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Moving Forward on Quan-Qual,

Perspectives on Using Tofs for Bioanalytical Work

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European Bioanalysis Forum

Understanding Role of HRMS / HRAM Technologies

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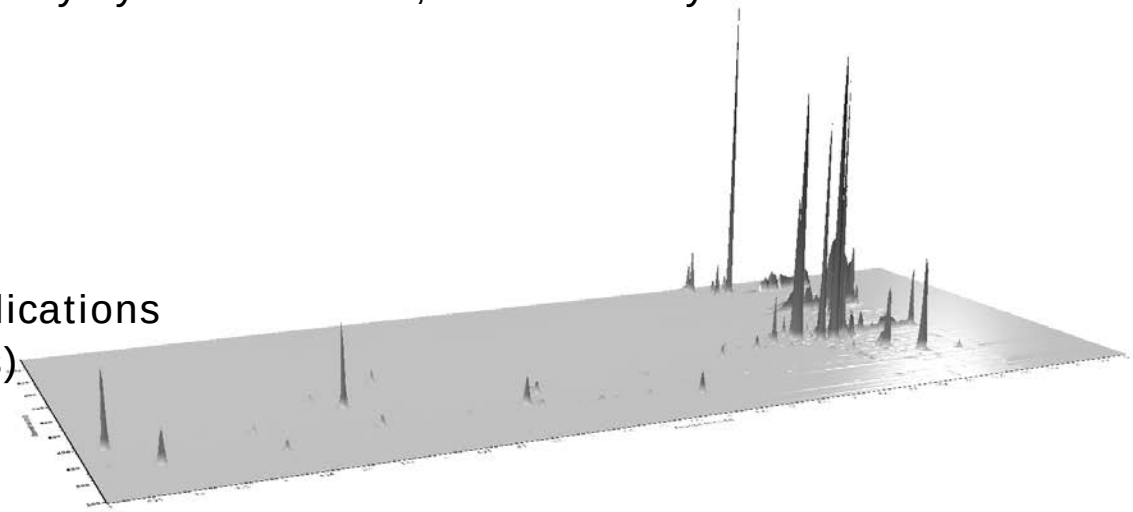
- **Advantages**
  - Ability to post mine/process data for additional information
  - Instrument specificity
    - Mass Resolution (Instrument Resolution) race is still ongoing
  - Data mining specificity/selectivity
    - Ambiguity of close masses *(ie: +14 = +CH2 or +O-2H)*
    - Tolerance window for XICs can be adjusted
    - Different charge states can be accessed
    - Isotopes can be accessed
    - Multiple fragment ions can be accessed in MSMS, MS^E (MS ALL), DDA data types
  - Seeing potential interference
  - Every time you add an MRM, duty cycle is reduced, full scan stays the same…

- **Considerations**
  - Complexity/size of data set
  - Sensitivity / Linearity
  - Regulatory
  - Patient consent – Clinical Applications
  - Retraining Labs (Departments)



Shootout

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- Compare head to head using same samples
  - Xevo G2-S versus Xevo TQ-S
- Where do we stand on raw performance factors?
  - Sensitivity
  - Linearity / Dynamic Range
  - Selectivity
  - Robustness/Reproducibilty
  - Flexibility?
- Discovery vs Regulated Bioanalysis?

We expect each platform to win some, lose some
BUT are we closer than we expect?
(and where are the gaps?)

Discovery Bioanalysis

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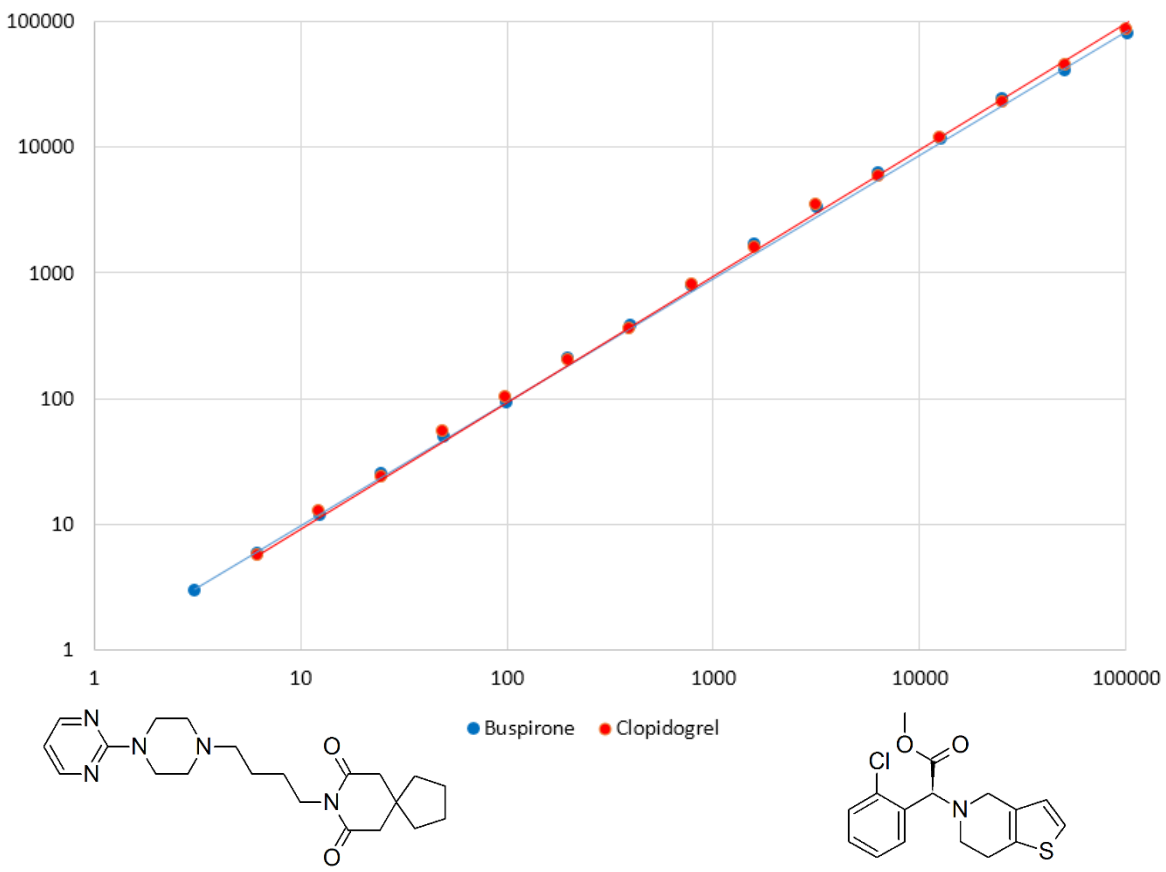
- Xevo G2-S Qtof / Acquity I-Class
- Buspirone and Clopidogrel
- Human Plasma Matrix
- No IS correction
- Linear, $1/x^2$ fit
- Log-Log Plot (base 10)



Discovery Bioanalysis

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- Xevo G2-S Qtof / Acquity I-Class
- No IS correction, linear
- $1/x^2$ fitting
- Human Plasma Matrix
- 3-100,000 pg/mL
- 4.5 order of dynamic range tested

No.	Standard conc. (pg/mL)	Buspirone observed conc (pg/mL)	Buspirone %deviation	Clopidogrel observed conc. (pg/mL)	Clopidogrel %deviation
1	3.05	3.05	-0.140	na	na
2	6.10	6.00	-1.67	5.79	-5.11
3	12.2	12.1	-0.961	13.0	6.52
4	24.4	26.0	6.42	24.1	-1.15
5	48.8	50.8	4.12	55.5	13.8
6	97.7	95.9	-1.82	103	5.61
7	195	216	10.6	203	4.00
8	391	392	0.462	360	-7.88
9	781	808	3.43	819	4.87
10	1560	1740	11.5	1630	4.51
11	3130	3370	7.86	3520	12.7
12	6250	6370	1.86	6000	-3.96
13	12,500	11,900	-5.12	12,000	-4.01
14	25,000	24,500	-2.08	23,100	-7.67
15	50,000	41,800	-16.3	45,300	-9.41
16	100,000	81,900	-18.1	87,200	-12.8

Between 15 and 20% → *Buspirone more sensitive and showing signs of saturation on the top end*

Discovery Bioanalysis - Summary

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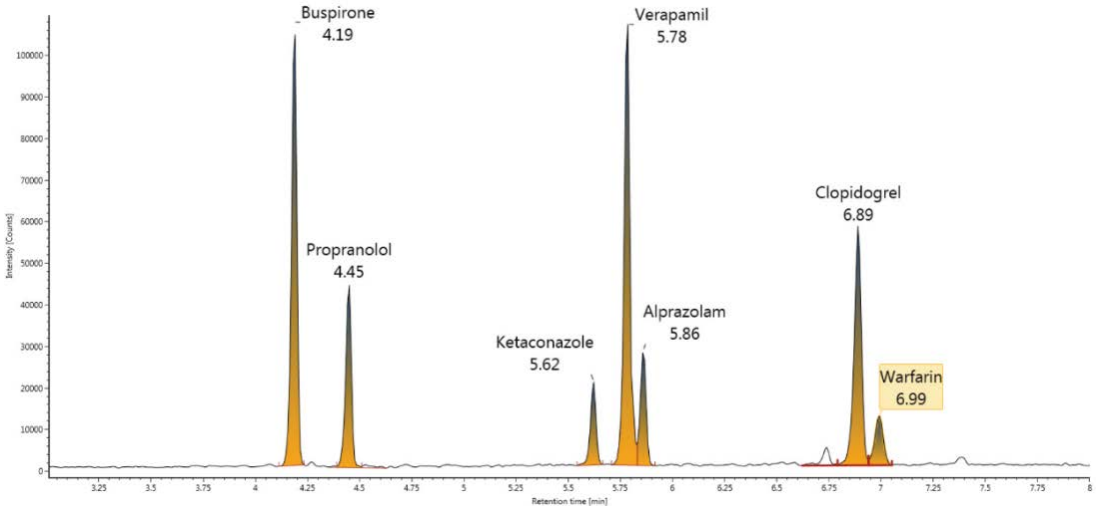
Xevo G2-S QTof in Fullscan vs Xevo TQS MRM S/N Comparison

	m/z	Mass resolution†	Peak width (s)*	Linear range (pg/mL)	Linear dynamic range (Log)	$R^{2‡}$	LOD pg/mL	S/N at LOD	TQS S/N at Tof LOD	TQS/Tof S/N ratio
Propranolol	260.1651	29330	1.5	6 to 25,000	3.6	0.994	3	4.6	9.5	2
Alprazolam	309.0908	39025	1.5	12 to 100,000	3.9	0.992	12	3.6	5.7	2
Warfarin	309.1128	26419	2.1	24 to 25,000	3.0	0.993	24	2.6	12	5
Clopidogrel	322.0669	32368	2.0	6 to 100,000	4.2	0.992	6	6.7	13	2
Buspirone	386.2557	40235	1.5	3 to 100,000	4.5	0.998	3	3.1	21	7
Verapamil	455.2911	39249	1.4	6 to 50,000	3.9	0.994	6	2.9	na	na
Ketoconazole	531.1567	36758	1.3	24 to 100,000	3.6	0.995	24	3.9	8	2



Precision of Tof Verapamil/Buspirone IS, 96 injections

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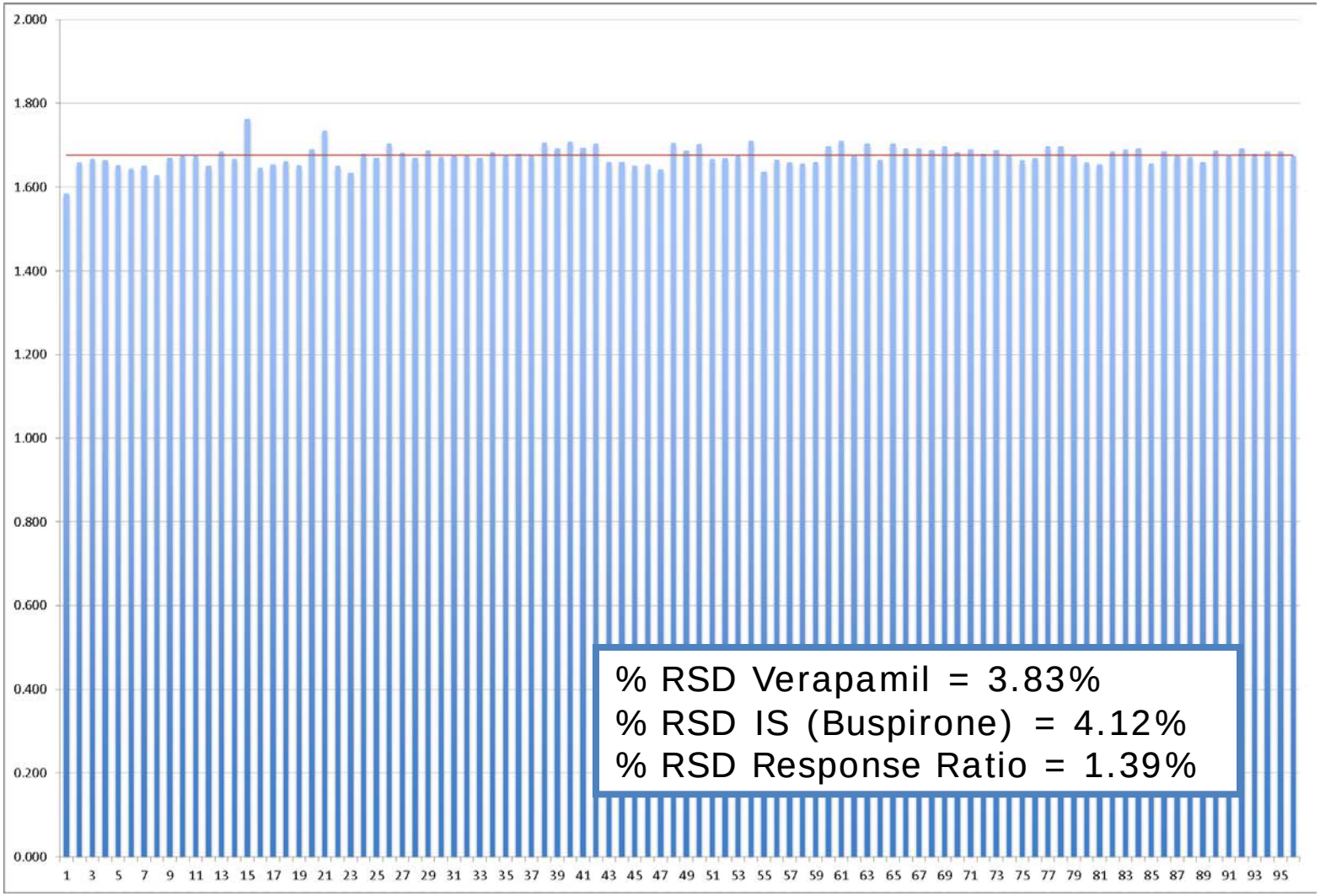

Validating Studies

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Next level (AND MOST CONTENTIOUS ONE) is moving into applications dominated by Triple Quadrupoles

Proceed with caution

BUT REMEMBER THIS IS ALSO WHERE TRIPLE QUADRUPOLE TECHNOLOGY ONCE WAS

Validating Studies
Reserpine in Human Plasma

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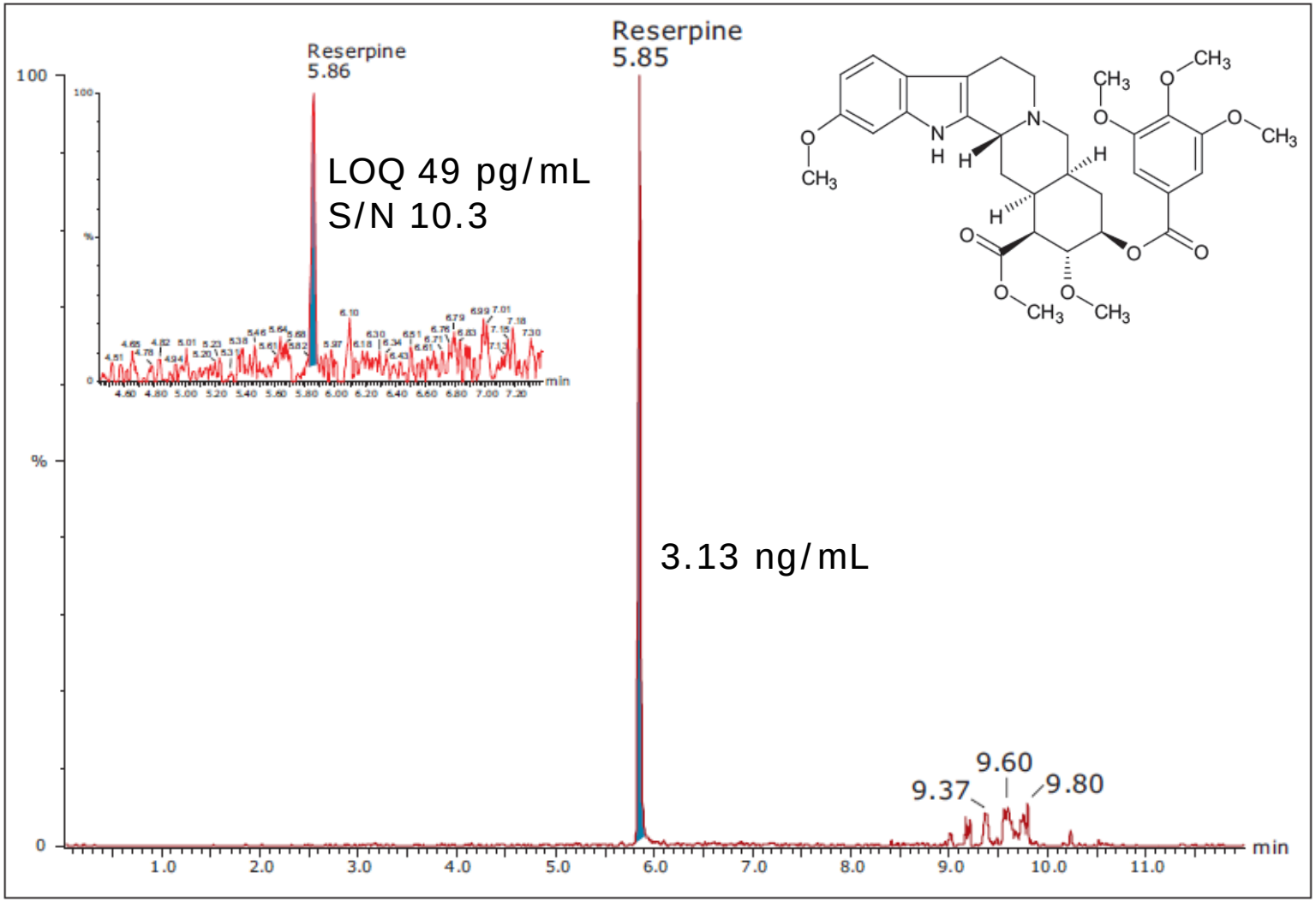

Xevo G2-S Qtof / Acquity I-Class

Validation - 3 Day Study
Resperpine in Human Plasma

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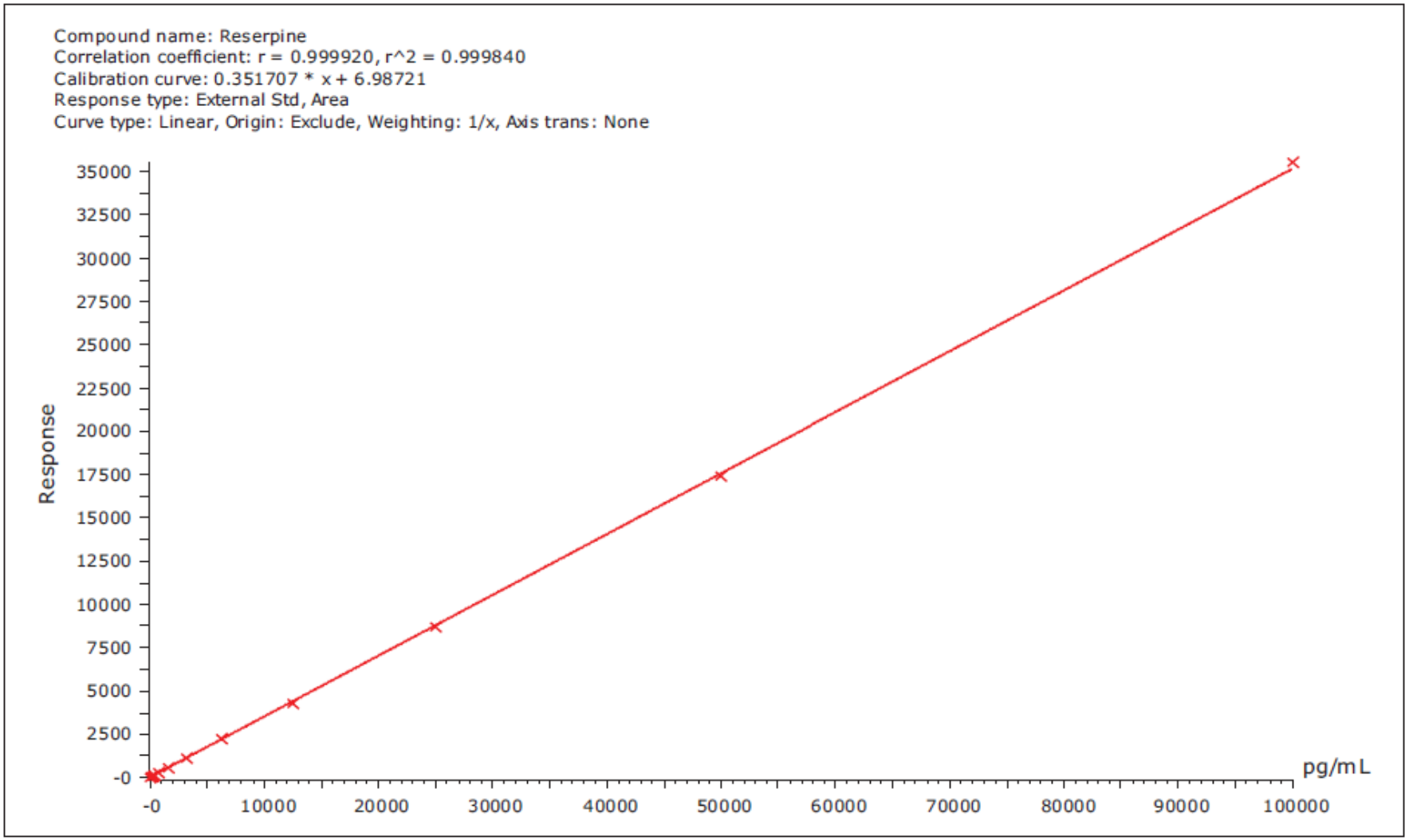

Xevo G2-S Qtof / Acquity I-Class

3 Day Study, Reported Conc / Biases Reserpine in Plasma

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Standard	Act Conc ng/mL	Day 1 Conc ng/mL	Day 1 %Bias	Day 2 Conc ng/mL	Day 2 %Bias	Day 3 Conc ng/mL	Day 3 %Bias
1	0.049	0.048	-1.2	0.042	-13.2	0.052	6.0
2	0.098	0.096	-1.6	0.104	6.7	0.098	0.2
3	0.20	0.20	0.6	0.19	-0.6	0.21	5.4
4	0.39	0.40	2.6	0.42	6.5	0.40	3.4
5	0.78	0.79	1.6	0.81	3.0	0.75	-3.7
6	1.56	1.59	1.6	1.56	-0.1	1.58	1.4
7	3.13	3.11	-0.4	3.12	-0.2	3.12	-0.2
8	6.25	6.31	0.9	6.24	-0.1	6.13	-1.9
9	12.5	12.1	-2.9	12.0	-3.6	11.5	-8.0
10	25.0	24.7	-1.4	25.0	1.1	24.0	-4.2
11	50.0	49.6	-0.8	50.5	1.0	49.5	-1.1
12	100.0	101.0	1.0	99.6	-0.4	102.7	2.7

Xevo G2-S Qtof / Acquity I-Class

Interday precision / accuracy Reserpine in Plasma

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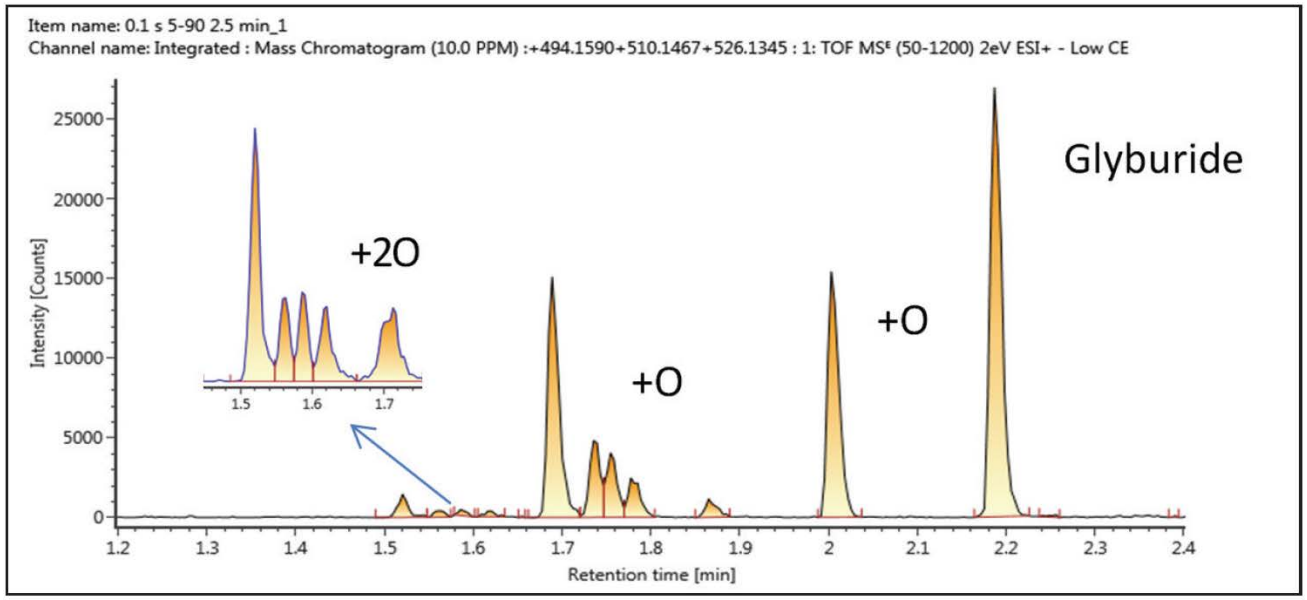
3 Day Study, Reported Conc/Biases, Reserpine in Plasma

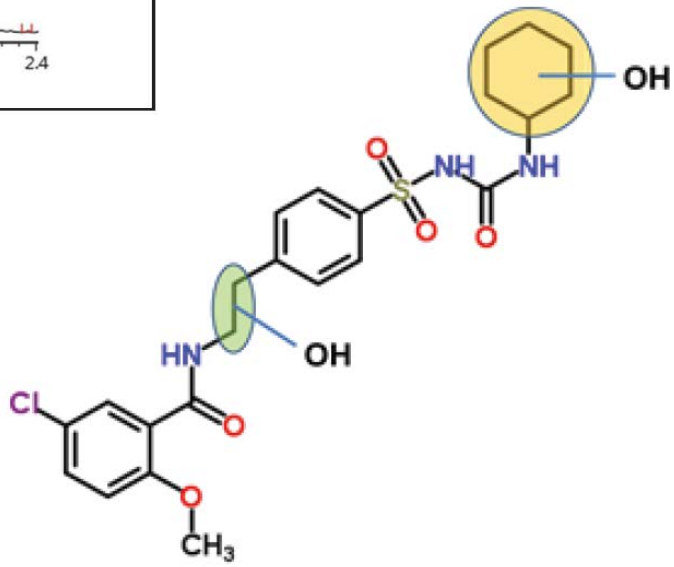
	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9	Std 10	Std 11	Std 12
	0.049 ng/mL	0.098 ng/mL	0.20 ng/mL	0.39 ng/mL	0.78 ng/mL	1.56 ng/mL	3.13 ng/mL	6.25 ng/mL	12.5 ng/mL	25 ng/mL	50 ng/mL	100 ng/mL
Day 1	0.048	0.096	0.20	0.40	0.79	1.59	3.11	6.31	12.1	24.7	49.6	101.0
Day 2	0.042	0.104	0.19	0.42	0.81	1.56	3.12	6.24	12.0	25.3	50.5	99.6
Day 3	0.052	0.098	0.21	0.40	0.75	1.58	3.12	6.13	11.5	24.0	49.5	102.7
Mean	0.047	0.099	0.20	0.41	0.78	1.58	3.12	6.23	11.9	24.6	49.9	101.1
St Dev	0.005	0.004	0.006	0.008	0.028	0.015	0.003	0.089	0.343	0.652	0.582	1.53
%CV	9.9	4.28	3.13	1.94	3.53	0.92	0.09	1.42	2.89	2.65	1.17	1.51
%Bias	-2.8	1.74	1.83	4.16	0.28	0.97	-0.27	-0.36	-4.86	-1.5	-0.29	1.12

Xevo G2-S Qtof / Acquity I-Class

Qualitative Analysis



- Putting all of the metabolism in perspective
- Through relative quan and/or quantitation with authentic standards
- HRMS/HRAM is what opens up this possibility in the most generic way possible



Tof Scan Speed Characteristics/Strengths

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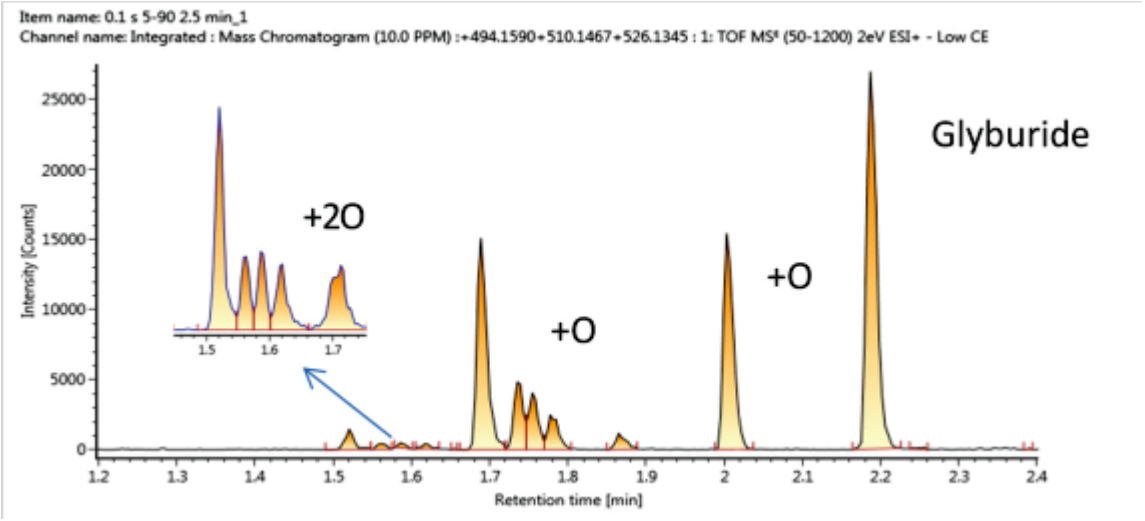
- The Tof itself actually runs at a *very* high scan (in the 10,000's of Hz)

- A single tof scan (ie: 10 Hz or 0.1 seconds/scan) is actually already a composite of 1000's of scans. The platform (maximum) scan rate is chosen primarily to balance good ion statistics and a reasonable sampling of the chromatography

- For a 1 second wide peak (pretty sharp) >30 Hz provides ~30 points across the peak



Scan Speed Considerations

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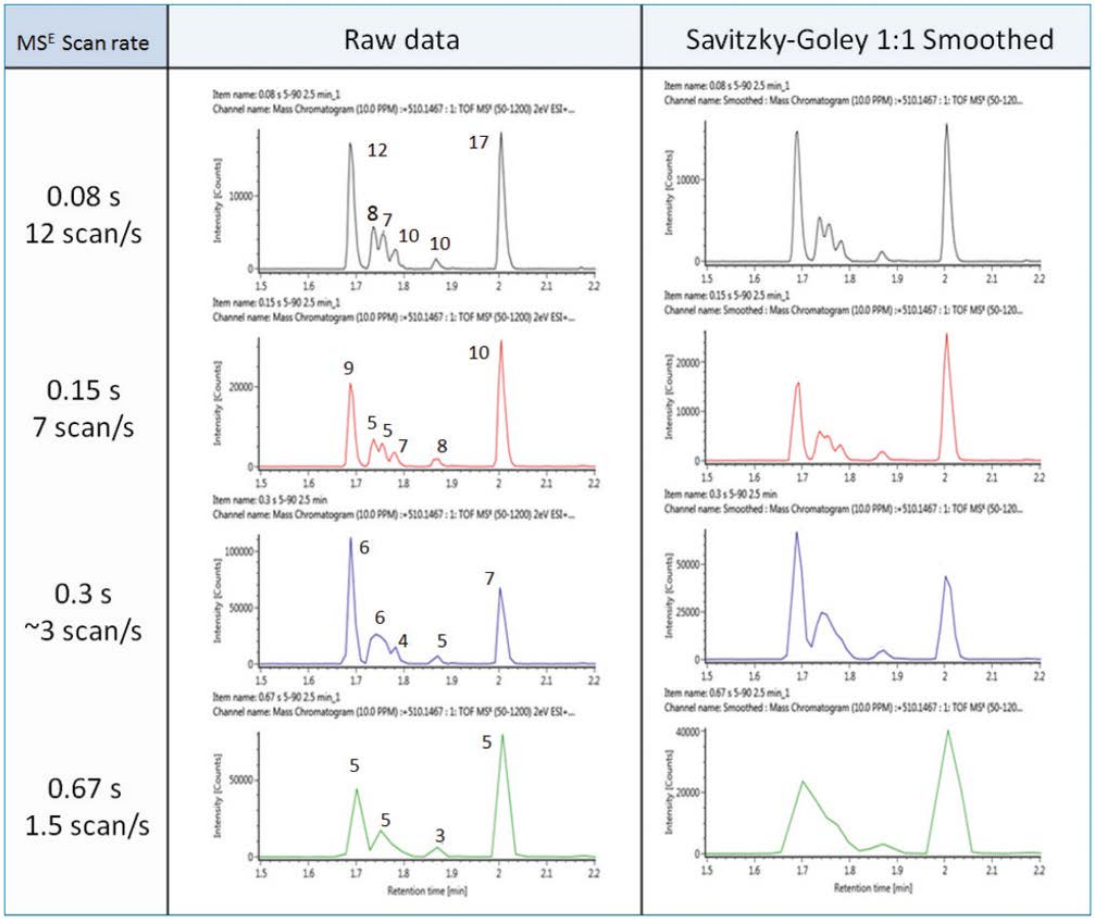

- UPLC Resolution of these peaks is ~ 0.9 s at half height and ~ 2.7 s at base
- This is shown using MS^E data mode (simultaneous acquisition of time-aligned fragment ions)
- Averages of points across the peak are 11, 7, 6 and 4 respectively

Tof Resolution – Characteristics/Strengths

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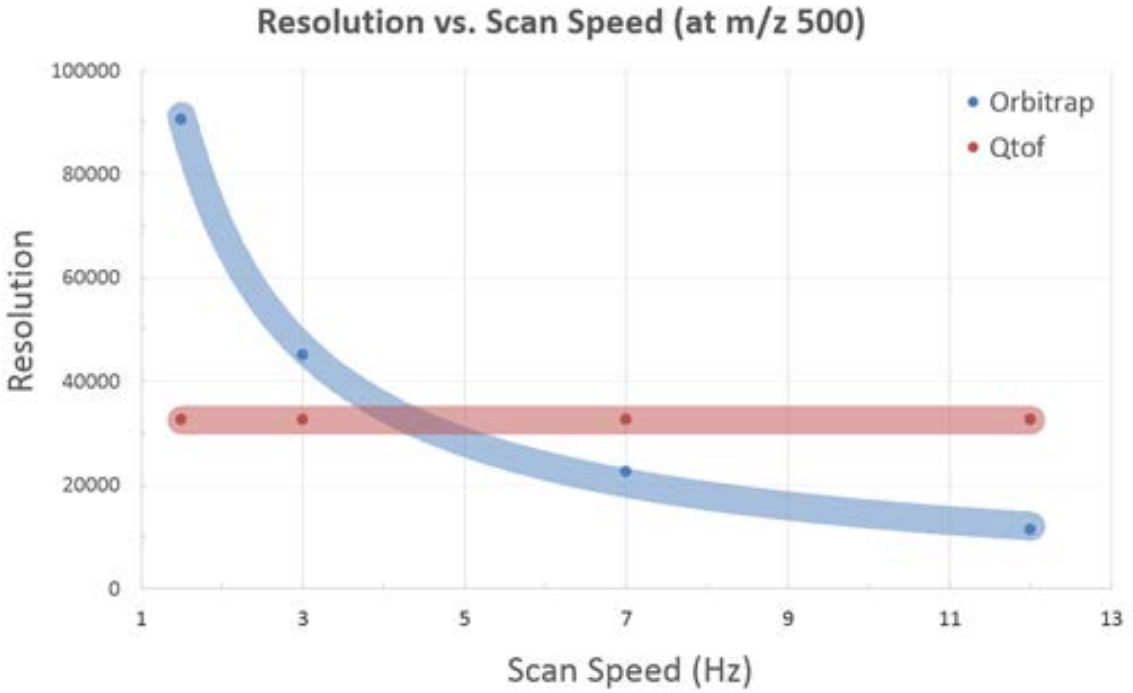
- Tof Resolution is relatively constant across the ENTIRE mass range



- Important to note that resolution reported at a low mass is also the same at mass 1000 or higher

Practical Consequences of Scan Rate on Tof Spectral Quality

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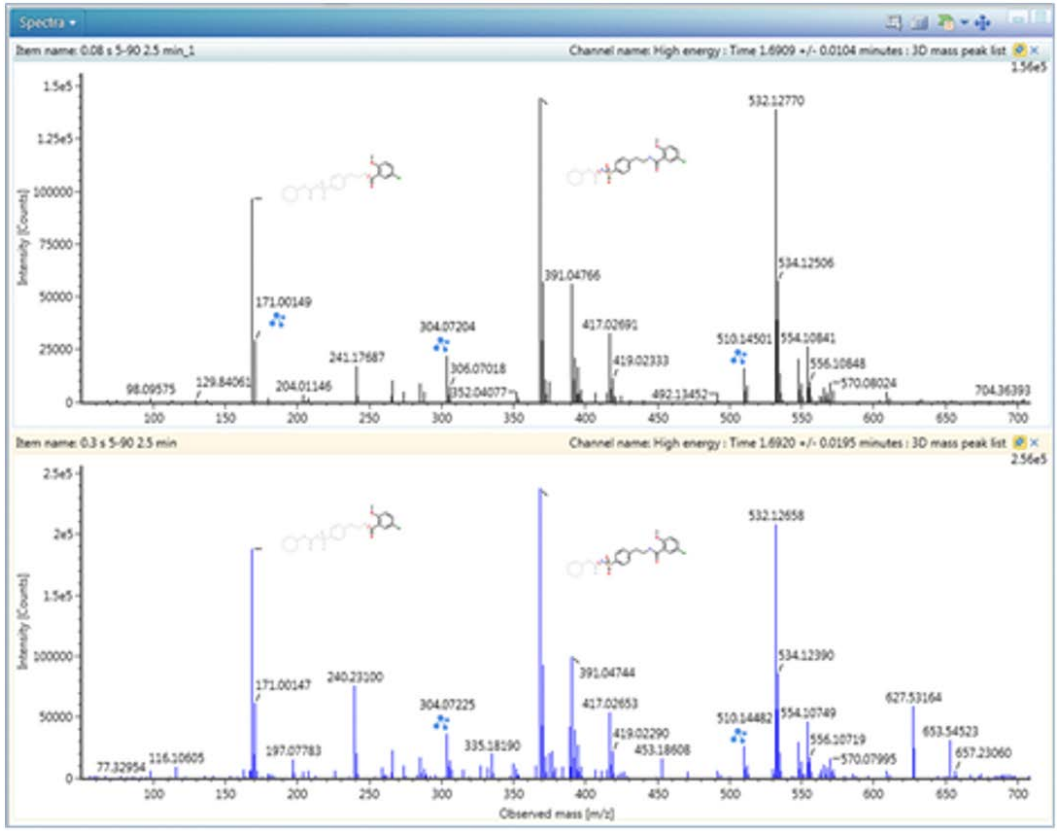

- The underlying Tof Data (scanning at > 10,000 Hz) is the same, so combining data from faster scan times is indistinguishable from slower scan rates.
- The underlying Tof resolution is unchanged at varying scan rates is the same, so resolution is ALSO the same for both spectra across all masses

Conclusions

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- Sensitivity
  - Fullscan is closer than you think
- Linearity / Dynamic Range
  - Fit for purpose
- Selectivity
  - Many options to improve selectivity
  - Acquisition and data processing based
- Robustness/Precision
  - Fit for purpose
- Flexibility
  - Scan types, Resolutions, Scan Rates

Next Steps

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- More data points! More labs….
  - Triple quadrupoles have a big head start

- Moving/testing the right assays into this space (first,
  - Discovery Applications - Rapid Screening/Stability
  - Peptides / Biomolecule assays
  - Small molecule assays that struggle with sensitivity on TQs and/or have poor fragmentation

- Many labs collecting this type of data now
  - beginning to generate confidence in these assays

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