International Journal of Pharmaceutical Biological and Chemical Sciences (IJPBCS)
ISSN: 2278-5191 (Online)
IJPBCS | VOLUME 14| ISSUE 1 | JAN-MAR 2025 | 01-11
Original Research Article – Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TADALAFIL BY USING RP-HPLC METHOD

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ABSTRACT

A new, simple, rapid, accurate and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Tadalafil, in Active pharmaceutical Ingredient form as well as in combined tablet dosage form. Chromatography was carried out on Symmetry ODS C18 (4.6mm × 250mm, 5µm) column using a mixture of Methanol: Acetonitrile (35:65v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 220 nm. The retention time of the Tadalafil was 2.885± 0.02min respectively. The method produces linear responses in the concentration range of 30-70mg/ml of Tadalafil. The mean % assay of marketed formulation was found to be 100.04%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise and rapid, making it suitable for estimation of Tadalafil in API and combined tablet dosage form. The method is useful in the quality control of bulk and pharmaceutical formulations.

Key words:
Tadalafil, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Reverse-Phase (RP) chromatography

Reverse phase chromatography is the most widely used form of HPLC. In reverse phase partition chromatography, the stationary phases are non-polar and thus polar mobile phases are required.

Stationary phase

The stationary phase is silica, chemically bonded through a siloxane linkage to low polar functional group. The surface of the support, e.g. the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support[18]. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system. Common reverse phase materials include octadecylsilane (ODS or C_{18}) and octylsilane (C_8).

Mobile Phase

The mobile phase generally comprises water and a less polar organic solvent modifier such as methanol or acetonitrile. The solutes in reverse phase chromatography are eluted in order of decrease their polarities. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solute, the less polar solute partitioning to a greater extent into the non-polar stationary phase and consequently being retained on the column longer than the more polar solute[19]. The rate of elution of the components is controlled by the polarity of the organic modifier and its proportion in the mobile phase. The rate of elution is increased by reducing polarity e.g. by increasing the proportion of the organic solvent or by using acetonitrile instead of methanol

High pressure liquid chromatography (HPLC)

High performance liquid chromatography is basically a highly improved form of column chromatography. It is the most widely used form of chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through the column under high pressures and this improves separation. The improvements of HPLC have enabled liquid

chromatography to match the great success of gas chromatography.

The separation principles involved May include[10]

- Adsorption
- Partition
- Ion exchange
- Gel permeation
- Affinity

Sample		LC mode	Column choice
		Reverse Phase-ion pair (allows neutral and charged compounds to be simultaneously analysed)	C_{18}, C_8, C_6, C_4, C_2, TMS, CN, amino (not for carbonyl compounds), phenyl, Hamilton PRP-1 (pH 1-13)
Basic or Acidic			
		Ion suppression	C_{18}, C_8, C_6, C_4, C_2, TMS, CN, amino (not for carbonyl compounds), phenyl, Hamilton PRP-1 (pH 1-13)
Ionizable		Ion Exchange	
		Anionic	Strong Anion exchange
		Cationic	Strong Cation exchange
		Normal phase	Increasing polarity of bonded phases diol
			CN
Neutral			NH_2
			Silica
		Reverse phase	Alumina, C_{-18}, C_{-8}, phenyl

Selection of Column:

Drug profile

Generic name : Tadalafil

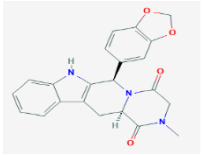
Trade name : Tadacip,Tadalis

Molecular formula : $C_{22}H_{19}N_3O_4$

Molecular weight : 389.4g/mole

Description : White crystalline power

Structure :



Chemical Name: (2*R*,8*R*)-2-(1,3-benzodioxol-5-yl)-6-methyl-3,6,17-triaza tetracyclo [8.7.0.0^{3,8}.0^{11,16}] heptadeca-1(10),11,13,15-tetraene-4,7-dione.

Mechanism:

In vitro, studies have shown that the effect of tadalafil is more potent on phosphodiesterase type 5 (PDE5) than on other phosphodiesterases. These studies have shown that tadalafil is >10,000-fold more potent for PDE5 than for PDE1, PDE2, PDE4, and PDE7 enzymes, which are found in the heart, brain, blood vessels, liver, leukocytes, skeletal muscle, and other organs. Tadalafil is >10,000-fold more potent for PDE5 than for PDE3, an enzyme found in the heart and blood vessels. Additionally, tadalafil is 700-fold more potent for PDE5 than for PDE8, PDE9, PDE10 and 14-fold more potent for PDE5 than for PDE11A1, an enzyme found in human skeletal muscle. Tadalafil inhibits human recombinant PDE11A1 activity at concentrations within the therapeutic range

Adverse drug reaction: Administration of tadalafil to patients who are using any form of organic nitrates, either regularly and/or intermittently, is contraindicated; in clinical pharmacology studies tadalafil was shown to potentiate the hypotensive effects of nitrates; this is thought to result from the combined effects of nitrates and tadalafil on the nitric oxide/cGMP pathway.

EXPERIMENTAL SECTION

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra Sonicator	Labman

S.No	Chemical	Brand names
1	TADALAFIL	Aligns international
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

METHOD VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of Mobile Phase:

Accurately measured 350 ml (35%) of Methanol, 650 ml of Acetonitrile (65%) were mixed and degassed in digital ultra sonicator for 20 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Tadalafil stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Tadalafil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.5ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (30ppm of Tadalafil**):**
Pipette out 0.3ml of Tadalafil stock solution was take in a 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – II (Tadalafil**):**
Pipette out 0.4ml of Tadalafil stock solution was take in a 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – III (Tadalafil**):**
Pipette out 05ml of Tadalafil stock solution was take in a 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – IV (Tadalafil**):**
Pipette out 0.6ml of Tadalafil stock solution was take in a 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – V (Tadalafil**):**
Pipette out 0.7ml of Tadalafil stock solution was take in a 10ml of volumetric flask dilute up to the mark with diluent.
Procedure: Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION
REPEATABILITY
Preparation of Tadalafil **Product Solution for Precision:**
Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5ml of the above Tadalafil solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.
Procedure:
DAY 1:
The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
DAY 2:
The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Accuracy:
For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.25ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.75ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Tadalafil and calculate the recovery and mean recovery values.

ROBUSTNESS:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .
For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Tadalafil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5ml of the above Tadalafil stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Acetonitrile (35:65 v/v) was taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Acetonitrile (35:65 v/v)
Column : Symmetry ODS C18 (4.6mm × 250mm, 5µm)
Flow rate : 1 ml/min
Wavelength : 220 nm
Column temp : Ambient
Injection Volume : 20 µl
Run time : 10 minutes

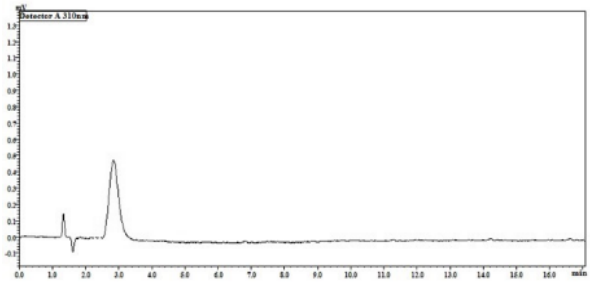


Figure 1: Optimized Chromatogram

Table 1: Optimized Chromatogram(standard)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tadalafil	2.89	187656	1876		1.58	5674

Observation: From the above chromatogram it was observed that the Tadalafil peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

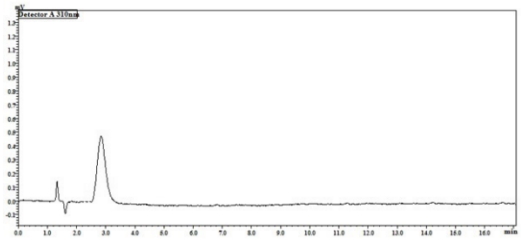


Figure 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tadalafil	2.89	187546	1856		1.59	5478

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

- **METHOD VALIDATION**
- **Blank:**

Figure 3: Chromatogram showing blank (mobile phase preparation)

System Suitability:

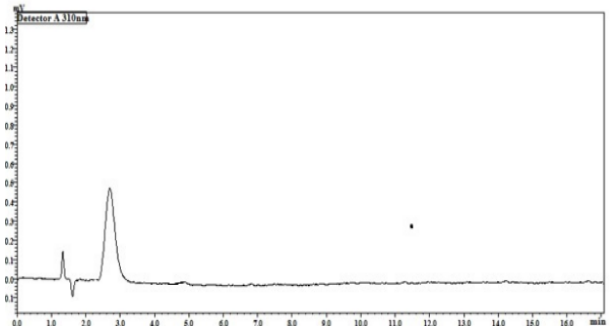


Figure 4: Chromatogram showing injection -1

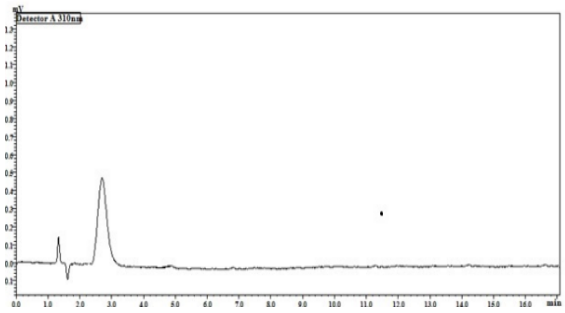


Figure 5: Chromatogram showing injection -2

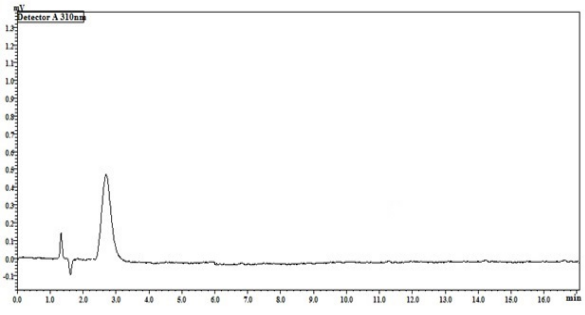


Figure 6: Chromatogram showing injection -3

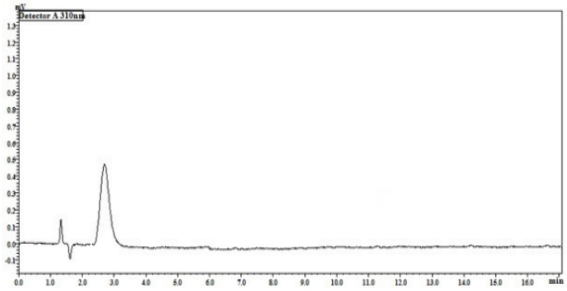


Figure 7: Chromatogram showing injection -4

Table 3: Results of system suitability for Tadalafil

S No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tadalafil	2.88	175678	1875	5436	1.60
2	Tadalafil	2.88	175547	1874	5428	1.59
3	Tadalafil	2.87	169989	1798	5347	1.56
4	Tadalafil	2.87	169876	1797	5345	1.56
Mean			**179865**			
Std. Dev			**1876,34**			
% RSD			**0.678453**			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

- The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.
- Analytical method was tested for specificity to measure accurately quantitate Tadalafil

Assay (Standard):

%ASSAY =The % purity of Tadalafil in pharmaceutical dosage form was found to be100.04%.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Tadalafil**:**

Concentration µg/ml	Average Peak Area
30	187546
40	175678
50	175547
60	169989
70	169876

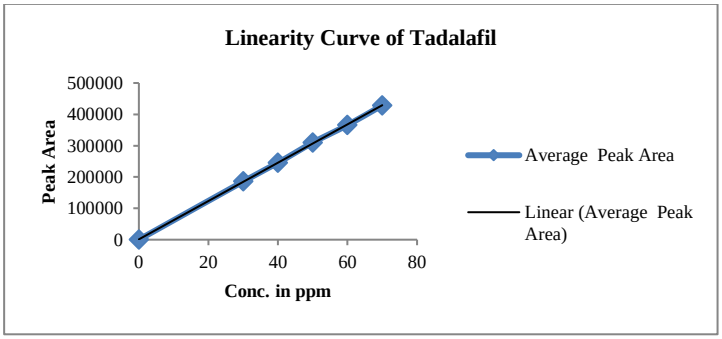


Figure 8: Linearity for Tadalafil

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Tadalafil is a straight line.

Y = mx + c

Slope (m) = 6106

Intercept (c) = 1024

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 1024. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

System Suitability:

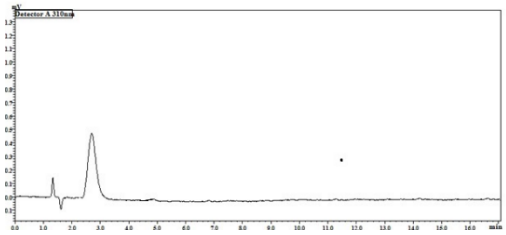


Figure 9: Chromatogram showing injection -1

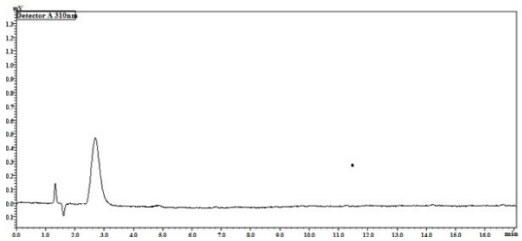


Figure 10: Chromatogram showing injection -2

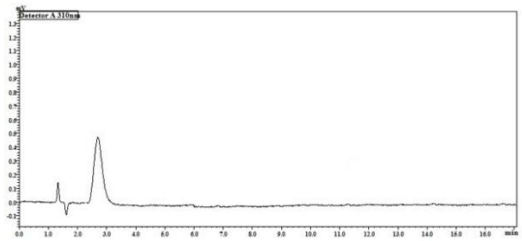


Figure 11: Chromatogram showing injection -3

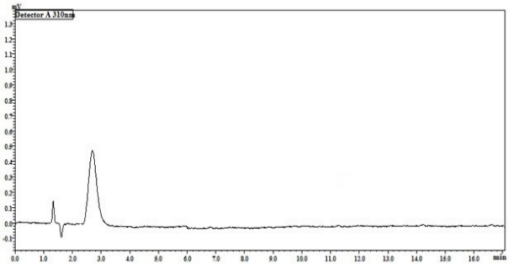


Figure 12: Chromatogram showing injection -4

Table 4: Results of system suitability for Tadalafil

S No	Name	Rt	Area	Height	USP plate count	USP Tailing
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3	Tadalafil	2.87	169989	1798	5347	1.56
4	Tadalafil	2.87	169876	1797	5345	1.56
Mean			**179865**			
Std. Dev			**1876,34**			
% RSD			**0.678453**			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

ACCURACY:

- Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.
- **Accuracy 50%:**

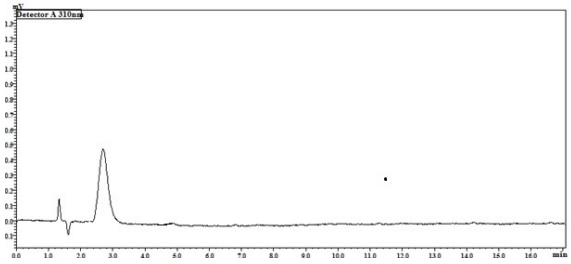

Figure 13: Chromatogram showing accuracy-50% injection

Accuracy 100%:

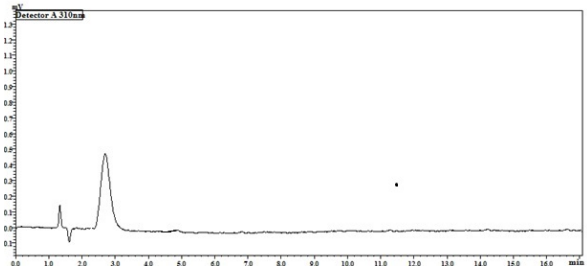

Figure 14: Chromatogram showing accuracy-100%

Accuracy 150%:

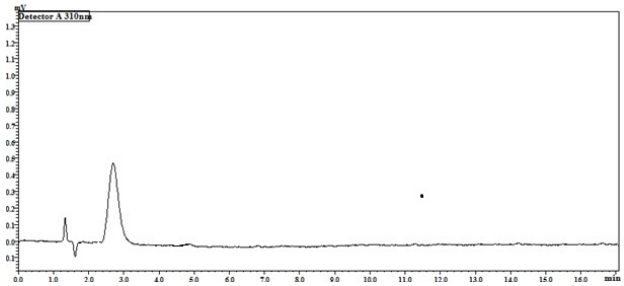

Figure 15: Chromatogram showing accuracy-150%

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s
Where,
σ = Standard deviation of the response
S = Slope of the calibration curve
Result:
Tadalafil: =0.8µg/ml

LIMIT OF QUANTITATION
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
LOQ=10×σ/S
Where,
σ = Standard deviation of the response
S = Slope of the calibration curve
Result:
Tadalafil: = 2.1µg/ml
Robustness
The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tadalafil. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Tadalafil were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 5: Results for Robustness (Tadalafil)

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	175678	2.89	5436	1.60
Less Flow rate of 0.9 mL/min	175434	2.88	5428	1.59
More Flow rate of 1.1 mL/min	177699	2.67	5347	1.59
Less organic phase	175434	2.78	5345	1.61
More organic phase	174567	2.86	5245	1.65

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

the developed RP-HPLC method for the estimation of Tadalafil in both active pharmaceutical ingredient (API) and combined tablet dosage form has proven to be simple, rapid, accurate, and precise. The method demonstrated excellent linearity, with a concentration range of 30-70 mg/mL, and a retention time of 2.885 ± 0.02 minutes. Validation results showed that the method is highly reliable, with a mean assay of 100.04% and recovery rates between 98-102%, along with a relative standard deviation of less than 2%, indicating strong precision. The method's robustness, along with its suitability for quality control of both bulk material and pharmaceutical formulations, makes it a valuable tool for routine analysis in the pharmaceutical industry. This approach can be easily adopted for the routine monitoring and assurance of the quality of Tadalafil in API and dosage forms.

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