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Spectrophotometric quantification of tadalafil by oxidative coupling reaction with MBTH reagent

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ABSTRACT

A simple and sensitive spectrophotometric method in visible region has been developed and validated for quantification of tadalafil bulk drug and pharmaceutical dosage forms. The method is based on the oxidative coupling reaction of 3-methyl-2-benzothiazoline hydrazone (MBTH) with tadalafil in the presence of ferric chloride to form green coloured chromogen with absorption maximum at 676 nm. Beer's law is obeyed in the concentration range of 2 – 12 µg/mL with correlation coefficient 0.999, limit of detection and quantification were 15.72 ng/mL and 52.63 ng/mL respectively. When marketed tablet formulations were analyzed, the results obtained by the proposed method were in good agreement with labeled amounts. The developed method was validated statistically as per ICH guidelines. Satisfactory recovery from the spiked samples suggests no interference of any excipients present in the formulations. The developed method is simple, sensitive and can be successfully employed in routine analysis of tadalafil pharmaceutical dosage forms.

KEYWORDS

Tadalafil;
MBTH;
Visible Spectrophotometry.

INTRODUCTION

Tadalafil, which is chemically (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxy- phenyl) pyrazino [20,10:6,1] pyrido [3,4-b] indole-1,4-dione, is an orally administered drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension[1].

A detailed literature survey revealed that several analytical methods are reported for the determination of tadalafil by high performance liquid chromatography (HPLC)[2-4], high performance thin layer chromatography (HPTLC)[5], gas chromatography-mass spectrometry (GC-MS)[6] and spectrofluorimetry[7]. The literature survey revealed that few visible spectrophotometric methods reported for quantification of tadalafil by using bromophenol blue (BPB) dye, folin ciocalteu reagent in the presence of 4% NaOH solution, sodium nitroprusside and hydroxyl amine [8, 9]. The reported spectrophotometric methods are not much sensitive; require high concentration of organic solvents and extraction procedures. Therefore, there is a need for new simple, specific and sensitive spectrophotometric method for quantification of tadalafil in the presence of low concentration of solvent without any extraction procedures[10,11]. From literature, we came to know that, there is no visible spectrophotometric method available for quantification of tadalafil by using MBTH reagent.

This work presents a simple, specific and sensitive visible spectrophotometric method for the quantification of tadalafil based on its oxidative coupling interaction with 3-methyl-2-benzothiazoline hydrazone (MBTH). The proposed method was validated as per ICH guidelines.

EXPERIMENTAL

Double-beam Shimadzu UV-Visible Spectrophotometer 1800, with spectral bandwidth of 1.0 nm, wavelength accuracy ± 0.1 nm and a pair of 1cm path length matched quartz cells were used to measure absorbance of the resulting solution.

Tadalafil standard gift sample was provided by Mylan Pharma Ltd, Hyderabad, India. The commercially available tablet dosage forms Tazzle 10 (Dr Reddy's Laboratories Ltd, Hyderabad) and Megalis 20 (Mecleods Pharamceutical Ltd, Hyderabad) were acquire from local pharmacies. The analytical grade MBTH reagent, ferric chloride and methanol were purchased from Sd fine chemicals, Mumbai.

Preparation of standard stock solution

The standard stock solution (1mg/mL) of tadalafil was prepared by transferring 10 mg of tadalafil in 10 mL volumetric flask and volume was made up to the mark with methanol.

Method validation

The method was validated for linearity, precision, accuracy, selectivity and sensitivity by the following procedures.[12]

Linearity

Aliquots of tadalafil stock solutions were transferred into a series of 10 mL volumetric flask and add aqueous solutions of 0.3% MBTH (2 mL) and 3% ferric chloride solution (2 mL). The volume was then made up to the mark with methanol to prepare a series of standard solutions containing 2-12 µg/mL. The solutions were kept aside for 5 min. Absorbance was measured at 676 nm against blank. The calibration curve was constructed by plotting the analyte absorbance against the concentration (µg/mL).

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

Precision

Precision of the method was determined by intra-day precision and inter-day precision variations as per ICH guidelines. For both intra-day precision and inter-day precision of the samples containing tadalafil 4, 8, and 12µg/mL were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter-day precision). The % RSD was calculated.

Accuracy

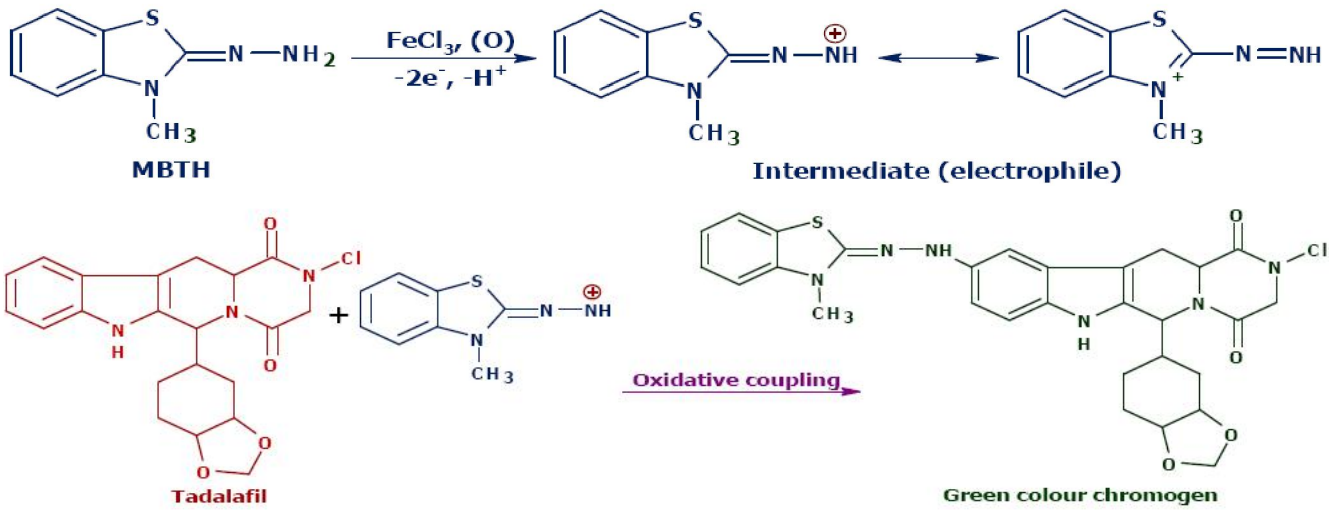
The accuracy of the method was determined by calculating recoveries of tadalafil by the method of standard additions. Tablet powder (TAZZLE-10 and MEGALIS 20) equivalent to 10 mg of tadalafil was transferred into three different 25 mL volumetric flasks and to it 80%, 100% and 120% of pure bulk drug was added respectively and to this 0.3% MBTH, 3% ferric chloride solutions were added. Finally, the volume was made up to the mark with methanol and analyzed by using methanol-reagent as blank. The amounts of tadalafil were estimated by measuring absorbance at appropriate wavelength 676 nm and recovery was verified by estimation of drug in triplicate preparations at each specified concentration level.

Assay of tadalafil in its dosage form:

The accurate quantity equivalent to 10 mg of active ingredient was dissolved in methanol and the volume was made up to 10 mL to get the stock solution of 1 mg/mL. Subsequent dilutions of this solution were made, to it aqueous solutions of 0.3% MBTH (2 mL) and 3% ferric chloride in distilled water (2 mL) were added. The solutions were kept aside for 10 min and stirred occasionally. The solutions were finally made up to the mark with distilled water and the absorbance of the green coloured chromogen was measured at 676 nm against the corresponding reagent blank and analyzed for drug content and their results were statistically validated.

RESULTS AND DISCUSSION

This method is based on the oxidative coupling



Scheme 1 : Coupling reaction mechanism between MBTH and Tadalafil.

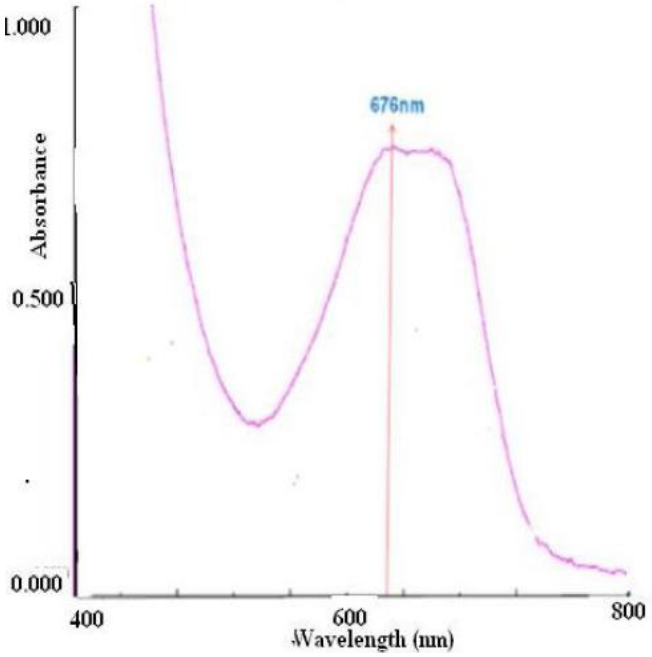


Figure 1 : UV Spectrum of tadalafi

reaction. MBTH gets oxidized and forms an electrophilic intermediate. This intermediate reacts with tadalafil in the presence of ferric chloride. MBTH on oxidation with ferric chloride looses two electrons and one proton to form an electrophilic intermediate, which is the active coupling species, reacts with the coupler by electrophilic attack on the most nucleophilic site of tadalafil to form a green colored species. (Scheme-1) and measured the absorbance of green colour chromogen at 676 nm (Figure-1).

The developed method was statistically validated as per ICH guidelines, follows the Beer's law in the concentration range of 2 - 12 µg/mL with regression

TABLE 1 : Optimum conditions of the proposed method

Parameter	Value
Absorption Wavelength (nm)	676
Range (µg/ml)	2-12
Limit of Detection (ng/ml)	15.72
Limit of Quantification (ng/ml)	52.63
Correlation Coefficient (r^2)	0.999
Slope (m)	0.038
Intercept (c)	0.28
Regression equation	Y=0.038x+0.28

TABLE 2 : Assay of tadalafil in commercial tablets

Brand name	Label claim per tablet (mg)	Amount of Mean drug found (mg) ± SD (n=3)	RSD (%)	% Drug estimated
Tazzle-10	10	9.98± 0.03	0.3	99.8
Megalis-20	20	19.8± 0.25	1.26	99

TABLE 3 : Precision of the proposed method

Concentration (µg/mL)	Intraday Precision (n=6)		Inter-day Precision (n=6)	
	Amount found (mg) ±SD	%RSD	Amount found (mg) ±SD	%RSD
4	4.2 ± 0.15	0.35	4.1±0.03	0.73
8	7.9 ± 0.04	0.5	8.1±0.52	0.64
12	11.8 ± 0.06	0.5	11.9±0.025	0.21

equation Y = 0.038 x + 0.28 and correlation value 0.999. The LOD and LOQ values are 15.72 and 52.63 ng/mL respectively (TABLE-1). The amount of drug was found to be 99.8 and 99 % for tablet formulations (TABLE-2). The sample recoveries in formulation were in good agreement with their respective label claim which suggested no interference of formulation excipients in the estimation. Also the % RSD for both the tablet analysis and recovery studies was less than 2% indicating high degree of precision (TABLE-3) and accuracy of the proposed method (TABLE-4).

TABLE 4 : Accuracy of the proposed method

Analyte	Recovery level	Theoritical content (mg)	Amount found(mg) ± SD (n=3)	%Recovered	RSD (%)
Tazzle 10 (10mg)	80	18	17.9 ±0. 02	99.9	0.11
	100	20	20.1 ±0. 04	100.1	0.19
	120	22	22.2 ±0.02	100.9	0.09
Megalis 20 (20mg)	80	18	18 ±0. 03	100	0.16
	100	20	19.4 ±0. 02	97	0.10
	120	22	23.5 ±0. 03	102	0.13

CONCLUSION

It is concluded that the proposed method was found to be simple, sensitive, accurate and precise for the quantification of tadalafil in tablet dosage forms. The assay values were in good agreement with their respective labelled claim. This spectrophotometric method has been found to be better because of its specificity, sensitivity, no extraction procedures and low concentration of solvent. These advantages encourage that the proposed method can be routinely employed in quality control for analysis of tadalafil in tablet dosage forms.

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REFERENCES

[1] CIMS; Current index of medical specialities. Bangalore: CMP Media India Pvt Ltd., **(2012)**.
[2] Y.Mohammad, D.Gowri Sankar, B.Pragati Kumar, S.Hameeduv; E-Journal of Chemistry, **7**, 3 **(2010)**.
[3] B.Prasanna Reddy, K.Amarnadh Reddy, M.S.Reddy; Res Pharma Biotechno., **2**, 1 **(2010)**.
[4] N.Kannappan, Y.Deepthi, Y.Divya, R.Shashikanth, Mannavalan; International Journal of Chem.Tech.Research, **2**, 1 **(2010)**.
[5] A.S.Sutar, S.Magduma, S.Patila, N.S.Naikawadia; Int.J.Chem.Sci., **6**, 2 **(2008)**.
[6] A.Satish, J.Natvarlal; American Journal of Pharm.Tech.Research, **1**, 3 **(2011)**.
[7] P.Nikolou, I.Papoutsis, S.Athanaselis, G.Alevisopoulos, A.Khraiwesh, C.Pistos, C.Spiliopoulou;. J.Pharm.Biomed.Anal., **256**, 3 **(2011)**.
[8] A.Kavitha, D.Vijaya Durga, S.Himabindu, K.Eshvendar, N.Khaleel, Panikumar D.Anumolu; Asian J.Pharm.Clin.Res., **6**, 2 **(2013)**.
[9] G.H.U.Raju, D.G.Sankar, P.Y.Naidu; Asian J.Research.Chem., **3**, 4 **(2010)**.
[10] K.Ramakrishna, D.Rebecca Shiffali, A.D.Panikumar, P.Manju Bhargavi; Current Pharma Research., **3**,1 **(2012)**.
[11] A.Salma, AL.Tamini, A.Faima and M.Al-Otaibi; Asian Journal of Chemistry. **24**, 1 **(2012)**.
[12] International Conference on Harmonization; ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2B, **(1996)**.