Journal of Pharmaceutical Research International

33(39B): 193-202, 2021; Article no.JPRI.71927
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

A Novel Analytical Method for Simultaneous Quantification of Silodosin and Tadalafil by RP-HPLC

Ajay Gupta[1*] and S. K. Mishra[2]

[1]Department of Chemistry, Jiwaji University, Gwalior, M.P. 474001, India.
[2]Department of Chemistry, Govt. P. G. College, Datia, M.P. 475661, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i39B32195
Editor(s):
(1) Prof. John Yahya I. Elshimali, Charles R. Drew University of Medicine and Science, USA.
Reviewers:
(1) Nadia Taha Ali Dawoud, Al-Azhar University, Egypt.
(2) Ashwani K. Dhingra, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71927

Original Research Article

Received 25 May 2021
Accepted 29 July 2021
Published 02 August 2021

ABSTRACT

The Reverse phase HPLC method was developed for simultaneous determination of Silodosin and Tadalafil in single analytical method. Chromatographic separation was achieved on a Supelco C8 (150mmx4.6mm, 5µm) column applying an isocratic elution based on premix of potassium phosphate dibasic buffer pH (4.3) and acetonitrile in the ratio of (70:30 v/v) as mobile. Validation parameters specificity, precision and robustness have been observed to be desirable over the concentration ranges of 80-240 µg/ml for Silodosin and 50-150 µg/ml for Tadalafil in accuracy parameter and 128-192 µg/ml for Silodosin and 80-120 µg/ml for Tadalafil in linearity parameter. All the variables have been studied to optimize the chromatographic conditions. The optimized approach verified through validation and confirmed to be intended purpose for the quality control of the mentioned drugs, as per ICH guidelines. For simultaneous quantification of Silodosin and Tadalafil, the developed method was found to be genuinely exact precise, accurate, linear, fast and cost effective.

Keywords: Silodosin; tadalafil; high-performance liquid chromatography; validation.

**Corresponding author: E-mail: ajayji2006@gmail.com;*

1. INTRODUCTION

In the pharmaceutical industry, all manufactured products need to be of the highest quality to ensure the least risk to patients. To guarantee that goods pass certain standards, researchers, manufacturers and developers use various technical equipment and analytical techniques, including liquid chromatography, during the development process [1]. It's possible that the medicine or drug combination isn't included in any pharmacopoeias. Due to patents, legislation, and other factors, a proper analysis process for the medicine may not be available in the literature. Due to the interference caused by the formulation excipients, analytical techniques for the drugs in the form of a formulation may not be available. Analytical methods for measuring drug concentrations in biological fluids may not be available [2].

Many multi-component medicines have been developed in the modern pharmaceutical business, and we can now quantify them with greater accuracy [3]. In quality control, liquid chromatography (LC) is the most extensively used analytical technique for determining the identification and amount of analytes and impurities in production batches. The pharmaceutical sector is heavily regulated. It is being scrutinized more closely by government regulatory agencies and public interest groups in order to reduce costs and ensure the timely delivery of safe and effective products to market. Both industry and regulatory agencies have turned their attention to the quality of drug products. Increased pressure on pharmaceutical analysts to supply accurate and precise analytical data in the quickest feasible time has resulted from faster drug research and drug product development processes, as well as increased requirements from industry and regulatory bodies [4]

A detailed literature review shown that individual analytical HPLC method is available for the determination of Silodosin and Tadalafil [5,6,7]. However few analytical methods are available for simultaneous determination of Silodosin in combination with other drugs except with Tadalafil [8]. In the same way few analytical methods are available for simultaneous determination of Tadalafil in combination with other drugs except with Silodosin [9,10]. But so far there is no single analytical HPLC method is available for simultaneous estimation of Silodosin and Tadalafil. The goal of this study is to establish a simple, reproducible, linear, precise single analytical method for simultaneous quantification of Silodosin and Tadalafil.

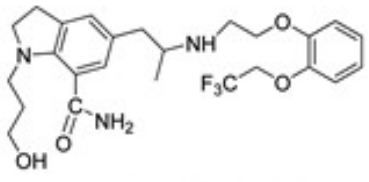
1.1 Silodosin

Silodosin is a kind of silodosin that (Brand Name Rapaflo) Silodosin is a drug used to treat the symptoms of benign prostatic hyperplasia. It has a strong uroselectivity and acts as a 1-adrenoreceptor antagonist (selectivity for the prostate) [11]. The great selectivity, on the other hand, appears to be the reason of silodosin's common side effect of loss of seminal emission. Silodosin is a prescription drug that is used to treat a variety of conditions [12]. Silodosin is a novel selective 1-adrenoreceptor antagonist with a high pharmacologic selectivity in the alpha-blocker class [13].

1.1.1 Silodosin IUPAC name

1-(3-hydroxypropyl)-5-[(2R)-2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylamino]propyl]-2,3-dihydroindole-7-carboxamide

1.1.2 Structure



1.1.3 Molecular formula

$C_{25}H_{32}F_3N_3O_4$

1.1.4 Molecular weight

495.534 g/mol

1.2 Tadalafil

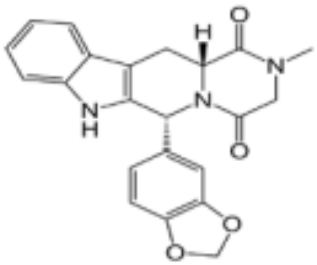
Tadalafil is used to treat male penile erectile dysfuctions (ED) by inhibiting the enzyme phosphodiesterase, which reduces the metabolism of cyclic guonosine monophosphate (cGMP), causing smooth muscle relaxation in the corpus cavereosus, with an onset time of 30-45 minutes [14].

1.2.1 Tadalafil IUPAC name

(2R,8R)-2-(1,3-benzodioxol-5-yl)-6-methyl-3,6,17-

triazatetracyclo[8.7.0.03,8.011,16]heptadeca-1(10),11,13,15-tetraene-4,7-dione

1.2.2 Structure



1.2.3 Molecular formula

$C_{22}H_{19}N_3O_4$

1.2.4 Molecular weight

389.404 g/mol

2. METHODS AND MATERIALS

The liquid chromatography consisted of a Shimadzu HPLC system model LC 2010 High-Performance Liquid Chromatography. For the RP-HPLC system, a Supelco C8 (150 mm x 4.6 mm, 5 µm) column was used. A Photodiode array detector (PDA) with an automated sample injector integrated with the system. Empower software was used to monitor and integrate the output signal. The hydrogen ion concentration (pH) of the buffer solutions was adjusted and determined using a digital pH meter. Active Pharmaceutical ingredient (API) of Silodosin and Tadalafil were supplied as gift from Metrochem API Pvt Ltd, Hyderabad. HPLC grade Methanol, Acetonitrile, Triethylamine, Miili-Q water, Ortho-Phosphoric acid (OPA) and Potassium Phosphate dibasic of analytical grade were obtained from Finar Chemicals Ltd.

2.1 Mobile Phase and Chromatographic Conditions

Potassium phosphate dibasic buffer solution was prepared by dissolving 3.48 gm of potassium phosphate dibasic in 1000 ml of HPLC grade water. Add accurately 2 mL of triethylamine in this solution and mix. The solution pH was adjusted to 4.3 with orthophosphoric acid. Mix buffer solution pH 4.3 and acetonitrile in the ratio of 70:30 v/v and use as a mobile phase. Diluent used mixture of milli-Q water and acetonitrile in the ratio of 50:50 v/v.

2.2 Standard Preparation

Standard solutions of Silodosin and Tadalafil were prepared by dissolving about 16 mg of silodosin working standard and 10 mg of Tadalafil working standard into a 100 mL volumetric flask, add about 70 mL of diluent, sonicate to dissolve and make up to volume to100 mL with diluent.

2.3 Sample Preparation

The average weight was derived after accurately weighing of 10 tablets. The tablets were crushed and powdered and a quantity of powder equivalent to 16 mg of Silodosin and 10 mg of Tadalafil was transferred into 100 mL volumetric flask and diluent was added about 70 ml. The solution was sonicate for 20 min. It was diluted to volume with diluent and mix well. A portion of the solution was filter with the 0.45 µm membrane filter.

2.4 Chromatographic Conditions

Shimadzu, Model: LC 2010, Photodiode array detector (PDA), with an auto sampler injector. The output signal was monitored and integrated using Empower software. The separation was successfully achieved on Supelco C8 (150x4.6mm, 5µm) column. The column temperature was maintained at 30°C and the eluent was monitored at 232 nm using a PDA detector. The injection volume was 10 µl. Mixture of phosphate buffer (pH 4.3) and acetonitrile in the proportion of 70:30 v/v as flow rate of 1.0 mL/min was used as a mobile phase with isocratic method.

2.5 Development and Optimization of Method

The main objective of chromatographic method is to develop a single RP-HPLC method for accurate quantification of Silodosin and Tadalafil. After detailed literature survey Silodosin available with two pKa (pKa1: 4.03, Nindoline ring and pKa2: 8.53, N-ethylaminopropyl group) [15]. The pKa values for Tadalafil were found to be 3.52 by potentiometry and 3.44 by spectrophotometry [16]. Tadalafil is very slightly soluble in water and slightly soluble in alcohol [17]. Method development started with different ratio of

potassium phosphate monobasic buffer as mobile phase A and acetonitrile as mobile phase B on Hypersil BDS C8 (150x4.6mm, 5µm) with 1.0 mL/min. flow rate. But chromatography was not achieved as desired. Therefore potassium phosphate dibasic buffer (pH 4.3) used as mobile phase A and acetonitrile is taken as mobile phase B on Supelco C8 (150x4.6mm, 5µm) with 1.0 mL/min. flow rate [18]. Peaks of both drugs were found satisfactory in this chromatography with different ratio of buffer and acetonitrile. But for shorter run time, Gaussian peak shape and better resolution premix of buffer (pH 4.3) and acetonitrile in the ratio of 70:30 v/v is used as mobile phase after optimization of method [18].

After optimization of method the final chromatographic conditions are Supelco C8 (150x4.6mm, 5µm) column. The column temperature was maintained at 30°C and the eluent was monitored at 232 nm using a PDA detector. The injection volume was 10 µl. Mixture of potassium phosphate dibasic buffer (pH 4.3) and acetonitrile in the proportion of 70:30 v/v as flow rate of 1.0 mL/min was used as a mobile phase with isocratic method with 15 minutes run time.

2.6 Method Validation

The parameters for method validation were carried out in accordance with ICH recommendations [19].

2.6.1 Specificity

The specificity was established by injecting blank, placebo, sample, spiked sample and individual impurities into the system. All samples solutions were prepared as per developed method. For analyte identification, chromatograms were recorded and retention times from sample and standard preparations were compared. No interference was observed from the blank, placebo & known impurities at the retention time of Silodosin and Tadalafil peak. As purity angle was found less than purity threshold for both drugs (Table-1). Blank, Standard solution and sample solution chromatograms are shown in Fig. 1, Fig. 2 & Fig. 3.

Table 1. Specificity: Peak purity data of Standard and Sample solution

	Silodosin		Tadalafil	
	Purity angle	**Purity threshold**	**Purity angle**	**Purity threshold**
Standard solution	0.735	0.968	0.489	0.865
Sample solution	0.785	0.977	0.520	0.885
Spiked Sample solution	0.776	0.974	0.514	0.878

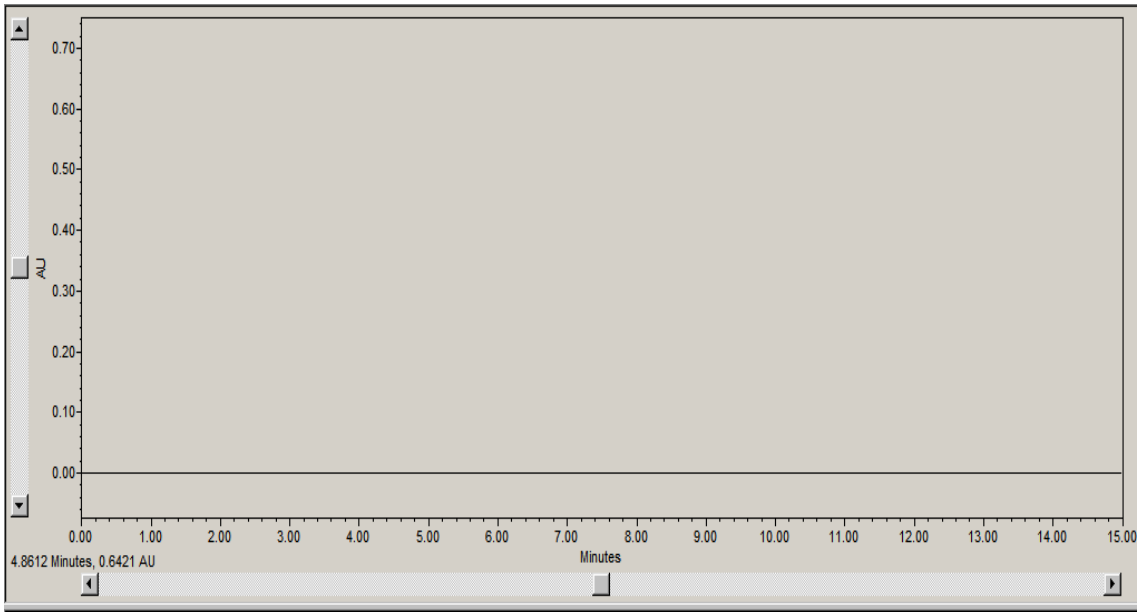


Fig. 1. Blank chromatogram

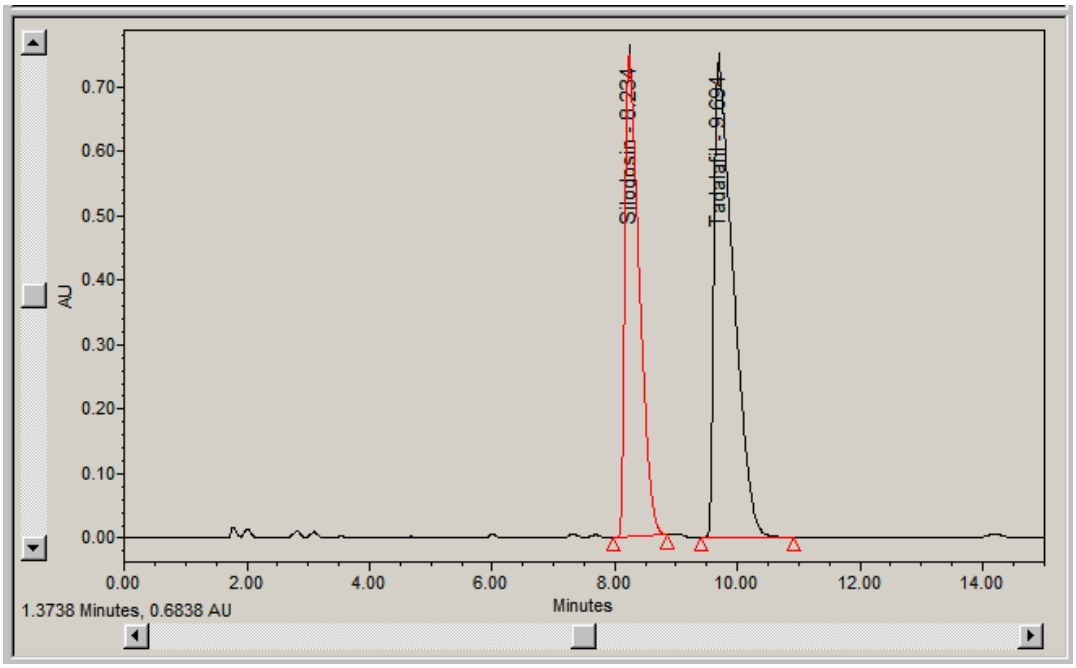


Fig. 2. Standard solution

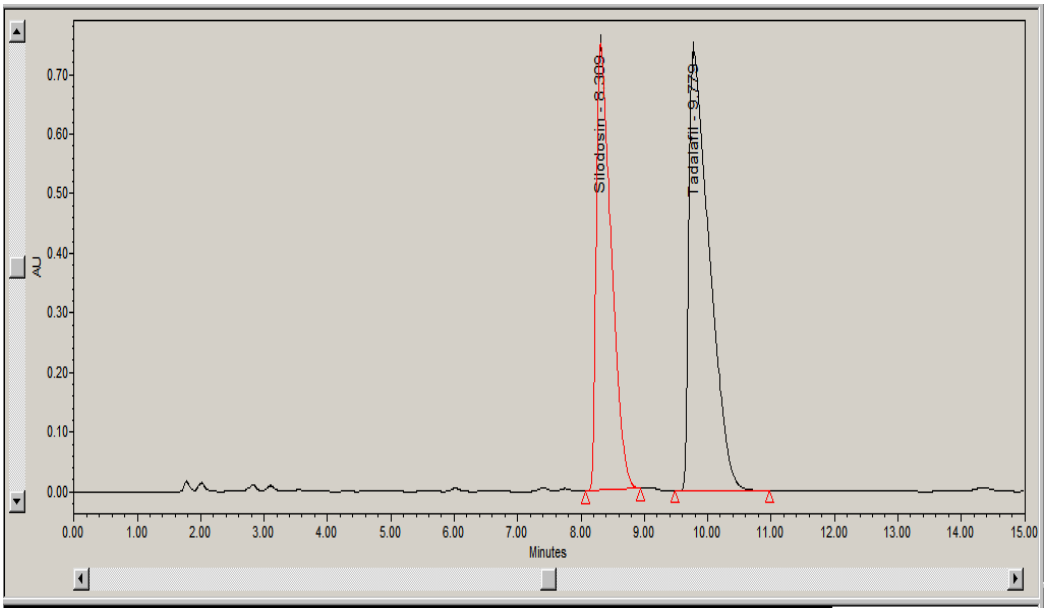


Fig. 3. Sample solution

2.6.2 Precision

The system precision was verified by injecting six replicate injections of standard solutions. Calculate the mean assay and percent relative standard deviation (%RSD) of area counts of Silodosin and Tadalafil peak (Table 2). The method precision was verified by injecting six replicate injections of sample solution. Calculate the mean assay and percent relative standard deviation (%RSD) of area counts of Silodosin and Tadalafil peak (Table 2). The intermediate precision was verified by injecting six replicate injections of sample solution on a different day by

a different analyst and analyse on a different instrument using the column of different serial number. Calculate the mean assay and percent relative standard deviation (%RSD) of area counts of Silodosin and Tadalafil peak (Table 3).

2.6.2.1 Linearity

In the linearity parameter, concentration of Silodosin and Tadalafil response were determined in the range of 80%-120% of standard solution. Calibration curves were plotted between analyte concentration and peak response. MS-Excel was used to determine the slope, intercept, and correlation coefficient. The calibration data of Silodosin and Tadalafil is given in Table 4, while Fig. 4 & Fig. 5 represents calibration curve of both drugs respectively.

2.6.3 Accuracy

The accuracy was determined by adding known quantities of the analyte to the placebo. A 3-fold measurement at 50% (Level 1), 100% (Level 2), and 150% (Level 3) of sample concentration respectively (9 Determinations in total) was carried out. Data for Silodosin and Tadalafil are shown in Tables 5-6.

Table 2. Summary of system precision data

Injection No.	Silodosin peak area	Tadalafil peak area
1	13158731	12258778
2	13128542	12210980
3	13273455	12193187
4	13076543	12143091
5	13186749	12316832
Mean	**13164804**	**12224574**
SD	**73192.283**	**66126.530**
%RSD (≤2%)	**0.56**	**0.54**

Table 3. Summary of method precision and intermediate precision data

Sample No.	Silodosin assay (% of label claim)		Tadalafil assay (% of label claim)	
	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
1	100.8	99.9	99.5	100.2
2	100.3	100.4	99.2	99.7
3	100.5	100.5	99.7	100.1
4	101.0	100.2	99.1	99.5
5	100.7	100.6	98.9	99.9
6	100.5	100.3	99.8	100.3
Overall Mean	**100.5**		**99.7**	
Overall SD	**0.290**		**0.440**	
Overall %RSD (≤2%)	**0.29**		**0.44**	

Table 4. Summary of linearity data

Silodosin		Tadalafil	
Concentration (µg/mL)	**Mean Area**	**Concentration (µg/mL)**	**Mean Area**
128.35	10830721	80.40	10089486
144.40	11876452	90.45	11499970
160.44	13287659	100.50	12568749
176.48	14540924	110.54	13787107
192.53	15837985	120.59	14970900
Peak Name	**Correlation Coefficient**	**Intercept**	**Slope**
Silodosin	0.9991	595748.20000	79026.72286
Tadalafil	0.9992	533277.40000	119906.11473

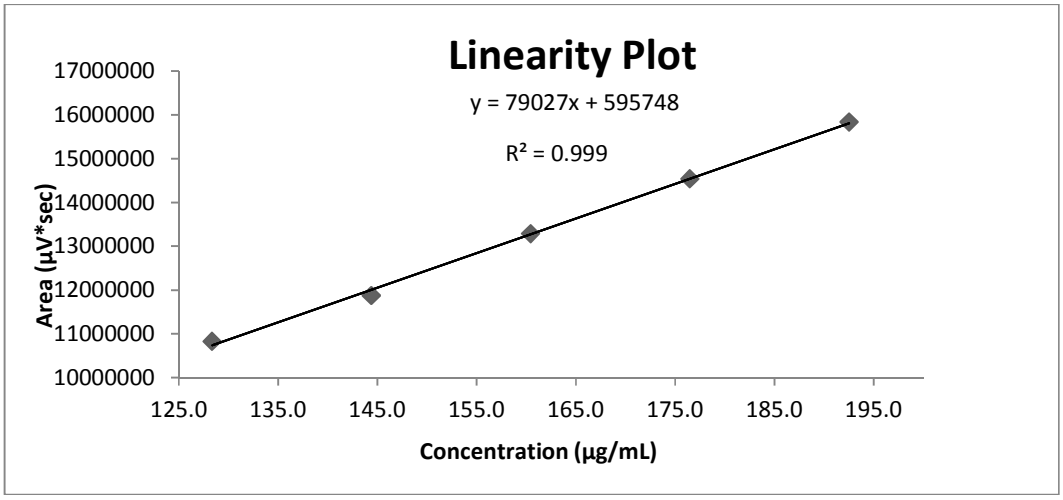


Fig. 4. Linearity graph of Silodosin

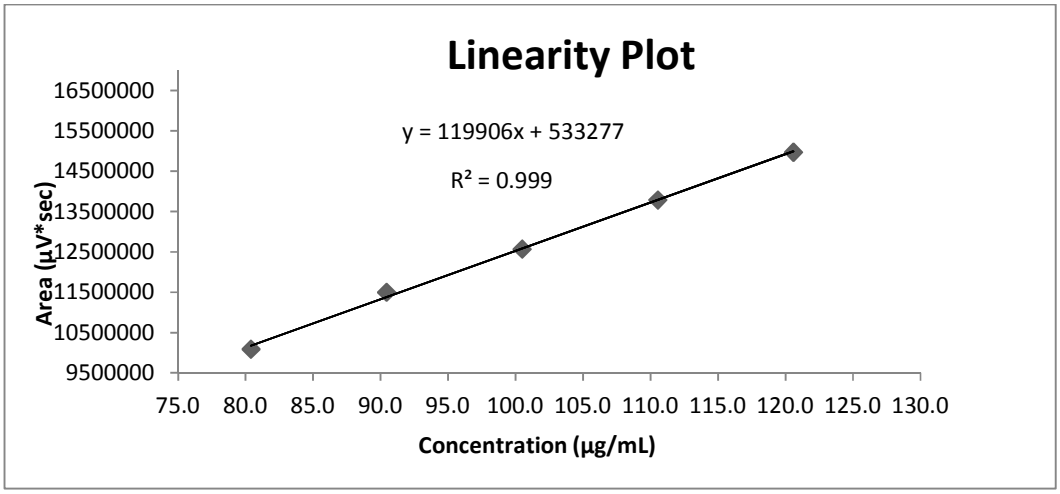


Fig. 5. Linearity graph of Tadalafil

Table 5. Summary of accuracy results of Silodosin

Level	Sample	Actual Amount added (mg)	Amount Recovered (mg)	% Recovery (98.0% - 102.0%)	% Mean Recovery
50%	1	80.269	79.686	99.3	99.4
	2	80.050	79.686	99.5	
	3	80.060	79.686	99.5	
100%	1	160.019	161.957	101.2	100.9
	2	159.790	160.528	100.5	
	3	160.199	161.957	101.1	
150%	1	240.378	239.650	99.7	100.0
	2	240.278	241.079	100.3	
	3	239.849	239.650	99.9	
Overall % Mean Recovery (Between 98.0% -102.0%)					100.1
Overall Standard deviation (SD)					0.70
Overall %Relative standard deviation (%RSD) (≤2%)					0.7

Table 6. Summary of accuracy results of Tadalafil

Level	Sample	Actual amount added (mg)	Amount recovered (mg)	% Recovery (98.0% - 102.0%)	% Mean recovery
50%	1	50.140	49.950	99.6	99.6
	2	50.259	49.950	99.4	
	3	50.010	49.950	99.9	
100%	1	100.030	100.626	100.6	100.6
	2	99.930	100.626	100.7	
	3	100.229	100.626	100.4	
150%	1	150.149	148.745	99.1	99.1
	2	149.930	148.745	99.2	
	3	150.329	148.745	98.9	
Overall % Mean Recovery (Between 98.0% -102.0%)					99.8
Overall Standard deviation (SD)					0.68
Overall %Relative standard deviation (%RSD) **(≤2%)**					0.7

Table 7. Summary of Robustness results of Silodosin and Tadalafil

Variation in chromatographic condition	Observed system suitability parameters in standard			
	USP Tailing (≤2.0)		%RSD (≤2%)	
	Silodosin	Tadalafil	Silodosin	Tadalafil
Method Precision	1.4	1.6	0.25	0.36
Column oven temperature (+5°C)	1.3	1.6	0.33	0.40
Column oven temperature (-5°C)	1.3	1.5	0.38	0.34
Buffer pH (+0.5 unit)	1.5	1.5	0.43	0.48
Buffer pH (-0.5 unit)	1.4	1.4	0.32	0.56
Wavelength (+3 nm)	1.6	1.6	0.67	0.43
Wavelength (-3 nm)	1.5	1.6	0.44	0.65
Flow rate (+10%)	1.7	1.8	0.39	0.28
Flow rate (-10%)	1.4	1.5	0.78	0.55

2.6.4 Robustness

Robustness of the method shall be demonstrated by deliberately changing the chromatographic parameters and monitoring system suitability parameters under each condition. Prepare standard solutions as described in method to be injected under each of the variable conditions such as wavelength of detection by ± 3 nm, flow rate by ± 10%, pH of buffer by ±0.2 unit and column oven temperature by ± 5°C.

3. RESULTS AND DISCUSSIONS

3.1 Specificity

There was no interference of blank and placebo at the retention time of Silodosin and Tadalafil peak. Also purity angle was found less than from purity threshold for both drugs. Hence the method was found to be specific.

3.2 Linearity and Range

The correlation coefficients for Silodosin and Tadalafil were found to be 0.9991 and 0.9992 respectively between 80%-120% range of the target concentration of analyte.

3.3 Precision

The % RSD was found 0.56 and 0.54 for system precision and 0.29 and 0.44 was found for repeatability study for Silodosin and Tadalafil respectively.

3.4 Accuracy

Percentage recovery for Silodosin was found to be 99.4%, 100.9% and 100.0% whereas for Tadalafil it was found to be 99.6%, 100.6% and 99.1% at three levels (50%, 100%, and 150%).

3.5 Robustness

All system suitability criteria were found within acceptance limit during small but deliberately changes in chromatographic conditions that indicates developed method is robust.

3.6 Stability of Analytical Solution

Prepare the standard and sample solution as per developed method, keep the solutions at 25°C. Inject at different time intervals. Solution stability of standard and sample solution was found for 24 hours.

4. CONCLUSION

Above results concluded that developed method is specific, precise, linear, reproducible and rugged. This method is validated according with ICH guideline. During analytical method validation, results were found satisfactory. Simultaneously quantification of Silodosin and Tadalafil in single analytical method with shorter run time shows this method cost-effective, time saving and can be used for routine analysis in industries.

DISCLAIMER

There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The authors are grateful to the Dr. Dishant Gupta (Asst. Professor) and management of college for providing amenities to carry out this work. I am very thankful to Metrochem API Pvt. Ltd providing API's of Silodosin and Tadalafil and also thankful to Finar chemicals Ltd for providing reagent and solvents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Masson P. Quality control techniques for routine analysis with liquid chromatography in laboratories. J Chromatogr A. 2007;1158(1-2):168-173. DOI:10.1016/j.chroma.2007.03.003
2. Nickerson B, Joseph RM, Palmer C, Opio AM, Beresford GH. Analytical method development: challenges and solutions for low-dose oral dosage forms. in: formulation and analytical development for low-dose oral drug products. John Wiley & Sons, Inc. 2008;239-263. DOI:10.1002/9780470386361.ch10
3. Taylor D. The Pharmaceutical Industry and the Future of Drug Development. In: Royal Society of Chemistry. 2015;1-33. DOI:10.1039/9781782622345-00001
4. Pisani J, Arlington S. Pharma 2020: Challenging business models. Innov Pharm Technol. 2009;(30):26-29.
5. Er E, Erk N. An effective and sensitive stability-indicating chromatographic approach based on HPLC for silodosin assay. J Anal Sci Technol. 2016;7(1). DOI:10.1186/s40543-016-0100-y
6. Patel DB, Patel NJ, Patel SK, Patel PU. Validated Stability Indicating HPTLC Method for the Determination of Dutasteride in Pharmaceutical Dosage Forms. Chromatogr Res Int. 2011;2011:1-5. DOI:10.4061/2011/278923
7. Bojanapu A, Subramaniam A, Munusamy J, et al. Validation and Method Development of Tadalafil in Bulk and Tablet Dosage Form by RP-HPLC. Drug Res (Stuttg). 2014;65(02):82-85. DOI:10.1055/s-0034-1372608
8. Ks N, A SR, Harshitha S, Pravallika K. Journal of drug metabolism & toxicology analytical method development and validation for the estimation of silodosin and dutasteride in capsule dosage form by RP-HPLC method. 2020;1-8. DOI:10.35248/2157-7609.20.11.245
9. Fidan AK, Bakirdere S. Simultaneous determination of sildenafil and tadalafil in legal drugs, illicit/counterfeit drugs, and wastewater samples by high-performance liquid chromatography. J AOAC Int.

2016;99(4):923-928.
DOI:10.5740/jaoacint.15-0320
10. Patel JK. Stability-indicating RP-HPLC method for determination of ambrisentan and tadalafil in pharmaceutical dosage form. Sci Pharm. 2014;82(4):749-763.
DOI:10.3797/scipharm.1403-22
11. Roumeguere T, Maxime Rossi. Silodosin in the treatment of benign prostatic hyperplasia. Drug Des Devel Ther. Published online October 2010:291.
DOI:10.2147/DDDT.S10428
12. Bozkurt O, Demir O, Sen V, Esen A. Silodosin causes impaired ejaculation and enlargement of seminal vesicles in sexually active men treated for lower urinary tract symptoms suggestive of benign prostatic hyperplasia. Urology. 2015;85(5):1085-1089.
DOI:10.1016/j.urology.2015.01.011
13. Yoshida M, Homma Y, Kawabe K. Silodosin, a novel selective α 1A - adrenoceptor selective antagonist for the treatment of benign prostatic hyperplasia. Expert Opin Investig Drugs. 2007;16(12):1955-1965.
DOI:10.1517/13543784.16.12.1955
14. Coward R, Carson C. Tadalafil in the treatment of erectile dysfunction. Ther Clin Risk Manag. 2008; 4:1315-1329.
DOI:10.2147/TCRM.S3336
15. Product information for AusPAR Urorec Silodosin Mayne Pharma International Pty Ltd 2017;(October): PM2016-00744-1-3,
16. Doğan A, Başcı NE, Polat MB. Spectrophotometry, potentiometry and HPLC in determination of acidity constant for Cabergoline and Tadalafil. J Res Pharm. 2019;23(2):177-186.
DOI:10.12991/jrp.2019.123
17. Ghadge O, Samant M and Khalel A. Determination of solubility of tadalafil by shake flask method by employing validated Hplc. 2015;4(7):1370-1382.
18. Snyder LR, Glajch JL, Kirkland JJ. Practical HPLC method second, Edition. 1997;2nd;.
19. Teasdale A, Elder D, Nims RW, eds. ICH quality guidelines. John Wiley & Sons, Inc.; 2017.
DOI:10.1002/9781118971147

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71927