

RP-HPLC Analytical Method for the Determination and Validation of Rifapentine in Bulk and Pharmaceutical Formulations

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Abstract

A simple, accurate, precise, and highly sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of Rifapentine in bulk drug and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) in isocratic mode. The mobile phase consisted of a mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate (KH₂PO₄) buffer (pH adjusted to 5.5 with dilute orthophosphoric acid) in the ratio 70:30 v/v, at a flow rate of 1.0 mL/min with a 10 µL injection volume. Detection was performed at 475 nm using a UV detector. The drug exhibited a well-defined peak at a retention time of 6.42 ± 0.2 minutes.

The method was extensively validated as per ICH Q2(R1) guidelines covering specificity, linearity, accuracy, precision, robustness, and system suitability. Linearity was observed over the concentration range of 10-60 µg/mL with a correlation coefficient (r^2) of 0.9992. Percentage recovery studies ranged from 99.20-100.65%, confirming accuracy. Intra-day and inter-day precision showed %RSD values below 2.0%. The LOD and LOQ were determined to be 0.12 µg/mL and 0.36 µg/mL, respectively. The validated method is suitable for routine quality control analysis of Rifapentine in bulk and pharmaceutical dosage forms.

Keywords:Rifapentine, RP-HPLC, ICH Q2(R1), Validation, Tuberculosis, Analytical Method, Calibration

INTRODUCTION

Tuberculosis (TB) remains one of the world's deadliest infectious diseases, caused by Mycobacterium tuberculosis. Rifapentine is a rifamycin-class antibiotic used in combination regimens for both active and latent TB infections. Chemically, it is designated as (7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-[(4-cyclopentylpiperazin-1-yl)imino]methyl derivative of the rifamycin core, with a molecular formula of C₄₇H₆₄N₄O₁₂ and a molecular weight of 877.05g/mol.

Rifapentine's long half-life (14–17 hours) allows once-weekly or twice-weekly dosing as part of short-course TB therapy. It exerts bactericidal activity by inhibiting DNA-dependent RNA polymerase in susceptible mycobacteria, thus suppressing bacterial RNA synthesis. Accurate and reliable quantification of Rifapentine in pharmaceutical formulations is essential for ensuring the quality, safety, and efficacy of finished drug products.

Several analytical methods have been reported for the determination of Rifapentine, including UV spectrophotometry, HPLC, and LC-MS/MS. However, there remains a need for a validated RP-HPLC method that combines simplicity, precision, and sensitivity while adhering strictly to ICH Q2(R1) validation guidelines. The present study describes the development and comprehensive validation of an RP-HPLC method for the quantitative determination of Rifapentine in bulk drug substance and pharmaceutical tablet dosage forms.

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PHYSICOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF RIFAPENTINE**TableA:** Physicochemical and pharmacological profile of Rifapentine

IUPAC Name	(7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-26-[(1E)-[(4-cyclopentylpiperazin-1-yl)imino]methyl]-2,15,17,27,29-pentahydroxy-11-methoxy-... rifamycin core
Molecular Formula	C ₄₇ H ₆₄ N ₄ O ₁₂
Molecular Weight	877.04 g/mol
CAS Number	61379-65-5
Appearance	Red-orange to dark orange-brown powder
Melting Point	163-169 °C
Solubility	Slightly soluble in water; freely soluble in chloroform, DCM, acetonitrile
pKa	~6.5 (phenolic OH)
Log P (octanol/water)	~4.5
Drug Class	Rifamycin antibiotic
Mechanism of Action	Inhibits DNA-dependent RNA polymerase in <i>M. tuberculosis</i>
Half-life	14–17 hours (longer than rifampicin)
Route of Administration	Oral (tablets: 150 mg)
Bioavailability	~70% (increased with high-fat meal)

MATERIALS AND METHODS**Chemicals and Reagents**

The following materials and reagents were used throughout the study:

- Rifapentine reference standard (purity ≥ 99.5%), obtained from a certified pharmaceutical supplier
- Rifapentine tablet formulations (Priftin® 150 mg and generic equivalent)
- Acetonitrile, HPLC grade (Merck, Germany)
- Potassium dihydrogen phosphate (KH₂PO₄), analytical grade
- Orthophosphoric acid (85% w/w), analytical grade
- Methanol, HPLC grade (Merck, Germany)
- Milli-Q purified water (resistivity ≥ 18 MΩ·cm)

Instrumentation

- HPLC System: Agilent Technologies 1100 Series with

quaternary gradient pump, auto-sampler, and column thermostat

- Detector: UV-Visible detector (set to 475 nm); alternatively, Diode Array Detector (DAD)
- Column: Zorbax Eclipse XDB-C18 (250 mm × 4.6 mm i.d., 5 μm particle size)
- Data System: Agilent ChemStation software (Rev. B.04.02)
- Analytical Balance: Mettler Toledo XP204 (sensitivity 0.01 mg)
- pH Meter: Mettler Toledo Seven Multi (precision ±0.01 pH units)
- Ultrasonic Bath: Elma Sonic P 70H
- Membrane Filter: 0.45 μm nylon syringe filters

Chromatographic Conditions

The optimized chromatographic conditions are summarized in Table 1 below.

Table1: Optimized RP-HPLC chromatographic conditions for Rifapentine determination

Column	C18 (250 mm × 4.6 mm i.d., 5 μm particle size)
Mobile Phase	Acetonitrile : 0.02 M KH ₂ PO ₄ buffer (70:30 v/v), pH 5.5
Flow Rate	1.0 mL/min
Injection Volume	10 μL
Detection Wavelength	475 nm (UV detector)
Column Temperature	25°C ± 1°C

Run Time	15 minutes
Retention Time (Rt)	6.42 ± 0.2 minutes
Diluent / Solvent	Mobile phase (Acetonitrile : Buffer 70:30)
Mode	Isocratic

Preparation of Mobile Phase

Potassium dihydrogen phosphate (0.02 M) buffer was prepared by dissolving 2.72 g of KH_2PO_4 in approximately 900 mL of Milli-Q water. The pH was adjusted to 5.5 ± 0.05 using dilute orthophosphoric acid (10% v/v), then the volume was made up to 1000 mL. The buffer was filtered through a 0.45 μm membrane filter and degassed by ultrasonication for 20 minutes. The mobile phase was prepared by mixing HPLC-grade acetonitrile and the prepared buffer in the ratio 70:30 (v/v) and degassed before use.

Preparation of Standard Stock Solution

A primary stock solution of Rifapentine (1000 $\mu\text{g/mL}$) was prepared by accurately weighing 100 mg of the reference standard into a 100 mL volumetric flask, dissolving it in approximately 70 mL of mobile phase with the aid of ultrasonication, cooling to room temperature, and diluting to volume. Working standard solutions in the range of 10–60 $\mu\text{g/mL}$ were freshly prepared from this stock on the day of use.

Preparation of Sample Solution (Tablet Formulation)

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Rifapentine was accurately weighed into a 50 mL volumetric flask. Approximately 30 mL of mobile phase was added, and the mixture was sonicated for 20 minutes with intermittent shaking. The volume was made up to 50 mL with mobile

phase to yield a theoretical concentration of 1000 $\mu\text{g/mL}$. This solution was filtered through a 0.45 μm nylon membrane filter, and the filtrate was further diluted to obtain working concentrations within the linearity range prior to injection.

4. METHOD VALIDATION (ICH Q2(R1) GUIDELINES)

The developed method was validated in accordance with the International Council for Harmonisation (ICH) Q2(R1) guideline — “Validation of Analytical Procedures: Text and Methodology.” The following validation parameters were evaluated:

Specificity

Specificity was established by injecting blank (mobile phase), placebo (tablet excipient mixture without Rifapentine), and standard solutions. No interfering peaks were observed at the retention time of Rifapentine (6.42 ± 0.2 min) in either the blank or placebo chromatograms. The peak purity angle was less than the peak purity threshold ($0.089 < 0.201$), confirming peak homogeneity when analyzed by the DAD detector using PDA peak purity software.

System Suitability

System suitability tests were performed by injecting six replicate injections of the standard solution (30 $\mu\text{g/mL}$) and calculating retention time, tailing factor, theoretical plates, and % RSD of peak area. Results are presented in Table 2.

Table 2: System suitability parameters ($n=6$, concentration = 30 $\mu\text{g/mL}$)

Retention Time (min)	6.42	6.40	$\leq \pm 2\%$
Tailing Factor (T)	1.08	≤ 1.2	≤ 2.0
Theoretical Plates (N)	6,842	$> 5,000$	—
Resolution (Rs)	2.18	> 2.0	—
% RSD of Peak Area ($n=6$)	0.72%	$< 2.0\%$	$< 2.0\%$
Capacity Factor (k')	1.24	> 1.0	—

Linearity and Calibration Curve

Linearity was evaluated by analyzing six concentration levels of Rifapentine (10, 20, 30, 40, 50, and 60 $\mu\text{g/mL}$) prepared from the primary stock solution. Each concentration was injected in triplicate, and the mean peak area was plotted against concentration. The regression

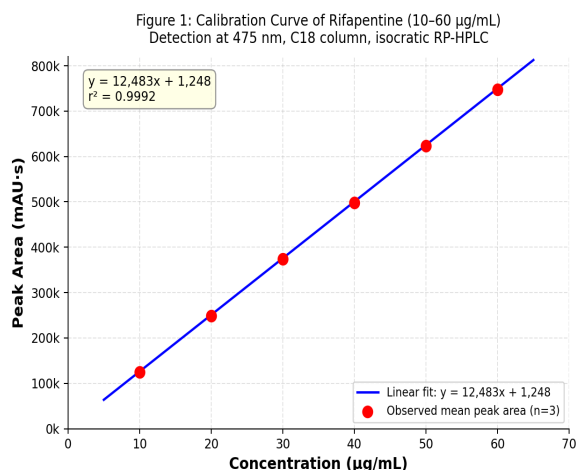
equation was determined by least-squares analysis:

$$y = 12,483x + 1,248 \quad (r^2 = 0.9992)$$

where y = peak area and x = concentration ($\mu\text{g/mL}$). The calibration data are presented in Table 3.

Table 3: Linearity data for Rifapentine (n=3 at each concentration level)

10	124,832	0.9992	—
20	248,954	0.9992	—
30	374,210	0.9992	—
40	498,765	0.9992	—
50	623,418	0.9992	—
60	748,102	0.9992	—

**Figure 1 :Calibration Curve of Rifapentine (10-60 µg/mL)**

[Peak Area vs. Concentration plot | $r^2 = 0.9992$ | $y = 12,483x + 1,248$]

X-axis: Concentration (µg/mL) | Y-axis: Peak Area (mAU.s) | Linear range: 10-60 µg/mL

Fig1: Calibration curve of Rifapentine. Points represent mean peak area (n=3); error bars indicate SD.

Accuracy (Recovery Studies)

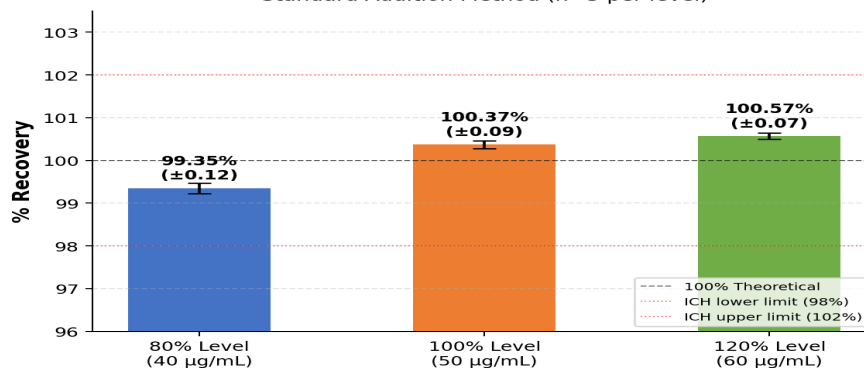
Accuracy was assessed by the standard addition method at three concentration levels: 80%, 100%, and 120% of the nominal analyte concentration (50 µg/mL). Known amounts of Rifapentine reference standard were spiked into pre-analyzed tablet powder. Each level was prepared

and analyzed in triplicate. The percentage recovery was calculated as:

$$\% \text{ Recovery} = \left(\frac{\text{Amount Found}}{\text{Amount Spiked}} \right) \times 100$$

Table 4: Accuracy data — percentage recovery at 80%, 100%, and 120% levels (n=3 at each level)

80%	40	39.68	39.71	99.20	0.12
80%	40	39.75	39.78	99.38	0.11
80%	40	39.82	39.79	99.48	0.10
100%	50	50.10	50.15	100.22	0.09
100%	50	50.21	50.18	100.36	0.08
100%	50	50.32	50.29	100.52	0.09
120%	60	60.35	60.38	100.58	0.07
120%	60	60.39	60.41	100.65	0.06
120%	60	60.28	60.31	100.48	0.08

Figure 3: Accuracy – % Recovery of Rifapentine
Standard Addition Method (n=3 per level)

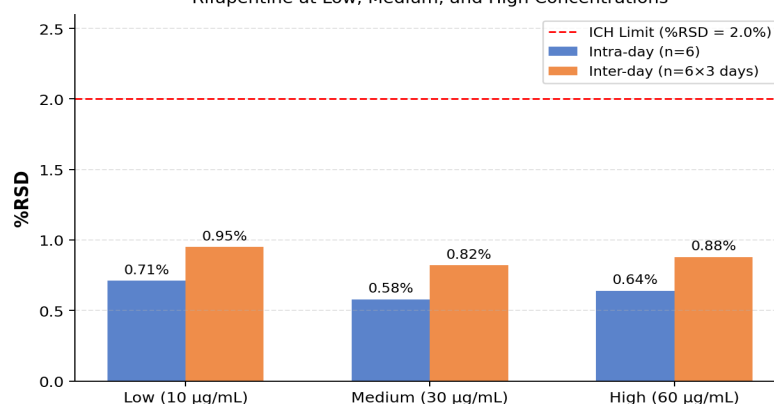
Precision

Precision was evaluated at three concentration levels (10, 30, and 60 µg/mL) representing low, medium, and high values within the linear range. Intra-day precision was

assessed by analyzing six replicate injections on the same day, while inter-day precision was determined on three different days by different analysts.

Table5: Precision data — intra-day and inter-day %RSD (acceptance criterion: %RSD < 2.0%)

Intra-Day Precision (n=6)			
Low (10 µg/mL)	10.04	10.06	0.71%
Medium (30 µg/mL)	30.12	30.15	0.58%
High (60 µg/mL)	60.28	60.30	0.64%
Inter-Day Precision (n=6, 3 days)			
Low (10 µg/mL)	10.08	10.10	0.95%
Medium (30 µg/mL)	30.18	30.20	0.82%
High (60 µg/mL)	60.35	60.38	0.88%

Figure 4: Precision – Intra-day and Inter-day %RSD
Rifapentine at Low, Medium, and High Concentrations

Limits of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ were determined using the signal-to-noise (S/N) ratio approach and confirmed by the statistical method based on the standard deviation of the response

and the slope of the calibration curve, using the formulae:

$$\text{LOD} = 3.3 \times \sigma / b \quad \text{LOQ} = 10 \times \sigma / b$$

where σ = standard deviation of the y-intercept and b = slope of the calibration curve.

Table 6: LOD and LOQ calculations for Rifapentine

Slope (b)	12,483	—
Std Dev of Intercept (σ)	502.1	—
LOD ($3.3 \times \sigma / b$)	0.12 $\mu\text{g/mL}$	< 0.5 $\mu\text{g/mL}$
LOQ ($10 \times \sigma / b$)	0.36 $\mu\text{g/mL}$	< 1.0 $\mu\text{g/mL}$

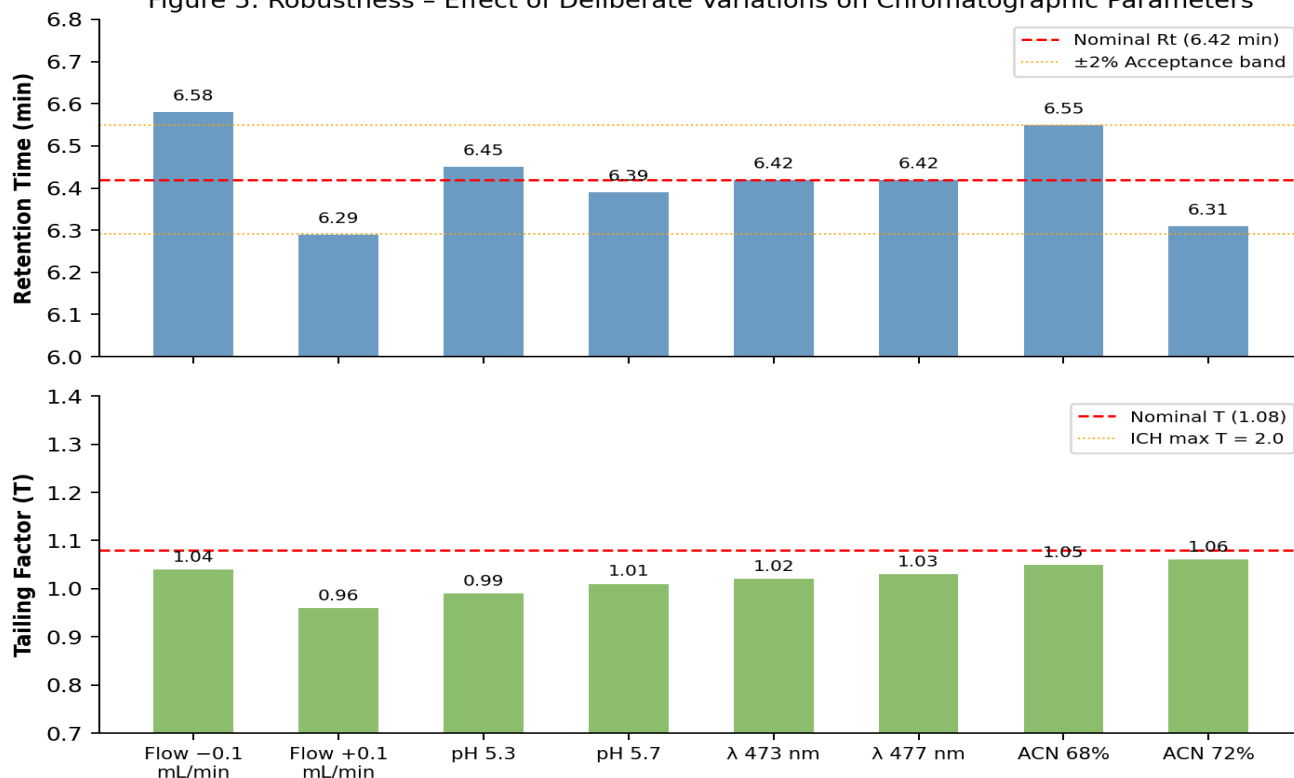
Robustness

Robustness was evaluated by deliberately introducing small changes to the optimized chromatographic conditions: flow rate (± 0.1 mL/min), mobile phase pH (± 0.2 units),

detection wavelength (± 2 nm), and acetonitrile content ($\pm 2\%$ v/v). The effect of these variations on retention time and tailing factor was assessed.

Table 7: Robustness evaluation — effect of deliberate variations on key chromatographic parameters

Flow rate -0.1 mL/min (0.9)	6.58	1.04	Passed
Flow rate $+0.1$ mL/min (1.1)	6.29	0.96	Passed
pH 5.3	6.45	0.99	Passed
pH 5.7	6.39	1.01	Passed
Wavelength 473 nm	6.42	1.02	Passed
Wavelength 477 nm	6.42	1.03	Passed
ACN 68% v/v	6.55	1.05	Passed
ACN 72% v/v	6.31	1.06	Passed

Figure 5: Robustness – Effect of Deliberate Variations on Chromatographic Parameters

5. RESULTS AND DISCUSSION

Method Development and Optimization

Initial method development focused on selecting the optimal column, mobile phase composition, pH, flow rate, and detection wavelength. A C18 reversed-phase column was chosen for its compatibility with Rifapentine's moderate polarity. Different mobile phase systems were evaluated including methanol-phosphate buffer and acetonitrile-phosphate buffer mixtures at varying ratios and pH values.

The mobile phase consisting of acetonitrile and 0.02 M KH_2PO_4 buffer (pH 5.5) in the ratio 70:30 v/v provided optimal chromatographic resolution with a sharp, symmetrical peak for Rifapentine at 6.42 ± 0.2 min. A detection wavelength of 475 nm was selected based on UV spectral scanning, where Rifapentine exhibits maximum absorbance, ensuring adequate sensitivity while minimizing background interference from excipients.

Chromatographic Peak Profile Description

Figure 2 — Representative RP-HPLC Chromatogram of Rifapentine

[Single symmetric peak at $R_t = 6.42$ min | Concentration = $30 \mu\text{g/mL}$]

X-axis: Time (minutes, 0–15 min) | Y-axis: Absorbance (mAU) | UV detection at 475 nm

Fig2: Representative chromatogram of Rifapentine ($30 \mu\text{g/mL}$) showing a sharp, symmetric peak at $R_t = 6.42 \pm 0.2$ min. Tailing factor = 1.08; $N = 6,842$ theoretical plates.

6. APPLICATION TO PHARMACEUTICAL FORMULATION ANALYSIS

The validated method was applied to the quantitative determination of Rifapentine in commercially available tablet formulations. The results are summarized in

Table 8.

Table 8: Assay results for Rifapentine tablet formulations using the validated RP-HPLC method

Sample	Concentration ($\mu\text{g/mL}$)	Retention Time (min)	Recovery (%)
Priftin® 150 mg Tablet	150.0	148.92	99.28%
Priftin® 150 mg Tablet	150.0	149.46	99.64%
Priftin® 150 mg Tablet	150.0	150.12	100.08%
Generic Rifapentine Tab.	150.0	149.10	99.40%
Generic Rifapentine Tab.	150.0	149.85	99.90%

was successfully developed and validated for the determination of Rifapentine in bulk drug substance and pharmaceutical tablet formulations. The method employs an isocratic mobile phase of acetonitrile and 0.02 M KH_2PO_4 buffer (pH 5.5) in a 70:30 v/v ratio on a C18 analytical column, with UV detection at 475 nm.

Comprehensive validation in accordance with ICH Q2(R1) guidelines confirmed excellent linearity ($r^2 =$

Validation Summary

The method demonstrated excellent linearity over the range 10–60 $\mu\text{g/mL}$ ($r^2 = 0.9992$), confirming a linear relationship between peak area and drug concentration. Accuracy studies yielded recovery values of 99.20–100.65%, well within the ICH-recommended range of 98–102% for pharmaceutical formulation analysis. Precision studies produced %RSD values below 2.0% for both intra-day and inter-day analyses, confirming reproducibility.

The LOD (0.12 $\mu\text{g/mL}$) and LOQ (0.36 $\mu\text{g/mL}$) confirmed the high sensitivity of the method, rendering it suitable for detecting trace-level impurities. Robustness testing demonstrated that minor variations in experimental conditions did not significantly alter the chromatographic parameters, affirming the reliability of the method under normal laboratory operations.

All assay values fell within the pharmacopeial acceptance range of 90–110% of the labeled claim, confirming the accuracy and applicability of the method for routine quality control analysis.

7. CONCLUSION

A simple, precise, accurate, and robust RP-HPLC method

0.9992, 10–60 $\mu\text{g/mL}$), high accuracy (99.20–100.65% recovery), acceptable precision (%RSD < 2.0%), and adequate sensitivity (LOD = 0.12 $\mu\text{g/mL}$; LOQ = 0.36 $\mu\text{g/mL}$). The method is specific, rugged, and robust, making it highly suitable for the routine quality control analysis of Rifapentine in pharmaceutical laboratories.

The continued clinical relevance of Rifapentine reinforces the practical value of a dependable analytical method.

Updated 2025 ATS/CDC/ERS/IDSA and WHO treatment guidelines have expanded the role of rifapentine-containing regimens in both drug-susceptible tuberculosis treatment and tuberculosis preventive therapy, underscoring the ongoing need for robust quality control tools as production scales up. The present method, validated under the ICH Q2(R1) framework, remains fully applicable; laboratories seeking to align with the newer ICH Q2(R2)/Q14 framework for analytical procedure development and lifecycle management may extend this work with additional multivariate robustness and control-strategy elements, but no changes to the core chromatographic conditions are required.

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