ゾフルーザ錠 10mg，同 20mg

第 2 部 (モジュール 2) : CTD の概要 (サマリー)

2.6 非臨床試験の概要文及び概要表

2.6.5 薬物動態試験概要表

塩野義製薬株式会社

目次

略号及び用語定義一覧表 5
2.6.5 薬物動態試験概要表 7
2.6.5.1 薬物動態試験： 一覧表 7
2.6.5.2 分析方法及びバリデーション試験 11
2.6.5.2.1 Specification (Lot Number, Specific Radioactivity, Radiochemical Purity, Chemical Purity, and Description) of [^{14}C]-S-033188 and [^{14}C]-S-033447 11
2.6.5.2.2 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (1) 12
2.6.5.2.3 Method for Determination of S-033188 and S-033447 in Monkey Plasma by LC/MS/MS (1) 13
2.6.5.2.4 Method for Determination of S-033188 and S-033447 in Rabbit Plasma by LC/MS/MS 14
2.6.5.2.5 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (2) 15
2.6.5.2.6 Method for Determination of S-033188 and S-033447 in Monkey Plasma by LC/MS/MS (2) 16
2.6.5.2.7 Method for Determination of S-033188 and S-033447 in Mouse Plasma by LC/MS/MS (1) 17
2.6.5.2.8 Method for Determination of S-033188 and S-033447 in Ferret Plasma by LC/MS/MS 18
2.6.5.2.9 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (3) 19
2.6.5.2.10 Method for Determination of S-033188 and S-033447 in Mouse Plasma by LC/MS/MS (2) 20
2.6.5.3 薬物動態試験： 吸収：単回投与 21
2.6.5.3.1 Plasma and Blood Concentrations of Radioactivity and Plasma Concentration of S-033447 after a Single Oral Administration of [^{14}C]-S-033188 in Rats 21
2.6.5.3.2 Dose-linearity and Food Effect of S-033447 after a Single Oral Administration of S-033188 in Rats 22
2.6.5.3.3 Pharmacokinetic Parameters of S-033447 in Plasma after Single Oral or Intravenous Administration of S-033447 in Rats 23
2.6.5.3.4 Plasma and Blood Concentrations of Radioactivity and Plasma Concentration of S-033447 after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys 24
2.6.5.3.5 Dose-linearity and Food Effect of S-033447 after a Single Oral Administration of S-033188 in Monkeys 25
2.6.5.3.6 Dose-linearity of S-033447 after a Single Intravenous Administration of S-033447 in Monkeys 26
2.6.5.3.7 Effect of Metal Ion on plasma concentration of S-033447 after a single oral administration of S-033188 in Monkey 27

2.6.5.3.8 Plasma concentrations of S-033188 and S-033447 after a single oral administration of S-033188 in Juvenile Rats 28
2.6.5.3.9 Plasma concentrations of S-033447 after a single intravenous administration of S-033447 in Juvenile Rats 29
2.6.5.3.10 BA Calculation in Juvenile Rats 30
2.6.5.4 薬物動態試験： 吸収：反復投与 31
2.6.5.5 薬物動態試験： 分布 31
2.6.5.5.1 Tissue Distribution after a Single Oral Administration of [^{14}C]-S-033188 in Rats 31
2.6.5.6 薬物動態試験： たん白結合及び血球移行 34
2.6.5.6.1 薬物動態試験： たん白結合及び血球移行 34
2.6.5.6.2 In Vitro Distribution of [^{14}C]-S-033447 to Blood Cells 35
2.6.5.6.3 Distribution to blood Cells after a Single Oral Administration of [^{14}C]-S-033188 in Rats 36
2.6.5.6.4 Distribution to blood Cells after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys 37
2.6.5.7 薬物動態試験： 妊娠又は授乳動物における試験 38
2.6.5.7.1 Tissue Distribution after a Single Oral Administration of [^{14}C]-S-033188 in Pregnant Rats 38
2.6.5.7.2 Excretion into Milk after a Single Oral Administration of [^{14}C]- S–033188 in Nursing Rats 41
2.6.5.8 薬物動態試験： その他の分布試験 42
2.6.5.9 薬物動態試験： In Vivo における代謝 43
2.6.5.9.1 Metabolite Profiling in Plasma, Urine, Feces, and Bile after a Single Oral Administration of [^{14}C]-S-033188 in Rats 43
2.6.5.9.2 Metabolite Profiling in Plasma, Urine, Feces, and Bile after a Single Oral Administration of [^{14}C]- S-033188 in Monkeys 45
2.6.5.10 薬物動態試験： In Vitro における代謝 47
2.6.5.10.1 In Vitro Hydrolysis of [^{14}C]-S-033188 47
2.6.5.10.2 Investigation of Responsible Enzyme for Hydrolysis of [^{14}C]-S-033188 48
2.6.5.10.3 In Vitro Metabolism of [^{14}C]-S-033188 and [^{14}C]-S-033447 in Human Hepatocyte 49
2.6.5.10.4 In Vitro Identification of Responsible Enzymes for Glucuronidation of S-033447 51
2.6.5.10.5 In Vitro Identification of Responsible Enzymes for Oxidation of S-033447 52
2.6.5.11 薬物動態試験： 推定代謝経路 53
2.6.5.12 薬物代謝酵素の誘導／阻害 54
2.6.5.12.1 In Vitro Inhibitory Effects of S-033188 and S-033447 on Human CYP Enzymes 54
2.6.5.12.2 In Vitro CYP1A2, CYP2B6, and CYP3A4 Induction by S-033188 and S-033447 in Human Hepatocytes 56
2.6.5.12.3 In Vitro UGT Inhibition by S-033188 and S-033447 in Human Liver Microsomes 58

2.6.5.12.4 Effects on Hepatic Drug Metabolizing Enzymes in Rat 59
2.6.5.13 薬物動態試験： 排泄 60
2.6.5.13.1 Urinary and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Rats 60
2.6.5.13.2 Urinary and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys 61
2.6.5.14 薬物動態試験： 排泄：胆汁中 62
2.6.5.14.1 Urinary, Biliary, and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Rats 62
2.6.5.14.2 Urinary, Biliary, and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys 63
2.6.5.14.3 Enterohepatic Circulation of Radioactivity after a Single Oral Administration of [^{14}C]-S-033188 in Rats 64
2.6.5.15 薬物動態試験： 薬物相互作用 65
2.6.5.15.1 In Vitro Substrate Assessments of [^{14}C]-S-033188 and [^{14}C]-S-033447 for Human Efflux Transporters 65
2.6.5.15.2 In Vitro Substrate Assessments of [^{14}C]-S-033447 for Human Uptake Transporters 66
2.6.5.15.3 In Vitro Inhibitor Assessments of S-033188 and S-033447 for Human Efflux Transporters 67
2.6.5.15.4 In Vitro Inhibitor Assessments of S-033447 for Human Transporters 68
2.6.5.15.5 In Vitro Inhibitor Assessments of S-033447 for BSEP 70
2.6.5.15.6 In Vitro Investigation for Protein Binding Site of [^{14}C]-S-033447 (Effect of Site-specific Drug) 71
2.6.5.15.7 In Vitro Investigation for Protein Binding Site of S-033447 (Effect of S-033447) 72
2.6.5.16 薬物動態試験： その他 73
2.6.5.16.1 In Vitro Solubility of S-033188 and S-033447 73
2.6.5.16.2 In Vitro Permeability of S-033188 and S-033447 74

略号及び用語定義一覧表

略号	用語定義
AADAC	arylacetamide deacetylase
AUC	area under the concentration-time curve
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time 0 to infinity
$AUC_{0\text{-}24hr}$	area under the concentration-time curve from time 0 to 24 hours post-dose
BA	bioavailability
BCRP	breast cancer resistance protein
BLQ	below the lower limit of quantification
BNPP	bis(4-nitrophenyl) phosphate
BSEP	bile salt export pump
CL_{tot}	total clearance
2.4CES	carboxylesterase
C_{max}	maximum concentration
CV	coefficient of variation
CYP	cytochrome P450
DFP	diisopropyl fluorophosphates
DTNB	5,5'-dithiobis (2-nitrobenzoic acid)
EROD	ethoxyresorufin O-deethylase
$E_2$17βG	estradiol 17β- D-glucuronide
ES	estrone sulfate
F	female
HPLC	high-performance liquid chromatography
HSA	human serum albumin
IC_{50}	50% inhibitory concentration
iso-OMPA	tetraisopropyl pyrophosphoramide
LC/MS/MS	high-performance liquid chromatography with tandem mass spectrometry
LC/MS^n	high-performance liquid chromatography with multistage mass spectrometry
LLOQ	lower limit of quantification
LSC	liquid scintillation counter
M	male
MATE	multidrug and toxin extrusions
MC	methylcellulose aqueous solution
mRNA	messenger RNA
N/A	not applicable
N.C.	not calculated
N.D.	Not detected
N.R.	not represented
N.S.	no sample
P_{app}	apparent permeability
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PAH	aminohippuric acid
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
PMSF	phenylmethylsulfonyl fluoride
p-NP UDPGT	p-nitrophenol uridine 5-diphosphoglucuronic acid glucuronosyltransferase
QC	quality control
QWBA	quantitative whole-body autoradiography
Radio-HPLC	high performance liquid chromatography with radio-detector
RE	relative error of determination
RSD	relative standard deviation
S9	supernatant fraction after centrifugation of homogenate at 9000 × g.

S.D.	standard deviation
T16α-OHase	testosterone 16α-hydroxylase
T16β-OHase	testosterone 16β-hydroxylase
T3 UDPGT	3,3',5-triiodo-L-thyronine uridine 5-diphosphoglucuronic acid glucuronosyltransferase;
T4 UDPGT	L-thyroxine uridine 5-diphosphoglucuronic acid glucuronosyltransferase
T6β-OHase	testosterone 6β-hydroxylase
$t_{1/2,z}$	terminal elimination half-life
TCA	taurocholic acid
T_{max}	time to maximum plasma concentration
UGT	uridine diphosphate glucuronosyltransferase
Vd_{ss}	volume of distribution at steady state

2.6.5 薬物動態試験概要表

2.6.5.1 薬物動態試験： 一覧表

Test Article: S-033188

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in CTD
Analytical Methods and Validation					
Radiolabeled compounds	[^{14}C]-S-033188 and [^{14}C]-S-033447	N/A	Shionogi & Co., Ltd.	R-033188-QB-006-N, S-033188-QB-126-N	4.2.2.1-01, 4.2.2.1-02
Method for Determination of S-033188 and S-033447 for TK Analysis	Plasma of rat, rabbit, and monkey	N/A	Shionogi & Co., Ltd.	R-033188-TB-045-N	4.2.2.1-03
Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS for Juvenile Toxicity Study	Rat	N/A	[redacted]	S-033188-TF-118-L	4.2.2.1-04
Method for Determination of S 033188 and S-033447 in Mouse Plasma by LC/MS/MS for Phototoxicity study	Mice	N/A	Shionogi & Co., Ltd.	S-033188-TB-142-N	4.2.2.1-05
Absorption					
Plasma and blood concentrations of radioactivity and plasma concentration of S-033188 and S-033447 after a single oral administration of [^{14}C]-S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-057-N	4.2.2.2-01
Dose-linearity of S-033188 and S-033447 after a single oral administration of S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-051-N	4.2.2.2-02
Plasma and blood concentrations of radioactivity and plasma concentration of S-033188 and S-033447 after a single oral administration of [^{14}C]-S-033188	Monkey	Oral	[redacted]	R-033188-PF-026-N	4.2.2.2-03
Dose-linearity of S-033188 and S-033447 after a single oral administration of S-033188	Monkey	Oral	Shionogi & Co., Ltd.	R-033188-PB-028-N	4.2.2.2-04
Mineral effect on plasma concentration of S-033447 after a single oral administration of S-033188	Monkey	Oral	Shionogi & Co., Ltd.	S-033188-PB-244-N	4.2.2.2-05
Plasma concentrations of S-033188 and S-033447 after a single oral administration of S-033188	Juvenile rat	Oral	[redacted]	S-033188-PF-148-N	4.2.2.2-06
Plasma concentrations of S-033447 after a single intravenous administration of S-033447	Juvenile rat	Intravenous	[redacted]	S-033188-PF-190-N	4.2.2.2-07
Plasma concentrations of S-033447 after a single administration of S-033188	A/WSN/33 (H1N1)-infected mice	Oral	Shionogi & Co., Ltd.	R-033188-EB-056-N	4.2.1.1-19
Plasma concentrations of 033447 after a single administration of S-033188	Ferret	Oral	Shionogi & Co., Ltd.	R-033188-EB-071-N	4.2.1.1-29

Two-Week Oral Toxicity Study of S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-TB-032-L	4.2.3.2.-01
One-Month Oral Toxicity Study of S-033188 in Rats	Rat	Oral	Shionogi & Co., Ltd.	S-033188-TB-131-L	4.2.3.2.-02
Two-Week Oral Toxicity Study of S-033188 in Monkeys	Monkey	Oral	[redacted]	R-033188-TF-043-L, R-033188-TF-083-L	4.2.3.2.-03, 4.2.3.2.-04
One-Month Oral Toxicity Study of S-033188 in Monkeys	Monkey	Oral	[redacted]	S-033188-TF-132-L	4.2.3.2.-05
Distribution					
Tissue distribution after a single oral administration of [^{14}C]-S-033188	Pigmented rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-020-N	4.2.2.3-01
In vitro protein binding of [^{14}C]-S-033447	Rat serum, Monkey serum, Human serum, Purified human serum protein	In vitro	Shionogi & Co., Ltd.	R-033188-PB-021-N	5.3.2.1-01
In vitro distribution of [^{14}C]-S-033447 to blood cells	Rat blood, Monkey blood, Human blood	In vitro	Shionogi & Co., Ltd.	R-033188-PB-021-N	5.3.2.1-01
Distribution to blood cells after a single oral administration of [^{14}C]-S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-057-N	4.2.2.2-01
Distribution to blood cells after a single oral administration of [^{14}C]-S-033188	Monkey	Oral	[redacted]	R-033188-PF-026-N	4.2.2.2-03
Tissue distribution after a single oral administration of [^{14}C]-S-033188	Pregnant rat	Oral	[redacted]	S-033188-PF-177-L	4.2.2.3-02
Excretion into milk after a single oral administration of [^{14}C]-S-033188	Nursing rat	Oral	[redacted]	S-033188-PF-176-N	4.2.2.3-03
Metabolism					
In vitro Hydrolysis of [^{14}C]-S-033188	Rat serum, liver S9, and intestinal S9, Monkey serum, liver S9, and intestinal S9, Human serum, liver S9, and intestinal S9	In vitro	Shionogi & Co., Ltd.	R-033188-PB-030-N	5.3.2.2-01
Investigation of the Responsible Enzymes for Hydrolysis of [^{14}C]-S-033188	Human liver S9 and intestinal S9	In vitro	Shionogi & Co., Ltd.	S-033188-PB-101-N	5.3.2.2-02
In vitro metabolism of [^{14}C]-S-033188	Human hepatocytes	In vitro	Shionogi & Co., Ltd.	R-033188-PB-050-N	5.3.2.2-03
In vitro identification of responsible enzymes for [^{14}C]-S-033447 metabolism	Human liver microsomes, Recombinant human UGT	In vitro	Shionogi & Co., Ltd.	S-033188-PB-109-N	5.3.2.2-04

	enzymes				
In vitro identification of responsible enzymes for [^{14}C]-S-033447 oxidative metabolism	Human liver microsomes, Recombinant human CYP enzymes	In vitro	Shionogi & Co., Ltd.	S-033188-PB-180-N	5.3.2.2-05
In vitro inhibitory effects of S-033188 and S-033447 on human CYP enzymes	Human liver microsomes	In vitro	Shionogi & Co., Ltd.	S-033188-PB-090-N	5.3.2.2-06
In vitro CYP1A2, CYP2B6 and CYP3A4 induction by S-033188 and S-033447	Human hepatocytes	In vitro	[redacted]	S-033188-PF-105-N	5.3.2.2-07
In vitro inhibitory effects of S-033188 and S-033447 on human UGTs	Human liver microsomes	In vitro	[redacted]	S-033188-PF-172-N	5.3.2.2-08
Metabolite profiling of S-033188 after a single oral administration of [^{14}C]-S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-033-N	4.2.2.4-01
Metabolite profiling of S-033188 after a single oral administration of [^{14}C]-S-033188	Monkey	Oral	Shionogi & Co., Ltd.	R-033188-PB-055-N	4.2.2.4-02
Effects of S-033188 on hepatic drug metabolizing enzymes in two-week oral toxicity study	Rat	Oral	[redacted]	R-033188-PF-080-N	4.2.2.4-03
Excretion					
Urinary and fecal excretion after a single oral administration of [^{14}C]-S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-013-N	4.2.2.5-01
Urinary, biliary, and fecal excretion after a single oral administration of [^{14}C]-S-033188	Rat	Oral	Shionogi & Co., Ltd.	R-033188-PB-013-N	4.2.2.5-01
Urinary and fecal excretion after a single oral administration of [^{14}C]-S-033188	Monkey	Oral	[redacted]	R-033188-PF-026-N	4.2.2.2-03
Urinary, biliary, and fecal excretion after a single oral administration of [^{14}C]-S-033188	Monkey	Oral	[redacted]	R-033188-PF-026-N	4.2.2.2-03
Enterohepatic circulation after a single oral administration of [^{14}C]-S-033188	Rat	Oral	[redacted]	S-033188-PF-151-N	4.2.2.5-02
Pharmacokinetic Drug Interactions					
In vitro substrate assessments of S-033188 and S-033447 for human transporters	Caco-2 cells, OATP 1B1, OATP1B3 transfected HEK-293 cells	In vitro	[redacted]	S-033188-PF-111-N	5.3.2.2-09
In vitro inhibitor assessments of S-033188 and S-033447 for human efflux transporters	Caco-2 cells, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 or MATE2-K expressing HEK293 cells	In vitro	[redacted]	S-033188-PF-113-N	5.3.2.2-10
In vitro inhibitor assessments of S-033447 for human transporters	Caco-2 cells, OATP1B1, OATP1B3, OAT1, OAT3, OCT1,	In vitro	[redacted]	S-033188-PF-113-N	5.3.2.2-10

	OCT2, MATE1 or MATE2-K expressing HEK293 cells				
In vitro inhibitor assessments of S-033447 for BSEP	BSEP expressing vesicle	In vitro	[redacted]	S-033188-PF-168-N	5.3.2.2-11
In vitro investigation for protein binding site of [^{14}C]-S-033447 (Effect of Site-specific Drugs)	HSA	In vitro	Shionogi & Co., Ltd.	S-033188-PB-181-N	5.3.2.1-02
In vitro investigation for protein binding site of [^{14}C]-S-033447 (Effect of S-033447)	HSA	In vitro	Shionogi & Co., Ltd.	S-033188-PB-181-N	5.3.2.1-02
Others					
Preliminary study on determination of membrane permeability and solubility of S-033188 and S-033447	pH 1.0, 6.8, 7.5 buffer, Caco-2 cells	In vitro	[redacted]	S-033188-PF-100-R	4.2.2.7-01
Abbreviations: BSEP, bile salt export pump; CYP, cytochrome P450; HSA, human serum albumin; MATE, multidrug and toxin extrusions; N/A, not applicable; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; UGT, uridine diphospho-glucuronosyl transferase; S9, supernatant fraction after centrifugation of homogenate at 9000 × g.					

2.6.5.2 分析方法及びバリデーション試験

2.6.5.2.1 Specification (Lot Number, Specific Radioactivity, Radiochemical Purity, Chemical Purity, and Description) of [^{14}C]-S-033188 and [^{14}C]-S-033447

Radiolabeled Compound	Lot Number	Specific Radioactivity	Radiochemical Purity	Chemical Purity	Description	Study Number	Location in CTD
[^{14}C]-S-033188	20[redacted]-0047-039-01	1.94 MBq/mg (52.4 µCi/mg)	≥98.0%	≥98.9%	White crystals	R-033188-QB-006-N	4.2.2.1-01
[^{14}C]-S-033188	20[redacted]-0221-006-01	1.91 MBq/mg (51.6 µCi/mg)	≥99.3%	≥99.4%	White crystals	S-033188-QB-126-N	4.2.2.1-02
[^{14}C]-S-033447	20[redacted]-0047-031-01	2.21 MBq/mg (59.8 µCi/mg)	≥98.1%	≥99.0%	White crystals	R-033188-QB-006-N	4.2.2.1-01
[^{14}C]-S-033447	20[redacted]-0221-025-01	2.23 MBq/mg (60.2 µCi/mg)	≥98.8%	≥99.1%	White crystals	S-033188-QB-126-N	4.2.2.1-02

2.6.5.2.2 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (1)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Rat/Crl:CD (SD) Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-TB-032-L, R-033188-TB-047-L, R-033188-TB-070-L, S-033188-TB-131-L, S-033188-PF-148-N			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (μg/mL)	0.00500		0.00500	
Quantification Range (μg/mL)	0.00500 to 10.0		0.00500 to 10.0	
Calibration Curve Range (μg/mL)	0.005 to 10		0.005 to 10	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (μg/mL) Within-run Precision (RSD, %) Within-run Accuracy (%) Between-run Precision (RSD, %) Between-run Accuracy (%)	0.00500 12.7 to16.4 92.2 to 95.4 13.2 94.2	0.0150, 0.150, 8.00 0.7 to7.9 92.7 to 102.6 2.7 to7.6 96.0 to 100.5	0.00500 12.3 to19.5 87.2 to 97.2 16.2 91.2	0.0150, 0.150, 8.00 0.2 to7.4 90.0 to 104.7 4.7 to8.4 95.3 to 99.3
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 14 days at −20°C, and at least for 2 hours on ice			
Processed Extract Stability	Stable at least for 141 hours at 15°C			
Abbreviations: LLOQ, lower limit of quantification; QC, quality control; RSD, relative standard deviation.				
Study Number	R-033188-TB-045-N			
Location in CTD	4.2.2.1-03			

2.6.5.2.3 Method for Determination of S-033188 and S-033447 in Monkey Plasma by LC/MS/MS (1)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Monkey/Cynomolgus Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-TF-043-L, R-033188-SF-035-L, R-033188-TF-083-L, S-033188-TF-132-L			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (μg/mL)	0.00500		0.00500	
Quantification Range (μg/mL)	0.00500 to 10.0		0.00500 to 10.0	
Calibration Curve Range (μg/mL)	0.005 to 10		0.005 to 10	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (μg/mL)	0.00500	0.0150, 0.150, 8.00	0.00500	0.0150, 0.150, 8.00
Within-run Precision (RSD, %)	3.2 to7.6	0.6 to5.1	5.0 to17.0	1.4 to6.0
Within-run Accuracy (%)	92.0 to 101.2	90.7 to 104.7	85.8 to 103.6	87.9 to 109.3
Between-run Precision (RSD, %)	6.5	5.2 to 6.8	12.6	6.6 to7.2
Between-run Accuracy (%)	97.8	97.3 to 98.7	95.2	95.6 to 101.3
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 65 days at −20°C, and at least for 2 hours on ice			
Processed Extract Stability	Stable at least for 120 hours at 15°C			
Study Number	R-033188-TB-045-N			
Location in CTD	4.2.2.1-03			

2.6.5.2.4 Method for Determination of S-033188 and S-033447 in Rabbit Plasma by LC/MS/MS

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Rabbit/Kbl:NZW Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-TF-066-L, S-033188-TF-135-L			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (μg/mL)	0.00500		0.00500	
Quantification Range (μg/mL)	0.00500 to 10.0		0.00500 to 10.0	
Calibration Curve Range (μg/mL)	0.005 to 10		0.005 to 10	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (μg/mL)	0.00500	0.0150, 0.150, 8.00	0.00500	0.0150, 0.150, 8.00
Within-run Precision (RSD, %)	5.5 to19.5	1.0 to 9.9	6.1 to12.9	1.6 to11.0
Within-run Accuracy (%)	99.0 to 105.4	87.1 to 98.0	110.0 to 120.4	95.9 to 111.3
Between-run Precision (RSD, %)	12.3	3.2 to 8.1	9.5	5.0 to8.1
Between-run Accuracy (%)	102.4	89.8 to 91.3	114.0	101.1 to 107.3
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 10 days at −20°C, 3 freeze (−80°C) –thaw (on ice) cycles, at least for 46 days at −80°C, and at least for 2 hours on ice			
Processed Extract Stability	Stable at least for 96 hours at 15°C			
Study Number	R-033188-TB-045-N			
Location in CTD	4.2.2.1-03			

2.6.5.2.5 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (2)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Rat/Crl:CD (SD) Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-PB-051-N, R-033188-PB-057-N, S-033188-PF-148-N, S-033188-PF-190-N			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (ng/mL)	0.500		0.500	
Quantification Range (ng/mL)	0.500 to 2500		0.500 to 2500	
Calibration Curve Range (ng/mL)	0.5 to 500		0.5 to 500	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (RSD, %) Within-run Accuracy (%)	0.500 11.7 106.2	1.50,15.0, 400 5.5 to 7.5 99.5 to 110.0	0.500 6.8 103.6	1.50,15.0, 400 1.9 to 4.3 97.8 to 106.7
Dilution Factor Within-run Precision (RSD, %) Within-run Accuracy (%)	10 5.2 106.8		10 4.9 90.0	
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 11 days at −20°C, and at least for 2 hours on ice			
Processed Extract Stability	Stable at least for 114 hours at 15°C			
Study Number	R-033188-PB-051-N, S-033188-PF-148-N			
Location in CTD	4.2.2.2-02, 4.2.2.2-06			

2.6.5.2.6 Method for Determination of S-033188 and S-033447 in Monkey Plasma by LC/MS/MS (2)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Monkey/Cynomolgus Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-PF-026-N, R-033188-PB-028-N, S-033188-PB-244-N			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (ng/mL)	0.500		0.500	
Quantification Range (ng/mL)	0.500 to 2500		0.500 to 2500	
Calibration Curve Range (ng/mL)	0.5 to 500		0.5 to 500	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (RSD, %) Within-run Accuracy (%)	0.500 2.9 95.2	1.50,15.0, 400 1.8 to 3.5 108.8 to 114.7	0.500 4.6 112.0	1.50,15.0, 400 1.3 to 2.5 101.3 to 105.3
Dilution Factor Within-run Precision (RSD, %) Within-run Accuracy (%)	10 3.0 106.8		10 3.8 85.2	
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 19 days at −20°C, and at least for 4 hours on ice			
Processed Extract Stability	Stable at least for 36 hours at 15°C			
Study Number	R-033188-PF-026-N			
Location in CTD	4.2.2.2-03			

2.6.5.2.7 Method for Determination of S-033188 and S-033447 in Mouse Plasma by LC/MS/MS (1)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Mouse/BALB/cAnNCrlCrlj Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	R-033188-EB-056-N, R-033188-EB-058-N			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (ng/mL)	0.500		0.500	
Quantification Range (ng/mL)	0.500 to 500		0.500 to 500	
Calibration Curve Range (ng/mL)	0.5 to 500		0.5 to 500	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (RSD, %) Within-run Accuracy (%)	0.500 5.4 111.0	1.50,15.0, 400 2.5 to 8.9 104.7 to 112.0	0.500 4.6 108.2	1.50,15.0, 400 1.2 to 1.3 103.3 to 106.7
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 14 days at −20°C, and at least for 4 hours on ice			
Processed Extract Stability	Stable at least for 73 hours at 15°C			
Study Number	R-033188-EB-056-N			
Location in CTD	4.2.1.1-19			

2.6.5.2.8 Method for Determination of S-033188 and S-033447 in Ferret Plasma by LC/MS/MS

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Ferret/- Plasma (Anticoagulant: heparin)			
Study Number Supported	R-033188-EB-071-N			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (ng/mL)	0.500		0.500	
Quantification Range (ng/mL)	0.500 to 1500		0.500 to 1500	
Calibration Curve Range (ng/mL)	0.5 to 500		0.5 to 500	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (RSD, %) Within-run Accuracy (%)	0.500 4.2 105.6	1.50,15.0, 400 1.3 to 2.8 98.5 to 105.3	0.500 2.5 103.8	1.50,15.0, 400 1.7 to 3.2 103.3 to 104.5
Dilution Factor Within-run Precision (RSD, %) Within-run Accuracy (%)	5 1.4 95.3		5 2.9 92.7	
Stability in Plasma	Stable after 3 freeze (−80°C) –thaw (on ice) cycles, at least for 12 days at −80°C, and at least for 4 hours on ice			
Processed Extract Stability	Stable at least for 65 hours at 15°C			
Study Number	R-033188-EB-071-N			
Location in CTD	4.2.1.1-29			

2.6.5.2.9 Method for Determination of S-033188 and S-033447 in Rat Plasma by LC/MS/MS (3)

Test Site	Envigo CRS Limited			
Species/Strain Matrix	Rat/Crl:CD (SD) Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	S-033188-TF-128-L			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (ng/mL)	5.00		5.00	
Quantification Range (ng/mL)	5.00 to 80000		5.00 to 80000	
Calibration Curve Range (ng/mL)	5 to 10000		5 to 10000	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL)	5.00	15.0, 150, 8000	5.00	15.0, 150, 8000
Within-run Precision (CV, %)	3.6 to10.2	1.3 to 6.4	5.6 to14.0	0.9 to 7.9
Within-run Accuracy (RE, %)	−7.8 to 17.8	−8.7 to 2.7	−7.6 to 6.2	−9.3 to 6.7
Between-run Precision (CV, %)	10.6	4.0 to 6.0	10.9	5.4 to 6.3
Between-run Accuracy (RE,%)	1.4	−4.5 to 1.3	−0.2	−1.3 to 4.0
Dilution Factor	20		20	
Within-run Precision (CV, %)	1.9		4.3	
Within-run Accuracy (RE,%)	−4.5		6.8	
Stability in Plasma	Stable after 3 freeze (−70°C) –thaw (on ice) cycles, at least for 46 days at −70°C, and at least for 2.5 hours on ice			
Processed Extract Stability	Stable at least for 5 days at 4°C			
Abbreviation: CV, coefficient of variation; RE, Relative error of determination.				
Study Number	S-033188-TF-118-L			
Location in CTD	4.2.2.1-04			

2.6.5.2.10 Method for Determination of S-033188 and S-033447 in Mouse Plasma by LC/MS/MS (2)

Test Site	Shionogi & Co., Ltd.			
Species/Strain Matrix	Mouse/ Crl:CD1(ICR), Hos:HR-1 Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)			
Study Number Supported	S-033188-TB-149-L			
Analyte	S-033188		S-033447	
Lower Limit of Quantification (μg/mL)	0.00500		0.00500	
Quantification Range (μg/mL)	0.00500 to 10.0		0.00500 to 10.0	
Calibration Curve Range (μg/mL)	0.005 to 10		0.005 to 10	
Crl:CD1(ICR)	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (μg/mL)	0.00500	0.0150, 0.150, 8.00	0.00500	0.0150, 0.150, 8.00
Within-run Precision (RSD, %)	4.9 to 6.3	0.6 to4.9	5.9 to10.4	1.4 to 6.1
Within-run Accuracy (%)	97.8 to 102.8	94.7 to 110.7	99.6 to 112.4	91.3 to 110.7
Between-run Precision (RSD, %)	5.4	2.7 to7.9	9.0	5.0 to 7.9
Between-run Accuracy (%)	100.0	99.3 to 101.0	104.8	99.9 to 106.0
Hos:HR-1	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (μg/mL)	0.00500	0.0150, 0.150, 8.00	0.00500	0.0150, 0.150, 8.00
Within-run Precision (RSD, %)	12.2	3.2 to4.7	3.3	1.4 to 3.1
Within-run Accuracy (%)	94.8	97.9 to 102.7	109.6	103.5 to 106.7
Stability in Plasma	Stable after 3 freeze (−20°C) –thaw (on ice) cycles, at least for 65 days at −20°C, and at least for 4 hours on ice			
Processed Extract Stability	Stable at least for 144 hours at 15°C			
Study Number	S-033188-TB-142-N			
Location in CTD	4.2.2.1-05			

2.6.5.3 薬物動態試験： 吸収：単回投与

2.6.5.3.1 Plasma and Blood Concentrations of Radioactivity and Plasma Concentration of S-033447 after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)		
Gender (M/F) Number of Animals	M/4		
Feeding Condition	Non-fasted		
Vehicle/Formulation	0.5 w/v% MC/suspension		
Method of Administration	Single oral administration		
Dose (mg/kg)	1		
Radionuclide	^{14}C		
Specific Activity	1.94 MBq/mg (52.4 μCi/mg)		
Sample	Plasma and Blood (15, 30 minutes, 1, 2, 4, 6, 8, 10, 24 hours post-dose)		
Analyte	Radioactivity (S-033447 equivalent), S-033188, and S-033447		
Assay	LSC for radioactivity, LC/MS/MS for S-033188, and S-033447		
Pharmacokinetic Parameters	Radioactivity in Plasma	S-033447 in Plasma	Radioactivity in Blood
C_{max} (ng/mL)	20.6 ± 3.1	16.6 ± 3.1	20.8 ± 3.3
T_{max} (hr)	0.813 ± 0.375	1.06 ± 0.72	0.813 ± 0.375
$t_{1/2,z}$ (hr)	6.42 ± 3.31	4.93 ± 1.47	10.1 ± 5.5
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	135 ± 14	121 ± 17	153 ± 16
$AUC_{0\text{-}inf}$ (ng·hr/mL)	149 ± 38	123 ± 22	200 ± 69
AUC Ratio to Radioactivity (%)		89.7 ± 7.2	

Abbreviations: AUC, area under the concentration-time curve; $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 to infinity; $AUC_{0\text{-}24hr}$, area under the concentration-time curve from time 0 to 24 hours post-dose; C_{max}, maximum concentration; LC/MS/MS, high performance liquid chromatography with tandem mass spectrometry; LSC, liquid scintillation counter; M/F, male/female; MC, methylcellulose aqueous solution; S.D., standard deviation; $t_{1/2,z}$, terminal elimination half-life; T_{max}, time to maximum plasma concentration

Data are expressed as the mean ± standard deviation (S.D.) (n=4)

The concentrations of radioactivity in blood and plasma were not detected at 24 hours after administration except in blood of one rat (1.68 ng eq./mL).

The pharmacokinetic parameters of S-033188 in plasma were not shown because the concentrations of S-033188 in all plasma samples were below the lower limit of quantification (BLQ: < 0.960 ng/mL).

Study Number	R-033188-PB-057-N
Location in CTD	4.2.2.2-01

2.6.5.3.2 Dose-linearity and Food Effect of S-033447 after a Single Oral Administration of S-033188 in Rats

Test Article: S-033188

Species/Strain	Rat/Crl:CD(SD)				
Gender (M/F)/Number of Animals	M/4 per group				
Feeding Condition	Non-fasted for 0.3, 1, 3, 10 mg/kg, fasted for 1 mg/kg				
Vehicle/Formulation	0.5 w/v% MC/suspension				
Method of Administration	Single oral administration				
Dose (mg/kg)	0.3, 1, 3, 10				
Sample	Plasma (15, 30 minutes, 1, 2, 4, 6, 8, 10, 24 hours post-dose)				
Analyte	S-033188 and S-033447				
Assay	LC/MS/MS				
Pharmacokinetic Parameters of S-033447	Non-fasted Rat				Fasted Rat
	0.3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	1 mg/kg
C_{max} (ng/mL)	4.82 ± 0.85	17.1 ± 4.9	68.9 ± 5.8	169 ± 34	34.3 ± 13.8
T_{max} (hr)	1.00 ± 0.00	1.25 ± 0.50	0.875 ± 0.250	0.875 ± 0.250	0.375 ± 0.144
$t_{1/2,z}$ (hr)	10.4 ± 5.7	4.36 ± 0.57	4.02 ± 0.62	3.21 ± 0.10	3.68 ± 0.84
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	34.7 ± 7.4	103 ± 20	468 ± 183	1110 ± 120	125 ± 11
$AUC_{0\text{-}inf}$ (ng·hr/mL)	48.9 ± 21.2	101 ± 18	479 ± 195	1120 ± 120	122 ± 13
BA (%)	10.9 ± 2.3	9.77 ± 1.93	14.7 ± 5.8	10.5 ± 1.1	11.9 ± 1.0
Abbreviations: $AUC_{0\text{-}24hr}$, area under the plasma concentration-time curve from time 0 to 24 hours post-dose; BA, bioavailability. Data are expressed as the mean ± S.D. (n=4). The pharmacokinetic parameters of S-033188 were not shown because the concentrations of S-033188 in all plasma samples were below the lower limit of quantification (BLQ: <0.500 ng/mL). BA was calculated by following equation: ($AUC_{0\text{-}24hr}$ of S-033447 after oral administration / $AUC_{0\text{-}24hr}$ of S-033447 after intravenous administration) × (dose for oral administration as S-033447 / 0.254 mg/kg for intravenous administration of S-033447) × 100.					
Study Number	R-033188-PB-051-N				
Location in CTD	4.2.2.2-02				

2.6.5.3.3 Pharmacokinetic Parameters of S-033447 in Plasma after Single Oral or Intravenous Administration of S-033447 in Rats

Test Article: S-033447

Species/Strain	Rat/Crl:CD(SD)		
Gender/Number of animals	M/4 per group		
Feeding Condition	Non-fasted		
Vehicle/Formulation	0.5 w/v% MC/suspension for oral administration, (*N*,*N*-Dimethylacetamide/Propylene glycol/20% [w/v] Hydroxypropyl-β-cyclodextrin aqueous solution = 2/2/1 [by vol.])/Solution for intravenous administration		
Method of Administration	Single oral or intravenous administration		
Dose (mg/kg)[a]	0.846 mg/kg for oral administration, 0.254 and 0.846 mg/kg for intravenous administration		
Sample	Plasma (15, 30 minutes, 1, 2, 4, 6, 8, 10, 24 hours post-dose for oral administration. 2, 5, 15, 30 minutes, 1, 2, 4, 6, 8, 24 hours post-dose for intravenous administration)		
Analyte	S-033447		
Assay	LC/MS/MS		
Pharmacokinetic parameter	Oral administration	Intravenous administration	
	0.846 mg/kg	0.254 mg/kg	0.846 mg/kg
T_{max} (hr)	5.00 ± 2.58	N/A	N/A
C_{max} (ng/mL)	0.833 ± 0.064	N/A	N/A
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	6.75 ± 2.70	318 ± 29	974 ± 98
$AUC_{0\text{-}inf}$ (ng·hr/mL)	N.C.	314 ± 38	985 ± 97
$t_{1/2\text{-}z}$ (hr)	N.C.	3.74 ± 0.27	4.03 ± 0.15
CL_{tot} (mL/hr/kg)	N/A	819 ± 97	865 ± 83
Vd_{ss} (mL/kg)	N/A	3690 ± 270	4040 ± 290
Abbreviations: CL_{tot}, total clearance; N/A, not applicable; N.C., not calculated; Vd_{ss}, volume of distribution at steady state. Data are expressed as the mean ± S.D. (n=4). a 0.254 and 0.846 mg/kg of S-033447 is equivalent to 0.3 and 1 mg/kg of S-033188, respectively			
Study Number	R-033188-PB-051-N		
Location in CTD	4.2.2.2-02		

2.6.5.3.4 Plasma and Blood Concentrations of Radioactivity and Plasma Concentration of S-033447 after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys

Test Article: [^{14}C]-S-033188

Species/Strain	Monkey/Cynomolgus (Hamri)		
Gender (M/F)/Number of Animals	M/3		
Feeding Condition	Non-fasted		
Vehicle/Formulation	0.5 w/v% MC/suspension		
Method of Administration	Single oral administration		
Dose (mg/kg)	3		
Radionuclide	^{14}C		
Specific Activity	1.94 MBq/mg (52.4 μCi/mg)		
Sample	Plasma and Blood (15, 30 minutes, 1, 2, 4, 6, 8, 12, 24 and 48 hours post-dose)		
Analyte	Radioactivity (S-033447 equivalent), S-033188, and S-033447		
Assay	LSC for radioactivity, LC/MS/MS for S-033188, and S-033447		
Pharmacokinetic Parameters	Radioactivity in Plasma	S-033447 in Plasma	Radioactivity in Blood
C_{max} (ng/mL)	119 ± 98	92.9 ± 75.6	118 ± 92
T_{max} (hr)	4.0 ± 2.0	4.0 ± 2.0	4.0 ± 2.0
$t_{1/2,z}$ (hr)	17.8 ± 4.0	16.7 ± 3.7	17.3 ± 2.7
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	1680 ± 820	1390 ± 800	1950 ± 1000
$AUC_{0\text{-}inf}$ (ng·hr/mL)	1990 ± 1020	1640 ± 1000	2270 ± 1150
AUC Ratio to Radioactivity (%)		80.0 ± 7.5	
Data are expressed as the mean ± S.D. (n=3) The concentrations of radioactivity in blood and plasma were detected at 48 hours after administration (blood: 12.7 ± 5.7 ng eq./mL, plasma: 11.0 ± 6.1 ng eq./mL). The pharmacokinetic parameters of S-033188 were not shown because the concentrations of S-033188 in all plasma samples were below the lower limit of quantification (BLQ: <0.998 ng/mL).			
Study Number	R-033188-PF-026-N		
Location in CTD	4.2.2.2-03		

2.6.5.3.5 Dose-linearity and Food Effect of S-033447 after a Single Oral Administration of S-033188 in Monkeys

Test Article: S-033188

Species/Strain	Monkey/Cynomolgus (Hamri)			
Gender (M/F)/Number of Animals	M/3 per group			
Feeding Condition	Non-fasted for 0.3, 1, 3 mg/kg, fasted for 1 mg/kg			
Vehicle/Formulation	0.5 w/v% MC/suspension			
Method of Administration	Single oral administration			
Dose (mg/kg)[a]	0.3, 1, 3 for non-fasted, 1 for fasted			
Sample	Plasma (15, 30 minutes, 1, 2, 4, 6, 8, 10, 24 hours post-dose)			
Analyte	S-033188 and S-033447			
Assay	LC/MS/MS			
Pharmacokinetic Parameters	Non-fasted Monkey			Fasted Monkey
	0.3 mg/kg	1 mg/kg	3 mg/kg	1 mg/kg
T_{max} (hr)	2.17 ± 1.76	5.33 ± 3.06	3.33 ± 1.15	2.67 ± 1.15
C_{max} (ng/mL)	5.81 ± 1.26	19.6 ± 4.2	57.6 ± 5.4	108 ± 48
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	86.2 ± 11.8	309 ± 59	957 ± 118	1450 ± 610
$AUC_{0\text{-}inf}$ (ng·hr/mL)	158 ± 86	438 ± 112	1340 ± 150	1770 ± 730
$t_{1/2,z}$ (hr)	19.6 ± 12.8	12.7 ± 2.0	12.5 ± 0.8	9.32 ± 0.32
BA (%)	10.4 ± 1.4	11.1 ± 1.6	11.5 ± 1.3	50.6 ± 15.1
Data are expressed as the mean ± S.D. (n=3) The pharmacokinetic parameters of S-033188 were not shown because the concentration of S-033188 was BLQ (< 0.500 ng/mL) in all plasma samples. BA was calculated using $AUC_{0\text{-}24hr}$ after intravenous dosing (836 ng·hr/mL at 0.254 mg/kg).				
Study Number	R-033188-PB-028-N			
Location in CTD	4.2.2.2-04			

2.6.5.3.6 Dose-linearity of S-033447 after a Single Intravenous Administration of S-033447 in Monkeys

Test Article: S-033447

Species/Strain	Monkey/Cynomolgus (Hamri)	
Gender (M/F)/Number of Animals	M/3 per group	
Feeding Condition	Non-fasted	
Vehicle/Formulation	(*N*,*N*-Dimethylacetamide/Propylene glycol/20% [w/v] Hydroxypropyl-β-cyclodextrin aqueous solution = 2/2/1 [by vol.])/Solution	
Method of Administration	Single intravenous administration	
Dose (mg/kg)[a]	0.254, 0.846	
Sample	Plasma (5, 15, 30 minutes, 1, 2, 4, 6, 8, and 24 hours post-dose)	
Analyte	S-033447	
Assay	LC/MS/MS	
Pharmacokinetic Parameters	0.254 mg/kg	0.846 mg/kg
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	836 ± 116	2810 ± 320
$AUC_{0\text{-}inf}$ (ng·hr/mL)	1050 ± 150	3480 ± 410
$t_{1/2\text{-}z}$ (hr)	12.0 ± 0.8	11.3 ± 0.7
CL_{ss} (mL/hr/kg)	245 ± 36	245 ± 27
Vd_{ss} (mL/kg)	3400 ± 550	3210 ± 370
Data are expressed as the mean ± S.D. (n=3) a 0.254 and 0.846 mg/kg of S-033447 is equivalent to 0.3 and 1 mg/kg of S-033188, respectively		
Study Number	R-033188-PB-028-N	
Location in CTD	4.2.2.2-04	

2.6.5.3.7 Effect of Metal Ion on plasma concentration of S-033447 after a single oral administration of S-033188 in Monkey

Test Article: S-033188

Species/Strain	Monkey/Cynomolgus (Hamri)				
Gender (M/F)/Number of Animals	M/3 per group				
Feeding Condition	Fasted				
Vehicle/Formulation	0.5% MC/suspension				
Method of Administration	Single oral administration				
Dose (mg/kg)	1				
Sample	Plasma (30 minutes, 1, 2, 4, 6, 8, 24 hours post-dose)				
Analyte	S-033447				
Assay	LC/MS/MS				
Pharmacokinetic Parameters	S-033188 alone	Calcium[a]	Mixture of magnesium and aluminum[b]	Iron[c]	Mixture of metal ion[d]
T_{max} (hr)	2.33 ± 1.53	2.00 ± 0.00	2.00 ± 0.00	1.33 ± 0.58	2.67 ± 1.15
C_{max} (ng/mL)	80.1 ± 46.3	50.1 ± 17.2	49.5 ± 20.1	38.3 ± 12.5	43.1 ± 18.6
AUC_{0-inf} (ng·hr/mL)	1220 ± 720	772 ± 247	755 ± 308	596 ± 217	757 ± 356
Data are expressed as the mean ± S.D. (n=3) a Calcium carbonate was dosed at 41.7 mg/kg. b Mixture of magnesium hydroxide (26.7 mg/kg) and aluminum oxide (14.9 mg/kg) was dosed. c Ferrous fumarate was dosed at 4.5 mg/kg. d Mixture of calcium carbonate (40.6 mg/kg), magnesium hydroxide (2.7 mg/kg), and ferrous fumarate (0.16 mg/kg) was dosed. These metal contents were mimicked for human food used for phase 1 clinical study (section 2.7.1.2.1.1)					
Study Number	S-033188-PB-244-N				
Location in CTD	4.2.2.2-05				

2.6.5.3.8 Plasma concentrations of S-033188 and S-033447 after a single oral administration of S-033188 in Juvenile Rats

Test Article: S-033188

Species/Strain		Rat/Crl:CD(SD)				
Gender (M/F)/Number of Animals		M/3 per group				
Feeding Condition		Non-fasted				
Vehicle/Formulation		0.5 w/v% MC/suspension				
Method of Administration		Single oral administration				
Dose (mg/kg)		3, 10, 30				
Sample		Plasma (15, 30 minutes, 1, 2, 4, 8, 24 hours post-dose)				
Analyte		S-033188, S-033447				
Assay		LC/MS/MS				
	Dose of S-033188 (mg/kg)	Pharmacokinetic Parameters				
		C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2,z}$ (hr)	$AUC_{0\text{-}inf}$ (ng·hr/mL)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)
Day 10 of age						
S-033188	3	5.15	0.500	N.C.	N.C.	2.91
	10	4.82	0.250	0.781	5.44	5.60
	30	14.8	0.250	1.17	17.3	17.6
S-033447	3	688	2.00	9.22	11200	9420
	10	1880	4.00	7.45	26800	23800
	30	4570	1.00	6.35	51800	48100
Day 20 of age						
S-033447	3	202	1.00	4.29	1470	1450
	10	707	1.00	3.61	3520	3490
	30	962	0.500	2.85	7670	7640
Day 30 of age						
S-033447	3	90.4	0.500	2.87	473	472
	10	313	1.00	2.86	1490	1490
	30	609	0.500	2.74	3160	3150
Pharmacokinetic parameters were calculated from the mean concentrations of three rats. In rats at day 20 and 30 of age, after oral administration at doses of 3, 10, and 30 mg/kg, the plasma concentration of S-033188 was BLQ (<0.500 ng/mL) at all the time points.						
Study Number		S-033188-PF-148-N				
Location in CTD		4.2.2.2-06				

2.6.5.3.9 Plasma concentrations of S-033447 after a single intravenous administration of S-033447 in Juvenile Rats

Test Article: S-033447

Species/Strain		Rat/Crl:CD(SD)				
Gender (M/F)/Number of Animals		M/3 per group				
Feeding Condition		Non-fasted				
Vehicle/Formulation		*N*,*N*-Dimethylacetamide/5 v/v% tween 80 containing saline = 1/99 (by vol.)/solusion				
Method of Administration		Single intravenous administration				
Dose (mg/kg)		0.254				
Sample		Plasma (5, 15, 30 minutes, 1, 2, 4, 8, 24 hours post-dose)				
Analyte		S-033447				
Assay		LC/MS/MS				
The day of age (days)	Pharmacokinetic Parameters					
	$AUC_{0\text{-}24hr}$ (ng·hr/mL)		$AUC_{0\text{-}inf}$ (ng·hr/mL)	$t_{1/2_z}$ (hr)	CL_{tot} (mL/hr/kg)	Vd_{ss} (mL/kg)
10	1460		1640	7.77	155	1540
20	410		417	4.15	610	2920
30	190		171	2.33	1490	4230
Pharmacokinetic parameters were calculated from the mean concentrations of three rats.						
Study Number		S-033188-PF-190-N				
Location in CTD		4.2.2.2-07				

2.6.5.3.10 BA Calculation in Juvenile Rats

Test Article: S-033447

Species/Strain	Rat/Crl:CD(SD)								
Gender (M/F)/Number of Animals	M/3 per group								
Feeding Condition	Non-fasted								
Vehicle/Formulation	*N,N*-Dimethylacetamide/5 v/v% tween 80 containing saline = 1/99 (by vol.)/solusion								
Method of Administration	Single intravenous administration								
Dose (mg/kg)	0.254								
Sample	Plasma (5, 15, 30 minutes, 1, 2, 4, 8, 24 hours post-dose)								
Analyte	S-033447								
Assay	LC/MS/MS								
Pharmacokinetic parameter (Mean):	Day 10 of age			Day 20 of age			Day 30 of age		
	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
BA (%)	68.3	49.0	31.6	35.3	25.3	18.4	27.7	26.1	18.5
BA was calculated using $AUC_{0\text{-}inf}$ after oral [2.6.5.3.8] and intravenous [2.6.5.3.9] administration.									
Study Number	S-033188-PF-190-N								
Location in CTD	4.2.2.2-07								

2.6.5.4 薬物動態試験： 吸収：反復投与

反復投与時の吸収は，2.6.7 項に TK として記載している．

2.6.5.5 薬物動態試験： 分布

2.6.5.5.1 Tissue Distribution after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]- S-033188

Species/Strain	Rat (Iar:Long-Evans)
Gender (M/F)/Number of Animals	M/1 per time point
Feeding Condition	Non-fasted
Vehicle/Formulation	0.5 w/v% MC/suspension
Method of Administration	Single oral administration
Dose (mg/kg)[a]	1
Radionuclide	^{14}C
Specific Activity	1.94 MBq/mg (52.4 μCi/mg)
Sample	Plasma, Adrenal gland, Blood in the heart, Bone, Bone marrow, Brown fat, Cerebellum, Cerebrum, Cervical lymph node, Connective tissue, Epididymis, Exorbital lacrimal gland, Eye ball, Harderian gland, Intestinal mucosa, Liver, Lung, Lymph fluid, Myocardium, Nasal mucosa, Pancreas, Parotid gland, Pigmented skin, Pineal body, Pituitary gland, Preputial gland, Prostate, Rectal mucosa, Renal cortex, Renal medulla, Seminal vesicle, Skeletal muscle, Spinal cord, Spleen, Stomach mucosa, Sublingual gland, Submaxillary gland, Testis, Thymus, Thyroid, Uveal tract (chorioidea), Uveal tract (iris), White fat, White skin, Xiphoid process
Analyte	Radioactivity (S-033447 equivalent)
Assay	LSC for plasma and QWBA for other tissues

Tissues	Radioactivity Concentration in Tissue (ng/g)							$t_{1/2}$ (hr)
	1 hr	2 hr	8 hr	24 hr	72 hr	168 hr	336 hr	
Plasma	12.9	10.7	5.77	N.D.	N.D.	N.D.	N.D.	N.C.
Adrenal gland	79.0	56.7	48.9	BLQ	BLQ	BLQ	BLQ	N.C.
Blood in the heart	12.6	12.6	3.93*	BLQ	BLQ	BLQ	BLQ	N.C.
Bone	64.1	78.0	57.5	56.8	14.9	11.0	3.98*	63.3[a]
Bone marrow	41.9	37.2	29.9	9.00	BLQ	BLQ	BLQ	10.4[b]
Brown fat	35.8	41.9	18.7	BLQ	BLQ	BLQ	BLQ	N.C.
Cerebellum	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
Cerebrum	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
Cervical lymph node	18.7	34.6	33.4	BLQ	BLQ	BLQ	BLQ	N.C.
Connective tissue	22.8	N.C.	16.2	BLQ	BLQ	BLQ	BLQ	N.C.
Epididymis	2.15*	12.4	15.3	BLQ	BLQ	BLQ	BLQ	N.C.

Tissues	Radioactivity Concentration in Tissue (ng/g)							$t_{1/2}$ (hr)
	1 hr	2 hr	8 hr	24 hr	72 hr	168 hr	336 hr	
Exorbital lacrimal gland	37.6	65.6	73.1	BLQ	BLQ	BLQ	BLQ	N.C.
Eye ball	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
Harderian gland	28.4	48.4	55.6	BLQ	BLQ	BLQ	BLQ	N.C.
Intestinal mucosa	3350	1050	588	BLQ	BLQ	BLQ	BLQ	N.C.
Liver	879	854	637	103	19.1	BLQ	BLQ	12.9°
Lung	39.6	44.5	23.0	BLQ	BLQ	BLQ	BLQ	N.C.
Lymph fluid	19.1	11.0	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
Myocardium	81.1	100	55.6	BLQ	BLQ	BLQ	BLQ	N.C.
Nasal mucosa	7.80*	6.50*	29.9	11.5	BLQ	BLQ	BLQ	N.C.
Pancreas	80.6	75.4	42.6	BLQ	BLQ	BLQ	BLQ	N.C.
Parotid gland	68.5	86.3	45.7	BLQ	BLQ	BLQ	BLQ	N.C.
Pigmented skin	14.4	20.4	21.1	BLQ	BLQ	BLQ	BLQ	N.C.
Pineal body	59.5	93.1	88.6	BLQ	BLQ	BLQ	BLQ	N.C.
Pituitary gland	58.5	60.3	22.2	BLQ	BLQ	BLQ	BLQ	N.C.
Preputial gland	26.9	N.C.	14.8	BLQ	BLQ	BLQ	BLQ	N.C.
Prostate	17.9	28.9	29.4	BLQ	BLQ	BLQ	BLQ	N.C.
Rectal mucosa	25.8	32.8	24.2	BLQ	BLQ	BLQ	BLQ	N.C.
Renal cortex	144	133	104	BLQ	BLQ	BLQ	BLQ	N.C.
Renal medulla	93.3	91.6	48.0	BLQ	BLQ	BLQ	BLQ	N.C.
Seminal vesicle	BLQ	1.97*	3.77*	BLQ	BLQ	BLQ	BLQ	N.C.
Skeletal muscle	21.8	30.0	30.0	BLQ	BLQ	BLQ	BLQ	N.C.
Spinal cord	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
Spleen	57.5	59.1	31.5	BLQ	BLQ	BLQ	BLQ	N.C.
Stomach mucosa	166	197	123	BLQ	BLQ	BLQ	BLQ	N.C.
Sublingual gland	59.7	83.3	43.6	BLQ	BLQ	BLQ	BLQ	N.C.
Submaxillary gland	82.1	98.2	52.2	BLQ	BLQ	BLQ	BLQ	N.C.
Testis	BLQ	BLQ	3.36*	BLQ	BLQ	BLQ	BLQ	N.C.
Thymus	8.77	22.2	26.8	BLQ	BLQ	BLQ	BLQ	N.C.
Thyroid	84.8	76.1	53.5	BLQ	BLQ	BLQ	BLQ	N.C.

Tissues	Radioactivity Concentration in Tissue (ng/g)							$t_{1/2}$ (hr)
	1 hr	2 hr	8 hr	24 hr	72 hr	168 hr	336 hr	
Uveal tract (chorioidea)	BLQ	170	N.C.	N.R.	BLQ	BLQ	BLQ	N.C.
Uveal tract (iris)	BLQ	73.8	77.0	BLQ	BLQ	BLQ	BLQ	N.C.
White fat	BLQ	2.73*	BLQ	BLQ	BLQ	BLQ	BLQ	N.C.
White skin	8.00*	16.6	14.2	BLQ	BLQ	BLQ	BLQ	N.C.
Xiphoid process	N.C.	11.9	35.7	4.61*	BLQ	BLQ	BLQ	N.C.
Abbreviations: BLQ, below the lower limit of quantification; QWBA, quantitative whole-body autoradiography; N.D., not detected (< 10 dpm/sample=0.0696 ng eq. of S-033447/sample for all LSC measurements); N.R., not represented. BLQ is < 8.51 ng eq. of S-033447/g or sample shapes were not discernible from background. When quantitative data for one tissue were obtained from multiple sections, data are expressed as the mean value of each data. In the calculation of mean values, BLQ was treated as zero. When the data on all sections were BLQ, the mean value is expressed as "BLQ". a $t_{1/2,8\text{-}168hr}$. b $t_{1/2,2\text{-}24hr}$. c $t_{1/2,2\text{-}72hr}$. * The mean values were less than LLOQ because they were calculated from the each value including BLQ (BLQ is calculated as 0).								
Study Number	R-033188-PB-020-N							
Location in CTD	4.2.2.3-01							

2.6.5.6 薬物動態試験： たん白結合及び血球移行

2.6.5.6.1 薬物動態試験： たん白結合及び血球移行

Test Article: [^{14}C]-033447

Test System	In vitro, Equilibrium dialysis method		
Radionuclide	^{14}C		
Specific Activity	2.21 MBq/mg (59.8 μCi/mg)		
Concentration (ng/mL)	50, 100, 1000		
Incubation	At 37°C for 24 hours		
Sample	Sera under fasted condition (rat [male, Sprague-Dawley], monkey [male, cynomolgus], human [male]), purified serum human proteins (albumin, α_1-acid glycoprotein, γ-globulin)		
Analyte	Radioactivity		
Assay	LSC		
	Protein Binding Ratio (%)		
Concentration	50 ng/mL	100 ng/mL	1000 ng/mL
Serum[a]			
Rat	92.1 ± 1.0	91.9 ± 0.5	92.0 ± 0.7
Monkey	89.5 ± 1.6	85.0 ± 1.9	88.8 ± 0.7
Human	92.9 ± 0.6	93.0 ± 0.4	93.9 ± 0.4
Purified human serum protein[b]			
4% Albumin	91.4 ± 0.3	91.2 ± 0.1	92.1 ± 0.1
0.08% α_1-Acid glycoprotein	59.3 ± 2.1	59.0 ± 0.5	52.2 ± 0.6
1% γ-Globulin	24.5 ± 2.2	23.6 ± 1.1	38.1 ± 0.7
a Data are expressed as the mean ± S.D. of 3 individual rat, monkey, or human sera. b Data are expressed as the mean ± S.D. of 3 incubations.			
Study Number	R-033188-PB-021-N		
Location in CTD	5.3.2.1-01		

2.6.5.6.2 In Vitro Distribution of [^{14}C]-S-033447 to Blood Cells

Test Article: [^{14}C]-S-033447

Test System	In vitro distribution in blood cells		
Radionuclide	^{14}C		
Specific Activity	2.21 MBq/mg (59.8 μCi/mg)		
Concentration (ng/mL)	50, 100, 1000		
Incubation	At 37°C for 15 min		
Sample	Blood under fasted condition (rat [male, Sprague-Dawley], monkey [male, cynomolgus], human [male])		
Analyte	Radioactivity		
Assay	LSC		
	Distribution ratio of radioactivity in blood cells (%)		
Concentration	50 ng/mL	100 ng/mL	1000 ng/mL
Rat	59.6 ± 0.3	58.7 ± 1.9	57.1 ± 1.1
Monkey	52.9 ± 4.0	52.9 ± 2.4	50.4 ± 3.2
Human	54.4 ± 1.8	53.0 ± 1.7	48.5 ± 1.4
Data are expressed as the mean ± S.D. of 3 individual rat, monkey, or human blood.			
Study Number	R-033188-PB-021-N		
Location in CTD	5.3.2.1-01		

2.6.5.6.3 Distribution to blood Cells after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)
Gender (M/F)/Number of Animals	M/4
Feeding Condition	Non-fasted
Vehicle/Formulation	0.5 w/v% MC/suspension
Method of Administration	Single oral administration
Dose (mg/kg)	1
Radionuclide	^{14}C
Specific Activity	1.94 MBq (52.4 μCi)/mg
Sample	Blood, Plasma
Analyte	Radioactivity
Assay	LSC
Time	Distribution of Radioactivity to Blood Cells (%)
15 min	34.2 ± 4.1
30 min	39.0 ± 7.5
1 hr	41.7 ± 2.6
2 hr	43.2 ± 5.2
4 hr	43.7 ± 7.4
6 hr	43.3 ± 3.0
8 hr	41.3 ± 2.1
10 hr	49.5 ± 18.8
24 hr	N.C.
Data are expressed as the mean ± S.D. (n=4).	
Study Number	R-033188-PB-057-N
Location in CTD	4.2.2.2-01

2.6.5.6.4 Distribution to blood Cells after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys

Test Article: [^{14}C]-S-033188

Species/Strain	Monkey/Cynomolgus (Hamri)
Gender (M/F)/Number of Animals	M/3
Feeding Condition	Non-fasted
Vehicle/Formulation	0.5 w/v% MC/suspension
Method of Administration	Single oral administration
Dose (mg/kg)	3
Radionuclide	^{14}C
Specific Activity	1.94 MBq (52.4 μCi)/mg
Sample	Blood, Plasma
Analyte	Radioactivity
Assay	LSC
Time	Distribution of Radioactivity to Blood Cells (%)
15 min	45.6 [a]
30 min	44.2 ± 5.0
1 hr	44.1 ± 2.4
2 hr	38.4 ± 5.6
4 hr	50.0 ± 4.7
6 hr	47.1 ± 1.4
8 hr	50.3 ± 0.9
12 hr	52.3 ± 1.2
24 hr	49.3 ± 4.9
48 hr	49.0 ± 5.9
Data are expressed as the mean ± S.D. (n=3). a Data are expressed as the mean (n = 2)	
Study Number	R-033188-PF-026-N
Location in CTD	4.2.2.2-03

2.6.5.7 薬物動態試験： 妊娠又は授乳動物における試験

2.6.5.7.1 Tissue Distribution after a Single Oral Administration of [^{14}C]-S-033188 in Pregnant Rats

Test Article: [^{14}C]- S-033188

Species/Strain	Rat/Crl:CD(SD)
Gender (M/F)/Number of Animals	F/1 per time point
Feeding Condition	Non-fasted
Vehicle/Formulation	0.5 w/v% MC/suspension
Method of Administration	Single oral administration
Dose (mg/kg) [a]	1
Radionuclide	^{14}C
Specific Activity	1.91 MBq/mg (51.6 μCi/mg)
Sample	Plasma, Blood, Adrenal cortex, Adrenal medulla, Aortic wall, Bone marrow, Bone surface, Brain, Brown fat, Choroid plexus, Clitoris, Exorbital lachrymal gland, Harderian gland, Intra-orbital lachrymal gland, Kidney cortex, Kidney medulla, Lens, Liver, Lung, Mammary tissue, Mandibular lymph node, Meninges, Muscle, Myocardium, Nasal mucosa, Ovary, Pancreas, Periodontal membrane, Pineal body, Pituitary gland, Salivary glands, Skin, Spinal cord, Spleen, Thymus, Thyroid gland, Tongue, Tooth pulp, Trachea, Urinary bladder wall, Urine, Uterus, Uveal tract/retina, White fat, Placenta, Amniotic fluid, Foetus, Foetal adrenal, Foetal blood, Foetal bone, Foetal brain, Foetal eye, Foetal git, Foetal kidney, Foetal liver, Foetal lung, Foetal myocardium, Foetal skin, Foetal uveal tract, Oesophageal wall, Stomach mucosa (fundic), Stomach mucosa (non fundic), Small intestine mucosa, Caecum mucosa, Large intestine mucosa, Rectum mucosa
Analyte	Radioactivity (S-033447 equivalent)
Assay	LSC for plasma and QWBA for other tissues

Tissues	Radioactivity Concentration in Tissue[a] (ng/g)				
	1 hr	2 hr	8 hr	24 hr	48 hr
Plasma	16.4	29.3	16.0	N.D.	N.D.
Blood	BLQ	42.5	BLQ	BLQ	BLQ
Adrenal cortex	165	265	347	BLQ	BLQ
Adrenal medulla	65.9	126	113	BLQ	BLQ
Aortic wall	77.6	56.1	104	BLQ	BLQ
Bone marrow	44.6	87.0	98.3	BLQ	BLQ
Bone surface	BLQ	51.5	97.4	75.4	170
Brain	BLQ	BLQ	BLQ	BLQ	BLQ
Brown fat	BLQ	85.0	88.3	BLQ	BLQ
Choroid plexus	55.6	118	184	BLQ	BLQ
Clitoris	46.5	106	188	BLQ	BLQ

Tissues	Radioactivity Concentration in Tissue[a] (ng/g)				
	1 hr	2 hr	8 hr	24 hr	48 hr
Exorbital lachrymal gland	41.5	126	129	BLQ	BLQ
Harderian gland	37.2	115	209	BLQ	BLQ
Intra-orbital lachrymal gland	50.1	128	110	BLQ	BLQ
Kidney cortex	144	175	261	BLQ	BLQ
Kidney medulla	89.0	230	203	BLQ	BLQ
Lens	BLQ	BLQ	BLQ	BLQ	BLQ
Liver	2670	974	864	BLQ	BLQ
Lung	60.3	86.4	114	BLQ	BLQ
Mammary tissue	BLQ	37.1	56.6	BLQ	BLQ
Mandibular lymph node	BLQ	82.2	86.4	BLQ	BLQ
Meninges	BLQ	BLQ	53.5	BLQ	BLQ
Muscle	BLQ	36.4	84.9	BLQ	BLQ
Myocardium	97.0	206	240	BLQ	BLQ
Nasal mucosa	BLQ	BLQ	42.1	BLQ	BLQ
Ovary	39.8	45.1	43.5	BLQ	BLQ
Pancreas	91.9	185	191	BLQ	BLQ
Periodontal membrane	52.3	89.0	106	36.7	BLQ
Pineal body	71.7	192	264	BLQ	BLQ
Pituitary gland	73.8	149	177	BLQ	BLQ
Salivary glands	101	199	204	BLQ	BLQ
Skin	BLQ	BLQ	50.4	BLQ	BLQ
Spinal cord	BLQ	BLQ	BLQ	BLQ	BLQ
Spleen	74.5	143	153	BLQ	BLQ
Thymus	BLQ	49.9	109	BLQ	BLQ
Thyroid gland	83.4	194	231	BLQ	BLQ
Tongue	65.9	119	195	BLQ	BLQ
Tooth pulp	65.8	125	172	BLQ	BLQ
Trachea	BLQ	62.4	81.5	77.6	BLQ
Urinary bladder wall	99.6	BLQ	316	BLQ	BLQ
Urine	177	BLQ	144	BLQ	BLQ
Uterus	38.8	39.0	83.2	BLQ	BLQ
Uveal tract/retina	BLQ	43.9	132	BLQ	BLQ
White fat	BLQ	BLQ	35.0	BLQ	BLQ
Placenta	43.0	77.8	74.6	BLQ	BLQ
Amniotic fluid	BLQ	BLQ	BLQ	BLQ	BLQ
Foetus	BLQ	BLQ	53.6	BLQ	BLQ

Tissues	Radioactivity Concentration in Tissue[a] (ng/g)				
	1 hr	2 hr	8 hr	24 hr	48 hr
Foetal adrenal	BLQ	55.5	107	BLQ	BLQ
Foetal blood	BLQ	BLQ	37.4	BLQ	BLQ
Foetal bone	BLQ	BLQ	286	186	327
Foetal brain	BLQ	BLQ	BLQ	BLQ	BLQ
Foetal eye	BLQ	BLQ	48.5	BLQ	BLQ
Foetal git	BLQ	BLQ	49.0	BLQ	BLQ
Foetal kidney	BLQ	49.0	45.7	BLQ	BLQ
Foetal liver	BLQ	36.7	50.1	BLQ	BLQ
Foetal lung	BLQ	BLQ	60.1	BLQ	BLQ
Foetal myocardium	BLQ	BLQ	42.5	BLQ	BLQ
Foetal skin	BLQ	BLQ	47.1	BLQ	BLQ
Foetal uveal tract	BLQ	BLQ	60.7	BLQ	BLQ
Oesophageal wall	BLQ	91.0	81.0	BLQ	BLQ
Stomach mucosa (fundic)	52.0	236	114	BLQ	BLQ
Stomach mucosa (non fundic)	136	286*	120	38.0	BLQ
Small intestine mucosa	117	268	172	BLQ	BLQ
Caecum mucosa	72.1	202	357*	BLQ	BLQ
Large intestine mucosa	44.5	166	122	BLQ	BLQ
Rectum mucosa	BLQ	62.7	82.6	BLQ	BLQ
BLQ is <33.5 ng eq. of S-033447/g or sample shapes were not discernible from background. N.D. is <1.32 ng eq. of S-033447/g Terminal half-lives could not be determined for any tissue, as none contained three data points on the elimination phase, where the linearity (R^2) was >0.8. When quantitative data for one tissue were obtained from multiple sections, data are expressed as the mean value of each data. In the calculation of mean values, BLQ was treated as zero. When the data on all sections were BLQ, the mean value is expressed as "BLQ". a Doses administered as S-033188. S-033188 is entirely converted into S-033447 after oral administration, hence tissue concentrations are reported in terms of ng equiv of S-033447/g. * Tissue measurement affected by high levels of radioactivity present in adjacent GIT contents.					
Study Number	S-033188-PF-177-L				
Location in CTD	4.2.2.3-02				

2.6.5.7.2 Excretion into Milk after a Single Oral Administration of [^{14}C]- S–033188 in Nursing Rats

Test Article: [^{14}C]-S–033188

Species/Strain	Rat/Crl:CD(SD)				
Gender (M/F)/Number of Animals	F/5 per time point				
Feeding Condition	Non-fasted				
Vehicle/Formulation	0.5 w/v% MC/suspension				
Method of Administration	Single oral administration				
Dose (mg/kg)[a]	1				
Radionuclide	^{14}C				
Specific Activity	1.91 MBq/mg (51.6 μCi/mg)				
Sample	Milk, Plasma				
Analyte	Radioactivity (S-033447 equivalent)				
Assay	LSC				
Radioactivity Concentration in Tissue	1 hr	2 hr	4 hr	8 hr	24 hr
Milk (ng/g)	79.6 ± 29.6	124 ± 52	109 ± 43	57.7 ± 15.0	N.D.[b]
Plasma (ng/g)	34.9 ± 6.8	24.3 ± 4.7	15.7 ± 3.2	8.06 ± 1.96	N.D.[c]
Milk/Plasma ratio	2.33 ± 0.86	5.11 ± 1.61	6.94 ± 2.16	7.39 ± 2.17	N.C.
Data are expressed as the mean ± S.D. (n=5) a Doses administered as S-033188. S-033188 is entirely converted into S-033447 after oral administration, hence tissue concentrations are reported in terms of ng equiv of S-033447/g. b <4.78 ng/g. c <4.02 ng/g					
Study Number	S-033188-PF-176-N				
Location in CTD	4.2.2.3-03				

2.6.5.8 薬物動態試験： その他の分布試験

該当する試験なし.

2.6.5.9 薬物動態試験： In Vivo における代謝

2.6.5.9.1 Metabolite Profiling in Plasma, Urine, Feces, and Bile after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)
Gender (M/F)/Number of Animals	M/8 (intact) or 2 (bile duct-cannulated)
Feeding condition	Non-fasted
Vehicle/Formulation	0.5 w/v% MC / suspension
Method of Administration	Single oral administration
Dose (mg/kg)	5
Radionuclide	^{14}C
Specific radio activity	1.94MBq (52.4 μCi)/mg
Sample	Plasma obtained from intact rats, Urine and feces obtained from intact rat, Bile obtained from bile duct-cannulated rats
Analyte	S-033188, S-033447 and its metabolites
Assay	LSC, Radio-HPLC, LC/MS[a]

Metabolites	Name of metabolite	Composition (% of run in HPLC sample)[a]		Cumulative Excretion (% of dose)[a]		
		1-hour plasma	4-hour plasma	0 to 48-hour urine	0 to 48-hour bile	0 to 48-hour feces
RPM1, RUM1, RBM1, RFM1	(12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide[b]	9.2	6.5	0.2	2.5	3.6
RUM2, RFM2 (unknown)	–	N.D.	N.D.	0.0	N.D.	0.0
RFM3 (unknown)	–	N.D.	N.D.	N.D.	N.D.	0.0
RBM2	Glucuronide of oxidized form of S-033447 pyrrole (proposed)	N.D.	N.D.	N.D.	0.1	N.D.
RUM3, RBM3, RFM4	(12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide[b]	N.D.	N.D.	0.0	0.9	1.8
RPM2, RUM4, RBM4	S-033447 glucuronide[c]	6.1	3.1	0.0	6.3	N.D.
RFM5 (unknown)	–	N.D.	N.D.	N.D.	N.D.	0.0
RBM5	Glucuronide of S-033447 pyrrole (proposed)	N.D.	N.D.	N.D.	0.6	N.D.
S-033447 pyrrole (RFM6)	S-033447 pyrrole	N.D.	N.D.	N.D.	N.D.	1.0
S-033447	S-033447	81.9	90.5	0.0	1.9	69.3
S-033188	S-033188	N.D.	N.D.	N.D.	N.D.	N.D.
Others	–	2.9	0.0	0.2	1.0	4.2

Abbreviations: Radio-HPLC, high performance liquid chromatography with radio-detector; HPLC, high-performance liquid chromatography; LC/MS[a], high-performance liquid chromatography with multistage mass spectrometry.

a The data of the plasma were determined from the pooled sample of 2 animals and data of the urine, bile, and feces were determined from the sample of 1 animal.

b Identified in the study of Metabolite Profiling and Identification of S-033188 after a Single Oral Administration in Humans (Section 2.7.2.2.1.1)

c Identified in human mass balance study (Section 2.7.2.2.1.2)

Study Number	R-033188-PB-033-N
Location in CTD	4.2.2.4-01

2.6.5.9.2 Metabolite Profiling in Plasma, Urine, Feces, and Bile after a Single Oral Administration of [^{14}C]- S-033188 in Monkeys

Test Article: [^{14}C]-S-033188

Species/Strain	Monkey/Cynomolgus (Charles River)
Gender (M/F)/Number of Animals	M/3 (intact) or 3 (bile duct-cannulated)
Feeding Condition	Non-Fasted
Vehicle/Formulation	0.5 w/v% MC / suspension
Method of Administration	Single oral administration
Dose (mg/kg)	3
Radionuclide	^{14}C
Specific Activity	1.94MBq (52.4 μCi)/mg
Sample	Plasma urine, and feces obtained from intact monkeys, Bile obtained from bile duct-cannulated monkeys (from another monkey PK study [R-033188-PF-026-N])
Analyte	S-033188, S-033447 and its metabolites
Assay	LSC, Radio-HPLC, LC/MS[a]

Metabolites	Name of metabolite	Composition (% of run in HPLC sample)[a]			Cumulative Excretion (% of dose)[a]		
		2-hr plasma	6-hr plasma	24-hr plasma	0–48 hr urine	0–72 hr bile	0–96 hr feces
MPM1 (unknown)	–	5.2	N.D.	N.D.	N.D.	N.D.	N.D.
MUM1, MBM1	Glucuronide of oxidized form of S-033447 (proposed)	N.D.	N.D.	N.D.	0.0	0.0	N.D.
MBM2	Glucuronide of oxidized form of S-033447 pyrrole (proposed)	N.D.	N.D.	N.D.	N.D.	0.1	N.D.
MPM2, MUM2, MBM3, MFM1	(12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide[b]	10.4	11.7	9.5	0.2	0.8	0.3
MBM4	Glucuronide of oxidized form of S-033447 pyrrole (proposed)	N.D.	N.D.	N.D.	N.D.	0.1	N.D.
MPM3, MUM3, MBM5, MFM2	(12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide[b]	4.8	4.4	5.0	0.1	0.4	0.6
MPM4, MUM4, MBM6	S-033447 glucuronide[c]	18.8	8.8	2.5	0.3	4.8	N.D.
MFM3	S-033447 oxide (proposed)	N.D.	N.D.	N.D.	N.D.	N.D.	0.1
MFM4	S-033447 oxide (proposed)	N.D.	N.D.	N.D.	N.D.	N.D.	0.5
MBM7	Glucuronide of S-033447 pyrrole (proposed)	N.D.	N.D.	N.D.	N.D.	1.1	N.D.
S-033447 pyrrole (MFM5)	S-033447 pyrrole	N.D.	N.D.	N.D.	N.D.	N.D.	1.7
S-033447	S-033447	60.9	75.1	83.0	0.1	1.2	60.0
S-033188	S-033188	N.D.	N.D.	N.D.	0.1	N.D.	2.5
Others	–	N.D.	N.D.	N.D.	0.2	0.6	3.4

Abbreviation: PK, pharmacokinetics. a The data of the plasma were determined from the pooled sample of 2 animals and data of the urine, bile, and feces were determined from the sample of 1 animal. b Identified in the study of Metabolite Profiling and Identification of S-033188 after a Single Oral Administration in Humans (Section 2.7.2.2.1.1) c Identified in human mass balance study (Section 2.7.2.2.1.2)	
Study Number	R-033188-PB-055-N
Location in CTD	4.2.2.4-02

2.6.5.10 薬物動態試験： In Vitro における代謝

2.6.5.10.1 In Vitro Hydrolysis of [^{14}C]-S-033188

Test Article: [^{14}C]-S-033188

Test System	Human, rat, and monkey sera, and human, rat, and monkey liver S9 and intestinal S9 (protein concentration: 1 mg/mL)
Incubation	At 37°C for 0.5 and 1 hour (human serum and liver S9 and intestinal S9) or 1 hour (rat and monkey sera and liver S9 and intestinal S9)
Concentration (μmol/L)	10 (human, rat, and monkey sera), 100 (rat,and monkey liver S9 and intestinal S9), 10 and 100 (human liver S9 and intestinal S9)
Evaluation items	In vitro hydrolysis of [^{14}C]-S-033188 in liver S9, intestinal S9, and sera from rat, monkey, and human. Hydrolysis ratios of [^{14}C]-S-033188 at the designed time point were calculated based on the peak area of S-033188 and S-033447 on a radio chromatogram.
Radionuclide	^{14}C
Specific Activity	1.94MBq (52.4 μCi)/mg
Analyte	S-033188, S-033447
Assay	LSC, Radio-HPLC

Species	[^{14}C]-S-033188 concentration (μmol/L)	Incubation time (hr)	Hydrolysis (%)
Serum			
Human	10	0.5	45.9 ± 2.9
		1	71.6 ± 1.8
Rat	10	1	100 ± 0
Monkey	10	1	87.1 ± 0.4
Liver S9			
Human	10	0.5	100 ± 0
		1	100 ± 0
	100	0.5	95.7 ± 0.1
		1	100 ± 0
Rat	100	1	98.1 ± 0.1
Monkey	100	1	100 ± 0
Intestinal S9			
Human	10	0.5	73.6 ± 2.0
		1	93.2 ± 0.4
	100	0.5	57.3 ± 0.7
		1	81.8 ± 0.0
Rat	100	1	51.1 ± 0.5
Monkey	100	1	43.0 ± 0.2
Abbreviation: Radio-HPLC, high performance liquid chromatography with radio-detector. Data are expressed as the mean ± S.D. (n=3)			
Study Number	R-033188-PB-030-N		
Location in CTD	5.3.2.2-01		

2.6.5.10.2 Investigation of Responsible Enzyme for Hydrolysis of [^{14}C]-S-033188

Test Article: [^{14}C]-S-033188

Test System	Intestinal and liver S9 from human (protein concentration : 1 mg/mL[intestine] and 0.5 mg/mL[liver])
Incubation	At 37°C for 1 hour
Concentration (μmol/L)	100
Evaluation items	Inhibitory effect of esterases inhibitors on S-033188 hydrolysis in human intestinal and liver S9 fraction
Radionuclide	^{14}C
Specific Activity	1.94MBq (52.4 μCi)/mg
Analyte	S-033188, S-033447
Assay	LSC, Radio-HPLC

Inhibitor	Inhibited Enzyme		Human liver S9		Human intestinal S9	
	Family	Member	Hydrolysis ratio (%)	Inhibition (%)	Hydrolysis ratio (%)	Inhibition (%)
Chemical hydrolysis enzyme inhibitor (concentration)						
Eserine (100 μmol/L)	Serine esterase	Non-specific	3.2	96.4	2.9	97.1
Paraoxon (100 μmol/L)			0.0	100.0	0.4	99.6
DFP (100 μmol/L)			0.0	100.0	0.9	99.1
PMSF (1000 μmol/L)			31.3	65.0	49.1	49.9
BNPP (100 μmol/L)		CES and AADAC	6.4	92.9	16.4	83.3
iso-OMPA (100 μmol/L)		Acetylcholinesterase and butyrylcholinesterase	88.8	0.9	93.6	4.5
EDTA 2Na (1000 μmol/L)	Ca-dependent A-esterase	Non-specific	83.9	9.0	93.8	5.0
DTNB (100 μmol/L)	Arylesterase	Non-specific	87.9	1.9	97.5	0.5
Specific Inhibitor (μmol/L)						
Digitonin (100 μmol/L)	CES1		91.8	0.5	99.4	−0.6
Telmisartan (100 μmol/L)	CES2		77.7	13.2	89.2	9.0
Vinblastine (100 μmol/L)	CES2 and AADAC		22.5	75.6	53.4	45.9
Abbreviations: AADAC, arylacetamide deacetylase; BNPP, bis(4-nitrophenyl) phosphate; CES, carboxylesterase; DFP, diisopropyl fluorophosphate; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); iso-OMPA, tetraisopropyl pyrophosphoramide; PMSF, phenylmethylsulfonyl fluoride.						
Study Number	R-033188-PB-101-N					
Location in CTD	5.3.2.2-02					

2.6.5.10.3 In Vitro Metabolism of [^{14}C]-S-033188 and [^{14}C]-S-033447 in Human Hepatocyte

Test Article: [^{14}C]- S-033188, [^{14}C]- S-033447

Test System	Cryopreserved human hepatocytes (2 Donors [Lot No. HC4-5, and HC5-23])
Incubation	At 37°C for 2 and 4 hours
Concentration (μmol/L)	5 and 50
Evaluation items	In vitro metabolite profiling of the reaction mixtures after incubation of [^{14}C]-S-033188 and [^{14}C]-S-033447 with cryopreserved human hepatocytes Identification and/or structural estimation of S-033188, S-0334447, and its metabolites in the reaction mixtures after incubation of [^{14}C]-S-033188 or [^{14}C]-S-033447 with cryopreserved human hepatocytes Evaluation of interconversion of S-033447 into its diastereomers in cryopreserved human hepatocytes
Radionuclide	^{14}C
Specific Activity	1.94MBq (52.4 μCi)/mg
Analyte	S-033188, S-033447, and its metabolites
Assay	Radio-HPLC, LC/MS[a], LSC

Metabolites[a]	Name of metabolite	Substrates							
		S-033188				S-033447			
		Composition, % in HPLC sample (% of run)				Composition, % in HPLC sample (% of run)			
		Lot. HC4-5[b]		Lot. HC5-23[b]		Lot. HC4-5[b]		Lot. HC5-23[b]	
		2 hours	4 hours	2 hours	4 hours	2 hours	4 hours	2 hours	4 hours
HHM1	(12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide[c]	1.38	1.56	2.21	2.02	0.34	0.44	1.04	1.36
HHM2	(12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide[c]	1.41	1.88	1.36	1.89	1.09	1.16	1.53	1.95
HHM3[d]	Dioxidized S-033447 (proposed)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
HHM4	S-033447 glucuronide[e]	1.18	1.70	N.D.	N.D.	1.27	1.47	0.58	0.90
HHM5	Glucuronide of S-033447 pyrrole (proposed)	0.76	0.85	1.05	0.92	0.91	0.83	0.67	1.58
HHM6[d]	Ketone form of S-033447 (proposed)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
S-033447 pyrrole (HHM7)[d]	S-033447 pyrrole	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
S-033447	S-033447	86.91	86.06	86.35	86.44	88.99	89.01	88.17	85.26
S-033188	S-033188	N.D.	N.D.	N.D.	N.D.	N/A	N/A	N/A	N/A

a The data at the concentration of 5 µmol/L are shown. b Lot numbers of cryopreserved human hepatocytes. c Identified in the study of Metabolite Profiling and Identification of S-033188 after a Single Oral Administration in Humans (section 2.7.2.2.1.1) d The metabolite detected at the concentration of 50 µmol/L. e Identified in human mass balance study (section 2.7.2.2.1.2)	
Study Number	R-033188-PB-050-N
Location in CTD	5.3.2.2-03

2.6.5.10.4 In Vitro Identification of Responsible Enzymes for Glucuronidation of S-033447

Test Article: [^{14}C]- S-033447

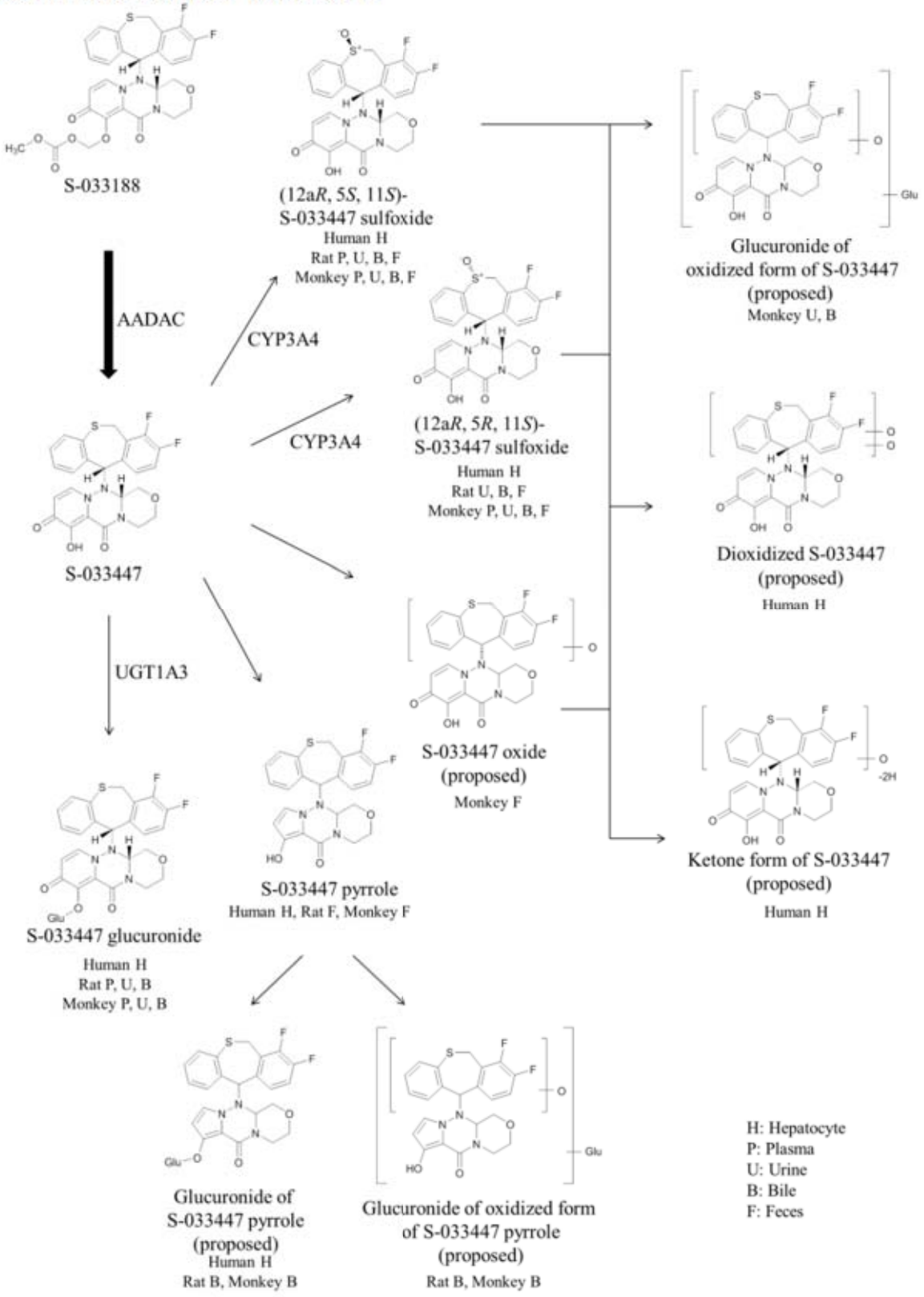
Test System		Human liver microsomes (a pool of 15 individuals), Recombinant human UGT enzymes						
Incubation		At 37°C for 60 minutes						
Concentration (μmol/L)		5 and 50						
Recombinant rhUGT Enzymes		UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, UGT2B15.						
Radionuclide		^{14}C						
Specific Activity		2.21 MBq (59.8 μCi)/mg						
Evaluation items		Identification of responsible enzyme for the glucuronidation of [^{14}C]-S-033447 by recombinant human UGT						
Analyte		S-033447 and its metabolites						
Assay		LSC, Radio-HPLC, LC/MS^n						
	Concentration of [^{14}C]- S-033447 (μmol/L)	UGT1A1	UGT1A3	UGT1A4	UGT1A6	UGT1A9	UGT2B7	UGT2B15
Formation of S-033447 glucuronide with recombinant UGT (pmol/min/mg protein)	5	0	11.3	0	0	0	0	0
	50	0	36.5	0	0	0	0	0
Data are expressed as the mean of duplicate assays.								
Study Number		S-033188-PB-109-N						
Location in CTD		5.3.2.2-04						

2.6.5.10.5 In Vitro Identification of Responsible Enzymes for Oxidation of S-033447

Test Article: [^{14}C]- S-033447

Test System	Human liver microsomes (a pool of 15 individual), Recombinant human CYP enzymes							
Incubation	At 37°C for 90 minutes							
Concentration (μmol/L)	50							
Recombinant Enzymes	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4							
CYP Inhibitors	α-Naphthoflavone (CYP1A2 inhibitor, 0.5 μmol/L), Thiotepa (CYP2B6 inhibitor, 100 μmol/L), Montelukast (CYP2C8 inhibitor, 5 μmol/L), Sulphaphenazole (CYP2C9 inhibitor, 5 μmol/L), Benzylnirvanol (CYP2C19 inhibitor, 5 μmol/L), Quinidine (CYP2D6 inhibitor, 1 μmol/L), Ketoconazole (CYP3A4 inhibitor, 1 μmol/L)							
Radionuclide	^{14}C							
Specific Activity	2.23 MBq/mg (60.2 μCi/mg)							
Evaluation items	Identification of responsible enzyme(s) for the oxidative metabolism of [^{14}C]-S-033447 to (12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide and (12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide							
Analyte	S-033447 and its metabolites							
Assay	LSC, Radio-HPLC, LC/MS[a]							
	Control[a]	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
(12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide Formation with Recombinant CYP (pmol/hour/pmol P450)	0	0	0	0	0	0	0	19.6
Effects of CYP Inhibitors on (12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide Formation (% Inhibition from Control)		−2.9	4.8	−5.8	−9.7	0.2	−10.6	74.2
	Control	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
(12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide Formation with Recombinant CYP (pmol/hour/pmol P450)	0.9	1.1	0.2	0.4	1.6	1.0	0.5	27.8
Effects of CYP Inhibitors on (12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide Formation (% Inhibition from Control)		0.7	16.8	−11.8	−5.4	6.6	3.9	77.3
Data are expressed as the mean of duplicate assays. a Control supersomes™ (microsomes obtained from insect cells infected with wild type baculovirus) was used.								
Study Number	S-033188-PB-180-N							
Location in CTD	5.3.2.2-05							

2.6.5.11 薬物動態試験： 推定代謝経路



2.6.5.12 薬物代謝酵素の誘導／阻害

2.6.5.12.1 In Vitro Inhibitory Effects of S-033188 and S-033447 on Human CYP Enzymes

Test Article: S-033188, S-033447

Test System	Human liver microsomes (a pool of 15 individual)
Incubation	At 37°C for 2 minutes (Midazolam), 5 minutes (Testosterone), 10 minutes (Phenacetin, Bupropion, Paclitaxel, Dextromethorphan), 20 minutes (Tolbutamide) ,30 minutes (S-Mephenytoin) after 0 or 30 minutes preincubation
Concentration (μmol/L)	0 (control), 0.1, 3, 10, 30, and 100
Substrates (Concentration)	Phenacetin (50 μmol/L) for CYP1A2, Bupropion (100 μmol/L) for CYP2B6, Paclitaxel (15 μmol/L) for CYP2C8, Tolbutamide (100 μmol/L) for CYP2C9, S-Mephenytoin (40 μmol/L) for CYP2C19, Dextromethorphan (5 μmol/L) for CYP2D6, Testosterone (50 μmol/L) for CYP3A4, Midazolam (2.5 μmol/L) for CYP3A4
Evaluation items	IC_{50} values of S-033188 and S-033447 toward CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 regarding direct inhibition and time-dependent inhibition
Analyte	Acetaminophen, Hydroxybupropion, 6α-Hydroxypaclitaxel, 4-Hydroxytolbutamide, 4-Hydroxymephenytoin, Dextrorphan, 6β-Hydroxytestosterone, 1'-Hydroxymidazolam
Assay	LC/MS/MS

	CYP isoform	Direct inhibition (0 min)		Time-dependent inhibition (30 min)		Potential for time-dependent inhibition[b]
		IC_{50} [a](μmol/L)	% of control at 100 μmol/L (%)	IC_{50} [a](μmol/L)	% of control at 100 μmol/L (%)	
S-033188	CYP1A2	>100	88.6	>100	110	None
	CYP2B6	46.0	32.8	44.2	33.3	None
	CYP2C8	63.2	42.6	>100	68.6	None
	CYP2C9	>100	72.1	>100	77.8	None
	CYP2C19	>100	82.2	>100	83.0	None
	CYP2D6	>100	63.5	> 100	72.4	None
	CYP3A4 (Teststerone)	50.2	32.5	77.4	40.9	None
	CYP3A4 (Midazolam)	23.2	16.5	31.7	16.0	None
S-033447	CYP1A2	>100	91.9	> 100	102	None
	CYP2B6	29.3	26.9	39.7	32.8	None
	CYP2C8	>100	64.4	>100	69.8	None
	CYP2C9	>100	80.3	>100	82.0	None
	CYP2C19	>100	73.1	>100	90.9	None
	CYP2D6	>100	66.6	>100	77.9	None
	CYP3A4 (Testosterone)	>100	51.8	95.8	49.7	None
	CYP3A4 (Midazolam)	43.2	26.8	31.7	20.2	None

Abbreviation: IC_{50}, 50% inhibitory concentration. a Average data (i.e., percent of control activity) obtained from duplicate samples for S-033188 or S-033447 concentration were used to calculate IC_{50} values. b When IC_{50} values were calculated, time-dependent inhibition was determined by comparison of IC_{50} values for 0-minutes pre-incubation and 30-minutes pre-incubation. If the observed inhibition was insufficient to calculate IC_{50} values, % of control values for each concentration were compared.	
Study Number	S-033188-PB-090-N
Location in CTD	5.3.2.2-06

2.6.5.12.2 In Vitro CYP1A2, CYP2B6, and CYP3A4 Induction by S-033188 and S-033447 in Human Hepatocytes

Test Article: S-033188, S-033447

Test System	Primary cultures of cryopreserved human hepatocytes (3 Donors [Lot No. Hu8160, Hu1389, and RSF])
incubation	At 37°C for 72 hours
Concentration (µmol/L)	0 (Control),0.1, 1, 3, 10, 30, and 60
Positive controls (Concentration)	Omeprazole (50 µmol/L) for prototypical inducer of CYP1A2, Phenobarbital (1000 µmol/L) for prototypical inducer of CYP2B6, Rifampicin (10 µmol/L) for prototypical inducer of CYP3A4
Substrates (Concentration)	Phenacetin (50 µmol/L) for CYP1A2, Bupropion (100 µmol/L) for CYP2B6, Midazolam (50 µmol/L) for CYP3A4
Sample	CYP1A2, 2B6 and 3A4 activities after incubation of substrate with human hepatocytes which were exposed with S-033188, S-033447 or positive control, RNA amount of CYP1A2, 2B6 and 3A4 from human hepatocytes which were exposed with S-033188, S-033447 or positive control
Analyte	Acetaminophen, Hydroxybupropion, 1'-Hydroxymidazolam, mRNA
Assay	LC/MS/MS, Quantitative real-time polymerase chain reaction

CYP isoform	Test Article or Positive controls	Concentration (µmol/L)	Fold induction of mRNA level[a] (fold)			Fold induction of Enzyme activity[a] (fold)		
			Hu8160	Hu1389	RSF	Hu8160	Hu1389	RSF
CYP1A2	S-033188	0.1	0.681	0.680	0.990	1.020	0.765	0.971
		1	0.752	0.791	1.067	0.866	0.716	0.987
		3	0.717	0.802	0.972	0.782	0.697	0.806
		10[b]	1.057	1.075	1.175	0.647	0.603	0.717
		30[b]	1.034	2.345	1.557	0.204	0.134	N.C.
		60[b]	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S-033447	0.1	0.895	0.930	1.035	0.993	0.888	1.031
		1	1.576	1.721	1.159	1.244	1.338	1.272
		3	1.464	1.500	1.090	1.613	1.458	1.637
		10	1.382	1.503	1.422	1.155	1.170	1.612
		30	1.761	2.510	1.482	0.518	1.176	0.854
		60[b]	1.861	2.665	1.249	0.351	0.781	0.330
	Omeprazole	50	61.560	65.682	32.547	13.023	17.571	13.655

CYP isoform	Test Article or Positive controls	Concentration (μmol/L)	Fold induction of mRNA level[a] (fold)			Fold induction of Enzyme activity[a] (fold)		
			Hu8160	Hu1389	RSF	Hu8160	Hu1389	RSF
CYP2B6	S-033188	0.1	0.825	1.172	0.936	1.052	0.979	0.851
		1	1.135	1.236	1.023	1.092	1.007	0.730
		3	0.969	1.462	1.151	0.981	1.020	0.757
		10[b]	1.631	1.667	1.255	0.804	0.964	0.749
		30[b]	1.029	1.988	3.085	N.C.	N.C.	N.C.
		60[b]	N.C.	8.418	7.900	N.C.	N.C.	N.C.
	S-033447	0.1	1.099	1.065	0.903	1.007	0.970	1.117
		1	1.188	1.105	1.024	1.039	0.939	1.115
		3	1.425	1.412	1.170	1.054	0.963	1.031
		10	1.128	1.577	1.498	1.040	0.945	1.175
		30	0.901	2.224	1.785	0.531	0.957	0.664
		60[b]	0.880	2.182	1.978	0.259	0.488	0.414
	Phenobarbital	1000	7.410	9.621	6.740	9.261	5.084	6.346
CYP3A4	S-033188	0.1	1.116	1.510	1.051	0.931	1.052	1.172
		1	1.032	1.228	0.950	0.887	1.117	1.175
		3	0.857	1.021	1.237	0.964	1.095	1.074
		10[b]	0.711	0.752	0.284	0.112	0.154	0.166
		30[b]	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
		60[b]	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
	S-033447	0.1	0.743	0.870	1.029	0.963	1.026	1.075
		1	0.818	1.043	1.045	0.969	1.135	1.233
		3	1.239	1.605	1.044	0.997	1.152	1.210
		10	1.492	1.916	1.361	0.953	1.250	1.108
		30	2.090	2.533	1.619	0.909	0.795	0.994
		60[b]	1.388	1.621	1.536	0.509	0.718	0.796
	Rifampicin	10	11.369	14.226	19.998	8.943	8.334	21.051

Abbreviation: mRNA, messenger RNA.
a Relative to the corresponding control.
b Data were shown as reference due to cytotoxicity

Study Number	S-033188-PF-105-N
Location in CTD	5.3.2.2-07

2.6.5.12.3 In Vitro UGT Inhibition by S-033188 and S-033447 in Human Liver Microsomes

Test Article: S-033188, S-033447

Test System	Human liver microsomes (mixed-gender pool of 200 individuals)		
Exposure	At 37°C for 5 minutes for UGT1A1, UGT1A4, 1A6, 1A9 and 2B7 and 10 minutes for UGT1A3 and 2B15.		
Concentration (μmol/L)	0, 0.1, 0.3, 1, 3, 10, 30, and 100 for S-033188. 0, 0.03, 0.1, 0.3, 1, 3, 10, 30 for S-033447.		
Positive controls (Concentration)	Troglitazone (12, 100, 3, 20, 20, 100, 50 μmol/L) for UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15, respectively.		
Substrates (Concentration)	17β-Estradiol (12 μmol/L) for UGT1A1, Chenodeoxycholic acid (160 μmol/L) for UGT1A3, Trifluoperazine (20 μmol/L) for UGT1A4, 1-Naphthol (2 μmol/L) for UGT1A6, Propofol (16 μmol/L) for UGT1A9, Morphine (400 μmol/L) for UGT2B7, Oxazepam (100 μmol/L) for UGT2B15.		
Evaluation items	IC_{50} values of S-033188 and S-033447 toward UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 regarding direct inhibition.		
Analyte	17β-Estradiol 3 glucuronide, Chenodeoxycholic acid 24O-glucuronide, Trifluoperazine glucuronide, 1-Naphthol glucuronide, Propofol glucuronide, Morphine 3-glucuronide, S-Oxazepam glucuronide.		
Assay	LC/MS/MS		
	UGT isoform	IC_{50} [a] (μmol/L)	Maximum inhibition observed[b] (%)
S-033188	UGT1A1	>100	39
	UGT1A3	>100	46
	UGT1A4	>100	39
	UGT1A6	>100	13
	UGT1A9	>100	17
	UGT2B7	>100	37
	UGT2B15	94	49
S-033447	UGT1A1	>30	15
	UGT1A3	>30	30
	UGT1A4	>30	7.7
	UGT1A6	>30	6.3
	UGT1A9	>30	14
	UGT2B7	>30	16
	UGT2B15	>30	12
a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. b Maximum inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures): Maximum inhibition observed (%) = 100% − the minimum percent solvent control for any test article concentration.			
Study Number	S-033188-PF-172-N		
Location in CTD	5.3.2.2-08		

2.6.5.12.4 Effects on Hepatic Drug Metabolizing Enzymes in Rat

Test Article: S-033188

Species/Strain	Rat/Crl:CD(SD)
Gender (M/F)/Number of Animals	M and F/10
Vehicle/Formulation	0.5 w/v% MC/suspension
Method of Administration	Oral administration for 2 weeks
Dose (mg/kg)	0 [vehicle control], 2000 mg/kg/day
Sample	Microsomes prepared from livers were transferred from the toxicity study [R-033188-TB-032-L])
Enzyme activity (main responsible CYP isoforms)	EROD activity (CYP1A), T6β-OHase activity (CYP3A), T16α-OHase activity (CYP2C11), T16β-OHase activity (CYP2B1), *p*-NP UDPGT activity, T3 UDPGT activity, T4 UDPGT activity

	Average activity [S.D.]								
	Male								
Dose (mg/kg/day)	Protein content (mg/g liver)	P450 content (nmol/mg protein)	EROD (nmol/min/mg protein)	T6β-OHase (nmol/min/mg protein)	T16α-OHase (nmol/min/mg protein)	T16β-OHase (nmol/min/mg protein)	*p*-NP UDPGT (nmol/min/mg protein)	T3 UDPGT (pmol/min/mg protein)	T4 UDPGT (pmol/min/mg protein)
0	31.2 [4.0]	0.717 [0.108]	0.0215 [0.0032]	1.42 [0.51]	1.19 [0.30]	0.0304 [0.0217]	15.6 [3.1]	10.4 [2.5]	3.13 [0.67]
2000	34.6 [4.3]	1.32** [0.12]	0.0239 [0.0035]	4.72** [0.45]	0.869* [0.202]	0.100** [0.032]	17.0 [5.7]	24.0† [6.4]	13.6† [3.8]
	Female								
Dose (mg/kg/day)	Protein content (mg/g liver)	P450 content (nmol/mg protein)	EROD (nmol/min/mg protein)	T6β-OHase (nmol/min/mg protein)	T16α-OHase (nmol/min/mg protein)	T16β-OHase (nmol/min/mg protein)	*p*-NP UDPGT (nmol/min/mg protein)	T3 UDPGT (pmol/min/mg protein)	T4 UDPGT (pmol/min/mg protein)
0	21.2 [3.5]	0.635 [0.080]	0.0314 [0.0068]	0.0536 [0.0205]	N/A	0.0224 [0.0122]	7.20 [2.80]	17.0 [3.9]	2.31 [0.42]
2000	25.0** [1.8]	0.985** [0.067]	0.0288 [0.0045]	1.64† [0.32]	N/A	0.102† [0.026]	10.3** [1.7]	40.8** [4.8]	12.7† [2.5]

Abbreviations: EROD, ethoxyresorufin O-deethylase; p-NP UDPGT, p-nitrophenol uridine 5-diphosphoglucuronic acid glucuronosyltransferase; T16α-OHase, testosterone 16α-hydroxylase; T16β-OHase, testosterone 16β-hydroxylase; T3 UDPGT, 3,3',5-Triiodo-L-thyronine uridine 5-diphosphoglucuronic acid glucuronosyltransferase; T4 UDPGT, L-thyroxine uridine 5-diphosphoglucuronic acid glucuronosyltransferase; T6β-OHase, testosterone 6β-hydroxylase.
Data are expressed as the mean ± S.D. (n=10)
*P < 0.05, and **P < 0.01 vs control group (Student's t test). †P < 0.01 vs control group (Aspin-Welch's t test).

Study Number	Study Number: R-033188-PF-080-N
Location in CTD	4.2.2.4-03

2.6.5.13 薬物動態試験： 排泄

2.6.5.13.1 Urinary and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)		
Gender (M/F)/Number of Animals	M/4		
Feeding Condition	Non-fasted		
Vehicle/Formulation	0.5 w/v% MC/suspension		
Method of Administration	Single oral administration		
Dose (mg/kg)	1		
Radionuclide	^{14}C		
Specific Activity	1.94 MBq (52.4 μCi)/mg		
Sample	Urine, Feces		
Analyte	Radioactivity		
Assay	LSC		
Sampling time (hours)	Cumulative Excretion Rate of Radioactivity (% of dose)		
	Urine	Feces	Total
6	0.3 ± 0.0	N.S.	N.C.
24	0.4 ± 0.0	90.1 ± 5.8	90.5 ± 5.8
48	0.4 ± 0.0	96.3 ± 1.1	96.8 ± 1.1
72	0.4 ± 0.0	96.5 ± 1.0	96.9 ± 1.0
96	0.4 ± 0.0	96.5 ± 1.0	97.0 ± 1.0
120	0.4 ± 0.0	96.6 ± 1.0	97.0 ± 1.0
144	0.4 ± 0.0	96.6 ± 1.0	97.0 ± 1.0
168	0.4 ± 0.0	96.6 ± 1.0	97.1 ± 1.0
Abbreviation: N.S., No sample. Data are expressed as the mean ± S.D. (n=4).			
Study Number	R-033188-PB-013-N		
Location in CTD	4.2.2.5-01		

2.6.5.13.2 Urinary and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys

Test Article: [^{14}C]-S-033188

Species/Strain	Monkey/Cynomolgus (Charles River)			
Gender (M/F)/Number of Animals	M/3			
Feeding Condition	Non-fasted			
Vehicle/Formulation	0.5 w/v% MC/suspension			
Method of Administration	Single oral administration			
Dose (mg/kg)	3			
Radionuclide	^{14}C			
Specific Activity	1.94 MBq (52.4 μCi)/mg			
Sample	Urine, feces, and cage washing			
Analyte	Radioactivity			
Assay	LSC			
Sampling time (hours)	Cumulative Excretion Rate of Radioactivity (% of dose)			
	Urine	Feces	Cage washing	Total
6	0.2 ± 0.1	N.S.	0.5 ± 0.5	0.7 ± 0.6
24	0.6 ± 0.2	21.5 ± 36.9	0.8 ± 0.8	22.9 ± 37.8
48	0.8 ± 0.2	61.7 ± 13.6	0.9 ± 0.9	63.4 ± 14.6
72	0.9 ± 0.2	89.2 ± 4.5	1.0 ± 1.0	91.1 ± 3.6
96	1.0 ± 0.1	92.4 ± 4.0	1.0 ± 1.0	94.4 ± 2.9
120	1.0 ± 0.1	93.7 ± 3.9	1.1 ± 1.1	95.9 ± 2.7
144	1.0 ± 0.1	94.2 ± 3.6	1.1 ± 1.1	96.3 ± 2.5
168	1.0 ± 0.2	94.4 ± 3.5	1.1 ± 1.1	96.5 ± 2.3
Data are expressed as the mean ± S.D. (n=3).				
Study Number	R-033188-PF-026-N			
Location in CTD	4.2.2.2-03			

2.6.5.14 薬物動態試験： 排泄：胆汁中

2.6.5.14.1 Urinary, Biliary, and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)					
Gender (M/F)/Number of Animals	M/5 (bile duct-cannulated)					
Feeding Condition	Non-fasted					
Vehicle/Formulation	0.5 w/v% MC/suspension					
Method of Administration	Single oral administration					
Dose (mg/kg)	1					
Radionuclide	^{14}C					
Specific Activity	1.94 MBq (52.4 μCi)/mg					
Sample	Urine, Bile, Feces, Stomach contents, Intestinal contents					
Analyte	Radioactivity					
Assay	LSC					
Sampling time (hours)	Cumulative Excretion Rate of Radioactivity (% of dose)					
	Urine	Bile	Feces	Stomach contents	Intestinal contents	Total
6	0.1 ± 0.1	11.0 ± 2.8	N.S.	N.S.	N.S.	N.C.
24	0.5 ± 0.1	16.7 ± 2.9	39.4 ± 28.0	N.S.	N.S.	56.7 ±27.3
48	0.6 ± 0.2	18.5 ± 3.2	55.7 ± 31.5	4.8 ± 9.3	15.7 ± 24.4	95.2 ± 1.2
Data are expressed as the mean ± S.D (n=5).						
Study Number	R-033188-PB-013-N					
Location in CTD	4.2.2.5-01					

2.6.5.14.2 Urinary, Biliary, and Fecal Excretion after a Single Oral Administration of [^{14}C]-S-033188 in Monkeys

Test Article: [^{14}C]-S-033188

Species/Strain	Monkey/Cynomolgus (Charles River)						
Gender (M/F)/Number of Animals	M/3 (bile duct-cannulated)						
Feeding Condition	Non-fasted						
Vehicle/Formulation	0.5 w/v% MC/suspension						
Method of Administration	Single oral administration						
Dose (mg/kg)	3						
Radionuclide	^{14}C						
Specific Activity	1.94 MBq (52.4 μCi)/mg						
Sample	Urine, bile, feces, cage washing, and gastro-intestinal tract contents						
Analyte	Radioactivity						
Assay	LSC						
Sampling time (hours)	Cumulative Excretion Rate of Radioactivity (% of dose)						
	Urine	Bile	Feces	Cage washing	Total	Gastro-intestinal tract contents	Total recovery
6	0.3 ± 0.1	2.3 ± 0.8	N.S.	0.1 ± 0.1	2.3 ± 0.8	N.S.	2.3 ± 0.8
24	0.8 ± 0.1	6.1 ± 0.5	45.6 ± 25.1	0.2 ± 0.1	52.8 ± 25.5	N.S.	52.8 ± 25.5
48	1.1 ± 0.1	8.0 ± 0.5	86.5 ± 3.7	0.3 ± 0.2	95.8 ± 3.5	N.S.	95.8 ± 3.5
72	1.2 ± 0.1	8.5 ± 0.6	89.5 ± 2.3	0.3 ± 0.2	99.5 ± 2.0	0.2 ± 0.1	99.6 ± 2.0
Data are expressed as the mean ± S.D (n=3).							
Study Number	R-033188-PF-026-N						
Location in CTD	4.2.2.2-03						

2.6.5.14.3 Enterohepatic Circulation of Radioactivity after a Single Oral Administration of [^{14}C]-S-033188 in Rats

Test Article: [^{14}C]-S-033188

Species/Strain	Rat/Crl:CD(SD)							
Gender (M/F)/Number of Animals	M/8 (4 donor rats and 4 recipient rats)							
Feeding Condition	Non-fasted							
Vehicle/Formulation	0.5 w/v% MC / suspension							
Method of Administration	Single oral administration							
Dose (mg/kg)	1							
Radionuclide	^{14}C							
Specific Activity	1.91 MBq/mg (51.6 μCi/mg)							
Sample	Urine, Bile, Feces, Gastrointestinal contents, Carcass							
Analyte	Radioactivity							
Assay	LSC							
Sampling time (hours)	Cumulative Excretion Rate of Radioactivity (% of dose)							
	Donor Rats				Recipient Rats			
	Urine	Bile	Feces	Total	Urine	Bile	Feces	Total
6	0.2 ± 0.2	N.S.	N.S.	N.C.	0.0 ± 0.0	0.2 ± 0.1	N.S.	N.C.
24	0.6 ± 0.4	N.S.	52.3 ± 23.2	N.C.	0.0 ± 0.0	0.7 ± 0.3	8.6 ± 6.4	N.C.
48	0.7 ± 0.3	N.S.	76.6 ± 2.1	78.9 ± 2.6 [a]	0.0 ± 0.0	0.9 ± 0.3	17.0 ± 6.7	21.2 ± 1.2[b]
Total recovery	100.1 ± 2.1							
Reabsorption (% of dose)	1.4 ± 0.7							
Reabsorption (% of radioactivity recovered in recipient rat)	6.7 ± 4.0							
Enterohepatic circulation (% of dose)	0.9 ± 0.3							
Enterohepatic circulation (% of radioactivity recovered in recipient rat)	4.2 ± 1.7							
[^{14}C]-S-033188 was administered to donor rats. Data are expressed as the mean ± S.D (n=4). a Including the radioactivity in gastrointestinal contents and carcass 48 hours post-dose (0.9 ± 1.4% and 0.8 ± 0.4%, respectively). b Including the radioactivity in gastrointestinal contents and carcass 48 hours post-dose (2.9 ± 5.0% and 0.5 ± 0.4%, respectively).								
Study Number	S-033188-PF-151-N							
Location in CTD	4.2.2.5-02							

2.6.5.15 薬物動態試験： 薬物相互作用

2.6.5.15.1 In Vitro Substrate Assessments of [^{14}C]-S-033188 and [^{14}C]-S-033447 for Human Efflux Transporters

Test Article: [^{14}C]-S-033188 and [^{14}C]-S-033447

Test System	Caco-2 cells				
Incubation	At 37°C for 2 hours				
Concentration (μmol/L)	2 or 10				
Radionuclide	^{14}C				
Specific Activity	[^{14}C]-S-033188 (1.94 MBq/mg), [^{14}C]-S-033447 (2.21 MBq/mg)				
Positive control (Concentration)	[^{3}H]-Digoxin (1 μmol/L) for P-gp and [^{3}H] ES (0.1 μmol/L) for BCRP				
Inhibitors (Concentration)	Verapamil (30 μmol/L) for P-gp and Ko143 (1 μmol/L) for BCRP				
Sample	Apical side solution, Basolateral side solution				
Analyte	Radioactivity				
Assay	LSC				
Substrates	Inhibitor	Apparent Permeability (× 10^{-6} cm/sec)		P_{app} ratio	% of control[a]
		Apical to Basolateral	Basolateral to Apical		
[^{14}C]-S-033188 (2 μmol/L)	None	4.75 ± 0.36	29.4 ± 1.4	6.2	100.0
	Verapamil	17.1 ± 0.7	20.4 ± 0.2	1.2	3.8
	Ko143	4.52 ± 0.12	30.8 ± 3.3	6.8	111.5
[^{14}C]-S-033188 (10 μmol/L)	None	6.34 ± 0.14	27.9 ± 0.4	4.4	100.0
	Verapamil	17.2 ± 0.6	20.5± 0.4	1.2	5.9
	Ko143	7.30 ± 0.12	27.5 ± 2.1	3.8	82.4
[^{14}C]-S-033447 (2 μmol/L)	None	0.503 ± 0.022	3.15 ± 0.59	6.3	100.0
	Verapamil	0.428 ± 0.370	1.04 ± 0.18	2.4	26.4
	Ko143	0.650 ± 0.071	3.03 ± 0.44	4.7	69.8
[^{14}C]-S-033447 (10 μmol/L)	None	0.495 ± 0.010	3.12 ± 0.18	6.3	100.0
	Verapamil	0.634 ± 0.037	1.25 ± 0.09	2.0	18.9
	Ko143	0.488 ± 0.014	3.03 ± 0.14	6.2	98.1
[^{3}H]-Digoxin (1 μmol/L)	None	0.737 ± 0.018	15.0 ± 0.5	20.4	100.0
	Verapamil	4.18 ± 0.11	5.45 ± 0.35	1.3	1.5
[^{3}H]-ES (0.1 μmol/L)	None	0.871 ± 0.054	37.7 ± 2.2	43.3	100.0
	Ko143	4.22 ± 0.11	5.34 ± 0.46	1.3	0.7
Abbreviations: BCRP, breast cancer resistant protein; ES, estrone sulfate; P_{app}, apparent permeability; P-gp, P-glycoprotein. Data are expressed as the mean ± S.D. of triplicate determinations. a The % of control was calculated by the following equation: % of control = (P_{app} ratio in the presence of inhibitor – 1) / (P_{app} ratio in the absence of inhibitor – 1) × 100.					
Study Number	S-033188-PF-111-N				
Location in CTD	5.3.2.2-09				

2.6.5.15.2 In Vitro Substrate Assessments of [^{14}C]-S-033447 for Human Uptake Transporters

Test Article: [^{14}C]-S-033447

Test System	HEK-293 cells transfected with recombinant human uptake transporter (OATP1B1 or OATP1B3)				
Incubation	At 37°C for 2, 5, or 10 minutes				
Concentration (μmol/L)	1 or 5				
Radionuclide	^{14}C				
Specific Activity	[^{14}C]-S-033447 (2.21 MBq/mg)				
Positive control (Concentration)	[^{3}H]$E_2$17βG (0.05 μmol/L)				
Inhibitors (Concentration)	Rifampicin (10 μmol/L) for OATP1B1 and OATP1B3				
Sample	Cell lysate				
Analyte	Radioactivity				
Assay	LSC				
Substrates	Inhibitor	Incubation Time (minutes)	Cleared volume (μL/mg protein)		
			Control cells	OATP1B1-expressing cells	OATP1B3-expressing cells
[^{14}C]-S-033447 (1 μmol/L)	–	2	148 ± 4	155 ± 2	152 ± 6
		5	245 ± 4	241 ± 9	267 ± 12
		10	348 ± 17	397 ± 23	399 ± 25
	Rifampicin	2	144 ± 10	134 ± 6	139 ± 9
		5	263 ± 11	237 ± 20	249 ± 11
		10	365 ± 4	436 ± 22	431 ± 31
[^{14}C]-S-033447 (5 μmol/L)	–	2	141 ± 8	133 ± 4	136 ± 5
		5	255 ± 9	225 ± 4	236 ± 7
		10	367 ± 17	405 ± 42	433 ± 23
	Rifampicin	2	126 ± 17	92.4 ± 4.4	111 ± 16
		5	232 ± 21	174 ± 16	191 ± 20
		10	325 ± 31	379 ± 21	491 ± 6
[^{3}H]$E_2$17βG (0.05 μmol/L)	–	2	0.267 ± 0.061	130 ± 8	18.7 ± 0.7
	Rifampicin	2	0.225 ± 0.029	22.5 ± 1.5	1.39 ± 0.07
Abbreviation: $E_2$17βG, estradiol 17β- D-glucuronide. Data are expressed as the mean ± S.D. of triplicate determinations.					
Study Number	S-033188-PF-111-N				
Location in CTD	5.3.2.2-09				

2.6.5.15.3 In Vitro Inhibitor Assessments of S-033188 and S-033447 for Human Efflux Transporters

Test Article: S-033188 and S-033447

Test System	Caco-2 cells		
Incubation	At 37°C for 2 hours		
Concentration (μmol/L)[a]	S-033188 (0.994, 2.00, 5.00, 10.0, 25.0, 50.0, 78.1), S-033447 (0.282, 0.637, 0.778, 3.14, 7.01, 9.54, 20.9)		
Substrate (Concentration)	[^{3}H]-Digoxin (1 μmol/L) for P-gp and [^{3}H] ES (0.1 μmol/L) for BCRP		
Radionuclide	^{3}H		
Positive control (Concentration)	Verapamil (30 μmol/L) for P-gp and Ko143 (1 μmol/L) for BCRP		
Sample	Apical side solution, Basolateral side solution		
Analyte	Radioactivity		
Assay	LSC		
Transporters	Inhibitor	IC_{50} (μmol/L)	% of control [b] (% at maximum concentration of inhibitors)
P-gp	S-033188	8.75	3.1
	S-033447	>20.9	62.7
	Verapamil	N.C.	1.9
BCRP	S-033188	>78.1	51.7
	S-033447	7.10	43.4
	Ko143	N.C.	1.4
a The value represents the concentration corrected with the adhesion ratio. b The % of control was calculated by the following equation: % of control = (P_{app} ratio in the presence of inhibitor – 1) / (P_{app} ratio in the absence of inhibitor – 1) × 100.			
Study Number	S-033188-PF-113-N		
Location in CTD	5.3.2.2-10		

2.6.5.15.4 In Vitro Inhibitor Assessments of S-033447 for Human Transporters

Test Article: S-033447

Test System	HEK293 cells transfected with recombinant human uptake transporter (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 or MATE2-K)
Incubation	At 37°C for 2 minutes for OATP1B1, OATP1B3, OAT3 and OCT2, for 0.5 minutes for OAT1, for 5 minutes for OCT1, MATE1 and MATE2-K.
Concentration (μmol/L)[a]	0.398, 0.789, 1.30, 3.46, 7.98, 11.3, 20.4 for OATP1B1 and OATP1B3, OAT3 and OCT2 0.439, 0.813, 1.07, 3.56, 7.33, 11.0, 20.4 for OAT1, 0.294, 0.686, 1.16, 2.95, 7.65, 9.80, 19.8 for OCT1 0.193, 0.554, 0.822, 2.01, 5.99, 13.0, 22.7 for MATE1 and MATE2-K.
Substrate (Concentration)	[^{3}H] $E_2$17βG (0.05 μmol/L for OATP1B1 and OATP1B3), [^{3}H] PAH (1 μmol/L for OAT1), [^{3}H] ES (0.05 μmol/L for OAT3), [^{14}C] Metformin (10 μmol/L for OCT1, OCT2, MATE1, MATE2-K)
Positive control (Concentration)	Rifampicin (10 μmol/L for OATP1B1 and OATP1B3), Probenecid (100 μmol/L for OAT1 and OAT3), Quinidine (100 μmol/L for OCT1, 300 μmol/L for OCT2), Cimetidine (10 μmol/L for MATE1, 100 μmol/L for MATE2-K)
Sample	Cell lysate
Analyte	Radioactivity
Assay	LSC

Transporters	Inhibitors	IC_{50} (μmol/L)	% of control[b] (% at maximum concentration of inhibitors)
OATP1B1	S-033447	6.81	35.5
	Rifampicin	N.C.	21.8
OATP1B3	S-033447	>20.4	67.6
	Rifampicin	N.C.	9.2
OAT1	S-033447	>20.4	95.6
	Probenecid	N.C.	16.9
OAT3	S-033447	>20.4	84.7
	Probenecid	N.C.	9.0
OCT1	S-033447	6.52	40.1
	Quinidine	N.C.	2.1
OCT2	S-033447	>20.4	73.0
	Quinidine	N.C.	1.6

Transporters	Inhibitors	IC_{50} (μmol/L)	% of control[b] (% at maximum concentration of inhibitors)
MATE1	S-033447	11.2	45.1
	Cimetidine	N.C.	7.8
MATE2-K	S-033447	1.91	17.3
	Cimetidine	N.C.	14.4
Abbreviation: PAH, para-aminohippuric acid. a The value represents the concentration corrected with the adhesion ratio. b The % of control was calculated by the following equation: % of control = (D − C) / (B – A) × 100. A: Cleared volume of control cells in the absence of inhibitor, B: Cleared volume of transporter expressing cells in the absence of inhibitor, C: Cleared volume of control cells in the presence of inhibitor, D: Cleared volume of transporter expressing cells in the presence of inhibitor.			
Study Number	S-033188-PF-113-N		
Location in CTD	5.3.2.2-10		

2.6.5.15.5 In Vitro Inhibitor Assessments of S-033447 for BSEP

Test Article: S-033447

Test System	BSEP expressing vesicles
Incubation	At 37°C for 5 minutes
Concentration (μmol/L)[a]	0, 0.472, 0.824, 2.11, 7.37, 17.2, 45.3, and 78.0
Substrate (Concentration)	[^{3}H] TCA (1 μmol/L)
Positive control (Concentration)	Cyclosporin A (10 μmol/L)
Sample	Vesicles on the glass fiber filter
Analyte	Radioactivity
Assay	LSC

Transporters	Inhibitors	IC_{50} (μmol/L)	% of control[b] (% at maximum concentration of inhibitors)
BSEP	S-033447	>78.0	105.6
	Cyclosporin A	N.C.	13.8

Abbreviation: TCA, taurocholic acid.

a The value represents the concentration corrected with the adhesion ratio.

b The % of control was calculated by the following equation:

% of control = (D − C) / (B – A) × 100.

A: Cleared volume of vesicles in the absence of inhibitor [AMP (+)], B: Cleared volume of vesicles in the absence of inhibitor [ATP (+)]

C: Cleared volume of vesicles in the presence of inhibitor [AMP (+)], D: Cleared volume of vesicles in the presence of inhibitor [ATP (+)]

AMP, adenosine monophosphate; ATP, adenosine triphosphate.

Study Number	S-033188-PF-168-N
Location in CTD	5.3.2.2-11

2.6.5.15.6 In Vitro Investigation for Protein Binding Site of [^{14}C]-S-033447 (Effect of Site-specific Drug)

Test Article: [^{14}C]-S-033447

Test System	4% HSA solution	
Evaluation Method	Equilibrium dialysis method	
Radionuclide	^{14}C	
Specific Activity	2.23 MBq/mg (60.2 μCi/mg)	
Concentration (μg/mL)	0.1	
Site-specific drug (Concentration)	Warfarin for Site I (1, 10, 100 μg/mL), Diazepam for Site II (1, 10, 100 μg/mL), Digoxin for Site III (0.001, 0.01, 0.1 μg/mL)	
Incubation	At 37°C for 24 hours	
Sample	HSA solution	
Analyte	Radioactivity	
Assay	LSC	
Site-specific drug (Concentration)	[^{14}C]-S-033447 (0.1 μg/mL)	
	Unbound fraction (%)	% of control (%)[a]
None	6.17 ± 0.10	100 ± 2
Warfarin (1 μg/mL)	6.08 ± 0.13	98.5 ± 2.2
Warfarin (10 μg/mL)	6.32 ± 0.04	103 ± 1
Warfarin (100 μg/mL)	6.78 ± 0.25	110 ± 4
Diazepam (1 μg/mL)	5.37 ± 1.12	87.1 ± 18.2
Diazepam (10 μg/mL)	5.94 ± 0.84	96.4 ± 13.6
Diazepam (100 μg/mL)	6.58 ± 0.08	107 ± 1
Digoxin (0.001 μg/mL)	6.10 ± 0.04	98.9 ± 0.7
Digoxin (0.01 μg/mL)	6.03 ± 0.06	97.8 ± 1.0
Digoxin (0.1 μg/mL)	6.20 ± 0.20	101 ± 3
Data are expressed as the mean ± S.D. of triplicate assays. a The % of control was calculated by the following equation: % of control = (unbound fraction in the presence of concomitant drug) / (mean unbound fraction in the absence of concomitant drug) × 100.		
Study Number	S-033188-PB-181-N	
Location in CTD	5.3.2.1-02	

2.6.5.15.7 In Vitro Investigation for Protein Binding Site of S-033447 (Effect of S-033447)

Test Article: S-033447

Test System	4% HSA solution	
Evaluation Method	Equilibrium dialysis method	
Concentration (μg/mL)	0.1, 1, 10	
Site-specific drug (Specific Activity)	[^{14}C]-Warfarin: 2168 MBq/mmol (58.6 mCi/mmol), [^{14}C]-Diazepam: 2035 MBq/mmol (55 mCi/mmol) [^{3}H]-Digoxin: 1473 GBq/mmol (39.8 Ci/mmol)	
Site-specific drug (Concentration)	[^{14}C]-Warfarin for Site I (1 μg/mL), [^{14}C]-Diazepam for Site II (1 μg/mL), [^{3}H]-Digoxin for Site III (0.001 μg/mL)	
Incubation	At 37°C for 24 hours	
Sample	HSA solution	
Analyte	Radioactivity	
Assay	LSC	
S-033447 Concentration (μg/mL)	[^{14}C]-Warfarin (1 μg/mL)	
	Unbound fraction (%)	Percent of control (%)
0	1.30 ± 0.02	100 ± 1
0.1	1.29 ± 0.01	98.6 ± 0.8
1	1.29 ± 0.02	98.8 ± 1.2
10	1.30 ± 0.00	99.6 ± 0.3
S-033447 Concentration (μg/mL)	[^{14}C]-Diazepam (1 μg/mL)	
	Unbound fraction (%)	Percent of control (%)
0	1.10 ± 0.01	100 ± 1
0.1	1.07 ± 0.00	96.7 ± 0.4
1	1.08 ± 0.01	98.0 ± 0.9
10	1.08 ± 0.01	98.0 ± 0.5
S-033447 Concentration (μg/mL)	[^{3}H]-Digoxin (0.001 μg/mL)	
	Unbound fraction (%)	Percent of control (%)
0	67.0 ± 0.7	100 ± 1
0.1	66.6 ± 0.9	99.5 ± 1.3
1	66.7 ± 0.5	99.6 ± 0.7
10	66.3 ± 0.7	99.1 ± 1.1
Data are expressed as the mean ± S.D. of triplicate assays.		
Study Number	S-033188-PB-181-N	
Location in CTD	5.3.2.1-02	

2.6.5.16 薬物動態試験： その他

2.6.5.16.1 In Vitro Solubility of S-033188 and S-033447

Test Article: S-033188 and S-033447

Test System		Shake-flask method for solubility	
Assay concentration		1 mg/mL for S-033188 and S-033447	
Incubation		At 37°C for 3 hours and over night	
pH		1.0, 6.8, 7.5	
Sample		Filtrate (using a pre-warmed 0.45 µm nylon syringe filter) of the solution	
Analyte		S-033188 and S-033447	
Assay		LC/MS/MS	
pH (Buffer)		S-033188	S-033447
		Measured Concentrations (mg/mL)	Measured Concentrations (mg/mL)
1.0 (HCl)	3 hours	0.0208	0.0117
	overnight	0.0192	0.0128
6.8 (Phosphate)	3 hours	0.0223	0.00242
	overnight	0.0209	0.00163
7.5 (Phosphate)	3 hours	0.0223	0.00306
	overnight	0.0207	0.00143
Data are expressed as the mean value of triplicate samples.			
Study Number		S-033188-PF-100-R	
Location in CTD		4.2.2.7-01	

2.6.5.16.2 In Vitro Permeability of S-033188 and S-033447

Test Article: S-033188 and S-033447

Test System		Caco-2 cells
Incubation		At 37°C, 15, 30, and 45 minutes for receivers
Concentration		2.80 µmol/L
Internal controls (Concentration)		Minoxidil (10 µmol/L), Atenolol (100 µmol/L)
Sample		Apical side solution, Basolateral side solution
Analyte		S-033188, S-033447, Minoxidil, Atenolol
Assay		LC/MS/MS
		Apical to Basolateral Apparent Permeability ($\times 10^{-6}$ cm/sec)
S-033188		5.09 ± 0.357
Reference	Minoxidil	4.88 ± 0.238[a]
	Atenolol	0.131 ± 0.0178[a]
S-033447		0.809 ± 0.319
Reference	Minoxidil	5.28 ± 0.271[a]
	Atenolol	0.146 ± 0.0128[a]
Data are expressed as the mean ± S.D. of triplicate samples. a: Minoxidil and Atenolol were co-incubated with S-033188 or S-033447 in the same cell-line.		
Study Number		S-033188-PF-100-R
Location in CTD		4.2.2.7-01