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Research Article

Stability indicating HPLC method for analysis of Avanafil using diode array detector.

Usama A. Fahmy* and Bader M. Aljaeid.

Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University,

Jeddah, Saudi Arabia.

ABSTRACT

The aim of this work is determinationof avanafil which a new FDA approved drug. By using a fast with wide concentrations range, accurate, sensitive and reproducible method. A developedreversed phase high performance liquid chromatographic (HPLC) technique for the quantitation of Avanafil. The drug was eluted from ThermoRP-C18 column. The mobile phase was composed of 0.1MAmmonium acetate buffer PH 2.5, methanol and acetonitrile with ratios (20:40:40) respectively. The flow rate was 1.2 mL/ minDAD- detector, set at 230 nm, was used to determine it. Results revealed that time needed for analysis required no longer than 3 min. The limit of quantitation was 1mcg /mL. Linearity range was between 10- 1000 mcg/ml. Recovery (%) for different concentrations ranged from 99.01% to 100.91%. This method can be summarized with fast technique, accurate for determination of low concentration of avanafil,many advantages like low-cost, rapid and its high specificity to avanafil. .

Keywords: 5-PDE-5 inhibitors; impotence; erectile dysfunction, DAD.

INTRODUCTION

Avanavil (AVA) is a second generation PDE-5 inhibitor for erectile dysfunction treatment[1].which recently approved by the international pharmaceutical regulating agencies, United States Foodand Drug Administration (U.S. FDA) and The EuropeanMedicines Agency (EMA)[2].AVAinhibits phosphodiesterase type-5 that increase cGMP in the penile vasculature so, mediate the antiproliferative and vasodilating effects of endogenous nitric oxide which excreated from endothelial cells that line the artries leading to maintain strong erection[3].AVA was developed for its highselectivity for the PDE5 isoenzyme relative to other PDE5inhibitors. AVA shows fast onset of action in the range of 30–45 min [4].AVA is a water-insoluble, soluble in organicsolvents like ethanol, methanol freely soluble in 0.1 N Hcl. Some published methods were practically tried but most of them were narrow linearity range[5], or with bad suitability system. However, some of these methods are time-consuming, while others are expensive and need special training for some techniques. The aim of this study is the assay method of AVA using reversed phase HPLC is suitable for its intended use.

MATERIALS

Avanafil was purchased from Jinlan Pharm-Drugs Technology Co., Ltd. (Hangzhou, China). Ammonium acetate (Fluka, Buchs, Germany). Glacial acetic acid,Acetonitrile and Methanol HPLC grade were purchased from (Sigma-Aldrich, Darmstadt, Germany).

Method Validation

This manuscript describes the validation of a reversed phase high performance liquid chromatographicmethod for the assay of .AVA by using (Agilient 1200 equipment, Agilent Technologies, CA, USA) equipped with diode array detector (DAD) and set at 230 nm, separation carried out by using Thermo RP-C18 (250 mm x 4.6 mm, 4.5µm particle size) column was used, 20µL injection

volume and 1.2 ml/min flow rate. During method development different mobile phases were tried, methanol, Acetonitrile, water and potassium di hydrogen phosphate buffer with different PHs, also, different wave lengths 254,230,210,254 nm were tried up on AVA UV- Spectrum maximum absorbance as in Fig. 1, at the end mobile phase which composed of 0.1 M Ammonium Acetate buffer, PH was adjusted by glacial acetic acid to 2.5, methanol and acetonitrile with ratios (20:40:40).
The selection of this HPLC condition was based on maximum absorbance and best system suitability criteria. All validation procedure was carried out under ICH guidelines.

Validation procedure

Linearity, Limit of detection (LOD) and limit of quantitation (LOQ):

Linearity is a relation between concentrations and peak area of the responses.the correlation coefficient, which should be found to be ≥ 0.99, Linearity is performed by preparing 6 different concentrations (1 mg / ml, 0.8 mg / ml, 0.4 mg / ml, 0.2 mg / ml, 0.1 mg / ml,0.01 mg / ml) of AVA and inject in HPLC 3 replicates of each concentration.Accuracy (%) was determined by the calculating the relative percentage between the mean measured concentrations and the spiked concentrations. The LOQ and LOD of the assay were determined at a noise to signal ratios of 1:10 and 1:3, respectively[6].

Selectivity / specificity: performing injctions to solvents and blank buffer to provide there is No interference between AVA peak and other solvents or buffer used. The method to be selective and stability indicating, the peak of AVA should be well resoluted from any other peak that may appear due to solvents used.

Degradation studies

From the prepared working standard of standard AVA and sample, 5 mL of aliquots were diluted separately up to 5mLwith5% H_2O_2 (v/v), distilled water, 0.1M HCl, and 0.1 M NaOH,Solutions wereheated at 70°C for 12 h. For oxidative degradation, drugs were storedat room temperature (RT) in 5% H2O2 (v/v) for 72 h[7].

Accuracy: Accuracy was determined by the calculating the relative percentage between the mean measured concentrations and the spiked concentrations. %RSD of the total number of replicates that have been made in this item should be≤ 3% .Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay time, different assay temperature, different days, etc. Day to Day: 3 replicates of a single sample of powder material or product (100% conc) are used for each determination. First day: 6 replicates, then on a second day: 3 replicates of freshly prepared test from the same sample are analyzed. The same analyst performs both analysis:

Precision: System precision was performed by performing 6 or more replicate injections of same solution sample, solution was prepared within ± 10 % of the working concentration, which is found to be about 0.1 mg/ml of AVA.The sample was prepared by dissolving 10 mg of AVA Working Standard in 10 ml of 0.1N HCL then dilute to 100ml with mobile phase. RSD of the 6 replicates should be ≤ 1%[8].

RESULTS

Economic, fast, specific and reproducible HPLC method has been developed and validated for quantitative determination of AVA. The representative chromatograms of AVA standard concentration are shown (Fig.2,3), the retention time of AVA 4.3min.

For system suitability for concentration 1µg/ml the USP acceptance criterion was less than 2 % relative standard deviation (RSD) for peak area, >2000 column plates, peak width < 0.25 minutes, a resolution factor > 3 and USP Tailing factor < 1.25. These results were used to evaluate critical operating parameters on the chromatographic system, assuring system acceptability each time the method was used.

Linearity, limit of detection (LOD) and limit of quantitation (LOQ).

Peak area of AVA was measured. A representative calibration graph of peak area versus concentration in the range of 0.5 µg/ml-100 µg/ml as shown in Fig. 3, resulted in regression equation of the calibration curve was calculated as y = 5.4333x - 21.329 (correlation coefficient, R^2 = 0.9994) where y is the peak area of AVA and x is the concentration of AVA as shown in Fig 2. These results demonstrated a good linearity between the peak area ratios versus concentrations. The limit of detection (LOD) and limit of quantitation (LOQ) was 2 ng/ml (S/N≥3) and 10 ng/ml.

Degradation studies

1.**Degradation in alkali:** AVA was found to be highly destructed at alkalinehydrolysis. Around 60% degradation of the drug was observed in 0.1MNaOH at 70°C within 2 h. The degradation peaks appeared at retention time 1.82 and 6.32 min as shown in Fig. 4.

Acidic conditions: AVAwas found torelatively stableto acidhydrolysis in 0.1NHCl at 70°C. Forming degradationproducts showing retention time 6 min as shown in Fig. 4.

Oxidative conditions: AVA was found to be subjected followingexposure to oxidative condition other peak appeared at 6.3 min. (5% H2O2 at RT for 72 h) as shown in Fig. 4.

2. Accuracy and Precision.

Accuracy is determined by reanalysis of samples containing known amounts of the AVA. It was measured using a minimum of four determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended.

In order to determine the intra-day and inter-day accuracy and precision, the concentration of AVA present in five replicates of cocentrations1,50,100,1000µg/ml AVA was estimated by HPLC within a day or on three consecutive days. 90-120% accuracy and coefficient of variation values <10 % except at LOQ (accuracy 90-110% and CV ≤10%) were considered acceptable.

The extraction efficiency for AVAfrom the solvent was accurate, precise, consistent, and reproducible. The results showed an absolute extraction recovery of AVA was more than 90% with the RSD values being less than 2% of the measured value at each concentration as shown in table 1.

System suitability

The system suitability standard solution containing 10ug/mL AVA was prepared by diluting AVA stock solution in mobile phase. System suitability was determined by making six replicate injections of the standard as shown in table 2.

Conclusion

Using mixed mobile phase composed of methanol, acetonitrile and 0.1 M ammonium acetate buffer PH 2.5 is achieving good separation, with wide linearity range, accurate and precise. Moreover, considered effective stability indicating method which separate AVA peak away from its degradation compounds so, it is recommended for measuring AVA in different dosage forms and so on could be used in stability studies.

Table 1
Interday validation parameters of AVA analysis.

Parameters and linearity data					
Standard Curves	**Range ug/ml**	**Calibrators**	**Slope**	**Y-intercept**	**R^2**
Day 1	1-1000	6	5.4333	21.329	0.9994
Day 2	1-1000	6	5.4231	21.432	0.9997
Day 3	1-1000	6	5.4532	21.324	0.9995
Accuracy (%, n=6)					
		1 ug/ml	**50ug/ml**	**100ug/ml**	**1000ug/ml**
Day 1		97.39	98.41	99.66	99.66
Day 2		96.13	98.66	99.15	98.66
Day 3		96.3	97.51	99.87	100.12
Precision (% RSD, n=6)					
		1 ug/ml	**50ug/ml**	**100ug/ml**	**1000ug/ml**
Day 1		1.30	1.34	1.86	0.85
Day 2		1.71	1.51	1.15	1.01
Day 3		1.55	1.93	1.31	1.01

Table 2
System suitability of 1 ug/mL of AVA (n = 6).

Parameters	**Specifications**	**Day 1**	**Day 2**	**Day 3**
Capacity factor (RSD)	**< 2.0**	0.02	0.03	0.01
Area (%RSD)	**≤ 2.0**	1.54	1.91	1.18
Plates (column)	**>2000**	7130	53320	6829
USP tailing	**< 2.0**	1.07	1.05	1.08
Retention time (min)	**< 4.2**	4.46	4.42	4.45

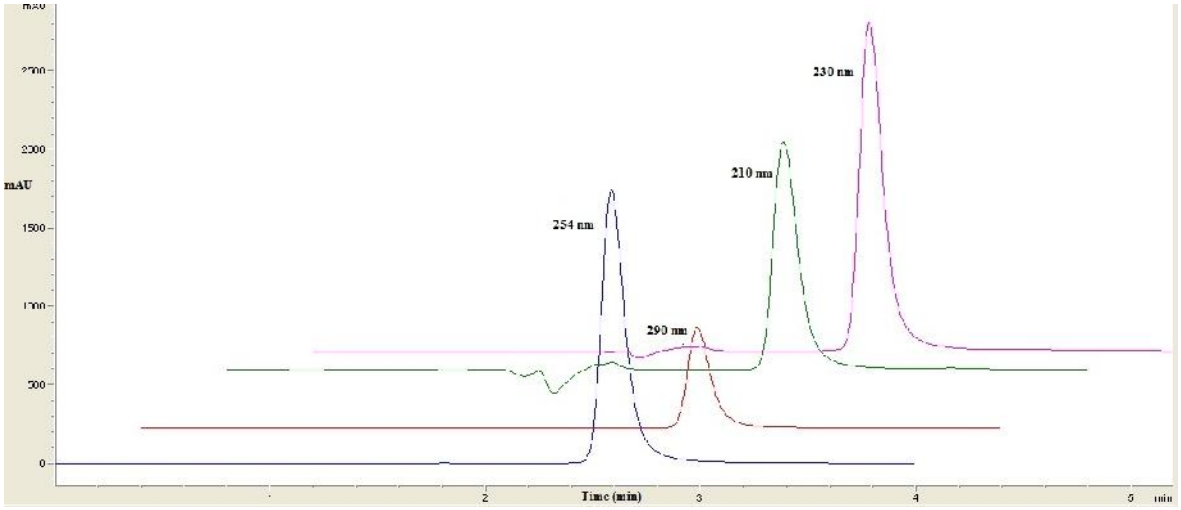

Fig.1
AVA absorbance at different wavelengths.

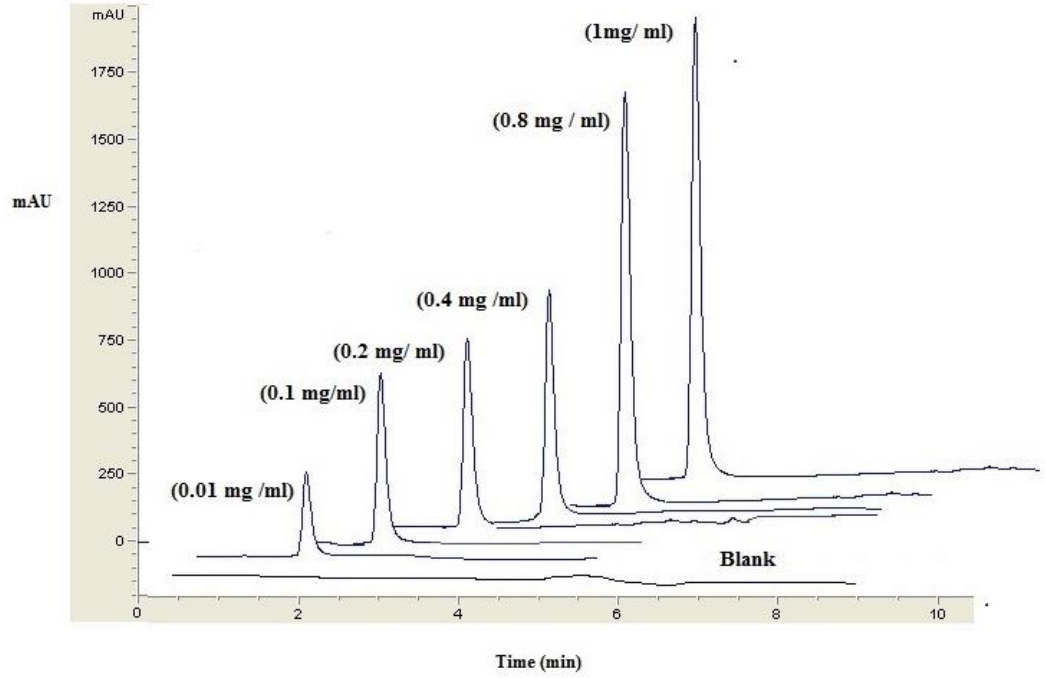

Fig.2
AVA HPLC spectrum at different concentration

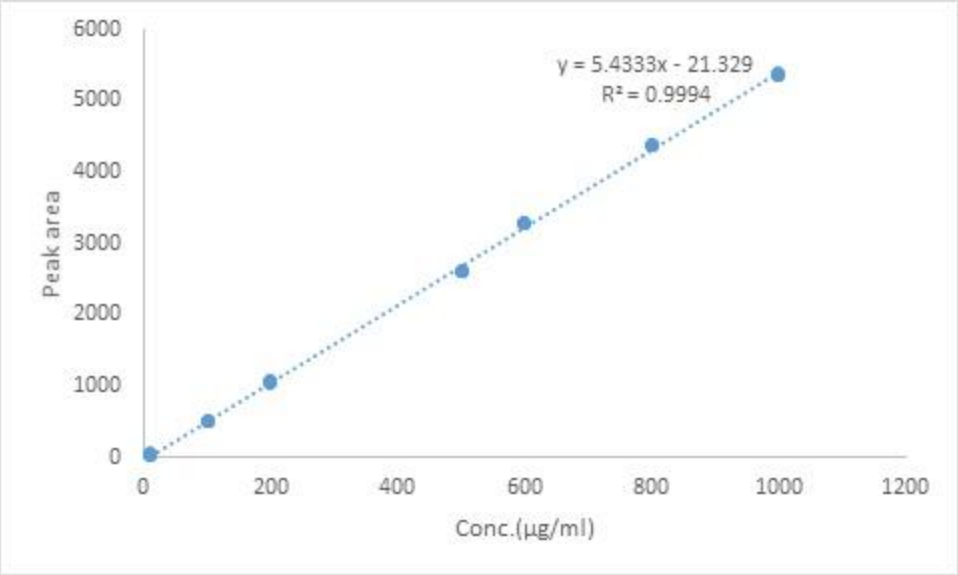

Fig.3
AVA calibration curve.

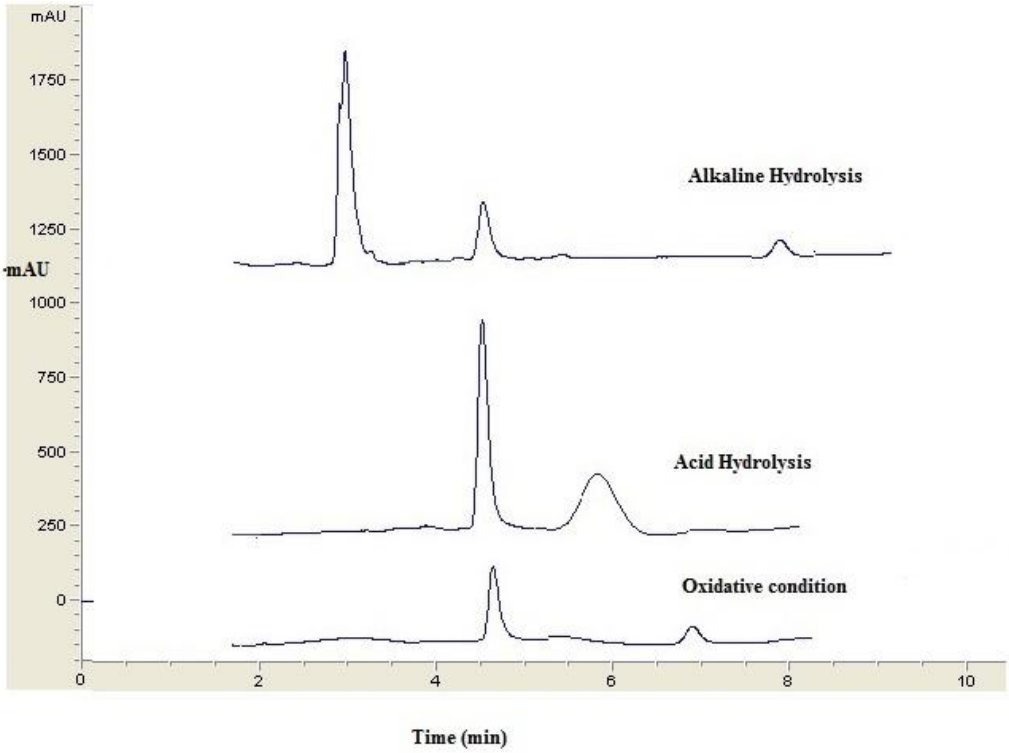

Fig 4
AVA degradation study peaks: Alkaline hydrolysis, acid hydrolysis and oxidative condition.

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