Research Article

Estimation of Tadalafil Using Derivative Spectrophotometry in Bulk Material and in Pharmaceutical Formulation

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Received 17 January 2014; Accepted 2 May 2014; Published 21 May 2014

Academic Editor: Craig J. Eckhardt

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Four simple, rapid, accurate, precise, reliable, and economical UV-spectrophotometric methods have been proposed for the determination of tadalafil in bulk and in pharmaceutical formulation. “Method A” is first order derivative UV spectrophotometry using amplitude, “method B” is first order derivative UV spectrophotometry using area under curve technique, “method C” is second order derivative UV spectrophotometry using amplitude, and “method D” is second order derivative UV spectrophotometry using area under curve technique. The developed methods have shown best results in terms of linearity, accuracy, precision, and LOD and LOQ for bulk drug and marketed formulation as well. In N,N-dimethylformamide, tadalafil showed maximum absorbance at 284 nm. For “method A” amplitude was recorded at 297 nm while for “method B” area under curve was integrated in the wavelength range of 290.60–304.40 nm. For “method C” amplitude was measured at 284 nm while for “method D” area under curve was selected in the wavelength range of 280.80–286.20 nm. For methods A and B, tadalafil obeyed Lambert-Beer’s law in the range of 05–50 $\mu\text{g/mL}$ while for “methods C and D”, tadalafil obeyed Lambert-Beer’s law in the range of 20–70 $\mu\text{g/mL}$, and-for “methods A, B, C, and D” the correlation coefficients were found to be > than 0.999.

1. Introduction

Tadalafil (TD) is (6R,12aS)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1',2':1,6] pyrido[3,4-b] indole-1,4-dione [1]. The chemical structure of tadalafil is shown in Figure 1. Tadalafil is a selective inhibitor of phosphodiesterase type 5 (PDE5) [2]. It is an impotence agent. It is indicated for the treatment of erectile dysfunction [3]. A detailed literature survey for tadalafil revealed that several analytical methods are reported for the determination of tadalafil by high-performance liquid-chromatography [4–8], high-performance thin-layer chromatography [9], spectrofluorimetry [10], and UV-spectrophotometry [11].

To our knowledge no methods were found in literature for determination of tadalafil in bulk and pharmaceutical formulation using derivative spectroscopic techniques. Therefore, our attempt is to develop first order and second

order derivative spectroscopy using amplitude and also area under curve (AUC) techniques.

The AUC method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths λ_1 and λ_2 . Selection of wavelength range is on the basis of repeated observations so as to get the linearity between AUC and concentration [12]. Further, methods were validated as per ICH guidelines [13].

2. Experimental Work

2.1. Material and Methods. Tadalafil working standard was obtained from Glenmark Pharmaceuticals Ltd., Mumbai,

FIGURE 1: Chemical structure of tadalafil.

India. The marketed formulation (TADACIP) 20 mg was purchased from local market. *N,N*-Dimethylformamide (DMF) (analytical grade) and R.O. water were used for the experiment.

2.2. Instrument. A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 400–200 nm; scan speed: medium; sampling interval: 1.0 nm; band width ($\Delta\lambda$): 1.0 nm; spectral slit width: 1 nm. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

2.3. Preparation of Stock Standard Solution and Selection of Wavelengths. The stock standard solution of tadalafil was prepared by dissolving accurately weighed 10 mg in 50 mL of DMF. It was further diluted with water to obtain concentration of 40 $\mu\text{g/mL}$ which was scanned in UV range 400–200 nm; tadalafil showed a maximum absorbance at 284 nm. For “method A” amplitude in first order derivative spectrum was determined at 297 nm while for “method B” AUC in first order derivative spectrum was selected in between 290.60 and 304.40 nm. In “method C” amplitude of second order derivative spectrum was recorded at 284 nm while in “method D” AUC of second derivative spectrum was selected in between 280.80 and 286.20 nm.

The selection of wavelengths in all four methods is shown in Figure 2.

2.4. Methods A and B. The zero order absorption spectra of tadalafil were derivatized in first order using software UV Probe 2.21 with delta lambda 4 and scaling factor 2. In “method A” the amplitudes were recorded at 297 nm while for “method B” area under curve between the two wavelengths 290.60 and 304.40 nm was selected. The calibration curves were constructed by plotting concentrations 05–50 $\mu\text{g/mL}$ versus amplitude/AUC between selected wavelengths for “methods A and B,” respectively.

2.5. Methods C and D. The zero order absorption spectra of tadalafil was derivatized in second order using software UV Probe 2.21 with delta lambda 4 and scaling factor 2. In “method C” the amplitudes were recorded at 284 nm while in “method D” area under curve was recorded in between the two wavelengths 280.80 and 286.20 nm. The calibration curves were constructed by plotting concentrations 20–70 $\mu\text{g/mL}$ versus amplitude/AUC for “method C and D,” respectively.

2.6. Preparation of Sample Solution. Ten tadalafil (TADACIP) tablets (label claim 20 mg) were weighed, transferred to a clean dry mortar, and grounded into a fine powder using a pestle. Tablet powder equivalent to 10 mg of tadalafil was transferred to a 50 mL volumetric flask and 30 mL DMF was added. After ultrasonic vibration for 10 min, volume was made up to be marked with DMF and filtered through Whatman filter paper (no. 41). From the filtrate, an appropriate volume was taken and diluted with water to get the final concentration of 20.0 $\mu\text{g/mL}$ for “methods A and B” and 40.0 $\mu\text{g/mL}$ for “methods C and D.” The responses measured and concentrations in the sample were determined from respective linearity equation.

3. Validation of Method

The proposed method was validated as per ICH guidelines [13].

3.1. Linearity. The linearity of the “methods A and B” was evaluated by analysis of six standard solutions of tadalafil of concentrations 05, 10, 20, 30, 40, and 50 $\mu\text{g/mL}$ while for “methods C and D” linearity was evaluated by analysis of six standard solutions of tadalafil of concentrations 20, 30, 40, 50, 60, and 70 $\mu\text{g/mL}$.

3.2. Accuracy. The accuracy of all methods was evaluated by measurement of recovery. To the preanalyzed sample solutions (10 $\mu\text{g/mL}$ in methods A and B and 30 $\mu\text{g/mL}$ in methods C and D), known amounts of stock standard solutions were added at different levels, that is, 80%, 100%, and 120%. The solutions were reanalyzed by the proposed methods. The experiments were repeated for three times at each level for each method.

3.3. Precision. Precision of the methods was studied as intra-day and inter-day variations. For “methods A and B,” precision was determined by analyzing the 10, 20, and 40 $\mu\text{g/mL}$ of tadalafil solutions as intra-day and inter-day variations. For “methods C and D,” precision was determined by analyzing the 30, 40, and 60 $\mu\text{g/mL}$ of tadalafil solutions as intra-day and inter-day variations.

3.4. Sensitivity. The sensitivity of measurements of tadalafil by the use of proposed methods was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) which were calculated using formulae “ $\text{LOQ} = 10 \times N/B$ ” and “ $\text{LOD} = 3.3 \times N/B$,” where “ N ” is standard deviation of

FIGURE 2: Zero order spectrum of tadalafil. (a) First order derivative spectrum. (b) First order derivative spectrum showing AUC between selected wavelengths. (c) Second order derivative spectrum. (d) Second order derivative spectrum showing AUC between selected wavelengths.

the amplitudes or peak areas of the tadalafil ($n = 3$), taken as a measure of noise, and “B” is the slope of the corresponding calibration curve.

3.5. Repeatability. In “methods A and B,” repeatability was determined by analyzing 20 $\mu\text{g/mL}$ concentration of tadalafil while for “method C and D,” it was studied by analyzing 40 $\mu\text{g/mL}$ concentration of tadalafil solution for six times.

3.6. Ruggedness. For “methods A and B” ruggedness of the proposed method was determined by analyzing 20 $\mu\text{g/mL}$ concentration of tadalafil while for “methods C and D” it was measured by analyzing 40 $\mu\text{g/mL}$ concentration of tadalafil by two different analysts using similar operational and environmental conditions.

4. Results and Discussion

4.1. Method Validation

4.1.1. Linearity. From the linear regression data it is clear that for “methods A and B” calibration curves showed good linear relationship over the concentration range of 05–50 $\mu\text{g/mL}$; similarly for “methods C and D” a good linear relationship was obtained over the concentration range 20–70 $\mu\text{g/mL}$ for tadalafil. The data of regression analysis is shown in Table 1.

4.1.2. Accuracy. The solutions were reanalyzed by proposed methods; results of recovery studies are reported in Table 2. The % RSD values that were determined and found to be less than 2 indicate that the method is accurate.

TABLE 1: Optical characteristics of tadalafil.

Parameters	Method A	Method B	Method C	Method D
Beer-Lambert's range ($\mu\text{g/mL}$)	05–50	05–50	20–70	20–70
λ max (nm)/wavelength range (nm)	297	290.60–304.40	284	280.80–286.20
Slope	0.0064	0.0380	0.0013	0.0015
Intercept	−0.0123	−0.0269	−0.0178	0.0151
Correlation coefficient	0.9993	0.9993	0.9990	0.9991

TABLE 2: Accuracy studies.

Drug	Methods	Initial amount [$\mu\text{g/mL}$]	Amount added [$\mu\text{g/mL}$]	Amount recovered [$\mu\text{g/mL}$, $n = 3$]	% Recovered	% RSD
Tadalafil	A	10	8	18.07	100.91	0.52
		10	10	20.16	101.63	0.65
		10	12	22.13	101.11	0.47
	B	10	8	18.06	100.75	0.89
		10	10	20.10	101.06	1.22
		10	12	22.06	100.50	0.29
	C	30	24	54.13	100.54	0.18
		30	30	60.13	100.44	0.16
		30	36	66.06	100.16	0.19
	D	30	24	54.11	100.48	0.16
		30	30	60.10	100.35	0.12
		30	36	66.07	100.21	0.29

n : number of estimations.

TABLE 3: Precision studies.

Drug	Methods	Concentration [$\mu\text{g/mL}$]	Intra-day [$n = 3$]	% RSD	Inter-day [$n = 3$]	% RSD
Tadalafil	A	10	9.93	0.32	9.95	0.31
		20	20.20	0.77	20.25	1.17
		40	40.71	0.10	40.66	0.07
	B	10	9.87	0.67	9.90	0.40
		20	20.03	0.06	20.05	0.13
		40	40.07	0.06	40.17	0.14
	C	30	30.08	0.12	30.33	1.32
		40	40.12	0.11	40.07	0.08
		60	60.07	0.07	60.11	0.11
	D	30	29.73	1.69	30.02	1.30
		40	39.91	0.94	39.68	1.41
		60	59.48	1.03	59.93	1.11

n : number of estimations.

TABLE 4: Repeatability studies.

Drug	Methods	Amount taken [$\mu\text{g/mL}$]	Amount found [$\mu\text{g/mL}$]	% Amount found [$n = 6$]	Mean $\pm$ SD	% RSD
Tadalafil	A	20	20.15	100.75	100.75 $\pm$ 1.06	1.06
	B	20	20.02	100.14	100.14 $\pm$ 0.37	0.37
	C	40	40.30	100.76	100.76 $\pm$ 0.86	0.85
	D	40	39.90	99.75	99.65 $\pm$ 0.71	0.70

n : number of estimations.

TABLE 5: Ruggedness studies.

Drug	Methods	Analyst I		Analyst II	
		% Amount found $\pm$ SD [$n = 3$]	% RSD	% Amount found $\pm$ SD [$n = 3$]	% RSD
Tadalafil	A	101.27 $\pm$ 0.90	0.89	100.75 $\pm$ 0.45	0.44
	B	100.55 $\pm$ 0.18	0.18	99.90 $\pm$ 0.11	0.11
	C	101.47 $\pm$ 0.40	0.39	101.41 $\pm$ 0.29	0.28
	D	99.05 $\pm$ 0.58	0.59	99.61 $\pm$ 1.10	1.11

n : number of estimations.

4.1.3. Precision. The precision of the developed methods was expressed in terms of % relative standard deviation % RSD. These results showed reproducibility of the assay. The % RSD values were found to be less than 2, so this indicates that the methods are precise for the determination of the tadalafil in pharmaceutical formulation. Results are shown in Table 3.

4.1.4. Sensitivity. The LOD and LOQ for tadalafil in “method A” were found to be 0.11 μg and 0.33 μg while in “method B” 0.15 μg and 0.45 μg . Similarly in “method C” values for LOD and LOQ were as 0.50 μg and 1.52 μg and in “method D” 0.49 μg and 1.49 μg .

4.1.5. Repeatability. For “methods A and B” repeatability was determined by analyzing 20 $\mu\text{g}/\text{mL}$ concentration of tadalafil while for “method C and D” repeatability was determined by analyzing 40 $\mu\text{g}/\text{mL}$ concentration of tadalafil solution for six times with % RSD values < 2 for all the methods. Results are shown in Table 4.

4.1.6. Ruggedness. Ruggedness was determined for solutions of tadalafil. The results are in acceptable range that is % RSD values < 2 for all the methods as shown in Table 5. The results showed no statistical differences between different operators suggesting that the developed methods are rugged.

4.2. Analysis of Tablet Formulation. The amounts of tadalafil estimated from tablet formulation using methods A, B, C, and D were found to be 99.32%, 100.18%, 99.61%, and 99.81%, respectively. The % amount estimated from tablet formulation indicates that there is no interference from excipients present in it.

5. Conclusion

All four methods were developed for the determination of tadalafil based on different analytical techniques, UV-spectrophotometric derivative, and AUC methods. The methods were validated and found to be simple, sensitive, accurate, and precise. Hence, the methods can be used successfully for routine analysis of pharmaceutical dosage form of tadalafil. The proposed spectrophotometric methods will not be substituted to the existing known methods available for the analysis of tadalafil. However, it can serve as an option

where advanced instruments (e.g., HPLC) are not available for routine analysis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors are thankful to the Principal, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, 425 405 (MS), India, for providing the laboratory facility.

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