Spectrophotometric microdetermination of tranexamic acid in pharmaceutical formulation

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Background and objectives
Tranexamic acid is used to treat various conditions in which there is bleeding or risk of bleeding, such as prostatectomy, dental extraction, menorrhagia, and thrombolytic overdose. Most of the currently available analytical techniques for Tranexamic acid measurement use high-performance liquid chromatography (HPLC) separation or the spectrophotometric method of detection. The aim of the present study was to describe the determination of Tranexamic acid as an active pharmaceutical ingredient in tablets using three accurate and sensitive spectrophotometric methods: the ion–air complex method (method A), the bromination complex method (method B), and the charge transfer complex method (method C).

Materials and methods
The first method (A) is based on the formation of an ion–pair complex between the basic nitrogen of Tranexamic acid and alizarin red S as an anionic acid dye. The absorbance of the formed complex was measured at λ_{max} equal to 300 nm. The second method (B) is based on the oxidation of Tramexamic acid by *N*-bromosuccinimide (NBS) and determination of the nonreacted NBS by measurement of the decrease in absorbance of liberated iodine at λ_{max} equal to 520 nm. The third method (C) is based on forming a charge transfer complex with chloranil in absolute ethanol at alkaline pH. The absorbance of the formed charge transfer complex was measured at λ_{max} equal to 330 nm. Linear calibration curves were obtained in the ranges of 2.00–26.00, 2.00–25.00, and 2.00–27.00 μg/ml. The methods showed relative standard deviations of 0.839, 0.952, and 0.984 for methods A, B and C, respectively.

Results and conclusion
The results showed the suitability, safety, accuracy, and simplicity of these methods for determination of Tranexamic acid as an active pharmaceutical ingredient. The results obtained by the three methods, A, B, and C, is in good agreement with those obtained by the official method. The developed methods were successfully applied to the determination of Tranexamic acid in pharmaceutical preparation (tablets).

Keywords:
alizarin red S, chloranil, *N*-bromosuccinimide, spectrophotometer, Tranxamic acid

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Introduction

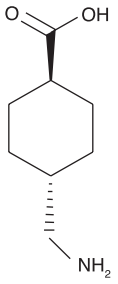
Tranexamic acid (TA) is a trans-4-aminomethyl-cyclohexane carboxylic acid (Fig. 1). It is a synthetic analog of the amino acid lysine. TA is an antifibrinolytic agent that poses difficulties in the achievement and maintenance of a therapeutic concentration [1–3]. It is a hydrophilic drug used as a potent hemostatic agent to control unwanted bleeding and reduce the amount of blood loss. TA shows high affinity for lysine binding sites of plasminogen and plasmin [3]. It acts as a competitive inhibitor of plasminogen activation, and at higher concentration it is a noncompetitive inhibitor of plasmin [4]. TA is a proved in British and European Pharmacopoeia [1–5]. Therefore, several methods have been reported for the determination of TA in pharmaceutical formulations: these methods include UV spectrophotometry [6–14], colorimetry [15,16], fluorimetry [17], high-performance liquid chromatography [18,19], LC-mass spectrometry [20], and atomic absorption spectrometry [21].

A dye reagent had been utilized for the spectrophotometric determination of TA in pharmaceutical preparations [22–25]. The method of using alizarin red S (ARS) (9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid monoso-dium salt) is based on the formation of a colored ion-association complex between the basic nitrogen of the drug and an anionic acid dye at a selected acidic pH [26,27].

N-bromosuccinimide (1-bromo-2,5-pyrolidinedione; NBS) is a brominating and oxidizing agent that is used as a source of bromine in radical reactions (e.g. allylic bromination) and various electrophilic additions [28–30].

P-chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone) is a molecular charge transfer (CT) complex. Chloranil is a quinonoid substituted in four positions by electron withdrawing chlorine atoms. Therefore, it acts as a good acceptor especially for weak donor secondary and

Figure 1



Structure of tranexamic acid.

tertiary nitrogen atoms such as aryl amines, amino acids, phenols, naphthalene, and many pharmaceuticals having low electron-donating ability. Chloranil CT complexes were reported to be useful for the identification and determination of several drug substances [14,23,31,32].

The aim of the present study was to investigate three simple, accurate, and sensitive spectrophotometric methods (A, B, and C) for the determination of TA as a pharmaceutical ingredient and its tablet formulation using ARS, NBS and chloranil.

Experiment

Materials and methods

Authentic TA [trans-4-(aminomethyl)cyclohexanecarboxylic acid] $C_8H_{15}NO_2$ (molecular weight 157.21 g/mol sample) was supplied by Amoun Company (El-Obour city, Cairo, Egypt). The contents of TA were assigned a purity of 97.82% (Fig. 1).

Apparatus

A Shimadzu self-recording double-beam computerized spectrophotometer UV-visible 1601 pc (Koyoto, Japan) with 10 mm quartz cells was used (pH meter, model 691 Metrohm).

Pharmaceutical preparation

The drug Kapron was purchased from Amoun Company. Ten 500 mg Kapron tablets were weighed and ground to a fine powder. An amount of powder containing 500 mg of TA was mixed with 50 ml of doubly distilled water (DW). The mixture was stirred well with a magnetic stirrer and filtered into a 100 ml volumetric flask and analyzed according to the official method, with purity of 95.84% [33].

Standard solutions

A standard stock solution of TA (3×10^{-3} mol/l) was prepared by dissolving 47.16 mg in 50 ml of DW in a 100 ml volumetric flask. The contents of the flask were shaken for 10 min and the level was brought up to the mark with DW. The solution was left at room temperature and then used for the determination of Tranexamic acid according to the procedure mentioned.

A standard solution of TA (1×10^{-3} mol/l) was prepared by dissolving 7.86 mg in the least amount of absolute ethanol (5 ml) and then transferring it into a 50 ml volumetric flask. The solution was brought up to the mark with absolute ethanol. The contents of the flask were shaken for 10 min. The solution was left to stand for a few minutes at room temperature according to the procedure used.

Reagents

ARS [9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid monosodium salt; $C_{14}H_7NaO_7S$; molecular weight (342.25)] was from Fluka (Gallen, Switzerland).

The stock solution of ARS (3×10^{-3} mol/l) was prepared by dissolving 102.7 mg of ARS in 50 ml DW and the level brought up to 100 ml with distilled water.

NBS [2,5-pyrolidinedione-1-bromo; Succinibromimide; NBS 99%, $C_4H_4BrNO_2$, molecular weight (177.99)] was from LOBA Chemie (Colaba, Mumbai, India).

The stock solution of NBS (3×10^{-3} mol/l) was freshly prepared by accurately weighing and dissolving 26.6 mg of NBS in 25 ml of warmed DW and bringing the level up to mark with distilled water in a 50 ml volumetric flask.

P-chloranil [tetrachloro-1,4-benzoquinone; 2,3,4,5-tetrachloro-2,5-cyclohexadiene-1,4-dione; 2,3,5,6-tetrachloro-p-benzoquinone; tetrachloro-p-quinone; $C_6Cl_4O_2$, molecular weight (245.88)] was from LOBA Chemie.

The stock solution of chloranil (1×10^{-3} mol/l) was freshly prepared each time by dissolving 12.3 mg of chloranil in 1 ml of absolute ethanol and then transferring into a 50 ml measuring flask. The volume was brought up to mark with absolute ethanol.

Acetic acid and hydrochloric acid, sodium hydroxide free carbonate, ethyl alcohol, and sodium acetate trihydrate were in analytical grade and purchased from Sigma Aldrich (Louis, MO, US). Potassium iodide (KI) was purchased from E. Merck (Hohenbrunn, Germany).

Preparation of acetate buffer solution

Acetate buffer, pH 4.5 (500 ml), of 100 mmol/l was prepared by dissolving 2.93 g sodium acetate trihydrate

in 400 ml of DI water in a 600 ml beaker. To this was added 1.62 ml of glacial acetic acid and mixed well. The pH was adjusted to 4.5 ± 0.1 with glacial acetic acid or 100 mmol/l acetic acid, and the level was brought up to mark in a 500 ml volumetric flask.

General procedures

Ion–pair complex method (method A)

In a calibrated 10 ml volumetric flask, 1 ml (3×10^{-3} mol/l) of standard solution, 5 ml of acetate buffer solution at pH 4.5 and 1 ml (3×10^{-3} mol/l) of ARS solution were mixed well. The volume was brought up to mark with DW. The absorption spectra of the complex product were scanned in the wavelength range of λ equal to 200–800 nm at 30–40 min, and then the suitable wavelength was selected against a reagent blank prepared in the same way without addition of the examined drug. The absorbance was measured at 300 nm (Table 1).

Bromonation complex method (method B)

In a calibrated 10 ml volumetric flask, 2 ml (3×10^{-3} mol/l) of standard solution was added to 5 ml (3×10^{-3} mol/l) of NBS reagent. The solution was left undisturbed for 10 min at room temperature, and then brought up to mark with 10% KI solution. After 10 min, the absorption spectrum of the complex product was scanned in the wavelength range of λ equal to 200–800 nm at 30–40 min. The absorbance was measured at 520 nm (Table 1).

Charge transfer complex method (method C)

Standard solution (1×10^{-3} mol/l) of 1.0 ml in absolute ethanol was taken in a calibrated 10 ml volumetric flask to which was added 2.0 ml of chloranil reagent (1×10^{-3} mol/l). Thereafter, 5.0 ml of acetate buffer at pH 8 was added and the volume was brought to mark with absolute ethanol as a solvent. The resulting solution was poured into a test tube and warmed at 60°C for 30 min in a water bath. A spectrum of the complex product was scanned at the wavelength range of λ equal to 200–800 nm at 30–40 min. The maximum wavelength was measured at 330 nm (Table 1).

Table 1 Quantitative parameters of the calibration graph for the developed methods A, B and C

Parameters	Methods		
	A	B	C
λ_{max} (nm)	300	520	330
Bee's law (μg/ml)	2.0–26.0	2.0–25.0	2.0–27.0
Molar absorptivity (ml/mol/cm)	0.665	0.961	0.400
Detection limits (μg/ml)	0.22	0.35	0.55
Regression equation (*A*)	*A = a + bC*	*A = a + bC*	*A = a + bC*
Intercept (*a*)	–0.0815	2.21	0.0588
Slope (*b*)	0.0055	–0.0118	0.0022
SD	0.839	0.952	0.984
Correlation coefficient (*r*)	0.999	0.956	0.987

With respect to *A = a* + bC; where *A* is the absorbance unit and *C* is the concentration (μg/ml). Confidence internal of the intercept at 95% confidence level.

Calibration graphs

Method A

In a 10 ml calibrated flask containing 1.0–31.6 mg/ml TA solution (equivalent to 0.2×10^{-4} to 0.6×10^{-5} mol/l), 5.0 ml of acetate buffer at pH 4.5 and 1.0 ml of ARS (3×10^{-3} mol/l) were added in typical sequence and mixed well. The volume was brought up to 10 ml with DW. The absorbance of the solutions was measured at 300 nm against a blank solution, which had been treated similarly without the drug. A calibration graph was prepared by plotting the absorbance of the complex formed against the drug concentration (Fig. 2a).

Method B

In a 10 ml volumetric flask, different concentrations of standard solution (1×10^{-3} mol/l) were added to 5.0 ml of 1×10^{-3} mol/l NBS, mixed well, and allowed to stand for 10 min at room temperature. The volumes were brought to mark with 10% KI solution, which was added in the typical sequence. After 5 min, absorbance of the solutions was measured at 520 nm against DW as a blank. A calibration graph was prepared by plotting the decrease of absorbance of the iodine liberated against the drug concentration (Fig. 2b).

Method C

In a 10 ml volumetric flask, 2.0 ml of 1×10^{-3} mol/l chloranil solution was added to different concentrations of standard solution (0.4 mg–0.77 mg) (equivalent to 4.4×10^{-5} to 7.7×10^{-5} mol/l) and mixed well. To this was added 5.0 ml of acetate buffer at pH 6 in typical sequence and the volume was brought to 10 ml with absolute ethanol. The absorbance of the solutions was measured at 330 nm against a blank solution, which had been treated similarly without the drug. A calibration graph was prepared by plotting the absorbance of the complex formed against the Tranexamic acid concentration (Fig. 2c).

Results and discussion

Ion–pair complex method (method A)

Absorption spectra of the complex formed between ARS and TA are shown in Fig. 3a. The band at 300.0 nm was attributed to the formation of the ion–pair complex. These bands are shifted by ~28.0 and 120 nm from the TA, and ARS bands occur at

270.0 and 420.0 nm, respectively. These differences were quite enough to be relied upon for determining TA in the pharmaceutical preparation (Table 1).

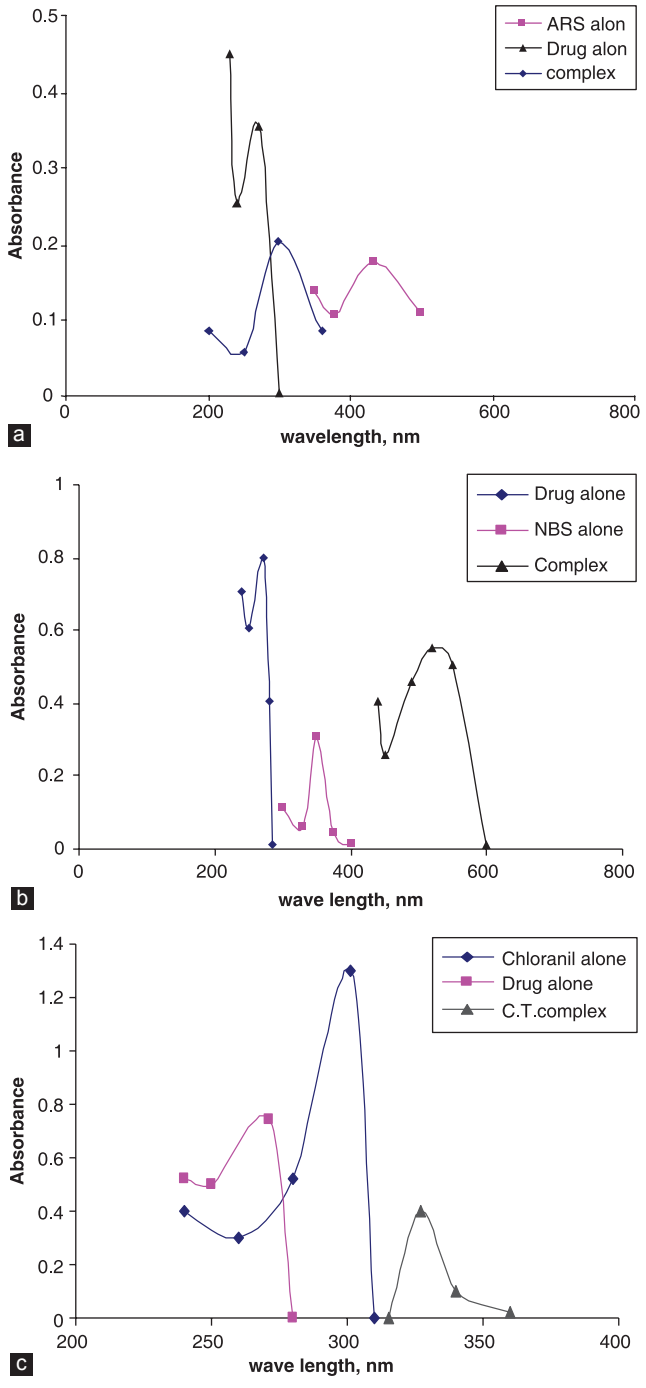
Effect of sequence of mixing

The most favorable sequence was reagent–drug–buffer acetate for the production of highest color intensity and the shortest time for developing maximum absorbance, whereas the other sequence required longer time and produced a lower absorbance value.

Effect of pH

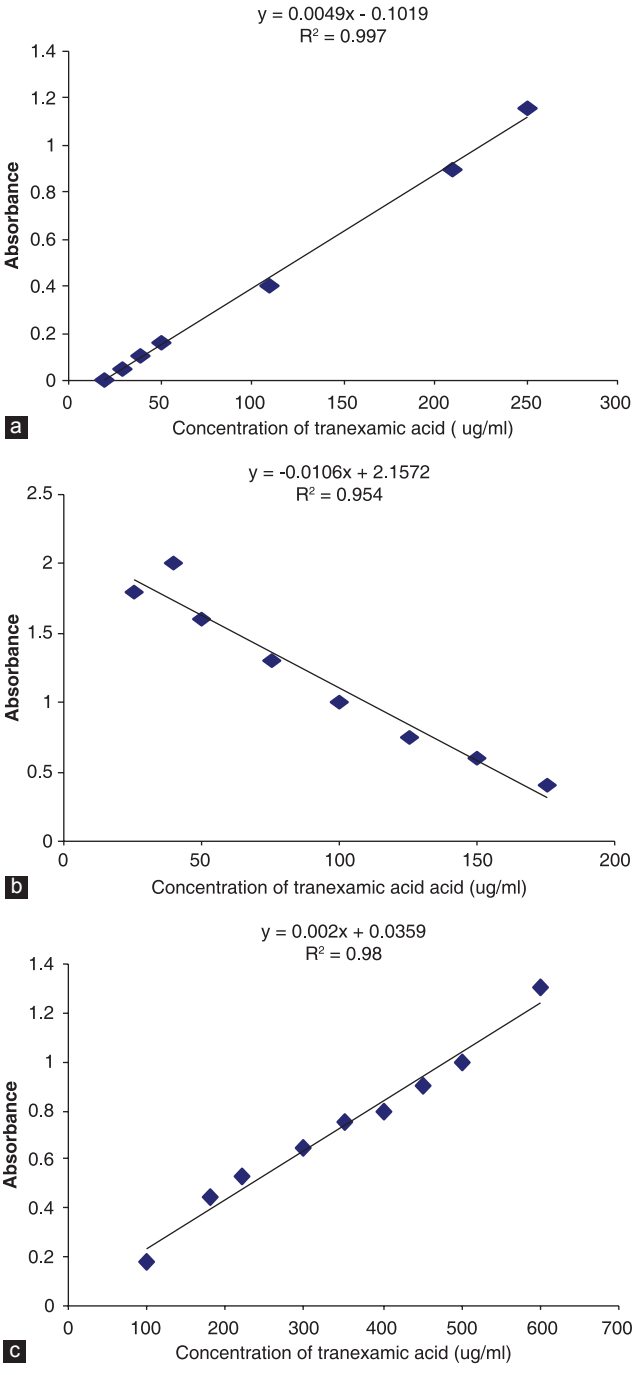
Buffer acetate proved to be the most favorable one, with the highest absorbance values. pH adjustment was necessary, especially in acidic medium, because the

Figure 2



(a) The absorption spectra of the ion–pair complex between Tranexamic acid and alizarin red S (ARS). (b) The absorption spectra for the reaction product between Tranexamic acid and *N*-bromosuccinimide. (c) The absorption spectra of the charge transfer complex formed between Tranexamic acid and chloranil.

Figure 3



(a) The linearity of absorbance to concentration of Tranexamic acid and alizarin. (b) The linearity of absorbance to concentration of Tranexamic acid and *N*-bromosuccinimide. (c) The linearity of absorbance to concentration of Tranexamic acid and chloranil.

reaction was affected by change in pH in the range of 3.0–8.0 and the optimum pH value was 4.5.

Effect of time

The reaction took place instantaneously with shaking to give reliable results. Significant absorbance was seen to gradually increase and remained constant up to 30–40 min, and then the complex started to decompose.

Effect of temperature

The optimum temperature was 25°C; on increasing the temperature a sound decrease in absorbance was observed.

Molecular ratio of the formed complex

The molecular ratio of the complex formed between TA and ARS at the predetermined conditions was investigated by applying the molar ratio and continuous variation methods [34–37]. The obtained results indicated that the molecular ratio of the reagent to Tranexamic acid was 1 : 1 for the ion–pair complex under investigation.

Suggested mechanism

The formed ion–pair complex between the anion parts was produced from ionization of acid dye containing the phenolic OH group, and a basic nitrogen of the drug. Scheme 1 represents the mechanism for the formation of the ion–pair complex [34].

Bromonation complex method (method B)

Absorption spectra of the complex formed between NBS and TA are shown in Fig. 3b. The band at 520 nm was attributed to the formation of the complex. These absorption bands were shifted by ~250.0 and 170.0 nm from the TA, and NBS absorption bands occurred at 270.0 and 350.0 nm, respectively. These differences were adequate to be relied upon for determining TA in the pharmaceutical preparation. [35] The effects of essential parameters are described in Table 1.

Effect of reagent concentration

The effect of varying the amount of NBS on the bromination of 2 ml of 0.5 mg/ml of TA was determined. The most pronounced effect was obtained by adding 5 ml of 0.18 mg/ml of NBS solution. The obtained results indicated that the absorbance was small upon using a reagent volume of 2.0–4.5 ml, whereas 5.0 ml gave the maximum absorbance. At greater than 5.0 ml the absorbance remained constant. For this reason 5.0 ml of 0.18 mg/ml NBS was chosen.

Effect of time

The time required for complete bromination reaction of TA with NBS was investigated at 520 nm. By allowing the reactants to stand for different time intervals, it was observed that the reaction takes place completely in the presence of 10% KI. After 10 min the highest absorbance was obtained, which then started to decrease. This was due to the decrease in the amount of liberated iodine [35]. Method B was based on the reaction of Tranexamic acid with NBS. The excess NBS was reacted with KI, and iodine was determined at a wavelength of 520 nm.

Effect of temperature

The effect of temperature was studied for Tranexamic acid and the blank at different temperatures (25–40°C). It was clear that room temperature (25°C ± 4) was the optimum one.

Sequence of addition

Drug–oxidant–KI is the optimum sequence of addition; other sequences gave lower absorbance values under the same experimental conditions.

Suggested mechanism

These methods involve two stages: (a) bromination of the TA drug by NBS and (b) estimation of unconsumed NBS with KI. It was clear that the bromination reaction takes place at a molecular ratio of 1 : 1 Scheme 2.

Charge transfer complex method (method C)

The absorption band of the formed CT complex was measured at λ_{max} 330 nm (Fig. 3c). These absorption bands were shifted by ~50.0 and 26.0 nm from both TA and chloranil bands, which occurred at 275 and 300 nm, respectively. These differences were sufficient for determining TA in the pharmaceutical preparation; the effects of essential parameters are described in Table 1.

Sequence of addition

Different sequences of addition were tested and the sequence drug–chloranil–buffer acetate was found to give the maximum absorption at λ_{max} of 330 nm for the determination of TA.

Effect of pH on the ion–pair complex formation

The effect of pH of the aqueous phase was studied by extracting the colored complex in the presence of either hydrochloric acid or acidic buffers of pH 3.0–4.5. Also, the formed ion–pair complex was found to be pH independent as no remarkable changes were observed while using different concentrations of HCl, such as

0.1, 0.3, and 0.5 mol/l. Further, 5.0 ml of 0.1 mol/l HCl gave reproducible results, and it was fixed throughout the study.

Effect of time

The reaction took place instantaneously with shaking to give reliable results. Significant absorbance was immediately obtained and remained constant up to 24 h. This means that the CT complex between TA and chloranil was highly stable.

Effect of temperature

Increasing or decreasing the temperature more than 60°C did not decompose the formed complex. This was relevant to the high stability of the formed complex.

Effect of reagent concentration

The effect of varying the amount of chloranil on the absorbance of the formed CT complex with TA at a constant concentration of 1.0×10^{-3} mol/l was obtained. Reasonable absorbance was observed using 1–5 ml of 1.0×10^{-3} mol/l chloranil solution, and its maximum was obtained by adding 2.0 ml of chloranil reagent. The molecular ratio of the complex formed between TA and chloranil at the predetermined conditions was investigated using molar ratio [36,37]. The results indicated that the molecular ratio of the reagent to Tranexamic acid was 1 : 1 for the CT complex under investigation.

Suggested mechanism

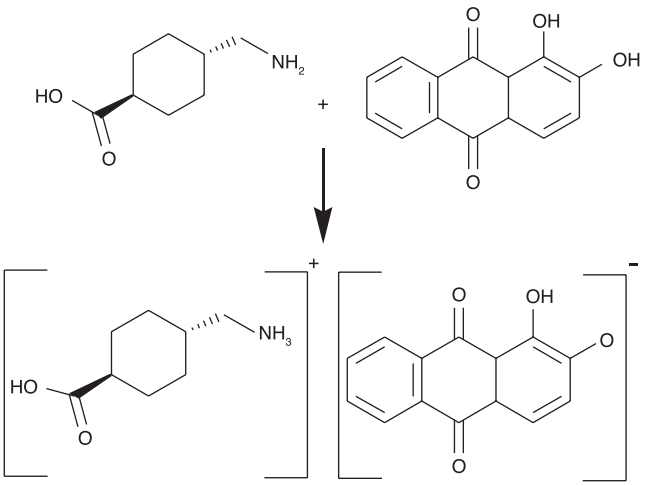
The aim of this work was to investigate the formation and stability of the new CT complex formed between chloranil (π acceptor) and TA (π donors). This stability is due to that the CT is formed by the donation of two nitrogen atom of TA to the C = O group of Chloranil Scheme 3 [38].

Effect of interference

To test the efficiency and selectivity of the developed analytical methods to pharmaceutical formulations, a systematic study under the optimum experimental conditions was made for the effect of the additives and excipients (e.g. lactose, glucose, fructose, calcium hydrogen phosphate, magnesium stearate, and starch) that are usually present in dosage forms. The criterion of interference was an error of not more than ± 2.0% in absorbance. Experiments showed that there was no interference from additives and excipients in the examined methods.

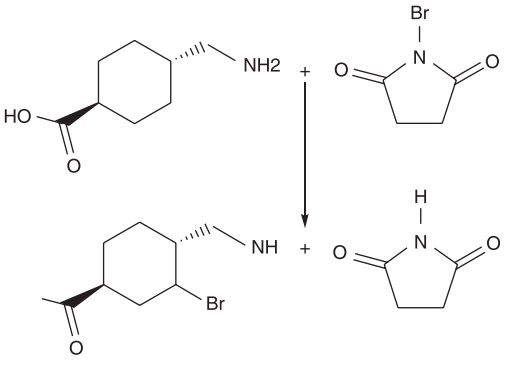
The applicability of the proposed methods for determining TA has been tested on pharmaceutical formulations. The concentration of TA was determined from its corresponding linear regression equations. The results of the proposed methods were compared with those obtained by the official method [5]. For microdetermination of TA, it is better to perform the *F*-test and *t*-test (Table 3). The calculated *F*-test and *t*-test [39] values at the 95% confidence level did not exceed the theoretical values, indicating that there is no significant difference between the developed methods and the official method. The small values of SD and

Scheme 1



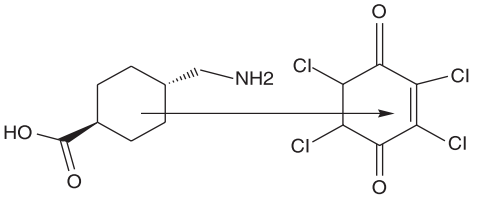
The proposed mechanism of the reaction of Tranexamic acid and alizarin red S.

Scheme 2



The mechanism of the reaction of Tranexamic acid and *N*-bromosuccinimide.

Scheme 3



Charge transfer complex formed between Tranexamic acid and chloranil.

RSD indicate the reliability, accuracy, and precision of the developed procedures.

Quantification

Beer–Lambert law limits, molar absorptive, regression equation, slope intercept, and correlation coefficient were calculated (Table 1) for the A, B, and C methods. The detection and quantitation limits were calculated from the SD of the absorbance measurements obtained from a series of six solutions for each procedure. To determine the accuracy and precision of the methods, solutions containing three different concentrations of TA were prepared and analyzed in four replicates. The analytical results obtained from this investigation have been summarized in Table 2. The relative average errors obtained by these developed methods ranged from 0.32 to 0.60, 0.17 to 0.26, and 0.44 to 1.15% with recovery percentage ranging from 99.50 to 99.70, 99.20 to 99.85, and 99.92 to 99.96% for methods A, B, and C, respectively. The relative standard deviations ranged from 0.45 to 1.25, 0.15 to 1.10, and 0.30 to 0.45 for methods A, B, and C, respectively. These values indicate high accuracy and precision.

Table 2 Evaluation of the accuracy and precision for the developed methods A, B and C

Methods	Concentration taken (μg/ml)	Recovery (%)	RSD (%)[a]	RE (%)	Confidence limits[b]
A	50.00	99.50	1.26	0.32	0.314
	100.0	98.50	1.20	0.60	1.915
	230.0	99.70	0.44	0.50	1.598
B	30.00	99.20	1.10	0.17	0.525
	120.0	99.65	0.43	0.26	0.826
	240.0	99.85	0.14	0.17	0.543
C	230.0	99.96	0.45	0.51	1.625
	350.0	99.92	0.28	0.44	1.555
	500.0	99.40	0.46	1.15	2.667

[a]RSD mean and relative SDs for four determinations; [b]Ninety-five percent confidence limits and five degrees of freedom.

Analytical application

The validity of the developed method was tested by the determination of TA in tablets. The performance of the developed method was assessed by comparison of *t* and *F*-values with the official method [14]. Mean values were obtained by using the Student *t*-test (for accuracy) and variance ratio *F*-tests (for precision) in the official method [33] and at 95% confidence limits for four degrees of freedom. The results showed that the calculated values did not exceed the theoretical ones.

Table 3 shows the statistical evaluation of the results obtained for assessment of the bias with true values using the Student *t*-test. The *t*-value for TA ranged from 1.53 to 4.38, 0.18 to 0.39, and 0.10 to 0.20 for methods A, B, and C, respectively. These values indicate at 95% confidence level that there is no significant difference or systematic error between the measured values obtained using the developed methods and the real values. The results of the developed method were also compared with the high-performance liquid chromatography official method using the statistical *F*-test at four degrees of freedom. *F*-values of 1.83–3.75, 0.66–5.56, and 0.80–2.06 were obtained for methods A, B, and C, respectively. These values indicate, at 95% confidence level, that there was no significant difference in precision between the developed methods A, B, and C and the official method. These findings indicate that the developed methods were accurate and precise and it can be safely used for routine work in drug control laboratories (Tables 4 and 5).

Table 3 Comparison of the developed method A with the reference method at 95% confidence level

Concentration taken (μg/ml)	Concentration found (μg/ml)	Recovery (%)* (SD)	*t*-value	Concentration found (μg/ml)	Recovery (%)** (SD)	*t*-value***	*F****
50.00	49.77	99.50 (0.63)	1.53	49.58	99.16 (0.46)	0.91	1.96
100.00	98.57	98.50 (1.20)	4.38	99.30	99.30 (0.62)	0.69	3.75
165.00	164.10	99.10 (1.10)	3.59	164.20	99.35 (0.68)	0.89	2.62
230.00	229.40	99.70 (1.00)	2.79	229.00	99.40 (0.739)	1.08	1.83

*Mean recovery ± SD standard deviation for four determinations in parenthesis for developed method, **Mean recovery ± SD standard deviation for four determinations in parenthesis for official method, *T**** value between the official and developed method in relation to the variation in the data, *F**** value between the official and developed method

Table 4 Comparison of the developed method B with the reference method at 95% confidence level

Concentration taken (μg/ml)	Concentration found (μg/ml)	Recovery (%)* SD	*t*-value	Concentration found (μg/ml)	Recovery (%)** SD	*t*-value***	*F****
30.00	29.98	99.20 (0.33)	0.18	29.96	99.35 (0.14)	0.29	5.56
75.00	74.82	99.50 (0.43)	0.33	74.76	99.51 (0.38)	0.37	1.30
120.0	119.65	99.65 (0.52)	0.39	119.55	99.67 (0.34)	0.59	2.34
240.0	239.65	99.60 (0.34)	0.23	239.60	99.55 (0.42)	0.24	0.66

*Mean recovery ± SD standard deviation for four determinations in parenthesis for developed method, **Mean recovery ± SD standard deviation for four determinations in parenthesis for official method, *T**** value between the official and developed method in relation to the variation in the data, *F**** value between the official and developed method

Table 5 Comparison of the developed method C with the reference method at 95% confidence level

Concentration taken (μg/ml)	Concentration found (μg/ml)	Recovery (%)[a] (SD)	*t*-value	Concentration found (μg/ml)	Recovery (%)[b] SD	*t*-value[c]	*F*[d]
230.0	229.25	99.93 (1.02)	0.10	229.20	99.94 (0.71)	0.14	2.06
350.0	348.90	99.92 (0.98)	0.20	348.80	99.93 (0.81)	0.25	1.2
425	423.65	99.76 (1.64)	0.11	423.56	99.80 (1.71)	0.12	0.92
500.0	498.40	99.6 (2.30)	0.17	498.20	99.65 (2.60)	0.20	0.80

[a]Mean recovery ± SD for four determinations in parenthesis for the developed method; [b]Mean recovery ± SD for four determinations in parenthesis for the official method [9]; [c]Value between the official and developed method in relation to the variation in the data; [d]Value between the official and developed method.

We used Student's *t*-test test for comparing the means of two samples (or treatments), even if they had different numbers of replicates. The *t*-test compares the actual difference between two means in relation to the variation in the data (expressed as the SD of the difference between the means).

Conclusion

A simple, rapid, selective, and highly sensitive spectrophotometric method has been described for the quantitative microdetermination of TA. The effect of different parameters was studied. The results obtained by the developed methods were compared with those obtained by the official method.

The results on applying the developed methods A, B, and C for microanalytical determination compared with the official method indicated that both ARS (method A) and NBS (method B) gave high absorbance reading at λ_{max} 300 and 520 nm at low concentration as the detection limit was 0.22 and 0.35 μg/ml, respectively. P-chloranil (method C) with a detection limit of 0.55 μg/ml at λ_{max} 330 nm may be used at a slightly higher TA concentration. Method C showed higher stability compared with both ARS and NBS.

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Conflicts of interest

There are no conflicts of interest.

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