Development and clinical validation of an LC-UV method to quantify dolutegravir in dried blood spots

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INTRODUCTION

- Dolutegravir (DTG) is now the preferred component of first-line antiretroviral therapy (ART) in all population groups.
- Facilitating clinical pharmacology studies of dolutegravir in key populations (e.g. neonates and pregnant women), quantitative analysis methods compatible with micro-sampling and adaptable in resource-limited settings are desirable.
- Here, a method to quantify dolutegravir in dried blood spots (DBS) using liquid chromatography with ultraviolet detection (LC-UV) was developed, validated and applied in a pharmacokinetic (PK) study in HIV-positive women receiving DTG-containing ART.

METHODS

- Validation samples (over the concentration range of 400 - 10000 ng/mL) were prepared by spotting 50 μL of DTG-spiked whole blood on DBS cards.
- Extraction was by simple protein precipitation using methanol.
- Chromatographic separation was achieved with a gradient elution of acetonitrile/potassium phosphate monobasic buffer (pH 5) on a reverse-phase C18 column, at a flow rate of 1 mL/min using pioglitazone as the internal standard. Detection was by UV at a wavelength of 260 nm.
- Clinical validation was conducted by collecting DBS from participants (n = 10) at 8 time points (0.25-24 hours) after dose (paired plasma at 1 and 12 hours) from finger prick.
- DBS-derived plasma concentrations were obtained from DBS concentrations using (DBS/(1-Hct)) x 0.99 (DBS: measured DTG in DBS; Hct: mean haematocrit for female (0.40 L/L)2; 0.99 = plasma bound ratio of DTG).
- The method was used to quantify DTG, and PK parameters were estimated from concentration-time data using non-compartmental analysis.
- DBS-derived and measured plasma concentrations correlation was evaluated using linear regression and Bland-Altman plots.

RESULTS

- Accuracy ranged between 102.4 and 114.8% and precision ranged between 3.4 and 14.7% (Table 1).
- The mean recovery was 41.3% (%CV: 13.6).
- The method was specific and selective for dolutegravir with no interference at its retention time.
- Compared with plasma, DBS concentration was 37.5% (%CV: 6.1) lower.
- DBS-derived concentrations were used to characterise PK of DTG (Figure 1 and Table 2).
- A strong predictable correlation exists between DBS-derived and measured DTG plasma concentration (Figure 3 & 4).

Table 1: Accuracy and Precision for the quantification of dolutegravir in dried blood spot

<table>
<thead>
<tr>
<th>QCs (ng/mL)</th>
<th>Inter-day (measured concentration)</th>
<th>Intra-day (measured concentration)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>Precision (%CV)</td>
</tr>
<tr>
<td>LQC (500)</td>
<td>112.3</td>
<td>12.5</td>
</tr>
<tr>
<td>MQC (4500)</td>
<td>114.8</td>
<td>8.42</td>
</tr>
<tr>
<td>HQC (8000)</td>
<td>107.5</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Table 2: DBS-derived vs measured plasma dolutegravir PK parameters. Data are presented as mean (%CV)

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>DBS-derived values (n = 10)</th>
<th>Plasma (measured) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/mL)</td>
<td>2.70 (24.7)</td>
<td>3.34 (16)</td>
</tr>
<tr>
<td>C48 (μg/mL)</td>
<td>1.34 (35.6)</td>
<td>0.83 (26)</td>
</tr>
<tr>
<td>AUC0-24 (μg.h/mL)</td>
<td>37.80 (23.2)</td>
<td>43.40 (20)</td>
</tr>
</tbody>
</table>

CONCLUSION

- The developed method is simple, accurate and precise. Its application will expand opportunities to undertake clinical PK studies of DTG in key populations especially in limited-resource settings.
- PK of DTG was successfully characterised using DBS method.
- The reasons for lower PK parameters for DTG compared to previous studies using plasma samples warrant further investigation.

REFERENCES

2. Reid, S. A. et al. (2004); Journal of American Medical Director Association, 5, 395-400