RESEARCH ARTICLE

ISSN: 2348-8948

Vol: 3; Issue: 6

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AVANAFIL IN PHARMACEUTICAL DOSAGE FORM

Bhatt Bhumik, Raval Kashyap, Sheetal Buddhadev

Noble Pharmacy College, Junagadh, India

Email: raval.kashyap999@gmail.com

Date Received:	Date of Accepted:	Date Published:
21-May-2015	29-May-2015	03-Jun-2015

Abstract:

Analysis of pharmaceutical product is very important as it concerned with life. A stability-indicating assay accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities. Stability of the pharmaceutical product is most important, so that work is carried out to develop a new, simple, precise, accurate, validated stability indicating Analytical method for estimation of Avanafil in Pharmaceutical Dosage Form. In this method Acetonitrile: Water: Triethylamine: Acetic Acid in the ratio (65:35:0.1:0.1) was used as a mobile phase, so that developed method becomes ecofriendly. The detection was carried out at 254 nm and a flow rate of 1 mL/min and retention time 4.7 min. and C18 column was used for the separation of drug with other degraded product and process impurities. The linearity was observed in the range of 50–150 ppm. The developed method was meets all the acceptance criteria for the validation of analytical method as per the ICH guideline. The degradation of the drug in acid, alkali, oxidation, Thermal was found to be 2.78%, 4.00%, 2.06%, 1.48% respectively and it meets acceptance criteria for the stability of the pharmaceutical product as per ICH guideline. The assay of drug was found in acceptance range.

Keywords: Avanafil, Acetonitrile, Water, Triethylamine, Acetic Acid.

Introduction

Avanafil as (S) -4-[(3-Chloro-4 methoxybenzyl) amino] - 2- [2- (hydroxymethyl)- 1 – pyrrolidinyl] – N - (2pyrimidinylmethyl) – 5 -pyrimidinecarboxamide. It is a practically White crystalline powder. It is freely soluble in Methanol & in Acetonitrile, Practically insoluble in water, soluble in 0.1 mol/L hydrochloric acid. Its molecular formula C23H26ClN7O3 and calculated molecular weight of 483.95 gm/mol. The physiologic mechanism of erection of the penis involves release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. NO then activates the enzyme guanylate cyclase, which results in increased levels of cGMP, producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. Avanafil has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of NO by inhibiting PDE5, which is responsible for degradation of cGMP in the corpus cavernosum. Because sexual stimulation is required to initiate the local release of nitric oxide, the inhibition of PDE5 has no effect in the absence of sexual stimulation. Studies in vitro have shown that avanafil is selective for PDE5. Its effect is more potent on PDE5 than on other known phosphodiesterases

(greater than 100-fold for PDE6; greater than 1,000-fold for PDE4, PDE8 and PDE10; greater than 5,000-fold for PDE2 and PDE7; greater than 10,000-fold for PDE1, PDE3, PDE9, and PDE11). Avanafil is greater than 100-fold more potent for PDE5 than PDE6, which is found in the retina and is responsible for phototransduction.

In addition to human corpus cavernosum smooth muscle, PDE5 is also found in other tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle, brain, heart, liver, kidney, lung, pancreas, prostate, bladder, testis, and seminal vesicle. Erectile dysfunction (ED) is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during sexual performance. A penile erection is the hydraulic effect of blood entering and being retained in sponge-like bodies within the penis. The process is often initiated as a result of sexual arousal, when signals are transmitted from the brain to nerves in the penis. Erectile dysfunction is indicated when an erection is difficult to produce**.** Avanafil is an effective treatment option for males suffering from ED. Its main advantage over the other available PDE5 inhibitors is its faster onset of action. **1-3** Till now only some methos published on Avanafil **4-7** but no stability indicating method published till now.

MATERIALS AND METHODS

Optimization of Chromatographic Parameters
Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimization of Mobile Phase Strength

The mobile phase chosen after several trials with Water: MeOH in various proportions which is shown in table. Finally the mobile phase consisted mixture Acetonitrile: Water: Triethylamine: Acetic Acid (65:35:0.1:0.1).which resolved the tailing of peak. The flow rate of 1 ml/min was selected as it gave good result, system suitability parameters and reasonable retention time. The retention time of Avanafil was observed 4.7 min at 254 nm wavelength with run time of 6 min.

Selection of Detector and Detection Wavelength
UV detector was selected, as it is reliable and wavelength was set to 254nm.

Preparation of solution

Preparation of Mobile Phase: Prepare a Mixture Acetonitrile: Water: Triethylamine: Acetic Acid in the Ratio of (65:35:0.1:0.1) and sonicated for 20 min in ultra sonicator.

Preparation of Diluent: Mobile phase is used as Diluent

Preparation of Standard Stock Solution (1000 ppm):
Take 100 mg of Avanafil working standard into 100 ml volumetric flask, add 25 ml of diluent, dissolve it and make up volume with diluent.

Standard Solution (100 ppm):
Take 5 ml of Avanafil standard stock solution into 50 ml volumetric flask, make up volume with diluent and sonicated for 5 min in ultra-sonicator.

Sample Solution (100 ppm):
Twenty tablets were accurately weighed and break it .Then transferred into 1000 ml volumetric flask and 700 ml of diluent was added. The volumetric flask was sonicated to disperse tablets completely for 30 minutes with intermittent shaking. The solution was cooled to the room temperature and made up to volume with diluent. 10 ml of this solution was diluted to 100 ml with diluents. The solution was filtered through 0.45 µ Millipore PVDF filter; filtrate was collected after discarding first few ml.

Validation studies:
The following parameters were considered for the analytical method validation for the quantification of avanafil in tablet dosage form as per ICH guideline.

Specificity:
The analyte should have no interference from other extraneous components and be well resolved from them. Specificity is theprocedure to detect quantitatively the analyte in presence of componentthat may be expected to be present in the sample matrix.

System suitability:
System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (100 ppm) was injected six times and thechromatograms were recorded.

Acceptance Criteria:
The % RSD for area response obtained from six replicate injections of Standard solution should be ≤ 2.0 %.
Tailing factor for Avanafil peak should be ≤ 2.0 in standard solution.
Theoretical plates of Avanafil peak should ≥ 2000 in Standard solution.

Linearity:
Different levels of standard solution were prepared by diluting out known volumes of standard stock solution (1000 ppm) with the diluents to get the required analyte concentrations in the range of 50-150 ppm (Table 4.5). A graph of Concentration (ppm) vs. Area was plotted and the regression coefficient „r2", y-intercept and slope of

the regression were calculated.

Acceptance Criteria
The Regression coefficient (r2) should not be less than 0.999 for Avanafil.

Accuracy (by recovery study):
Accuracy was determined over the range 80 % to 120 % of the sample concentration. Calculated amount of Avanafil API was added in placebo to attain 80 %, 100 % and 120 % of sample concentration

Amount as shown above was weighed and transferred into 50 ml volumetric flask and 25 ml of diluent was added. Dissolve it and make up the volume up to the mark with diluent. Sonicated for 5 min in ultra sonicator. Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated.

Acceptance Criteria
Mean % recovery at each level should be between 98.0 % and 102.0 % for Avanafil.

Precision:
System Precision:
The system precision was checked by using standard substance to ensure that the analytical system was working properly. Standard solution (100 ppm) was injected six times and chromatograms were recorded. The retention time and area of six determinations was measured and % RSD calculated.

Acceptance Criteria:
The % RSD of the peak area obtained from six replicate injections should be ≤ 2.0 %.

Method Precision (Repeatability):
The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. It provides an indication of random error results and is expressed as coefficient of variation (CV). This was performed to indicate whether the method was giving consistent results for a single batch. Method precision was carried out by analyzing six replicate injections of assay concentration (100 ppm) of standard and sample solutions. Percentage assay of sample to that of label claim was calculated by comparing the sample solution response to that of standard solution response. % RSD was calculated.

Intermediate Precision (Ruggedness)
Intermediate precision was assessed by analyzing the standard solution and sample solution on different days. The % assay and % RSD were calculated.

Acceptance Criteria:
The % RSD calculated on 6 determinations for assay value of Avanafil should be ≤ 2.0 %.

ROBUSTNESS:
The robustness of an analytical method was carried out to confirm that the method remained unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected five times for each varied conditions of flow, column temperature, pH, and mobile phase ratio and chromatograms were recorded.

DEGRADATION STUDIES
Forced degradation study was carried out to confirm that during stability study or through its shelf life, any degradation product if found would not interfere with the peak of Avanafil Stress studies were carried out under the condition of acid, alkali, oxidation, thermal and light as mentioned in ICH guidelines.

Result of Acid Degradation:
Degradation of AVANAFIL was carried out in 1 N HCl at 40ºC in Water bath. Measurable degradation was obtained after 24 hrs. It showed multiple peaks of degraded products. Major degraded peak was found at 4.927 min. in drug substance. This study indicates that the drug is practically labile to acidic stress. The developed method could resolve all the peaks, so quantitation of drugs is possible with developed method in presence of acid degraded products.

Degradation of AVANAFIL was tried in 0.1N NaOH at RT. But no degradation was found. After that degradation was carried out in 1N NaOH at 40ºC in Water bath. Measurable degradation was obtained after 24 hr. Degradation peak was found at 4.82 min. in drug substance. This study indicates that the drug is practically labile to alkaline stress. The developed method could resolve all the peaks, so quantitation of the drugs is possible with developed method in presence of base degraded products.

Drug substances were treated with 30% solution of H2O2 and kept at RT. Measurable degradation was obtained after 24 hr. Degradation peak was found at 4.832 min. This study indicates that the drug is practically labile to oxidative stress. The developed method could resolve all the peaks, so quantitation of the drugs is possible with developed method in presence of peroxide degraded products.

Drug substances were kept 70ºC. Measurable degradation was obtained after 24 hr. Degradation peak was found at 4.812 min. This study indicates that the drug is practically labile to Thermal stress. The

Final Chromatographic Conditions for Assay Method

Chromatographic Mode	Chromatographic Condition
Stationary phase	J & J C18 (250X4.6mm) 5 µ
Mobile phase	Acetonitrile:Water:Triethylamine:Acetic Acid(65:35:0.1:0.1)
Detection wavelength	254 nm
Flow rate	1 ml/min
Injection volume	20 µL
Diluent	Mobile Phase

Preparation of Linearity Samples for Assay Method

Level	PPM	Dilution (ml)	Diluted (ml)
L1	50	5	100
L2	75	7.5	100
L3	100	10	100
L4	125	12.5	100
L5	150	15	100

Sample Preparations for Accuracy for Assay Method

Sample	Level 1 (80 %)	Level 2 (100 %)	Level 3 (120 %)
Avanafil (mg)	50	50	50

Change in Flow rate (±0.2 ml/min)

Condition of Change in Flow Rate for Assay Method

Condition	Flow rate (ml/min)
Original flow	1.0
Increased flow	1.2
Decreased flow	0.8

Change in Column Temperature (± 2 oC)

Condition of Change in Temperature for Assay Method

Condition	Temperature (C)
Original temperature	25
Increased temperature	27
Decreased temperature	23

Stability Indicating Assay Method
Apparatus and Equipment

Apparatus and Equipments for SIAM

Sr. No.	Instrument Name	Make and Model of Instrument
1	Electronic Analytical Balance	Shimadzu AX-200
2	HPLC	Waters 996
3	Ultra-Sonicator	PEI, Ultrasonic bath
4	Filter	0.45 µ Millipore PVDF
5	P Meter	Chemiline, CL-180, Labline technology PVT LTD (AL)

Final chromatographic condition

Final Chromatographic Conditions for Assay Method

Chromatographic Mode	Chromatographic Condition
Stationary phase	J & J C18 (250X4.6mm) 5 µ
Mobile phase	Acetonitrile:Water:Triethylamine:Acetic Acid(65:35:0.1:0.1)
Detection wavelength	254 nm
Flow rate	1 ml/min
Injection volume	20 µL
Diluent	Mobile Phase

Acid Degradation

Acid Degradation Procedure for SIAM

Sample (ml)		Degradation with 1N HCl (ml)	Neutralization with 1N NaOH (ml)
Diluent	2	1	1
Standard solution (100 ppm)	2	1	1

After degradation, make up the volume 5 ml with diluent and then go for the HPLC analysis.

Alkali Degradation

Alkali Degradation Procedure for SIAM

Sample (ml)		Degradation with 1N NaOH (ml)	Neutralization with 1N HCl (ml)
Diluent	2	1	1
Standard solution (100 ppm)	2	1	1

After degradation, make up the volume 5 ml with diluent and then go for the HPLC analysis.

Peroxide Degradation

Peroxide Degradation Procedure for SIAM

Sample (ml)		Degradation with 30 % H2O2 (ml)
Diluent	2	1
Standard solution (100 ppm)	2	1

After degradation, make up the volume 5 ml with diluent and then go for the HPLC analysis.

Thermal Degradation

Thermal Degradation Procedure for SIAM

Sample (ml)		Kept at 70°C
Diluent	2	1
Standard solution (100	2	1

After degradation, make up the volume 5 ml with diluent and then go for the HPLC analysis.

RESULT & DISCUSSION:

TRIAL FOR METHOD DEVELOPMENT OF AVANAFIL

Table 1: Method Development Trials

Sr. No.	Trials Taken	Observation	Remarks
1	Water: MeOH (50:50)	Peak shape was not good. Broad peak was there.	Not Satisfactory
2	Water: MeOH (70:30)	Peak shape was not good.	Not Satisfactory
4	Acetonitrile:Water:Triethylamine:Acetic Acid(65:35:0.1:0.1)	Peak shape was good, as well as Retention time was good.	Satisfactory

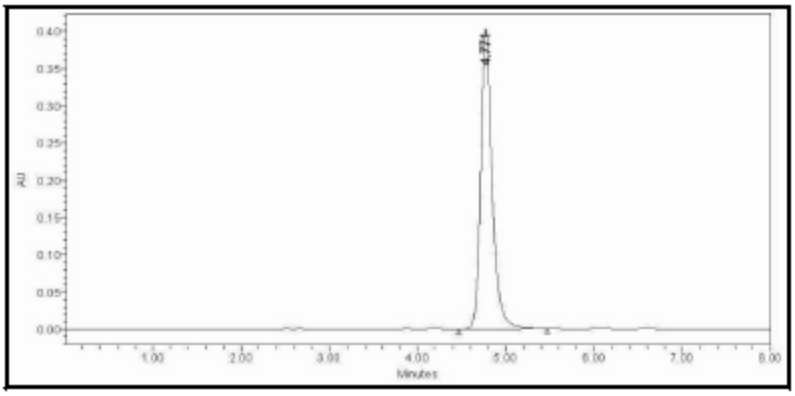

Fig 1: Method Development Trial 3 Acetonitrile:Water:Triethylamine:Acetic Acid (65:35:0.1:0.1)

RESULT & DISCUSSION OF STABILITY INDICATING ASSAY METHOD

FORCE DEGRADATION

ACID DEGRADATION

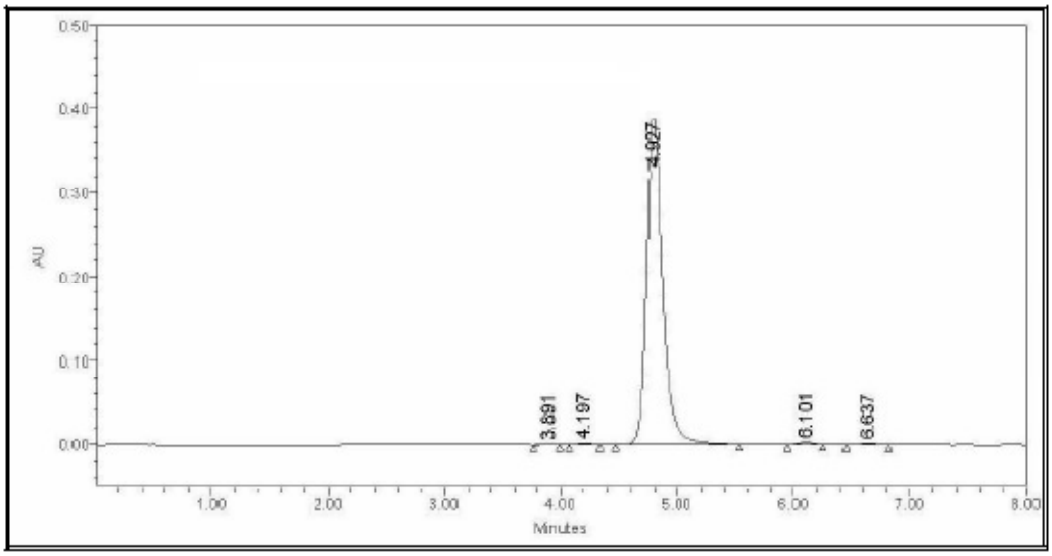

Fig 2: Chromatogram of Acid Degradation of Avanafil Standard

	Rt (min)	Area (mAU*S)	% Area	% Degradation	Resolution
Standard	4.786	3713564			
AVANAFIL	4.927	3610472	97.22		2.87
DP 1	3.891	4782	0.13		
DP 2	4.197	13160	0.72	2.78	1.70
DP 3	6.101	19332	1.03		5.81
DP 4	6.637	13345	0.90		2.20

ALKALI DEGRADATION

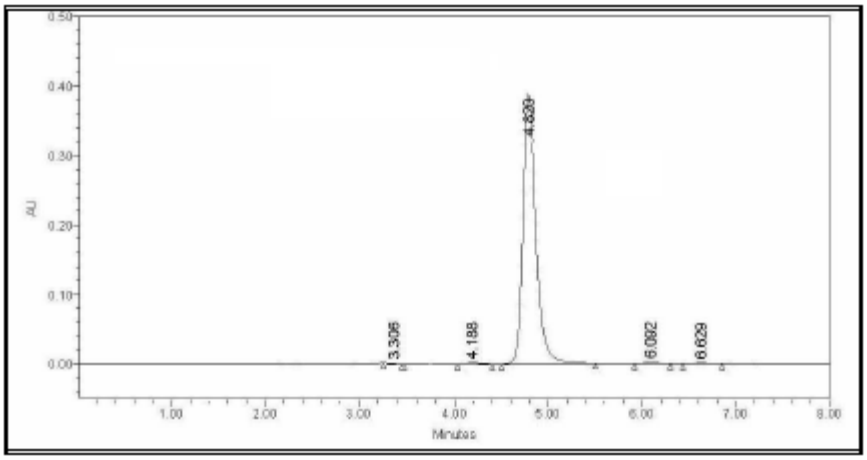

Fig 3: Chromatogram of Alkali Degradation of Avanafil Standard

Table 2: Result of Alkali Degradation

	Rt (min)	Area (mAU*S)	% Area	% Degradation	Resolution
Standard	4.786	3713564	100	4.00	
AVANAFIL	4.820	3565221	96.00		2.78
DP 1	3.306	5562	0.15		
DP 2	4.188	15268	0.81		1.59
DP 3	6.092	21841	2.38		5.62
DP 4	6.629	14310	0.66		2.11

PEROXIDE DEGRADATION

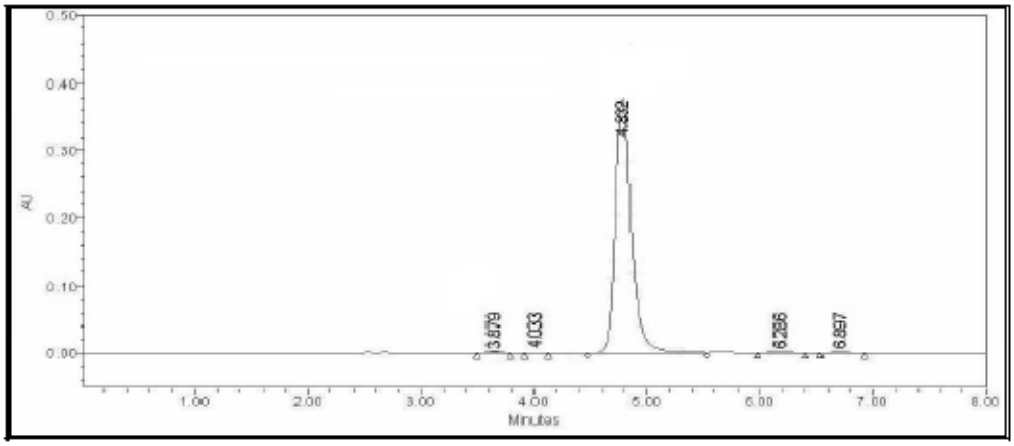

Fig 4: Chromatogram of Peroxide Degradation of Avanafil Standard

Table 3: Result of Peroxide Degradation

	Rt (min)	Area (mAU*S)	% Area	% Degradation	Resolution
Standard	4.786	3713564	100	2.06	
AVANAFIL	4.832	3637271	97.94		2.78
DP 1	3.879	5518	0.11		
DP 2	4.033	15239	0.30		1.58
DP 3	6.286	21921	1.40		6.03
DP 4	6.897	14242	0.25		2.11

□□THERMAL DEGRADATION

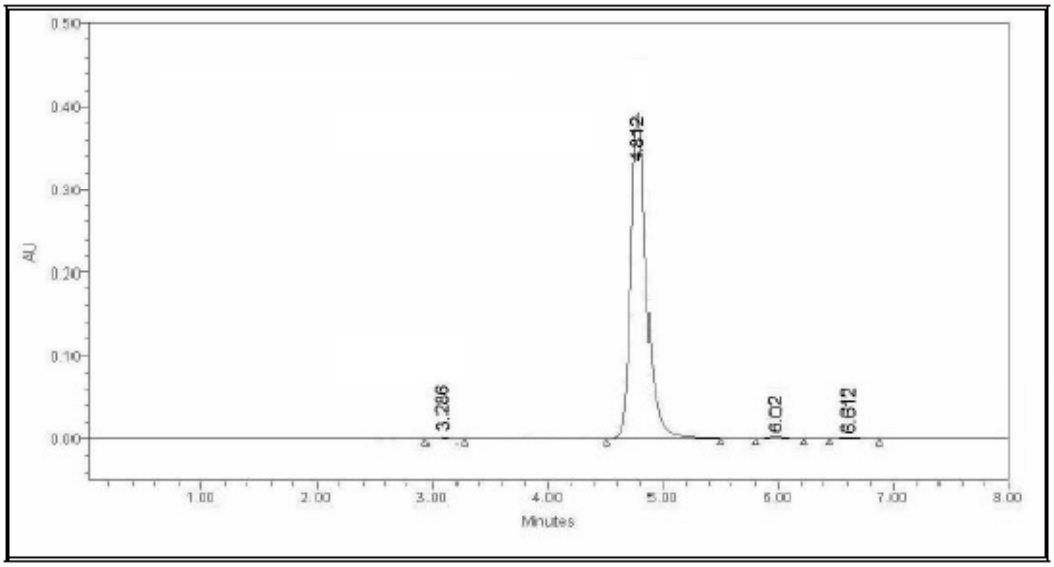

Fig 5: Chromatogram of Thermal Degradation of Avanafil Standard

Table 4: Result of Thermal Degradation

	Rt (min)	Area (mAU*S)	% Area	% Degradation	Resolution
Standard	4.786	3713564	100	1.48	
AVANAFIL	4.812	3661670	98.52		2.80
DP 1	3.286	5939	0.18		
DP 2	6.020	21799	0.85		5.70
DP 3	6.612	14368	0.45		2.10

VALIDATION OF ASSAY METHOD

SPECIFICITY

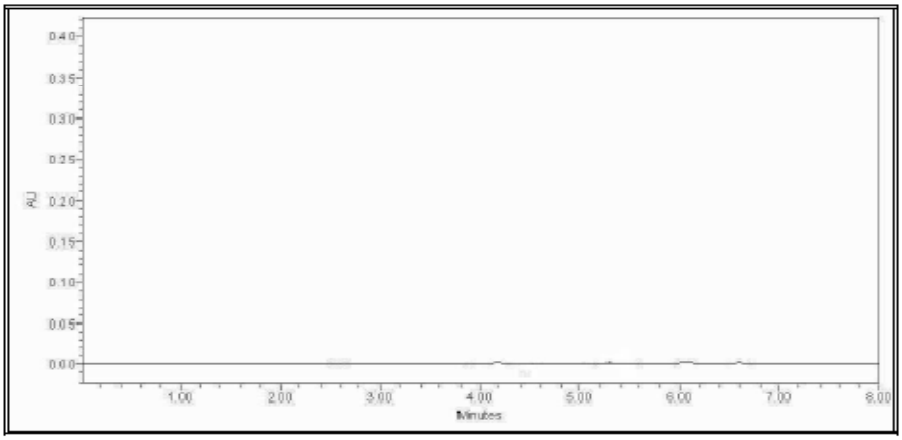

Fig 6: Chromatogram of Diluent

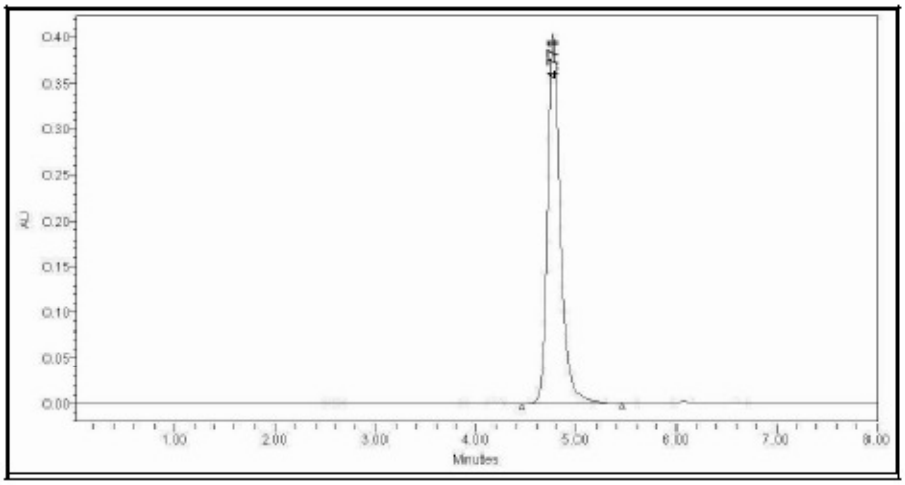

Fig 7: Chromatogram of Sample

Table 5: Result of System Suitability for Assay Method

Injection No.	Standard Response (mAU*S)
1	3713682
2	3721621
3	3718772
4	3701976
5	3711769
Average	3713564
SD	7575.556
% RSD	0.203
Theoretical plate	7163.6
Tailing Factor	1.29

LINEARITY, LOD AND LOQ

Table 6: Result of Linearity, LOD and LOQ for Assay Method

Sr No.	Conc. (ppm)	Mean Area (mAU*S)
1	50	1923193
2	75	2944032
3	100	3704692
4	125	4872290
5	150	5874995
Mean Intercept		-68904
Mean Slop		39327
r^2		0.996
LOD	0.0005 mcg/ml	**LOQ** 0.0016mcg/ml

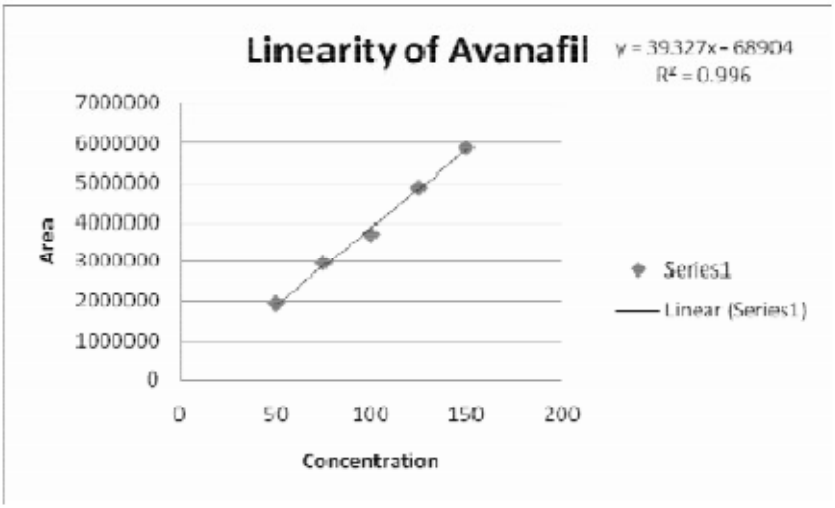

Fig 8: Calibration Curve of Avanafil (50 – 150 ppm) for Assay Method

PRECISION

Method Precision (Repeatability)

Table 7:- Result of Method Precision (Repeatability) for Assay Method

Sr. No.	Peak Area (mAU*S)	Mean Area (mAU*S)	SD	% RSD
1	3714095	3721641.667	29324.98598	0.788
2	3705281			
3	3756807			
4	3758503			
5	3687045			
6	3708119			

Intermediate Precision

Table 8: Result of Intermediate Precision for Assay Method

Sr. No.	Conc. (ppm)	Area (mAU*S)	Mean Area (mAU*S)	SD	% RSD
1	50	1929376	1928634	950.222	0.049
2	50	1927563			
3	50	1928963			
4	100	3738862	3743761.667	6537.641	0.174
5	100	3741238			
6	100	3751185			
7	150	5873897	5875146.333	1481.475	0.025
8	150	5876783			
9	150	5874759			

ACCURACY

Table 9: Result of Accuracy for Assay Method

Spiked level (%)	Conc. in sample (µg/ml)	Conc. Std. added in (µg/ml)	Total conc. (µg/ml)	Conc. Recovered (µg/ml)	% Recovery	SD	% RSD
80	65	52	117	51.00	98.07	0.385	0.391
80	65			50.49	98.79		
80	65			51.06	98.19		
100	65	65	130	64.70	99.53	0.992	0.986
100	65			65.97	101.49		
100	65			65.51	100.78		
120	65	78	143	78.79	101.01	1.210	1.214
120	65			76.99	98.70		
120	65			77.4	99.23		

ROBUSTNESS

Change in Flow rate (± 10%)

Table 10: Result of Change in Flow Rate for Assay Method

Sr. No.	Flow rate 1.2 ml/min (+10%) Area (mAU*S)	Flow rate 0.8 ml/min (-10%) Area (mAU*S)
1	3613172	4806692
2	3618169	4806397
3	3623135	4805781
4	3605207	4805186
5	3628129	4807292
6	3616234	4804567
Mean	3617341	4805986
% RSD	0.219	0.02

Change in Column Temperature (± 2 oC)

Table 11: Result of Change in Column Temperature for Assay Method

Sr. No.	Temperature 27oC (+2%) Area (mAU*S)	Temperature 23oC (-2%) Area (mAU*S)
1	3775425	3758684
2	3789623	3767572
3	3767239	3773237
4	3771397	3761487
5	3782276	3751523
6	3793467	3755573
Mean	3779904.5	3761346
% RSD	0.274	0.211

ASSAY OF AVANAFIL TABLET

Table 12:- Result of Assay of Avanafil Tablet by Assay Method

Sr No.	Area (mAU*S)	% Assay
1	3684477	98.71
2	3643673	97.62
Std. 100 ppm	**3732451**	

developed method could resolve all the peaks, so quantitation of the drugs is possible with developed method in presence of peroxide degraded products

SYSTEM SUITABILITY

Chromatograph the standard solution preparation and record the peak responses. The column efficiency is NLT 2000 theoretical plates and the tailing factor is NMT 2.0 for Avanafil peak. The relative standard deviation for five replicate standard injections is not more than 2.0 %.

REFERENCE:

1. "Avanafil", 2012

http://en.wikipedia.org/wiki/Avanafil

2. Drug Information online Drugs.com, 2012, Avanafil

http://www.drugs.com/search.php?searchterm=avanafil

3. Pharmacokinetics of Avanafil,

http://www.rxlist.com/stendra-drug.htm

4. Mangukiya MA, Patel GF et al. "Development and validation of spectroscopic methods for simultaneous estimation of avanafil and dapoxetine hydrochloride in combined dosage form." Inventi Rapid: Pharm Analysis & Quality Assurance, 2012, 1-5.

5. Sharma N, Rajput P, "RP-HPLC Method Development for Estimation of Sildenafil Citrate in Tablets and in Seminal Fluid." Journal of Applied Pharmaceutical Science, 2012, 172-178

6. Prasanna Reddy B, Amarnadh Reddy K. "Validation and Stability indicating RP- HPLC method for the determination of Tadalafil API in pharmaceutical formulations." Research in Pharmaceutical Biotechnology, 2010, 2(1), 1-6.

7. Kannappan N, Deepthi Y, "Method development and validation of stability indicating methods for assay of Tadalafil and sildenafil citrate by HPLC."