2.6.5.7 薬物動態試験：妊娠又は授乳動物における試験

Test Article: [^{14}C]lemborexant

Pregnant Rats **Study Number:** AE-7524-G **Location in CTD:** 4.2.2.3.4

Strain/Gender/Number of Animals	Sprague Dawley/Female/3 (Days 13 and 18 of pregnancy)							
Feeding Condition	Food and water were given freely.							
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution							
Method of Administration / Dose	Oral, using a syringe with oral tube / 10 mg/kg							
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (E-150728)							
Analyte/Assay	Radioactivity/Liquid scintillation counting							
Tissue	**Radioactive Content (% of Dose)**							
	Day 13 of Pregnancy				**Day 18 of Pregnancy**			
	1 h	**4 h**	**8 h**	**24 h**	**1 h**	**4 h**	**8 h**	**24 h**
Cerebrum	0.01±0.00	0.00±0.01	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00
Heart	0.03±0.01	0.02±0.01	0.01±0.00	0.00±0.00	0.04±0.01	0.02±0.00	0.01±0.01	0.00±0.00
Lung	0.03±0.02	0.02±0.01	0.01±0.00	0.00±0.00	0.03±0.01	0.02±0.01	0.01±0.01	0.00±0.00
Liver	3.73±0.46	2.13±0.40	1.36±0.25	0.43±0.07	3.41±0.67	2.14±0.31	1.03±0.20	0.52±0.04
Kidney	0.14±0.04	0.08±0.03	0.05±0.01	0.00±0.00	0.11±0.01	0.08±0.01	0.03±0.01	0.01±0.01
Adrenal gland	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00
Uterus	0.04±0.01	0.03±0.01	0.02±0.01	0.00±0.00	0.05±0.02	0.05±0.02	0.02±0.01	0.00±0.00
Ovary	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01	0.00±0.00	0.00±0.00
Fetus (Whole body)[a]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.00	0.00±0.01	0.00±0.00

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Test Article: [^{14}C]lemborexant

Pregnant Rats **Study Number: AE-7524-G** **Location in CTD: 4.2.2.3.4**

Tissue	Ratio to the Concentration of Radioactivity in Plasma							
	Day 13 of Pregnancy				Day 18 of Pregnancy			
	1 h	4 h	8 h	24 h	1 h	4 h	8 h	24 h
Fetus (Whole body)[b]	0.34±0.10	0.32±0.02	0.28±0.02	0.19±0.04	0.59±0.07	0.60±0.01	0.52±0.09	0.36±0.16

Values represent the mean ±SD of 3 animals.

CTD = Common Technical Document.

a: For the fetus (whole body), percentage distribution per fetus was calculated.

b: Eight fetuses from each of 3 dams were used on the Day 13 of pregnancy. One fetus from each of 3 dams was used on the Day 18 of pregnancy.

Additional Information: These results indicated low placental transfer of the radioactivity in rats after a single oral administration of [^{14}C]lemborexant. Major radioactive components in fetus, plasma from fetus (only in the rats dosed on Day 18 of gestation) and maternal rat were lemborexant and co-elution of M3 and M9, indicating the fetal transfer of M3, M9, and lemborexant. The major metabolic pathways of lemborexant in pregnant rats were oxidation of dimethylpyrimidine moiety or fluorophenyl moiety of lemborexant and subsequent further oxidation, glucuronidation, or glutathione conjugation, which are similar to those in male Sprague Dawley rats (Section 2.6.5.11).

2.6.5.7 薬物動態試験：妊娠又は授乳動物における試験（続き）

Test Article: **[^{14}C]lemborexant**

Lactating Rats **Study Number: AE-7524-G** **Location in CTD: 4.2.2.3.4**

Strain/Gender/Number of Animals	Sprague Dawley/Female/3 (Postnatal day 10)	
Feeding Condition	Food and water were given freely.	
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution	
Method of Administration / Dose	Oral, using a syringe with oral tube / 10 mg/kg	
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (E-150728)	
Analyte/Assay	Radioactivity/Liquid scintillation counting	
Time (h)	**Concentration of Radioactivity (ng eq./mL)**	
	Plasma	**Milk**
0.25	734±186	4240±700
1	485±201	2780±1280
2	360±200	1790±1150
4	210±58	798±44
8	250±58	907±713
24	106±10	124±44
48	27.0±12.3	34.6±7.5
72	14.6±10.0	16.7±7.0

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Test Article: [^{14}C]lemborexant

Lactating Rats **Study Number: AE-7524-G** **Location in CTD: 4.2.2.3.4**

Pharmacokinetic Parameters	Plasma	Milk	Milk/Plasma Ratio
C_{max} (ng eq./mL)	734±186	4240±700	5.93±1.22
t_{max} (h)	0.25 (0.25 – 0.25)	0.25 (0.25 – 0.25)	1.00 ± 0.00
$AUC_{(0\text{-}inf)}$ (ng eq.·h/mL)	7830±1520	22,700±5500	3.06±1.33
$t_{1/2}$ (h)	16.9±6.5	17.3±5.1	1.06±0.20

Concentration of radioactivity was converted to the equivalent of lemborexant (ng eq./mL). Data except t_{max} represent the mean ±SD of 3 animals and t_{max} represents the median values and range (in parentheses) of 3 animals.

$AUC_{(0\text{-}inf)}$ = AUC from zero time extrapolated to infinite time, CTD = Common Technical Document, eq. = equivalent, t_{max} = time at which the highest drug concentration occurs, $t_{1/2}$ = terminal elimination phase half-life.

Additional Information: The terminal elimination phase half-life of the radioactivity in milk was comparable to that in plasma, suggesting no tendency for the radioactivity to remain in milk. The major radioactivity components detected in milk were lemborexant and co-elution of M3 and M9, indicating the excretion of M3, M9, and lemborexant into the milk. The major metabolic pathways of lemborexant in lactating rats were oxidation of dimethylpyrimidine moiety or fluorophenyl moiety of lemborexant and subsequent further oxidation, glucuronidation, or glutathione conjugation, which are similar to those in male Sprague Dawley rats (Section 2.6.5.11).

2.6.5.8 薬物動態試験：その他の分布試験

Test Article: lemborexant, M4, M9, M10

Blood to Plasma Concentration Ratio of Lemborexant and Its Metabolites　　Study Number: 16-560　　Location in CTD: 5.3.2.3.1

Study System	in vitro			
Test System	Blood			
Method	Incubation for 30 minutes at 37 °C			
Assay / Analyte	LC-MS/MS / Lemborexant and its metabolites, M4, M9, and M10			
Species	**Compound**	**R_B**		
		Concentration (ng/mL)		
		100	**300**	**1000**
Mouse	Lemborexant	0.824±0.026	0.736±0.004	0.766±0.017
Rat		0.964±0.003	0.941±0.008	0.940±0.025
Monkey		0.879±0.028	0.859±0.006	0.816±0.012
Human		0.643±0.004	0.656±0.024	0.610±0.007
Mouse	M4	0.798±0.003	0.754±0.017	0.776±0.011
Rat		1.06±0.03	1.02±0.04	1.03±0.03
Monkey		0.759±0.009	0.731±0.011	0.745±0.007
Human		0.705±0.018	0.650±0.043	0.697±0.029
Mouse	M9	0.976±0.018	0.900±0.010	0.961±0.033
Rat		1.13±0.04	1.20±0.03	1.17±0.03
Monkey		0.945±0.016	0.886±0.029	0.885±0.009
Human		0.766±0.020	0.742±0.014	0.738±0.023

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Test Article: lemborexant, M4, M9, M10

Blood to Plasma Concentration Ratio of Lemborexant and Its Metabolites **Study Number: 16-560** **Location in CTD: 5.3.2.3.1**

Species	Compound	R_B		
		Concentration (ng/mL)		
		100	300	1000
Mouse	M10	0.886±0.038	0.842±0.012	0.890±0.015
Rat		0.958±0.007	0.930±0.014	0.955±0.018
Monkey		0.765±0.008	0.689±0.003	0.700±0.015
Human		0.616±0.014	0.567±0.006	0.562±0.025

Each value represents the mean ±SEM (n=3). Pooled blank blood was used in mice, and individual blank blood was used in rats, monkeys, and humans.

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, R_B = blood to plasma concentration ratio.

Additional Information: No apparent concentration dependency of R_B values for lemborexant and its metabolites was observed in all species examined.

2.6.5.9 薬物動態試験：代謝：in vivo

Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats **Study Number: AE-7077-G** **Location in CTD: 4.2.2.4.1**

Strain/Gender/Number of Animals	Sprague Dawley/Male/3
Feeding Condition	Fasted
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution
Method of Administration / Dose	Oral / 10 mg/kg
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (K0110-030)
Assay / Analyte	LC-MS(MS^n) / Metabolites derived from lemborexant

Lemborexant and Its Metabolites in Plasma at 1, 4, and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	8 h	1 h	4 h	8 h
mR1	–	ND	6.0±0.9	ND	ND	6.1±1.1	ND
mR4	–	ND	ND	0.6±0.3	ND	ND	2.3±1.2
mR9	–	1.5±0.3	ND	ND	6.1±0.6	ND	ND
mR11	–	ND	1.8±0.3	3.0±1.5	ND	1.8±0.3	11.9±6.0
mR13	–	ND	2.8±0.7	9.7±0.9	ND	2.8±0.6	37.8±3.3
mR17	–	ND	ND	0.9±0.1	ND	ND	3.6±0.3
mR19	–	2.7±0.4	9.7±1.7	2.7±0.3	11.2±1.1	9.6±1.3	10.6±1.4
mR25	–	ND	ND	2.7±0.6	ND	ND	10.1±2.0
mR29	–	ND	ND	43.8±6.7	ND	ND	173.3±37.2
mR30	–	1.4±0.2	11.9±3.7	ND	5.8±0.6	11.6±3.1	ND
mR32	–	ND	10.9±1.0	3.0±0.6	ND	11.1±1.7	11.4±1.8
mR35	–	ND	ND	0.6±0.3	ND	ND	2.3±1.2
mR38	–	ND	ND	3.3±1.7	ND	ND	12.1±6.1
mR40	–	ND	ND	1.9 ± 1.0	ND	ND	7.0 ± 3.6
mR47b	347	14.4±1.1	3.5±0.6	ND	59.7±2.9	3.6±0.7	ND

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Plasma at 1, 4, and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	8 h	1 h	4 h	8 h
mR57b (M11)	317	1.9±0.8	ND	ND	7.6±2.7	ND	ND
mR57c-1 (M14)	443	4.3±0.5	1.9±0.1	1.4±0.8	18.4±4.2	1.9±0.1	5.2±2.8
mR57c-2	443	1.5±1.2	0.5±0.4	ND	7.5±6.4	0.5±0.4	ND
mR63	–	ND	ND	0.8±0.4	ND	ND	2.8±1.4
mR65a (M12)	441	8.4±1.0	ND	ND	36.3±8.7	ND	ND
mR65b (M4)	427	1.1±0.4	ND	ND	5.1±2.3	ND	ND
mR66-1 (M3)	427	2.4±0.6	ND	0.6±0.3	10.6±3.8	ND	2.1±1.2
mR66-2 (M9)	427	34.9±3.9	7.4±3.4	0.6±0.3	145.6±15.5	7.9±4.0	2.2±1.2
mR72a (Lemborexant)	411	4.5±0.4	5.2±3.6	ND	19.6±4.3	5.6±4.2	ND
Others		12.6±1.7	17.1±1.1	13.3±1.4	–	–	–
Recovery (%)		91.6±0.4	78.6±2.7	88.9±1.3	–	–	–
Total Concentration[a]		–	–	–	422.4±49.4	101.0±5.6	389.2±21.8

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: Concentration of total radioactivity in plasma (Study No.14-074).

Lemborexant and Its Metabolites in Blood Cells at 1, 4, and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	8 h	1 h	4 h	8 h
mR13	–	ND	ND	2.1±0.4	ND	ND	5.9±0.5
mR25	–	ND	ND	5.1±1.5	ND	ND	18.0±8.2
mR29	–	ND	ND	14.5±4.8	ND	ND	38.4±4.3
mR38	–	ND	ND	5.6±1.6	ND	ND	18.3±6.7
mR57c-1 (M14)	443	6.6±1.7	2.2±1.1	7.3±1.1	83.4±24.1	3.1±1.6	22.9±6.3
mR63	–	ND	ND	2.0±0.6	ND	ND	7.1±3.8

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Blood Cells at 1, 4, and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	8 h	1 h	4 h	8 h
mR66-2 (M9)	427	64.8±2.4	41.8±2.5	6.1±0.5	817.4±63.9	53.6±6.7	19.3±6.2
mR72a (Lemborexant)	411	ND	2.2±0.6	0.5±0.3	ND	2.7±0.4	1.9±0.9
Others		5.8±0.1	21.1±2.2	19.0±1.9	–	–	–
Recovery (%)		77.2±1.2	67.3±2.3	62.3±1.9	–	–	–
Total concentration[a]		–	–	–	1259.4 ± 74.2	128.6 ± 14.4	309.5 ± 82.9

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: Concentration of total radioactivity in blood cells (Study No.14-074).

Lemborexant and Its Metabolites in Cerebrum at 1 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	Concentration of Radioactivity (ng eq./g)
mR38	–	1.9±0.1	1.6±0.1
mR46	–	3.0±0.3	2.4±0.3
mR55	–	1.4±0.5	1.1±0.4
mR62	–	1.2±0.1	1.0±0.1
mR63	–	0.7±0.4	0.6±0.3
mR66-2 (M9)	427	30.0±4.2	24.2±2.8
mR71	–	28.3±2.2	23.0±2.2
mR72a (Lemborexant)	411	9.8±1.4	8.1 ± 1.6
Others		23.0±2.9	–
Recovery (%)		99.3±0.1	–
Total concentration[a]		–	81.5±4.9

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: Concentration of total radioactivity in cerebrum (Study No.14-074).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Liver at 1 and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample		Concentration of Radioactivity (ng eq./g)	
		1 h	8 h	1 h	8 h
mR3	–	1.3±0.1	ND	198.9±38.0	ND
mR4	–	ND	1.7±0.3	ND	81.9±10.8
mR6	–	0.7±0.1	ND	109.0±30.0	ND
mR8	750	3.6±0.3	ND	550.0±115.3	ND
mR9	–	ND	3.2±1.3	ND	154.2±63.3
mR11	–	ND	6.9±0.5	ND	325.2±19.4
mR12	730	1.8±0.4	ND	286.8±96.9	ND
mR16b	734	1.7±0.2	2.1±0.3	265.2±68.7	99.3±13.4
mR21	714	1.3±0.2	ND	205.1±55.4	ND
mR23	–	ND	2.8±0.4	ND	129.2±14.1
mR28	–	ND	8.8±1.4	ND	409.0±38.0
mR30	–	1.4±0.2	2.1±0.4	211.7±39.7	103.7±25.0
mR32	–	ND	2.4±0.2	ND	117.2 ± 17.6
mR37	539	1.0±0.2	1.5±0.5	148.8±37.3	70.1±25.1
mR40	–	ND	2.1±0.5	ND	104.9±32.4
mR42 (M18)	603	0.5±0.1	1.5±0.8	86.8±33.5	72.7±36.6
mR45	457, 459, 443	1.4±0.1[a]	3.1±0.5[b]	208.6±39.8[a]	145.3±23.6[b]
mR47[c]	333, 347, 457	1.4±0.2	ND	217.2±48.4	ND
mR48a (M20)	603	0.5±0.3[d]	0.4±0.2	81.8±61.7[d]	18.2±9.1
mR48b	617		0.1±0.1		6.7±3.4
mR49	459	1.0±0.2	ND	164.5±50.7	ND
mR52	617	1.4±0.2	ND	213.7±56.6	ND
mR53 (M21)	603	0.8±0.1	ND	121.9±25.4	ND
mR56	457	0.7±0.1	1.1±0.1	109.4±28.4	50.5±2.1

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Liver at 1 and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample		Concentration of Radioactivity (ng eq./g)	
		1 h	8 h	1 h	8 h
mR57b (M11)	317	0.8±0.1	ND	123.4±26.5	ND
mR57c-1 (M14)	443	1.3±0.2	ND	197.6±32.3	ND
mR57c-2	443	3.4±2.2	ND	577.6±430.7	ND
mR58 (M1)	427	0.3±0.1	0.5±0.2	53.3±16.2	23.1±11.7
mR61	443	2.0±0.3	1.6±0.8	311.2±76.2	79.2±43.5
mR63	–	ND	1.9±0.4	ND	87.9±18.8
mR65a (M12)	441	36.8±2.0	ND	5473.2±589.1	ND
mR66-1 (M3)	427	0.2±0.0	0.4±0.2	35.6±7.9	17.8±10.8
mR66-2 (M9)	427	4.8±2.2	0.7±0.1	630.3±271.0	34.4±6.1
mR69 (M8)	427	4.2±1.1	6.5±1.9	656.5±215.9	320.4±115.9
mR70	457	1.0±0.1	ND	153.8±18.5	ND
mR72a (Lemborexant)	411	2.7±0.2	ND	417.9±85.1	ND
mR72b	455	0.3±0.1	ND	53.3±16.2	ND
Others		8.9±0.6	11.5±1.1	–	–
Recovery (%)		87.4±0.7	62.7±1.1	–	–
Total Concentration[e]		–	–	15,100.2±2304.9	4761.8±317.4

Values represent the mean ±SEM of 3 animals.　–: Not available.

a: mR45 was a mixture of mR45a, mR45b-1, mR45b-2, mR45b-3, and mR45c at 1 hour.

b: mR45 was a mixture of mR45b-1, mR45b-2, mR45b-3, and mR45c at 8 hours.

c: mR47 was a mixture of mR47a, mR47b, and mR47c.

d: These values were for a mixture of mR48a (M20) and m48b.

e: Concentration of total radioactivity in liver (Study No.14-074).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Kidney at 1 and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats					
Metabolite	**[M+H]$^+$ (*m/z*)**	**% in Sample**		**Concentration of Radioactivity (ng eq./g)**	
		1 h	**8 h**	**1 h**	**8 h**
mR1	–	8.2±2.6	ND	118.2±32.0	ND
mR4	–	ND	2.5±0.2	ND	23.0±1.4
mR6	–	3.3±0.8	ND	50.0±12.3	ND
mR9	–	2.0±1.3	2.9±1.4	29.4±16.6	26.4±13.2
mR11	–	ND	1.5±0.4	ND	13.8±3.7
mR13	–	1.0±0.5	12.0±2.6	16.0±8.0	112.5±26.0
mR15	–	ND	1.3 ± 0.8	ND	12.4 ± 7.2
mR19	–	ND	1.4±0.1	ND	13.4±1.2
mR27	–	ND	5.1±1.0	ND	47.5±8.1
mR29	–	ND	38.6±4.9	ND	358.9±39.8
mR30	–	ND	3.4±1.7	ND	31.1±15.7
mR35	–	ND	1.1±0.6	ND	10.3±5.8
mR40	–	ND	2.0±0.4	ND	18.8±4.1
mR47b	347	4.9±1.3	2.1±1.1	76.7±28.2	19.9±10.9
mR57b (M11)	317	2.6±1.1	ND	37.7±13.6	ND
mR57c-1 (M14)	443	3.3±1.0	0.6±0.4	52.2±20.2	6.1±3.6
mR57c-2	443	4.3±2.3	ND	70.6±42.6	ND
mR65a (M12)	441	17.3±0.4	ND	258.4±28.8	ND
mR66-1 (M3)	427	1.2±0.1	0.2±0.1	17.2±1.2	1.9±1.0
mR66-2 (M9)	427	22.2±4.3	0.9±0.7	327.5±58.2	9.0±6.3

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Kidney at 1 and 8 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample		Concentration of Radioactivity (ng eq./g)	
		1 h	8 h	1 h	8 h
mR67	–	ND	1.1±0.6	ND	10.9±5.9
mR72a (Lemborexant)	411	5.3±0.9	ND	80.5±18.9	ND
Others		21.5±2.3	18.6±2.1	–	–
Recovery (%)		97.1±0.1	95.4±0.3	–	–
Total Concentration[a]		–	–	1492.4±158.0	934.2±28.1

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: Concentration of total radioactivity in kidney (Study No.14-074).

Lemborexant and Its Metabolites in Bile at 0 to 12 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mR1	–	1.1±0.2	1.1±0.2
mR3	–	1.4±0.3	1.4±0.3
mR4	–	1.4±0.1	1.4±0.1
mR7	621	1.2±0.2	1.2±0.2
mR8	750	4.4±0.6	4.4±0.5
mR10[a]	601, 734	2.4±0.4	2.4±0.5
mR12	730	2.6±0.5	2.5±0.4
mR16[b]	605, 734	20.7±5.3	20.5±5.3
mR18	585	3.2±0.9	3.1±0.9
mR21	714	7.3±1.8	7.2±1.8

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Bile at 0 to 12 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mR22	619	0.6±0.3	0.6±0.3
mR33[c]	603, 619	5.1±1.0	5.1±1.0
mR42 (M18)	603	3.7±0.8	3.7±0.8
mR44	619	0.6±0.2	0.6±0.2
mR48a (M20)	603	2.7±0.7	2.7±0.7
mR48b	617	0.5±0.0	0.5±0.0
mR53 (M21)	603	2.7±1.4	2.7±1.4
mR65a (M12)	441	30.1±10.2	29.8±10.1
mR70	457	0.4±0.2	0.4±0.2
mR72b	455	1.4±0.5	1.3±0.5
Others		6.6±0.9	–
Recovery (%)		100.0±0.0	–
Excretion (%)[d]		–	99.0±1.1

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: mR10 was a mixture of mR10a and mR10b.
b: mR16 was a mixture of mR16a and mR16b.
c: mR33 was a mixture of mR33a (M16), mR33b-1, and mR33b-2.
d: Total radioactivity excretion in bile (Study No.14-074).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Urine at 0 to 24 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mR2	539	2.2±0.6	0.1±0.0
mR5	363	5.2±0.5	0.3±0.0
mR11	–	3.9±0.5	0.2±0.1
mR14	495	14.7±1.2	0.8±0.1
mR20	–	2.9±0.9	0.2±0.0
mR26[a]	445, 523	12.8±1.2	0.7±0.1
mR28	–	1.9±0.1	0.1±0.0
mR31[b]	427, 523	42.0±1.2	2.3±0.3
mR47[c]	333, 347, 443	2.8±0.4	0.2±0.0
mR57c-1 (M14)	443	1.2±0.6	0.1±0.1
Others		10.4±0.6	–
Recovery (%)		100.0±0.0	–
Excretion (%)[d]		–	5.6±0.7

Values represent the mean ±SEM of 3 animals.　–: Not available.
a: mR26 was a mixture of mR26a and mR26b.
b: mR31 was a mixture of mR31a and mR31b.
c: mR47 was a mixture of mR47a, mR47b, and mR47d (M13).
d: Total radioactivity excretion in urine (Study No.14-074).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats　　Study Number: AE-7077-G　　Location in CTD: 4.2.2.4.1

Lemborexant and Its Metabolites in Feces at 0 to 24 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats			
Metabolite	**$[M+H]^+$ (*m/z*)**	**% in Sample**	**% of Dose**
mR24	487	1.2±0.2	1.0±0.2
mR29	–	0.5±0.2	0.4±0.2
mR32	–	1.0±0.2	0.9±0.2
mR34	471	2.1±0.5	1.9±0.4
mR36	471	1.4±0.4	1.2±0.3
mR41[a]	425	1.8±0.4	1.6±0.3
mR43	–	1.0±0.5	0.9±0.4
mR45c	443	2.7±0.3	2.4±0.2
mR47a	333	0.7±0.4	0.6±0.3
mR51[b]	409, 487	1.1±0.6	1.0±0.6
mR54	471	1.3±0.1	1.2±0.0
mR57[c]	443, 317	0.6±0.1	0.6±0.1
mR58 (M1)	427	3.0±0.7	2.7±0.5
mR59[d]	471	1.4±0.1	1.2±0.1
mR61	443	1.2±0.1	1.1±0.1
mR64	455	2.9±0.5	2.6±0.4

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Cerebrum, Liver, Kidney, Bile, Urine, or Feces of Rats **Study Number: AE-7077-G** **Location in CTD: 4.2.2.4.1**

Lemborexant and Its Metabolites in Feces at 0 to 24 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg/kg in Male Rats

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mR65a (M12)	441	35.5±6.8	31.8±6.5
mR66[e] (M3 + M9)	427	2.0±0.6	1.7±0.5
mR68	455	1.1±0.3	0.9±0.3
mR69 (M8)	427	2.3±0.7	2.0±0.6
Others		12.6±1.4	–
Recovery (%)		77.2±1.1	–
Excretion (%)[f]		–	88.8±1.8

Values represent the mean ±SEM of 3 animals. –: Not available.
a: mR41 was a mixture of mR41-1 and mR41-2.
b: mR51 was a mixture of mR51a and mR51b.
c: mR57 was a mixture of mR57a-1, mR57a-2, and mR57b (M11).
d: mR59 was a mixture of mR59-1 and mR59-2.
e: mR66 was a mixture of mR66-1 (M3) and mR66-2 (M9).
f: Total radioactivity excretion in feces (Study No.14-074).

The metabolites were assigned serial numbers with the prefix "mR". The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies.
CTD = Common Technical Document, eq. = equivalent, LC-MS(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of protonated molecular ion, ND = not detected.
Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Strain/Gender/Number of Animals	Cynomolgus/Male/3
Feeding Condition	Fasted
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution
Method of Administration / Dose	Oral / 3 mg/kg
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (K0110-030)
Assay / Analyte	LC-MS(MS^{n}) / Metabolites derived from lemborexant

Lemborexant and Its Metabolites in Plasma at 1, 4, and 6 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	6 h	1 h	4 h	6 h
mM2	–	ND	0.6±0.3	ND	ND	2.8±2.1	ND
mM3	–	ND	0.3±0.2	ND	ND	1.2±0.8	ND
mM7	–	ND	0.7±0.4	ND	ND	2.3±1.2	ND
mM8	–	ND	1.6±0.7	2.8±0.5	ND	3.6±0.4	4.9±0.4
mM9	–	2.2±1.1	1.8±0.6	ND	10.2±6.3	4.9±2.1	ND
mM10	–	ND	0.7±0.3	1.1±0.6	ND	2.5±1.5	2.6±1.6
mM11	–	ND	ND	1.6±0.3	ND	ND	2.9±0.4
mM12[a]	619	ND	1.6±0.1	2.4±0.8	ND	4.7±1.6	4.1±0.4
mM13	459	ND	0.8±0.4	ND	ND	1.4±0.7	ND
mM14	–	ND	0.6±0.3	1.2±0.6	ND	2.3±1.7	2.3±1.5
mM17[b]	427, 619, 523	ND	1.7±0.6	3.0±0.5	ND	3.6±0.1	5.5±0.6
mM19a (M16)	603	1.8±1.0	9.2±2.1	15.8±1.3	7.5±3.8	28.7±14.9	29.8±5.0
mM22	–	ND	0.3±0.2	0.7±0.3	ND	0.8±0.5	1.6±0.8
mM25	619	ND	2.4±0.3	4.1±0.9	ND	6.9±2.8	7.1±0.3
mM27 (M18)	603	ND	ND	1.6±0.5	ND	ND	3.4±1.8

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Plasma at 1, 4, and 6 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys							
Metabolite	**[M+H]$^+$ (*m/z*)**	**% in Sample**			**Concentration of Radioactivity (ng eq./mL)**		
		1 h	**4 h**	**6 h**	**1 h**	**4 h**	**6 h**
mM28b	459	1.6±0.8	3.2±0.8	ND	9.8±4.9	12.0±7.6	ND
mM29	–	ND	0.6±0.3	2.3±0.6	ND	1.1±0.5	4.0±1.1
mM31	–	ND	0.3±0.2	ND	ND	1.4±1.0	ND
mM34a (M13)	443	11.4±0.9	6.9±0.8	4.5±1.1	59.0±12.5	22.6±10.9	9.9±4.6
mM35 (M20)	603	1.5±1.0	2.6±0.5	4.1±0.4	6.0±3.2	7.8±3.8	7.8±1.3
mM36	–	ND	0.3±0.2	0.3±0.1	ND	1.3±0.8	0.6±0.3
mM38 (M21)	603	ND	0.7±0.5	1.2±0.2	ND	3.7±3.3	2.4±0.9
mM40b (M11)	317	1.1±0.1	2.2±0.2	2.9±0.2	5.4±0.8	7.0±3.3	5.8±1.5
mM40c (M14)	443	13.5±0.6	7.4±1.5	3.9±0.8	71.6±16.3	22.8±9.4	8.3±3.0
mM42	–	ND	0.5±0.3	ND	ND	2.3±1.7	ND
mM43 (M2)	427	0.6±0.4	0.6±0.3	ND	3.3±2.5	2.5±1.7	ND
mM45b-1 (M4)	427	21.4±1.5	9.9±1.8	4.9±1.1	114.5±29.3	34.4±18.7	10.5±4.4
mM45b-2 (M7)	427	1.9±0.1	0.6±0.4	0.6±0.3	9.7±1.6	2.9±2.3	1.3±0.7
mM46	427	3.8±1.5[c]	2.6±0.2[c]	3.6±0.9[d]	22.7±11.3[c]	8.2±3.7[c]	6.7±1.9[d]
mM47 (M10)	427	1.7±0.2	2.9±0.7	1.4±0.8	9.3±2.6	10.4±6.2	3.4±2.1

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Plasma at 1, 4, and 6 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	6 h	1 h	4 h	6 h
mM50	–	ND	0.8±0.4	ND	ND	3.6±2.5	ND
mM53 (Lemborexant)	411	25.4±0.7	15.9±0.7	9.7±0.6	130.7±23.4	50.4±23.6	19.4±5.7
Others		9.8±0.8	12.8±3.5	16.3±0.6	–	–	–
Recovery (%)		97.7±0.1	93.1±1.7	89.8±1.4	–	–	–
Total Concentration[e]		–	–	–	521.3±105.1	306.9±132.0	195.6±46.9

Values represent the mean ±SEM of 3 animals. –: Not available.
a: mM12 was a mixture of mM12-1 (M23), mM12-2, and mM12-3.
b: mM17 was a mixture of mM17a, mM17b-1, mM17b-2, and mM17c.
c: mM46 was a mixture of mM46-1 (M3) and mM46-2 (M9).
d: mM46-1 (M3) only. mM46-2 (M9) was not detected at 6 hours postdose.
e: Concentration of total radioactivity in plasma (Study No.14-075).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Blood Cells at 1, 4, and 6 Hours After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample			Concentration of Radioactivity (ng eq./mL)		
		1 h	4 h	6 h	1 h	4 h	6 h
mM19a (M16)	603	ND	ND	2.7±1.6	ND	ND	7.0±3.8
mM28b	459	1.3±0.7	ND	ND	4.4±2.2	ND	ND
mM34a (M13)	443	20.7±2.5	10.3±3.1	ND	60.8±10.5	34.7±9.4	ND
mM40c (M14)	443	20.4±2.2	13.0±7.3	6.6±3.8	65.0±19.9	42.8±22.8	17.6±9.2
mM43 (M2)	427	0.9±0.5	ND	ND	1.9±1.0	ND	ND
mM45b-1 (M4)	427	21.7±2.4	8.1±4.6	ND	71.2±27.3	26.7±14.2	ND
mM46-1 (M3)	427	3.1±0.8	ND	ND	10.7±4.5	ND	ND
mM47 (M10)	427	1.2 ± 0.5	ND	ND	2.9 ± 0.8	ND	ND
mM53 (Lemborexant)	411	23.6±0.3	10.9±3.7	4.7±2.5	74.5±21.8	36.6±11.3	12.8±6.4
Others		4.5±0.7	21.2±4.6	35.3±3.2	–	–	–
Recovery (%)		97.3±0.2	63.5±17.6	49.4±6.8	–	–	–
Total Concentration[a]		–	–	–	313.0±87.6	340.8±18.5	293.6±27.2

Values represent the mean ±SEM of 3 animals. –: Not available.
a: Concentration of total radioactivity in blood cells (Study No.14-075).

Lemborexant and Its Metabolites in Urine at 0 to 96 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mM6	495	1.0±0.6	0.2±0.1
mM9	–	4.2±0.6	0.9±0.2
mM10	–	0.8±0.4	0.2±0.1
mM12[a]	619	4.8±0.4	1.0±0.1
mM13	459	2.3±0.4	0.5±0.1
mM17[b]	427, 619, 523	5.0±0.3	1.0±0.1
mM19[c]	603, 619, 459	16.1±1.4	3.4±0.2

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Urine at 0 to 96 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mM24 (M17)	603	2.0±0.5	0.4±0.1
mM25	619	2.5±0.1	0.5±0.0
mM27 (M18)	603	12.8±2.1	2.7±0.6
mM28[d]	507, 459, 619	5.6±0.2	1.2±0.1
mM34a (M13)	443	11.2±1.0	2.4±0.1
mM34b	603	0.8±0.3	0.2±0.1
mM35 (M20)	603	7.4±0.7	1.6±0.2
mM38 (M21)	603	1.9±0.7	0.4±0.2
mM40[e]	317, 443	1.6±0.5	0.3±0.1
mM45a (M12)	441	1.1±0.4	0.2±0.1
mM51	457	3.0±1.1	0.7±0.2
Others		15.4±0.7	–
Recovery (%)		99.7±0.2	–
Excretion (%)[f]		–	21.1±1.1

Values represent the mean ±SEM of 3 animals. –: Not available.
a: mM12 was a mixture of mM12-1 (M23), mM12-2, and mM12-3.
b: mM17 was a mixture of mM17a, mM17b-1, mM17b-2, and mM17c.
c: mM19 was a mixture of mM19a (M16), mM19b-1, mM19b-2, and mM19c.
d: mM28 was a mixture of mM28a, mM28b, and mM28c (M24).
e: mM40 was a mixture of mM40b (M11) and mM40c (M14).
f: Total radioactivity excretion in urine (Study No.14-075).

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Feces at 0 to 96 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys			
Metabolite	**$[M+H]^+$ (*m/z*)**	**% in Sample**	**% of Dose**
mM16	523	0.7±0.0	0.5±0.1
mM18	–	0.7±0.1	0.5±0.1
mM20	523	0.5±0.1	0.4±0.0
mM21[a]	425, 459	1.0±0.1	0.7±0.1
mM23	459	0.9±0.1	0.6±0.1
mM26	425	1.4±0.1	1.0±0.0
mM28a	507	2.5±0.3	1.7±0.2
mM30	425	0.5±0.1	0.3±0.0
mM32[b]	443, 459	2.9±0.0	2.0±0.1
mM33	459	1.1±0.1	0.7±0.1
mM37a	507	3.4±0.7	2.3±0.5
mM37b	409	0.3±0.0	0.2±0.0
mM39	457	1.6±0.2	1.1±0.1
mM40[c]	443, 317	3.2±0.3	2.2±0.3
mM41[d]	427, 443	1.3±0.2	0.8±0.1
mM44	441	1.6±0.1	1.0±0.0
mM45[e]	441, 427	26.2±2.6	17.7±1.7
mM46-1(M3)	427	1.6±0.5	1.1±0.3
mM46-2 (M9)	427	4.0±0.5	2.8±0.4
mM48	455	0.8±0.1	0.5±0.1
mM49 (M8)	427	1.1±0.0	0.8±0.1

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Blood Cells, Urine, or Feces of Monkeys **Study Number: AE-7078-G** **Location in CTD: 4.2.2.4.2**

Lemborexant and Its Metabolites in Feces at 0 to 96 Hour After a Single Oral Administration of [^{14}C]Lemborexant at 3 mg/kg in Male Monkeys

Metabolite	$[M+H]^+$ (*m/z*)	% in Sample	% of Dose
mM52 (M5)	427	0.2±0.0	0.1±0.0
mM54	–	0.2±0.0	0.1±0.0
Others		7.1±0.5	–
Recovery (%)		64.9±1.4	–
Excretion (%)[f]		–	67.8±3.6

Values represent the mean ±SEM of 3 animals. –: Not available.
a: mM21 was a mixture of mM21a and mM21b.
b: mM32 was a mixture of mM32a-1, mM32a-2, and mM32b.
c: mM40 was a mixture of mM40a and mM40b (M11).
d: mM41 was a mixture of mM41a (M1), mM41b-1, mM41b-2, and mM41b-3.
e: mM45 was a mixture of mM45a (M12) and mM45b-2 (M7).
f: Total radioactivity excretion in feces (Study No.14-075).

The metabolites were assigned serial numbers with the prefix "mM". The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies.
CTD = Common Technical Document, eq. = equivalent, LC-MS(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of protonated molecular ion, ND = not detected.

Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Urine, or Feces of Humans **Study Number: AE-7433-G** **Location in CTD: 5.3.2.2.11**

Race	White, Black, African American, Other Asian, or Others	
Gender/Number of Subjects	Male/8	
Feeding Condition	Fasted	
Vehicle / Formulation	Water / Capsule	
Method of Administration / Dose	Oral / 10 mg	
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / 4.02 mCi/mmol (60308-14-001)	
Assay / Analyte	HPLC with liquid scintillation counting or accelerator mass spectrometry, or LC-MS(MS^{n}) / Metabolites derived from [^{14}C]lemborexant	
Lemborexant and Its Metabolites in Plasma After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg in Healthy Male Subjects		
Metabolite	**$[M+H]^+$ (*m/z*)**	**% of Total Drug Related Exposure**
mH1	–	1.1
mH2	–	1.2
mH4	–	1.6
mH8	445	2.8
mH12	–	3.2
mH13	–	2.2
mH17b-1	619	2.0
mH19 (M18)	603	6.0
mH24b (M13)	443	1.6
mH25 (M20)	603	1.5
mH26	–	1.4
mH30[a] (M11 + M14)	317, 443	3.0
mH32 (M15)	443	2.6
mH36a (M12)	441	3.1

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Urine, or Feces of Humans　　**Study Number: AE-7433-G**　　**Location in CTD: 5.3.2.2.11**

Lemborexant and Its Metabolites in Plasma After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg in Healthy Male Subjects

Metabolite	$[M+H]^+$ (*m/z*)	% of Total Drug Related Exposure
mH36b (M4)	427	6.3
mH38-2 (M9)	427	6.6
mH40 (M10)	427	12.5
mH46a (Lemborexant)	411	26.5
Others		6.2
Recovery (%)[b]		91.4

Each value represents % of total drug related exposure which was determined using a single zero time to 96 hours AUC pooled plasma sample. Data were obtained from the pooled sample of 8 subjects. –: Not available

a: mH30 was a mixture of mH30a (M11) and mH30b (M14).

b: Recovery of total radioactivity from plasma.

Lemborexant and Its Metabolites in Urine and Feces After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg in Healthy Male Subjects

Metabolite	$[M+H]^+$ (*m/z*)	% of Dose		
		Urine	Feces	Total[a]
mH5	–	0.5±0.4	ND	0.5±0.4
mH8	445	0.7±0.4	ND	0.7±0.4
mH9 (M23)	619	0.5±0.4	ND	0.5±0.4
mH12	–	0.2±0.3	ND	0.2±0.3
mH14[b]	603, 619, 493	1.7±0.4	ND	1.7±0.4
mH15	–	ND	0.3±0.4	0.3±0.4
mH17[c]	603, 619	0.6±0.3	ND	0.6±0.3
mH19 (M18)	603	11.0±2.5	ND	11.0±2.5
mH21 (M24)	619	0.9±0.2	ND	0.9±0.2
mH22[d]	507, 457	ND	0.8±0.5	0.8±0.5
mH23[e]	443	ND	1.7±0.4	1.7±0.4
mH24[f]	333, 443	0.9±0.2	ND	0.9±0.2
mH25 (M20)	603	0.5±0.4	ND	0.5±0.4

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Test Article: [^{14}C]lemborexant

Metabolites in Plasma, Urine, or Feces of Humans **Study Number:** AE-7433-G **Location in CTD:** 5.3.2.2.11

Lemborexant and Its Metabolites in Urine and Feces After a Single Oral Administration of [^{14}C]Lemborexant at 10 mg in Healthy Male Subjects

Metabolite	$[M+H]^+$ (*m/z*)	% of Dose		
		Urine	Feces	Total[a]
mH27	507	ND	1.1±0.4	1.1±0.4
mH28 (M21)	603	1.4±0.7	ND	1.4±0.7
mH30[g] (M11 + M14)	317, 443	0.8±0.3	ND	0.8±0.3
mH31[h]	427, 443	ND	2.5±0.7	2.5±0.7
mH36[i] (M12 + M4)	441, 427	4.0±1.3	12.0±2.1	16.0±1.8
mH37 (M7)	427	ND	1.4±0.3	1.4±0.3
mH38[j] (M3 + M9)	427	ND	4.7±1.8	4.7±1.8
mH41 (M8)	427	ND	0.7±0.5	0.7±0.5
mH46[k]	411, 455	ND	13.0±5.3	13.0±5.3
Excretion (%)[l]		25.9±4.2	55.9±7.7	81.8±5.9

Urine samples at 0 to 120 hours and fecal samples at 0 to 264 hours postdose were used. Values represent the mean ±SD of 8 subjects. –: Not available, ND: Not detected.

a: Sum of excretion in urine and feces.
b: mH14 was a mixture of mH14a (M16), mH14b-1, mH14b-2, and mH14c (M22).
c: mH17 was a mixture of mH17a (M17), mH17b-1, and mH17b-2.
d: mH22 was a mixture of mH22a and mH22b.
e: mH23 was a mixture of mH23-1 and mH23-2.
f: mH24 was a mixture of mH24a and mH24b (M13).
g: mH30 was a mixture of mH30a (M11) and mH30b (M14).
h: mH31 was a mixture of mH31a (M1) and mH31b.
i: mH36 was a mixture of mH36a (M12) and mH36b (M4). Since mH36b (M4) was not detected in feces, the value in feces represents excretion of mH36a (M12).
j: mH38 was a mixture of mH38-1 (M3) and mH38-2 (M9).
k: mH46 was a mixture of mH46a (lemborexant) and mH46b.
l: Cumulative excretion of total radioactivity in urine and feces (Study E2006-A001-007).

The metabolites were assigned serial numbers with the prefix "mH". The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies.

CTD = Common Technical Document, eq. = equivalent, HPLC = high performance liquid chromatography, LC-MS(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of protonated molecular ion.

Additional Information: Lemborexant was the most dominant component in plasma. M10 was the only metabolite that exceeded 10% of total drug-related exposure. Lemborexant was not detected in human urine, but was found as the major component (13.0% of the dose) in feces, which was likely derived from the unabsorbed fraction of [^{14}C]lemborexant administered to healthy subjects. Defluorinated metabolites were not found in human plasma and excreta.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: lemborexant

Metabolites Comparison in Plasma at Steady State of Human and Animals **Study Number: DMPKT2013-005** **Location in CTD: 5.3.2.2.6**

Race	White, Black or African American					
Gender/Number of Subjects	Male/5					
Feeding Condition	Fasted					
Vehicle / Formulation	2×10-mg and 2×2.5-mg capsule					
Method of Administration/Dose[a]	Oral/25 mg, once daily for 14 days					
Assay / Analyte	LC-MS/MS / Metabolites derived from lemborexant					
Exposure Ratios of Circulating Metabolites Between Human and Monkey						
	Exposure Ratio[b, c]					
	Male			**Female**		
Metabolite	**20 mg/kg**	**100 mg/kg**	**1000 mg/kg**	**20 mg/kg**	**100 mg/kg**	**1000 mg/kg**
M2	NA	NA	NA	NA	NA	NA
M3	NA	NA	NA	NA	NA	NA
M4	34.13	125.73	230.30	24.99	85.17	115.03
M7	NA	NA	NA	NA	NA	NA
M8	NA	NA	NA	NA	NA	NA
M9	10.77	30.85	91.17	6.82	42.29	47.79
M10	2.46	7.61	15.10	1.83	6.67	7.58
M11	NA	NA	NA	NA	NA	NA
M12	NA	NA	NA	NA	NA	NA

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Test Article: lemborexant

Metabolites Comparison in Plasma at Steady State of Human and Animals　**Study Number: DMPKT2013-005**　**Location in CTD: 5.3.2.2.6**

Exposure Ratios of Circulating Metabolites Between Human and Monkey						
	Exposure Ratio[b, c]					
	Male			**Female**		
Metabolite	**20 mg/kg**	**100 mg/kg**	**1000 mg/kg**	**20 mg/kg**	**100 mg/kg**	**1000 mg/kg**
M13	NA	NA	NA	NA	NA	NA
M14	53.83	125.56	201.23	39.90	96.11	123.69
M15	1.68	3.44	9.19	1.48	3.68	5.54
M16	219.89	991.36	1320.98	165.23	615.39	901.63
M17	NA	NA	NA	NA	NA	NA
M18	13.34	78.89	109.50	6.12	35.94	55.17
M19	NA	NA	NA	NA	NA	NA
M20	155.63	676.45	735.11	95.43	363.73	577.54
M21	NA	NA	NA	NA	NA	NA

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Test Article: lemborexant

Metabolites Comparison in Plasma at Steady State of Human and Animals　**Study Number: DMPKT2013-005**　**Location in CTD: 5.3.2.2.6**

Exposure Ratios of Circulating Metabolites Between Human and Rat

	Exposure Ratio[b, c]					
	Male			Female		
Metabolite	**30 mg/kg**	**100 mg/kg**	**1000 mg/kg**	**30 mg/kg**	**100 mg/kg**	**1000 mg/kg**
M2	NA	NA	NA	NA	NA	NA
M3	NA	NA	NA	NA	NA	NA
M4	0.21	4.16	16.75	NA	0.87	22.57
M7	NA	NA	NA	NA	NA	NA
M8	NA	NA	NA	NA	NA	NA
M9	17.98	79.85	148.42	62.61	138.64	217.44
M10	0.05	0.14	1.60	0.05	0.13	3.11
M11	NA	NA	NA	NA	NA	NA
M12	NA	NA	NA	NA	NA	NA
M13	NA	NA	NA	NA	NA	NA
M14	10.38	44.52	67.43	3.82	17.09	78.59
M15	NA	2.37	12.36	NA	NA	19.58
M16	201.94	801.95	2566.37	258.19	1464.35	9354.95
M17	NA	NA	NA	NA	NA	NA
M18	NA	0.91	2.82	NA	NA	4.57
M19	NA	NA	NA	NA	NA	NA

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Test Article: lemborexant

Metabolites Comparison in Plasma at Steady State of Human and Animals **Study Number: DMPKT2013-005** **Location in CTD: 5.3.2.2.6**

Exposure Ratios of Circulating Metabolites Between Human and Rat						
	Exposure Ratio[b, c]					
	Male			**Female**		
Metabolite	**30 mg/kg**	**100 mg/kg**	**1000 mg/kg**	**30 mg/kg**	**100 mg/kg**	**1000 mg/kg**
M20	NA	1.91	4.37	NA	NA	6.89
M21	NA	NA	NA	NA	NA	NA

The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies. Exposure ratios are shown as the mean values of 4 or 5 subjects. NA; Not applicable because the identified metabolite was either only detected in humans or animals but not both, and in monkey sample, some identified metabolites were not detected in both humans and monkeys.

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NA = not applicable.

a: Multiple oral dose of lemborexant at 25 mg (Cohort 4) in Study E2006-A001-002 (5.3.3.1.2), Module 2.7.2.2.2.2.1

b: Exposure ratios between humans and animals were calculated by the following equation:

Exposure ratio = PAR_{animal} / PAR_{human},

Where PAR_{animal} is the peak area ratio of a metabolite in mixed animal plasma sample, and PAR_{human} is the peak area ratio of a metabolite in mixed human plasma sample.

c: Refer to Study No. DMPKT 2013-009 for the actual AUC value of M2, M3, M4, M7, M8, M9, M10, M11, M12, M13, M14, and M15 exposure in the clinical study.

Additional Information: Human plasma samples collected from healthy subjects receiving 25 mg of lemborexant once daily for 14 days in multiple ascending dose study (Study E2006-A001-002 [5.3.3.1.2]), and animal plasma samples from repeat-dose toxicity studies in monkeys (20, 100, and 1000 mg/kg, once daily on day 91, Study No. SBL038-067) and rats (30, 100, and 1000 mg/kg, once daily on day 71, Study No. K12033) were used The plasma samples collected were individually pooled across the time points according to the procedure referred to as Hamilton method (Hamilton, et al., 1981).

Eight metabolites, M4, M9, M10, M14, M15, M16, M18, and M20, were detected in human plasma. Even at the lowest dose in the animal toxicity studies (20 mg/kg in monkeys and 30 mg/kg in rats), plasma exposures of the 8 metabolites were higher in monkeys or rats or both than those in humans at 25 mg. The other 10 metabolites not detected in human plasma were observed in plasma samples from monkeys, rats, or both. These results suggested that plasma exposures of these 18 metabolites would be higher in at least one animal species used for toxicity studies than those in humans.

References:

Hamilton RA, Garnett WR, Kline BJ. Determination of mean valproic acid serum level by assay of a single pooled sample. Clin Pharmacol Ther. 1981;29(3):408-13.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: lemborexant

Metabolites Concentrations in Human Plasma **Study Number: DMPKT2013-009** **Location in CTD: 5.3.2.2.7**

Race	White, Black or African American			
Gender/Number of Subjects	Male/5			
Feeding Condition	Fasted			
Vehicle / Formulation	2×10-mg and 2×2.5-mg capsule			
Method of Administration/Dose	Oral/25 mg, once daily for 14 days			
Assay / Analyte	LC-MS/MS / Lemborexant and metabolites derived from lemborexant			
Pharmacokinetic Parameters of 12 Metabolites and Lemborexant in Human Plasma Samples From the Clinical Study E2006-A001-002				
Analyte	**$AUC_{(0-24)}$ (ng·h/mL)**	**C_{max} (ng/mL)**	**t_{max} (h)**	**$t_{1/2}$ (h)**
M2	3.25±2.12	0.718±0.154	4.00 (2.00 – 8.00)	NC
M3	33.8±18.7	2.90±1.65	4.00 (1.50 – 8.00)	NC
M4	351±75.2	24.8±2.33	4.00 (2.00 – 8.00)	39.3±20.7 (3 – 7)
M7	8.84±3.03	1.64±0.320	3.00 (1.50 – 5.00)	2.40±0.417 (3 – 4)
M8	NC	NC	NC	NC
M9	219±66.5	18.0±5.22	4.00 (2.00 – 8.00)	41.6±19.7 (3 – 6)
M10	632±142	31.8±4.66	8.00 (5.00 – 10.00)	41.8±15.3 (3 – 12)
M11	4.68±2.99	0.919±0.202	3.00 (1.50 – 8.00)	NC
M12	53.4±19.5	5.06±0.961	4.00 (2.00 – 8.00)	NC

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Test Article: lemborexant

Metabolites Concentrations in Human Plasma **Study Number: DMPKT2013-009** **Location in CTD: 5.3.2.2.7**

Pharmacokinetic Parameters of 12 Metabolites and Lemborexant in Human Plasma Samples From the Clinical Study E2006-A001-002				
Analyte	**$AUC_{(0-24)}$ (ng·h/mL)**	**C_{max} (ng/mL)**	**t_{max} (h)**	**$t_{1/2}$ (h)**
M13	44.4±12.5	3.50±1.57	5.00 (2.00 – 8.00)	11.5±5.68 (3 – 7)
M14	119±10.4	7.17±0.989	6.00 (2.00 – 10.00)	25.7±12.0 (5 – 13)
M15	124±28.6	6.15±1.44	8.00 (6.00 – 12.00)	40.0±15.0 (3 – 6)
Lemborexant[a]	1100±387	107±38.9	3.00 (1.50 – 5.00)	50.6±10.7

Each parameter except for t_{max} represents mean ±SD of 3 to 5 subjects, and t_{max} is shown as the median and range of 3 to 5 subjects. Terminal elimination phase half-life was calculated from the time points shown in parentheses.

$AUC_{(0-24)}$ = AUC from zero time to 24 hours, CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NC = not calculated, $t_{1/2}$ = terminal elimination phase half-life, t_{max} = time at which the highest drug concentration occurs.

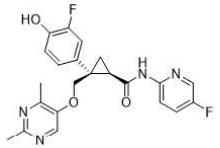
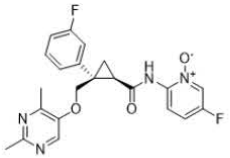
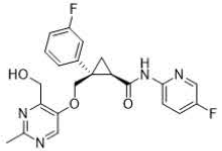
a: Cited from Study E2006-A001-002 (5.3.3.1.2) (Module 2.7.2.2.2.2.1).

Additional Information: Human plasma samples collected from healthy subjects receiving 25 mg of lemborexant once daily for 14 days in multiple ascending dose study (Study E2006-A001-002 [5.3.3.1.2]) were used.

2.6.5.10 薬物動態試験：代謝：in vitro

Test Article: lemborexant

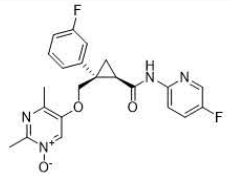
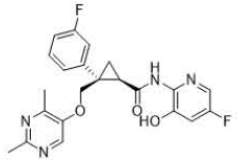
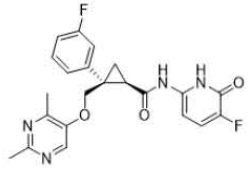
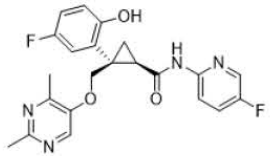
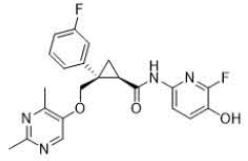
Metabolite Identifications **Study Number: C12062** **Location in CTD: 5.3.2.2.1**

Study System	in vitro				
Test System	Mouse, rat, monkey, and human liver microsomes (protein concentration: 2 mg/mL), or rCYP3A4 and rCYP3A5 (CYP content: 10 pmol/mL) in the presence of NADPH-generating system				
Method	The reactions using mouse, rat and monkey microsomes were continued for 15 minutes at 37 °C, while the reactions using human microsomes were continued for 60 minutes at 37 °C. The reactions using recombinant human CYP3A4 and CYP3A5 were continued for 30 minutes at 37 °C.				
Assay/Analyte	LC-MS(MS^n)/Metabolites derived from lemborexant				
Concentration	Lemborexant: 50 μmol/L				
Metabolite	**ER-Number**	**Matrix Found**	**Structure**	**$[M+H]^+$ (*m/z*)**	**Description**
M1	ER-571796	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated
M2	ER-571788	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	*N*-oxidated
M3	ER-571795	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated

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Test Article: lemborexant

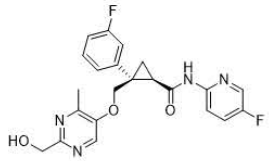
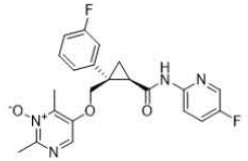
Metabolite Identifications **Study Number: C12062** **Location in CTD: 5.3.2.2.1**

Metabolite	ER-Number	Matrix Found	Structure	$[M+H]^+$ (*m/z*)	Description
M4	ER-571789	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	*N*-oxidated
M5	ER-571797	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated
M6	ER-583148	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Oxidized
M7	ER-583149	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated
M8	ER-583153	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated

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Test Article: lemborexant

Metabolite Identifications **Study Number: C12062** **Location in CTD: 5.3.2.2.1**

Metabolite	ER-Number	Matrix Found	Structure	$[M+H]^+$ (*m/z*)	Description
M9	ER-577483	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	Hydroxylated
M10	ER-571792	Mouse, rat, monkey, and human liver microsomes rCYP3A4 and rCYP3A5		427	*N*-oxidated

The M-numbers mean metabolites identified or proposed in in vitro or in vivo metabolism studies. The ER-number indicates the original sample number.

CTD = Common Technical Document, CYP = cytochrome P450, LC-MS(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of protonated molecular ion, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP.

Additional Information: No human specific metabolites were observed.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: lemborexant

CYP Isoforms for Metabolism of Lemborexant **Study Number: DMPKT2012-005** **Location in CTD: 5.3.2.2.2**

Study System	in vitro	
Test System	Lemborexant was spiked into each rCYP in the presence or absence of an NADPH-generating system	
Method	Incubation of lemborexant with each rCYP for 0 or 20 minutes at 37 °C	
Assay / Analyte / Concentration	LC-MS/MS / Lemborexant / 100 ng/mL	
rCYP Isoform	**Residual Ratio (%)**	
	Incubation Sample With NADPH Generating System	**Incubation Sample Without NADPH Generating System**
CYP1A2	97.9	98.8
CYP2A6	103.7	102.0
CYP2B6	100.6	97.3
CYP2C8	100.8	98.4
CYP2C9	98.5	99.8
CYP2C19	96.2	100.6
CYP2D6	94.8	98.1
CYP2E1	99.2	100.2
CYP2J2	100.5	102.5
CYP3A4	30.2	101.5
CYP3A5	80.9	98.5
CYP4F12	98.4	100.9

Microsomal protein content and rCYP content were 0.75 mg/mL and 30 pmol rCYP/mL, respectively.

Residual ratio (%) = (mean peak area ratio after 20-minute incubation) / (mean peak area ratio at 0 minute) × 100.

CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP.

Additional Information: The results suggested that CYP-dependent lemborexant metabolism is mediated by CYP3A4 predominantly and to a lesser extent by CYP3A5.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: M4, M9, M10

CYP Isoforms for Metabolism of Lemborexant Metabolites **Study Number: DMPKT2013-011** **Location in CTD: 5.3.2.2.3**

Study System	in vitro					
Test System	Each metabolite was spiked into each rCYP in the presence or absence of an NADPH-generating system					
Method	Incubation of each metabolite with each rCYP for 0 or 30 minutes at 37 °C					
Assay / Analyte / Concentration	LC-MS/MS / M4, M9, or M10 / 100 ng/mL					
rCYP Isoform	**Residual Ratio (%)**					
	M4		**M9**		**M10**	
	NADPH (+)	**NADPH (–)**	**NADPH (+)**	**NADPH (–)**	**NADPH (+)**	**NADPH (–)**
CYP1A2	96.3	98.2	99.9	101.4	98.3	100.9
CYP2A6	96.5	98.8	97.0	95.4	99.0	99.8
CYP2B6	99.9	100.9	93.3	94.8	99.2	100.8
CYP2C8	97.6	98.1	96.6	99.7	100.6	100.1
CYP2C9	100.6	99.1	99.2	99.8	98.8	99.3
CYP2C19	97.3	99.8	96.5	98.7	94.3	99.3
CYP2D6	107.8	98.4	94.3	94.0	100.0	97.4
CYP2E1	99.7	98.1	97.9	95.8	98.2	98.0
CYP3A4	21.9	98.8	37.1	99.8	45.8	98.0
CYP3A5	48.1	99.4	89.3	98.4	74.1	99.2

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Test Article: M4, M9, M10

CYP Isoforms for Metabolism of Lemborexant Metabolites **Study Number: DMPKT2013-011** **Location in CTD: 5.3.2.2.3**

rCYP Isoform	Residual Ratio (%)					
	M4		M9		M10	
	NADPH (+)	NADPH (–)	NADPH (+)	NADPH (–)	NADPH (+)	NADPH (–)
CYP2J2	96.9	97.6	96.8	97.3	101.4	100.0
CYP4F12	99.9	100.0	97.0	96.1	99.0	100.8

Microsomal protein content and rCYP content were 1.05 mg/mL and 75 pmol rCYP/mL, respectively.

Residual ratio (%) = (mean peak area ratio after 30-minute incubation) / (mean peak area ratio at 0 minute) × 100.

NADPH (+): Incubation sample with NADPH-generating system.

NADPH (–): Incubation sample without NADPH-generating system.

CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP.

Additional Information: The results suggested that CYP-dependent metabolism of M4, M9, and M10 are predominantly mediated by CYP3A4 and that CYP3A5 is also involved in the metabolism of M4 and M10.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: lemborexant

Identification of Metabolic Enzymes **Study Number: DMPKT2017-002** **Location in CTD: 5.3.2.2.4**

Study System	in vitro	
Test System	Cryopreserved human hepatocytes (1×10^6 cells/mL)	
Method	Incubation with hepatocyte suspension for 0 to 2 hours in the absence and presence of inhibitors	
Assay / Analyte	LC-MS/MS / Lemborexant	
Concentration	300 nmol/L	
Inhibitor (Concentration)	**CYP Isoform**	**Inhibition Ratio (%)**
1-Aminobenzotriazole (1.0 mmol/L)	All CYPs	97.7
Troleandomycin (10 μmol/L)	CYP3A	96.4
Ketoconazole (1.0 μmol/L)	CYP3A	97.8

Lemborexant (300 nmol/L) was incubated with cryopreserved human hepatocytes (1×10^6 cells/mL) in the absence (control) or presence of inhibitors (1-aminobenzotriazole, troleandomycin, or ketoconazole) at 37 °C for 0, 0.5, 1, 1.5, and 2 hours. Inhibition ratio was calculated from elimination rate constants in the absence or presence of each inhibitor which were estimated based on the plots of residual ratios versus incubation times.

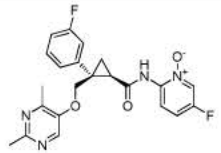
CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry.

Additional Information: The residual ratio of lemborexant in the absence of inhibitors decreased time-dependently, resulting in 46.3% after incubation at 37 °C for 2 hours.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: lemborexant

Metabolite Identifications in Rat Activated Liver S9　　**Study Number: DMPKT2011-014**　　**Location in CTD: 4.2.2.4.3**

Study System	in vitro				
Test System	Rat activated liver S9 (Rat liver 9000×g supernatant fraction induced with phenobarbital and 5,6-benzoflavone)				
Method	Incubation of lemborexant with rat activated liver S9 in the presence of Cofactor-I for 20 minutes at 37 °C				
Assay / Analyte	LC-MS/(MS^n) / Metabolites derived from lemborexant				
Concentration	Lemborexant: 100 μmol/L				
Metabolite	**ER-Number**	**Species Found**	**Structure**	**$[M+H]^+$ (*m/z*)**	**Description**
M2	ER-571788	Rat		427	*N*-oxidated

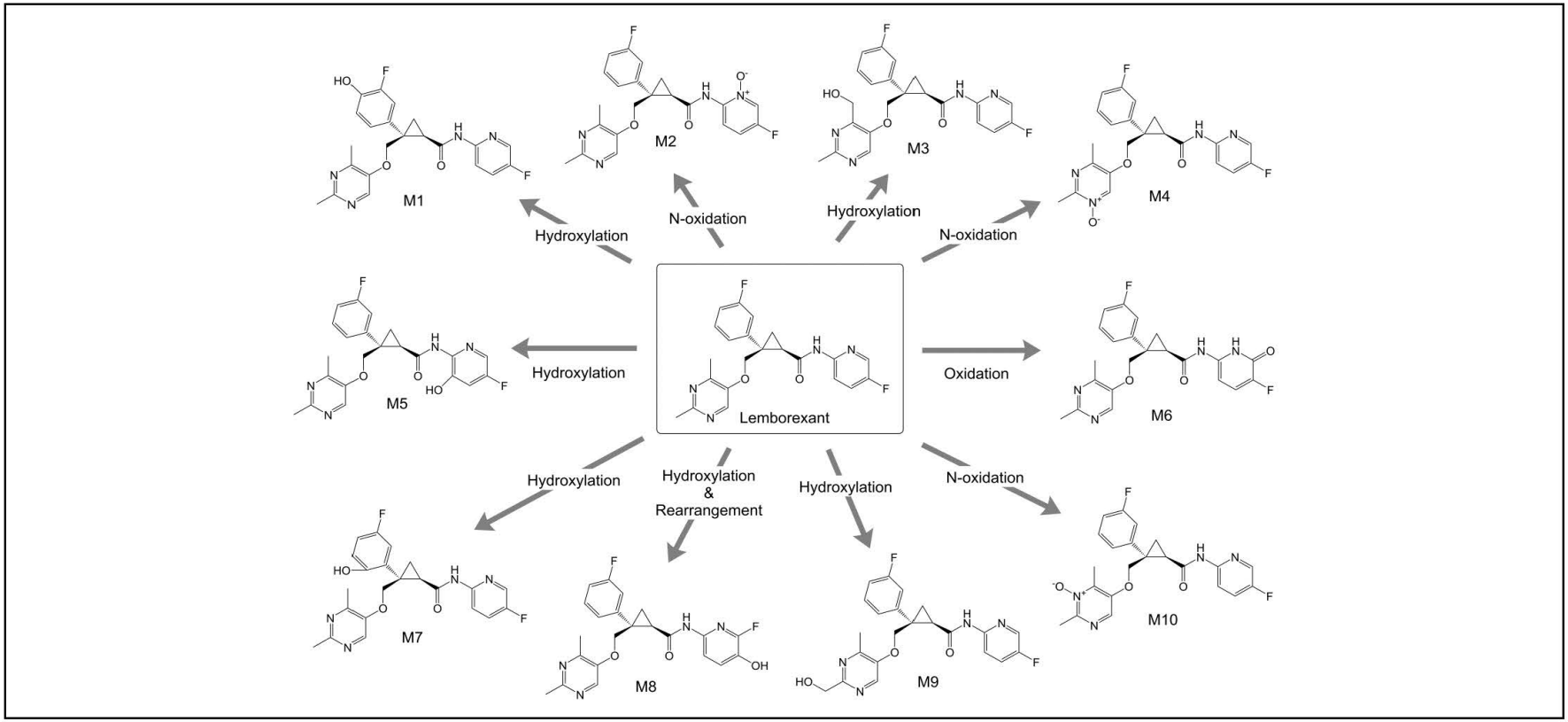
The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies. The ER-number indicates the original sample number.

CTD = Common Technical Document, LC-MS/(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of protonated molecular ion, S9 = 9000×g supernatant of homogenate (subcellular fraction containing microsomes and cytosol).

Additional Information: M2 was produced by rat activated liver S9 used in the Ames test (Study No. S11002).

2.6.5.11 薬物動態試験：推定代謝経路

Proposed Metabolic Pathways Based on the Metabolites Identified In Vitro



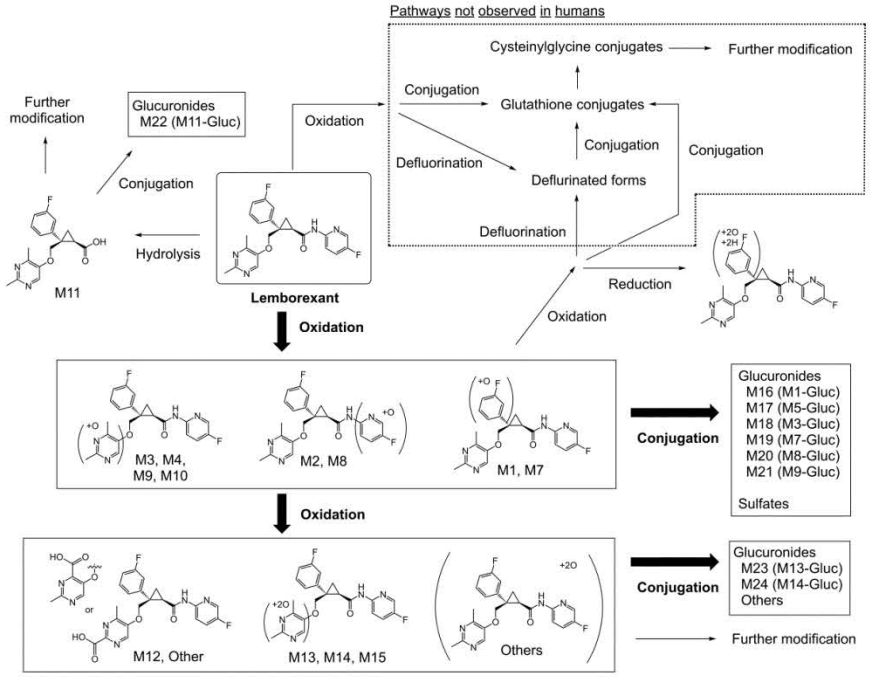
The proposed metabolic pathways for lemborexant are shown based on the 10 metabolites identified in in vitro studies. The M-numbers mean metabolites identified in in vitro (Section 2.6.5.10) or in vivo (Section 2.6.5.9) metabolism studies.

S9 = 9000×g supernatant of homogenate (subcellular fraction containing microsomes and cytosol).

Additional Information: The metabolic pathways were prepared based on Study No. C12062. No human specific metabolite was observed. It was confirmed that M2 was produced by rat activated liver S9.

2.6.5.11 薬物動態試験：推定代謝経路（続き）

Proposed Metabolic Pathways Based on the Metabolites Observed In Vivo



The M-numbers mean metabolites identified in in vitro (Section 2.6.5.10) or in vivo (Section 2.6.5.11) metabolism studies.

Gluc = glucuronide.

Additional Information: The metabolic pathways were prepared based on Study Nos. AE-7077-G, AE-7078-G, AE-7433-G, and DMPKT2012-008. Considering the metabolic profiles in human plasma and excreta, primary metabolic pathways of lemborexant were mono- or multiple oxidation and subsequent glucuronidation. Neither lemborexant enantiomer nor diastereomers was observed in humans, suggesting that conversion of lemborexant to its stereoisomers is negligible in humans. Bold arrows represent major metabolic pathways in humans. Some defluorinated metabolites were detected in rats and monkeys, but not detected in humans. No human specific circulating metabolites were observed.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害

Test Article: lemborexant

Reversible Inhibition of CYPs In Vitro **Study Number: DMPKT2012-001** **Location in CTD: 5.3.2.2.8**

Study System	in vitro
Test System	Human liver microsomes (0.2 mg protein/mL) in the presence of an NADPH-generating system
Method	Incubation of lemborexant with each probe substrate in microsomal matrix at 37 °C for 10 minutes (3 minutes for midazolam)
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP mediated metabolism
Concentration	3, 10, and 30 µmol/L

Percentage Inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, and CYP3A Activity by Lemborexant at 30 µmol/L and Specific Positive Control Inhibitors

CYP Isoform (Substrate)	**Lemborexant (30 µmol/L)**	**Positive Control Inhibition**		
	Inhibition (%)[a]	**Inhibitor**	**Conc. (µmol/L)**	**Inhibition (%)[a]**
CYP1A2 (Phenacetin)	5.7	α-Naphthoflavone	0.0071	51.8
CYP2B6 (Bupropion)	14.9	Ticlopidine	6.1	62.1
CYP2C8 (Paclitaxel)	39.3	Montelukast	0.21	53.1
CYP2C9 (Tolbutamide)	39.7	Sulfaphenazole	2.9	55.4
CYP2D6 (Bufurarol)	7.1	Quinidine	0.29	56.9
CYP3A (Midazolam)	48.2	Ketoconazole	0.026	53.8
CYP3A (Nifedipine)	9.6	Ketoconazole	0.027	55.3
CYP3A (Testosterone)	38.6	Ketoconazole	0.032	54.7

a: Inhibition (%) = $(C_{control} - C_{inhibitor}) / C_{control} \times 100$.
$C_{control}$: Mean concentration of the marker metabolite in the control sample.
$C_{inhibitor}$: Mean concentration of the marker metabolite in the inhibition sample.

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Test Article: lemborexant

Reversible Inhibition of CYPs In Vitro　　**Study Number: DMPKT2012-001**　　**Location in CTD: 5.3.2.2.8**

IC_{50} Values of Lemborexant and Inhibition Percentage of Positive Control Inhibitors on CYP2A6 and CYP2C19 Activity

CYP Isoform (Substrate)	IC_{50} of Lemborexant[b] (μmol/L)	Positive Control Inhibition		
		Inhibitor	Conc. (μmol/L)	Inhibition (%)[a]
CYP2A6 (Coumarin)	7.8	8-Methoxypsoralen	0.52	55.7
CYP2C19 ((*S*)-mephenytoin)	24.6	Ticlopidine	0.92	45.5

a: Calculation formula was in the footnote of the previous page.
b: IC_{50} (μmol/L) = (50% – High%) / (High% – Low%) × (High conc. – Low conc.) + (High conc.).
Low%: The highest inhibition (%) less than 50%; High%: The lowest inhibition (%) more than 50%.
Low conc.: Lemborexant concentration showing Low%; High conc.: Lemborexant concentration showing High%.

Effect of Lemborexant and Positive Control Inhibitor on CYP2E1 Activity

CYP Isoform (Substrate)	Relative Activity (%)[c]			Inhibition (%)[a]
	Lemborexant			Diethyldithiocarbamic Acid
	3 μmol/L	10 μmol/L	30 μmol/L	53 μmol/L
CYP2E1 (Chlorzoxazone)	125.1	156.5	212.6	50.6

a: Calculation formula was in the footnote of the previous page.
c: Relative activity (%) = $C_{Lemborexant}$ / $C_{control}$ × 100.
$C_{control}$: Mean concentration of the marker metabolite in the control sample.
$C_{Lemborexant}$: Mean concentration of the marker metabolite in the lemborexant sample.

CTD = Common Technical Document, CYP = cytochrome P450, IC_{50} = half-maximal inhibitory concentration, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

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Test Article: lemborexant

Inhibition of CYPs In Vitro (Time-Dependent Inhibition)　**Study Number: DMPKT2012-001**　**Location in CTD: 5.3.2.2.8**

Study System	in vitro
Test System	Human liver microsomes in the presence of a NADPH-generating system
Method	Lemborexant was pre-incubated with human liver microsomes at 37 °C for 30 minutes prior to addition of the appropriate CYP substrate. In follow-up assay, pre-incubation time were 10, 20, and 30 minutes.
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP mediated metabolism
Concentration	Initial assay: 30 µmol/L. Follow-up assay for CYP3A (Midazolam 1'-Hydroxylation): 3, 6, 10, 30, and 50 µmol/L.

Time-dependent Inhibitory Potency of Lemborexant and Positive Control Inhibitor on CYP Activity

CYP Isoform (Substrate)	**Lemborexant (30 µmol/L)**	**Positive Control**		
	Ratio (%)[a]	**Inhibitor**	**Conc. (µmol/L)**	**Ratio (%)[a]**
CYP1A2 (Phenacetin)	87.5	Furafylline	0.4	52.2
CYP2A6 (Coumarin)	121.1	8-Methoxypsoralene	0.3	53.1
CYP2B6 (Bupropion)	103.4	Ticlopidine	0.7	51.2
CYP2C8 (Paclitaxel)	101.7	Gemfibrozil 1-*O*-β-glucuronide	20	40.6
CYP2C9 (Tolbutamide)	98.3	Tienilic acid	5	37.4
CYP2C19 ((*S*)-mephenytoin)	107.3	Ticlopidine	3	48.7
CYP2D6 (Bufurarol)	91.9	Paroxetine	1	44.1
CYP2E1 (Chlorzoxazone)	87.5	Diethyldithiocarbamic acid	10	43.8
CYP3A (Midazolam)	38.5	Troleandomycin	0.8	39.7

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Test Article: lemborexant

Inhibition of CYPs In Vitro (Time-Dependent Inhibition)　　**Study Number: DMPKT2012-001**　　**Location in CTD: 5.3.2.2.8**

Time-dependent Inhibitory Effect and Inactivation Parameters of Lemborexant on CYP3A (Midazolam 1'-Hydroxylation) Activity

Concentration (μmol/L)	Remaining Activity, t[b]			K_{obs}[c] (min^{-1})	k_{inact}[c] (min^{-1})	K_I[c] (μmol/L)
	10 minutes	20 minutes	30 minutes			
3	0.932	0.989	0.853	0.0044	0.0503	25.2
6	0.878	0.844	0.732	0.0091		
10	0.805	0.741	0.614	0.0135		
30	0.638	0.456	0.349	0.0302		
50	0.481	0.366	0.255	0.0317		

a: Ratio (%) = ($C_{inhibitor\ (+NADPH)}$ / $C_{control\ (+NADPH)}$) / ($C_{inhibitor\ (-NADPH)}$ / $C_{control\ (-NADPH)}$) × 100.
$C_{inhibitor\ (+NADPH)}$: Mean concentration of the marker metabolite with NADPH generating system in the presence of inhibitor.
$C_{control\ (+NADPH)}$: Mean concentration of the marker metabolite with NADPH generating system in the absence of inhibitor.
$C_{inhibitor\ (-NADPH)}$: Mean concentration of the marker metabolite without NADPH generating system in the presence of inhibitor.
$C_{control\ (-NADPH)}$: Mean concentration of the marker metabolite without NADPH generating system in the absence of inhibitor.

b: Remaining activity, t = ($C_{inhibitor,\ t}$) / ($C_{control,\ t}$).
$C_{inhibitor,\ t}$: Mean concentration of the marker metabolite after pre-incubation for t minutes in the presence of inhibitor.
$C_{control,\ t}$: Mean concentration of the marker metabolite after pre-incubation for t minutes in the absence of inhibitor.

c: K_I, k_{inact}, and k_{obs} were calculated by following formulas.:
Remaining activity, t = – (k_{obs}) × (preincubation time (t)).
$k_{obs} = k_{inact} \times I / (K_I + I)$,
Where I = nominal concentration of lemborexant in the inhibition sample, K_I = half-maximal inhibitory concentration of the maximal inactivation rate constant, k_{inact} = maximal inactivation rate constant, and k_{obs} = observed inactivation rate constant.

CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

Details of the time-dependent inhibition measurement method: Microsomal matrix was pre-incubated with lemborexant at 37 °C for the designated time, and then an aliquot of the reaction mixture was subjected to a 5-fold dilution with buffer containing an NADPH-generating system and a probe substrate, except paclitaxel which was spiked after the dilution. After the incubation at 37 °C for 10 minutes (3 minutes for midazolam), remaining activities were measured by determining the concentration of the marker metabolites for each CYP isoform.