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A validated stability indicating LC method for vardenafil hydrochloride trihydrate and its related impurities

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ABSTRACT

A gradient reversed phase liquid chromatographic method was developed for the quantitative determination of Vardenafil hydrochloride trihydrate process related impurities and intermediates in bulk drugs. The chromatographic separation was achieved on Waters Symmetry shield RP-8, 250 × 4.6mm, 5µm, column. The gradient liquid chromatographic method employs two solutions as mobile phase A and B. The solution A contains a mixture of 0.025 mM of $NaH_2PO_4 H_2O$, 1.0 mL of Tri ethylamine in 1000 mL of water (pH 7.5): Acetonitrile (85:15, *v/v*) and solution B contains a mixture of water: Acetonitrile (22:78, *v/v*). The flow rate was 1.0 $mLmin^{-1}$ and the detection wavelength was 240 nm. The method is found to be precise, linear, accurate and also robust. The LOD and LOQ of impurities were ranged from 0.0024 to 0.0059µgmL^{-1} and 0.088 to 0.26µgmL^{-1}. Vardenafil hydrochloride trihydrate solutions were found to be stable for at least 48 hours. The method was also found to be stability indicating.

KEYWORDS

Vardenafil hydrochloride trihydrate;
HPLC;
Validation;
Specificity;
Solution stability;
Stability indicating.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. Isocratic and gradient reverse-phase high-performance liquid chromatography (HPLC) have evolved as the primary techniques for the analysis of non-volatile active pharmaceutical ingredients and impurities. For HPLC methods used in the pharmaceutical analysis the stability indicating ability is a critical aspect that needs to be addressed in method development and validation. The HPLC detector of choice for many types of methods development is the photodiode array (PDA) detector because it can be used for both quantitative and qualitative analysis. The use of a PDA detector to determine peak purity of the active ingredient in stressed samples greatly facilitates the development of stability-indicating assays.

Vardenafil hydrochloride trihydrate (Piperazine, 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo [5,1-*f*][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethyl-, monohydrochloride trihydrate) is an oral therapy for the treatment of erectile dysfunction. This monohydrochloride salt of vardenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5).

There are several reports in the literature for the quantification of Vardenafil in tablet formulations[1,2]. Liquid chromatography coupled with electrospray ionization mass spectrometry or tandem mass spectrometry methods were used for simultaneous determination of undeclared PDE5 inhibitors Sildenafil, Vardenafil and

Tadalafil in dietary supplements[3-5]. Recently HPLC coupled with electrospray ionization tandem mass sepectrometer (LC-MS/MS) method was developed and validated in 1 ml of human plasma and urine[6]. Another LC-MS/MS method without given full validation details, was also reported to measure plasma levels of Vardenafil and its metabolite in food effect study[7]. Recently, Cheng et al. also reported the method development and validation for the determination of Vardenafil with HPLC using fluorescence detection[8].

In the present case we have reported a simple and effective method for the determination and validation of Vardenafil hydrochloride trihydrate and its related impurities. This method is also capable to detect the metabolite impurities. Herein we discuss stability indicating method for identification of the process impurities and intermediates of API Vardenafil hydrochloride trihydrate synthesized in our group according to the ICH (International Conference on Harmonization) guidelines[9-12].

EXPERIMENTAL

Chemicals

Vardenafil hydrochloride trihydrate and related impurities were received from process research department of Dr. Reddy's laboratories, Hyderabad, India (Figure 1). HPLC grade acetonitrile, Analytical grade ortho phosphoric acid (Merck, Germany) and Analytical grade Triethyl amine (Fluka) were used as received. Water purified by a Millipore system was used for making the solutions.

Chromatographic conditions and equipment

The stationary phase used was a Waters Symmetry shield RP-8, 250 × 4.6mm, 5 μm particles. The solution A contains a mixture of 0.025 mM of NaH_2PO_4 H_2O, 1mL of Tri ethylamine in 1000 mL of water, pH-7.5:Acetonitrile (85:15, v/v) and solution B contains a mixture of Water:Acetonitrile (22:78, *v/v*). The flow rate was 1.0 $mLmin^{-1}$. The HPLC gradient program was set as: time in minutes per % solution B: 0.01/15, 5/15, 37/70, 55/70, 57/15 and 60/15 with a post run time of 5 minutes. The column temperature was maintained at 27^0C and the detection was monitored at a wavelength of 240 nm. The injection volume was 10 μL. A mixture of Water: Acetonitrile (22:78) was used as diluent.

The liquid chromatography systems used for method development and method validation were Waters 515 binary pump, 717 plus auto sampler with a 2996 PDA and Agilent (Wilmington, Delaware, USA) 1100 system equipped with PDA .The output signal was monitored and processed using Waters (Milford, MA, USA) Empower software.

Preparation of solutions

Sample preparation

8.0 mg of the Vardenafil hydrochloride trihydrate test sample was taken in the 100 mL volumetric flask and diluted up to the mark with diluent. 0.1 mL of the solution was further dilutes to 10 mL with the diluent.

Preparation of system suitability solution

8.0 mg of Vardenafil hydrochloride trihydrate standard was taken in a 10mL volumetric flask containing 5mL of diluent, to this 15 μL of VAR-4A and VAR-4iA solution (8.0 mg of each standard in 10mL of volumetric flask containing 5 mL of diluent. The sample was dissolved and diluted upto the mark) was dissolved and diluted up to the mark with diluent.

Method validation

System suitability criteria

System suitability solution was prepared as mentioned above. The system suitability test solution was injected and the chromatographic parameters like resolution between VAR-4A and VAR4iA, the USP Tailing for Vardenafil peak were evaluated for proving the system suitability.

Specificity-forced degradation studies

Forced degradation studies were performed on Vardenafil hydrochloride trihydrate to prove the stability indicating property of the method. The stress conditions employed for degradation study of Vardenafil hydrochloride trihydrate includes light exposure (carried out as per ICH Q1B), heat (60^0C), acid hydrolysis (2 N HCl), base hydrolysis (2 N NaOH), water hydrolysis and oxidation (3% H_2O_2). For heat and light studies, the monitoring period was 10 days whereas for acid, base, water hydrolysis it was 48 hours. Oxidation was carried out for 3hrs. Peak purity of the principal peak in the chromatogram of stressed samples of Vardenafil hydrochloride trihydrate was checked using

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photo diode array detector.

Linearity

Linearity of the response for all impurities was carried out at eight (n=8) concentration levels from LOQ to 200% to the specification limit (0.0012mgmL^{-1}) each with respect to concentration of Vardenafil hydrochloride trihydrate (LOQ, 0.0003,0006 , 0.0009, 0.0012 , 0.0015,0.0018 and 0.0024mgmL^{-1}).

Precision

The precision of the related substance method was checked by injecting six individual preparations of Vardenafil hydrochloride trihydrate spiked with 0.0012mgmL^{-1} each impurity (VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl impurity and VAR-Dimer impurity).

Accuracy

Standard addition and recovery experiments were conducted to determine accuracy of the method for the quantification of impurities in Vardenafil hydrochloride trihydrate sample. The study was carried out in triplicate (n=3) at 0.06, 0.12, 0.15 and 0.18μgmL^{-1} of the analyte concentration (0.8mg mL^{-1}).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl impurity and VAR-Dimer impurity were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration.

Robustness

To determine the robustness of the developed method experimental conditions were purposely altered and the resolution between VAR-4A and VAR-4iA peaks was evaluated. The flow rate of the mobile phase was 1.0 mLmin^{-1}. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mLmin^{-1}. The effect of pH on resolution of impurities was studied by varying ± 0.2 pH units (at 7.3, 7.7 Buffer pH). The effect of column temperature on resolution was studied at 22^{0}C and 32^{0}C instead of 27^{0}C. In all the above-varied conditions, the components of the mobile phase were held constant as stated in section 2.2.

Solution stability and mobile phase stability

Vardenafil hydrochloride trihydrate solutions prepared in diluent were injected at 0 hours, 24 hours and 48 hours of time intervals, calculated the impurity content (VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl and VAR-Dimer impurity) and checked the consistency in the % area of the principal peak at each interval. Mobile phase prepared was kept constant during the study period.

The mobile phase study was demonstrated by injecting the freshly prepared sample solution at different time intervals. (0 hours, 24 hours and 48 hours).

Relative response factor

The relative response factors for VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl and VAR-Dimer impurity relative to the response of Vardenafil was established by injecting different known concentrations (0.08, 0.16, 0.24, 0.32 and 0.40μgmL^{-1}) of the impurities (the potencies of the impurities used for the RRF calculations).

RESULTS AND DISCUSSION

System suitability

The system suitability test solution was injected and the chromatographic parameters like resolution, tailing factor and number of USP theoretical plates were evaluated. The resolution between VAR-4A and VAR-4iA was found to be more than 1.5, the USP Tailing for Vardenafil peak was found to be less than 1.08 and USP theoretical plates for Vardenafil peak was found to be more than 62000.

Specificity-forced degradation studies

Degradation was not observed in Vardenafil hydrochloride trihydrate stressed samples that were subjected to light, heat, base, acid and water hydrolysis. However, the degradation was observed under oxidative conditions that impurity is N-Oxide impurity and confirmed by LCMS/MS (TABLE 1). The peak purity test results derived from DAD confirmed that the Vardenafil peak was pure and homogeneous in all the analyzed stress samples and also peak purity of impurities was checked and found that no other peaks were co-eluted with impurities peaks. This indicates that the method is specific and stability indicating.

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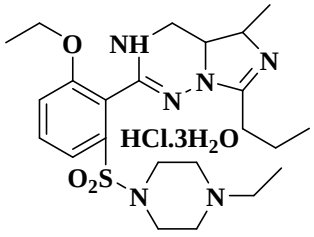
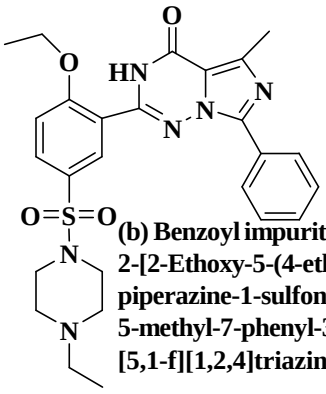
TABLE 1 : Specificity results (Forced degradation studies)

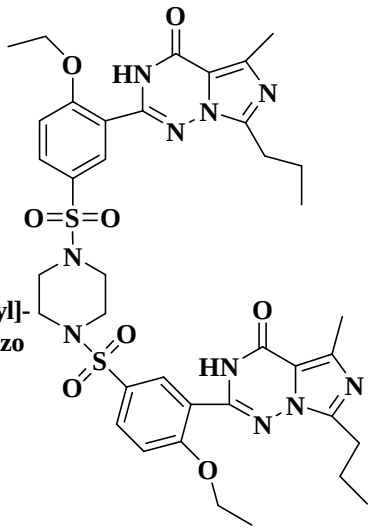
Stressed condition	%VAR-4A	%VAR-4iA	% Benzoyl	% Dimer	%VAR-6A	% Unk-(RRT)	%Deg
RRT	(~0.29)	(~0.31)	(~1.30)	(~1.48)	(~1.20)	-	-
Normal	ND	ND	0.02	0.03	ND	0.06(1.03)	-
Thermal Deg. (60°C for 10days)	ND	ND	0.02	0.03	ND	0.08(1.03)	0.29
Photo Deg.(UV light)	ND	ND	0.02	0.03	0.01	0.18(0.15)	0.48
Acid Hydrolysis(2N HCl)	ND	ND	0.02	0.03	ND	0.07(1.03)	0.32
Oxidation(3% H_2O_2)	ND	ND	0.02	0.04	ND	6.24 (0.32)	6.47
Water hydrolysis	ND	ND	0.02	0.02	ND	0.07(1.04)	0.26
Base hydrolysis(2N NaOH)	ND	ND	0.02	0.03	ND	0.48 (0.17)	0.97



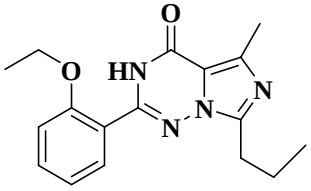
(a) Vardenafil hydrochloride trihydrate. Piperazine, 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo [5,1-*f*][1,2,4] triazin-2-yl)-4-ethoxyphenyl] sulfonyl]-4-ethyl-, monohy drochloride



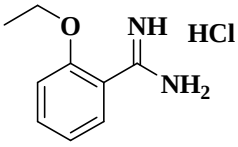
(b) Benzoyl impurity 2-[2-Ethoxy-5-(4-ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-phenyl-3H-imidazo [5,1-f][1,2,4]triazin-4-one.



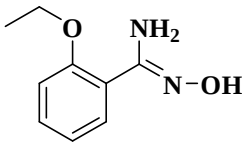
(c) Dimer impurity : Bis 2-[2-Ethoxy-5-(piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one



(d) VAR-6A: 2-(2-Ethoxy-phenyl)-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one



(e) VAR-4A: 2-Ethoxy-benzamidine hydrochloride



2-Ethxoy-N-hydroxybenzamidine

(f) VAR-4(i)A

Figure 1:Chemical structure and name of vardenafil and it impurities (a) Vardenafil (b) VAR-Benzoyl impurity (c)Dimer impurity (d)VAR-6A(e)VAR-4A(f)VAR-4(i)A

Linearity of response

Linear calibration plot of the method was obtained over the calibration ranges tested, i.e. LOQ to 200% for VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl and VAR-Dimer impurity. The correlation coefficient obtained was greater than 0.999 indicating linear response of the impurities.

Precision

The % RSD of % area of impurities VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl impurity and VAR-Dimer impurity was found to be less than 2.2, confirming good precision of the method.

Accuracy

The percentage recovery of VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl impurity and VAR-Dimer impurity ranged from 93 to 107. All the impurities are within the acceptance limit.

LOD and LOQ

The LODofVAR-4A, VAR-4iA, VAR-6A, VARBenzoyl impurity and VAR-Dimer impurity was 0.044,0.059, 0.026, 0.024 and 0.029μgmL^{-1} .The LOQ of VAR-4A,VAR-4iA,VAR-6A,VAR-Benzoyl and VAR-Dimer impurity were 0.16, 0.26, 0.11,0.088 and 0.104 μgmL^{-11}

From the LOD and LOQ analysis infer that the method is highly sensitive towards the impurities present in Vardenafil.

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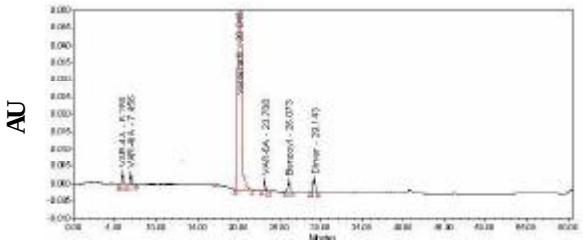

Figure 2 : Blend chromatogram of Vardenafil and it impurities

Robustness

During the robustness study the resolution between VAR-4A and VAR-4iA was greater than 1.5, the USP Tailing for Vardenafil peak was found to be less than 1.15 and USP theoretical plates for Vardenafil peak was found to be more than 51000.

No significant changes were observed in the content of VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl impurity and VAR-Dimer impurity with column Oven temperature, Flow rate of mobile phase and Mobile phase pH variation.

Solution stability and mobile phase stability

Consistency was observed in impurities levels and the principal peak area at each interval. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase were stable up to 48 hours.

Relative response factor

A typical LC chromatogram of Vardenafil hydrochloride trihydrate and its impurities (spiked) is shown in figure 2. Relative response factors of impurities VAR-4A, VAR-4iA, VAR-6A, VAR-Benzoyl and VAR-Dimer are 0.54, 0.41, 1.06, 1.16 and 1.14, respectively. The low relative responses for VAR-4A and VAR-4iA could be due to less conjugation present in their molecular structures compared to other impurities.

CONCLUSION

In this manuscript the simple, accurate and well defined stability indicating gradient LC method for the determination of Vardenafil Hydrochloride Trihydrate in the presence degradation products was described for the first time. This method is validated and it is found to be specific, precise, accurate, linear and rugged for the detection and quantification of related impurities of API. The behavior of Vardenafil Hydrochloride Trihydrate under various stress conditions were studied and presented. The information presented herein could be very useful for quality monitoring of bulk, premix samples, finished dosage forms and as well as employed to check the quality during the stability studies.

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