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

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Novel Aspect

A rapid, selective, and sensitive ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed and successfully validated for quantitation of tebipenem pivoxil (TBP-PI) and tebipenem (TBP) in human whole blood.

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 quantitation of TBP-PI and TBP in human whole blood.
- To evaluate the long-term stability of TBP and TBP-PI ~~in~~ under 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 pivoxil-D5) and TBP-PI was approximately 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:
- Stock 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 the stored solutions to 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 quantitation (**Table 1**).

Table 1. Validation Assay Summaries for Tebipenem and Tebipenem Pivoxil

	Tebipenem	Tebipenem Pivoxil
Matrix and Anticoagulant	Human whole blood KOx/NaF)	
Matrix Processing	Protein precipitation of matrix at a ratio of 1:1 (v:v) human whole blood (KOx/NaF): IPA followed by centrifugation for supernatant	
Extraction Volume	25 µL	
Extraction Procedure	Protein Precipitation	
Instrumentation	SCIEX API-5500	
Detection	Electrospray ionization (positive-ion mode) Multiple-reaction-monitoring scan mode	
Regression, weighting	Linear, $1/x^2$	
Standard Curve Range	2.00 to 1000 ng/mL	
Quality Control Concentration	2.00 ng/mL (QC-LLOQ), 6.00 ng/mL (QC-Low), 40.0 ng/mL (QC-Mid) and 800 ng/mL (QC-High)	
Accuracy and Precision		
Intra-batch accuracy (% bias)	-7.0 → 5.1	-4.2 → 5.5
Intra-batch precision (% CV)	2.5 → 7.4	2.3 → 7.9
Inter-batch accuracy (% bias)	-4.0 → 1.0	-0.6 → 2.3
Inter-batch precision (% CV)	4.4 → 6.2	3.7 → 7.0
Dilution Linearity	5000 ng/mL (Dilution Factor = 10)	
Stability in Whole Blood	120 minutes on ice	30 minutes on ice
Short-Term Stability in Whole Blood Supernatant	24 hours on ice	19 hours on ice
Freeze/Thaw Stability in Whole Blood Supernatant	4 Cycles (-80°C/on ice)	
Reinjection Reproducibility	6 day refrigerated	

Validation Assay Summary

- Intra- and inter-day accuracy and precision were within 85%–115% and 15% CV, respectively.
- Recoveries were >75% for TBP-PI and TBP and their respective deuterated internal standards, in human whole blood, with matrix effects less than 20% over six batches of human whole blood.
- Carryover was evaluated on each run during the validation, and no significant carryover of peaks was observed.
- Dilution integrity quality control (QC) samples were analyzed to show that the method was suitable for analyzing human whole blood with analyte concentrations higher than upper limit of quantification.
- The lower limit of quantification for TBP was 2 ng/mL in treated whole blood.
- The analytical method showed excellent sensitivity, precision, and accuracy
- Chromatograms under various conditions characterized TBP and TBP-PI (**Figures 1, 2, and 3**).
- Standard curve regression, for TBP and TBP-PI (**Figures 4**)

Stability Studies

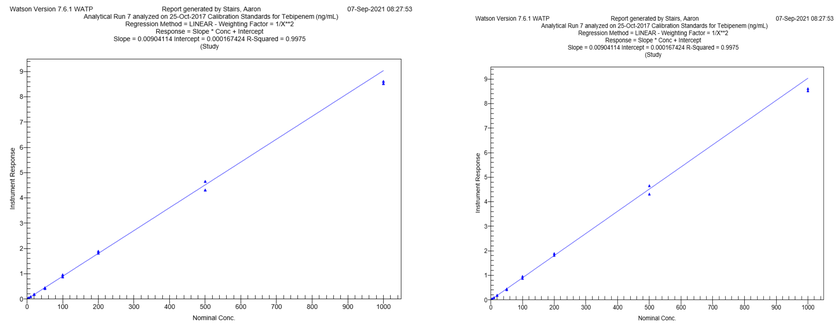
- TBP-PI and TBP in human whole blood remained stable at typical refrigerator temperatures (4°C) short term and under typical freezer conditions long term (-80°C).
- Long-term stability of TBP and TBP-PI in stock solution was established for 128 days and 203 days, respectively, at -20°C.
- Long-term stability of TBP and TBP-PI in 1:1 (v:v) isopropanol:human whole blood (NaF/KOx) supernatant was established for 398 days at -80°C.
- Long-term stability of TBP in 1:1 (v:v) isopropanol:human whole blood (NaF/KOx) supernatant was established for 3 days at -20°C.
- Stability of TBP-PI at -20°C could not be established.
- Stability of TBP and TBP-PI in 1:1 (v:v) isopropanol:human whole blood (NaF/KOx) was successfully monitored for 147 days at -80°C
- 2 hours of stability in whole blood on-ice was established for TBP
- 0.5 hour of stability in whole blood on-ice was established for TBP-PI
- Multiplexing was validated for TBP and TBP-PI.

Figure 1. Chromatograms for TBP (left) and TBP-PI (right) under control conditions

Figure 2. Chromatograms for TBP (left) and TBP-PI (right) under matrix conditions

Figure 3. Chromatograms for TBP (left) and TBP-PI (right) under standard conditions – LLOQ 2 ng/mL

Figure 4. Standard curve regression for TBP (left) and TBP-PI (right) in 1:1 (v:v) isopropanol:human whole blood (NaF/KOx)



SUMMARY AND CONCLUSIONS

- Validation results indicated that the assay is sufficiently linear, specific, reproducible, and accurate to support the analysis of TBP and TBP-PI in human whole blood (KOx/NaF).
- 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-PI HBr.
- The stability under different storage conditions could allow for its use in diverse clinical settings.

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