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

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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

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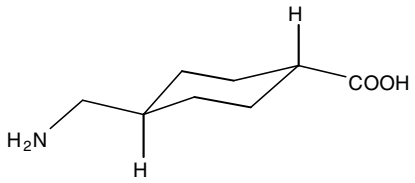


Fig. 1. Tranexamic acid.

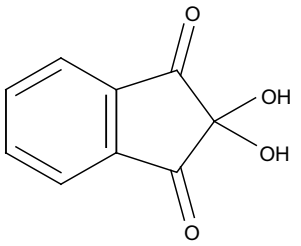


Fig. 2. Ninhydrin.

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

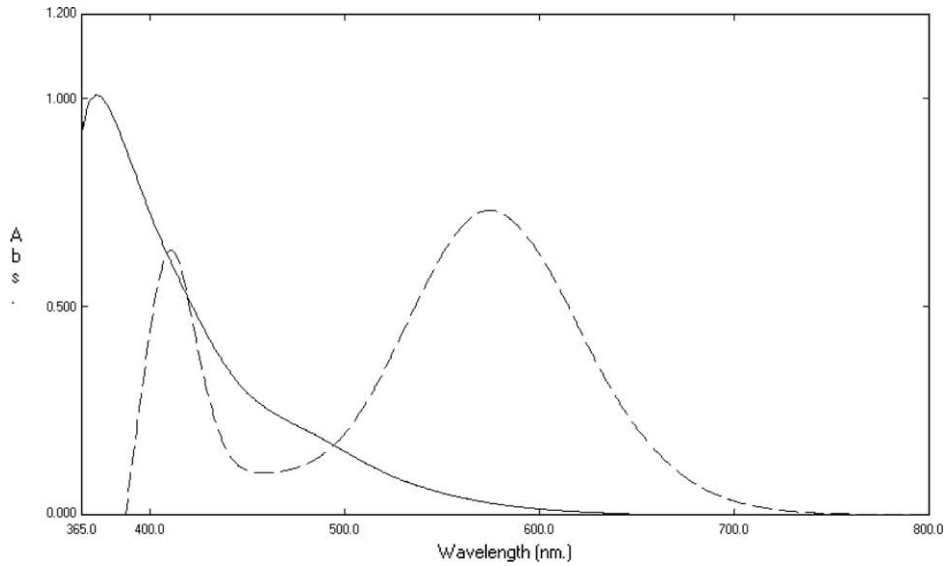
Different aliquots (50–800 μg mL^{-1}) of tranexamic acid were transferred into a series of 25 mL volumetric flasks. To each flask, 3 mL of 1% ferric chloride solution was added. The yellowish orange color was measured at 375 nm against reagent blank at room temperature (Spectra 1).

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.

under the specified experimental conditions. Similarly tranexamic acid reacts with ferric chloride solution (1%) at room temperature (25 °C) and gives a yellowish complex with absorbance at 375 nm. There is no report in literature on the interaction of tranexamic acid with iron. On the contrary, there are reports on the co administration of tranexamic acid with iron [19].

4.2. Method A

4.2.1. Optimization of reaction variables

The reaction between tranexamic acid and ninhydrin resulted in the formation of blue colored complex. At boiling temperature, the intensity of color increased with time and became stable after 120 min. The effect of ninhydrin concentration on reaction rate was investigated using 1–5 mL of 1% ninhydrin, it was found that increasing the volume of 1% ninhydrin solution would increase the absorbance of the reaction product up to 2 mL, after which further increase in the volume of ninhydrin resulted in no change in the absorbance of reaction product. Thus, 2 mL of 1% ninhydrin was adopted as the most suitable volume for maximum absorbance. Optical characteristics and statistical data for the regression equation of the proposed method are given in Table 1.

4.2.2. Kinetic study using ninhydrin

4.2.2.1. Initial rate method. The initial rate of the reaction was determined from the measurement of the slope of the initial tangent to the absorbance – time curve. The order with respect to tranexamic acid was ascertained by studying the reaction at different concentrations of tranexamic acid keeping constant concentration of ninhydrin. For each run, a plot of $\log A_{¥}/A_{¥}-A_t$ versus time was a straight line confirming the first order reaction (Fig. 4). The first order rate constant was also evaluated from the slopes of the above plot. All subsequent investigations were performed with different concentrations of ninhydrin with a fixed concentration of tranexamic acid. The plot of $\log A_{¥}/A_{¥}-A_t$ versus time also indicated

Table 1
Linear regression functions and their statistical parameters of different methods

	Method A			Method B
	Initial rate	Rate constant	Fixed time	
Intercept	−0.0004	−0.22456	0.00017	−0.0087
Slope	0.01008	0.00984	0.02852	0.0007
r^a	0.9997	0.996	0.9999	0.9997
LOQ[b]	6.968	7.138	2.462	6.227
LOD[c]	2.090	2.141	0.739	1.868

[a] Correlation coefficient.
[b] Limit of quantification in μg mL^{-1}.
[c] Limit of detection in μg mL^{-1}.

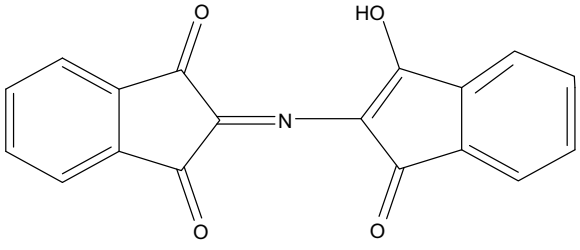


Fig. 3. Tranexamic acid ninhydrin complex.

Table 2
Rate constant under pseudo-first order condition

Concentration (μg mL^{-1})	Rate constant (slope) (min^{-1})
16	0.0098
21	0.0098
26	0.0098
32	0.0098
37	0.0098

Table 3
Statistical data of different concentration for fixed times method

	30 min	60 min	90 min	120 min
Intercept	0.0026	0.0052	−0.004	−0.0031
Slope	0.0113	0.0226	0.0345	0.0457
r^a	0.9999	0.9999	0.9998	0.9999

[a] Correlation coefficient.

Table 4
Precision and accuracy of the proposed method (Method A)

Amount taken (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%)	Amount found (μg mL^{-1})	Recovery (%)	Amount found (μg mL^{-1})	Recovery (%)
16	15.96	99.75	15.83	98.94	15.71	98.20
21	21.3	101.43	20.74	98.76	20.84	99.22
26	25.7	98.85	25.64	98.62	25.75	99.05
32	32.3	100.94	32.27	100.84	31.71	99.10
37	37.5	101.35	36.48	98.59	36.89	99.70
Mean		100.46		99.15		99.05
STd[a]		1.1257		0.956		0.543
Rsd[b]		1.1206		0.965		0.548

%, Percent recovery.
[a] Standard deviation.
[b] Relative standard deviation.

Table 5
Determination of tranexamic acid in pharmaceutical formulation

	Taken concentration (μg)	Found (μg)	Found (μg)	Found (μg)
	500	498.750	494.69	491.00
	500	507.143	493.81	496.09
	500	494.231	493.08	495.24
	500	504.688	504.22	495.52
	500	506.757	492.97	498.51
Mean		502.314	495.75	495.27
STd[a]		5.629	4.78	2.71
Rsd[b]		1.121	0.96	0.5479

Lable claim 500 mg.
[a] Standard deviation.
[b] Relative standard deviation.

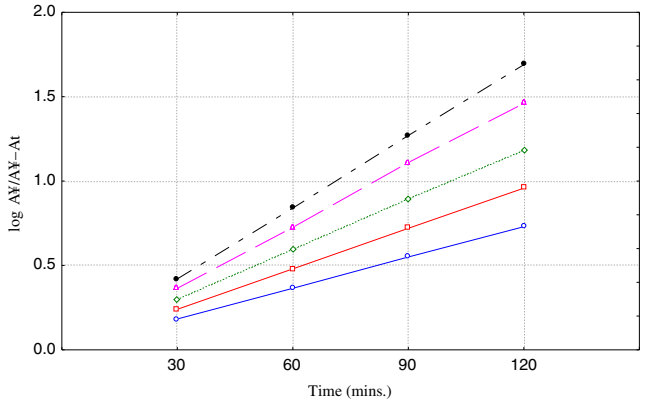
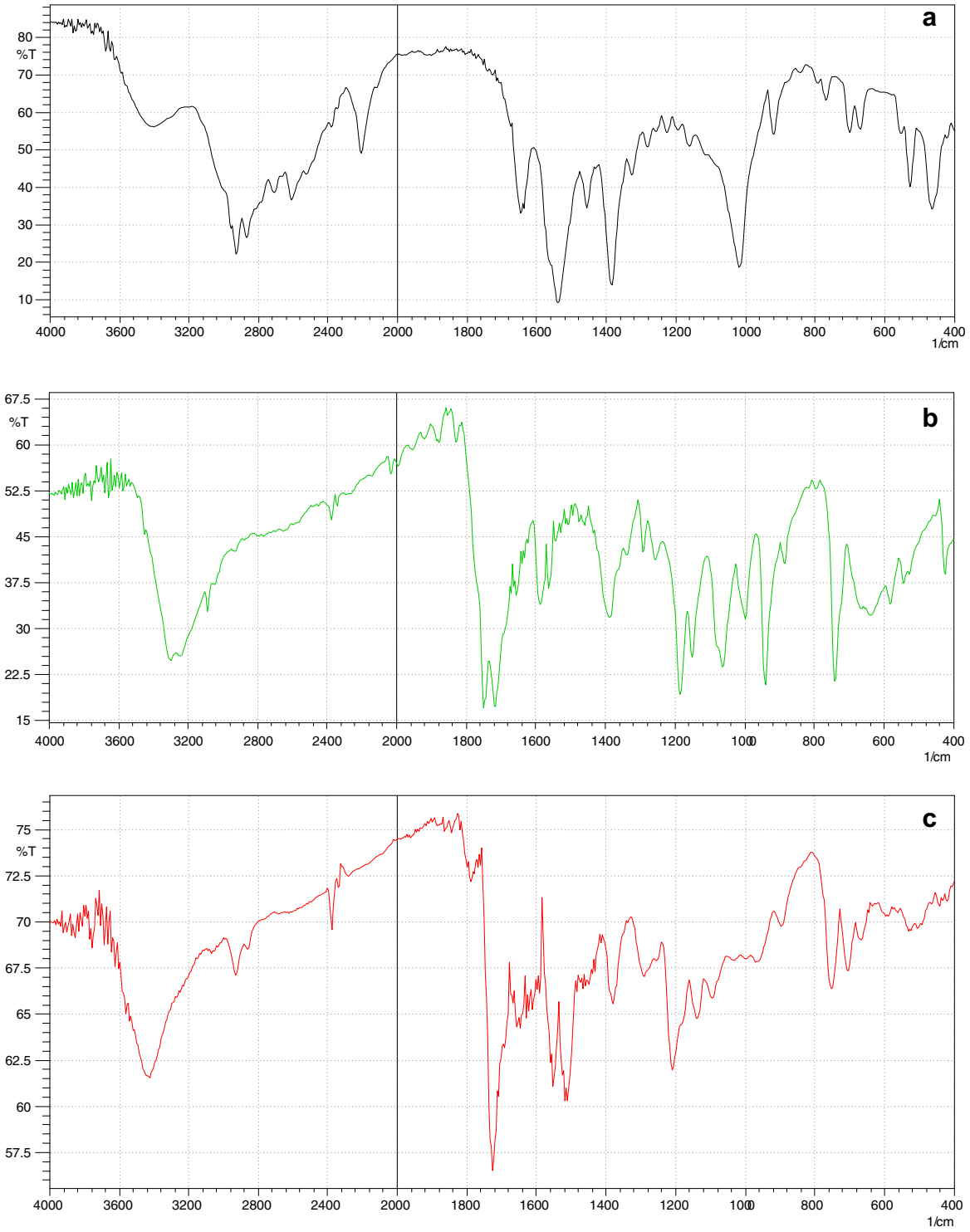


Fig. 4. Plot of $\log A_{¥}/A_{¥}-A_t$ versus time is a straight line confirming the first order reaction.



Spectra 2. (a) Representative IR spectra of tranexamic acid. (b) Representative IR spectra of ninhydrin. (c) Representative IR spectra of complex.

a first order kinetics with respect to ninhydrin concentration. The initial rate of reaction under pseudo-first order conditions would obey the following equation:

$$\text{Rate} = \mathrm{d}A/\mathrm{d}t = k\prime C^n$$

where, "$k\prime$" is the pseudo-first order rate constant, "C" is the concentration of tranexamic acid, "n" is the order of reaction. The above equation may be written in the logarithmic form as,

$$\text{Log rate} = \log k\prime + n \log C$$

The slope, intercept and correlation coefficient were evaluated by linear regression analysis of calibration data (Table 1). The regression of log rate versus log C gave a linear regression equation,

$$\text{Log rate} = 0.0004 + 0.01008 \log C$$

The value of "n" in the regression equation also indicated the first order reaction with respect to tranexamic acid concentration. The calibration curve constructed by plotting absorbances of different concentrations of tranexamic acid versus time showed a linear relationship over the concentration range of 16–37 μg mL^{-1}.

4.2.3. Rate constant method

Under pseudo-first order condition, the rate constants corresponding to different concentrations of tranexamic acid were calculated from the slopes of $\log A_{\infty}/A_{\infty}-A_t$ versus time and are summarized in Table 2. The calibration graph was constructed by plotting rate constant against the tranexamic acid concentration, which showed a linear response over the concentration range of 16–37 μg mL^{-1}. The regression values, correlation coefficient and detection limit are shown in Table 1.

4.2.4. Fixed time method

At a pre-selected fixed time, the absorbance was measured at 575 nm against reagent blank. The calibration graphs of absorbance versus initial concentration of tranexamic acid were established at fixed time of 30, 60, 90 and 120 min. Under the established working condition, regression equations were developed and important analytical parameters have been calculated (Table 3). It is evident from Table 3 that the acceptable values of correlation coefficient, intercept and slope were obtained at all fixed time therefore, any fixed time can be used for assay of tranexamic acid concentration.

The values of slopes of the regression equations of the proposed methods indicate good sensitivity. The small values of the standard deviation speak about the negligible scattering of the calibration data points around the line of regressions for all the proposed procedures. The high values of correlation coefficients obtained for regression equations exhibit good linearity of the methods. To check the precision as well as accuracy of the proposed methods, independent repeatability studies were performed with five repetitions for each method. The results are summarized in Table 4. The obtained data show that the methods are accurate as well as applicable for dosage formulation (Table 5).

5. Spectroscopic studies

5.1. Infrared spectra

Many primary amines give Ruhemann's Purple with ninhydrin due to complex formation (Fig. 3) [20]. The absorbance bands from the IR spectra (Spectra 2a, b and c) of the pure drug and complex with ninhydrin are described. In tranexamic acid the spectrum showed the NH stretching band at 2980 cm^{-1} and at 1600 cm^{-1} N–H bending vibrations. The 2850 cm^{-1} band indicated C–H stretching, band at 1650 cm^{-1} is assigned to the presence of carbonyl group. Methylene group showed characteristics absorption at 1450 cm^{-1}. The 2609 cm^{-1} band is assigned to O–H stretching, the 800 and 930 cm^{-1} frequencies showed out of plane bending of N–H and O–H bond, respectively [21]. Similarly ninhydrin which has two OH groups in the structure showed two broad bands at 3300 and 3250 cm^{-1} and also absorption band at 1061 cm^{-1} owing to 2 alcohol C–O stretching. One strong band near 750 cm^{-1} is obtained due to aromatic ring. The ketone absorbs in the region 1660–1760 cm^{-1} [21]. By comparing the spectra of the product with that of the pure reagents, the characteristic bands of pure reagents were found absent, evidently because of formation of complex. The two sharp broad bands at 3300 and 3250 cm^{-1} owing to O–H stretching of ninhydrin completely diminished in the complex spectra. C=C group absorption at 1560 cm^{-1} remained in its position.

5.2. Nuclear magnetic resonance spectra

The ^1H NMR spectra of drug and complex confirmed the above results. The spectrum of ninhydrin showed a singlet at δ 8.07 ppm for the four protons of the aromatic group and a singlet at δ 2.5 ppm for two protons of the OH group [22,23]. The spectrum of tranexamic acid showed a singlet at δ 12.0 ppm for proton of carboxylic acid and at 2.0 ppm for the protons of amine group. Two singlets occured at δ 2.75–2.73 ppm for two protons in the CH_2 near the N in CH_2N. The multiplet at δ 0.9–1.6 ppm is due to ten protons of the aliphatic rings. In the spectrum of the complex it is apparent that an effect is exerted on the NH_2 protons, which completely diminished. The broad multiplet appearing between δ 7.23 and 7.92 ppm showing four aromatic CH protons. A singlet at δ 6.80 ppm marks the OH proton. The above results confirm the UV and IR spectra, also confirming the proposed structure.

As far as the selectivity of the method is concerned, the kinetic approach based on the reaction of tranexamic acid–ninhydrin was found to be highly selective for the determination of tranexamic acid at boiling temperature.

Table 6
Precision and accuracy of the proposed method (Method B)

	Amount μg mL^{-1}	Recovery μg mL^{-1}	% Recovery
	50	98.16	49.08
	80	98.71	78.97
	100	98.54	98.54
	140	101.40	141.97
	180	99.49	179.08
	300	99.81	299.42
	400	99.79	399.16
	600	100.05	600.29
	800	99.78	798.27
Mean	99.53		
STd[a]	0.97		
Rsd[b]	0.01		

[a] Standard deviation.
[b] Relative standard deviation.

5.2.1. For method B

The reaction between tranexamic acid and ferric chloride in water resulted in the formation of yellowish orange colored complex. To optimize the reaction conditions, different parameters such as temperature, reaction time, reagent concentration and color stability have been investigated. It was observed that the reaction occurred at room temperature (25 ± 2 °C). The optimum reaction time to develop maximum color was obtained in one minute at room temperature. The effect of ferric chloride concentration on color development was investigated using 1–4 mL of 1% ferric chloride. Absorbance remained constant after addition of 3 mL of 1% ferric chloride. Hence, the later concentration was adopted as the most suitable volume for maximum absorbance. Optical characteristics and statistical data for the regression equation of the proposed method are given in Table 1.

Under optimum experimental condition, the values of slope of the regression equations of the proposed method indicate good sensitivity. The values of standard deviation and correlation coefficient obtained for regression equation exhibited good linearity of the method. To check the precision as well as accuracy of the proposed method, independent repeatability studies were performed with five repetitions (Table 6). The results of the recovery analysis are also represented in Table 6. High recovery and low standard deviation confirmed the suitability of the proposed method.

6. Conclusion

The proposed methods are simple, rapid, accurate, precise and economical for the routine analysis of tranexamic acid in pharmaceutical quality control laboratories. Method in which ferric chloride was used, was less time consuming than the method in which ninhydrin was used. However, the ninhydrin method is

selective for tranexamic acid and is suitable for the analysis of tranexamic acid in pure as well as commercial dosage forms.

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