Increasing Efficiency of ADME Assays Using the High-throughput RapidFire System

MS Sample Analysis in <10 seconds

Life Sciences Group

Vaughn Miller, Ph.D.
Applications Manager
RapidFire
Oct. 6, 2011

For Research Use Only. Not for Use in Diagnostic Procedures.
Today’s Agenda

• SPE/MS (RapidFire/MS) technology overview

• Select application case studies
  • In vitro ADME assays
  • Bioanalysis
  • RapidFire/QQQ & RapidFire/TOF

• Summary
SPE-MS/MS Analysis

Aqueous wash → Organic reverse elution → waste → Mass spec
RapidFire Mass Spectrometry

Ultra-fast autosampler & in-line SPE system

- Replaces LC in LC/MS
- Reusable SPE cartridge
- Integrates with standard ESI MS instruments
- **Cycle time = 6-10 s/sample**

Compatible with biological matrices

- Microsomal preparations
- Cell culture supernatants
- Tissue extracts
- Serum, plasma, urine, whole blood
RapidFire Removes the LC Bottleneck

- Run Assay
- Data Acquisition
- Data Analysis

1-5 min/sample

LC-MS

8-10 sec/sample

Run Assay
Data Acquisition
Data Analysis

RF-MS
Drug Discovery with RapidFire/MS

Target Selection
- Genomics
- Proteomics
- Metabolomics
- Lipidomics
- Animal models
- Knockouts

High-throughput Target Screening (HTS)
- Label-free HTS using native substrate
- Secondary screen
- Selectivity screen

Lead Optimization
- ADMET
  - DDI
  - Met Stab
  - Permeability
  - Protein binding
  - Pgp inhibition
  - CYP induction

Preclinical Testing
- Bioanalysis

Clinical Trials
- Bioanalysis

Therapeutic Areas
- Metabolic disease/diabetes
- Epigenetics
- Cardiovascular disease
- Kinases
- Oncology
- Anti-infectives
- Inflammation/Pain
- Neurology
Select Application Case Studies

- CYP inhibition
- Permeability
- Pgp inhibition
- Bioanalysis
- Metabolic Stability
Direct CYP Inhibition

Validation Experiment with BD Gentest

• Samples split and analyzed by RapidFire and LC-MS/MS by BD Gentest
• 8 different isoforms/probe pairs

IC$_{50}$ (uM) by RapidFire @ BioTrove vs. IC$_{50}$ (uM) by Traditional LCMS @BD Gentest

R$^2 = 0.986$

>20-Fold Faster Analysis
• Rapid data delivery to clients.
• Acquire more data points per unit time
• Option to conduct robust, multipoint assays typical of drug development
Time-Dependent Inhibition (TDI) of CYP3A4 with RapidFire: IC\textsubscript{50} shift

RapidFire-MS/MS

**Ritonavir/Midazolam RapidFire**

- w/NADPH
- w/o NADPH

**Azamulin/Midazolam RapidFire**

- w/NADPH
- w/o NADPH

**Diltiazem/Testosterone RapidFire**

- w/NADPH
- w/o NADPH

LC-MS/MS

**Ritonavir/Midazolam Trad. LC/MS**

- w/NADPH
- w/o NADPH

**Azamulin/Midazolam Trad. LC/MS**

- w/NADPH
- w/o NADPH

**Diltiazem/Testosterone Trad. LS/MS**

- w/NADPH
- w/o NADPH

---

Confidential
LC-MS/MS vs. RF-MS/MS P450 Inhibition
Published Data

Novartis
• 2010 RCMS letter to the editor

Takeda
• 2007 ASMS presentation & 2010 J. Biomol. Screening

AstraZeneca
• 2007 AAPS & 2009 ISSX poster

Boehringer Ingelheim
• 2009 ASMS presentation

Merck
• 2009 ASMS presentation

BD Gentest
• 2009 ISSX posters

Exelixis
• 2008 SBS poster
Correlation of Permeability Coefficients For 110 Caco-2 Experiments

Blue squares indicate same day sample measurement and red squares indicate a measurement delay of > one day and one additional freeze-thaw cycle.

Correlation of Permeability Coefficients For 35 PAMPA experiments

Compounds were investigated at different pH values and display low (<1 × 10⁻⁸ cm/s), medium (>1 × 10⁻⁸, but <1 × 10⁻⁷ cm/s), or high permeability in the assay.

P-gp inhibition Assay Using a Caco-2 Cell Model

- Digoxin Only

Caco-2 cells (with P-gp expression) on membrane support

- Digoxin + Inhibitor

Correlation Plots Comparing % P-gp Inhibition of Test Compounds

RF/MS/MS v. LC/MS/MS % Inhibition

\[ y = 0.997x + 3.4616 \]
\[ R^2 = 0.9268 \]

LC/MS/MS v. Radiolabeled % Inhibition

\[ y = 0.8804x + 6.6524 \]
\[ R^2 = 0.9076 \]

RF/MS/MS v. Radiolabeled % Inhibition

\[ y = 0.9035x + 9.164 \]
\[ R^2 = 0.8953 \]
Comparison of Cyclosporin A P-gp Inhibition Assay Methods

Radioactivity

LC-MS/MS

RF-MS/MS

IC$_{50}$ = 3.1 µM

IC$_{50}$ = 2.7 µM

IC$_{50}$ = 2.7 µM
Published In Vitro ADME Applications

Novartis
• Metabolic Stability, PAMPA, CYP inhibition & Solubility
  – AAPS 2010 poster, RCMS 2010
  – ASMS 2010 presentation & poster

Boehringer Ingelheim
• Metabolic Stability, Caco-2, PAMPA
  – J. Biomolecular Screening, 2011

Agilent/BIOCIUS Life Sciences
• Metabolic Stability, PAMPA, Plasma protein binding
  – SBS & ASMS 2010/1 posters

Bristol-Myers Squibb
• Metabolic Stability, Caco-2, P-gp inhibition
  – RCMS 2011
  – ASMS 2009 & 2010 posters

Takeda
• Metabolic Stability, PAMPA, Plasma protein binding
  – ASMS 2008 poster
Analysis of Levetiracetam in Serum

ASMS 2011 Poster Session: WP10 - Diagnostic Clinical Chemistry II
poster number: 161, Wednesday

High-Throughput Analysis of Levetiracetam in Serum Using Ultra-fast SPE-MS/MS

Michelle Romm¹; Eric Korman²; Vaughn Miller¹; Christine Snozek²; Frank W. Crow²; Loralie Langman²; William A. Lamarr¹
¹Agilent Technologies, Inc., Wakefield, MA; ²Mayo Clinic, Rochester, MN
Prepared Levetiracetam Calibration Standards and Commercially Available Quality Controls

<table>
<thead>
<tr>
<th>Levetiracetam Conc (ug/ml)</th>
<th>Accuracy (%) Interday (n=4)</th>
<th>Precision (%) Interday (n=4)</th>
<th>Accuracy (%) Intraday (n=3)</th>
<th>Precision (%) Intraday (n=3)</th>
<th>Quant/Qual AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>106.9</td>
<td>2.8</td>
<td>106.5</td>
<td>1.3</td>
<td>19.1</td>
</tr>
<tr>
<td>5</td>
<td>92.9</td>
<td>1.3</td>
<td>93.8</td>
<td>0.9</td>
<td>18.2</td>
</tr>
<tr>
<td>25</td>
<td>98.4</td>
<td>3.4</td>
<td>98.1</td>
<td>0.9</td>
<td>18.3</td>
</tr>
<tr>
<td>50</td>
<td>102.3</td>
<td>1.8</td>
<td>101.9</td>
<td>0.3</td>
<td>17.9</td>
</tr>
<tr>
<td>100</td>
<td>99.5</td>
<td>1.1</td>
<td>99.8</td>
<td>0.2</td>
<td>17.1</td>
</tr>
<tr>
<td>UTAK 1 (15.5)</td>
<td>93.1</td>
<td>3.4</td>
<td>94.6</td>
<td>0.7</td>
<td>17.8</td>
</tr>
<tr>
<td>UTAK 2 (39.7)</td>
<td>102.8</td>
<td>3.8</td>
<td>102.6</td>
<td>0.8</td>
<td>17.3</td>
</tr>
<tr>
<td>UTAK 3 (73.7)</td>
<td>105.1</td>
<td>3.5</td>
<td>104.1</td>
<td>0.6</td>
<td>17.3</td>
</tr>
</tbody>
</table>

* 1/x weighing factor was applied.

• **Sample Preparation**
  • Protein precipitation

• **RapidFire analysis**
  • RapidFire 300 + Agilent 6460 Triple quad
  • Total sample cycle time = 9.5 sec

For Research Use Only. Not for Use in Diagnostic Procedures.
Levetiracetam – Blinded Human Samples

Correlation between RapidFire-MS and LC-MS

- **HPLC-MS Workflow**
  - 7.5 min/sample
  - multiplex X4 (~2 min/sample)
  - 768 samples/day/mass spec

- **RapidFire-MS Workflow**
  - 9.5 sec/sample
  - no multiplexing
  - > 9000 samples/day/mass spec

> 10X throughput enhancement

For Research Use Only. Not for Use in Diagnostic Procedures.
RapidFire/MS Minimizes the LC Bottleneck

- Run Assay
  - Data Acquisition
  - Data Analysis
  - 1-5 min/sample

- Run Assay
  - Data Acquisition
  - Data Analysis
  - 8-10 sec/sample
Advantages of TOF MS

- Survey all masses within a defined range
  - For example: 100-1000amu
- High mass resolution & accuracy
  - ~20,000
  - 1-2 ppm
- Sensitive & Quantitative
  - Comparable to mid-range QQQ
- Retrospective analysis
  - Ability to reanalyze data set

Schematic of Agilent QTOF
RapidFire 360 High Resolution System

Eliminates bottlenecks in high-throughput in vitro ADME analysis
RapidFire 360 Workflow

Load Assay Plates and Acquire Full-Scan ESI-TOF data
- 96 or 384-well compatible, no *a priori* knowledge of analytes required

Automatically Extract and Integrate masses of interest
- Compound molecular formulas provided as a text file

Automated Report Generation
- Integrated peak areas and chromatograms for each analyte are generated
Data: Microsomal Stability Assay with Midazolam

Extraction and peak integration of analytes from TIC is fully automated.
RapidFire 360 Removes the MRM Bottleneck

RF-MS/MS

- Run Assay
- QqQ method development
- Data Acquisition
- Data Analysis

4-10 min/analyte

RF360

- Run Assay
- Data Acquisition
- Data Analysis
- Generic Methods & Full MS Scan

6-10 sec/sample (1-2 min/analyte)

Agilent Technologies
Metabolic Stability Experimental Conditions
RapidFire/TOF vs. LC/MS/MS

• Diverse set of substrates
  • 39 commercial drug-like compounds
  • MW 218-734; XlogP3 values 0.4-7.1
• Microsomal incubations
  • Human liver microsomes, time course 0-60 min.
  • Measure substrate depletion and calculate $t_{1/2}$ values
• Analytical conditions

<table>
<thead>
<tr>
<th>RapidFire/TOF</th>
<th>LC/MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent RapidFire 360</td>
<td>Agilent 1260 LC</td>
</tr>
<tr>
<td>Agilent 6530 Q-TOF</td>
<td>Agilent 6460 QQQ</td>
</tr>
<tr>
<td>Generic SPE method</td>
<td>Generic LC method</td>
</tr>
<tr>
<td>Generic MS source parameters</td>
<td>Optimized MRM transitions</td>
</tr>
<tr>
<td>9.5 seconds per sample</td>
<td>2.2 min per sample</td>
</tr>
</tbody>
</table>
Metabolic Stability Curves

Nicardipine

T_{1/2} = 6.32  LC/MS/MS
T_{1/2} = 6.02  RapidFire/TOF

Midazolam

T_{1/2} = 6.25  LC/MS/MS
T_{1/2} = 6.75  RapidFire/TOF
Correlation Data for Half-life Values 0-60 min

R² = 0.9651
Summary of Results

• Half-life correlation
  • All compounds binned in same manner
• Carry-over
  • No significant carry-over seen for RapidFire/TOF analysis
• Analysis Speed
  • RapidFire/MS 13x faster than LC/MS/MS
• Data table for compounds with \( t_{1/2} < 60 \) min

### Table 1. Summary of Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>RapidFire-Q-TOF</th>
<th>LC-MS/MS</th>
<th>Mol Formula</th>
<th>MW</th>
<th>XLogP3</th>
<th>% Carryover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicardipine</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>478,5250</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td>Ne azidone</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{Cl}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>470,0089</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>Midazolam</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{ClF}_{6} )</td>
<td>325,7873</td>
<td>2.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>418,4403</td>
<td>3.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Diofafenac</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{Cl}<em>{2}\text{N}</em>{12}\text{O}_{6} )</td>
<td>296,1486</td>
<td>4.4</td>
<td>ND</td>
</tr>
<tr>
<td>Pyriamine</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>285,3840</td>
<td>3.3</td>
<td>ND</td>
</tr>
<tr>
<td>Propafenone</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>341,4440</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Tiolodipine</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>263,7857</td>
<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td>Verapamil</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>454,6016</td>
<td>3.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>471,6734</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td>Buspirone</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>386,5031</td>
<td>2.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Chlopromazine</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{Cl}<em>{2}\text{N}</em>{12}\text{O}_{6} )</td>
<td>318,8642</td>
<td>5.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>20-60</td>
<td>20-60</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>437,5216</td>
<td>4.4</td>
<td>ND</td>
</tr>
<tr>
<td>Promazine</td>
<td>20-60 (53)</td>
<td>&gt;80</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>284,4191</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>Thiordazine</td>
<td>20-60</td>
<td>&gt;80</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>370,5745</td>
<td>5.9</td>
<td>0</td>
</tr>
<tr>
<td>Promethazine</td>
<td>20-60 (56)</td>
<td>&gt;80</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>284,4191</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>20-60 (59)</td>
<td>&gt;80</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>271,3972</td>
<td>3.4</td>
<td>0</td>
</tr>
<tr>
<td>Cinnarizine</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>( \text{C}<em>{42}\text{H}</em>{27}\text{N}<em>{12}\text{O}</em>{6} )</td>
<td>368,5139</td>
<td>5.8</td>
<td>0</td>
</tr>
</tbody>
</table>

ND = not determined
Correlation Data for a Large Set of Med Chem Leads

$y = 0.9118x - 6.0016$

$R^2 = 0.927$

$n = 597$

RapidFire Streamlines Workflow

Traditional LCMS Workflow

- **Samples Received**
  - 0.5 hr
  - 12 hr
  - 24 hr
  - 2 hr
- **Analyte Tuning**
- **Sample Analysis**
- **Data Delivery**

38.5 hr

RapidFire 300 MS QqQ Work Flow

- **Samples Received**
  - 0.5 hr
  - 12 hr
  - 2 hr
  - 2 hr
- **Data Delivery**

16.5 hr

RapidFire 360 MS TOF Work Flow

- **Samples Received**
  - Data Delivery

4.5 hr

Adapted from oral presentation by Panos Hatsis, Novartis, 58th ASMS Conference on Mass Spectrometry and Allied Topics, May 23 - 27, 2010
Metabolite Analysis – Data Extraction Using RapidFire Integrator Software

Screen for ID of common metabolites

- New RapidFire 360 software feature
- Intuitive, automated workflow

- Monoxidation
- Dioxidation
- Demethylation
- Deethylation
- Reduction
- Desaturation
- Glucuronadation
- Sulphation
- Acetylation
## Metabolite Analysis Data

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Slip</td>
<td>Sequence</td>
<td>Plate Name</td>
<td>Row</td>
<td>Column</td>
<td>Injection Mass IS (m/z)</td>
<td>Abundance IS (m/z)</td>
<td>Mass 1</td>
<td>Abundance 1 (m/z +15.995)</td>
<td>Mass 1</td>
<td>Abundance 1 (m/z +15.995)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>MET STAB - Midazolam - TOF</td>
<td>1</td>
<td>1</td>
<td>9.92</td>
<td>289.2202</td>
<td>33285</td>
<td>326.0782</td>
<td>24326</td>
<td>342.0731</td>
<td>674</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>MET STAB - Midazolam - TOF</td>
<td>2</td>
<td>1</td>
<td>26.2</td>
<td>289.2202</td>
<td>24532</td>
<td>326.0782</td>
<td>19041</td>
<td>342.0731</td>
<td>2215</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>MET STAB - Midazolam - TOF</td>
<td>3</td>
<td>1</td>
<td>29.29</td>
<td>289.2202</td>
<td>222010</td>
<td>326.0782</td>
<td>18801</td>
<td>342.0731</td>
<td>2930</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>MET STAB - Midazolam - TOF</td>
<td>4</td>
<td>1</td>
<td>37.9</td>
<td>289.2202</td>
<td>22279</td>
<td>326.0782</td>
<td>15347</td>
<td>342.0731</td>
<td>1376</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>MET STAB - Midazolam - TOF</td>
<td>5</td>
<td>1</td>
<td>46.7</td>
<td>289.2202</td>
<td>21361</td>
<td>326.0782</td>
<td>15810</td>
<td>342.0731</td>
<td>1836</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>MET STAB - Midazolam - TOF</td>
<td>6</td>
<td>1</td>
<td>55.8</td>
<td>289.2202</td>
<td>22154</td>
<td>326.0782</td>
<td>16192</td>
<td>342.0731</td>
<td>1668</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>MET STAB - Midazolam - TOF</td>
<td>7</td>
<td>1</td>
<td>64.79</td>
<td>289.2202</td>
<td>0</td>
<td>326.0782</td>
<td>0</td>
<td>342.0731</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>MET STAB - Midazolam - TOF</td>
<td>8</td>
<td>1</td>
<td>73.49</td>
<td>289.2202</td>
<td>0</td>
<td>326.0782</td>
<td>0</td>
<td>342.0731</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>MET STAB - Midazolam - TOF</td>
<td>1</td>
<td>2</td>
<td>62.43</td>
<td>289.2202</td>
<td>31275</td>
<td>326.0782</td>
<td>23786</td>
<td>342.0731</td>
<td>667</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>MET STAB - Midazolam - TOF</td>
<td>2</td>
<td>2</td>
<td>51.27</td>
<td>289.2202</td>
<td>22507</td>
<td>326.0782</td>
<td>27659</td>
<td>342.0731</td>
<td>3002</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>MET STAB - Midazolam - TOF</td>
<td>3</td>
<td>2</td>
<td>100.38</td>
<td>289.2202</td>
<td>22890</td>
<td>326.0782</td>
<td>18244</td>
<td>342.0731</td>
<td>1928</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>MET STAB - Midazolam - TOF</td>
<td>4</td>
<td>2</td>
<td>108.96</td>
<td>289.2202</td>
<td>20580</td>
<td>326.0782</td>
<td>15669</td>
<td>342.0731</td>
<td>1639</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>MET STAB - Midazolam - TOF</td>
<td>5</td>
<td>2</td>
<td>117.54</td>
<td>289.2202</td>
<td>20170</td>
<td>326.0782</td>
<td>10033</td>
<td>342.0731</td>
<td>1060</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>MET STAB - Midazolam - TOF</td>
<td>6</td>
<td>2</td>
<td>126.66</td>
<td>289.2202</td>
<td>21659</td>
<td>326.0782</td>
<td>16270</td>
<td>342.0731</td>
<td>1961</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>MET STAB - Midazolam - TOF</td>
<td>7</td>
<td>2</td>
<td>135.38</td>
<td>289.2202</td>
<td>0</td>
<td>326.0782</td>
<td>0</td>
<td>342.0731</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>16</td>
<td>MET STAB - Midazolam - TOF</td>
<td>8</td>
<td>2</td>
<td>143.97</td>
<td>289.2202</td>
<td>38</td>
<td>326.0782</td>
<td>35</td>
<td>342.0731</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>17</td>
<td>MET STAB - Midazolam - TOF</td>
<td>1</td>
<td>3</td>
<td>152.58</td>
<td>289.2202</td>
<td>31109</td>
<td>326.0782</td>
<td>23659</td>
<td>342.0731</td>
<td>924</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>18</td>
<td>MET STAB - Midazolam - TOF</td>
<td>2</td>
<td>3</td>
<td>161.54</td>
<td>289.2202</td>
<td>20335</td>
<td>326.0782</td>
<td>18175</td>
<td>342.0731</td>
<td>1432</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>19</td>
<td>MET STAB - Midazolam - TOF</td>
<td>3</td>
<td>3</td>
<td>170.32</td>
<td>289.2202</td>
<td>21269</td>
<td>326.0782</td>
<td>19609</td>
<td>342.0731</td>
<td>1975</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>MET STAB - Midazolam - TOF</td>
<td>4</td>
<td>3</td>
<td>178.88</td>
<td>289.2202</td>
<td>21973</td>
<td>326.0782</td>
<td>14951</td>
<td>342.0731</td>
<td>1745</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>21</td>
<td>MET STAB - Midazolam - TOF</td>
<td>5</td>
<td>3</td>
<td>187.47</td>
<td>289.2202</td>
<td>19944</td>
<td>326.0782</td>
<td>11223</td>
<td>342.0731</td>
<td>2044</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>22</td>
<td>MET STAB - Midazolam - TOF</td>
<td>6</td>
<td>3</td>
<td>196.59</td>
<td>289.2202</td>
<td>21645</td>
<td>326.0782</td>
<td>16923</td>
<td>342.0731</td>
<td>1815</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>23</td>
<td>MET STAB - Midazolam - TOF</td>
<td>7</td>
<td>3</td>
<td>203.71</td>
<td>289.2202</td>
<td>0</td>
<td>326.0782</td>
<td>0</td>
<td>342.0731</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>24</td>
<td>MET STAB - Midazolam - TOF</td>
<td>8</td>
<td>3</td>
<td>214.43</td>
<td>289.2202</td>
<td>0</td>
<td>326.0782</td>
<td>0</td>
<td>342.0731</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>25</td>
<td>MET STAB - Midazolam - TOF</td>
<td>1</td>
<td>4</td>
<td>222.99</td>
<td>289.2202</td>
<td>20194</td>
<td>326.0782</td>
<td>21271</td>
<td>342.0731</td>
<td>2408</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>26</td>
<td>MET STAB - Midazolam - TOF</td>
<td>2</td>
<td>4</td>
<td>232.11</td>
<td>289.2202</td>
<td>22814</td>
<td>326.0782</td>
<td>16482</td>
<td>342.0731</td>
<td>2598</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>27</td>
<td>MET STAB - Midazolam - TOF</td>
<td>3</td>
<td>4</td>
<td>240.95</td>
<td>289.2202</td>
<td>21227</td>
<td>326.0782</td>
<td>15075</td>
<td>342.0731</td>
<td>3640</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>28</td>
<td>MET STAB - Midazolam - TOF</td>
<td>4</td>
<td>4</td>
<td>249.65</td>
<td>289.2202</td>
<td>18233</td>
<td>326.0782</td>
<td>4528</td>
<td>342.0731</td>
<td>5090</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>29</td>
<td>MET STAB - Midazolam - TOF</td>
<td>5</td>
<td>4</td>
<td>258.13</td>
<td>289.2202</td>
<td>20417</td>
<td>326.0782</td>
<td>2249</td>
<td>342.0731</td>
<td>6933</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>30</td>
<td>MET STAB - Midazolam - TOF</td>
<td>6</td>
<td>4</td>
<td>267.26</td>
<td>289.2202</td>
<td>21351.5</td>
<td>326.0782</td>
<td>1617</td>
<td>342.0731</td>
<td>4578</td>
<td></td>
</tr>
</tbody>
</table>
Metabolite Analysis Results - Midazolam

Midazolam

Time, min

Prod/IS, AUC

Midazolam

Oxidation

oxidation
Metabolite Analysis Results - Chloropromazine

Chlorpromazine

0 0.2 0.4 0.6 0.8 1 1.2
Prod/IS, AUC

Time, min

Chlorpromazine Metabolites

0 0.01 0.02 0.03 0.04 0.05
Prod/IS, AUC

Time, min

- oxidation
- demethylation
- 2 demethylations

1\textsuperscript{st} demethylation

oxidation

2\textsuperscript{nd} demethylation
Do more with less... Be more efficient... Faster, faster!!

RapidFire-MS is the Solution

Shorter data turnaround
• No off-line sample prep required
• Sample analysis cycle times of 6-10s for a variety of assays

More efficient use of resources
• Capital equipment, reagents and personnel
  – No need for expensive consumables
  – Lower operational costs
  – Complements existing work flows
  – ~10x more data generated per FTE vs. LC-MS

Maintain assay data quality
• Many examples of strong correlation to LC-MS/MS
  – CYP inhibition, microsomal stability, Caco-2 permeability & other assays
• No need to compromise assay design
  – Easily perform full IC_{50}s and multiple data points
Contact Us to Learn More

Agilent Technologies

www.Agilent.com
781-928-2750

Vaughn Miller, Ph.D.
Applications Manager, RapidFire
vaughn.miller@agilent.com

For Research Use Only. Not for Use in Diagnostic Procedures.