HPLC System Suitability Report

NXA-4471 Tablets, 50 mg — Assay & Related Substances Method

Apex Analytical Laboratories ▪ Quality Control Chemistry — HPLC Section ▪ Report No. AAL-HPLC-2026-0143 ▪ 04 August 2026

1 Report Overview & Scope

Purpose & Scope

This report documents the system suitability test (SST) performed on the reversed-phase HPLC method used for assay and related-substances testing of NXA-4471 Tablets, 50 mg, manufactured by Nexa Therapeutics, Inc. The SST was executed prior to sample analysis of Batch B26-0417 to verify that the chromatographic system, method, and column combination deliver data of acceptable quality, in accordance with SOP-QC-014 [1] and the general requirements of USP General Chapter <621> Chromatography [2].

Report Information

Report No.:	AAL-HPLC-2026-0143
Batch No.:	B26-0417
Test Date:	04 August 2026
Method Ref.:	SOP-QC-014
Status:	SST Passed
Client:	Nexa Therapeutics, Inc.

Personnel & Authorization

- **Analyst:** Priya Raman, QC Analyst II
- **Reviewer:** David Chen, QC Supervisor
- **Facility:** HPLC Section, Quality Control Chemistry, Apex Analytical Laboratories

2 Instrumentation & Method Parameters

The following instrumentation and chromatographic conditions were used for the system suitability test and the subsequent sample analysis of Batch B26-0417.

Instrument & Column

- **HPLC System:** Apex LC-3200 Quaternary System
- **Detector:** UV-Vis Diode Array, 254 nm
- **Data System:** ApexChrom CDS v4.2
- **Column:** Octadecylsilane (C18), 150 × 4.6 mm, 5 µm
- **Column ID:** COL-118 (Lot C18-0552)
- **Column Temp.:** 30°C

Mobile Phase & Run Conditions

- **Mobile Phase A:** 0.1% phosphoric acid in water, pH 3.0
- **Mobile Phase B:** Acetonitrile, HPLC grade
- **Elution Mode:** Isocratic, 55:45 (A:B, v/v)
- **Flow Rate:** 1.0 mL/min
- **Injection Volume:** 10 µL
- **Run Time:** 15 min per injection

Mobile Phase Handling & Stability

Mobile phase A is buffered to pH 3.0 with phosphoric acid and is stable for 48 hours at room temperature; solutions older than 48 hours were discarded prior to use. Both mobile phase components were filtered through a 0.45 µm membrane and degassed by sonication for 15 minutes before use to prevent baseline noise and pump cavitation.

3 System Suitability Test Design

Reference Standard & SST Solution

The system suitability solution was prepared by combining NXA-4471 Working Reference Standard (WRS Lot RS-0192) with Impurity-A reference material (*N*-desmethyl-NXA-4471) at a target level of 0.5% relative to the nominal NXA-4471 concentration, in diluent (mobile phase A : methanol, 50:50). Six replicate 10 µL injections of the SST solution were made under equilibrated system conditions, following two priming injections that were excluded from the calculated statistics [3].

Acceptance Criteria (SOP-QC-014)

- Resolution (R_s), Imp-A/NXA-4471 $\geq$ 2.0
- Tailing factor (T), main peak $\leq$ 2.0
- Theoretical plates (N), main peak $\geq$ 2000
- %RSD retention time (n=6) $\leq$ 2.0%
- %RSD peak area (n=6) $\leq$ 2.0%

Critical Resolution Pair

Impurity-A elutes immediately before the NXA-4471 main peak (relative retention time, RRT $\approx$ 0.54) and represents the closest-eluting, and therefore most critical, resolution pair for this method.

Criteria are defined per USP <621> [2] and ICH Q2(R2) [4], and are applied to the peaks in the representative SST chromatogram (Figure 1, Section 4) as detailed in the results in Section 5.

4 Representative SST Chromatogram

Figure 1 shows the chromatogram obtained from injection 3 of the system suitability solution, representative of the six replicate injections summarized in Table 1 (Section 5).

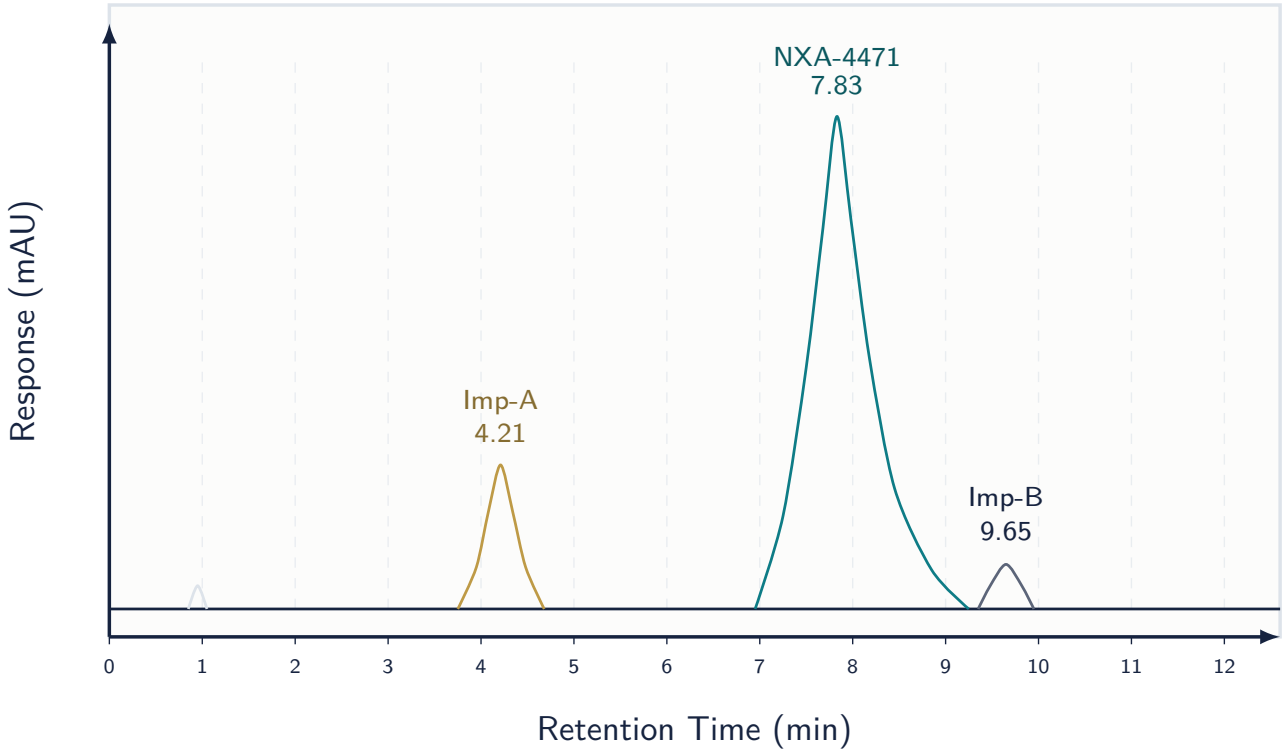

Figure 1: Representative SST chromatogram (injection 3 of 6) showing Impurity-A (RT 4.21 min), the NXA-4471 main peak (RT 7.83 min), and Impurity-B (RT 9.65 min). Detection at 254 nm; total run time 15 min.

5 Results: Retention Time, Resolution & Efficiency

Table 1 summarizes retention time, resolution, tailing factor, theoretical plate count, and main-peak area for the six replicate SST injections.

Inj.	RT Imp-A (min)	RT NXA-4471 (min)	R_s	T	N	Area (main, mAU·s)
1	4.19	7.81	3.46	1.28	6910	284655
2	4.20	7.82	3.44	1.29	6875	284102
3	4.21	7.83	3.41	1.31	6842	285210
4	4.22	7.85	3.38	1.97	6790	283958
5	4.21	7.84	3.43	1.30	6861	284530
6	4.20	7.83	3.45	1.29	6903	284877
Mean	4.21	7.83	3.43	1.41	6864	284555
SD	0.010	0.014	0.029	0.273	44.1	470
%RSD	0.25%	0.18%	0.85%	19.4%	0.64%	0.17%

Table 1: R_s = resolution between Impurity-A and the NXA-4471 main peak; T = USP tailing factor at 5% peak height; N = theoretical plates (USP plate-count equation), main peak. %RSD for T and N is reported for information only and is not a pass/fail criterion under SOP-QC-014.

Acceptance Criteria Summary

Parameter	Criterion	Result	Status
Resolution (R_s), Imp-A / NXA-4471	≥ 2.0	3.43 (mean)	PASS
Tailing factor (T), main peak	≤ 2.0	1.28–1.97 (range)	PASS
Theoretical plates (N), main peak	≥ 2000	6790–6910 (range)	PASS
%RSD retention time (n=6)	$\leq 2.0\%$	0.18%	PASS
%RSD peak area (n=6)	$\leq 2.0\%$	0.17%	PASS

Table 2: All five SST acceptance parameters met specification; the method is suitable for use in the assay and related-substances determination of NXA-4471 Tablets, Batch B26-0417.

6 Discussion & Observations

1. **Elevated Tailing Factor, Injection 4:** Injection 4 showed a tailing factor of 1.97, compliant with the ≤2.0 acceptance limit but visibly elevated relative to the remaining five injections (range 1.28–1.31). Review of the pump pressure trace showed a transient 4% pressure dip coincident with this injection, consistent with a small air bubble in the mobile phase line that resolved after re-priming. No corrective action was required, as the injection met the individual acceptance criterion, but the analyst flagged the observation in the instrument logbook per SOP-QC-014 §7.3 for trend monitoring.
2. **Resolution Margin:** The mean resolution between Impurity-A and the NXA-4471 main peak (3.43) provides a comfortable margin above the ≥2.0 requirement, indicating the method is robust to minor retention-time drift over the course of a sample analysis sequence.
3. **Plate Count Trend:** Theoretical plate counts (6790–6910) are well above the 2000-plate minimum and consistent with historical qualification data for column Lot C18-0552, indicating no loss of column efficiency since installation.
4. **Impurity-B:** Impurity-B (RT 9.65 min, RRT ≈ 1.23) was detected in the SST solution as expected from the reference material blend; it is not part of the critical resolution pair and carries no independent acceptance criterion under this method [5].

7 Conclusion & Disposition

System Suitability Determination

The HPLC system, column, and method met all five acceptance criteria defined in SOP-QC-014 for the assay and related-substances determination of NXA-4471 Tablets:

- **Resolution:** 3.43 (mean, n=6), exceeds the ≥2.0 requirement.
- **Tailing Factor:** 1.28–1.97 (range), within the ≤2.0 limit.
- **Theoretical Plates:** 6790–6910, exceeds the ≥2000 requirement.
- **Overall System Suitability:** **PASS**

The chromatographic system is confirmed suitable for its intended use. Sample analysis of NXA-4471 Tablets, Batch B26-0417, proceeded immediately following this SST under the same instrument sequence, per SOP-QC-014 [1].

Approvals & Signatures

By signing below, the analyst, reviewer, and approver certify that the data presented in this report is authentic and has been verified against the raw chromatographic data files retained in ApexChrom CDS.

Priya Raman
Analyst — QC Analyst II
Date: 04 August 2026

David Chen
Reviewer — QC Supervisor
Date: 04 August 2026

Elena Vasquez
Approver — QA Manager
Date: 04 August 2026

References

[1] Apex Analytical Laboratories. Sop-qc-014: Hplc system suitability testing. Internal standard operating procedure, Apex Analytical Laboratories, Quality Control Chemistry, 2025.

[2] United States Pharmacopeial Convention. General chapter <621> chromatography. Technical report, USP–NF, 2023.

[3] Lloyd R. Snyder, Joseph J. Kirkland, and John W. Dolan. *Introduction to Modern Liquid Chromatography*. Wiley, Hoboken, NJ, 3rd edition, 2010.

[4] International Council for Harmonisation. Ich harmonised guideline q2(r2): Validation of analytical procedures. Technical report, ICH, 2023.

[5] John W. Dolan. Peak tailing and resolution. *LCGC North America*, 20(5):430–436, 2002.