PHARMACOKINETIC REPORT

Pharmacokinetics of OCT-598 Following intravenous Administration to Male Cynomolgus Monkeys

Study Number:	PH-DMPK-OSCO-M-24-007
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Sponsor:	Oscotec Inc.
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1 SIGNATURE PAGE

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2 ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
API	Active pharmaceutical ingredient
$AUC_{(0-t)}$	Area under the time-concentration curve from zero to the last time point
$AUC_{(0-\infty)}$	Area under the time-concentration curve from zero to infinity
BLOQ	Below the limit of quantification
CE	Collision energy
CL	Clearance
C_0	Initial concentration
CV	Coefficient of variation
CXP	Collision cell exit potential
DP	Declustering potential
EP	Entrance Potential
ESI	Electrospray ionization
g	Acceleration of gravity
HPLC	High performance liquid chromatography
h	Hour
IS	Internal Standard
IV	Intravenous
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower limit of quantification
mg	Milligram
min	Minute
MRM	Multiple reaction monitoring
m/z	Ratio of mass to charge
NA	Not available
ng/mL	Nanogram per milliliter
QC	Quality control
R	Correlation coefficient
RPM	Revolutions per minute
RS	Reference Standard
SD	Standard deviation
sec	Second
$t_{1/2}$	Terminal half life
µL	Microliter
ULOQ	Upper limit of quantification

Abbreviation	Definition
v/v	Volume/volume
Vss	The apparent volume of distribution at steady state of the parent test item in the test system following IV dosing

3 SUMMARY

The purpose of this study is to evaluate the pharmacokinetic profiles of OCT-598 following single intravenous administration to Cynomolgus Monkeys.

PK parameters of OCT-598 in plasma were determined following intravenous administration of 3 mg/kg OCT-598 to male Cynomolgus Monkeys according to the design in Table 3-1.

Table 3-1 Study Design

Group No.	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Conc. (mg/mL)	No. of Animals
1	OCT-598	3	1	3	3 Males

Intravenous administration of dose group was performed without incident. No abnormal clinical symptoms were observed during the entire experiment.

Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h post-dose. The concentrations of OCT-598 were determined in plasma and the mean PK parameters of OCT-598 are summarized in Table 3-2.

Table 3-2 Mean Plasma Pharmacokinetic Parameters of OCT-598 Following IV Administration of OCT-598 to Male Cynomolgus Monkeys at 3 mg/kg

Treatment	Dose Level (mg/kg)	C_0 (ng/mL)	$AUC_{(0\text{-}24h)}$ (h.ng/mL)	$AUC_{(0\text{-}\infty)}$ (h.ng/mL)	$t_{1/2}$ (h)	CL (mL/min/kg)	Vss (L/kg)
OCT-598	3	9014	3753	3857	8.36	13.4	1.74

Following single intravenous administration of OCT-598 to male Cynomolgus Monkeys at 3 mg/kg, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 8.36 hours. Mean clearance (CL) and mean volume of distribution at stead-state (Vss) values were 13.4 mL/min/kg and 1.74L/kg, respectively.

4 OBJECTIVE

The purpose of this study is to evaluate the pharmacokinetic profiles of OCT-598 following single intravenous administration to Cynomolgus Monkeys.

5 MATERIALS AND METHODS

5.1 Test article information

OCT-598 provided by the Sponsor was used for the dose formulation and standard stock solution preparation as listed in Table 5-1:

Table 5-1 Test materials information

Formulation Identification	Lot No	MW	FW	Salt Factor	Correction Factor	Storage Condition
OCT-598	EW40829-43-P1	507.62	-	1	NA	2-8℃
OCT-598-D3[1]	P-0003800-002	510.64	-	1	NA	2-8℃

1 Internal standard

5.2 Animals

Male Cynomolgus Monkeys were purchased from AniKeeper (Zhanjiang) Biotech Co., Ltd. The animals were ~4 years old with body weights of 3.9-4.45 kg on the dosing date. This study was approved by the Pharmaron Institutional Animal Care and Use Committee (IACUC).

5.3 Study design

Total of 3 male Cynomolgus Monkeys were dosed via a design as shown in Table 5-2.

Table 5-2 Dosing information

Group No.	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Conc. (mg/mL)	No. of Animals
1	OCT-598	3	1	3	3 Males

Feeding condition: Animals will have free access to food and water

5.4 Formulation preparation

Preparation of dosing formulation for IV administration (3 mg/kg OCT-598):

Dissolved 52.46 mg of OCT-598 in 17.487 mL of 10% SBE-β-CD in 50 mM potassium phosphate (pH 8.0) with vortexing and sonification to obtain a solution.

The concentration of each dose formulation was measured by LC-MS, and the results shown in Appendix III.

5.5 Dosing procedure

The cephalic (forelimb) is the vein of choice for intravenous injection in monkeys. The monkey will be restrained using the monkey chair. One technician restrains the monkey's arms. The other technician disinfects the injection site with alcohol wipes or other disinfectant. The first technician occludes the cephalic vein at the elbow while extending the monkey's forelimb toward the second technician. The second technician inserts the needle at a shallow angle into the vein. Aspirate syringe to ensure proper needle placement (blood at the tip of the syringe indicates proper placement). Once the needle has been inserted properly into the vein, gently push the syringe plunger to deliver the formulation in a smooth, even flow.

5.6 Sample collection

Blood samples (0.3 mL) were collected from each animal via cephalic vein on the following timepoints: 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h post-dose. Blood samples were placed into tubes containing EDTA-K2, and then centrifuged at 2000 g for 10 minutes at 20-25 ℃ to prepare plasma. All samples were stored at -75 ±15 ℃ until analysis.

5.7 Preparation of standard solutions and internal standard solution for LC-MS/MS Analysis

OCT-598 was prepared in DMSO with vortexing at 1 mg/mL (free form) to make the standard stock solution.

Calibration standard working solutions were prepared at concentrations of 5, 10, 20, 50, 100, 500, 1000, 5000, and 10000 ng/mL by serial dilution of the standard stock solution by 50% acetonitrile in water. Quality control working solutions at concentrations of 10, 20, 50, 500, and 8000 ng/mL were prepared by serial dilution of the standard stock solution by 50% acetonitrile in water. These QC samples were prepared on the day of analysis in the same way as calibration standards.

OCT-598-D3 was prepared in DMSO with vortexing at 1 mg/mL (free form) to make the internal standard stock solution.

The internal standard working solutions were prepared at concentrations of 20 ng/mL by dilution of the internal standard stock solution by 100% acetonitrile for precipitating protein respectively.

5.8 Sample treatment

5 μL of each calibration standard working solution (5, 10, 20, 50, 100, 500, 1000, 5000, and 10000 ng/mL) was added to 50 μL of blank Cynomolgus Monkeys plasma to achieve calibration standards of 0.5-1000 ng/mL (0.5, 1, 2, 5, 10, 50, 100, 500, and 1000 ng/mL) in a total volume of 55 μL. Quality Control (QC) samples at 1 ng/mL (low-1), 2 ng/mL (low-2), 5 ng/mL (low-3), 50 ng/mL (mid) and 800 ng/mL (high) in blank plasma were prepared independently from those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards.

55 μL of standards, 55 μL of QC samples or 55 μL of unknown samples (50 μL of plasma sample with 5 μL 50% acetonitrile in water) were mixed with 200 μL of acetonitrile containing IS (OCT-598-D3 with concentration at 20 ng/mL) to precipitate proteins. Then the samples were vortexed for 30 sec. After centrifugation at 4 ℃, 3900 rpm for 15 min, the supernatant was diluted at a ratio of 1:2 with water (v/v: 1/2). 10 μL of diluted supernatant was injected into the LC-MS/MS system for quantitative analysis.

5.9 LC-MS/MS conditions

The LC-MS/MS system consisted of Degasser DGU-20A5R, S, Liquid Chromatograph LC-30AD, Communications Bus Module CBM-20A, Auto Sampler SIL-30AC, Rack changer II and an AB API 5500 LC/MS/MS instrument (Serial No. EF20361804).

Chromatographic separation was performed on a Raptor Biphenyl 2.7μm (50*2.1 mm) at room temperature. The mobile phase was composed of A: 5% acetonitrile (0.1% formic acid) in water; B: 95% acetonitrile (0.1% formic acid) in water. The flow rate was 0.6 mL/min. The injection volume was 10 μL.

Positive mode electrospray ionization (ESI) was performed on a Turbo V® ion source to obtain a protonated ion of OCT-598 (RS) and OCT-598-D3 (IS). A multiple reaction monitoring (MRM) method was selected for quantitative analysis. The optimized transitions were 508.102/306.00 and 511.081/ 168.00 for OCT-598 and OCT-598-D3, respectively. The instrument parameters were set as follows: ion spray voltage: 5500 V; curtain gas: 40 psi; nebulizer gas: 50 psi; turbo gas: 50 psi; collision gas: 9 psi; temperature: 500 ℃. The compound dependent parameters are listed in Table 5-3.

Table 5-3 Compound-dependent parameters on MS

Compound ID	OCT-598 (RS)	OCT-598-D3 (IS)
Transition	508.102/306.00	511.081/168.00
DP	256	256
CE	31	49
CXP	12	16

5.10 Data acceptance criteria

Acceptance criteria of standard calibration samples:

At least 6 samples should be analyzed to obtain a calibration curve. Acceptance of calibration standards requires calculated concentration within 80%-120% of the nominal concentration. 75% of the calibration standards should be within the acceptable range.

Acceptance criteria of quality control samples:

At least 3 concentrations of quality control samples (QCs) should be analyzed in a run. Each concentration should include at least 2 individual samples. Acceptance of QCs requires calculated concentration within 80%-120% of the nominal concentration. QCs should be analyzed amongst all unknown samples and 2/3 of the QCs should be within the acceptable range, including at least 1 sample at each concentration level in an analytical run.

Acceptance criteria of unknown samples:

Unknown samples with normal peak shape of analyte and calculated concentration within the calibration range should be accepted. Samples with calculated concentration below LLOQ should be recorded as BLOQ. Samples with calculated concentration above 100% of ULOQ should be diluted with blank matrix and re-assayed. The re-assayed concentration should be multiplied by the dilution factor to obtain the final data. In cases of abnormality, such as equipment malfunction, power outage, sample treatment failure and/or sample injection failure, re-assay should be done in an individual analytical run.

5.11 Statistical analysis

Data acquisition was performed by Sciex Analyst 1.7.2 software (AB Sciex, Forster City, CA). All concentration data and Pharmacokinetic parameters were reported with 3 significant figures. BLOQ was set to zero in calculation. Data statistics were performed using Excel 2010 software.

5.12 Quality standard

This study has been performed in compliance with Pharmaron standard operating procedures. This report represents a true and accurate record of the results obtained.

This report has been reviewed for scientific content and consistency against the Working Practice.

5.13 Study plan and working practice deviations

There were no deviations from the study plan or working practice.

5.14 Data Storage

All raw data, the study plan, and the final report will be retained at Pharmaron for five years after issue of the final report. After this period, the Sponsor will be contacted to determine whether further storage of the data is required, whether the data should be sent to the Sponsor, or if it should be destroyed.

6 RESULTS

6.1 Clinical observations

Intravenous administration of dose group was performed without incident. No abnormal clinical symptoms were observed during the entire experiment.

6.2 Linearity

In this study, a calibration standard was used for the regression of a $1/x^2$ weighted calibration curve. The curve was plotted using peak area ratio of OCT-598 (RS) and OCT-598-D3 (IS) versus the nominal concentration. The back-calculated concentrations of the calibration standards and calibration curve parameters are shown in Appendix II for plasma samples. The accuracy of the accepted standard samples was 94.4%-105% for male Cynomolgus Monkeys plasma samples. The accuracy of quality control samples was within acceptance criteria (80-120%).

6.3 Quality control samples

The results of QC samples for plasma are shown in Appendix II. The accuracy of the accepted QC samples was 98.6%-109% for male Cynomolgus Monkeys plasma. The accuracy of quality control samples was within acceptance criteria (80-120%).

6.4 Bioanalytical sample analysis

OCT-598 plasma concentration in Cynomolgus Monkeys is shown in Table 8-1,. The OCT-598 plasma concentrations vs. time profiles are shown in Figure 9-1 and Appendix I.

6.5 Pharmacokinetic analysis

OCT-598 plasma concentrations for each animal following intraveneous administration were used to calculate pharmacokinetic parameters by employing a non-compartmental analysis (Phoenix TM WinNonlin® 8.3). The linear trapezoidal algorithm was used for AUC calculation. Plasma pharmacokinetic parameters are shown in Table 8-2.

Following single intravenous administration of OCT-598 to male Cynomolgus Monkeys at 3 mg/kg, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 8.36 hours. Mean clearance (CL) and mean volume of distribution at stead-state (Vss) values were 13.4 mL/min/kg and 1.74L/kg, respectively.

7 CONCLUSIONS

Intravenous administration of dose group was performed without incident. No abnormal clinical symptoms were observed during the entire experiment.

Following single intravenous administration of OCT-598 to male Cynomolgus Monkeys at 3 mg/kg, plasma concentration of OCT-598 decreased with a terminal half-life (t1/2) of 8.36 hours. Mean clearance (CL) and mean volume of distribution at stead-state (Vss) values were 13.4 mL/min/kg and 1.74L/kg, respectively.

8 TABLES

Table 8-1: OCT-598 Plasma Concentrations Following IV Administration of OCT-598 to Male Cynomolgus Monkeys at 3 mg/kg

Time	Concentration (ng/mL)			Mean	SD
(h)	20C05001	20C05043	20C05093	(ng/mL)	(ng/mL)
0.083	7850	5750	6670	6757	1053
0.25	4790	3550	3140	3827	859
0.5	2380	1610	1630	1873	439
1	862	472	495	610	219
2	220	112	94.7	142	67.9
4	50.5	41.1	22.8	38.1	14.1
8	22.1	27.7	7.46	19.1	10.5
24	10.1	10.7	1.01	7.3	5.4

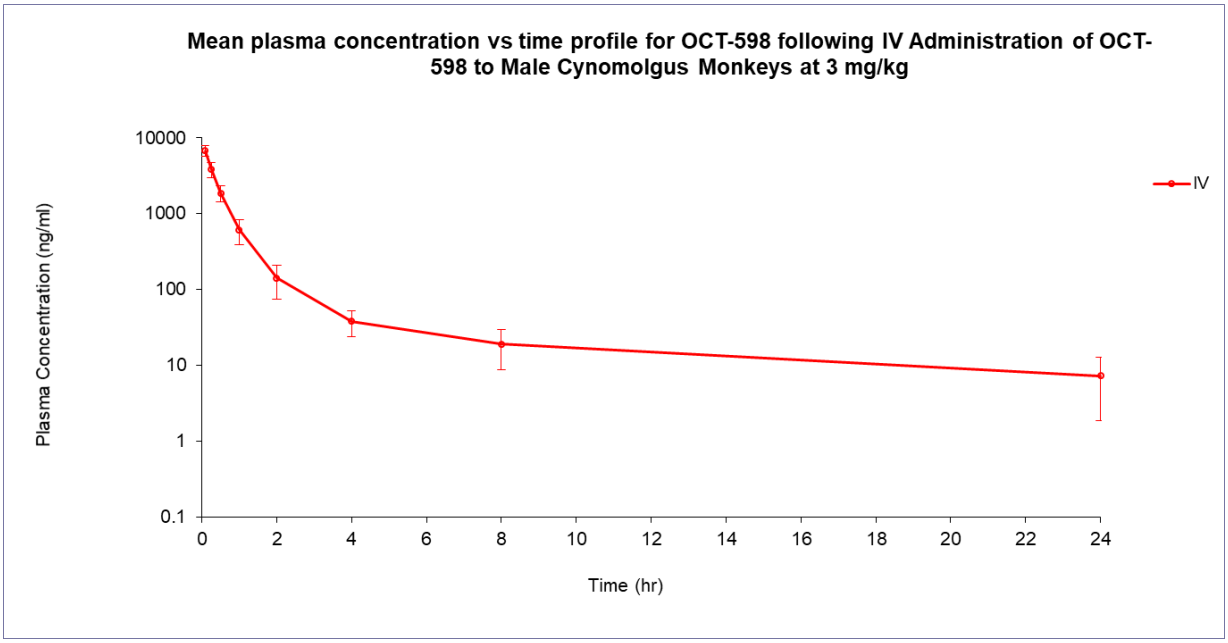
BLOQ = below quantifiable limit of 0.5 ng/mL.

Table 8-2: Plasma Pharmacokinetic Parameters of OCT-598 Following IV Administration of OCT-598 to Male Cynomolgus Monkeys at 3 mg/kg

Animal	Cl_obs (mL/min/kg)	$T_{1/2}$ (h)	C_0 (ng/mL)	$AUC_{(0\text{-}24h)}$ (h*ng/mL)	$AUC_{(0\text{-}\infty)}$ (h*ng/mL)	MRT_{Inf_obs} (h)	$AUC_{(0\text{-}24h)}/D$ (h*ng/mL)	V_{ss_obs} (L/kg)
20C05001	10.3	9.70	10034	4719	4860	2.42	1573	1.49
20C05043	14.1	10.7	7307	3374	3538	3.53	1125	2.99
20C05093	15.8	4.71	9699	3167	3173	0.778	1056	0.736
Mean	13.4	8.36	9014	3753	3857	2.24	1251	1.74
SD	2.8	3.2	1487	843	887	1.38	281	1.15

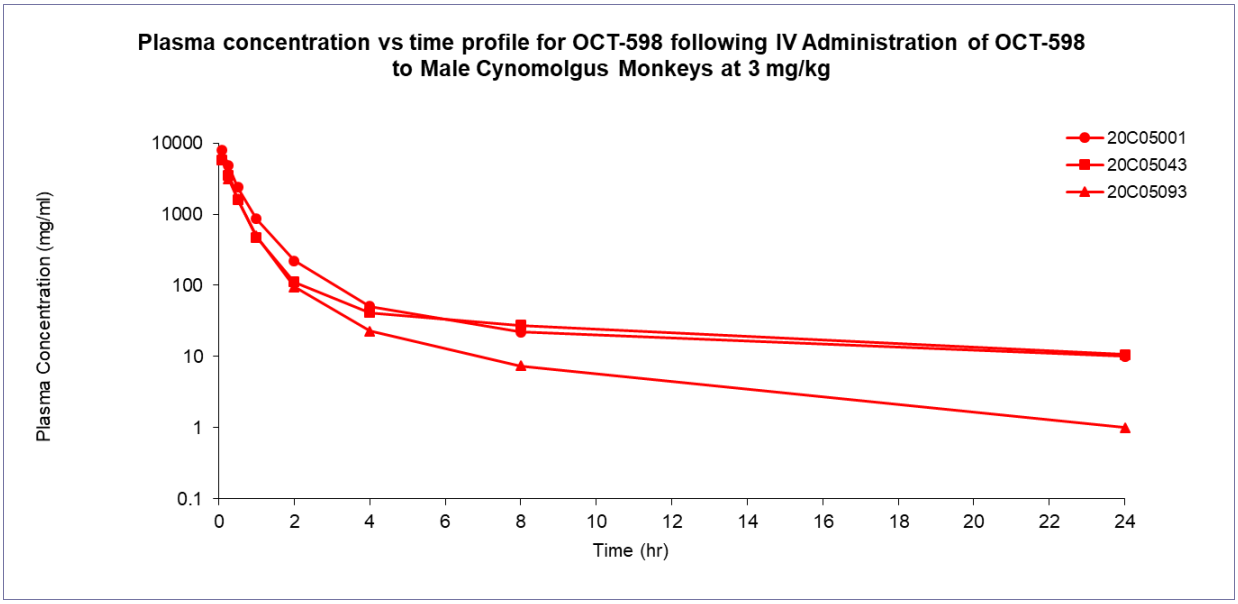
9 FIGURES

Figure 9-1: Mean (± SD, n = 3) Plasma Concentration Vs Time Curves of OCT-598 Following IV Administration of OCT-598 to Male Cynomolgus Monkeys at 3 mg/kg



Appendix I: Plasma vs Time-Concentration Curves

Plasma Semi-log Time-Concentration Curves of OCT-598 Following IV Administration of OCT-598 to Male Cynomolgus Monkeys at 3 mg/kg



Appendix II: LC-MS/MS Validation Data

Back-Calculated Concentration of Calibration Standards of OCT-598 to Male Cynomolgus Monkeys Plasma

Expected Concentration (ng/mL)	Number of Values	Calculated Concentration (ng/mL)	% Accuracy
0.5	1	0.524	105
1	2	0.944	94.4
2	2	2.02	101
5	2	5.02	100
10	2	10.2	102
50	2	50.7	102
100	2	101	101
500	2	501	100
1000	2	969	96.9

$y = 0.0133 x + 0.00123$ ($r = 0.9989$)

Precision and Accuracy of QC Samples IV of OCT-598 to Male Cynomolgus Monkeys Plasma

Nominal Concentration (ng/mL)	Number of Values	Calculated Concentration (ng/mL)	% Accuracy
1	2	1.09	109
2	2	1.98	99.1
5	2	5.16	103
50	2	49.9	100
800	2	789	98.6

Appendix III: Dose Formulation Concentration

Route	Sample Name	Dilution Factor	Nominal (mg/mL)	Measured (mg/mL)	Mean (mg/mL)	Accuracy (%)	SD (mg/mL)
IV_	DOSE_IV_1	100000	3	3.02	2.92	97.3	0.100
	DOSE_IV_2	100000		2.92			
	DOSE_IV _3	100000		2.82			

Appendix IV: CoA

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CERTIFICATE OF ANALYSIS		
Product	**OCT-598**	
Lot No	EW40829-43-P1	
Appearance	White solid	
Identification	^{1}H-NMR, Mass	confirm
Assay (HPLC)	min, 90.0%	98.9%

Analysis Date: 2023. 11. 30

Expiry date: 2024. 11. 29

Prepared by:

Name: Kwon Soonsang Signature: Date: 05. Dec. 2023

Approved by:

Name: Jung Dongsik Signature: Date: 05. Dec. 2023

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CERTIFICATE OF ANALYSIS

Product	**OCT-598-D3**	
Lot No	P-0003800-002	
Appearance	White solid	
Identification	^{1}H-NMR, Mass	confirm
Assay (HPLC)	min, 90.0%	98.0%

Analysis Date: 2023. 11. 30

Expiry date: 2024. 11. 29

Prepared by:

Name: Kwon Soonsang Signature: Date: 05. Dec. 2023

Approved by:

Name: Jung Dongsik Signature: Date: 05. Dec. 2023

Oscotec Inc.

CERTIFICATE OF ANALYSIS

Product	**Soluplus (OSCO-SDP_C2)**	
Quantity	**2.12g**	
Lot No.	Bat#23K16	
Analytical tests	**Specifications**	**Results**
Appearance	Off-white powder	Off-white powder
Identification	1H NMR, Mass	confirmed
Assay (HPLC)	Min. 80%	99.1%
Analysis Date	12/14/2023	
Expiry Date	12/14/2024	

Prepared	Soonsang Kwon
Signature	Soonsang Kwon
Date	18-Dec-23

Oscotec Inc.

CERTIFICATE OF ANALYSIS

Product	**HPMC (OSCO-SDP_C4)**	
Quantity	**2.2g**	
Lot No.	Bat#23K17	
Analytical tests	**Specifications**	**Results**
Appearance	Off-white powder	Off-white powder
Identification	1H NMR, Mass	confirmed
Assay (HPLC)	Min. 80%	91.0%
Analysis Date	12/14/2023	
Expiry Date	12/14/2024	

Prepared	Soonsang Kwon
Signature	Soonsang Kwon
Date	18-Dec-23

Oscotec Inc.

CERTIFICATE OF ANALYSIS

Product	**Kollidon VA 64 (OSCO-SDP/C6)**	
Quantity	**2.1g**	
Lot No.	Bat#23K18	
Analytical tests	**Specifications**	**Results**
Appearance	Off-white powder	Off-white powder
Identification	1H NMR, Mass	confirmed
Assay (HPLC)	Min. 80%	108.2%
Analysis Date	12/14/2023	
Expiry Date	12/14/2024	

Prepared	Soonsang Kwon
Signature	Soonsang Kwon
Date	18-Dec-23