The Q Exactive – A Benchtop Orbitrap Mass Spectrometer for DMPK

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Simon Szwandt Ph.D.
Market Development Manager– Pharma & CRO
Agenda

- Triple vs HR/AM
- Instrumentation and HR/AM
- Q Exactive Bioanalytical Data – Small Molecule and Biologics
- Hepcidin Quantitation
The Exactive Family

- The Exactive Plus
  - HCD cell optional
  - Upgradeable to Q Exactive
  - Mass range to m/z 6000
  - Faster polarity switching
  - Rotated flatapole
  - New scan modes

- The Q Exactive
  - Resolving power >140,000
  - Parent ion selection capability
  - Spectral multiplexing
  - UHPLC and nanoflow capable

Superior selectivity & specificity due to HR/AM-MS and MS/MS
Why HR/AM in Bioanalysis?
Full Scan – The Most Information

**Triple Quadrupole**

**Q Exactive**

**NL:** 2.08E4
m/z = 329.58-330.58 F: FTMS + p ESI Full ms
[150.00-400.00] MS Paroxetine-FS-002

**NL:** 3.05E6
m/z = 330.14835-330.15165 F: FTMS + p ESI Full ms [150.00-400.00] MS Paroxetine-QE-FS-03
SIM – The Highest Possible Signal

Triple Quadrupole

Q Exactive
MSMS (SRM Vs. MSMS)

Triple Quadrupole

Q Exactive

[Graph showing comparison between Triple Quadrupole and Q Exactive]

[Thermo Fisher Scientific logo]
Specificity = Resolution + Mass Accuracy

Resolution: 10k, 30k, 50k, 100k

Butyl-Phthalate, 279.15909
(ubiquitous background ion)

54 ppm apart

Ethyl-Estradiol, 279.17434
This permitted a direct comparison between the LC-HRMS and LC-MS/MS data. The data indicated that the selectivity of LC-HRMS exceeds LC-MS/MS, if high resolution mass spectrometry (HRMS) data is recorded with a resolution of 50,000 full width at half maximum (FWHM) and a corresponding mass window. This conclusion was further supported by experimental data (MS/MS based trace analysis).

This is to demonstrate that Exactive and HRAM with 50K resolution is equivalent or better than QQQ.
Why HR/AM Q Exactive Large Molecule
# Small vs Large Chemical Characteristics

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B. De Silva, Bristol-Myers Squibb
Real High Resolution to Increased Selectivity

Multiplex SIM Mode: SDLAVPSELALLK

Resolution : 70 000

Resolution : 140 000

> Separation of interfering signals: improvement of LOQ (200 x)
> Increase in cycle time (~ 2 x)
Sensitivity Gain by Selected Ion Monitoring (SIM)

XIC for DIKCSNILLNNSGQIK at m/z 1007.0435(2+)

- Spectrum noise is dependent on the ratio of compound within a certain ion population
- Spectrum noise is much less in SIM mode
- Sensitivity gain 5 – 10 x with SIM mode
- The gain will be higher in more complex matrices
Alprazolam, Full Scan Experiment

Alprazolam

\[ Y = 6366.31 + 514.015 \times X \quad R^2 = 0.9967 \quad W: 1/X \]

50 ppt – 10 ppb
250 fg oc - 50 pg oc

Zoom in 50 ppt- 100ppt
Alprazolam SIM Experiment

Alprazolam

$Y = -3135.8 + 552.216 \times X$  \hspace{0.5cm} R^2 = 0.9982 \hspace{0.5cm} W: 1/X

50 ppt – 10 ppb
250 fg oc - 50 pg oc

10 ppt – 10 ppb
50 fg oc - 50 pg oc

Zoom 10 ppt - 100 ppt
Q Exactive
High Throughput HR/AM Quantitation

QQ-Orbi

[Diagram showing the process of high throughput HR/AM quantitation]
The Q Exactive: Hardware Innovations

- HCD Cell
- Orbitrap Mass Analyzer
- Enhanced FT
- C-Trap
- Quadrupole Mass Filter
- S-lens Ion Source
Benchtop Orbitraps vs QToFs

• All high-resolution, accurate mass instruments trade in three “currencies”
  • Scan speed (Hz) – also called “dwell time” which is familiar to triple quad users
  • Resolution
  • Sensitivity
Benchtop Orbitraps vs QToFs

- ToF based instruments trade **speed** for **sensitivity**
- Resolution is based on flight path so it is “fixed” – with a mass dependence
- You can scan at 100 Hz… but data compromised
- Typical recommended full scan speed – 150-250 ms
- This means ToF scan speeds are 4-10 Hz.
Benchtop Orbitraps vs QToFs

- Orbitrap based instruments trade speed and resolution
- Resolution comes from more time spent measuring the transient
- Slow down to get more resolution
- Sensitivity is “fixed” since we pre-fill the C-trap for up to the Orbitrap scan time (it doesn’t “miss” any ions)
Plecanatide Peptide Analysis – Pyxant Laboratories
Plecanatide at 35K Resolution

%CV = Variability based on Peak Area
%RSD = Variability based on Calculated Concentration

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%CV is good
%Diff is not good
%RSD is good
### Plecanatide at 70K Resolution

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<td>3.36 ng/mL</td>
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%CV = Variability based on Peak Area
%RSD = Variability based on Calculated Concentration

%CV is good
%Diff is good
%RSD is good
Plecanatide at 35K Resolution Chromatograms

Blank

1 ng/mL

250 ng/mL

QC 0

QC Low

QC High
Plecanatide at 70K Resolution Chromatograms

Blank

1 ng/mL

250 ng/mL

QC 0

QC Low

QC High
High Resolution Ensures Accurate Quantification Using SIM

Resolution = 35 K

Resolution = 70 K

Actual

Theoretical

Theoretical Mass $[M+2H]^+ = 841.31987$
• Validated Quantitation of Hepcidin
A Validated Method for the Definitive Quantitation of Hepcidin-25 in Human Serum by LC/MS using high resolution, accurate mass MS

• John E. Buckholz, Gary A. Schultz, Barry R. Jones, Kristen M. Bearup, Kathlyn M. Porter, Danielle J. Strong, Johnson Zhang
• Advion BioServices, Inc., Ithaca, NY
Hepcidin is a 25-amino acid peptide hormone and is the central regulator of iron metabolism making it an interesting biomarker for many applications.

25 amino acids, folded over in a hairpin shape with 4 disulfide bridges at Cys7-Cys23, Cys10-Cys22, Cys11-Cys19, Cys13-Cys14

**Internal Standard**

Mouse hepcidin molecular weight = 2752.02 g/mol

Mouse Hepcidin Sequence = DTNFPICIFCCKCCNNSQCGICCKT
Methods
Preparation and Extraction Procedure

• Protein precipitation extraction
• Ostro block for phospholipid removal
• 96 well format
• Reversed phase chromatography
• Q-Exactive
• Human Hepcidin Sequence = DTHFPICICIFCCGCHRSKCGMCCKT
Hepcidin Product Ion Mass Spectrum

Q1 Scan
[M+4H]$^4+$
Isotope pattern confirmation
human hepcidin

Theoretical mass spectrum @ 40,000 FWHM resolution
parameter

Experimental mass spectrum at 256 ng/mL
HRAM hepcidin @ 30,000 FWHM

Top 6 isotopes summed using +/- 5 ppm mass tolerance

\[ [M+5H]^{5+} \]

\[ [M+4H]^{4+} \]

\[ [M+3H]^{3+} \]
Human Hepcidin extracts from serum

QQQ m/z 698.2 > m/z 1040.0

Q-E SIM – [M+4H]^4+ Top 6 Sum

Blank

Std 1 2ng/mL

Std 2 4ng/mL

Blank

Std 1 2ng/mL

Std 2 4ng/mL
Linear Dynamic Range HRAM-MS
human hepcidin using 140,000 FWHM resolution

- Instrument Response
- Nominal Conc.

Linear - Weighting Factor = 1/X**2
Slope = 0.005439
Intercept = -0.003694
r-Squared = 0.9927
LLOQ = 2.0 ULOQ = 2048.0
Mean bias = 5.3
Standard deviation = 4.9
## Accuracy and Precision HRAM-MS

Human hepcidin using 140,000 FWHM acquisition parameter

<table>
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<th>Theor. Conc., ng/mL</th>
<th>LLOQQC</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
<th>ULOQQC</th>
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<tr>
<td>Mean</td>
<td>2.2</td>
<td>6.7</td>
<td>1067.5</td>
<td>1589.3</td>
<td>2160.2</td>
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<td>S.D.</td>
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<td>38.7</td>
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<td>%CV</td>
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<td>%RE</td>
<td>110</td>
<td>111.7</td>
<td>104.2</td>
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<td>Replicates, n</td>
<td>6</td>
<td>6</td>
<td>6</td>
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</table>
• Same instrument for qualitative and quantitative work.
• Operated under multiple modes, i.e., full scan, SIM and MS/MS.
• MS tuning simpler than triple quadrupoles.
• Enhanced selectivity by exact mass rather than fragment ions.
• Increased sensitivity by noise-free baseline and no signal loss from fragmentation.
• Increased mass range good for large molecule quantitation.
• Q Exactive can be used for quantitative bioanalysis
• Sensitivity (S/N) can be improved
• Easy to operate and proven to be potentially beneficial for assays including:
  – Difficult to fragment
  – High background
  – Multiple charges
  – High mass
Industry Trends
High Throughput HR/AM Quantitation

Thermo Posters at ASMS 2012

- **Protein Quan**
  - Th625, Zhang

- **IS drugs in blood**
  - TP36, Van Natta, Clark

- **Toxins in food**
  - T576, Wang

- **Pesticides in matrix**
  - T590, Scheibner
  - W569, Yang

- **In vitro metabolism**
  - Th379, Murphy

- **Antibiotics in water**
  - M542, Beck

- **Vitamin D in plasma**
  - T698, Berube

- **Drug stability**
  - Th347, Duczak

- **PK drug discovery**
  - TP36, Gao

- **Library Screening**
  - W107, Brant

- **Bioanalysis**
  - Th342, Cunniff

- **Drug metabolites**
  - Th342, Yang

- **Drug stability**
  - Th347, Duczak

- **Ligand binding assay**
  - M115, Murphy

- **rHuEPO in horse plasma**
  - M121, Dauley

- **Drugs in blood**
  - TP36, Van Natta

- **Melamine in pharma**
  - ThP28, Comstock

- **PK drug discovery**
  - TP36, Gao

- **Library Screening**
  - W107, Brant
• Oral: WOC pm - Regulated Bioanalysis using High Resolution LC/MS: Headache or Opportunity?
  
  • Quantitative Pharmacokinetic Sample Analysis and Metabolite Identification of Buspirone using High Resolution Accurate Mass Spectrometry, Kevin Cook¹; Rose Herbold¹; Keeley Murphy ¹; Panos Hatsis ², ¹Thermo Fisher Scientific, San Jose, CA; ²Novartis, Cambridge, MA
  
  • Evaluation of Resolving Power and Extraction Window with Comparison of Profile and Centroid Modes for High-Resolution Mass Spectrometry Phospholipid Quantitation, Mingkun Fu; Qing Lu, Millennium: the Takeda Oncology Company, Cambridge, MA

• Oral: MOC pm - Integrated Qualitative and Quantitative LC-MS for Small Molecule Analysis
  
  • Development of a Higher Throughput Metabolite Screening Assay in Early ADME Profiling Using Generic HRMS Acquisition and Automated Data Processing, Anthony Paiva¹; Cheryl Klakouski¹; Tatyana Zvyaga¹; Dieter Drexler¹; Jonathan Josephs ¹; Harold Weller¹; Wilson Shou ¹; Ismael Zamora², ¹Bristol-Myers Squibb Company, Wallingford, CT; ²Lead Molecular Design, San Cugat del Valles, Spain
ASMS – Q Exactive Pharma/Biopharma

- Drug discovery PK analysis using a quadrupole high resolution orbitrap mass spectrometer (Q Exactive): an alternative approach to triple quadrupole, Hongying Gao; Andre Negahban, Pfizer, Inc, Groton, CT
- The Opening of Bioanalytical Space Through Benchtop Accurate Mass Spectrometry: The Analysis of Propofol and its Four Metabolites, Beijing Tan¹; Yizhong Zhang²; Andre Negahban³; Hongying Gao⁴; Christopher Holliman⁵, ¹Pfizer, Inc., Groton, CT; ²Pfizer Inc., Groton, CT; ³Pfizer, Groton, CT; ⁴Pfizer, Inc, Groton, CT; ⁵Pfizer Inc, Groton, CT
- High Throughput Bioanalysis of Bile Acids and Bile Salts Using UHPLC Coupled with High Resolution Mass Spectrometers (HR-MS), Jie Ding; Eric Lund; Donald Mckenzie; John Lindsay, Covance Laboratories Inc., Madison, WI
ASMS – Q Exactive Pharma/Biopharma

• Utilizing a Non-Targeted HR/AM-MS Method to Accelerate Quantitative Throughput for In-Vitro Metabolic Profiling, Keeley Murphy; Kevin Cook; Tim Stratton; Patrick Bennett, Thermo Fisher Scientific, San Jose, CA

• Ultra High-throughput MS Methods for the Discovery of Histone Demethylase Inhibitors, John M. Peltier¹; Nicole White¹; Priti Gaitonde¹; Wendy Broom¹; Zhao Bin Kang¹; Ji-Hu Zhang¹; Michael Acker¹; Keeley Murphy²; David Farley¹; W. Adam Hill¹, ¹Novartis Institutes for Biomedical Research, Cambridge, MA; ²Thermo-Fisher Scientific, San Jose, US

• Quantitation of Oligonucleotides In Human Plasma Using Q-Exactive Orbitrap High Resolution MS, Weiwei Yuan¹; Laixin Wang¹; Min Meng¹; Jessica Wang²; Kevin Cook ²; Patrick Bennett², ¹Tandem Labs, Salt Lake City, UT; ²Thermo Fisher Scientific, San Jose, CA

• Robust Ultra High Sensitivity Quantitative Analysis using Low Flow LC/MS/MS and Reduced Phospholipid Matrix Effects. Myth or Reality?, David Humphries¹; Roger N. Hayes¹; Kevin Cook²; Mark Dreyer²; Xiang He²; Subodh Nimkar²; Patrick Bennett², ¹MPI Research, Mattawan, MI; ²Thermo Fisher Scientific, San Jose, CA
ASMS – Q Exactive Pharma/Biopharma

- **Evaluation of Resolution/Scan Speed Limitations for Quantitation of Small Molecules by UHPLC-HRMS on the Q-Exactive Platform**, Allysen Meymaris\(^1\); Richard Lelacheur\(^1\); Patrick Bennett\(^2\); Kevin Cook\(^2\); Xin Zhang\(^1\), \(^1\)Agilux Labs, Worcester, MA; \(^2\)Thermo Fisher, San Jose, CA

- **Quantification of Plant Expressed Proteins Using High Resolution LC/MS**, Jeffrey Gilbert; Trent Oman; Debbie Schwedler; John Lawry; Jesse Balcer; Suresh Babu Annangudi Palani; Yelena A. Adelfinskaya; Brian Wendelburg; Barry Schafer, Dow AgroSciences, Indianapolis, IN

- **Quantitative Bioanalysis using the Q Exactive HRMS: Factors in choosing Resolution and Scan Type**, Jack Cunniff\(^1\); Chris Yang\(^2\); Yujin Wang\(^2\); Kevin Cook\(^1\); Patrick Bennett\(^1\), \(^1\)Thermo Fisher Scientific, San Jose, CA; \(^2\)Gilead Sciences, Foster City, CA

- **Improving the Bioanalysis of Endogenous Bile Acids as Biomarkers for Hepatobiliary Toxicity**, Troy Voelker\(^1\); Kevin Cook\(^2\); Min Meng \(^1\); Patrick Bennett\(^2\), \(^1\)Tandem Labs, Salt Lake City, UT; \(^2\)Thermo Fisher Scientific, San Jose, CA
ASMS – Q Exactive Pharma/Biopharma

• UHPLC-ESI-HRMS quantitation of metabolites without using reference standards-Impact of mobile phase composition on MS response, Sumithra Katragadda; Dil Ramanthan, Kean University, Iselin, NJ

• LC/SRM Has Come of Age to Quantitate Proteins: Amyloid β isoforms measured in Human Cerebrospinal Fluid and Compared to ELISA, Kwasi Mawuenyega; Tom Kasten; Vitaliy Ovod; Kelly Moor; Ling Munsell; Rose Connors; Wendy Sigurdson; Randall Bateman, Washington University School of Medicine, Saint Louis, MO

• Quantitation of Melatonin and N-Acetylserotonin in Human Plasma by Nanoflow LC-MS/MS and Electrospray LC-MS/MS, Melissa D. Carter; M. Wade Calcutt; Beth A. Malow; Kristie Lindsey Rose; David L. Hachey, Vanderbilt University, Nashville, TN
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  - Patrick Bennett
  - Kevin Cook
  - Zhiqi Hao
  - Keeley Murphy
  - Jessica Wang
  - TFS Demo Labs

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  - John Buckholz
  - Gary Schultz
  - Barry Jones
  - Kristen Bearup
  - Kathlyn Porter
  - Danielle Strong
  - Johnson Zhang

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