www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 329-333, Jan-Mar 2010

METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHODS FOR ASSAY OF TADALAFIL AND SILDENAFIL CITRATE BY HPLC

N. Kannappan, Deepthi Yada, *Divya Yada, Shashikanth, Mannavalan. R

Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram,

Post Code No. 608002, Tamil Nadu, India.

***E-mail: divya_yada@yahoo.co.in**
E-mail: kannappan70@yahoo.co.in

ABSTRACT: The simple, reliable and reproducible HPLC methods were developed for the analysis of Tadalafil and Sildednafil citrate(API). The column used was YMC-Pack ODS AQ (150 mm x 4.6 mm,i.d.).The mobile phase used was phosphate buffer (10mM,pH 3.0) acetonitrile gradient run at the flow rate of 1mL/min with UV (PDA) detector at 220nm at ambient temperature. Extraction of Tadalafil and Sildenafil citrate from tablet was carried out using methanol. Linearity was observed in the range from 50 to 150µg/ml for tadalafil with a correlation coefficient (R^2) 0.99 and 10ng/ml as the limit of detection. The values of linearity range, correlation coefficient(R^2) and limit of detection were 50 to 150µg/ml, 0.99 and 20ng/ml respectively for sildenafil. Parameters of validation prove the precision and stability of the method and it's applicability for the Assay of tadalafil and sildenafil citrate. The method is suitable for routine analysis of the drug.

KEY WORDS: Sildenafil citrate API, Tadalafil, HPLC analytical method.

INTRODUCTION

The chemical name of sildenafil(SLD) is 5[2-ethoxy-5-(4-methyl piperazin-1-ylsulfonyl) phenyl]-1-methyl-3 -propyl-1,6-dihydro-7H-pyrazolo [4,3-d]pyrimidin-7-one and it's empirical formula is $C_{22}H_{30}N_6O_4S$.

The chemical name of tadalafil is (6R-trans)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexa hydro-2-methyl-pyrazino[1',2':1,6.]pyrido[3,4-b]indole 1,4-dione and it's empirical formula is $C_{22}H_{19}N_3O_4$.

Sildenafil and Tadalafil works by inhibiting the enzyme PDE5[1-4] by occupying its active site. This means that cGMP is not hydrolysed as fast and this allows the smooth muscle to relax leading to increased blood flow into the organ and therefore penile erection.

EXPERIMENTAL WORK

Apparatus and chromatographic condition

The analysis was performed by using HPLC, column used is YMC-Pack ODS AQ column (150mm x 4.6mm, i.d), with flow rate of 1ml/min and kromasil(150mm x 4.6mm). The mobile phase was phosphate buffer (10 mM).pH adjusted to 3.0, an injection volume is 20µl from a solution of 1mg/ml and UV (PDA) detector was used at 220nm.

Reagents and Solutions

Pure sample and sildenafil and tadalafil were collected from Cassel Research Lab,Chennai. Acetonitrile and water used were of HPLC and milli-q-grade, potassium dihydrogen-ortho-phosphate-GR. Optimized chromatographic conditions are in table.1.

Standard Preparation

Standard solution containing 100µg/ml mixture of water: acetonitrile (1:1v/v).

Sample Preparation

100µg/ml Tadalafil and 100µg/ml Sildenafil citrate in a mixture of water: acetonitrile (1:1v/v)

Linearity

Several aliquots of standard solutions of Tadalafil and Sildenafil were taken in different10ml volumetric flasks and diluted upto the mark with mobile phase

such that the final concentration of Sildenafil and Tadalafil is 50-150µg/ml respectively.

Assay:

20µl of standard and sample solution were injected into an injector of liquide chromatography, from the peak area of Sildenafil and Tadalafil, amount of drug in sample were computed[5,6]..

METHOD VALIDATION

Limit of detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentration of standard solution using the HPLC method. The LOD is the smallest concentration of analyzed that gives a measurable response (signal to noise ratio of 3 to 1).The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy (signal to noise ratio of 10 to 1).

Ruggedness and Robustness

Ruggedness of a method was determined by carrying out experiment on different HPLC instruments by different analysts using different columns like kromasil (150mm x 4.6mm),YMC-Pack ODS AQ (150 mm x 4.6 mm) in different laboratories by different ruggedness etc.,Robustness of a method was determined by making slight changes in the chromatographic conditions.

Solution stability

The both samples were found to be stable at 25'C for 12 hrs[7].

Recovery studies

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop accurate and stable assay method.

A YMC-Pack ODS AQ column (150 mm x 4.6 mm) in isocratic method with mobile phase phosphate buffer (10mM) in acetonitrile : water (1/1 v/v), pH 3.0.The flow rate was 1 ml per min, ideal compounds were analyzed at 220nm. Linearity was assessed by plotting concentration Vs area which is shown in figure 1 and 2 respectively with a linear in the range of 50 to 150µg/ml respectively for sildenafil and tadalafil with correlation co-efficient 0.9899 and 0.9949 respectively with a good linearity response greater than 0.999.Evaluation of two drugs was performed with UV (PDA) detector at 220nm, peak area recorded for all peaks. The slope and intercept value for caliberation curve was y = 45655x + 572159(R2 = 0.9899) for sildenafil and y = 92427x +06 +1E(R2 = 0.9949) for Talandafil. The results show that an excellent correlation exist between peak area & concentration of drugs within the correlation range and regression graphs were plotted.

The pertcentage of recovery was found to be within the limits of acceptance criteria with % RSD 0.39 for sildenafil and 0.28 for tadalafil. The LOD for Sildenafil and Tadalafil was found to be 0.02µg/ml and 0.01µg/ml respectively The LOQ for Sildenafil and Tadalafil was found to be 0.05µg/ml and 0.025µg/ml respectively. The sensitivity of the method shows that LOD and LOQ are shown in Table 3.

The assay procedures were repeated for six times and the percentage of drugs were found to give 97.87% of sildenafil and 98.85% of tadalafil and the recovery assay values are given in Table.2. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method developed, are rugged and robust. To check the stability of analyte on stationary phase stability studies were performed at 0,2.5,4,6,8,12 hrs respectively.

CONCLUSION

The proposed HPLC method gives good resolution between sildenafil citrate and tadalafil within short analysis time (less than 8min). The method is very simple,rapid and no complicated sample preparation is needed. High percentage of recovery shows the method is free from interference of excipients present in formulation.

Table1: System suitability parameters

Parameter	Sildenafil	Tadalafil
Retention time	2.695	7.841
Purity Angle	0.453	0.269
Purity Threshold	0.938	1.032
Tailing factor(T)	1.98	1.26
Theoretical plates(N)	2986	3254

HPLC CHROMATOGRAM

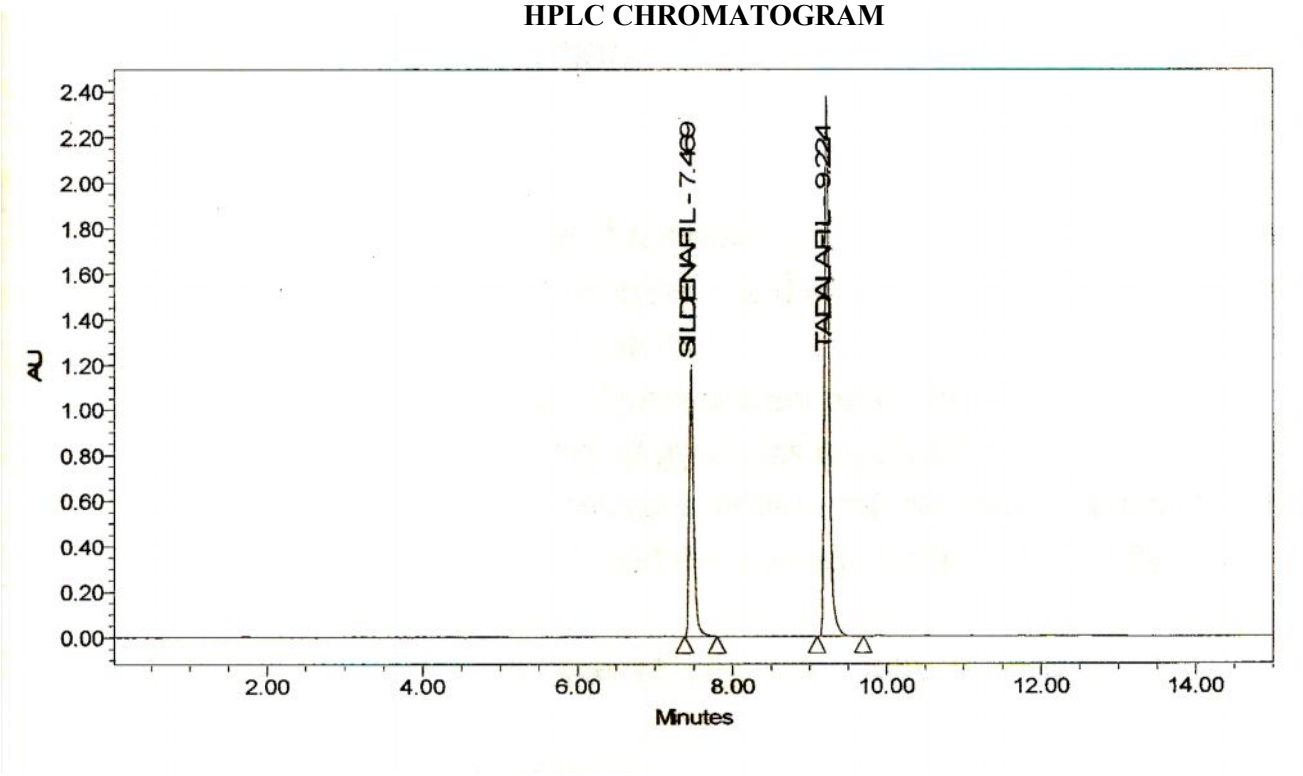


Table 2: Recovery Analysis of Sildenafil and Tadalafil

Sl. no	Drug	Amount taken (µg/ml)	Amountfound (µg/ml)	Amont found(%)	%RSD
1	Sildenafil	50.1	49.5	98.80	0.11
2	Tadalafil	50.08	49.5	98.84	0.43

Table 3: Validation parameter

Parameter	Sildenafil	Tadalafil
Linearity range	50-150µg/ml	50-150µg/ml
Correlation co-efficient (R^2)	0.9899	0.9949
LOD	0.02µg/ml	0.01µg/ml
LOQ	0.05µg/ml	0.025µg/ml
%RSD for Recovery	% 0.39	% 0.28

Fig:-1

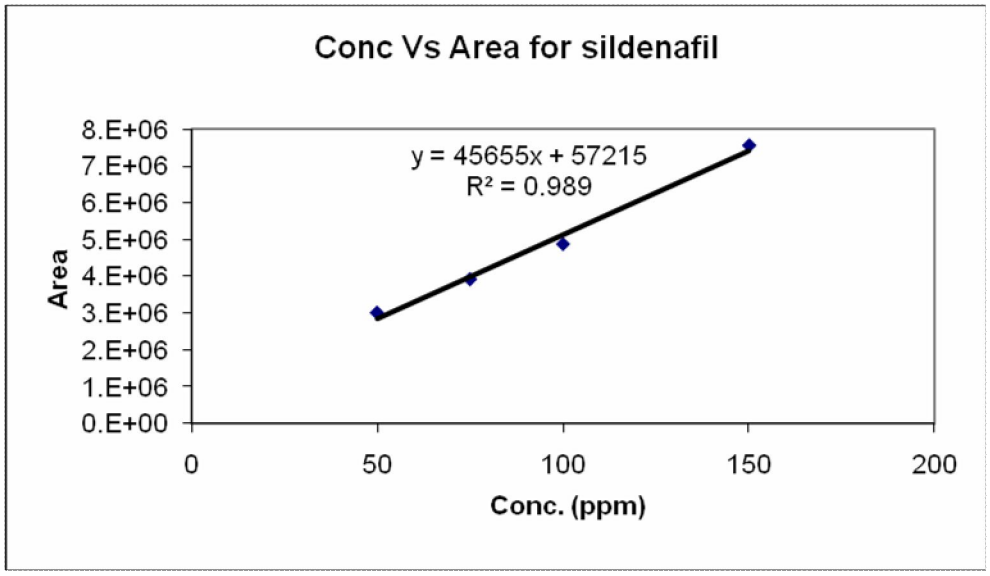
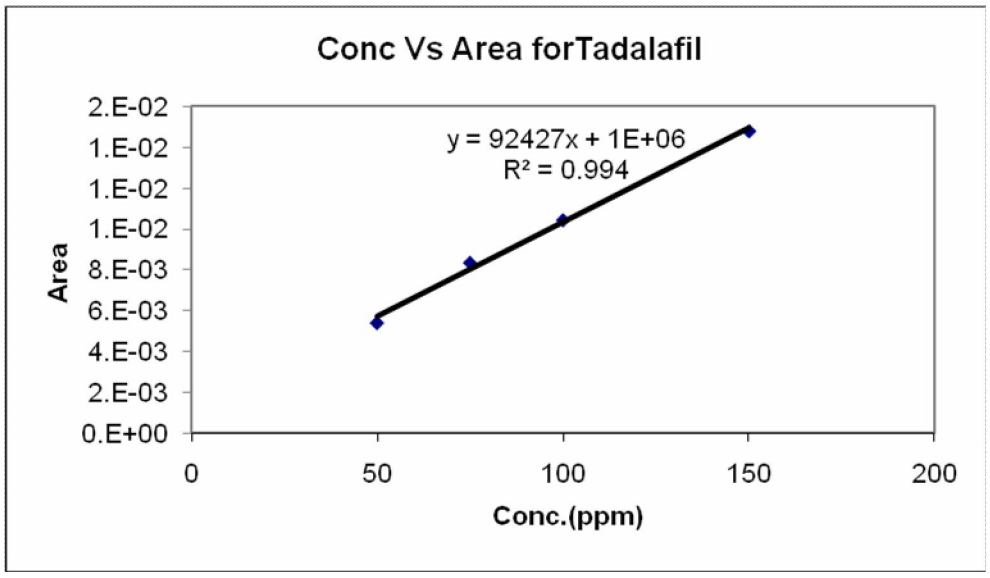


Fig:-2



REFERENCES

1. Webb, D.J.; Freestone, S.; Allen, M.J.; Muirhead, G.J. (March 4, 1999). "Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist". *Am. J. Cardiol.* **83** (5A): 21C–28C. doi:10.1016/S0002-9149(99)00044-2. PMID 10078539
2. *The Journal of Urology*, Volume 177, Issue 3, Pages 1071-1077 K. McVary, W. Monnig, J. Camps Jr., J. Young, L. Tseng, G. van den Ende
3. *Journal of the American College of Cardiology*, Volume 36, Issue 3, Pages 845-851
4. J. D. Corbin and S. H. Sharron. "Molecular Biology and Pharmacology of PDE-5-Inhibitor Therapy for Erectile Dysfunction". *J. Androl.* **24**: S38-S41.
5. L. Chaletz, stability –indicating Assay Methods for Drug and their dosage form,J.Pharm.sci 60(1971)335-349.
6. C.Ho and G.L.Chen, stability indicating HPLC Assay methods for Drug in Pharmaceutical dosage forms: Part-I, J Food Drug Anal.4(1996)271-292.
7. ICH, Stability testing of new drug substances and products, International Conference of Hormonisation, IFPMA, Geneva(2000).
8. FDA Guideline for submitting documentation for stability of human grugs and biologics, Food and Drug Administration, Rockvile,MD,1987.
9. K.Florey(ED).Analytical profile of drug substances,Academic presses,LONDON.
10. Sharma B.K., Insturmental Methods of Chemical Analysis,10th edition, Goal Publishing House.

*****