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Short Communication

# HPLC DETERMINATION OF SILDENAFIL IN TABLETS

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**ABSTRACT**

**Objective:** The popularity of Sildenafil, the widespread distribution of various products and dietary supplements with added synthetic drugs, requires reliable analysis methods. This research study aimed to develop a simple isocratic HPLC method for the determination of Sildenafil in tablet dosage forms from the local market.

**Methods:** Separation was carried out at 30 °C, using column LiChrosorb® RP-18 (150 x 4.0 mm, 5 µm) with mobile phase consisting of acetonitrile: methanol: 0.5% triethylamine (15: 26: 59 v/v/v). The detector was set at 290 nm. The flow rate was 1.0 ml/min and the injection volume was 20 µl.

**Results:** Linear correlation was obtained within the range 6.25–50.0 µg/ml with correlation coefficient ($R^2$) 0.9998. The achieved limits of detection and quantitation were 0.7 and 2.2 µg/ml, respectively.

**Conclusion:** The developed method can be applied for the quality control of Sildenafil preparations.

**Keywords:** Erectile dysfunction, Sildenafil, HPLC, Phosphodiesterase inhibitors, Tablets, Drugs


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Erectile dysfunction is a socially significant disorder. It can be caused by both various medical conditions [1] and psychogenic factors [2]. Most of erectile dysfunction treatment approaches are based on phosphodiesterase inhibitors [3]. Sildenafil citrate (chemically known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methyl piperazine citrate) is a potent and selective phosphodiesterase inhibitor. It is the first phosphodiesterase inhibitor introduced on the market (Viagra®, Pfizer) [4].

The social importance of erectile dysfunction, drug efficacy and easy internet access to Sildenafil (even without a prescription) can lead to unreasonable and uncontrolled use. Moreover, online drug purchases are always a risk, particularly in the case of illegal products that may contain poor-quality drugs or unknown analogs. This is especially dangerous for patients with preconditions for cardiovascular disease and/or concomitantly receiving cardiovascular drugs. On the other hand, healthy foods and dietary supplements have become increasingly popular in recent years. Many dietary supplements (advertised as "natural" or "herbal") are available on the market to help combat erectile dysfunction or improve sexual activity. Low control on such products makes it possible to insert medicines of unclear origin and quality, which in turn lead to health risks [5-8].

The popularity of Sildenafil, the widespread distribution of various counterfeit products and dietary supplements with added synthetic drugs requires analysis methods for analysis.

Various analytical techniques like spectrophotometry [9, 11], Raman spectroscopy [11, 12], thin layer chromatography [13], high performance liquid chromatography [14-23], high performance liquid chromatography-mass spectrometry [24-27] are reported for Sildenafil determination in pharmaceutical preparations [10, 12, 18-20, 22, 23, 25], biological samples [16, 20, 21, 24-27], dietary supplements [3, 5, 13], herbal products [15] and drinks [11].

Where the determination of traces or unknown analogs is necessary, then a highly sensitive technique, capable to provide information on the structure of the target substances is required. On the other hand, when a single substance is determined, conventional techniques are preferable due to their simplicity, accessibility, and relatively lower costs.

This article presents the development of a simple isocratic HPLC method for the determination of Sildenafil in tablet dosage forms obtained randomly from the local market.

The reagents used in this study were Sildenafil citrate (reference substance was obtained by Sigma Aldrich), HPLC grade acetonitrile, and methanol (Merck Ltd., Germany). Sildenafil tablets (50 mg) were obtained randomly from the local market. All other chemical reagents were of analytical grade.

For the preparation of the reference solution, 25 mg (accurately weighed) of Sildenafil were dissolved with the mobile phase in a 100.0 ml volumetric flask. Ten milliliters of this solution were diluted to 100.0 ml with the mobile phase (C = 25 µg/ml).

For sample solution preparation, ten tablets of each formulation were weighed and were crushed to a fine powder. An amount equivalent to 50 mg Sildenafil (1 tablet) was weighed accurately and transferred in a 100.0 ml volumetric flask. Around 50 ml of the mobile phase were added and the mixture was sonicated for 10 min. Then the sample was diluted to 100.0 ml with the mobile phase. The solution was filtered and 5.00 ml were diluted to 100.0 ml with the mobile phase (C = 25 µg/ml).

In this study, a high performance liquid chromatographic system (SHIMADZU Corporation) equipped with an LC-20 AD quaternary pump and autosampler, Shimadzu DGU-20$A_5$ vacuum degasser, and a Shimadzu SPD-20A UV/VIS detector was used for analysis. To optimize the chromatographic conditions, some preliminary studies were conducted.

In these studies, different stationary phases (C8 and C18) were used, as well as mobile phases with different compositions and ratios of the organic and aqueous phases. The combinations of acetonitrile: water and methanol: water in different ratios did not lead to satisfactory results. The effects of organic modifier (triethylamine, 0.3-0.7%), flow rate, column temperature, and wavelength were also investigated. In the end, the optimal conditions achieved were as follows: C18 column (LiChrosorb® RP-18 (150 x 4.0 mm, 5 µm)), at 30 °C and a mobile phase consisting of acetonitrile: methanol: 0.5% triethylamine (15: 26: 59 v/v/v). The detector was set at 290 nm. The flow rate was 1.0 ml/min and the injection volume was 20 µl. The mobile phase and the samples were filtered through a 0.45 µm membrane filter. The data was recorded using Lab Solutions Software. The retention time of Sildenafil was approximately 10 min and was comparable to the retention times from previously published reports [18, 23].

The analytical method was validated according to the International Conference on Harmonization (ICH) guidelines [28].

The specificity of the HPLC method was determined by analyzing the standard drug solution and sample solution. As shown in fig. 1 (standard solution) and fig. 2 (sample solution), there was no interference by the formulation excipients since no other peaks were corresponding to the retention time of the Sildenafil.

The chromatographic parameters for Sildenafil, obtained at optimal experimental conditions, are listed in table 1.

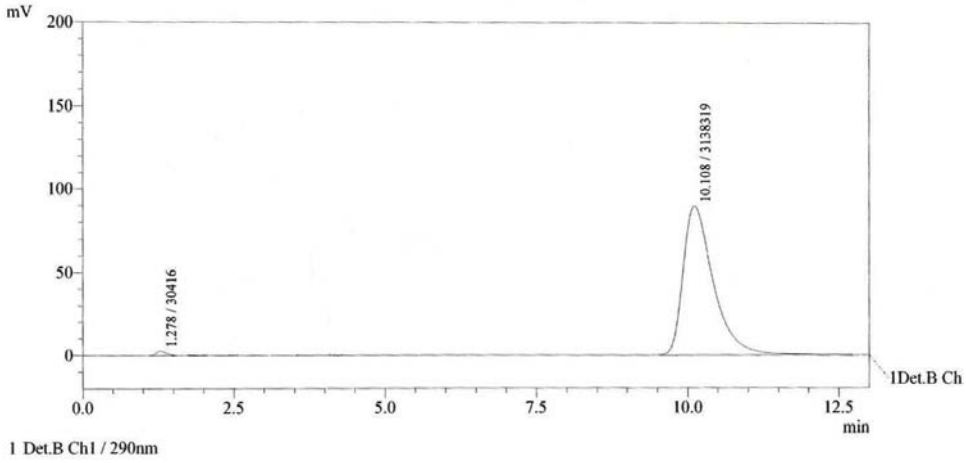


**Fig. 1: Chromatogram of Sildenafil standard solution**

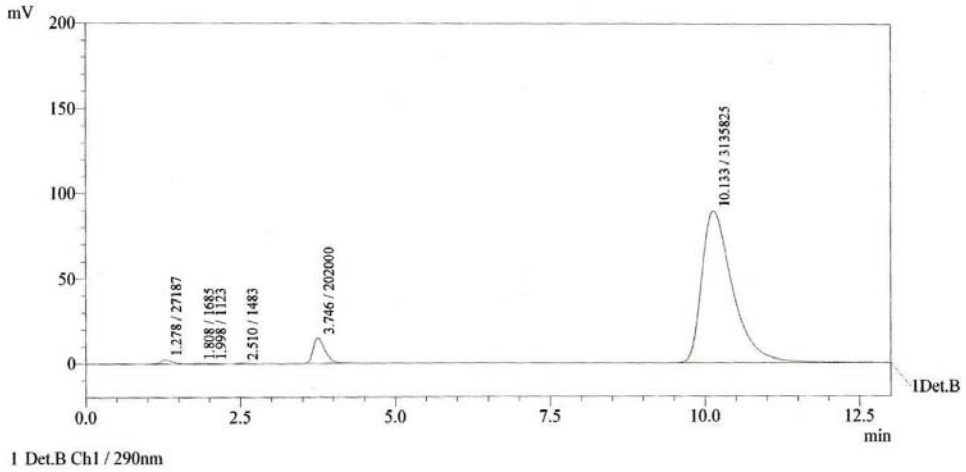


**Fig. 2: Chromatogram of Sildenafil sample solution**

**Table 1: Chromatographic data for the HPLC method**

| Parameter | Sildenafil |
|---|---|
| Theoretical plates | 2126±3.6 |
| Tailing factor | 1.64±4.71x$10^{-4}$ |
| Resolution factor | 10.37±0.01 |
| Retention factor | 6.92±0.01 |

*The values are expressed as mean±SD, n=3

Five standard solutions with concentrations within the range 6.25–50.0 µg/ml were used for calibration and linearity study. Each solution was injected five times. The response (peak area) was plotted against the concentration of standard solutions. A linear correlation was obtained and the regression equation was y = 126217.2x–29536.0 with correlation coefficient ($R^2$) 0.9998. Limit of Detection (LOD) and Limit of Quantitation (LOQ) were evaluated based on signal-to-noise ratio and were found to be 0.7 and 2.2 µg/ml, respectively, which are similar to earlier reported [16, 23]. The precision was determined by six successive injections of a sample at the 100% concentration level of the Sildenafil. The RSD (%) values (table 2) for intra-and inter-day precision were found to be 0.16% and 0.65%, respectively, thus indicating that the method is precise. The method accuracy was evaluated by recovery studies. Samples at a concentration range of 50%-150% were prepared and each concentration level was injected in triplicate. The accuracy was expressed as the percentage of analyte recovered (>98%) and RSD (<1%). The results listed in table 3 show good accuracy and indicate that the proposed method is suitable for the quantitative determination of Sildenafil.

**Table 2: Precision of the method**

| Amount taken (mg/tablet) | Intra-day | | Inter-day | |
|---|---|---|---|---|
| | Amount found (mg/tablet) | Amount found (%) | Amount found (mg/tablet) | Amount found (%) |
| | 50.09 | 100.2 | 50.15 | 100.3 |
| 50.00 | 49.90 | 99.80 | 49.95 | 99.90 |
| | 49.96 | 99.92 | 49.86 | 99.72 |
| | 50.04 | 100.1 | 50.07 | 100.1 |
| | 49.89 | 99.78 | 49.79 | 99.58 |
| | 49.93 | 99.86 | 49.23 | 98.46 |
| Mean | 49.97 | 99.94 | 49.84 | 99.68 |
| ±SD | 0.081 | 0.170 | 0.327 | 0.649 |
| RSD % | 0.161 | 0.171 | 0.657 | 0.652 |

*The values are expressed as mean±SD, n=6

**Table 3: Recovery studies of Sildenafil**

| Level (%) | Amount taken (mg) | Amount found (mg) | Amount found (%) | ±SD | RSD (%) |
|---|---|---|---|---|---|
| 50 | 25 | 24.96 | 99.84 | 0.048 | 0.194 |
| 100 | 50 | 49.43 | 98.86 | 0.097 | 0.194 |
| 150 | 75 | 74.96 | 99.95 | 0.121 | 0.161 |

*The values are expressed as mean±SD, n=3

The method was applied for the determination of Sildenafil in tablet dosage forms, randomly obtained from a local market. No significant differences were found between achieved results (table 4) and label claim.

**Table 4: Determination of Sildenafil in tablet formulations**

| Product | Amount declared (mg/tablet) | Amount found (mg/tablet) | Results (%) |
|---|---|---|---|
| Product 1 | 50 | 50.08±0.022 | 100.2 |
| Product 2 | 50 | 49.56±0.036 | 99.12 |
| Product 3 | 50 | 49.83±0.046 | 99.66 |

*The values are expressed as mean±SD, n=3

## CONCLUSION

The isocratic HPLC-UV method, described in this paper, was developed for determination and quantity control of the Sildenafil in tablets. The procedure showed satisfactory run time (12 min), good linearity range (6.25–50.0 µg/ml), and a high degree of accuracy and precision (RSD<1%). The method is simple, easy for implementation, and requires common equipment; therefore, it is cost-effective. It was successfully applied to real samples.

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Nil

## AUTHORS CONTRIBUTIONS

All authors have contributed equally.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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