Informatics and High Resolution QTof MS

How can we ask better questions and get better answers in DMPK?

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Overview

- **HRMS in DMPK**
  - Where are we today?

- **Quantitation using HRMS**
  - Bridging Qualitative Information and Quantitative Information

- **Ion mobility**
  - Extra dimension of information and selectivity

- **Informatics**
  - How do we track information and store information for DMPK?
  - Bridging this information across disciplines and institutions
  - How do we move from (lot’s of) information to knowledge?
Challenges in DMPK/PDM

in vivo (blood, tissues, urine, bile, etc)
in vitro (microsomes, hepatocytes, S9)

Rat  
Dog  
Monkey  
Human

Keeping track of information and putting all of this into perspective

Characterized Relationship
Predicted/Modeled
Instrument Needs

- **High throughput**
  - Medium/High sensitivity assays
  - Simple validation required

- **Moderate throughput**
  - Highest sensitivity assay
  - Full validation required

- **High throughput**
  - Medium/High sensitivity assay
  - Full validation required

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**Pre-clinical testing R&D**

- **Lead Optimization**
- **Candidate Selection & Confirmation**
- **Phase 1**
- **Phase 2**
- **Phase 3**

**Submit IND**

**Submit NDA**

**QTof**

**Quad**

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Changing Dynamic of Science and Industry

Academia

Contract Research Organizations

Biotech Pharma

Traditional Pharma

Hardware / Software Companies

How do we (better) build and transfer scientific knowledge across these groups?
Quantitation with Accurate Mass?

- ASMS 2012
  - 2 Key Oral Sessions on developments in Quan and Quan/Qual this year

- Both sessions dominated by high-resolution mass spectrometry based workflows
Why the desire for HRMS to expand its utility?

- Many labs have made huge investments in HRMS
  - desire to get maximum impact out of the platform

- Critical data needed early. BEFORE we initiate costly:
  - Radiolabel studies
  - Authentic standard synthesis
  - Initiating larger preclinical and clinical studies

- Quantitative information drives decision making and allows us to test scientific hypotheses and questions

Does routine quantitative and quantitative data collection together make a difference?

Does it make sense?
2 Scenarios

- If you’re already doing qualitative work **ABSOLUTELY**, helps put this data in context across datasets. As we begin to understand and put in place better data integration systems, this will only provide benefits.

- If you’re (thinking of) shifting traditional quantitative work into this space, the benefit of doing so must outweigh the negatives
  - Sensitivity/Specificity
  - Accuracy
  - Reproducibility
  - Datasize Processing Time

Must be “fit-for-purpose”
4 Rats Dosed 75mg/kg
Plasma collected at t = 0 (predose), 1, 1.5, 2, 4, 6, 24hr

Pool Time Points,
Protein Precipitate with Acetonitrile and dilute 1:1 w/ Water

Inject on Xevo G2 Systems equipped with Acquity UPLCs
Standard Curves – QqQ vs QTof
1ng/mL-10000ng/mL

R² = 0.9986
R² = 0.9973

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Standard Curve – No IS Correction
1ng/mL – 10000ng/mL

Propranolol in Plasma Raw Area Counts vs Concentration
QToF

Propranolol in Plasma Raw Area Counts vs Concentration QqQ

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Standard Curve – No IS Correction
Zoomed in on Dynamic Range

Propranolol in Plasma Raw Area Counts vs Concentration QToF

Propranolol in Plasma Raw Area Counts vs Concentration QqQ

R² = 0.9901

R² = 0.9929
PK Data - QqQ vs QTof

- Laval QqQ
- Laval QTof

Measured Concentration vs Time

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Sensitivity – Historical Perspective

QToF Sensitivity Improvements

Relative Transmission (Output Current)

Date

October 1995
July 1998
April 2001
January 2004
October 2006
July 2009

QToF 1
QToF 2
QToF Ultima
Synapt G1
Synapt G2
Xevo G2 QTof

QToF Premier

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How to address issues with larger sampling orifice

- Maximise signal
- Eliminate Noise

Diffuse Ion Cloud
Ion Path Modelling
SIMION

1 mbar N$_2$

With Electric Field (25 V between guides)
Specificity Considerations

- Does the extra specificity increase detection limits in samples (in particular with high matrix burden?)

- Do any the components of interest not separate easily on the basis of rt or m/z?
  - Many compounds we want to discriminate in fact are isobaric
    - Biological isomers
    - Drugable compounds – stereoisomers, structural isomers
  - What can I do about this?

- How can I get more information on structure?
  - Accurate mass yields only part of the picture
What is ion mobility?

- The separation of compounds by size, shape, charge and mass

What does this bring to a DMPK analysis?
Raw Bile Data
m/z versus RT
Raw Bile Data
m/z versus Drifttime

Doubly Charged Species
And less compact molecules

Singly Charged Species

Different Classes of related molecules tend to have similar structural properties
- Xenobiotics
- Bile Salts
- Lipids
- Endogenous Molecule Classes
Unique Applications of IMS

Sites of Protonation
understanding matrix effects on product ion/MRM ratios

Collisional Cross Section
Of Metabolites
Correlating Drifttime to theoretical cross section of isomeric metabolites

Collisional Cross Section
Of Metabolite Product Ions
Correlating Drifttime to theoretical cross section to key product ions
Challenges in DMPK/PDM

in vivo (blood, tissues, urine, bile, etc)

Can we do better than cutting and pasting into Excel?

in vitro (microsomes, hepatocytes, etc) -> Characterized Relationship -> Predicted/Modeled

Rat
Dog
Monkey
Human
Software, Putting it all together Intelligently

Cleavages unique to molecule itself

Potential Biotransformations

+O  +glucuronide
+H₂O  +sulfate
+O₂  +glutathione
+.  +.
+.  +.

Data (Fragmentation Spectra)

Need to bring all this knowledge together in a meaningful way and have a clear way of visualizing this to the user
The need for Integrated Software

- A single software processing model to enable integrated datasets
- A platform built incorporating the quantitative tools already built for regulated bioanalysis
Components can span multiple channels of data
The software organizes the data across all channels into components
Peaks are detected and information organized as components.
The components can now be analyzed.

<table>
<thead>
<tr>
<th>ID</th>
<th>Mass</th>
<th>RT</th>
<th>Area</th>
<th>Isotopes</th>
<th>Fragments</th>
<th>Adducts</th>
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<tbody>
<tr>
<td>1</td>
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<td>1.53</td>
<td>1220</td>
<td>2</td>
<td>4</td>
<td>H⁺</td>
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<td>3029</td>
<td>3</td>
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<td>...</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data can be sorted, filtered, pivoted, plotted
AND SEARCHED
By sample, by time, by species, historically, etc.
New ways of looking at data

Software needs to be built from the ground up to handle
Quantitative information
(Quantitation can not be a special case)
Interaction with data needs to be flexible
Quantitative and Qualitative Information displayed in a table OR visually
Visulizing Metabolic Hotspots
### Filter/Sort/Interrogate Structural Data using Tables

#### Component Summary

<table>
<thead>
<tr>
<th>Component name</th>
<th>Formula</th>
<th>m/z (Da)</th>
<th>Passed Mass Defect Filter</th>
<th>Passed Halogen Match Filter</th>
<th>Passed Fragment Search Filter</th>
<th>Mass error (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nefazodone</td>
<td>C25H32CIN5O2</td>
<td>476.2315</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-0.0002</td>
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<tr>
<td>Nefazodone + Hydroxylation</td>
<td>C25H32CIN5O3</td>
<td>486.2265</td>
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<td>✓</td>
<td>✓</td>
<td>-0.0001</td>
</tr>
<tr>
<td>Nefazodone + Hydroxylation</td>
<td>C25H32CIN5O3</td>
<td>486.2267</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>0.0001</td>
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<tr>
<td>Nefazodone + 2x(Hydroxylation)</td>
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<td>C15H21N3O3</td>
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</tbody>
</table>

#### Diagram

![Chemical structure of Nefazodone](image1.png)

- **View:** Filters and Limits
- **Columns:**
  - Mass error (ppm)
  - Isotope Match Intensity RMS Percent
  - Isotope Match Mz RMS PPM
  - Identified High Energy Fragments
  - Detector counts
  - Adducts

<table>
<thead>
<tr>
<th>Mass error (ppm)</th>
<th>Isotope Match Intensity RMS Percent</th>
<th>Isotope Match Mz RMS PPM</th>
<th>Identified High Energy Fragments</th>
<th>Detector counts</th>
<th>Adducts</th>
</tr>
</thead>
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<tr>
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<td>10</td>
<td>33278 +H</td>
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<tr>
<td>0.00</td>
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<td>0.72</td>
<td>13</td>
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<tr>
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<td>0.57</td>
<td>1</td>
<td>1760 +H, +Na</td>
</tr>
<tr>
<td>0.12</td>
<td>1.28</td>
<td>2.97</td>
<td>1.19</td>
<td>4</td>
<td>1651 +H</td>
</tr>
</tbody>
</table>

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DMPK/PDM Informatics Challenges

- Drug Metabolism and Pharmacokinetics (DMPK) scientists are continually looking ways to adapt technology and software to bring safer, more efficacious treatments to patients.

**Discovery DMPK**

- Innovation, productivity, weeding through lots and lots of information quickly
- *Help screen great drugs!*

**Development DMPK**

- Need to manage a complex information stream and retrieve and compile this information. Regulatory and safety driven
- *Help bring great safe and efficacious drugs to market*
How we share data is the key
Scaling Informatics

Seamless data and method sharing

Control of local and networked instrument systems

GLP/GMP and 21 CFR Part 11 compliance

Archive, backups, and redundancy

Version control and software maintenance

How does this enable us to go further scientifically?

Share data with our Colleagues and Collaborators more effectively!
The average scientist spends more time replotting/reporting/writing up data than actually analyzing it.

Reports need to be seamless with interpretation.
Scientific Needs

Pre-clinical testing R&D

High throughput
Medium/High sensitivity assays
Simple validation required

Moderate throughput
Highest sensitivity assay
Full validation required

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Full validation required

Pre-clinical testing R&D

Lead Optimization

Candidate Selection & Confirmation

Phase 1

Submit IND

Phase 2

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Hardware + Informatics + Core Scientific Expertise
Acknowledgements

- **Vertex**
  - Julie Laterreur
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QUESTIONS?