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Simultaneous Determination of N-Acetyl Cysteine and Taurine by HPTLC Method in Active Pharmaceutical Ingredient and Pharmaceutical Dosage Form

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Abstract

Specific, precise and sensitive TLC-Densitometric method was developed and validated for the simultaneous estimation of N-Acetyl cysteine and Taurine in active pharmaceutical ingredient and pharmaceutical dosage form. An effective separation was achieved on pre-coated silica gel HPTLC plates by using n-butanol:acetic acid:water (8:0.5:1.5 v/v/v). The spots were scanned densitometrically at 295 nm. The RF values of N-Acetyl cysteine and Taurine were found to be 0.29 and 0.52, respectively. Calibration curves were linear in the range of 30 - 180 and 100 - 600 ng/band for N-Acetyl cysteine and Taurine, correspondingly with correlation coefficients of 0.999. The developed method was validated as per ICH guidelines. The limits of detection were 11.24 and 63.40 ng/spot for NAC and TAU respectively. The method developed was found to be precise and specific for the simultaneous analysis of N-Acetyl cysteine and Taurine in pure and tablet dosage form.

Keywords

High Performance Thin Layer Chromatography, N-Acetyl Cysteine, Taurine, Validation

1. Introduction

Acetyl cysteine is chemically known as (2R)-2-(acetyl amino)-3-sulfanyl propanoic acid, and is used as a mucolytic agent to reduce the viscosity of secretions probably by means of disulphide bond splitting in mucoproteins. Acetyl cysteine in addition is able to promote the detoxification of an intermediate paracetamol

metabolite and has a chief role in the supervision of paracetamol over dosage. It is in use for its mucolytic action in respiratory disorders coupled with productive cough. Taurine is chemically called as 2-aminoethane sulfonic acid, and is an amino acid which involves in bile acid conjugation and other physiological functions [1].

The reported analytical methods for N-Acetyl cysteine include colorimetric [2], spectrophotometric [3], Chemiluminescence [4], HPLC with electrochemical [5] [6] [7], fluorimetric detection [8] [9] [10], mass [11] [12], ultraviolet detection [13] [14] [15] and Gas chromatographic [16].

For the estimation of Taurine it is quantified by means of HPLC employing post column derivatisation [17] [18] [19], pre-column [20] [21], and on-column [22].

The methods developed for taurine comprises detection by means of evaporative light scattering [23] amperometric detection [24], Chemiluminescence detection [25], mass spectrometric [26] [27] and planar chromatography with multiple detection [28].

As per the literature survey there have been no reports on High Performance Thin Layer Chromatographic method for quantitative analysis of N-Acetyl cysteine and Taurine in both active pharmaceutical ingredients (APIs) and marketed tablet formulation. Hence the present study was intended for the development of a method for the simultaneous estimation of N-Acetyl cysteine and Taurine in active pharmaceutical ingredient and tablet dosage form and to validate as per ICH guidelines.

2. Materials

2.1. Chemicals and Drugs

Analytical pure samples of N-Acetyl cysteine (NAC) and Taurine (TAU) obtained as a gift sample from FOURRTS Laboratories, India were used in the study. The Pharmaceutical dosage form employed in this study was Nefrosave (Fourrts Laboratories limited, India) procured from the local market and labeled to contain 150 mg of N-Acetyl cysteine and 500 mg of Taurine for each tablet.

2.2 Instrumentation

It comprises of Linomat-IV sample applicator and TLC Scanner with UV detector (Camag, Switzerland).The software employed in the study was winCATS. The sample was spotted by using micro syringe of 100 µL capacity and the development of the plate was done in twin trough chamber.

2.3. Standard Solution Preparation

Solutions of standard were prepared by dissolving individually 10 mg of N-Acetyl cysteine and 10 mg of Taurine each in volumetric flask containing 10 mL of methanol so as to obtain a concentration of 1000 µg/mL. The Standard stock solutions were suitably diluted with methanol to obtain the working standard solutions of both N-Acetyl cysteine and Taurine (100 µg/mL).

2.4. Marketed Sample Preparation

Weigh and powder twenty tablets so as to obtain a fine powder. An accurately weighed powder sample equivalent to 30 mg of N-Acetyl cysteine and 100 mg of Taurine was weighed, transferred to a 50 mL volumetric flask consisting 40 mL of methanol to solubilise the drug and then sonicated, made up to the volume and then filtered. From the filtrate 2.5 mL of the solution was diluted up to 10 ml using the same solvent so as to obtain a concentration of 150 µg/mL of N-Acetyl cysteine and 500 µg/mL of Taurine respectively. Appropriate working sample solutions (1.0 µL) containing N-Acetyl cysteine and Taurine in the concentration ratio of 150 ng/band of N-Acetyl cysteine and 500 ng/band of Taurine, correspondingly were employed for the quantitative analysis.

3. Development of Chromatographic Method

For the assortment of suitable mobile phase for the efficient separation of N-Acetyl cysteine and Taurine, numerous runs were made by using mobile phase containing solvents of altering polarity and at various ratio levels. In the midst of the diverse mobile phase combinations employed, the mobile phase comprising of n-butanol: acetic acid: water in the ratio of 8:0.5:1.5 v/v/v/ produced a sharp well defined peaks with R_F values of 0.29 and 0.52 for N-Acetyl cysteine and Taurine, respectively.

For the choice of analytical wavelength, the standard spots were applied on silica gel were scanned to obtain their overlain spectra. From the overlain spectra, it was observed that both N-Acetyl cysteine and Taurine exhibited strong absorbance at about 295 nm which was selected as the analytical wavelength.

4. Method Validation

The following parameters were validated for the HPTLC determination of NAC and TAU in dosage forms as per ICH guidelines [29].

4.1. Linearity

Peak areas were found to have improved linear relationship with the concentration than the peak heights. For N-Acetyl cysteine and Taurine the r^2 was found to be 0.999. Calibration graphs were constructed in the concentration range of 30 - 180 ng/band for N-Acetyl cysteine and 100 - 600 ng/band for Taurine.

4.2. Accuracy

The accuracy studies were performed out at 80%, 100% and 120% of the sample concentration as per the recommendations of ICH guidelines. The recovery percentage of N-Acetyl cysteine and Taurine at all the three levels was found to be acceptable.

For N-Acetyl cysteine, the percentage recovery was found between 99.88% and 100.10% and for Taurine between 99.92% and 100.17% respectively.

4.3. Precision Study

Precision Study was calculated in terms of RSD. The results revealed that the statistical datas were within acceptable range at concentration of 150 ng/ band for N-Acetyl cysteine and 500 ng/band for Taurine.

The precision studies were determined by means of% RSD and the values were found to be less than 2% which is in the acceptable range indicates the developed method is precise.

4.4. Robustness Study

Robustness for the developed method was evaluated by implementing slight variations in composition of mobile phase, saturation time of the chamber and minimal variation in migration distance of the solvent. Triplicate analysis was performed for robustness determination and% RSD values of peak area were calculated.

5. Results and Discussion

5.1. Method Optimization

Several solvent system were tried to obtain efficient separation of NAC and TAU. Finally the solvent system comprising n-butanol: acetic acid: water 8:0.5:1.5 v/v/v provided the best separation with RF values of N-Acetyl cysteine and Taurine was found to be 0.29 and 0.52 respectively. Chromatography was carried out on precoated plates using silica gel 60 F254. Before developments the plates were prewashed using methanol and then activated for 6 min at 110˚C. The solutions were applied on to the plates by using Linaomat sample applicator and developed by ascending method in twin trough chamber using n-butanol: acetic acid: water (8:0.5:1.5 v/v/v). After the development, the plates were dried in air by air dryer. The scanning was done by using TLC Scanner at 295 nm. The plate was developed by employing ascending method in twin trough chambers and the saturation was done at room temperature. The densitogram of NAC and TAU is shown in **Figure 1**. The RF values of N-Acetyl cysteine and Taurine was found to be 0.29 and 0.52 with good resolution between the components. The summary of validation parameters is represented in **Table 1**.

The proposed HPTLC method was used for the simultaneous estimation of NAC and TAU, in tablets. Results obtained were satisfactory and each compound is in accord with label claim and the results were imparted in the **Table 2**.

5.2. Method Validation

5.2.1. Linearity

Linearity plot of N-Acetyl cysteine and Taurine was plotted using peak area versus concentration as shown in **Figure 2** and **Figure 3**.

Linearity was found in the concentration range of 30 - 180 ng/spot for N-Acetyl cysteine and 100 - 600 ng/spot for Taurine respectively, which is represented in 3D display as represented in **Figure 4**. The correlation coefficient

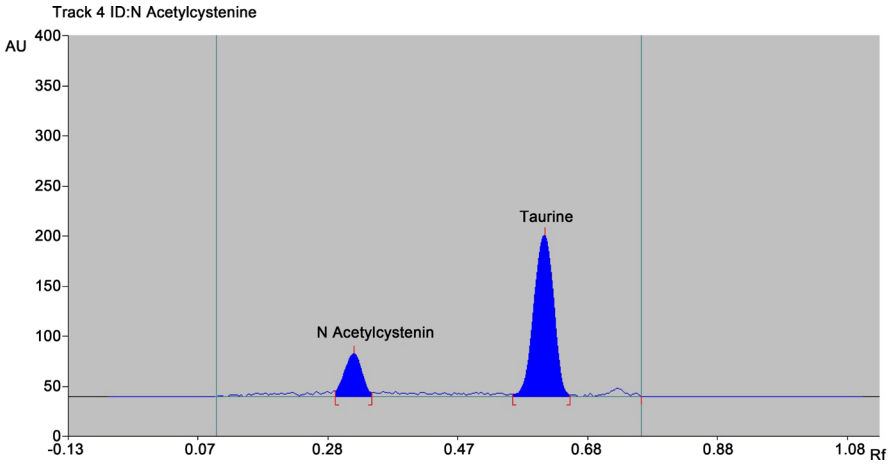

Figure 1. Densitogram of NAC and TAU detected at 295 nm.

Table 1. Summary of validation parameters for N-Acetyl cysteine and taurine.

Parameters	NAC	TAU
Linearity range	30 - 180 ng/band	100 - 600 ng/band
Linear regression equation	13.00x + 4.107	15.61x – 25.39
Slope ± SD	13.00 ± 0.327	15.61 ± 0.275
Intercept ± SD	4.107 ± 0.914	25.39 ± 0.964
Correlation coefficient	0.999	0.999
Limit of detection (ng)	11.24	63.40
Limit of quantification (ng)	34.07	192.12
Repeatability	0.070	0.130
Intra-day	0.122	0.102
Inter-day	0.072	0.266

Table 2. Results of HPTLC assay of NAC and TAU.

Drug	Amount present (mg per tablet)	Percentage amount found	S.D	% SD
NAC	150	149.79	0.050	0.033
TAU	500	499.80	0.146	0.029

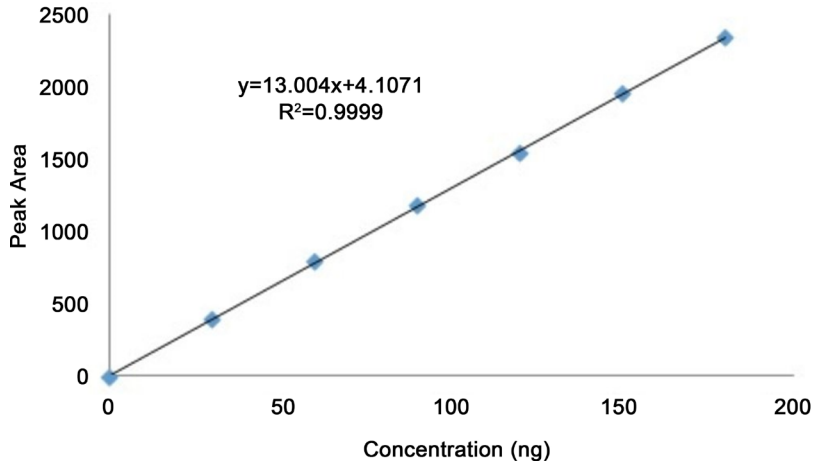

Figure 2. Linearity plot of NAC by HPTLC.

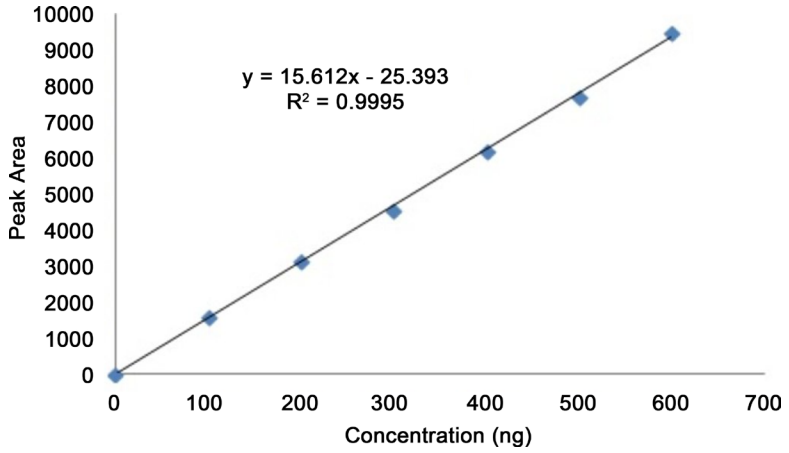

Figure 3. Linearity plot of TAU by HPTLC.

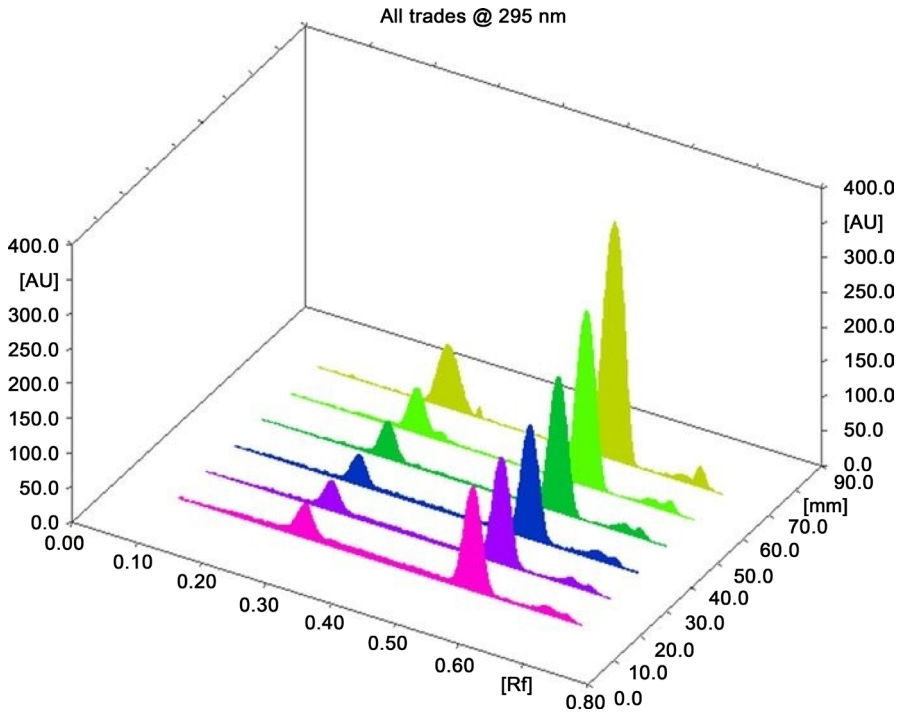

Figure 4. Typical 3-D view of the linearity study of NAC and TAU.

values were found to be 0.999 for both the drugs indicates the developed method is linear.

5.2.2. Accuracy

Mean percentage recovery values at three different concentrations of N-Acetyl cysteine and Taurine were calculated and the results were tabulated in Table 3. As per ICH guidelines, the percentage recovery of N-Acetyl cysteine and Taurine at each level was within the prescribed limits of 98% to 102%. Hence, the developed method proved to be accurate.

5.2.3. Precision

The precision data of N-Acetyl cysteine and Taurine was shown in Table 4.

Table 3. Determination of recovery studies of NAC and TAU.

Drug	Recovery Level (%)	Initial Amount (ng/band)	Amount Found (ng/band)	% Recovery	% RSD
NAC	80	120	119.84	99.88	0.112
	100	150	149.96	99.97	
	120	180	180.19	100.10	
TAU	80	400	399.68	99.92	0.135
	100	500	499.79	99.95	
	120	600	600.98	100.17	

Table 4. Determination of precision study of NAC and TAU.

S. No	NAC		TAU	
	Concentration (ng/spot)	Peak Area	Concentration (ng/spot)	Peak Area
1	150	1958	500	7679
2	150	1927	500	7642
3	150	1945	500	7659
4	150	1960	500	7635
5	150	1933	500	7648
6	150	1950	500	7663
Mean		1945.5		7654.33
% RSD		0.68		0.21

Percentage RSD values of N-Acetyl cysteine and Taurine in precision studies were found to be less than 2%, which were within the limits as recommended by ICH guidelines, hence found to be precise. 3-D Precision densitogram of N-Acetyl cysteine and Taurine is shown in **Figure 4**.

5.2.4. LOD

It is the smallest concentration that can be detected but not be quantified as exact value. LOD was calculated by using the formula

$$\text{LOD} = 3.3 \times \text{S.D/Slope}$$

5.2.5. LOQ

It is the smallest amount of analyte that can be quantitated with appropriate accuracy and precision.LOQ was calculated by using the formula

$$\text{LOQ} = 10 \times \text{S.D/Slope}$$

5.2.6. Robustness

The developed method is very sensitive to both mobile phase composition and chamber saturation time which is to be controlled, so slight changes in Rf values are observed if these parameters are not being controlled and the data is shown in **Table 5**.

Table 5. Robustness of chromatographic method.

Parameters	Drug	% S.D	% RSD
Mobile phase composition (±0.1 ml)	NAC	0.231	0.245
	TAU	0.462	0.458
Amount of mobile phase (±5%)	NAC	0.836	0.824
	TAU	0.792	0.773
Time from spotting to chromatography (±10 min)	NAC	0.573	0.568
	TAU	0.924	0.916
Time from chromatography to scanning (±10 min)	NAC	0.635	0.628
	TAU	0.527	0.523

6. Conclusions

The present work was intended for the development and validation of an HPTLC method for the simultaneous determination of N-Acetyl cysteine and Taurine in pure and pharmaceutical dosage forms. After performance of numerous trials, a specific and sensitive method was developed, which was validated as per ICH recommendation.

The developed method proved to be specific, precise, accurate and robust for the simultaneous determination of N-Acetyl cysteine and Taurine in both pure and pharmaceutical dosage forms. The developed method does not experience any interference from excipient which is expected to be present in the pharmaceutical dosage forms. Hence the proposed method can be suitably adopted for routine quality control analysis of N-Acetyl cysteine and Taurine in pure and tablet dosage forms.

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