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Research Article

DEVELOPMENT AND VALIDATION OF NEW COLORIMETRIC METHOD FOR THE ESTIMATION OF AVANAFIL IN BULK AND DOSAGE FORM

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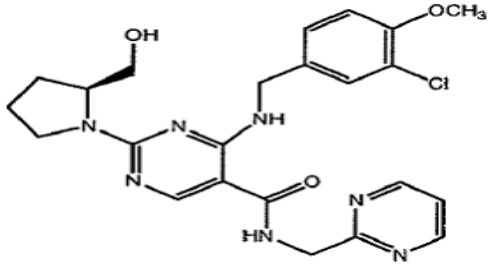
Abstract:

A simple, sensitive, rapid and accurate colorimetric method has been developed for the estimation of Avanafil in bulk and pharmaceutical dosage forms. Here Method was based on Ion complexreaction involving the formation of yellow colored complex between Avanafil and Bromophenol blue. The complex formed was extracted into chloroform and themaximum absorbance of the solution was measured at 417 nm against blank. The calibration curve calculated obeys Beer's law over the concentration range of 1-5 µg/ml.The method was validated based on ICH guidelines. It is simple, sensitive, and reliable and results are reproducible. The high recovery and low relative standard deviation confirms the suitability of the method for determination Avanafil in pharmaceutical dosage forms.Hence it is useful for the routine analysis of Avanafil.

Key words: Avanafil, 0.1M HCL,Bromophenol blue, UV spectrophotometric and validation parameters.

Introduction:A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1]Avanafil is a selective inhibitor of cGMP-specific PDE5. Avanafil is designated chemically as (S)-4-[(3-Chloro-4-methoxybenzyl) amino]-2-[2-(hydroxymethyl)-1-pyrrolidinyl]-N-(2-pyrimidinylmethyl)-5-pyrimidinecarboxamide. Avanafil occurs as white crystalline powder. Its Molecular formula and molecular weight is $C_{23}H_{26}C_lN7O_3$ and 483.95 respectively. The structural formula is:

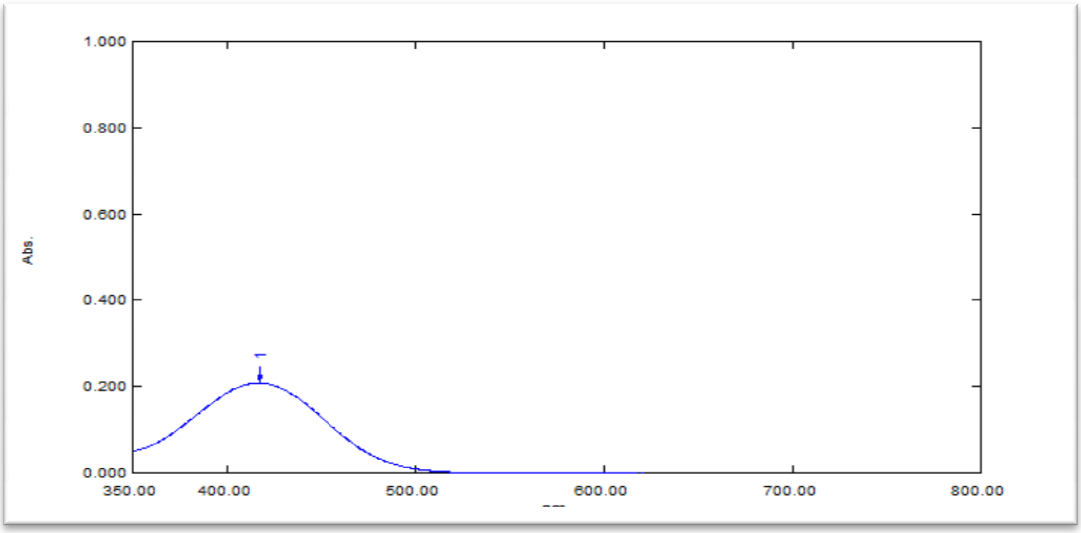


It is slightly soluble in ethanol, practically insoluble in water, soluble in 0.1 mol/L hydrochloric acid. [2, 3]Up to now no colorimetric method developed on Avanafil. Only colorimetric method developed on Tadanafil and Sildenafil. [4, 5]The aim of study is here to developed new colorimetric method. Estimation of Avanafil was carried out by the reaction of functional group present in it with suitable agent like Bromophenol blueto formed colored products which are determined calorimetrically. Analysis of the drug is important for development of drugs in their formulation and their use in therapies, for which we require standard analytical procedures.The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Ruggedness, Robustness[6,7,8]As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

Materials and Methods:

A colorimetric method was developed for Avanafil by using Bromophenol blue dye and extraction with $CHCL_3$.

Figure No.1: Bromophenol blue with drug & $CHCL_3$



Conclusion: The λmax of the colour sample was found to be 417nm.

1.0 Experimental:

1.1 Instrumentation:

All the experiments were carried out on Jasco1800 UV-Vis spectrophotometer using 1cm matched quartz cuvettes.

1.2 Preparation of standard stock solution of Avanafil (1000µg/ml):

Stock solution of avanafil was prepared by weighing accurately 100mg of pure drug into a 100ml volumetric flask and dissolved it and the volume was made up to mark with 0.1M HCL to get a concentration of 1000 µg/ml.

1.3 Preparation of working standard solution of Avanafil (100µg/ml):

The working standard solution of Avanafil was prepared by dilution of the stock solution suitably with 0.1M HCL to get a concentration of 100µg / ml.

1.0 Preparation of reagents:

2.1 Solution of Bromophenol blue (1%):

The solution of Ammonium molyBromophenol blue was prepared by dissolving 1gm of Bromophenol blue in 100 ml of dist. water.

2.2 0.1M hydrochloric acid:

0.86ml conc. HCL + 100ml distil water [9]

3.0 Preliminary investigation:

To 1ml of the drug solution containing 1µg/ ml, 1 ml of 1% of Bromophenol blue was added, followed by 10ml $CHCL_3$ and make up volume till 100ml with 0.1M HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take out Chloroform layer which is yellow in colour and measure the λmax. Corresponding reagent blank was prepared in the same manner omitting the drug in which no colour was seen in chloroform layer.

4.0 Parameter Fixation:

4.1 Determination of λmax:

An absorption maxima (or) λmax are the wavelengths at which maximum absorption takes place. It is important to know the absorption maxima of the substance under study, since it helps to avoid any interfering impurities.

- λmax of coloured sample

Model : 1800

Band width : 2nm.

Response : Medium.

Measurement : 800-400nm.

No. of cycle : 1.

Sample : Avanafil

λmax : 417 nm.

4.2 Stability of colour:

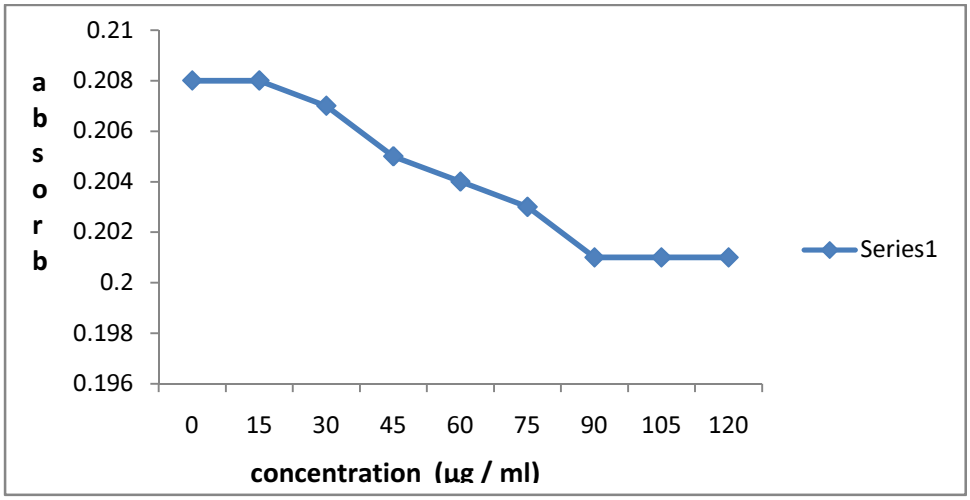
The time taken for color development and stability was studied.

Procedure: To 1ml of the solution containing Avanafil taken in 100ml volumetric flasks, 1 ml of 1% of Bromophenol blue was added, followed by 10ml $CHCL_3$ and make up volume till 100ml with 0.1M HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take the below Chloroform which contain yellow colour. The absorbance was measured at different time intervals after dilution. The absorbance was measured against a reagent blank at 417 nm. The results are recorded in Table No: 1 and graph is given in figure No: 2.

Table No. 1: Stability of colored species.

SL. No.	Volume of drug solution (100µg / ml)	Time (Minutes)	Absorbance At 417 nm
1.	1 ml	0 min	0.208
2.	1 ml	15 min	0.208
3.	1 ml	30 min	0.207
4.	1 ml	45 min	0.205
5.	1 ml	60 min	0.204
6.	1 ml	75 min	0.203
7.	1 ml	90 min	0.201
8.	1 ml	105 min	0.201
9.	1 ml	120 min	0.201

Figure No. 2: Stability of color



Conclusion: The stability of color was found to be stable for 2 hr.

5.0 Investigation:

5.1 Effect of concentration of reagent (Bromophenol blue in $CHCL_3$)

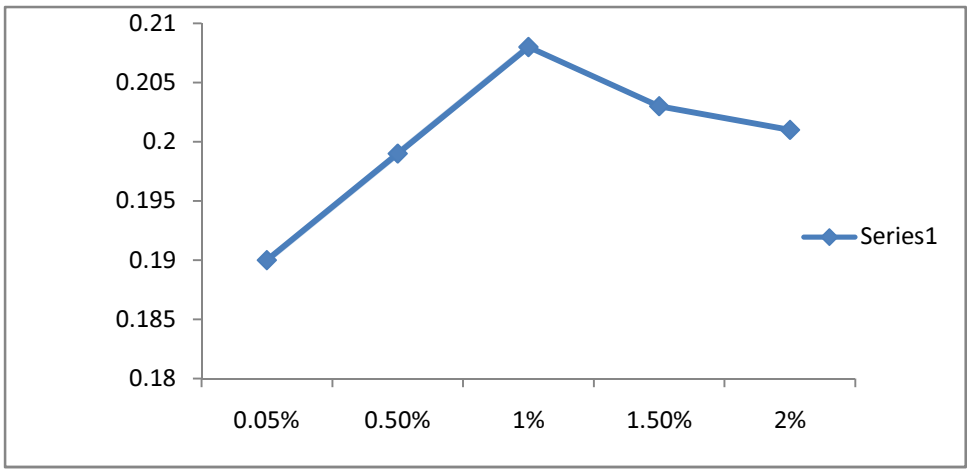
Experiment was carried out to ascertain the optimum concentration of reagents needed for rapid and quantitative formation of yellow colour by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

Procedure: 1ml of the drug solution was placed in 5 different 100 ml volumetric flasks, to this different concentrations of Bromophenol bluefollowed by 10ml $CHCL_3$ then volume was made up to 100ml with 0.1m HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take the Chloroformlayer which contains yellow colour. The absorbance was measured against a reagent blank at 417 nm. The results were given is Table No.2 and the graph was given in figure No.3.

Table No. 2: Effect of Bromophenol blue in $CHCL_3$

SL. No.	Concentration Of Bromophenol blue in $CHCL_3$	Absorbance At417 nm
1.	0.05 %	0.190
2.	0.5 %	0.199
3.	1 %	0.208
4.	1.5%	0.203
5.	2 %	0.201

Figure No. 3: Effect of concentration of Bromophenol blue in $CHCL_3$



Conclusion: The maximum absorbance was obtained at the conc. of 1% of Bromophenol blue in $CHCL_3$

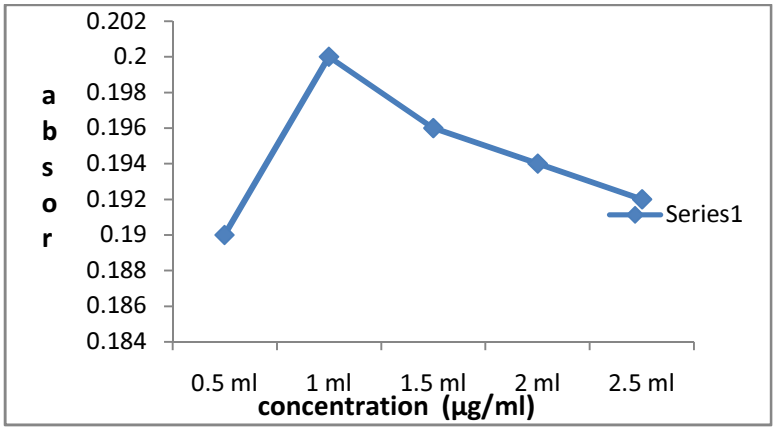
5.2 Effect of volume of Bromophenol blue in $CHCL_3$:

Procedure: 1ml of the drug solution was placed in 5 different 100 ml volumetric flasks, to this different volume of 1% Bromophenol bluefollowed by 10ml $CHCL_3$ then volume was made up to 100ml with 0.1m HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take the Chloroform layer which contains yellow colour. The absorbance was measured against a reagent blank at 417 nm. The results were given in table No .3 and graph was given in fig No.4.

Table No. 3: Effect of Volume of Bromophenol blue in $CHCL_3$

SL. No.	Volume of Bromophenol blue in $CHCL_3$	Absorbance At417 nm
1.	0.5 ml	0.190
2.	1 ml	0.200
3.	1.5 ml	0.196
4.	2 ml	0.194
5.	2.5 ml	0.192

Figure No.4: Effect of Volume of Bromophenol blue in $CHCL_3$



Conclusion: The maximum absorbance was obtained at the 1.0 ml of 1% of Bromophenol blue in $CHCL_3$

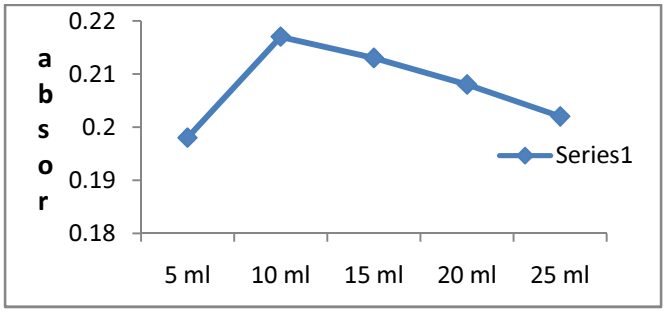
5.3 Effect of volume of $CHCL_3$:

Procedure:1ml of the drug solution was placed in 5 different 100 ml volumetric flasks, to this 1ml of 1% Bromophenol bluefollowed by different volume of $CHCL_3$ then volume was made up to 100ml with 0.1m HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take the Chloroform layer which contains yellowcolour. The absorbance was measured against a reagent blank at 417 nm. The result was given in table No.4 and graph was given in Figure No.5

Table No. 4: Effect of volume of $CHCL_3$

SL. No.	Volume of $CHCL_3$	Absorbance At 417nm
1.	5 ml	0.198
2.	10 ml	0.217
3.	15 ml	0.213
4.	20 ml	0.208
5.	25 ml	0.202

Figure No. 5: Effect of volume of $CHCL_3$



Conclusion: The maximum absorbance was obtained at the 10 ml of $CHCL_3$

6.0 Optical Characters:

6.1 Determination of concentration range:

For spectrophotometric analysis, determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility.

6.2 Preparation of Standard Curve:

A standard curve was prepared by using pure Avanafil in the concentration of 1-5 µg/ml by this method and selecting the absorption maxima at 417 nm.

Procedure: From the working standard drug solution of 1, 2,3,4,5 ml (which gives 1-5µg/ml) drug solution was placed in 5 different 100 ml volumetric flasks. Into this 1 ml of 1% of Bromophenol blue was added, followed by 10ml $CHCL_3$ and make up volume till 100ml with 0.1M HCL then take the solution in separating funnel. Shake it for some time and keep aside for 5 min. Take the Chloroform layer which contains yellow colour. Corresponding reagent blank was prepared in the same manner omitting the drug in which no colour was seen in Chloroform layer. The absorbance was measured against a reagent blank. And the results were recorded in table No: 5 and the graph was given in the figure No: 6 and figure no.7.

Table No. 5: STD Curve

SL. No.	Volume of drug 100µg/ml	Concentration of drug taken in µg/ml	Absorbance At 417 nm
1.	1 ml	1 µg	0.208
2.	2 ml	2 µg	0.4
3.	3 ml	3 µg	0.583
4.	4 ml	4 µg	0.819
5.	5 ml	5 µg	0.997

Figure No. 6STD curve Linearity of Avanafil

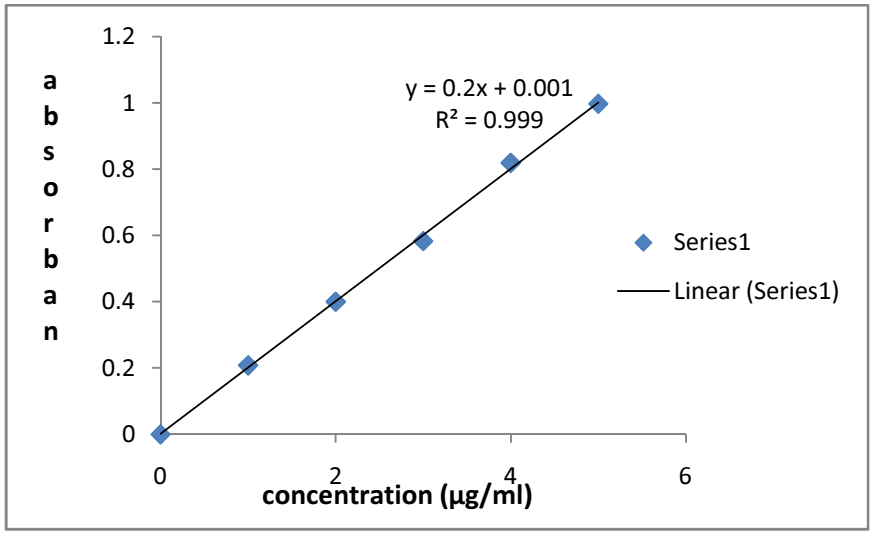
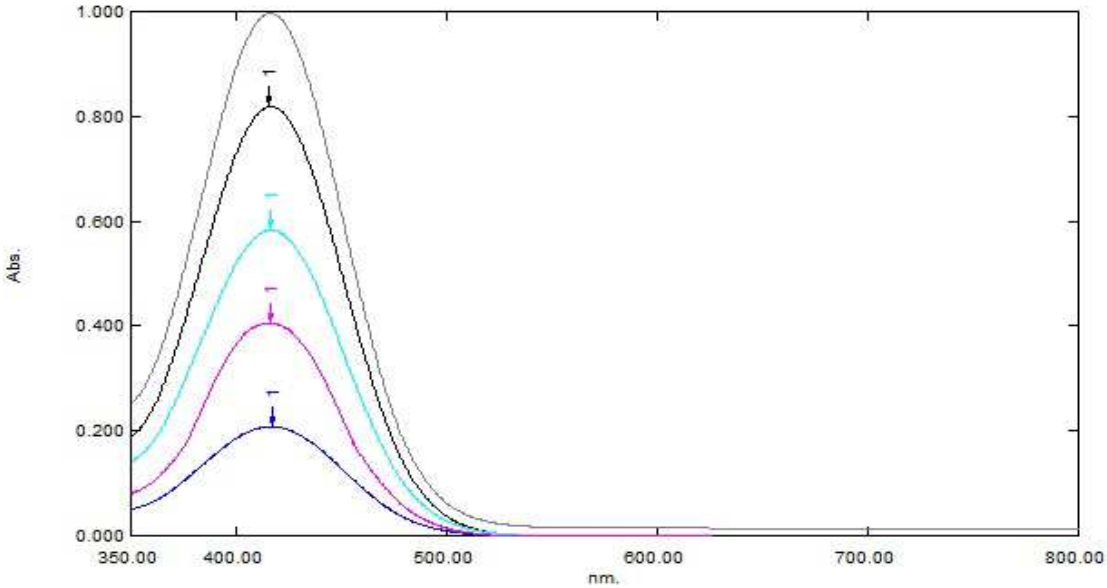


Figure no.7: STD curve Graph



7.0 Analysis of Formulation:

Avanafil was procured from the local market as tablets of strength 25 mg and marketed with brand name of STENDRA.

7.1 Preparation of sample solution:

20 tablets were weighed and crushed properly using a mortar and pestle. Then Powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in 0.1M HCL and filtered through whatmann filter paper in to another 100ml volumetric flask and made up to mark with same diluent which give the solution of 1000µg/ml conc., Further dilution was performed to get a concentration of 100µg/ml.

Table no. 6: Assay Results of Marketed Formulation

Formulation	Label claim of Avanafil (µg/ml)	Amount obtained of Avanafil (µg/ml)	% Avanafil
Tablet	25	24.85	99.4

8.0 Validation Parameter: [8]

1. Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure.

This method obeys the Beer- Lambert's law in the concentration range of 1-5 µg/ml. as given in table no.7.

Table no. 7: Linearity data

Concentration (µg/ml)	Absorbance mean ± SD	RSD
1	0.208667 ± 0.002338	1.1153
2	0.402833 ± 0.003061	0.7598
3	0.583333 ± 0.001366	0.2341
4	0.818333 ± 0.002582	0.3155
5	0.994833 ± 0.001941	0.1951

***n=6**

2. Accuracy

Accuracy was established across the specified range of the analytical procedure.

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

3. Repeatability: Standard solutions of Avanafil (1, 2, 3, 4 and 5 µg/ml) were prepared and a spectrum was recorded. Absorbance was measured at 417 nm taking the mixture of Bromophenol bluefollowed by $CHCL_3$ as the blank. The absorbance of the same concentration solution was measured six times and RSD was calculated.

Table no.8: Determination of Accuracy

Amt of sample(µg/ml)	Amt. of drug added (µg/ml)	Amt. of drug recovered (µg/ml)	% Recovery
1	0.5	0.48	96
1	1	0.9	90
1	1.5	1.4	93.33

Table 9: Repeatability data for Avanafil at 417 nm

Concentration	1µg/ml	2µg/ml	3µg/ml	4µg/ml	5µg/ml
Absorption	0.208	0.4	0.583	0.819	0.997
	0.208	0.399	0.583	0.82	0.995
	0.207	0.4	0.581	0.818	0.993
	0.205	0.402	0.581	0.818	0.997
	0.207	0.4	0.579	0.82	0.996
	0.206	0.399	0.58	0.819	0.997
Mean.	0.20683333	0.4	0.58116667	0.819	0.99583333
Std. Dev.	0.001169045	0.00109545	0.00160208	0.00089443	0.00160208
Coefficient variation	0.005652	0.002738	0.002756	0.001092	0.001608
% RSD	0.5652	0.2738	0.2756	0.1092	0.1608

***n = 6**

Table no. 10: Repeatability of sample application data for Avanafil.

Concentration	Avanafil2 µg/ml
Absorption	0.399
	0.398
	0.4
	0.399

	0.398
	0.397
Mean.	0.3985
Std. Dev.	0.001048809
Coefficient variation	0.002631
% RSD	0.2631

***n = 6**

3. Limit of detection (LOD) & limit of quantitation (LOQ):

- The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

- The LOQ is estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as;

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

LOD and LOQ show in table no. 11.

Table no. 11: LOD AND LOQ

LOD	LOQ
0.06378	0.1932

4. Specificity and selectivity:

Data were recorded in table np.12.

Table no. 12: Specificity and Selectivity study

Study	Avanafil
Specificity	Specific
Selectivity	Selective

5. Reproducibility:

Reproducibility is assessed by means of an inter-laboratory trial.

The absorbance readings were measured at 417nm at different laboratory using another spectrophotometer. This recorded in table no. 13.

Table no. 13: Reproducibility data for Avanafil at 417 nm.

Instrument 1 SIMADZU	Instrument 2 JASCO	Inference
0.2063± 0.0008464	0.2060 ± 0.0008944	Not significant difference

* At 95% confidence interval

6. Intra and inter day precision :

Variation of results within the day (intra-day), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing Avanafil for three times in the same day at 417 nm.

Inter day precision was determined by analyzing the drug different day for three days at 417 nm.

All Summary of Validation Parameters of Spectrophotometry was noted in table no. 15.

Table no. 14: Precision data for Avanafil at 417nm

Conc. µg/ml	Intra-day (n=3)	CV	%RSD
1	0.206333333 ± 0.00057735	0.002798	0.2798
2	0.399 ± 0.001	0.002506	0.2506
3	0.580 ± 0.001	0.001727	0.1727

Conc. µg/ml	Inter day (n=3)	CV	%RSD
1	0.205± 0.001	0.004878	0.4878

2	0.398333333 ±0.002516611	0.006317	0.6317
3	0.578333333 ± 0.001527525	0.002641	0.2641

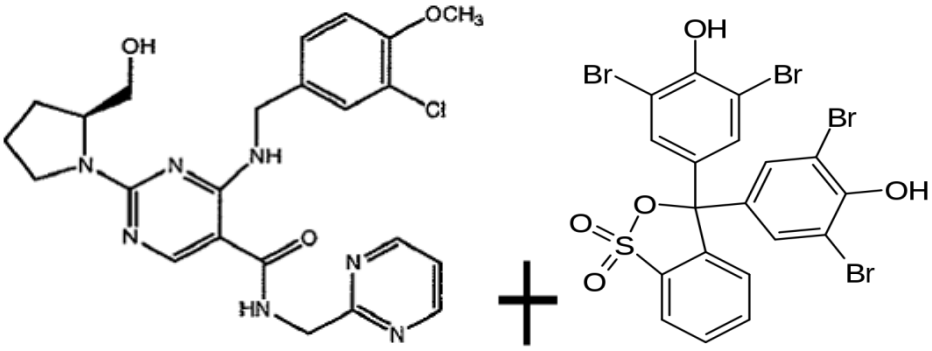
Table No: 15: Summary of Validation Parameters of Spectrophotometry.

Parameter	Result
λmax(nm)	417.0
Beer's law limits (µg/ml)	1-5
Molar absorptivity (1/mol.cm)	$9.7417X10^4$
Sandell's sensitivity (µg.cm^2/0.001 Au)	0.004965
Regression equation (y=a+bc) Slope (b) Intercept (a)	 0.2 0.0011
Correlation coefficient (r^2)	0.999
% Relative Standard deviation	0.5239
Limit of Detection (µg/ml)	0.06378
Limit of Quantitation (µg/ml)	0.1932

Results and Discussion:

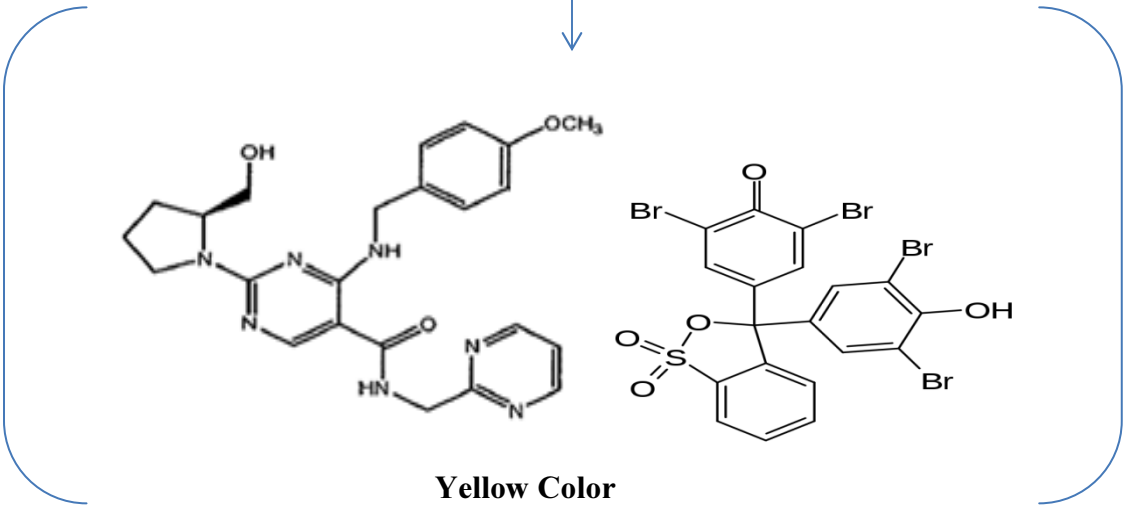
Avanafil was estimated based on the reaction of Bromophenol blueand extraction with Chloroform. The probable reaction takes place was Ion pair complex reaction, resulting in the formation of yellow colour in chloroform layer which showed λmax at 417 nm. The colour was stable for more than 2 hour. The method obeyed Beer-Lambert's law in the concentration range of 1-5 µg/ml.

The probable reaction is as per follow



Avanafil Bromophenol Blue

-HCL



Yellow Color

Conclusion:

The proposed method is simple, selective and sensitive. And the λmax is 417nm. HPLC method is costly as it requires sophisticated instruments & high grade chemicals so here method is considered for colorimetric method. Hence the proposed method can be used for routine analysis of Avanafil

Acknowledgement:

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Reference:

1. Chemistry 111 Lab: Intro to Spectrophotometry. Spectrophotometry 2005, E1-8
2. Super Avana: http://www.indiapharmaexport.com/generic-stendra-drug.html

3. Avanafil: http://www.rxlist.com/stendra-drug.htm
4. Vivek Jain, AnujModi, Anurekha Jain, et al. A Validated UV spectrophotometric method for dosage form determination of Tadanafil in bulk and solid. Journal of Advances in Drug Research 2012; 1(2): 13-8.
5. Hesham Salem. Spectrochemical Methods for the Determination of Sildenafil Citrate (Viagra) in Bulk Powder and in Pharmaceutical Dosage Form. J. J. Appl. Science 2006; 8(1): 28-43
6. H. Ludwig, Validation of Analytical Methods and Procedures, Labcompliance News, USA, 2007
7. Devid G. Watson, Pharmaceutical analysis, 1st edition.
8. International Conference on Harmonisation, Guidance for industry in; Q2B Validation on Analytical Procedures: Methodology. Switcherland: IFPMA, 1996, pp1-8.
9. Indian pharmacopoeia 2007; vol 1: 550

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