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Original Article

# DEVELOPMENT AND VERIFICATION OF UV SPECTROPHOTOMETRIC TECHNIQUE FOR DETERMINING N-ACETYLCYSTEINE IN TABLET FORMULATIONS

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**ABSTRACT**

**Objective:** This study aimed to determine the concentration of N-Acetyl Cysteine (NAC) in tablet formulations using UV-Visiblespectroscopy.

**Methods:** A precise and accurate UV spectrophotometric method was developed for determining N-acetylcysteine in tablets, using 0.1N NaOH as a diluent. The drug's purity was assessed using UV-visible spectrophotometry, which validated linearity, accuracy, precision, specificity, limit of detection, and quantification.

**Results:** The calibration curve had a high correlation coefficient ($r^2$ = 0.9992) and maximum absorbance at 235 nm. There was no interference in dosage. The results showed 100.17% mean % recovery and 100.27% tablet assay, with a precision of 0.60% and 0.57%, respectively. The method was robust and rugged.

**Conclusion:** The method was evaluated using statistical parameters, including precision, accuracy, linearity, recovery, and robustness. The results showed no significant differences compared to other methods. The method can be used to analyse pharmaceutical formulations quickly, and no significant differences in mean values and standard deviations were found.

**Keywords:** N-acetylcysteine, UV-Visiblespectroscopy, ICH guidelines, Dosage form, Validation


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## INTRODUCTION

N-acetyl cysteine (NAC) is a medication used to decrease mucus thickness in the lungs, which underpins respiratory conditions. It also functions as an antidote for paracetamol toxicity when administered intravenously [1-5]. It is produced from the amino acid l-cysteine and acts as a glutathione (GSH) precursor, which affects various disease mechanisms and oxidative stress. These mechanisms, in turn, affect neurotransmitters glutamate and dopamine and apoptosis, inflammation, and mitochondrial function. NAC is widely available as a nutraceutical in several countries, including the US, Canada, and Australia, and has been studied for its potential therapeutic benefits in laboratory and animal settings. Its antioxidant properties make it a popular choice for treating paracetamol overdose, a common occurrence in countries such as Australia, the United Kingdom, and the United States, where instances of paracetamol poisoning have increased by 40% in the past decade. The NAC market, valued at USD 1083.78 million in 2021, is projected to grow steadily, reaching USD 7598.08 million by 2030, with a robust Compound Annual Growth Rate (CAGR) of 21.5% between 2023 and 2030 [6, 7]. Several methods for measuring N-acetyl-L-cysteine have been documented in the literature, including spectrophotometric and differential pulse polarography [8], liquid chromatography-tandem mass spectrometry [9-13], HPTLC [14, 15], HPLC [16-31], UV-VIS spectroscopy [32-39], chemiluminescence [40-42], Electrochemical [43-48], turbidimetry and nephelometry [49], capillary electrophoresis [50-52], and. These methods are commonly used to quantify N-acetyl-L-cysteine and other drugs, such as clomiphene citrate, arginine, and cefixime trihydrate. Reverse-phase HPLC and ion pair chromatography are commonly used to test substances related to NAC in both bulk and drug products. However, more expensive and less readily available techniques such as LC-UV-MS and capillary electrophoresis-mass spectrometry have also been reported for quantifying substances related to N-acetylcysteine. Our study aimed to develop a cost-effective, precise, and specialized UV-Visible spectroscopy method for analysing NAC, as existing methods are expensive and laborious. Our process addresses the market demand for NAC analysis and has been validated by the International Conference on Harmonization's Q2R1 guidelines. Although a literature review showed no UV-visible spectroscopic technique available for the analysis of NAC, our method, which uses 0.1N NaOH as a diluent at a detection wavelength of 235 nm, is more precise than previous studies of a similar nature to the existing methods.

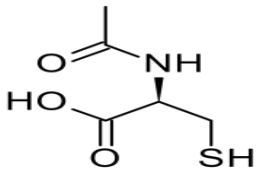


**Fig. 1: Structure of N-acetylcysteine**

## MATERIALS AND METHODS

### Materials

N-acetylcysteine was generously provided as a gift sample using Alphamed Formulations (Hyderabad). The investigational study utilized tablet formulation of N-acetylcysteine (NAC) manufactured by Sun Glow Ltd (India). Sodium hydroxide was procured from Thermo Fisher Scientific India Pvt., Ltd. (Mumbai, India).

### Instrumentation

Experiments were conducted using a UV-Visibledouble-beam spectrophotometer (Labindia 3092, UV-WIN software) and an

analytical balance (Shimadzu AY220) for weighing; we also used an ultrasonicator (Oscar Microclean 103) to sonicate the standard and product sample solutions.

**Methods**

**Method development**

**Selection of solvent**

NAC was dissolved in several solvents to identify the solvent with the optimal solubility. Specifically, methanol, ethanol, acetonitrile, and diluted acidic and basic buffers were used. Consequently, N-acetylcysteine demonstrated solubility more in 0.1N NaOH.

**Determination of wavelength of maximum absorbance (λmax)**

The standard working solution containing 100 µg/ml NAC was scanned using the full scan mode and a medium scanning speed across the entire range of UV-visible spectrophotometers, from 200-800 nm, with methanol as the blank. Following the acquisition of the spectrum, the λmax was determined to be 235 nm (fig. 2).

**Preparation of the standard solution**

Solubility tests confirmed the suitability of a 0.1N NaOH solution as the solvent. NAC (100 mg) was dissolved in 100 ml of 0.1N NaOH, resulting in a 1000 µg/ml concentration. Next, 10 ml of the resulting solution was dispensed and diluted to 100 ml with NaOH (using 0.1N) NaOH. Afterward, 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml aliquots were pipetted, and each was topped up to 10 ml.

**Preparation of sample solutions**

Twenty tablets with an average weight were crushed into a fine powder, and 1385.54 mg of this powder was weighed and transferred into a 100 ml volumetric flask. Next, three-quarters of the diluent was added, and the mixture was sonicated for 30 min. The volume was then diluted with the diluent and filtered. From the resulting stock solution, 5 ml was pipetted and adjusted to 25 ml with diluent. Subsequently, 0.1 ml was pipetted and made up to 10 ml with diluent, resulting in a 15 µg/ml concentration. This study estimated NAC using a UV-Visiblespectrophotometer at a wavelength of maximum absorbance of 235 nanometers in 0.1N NaOH solution.

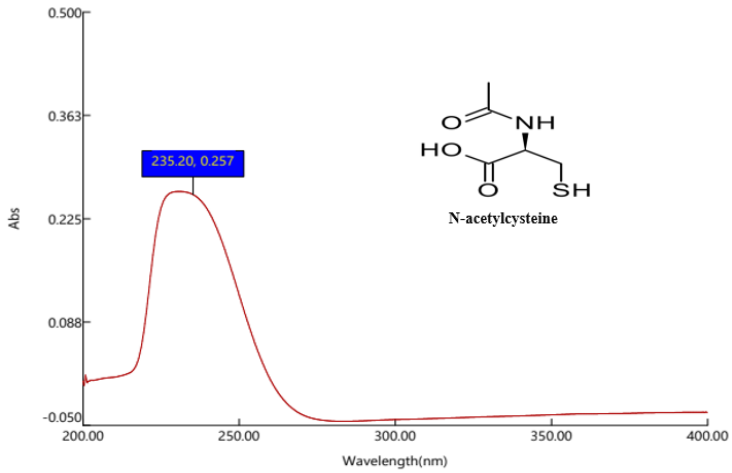


**Fig. 2: N-acetylcysteine wavelength of maximum absorbance at 235 nm**

**Validation of method parameters**

The International Council for Harmonisation (ICH) guidelines were followed to validate the method's suitability, linearity, precision, and accuracy [53].

**System suitability**

Six identical standard solutions were injected into the UV system at 235 nm, and the percentage Relative Standard Deviation (%RSD) was determined.

**Linearity**

The N-acetylcysteine working standard solutions were prepared by adding appropriate volumes to several 10 ml volumetric flasks, followed by dilution with distilled water to achieve concentrations of 5, 10, 15, 20, and 25 µg/ml. Calibration curves were created by relating the absorbance values to the concentrations, and regression equations were established to analyze the behaviour of the drug.

**Range**

The range of analytical methods was established by determining the interval between the upper and lower levels of the calibration curve plotted on a graph.

**Intraday precision (repeatability) and inter-day precision (intermediate precision)**

Intraday accuracy involves examining the drug at a concentration of 15 µg/ml six times throughout the day, whereas inter-day accuracy stretches the analysis over two consecutive days. Repeatability (intraday) was assessed by analyzing six samples with the same drug concentration (15 µg/ml) and reporting the absorbance fluctuations in terms of relative standard deviation. This method allows the evaluation of the dependability and uniformity of the analytical technique.

**Accuracy/recovery study**

To evaluate the method's reliability, the accuracy of NAC concentration was determined using the standard addition technique at three different concentrations (50%, 100%, and 150%). The mean percentage recoveries were calculated, and the recovery values are summarised in table 4. This approach offers valuable information regarding the ability of the method to measure N-acetylcysteine concentrations at different levels accurately.

**Limit of detection and quantification (LOD and LOQ)**

**LOD**

The lowest analyte concentration in a test sample can be clearly distinguished from zero.

**LOQ**

UV spectroscopy was used to compare the signals from the samples with those of the blank samples to accurately determine the minor concentration of analyte in a test sample while maintaining acceptable repeatability.

**RESULTS**

**Linearity and range**

To confirm the dependability of the proposed method, calibration curves were constructed in the concentration range of 5–25 µg/ml. NAC followed Beer's law within this 5–25 µg/ml range,

demonstrating exceptional adherence, with a regression equation of Y = 0.0347x+0.0029. The correlation coefficient (r) was more significant than 0.9992, indicating the robustness of the standard curve. All stock solutions and working standards were prepared in 0.1N NaOH, and the calibration curve data are presented in table 1 and fig. 3.

**Precision**

The accuracy of the technique was assessed by conducting six separate tests on the sample preparation, calculating the standard deviation, and evaluating precision and reproducibility. To determine precision and reproducibility, replicate analyses of standard solutions were performed. Concentrations within the calibration range were prepared using 0.1N NaOH and analyzed using respective calibration curves. The results showed that the technique demonstrated excellent precision and accuracy, with low intra-and inter-day variability, as indicated by the Relative Standard Deviation (RSD %) in table 2.

**Table 1: Linearity data**

| Concentration (µg/ml) | *Absorbance |
|---|---|
| 5 | 0.182 |
| 10 | 0.359 |
| 15 | 0.508 |
| 20 | 0.694 |
| 25 | 0.878 |
| $r^2$ | 0.9992 |
| Range | 5 to 25 µg/ml |
| Slope | 0.0347 |
| Intercept | 0.0029 |

*n=6

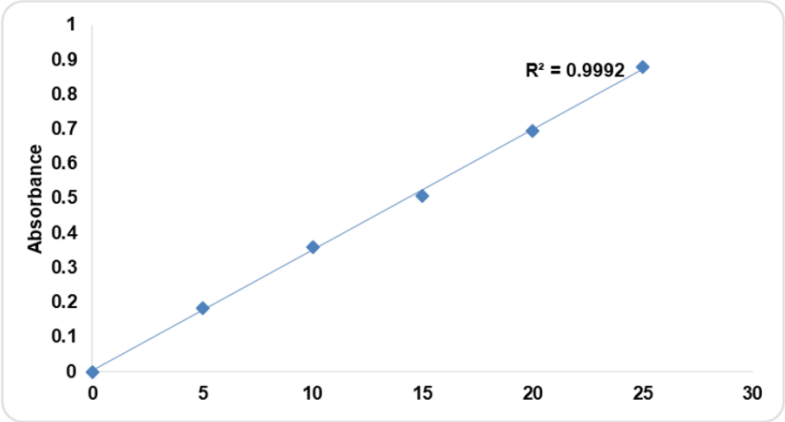


**Fig. 3: Linearity of N-acetylcysteine**

**Table 2: Precision table**

| S. No. | *Intra-day precision | *Inter day precision | |
|---|---|---|---|
| | | Day-1 | Day-2 |
| 1 | 0.506 | 0.507 | 0.507 |
| 2 | 0.511 | 0.512 | 0.512 |
| 3 | 0.513 | 0.516 | 0.516 |
| 4 | 0.508 | 0.512 | 0.512 |
| 5 | 0.509 | 0.511 | 0.511 |
| 6 | 0.514 | 0.513 | 0.513 |
| Mean | 0.510 | 0.512 | 0.5118 |
| SD | 0.0030 | 0.2930 | 0.0029 |
| % RSD | 0.60 | 0.57 | 0.57 |

*n= 6, SD: Standard deviation %RSD= Relative standard deviation, %RSD value for intra-and inter-day precision was<2% (0.60 and 0.57%, respectively), which is within the limit; hence, the precision parameter was validated.

**Accuracy/recovery**

Experiments involving the addition of known quantities of the pure drug to pre-analyzed formulations were carried out to confirm the accuracy of the developed method. The data obtained for the drug's accuracy demonstrated a high percentage recovery, ranging from 99.97 to 100.53. Notably, the developed method exhibited excellent precision and accuracy, making it applicable for determining NAC tablets in pharmaceuticals without interference from excipients (table 3).

**Table 3: Accuracy data**

| % Level | Standard absorbance | Sample absorbance | % Recovery | *Average % recovery | *mean % recovery |
|---|---|---|---|---|---|
| 50% | 0.509 | 0.206 | 100.69 | 100.53 | 100.17 |
| | | 0.207 | 101.18 | | |
| | | 0.204 | 99.71 | | |
| 100% | | 0.512 | 100.11 | 99.97 | |
| | | 0.508 | 99.31 | | |
| | | 0.514 | 100.49 | | |
| 150% | | 0.904 | 100.44 | 100.02 | |
| | | 0.902 | 100.19 | | |
| | | 0.892 | 99.43 | | |

*n= 3

**Table 4: Short-term stability determined by the proposed method**

| Stability duration (h) | Concentration (µg/ml) | *Conc. found (mean±SD, µg/ml) | RSD (%) | % Potency |
|---|---|---|---|---|
| 0.0 | 15 | 14.96±0.0051 | 0.234 | 99.89 |
| 1.0 | | 15.06±0.0013 | 0.532 | 98.41 |
| 5.0 | | 14.83±0.0021 | 0.631 | 99.74 |
| 10.0 | | 14.92±0.0047 | 0.832 | 99.87 |
| 24.0 | | 15.09±0.0034 | 0.725 | 98.95 |

*n= 6, SD: Standard deviation, %RSD= Relative standard deviation

**Table 5: Content of NAC in marketed products determined by the proposed method.**

| Formulation | Drug | Label claim (mg) | *%Amount found±SD | %RSD |
|---|---|---|---|---|
| Tablet | N-acetylcysteine | 600 mg | 101.27±0.0019 | 0.268 |

*n= 6, SD: Standard deviation, %RSD= Relative standard deviation

**Limit of detection (LOD) and quantitation (LOQ)**

The sensitivity parameter was determined by the LOD and LOQ for NAC and was found to be 0.35 µg/ml and 1.0 µg/ml, respectively.

**Stability study**

Table 4 shows the results of the repeatability study, where the samples were stored at room temperature for 24 h and then analyzed the next day to gauge their short-term stability.

**Content of NAC in marketed brands**

The proposed method effectively measured NAC in tablet formulations without any interference. The outcomes of our assay were consistent with this claim, which validates our approach [table 5]. To guarantee the long-term dependability of the method, we conducted a stability assessment of NAC in a 0.1N NaOH solution.

## DISCUSSION

Developing of a new spectroscopic method for the estimation of drugs in their dosage forms has raised recently due to its important role in pharmaceutical analysis and development [48]. Different solvents like methanol, ethanol, acetonitrile, and diluted acidic and basic buffers were used to determine the studied drug's solubility peak quality and peak shape. Among these, 0.1 NaoH fulfilled all criteria in showing better solubility and giving better peak quality. NAC wavelength was selected at 235 nm, whereas no absorption was detected due to excipients at NAC wavelength. This shows no interference from the excipients at the selected wavelength. This innovation promises to significantly reduce the sample preparation and material costs typically associated with routine analyses.

Lea Kukoc-Modun *et al.* [49] method solutions of NAC used in the method are only stable for a short period of time. They should be analysed within 24 h to ensure method stability and accuracy. In the present study, the solution stability study led that the absorbance of NAC was 98% to 99% of the initial value (table 4) with a %RSD of 0.99, which indicates that the NAC solution in the 0.1N NaoH was stable for at least 24 h. Solution stability is an important criterion to ensure that the prepared samples are stable and that no changes could affect the samples during the test period.

Linearity was calculated using six different 5-25 µg/ml concentrations. These data showed linear absorbance readings through the selected range. The same $R^2$ was found by Swati S. Agawane 1 *et al.* for the determination of Clomiphene citrate and Acetylcysteine in its dosage forms by using the UV-visible spectroscopic method [50].

For comparison, the spectrophotometric methods were reported by Bondarenko N *et al.* [51] and Maja Biocic *et al.* [52]. The articles may not provide results from the analysis of pharmaceutical samples containing NAC and comprehensive information on the validation parameters of the developed method, such as accuracy, precision, linearity, and robustness, which are essential for method reliability. In this contrast, the precision test results are shown in table 2. The % RSD was less than 2%, indicating that the precision values of the validated method were within the accepted limits.

The accuracy of the method was further ascertained through the recovery studies. To the drug solutions of the granules or the tablet powder, the standard solutions of the synthetic NAC were added at four three different concentrations. The recovery of added NAC was 99–100% (table 3), with the relative standard deviation (% RSD) less than 2%. This indicates that the developed method had a higher recovery than the reported method [53, 54].

Unlike some reported methods, the proposed method is free from drastic experimental conditions such as flow injection, solvent effect, heating, and reagents [52-54]. It is also worth mentioning that the proposed method was performed in the UV region ($\lambda$ = 235 nm) away from UV-absorbing interfering excipient materials, which might be dissolved from pharmaceutical formulations.

## CONCLUSION

Using UV-visible spectroscopy, a method for determining NAC in tablet form was developed and validated. The technique was accurate and cost-effective, making it suitable for the routine quality control analysis of commercially available formulations.

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Nil

## AUTHORS CONTRIBUTIONS

All the authors played an equal role in this study. Mounika, Venkata Sai Ranjitha Dubba, Mandava Jahnavi, Sowjanya Sonti, and Yakshitha Yarra collected the necessary chemicals and reagents for the experimental work in our laboratories. Dr Ramarao Nadendla contributed to the interpretation of the data. Dr. Venkata Suresh and Siva Prasad Morla drafted and revised the manuscript to ensure its quality.

## CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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