Sai Life Sciences Limited

PHARMACOKINETIC REPORT

Study Number: SAIDMPK/PK-19-11-918

Study Title

Plasma pharmacokinetics and brain distribution of Compound-781 following single intraperitoneal (Dose: 10 and 30 mg/kg) dose administration in male C57BL/6 mice

Sponsor

Theodore Price PhD
University of Texas at Dallas
BSB 14.102G 800 W Campbell Rd
Richardson
TX 75080 phone: 972-883-4311
cell: 520-471-0360 fax: 972-883-2491
theodore.price@utdallas.edu

Test Facility

DMPK, Sai Life Sciences Limited
Building 1, Plot 2, Chrysalis Enclave
International Biotech Park, Phase II Hinjewadi
Pune - 411 057 INDIA
Phone: +91-20-30125000

Date of Completion of Report: November 2019

[redacted]

Contents

List of Tables

List of Figures

List of Abbreviations

LLOQ	Lower Limit of Quantitation
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CV	Coefficient of Variation
IAEC	Institutional Animal Ethics Committee
IS	Internal Standard
LC-MS/MS	Liquid Chromatography Mass Spectrometry
NA	Not Applicable
IP	Intraperitoneal
SD	Standard Deviation
SOP	Standard Operating Procedure
C_{max}	Maximum concentration
T_{max}	Time to reach maximum concentration
AUC	Area under Plasma concentration - Time curve

Study Responsibilities

Study Director :	Aslam Burhan, Ph.D.
Formulation:	Anandkumar Yadav, M. Pharm.
In-Life Phase:	Mahesh Rahinj, M. Pharm. Amol S. Rasal, M. Pharm. Nilkanth Naik, M. Pharm. Anandkumar Yadav, M. Pharm. Suraj Lanke, M. Pharm. Sanjay Bedare, M. S. Pharm. Naveenkumar Sapa, M. Pharm.
Bioanalysis:	Srikant Jadhav, M. Pharm.
Data Analysis and Report Prepared By:	Mahesh Rahinj, M. Pharm.
QC By:	Sapana Chavan, M.Sc.
Report Reviewed By:	Aslam Burhan, Ph.D.

1.0 Summary

The objective of this study was to investigate the plasma pharmacokinetics and brain distribution of Compound-781 following single intraperitoneal dose administration in male C57BL/6 mice at 10 and 30 mg/kg dose. A group of twelve male mice were divided into two groups as:

Group 1; 10 mg/kg/IP; (n = 6; Animal # 1-6),

Group 2; 30 mg/kg/IP; (n = 6; Animal # 7-12).

Animals in Group 1 and Group 2 were administered intraperitoneally with Compound-781 solution formulation in normal saline at 10 and 30 mg/kg dose respectively.

Blood samples (approximately 60 μL) were collected under light isoflurane anesthesia from retro orbital plexus such that samples were obtained at 0.08, 0.5, 1, 2, 4 and 8 hr (IP). At each time point blood samples were collected from three mice. Immediately after collection, plasma was harvested by centrifugation and stored at -70ºC until analysis..

Immeadiately after collection of blood, animals were euthanized with excess CO_2 asphyxiation. Brain samples were collected from mice at 4 and 8 hr from respective animal. Brain samples were homogenized using ice-cold phosphate buffer saline (pH-7.4). Total homogenate volume was three times the brain weight.

All samples were processed for analysis by protein precipitation using acetonitrile and analyzed with fit-for-purpose LC/MS/MS method (LLOQ = 1.01 ng/mL for plasma, 3.03 ng/g for brain).

Pharmacokinetic parameters were calculated using the non-compartmental analysis tool of Phoenix WinNonlin (Version 7.0). The overall pharmacokinetic parameters are summarized below:

Matrix	Route	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)
Plasma	IP	10	0.50	4349.71	3322.51	3365.94
		30	0.08	24784.83	17285.49	17310.36

10 mg/kg/IP: Following a single intraperitoneal dose administration of Compound-781 at 10 mg/kg to male C57BL/6 mice, the plasma concentrations were observed up to 8 hr with T_{max} at 0.5 hr. Brain concentrations were quantifiable at 4 and 8 hr, Brain-to-plasma ratio were 1.05 (4 hr) and 0.71 (8 hr).

30 mg/kg/IP: Following a single intraperitoneal dose administration of Compound-781 at 30 mg/kg to male C57BL/6 mice, the plasma concentrations were observed up to 8 hr with T_{max} at 0.08 hr. Brain concentrations were quantifiable at 4 and 8 hr, Brain-to-plasma ratio were 0.54 (4 hr) and 8.42 (8 hr).

2.0 Study Objective

To investigate the plasma pharmacokinetics and brain distribution of Compound-781 following single intraperitoneal dose administration in male C57BL/6 mice at 10 and 30 mg/kg dose..

3.0 Test Guidelines / SOPs / Compliance

The study was conducted at Sai Life Sciences Limited, Pune, India, in accordance with the Study Plan SAIDMPK/PK-19-11-918.

This study was performed with approval of Institutional Animal Ethics Committee (IAEC) in accordance with requirement of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The study was not performed as per GLP regulations and not audited by QA; however all appropriate documentation is maintained in study file. Study phases, data generated and the report have been verified for the accuracy by the study group.

4.0 Animal Welfare

All procedures of the present study were in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study.

5.0 Experimental

5. 1 Test Compound

The test compound Compound-781 (Mol. Wt: 515.30, Purity: 98.142%) was received from Sponsor.

5. 2 Test System

Healthy male C57BL/6 mice (8-12 weeks old) weighing between 20 to 35 g were procured from ACTREC, India. Three mice were housed in each cage. Temperature and humidity were maintained at 22 ± 3 ºC and 30-70%, respectively and illumination was controlled to give a sequence of 12 hr light and 12 hr dark cycle. Temperature and humidity were recorded by auto-controlled data logger system. All

the animals were provided laboratory rodent diet (Envigo Research private Ltd, Hyderabad). Reverse osmosis water treated with ultraviolet light was provided *ad libitum*.

5. 3 Study Design

A group of twelve male mice were divided into two groups as:

Group 1; 10 mg/kg/IP; (n = 6; Animal # 1-6),

Group 2; 30 mg/kg/IP; (n = 6; Animal # 7-12).

Animals in Group 1 and Group 2 were administered intraperitoneally with Compound-781 solution formulation in normal saline at 10 and 30 mg/kg dose respectively.

The dosing volume administered was 10 mL/kg. The assignment of animals was shown in the table below:

Group	Route	Dose (mg/kg)	Animal ID
1	IP	10	6 (1- 6)
2	IP	30	6 (7-12)

5. 4 Formulation Preparation

The strengths of intraperitoneal solution formulations were 1 mg/mL and 3 mg/mL.

Ingredients	IP (1 mg/mL)	IP (3 mg/mL)
Compound-781	2.77 mg	7.50 mg
Normal saline (100%)	2.718 mL	2.453 mL

1 mg/mL: Accurately weighed quantity 2.77 mg of Compound-781 for IP dosing was added in a labeled bottle. The volume 2.718 mL of normal saline was added. The formulation was vortexed for 2 minutes to get clear solution.

3 mg/mL: Accurately weighed quantity 7.50 mg of Compound-781 for IP dosing was added in a labeled bottle. The volume 2.453 mL of normal saline was added. The formulation was vortexed for 2 minutes to get clear solution.

5.4.1 Formulation Results

After preparation of formulations, a volume of 200 µL was aliquoted for analysis. The formulations were analyzed and found to be within the acceptance criteria (in-house acceptance criteria is ± 20% from the nominal value). Formulations were prepared freshly prior to dosing.

Compound	Formulation	Theoretical Conc. (mg/mL)	Conc. Found (mg/mL)	% Change
Compound-781	IP	1.00	1.03	3.00
		3.00	3.11	3.67

5. 5 Observations

All the animals were found to be normal without showing any clinical signs after intraperitoneal dose administration at 10 and 30 mg/kg dose.

5. 6 Sample Collection

Blood: Blood samples (approximately 60 µL) were collected under light isoflurane anesthesia from retro orbital plexus such that samples were obtained at 0.08, 0.5, 1, 2, 4 and 8 hr (IP). At each time point blood samples were collected from three mice. Immediately after collection, plasma was harvested by centrifugation and stored at -70ºC until analysis..

Brain: Immeadiately after collection of blood, animals were euthanized with excess CO_2 asphyxiation. Brain samples were collected from mice at 4 and 8 hr from respective animal. Brain samples were homogenized using ice-cold phosphate buffer saline (pH-7.4). Total homogenate volume was three times the brain weight.

5. 7 Bioanalysis

Concentrations of Compound-781 in mouse plasma and brain samples were determined by fit for purpose LC-MS/MS method. The sample processing and extraction procedure, chromatographic and mass spectrometric conditions were presented in Annexure I.

6.0 Data Analysis

Non-compartmental analysis module in Phoenix WinNonlin® (Version 7.0) was used to assess the pharmacokinetic parameters. Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) were the observed values. The areas under the concentration time curve (AUC_{last} and AUC_{inf}) and elimination half-life was calculated by linear trapezoidal rule. The terminal elimination rate constant, ke was determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve.

7.0 Results

10 mg/kg/IP: Following a single intraperitoneal dose administration of Compound-781 at 10 mg/kg to male C57BL/6 mice, the plasma concentrations were observed up to 8 hr with T_{max} at 0.5 hr. Brain concentrations were quantifiable at 4 and 8 hr, Brain-to-plasma ratio were 1.05 (4 hr) and 0.71 (8 hr).

30 mg/kg/IP: Following a single intraperitoneal dose administration of Compound-781 at 30 mg/kg to male C57BL/6 mice, the plasma concentrations were observed up to 8 hr with T_{max} at 0.08 hr. Brain concentrations were quantifiable at 4 and 8 hr, Brain-to-plasma ratio were 0.54 (4 hr) and 8.42 (8 hr).

8.0 Data Archiving

All raw data, study protocol, and final report were documented and will be archived. The materials (hard and soft copies) will be retained for 1 year from the date of approval of final report. Thereafter, the archived material will be destroyed or stored for extended period as per written consent from the sponsor.

Table 1: Plasma pharmacokinetic parameters of Compound-781 following a single intraperitoneal dose administration to male C57BL/6 mice (Dose: 10 and 30 mg/kg)

Matrix	Route	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)
Plasma	IP	10	0.50	4349.71	3322.51	3365.94
		30	0.08	24784.83	17285.49	17310.36

Table 2: Individual plasma concentration-time data of Compound-781 following a single intraperitoneal administration to male C57BL/6 mice (Dose: 10 mg/kg)

Animal ID	Plasma concentration (ng/mL)					
	Time (hr)					
	0.08	**0.5**	**1**	**2**	**4**	**8**
1	3317.75		102.90		13.63	
2	3309.46		119.85		13.17	
3	3259.79		102.13		12.48	
4		4337.01		232.58		13.23
5		4414.25		225.41		14.40
6		4297.88		240.20		14.57
Mean	**3295.67**	**4349.71**	**108.29**	**232.73**	**13.09**	**14.07**
SD	31.35	59.22	10.02	7.40	0.58	0.73
CV%	0.95	1.36	9.25	3.18	4.42	5.19

LLOQ = 1.01 ng/mL.

Table 3: Individual plasma concentration-time data of Compound-781 following a single intraperitoneal administration to male C57BL/6 mice (Dose: 30 mg/kg)

Animal ID	Plasma concentration (ng/mL)					
	Time (hr)					
	0.08	**0.5**	**1**	**2**	**4**	**8**
7	26566.26		2081.97		88.93	
8	24204.45		2049.00		87.70	
9	23583.77		1987.90		83.69	
10		17713.37		943.75		19.22
11		17959.73		1060.84		20.26
12		15151.17		930.26		18.67
Mean	**24784.83**	**16941.42**	**2039.62**	**978.28**	**86.77**	**19.38**
SD	1573.67	1555.29	47.73	71.81	2.74	0.81
CV%	6.35	9.18	2.34	7.34	3.16	4.17

LLOQ = 1.01 ng/mL.

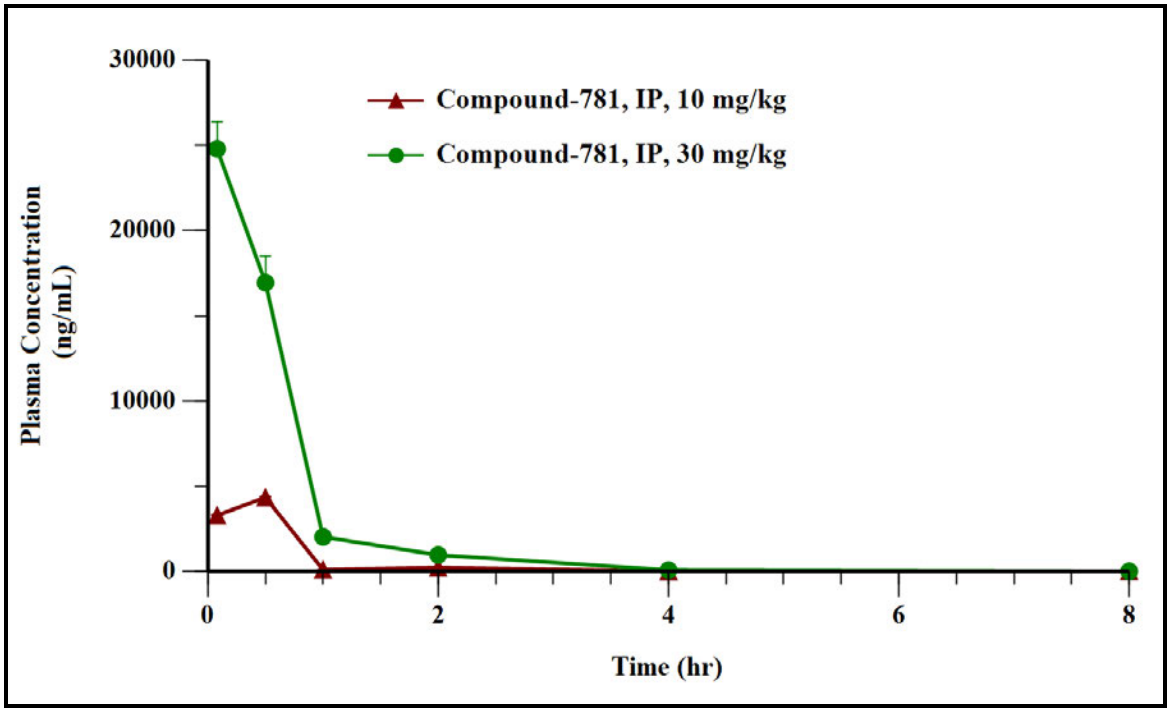
Table 4: Mean brain-to-plasma concentration ratio of Compound-781 following a single intraperitoneal administration to male C57BL/6 mice (Dose: 10 and 30 mg/kg)

Dose (mg/kg)	Time (hr)	Animal ID	Plasma concentration (ng/mL)	Brain concentration (ng/g)	Brain-to-Plasma Ratio	Mean Ratio
10	4	1	13.63	6.33	0.46	**1.05**
		2	13.17	19.86	1.51	
		3	12.48	14.61	1.17	
	8	4	13.23	9.57	0.72	**0.71**
		5	14.40	8.43	0.59	
		6	14.57	12.15	0.83	
30	4	7	88.93	45.72	0.51	**0.54**
		8	87.70	49.68	0.57	
		9	83.69	45.12	0.54	
	8	10	19.22	26.82	1.40	**8.42**
		11	20.26	336.66	16.62	
		12	18.67	135.15	7.24	

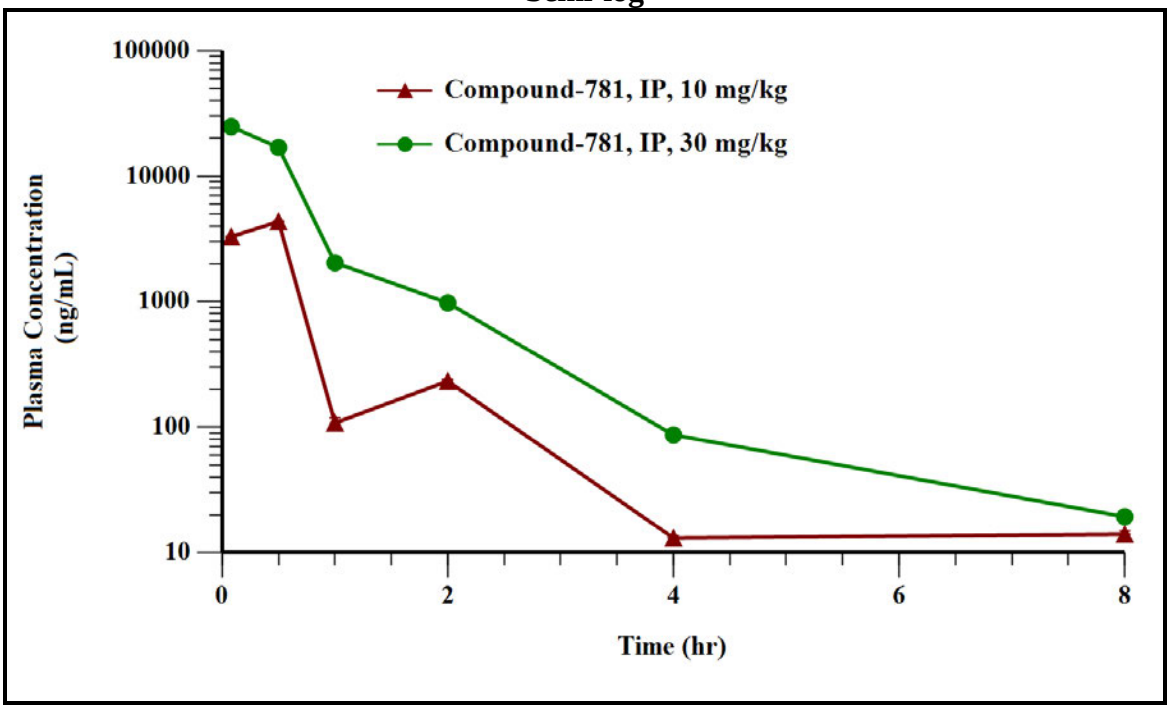
LLOQ = 3.03 ng/g for brain and 1.01 ng/mL for plasma.

Figure 1: Mean plasma concentration-time profiles of Compound-781 following a single intraperitoneal dose administration to male C57BL/6 mice (Dose: 10 and 30 mg/kg)

Linear



Semi-log



9.0 Annexure I

Bioanalytical Summary

LC Conditions:

Mobile Phase A: 0.1% Formic acid in Acetonitrile

B: 10 Mm Ammonium Formate

Column : Accucore Phnyl X, 2.6µ, 50 X 2.1 mm

Injection Volume (µL) **:** 5

Column Oven Temperature (ºC) **:** 45

Retention Time (in min) **: Analyte:** Compound-781 : 0.97

IS: Glipizide: 1.07

LC Gradient Used

Time (Minutes)	Flow Rate (mL/min)	PUMP A (% Conc)	PUMP B (% Conc)
Initial	0.7	0	100
0.30	0.7	0	100
0.50	0.7	95	5
1.30	0.7	95	5
1.40	0.7	0	100
1.80	0.7	0	100

Mass Conditions

MRM Transitions:

Analyte ID / IS ID	Q1	Q3	DP	CE	CXP	Dwell time (msec)
COMPOUND-781 252	516.4	252.2	108	37	18	50
Glipizide	446.3	347.0	40	22	12	50

Source Parameter:

Polarity	Positive
CAD	8
CUR	25
GS1	40
GS2	60
Ion Spray Voltage	5500
Temperature	550
Interface Heater	ON
EP	10

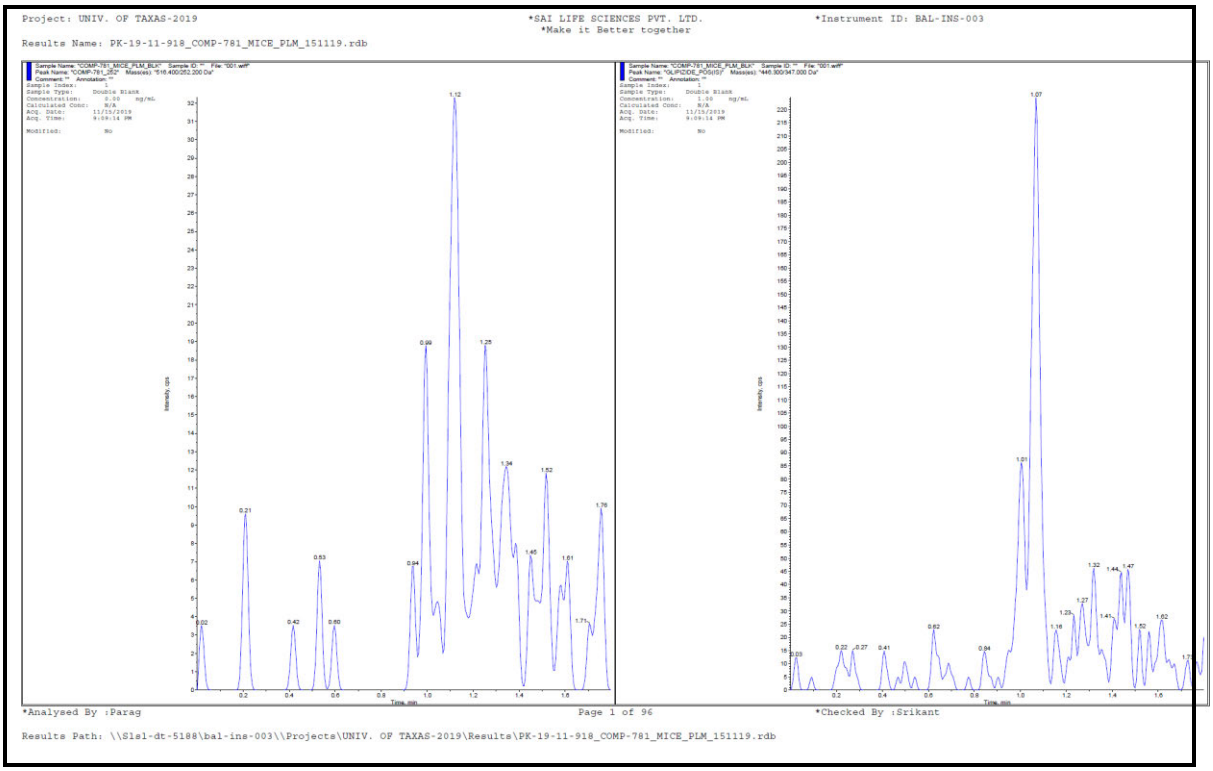
Extraction Procedure:

The extraction procedure for plasma/brain samples and the spiked plasma/brain calibration standards were identical:

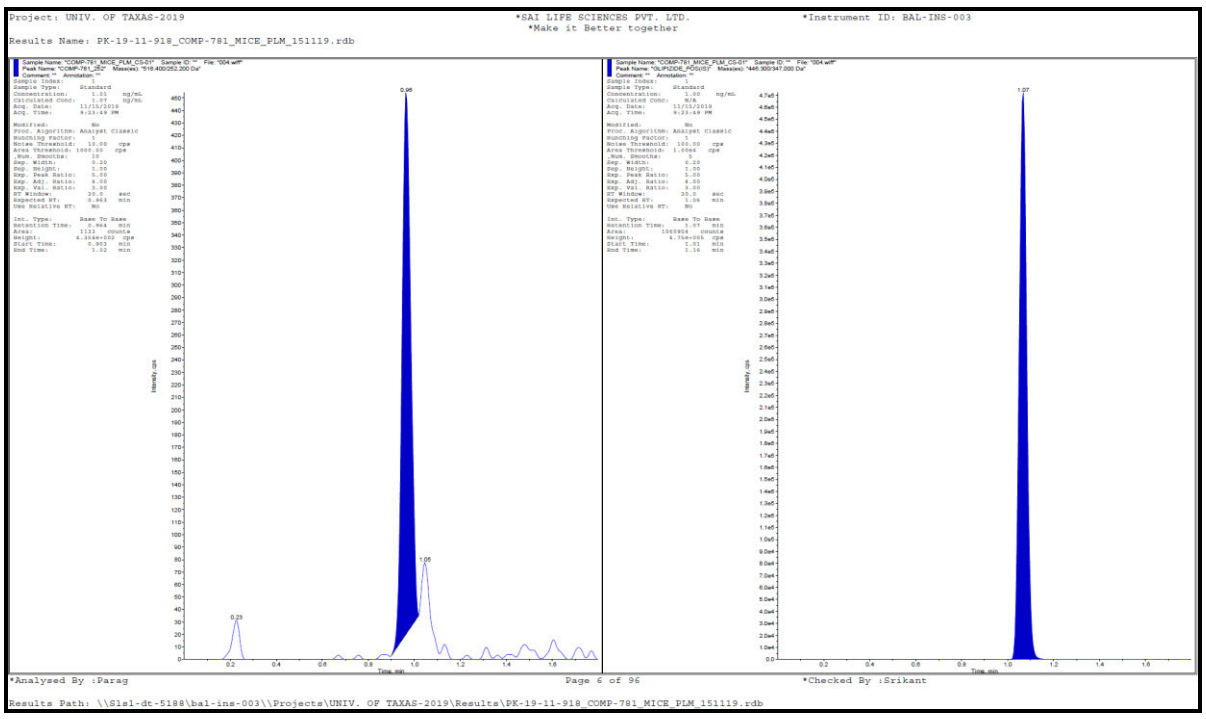
A 25 µL of study sample or spiked plasma/brain calibration standard was added to individual pre-labeled micro-centrifuge tubes followed by 100 µL of internal standard prepared in Acetonitrile (Glipizide, 500 ng/mL) was added except for blank, where 100 µL of Acetonitrile was added. Samples were vortexed for 5 minutes. Samples were centrifuged for 10 minutes at a speed of 4000 rpm at 4 °C. Following centrifugation, 100 µL of clear supernatant was transferred in 96 well plates and analyzed using LC-MS/MS.

Representative Chromatograms

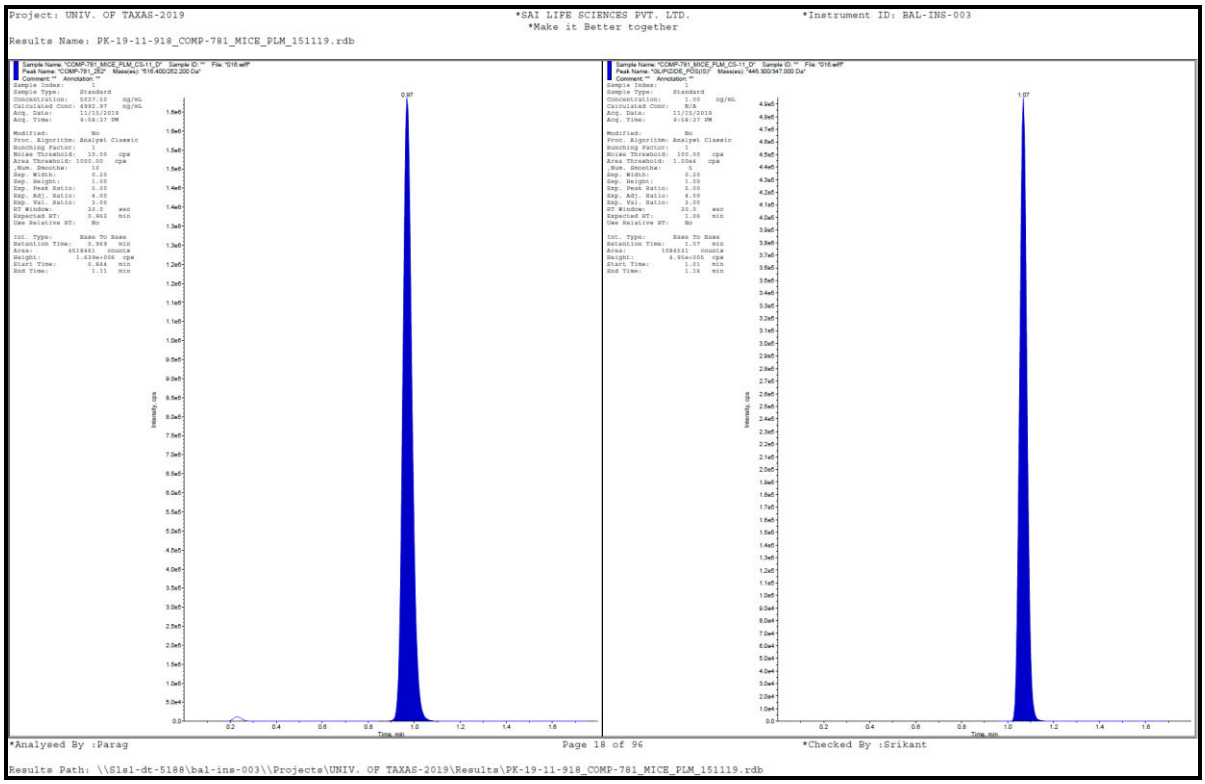
Representative chromatogram of blank mice plasma in the LC-MS/MS analysis of COMPOUND-781



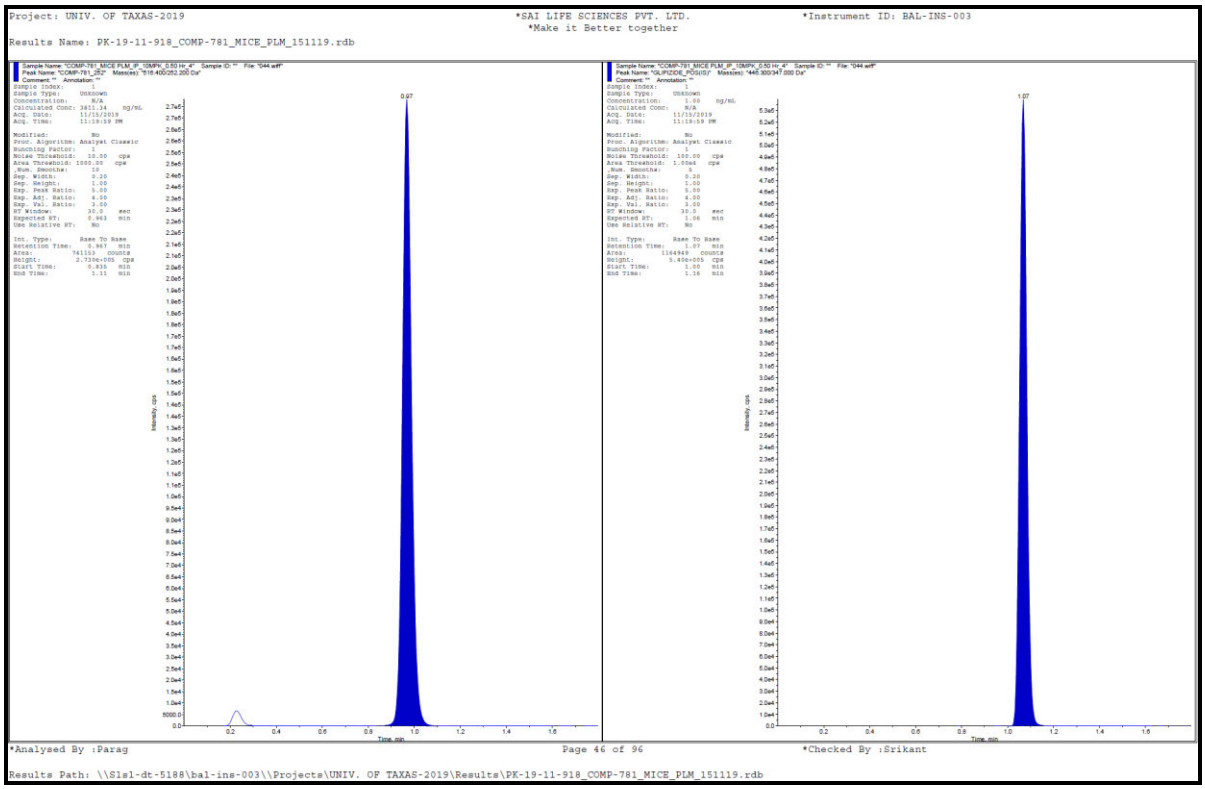
Representative LC-MS/MS chromatogram of COMPOUND-781 LLOQ standard and Glipizide (IS) in mice plasma



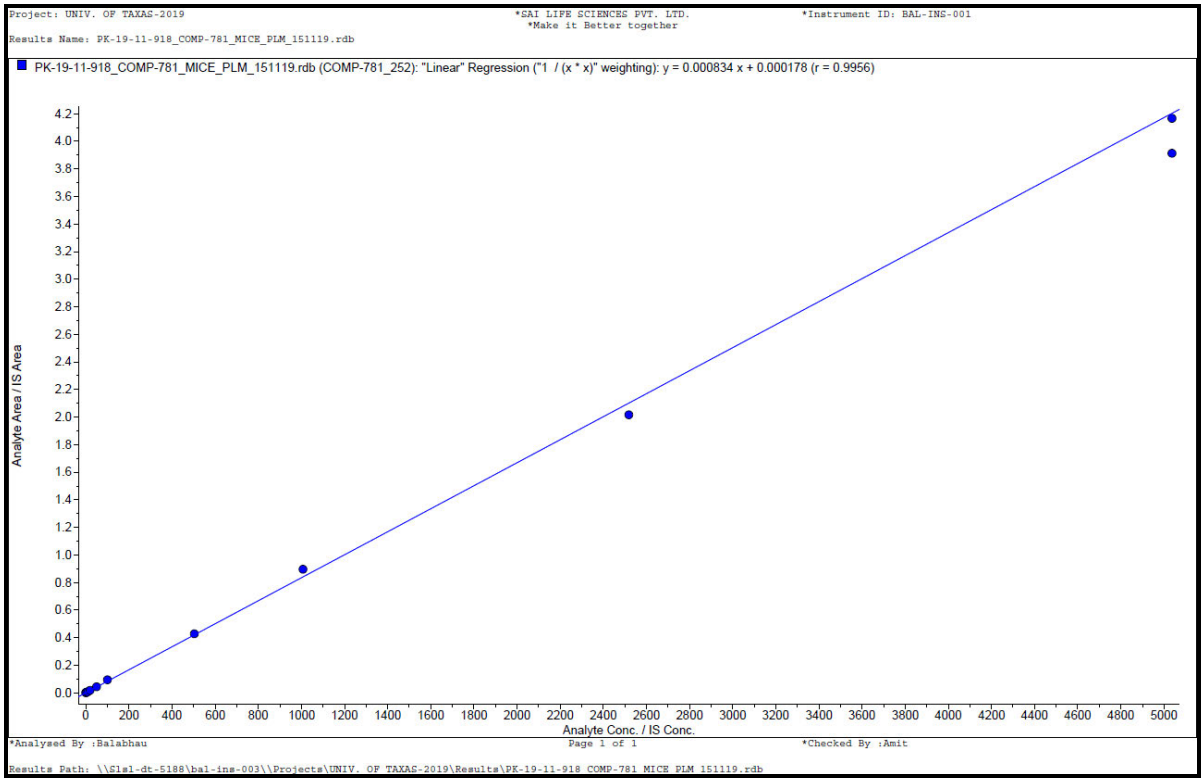
Representative LC-MS/MS chromatogram of COMPOUND-781 ULOQ standard and Glipizide (IS) in mice plasma



Representative LC-MS/MS chromatogram of COMPOUND-781 study sample (plasma) and Glipizide (IS)



Calibration curve of COMPOUND-781 in mice plasma



Calibration curve of COMPOUND-781 in mice brain