A Validated Sensitive and Selective Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) Method for Quantitative Analysis of Tebipenem Pivoxil and Tebipenem, in Human Whole Blood and its Application in a Pharmacokinetic Study in Healthy Human Volunteers

Praveen Srivastava¹, Erika Manyak², Luke Utley², and Vipul Gupta¹
¹Spero Therapeutics, Cambridge MA, and ²Ribon Therapeutics, Cambridge MA

INTRODUCTION

Tebipenem pivoxil hydrobromide (TBP-PI-HBr) is an oral prodrug that is converted to tebipenem, the active moiety. TBP is a carbapenem with activity against multidrug-resistant Gram-negative pathogens, including extended-spectrum β-lactamase-producing Enterobacterales and is being developed for treating complicated urinary tract infections (cUTI) and acute pyelonephritis (AP).

OBJECTIVES

- To develop and validate an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay for quantification of TBP-PI and TBP in human whole blood.
- To evaluate the long-term stability of TBP and TBP-PI in various conditions.

METHODS

Analytical Method Details

An ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed for quantification of TBP-PI and TBP in whole blood.

- Sample preparation involved addition of isopropyl alcohol (IPA) as a stabilizer during whole blood sample collection to prevent conversion of TBP-PI to TBP following sample collection.
- 25 µL of mixed matrix (Potassium Oxalate/Sodium Fluoride [KOx/NaF]) Whole blood: IPA, (1:1), v/v) samples were extracted with 100% acetonitrile protein precipitation followed by dilution (1:4, v/v), with milli-Q water.
- A gradient program was used to elute the analytes using 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phase solvents, at a flowrate of 0.65 mL/min.
- Total run time was 2.75 min and the retention times for the internal standard (tebipenem-D5) and TBP were 1.05-1.15 minutes.
- Retention time for the internal standard (tebipenem-D5) and TBP was approximately 0.55-0.65 minutes.
- Accuracy and intra- and inter-assay precision of the method was determined by assaying 6 replicates of each of the validation samples at the lower limit of quantification (LLOQ), low, mid, and high concentration ranges, in three separate runs.

Stability

- Stability of TBP and TBP-PI was evaluated in:
  - Block solutions stored at -20°C.
  - 1:1 (v:v) isopropanol:human whole blood (NaF/KOx) supernatant stored at -80°C and -20°C.
  - 1:1 (v:v) isopropanol:human whole blood (NaF/KOx) stored at -80°C.
- Stability in solutions was evaluated by comparing the mean instrument response (peak area ratio) of freshly prepared solutions.
- Stability in matrix was evaluated based on measured concentrations relative to nominal concentrations.

RESULTS

Validation Studies

- Standard curves were linear over a large dynamic range (2-1000 ng/mL) with sufficient limits of quantification (Table 1).

Table 1. Validation Assay Summaries for Tebipenem and Tebipenem Pivoxil

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Human whole blood KOx/NaF</td>
<td>Protein precipitation of matrix at a ratio of 1:1 (v:v) human whole blood (KOx/NaF)</td>
<td>25 µL</td>
<td>Protein Precipitation</td>
<td>SCIEX API-5500</td>
<td>Electrospray ionization (positive-ion mode)</td>
<td>Multiple-reaction-monitoring scan mode</td>
<td>Linear, 1/x²</td>
<td>2.00 to 1000 ng/mL</td>
<td>7.0 → 5.1</td>
<td>0.0 ng/mL</td>
<td>120 minutes on ice</td>
<td>24 hours on ice</td>
<td>4 Cycles (-80°C/on ice)</td>
</tr>
</tbody>
</table>

- The UPLC-MS/MS assay method is robust and will be applied for clinical pharmacology studies to characterize the pharmacokinetics of TBP following oral administration of TBP-HBr.
- The stability under different storage conditions could allow for its use in diverse clinical settings.

SUMMARY AND CONCLUSIONS

- Validation results indicated that the assay is sufficiently linear, specific, reproducible, and accurate to support the analysis of TBP-PI and TBP in human whole blood (KOx/KOx).
- The UPLC-MS/MS assay method is robust and will be applied for clinical pharmacology studies to characterize the pharmacokinetics of TBP following oral administration of TBP-HBr.
- The stability under different storage conditions could allow for its use in diverse clinical settings.

Funded by Spero Therapeutics, Inc., Cambridge, MA

Phone: (857) 242-1600
psrivastava@sperotherapeutics.com

Spero Therapeutics
675 Massachusetts Ave
14th Floor
Cambridge, MA 02139

15th WRIB Virtual Meeting
Sept. 27 – Oct. 1, 2021

1Spero Therapeutics, Cambridge MA, and 2Ribon Therapeutics, Cambridge MA

Figures 1, 2, 3, and 4