



Stability-Indicating Micelle-Enhanced Spectrofluorimetric Method for Determination of Tamsulosin Hydrochloride in Dosage Forms.

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ABSTRACT:

A rapid, simple and highly sensitive spectrofluorimetric method is developed for the determination of Tamsulosin hydrochloride (Tams.HCl) in pharmaceutical formulations. The proposed method is based on investigation of the fluorescence spectral behavior of Tams.HCl in a sodium dodecyl sulphate (SDS) micellar system. In aqueous solution of Tris buffer of pH 7 ± 0.2 , SDS causes marked enhancement in the fluorescence intensity of Tams.HCl (about +110%). The fluorescence intensity is measured at 328 nm after excitation at 280 nm and the fluorescence-concentration plots are rectilinear over the range $0.1-1.2 \mu\text{g ml}^{-1}$, with lower detection limit of $0.027 \mu\text{g ml}^{-1}$ and quantification limit of $0.09 \mu\text{g ml}^{-1}$. The method is successfully applied to the analysis of the studied drug in its commercial capsules, and the results are in good agreement with those obtained with the official method. The application of the proposed method is extended to stability studies of Tamsulosin hydrochloride after exposure to different forced degradation conditions, such as acidic, alkaline and oxidative conditions, according to ICH guidelines.

Keywords: Tamsulosin hydrochloride, sodium dodecyl sulphate, micellar system



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INTRODUCTION:

Tamsulosin 5-[(2R)-2-[[2-(2-Ethoxyphenoxy)ethyl]amino]propyl]-2-ethoxybenzene-sulfonamide [1] (Fig. 1). Tamsulosin is an α_1 -adrenoceptor blocker; it is reported to be more selective for the α_1A -adrenoceptor subtype. It is used in benign prostatic hyperplasia to relieve symptoms of urinary obstruction. Tamsulosin is given by mouth as the hydrochloride salt in a modified-release formulation, in a dose of 400 μ g once daily [2]. Several analytical procedures describing the determination of Tamsulosin have been published; these methods include non-aqueous titration [3], voltammetry [4] and spectrophotometry [5]. Several chromatographic methods were described for determination of Tamsulosin, such as high-performance liquid chromatography (HPLC) with UV detection [3, 6–9], fluorescence detection [10], mass spectrometric detection [11–15], and capillary electrophoresis [16].

The key characteristic of fluorescence spectrometry is its high sensitivity. Fluorometry may achieve limits of detection several orders of magnitude lower than those of most other techniques. Because of the low detection limits, fluorescence is widely used for quantification of trace constituents of biological and environmental samples. Most complex samples contain one or more components that can quench the fluorescence of the analyte. Thus, it often is necessary to subject complex samples to be analyzed by fluorometry to extensive prior cleanup to remove potential quenchers. Alternatively, one may try to provide a uniform microenvironment for the analyte and thus a reproducible fluorescence yield from sample to sample by adding a micelle forming surfactant to each sample [17]. This is based on the fact that fluorescent molecules in solution may be partially or fully hidden from quenchers by incorporating them into organized media such as surfactant micelles or cyclodextrin cavities [18]. The environment seen by a micelle-solubilized solute differs from that encountered in aqueous solution in having a higher local viscosity which decreases collision-induced non-radiative decay or possible quenching reactions, resulting in increased fluorescence quantum yield with a subsequent increase in analytical sensitivity [19]. Recently, micelle-enhanced fluorescence using sodium dodecyl sulfate (SDS) has been utilized for the spectrofluorometric determination of Ezetimibe [20], loratadine and desloratadine [21], galantamine [22], verapamil hydrochloride [23], gatifloxacin [24], garenoxacin in human urine and serum [25], naproxen [26], Levofloxacin [27], ellipticine [28], carbendazim [29] and fenproporex [30]. Surveying the literature revealed that there is no reported spectrofluorometric method for the determination of Tams.HCl using organized media. Tams. HCl is considered as a minor pharmaceutical active ingredient which needs highly sensitive methods to be determined in its dosage forms and biological fluids. The objective of the present work is to study the effect of SDS micellar medium on enhancing the native luminescence characters of Tams.HCl in order to develop a new simple and sensitive micro analytical methodology for determination of the studied drug in its dosage forms.

EXPERIMENTAL:

Apparatus

All the fluorescence spectra were recorded using a JASCO FP-6200 Spectrofluorometer, equipped with 150 W Xenon lamp, grating excitation and emission monochromators, and a recorder. Slit widths for both monochromators were set at 10 nm; they were set at 5 nm for fluorescence quantum yield measurements. A 1 cm quartz cell was used. Spectra were evaluated using Spectra Manager FP-6200 Control Driver software, Version 1.54.03 [Build 1], JASCO Corporation.

Materials and reagents

a) Pure materials:

- Tams.HCl, was purchased from SIGMA pharmaceutical industries (Osmopham, Switzerland), its percent purity was 100.68% as received.

b) Pharmaceutical preparations:

-Tamsulin capsules Manufactured by (Marcyl Pharmaceutical industries, Elobour City, Egypt), were purchased from commercial sources in the local market. Each capsule is labeled to contain 0.4 mg of Tamsulosin HCl. Batch Number1020774

-Tamic capsules Manufactured by (Sigma Pharmaceutical industries, Egypt), were purchased from commercial sources in the local market. Each capsule is labeled to contain 0.4 mg of Tamsulosin HCl. Batch Number 81774.

c) Reagents:

All chemicals used were of analytical reagent grade and were used without further purification.

- SDS (Rediel –De- Haen, Germany), 1.0 % (W/V) aqueous solution was prepared by dissolving 10 g SDS in distilled water, and then it was diluted to 1 L with the same solvent. It is stable for 7 days when left in the refrigerator.
- B-cyclodextrin was obtained from Merck (Germany), 0.05 % (W/V) aqueous solution was prepared by dissolving 0.5 g B-cyclodextrin in distilled water, and then it was diluted to 100 ml with the same solvent.
- Tris-Chloride buffer [Tris (hydroxyl methyl) amino methane] was obtained from (Fluka Bio Chemika), 0.1 M aqueous solution pH 7 \pm 0.2 was prepared according to the B.P.



- McIlvaine buffer[31], pH (2.2-7) was prepared by mixing appropriate volumes of 0.1M citric acid and 0.2 M disodium hydrogen orthophosphate, both citric acid and disodium hydrogen orthophosphate were obtained from El-Nasr Pharmaceutical Chemical Co. (ADWIC; Egypt).
- Methanol HPLC grade 99.9 % (Sigma, Germany)
- Hydrochloric acid HPLC grade 37 % (Rediel –De- Haen, Germany), 2.0 M aqueous solution was prepared.
- Sodium chloride (Winlab, Leicestershire, U.K.)
- Sodium hydroxide pellets (Winlab, Leicestershire, U.K.) 2.0 M aqueous solution was prepared.

Standard solutions

Tams. HCl Stock solutions:

Stock solution 1 (250.0 µg ml⁻¹) was prepared by dissolving 25.0 mg of Tams. HCl in 100.0 ml of methanol.

Stock solution 2 (20.0 µg ml⁻¹) was prepared by diluting 8.0 ml of **Stock solution 1** to 100.0 ml with distilled water.

Tams. HCl working solution:

A working solution (4 µg ml⁻¹) was prepared by diluting 20.0 ml of Stock solution 2 to 100.0 ml with distilled water.

General procedures

a) Procedure for calibration graph.

Aliquots from Tams.HCl working solution equivalent to (1.0-12.0 µg) Tams.HCl were transferred into a series of 10-ml volumetric flasks. Then 2.0 ml 0.1 M Tris-Chloride buffer solution, pH 7±0.2, were added to each flask, followed by 2.0 ml 1.0% w/v SDS solution. The volume was completed with distilled water; the contents of the flasks were mixed well. A blank experiment was performed simultaneously and the relative fluorescence intensity was measured at 328 nm after excitation at 280 nm. The corrected fluorescence intensities were plotted against the final drug concentrations (µg ml⁻¹) to obtain the calibration graph. Alternatively, the corresponding regression equation was derived.

b) Procedure for capsules.

The contents of not fewer than ten capsules were weighed and mixed well. An amount of the capsule pellets equivalent to 1 mg Tams.HCl was accurately weighed, transferred to 25-ml volumetric flask and dissolved in methanol. The flask was sonicated for 30 minutes then, completed to volume with the same solvent. The produced solution (40 µg ml⁻¹) was used to prepare aqueous working solution (4 µg ml⁻¹). Aliquots from working solution covering the concentration range cited in table (1) were transferred into a series of 10-ml volumetric flasks, the 'Procedure for calibration graph' (above) was then performed. The capsule content was determined either from a previously plotted calibration graph or using corresponding regression equation.

c) Procedure for biological fluids.

Aliquots of human plasma equivalent to 1.0 ml were transferred into centrifugation tubes and spiked with 1.0 ml of Tams.HCl. Then, 2.0 ml acetonitrile were added to each tube. The mixture was centrifuged at 6000 rpm for 20 minutes. 1.0 ml of the supernatant was carefully transferred to a measuring flask and the 'Procedure for calibration graph' was then applied. A blank experiment was carried out simultaneously. The drug content in plasma was determined using corresponding regression equation.

d) Procedures for stability indicating assay

- **Alkaline and acidic degradation.**

Aliquots of Tams.HCl Stock solution 1 (equivalent to 250.0 µg of the drug) were transferred into a series of small conical flasks; aliquots of 2 M NaOH or 2 M HCl equivalent to 5.0 ml were added. The flasks were heated in a boiling water bath for different time intervals (5–60 min). At the specified time intervals, the contents of each flask were cooled and neutralized to pH 7.0 with 2 M HCl or 2 M NaOH, respectively. The solutions were then transferred into 25-ml volumetric flasks and completed to volume with water. One ml of the resulting solutions was then transferred into 10-ml volumetric flasks and the procedure 'Procedure for calibration graph' was performed.

- **Oxidative degradation.**

Aliquots of Tams.HCl Stock solution 1 (equivalent to 250.0 µg of the drug) were transferred into a series of 25-ml volumetric flasks; different volumes of 10% H₂O₂ were added and the volume was completed with water. One ml of these solutions was transferred into 10-ml volumetric flasks and the procedure 'Procedure for calibration graph' was performed.

- **Photo-degradation.**

Aliquots of Tams.HCl Stock solution 1 (containing 250.0 µg of the drug) were transferred into 25-ml volumetric flasks and completed to volume with distilled water. The volumetric flasks were exposed to UV light at a wavelength of 254 nm and at



a distance of 15 cm, placed in a wooden cabinet, for 5 hours. one ml of the solutions was then transferred into 10-ml volumetric flasks and the procedure 'Procedure for calibration graph' was performed.

RESULTS AND DISCUSSION

Tams.HCl was found to exhibit a weak emission band at 328nm in aqueous Tris-Chloride buffer solution of pH 7 after excitation at 280 nm. This work aimed to enhance this emission band, in order to develop a new methodology for the analysis of Tams.HCl in its pharmaceutical preparations. The use of different types of surfactants to enhance the fluorescence of many drugs has found wide applications in the field of analysis. When a surfactant at a concentration above its critical micellar concentration is added to a given fluorophore solution, it increases the molar absorptivity and/or the fluorescence quantum yield of the fluorophore in many cases [32]. This fact has been used to improve the performance of spectrofluorimetric methods of various analytes. The fluorescence properties of Tams.HCl were studied in different types of organized media (SDS, β - cyclodextrin and Tween 80). Tween 80 caused severe decrease in the RFI of the studied drug while the addition of SDS caused an enhancement (about 110%) of its fluorescence intensity compared with aqueous solution (Fig. 2). On the other hand β - cyclodextrin showed no marked effect on the fluorescence intensity of Tams. HCl (Fig. 3), therefore SDS was used as a fluorescence enhancer in order to develop a new spectrofluorimetric method for the determination of Tams. HCl in its dosage form.

Optimization of the reaction conditions

a) Effect of pH and type of buffer

The influence of pH on the micelle enhanced fluorescence of Tams. HCl was studied using different types of buffers covering wide pH range, such as McIlvaine buffer over the pH range 3–7 and 0.1 M Tris-Chloride buffer over the pH range 7–10. It was found that maximum RFI was achieved over the pH range 4.0–8.0; increasing the pH value above pH 8.0 caused gradual decreases in the RFI (Fig. 4). Therefore, pH 7 was chosen as the optimum pH value for measurement of RFI of the studied drug in SDS medium. Comparison between the RFI of the studied drug in SDS medium of pH 7 was carried out using McIlvaine and Tris-Chloride buffer. No significant difference between the RFI of the drug in the two buffer media, so Tris-Chloride buffer was used to adjust the optimum pH for the reaction.

The pKa value of Tams. HCl is reported to be 8.4 (secondary amine) [1]. This may explain the reason for the maximum RFI observed over the pH range 4.0–8.0 at which the studied drug is mostly present in the protonated form which interact more strongly with the anionic micelles of SDS than the neutral form. It is also reported that the fluorescence intensity for protonated species is always higher than that for neutral species [33].

b) Effect of the volume of SDS

The influence of SDS on the RFI of Tams. HCl was studied using increasing volumes of 1.0% w/v SDS. It was found that increasing volumes of SDS solution resulted in a consequent increase in RFI up to 1.0 ml, after which no further increase in RFI was observed (Fig. 5). Therefore, 2ml 1.0% w/v SDS solution was chosen as the optimum volume to achieve maximum enhancement in RFI of Tams. HCl.

c) Effect of Molar concentration of Tris buffer

The effect of Molar concentration of Tris buffer (pH = 7) on the RFI of Tams. HCl in the presence of SDS was studied. Different Molar concentrations ranging from 0.025 M up to 0.2 M were used (Fig. 6). No significant difference in the RFI of the studied drug was observed upon changing the Molar concentration of buffer, so 0.1 M Tris buffer was used to adjust pH of the reaction.

d) Effect of time

The effect of time on the RFI of the studied drug was studied over time intervals ranging from five minutes up to two hours. It was found that enhancement of the fluorescence intensity was immediately developed and remained stable for two hours as shown in (Fig.7).

e) Effect of diluting solvent

The effect of different diluting solvents on the RFI of Tams. HCl in the presence of SDS was investigated using water, methanol, n-propanol and acetonitrile. It was found that water was the best solvent for dilution, as it gave the highest RFI and the lowest blank reading, also it is cheap, safe and available solvent. Other solvents showed no marked change in the RFI of Tams. HCl (Fig. 8).

Validation of the proposed method

The proposed method was tested for linearity, LOQ, LOD, accuracy, precision and robustness.

a) Linearity

After optimizing the conditions, evaluation of linearity of the assay method was performed by analysing seven concentrations for the studied drug (standard calibration plots). The calibration graph of Tams. HCl was constructed by plotting the enhanced RFI of the studied drug in SDS medium against final concentration in $\mu\text{g ml}^{-1}$ (Fig.9). The



fluorescence – concentration plot was linear over the concentration range 0.10 -1.20 $\mu\text{g ml}^{-1}$. Analysis of the data gave the following regression equation:

$$\text{RFI} = 829.55 C - 2.150 \quad (r = 0.9998)$$

Where RFI is the relative fluorescence intensity, C is the concentration of the studied drug in ($\mu\text{g ml}^{-1}$) and (r) is the correlation coefficient.

The limit of quantitation (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendations [34], below which the calibration graph is non-linear. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected.

The values of LOQ and LOD were calculated according to the following equation

$$\text{LOQ} = 10 \sigma / S \quad \text{LOD} = 3.3 \sigma / S$$

Where σ = the residual standard deviation of the regression line

And S = slope of the calibration curve

The results of LOQ and LOD are summarized in Table (1). The proposed method was evaluated by calculating accuracy as percent relative error and precision as percent relative standard deviation, the results are abridged in Table (1). Calibration data of Tams. HCl in pure form by the proposed method are presented in Table (1).

b) Accuracy and Precision

The results of the proposed method were compared with those obtained using the official method [3]. Statistical analysis [35] of the results using Student's t-test and Variance ratio F-test revealed no significant difference between the two methods regarding accuracy and precision, respectively Table (2).

c) Repeatability

The repeatability (intra day precision) was evaluated through replicate analysis of the studied drug in pure form and in Tamsulin capsule using three different concentrations (0.40, 0.60 and 0.80 $\mu\text{g ml}^{-1}$) and each concentration was measured three successive times. The results are summarized in Table (3).

d) Intermediate Precision

The Intermediate Precision (inter-day precision) was evaluated through replicate analysis of the studied drug in pure form and in Tamsulin capsule on three successive days. The results are abridged in Table (3). The precision of the proposed method was fairly high, as indicated by the low values of SD and %RSD, respectively. Also the inter-day and intra-day accuracy was proved by the low values of %Er.

e) Robustness of the method

The robustness of the proposed method was demonstrated by the constancy of the RFI with minor changes in the experimental conditions, such as the pH (7 ± 0.5) and the change in the volume of SDS ($2.0 \pm 0.5\text{mL}$). These minor changes that may take place during the experimental operation did not affect the RFI.

Pharmaceutical application

The proposed method was applied to the determination of Tams. HCl in its commercial dosage forms (Tamsulin capsule, Tamic capsule). Tams.HCl is official in the USP and so are its capsules. The USP states that Tams. HCl capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount. The results obtained by the proposed method were within the USP stated limit and they were compared with those obtained using the reference method [3] Table (4, 5). Statistical analysis of the results obtained using Student's t-test and Variance ratio F-test revealed no significant difference between the performance of two methods regarding accuracy and precision, respectively.

Biological application

The proposed method was applied to the determination of Tams.HCl in spiked human plasma. The intra-day precision was evaluated through replicate analysis of plasma samples spiked with different concentrations of the studied drug (0.4 and 0.8 $\mu\text{g ml}^{-1}$). The mean percentage recovery based on the average of five separate determinations of each concentration was 99.51 ± 5.1 .The results are abridged in Table (6).

Results of stability indicating assay

Tams.HCl was found to be susceptible to acid degradation after boiling with 2M HCl for 1 hour 18% of the drug was degraded. The studied drug was found to be less susceptible to alkaline degradation as only 7% of the drug was degraded after boiling with 2M NaOH for 1 hour.

Oxidative degradation of Tams.HCl with hydrogen peroxide was also studied. It was found that treating the drug with 30% H_2O_2 at room temperature, according to the procedure described above, resulted in immediate degradation of Tams.HCl. The amount of the degraded drug was dependent on the volume of H_2O_2 added; 84% degradation was observed upon the addition of 7.0 ml 30% H_2O_2 to the drug (Fig. 9).

The effect of 10% H₂O₂ on Tams. HCl was also studied, as shown in Fig. (10), addition of 6.0 ml 10% H₂O₂ caused 41% degradation of the studied drug. The results of stability studies were calculated from fluorescence measurement of Tams.HCl in both aqueous and micellar media and they were so close in both conditions.

The effect of UV light on the stability of Tams.HCl was studied by exposing the aqueous drug solutions to the UV light. No considerable degradation was observed upon exposure of the drug to UV light up to 3 hours.

Mechanism of the micellar enhancement effect of SDS on Tams. HCl fluorescence:

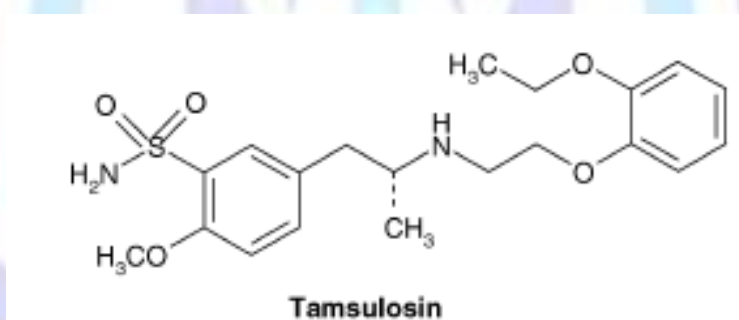
In order to establish whether the sensitization process of Tams.HCl fluorescence was only due to an increase in quantum yield, or whether it was affected by an increase in absorption at the excitation wavelength, the molar absorptivity of Tams.HCl in the presence of SDS was determined at 280nm (λ_{ex}). Table (7) shows that the ϵ micellar: ϵ water ratio was approximately 1.0, which indicates that the increase in sensitivity is not affected by an increase in the absorption of the studied drug at its λ_{ex} , but it is due to an increase in the quantum yield of Tams.HCl by protection of the lowest excited singlet state in the micellar microenvironment from non-radiative processes. The quantum yield of Tams.HCl was found to be 0.029 in aqueous medium and 0.051 in the presence of SDS as shown in Table (8). The quantum yield was calculated according to the following equation [36]:

$$\Phi_u = [\Phi_s F_u / F_s] \times [A_s / A_u]$$

where Φ_u and Φ_s referred to the fluorescence quantum yields of the drug and quinine sulphate (Q.S.), respectively; F_u and F_s represented the integral fluorescence intensity of the drug and quinine, respectively; A_u and A_s referred to the absorbance of the drug and quinine at the excitation wavelength, respectively.

CONCLUSION

The micellar enhanced spectrofluorimetric method proposed for the determination of Tams. HCl, has the advantages of simplicity, speed, accuracy, low detection limit and the use of inexpensive equipment. It can be considered as an attractive alternative to numerous other more complicated methods. The proposed method could be successfully applied for the determination of the studied drug in pure form and in pharmaceutical preparations; the results are in good agreement with the official methods. Moreover, it has been adapted for stability studies of the studied drug as a rapid and simple alternative to the reported stability-indicating HPLC methods.



C₂₀H₂₈N₂O₅S M.Wt = 408.5

C₂₀H₂₈N₂O₅S . HCl M.Wt = 445

Fig. (1): Tamsulosin Hydrochloride

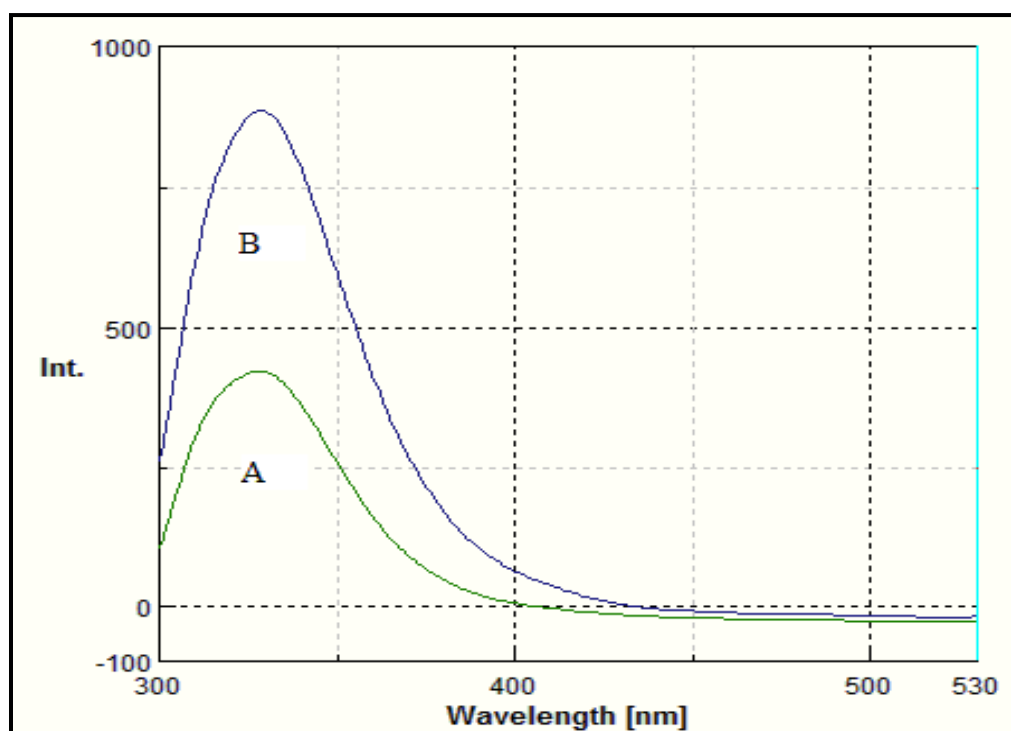


Fig. (2): Emission spectra of Tams. HCl ($1.0 \mu\text{g ml}^{-1}$) in presence of
(A) : aqueous Tris buffer of pH 7.
(B) : 1% SDS medium of pH 7.

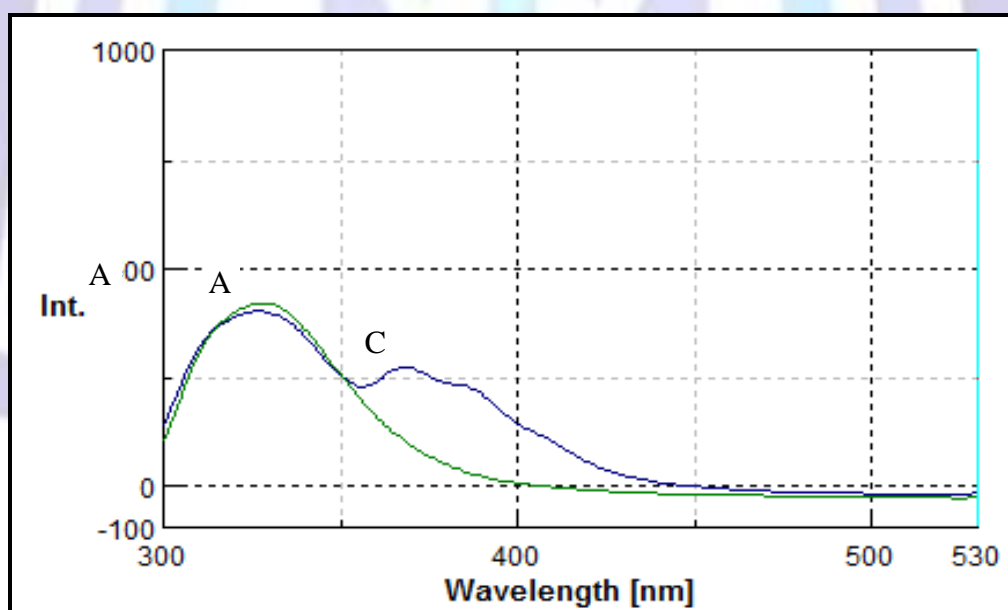


Fig. (3): Emission spectra of Tams. HCl ($1.0 \mu\text{g ml}^{-1}$) in presence of
(A) : aqueous Tris buffer of pH 7.
(C) : 0.5% β -Cyclodextrine of pH 7.

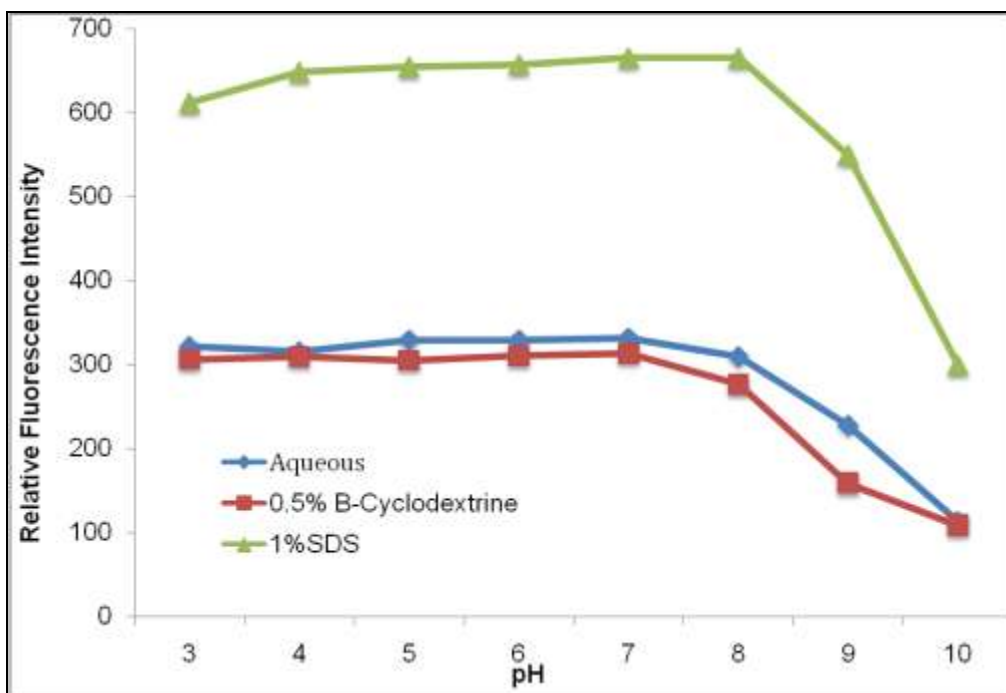


Fig. (4): Effect of pH on the fluorescence intensity of Tams. HCl ($0.8 \mu\text{g ml}^{-1}$) in presence of : \blacklozenge Aqueous , \blacksquare 0.5% β -Cyclodextrine and \blacktriangle 1% SDS media.

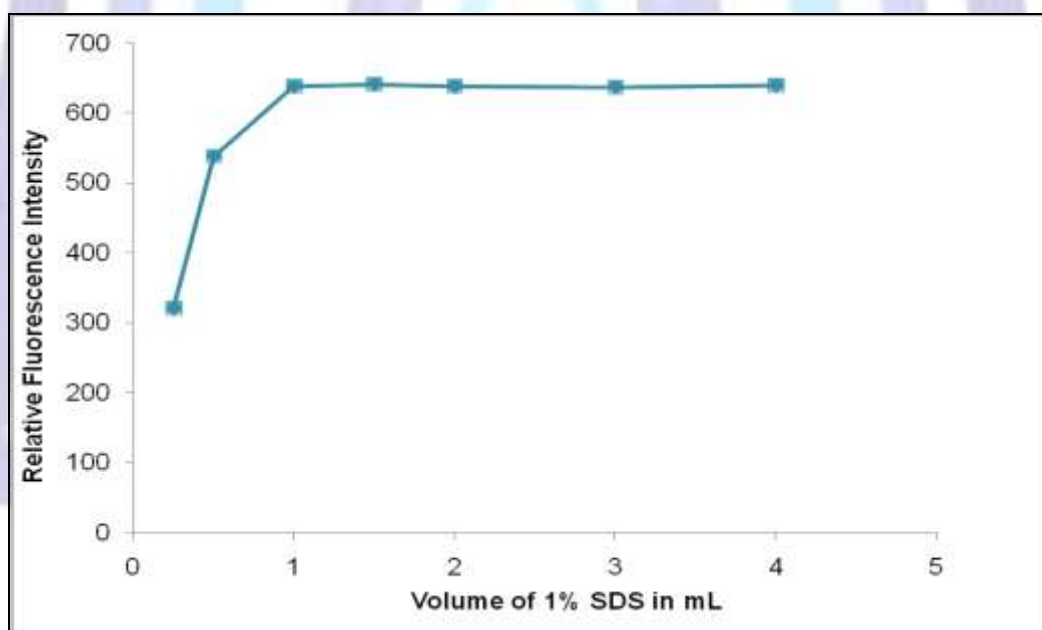


Fig. (5):Effect of volume of 1%SDS solution on the relative fluorescence intensity of Tams. HCl ($0.8 \mu\text{g ml}^{-1}$).

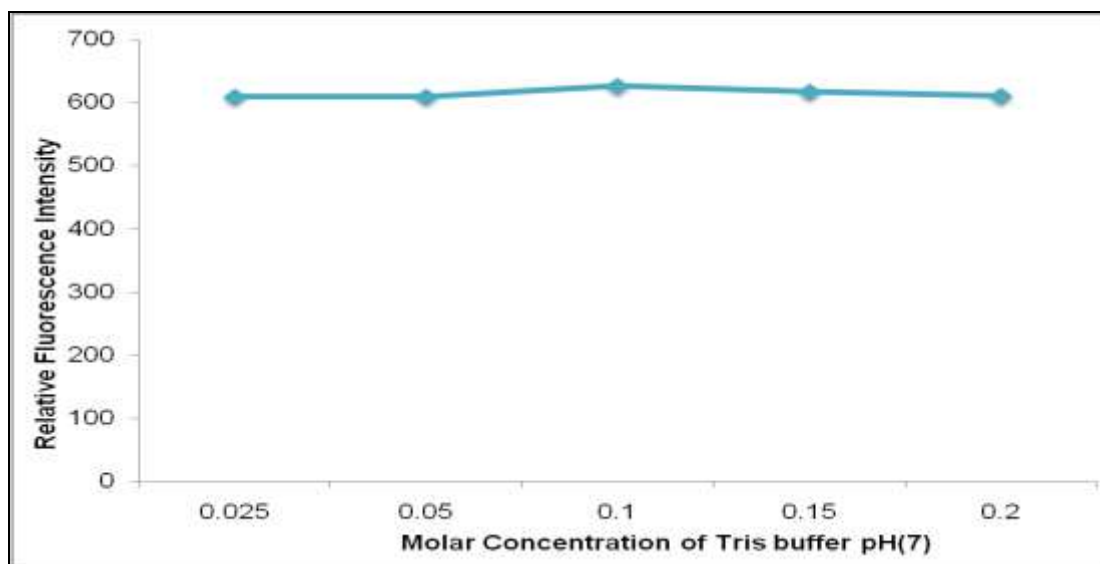


Fig. (6): Effect of Molar concentration of Tris buffer (pH = 7) on the relative fluorescence intensity of Tams. HCl ($0.8 \mu\text{g ml}^{-1}$).

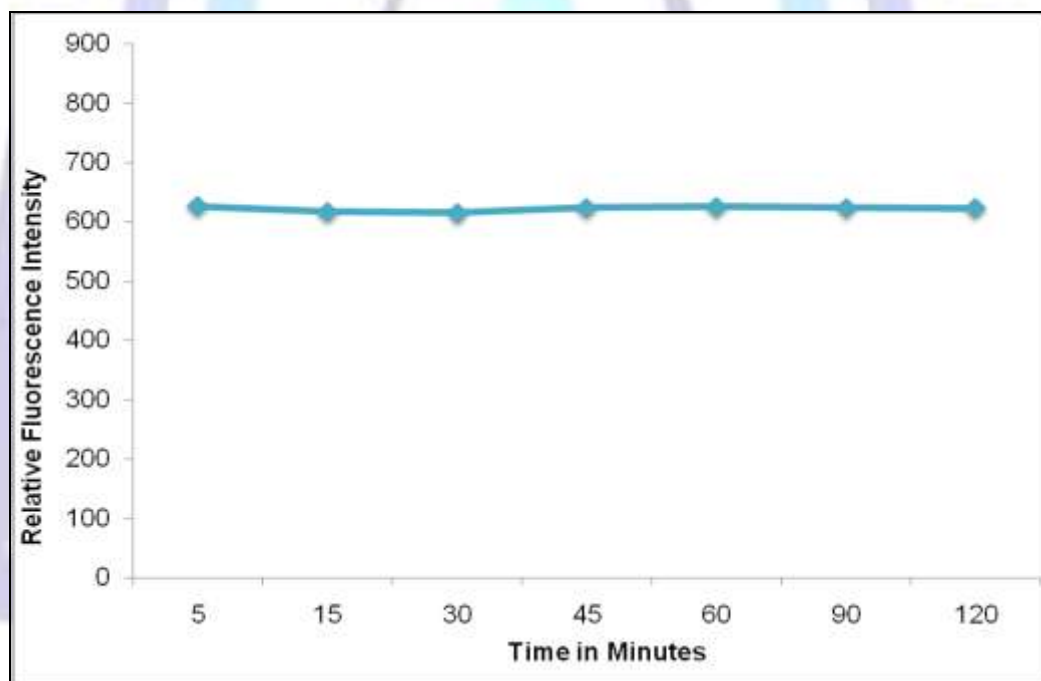


Fig. (7): Effect of time on the relative fluorescence intensity of Tams. HCl ($0.8 \mu\text{g ml}^{-1}$).

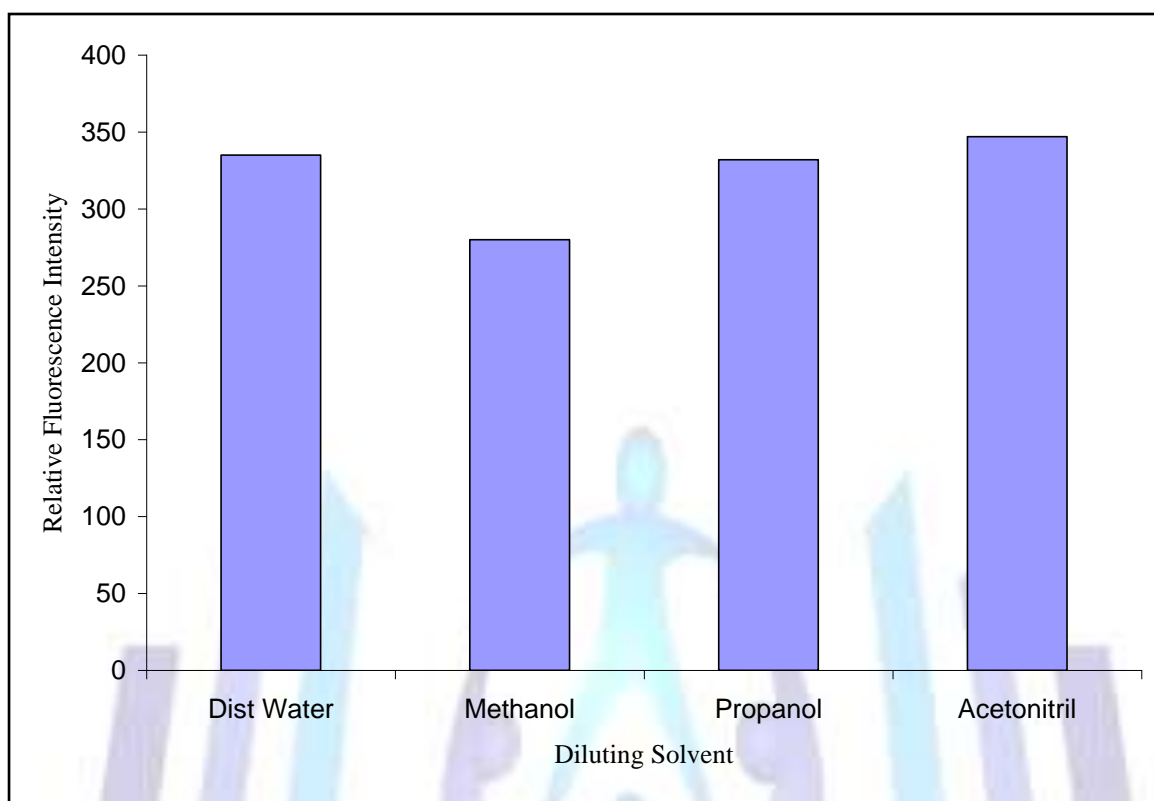


Fig. (8): Effect of diluting solvent on the relative fluorescence intensity of Tams. HCl ($0.4 \mu\text{g ml}^{-1}$).

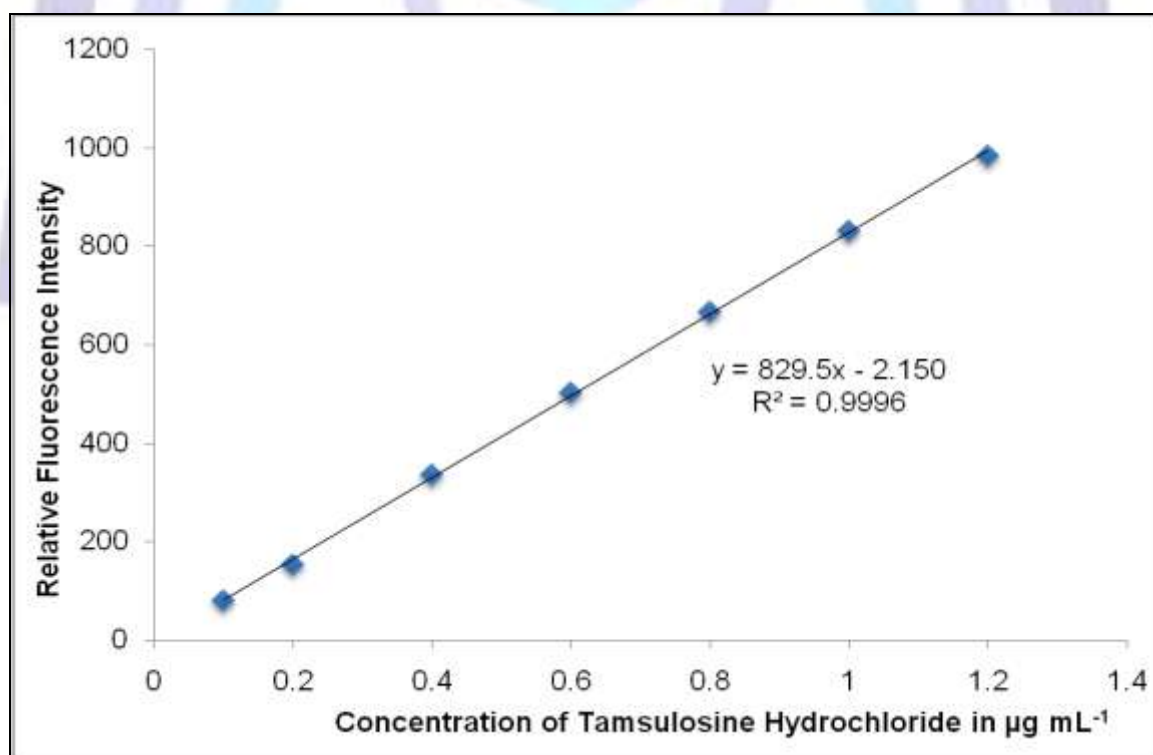


Fig. (9): Calibration graph of Tams. HCl in pure form using SDS system

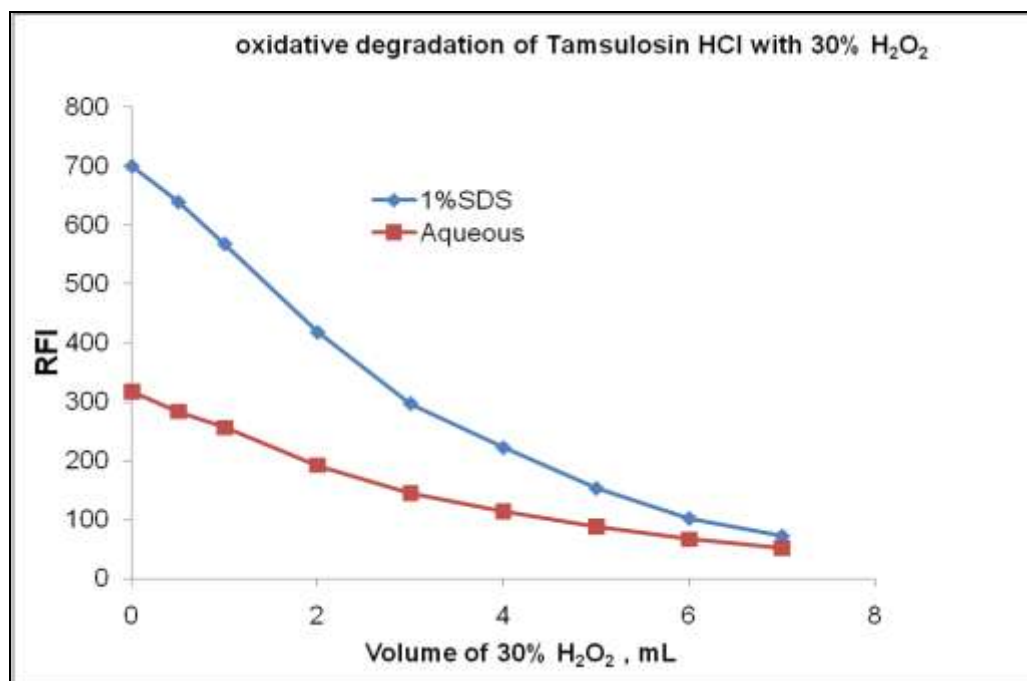


Fig. (10): Plot of relative fluorescence intensity of Tams. HCl ($1.0 \mu\text{g ml}^{-1}$) versus volume of 30% H₂O₂ in presence of : ■ Aqueous and ♦ 1% SDS media.

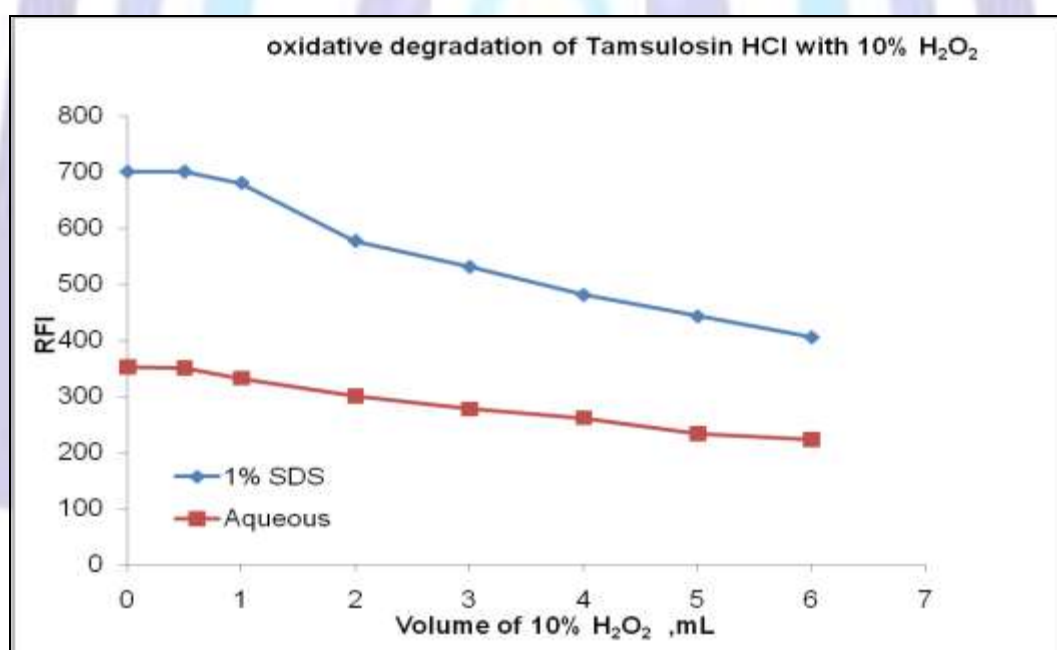


Fig.(11):Plot of relative fluorescence intensity of Tams. HCl ($1.0 \mu\text{g ml}^{-1}$) versus volume of 10% H₂O₂ in presence of : ■ Aqueous and ♦ 1% SDS media.



Table (1) : Calibration data and Performance data of the proposed method for determination of Tams.HCl in pure form.

Parameter	Concentration $\mu\text{g ml}^{-1}$	RFI at 328 nm
	0.1	80
	0.2	154
	0.4	335
	0.6	502
	0.8	665
	1.0	831
	1.2	984
Concentration range ($\mu\text{g ml}^{-1}$)	0.10 -1.20 $\mu\text{g ml}^{-1}$	
Regression equation	RFI = 829.55 C- 2.150	
Correlation coefficient (r)	0.9998	
Standard deviation of the residuals ($S_{y/x}$)	7.557	
% Recovery	99.42	
\pm S D	2.58	
%RSD	2.60	
% Er	0.58	
(LOQ)	0.09 $\mu\text{g ml}^{-1}$	
(LOD)	0.03 $\mu\text{g ml}^{-1}$	



Table (2) : Statistical analysis of the results of Tams.HCl in pure form by the proposed method, compared with Reference method [3].

Parameter	proposed method			Official method USP [3]
	$\mu\text{g ml}^{-1}$ taken	$\mu\text{g ml}^{-1}$ found	% Recovery	% Recovery
	0.10	0.099	99.00	99.0 100.7 101.2 101.8
	0.20	0.188	94.00	
	0.40	0.406	101.50	
	0.60	0.608	101.33	
	0.80	0.805	100.63	
	1.00	1.004	100.40	
	1.20	1.189	99.08	
Mean (X)	99.42			100.68
\pm SD	2.58			1.20
No of experiments	7			4
Variance	6.656			1.44
F-test	4.62 (14,73)*			
Students t-test	0.906 (2.26)*			

N.B. Figures between parentheses are the tabulated F and t values respectively at $P = 0.05$ [35].



Table (3): Accuracy and Precision data of the proposed method for the determination of Tams.HCl in pure and dosage forms (Tamsulin capsule).

Parameter	Intra-day precision (Repeatability)			Inter-day precision (intermediate precision)		
	0.40	0.60	0.80	0.40	0.60	0.80
Concentration $\mu\text{g mL}^{-1}$						
%Recovery Of pure Tams	101.50	101.33	100.63	102.75	99.17	100.75
	100.00	102.08	100.85	101.50	100.20	101.30
	101.00	99.70	98.25	99.25	97.80	101.13
– Mean (X)	100.83	101.04	99.91	101.17	99.06	101.06
± SD	0.76	1.20	1.44	1.77	1.20	0.28
%RSD	0.75	1.19	1.44	1.75	1.21	0.28
%Er	0.83	1.04	0.09	1.17	0.94	1.06
%Recovery Tamsulin capsule	101.38	99.5	99.88	102.50	100.00	102.00
	99.01	102.83	100.63	102.00	102.20	102.50
	101.8	102.17	100.38	99.80	101.8	99.00
– Mean (X)	100.73	101.50	100.30	101.43	101.33	101.17
± SD	1.50	1.76	0.38	1.44	1.17	1.89
%RSD	1.49	1.73	0.38	1.42	1.15	1.87
%Er	0.73	1.50	0.30	1.43	1.33	1.17



Table (4): Application of the proposed method for the analysis of Tams.HCl in dosage form (Tamsulin capsule):

Parameter	proposed method			Official method USP ^[3]
Concentration taken ($\mu\text{g mL}^{-1}$)	Concentration found ($\mu\text{g mL}^{-1}$)	% Recovery	– Mean (X)	% Recovery
0.40	0.405	101.38	100.73	99.60
	0.396	99.01		
	0.407	101.80		
0.60	0.597	99.50	101.50	101.4
	0.617	102.83		
	0.613	102.17		
0.80	0.799	99.88	100.30	101.9
	0.805	100.63		
	0.803	100.38		
1.00	0.988	98.80	100.13	100.7
	1.005	100.50		
	1.011	101.10		
– Mean (X)	100.67			100.90
Nominal content of tamsulin capsule (mg)	0.403			0.404
± SD	0.611			0.997
%RSD	0.807			0.988
% ER	0.67			0.90
No of experiments	4			4
Variance	0.37			0.994
F - test	2.69 (15.44)*			
Student's t-test	0.402 (2.45)*			

N.B. Figures between parentheses are the tabulated F and t values respectively at $P = 0.05$ ^[35].



Table (5): Application of the proposed method for the analysis of Tams.HCl in dosage form (Tamic capsule):

Parameter	proposed method			Official method USP ^{B]}
	Concentration taken ($\mu\text{g mL}^{-1}$)	Concentration found ($\mu\text{g mL}^{-1}$)	% Recovery	Mean (X)
0.20	0.201	100.50	100	99.65
	0.198	98.00		
	0.203	101.50		
0.40	0.409	102.25	101.33	98.70
	0.405	101.25		
	0.402	100.50		
0.60	0.611	101.83	101.50	98.10
	0.603	100.50		
	0.613	102.17		
0.8	0.790	98.75	98.79	98.90
	0.797	99.63		
	0.784	98.00		
Mean (X)	100.41			98.84
Nominal content of tamic capsule (mg)	0.402			0.395
\pm SD	1.27			0.64
%RSD	1.26			0.65
% ER	0.41			1.16
No of experiments	4			4
Variance	1.61			0.41
F - test	3.93 (15.44)*			
Student's t-test	2.21 (2.45)*			

N.B. Figures between parentheses are the tabulated F and t values respectively at $P = 0.05$ ^[35].



Table (6): Application of the proposed method for the analysis of Tams.HCl in spiked human plasma:

Concentration added ($\mu\text{g ml}^{-1}$)	Concentration found ($\mu\text{g ml}^{-1}$)	% Recovery
0.4	0.418	104.50
	0.430	107.50
	0.366	91.50
	0.375	93.75
	0.387	96.75
0.8	0.777	97.13
	0.815	101.90
	0.806	100.75
	0.824	103.00
	0.775	96.90
0.8	0.844	105.4
	0.828	103.50
	0.813	101.63
	0.724	90.50
	0.784	98.00
– Mean (X)		99.51
± SD		5.1
%RSD		5.13
% ER		0.49

Table (7): Determination of the molar absorbtivity of Tams.HCl in aqueous and micellar media:

Concentration taken ($\mu\text{g ml}^{-1}$)	Absorbance In aqueous medium at 280 nm	ϵ water	Absorbance In micellar medium at 280 nm	ϵ micellar	ϵ micellar
					ϵ water
12.5	0.120	4270	0.124	4412.8	1.03
25	0.240	4270	0.248	4412.8	1.03

**Table (8): Determination of the fluorescence quantum yield of Tams.HCl in aqueous and micellar media:**

Concentration Taken of (Q.S.) ($\mu\text{g ml}^{-1}$)	Absorbance at 280 nm	Integral fluorescence of (Q.S.)	Concentration Taken of studied drug ($\mu\text{g ml}^{-1}$)	Integral fluorescence of studied drug In aqueous medium	Integral fluorescence of studied drug In micellar medium
5.0	0.01	14491	12.5	9072	16098
10.0	0.02	29599	25	15871	26654

N.B. Dilute solution of quinine sulfate dissolved in 0.05 M sulfuric acid with fluorescence quantum yield of 0.55 was used as reference reagent ^[36].

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