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A Stability Indicating Rp-Hplc Method for the Estimation of Tadalafil in Oral Jelly Dosage Forms

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ABSTRACT

A simple, selective, precise and stability indicating RP-HPLC method for the determination of tadalafil was developed and validated in oral jelly dosage forms. The chromatographic parameters comprised of Zorbax C18 column (250X4.6 mm, 5µ) and mixture of buffer: acetonitrile (55: 45 v/v) as mobile phase. The detection was observed at 225nm with 1.5 ml/min flow rate. The developed method has been validated according to ICH and USP guidelines. The linearity studies showed a good correlation over the range of 10 to 75 µg/ml with correlation coefficient (r2) of 0.998. The drug was subjected to forced degradation analysis on varied conditions of acidic, basic, peroxide, thermal, light and UV radiation. All the results have proved that the method was selective and reproducible for the determination of tadalafil. The proposed stability indicating RP-HPLC method can be effectively employed for the determination of tadalafil in routine drug analysis of oral jelly dosage forms.

Keywords : Tadalafil, HPLC, stability indicating, and validation

1. INTRODUCTION

Tadalafil ((6R-trans)-6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12 a-hexahydro-2-methyl-pyrazino [1', 2':1,6] pyrido [3,4-b] indole-1,4-dione or $C_{22}H_{19}N_3O_4$) (Figure 1) is a phosphodiesterase type 5 (PDE5) inhibitor used to treat the erectile dysfunction (ED) by increasing the levels of cGMP[1]. It is yellowish white crystalline powder, and ease of soluble in water. The literature survey reveals various methods like Spectrophotometric[2-4], HPLC[5-17], LC/MS[18, 19] have been studied for tadalafil. So far, there is no stability indicating method has not revealed. Hence, an attempt has been made to confirm the stability indicating method for the determination of tadalafil in pharmaceutical dosage forms. It was optimized and validated according to International Conference on Harmonization (ICH) guidelines[20, 21] and USP [22] guidelines. The present study was aimed to develop a simple, rapid, precise, accurate, stable and selective reversed phase chromatographic method along with the stability indicating studies to estimate the tadalafil in oral jelly dosage forms.

2. MATERIALS AND METHODS

2.1 Chemicals

The HPLC grade of methanol and acetonitrile (Qualigens) was used for the analysis. The gift sample of tadalafil oral jelly and its placebo was obtained from Caplin Point Laboratories Limited, Chennai. Commercially available gel formulation Tadaga oral jelly, Cialis was used for verifying the effectiveness of the method. The experimental procedure was carried out completely using Pall Cascada AN water, New York. All other chemicals were used with their respective grade.

2.2 Instrumentation and Analytical Conditions

The chromatographic method was carried out using the Agilent 1260 equipped with DAD and VWD detector. The software of open lab was used to monitor the data acquisitions and other proceedings. The freshly prepared mobile phase was vaccum filtered through a 0.45 µm Millipore nylon filter, used for the entire study.

2.3 Preparation of 0.05 M Phosphate Buffer

Weigh accurately about 7.098 grams of Disodium hydrogen orthophosphate into the 1000 ml volumetric flask and dissolve it using 100 ml of HPLC water and then made up the mark with HPLC water.

2.4 Preparation of standards

Transfer accurately weighed quantity of 25 mg of Tadalafil standard in 100 ml volumetric flask, and add 50 ml of mobile phase, sonicate for 15 mins using PCI Analytical sonicator, Mumbai to dissolve it and make upto the mark with mobile phase. Take 5 ml of the resulting solution into 25 ml volumetric flask, and make upto the mark with the mobile phase.

2.5 Preparation of sample

Transfer the weight of 25 mg equivalent of Tadalafil oral jelly sample in a 100 ml volumetric flask, and add 50 ml of mobile phase, sonicate for 15 mins using PCI Analytical sonicator, Mumbai to dissolve it and make upto the mark with mobile phase. Take 5 ml of the resulting solution into 25 ml volumetric flask, and make upto the mark with the mobile phase.

2.6 Preparation of placebo

Transfer 6.25g of Tadalafil oral jelly placebo in a 100 ml volumetric flask, add 50ml of mobile phase, sonicate for 15 mins using PCI Analytical sonicator, Mumbai to dissolve it and make upto the mark with mobile phase. Take 5 ml of the resulting solution into 25 ml volumetric flask, and make upto the mark with the mobile phase.

2.7 Forced / Stress degradation studies

The Tadalafil standard was exposed to various stress condition like Acidic hydrolysis (1 N and 2 N HCl), Basic Hydrolysis (1 N and 2 N NaOH), Peroxide (5% and 10% H_2O_2), and the thermal degradation of tadalafil was carried out by using hot air oven and monitored at the temperature of 50 ºC, 60 ºC and 70 ºC after 2 hours exposure. The light and UV exposure study carried out for 15 days.

Take 25mg of tadalafil standard in a 100 ml flask and add 5ml of 1N NaOH and heat it for one hour at 60°C .Cool to room temperature, neutralize and transfer the whole contents exactly into a 100ml volumetric flask, make upto the mark with mobile phase. Take 5ml from the above solution and transfer into a 25ml volumetric flask, make upto the mark with mobile phase.

Perform the same manner for rest of the acidic (1N and 2N HCl), basic (2N NaOH) and peroxide (5% and 10% H_2O_2) treatments for standard tadalafil. Perform and continue the degradation study with the sample and placebo of tadalafil oral jelly in the same manner. Run the chromatogram upto 25 minutes for all stress study samples analysis to monitor the degraded or matrix peaks in higher retention time.

3. RESULTS AND DISCUSSION

The study was aimed to develop a stability indicated RP-HPLC method for the determination of tadalafil in tadalafil oral jelly dosage form. The initial trails were conducted based upon the peak symmetry and time reduction in the chromatographic analysis. The C_{18} column was selected to conduct the method development study based on the polarity of tadalafil. All the trails were done using Zorbax C_{18} (250 X 4.6mm, 5 µm) column as stationary phase. The mobile phase was assessed after conducting the various trails using the solvents of methanol, acetonitrile and phosphate buffer. The mobile phase of Phosphate buffer: methanol (50: 50 v/v) was used and it shows a poor peak shape with long retention time. Hence, decided to modify the mobile phase on the basis of the polarity chosen the solvents of phosphate buffer: acetonitrile. The different proportions of mobile phase was mixed and analyzed in the HPLC system which include 40:60 v/v, 45:55 v/v, 50:50 v/v, 55: 45 v/v, 60:40 v/v, 70:30 v/v, and 75:25 v/v. When the content of acetonitrile proportion was decreased in the mobile phase, the result showed a good peak shape. The ratio of mobile phase buffer: acetonitrile (60: 40 v/v) showed a concise peak due to decreased organic content. The ratio of the organic modifier was adjusted to 55: 45 v/v showed a good peak shape with limits of system suitability. The method was furtherly proceeded using the mobile phase of buffer: acetonitrile (55: 45 v/v) and it showed in Figure 2. The results found to be specific and it shows there is no interference and co-elution of any other peaks with the retention of tadalafil. The optimized conditions are showed below.

Optimized Chromatographic Conditions:

Column	:	Zorbax C_{18} [250 X 4.6mm, 5 µm]
Mobile phase	:	Buffer: Acetonitrile [55: 45 v/v]
Diluent	:	Mobile phase
Temperature	:	40 °C
Inject volume	:	20 µL
Wave length	:	225 nm
Flow rate	:	1.0 ml/min
Run time	:	10 min.

3.1 Stress Degradation Studies

The stress degradation studies are conducted in the conditions of acidic, basic, peroxide, thermal, light and UV radiation. The chromatograms obtained after stress degradation studies of tadalafil are showed in Figure 3-8. The results are compared with the untreated standard and summarized in Table 1.

3.1.1 Degradation in acid hydrolysis

It was performed using 1 N and 2 N HCl at 60 ºC for 1 hour. The results showed a significant degradation.

3.1.2 Degradation in basic hydrolysis

The analysis conducted using of 1 N and 2 N NaOH at 60 ºC for 1 hour. The result showed a moderate degradation.

3.1.3 Peroxide Conditions

The degradation was carried out using the 5% and 10% H_2O_2 and the result showed the prominent degradation.

3.1.4 Thermal Conditions

The tadalafil exposed at different temperature conditions using the thermostat at 50ºC, 60ºC and 70ºC for 2 hours and the results found to be less moderate degradation.

3.1.5 Light and UV radiation

The sample of tadalafil was exposed for 15 days and the results showed to be stable.

3.2 Method Validation

3.2.1 Specificity

The results of the specificity were showed there was no interference and co-elution of any other peaks with the retention of tadalafil. The peak purity of tadalafil and sample of oral jelly found within the limit which proved that there was no interference between the blank and placebo peaks.

3.2.2 Linearity

The linear calibration plot was constructed by analyzing the concentrations over the range of 10-75 µg/ml. The sample volume of 20 µl was injected three times into the column and it was used for the determination of peak area. The standard graph has drawn taking the concentration samples of drug on x-axis and peak area of absorption on y-axis and showed in Figure 9. The results are interpolated using the linear regression correlation method. The correlation coefficient obtained from the linearity studies showed a good linear response with limits of correlation between the peak area and concentration of the analytes.

3.2.3 Precision

The intra-day and inter-day precision was determined by injecting the six replicates of standard concentration into the HPLC system. The % RSD was calculated from the peak area responses of the concentration on the same day and it on consecutive days (n=3). The result showed the % RSD was found to be less than 2.0 and it indicates the precise method. The summarized results were showed in Table 2 and 3.

3.2.4 Accuracy

The accuracy was estimated by using the standard addition method. It was determined by spiking the concentration with levels of 50%, 80%, 100%, 120% and 150%. The known quantity of tadalafil standard concentration was spiked with placebo. The results showed a good recovery and showed in Table 4.

3.2.5 Limit of Detection and Limit of Quantification

The LOD and LOQ were evaluated on the basis of the linearity curve. The LOD and LOQ of tadalafil were found to be 0.42 µg/ml and 1.28 µg/ml respectively. The results showed that the method can be efficiently employed for the estimation of tadalafil.

3.2.6 Robustness

The robustness was conducted by deliberate changing in the optimized chromatographic conditions. The mobile phase variation (± 3% v/v), flow rate (±0.2 ml/min), wave length (±2 nm), column oven temperature (±5 ºC), were slightly changed. The reproducible results were obtained which proves the robust method and the summarized data was showed in Table 5.

3.2.7 Stability of the solution

The stability of the solution was performed for 24 hours period at each interval of 4 hours. The percentage stability was showed in Table 6.

3.2.8 Analysis of Commercial Formulation

The method was verified using the commercial formulation of TADAGA Oral Jelly which it was injected six replicate preparation of samples into the LC system and the results found between 100.16 to 100.80% and summarized in the Table 7.

4. CONCLUSIONS

A simple, selective, accurate, precise and stability indicating RP-HPLC method has been developed for the determination of tadalafil in tadalafil oral jelly. The method was validated successfully with significant results according to ICH and USP guidelines. The present study can be effectively employed for the determination of tadalafil in oral jelly dosage form for routine drug analysis.

5. ACKNOWLEDGEMENTS

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Tamil Nadu, India for providing the necessary facilities for the completion of work successfully.

Table 1: Summarized data of forced degradation studies

	Standard		Sample	
Degradative condition	Peak Area	% Assay	Peak Area	% Assay
Acidic degradation				
1 N HCl	521318992	97.85	524719757	98.60
2 N HCl	484589543	91.89	479888208	90.30
Basic degradation				
1 N NaOH	511459785	96.99	504689522	95.70
2 N NaOH	499658217	94.05	498657845	94.56
H_2O_2 Effect				
5 % H_2O_2	465897541	84.51	444685051	79.85
10 % H_2O_2	395487952	71.74	416665842	74.95
Thermal Degradation after 2 Hours				
50 °C	524736758	95.18	528822778	95.05
60 °C	526974411	94.75	521413880	93.97
70 °C	517375958	93.15	521792627	93.51
Light				
15 days	521613305	98.35	524708494	98.90
UV Radiation				
15 days	539721126	100.55	513987498	97.21

Table 2: Summarized data of Repeatability sample

Conc. (µg/ml)	Repeatability of sample	
	Peak Area	% Assay
50	544461991	100.25
50	548304855	101.10
50	547870569	101.15
50	547992239	101.15
50	542322318	99.95
50	548269277	101.15
Mean		100.79
SD		0.5159
%RSD		0.51

Table 3: Summarized Inter-mediate Precision data of standard and sample dosage forms

Conc. (µg/ml)	Peak area					
	Day-1		Day-2		Day-3	
	Sample	% Assay	Sample	% Assay	Sample	% Assay
50	545548712	100.25	545574892	100.30	545589652	100.65
50	546489245	100.20	549325404	100.75	545362547	100.36
50	544879562	99.90	545487914	100.05	543254892	100.04
50	547546894	100.55	545992200	100.30	544879546	100.45
50	546154879	100.00	546022324	99.95	548754821	99.69
50	545584970	100.30	544269225	100.05	547754895	100.02
Mean	546034043	100.20	546111993	100.23	545932726	100.20
SD	925264	0.1953	1698361	0.3052	2000365	0.3489
%RSD	0.16	0.19	0.31	0.30	0.36	0.34

Table 4: Accuracy results of tadalafil sample

Level (%)	Area Obtained	Amount Recovered (mg)	Amount Added (mg)	% Recovery	(%) Avg. Recovery
50	273855245	25.04	25.06	99.93	99.89
	274854879	25.09	25.02	100.29	
	272548751	24.83	24.97	99.45	
80	435445681	39.82	40.10	99.30	99.56
	437648520	39.95	40.03	99.81	
	436547892	39.77	39.95	99.56	
100	546682154	49.99	50.12	99.74	99.76
	547895426	50.02	50.04	99.96	
	545847562	49.73	49.94	99.59	
120	659452045	60.30	60.14	100.26	100.05
	658425874	60.11	60.05	100.10	
	656282246	59.80	59.93	99.78	
150	816216900	74.63	75.18	99.28	99.34
	818542713	74.73	75.06	99.56	
	815487520	74.30	74.91	99.19	
				Average	99.72

Table 5: Robustness study of tadalafil standard

Parameter	Condition	Retention time	HETP	Asymmetric factor	% RSD
Flow rate (ml)	1.35	4.973	9559	0.95	0.20
	1.65	4.093	7862	1.15	0.22
Mobile Phase Variation (v/v)	52: 48	3.582	7317	0.92	0.24
	58: 42	6.413	8541	0.94	0.24
Column Temperature (ºC)	35	4.961	9064	0.93	0.12
	45	4.965	9564	0.97	0.24
Wave length (nm)	223	4.843	9265	0.78	0.19
	227	4.821	9185	0.82	0.14

Table 6: Stability data of tadalafil standard and sample dosage form

Time (Hrs.)	Standard		Sample	
	Average Peak Area	% Difference	Average Peak Area	% Difference
0	537784873	0.00	539961970	0.00
4	538135314	0.07	538658606	-0.24
8	538128610	0.06	537970286	-0.37
12	538054557	0.05	537804706	-0.40
16	538772985	0.18	536423466	-0.66
20	53878038	0.19	538247270	-0.32
24	537416772	-0.07	536444170	-0.65

Table 7: Summarized data of Tadaga oral jelly

Conc. (µg/ml)	Repeatability of sample	
	Peak Area	% Assay
50	536578421	100.16
50	538954745	100.60
50	538754891	100.57
50	539478412	100.70
50	540024875	100.80
50	542487543	101.26
Mean		100.68
SD		0.3591
%RSD		0.35

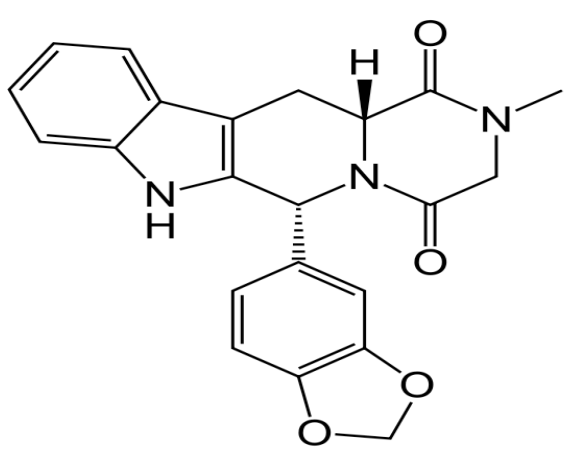

Figure 1: Structure of tadalafil

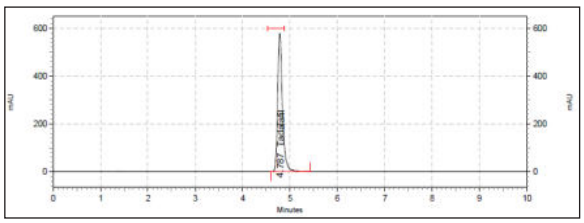

Figure 2: Standard Chromatogram of tadalafil

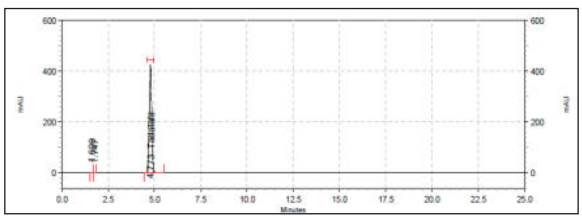

Figure 3: Chromatogram of tadalafil using 1N HCl

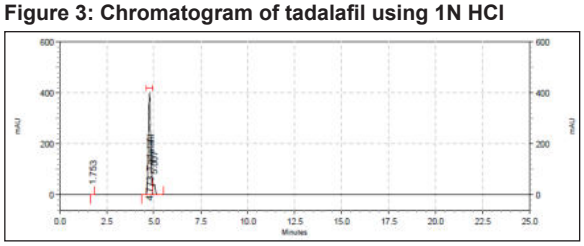

Figure 4: Chromatogram of tadalafil using 1N NaOH

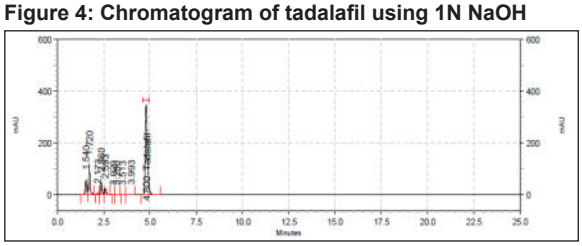

Figure 5: Chromatogram of tadalafil using 10% H_2O_2

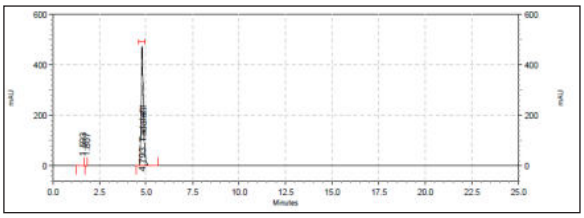

Figure 6: Chromatogram of tadalafil using Thermal degradation

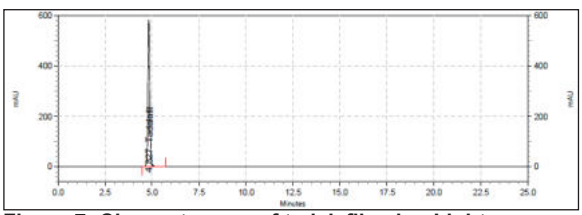

Figure 7: Chromatogram of tadalafil using Light

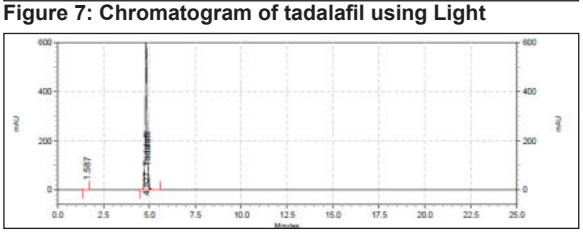

Figure 8: Chromatogram of tadalafil using UV radiation

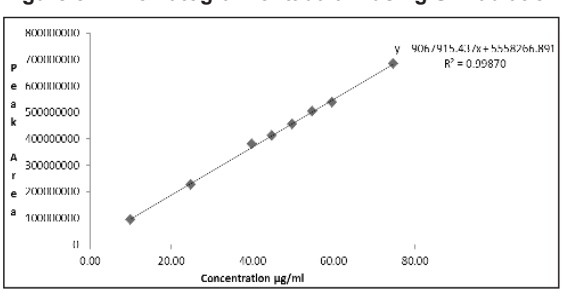

Figure 9: Linearity of tadalafil

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