

Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource and St. Jude Children's Research Hospital, Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

Childhood Solid Tumor Network



Sai Life Sciences Limited

BIOANALYTICAL AND PHARMACOKINETICS REPORT

Date: 20th September 2016

Study Number: U5-DMPK-PK-16-Cabozantinib

Bioanalysis and Pharmacokinetics of Cabozantinib in CD1 Mouse plasma & Tumor Homogenate using Ultra Performance Liquid Chromatography and Tandem Mass Spectroscopy Using Glipizide as an Internal Standard

Study Number : U5-DMPK-PK-16-Cabozantinib

Sponsor : St. Jude

Testing Facility : DMPKT,
Sai Life Sciences Limited,
Building 1, Plot 2, Chrysalis Enclave,
International Biotech Park, Phase II, Hinjewadi,
Pune - 411 057, India

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1. UPLC and MS Conditions:

Chromatographic mode	:	AQUITY UPLC
MS System Used	:	AB Sciex API-4000
Software Version	:	Analyst 1.6
Scan Type	:	MRM
Polarity	:	Positive
Ion Source	:	Turbospray
Mobile Phase	:	A: 0.1% Formic acid in acetonitrile B: 10 mM Ammonium formate
Probe Position	:	5 mm vertical, and 5 mm horizontal
Injection volume (µL)	:	2
Auto sampler temperature (°C)	:	10
Column Oven temperature (°C)	:	45
Column Used (length x width in mm, Particle size)	:	Waters, XSelect CSH,C18, 2.1 X 50 mm, 2.5µm
Run times (in minutes)	:	Cabozantinib: 0.69 Glipizide:0.69



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UPLC Gradient used:

Pump A - 0.1 % Formic acid in acetonitrile

Pump B - 10 mm Ammonium formate

Time (Minutes)	Flow Rate (mL/min)	PUMP A (% Conc)	PUMP B (% Conc)
Initial	0.8	5	95
0.3	0.8	5	95
0.5	0.8	95	5
1.2	0.8	95	5
1.4	0.8	5	95
1.8	0.8	5	95

MRM Transitions used:

Analyte ID / IS ID	Q1	Q3	DP	CE	CXP	Dwell time (msec)
CABOZANTINIB_323	502.2	323.2	130	51	20	30
GLIPIZIDE	446.3	347.0	40	22	12	30

Source Parameters used:

Curtain Gas	30
Collision Gas	8
IS Voltage	5500
Temp	550
GS1	30
GS2	60
Interface Heater (ihe)	ON

2. Extraction Procedure:

The extraction procedure for plasma samples, tumor homogenate samples and the spiked plasma and tumor homogenate calibration standards were identical:

A 10 μL of study sample or spiked calibration standard was added to individual pre-labeled micro-centrifuge tubes followed by 200 μL of internal standard prepared in acetonitrile (Glipizide, 500 ng/mL) was added except for blank, where 200 μL of acetonitrile was added. Samples were vortexed for 5 minutes. Samples were centrifuged for 5 minutes at a speed of 4000 rpm at 4 $^{\circ}\text{C}$. Following centrifugation, 180 μL of clear supernatant was transferred in 96 well plates and analyzed using LC-MS/MS.

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3. Calibration Curve Details

Table 1: Calibration Curve of Cabozantinib in CD1 Mice Plasma

Calibration Standards	CS1		CS1_D		CS2		CS3		CS4		CS5	
Nominal Conc.	8.08		8.08		16.49		38.34		81.57		148.30	
Result Name	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc
CABOZANTINIB_01_MOUSE_PLM+TH_190916	8.42	104.24	8.13	100.56	18.08	109.65	36.98	96.45	85.04	104.26	154.27	104.03
CABOZANTINIB_01_MOUSE_PLM+TH_190916_BACK	7.45	92.15	8.03	99.40	16.41	99.49	37.10	96.75	82.07	100.61	147.79	99.66
CABOZANTINIB_REPEATS_MOUSE_PLM+TH_200916	7.61	94.13	7.95	98.34	16.95	102.79	36.13	94.24	80.93	99.22	147.74	99.62
CABOZANTINIB_REPEATS_MOUSE_PLM+TH_200916_BACK	7.77	96.15	9.02	111.64	16.38	99.31	37.55	97.95	83.53	102.41	150.68	101.61

Calibration Standards	CS6		CS7		CS8		CS8_D	
Nominal Conc.	247.17		352.10		493.13		493.13	
Result Name	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc
CABOZANTINIB_01_MOUSE_PLM+TH_190916	262.31	106.13	372.45	105.78	498.3	101.05	481.04	97.55
CABOZANTINIB_01_MOUSE_PLM+TH_190916_BACK	236.25	95.58	338.46	96.12	476.04	96.53	463.59	94.01
CABOZANTINIB_REPEATS_MOUSE_PLM+TH_200916	253.27	102.47	364.49	103.52	478.95	97.12	490.71	99.51
CABOZANTINIB_REPEATS_MOUSE_PLM+TH_200916_BACK	251.00	101.55	351.14	99.73	491.75	99.72	488.11	98.98

Result Name	Slope	Intercept	Regression Coefficient
CABOZANTINIB_01_MOUSE_PLM+TH_190916	0.00294	0.000802	0.9984
CABOZANTINIB_REPEATS_MOUSE_PLM+TH_200916	0.00252	0.000168	0.9989

Table 2: Quality control samples of Cabozantinib in CD1 Mice Plasma

Result Name	QC Sample Name	Nominal Conc (ng/mL)	Calculated Conc (ng/mL)	% Accuracy
Cabozantinib_01_Mouse_PLM+TH_190916	Cabozantinib_STABILITY_PLM_LQC-01	24.13	21.60	89.51
	Cabozantinib_STABILITY_PLM_LQC-02	24.13	21.77	90.24
	Cabozantinib_STABILITY_PLM_LQC-03	24.13	22.24	92.15
	Cabozantinib_STABILITY_PLM_MQC-01	305.41	281.40	92.14
	Cabozantinib_STABILITY_PLM_MQC-02	305.41	308.90	101.14
	Cabozantinib_STABILITY_PLM_MQC-03	305.41	284.42	93.13
	Cabozantinib_STABILITY_PLM_HQC-01	407.22	370.50	90.98
	Cabozantinib_STABILITY_PLM_HQC-02	407.22	366.05	89.89
	Cabozantinib_STABILITY_PLM_HQC-03	407.22	365.47	89.75
	Cabozantinib_PLM_LQC-01	24.16	20.55	85.04
	Cabozantinib_PLM_MQC-01	305.84	287.87	94.13
	Cabozantinib_PLM_HQC-01	407.79	371.02	90.98
	Cabozantinib_PLM_LQC-02	24.16	23.74	98.27
	Cabozantinib_PLM_MQC-02	305.84	266.74	87.22
	Cabozantinib_PLM_HQC-02	407.79	345.44	84.71
	Cabozantinib_PLM_LQC-03	24.16	20.58	85.20
	Cabozantinib_PLM_MQC-03	305.84	265.96	86.96
	Cabozantinib_PLM_HQC-03	407.79	353.80	86.76
Cabozantinib_Repeats_Mouse_PLM+TH_200916	Cabozantinib_PLM_LQC-01	24.16	20.95	86.71
	Cabozantinib_PLM_MQC-01	305.84	283.39	92.66
	Cabozantinib_PLM_HQC-01	407.79	368.47	90.36
	Cabozantinib_PLM_LQC-02	24.16	22.12	91.57
	Cabozantinib_PLM_MQC-02	305.84	278.35	91.01
	Cabozantinib_PLM_HQC-02	407.79	355.40	87.15
	Cabozantinib_PLM_LQC-03	24.16	22.56	93.36
	Cabozantinib_PLM_MQC-03	305.84	278.90	91.19
Cabozantinib_PLM_HQC-03	407.79	368.30	90.32	

Figure 1: Calibration Curve of Cabozantinib in CD1 Mice Plasma (Result Name: Cabozantinib_01_Mouse_PLM+TH_190916)

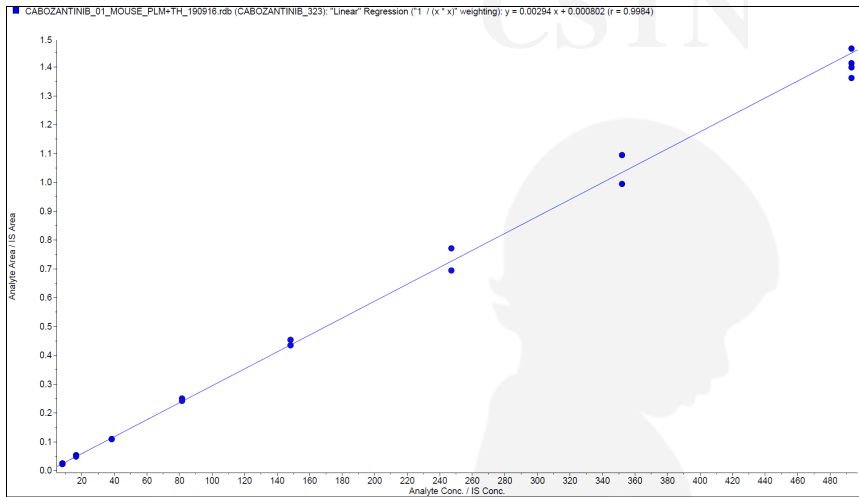
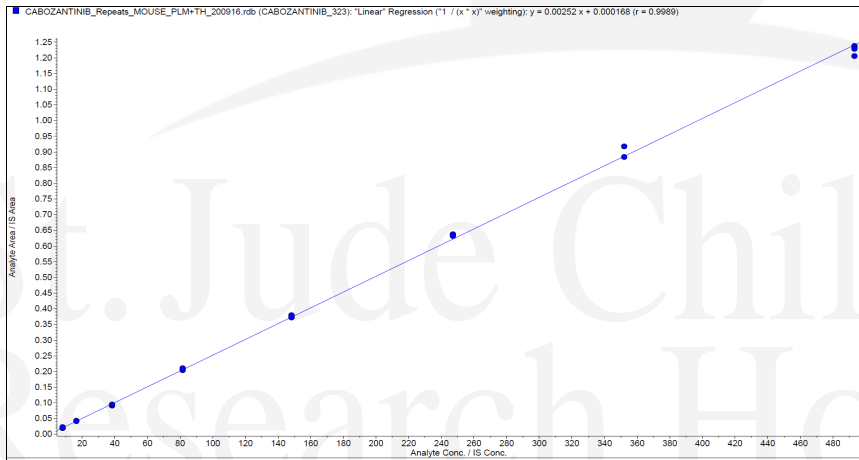


Figure 2: Calibration Curve of Cabozantinib in CD1 Mice Plasma (Result Name: Cabozantinib_Repeats_Mouse_PLM+TH_200916)



Study Sample Concentration Data

Table 3: Study Sample Concentration of Cabozantinib plasma sample
CC Range – 8.08-493.13 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Cabozantinib_PLM_10 Min-Mouse-01*	4300.80	1.00
Cabozantinib_PLM_10 Min-Mouse-02*	4387.96	1.00
Cabozantinib_PLM_10 Min-Mouse-03*	686.58	1.00
Cabozantinib_PLM_1 Hr.-Mouse-04*	3991.98	1.00
Cabozantinib_PLM_1 Hr.-Mouse-05*	4022.43	1.00
Cabozantinib_PLM_1 Hr.-Mouse-06*	3476.42	1.00
Cabozantinib_PLM_4 Hr.-Mouse-07*	3765.15	1.00
Cabozantinib_PLM_4 Hr.-Mouse-08*	4891.51	1.00
Cabozantinib_PLM_4 Hr.-Mouse-09*	6586.90	1.00
Cabozantinib_PLM_8 Hr.-Mouse-10*	1956.10	1.00
Cabozantinib_PLM_8 Hr.-Mouse-11*	1914.49	1.00
Cabozantinib_PLM_8 Hr.-Mouse-12*	3069.77	1.00
Cabozantinib_PLM_24 Hr.-Mouse-13	109.78	1.00
Cabozantinib_PLM_24 Hr.-Mouse-14	22.06	1.00
Cabozantinib_PLM_24 Hr.-Mouse-15	241.11	1.00

Note: * = Samples were above ULOQ hence analysis was repeated and repeat values are reported below.

Table 4: Repeat Study Sample Concentration of Cabozantinib plasma sample
CC Range – 8.08-493.13 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Cabozantinib_PLM_10 Min-Mouse-01_REP	4555.96	20.00
Cabozantinib_PLM_10 Min-Mouse-02_REP	4567.06	20.00
Cabozantinib_PLM_10 Min-Mouse-03_REP	662.59	20.00
Cabozantinib_PLM_1 Hr.-Mouse-04_REP	3942.97	20.00
Cabozantinib_PLM_1 Hr.-Mouse-05_REP	4323.99	20.00
Cabozantinib_PLM_1 Hr.-Mouse-06_REP	3238.98	20.00
Cabozantinib_PLM_4 Hr.-Mouse-07_REP	3628.94	20.00
Cabozantinib_PLM_4 Hr.-Mouse-08_REP	5290.39	20.00
Cabozantinib_PLM_4 Hr.-Mouse-09_REP	5933.43	20.00
Cabozantinib_PLM_8 Hr.-Mouse-10_REP	1938.26	20.00
Cabozantinib_PLM_8 Hr.-Mouse-11_REP	1875.12	20.00
Cabozantinib_PLM_8 Hr.-Mouse-12_REP	3167.79	20.00

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Table 5: Study Sample Concentration of Cabozantinib tumour sample
CC Range – 8.08-493.13 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Cabozantinib_TH_10 Min-Mouse-01	32.34	1.00
Cabozantinib_TH_10 Min-Mouse-02	67.48	1.00
Cabozantinib_TH_10 Min-Mouse-03#	4.79	1.00
Cabozantinib_TH_1 Hr.-Mouse-04	185.65	1.00
Cabozantinib_TH_1 Hr.-Mouse-05	251.67	1.00
Cabozantinib_TH_1 Hr.-Mouse-06	122.17	1.00
Cabozantinib_TH_4 Hr.-Mouse-07*	839.22	1.00
Cabozantinib_TH_4 Hr.-Mouse-08*	594.13	1.00
Cabozantinib_TH_4 Hr.-Mouse-09*	759.54	1.00
Cabozantinib_TH_8 Hr.-Mouse-10	393.33	1.00
Cabozantinib_TH_8 Hr.-Mouse-11	300.85	1.00
Cabozantinib_TH_8 Hr.-Mouse-12	368.29	1.00
Cabozantinib_TH_24 Hr.-Mouse-13	62.23	1.00
Cabozantinib_TH_24 Hr.-Mouse-14	32.88	1.00
Cabozantinib_TH_24 Hr.-Mouse-15	42.69	1.00

Note: * = Samples were above ULOQ hence analysis was repeated and repeat values are reported below.

Note: * = Samples were above ULOQ so will be repeated.

#= Sample repeated due to not matching concentration profile in 10 min samples.

Table 6: Repeat Study Sample Concentration of Cabozantinib tissue sample
CC Range – 8.08-493.13 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Cabozantinib_TH_10 Min-Mouse-03_REP	4.28	1.00
Cabozantinib_TH_4 Hr.-Mouse-07_REP	793.49	10.00
Cabozantinib_TH_4 Hr.-Mouse-08_REP	459.79	10.00
Cabozantinib_TH_4 Hr.-Mouse-09_REP	767.16	10.00

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Sponsor: St. Jude
Prepared by: DMPK, Sai Life Sciences Ltd.

Study No: U5-DMPK-PK-16-Cabozantinib

Childhood Solid Tumor Network CSTN



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Pharmacokinetic Analysis:

Plasma concentration and tumor concentration time data was received from bio-analytical team and subjected to Pharmacokinetics analysis using the non-compartmental analysis tool of Phoenix WinNonlin (Version 6.3). In-life phase was performed at St. Jude facility and samples were submitted for analysis at SAI life sciences limited. The brief study design was summarized as:

Dose (mg/kg): Cabozantinib 30 mg/kg

Route of administration: Oral gavage at dose volume of 10 mL/kg

Dilution factor (DF) for tumor samples (or any other criteria): DF of 6 for all tumor homogenates (1 part of tumor: 5 parts aqueous by w/v)

Number of animals: Total fifteen animals (n =3 per time points)

Time points (hr): 0.17, 1, 4, 8 and 24 (plasma and tumor)

Table 7: Mean oral plasma and tumor pharmacokinetic parameters of Cabozantinib

Compound		Cabozantinib		
Pharmacokinetics Parameter/Matrix		Plasma	Tumor	Kp_tumor
T _{max}	(hr)	4.00	4.00	-
C _{max}	(ng/mL)	4950.92	4040.88	0.82
T _{last}	(hr)	24.00	24.00	-
C _{last}	(ng/mL)	124.32	275.60	-
AUC _{last}	(hr*ng/mL)	50568.88	39889.86	0.79
AUC _{INF_pred}	(hr*ng/mL)	51243.42	41945.77	0.82
AUC%Extrap _{pred}	(%)	1.32	4.90	-
AUM _{clast}	(hr*hr*ng/mL)	286968.10	281651.02	-
T _{1/2}	(hr)	3.77	5.24	-
Cl _{Fpred}	(mL/min/kg)	9.76	11.92	-
Vz _{Fpred}	(L/kg)	3.18	5.40	-
Rsq_adjusted		0.99	0.99	-

Tumor concentrations were expressed as ng/g or hr*ng/g

Following single oral administration of Cabozantinib at 30 mg/kg dose, plasma and tumor concentrations were quantifiable till 24 hr with T_{max} at 4 hr for both the matrices.

In general, the K_p values for tumor was ranged from 0.79 to 0.82 considering the C_{max},

AUC_{last} and AUC_{INF_pred} exposures.

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Table 8: Individual plasma concentration-time data of Cabozantinib following single oral administration (Dose: 30 mg/kg)

Compound	Route	Matrix	Animal	Time (hr)					
				0.17	1	4	8	24	
				Concentration (ng/mL)					
Cabozantinib	Oral	Plasma	1	4555.96					
			2	4567.06					
			3	662.59					
			4	3942.97					
			5	4323.99					
			6	3238.98					
			7	3628.94					
			8	5290.39					
			9	5933.43					
			10	1938.26					
			11	1875.12					
			12	3167.79					
			13	109.78					
			14	22.06					
			15	241.11					
Mean				3261.87	3835.31	4950.92	2327.06	124.32	
SD				2251.05	550.46	1189.16	728.78	110.25	
CV%				69.01	14.35	24.02	31.32	88.68	

LLOQ- 8.08 ng/mL

Table 9: Individual tumor concentration-time data of Cabozantinib following single oral administration (Dose: 30 mg/kg)

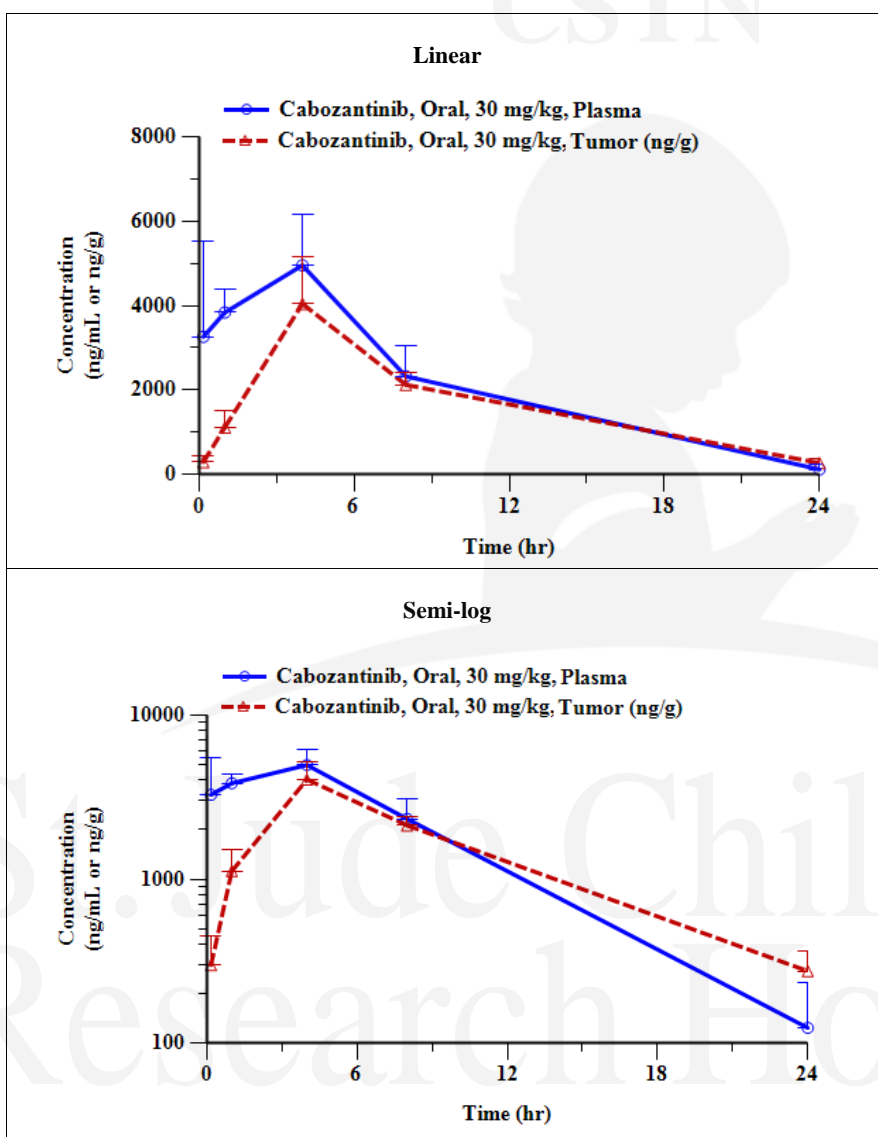
Compound	Route	Matrix	Animal	Time (hr)					
				0.17	1	4	8	24	
				Concentration (ng/g)					
Cabozantinib	Oral	Tumor	1	194.04					
			2	404.88					
			3	0.00					
			4		1113.9				
			5		1510.02				
			6		733.02				
			7			4760.94			
			8			2758.74			
			9			4602.96			
			10					2359.98	
			11					1805.1	
			12					2209.74	
			13						373.38
			14						197.28
			15						256.14
Mean				299.46^a	1118.98	4040.88	2124.94	275.6	
SD				NA	388.52	1113.17	287	89.65	
CV%				NA	34.72	27.55	13.51	32.53	

LLOQ = 48.48 ng/g; NA- not applicable; ^a - Average of two values reported and considered for PK data analysis; NA- not applicable; Value below LLOQ was considered as zero.

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Figure 3: Mean plasma and tumor concentration-time (ng/mL for plasma and ng/g for tumor) profile of following single oral administration (Dose: 30 mg/kg)



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Annexure:

Representative Chromatograms

Figure 4: Representative Chromatogram of Cabozantinib in Blank plasma

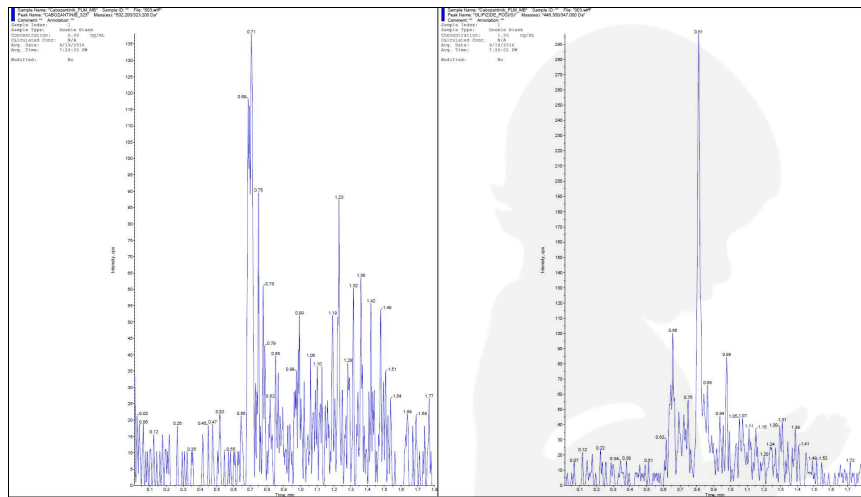


Figure 5: Representative Chromatogram of Cabozantinib Plasma LLOQ Standard

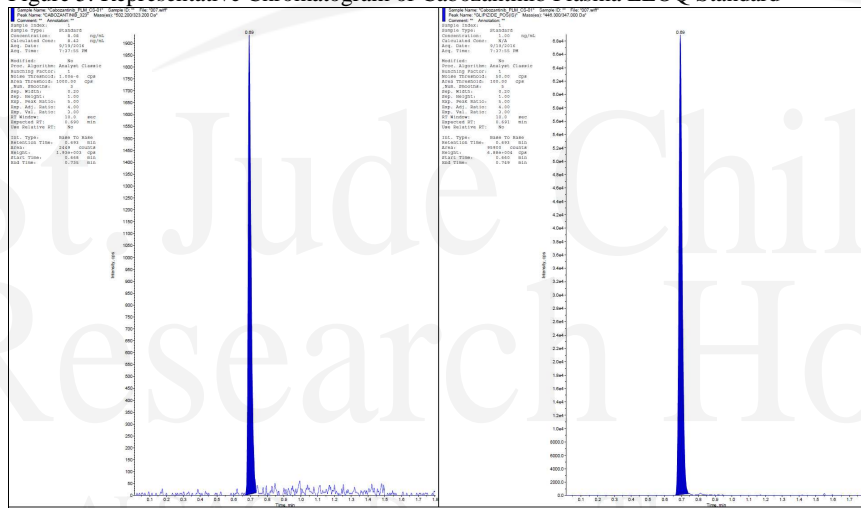


Figure 6: Representative Chromatogram of Cabozantinib Plasma ULOQ Standard

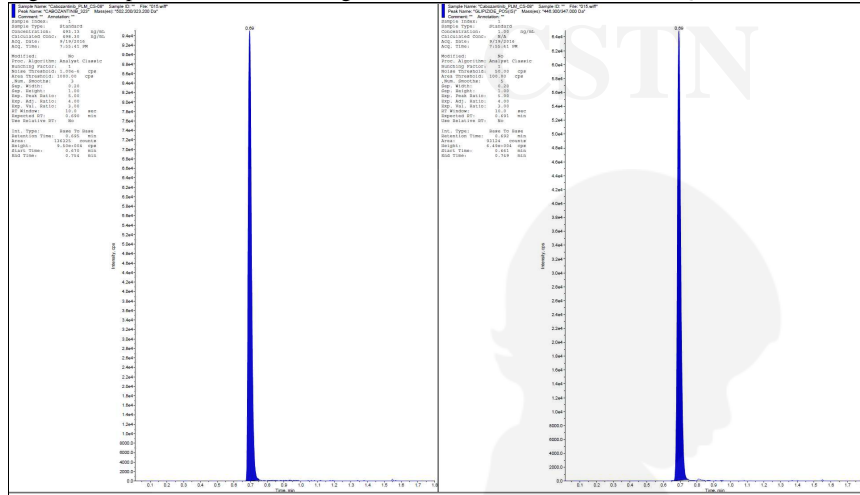
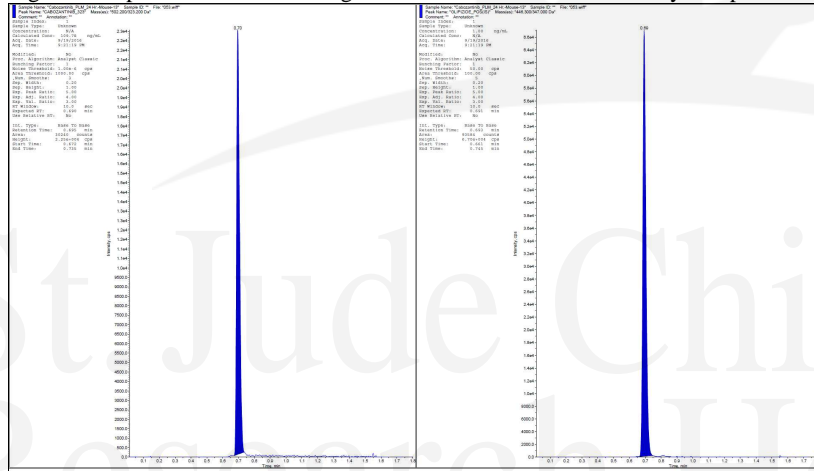


Figure 7: Representative Chromatogram of Cabozantinib Plasma Study Sample



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