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DEVELOPMENT OF COLORIMETRIC METHOD FOR THE ANALYSIS OF AMINOCAPROIC ACID USING DCQ

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ABSTRACT

A simple, sensitive, accurate and cost-effective colorimetric method was developed for the analysis of aminocaproic acid in bulk and injectable forms. The developed method was based on the generation of a colored complex through utilizing the known reaction of 2, 6-dichloroquinone 4-chloroimide (DCQ) with phenols, primary and secondary amines. A colored product with λ_{max} at 680nm was formed after heating aminocaproic acid with DCQ in dimethylsulfoxide (DMSO) for 10 minutes. Stoichiometry of the reaction was studied and revealed 1:1 ratio. Beer's law was found to be valid over the concentration range 3-15µg/ml. Regression analysis showed good correlation coefficient (not less than 0.998) with detection limit and quantification limit of 1.51µg/ml and 5.00µg/ml respectively. The mean percentage recovery was found to be 100.30 ±1.84, n=4.

KEYWORDS: Colorimetry; Aminocaproic acid; DCQ.

1. INTRODUCTION

Aminocaproic acid (Fig. 1) is a derivative and analogue of the amino acid lysine. It is indicated for the treatment of fibrinolysis and blood loss,[1] intracranial hemorrhage,[2] cirrhosis, hyperfibrinolysis[3] and as adjunctive therapy in hemophilia.

Many liquid chromatographic methods were reported for analysis of ACA in plasma[4] and urine.[5]

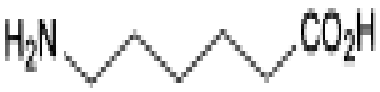


Fig.1 Chemical structure of Aminocaproic acid.

DCQ has been utilized as chromogenic reagent for the spectrophotometric determination of some thiol containing drugs,[6, 7] amine containing drugs[8, 9] and phenolic drugs.[10, 11]

In the present work, DCQ (Gibbs reagent) was used as chromogen for the colorimetric determination of aminocaproic acid in bulk and pharmaceutical formulations.

2. EXPERIMENTAL

2.1. MATERIALS AND INSTRUMENTS

All materials and reagents used were of analytical grade.

Aminocaproic acid injection (Amicar® 250mg/ml) was obtained from Hospira, USA. The reference standard obtained from Saudi Arabia Central lab, KSA. The chromogen reagent; 2, 6-dichloroquinone-4-chlorimide (DCQ); was obtained from Fluka Analytical, Austria; 0.4%w/v of the reagent was freshly prepared in dimethylsulfoxide. Dimethylsulfoxide (DMSO); Loba Chemie, India. Sodium acetate; British Drug House, England.

UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, (Koyoto, Japan).

2.2. Buffer solutions

Sodium acetate buffer of pH 3.5 and 5.5 were prepared in distilled water.

2.3. Stock solution of standard aminocaproic acid (ACA)

0.01 g of aminocaproic acid RS was accurately weighed and dissolved in 1 ml of water. The solution was transferred to 50ml volumetric flask and volume was then completed to mark with dimethylsulphoxide. 3ml of the resultant solution was further diluted to 10ml using dimethylsulphoxide (60µg/ml; solution A)

2.4. Stock solution of aminocaproic acid injection (AMICAR®)

One ml of the injection was transferred into 25 ml volumetric flask and volume completed to mark with distilled water. 1 ml was then transferred into 50 ml volumetric flask and volume was then completed with dimethylsulphoxide. 3ml were further diluted to 10ml using dimethylsulphoxide (60µg/ml; solution B).

3. Procedures

3.1. Calibration curve

Serial dilutions were made from solution A. Aliquot volumes of 0.5, 1.0, 1.5, 2.0 and 2.5ml were transferred to five stoppered glass tubes A volume of dimethylsulfoxide was added to

each tube to adjust the volume to 2.5ml. 2ml of 0.4%w/v freshly prepared DCQ and 1ml of sodium acetate buffer were added to each tube. The volumes were then completed to 10ml with DMSO before heating for 10 minutes at 50 °C. After cooling, the absorbance values of the resultant solutions were measured at 680nm against a blank reagent prepared similarly using 2.5ml of DMSO instead of solution A.

The calibration curve was constructed by plotting the absorbance values versus the aminocaproic acid concentration.

3.2. Assay of ACA injection

1.5ml of solution B was treated as under the calibration curve. The content of the injection was then calculated either from the calibration curve or the corresponding regression equation.

4. RESULTS AND DISCUSSION

Aminocaproic acid exhibits weak UV absorption. Therefore, a suitable chromogen is needed to react with ACA to obtain a colored product that can increase the selectivity and sensitivity of the drug analysis.

ACA contains an amino group, which is susceptible for the reaction with DCQ to produce a colored product. Fig. 2 shows the UV/VIS spectrum of the reaction complex which has an analytically useful peak at 680nm.

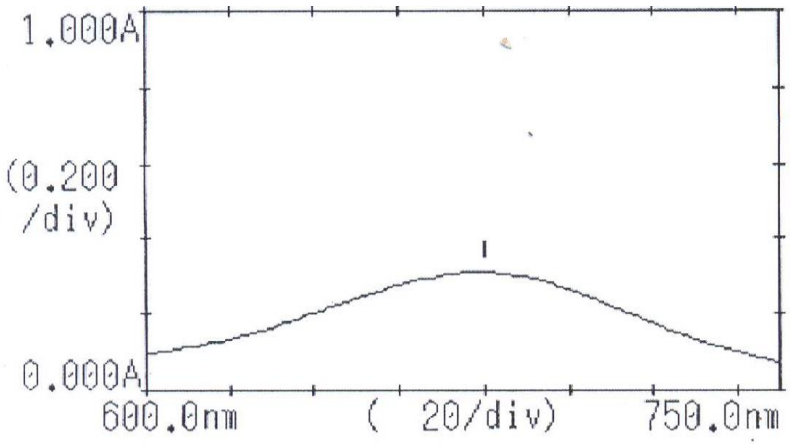


Figure 2. UV/VIS spectrum of the ACA-DCQ complex

Reaction stoichiometry was determined by the Molar ration method using different aliquot volumes of 3×10^{-3} M and constant volume of DCQ 3×10^{-3} M. A plot of absorbance as a

function of ACA/DCQ molar ratio gave two branches that intersect at a mole ratio (1:1) corresponding to the formula of the colored complex (Figure 3).

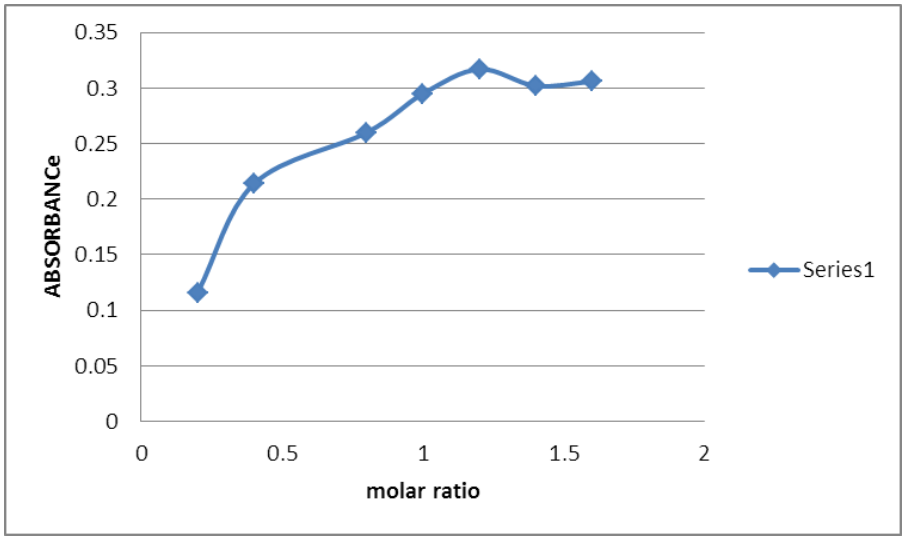
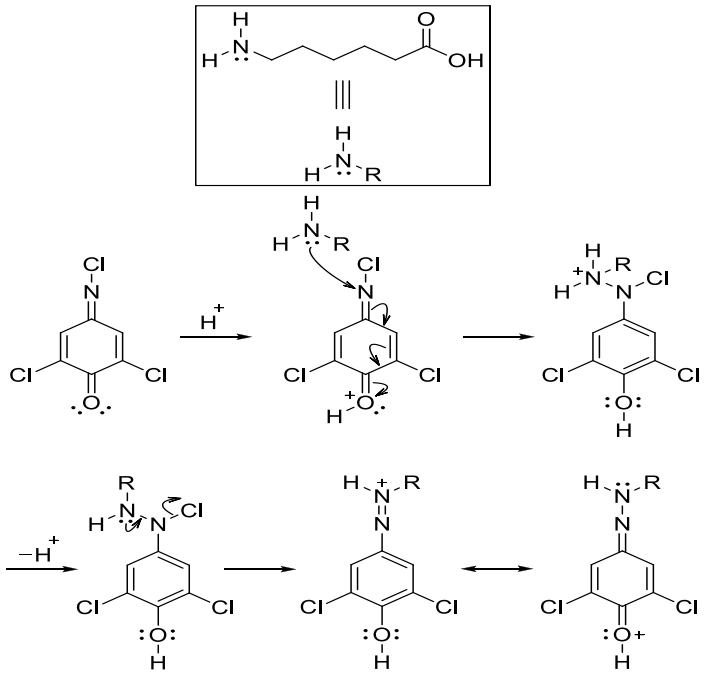


Figure 3. Molar ratio plot for the stoichiometry determination

Accordingly, the reaction between ACA and DCQ was suggested to proceed as illustrated in scheme 1.



Scheme 1. Proposed Pathway for the reaction of ACA with DCQ

3.1. Optimization of the reaction conditions

Experimental parameters that affecting the color development and stability were studied to determine the optimum conditions. These include solvents, reagent concentration and volume, reaction time and temperature.

The effect of solvents with different dielectric constants (DE) namely water (DE= 78), ethanol (DE= 24.6) and DMSO (DE= 47) were studied (Table 1). More stable and intense color was developed when using DMSO as a solvent.

Table 1. Effect of different solvent on the reaction mixture intensity and wavelength

Solvents	A(mean)	λ_{max}	R
Water	0.105	670	0.95
DMSO	0.33	680	0.998
Ethanol	0.06	670	0.00

The effect of reagent concentration was investigated by using 2ml of 0.2%, 0.4% and 1% w/v DCQ solution. It was found that increasing the concentration of DCQ up to 0.4% would increase the absorbance of the reaction complex, after which further increase in the concentration of DCQ resulted in no change in the absorbance. Thus, 2ml of 0.4% w/v DCQ was adopted as the suitable concentration for maximum absorbance.

The influence of pH on the absorption value of the reaction product was evaluated at neutral and buffered media. The developed color in neutral media and pH 5.5 was found to be unstable. Maximum color intensity was obtained with sodium acetate pH 3.5.

The effect of heating time on the reaction rate and color intensity was also studied. It was found that increasing heating time gives faster color development and better r-values. Thus, heating for 10minutes at 50 °C was established as the optimum condition for reproducible absorbance values.

The sequence of addition of the reagents as described under calibration graph was essential for good reproducibility. The formed color was found to remain stable for at least 1hour.

3.2. Analytical data

Beer's law was found to be valid in the concentration range 3 – 15 µg/ml of aminocaproic acid. Linear regression analysis of the data gave the following equation:

A= -0.0137+ 0.036C (r= 0.9989).

Where A is the absorbance in 1cm cell, C is the concentration of the drug in µg/ml and R is the correlation coefficient.

Optical characteristics and statistical data for the regression equation of the proposed method are given in Table 2.

The statistical calculation of the limit of detection and quantification was performed using the following equation (12)

LOD= 3.3 δ/ S and LOQ = 10δ/ S

Where δ= standard deviation of the intercept of the regression line; *S* = the slope of the calibration line.

Table2. Spectral data for the prosposed method (n=5)

Parameter	Proposed method
λ_{max}	680nm
Range	3-15µg/ml
Slope	0.036
Standard deviation of slope (S_b)	1.89×10^{-3}
95% confidence limit of slope (tS_b)	6.01×10^{-3}
Intercept	-0.0137
Standard deviation of slope (S_a)	0.0188
95% confidence limit of slope (tS_a)	0.60
Correlation coefficient	0.9989
Limit of detection (LOD)	1.51
Limit of quantification (LOQ)	5.00

The proposed method was applied to the determination of aminocaproic acid in injections labeled to contain 250mg/ml. The mean content percent of three independent analyses was found to be 99.4±1.07, n=3.

The accuracy of the proposed method was checked out by added recovery testing. The results showed good recovery (100.3 ±1.84, n=3), which indicates no interference with the injection excipients.

In order to evaluate between days variation (reproducibility) and within day variation (repeatability), three different conc. of ACA solution within the linearity range were assessed. The calculated RSD values were found to be within the accepted limit(less than 2%), Table 3.

Table3. Precision of the developed method

Conc. µg/ml	RSD% (n=3)	
	Repeatability	Reproducibility
3	1.23	1.76
9	1.20	1.80
12	0.96	1.00

The accuracy of the method was evaluated through calculation of the Student-t-value at 95% confidence limit utilizing the formula;[12]

t= (x-μ) √n /SD

t= (99.4-100).√3 /1.06= 1.01

Where;

t= Student-t-value

μ=known mean (considering injection content as 100%)

x=mean content of the sample

n= number of samples

SD= standard deviation of the assay results

The calculated t-value (1.01) was found to be less than the tabulated value (4.3). This indicates the accuracy of the developed method.

4. CONCLUSION

Unlike the developed method, most of the reported methods for the assay of aminocaproic acid require expensive or sophisticated instruments or involve procedures with rigorous control of experimental conditions. It can be concluded that the developed method is suitable for routine analysis of aminocaproic acid because of its simplicity, accuracy and sensitivity.

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