Physicochemical characteristics and ex vivo skin permeability for three phosphodiesterase 5 inhibitors (sildenafil, tadalafil and vardenafil): a proof-of-concept study for topical penile therapy

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Abstract

Efficient topical drug delivery for phosphodiesterase inhibitors (PDE inhibitors) as a potential alternative to the oral therapy could revolutionize the treatment strategy of erectile dysfunction for decades to come. This work aims at investigating three selected members of PDE 5 inhibitors for potential topical penile therapy through studying preformulation characteristics such as pH solubility, partition coefficient, chemical stability and ex vivo permeation. Results showed that the three drugs showed pH-dependent solubility and lipid solubility (log P). Sildenafil showed the least solubility:dose ratio and least lipid solublility, compared to both vardenafil and tadalafil. The roles of two solubilizing agents and penetration enhancers (Solulan C24 and Pluronic F127) were studied. Pluronic F127 seemed to be superior and significantly enhanced skin permeability of sildenafil by 1.7. The apparent permeability coefficient (P_{app}) for the three drugs (silda, varda and tada) was

14, 76 and 90 cm/h, respectively. The forced degradation studies indicated that the three drugs were highly sensitive to both acid- and base-forced degradation; nevertheless, they are chemically stable against oxidative and photolytic degradations. In conclusion, topical penile therapy for tada and varda seems to more viable options than silda as far as skin permeation and solubility/dose ratio are concerned.

Key words: Penile, therapy, preformulation, sildenafil, vardenafil, tadalafil, permeation

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1. Introduction

Erectile dysfunction (ED) is a prevalent medical issue affecting a significant portion (15%) of men each year. In the late 1990s, the statistics revealed that > 150 million men globally affected by ED. This is expected to increase to >300 million by 2025 [1]. ED has been associated with many common chronic diseases such as diabetes, hypertension and depression [2]. The epidemiology of ED differs widely among countries. It was recorded that 18% in men ≥ 20 years in the USA [3], while it was estimated to be approximately 50% and 64% in Canada [5] and in Hong Kong [6],

respectively. In Arab countries, ED was estimated to be 66% among men with chronic hypertension and 24% among non-hypertensive male patients (Bener, 2007).

The good news is that ED can be treated. The treatment depends on general health and the underlying medical conditions. There are invasive and more recently non-invasive oral phosphodiesterase 5 (PDE5) inhibitors treatments. The invasive treatment can range from penile prostheses, vacuum devices or implants to local penile injections [3]. These modes of treatments are effective treatment for severe ED. For example, penile implants require minor surgeries and are typically performed in an outpatient center. However, invasive treatments are patients unfriendly and associated with poor compliance, high cost and complications such as trauma to the penile tissues or priapism [3, 4].

Non-surgical medicinal therapy includes injectable testosterone-replacement therapy and oral 5-phosphodiesterase inhibitors (PDE5); PDE5 members include sildenafil, vardenafil, tadalafil, and avanafil.

The PDE5 inhibitors are grossly well tolerated for the treatment of ED and have dramatically changed the treatment strategy of ED. This drug class is inhibitors of cyclic guanosine monophosphate (cGMP) phosphodiesterase in the corpus cavernosum of the penis. This inhibition results in reducing smooth muscle tone with vasodilatation required for erection [5].

The chemical structures of sildenafil (silda), vardenafil (varda) and tadalafil (tada) are shown in Figure 1. These three drugs are among the PDE5 inhibitors. Sildenafil is the first member in the PDE5 inhibitors class approved for treatment of ED in 1998. Five years later, vardenafil was approved for the same indication. Several months later,

tadalafil was approved in 2003 lending the patients two more alternatives [5]. According to the manufacturer, oral bioavailability (rate and extent of absorption) of the PDE5 inhibitors has been reported to be affected by fatty meal [5]. For example, peak plasma concentration of sildenafil can be reduced by 29% and time to peak concentration is delayed by 60 minutes. This is likely to affect patients' spontaneity and overall therapeutic benefits.

Topical therapy of PDE5 inhibitors could offer some advantages of being non-invasive, fast onset of action, ease of use, lack of interactions with food or alcohol intake and more importantly, topical therapy could avoid the systemic side effects of PDE5 inhibitors. These systemic unwanted effects encompass nasal congestion, pharyngitis, headache and maldigestion. Visual abnormalities, loss of vision and sudden hearing loss are among the serious side effects [5]. Transdermal sildenafil delivery was studied using nano-transferosomal films [6]. Extremely delayed effects (T_{max} = 16 h) were reported for transdermal formulation compared to oral suspension (T_{max} = < 2 h). These findings could highlight the need of topical delivery of PDE5 members that could offer faster/comparable onset of action to oral PDE5 with minimal adverse effects .

Future treatments for ED should focus on providing non-invasive and more patient friendly that are more effective, work faster with fewer side effects. While there are very scarce published reporst on topical PDE5 therapy, alprostadil cream has been reported to show a potential alternative for penile injections for treatment of ED {Anaissie, 2016 #158}. Sildenafil-loaded into nanosized bilosomes (bile slats

modified liposomes) have been promising as a topical penile therapy for treatment of ED in aged rat models {Abdelalim , 2020 #159}.

There are limited reports on the physicochemical properties of PDE5 inhibitors such as water solubility, lipophilicity and stability in liquid or semisolid dosage forms for topical formulations. Therefore, this work aims at investigating preformulation characteristics (pH-solubility, partition coefficient, permeability and stability studies) of three commonly used members of PDE5 inhibitors for local penile delivery (Figure 1).

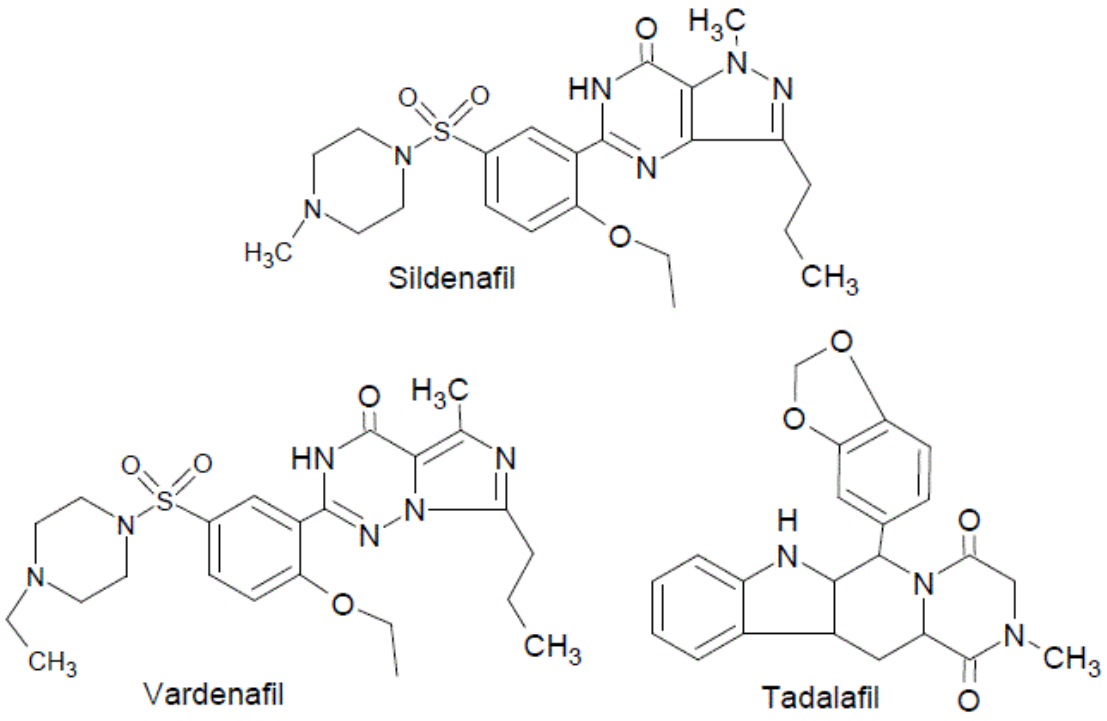

Figure 1. Chemical structures of three PDE5 inhibitors members selected for this study.

2. Materials and methods

Sildenafil citrate (B. N. 052125) was given by South Egypt Drug Industries Company (SEDICO). Tadalafil (B. N. 2020110) was given by Penta Pharm Egypt. Vardenafil (B. N. 202275) was given by Egyptian Group for Pharmaceutical Industries (EGPI). Pluronic F127 was bought from Sigma Aldrich (St. Louis, MO, USA). Solulan C 24 was obtained from Lubrizol Inc., France.

2.1. Methods

2.1.1. Solubility studies

The solubility of silda, varda and tada was studied in aqueous solutions with different pH values of 3, 5, 7, 9 and 12. In addition, the solubility in two different surfactant (Pluronic F127 and Solulan C24) solutions with two different concentrations (0.01% and 0.1% w/v) was studied. Excess amounts of the three drugs under investigation were added to the above-mentioned solutions and placed in a shaker water bath (Jeio Tech, South Korea) agitated at 100 strokes per minute (min) at 37±1°C for 48 h. Samples (5 ml each) were withdrawn, filtered and analyzed for silda, varda and tada using a UV-visible spectrophotometer (Shimadzu 16001, Japan) by measuring the absorbance at 291, 284 and 270 nm, respectively. Experiments were repeated 3 times and the mean was calculated for equilibrium solubility.

2.1.2. Partition Coefficient

The partitioning of silda, varda and tada between equal volumes of octanol (oil) and buffer solutions (aqueous) was analyzed at the previously mentioned pH systems. The method of calculation partition coefficient (expressed as log P) for the three drugs was mentioned in full details in the previously published method [7]. Briefly, an appropriate amount of each drug (5 mg) was dissolved in 2 ml of n-octanol. Equal volumes of the buffer solutions were vortexed in glass vials for 1 minute and incubated for 24 h at 37º C. The two layers were separated by centrifugation (10000 rpm for 10 min). the drug content in the buffer solutions was determined using UV spectrophotometry (Shimadzu 16001, Japan) at 291, 284 and 270 nm respectively. The P was calculated through equation 1.

$$\mathbf{P = \frac{C_o}{C_w}} \qquad \textbf{Equation 1}$$

Where the C_0 is the concentration of the drug in octanol and C_w is the concentration of drug in the buffer solutions .

After that, the same procedure was repeated for each drug at different surfactants-water media (phosphate buffer pH 7 alone, Solulan C24 and Pluronic 127) with concentrations 0.01 and 0.1% alternatively for each drug.

2.1.3. Ex-vivo permeation studies

Permeation of silda, varda and tada across full thickness excised rat skin from drug suspensions and solutions of Pluronic F127 (0.01%). The dermal permeation studies were studied using the simulated Franz diffusion cell apparatus. Sprague Dawley rats were housed and euthanized using ketamine injection based on the protocol approved by Animals and Ethics Committee, Deraya University. Abdomen skin from Sprague Dawley rats were shaved and excised. The excised skin was placed between the donor and receptor compartments providing a diffusional surface area of 1.77 cm^2 and receptor volume of 25 ml filled with a citrate buffer pH 5.8 containing 0.1% Tween 80 and temperature adjusted at 32 °C ± 1 °C. Samples (1 ml each) of drug suspensions containing 10 mg, 2 mg and 2 mg of silda, varda and tada, respectively were applied to the donor compartment. The whole setup was kept in a water bath model 3047(Köttermann, Hänigsen, Germany) at 32 °C ± 1 °C, shaking at 100 strokes per minute. Aliquots (3 ml each) were collected at 0.25, 0.5, 1, 2, 3, 4 and 5 h. The withdrawn samples were determined using UV spectrophotometry (Shimadzu 16001, Japan) at 291, 284 and 270 nm respectively. Drug permeability via the excised abdominal rat skin was calculated via equation 2.

$$\boldsymbol{P_{app}= \frac{F}{C_0}} \qquad \textbf{Equation 2}$$

Where F is the flux and was equal to the slope of the cumulative amount/cm^2 versus time curve; Co is the initial drug concentration (mg/ml).

2.1.4. Stability studies

2.1.4.1. HPLC conditions and validations

The HPLC assay was performed using HPLC Dionex UltiMate 3000 RS system (Thermo Scientific, Dionex Sunnyvale, CA, the USA). The HPLC system was equipped with quaternary pump and an RS auto-sampler injector. A reverse phase column Inertsil ® ODS- 3 (25cm x 0.46 cm, 5 µm) at a temperature of 25ºC was used. A mobile phase consisted of deionized water (pH 2.6 adjusted with phosphoric acid): methanol at 40:60 v/v. An isocratic flow rate of 1 ml/min was used; an RS diode array detector (DAD) was employed at 254 nm; an injection volume of 20 µl was used.

The limits of detection (LOD) and quantitation (LOQ) were estimated from equation 3 and equation 4 [8]:

$$\mathbf{LOD = 3.3}x\frac{\sigma}{S} \qquad \textbf{Equation 3}$$

$$\mathbf{LOQ = 10}\ x\ \frac{\sigma}{S} \qquad \textbf{Equation 4}$$

Where S is mean of the slope; σ is the standard deviation (SD) of the best fitting line of the calibration curve.

The HPLC method was validated for linearity, repeatability, accuracy and specificity according to International Conference on Harmonization (ICH) guidelines [14, 15].

2.1.4.2. Forced degradation

Acid and alkali-forced degradation

Standard stock solutions of silda, tada or varda containing 10 mg/ml each was prepared in methanol. An aliquot of the stock solutions from each drug methanolic solution was transferred into a 10 m-volumetric flask and volume was completed using 0.01 N and 0.1 N HCl; and 0.01 N and 0.1 N NaOH for acid and alkali degradation; respectively. The solutions were stored at 40◦C for specific time periods as outlined in Table 4. One ml of each solution was withdrawn, cooled, neutralized and transferred into a 10-ml volumetric flask. Volumes were made up with water to obtain a concentration of 0.1 mg/ml.

Thermal hydrolysis

One ml of stock standard (10 mg/ml) of each silda, tada and varda was transferred into a 100 ml-volumetric flask. The volumes were completed to the mark with distilled water to a concentration of 0.1 mg/ml. The solution was kept at specified conditions in Table 4.

Oxidative degradation

One ml of stock standard (10 mg/ml) for silda, tada and varda was transferred into a 10 ml-volumetric flask. the volumes were made up with H_2O_2 solutions of different concentrations 3, 10, and 30% and diluted as mentioned above

Thermal degradation on solid drugs

A sample (50 mg each) of drug powder was exposed to dry heat at 50 ˚C for 5 days. Twenty-five mg sample for each drug was weighed and transferred into a 25 ml-volumetric flask. The sample was dissolved in methanol. One ml of this methanolic solution was transferred into a 10 ml-volumetric flask and diluted as previously mentioned.

Photolytic degradation

Specific amounts (50 mg each) of drug powders were exposed to light with intensity of 1.2×10^6 lux for 5 days in a photo stability chamber equipped with 1500 W air-cooled xenon lamp (ATLAS Material Testing Technology, Illinois, USA) as per ICH guidelines. Twenty-five mg of each drug was transferred into a 25 ml-volumetric flask; the procedure was completed as mentioned above.

2.2. Statistical analysis

Unpaired t-test and One-way ANOVA followed by Tuckey's comparisons were studied using Minitab 17® software for analysis of statistical differences of solubility data, flux and apparent permeability coefficients and % recovery from forced degradation studies. P value < 0.05 indicates significant differences; while $P > 0.05$ indicates insignificant differences.

3. Results and discussion

3.1. Effect of pH on solubility of silda, varda and tada

The equilibrium solubility of the three PDE5 inhibitors silda, varda and tada at different pH values is shown in Figure 2. The solubility of both silda and varda showed pH-dependent solubility. For example, the solubility of silda gradually decreased with pH from 3 to 9 (Figure 2) and then abruptly increased at pH 12 by 4-fold, compared to pH 3. Similar behaviour was recorded for varda with significantly ($P < 0.001$) higher solubility values than silda. Silda has two basic pKa values of 8.7 and 9.6 to 10.1 due to N-CH_3 - piperazine and NH-amide functional groups respectively [9]. Varda has two pKa values of 4.72 and 6.21 due to tertiary amine [10]. Both silda and varda showed weak basic properties; however, varda could demonstrate relatively stronger basic properties than silda. This could explain the solubility data and pH-dependent solubility of both silda and varda. The greater solubility of Varda could be attributed to being more ionizable. The estimated pKa value for tada is 15.17 [11]. This is a very weak basic drug and the solubility was relatively higher in acidic conditions (Figure 2). It is worth mentioning that non-physiologically relevant high pH values such as pH 9 and pH 12 were employed in this study merely used to show complete pH-solubility profiles for these very weak ionizable compounds.

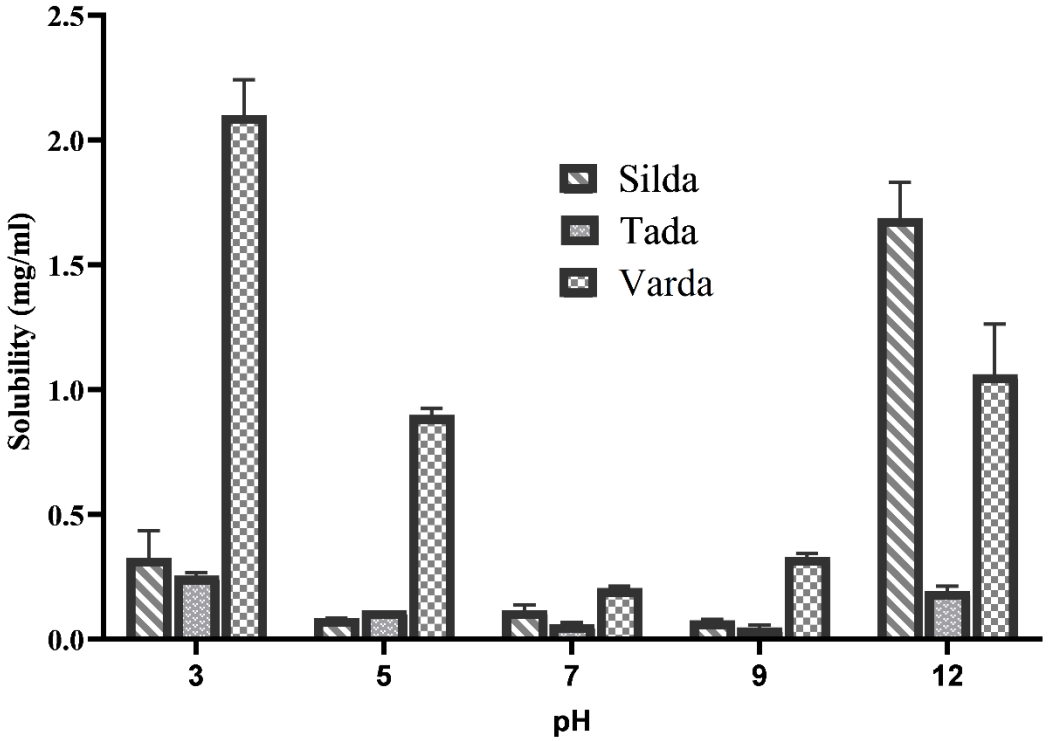

Figure 2. Equilibrium solubility of the three PDE5 inhibitors silda, varda and tada at different pH values.

3.2. Effect of different type and concentrations of surfactant solutions on solubility of silda, varda and tada

Figure 3 shows the solubility of the three different drugs (silda, tada and varda) with two different concentrations (0.01% and 0.1% w/v) of two different surfactants (Solulan C24 and Pluronic F127).

The results showed that the solubility of the three drugs was significantly ($P < 0.01$) enhanced compared the solubility in phosphate buffer pH 7 with 0% surfactants. Both sol and P are two non-ionic surfactant that is likely to enhance solubility of the three hydrophobic drugs by micellar solubilization. That is to say that the hydrophobic drug molecules are happily dissolved in the hydrophobic core of the micelles formed. The hydrophobic core is due to the hydrocarbon chains and steroidal nucleus of P and sol.

With the exception of varda, the concentration of surfactant has little effects on the solubilization of the silda and tada. On the other hand, the solubility of varda markedly increased with increasing P from 0.01% to 0.1%. The two surfactants used are both non-ionic and did not show any statistical ($P > 0.05$) differences in their solubilizing capacity.

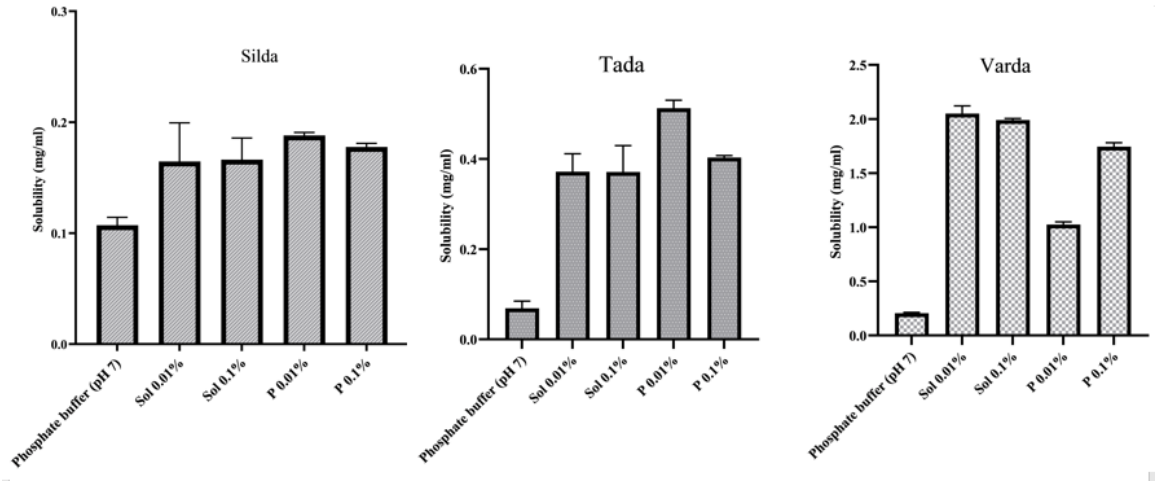

Figure 3. Equilibrium solubility of silda, tada and varda in different concentrations (0% to 0.1%) of Solulan C24 (sol) and Pluronic F127 (P) at pH 7.

3.3. Effect of pH on partition coefficient (log P) of silda, varda and tada

Partition coefficient is an important physicochemical parameter for assessing lipophilicity of compounds and predicting their permeability through lipophilic membranes [12]. Therefore, the effects of pH on the partition coefficient were studied for the three different drugs (silda, tada and varda), as shown in Figure 4, in order to choose the optimum pH that is near to physiological conditions and shows maximum lipophilic characteristics of the studied drugs for dermal delivery.

The ranking of log P values for the three drugs was in the following order: tada > varda > silda. This indicated that tada is the most lipophilic drug among the three investigated members: while silda is the most hydrophilic one. Further, log P values of

silda showed significant changes with pH. The log P values for silda were -0.12, -0.03, 0.4 and -0.1 at pH 3, pH 5, pH 7 and 9, respectively. The log P values for tada and varda were 3 and 1.8 at pH 7, respectively. At pH 7, silda showed a markedly lower (log P =0.4) value. Both tada and varda recorded log P values 7.5- and 4.5-fold greater that that measured for silda at pH 7. These findings could indicate that both tada and varda can be considered as lipophilic drugs and their permeation through lipophilic membranes is likely to be less restricted than silda.

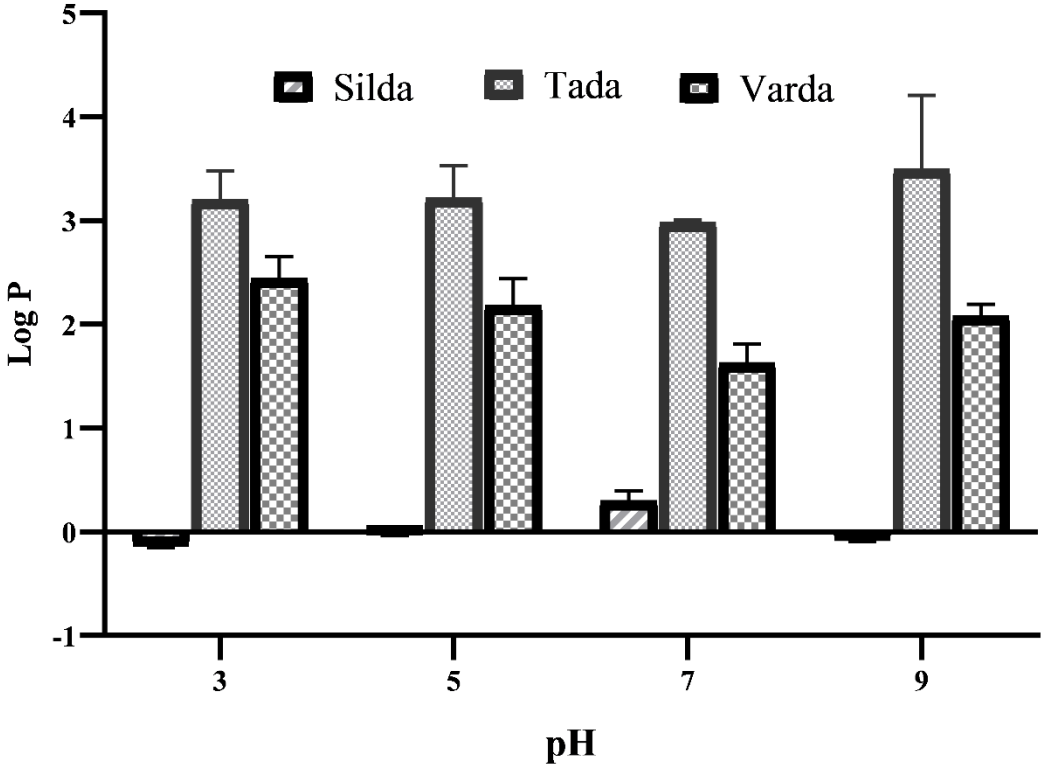

Figure 4. partition coefficients expressed as log values for the three different drugs measured at different pH values (pH 3- pH 9).

3.4. Effect of surfactant type and concentration on partition coefficient (log P) of silda, varda and tada

The effects of the two surfactants used Pluronic F127 and Solulan C24 on log P for the three different drugs (silda, tada and varda), as shown in Figure 5. The addition of the two surfactants showed marked enhancement of log P values by 1.14- to 7.4-fold. The greatest enhancement factor was obviously in favour of silda, compared to varda and tada. Additionally, Pluronic F127 was superior over Solulan C24 for increasing log P values for the three different drugs. Technically, measurement of log P for tada in presence of Pluronic F127 was too difficult to separate organic layer from aqueous due to emulsification of the two layers. The enhancement of log p values for three different drugs could be ascribed that the two surfactants were able to decrease interfacial tension between the aqueous and organic layers and increasing the diffusion coefficients of drugs and hence partitioning of drugs into the organic layer. These findings could indicate that both Solulan C24 and Pluronic F127 could be potential chemical enhancers for silda, varda and tada. Being non-ionic surfactants, they are better tolerated and are unlikely to cause significant irritation on skin surface [13].

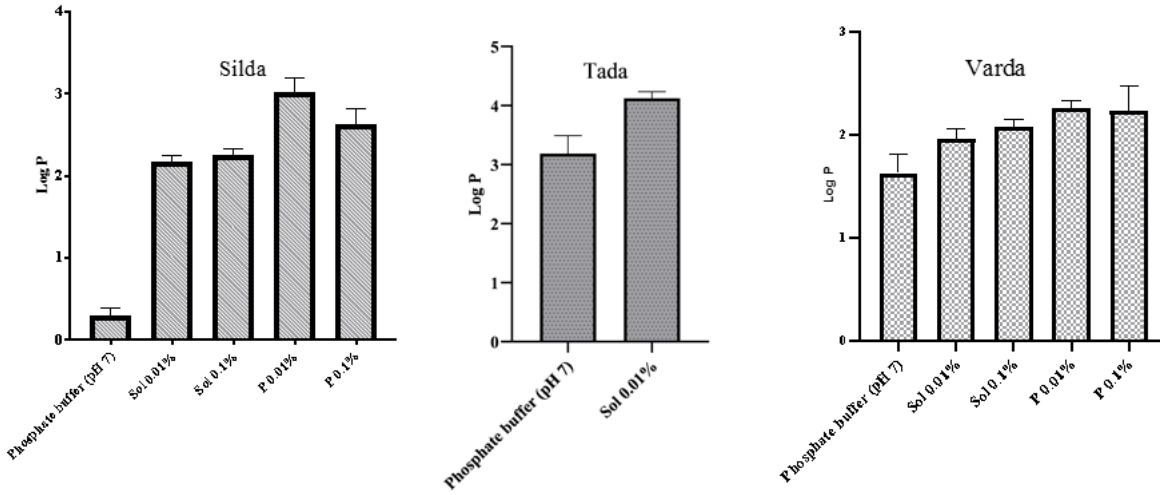

Figure 5. Partition coefficients (log P) for silda, tada and varda measured in different concentrations (0% to 0.1%) of Solulan C24 (sol) and Pluronic F127 (P) at pH 7.

3.5. Skin permeability studies

The permeability of the three different drugs silda, varda and tada in absence and presence of Pluronic F127 (P) through excised abdominal rat skin (as a model of biological membrane) was studied in Figure 6 (A-D); flux and apparent permeability (P_{app}) were estimated and presented in Table 1.

Skin permeation profiles for varda and tada suspensions showed superior permeation rates (flux), compared to silda after normalizing the amount permeated/cm^2 per applied dose, Figure 6 (A). The estimated P_{app} for varda and tada was 5.4- to 6-fold greater than silda. This significant ($P < 0.01$) enhancement of permeability attributed to two fundamental physicochemical properties: solubility/dose ratio and lipid solubility as estimated from calculations of log P (Figure 2 and Figure 4, respectively). The solubility of silda, varda and tada at pH 7 was 0.11, 0.2 and 0.06; when normalized per dose, it would demonstrate solubility:dose ratio of 1:100, 1:10 and 1:33, respectively. It was clear that greater solubility:dose ratio was in favour of both varda and tada than silda. In addition, the log P values estimated for silda, varda and tada at pH 7 were 0.3, 1.8 and 2.9, respectively. Greater log P indicates better lipid solubility; and hence superior permeability across biological membranes ensues.

The effect of addition of the solubilizing agent and penetration enhancer Pluronic F127 on both flux and P_{app} was also studied. Greater enhancement for the two permeation parameters (flux and P_{app}) was recorded for all three drugs silda (Figure 6 (B)), varda (Figure 6 (C)) and tada (Figure 6 (D)); nevertheless, silda benefited the much from inclusion of the solubilizing agent and penetration enhancer Pluronic F127.

Dermal penetration enhancers can reduce the barrier properties and hence decrease resistance of the stratum corneum to drug penetration. Inclusion of Pluronic F127 is likely to allow drugs to penetrate more happily into skin tissue [13].

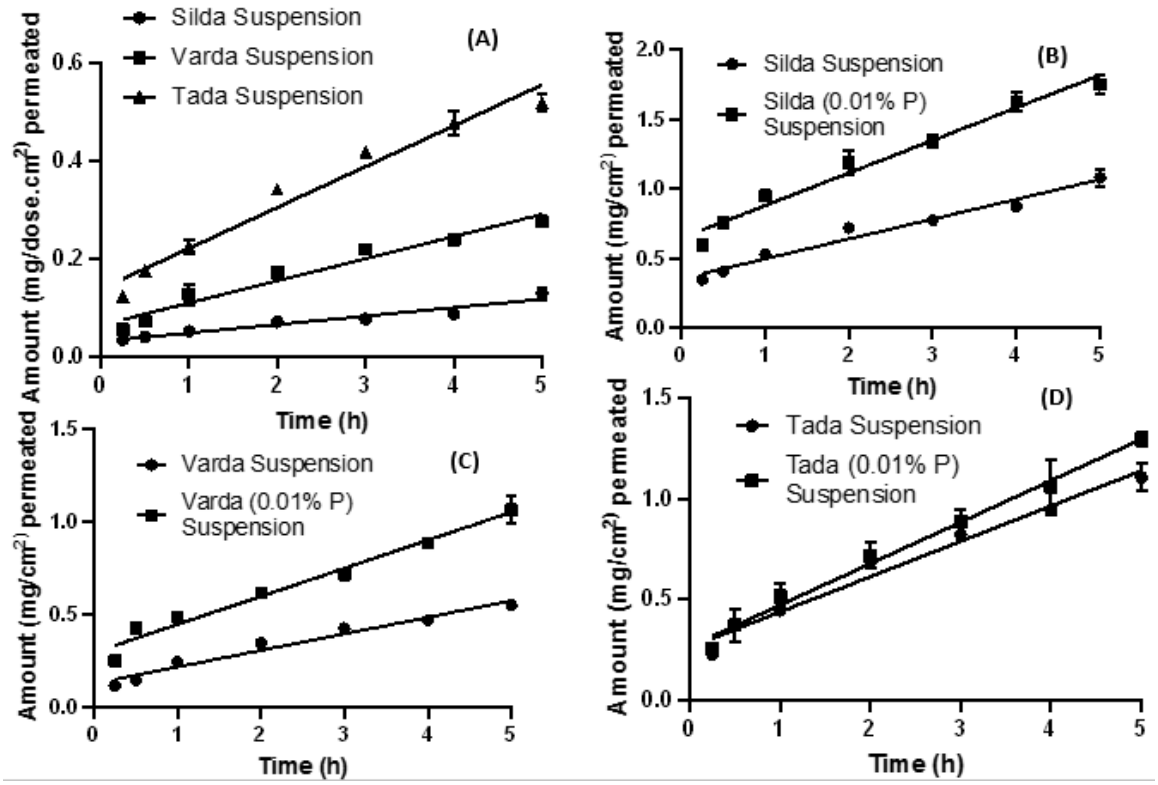

Figure 6. Ex-vivo permeation profiles from silda, tada and varda suspensions (A) and from suspension and suspension containing the penetration enhancer Pluronic F127 (P) for Silda (B), Varda (C) and Tada (D) using excised abdominal rat skin.

Table 1. Flux and apparent permeability (P_{app}) coefficient through excised rat skin for the three PDE5 inhibitors (silda, varda and tada) suspensions in absence and presence of the penetration enhancer Pluronic F127 (P).

Formulation	Flux ($mg.cm^{-2}\ h^{-1}$)	$P_{app} \times 10^{-3}$ ($cm.\ h^{-1}$)
Silda Suspension	0.24 ± 0.01	14 ± 2.8
Silda (P 0.01%) suspension	0.43 ± 0.02	24 ± 1.4
Varda suspension	0.27 ± 0.01	76 ± 4.2
Varda (0.01%) suspension	0.29 ± 0.01	81 ± 4.2
Tada suspension	0.32 ± 0.02	90 ± 2.8
Tada (P 0.01%) suspension	0.38 ± 0.02	104 ± 4.3

3.6. Stability studies

3.6.1. HPLC-stability indicating assay

The three drugs silda, tada and varda were determined using the optimized mobile phase composed of water (pH 2.6): methanol at ratio of 40:60 v/v. The flow rate was 1 ml/min. Detection was at 210 nm for the three drugs using a photodiode array detector; the temperature was set at 25◦C. Chromatograms showing peaks of silda, tada and varda alone and resolved from the peaks of forced degradation products at stress conditions of acid, base, thermal, oxidative and photolytic were shown in Figure 7-9.

Method validation

The developed HPLC method was validated; Table 2 shows the validation parameters such as linearity, repeatability, accuracy and specificity according to the ICH guidelines

[14, 15]. Table 3 shows system suitability parameters including capacity factor, symmetry factor, resolution and selectivity.

Linearity and limits of detection and quantitation

The linearity recorded high value of coefficient of determination (r^2 = 0.9998) for the three drugs; the linearity range recorded for silda, tada and varda were 1-15, 2-16 and 1-16 µg/ml, respectively (Table 2). LOD and LOQ range of the three drugs were 0.2 – 0.33 µg/ml and 0.6 – 1.03 µg/ml, respectively.

Table 2 shows the method accuracy with % recovery of 99.97 ± 1.12, 99.92 ± 1.09 and 99.89 ± 1.17 for silda, tada and varda, respectively.

Precision

The intra-day precision for silda, tada and varda was studied by analyzing three different concentrations in the same day [15]. Percentage relative standard deviation (%RSD) was calculated and was found to < 2%.

The inter-day precision for silda, tada and varda determination were performed by determining 3 different concentrations throughout the linear range on 3 consecutive days. Precision was estimated as the RSD %. The % RSD was found to be > 1.1% indicating precision of the developed method.

Table 2. Quantitative parameters for the determination of silda, tada and varda using the proposed HPLC method. Results are expressed as mean (n = 9).

Validation parameters	Silda	Tada	Varda
Linearity (µg/ml)	1-15	2-16	1-16
Slope	0.1299	0.1132	0.1290
Intercept	-0.0765	0.0875	0.0512
Coefficient of determination (r^2)	0.9999	0.9999	0.9998
Accuracy (mean ± SD)	99.97 ± 1.1	99.92 ± 1.1	99.89 ± 1.2
Precision (% RSD):			

Repeatability*	1.55	1.49	1.67
Intermediate precision*	1.74	1.87	2.03
LOD (µg/ml)	0.20	0.33	0.24
LOQ (µg/ml)	0.60	1.03	0.72

*The intra-day and inter-day relative standard deviation of the average concentrations (4, 8, 12 µg/ml) of the three studied drugs.

Table 3. System suitability of the proposed HPLC method for the determination of silda, tada and varda.

Parameters	**Silda**	**Tada**	**Varda**	**Reference value** [15]
Capacity factor	1.03	1.45	1.33	1-10
Symmetry factor	0.95	1.06	1.01	Near 1
Resolution	2.75	2.75	2.75	$R > 2$
Selectivity	1.21	1.21	1.21	$\alpha > 1$

Number of theoretical plates	4229.813	5584.666	5432.764	
HETP Height equivalent to theoretical plate (cm/plate)	5.9×10^{-3}	4.5×10^{-3}	5.1×10^{-3}	

Forced degradation

The three drugs silda, tada and varda were exposed to standard stress degradation to force their chemical degradations under acid, base, oxidative, thermal and photolytic conditions for a certain time period to produce % degradation of 5 to 20%; and to ensure that the HPLC method could offer a stability-indicating assay by resolving the drug peak from the produced degradation [16]. Figures 7-9 shows three different chromatograms for the tree drugs silda, tada and varda, respectively under different stress conditions and Table 4 summarizes the conditions and degradation percentage (%).

Acid and alkali degradation

Acid forced degradation of the three drugs was studied by exposing the three drugs under 0.01 N HCl, 0.1 N HCl at room temperature (25ºC), elevated temperature (40ºC) and refluxing (boiling 100 ºC) for 6 h. Extensive degradation (up to 92%) was recorded for the three drugs under refluxing in the acidic conditions of 0.1 N HCl. Percentage (%) degradation of 5 to 12% was recorded for the three drugs under milder acidic conditions of 0.01 N HCl at room temperature and at 40ºC.

For alkali-forced degradation, silda, tada and varda were found to be degraded completely under both concentrations of 0.1 N NaOH and 0.01 N NaOH. These findings indicate that the three drugs can be classified to be extremely sensitive to alkaline conditions.

Thermal hydrolysis

Significant chemical degradation (20-23%) were recorded for the three drugs under refluxing for 6 h; while relatively non-significant degradation (≈1%) at 40ºC.

Oxidative degradation

The oxidative forced degradation on the three drug silda, tada and varda were performed under different concentrations of hydrogen peroxide 3%, 10% and 30% for 6 and 24 h. No appreciable degradations for the three different drugs were recorded at the concentration of 3% for either 6 or 24 h. Under higher concentrations (10% and 30%), the degradation % for the three drugs did not exceed 5% and 20%, respectively. These findings indicate that the three drugs are fairly stable under oxidative conditions.

Thermal and photolytic degradation

Thermal degradation for the solid drug powders exposed to elevated temperature of 50ºC for 5 days exhibited no degradation and this indicates that the three drugs in solid states found to be thermally stable.

Photodegradation was also performed on solid drug powders by exposing the samples under light with intensity of 1.2 million lux for 5 days. No degradation was recorded and this indicates that the three drugs are not photo-labile.

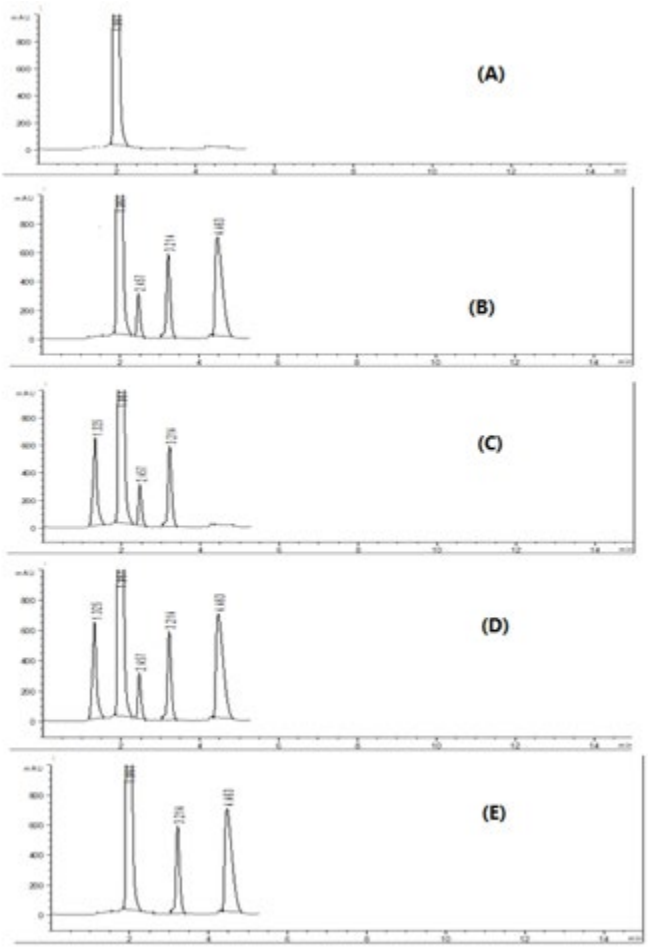

Figure 7. HPLC chromatogram of silda (A), acid degradation (B), alkali degradation (C), thermal hydrolysis (D) and oxidative degradation (E).

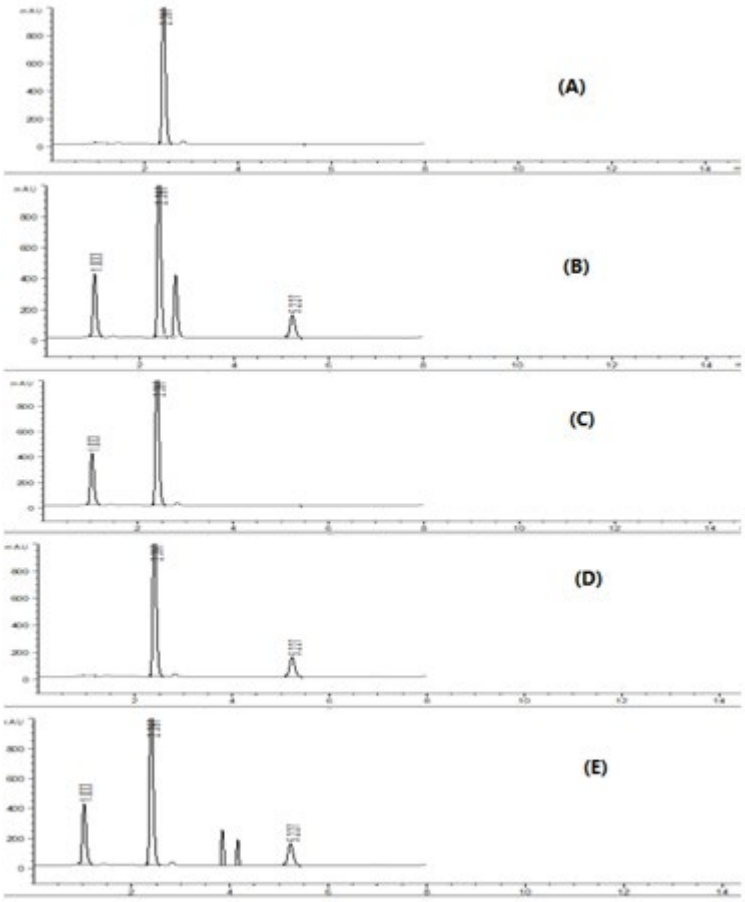

Figure 8. HPLC chromatogram of tada (A), acid degradation (B), alkali degradation (C), thermal hydrolysis (D) and oxidative degradation (E).

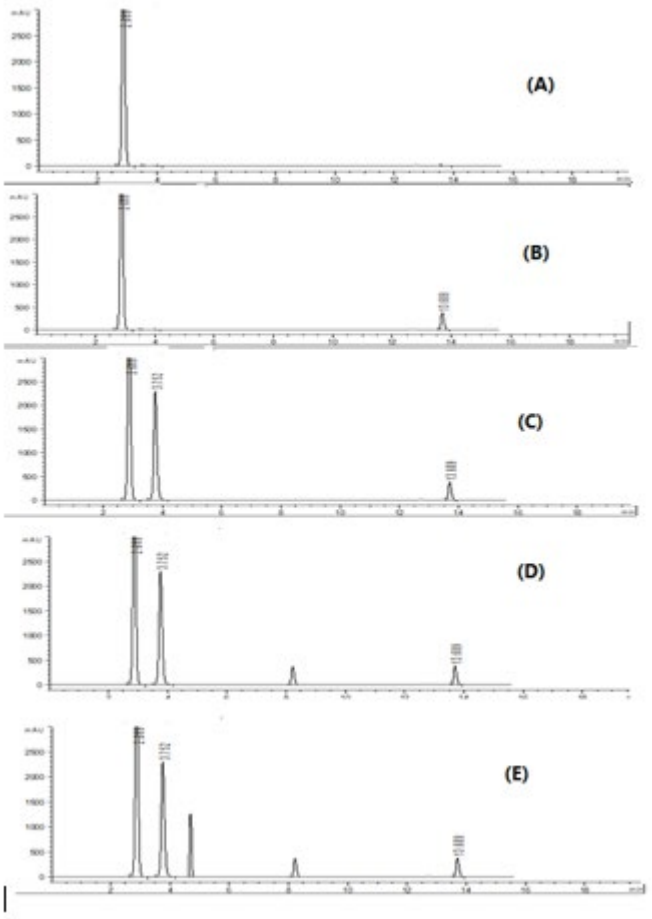

Figure 9. HPLC chromatogram of varda (A), acid degradation (B), alkali degradation (C), thermal hydrolysis (D) and oxidative degradation (E).

Table 4. Percentage (%) degradation of silda, tada and varda under various stress conditions.

Conditions		Silda % Deg.	Tada % Deg.	Varda % Deg
Acidic degradation	0.1N HCl – reflux – 6 hours	92 %	90 %	91 %
	0.01N HCl – 40 ºC – 6 hours	11 %	10 %	12 %
	0.01N HCl – room temp. – 2 hours	6 %	5 %	5 %
Alkali degradation	0.1N NaOH – room temp-2 hours.	100 %	100 %	100 %
	0.01 N NaOH – 40 ºC – 2 hours	100 %	100 %	100 %
	0.01N NaOH – room temp. – 2 hours	98 %	99 %	98 %
Thermal hydrolysis	Reflux – 6 hours	20 %	22 %	23 %
	40 ºC – 6 hours	1 %	2 %	2 %
	Room temp. – 2 hours	No Deg	No Deg	No Deg
Oxidative degradation	3 % H_2O_2 – 6 hours	No Deg	No Deg	No Deg
	3 % H_2O_2 – 24 hours	No Deg	No Deg	No Deg
	10 % H_2O_2 – 24 hours	4 %	5 %	5 %
	30 % H_2O_2 – 24 hours	17 %	19 %	20 %
Thermal degradation	50 ºC – 5 days	No Deg	No Deg	No Deg
Photolytic degradation	1.2 x 10^6 lux- 5 days	No Deg	No Deg	No Deg

4. Conclusion

In this study, three members of PDE5 inhibitors (silda, tada and varda) were selected for being the most widely used and available commercially in many countries. The three

drugs were investigated physiochemically to explore the possibility of being applied for topical penile therapy. Water and lipid solubility at different pH values, the effects of two solubilizing agents and penetration enhancers (Solulan C24 and Pluronic F127) were studied. The three drugs showed pH-dependent solubility with relatively higher solubility at both acidic and basic pH. Similarly, the three drugs demonstrated pH-dependent log P values. According to lipid solubility, the three drugs can be ranked as follows: tada > varda >> silda. Pluronic F127 seemed superior compared to Solulan C24 as a chemical penetration enhancer. An HPLC-indicating assay was developed and forced degradation studies indicated that the three drugs exhibited good stability under oxidative and photodegradation. From the preformulation characteristics, topical penile therapy seems to be a viable option; future research will look into in preclinical efficacy and safety in a proper animal model.

Credit Authors Statements

Soad A Mohamed: Data collection, Methodology, Writing. **Wessam W Mustafa:** Methodology, Editing and Writing. **Hesham Salem:** Methodology, Data collection, Data analysis. **Mahmoud Elrehany:** Methodology and editing. **Remon Roshdy Rofaeil:** Methodology, Data collection. **Hamdy Abdelkader:** Conceptualization, Methodology, Formal data analysis, Editing and writing the first draft.

Conflict of interest

The authors report no conflict of interest

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