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Dual-purpose vardenafil hydrochloride/dapoxetine hydrochloride orodispersible tablets: in vitro formulation/evaluation, stability study and in vivo comparative pharmacokinetic study in healthy human subjects

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Introduction

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection for good sexual activity [1]. Current interest in a safe and effective oral therapy for erectile dysfunction (ED) has resulted in men seeking help [2]. Vardenafil is a new phosphodiesterase (PDE) inhibitor which is potent selective inhibitor of cyclic GMP (cGMP), specifically phosphodiesterase type 5 (PDE5), causes relaxation in smooth muscle and blood vessels in penis resulting in dilation in blood vessel and penile erection [3]. Vardenafil has less side-effect profile and improved specificity with duration of action up to 36 h [4]. Vardenafil has also been shown to be effective and safe for the treatment of ED in a 12-week, multicenter, randomized, double-blind, placebo controlled trial that included 601 volunteer with severe to mild ED [5]. Oral Vardenafil has absolute bioavailability of 15% in the fasting state and with a high-fat meal reduced the rate of absorption with reduction in the Cmax by 18% and increase in time to maximum plasma concentration (Tmax) of 1 h [6]. Orally disintegrating tablets (ODT) of Vardenafil hydrochloride is preferred as they are designed to be dissolved on the tongue and pass hepatic metabolism. Vardenafil 5 mg is preferred for elderly men with ED [7]. ODT designed to perform better bioavailability and faster onset of action due to saliva in oral cavity causes pre-gastric absorption where drug dissolves quickly. Pre-gastric absorption avoids hepatic metabolism and can be a great advantage in drugs that undergo a great deal of first pass metabolism, such as Vardenafil [8]. Not all ODTs have the same advantages and may have similar absorption and bioavailability to standard oral dosage forms with the primary route remaining GI absorption.

Erectile dysfunction can cause premature ejaculation (PME); both problems of PME and ED are the most important challenges in sexology. PME is defined as persistent or recurrent ejaculation before man wants it with the sexual stimulation before, or shortly after penetration. Dapoxetine hydrochloride (DH) is a new fast acting serotonin transporter inhibitor used for the treatment of premature ejaculation [9]. DH is the first drug approved for the treatment of premature ejaculation (PME) in men as a short-acting selective serotonin reuptake inhibitor (SSRI) [10]. SSRIs blocks presynaptic membranes of 5-HT transporters, leading to higher serotonin levels in the synaptic cleft. The serotonin then binds to 5-HT2c and 5-HT1a receptors resulting in delay in ejaculation [11]. Therefore, the aim of this work is to formulate a combination of VH and DH in oral disintegrating tablet that may be very promising dosage form for treating ED and PME simultaneously with faster onset of action.

Materials

The materials used were as follows: Vardenafil hydrochloride (Rakshit Drugs Pvt Ltd., India); Dapoxetine hydrochloride (Shanghai Worldyang Chemical Co., Ltd., China); Mannitol (Roquette Pharma, France); Gelatin, Glycine, Tween80, Sodium chloride, and Potassium chloride (El-Nasr Pharmaceutical Chemicals Co., Egypt); Xanthan gum (MP Biomedicals, Inc., France); Gelatin, Glycine, Tween80, Sodium chloride, and Potassium chloride (El-Nasr Pharmaceutical Chemicals Co., Egypt); Xanthan gum (MP Biomedicals, Inc., France); Poly ethylene glycol (PEG 400 and PEG 6000) and polyvinyl pyrrolidone (PVP...
K30, PVPK90) (Fluka AG, Buchs. Switzerland); Disodium hydrogen phosphate and potassium dihydrogen phosphate and N-Methylpyrrolidone (Riedel-de Haen, Sigma-Aldrich, GmbH, Germany); HPLC grade Sodium dihydrogen phosphate (NaH2PO4), Disodium hydrogen phosphate (Na2HPO4), and Acetonitrile (Merck, India).

**Methods**

**Drug-excipients compatibility studies**

**Differential scanning calorimetry (DSC)**

Approximately 2 mg samples of Vardenafil hydrochloride (VH), Dapoxetine hydrochloride (DH) individually and in binary mixtures of a 1:1 ratio were analyzed using DSC; binary (1:1) physical mixtures of VH with Gelatin, Xanthan gum, Aerosil 200, PEG 6000, PVPK30, Glycine, Mannitol, Aspartame, and Croscarmellose sodium were analyzed; binary (1:1) physical mixtures of DH with Gelatin, Glycine, Mannitol, Aspartame, and Croscarmellose sodium were also analyzed. DSC thermograms were generated at temperatures between 30 and 400 °C using a Model DS-60 (Shimadzu®; Tokyo, Japan) with equipment and PC control unit TAC 60 (Shimadzu®; Tokyo, Japan) a nitrogen flow rate of 20 ml/min and a heating rate of 10 °C/min. DSC thermograms of pure drugs and binary mixtures were recorded [12].

**Fourier-transform Infrared spectroscopy (FT-IR)**

The IR absorption spectra of Vardenafil hydrochloride (VH) and Dapoxetine hydrochloride (DH) individually and in binary mixtures of a 1:1 ratio were analyzed using FTIR and physical mixtures of VH with Gelatin, Xanthan gum, Aerosil 200, PEG 6000, PVPK30, Glycine, Mannitol, Aspartame, and Croscarmellose sodium, also, DH with Gelatin, Glycine, Mannitol, Aspartame and Croscarmellose sodium were also analyzed using a Spectrum 100 FT-IR ATR Spectrophotometer (Perkin Elmer™ Ltd, Beaconsfield, United Kingdom). Samples of binary mixtures were prepared by weighing approximately 0.5 g of pure drug and other components and gently blending the mixture using a mortar and pestle. A small amount of the mixture was then placed on a diamond crystal and analyzed in wave range, 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹ [13].

**Construction of calibration curve of (VH) in simulated saliva fluid**

Stock solution of VH was prepared by dissolving 11.85 mg Vardenafil hydrochloride (eq. to 10 mg Vardenafil base) in 250 ml Simulated Saliva Fluid (Phosphate buffer, pH = 6.8) to get a final concentration of 40 mcg/ml. Serial dilutions were prepared to obtain samples having concentrations 4, 8, 12, 16, 20, 24, 28, 32, and 36 mcg/ml. The samples were scanned in the UV range at ƛmax of 233 nm, the experiment was done in triplicate and the mean absorbance of the three readings was calculated and the data was used to construct the standard calibration curve.

**Preparation of vardenafil hydrochloride orally disintegrating tablets (ODTs)**

Vardenafil hydrochloride (VH) ODTs were prepared using four matrix formers, along with some other excipients, and collapse protectant; the four matrix formers were: Gelatin (2% w/w) [14–17], Xanthan gum (2% w/w) [14,17], Na alginate (2% w/w) [14], Aerosil 200 (2% w/w) [17,18]. Other excipients used were croscarmellose sodium as a super disintegrant [19], mannitol as filler, Aspartame as a sweeter as VH has bitter taste [20], and glycine as a collapse protectant [17].

An accurately weighed amount of VH powder was dispersed in an aqueous solution (500 mg water, 0.5 ml distilled water for every tablet) contain the matrix former and other excipients using magnetic stirrer to result in a dose of 11.85 mg of VH per 700 mg of suspension. About 700 mg of suspension was then poured into each of pockets of a Polystyren chloride (PVC) blister pack resulting in a dose of 11.85 mg VH per orally disintegrating tablet.

The ODTs blister packs were then transferred to a freezer at −22 °C and kept in the freezer for 24 h. The frozen ODTs were then transferred to lyophilizer for 24 h using a Novaserve NL 500 freeze dryer with a condenser temperature of −45 °C and a pressure of 0.07 mbar.

Selected formulae based on percentage of VH dissolved after 5 min, were also prepared after adding different solubilizers to the previously described aqueous solution such as: PEG 400, PEG 6000, PVPK30, PVPK90, Tween80, and N-methyl pyrrolidone (NMP) (Table 1).

**Preparation of VH/DH orally disintegrating tablets (VH/DH-ODTs)**

The best selected formula (G2, G6, and G7) ODTs based on percentage of VH dissolved after 5 min, were used to prepare (VH/DH) ODTs using gelatin as a matrix former (2% w/w), along with some other excipients. Other excipients used were croscarmellose sodium as a super disintegrant, mannitol as a filler, Glycine as a collapse protectant, Aspartame as a sweeter, Tween80 or PEG 400 or N-Methylpyrrolidone as a solubilizer as shown in Table 1. D1, D2, D3 ODTs will be examined for: drug content uniformity, uniformity of weight, friability test, in vitro disintegration testing, wetting time, moisture analysis, and in vitro dissolution studies.

**Characterization of the prepared VH and VH/DH ODTs**

(a) Determination of Vardenafil hydrochloride in VH ODT by UV-spectrophotometry. Ten tablets of VH-ODTs were selected from each formulation and assayed individually for drug content uniformity. VH in VH-ODTs was assayed by dissolving each tablet in 250 ml SSF (pH = 6.8). The solution was then filtered, properly diluted, and the absorbance was spectrophotometrically measured at the ƛmax 233 nm of VH. The mean value of ten tablets was estimated to calculate the percentage of VH content of the tablets [21]. Vardenafil hydrochloride and Dapoxetine hydrochloride content in the prepared VH/DH-ODTs were determined by validated HPLC method ( Waters Alliance, Waters Corporation, Milford, MA) according to ICH guidelines.

**Chromatographic conditions.** Mobile phase: Potassium dihydrogen phosphate buffer pH = 4 (6.8 g in 1 l): Acetonitrile (50:50), Column: C-18 silica based column, particle size 5 μm Stainless steel (250 × 4.6 mm) ODS (Kromasil equivalent), wave length: 230 nm, flow rate: 1.0 ml/min, inj. volume: 20 μl, run time: 4 min and temperature: 30 °C.

**Standard solution:** Accurately weigh 11.85 mg VH and 33.58 mg DH standard and dilute to 100 ml with diluent. **Standard stock**
All formulations contain 11.85 mg of vardenafil hydrochloride. 

In a petri dish of 10 cm diameter, ten milliliters of distilled water containing eosin (a water-soluble dye) were placed. The tablet was carefully placed in the petri dish and the wetting time is the time required for the dye to reach the upper surface of the tablet and the test results presented are the average of three determinations \((n = 3) [22]\).

**Moisture analysis**
Using a Karl Fischer titrator (Veego Matic-MD, Bombay, India), three tablets were analyzed for their residual moisture content after lyophilization for each formula and the test results presented are the average of three determinations \((n = 3) [22]\).

**In vitro dissolution studies**
The dissolution profiles of VH in VH-ODTs compared with the plain powder and the corresponding market product (Levitra®) were determined using the USP dissolution tester type II. According to FDA dissolution guidance for VH ODT, dissolution media were 900 ml SSF (pH 6.8) maintained at temperature of 37 ± 0.5 °C with a paddle rotation speed at 50 rpm to maintain sink condition. The amount of vardenafil hydrochloride used was 11.85 mg equivalent to 10 mg vardenafil base in VH-ODT and VH/DH ODT, the amount of DH was 33.58 mg equivalent to 30 mg Dapoxetine base in the VH/DH-ODT. At specified time intervals of 1, 2, 3, 5, 7, 10, and 15 min, a 3 ml of dissolution media were withdrawn, and replaced with an equal volume of the fresh medium to maintain a constant total volume and maintain sink condition. Samples from VH-ODT were filtered through 0.45 μm Millipore filter and assayed for drug content spectrophotometrically at \(\lambda_{\text{max}} 233 \text{nm}\) after appropriate dilution. Samples from VH/DH-ODT were filtered through 0.45 μm Millipore filter and assayed for drug content using aforementioned validated method HPLC method. The best formula was selected to be further tested with different solubilizers. Solubilizers used were: PEG 400, PEG 6000, PVPK30, PVPK90, Tween 80, and N-methyl pyrrolidone (each in concentration of 2% w/w).

Dissolution profiles of each of the prepared formulae will be compared to that of the corresponding commercial product using difference factor \((f_1)\) and similarity factor \((f_2) [23, 24]\) using DDSolver software excel Add-Ins [25].

**Effect of storage on the prepared VH/DH ODTs**
The selected VH/DH-ODT formula was stored at 40 ± 2 °C and 75 ± 5% RH in a PVC blisters covered with aluminum foil, aluminum Foil was applied on the PVC through blister packing machine.
which apply softening the PVC margins and pressing the aluminium foil against it, during a period of 6 months (accelerated stability). Samples were withdrawn and evaluated for drug content uniformity, *in vitro* disintegration, and dissolution studies, as well as residual moisture content analysis experiments after 0, 1, 3, and 6 months’ storage. One-way analysis of variance (ANOVA) was applied for testing the equality of several means and *p* values <.05 was considered statistically significant.

**Pharmacokinetic study of VH/DH-ODTs on healthy volunteers**

**Protocol approval**

The protocol of the study was reviewed and approved by the clinical research ethics committee of the Faculty of Pharmacy, Cairo University, Cairo, Egypt with approval number: PI (1057), which is valid from 31/03/2014. The research was carried out according to the ICH guidelines, enunciated in the Declaration of Helsinki [26].

**Volunteer selection**

The volunteers were males with age between 20 and 40 years. The volunteers have no known history of alcohol, drug abuse, chronic gastrointestinal, cardiac, hepatic, vascular, or renal diseases and were nonsmokers. Special medical investigations were done before the study and all volunteers show normal physiological examination. The aim of the study was explained and each volunteer signed a written consent before enrolling in the study.

**Study design**

The scientific integrity of the study and the validity of the obtained results and the conclusions derived from the study depend primarily on the study design.

The study was performed using three formulae, namely: (Levitra® 20 mg tablet) market reference product containing VH, (Priligy® 30 mg tablet) market reference product containing DH, and the prepared formula (D3) VH/DH-ODT.

*In vivo* study performed on six male volunteers aged between 20 and 40 years. All volunteers were fasted for 12 h with access to water. The study was conducted according to a two-period, two-sequence crossover design with 1 week wash out period between each phase. Six male volunteers were randomly divided into two groups of three volunteers each. In period I, Test group received ODTs (D3) whereas the Reference group received market products (Levitra®) and (Priligy®) then crossover was done in period II.

**Sample collection and preparation**

After drug administration, blood samples were withdrawn from volunteers at time 0 (pre-dose), 5, 10, 15, 30, and 45 min, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h, each sample is 5 ml. To avoid coagulation of blood the blood samples were then transferred into heparinized tubes. The blood samples were then centrifuged at 3000 rpm for 10 min and clear plasma was then collected in polyethylene capped tubes and plasma was stored at −20°C and clear plasma was then collected in polyethylene capped tubes and deep frozen at −80°C until LC/MS/MS analysis. About 0.3 ml of heparinized normal saline injectable solution is flushed into the cannula to prevent blood clotting after each blood sampling.

**Determination of VH and DH in human plasma**

A selective, sensitive, and accurate Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC-MS/MS) method was developed and validated (data not shown) before the study for determination of VH and DH in human plasma. The validation of the method was according to international guidelines [27].

**Validation of analytical method.** The determination of VH and DH in Human Plasma by LC-MS/MS.

**Vardenafil hydrochloride standard solution.** Accurately weigh 11.85 mg Vardenafil Hydrochloride (equiv. to 10 mg Vardenafil base). Transfer into a 100 ml volumetric flask, add about 80 ml methanol, sonicate for 10 min and complete to volume with methanol. This solution contains 100 μg/ml vardenafil citrate ‘Solution A’.

Transfer 0.5 ml of “Solution A” into a 100 ml volumetric flask and complete to volume with water to obtain 500 ng/ml ‘Solution B’.

**Dapoxetine hydrochloride master standard solution.** Accurately weigh 10 mg of Dapoxetine standard. Transfer into a 100 ml volumetric flask, add about 80 ml methanol, sonicate for 10 min, and complete to volume with methanol. This solution contains 100 μg/ml Dapoxetine ‘Solution A’.

Transfer 5 ml of Solution A into a 100 ml volumetric flask and complete to volume with methanol to obtain 5000 ng/ml ‘Solution B’.

Transfer 0.5 ml of Solution A into a 100 ml volumetric flask and complete to volume with Methanol to obtain 500 ng/ml ‘Solution C’.

**Sildenafil standard solution.** Accurately weigh 10 mg of sildenafil standard. Transfer into a 100 ml volumetric flask, add about 80 ml of methanol, sonicate for 10 min, and complete to volume with methanol. This solution contains 100 μg/ml sildenafil solution (A) from solution (A).

Transfer 0.7 ml of prepared solution into a 100 ml volumetric flask and complete to volume with Methanol to obtain 700 ng/ml sildenafil solution (B).

**Preparation of calibration standards.** Calibration curve consisted of blank sample (matrix sample processed with internal standard) and eight non-zero plasma standards, in duplicate, covering the expected range of concentrations to be quantified. Calibration standards were prepared by spiking 0.5 ml control human plasma with 0.5ul working solutions containing the analyte to be quantified. Standards were prepared daily in the amounts required for the assay. They were extracted along with plasma samples.

**Mass spectrometric conditions.** The Agilent 6410 operated in positive electrospray ionization mode (ESI). General MS parameters were: Vardenafil: Precursor Ion (489), Product Ion (151), Delta EMV (500), Dwell Time (200), Fragmentor (135) and Collision Energy (30). Dapoxetine: Precursor Ion (306.2), Product Ion (157.2), Delta EMV (500), Dwell Time (200), Fragmentor (120) and Collision Energy (31). Sildenafil: Precursor Ion (475), Product Ion (283), Delta EMV (500), Dwell Time (200), Fragmentor (135), and Collision Energy (40).

The analytical method that would be used in the *in vivo* study would be well characterized; the applied analytical method for the determination of VH and DH in human plasma would be LC/MS/MS. The method was fully validated and documented. Chromatographic Conditions: Mobile phase (0.1% Formic acid:...
Methanol (20:80) V/V), Analytical column (Phenomenex C18, 4.6 × 50 mm, 5.0 micron), Guard column (Uniguard), Autosampler temperature (Ambient), Flow rate (0.35 ml/min), Column Temperature (Ambient), Injection volume (15 μl) and Total run time (2.8 min). Ion source parameters: gas temperature (350°C), gas flow (6.3 l/min), Nebulizer Pressure (23 PSI) and Capillary Voltage (5000 V).

**Plasma sample preparation.** Appropriate numbers of disposable glass test tubes were placed in a rack. The tubes are numbered according to the order of the analytical runs and then blank and the volunteers' human plasma samples (500 ml) added into appropriate tubes then the internal standard (50 μl of omeprazole working solution 500 ng/mL) was dispersed and vortexed for 1 min. Then 1 ml of acetonitrile was added to each and vortexed for 1 min then centrifuge the samples at 4000 rpm for 5 min and transfer clear supernatant layer to auto sampler vial and (10 μl) was injected in to LC/MS/MS.

This analysis was conducted in conformity with the study protocol and FDA Guidance for analytical methods validation. The individual and mean data for re-study validation, recovery and stability tests results are expressed using three significant figures.

Major validation steps required according to (FDA Guidance for Analytical Methods Validation) are: Specificity, linearity and range, accuracy, precision, and detection limit.

**Pharmacokinetic and statistical analyzes**

One-way ANOVA using SAS 9 software, was applied to compare the mean values of pharmacokinetic parameters to assess the significance of the effect of formulation and subject factors on the pharmacokinetic parameters of the selected formulation and market products (Levitra®) and (Priligy®). Parameters were considered statistically significant for p values < .05. Relative bioavailability of VH/DH-ODT compared to the market products (Levitra®) and (Priligy®) was calculated according to the following equation:

Relative bioavailability (%) = AUCo-12 (oral disintegrating tablets)/AUCo-12 (market products) × 100 ****.

**Results and discussion**

**Drug-excipient compatibility studies**

**Differential scanning calorimetry (DSC)**

Figures 1 and 2 showed the DSC thermograms of VH, DH, binary mixture of VH and DH, and 1:1 w/w drug-excipients mixtures. It is clear from DSC thermograms that VH and DH show their characteristic endothermic peak around 215 and 177°C, respectively, corresponding to their melting points [10], there was no shift in VH or DH peak upon mixing with Gelatin, Xanthan gum, Aerosil 200, croscarmellose sodium, aspartame, PEG 6000, PVPK30, Glycine, mannitol, maltodextrin, and sodium carboxymethyl cellulose indicating the absence of drug-excipient interaction.

**Fourier-transform infrared spectroscopy (FT-IR)**

VH exhibited the peaks at 2900 cm⁻¹ for C–H aromatic stretching, 1730 cm⁻¹ stretch for carbonyl group, 3330 cm⁻¹ stretch for NH₂, and 1100-1300 cm⁻¹ stretch for SO₂.

Figures 3 and 4 showed the FTIR spectra of VH, DH, binary mixture of VH and DH and 1:1 w/w drug-excipients mixtures. It is clear from FTIR that there was no shift in VH or DH peak upon mixing with Gelatin, Xanthan gum, Aerosil 200, croscarmellose sodium, aspartame, PEG 6000, PVPK30, Glycine, and mannitol. This clearly indicates that there is no drug excipient interaction.

**Uniformity of VH and DH content in VH/DH-ODTs**

The mean percentage of VH and DH content in ODTs from all formulations is presented in Table 2. The drug content of VH and DH in the prepared VH/DH-ODTs fall within the acceptable range according to the European Pharmacopeia [21].

**Uniformity of weight**

The average weight for all tablet formulations ranged from (198.00 ± 1.0 mg to 200.7 ± 1.3 mg), presented at Table 2, therefore all the tablets fall within the acceptable weight variation range; according to the European Pharmacopeia [21].
Friability test

Results in Table 2 showed that all tablets formulae VH and VH/DH ODTs show percentage friability within the acceptable range for tablets less than 0.8%. All formulae showed percentage weight loss ranged from 0.45% to 0.90%.

**In vitro disintegration time**

Results showed also ODTs containing matrix former xanthan gum has longest disintegration times than ODTs containing Gelatin or Aerosil200 or Na alginate as a matrix former. G1, X1, A1, and N1 showed an average disintegration time of 17.17 ± 0.98, 18.83 ± 1.17, 17.50 ± 0.54, and 18.17 ± 0.75 s, respectively. The shortest disintegration time was taken by the formulations containing gelatin as a matrix former (G1) where (G1) disintegrated within 17.17 ± 0.98 s.

Table 2 showed the average values of the in vitro disintegration times from the different tablet formulations. According to the compendial standards, orodispersible tablets should disintegrate within 3 min when examined by the test of disintegration of tablets [21]. The disintegration time ranged from 17.17 ± 0.98 to 18.83 ± 1.17 s; this may be due to the effect of super disintegrants which were added to the drug formulations, they facilitated the breakup or disintegration of tablet content into smaller particles that dissolved more rapidly [28]. Statistical analysis using ANOVA test revealed that changing the type of matrix former had no significant effect on the average in vitro disintegration times (p > .05).

ODTs formulae G2 and G3 showed significantly shorter disintegration times (10.00 ± 1.09 and 9.16 ± 0.75 s) compared to ODT G1 (17.17 ± 0.98 s) (p < .05). This could be due to the hydrophilic nature of the used solubilizers, which facilitates the penetration of water which caused a faster disintegration to tablets. These results are in accordance with the results obtained by Ciper and Bodmeier [29], where they found that the addition of hydrophilic additives, such as PEG 400, PEG1500, and xylitol decreased the disintegration time, and increased the rate of dissolution of gelatin fast dissolving films. While the addition of other solubilizers to G1 formula (G4–G7) did not affect disintegration time significantly with values ranged from 17.17 ± 0.40 to 18.00 ± 0.63 s. D1, D2, and D3 ODTs disintegrated in 16.17 ± 0.41, 15.33 ± 0.52, and 15.50 ± 0.55 s, respectively.

**Wetting time**

Table 2 showed the average wetting times of the different formulations. The wetting time of the formulae G1, A1, X1, and N1 was 6.00 ± 1.00, 8.33 ± 0.577, 8.00 ± 1.00, and 8.00 ± 1.00 s, respectively and the wetting time of all formulations did not exceed 10 s. Statistical analysis using ANOVA revealed that changing the type of matrix former had no significant effect on the wetting time of tablets (p > .05).

Statistical analysis revealed that tablet formulations containing PEGs, PVP K30, Tween, and NMP as solubilizer showed significantly shorter wetting times compared to ODT G1 (p < .05). Due to the surfactant adsorbing effect at solid surface, which reduces interfacial tension and modifies the ability of water or oil to wet the solid surface, a number of studies have shown wettability shifts.
from oil-wetting towards water-wetting due to surfactant adsorption [30]. It is clear that D1, D2, and D3 ODTs showed rapid wetting time 4.67 ± 0.58, 5.33 ± 0.58, 6.00 ± 0.00 s, respectively with average wetting time not exceeding 7 s.

 Moisture content

Table 2 showed the average percentage moisture content of different tablet formulations. The residual moisture content in the lyophilized tablets was very small, not exceeding 1% ranging from 0.83 ± 0.15 to 0.93 ± 0.25. Statistical analysis using ANOVA test revealed no significant difference between the tablet formulations (p > .05).

 In vitro dissolution studies

The saliva ordinarily maintains the pH of the mouth between 5.6 and 7.6. Therefore, in the dissolution studies, SSF that has a pH of 6.8 ± 0.5 was chosen as the dissolution medium. Figure 5(A) shows the percentage VH dissolved as a function of time from ODTs containing 2% Gelatin, 2% Xanthan gum, 2% Aerosil 200, and 2% Na-alginate compared to VH plain powder and the market product (Levitra®). During first 5 min, the percentage of drug dissolved from formulations G1, X1, A1, and N1, the market product and VH plain powder were 76.43 ± 1.25, 64.10 ± 0.59, 64.10 ± 1.15, 67.40 ± 0.69, 10.63 ± 0.32, and 4.87 ± 0.13%, respectively. VH Plain powder yielded the slowest dissolution rate, with only 4.87 ± 0.13% dissolved after 5 min. On the other hand, VH in the lyophilized tablet was immediately dispersed and almost completely dissolved in 15 min in formulation containing gelatin as a matrix former.

Pairwise comparison of dissolution profiles of formulae G1, X1, A1, and N1 to that of Levitra revealed that the difference factor, \( f_1 \) were 533.87, 455.46, 467.09, and 470.16, respectively and similarity factor, \( f_2 \) were 10.56, 14.31, 13.70, and 13.53, respectively. These results show that the prepared formula (G1) has the greatest difference factor, \( f_1 \) and smallest similarity factor, \( f_2 \) so formula (G1) will be chosen for further investigation.

Also formulation containing gelatin (G1) showed faster drug release than (A1, X1, and N1). Similarly obtained by Abdelet al. [17] in her study on the influence of gelatin on the solubility of sildenafil citrate using freeze-drying method. Statistical analysis revealed that formulation containing 2% aerosil 200 showed a significant decrease in the percentage of the drug dissolved after 5 min compared to the formulation containing 2% gelatin (G1) (p < .05). Similarly as Sadeghi et al. [31] who studied that the
addition of aerosol 200 into formulation of matrices, caused considerable reduction in drug release at different sampling time and also, drug release rates.

Formulation containing 2% xanthan gum showed a significant decrease in the percentage of the drug dissolved after 5 min compared to the formulation containing 2% gelatin (G1) \( p < .05 \). This could be attributed to the hydration of individual xanthan gum particles results in extensive swelling and as a result of the rheological nature of the hydrated matrix, the swollen particles would coalesce. This results in a continuous viscoelastic matrix that fills the interstices, maintaining the integrity of the tablet and retarding further penetration of the dissolution medium [32].

Statistical analysis revealed that formulation containing 2% sodium Alginate (N1) showed a significant decrease in the percentage of the drug dissolved after 5 min compared to the formulation containing 2% gelatin (G1) \( p < .05 \). This effect may be due to easy hydration and gelation of sodium alginate, leading to early clogging of available pores in the matrix and, therefore, much slower tablet disintegration and drug dissolution [33].

Using six different solubilizers (PEG400, PEG6000, PVPK30, PVPK90, Tween80, and N-methyl pyrrolidone) to increase the solubility of VH and increase its dissolution. As polyethylene glycols (PEG) are polymers of ethylene oxide, with a molecular weight (MW) usually falling in the range 200-300,000. Their solubility in water is generally good, but reduces with increasing MW. A meticulous advantage of PEGs for the solid dispersions is that they have good solubility in numerous organic solvents. Additional attractive features of the PEGs include their ability to solubilize some compounds and also to improve compound wettability. Even the dissolution rate of a relatively soluble drug like aspirin can be improved by formulating it as a solid dispersion in PEG 6000 [34]. Similar to PEGs, PVPs have good water solubility and can improve the wettability and thereby the dissolution of the dispersed compound in many cases. PVPs have been reported to improve the solubility and to enhance the rate and extent of poorly soluble drugs [35].

Tween80 increase the micellar concentration, which enhances the drug release and lower the surface tension, in order to make the drug to distribute evenly and prevent further aggregation of drug particles [36]. NMP acts as a cosolvent and complexing agent, nonpolar carbons, which can weaken the hydrogen-bonded structure of water, thus enabling it to act as a cosolvent and the presence of a large planar nonpolar region can lead to hydrophobic interactions between NMP and drugs [37].

Figure 5 also shows the Percentage of VH dissolved from ODTs (G2, G3, G4, G5, G6, and G7) compared to VH dissolved from ODTs (G1) in simulated saliva fluid (PH 6.8) at 37°C. After 5 min, VH dissolved from formula G2 (PEG 400) and G3 (PEG 6000) were 88.00 ± 1.00% and 78.50 ± 0.50%, respectively, compared to 76.43 ± 0.50% from G1 ODT. The improved dissolution of VH is mainly attributed also to increased wettability and accordingly solubility due to the higher level of hydrophilicity by the use of polymeric carriers as Lannie et al. [38] in his study for enhancing the dissolution of ibuprofen using PEG 400.

The results also show that the drug dissolution was inversely proportional with chain length of PEG. This may be attributed to the fact that the water solubility of PEGs decreasing with increasing molecular weight. Similar results were obtained with Bashiri-Shahroodi [39] in his study dropping method as a new possibility in preparation of solid dispersions. After 5 min,
the percentage drug dissolved from formula G4 and G5 were 86.83 ± 0.47% and 87.73 ± 0.64, respectively, compared to 76.43 ± 0.50% from G1 ODT. Additions of PVPs improved the solubility and enhance the rate and extent of VH. Rahamathulla [40] studied solubility and dissolution improvement of rofecoxib using solid dispersion technique and he found that PVPK30 was effective in improving the dissolution of Rofecoxib to 98.57% after 90 min. After 5 min, the percentage of drug dissolved from formula G6 (Tween80) was 86.33 ± 0.58% compared to 76.43 ± 0.50% from G1 ODT. Addition of Tween80 showed a significant increase in the percentage of VH dissolved after 5 min compared to G1 ODT (p < 0.05).

These results are in agreement with those obtained by Ibrahim et al. [41] in his study enhancement of solubility and dissolution rate of domperidone by utilizing different techniques by using surfactant (10% Tween80), resulting in an increase in dissolution of domperidone over that obtained for pure drug. After 5 min, the percentage of drug dissolved from formula G7 was 88.34 ± 0.57% compared to 76.43 ± 0.50% from G1 ODT. The addition of N-methylpyrrolidone showed a significant increase in the percentage of VH dissolved after 5 min when compared to G1 ODT (p < 0.05).

Pairwise comparison of dissolution profiles of formulae (G2, G3, G4, G5, G6, and G7) to that of Levitra revealed that the difference factor, \( f_1 \) were 583.48, 547.84, 575.44, 578.88, 595.43, and 599.16, respectively, and similarity factor, \( f_2 \) were 8.42, 10.00, 8.77, 8.66, 8.06, and 7.93, respectively. These results showed that the prepared formulae (G2, G6, G7) have the greatest difference factor, \( f_1 \) and smallest similarity factor, \( f_2 \) so formulae (G2, G6, G7) will be chosen for further investigation.

Results showed that three solubilizers showed the highest significant enhancement in the dissolution of VH from the lyophilized tablets containing gelatin as a matrix former during 5 min, namely, (G2, G6, and G7) PEG 400, Tween80, and N-Methylpyrrolidone.

Figure 5(D) showed the dissolution profile of VH from ODTs (D1, D2, and D3). Statistical analysis using ANOVA showed that no significant difference in the percentage of VH dissolved after 1, 2, 3, 5, 7, 10, and 15 min compared to ODT (G2, G6, G7) (p > 0.05), indicating that addition of Dapoxetine hydrochloride did not affect the rate or extent of release of VH. Pairwise comparison of dissolution profiles of formulae (D1, D2, and D3) to that of Levitra revealed that the difference factor, \( f_1 \) were 585.28, 598.74, and 607.78, respectively, and similarity factor, \( f_2 \) were 8.39, 7.94, and 7.68, respectively. These results infer that the prepared formulae gave significantly higher dissolution results compared to the commercial product.

Figure 6(B) showed the dissolution profile of Dapoxetine hydrochloride from ODTs (D1, D2, D3) tablets. Results show high percentage of dapoxetine hydrochloride released during the first 5 min and after 15 min, it was 100.00 ± 0.00, 100.03 ± 0.58, and 100.43 ± 0.32 of Dapoxetine hydrochloride for ODTs (D1, D2, D3), respectively.

**Effect of storage on the prepared VH/DH ODTs (accelerated stability study)**

Results show non-significant difference in the mean percentage of VH and DH content and in vitro disintegration time, in ODTs D1, D2, and D3 during a storage period of 6 months (p > 0.05). Also, statistical analysis showed that no significant difference in the residual moisture content of D3 ODTs during a storage period of 6 months (p > 0.05), but D1 and D2 ODTs showed increase in the residual moisture content after 6 months’ storage (p = .001).

Also, results show no significant difference in the percentage of VH and DH from D3 ODTs dissolved after 1, 2, 3, 5, 7, 10, and 15 min during storage for 6 months (p > .05), Figure 6(A,B).

The percentage of VH and DH dissolved from D1 and D2 ODTs after 5 min was significantly decreased after storage for 6 months (p = .019). These results are consistent with the work of Li et al. [42] in his study on the Correlation and prediction of moisture-mediated dissolution stability for benazepril hydrochloride tablets, resulting in linear decrease in dissolution was observed as a function of increase in moisture content. The previous results showed that ODT D3 can be selected for further in vivo comparison against market product (Levitra® and Priligy®).
Pharmacokinetic study of VH/DH ODTs on healthy volunteer

Validation of procedures for the determination of vardenafil and dapoxetine in human plasma by LC-MS/MS

Vardenafil hydrochloride and Dapoxetine hydrochloride was determined in plasma using a validated LC-MS/MS technique, a sample of chromatograms and calibration curves is presented at Figure 7(A,B).

Pharmacokinetic study in healthy volunteers

Figure 7(C) shows the mean plasma VH concentrations versus time for D3-ODT and market product (Levitra®) to human volunteers, and (D) Mean plasma concentration–time profiles of DH after oral administration of (D3-ODT) and market product (Priligy®) to human volunteers.

Figure 7. (A) LC-MS/MS Chromatograms of VH and DH in plasma, (B) Calibration curves of vardenafil hydrochloride and dapoxetine hydrochloride in plasma, (C) Mean plasma concentration–time profiles of VH after oral administration of (D3-ODT) and market product (Levitra®) to human volunteers, and (D) Mean plasma concentration–time profiles of DH after oral administration of (D3-ODT) and market product (priligy®) to human volunteers.
Table 3. Pharmacokinetic parameters of (VH and DH) after administration of D3 and market products (Levitra®) and (Priligy®) to human volunteers.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>VH in D3-ODT</th>
<th>VH in (Levitra®)</th>
<th>DH in D3-ODT</th>
<th>DH in (Priligy®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/l)</td>
<td>13.116 ± 1.096</td>
<td>9.524 ± 0.757</td>
<td>177.799 ± 8.703</td>
<td>150.326 ± 6.592</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.583 ± 0.129</td>
<td>1.25 ± 0.274</td>
<td>0.625 ± 0.137</td>
<td>1.167 ± 0.258</td>
</tr>
<tr>
<td>AUC0–t (ng/ml h)</td>
<td>40.053 ± 11.096</td>
<td>39.937 ± 10.548</td>
<td>353.673 ± 129.967</td>
<td>625.914 ± 80.879</td>
</tr>
<tr>
<td>AUC0–t2 (ng /ml h)</td>
<td>39.234 ± 10.932</td>
<td>38.867 ± 10.548</td>
<td>531.681 ± 129.544</td>
<td>623.217 ± 80.917</td>
</tr>
<tr>
<td>Kt (h−1)</td>
<td>0.173 ± 0.023</td>
<td>0.175 ± 0.025</td>
<td>0.251 ± 0.011</td>
<td>0.249 ± 0.027</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>4.069 ± 0.567</td>
<td>4.028 ± 0.661</td>
<td>2.774 ± 0.154</td>
<td>2.807 ± 0.289</td>
</tr>
<tr>
<td>MRTINF</td>
<td>4.921 ± 0.601</td>
<td>5.329 ± 1.102</td>
<td>3.52 ± 0.467</td>
<td>4.016 ± 0.445</td>
</tr>
</tbody>
</table>

Relative bioavailability (%)a 100.9%

aRelative bioavailability is based on comparison of AUC0–t.

Table 4. ANOVA test showing p values for pharmacokinetic parameters of (VH and DH) after administration of D3 and market products (Levitra®) and (Priligy®) to human volunteers.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>VH</th>
<th>DH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>.0001*</td>
<td>.0007*</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>.0001</td>
<td>.0002*</td>
</tr>
<tr>
<td>AUC0–t (ng h/ml)</td>
<td>.7147</td>
<td>.2154</td>
</tr>
<tr>
<td>AUC0–t2 (ng h/ml)</td>
<td>.6772</td>
<td>.2143</td>
</tr>
</tbody>
</table>

*p value

D3-ODT and (Levitra®) tablets and (Priligy®) tablets are shown in Table 3.

Statistical analysis using Analysis of variance (ANOVA) of log transformed pharmacokinetic parameters and includes formulation, period, sequence, and subject factors was done. The results of ANOVA are calculated at 5% level of significance (α = 0.05) and p values is presented at Table 4. The mean values for the maximum plasma concentration (Cmax) of VH were 13.116 ± 1.096 and 9.524 ± 0.757 ng/ml after the oral administration of the market product D3-ODT and (Levitra®) respectively. In addition, the mean values for the time to peak plasma concentration (tmax) were 0.583 ± 0.129 and 1.25 ± 0.274 h, while the mean values for the area under plasma concentration-time curve AUC0–t were 40.053 ± 11.096 and 39.937 ± 11.106 ng h/ml, AUC0–t2 were 39.234 ± 10.932 and 38.867 ± 10.548 ng h/ml for the two aforementioned formulations, respectively. The mean values for the maximum plasma concentration (Cmax) of DH were 177.799 ± 8.703, and 150.326 ± 6.592 ng/ml after the oral administration of D3-ODT and (Priligy®) respectively. In addition, the mean values for the time to peak plasma concentration (tmax) were 0.625 ± 0.137 and 1.167 ± 0.258 h, while the mean values for the area under plasma concentration-time curve AUC0–t were 353.673 ± 129.967 and 625.914 ± 80.879 ng h/ml and AUC0–t2 were 353.681 ± 129.544 and 623.217 ± 80.917 ng h/ml for the two aforementioned formulations, respectively.

The shorter Tmax of VH and DH obtained from the ODT formulation suggests a more rapid onset of action compared to the commercial products (Levitra®) and (Priligy®), respectively. These results obtained from our work is consistent with the aim of work and conclusion of the results obtained by Debruyne et al. [43], where they found Vardenafil ODT shows a rapid onset of action comparable with that of vardenafil film-coated tablet and Berry et al. [44], where they found that vardenafil absorbed faster and with less variability using the nebulizer for drug delivery, this means that rate of absorption is a priori.

Conclusion

In light of results of the current work, it is conclusive that lyophilization technique is suitable for the preparation of ODTs of VH and VH/DH ODTs. Gelatin is a suitable matrix former for the preparation of ODTs that complies with suitable weight uniformity, friability, drug content uniformity, disintegration time, and release of VH from ODT. Addition of DH did not affect significantly the percent released of VH in SSF. Stability studies showed that D3-ODT maintained its initial properties with respect to disintegration time, residual moisture, and dissolution characteristics after 6 months’ storage at 40 ± 2 °C and 75 ± 5% RH. Formula of choice (D3-ODT) performed significant higher Cmax signiﬁcant shorter Tmax, higher comparable AUC0–t and hence enhanced rate of bioavailability for VH compared to the market product (Levitra®). Formula of choice (D3-ODT) performed significant higher Cmax signiﬁcant shorter Tmax, comparable AUC0–t and hence enhanced rate of bioavailability for DH compared to the market product (Priligy®).

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Disclosure statement
The authors alone are responsible for the content and writing of this article and reports that there is no conﬂict of interest.

References

