Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 54 (2013) 12331-12334

ISSN: 2229-712X

Assay of Tranexamic Acid via Coupling with Ascorbic Acid Using Kinetic Methods

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ARTICLE INFO

Article history:

Received: 21 November 2012;

Received in revised form:

27 December 2012;

Accepted: 3 January 2013;

Keywords

Kinetic determination,

Tranexamic acid,

Ascorbic acid,

Spectrophotometric analysis.

ABSTRACT

A simple, precise and accurate kinetic spectrophotometric method has been developed for determination of tranexamic acid. The method was based on a kinetic investigation of the coupling reaction between tranexamic acid and ascorbic acid after heating to form a purple-pink colored complex. The reaction was followed up spectrophotometrically by measuring the increase in absorbance at 530nm & 390nm as a function of time. The initial rate, rate constant, fixed time and fixed absorption time methods were adopted for constructing the calibration curves and examined for their suitability for the assay of tranexamic acid in pure and capsules forms.

Introduction

Trans-4-(amino methyl) cyclo hexane carboxylic acid (fig.1), pharmacologically known as tranexamic acid, is antifibrinolytic drug inhibits plasminogen activation and thus prevents fibrin degradation by active plasmin. It is used to treat various conditions in which there is bleeding or risk of bleeding, such as prostatectomy, dental extraction, menorrhagia and thrombolytic overdose [1].

The published reported methods for the determination of this drug included either chromatographic methods such as RP-HPLC [2], HPLC-ELS [3], LC-MS [4] and TLC-desitometry [5] or spectrophotometric methods such as fluorimetry [6, 7] and UV/VIS spectrophotometry [8-13]. Most of these methods are either requires sophisticated instruments or tedious and material consuming.

Literature revealed that the colorimetric determination of Alfuzocin [14], Tobramycin [15], Penicillins [16], Cephalosporins [16] and Lisinopril [17] using ascorbic acid based on the reaction of their amino groups with carboxylic group of dehydro ascorbic acid. Accordingly, tranexamic acid, amine containing drug, can form colored complex through coupling with oxidized form of ascorbic acid.

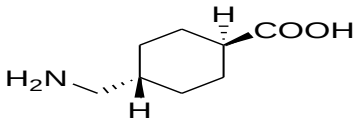

Fig (1): Structure of tranexamic acid

In this work, coupling of tranexamic acid with ascorbic acid as chromogenic reagent was studied kinetically in an attempt to develop a reliable kinetic spectrophotometric method for determination of tranexamic acid in pure form and in pharmaceutical formulations.

Experimental

Apparatus:

Shimadzu UV-1800 spectrophotometer, Japan, Gesellschaft fur Labotechink 1032 water bath, Germany and Shimadzu A × 120 electronic balances, Japan.

Materials:

Reference tranexamic acid (purity100%) was supplied by the Central Laboratory, Sudan. Trexamin® capsules labeled to contain 250mg tranexamic acid were purchased from local market. A stock solution was prepared by dissolving 200mg of tranexamic acid in 100ml of distilled water and was further diluted to give a working standard solution with final concentration 500µg/ml. L-ascorbic acid was obtained from S.d.fine-chem limited, LR 99%, India. A 2% w/v of the reagent was freshly prepared in dimethyl sulfoxide (DMSO). DMSO was supplied from S.d.fine-chem limited, AR 99.5%, India.

Procedure:

Different aliquot volumes from working standard solution (0.2-0.5ml) were transferred into a series of 10ml volumetric flasks. A volume of distilled water was added to each flask to adjust the volume to 0.5ml. Then 1ml of ascorbic acid solution followed by 3ml of DMSO were added. These solutions were heated in a boiling water bath for different time intervals. After cooling to room temperature, the volumes were completed to mark with DMSO and the absorbance of each solution was measured at 390nm & 530nm against the reagent blank. The absorbance values for each concentration of tranexamic acid (10-25µg/ml) was plotted against heating time to illustrate the reaction rate dependency and the four kinetic methods namely; initial rate, rate constant, fixed time and fixed absorption time were used for construction of the calibration curves for determination of the studied drug.

Results and discussion

The absorption spectrum (fig. 2) of the purple-pink colored complex produced by coupling of tranexamix acid with oxidized form of ascorbic acid (dehydroascorbic acid) gave two peaks at 390nm & at 530nm.

Different experimental parameters affecting the color development and its stability were carefully studied and the optimum conditions were outlined in the procedure. Under optimal conditions, the rate of the reaction was followed with various concentrations of tranexamic acid keeping constant

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concentration of ascorbic acid. The absorbance was measured, at 390nm & 530nm, at different heating time intervals for 45minutes starting from 10minutes. The assay was carried out in presence of excess concentration of ascorbic acid. Therefore, a pseudo-zero order reaction condition was worked out with respect to the concentration of the reagent. Plots of absorbance versus time showed that the reaction rate is concentration dependant (fig. 3).

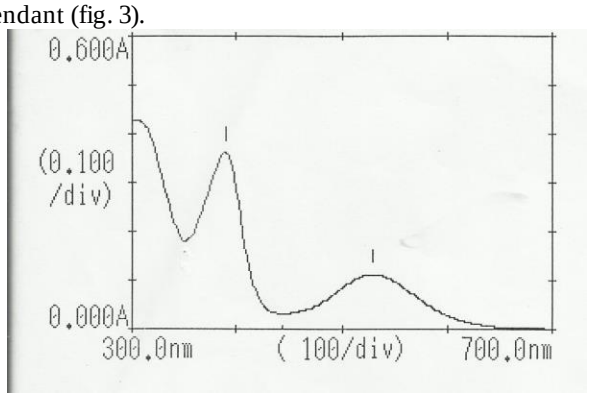
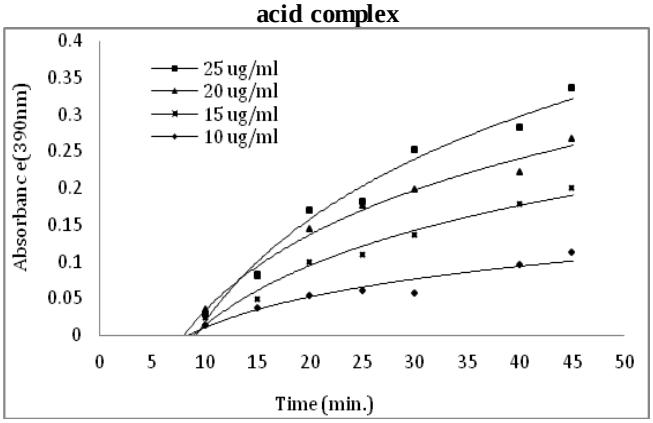


Fig (2): Absorption spectrum of tranexamic acid – ascorbic acid complex



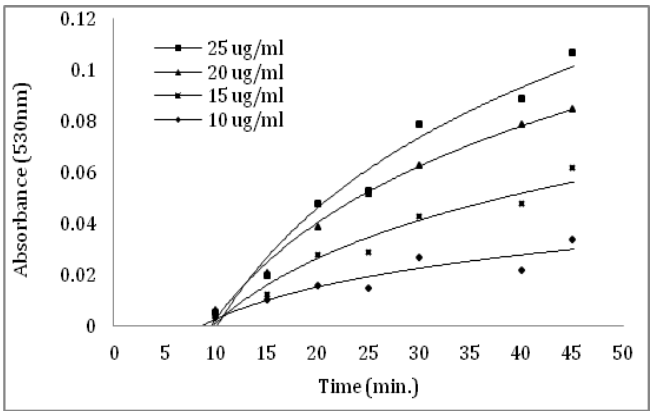


Fig (3): Absorbance-time curves of different tranexamic acid concentrations

1-Initial rate method:

The initial rate of reaction was obtained by measuring the slopes ($\Delta A/\Delta t$) of the initial tangent to the absorbance-time curves at different concentrations (10-25µg/ml) of the investigated drug. The initial rate of reaction would follow a pseudo-first order and obeyed the following rate equation:

$$r = \frac{\Delta A}{\Delta T} = K' C^n$$

Whereas r is the reaction rate, A is the absorbance, T is the measuring time (min.), k' is the pseudo-first order rate constant, C is the concentration of the drug (µg/ml) and n is the order of the reaction. The logarithmic form of the above equation is written as follows:

$$\log r = \log \frac{\Delta A}{\Delta T} = \log K' + n \log C$$

A calibration curve (fig. 4) was constructed by plotting the logarithm of the initial rate of reaction (*log r)* versus logarithm of initial concentration of the tranexamic acid (*log C*), which showed a linear relationship over concentration range of 10-25µg/ml. The regression equations of *log r* versus *log C* are given in table 1.

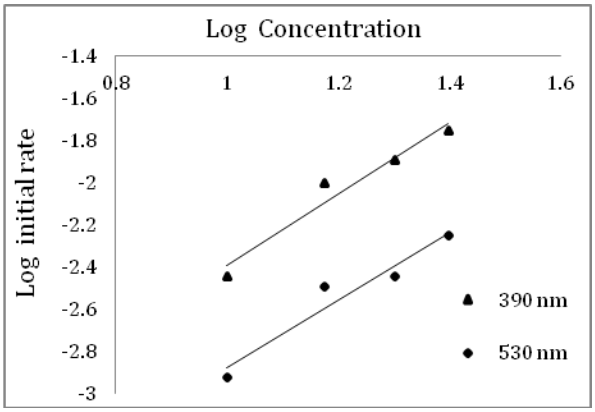


Fig (4): Calibration curve for initial rate method

Table (1): Relation between initial rates of reaction and tranexamic acid concentrations

$\log \frac{\Delta A}{\Delta T}$ (λ 390)	$\log \frac{\Delta A}{\Delta T}$ (λ 530)	log C (µg/ml)	Calibration equation	R
-2.443	-2.920	1	λ 390:	
-2	-2.494	1.176	Log r = - 4.08+1.69 logC	0.951
-1.892	-2.443	1.301	λ 530:	
-1.754	-2.251	1.397	Log r = - 4.47+1.59 logC	0.939

The initial rate method has low correlation coefficient (R: 0.95-0.93). Therefore, it is not considered suitable method for the assay.

2- Rate constant method:

Graphs of log absorbance at 390nm & 530nm versus time in minutes for each concentration of tranexamic acid (10-25µg/ml) were constructed. Pseudo first-order rate constants (*k'*) corresponding to different concentrations (*C*) were calculated from the slopes, multiplied by -2.303. Pseudo first-order rate constant (*k'*) versus the initial concentration of the drug was then plotted (fig. 5) and the regression equations of the calibration graphs are given in table 2.

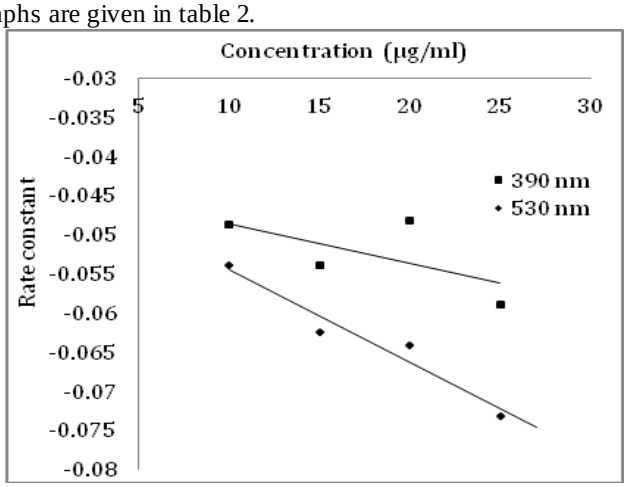


Fig (5): Calibration curve of rate constant method

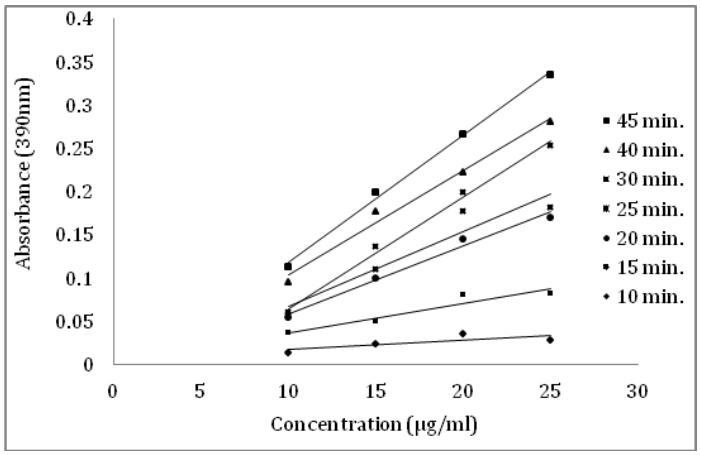
Table (2): Relation between k' values for different concentrations of the studied drug.

k' (min^{-1}) (λ 390)	k' (min^{-1}) (λ 530)	Conc. µg/ml	Calibration equation	R
-0.0486	-0.0539	10	λ 390: $k' = -5X10^{-4}C - 0.0436$	0.418
-0.0538	-0.0624	15		
-0.0482	-0.0641	20	λ 530: $k' = -12X10^{-3}C - 0.0427$	0.945
-0.0589	-0.0731	25		

It was found that there is no linear response over the concentration range of 10-25µg/ml at 390nm, and poor linarity at 530nm. Thus, rate constant method was found not suitable for estimation of tranexamic acid concentration over this range. This poor linearity is propably due to differences of k' value as a result of changes in one of the reaction conditions.

3- Fixed time method:

At a preselected fixed time, the absorbance value of different concentration of tranexamic acid (10-25µg/ml) was recorded and plotted against the corresponding initial concentration (fig 6). The linear regression equations with correlation coefficients are summarized in table 3 at fixed time starting from 10 to 45 min. with interval of 5min.



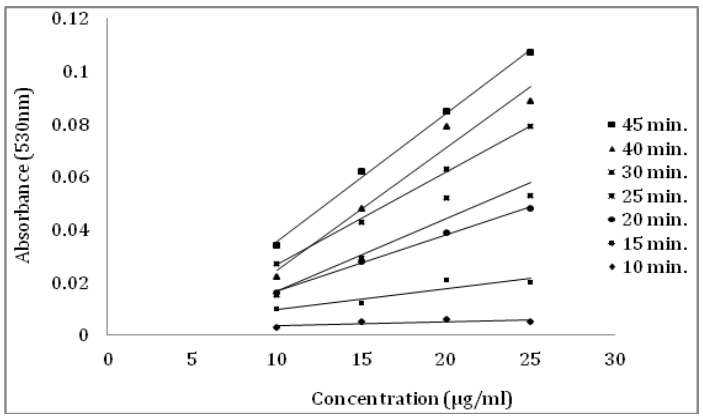


Fig (6): Abs.–Conc. curves of tranexamic acid-ascorbic acid complex at different time interval.

It is evident from the table that the slope increases with time and the highest value of the slope and correlation coefficient were obtained at a fixed time of 45minutes, which was therefore chosen as the most suitable fixed time for the measurement.

4- Fixed absorption time method:

The general procedure was followed up by recording the time in minutes at different drug concentration levels required for the absorbance to reach a preselected value. The absorbance value 0.113 at 390nm was fixed and the time at which each concentration of tranexamic acid can take to attain was determined (table 4).

Table (4): Results for fixed absorption time method.

Concentration (µg/ml)	Heating time (minutes)
10	45
15	25
20	16

The reciprocal of time (1/t) versus the initial concentration of the drug was plotted (fig. 7) and the regression equations of the calibration graphs are given in table 5.

Table (5): Relation between drug concentration and time to attain preselected absorbance value

1/t (s^{-1})	Conc.(µg/ml)	Calibration equation	R
$3.7X10^{-4}$	10	$1/t = 6.71X10^{-5}C - 3.14X10^{-4}$	0.995
$6.7X10^{-4}$	15		
$10X10^{-4}$	20		

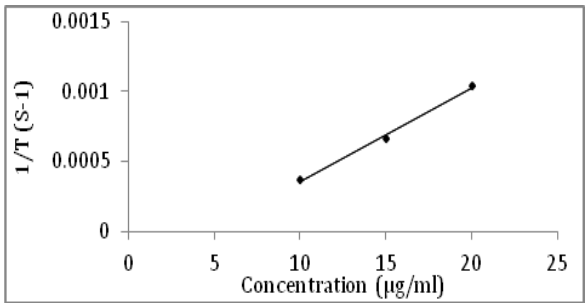


Fig (7): Calibration curve for fixed absorption time method

Despite the good R value (0.995; for only three points), the method suffers from the narrow concentration range (10-20µg/ml) that can be used, besides insufficient repeatabilities and reproduceabilities.

5-Application:

Fixed time method at 45minutes was chosen for determination of tranexamic acid in pure and capsules forms. Over the tranexamic acid concentration range 10-25µg/ml, linear regression analysis of the data gave the following equations with limit of detection 1.83µg/ml (390nm) & 1.09µg/ml (530nm) and limit of quantification 6.09µg/ml (390nm) & 3.65µg/ml (530nm).

At 390nm: A=0.01472C - 0.0286. (R= 0.998).

At 530nm: A=4.8×10-3C- 0.0127. (R= 0.998).

The proposed method was applied to the determination of tranexamic acid in capsules labeled to contain 250mg. Statistical analysis of the results obtained by the proposed and reference method (BP2002) [18] using t-test and F-test [19] revealed no significant difference between the performance of the two methods regarding the accuracy and precision (table 6).

Table (6): Determination of tranexamic acid in capsules by the proposed and official methods

Parameter	Proposed method (390nm)	Proposed method (530nm)	Official method
Mean content percent	99.77	101.01	100.08
Standard deviation	1.02	0.97	0.91
Relative standard deviation	1.02%	0.96%	0.91%
Calculated t-value	0.39(2.78*)	0.97(2.78*)	
Calculated F-value	1.26(9.28*)	1.14(9.28*)	

*Tabulated t-value and F-value at 95% confidence level.

Table (3): Calibration equations for relation between Abs. and Conc. of tranexamic acid at fixed times

Fixed time(min.)	Calibration equation (390nm)	R	Calibration equation (530nm)	R
10	$A=1.1\times10^{-3}C+5.8\times10^{-3}$	0.79	$A=1.4\times10^{-4}C+2.3\times10^{-3}$	0.71
15	$A=3.4\times10^{-3}C+3.6\times10^{-3}$	0.95	$A=7.8\times10^{-4}C+2.1\times10^{-3}$	0.90
20	$A=7.9\times10^{-3}C-0.0198$	0.993	$A=2.1\times10^{-3}C-4.7\times10^{-3}$	0.997
25	$A=8.6\times10^{-3}C-0.0180$	0.95	$A=2.7\times10^{-3}C-0.0107$	0.95
30	$A=0.01294C-0.0647$	0.996	$A=3.5\times10^{-3}C-8.6\times10^{-3}$	0.998
40	$A=0.01206C-0.0163$	0.992	$A=4.6\times10^{-3}C-0.0217$	0.98
45	$A=0.01472C-0.0286$	0.998	$A=4.8\times10^{-3}C-0.0127$	0.998

Conclusion

The data given above revealed that the initial rate, rate constant, fixed absorption time methods are not suitable for estimation of tranexamic acid over the range 10-25µg/ml under the selected reaction conditions. The fixed time method at 45minutes is simple, accurate and precise for assay of tranexamic acid in bulk and in capsules form.

Acknowledgement

The authors are thankful for the support provided by the department of pharmaceutical chemistry, University of Khartoum.

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