VALIDATED BIOANALYTICAL METHOD

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

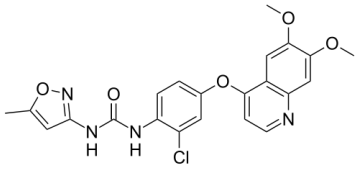
Director: Dr. James Mohler

Tivozanib in Human EDTA Plasma

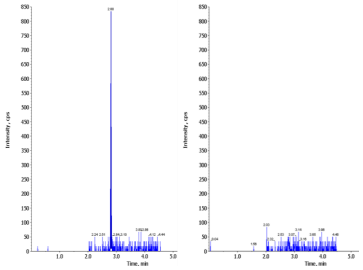
(Sensitivity: 0.500 ng/mL)

BMPK has validated a highly sensitive liquid chromatographic tandem mass spectrometric assay (LC-MS/MS) for the analysis of tivozanib in human EDTA plasma. Tivozanib is an oral, once-daily vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor, which is active against all three VEGF receptors. Tivozanib was approved in August, 2017 by the European Commission (EC) for use in the European Union, Norway and Iceland as the first line treatment for adult patients with advanced renal cell carcinoma (RCC) and those who are VEGFR and mTOR pathway inhibitor-naïve following disease progression after prior treatment with cytokine therapy for advanced RCC. The validated method was used to support a clinical trial conducted at Roswell Park Comprehensive Cancer Center entitled "Multicenter Phase 1b/2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma".

Specifications and Validation Performance	
Matrix (Anticoagulant):	Human Plasma (Dipotassium EDTA)
Required Volume:	100 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	C18
Mobile Phase:	Acetonitrile with Ammonium Acetate
Flow Rate:	500 µL/min
Detection Type:	Tandem Mass Spectral (MS/MS)
Calibration Ranges:	0.500 - 150 ng/mL
Calibrator Accuracy:	100% (97.1 - 102%; n=5)
Calibrator Precision:	2.60% CV (1.20 - 4.45%; n=5)
QC Concentrations:	1.50, 15.0 and 115 ng/mL
QC Accuracy:	106% (102 - 109%; n=18)
QC Precision:	3.85% CV (3.23 - 4.62%; n=18)



Tivozanib
Formula: $C_{22}H_{19}ClN_4O_5$
MW: 454.8698 g/mol



LLOQ (S/N = 16) Matrix Blank

Human Pharmacokinetic Parameters of Tivozanib[1,2]	
Recommended Dosing	0.50 - 1.5 mg/day
Maximum Tolerated Dose (MTD)	1.5 mg/day for 21 days followed by 7 day rest period
Bioavailability	71.8 - 82.4% in rats
Active Metabolites	None
Metabolism	~91% of the drug circulates unchanged (79% is eliminated in feces and 12% in urine unmetabolized)
Plasma Protein Binding	99.3% in humans; no gender effect
Maximum Plasma Concentration (C_{max})	10.2 - 25.2 ng/mL (1.34 mg single dose); accumulates 6-7-fold at steady state
Time to Maximum Plasma Concentration (T_{max})	2 - 24 hrs; variable due to enterohepatic recirculation
Terminal Half-Life ($t_{1/2}$)	4.5 - 5.1 days

[1]Tivozanib (AV-951) Investigator's Brochure, Aveo Pharmaceuticals, Version 12.1, and [2]EMA/CHMP/437168/2017

BMPK offers a wide range of bioanalytical and PK/PD modeling services to assist investigators in their basic research, preclinical, and clinical study objectives. For information on services and pricing, contact John Wilton, Ph.D., Associate Director, at (716) 845-3258 or John.Wilton@RoswellPark.org.

ROSWELL PARK COMPREHENSIVE CANCER CENTER

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