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Development and Validation Method for the Determination of Sildenafil Citrate Tablets Drugs by Using High Performance Liquid Chromatography (HPLC) in Pharmaceutical Formulation

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Abstract: ***The objective of this is to describe the optimization, validation, and application of spectrochromatographic techniques for determination of Sildenafil Citrate in their pharmaceutical formulation (tablets). A simple, rapid, accurate and sensitive c spectrochromatographic has been developed and validated. The method is a direct spectrochromatographic method based on the chromatographic separation of Sildenafil citrate compound. This chromatographic method was developed by using inertstil C18 (250 *4.6 mm), 5µm column with a mobile phase consisting of 0.2 M ammonium acetate and Acetonitrile in (1:1) ratio. The flow rate was adjusted at 1.0 ml/min, injection volume 20 µL, with UV-Detector the maximum absorption peak (λ_{max}) at 240 nm, temperature, at ambient temperature, and retention time was found to be 6.002 min. Under the optimized condition, beer's law correlating the absorbance (Y) with concentration (X) was obeyed in the range of 0.014 to 0.035 µg/ml.***

1. Introduction

Sildenafil citrate (SIL) was patented in1996 and launched in May 1998 as first oral drug approved by Food and Drug Administration (FDA) to treat erectile dysfunction (ED) in the United States. It is also effective for treatment of pulmonary arterial hypertension (PAH) [1, 2].

Sildenafil Citrate is a white to off-white crystalline powder [3] with a solubility of 3.5 mg/mL in water and a molecular weight of 666.7 Dalton Molecular formula is $C_{22}H_{30}N_6O_4S$. .Chemically, designated as 1-[[3- (6, 7-dihydro -1- methyl – 7 – oxo – 3 – propyl -1Hpyrazolo [4,3 - d] pyrimidine – 5 - lye) - 4ethoxyphenyl] sulfonyl] - 4 methylpiperazine as result of release nitric oxide (NO) which works by activation of the enzyme guanylatecyclase responsible for converting guano sine triphosphate (GTP) to 3'5' cyclic guanosine monophosphate (cGMP) [4].

The cGMP is a potent vasodilator vital erection of the penis. Sildenafil Citrate selectively inhibits the enzyme PDE-5A (phosphodiesterase-5A) that hydrolyzes cGMP. Thus it increases level of cGMP by preventing it from breaking down.

Consequently smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis causing an erection by fascinating the signaling actions of nitric oxide (NO) in penile smooth muscle. The most common side effects of Sildenafil citrate are headache, facial flushing, and upset stomach. Less commonly cyanosis (bluish vision), blurred vision, or sensitivity to light may briefly occur [5].

Dapoxetine HCl (DAP) is designated chemically as (S)-N,N-dimethyl-3-(naphthalen-1-yloxy)-1 phenylpropan-1-amine with an empirical formula of $C_{21}H_{23}NO$ and molecular weight of 305.413g/mol.

This drug is mainly useful in erectile dysfunction as selective serotonin reuptake inhibitor (SSRI) [6].

SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders.

They can also sometimes be effective and useful for treating premature ejaculation problems, impotence and some cases of insomnia. The drug's mechanism of action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time [7]. Its structural formula is given below in Figure.

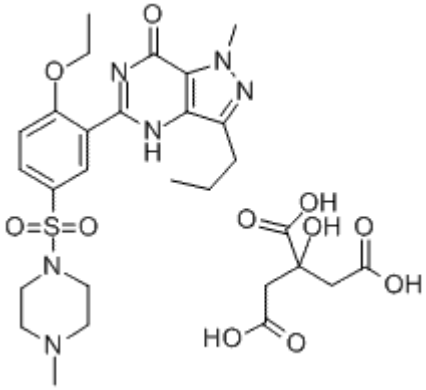

Figure 1: Structure of sildenafil citrate

Sildenafil citrate, a specific phosphodiesterase-5 inhibitor [8], is increasingly used for pulmonary hypertension in pregnancy [9]. Sildenafil Citrate is also emerging as a potential candidate for the treatment of intra-uterine growth retardation and for premature labor [10]. Its effects in the fetoplacental circulation are not known [11]. Our objectives were to determine whether phosphodiesterase-5 is present in the human feto-placental circulation [12], and to characterize the effects and mechanisms of action [13] of Sildenafil Citrate in this circulation.

1.1 Pharmacology of sildenafil citrate

1.1.1 Pharmacodynamics

Studies in vitro have shown that sildenafil is selective for PDE5, which is involved in the erection process. Its effect is more potent on PDE5 than on other known phosphodiesterases [14, 15]. There is a 10-fold selectivity over PDE6 which is involved in the phototransduction pathway in the retina. At maximum recommended doses, there is an 80-fold selectivity over PDE1, and over 700-fold over PDE2, 3, 4, 7, 8, 9, 10 and 11. In particular, sildenafil has greater than 4,000-fold selectivity for PDE5 over PDE3, the cAMP-specific phosphodiesterase isoform involved in the control of cardiac contractility [16].

Sildenafil Citrate is rapidly absorbed. Maximum plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasted state. The mean absolute oral bioavailability is 41% (range 25–63%). The oral pharmacokinetics of sildenafil is proportional over the recommended dose range (25–100 mg) [17]. When sildenafil is taken with a high fat meal, the rate of absorption is reduced with a mean delay in T_{max} of 60 minutes and a mean reduction in C_{max} of 29%. Patients may need to individual their dosing relative to their food intake based on their own experienced clinical response [18].

The total body clearance of sildenafil is 41 L/h with a resultant terminal phase half-life of 3–5 hours. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the faces (approximately 80% of the administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose) [19].

Sildenafil is an oral therapy for erectile dysfunction which restores impaired erectile function by increasing blood flow to the penis, resulting in a natural response to sexual stimulation. The physiological mechanism responsible for erection of the penis involves the release of nitric oxide (NO) in the corpus cavernous during sexual stimulation. Nitric oxide then activates the enzyme, guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the corpus cavernosam and allowing inflow of blood [7].

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories [20].

Validation is defined as finding or testing the truth of something. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [21].

Validation of analytical method is an important regulatory requirement in pharmaceutical analysis. Method validation provides documented evidence, and high degree of assurance that an analytical method employed for a specific test is suitable for intended use. Over recent years regulatory authorities have become increasingly aware of the necessity of ensuring that the data submitted have been acquired for marketing authorization using validated analytical methodology. The international conference on harmonization (ICH) has introduced guideline for analytical methods validation [22].

Analytical methods need to be validated, verified, or revalidated in the following instances:

- Before initial use in routine testing
- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method [20].

Method validation has received considerable attention in literature from industrial committees and regulatory agencies. This chapter outlines how method validation helps to achieve high quality data [20].

2. Materials and methods

2.1 Reagents and Solutions

All chemicals used are analytical grade (AG), and spectroscopic organic solvents. Deionized water was used for preparing solutions.

- Atorvastatin working standard, Batch No.: ATC/1104B/0014,purity100.2%, Water content: 5.07% were obtaining from CHROMO LABORATORIES.

- Sildenafil citrate working standard, (purity 99.57%) was purchased from RAKSHIT DRUGS PRIVATE LIMITED.
- Methanol HPLC grade (DUKSAN, pure chemical, Korea).
- Acetonitrile HPLC grade reagents were supplied by (scharlau, Spain).
- Dichloromethane pure (scharlau, Spain).
- Sodium hydroxide pellets purified (SDFCL, India).
- Orthophosphoric acid A.R (CDH).
- Potassium dihydrogen orthophosphate anhydrous.
- Tetrahydrofuran (scharlau, Spain).
- Chloroform, (ROMIL pure chemical).
- Hydrochloric Acid (SDFCL, India), sp.gr:1.18 g/ml, percentage: 37.5%.
- Phosphate buffer PH 7.4.
- Hydrochloric acid 0.1 M.
- Sodium hydroxide solution 0.1 M, hydrogen peroxide30%. - Ammonium acetate buffer, glacial acetic acid)ROMIL pure chemical), -whatmann filter paper No. 41.

2.3 Instrumentation

The following instruments and equipment's or apparatus were used during the course of this work:

HPLC with degasser (SHIMADZU, KYOTO,JAPAN), column C18, Dimensions : 4.6 x 250 mm Model of pump: LC-20 AB, Model of detector SPD-20A, Model of oven CTO – 20 AC, Model of degasser DGU-20A5, Model of prominence communication Bus CBM-20A, Software LC Solution.

FTIR (Fourier Transform Infra-Red spectrophotometer), (SHIMADZU, KYOTO, JAPAN), Model FTIR-8400s.
PH-Meter, Model (HANNA instrument, INDIA), PH/mV/EC/TDS/ NaCl.
Electronic Sensitive balance (SHIMADZU, KYOTO, JAPAN), Type A*120, Capacity 120g, Read ability 0.1 mg.
Rotary vacuum pump (SHIMADZU, JAPAN). , Model SA18.
Melting Point, Model (PHARMATEST, INDIA).
Friability tester, Model (PHARMATEST, INDIA).
Thickness tester, Model (2.8.1.5 PH2.8.1.5 ARMATEST, INDIA).
Hardness tester, Model (PHARMATEST, INDIA).
Dissolution Tester, Model (PHARMATEST, INDIA)
Disintegration tester, Model (ELECTROLAB GERMANY).

2.4 Preparation of standard stock solution of Sildenafil Citrate for HPLC assay

50 mg of sildenafil Citrate working standard was accurately weighed and transferred to 50ml volumetric flask. 30 ml of diluent was added to the weight and sonicate for one minute for dissolution, left to reach room temperature, completed to the required volume with diluent solution and mixed very well, 5ml of this solution was transferred to 50 ml volumetric flask and diluted with the same diluent to the mark. Filtered through 0.45µm PVDF syringe filter into HPLC vial, 2.0 ml of this filtrate will discarded firstly.

2.5 Preparation of sample solution of Sildenafil Citrate for HPLC analysis

20 tablets of SIL drugs were selected randomly from provided samples and finely powdered. 620.0 mg of this powder accurately weighed and transferred into 50 ml volumetric flask, 30 ml of diluent were added to the weight and sonicated in ultrasonic to completely dissolution, left to reached room temperature, completed to the required volume with diluent solution and mixed very well. 5ml of this solution was transferred into 50ml volumetric flask and diluted with the same diluent solution to the mark, filtered through 0.45µm PVDF syringe filter into HPLC vial. 2ml of this filtrate will discarded firstly.

2.6 Preparation of mobile phase

0.5 M Sodium hydroxide solution
Weight 20 g of sodium hydroxide pellets were dissolved it in 1000 ml of deionized water.

2.7 Phosphate buffer pH 7.4 (media)

6.80 g of potassium dihydrogen phosphate was weighted and transferred to 1000 ml volumetric flask; adjust the pH to 7.4 by using solution of 0.5 M sodium hydroxide.

2.8 Buffer solution

1.0 ml of phosphoric acid was transferred in 100 ml volumetric flask, diluted with deionized water, mixed and complete to the mark with the same solvent.

2.9 Mobile phase (MP)

Buffer solution and acetonitrile (50: 50), were mixed, filtered and degassed by using degasser instrument.

3. Results and Discussions

3.1 Method Development

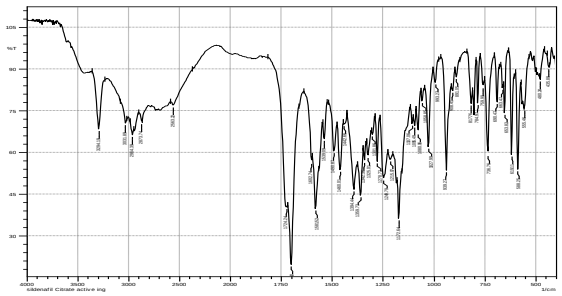

Figure 2: FTIR spectra of Sildenafil Citrate STD (reference)

3.2. Identification of sildenafil Citrate (Active Ingredient)

The FTIR absorption spectra of atorvastatin was obtained using KBr pellet technique and the spectra was found to exhibit characteristics absorption bands at 3240, 1627, 1620, 1180,1100,3600, 828 cm-1, [23]showing N-H, C=O, C=C, C-O, C-N, O-H and aromatic substitution Bands respectively of ATV Calcium. The chemical structure of SIL was shown in the Figure 3.1.

Identification of Sildenafil Citrate and excipient (Tablets 25 mg)

Table 1: Infrared frequencies SIL

No.	Functional group(s)	Characteristic Absorption(s) range (cm-1)	Identified in this study (cm-1)
1	SO2 stretch	1200 - 1100	1172.74
2	Aromatic C=C bond	1700 – 1500	1582.62
3	C=O stretch	1750 – 1680	1702.21
4	Saturated C–H stretch	2950 – 2850	2962.71
5	Unsaturated C–H stretch	3100 - 3010	3029.26
6	Secondary N-H stretch	3500 – 3300	3299.3
7	O-H stretch	3550 – 3200	3617

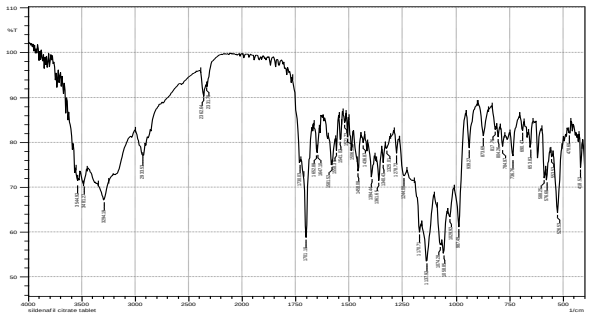

Figure 3: FTIR spectra of Sildenafil Citrate tablets (sample)

The Infrared (IR) absorption spectrum of SIL tablets was generated using a Spectrum 100 Fourier transform-infrared attenuated total reflectance spectrometer (SHIMADZU 8400s). The spectrum, obtained at a wave-number range of 4000 –418 cm-1 and a resolution of 4cm-1, [24] is descripted in Figure 3.1.3 Band assignments for the resultant spectrum are summarized in Table 3.1.1 below.

3.3. Methodology

3.3.1. Development and validation methods of sildenafil citrate tablet drug (50mg)

The validation on an HPLC method incorporates the following requirements: confirmation of the process by means of experimental studies, and ensuring that the performance of the analytical method meets the accuracy and precision standards required to achieve an acceptable uncertainty limit [25]. The importance of validating the proposed HPLC method is to ensure the reliability and reproducibility of results generated when using that method, even when carried out by different operators using the same analytical equipment.

Although an important aspect of the validation process is to ensure the acceptability of the method that has been developed, it is also to investigate the method thoroughly to determine the limits of allowed variability for the conditions to achieve a desired outcome during analysis [26]. In the present work section spectrophotometric methods has been developed and validated for quantitative determination of active ingredient spectrophotometric and high performance liquid chromatography (HPLC) analytical methods were developed and validated as assay methods for determination of spironolactone in raw materials and tablet dosage form. The method (1:1) acetonitrile: buffer, 0.2 M KH_2PO_4 with pH 7.4 was the best one for this group of drugs regarding symmetry of peaks, resolution, and their retention time, when they are analyzed by HPLC system.

3.3.2 Development and optimization of the HPLC method

In the present work, an analytical method based on LC was developed and validated for assay and content uniformity determination of lercanidipine hydrochloride in tablet formulation. The basic chromatographic conditions used for this method were designed to be simple and easy to use and reproduce. The analytical conditions were selected after testing the different parameters that influence LC analysis, such as column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters. Inertstil C18, 250×4.6 mm column having 5 µm particle size was used because of its advantages of high degree of retention, high resolution capacity, better reproducibility, Ability to produce lower back pressure and low degree of tailing [27]. For mobile phase selection, the preliminary trials using different compositions of mobile phases consisting of water and methanol (50:50 v/v) gave poor peak shape. The representative chromatogram for the same is shown as under (Figure 3.4.1).

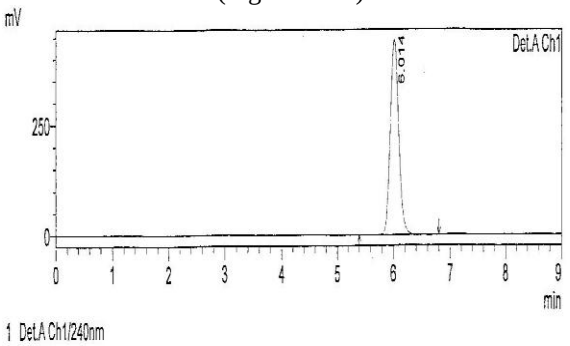

Figure 4: Chromatogram of Sildenafil citrate optimization of the HPLC method

Table 2: Chromatogram values of sildenafil citrate optimization of the HPLC method

Peak #	Name	Ret. Time	Area	Height	Area %
1	Sildenafil citrate	6.014	4412978	443515	100.00
Total	-	6.014	4412978	443515	100.00

Table 3: Optimization of the HPLC method Parameters for method (A)

Peak #	Name	Theoretical plate	K^1	Tailing factor	Resolution
1	Sildenafil citrate	7976.274	11.029	1.159	00.00
Total	-	7976.274	11.029	1.159	00.00

3.3.3 Specificity and selectivity

The specificity of an analytical method is defined as its capacity to accurately measure the concentration of an analyte in a sample, in the presence of impurities or pharmaceutical excipients [28].

Table 4: Specificity and selectivity values of condition of SILC under study

W						
Tests	Presence of Mean Peak	Resolution between mean peak and nearest peak	Tailing/Symmetry	No. Of Theoretical Plates	Area% of mean peak in Chromatogram	Total No. of Peaks
Blank	-ve	-ve	-ve	-ve	-ve	3
Placebo solution	-ve	-ve	-ve	-ve	-ve	3
Standard solution	4072493	25.346	1.08	12059	99.6	3
Sample solution	5526837	25.362	1.07	12207	99.45	7

Specificity studies are a crucial aspect of an HPLC method if a method lacks the capacity to produce sample peaks that are clearly resolved from interference(s) from other materials, the accuracy of the method is likely to compromise the accuracy of the overall results. Specificity tests [29] were performed by comparison of chromatograms developed from the analysis of a standard solution of SIL and results based on the analysis of commercially available SIL tablets.

The peaks of interest were adequately resolved, as shown in Figure 2.5, and it was noted that there were no other peaks present in the chromatogram suggesting that the proposed method can be considered specific for the analysis of SIL [30].

Study Conducted Through Chromatograph Standard, Test, placebo and Blank Solution; Also, Standard and Sample under stress condition chromatographed to prove that there is no peak Interference, and test method able to separate Sildenafil Citrate Clearly "calculated as base" and Related Substance [31].

Sildenafil Citrate Peak appears clearly in Standard and Test Solutions at Retention Time 6.014 minute. With good resolution and tailing factor, doesn't appear in Blank Solutions and placebo solutions, "There is No. Interference with blank or placebo."

Standard under Stress Conditions show degradation peaks doesn't interface with Sildenafil Citrate peak with 0.1N HCL & 0.1N NaOH & 80°C and calculated content doesn't differ than expected by not more than 0.62 mg but in case of 30% H_2O_2 sever decomposition take place and Calculated Content decreased to 0.13 mg which approve That degradation products due to acid, base hydrolysis and oxidation Practically doesn't interface with main peak. Sample under stress conditions show same behavior as standard, also degradation due to excipient doesn't interface with main peak and calculated Content doesn't differ than expected by not more than 0.79 mg while in case of 30%

H_2O_2 same behavior take place and calculated content decreased to 0.00 mg and that approve degradation due to excipient practically doesn't interface with main peak [32]

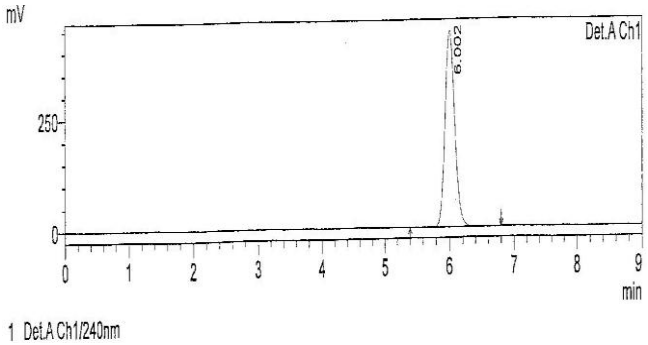

Figure 5: Chromatogram of Sildenafil citrate optimization of the HPLC method

Table.5 Chromatogram values of sildenafil citrate system suitability

Peak #	Name	Ret .Time	Area	Height	Area %
1	Sildenafil citrate	6.002	4416781	443792	100.00
Total	-	6.002	4416781	443792	100.00

Table 6: System suitability parameters for method (A)

Peak #	Name	Theoretical plate	K^1	Tailing factor	Resolution
1	Sildenafil citrate	7938.825	11.003	1.158	00.00
Total	-	7938.825	11.003	1.158	00.00

Table 7: System suitability values for SIL standard under stress conditions

1. Specivity / Selectivity						
Potency 99.27 %				Water content 0.69 %		
	Standard	Standard 2	Standard with 0.1 N NaOH	Standard with 30% H_2O_2	Standard after heating at 80 for I Hour	Standard with 0.1 N HCL
Standard Wt.	49.9	50.1	50	50.5	50.8	50.3
Actual Sildenafil Citrate content on dried basis (mg)	35.02	35.16	35.09	35.44	35.65	35.30
Injection#1	4078263	4081356	4147198	9150	4122947	4223354
Injection#2	4062991	4065910	4157744	20575	4152484	4119974
Injection#3	4074806	4088067	-	-	-	-
Injection#4	4069856	-	-	-	-	-
Injection#5	4076549	-	-	-	-	-
AVR	4072493	4078444	4152471	14862.5	4137716	4171664
RSD%	0.16	0.28	0.18	54.36	0.50	1.75
Calculated content as Sildenafil Citrate on dried bases"mg"		35.07	35.71	0.13	35.58	35.87
Difference "mg"		-0.09	0.62	-35.31	-0.07	0.57

Due to there is no sildenafil Citrate impurity standard and with reference to analytical profile 27 we detected 4 impurity products and according to same reference; limit of degradation products approved [33].

Table 8: System suitability values for SIL samples under stress conditions

	Sample with 0.1N NaOH	Sample with 0.1N HCl	Sample with 30% H2O2	Sample with 80°c
Sildenafil Citrate Wt.	50.1	50.3	50	50.5
Actual content Sildenafil Citrate on dried bases"mg"	35.16	35.30	35.09	35.44
Injection#1	3974937	0	4102214	4160795
Injection#2	4020118	0	4185824	4146552
Average	3997528	0	4144019	4153674
RSD%	0.01	#DIV/0!	0.014	0.002
calculated content as Sildenafil Citrate on dried basis' 'mg"	34.37	0.00	35.63	35.72
Difference "mg"	-0.78	-35.30	0.54	0.28

Specify of SIL approved by absence of main peak in placebo, blank, and presence of it in standard and sample solution, there is no interference between degradation products and main peak. SIL show good resolution between main peak and degradation peaks, using standard and product under stress conditions.

3.4 Linearity, LOD, LOQ and Range

Through serial dilution from working standard to conduct linear calibration curve, and calculate correlation coefficient " R" coefficient of determination "R2" , slope, Y-intercept and relationship equation. LOD, LOQ and Range calculated.

3.4.1 Linearity

From Calibration Curve:

Table 9: Summary of linearity values of SILC of standard curve

Correlation Coefficient "R"	0.9995
Coefficient of determination "R²"	0.9991
Slope"S"	58425645.11
Y- intercept"b"	8275.39558
Regression equation Y= mX+C	58425645.11x + 98275.3956
Residual sum of Squares	59284730

Linearity of SIL approved by calibration curve; linearity study start from concentration of 0.0143mg/ml to 0.2137 mg/ml as Sildenafil base, its correlation coefficient ''R'' = 0.9995 and coefficient of Determination "R²"= 0.9991, Equation X= (Y-98275.39558)/58425645.11

3.4.2 LOD

0.020 µg/ml Calculated with reference to blank standard deviation and slope, S/N = 19.7

3.4.3 LOQ

0.05 µg/ml Calculated with reference to blank standard deviation and slope, S/N = 17.6 Linearity of ATV approved by calibration curve; Linearity study start from concentration of 0.0143mg/ml to 0.2137 mg/ml as Sildenafil base, its correlation coefficient ''R'' = 0.9995 and coefficient of Determination "R²"= 0.9991, Equation X= (Y-98275.39558)/58425645.11

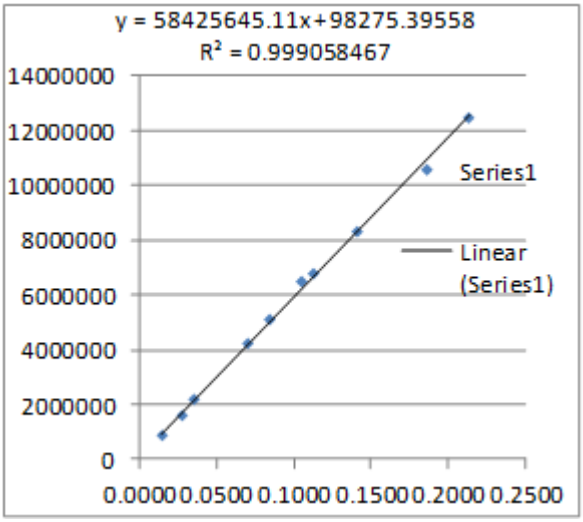

Figure 6: Calibration curve of proposed method (A)

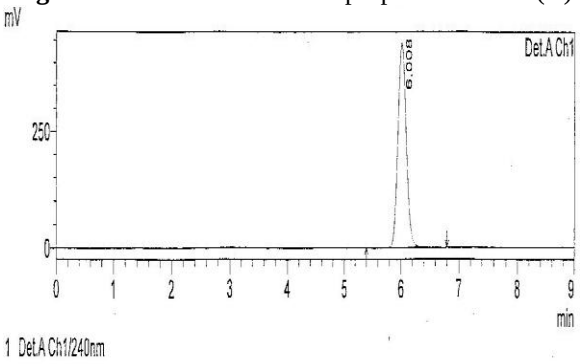

Figure 7: Chromatogram of Sildenafil citrate standard

Table 10: Chromatogram values of sildenafil citrate standard

Peak #	Name	Theoretical plate	K^1	Tailing factor	Resolution
1	Sildenafil citrate	7961.440	11.017	1.158	00.00
Total	-	7961.440	11.017	1.158	00.00

Table 11: System suitability parameters for SILC STD

Peak #	Name	Ret.Time	Area	Height	Area %
1	Sildenafil citrate	6.008	4414434	443150	100.00
Total	-	6.008	4414434	443150	100.00

3.4.4 Range

Range of SIL established through linearity measurement to be from 14.1 % to 206.0 % Assay

Table 12: Summary result of linearity for standard curve recovery

Calculated content " mg "	Calculated %"Assay"
7.03	14.05
13.40	26.81
17.77	35.55
35.14	70.29
42.22	84.44
53.49	106.98
56.18	112.35
68.57	137.14
103.02	206.03

Range
From 7.0 mg to 103.0 mg
From 14.1 mg to 206.0 mg

3.5 Accuracy

Data Collected from Linearity Measurements: Contents of solutions Calculated "Found contents calculated using Average of instrument response" and Compared with Actual content of prepared solutions. By Data Collected from Precision; Repeatability measurements: between prepared concentrations and result found.

1) From Linearity and within 9 different concentrations from 14.0 to % to 206.0 %; %Recovery found to be 98.78% with %RSD 2.34%.
2) From Repeatability and within 3 different concentration from 50%, 100% and 150% through 9 preparations; % Recovery found to be 99.40% with 1.18 %RSD.
3) Average of Recovery % through both kinds of preparations found 99.09% with RSD 0.44%. Method found accurate with % of Recovery = 99.09 %; with +/- 0.44

3.6 Precision

Precision of analytical method developed through repeatability and intermediate precision" Ruggedness" Repeatability approved through 9 preparations from 3 different concentrations, while intermediate precision approved through Day to Day and Analyst to analyst analysis.

3.7 Repeatability

1) %RSD for Instrument response for individual preparations"5 injections" found to be 0.11 % to 0.47 %
2) %RSD overall for instrument response "45 injection'' 0.28%
3) %RSD of Retention time for individual preparations " 5 injections" found to be between 0.02% to 0.26%
4) %RSD overall for retention time "45 injections'' is 0.85% and average retention time is 8.627 minutes.
5) Method found Repeatable with RSD% doesn't exceed 0.5% when testing instrument response and retention time;

3.8 Intermediate Precision

1) Day to Day Analysis: Day1 assay: 98.21% and Day 2: 97.87%, with average: 98.03% and %RSD: 0.26%
2) Analyst to Analyst: Analyst 1 assay: 98.21% and Analyst 2: 95.66%, with average: 96.94% and %RSD: 1.86%

Method found precise with RSD% doesn't exceed 0.5% when testing Instrument response and retention time; method found precise with RSD% doesn't exceed 2.0 % when testing intraday and intra analyst precision.

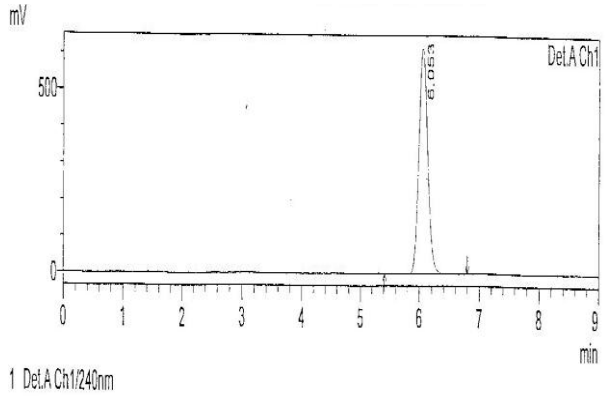

Figure 8: Chromatogram of Sildenafil citrate for intermediate precision

Table 13: Values chromatogram values of sildenafil citrate for intermediate precision

Peak #	Name	Ret.Time	Area	Height	Area %
1	Sildenafil citrate	6.053	6097960	616707	100.00
Total	-	6.053	6097960	616707	100.00

Table 14: Values chromatogram values of sildenafil citrate for intermediate precision parameters for SIL

Peak #	Name	Theoretical plate	K^1	Tailing factor	Resolution
1	Sildenafil citrate	8179.9	11.107	1.157	00.00
Total	-	8179	11.107	1.157	00.00

Table 15: Repeatability of retention time for different drug doses

A- Repeatability: "Retention Time "In Case of Chromatographic Method										
	STD	Assay 50%			100% Assay			150%Assay		
		1st Test	2nd Test	3rd Test	4th Test	5th Test	6th Test	7th Test	8th Test	9th Test
1st Retention	8.598	8.595	8.577	8.566	8.561	8.558	8.606	8.672	8.706	8.741
2 ed Retention	8.604	8.587	8.571	8.568	8.564	8.558	8.641	8.675	8.715	8.741
3 rd Retention	8.602	8.589	8.575	8.568	8.564	8.556	8.647	8.672	8.728	8.764
4 th Retention	8.604	8.585	8.575	8.565	8.559	8.561	8.654	8.67	8.727	8.749
5 th Retention	8.597	8.587	8.575	8.567	8.564	8.562	8.664	8.693	8.721	8.75
AVR	8.601	8.5886	8.5746	8.5668	8.5624	8.559	8.6424	8.6764	8.7194	8.749
STDEV	0.00332	0.00385	0.00219	0.00130	0.00230	0.00245	0.02207	0.00945	0.00913	0.00941
% RSD	0.04	0.04	0.03	0.02	0.03	0.03	0.26	0.11	0.10	0.11
Over all Avg		8.627					-	-	-	-
Over all RSD%		0.85					-	-	-	-

Table 16: Intermediate precision and ruggedness values

B. Intermediate Precision/ Ruggedness				
Claim 50 mg		WC 0.69 %	Tablet Av Wt. 308 mg	
Dilution Factor	1P 99.27%			
	Day to Day		Analyst to Analyst	
Day	1	1	30	30
WS1	49.9	50.2	50	49.9
WT1	300.5	300.2	300.1	300.5
S1 injt#1	4078263	4109230	4069025	4078263
S1 injt#2	4062991	4102421	4128121	4062991
S1 injt#3	4074806	4102424	4102967	4074806
S1 injt#4	4069856	4095498	4252546	4069856
S1 injt#5	4076549	4086280	4145874	4076549
STD1 average	4072493	4099170.6	4139706.6	4072493
RSD%	0.15	0.21	1.68	0.15
T1 inj#1	5571864	5486109	5509966	5571864
T1 inj#2	5586941	5611579	5486342	5469809
S1 inj#6	3990419	4080070	2128827	2210183
T1 Average	5571864	5548844	5498154	5571864
all std1 average	4072493	4089620	3134266.8	4072493
t1 assay	98.21	97.85	95.66	98.21
Av assay per day	98.03		Av Assay Per Analyst	96.94
%RSD	0.26		%RSD	1.86
Over all Assay AVR	97.48%			
%RSD	0.80 %			

Table 17: Summary values of precision repeatability result for different dosages

* From Precision Repeatability Results									
	50% Assay			100% Assay			150% Assay		
Actual Assay	50.36	50.36	50.21	101.00	100.71	100.86	151.50	150.93	151.50
Assay Found	50.71	50.67	50.41	100.76	100.27	99.19	147.92	147.71	151.01
Difference Between Actual Assay and found Assay	0.36	0.31	0.20	-0.24	-0.44	-1.66	-3.58	-3.21	-0.48
% Recovery	100.71	100.62	100.39	99.77	99.56	98.35	97.64	97.87	99.68
Average % Recovery	99.40 %								
%RSD	1.18%								
Average over all % Recovery	99.09%					RSD % = 0.44 %			

Method found accurate with % of Recovery = 99.09 %; with ± 0.44%

Table 18: Repeatability of Retention Time values for of Chromatographic Method

Repeatability: "Retention Time "In Case of Chromatographic Method										
STD		Assay 50%		100% Assay				150%Assay		
		1st Test	2ndTest	3rd Test	4th Test	5th Test	6th Test	7th Test	8th Test	9th Test
1st Retention	8.598	8.595	8.577	8.566	8.561	8.558	8.606	8.672	8.706	8.741
2ed Retention	8.604	8.587	8.571	8.568	8.564	8.558	8.641	8.675	8.715	8.741
3rd Retention	8.602	8.589	8.575	8.568	8.564	8.556	8.647	8.672	8.728	8.764
4th Retention	8.604	8.585	8.575	8.565	8.559	8.561	8.654	8.67	8.727	8.749
5th Retention	8.597	8.587	8.575	8.567	8.564	8.562	8.664	8.693	8.721	8.75
AVR	8.601	8.5886	8.5746	8.5668	8.5624	8.559	8.6424	8.6764	8.7194	8.749
STDEV	0.00332	0.00385	0.00219	0.00130	0.00230	0.00245	0.02207	0.00945	0.00913	0.00941
% RSD	0.04	0.04	0.03	0.02	0.03	0.03	0.26	0.11	0.10	0.11
Over all Av		8.627								
Over all RSD%		0.85								

3.9 Robustness

Robustness tested through changing in Flow rate and mobile phase Composition, and record its effect on retention time, peak shape and system suitability parameters [34].

Table 19: Robustness tests conditions of methodology

Results		
Change in	Flow rate	Mobile phase
Parameter	-10%	_4% acetonitrile
Retention Time 1	9.71	10.593
Retention Time 2	9.888	10.642
AVR	9.799	10.6175
RSD%	1.28	0.33
Tailing Factor 1	1.086	1.084
Tailing Factor 2	1.088	1.086
AVR	1.087	1.085
RSD%	0.13	0.13
N1	12657	14585
N2	13256	14520
AVR	12956.5	14552.5
RSD%	3.27	0.32

Robustness Approved by Flow rate -10% variation and mobile phase chemical composition variation Influence of variation measured from system suitability parameters through Tailing Factor, No. of theoretical plates and Retention time; and found acceptable.

Decrease flow rate to 0.9 ml/min. increase peak retention time to 9.7 min. decrease acetonitrile ratio in mobile phase increase the retention time to 10.6 min

3.10 System suitability

System Suitability Checked from data collected through Precision Repeatability Measurements, Parameters checked is Tailing factor "AVR: 1.104", No. Of Theoretical Plates"AVR: 12335" and Repeatability "RSD%: 0.28%" and found acceptable

Table 20: Repeatability of system suitability of doses of SIL drug

9. System Suitability									
	50 % assay			100 % assay			150 % assay		
	1st test	2nd test	3rd test	4th test	5th test	6th test	7th test	8th test	9thtest
Repeatability RSD %									
RSD%	0.26%	0.25%	0.11%	0.26%	0.18%	0.21%	0.29%	0.47%	0.47%
Av	False								

Table 21: Tailing factor values for SILC samples

Tailing factor									
Tailing factor 1	1.109	1.106	1.106	1.108	1.106	1.102	1.103	1.1	1.097
Tailing factor 2	1.108	1.106	1.106	1.106	1.106	1.102	1.102	1.1	1.099
Tailing factor 3	1.108	1.107	1.108	1.107	1.106	1.102	1.102	1.1	1.096
Tailing factor 4	1.107	1.107	1.108	1.105	1.107	1.103	1.102	1.099	1.096
Tailing factor 5	1.107	1.106	1.108	1.107	1.108	1.104	1.1	1.1	1.097
AVR	1.104								
RSD %	0.340								

Table 22: No. of Theoretical plates values

No. of Theoretical plates									
Theoretical plates 1	12050	12017	12090	12166	12240	12380	12535	12665	12880
Theoretical plates 2	12032	12081	11995	12208	12228	12429	12572	12684	12810
Theoretical plates 3	12019	11979	11964	12217	12189	12467	12542	12735	12916
Theoretical plates 4	12019	12006	11947	12238	12152	12482	12525	12703	12888
Theoretical plates 5	12045	12012	11956	12178	12150	12497	12601	12709	12871
Av	12335								
RSD %	2.51								

Table 23: Determination of sildenafil citrate tablets by the proposed method (A)

Brand name and dosage form	Labeled claim (mg/tablet)	Amount found (mg/tablet)	(% found ± RSD)
Sildenafil citrate tablets	50	49.545	99.09 ± 0.44

*RSD, relative standard deviation

3.11 Application of method (A)

The proposed method was applied to the pharmaceutical formulation containing sildenafil citrate. The result is shown in table 3.4.25 indicate that the high accuracy of the proposed method for the determination of the drug studied. The proposed method has the advantages of being virtually free from interference by excipient. The percentage was 99.09

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