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IJPBS |Volume 2| Issue 1 |JAN-MARCH |2012|12-19

Simple and Inexpensive Methods Development for the estimation of Tamsulosin Hydrochloride as a single component from Its Solid Dosage Forms by Visible Spectrophotometry

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BIOLOGICAL SCIENCES

Research Article

RECEIVED ON 02-12-2011

ACCEPTED ON 12-1-2012

ABSTRACT

Tamsulosin hydrochloride (TAM) is an uroselective alpha1A-adrenergic receptor antagonist which is used in benign prostatic hyperplasia (BPH).The drug is official in European Pharmacopoeia. Two direct, simple and sensitive visible spectrophotometric methods are described for the assay of tamsulosin hydrochloride in pure and solid dosage forms. The first method M_1 is based on the formation of yellowish brown colored species by the drug with Folin reagent and exhibits absorption maxima at 440 nm. Second method (M_2) is based on the formation of purple red colored species with sodium nitroprusside–acetaldehyde reagent exhibiting maximum absorption at 560 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (16-48) µg/ml for method M_1, and (8.0-24) µg/ml for method M_2 respectively. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the tamsulosin hydrochloride in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

KEYWORDS: *Assay, BPH, Folin reagent, Sodium nitroprusside, Acetaldehyde, Nucleophillic substitution, Statistical analysis.*

INTRODUCTION

Tamsulosin hydrochloride (TAM) (**Fig.1**), 5-[(2R)-2-[2-(2-ethoxyphenoxy) ethylamino] propyl]-2-methoxybenzenesulfonamide hydrochloride is a new type of highly selective α_{1A} adrenergic receptor antagonist for treatment of benign prostatic hyperplasia (BPH)[1]. The drug exits in two enantiomeric forms, but only R-isomer is the pharmaceutically active component. It works by blocking α-receptors that are found in the muscle of the prostate gland, which causes the muscle in the prostate to relax. This allows urine to flow freely post the prostate and relieves the urinary symptoms. The drug is extensively metabolized by cytochrome P450 enzymes in the liver and the most frequently prescribed medication for the treatment of lower urinary tract symptoms associated with BPH. Compared to other α-antagonists, TAM has greater specificity for α-1-receptors in the human prostate and does not affect receptors on blood vessels. The drug is official in European Pharmacopoeia [2].

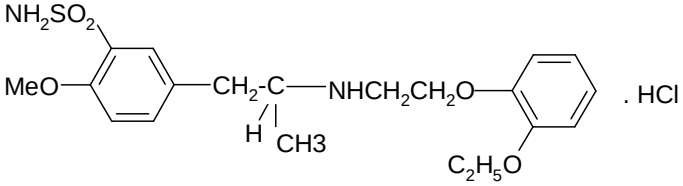

Fig.1: Chemical structure of TAM

Some analytical methods which include HPLC [3-11], LC-MS-MS [12-18], HPTLC [19-21] Radioreceptor assay [22-23], Non aqueous potentiometric[24], voltametry[25], Capillary electrophoresis [26], spectroflorimetric[27] UV[28-29] and visible spectrophotometric [30-31] have been reported in the literature for Determination of TAM in biological fluids (more) and pharmaceutical preparations(less). The main purpose of the present study was to establish relatively simple, sensitive, validated and inexpensive extraction free Visible spectrophotometric methods for the determination of TAM in pure form and in pharmaceutical preparations, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity. So the authors have made some attempts in this direction and succeeded in developing these methods based on the reaction between the drug and folin reagent [32] (M_1) or sodium nitro prusside-acetaldehyde reagent[33] (M_2) under specified experimental conditions. The proposed methods for TAM determination have many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. These methods can be extended for the routine quality control analysis of pharmaceutical products containing TAM.

MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals: A Shimadzu UV-Visible spectrophotometer 1601 with10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361 was used for pH measurements. All the chemicals used were of analytical grade. Pure TAM drug was obtained as a gift sample from M/s Tychy Industries, Hyderabad (AP). Tablets were purchased from local market.

Folin reagent (NQS) solution (Loba, 0.5%, $1.92x10^{-2}$M prepared by dissolving 500mg of NQS in 100 ml of distilled water), aqueous solutions of sodium nitro prusside (SNP, E. Merck, 1.0%, $3.35x10^{-2}$M), acetaldehyde (10%), phosphate buffer of pH 8.0 (prepared by mixing 30 ml of 0.067M potassium hydrogen phosphate and 970 ml of 0.067M disodium hydrogen phosphate and pH adjusted to 8.0) were prepared.

Preparation of Standard stock solution: The standard stock solution (1mg/ml) of TAM was prepared by dissolving 100mg of TAM in 10 ml 0.1M sodium hydroxide and the volume was brought to 100 ml with distilled water. The working standard solutions of TAM were obtained by appropriately diluting the standard stock solution with the same solvent (M_1- 400 µg/ml & M_2-200 µg/ml). The prepared stock solution was stored at 4° C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Preparation of Sample solution: About 20 tablets or capsules were weighed to get the average tablet or capsule weight and pulverized. The powder equivalent to 100mg of TAM was weighed, dispersed in 25ml of Isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No 41.The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Determination of wavelength maximum (λ_{max}):

Method M_1: The 3.0 ml of working standard solution of TAM (400µg/ml) was taken in 25ml standard flask. To this, 1.0ml of folin reagent ($1.092x10^{-2}$M), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 min. to get a concentration of 48µg/ml. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 360-560nm by UV-Visible spectrophotometer. From the spectra (**Fig.2**), it was concluded that 440nm is the most appropriate wavelength for analyzing TAM with suitable sensitivity.

Method M_2: The 3.0 ml of working standard solution of TAM (200µg/ml) was taken in 25 ml calibrated tubes containing 15ml of buffer pH 8.0. To this, 1.0 ml each of SNP solution and acetaldehyde were added successively and shaken

for 2 minutes and kept aside for 15 minutes at room temperature and made up to the mark with distilled water and sonicated for 1 min, to get a concentration of 24µg/ml. In order to investigate the wavelength maximum, the colored solution was scanned in the range of 400-700nm by UV-Visible spectrophotometer. From the UV spectra (**Fig.4**), it was concluded that 560nm is the most appropriate wavelength for analyzing TAM with suitable sensitivity.

Preparation of calibration curve:

Aliquots of the standard TAM solution [1.0-3.0ml, 400µg/ml (M_1)] were placed in a series of 25ml standard flask. Then 1.0ml of folin reagent ($1.092x10^{-2}M$), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 min. The absorbance was measured at 440 nm (M_1) against a reagent blank within the stability period (5minutes to 30min). The calibration graph was constructed by plotting the drug concentration versus absorbance. The amount of drug was computed from its calibration graph (**Fig. 3**).

Aliquots of working standard TAM drug solution (200µg/ml) such as 1.0, 1.5, 2.0, 2.5, and 3.0 ml were taken separately in a series of 25ml calibrated tubes containing 15ml of buffer pH 8.0. Then 1.0ml each of SNP solution and acetaldehyde were added successively and shaken for 2 minutes and kept aside for 5 minutes at room temperature and made up to the mark with distilled water and sonicated for 1 min. The absorbance was measured at 560 nm (M_2) against a reagent blank within the stability period (5minutes to 30min).The calibration graph was constructed by plotting the drug concentration versus absorbance. The amount of drug was computed from its calibration graph (**Fig. 5**).

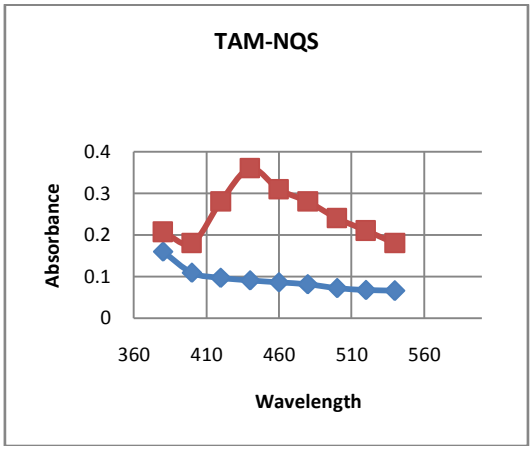

Fig.2: Absorption spectra of TAM-NQS

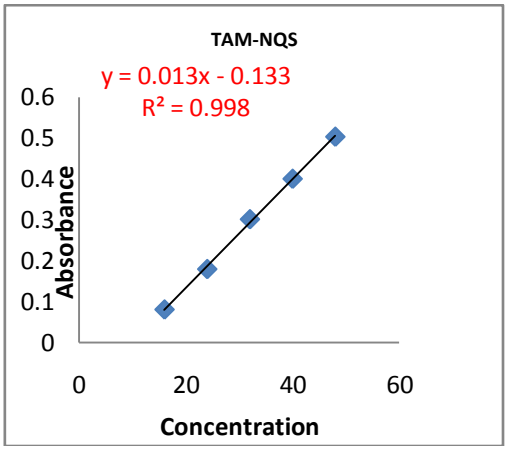

Fig.3: Beer's Law plot of TAM-NQS

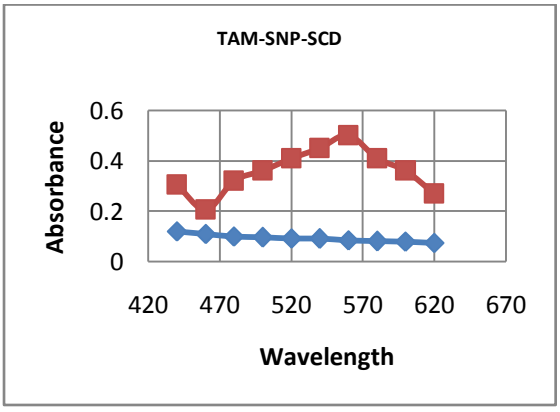

Fig.4: Absorption spectra of TAM-SNP-ACD

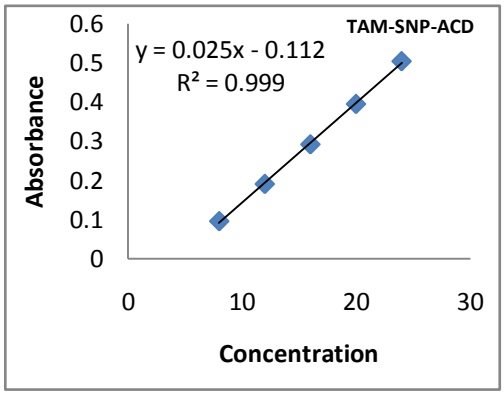

Fig.5: Beer's Law plot of TAM-SNP-ACD

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents, pH buffer solutions and solvent for final dilution of the colored species were studied. Distilled water was found to be best solvent for final dilution. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile have no additional advantage in increasing the intensity of the color in both methods. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in **Table-1**.

Commercial formulations containing TAM were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in **Table-2**.

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS

Parameter	Method M_1	Method M_2
λ_{max}	440 nm	**560 nm**
Beer's law limit(µg/ml)	16-48	**8-24**
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.004238411	**0.002191781**
Molarabsorptivity (Litre/mole/cm)	104987.4688	**203022.125**
Correlation coefficient Regression equation (Y)*	0.998	**0.999**
Intercept (a)	-0.133	**-0.112**
Slope(b)	0.013	**0.025**
%RSD	1.114	**0.927**
% Range of errors(95% Confidence limits) **0.05 significance level**	1.17	**0.973**
0.01 significance level	**1.83**	**1.53**

*Y = a + b x, where Y is the absorbance and x is the concentration of TAM in µg/ml.

TABLE-2: ANALYSIS OF TAMSULOSIN HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS BY PROPOSED AND REFERENCE METHODS.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
A	Batch-1	0.2	0.199 ± 0.001	0.513	4.57	0.199 ± 0.0007	**99.54 ± 0.73**
	Batch-2	0.4	0.398 ± 0.002	0.38	3.72	0.397± 0.0044	**99.41 ± 0.56**
B	Batch-1	0.2	0.199 ± 0.0007	0.164	1.32	0.199 ± 0.0007	**99.406 ± 0.389**
	Batch-2	0.4	0.398 ± 0.002	0.95	3.87	0.397± 0.0044	99.59 ± 0.553

*** Different batches from two different companies **Average ± Standard deviation of six determinations, the t- and F-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t =2.57 and F = 5.05.**

 # Recovery of 10mg added to the pre-analyzed sample (average of three determinations). Reference method (reported UV method) using distilled water (λ_{max}=279nm).

Chemistry of colored species:

In the present investigation, the presence of aliphatic secondary amino group of TAM permits the development of visible spectrophotometric methods for its determination. In method M_1, yellowish brown colored species (N-alkyl amino napthaquinone) was formed by replacement of the sulphonate group of the napthaquinone sulphonic acid by a secondary amino group of drug.

Cullies and Waddington[34] found that many secondary but not primary or tertiary amines react with sodium nitro prusside and acetaldehyde under mild alkaline conditions. Wolfe and Swine hart[35] have reported the formation of $[Fe(CN)_5H_2O]^{3-}$ in aqueous solution of sodium nitro prusside. The proposed method M_2 exploits structural features aliphatic secondary amine of the TAM molecule. The nature of colored species formation with sodium nitro prusside-acetaldehyde reagent is initial N-alkyl vinyl amine formation with acetaldehyde then followed by formation of colored inner molecular complex with sodium nitro prusside. The formation of colored species with these reagents may be assigned through above analogy as shown in schemes (**Fig.6**).

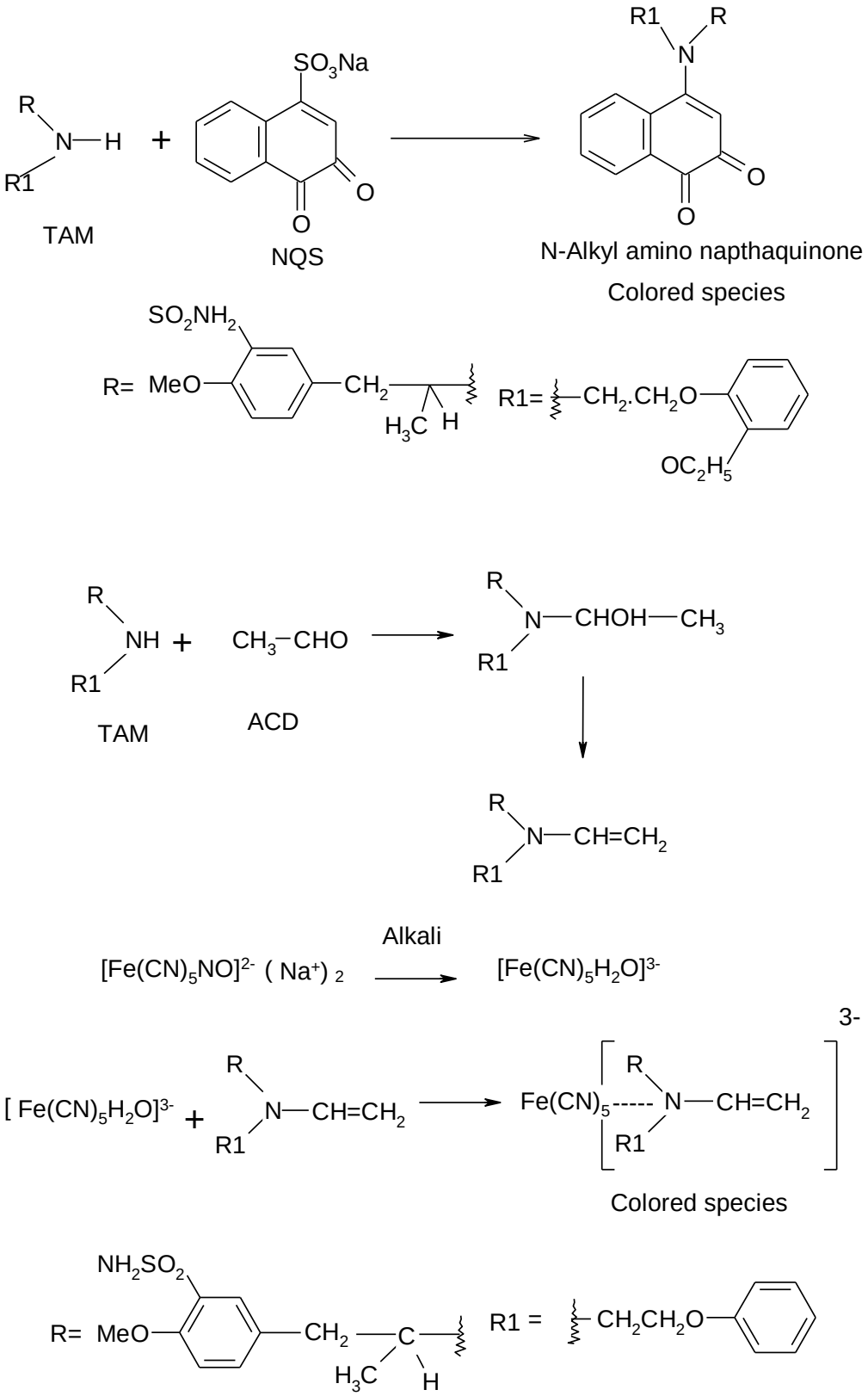

Fig.6: Scheme of the proposed methods

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed colorimetric methods possesses reasonable precision, accuracy, and are simple, sensitive and can be used as alternative methods to the reported ones for the routine determination of TAM depending on the need and situation.

ACKMOWLEDGEMENTS

The authors are very much thankful to the m/s Tychy Industries, Hyderabad, Andhra Pradesh (India) for providing gift sample of the drug and also thanks to the University authorities for providing necessary facilities.

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