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Development of Colorimetric Method for the Assay of N-acetylcysteine in Dosage Forms using 2, 6-Dichloroquinone-4-Chlorimide.

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ABSTRACT

A simple colorimetric method was developed for the determination of N-acetylcysteine (NAC) in pharmaceutical preparations. The method is based on the coupling N-acetylcysteine with 2,6-dichloroquinone-4-chlorimide (DCQ) in dimethylsulphoxide to give a yellow colored product absorbing at 438 nm. The absorbance–concentration plot was rectilinear over the range of 10-50 µg/mL with minimum detection limit (LOD) of 0.769 µg/mL. The recovery results ($100.90 \pm 0.00\%$, $n=3$) reflected no interference by the formulation excipients. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. The developed method was successfully applied to the analysis of NAC in injection and capsule dosage forms. The results obtained were statistically compared with those of the official titrimetric methods. A pathway for the reaction of NAC with DCQ was suggested.

Keywords: N-acetylcysteine, 2,6-dichloroquinone-4-chlorimide (DCQ), spectrophotometry, dosage forms

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INTRODUCTION

Acetylcysteine (Figure 1): N- acetyl – L- cysteine ($C_5 H_9 NO_3 S$), L- alpha – acetamido – beta – mercaptopropionic acid [L- α - acetamido - β mercaptoproionic acid, N – acetyl – 3 – mercaptoalaline] has many pharmaceutical uses. It is the drug of choice for the treatment of an acetaminophen overdose [1], management of paracetamol (acetaminophen) poisoning [2] and a mucolytic agent, a precursor of L-cysteine and reduced glutathione [3].

Several methods have been reported for the quantitative determination of NAC in formulations. These methods include titrimetric methods [4,5], spectrophotometric methods [6,7] and chromatographic methods [8,9]. 2, 6-Dichloroquinone-4-chlorimide (DCQ) has been used as an analytical reagent in pharmaceutical analysis of drugs in bulk and formulation. It has been used for the colorimetric determination of a number of drugs having certain functional groups including OH, NH_2 and SH [10-13].

The aim of the present work is to develop a simple, sensitive and accurate colorimetric method for the rapid measurement of NAC in bulk and dosage forms using DCQ as a coloring reagent utilizing its SH group.

EXPERIMENTAL

Materials

N- N-Acetylcysteine RS Gehalt as 99.4% Merck KGA, Germany.

Parvolex[®] solution for infusion 10 ml acetyl cysteine ph. Eur. (200 mg/ml), Batch No. 517774, UCB Pharma United, UK.

NAC (N-Acetylcysteine 600 mg vegetable capsules), Batch No. 60384503 SOLGAR Vitamins and Herb, Leonia, N.J., USA.

Reagents

2,6- dichloroquinone-4-chloroimide DCQ Merck KGA, Germany

Potassium Iodine (extra pure) S.D fine chem. Limited, India

Ethyl alcohol (absolute) Media lab, India

Acetic acid glacial (extra pure) S.D fine chem. Limited, India

Iodine resublimed, BDH, England

Glycine, Blulux[®] Regent Blulux[®] Regent, India

Diluent solvent was prepared by mixing 50:50 v/v absolute ethanol and water.

Instrument

Ultraviolet/visible spectrophotometer, Perkin Elmer Ltd. Beaconsfield, Buckinghamshire
pH meter, model: 3510, Barlow World Scientific, Europe.

Preparation of NAC RS solution (150 μ g/ml)

0.015g of NAC standard was accurately weighed, dissolved in about 50ml absolute ethanol and transferred into 100 ml volumetric flask. The volume was then completed to the mark with distilled water (0.015% w/v, solution A).

Preparation of NAC RS solution (250 μ g/ml)

0.025g of NAC RS was accurately weighed, dissolved in about 50ml absolute ethanol and transferred into 100 ml volumetric flask. The volume was then completed to the mark with distilled water (0.025% w/v, solution B).

Preparation of Parvolex® sample solution (250 µg/ml)

2.5 ml of sample solution was transferred into 100 ml volumetric flask; the volume was completed to mark with the diluent solvent. 5 ml of the resultant solution was further diluted to 100ml with the same solvent (0.025%w/v, solution C)

Preparation of NAC 600 mg vegetable capsules sample solutions

A weight equivalent to 25mg of the capsule powder content was accurately weighed and transferred into 100ml volumetric flask. The volume was then completed to mark with the diluent solvent (0.025%w/v, solution D).

Procedures

Construction of calibration Curve

Aliquot volumes (1-5 ml) of solution B were transferred into five volumetric flasks (25ml). A volume of the diluent solvent was added to each flask to adjust the volume to 5 ml using volumetric pipette, then 1 ml of DCQ solution in absolute ethanol (0.25% w/v) was added to each flask. 1ml of sodium acetate 1%w/v was added to each flask and the volumes were then completed to mark with the diluent solvent.

The absorbance was then measured at 438 nm against its blank (prepared similarly but without adding NAC standard). The absorbance values were plotted against the final concentration in order to obtain a calibration graph.

Effect of pH on absorption spectra

Five ml of each solution B was transferred into two separate 25 ml volumetric flask. 2 ml of the diluent solvent was added. 1 ml of DCQ solution in absolute ethanol (0.25% w/v) was added to each flask. 1 ml of sodium acetate (pH 3.5) to one flask and 1ml of phosphate buffer (pH 7) was added to the other flask. The volumes were then completed to mark with distilled water. The wavelength of the maximum absorbance and stability was then recorded.

Assay of Parvolex® and NAC 600 mg vegetable capsules

Three different solutions of NAC RS, parvolex and solgar NAC 600 mg vegetable capsules were prepared and treated as under the calibration curve.

Added recovery

Two ml of each of solution B and C were transferred into two separate stoppered glass tubes. In a third stoppered glass tube 2ml of solution B and 2ml of solution C were mixed. Solutions in the three tubes were treated as under calibration graph and the percent recovery was calculated using the adopted formula reported [12].

Titrimetric method (Official BP Method)

2 ml of parvolex® injection was transferred into 100 ml volumetric flask; 20 ml of glacial acetic acid was then added. The solution was titrated against 0.049 M iodine VS slowly till a permanent pale yellow color is obtained. The volume of the added iodine was recorded.

Interference

Small amounts of each of L-cysteine, L-phenylalanine and L- tyrosine were taken, dissolved in aliquot volumes of the diluents solvent, then 1 ml of DCQ in absolute ethanol was added and results were then recorded.

RESULTS AND DISCUSSION

Colorimetric analysis of organic compounds and drugs has been used extensively in numerous fields. It can be stated that they will remain of great value in future, in spite of the steadily growing resort to purely physical methods. The later often necessitate very sophisticated and expensive instrumentation. Routine colorimetric analysis can be performed with very simple instrumentation, resulting nevertheless in sensitive and accurate measurements with the advantages of less cost, higher speed, simplicity and lack of interference [14,15].

Most of the reported analysis methods of NAC are time consuming, lack selectivity, require sophisticated equipment or not simple for routine analysis.

Therefore the need for a fast, sensitive, simple and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing NAC. Due to the lack of chromophore and/or auxochrome in NAC molecule, direct spectrophotometry cannot be used for its analysis. 2, 6-Dichloroquinone-4-chlorimide (DCQ) has numerous applications as an analytical reagent. It has been used for the colorimetric determination of some phenolic and thiol containing drugs [11]. NAC contains a thiol group that can develop color upon addition of DCQ. NAC reacts with DCQ to give colored complex absorbing at 438nm. Under the same conditions NAC does not absorb at this wavelength due to lack of chromophore in its structure Fig. 1

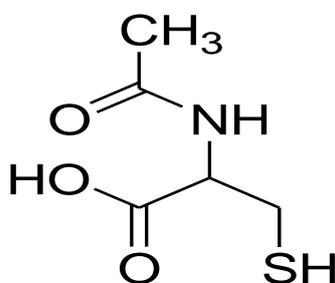


Figure 1: Acetylcysteine chemical structure

The various experimental conditions influencing the color development were extensively studied to determine the optimal conditions for assay procedure. All conditions studied were optimized at room temperature (25 °C ± 1).

Effect of pH

The effect of pH on the reaction time, color intensity and stability was also studied and evaluated. Two pH values (3.5 (acetate 1%w/v) and 7.0 (phosphate buffer)) were used for this study. The most optimum pH value for the reaction was found to be pH 3.5 using 1%w/v acetate buffer (Table 1).

Table1: effect of pH on formation, intensity and stability of the reaction product

pH	λ_{\max}	Drug ABS	Standard ABS	Stability
3.5	438	0.406	0.351	Stable
7	438	0.720	0.669	Unstable

Effect of solvent

To study the effect of solvent, two solvents of different dielectric constant (DMSO and absolute ethanol) were used. The obtained ABS and λ_{\max} values revealed that the color was unstable in DMSO while it was stable in absolute ethanol (Table2). Therefore, absolute ethanol was selected as the appropriate solvent for the reaction.

Table 2: Effect of some solvents on the color formation and intensity

Solvent	Color	Absorbance	λ_{max}	Dielectric constant	Blank color
Ethanol (absolute)	Immediate	0.532	438	24.6	Clear
DMSO	colored after a while	0.697	450	46.7	Greyish

Concentration effect

The optimal concentration of DCQ that can give maximum color development was studied. The concentration of NAC was kept constant while reacting with three different concentrations of DCQ (0.25%, 0.33% & 0.35% w/v). The DCQ concentration of 0.25%w/v was selected as an optimum chromogen's concentration suitable for the generation of the colored product with maximum absorbance and stability.

Analytical curves

Under the optimum experimental conditions employed above, Beer's law was found to be valid over concentration range (10-50µg/ml). The molar absorptivity (ϵ) of the complex was 2130.96 L mole⁻¹cm⁻¹. This value being relatively high confirms the sensitivity of the method. The relationship between the Absorbance (A) at 438nm and concentration (C) was $A=0.0121+ 0.0142C$ with a correlation coefficient ($r=0.999$; $n=5$) which indicating suitable linearity. The detection limit was 0.768ug/ml which represents the minimum absorbance value that can be measured for the colored product.

Accuracy and Precision

The validity of the developed method was evaluated by comparing the statistical results obtained with those of the official titrimetric method (Table 3). As the calculated t- and F-values were less than the tabulated one, the results of the developed method can be considered as accurate and precise as the official method.

Table 3: Validation results of the developed method compared to the official method

	Content% of NAC \pm SD%	*t cal, t(tab)	*F cal, F(tab)
NAC Capsules	102.87 \pm 1.82	2.17 (2.78)	3.12 (19)
Parvolex	101.56 \pm 1.39	1.89 (2.78)	1.82 (19)
Official method	99.70 \pm 1.03	-	-

* = t and F calculated and tabulated

Reproducibility

The reproducibility of the method was determined on three different concentrations of NAC. The results obtained showed a low relative standard deviation values (RSD) varying from 2.47 to 1.66% which reflects that the method is satisfactory reproducible.

Application of the developed method

The developed method was applied for the determination of NAC in pure and commercial formulations (injections and capsules). The results of assay of NAC in capsules and Parvolex sample solution were found to be 102.87 \pm 1.82 % and 101.56 \pm 0.89% (mean \pm SD, $n=3$) using double point standardization method. The obtained result of the added recovery was found to be 100.90 \pm 0.23%, $n=3$.

Both results of the assay and recovery confirm the accuracy of the method and the freedom of interference by the excipients.

Interference

The interference of L- cysteine, L-phenylalanine and L- tyrosine in the reaction was also studied. Their reaction DCQ was found negligible.

Molar Ratio Method

To study the stoichiometry of the reaction, the molar ratio of NAC to DCQ was determined in the reaction mixture by the mole ratio method using concentration of $1.57 \times 10^{-4} \text{M}$ for both DCQ and NAC. The absorbance readings were then plotted against the molar ratio of NAC/ DCQ (Fig.2).The molar ratio was found to be 1/1.

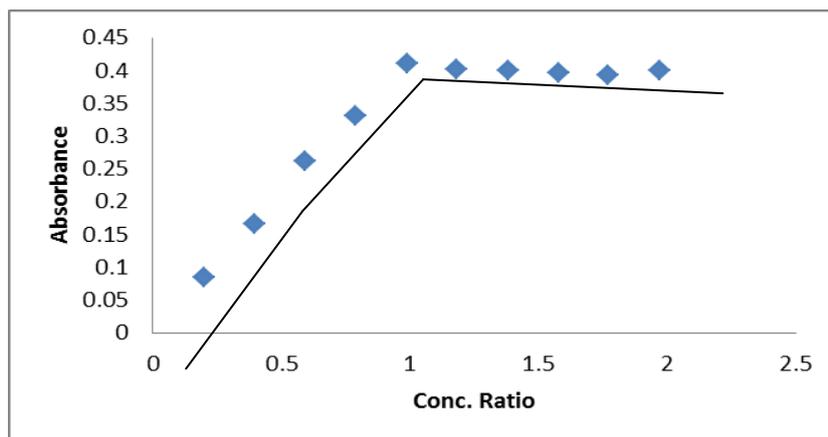
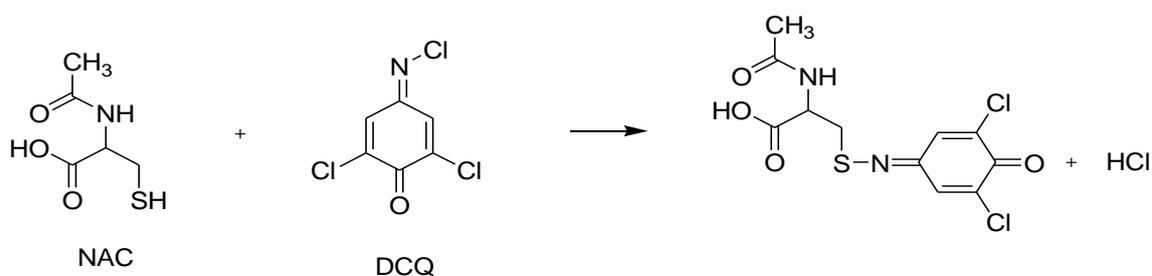


Figure 2: Molar ratio method

According to Karmar and Gamson, DCQ reacts via the chlorine atom of the chlorimide with thiol to give a yellow to brown color of dichloroquinone sulfenimide when reacted with D-penicillamine [15]. By analogy to such reaction, it is proposed that the reaction pathway between NAC and DCQ will be as outlined in Scheme 1



Scheme 1: Proposed reaction pathway between DCQ and NAC

CONCLUSION

The proposed method was proved to be simple, accurate, precise and time saving. 2,6-dichloroquinone-4-chlorimide (DCQ) proved to be a suitable reagent for the determination of NAC in pure form and its dosage forms without interference from excipients. It can be used for the determination of NAC in quality control and industry. Moreover, no interference was encountered from the commonly formulated drug, hydrochlorothiazide.

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