RESEARCH ARTICLE

Development and Validation of Analytical Method for Estimation of Sildenafil Citrate in Swab Samples

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Received: 19th November, 2023; Revised: 17th December, 2023; Accepted: 15th April, 2024; Available Online: 25th June, 2024

ABSTRACT

Among the most significant issues with pharmaceutical production in shared facilities is the risk of cross-contamination, which lowers product quality and costs the business a lot of money. This work set out to evaluate a cleaning approach by creating and testing a straightforward UV spectrophotometric method for estimating sildenafil citrate in swab samples. To get a good recovery from a stainless steel surface utilising a sterile cotton swab stick, the swabbing technique was fine-tuned. A wavelength of 292.20 nm was chosen for detection. Linearity, precision, accuracy, detection limit, and quantification limit were the criteria used to validate the suggested approach. A correlation coefficient of 0.9993 was determined for the regression line after studying linearity throughout a concentration range of 5 to 25 µg/mL. An average of 88.23% was recovered.

Keywords: Sildenafil citrate, Swab testing, UV- Spectroscopy, Cleaning validation.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.38

How to cite this article: Gaikwad D, Dhobale G, Kakade R, Tare H. Development and Validation of Analytical Method for Estimation of Sildenafil Citrate in Swab Samples. International Journal of Drug Delivery Technology. 2024;14(2):858-861.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cleaning validation is the process of documenting the results of an approved cleaning method. It ensures that the equipment will be suitable for processing pharmaceutical goods or APIs. Swab sampling, which involves drawing blood from the surface of the body, rinse solutions, and a placebo were all deemed suitable sample methods for validating the cleaning procedure.[1]

As a means of controlling the possibility of microbiological contamination, cleaning validation ensures that the cleaning process successfully removes product residues, degradation products, additives, and cleaning chemicals.[2] In addition, it is crucial to ensure that there is absolutely no chance of active components becoming cross-contaminated. Establishing a dependable cleaning process is the primary objective of the cleaning validation, which aims to minimise or eliminate analytical monitoring during the routine phase.[3] It is now required by law to validate cleaning processes. Manufacturing equipment must be kept clean and organized according to GMP guidelines (part 133.4). Processing apparatus should designed to be visually inspectable and to facilitate cleanability.[4] When possible, it should prepared from nonreactive materials with uneven surfaces. It is important to identify critical sites, that are the most challenging to clean.[5]

If direct surface sampling might be hindered by a lack of access to equipment parts, a combination of the first two processes is usually the way to go. Before using the sampling medium and solvent, make sure they are suitable and easy to use. You can sample a lot of ground with rinse samples. In addition, difficult-to-reach parts of machinery that aren't dismantled often can be examined. Nevertheless, the solubility of the contaminant needs to be considered.[6] It is important to directly test the product remainder proper solvent. To practise making future batches, the placebo sampling method is useful. Due to the large amount of variety in the apparatus/methods utilized across ended & bulk items, there are no clear recommendations to verify the validity of a cleaning procedure.[7] Therefore, it is expected that pharmaceutical corporations will set acceptance criteria that are well-thought-out, reasonable, achievable, empirical, and supported by science. The analytical procedures employed to ascertain contaminants or residuals should be substance-specific & deliver a perspective that mirrors hygiene deemed respectable by the organization. To establish acceptable boundaries, it is necessary to specify the analytical method's sensitivity.[8] Products or medications treated, processing equipment, drug strength, toxicity levels, and acceptability criteria can all change. The validation

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of the cleaning process needs to be defined, and for that, a confirmation protocol is required. The document lays out the goals of the validation process, who is responsible for carrying them out and giving their stamp of approval to the study, what tools will be utilized, how long it will be until production is finished and cleaning can begin, how many lots of the same product can be made in a drive before cleaning is complete, and so on. Specific methods for cleaning every product, system, recommended sequence of washing cycles, the need for regular monitoring, methods for sampling, including the rationale behind the selection of a particular sampling technique, clearly specified sampling locations, information pertaining to research on possible recoveries, analysis methods that have been validated, including detection and quantification limits for those styles; acceptance criteria, including an explanation of how specific limits were set.[9,10]

One such phosphodiesterase type 5 inhibitor is sildenafil citrate, which is both powerful and selective. Its chemical formula is 5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulonylphenyl](1) methyl-3-propyl-6H-pyrazilo[4,3-d]pyrimidin-7-one. One structurally comparable pyrazolopyrimidinone derivative of zaprinast is the salt of this compound, which is known as sildenafil citrate. Vasodilation in the corpus cavernosum of the penis and penile erection are the outcomes of sildenafil's selective inhibition of 5 phosphodiesterase. Since it dissolves easily in water, distilled water was used as the solvent.[11] So, the idea to use water as a cleaning solution was born. A review of the literature found that there are not many spectroscopic or chromatographicapproaches described to quantify sildenafil citrate at either formulation or bulk form. The amount of sildenafil citrate in swab samples has not been documented using any method. For this reason, sildenafil citrate is now available through a UV technique. Linearity, accuracy, precision, limit of detection (LoD), and limit of quantitation (LoQ) have all been checked for in the suggested analytical procedure.[12] The work goals is to develop and authenticate a spectroscopic approach for the analysis of sildenafil citrate swab samples.

MATERIAL AND METHOD

Reagent and Chemicals

The gift sample was obtained from Flemigo Pharmaceutical and is a working standard of sildenafil citrate (Figure 1). We just utilized analytical-grade chemicals for the rest of the operation. The solvent used for swab testing was water of an AR grade. The Whatman filter paper was used to first pass the sample solution. We used sterile swabs made of 100% cotton to collect swab samples.

Instrumentation

We used a Shimadzu UV 1800 double beam spectrophotometer with a UV probe 2.33, a spectral width of 2 nm, a wavelength precision of 0.5 nm, and a couple of matched quartz cells that were 1-cm in diameter. Whatman filter paper, an analytical balance made by Shimadzu. The swab testing was conducted using a tablet compression device.

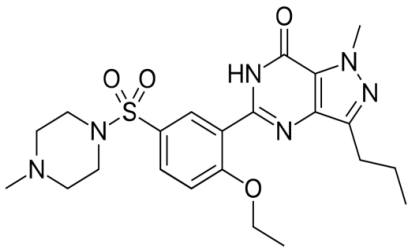

Figure 1: Elemental arrangement of sildenafil citrate

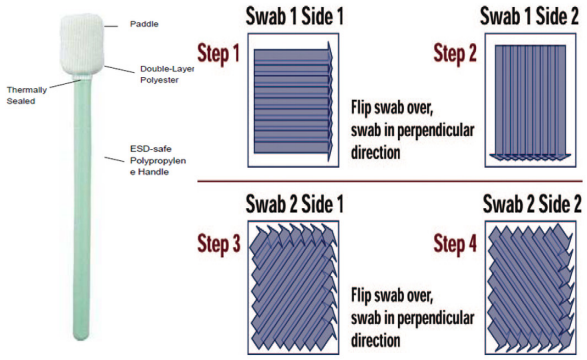

Figure 2: Swab wipes sampling protocol

Recovery Studies of Sildenafil Citrate from Clean Tip Swabs and Stainless Steel Plate

The surface testing was conducted using a stainless steel plate with dimensions of 30 x 15 cm. A conc. of 1000 µg/mL was achieved in spiking solvent by solubilizing 50 mg of citrate in 50 mL water. In order to get 10 µg/mL, it was further diluted. Each 100% cotton swab stick's head was washed with AR-grade water. The three designated sections of the recovery plate were treated with solutions of different concentrations (1.6, 2, and 2.2 mL) using a calibrated graduated pipette. The recovery plate was covered with these solutions in an area measuring 5 x 5 cm, and they were left to dry. The stainless steel plate was swabbed using swab sticks that had been previously inserted into a glass test tube with 5 mL water. Swab process began with a horizontal motion and progressed to a vertical one.[13]

Last but not least, AR-grade water was added to the swab sticks before being sonicated for 10 minutes at room temperature (Figure 2). It was lastly measured at a detection wavelength of 292.20 nm the absorbance of these sample solutions.

Method for Cleaning the Instrument

A dry cloth was used to clean the tablet compression machine. Following two rounds of cleaning with a 2% SLS solution, the machine was wiped down with water using a cotton plug to eliminate any remaining drug residue.

Method for Swab Testing

Table 1 shows the locations of the critical sites, which were designated with area. We soaked every swab in 5 mL of water. Carefully, swabs were obtained from the designated

Table 1: Critical sites and area selected for analytical readings

Critical sites selected	*Area for swab testing (Cm x Cm)*	*Absorbance*
Turret	2 × 2	Not detected
Platform	2 × 2	Not detected
Upper camp tract	2c× 2	Not detected
Lower punch (12.5 mm)	1 × 1	0.0021
Die	1 × 1	Not detected
Upper punch (12.5 mm)	1 × 1	0.0001

Table 2: Linearity for sildenafil citrate

Concentration (µg/mL)	*Absorbance*
5	0.109
10	0.211
15	0.323
20	0.431
25	0.526

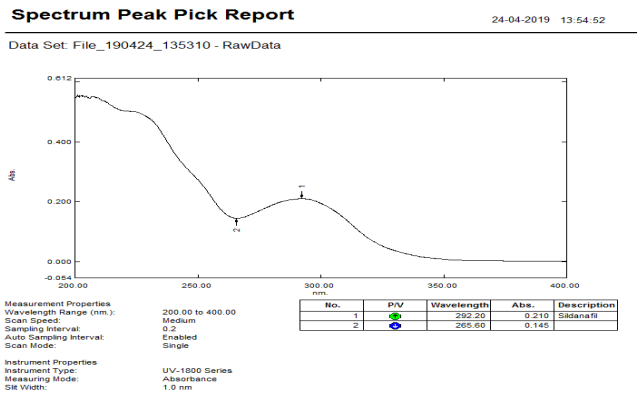

Figure 3: Absorption maxima for sildenafil citrate

areas using separate swabs. Both the horizontal and vertical directions should be swabbed initially. The swabs were then re-immersed in 5 mL of the water that was in the 10 mL test tube. Following a 10-minute sonication, the volume was increased to the specified level in the test tubes. Analyzed at 292.20 nm, the filtrated solutions were passed through the Whatmann filter paper.

Preparation of Standard Solution

To make a 50 mg stock preparation, 50 mL of water was mixed with 50 mg of sildenafil citrate. To obtain a 10 µg/mL, the mixture was appropriately diluted further.[14]

Determination of Absorption Maxima

Between 200 and 400 nm, a standard solution containing 10 µg/mL was analyzed. The optimum absorption maximum that was determined from the measured spectrum was 292.20 nm (Figure 3).[15]

Accuracy

Measurements were taken at 80, 100, and 120% of the LoQ concentration, and the analytical quantity measured was compared to the recorded amount spiked at each level to determine the procedure's accuracy.

Table 3: Result for validation parameter

Validation parameter	*Results*
Precision	(%RSD)
Inter-day	1.30
Intermediate	1.00
Intraday	1.62
Accuracy	Percentage recovery (%)
80%	86.14
100%	89.62
120%	84.25
Linearity	R^2= 0.9993
LoD (µg/mL)	0.0002651
LoQ (µg/mL)	0.0025371

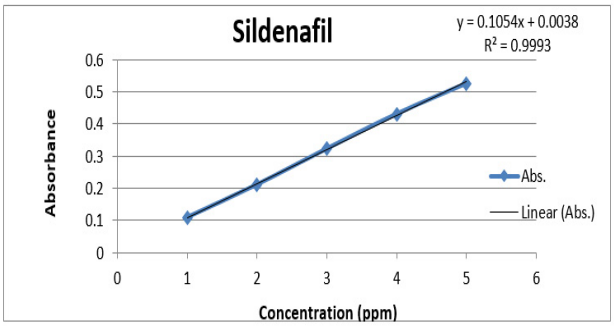

Figure 4: Calibration curve of pure sildenafil citrate

Limit of detection and quantitation

Using the response standard deviation and calibration curve (Table 2 and Figure 4) slope, the following formula was used to estimate the LoD and LoQ of sildenafil citrate.

$$LoD = \frac{3.3 \times \sigma}{S}$$

$$LoQ = \frac{10 \times \sigma}{S}$$

Where σ = the standard deviation

S = slope

We observed that the LoD was 0.0002651 µg/mL [0.2651 ng/mL] and LoQ was 0.0025371 µg/mL [2.5361 ng/mL]. The outcomes can be shown in Table 3.

RESULT AND DISCUSSION

Newly-developed cleaning procedure gets rid of any and all drug residue on the device. The established analytical approach was discovered to be sensitive enough to detect minute amounts of drug residue, as well as linear, exact, and accurate.[16]

CONCLUSION

It is possible to utilize the suggested method for routine swab analysis because it is easy, quick, sensitive, and inexpensive.

REFERENCES

1. Tracqui A, Ludes B. HPLC-MS for the determination of sildenafil citrate (Viagra®) in biological fluids. Application to the salivary excretion of sildenafil after oral intake. Journal of Analytical toxicology. 2003 Mar 1;27(2):88-94.
2. Asfaram A, Ghaedi M, Purkait MK. Novel synthesis of nanocomposite for the extraction of Sildenafil Citrate (Viagra) from water and urine samples: process screening and optimization. Ultrasonics Sonochemistry. 2017 Sep 1;38:463-72.
3. Lanzarotta A, Lorenz L, Batson JS, Flurer C. Development and implementation of a pass/fail field-friendly method for detecting sildenafil in suspect pharmaceutical tablets using a handheld Raman spectrometer and silver colloids. Journal of Pharmaceutical and Biomedical Analysis. 2017 Nov 30;146:420-5.
4. Ouranidis A, Tsiaxerli A, Vardaka E, Markopoulou CK, Zacharis CK, Nicolaou I, Hatzichristou D, Haidich AB, Kostomitsopoulos N, Kachrimanis K. Sildenafil 4.0 – Integrated synthetic chemistry, formulation and analytical strategies effecting immense therapeutic and societal impact in the fourth industrial era. Pharmaceuticals. 2021 Apr 15;14(4):365.
5. Shaw A, Lawrence TE, Yan T, Liu M, Summers N, Daggumati V, Austria ST, Rondon JC, Hackley S, Vignesh SO, Hassan TA. Bioequivalence Studies of Sildenafil Citrate Orodispersible Film Administered with and without Water vs Viagra Film-Coated Tablets in Healthy Male Volunteers. Current Therapeutic Research. 2023 Jan 1;99:100708.
6. Alkharfy KM. Simple and sensitive LC-ESI-MS method for the quantitation of sildenafil in plasma samples. Journal of separation science. 2009 Nov;32(22):3866-70.
7. Mujawar T, Ahmad S, Tajane P, Sable V, Gaikwad A, Tare H. Development and validation of ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form. Multidisciplinary Science Journal. 2023 Apr 9;5(2):2023020.
8. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2018 Nov 7;10(6):8-15.
9. Ahmad S, Mujawar T, Pawara J, Sable V, Patil K, Tare H. Analytical Method Development and Validation for the Simultaneous Estimation of Olmesartan and Hydrochlorothiazide by RP-HPLC in Bulk and Tablet Dosage Forms. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):30-35
10. Mane V, Killedar S, More H, Gaikwad A, Tare H. A Novel Rp-Hplc Gradient Elution Technique For Bioanalytical Method Development And Validation For Estimating Gallic Acid In Wistar Rat Plasma. International Journal of Applied Pharmaceutics. 2023;15(2):153-60.
11. Al-Rimawi F. Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV. Talanta. 2009 Oct 15;79(5):1368-71.
12. Shinde KP, Rajmane AD. A Review UV Method Development and Validation. Asian Journal of Pharmaceutical Analysis. 2023;13(2):122-30.
13. Ahmad S, Mujawar T, Batewal B, More P, Gaikwad A, Chumbhale D, Tare H. RP-UHPLC and HPTLC method development and validation for analysis of andrographolide from herbal hepatoprotective formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14:96-104.
14. Chudzik GM. General guide to recovery studies using swab sampling methods for cleaning validation. Journal of Validation Technology. 1998;5:77-81.
15. Kharate V, Kuchekar M, Harde M, Pimple B, Patole V, Salunkhe M, Wadgave P, Bhise M, Gaikwad A, Tare H. Development of Validated Stability Indicating HPTLC Method for Estimation of Febuxostat in Bulk and Tablet Dosage Form by Using QBD Approach. International Journal of Drug Delivery Technology. 2023;13(2):542-50.
16. Ahmad S, Shaikh TJ, Patil J, Meher A, Chumbhale D, Tare H. Osmotic Release Oral Tablet Formulation, Development, and Evaluation of an Anti-epileptic Drug. International Journal of Drug Delivery Technology. 2023;13(1):305-312.