Advance Journal of Pharmaceutical Research & Review
Volume 1, Issue 3, September 2024, PP: 97-107, ISSN No: 3048-491X

HPLC Method Development and Validation for Determination of Verdenafil as Bulk Drug and In Tablet Dosage Form

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Abstract
Vardenafil is separated using RP-high-performance liquid chromatography (HPLC) and quantified using a photo diode array (PDA) detector in bulk form. The Kromasil® C18 (5µm, 250mm×4.6mm) separation chromatographic column was used. The 30:70 MEOH: POT.HYDROGEN PHOSPHATE (20 MM) mobile phase was utilized, and the detection wavelength was 268 nm. Vardenafil retention duration was measured at 2.31 minutes with a flow rate of 1 milliliter per minute. A sample volume was injected to facilitate method development and validation, taking into account criteria like accuracy, precision (both intraday and interday), system appropriateness, specificity, robustness, and ruggedness. It was discovered that the limits of detection and quantification (LOD and LOQ) were, respectively, 0.56 ppm and 1.73 ppm. It was determined that the devised approach complied with the ICH criteria. As a result, the suggested RP-HPLC technique Consequently, vardenafil may be satisfactorily determined both qualitatively and quantitatively using the suggested RP-HPLC approach

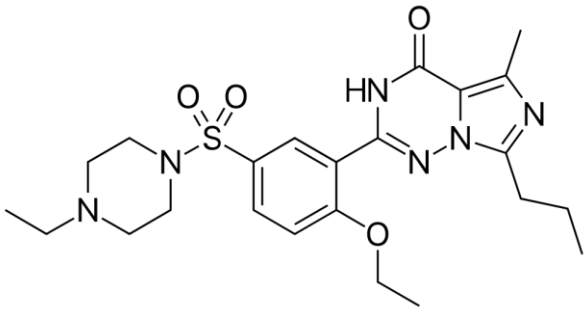
Keywords: RP-HPLC, Vardenafil , Validation

I. INTRODUCTION

Vardenafil is a robust and exact inhibitor of phosphodiesterase type 5 (PDE5), an enzyme that interruptions down recurring guanosine monophosphate (cGMP) in the corpus cavernosum. Smooth muscle relaxation, an growth in blood movement, and an erection are caused by the presence of cGMP in the corpus cavernosum. Consequently, normal sexual excitement will raise cGMP levels in the corpus cavernosum of vardenafil-treated erectile dysfunction patients. Vardenafil shouldn't produce cGMP or generate an erection in the absence of sexual stimulation..

Men whose underlying cardiovascular condition precludes them from engaging in sexual activity should not take vardenafil. Prolonged erections lasting more than four hours and priapism are potentially possible side effects. Patients should cease taking vardenafil immediately if they have sudden blindness in one or both eyes. PDE5 inhibitor users, such as those on vardenafil, may also experience a longer QT interval and abrupt hearing loss.

Vardenafil reduces PDE5, a phosphodiesterase that is restricted to cyclic guanosine monophosphate (GMP). In the corpus cavernosum, which is located around the penis, PDE5 is in charge of degrading cyclic GMP. Penile erection when sexually stimulated is due to increased penile blood flow caused by the relaxation of the penile arteries and corpus cavernosal smooth muscle. Nitric oxide (NO) is released from nerve terminals and endothelial cells, mediating this response and stimulating smooth muscle cells to produce cyclic GMP. Cycle-induced smooth muscle relaxation and improved blood flow into the corpus cavernosum are the results of GMP. The tissue distribution of this protein is regulated by the rates of cyclic GMP synthesis and breakdown by phosphodiesterases (PDEs



IUPAC NAME

2-{2-ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl}-5-methyl-7-propyl-1H,4H-imidazo[4,3-f][1,2,4]triazin-4-o Instruments and equipments

Table no 1: Instrumentation

S.No	Equipments	Make	Model
1	Electronic balance	Shimadzu Corporation	BL-220H
2	High Performance Liquid Chromatography	Waters HPLC	2695
3	Digital pH Meter	ELICO® LI 120	---
4	UV-Visible spectrophotometer	Shimadzu	UV 1800

Results for method development

Preliminary study: Screening of columns and mobile phases

Table no 2 : Various optimization trials and chromatograms are presented in the following table

Trail no	Coloum	Mobile phase (% v/v)	tR (min)	Theoretical plates	Tailing
1	Discovery 250	40:60 ACN: OPA	2.8	2980	1.3
2	BDS 250	30:70 ACN: MEO	2.676	2985	1.25
3	Kromasil 250	50:50 ACN: OPA	2.174	7692	1.29
4	Kromasil 250	30:70 MEOH: POT.HYDROGEN PHOSPHATE(20 MM)	2.31	6590	1.4

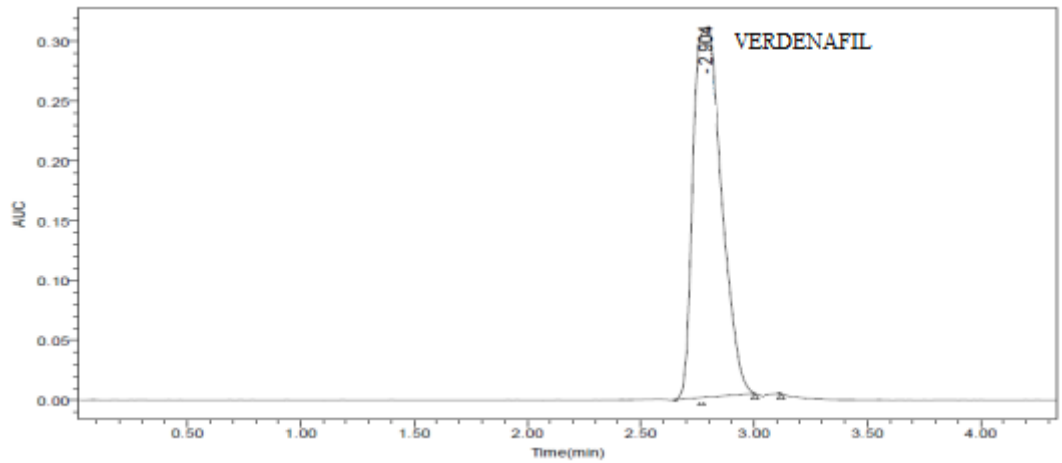


Figure 1 : Trail 1 of [ACN: OPA (50:50% v/v)]

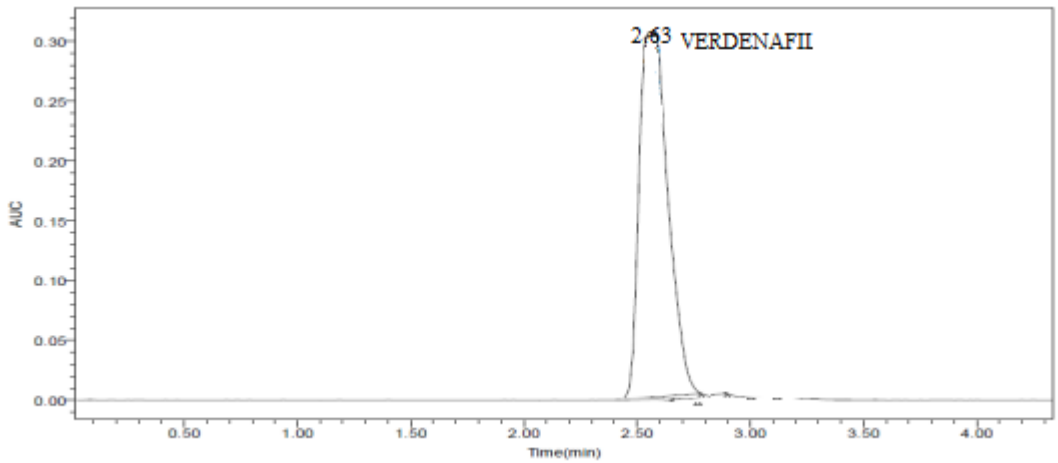


Figure 2 : Trail 2 of [ACN: MEOH (50:50% v/v)]

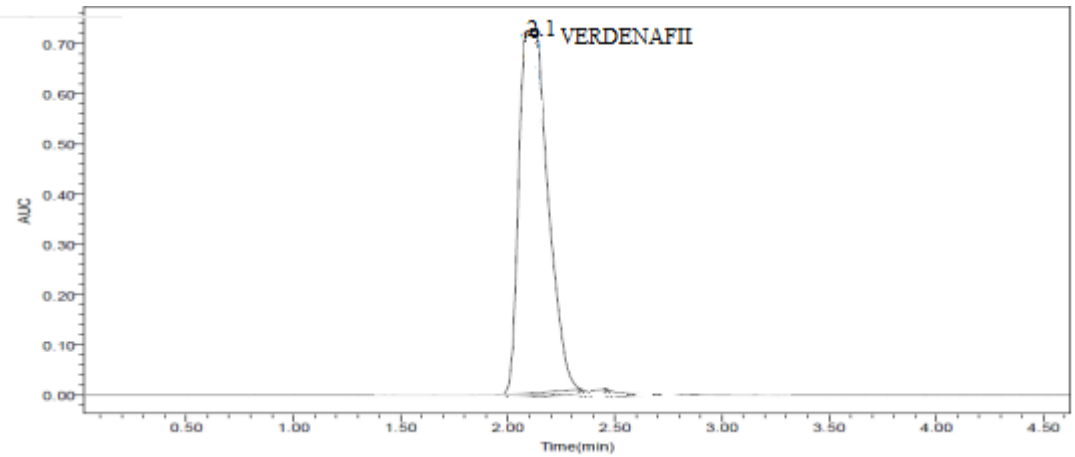


Figure 3 : Trail 3 of [ACN: OPA (50:50% v/v)]

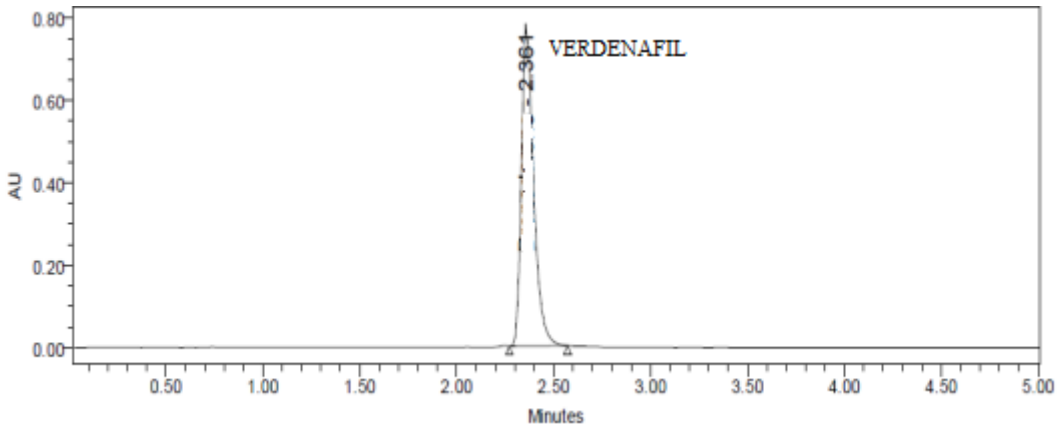


Figure 4 : Trail 4 of [MEOH: KHP (30:70% v/v)]

Table no 3 : Optimized conditions for Verdenafil

Parameters	Optimized conditions
Mobile Phase	MEOH: KHP (30:70% v/v)]
Column	Kromasil® C18
Flow Rate	1ml/min
Temperature	30°C
Injection Volume	10µL
Detector	268 nm

Preparation of stock solution

After carefully measuring and transferring 25 mg of verdenafil along with the standard into a 25 ml dry volumetric flask, you should add 15 ml of solvent, sonicate the solution for 30 minutes, and then use diluents to bring the volume up to the final amount. (1 000 ppm Verdenafil).

Preparation of working standard solution

1 ml from the over stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml with the diluents to get 100 ppm concentration.

Results for validation of Method

Table no 4 : System suitability data for Verdenafil

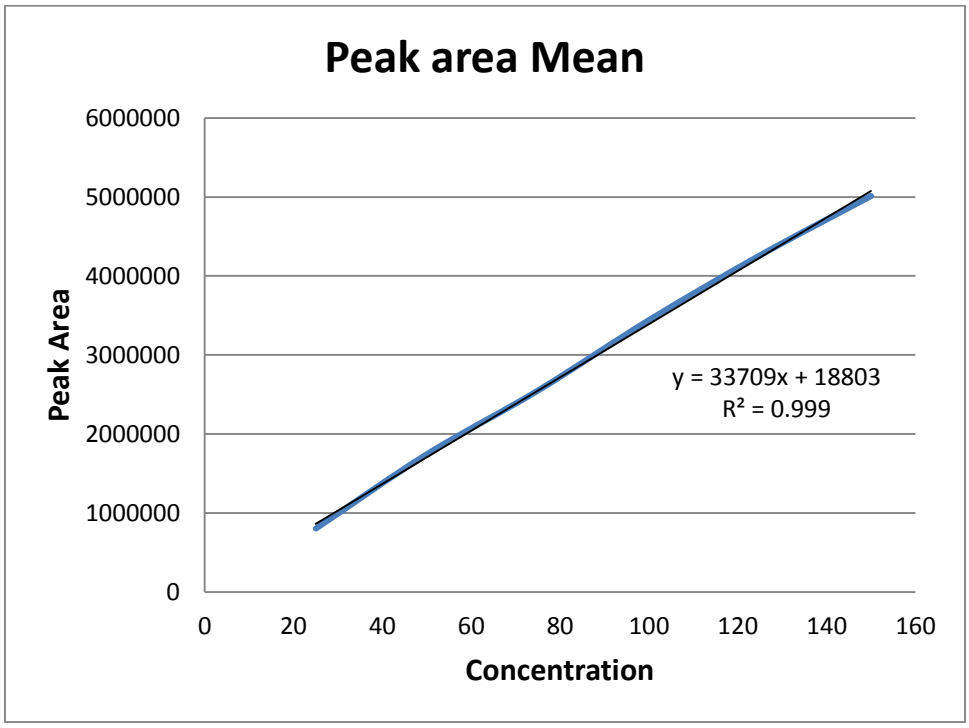
S.No.	Parameter	Verdenafil	Acceptance Limit
1	Retention time	2.31	-
2	Number of theoretical plates (N)	6590	More than 2000
3	Tailing factor (T)	1.4	Less than 2
4	Capacity factor (K)	1.35	0.5<K<20

Linearity

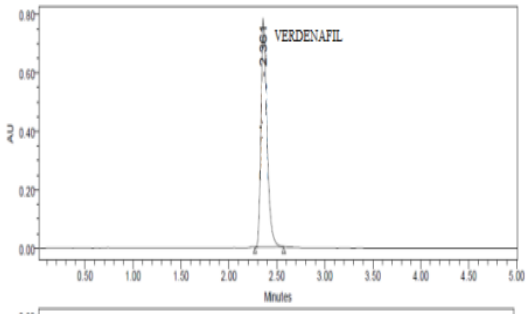
The calibration curves were created using six distinct standard Verdenafil concentrations, ranging from 25 to 150 ppm. At each concentration, the samples were examined in triplicate, and mean values were determined. Plotting concentration against peak area allowed for the determination of the analytes' calibration curve. According to ICH criteria, the correlation coefficient value of 0.999 was deemed to be within the permissible bounds.

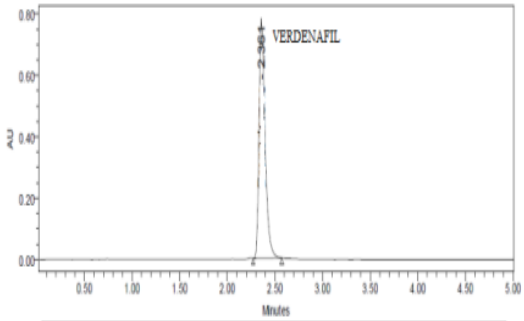
Table no 5 : Linearity

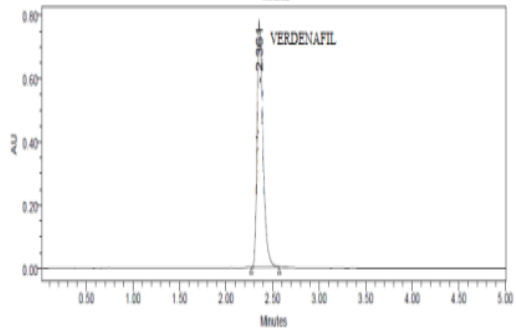
Linearity Range	Concentration(ppm) Verdenafil	Peak Area Mean ± S.D. (n=3)
25	25	802411± 8686.10
50	50	1743366± 10281.42
75	75	2549446± 40321.49
100	100	3441280±32089.11
125	125	4261237±28806.57
150	150	5012442±17442.28
Slope	33709	
Intercept	18803	
Correlation coefficient	0.999	



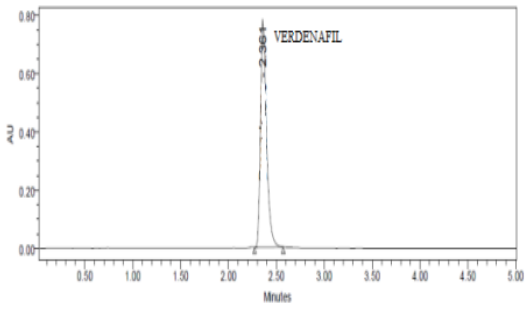
Calibration Curve for Verdenafil

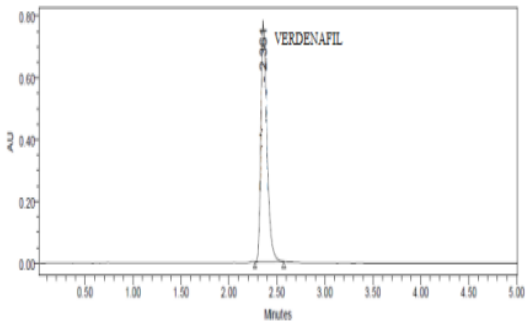


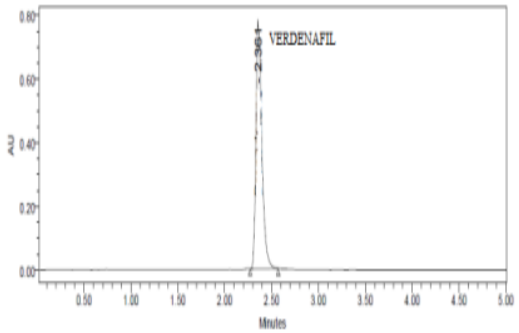




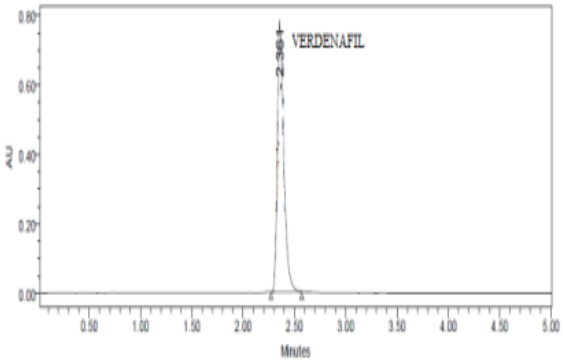
Linearity Chromatograms of Verdenafil at 25 % range

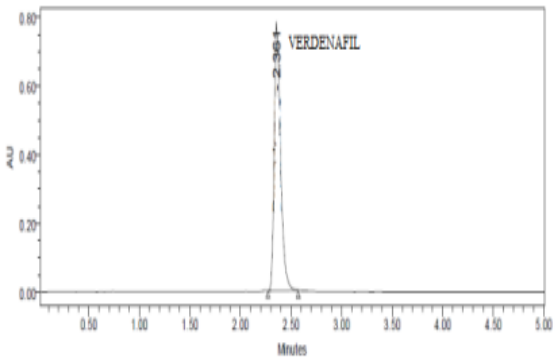


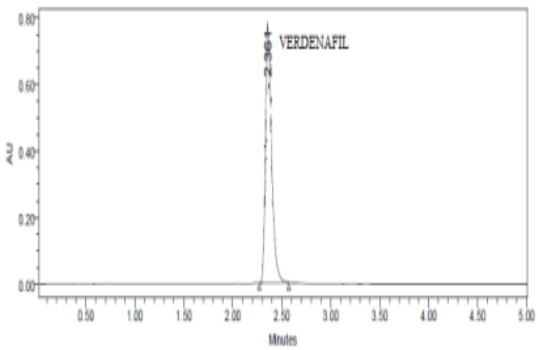




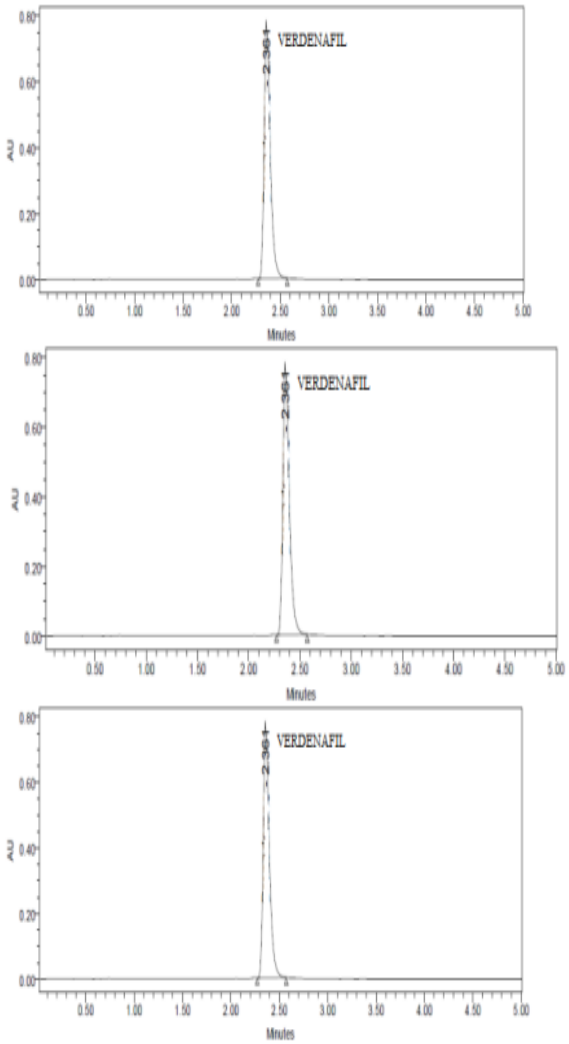
Linearity Chromatograms of Verdenafil at 50 % range



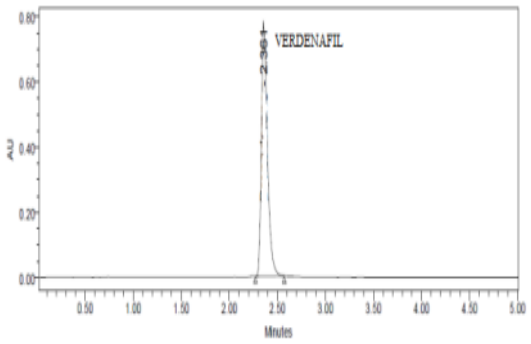


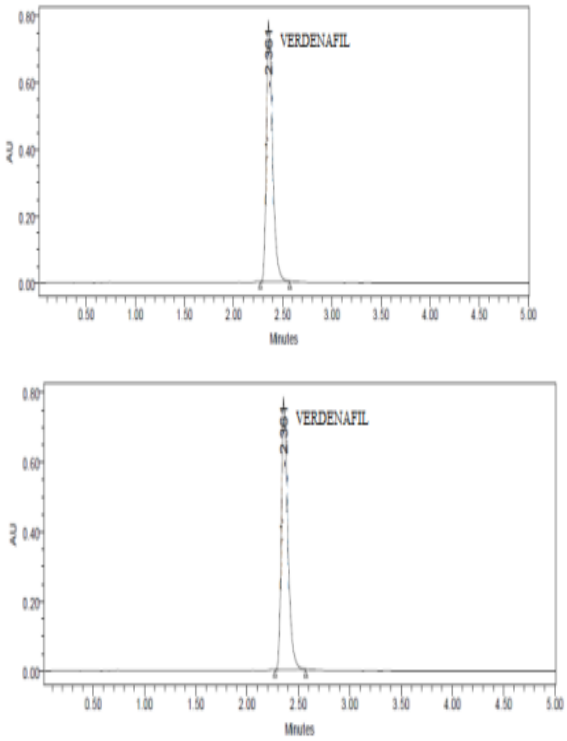


Linearity Chromatograms of Verdenafil at 75 % range

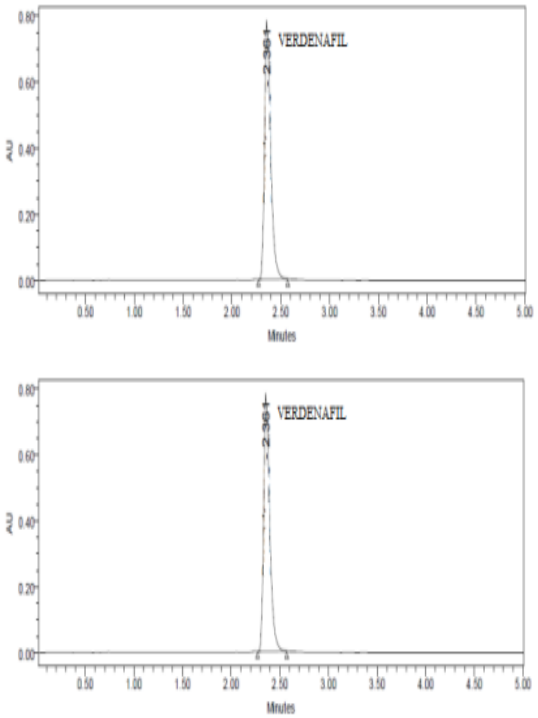


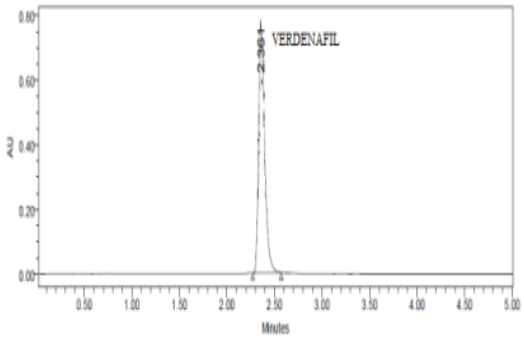
Linearity Chromatograms of Verdenafil at 100 % range





Linearity Chromatograms of Verdenafil at 125 % range

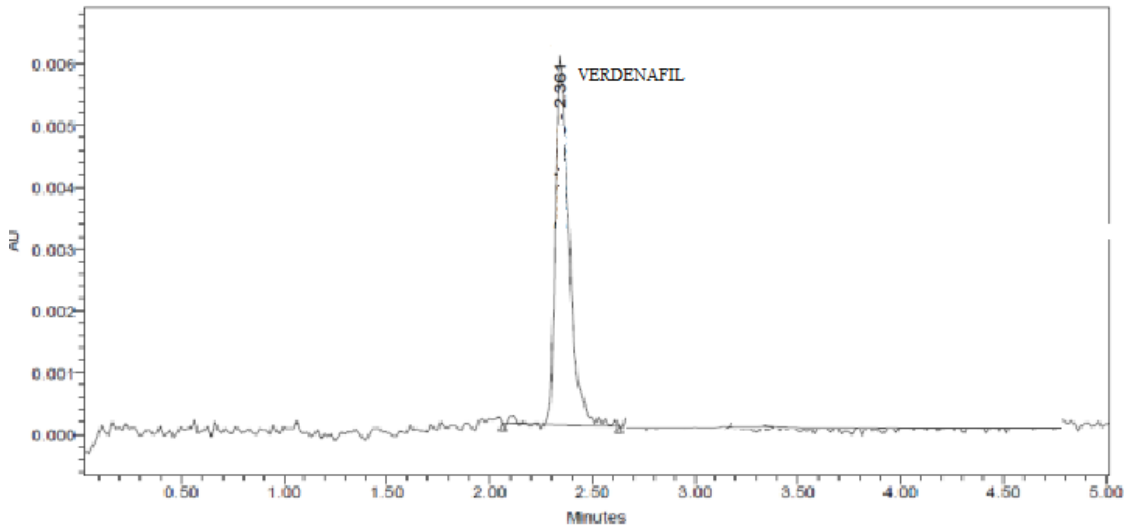




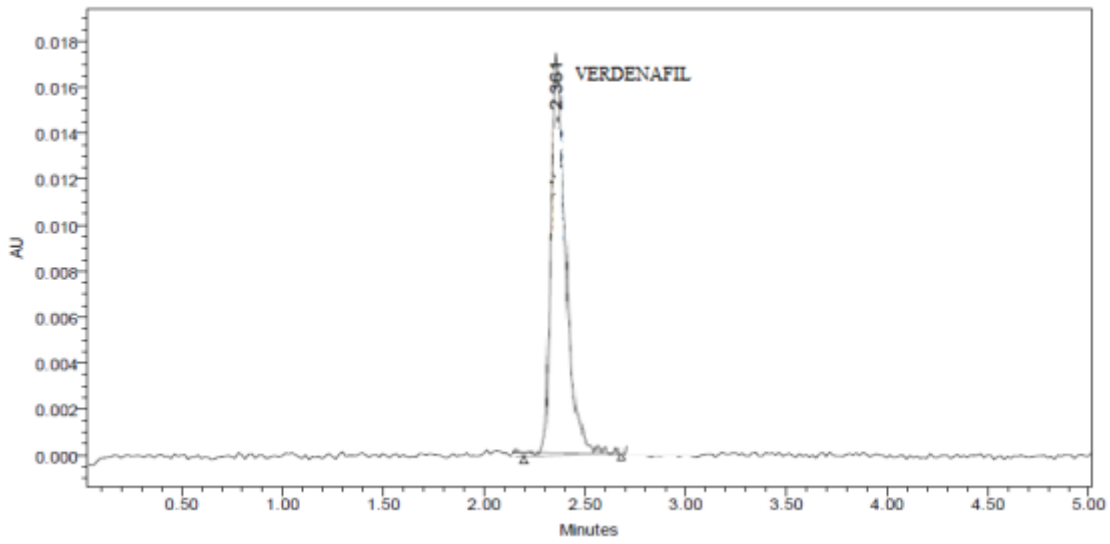
Linearity Chromatograms of Verdenafil at 150 % range

LOD & LOQ

The limits of detection and quantification were determined using the response standard deviation and calibration curve slope. The recommended approach's verdenafil LOD and LOQ were found to be 0.56 ppm and 1.73 ppm, respectively, based on the chromatograms shown in Figure.



LOD Chromatogram for verdenafil.



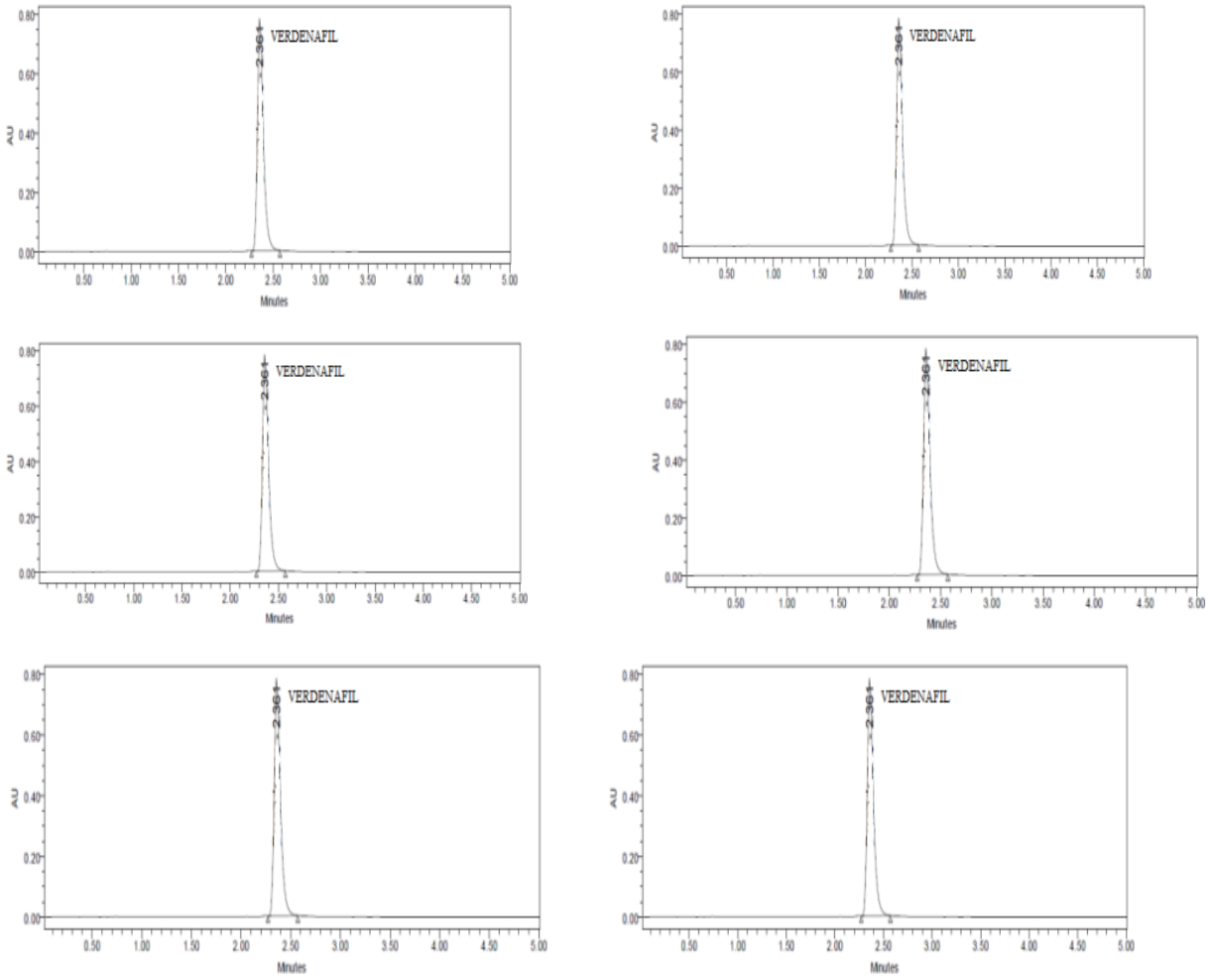
LOQ Chromatogram for verdenafil

Precision

Table no 6 : Precision results for Verdenafil

Injection replicates	PA of Standard	PA of Sample (Intraday precision)	PA of Sample (Interday Precision)
1	3413451	3408491	3330411
2	3401319	3418311	3344421

3	3421652	3434262	3347113
4	3424976	3417142	3342556
5	3321256	3437345	3354517
6	3334131	3411639	3379361
Mean	3311497	3411649	3322144
SD	37931.8	13256.2	8587.4
% RSD	0.8	0.4	0.3



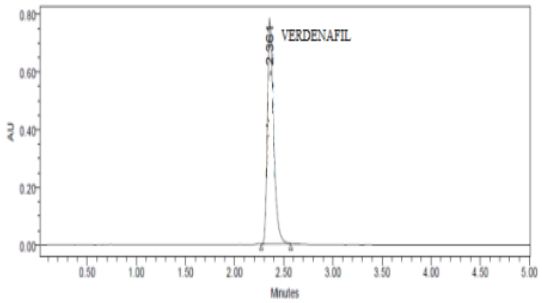
Precision Chromatograms for Verdenafil

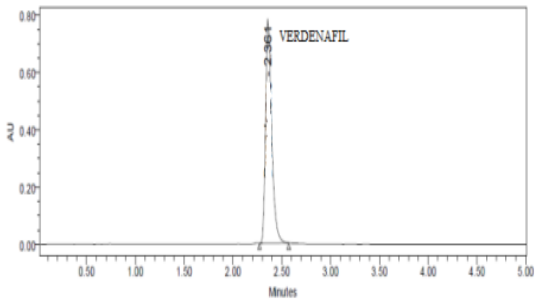
Accuracy

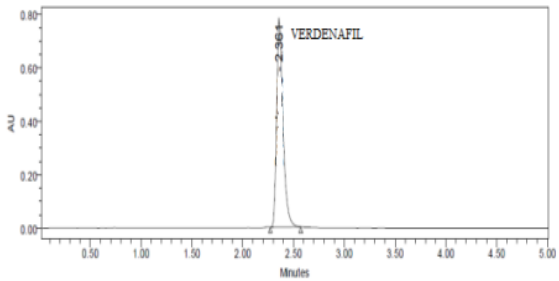
Table no 7 :Accuracy for Verdenafil

Recovery level	**Verdenafil**	
	Mean % recovery± SD *	**% RSD**
50	99.29±0.06	0.07
100	100.92±0.32	0.32
150	99.25±0.51	0.51

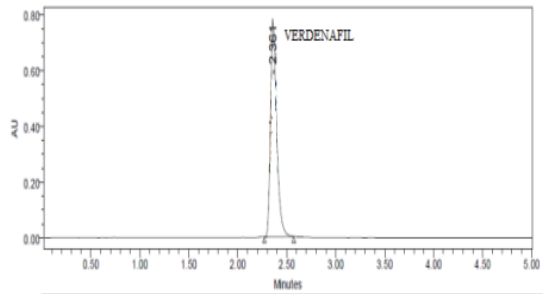
*** % recovery from triplicate determinations**

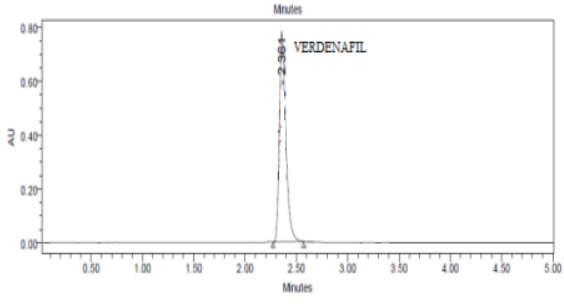


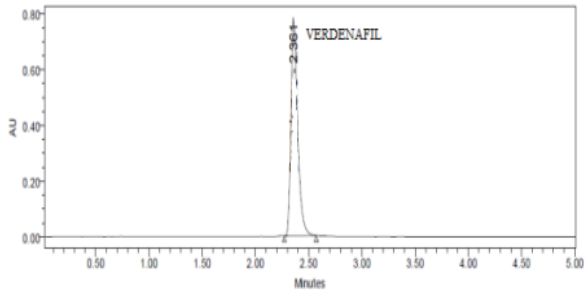




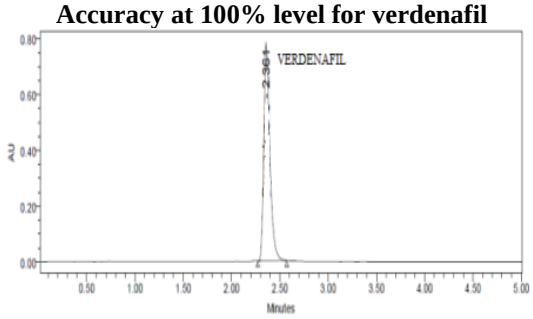
Accuracy at 50% level for Verdenafil

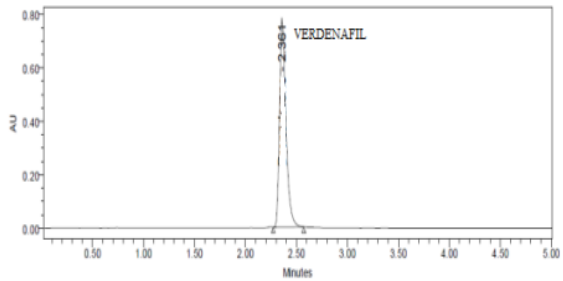


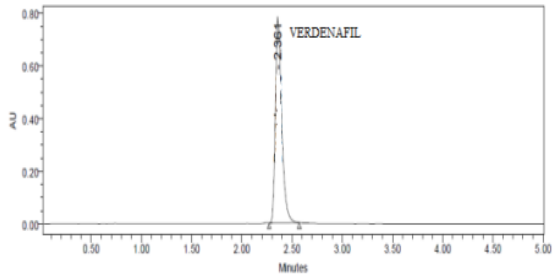




Accuracy at 100% level for verdenafil







Accuracy at 150 % level for verdenafil

Table no 8 :Robustness for Verdenafil

Parameters	Value	Plate count± SD	Tailing± SD	% RSD for peak area
Flow rate (mL/ min)	0.8	6034.83±414.80	1.42±0.03	0.94
	1	6224.83±584.80	1.39±0.03	0.72
	1.2	6470.33±794.91	1.37±0.04	0.49

Mobile phase (%POT.HYDROGEN PHOSPHATE	70	5579.16±630.62	1.37±0.03	0.93
	80	5169.16±6430.64	1.55±0.20	0.73
	90	4729.16±2242.24	1.75±0.20	0.56
Column Temperature (°C)	28	7674.00±33.79	1.39±0.01	0.41
	30	7132.83± 329.29	1.40±0.05	0.62
	32	6732.83± 389.29	1.42±0.05	0.82

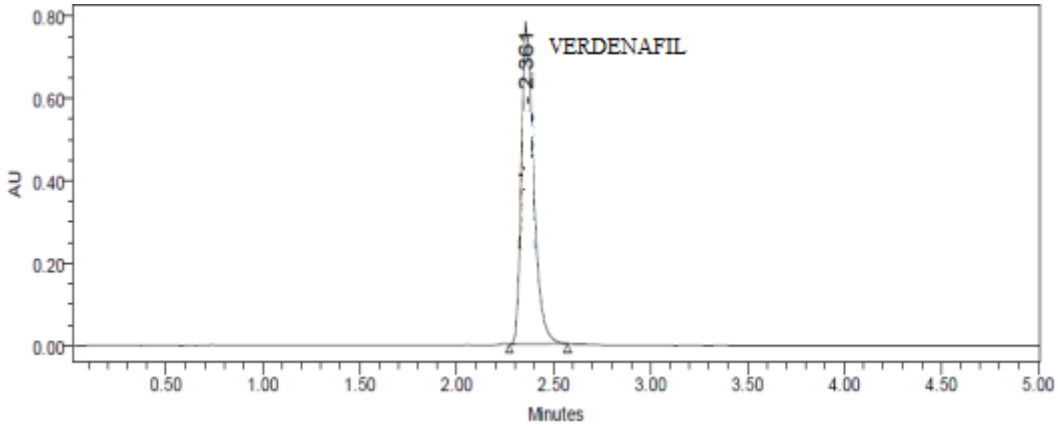
Application to tablet dosage form

Assaying involves injecting 10µl of standard and sample solutions into the RP-HPLC system in order to determine the percentage purity of a particular chemical using an appropriate procedure. This calculation of the assay percentage in the prepared solution was done using the peak area of the detector response. The suggested approach was used for the Lavitra pill, and it was found that the mean assay percentage for verdenafil was 100.64%. The chromatogram showed that excipients were not causing any blockage.

The drug's assay findings are reported in the pharmaceutical product [(Lavitra Tablet, Label Claim 10 mg Verdenafil): (n=6)].

Table no 10 : The drug's assay

Parameter	Verdenafil
Mean Peak area± SD n	3351185±8346.5
Recovery (%)	100.63±0.22
RSD (%)	0.24
System Suitability Parameters	
Retention time (min)	2.31
Tailing factor	6590
Plate count	1.4
Capacity factor	1.35



Sample chromatogram for verdenafil

II. CONCLUSION

For the estimation of vardenafil in bulk form, a straightforward, affordable, accurate, and reliable RP-HPLC method has been devised and validated. The ICH rules state that all of the validation parameters fall within the acceptance requirements. Thus, routine qualitative and quantitative examination of vardenafil in pharmaceutical bulk form can be effectively conducted using the suggested method.

ACKNWOLEDGEMENTS

The authors wish to thank Sigma-aldrich (USA) for supplying the samples of vardenafil within the required time so as to enable us to complete this research paper quickly. Special thanks to SSJIPER College of Pharmacy jammer .

REFERENCES

[1]. Young JM. Vardenafil. Expert Opin Investig Drugs. 2002; 11(10): 1487-96. doi: 10.151 7/13543784.11.10.1487, PMID 12387708.

[2]. Ashour AE, Rahman AM, Kassem MG. Vardenafil dihydrochloride. Profiles Drug

Subst Excipients Relat Methodol. 2014; 39: 515-44.

[3]. Abu Lila AS, Gomaa E, Ghazy FES, Hasan AA. Treatment of pulmonary arterial hypertension by vardenafil-solid dispersion lozenges as a potential alternative drug S542 Indian Journal of Pharmaceutical Education and Research, Vol 58, Issue 2 (Suppl), Apr-Jun, 2024 Gadekar, et al.: Fast and Accurate HPLC Technique for Quantifying Vardenafil delivery system. J Drug Deliv Sci Technol. 2020; 55: 101444. doi: 10.1016/j.jddst.201 9.101444.

[4]. Jing ZC, Yu ZX, Shen JY, Wu BX, Xu KF, Zhu XY, et al. Vardenafil in pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled study. Am J Respir Crit Care Med. 2011; 183(12): 1723-9. doi: 10.1164/rccm.201101-0093OC, PMID 21471085.

[5]. Kumar AV, Reddy T, Sekharan C. Spectrophotometric analysis of vardenafil in tablet dosage forms by using electrophilic coupling reagents, Anal Bioanal. Chem Res. 2016; 3(1): 29-39.

[6]. Ahmed NR, Ahmed IA, Qasim HY. Ultrasensitive estimation of vardenafil. HCl in pharmaceutical preparations and environmental waste water samples. Eur J Biomed PharmSci. 2020; 7: 85-9.

[7]. Gratz SR, Flurer CL, Wolnik KA. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. J Pharm Biomed Anal. 2004; 36(3): 525-33. doi: 10.1016/j.jpba.2004.07.004, PMID 15522526.

[8]. Limin, M., Johnsen, N.and Hellstrom, W.J. A new rapid-onset phosphodiesterase 5 inhibitor for the treatment of erectile dysfunction. Expert Opin Investig Drugs, 2010. 19(11): p. 1427-37.

[9]. Levitra (vardenafil hydrochloride) package insert. Whippany, NJ: Bayer HealthCare Pharmaceuticals Inc; April 2014. 2014.

[10]. Abdelshakour, M.A., et al., HPLC-UV and UPLC-MS/MS methods for the simultaneous analysis of sildenafil, vardenafil, and tadalafil and their counterfeits dapoxetine, paroxetine, citalopram, tramadol, and yohimbine in aphrodisiac products. RSC Advances, 2021. 11(14): p. 8055-8064.1