VALIDATED BIOANALYTICAL METHOD

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Director: Dr. James Mohler

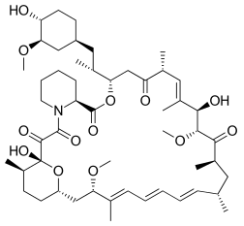
Rapamycin in Human Whole Blood

(Sensitivity: 1.25 ng/mL)

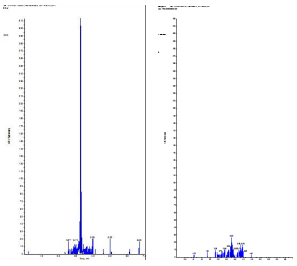
BMPK has validated a highly sensitive liquid chromatographic tandem mass spectrometric assay (LC-MS/MS) for the analysis of rapamycin, also known as sirolimus, in K2EDTA human whole blood (WB). Sirolimus is an mTOR inhibitor enhancing the immune response to tumor targeting. Sirolimus was initially approved by the FDA in 1999 as a potent immunosuppressive for transplant patients to minimize organ rejection. Oncology studies have utilized its immunosuppressive properties to sensitize cancer cells to be more responsive to various chemotherapeutic treatments. The validated method has been used to support Roswell Park clinical trials entitled "A Phase I Clinical Trial of mTOR Inhibition with Sirolimus for Enhancing AL VAC(2)-NY-ESO-I(M)ffRICOM Vaccine Induced Anti-Tumor Immunity in Ovarian, Fallopian Tube and Primary Peritoneal Cancer" and "A Phase I Clinical Trial of mTOR Inhibition with Rapamycin for Enhancing Intranodal Dendritic Cell Vaccine Induced Anti-Tumor Immunity in Patients with NY-ES0-1 Expressing Solid Tumors".

Specifications and Validation Performance

Matrix (Anticoagulant):	Whole Blood (K2EDTA)
Required Volume:	40.0 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	C18
Mobile Phase:	Aqueous methanol with Ammonium Acetate \ Formic Acid
Flow Rate:	500 µL/min
Detection Type:	Tandem Mass Spectral (MS/MS)
Calibration Ranges:	1.25 - 100 ng/mL
Calibrator Accuracy:	100% (97.6 - 105%; n=6)
Calibrator Precision:	2.63% CV (1.16 - 4.15%; n=6)
QC Concentrations:	3.75, 20.0 and 75.0 ng/mL
QC Accuracy:	102% (101 - 103%; n=21)
QC Precision:	6.08% CV (5.86 - 6.24%; n=21)



Rapamycin (Sirolimus)
$C_{51}H_{79}NO_{13}$
MW: 914.172



LLOQ Calibrator S/N = 14.5

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Human Pharmacokinetic Parameters of Rapamycin[1]

Recommended Dosing	2 - 5 mg; oral dose per day dependent on treatment type
Mechanism of Action	Binds to FKBP-12 creating a complex that binds to mTOR, which inhibits activation of mTOR and disrupts the cell cycle
Active Metabolites	None; 7 inactive metabolites
Metabolism	Substrate for CYP3A4 and P-gp. Metabolized in the intestinal wall and liver by O-demethylation and hydroxylation
Plasma Protein Binding	92% in humans
Maximum WB Concentration (C_{max})	15.0 ± 4.9 ng/mL with dose of one, 2 mg tablet per day
Terminal Elimination Half-Life ($t_{1/2}$)	62 ± 16 hours in stable renal transplant patients after multiple dosing

[1]Rapamune® Package Insert, 08/2018

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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