Spectrophotometric Methods for Determination of Tranexamic Acid and Etamsylate in Pure Form and Pharmaceutical Formulation

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ABSTRACT

Background: Tranexamic acid and etamsylate drugs belong to a group of antifibrinolytics. Tranexamic Acid (TRA) is the most potent antifibrinolytic lysine analogue used in a broad spectrum of peri- and postoperative interventions and bleeding disorders. The administration of TRA is associated with a reduction in bleeding due to its inhibitory effect on clot breakdown (fibrinolysis). Etamsylate (ESL) is a haemostatic agent that appears to maintain the stability of the capillary wall and correct abnormal platelet adhesion. It is given for the prophylaxis and control of haemorrhages from small blood vessels. **Objective:** Two simple and sensitive spectrophotometric methods are described for the determination of tranexamic acid (TRA) and etamsylate (ESL) drugs in pure form and pharmaceutical preparations. **Materials and Methods** Method (A) is used for the determination of TRA and based on condensation reaction of the primary amino group of TRA with salicylaldehyde reagent (SA) (Schiff base formation) producing a yellow coloured product which is measured spectrophotometrically at 400 nm. Method (B) is utilized for the determination of ESL and based on the oxidation-reduction reaction of ESL using iodic acid (HIO_3). The liberated iodine was extracted in chloroform and the absorbance of the red coloured product is measured spectrophotometrically at 510 nm. For method A and B, different variables affecting the reactions were studied and optimized. **Results:** Under the optimum conditions, linear relationships with good correlation coefficients 0.9989 and 0.9959 were found between the absorbance and the concentration of the drug in the range from 5 to 500 and 2.5 to 275 $\mu\text{g mL}^{-1}$ for ESL and TRA drugs, respectively. The limit of detection ($\mu\text{g mL}^{-1}$), limit of quantification ($\mu\text{g mL}^{-1}$) and molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$) values were found to be 1.442, 4.80 and 5.39×10^2 for TRA drug and 2.20, 7.25 and 3.0×10^2 for ESL drug, respectively. **Conclusion:** The method was applied for determination of the investigated drugs in tablets. The method was validated and can be suggested for routine analysis of both drugs.

Key words: Etamsylate, tranexamic acid, salicylaldehyde, Schiff base reaction, oxidation-reduction reaction, iodic acid

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INTRODUCTION

Tranexamic acid and etamsylate drugs belong to a group of antifibrinolytics. Tranexamic acid [trans-4-(aminomethyl) cyclohexane carboxylic acid] (TRA) is the most potent antifibrinolytic lysine analogue (Fig. 1a) used in a broad spectrum of peri- and postoperative interventions and bleeding disorders^{1,2}. The administration of TRA is associated with a reduction in bleeding due to its inhibitory effect on clot breakdown (fibrinolysis). Etamsylate (ESL), chemically known as 2,5-dihydroxybenzene sulphonic acid with ethylene diamine. Etamsylate (Fig. 1b) is a haemostatic agent that appears to maintain the stability of the capillary wall and

correct abnormal platelet adhesion. It is given for the prophylaxis and control of haemorrhages from small blood vessels³.

Fig. 1(a-b): Structures of (a) Tranexamic acid and (b) Etamsylate drugs

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Several methods have been reported for the determination of ESL drug in pharmaceutical dosage forms and in biological fluids including high-performance liquid chromatography⁴⁻⁶, high-performance thin layer chromatography^{7,8}, flow injection potentiometry⁹, chemiluminescence methods¹⁰⁻¹², UV spectrophotometry^{13,14} and titrimetric method¹⁵. The methods of determination of TRA drug in pharmaceutical dosage forms and in biological fluids are high-performance Liquid Chromatography (LC)¹⁶⁻¹⁸, high-performance liquid chromatography-fluorescence method¹⁹, spectrophotometric methods²⁰⁻²² and flow-injection methods²³.

This study describes the oxidation-reduction and condensation reactions for the spectrophotometric determination of ESL and TRA drugs, respectively. Different experimental conditions were optimized and Beer's law is carried out. The methods are successfully applied for the determination of ESL and TRA drugs in tablets.

MATERIALS AND METHODS

Reagents: All reagents and chemicals used were of analytical grade and all solutions were freshly prepared daily. The TRA and ESL drugs were supplied from the National Organization for Drug Control and Research, Egypt. Salicylaldehyde (SA) and iodic acid (IO_3) reagents were supplied from Riedel-deHaen and B.D.H, respectively. Absolute ethanol (supplied from Adwic) and all solvents used throughout this study were of analytical grade including methylene chloride, chloroform and petroleum ether (supplied from El-Nasr Company).

Procedure for stock solution preparation: Standard solutions of ESL drug ($3.79 \times 10^{-3} \text{ mol L}^{-1}$) were prepared by dissolving an accurately weighed quantity of drug in a definite volume of methanol. Standard solutions of TRA drug ($6.4 \times 10^{-3} \text{ mol L}^{-1}$) were prepared by dissolving an accurately weighed quantity of it in bidistilled water and completed by absolute ethanol in the percent 1:2.33 (H_2O /absolute ethanol) ratio. About 10 mg mL^{-1} of salicylaldehyde reagent was prepared in absolute ethanol and 20 mg mL^{-1} of iodic acid (HIO_3) was prepared in bidistilled water. Tablets containing etamsylate (Haemostop, 250 mg/tablet) and tranexamic acid (Kapron, 500 mg/tablet) were supplied from Amoun Pharmaceutical Company, Egypt.

Apparatus: All absorbance measurements were carried out using the manual Unico 1200 (United Products and

Instruments, Inc., Germany) in the wavelength range from 325-1000 nm. Small volumes were taken using micropipette.

General procedure

Batch measurements

Method A: In calibrated 5 mL volumetric flask, different aliquots containing 2.50-275.0 $\mu\text{g mL}^{-1}$ of TRA was added to 1 mL of 1% (w/v) salicylaldehyde solution (SA). The volume was completed to the mark with absolute ethanol. The complete colour development was attained after 45 min. Then, the absorption spectra of the resulted Schiff base product was measured at $\lambda = 400 \text{ nm}$ against SA reagent blank.

Method B: Aliquots containing ESL drug ($5\text{-}500 \mu\text{g mL}^{-1}$) was added to 1 mL of 2% (w/v) HIO_3 and the mixture was completed to 10 mL by bidistilled water. After 30 min, 5 mL chloroform was added and shaken well for 1 min. Complete extraction of the liberated iodine was done and the chloroform layer was separated and collected in 10 mL measuring flask. The absorbance of the colored product was measured at $\lambda = 510 \text{ nm}$ against chloroform as a blank.

In both spectrophotometric methods (A and B), a calibration curve was prepared by plotting the absorbance values, increasing absorbance values versus concentration of TRA and ESL drugs ($\mu\text{g mL}^{-1}$). The concentration of TRA and ESL drugs in drug formulations was calculated from the equations of the calibration graph.

RESULTS AND DISCUSSION

Spectral characteristics: Figure 2 shows the absorption spectra of TRA and ESL. The TRA drug condenses with salicylaldehyde giving a yellow Schiff base product of stable yellow colour exhibiting maximum wavelength at 400 nm and the reaction proceeds in ethanol (Fig. 2a). TRA drug contains a primary amine which reacts with an active carbonyl group in salicylaldehyde forming Schiff bases, compounds containing an azomethine group ($-\text{RCH} = \text{N}-$). The proposed structure is shown in Fig. 3.

The ESL drug reacts with HIO_3 , an oxidation-reduction reaction occurs with the liberation of iodine. The liberated iodine is extracted in chloroform and the absorbance was measured in the wavelength = 510 nm (Fig. 2b). The mechanism of oxidation of ESL drug is given in Fig. 4.

Fig. 2(a-b): Absorption spectra of (a) TRA drug condenses with salicylaldehyde in ethanol (b) ESL oxidation-product resulted from its reaction with IO_3^- in ethanol

Fig. 3: Reaction of tranexamic acid with salicylaldehyde forming Schiff base

Fig. 4: Mechanism of oxidation reduction reaction between periodic acid and ESL drug

Optimization of reaction conditions

Effect of reagent concentration: The ESL and TRA drugs under investigation ($100 \mu\text{g mL}^{-1}$) are allowed to react with varying volumes of HIO_3 and salicylaldehyde, respectively. The maximum absorbance is obtained with 0.3 and 0.4 mg mL^{-1} of HIO_3 and salicylaldehyde, respectively. Higher concentrations of the reagents have no effect that may be useful for rapidly reaching equilibrium, thus minimizing the time required for attaining maximum absorbance at the corresponding wavelengths.

Effect of solvents: The reaction of ESL drug under investigation with HIO_3 reagent is made in different solvents. Chloroform shows high absorbance over petroleum ether and methylene chloride (Fig. 5), hence, it was chosen as an extraction solvent for the subsequent steps.

Effect of reaction time and temperature: For TRA drug, the effect of temperature ($0-90^\circ\text{C}$) and time on the condensation reaction is optimized. The results obtained indicate that, complete colour development is attained immediately at 40°C after 45 min (Fig. 6). The absorbance of the reaction products is increased with the increase of temperature up to 40°C .

For ESL drug, the effect of temperature ($0-60^\circ\text{C}$) and time on the reaction is optimized. The results obtained indicate that, complete colour development is

Fig. 5: Effect of solvents in the reaction of ESL oxidation-product resulted from its reaction with IO_3^- in chloroform

Fig. 6(a-b): Effect of (a) Time and (b) Temperature in the reaction between TRA drug and SA reagent in ethanol

attained immediately at 25°C and 30 min time interval (Fig. 7). The absorbance of the reaction products is increased with the increase of temperature up to 30°C. Higher temperatures are avoided in quantitative

Fig. 7(a-b): Effect of (a) Time and (b) Temperature in the reaction between ESL and IO_3^- in chloroform

determination of the drug under investigation in their reaction with HIO_3 due to the loss of iodine at high temperature.

Validity of Beer's law: Under the optimum conditions described above, the calibration graphs are constructed for the investigated TRA and ESL drugs applying SA and HIO_3 reagents, respectively. The molar absorptivities, standard deviations, concentration ranges, regression equations, limits of detection and quantification for each drug using specified reagent is tabulated in Table 1. Beer's law is valid over the concentration range from 5-500 and 2.5 to 275 $\mu\text{g mL}^{-1}$ of ESL and TRA drugs using HIO_3 and SA reagents, respectively.

Between-day determination of ESL and TRA drugs: The between day availability of the proposed method is given in Table 2 and shows the values of the between-day relative standard deviations for different concentrations of the drugs, obtained from experiments carried out over a period of four days. It gives a SD of 0.014-0.08 and 0.03-0.09 for ESL and TRA drugs,

respectively and RSD% of 2.83-4.97 and 0.93-3.79 for ESL and TRA drugs, respectively, referring to the high accuracy and precision of the applied procedures.

Spectrophotometric determination of ESL and TRA drugs in pharmaceutical preparations: The results obtained are given in Table 3. The data show that, the determined concentrations of the ESL and TRA drugs by the proposed methods are closed to that calculated from the official titrimetric methods. In order to check the confidence and correlation between the

suggested spectrophotometric procedures and the official method for determination of ESL and TRA drugs, the percent recovery for all the results are calculated. The percentage recovery values obtained by the proposed methods ($\mu\text{g mL}^{-1}$) are higher than or close to those obtained by the official titrimetric methods (mg mL^{-1}). In addition, the standard deviation values obtained by the proposed methods are close to those obtained by the official titrimetric methods. The small values of standard deviation and relative standard deviation indicate the reliability, accuracy and precision of the suggested procedures.

Table 1: Spectral characteristics of reaction between ESL and TRA drugs using HIO_3 and SA reagents, respectively and the analytical statistics (accuracy and precision) of these reactions

Parameters	Drug	
	ESL	TRA
λ_{max} (nm)	510	400
Drug ($\mu\text{g mL}^{-1}$)	5-500	2.5-275
ϵ ($\text{L mol}^{-1} \text{cm}^{-1}$)	3.0×10^3	5.39×10^2
S ($\mu\text{g cm}^{-2}$)	0.0126	0.0118
A = mC + z		
m	0.0012	0.0072
z	0.0101	0.0277
r^2	0.9959	0.9989
Recovery (%)	98.70-104	99.1-102.0
LOD ($\mu\text{g mL}^{-1}$)	2.20	1.442
LOQ ($\mu\text{g mL}^{-1}$)	7.25	4.80
SD	0.005-0.042	0.03-0.082
RSD (%)	1.17-2.64	0.93-2.40

*A = $z + mC$; where, C is the concentration in $\mu\text{g mL}^{-1}$, RSD: Relative standard deviation, SD: Standard deviation

Table 2: Between-day precision of the determination of ESL and TRA drugs using HIO_3 and SA reagents

Drug	Drug taken ($\mu\text{g mL}^{-1}$)	Drug found ($\mu\text{g mL}^{-1}$)	Percentage recovery (%)	SD*	RSD%*
ESL	20	19.85	99.25	0.06	1.97
	50	52.00	104.00	0.014	4.97
	200	202.00	101.00	0.05	3.61
	400	395.00	98.75	0.08	2.83
TRA	10	9.91	99.10	0.03	3.76
	50	51.00	102.00	0.05	3.79
	150	150.20	100.10	0.07	0.93
	250	249.80	99.92	0.09	1.23

*Average of four replicates

Method validation

Linearity: Under optimum experimental conditions for determination of TRA and ESL drugs under investigation, the absorbance versus concentration plots were found to be linear over the concentration ranges stated in Table 1. The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope (b) and the intercept (a) are presented in Table 1. The linearity of the calibration graphs was demonstrated by the high values of the correlation coefficient (r) and the small values of the intercepts of the regression equations²⁴. The molar absorptivity and Sandell sensitivity are also shown in Table 1.

Accuracy and precision: In order to determine the precision of the proposed method, the results of the assay of the studied drug in pharmaceutical preparation were compared with the reference method. Data shown in Table 3 showed no significant differences between them regarding accuracy and precision. Intra- and inter-day precisions were assessed using four concentrations of TRA and ESL were prepared and analyzed in four replicates and the analytical results are summarized in Table 2. The low values of the relative standard deviation (%RSD) indicate the high precision and the good accuracy of the proposed methods²⁴⁻²⁶. RSD (%) and SD values were obtained within the same

Table 3: Spectrophotometric determination of ESL and TRA drugs in different pharmaceutical preparations using HIO_3 and SA reagents and official method

Drug and dose	Drug found		Recovery (%)			
	Proposed method ($\mu\text{g mL}^{-1}$)	Official method (mg mL^{-1})	Proposed method	Official method	SD*	SD**
ESL						
100	101.0	99.0	101.00	99.00	0.06	0.05
200	199.3	198.0	99.65	99.00	0.04	0.06
TRA						
100	99.0	102.0	99.00	102.02	0.04	0.05
150	149.0	149.5	99.30	99.60	0.03	0.08

*Standard deviation (SD) values using proposed method, **Standard deviation (SD) values using official method

day to evaluate repeatability (intra-day precision) and over four days to evaluate intermediate precision (inter-day precision)²⁴⁻²⁶.

Limits Of Detection (LOD) and quantitation (LOQ): Sensitivity of the method can be determined, through the Limit of Detection (LOD) and Limit Of Quantification (LOQ). The LOD for the proposed method was calculated using the following equation²⁷:

$$\text{LOD} = 3 \times \sigma / S$$

where, σ is the standard deviation of replicate determination values and S is the slope of the calibration graph.

The LOQ defined as²⁷:

$$\text{LOQ} = 10 \times \sigma / S$$

Based on the above equations, the limits of detection and quantification were calculated and recorded in Table 1.

Quantification: Under the specified reaction conditions, the molar absorptivity at λ_{max} was found to be a function of concentration of the investigated drug. Beer's law plots were linear with small intercept (0.0101-0.0277) and slopes (0.0012-0.0072) values in the concentration ranges were presented in Table 1. The correlation coefficient, intercepts and regression equation for the proposed procedures were listed in Table 1²⁸. The apparent molar absorptivities found to be in the order of 3.0×10^2 and $5.39 \times 10^2 \text{ L mol}^{-1} \text{ cm}^{-1}$ with the Sandell sensitivities of 0.0126 and $0.0118 \mu\text{g cm}^{-2}$ for ESL and TRA drugs, respectively, as calculated from Beer's law (Table 1). The correlation coefficients of the data obtained are 0.9959 and 0.9989 for ESL and TRA drugs, respectively. Beer's law is obeyed over the concentration ranges from 5-500 and 2.5-275 $\mu\text{g mL}^{-1}$ for ESL and TRA drugs, respectively.

CONCLUSION

This study demonstrated that HIO_3 and SA can be utilized as useful reagents for the spectrophotometric determination of ESL and TRA drugs, respectively, under investigation. Rapid formation of stable colour products is advantage of the suggested methods over the previously official titrimetric method. The proposed spectrophotometric methods are simpler, time saving and they involve very simple procedures, that can be applied in routine analysis.

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