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Validated spectrophotometric methods for the determination of Tadalafil in pharmaceutical formulations

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ABSTRACT

Two simple and sensitive UV-Visible spectrophotometric methods A and B for the determination of *tadalafil* have been described. These methods are based on the formation of bluish green color and blue colored chromogens obtained when the drug was condensed with Isatin and Xanthydrol exhibiting λ_{max} at 665 and 640nm respectively. These methods obey Beer's Law in the concentration ranges of 0.4-2.0 and 4-20 µg/mL. The optimum conditions for all color development are described and the proposed methods have been successfully applied for the determination of tadalafil in bulk drug and pharmaceutical formulations. The common execipients and additives did not interfere in this method.

Key words: Tadalafil, chromogen, spectrophotometry, pharmaceutical.

INTRODUCTION

Tadalafil (CIALIS), an oral treatment for erectile dysfunction, is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Tadalafil is chemically designated as pyrazino[12 ,22 :1,6]pyrido [3,4-b] indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a- hexahydro-2-methyl-, (6R,12aR). In view of the great importance and wide use of tadalafil, different analytical methods have been reported for its determination in biological fluids, which include high performance liquid chromatography (HPLC),[1-3] liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)[4-6] and capillary electrophoresis (CE)[7]. These reported methods such as HPLC, LC-MS/MS and CE are sensitive but expensive due to high cost. The main problem associated with these determinations is the laborious cleanup procedure required prior to analysis of drug when compared with spectrophotometry is attractive because of speed, and simplicity.

The aim of this study was to develop and validate two UV-Visible spectrophotometric methods for the determination of tadalafil in the presence of formulation. Method A is based on the condensation reaction of tadalafil with isatin to form colored species which absorbs maximally at 665 nm. Method B utilizes the condensation reaction of tadalafil with xanthydrol to form blue colored chromogens peaking at 640nm. The reaction conditions are optimized and validated .The proposed method was applied to determine the tadalafil in its drug formulations including tablet and injection and satisfactory results were obtained in comparison with official method.

MATERIAL AND METHODS

Apparatus

All spectral measurements were made on ELICO SL-159 double beam, UV – visible spectrophotometer with 1 cm quartz cells was used for all absorbance measurements.

Reagents and Standards

Standard stock solution (1.0 mg/mL)

An stock solution of Tadalafil was prepared by dissolving 100 mg of it in 100 mL 5% acetic acid solution.

Isatin solution (0.04%, w/v)

Prepared by dissolving 40mg of Isatin in 100mL of acetic acid.

Xanthydrol solution (0.05%, w/v)

Prepared by dissolving 50mg of Xanthydrol in 100mL of acetic acid.

Analysis of Pharmaceutical formulation

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 20 mg of tadalafil was weighed accurately and extracted with methanol to eliminate any interference from excipients. It was filtered through Whatmann No. 42 filter paper and the residue was washed well with methanol for complete recovery of the drug. The methanol was evaporated to dryness and the drug was dissolved in doubly distilled water and diluted to 100 mL with doubly distilled water. It was further diluted if needed and then analyzed following the recommended procedures.

Method A

Aliquots of standard tadalafil solution (0.5-2.5mL; 100ìg.mL^{-1}) was transferred into a series of 10mL calibrated tubes and volume of each test tube adjusted to 3.0mL with methanol. To each test tubes 1.0mL of Isatin (2.718×10^{-2}M) and 1.0mL of Conc.H_2SO_4 were added, while cooling under a tap with constant shaking and the volume was made up to the mark with methanol. The absorbance was read against reagent blank at 665nm between 1-30 min. The amount of drug present was calculated from its calibration graph.

Method B

Aliquots of standard Tadalafil solution (0.5-2.5mL; 200µg.mL^{-1}) was transferred into a series of 10mL calibrated tubes and volume of each of test tube adjusted to 3.0mL with methanol. To each of these tubes 1.0mL of xanthydrol (2.32×10^{-2}M) and 1mL Conc.H_2SO_4 were added, while cooling under a tap with constant shaking and the volume was made up to the mark with methanol. The absorbance was read against reagent blank at 640nm between 1-30min. The amount drug present was calculated from its calibration graph.

In all the above methods, a calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve or deduced using a regression equation calculated from Beer's law data.

RESULTS AND DISCUSSION

The presence of secondary amine group in Tadalafil enabled the use of condensation with isatin, to form blue green colored chromogen in method A (Scheme I) exhibiting λ_{max} at 665nm. In method B (Scheme II) same condensation reaction was followed by xanthydrol, to form blue colored chromogens exhibiting λ_{max} at 640nm. The Beer's law was obeyed by these two methods in the concentration ranges 2.0-10.0 and 4.0-20.0ìg.mL respectively. The optical characteristics such as Beer's law limits, absorption maxima, molar absorptivity, sandell's sensitivity, percent relative standard deviation (%RSD) calculated from six measurements containing 3/4th of the amount of the upper Beer's law limits and percent range of errors (0.05 levels and 0.01 confidence limits) were calculated for the two methods of tadalafil are reported in Table 1.

The optimum concentration for the estimation of tadalafil was established by varying the drug concentration, keeping reagent concentration fixed. After establishing the optimum concentration for the drug, the reagent concentration was varied. The above ranges of drug and reagent concentrations were chosen because the colored species formed gave better absorbance and obeyed Beer's law. The values obtained for the

Table 1: Optical Characteristics and Precision

Optical Characteristics	Method A	Method B
λmax (run)	665	640
Beer's law limits(μg/mL)(C)	2.0-10.0	4.0-20.0
Molar absorptivity (lit.mol^{-1}cm^{-1})	7.70×10^3	2.59×10^4
Sandell's sensitivity (μg/cm^2)-0.001 ab units	0.1456	0.0643
Regression equation(Y = a + bc)*		
Slope (b)	0.2357	0.0194
Intercept (a)	-0.0001	0.0034
Correlation coefficient (r)	0.9991	0.9993
% RSD	1.0033	1.2997
Range of errors**	1.0530	13642
Confidence limits with 0.05 level	1.6514	2.1395
Confidence limits with 0.01 level		

* Y is the absorbance and C is the concentration μg/mL

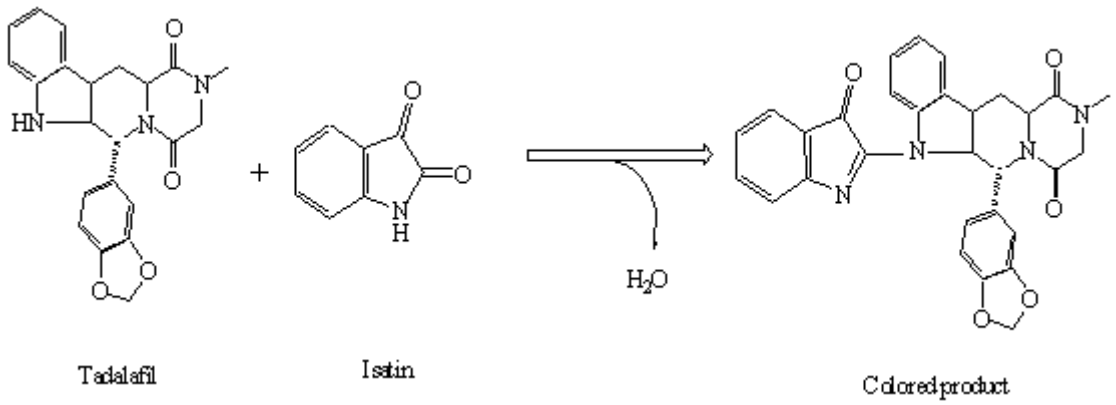
**For six measurements

Table 2: Estimation of Tadalafil in Pharmaceutical formulations

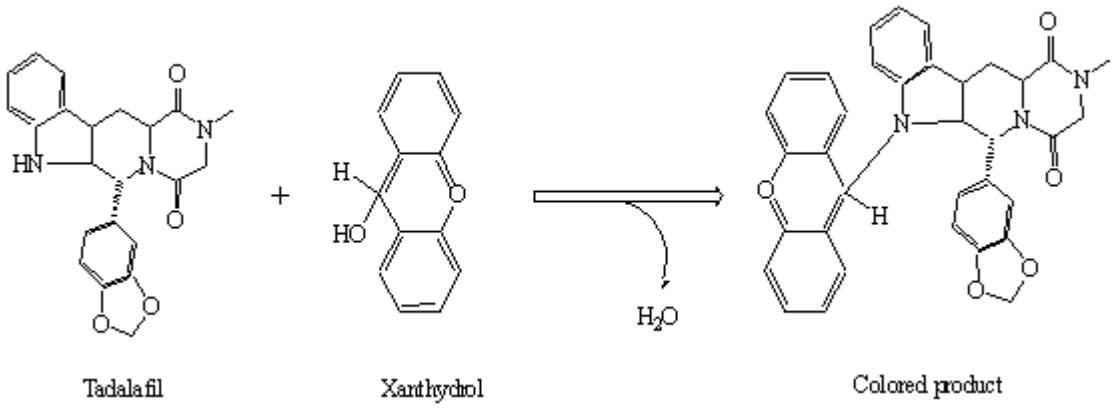
Sample	Labelled amount (mg)	Amount obtained (mg) Proposed methods*		UV method	%Recovery of Proposed methods**	
		MethodA	MethodB		MethodA	MethodB
Tablet - 1	2.5	2.47	2.46	2.48	99.59	99.19
Tablet - 2	5.0	4.94	4.95	4.98	99.19	99.39
Tablet - 3	10.0	9.96	9.94	9.97	99.89	99.69

*Average of six determinations

** Mean and standard deviation of six determinations



Scheme 1



Scheme 2

determination of tadalafil in different brands of Tablet samples 1, 2and 3 by the proposed and U.V methods, are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table 2.

The methods reported here are found to be simple, sensitive, accurate and precise. The reaction occurs at 0-5° C temperature and no extractions procedure is involved as compared with other established methods. The present methods involve the formation of highly stable colored species which makes it easier for the determination of tadalafil from pharmaceutical dosage forms in a routine manner.

These studies revealed that the common execipients usually present in the tablet form, did not interfere at their regularly added levels.

CONCLUSIONS

The developed method is simple, rapid, selective, and inexpensive and exhibits a fair degree of precision and accuracy. The method does not involve any critical reaction conditions. The proposed method can serve as an alternative method for the routine analysis of tadalafil in pure drugs and in pharmaceutical formulations.

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