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Spectrophotometric Method for the Determination of Tranexamic Acid in Bulk and Dosage Forms

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Abstract: A simple spectrophotometric method for the determination of tranexamic acid in pure form and pharmaceutical preparations was developed. The method is based on coupling tranexamic acid with 2,6-dichloroquinone-4-chlorimide (DCQ) in dimethyl sulfoxide (DMSO) to produce a colored product which absorbs maximally at wavelength (λ_{max}) 670nm. The molar ratio of tranexamic acid: DCQ is 2:1. Beer's law is obeyed in the concentration range 50-250 µg/ml of tranexamic acid with molar absorbtivity (ε) of 0.52×10^3 $Lmol^{-1}$ cm^{-1}. Different experimental parameters affecting the development and stability of the color were studied and optimized. The proposed method was validated against an adopted B.P formal titration method. The mean percentage recovery for the assay of commercial capsules was found to be 101.13±1.04.

Key words: Spectrophotometry · Assay · Tranexamic acid · 2,6-dichloroquinone-4-chlorimide (DCQ) · Pharmaceutical formulations

INTRODUCTION

Tranexamic acid, trans-4-(aminomethyl) cyclo hexane carboxylic acid (Figure 1), is a synthetic lysine analogue antifibrinolytic drug. It competitively inhibits the binding of plasminogen to fibrin at lysine binding site. Therefore, tranexamic acid is used to treat bleeding associated with excessive fibrinolysis, such as haemorrhage following prostatectomy, tonsillectomy and menorrhagia [1].

Several methods have been reported for the assay of tranexamic acid in pharmaceutical formulations and biological fluids. These methods include UV spectrophotometry [2-5], colorimetry [6-9], fluorimetry [10, 11], HPLC [12, 13], LC-mass spectrometry [14] and TLC-desitometry [15].

DCQ has been utilized as chromogenic reagent for the spectrophotometric determination of some thiol drugs (Captopril [16] and D-penicillamine [17]), amine containing drugs (Pregabalin [18] and Methyl dopa [19]) and phenolic drugs (Propofol [20] and Cefadroxil [21]).

The aim of the present work was to develop a simple spectrophotometric method for accurate and rapid determination of tranexamic acid in pure bulk and dosage forms using DCQ as chromogenic reagent.

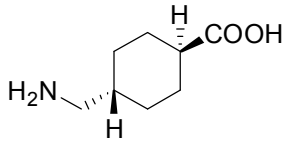

Fig. 1: Structure of tranexamic acid

Experimental

Apparatus: All spectrophotometric measurements were carried out using a Shimadzu UV-1800 spectrophotometer, Japan, a Shimadzu A×120 electronic balance, Japan and Gesellschaft fur Labotechink 1032 water bath, Germany.

Materials: Reference tranexamic acid (purity100%) was supplied by the Central Laboratory, Sudan. Trexamin® capsules (Batch No.8838) labeled to contain 250mg tranexamic acid, product of Hilton Pharma (Pvt.) Ltd., Pakistan, were obtained from commercial sources. A stock solution was prepared by dissolving 400mg of tranexamic acid in 100ml of distilled water and was further diluted to give a working standard solution with final concentration 1000µg/ml. DCQ was obtained from Lobal chemie, GR 99%, India. A 0.25% w/v of the reagent was freshly prepared in methanol. Dimethyl sulfoxide was supplied from S.d.fine-chem limited, AR 99.5%, India.

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Construction of Calibration Curve: Different aliquot volumes from working standard solution (0.5-2.5ml) were transferred into a series of 10ml volumetric flasks. A volume of distilled water was added to each flask to adjust the volume to 2.5ml. Then 5ml of DMSO followed by 0.7ml of DCQ solution were added. The volume was completed to mark with distilled water and heated in a boiling water bath for 3minutes. After cooling to room temperature, the absorbance of each solution was measured at 670nm against the reagent blank. The absorbance was plotted against final tranexamic acid concentration (50-250µg/ml) in order to obtain a calibration curve.

Assay of Tranexamic Acid Capsules by the Proposed Method: A quantity of the mixed contents of 20 capsules (Trexamin®) containing the equivalent of 400mg of tranexamic acid was transferred into 100ml volumetric flask. About 80ml of distilled water was added and the solution was shaken for 15minutes. After completion the volume to mark with water, the solution was filtered. Then 25ml of the filtrate was diluted to 100ml with water to give tranexamic acid sample solution with final concentration of 1000µg/ml.

To 1.5ml of the sample solution in a 10ml volumetric flask, 1ml of distilled water, 5ml DMSO and 0.7ml freshly prepared DCQ solution were added. This was followed by volume completion to mark with distilled water and heating in a boiling water bath for 3minutes. Then, the solution was allowed to cool at room temperature and the absorbance was measured at 670nm against the reagent blank. The content of tranexamic acid capsules was calculated either from the calibration curve or the corresponding regression equation.

Assay of Tranexamic Acid Capsules by the Formal Titration Method: A quantity of the mixed contents of 20 capsules containing the equivalent of 0.5g of tranexamic acid was dissolved in 20ml distilled water. The solution was then shaken for 15 minutes and the volume was completed to 25ml. Ten ml of formaldehyde solution previously neutralized by sodium hydroxide was added. Titration was then carried out using 0.5N sodium hydroxide as titrant in presence of phenolphthalein as indicator. The end point was determined by formation of stable slight pink color and the required volume of titrant was determined.

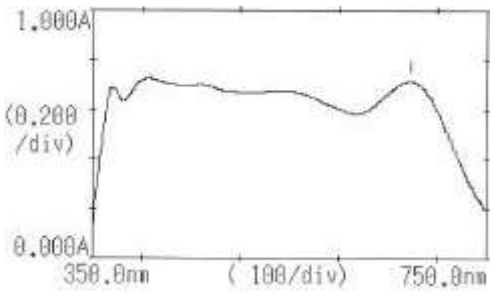


Fig. 2: Absorption spectrum of tranexamic acid (250µg/ml) -DCQ complex

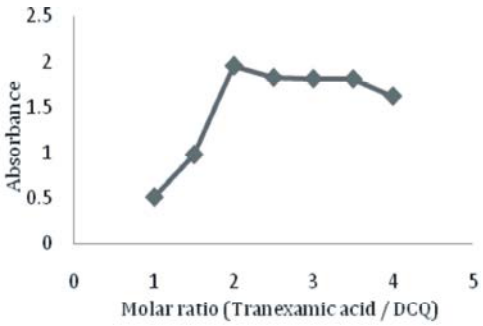


Fig. 3: Mole ratio plot for reaction stoichiomeetry determination

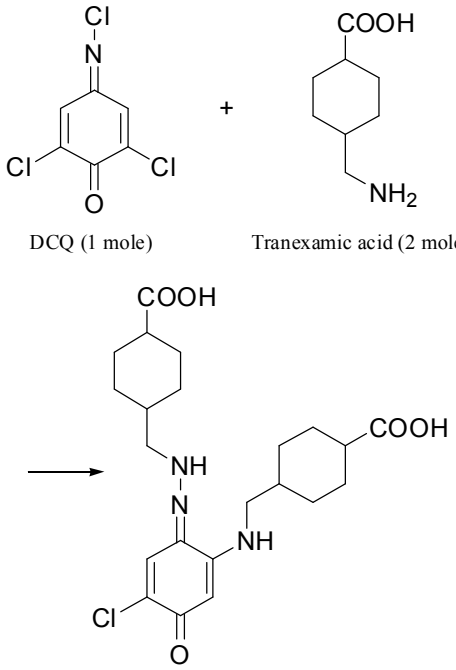
RESULTS AND DISCUSSION

Tranexamic acid contains amino group which is susceptible for the reaction with DCQ to give a colored complex. Figure 2 shows the absorption spectrum of the formed colored product which has an analytically useful peak at 670nm and a non-stable rather flat peak at about 412nm.

Reaction stoichiometry was determined by the molar ratio method [22] using different aliquot volumes of tranexamic acid 24×10^{-3} M and constant volume of DCQ 12×10^{-3} M. A plot of absorbance as a function of the tranexamic acid-to-DCQ mole ratio (TA/DCQ) gave two linear branches that intersect at a mole ratio (2:1) corresponding to the formula of the colored complex (Figure 3).

Based on this data of the reaction ratio, a possible structure of the formed derivative is shown in scheme 1.

Optimization of Reaction Conditions: Different experimental parameters affecting the color development were studied to determine the optimal conditions for assay procedure.



Scheme 1: Reaction of tranexamic acid with DCQ

The colored product was formed under both heating and non-heating conditions. However, heating increases both the reaction rate and absorbance intensity markedly (Table 1). Increasing heating time in a boiling water bath more than 3minutes causes slight or no change in absorbance value. Therefore, 3minutes was chosen as optimal heating time.

Reaction was carried out at both alkaline media (1ml of 1%-10% sodium acetate aqueous solution, pH 8.5) and neutral media in non-heating conditions. The color intensity was increased with increase in sodium acetate concentration. However, the developed color in alkaline media was unstable. The color developed in neutral media was stable but had low absorbance value (Table 2).

The effect of DCQ concentration on reaction rate was investigated using 0.7±0.1ml of 0.1%, 0.25% and 0.5% DCQ solutions. It was found that increasing the concentration of 0.7±0.1ml DCQ solution would increase the absorbance of the reaction product up to 0.25%, after which further increase in the concentration of DCQ resulted in no change in the absorbance of reaction product. Thus, 0.7ml of 0.25% DCQ was adopted as the suitable concentration for maximum absorbance (Figure 4).

The effect of different solvents with different dielectric constants (DE), namely water (DE 78), isopropanol (DE 19.9) and DMSO (DE 47), on the color development was studied. Faster and intense color was obtained with DMSO.

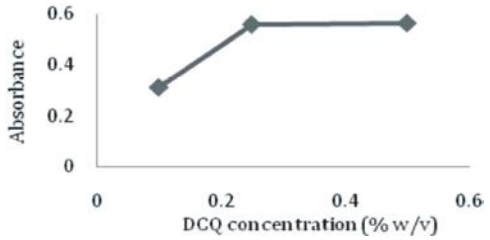


Fig. 4: Effect of DCQ concentration on the absorbance value of the reaction product (tranexamic acid 200µg/ml) at 670nm

Table 1: Effect of heating on the absorbance value (Abs.) of the reaction product at 670nm (tranexamic acid 200µg/ml)

Condition	Time (min.)	Abs.
Heating in a boiling water bath	3	0.554
Waiting at room temperature	3	0.093
Waiting at room temperature	120	0.156

Table 2: Effect of alkaline media on the absorbance intensity of the reaction product (tranexamic acid 60µg/ml)

Condition	λ max	Absorbance after 1 hour
10% sodium acetate	682	2.780
1% sodium acetate	680	1.041
No sodium acetate	674	0.062

Addition of DMSO before heating was essential to obtain maximum color intensity.

Under the experimental conditions employed, the color intensity of the system remained stable for 2 hours which was considered sufficient time for absorbance measurements.

Analytical Data: Beer's law was found to be valid over the tranexamic acid concentration range 50-250µg/ml. Linear regression analysis of the data gave the following equation:

$$A = -0.1003 + 3.2985 \times 10^{-3} C \quad (R = 0.996).$$

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 [23] data for the regression equation of the proposed method are given in Table 3.

To test the accuracy of the proposed method a volume of reference tranexamic acid solution (100µg/ml) was added to the capsules solution (100µg/ml) and

Table 3: Optical and regression characteristics of the proposed method

Parameter	Value*
λ_{max}	670nm
Beer's law limit (µg/ml)	50-250
Limit of detection (µg/ml)	24.01
Limit of quantification (µg/ml)	80.04
Molar absorbtivity ($Lmole^{-1}$ cm^{-1})	0.52×10^{3}
Slope (b)	3.3×10^{-3}
Standard deviation of slope (S_b)	1.7×10^{-4}
95% confidence limit of slope (tS_b)	5.3×10^{-4}
Intercept (a)	-0.10028
Standard deviation of intercept (S_a)	0.0277
95% confidence limit of intercept (tS_a)	0.0881
Correlation coefficient (R)	0.996

*Average of four independent analyses with RSD values not more than 2%.

Table 4: Determination of tranexamic acid in Trexamin® capsules by the proposed and titrimetric method

Parameter	Proposed method	Titrimetric method
Mean content percent	101.13	100.08
Standard deviation	1.04	0.91
Relative standard deviation	1.03%	0.91%
Calculated t-value	1.31(2.78*)	
Calculated F-value	1.30(9.28*)	

*tabulated t-value and F-value at 95% confidence level

subsequently assayed by the proposed method. The mean percentage added recovery using regression equation was found to be 100.64±1.2.

The repeatability and reproducibility of the method were carried out using 200 and 150µg/ml tranexamic acid, respectively. The mean percentage recovery of four replicate analyses was found to be 99.99±0.86 and 100.01±0.94, respectively.

The proposed method was applied to the determination of tranexamic acid in capsules labeled to contain 250mg. The mean content percent of four independent analyses was found to be 101.13±1.04.

Statistical analysis of the results, obtained by an adopted B.P formal titration method for assay of tranexamic acid injection [24] and the proposed method using t-test and F-test [23], showed no significant difference between the performance of the two methods regarding the accuracy and precision (Table 4).

CONCLUSION

The proposed method is simple, rapid, accurate and precise. Therefore, it can be used for the routine analysis of tranexamic acid in pure as well as in commercial dosage forms.

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