付録 3g. ヒト尿中 Leniolisib 濃度測定に用いた分析法の性能の概要（1100679g）

Bioanalytical method validation report name, amendments, and hyperlinks	Quantitative determination of CDZ173 in human urine by LC-MS/MS Method Validation Report DMPK R1100679g		
Method description	Human urine samples were diluted with ethanol and water. Then, the supernatant was analyzed by LC-MS/MS in SRM mode using ESI in positive mode as the ionization technique.		
Materials used for standard calibration curve and concentration	Leniolisib phosphate, [redacted] Batches 1251002.REF and 1010006837 Calibration standards: 10.0, 50.0, 100, 500, 1000, 5000, and 10000 ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE 261-1		
Validated assay range	10.0 to 10000 ng/mL		
Material used for QCs and concentration	Leniolisib phosphate, [redacted] Batches 1251002.REF and 1010006837, QC levels: 10.0 (LLOQ), 30.0 (LQC), 5000 (MQC), 7500 (HQC), and 100000 (Dilution QC) ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE 261-1		
Minimum required dilutions	NA		
Source and lot of reagents	Human urine Ethanol, Lichrosolv® gradient grade (Merck) Formic acid (Fluka) Tetrahydrofuran, Chromasolv® grade (Sigma-Aldrich) Purified deionized water using Integra UV Plus (SG)		
Regression model and weighting	Quadratic ($y=ax^2+bx+c$), weighted ($1/x^2$)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	7	DMPK R1100679g, Table 6-13
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-2.4% to 2.0%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤4.8%	

付録 3g. ヒト尿中 Leniolisib 濃度測定に用いた分析法の性能の概要（1100679g）（続き）

Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	-5.3% to 9.3%	DMPK R1100679g, Table 6-14
	Interbatch %CV	≤7.9%	
	Total error	NR	NA
Selectivity and matrix effect	Selectivity for leniolisib and IS was considered acceptable in 6 batches of urine tested. Interference from blank matrix was ≤12.3% of response at LLOQ for leniolisib and ≤0.3% of response at working concentration for IS. Matrix effect from LQC to HQC: Mean matrix factor: 1.61 to 1.95, precision (%CV): ≤17.9% Mean IS-normalized matrix factor: 1.25 to 1.37, precision (%CV): ≤10.4%		DMPK R1100679g, Table 6-1, Table 6-2, Table 6-5, Table 6-6, and Table 6-7
Interference and specificity	Contribution of IS to the signal of leniolisib (8.0% interference) and of leniolisib to the signal of IS (0.3% interference) was not significant.		DMPK R1100679g, Table 6-3 and Table 6-4
Hemolysis effect	NA		NA
Lipemic effect	NA		NA
Dilution linearity and hook effect	100-fold dilution of 100000 ng/mL, accuracy (%bias): -14.3%, precision (%CV): 3.9% Hook effect: NA		DMPK R1100679g, Table 6-16
Additional experiments in urine with 1% Tween: Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	7	DMPK R1100679g, Table 7-5
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.8% to 3.2%	
Additional experiments in urine with 1% Tween: Performance of QCs	**Cumulative accuracy (%bias) in 4 QC levels**	-10.2% to 8.4%	DMPK R1100679g, Table 7-6
	Intrabatch %CV	≤7.0%	
Additional experiments in urine with 1% Tween: Selectivity and matrix effect	Selectivity for leniolisib and IS was considered acceptable in 6 batches of urine tested. Interference from blank matrix was ≤2.3% of response at LLOQ for leniolisib and 0.0% of response at working concentration for IS.		DMPK R1100679g, Table 7-1 and Table 7-2
Additional experiments in urine with 1% Tween: Interference and specificity	Contribution of IS to the signal of leniolisib (0.0% interference) and of leniolisib to the signal of IS (0.0% interference) was not significant.		DMPK R1100679g, Table 7-3 and Table 7-4
Bench-top/process stability	5 h in urine at room temperature 160 h for processed extract in autosampler at 5°C		DMPK R1100679g, Table 6-17 and Table 6-20
Freeze-thaw stability	13 cycles at <-15°C in urine 13 cycles at <-65°C in urine 2 cycles at ≤-15°C in urine with 1% Tween 20		DMPK R1100679g, Table 6-18, Table 6-19, and Table 7-7

付録 3g. ヒト尿中 Leniolisib 濃度測定に用いた分析法の性能の概要（1100679g）（続き）

Long-term storage	67 days in urine with 1% Tween 20 at ≤-15°C	DMPK R1100679g, Table 7-8
Parallelism	NR	NA
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. There was no significant carryover evident for leniolisib (≤6.5% carryover) or IS (≤0.2% carryover).	DMPK R1100679g, Table 6-11
	Method Performance in CCDZ173X2101 Bioanalytical Report: DMPK RCCDZ173X2101	
Assay passing rate	16 of 18 runs (89%) in urine met acceptance criteria (excluding runs analyzed with Method 1100679f rather than Method 1100679g).	DMPK RCCDZ173X2101, Table 5-1
Standard curve performance	Cumulative accuracy (%bias): -1.1% to 1.2% Cumulative precision (%CV): ≤6.6%	DMPK RCCDZ173X2101, Table 5-8
QC performance	Cumulative accuracy (%bias): -1.7% to 6.2% Cumulative precision (%CV): ≤21.7% Total error: NR	DMPK RCCDZ173X2101, Table 5-10
Method reproducibility	Incurred sample reanalysis was performed on 28 samples, and 23 (82%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2101, Table 9-5
Study sample analysis stability	Samples were received frozen and stored at ≤-15°C. Long-term stability could not be demonstrated in samples not treated with Tween 20. Samples treated with Tween 20 were analyzed within the established stability limit of 67 days at <-15°C.	DMPK RCCDZ173X2101, Section 1 and Section 6
Standard calibration curve performance during accuracy and precision	16 calibrator sets with 2 replicates at each of 7 concentration levels from 10.0 to 1000 ng/mL (total 224 datapoints) – 4 excluded from curve fitting due to deviation ≥15% of nominal.	

CV=coefficient of variation; ESI=electrospray ionization; HQC=high QC; IS=internal standard; LC-MS/MS=liquid chromatography coupled with tandem mass spectroscopy; LLOQ=lower limit of quantification; LQC=low QC; MQC=middle QC; NA=not applicable; NR=not reported; QC=quality control; SRM=selective reaction monitoring; ULOQ=upper limit of quantification.

付録 3h. ヒト血漿中イトラコナゾール濃度測定に用いた分析法の性能の概要（15BAS0302）

Bioanalytical method validation report name, amendments, and hyperlinks	Quantitative determination of itraconazole in human plasma by LC-MS/MS Method Validation Report 15BAS0302 Details of bioanalytical method validation and the validation report are available upon request.	
	Method Performance in CCDZ173X2102 **Bioanalytical Report: DMPK RCCDZ173X2102**	
Assay passing rate	5 of 6 runs (83%) met acceptance criteria	DMPK RCCDZ173X2102, Table 5-1 and Table 5-3
Standard curve performance	Cumulative accuracy (%bias): -5.6% to 4.0% Cumulative precision (%CV): ≤10.3%	DMPK RCCDZ173X2102, Table 5-7
QC performance	Cumulative accuracy (%bias): 2.3% to 9.7% Cumulative precision (%CV): ≤4.1% Total error: NR	DMPK RCCDZ173X2102, Table 5-11
Method reproducibility	Incurred sample reanalysis was performed on 36 samples, and 36 (100%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2102, Table 9-8
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples were stored at -80°C for up to 41 days (14 Aug 2015 to 24 Sep 2015).	DMPK RCCDZ173X2102, Section 1 and Section 6
Standard calibration curve performance during accuracy and precision	5 calibrator sets with 2 replicates at each of 7 concentration levels from 10.0 to 10000 ng/mL (total 70 datapoints) – 1 datapoint failed to meet acceptance criteria and was excluded from curve fitting	

CV=coefficient of variation; LC-MS/MS=liquid chromatography coupled with tandem mass spectroscopy; NR=not reported; QC=quality control.

付録 3i. ヒト血漿中エチニルエストラジオール及びレボノルゲストレル濃度測定に用いた分析法の性能の概要（Draft10BASM032V2）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an HPLC-MS/MS assay method for the determination of ethynyl estradiol (EE) and levonorgestrel (LN) in human plasma Method Validation Report 09BAS0248 Amendment 1 09BAS0248 Amendment 1 Assessment of interference from leniolisib was performed as within-study validation and is reported in DMPK RCCDZ173X2104.
Method description	Liquid-liquid extraction of human plasma (K_2EDTA) samples and analysis using HPLC for separation and MS/MS for detection.
Materials used for standard calibration curve and concentration	Ethinylestradiol, Toronto Research Chemicals Inc., Lot 2-QL-193-1 Calibration standards: 5.00, 10.0, 25.0, 100, 200, 800, 2000, and 2500 pg/mL Levonorgestrel, United States Pharmacopeia, Lot F0H323 Calibration standards: 50.0, 100, 250, 1000, 2000, 8000, 20000, and 25000 pg/mL Internal standards: 17-α-ethynylestradiol-2,4,16,16-D4 Toronto Research Chemicals Inc., Lot 1-ARP-10-1 Norgestrel-D6 Toronto Research Chemicals Inc., Lot 2-QFY-17-6
Validated assay range	Ethinylestradiol: 5.00 to 2500 pg/mL Levonorgestrel: 50.0 to 25000 pg/mL
Material used for QCs and concentration	Ethinylestradiol, Toronto Research Chemicals Inc., Lot 2-QL-193-1 QC levels: 5.00 (LLOQ), 15.0 (LQC), 125 (MQC), 1900 (HQC), and 25000 (Dilution QC) pg/mL Levonorgestrel, United States Pharmacopeia, Lot F0H323 QC levels: 50.0 (LLOQ), 150 (LQC), 1250 (MQC), 19000 (HQC), and 250000 (Dilution QC) pg/mL Internal standards: 17-α-ethynylestradiol-2,4,16,16-D4 Toronto Research Chemicals Inc., Lot 1-ARP-10-1 Norgestrel-D6 Toronto Research Chemicals Inc., Lot 2-QFY-17-6
Minimum required dilutions	NA
Source and lot of reagents	Human plasma K_2EDTA (Bioreclamation, Inc.) Methanol, HPLC grade (Merck) Acetonitrile, HPLC grade (Merck) Isopropanol, HPLC grade (Sigma) Sodium carbonate, AR grade (Ling Feng) Formic acid, HPLC grade (Tedia)

付録 3i. ヒト血漿中エチニルエストラジオール及びレボノルゲストレル濃度測定に用いた分析法の性能の概要（Draft10BASM032V2）（続き）

	Acetone, HPLC grade (Sigma) Ethyl acetate, HPLC grade (Sigma) 1-butylchloride, HPLC grade (Sigma) Dansyl chloride, HPLC grade (Sigma)		
Regression model and weighting	Linear, 1/conc, squared		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	Ethinylestradiol: 8 Levonorgestrel: 8	09BAS0248, Table 5
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Ethinylestradiol: -4.4% to 3.0% Levonorgestrel: -2.4% to 2.0%	
	Cumulative precision (%CV) from LLOQ to ULOQ	Ethinylestradiol: ≤6.7% Levonorgestrel: ≤8.7%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	Ethinylestradiol: -7.2% to -3.2% Levonorgestrel: -9.0% to -4.0%	09BAS0248, Table 8
	Interbatch %CV	Ethinylestradiol: ≤9.0% Levonorgestrel: ≤12.2%	
	Total error	NR	NA
Selectivity and matrix effect	Selectivity for ethinylestradiol and levonorgestrel was considered acceptable in 6 individual lots of plasma tested. Interference from blank matrix was ≤15.5% (ethinylestradiol) and 0% (levonorgestrel) of response from LLOQ standard. All matrix lots had 2 of 3 (67%) samples spiked to LQC show ≤15% bias for both ethinylestradiol and levonorgestrel.		09BAS0248, Table 11 and Table 12
Interference and specificity	No significant chromatographic interference from leniolisib (6000000 pg/mL) was detected at the retention time of ethinylestradiol and levonorgestrel.		DMPK RCCDZ173X2104, Table 5-6 and Table 5-7
Hemolysis effect	NR		NA
Lipemic effect	NR		NA
Dilution linearity and hook effect	Ethinylestradiol: 20-fold dilution of 25000 pg/mL. Hook effect: NA Levonorgestrel: 20-fold dilution of 250000 pg/mL. Hook effect: NA		09BAS0248, Table 10
Bench-top/process stability	Ethinylestradiol and Levonorgestrel: 20 h in plasma at 15°C to 30°C 57 h for processed extract in Na_2CO_3 buffer and dansyl chloride solution at 2°C to 8°C 61 h reinjection reproducibility in Na_2CO_3 buffer and dansyl chloride solution at 2°C to 8°C		09BAS0248, Table 15, Table 18, and Table 19,

付録 3i. ヒト血漿中エチニルエストラジオール及びレボノルゲストレル濃度測定に用いた分析法の性能の概要（Draft10BASM032V2）（続き）

Freeze-thaw stability	Ethinylestradiol and Levonorgestrel: 3 cycles at -20°C and -80°C 6 cycles at -20°C	09BAS0248, Table 17
Long-term storage	Ethinylestradiol and Levonorgestrel: 191 days in plasma at -20°C 191 days in plasma at -80°C	09BAS0248, Table 16
Parallelism	NR	NA
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. There was no significant carryover evident for ethinylestradiol (≤17.7% carryover) or levonorgestrel (≤3.4% carryover) or their internal standards.	09BAS0248, Validation Summary
	Method Performance in CCDZ173X2104 **Bioanalytical Report: DMPK RCCDZ173X2104**	
Assay passing rate	Ethinylestradiol: 15 of 15 runs (100%) met acceptance criteria; stock checks and 19 runs with wash solvent not used/interference to ethynylestradiol-2,4,16,16-d_4/injection valve blocking/failed autosampler stability criteria/only 1 set of calibration standards and no QC samples/instrument error excluded from calculation. Levonorgestrel: 21 of 21 runs (100%) met acceptance criteria; stock checks and 6 runs with wash solvent not used/injection valve blocking/failed autosampler stability criteria/only 1 set of calibration standards and no QC samples/instrument error excluded from calculation.	DMPK RCCDZ173X2104, Table 5-1 and Table 5-2
Standard curve performance	Ethinylestradiol: Cumulative accuracy (%bias): -1.8% to 1.6% Cumulative precision (%CV): ≤3.1% Levonorgestrel: Cumulative accuracy (%bias): -1.0% to 1.0% Cumulative precision (%CV): ≤6.4%	DMPK RCCDZ173X2104, Table 5-3
QC performance	Ethinylestradiol: Cumulative accuracy (%bias): -2.1% to 0.7% Cumulative precision (%CV): ≤4.1% Total error: NR Levonorgestrel: Cumulative accuracy (%bias): -2.7% to -0.8% Cumulative precision (%CV): ≤5.4% Total error: NR	DMPK RCCDZ173X2104, Table 5-4
Method reproducibility	Incurred sample reanalysis was performed on 30 samples, and 30 (100%) of which were within ±20% difference from original results for both ethinylestradiol and levonorgestrel.	DMPK RCCDZ173X2104, Table 9-3
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples for ethinylestradiol and levonorgestrel were stored at -80°C for up to 85 days (08 Apr 2016 to 02 Jul 2016).	DMPK RCCDZ173X2104, Section 1 and Section 6

付録 3i. ヒト血漿中エチニルエストラジオール及びレボノルゲストレル濃度測定に用いた分析法の性能の概要（Draft10BASM032V2）（続き）

Standard calibration curve performance during accuracy and precision	Ethinylestradiol: 14 calibrator sets with 2 replicates at each of 7 concentration levels from 5.00 to 2500 ng/mL (total 196 datapoints) – none excluded from curve fitting Levonorgestrel: 20 calibrator sets with 2 replicates at each of 7 concentration levels from 50.0 to 25000 ng/mL (total 280 datapoints) – 2 datapoints (1 failed to meet acceptance criteria; 1 with no analyte and IS response) were excluded from curve fitting

CV=coefficient of variation; HPLC=high-performance liquid chromatography; HQC=high QC; IS=internal standard; LLOQ=lower limit of quantification; LQC=low QC; MQC=middle QC; MS/MS=tandem mass spectroscopy; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3j. ヒト血漿中カフェイン、パラキサンチン、ミダゾラム及びα-ヒドロキシミダゾラム濃度測定に用いた分析法の性能の概要（23302）

Bioanalytical method validation report name, amendments, and hyperlinks	The validation of an LC-MS/MS bioanalytical method for the determination of caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam in human K2-EDTA plasma samples
Method description	Caffeine, Paraxanthine, Midazolam & Alpha-hydroxymidazolam and their internal standards are isolated from human K2-EDTA plasma by a protein precipitation. After precipitation the samples are diluted in injection solvent. After preparation, all samples are injected into the chromatographic system. Chromatographic separation is performed on an Acquity BEH C18 column using gradient elution. A QTRAP6500+ tandem mass spectrometer equipped with a Turbo Ion Spray probe operating in the positive multiple reaction monitoring mode is used for quantification.
Materials used for standard calibration curve and concentration	Caffeine Sigma-Aldrich, Lot 0000181333 Calibration standards: 5.00, 10.0, 25.0, 50.0, 100, 250, 500, 1000, 1600 and 2000 ng/mL Paraxanthine TRC, Lot 2-AAH-100-1 Calibration standards: 5.00, 10.0, 25.0, 50.0, 100, 250, 500, 1000, 1600 and 2000 ng/mL Midazolam Duchefa, Lot 111.1B0.6 Calibration standards: 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 20.0, 80.0 and 100 ng/ml Alpha-hydroxymidazolam Duchefa, Lot 330.1B15.1L2 Calibration standards: 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 20.0, 80.0 and 100 ng/ml <u>Internal standards:</u> Caffeine-d3 C/D/N Isotopes Inc., Lot FG-128 Paraxanthine-1-methyl-d3 TRC, Lot 4-RUS-48-2 Midazolam-d4 Duchefa, Lot 1767.1B3.2L1 Alpha-hydroxymidazolam-d4 Sigma-Aldrich, Lot FN03012235
Validated assay range	5.00 to 2000 ng/mL (caffeine and paraxanthine) 0.100 to 100 ng/ml (midazolam and alpha-hydroxymidazolam)
Material used for QCs and concentration	Caffeine Sigma-Aldrich, Lot 0000181333 Calibration standards: 5.00, 10.0, 25.0, 50.0, 100, 250, 500, 1000, 1600 and 2000 ng/mL

付録 3j. ヒト血漿中カフェイン、パラキサンチン、ミダゾラム及びα-ヒドロキシミダゾラム濃度測定に用いた分析法の性能の概要（23302）（続き）

	Paraxanthine TRC, Lot 2-AAH-100-1 Calibration standards: 5.00, 10.0, 25.0, 50.0, 100, 250, 500, 1000, 1600 and 2000 ng/mL Midazolam Duchefa, Lot 111.1B0.6 Calibration standards: 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 20.0, 80.0 and 100 ng/ml Alpha-hydroxymidazolam Duchefa, Lot 330.1B15.1L2 Calibration standards: 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 20.0, 80.0 and 100 ng/ml Internal standards: Caffeine-d3 C/D/N Isotopes Inc., Lot FG-128 Paraxanthine-1-methyl-d3 TRC, Lot 4-RUS-48-2 Midazolam-d4 Duchefa, Lot 1767.1B3.2L1 Alpha-hydroxymidazolam-d4 Sigma-Aldrich, Lot FN03012235		
Minimum required dilutions	NA		
Source and lot of reagents	Methanol, ULC/MS grade (Biosolve) Acetonitrile, ULC/MS grade (Biosolve) Ethanol, HPLC-gradient Grade >99.8% (VWR Chemicals) Ammonium formate, LC-MS grade (Sigma) Formic acid, LC-MS grade (Biosolve) Ultra-Pure water (Veolia/Millipore)		
Regression model and weighting	Linear, weighted ($1/x^2$)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	Caffeine: 10 Paraxanthine: 10 Midazolam: 10 Alpha-hydroxymidazolam: 10	23302, Table 24 – Table 27
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Caffeine: -1.80% to 2.60% Paraxanthine: -1.70% to 2.90% Midazolam: -5.20% to 2.60% Alpha-hydroxymidazolam: -3.80% to 3.20%	
	Cumulative precision (%CV) from LLOQ to ULOQ	Caffeine: ≤4.40% Paraxanthine: ≤6.10% Midazolam: ≤3.50%	

付録 3j. ヒト血漿中カフェイン、パラキサンチン、ミダゾラム及びα-ヒドロキシミダゾラム濃度測定に用いた分析法の性能の概要（23302）（続き）

		Alpha-hydroxymidazolam: ≤4.90%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	Caffeine: -4.00% to 5.0% Paraxanthine: -3.70% to 5.60% Midazolam: -5.20% to 9.90% Alpha-hydroxymidazolam: -3.10% to 15.0%	23302, Table 29 – Table 32
	Overall CV (%CV)	Caffeine: ≤10.7% Paraxanthine: <11.3% Midazolam: <15.0% Alpha-hydroxymidazolam: <10.3%	
	Total error	NR	NA
Selectivity and matrix effect	Selectivity for caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam was considered acceptable in 6 individual lots of plasma tested. Interference from blank matrix was ≤10.4% (caffeine), ≤9.6% (paraxanthine), ≤5.6% (midazolam) and ≤18.2% (alpha-hydroxymidazolam) of response from LLOQ standard		23302, Table 6 - 9
Hemolysis effect	No significant effect of hemolysis (2%[v/v] whole blood) at LLOQ for caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam		23302, Table 6 - 9
Lipemic effect	No significant effect of lipemia (≥240 mg/dl triglycerides) at LLOQ for caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam.		23302, Table 6 - 9
Dilution linearity	Caffeine: 10-fold dilution of 16000 ng/mL. Paraxanthine: 10-fold dilution of 16000 ng/mL. Midazolam 10-fold dilution of 800 ng/ml Alpha-hydroxymidazolam 10-fold dilution of 800 ng/ml		23302, Table 34 - 37
Bench-top/process stability	Caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam: 65h at +10°C in human plasma 19h at room temperature in human plasma Caffeine and paraxanthine 22h at room temperature for stock solution in methanol		23302, Table 50 – 53; Table 58 – 61, Table 62 - 63
Freeze-thaw stability	Caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam: 4 cycles at ≤-18°C		23302, Table 42 - 49
Long-term storage	Caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam: 100 days in plasma at ≤-18°C for QC-low and QC-high 42 days spike solutions in methanol at 2-8°C Caffeine, midazolam and alpha-hydroxymidazolam:		23302, Table 54 – 57; Table 64 - 67

付録 3j. ヒト血漿中カフェイン、パラキサンチン、ミダゾラム及びα-ヒドロキシミダゾラム濃度測定に用いた分析法の性能の概要（23302）（続き）

	94 days in plasma at ≤-18°C for OQC-2	
Parallelism	NR	NA
Carryover	Carryover was evaluated by injecting two blank matrix samples after the ULOQ standard. For four out of five runs, no significant interference was observed for caffeine and paraxanthine. For midazolam and alpha-hydroxymidazolam the acceptance criteria for carryover where not met. Therefore, monitoring of carryover will be needed when analyzing clinical samples.	23302, Table 33
	Method Performance in LE5101 **Bioanalytical Report: DMPK 23303**	
Assay passing rate	Caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam: 10 of 10 runs (100%) met acceptance criteria;	23303, Table 5
Standard curve performance	Caffeine: Cumulative accuracy (%bias): -1.4% to 2.0% Cumulative precision (%CV): ≤1.2% Paraxanthine: Cumulative accuracy (%bias): -0.8% to 2.3% Cumulative precision (%CV): ≤2.0% Midazolam: Cumulative accuracy (%bias): -3.3% to 2.1% Cumulative precision (%CV): ≤4.1% Alpha-hydroxymidazolam: Cumulative accuracy (%bias): -2.5% to 2.0% Cumulative precision (%CV): ≤6.4%	23303, Table 8 - 11
QC performance	Caffeine: Cumulative accuracy (%bias): -2.3% to 0.1% Cumulative precision (%CV): ≤3.0% Paraxanthine: Cumulative accuracy (%bias): 2.7% to 6.1% Cumulative precision (%CV): ≤3.2% Midazolam: Cumulative accuracy (%bias): -2.2% to 6.3% Cumulative precision (%CV): ≤3.8% Alpha-hydroxymidazolam: Cumulative accuracy (%bias): 0.2% to 7.9% Cumulative precision (%CV): ≤6.3%	23303, Table 13 - 16
Method reproducibility	Incurred sample reanalysis was performed on 51 samples, and 50 (98.0%) of which were within ±20% difference from original results for caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam.	23303, Table 19 - 22
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples for caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam were stored at ≤-18°C for up to 66 days (12 Feb 2024 to 18 Apr 2024).	23303, Section 1

付録 3j. ヒト血漿中カフェイン、パラキサンチン、ミダゾラム及びα-ヒドロキシミダゾラム濃度測定に用いた分析法の性能の概要（23302）（続き）

Standard calibration curve performance during accuracy and precision	Caffeine, paraxanthine, midazolam and alpha-hydroxymidazolam: 10 calibrator sets at 10 concentration levels from 5.00 to 2000 ng/mL of which none were excluded from curve fitting

CV=coefficient of variation; HPLC=high-performance liquid chromatography; HQC=high QC; IS=internal standard; LLOQ=lower limit of quantification; LQC=low QC; MQC=middle QC; MS/MS=tandem mass spectroscopy; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3k. ヒト血漿中フロセミド及びロスバスタチン濃度測定に用いた分析法の性能の概要（23452）

Bioanalytical method validation report name, amendments, and hyperlinks	The validation of an LC-MS/MS bioanalytical method for the determination of furosemide and rosuvastatin in human K2-EDTA plasma samples
Method description	Furosemide and Rosuvastatin and their internal standards are isolated from Human K2-EDTA plasma using solid phase extraction. After elution the samples are evaporated under a stream of nitrogen and reconstituted in injection solvent. After preparation, all samples are injected into the chromatographic system. Chromatographic separation is performed on an Acquity UPLC HSS T3 column using gradient elution. A QTRAP6500+ tandem mass spectrometer equipped with a Turbo Ion Spray probe operating in the negative multiple reaction monitoring mode is used for quantification.
Materials used for standard calibration curve and concentration	Furosemide TRC, Lot 2023-CPO-070 Calibration standards: 0.200, 0.400, 0.800, 2.00, 4.00, 8.00, 20.0, 40.0, 80.0, 160 and 200 ng/mL Rosuvastatin TRC, Lot 711865109-1-1 Calibration standards: 0.100, 0.200, 0.400, 1.00, 2.00, 4.00, 10.0, 20.0, 40.0, 80 and 100 ng/mL Internal standards: Furosemide-D5 TRC, Lot C-197 Rosuvastatin-D6 TRC, Lot 2-WEN-131-1
Validated assay range	0.200 to 200 ng/mL (furosemide) 0.100 to 100 ng/ml (rosuvastatin)
Material used for QCs and concentration	Furosemide TRC, Lot 2023-CPO-070 Calibration standards: 0.200, 0.400, 0.800, 2.00, 4.00, 8.00, 20.0, 40.0, 80.0, 160 and 200 ng/mL Rosuvastatin TRC, Lot 711865109-1-1 Calibration standards: 0.100, 0.200, 0.400, 1.00, 2.00, 4.00, 10.0, 20.0, 40.0, 80 and 100 ng/mL Internal standards: Furosemide-D5 TRC, Lot C-197 Rosuvastatin-D6 TRC, Lot 2-WEN-131-1
Minimum required dilutions	NA

付録 3k. ヒト血漿中フロセミド及びロスバスタチン濃度測定に用いた分析法の性能の概要（23452）（続き）

Source and lot of reagents	Methanol, ULC/MS grade (Biosolve) Acetonitrile, ULC/MS grade (Biosolve) Ammonium formate, LC-MS grade (Sigma) Formic acid, LC-MS grade (Biosolve) Ultra-Pure water (Veolia/Millipore) Isopropylalcohol, LC-MS grade (Sigma) Phosphoric acid, LC-MS grade (Boom)		
Regression model and weighting	Linear, weighted ($1/x^2$)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	Furosemide: 11 Rosuvastatin: 11	23452, Table 14 – Table 15
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Furosemide: -4.2% to 2.6% Rosuvastatin: -3.0% to 6.3%	
	Cumulative precision (%CV) from LLOQ to ULOQ	Furosemide: ≤8.1% Rosuvastatin: ≤7.0%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	Furosemide: -1.1% to 5.5% Rosuvastatin: 0.7% to 4.6%	23452, Table 17 – Table 18
	Overall CV (%CV)	Furosemide: ≤7.4% Rosuvastatin: <6.3%	
Selectivity and matrix effect	Selectivity for furosemide and rosuvastatin was considered acceptable in 6 individual lots of plasma tested. Interference from blank matrix was ≤0.0% (furosemide and rosuvastatin) of response from LLOQ standard		23452, Table 6 – Table 7
Hemolysis effect	No significant effect of hemolysis (2%[v/v] whole blood) at LLOQ for furosemide and rosuvastatin		23452, Table 6 – Table 7
Lipemic effect	No significant effect of lipemia (≥240 mg/dl triglycerides) at LLOQ for furosemide and rosuvastatin		23452, Table 6 – Table 7
Dilution linearity	Furosemide: 10-fold dilution of 1600 ng/mL. Rosuvastatin: 10-fold dilution of 800 ng/mL.		23452, Table 20 – Table 21
Bench-top/process stability	Furosemide and rosuvastatin: 152h at +10°C in human plasma 25h at room temperature in human plasma 21h at room temperature for stock solution in methanol		23452, Table 26 – Table 27, Table 30-33
Freeze-thaw stability	Furosemide and rosuvastatin: 4 cycles at ≤-18°C		23452, Table 24 – Table 25
Long-term storage	Furosemide and rosuvastatin: 91 days in plasma at ≤-18°C for QC-low, QC-high and OQC-2		23452, Table 28 – Table 29, Table 34-37

付録 3k. ヒト血漿中フロセミド及びロスバスタチン濃度測定に用いた分析法の性能の概要（23452）（続き）

	91 days spike solutions in methanol at ≤-18°C 91 days spike solutions in methanol at 2-8°C	
Parallelism	NR	NA
Carryover	Carryover was evaluated by injecting two blank matrix samples after the ULOQ standard in three runs. No significant interference was observed for furosemide and rosuvastatin.	23452, Table 19
	Method Performance in LE5101 **Bioanalytical Report: DMPK 23307**	
Assay passing rate	Furosemide and rosuvastatin: 25 of 26 runs (93.1%) met acceptance criteria; One (of 26) run was cancelled due to an error in sample preparation procedure	23307, Table 5
Standard curve performance	Furosemide: Cumulative accuracy (%bias): -2.8% to 2.6% Cumulative precision (%CV): ≤5.6% Rosuvastatin: Cumulative accuracy (%bias): -1.4% to 1.7% Cumulative precision (%CV): ≤6.7%	23307, Table 11 - Table 12
QC performance	Furosemide: Cumulative accuracy (%bias): -3.0% to 5.9% Cumulative precision (%CV): ≤6.6% Rosuvastatin: Cumulative accuracy (%bias): -1.0% to 9.7% Cumulative precision (%CV): ≤12.9%	23307, Table 15 - 16
Method reproducibility	Incurred sample reanalysis was performed on 56 samples, and 55 (98.2%) for furosemide and 46 (82.1%) for rosuvastatin were within ±20% difference from original results.	23307, Table 21 - 22
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples for furosemide and rosuvastatin were stored at ≤-18°C for up to 60 days (20 Mar 2024 to 06 Jun 2024).	23307, Section 1
Standard calibration curve performance during accuracy and precision	9 calibrator sets at 11 concentration levels, from 0.200 to 200 ng/mL for furosemide and 0.100 to 100 ng/ml for rosuvastatin, of which none were excluded from curve fitting	

CV=coefficient of variation; HPLC=high-performance liquid chromatography; HQC=high QC; IS=internal standard; LLOQ=lower limit of quantification; LQC=low QC; MQC=middle QC; MS/MS=tandem mass spectroscopy; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3l. ヒト血漿中メトホルミン濃度測定に用いた分析法の性能の概要（23306）

Bioanalytical method validation report name, amendments, and hyperlinks	The validation of an LC-MS/MS bioanalytical method for the determination of metformin in human K2-EDTA plasma samples		
Method description	Metformin and its internal standard are isolated from human K2-EDTA plasma using protein precipitation. After preparation, all samples are injected into the chromatographic system. Chromatographic separation is performed on an XBridge BEH Amide column using gradient elution. An QTRAP6500+ tandem mass spectrometer equipped with a Turbo Ion Spray probe operating in the positive multiple reaction monitoring mode is used for quantification.		
Materials used for standard calibration curve and concentration	Metformin TRC, Lot 1-MJS-112-1 Calibration standards: 5.00, 10.0, 20.0, 50.0, 100, 200, 500, 1000, 1600 and 2000 ng/mL Internal standards: Metformin-D6 TRC, M-206		
Validated assay range	5.00 to 2000 ng/mL		
Material used for QCs and concentration	Metformin TRC, Lot 1-MJS-112-1 Calibration standards: 5.00, 10.0, 20.0, 50.0, 100, 200, 500, 1000, 1600 and 2000 ng/mL Internal standards: Metformin-D6 TRC, M-206		
Minimum required dilutions	NA		
Source and lot of reagents	Methanol, ULC/MS grade (Biosolve) Acetonitrile, ULC/MS grade (Biosolve) Ammonium acetate, LC-MS grade (Merck) Ultra-Pure water (Veolia/Millipore)		
Regression model and weighting	Linear, weighted ($1/x^2$)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	10	23306, Table 11
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-2.6% to 1.4%	

付録 3l. ヒト血漿中メトホルミン濃度測定に用いた分析法の性能の概要（23306）（続き）

	Cumulative precision (%CV) from LLOQ to ULOQ	≤2.2%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	1.7% to 8.5%	23306, Table 13
	Overall CV (%CV)	≤3.1%	
Selectivity and matrix effect	Selectivity for metformin was considered acceptable in 6 individual lots of plasma tested. Interference from blank matrix was ≤17.7% of response from LLOQ standard		23306, Table 6
Hemolysis effect	No significant effect of hemolysis (2%[v/v] whole blood) at LLOQ		23306, Table 6
Lipemic effect	No significant effect of lipemia (≥240 mg/dl triglycerides) at LLOQ		23306, Table 6
Dilution linearity	10-fold dilution of 16000 ng/mL.		23306, Table 15
Bench-top/process stability	56h at +10°C in human plasma 26h at room temperature in human plasma 26h at room temperature for stock solution in methanol		23306, Table 18, Table 20 - 21
Freeze-thaw stability	4 cycles at ≤-18°C		23306, Table 17
Long-term storage	92 days in plasma at ≤-18°C for QC-low, QC-high and OQC-2 117 days spike solutions in methanol at ≤-18°C 49 days spike solutions in methanol at 2-8°C		23306, Table 19, Table 22 - 23
Parallelism	NR		NA
Carryover	Carryover was evaluated by injecting two blank matrix samples after the ULOQ standard in three runs. No significant interference was observed		23306, Table 14

	Method Performance in LE5101 Bioanalytical Report: DMPK 23307	
Assay passing rate	25 of 26 runs (93.1%) met acceptance criteria; One (of 26) run was cancelled due to an error in sample preparation procedure	23307, Table 5
Standard curve performance	metformin: Cumulative accuracy (%bias): -2.8% to 1.9% Cumulative precision (%CV): ≤4.5%	23307, Table 10
QC performance	metformin: Cumulative accuracy (%bias): -5.2% to -2.2% Cumulative precision (%CV): ≤6.4%	23307, Table 14
Method reproducibility	Incurred sample reanalysis was performed on 56 samples, and 51 (91.1%) were within ±20% difference from original results.	23307, Table 20

付録 3l. ヒト血漿中メトホルミン濃度測定に用いた分析法の性能の概要（23306）（続き）

Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples for metformin were stored at ≤-18°C for up to 60 days (20 Mar 2024 to 06 Jun 2024).	23307, Section 1
Standard calibration curve performance during accuracy and precision	8 calibrator sets at 10 concentration levels, from 5.00 to 2000 ng/mL for metformin of which none were excluded from curve fitting	

CV=coefficient of variation; HPLC=high-performance liquid chromatography; HQC=high QC; IS=internal standard; LLOQ=lower limit of quantification; LQC=low QC; MQC=middle QC; MS/MS=tandem mass spectroscopy; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 4 生物薬剤学試験及び食事の影響に関する試験における血漿中 Leniolisib の薬物動態パラメーターの概要

Study Number; Phase; Study Design	No. of Participants (Male/Female) Age Mean [Range]	Treatment	Geometric Mean (Geometric CV%) [n][a]					
			C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	CL/F (L/h)	V_z/F (L)
LE1101 (CSR Study LE1101); Phase 1; Single-center, randomized, open-label, 2-way crossover study	20 healthy participants (17 M/3 F) 32 [19, 58] years	Oral leniolisib 70 mg, capsule, fasted state	2873 (21.5) [n=18]	0.75 [0.50, 2.00] [n=18]	16600 (35.9) [n=18]	16701 (36.0) [n=18]	4.19 (36.0) [n=18]	39.9 (26.1) [n=18]
		Oral leniolisib 70 mg, tablet, fasted state	3102 (21.8) [n=18]	0.63 [0.50, 1.25] [n=18]	17222 (33.8) [n=18]	17327 (33.8) [n=18]	4.04 (33.8) [n=18]	38.9 (24.4) [n=18]
CCDZ173X2101 Part 2 (CSR Study 2101); Phase 1; Randomized, open-label, 2-way crossover, food effect study	12 healthy participants (9 M/3 F) 33.3 [24, 42] years	Oral leniolisib 70 mg, capsule, fed state	1890 (21.2) [n=12]	3.51 [2.50, 6.00] [n=12]	19100 (33.3) [n=12]	19200 (33.2) [n=12]	3.64 (33.2) [n=12]	35.4 (15.1) [n=12]
		Oral leniolisib 70 mg, capsule, fasted state	3190 (18.0) [n=12]	0.642 [0.500, 2.02] [n=12]	19200 (42.0) [n=12]	19300 (42.0) [n=12]	3.63 (42.0) [n=12]	34.6 (27.2) [n=12]
LE1102 (CSR Study LE1102); Phase 1 Single-center, randomized, open-label, 6-way crossover study[b,c]	18 healthy participants (14 M/4 F) 55 [35, 60] years	Oral leniolisib 70 mg, tablet, fasted state	43.7 (18.6) [n=17]	0.66 (46.0) [n=17]	242 (26.6) [n=17]	244 (26.7) [n=17]	4.104 (26.7) [n=17]	ND
		Oral leniolisib 30 mg (3×10 mg), tablet, fasted state	42.2 (16.4) [n=18]	0.66 (44.4) [n=18]	228 (30.0) [n=18]	230 (30.1) [n=18]	4.343 (30.1) [n=18]	ND
		Oral leniolisib 30 mg (1×30 mg), tablet, fasted state	42.0 (15.3) [n=18]	0.64 (40.2) [n=18]	230 (27.6) [n=18]	233 (27.5) [n=18]	4.297 (27.5) [n=18]	ND

Sources: 5.3.1.2 CSR Study LE1101, Table 15.2.2; 5.3.3.1 CSR Study 2101, Table 14.2-2.1b; CSR LE 1102 Table 14.2.12.

AUC=area under the concentration-time curve; $AUC_{0-\infty}$=AUC from time 0 to infinity; AUC_{0-t}=AUC from time 0 to the last measurable concentration; CL/F=apparent clearance; C_{max}=maximum concentration; CV=coefficient of variation; F=female; M=male; n=number with estimable parameter; ND=not determined; T_{max}=time to maximum concentration; V_z/F=apparent volume of distribution.

[a] Median [minimum, maximum] [n] presented for T_{max}.

付録 4 生物薬剤学試験及び食事の影響に関する試験における血漿中 Leniolisib の薬物動態パラメーターの概要（続き）

Study Number; Phase; Study Design	No. of Participants (Male/Female) Treatment Age Mean [Range]	Geometric Mean (Geometric CV%) [n][a]					
		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng*h/mL)	$AUC_{0-\infty}$ (ng*h/mL)	CL/F (L/h)	V_z/F (L)

[b] Study LE1102 included three additional formulations (40 mg FCT, 50 mg FCT, and 30 mg film-coated granules) that are not described here as they were completed for other regions and are not applicable to this submission.

[c] Study LE1102 parameters are dose normalized (C_{max}/D [ng/mL/mg], AUC_{0-t} /D, [ng*h/mL/mg], $AUC_{0-\infty}$/D, [ng*h/mL/mg])