Analytical Method Validation Report

Assay of Nimbatinib Tablets, 50 mg by Reversed-Phase HPLC

Validated per ICH Q2(R1)/Q2(R2) — Specificity, Linearity, Accuracy, Precision, Range, Detection/Quantitation Limits, Robustness

Report No.: VAL-AM-2026-0417
Protocol No.: VP-AM-0142-03
Method No. / Version: AM-HPLC-0142, v3.0
Product: Nimbatinib Tablets, 50 mg, film-coated
Drug Substance Lot: Nimbatinib RS, Lot RS-2214 (assigned purity 99.8%)

Tablet Batch Used: NMB-0623B (placebo blend PLB-0091)
Sponsor: Meridian Pharmaceuticals, Inc.
Testing Facility: Vertex Analytical Laboratories, Method Validation Group
Study Dates: 2026-04-13 to 2026-05-08
Regulatory Basis: ICH Q2(R1) [1]; ICH Q2(R2) [2]; USP ⟨1225⟩ [4]

Purpose

This report documents the validation of analytical method **AM-HPLC-0142, Version 3.0**, used for the quantitative determination of Nimbatinib in Nimbatinib Tablets, 50 mg, by reversed-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet detection. Validation was executed against approved protocol VP-AM-0142-03 and evaluates specificity, linearity, range, accuracy, precision, detection/quantitation limits, and robustness, in accordance with ICH Q2 guidance and the sponsor's Analytical Quality Agreement. The method is intended for routine release and stability testing of the drug product.

Approval

Role	Name	Signature	Date
Principal Analyst	Priya Nandakumar, M.Sc.	_____	2026-05-08
Study Director	Elena Kowalski, Ph.D.	_____	2026-05-08
QA Reviewer	Rajesh Patel	_____	2026-05-09
Sponsor Representative	Marcus Webb, Dir. Analytical Dev. (Meridian)	_____	2026-05-12

1 Scope and Method Summary

1.1 Scope

This validation applies to the assay determination of Nimbatinib in Nimbatinib Tablets, 50 mg, across the labelled dosage strength and manufacturing process registered under Meridian Pharmaceuticals product code MER-NMB-050. The method is stability-indicating: the same chromatographic conditions are used to resolve and quantify the principal specified degradation product, Impurity A (des-methyl nimbatinib), from the active moiety. Results in this report were generated on the Nimbus LC-3200 platform; a bridging verification on an alternate HPLC platform is documented separately under transfer protocol VT-AM-0142-01 and is out of scope here.

1.2 Method Conditions

Parameter	Condition
Instrument	Nimbus LC-3200 HPLC with photodiode-array (PDA) detector, S/N NX-LC-0417
Column	Apex C18, 150 × 4.6 mm, 3.5 µm (Lot AC-2291)
Mobile Phase A	0.1% v/v phosphoric acid in water, pH 2.3 ± 0.1
Mobile Phase B	Acetonitrile, HPLC grade
Gradient	20% B → 65% B over 12 min, hold 2 min, re-equilibrate 5 min
Flow Rate	1.0 mL min^{-1}
Column Temperature	30 °C
Detection Wavelength	268 nm (PDA scan 200–400 nm acquired for peak-purity assessment)
Injection Volume	10 µL
Run Time	15 min
Diluent	Mobile Phase A : Acetonitrile (70:30, v/v)
Needle Wash	Diluent, 400 µL

1.3 System Suitability

System suitability was assessed from six replicate injections of the Nimbatinib working standard solution (0.500 mg mL^{-1} nominal) at the start of each analytical sequence.

Parameter	Result	Criterion	Verdict
Retention time, mean (min)	8.42	Report only	—
Retention time %RSD (n = 6)	0.18	≤ 1.0%	✔ PASS
Tailing factor	1.12	≤ 2.0	✔ PASS
Theoretical plates	8650	≥ 2000	✔ PASS
Resolution vs. Impurity A	3.80	≥ 2.0	✔ PASS
Peak area %RSD (n = 6)	0.31	≤ 2.0%	✔ PASS

Acceptance Criteria

System suitability must pass at the head of every analytical sequence before sample results are reportable; a failing sequence is invalidated and re-injected per SOP QC-INJ-009 prior to any deviation being raised.

2 Specificity and Stability-Indicating Capability

2.1 Forced Degradation Design

Specificity was demonstrated by subjecting Nimbatinib drug substance and placebo blend PLB-0091 to forced degradation, then confirming that the Nimbatinib peak was free of co-eluting interference and chromatographically resolved from all degradation products and excipient peaks. Peak purity was evaluated from PDA spectral data (200–400 nm) using the purity-angle/threshold algorithm in Vertex Chrom-Suite v9.2; a peak is considered pure when the purity angle does not exceed the purity threshold.

Stress Condition	Duration	%Degrad.	Angle	Threshold	Pure
Acid hydrolysis, 1 N HCl, 60 °C	2 h	8.4	0.18	0.42	✔ PASS
Base hydrolysis, 1 N NaOH, 60 °C	1 h	12.1	0.21	0.42	✔ PASS
Oxidative, 3% H_2O_2, RT	1 h	6.7	0.15	0.42	✔ PASS
Thermal, dry heat, 105 °C	24 h	3.2	0.12	0.42	✔ PASS
Photolytic, ICH Q1B (1.2 Mlux·h, 200 W·h/m^2 UV)	—	4.5	0.19	0.42	✔ PASS
Humidity, 75% RH / 40 °C	7 d	2.1	0.10	0.42	✔ PASS

2.2 Placebo and Blank Interference

Six independent placebo preparations (PLB-0091) were extracted and injected in accordance with the method. No peak was observed at the Nimbatinib retention time (8.42 min ± 2%) above the reporting threshold of 0.05%. Diluent blank injections showed no carryover exceeding 0.03% of the working standard response.

✔ Conclusion

The Nimbatinib peak remained chromatographically resolved and spectrally pure under all six stress conditions, and neither placebo excipients nor diluent contributed interference at the analyte retention time. The method is specific and stability-indicating for its intended use.

3 Linearity and Range

3.1 Linearity

Linearity was assessed across five concentration levels spanning 50% to 150% of the nominal assay concentration (0.500 mg mL^{-1}), prepared independently in triplicate from Nimbatinib RS Lot RS-2214. Each preparation was injected once; peak area (mAU·s) at 268 nm was plotted against concentration and evaluated by unweighted least-squares linear regression.

Level	Conc. (mg mL^{-1})	Mean Area (n=3)	%RSD	% of Nominal
1	0.250	1 220 800	0.42	50%
2	0.375	1 828 300	0.31	75%
3	0.500	2 435 800	0.28	100%
4	0.625	3 043 300	0.35	125%
5	0.750	3 650 800	0.39	150%

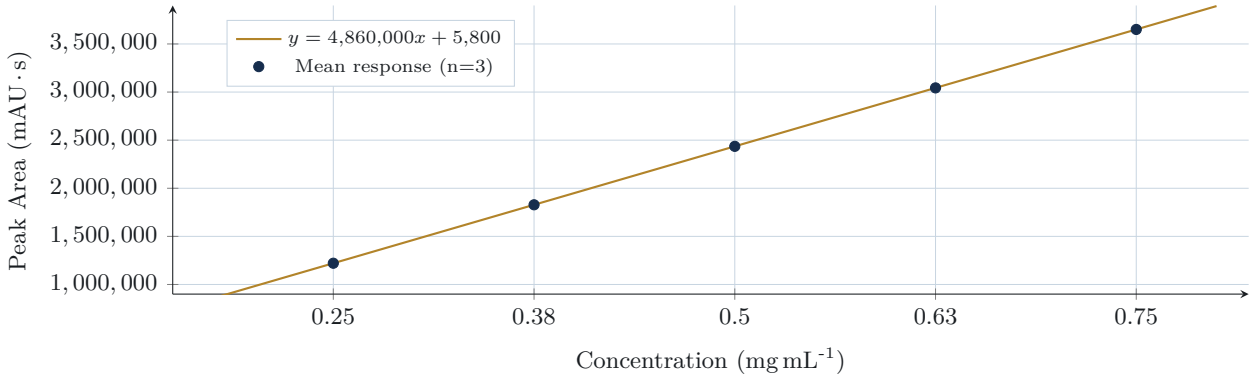

Figure 1. Linearity regression, Nimbatinib peak area versus concentration, 50–150% of nominal (n = 15 preparations across 5 levels).

3.2 Regression Statistics and Acceptance

Statistic	Result	Criterion	Verdict
Correlation coefficient, r	0.9999	≥ 0.999	✔ PASS
Coefficient of determination, r^2	0.9998	Report only	—
Slope	4 860 000	Report only	—
Y-intercept	5800	Report only	—
Y-intercept, % of 100% response	0.24%	$\leq 2.0\%$	✔ PASS

3.3 Range

Acceptance Criteria

Range is established as the interval over which linearity, accuracy, and precision jointly meet their acceptance criteria (ICH Q2(R1) §1.3) [1].

Linearity (Section 3.1), accuracy (Section 4.1), and precision (Section 4.2) each met their acceptance criteria across 50%–150% of the nominal assay concentration. The validated range for method AM-HPLC-0142 is therefore **50% to 150% of the labelled Nimbatinib content** (0.250–0.750 mg mL^{-1} as injected concentration), which fully brackets the 80%–120% window required for routine assay release and the extended window needed for accelerated and long-term stability testing.

4 Accuracy and Precision

4.1 Accuracy (Recovery)

Accuracy was determined by spiking Nimbatinib RS Lot RS-2214 into placebo blend PLB-0091 at three levels bracketing the assay specification (80%, 100%, and 120% of nominal), with three independent preparations per level (n = 9 total).

Level	Added (mg mL^{-1})	Rec. 1 (%)	Rec. 2 (%)	Rec. 3 (%)	Mean (%)	%RSD
80%	0.400	99.4	100.1	99.7	99.73	0.35
100%	0.500	100.3	99.6	100.0	99.97	0.35
120%	0.600	99.8	100.4	99.5	99.90	0.46

Acceptance Criteria

Mean recovery per level: 98.0%–102.0%. %RSD per level: $\leq 2.0\%$. Overall mean recovery (n = 9): 98.0%–102.0%.

Overall mean recovery across all nine preparations was **99.87%** with an overall %RSD of **0.42%**, both within the pre-specified acceptance range. ✔ **PASS**

4.2 Precision

4.2.1 Repeatability

Six preparations of a single homogeneous tablet composite (Batch NMB-0623B) at the 100% level were independently assayed by one analyst on one day, on one instrument.

Rep.	1	2	3	4	5	6	Mean	%RSD
%Assay	99.6	100.2	99.8	100.5	99.4	100.1	99.93	0.40

4.2.2 Intermediate Precision

The repeatability set was repeated on a second day, by a second analyst, using an alternate column lot (AC-2305) on the same instrument, per the intermediate-precision design in protocol VP-AM-0142-03.

Rep.	1	2	3	4	5	6	Mean	%RSD
%Assay	100.4	99.7	100.6	99.9	100.8	99.5	100.15	0.50

Precision Statistic	Result	Criterion	Verdict
Repeatability %RSD (n = 6)	0.40	≤ 2.0%	✔ PASS
Intermediate precision %RSD (n = 6)	0.50	≤ 2.0%	✔ PASS
Pooled %RSD, both days (n = 12)	0.46	≤ 2.0%	✔ PASS
Absolute difference between day means	0.22	≤ 2.0%	✔ PASS

5 Robustness and Sensitivity

5.1 Robustness

Deliberate small variations were introduced, one factor at a time, around the nominal method conditions to evaluate their effect on system suitability. The working standard solution (0.500 mg mL^{-1}) was injected in duplicate at each condition.

Parameter	Variation	Resolution	Tailing	Area %RSD	Verdict
Flow rate	0.9 and 1.1 mL min^{-1} (nominal 1.0)	3.62	1.15	0.38	✔ PASS
Column temperature	25 and 35 °C (nominal 30 °C)	3.71	1.13	0.29	✔ PASS
Mobile phase A pH	2.1 and 2.5 (nominal 2.3)	3.44	1.18	0.41	✔ PASS
%Organic at start of gradient	18% and 22% B (nominal 20%)	3.35	1.16	0.44	✔ PASS
Column lot	Alternate lot AC-2305 vs. AC-2291	3.58	1.14	0.33	✔ PASS

Acceptance Criteria

Under every deliberate variation: resolution vs. Impurity A ≥ 2.0; tailing factor ≤ 2.0; peak area %RSD ≤ 2.0%.

5.1.1 Solution Stability

Standard and sample solutions, stored capped at 2–8 °C and protected from light, were re-injected at 24 h and 48 h against freshly prepared reference solutions. Peak area for the Nimbatinib peak differed from the initial (0 h) result by no more than 0.8% at 24 h and 1.4% at 48 h (criterion: ≤ 2.0% difference). Both solutions are assigned a validated stability hold time of **48 hours at 2–8 °C**.

5.2 Limit of Detection and Limit of Quantitation

LOD and LOQ were established for Impurity A, the principal specified degradation product co-quantified by this method, using the standard-deviation-of-response/slope approach on the Section 3 calibration curve (residual standard deviation $\sigma = 2150$; slope $S = 4{,}860{,}000$) [1].

$$\mathrm{LOD} = \frac{3.3\,\sigma}{S} = 0.00146\,mg\,mL^{-1}\ (0.29\%\ \text{of nominal}) \qquad \mathrm{LOQ} = \frac{10\,\sigma}{S} = 0.00442\,mg\,mL^{-1}\ (0.88\%\ \text{of nominal})$$

Both limits were confirmed experimentally: six replicate injections at the calculated LOQ level gave a mean peak area with %RSD of 4.8% (criterion: ≤ 10% at LOQ, ✔ **PASS**); the LOD-level solution produced a signal-to-noise ratio of 3.4:1 (criterion: ≥ 3:1, ✔ **PASS**).

6 Summary and Conclusion

6.1 Summary of Validation Characteristics

Characteristic	Acceptance Criterion	Result	Verdict
Specificity	No interference at analyte RT; peak purity within threshold under all stress conditions	Confirmed across 6 stress conditions and placebo/blank	✔ PASS
Linearity	$r \geq 0.999$; y-intercept $\leq 2.0\%$ of 100% response	$r = 0.9999$; y-intercept = 0.24%	✔ PASS
Range	Established from linearity/accuracy/precision	50%–150% of nominal	✔ PASS
Accuracy	Mean recovery 98.0%–102.0% per level and overall	99.73%–99.97% per level; 99.87% overall	✔ PASS
Repeatability	%RSD $\leq 2.0\%$ (n = 6)	0.40%	✔ PASS
Intermediate Precision	%RSD $\leq 2.0\%$; pooled %RSD $\leq 2.0\%$	0.50%; pooled 0.46%	✔ PASS
LOD (Impurity A)	S/N $\geq$ 3:1	0.29% of nominal, S/N 3.4:1	✔ PASS
LOQ (Impurity A)	%RSD $\leq 10\%$ at LOQ	0.88% of nominal, %RSD 4.8%	✔ PASS
Robustness	Resolution ≥ 2.0; tailing ≤ 2.0; %RSD $\leq 2.0\%$ under all variations	Met at every deliberate variation tested	✔ PASS
System Suitability	Per Section 2.3 criteria	All parameters within limits	✔ PASS

6.2 Deviations

No deviations from protocol VP-AM-0142-03 were recorded during execution of this validation. All raw data, chromatograms, and instrument audit trails are retained in the Vertex LIMS study folder VAL-AM-2026-0417 and are available for sponsor audit upon request.

✔ Conclusion

Method AM-HPLC-0142, Version 3.0, is validated in accordance with ICH Q2(R1) [1] and ICH Q2(R2) [2] for the assay of Nimbatinib in Nimbatinib Tablets, 50 mg, over the range 50%–150% of the labelled content. The method is specific, linear, accurate, precise, and robust, and is suitable for its intended use in routine release and stability testing of the drug product. Vertex Analytical Laboratories recommends the method be adopted as the registered assay procedure and issued as a controlled document under Meridian's Analytical Method Master List.

6.3 References

This validation was designed and evaluated against the compendial and regulatory sources cited throughout this report [1–6].

References

[1] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonised tripartite guideline: Validation of analytical procedures: Text and methodology Q2(R1). Technical report, ICH, Geneva, Switzerland, 2005.

[2] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonised guideline: Validation of analytical procedures Q2(R2). Technical report, ICH, Geneva, Switzerland, 2023.

[3] Börje Magnusson and Ulla Örnemark. Eurachem guide: The fitness for purpose of analytical methods — a laboratory guide to method validation and related topics. Technical report, Eurachem, 2014.

[4] United States Pharmacopeial Convention. General chapter ⟨1225⟩: Validation of compendial procedures. United States Pharmacopeia–National Formulary (USP–NF), 2024.

[5] United States Pharmacopeial Convention. General chapter ⟨621⟩: Chromatography. United States Pharmacopeia–National Formulary (USP–NF), 2024.

[6] U.S. Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: Analytical procedures and methods validation for drugs and biologics. Technical report, U.S. Department of Health and Human Services, FDA, Silver Spring, MD, 2015.