

Review Article

Spectrophotometric and HPLC – MS / MS for Determination of Silodosin in Pharmaceutical Formulation

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Abstract

A simple, selective, and sensitive spectrophotometric and HPLC – MS / MS method for determining microgram amounts of silodosin is described. Silodosin is converted into (R)-1-(3-hydroxypropyl)-5-(2-((2-(2,2,2-trifluoroethoxy)phenoxy)ethyl)amino)propyl)-2,3-dihydroindoline-7-carboxyhydroxamic acid, which gives a purple violet colour ($\lambda_{\max} = 510 \text{ nm}$) with ferric chloride in an acidic medium. This forms the basis for the quantitative determination of ethionamide in both its pure form and tablet form. The influence of commonly used excipients is also studied. The HPLC determination was performed on a C18 column using an acetonitrile and water mobile phase. Both methods were validated for linearity, precision, accuracy, and robustness. The HPLC method demonstrated good linearity over the spectrophotometric range of 3–18 $\mu\text{g/mL}$.

Keywords: Silodosin; HPLC – MS / MS; Spectrophotometric; Hydroxamic acid

Introduction

Silodosin is used to treat bothered by Lower Urinary Tract Symptoms (LUTS) secondary to Benign Prostatic Hyperplasia (BPH) [1]. Silodosin determines smooth muscle relaxation in bladder and prostate tissues, increases bladder blood flow in conditions of chronic bladder ischemia and regulates the activity of transcriptional factors responsible for stromal growth and prostate hyperplasia [2].

Silodosin, a potent α_{1A} -adrenergic receptor antagonist, stands out for its unprecedented selectivity. Unlike its counterparts, it specifically targets α_{1A} receptors, making it an attractive therapeutic option. Silodosin effectively tackles Lower Urinary Tract Symptoms (LUTS) associated with Benign Prostatic Hyperplasia (BPH). Its rapid onset of action improves voiding and storage symptoms, enhancing overall quality of life. Notably, this medication prioritizes cardiovascular tolerability, making it a promising choice for patients in need. In summary, silodosin holds the key to alleviating BPH-related urinary woes [3-11].

Silodosin is a new type of alpha-blocker that has a unique ability to selectively target α_{1A} -adrenergic receptors, compared to α_{1B} and α_{1D} receptors [3,4]. It works effectively as an α_{1A} -adrenergic receptor blocker and does not have major side effects [5]. Silodosin is a safe and effective treatment for relieving symptoms of lower urinary tract issues related to Benign Prostatic Hyperplasia (BPH) [6]. When taken orally, silodosin quickly improves symptoms in men with BPH, including voiding and storage symptoms, and increases

the maximum urinary flow rate [7-9]. It works by improving bladder storage function and relieving the obstruction caused by an enlarged prostate [10]. Silodosin is particularly beneficial for patients who need a treatment that is gentle on the cardiovascular system [11].

Literature shows that various methods have been developed and validated for estimating silodosin in bulk and pharmaceutical forms. These methods include UV-Vis Spectrophotometry, Uv-Spectroscopic and RP-HPLC method, and a Spectrofluorimetric method. These techniques have been used to accurately measure silodosin in pure form, pharmaceuticals, and biological samples [12-14].

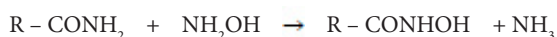
Several innovative methods have been developed for the analysis of silodosin. A new, specific, and stable method using Ultra-High-Performance Liquid Chromatography (UHPLC) was developed and validated. This method, compatible with mass spectrometry, accurately determines silodosin and its related substances. In addition, a simple, sensitive, precise, and specific method using high-performance thin layer chromatography was developed and validated. This method is particularly useful for determining silodosin in bulk and in pharmaceutical dosage forms. Lastly, a new eco-friendly method using High-Performance Liquid Chromatography (HPLC) with dual detection modes was presented. This method allows for the versatile quantification of dutasteride and silodosin in pharmaceutical formulations. These advancements significantly contribute to the field of pharmaceutical analysis. [15-17].

Various analytical methods are described in the literature for examining Silodosin. These include a selective spectrofluorimetric method with enhanced sensitivity for determining Silodosin in dosage forms and human plasma, RP-HPLC for analysing the kinetics of Silodosin degradation, and liquid chromatography/Fourier transform mass spectrometry for characterizing the degradation products of Silodosin under stress conditions. Stability-indicating spectrophotometric methods are used to determine Silodosin in the presence of its degradation product. Additionally, an LC-ESI-MS/MS method is employed to study the forced degradation behaviour of Silodosin and evaluate the anticancer activity of Silodosin and its major degradation products [18-21].

In simpler terms, researchers have developed two methods for analysing silodosin, a drug used to treat urinary problems. The first method uses capillary gas chromatography to detect and measure the amount of two genotoxic impurities in silodosin, which are harmful substances that can damage genetic material. This method also includes an in-silico toxicity assessment, which is a computer-simulated evaluation of the potential harmful effects of these impurities. The second method, guided by a quality by design approach, uses capillary electrophoresis to determine the chiral purity of silodosin, which is a measure of the balance between its two mirror-image forms. These methods ensure the safety and effectiveness of silodosin [22-23].

Chromatography is known as the most effective method for analysis.

In the present spectrophotometric method is based on the ferric hydroxamate method for determination of amides as



The HPLC is a more commonly used tool in quality control as a result spectrophotometric and HPLC methods are developed for silodosin.

Materials and Methods

Chemicals and Reagents

Aristo Pharmaceutical gave a free Silodosin API sample. All analysis reagents were AR grade. SDFCL (Mumbai) supplied Ortho Phosphoric acid.

Reagents Preparation for UV method

- **Hydroxylamine hydrochloride (2.0 M):** Dissolve 35 gm of hydroxylamine in 250 mL of distilled water.
- **NaOH Solution (3.5 M):** Dissolve 14 gm of NaOH in 100 mL of distilled water.
- **HCl Solution (3.5 M):** Dissolve 30 mL of HCl in 100 mL of distilled water.
- **Drug solution:** Dissolve 1 mg of the drug in 100 mL of ethanol.
- **FeCl₃ (0.1 M) in 0.1 M HCl:** Dissolve 8 gm of FeCl₃ in 100 mL of 0.1 M HCl solution.
- **Alkaline solution:** Mix 50 mL of Hydroxylamine hydrochloride with 50 mL of NaOH solution. The ratio is 50:50.

Apparatus

A Shimadzu HPLC-LC-2010CHT with a UV detector to develop, optimize, and validate Silodosin. Separation was done using a C18 column (250 × 4.6 mm, 5 μm). The solution's pH was adjusted using a Systronics MK VI pH meter. Filtration used 0.45 μm nylon membranes.

Solvents were degassed with an ultrasonicator. The Silodosin API was weighed with a WENSARTM DAB 220 balance. The optimal mobile phase was a mix of Methanol, Acetonitrile, and 10 mM Phosphate buffer (pH adjusted to 4 using 1% OPA) in a 40:40:20 ratio. The flow rate was 1 mL/min, the column temperature was 25 ± 1, the injection volume was 10 μL, and the detection wavelength was 225 nm. A Shimadzu UV-1800 spectrophotometer with UV Probe software was used, with a double beam and 1.0 cm quartz cells.

Preparation of the Standard Solutions

Accurately weigh 10 mg of Silodosin and transfer it into a 100 mL volumetric flask. Dissolve it in Mobile Phase and dilute to mark with the same solvent to obtain a standard stock solution of 100 μg/mL. From this solution, pipette out volumes of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL and transfer each to a 10 mL volumetric flask. Fill each flask up to the mark with Mobile Phase, resulting in concentrations of 5, 10, 15, 20, 25, and 30 μg/mL respectively. For the Spectrophotometric method, accurately weigh 10 mg of Silodosin reference standard and transfer it to a 100 mL volumetric flask. Dissolve it in a solution. Prepare concentrations of 3, 6, 9, 12, 15, and 18 μg/mL.

Preparation of the Sample Solutions

In the HPLC method, an accurately weighed amount of Silodosin capsule powder, equivalent to 8.0 mg of Silodosin, was transferred to a 100 mL volumetric flask and dissolved in the mobile phase. The final prepared solution had a concentration of 80 μg/mL. For the UV Spectrophotometric method, 12.5 mL of alkaline solution is added to 1 mL of the drug solution. This mixture is heated for 5 minutes and then cooled in an ice bath. Next, 5 mL of HCl (3.5M) and 5 mL of FeCl₃ (0.1M) are added. The absorbance is then measured at 591 nm using UV Spectrometry (UV-1800 Shimadzu).

Method Validation

The methods were validated according to International Conference on Harmonization (ICH) guidelines Q2 (R1) for validation of analytical procedures [9]. The System Suitability Test was carried through for both the methods evaluating theoretical plates and asymmetry.

Linearity

The calibration curve was generated using five concentrations of the standard solution (5-30 μg/ml for the HPLC method and 3-18 μg/ml for the UV spectrophotometric method). Linearity was assessed using linear regression analysis, calculated by the least square regression method.

Precision

Assay precision was assessed through repeatability (within-day), intermediate precision (intraday), and reproducibility (inter-day). Repeatability involved analysing samples at the same concentration

on the same day, with a repeatability of 10 µg/mL for the HPLC method and 6 µg/mL for the UV spectrophotometric method ($n = 6$). Intermediate precision was evaluated by analysing samples at three different concentrations and three different times within the same day, with concentrations of 5, 10, and 15 µg/mL for the HPLC method and 3, 6, and 9 µg/mL for the UV spectrophotometric method ($n = 3$). Reproducibility was assessed by analysing samples at three different concentrations on three different days, with concentrations of 5, 10, and 15 µg/mL for the HPLC method and 3, 6, and 9 µg/mL for the UV spectrophotometric method ($n = 3$).

Accuracy

Accuracy in an analytical method reflects how closely the test results match the true value. In the UV spectrophotometric method, accuracy was gauged by recovering known amounts of silodosin reference standard added to the samples initially. The accuracy was evaluated through three replicate determinations at three concentration levels: 11.08, 12, and 13.02 µg/mL. The absolute means obtained were 99.83%, 99.66%, and 100.22%, indicating the method's accuracy within the desired range, as depicted in Table 2. For the HPLC method, accuracy was determined by assaying three concentrations of the sample solution (18, 20, and 22 µg/mL) in triplicate. The absolute means obtained were 100.16 %, 100.41 %, and 99.92 %, as illustrated in Table 3.

Robustness

The HPLC method's robustness was evaluated by analysing samples under different conditions, including slight variations in pH (ranging from 3.5 to 4.0) and flow rate (0.8 to 1 mL/min). This assessment included studying the impact on retention time, Silodosin quantification, and peak parameters. Meanwhile, the robustness of the UV spectrophotometric method was determined by making small adjustments to the wavelength.

Results and Discussion

HPLC Method

The choice of mobile phase was guided by factors like peak symmetry, run time, ease of preparation, and cost. Figure 2 displays a typical chromatogram resulting from analysing both a standard and a sample solution of Silodosin using this method. The chromatogram illustrates a symmetrical peak for Silodosin, with a retention time of 2.9 minutes, enabling quick drug determination, vital for routine analyses. Calibration curves were constructed by plotting peak area against concentration, showing excellent linearity within the 5-30 µg/mL range. The linear equation, $y = 18104x + 133239$, and the highly significant correlation coefficient ($r = 0.9976$) underscore the method's reliability (Table 1).

Table 1: Linearity study Data.

Parameter	HPLC Method	UV Method
Concentration range	5 – 30 µg/mL	3 – 18 µg/mL
Regression equation	$y = 18104x + 133239$	$y = 0.0357x - 0.0702$
Correlation coefficient (R^2)	0.9976	0.9972
Standard error of slope	18104	0.0357
Standard error of intercept	133329	0.0702
Limit of detection (µg/ml)	0.39	0.04
Limit of quantification (µg/mL)	1.18	0.14

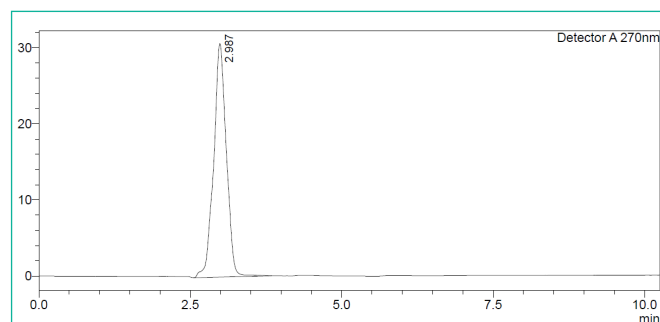


Figure 1: chromatogram of sample solution (80 µg/mL).

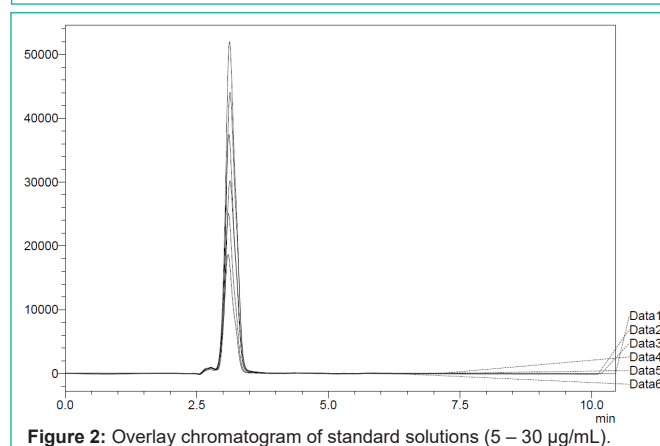


Figure 2: Overlay chromatogram of standard solutions (5 – 30 µg/mL).

The sensitivity of method was indicated by LOD and LOQ values of 0.3 and 1.18 µg/mL, respectively, reflecting high sensitivity. Method precision was assessed through repeatability, intermediate precision, and reproducibility, expressed as R.S.D (%) of multiple measurements. Results showed a repeatability R.S.D of 0.81%, an intermediate precision mean R.S.D of 0.77%, and a reproducibility mean R.S.D of 0.87%. The method's accuracy was determined, with a mean recovery ranging from 99.92% to 100.41%, indicating agreement between the true and found values (Table 3). Specifically designed for assessing Silodosin in tablets, the HPLC method exhibited specificity, with no interfering peaks observed even when simulated excipient samples were added to the solutions. The method was also found to be robust against variations in pH and flow rate.

Spectrophotometric Method

The impact of altering the concentration of hydroxylamine hydrochloride was examined at 591 nm. During this investigation, the concentration of silodosin was maintained constant, while the concentration of hydroxylamine hydrochloride was varied. The proposed spectrophotometric method offers quick and convenient quantification of Silodosin in capsules without needing extensive sample preparation. Additionally, it employs simple instrumentation compared to other techniques. The absorption spectra of Silodosin revealed a λ_{max} of 270 nm, which was utilized in the analysis. Calibration curves were established within the expected concentration range (3 - 18 µg/mL), yielding the representative equation $y = 0.0357x - 0.0702$, with a correlation coefficient of 0.9972 (Table 1). LOD and LOQ were determined as 0.04 and 0.14 µg/mL, respectively, confirming the method's efficacy for Silodosin determination. Precision assessment resulted in a repeatability R.S.D of 0.57%, an intermediate precision

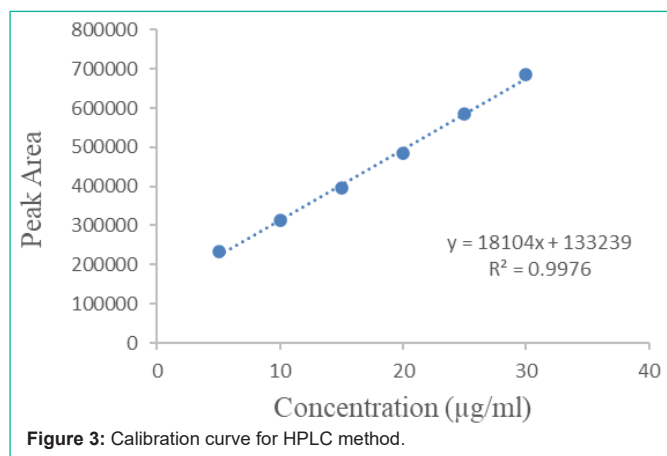


Figure 3: Calibration curve for HPLC method.

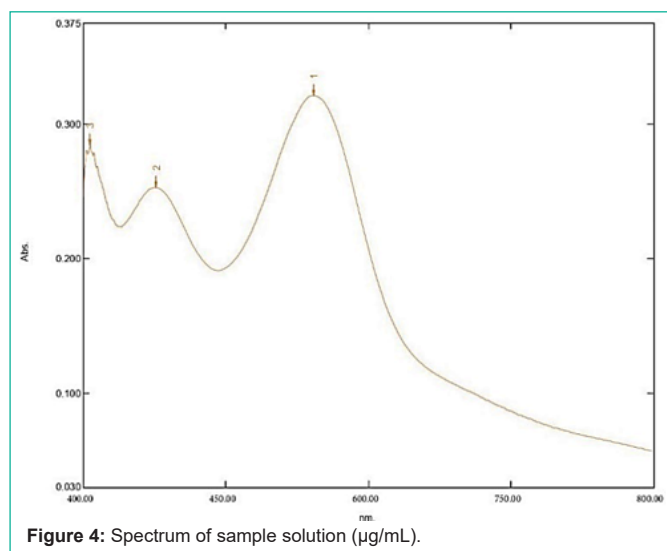


Figure 4: Spectrum of sample solution (µg/mL).

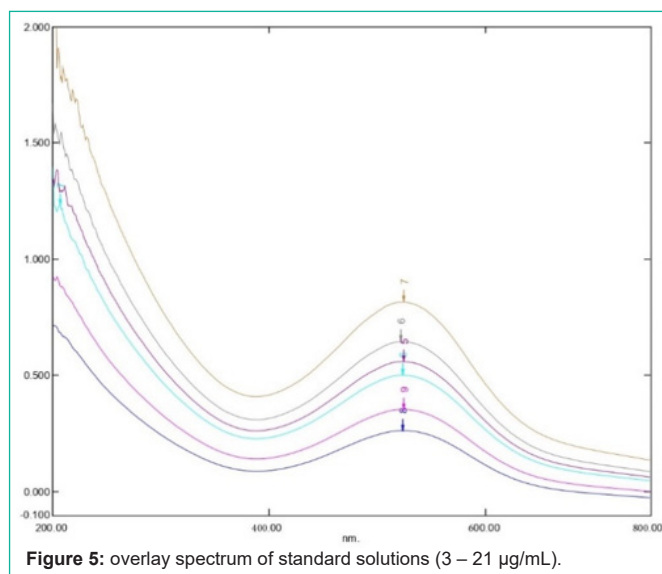


Figure 5: overlay spectrum of standard solutions (3 – 21 µg/mL).

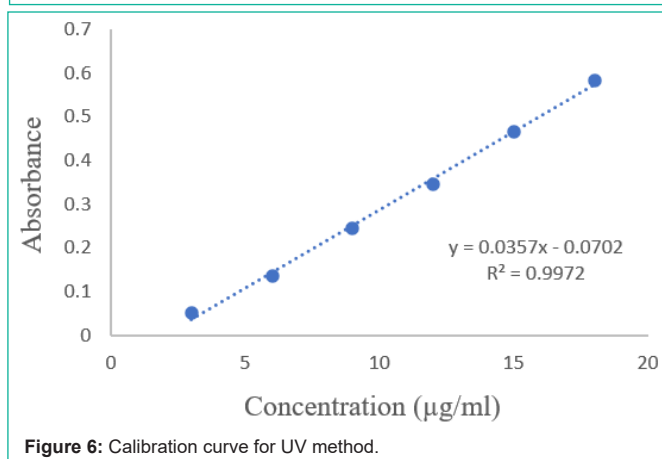


Figure 6: Calibration curve for UV method.

Table 2: Precision tests Data.

Precision Parameter	HPLC Method			UV Method		
	Con. (µg/mL)	Mean Peak Area	R.S.D. (%)	Con. (µg/mL)	Mean Absorbance	R.S.D. (%)
Repeatability (n = 6)	10	314613	0.811	6	0.136	0.579
Intermediate precision (n = 3)	5	234991	0.883	3	0.0517	0.678
	10	314201	0.868	6	0.1367	0.335
Reproducibility (n = 3)	15	394773	0.564	9	0.2463	0.204
	5	235205	0.965	3	0.0605	0.595
	10	315897	0.957	6	0.1403	0.653
	15	396381	0.675	9	0.2371	0.345

Table 3: Recovery tests Data.

Method	Level of drug taken (%)	Mean percent recovery (%)	S. D
HPLC	80	100.16	0.257
	100	100.41	0.276
	120	99.92	0.44
UV	80	99.83	0.03
	100	99.66	0.215
	120	100.22	0.047

Table 4: Robustness study Data.

Method	Condition	Variation	% Assay
HPLC	pH	4	99.84
		3.5	99.51
	Flow rate	1.0 mL/min	100.2
0.8 mL/min		99.3	
UV	Detection wavelengths	591	100.1
		594	99.7

mean R.S.D of 0.40%, and a reproducibility mean R.S.D of 0.53%. Accuracy evaluation revealed a mean recovery ranging from 99.66% to 100.22%, indicating agreement between the true and measured values (Table 3). Lastly, the method demonstrated specificity in determining Silodosin in capsules (Figure 4).

Conclusion

In this study, High-Performance Liquid Chromatography (HPLC) and UV-Spectrophotometric methods were successfully developed and validated for analysing silodosin, an α 1-adrenoceptor antagonist used in treating Benign Prostatic Hyperplasia (BPH). Both methods demonstrated excellent linearity, precision, accuracy, and robustness, meeting ICH guidelines. The HPLC method showed superior specificity and resolution, making it suitable for complex matrices and trace-level silodosin analysis in pharmaceutical formulations. Conversely, UV-Spectrophotometry proved simpler and more cost-effective for routine analysis, providing rapid quantification at Silodosin's maximum absorbance wavelength of 280 nm. Comparative evaluation of HPLC and UV-Spectrophotometric methods revealed HPLC's enhanced sensitivity and selectivity, while UV-Spectrophotometry offered practical advantages in instrument availability, ease of operation, and affordability. Therefore, the method choice should consider specific analytical needs, sample complexity,

and resource availability. Both HPLC and UV-Spectrophotometric methods are suitable for silodosin quantification in pharmaceutical formulations, ensuring quality control and regulatory compliance. This research contributes valuable insights to analytical chemistry, aiding analysts and researchers in selecting the most appropriate method for silodosin analysis based on their specific needs and constraints.

Author Statements

Conflicts of Interest

Authors have declared that there is no conflict of interest.

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