# Estimation and determination of spectrophotometric determination for tranexamic acid in pharmaceutical preparation by using chelating agent

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## *Introduction:*

Tranexamic acid (TXA) is used off-label (without approval by the U.S. Food and Drug Administration), most often intravenously for a variety of conditions including prevention or reduction of blood loss during hip, knee, cardiac, facial, and spinal surgery as well as cesarean section and trauma. Used orally off-label for hereditary angioedema (HAE), to reduce blood loss during and after prostate surgery, and traumatic hyphema (bleeding in the anterior chamber of the eye).

Some surgeons use Tranexamic acid soaked sponges in the surgical field to reduce diffuse bleeding. An oral solution can be used (also off-label) to prevent bleeding in anticoagulated patients undergoing dental procedures.

Chemical Names: Tranexamic acid; 1197-18-8; Cyklokapron; Tranexamsaeure; Trans AMCHA; Tranhexamic acid , others, see figur-1

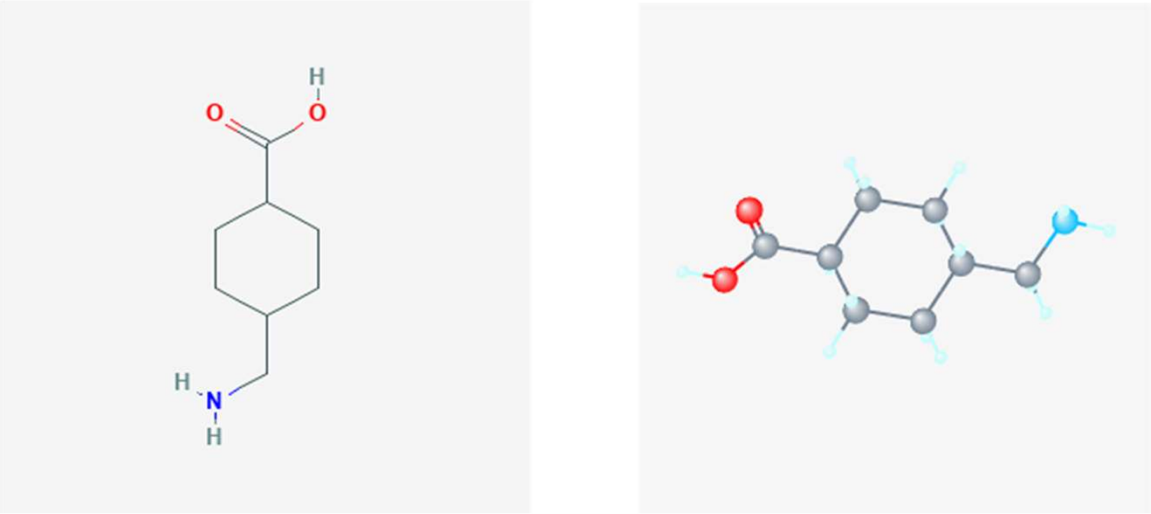



***Fig.1.chemical structure of Tranexamic acid***

## ***Medicinal uses of Tranexamic Acid Include:***

1. Post-partum bleeding

A large, international study was conducted on the use of tranexamic acid after childbirth to prevent hemorrhaging. Post-partum hemorrhage is the leading cause of maternal death worldwide.

2. Mouthwash for oral procedures

An antifibrinolytic mouthwash can be used for controlling bleeding after an oral procedure such as a tooth extraction, This treatment is especially effective for patients with blood clotting disorders.

3. Heavy menstruation

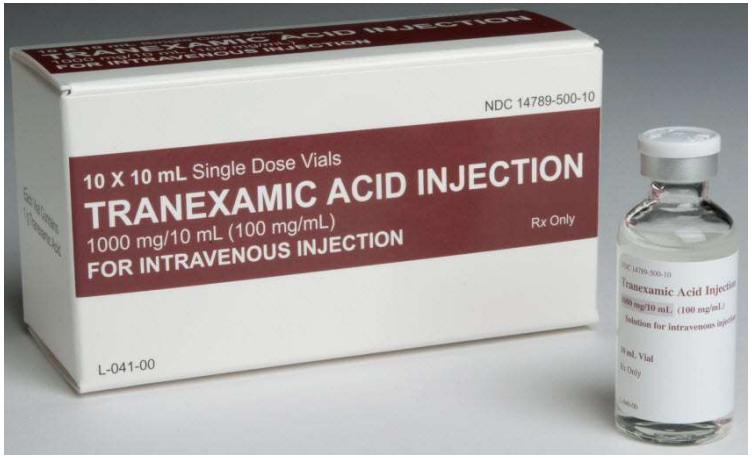
Tranexamic acid can help with heavy menstruation. It is available commercially in tablet form and sold under the brand name Lysteda for this indication.

4. Nose bleeds

A topically applied tranexamic acid solution can help reduce nose bleeds. Gauze can be soaked in the solution then held inside the nose for about an hour.

5. Melasma

Topically applied tranexamic acid has been shown to significantly improve the appearance of melasma. When compared to a combination of hydroquinone and dexamethasone, it produced no noticeable difference in results but had less side effects.



## ***Side effects:***

Get emergency medical help if you have any of these **signs of an allergic reaction:** hives; difficulty breathing; swelling of your face, lips, tongue, or throat. Serious side effect such as:

- problems with your vision (including color vision);
- sudden numbness or weakness, especially on one side of the body;
- sudden headache, confusion, problems with vision, speech, or balance;
- sudden chest pain or trouble breathing;
- pain or swelling in one or both legs;
- migraine headache;
- pale skin, feeling light-headed or short of breath, rapid heart rate, trouble concentrating; or
- feeling like you might pass out.

## ***Special Population:***

Renal Impairment:

Intravenous: Adjust dose based on the serum-creatinine concentration:

- 120-250 micromol/l: 10 mg/kg bid daily;
- 250-500 micromoles/l: 10 mg/kg once daily;
- >500 micromol/l: 5 mg/kg once daily or 10 mg/kg once every 48 hr.

Oral: Adjust dose based on serum creatinine concentration:

- 120-250 micromol/l: 15 mg/kg bid daily;
- 250-500 micromol/l: 15 mg/kg once daily;
- >500 micromol/l: 7.5 mg/kg once daily or 15 mg/kg once every 48 hr.

Pediatric Use: The drug has had limited use in pediatric patients, principally in connection with tooth extraction.

## Mechanism of action:

Tranexamic acid is a synthetic analoug of the amino acid lysine. It serves as an antifibrinolytic by reversibly binding four to five lysine receptor sites on plasminogen. This reduces conversion of plasminogen to plasmin, preventing fibrin degradation and preserving the framework of fibrin's matrix structure.

## Pharmacokinetics:

Tranexamic acid 1 g was given intravenously to three healthy volunteers, most elimination took place during the first eight hours, giving an apparent elimination half-life of approximately two hours. Plasma clearance ranged between 110–116 ml/min. The urinary recovery of tranexamic acid exceeded 95% of the dose.

Ten healthy volunteers were given tranexamic acid 2 g orally on an empty stomach, and together with a meal. Food had no influence on the absorption of tranexamic acid, the time required to reach the peak, the AUC from zero to six hours.

## *properties:*

| | |
|---|---|
| Appearance | White Crystalline Powder |
| Boiling Point | 300.2°C |
| Melting Point | 300 °C |
| Molar Mass | 157.21 g/mol |
| Molecular Formula | $C_8H_{15}NO_2$ |

Water Solubility 167000 mg/L

## *There are many methods for determination of tranexamic acid :*

### By RP-HPLC Method in Bulk and Pharmaceutical Dosage Form:

The chromatographic conditions were successfully developed for the separation of Tranaxamic acid and Ethamsylate by using Thermosil C18 column (4.6×100mm) 5µ, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer $P^H$ 3 (35:65 v/v), detection wavelength was 256 nm. The Spectroscopic method was done in solvent using methanol and the instrument used was WATERS HPLC Auto Sampler.

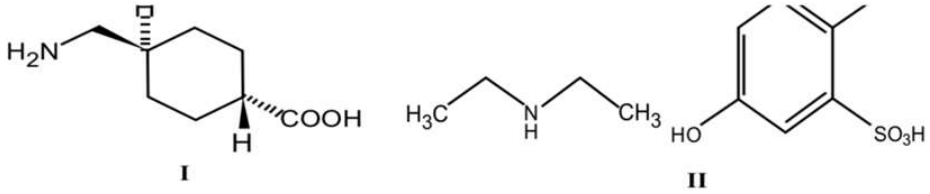



**Figure 1:** Chemical structures: I) Tranexamic acid and II) Ethamsylate.

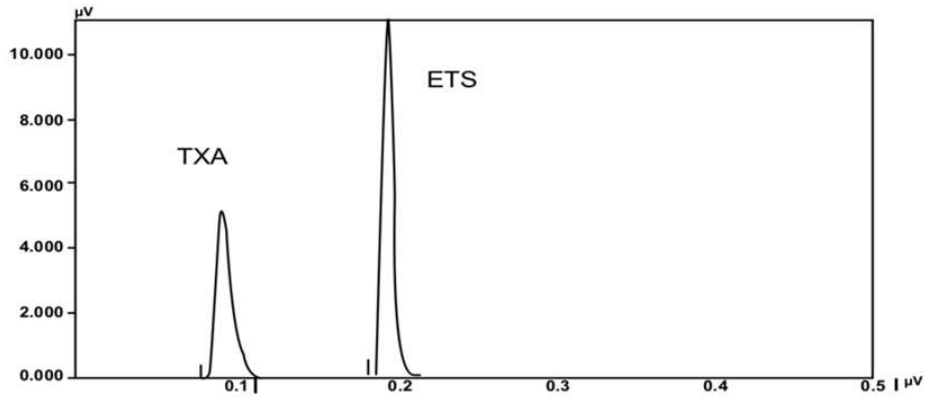
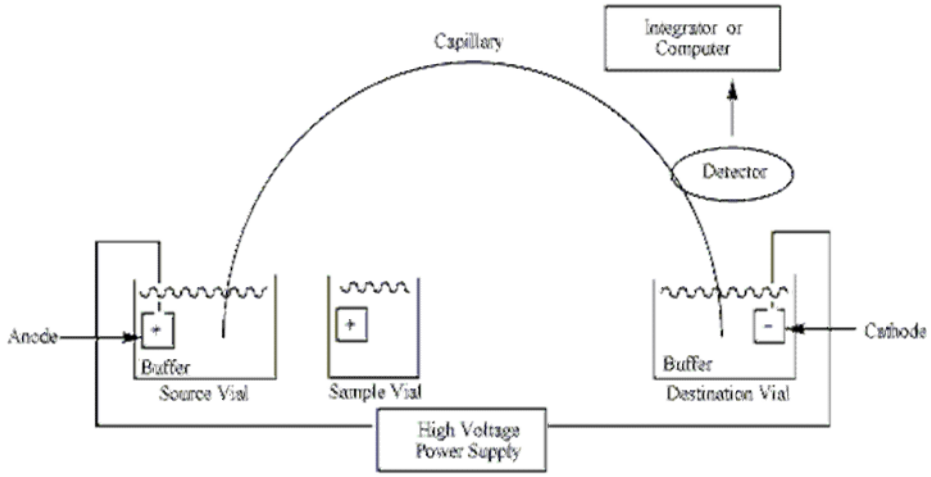



**Figure 2:** Separation of Tranexamic acid and Ethamsylate in selected mobile phase.

## By Capillary electrophoresis (CE):

analytes migrate through electrolyte solutions under the influence of an electric field. Analytes can be separated according to ionic mobility and/or partitioning into an alternate phase via non-covalent interactions. Additionally, analytes may be concentrated or "focused" by means of gradients in conductivity and pH.



## By Spectrophotometric determination of tranexamic acid in pharmaceutical bulk and dosage forms:

In this study, a simple, fast, accurate and sensitive spectrophotometric method has been developed for the determination of tranexamic acid in bulk and pharmaceutical preparations. The method is based on the reaction of ninhydrin with the primary amino group of tranexamic acid in the basic medium at PH 8.0. The reaction produces a bluish-purple colour which absorbs maximally at 565nm. Beer's law was obeyed in the range of 3-40 $\mu g.ml^{-1}$ with molar absorptivity of $5.093x10^{3} L\ mol^{-1}\ cm^{-1}$. The effects of various factors such as temperature, heating time, concentration of reagent, color stability and interferences were investigated to optimize the procedure. The results have been validated analytically and statistically. The proposed method has been applied for the determination of tranexamic acid in bulk and pharmaceutical preparations with good results.

## *The work aims:*

To demonstrate two simples, accurate and sensitive spectrophotometric methods for determination of tranexamic acid in pure form and in pharmaceutical formulations. The present work describes two spectrophotometric methods,which are less expensive than the published HPLC and capillary electrophoresis.

The present analytical procedures involved oxidation of tranexamic acid with ferric chloride and determining the iron II produced by complexing either with α,α'-Dipyridyl or 1,10-phenanthroline. The possible reaction was showed in scheme(1) for the proposed methods

Methods A and B are based on the oxidation of TXA by excess of ferric salt $Fe^{+3}$ and the reduced state was utilized besides the unreacted $Fe^{+3}$.

The $Fe^{+3}$ has tendency to give colored complex on treatment with α,α'-Dipyridyl (method A) scheme(1) or 1,10-phenanthroline (method B).

## *Experimental part*

### *Apparatus:*

All spectrophotometric measurements were carried out using a Shimadzu UV-1800 spectrophotometer,

## *Materials:*

Reference tranexamic acid (purity100%) was supplied by the Central Laboratory. Trexamin capsules labeled to contain 250mg tranexamic acid.

## *Solutions:*

1. Tranexamic acid solution (400 µg/ml)

A stock solution was prepared by dissolving 400mg of tranexamic acid in 100ml of distilled water and was further diluted to give a working standard solution with final concentration 1000µg/ml.

2. Ferric chloride (0.1%)

This solution is prepared by dissolving 0.1g of ferric chloride in 100 distal water.

3. 1,10-phenanthroline solution (0.1%)

The 0.1% solution of 1,10-phenanthroline reagent was in distilled water by dissolving 0.1g of 1,10-phenanthroline in 100ml distilled water.

4. α,α'-Dipyridyl (0.1%)

Prepared by dissolving 0.1g of α,α'-Dipyridyl reagent in 100ml distilled water.

## ***General procedure:***

In methods A and B different aliquots of work standard solution (200µg/ml for method A and 300µg/ml method B) were transferred into a series of 10 ml standard flasks. To each flask 1ml of ferric chloride (method A) or 1.5ml of FeCl3 (in method B) were added and kept in a water bath (80 -/+ 1c) for 15 min, then immediately cooled to room temperature (25 -/+ 1c) and 1ml of o-phosphoric acid was added. The solution were made up to volume with distilled water. The absorbance of each solution was measured at 295nm (method A) and 310nm (method B) with reagent blank. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug.

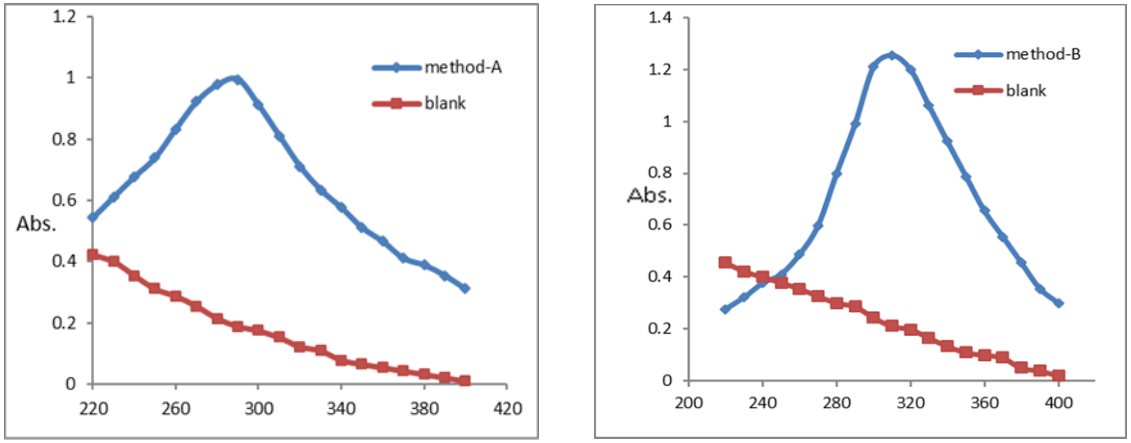
# ***Result & discussion:***

## *Optimization of the reaction conditions*

The proposed methods were optimized to achieve complete reaction formation, highest sensitivity and maximum absorbance.

## *Absorption spectrum*

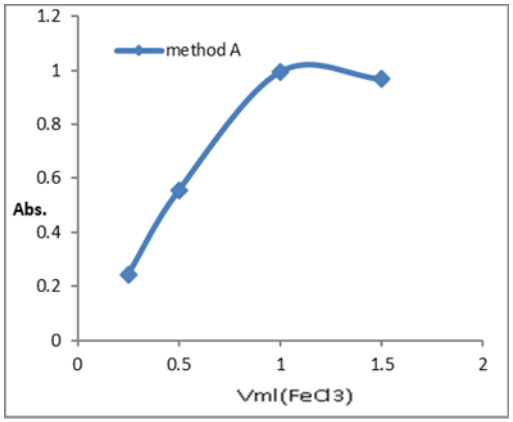
When the tranexamic acid is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption 295nm for method A & 310nm for method B. As show in figures 3.



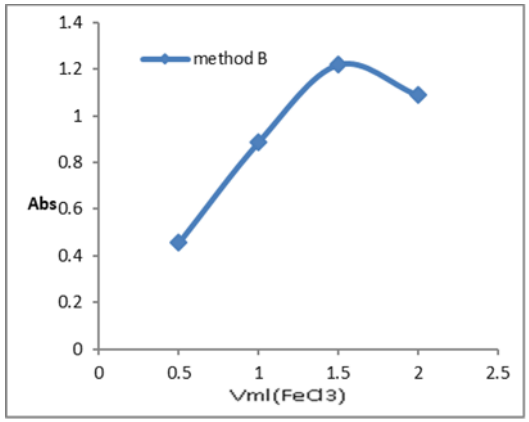

Fig(3) the absorption spectra of tranexamic acid complexes with 1,10-phenanthroline (method A) and α,α'-Dipyridyl (method B) . With 20 µg/ml concenteration for tranexamic acid.

## *Effect of ferric chloride oxidizing agent*

The effect of different amounts of ferric chloride oxidizing agent on the absorbance of solution was studied to be (0.25-1.5 ml method A) & (0.5-2 ml method B), the result indicated that the absorbance increase with increasing ferric chloride concenteration . 1ml and 1.5ml of ferric chloride in a total volume of 10ml were found optimum in methods A & B respectively and used in through out the experiment as show in fig 4 & 5.




Fig(4) effect of $Fecl_3$ volume on the complex absobance method A

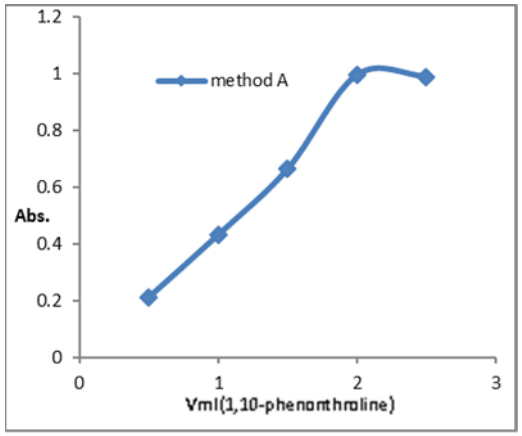



fig(5) effect of $Fecl_3$ volume on the complex absobance Method B

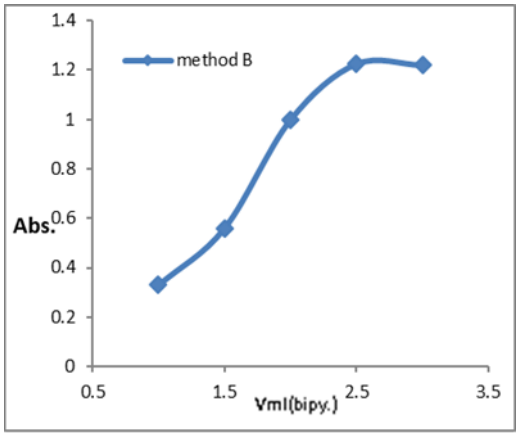
## *Effect of reagent amounts*

several experiments were carried out to study the influence of reagents amounts on the colour development by keeping the concenteration of tranexamic acid drug & $Fecl_3$ constant and changing reagent concenteration .

The optimum volume of 1,10-phenanthroline reagent and α,α'-Dipyridyl used for the production of maximum and reproducible colour intensity was found to be 2ml of 1,10-phenanthroline (method A) and 2.5ml of α,α'-Dipyridyl (method B) in total volume 10ml as fig(6) and (7).




Fig(6)the effect of 1,10-phenanthroline volume on absorbance of complex with method A

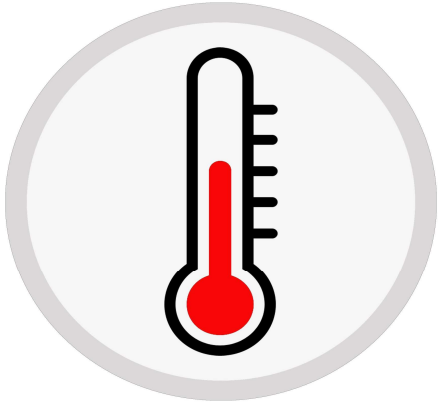



fig(7) effect of α,α'-Dipyridyl volume on absorbance of complex with method B

## *Effect of time and temperature*

The effect of time on the development and stability of the determination of tranexamic acid was studied . The standing times for full colour development were found to be 15min for both methods A and B .The absorbance remained constant at least for 2hours .

At higher temperature the time was reduced to 5min . maximum colour was obtained by heating on a boiling water bath for 5min.



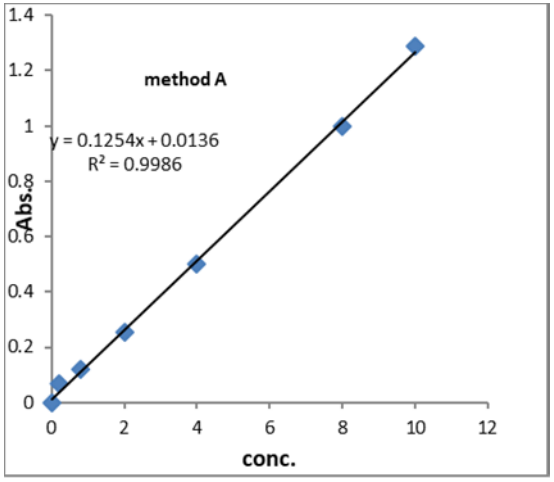
## *Method validation*

In order to know the beer's law limits of the proposed methods A & B , the absorbance of a series of solutions containing varying amounts of tranexamic acid (0.2ml-1ml) for method A & (0.2ml-2ml) for method B and specified concenteration of the remaining as given in the procedure (0.2µg/ml-10µg/ml) for method A and (0.2µg/ml-20µg/ml) for method B. In a total volume of 10ml were measured at 395nm and 310nm for both methods respectively against a reagent blank . calibration curve for tranexamic acid was constructed by plotting absorbance versus concenteration in µg/ml (fig 8 & 9). Under optimum conditions various analytical parameters were obtained and presented in table 1.

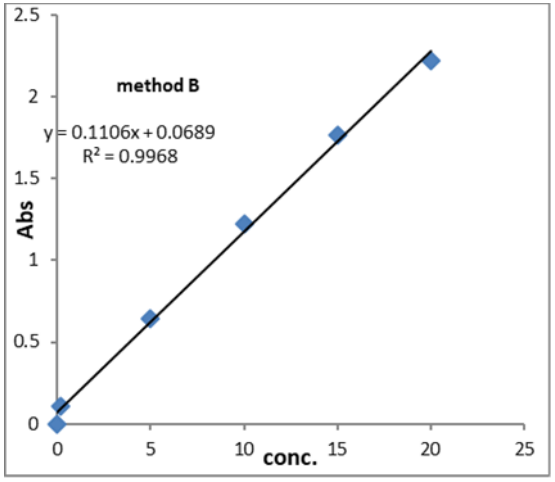
The value of correlation coefficient indicates good linearity of the present methods. High value of molar absorptivity and lower value of sandell's sensitivity reflect good and high sensitivity of the method.

***Table (1) analytical parameters***

| Parameters | Method A | Method B |
|---|---|---|
| Beer's law (µg/ml) | 0.2 - 10 | 0.2 - 20 |
| λ max (nm) | 395 | 310 |
| Molar absorptivity | $2.178 \times 10^{4}$ | $0.594 \times 10^{4}$ |
| Sandell's sensitivity ($\mu g.cm^{2}$) | $0.077 \times 10^{-3}$ | $1.4133 \times 10^{-3}$ |
| Detection limit (µg/ml) | 0.1 | 0.15 |
| Correlation coefficient $R^{2}$ | 0.9986 | 0.9968 |



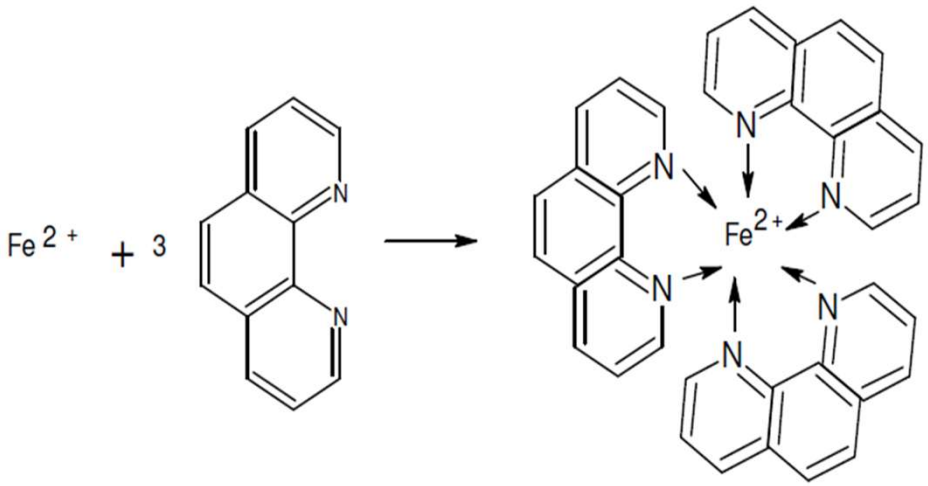

Fig(8) calibration curve of method A



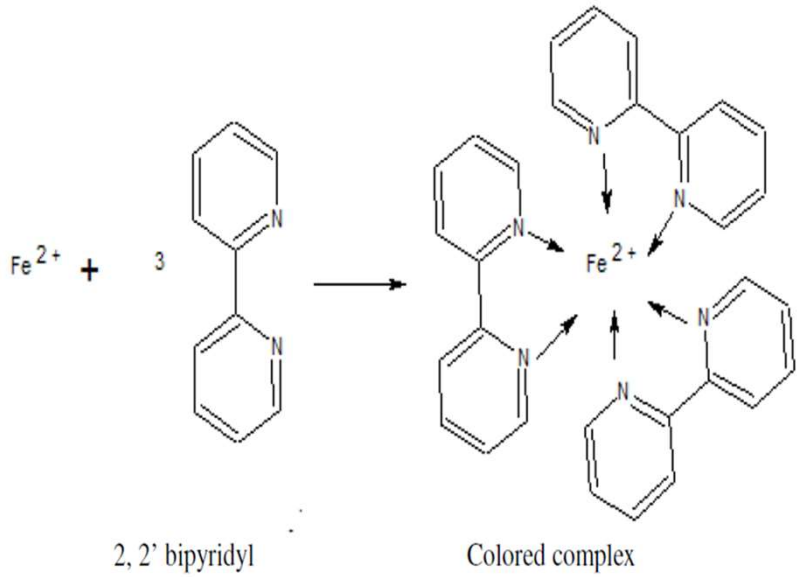

fig(9) calibration curve of method B

***According to the procedure the reaction complex presented by:***

***Tranexamic acid + $Fe^{+3}$ ⟶ reduced form of tranexamic acid + $Fe^{+2}$***




***Fig(10) Method A***




***Fig(11) Method B***


THANK
YOU!