付録 3b. ヒト血漿中 Leniolisib 濃度測定に用いた分析法の性能の概要（15BASM071V1）

Bioanalytical method validation report name, amendments, and hyperlinks	Quantitative determination of CDZ173 in human plasma by LC-MS/MS Method Validation Report DMPK R[redacted] Amendment 1 DMPK R[redacted]-01 Assessment of interference from itraconazole, quinidine, ethinylestradiol, and levonorgestrel were performed as within-study validation and are reported in DMPK RCCDZ173X2102 and DMPK RCCDZ173X2104.		
Method description	Protein precipitation of human plasma (K_3EDTA) samples and analysis of diluted samples by LC-MS/MS in MRM mode using ESI as the ionization technique		
Materials used for standard calibration curve and concentration	Leniolisib phosphate, [redacted] Batches 1251002.REF, 1251002, and 1010006837 Calibration standards: 3.00, 6.00, 15.0, 75.0, 300, 800, and 1000 ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE 261-1		
Validated assay range	3.00 to 1000 ng/mL		
Material used for QCs and concentration	Leniolisib phosphate, [redacted] Batches 1251002.REF, 1251002, and 1010006837 QC levels: 3.00 (LLOQ), 7.50 (LQC), 60.0 (GMQC), 500 (MQC), 750 (HQC), and 5000 (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 plasma, K_3EDTA-treated (Bioreclamation IVT) Lots 15BAS0051B.0001, 15BAS0051B.0002, 15BAS0075B.0006, 15BAS0051B.0003, 14BAS0178B.0001, 15BAS0129B.0002, and 16BAS0251B.0001 Itraconazole (Tokyo Chemical Industry) lot IRNNI Methanol, HPLC grade (Merck) Acetonitrile, HPLC grade (Merck) Isopropanol, HPLC grade (J.T. Baker) Formic acid, ACS grade (Sigma) Purified deionized water using ELGA Lab Water System (Lowell)		
Regression model and weighting	Linear (y=ax+b), weighted ($1/x^2$)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	7	DMPK R[redacted]-01, Table 4-16

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during accuracy and precision runs	Cumulative accuracy (%bias) from LLOQ to ULOQ	-0.7% to 1.2%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤3.4%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QC levels**	1.7% to 12.0%	DMPK R[redacted] Table 4-17
	Interbatch %CV	≤4.9%	
	Total error	NR	
Selectivity and matrix effect	Selectivity for leniolisib was considered acceptable in 6 individual lots of plasma tested. Interference from blank matrix was ≤1.0% of response at LLOQ for leniolisib and 0% of response at working concentration for IS. Matrix effect from LQC to HQC: Mean matrix factor: 1.07 to 1.09, precision (%CV): ≤4.8% Mean IS-normalized matrix factor: 0.95 to 1.03, precision (%CV): ≤1.4%		DMPK R[redacted] Table 4-1, Table 4-2, Table 4-6, Table 4-7, and Table 4-8
Interference and specificity	No significant chromatographic interference from IS, itraconazole (2500 ng/mL), quinidine (5000 ng/mL), ethinylestradiol (5.00 ng/mL), or levonorgestrel (0.500 ng/mL) was detected at the retention time of leniolisib.		DMPK R[redacted] Table 4-3, Table 4-4, and Table 4-5 DMPK RCCDZ173X2102, Table 5-14 DMPK RCCDZ173X2104, Table 5-5
Hemolysis effect	No significant effect of hemolysis (2%[v/v] whole blood) at LQC (7.5 ng/mL) and HQC (750 ng/mL). Accuracy (%bias): 1.7% to 2.9% Precision (%CV): ≤1.7%		DMPK R[redacted] Table 4-9
Lipemic effect	No significant effect of lipemia (≥20 mg/mL lipid concentration) at LQC (7.5 ng/mL) and HQC (750 ng/mL). Accuracy (%bias): 1.5% to 5.5% Precision (%CV): ≤3.2%		DMPK R[redacted] Table 4-10
Dilution linearity and hook effect	10-fold dilution of 5000 ng/mL, accuracy (%bias): 4.8%, precision (%CV): 5.2% Hook effect: NA		DMPK R[redacted] Table 4-19
Bench-top/process stability	21 h in plasma at room temperature. 115 h for processed extract in autosampler at 4°C. 2 h in whole blood at 37°C and in ice bath.		DMPK R[redacted] Table 4-23, Table 4-26, and Table 4-30
Freeze-thaw stability	6 cycles at -20°C and -80°C.		DMPK R[redacted] Table 4-24 and Table 4-25

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Long-term storage	104 days in plasma at -20°C. 783 days in plasma at -80°C.	DMPK R[redacted] Table 4-28 DMPK R[redacted]-01, Table 4-29
Parallelism	NR	NA
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. There was no significant carryover evident for leniolisib (≤16.8% carryover) or IS (≤0.3% carryover) except for Run 18 where leniolisib carryover was 30.9%.	DMPK R[redacted]-01, Table 4-13 and Table 4-14
Cross-validation	Comparison of 50 samples analyzed: 39 of 50 (78%) samples were within ±20.0% difference from the reference laboratory (Novartis Pharma AG) determined value.	DMPK R[redacted] Section 5
	Method Performance in CCDZ173X2102 **Bioanalytical Report: DMPK RCCDZ173X2102**	
Assay passing rate	16 of 17 runs (94%) met acceptance criteria; stock checks and 1 run with syringe wash malfunction excluded from calculation.	DMPK RCCDZ173X2102, Table 5-1 and Table 5-2
Standard curve performance	Cumulative accuracy (%bias): -1.1% to 0.7% Cumulative precision (%CV): ≤4.7%	DMPK RCCDZ173X2102, Table 5-6
QC performance	Cumulative accuracy (%bias): -0.2% to 2.5% Cumulative precision (%CV): ≤4.9% Total error: NR	DMPK RCCDZ173X2102, Table 5-10
Method reproducibility	Incurred sample reanalysis was performed on 50 samples, and 49 (98.0%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2102, Table 9-7
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 43 days (06 Aug 2015 to 18 Sep 2015).	DMPK RCCDZ173X2102, 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 3.00 to 1000 ng/mL (total 224 datapoints) – none excluded from curve fitting	
	Method Performance in CCDZ173X2104 **Bioanalytical Report: DMPK RCCDZ173X2104**	
Assay passing rate	16 of 18 runs (89%) met acceptance criteria; stock checks and 1 run using incorrect column excluded from calculation.	DMPK RCCDZ173X2104, Table 5-1 and Table 5-2
Standard curve performance	Cumulative accuracy (%bias): -3.8% to 3.3% Cumulative precision (%CV): ≤4.0%	DMPK RCCDZ173X2104, Table 5-3
QC performance	Cumulative accuracy (%bias): -2.6% to 0.8% Cumulative precision (%CV): ≤3.8% Total error: NR	DMPK RCCDZ173X2104, Table 5-4

付録 3b. ヒト血漿中 Leniolisib 濃度測定に用いた分析法の性能の概要（15BASM071V1）

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Method reproducibility	Incurred sample reanalysis was performed on 33 samples, and 32 (96.97%) of which were within ±20% difference from original results.	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 were stored at -80°C for up to 82 days (08 Apr 2016 to 29 Jun 2016).	DMPK RCCDZ173X2104, Section 1 and Section 6
Standard calibration curve performance during accuracy and precision	15 calibrator sets with 2 replicates at each of 7 concentration levels from 3.00 to 1000 ng/mL (total 210 datapoints) – none excluded from curve fitting	
Method Performance in CCDZ173X2203 Bioanalytical Report: DMPK RCCDZ173X2203		
Assay passing rate	10 of 10 runs (100%) met acceptance criteria; stock checks excluded from calculation.	DMPK RCCDZ173X2203, Table 5-1 and Table 5-2
Standard curve performance	Cumulative accuracy (%bias): -1.8% to 1.3% Cumulative precision (%CV): ≤5.7%	DMPK RCCDZ173X2203, Table 5-3
QC performance	Cumulative accuracy (%bias): -0.3% to 4.3% Cumulative precision (%CV): ≤5.7% Total error: NR	DMPK RCCDZ173X2203, Table 5-4
Method reproducibility	Incurred sample reanalysis was performed on 30 samples, and 29 (96.7%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2203, Table 9-3
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples were stored at ≤-70°C for up to 329 days (05 Jul 2016 to 30 May 2017).	DMPK RCCDZ173X2203, Section 1 and Section 6
Standard calibration curve performance during accuracy and precision	10 calibrator sets with 2 replicates at each of 7 concentration levels from 3.00 to 1000 ng/mL (total 140 datapoints) – none excluded from curve fitting.	

CV=coefficient of variation; ESI=electrospray ionization; GMQC=geometric QC; HPLC=high-performance liquid chromatography; 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; MRM=multiple reaction monitoring; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3c. ヒト血漿中 Leniolisib 濃度測定に用いた分析法の性能の概要（PKH/MOA/1031）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of a LC-MS/MS analytical method for CDZ173 assay in human plasma samples Method Validation Report DMPK R[redacted] Amendment 1 DMPK R[redacted]-01 Partial validation to extend the quantitation range from 3.00-1000 ng/mL to 5.00-5000 ng/mL is summarized in Appendix 3d.		
Method description	Protein precipitation of human plasma (K_3EDTA) samples and analysis of diluted samples by LC-MS/MS in MRM mode using ESI as the ionization technique		
Materials used for standard calibration curve and concentration	Leniolisib phosphate, [redacted] Batches 1010013786 and 1010013796 Calibration standards: 3.00, 6.00, 25.0, 125, 250, 500, 900, and 1000 ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE261-1/SVI-ALS-18-056		
Validated assay range	3.00 to 1000 ng/mL		
Material used for QCs and concentration	Leniolisib phosphate, [redacted] Batch 1010013786 QC levels: 3.00 (LLOQ), 9.00 (LQC), 500 (MQC), 800 (HQC), and 5000 (Dilution QC) ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE261-1/SVI-ALS-18-056		
Minimum required dilutions	NA		
Source and lot of reagents	Methanol, HPLC grade (Carlo Erba) Acetonitrile, HPLC grade (Carlo Erba) Isopropanol, HPLC grade (Carlo Erba) Formic acid, HPLC grade (Carlo Erba) Purified deionized water using Milli-Q Gradient System (Millipore) Human plasma, K_3EDTA-treated (Valley Biomedical and Bio IVT) batches 8D0779-01 to 06 R270418 and BRH1490291 to 296 R30518, BRH1593242 and 244 R291118, 0383975 R050320, BRH 1593242 R291118, 0383842 R05032020, and BRH1515308 R260618, respectively		
Regression model and weighting	Linear (y=ax+b), 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	8	DMPK R[redacted]01, Table 5-14
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.10% to 2.70%	

付録 3c. ヒト血漿中 Leniolisib 濃度測定に用いた分析法の性能の概要（PKH/MOA/1031）
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	Cumulative precision (%CV) from LLOQ to ULOQ	≤4.61%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	-2.42% to -1.11%	DMPK R[redacted]01, Table 5-15
	Interbatch %CV	≤7.07%	
	Total error	NR	
Selectivity and matrix effect	Selectivity for leniolisib was considered acceptable in 6 batches of plasma tested. No peak for leniolisib or IS was detected in blank samples. Matrix effect from LQC (9.00 ng/mL) to HQC (800 ng/mL): Mean matrix factor: 1.0 to 1.1, precision (%CV): ≤2.29% Mean IS-normalized matrix factor: 1.0, precision (%CV): ≤3.12%		DMPK R[redacted]-01, Table 5-1, Table 5-2, Table 5-5, Table 5-6, and Table 5-7
Interference and specificity	No significant chromatographic interferences from IS on the detection of leniolisib (no interfering peaks) and from leniolisib on detection of IS (interference ≤0.1%).		DMPK R[redacted]-01, Table 5-3 and Table 5-4
Hemolysis effect	No significant effect of hemolysis (5% hemolyzed plasma) from LQC (9.00 ng/mL) to HQC (800 ng/mL). Accuracy (%bias): -1.69% to -0.99% Precision (%CV): ≤3.97%		DMPK R[redacted]01, Table 5-20
Lipemic effect	No significant effect of lipemia (hyperlipidemic plasma) from LQC (9.00 ng/mL) to HQC (800 ng/mL). Accuracy (%bias): -0.83% to 0.11% Precision (%CV): ≤3.86%		DMPK R[redacted]-01, Table 5-21
Dilution linearity and hook effect	10- and 100-fold dilution of 5000 ng/mL, accuracy (%bias): -6.46% to -2.44%, precision (%CV): ≤6.67% Hook effect: NA		DMPK R[redacted]-01, Table 5-17
Bench-top/process stability	4 h 7 min in plasma at room temperature. 57 h 59 min for processed extract at 4°C. 1 h in whole blood at room temperature.		DMPK R[redacted]-01, Table 5-23, Table 5-24, and Table 5-28
Freeze-thaw stability	3 cycles at -20°C and 4 cycles at -80°C.		DMPK R[redacted]-01, Table 5-25 and Table 5-26
Long-term storage	733 and 1183 days in plasma at -20°C and -80°C, respectively.		DMPK R[redacted]-01, Table 5-29 and Table 5-30
Parallelism	NR		NA
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. There was no carryover evident for leniolisib and IS (no peaks observed).		DMPK R[redacted]-01, Table 5-11 and Table 5-12

付録 3c. ヒト血漿中 Leniolisib 濃度測定に用いた分析法の性能の概要（PKH/MOA/1031）

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Cross-validation	Comparison of 24 samples analyzed: 20 of 24 (83%) samples were within ±15.0% difference (±20.0% difference at LLOQ) from the reference laboratory (WuXi AppTec) determined value.	DMPK R[redacted]01, Appendix C
	Method Performance in LE1101 **Bioanalytical Report: LE 1101 BAR LE**	
Assay passing rate	10 of 10 runs (100%) met acceptance criteria (excluding a run for preparation of standards and QCs from calculation)	LE 1101 BAR LE, Table 3
Standard curve performance	Cumulative accuracy (%bias): -1.04% to 0.94% Cumulative precision (%CV): ≤5.39	LE 1101 BAR LE, Table 4
QC performance	Cumulative accuracy (%bias): -2.40% to -0.22% Cumulative precision (%CV): ≤5.02% Total error: NR	LE 1101 BAR LE, Table 7
Method reproducibility	Incurred sample reanalysis was performed on 79 samples, and 76 (96.20%) of which had <20% difference from the original results.	LE 1101 BAR LE, Table 8
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples were stored at ≤-20°C for up to 84 days (14 Sep 2021 to 13 Jan 2022).	LE 1101 BAR LE, Table 9
Standard calibration curve performance during accuracy and precision	11 calibrator sets with 1 replicate at each of 8 concentration levels from 5.00 to 5000 ng/mL (total 88 datapoints) – none excluded from curve fitting.	
	Method Performance in LE2101 **Bioanalytical Report: LE 2101 BAR LE**	
Assay passing rate	4 of 4 runs (100%) met acceptance criteria (excluding a run for preparation of frozen QCs from calculation)	LE 2101 BAR LE, Table 2
Standard curve performance	Cumulative accuracy (%bias): -2.56% to 3.99% Cumulative precision (%CV): ≤3.90%	LE 2101 BAR LE, Table 3
QC performance	Cumulative accuracy (%bias): -2.59% to 0.87% Cumulative precision (%CV): ≤4.55% Total error: NR	LE 2101 BAR LE, Table 6
Method reproducibility	Incurred sample reanalysis was performed on 32 samples, and 23 (71.88%) of which were within ±20% difference from original results.	LE 2101 BAR LE, Table 9
Study sample analysis stability	Stability data in matrix cover the sample collection, storage, and analysis period in this study. Samples were stored at -20°C for up to 32 days (12 Nov 2021 to 14 Dec 2021).	LE 2101 BAR LE, Table 10
Standard calibration curve performance during accuracy and precision	4 calibrator sets with 1 replicate at each of 8 concentration levels from 5.00 to 5000 ng/mL (total 32 datapoints) – none excluded from curve fitting.	

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Method Performance in CCDZ173X2201 Part 2 Bioanalytical Report: DMPK RCCDZ173X2201a		
Assay passing rate	8 of 8 runs (100%) met acceptance criteria	DMPK RCCDZ173X2201a, Table 9-3
Standard curve performance	Cumulative accuracy (%bias): -2.14% to 1.39% Cumulative precision (%CV): ≤8.67%	DMPK RCCDZ173X2201a, Table 5-4
QC performance	Cumulative accuracy (%bias): -3.26% to -1.86% Cumulative precision (%CV): ≤6.90% Total error: NR	DMPK RCCDZ173X2201a, Table 5-6
Method reproducibility	Incurred sample reanalysis was performed on 30 samples, and 25 (83.3%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2201a, Table 9-4
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 727 days (11 Sep 2018 to 07 Sep 2020).	DMPK RCCDZ173X2201a, Section 6 and Table 9-5
Standard calibration curve performance during accuracy and precision	8 calibrator sets with 1 replicate at each of 8 concentration levels from 3.00 to 1000 ng/mL (total 64 datapoints) – none excluded from curve fitting.	
Method Performance in CCDZ173X2201E1 Bioanalytical Report: DMPK RCCDZ173X2201E1		
Assay passing rate	11 of 11 runs (100%) met acceptance criteria	DMPK RCCDZ173X2201E1, Table 9-3
Standard curve performance	Cumulative accuracy (%bias): -1.15% to 1.25% Cumulative precision (%CV): ≤5.53%	DMPK RCCDZ173X2201E1, Table 5-4
QC performance	Cumulative accuracy (%bias): -1.82% to -1.41% Cumulative precision (%CV): ≤5.22% Total error: NR	DMPK RCCDZ173X2201E1, Table 5-6
Method reproducibility	Incurred sample reanalysis was performed on 30 samples, and 25 (83.3%) of which were within ±20% difference from original results.	DMPK RCCDZ173X2201E1, Table 9-4
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 1088 days (07 Sep 2018 to 30 Aug 2021).	DMPK RCCDZ173X2201E1, Section 6 and Table 9-5
Standard calibration curve performance during accuracy and precision	11 calibrator sets with 1 replicate at each of 8 concentration levels from 3.00 to 1000 ng/mL (total 88 datapoints) – none excluded from curve fitting.	

CV=coefficient of variation; ESI=electrospray ionization; HPLC=high-performance liquid chromatography; 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; MRM=multiple reaction monitoring; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3d. ヒト血漿中 Leniolisib 濃度測定法のパーシャルバリデーションの概要
（PKH/MOA/1464）

Bioanalytical method validation report name, amendments, and hyperlinks	Partial validation of a LC-MS/MS analytical method to measure leniolisib in human plasma Validation Report 21-0315A		
Changes in method	The quantitation range was extended from the 3.00 to 1000 ng/mL in the original validation to 5.00 to 5000 ng/mL in this partial validation.		
New validated assay range	5.00 to 5000 ng/mL		
Validation Parameters	**Partial Validation Performance**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Inter-run accuracy (%bias) from LLOQ to ULOQ	-3.33% to 4.59%	21-0315A, Table 4
	Inter-run precision (%CV) from LLOQ to ULOQ	≤5.24%	21-0315A, Table 4
Performance of QCs during accuracy and precision runs	Inter-run accuracy (%bias) in 4 QC levels	-3.60% to 2.23%	21-0315A, Table 6
	Inter-run %CV	≤5.18%	21-0315A, Table 6
	Total error	NR	NA
Interference and specificity	Contribution of IS to the signal of leniolisib (≤7.44% interference) and of leniolisib to the signal of IS (≤0.17% interference) was not significant.		21-0315A, Table 3
Dilution linearity	5-fold dilution of 10000 ng/mL, accuracy (%bias): -6.14%, precision (%CV): 2.20%		21-0315A, Table 10
Bench-top/process stability	12 days 9 h 15 min for processed extracts in autosampler at 4°C. 1 h in whole blood at room temperature. 4 h 7 min in plasma at room temperature.		21-0315A, Table 12, Table 14, and Table 15
Freeze-thaw stability	4 cycles at -20°C and -80°C.		21-0315A, Table 17
Long-term storage	138 days at -20°C and -80°C.		21-0315A, Table 18 and Table 19
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. There was no significant carryover evident for leniolisib (≤13.82% carryover) or IS (0% carryover).		21-0315A, Table 8 and Table 9
	Method Performance in LE1102 **Bioanalytical Report: LE 1102 BAR LE**		
Assay passing rate	20 of 20 runs (100%) met acceptance criteria		LE 1102 BAR LE, Table 2
Standard curve performance	Cumulative accuracy (%bias): -2.27% to 3.57% Cumulative precision (%CV): ≤4.15		LE 1102 BAR LE, Table 4

付録 3d. ヒト血漿中 Leniolisib 濃度測定法のパーシャルバリデーションの概要（PKH/MOA/1464）（続き）

QC performance	Cumulative accuracy (%bias): -4.13% to -0.36% Cumulative precision (%CV): ≤4.78% Total error: NR	LE 1102 BAR LE, Table 8
Method reproducibility	Incurred sample reanalysis was performed on 160 samples, and 157 (98.125%) of which had <20% difference from the original results.	LE 1102 BAR LE, Table 9
Study sample analysis stability	Samples were received frozen and stored at -80°C. Samples were analyzed within 83 days, which is within the established stability limit of 978 days at -80°C.	LE 1102 BAR LE, Table 1
Standard calibration curve performance during accuracy and precision	20 calibrator sets with 1 replicate at each of 8 concentration levels from 5.00 to 5000 ng/mL (total 160 datapoints) – none excluded from curve fitting.	
	Method Performance in LE4301 **Bioanalytical Report: LE 4301 BAR LE**	
Assay passing rate	2 of 4 runs (50%) met acceptance criteria	LE 4301BAR LE, Table 2
Standard curve performance	Cumulative accuracy (%bias): -3.06% to 3.39% Cumulative precision (%CV): ≤8.72	LE 4301BAR LE, Table 4
QC performance	Cumulative accuracy (%bias): -4.64% to -1.33% Cumulative precision (%CV): ≤9.84% Total error: NR	LE 4301BAR LE, Table 10
Method reproducibility	Incurred sample reanalysis was performed on 21 samples, and 21 (100%) of which had <20% difference from the original results.	LE 4301BAR LE, Table 13
Study sample analysis stability	Samples were received frozen and stored at -80°C. Samples were analyzed within 281 days, which is within the established stability limit of 978 days at -80°C.	LE 4301BAR LE, Table 15
Standard calibration curve performance during accuracy and precision	2 calibrator sets with 1 replicate at each of 8 concentration levels from 5.00 to 5000 ng/mL (total 16 datapoints) – none excluded from curve fitting.	
	Method Performance in LE5101 **Bioanalytical Report: LE 5101 BAR LE**	
Assay passing rate	3 of 3 runs (100%) met acceptance criteria	LE 5101 BAR LE, Table 2
Standard curve performance	Cumulative accuracy (%bias): -2.97% to 2.37% Cumulative precision (%CV): ≤5.84	LE 5101 BAR LE, Table 4
QC performance	Cumulative accuracy (%bias): -7.73% to -2.16% Cumulative precision (%CV): ≤5.58% Total error: NR	LE 5101 BAR LE, Table 8

付録 3d. ヒト血漿中 Leniolisib 濃度測定法のパーシャルバリデーションの概要（PKH/MOA/1464）（続き）

Method reproducibility	Incurred sample reanalysis was performed on 21 samples, and 19 (90.48%) of which had <20% difference from the original results.	LE 5101 BAR LE, Table 9
Study sample analysis stability	Samples were received frozen and stored at -80°C. Samples were analyzed within 41 days, which is within the established stability limit of 399 days at -80°C.	LE 5101 BAR LE, Table 11
Standard calibration curve performance during accuracy and precision	3 calibrator sets with 1 replicate at each of 8 concentration levels from 5.00 to 5000 ng/mL (total 24 datapoints) – none excluded from curve fitting.	

CV=coefficient of variation; IS=internal standard; LC-MS/MS=liquid chromatography coupled with tandem mass spectroscopy; LLOQ=lower limit of quantification; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3e. ヒト血漿中 Leniolisib 濃度測定法のパーシャルバリデーション（別の内標準物質を使用）の概要（PKH/MOA/1464）

Bioanalytical method validation report name, amendments, and hyperlinks	Partial validation of a LC-MS/MS analytical method to measure leniolisib in human plasma Validation Report 23-0395		
Changes in method	The isotopic IS was changed from [13C,2H3]-CDZ173 in the original validation to [$^{13}CD_3$] CDZ173-AZA in this partial validation.		
New validated assay range	5.00 to 5000 ng/mL		
Validation Parameters	**Partial Validation Performance**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Inter-run accuracy (%bias) from LLOQ to ULOQ	NR	NA
	Intra-run precision (%CV) from LLOQ to ULOQ	NR	NA
Performance of QCs during accuracy and precision runs	Intra-run accuracy (%bias) in 4 QC levels	-13.29% to -1.95%	23-0395, Table 8
	Intra-run %CV	≤9.05%	23-0395, Table 8
	Total error	NR	NA
Interference and specificity	Contribution of IS to the signal of leniolisib (≤5.04% interference) and of leniolisib to the signal of IS (≤0.05% interference) was not significant.		23-0395, Table 5
Carryover	Carryover was evaluated by injecting blank matrix samples after the ULOQ standard. Carryover was observed in the first two S0. No interference greater than 20.00% (≤11.85%) was observed at the retention time and in the MRM trace of Leniolisib after two blanks were injected after the highest calibration standard. Therefore, two blanks will be injected after the highest calibration standard during sample analysis. No interference was observed at the retention time and in the MRM trace of [13C, 2H3]- CDZ173, in any of the three blanks injected after the highest calibration standard.		23-0395, Table 10 and Table 11

CV=coefficient of variation; IS=internal standard; LC-MS/MS=liquid chromatography coupled with tandem mass spectroscopy; LLOQ=lower limit of quantification; MRM=multiple reaction monitoring; NA=not applicable; NR=not reported; QC=quality control; ULOQ=upper limit of quantification.

付録 3f. ヒト血漿中 Leniolisib のキラル測定の分析法の性能の概要（PKH/MOA/1411）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of a LC-MS/MS analytical chiral method to measure PH-IMP06 and leniolisib in human plasma including sample analysis Method Validation Report DOC-2946
Method description	Protein precipitation of human plasma (K_2EDTA) samples and analysis of diluted samples by LC-MS/MS in MRM mode using ESI as the ionization technique
Materials used for standard calibration curve and concentration	*(R)*-enantiomer of leniolisib, [redacted] Batch SB075-13 Calibration standards: 3.00, 6.00, 25.0, 125, 250, 500, 900, and 1000 ng/mL *(S)*-enantiomer of leniolisib, [redacted] Batch 1010013796 Calibration standards: 3.00, 6.00, 25.0, 125, 250, 500, 900, and 1000 ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE261-1/SVI-ALS-18-056
Validated assay range	3.00 to 1000 ng/mL (both enantiomers)
Material used for QCs and concentration	*(R)*-enantiomer of leniolisib, [redacted] Batch SB075-13 QC levels: 3.00 (LLOQ), 9.00 (LQC), 500 (MQC), 800 (HQC), and 2000 (Dilution QC) ng/mL *(S)*-enantiomer of leniolisib, [redacted] Batch 1010013796 QC levels: 3.00 (LLOQ), 9.00 (LQC), 500 (MQC), 800 (HQC), and 2000 (Dilution QC) ng/mL Internal standard: $^{13}CD_3$-leniolisib, [redacted] Batch SSE261-1/SVI-ALS-18-056
Minimum required dilutions	NA
Source and lot of reagents	Human plasma (Bio IVT) Batches 0391695 R080721, 0391745 R070721, 0391651 R080721, 0391675 R080721, 0391684 R080721, 0391697 R080721, 0391726 R080721, 0393860 R121021, 0393867 R121021, and HMN716353 R151221 Hemolyzed plasma (Eurofins|ADME Bioanalyses) Batches MACA P180821 and MECA P100521-2 Human blood (EFS) Batch 72212157230 R170821 Methanol (Carlo Erba) Acetonitrile (Carlo Erba) Isopropanol (Carlo Erba) Formic acid (Carlo Erba) Ammonium formate (Sigma) Purified deionized water using Milli-Q Gradient System
Regression model and weighting	Linear (y=ax+b), weighted ($1/x^2$)

付録 3f. ヒト血漿中 Leniolisib のキラル測定の分析法の性能の概要（PKH/MOA/1411）（続き）

Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8 (both enantiomers)	DOC-2946, Table 13 and Table 14
	Cumulative accuracy (%bias) from LLOQ to ULOQ	*(R)*-enantiomer of leniolisib: -1.02% to 1.53% *(S)*-enantiomer of leniolisib: -0.84% to 0.99%	
	Cumulative precision (%CV) from LLOQ to ULOQ	*(R)*-enantiomer of leniolisib: ≤5.29% *(S)*-enantiomer of leniolisib: ≤5.07%	
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QC levels**	*(R)*-enantiomer of leniolisib: 2.83% to 3.80% *(S)*-enantiomer of leniolisib: 2.32% to 4.30%	DOC-2946, Table 17 and Table 18
	Interbatch %CV	*(R)*-enantiomer of leniolisib: ≤4.91% *(S)*-enantiomer of leniolisib: ≤3.17%	
	Total error	NR	
Selectivity and matrix effect	Selectivity for leniolisib was considered acceptable in 6 batches of plasma tested. No peaks for *(R)*- or *(S)*-enantiomers of leniolisib or IS were detected in blank samples except in 2 batches where interference from *(R)*-enantiomer of leniolisib was ≤1.49%. Matrix effect from LQC (9.00 ng/mL) to HQC (800 ng/mL): Mean matrix factor: *(R)*-enantiomer of leniolisib: 0.8; *(S)*-enantiomer of leniolisib: 0.9 Mean IS-normalized matrix factor: *(R)*-enantiomer of leniolisib: 0.9; *(S)*-enantiomer of leniolisib: 1.0		DOC-2946, Table 6, Table 7, Table 8, Table 24, Table 26, Table 27, and Table 29
Interference and specificity	No significant chromatographic interferences from IS on the detection of *(R)*- or *(S)*-enantiomers of leniolisib (no interfering peaks) and from *(R)*- or *(S)*-enantiomers of leniolisib on detection of IS (no interfering peaks). No significant chromatographic interferences from *(R)*-enantiomer of leniolisib on the detection *(S)*-enantiomer of leniolisib (no interfering peaks) and from *(S)*-enantiomer of leniolisib on the detection *(R)*-enantiomer of leniolisib (<6% interference).		DOC-2946, Table 9, Table 10, Table 11, and Table 12
Hemolysis effect	No significant effect of hemolysis (5% hemolyzed plasma) from LQC (9.00 ng/mL) to HQC (800 ng/mL) for both enantiomers. *(R)*-enantiomer of leniolisib: Accuracy (%bias): 0.79% to 3.79% Precision (%CV): ≤4.40% *(S)*-enantiomer of leniolisib: Accuracy (%bias): -0.21% to -0.07% Precision (%CV): ≤1.44%		DOC-2946, Table 36 and Table 37

付録 3f. ヒト血漿中 Leniolisib のキラル測定の分析法の性能の概要（PKH/MOA/1411）（続き）

Lipemic effect	NA	NA
Dilution linearity and hook effect	Dilution integrity was maintained at 5-fold dilution of 2000 ng/mL for both enantiomers. *(R)*-enantiomer of leniolisib: Accuracy (%bias): -3.21% Precision (%CV): 3.10% Hook effect: NA *(S)*-enantiomer of leniolisib: Accuracy (%bias): -0.60% Precision (%CV): 1.64% Hook effect: NA	DOC-2946, Table 38 and Table 39
Bench-top/process stability	*(R)*- and *(S)*-enantiomer of leniolisib: 4 h 15 min in plasma at room temperature 110 h 56 min for processed extract at 4°C 1 h in whole blood at room temperature	DOC-2946, Table 46, Table 47, Table 50, Table 51, Table 52, and Table 53
Freeze-thaw stability	*(R)*- and *(S)*-enantiomer of leniolisib: 4 cycles at -80°C	DOC-2946, Table 56 and Table 57
Long-term storage	*(R)*-enantiomer of leniolisib: 33 days in plasma at -20°C and -80°C *(S)*-enantiomer of leniolisib: 146 days in plasma at -20°C and -80°C	DOC-2946, Table 58, Table 59, Table 61, and Table 62
Parallelism	NR	NA
Carryover	Carryover was evaluated in 3 validation runs by injecting 3 blank matrix samples after the ULOQ standard. There was no significant carryover evident for *(R)*- or *(S)*-enantiomers of leniolisib or the IS (7.04% interference).	DOC-2946, Table 21, Table 22, and Table 23
	Method Performance in CCDZ173X2201E1 **Bioanalytical Report: DOC-2946**	
Assay passing rate	*(R)*-enantiomer of leniolisib: 2 of 4 runs (50%) met acceptance criteria *(S)*-enantiomer of leniolisib: 4 of 4 runs (100%) met acceptance criteria	DOC-2946, Table 67
Standard curve performance	*(R)*-enantiomer of leniolisib: Cumulative accuracy (%bias): -2.19% to 3.78% Cumulative precision (%CV): ≤2.17% *(S)*-enantiomer of leniolisib: Cumulative accuracy (%bias): -3.37% to 2.16% Cumulative precision (%CV): ≤7.83%	DOC-2946, Table 13 and Table 14
QC performance	*(R)*-enantiomer of leniolisib: Cumulative accuracy (%bias): -2.01% to 1.27% Cumulative precision (%CV): ≤4.71% Total error: NR *(S)*-enantiomer of leniolisib: Cumulative accuracy (%bias): -2.19% to 3.84% Cumulative precision (%CV): ≤4.98% Total error: NR	DOC-2946, Table 19 and Table 20

付録 3f. ヒト血漿中 Leniolisib のキラル測定の分析法の性能の概要（PKH/MOA/1411）（続き）

Method reproducibility	*(R)*-enantiomer of leniolisib: 15 of 34 samples were injected nondiluted and diluted in 2 separate runs. All samples from both runs were BLQ. *(S)*-enantiomer of leniolisib: 10 of 34 samples were reanalyzed in a separate run from the original assay. Results from reanalysis matched results from the original assays.	DOC-2946, Section 7.2
Study sample analysis stability	Samples were collected starting 12 Jan 2021 and analyzed by 13 Jan 2022. Validation of long-term stability at >146 days at -20°C and -80°C remain ongoing.	DOC-2946, Section 7, Table 67, and Table 68
Standard calibration curve performance during accuracy and precision	*(R)*-enantiomer of leniolisib: 2 calibrator sets with 1 replicate at each of 8 concentration levels from 3.00 to 1000 ng/mL (total 16 datapoints) – none excluded from curve fitting. *(S)*-enantiomer of leniolisib: 4 calibrator sets with 1 replicate at each of 8 concentration levels from 3.00 to 1000 ng/mL (total 32 datapoints) – none excluded from curve fitting.	

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