FINAL REPORT

A Pharmacokinetic Study of OCT-598 Following Single Intravenous and Oral Gavage Administration to Beagle Dogs

Study Number: 0040523197

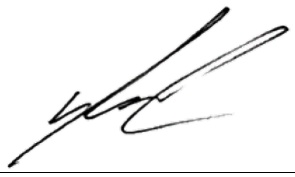
Nonclinical Research Center

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:  Nov-15-2023

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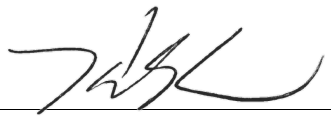
Management:  Nov-15-2023

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1. UNITS AND ABBREVIATIONS

The following lists of codes, abbreviations and units are used by QuBEST BIO Co., Ltd.

All of the abbreviations listed on these pages may not be applicable to this Protocol.

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

%	Percent	BLQ	Below the limit of quantification
℃	Degree Celsius	Conc.	Concentration
kg	Kilogram	LC-MS/MS	HPLC-tandem mass spectrometry
g	Gram	LLOQ	Lower limit of quantification
mg	Milligram (10^{-3} grams)	NA	Not applicable
μg	Microgram (10^{-6} grams)	ND	Not detected/Not determined
ng	Nanogram (10^{-9} grams)	No.	Number
L	Liter	SD	Standard deviation
mL	Milliliter (10^{-3} liters)	TBD	To be determined
μL	Microliter (10^{-6} liters)	ULOQ	Upper limit of quantification
min	Minute	CV (%)	Percent coefficient of variation
hr	Hour		

PHARMACOKINETIC ABBREVIATIONS

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
C0	Initial concentration
Cmax	The maximum measured concentration
CL	Total clearance after intravenous administration
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.
Vdss	Volume of distribution at steady-state after intravenous administration
Vd/F	Apparent volume of distribution after extravascular administration
Vz	Volume of distribution based at the terminal phase
%AUCexp	Percentage of the AUCinf that is contributed by the extrapolation from the last sampling time to infinity
BA	$\text{Bioavailability (\%)} = \frac{\text{AUC}extravescular}{\text{AUC}intravenous} \times \frac{\text{Dose}intravenous}{\text{Dose}extravescular} \times 100$

2. SUMMARY

The objective of this study was to evaluate the pharmacokinetic profiles of OCT-598 following single intravenous and oral gavage administration to Beagle dogs.

OCT-598 was administered to male and female Beagle dogs (fasted and fed) at 3, 10 and 30 mg/kg via oral gavage and 3 mg/kg via intravenous route. The blood samples were collected at the scheduled times up to 24 hours post-dose. Determination of OCT-598 in plasma was analyzed by UHPLC-MS/MS. Pharmacokinetic parameters were calculated by noncompartmental analysis using Phoenix® WinNonlin® (Ver. 8.4, Certara).

Following single intravenous administration of OCT-598 to male dogs at 3 mg/kg, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 0.90 hours. Total clearance (CL) of OCT-598 was 1624.35 mL/hr/kg. Volume of distribution at steady-state (Vdss) of OCT-598 was 371.42 mL/kg, which is similar to total body water, indicating that OCT-598 evenly distributed throughout the blood and tissues.

Following single oral gavage administration of OCT-598 to fasted male dogs at 3, 10 and 30 mg/kg, OCT-598 was absorbed with a median Tmax of 0.5 hours. After reaching Cmax, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) ranged from 2.55 to 4.32 hours. Cmax ranged from 886.98 to 14833.75 ng/mL and AUClast ranged 809.20 to 29151.32 ng·hr/mL. Systemic exposure (Cmax and AUClast) increased more than dose-proportionally. Specifically, as the dose increased in a ratio of 1.0 : 3.3 : 10.0, the Cmax increased in a ratio of 1.0 : 6.6 : 16.7 and the AUClast increased in a ratio of 1.0 : 5.9 : 36.0. The oral bioavailability of OCT-598 at 3 mg/kg was 41.89%.

Following single oral gavage administration of OCT-598 to fasted female dogs at 10 mg/kg, OCT-598 was absorbed with a median Tmax of 0.5 hours. After reaching Cmax, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 1.93 hours. Cmax and AUClast were 4844.87 ng/mL and 3422.66 ng·hr/mL, respectively.

Following single oral gavage administration of OCT-598 to fed male dogs at 10 mg/kg, OCT-598 was absorbed with a median Tmax of 0.5 hours. After reaching Cmax, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 2.77 hours. Cmax and AUClast were 8472.33 ng/mL and 6865.62 ng·hr/mL, respectively.

No notable gender differences and food effects (<2-fold) were observed.

3. STUDY INFORMATION

3.1 Objectives

The objective of this study was to evaluate the pharmacokinetic profiles of OCT-598 following single intravenous (slow injection) and oral gavage administration to Beagle dogs.

3.2 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.

3.3 Sponsor

Oscotec Inc.

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

TEL: +82-31-628-7631, FAX: +82-31-628-7668

3.4 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

3.5 Test Site

HLB bioStep Co., Ltd.

38, Academy-ro 79beon-gil, Yeonsu-gu, Incheon-si, 22014, Republic of Korea

TEL: +82-32-833-8899, FAX: +82-507-0322-9594

3.6 Study Schedule

Study Initiation:	September 11, 2023
Experimental Start:	September 11, 2023
Administration:	September 14, 2023
Sample Collection:	September 14, 2023 - September 15, 2023
Pharmacokinetic Analysis:	September 18, 2023 - September 21, 2023
Submission of Final Report:	November 15, 2023

3.7 Responsible Personnel

Storage/Preparation of the Test Article:	Su-Chan Lee (HLB bioStep Co., Ltd.)
Animal Experiment:	Sung-Jin Park, MS (HLB bioStep Co., Ltd.)
Bioanalysis:	Sungyong Choi, MS Jaeyoung Seo, BS
Internal Scientific Review:	Soohyeon Kim, MS
Archives:	Myeongseok Gu, BS

4. MATERIALS AND METHODS

4.1 Test Article and Vehicle Information

4.1.1 Test Article

Name:	OCT-598
Batch/Lot Number:	CPo131731-01-08-01
Appearance:	White solid
Purity/Assay:	97.3%
Molecular Weight:	507.62
Exact Mass:	507.19
Correction Factor:	Not applied
Expiration Date:	May 26, 2024
Storage Conditions:	Refrigerate (2-8℃), protected from light and dehumidification
Supplier:	Oscotec Inc.
Unused Test Article:	Returned to the Sponsor

4.1.2 Vehicle

Name:	10% SBE-β-CD in 50 mM Potassium phosphate buffer (pH 8.0)
Storage Conditions:	Room temperature
Manufacturer:	HLB bioStep Co., Ltd.

4.1.2.1 Vehicle Component

Name	Batch/Lot Number	Supplier
SBE-β-CD (Sulfobutylether-beta-cyclodextrin)	OC15791801	BIOSYNTH (Carbosynth)
Potassium phosphate dibasic	SLCK8159	Sigma-Aldrich
Potassium phosphate monobasic	NKCR5040	Sigma-Aldrich
DW (Distilled water)	S5X8B21	DAI HAN PHARM

4.2 Preparation of Dose Formulations

The dose formulations were freshly prepared in clean bench on the day of each dosing according to the procedure provided by the Sponsor and a correction factor was applied to prepare the dose formulations.

1) Required amount of test article was weighed and transferred to appropriate container.
2) 10% SBE-β-CD in 50 mM potassium phosphate buffer (approximately 80% of total volume) was added into the container and stirred/sonicated until clear solution by visual check (The dosing formulations were sonicated at least 30 minutes).
3) Finally, 10% SBE-β-CD in 50 mM potassium phosphate buffer (approximately 20% of total volume) was added into the container to the final formulation volume and stirred/vortexed until clear solution by visual check.

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

4.2.1 Analysis of Dose Formulations

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

4.3 Test System

4.3.1 Animal Information

Species and Strain:	Beagle dogs (non-naive) (*Canis familiaris*)
Breeder/Supplier:	Xi'an Dilepu Biology & Medicine Co., Ltd./SAERON BIO Inc. (2, Hanjikgollam-ro, Uiwang-si, Gyeonggi-do, Republic of Korea)
Sex and Number of Animals Ordered:	Males, 15 and Females, 3
Age at the Initiation of Dosing:	Approximately 17 - 22 months
Weight Range at the Initiation of Dosing:	8.6 - 11.7 kg

4.3.2 Justification for Selection

The Beagle dogs were chosen in this study because they were widely used in the pharmacokinetic study of drugs. The number of animals to be used in this study was considered the minimum number required to evaluate the pharmacokinetics of the test article.

4.3.3 Identification

During the study, each animal was identified by cage label card displaying the study number, group, animal number and tattoo number.

4.3.4 Animal Welfare

The Protocol and procedures involving the care and use of animals in this study was reviewed and approved by IACUC of HLB bioStep Co., Ltd. prior to conduct (Approval No.: HLB bioStep IACUC 23-KE-0267). During the study, the care and use of animals were conducted in accordance with all applicable guidelines of Animal Protection Law.

4.3.5 Husbandry and Environmental Conditions

This study was performed in the barrier animal facility area of HLB bioStep Co., Ltd.

HVAC Conditions:	100% HEPA-filtered air, at least 10~20 air changes/hr
Temperature and Humidity:	24.7 - 25.1℃, 57 - 61% (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 a single exclusive stainless-steel cage [800W x 900L x 800H (mm)]. Animal room and each cage were cleaned at regular intervals per SOPs of HLB bioStep Co., Ltd.

4.3.6 Food and Water

All animals were offered to standard irradiated pelleted commercial laboratory diet (Cargill Agri Purina, Inc.) for laboratory animals (300 g/dog/day) 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 HLB bioStep Co., Ltd., 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.

4.3.7 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. And then animals were randomly assigned to study group based on the body weights.

4.3.8 Food Restriction

Dosing Route (Group)	Fasting Time	Food Supply
Intravenous (G1)	Not applicable	*Ad libitum*
Oral gavage (G2 to G5)	Approximately 16 hours	Approximately 4 hours post-dose
Oral gavage (G6)	Not applicable	*Ad libitum*

4.4 Study Method

4.4.1 Group Assignment

Group	Treatment	Dosing Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	No. of Animals	Animal ID		Food Restriction
						Males	Females	
G1	OCT-598	IV	3	1	3	M1 - M3		Fed
G2		PO	3	5	3	M4 - M6		Fasted
G3			10		3		F7 - F9	
G4			10		3	M10 - M12		
G5			30		3	M13 - M15		
G6			10		3	M16 - M18		Fed

4.4.2 Justification of Administration Routes

The oral gavage administration route was selected because this is the intended route of human exposure. The intravenous administration was selected to investigate the total clearance, volume of distribution and oral bioavailability of test article.

4.4.3 Justification of Dose Levels

Dose levels were selected by the Sponsor.

4.4.4 Justification of Dose Volumes

Dose volumes were selected by the Sponsor.

4.4.5 Method of Administration

4.4.5.1 Intravenous Administration

The dose formulation was administered intravenously into the cephalic vein via a disposable syringe with 23-gauge needle (slow injection at approximately 5 mL/minute of dosing rate). The dose volume was individual dose volumes were based on the most recent body weights.

4.4.5.2 Oral Gavage Administration

The dose formulation was administered into the stomach using enteral feeding tube (French size 12) followed by 5 mL vehicle flush. The individual dose volume was based on the most recent body weights.

4.4.6 Dosing Regimen

The dose formulation was administered once. The day of dosing was designated as Day 1

4.5 In-life Observations and Examinations

4.5.1 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.

4.5.2 Body Weights

Individual body weights were measured prior to dosing for all animals.

4.6 Sample Collection

4.6.1 Blood Collection

Sampling Method:	Serial sampling
Sampling Schedule:	IV: 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hours post-dose (total 8 time points) PO: 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose (total 7 time points)
Sample Volume:	Approximately 1 mL/time point
Sampling Site:	Jugular vein
Anticoagulant:	Sodium heparin
Sample Handling:	Keep samples chilled (wet ice, as appropriate) during collection and processing

4.6.2 Plasma 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. Plasma was divided into two approximately equal aliquots.
Label Information:	Study group, animal number and time point

4.6.3 Sample Storage

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

4.7 Bioanalysis

The concentrations of OCT-598 in plasma was analyzed at Bioanalysis Center, QuBEST BIO using a qualified UHPLC-MS/MS method.

4.8 Data Processing and Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated at Nonclinical Research Center, QuBEST BIO. Non-compartmental analysis of OCT-598 concentrations in plasma was performed by using the Phoenix® WinNonlin® software (Ver. 8.4, Certara).

Plasma concentrations below the LLOQ were replaced with zero for calculation purposes.
The area under the concentration versus time curve (AUC) was calculated using linear trapezoidal method with linear interpolation. The slope of the terminal elimination phase was determined using the best fit for λz and log-linear regression on the unweighted concentration data.

5. 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.

6. RESULTS AND DISCUSSION

During the in-life period, vomiting and diarrhea were observed in the study animals (Table 1 and Appendix 1).

The mean pharmacokinetic parameters and concentration vs. time profiles of OCT-598 are presented in Text Table 1 to Text Table 7 and Figure 1 to Figure 4.

Individual pharmacokinetic parameters of OCT-598 are presented in Appendix 2 to Appendix 7.

Text Table 1. Mean (± SD) Pharmacokinetic Parameters of OCT-598 Following Single Intravenous Administration to Male Dogs at 3 mg/kg

PK Parameters	Male (Fed)
	3 mg/kg
C0 (ng/mL)	10043.81 ± 930.13
AUClast (ng·hr/mL)	1931.67 ± 261.28
AUCinf (ng·hr/mL)	1878.48 ± 344.42
t1/2 (hr)	0.90 ± 0.93
CL (mL/hr/kg)	1624.35 ± 297.83
Vdss (mL/kg)	371.42 ± 77.31
Vz (mL/kg)	1892.57 ± 1794.69
Rsq_adjusted	1.00 ± 0.01
%AUCexp (%)	0.11 ± 0.01

Text Table 2. Mean (± SD) Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 3, 10 and 30 mg/kg

PK Parameters	Male (Fasted)		
	3 mg/kg	10 mg/kg	30 mg/kg
Cmax (ng/mL)	886.98 ± 377.30	5810.79 ± 2573.74	14833.75 ± 9309.88
Tmax (hr)	0.5 [0.5 - 0.5]	0.5 [0.5 - 0.5]	0.5 [0.5 - 0.5]
AUClast (ng·hr/mL)	809.20 ± 395.27	4751.25 ± 2326.34	29151.32 ± 29955.87
AUCinf (ng·hr/mL)	830.23 ± 375.32	4769.58 ± 2331.80	29325.53 ± 30183.59
t1/2 (hr)	3.20 ± 1.97	2.55 ± 1.22	4.32 ± 2.97
CL/F (mL/hr/kg)	4240.35 ± 2148.10	2534.21 ± 1395.34	3452.47 ± 4402.15
Vd/F (mL/kg)	23564.16 ± 25006.33	7773.47 ± 304.23	32444.29 ± 51164.08
Rsq_adjusted	0.94 ± 0.10	0.92 ± 0.04	0.94 ± 0.08
AUCexp (%)	4.05 ± 5.87	0.40 ± 0.12	0.57 ± 0.31
Bioavailability (%)	41.89 ± 20.46	NA	NA

Tmax presented as median [min-max]

Text Table 3. Mean (± SD) Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Male and Female Dogs at 10 mg/kg

PK parameters	Male (Fasted)	Female (Fasted)
	10 mg/kg	10 mg/kg
Cmax (ng/mL)	5810.79 ± 2573.74	4844.87 ± 436.97
Tmax (hr)	0.5 ± 0.0	0.5 [0.5 - 0.5]
AUClast (ng·hr/mL)	4751.25 ± 2326.34	3422.66 ± 385.37
AUCinf (ng·hr/mL)	4769.58 ± 2331.80	3430.79 ± 385.35
t1/2 (hr)	2.55 ± 1.22	1.93 ± 0.53
CL/F (mL/hr/kg)	2534.21 ± 1395.34	2938.90 ± 322.69
Vd/F (mL/kg)	7773.47 ± 304.23	8338.16 ± 3034.33
Rsq_adjusted	0.92 ± 0.04	0.92 ± 0.12
%AUCexp (%)	0.40 ± 0.12	0.24 ± 0.07

Tmax presented as median [min-max]

Text Table 4. Mean (± SD) Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted and Fed Male Dogs at 10 mg/kg

PK parameters	Male (Fasted)	Male (Fed)
	10 mg/kg	10 mg/kg
Cmax (ng/mL)	5810.79 ± 2573.74	8472.33 ± 3180.49
Tmax (hr)	0.5 [0.5 - 0.5]	0.5 [0.5 - 0.5]
AUClast (ng·hr/mL)	4751.25 ± 2326.34	6865.62 ± 2922.99
AUCinf (ng·hr/mL)	4769.58 ± 2331.80	6894.91 ± 2919.08
t1/2 (hr)	2.55 ± 1.22	2.77 ± 0.44
CL/F (mL/hr/kg)	2534.21 ± 1395.34	1671.62 ± 804.67
Vd/F (mL/kg)	7773.47 ± 304.23	6345.29 ± 1980.09
Rsq_adjusted	0.92 ± 0.04	0.97 ± 0.02
%AUCexp (%)	0.40 ± 0.12	0.47 ± 0.44

Tmax presented as median [min-max]

Text Table 5. Dose Proportionality of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 3, 10 and 30 mg/kg

Ratio	3 mg/kg	10 mg/kg	30 mg/kg
Dose ratio	1.0	3.33	10.00
Cmax ratio	1.0	6.55	16.72
AUClast ratio	1.0	5.87	36.03

Text Table 6. Gender Difference of OCT-598 Following Single Oral Gavage Administration to Male and Female Dogs at 10 mg/kg

PK Parameters	Male	Female	Ratio[1)]
Cmax (ng/mL)	5810.79	4844.87	1.20
AUClast (ng·hr/mL)	4751.25	3422.66	1.39

1): Male/Female

Text Table 7. Food Effect of OCT-598 Following Single Oral Gavage Administration to Male Dogs at 10 mg/kg

PK Parameters	Fasted	Fed	Ratio[1)]
Cmax (ng/mL)	5810.79	8472.33	1.46
AUClast (ng·hr/mL)	4751.25	6865.62	1.45

1): Fed/Fasted

7. CONCLUSIONS

Pharmacokinetic studies of OCT-598 were assessed to determine the dose-dependent exposure levels and bioavailability by single intravenous or oral gavage administrations. Further pharmacokinetic studies also performed to assess gender difference and food effect of OCT-598 in Beagle dogs.

Following single intravenous administration of OCT-598 to male dogs at 3 mg/kg, total clearance (CL), volume of distribution at steady-state (Vdss) and terminal half-life (t1/2) were 1624.35 mL/hr/kg, 371.42 mL/kg and 0.90 hours, respectively.

Following single oral gavage administration of OCT-598 to fasted male dogs at 3, 10 and 30 mg/kg, systemic exposure (Cmax and AUClast) increased more than dose-proportionally and oral bioavailability of OCT-598 at 3 mg/kg was 41.89%.

No notable gender differences and food effects (<2-fold) were observed.

8. 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.

3) Kwon, Younggil, “Handbook of Essential Pharmacokinetics, Pharmacodynamics, and Drug Metabolism for Industrial Scientists”, Springer, 2002, p74

9. FIGURES

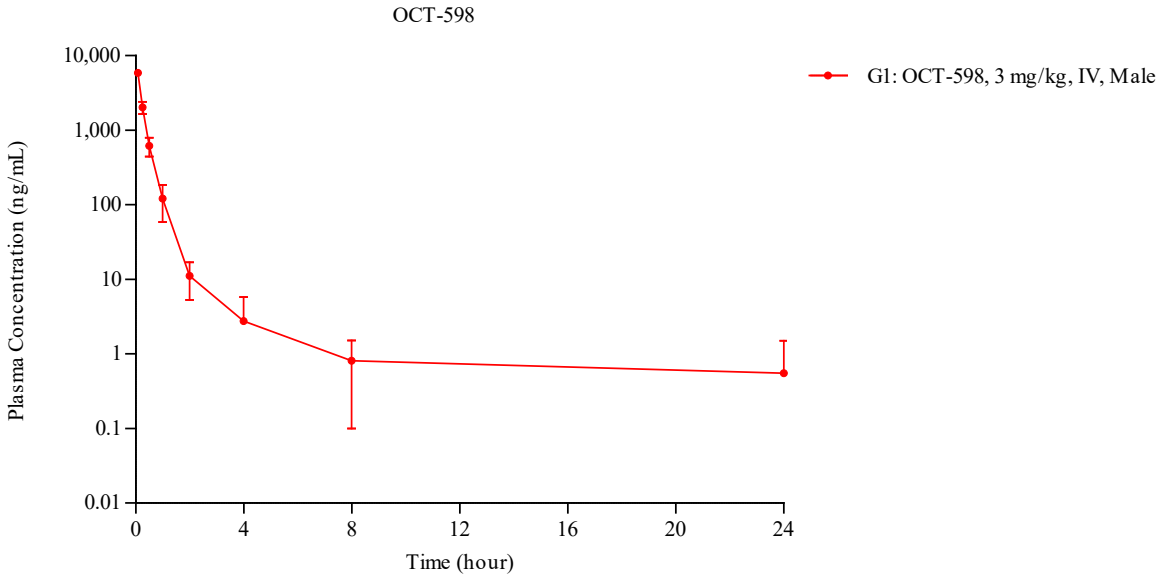


Figure 1. Mean (±SD) Plasma Concentrations-Time Profiles of OCT-598 Following Single Intravenous Administration to Male Dogs at 3 mg/kg

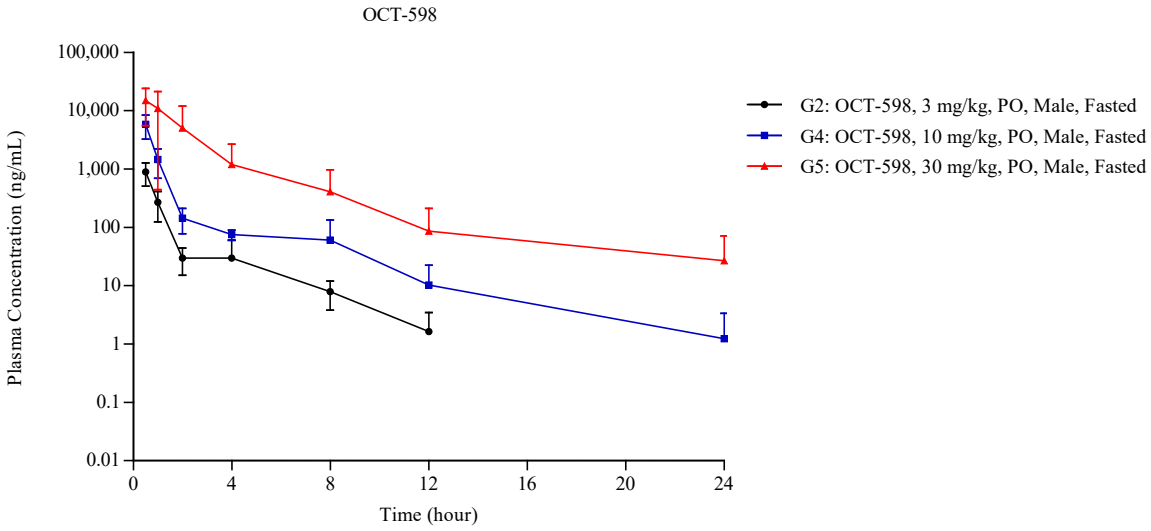


Figure 2. Mean (±SD) Plasma Concentrations-Time Profiles of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 3, 10 and 30 mg/kg

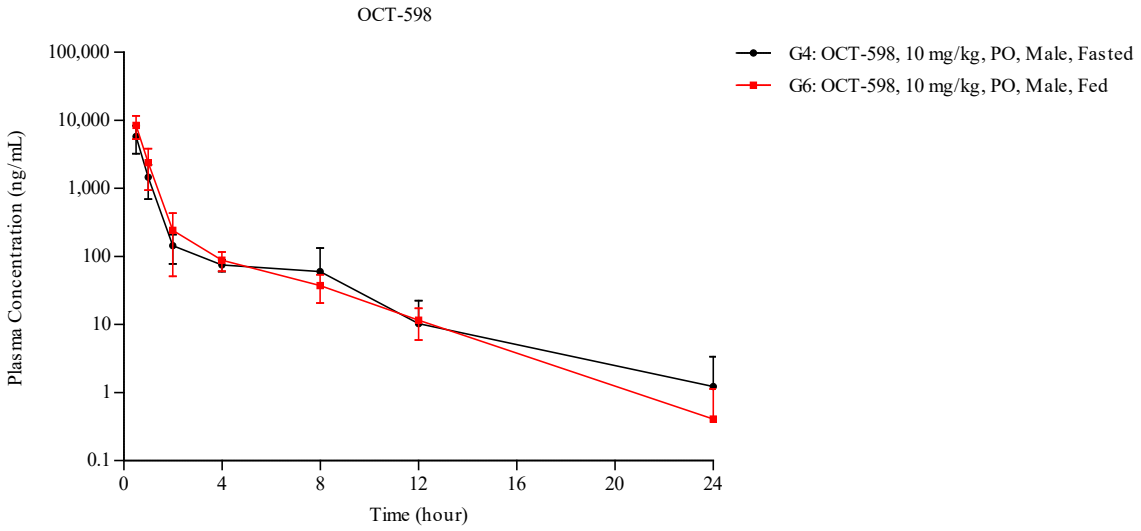


Figure 3. Mean (±SD) Plasma Concentrations-Time Profiles of OCT-598 Following Single Oral Gavage Administration to Fasted and Fed Male Dogs at 10 mg/kg

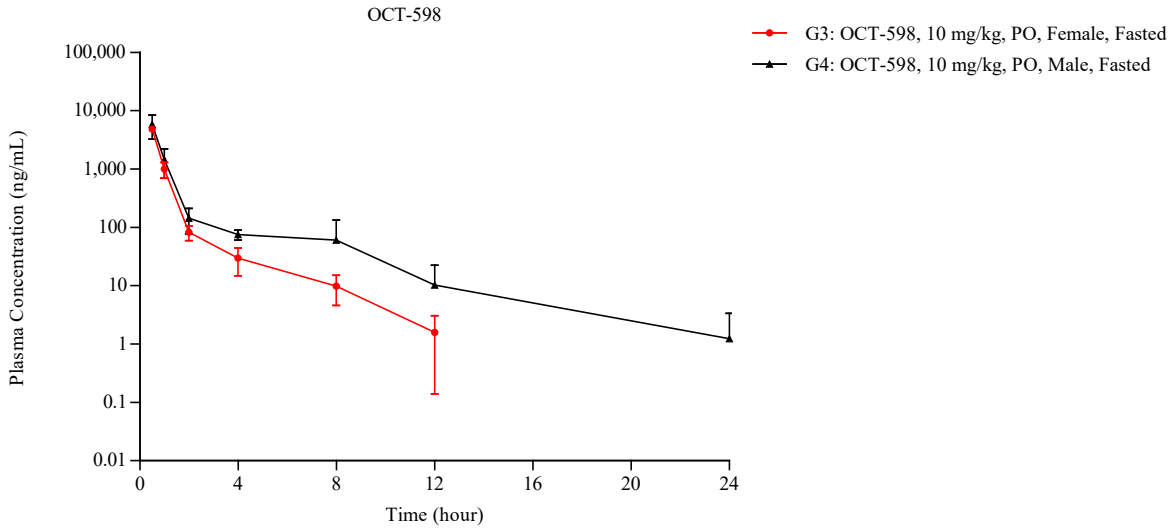


Figure 4. Mean (±SD) Plasma Concentrations-Time Profiles of OCT-598 Following Single Oral Gavage Administration to Fasted Male and Female Dogs at 10 mg/kg

10. TABLE

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

Group/ Treatment	Dose Level (mg/kg)	Dosing Route	Gender	Food Condition	No. of Animals	Clinical Observations	Body Weights (Mean ± SD, kg)
G1 OCT-598	3	IV	Male	Fed	3	Appears normal	9.4 ± 0.7
G2 OCT-598	3	PO	Male	Fasted	3	Appears normal	9.4 ± 0.6
G3 OCT-598	10	PO	Female	Fasted	3	Diarrhea	10.6 ± 1.0
G4 OCT-598	10	PO	Male	Fasted	3	Diarrhea	9.7 ± 0.6
G5 OCT-598	30	PO	Male	Fasted	3	Vomiting and Diarrhea	9.7 ± 0.9
G6 OCT-598	10	PO	Male	Fed	3	Appears normal	9.8 ± 1.2

11. APPENDICES

Appendix 1. Individual Clinical Observations and Body Weights

Group/ Treatment	Dose Level (mg/kg)	Dosing Route	Gender	Food Condition	Animal No.	Clinical Observations	Body Weights (kg)
G1 OCT-598	3	IV	Male	Fed	M1	Appears normal	8.6
					M2	Appears normal	9.7
					M3	Appears normal	10.0
G2 OCT-598	3	PO	Male	Fasted	M4	Appears normal	8.8
					M5	Appears normal	9.5
					M6	Appears normal	10.0
G3 OCT-598	10	PO	Female	Fasted	F7	Diarrhea (Approximately 5 hours post-dose)	9.8
					F8	Appears normal	10.2
					F9	Appears normal	11.7
G4 OCT-598	10	PO	Male	Fasted	M10	Appears normal	9.0
					M11	Diarrhea (Approximately 5 hours post-dose)	9.5
					M12	Appears normal	10.5
G5 OCT-598	30	PO	Male	Fasted	M13	Diarrhea (Approximately 7 hours post-dose)	9.1
					M14	Vomiting (Approximately 30 minutes post-dose)	9.3
					M15	Appears normal	10.8
G6 OCT-598	10	PO	Male	Fed	M16	Appears normal	9.1
					M17	Appears normal	9.2
					M18	Appears normal	11.2

Appendix 2. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Intravenous Administration to Fed Male Dogs at 3 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	M1	M2	M3			
0.083	5381.64	6287.27	5982.49	5,883.80	460.81	7.8
0.25	1664.38	2000.81	2419.81	2,028.33	378.47	18.7
0.5	426.48	658.25	773.18	619.30	176.60	28.5
1	68.07	105.66	191.25	121.66	63.13	51.9
2	4.80	12.31	16.30	11.14	5.84	52.4
4	0.00	2.34	5.97	2.77	3.01	108.6
8	0.00	1.34	1.09	0.81	0.71	88.0
24	0.00	1.64	0.00	0.55	0.95	173.2
C0 (ng/mL)	9643.17	11107.12	9381.14	10043.81	930.13	9.3
AUClast (ng·hr/mL)	1633.3	2042.12	2119.58	1931.67	261.28	13.5
AUCinf (ng·hr/mL)	1634.93	Not reported	2122.02	1878.48	344.42	18.3
t1/2 (hr)	0.24	Not reported	1.55	0.90	0.93	103.5
CL (mL/hr/kg)	1834.94	Not reported	1413.75	1624.35	297.83	18.3
Vdss (mL/kg)	316.75	Not reported	426.08	371.42	77.31	20.8
Vz (mL/kg)	623.53	Not reported	3161.61	1892.57	1794.69	94.8
Rsq_adjusted	0.99	Not reported	1.00	1.00	0.01	0.7
%AUCexp (%)	0.1	Not reported	0.11	0.11	0.01	6.7

M2 Pharmacokinetic Parameters (AUCinf, T1/2, CL, Vdss and %AUCexp): The values were unreasonable and were not used for mean value calculation, since Rsq_adjusted <0.85.

Appendix 3. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 3 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	M4	M5	M6			
0.5	533.13	1284.02	843.80	886.98	377.30	42.5
1	102.88	335.73	367.88	268.83	144.61	53.8
2	13.90	32.52	42.43	29.62	14.48	48.9
4	8.64	64.23	16.17	29.68	30.16	101.6
8	6.21	12.50	4.90	7.87	4.06	51.6
12	0.00	3.59	1.32	1.64	1.82	110.9
24	0.00	0.00	0.00	0.00	0.00	0.00
Cmax (ng/mL)	533.13	1284.02	843.8	886.98	377.30	42.5
Tmax (hr)	0.5	0.5	0.5	0.5	[0.5 - 0.5]	NA
AUClast (ng·hr/mL)	402.92	1192.46	832.21	809.20	395.27	48.8
AUCinf (ng·hr/mL)	451.85	1202.41	836.42	830.23	375.32	45.2
t1/2 (hr)	5.46	1.92	2.21	3.20	1.97	61.5
CL/F (mL/hr/kg)	6639.34	2494.98	3586.72	4240.35	2148.10	50.7
Vd/F (mL/kg)	52320.19	6920.12	11452.18	23564.16	25006.33	106.1
Rsq_adjusted	0.83	0.99	1.00	0.94	0.10	10.1
%AUCexp (%)	10.83	0.83	0.50	4.05	5.87	144.8
Bioavailability (%)	20.86	61.73	43.08	41.89	20.46	48.8

Tmax presented as median [min-max]

Appendix 4. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Female Dogs at 10 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	F7	F8	F9			
0.5	5073.50	4341.02	5120.09	4,844.87	436.97	9.0
1	1338.01	867.43	789.10	998.18	296.90	29.7
2	100.00	91.59	55.70	82.43	23.53	28.5
4	45.53	16.36	26.80	29.56	14.78	50.0
8	4.66	15.07	9.66	9.80	5.21	53.1
12	0.00	2.85	1.91	1.59	1.45	91.5
24	0.00	0.00	0.00	0.00	0.00	0.0
Cmax (ng/mL)	5073.5	4341.02	5120.09	4844.87	436.97	9.0
Tmax (hr)	0.5	0.5	0.5	0.5	[0.5 - 0.5]	NA
AUClast (ng·hr/mL)	3836.17	3073.53	3358.28	3422.66	385.37	11.3
AUCinf (ng·hr/mL)	3845.14	3083.14	3364.1	3430.79	385.35	11.2
t1/2 (hr)	1.33	2.34	2.11	1.93	0.53	27.5
CL/F (mL/hr/kg)	2600.69	3243.44	2972.56	2938.90	322.69	11.0
Vd/F (mL/kg)	5006.83	10943.96	9063.69	8338.16	3034.33	36.4
Rsq_adjusted	0.99	0.79	0.99	0.92	0.12	12.5
%AUCexp (%)	0.23	0.31	0.17	0.24	0.07	29.7

Tmax presented as median [min-max]

Appendix 5. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 10 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	M10	M11	M12			
0.5	6529.46	2954.10	7948.81	5,810.79	2,573.74	44.3
1	1218.25	840.93	2308.31	1,455.83	761.99	52.3
2	130.39	85.58	216.66	144.21	66.62	46.2
4	71.28	62.37	90.78	74.81	14.53	19.4
8	33.43	4.73	142.41	60.19	72.64	120.7
12	7.10	0.00	23.81	10.30	12.22	118.6
24	0.00	0.00	3.70	1.23	2.14	173.2
Cmax (ng/mL)	6529.46	2954.1	7948.81	5810.79	2573.74	44.3
Tmax (hr)	0.5	0.5	0.5	0.5	[0.5 - 0.5]	NA
AUClast (ng·hr/mL)	4735.76	2432.69	7085.29	4751.25	2326.34	49.0
AUCinf (ng·hr/mL)	4761.09	2442.04	7105.61	4769.58	2331.80	48.9
t1/2 (hr)	2.47	1.37	3.81	2.55	1.22	47.9
CL/F (mL/hr/kg)	2100.36	4094.94	1407.34	2534.21	1395.34	55.1
Vd/F (mL/kg)	7492.56	8096.61	7731.25	7773.47	304.23	3.9
Rsq_adjusted	0.97	0.89	0.90	0.92	0.04	4.7
%AUCexp (%)	0.53	0.38	0.29	0.40	0.12	30.3

Tmax presented as median [min-max]

Appendix 6. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fasted Male Dogs at 30 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	M13	M14	M15			
0.5	17321.30	4532.77	22647.18	14,833.75	9,309.88	62.8
1	9681.07	1088.97	21831.64	10,867.23	10,422.08	95.9
2	2160.30	117.37	12894.72	5,057.46	6,863.70	135.7
4	682.40	40.11	2839.87	1,187.46	1,466.62	123.5
8	157.75	11.77	1053.08	407.53	563.80	138.3
12	21.77	7.01	229.09	85.96	124.18	144.5
24	0.00	2.55	78.05	26.87	44.34	165.1
Cmax (ng/mL)	17321.3	4532.77	22647.18	14833.75	9309.88	62.8
Tmax (hr)	0.5	0.5	0.5	0.5	[0.5 - 0.5]	NA
AUClast (ng·hr/mL)	21883.64	3497.96	62072.35	29151.32	29955.87	102.8
AUCinf (ng·hr/mL)	21932.42	3525.38	62518.78	29325.53	30183.59	102.9
t1/2 (hr)	1.55	7.45	3.96	4.32	2.97	68.7
CL/F (mL/hr/kg)	1367.84	8509.71	479.86	3452.47	4402.15	127.5
Vd/F (mL/kg)	3064.97	91523.19	2744.71	32444.29	51164.08	157.7
Rsq_adjusted	0.99	0.98	0.85	0.94	0.08	8.3
%AUCexp (%)	0.22	0.78	0.71	0.57	0.31	53.5

Tmax presented as median [min-max]

Appendix 7. Individual Plasma Concentrations and Pharmacokinetic Parameters of OCT-598 Following Single Oral Gavage Administration to Fed Male Dogs at 10 mg/kg

Time (hr)	Plasma Concentrations (ng/mL)			Mean	SD	CV (%)
	M16	M17	M18			
0.5	7711.94	5740.95	11964.09	8,472.33	3,180.49	37.5
1	2733.95	802.02	3639.84	2,391.94	1,449.49	60.6
2	452.28	75.68	201.20	243.05	191.76	78.9
4	92.47	58.79	114.56	88.61	28.09	31.7
8	32.15	24.03	56.16	37.45	16.71	44.6
12	15.01	5.05	14.80	11.62	5.69	49.0
24	0.00	0.00	1.24	0.41	0.72	173.2
Cmax (ng/mL)	7711.94	5740.95	11964.09	8472.33	3180.49	37.5
Tmax (hr)	0.5	0.5	0.5	0.5	[0.5 - 0.5]	NA
AUClast (ng·hr/mL)	7020.88	3868.1	9707.89	6865.62	2922.99	42.6
AUCinf (ng·hr/mL)	7086.93	3884.56	9713.23	6894.91	2919.08	42.3
t1/2 (hr)	3.05	2.26	2.99	2.77	0.44	15.9
CL/F (mL/hr/kg)	1411.05	2574.29	1029.52	1671.62	804.67	48.1
Vd/F (mL/kg)	6208.66	8390.16	4437.06	6345.29	1980.09	31.2
Rsq_adjusted	0.98	0.95	0.99	0.97	0.02	2.1
%AUCexp (%)	0.93	0.42	0.06	0.47	0.44	93.0

Tmax presented as median [min-max]

Appendix 8. Certificate of Analysis (COA)

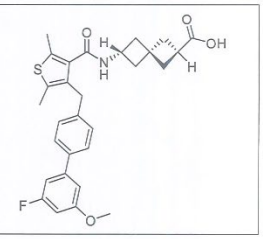
ASYMCHEM Controlled

Certificate of Analysis

Manufacturer Address and Contact	QF04168.V06 (03/09/2021) Asymchem Life Science (Tianjin) Co., Ltd. No. 71, 7th Avenue, TEDA Tianjin, 300457, P.R. China Tel.: +86 (0)22 66252888
COA No.	TJ2-CP-202305-074.02

Basic information

Asymchem CCL No.	131731	Client product code	OCT-598	CAS No.	N/A
Chemical Name	(2R,4r,6R)-6-(4-((3'-fluoro-5'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-2,5-dimethylthiophene-3-carboxamido)spiro[3.3]heptane-2-carboxylic acid				
Molecular Formula	$C_{29}H_{30}FNO_4S$		Molecular Weight	507.62	
Lot No.	CPo131731-01-08-01		Batch size	1.740 kg	
Date of manufacture	May 27, 2023		Testing date	May 30, 2023	
Release testing site and address	Asymchem Life Science (Tianjin) Co., Ltd. No. 71, 7th Avenue, TEDA Tianjin, 300457, P.R. China				
Storage condition	5°C (2°C ~8°C)				
Retest date	May 26, 2024				



Testing results

TEST ITEM	Target Quality*	ANALYTICAL RESULTS	TEST METHODS	NOTEBOOK REFERENCE	Pass / Fail	Comment
Appearance	Report	Off-white solid	AM-001.03	TJ2-N01173 page 256	-	-
Appearance-solution	Report	Light yellow clear liquid	AM-131731-018.01	TJ2-N03114 page 20-21	-	-
Identification (FTIR)	Report	Refer to attachment	AM-023.04	TJ2-N00876 page 201-202	-	-
Identification (1H-NMR)	Conforms to structure	Conforms to structure	AM-014.04	TJ2-N01120 page 53-54	Pass	-
Identification (HPLC)	RT matches to that of reference standard	RT matches to that of reference standard	AM-131731-016.02	TJ2-N02137 page 95-102	Pass	-
Identification (UV)	UV spectrum (diode array) matches to that of reference standard	UV spectrum matches to that of reference standard	AM-131731-024.01	TJ2-N01903 page 236-240	Pass	-
Polymorph	Conforms to the reference standard (Form A)	Conforms to the reference standard (Form A)	AM-029.08	TJ2-N01297 page 193-195	Pass	-
Assay (% w/w) As is basis	Report	96.6%	AM-131731-016.02	TJ2-N02137 page 95-102	-	-
As dry basis	Report	96.8%	AM-131731-016.02	TJ2-N00867 page 297-298	-	-
Purity (% area)	Report	97.3%	AM-131731-016.02	TJ2-N02137 page 95-102	-	-
Specified Impurities	Impurity B: Report	RRT 0.96: 0.68%			-	-

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Certificate of Analysis

Manufacturer Address and Contact: QF04168.V06 (03/09/2021) Asymchem Life Science (Tianjin) Co., Ltd. No. 71, 7th Avenue, TEDA Tianjin, 300457, P.R. China Tel.: +86 (0)22 66252888

COA No. TJ2-CP-202305-074.02

TEST ITEM	Target Quality*	ANALYTICAL RESULTS	TEST METHODS	NOTEBOOK REFERENCE	Pass / Fail	Comment
Any single unspecified impurity (% area)	Report RRT and % area for all impurities at or above 0.05% Target 0.5% for maximum for a single impurity	RRT 0.42: 0.11% RRT 0.93: 0.12% RRT 1.22: 0.97% RRT 1.33: 0.15% RRT 1.37: 0.44% RRT 1.38: 0.19%	AM-131731-016.02	TJ2-N02137 page 95-102	-	-
Total impurity (% area)	Report Target about 3%	2.7%			-	-
Chiral purity (% area)	NLT 99.0%	99.6%	AM-131731-021.01	TJ2-N01324 page 117-122	Pass	-
Residual solvent						
Dichloromethane	NMT 600ppm	<60 ppm	AM-131731-025.01	TJ2-N01300 page 238-249	Pass	-
2-Methyl THF	NMT 5000ppm	ND (not detected)			Pass	-
n-Heptane	NMT 5000ppm	<500 ppm			Pass	-
Methanol	NMT 3000ppm	ND (not detected)			Pass	-
THF	NMT 720ppm	ND (not detected)			Pass	-
MTBE	NMT 5000ppm	ND (not detected)			Pass	-
Ethyl acetate	NMT 5000ppm	735 ppm			Pass	-
NMP	NMT 530ppm	ND (not detected)			Pass	-
Triethylamine	Report	<500 ppm			-	-
DIPEA	Report	<150 ppm			-	-
Water Content (% w/w)	Report	0.14%	AM-003.08	TJ2-N01173 page 257-259	-	-
Residue on ignition	NMT 0.5%	0.0% (0.01%)	USP <281>	TJ2-N02354 page 77-84	Pass	-
Elemental impurities (ppm)	Cd ≤0.5 ppm	ND, <LOD, LOD=5 ppb	AM-066.05	TJ2-2023-00309 page 1-10	Pass	-
	Pb ≤0.5 ppm	0.0 ppm (0.005 pm)			Pass	-
	As ≤1.5 ppm	ND, <LOD, LOD=30 ppb			Pass	-

ASYMCHEM Controlled

Certificate of Analysis	Manufacturer Address and Contact	QF04168.V06 (03/09/2021) Asymchem Life Science (Tianjin) Co., Ltd. No. 71, 7th Avenue, TEDA Tianjin, 300457, P.R. China Tel.: +86 (0)22 66252888
	COA No.	TJ2-CP-202305-074.02

TEST ITEM	Target Quality*		ANALYTICAL RESULTS	TEST METHODS	NOTEBOOK REFERENCE	Pass / Fail	Comment
Elemental impurities (ppm)	Hg	≤3 ppm	0 ppm (0.09 pm)	AM-066.05	TJ2-2023-00309 page 1-10	Pass	-
	Co	≤5 ppm	0 ppm (0.02 pm)			Pass	-
	V	≤10 ppm	ND, <LOD, LOD=3 ppb			Pass	-
	Ni	≤20 ppm	1 ppm			Pass	-
	Pd	≤10 ppm	8 ppm			Pass	-
	Li	≤55 ppm	ND, <LOD, LOD=9 ppb			Pass	-
Particle size distribution	Report d_{10} , d_{50} and d_{90}		d_{10}: 2 μm d_{50}: 108 μm d_{90}: 467 μm	AM-030-01.04	TJ-03531 page 172-173	-	-
Microbial Examination							
TAMC	≤10^3 CFU/g		<50 CFU/g	AM-131731-026.01	TJ2-P01147 page 177-187	Pass	-
TYMC	≤10^2 CFU/g		<50 CFU/g			Pass	-
E.coli	Absence in 1g		Absence in 1g			Pass	-

Note: *These are target quality which means there will no OOS investigation if any test out of the target limits listed in the table above.

Specification reference

Pharmacopeia reference	☐USP, ☐EP, ☐BP, ☐ JP, ☐ ChP, ☒ N/A
Asymchem specification reference	N/A
Related customer's document reference	N/A

Revision History

Version	Description of Change
V01	Interim release (Will provide the full COA after the testing items are all completed)
V02	1. Full release(update the results of Appearance-solution, Identification (FTIR), Identification (UV), Assay (% w/w) As dry basis, Chiral purity (% area), Residual solvent, Water Content (% w/w), Elemental impurities (ppm), Particle size distribution and Microbial Examination) 2. Update storage condition to "5°C (2°C ~8°C)"

Approval

ASYMCHEM Controlled

Certificate of Analysis	Manufacturer Address and Contact	QF04168.V06 (03/09/2021) Asymchem Life Science (Tianjin) Co., Ltd. No. 71, 7th Avenue, TEDA Tianjin, 300457, P.R. China Tel.: +86 (0)22 66252888
	COA No.	TJ2-CP-202305-074.02

Product release testing principal	
Comment:	N/A
Signature/Date:	Jingjin Cui. 07/03/2023

Reviewed by Lab Director	
Comment:	N/A
Signature/Date:	Yongfei Tan 07/03/2023

Approved by QA	
QA Conclusion	☒Pass ☐QA Hold
Comment:	N/A
Signature/Date:	黄晓宇 07/03/2023

Appendix 9. Analytical Method

1. METHOD SUMMARY

Analyte / Metabolite	OCT-598 (Lot no. CPo131731-01-08-01)
Internal Standard	OCT-598-D3 (Lot no. P-0003800-002)
Species (Strain) / Matrix	Dog, Beagle / Plasma
Anticoagulant	Sodium heparin
Sample Volume	20 µL
Calibration Range	1 to 1000 ng/mL
Regression Type / Weighting	Linear / $1/x^2$
Extraction	Acetonitrile protein precipitation
LC-MS/MS	1290 Infinity II, Agilent / Triple Quad 4500, Sciex (QuBEST Instrument ID: 4500-2)
Sample Processing Temperature	Room temperature
Sample Storage Temperature	-80ºC
Special Storage / Treatment Requirements	None

2. LC CONDITION

Column	ZORBAX RRHD Eclipse Plus C8 (2.1 x 100 mm, 1.8 µm), Agilent (ID: C8-119)			
Column Temperature	40ºC			
Autosampler Temperature	15ºC			
Injection Volume	2 µL			
Needle Wash Solution	Isopropyl alcohol:Ultrapure water (UPW), 9:1 (v/v) (Flush Port, 30sec.)			
Mobile Phase A	10 mM Ammonium acetate in UPW			
Mobile Phase B	0.1% Formic acid in acetonitrile			
Mobile Phase Time Program	Time (min)	Flow (mL/min)	A (%)	B (%)
	0	0.4	35	65
	2.5	0.4	35	65

3. MASS SPECTROMETER CONDITION

Parameter	OCT-598	OCT-598-D3 (IS)
MRM (m/z)	508.2 > 306.0	511.2 > 306.0
DP (V)	121	126
EP (V)	10	10
CE (V)	31	31
CXP (V)	10	12
Polarity	ESI Positive	
CUR (psi)	30	
GS1 (psi)	30	
GS2 (psi)	40	
CAD (psi)	High	
Ion spray voltage (V)	5500	
Ion spray temp. (°C)	550	
Nebulizing gas	Nitrogen	
Data processing	Analyst 1.7.0	

4. EXTRACTION PROCEDURE

No.	Procedure
1	The samples exceeded the ULOQ concentration of the calibration curve at each concentration were used after 10, 20, 30 fold dilution by adding blank sample.
2	20 µL of study sample was transferred into a microtube. However, tubes containing 20 µL of standards (CAL or QC sample) were prepared.
3	200 µL of IS working solution (ISW3, 10 ng/mL) was added into the microtube. However, for DB sample, added 200 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 at 4ºC for 5 minutes.
5	100 µL of supernatant was transferred into a sample vial.