RESEARCH PAPER

Simultaneous Estimation Of N-Acetyl Cysteine And Pyridoxamine Dihydrochloride In It'S Bulk Drug And Pharmaceutical Dosage Form By Hptlc Method

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Received: *20th Feb, 2026;* ***Revised:*** *4th Mar, 2026;* ***Accepted:*** *25th Mar, 2026;* ***Available Online:*** *10th Apr, 2026*

Abstract

A simple, precise, and cost-effective high-performance thin-layer chromatographic (hptlc) method was developed and validated for the simultaneous estimation of n-acetyl cysteine (nac) and pyridoxamine dihydrochloride (pyri 2hcl) in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using silica gel 60f254 plates with a mobile phase consisting of methanol:toluene:formic acid (3:7:0.1 v/v/v). Densitometric analysis was performed at 212 nm. The rf values for nac and pyri 2hcl were found to be 0.42 and 0.13, respectively. The method showed good linearity in the range of 30–180 ng/band for nac and 5–30 ng/band for pyri 2hcl with correlation coefficients of 0.997 and 0.994. The method was validated as per ich guidelines and demonstrated acceptable accuracy, precision, sensitivity, robustness, and specificity. The developed method was successfully applied for the analysis of marketed formulations. Hence, the method can be used for routine quality control analysis.

Keywords: N-Acetyl Cysteine, Pyridoxamine Dihydrochloride, High Performance Thin Layer Chromatography, Validation.

How To Cite This Article: Bhavale Gh, Shelar Mu, Kadam Jn, Andhale Gs, Gondhale-Karpe P, Jadhav Ny, Dhumal Js, Dighe Rd. Simultaneous Estimation Of N-Acetyl Cysteine And Pyridoxamine Dihydrochloride In It'S Bulk Drug And Pharmaceutical Dosage Form By Hptlc Method. Int J Drug Deliv Technol. 2026;16(4):425-432. Doi: 10.25258/ijddt.16.4.41

1. Introduction

N-Acetyl-L-cysteine (NAC) is chemically designated as (2R)-2-acetamido-3-sulfanylpropanoic acid and is a derivative of the amino acid cysteine, in which the amino group is acetylated[1]. It is widely recognized for its mucolytic activity, attributed to its ability to cleave disulfide bonds in mucoproteins, thereby reducing the viscosity of secretions and facilitating their removal[2]. NAC is commonly used in ophthalmic preparations and bronchial nebulizing solutions for respiratory disorders[3]. Furthermore, it is an established antidote for paracetamol overdose, where it replenishes intracellular glutathione levels and mitigates the severity of acute liver injury[4]. Recent studies have also highlighted its antioxidant, anti-inflammatory, and cytoprotective properties, expanding its therapeutic applications[5–7]

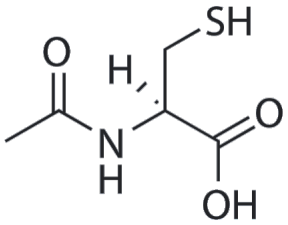

Fig. No. 1 Structure of N-Acetyl-L-cysteine

Pyridoxamine Dihydrochloride is a water-soluble vitamin B6 analog, chemically known as 4-(aminomethyl)-5-(hydroxymethyl)-2-methylpyridin-3-ol dihydrochloride. It is formed by the reaction of pyridoxamine with two equivalents of hydrochloric acid. Pyridoxamine plays multiple biological roles as a metabolite in microorganisms and higher organisms and exhibits pharmacological activities such as iron chelation and nephroprotection, making it useful in the management of diabetic nephropathy[8].

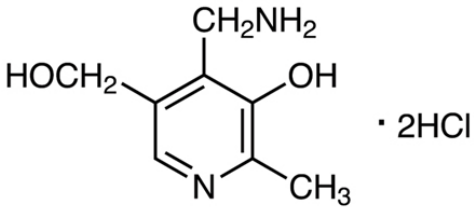

Fig. No. 1 Structure of Pyridoxamine Dihydrochloride

Several analytical methods have been reported for the estimation of NAC, including UV spectrophotometry, reverse-phase high-performance liquid chromatography (RP-HPLC), chemiluminescence, mass spectrometry, and gas chromatography techniques[9–13]. Similarly, analytical methods for Pyridoxamine dihydrochloride have been developed using high-performance thin-layer chromatography (HPTLC) in combination with other drugs and RP-HPLC methods[14–15].

However, based on an extensive literature survey, no HPTLC method has been reported for the simultaneous quantitative analysis of NAC and Pyridoxamine dihydrochloride in active pharmaceutical ingredient (API) and marketed tablet dosage forms. Therefore, the present study aims to develop and validate a simple, precise, and reliable HPTLC method for simultaneous estimation of these drugs in accordance with International Council for Harmonisation guidelines.

2. Materials and Methods

2.1 Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed using a CAMAG High-Performance Thin Layer Chromatography (HPTLC) system equipped with a Linomat V semi-automatic sample applicator and TLC Scanner III for densitometric evaluation. Sample application was carried out using a 100 µL Hamilton microsyringe under controlled conditions. Plate development was performed in a twin-trough glass chamber previously saturated with the mobile phase. Data acquisition and processing were accomplished using WinCATS software (version 3.17).

Pre-coated silica gel 60F254 aluminum-backed HPTLC plates (10 × 10 cm, 0.2 mm thickness; Merck KGaA, Germany) were used as the stationary phase. Accurate weighing of samples was carried out using a WENSAR high-precision analytical balance. Similar instrumentation setups are widely used in modern planar chromatographic analysis due to their reliability and reproducibility[15–17].

2.2 Chemicals and Reagents

Reference standards of N-acetyl cysteine (NAC) and pyridoxamine dihydrochloride (PYRI 2HCl), both with purity of 99%, were procured from Research Lab Fine Chem Industries (Mumbai, India). Analytical grade solvents including methanol, toluene, and formic acid were obtained from Oswal Scientific (Pune, India).

Commercially available tablet formulations containing NAC (300 mg) and PYRI 2HCl (50 mg) were purchased from the local pharmaceutical market and used for analysis. The use of analytical grade reagents ensures method accuracy and minimizes interference during chromatographic separation[18,19].

2.3 Mobile Phase Selection

To achieve optimal resolution, several mobile phase compositions consisting of solvents of varying polarity were evaluated. The optimized mobile phase system was found to be methanol: toluene : formic acid (3:7:0.1, v/v/v), which provided well-resolved and symmetrical peaks for both analytes.

The selection of an appropriate mobile phase is critical in HPTLC analysis as it directly influences separation efficiency, Rf values, and peak shape[16,20].

2.4 Preparation of Standard Solutions

Standard stock solutions were prepared by accurately weighing 30 mg of NAC and 5 mg of PYRI 2HCl, each dissolved separately in 10 mL of methanol to obtain final concentrations of 3000 µg/mL and 500 µg/mL, respectively.

These stock solutions were further diluted as required to obtain working concentrations within the linearity range. Preparation of standard solutions in methanol ensures good solubility and stability of analytes during analysis[21].

2.5 Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. An amount of powder equivalent to 30 mg of NAC and 5 mg of PYRI 2HCl was transferred into a 10 mL volumetric flask. Approximately 8 mL of methanol was added, and the mixture was sonicated to ensure complete extraction of the drugs. The solution was then diluted to volume with methanol and filtered through suitable filter media.

A working sample solution was prepared and 1 µL was applied per band, corresponding to 3000 ng/band for NAC and 500 ng/band for PYRI 2HCl. Proper sample preparation is essential to achieve accurate quantification and reproducible chromatographic results[22].

2.6 Chromatographic Procedure

Samples were applied as bands on the HPTLC plate using the Linomat V applicator under controlled conditions. The plates were developed in a saturated twin-trough chamber containing the optimized mobile phase. After development, plates were air-dried and subjected to densitometric scanning at 212 nm, which was selected based on maximum absorbance of both analytes.

The developed method produced well-defined peaks with Rf values of 0.42 for NAC and 0.13 for PYRI 2HCl, indicating effective separation. Selection of appropriate detection wavelength enhances sensitivity and selectivity in HPTLC analysis[20],[25].

2.7 Method Validation

The developed analytical method was validated in accordance with ICH Q2(R1) guidelines, including parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and specificity.

Validation ensures the reliability, reproducibility, and suitability of the analytical method for its intended purpose in pharmaceutical analysis[26,27].

3. Result & Discussion

3.1 Development of Chromatographic Method

The primary objective of method development was to achieve effective separation of N-acetyl cysteine (NAC) and pyridoxamine dihydrochloride (PYRI 2HCl) with good resolution and peak symmetry. Several solvent systems of varying polarity were evaluated. The optimized mobile phase consisting of methanol : toluene : formic acid (3:7:0.1 v/v/v) produced well-defined and compact spots with Rf values of 0.42 (NAC) and 0.13 (PYRI 2HCl).

Densitometric detection at 212 nm provided sufficient sensitivity for simultaneous estimation. Similar HPTLC-based analytical methods employing silica gel plates and optimized solvent systems have been widely reported for pharmaceutical analysis due to their simplicity and cost-effectiveness.

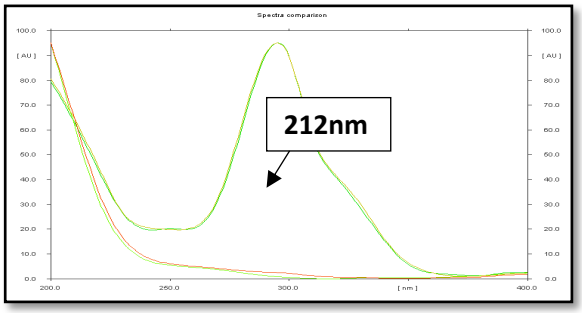

Fig No.3 UV spectra scanning of Drug NAC & PYRI 2 HCl.

3.2 Method Validation

The HPTLC determination of NAC and PYRI 2HCl in API and in dosage forms were validated as per ICH guidelines.

3.1.1 Linearity

The calibration curves demonstrated linearity over the concentration ranges of 30–180 ng/band for NAC and 5–30 ng/band for PYRI 2HCl, with correlation coefficients of 0.997 and 0.994, respectively. These results confirm a strong linear relationship between concentration and peak area, consistent with validated HPTLC analytical procedures.

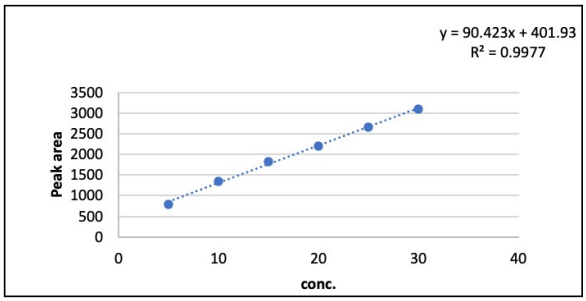

Fig No.4 Calibration Curve of PYRI 2HCl

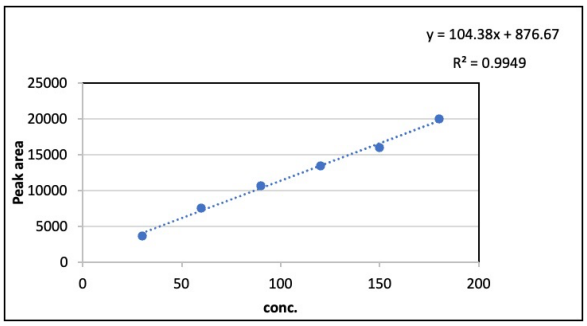

Fig No.5 Calibration Curve of NAC

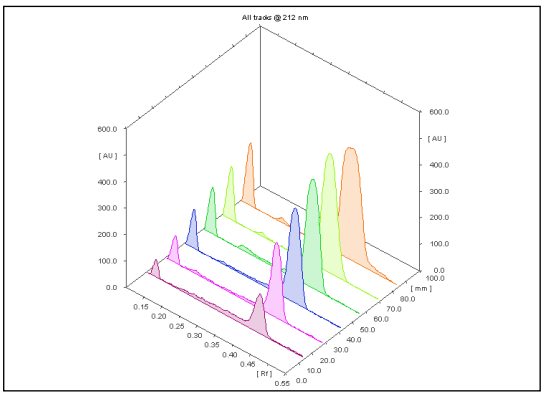

Fig No.4 Linearity of PYRI 2HCL and NAC

3.1.2 Accuracy

Accuracy was evaluated using recovery studies at three levels (80%, 100%, and 120%). The percentage recovery ranged from 100.69–101.17% for NAC and 99.5–100.67 % for PYRI 2HCl, indicating excellent accuracy.

Such recovery ranges are in agreement with ICH validation requirements and previously reported HPTLC methods. .

Table 1: Accuracy Data for PYRI 2HCl and NAC

Drug	Recovery Level (%)	Initial amount (ng/band)	Amount found (ng/band)	% Recovery	% RSD
PYRI 2HCl	80	40	39.8	99.5	0.65
	100	50	50.3	100.6	
	120	60	60.4	100.67	
NAC	80	240	242.8	101.17	0.24
	100	300	302.8	100.93	
	120	360	362.5	100.69	

3.1.3 Precision

Precision studies, expressed in terms of %RSD, demonstrated values of 1.63% for NAC and 1.16% for PYRI 2HCl, which are well within the acceptable limit of less than 2% as per ICH guidelines. This indicates that the method has excellent repeatability and consistency. The low variability in peak area suggests that the method is not significantly affected by minor experimental variations during analysis.

Table 2: Precision Data for PYRI 2HCl and NAC

Sr. No	PYRI 2HCL		NAC	
	Conc.(ng/spot)	Peak area	Conc.(ng/spot)	Peak area
1	50	2614	300	13908
2	50	2687	300	14003
3	50	2622	300	13360
4	50	2667	300	13364
5	50	2601	300	13265
6	50	2621	300	14847
MEAN	**2635.333**		**13507.83**	
%RSD	**1.16**		**1.63**	

3.1.4 LOD & LOQ

The sensitivity of the method was confirmed by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD values were found to be 1.65 ng/band for NAC and 14.85 ng/band for PYRI 2HCl, while the LOQ values were 5.02 ng/band and 45 ng/band, respectively.

Table 3: LOD & LOQ of PYRI 2HCl and NAC

Drug	LOD	LOQ
NAC	1.65	5.02
PYRI 2HCl	14.85	45

3.1.5 Robustness

Robustness studies revealed that small deliberate changes in chromatographic conditions such as mobile phase composition, chamber saturation time, and time intervals between spotting and scanning resulted in slight variations in Rf values. This indicates that the method is somewhat sensitive to these parameters and emphasizes the need for strict control of experimental conditions to ensure reproducibility.

Table 4: Robustness data of PYRI 2HCl and NAC

Parameters	Drug	RF
Amount of mobile phase (±5%)	PYRI 2HCl	0.63

	NAC	0.74
Mobile phase composition (±0.1ml)	PYRI 2HCl	0.34
	NAC	0.54
Time for spotting to scanning (±10min)	PYRI 2HCl	0.40
	NAC	0.49
Time for spotting to chromatography (±10min)	PYRI 2HCl	0.32
	NAC	0.48

3.1.6 Specificity

The method demonstrated good specificity, as no interference from excipients, solvents, or mobile phase components was observed at the Rf values of NAC and PYRI 2HCl. This confirms the method ability to selectively analyze both analytes in the presence of other components. For specificity study spot of Methanol, Mobile phase, Standard solution and tablet solution of both PYRI 2HCl and NAC are recorded *(13).*

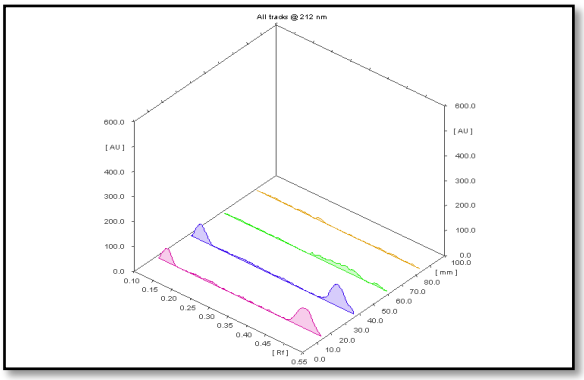

Fig. No.5 Specificity Densitogram of NAC and PYRI 2HCl

3.1.7 Assay

The HPTLC method was applied for estimation of Pyridoxamine dihydrochloride and N-Acetyl Cysteine in tablets. The content of Pyridoxamine dihydrochloride was found to be 49.12 mg (98.24%), while N-Acetyl Cysteine was 281.25 mg (93.75%) of the labeled claim.

Table 5: Analysis of marketed formulation

Drug	Amount present (mg/tab)	% amount found	S.D	%RSD
PYRI 2HCl	50	49.12	13.19	0.506
NAC	300	281.25	158.81	1.58

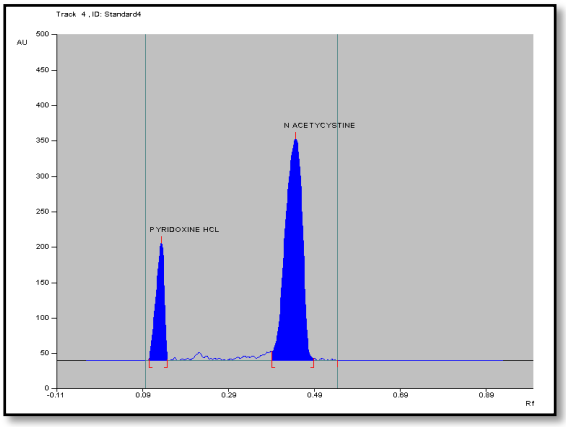

Fig. No.6 Densitogram of PYRI 2HCl and NAC

4.0 Conclusion

A simple, rapid, and reliable HPTLC method was successfully developed and validated for the simultaneous estimation of N-Acetyl Cysteine and Pyridoxamine dihydrochloride in bulk and pharmaceutical dosage forms. The optimized mobile phase consisting of methanol:toluene:formic acid (3:7:0.1 v/v/v) provided well-resolved and sharp peaks with distinct Rf values, indicating effective separation of both analytes.

The method exhibited excellent linearity over the selected concentration ranges with high correlation coefficients ($r^2 > 0.99$). Accuracy studies demonstrated satisfactory recovery within acceptable limits, confirming the reliability of the method. Precision results, expressed as %RSD, were found to be less than 2%, indicating good repeatability and reproducibility. The LOD and LOQ values confirmed the adequate sensitivity of the method for trace-level detection and quantification.

Furthermore, the method was found to be specific, as no interference from excipients or other components was observed at the respective Rf values. Although slight variations in chromatographic conditions affected Rf values, the method remained consistent under controlled conditions, indicating acceptable robustness.

Overall, the developed HPTLC method is economical, precise, accurate, and suitable for routine quality control analysis of N-Acetyl Cysteine and Pyridoxamine dihydrochloride in combined pharmaceutical formulations, complying with ICH validation guidelines.

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