"DEVELOPMENT OF NEW ANALYTICAL METHODS AND THEIR VALIDATION FOR THE DETERMINATION OF AND N-ACETYLCYSTEINE IN BULK AND MARKETED FORMULATIONS"

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ABSTRACT:

N-Acetyl-L-cysteine is an acetylated amino acid with antioxidant and mucolytic properties. Besides its mucolytic antioxidant effect it also has anti-inflammatory effect and as an antidote in paracetamol poisoning.

Purpose: Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Development of methods to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behavior and physicochemical properties of the drug substance. This determination requires highly sophisticated instruments and methods like HPLC, HPTLC and Spectrophotometer. Hence there was a need for the development of newer, simple, sensitive, rapid, accurate and reproducible analytical methods for the routine estimation of N-acetylcysteine in bulk and pharmaceutical dosage form

Methods: In the present work, two simple, sensitive, specific and validated methods (method IA and method IB) have been developed for the quantitative estimation of NAC in bulk and pharmaceutical dosage form. Method IA, NAC gives light brown colour with Ninhydrine in alkaline medium, which showed λmax at 485.2 nm. In Method IB, the drug was reacted with Fehling's solutions (A&B in equal volume) and ferric chloride, which produce yellow colour chromogen which showed λmax at 537.2 nm.

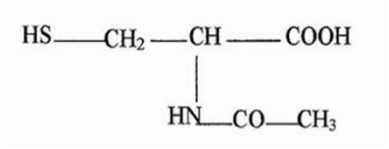
Results: The linearity was found in concentration range of 50-300µg/ml for both method IA and method IB. The correlation coefficient was found to be 0.9990 and 0.9991 for method IA and method IB respectively. The methods were validated as per ICH guidelines. The LOD and LOQ for estimation of NAC were found as 0.0773, 0.2343 for method IA and 0.0667, 0.2021 for method IB respectively.

Conclusion: Proposed methods were successfully applied for the quantitative estimation of NAC in marketed formulations.

KEY WORDS: N-acetylcysteine, Ninhydrine, Fehling's solution, Validation and Colourimetry.

INTRODUCTION

N-Acetylcysteine is derived from the sulfur-containing amino acid, cysteine. It is produced naturally in the body and is also obtained from the diet. Along with glutamic acid and glycine NAC is a precursor to glutathione, which is the body's most important cellular antioxidant. N-Acetylcysteine (NAC) is mainly used as a mucolyticum in bronchitis or pulmonary diseases. By depolymerising mucopolysaccha- rides it reduces the viscosity of pulmonary secretions. Besides its mucolytic effect it also has anti-oxidant and anti-inflammatory effects, is used as an antidote in paracetamol poisoning.



Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Development of methods to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behaviour and physicochemical properties of the drug substance. This determination requires highly sophisticated instruments and methods like HPLC, HPTLC and Spectrophotometer etc. Hence there was a need for the development of newer, simple, sensitive, rapid, accurate and reproducible analytical methods for the routine estimation of N-acetylcysteine bulk and pharmaceutical dosage form.

The literature survey on the analytical applications of Ninhydrin and Fehling's solution indicates that these compounds have not been earlier reported as reagents for the spectrophotometric determination of N-acetylcysteine in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop simple and rapid methods for the estimation of the sited drug in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

N-acetylcysteine was obtained in highly pure form (pharmaceutical grade) from the local pharmaceutical industry. Its pharmaceutical preparations obtained from different commercial sources. All other reagents were of analytical grade. Distilled water was used for preparation of all solutions. Ultraviolet and visible spectrophotometry were carried out through Systronics PC based Double Beam Spectrophotometer 2202 and JascoV-630 spectrophotometer

Standard drug solution:

Accurately weighed 100mg of N-acetylcysteine was dissolved in 100ml PBS to give a concentration of 1000 µg /ml. The final concentration was brought to 100 µg/ml for Methods A and B.

Reagents:

Method A:

- 0.5% Ninhydrin
- 25% Sodium bicarbonate

Method B:

- Fehling's solution
- 0.5% Ferric Chloride

Assay procedure for the determination of N-acetylcysteine:

Method I: Seven 10ml volumetric flasks were taken. Then 0.5, 1, 1.5, 2, 2.5, 3, ml working standard solution of NAC was added. 2.0 ml of Ninhydrin solution and 2.0 ml of 25% $NaHCO_3$ were added, heated for 10 minutes. Made up the volume with PBS. Absorbance was taken at 485.2nm.

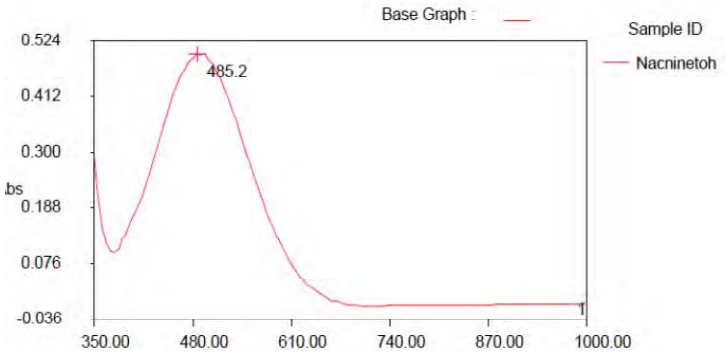


Fig: 1 Absorption spectrum of colored chromogen in method I

Method II: Seven 10ml volumetric flasks were taken. Then 0.5, 1, 1.5, 2, 2.5, 3, ml working standard solution of NAC was added. 1.0 ml of Ferric chloride solution and 1.0 ml of Fehling's solution were added. Made up the volume with PBS. Absorbance was taken at 537.2 nm.

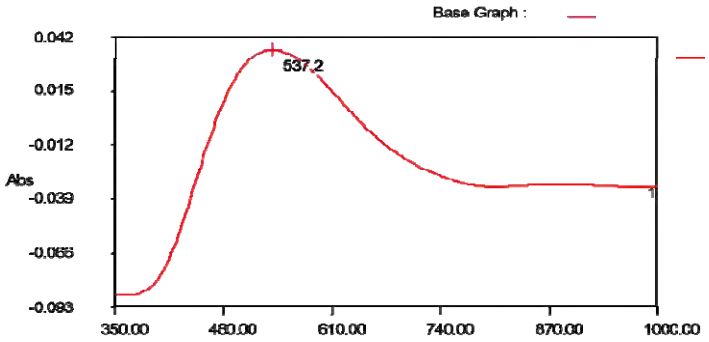


Fig: 2 Absorption spectrum of colored chromogen in method II

Assay of pharmaceutical formulations

Weighed accurately capsule powder equivalent to 100mg and transferred into 100ml volumetric flask and made up the volume with PBS to get 1000µg/ml concentration. This solution was further diluted to get concentration of 100µg/ml.

Appropriate aliquots of drug solution were taken. The individual assay procedures was carried out for the estimation of drug contents in tablets. The concentration of the drug in the capsules was calculated using calibration curve. The recovery experiment was carried out by standard addition method. The values of optical and regression terms of analysis are given in table no I

RESULTS AND DISCUSSION

Method I, NAC gives light brown colour with Ninhydrine in alkaline medium, which showed λmax at 485.2 nm. In Method II, the drug was reacted with Fehling's solutions (A&B in equal volume) and ferric chloride, which produce yellow colour chromogen which showed λmax at 537.2 nm.

The optical characteristics such as absorption maxima and Beer's law limits for these methods are presented in Table-1.The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1

Table no. 1

Parameter	Result of Method I	Result of Method II
λmax (nm)	485.2	537.2
Beer's law limit	50-300	50-300
Regression Equation* (y)	y=bx+a : y = 0.0032x + 0.0172	y=bx+a : y = 0.0032x + 0.0177
Slope (b)	0.0032	0.0032
Intercept (a)	0.0172	0.0177
Correlation coefficient (R^2)	0.9990	0.9991
Limit of Detection (µg/ml)	0.1031	0.2062
Limit of quantitation(µg/ml)	0.3125	0.6250
Accuracy (%Recovery±SD)	99.22±0.0208	98.60±0.0152
Precision (Reproducibility) Intraday(%Recovery±SD) Interday(%Recovery±SD)	 0.3395±0.00251 0.3395±000251	 0.3473±0.00030 0.3493±0.00028

y = bx + a, where y is the absorbance and x is the concentration of N-acetylcysteine in µg/ ml.

RECOVERY STUDIES:

Weighed accurately capsule powder equivalent to 100mg and transferred into 100ml volumetric flask and dissolve in small volume of PBS. Then filter the solution and filtrate is made up the volume with PBS to get 1000µg/ml concentration. This solution was further diluted to get concentration of 100µg/ml. To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentrations like 5, 10 and 15 µg/ml were prepared in case of both pure drug solution and the formulation extract solution and these solutions were subjected to analysis by above developed method. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in following table no. 2.

TABLE - 2.Assay and Recovery of N-acetylcysteine in Pharmaceutical Formulations

Method	Sample	Labeled amount (mg)	Amount found (mg)	% Recovery
I	NAC	600	594.86	99.14
II	NAC	600	591.39	98.565

CONCLUSION

It could be concluded that the developed methods for N-acetylcysteine assay is simple, sensitive, precise, accurate and can be satisfactorily applied to the analysis of N-acetylcysteine in bulk and pharmaceutical formulations. The proposed methods are used for the routine analysis of the drug in the quality control.

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