Research Article

An Improved RP-HPLC Method for the Quantitative Determination and Validation of Sildenafil Citrate in Bulk and Pharmaceutical Formulation

Panchumarthy Ravisankar*, Saranu Anusha, A. Dihitha Chowdary, P. Siva krishna
Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, Guntur, A.P, India.
***Corresponding author's E-mail:** banuman35@gmail.com

Received: 18-11-2018; **Revised:** 22-12-2018; **Accepted:** 04-01-2019.

ABSTRACT

The present work describes a simple, selective, specific, accurate, precise and inexpensive isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Sildenafil citrate in bulk and pharmaceutical tablet dosage form. A Welchrom C18 Column having 4.6 mm i.d X 250 mm, 5 µm particle size in isocratic mode with mobile phase containing 10 mM Phosphate buffer: acetonitrile (50:50 v/v) adjusted to pH-3.0 utilizing orthophosphoric acid. The flow rate was set to 1.0 ml/min with the responses monitored at 230 nm using Shimadzu SPD-20A Prominence UV-Visible detector. The retention time of Sildenafil citrate was found to be 3.473 minutes. Linearity was established for Sildenafil citrate in the range of 10-50 µg/ml with correlation coefficient 0.999. The LOD and LOQ were found to be 0.4221 µg/ml and 1.2792 µg/ml respectively. The amount of Sildenafil citrate present in the formulation was found to be 99.88 %. The developed method was validated and successfully applied to the estimation of Sildenafil citrate in pharmaceutical dosage form.

Keywords: Sildenafil citrate, RP-HPLC, Determination, Validation, Pharmaceutical dosage form.

INTRODUCTION

Sildenafil citrate marketed as Viagra™ (Pfizer) was approved as a drug for treating male erectile dysfunction (ED) by the US Food and Drug Administration on 27 March 1998. Sildenafil citrate is 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methyl piperazine citrate is a PDE5 inhibitor used for treatment of ED. Nitric Oxide (NO) is released with sexual stimulation from nerve endings and endothelial cells in the spongy erectile tissue, the corpus cavernosum of the penis. This release of NO activates the enzyme guanylate cyclase. The enzyme guanylate cyclase then converts guanosine triphosphate (GTP) into cGMP causing the smooth muscle to relax, which causes an inflow of blood, which then leads to an erection. Cyclic guanosine monophosphate (cGMP) is then hydrolyzed back to the inactive GMP by phosphodiesterase type 5 (PDE5). The level of cGMP is therefore controlled by the activation of cyclic nucleotide cyclase and the breakdown by PDE5.

Sildenafil citrate works by inhibiting the enzyme PDE5 by occupying its active site. Men who suffer from erectile dysfunction often produce too little amount of NO. Small amount of cGMP produced is broken down at the same rate and don't accumulate to cause a prolonged vasodilatation effect. cGMP is not hydrolyzed as fast, which allows the smooth muscle to relax leading to increased blood flow into the organ and therefore penile erection. Sildenafil is rapidly and incompletely absorbed after oral administration, with absolute bioavailability of approximately 40 %. According to Lee and Min, grape fruit juice appears to increase the C_{max} of Sildenafil by 42 % without significantly increasing area under the curve (AUC).

Extensive literature studies on the developed analytical methods on Sildenafil citrate revealed that very few analytical methods have been forthcoming to determine this drug in pharmaceutical dosage form. Some of them are HPLC[2-4], RP-HPLC[5], Electrokinetic chromatography[6], LC-MS[7-9], GC-MS[10] for the estimation of Sildenafil citrate were reported. But very few RP-HPLC methods have been developed for determination of Sildenafil citrate in the tablet form. However, the requirement of very simple, fast, efficient, accurate, precise, time-saving and highly reliable analytical RP-HPLC method for routine quality control purpose always necessities to see a new and better method. Thus the author has aimed to develop an efficient method that could determine Sildenafil citrate in its formulations. Therefore keeping all these in view, there is ever imperative need to develop a method which is simple, fast and economical. The structural formula of the Sildenafil citrate drug is shown in figure 1.

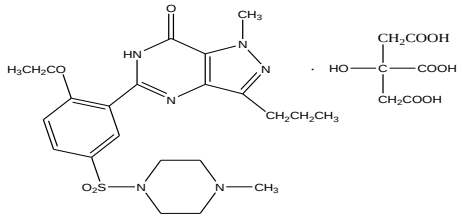

Figure 1: Chemical structure of Sildenafil citrate

MATERIALS AND METHODS

Chemicals and Reagents

Sildenafil citrate standard drug was kindly supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Telangana, India. All the chemicals were analytical grade from

Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) potassium dihydrogen phosphate, Potassium hydrogen phosphate and triethylamine (HPLC grade) from Merck pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of Sildenafil citrate formulation was procured from local market. Caverta 100 mg were manufactured by Ranbaxy Laboratories Ltd., Mumbai.

Instrumentation

Quantitative HPLC was performed on an isocratic high performance liquid chromatography (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µl (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250 mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX-223L), digital pH meter (Systronics model-802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this study.

Chromatographic conditions

Sildenafil citrate was analyzed by various reversed phase columns like C_8 and C_{18} columns. Among C_8 and C_{18} columns, C_{18} (250 mm X 4.6 mm, 5 µm) column was selected. Various combinations of acetonitrile, phosphate buffer and methanol with triethylamine as column modifier were tested. The mixture of 10 mM Phosphate buffer (pH adjusted to 3.0 using orthophosphoric acid) and acetonitrile in ratio of 50:50 v/v was selected as mobile phase and UV detection wavelength was 230 nm with a flow rate of 1 ml/min. Injection volume was 20 µ, with ambient temperature, run time was 7 minutes and retention time was 3.473 minutes.

Mobile phase preparation

Buffer preparation

Weigh accurately 1.488 g of KH_2PO_4 and 0.288 g of K_2HPO_4 dissolved in 500 ml of water to get phosphate buffer. pH was adjusted to 3.0 with orthophosphoric acid. Above prepared buffer and Acetonitrile were mixed in the proportion of 50:50 v/v. The mobile phase so prepared was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of standard and working standard drug solution

10 mg of Sildenafil citrate was correctly weighed and dissolved in 10 ml of mobile phase. This is 1000 µg/ml standard drug solution. This solution was filtered through membrane filler (0.2 µm) and sonicated for five minutes. The above prepared solution was further diluted with the mobile phase in different calibrated flasks to obtain working standard solutions of suitable concentrations (10-50 µg/ml) of Sildenafil citrate.

Preparation of Sample solution

The content of 20 tablets were exactly weighed and transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 100 mg of Sildenafil citrate was taken and the drug was extracted in 100 ml of mobile phase. The resulting solution of Sildenafil citrate was filtered using 0.45 µm membrane filter paper and degassed by sonication. This solution was further suitably diluted for chromatography.

Selection of detection wavelength

For the selection of analytical wavelength 10 µg/ml Sildenafil citrate solution was prepared from standard drug solution and scanned in the range of 200 - 400 nm. From the UV spectra, the maximum λ_{max} of Sildenafil citrate is found to be 230 nm. So this wavelength was selected as the detection wavelength for analysis.

Optimization of mobile phase and method development

Optimization of mobile phase was done based on trail and error method. A series of trials were conducted in order to obtain correct optimized HPLC conditions. In the first instance a number of mobile phase trials were done such as methanol : water, acetonitrile : HPLC grade water, methanol : acetonitrile : water in different ratio without adjusting pH, infact there are different problems such as high tailing factor, good symmetry and proper chromatographic peak were not obtained. Finally after reviewing the results, a mobile phase comprising of phosphate buffer mixture duly adjusted to pH 3.0, acetonitrile in the proportion of 50:50 v/v which full fill all the criteria of system suitability and also obtained sharp, well- gaussian shape peak. This mobile phase was selected as the diluent because the drug is freely soluble in the mobile phase. The stationary phase made up of Welchrom C_{18} column with 4.6 X 250 mm, 5 µm were observed and they are found to be utmost suitable for Sildenafil citrate. The ultra violet spectrum of diluted solutions of various concentrations of Sildenafil citrate in mobile phase was recorded by utilizing UV spectrophotometer ie., systronic double beam SL 2203. The optimum wavelength for the detection of Sildenafil citrate is 230 nm.

Table 1: Optimized chromatographic conditions and system suitability parameters for proposed HPLC method for Sildenafil citrate

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 X 250 mm, 5 µm)
Detector	SHIMADZU SPD-20A prominence UV-Visible detector

Diluents	10 mM Phosphate Buffer (pH-3): Acetonitrile (50:50 v/v)
Mobile phase	10 mM Phosphate Buffer (pH-3): Acetonitrile (50:50 v/v)
Flow rate	1 ml/min
Detection wave length	By UV at 230 nm
Run time	7 Minutes
Column back pressure	99 kgf
Temperature	Ambient temperature (25°C)
Volume of injection loop	20 µl
Retention time (R_t)	3.473 Minutes
Theoretical plates [th.pl] (Efficiency)	17803
Theoretical plates per meter [t.p/m]	356053
Tailing factor (asymmetry factor)	1.23

Calibration curve of Sildenafil citrate

Replicates of each calibration standard solutions (10, 20, 30, 40, 50 µg/ml) were injected utilizing a 20 µl fixed loop system and the chromatograms were recorded. Calibration curves were constructed by plotting concentration of Sildenafil citrate on X-axis and peak areas of standard Sildenafil citrate on Y-axis. The least square analysis method was followed for attaining the slope, intercept and correlation coefficient, regression data values. Linear regression data of the proposed method of Sildenafil citrate is computed in table 2. Calibration curve of Sildenafil citrate is shown in figure 2.

Table 2: Linear regression data of the proposed HPLC method of Sildenafil citrate

Parameter	Method
Detection wavelength (λ_{max})	230 nm
Linearity range (µg/ml)	10-50 µg/ml
Regression equation (Y=a+bx)	Y = 23.408x -17.454
Slope (b)	23.408
Intercept (a)	-17.454
Standard deviation of slope (S_b)	10.948
Standard deviation of intercept (S_a)	0.3616
Standard error of estimation (S_e)	15.127
Correlation coefficient (r^2)	0.999

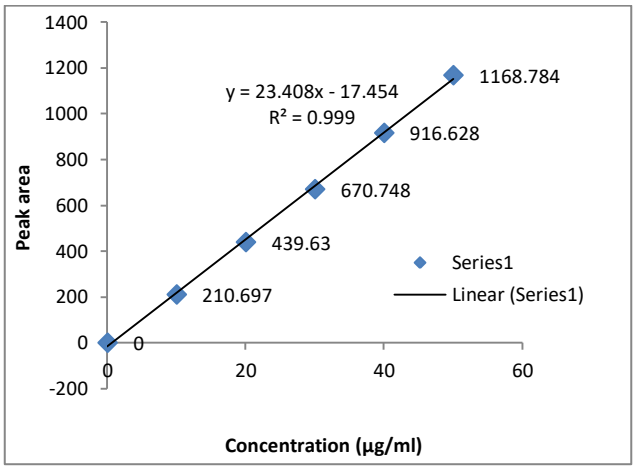

Figure 2: Calibration curve of Sildenafil citrate

Validation of the proposed method

The developed method of analysis was validated as per the ICH Q2 (R1)[11-14] for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

System suitability tests are an integral part of chromatographic method which was utilized to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg/ml for Sildenafil citrate to check the reproducibility of the system.

At first the HPLC system was stabilized for 30 minutes. One blank followed by six replicates of a single calibration standard solution of Sildenafil citrate was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as theoretical plate/meter, peak asymmetric factor, retention time and parameters were taken and results are presented in table 1.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Sildenafil citrate in the determinations under optimum conditions was investigated. The specificity of RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the placebo solution and injected and tested. The commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method.

Linearity

The linearity for RP-HPLC method was determined at 5 concentration levels ranging from 10-50 µg/ml for

Sildenafil citrate. The calibration curve was constructed by plotting peak area i.e., response factor against concentration of drugs. The slope and intercept value for calibration curve were y=23.408x -17.454 and (r^2=0.999) for Sildenafil citrate, where Y represents the peak area of drug and X represents the analyte concentration. The significant correlation between response factor and concentration of the drug within the concentration range. The representative chromatograms of 10-50 µg/ml indicating the Sildenafil citrate are shown in figures 3a to 3e and the typical and sample chromatograms of Sildenafil citrate are shown in figures 4a and 4b respectively.

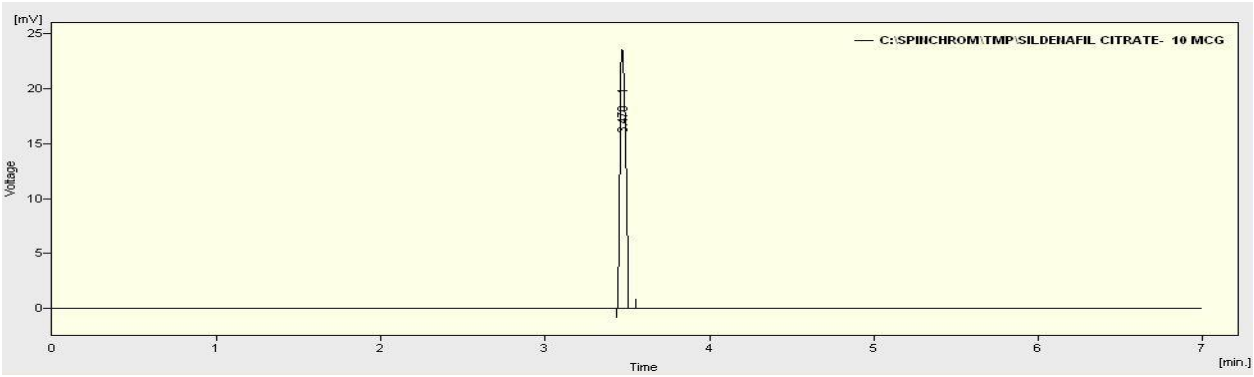

Figure 3a: Standard chromatogram of Sildenafil citrate (10 µg/ml)

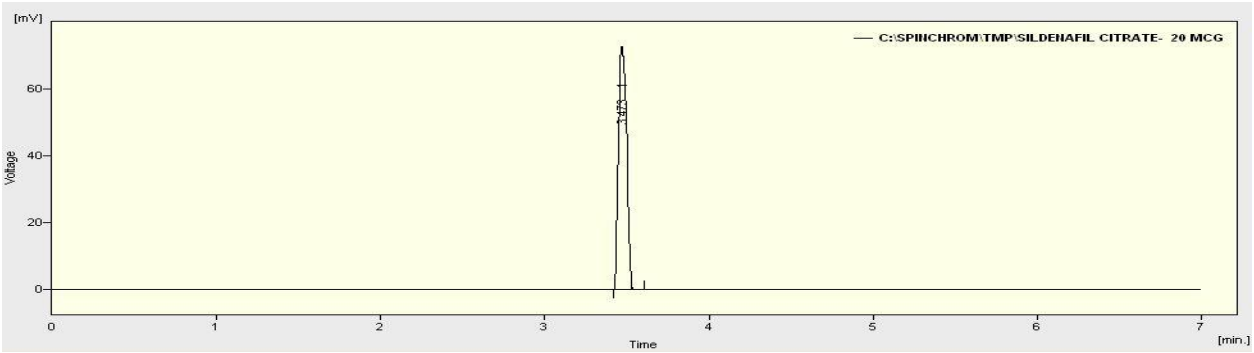

Figure 3b: Standard chromatogram of Sildenafil citrate (20 µg/ml)

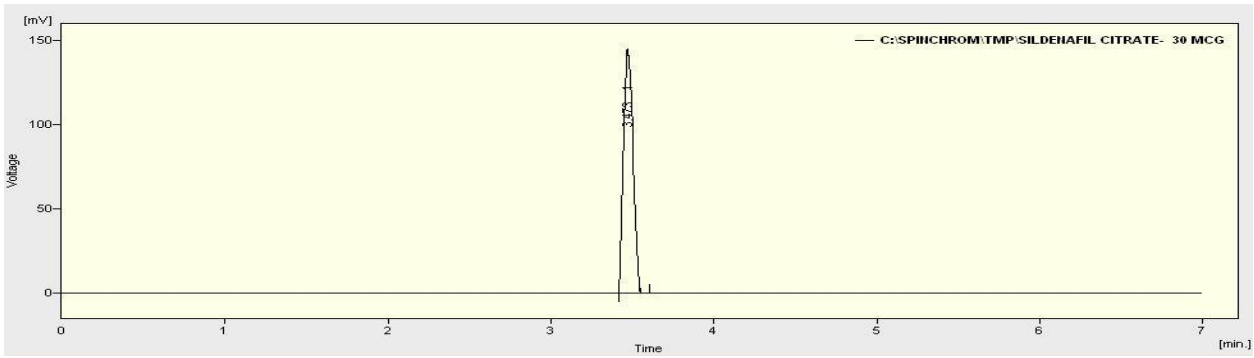

Figure 3c: Standard chromatogram of Sildenafil citrate (30 µg/ml)

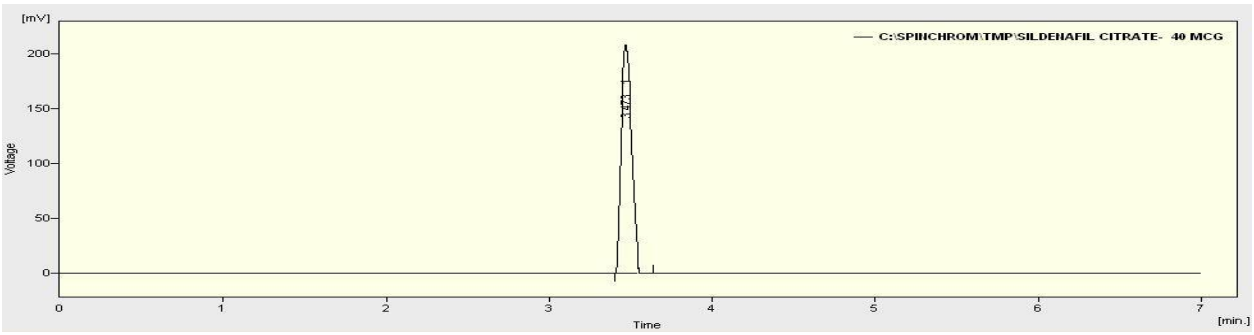

Figure 3d: Standard chromatogram of Sildenafil citrate (40 µg/ml)

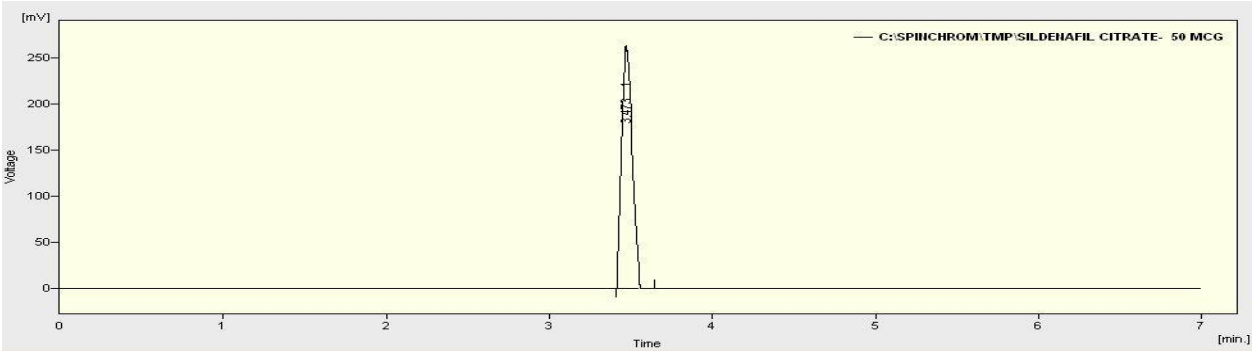

Figure 3e: Standard chromatogram of Sildenafil citrate (50 µg/ml)

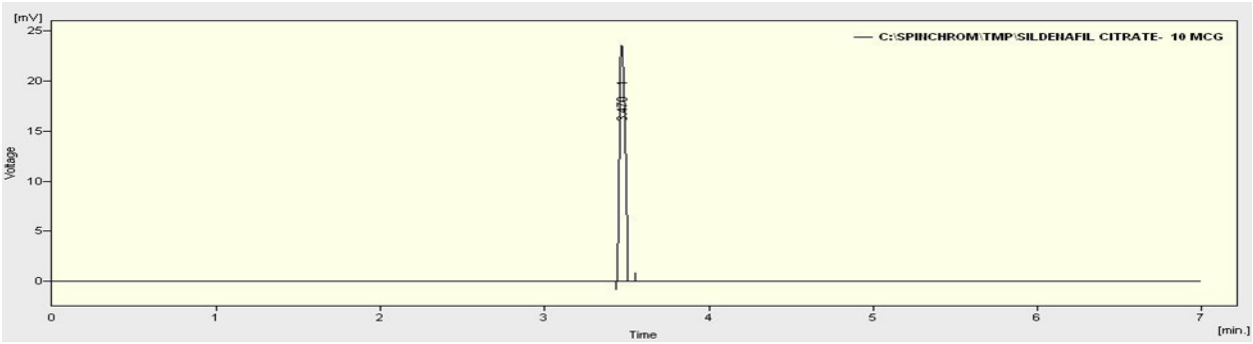

Figure 4a: A typical chromatogram of Sildenafil citrate

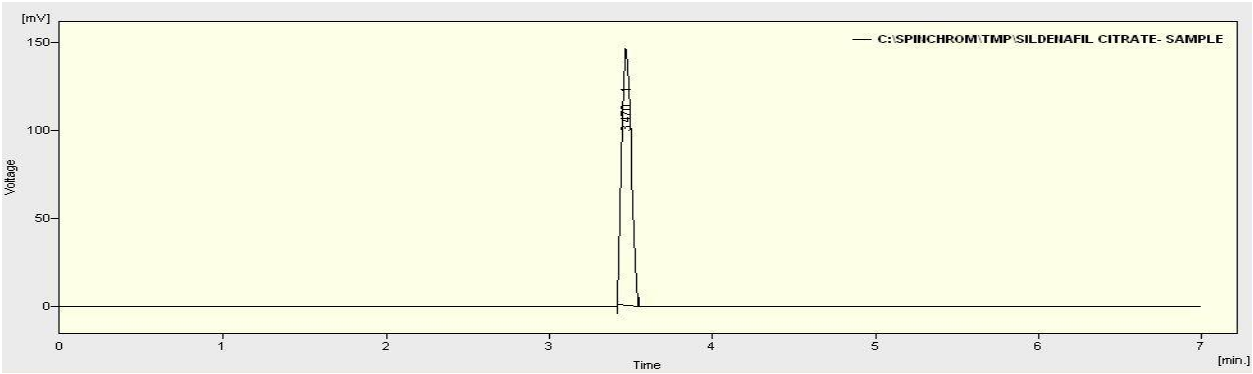

Figure 4b: Chromatogram of marketed formulation of Sildenafil citrate

Precision

The precision of the method was demonstrated by inter-day Intra-day variation studies. In the intra-day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage relative standard deviation was calculated. In inter-day precision, 6 repeated injections of standard and sample solutions were made for 3 consecutive days and response factor of drug peaks and % RSD were calculated which is within the acceptable criteria of not more than 2.0. From the data obtained, the developed RP-HPLC method was found to be precise. The results for intra-day and inter-day precision are presented in tables 3a and 3b respectively.

Table 3a: Results of Precision study (Intra-day)

Sample	Concentration (µg/ml)	Injection number	Peak area	% RSD
Sildenafil citrate	30	1	670.748	0.233
		2	670.745	
		3	670.748	
		4	670.746	
		5	670.748	
		6	670.748	

Table 3b: Results of Precision study (Inter-day)

Sample	Concentration (µg/ml)	Injection number	Peak area	% RSD
Sildenafil citrate	30	1	670.747	0.452
		2	670.748	
		3	670.746	
		4	670.747	
		5	670.747	
		6	670.748	

Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of Sildenafil citrate by the method of addition. Recovery was determined by spiking the formulation with standards of each drug equivalent to 50 %, 100 % and 150 % of the amount originally present. % Recovery calculated by comparing the area before and after addition of the working standard. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in table 4. The results of analysis showed that the amount of drug found were in good agreement with the label claim of the formulation.

Table 4: Recovery results of Sildenafil citrate

S. No.	Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	Mean ± SD	% RSD[#]
1	50 %	5	4.98	98.63 ± 0.305	0.265
		5	4.99		
		5	5.03		
2	100 %	10	9.98	99.63 ± 0.208	
		10	9.94		
		10	9.97		
3	150 %	15	14.98	99.72 ± 0.279	
		15	14.91		
		15	14.98		

[#]acceptance criteria < 2.0

Robustness

The Robustness was evaluated by the analysis of Sildenafil citrate under different experimental conditions such as making small changes in flow rate (± 0.2 m/min), detection wavelength (± 5 nm), and Mobile phase composition (± 5 %). The results are presented in table 5.

Table 5: Robustness results of Sildenafil citrate

S. No	Parameter	Optimized	Used	Retention time, (R_t)(min)	Plate count/meter	Peak asymmetry[#]	Remark
1.	Flow rate (± 0.2 ml/min)	1.0 Ml/min	0.8 ml/min	3.530	9510	1.263	*Robust
			1.0 ml/min	3.473	9503	1.256	*Robust
			1.2 ml/min	2.930	9458	1.246	*Robust
2.	Detection wavelength (± 5 nm)	230 nm	225 nm	3.473	9504	1.225	Robust
			230 nm	3.473	9503	1.252	Robust
			235 nm	3.473	9503	1.263	Robust
3.	Mobile phase composition (± 5 %)	50:50 v/v	55:45 v/v	3.228	9402	1.258	*Robust
			50:50 v/v	3.473	9503	1.224	*Robust
			45:55 v/v	3.489	9601	1.226	*Robust

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3 (SD)/S and LOQ= 10 (SD)/S, where SD = the standard deviation of response (peak area) and S = the slope of the calibration curve. The LOD and LOQ values were calculated.

RESULTS AND DISCUSSION

The mobile phase consisting of 10 mM phosphate buffer (pH-3.0): acetonitrile (50:50 % v/v) at 1 ml/min flow rate was optimized which gave sharp peak, minimum tailing factor. UV spectra of Sildenafil citrate showed that the drug absorbed maximum at 230 nm, so this wavelength was selected as the detection wavelength. The retention time for Sildenafil citrate was 3.473 min. The calibration curve for Sildenafil citrate was found to be linear over the range of 10-50 µg/ml. System suitability parameters and optimized chromatographic conditions are shown in table 1. The regression equation was found to be Y=23.408x - 17.454 with correlation coefficient r^2=0.999 which indicates this method has good linearity. The linear regression data is shown in table 2. The linearity of the graph is shown in figure 2. The specificity was studied for the examination of the presence of interfering components. They do not disturb the elution or quantification of Sildenafil citrate, furthermore the well-shaped peaks also indicate the specificity of the method. Therefore, it was concluded that the method is specific.

The representative standard chromatograms indicating the Sildenafil citrate are shown in figures 3a to 3e. The representative typical and sample chromatograms of Sildenafil citrate are shown in figures 4a and 4b respectively. Precision was studied to find out intra and inter day variations in the test methods of Sildenafil citrate for three times on the same day and different days. The intra-day and inter-day precision obtained was % RSD (< 2.0) indicates that the proposed method is quite precise and reproducible and results are shown in tables 3a and 3b. Recovery studies of the drug were carried out for the accuracy parameter at three different concentration levels i.e., multiple level recovery studies. A known amount of Sildenafil citrate standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in table 4. The percentage recovery of Sildenafil citrate was found to be in the range of 98.63% to 99.72 %. The method precision was done and the low % RSD values indicates that the proposed method which was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, detection wavelength, mobile phase composition etc. It was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limits which indicates that the method has robustness and suitable for routine use. The Robustness results are presented in table 5. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ. The limit of detection (LOD) was 0.4221 µg/ml and the limit of quantitation (LOQ) was 1.2792 µg/ml which shows that this method is very sensitive.

CONCLUSION

This paper Sildenafil citrate RP-HPLC method developed and fully validated for the quantitative determination of Sildenafil citrate in bulk and pharmaceutical tablet dosage forms. Statistical analysis of the results shows that the developed procedure has decorous precision and accuracy. The method was completely validated and shows satisfactory results and free from interference of the other active ingredients and additives used in the formulation. In fact the results of the study indicate that the developed method was found to be rapid, simple, reliable, accurate, linear, selective, sensitive, economical, and reproducible, have short run time and only requires low cost technology which makes this method economically good for most clinical laboratories. Hence it can be concluded that this method may be employed for the routine quality control analysis of Sildenafil citrate in active pharmaceutical ingredient API and pharmaceutical preparations.

Acknowledgement: The authors thank to Hetero Labs Limited, Jeedimetla, and Hyderabad for providing Sildenafil citrate as gift sample for this work. They also thank Chairman Dr. L. Rathaiah, Vignan group of institutions and Dr. P. Srinivasa Babu, Principal of Vignan Pharmacy College for providing necessary facilities to carry out this research work.

REFERENCES

1. Current Index of Medical Specialties (CIMS-133, Apr-July [update 2]) UBM Medica India Private Limited, Bangalore, 2016, 366.
2. Guermouchea M.H, Bensalah K, Solid phase extraction and liquid chromatographic determination of sildenafil and *N*-demethylsildenafil in rat serum with basic mobile phase, J. Pharm. Biomed. Anal, 40, 2006, 952-957.
3. Jeong C.K, Lee H.Y, Jang M.S, Kim W.B, Lee H.S, Narrow bore high-performance liquid chromatography for the simultaneous determination of sildenafil and its metabolite UK-103,320 in human plasma using column switching, J. Chromatogr. B, 752, 2001, 141-147.
4. Cooper J.D.H, Muirhead D.C, Taylor J.E, Baker R.P, Development of an assay for the simultaneous determination of sildenafil (viagra) and its metabolite (UK-103,320) using automated sequential trace enrichment of dialysates and high-performance liquid chromatography, J. Chromatogr. B, 701, 1997, 87-95.

5. Liaw J, Chang T.W, Determination of transdermal sildenafil in nude mouse skin by reversed-phase high-performance liquid chromatography, J. Chromatogr. B, 765, 2001, 161-166.

6. Nevado J.J.B, Flores J.R, Penalvo G.C, Farinas N.R, Determination of sildenafil citrate and its main metabolite by sample stacking with polarity switching using micellar electrokinetic chromatography, J. Chromatogr. A, 953, 2002, 279-286.

7. Weinmann W, Bohnert M, Wiedemann A, Renz M, Lehmann N, Pollak S, Post-mortem detection and identification of sildenafil (viagra) and its metabolites by LC/MS and LC/MS/MS, Int. J. Legal Med, 114, 2001, 252-258.

8. Kim J, Ji H, Kim S, Lee H, Lee S, Kim D, Yoo M, Kim W, Lee H, Simultaneous determination of sildenafil and its active metabolite UK-103,320 in human plasma using liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal, 32, 2003, 317-322.

9. Wang L, Wang J, Cui Y, Fawcett J, Gua J, Liquid chromatographic-tandem mass spectrometric method for the quantitation of sildenafil in human plasma, J. Chromatogr. B, 828, 2005, 118-121.

10. Saisho K, Scott K.S, Morimoto S, Nakahara Y, Effective Extraction and Determination of Sildenafil (Viagra®) and Its N-Desmethyl Metabolite in Rat and Human Hair by GC-MS, Biol. Pharm. Bull, 24, 2001, 1384-1388.

11. Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D, IOSR Journal of Pharmacy, 5 (10), 2015, 7-19.

12. Ravisankar P, Gowthami S, Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and Biotechnology, 2, 2014, 1183-1195.

13. Ravisankar P, Swathi V, Srinivasa Babu P, Shaheem Sulthana Md, Gousepeer SK, Current trends in performance of forced degradation studies and stability indicating studies of drugs, IOSR Journal of Pharmacy and Biological Sciences, 12 (6), 2017, 17-36.

14. ICH Q2 (R1) Validation of analytical procedures, Text and methodology International conference on Harmonization, Geneva, 2005, 1-17.

Source of Support: Nil, **Conflict of Interest:** None.