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SIMPLE COST-EFFECTIVE STABILITY INDICATING METHOD FOR THE ESTIMATION OF AVANAFIL IN BULK OR FORMULATION BY RP-HPLC

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ABSTRACT

This research presents a comprehensive analytical approach for the estimation of avanafil, a phosphodiesterase type 5 (PDE5) inhibitor used in the treatment of erectile dysfunction. The developed method utilizes Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and focuses on stability indication, linearity, precision, and robustness. System suitability parameters, linearity, recovery studies, and forced degradation studies were conducted to validate the method's efficacy. The results indicate a reliable and cost-effective approach for the estimation of avanafil in both bulk and pharmaceutical formulations. The method demonstrates sensitivity, precision, and stability-indicating characteristics, making it a valuable tool for routine quality control processes in the pharmaceutical industry.

Keywords: Avanafil, RP-HPLC, Erectile Dysfunction, Stability-Indicating Method, Linearity, Recovery Studies, Forced Degradation, Pharmaceutical Analysis.

INTRODUCTION

Avanafil, a selective phosphodiesterase type 5 (PDE5) inhibitor, has emerged as a potent therapeutic agent for the treatment of erectile dysfunction (ED) [1]. With its rapid onset of action and favorable side-effect profile, avanafil has gained prominence in the pharmaceutical market as an effective and well-tolerated option for individuals with ED. As the demand for avanafil-containing formulations continues to rise, there is an increasing need for robust and cost-effective analytical methods to ensure the quality, stability, and accurate dosage of pharmaceutical products [2].

Several analytical techniques have been employed for the quantification of avanafil, with high-performance liquid chromatography (HPLC) being widely recognized for its precision and sensitivity. However, the development of a stability-indicating method for avanafil estimation is crucial to assess its stability under various environmental conditions and to detect potential degradation products that may affect the quality of pharmaceutical formulations [3-7].

Avanafil's unique chemical structure, coupled with the necessity for accurate quantification, presents challenges in developing an analytical method that is not only sensitive but also cost-effective [8].

The identification and quantification of avanafil in the presence of its potential degradation products require a method capable of distinguishing between the intact drug and its various forms.

The primary objective of this study is to develop a reliable and cost-effective RP-HPLC method for the accurate estimation of avanafil in bulk and pharmaceutical formulations. The developed method aims to be stability-indicating, allowing for the differentiation of avanafil from its potential degradation products. This is essential for assessing the drug's stability during storage, transportation, and manufacturing processes. Recognizing the economic constraints in pharmaceutical industries, the study strives to design a method that is both simple and cost-effective, making it accessible and practical for routine analysis.

The method will be validated for its applicability in the estimation of avanafil content in commercially available formulations, providing a practical tool for quality control in the pharmaceutical industry. This study is significant in addressing the analytical needs of the pharmaceutical industry by introducing a stability-indicating RP-HPLC method for avanafil estimation. By emphasizing simplicity and cost-effectiveness, the method becomes a valuable tool for routine quality control processes. Furthermore, the stability-indicating nature of the method ensures its relevance in monitoring the quality and stability of avanafil-containing formulations throughout their shelf life.

MATERIAL AND METHODS

Mobile Phase Selection

Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 10mM KH_2PO_4 and Acetonitrile in the ratio of 15:85 adjust the pH 3.5 with OPA. The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min.

Selection of wavelength

100 mg of Avanafil was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10

ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 224.00 nm.

Selection of Separation Variable

Standard drug solution of Avanafil was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Avanafil 10µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard Stock Solution

10mg of Avanafil was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Avanafil 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of the Drug

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Validation [9]

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision

Day to Day

The statistical analysis of method was carried out.

Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different days, different analysts, different equipment etc). The standard dilution was prepared and three replicates of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Avanafil was studied.

Detection limit and quantitation limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10mg of Avanafil was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL for Avanafil.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Alkaline hydrolysis:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Oxidative degradation:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Thermal degradation:

50 mg of the drug sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

RESULTS AND DISCUSSION

The system suitability parameters, including retention time (RT), area under the curve (AUC), theoretical plates, and tailing factor, were evaluated for three replicates. The mean values indicate a consistent and reproducible chromatographic performance. The low standard deviations (S.D.) suggest precision in the measurement of these parameters, reinforcing the reliability of the method (Table 1).

The linearity study, represented in Table 2, demonstrates the correlation between the concentration of avanafil and its response. The mean values and low relative standard deviations (% RSD) affirm the linear relationship over the specified concentration range, ensuring accurate quantification of avanafil in diverse samples.

The recovery studies at three different levels provide insights into the accuracy of the method. The % recovery, standard deviation, and % RSD values (Table 3) suggest the method's reliability in quantifying avanafil in the presence of excipients and matrix components.

The repeatability study (Table 4) assesses the precision of the method within a single laboratory. The % RSD values for various concentrations indicate low variability, emphasizing the method's repeatability and reliability for routine analysis.The day-to-day variation study (Table 5) evaluates the method's consistency over different days. The mean values and % RSD indicate minimal variation, supporting the reliability of the method for daily use in analytical laboratories.

Analyst-to-analyst variation is assessed in Table 6, demonstrating the method's robustness and reproducibility across different operators. The low % RSD values indicate the method's consistency, irrespective of the analyst performing the analysis.

The robustness study (Table 7) evaluates the method's sensitivity to small variations in experimental conditions. The consistent concentration found and low % RSD values indicate the method's robust performance, reinforcing its reliability under different conditions.

The limits of detection (LOD) and quantification (LOQ) are essential parameters for method sensitivity. The low values (Table 8) suggest that the method can reliably detect and quantify avanafil at low concentrations.

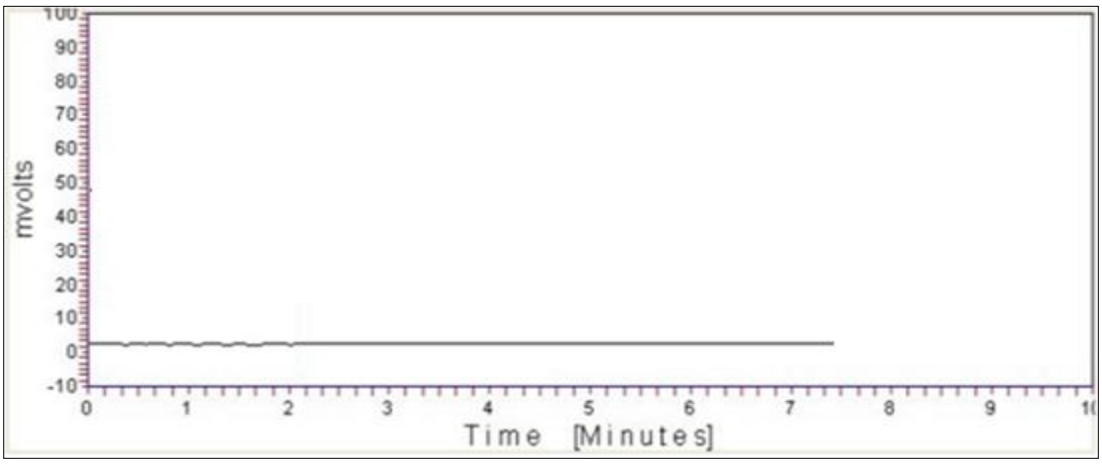
The assay results for a tablet formulation (Table 9) show a close match between the labeled and found amounts of avanafil, indicating the method's accuracy in real pharmaceutical samples.

Forced degradation studies provide insights into the drug's stability under various stress conditions. The results (Table 10) reveal the drug's susceptibility to acidic and alkaline hydrolysis, oxidative degradation, and thermal degradation, highlighting potential degradation pathways.

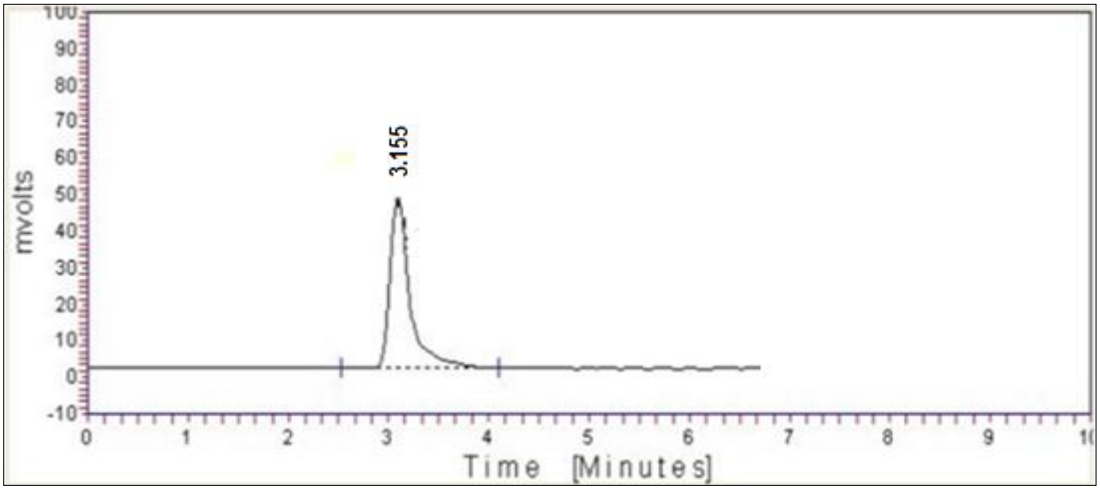
Table 1: Result of System Suitability Parameters for Avanafil

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	3.154	685.598	3256	1.15
Rep-2	3.165	692.254	3245	1.25
Rep-3	3.147	690.254	3265	1.28
Mean	3.1553	689.369	3255.33	1.2267
S.D.	0.0091	3.4152	10.017	0.0681

Linearity and Calibration Graph



(a)



(b)

Figure 1: (a) Chromatogram of Blank (b) Chromatogram of Standard

Table 2: Result of Linearity of Avanafil

Conc. µg/ml	5	10	15	20	25
Rep.	0	0	0	0	0
1	347.658	685.598	1055.365	1385.57	1730.66
2	352.658	692.254	1050.254	1380.25	1736.66
3	340.254	690.254	1046.658	1375.66	1725.66
Mean	346.857	689.369	1050.759	1380.494	1730.991
S.D.	6.241	3.415	4.375	4.960	5.508
R.S.D%	1.799	0.495	0.416	0.359	0.318

Table 3: Statistical validation of recovery studies

Level of Recovery (%)	% Recovery	Standard Deviation*	% RSD
80	99.34	0.330	0.332
100	98.85	0.664	0.672
120	98.28	0.631	0.642

*Denotes average of three determinations

Table 4: Repeatability of Avanafil

Replicate	Concentration found (μg/ml)					
	5	10	15	20	25	
Replicate-1	4.85	9.98	14.65	19.98	24.85	
Replicate-2	4.98	9.87	14.78	19.85	24.65	
Replicate-3	4.75	10.01	14.69	19.96	24.78	
Replicate-4	4.69	9.98	14.85	19.95	24.85	
Replicate-5	4.92	9.87	14.96	19.78	24.96	
Mean	4.838	9.942	14.786	19.904	24.818	
% Mean	96.76	99.42	98.573	99.52	99.272	98.709
SD	0.119	0.067	0.125	0.086	0.114	0.102
% RSD	0.123	0.067	0.126	0.086	0.115	0.103

Table 5: Day-to-Day variation of Avanafil

Replicate	Concentration found (μg/ml)					
	5	10	15	20	25	
Day 1	4.98	9.98	14.65	19.98	24.85	
Day 2	4.98	9.87	14.78	19.85	24.65	
Day 3	4.75	10.01	14.69	19.96	24.78	
Mean	4.903	9.953	14.707	19.930	24.760	
% Mean	98.067	99.533	98.044	99.650	99.040	98.867
SD	0.133	0.074	0.067	0.070	0.101	0.089
% RSD	0.135	0.074	0.068	0.070	0.102	0.090

Table 6: Analyst to analyst variation of Avanafil

Replicate	Concentration found (μg/ml)					
	5	10	15	20	25	
Analyst 1	5.01	9.85	14.85	19.87	24.78	
Analyst 2	4.96	9.65	14.68	19.85	24.96	
Mean	4.985	9.750	14.765	19.860	24.870	
% Mean	99.700	97.500	98.433	99.300	99.480	98.883
SD	0.035	0.141	0.120	0.014	0.127	0.088
% RSD	0.035	0.145	0.122	0.014	0.128	0.089

Table 7: Robustness of Avanafil

Replicate	Concentration found (μg/ml)					Mean
	5	10	15	20	25	
Replicate-1	4.95	9.95	14.78	19.98	24.78	
Replicate-2	4.78	9.98	14.65	19.98	24.68	
Replicate-3	4.96	9.78	14.35	19.78	24.78	
Replicate-4	4.85	9.65	14.95	20.01	24.68	
Replicate-5	5.01	9.85	14.85	19.98	24.78	
Mean	4.91	9.842	14.716	19.946	24.74	
% Mean	98.2	98.42	98.107	99.73	98.96	98.683
SD	0.093	0.134	0.232	0.094	0.055	0.121
% RSD	0.095	0.136	0.236	0.094	0.055	0.123

Table 8: LOD and LOQ of Avanafil

Name	LOD (μg/ml)	LOQ (μg/ml)
Avanafil	0.15	0.50

Table 9: Result of assay of tablet formulation

	Avanafil
Label Claim (mg)	100mg
% Found (mg)	99.85
% Assay	99.85
% RSD	0.125

*Average of three determination

Table 10: Results of forced degradation studies of Avanafil

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.99	0
Acidic hydrolysis	85.65	14.35
Alkaline hydrolysis	83.32	16.68
Oxidative degradation	93.32	6.68
Thermal degradation	96.65	3.35

CONCLUSION

In conclusion, the developed RP-HPLC method for the estimation of avanafil proves to be a robust and reliable analytical tool with significant applicability in pharmaceutical quality control. The comprehensive evaluation of various parameters confirms the method's efficacy and suitability for routine analysis. The RP-HPLC method for avanafil estimation stands as a cost-effective, stability-indicating, and precise analytical technique. Its robust performance in various validation parameters positions it as a valuable tool for routine quality control in the pharmaceutical industry, contributing to the assurance of avanafil-containing formulations' quality and efficacy.

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