Journal of Pharmaceutical and Biomedical Analysis Letters
ISSN: 2347-4742 | https://doi.org/10.30904/j.jpbmal.2026.4921
J. Pharm, Biomed. A. Lett., 2026; 14(1): 01-05

QBD-based RP-HPLC Method Development and Validation for Thioacetazone and Isoniazide in Pharmaceutical Dosage Forms as per ICH Guidelines

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Keywords
RP-HPLC
Isoniazid
Thioacetazone
Platisil C18 column
Orthophosphoric acid buffer

Received 24 Nov 2025
Revised 27 Dec 2025
Accepted 29 Jan 2026
Published 25 Feb 2026

Abstract
A novel and comprehensive High-Performance Liquid Chromatography (RP-HPLC) method was developed and optimized for the simultaneous estimation of Isoniazid and Thioacetazone in pharmaceutical formulations. Method optimization was achieved using a Box-Behnken design coupled with response surface methodology, evaluating critical chromatographic parameters such as flow rate, buffer ratio, and buffer pH. The optimized conditions employed a Platisil C18 column (150×3.0 mm, 3µm) with a mobile phase of 0.1% orthophosphoric acid buffer (pH 3.5) and methanol (40:60 v/v), at a flow rate of 0.8 mL/min and detection at 258 nm. System suitability tests confirmed acceptable resolution (≥2), tailing factor (≤2), and theoretical plate count (≥2000), ensuring reliable separation and peak symmetry for both analytes. The method was rigorously validated in accordance with ICH guidelines, demonstrating excellent linearity for Isoniazid (10–50 µg/mL, R^2 = 0.9998) and Thioacetazone (5–25 µg/mL, R^2 = 0.9995). Precision studies yielded %RSD values below 2%, confirming reproducibility under varied conditions. Sensitivity was established through low LOD and LOQ values, while recovery studies at multiple concentration levels (50%, 100%, 150%) showed accuracy within 98–102%. Robustness was maintained despite deliberate variations in chromatographic parameters. Overall, the validated method is accurate, precise, sensitive, and robust, making it highly suitable for routine quality control of Isoniazid and Thioacetazone in pharmaceutical dosage forms.

INTRODUCTION

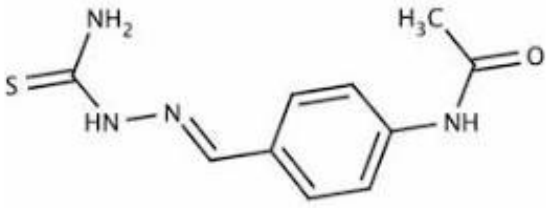

Fig.1: Thioacetazone

IUPAC Name: N-{4-[(Ethanethioamidoimino) methyl] phenyl} acetamide
Molecular Formula: $C_{10}H_{12}N_4OS$
Molecular Weight: 236.29 g/mol
Category: Antituberculosis agent
Physical Properties
Melting Point: Approximately 204-205°C
pKa: 9.38(acidic), 11.42 (acidic), 13.37(acidic), 2.45 (basic)
Solubility: Sparingly soluble in water; soluble in ethanol and acetone.

Description
Thioacetazone is an oral antibiotic used in the treatment of tuberculosis. It has largely fallen out of use due to its toxicity and the availability of more effective drugs like isoniazid.

Mechanism of Action
Thioacetazone is believed to interfere with mycolic acid synthesis in Mycobacterium tuberculosis, although its exact biological target remains elusive1.

Pharmacodynamics and Pharmacokinetics
Pharmacodynamics: Thioacetazone has weak activity against Mycobacterium tuberculosis and is primarily used to prevent resistance to more potent drugs.
Pharmacokinetics: Detailed pharmacokinetic data is limited.

Metabolism and Elimination
Metabolism: Not well-documented.
Route of Elimination: Primarily renal.

Protein Binding and Half-Life
Protein Binding: Not well-documented.
Half-Life: Not well-documented.

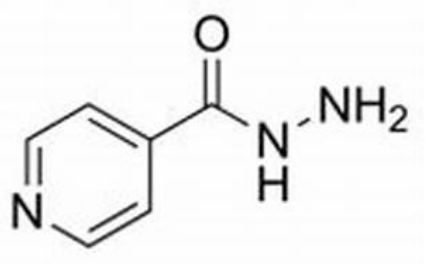

Fig.2: Isoniazide

IUPAC Name: Pyridine-4-carbohydrazide
Molecular Formula: $C_6H_7N_3O$
Molecular Weight: 137.14 g/mol
Category: Antituberculosis agent

Physical Properties

Melting Point: 171-173°C
pKa: 1.8 (acidic), 3.5 (acidic), 10.8 (basic)
Solubility: Soluble in water, ethanol, and methanol.

Description

Isoniazid, also known as isonicotinic acid hydrazide (INH), is an antibiotic used for the treatment and prevention of tuberculosis (TB). It is often used in combination with other TB medications to prevent resistance.

Mechanism of Action

Isoniazid works by inhibiting the synthesis of mycolic acids, which are essential components of the bacterial cell wall in Mycobacterium tuberculosis.

Pharmacodynamics: Isoniazid is bactericidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms.

Pharmacokinetics:

Absorption: Rapidly absorbed from the gastrointestinal tract.
Distribution: Widely distributed in body fluids and tissues.
Metabolism: Metabolized in the liver by acetylation.
Excretion: Primarily excreted in the urine.

Metabolism and Elimination

Metabolism: Metabolized in the liver by acetylation.
Route of Elimination: Primarily renal.

Uses and Dosage

Uses: Treatment and prevention of tuberculosis.
Dosage: Typically 5 mg/kg daily (maximum 300 mg daily) for adults; specific dosage should be determined by a healthcare provider

MATERIALS AND METHODS

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Betamethasone	Supplied by KP LBAS
2	Calcipotriene	Supplied by KP LBAS
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

Optimization of Column:

Platisil C18, (150×3.0mm, 3µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Temperature : Ambient
Column : Platisil C18, (150×3.0mm, 3µm)
Buffer : 0.1% OPA (pH-3.5)
Mobile phase: 40% 0.1% OPA (pH-3.5): 60% Methanol
Flow rate : 0.8ml per min
λmax : 258 nm.
Injection volume : 20 µl
Run time : 8min.

Preparation of buffer and mobile phase:

Preparation of OPA pH 3.5:

To prepare OPA solution, by adding 1ml OPA in 1000 ml water. Adjust this solution to pH 3.5 by using acid / base based on the PH of the resulted solution.

Preparation of mobile phase:

Mix a mixture of above 350 ml OPA (35%), MEOH 650ml (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Methanol: OPA (65:35) ratio.

System Suitability:

Tailing factor for the peaks due to Thiaacetazone and Isoniazide in Standard solution should not be more than 2.0
Theoretical plates for the Thiaacetazone and Isoniazide peaks in Standard solution should not be less than 2000

Method validation parameters:

Assay:

Standard Solution Preparation:

Accurately weigh and transfer mg of 20 mg Isoniazid and 10mg Thioacetazone working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (30ppm of isoniazide & 15ppm of thiaacetazone).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20mg Isoniazid and 10mg of Thioacetazone equivalent weight of the sample into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (30ppm of isoniazide & 15ppm of thiaacetazone).

Procedure:

Inject 10µL of the standard, sample into the chromatographic system and measure the areas for the Thioacetazone and Isoniazid peaks.

Calculation: (For Thiaacetazone and Isoniazide)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

Acceptance criteria of System Suitability
Tailing factor should be less than 2
Theoretical Plates should be above 2000

RESULTS AND DISCUSSION

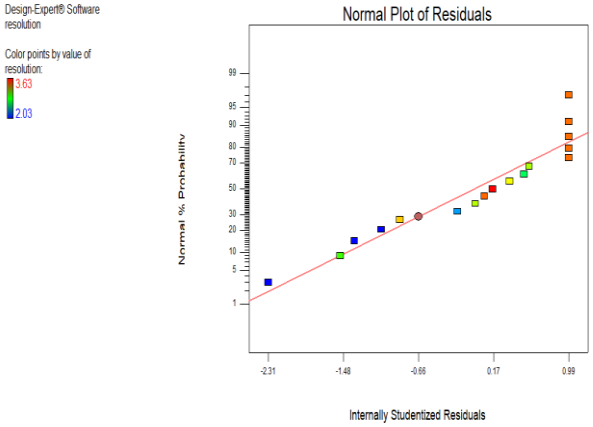

Fig.3: Normal plot of residuals for Isoniazid and Thioacetazone

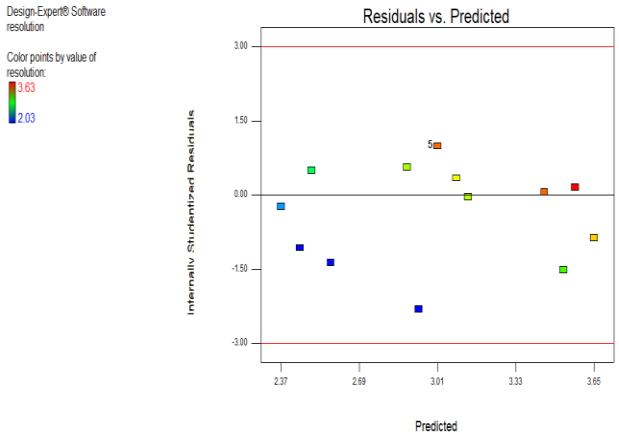

Fig.4: Residuals vs. Predicted for Isoniazid and Thioacetazone

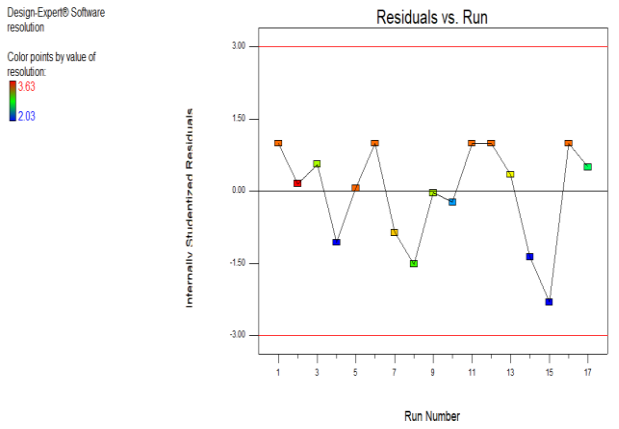

Fig.5: Residuals vs. Run for Isoniazid and Thioacetazone

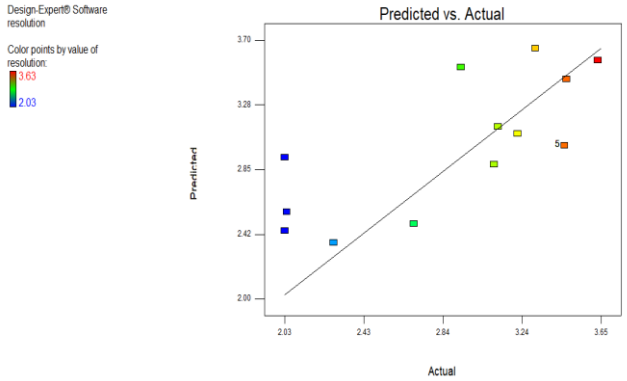

Fig.6: Predicted vs. Actual for Isoniazid and Thioacetazone

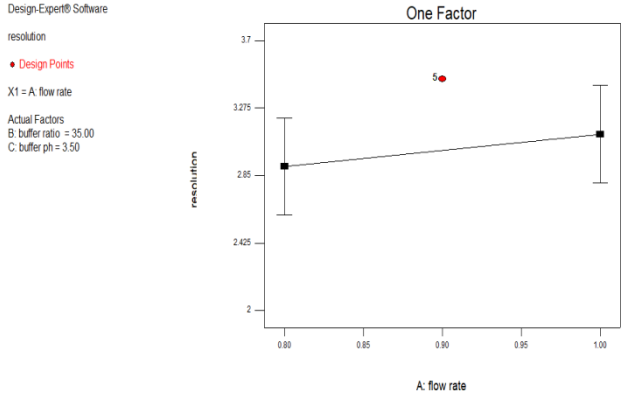

Fig.7: Resolution for Isoniazid and Thioacetazone

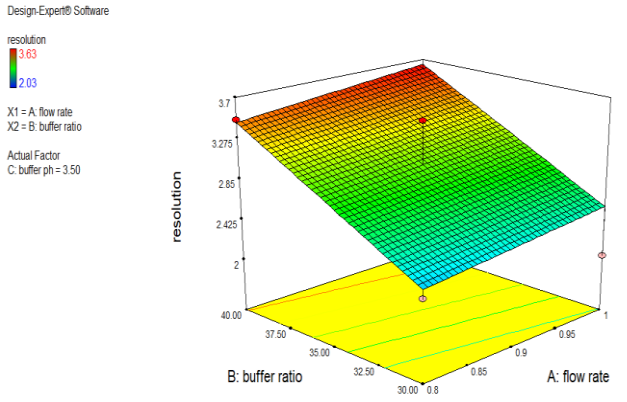

Fig.8: 3D Surface for Isoniazid and Thioacetazone

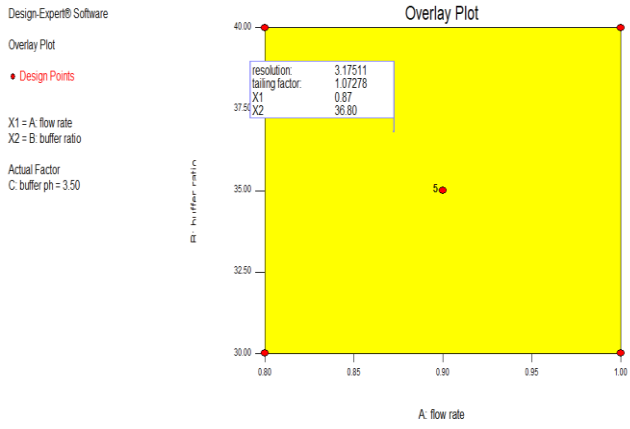

Fig.9: Overlay plot for Isoniazid and Thioacetazone

Table 1: FIT Summary (R_2) Tailing Factor of Isoniazid and Thioacetazone

Source	Sum of Squares	df	mean Square	F-Value	p-value Prob > F	
Mean vs Total	22.14	1	22.14	**Suggested**		
Linear vs Mean	0.075	3	0.025	3.46	0.0482	**Suggested**
2FI vs Linear	4.550E-003	3	1.517E-003	0.17	0.9146	
Quadratic vs 2FI	0.073	3	0.024	10.16	0.0061	**Suggested**
Cubic vs Quadratic	0.017	3	5.575E-003	6.366E+007	< 0.0001	**Aliased**
Residual	0.000	4	0.000			
Total	22.31	17	1.31			

Table 2: Response 2: Tailing factor ANOVA for Response Surface Quadratic Model

Souces	Sum of square	df	Mean square	F value	P value Prob>F	
Model	0.15	9	0.017	7.09	0.0086	significant
A-flow rate	0.011	1	0.011	4.40	0.0741	
B-buffer ratio	8.450E-003	1	8.450E-003	3.54	0.1021	
C-buffer ph	0.056	1	0.056	23.49	0.0019	
Residual	0.017	7	2.389E-003			
Lack of Fit	0.017	3	5.575E-003			
Pure Error	0.000	4	0.000			

Table 3: Results of system suitability parameters

Name	Rt	Area	Height	Resolution	Tailing factor	Plate count
Isoniazid	2.765	46351	5728	3.90	1.02	8873
Thioaceazone	3.552	19934	2864		1.01	2581

Table 4: Area of different concentration of Thiaacetazone and Isoniazide

S.NO	Concentration (µg/ml)	Areas of Isoniazide
1	10	15410
2	20	30802
3	30	46214
4	40	60635
5	50	77015

Table 5: Area of different concentration of Thiaacetazone and Isoniazide

S.NO	Concentration (µg/ml)	Areas of Thiaacetazone
1	5	6651
2	10	13285
3	15	19921
4	20	25871
5	25	33243

Table 6: Results of LOD

Drug name	Baseline noise(µV)	ignal obtained(µV)	S/N ratio	Conc. In ppm
Isoniazide	74	210	2.84	1.10
Thiaacetazone	74	215	2.91	1.13

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc. In ppm
Isoniazide	74	720	9.73	3.77
Thiaacetazone	74	734	9.92	3.84

4. Conclusion

The method was thoroughly validated as per ICH guidelines for parameters including linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). The resolution and tailing factors were used as response parameters to assess method performance. The optimized chromatographic conditions included the use of a Platisil C18 column (150 × 3.0 mm, 3 µm), with a mobile phase consisting of 0.1% OPA buffer (pH 3.5) and methanol in the ratio of 40:60, at a flow rate of 0.8 mL/min, and detection wavelength at 258nm. The method exhibited excellent linearity for Isoniazid (10–50 µg/mL, $R^2 = 0.9998$) and Thioacetazone (5–25 µg/mL, $R^2 = 0.9995$). Precision studies showed %RSD values below 2%, confirming reproducibility and robustness even with deliberate changes in chromatographic conditions. The LOD and LOQ values confirmed the method's sensitivity, while recovery studies at 50%, 100%, and 150% levels demonstrated accuracy within 98–102%. Overall, the validated method proved to be accurate, precise, robust, and suitable for the routine analysis of Isoniazid and Thioacetazone in pharmaceutical dosage forms.

5. References

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