Towards High Resolution MS in Regulated Bioanalysis

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MSD

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Outline

• Quads vs HRMS
• HRMS: Quantitative applicability
• Quan – Qual workflows
• Future considerations
Triple Quadrupole Mass Spectrometry

- Selective
  - Based on two stages of mass selection, parent m/z and fragment ion m/z
- Sensitive
  - Beam instrument with high duty cycle
- Robust
  - Calibration stable over long period
- Compact Data Sets
  - MRM data files are very small and easy to process
- 25+ years of constant development for quantitative applications
High Resolution Mass Spectrometry

- Selective?
  - Based on resolving power
- Sensitive?
  - Pulsed (TOF) or Indirect Detection (FT traps) based instruments
- Robust?
  - Calibration stable over long period (using internal calibration protocols)
- Large Data Sets
  - Full scan data files are large and time consuming to process
- ~3-5 years of recent development for quantitative applications
Selectivity: SRM Based Quantitation

Haloperidol
1 nM in plasma
Why Mass Accuracy and Stability Matters: Narrow Window XIC from Rat Plasma t=15 min
Full Scan Based Quantitation

Standard, 6 compound Mix, 0.001

24feb2010_PKTest_010

1: TOF MS ES+
376.146 0.00Da
1.01e3

Haloperidol
1 nM in plasma
XIC 3 mDa window
Synapt G2

Standard, 6 compound Mix, 0.001

24feb2010_PKTest_010 149 (1.416)

1: TOF MS ES+
371.1472
4.56e4

Haloperidol
1 nM in plasma
XIC 3 mDa window
Synapt G2
Plasma is a Dirty Matrix

Standard, 6 compound Mix, 0.001
24feb2010_PKTest_010 150 (1.425)

1: TOF MS ES+
3.99e4

-1.9 ppm
Why Resolution Matters:
Rat Plasma t=15 min

Experimental Data

At a resolution <20,000 FWHM the compound will be over quantified due to un-resolved contaminants.

Both improved mass resolution and chromatographic resolution are needed for accurate quantification.
Outline

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Challenges in Analytical Workflows

1. Perform Quantitative analysis. 
2. Increase Method Selectivity. 
3. Reduce Method development time. 
4. Increase laboratory throughput.

Resolving Challenges in Regulated Bioanalysis

**Single Reaction Monitoring**

(New Generation Triple Quads)

**High Resolution Mass Spectrometer**

(New Generation of High Resolution detector)
Challenges in Analytical Workflows

1. Quantitative analysis:
   - Able to reach level of sensitivity given by the SRM.
   - Good Linearity.

2. Method selectivity:
   - Can resolve chromatographic interference.
   - Decrease baseline noise (↑ signal to noise).

3. Method development:
   - Reduce method development working days.
   - Methods (extraction and chromatographic) simplified.

4. Laboratory throughput:
   - Decrease total injection runtime.
   - Decrease total extraction time.
Small Molecule - MK-A

- Clinical method- monoisotope 821, Human plasma, Isocratic, 2x50 mm UPLC, L/L cleanup

<table>
<thead>
<tr>
<th>Expected Conc. (ng/mL)</th>
<th>No. Of Values Used</th>
<th>API 4000</th>
<th>API 5000</th>
<th>AB 5500</th>
<th>AB 5600&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AB 5600&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>% C.V.</td>
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<td>% C.V.</td>
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<td>96</td>
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<td>103</td>
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<td>1.4</td>
<td>101</td>
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<td>0.7</td>
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<td>Range of Values</td>
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<td>1.3-6.0</td>
<td>96-103</td>
<td>0.7-8.7</td>
<td>97-102</td>
<td>0.6-4.2</td>
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</table>

<sup>a</sup> - AB 5600 TOF quant with 40 mDa width
<sup>b</sup> - AB 5600 MRM quant with 40 mDa width
### Small Molecule - MK-A

#### Sensitivity of Detection:

<table>
<thead>
<tr>
<th>MS</th>
<th>Vol (uL) Injection</th>
<th>Intensity at LLOQ</th>
<th>Average Noise</th>
<th>S/N</th>
<th>Intensity at ULOQ</th>
<th>Range of IS Response</th>
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<tr>
<td>API 4000</td>
<td>8</td>
<td>600</td>
<td>20</td>
<td>30</td>
<td>8.0 x 10^5</td>
<td>3.8x10^4 - 4.8x10^4</td>
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<td>API 5000</td>
<td>2</td>
<td>850</td>
<td>30</td>
<td>28</td>
<td>7.5 x 10^5</td>
<td>7.5x10^4 - 1.2x10^5</td>
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<tr>
<td>AB 5500</td>
<td>2</td>
<td>1250</td>
<td>50</td>
<td>25</td>
<td>2.3 x 10^5</td>
<td>1.3x10^5 - 1.6x10^5</td>
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<td>AB 5600^a</td>
<td>8</td>
<td>450</td>
<td>100</td>
<td>5</td>
<td>8.0 x 10^5</td>
<td>5.0x10^4 - 7.0x10^4</td>
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<tr>
<td>AB 5600^b</td>
<td>8</td>
<td>42</td>
<td>4</td>
<td>11</td>
<td>7.0 x 10^3</td>
<td>2.0x10^4 - 3.0x10^4</td>
</tr>
</tbody>
</table>

^a - AB 5600 TOF quant with 40 mdalton width

^b - AB 5600 MRM quant with 40 mdalton width
Progesterone Quantitation (2.5 nM)  
API 4000 vs Xevo G1
Outline

- Quads vs HRMS
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Correlation of Data (30 cpds) from Qtof and QqQ

± 20% range

% Parent remaining (data from Xevo)

% Parent remaining (data from API)
Guiding LO

Extensive O-demethylation dramatically increased clearance

Chemistry optimisation

Extension side-chain minimizes O-demethylation
Guiding LO

Monitoring undesired dealkylation

![Graph showing the concentration over time for ER-ant and ER-ago](image-url)
In vivo Metabolites (4 hr rat plasma time point)

Parent

M1

M2

M3

M4
Metabolite Profiles in Plasma

![Graph showing metabolite profiles in plasma over time with peak area ratios for L-873,724, M1, M2, M3, and M4.](image-url)
Multiplexed Analysis

Drug Measurements

Biomarker Measurements

Metabolite Identification
Outline

- Quads vs HRMS
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Future Drug Development Workflows

- When compared to a SRM, will HRMS instruments:
  - Resolve chromatographic problems?
    - Can have improved selectivity with HRMS, but will not fix "ionization" issues
  - Perform bioanalytical assays?
    - Yes
  - Simplify method development time?
    - Yes
  - Be robust and reliable?
    - Yes
  - Generate manageable data files?
    - ?, yet to be done – significant hurdle
  - Enable data processing throughput?
    - ?, need suitable software
Future Challenges

• HRMS hardware is ready for routine assay support
  – Sensitivity, Linearity, Selectivity are acceptable and improving

• Software continues to lag hardware
  – How to handle large datasets?
  – Validate data processing steps?
  – Centroid vs. Profile data?
  – Data reduction acceptable or not?

• Cost vs. Benefit of ownership
  – QQQ’s are a commodity with large user base
  – HRMS are more complex with fewer users
  – Future developments in hardware and software will narrow the gap between QQQ and HRMS
Ion Mobility with HRMS

- Waters G2-S using Ion Mobility
  - Additional level of selectivity beyond mass resolving power by using ion mobility
  - Early data looks promising, but much more work is needed
  - Data file size and data processing software will need to be addressed
Tamoxifen
20mDa XIC, MS Only, IMS OFF

0.25 ng/mL

0.5 ng/mL

1 ng/mL
Tamoxifen 20mDa XIC, IMS full scan DT 1-200 (no filter)

0.25 ng/mL

0.5 ng/mL

1 ng/mL
Tamoxifen
20mDa XIC + 92-102 DT filter

0.25 ng/mL

0.5 ng/mL

1 ng/mL
What if…

• HRMS was developed instead of QQQ technology?
  – Full scan based data acquisition with sensitivity and selectivity equal to today’s modern QQQ with fast data processing used routinely in today’s bioanalytical labs.

• What would the discussion be like if QQQ’s just came along now?
  – “I have to figure out how the compound fragments to analyze it?”
  – “I need a different MS method for every compound?”
  – “I have to know what I am looking for before I run my samples?”
  – “Nominal mass? Really? You must be joking?”
  – “What about all the other things in my samples?”
THIS IS TAKING TOO LONG - WE'LL HAVE TO INDUCE HATCHING.