Quantification of Rifapentine Concentrations in Dried Blood Spot Samples Using Liquid Chromatographic-Tandem Mass Spectrometric Analysis

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Objective

- Validation of Plasma and Dried Blood Spot (DBS) assays for the efficient and accurate testing of Rifapentine (RPT) and its active metabolite desacetyl-rifapentine (des-RPT) by liquid chromatographic-tandem mass spectrometric (LC-MS/MS) analysis.
Background: RPT for TB

• Treatment shortening for drug-sensitive TB
  – Daily RPT as part of multidrug therapy has great promise to reduce duration of Rx needed for cure
  – Phase 3 multi-site international RPT treatment-shortening trial planned

• RPT for treatment of latent TB infection (LTBI)
  – RPT plus isoniazid once weekly for a total of 12 doses was recently recommended for treatment of LTBI
  – Dose-finding trial of RPT planned to extend this therapy to pediatric population, including infants
Background: Drug Assays

• Measuring Drug Concentrations
  – Typically done in serum or plasma
  – Need adequate sample volume
  – Rapid sample preparation
  – Freezer storage
  – Shipping of potentially hazardous materials
Dried Blood Spots (DBS): An Alternative?

• Advantages
  – Reduction in blood volume required
  – No sample processing required
  – No freezer storage
  – Shipping – less volume, no dry ice, easier

• Disadvantages
  – Hematocrit effect

| 70% | 45% | 20% |
## Assay Validation: FDA Guidelines

<table>
<thead>
<tr>
<th>Validation test</th>
<th>Precision Requirement (CV%)</th>
<th>Accuracy Requirement (%error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interassay – calibration standards</td>
<td>≤15%, (≤20% at LLOQ)</td>
<td>≤ ±15%, (≤ ±20% at LLOQ)</td>
</tr>
<tr>
<td>Intraassay</td>
<td>≤ 15%</td>
<td>≤ ± 15%</td>
</tr>
<tr>
<td>Interassay</td>
<td>≤ 15%</td>
<td>≤ ± 15%</td>
</tr>
<tr>
<td>Stability – control versus treated</td>
<td>&lt;15% difference between treated and untreated group</td>
<td></td>
</tr>
<tr>
<td>Stability – in prepared sample for chromatographic injection</td>
<td>&lt;15% difference between original and re-injected value</td>
<td></td>
</tr>
<tr>
<td>Long term stability – of analyte in matrix</td>
<td>&lt;15% difference from original value</td>
<td></td>
</tr>
<tr>
<td>Stability – Stock solutions of reference standards</td>
<td>&lt;10% difference from nominal</td>
<td></td>
</tr>
<tr>
<td>Recovery (extraction efficiency)</td>
<td>Recovery of analyte should be consistent, precise, and reproducible, but need not be 100%, nor does the FDA give specific limits. Note: any inconsistencies in recovery would be reflected in the interassay and intrassay variation measured by the validation samples, unless IS corrects for inconsistencies, which is appropriate.</td>
<td></td>
</tr>
<tr>
<td>Matrix testing</td>
<td>All matrices within &lt;15% difference from each other and from theoretical</td>
<td></td>
</tr>
<tr>
<td>Concomitant Medications</td>
<td>Any interference or co-elution noted in the SOP as a limitation</td>
<td></td>
</tr>
</tbody>
</table>
LC-MS/MS monitored anti-TB drugs

D3-RIF
826.5 > 794.5

RPT
877.6 > 845.5

des-RPT
835.5 > 803.5
Quinone Reduction measure all RPT and des-RPT species
Sample Preparation

Specimen Pre-treatment

- 20 µl plasma aliquoted for analysis
- 6 mm DBS punched

Extraction

- Added Internal Standard
- Added Extracting Solvent (containing ascorbic acid)
- Isolation of analytes of interest

Analysis

- Dilution with analytical mobile phase
- Chromatographic separation
- Mass spectrometric monitoring of ions (MRM)
Representative Drug Chromatogram
Quadratic Regression (1/x^2 weighting): y = -1.84e-009 x^2 + 0.00108 x + -0.00829 (r^2 = 0.9978)

- RPT and des-RPT - 50-80,000 ng/ml
- r^2 values of ≥ 0.996
- Precisions ≤13%
- Accuracies ± 9.7%
## Method Stability Comparison

<table>
<thead>
<tr>
<th>Stability Condition</th>
<th>Plasma</th>
<th>DBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Freeze Thaw</strong></td>
<td>3 Cycles</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Sample Matrix</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LightExposed @ RT</td>
<td>1 day</td>
<td>2 days</td>
</tr>
<tr>
<td>LightProtected @ RT</td>
<td>2 day</td>
<td>≥ 11 weeks</td>
</tr>
<tr>
<td>LightExposed @ 40°C w/ 100% humidity</td>
<td>N/A</td>
<td>Unstable</td>
</tr>
<tr>
<td>LightProtected @ 45-65°C w/ 100% humidity in bag with desiccant</td>
<td>N/A</td>
<td>Unstable*</td>
</tr>
<tr>
<td><strong>Injection Matrix</strong></td>
<td>5 days</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Long Term Storage</strong></td>
<td>5 months</td>
<td>≥ 11 weeks</td>
</tr>
<tr>
<td><strong>Stock Solution</strong></td>
<td>2.5 years</td>
<td></td>
</tr>
</tbody>
</table>
Application of Method to a Clinical Trial

- Plasma and DBS samples obtained from TBTC Study 29B
  - Phase I dose escalation study of RPT
    - 1st and 14th daily drug dose
      - Heparinized plasma
      - Venous whole blood on Whatman 903 ®
  - Hematocrit obtained pre- and post-PK collection
Method Concordance

For DBS [RPT] / Plasma [RPT]

- Identity
- Bias (0.630)
- 95% Limits of agreement (0.310 to 0.950)

n = 38; 95% CI = 0.576 – 0.683

For DBS [des-RPT] / Plasma [des-RPT]

- Identity
- Bias (0.533)
- 95% Limits of agreement (0.097 to 0.969)

n = 36; 95% CI = 0.458 – 0.608

LOA = Mean ratio ± 1.96 standard deviation of the ratio
**DBS vs. Plasma**

**Identity**

Persisting & Bablok (I) fit

- DBS [RPT] (ng/ml)
  - (303.13 + 0.56x)

- Plasma [RPT] (ng/ml)
  - 95% CI bands

**Identity**

Persisting & Bablok (I) fit

- DBS [des-RPT] (ng/ml)
  - (-8.84 + 0.50x)

- Plasma [des-RPT] (ng/ml)
  - 95% CI bands
Can we correct?

- Average pre- and post-hematocrits
  - Mean 41.6% (Range 33.2% - 47.4%)

- \([DBS]_{\text{hematocrit}} = \frac{[DBS]}{(1 - \frac{\text{hematocrit}}{100})}

- \([\text{plasma}] \approx \frac{[DBS]}{(1 - \frac{\text{hematocrit}}{100})}\)
DBS$_\text{hematocrit}$ vs Plasma

- Identity
- Passing & Bablok (I) fit
  - (355.77 + 0.98x)
- 95% CI bands

Plots showing the correlation between DBS$_\text{hematocrit}$ and Plasma [RPT] (left) and DBS$_\text{hematocrit}$ [des-RPT] (right). The Passing & Bablok (I) fit equations are noted for each dataset.
DBS_{hematocrit} vs Plasma

\[ \text{DBS}_{\text{hematocrit}} \div \text{Plasma} \]

Identity

Bias (1.074)

95% Limits of agreement
(0.565 to 1.583)

\[ \text{LOA} = \text{Mean ratio} \pm 1.96 \times \text{standard deviation of the ratio} \]

\( n = 38; \text{ 95\% CI} = 0.989 – 1.160 \)

\( n = 36; \text{ 95\% CI} = 0.786 – 1.026 \)
Can we correct without Hematocrit?

- **Population based Bland-Altman Bias**
  
  - $[\text{DBS}]_{\text{corrected}} = \frac{[\text{DBS}]}{(\text{Bias})}$
  
  - $[\text{plasma}] \approx \frac{[\text{DBS}]}{(\text{Bias})}$

  - **Bias** = mean geometric drug concentration ratio in DBS versus plasma
DBS_{corrected} vs Plasma

Identity

Passing & Bablok (l) fit
(442.77 + 0.90x)

95% CI bands

DBS_{corrected} [RPT] (ng/mL)

Plasma [RPT] (ng/mL)

Passing & Bablok (l) fit
(-31.54 + 0.95x)

95% CI bands

DBS_{corrected} [des-RPT] (ng/mL)

Plasma [des-RPT] (ng/mL)
Summary

• RPT and des-RPT may be:
  – Measured in plasma
  – Measured in DBS
    • For concordance, must normalize to hematocrit, or use a population-based correction factor

• DBS offer:
  – Low blood volume requirements
  – Simple storage at Room Temperature
  – Straightforward sample preparation
Future Work

• Repeat 45ºC 100% Humidity Desiccant
• Hematocrit Experiment
  – Spiked whole blood, DBS, Plasma
• Paired subject whole blood, DBS samples
• DBS on FTA DMPK-C Paper
  – Fingerstick Spots
  – Capillary Spots - venous whole blood
  – Disposable pipet Spots - venous whole blood
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