Journal of Molecular Structure 891 (2008) 475–480

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Spectrophotometric techniques to determine tranexamic acid: Kinetic studies using ninhydrin and direct measuring using ferric chloride

M. Saeed Arayne [a,*], Najma Sultana [b], Farhan Ahmed Siddiqui [a], Agha Zeeshan Mirza [a], M. Hashim Zuberi [a]

[a] Department of Chemistry, University of Karachi, Karachi 75270, Pakistan
[b] Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan

ARTICLE INFO

Article history:
Received 1 December 2007
Received in revised form 12 April 2008
Accepted 15 April 2008
Available online 22 April 2008

Keywords:
Tranexamic acid
Ninhydrin
Color development
Spectrophotometric method
Ferric chloride

ABSTRACT

Two simple and sensitive spectrophotometric methods in ultraviolet and visible region are described for the determination of tranexamic acid in pure form and pharmaceutical preparations. The first method is based on the reaction of the drug with ninhydrin at boiling temperature and by measuring the increase in absorbance at 575 nm as a function of time. The initial rate, rate constant and fixed time (120 min) procedures were used for constructing the calibration graphs to determine the concentration of the drug, which showed a linear response over the concentration range 16–37 μg mL^{-1} with correlation coefficient "*r*" 0.9997, 0.996, 0.9999, LOQ 6.968, 7.138, 2.462 μgmL^{-1} and LOD 2.090, 2.141 and 0.739 μgmL^{-1}, respectively. In second method tranexamic acid was reacted with ferric chloride solution, yellowish orange colored chromogen showed λ_{max} at 375 nm showing linearity in the concentration range of 50–800 μg mL^{-1} with correlation coefficient "*r*" 0.9997, LOQ 6.227 μgmL^{-1} and LOD 1.868 μgmL^{-1}. The variables affecting the development of the color were optimized and the developed methods were validated statistically and through recovery studies. These results were also verified by IR and NMR spectroscopy. The proposed methods have been successfully applied to the determination of tranexamic acid in commercial pharmaceutical formulation.

1. Introduction

Tranexamic acid or *trans*-4-(aminomethyl) cyclohexanecarboxylic acid is a synthetic derivative of lysine (Fig. 1). Due to its potent antifibrinolytic activity and lack of effect on blood clotting parameters, tranexamic acid has been used in a wide range of haemorrhagic conditions [1,2] and reduces the need for replacement of blood factors. Its most interesting use has been in the treatment of malignant ovarian tumors, to promote formation of fibrin caps to wall off and inhibit growth of the tumor [3]. It reduces postoperative blood losses and transfusion requirement in a number of types of surgeries. It also reduces menstrual blood loses and is a possible alternative to surgery in menorrhagia and has been used successfully to control bleeding in pregnancy [4].

Reactions with ninhydrin (Fig. 2) are widely used to analyze and characterize amino acids, peptides and proteins as well as numerous other ninhydrin positive compounds in biomedical, clinical, food, forensic, histochemical, microbiological, nutritional and plant studies [5]. It has been extensively used in the determination of the compounds of pharmaceutical importance and in kinetic studies [6,7]. Although the ninhydrin reaction is used daily in thousands of laboratories and may very well be, the most widely used organic reaction, several features associated with it appear to be anomalous. Thus, in many cases the amount of color formed is not always stoichiometric [5].

The kinetic approach for determining tranexamic acid in commercial dosage form, using ninhydrin as a reagent, reduces the time of analysis as it requires simply heating and cooling of the reaction mixture. The published analytical methods for determining tranexamic acid include spectrophotometry [8,9], colorimetry [10,24], HPLC [11–14,25], LCMS [2], AAS [15], gas chromatography [16] and spectrofluorometry [17]. In the present manuscript, sensitive spectrophotometric methods for the determination of tranexamic acid have been described. These methods were based on the reaction of tranexamic acid with ninhydrin at boiling temperature for which a kinetic approach has been adopted and complex formation with ferric chloride. The proposed methods have been successfully applied to the determination of tranexamic acid in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

Shimadzu 1601 double beam UV–visible spectrophotometer possessing a fixed slit width (2 nm) with quartz cells of 10 mm

* Corresponding author. Tel.: +92 21 4610132.
E-mail addresses: msarayne@gmail.com, farhanchemist@hotmail.com (M.S. Arayne).

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doi:10.1016/j.molstruc.2008.04.026

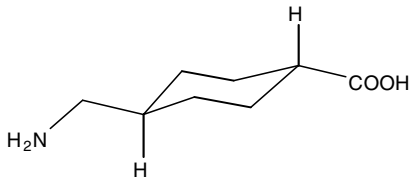


Fig. 1. Tranexamic acid.

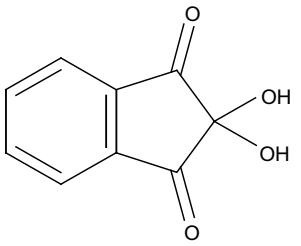


Fig. 2. Ninhydrin.

path length connected to a P IV computer loaded with Shimadzu UVPC version 3.9 software and a HP DeskJet 1200 printer were used to record the absorption spectra. IR spectra were recorded on a Shimadzu Model FTIR Prestige-21 spectrophotometer.

2.2. Reagents and materials

Tranexamic acid was obtained from S.D. Fine Chemicals, India. Ninhydrin, ferric chloride and methanol were purchased from Merck-Schuchardt, Germany. Maxna tablets 500 mg were purchased from local market. The 200 μg mL^{-1} solution of tranexamic acid was prepared in double distilled water and diluted as required. For second method 1000 μg mL^{-1} solution of tranexamic acid was prepared in double distilled water. One percent ninhydrin solution was prepared in methanol and 1% ferric chloride solution in water.

2.2.1. Method A

Aliquots of 200 μg mL^{-1} tranexamic acid were transferred into heating tubes. 2 mL of 1% ninhydrin solution was added and heated on boiling water bath for 2 h, after cooling the mixture was transferred into 25 mL volumetric flask and diluted to volume with distilled water. Increase in absorbance at 575 nm was recorded as a function of time against the reagent blank at room temperature (Spectra 1). The initial rate of reaction at different concentrations was calculated from the initial slope of absorbance time curve. The calibration curves were constructed by plotting (i) logarithm of initial rate of reaction versus logarithm of molar concentration, (ii) rate constant versus final concentration and (iii) absorbance measured at a fixed time versus final concentration of tranexamic acid.

2.3. Analysis of pharmaceutical formulations

Twenty capsules were weighed and content equivalent to 10 mg of the tranexamic acid was dissolved in small amount of doubled distilled water by stirring, diluted to 100 mL and filtered. The filtrate was used for the derivatization with ninhydrin or ferric chloride.

2.3.1. Method B

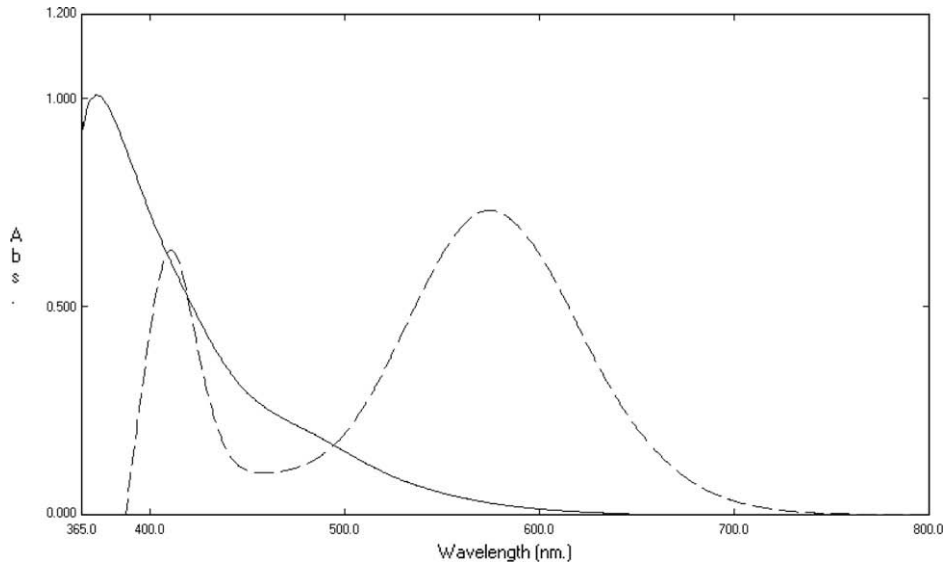
Different aliquots (50–800 μg mL^{-1}) of tranexamic acid were transferred into a series of 25 mL volumetric flasks. To each flask, 3 mL of 1% ferric chloride solution was added. The yellowish orange color was measured at 375 nm against reagent blank at room temperature (Spectra 1).

2.3.2. Spectral data

UV–vis (MeOH) λ_{max}: 575, 375 nm; ninhydrin; 1H NMR (MeOD, 300 MHz), δ: 8.07 (s, 3H), 2.5 (s, 2H), IR (KBr) *n*: 3300, 3250, 1760, 1660, 1061, 750 cm^{-1}; tranexamic acid; 1H NMR (MeOD, 300 MHz) 12.00 (s, 1H), 2.00 (s, 2H), 2.73–2.75 (2s, 2H), 0.9–1.6 (s, 10H) IR (KBr) *n*: 2980, 2850, 2609, 1650, 1600, 1450, 930, 800 cm^{-1}; complex; 1H NMR ($CDCl_3$, 300 MHz), δ: 7.23–7.92 (m, 4 H), 6.80 (s, 1H), IR (KBr) *n*: 3300, 3250, 1560.

3. Results and discussion

Ninhydrin is a well-established reagent for the determination of certain amines, amino acids and thiophenes [18]. Tranexamic acid does not absorb above 250 nm, therefore derivatization with ninhydrin and ferric chloride was carried out to increase the spectrophotometric sensitivity with bathochromic shift to visible region. In tranexamic acid primary amine, reacts with ninhydrin to produce a blue colored product, which absorbs maximally at 575 nm



Spectra 1. (---) Tranexamic acid–ninhydrin complex. (–) Tranexamic acid–iron complex.