STUDY REPORT

A PHARMACOKINETIC STUDY OF KT-00478 IN MALE CD-1 (ICR) MICE FOLLOWING SINGLE INTRAVENOUS AND ORAL ADMINISTRATIONS

TESTING FACILITY STUDY NUMBER: 421139-20210128B01-MPK

SPONSOR STUDY NUMBER: NA

DOCUMENT STATUS: FINAL

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SUMMARY

The purpose of this study was to determine the pharmacokinetic (PK) properties of KT-00478 following a single intravenous (IV) bolus and oral gavage (PO) administration of KT-00478 in male CD-1 (ICR) mice.

Six male CD-1 (ICR) mice were divided into two groups with 3 animals/group. Animals in Group 1 were administered KT-00478 by single intravenous bolus administration at 5 mg/kg. Animals in Group 2 were administered KT-00478 by single oral administration at 10 mg/kg. Plasma samples were collected at 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dose. Concentrations of KT-00478 in plasma samples were determined by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

The PK parameters of KT-00478 are summarized in the table below.

Group	**1**		**2**	
Dose Route	**IV bolus**		**PO**	
Dose Level (mg/kg)	**5**		**10**	
PK Parameters	**Mean**	**SD**	**Mean**	**SD**
C_0 or C_{max} (ng/mL)	4467	408	1583	812
T_{max} (h)	--	--	0.667	0.289
$T_{1/2}$ (h)	4.65	0.615	3.16	0.278
Vd_{ss} (L/kg)	7.07	1.05	--	--
Cl (mL/min/kg)	32.2	4.97	--	--
$AUC_{0\text{-last}}$ (ng·h/mL)	2594	445	2563	850
$AUC_{0\text{-inf}}$ (ng·h/mL)	2629	448	2574	854
Bioavailability (%)[a]	--	--	49.0	--

[a]: Bioavailability was calculated with $AUC_{0\text{-inf}}$ and nominal dose.
"--" means not applicable.

For the IV administration of KT-00478 at 5 mg/kg in male CD-1 (ICR) mice, KT-00478 showed a plasma clearance (Cl) of 32.2±4.97 mL/min/kg, half-life ($T_{1/2}$) at 4.65±0.615 h. The volume of distribution (Vd_{ss}) was 7.07±1.05 L/kg, the area under the plasma concentration-time curve from time zero to the last quantifiable concentration ($AUC_{0\text{-last}}$) value was 2594±445 ng·h/mL.

Following a single oral administration of KT-00478 at 10 mg/kg in male CD-1 (ICR) mice, $AUC_{0\text{-last}}$ value of KT-00478 was 2563±850 ng·h/mL. The C_{max} value of KT-00478 was 1583±812 ng/mL, while C_{max} was reached at 0.667±0.289 h. The oral bioavailability was 49.0% at 10 mg/kg.

All animals had tolerated the KT-00478 at the dosing levels during the entire course of the study. No adverse effect was observed during the in-life phase of the study.

STUDY IDENTIFICATION

Study Title:	A Pharmacokinetic Study of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous and Oral Administrations
Test Article:	KT-00478
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Sponsor's Representative:	Donggeon Kim Kanaph Therapeutics, Inc. E-mail Address: dkim@kanaphtx.com
Testing Facility:	WuXi AppTec (Shanghai) Co., Ltd. 31 Yiwei Road, Waigaoqiao Free Trade Zone, Pudong New Area, Shanghai 200131, China
Study Director:	Jia Lun, M.S. DMPK Service Department WuXi AppTec (Suzhou) Co., Ltd. E-mail Address: lun_jia@wuxiapptec.com
Testing Facility Study No.:	421139-20210128B01-MPK
Sponsor Study No.:	NA
In-life Dosing Date:	Feb. 24, 2021
PK Data Release Date:	Sep. 21, 2023

COMPLIANCE STATEMENT

This study was performed at WuXi AppTec (Shanghai) Co., Ltd. and adhered to the study protocol and Standard Operating Procedures (SOPs), but was not intended to be in full compliance with international good laboratory practice (GLP) regulations.

GLOSSARY OF ABBREVIATIONS

AUC	The area under the plasma concentration-time curve
$AUC_{0\text{-last}}$	The area under the plasma concentration-time curve from time zero to the last quantifiable concentration
$AUC_{0\text{-inf}}$	The area under the plasma concentration-time curve from time zero extrapolated to infinity were calculated using the linear/log trapezoidal rule
BQL	Below the quantifiable limit
Cl	Total body clearance
C_{max}	Peak plasma concentration
C_0	Initial plasma concentration
Conc.	Concentration
CV%	Coefficient of variation %
DMSO	Dimethyl sulfoxide
F.W.	Formula weight
GLP	Good laboratory practice
h (r)	Hour (s)
HPLC-UV	High-performance liquid chromatography/ultraviolet
IV	Intravenous
K_2-EDTA	Ethylene diaminetetra acetic acid dipotassium salt
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower limit of quantitation
min	Minute (s)
MRT	Mean residence time
$MRT_{0\text{-last}}$	Mean residence time from time zero to the last quantifiable concentration

$MRT_{0\text{-}inf}$	Mean residence time from time zero to infinity
M.W.	Molecular weight
PK	Pharmacokinetic
SOP	Standard operating procedure
T_{max}	Time to reach the maximum plasma concentration
$T_{1/2}$	Terminal half-life
Vd_{ss}	Volume of distribution at steady state

1 INTRODUCTION

The purpose of this study was to determine the pharmacokinetic (PK) properties of KT-00478 following a single intravenous (IV) bolus and oral gavage (PO) administration of KT-00478 in male CD-1 (ICR) mice.

2 MATERIALS AND METHODS

2.1 Test Article

Test Article	Lot or Batch No.	M.W.	F.W.	Formula	Exact mass	Purity%	Storage condition
KT-00478	KT-00478_1	507.62	507.62	$C_{29}H_{30}FNO_4S$	507.188	98.3	-20°C

2.2 Study Design

Six male CD-1 (ICR) mice were divided into two groups with 3 animals/group. Animals in Group 1 were administered KT-00478 by single intravenous bolus administration at 5 mg/kg. Animals in Group 2 were administered KT-00478 by single oral administration at 10 mg/kg. The vehicle was 10% DMSO/10% Solutol/80% Water. The detailed dosing and sampling regimens are described in the following tables.

Group No.	Test Article	No. of animals	Sex	Dose Route	Dose (mg/kg)	Conc (mg/mL)	Dose Volume (mL/kg)	Vehicle
1	KT-00478	3	Male	IV bolus	5	2.5	2	10% DMSO/10% Solutol/80% Water
2	KT-00478	3	Male	PO	10	1	10	10% DMSO/10% Solutol/80% Water

Group	Animal Number	Sampling Time Points
	Male	
1	M01, M02, M03	0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dose
2	M04, M05, M06	0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dose

2.3 Formulation

2.3.1 Intravenous (IV) Dose

The vehicle was 10% DMSO/10% Solutol/80% Water.

a. 1.76 mg of KT-00478 was weighed into a glass vial.

b. 69 µL of DMSO was added into the vial and stirred for 3 min to obtain a clear solution.

c. 69 µL of Solutol was added into the vial and stirred for 3 min to obtain a clear solution.

d. 554 µL of water was added into the vial and stirred for 3 min to obtain a clear solution.

e. Filtered the solution through formulation filter.

2.3.2 Oral (PO) Dose

The vehicle was 10% DMSO/10% Solutol/80% Water.

a. 1.71 mg of KT-00478 was weighed into a glass vial.

b. 168 µL of DMSO was added into the vial and stirred for 3 min to obtain a clear solution.

c. 168 µL of Solutol was added into the vial and stirred for 3 min to obtain a clear solution.

d. 1345 µL of water was added into the vial and stirred for 3 min to obtain a clear solution.

2.3.3 Formulation Analysis

Three aliquots (top, middle and bottom) were taken from dosing solution. Each aliquot was analyzed in duplicates for each dose using HPLC-UV methodologies with a six-point calibration curve.

Acceptance criteria: The measured concentrations of test article in each dose formulation must fall within 80% to 120% of the nominal concentrations.

2.4 In-Life Work

2.4.1 Animal Husbandry

Six male CD-1 (ICR) mice supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd. were used in this study. The animals were confirmed to be healthy before being assigned to the study. Each animal was given a unique identification number which was marked on the tail and written on the cage card. The 6 mice were divided into two groups (n=3/group, see Section 2.2).

The room (s) was controlled and monitored for relative humidity (targeted mean range 40% to 70%) and temperature (targeted mean range 20 to 26℃) with 15 or above air changes/hour. The room was on a 12-hour light/dark cycle except when interruptions were necessitated by study activities.

2.4.2 Feeding

Fresh drinking water (reverses osmosis) was available to all animals, *ad libitum*.

Certified animal diet from certified vendor was available to animals in IV group, *ad libitum*.

For animals in PO group, animals were fasted at least 12 hours prior to the administration. Certified rodent diet food was withheld until 4 hours post-dose. The fasting time did not exceed 20 hours.

2.4.3 Dose Administration

Animals were weighed prior to dose administration on the day of dosing to calculate the actual dose volume.

The body weights were in the range from 29.06 to 33.98 g for males on the dosing day. All animals in Group 1 received a single intravenous bolus administration of KT-00478 from the tail vein.

The animals in Group 2 received a single oral gavage administration of KT-00478.

2.4.4 Clinical Observation

Cage-side observations for the general health condition and appearance of the animal were performed before and after dosing and at each time point of sample collection. Unusual observations were recorded throughout the duration of the study.

2.4.5 Blood Collection and Plasma Preparation

Blood (about 0.03 mL) was collected at each time point via saphenous vein from each study animal. The actual time for each sample collection was recorded. The deviations on sampling time were less than 1 minute for the time points pre-dose through 1 hour post-dose, and less than 5% of the nominal time for time points after 1 hour post-dose.

All blood samples were transferred into commercial tubes containing K_2-EDTA. Plasma samples were then prepared by centrifuging the blood samples at approximately 4℃ , 3200×g for 10 minutes, and then quickly frozen over dry ice and kept at -60℃ or lower until LC-MS/MS analysis.

2.5 Bioanalytical Analysis

The concentrations of KT-00478 in plasma were determined by using an LC-MS/MS method. The bioanalytical assay conditions are reported in the APPENDIX II.

2.6 Pharmacokinetics Data Analysis

The plasma concentrations of KT-00478 in study animals were subjected to a non-compartmental pharmacokinetic analysis by using the Phoenix WinNonlin software program (version 6.3, Certara). The linear/log trapezoidal rule was applied in obtaining the PK parameters.

Individual plasma concentration values that were below the lower limit of quantitation (LLOQ) were excluded from the PK parameter calculation. The nominal dose levels and nominal sampling times were used in the calculation of all pharmacokinetic parameters.

3 RESULTS AND DISCUSSION

3.1 Clinical Observations

All animals had tolerated the KT-00478 at the dosing levels during the entire course of the study. No adverse effect was observed during the in-life phase of the study.

3.2 Dose Verification

The actual doses were determined by HPLC-UV and the data in Table 1 confirmed that the doses in all dosing formulations were within ±20% of the nominal target dosing levels and were acceptable per the study protocol.

3.3 Pharmacokinetics of KT-00478 in Animals

Following a single IV bolus administration of KT-00478 at 5 mg/kg and oral administration of KT-00478 at 10 mg/kg in male animals, the individual and mean plasma concentrations of KT-00478 are shown in Table 2 and Table 3, and are illustrated in Figure 1 to Figure 3. The individual and mean plasma pharmacokinetic parameters of KT-00478 are shown in Table 4 and Table 5. The data tables and figures converted to μM are shown in APPENDIX I.

The PK parameters of KT-00478 are summarized in the table below.

Group	**1**		**2**	
Dose Route	**IV bolus**		**PO**	
Dose Level (mg/kg)	**5**		**10**	
PK Parameters	**Mean**	**SD**	**Mean**	**SD**
C_0 or C_{max} (ng/mL)	4467	408	1583	812
T_{max} (h)	--	--	0.667	0.289
$T_{1/2}$ (h)	4.65	0.615	3.16	0.278
Vd_{ss} (L/kg)	7.07	1.05	--	--
Cl (mL/min/kg)	32.2	4.97	--	--
$AUC_{0\text{-last}}$ (ng·h/mL)	2594	445	2563	850
$AUC_{0\text{-inf}}$ (ng·h/mL)	2629	448	2574	854
Bioavailability (%)[a]	--	--	49.0	--

[a]: Bioavailability was calculated with $AUC_{0\text{-inf}}$ and nominal dose.
"--" means not applicable.

For the IV administration of KT-00478 at 5 mg/kg in male CD-1 (ICR) mice, KT-00478 showed a plasma clearance (Cl) of 32.2±4.97 mL/min/kg, half-life ($T_{1/2}$) at 4.65±0.615 h. The volume of distribution (Vd_{ss}) was 7.07±1.05 L/kg, the area under the plasma concentration-time curve from time zero to the last quantifiable concentration ($AUC_{0\text{-last}}$) value was 2594±445 ng·h/mL.

Following a single oral administration of KT-00478 at 10 mg/kg in male CD-1 (ICR) mice, $AUC_{0\text{-last}}$ value of KT-00478 was 2563±850 ng·h/mL. The C_{max} value of KT-00478 was 1583±812 ng/mL, while C_{max} was reached at 0.667±0.289 h. The oral bioavailability was 49.0% at 10 mg/kg.

4 DEVIATION

The study was conducted in accordance with the study protocol and relevant SOPs. No deviation was found.

5 ARCHIVING

Leftover bioanalytical samples from this study will be retained in WuXi AppTec (Shanghai) Co., Ltd. for at least 6 months after which the study is completed. The Sponsor will be contacted to determine disposition of the samples prior to the expiration; disposition options include shipment to the Sponsor or retention by the test facility. If

disposition of samples is not resolved within 60 days of notification, the test facility will dispose of the samples. The supporting documentation related to formulation and in-life, including raw data and written records generated in this study will be archived in WuXi AppTec (Shanghai) Co., Ltd. for a period of 5 years following submission of the final report to the sponsor. At the end of this period, all supporting documentation will be transferred, retained or destroyed according to the relevant SOP in WuXi AppTec DMPK.

DATA TABLES

Table 1 The Concentrations of KT-00478 in Dosing Formulations

Group No.	Dose Route	Sample No.	Determination	Dilution Factor	Measured Conc. (μg/mL)	Calculated Conc. (mg/mL)	Mean Conc. (mg/mL)	Nominal Conc. (mg/mL)	Accuracy[a] (%)
1	[Pre]IV bolus	Top	1	25	89.3	2.23	2.22	2.50	88.8
1	[Pre]IV bolus	Top	2	25	89.8	2.25	2.22	2.50	88.8
1	[Pre]IV bolus	Middle	1	25	88.4	2.21	2.22	2.50	88.8
1	[Pre]IV bolus	Middle	2	25	88.2	2.21	2.22	2.50	88.8
1	[Pre]IV bolus	Bottom	1	25	88.2	2.20	2.22	2.50	88.8
1	[Pre]IV bolus	Bottom	2	25	89.2	2.23	2.22	2.50	88.8
2	[Pre]PO	Top	1	10	94.4	0.944	0.942	1.00	94.2
2	[Pre]PO	Top	2	10	93.9	0.939	0.942	1.00	94.2
2	[Pre]PO	Middle	1	10	94.0	0.940	0.942	1.00	94.2
2	[Pre]PO	Middle	2	10	94.4	0.944	0.942	1.00	94.2
2	[Pre]PO	Bottom	1	10	93.7	0.937	0.942	1.00	94.2
2	[Pre]PO	Bottom	2	10	94.7	0.947	0.942	1.00	94.2

[a]: Accuracy (%) = Mean Concentration (mg/mL) / Nominal Concentration (mg/mL) × 100.

Table 2 Individual and Mean Plasma Concentrations (ng/mL) of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg

Time (h)	M01	M02	M03	Mean	SD	CV (%)
0.0830	2991	3512	3409	3304	276	8.35
0.250	1597	1799	2023	1806	213	11.8
0.500	816	1098	908	941	144	15.3
1.00	364	452	366	394	50.2	12.8
2.00	121	158	121	133	21.4	16.0
4.00	94.9	134	81.0	103	27.5	26.6
6.00	72.1	110	55.6	79.2	27.9	35.2
8.00	49.9	81.2	47.4	59.5	18.8	31.7
12.0	30.0	68.1	35.0	44.4	20.7	46.7
24.0	6.24	6.26	2.66	5.05	2.07	41.0

"M#" means animal number.

Table 3 Individual and Mean Plasma Concentrations (ng/mL) of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg

Time (h)	M04	M05	M06	Mean	SD	CV (%)
0.250	104	1885	629	873	915	105
0.500	246	2509	991	1249	1153	92.4
1.00	1250	1209	617	1025	354	34.5
2.00	310	263	117	230	101	43.8
4.00	155	144	104	134	26.8	20.0
6.00	87.9	119	81.7	96.2	20.0	20.8
8.00	79.5	75.6	66.7	73.9	6.56	8.87
12.0	40.0	33.2	32.3	35.2	4.21	12.0
24.0	2.02	3.10	1.85	2.32	0.678	29.2

"M#" means animal number.

Table 4 Individual and Mean Pharmacokinetic Parameters of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg

PK Parameters	M01	M02	M03	Mean	SD	CV (%)
Rsq_adj	1.00	0.955	0.970	--	--	--
No. points used for $T_{1/2}$	3.00	5.00	6.00	ND	--	--
C_0 (ng/mL)	4086	4897	4418	4467	408	9.13
$T_{1/2}$ (h)	5.33	4.50	4.13	4.65	0.615	13.2
Vd_{ss} (L/kg)	8.24	6.76	6.20	7.07	1.05	14.9
Cl(mL/min/kg)	35.5	26.5	34.7	32.2	4.97	15.4
T_{last} (h)	24.0	24.0	24.0	24.0	--	--
$AUC_{0\text{-}last}$ (ng.h/mL)	2293	3105	2385	2594	445	17.1
$AUC_{0\text{-}inf}$ (ng.h/mL)	2341	3146	2401	2629	448	17.1
$MRT_{0\text{-}last}$ (h)	3.28	3.91	2.80	3.33	0.557	16.7
$MRT_{0\text{-}inf}$ (h)	3.86	4.25	2.98	3.70	0.651	17.6
AUC_{Extra} (%)	2.05	1.29	0.659	1.33	0.696	52.3
$AUMC_{Extra}$ (%)	16.8	9.28	6.63	10.9	5.28	48.4

"--" means not applicable.

"ND" means not determined.

"M#" means animal number.

Table 5 Individual and Mean Pharmacokinetic Parameters of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg

PK Parameters	M04	M05	M06	Mean	SD	CV (%)
Rsq_adj	0.991	1.00	0.995	--	--	--
No. points used for $T_{1/2}$	3	3	3	3	--	--
C_{max} (ng/mL)	1250	2509	991	1583	812	51.3
T_{max} (h)	1.00	0.500	0.500	0.667	0.289	43.3
$T_{1/2}$ (h)	2.96	3.48	3.05	3.16	0.278	8.79
T_{last} (h)	24.0	24.0	24.0	24.0	--	--
$AUC_{0\text{-}last}$ (ng.h/mL)	2339	3502	1847	2563	850	33.2
$AUC_{0\text{-}inf}$ (ng.h/mL)	2347	3518	1856	2574	854	33.2
$MRT_{0\text{-}last}$ (h)	4.07	2.84	3.92	3.61	0.671	18.6
$MRT_{0\text{-}inf}$ (h)	4.16	2.96	4.02	3.71	0.656	17.7
AUC_{Extra} (%)	0.368	0.442	0.439	0.416	0.0419	10.1
$AUMC_{Extra}$ (%)	2.50	4.34	3.10	3.31	0.938	28.3
Bioavailability (%)[a]	-	-	-	49.0	--	--

[a]: Bioavailability (%) was calculated with $AUC_{0\text{-}inf}$ and nominal dose.

"--" means not applicable.

"M#" means animal number.

DATA FIGURES

Figure 1 Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous and Oral Administration of KT-00478

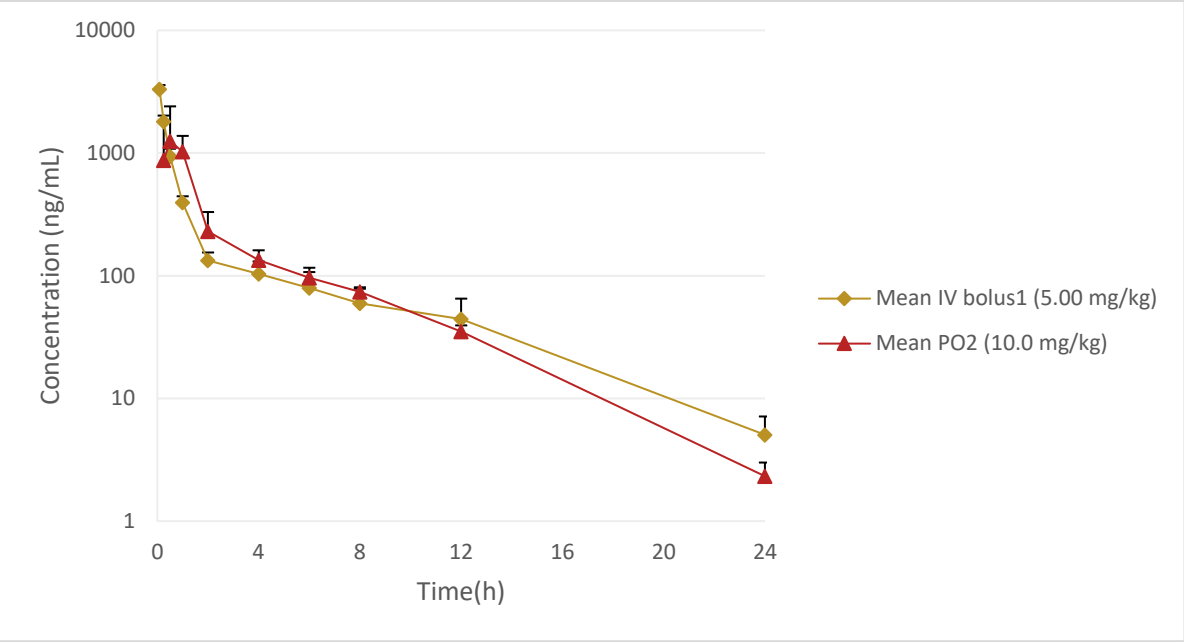
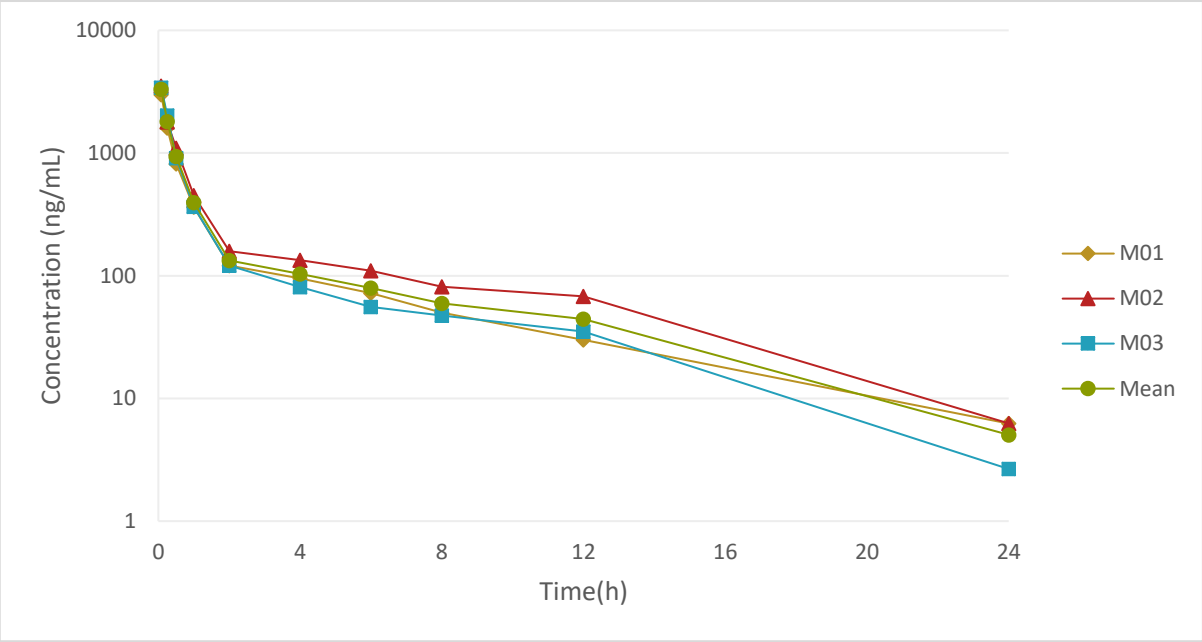
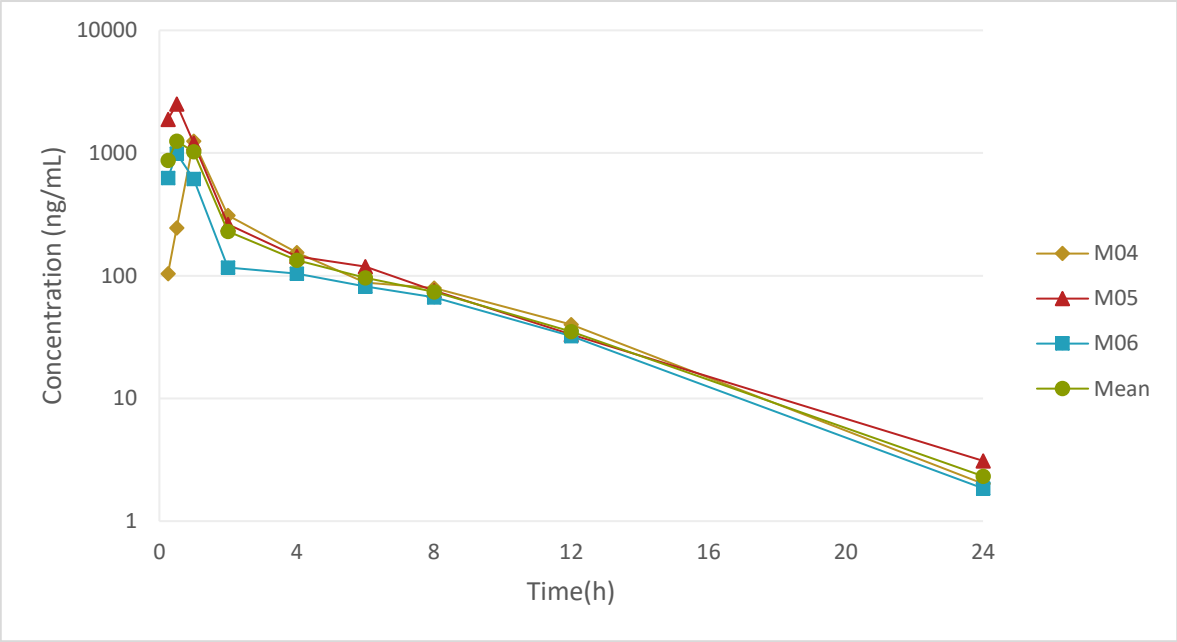


Figure 2 Individual and Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg



"M#" means animal number.

Figure 3 Individual and Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg



"M#" means animal number.

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APPENDIX I: TABLES AND FIGURES CONVERTED TO µM

Table 1 Individual and Mean Plasma Concentrations (µM) of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg

Time (h)	M01	M02	M03	Mean	SD	CV (%)
0.0830	5.89	6.92	6.72	6.51	0.546	8.39
0.250	3.15	3.54	3.98	3.56	0.415	11.7
0.500	1.61	2.16	1.79	1.85	0.280	15.1
1.00	0.717	0.890	0.721	0.776	0.0987	12.7
2.00	0.239	0.312	0.239	0.263	0.0421	16.0
4.00	0.187	0.263	0.160	0.203	0.0534	26.3
6.00	0.142	0.217	0.109	0.156	0.0553	35.5
8.00	0.0983	0.160	0.0934	0.117	0.0371	31.7
12.0	0.0590	0.134	0.0689	0.0873	0.0407	46.7
24.0	0.0123	0.0123	0.00525	0.00995	0.00407	40.9

"M#" means animal number.

Table 2 Individual and Mean Plasma Concentrations (μM) of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg

Time (h)	M04	M05	M06	Mean	SD	CV (%)
0.250	0.205	3.71	1.24	1.72	1.80	105
0.500	0.485	4.94	1.95	2.46	2.27	92.4
1.00	2.46	2.38	1.21	2.02	0.700	34.7
2.00	0.611	0.519	0.230	0.453	0.199	43.9
4.00	0.306	0.284	0.206	0.265	0.0525	19.8
6.00	0.173	0.235	0.161	0.190	0.0397	20.9
8.00	0.157	0.149	0.131	0.146	0.0133	9.14
12.0	0.0788	0.0653	0.0637	0.0693	0.00829	12.0
24.0	0.00397	0.00611	0.00364	0.00457	0.00134	29.3

“M#” means animal number.

Table 3 Individual and Mean Pharmacokinetic Parameters of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg

PK Parameters	M01	M02	M03	Mean	SD	CV (%)
Rsq_adj	1.00	0.955	0.970	--	--	--
No. points used for $T_{1/2}$	3	5	6	ND	--	--
C_0 (umol/L)	8.04	9.66	8.72	8.81	0.813	9.24
$T_{1/2}$ (h)	5.33	4.50	4.13	4.65	0.615	13.2
Vd_{ss} (L/kg)	8.23	6.76	6.20	7.06	1.05	14.8
Cl(mL/min/kg)	35.5	26.5	34.7	32.2	4.97	15.4
T_{last} (h)	24.0	24.0	24.0	24.0	--	--
$AUC_{0\text{-}last}$ (h*umol/L)	4.52	6.11	4.70	5.11	0.871	17.0
$AUC_{0\text{-}inf}$ (h*umol/L)	4.61	6.19	4.73	5.18	0.880	17.0
$MRT_{0\text{-}last}$ (h)	3.27	3.91	2.80	3.33	0.557	16.7
$MRT_{0\text{-}inf}$ (h)	3.86	4.25	2.98	3.70	0.651	17.6
AUC_{Extra} (%)	2.05	1.29	0.660	1.33	0.696	52.2
$AUMC_{Extra}$ (%)	16.8	9.26	6.64	10.9	5.27	48.4

"--" means not applicable.

"ND" means not determined.

"M#" means animal number.

Table 4 Individual and Mean Pharmacokinetic Parameters of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg

PK Parameters	M04	M05	M06	Mean	SD	CV (%)
Rsq_adj	0.991	1.00	0.995	--	--	--
No. points used for $T_{1/2}$	3	3	3	3	--	--
C_{max} (umol/L)	2.46	4.94	1.95	3.12	1.60	51.3
T_{max} (h)	1.00	0.500	0.500	0.667	0.289	43.3
$T_{1/2}$ (h)	2.96	3.48	3.05	3.16	0.278	8.79
T_{last} (h)	24.0	24.0	24.0	24.0	--	--
$AUC_{0\text{-}last}$ (h*umol/L)	4.61	6.90	3.64	5.05	1.67	33.1
$AUC_{0\text{-}inf}$ (h*umol/L)	4.63	6.93	3.65	5.07	1.68	33.2
$MRT_{0\text{-}last}$ (h)	4.07	2.84	3.92	3.61	0.671	18.6
$MRT_{0\text{-}inf}$ (h)	4.16	2.96	4.03	3.72	0.659	17.7
AUC_{Extra} (%)	0.366	0.443	0.438	0.416	0.0431	10.4
$AUMC_{Extra}$ (%)	2.49	4.34	3.09	3.31	0.944	28.5
Bioavailability (%)[a]	-	-	-	48.9	--	--

[a]: Bioavailability (%) was calculated with $AUC_{0\text{-}inf}$ and nominal dose.

"--" means not applicable.

"M#" means animal number.

Figure 1 Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous and Oral Administration of KT-00478

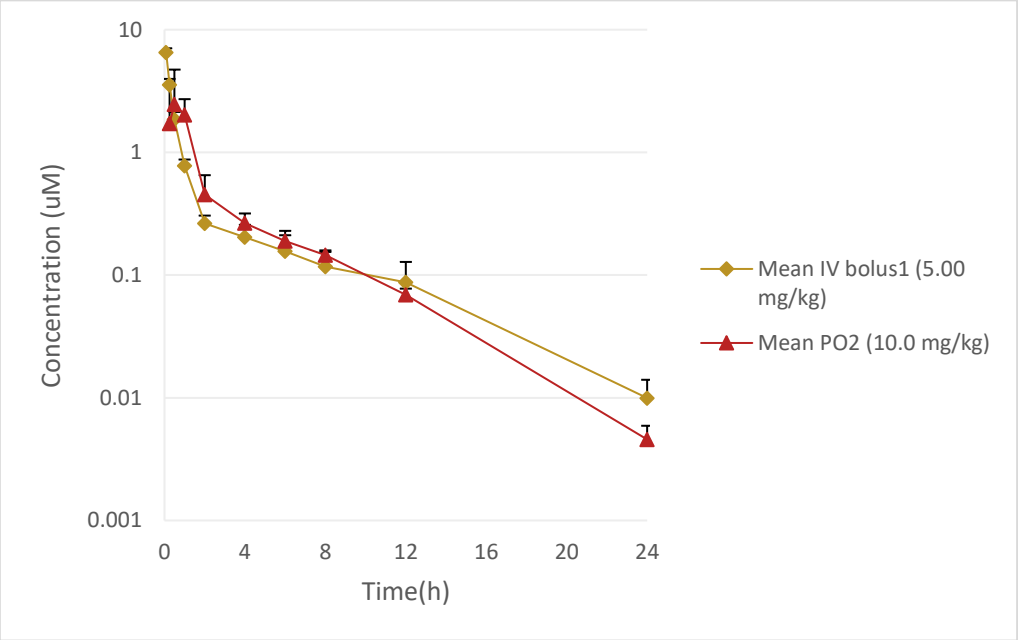
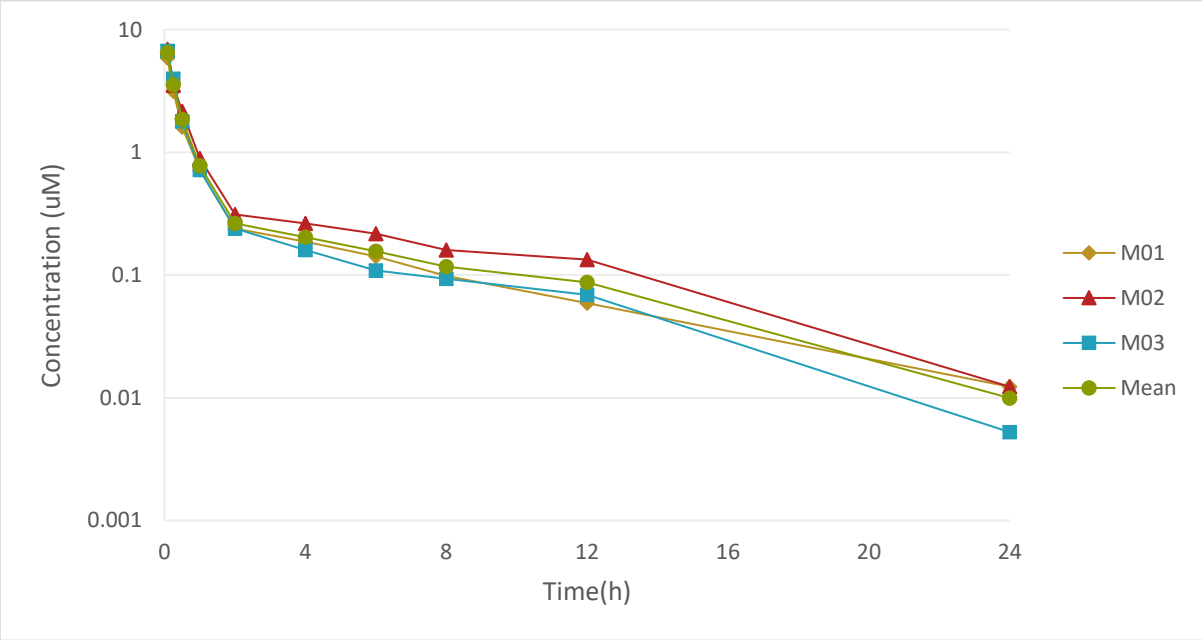
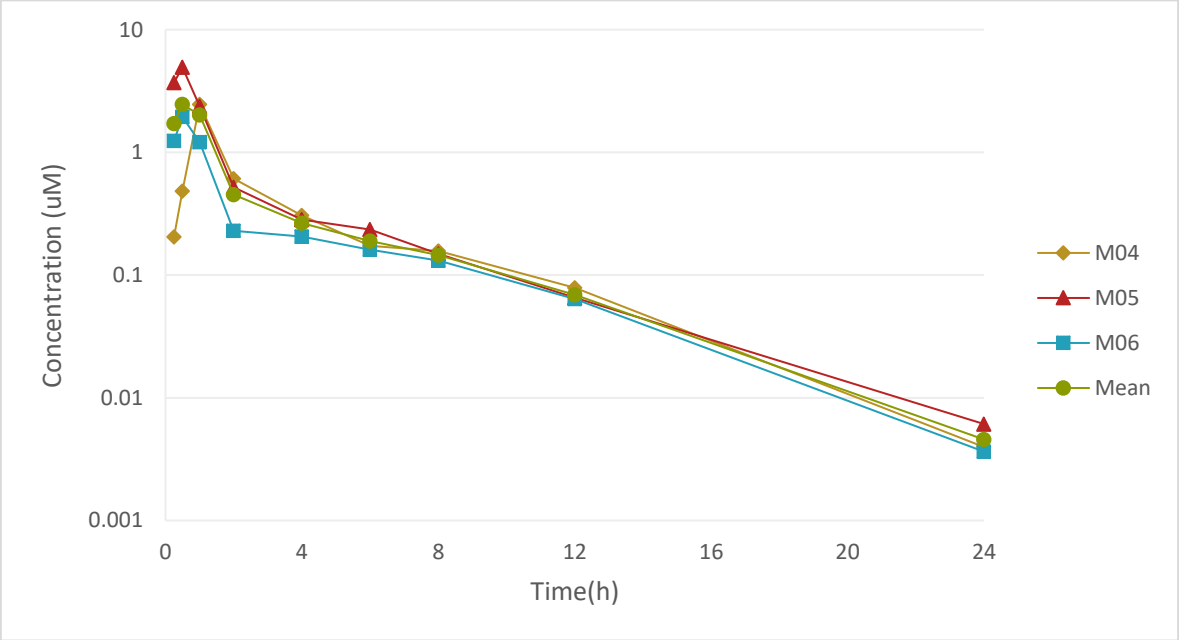


Figure 2 Individual and Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Intravenous Administration of KT-00478 at 5 mg/kg



"M#" means animal number.

Figure 3 Individual and Mean Plasma Concentration Profiles of KT-00478 in Male CD-1 (ICR) Mice Following Single Oral Administration of KT-00478 at 10 mg/kg



"M#" means animal number.

APPENDIX II: METHOD SUMMARY FOR KT-00478

1. ACCEPTANCE CRITERIA FOR A BIOANALYTICAL RUN

Calibration curve (C): The sample analysis should be performed concurrently with one set of calibration standards using the established LC-MS/MS method. A minimum of 6 calibration standards is back calculated to within ±20% of their nominal values in plasma.

Quality control (QC): The sample analysis should be performed concurrently with two sets of QC samples using the established LC-MS/MS method. A set of QC samples for the method consists of low, middle and high concentrations. A minimum of 4 out of 6 QC samples is back calculated to within ±20% of their nominal values in plasma.

Specificity and Sensitivity: A standard curve consists of 8 non-zero calibration standards for each LC-MS/MS method with a target LLOQ at ≤3 ng/mL. The mean calculated concentration in the single blank matrix should be ≤0.5 times the LLOQ.

Carryover: The mean calculated carry-over concentration in the single blank matrix immediately after the highest standard injection should be ≤LLOQ. If the carryover couldn't meet the criteria, then the carryover factor should be estimated following bioanalytical SOP.

2. BIOLOGICAL MATRIX

Plasma: Blank male CD-1(ICR) mouse plasma with EDTA-K_2 as anti-coagulant was used for the preparation of calibration standards (C) and QC samples.

3. TEST COMPOUNDS

Compound	Batch	Purity (%)	Salt Factor	Shipped from
KT-00478	KT-00478_1	98.3	1.00	Sponsor

4. PREPARATION OF STOCK AND C AND QC SAMPLES IN BIO-MATRICES

4.1 Preparation of standard stock solutions

4.1.1 Preparation of stock solution

KT-00478 stock solution in dimethylsulfoxide (DMSO) (2000 μg/mL):

After 1.78 mg of KT-00478 was weighed accurately and transferred into a vial, 0.875 mL of DMSO was added into it and mixed well to obtain a stock solution of 2000 μg/mL (Stock A).

An aliquot of 10 μL of stock A was added with 40 μL of DMSO, then mixed well to obtain a substock solution of 400 μg/mL (Sub stock)

4.2 Preparation of C And QC samples in Bio-Matrix

4.2.1 The preparation procedures of KT-00478 C and QC samples in plasma (HP D300e)

Source Solution			Calibration Standard		
Source Solution ID	Conc. (μg/mL)	Volume (nL)	Matrix Volume (nL)	Final Conc. (ng/mL)	Calibration Standard ID

Sub stock	400	302.4	40000	3000	C1
Sub stock	400	100.4	40000	1000	C2
Sub stock	400	50	40000	500	C3
Sub stock	400	10	40000	100	C4
Sub stock	400	4.8	40000	48.0	C5
Sub stock	400	1	40000	10.0	C6
Sub stock	400	0.2	40000	2.00	C7
Sub stock	400	0.1	40000	1.00	C8
Source Solution ID	**Conc. (µg/mL)**	**Volume (nL)**	**Matrix Volume (nL)**	**Final Conc. (ng/mL)**	**QC ID**
Sub stock	400	241.6	40000	2400	QC1
Sub stock	400	80	40000	800	QC2
Sub stock	400	4	40000	40.0	QC3
Sub stock	400	0.3	40000	3.00	QC4
Sub stock	400	404	40000	4000	DQC

4.2.2 Valid concentrations of C and QC samples

Compound	Matrix	Calibration Standard Samples Conc. (ng/mL)	Quality Control Samples Conc. (ng/mL)
KT-00478	Male CD-1(ICR) mouse plasma	1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000	3.00, 40.0, 800, 2400, 4000(DQC)

5. SAMPLE PROCESSING

5.1 Plasma samples preparation for KT-00478

An aliquot of 8 µL sample with 160 µL internal standard (Labetalol & Tolbutamide & Verapamil & Dexamethasone & Glyburide & Celecoxib 100 ng/mL for each in ACN), the mixture was vortex-mixed for 10 min at 800 rpm and centrifuged centrifuged for 15 min at 3220×g, 4°C. 30 µL supernatant was transferred to another clean 96-well plate and diluted with 30 µL of ACN, vortex-mixed for 10 min at 800 rpm and centrifuged for 5 min at 3220×g, 4°C. 2 µL sample was injected for LC-MS/MS analysis.

Dilution procedure description:

Dilution factor as 10-- An aliquot of 4 µL sample was added with 36 µL blank plasma. (0.083h-2h of G1&0.25h-2h of G2)

6. INSTRUMENTATION AND CONDITIONS

6.1 Instrumentation and conditions for KT-00478 in plasma

LC parameters

Equipment: ACQUITY UPLC System

Analytical column: ACQUITY UPLC HSS T3 1.8 µm 2.1 × 50 mm

Inject volume: 2 µL

Mobile phase A: 0.1% FA in water

Mobile phase B: 0.1% FA in ACN

Elution Mode: Gradient

KT-00478 in Plasma

Time (min)	Flow Rate (mL/min)	A (%)	B (%)
Initial	0.650	90	10
1.00	0.650	0	100
1.30	0.650	0	100
1.31	0.650	90	10
1.40	0.650	90	10

Mass spectrometer: Triple Quad 5500

Ionization mode: ESI (+)

Detective mode: MRM

The Collision Energy and UPLC Retention Time for KT-00478

Compound Name	Ion Transition	Collision Energy (V)	Declustering Potential (V)	Retention Time (min)
KT-00478	508.2/306.1	30	110	1.09
Dexamethasone	393.0/373.1	19	80	0.800

7. DATA PROCESSING

MultiQuant 3.0 software was used for processing the data of all samples. The regression mode was linear with $1/x^2$ as weighting factor.