Spectrophotometric and Spectrofluorimetric Methods for the Determination of Tranexamic Acid in Pharmaceutical Formulation

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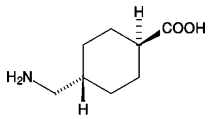
Received July 5, 2006; accepted November 15, 2006

Two simple and sensitive spectrophotometric and fluorimetric methods for the determination of tranexamic acid in tablets are developed. The methods are based on condensation the primary amino group of tranexamic acid with acetyl acetone and formaldehyde producing a yellow coloured product, which is measured spectrophotometrically at 335 nm or fluorimetrically at 480 nm the colour was stable for at least 1 h. Beer's law was valid within a concentration rang of 0.05—2.0 μg ml^{-1} spectrophotometrically and 0.05—0.25 μg ml^{-1} fluorimetrically. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipints. The validity of both methods was tested by analyzing tranexamic acid in its pharmaceutical preparations. Good recoveries were obtained and the results were comparable with those obtained by standard method.

Key words tranexamic acid; spectrophotometric; fluorimetric analysis; acetyl acetone/formaldehyde reagent; dosage form

Tranexamic acid, *trans*-4-(aminomethyl)cyclohexane carboxylic acid is Antifibrinolytic; haemostatic agent The BP[1] specifies non-aqueous titration technique detecting the end point potentiometrically in bulk tranexamic acid and using crystal violet indicator for tablets. This non-aqueous titration technique cannot provide the high degree of sensitivity required for pharmaceutical preparation analysis.



Tranexamic acid

The reported methods in the literature for the determination of tranexamic acid are capillary electrophoresis,[2] flow-injection determination[3] and HPLC.[4—8] Various colorimetric methods have reported for determination of tranexamic acid in bulk and pharmaceutical formulations using 2,4,6-trinitrobenzenesulphonic acid,[9] 7-chloro-4-nitrobenzofurazan,[10] 1-fluoro-2,4-dinitrobenzene,[11] 4-dimethylamino benzaldehyde.[12] The drug content in tablets has been determined spectrophotometrically in visible region based on the charge transfer reaction of tranexamic acid with aqueous alcoholic chloranil and subsequently measuring the absorbance at 346 nm[13] or with tetracyanoethylene (TCNE) measuring the colour at 330 nm.[14] Recently tranexamic acid was determined based on the condensation of the primary amino group with ninhydrin in the basic medium at pH 8.0 with the product of bluish purple colour measured at 565 nm.[15]

Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents new spectrophotometric methods for the determination of tranexamic acid in tablets. The methods are based on the reaction of tranexamic acid with acetyl acetone and formaldehyde producing Hantzsch product.

Experimental

Apparatus Measurements were carried out by using Shimadzu 1201 UV–visible spectrophotometer and Shimadzu RF 1501 spectrofluorophotometer.

Materials and Reagents All materials and reagents were of analytical grade and bidistilled water was used.

1) Tranexamic acid pure drug and Exacyl® 500 mg tablets (labeled to contain 500 mg tranexamic acid per tablet) were obtained from Sanofi Winthrop–France.
2) Acetylacetone (El-Nasr Chemical Co. Egypt), 8.4% v/v solution was freshly prepared by mixing 2.1 ml of acetylacetone with 10 ml of acetate buffer pH 5 and dilution to 25 ml with distilled water.
3) Formaldehyde (34—40%) (El-Nasr Chemical Co. Egypt). Twenty percent solution was prepared by mixing 5 ml of formaldehyde with distilled water to 25 ml acetate buffer pH 5.
4) Acetate buffer pH 5 (dissolve 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml).

Standard Solutions A 0.1 mg ml^{-1} aqueous solution of tranexamic acid for spectrophotometric method and 10 μg ml^{-1} solution prepared by dilution of the above solution with water for fluorimetric determination.

Construction of Calibration Curves. Spectrophotometric Method To different aliquots of standard solutions containing 0.05—0.2 mg tranexamic acid, 1 ml of 8.4% v/v acetylacetone solution and 0.3 ml of 20% formaldehyde reagents were added in a series of 10 ml test tubes. The mixture was heated for 5 min then cooled and diluted to 10 ml with distilled water. The absorbance was measured at 335 nm using the experiment as a blank.

Spectrofluorimetric Method To different aliquots of standard solutions containing 0.5—0.25 μg tranexamic acid, 0.5 ml of 8.4% v/v acetylacetone solution and 1 ml of 20% formaldehyde reagents were added in a series of 10 ml test tubes. The mixture was heated for 30 min then cooled and diluted to 10 ml with distilled water. The fluorescence intensity was measured at excitation wavelength of 415 nm and emission wavelength of 480 nm using the experiment as a blank.

Procedures for Assay of Tablets Twenty tablets of Exacyl 500 mg tablets were powdered and quantity of the powder equivalent to 100 mg of tranexamic acid was dissolved by shaking with 100 ml of water. The solution was filtered through filter paper. Further dilution was made to suit each method.

Result and Discussion

Hantzsch reaction is a known condensation reaction that was reported in the literatures as a useful pathway for pyrrole and pyridine synthesis.[16] In the same manner, acetylacetone together with formaldehyde react with aliphatic amines by Hantzsch reaction forming a yellow product that can be measured spectrophotometrically or spectrofluorimetrically. The reaction was applied for the determination of certainsulpha-drug[17] mesalamine[18] different β-lactam antibiotics,[19—22] aminoglycosides,[23,24] lisinopril[25] and more recently gaba-

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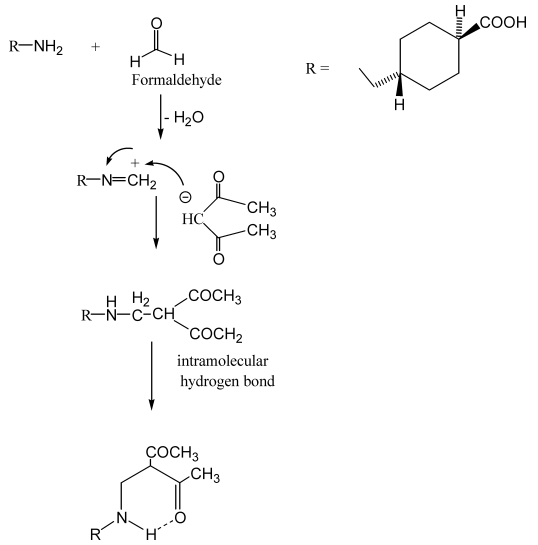

Chart 1. The Suggested Mechanism for the Hantzsch Reaction between Tranexamic Acid and Acetylacetone/Formaldehyde Reagent

pentin and cefprozil.[26)]

The proposed methods for determination of tranexamic acid (primary amine compound) was based on Hantzsch condensation reaction using acetylacetone as β-diketone and formaldehyde as an aldehyde to form a colored condensation product (Chart 1). The formed yellow color showed maximum absorption at 335 nm (Fig. 1). Moreover, the reaction product exhibited strong fluorescence at 480 nm (excitation at 415 nm), Fig. 2.

Investigation of Assay Parameters Effect of Heating Time: Heating at 100 °C for 5 min was sufficient to produce maximum color intensity, while 30 min were the optimum heating time using the spectrofluorimetric method and the produced color and fluorescence were stable for at least 1 h.

Effect of Reagent Concentration: One and 0.5 ml of 8.4% v/v acetylaceone, 0.3 and 1 ml of 20% v/v formaldehyde were the most suitable concentration using the spectrophotometric and spectrofluorimetric method, respectively.

Effect of pH: Different acetate bufferes with pH range of 3.7—5.3 were tried and pH 5 was the pH of choice for both methods.

Effect of Solvents: Different diluting solvents were used, such as water, ethanol, methanol, acetonitrile and acetone. Best color and fluorescence intensities were obtained using the first three solvents; water was used, being the most available solvent.

Stoichiometric Relationship Job's method for continuous variation using equimolar (2×10^{-2} M) solutions of tranexamic acid, acetylacetone and formaldehyde were used so that each two components were introduced in volume totaling 2 ml in presence of a constant concentration of the third one and absorbance was measured at 335 nm (Fig. 3). The ratio of tranexamic acid to acetylacetone and formaldehyde was found (1 : 1 : 1).

Quantification, Accuracy and Precision of the Proposed Methods A linear correlation was found between absorbance and concentration in the ranges given in Table 1. The correlation coefficients, intercepts and slopes for the calibration data for the cited drug are calculated using the last-squares method.

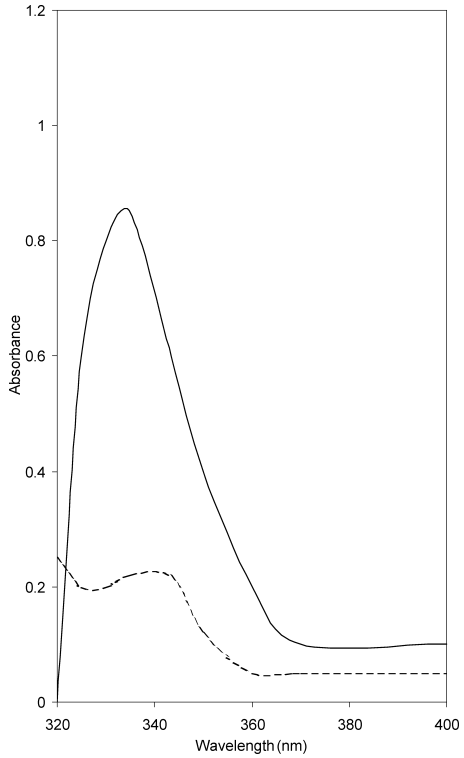

Fig. 1. Absorption Spectra of the Hantzsch Product of Acetylacetone (8.4% v/v)/Formaldehyde (20% v/v) Reagent and 1 μg ml^{-1} Tranexamic Acid

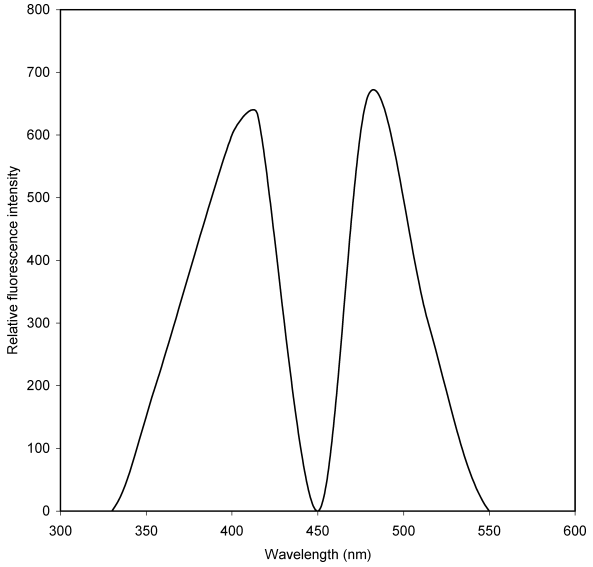

Fig. 2. Excitation and Emission Spectra of the the Hantzsch Product of Acetylacetone (8.4% v/v)/Formaldehyde (20% v/v) Reagent and 0.25 μg ml^{-1} Tranexamic Acid

The accuracy and precision of the proposed methods was established by measuring the content of tranexamic acid in pure form at three different concentration levels. The intra day precision of the proposed methods was performed by carrying out five independent analyses at each concentration level within one day Table 2. In the same manner, the inter day precision was also evaluated by measuring the cited drugs content at each concentration level on five consecutive days by the proposed methods Table 3. The results of stan-

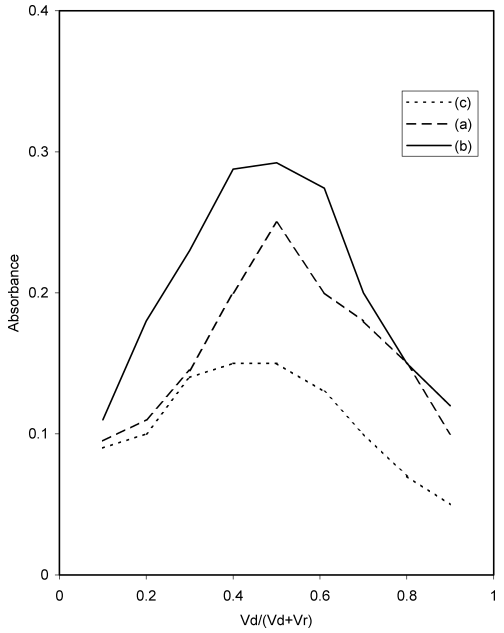

Fig. 3. Continuous Variation Plots of the Reaction between (a) 2×10^{-2} Tranexamic Acid and 2×10^{-2} M Acetylacetone (in Presence of 1 ml of Formaldehyde), (b) 2×10^{-2} M Tranexamic Acid and 2×10^{-2} M Formaldehyde (in Presence of 1 ml Acetyl Acetone) and (c) 2×10^{-2} M Acetylacetone and 2×10^{-2} M Formaldehyde (in Presence of 0.4 ml Tranexamic Acid)

dard deviation (S.D.), relative standard deviation (RSD) and recoveries by the proposed methods in Tables 2 and 3 can be considered to be very satisfactory. Thus the proposed methods are very effective for the assay of tranexamic acid in tablets.

The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of drug was estimated by the proposed methods. Each level was repeated five times. The results (Table 4) were reproducible with low S.D. and RSD. No interference from the common excipients was

Table 1. Optical Characteristics and Statistical Data of the Regression Equations for Determination of Tranexamic Acid Using the Proposed Methods

Parameters	Photometric methods	Fluorimetric method
Linearity range (μg ml^{-1})	5.00—20.00	0.05—0.25
Molar absorptivity (mol^{-1} l·cm^{-1})	7.321×10^{4}	—
Sandell's sensitivity (μg cm^{-1})	6.3×10^{-2}	—
Regression equation[a]:		
Intercept (a)	−0.0012	−16.021
Slope (b)	0.4533	2711
Correlation coefficient (r)	0.9995	0.9998

a) A=a+bC.

Table 2. Intra Day Assay: Test of Precision of the Proposed Methods for the Determination of Tranexamic Acid

Proposed methods	Concentration (μg ml^{-1})		Recovery±RSD[a] (%)	SAE[b]	CL[c]
	Theoretical	Nominal±S.D.[a]			
Photometric methods	7	7.01±0.112	100.14±1.60	0.05	0.138
	10	9.93±0.201	99.30±2.024	0.09	0.248
	15	14.90±0.223	99.33±1.50	0.10	0.275
Fluorimetric method	0.1	0.099±0.0010	99.00±1.01	0.0004	0.00123
	0.15	0.151±0.0016	100.67±1.06	0.0007	0.00197
	0.2	0.205±0.0021	102.5±1.024	0.0009	0.00259

a) Mean for five determinations. *b*) SAE, standard analytical error. *c*) CL, confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

Table 3. Inter Day Assay: Test of Precision of the Proposed Methods for the Determination of Tranexamic Acid

Proposed methods	Concentration (μg ml^{-1})		Recovery±RSD[a] (%)	SAE[b]	CL[c]
	Theoretical	Nominal±S.D.[a]			
Photometric methods	7	6.99±0.106	99.86±1.520	0.047	0.129
	10	9.94±0.119	99.40±1.197	0.053	0.146
	15	14.91±0.203	99.40±1.353	0.09	0.251
Fluorimetric method	0.1	0.099±0.0010	99.00±1.01	0.0004	0.00123
	0.15	0.149±0.0013	99.33±0.87	0.0006	0.00160
	0.2	0.198±0.0019	99.00±0.96	0.0009	0.00234

a) Mean for five determinations. *b*) SAE, standard analytical error. *c*) CL, confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

Table 4. Determination of Tranexamic Acid by Standard Addition Method

Proposed methods	Concentration (μg ml^{-1})			Recovery±RSD[a] (%)	SAE[b]	CL[c]
	Theoretical	Spiked	Nominal±S.D.[a]			
Exacyl tablets						
Photometric methods	10	10	19.96±0.053	99.8±0.266	0.024	0.065
Fluorimetric method	0.1	0.1	0.1978±0.0033	98.9±1.668	0.00147	0.00407

a) Mean for five determinations. *b*) SAE, standard analytical error. *c*) CL, confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

Table 5. Determination of Tranexamic Acid Using the Proposed Methods Compared with B.P Method [1]

	Proposed methods		Official method[a,1)]
	Photometric methods	Fluorimetric method	
Mean±S.D.	99.61±1.202	99.08±1.337	99.62±0.98
N	5	5	5
t:	0.029 (2.306)	0.622 (2.306)	
F:	1.512 (6.390)	1.782 (6.390)	

a) Titration method. Values in parentheses are the tabulated values of *t*- and *F*- at $p=0.05$.

observed. The proposed methods were compared with the official titration method.[1)] The results obtained showed that the calculated *t*- and *F*-values did not exceed the theoretical values (95% confidence limits for five degree of freedom), (Table 5) from which we can conclude that the proposed methods do not differ significantly from reference method. The proposed methods were also, applied to commercial tablets contain tranexamic acid. The results show that there is no interference from any excipients.

Conclusion

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by Spectrophotometric and spectrofluorimetric methods for the determination of tranexamic acid in its pure and dosage form demonstrate that the proposed method is accurate, precise and linear and thus can be extended for routine analysis of tranexamic acid in pharmaceutical industries, hospitals and research laboratories.

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