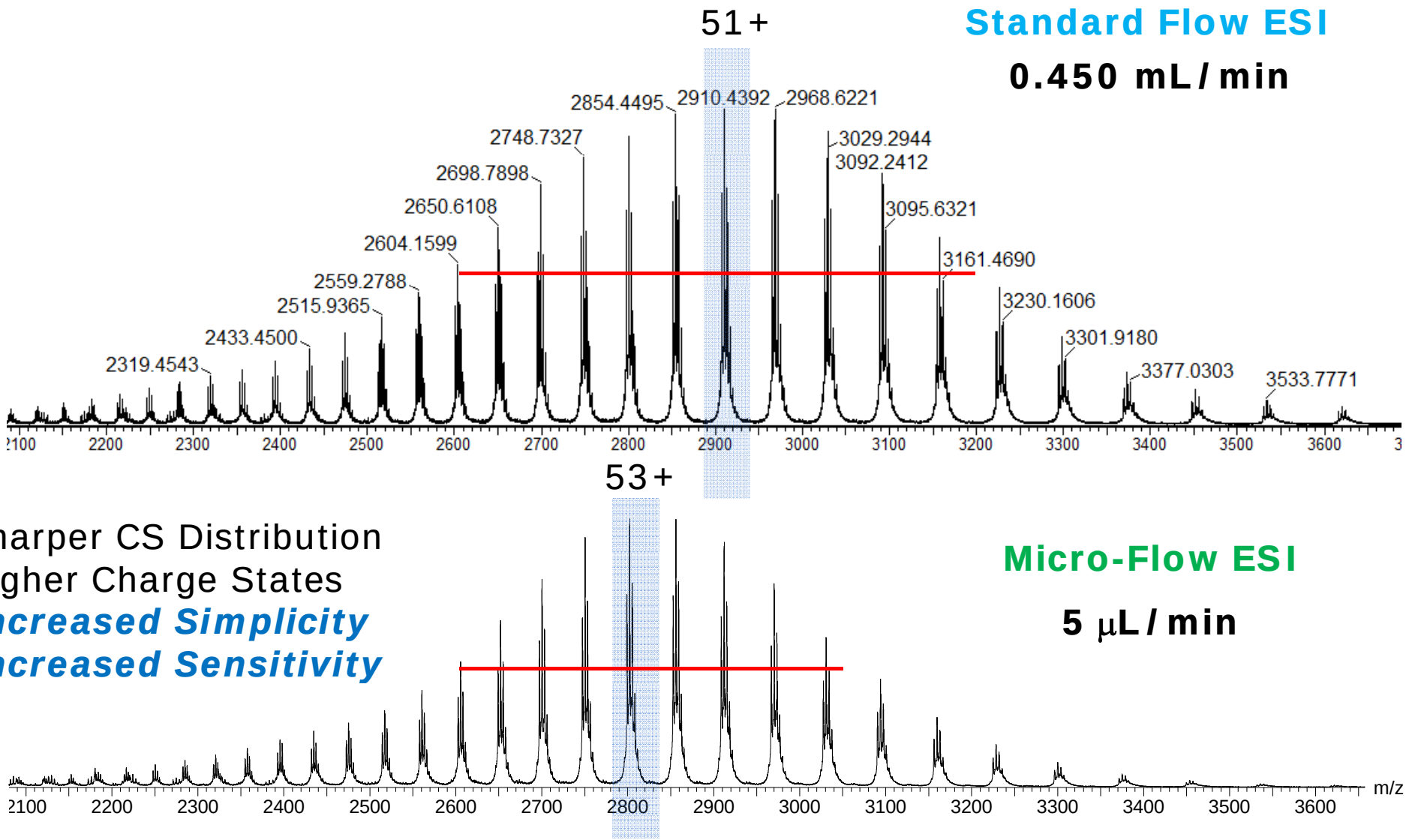

- Sharper CS Distribution
- Higher Charge States
- ***Increased Simplicity***
- ***Increased Sensitivity***

R.R. Ogerzaiek Loo; J. Am Soc. Mass Spectrom. (2014) 25:1675-1693

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Generic Affinity Capture of Humira from Rat Plasma

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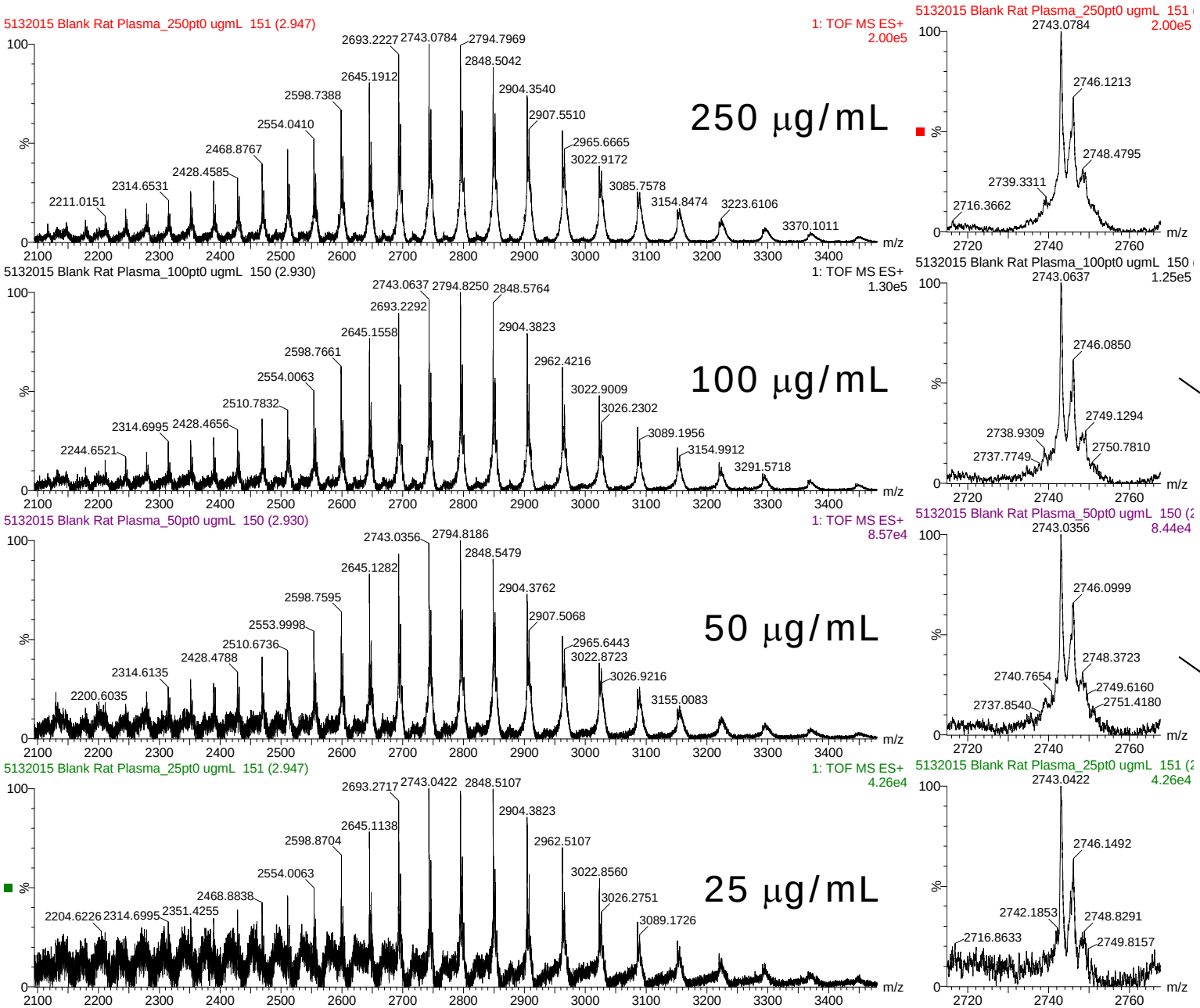
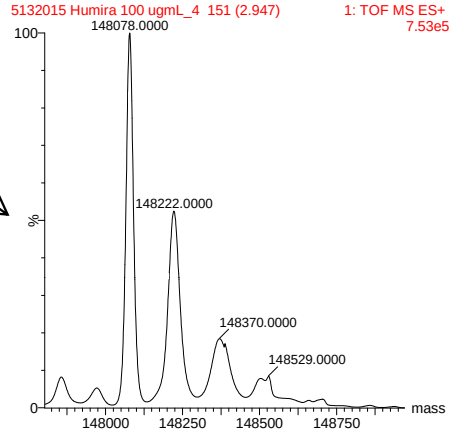
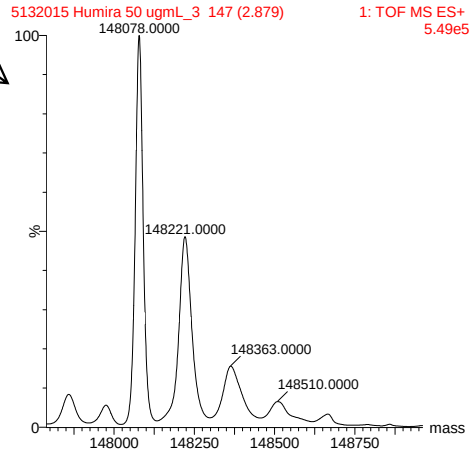


Humira Linear Range: 25-500 µg/mL

Deconvoluted Spectra





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Trastuzumab ADC (De-Glycosylated) High Sensitivity CSD Spectra

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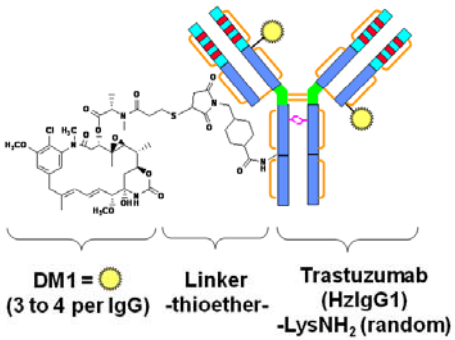
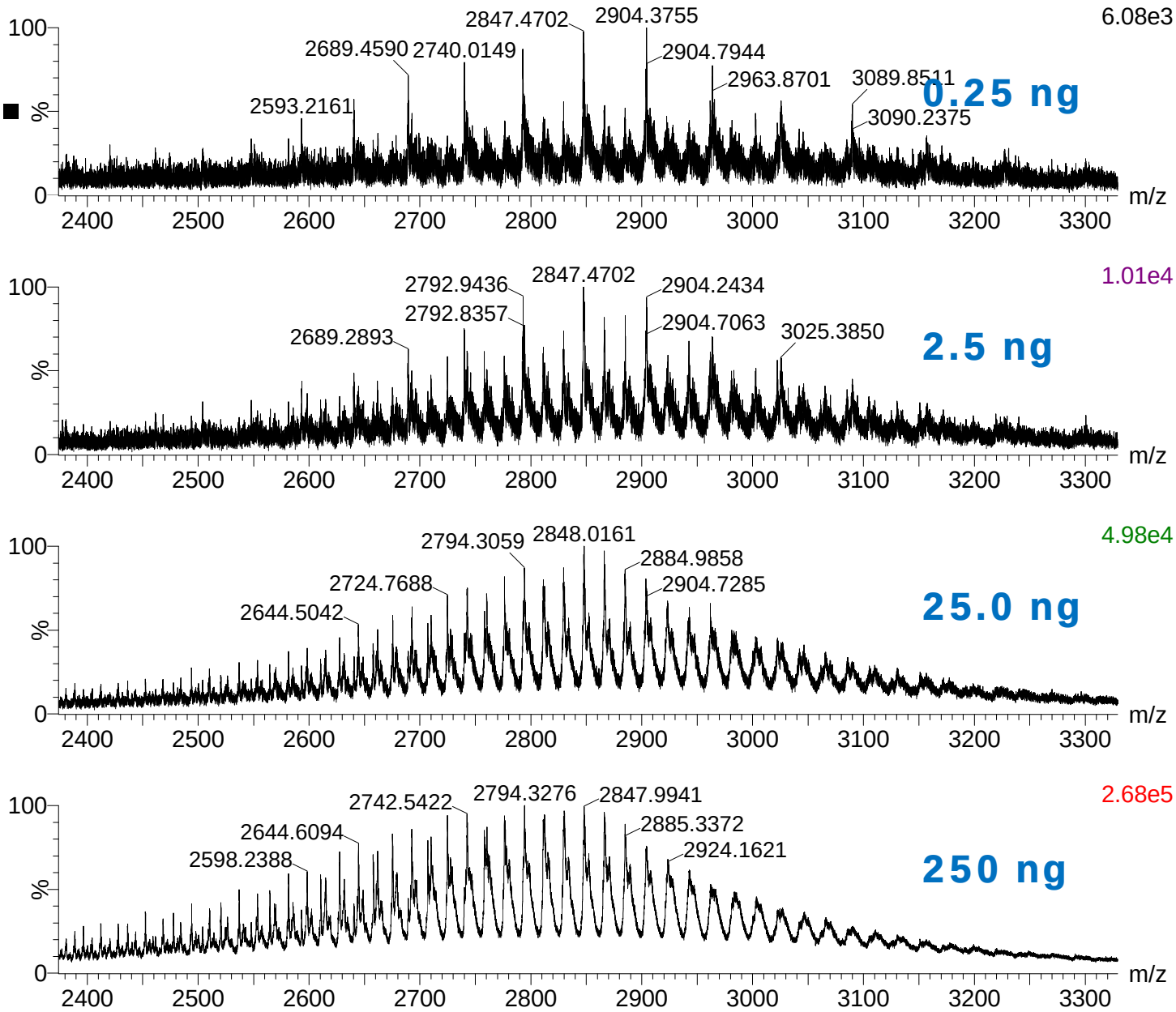
Trastuzumab MW:
145165.1575 g/mol

Linker and Drug:
958.5322 g/mol

1 Drug + 1 Antibody:
146123.6 g/mol





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Trastuzumab ADC (De-Glycosylated) Deconvolution on ionKey/MS

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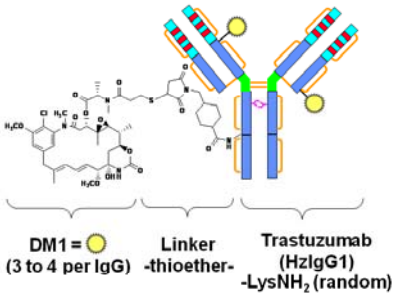
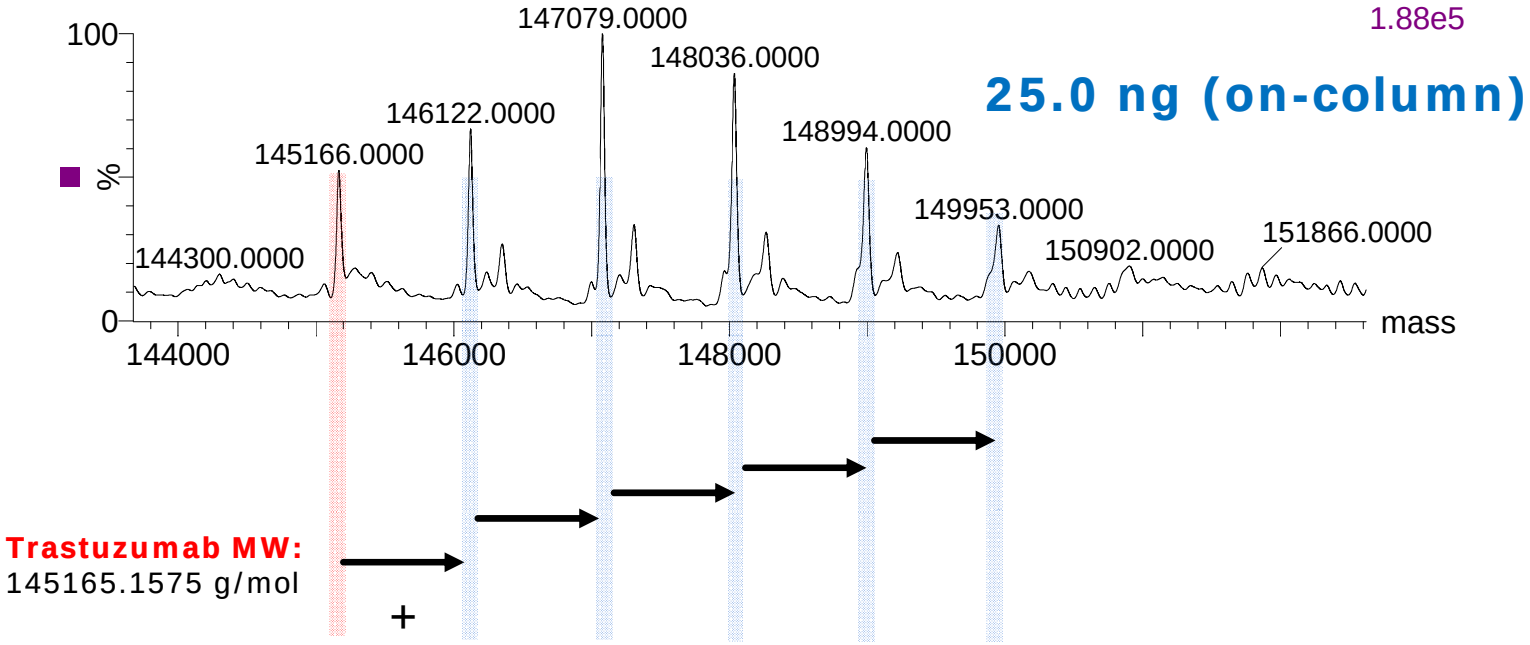
Trastuzumab MW:
145165.1575 g/mol

Linker and Drug:
958.5322 g/mol

1 Drug + 1 Antibody:
146123.6 g/mol





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April 05-07 ▫ Wilmslow, UK

Waters Corporation invites you to a 3 days Symposium.

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Conclusions

- **Selectivity can be exploited through**
  - Precursor selection – Quadrupole
    - Full scan modes vs MSMS or Tof MRM (not shown in this ppt)
  - Resolution – Instrument @ 32000 (for sensitivity)
  - Mass Tolerance of Peak, +/- ppm or mDa
  - *CCS Tolerance of Peak, +/- drift time*

- **Enabled by simple software tools to collect, process and use data effectively**

- **IonKey offers an extra step in sensitivity**

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Acknowledgements

- Nick Tomczyk
- Martin Palmer
- Yun Alelyunas
- Russell Mortishire-Smith
- Henry Shion

- Craig Dorschel
- Jennifer Simeone
- Catalin Doneanu
- Stephen McDonald
- Alistair Wallace

- Keith Richardson
- Jason Wildgoose
- Kevin Giles
- Paul Rainville
- Martin Green
- Kevin Cook
- Alan Millar
- Diane Diehl
- Simon Cubbon
- Steve Cubbedge
- Gordon Kearney

- Emma Marsden-Edwards
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- Jeff Robataille
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- Liuxi Chen

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