Spectrophotometric Determination of Tranexamic acid in Dosage forms by Derivatization

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Summary: Tranexamic acid (TA) was determined spectrophotometrically after derivatizing with salicylaldehyde (SA) or 5, 5'-methylene-bis-salicylaldehyde (BSA) at neutral pH in aqueous-methanolic solution. The reaction conditions were optimized and the derivatives absorbed maximally at 410 nm and 412 nm for SA and BSA respectively. The Beer's law was obeyed in the range 7.8-78.6 μg/ ml tranexamic acid, with coefficient of determination of 0.9995 and 0.9998. The methods were applied for the determination of tranexamic acid in pharmaceutical preparations with coefficient of variation 0.76-0.97 %.

Introduction

Tranexamic acid (trans-4-aminomethylcyclohexane carboxylic acid) (TA) is a synthetic amino-acid commonly used for controlling abnormal bleeding in a number of diseases [1,2]. TA is a cyclohexyl analogue of aminocaproic acid and is more potent than aminocaproic acid as an inhibitor of plasmin.

The analysis of TA is based on spectrophotometry [3-6], spectrofluorimetry [7], gas [8,9] and liquid chromatography [10-17]. The spectrophotometric methods aresimple and involve less expensive equipment. The sensitivity and selectivity of the determination could be improved by derivatization with suitable reagents. The derivatizing reagents for spectrophotometric methods of TA comprises of 2,4,6-trinitrobenzenesulphonic acid [6], 2-nitrophenyl hydrazine [18], 1-fluoro-2,4-dinitrobenzene [19], p-dimethylaminobenzaldehyde [4], and 2-hydroxynaphthaldehyde [20]. The present work examines the use of salicylaldehyde (SA) and 5,5'-methylene-bis-salicylaldehyde (BSA) as derivatizing reagents (Fig. 1).

Results and Discussion

Tranexamic acid (TA) does not absorb above 250 nm, therefore derivatization with SA and BSA were carried out to increase the spectrophotometric sensitivity with bathochromic shift to visible region. TA as derivatives of SA and BSA indicated molar absorptivities of 1648 L. mole^{-1}cm^{-1} and 1735 L. mole^{-1}cm^{-1} at 410 nm and 412 nm respectively. The effects of pH, reagent concentration and heating time and temperature on the derivatization reactions were examined. The pH effects within 1 to 10 at unit-interval were studied and it was observed that maximum absorbance was observed at pH 7 (Fig. 2) and sodium acetate buffer pH 7 covered pH range satisfactorily. The amount of derivatizing reagent added (0.3 % in methanol) was varied from 0.5 to 3.0 ml at an interval of 0.5 ml and addition of 2.0 ml was found to be optimal. The heating time and temperature were examined from 5 to 25 min at 70 °C at an interval of five min. and a similar response was observed after heating for 10 min. Therefore heating time for 15 min was considered as optimum (Fig. 3). The effect of variation in the concentration of TA on the

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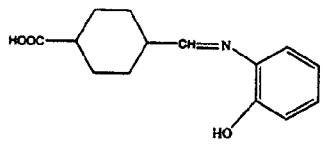

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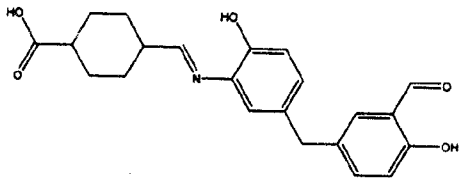

Fig. 1. Structural diagram of TA derivatives with (1) SA and (2) BSA.

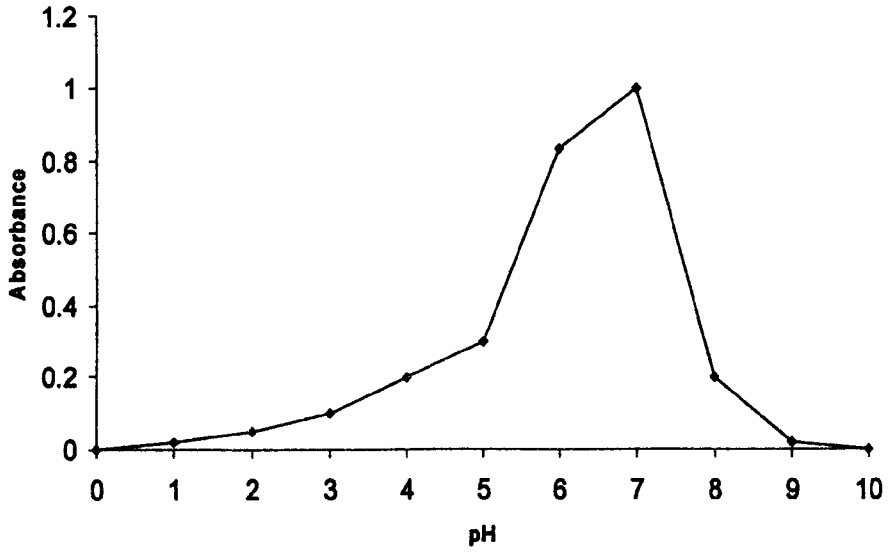

Fig. 2. Effect of pH on the derivatization of TA with SA.

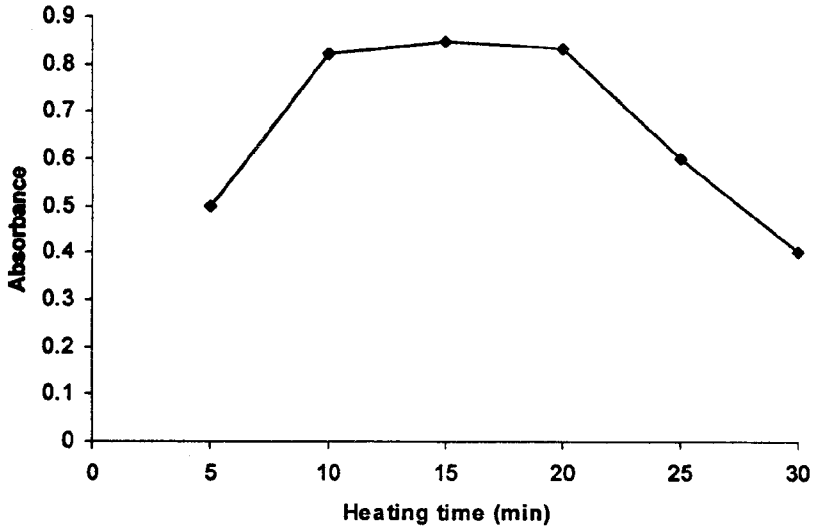

Fig. 3. Effect of heating time on the derivatization of TA and SA.

absorbance was examined and linear calibration curves were observed, which obeyed Beer's law within the concentration range 7.8-78.6 μg/ml with coefficient of determinations R^2 0.9995 and 0.9998 using SA or BSA respectively. The detection limit measured as 0.005 absorbance unit was observed 0.38 μg/ml TA.

The effect of the possible presence of additives such as methylparaben, propylparaben, gum acacia, magnesium stearate, lactose, glucose and starch in the pharmaceutical preparations were investigated at ten times the concentration of TA and none of these substances interfered.

The methods were applied for the determination of TA in pharmaceutical preparations. The results obtained were found in good agreement with the labeled values reported by the manufacturers. The coefficient of variations were obtained within 0.89-0.97 % and 0.76-0.78 % with relative deviation of 2.92-2.7 % and 2.92-2.6 % using SA or BSA respectively (Table-1 and 2).

Table-1: Analysis of TA in Pharmaceutical preparations using BSA

S. No.	Name of Preparation	Amount reported by the manu-facturer mg tablet^{-1}	Amount found mg tablet^{-1} (CV %)	% Relative Deviation
1	Transamine	250	243.2 (0.96)	2.72
2	Zataranax	250	242.8 (0.89)	2.88
3	Zamig	250	242 (0.97)	3.2

Table-2: Analysis of TA in Pharmaceutical preparations using SA

S. No.	Name of Preparation	Amount reported by the manu-facturer mg tablet^{-1}	Amount found mg tablet^{-1} (CV %)	% Relative Deviation
1	Transamine	250	242.7 (0.76)	2.92
2	Zataranax	250	242.6 (0.78)	2.96
3	Zamig	250	242.3 (0.77)	3.08

Experimental

Material and Reagents

All the chemicals and reagents used were of analytical or pharmaceutical grades. The double distilled water from all glass was used throughout the study. Pure TA was obtained from Hilton Pharma (Pvt) Ltd, Karachi. Acetic acid (E-Merck, Germany), sodium acetate (Fluka, Switzerland), ethanol (BDH U.K) trioxane (Fluka), sulphuric acid (98 %) (E. Merck) and salicylaldehyde (SA) (E-Merck Germany) were used. 5,5'-methylene-bis-(salicyl-aldehyde) (BSA) was prepared as reported [21] as under.

To the solution of salicylaldehyde (34 ml, 40 g) in glacial acetic acid (25 ml) at 90-95 °C in nitrogen atmosphere was added trioxane (3.5 g) in a mixture of sulphuric acid (0.25 ml) and glacial acetic acid (1.3 ml) dropwise with magnetic stirring. The temperature was maintained for 22 hrs and stirring was continued over the whole reaction period.

The reaction mixture was then poured into 1L of ice cold water and allowed to stand overnight. The solid was filtered and recrystallized from acetone m.p 145-148 °C. Buffer solutions between pH 1-10 at unit interval were prepared from hydrochloric acid (0.1 M), potassium chloride (1 M), acetic acid (1.0 M), sodium acetate (1 M), sodium bicarbonate (1M), sodium carbonate (saturated), ammonium chloride (1 M) and ammonia solution (1 M).

The solutions of SA (0.15 ml, 0.3 % v/v) and BSA (0.15 g, 0.3 % w/v) were prepared by dissolving in warm methanol (50 ml).

Spectrophotometric studies were carried out with a double beam Hitachi 220 (Hitachi (Pvt) Ltd, Tokyo, Japan) spectrophotometer with dual quartz cuvettes. pH measurements were made with Orion model 420A pH meter with glass electrode and combined reference electrode (Orion Research Inc. Boston,USA)

Analytical Procedure

The solution (1-2 ml) containing TA (78-785 μg) was transferred to 10 ml volumetric flask and was added either SA or BSA (2ml, 0.3 % in methanol), followed by acetate buffer pH 7 (1 ml). The contents were heated on water bath at 70 °C for 15 min. The solution was cooled at room temperature and volume was adjusted to mark with methanol. The absorbance was measured at 410nm or 412 nm respectively against reagent blank, prepared in a similar way, omitting the addition of TA.

Analysis of Tranexamic acid in Pharmaceutical Preparations

Twenty capsules each Transamine (Hilton Pharma. Co. Karachi, Pak.), Zataranax (Zafa pharma lab. Karachi, Pak) and Zamig(Highnoon Labs., Lahore, Pakistan) were thoroughly ground to fine powder and the amount 0.39031 g,0.33652 g and 0.35423 corresponding to 250 mg of TA were transferred to separate beakers and dissolved in water. The solution was filtered and the volume was adjusted to 100ml. The solution 5 ml was further diluted to 100ml and solution 2 ml was transferred to 10ml volumetric flask. The above derivatization procedure was followed and the amount of TA in pharmaceutical preparations was evaluated from external calibration curve.

Conclusions

Simple spectrophotometric methods have been developed for the determination of TA using SA or BSA as derivatizing reagents with linear calibration range within 7.8-78.6 μg/ml. Both the reagents indicated similar coefficient of variation for the analysis of TA in pharmaceutical preparations.

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