DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF AVANAFIL IN TABLET DOSAGE FORM

Akhilesh S. Tokey*, Gadde Shareena and Sanika S. Chindarkar

Department of Pharmaceutics, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Erandwane, Pune (MH), INDIA 411038.

*Corresponding Author: Akhilesh S. Tokey
Department of Pharmaceutics, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Erandwane, Pune (MH), INDIA 411038.

ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Avanafil in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Avanafil in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, slightly soluble in ethanol and practically insoluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Avanafil was found to be 151-152˚C (uncorrected). It showed absorption maxima 246 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 10μg/ml (PPM). The linearity was observed between 6-14 μg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.85% and for Ruggedness is 0.57%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Avanafil in tablet dosage form. The method could be considered for the determination of Avanafil in quality control laboratories.

KEYWORDS: Avanafil, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Avanafil is in a class of medications called phosphodiesterase (PDE) inhibitors. Avanafil is a phosphodiesterase type 5 (PDE5) inhibitor approved for erectile dysfunction by the FDA. It acts by inhibiting a specific PDE5 enzyme which is found primarily in the corpus cavernosum penis,[1] as well as the retina. It is chemically (S)-4-((3-chloro-methoxybenzyl)amino) 2-(2 (hydroxymethyl) pyrrolidin-1-yl)-N-(pyrimidin-2-ylmethyl) pyrimidine -5-carboxamide.[2] Avanafil is a selective inhibitor of cGMP-specific PDE5. Avanafil occurs as white crystalline powder. Its Molecular formula and molecular weight is $C_{23}H_{26}C_lN_7O_3$ and 483.95 respectively. Avanafil is used to treat erectile dysfunction (ED: impotence; inability to get or keep an erection in men).[3] It works by increasing blood flow to the penis during sexual stimulation. This increased blood flow can cause an erection. It is not known whether avanafil would be harmful if used by a woman during pregnancy or while breast-feeding but also Avanafil should not be taken by a woman or a child. Avanafil does not prevent pregnancy or the spread of sexually transmitted diseases such as human immunodeficiency virus (HIV). Avanafil may differentiate itself from the other phosphodiesterase type 5 inhibitors with its quicker onset and higher specificity for phosphodiesterase type 5 versus other phosphodiesterase subtypes, but may lead to complications of therapy. Approval of AVA by the US Food and Drug Administration was realized in 2012.[4]
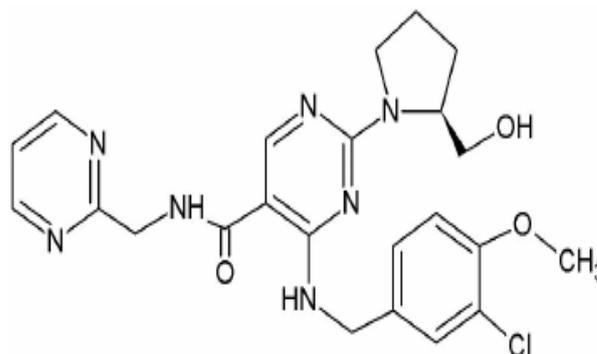

Fig. 1: Chemical Structure of avanafil.

Literature survey revealed that a limited number of HPLC,[5-10] fluorometric[11] methods were reported for the assay of avanafil alone and in combination with other drugs. HPTLC[12-14] method is also reported for avanafil alone and combine dosage form. Screening of the literature revealed there are only one LC-MS/MS[15] identified the degradation products of avanafil. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Avanafil in tablet dosage form.

MATERIALS AND METHODS
- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Avanafil was obtained as a gift sample. Avanafil tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development
A. Determination of λ max (10 PPM)[16,17]

50 mg weighed amount of Avanafil was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.
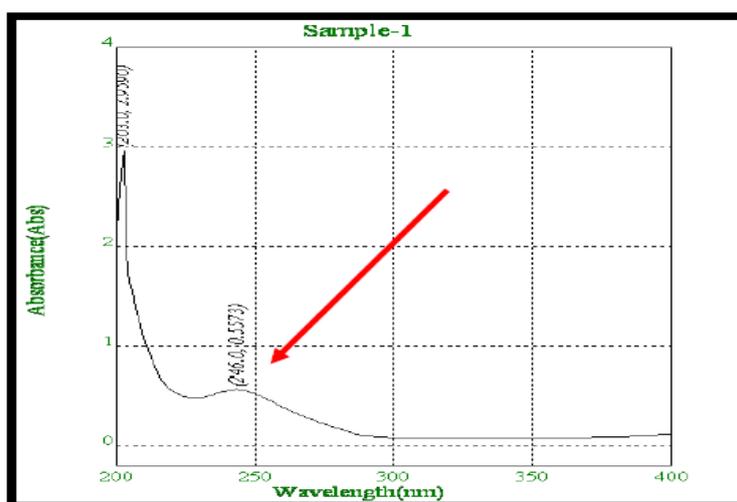

Fig. 2: Calibration curve.

B. Preparation of working concentration
Preparation of standard stock solution

Standard stock was prepared by dissolving 100 mg of Avanafil in 100 ml of analytical grade methanol to get concentration of 1000 µg/ml (PPM).

Preparation of standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade methanol to get concentration of 10 µg/ml (PPM).

C. Procedure for UV reading
Blank solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[18,19,20]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Avanafil was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 246 nm. The absorbance was recorded:

Table 1: Absorbance of dosage form.

Sunrise Remedies Pvt. Ltd. (100 mg)		
Sr. no.	Sample	Absorbance
1	Blank	0.0000
2	Standard	0.5274
3	Sample	0.5241

Table 2: Dosage form specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	AVANA – 100 Sunrise Remedied Pvt LTD (100mg)	05/2021	04/2023	A55121	0.2801	99.37

E. Method of validation[18,20,21]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 6 - 14 PPM at 246 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	6 PPM	0.3254
2	8 PPM	0.4188
3	10 PPM	0.5204
4	12 PPM	0.6121
5	14 PPM	0.7245
Correlation coefficient		0.9989 ~ 0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade methanol and measuring the absorbance at 246nm.

Table 4: Accuracy studies.

Spectrophotometric method			
Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5304
2	Sample Solution 2	0.5254
3	Sample Solution 3	0.5311
4	Sample Solution 4	0.5316
5	Sample Solution 5	0.5212
MEAN		0.5279
SD		0.0045
% RSD		0.8540

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for ruggedness studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5219	0.5223	99.03	0.5689
		0.5226			
2	Analyst 2	0.5245	0.5265	99.83	
		0.5284			

RESULTS
1. Solubility of avanafil
Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Analytical grade Methanol	Freely Soluble
2	Ethanol	Slightly soluble
3	Water	Practically insoluble

2. Melting point of avanafil
The melting point of Avanafil was found to be 151-152˚C (uncorrected).

3. Results for linearity for assay method of avanafil
The linearity of method was determined at concentration level ranging from 6 to 14 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9989 ~ 0.999**
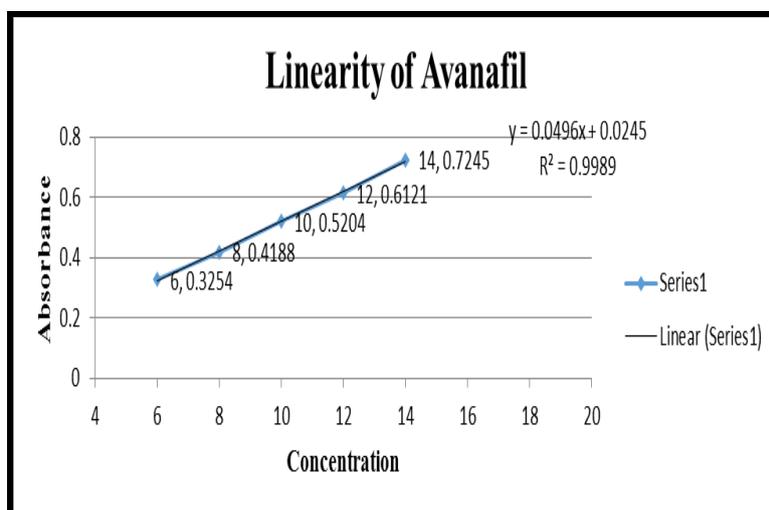
Fig. 3: Avanafil standard curve.

4. Results for accuracy for assay method of avanafil
The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of avanafil
The % RSD for different sample of precision was found to be 0.8540 ~ 0.85 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of avanafil
The %RSD for different sample of ruggedness was found to be 0.5689 ~ 0.57 and it is within acceptance criteria represented in Table - 6.

CONCLUSION
A method for the estimation of Avanafil in tablet form has been developed. From the spectrum of Avanafil, it was found that the maximum absorbance was 246 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 6-14 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Avanafil in solid dosage forms. Hence, the method could be considered for the determination of Avanafil in quality control laboratories.

ABBREVARTIONS
1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet

5. MS – Mass Spectroscopy
6. LC - Liquid Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml - Microgram per milliliter
15. Avg - Average
16. Wt - Weight
17. g - gm
18. PDE - Phosphodiesterase
19. ED - Erectile Dysfunction
20. HIV - Human Immunodeficiency Virus
21. AVA - Avanafil

REFERENCES

1. *en.wikepedia.org/wiki/Avanafil* accessed on 15-01-2022.
2. *https://go.drugbank.com/drugs/DB06237* accessed on 15-01-20122.
3. Burke, R.M.; Evans, J.D. Avanafil for treatment of erectile dysfunction: Review of its potential. Vasc. Health Risk Manag, 2012; 8: 517-523.
4. Kedia, G.T.; Uckert, S.; Assadi-Pour, F.; Kuczyk, M.A.; Albrecht, K. Avanafil for the treatment of erectile dysfunction: Initial data and clinical key properties. Ther. Adv. Urol, 2013; 5: 35-41.
5. Kashyap R, Srinivasa U, Badodaria K. Development and validation of RP-HPLC method for simultaneous estimation of avanafil and dapoxetine HCL in tablet dosage form. WJPPS, 2014; 3(7): 1697-1719.
6. Bhumik B, Kashyap R, Buddhadev S. RP-HPLC Stability indicating analytical method development and validation for estimation of avanafil in pharmaceutical dosage form. Int. J. Pharm. Drug Anal, 2015; 3(6): 181-194.
7. Hegazy M, Kessiba A, Abdelkawy M, El- Gindy AE. RP-HPLC with time programmed fluorescence detection for quantitation of avanafil and dapoxetine hydrochloride: Application to pharmaceutical dosage form and biological fluid. J Liq. Chromatogr. Relat. Technol, 2015; 38(18): 1660-1665.
8. Kumar N, Sangeetha D, Kalyanraman L, Sainath K. Stability-indicating HPLC method for simultaneous determination of degradation products and process-related impurities of avanafil in avanafil tablets. Acta Chromatogr, 2018; 30(3): 158-163.
9. Tol T, Kadam N, Raotole N, Desai A, Samanta G. A simultaneous determination of related substances by high performance liquid chromatography in a drug product using quality by design approach. J Chromatogr A, 2016; 1432: 26-38.
10. Schmidt AH, Molnár I. Using an innovative quality-by-design approach for the development of a stability-indicating UHPLC method for ebastine in the API and pharmaceutical formulations. J Pharm Biomed Anal, 2013; 78: 65-74.
11. Tailor PM, Patel VV, Mishra AD, Shah SA, Shah DR. Spectrofluorimetric method for the estimation of avanafil in bulk and their tablet dosage form. RRJODFDP, 2016; 3(3): 1-5.
12. Soliman MM, Darwish MK, Abdel-Razeq SAM. Validated stability indicating HPTLC, UHPLC and UV-spectrophotometric techniques for the determination of avanafil in presence of its oxidative degradation. Asian Journal of Applied Chemistry Research, 2019; 3(1): 1-14.
13. Chaudhari HH, Sen DJ, Patel CN. Development and validation of HPTLC method for simultaneous estimation of avanafil and dapoxetine HCL in tablet dosage form. WJPPS, 2015; 4(5): 1566-1575.
14. Shah DA, Vegad KL, Patel ED, Prajapati HK, Patel RN, Patel YK. Analytical method validation for estimation of avanafil and dapoxetine hydrochloride tablet dosage form by HPTLC method. Pharm. Biol. Eval, 2017; 4(3): 171-179.
15. Can NO. Development of validated and stability-indicating LC-DAD and LC-MS/MS methods for determination of avanafil in pharmaceutical preparations and identification of a novel degradation product by LCMS-IT-TOF. Molecules, 2018; 23(7): 1771.
16. Jung, J.; Choi, S.; Cho, S.H.; Ghim, J.L.; Hwang, A.; Kim, U.; Kim, B.S.; Koguchi, A.; Miyoshi, S.; Okabe, H.; et al. Tolerability and pharmacokinetics of avanafil, a phosphodiesterase type 5 inhibitor: A single- and multiple-dose, double-blind, randomized, placebo-controlled, dose-escalation study in healthy Korean male volunteers. Clin. Ther, 2010; 32: 1178-1187.
17. ICH draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, IFPMA, Switzerland, 1995; 60: 1246.
18. Beckeet.A.H, Stenlak. J. B, "Practical pharmaceutical chemistry edn CBS Publisher & Distribution, New Delhi, 2004; 4: 275-337.
19. United States Pharmacopoeia. In Validation of Compendial Methods. Pharmacopoeial Convention Inc., Rockville, 2003; 26: 2439-2442.
20. Indian Pharmacopoeia Ministry of Health and Family Welfare Government of India: Published by Indian Pharmacopoeia Commission, Ghaziabad, 2007; 2: 692- 693.