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RP-HPLC method development and validation for phosphodiesterase (PDE) inhibitors Avanofil in pharmaceutical dosage form

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ABSTRACT: A simple rapid, precise, and accurate RP-HPLC method was developed for the determination of precise in the pharmaceutical dosage form. According to the ICH criteria, the devised approach was validated.The linearity, precision, range, and robustness were within the limits as specified by the ICH guidelines. The main proposed method development has a simple and rapid procedure. In this reported RP-HPLC method samples were resolved on Younglin (S.K) Gradient System UV Detector using Methanol: Water (65:35 %v/v) pH 3 was used as a mobile phase with 0.7 mL/min flow rate on HPLC with Cosmosil C18 (150 ×4.6mm 5µm) column using Autochro-3000 software detector for the study. The detection was done at a wavelength of 246 nm. This method gave a good resolution and suitable retention time.

KEYWORDS:RP-HPLC, Avanafil, ICH guidelines, mobile phase, validation.

I. INTRODUCTION:

The chemical name of Avanafil is 4-(3-chloro-4-methoxybenzylamino)-2-((S)-2-(hydroxymethyl)pyrrolidin-1-yl)-N-((pyrimidin-2-yl)methyl)pyrimidine-5-carboxamide and its Molecular formula is $C_{23}H_{26}ClN_7O_3$.[1]Avanafil is used in the treatment of erectile dysfunction [2]–[11]It was approved on April 27, 2012, by the Food and Drug Administration.[12] The structure of Avanafil is shown in Figure 1.[13]

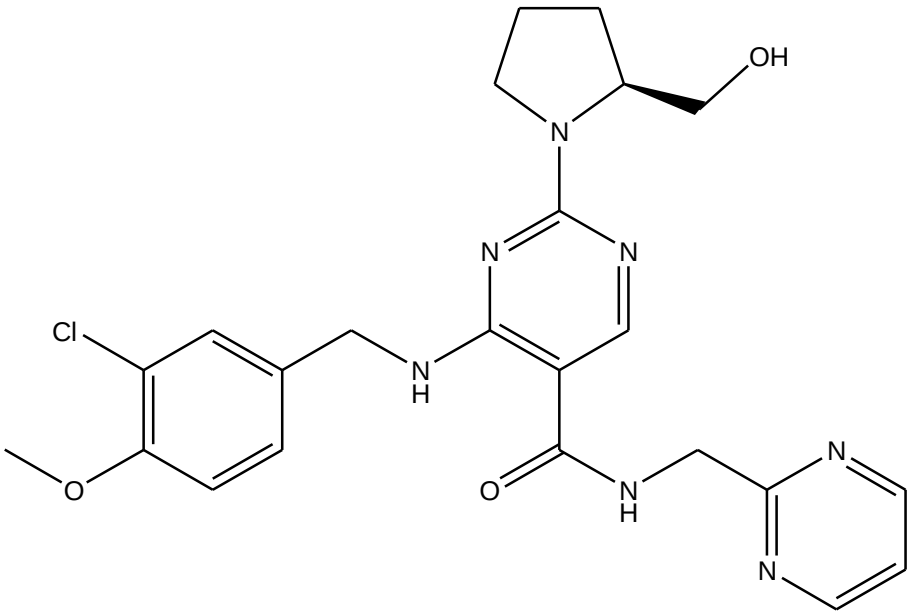

Fig. 1: Chemical structure of Avanafil

A literature survey reveals that very few chromatographic methods were developed for the estimation of Avanafil in Tablet dosage forms.[14]–[18] Hence, attempts were made to develop a simple, rapid, precise and to estimate Avanafil in Tablet dosage form, an accurate reverse

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phase chromatographic technique is used. The developed method is better than earlier published articles with respect to superior System Suitability Parameter such as Theoretical plates, tailing factor. This approach has been effectively employed for Avanafil and its formulation quality control analysis.

The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines[19]–[22]. The objective is to give anoverview of the mechanism of Reversed Phase - High Performance Liquid Chromatography (RP-HPLC)[23] of Avanafil and explain the basis of the retention mechanism and achieve high-speed separation without any loss of reproducibility.

II. MATERIAL AND METHODS

Chemicals and Reagents: An analytically pure Avanafil working standard was procured from Swapnaroop Drugs and Pharmaceutical, Aurangabad with defined potency [99.44 % as is basis]. AVANA (100 mg) Avanafil tablets were received as a gift sample from Lotus International, Goregaon (West), Mumbai. HPLC Grade Methanol and acetonitrile from Sigma Aldrich and Water-milli-Q Grade were used.

Instrumentation: Analytical Technologies® Limited UV-VIS double beam Spectrophotometer is used for study. The light source used is Deuterium lamp of spectrophotometer, a computer is attached which helps in data processing. A Quartz cuvette with path length 1cm was used.
The analysis of the drug was carried out on Younglin (S.K.) Gradient System UV Detector, equiped with Reverse Phase (Cosmosil) C18 column (4.6 mm x 150mm; 5µm), SP930D pump,20µL injection loop, UV730D absorbance detector, and running autochro-3000 software.

Selection of Wavelength: Standard Stock Solution About 10.0 mg of Avanafil was transferred to the 100 mL volumetric flask and the volume was made up to the mark with diluent. An aliquot portion of standard stock solutions of Avanafil was diluted appropriately with diluent to obtain the concentration of 10µg/mL of the drug. The solution was scanned in the 200–400 nm range. The absorbance maximum of Avanafil was found at 246nm as shown in Figure 2.

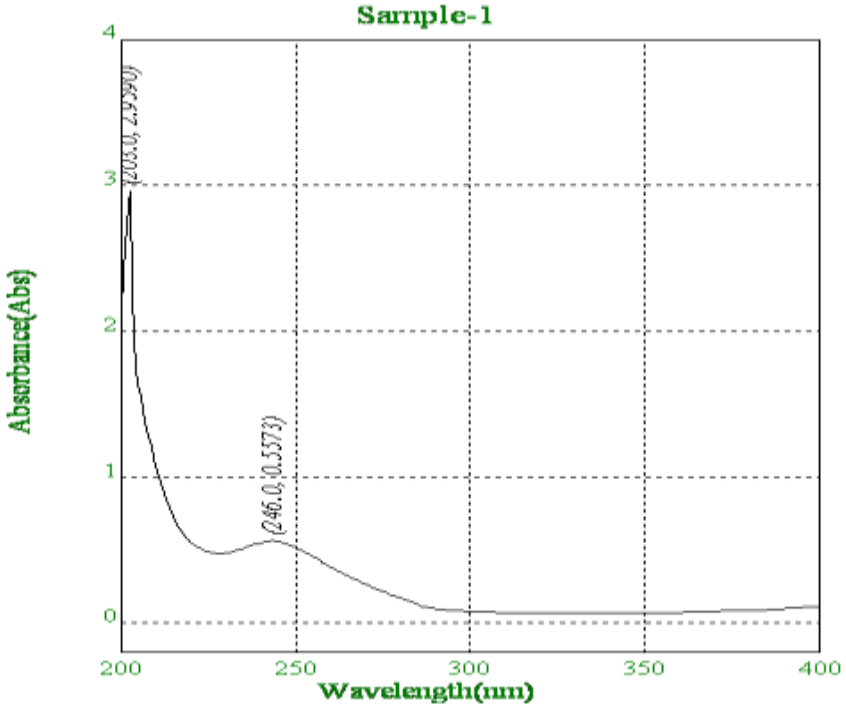

Fig. 2: UV spectrum of Avanafil scanned in the range of 200 – 400 nm

III. EXPERIMENTATION

Preparation of Standard Drug Solution: Accurately weight and transfer 10 mg Avanafil working standard into 10 mL volumetric flask as about diluent Methanol completely and make volume up to the mark with the same solvent to get 1000 µg/ml standard (stock solution) and 15

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min sonicates to dissolve it and the resulting stock solution 0.1 mL was transferred to 10 mL volumetric flask and the volume was made up to the mark with mobile phase Methanol: Water pH 3, prepared in (65 mL Methanol: 35 mL WATER %v/v) solvent.

Preparation of Sample Solution: Twenty tablets of AVANA (100 mg) were weighed and the average weight was calculated (0.133 g). The contents of the tablet were crushed into a fine powder.An accurately weighed 13.3 mg quantity of tablet powder equivalent to 10 mg of Avanafil was dissolved in a diluent. The drug was extracted from the tablet powder with 10 mL Methanol to ensure complete extraction then sonicated for 15 min.The 0.2 mL of supernatant was then diluted up to 10 mL mobile phase.

Method Optimization:[24] Chemical structure of the Avanafil shows that the drug is a basic and non-polar molecule. An end-capped column that provides strong hydrophobic interaction like the Cosmosil C18 column was selected for the retention of Avanafil. Initial trials were started with Water:Acetonitrileand Water:Methanolof different proportions out of different C18 columns, good peak shape with better SST parameters with Inert sustain column was found to be sensitive when compared to other C18 columns. The mobile phase consisting of mixture MeOH:Water (65:35 %v/v) was used, the flow rate was kept at 0.7 mL/min and UV detection wavelength of 246 nm and the column oven temperature was maintained at 30˚C. Optimization parameters were discussed in Table 1.

Table 1: Different Trials of Chromatographic Condition

Sr. No.	Column used	Mobile phase, Flow Rate and Wavelength	Inj. Vol.	Observation	Conclusion
1	CosmosilC18 (250 ×4.6mm, 5µ)	Methanol : Water pH 3 (90:10 %v/v), 246nm, Flow rate 0.7 mL/min	20µL	Sharp peaks were not obtained	Hence rejected
2	Cosmosil C18 (250 ×4.6mm, 5µ)	Methanol : Water pH 3 (75:25 %v/v), 246nm, Flow rate 0.7 mL/min	20 µL	Sharp peaks were not obtained	Hence rejected
3	Cosmosil C18 (250 ×4.6mm, 5µ)	Acetonitrile : Water pH 3 (75:25 %v/v), 246nm, Flow rate 0.7 mL/min	20 µL	Sharp peaks were not obtained	Hence rejected
4	Cosmosil C18 (150 ×4.6mm, 5µ)	Methanol : Water pH 3 (65:35 %v/v), 246nm, Flow rate 0.7 mL/min	20 µL	Sharp and well-resolved peaks were obtained	Hence selected

Thus, from the above, it has been observed that using the mobile phase of Methanol: Water pH 3 (65:35 %v/v), 246nm, Flow rate 0.7 mL/min gave adequate retention at 6.7333 min with good peak shape. (Theoretical plates for Avanafil 6285.2).

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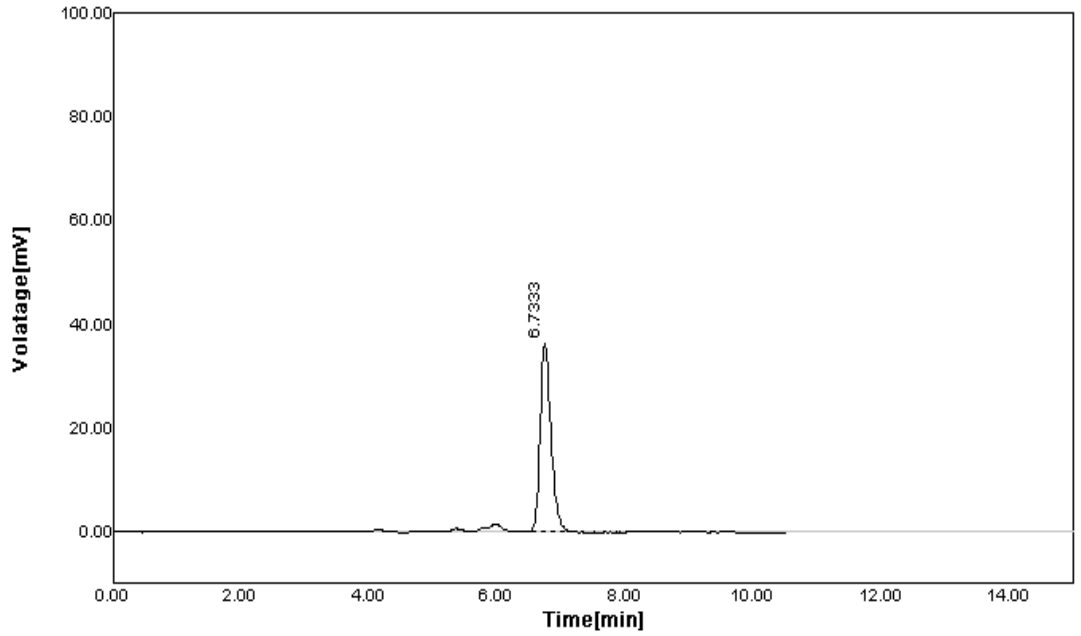

Fig. 3: Chromatogram of Avanafil standard solution

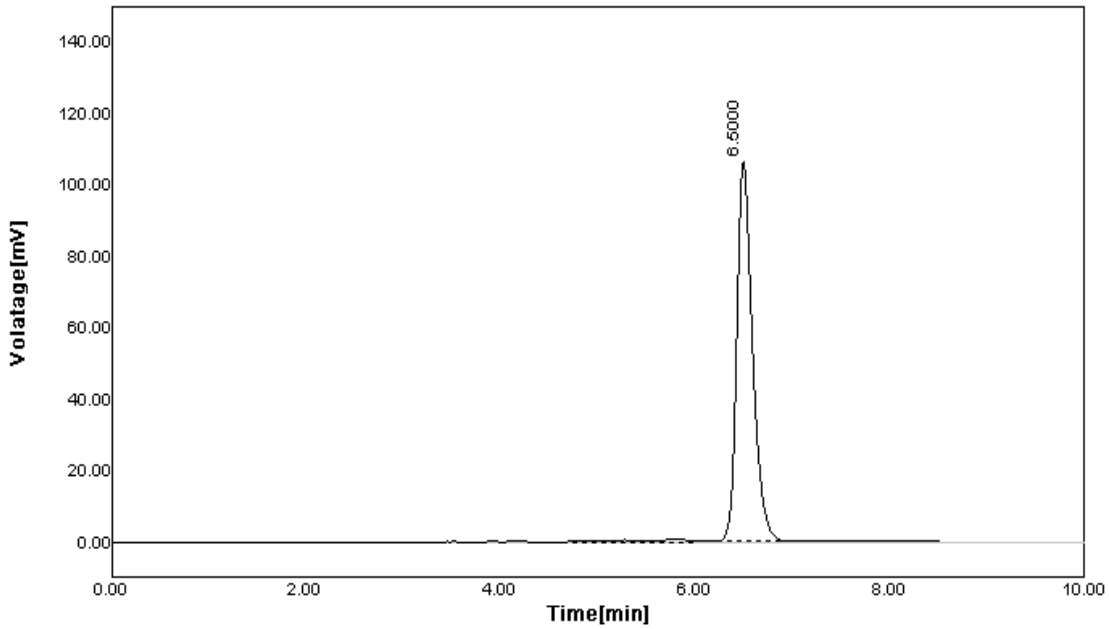

Fig. 4: Chromatogram of Avanafil sample solution 50 µg/ml.

Validation Method:[25], [26] Validation of the developed method was done as per the ICH Q2 (R1) 22 guidelines[27] with respect to various parameters such as linearity, accuracy, specificity, precision, and robustness. A standard solution of Avanafil was used for the comparison of results.

Linearity: From Avanafil standard stock solution, different working standard solutions (10-50µg/mL) were prepared in the mobile phase20 µL of sample solution was injected into the chromatographic systemusing a mixedvolume loop injector chromatograms were recorded. The data is as shown in table 2.Figure 5

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depicts the calibration curves.The linear calibration plot was created by plotting the concentrations over the chosen range against the sample's peak area. The response to the drug was linear in the concentration range between 10-50 µg/mL. The linear calibration plot was created by plotting the concentrations over the chosen range against the sample's peak area.

Table 2: Linearity data for validation

Linearity level	Concentration (µg/mL)	Area	r2
1	10	255.59	
2	20	498.61	
3	30	772.97	**0.999**
4	40	1018.97	
5	50	1239.98	

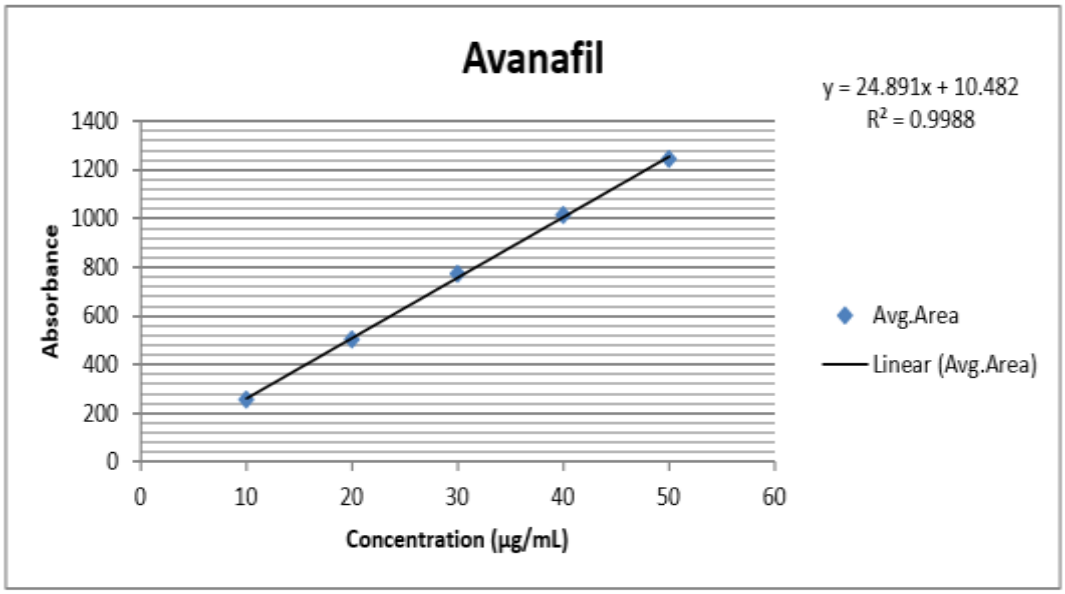

Fig. 5: Linearity study of Avanafil in the range of 10-50 µg/mL

Accuracy (Standard Addition method): [28]Recovery studies were carried out to ensure that the developed approach was accurate.At each additional concentration, the spiked substance recovered well, demonstrating that the procedure was accurate. A specific concentration of standard medicine (80 percent, 100 percent, and 120 percent) was added to the pill solution to pre-analyze it, and the recovery was then examined.Table No. 3 shows the statistical confirmation of recovery studies. To acquire data, the experiment was done three times at each concentration. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value as shown in Table 3 and 4.

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Table 3: Accuracy Study of Avanafil by Standard Addition Method.

Drug	Sr. No.	Level (%)	Amt. taken (µg/mL)	Amt. Added (µg/mL)	Amt. found mean ± S.D.	Amt. recovered mean ±S.D.
AVANA	1	80%	20	16	36.3±0.16	16.30±0.16
	2	100%	20	20	39.50±0.25	20.58±0.25
	3	120%	20	24	43.93±0.05	23.93±0.05

Table 4: Statistical Validation of Recovery Studies of Avanafil

Drug	Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
Avanafil	80%	101.89	0.98	0.96
	100%	97.53	1.27	1.30
	120%	101.58	0.21	0.20

Precision: The method was established by analyzing various replicates analysis of 20, 30, and 40 µg/mL standard solution of Avanafil.All of the solutions were analyzed three times to see if there was any intra-day or inter-day variation in the final outcome. The intraday result is displayed in (Table 5**)** respectively.Intraday and Inter day Precision for Avanafil which shows the high precision %amount in between 98% to 100% indicates to the analytical method that concluded.

The precision of the System was ascertained by a five-replicate analysis of 50 µg/mL Standard solution of Avanafil. The precision of the method was ascertained by three replicate analyses of homogeneous samples of tablet powder at a concentration of 50µg/mL.The intermediate precision was studied by injecting a freshly prepared working standard solution of Avanafil on two different days (interday) and on the same day but at three different times (intraday) Table 5.

Table 5: Study of system precision (Intraday and Interday) for Avanafil

METHOD	Drug	Conc (µg/mL)	Intraday Precision		Interday Precision	
			Mean*± SD	%Amt Found	Mean*± SD	%Amt Found
UV	Avanafil	20	498.82±5.52	98.05	494.28±2.76	97.15
		30	773.49±3.36	102.17	773.48±14.02	102.17
		40	994.87±3.24	98.85	1000.69±2.28	99.45

***Mean of each 3 reading**

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Robustness: The ability of a procedure to remain unaffected by tiny deliberate changes in parameters is known as robustness. Small but deliberate adjustments in the optimal technique parameters were used to test the robustness of the suggested method. Changes in mobile phase composition and flow rate, wavelength on retention time, and drug peak tailing factor were investigated.

The mobile phase composition was changed in (±1 mL/min^{-1}**)** proportion and the flow rate was varied by optimized chromatographic conditions. The results of robustness studies are shown in table 6.The robustness parameters were also found to be satisfactory, so the analytical technique was completed.

The changes were done throughflow rate (±1 mLmin^{-1}), pH of mobile phase composition and wavelength. The %RSD for peak area was calculated which should be less than 2%. The result is shown in analytical method concluded. The reproducible results were obtained which proves that method is robust as shown in table 6.

Table 6: Result of Robustness Study of Avanafil

Parameters	Conc. (µg/mL)	Area (mean ±SD)	% RSD
MP composition (MeOH 66 % + Water 34 %)	30	740.8±2.56	0.35
MP composition (MeOH 64 % + Water 36 %)	30	724.18±2.28	0.32
Wavelength change 244 nm	30	749.1±7.85	1.05
Wavelength Change 247 nm	30	775.65±1.45	0.19
Flow rate change (0.69 mL)	30	815.2±6.90	0.85
Flow rate change (0.71 mL)	30	816.04±3.47	0.43

Suitability: To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of the Avanafil system suitability parameters were studied. The result is shown in table 7. Test As a system suitability test was an integral part of chromatographic methods development and was used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Avanafil were evaluated. The chromatographic system's applicability was proved by comparing the acquired parameter values to the ICH guidelines' acceptance requirements, such as Area, tailing factor, theoretical plates, and retention time as shown in table 7.

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Table7: Repeatability studies on Avanafil

Sr. No.	Concentration of Avanafil (mg/mL)	Area	RT	TF	TP	Amount found (mg)	% Amount found
1	40	997.16	6.6167	1.2000	7222.9	39.25	98.13
2	40	981.18	6.6000	1.2444	6038.7		
3	40	984.27	6.6500	1.2500	7295.9		
	Mean	987.54	6.6222	1.2315	6852.5		
	SD	8.48	0.02	0.03	705.7		
	%RSD	0.86	0.38	2.22	10.30		

In the repeatability studies of Avanafilwas found that the %RSD was less than 2, which shows high percentage amount found in between 98% to 100% indicates the analytical method that concluded

Assay: For the quantitative estimation of Avanafil in tablet dosage form, samples of Avanafil were prepared and injected into the HPLC. The Mean, Standard deviation, and % RSD of Assay of Avanafil sample solution were calculated. Weigh 20 Avanafil tablets and calculated the average weight accurately. Weight and transfer the sample equivalent to 13.3 mg Avanafil into 10 mL volumetric flask. Add about 10 mL of diluent and sonicate to dissolve it completely and make the volume up to the mark with diluents. Filter through a 0.45 m filter after thoroughly mixing. Further pipette out 0.2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluents (20µg/mL). The amounts of Avanafil per tablet were calculated by extrapolating the value of area from the calibration curve. With tablet formulation, the analysis technique was done five times.Tablet Assay for % label claim for %RSD calculated. The result was shown in Table 8.

Table 8: Quantitative Study of Avanafil in Tablet Dosage Form

Sr. No	Amount present in mg	Area (I)	Amount found in mg	% Label claim
1	20	503.33	19.80	99.00
2	20	493.83	19.41	97.05
Mean	-	498.58	19.60	98.03
SD	-	6.72	0.28	0.27
%RSD	-	1.35	0.70	0.28

*Average of 2 determinations; SD is standard deviation; %RSD is relative standard deviation

IV. RESULT AND DISCUSSION

Novel and simple RP HPLC methods have been developed for the determination of Avanafil in Tablet dosage forms. The chromatographic conditions were optimized by taking into consideration the chemical structures of Avanafil, choice of the column with respect tothe flow rate of the mobile phase, and wavelength of detection. The optimized chromatographic condition was found

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satisfactory to yield a well retained, sharp, and symmetrical peak at 6.6222 min. The number of Average theoretical plates was 6852.5 and the tailing factor was 1.2315 for Avanafil, which indicates the efficient performance of the column. The results of linearity studied over the concentration range 10-50µg/mL showed the linear detector response with a correlation coefficient of 0.9988 and the regression equation of y=24.891x + 10.482. At each additional concentration, the spiked medication recovered well, demonstrating that the procedure was accurate. Percent Recovery was observed to be 98-100 % representing the accuracy of the method and non-interference of excipients. Replicate estimations of Avanafil by proposed method have yielded in range of 98-100 % indicating substantially high precision of the method for standard drug solution, sample solution, Excipients solution and blank reveals that the peak obtained in the respective chromatogram at working concentration are only because of the drug. The intermediate precision study was ascertained based on intra-day and interday data obtained by analyzing Avanafil by the proposed method and it is found to be very much reproducible.This represents the method's precision and repeatability. The method was sufficiently robust for normally expected variations in chromatographic conditions as per ICH guidelines such as wavelength, solvent system, and Flow rate. The developed HPLC method is simple, specific, accurate, and precise for Avanafil in the tablet dosage form.

V. CONCLUSION:

The developed method is better than earlier published articles with respect to superior system suitability parameters such as Theoretical plates and tailing factor. It was successfully validated in terms of linearity, accuracy, precision, repeatability/suitability,and recovery in accordance with ICH Q2 (R1) guidelines. Thus, the described method is suitable for routine analysis and quality control of Avanafil in Tablet dosage forms.

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