FINAL REPORT

A Pharmacokinetic Study of OCT-3598 following Single Oral Gavage Administration to C57BL/6 Mice

Study Number: 0040522413

Nonclinical Research Center
QuBEST BIO Co., Ltd.

COMPLIANCE STATEMENT AND APPROVAL

I, the undersigned, hereby declare that the work was performed under my supervision and that the report represents a true and accurate record of the results obtained.

This study was performed in accordance with the agreed protocol and with Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.
This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

Study Director:  Dec-13-2022

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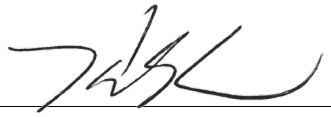
Management:  Dec-13-2022

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1. SUMMARY

The objective of this study was to evaluate the pharmacokinetic profiles of OCT-3598 following single oral gavage administration to C57BL/6 mice.

OCT-3598 was administered to C57BL/6 mice at 30 mg/kg and 100 mg/kg via oral gavage route. The blood samples were collected at the scheduled times up to 24 hours post-dose. Determination of OCT-3598 in plasma was performed by UHPLC-MS/MS. Pharmacokinetic parameters were calculated by noncompartmental analysis using Phoenix® WinNonlin® (Ver. 8.3, Certara).

Following single oral gavage administration of OCT-3598 to C57BL/6 mice at 30 and 100 mg/kg, OCT-3598 was absorbed with a Tmax of 0.25 hours. After reaching the maximum concentration (Cmax), plasma concentration of OCT-3598 decreased with a terminal half-life (t1/2) of 2.29 and 3.5 hours, respectively. Exposure of OCT-3598 increased less than dose-proportionally (Cmax) and more than dose-proportionally (AUClast) from 30 to 100 mg/kg. Specifically, as dose increased in a ratio of 1 : 3.3, the Cmax increased in a ratio of 1 : 2.2 and the AUClast increased in a ratio of 1 : 5.4.

Text Table 1. Mean Pharmacokinetic Parameters of OCT-3598 following Single Oral Gavage Administration to Mice at 30 and 100 mg/kg

PK parameters	OCT-3598	
	30 mg/kg	100 mg/kg
Cmax (ng/mL)	4397.70	9547.30
Tmax (hr)	0.25	0.25
AUClast (ng·hr/mL)	8080.63	43558.49
t1/2 (hr)	2.29	3.50

Text Table 2. Dose Proportionality of OCT-3598 following Single Oral Gavage Administration of OCT-3598 to Mice at 30 and 100 mg/kg

PK parameters	OCT-3598	
	30 mg/kg	100 mg/kg
Dose ratio	1	3.3
Cmax ratio	1	2.2
AUClast ratio	1	5.4

2. STUDY INFORMATION

2.1 Objectives

The objective of this study was to evaluate the pharmacokinetic profiles of OCT-3598 following single oral gavage administration to C57BL/6 mice.

2.2 Regulatory Test Guidelines

This is non-GLP study and was not conducted in compliance with GLP regulation; however, it was conducted according to the protocol and protocol amendment (if any) approved by the Sponsor and SOPs of Nonclinical Research Center, QuBEST BIO.

2.3 Sponsor

Oscotec Inc.

Korea Bio-Park Building A, 9th Floor, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13488, Republic of Korea

TEL: +82-31-628-7625, FAX: +82-31-628-7607

2.4 Test Facility

Nonclinical Research Center, QuBEST BIO Co., Ltd.

#301, Daewoo Frontier Valley I, 16-25, Dongbaekjungang-ro 16beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do, 17715, Republic of Korea

TEL: +82-31-5181-8700, FAX: +82-31-5181-8701

2.5 Study Schedule

Study initiation:	October 21, 2022
Experimental start (animal arrival):	October 21, 2022
Administration:	October 25, 2022
Sample collection:	October 25, 2022 - October 26, 2022
Pharmacokinetic analysis:	October 26, 2022- November 11, 2022
Submission of Final Report:	December 13, 2022

2.6 Responsible Personnel

Storage/preparation of the test article:	Sohee Kim, BS
Animal experiment:	Woontak Han, BS Jiyu Oh, BS, KLAT
Bioanalysis:	Sungyong Choi, MS Jaeyoung Seo, BS
Internal scientific review:	Soohyeon Kim, MS
Archives:	Jinyoung Jang, BS, KLAT

3. MATERIALS AND METHODS

3.1 Test Article

Name:	OCT-3598 (also referred to as KT-00478)
Batch/Lot Number:	EW36618-31-P1
Appearance:	Off-white solid
Purity/Assay:	99.3% (254nm)
Molecular weight (free form):	507.62
Exact mass:	507.19
Correction factor:	Will not be applied
Expiration date:	August 14, 2023
Storage conditions:	Room temperature, protected from light and moisture
Supplier:	Oscotec Inc.
Unused test article:	Returned to Sponsor

3.2 Vehicle

Name:	10% SBE-β-CD in 50 mM Potassium phosphate buffer (pH 8.0)
Appearance:	Clear liquid
Storage conditions:	Room temperature
Manufacturer:	Nonclinical Research Center, QuBEST BIO

3.2.1 Vehicle Component

Name	Batch/Lot Number	Supplier
SBE-β-CD (Sulfobutylether-beta-cyclodextrin)	C2110136	Aladdin
Potassium phosphate dibasic	SLCK8159	Sigma-Aldrich
Potassium phosphate monobasic	MKCR5040	Sigma-Aldrich
Distilled water	KAI20022 2B	JW Life Science

3.3 Preparation of Dose Formulations

The dose formulations were freshly prepared in clean bench on the day of dosing according to the procedure provided by the Sponsor and a correction factor was not applied to prepare the dose formulations.

3.3.1 50 mM Potassium phosphate buffer (pH 8.0)

1) Prepare 800 mL of DW in a suitable container.
2) Add 8.141 g of Potassium phosphate dibasic into the container and stirred until clear solution.
3) Add 443.9 mg of Potassium phosphate monobasic into the solution.
4) Finally, DW was added until the final volume is 1 L.

3.3.2 10% SBE- β-CD in 50 mM Potassium phosphate buffer

1) The 100 g of the SBE-β-CD (Sulfobutylether-beta-cyclodextrin) was weighed in an appropriate container.
2) 700 mL of 50 mM Potassium phosphate buffer was added into the container and stirred until dissolved. And 50 mM Potassium phosphate buffer was added until the final volume is 1 L.

3.3.3 Dose Formulations

1) The required amount of the test article was weighed in an appropriate container.
2) Appropriate volume of 10% SBE-β-CD in 50 mM Potassium phosphate buffer (~70% of total volume) was added into the container and sonicated for approximately 20 minutes and stirred (and/or vortexed) for approximately 10 minutes.
3) Finally, 10% SBE-β-CD in 50 mM Potassium phosphate buffer was added to the final formulation volume and sonicated and stirred (and/or vortexed) until well suspension solution.

During the dosing, formulations were handled at room temperature, the remaining formulations were discarded.

3.3.4 Analysis of Dose Formulations

Analysis of homogeneity, concentration verification and stability of the dose formulations were not performed in the Test Facility.

3.4 Test System

3.4.1 Animal Information

Species and strain:	Specific pathogen free (SPF) mouse, C57BL/6 [C57BL/6TacSam]
Breeder/supplier:	SAMTAKOBIOKOREA, Inc. (105, Seorang-ro, Osan-si, Gyeonggi-do, 18100, Republic of Korea)
Number of ordered:	Females, 50
Age at receipt:	Approximately 6 weeks
Age at the initiation of dosing:	Approximately 7 weeks
Weight range at the initiation of dosing:	12.7-15.7 g

3.4.2 Justification for Selection

The C57BL/6 mice were chosen in this study because they were widely used in the pharmacokinetic study of drugs. The number of animals used in this study was considered the minimum number required to evaluate the pharmacokinetics of test articles.

3.4.3 Animal Receipt and Acclimation

Upon receipt, each animal was inspected by a qualified study personnel and/or Study Director to assess health status. Animals judged to be in good health were placed in acclimation for at least 5 days. During the acclimation, animals were weighed, observed once daily and cared as same as the study period.

3.4.4 Identification

All animals were individually identified by tail marking method using indelible red marker during acclimation period. During the study, each animal was identified by tail marking method using indelible black marker and a cage label card displaying the study number, group, and animal number.

3.4.5 Animal Welfare

The Protocol and procedures involving the care and use of animals in this study was reviewed and approved by QuBEST BIO IACUC prior to conduct (Approval No.: QBIACUC-A22413). During the study, the care and use of animals were conducted in accordance with all applicable guidelines of Animal Protection Law.

3.4.6 Husbandry and Environmental Conditions

This study was performed in the barrier animal facility area of Nonclinical Research Center, QuBEST BIO.

HVAC conditions:	100% HEPA-filtered air, at least 10 air changes/hr
Temperature and humidity:	20.4-23.5℃, 49.5-59.6% (relative humidity)
Light cycle and intensity:	12 hours light and 12 hours dark (on 08:00-20:00, except during designated procedures in the Protocol), intensity 150-300 Lux

The animals were housed in polycarbonate cages [260W x 420L x 180H (mm)] with irradiated bedding material, up to three per cage. Animal room and each cage/bottle was cleaned at regular intervals per SOPs of Nonclinical Research Center, QuBEST BIO.

3.4.7 Food and Water

All animals were offered to standard irradiated pelleted commercial laboratory diet (Purina Rodent Chow 38057, Republic of Korea) and water during the study. Nutritional components and environmental contaminants in the food were analyzed routinely by the manufacturer. No contaminants were reasonably expected to be present that would interfere with the objectives of the study; therefore, no testing was conducted as part of the study. According to SOPs of Nonclinical Research Center in QuBEST BIO, the drinking water was analyzed for contaminants semiannually by an independent laboratory. Results of the analyses of food and water are on file at the Test Facility.

3.4.8 Randomization

On the last day of acclimation period, all animals were weighed, evaluated for general health and suitability of testing and those considered suitable for the study were released to the study. The animals were randomly assigned to study groups by a stratified randomization scheme designed to achieve similar group mean body weights. Prior to the initiation of dosing and/or immediately after dosing, any assigned animals considered unsuitable for use and/or accidental events were replaced from the remaining unassigned animals.

3.4.9 Food Restriction

Dosing Route (Group)	Fasting Time	Food Supply
Oral gavage (G1and G2)	Approximately 16 hours	Approximately 4 hours post-dose

3.5 Study Method

3.5.1 Group Assignment

Group	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	No. of Animals	Animal ID
G1	OCT-3598	30	10	3	21	F1-F21
G2		100	10	10	21	F22-F42

3.5.2 Justification of Administration Route

Oral gavage administration route was selected by the Sponsor.

3.5.3 Justification of Dose Levels

Dose levels were selected by the Sponsor.

3.5.4 Justification of Dose Volume

Dose volume was selected by the Sponsor.

3.5.5 Method of Administration

The dose formulations were administered orally via a syringe equipped with a disposable feeding tube [straight, 18 gauge (2.0 mm bulb diameter x 51 mm length)]. Individual dose volumes were based on the most recent body weights. The day of dosing was designated as study Day 1.

3.6 In-life Observations and Examinations

3.6.1 Mortality and Clinical Observations

All animals were observed twice daily for mortality, abnormalities, and signs of pain and stress, once in the morning and once in the afternoon.

3.6.2 Body Weights

Individual body weights were measured for all animals on the day of animal receipt, randomization, prior to dosing (Day 1).

3.7 Sample Collection

3.7.1 Blood Collection

Sampling method:	Terminal sampling (3 mice/time point)
Sampling schedule:	0.25, 0.5, 1, 2, 4, 8, and 24 hours post-dose (total 7 time points)
Sample volume:	Approximately 500 μL/time point
Sampling site:	Cardiac
Anesthetic:	Isoflurane inhalation
Anticoagulant:	Sodium heparin (heparinized capillary tube)
Sample handling:	Keep samples chilled (wet ice, as appropriate) during collection and during processing.

3.7.2 Sample Processing

Centrifugation:	12,000 rpm for approximately 2 minutes at 4°C
Aliquots:	The maximum amount of plasma was recovered and placed in uniquely labeled micro tubes.
Label information:	Study group, animal number and time point

3.7.3 Sample Storage

Plasma samples were stored frozen (below -70°C) unless validated method indicates otherwise.

3.8 Bioanalysis

The concentrations of OCT-3598 in plasma were analyzed at Bioanalysis Center, QuBEST BIO using a qualified UHPLC-MS/MS method.

3.9 Data Processing and Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated at Nonclinical Research Center, QuBEST BIO. Non-compartmental analysis of OCT-3598 concentrations in plasma was performed by using the Phoenix® WinNonlin® software (Ver. 8.3, Certara).

Plasma concentrations below the LLOQ were replaced with zero for calculation purposes.

The following configuration was used for the analysis:

Calculation method:	Linear Trapezoidal with Linear Interpolation
Lambda Z (λz) method:	Best fit for λz, Log regression
Weighting (λz calculation):	Uniform

The following parameters (including abbreviation and description for each parameter) were calculated and reported.

Parameters	Definition
AUClast	Area under the curve from the time of dosing to the last measurable concentration
AUCinf	Area under the curve from dosing time extrapolated to infinity
Cmax	The maximum measured concentration
CL/F	Apparent total clearance after extravascular administration
Tmax	The time to reach maximum concentration
t1/2	Terminal half-life
Rsq_adjusted	Goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of Lambda Z.
Vd/F	Apparent volume of distribution after extravascular administration
%AUCexp	Percentage of the AUCinf that is contributed by the extrapolation from the last sampling time to infinity

4. RETENTION OF RECORDS, SAMPLES AND SPECIMENS

The study-specific raw data, documents, correspondences, Protocol and Final Report will be archived at the test facility for six months after issuance of Final Report. Remained test or reference articles, specimens and study samples will be retained under appropriate condition by the issuance of Final Report but not later than six months after submission of Draft Report. The Sponsor will be informed the list of the specimens and study samples remained prior to discard.

5. RESULTS AND DISCUSSION

During the in-life period, no test article-related abnormality was observed in all animals (Table 1 and Appendix 1).

The mean pharmacokinetic parameters and concentration vs. time profiles of OCT-3598 are presented in Text Table 3 and Figure 1.

Individual plasma concentrations of OCT-3598 are shown in Appendix 2 and Appendix 3.

Text Table 3. Mean Pharmacokinetic Parameters of OCT-3598 following Single Oral Gavage Administration to Mice at 30 and 100 mg/kg

PK parameters	OCT-3598	
	30 mg/kg	100 mg/kg
Cmax (ng/mL)	4397.70	9547.30
Tmax (hr)	0.25	0.25
AUClast (ng·hr/mL)	8080.63	43558.49
AUCinf (ng·hr/mL)	9071.93	44008.83
t1/2 (hr)	2.29	3.50
CL/F (mL/hr/kg)	3306.90	2272.27
Vd/F (mL/kg)	10901.71	11484.94
Rsq_adjusted	0.62	1.00
%AUCexp (%)	10.93	1.02

Tmax presented as median

6. CONCLUSIONS

After a single oral gavage administration to mice, all animals were exposed to OCT-3598, thereby enabling pharmacokinetic profile evaluation at dose level administered (30 and 100 mg/kg).

In addition, single oral gavage doses up to 100 mg/kg of OCT-3598 was clinically well tolerated.

7. REFERENCES

1) B. Davies and T. Morris, “Physiological Parameters in Laboratory Animals and Humans”, Pharmaceutical Research, Vol. 10, No. 7, 1993, pp. 1093-1095.

2) Katya Tsaioun and Steven A. Kates, “ADMET for Medicinal Chemists: A Practical Guide”, John Wiley & Sons, 2011, p159.

8. FIGURE

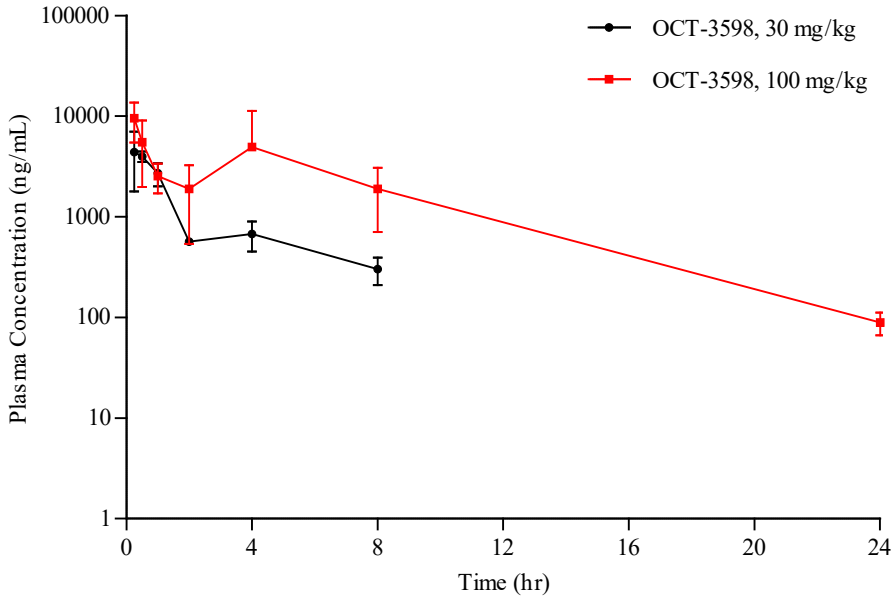

Figure 1. Mean (±SD) Plasma Concentrations-Time Profiles of OCT-3598 following Single Oral Gavage Administration to Mice at 30 and 100 mg/kg

9. TABLE

Table 1. Clinical Observations and Body Weights (Group Summary)

Group/	Dose Level (mg/kg)	Clinical Observations	Body Weights (Mean ± SD, g)
G1 OCT-3598	30	Appears normal	13.97 ± 0.56
G2 OCT-3598	100	Appears normal	13.89 ± 0.80

10. APPENDICES

Appendix 1. Individual Clinical Observations and Body Weights

Group/ Treatment	Dose Level (mg/kg)	Animal No.	Clinical Observations	Body Weights (g)
G1 OCT-3598	30	F1	Appears normal	14.3
		F2	Appears normal	13.8
		F3	Appears normal	14.9
		F4	Appears normal	14.3
		F5	Appears normal	14.4
		F6	Appears normal	13.6
		F7	Appears normal	13.7
		F8	Appears normal	14.2
		F9	Appears normal	13.6
		F10	Appears normal	14.0
		F11	Appears normal	14.5
		F12	Appears normal	14.2
		F13	Appears normal	13.1
		F14	Appears normal	14.0
		F15	Appears normal	12.9
		F16	Appears normal	14.0
		F17	Appears normal	14.6
		F18	Appears normal	13.1
		F19	Appears normal	13.7
		F20	Appears normal	13.6
		F21	Appears normal	14.9

Group/ Treatment	Dose Level (mg/kg)	Animal No.	Clinical Observations	Body Weights (g)
G2 OCT-3598	100	F22	Appears normal	14.8
		F23	Appears normal	14.0
		F24	Appears normal	13.4
		F25	Appears normal	13.7
		F26	Appears normal	14.4
		F27	Appears normal	15.7
		F28	Appears normal	15.2
		F29	Appears normal	14.4
		F30	Appears normal	12.8
		F31	Appears normal	13.3
		F32	Appears normal	13.9
		F33	Appears normal	13.8
		F34	Appears normal	13.3
		F35	Appears normal	13.3
		F36	Appears normal	14.5
		F37	Appears normal	13.3
		F38	Appears normal	13.5
		F39	Appears normal	13.7
		F40	Appears normal	13.1
		F41	Appears normal	12.7
		F42	Appears normal	14.8

Appendix 2. Plasma Concentrations of OCT-3598 following Single Oral Gavage Administration to Mice at 30 mg/kg

Time (hr)	Animal No.			Mean	SD	CV (%)
	Plasma concentrations (ng/mL)					
0.25	F1	F2	F3			
	4105.0	7149.0	1939.0	4397.7	2617.3	59.5
0.5	F4	F5	F6			
	4230.3	4267.1	3413.1	3970.2	482.8	12.2
1	F7	F8	F9			
	2123.5	2496.2	3475.9	2698.5	698.5	25.9
2	F10	F11	F12			
	574.8	559.8	558.7	564.4	9.0	1.6
4	F13	F14	F15			
	929.2	511.1	580.1	673.5	224.1	33.3
8	F16	F17	F18			
	393.0	208.9	300.1	300.7	92.1	30.6
24	F19	F20	F21			
	3.8	5.4	6.2	5.1	1.2	23.8

BLQ: < LLOQ (10 ng/mL); NA: Not applicable

Appendix 3. Plasma Concentrations of OCT-3598 following Single Oral Gavage Administration to Mice at 100 mg/kg

Time (hr)	Animal No.			Mean	SD	CV (%)
	Plasma concentrations (ng/mL)					
0.25	F22	F23	F24			
	8195.5	14153.2	6293.2	9547.3	4100.7	43.0
0.5	F25	F26	F27			
	9014.0	5501.0	1975.2	5496.7	3519.4	64.0
1	F28	F29	F30			
	1695.2	2553.1	3360.2	2536.2	832.6	32.8
2	F31	F32	F33			
	1451.8	807.9	3406.3	1888.7	1353.2	71.6
4	F34	F35	F36			
	12217.6	951.9	1616.8	4928.8	6321.1	128.2
8	F37	F38	F39			
	3250.8	1220.3	1191.8	1887.6	1180.6	62.5
24	F40	F41	F42			
	112.7	86.9	67.6	89.1	22.6	25.4

BLQ: < LLOQ (10 ng/mL); NA: Not applicable

Appendix 5. Certificate of Analysis

药明康德 WuXi AppTec	药明康德新药开发有限公司核心分析部 Core Analytical Services of WuXi AppTec (Wuhan) Co., Ltd.			
文件名称 Title	分析证书 Certificate of Analysis		页码 Page	1 of 2
文件编号 Doc. No.	CAS-WH-2022-COA-185		版本号 Version No.	00
起草人/日期 Prepared by/Date	邓梦婷 19-Aug-2022	复核人/日期 Reviewed by/Date	张强 19-Aug-22	
项目负责人/日期 SD/Date	崔世星 19-Aug-2022			

化合物编号(Compound ID): KT-00478

分子式(Empirical Formula):C29 H30 F N O4 S

精确分子量(Exact Mass): 507.19

摩尔分子量(Molecular Weight): 507.62

生产日期(Manufacturing Date): 12-Aug-2022

重量(Amount): 505.00g

样品批号(Batch No.): EW36618-31-P1

样品编号(Sample ID): EW36618-31-P1A

检测日期(Test date): 15-Aug-2022

复检日期(Retest date): 14-Aug-2023

检测项 Testing Item	方法编号 Method No.	接受指标 Criteria	结果 Result
Appearance	Visual Inspection	Report	Off-white solid
^{1}HNMR	CAS-QMS-025.03	Match with structure	Match with structure
^{13}CNMR	CAS-QMS-025.03	Match with structure	Match with structure
^{19}FNMR	CAS-QMS-025.03	Match with structure	Match with structure
MS	30-90AB_30MIN_M1	Match with structure	Match with structure
Water Content	CAS-GTM-005.00	Report	0.26%
ROI	CAS-STM-009.00	Report	0.07%
ICP-MASS	20220817-01(2022-08-17_16-58-18)	Report	Li:<2.00ppm Fe:8.51ppm Pd:145.59ppm
Residual Solvent	HS(110-120-130_23min)_1.0_20_40(0)-10-100(3)-25-240(2)_260_CP-Volamine_160Kpa	Report	MeOH: <619ppm(11ppm) MeCN:<80ppm(N.D.) DCM:<119ppm(N.D.) EtOAc:<1002ppm(16ppm) THF:<144ppm(N.D.) Dioxane:<77ppm(N.D.) DMF:<188ppm(N.D.)
HPLC	30-90AB_30MIN_M1	Purity :>98.0%	98.7% (220nm) 99.3% (254nm)
QNMR	CAS-QMS-025.03	Report	98.1%
SFC	OJ-3-MeOH(DEA)-5-40-3ML-35T	Report	E.E%=100%

Address: No.666 Gaoxin Road, Wuhan East Lake High-tech Development Zone, Hubei, China
Tel: +86-27-6539 0001 Fax: +86-27-6539 0010

声明（Statement）

检测方法未经验证，结果仅供参考。
Test results in this document are presented for information only as the test methods used to generate the data have not been validated.

贮存条件(Storage Condition):

室温, 避光, 干燥
Ambient temperature, Protect from light, Dry

附件清单（List of appendixes）

检测项 Testing Item	参考记录 Reference
Appearance	WH-LCMS-2022001-19
^{1}HNMR	WH-NMR-2022002-17
^{13}CNMR	WH-NMR-2022002-17
^{19}FNMR	WH-NMR-2022002-17
MS	WH-LCMS-2022001-19
Water Content	WS-WH-2022-3582
ROI	WS-WH-2022-1649
ICP-MASS	WS-WH-2022-2580
Residual Solvent	WH-GCRS-2022001-05
HPLC	WH-LCMS-2022001-19
QNMR	WH-QNMR-2022002-15
SFC	WS-WH-2022-3510

变更历史(Change History):

版本 Version	变更描述 Description of Change
00	初始发行（Newly established）

Appendix 6. Analytical Method

1. METHOD SUMMARY

Analyte/Metabolite Name(s)	OCT-3598 (Lot no. EW36618-31-P1)
Internal Standard Name(s)	OCT-3598-D3 (Lot no. P-0003800-002)
Species (Strain)/Matrix	Mouse (C57BL/6) / Plasma
Anticoagulant (if applicable)	Sodium heparin
Sample Volume	20 µL
Calibration Range(s)	10 to 10000 ng/mL
Regression Type	Linear, 1/x2
Extraction Type	Acetonitrile protein precipitation
Instrumentation/Detection	1290 Infinity II (Agilent, California) and QTrap 4500 (SCIEX, Massachusetts) with ESI +
Sample Preparation Temperature:	Room temperature
Sample Storage Temperature	-80ºC
Special Storage/Treatment Requirements	None

2. HPLC CONDITION

Column	ZORBAX RRHD Eclipse Plus C8 (2.1 x 100 mm, 1.8 µm), Agilent (ID: C18-119)			
Column temperature	40 ºC			
Autosampler temperature	15 ºC			
Injection volume	1 µL			
Needle wash solution	90% Isopropyl alcohol in type 1 water (flush port, 30 sec.)			
Mobile phase A	10 mM Ammonium acetate in type 1 water			
Mobile phase B	0.1% Formic acid in acetonitrile			
Mobile phase	Time (min)	Flow (mL/min)	A (%)	B (%)
	0.00	0.400	35	65
	2.50	0.400	35	65

3. MASS SPECTROMETER CONDITION

Parameters	OCT-3598	OCT-3598-D3
Polarity	ESI Positive	
MRM (m/z)	508.2 > 153.0	511.2 > 218.1
DP	170	170
EP	10	10
CE	41	43
CXP	14	14
CUR	30	
GS1	45	
GS2	50	
CAD	High	
Ion spray voltage	5500	
Ion spray temp.	550	
Nebulizing gas	Nitrogen	
Data processing	Analyst 1.7.0	

4. SAMPLE PROCESSING PROCEDURES

No.	Procedure
1	The samples exceeded the ULOQ concentration of the calibration curve at each concentration were used after 10 folds dilution by adding blank sample (sample : blank matrix = 2 µL : 18 µL).
2	20 µL of study sample was added into a microtube.
3	1000 µL of IS working solution (ISW2, 100 ng/mL) was added into the microtube, which was contained STD or sample. However, for Blank or Blank + Drug sample, added 1000 µL of acetonitrile instead of IS working solution.
4	The mixture was mixed at 2000 rpm for 2 minutes using vortex mixer, and then centrifuged at 15000 RCF for 5 minutes at 4ºC.
5	The supernatant was transferred into a sample vial.