A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF TADALAFIL IN FORMULATIONS

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Tadalafil tablet dosage form. Isocratic elution at a flow rate of 1.3ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile 65:35 v/v. The UV detection wavelength was 222 nm and 20µl sample was injected. The retention time for Tadalafil was 7.8 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Tadalafil tablet dosage form and bulk drug.

Key Words: Tadalafil, RP-HPLC, UV detection, recovery, precise, 222 nm

INTRODUCTION

Tadalafil is a PDE5 inhibitor, currently marketed in pill form for treating erectile dysfunction (ED) under the name Cialis; and under the name Adcirca for the treatment of pulmonary arterial hypertension. The approved dose for pulmonary arterial hypertension is 40 mg (two 20-mg tablets) once daily. Tadalafil is also manufactured and sold under the name of *Tadacip* by the Indian pharmaceutical company Cipla in doses of 10 mg and 20 mg.pharmacologic distinction is its longer half-life (17.50 hours) – compared to Viagra (4.0–5.0 hours) and Levitra (4.0–5.0 hours) – resulting in longer duration of action, and so partly responsible for "The Weekend Pill" sobriquet. Furthermore, the longer half-life is the basis for current investigation of tadalafil's daily therapeutic use in relieving pulmonary arterial hypertension. Currently, sildenafil (trade name Revatio) is approved in several world regions as a thrice-daily therapy for pulmonary arterial hypertension. Penile erection during sexual stimulation is caused by increased penile blood flow resulting from the relaxation of penile arteries and the smooth muscle of the corpus cavernosum. This response is mediated by the release of nitric oxide (NO) from nerve terminals and endothelial cells, which stimulates the synthesis of cGMP in smooth muscle cells. Cyclic GMP relaxes smooth muscle and increases blood flow to the corpus caver nosum.

The inhibition of phosphodiesterase type 5 (PDE5) enhances erectile function by increasing the amount of cGMP. Tadalafil (and sildenafil and vardenafil) inhibits PDE5. However, because sexual stimulation is required to initiate the local penile release of nitric oxide, tadalafil's inhibition of PDE5 will have no effect without direct sexual stimulation of the penis. The recommended tadalafil starting dose for most men is 10 mg, taken as needed before sexual activity (but not more than once daily). The dose may be increased to 20 mg or decreased to 5 mg, per its efficacy and the man's personal tolerance of the drug. To avoid the

inconvenience of a man having to program and plan using tadalafil around the time of his anticipated sexual activity, Lilly ICOS began a clinical development program to evaluate the risks and benefits of chronic, once-daily use of the drug. In June 2007, the European Commission approved low-dose (2.5 mg and 5 mg) Cialis to be used as single-daily ED therapy.

In patients with pulmonary arterial hypertension, the pulmonary vascular lumen is decreased as a result of vasoconstriction and vascular remodeling, resulting in increased pulmonary artery pressure and pulmonary vascular resistance. Tadalafil is believed to increase pulmonary artery vasodilation, and inhibit vascular remodeling, thus lowering pulmonary arterial pressure and pulmonary vascular resistance. Right heart failure is the principal consequence of pulmonary arterial hypertension.

Tadalafil has been used in approximately 15,000 men participating in clinical trials, and over 8 million men worldwide (primarily in the post-approval/post-marketing setting). The most common side effects when using tadalafil are headache, indigestion, back pain, muscle aches, flushing, and stuffy or runny nose. These side effects reflect the ability of PDE5 inhibition to cause vasodilation (cause blood vessels to widen), and usually go away after a few hours. Back pain and muscle aches can occur 12 to 24 hours after taking the drug, and the symptom usually disappears after 48 hours.

The U.S. Food and Drug Administration found that Tadalafil (along with other PDE5 inhibitors) was associated with vision impairment related to NAION (non arteritic anterior ischemic optic neuropathy) in certain patients taking these drugs in the post-marketing (outside of clinical trials) setting. Most, but not all, of these patients had underlying anatomic or vascular risk factors for development of NAION unrelated to PDE5 use, including: low cup to disc ratio ("crowded disc"), age over 50, diabetes, hypertension, coronary artery disease, hyperlipidemia and smoking. Given the small number of NAION events with PDE5 use (less than 1 in 1 million), the large number of users of PDE5 inhibitors (millions) and the fact that this event occurs in a similar population to those who do not take these medicines, the FDA concluded that they were not able to draw a cause and effect relationship, given these patients underlying vascular risk factors or anatomical defects. However, the label of all three PDE5 inhibitors was changed to alert clinicians to a possible association.

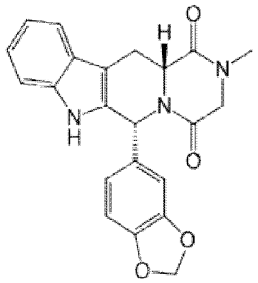

Figure 1: Structure of Tadalfin

EXPERIMENTAL

Materials

Working standard of Tadalafil was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Tadalafil were scanned in the range of 200 -400 nm against mobile phase as a blank. Tadalafil showed maximum absorbance at 218nm. So the wavelength selected for the determination of Tadalafil was 222 nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Tadalafilan isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Acetonitrile 65:35 v/v.(P^H 5.2 with 0.1M Phosphate buffer). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.0 ml/min. Detection was performed at 222 nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Tadalafilreference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (INTELENCE) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Tadalafilwas accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 30ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Api Concentration	40 ppm
Mobile Phase	Methanol: Acetonitrile 65:35
Wavelength	222 nm
Column	C_{18} Column
P^H	5.2
Concentration	40ppm
Retention Time	2.23min
Run Time	7.8 min
Area	201103
Th. Plates	3405
Tailing Factor	1.88
Pump Pressure	09.8MPa

Table.1 System suitability parameters of Tadalfin

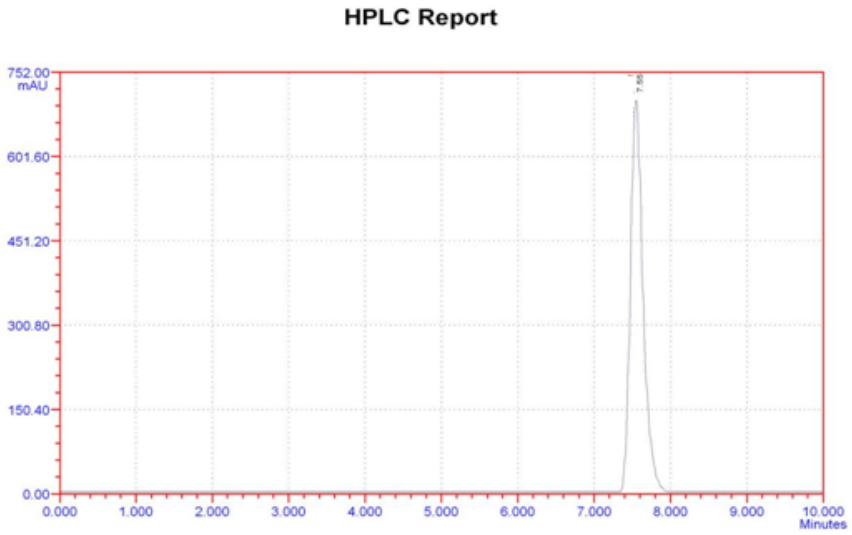

Figure.2: Standard chromatogram of Tadalafil

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 10, 20, 40, 60, 80 and 100ppm for Tadalafil. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -4605.1 + 6987.118x (r= 0.999). Linearity values can show in Table: 2

S.No	Concentration (µg/ml)	Area
1	10	48903
2	20	93279
3	40	201103
4	60	298970
5	80	406880
6	100	514682
	Slope Intercept CC	5148.557 -4605.1 0.99976

Table.2: Linearity results of Tadalafil

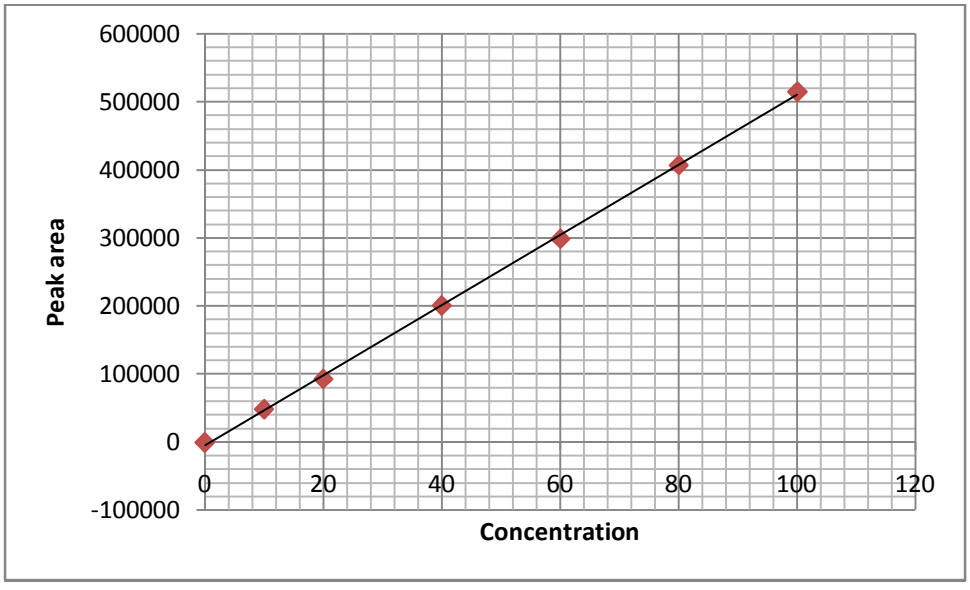

Figure 3: Calibration curve of Tadalafil

Precision

To study precision, six replicate standard solutions of Tadalafil(40ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated

and it was found to be which is well within the acceptance criteria of not more than 2.0%. The % RSD Was found to be both intraday and inter day was 1.42 and 0.95.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.80ppm dilution Peak was not clearly observed, based on which 0.5 ppm is considered as Limit of Detection and Limit of Quantification is 1.0 ppm.

Parameter	**Measured Value**
Limit of Quantification	1.0 ppm
Limit of Detection	0.5 oppm

Table.3: LOD and LOQ results of Tadalafil

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Tadalafil at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 4.

S.NO	Parameter	Change	Area	% of Change
1	Standard	.....	201103	.....
2	MP	Methanol :ACN: 45:55 85:15	201452 201231	0.17 0.06
3	PH	5.4 5.0	201521 201624	0.20 0.25
4	WL	220 nm 224 nm	201356 201259	0.12 0.07

Table.4: Robustness results of Tadalafil

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation. The %RSD was found to be 0.83

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e.60ppm, 80ppm, and 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.6 to 101. 6 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.5

% Recovery	Tadalafil				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	40	20	60	59.2	98.66667
	40	20	60	60.1	100.1667
	40	20	60	60.8	101.3333
100%	40	40	80	79.8	99.75
	40	40	80	80.6	100.75
	40	40	80	81.2	101.5
150%	40	60	100	98.9	98.9
	40	60	100	99.2	99.2
	40	60	100	100.5	100.5

Table.5: Recovery results of Tadalafil

Formulation	Dosage	Concentration	Amount found	% Assay
Zydalis	10mg	40 ppm	39.86	99.65

Table. 6: Formulation Analysis

CONCLUSION

The proposed method for the assay of Tadalafil tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES

1. http://www.forbes.com/2003/11/24/cx_mh_1124cialis.html
2. Daugan, A; Grondin P, Ruault C, Le Monnier de Gouville AC, Coste H, Kirilovsky J, Hyafil F, Labaudinière R (October 9, 2003). "The discovery of tadalafil: a novel and highly selective PDE5 inhibitor. 1: 5,6,11,11a-tetrahydro-1H-imidazo[1',5':1,6]pyrido[3,4-b]indole-1,3(2H)-dione analogues". Journal of Medicinal Chemistry **46** (21): 4525–32.
3. Richards, Rhonda (September 17, 1991). "ICOS At A Crest On Roller Coaster". USA Today: p. 3B.
4. Ervin, Keith (June 21, 1998). "Deep Pockets + Intense Research + Total Control = The Formula -- Bothell Biotech Icos Keeps The Pipeline Full Of Promise". The Seattle Times: p. F1. Retrieved January 10, 2009.
5. "Sildenafil Pharmaco-Cinétique". BIAM. April 20, 2001. Retrieved 2007-04-06.
6. Revill, Jo (February 2, 2003). "Drugs giant says its new pill will pack more punch than rival Viagra". The Observer. Retrieved 2007-04-06.
7. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm274642.htm
8. "FDA Announces Revisions to Labels for Cialis, Levitra and Viagra". Food and Drug Administration. 2007-10-18. Retrieved 2009-09-28.
9. Cialis: Warnings, Precautions, Pregnancy, Nursing, Abuse". RxList. 2007. Retrieved 2007-04-06.
10. Bischoff, E (June 2004). "Potency, selectivity, and consequences of non selectivity of PDE inhibition". International Journal of Impotence Research **16**: S11–4
11. Elliott, Stuart (January 10, 2006). "For Impotence Drugs, Less Wink-Wink". The New York Times: p. C2. Retrieved January 15, 2009.
12. Elliott, Stuart (April 25, 2004). "Viagra and the Battle of the Awkward Ads". The New York Times: p. 1. Retrieved January 15, 2009.
13. McCarthy, Shawn (March 5, 2005). "First they tried to play it safe; Ads for erectile dysfunction drug Cialis bared all - including a scary potential side effect. It was risky but it has paid off". The Globe and Mail: p. B4.
14. Loyd, Linda (July 6, 2003). "Two Pills Look to Topple Viagra's Reign in Market; Levitra Expects Approval Next Month, Cialis Later This Year". The Philadelphia Inquirer: p. E01.