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RESEARCH ARTICLE

Analysis of Sildenafil Citrate in Male Stamina Supplements with Densitometry TLC Method

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Abstract

Introduction: Sildenafil citrate is a chemical drug that is prohibited by Indonesia National Agency of Drug and Food Control to being added in male stamina supplements. Recently, several male health supplements have been found to contain sildenafil citrate. Objection: The purpose of this study was to validate the TLC-densitometry method used in the quantitative analysis of sildenafil citrate. Methods: Validation of the method determined is selectivity, detection limit, quantitation limit, linearity, accuracy, and precision. A good solvent for extracting sildenafil citrate from the matrix sample is methanol. Sildenafil citrate was separated from the matrix component in silica gel F_{254} using methanol: ethyl acetate (1: 2) as an eluent. The retardation factor of sildenafil citrate is 0.41, the resoluteion factor between sildenafil citrate and the closest matrix compound is 3.0. The response is linear in the range of sildenafil citrate concentrations 500 - 1500 ppm. A good solvent for extracting sildenafil citrate from the matrix sample is methanol. Results: The results showed that the detection limit of sildenafil citrate was 4.96 and the quantization limit was 15.03 ppm. Sildenafil citrate recovery was found 98.46-101.09% with an average of 99.83% and precision results of 0.99%. Conclusion: The Densitometry TLC method can be used for quantitative analysis of sildenafil citrate because the validation parameters have met the requirements.

Keywords: *Sildenafil Citrate, TLC-Densitometry, Validation method.*

Introduction

Erectile dysfunction that occurs in men is defined as the inability to maintain a penile erection for satisfying sexual activity. This can affect the quality of a person's life such as depression, an increase in one's anxiety, and affect a man's self-esteem. Medical therapy to treat erectile dysfunction is still not very effective before the introduction of sildenafil [1]. In November 2014 to August 2015 a total of 50 male supplement stamina products were found to contain medicinal chemicals. In these findings, the medicinal ingredients identified in male supplement stamina products were dominated by sildenafil citrate and its derivative [2].

Taking male stamina supplements that contain hard drugs can endanger the health and cause side effects, including headaches, dizziness, vision problems, stomach ulcers, abdominal pain, nasal inflammation, chest pain, palpitations, difficult to stop erections, and death [3].

Testing the drug content is an important stage of the product development process of a drug. The aim of drug stability is to find out how the quality of the substances contained in the drug can be influenced by several factors such as the environment, temperature, humidity, sunlight, and storage conditions [4]. Sildenafil citrate works by inhibiting triphosphate guanylate conversion into cGMP. When sexual arousal occurs, nitric oxide is released by neurons in penile tissue thereby increasing the activity of the enzyme guanylate cyclase, which is an enzyme responsible for converting guanylate triphosphate to cGMP which is a vasodilator neurotransmitter on a tissue [4].

Sildenafil citrate chemically has the name IUPAC 1- [4-etoxy-3- (6,7-dihydro-1-methyl-7-oxo-3-propyl 1H pyrazolo [4,3-d] pyrimidin-5-yl) Phenyl Sulfonyl] -4-metilpiperazin with a molecular weight of 666.7 g/mol ($C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$) [5].

Sildenafil increases the relaxation of the corpus cavernous smooth muscle which is responsible for increasing blood flow to the cavernous space which causes an increase in intracavernosal pressure. This causes erection of the penis [6]. Sildenafil citrate is the main component of the active ingredient known as Viagra in the form of white powder with a solubility of 3.5 mg/ml in water [7]. There are several methods for detecting sildenafil citrate found in male stamina supplements, one of which is the Densitometry TLC method [8]. The TLC method was developed to identify sildenafil as an illegal additive contained in food and beverages [9]. Chromatography is the most common separation technique in the field of chemical analysis and can be used to carry out analysis, both qualitative analysis, quantitative analysis, and preparative in the pharmaceutical and industrial fields. Chromatography is divided into three based on the tools used, namely thin layer chromatography, high-performance liquid chromatography, and gas chromatography [9]. In this study, the type of chromatography used was thin layer chromatography. Separation in thin layer chromatography is generally terminated before all phases pass through the entire surface of the stationary phase. Solute on both chromatographies was characterized by solute migration distance to the distance of the end of the mobile phase [10]. Rf values are calculated using equations:

$$Rf = \frac{\text{Distance taken by solute}}{\text{Distance traveled by the mobile phase}}$$

Quantitative analysis of a compound that has been separated by the TLC method is usually done with a densitometer that works in absorption or fluorescence. Based on the background above, this study aims to validate the TLC-Densitometry method which is able to analyze sildenafil citrate in supplementation of male stamina health which includes selectivity (specificity), linearity, detection limit, quantitation limit, accuracy, precision, and range and obtaining eluent which is able to separate sildenafil citrate from the male supplement stamina matrix. The hypothesis in this study is the validation of the TLC Densitometry method for the analysis of sildenafil citrate in male stamina supplements meeting the requirements.

Materials and Methods

Qualitative analysis on this method uses the calculation of the value of the Rf stain distance seen on the TLC plate, while the quantitative analysis uses a densitometer by comparing the stained area of sildenafil citrate in supplementing male stamina with the standard, then determining the maximum raw wavelength of sildenafil citrate. Male stamina supplements contain several matrices and sildenafil citrate which in the analysis process needs to be separated by Densitometry TLC method. In this study, the stationary phase used was the polar TLC silica gel plate, while for the mobile phase using a non-polar eluent.

Making of Standard Soluteion for Sildenafil Citrate

The sildenafil citrate soluteion weighed 125 mg was dissolved with methanol to dissolve, then put it in a 25 ml volumetric flask quantitatively and added methanol to the marked line. The main raw soluteion obtained is 5000 ppm.

Making Male Stamina Supplement Matrix Soluteion

Male supplement stamina capsules were weighed first to determine the diversity of their weights and then remove the contents from each capsule. Then the net weight of each capsule content was calculated by reducing the weight of the capsule with the weight of the empty capsule shell. The results of calculating the matrix weight of male stamina supplements were found with an average of 0.8036 grams. Then the soluteion is put into Erlenmeyer and 10 ml of ethanol is added and shaken until homogeneous and then vibrated in an ultrasonic shaker for 5 minutes. The soluteion is then put into a 25 ml volumetric flask and filtered twice and added methanol to the marked line.

Selectivity Test

The soluteion extracted from the male supplement stamina matrix with the addition of sildenafil citrate and without the addition of sildenafil citrate each was bottled on 2 µl

of 3 TLC silica gel F254 plates and put into a chromatographic vessel which had been saturated with eluent. When the increase in eluent has reached the upper limit of the TLC plate, the plate will be lifted and dried at room temperature. Subsequently, stains were observed under ultraviolet light. The selectivity test uses the mobile phase as follows:

- Methanol: ethyl acetate = 1: 2
- Ethyl acetate: n-propanol: 25% NH3 soluteion = 45: 5: 1
- Methanol: chloroform: dietilamine = 10: 90: 1

The three mobile phases were chosen which were able to separate sildenafil citrate from the male stamina supplement matrix which had the best Rf value and Rs > 1.5 - 2.0.

Determination of Selected Wavelengths

1000 ppm sildenafil citrate soluteion was bottled as much as 2 µl on the TLC silica gel F254 plate, then released with the selected mobile phase to the signing line. The stains obtained were observed with densitometers at wavelengths of 200-400 nm.

Linearity

The linearity test is carried out by making 5 kinds of standard soluteions of sildenafil citrate as follows:

- Working standard soluteion of 500 ppm

The standard soluteion of 5000 ppm sildenafil citrate parent was taken with a 1 ml pipette and then put in a 10 ml volumetric flask and added methanol to the mark line.

- 800 ppm standard working soluteion

The standard soluteion of 5000 ppm sildenafil citrate parent was taken with a 4 ml pipette and then put in a 25 ml volumetric flask and added methanol to the mark line.

- Standard working soluteion of 1000 ppm

The standard soluteion of 5000 ppm sildenafil citrate parent was taken with a 1 ml pipette and then put into a 5 ml volumetric flask and added methanol to the mark line.

- Working standard soluteion of 1200 ppm

The standard soluteion of 5000 ppm sildenafil citrate parent was taken with a 6 ml pipette and then put in a 25 ml volumetric flask and added methanol to the mark line.

- The standard working soluteion is 1500 ppm

The standard soluteion of 5000 ppm sildenafil citrate parent was taken with a 3 ml pipette and then put in a 10 ml volumetric flask and added methanol to the mark line.

Each standard soluteion was bottled on 2 µl silica gel F254 plate and then put into a chromatographic vessel which had been saturated beforehand and released to the final mark limit. Furthermore, the stain produced is measured by its maximum wavelength with a densitometer.

Determination of Detection Limits and Quantitative Limits

Determination is carried out by observing the absorbance of methanol blanks and standard work absorptions at various levels from the lowest standard to the highest levels. The standard work soluteion for determining detection limits and quantitation limits is made as follows:

- 25 ppm standard working soluteion

A standard soluteion of 500 ppm was taken with a pipette of 0.5 ml and then put in a 10 ml volumetric flask and added with methanol to the sign line

- Working standard soluteion of 40 ppm

The standard 500 ppm soluteion was taken with a 2 ml pipette and then put in a 25 ml volumetric flask and added methanol to the mark line

- 50 ppm standard working soluteion

The standard 500 ppm soluteion was taken with a 1 ml pipette and then put into a 10 ml volumetric flask and added methanol to the mark line

- 80 ppm standard working soluteion

The standard 500 ppm soluteion was taken with a 4 ml pipette then put in a 25 ml volumetric flask and added methanol to the mark line

- 100 ppm standard working soluteion

The 500 ppm standard soluteion was taken with a 1 ml pipette and then put in a 5 ml volumetric flask and added methanol to the mark line

- Each working standard soluteion is bottled on the TLC silica gel F254 plate as much as

2 µl each which is then fed into the developer vessel which has been saturated with the selected mobile phase and eluted to the marked line. The resulting stain is measured by the selected maximum wavelength using a densitometer and measured by noise measurements from blank along the chromatogram base width.

Determination of Accuracy and Precision

Determination of accuracy is done by calculating the percent acquisition of sildenafil citrate with 3 different concentrations of 80%, 100%, and 120%. The previously made soluteion was each bottled as much as 2 µl on the TLC plate in each composition and then eluted. The stains produced then measured the area with a densitometer and calculated the percent price and the KV price for accuracy and precision parameters.

% Revenue = A/B x 100%

Information:

A: The results of analyte content determination in the simulation sample

B: The weighing results of the analytes in the simulation sample

KV = SD/χ x 100%

Information:

SD: Standard Deviation

χ: Average value

Results

Selectivity

Based on the data obtained it is known that the mobile phase of methanol: ethyl acetate with a ratio of 1: 2 gives better separation results than the other two mobile phases with an Rf value of 0.41 and Rs of 3. The Rf and Rs values meet the required range, namely Rf 0.2 - 0.8 and Rs> 1.5. The results of the selectivity test on several types of mobile phases of sildenafil citrate in the male stamina supplementary matrix obtained Rf and Rs values as follows:

Table 1: Sildenafil Citrate Selectivity Test Results on the Male Stamina Supplement Matrix

Eluent	R_f			R_s
	Sildenafil Citrate	Sildenafil Citrate in Male Stamina Supplement Matrix	Male Stamina Supplement Matrix	
Methanol : Ethyl Acetate (1 : 2)	0,43	0,41	0,6	3
Ethyl Acetate : n-propanol : NH_3 25% (45 : 5 : 1)	0,15	0,15	0,52	7,1
Methanol : Chloroform : Diethylamine (10 : 9 : 1)	0,54	0,58	0,54	1,5

Determination of Detection Limits and Quantitative Limits

The sildenafil citrate soluteion which has been released with the selected mobile phase is then determined by the slope value of the regression equation between the concentration of sildenafil citrate and the area measured by the densitometer.

The slope value obtained from the regression equation y = 34.375 x + 141.111 which is equal to 34.375. Based on the data slope, the regression equation and the standard deviation of the blank are determined by the detection limit and the quantity limit. The results obtained were 4.96 ppm and 15.03 ppm respectively.

Table 2: Concentration Data and Sildenafil Citrate Area for Determination of Detection Limits and Quantitative Limits

Concentration (ppm)	Area
25	928,7
40	1412,8
50	1955,8
80	3090,9
100	3514,8
150	5240,9

Linearity

The results of the linearity test of sildenafil citrate found a regression equation y = 8.4820 x + 8384.4724 with the value of the correlation coefficient (r) of 0.9989 and Vxo of 2.02%. These results meet the linearity requirements, namely the value of Vxo is not more than 5%.

Accuracy and Precision

Testing the accuracy and precision of sildenafil citrate on the male supplement stamina matrix is done by addition. The results of accuracy and precision with three concentrations with three replications were obtained as a percentage with a range of 98.46% - 101.09% and an average of 99.87% and a KV of 0.97%.

Table 3: Data on Accuracy and Precision of Sildenafil Citrate

Sildenafil Citrate	Replication	Initial Level (ppm)	Obtained Level (ppm)	% recovery	Average % recovery
80%	1	800	787,68	98,46	99,40
	2	804	796,25	99,04	
	3	798	803,39	100,68	
100%	1	100	998,52	98,83	99,47
	2	100	1010,94	101,09	
	3	1006	1003,06	99,71	
120%	1	1200	1200,42	101,02	100,19
	2	1200	1203,23	100,32	
	3	1206	1201,65	99,64	
Mean					99,87
Standard Deviation					0,97
KV					0,97%

The percentage results for the acquisition value and the KV obtained to meet the requirements according to AOAC, namely 97 - 103% for the percentage of recovery and <2.8% for the price of KV [12]

Discussion

Based on USP 38 of 2015, the analysis of sildenafil citrate in male health supplement stamina was included in category I, so the measured validation parameters included selectivity (specificity), linearity, accuracy, precision, and range. In this study also measured detection limits and quantitation limits to provide additional information about the smallest levels that can still be detected and quantified by the TLC-Densitometry method. Among the three phases that are able to optimally separate sildenafil citrate from the supplement matrix of male stamina, health is methanol: ethyl acetate (1: 2).

Stained sildenafil citrate and separate male stamina health supplementary matrix have Rf values of 0.41 and 0.60 with Rs 3.0, respectively. Based on the results obtained, the values of Rf and Rs have met the requirements so that the mobile phase of methanol: ethyl acetate (1: 2) can be used for further analysis.

The mobile phase composition of methanol: ethyl acetate (1: 2) which is relatively semi-polar and the stationary phase of silica gel F254 is relatively polar causing the polar component to be retained in the stationary phase and the Rf price is smaller than the non-polar component [10]. Sildenafil citrate has a higher polarity compared to the supplementary matrix of male stamina health so that the price of Sildenafil citrate Rf is lower than the price of Rf matrix supplementing men's stamina health. The next stage is determining the maximum wavelength of sildenafil citrate. Previously, the baseline was carried out first in areas with no stains. The maximum wavelength of sildenafil citrate is determined by looking for absorptions in the greatest range of 200-400 nm. The biggest absorbance obtained is 304 nm, so it is determined as the maximum wavelength of sildenafil citrate.

The maximum wavelength obtained can be used in determining the next analysis. Determination of detection limits is the determination of the smallest number of analytes in a sample that can still be detected by a tool but cannot always be quantified, while the limit of quantitation is a quantitative concentration parameter test with a low concentration [11].

The detection limit test and the limit of quantization of sildenafil citrate were bottled in a range of 25-150 ppm as much as 2 µl and blank. The next stain on the TLC plate was measured by a densitometer to observe the visible stain area. The calculation results show a detection limit of 4.96 ppm and a quantization limit of 15.03 ppm.

Accuracy and precision tests were carried out by addition method, namely adding sildenafil citrate to the supplement matrix of male stamina health.

This is because the matrix used does not contain sildenafil citrate. The concentration of sildenafil citrate added to the matrix is 80%, 100%, and 120%. Each concentration was replicated three times. The results of calculations, then obtained percent recovery of sildenafil citrate and the price of KV respectively were 98.46-101.09% and the average (99.87 ± 0.97)% and 0.97%. Percent of recovery and the price of KV obtained to meet the requirements, namely 97-103% for analytes in the sample and KV <2.8% or ≤ 2% [16]. So it can be concluded that the test of accuracy and precision is acceptable.

Determination of the mobile phase capable of separating sildenafil citrate from the supplementary matrix of male stamina health and method validation, it can be concluded that the mobile phase of methanol: ethyl acetate (1: 2) and wavelength 304 nm can be used for quantitative analysis of sildenafil citrate in supplement preparations male stamina health because it meets the validation requirements.

This method of analysis is expected to be used as a reference for the analysis and determination of the levels of sildenafil citrate in supplements of male stamina health.

Conclusion

The TLC-densitometry method for analysis of sildenafil citrate in male stamina health supplements fulfills the requirements of method validation based on selectivity parameters, linearity, detection limits and limit of quantitation, accuracy, precision, and range. Eluent methanol: ethyl acetate (1: 2) is able to optimally separate sildenafil citrate from the supplement matrix of male stamina health with Rf prices in a row of 0.41 and 0.60 and Rs 3.0. The TLC-densitometry method for analysis of sildenafil citrate in stamina health supplements can be used as a reference for assay.

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