RESEARCH ARTICLE

HPLC Determination of Sildenafil in Bulk and Tablet Dosage Form by Applying Green Analytical Chemistry

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ABSTRACT

An eco-friendly reversed-phase High-performance liquid chromatography (RP-HPLC) method has been evolved and proven for the fast analysis of Sildenafil (SIL) in both bulk drug substances and commercial tablet formulations. The separation turned into the usage of a Lichrosphere RP C8 column (250 × four.0 mm, 5 µm particle length) as the stationary phase. The cellular segment consisted of a blend of ethyl acetate and ethanol (60:forty, v/v), selected as inexperienced solvents, with a waft charge of 1.0 mL/min and UV detection at 290 nm.The technique underwent complete validation, inclusive of assessments for linearity, selectivity, accuracy, precision, reproducibility, robustness, sensitivity, and specificity. The technique's suitability was confirmed by reading Sildenafil in each bulk drug and pill paperwork. It demonstrated incredible overall performance in phrases of selectivity, precision, reproducibility, accuracy, robustness, sensitivity, and specificity. For industrial pill formulations, the Sildenafil content change was found to be 101.25% and 98.67%, respectively. Additionally, the approach effectively separated the Sildenafil peak from its degradation products, confirming its capacity as a stability-indicating approach. These findings propose that the developed technique is good for the recurring analysis of Sildenafil in each bulk drug substance and finished pharmaceutical product.

Key words-Sildenafil, HPLC, Validation, Green solvent

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INTRODUCTION

The discipline of inexperienced chemistry has been advancing unexpectedly in response to the growing international call for sustainable solutions that stabilise environmental, economic, and social goals. This shift is becoming greater critical as attention grows regarding ecological safety, pollutants discount, and the need for purifier production technology in industrial tactics. Many usually used solvents in analytical methods are risky natural compounds (VOCs), which might be unsafe air pollution (HAPs) and pose great risks, consisting of flammability, toxicity, and carcinogenicity. Analytical tactics authorised using corporations like the US Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) often rely on corrosive and toxic chemicals, with restrained secure alternatives. These solvents raise serious worries regarding human and environmental health, and manner safety, in addition to waste control and disposal demanding situations. Sildenafil is a crystalline substance, usually acting white or off-white in colouration. It is soluble in methanol but simplest barely soluble in water. With a molecular weight of 474.6 g/mol, its chemical method is C22H30N6O4S. After oral intake, sildenafil is hastily absorbed, reaching top plasma concentrations within 30 to 120 minutes. It is specifically metabolized inside the liver via the cytochrome P450 enzyme machine, and its removal is 1/2-life degrees over three to 5 hours. Sildenafil is assessed as a phosphodiesterase kind 5 (PDE5) inhibitor. It works with the aid of blocking the motion of the PDE5 enzyme, which usually breaks down cyclic guanosine monophosphate (cGMP). By stopping this breakdown, sildenafil enables elevate cGMP levels in the penis, facilitating the system of achieving and preserving an erection. Recent studies on the analysis of SIL (silymarin) emphasize the use of each natural and combined aqueous-organic solvent system. However, a lot of those solvents are risky natural compounds (VOCs), which pose environmental challenges. VOCs contribute to the formation of ground-degree ozone and smog by undertaking unfastened radical reactions inside the surroundings. To mitigate such environmental worries, the principles of inexperienced analytical chemistry inspire the discount of dangerous chemicals, minimizing waste, and reducing the ecological footprint at some stage in method improvement (Anastas & Warner, 1998; Sheldon, 2005). Green solvents, including bio solvents from renewable agricultural assets like ethanol and ethyl acetate, alongside water, provide an extra sustainable alternative to conventional petrochemical-based solvents. A new approach has been advanced that utilizes an eco-friendly cell segment comprising ethyl acetate, ethanol, and water. This, while paired with a C8 opposite-segment HPLC column, permits the speedy analysis of SIL. Moreover, this technique allows for the evaluation of SIL stability in both

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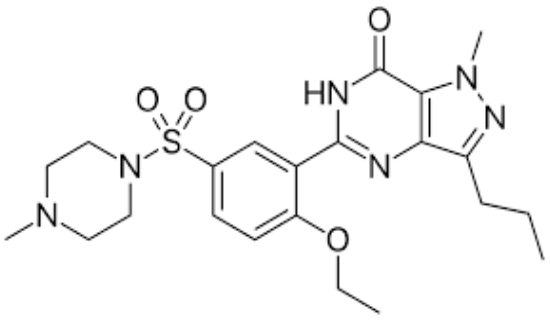

Fig no-1 Chemical structure for sildenafil. IUPAC-5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-d]pyrimidin-7-one;2-hydroxypropane-1,2,3-tricarboxylic acid

commercially to-be-had and in-house pharmaceutical formulations. An exceptional gain of this technique is its capacity to lessen exposure to unstable and corrosive organic solvents, improving protection conditions for laboratory personnel throughout the analytical method.

EXPERIMENTS

HPLC grade Ethanol, Ethyl acetate, distilled water &other chemical substances and reagents used were of analytical reagent (AR) grade purchased from Jalgaon. The analytical requirements for Sildenafil had been graciously supplied as a donation from Sigma-Aldrich (USA). Viagra 100mg Tablet was acquired from the nearby commercial market, produced by Torrent Pharmaceutical Company.

Instrumentation and chromatogram.

The observer applied a UV spectrophotometer with a Photodiode Array (PDA) detector, specifically the Alliance 2996 version, to perceive the λ max values of the pharmaceutical compounds. For technique improvement, a non-end capped ZORBAX C18 column (250 x 4.6 mm, five µm) was hired. The chromatographic system was governed by using Empower analytical software. UV detection turned into carried out at a wavelength of 290 nm, with the cellular segment composed of an inexperienced solvent aggregate of ethyl acetate and ethanol in a 60:40 (v/v) ratio. The evaluation was performed at a glide price of 0.0 mL/min, with UV detection set to 290 nm. The drift charge changed later adjusted to 0.7 mL/min, while the attention price remained constant at 1.0 mL/min, and absorbance measurements were taken at 290 nm. The temperature of the device turned into maintained at 37°C, and the total run time for the evaluation become set to 8 mins. The ZORBAX C18 column (250 x 4.6 mm, 5 µm) was specifically chosen for approach improvement in this observation.

Preparation Of Stock Solution For Calibration Curve

A calibration curve for SIL was changed to prepare the usage of concentrations from 0.1 to 200 µg/mL. A 2 hundred µg/mL inventory answer was organized with the aid of dissolving 20 mg of SIL in the cell section. To reap the target concentrations inside the variety of 0.1 to two

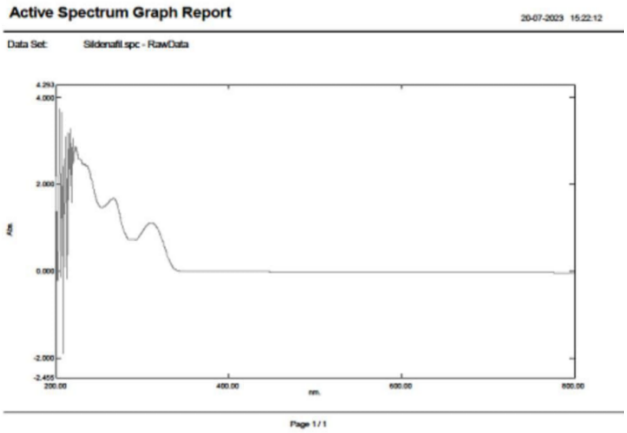

Figure 2: UV spectra for Sildenafil

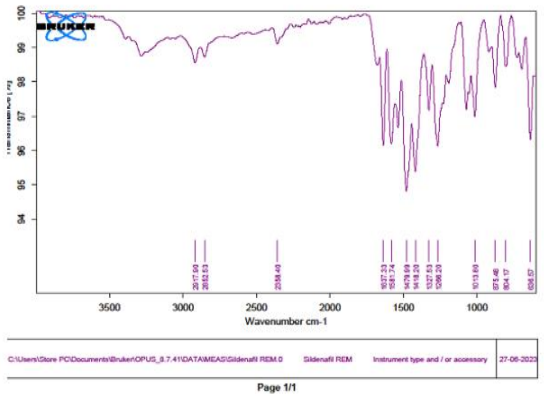

Figure 3: FTIR spectra for Sildenafil

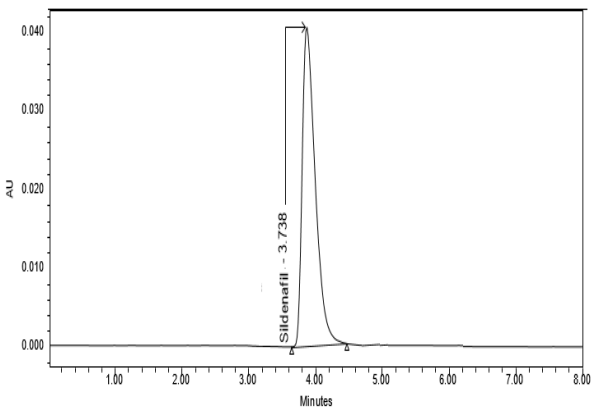

Figure 3: HPLC Chromatogram for sildenafil SAM

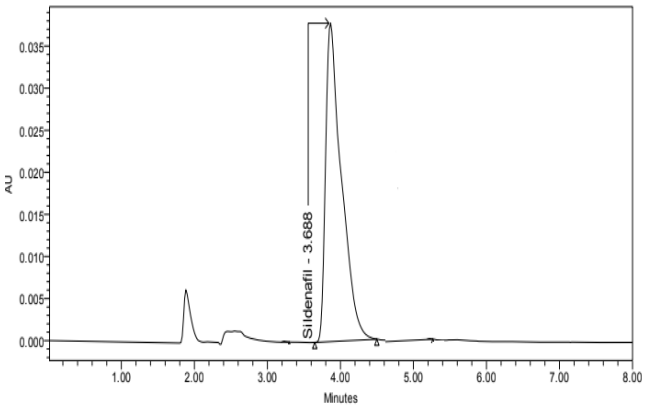

Figure 4: HPLC Chromatogram for sildenafil STD

Table 1:Selectivity of green HPLC method n=6

Sr no	concentration (microgram/ml)	Peak area	Mean area± SD	RSD%	RT(mean)	Mean Rt±SD (% RSD)
1	20	175532			3.73	
2	20	174051			3.68	
3	20	178090	176261.5±2562.565258	1.453843	3.69	3.705±0.018708287
4	20	179532			3.71	(0.504947016)
5	20	177532			3.72	
6	20	172832			3.70	

Table 2: Accuracy of green HPLC method(% of recovery n=3)

% of drug added to the analyte	Theoretical conc .(µg/ml)	Measured concentration µg/ml ±SD	RSD	% Recovery
0	20	19.67±0.020	0.105	98.35
50	30	30.33±0.22	0.7322	101.10
100	40	40.11±0.22	0.560	100.20
150	50	49.71±0.52	1.04	99.42

Table 3: Precision of green HPLC method(% of recovery n=3)

concentration µg/ml	Repeatability (intra-day precision)		Intermediate precision (inter-day)	
	Mean area±SD	RSD%	Mean area±SD	RSD%
20	177439±1987.61	1.120	172829±453.8369751	0.262593069
30	265479±1834.88	0.69	257088±263.4444913	0.102472496
40	365179±1905.26	0.512	358685±731.7403911	0.20400641
50	431279±1010.26	0.023	438688±754.4216328	0.171972252

hundred µg/mL, serial dilutions were achieved by appropriately diluting the inventory answer with the cell section.

Method development

The choice of the solvent system became based totally on several key issues, together with assay sensitivity, balance trying out criteria, evaluation time, top traits, ease of practice, and the desire for fee-effective, environmentally pleasant solvents. Different solvent mixtures were tested, together with ethanol, ethyl acetate, ethanol-water, ethyl acetate-ethanol, Tween 80, Tween eighty-water, glycerol, and glycerol-water, at various proportions. After a comprehensive evaluation, an aggregate of ethyl acetate and ethanol in a 60:40 (v/v) ratio became identified as the ultimate and environmentally conscious cellular phase for the following experiments.

Validation

The inexperienced RP-HPLC technique changed into demonstrated according to ICH guidelines, comparing key parameters inclusive of linearity, selectivity, accuracy, precision, reproducibility, sensitivity, robustness, and specificity. Calibration curves had been built with the use of trendy solutions with concentrations ranging from 0.1 to 200 µg/mL, freshly organized for each evaluation. The cell segment turned into a 60:40 (v/v) aggregate of ethyl acetate and ethanol, with a flow fee of 1.0 mL/min to equilibrate the column, while non-stop baseline tracking was maintained. Detection became accomplished at 290 nm. Each awareness was injected in triplicate, and peak regions were measured to generate a calibration curve by way of plotting awareness in opposition to the top area.

The technique's selectivity turned into determined by means of injecting several samples of a target awareness of SIL (20 µg/mL), watching any adjustments in retention time and top region to confirm its selectivity, with the consequences summarized in Table 1.

Accuracy is assessed with the usage of the usual addition approach, where a 20 µg/mL SIL solution changed into spiked with 0%, 50%, 100 %, and one hundred 50% of additional SIL answer. The samples were analyzed in triplicate, and the recovery (%), relative well-known deviation (RSD%), and fashionable errors for every spiking stage were computed.

Precision was evaluated through each intraday repeatability and intermediate precision. Repeatability was decided by studying 4 concentrations (20, 30, 40, and 50 µg/mL) in triplicate on the same day. Intermediate precision was evaluated through the evaluation on three unique days to assess the approach's consistency through the years.

The limits of detection (LOD) and quantification (LOQ) were calculated with the usage of the usual deviation (SD) of the clean samples, injected 3 times, and the slope (S) of

Table 4: Reproducibility of green HPLC method(% of recovery n=3)

concentration µg/ml	Repeatability (intra-day precision)		Intermediate precision (inter-day)	
	Mean area±SD	RSD %	Mean area±SD	RSD%
20	176322±2762.138845	1.56	169860±383.8528364	0.225981889
30	266447±4476.829347	1.68	266344±2403.05826	0.902238556
40	367817.6667±2101.123588	0.57	367065±2478.62	0.675
50	427207.6667±428.3051871	0.100	437515±1682.4216328	0.344

Table 5: Robutness of green HPLC method(% of recovery n=3)

Parameter	Mean area± SD	RSD%	RT(mean)	Mean Rt±SD (% RSD)
Mobile Phase Composition				
65:35 % v/v	178887± 873.69	0.488	3.69 ±0.011	0.31
55:45 % v/v	175252± 230.68	0.131	3.7 ±0.01	0.27
Mobile phase flow rate				
1.25 ml/min	171163± 100.74	0.058	3.73 ±0.021	0.56
0.75 ml/min	176530± 2817.23	1.51	3.70 ±0.025	0.678
Detection wavelength				
288 nm	159132± 473.10	0.296	3.7 ±0.026	0.71
292 nm	174851± 2276.12	1.301	3.73 ±0.015	0.408

Table 6: Assay of marketed formulation

Brand	Drug	Sample peak area	Standard peak area	Labelled amount (mg/tab)	% Assay	RSD
Viagra 100mg	Sildenafil	175532	176042	100	99.73	0.91

the calibration curve. The formulas used for LOD and LOQ had been:

LOD = 3.3 × (SD/S)

LOQ = 10 × (SD/S)

The robustness of the approach was tested using intentionally altering key chromatographic parameters. For those assessments, a target concentration of 20 µg/mL was used, and the effect of modifications in cellular section composition (from 60:40 to 55:45 and 65:35), flow rate (from 1.0 mL/min to 0.75 and 1.25 mL/min), and detection wavelength (from 290 nm to 288 nm and 292 nm) became evaluated to assess the robustness of the inexperienced RP-HPLC technique.

Results of assay of marketed formulation

To decide the mass of a person's pill, the hundreds of twenty pills were first measured, and the average weight was calculated. The mass of 1 tablet was then transferred into a 100 mL volumetric flask, to which 50 mL of diluent was introduced. The flask was sonicated for 25 minutes and then filtered. From the filtered answer, 1 mL was carefully pipetted into a 10 mL volumetric flask, and the quantity was made up to the mark with the same diluent.

RESULT AND DISCUSSION

To attain a sharp and symmetrical peak, ethyl acetate and ethanol were tested as capacity cell stages. The most effective mixture, inclusive of 60% ethyl acetate and 40% ethanol (v/v), provided the premier overall performance. This aggregate produced a well-defined height with a tailing factor of 1.09 and a retention time of 2.73 ± 0.50 mins. Additionally, it established desirable sensitivity and a reasonable general evaluation time of 5 minutes. The detection wavelength for SIL became set at 290 nm. Consequently, the ethyl acetate-ethanol aggregate become decided on because of the favoured mobile segment for the speedy and green evaluation of SIL in tablet formulations.

Method validation

The calibration curve for the same old SIL changed into mounted the usage of linear regression primarily based on the least squares approach. A robust linear relationship between height location and awareness was located within the concentration range of 10 to 50 µg/mL. The derived equation from the calibration curve was y = 9930x – 19117, with a super correlation coefficient (r^2) of 0.999 ± 0.0005.

To determine the selectivity of the inexperienced HPLC technique, more than one injection of SIL has been completed. The results confirmed that the usual deviation (SD) and relative well-known deviation (% RSD) for each retention time and peak place were minimal (as detailed in Table 1). Specifically, the % RSD values for retention time and height place were 0.50 and 1.14, respectively, indicating excessive selectivity of the method.

Accuracy was evaluated using figuring out the share recovery, as proven in Table 2. The restoration probabilities for the spiked drug ranged from 98.02% to one hundred and 1.73% across various concentrations, indicating appropriate precision. The general deviation values for the concentrations remained low, between 0.020 and 0.53, even as the % RSD values varied from 0. A 105% to 1.4%, further confirming the accuracy of the approach.

Intraday precision and intermediate precision were assessed with the aid of calculating the % RSD values, as supplied in Table 3. The approach confirmed extraordinary precision, with % RSD values ranging from 9.03 to at least 1.12 for intraday precision and 0.17 to 0.26 for intermediate precision. These low % RSD values endorse an excessive level of precision inside the method.

Reproducibility becomes tested by trying out the method's overall performance in an extraordinary laboratory, using a separate Waters HPLC machine and analyst. Both intraday and intermediate precision have been evaluated, without a giant variation in RSD values among the 2 laboratories, confirming the reproducibility of the approach (Table 4).

For robustness testing, the standard deviation (SD), % RSD, and standard mistakes of the height areas had been decided at the attention of 20 µg/mL beneath various situations which include cell segment composition, detection wavelength, and flow price, as proven in Table 5. The low % RSD and widespread mistake values discovered beneath these minor variations in parameters reveal the robustness and reliability of the technique.

CONCLUSION

The proposed RP-HPLC technique is green, selective, and accurate, imparting excessive precision, reproducibility, and robustness. It showed extraordinary sensitivity while used to research SIL in commercial tablet formulations, handing over steady and reliable assay consequences. The technique is notably effective, putting off the need for sample extraction, and making use of an environmentally friendly mobile segment. With a short retention time, it allows rapid evaluation and UV detection is used without requiring internal popularity. These blessings make the method ideal for routine high-quality control of SIL in each bulk substance and quite a few industrial products. Furthermore, the technique employs safer, much less poisonous solvents and reagents, presenting a greater sustainable alternative to traditional, hazardous materials commonly used in pharmaceutical evaluation.

Conflict of interest

The authors file no warfare of hobby

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