Asian Journal of Chemistry; Vol. 23, No. 3 (2011), 1219-1222

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Liquid-Chromatography Determination of Impurities in Sildenafil Citrate

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(*Received*: 21 April 2010; *Accepted*: 6 November 2010) AJC-9271

A simple, economic and time-efficient stability-indicating, reversed phase liquid chromatographic (RP-LC) method has been developed for the analysis of sildenafil citrate in the presence of both process related impurities and degradation products generated by decomposition. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in alkali and oxidative stress conditions. The drug was found to be stable to other stress conditions attempted. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.6 %. The method was validated with respect to specificity, linearity, accuracy, precision, robustness, limit of detection and quantification. The method is simple, rapid, selective, accurate and stability indicating, useful in the quality control of bulk manufacturing of sildenafil.

Key Words: Column liquid chromatography, Sildenafil, Stability indicating, Impurities, Validation.

INTRODUCTION

Sildenafil citrate, marketed as Viagra, Revatio and under various other trade names, is a drug used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). It was developed and is being marketed by the pharmaceutical company Pfizer. It acts by inhibiting cGMP specific phosphodiesterase type 5 (PDE 5), an enzyme that regulates blood flow in the penis. Since becoming available in 1998, sildenafil has been the prime treatment for erectile dysfunction. Its primary competitors on the market are tadalafil (cialis) and vardenafil (levitra).

Till now, a few analytical methods have been reported[1-7] for the estimation of sildenafil citrate in dosage form as well as in pure substance include determination of sildenafil citrate in the presence of its oxidative-induced degradation products[1], using monolithic silica HPLC column[2], a micellar electrokinetic chromatographic method[3], a stability indicating reversed phase liquid chromatographic determination of sildenafil citrate in pure form and in pharmaceutical samples[4] another in bulk and formulations[5] are previously published. Determination of sildenafil and desmethyl sildenafil in plasma by HPLC using electro chemical detection[6] is also reported. These methods are limited to quantification of active drug in the respective samples. However, Daraghmeh *et al.*[7] reported a chromatographic method for the determination of sildenafil citrate and related substances in the commercial products and tablet dosage form. This method was developed based on certain related substances whereas the author considered theprecursers are as impurities. Moreover, impurity D is a staring material and impurity A, B, C are the precursors of staring material. In the present study, other related impurities namely SLC-Imp-A (Des-piperazine derivative), SLC-Imp-B (Des-methyl sildenafil, metabolite), along with impurity C and impurity D described in the publication[7] were also considered for the validation. Organic impurities can arise during the manufacturing process and storage of the drug substances and the criteria for their acceptance up to certain limits are based on regulatory guidelines[8] or known safety data. It is, therefore, essential to isolate and characterize process related impurities present in the bulk active pharmaceutical ingredient (API). During the development of an analytical procedure, the liquid-chromatography method was developed for the determination of in-house synthesized sildenafil citrate and the related impurities arising during its manufacturing. In the method, developed, herein, all the process related impurities were well resolved. The method has been thoroughly validated as per the ICH guidelines[9].

EXPERIMENTAL

Reference standard of sildenafil citrate and four impurities namely SLC-Imp-A, SLC-Imp-B, SLC-Imp-C and SLC-Imp-D (Fig. 1) were synthesized and characterized in M/s SMS Pharma Research Centre, Hyderabad, India. The commercial samples of sildenafil citrate are also manufactured by M/s SMS Pharmaceuticals Limited. HPLC grade acetonitrile was obtained from Merck, India. Analytical grade sodium dihydrogen orthophosphate dihydrate, triethylamine was purchased from SD Fine Chem., India. High purity water was prepared by using Milli-Q Elix and then using Milli-Q Academic purification system (Millipore, USA).

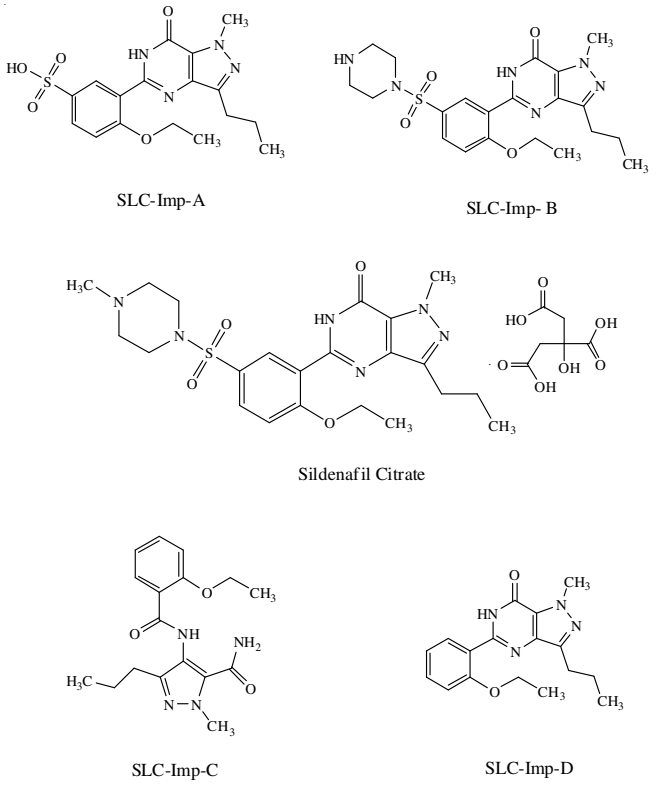

Fig. 1. Chemical structures of sildenafil citrate and impurities

The liquid-chromatography system was equipped with quaternary gradient pump, auto sampler and a column oven connected with a ultra-violet detector (1200 series, Agilent, USA). The output signal was monitored and integrated using EZ Chrom Elite chromatography manager software.

Preparation of standard solutions: A stock solution of sildenafil citrate (5.0 mg/mL) was prepared by dissolving appropriate amount in the diluent. Working solutions of 250 µg/mL was prepared from the above stock solution for both assay and related substances determinations. A stock solution of impurity mixture (SLC-Imp-A, B, C and D) at 0.25 mg/mL was also prepared in diluent. As per the approved in-house protocol, different dilutions were made to evaluate specificity, precision, linearity, LOD, LOQ, accuracy and robustness.

Forced degradation samples for specificity study: For acid degradation, sildenafil citrate sample was refluxed with 5 N HCl at 80 ºC for 5 h and the solution was further diluted to required concentration with the diluent. For basic degradation, sildenafil citrate sample was refluxed with 5 N NaOH at 80 ºC for 5 h and the solution was further diluted to required concentration with diluent. For oxidative degradation, sildenafil citrate sample was refluxed with 5 % H_2O_2 at 80 ºC for 3 h and then diluted to required concentration with diluent. For photo and thermal degradations, sildenafil citrate sample was exposed to ultraviolet light (254 nm) for 24 h and another sample was kept at 105 ºC temperature for 24 h, respectively. These samples solutions were prepared to required concentration with diluent.

Chromatographic conditions: A Waters XTerra, RP-18 analytical column (250 mm × 4.6 mm, 5 µm) was used. The photodiode array detector was set to a wavelength of 230 nm for the detection. A mixture of aqueous 0.05 M sodium dihydrogen phosphate dihydrate (pH of the buffer adjusted to 5.6 ± 0.1 with triethyl amine) and acetonitrile in the ratio of 70:30, (v/v) was used as mobile phase-A and a mixture of aqueous 0.05 M sodium dihydrogen phosphate dihydrate (pH of the buffer adjusted to 5.6 ± 0.1 with triethyl amine) and acetonitrile in the ratio of 40:60, (v/v) was used as mobile phase-B. A gradient program is used for the separation of the impurities. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20 µL. It was filtered through a 0.45 µm nylon membrane filter prior to use.

Validation of the method

Specificity: The specificity of the developed liquid-chromatography method for sildenafil citrate was carried out in the presence of its impurities namely SLC-Imp-A, SLC-Imp-B, SLC-Imp-C and SLC-Imp-D. Stress studies were performed for sildenafil citrate bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of photolytic (UV light at 254 nm), heat (at 105 ºC), acid (5 N HCl at 80 ºC), alkali (5 N NaOH at 80 ºC) and oxidation (5 % H_2O_2 at 80 ºC) to evaluate the ability of the proposed method to separate sildenafil from its degradation products. For thermal and photolytic studies, the study period was 24 h, for the acid, alkali and oxidation the study period was 5 h. Peak purity test was carried out for the sildenafil peak by using a PDA detector in stress samples. Assay studies were carried out for stress samples against a qualified sildenafil citrate reference standard.

Precision: The precision of the assay method was evaluated by carrying out six independent assays of sildenafil citrate test samples against a qualified reference standard and calculated the percentage RSD of the assay. The precision of the related substances method was checked by injecting six individual spiked test preparations of sildenafil citrate (0.25 mg/mL) spiked with 0.10 % of each impurity with respect to sildenafil citrate analyte concentration. The percentage RSD of the area responses of each impurity was calculated. Similarly, the intermediate precision of the method was demonstrated by different analyst on different day using different instrument in the same laboratory.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ concentrations were determined by measuring the magnitude of analytical background. The LOD and LOQ concentrations were calculated from the linearity

data using residual standard deviation of the response and slope of the calibration curve for each impurity. The LOD and LOQ values for all four impurities were determined by injecting a series of dilute solutions with known concentrations.

Linearity: Linearity solutions for the assay method were prepared from sildenafil citrate stock solution at six concentration levels from 80-120 % of the assay analyte concentration (200, 225, 250, 275 and 300 µg/mL). The peak area *versus* concentration data was treated by least-squares linear regression analysis. Linearity solutions for the related substances method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared from LOQ to 150 % of specification level.

Accuracy: The accuracy study of impurities was carried out in triplicate at three concentration levels *i.e.*, 0.05, 0.10 and 0.15 % of the sildenafil citrate analyte concentration (250 µg/mL). The percentagte mean recoveries for all the four impurities were calculated.

Robustness: To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between sildenafil and all four impurities were recorded. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, flow was changed by ± 0.1 units as 0.9 and 1.1 mL/min. The pH of the buffer was 5.6. To study the effect of pH of the buffer on the resolution, buffer pH was changed by ± 0.2 units as 5.4 and 5.8.

Solution stability: The solution stability of sildenafil citrate in the assay method was carried out by leaving both solutions of sample and reference standard in tightly capped volumetric flask at room temperature for 24 h. The same solutions were assayed for 6 h intervals up to the study period. The percentage RSD for the assay of sildenafil citrate was calculated during solution stability experiments. The solution stability of sildenafil citrate and its impurities in the related substances method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 24 h. The content of each impurity was determined for every 6 h interval up to the study period.

RESULTS AND DISCUSSION

Method development: The main objective of the chromatographic method is to separate sildenafil citrate from its related impurities. Different mobile phases and columns were employed to achieve the best separation and resolution. Finally, a mixture of aqueous 0.05 M sodium dihydrogen phosphate dihydrate (pH of the buffer adjusted to 5.6 ± 0.1 with triethyl amine) and acetonitrile in the ratio of 70:30, (v/v) was used as mobile phase-A and a mixture of aqueous 0.05 M sodium dihydrogen phosphate dihydrate (pH of the buffer adjusted to 5.6 ± 0.1 with triethyl amine) and acetonitrile in the ratio of 40:60, (v/v) was used as mobile phase-B. It was filtered through a 0.45 µm nylon membrane filter prior to use. Gradient program is (time/% B) 0/0, 30/100, 35/100, 40/0, 45/0 with a flow rate of 1.0 mL/min using Waters XTerra RP-18, 250 mm × 4.6 mm, 5 µm column was found to be appropriate, allowing good separation and symmetrical peaks. Sildenafil citrate and its process related impurities show significant UV absorbance at wavelength 230 nm. Hence this wavelength has been chosen for detection in the analysis. The peak shape of sildenafil was found to be symmetrical. In optimized chromatographic conditions sildenafil, SLC-Imp-A, SLC-Imp-B, SLC-Imp-C and SLC-Imp-D were separated with a resolution greater than 2, typical retention times were about 13.07, 4.18, 7.18, 11.08 and 24.48 min, respectively. System suitability results are shown in Table-1.

TABLE-1
SYSTEM SUITABILITY REPORT

Name	RT	RRT	Resolution	Tailing factor	Relative response factor
SLC-Imp-A	4.18	0.32	0.0	1.1	1.3
SLC-Imp-B	7.19	0.55	14.1	1.0	1.3
SLC-Imp-C	11.08	0.85	16.4	1.0	1.0
SLC-Imp-D	24.48	1.88	36.7	1.0	1.3
Sildenafil	13.07	1.00	7.3	1.0	–

Specificity: No degradation was observed in sildenafil citrate samples when subjected to stress conditions like heat, photolytic and acid conditions. Sildenafil citrate was degraded under alkali and oxidative conditions. Peak purity test results obtained by using a PDA detector confirmed that the sildenafil peak is homogenous and pure in all the analyzed stress samples. The assay of sildenafil citrate is unaffected in the presence of all the four impurities and its degradation products confirm the stability indicating power of the method. The summary of forced degradation studies is given in Table-2. The LC chromatograms of bulk sample and the bulk sample spiked with 0.10 % of all four impurities are shown in Fig. 2.

TABLE-2
SUMMARY OF FORCED DEGRADATION RESULTS

Stress condition	Time (h)	Assay of active substance (%)	Total impurities (%)	Mass balance (%) (% assay + % impurities)
Real sample	Initial	99.6	0.02	99.62
Thermal (105 ºC)	24	99.5	0.06	99.56
Photolytic (UV at 254 nm)	24	99.6	0.06	99.66
Acid hydrolysis (5 N HCl) at 80 ºC	5	98.8	0.04	98.84
Alkali hydrolysis (5 N NaOH) at 80 ºC	5	90.2	9.75	99.95
Oxidation (5 % H_2O_2) at 80 ºC	3	71.8	27.37	99.17

Precision: The percentage RSD of the assay of sildenafil citrate during the precision study was within 0.07 % and the percentage RSD for the area responses of each impurity in the related substances during the precision study was within ± 4.0 %. The overall percentage RSD of the assay results obtained in the intermediate precision study was within ± 0.09 % and the overall percentage RSD for the area responses of each impurity was well within ± 1.2 %, conforming good precision of the method. Results are shown in Table-3.

Limit of detection and limit of quantification: The limit of detection of a compound is defined as the lowest concentration that can be detected. The limit of quantification is the lowest concentration of a compound that can be quantified

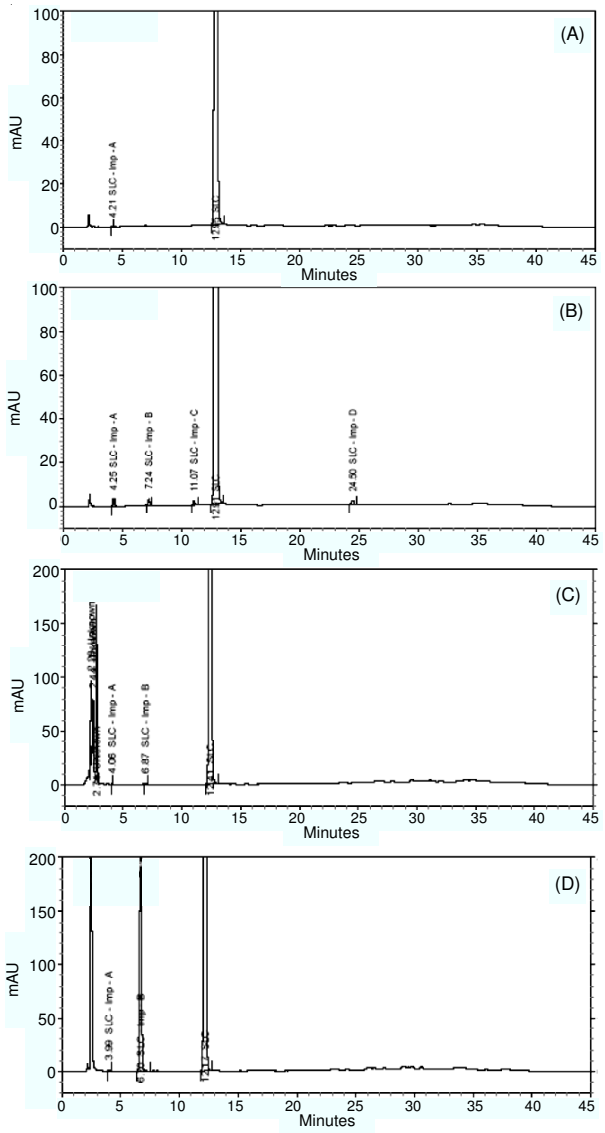

Fig. 2. Chromatograms obtained from (A) sildenafil citrate bulk sample, (B) bulk sample spiked with all the impurities (0.10 %), (C) and (D) samples obtained from stress studies

TABLE-3
METHOD VALIDATION DATA

Parameter	SLC-Imp-A	SLC-Imp-B	SLC-Imp-C	SLC-Imp-D
Linearity (0.005-0.10 %)				
Correlation coefficient	0.9999	0.9999	0.9999	1.0000
Slope	70758	71252	55072	70829
Y-Intercept	-120	-434.9	-56.8	296.4
Limit of detection and quantitation (%)				
LOD	0.0020	0.0024	0.0025	0.0018
LOQ	0.0067	0.0080	0.0082	0.0059
Accuracy (recovery %)				
0.050 (n = 3)	93.7	96.7	97.9	100.3
0.100 (n = 3)	94.1	96.9	107.0	101.2
0.150 (n = 3)	94.8	95.1	97.3	97.9
Precision (RSD %)				
Intra-day (n = 6)	0.0	0.0	4.0	0.0
Intermediate precision (RSD %)				
Inter-analyst (n = 6)	0.0	0.0	4.0	0.0
Overall (n = 12)	4.7	0.0	5.1	0.0
Inter-day (n = 6)	0.0	4.0	0.0	0.0
Overall (n = 12)	0.0	2.9	2.9	0.0

with acceptable precision and accuracy. A typical S/N ratio of 2-3 and 9-10 are generally considered to be acceptable for LOD and LOQ, respectively. The precision at the LOQ concentrations for all the four impurities were below 6.5 %. LOD and LOQ values for all the four impurities are shown in Table-3.

Linearity: The linearity calibration plot for the assay method was obtained over the calibration ranges tested, *i.e.*, 200-300 µg/mL and correlation coefficient was 0.9999.

Linear calibration plot for the related substances method was obtained over the calibration ranges tested, *i.e.*, LOQ to 0.15 % for each impurity. The correlation coefficients obtained were greater than 0.9999. The result shows that an excellent correlation existed between the peak area and concentration in both the methods. Results are shown in Table-3.

Accuracy: The percentage mean recovery (n = 3) of the impurities SLC-Imp-A, SLC-Imp-B, SLC-Imp-C and SLC-Imp-D in sildenafil citrate sample varied from 94-95, 95-97, 97-107 and 98-101 %, respectively. Results are shown in Table-3.

Robustness: In all the deliberate varied chromatographic conditions (flow rate and pH of buffer), the resolution between the critical pairs, *i.e.*, SLC-Imp-C and sildenafil was greater than 3.1, illustrating the robustness of the method.

Solution stability: The percentage RSD (n = 6) of the assay of sildenafil citrate during solution stability experiments are less than 0.8 %. No significant changes were observed in the content of the four impurities during solution stability experiments. The solution stability experiment data confirms that the sample solutions used during assay and the related substances determination were stable for at least 24 h.

Conclusion

The RP-LC method developed for assay and related substances determination of sildenafil citrate is precise, accurate, rapid and specific. The method was fully validated, stability indicating and can be conveniently used by quality control departments to determine the related substances and assay in production samples as well as stability samples.

ACKNOWLEDGEMENTS

The authors wish to thank the management of SMS Pharma group for supporting this work. Authors wish to acknowledge the process development group for providing the samples for our research work.

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