NEXA ANALYTICAL LABORATORIES | Quality Control Division

Limit of Detection and Limit of Quantitation Report

HPLC-UV Determination of Zolarvex (Related Substance C) in Nimbufen Tablets, 50 mg

Report No.	NX-LOD-2026-0147	**Date Issued**	22 July 2026
Study Protocol	STU-VLD-118	**Report Status**	Final — Rev. 1
Client	Vertex Pharmaceuticals, Inc.	**Product**	Nimbufen Tablets, 50 mg
Analyte	Zolarvex (Impurity C)	**Method Ref.**	Nexa SOP QC-042 (HPLC-UV)
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Study Purpose

This report establishes the Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the validated HPLC-UV method for Zolarvex, the principal degradation-related impurity of Nimbufen, in support of the impurity control strategy defined for the finished tablet product.

Headline Result

LOD = 0.11 ng/mL
LOQ = 0.33 ng/mL
Confirmed by signal-to-noise assessment and a 10-replicate precision study at LOQ (see Section 6).

Document Control

Rev.	Date	Description	Author
0	15 Jul 2026	Initial draft, issued for QA review	P. Anand
1	22 Jul 2026	Finalised following QA comment resolution; approved for use	P. Anand

1 Introduction

Zolarvex is the principal thermal degradation product of Nimbufen and is monitored as a specified impurity in Nimbufen Tablets, 50 mg, manufactured by Vertex Pharmaceuticals, Inc. Before Nexa Analytical Laboratories' stability-indicating HPLC-UV method (SOP QC-042) can be relied upon for release and stability decisions at trace impurity levels, its sensitivity must be characterised in quantitative terms.

Two figures of merit describe that sensitivity. The **Limit of Detection (LOD)** is the lowest concentration reliably distinguished from background noise, without any claim of accurate quantitation. The **Limit of Quantitation (LOQ)** is the lowest concentration measurable with acceptable precision and accuracy, and is the figure against which reporting and specification thresholds are set. Both are required elements of a validation package under ICH Q2(R2) [3] and are referenced

by the impurity thresholds of ICH Q3B(R2) [2].

This report documents the regression-based determination of LOD and LOQ for Zolarvex (Section 5), cross-checked against a signal-to-noise assessment and confirmed by a ten-replicate precision and recovery study at the calculated LOQ. The study was executed under Study Protocol STU-VLD-118 on a single instrument, reference standard lot, and column, consistent with the scope defined in Section 2.

Relationship to Specification Limits

The LOD and LOQ reported here are properties of the analytical procedure, not of the product. Any reporting, identification, or qualification threshold applied to Zolarvex in the finished product specification is established separately by Vertex Pharmaceuticals' Quality organisation and is out of scope for this report; this study only confirms that the method is analytically capable of supporting whatever threshold is ultimately specified.

2 Scope, Instrumentation and Materials

2.1 Scope

This study applies to the determination of Zolarvex in Nimbufen Tablets (50 mg and 100 mg strengths, identical core formulation) by the HPLC-UV method of Nexa SOP QC-042. The working range investigated, 0.5–50 ng/mL, brackets the anticipated LOD and LOQ and lies well below the routine assay range (2–150 µg/mL for the Nimbufen active), so no interaction with the primary assay calibration is implied.

2.2 Instrumentation

- Nexa LC-3200 UHPLC system with quaternary pump, thermostatted autosampler, and photodiode array (PDA) detector
- Column: Vertex C18, 100 × 2.1 mm, 1.8 µm particle size, thermostatted at 40 °C
- Detection wavelength: 254 nm (4 nm bandwidth), reference off
- Injection volume: 5 µL; flow rate: 0.4 mL/min
- Data system: Nexa Chromatics CDS v11.2, 21 CFR Part 11 compliant

2.3 Mobile Phase and Reagents

- Mobile Phase A: 0.1 % v/v formic acid in water (LC-MS grade)
- Mobile Phase B: acetonitrile (LC-MS grade)
- Diluent: Mobile Phase A / Mobile Phase B, 80:20 v/v
- Placebo matrix: Nimbufen Tablet placebo blend, Lot PLB-0472, prepared and dispensed by Nexa Formulation Support
- Zolarvex Reference Standard, Lot ZLX-RS-0912, certified purity 99.6 % (w/w), stored at 2–8 °C, protected from light

2.4 Statistical Basis

Regression parameters were computed by unweighted ordinary least-squares linear regression of detector response against nominal concentration, following the treatment of Miller and Miller [4] and the regression-based approach of ICH Q2(R2) [3]. The resulting LOD and LOQ are treated as fit-for-purpose performance characteristics of the method in this matrix [1].

3 Experimental Procedure

3.1 Standard Preparation

A stock solution of Zolarvex Reference Standard was prepared at 1.00 mg/mL in diluent. This stock was serially diluted in placebo-matrix extract to yield seven working calibration standards spanning the anticipated LOD/LOQ region: 0.5, 1, 2, 5, 10, 20 and 50 ng/mL. Each level was prepared independently in triplicate (21 discrete solutions) to allow both regression fitting and an estimate of level-wise precision.

3.2 Injection Sequence

System suitability was demonstrated before sample analysis using five replicate injections of the 10 ng/mL standard: %RSD of peak area $\leq$ 2.0 %, USP tailing factor $\leq$ 1.5, and resolution from the nearest matrix-related peak $\geq$ 2.0. The analytical sequence then consisted of:

1. Six blank (unspiked placebo extract) injections, interspersed through the run, used to characterise baseline noise (Section 5.2)
2. Twenty-one calibration injections (seven levels $\times$ three independent preparations), randomised across the sequence
3. Ten independent replicate preparations of placebo extract spiked at the candidate LOQ concentration (0.330 ng/mL), used for the precision and recovery confirmation in Section 6
4. A closing system suitability check (10 ng/mL standard, single injection) to confirm no drift occurred over the sequence

3.3 Data Treatment

Peak areas were integrated automatically by the CDS with a threshold of 0 and manually verified against raw chromatograms by a second analyst. Calibration data were fitted by unweighted linear regression, and LOD/LOQ calculated per Section 5, then cross-checked by signal-to-noise comparison at the blank, near-LOD and near-LOQ concentrations. In total, 39 injections (6 blanks + 21 calibration + 10 LOQ confirmation + 2 system suitability) were completed within a single 24-hour autosampler qualification window.

4 Calibration Curve and Regression Analysis

4.1 Raw Response Data

Table 1 summarises the triplicate peak-area response at each of the seven calibration levels.

Table 1: Calibration response data, peak area in mAU·s (n = 3 per level)

Level	Conc. (ng/mL)	Rep. 1	Rep. 2	Rep. 3	Mean	%RSD
1	0.500	9312.0	9587.0	9405.0	9434.7	1.48
2	1.00	18420.0	18705.0	18190.0	18438.3	1.40
3	2.00	37650.0	36980.0	37420.0	37350.0	0.91
4	5.00	92100.0	93850.0	91700.0	92550.0	1.23
5	10.0	186500.0	184200.0	187300.0	186000.0	0.86
6	20.0	369800.0	372100.0	368500.0	370133.3	0.49
7	50.0	924000.0	918500.0	927200.0	923233.3	0.48

4.2 Calibration Curve

4.3 Regression Summary

The response is linear across the tested range, with $r^2 = 0.9998$ exceeding the SOP QC-042 acceptance criterion of $r^2 \geq 0.995$, and no trend in residuals with concentration, supporting the

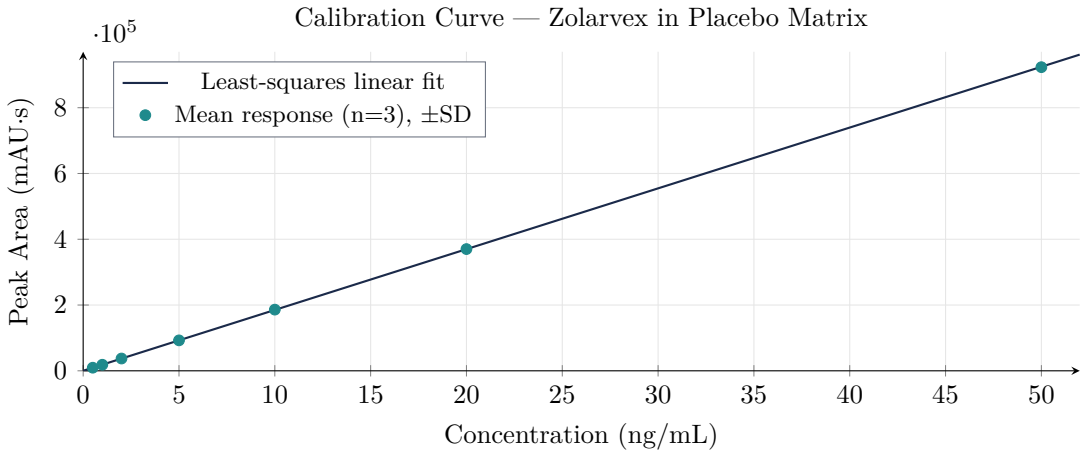

Figure 1: Mean peak area versus nominal concentration, with error bars showing ±1 standard deviation of the triplicate preparations at each level.

Table 2: Linear regression statistics for the calibration curve

Parameter	Value
Slope, S (mAU·s per ng/mL)	18486
Intercept, a_0 (mAU·s)	187.4
Correlation coefficient, r^2	0.9998
Residual standard deviation, $S_{y/x}$	612
Calibration range	0.5–50 ng/mL
Levels × replicates	7 × 3 ($n = 21$)

unweighted regression model used for the LOD/LOQ calculation in Section 5.

5 LOD and LOQ Determination

5.1 Regression-Based Calculation

Per the regression-based approach of ICH Q2(R2) [3], LOD and LOQ are derived from the residual standard deviation of the calibration line, $S_{y/x}$, and the slope of the calibration curve, S:

$$\mathrm{LOD} = \frac{3.3\, S_{y/x}}{S} \tag{1}$$

$$\mathrm{LOQ} = \frac{10\, S_{y/x}}{S} \tag{2}$$

Substituting the regression statistics from Table 2 ($S_{y/x} = 612$ mAU·s, $S = 18486$ mAU·s per ng/mL):

$$\mathrm{LOD} = \frac{3.3 \times 612}{18486} = 0.1092 \text{ ng/mL} \tag{3}$$

$$\mathrm{LOQ} = \frac{10 \times 612}{18486} = 0.3311 \text{ ng/mL} \tag{4}$$

Per Nexa SOP QC-042 rounding convention (two significant figures for trace-level results):

Reported Sensitivity

LOD = 0.11 ng/mL LOQ = 0.33 ng/mL

5.2 Signal-to-Noise Confirmation

As an orthogonal check, six blank placebo-extract injections established mean baseline noise (peak-to-peak amplitude, Zolarvex retention-time window), and spiked-matrix injections near the calculated LOD and LOQ were evaluated for signal-to-noise (S/N) ratio using peak height, consistent with common chromatographic practice for low-level noise assessment.

Table 3: Signal-to-noise confirmation (n = 6 blank injections)

Sample	Mean Peak Height (mAU)	Mean Baseline Noise (mAU)	S/N
Blank (unspiked)	not detected	0.0058	—
Near LOD (0.11 ng/mL spike)	0.0180	0.0058	3.1
Near LOQ (0.33 ng/mL spike)	0.0592	0.0058	10.2

The observed S/N of 3.1 at the calculated LOD and 10.2 at the calculated LOQ closely match the conventional S/N benchmarks of 3 and 10 respectively, corroborating the regression-based estimates in Section 5.1.

Interpretation Note

LOD confirms detectability only; it must not be used to report a quantitative result. Any Zolarvex signal observed between LOD and LOQ should be reported as "detected, below LOQ" per Nexa SOP QC-042 Section 9.3, not as a numeric value.

6 Precision and Recovery Confirmation at LOQ

To confirm that the calculated LOQ (0.330 ng/mL) is a concentration at which Zolarvex can be measured with acceptable precision and accuracy, ten independent placebo-matrix preparations were spiked at the nominal LOQ level and analysed within the same sequence. Calculated concentration was back-derived from the calibration equation $y = 187.4 + 18486x$ established in Section 4.

Table 4: LOQ precision and recovery, n = 10 independent preparations at 0.330 ng/mL

Replicate	Peak Area (mAU·s)	Calc. Conc. (ng/mL)	% Recovery
1	6694.5	0.352	106.7
2	6491.1	0.341	103.3
3	6805.4	0.358	108.5
4	6398.7	0.336	101.8
5	6639.0	0.349	105.8
6	6879.3	0.362	109.7
7	6343.2	0.333	100.9
8	6749.9	0.355	107.6
9	6546.6	0.344	104.2
10	6472.6	0.340	103.0

The ten replicates gave a mean calculated concentration of 0.347 ng/mL (SD 0.0097 ng/mL, %RSD 2.80 %) and a recovery range of 100.9 %–109.7 % (mean 105.2 %). Acceptance criteria for trace-level determinations per Nexa SOP QC-042 (%RSD $\leq$ 15 %; mean recovery within 80–120 %) were met with margin, confirming that the calculated LOQ is a concentration at which Zolarvex can be quantified with acceptable precision and accuracy in the tablet placebo matrix.

7 Conclusion and Recommendations

The HPLC-UV method of Nexa SOP QC-042 was demonstrated to have a Limit of Detection of **0.11 ng/mL** and a Limit of Quantitation of **0.33 ng/mL** for Zolarvex in Nimbufen Tablet placebo matrix, calculated from calibration-curve regression statistics per ICH Q2(R2) [3]. These values were corroborated by a signal-to-noise assessment (Section 5.2) and confirmed by a ten-replicate precision and recovery study at the calculated LOQ (Section 6, %RSD 2.80 %, mean recovery 105.2 %), both comfortably within the SOP's trace-level acceptance criteria.

Considered together with the linearity data of Section 4 ($r^2 = 0.9998$ across 0.5–50 ng/mL), the method is judged fit for its intended purpose [1]: supporting identification, quantitation and trending of Zolarvex at levels relevant to the impurity control strategy for Nimbufen Tablets. Applicability to any specific reporting or qualification threshold under ICH Q3B(R2) [2] remains the responsibility of Vertex Pharmaceuticals' Quality organisation, informed by the sensitivity figures established here.

7.1 Recommendations

- Adopt LOD = 0.11 ng/mL and LOQ = 0.33 ng/mL as the reportable sensitivity limits for Zolarvex under Nexa SOP QC-042.
- Re-verify LOD/LOQ upon any change of HPLC column lot, detector lamp, or instrument platform, and in any case no less frequently than every 12 months, consistent with USP ⟨1225⟩ lifecycle expectations [5].
- Retain raw chromatographic data and this report for the retention period defined in the Nimbufen Tablets product file.

Approval

Role	Name / Title	Date	Signature
Prepared by	Priya Anand, Analyst II	2026-07-22	_____
Reviewed by	Marcus Ferreira, QA Reviewer	2026-07-23	_____
Approved by	Dr. Elena Kowalski, Laboratory Director	2026-07-24	_____

References

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