

Sai Life Sciences Limited

**PHARMACOKINETIC REPORT**

**Study Number: SAIDMPK/PK-21-06-563 and 566**

**Study Title**

**Pharmacokinetics and Brain Distribution of ZD-3-188 in Male C57BL/6 Mice Following a Single Intravenous and Oral Administration**

**(Dose: 3 mg/kg; IV and 3, 10 and 30 mg/kg; PO)**

|  |
| --- |
| **Sponsor** |
|  | Miller, Eric J. Emory UniversityAtlanta, GA 30322 |  |
| **Test Facility** |
|  | DMPK, Sai Life Sciences LimitedBuilding 1, Plot 2, Chrysalis EnclaveInternational Biotech Park, Phase II HinjewadiPune - 411 057 INDIAPhone: +91-20-30125000 |  |
| **Date of Completion of Report: July 2021** |
| **Confidential**This is a **CONFIDENTIAL** document. Any distribution beyond the parties listed within must be authorized by the sponsoring or requisitioning company/function. Reference to this document should only be made in documents having the same or a higher security classification. |

# CONTENTS

[CONTENTS 2](#_Toc77066583)

[LIST OF TABLES 3](#_Toc77066584)

[LIST OF FIGURES 4](#_Toc77066585)

[LIST OF ABBREVIATIONS 5](#_Toc77066586)

[REPORT ACCEPTANCE 6](#_Toc77066587)

[SUMMARY 7](#_Toc77066588)

[1.0 STUDY OBJECTIVE 9](#_Toc77066589)

[2.0 STUDY PERSONNEL 9](#_Toc77066590)

[3.0 COMPLIANCE AND ANIMAL WELFARE 9](#_Toc77066591)

[4.0 MATERIAL AND METHOD 9](#_Toc77066592)

[4.1 Test Item 9](#_Toc77066593)

[4.2 Test System 9](#_Toc77066594)

[4.3 Study Design 10](#_Toc77066595)

[4.4 Formulation Preparation 11](#_Toc77066596)

[4.5 Formulation Analysis Results 11](#_Toc77066597)

[4.6 Clinical Observations 11](#_Toc77066598)

[4.7 Sample Collection 12](#_Toc77066599)

[4.8 Bioanalysis 12](#_Toc77066600)

[5.0 DATA ANALYSIS 12](#_Toc77066601)

[6.0 RESULTS 13](#_Toc77066602)

[7.0 DATA ARCHIVING 13](#_Toc77066603)

[8.0 REFERENCES 13](#_Toc77066604)

[9.0 ANNEXURE I 20](#_Toc77066605)

# LIST OF TABLES

[Table 1: Pharmacokinetics data of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration (Dose: 3 mg/kg; IV and 3, 10, 30 mg/kg; PO). 14](#_Toc77066606)

[Table 2: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single intravenous administration (Dose: 3 mg/kg) 15](#_Toc77066607)

[Table 3: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 3 mg/kg) 16](#_Toc77066608)

[Table 4: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 10 mg/kg) 16](#_Toc77066609)

[Table 5: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 30 mg/kg) 17](#_Toc77066610)

[Table 6: Individual plasma and brain concentrations and brain-Kp of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 3, 10 and 30 mg/kg) 18](#_Toc77066611)

# LIST OF FIGURES

[Figure 1: Plasma concentrations-time profiles (mean ± SD) of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration of ZD-3-188 (Dose: 3 mg/kg, IV; 3, 10 and 30 mg/kg, PO) 19](#_Toc77066612)

# LIST OF ABBREVIATIONS

|  |  |
| --- | --- |
| AUC | Area under the plasma concentration-time profile |
| BLQ | Below limit of quantitation |
| C0 | Back extrapolated concentration |
| Cmax | Peak plasma concentrations |
| Conc. | Concentration(s) |
| CPCSEA | Committee for the Purpose of Control and Supervision of Experiments on Animals |
| CV  | Coefficient of Variation |
| CL | Clearance |
| °C | Degree Celsius |
| DMPK | Drug Metabolism and Pharmacokinetics |
| g | gram |
| h | hour |
| K2-EDTA | Di-potassium ethylenediamine tetra acetic acid |
| kg | kilo-gram |
| IAEC | Institutional Animal Ethics Committee |
| IS | Internal Standard |
| IV | Intravenous |
| PO | Per oral |
| Kp | Tissue-to-plasma ratio |
| LC-MS/MS | Liquid Chromatography Mass Spectrometry |
| LLOQ | Lower Limit of Quantitation |
| mg | Milligram |
| min | Minute |
| mL | Milliliter |
| µL | Microliter |
| NA | Not Applicable |
| NMP | N-Methyl-Pyrrolidone |
| NC | Not calculated |
| ng | Nano-gram |
| rpm | Rotations Per Minute |
| T1/2 | Half life |
| SD | Standard Deviation |
| SOP | Standard Operating Procedure |
| Tmax | Time to reach peak plasma concentrations |
| v/v | Volume/volume |
| w/v | Weight/volume |
| VSS | Volume of distribution |

# REPORT ACCEPTANCE

**Study Title:** Pharmacokinetics and brain distribution of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration (Dose: 3 mg/kg; IV and 3, 10, 30 mg/kg; PO)

We the undersigned herewith accept responsibility for the conduct of the study and hereby declare that the study was performed according to the procedures described herein. This report represents a true and accurate record of the results obtained.

**Nilkanth Naik, M. Pharm.**

**Associate Senior Research Scientist,**

DMPK, Sai Life Sciences Limited

Building 1, Plot 2, Chrysalis Enclave

International Biotech Park, Phase II Hinjewadi,

Pune - 411 057, Maharashtra, India

Phone: +91- 20-30125000

**Vishwanath K, M. Pharm.**

**Associate Director,**

DMPK, Sai Life Sciences Limited

Building 1, Plot 2, Chrysalis Enclave

International Biotech Park, Phase II Hinjewadi,

Pune - 411 057, Maharashtra, India

Phone: +91- 20-30125000

# SUMMARY

The objective of this study was to investigate the pharmacokinetics and brain distribution of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration (Dose: 3 mg/kg; IV and 3, 10, 30; PO). Study was conducted in two parts. Total thirty six male mice were included into the study and divided in to four groups as Group 1 to Group 4 with nine mice in each group.

**PK-21-06-563:** Animals in Group 1 (n=9) were administered intravenously with solution formulation of ZD-3-188 at 3 mg/kg dose. The formulation vehicle was 5% *v/v* NMP, 5% *v/v* Solutol HS-15, 30% *v/v* PEG-300 and 60% *v/v* normal saline.

**PK-21-06-566:** Animals in Group 2 (n=9), Group 3 (n=9) to Group 4 (n=9) were administered orally with solution formulation of ZD-3-188 at 3, 10 and 30 mg/kg dose, respectively. The formulation vehicle was 5% *v/v* NMP, 5% *v/v* Solutol HS-15, 30% *v/v* PEG-300 and 60% *v/v* normal saline.

Blood samples (approximately 60 μL) were collected under light isoflurane anesthesia (Surgivet®) from retro orbital plexus from a set of three mice at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (IV) and Pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (PO; Group 2 to Group 4). Immediately after blood collection, plasma was harvested by centrifugation at 4000 rpm, 10 min at 40C and samples were stored at -70±10ºC until bioanalysis.

Following blood collection from Group 2 to Group 4, animals were sacrificed immediately followed by abdominal vena-cava was cut open and whole body was perfused from heart using 10 mL of normal saline. Brain samples were collected from set of three mice at 1, 4, and 24 h (PO) from respective mice. After isolation, brain samples were rinsed three times in ice cold normal saline (for 5-10 seconds/rinse using ~5-10 mL normal saline in disposable petri dish for each rinse) and dried on blotting paper. Brain samples were homogenized using ice-cold phosphate buffer saline (pH-7.4). Total homogenate volume was three times the brain weight. All homogenates were stored below -70±10 ºC until bioanalysis. All samples were processed for analysis of ZD-3-188 by protein precipitation method and analyzed with fit-for-purpose LC-MS/MS method (LLOQ = 1.03 and 2.07 ng/mL for plasma and 1.03 ng/mL for brain). The pharmacokinetic parameters were estimated using non-compartmental analysis tool of Phoenix® WinNonlin software (Ver 8.0) and parameters are summarized below:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Route** | **Dose****(mg/kg)** | **Tmax****(h)** | **aC0/Cmax** **(ng/mL)** | **AUClast** **(h\*ng/mL)** | **T1/2****(h)** | **CL****(mL/min/kg)** | **Vss****(L/kg)** | **%F** |
| Plasma | IV | 3 | 0.08 | 16152.10 | 12325.03 | 2.78 | 4.05 | 0.59 | - |
| PO | 3 | 0.50 | 1300.80 | 6475.22 | - | - | - | 53 |
| 10 | 4.00 | 3557.68 | 30597.59 | - | - | - | 74 |
| 30 | 8.00 | 9799.59 | 148877.32 | - | - | - | >100 |

a – Back extrapolated concentration in IV group;

Following a single intravenous administration of ZD-3-188 min in male C57BL/6 mice at 3 mg/kg dose, compound showed low plasma clearance (~5% of the normal liver blood flow in mice: 90 mL/min/kg) and low Vss (less than the total body water content: 0.7 L/kg) with terminal elimination plasma half-life of 2.78 h.

Following a single oral administration of ZD-3-188 in male C57BL/6 mice at 3, 10 and 30 mg/kg dose, peak plasma concentrations were observed in between 0.5 to 8 h, suggesting rapid to prolonged absorption.

The dose releted increase in plasma exposure was observed from 3 to 10 mg/kg and 10 to 30 mg/kg.

In summary, ZD-3-188 exhibited low clearance, low Vss and adequate elimination half-life, good oral bioavailability and dose related increase in plasma exposure at 3, 10 and 30 mg/kg in male C57BL/6 mice.

# STUDY OBJECTIVE

To determine the pharmacokinetics and brain distribution of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration (Dose: 3 mg/kg; IV and 3, 10 and 30 mg/kg; PO).

# STUDY PERSONNEL

|  |  |  |
| --- | --- | --- |
|  | Study Director : | Nilkanth Naik, M. Pharm. |
|  | Formulation: | Amol Rasal, M. Pharm. |
|  | In-Life Phase: | Ananadkumar Yadav, M. Pharm.Gangadhar Kedar, M. Pharm.Yogesh Dalvi, M. Pharm.Raj Katariya, M. Pharm. |
|  | Bioanalysis: | Prashant Devkar, M. Pharm. |
|  | Data Analysis and Report  | Mahesh Rahinj, M. Pharm. |
|  | Report Reviewed By: | Vishwanath K, M. Pharm. |

# COMPLIANCE AND ANIMAL WELFARE

The study was conducted at AAALAC accredited facility of Sai Life Sciences Limited, Pune, India, in accordance with the Study Protocol SAIDMPK/ PK-21-06-564 and 568. All procedures of the present study will be 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. The study was conducted as non-GLP; however all appropriate documentation were maintained in study file.

# MATERIAL AND METHOD

## Test Item

The test item ZD-3-188; Mol. Wt: 529.53, Purity: Considered as 95% was received from sponsor.

## Test System

Healthy male C57BL/6 mice (8-12 weeks old) weighing between 25 ± 5 g were procured from Global, 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 h light and 12 h 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*.

## Study Design

Study was conducted in two parts. Total thirty six male mice were included into the study and divided in to four groups as Group 1 to Group 4 with nine mice in each group.

**PK-21-06-563:** Animals in Group 1 (n=9) were administered intravenously with solution formulation of ZD-3-188 at 3 mg/kg dose. The formulation vehicle was 5% v/v NMP, 5% v/v Solutol HS-15, 30% v/v PEG-300 and 60% v/v normal saline.

**PK-21-06-566:** Animals in Group 2 (n=9), Group 3 (n=9) to Group 4 (n=9) were administered orally with solution formulation of ZD-3-188 at 3, 10 and 30 mg/kg dose, respectively. The formulation vehicle was 5% v/v NMP, 5% v/v Solutol HS-15, 30% v/v PEG-300 and 60% v/v normal saline.

Blood samples (approximately 60 µL) were collected under light isoflurane anesthesia (Surgivet®) from retro orbital plexus from a set of three mice at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (IV) and 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (PO; Group 2 to Group 4). Immediately after blood collection, plasma was harvested by centrifugation at 4000 rpm, 10 min at 40C and samples were stored at -70±10ºC until bioanalysis.

The dosing volume for intravenous administration was 5 mL/kg and for oral administration was 10 mL/kg. The assignment of animals was shown in the table below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | **Route** | **Dose** **(mg/kg)** | **Matrix** | **Animal ID** |
| Group 1 | IV | 3 | Plasma  | 1-9 |
| Group 2 | PO | 3 | Plasma and brain  | 10-18 |
| Group 3 | PO | 10 | Plasma and brain | 19-27 |
| Group 4 | PO | 30 | Plasma and brain | 28-36 |

## Formulation Preparation

**IV (0.6 mg/mL):** Accurately weighed quantity (1.66 mg) of ZD-3-188 for IV dosing was added in a labeled bottle. Compound weight was corrected for purity and individual excipient volumes were calculated to prepare solution formulation of ZD-3-188 at strength of 0.6 mg/mL. The volume 0.131 mL, 0.131 mL of Solutol HS-15, 0.788 mL of PEG-300 and 1.577 mL of normal saline were added followed by vortexing aftereach addition. Final formulation was vortexed for 2 minutes to get clear solution. The amount weighed and calculation details are as below.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients** | **% content** | **IV** **(0.6 mg/mL)** | **PO** **(0.3 mg/mL)** | **PO** **(1 mg/mL)** | **PO** **(3 mg/mL)** |
| ZD-3-188  | - | 1.66 mg | 1.64 mg | 3.36 mg | 10.14 mg |
| NMP | 5 | 0.131 mL | 0.259 mL | 0.159 mL | 0.160 mL |
| Solutol HS-15 | 5 | 0.131 mL | 0.259 mL | 0.159 mL | 0.160 mL |
| PEG-300 | 30 | 0.788 mL | 1.557 mL | 0.957 mL | 0.963 mL |
| Normal saline | 60 | 1.577 mL | 3.116 mL | 1.916 mL | 1.927 mL |

Other solution formulations were prepared by same procedure with respective weights and vehicles.

## Formulation Analysis 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** | **Route** | **Theoretical Conc.****(mg/mL)** | **Conc. Found****(mg/mL)** | **% Change** |
| ZD-3-188  | IV | 0.60 | 0.64 | 6.67 |
| PO | 0.30 | 0.34 | 13.33 |
| 1.00 | 1.05 | 5.00 |
| 3.00 | 3.41 | 13.67 |

## Clinical Observations

Following a single intravenous and oral dose administration of ZD-3-188, all the animals were normal without any clinical signs.

## Sample Collection

Blood samples (approximately 60 µL) were collected under light isoflurane anesthesia (Surgivet®) from retro orbital plexus from a set of three mice at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (IV) and Pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (PO; Group 2 to Group 4). Immediately after blood collection, plasma was harvested by centrifugation at 4000 rpm, 10 min at 40C and samples were stored at -70±10ºC until bioanalysis.Following blood collection from Group 2 to Group 4, animals were sacrificed immediately followed by abdominal vena-cava was cut open and whole body was perfused from heart using 10 mL of normal saline. Brain samples were collected from set of three mice at 1, 4, and 24 h (PO) from respective mice. After isolation, brain samples were rinsed three times in ice cold normal saline (for 5-10 seconds/rinse using ~5-10 mL normal saline in disposable petri dish for each rinse) and dried on blotting paper. Brain samples were homogenized using ice-cold phosphate buffer saline (pH-7.4). Total homogenate volume was three times the brain weight. All homogenates were stored below -70±10 ºC until bioanalysis.

## Bioanalysis

Concentrations of ZD-3-188 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.

# DATA ANALYSIS

Non-Compartmental-Analysis tool of Phoenix WinNonlin® (Version 8.0) was used to assess the pharmacokinetic parameters. Peak plasma concentration (Cmax) and time for the peak plasma concentration (Tmax) were the observed values. The areas under the concentration time curve (AUClast and AUCinf) were 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. The terminal half-life (T1/2) was estimated as 0.693/ke. CLIV= Dose/AUCinf; Vss= MRT X CLIV; %F = [(AUCPO × DoseIV) / (AUCIV × DosePO)] × 100. Mean, SD and %CV calculated for each analyte. Brain-Kp ratios were calculated using microsoft excel.

# RESULTS

Following a single intravenous administration of ZD-3-188 min in male C57BL/6 mice at 3 mg/kg dose, compound showed low plasma clearance (~5% of the normal liver blood flow in mice: 90 mL/min/kg) and low Vss (less than the total body water content: 0.7 L/kg) with terminal elimination plasma half-life of 2.78 h.

Following a single oral administration of ZD-3-188 in male C57BL/6 mice at 3, 10 and 30 mg/kg dose, peak plasma concentrations were observed in between 0.5 to 8 h, suggesting rapid to prolonged absorption.

The dose releted increase in plasma exposure was observed from 3 to 10 mg/kg and 10 to 30 mg/kg.

In summary, ZD-3-188 exhibited low clearance, low Vss and adequate elimination half-life, good oral bioavailability and dose related increase in plasma exposure at 3, 10 and 30 mg/kg in male C57BL/6 mice.

# 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.

# REFERENCES

Study Protocol number: SAIDMPK/ PK-21-06-563 and 566

Table 1: Pharmacokinetics data of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration (Dose: 3 mg/kg; IV and 3, 10, 30 mg/kg; PO).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Route** | **Dose****(mg/kg)** | **Tmax****(h)** | **aC0/Cmax** **(ng/mL)** | **AUClast** **(h\*ng/mL)** | **T1/2****(h)** | **CL****(mL/min/kg)** | **Vss****(L/kg)** | **%F** |
| Plasma | IV | 3 | 0.08 | 16152.10 | 12325.03 | 2.78 | 4.05 | 0.59 | - |
| PO | 3 | 0.50 | 1300.80 | 6475.22 | 2.63 | - | - | 53 |
| 10 | 4.00 | 3557.68 | 30597.59 | 1.87 | - | - | 74 |
| 30 | 8.00 | 9799.59 | 148877.32 | 2.82 | - | - | >100 |

a – Back extrapolated concentration in IV group;

Table 2: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single intravenous administration (Dose: 3 mg/kg)

|  |  |
| --- | --- |
| **Animal****ID** | **Plasma concentration (ng/mL)** |
| **Time (h)** |
| **0.08** | **0.25** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** |
| 1 | 11921.25 |  |  | 3778.85 |  |  | 256.46 |  |  |
| 2 | 8578.22 |  |  | 2903.84 |  |  | 219.26 |  |  |
| 3 | 10044.82 |  |  | 3498.90 |  |  | 79.10 |  |  |
| 4 |  | 3120.36 |  |  | 1399.72 |  |  | 41.96 |  |
| 5 |  | 4256.40 |  |  | 1412.18 |  |  | 26.84 |  |
| 6 |  | 4079.34 |  |  | 1198.01 |  |  | 70.02 |  |
| 7 |  |  | 2459.36 |  |  | 801.99 |  |  | 3.06 |
| 8 |  |  | 3205.64 |  |  | 1106.90 |  |  | BLQ |
| 9 |  |  | 2851.57 |  |  | 952.22 |  |  | BLQ |
| **Mean** | **10181.43** | **3818.70** | **2838.86** | **3393.86** | **1336.64** | **953.70** | **184.94** | **46.27** | **3.06c** |
| SD | 1675.70 | 611.23 | 373.30 | 446.86 | 120.22 | 152.46 | 93.53 | 21.91 | NA |
| CV% | 16 | 16 | 13 | 13 | 9 | 16 | 51 | 47 | NA |

LLOQ = 1.03 ng/mL; BLQ- Below limit of quantitation; NA- Not applicable; c- Single value reported and not considered for data anlysis and graphical representation.

Table 3: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 3 mg/kg)

|  |  |
| --- | --- |
| **Animal****ID** | **Plasma concentration (ng/mL)** |
| **Time (h)** |
| **Pre-dose** | **0.25** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** |
| 1 | BLQ | 897.12 |  | 1009.01 |  |  |  |  |  |
| 2 | BLQ | 1405.37 |  | 924.85 |  |  |  |  |  |
| 3 | BLQ | 1240.05 |  | 1286.65 |  |  |  |  |  |
| 4 |  |  | 1395.64 |  | 1044.63 | 501.73 |  |  |  |
| 5 |  |  | 1395.87 |  | 738.56 | 643.78 |  |  |  |
| 6 |  |  | 1110.89 |  | 1275.15 | 951.49 |  |  |  |
| 7 |  |  |  |  |  |  | 279.67 | 105.34 | BLQ |
| 8 |  |  |  |  |  |  | 341.98 | 98.14 | BLQ |
| 9 |  |  |  |  |  |  | 196.62 | 51.35 | BLQ |
| **Mean** | **NA** | **1180.85** | **1300.80** | **1073.50** | **1019.45** | **699.00** | **272.76** | **84.94** | **NA** |
| SD | NA | 259.25 | 164.47 | 189.33 | 269.18 | 229.91 | 72.93 | 29.31 | NA |
| CV% | NA | 22 | 13 | 18 | 26 | 33 | 27 | 35 | NA |

LLOQ = 1.03 ng/mL; BLQ- Below limit of quantitation; NA- Not applicable.

Table 4: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 10 mg/kg)

|  |  |
| --- | --- |
| **Animal****ID** | **Plasma concentration (ng/mL)** |
| **Time (h)** |
| **Pre-dose** | **0.25** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** |
| 10 | BLQ | 3511.71 |  | 2906.75 |  |  |  |  |  |
| 11 | BLQ | 2212.37 |  | 3914.48 |  |  |  |  |  |
| 12 | BLQ | 3067.73 |  | 2807.23 |  |  |  |  |  |
| 13 |  |  | 2974.36 |  | 2656.55 | 3450.46 |  |  |  |
| 14 |  |  | 3946.44 |  | 3562.05 | 4214.91 |  |  |  |
| 15 |  |  | 3067.02 |  | 3809.85 | 3007.67 |  |  |  |
| 16 |  |  |  |  |  |  | 1384.58 | 554.58 | 5.64 |
| 17 |  |  |  |  |  |  | 1586.32 | 570.61 | BLQ |
| 18 |  |  |  |  |  |  | 1624.21 | 512.73 | 3.52 |
| **Mean** | **NA** | **2930.60** | **3329.27** | **3209.49** | **3342.82** | **3557.68** | **1531.70** | **545.97** | **4.58d** |
| SD | NA | 660.43 | 536.49 | 612.57 | 607.10 | 610.72 | 128.81 | 29.88 | NA |
| CV% | NA | 23 | 16 | 19 | 18 | 17 | 8 | 5 | NA |

LLOQ = 2.07 ng/mL; BLQ- Below limit of quantitation; NA- Not applicable; d- Averege of two values reported and considered for data anlysis and graphical representation.

Table 5: Individual plasma concentration-time data of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 30 mg/kg)

|  |  |
| --- | --- |
| **Animal****ID** | **Plasma concentration (ng/mL)** |
| **Time (h)** |
| **Pre-dose** | **0.25** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** |
| 19 | BLQ | 7800.94 |  | 9634.01 |  |  |  |  |  |
| 20 | BLQ | 6164.14 |  | 9782.59 |  |  |  |  |  |
| 21 | BLQ | 7728.26 |  | 8741.04 |  |  |  |  |  |
| 22 |  |  | 11547.46 |  | 8862.59 | 11605.76 |  |  |  |
| 23 |  |  | 8771.07 |  | 8299.41 | 8046.99 |  |  |  |
| 24 |  |  | 9049.75 |  | 9684.29 | 6695.47 |  |  |  |
| 25 |  |  |  |  |  |  | 11303.33 | 8702.83 | 421.90 |
| 26 |  |  |  |  |  |  | 9045.33 | 4831.03 | 1.93e |
| 27 |  |  |  |  |  |  | 9050.11 | 7464.40 | 42.77 |
| **Mean** | **NA** | **7231.11** | **9789.43** | **9385.88** | **8948.76** | **8782.74** | **9799.59** | **6999.42** | **232.34d** |
| SD | NA | 924.74 | 1528.86 | 563.37 | 696.45 | 2536.48 | 1302.28 | 1977.34 | NA |
| CV% | NA | 13 | 16 | 6 | 8 | 29 | 13 | 28 | NA |

LLOQ = 2.07 ng/mL; BLQ- Below limit of quantitation; NA- Not applicable; e – Value excluded from data analysis and graphical presentation; d-Average of two values reported and considered for data analysis and graphical representation.

**Table 6: Individual plasma and brain concentrations and brain-Kp of ZD-3-188 in male C57BL/6 mice following a single oral administration (Dose: 3, 10 and 30 mg/kg)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Dose (mg/kg)** | **Time****(h)** | **Animal ID** | **Plasma Concentration****(ng/mL)** | **Brain****Concentration****(ng/g)** | **Brain-Kp** | **Mean****Brain-Kp** |
| 3 mg/kg | 1 | 1 | 1009.01 | 31.23 | 0.03 | 0.03 |
| 2 | 924.85 | 35.31 | 0.04 |
| 3 | 1286.65 | 29.94 | 0.02 |
| 4 | 4 | 501.73 | 17.97 | 0.04 | 0.03 |
| 5 | 643.78 | 25.65 | 0.04 |
| 6 | 951.49 | 14.82 | 0.02 |
| 24 | 7 | BLQ | BLQ | NC | NA |
| 8 | BLQ | BLQ | NC |
| 9 | BLQ | BLQ | NC |
| 10 mg/kg | 1 | 10 | 2906.75 | 174.18 | 0.06 | 0.05 |
| 11 | 3914.48 | 112.20 | 0.03 |
| 12 | 2807.23 | 128.07 | 0.05 |
| 4 | 13 | 3450.46 | 85.44 | 0.02 | 0.03 |
| 14 | 4214.91 | 105.30 | 0.02 |
| 15 | 3007.67 | 123.06 | 0.04 |
| 24 | 16 | 5.64 | BLQ | NC | NA |
| 17 | BLQ | BLQ | NC |
| 18 | 3.52 | BLQ | NC |
| 30 mg/kg | 1 | 19 | 9634.01 | 520.89 | 0.05 | 0.05 |
| 20 | 9782.59 | 439.29 | 0.04 |
| 21 | 8741.04 | 427.77 | 0.05 |
| 4 | 22 | 11605.76 | 635.85 | 0.05 | 0.03 |
| 23 | 8046.99 | 278.55 | 0.03 |
| 24 | 6695.47 | 146.94 | 0.02 |
| 24 | 25 | 421.90 | BLQ | NC | NA |
| 26 | 1.93 | BLQ | NC |
| 27 | 42.77 | BLQ | NC |

LLOQ: 1.03 ng/mL for plasma and brain; BLQ- Below limit of quantitation; NC- Not calculated.

**Figure 1: Plasma concentrations-time profiles (mean ± SD) of ZD-3-188 in male C57BL/6 mice following a single intravenous and oral administration of ZD-3-188 (Dose: 3 mg/kg, IV; 3, 10 and 30 mg/kg, PO)**

|  |
| --- |
| **Linear** |
| **Semi-log** |

# ANNEXURE I

**Bioanalytical Summary**