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Development and Validation of RP-HPLC Method for Estimation of Vardenafil in Bulk and Pharmaceutical Formulation

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ABSTRACT

A specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of vardenafil in bulk and pharmaceutical formulation. A Hypersil BDS C 8, 5µ column having 250 x 4.6 mm internal diameter in isocratic mode with mobile phase containing buffer and acetonitrile (60:40, v/v) was used. The flow rate was 1.0 ml/min and effluents were monitored at 240.0 nm over the concentration range of 50-250 µg/ml. The retention time obtained for vardenafil was 5.4 min. The method was validated for linearity, accuracy, repeatability, precision, specificity (in terms of acid, alkali, peroxide and thermal degradation), limit of detection, limit of quantification and ruggedness. Chromatographic peak purity data demonstrated specificity of the method to estimate vardenafil in presence of degradation products. Limit of detection and limit of quantification were found 0.0125 µg/ml and 0.025 µg/ml respectively. Accuracy in terms of recovery of vardenafil from tablet formulation was found to be above 98 % indicating that vardenafil can be estimated from tablet dosage form without interference from the excipients. The validation data proves the potential utility of the proposed method for the quantitative determination of vardenafil in tablet formulation.

Keywords: Vardenafil, RP-HPLC, Validation

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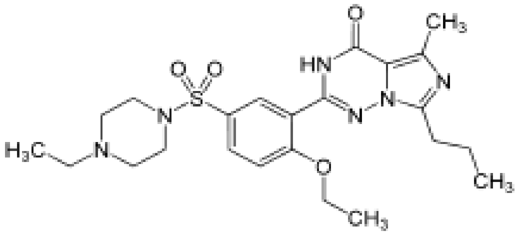
INTRODUCTION

Vardenafil[1, 2] is a phosphodiesterase type 5 (PDE5) inhibitor used to treat erectile dysfunction (ED). It is chemically 2-[2-ethoxy-5-(4-ethylpiperazine-1-sulfonyl)phenyl]-5-methyl-7-propyl-1H,4H-imidazo[4,3-f][1,2,4]triazin-4-one. Vardenafil was approved by the FDA in September 2003. It is marketed under the trade name LEVITRA and STXYN. Vardenafil inhibits the cGMP specific phosphodiesterase type 5 (PDE5) which is responsible for degradation of cGMP in the corpus cavernosum located around the penis. Penile erection during sexual stimulation is caused by increased penile blood flow resulting from the relaxation of penile arteries and corpus cavernosal smooth muscle. 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 causes smooth muscle relaxation and increased blood flow into the corpus cavernosum. The inhibition of phosphodiesterase type 5 (PDE5) by vardenafil enhances erectile function by increasing the amount of cGMP. Literature reveals many methods for the estimation of vardenafil that include HPLC[3], HPLC method with UV detection (Human plasma)[4], HPLC-Chemiluminescence (Dietary Supplement)[5], Stability indicating RP-LC Method[6,10], HPLC method using fluorescence detection (Rat plasma and bile)[7], GC-MS(Blood)[8], Stability-Indicating RP-LC (UPLC)[9], LC/MS (Biological specimen)[11] and Capillary Electrophoretic Method[12]. The present study is an attempt to explore the use of C8 chromatographic column in development of RP-HPLC method and to validate the proposed method. The specificity study was carried out in API and tablet dosage form, in terms of acid, alkali, peroxide and thermal degradation, and it revealed satisfactory results.

Structure of vardenafil:



2-[2-ethoxy-5-(4-ethylpiperazine-1-sulfonyl) phenyl]-5-methyl-7-propyl-1H, 4H-imidazo [4, 3-f] [1, 2, 4] triazin-4-one

MATERIALS AND METHODS

Apparatus

HPLC measurements were made on Agilent 1100 series, PDA detector and ruggedness was checked on Waters Alliance series, UV detector.

Reagents and solutions

All chemicals were of analytical reagent grade and solutions were prepared with HPLC water. Vardenafil gift sample was obtained from Centurion Laboratories, Vadodara, Gujarat.

Chromatographic condition

The analysis was performed by using HPLC instrument. The column used was Hypersil BDS C 8, 250 x 4.6 mm, 5 µ (Thermo scientific, U.K.). The mobile phase consisted of buffer (Dissolve 4.5 gm of potassium dihydrogen phosphate in 1000 ml water. Adjust the pH to 4.5, if necessary) and acetonitrile and was filtered through membrane filter (0.45µ), degassed and pumped from the solvent reservoir into the column in the ratio of 60:40(v/v). The flow rate of mobile phase was maintained at 1.0 ml/min and detection wavelength was set at 240.0 nm with a run time of 10 min. The volume of injection loop was 20µl. Prior to injection of the drug solution, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of standard stock solution

Vardenafil, 25 mg, was accurately weighed in to a 50 ml volumetric flask, dissolved and diluted to volume with mobile phase. Further 5 ml of this solution was diluted to 25 ml with mobile phase.

Preparation of sample solution

Ten tablets were accurately weighed and ground to fine powder. The powder equivalent to 10 mg of vardenafil was accurately weighed and transferred to a 100 ml volumetric flask. To this was added, 80 ml of mobile phase and sonicated for about 15 minutes. The volume was made with mobile phase, mixed well and centrifuged at 4000 rpm for 10 minutes to get clear supernatant.

Calibration curve

Appropriate aliquots of standard stock solution of vardenafil were taken into 50 ml volumetric flasks separately and were diluted up to the mark with mobile phase to obtain the final concentration of 50, 75, 100, 125, 150, 175, 200 and 250 µg/ml of vardenafil. These solutions were injected into chromatographic system, chromatograms were obtained and peak area was determined for each concentration of drug solution. Calibration curve of vardenafil was constructed by plotting peak area versus applied concentration of vardenafil and regression equation was computed.

METHOD VALIDATION

The proposed method was validated in terms of linearity, range, repeatability, method precision, intermediate precision, accuracy, LOD, LOQ, solution stability, specificity, ruggedness and system suitability. The results of all validation parameters are shown in table 2 to table 7 and graphically depicted in figure 1 to 4.

Linearity and range

The linearity of response for vardenafil was determined by preparing and injecting solutions with concentrations of 50 µg/ml to 250 µg/ml.

Repeatability

Intraday and interday repeatability of the proposed method was determined on the API.

(A) Intraday

The solutions of three different concentrations viz. 50 µg/ml, 100 µg/ml and 150 µg/ml were prepared and six replicates of each were injected into HPLC system on the same day. The % relative standard deviation was calculated.

(B) Interday

Fresh solutions of three different concentrations viz. 50 µg/ml, 100 µg/ml and 150 µg/ml were prepared and six replicates of each were injected into HPLC system on 3 consecutive days. The % relative standard deviation was calculated.

Precision

Precision was checked in terms of method precision and intermediate precision.

Method precision

Six sample solutions of 100 µg/ml were prepared from the tablet formulation and analysed by the proposed method to check the method precision.

Intermediate precision (Interday)

Three fresh sample solutions, each of 100 µg/ml, were prepared from the tablet formulation and analysed on 3 consecutive days to check intermediate precision.

Limit of Detection and Limit of Quantitation

Limit of Detection (LOD) and limit of Quantitation (LOQ) for vardenafil was determined by visual method. In this, subsequent dilutions of different concentrations of vardenafil were prepared and injected into HPLC system. The limit of Quantitation (LOQ) was determined as the minimum concentration at which the % RSD of three replicates were below 10.0 %. The limit of detection (LOD) was determined as the concentration at which the peak of vardenafil was detectable.

Accuracy

Accuracy (recovery) studies were performed by spiking vardenafil API in tablet powder at levels

50, 100 and 150 % of label claim.

Solution stability (room temperature)

The stability of vardenafil solution (100 µg/ml) was evaluated by injecting a freshly prepared standard solution at zero time and subsequently at different time intervals. The peak response data are given in table 3.

Specificity

The specificity of method was checked by carrying out forced degradation of the API and tablet sample with 1 N HCl, 1 N NaOH, 30 % hydrogen peroxide and heating in water bath at 80°C for 30 minutes. The samples were prepared as described in preparation for sample solution and injected into HPLC column with a photodiode array detector. In each case, vardenafil peak was observed for specificity of the proposed method.

Ruggedness

Method ruggedness was determined by analysing the sample at normal operating conditions and also by changing some of the operating analytical conditions such as column, instrument, mobile phase composition and flow rate. The changed conditions are described as follows.

Table 1: Ruggedness Study

Parameter	Normal condition	Changed condition
Column	Hypersil BDS C8, 250 x 4.6 mm, 5 µ (Thermo scientific, UK)Sr No. : 0583870M	Hypersil BDS C8, 250 x 4.6 mm, 5 µ.(Thermo scientific, UK) Sr No. : 0583834M
Flow rate	1.0 ml/min	1.2 ml/min
Mobile phase ratio	Buffer : Acetonitrile – 60 : 40	Buffer : Acetonitrile – 65 : 35
System	Agilent 1100 series, PDA detector	Waters Alliance series, UV detector

RESULTS AND DISCUSSION

RP-HPLC method has been developed and validated for estimation of vardenafil using C 8 column. The results are summarized in table 2 to 7 and figure 1 to 4. The mobile phase was optimized taking different ratios of buffer (pH 4.5) and acetonitrile. In the first trial, 50:50 v/v ratio of buffer: acetonitrile was used and methanol was used as a diluent. The result obtained suffered from fronting of the peak. However, by changing the ratio of mobile phase to 60: 40 v/v and using the same mobile phase as a diluent, this problem could be solved and well defined peak was obtained at 5.4 min. Typical chromatogram of vardenafil is depicted in figure 1.

The proposed method was found to be linear in the concentration range of 50 to 250 µg/ml with correlation coefficient (r^2) 0.9998, slope 27.7756 and intercept 1.0522 as shown in table 2. The calibration curve is shown in figure 2. The method was validated in terms of accuracy, precision, repeatability, LOD, LOQ, solution stability, specificity, ruggednes, system suitability and the results are recorded in table 3 to table 7. The results of interday and intraday precision (% RSD less than 2), method precision and intermediate precision as shown in table 3 indicate that the proposed method is precise enough for the analysis of drug. Also, satisfactory stability of solution was found up to 48 hrs and % RSD was found to be 0.81%. Limit of detection and limit of Quantitation were found to be 0.0125 µg/ml and 0.025 µg/ml respectively. The accuracy of the method was determined by performing recovery studies by standard addition method. Values of recovery ± SD greater than 98.0% (table 4) indicate that the proposed method is accurate for the analysis of drug.

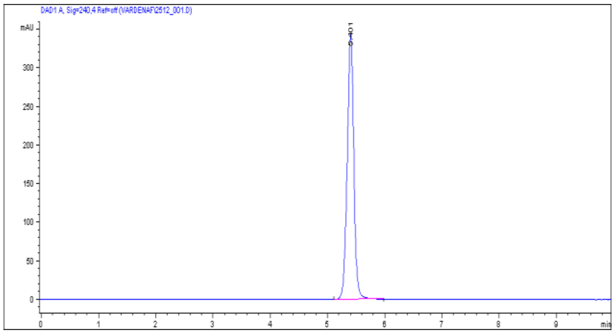


Figure 1: Typical Chromatogram of Vardenafil

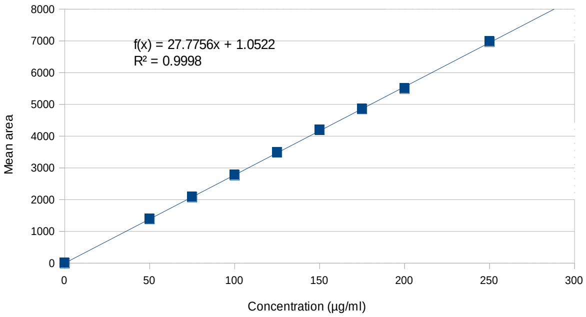
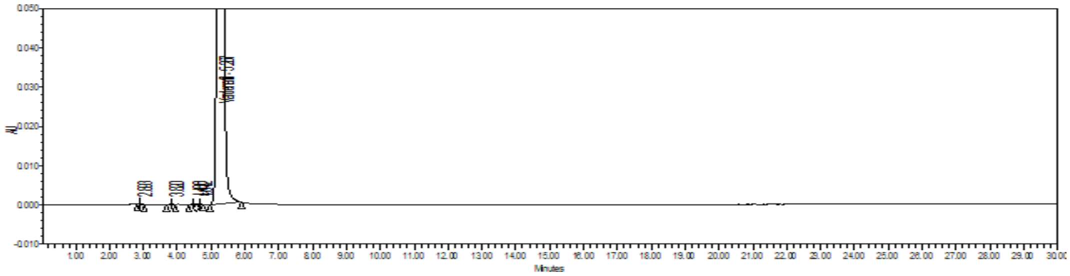
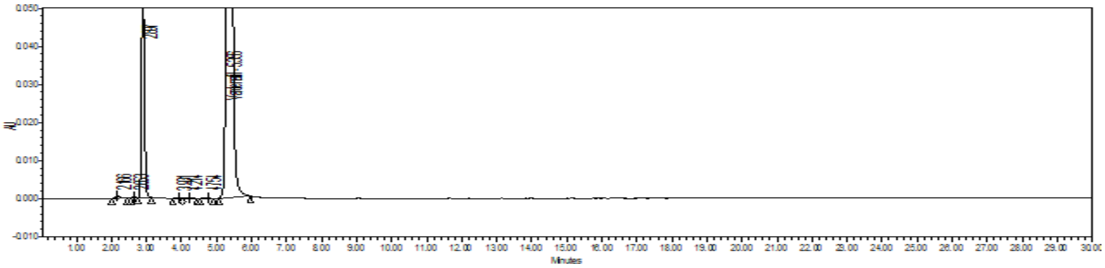


Figure 2: Calibration curve of Vardenafil

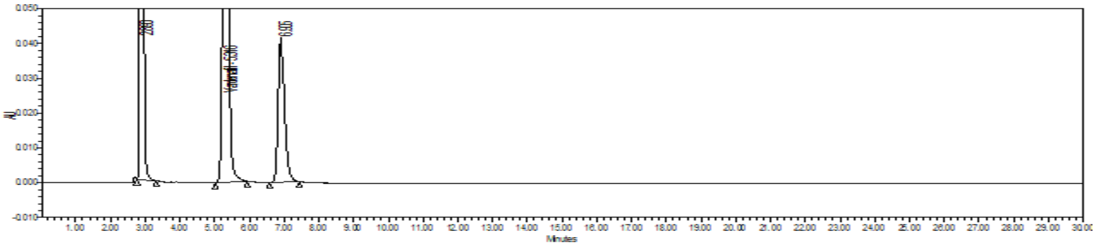
The specificity of method was checked by carrying out forced degradation study of API and tablet sample in terms of acid degradation using 1 N HCl, alkali degradation using 1 N NaOH, peroxide degradation using 30 % hydrogen peroxide and thermal degradation by subjecting the sample to 80^{o}C for 30 min. The results shown in table 5 and figure 3 and 4 reveal that in each case, vardenafil peak was passing in terms of peak purity criteria. Chromatographic peak purity results indicated the absence of co-eluting peaks with the main peak of vardenafil, which demonstrated the specificity of assay method for estimation of vardenafil in presence of degradation products.



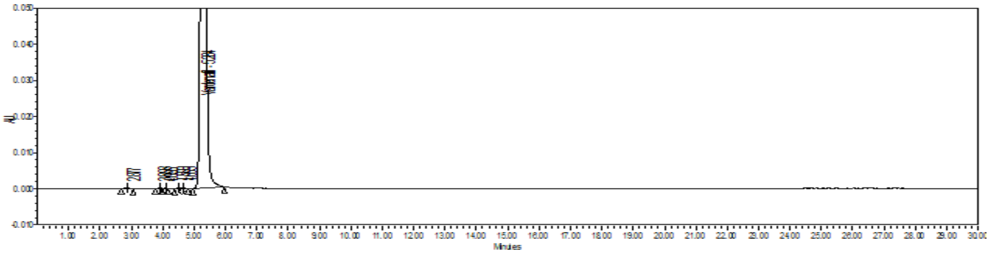
(A)Acid degradation



(B)Alkali degradation

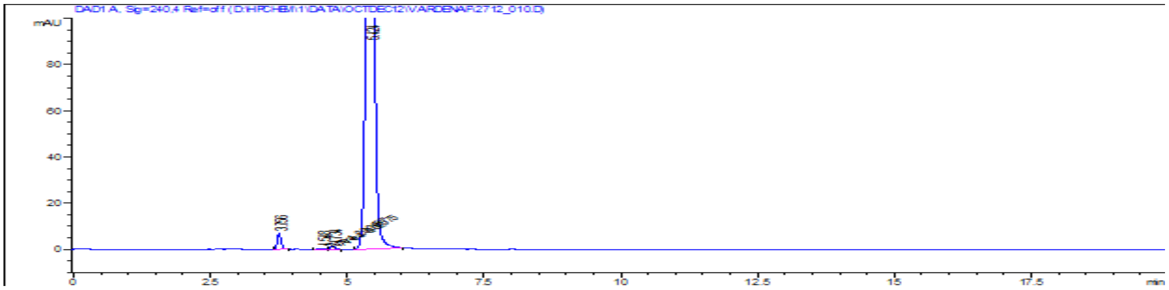


(C)Peroxide degradation

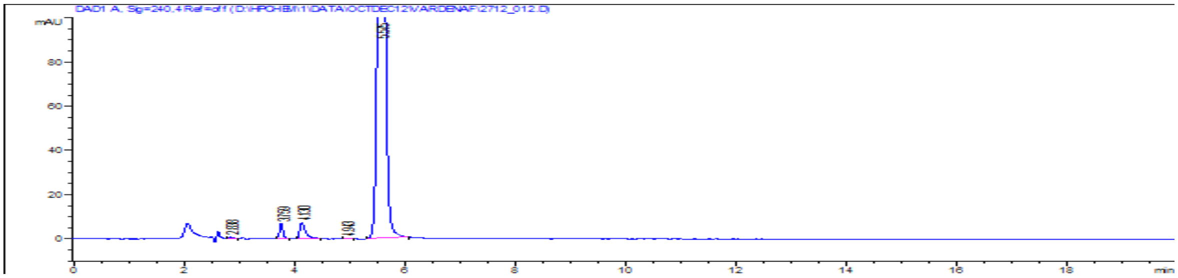


(D)Thermal degradation

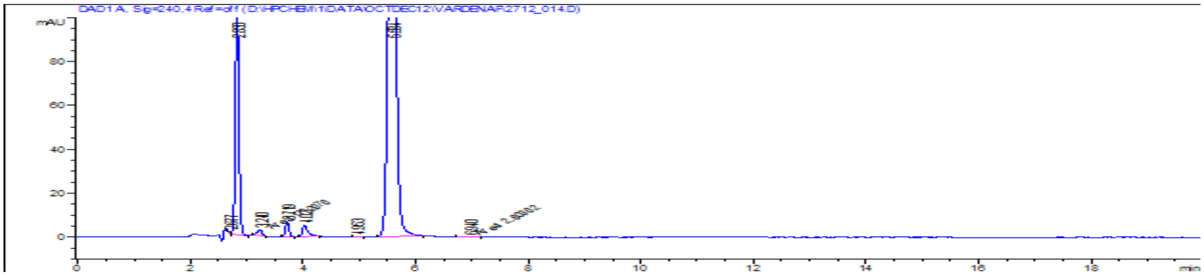
Figure 3: Typical chromatograms of API specificity



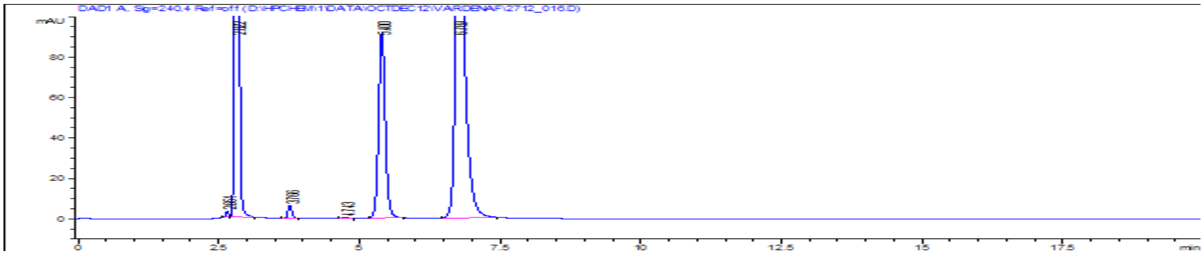
(A)Sample – As such



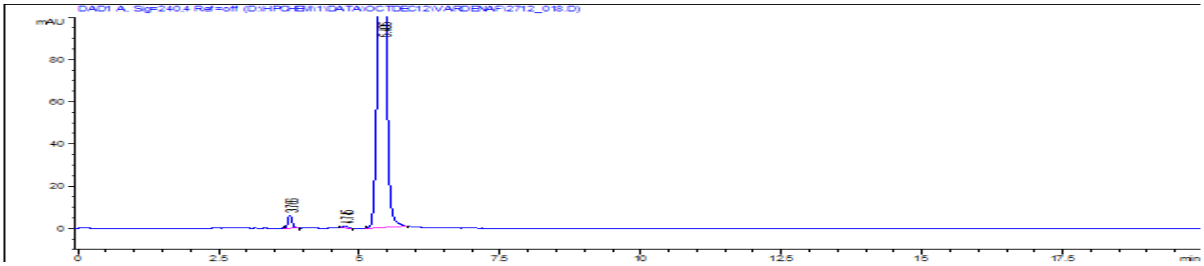
(B)Acid degradation



(C)Alkali degradation



(D)Peroxide degradation



(E)Thermal degradation

Figure 4: Typical chromatograms of sample specificity

Ruggedness of the method was determined by changing some of the operating conditions. The lower value of % deviation of 0.16 % as seen in table 6 indicates that the proposed method is rugged. The system suitability test parameter shown in table 7 also shows satisfactory results.

The liquid chromatographic method was applied to the determination of vardenafil in tablet dosage form. Percentage recovery results (table 4) shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of vardenafil in bulk and in tablet dosage form.

Table 2: Regression Analysis data for the Proposed Method

Parameter	Values
λ_{max}	240.0 nm
Range	50–250 µg/ml
Regression equation (y = mx+ c)	Y = 27.7756x+1.0522
Slope (m)	27.7756
Intercept (c)	+1.0522
Correlation coefficient	0.9998

Table 3: Summary of Validation Parameters for the Proposed Method

Parameters	Result
Linearity	50-250 µg/ml
Repeatability: Intraday precision	0.11 %
Interday precision	0.15 %
Method Precision	98.84±0.80
Intermediate precision	99.26±0.35
Accuracy : 50%	98.23±0.56
100%	98.27±1.07
150%	98.02±0.29
Assay	98.84 %
Limit of Detection	0.0125 µg/ml
Limit of Quantitation	0.025 µg/ml
Stability of solution	Stable up to 48 hrs

Table 4: Recovery Study for Vardenafil

Level	Amount of drug present in sample (µg/ml)	Amount of drug spiked (µg/ml)	Mean Area*	Total Amount of Drug recovered (µg/ml)	Amount of spiked drug in final solution (µg/ml)	%Recovery
50%	105.967	49.6	4297.720	154.692	48.72	98.24
50%	105.772	49.9	4294.292	154.569	48.80	98.78
50%	105.577	49.9	4287.247	154.315	48.74	97.67
100%	105.188	98.4	5591.543	201.273	96.79	97.65
100%	105.382	98.2	5636.373	201.273	95.89	97.65
100%	105.577	97.8	5650.373	202.896	97.32	99.51
150%	105.188	150.1	7003.780	252.118	146.93	97.89
150%	104.993	149.4	6999.063	251.948	146.96	98.36
150%	104.408	149.0	6949.317	250.157	145.75	97.82
					Mean Recovery	**98.17%**

*n=3 determinations

Table 5: Specificity Study

Condition applied	% Assay*	% Assay**
Acid degradation (1 N HCl)	100.77 %	98.32 %
Alkali degradation (1 N NaOH)	101.57 %	98.43 %
Peroxide degradation (30 % H_2O_2)	82.85 %	60.28 %
Thermal degradation (80^{o}C)	101.48 %	98.36 %

*Results of API, **Results of Sample

Table 6: Results of Ruggedness of the Method

Parameter	Assay with normal condition	Assay with changed condition
Column	98.84 %	99.00 %
Flow rate	98.90 %	99.00 %
Mobile phase ratio	99.00 %	98.98 %
System	98.80 %	99.00 %

Table 7: System Suitability Parameters

Parameter	Result
Retention time	5.4 min
Tailing factor (T)	0.94
Theoretical plates (N)	11690

CONCLUSION

The present study is an attempt to explore the use of Hypersil BDS C8 chromatographic column in the development of RP-HPLC method for estimation of vardenafil. The method was developed using simple mobile phase of buffer pH 4.5: acetonitrile in the ratio 60:40 v/v with the flow rate of 1.0 ml/min and the detection wavelength of 240.0 nm. Vardenafil was obtained at the retention time of 5.4 min. The proposed method was found to be linear in the range of 50 to 250 µg/ml. The method was validated in terms of linearity, range, repeatability, precision, accuracy, LOD, LOQ, solution stability, specificity, ruggedness and system suitability. The results of method validation clearly indicate that the proposed method is accurate, precise, specific, rugged and can be satisfactorily applied to the tablet dosage form without any interference of excipients. This reveals potential utility of the proposed method for quality control laboratories.

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