Moving Forward on Quan-Qual,
Perspectives on Using Tofs for Bioanalytical Work

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Understanding Role of HRMS/HRAM Technologies

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 \((ie: +14 = +CH2 \text{ or } +O-2H)\)
  - 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

- 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/Reproducibility
  - 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?)*
- 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

- **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

#### Table: Buspirone and Clopidogrel Concentrations

<table>
<thead>
<tr>
<th>No.</th>
<th>Standard conc. (pg/mL)</th>
<th>Buspirone observed conc (pg/mL)</th>
<th>Buspirone %deviation</th>
<th>Clopidogrel observed conc. (pg/mL)</th>
<th>Clopidogrel %deviation</th>
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**Between 15 and 20%**

*Buspirone more sensitive and showing signs of saturation on the top end*
## Discovery Bioanalysis - Summary

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

<table>
<thead>
<tr>
<th></th>
<th>m/z</th>
<th>Mass resolution†</th>
<th>Peak width (s)*</th>
<th>Linear range (pg/mL)</th>
<th>Linear dynamic range (Log)</th>
<th>R²‡</th>
<th>LOD pg/mL</th>
<th>S/N at LOD</th>
<th>TQS S/N at Tof LOD</th>
<th>TQS/Tof S/N ratio</th>
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![Graph showing retention time vs intensity for various compounds](image-url)
Precision of Tof
Verapamil/Buspirone IS, 96 injections

% RSD Verapamil = 3.83%
% RSD IS (Buspirone) = 4.12%
% RSD Response Ratio = 1.39%
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

LOQ 49 pg/mL
S/N 10.3

3.13 ng/mL

Xevo G2-S Qtof / Acquity I-Class
Validation - 3 Day Study
Reserpine in Human Plasma

Compound name: Reserpine
Correlation coefficient: $r = 0.999920$, $r^2 = 0.999840$
Calibration curve: $0.351707 \times x + 6.98721$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

Xevo G2-S Qtof / Acquity I-Class
# 3 Day Study, Reported Conc/Biases
*Reserpine in Plasma*

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<tr>
<th>Standard</th>
<th>Act Conc ng/mL</th>
<th>Day 1 Conc ng/mL</th>
<th>Day 1 %Bias</th>
<th>Day 2 Conc ng/mL</th>
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**Xevo G2-S Qtof / Acquity I-Class**
Interday precision/accuracy
Reserpine in Plasma

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

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<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
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<td>Day 1</td>
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<td>0.39</td>
<td>0.78</td>
<td>1.56</td>
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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**

- 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

- UPLC Resolution of these peaks is ~ 0.9 s at half height and ~ 2.7 s at base

- This is shown using MSE 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

- 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
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

- 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

- 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
Acknowledgements

- Yun Alelyunas
- Craig Dorschel
- Jennifer Simeone
- Paul Rainville
- Kevin Cook
- Stephen McDonald
- Russell Mortishire-Smith
- Alan Millar