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*Article*

# Optimization of the Factors Affecting the Absorption of Vardenafil from Oral Disintegrating Tablets: A Clinical Pharmacokinetic Investigation

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**Abstract:** Because of lower solubility and considerable metabolism, vardenafil (VRD) bioavailability is 15 %. To get over this obstacle, this study aimed to increase the solubility, hasten the onset of action, and mask the unpleasant taste of VRD utilizing β-cyclodextrin (β-CD) and formulation of the inclusion complex as oral disintegrating tablets (ODTs). The solubility of the obtained complexes in various ratios has been studied. A Box-Behnken design (BBD) was utilized to investigate the influence of excipients on the quality of ODTs. The solubility of VRD was improved at 1:2 drug: β-CD ratio. The formulated VRD-ODTs exhibited satisfying results regarding the hardness and disintegration time. In addition, in vivo taste masking and disintegration time showed improved results, after placing the tablets in the oral cavity of the healthy volunteers. The pharmacokinetic parameters for the optimized VRD–ODTs exhibited a significant improvement with $P < 0.05$ in the maximum plasma concentration and reduction in the time needed to reach this concentration when compared with the marketed tablets. Finally, the optimized VRD-ODTs exhibited increased oral absorption of VRD and subsequent decreasing the time of onset of clinical effect and masking the unpleasant taste, which is favored for patients with erectile dysfunction.

**Keywords:** Bioavailability; Box-Behnken design; β-cyclodextrin; erectile dysfunction; taste masking; vardenafil.

## 1. Introduction

Vardenafil (VRD), a potent phosphodiesterase (type V) inhibitor, used for the treatment of the erectile dysfunction disease [1]. Its mechanism of action depends on prevention of the degradation of cyclic GMP (cGMP) in the smooth muscle tissues located on the internal surface of the blood vessels that supply the corpus cavernosum of the penis. This accumulation of cGMP in the corpus cavernosum leads to the release of nitric oxide that causes dilation of the blood vessels then, the erection occurs successfully [2]. Furthermore, VRD has been utilized in patients with pulmonary hypertension due to the presence of PDE5 in the smooth muscle of the arterial wall within the lungs [1]. According to the biopharmaceutical classification system (BCS), VRD was classified as a class II drug [3] that suffers from drawbacks of low bioavailability (15%) and bitter taste. It is also subjected to extensive first-pass metabolism which is the major reason for low oral bioavailability. VRD is mainly metabolized by cytochrome P450 [4].

Oral drug delivery is the favored delivery system for numerous drugs. In comparison to different delivery systems, the oral drug delivery has arising benefits towards the ease of administration and the bioavailability [5]. However, due to the difficulties of swallowing facing

pediatric, geriatric, and mentally retarded patients, scientists developed oral disintegrating tablets (ODTs) as a convenient way of administration [6]. Therefore, ODTs improved patient compliance and convenience [7] beside enhanced the absorption and bioavailability comparing to conventional tablets [8].

Most of the active ingredients have unacceptable taste in which taste masking has an important role in the formulation of ODTs. The unacceptable taste of the active ingredients can be eliminated by several methods, e.g., the addition of sweeteners and flavors, blending with cyclodextrins (CDs), and encapsulating the unpleasant drug into microparticles [9]. Unacceptable taste is one of the significant drawbacks of the orally administrating drugs, which encountered with numerous medications. Administration of medications by oral route with pleasant taste is a critical issue for health providers and in the commercial success of ODTs [10]. Therefore, the unacceptable tasting drugs often affect the compliance of patients [11]. Masking the unpleasant drug taste could be reached by several techniques. These techniques contain inclusion of complex by using CD, ion exchange resins, polymers, and microencapsulation technology [12–16]. CD complexation ability have been broadly used in the pharmaceutical area for several implementations, involve taste-masking of bitter taste [17–19] and improve solubility, stability, and bioavailability of the drug [20,21]. β-cyclodextrin (β-CD) is broadly utilized as taste covering agents of unacceptable taste drugs due to the solubility (it has the least solubility in comparison to other kinds of CDs), the sweet taste, and the good safety profile [22,23]. The inhibition of unpleasant taste by CD was superior in beta-CD in comparison with gamma and alpha CDs stable complex [15,19]. However, the utilize of β-CD as a taste-masking agent is broadly reported [24–26].

Therefore, the aim was the investigation of the factors affecting the oral absorption of VRD from taste-masked VRD-ODTs. In addition, the pharmacokinetic parameters of the optimized VRD-ODTs were studied on human volunteers.

## 2. Materials and Methods

### *2.1. Materials*

Vardenafil (VRD) was purchased from Jinlan Pharm-Drugs Technology Co., Ltd. (Hangzhou, China); β-Cyclodextrin (β-CD) was kindly gifted from Nihon Shokuhin Kako Co., Ltd., (Tokyo, Japan); Crospovidone was from BASF (Ludwigshafen, Germany); lactose, microcrystalline cellulose (Avicel PH 101), and sodium starch glycolate (Explotab) were kindly gifted from Jamjoom Pharmaceuticals Co. (Jeddah, Saudi Arabia); mannitol, magnesium stearate and talc from SAJA Pharmaceutical Co. Ltd. (Jeddah, Saudi Arabia), and methanol from Sigma-Aldrich (St. Louis, MI, USA).

### *2.2. Pre-formulation studies:*

#### 2.2.1. Preparation of VRD-β-CDs inclusion complexes

The inclusion complexes of VRD with β-CD at different molar ratios 1:1, 1:1.5, and 1:2 were formed by the kneading method as reported previously [27–30]. The calculated amounts of VRD and the polymer were triturated with a small volume of methanol to prepare a homogenous slurry, then kneaded for 45 min and dried for 24 h at room temperature. The dried mass was pulverized and passed through mesh No. 200 and stored at 4 °C until further utilization.

#### 2.2.2. Solubility study

The effect of inclusion complexes on the solubility of VRD was evaluated according to the method reported by Higuchi and Connors [31]. Briefly, an excess of raw VRD and VRD- β-CD were added to vials containing distilled water then placed in a shaking water bath at 25± 0.5°C. Samples were analyzed for VRD content at 230 nm until equilibrium is attained.

#### 2.2.3. Compatibility study

Drug-polymer interactions were examined utilizing Fourier transform infrared spectroscopy (FTIR). The spectra were recorded on a Nicolet IZ 10 (Thermo Fisher Scientific, Waltham, MA, USA) in the range of 4000–400cm$^{-1}$.

*2.3. Formulation of ODTs*

2.3.1. Application of Box Behnken experimental design

A three-level three-factor BBD was utilized. These factors are the percentage of a bulking agent (mannitol) as $X_1$, the percentage of superdisintegrant (sodium starch glycolate; Explotab) as $X_2$, and the percentage of binding agent (microcrystalline cellulose; Avicel) as $X_3$. The ODTs hardness ($Y_1$), and the disintegration time ($Y_2$) were the evaluated responses. Construction and estimation of the statistical design were achieved with Statgraphics Centurion XV version 15.2.05 software, StatPoint Technologies Inc., (Warrenton, VA, USA). To produce formulations displaying maximum hardness with minimum disintegration time, 15 experimental formulations were suggested by BBD (Table 1).

**Table 1.** Composition of VRD-ODTs formulations based on Box-Behnken design

| Formula # | Drug complex * | Manni-tol | Explotab | Avicel | Crospo-vidone | Lactose | Magnesium stearate | Talc |
|---|---|---|---|---|---|---|---|---|
| **F1** | 30 | 70 | 20 | 30 | 10 | 36 | 2 | 2 |
| **F2** | 30 | 70 | 20 | 50 | 10 | 16 | 2 | 2 |
| **F3** | 30 | 60 | 12 | 40 | 10 | 44 | 2 | 2 |
| **F4** | 30 | 70 | 12 | 30 | 10 | 44 | 2 | 2 |
| **F5** | 30 | 60 | 16 | 50 | 10 | 30 | 2 | 2 |
| **F6** | 30 | 80 | 16 | 30 | 10 | 30 | 2 | 2 |
| **F7** | 30 | 70 | 12 | 50 | 10 | 24 | 2 | 2 |
| **F8** | 30 | 60 | 20 | 40 | 10 | 36 | 2 | 2 |
| **F9** | 30 | 80 | 20 | 40 | 10 | 16 | 2 | 2 |
| **F10** | 30 | 80 | 16 | 50 | 10 | 10 | 2 | 2 |
| **F11** | 30 | 60 | 16 | 30 | 10 | 50 | 2 | 2 |
| **F12** | 30 | 80 | 12 | 40 | 10 | 24 | 2 | 2 |
| **F13** | 30 | 70 | 16 | 40 | 10 | 30 | 2 | 2 |
| **F14** | 30 | 70 | 16 | 40 | 10 | 30 | 2 | 2 |
| **F15** | 30 | 70 | 16 | 40 | 10 | 30 | 2 | 2 |

* Equivalent to 5 mg VRD

2.3.2. Preparation of ODTs

Direct compression method was utilized for the preparation of the suggested VRD-ODTs as displayed in Table 1. The tablet blend was compressed using 7 mm punches into 200 mg tablets using a tablet press (Erweka, GmbH, Heusenstamm, Germany).

*2.4. Evaluation of the prepared VRD-ODTs*

Evaluation of VRD-ODTs was performed on the tablets of all batches considering the visual inspection, weight and content uniformity, thickness, hardness and friability according to the Pharmacopeial requirements.

*2.5. In vitro disintegration of VRD-ODTs*

VRD-ODTs (6 tablets/batch) were placed in the baskets of USP disintegration apparatus (Pharmatest, PT-DT7, Germany). The apparatus run utilizing distilled water as the immersion fluid at 37 ± 0.5°C. The tablets were observed, and the time taken for complete disintegration of all tablets was determined.

*2.6. In vitro dissolution of VRD-ODTs*

USP dissolution apparatus II (paddle method) of Erweka GmbH, (Heusenstamm, Germany) was used in the dissolution of VRD from the ODTs. The study was achieved with 900 ml distilled water at 50 rpm and equilibrated at 37 ± 0.5°. Samples of 5 ml were withdrawn at the predetermined time intervals 5, 10, 15, 20, 30, 45, and 60 minutes and replaced with a fresh preheated medium for each time point, then analyzed by UV spectrophotometer at 230 nm. The experiment was performed three times for each formula and the mean values of the cumulative % release of VRD after 60 minutes were determined.

*2.7. VRD-ODTs formulation data analysis by BBD*

$Y_1$ and $Y_2$ were analyzed using the experimental design software. Significance of the analysis was set for any factor at $p < 0.05$. The optimized VRD-ODT formulation suggested was prepared and checked for the hardness and disintegration time results. The observed values were compared with the predicted ones and the residuals were calculated.

*2.8. In vivo evaluation of the optimized VRD-ODTs on human volunteers*

2.8.1. In vivo taste masking and disintegration time evaluation

A single-blind study was intended for disintegration time and the taste masking tests in the buccal cavity of six healthy human volunteers. The human subjects were asked to rate the bitter taste of the optimized formula utilizing a scale of 0–3. When the score ≤ 1, the taste was acceptable while if the score > 1, indicates the tablet is bitter and not acceptable [14]. Also, the disintegration time of the tablet in the oral cavity was recorded.

2.8.2. Pharmacokinetic parameters evaluation

An open-label, single dose, randomized, one-period, parallel design comprising fourteen days of screening preceding 24 h study periods was used. The participants were administered an oral 10 mg of VRD either in the optimized formulation (test) or in the marketed tablets (reference) with water. The study was carried out at the Egyptian Research and Development Company (ERDC), Cairo, Egypt. ERDC Research Ethical Committee had formally approved the study design protocol on its expedited meeting on August 30, 2017 (Ethical Approval Code is Verd-p 0566/449). The study was accomplished in agreement with the Declaration of Helsinki and the International Conference on "Harmonization of Good Clinical Practices."

*2.8.2.1. Population and sampling*

Healthy male volunteers (25 and 43 years of age) at the time of screening were selected for the study. The selected subjects signed written informed consent, were willing to participate in this clinical trial, and to comply with the study requirements. Complete medical history, laboratory analysis, and physical examination were performed for the selected candidates to ensure their eligibility for participation. Subjects were divided into two groups (6 each). The first group was administered the optimized formulation while the second one was given the marketed VRD tablets. Blood samples (5 mL) were collected at 0, 0.16, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h in heparinized tubes. The tubes were centrifuged at 3500 rpm for 10 min (Centurion, West Sussex, UK) then stored at -80 °C until analysis.

*2.8.2.2. Chromatographic conditions*

VRD detection in human plasma was conducted using a high-performance liquid chromatography coupled with MS/MS method (HPLC-MS/MS) that was developed at the ERDC. Validation of the method was based on the FDA Bio-analytical Method Validation Guidelines 2003. Assay linearity was verified for VRD at a concentration range of 3-350 ng/ml with regression coefficients (R2) of 0.996 and 0.994 for VRD. The lower limits of quantification were 3 ng /ml for VRD. The HPLC-MS/MS system consisted of HPLC, Agilent series 1200 (Agilent Technologies Deutschland GmbH, Waldbronn, Germany), used with a Triple Quad G1311A quaternary pump and mass hunter software. Chromatography was performed (75% acetonitrile: 25% buffer (ammonium formate 20 mM + 0.2 % (v/v) formic acid in water) as mobile phase at a flow rate of 0.35 ml/min and a reversed phase column Intersil ODS -3 (4.6 mm x 50 cm, dp 5µm) at 25 ◦C. Sildenafil was selected as an internal standard (IS).

*2.8.2.3. Pharmacokinetic analysis*

A noncompartmental analysis of the pharmacokinetic parameters was achieved by unpaired t-test (two-tailed) using Kinetica® software. Any significant difference in drug plasma concentration between the two groups was assessed with two-way ANOVA followed by Sidak's multicomparison test using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA). Results were considered significant at $P < 0.05$. The peak plasma concentration achieved by the drug (Cmax), the time after administration of a drug when the maximum plasma concentration is reached (tmax), the area under curve (AUC), elimination rate constant (Kel) and mean drug residence time (MRT) was calculated to allow the relative bioavailability [(AUCformulation/AUCtablets) ×100] to be determined.

## 3. Results and Discussion

VRD-ODTs were developed to deliver the drug rapidly. A pre-formulation study involving complexation of VRD with β-CD was carried out. A direct compression method was used for the formulation of 15 formulae of VRD-ODTs according to BBD. All the prepared formulations were evaluated for weight uniformity, thickness, friability, hardness, content uniformity, and in vitro disintegration as well as in vitro dissolution. The results of all experiments were used to correlate the independent variables that constitute the combination of excipients of tablets with the dependent variables that represent the quality parameters of the ODTs. BBD utilized these relations to statistically optimize the process to produce VRD-ODTs with maximum hardness and minimum disintegration time. Finally, the obtained optimized formulation was evaluated in vivo on healthy human volunteers compared with the marketed Levitra tablets. The details of the results and their discussion are given in the following sections.

*3.1. Saturation solubility studies of the prepared complexes*

The data represented in Figure 1 revealed that the solubility of raw VRD was 0.13 mg/ml. While the solubility of VRD in solid dispersion using β-CD in different molar ratios 1:1, 1:1.5, and 1:2 was increased to reach 13.7 mg/ml for VRD-β-CD 1:2 molar ratio. This finding confirms the formation of a complex with β-CD and improves VRD solubility which in a good agreement with the previously reported results of carvedilol [32], piroxicam [33], ketoconazole [34], gliclazide [35], Zafirlukast [36], and aripiprazole [37].

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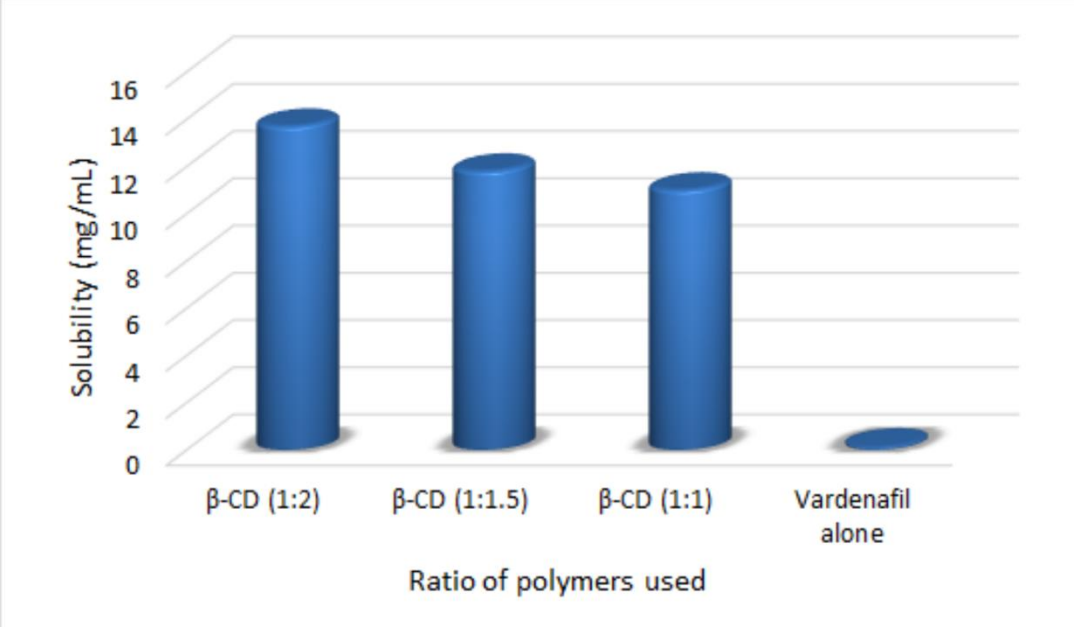


**Figure 1.** The solubility of VRD in complexes with the β-CD polymer in different ratios

*3.2. Drug-excipients compatibility study using FTIR*

VRD- β-CD and VRD-excipients interactions were evaluated using FTIR spectra. Figure 2 showed the characteristic amidic carbonyl group of VRD (1685 cm-1) with the absence of N-H group at (3000 or more) as it is tautomeric amidic N-H. β-CD showed O-H groups as a broad and intense peak at 3220-3500. The VRD- β-CD complex showed the reduced intensity of O-H group that could be attributed to the participation of part of O-H groups, in the core of β-CD were involved with N-H group of VRD. In addition, carbonyl group intensity for VRD was reduced that could be attributed to VRD is tautomerized into keto-enol form. In keto form, carbonyl group act as hydrogen bond acceptor. In enol form, the carbonyl group is completely disappeared and replaced with O-H group. Further confirmation of results, in case of VRD there was a very weak peak at 2400 cm-1 that indicated the absorption band of C=N (existed in enol form) which means VRD was in the isolated form mostly existing in keto form. While, in the case of the complex the percentage of keto form decreased, and the percentage of enol form increased that is attributed to the involvement of VRD with hydrogen bonding with β-CD.

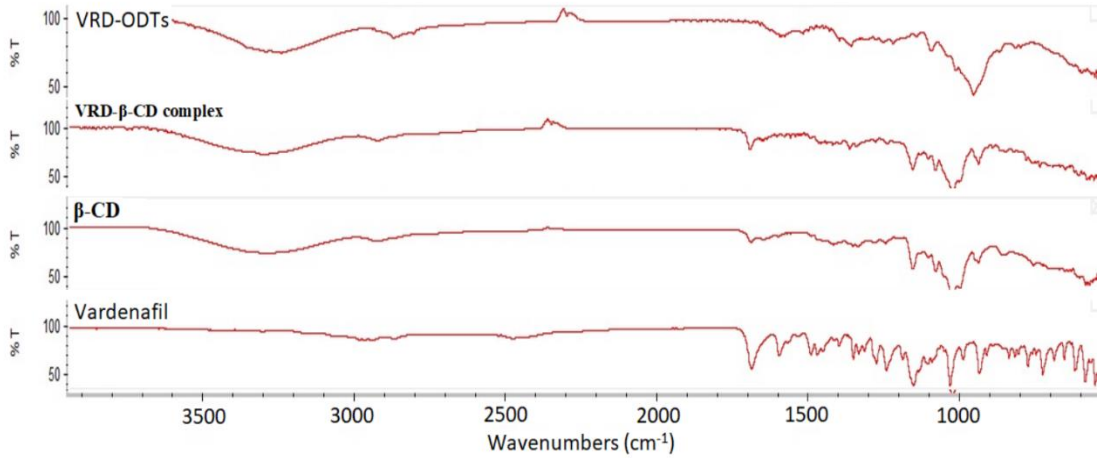


**Figure 2.** FTIR spectra of VRD alone, β-CD alone, VRD β-CD complex, and VRD-ODTs

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*3.3. Development of VRD–ODTs*

Development of the formulation in the present study was mainly based on three factors namely mannitol percentage as a diluent, Explotab percentage as a super disintegrant, and Avicel percentage as a binder. Various ratios of each component combinations were used to get oral disintegrating tablets with good quality attributes. All formulations were suggested by BBD as described in Table 1.

*3.4. Evaluation of VRD-ODTs*

All batches of VRD-ODTs were evaluated and results are shown in Table 2. The tablet weights of the prepared batches were less than 2 %, in accordance with USP requirements. The tablets formulations met the European Pharmacopeia limits for disintegration of oral disintegration tablets (> 3 min). Friability, hardness and thickness of the prepared tablets were in the acceptable limits as indicated in Table (2).

**Table 2.** Characteristics of VRD-ODTs, data expressed as mean ± SD (n = 10)

| Formula No. | Weight (mg) | Thickness (mm) | Hardness ($kg/cm^2$) | Friability (%) | Drug content (%) | Disintegration time (s) |
|---|---|---|---|---|---|---|
| F1 | 203.0 | 2.64 | 3.72 | 0.635 | 97.77 | 38.32 |
| F2 | 201.6 | 2.62 | 5.58 | 0.496 | 101.07 | 48.67 |
| F3 | 203.3 | 2.60 | 4.74 | 0.147 | 100.98 | 88.33 |
| F4 | 204.3 | 2.60 | 3.44 | 0.645 | 99.37 | 52.67 |
| F5 | 202.3 | 2.69 | 5.31 | 0.494 | 101.7 | 46.67 |
| F6 | 202.6 | 2.69 | 4.57 | 0.493 | 98.7 | 72.67 |
| F7 | 200.0 | 2.60 | 6.43 | 0.301 | 96.84 | 76.33 |
| F8 | 201.6 | 2.60 | 3.17 | 0.297 | 99.86 | 36.33 |
| F9 | 203.0 | 2.57 | 6.27 | 0.197 | 99.73 | 41.43 |
| F10 | 203.0 | 2.59 | 6.72 | 0.492 | 100.09 | 88.49 |
| F11 | 199.3 | 2.60 | 3.75 | 0.051 | 100.45 | 65.0 |
| F12 | 203.0 | 2.60 | 4.94 | 0.977 | 97.59 | 97.67 |
| F13 | 201.0 | 2.60 | 4.3 | 0.299 | 95.43 | 66.43 |
| F14 | 202.0 | 2.60 | 4.54 | 0.098 | 98.84 | 73.33 |
| F15 | 202.0 | 2.61 | 4.67 | 0.198 | 100.36 | 63.45 |

In addition, drug content was in the range of 96.84 to 101.7 %. To avoid delaying the disintegration of ODTs, the hardness usually planned to be lower than the conventional tablets. The hardness is an essential factor which affects the disintegration and dissolution times that influence bioavailability [38]. Some challenges encountered in the formulation and production of ODTs such as the disintegration time and mechanical strength. The ideal ODTs should have concise disintegration time that is usually about one min or less which require low mechanical strength. The disintegration time is directly proportional with the mechanical strength of the tablets. Therefore, it is essential to have a good compromise between mechanical strength and disintegration time [9].

*3.5. In vitro dissolution studies*

Figure 3 displayed the release profiles of the 15 formulations proposed by BBD. Most of the formulations released all VRD content within 20 min of the study period and all of them showed more than 98.56% within 60 min.

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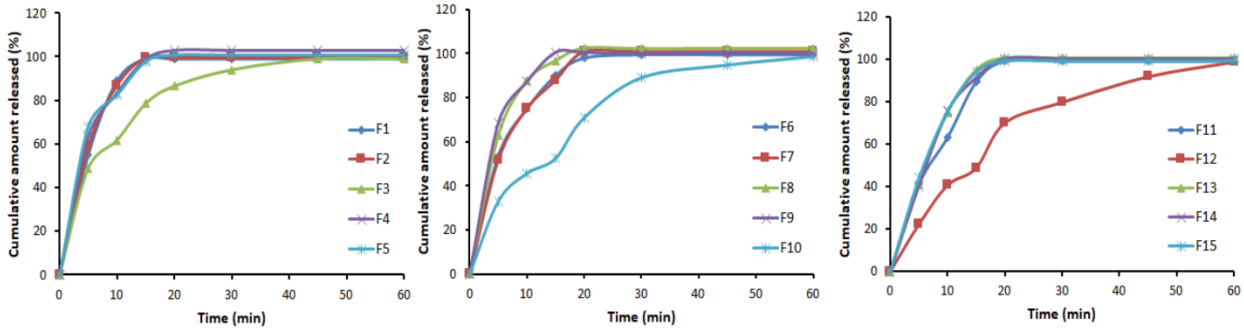


**Figure 3.** In vitro dissolution profile of formulations (F1-F15)

*3.6. Quantitative estimation of the factors affecting VRD-ODTs*

Table 3 revealed the estimate effect of each factor and p-values for $Y_1$ and $Y_2$ resulted from two-way analysis of variance (ANOVA). From the obtained analysis, Explotab percentage ($X_2$) had no significant effect on the hardness of tablets ($Y_1$) but had a significant antagonistic effect on the disintegration time of the tablets ($Y_2$) with a *P*-value of 0.0001. Also, it was found that mannitol percentage ($X_1$) and avicel percentage ($X_3$) had significant synergistic effects on the hardness ($Y_1$) with *P*-values of 0.0009 and 0.0001, respectively. In addition, the interaction term $X_1X_2$ showed a significant synergistic effect on the hardness with a *P*-value of 0.0035.

**Table 3.** Estimated effects of factors, *F*-ratios and associated *p*-values for ($Y_1$ and $Y_2$) responses

| | Hardness ($Y_1$) | | | Disintegration time ($Y_2$) | | |
|---|---|---|---|---|---|---|
| Factor | Estimate | *F*-ratio | *P*-value | Estimate | *F*-ratio | *P*-value |
| A: Mannitol | 1.3825 | 49.05 | 0.0009* | 15.9825 | 2.78 | 0.1565 |
| B: Explotab | -0.2025 | 1.05 | 0.3520 | -37.5625 | 15.34 | 0.0112* |
| C: Avicel | 2.14 | 117.53 | 0.0001* | 7.875 | 0.67 | 0.4490 |
| AA | 0.5717 | 3.87 | 0.1063 | 12.4133 | 0.77 | 0.4195 |
| AB | 1.45 | 26.98 | 0.0035* | -2.12 | 0.02 | 0.8819 |
| AC | 0.295 | 1.12 | 0.3390 | 17.075 | 1.58 | 0.2637 |
| BB | -0.0183 | 0.00 | 0.9521 | -16.0067 | 1.29 | 0.3083 |
| BC | -0.565 | 4.10 | 0.0989 | -6.655 | 0.24 | 0.6445 |
| CC | 0.5967 | 4.22 | 0.0952 | -11.4717 | 0.66 | 0.4534 |

*Significant effect of factors on the investigated response

3.6.1. Mathematical modeling of the data

Mathematical modelling for $Y_1$ and $Y_2$ of VRD-ODTs were generated after analysis of the data using the Statgraphics® software (Eqs. 1 and 2).

Hardness ($Y_1$) = 34.332 - 1.360 $X_1$ - 1.986 $X_2$ - 0.244 $X_3$ + 0.011 $X_1^2$ + 0.073 $X_1X_2$ + 0.006 $X_1X_3$ - 0.002 $X_2^2$ - 0.028 $X_2X_3$ + 0.0119 $X_3^2$ (1)

In vitro disintegration time ($Y_2$) = 311.603 - 21.762 $X_1$ + 32.988 $X_2$ + 0.674 $X_3$ + 0.248 $X_1^2$ - 0.106 $X_1X_2$ + 0.342 $X_1X_3$ - 2.001 $X_2^2$ - 0.333 $X_2X_3$ - 0.229 $X_3^2$ (2)

Equations 1 and 2 reflect the quantitative effect of formulation factors; mannitol % ($X_1$), Explotab % ($X_2$), and Avicel % ($X_3$) and their interactions on the responses; the hardness ($Y_1$) and the in vitro disintegration time ($Y_2$). Figure 4, 2D Pareto charts, showed the effect of $X_1$-$X_3$ and their interactions on the $Y_1$ and $Y_2$.

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3.6.2 Effect of the factors on $Y_1$ and $Y_2$

Figure 4 displayed that $X_1$ and $X_3$ have significant synergistic effects on $Y_1$, and the interaction between $X_1$ and $X_2$ have also a significant synergistic effect on $Y_1$. While $X_2$ has no significant effect on $Y_1$. Figure 5, 3D surface plots, confirmed the understanding the influence of each factor on $Y_1$, Similar findings were reported in the literature for the effect of excipients on the prepared tablet properties [39–45].

Also, Figure 4 displayed that $X_2$ has a significant antagonistic effect on $Y_2$. This finding means that increasing $X_2$ level will lead to reduction in the disintegration time. Other studies have investigated the effect of Explotab on tablet disintegration times [42,46,47]. Both $X_1$ and $X_3$ have no significant effect on $Y_2$. Also, 3D response surface plots (Figure 5) were graphically established utilizing the software.

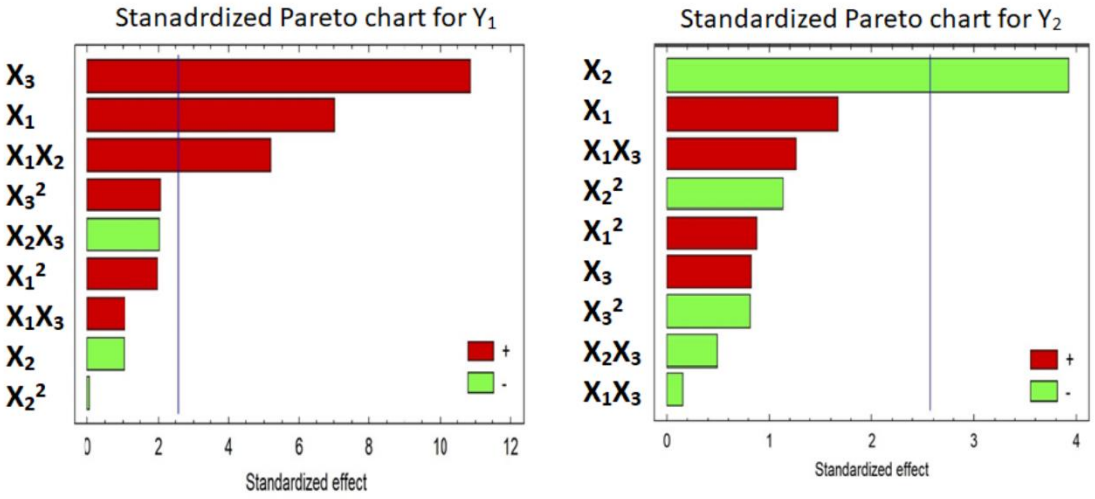


**Figure 4.** Standardized Pareto charts for $Y_1$ and $Y_2$

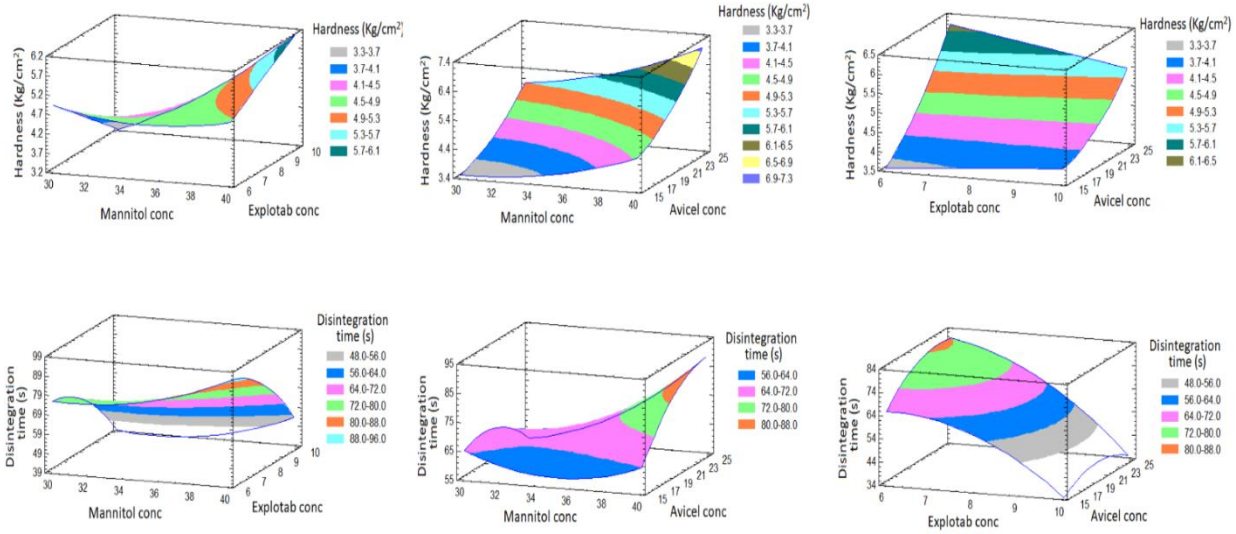


**Figure 5.** Response surface plots (3D) showing the effects of $X_1$, $X_2$ and $X_3$ on $Y_1$ and $Y_2$

*3.7. Prediction of the optimized formulation*

After the data analysis, the combination of factor levels that achieve maximum desirability function were detected to be used in the preparation of the optimized formulation. The optimum combination of factors was 38.52 % of Mannitol concentration, 9.99 % of Explotab concentration, and

25 % of Avicel concentration. The observed, predicted and residual values for $Y_1$ were 6.98 kg/cm$^2$, 6.72 kg/cm$^2$ and 0.27, respectively while, for $Y_2$ were 51.77 s, 49.78 s and 1.99, respectively. This finding proved the mathematical experimental design reliability in maximizing $Y_1$ and minimizing $Y_2$ that fulfills the Pharmacopoeial requirements via the direct compression method.

### *3.8. In vivo evaluation of the optimized VRD-ODTs on human volunteers*

#### 3.8.1. In vivo taste masking and disintegration time

The results of the in vivo taste masking test were listed in Table 4. The scores of the six volunteers were equal or less the one which indicate the formulation has an acceptable taste masking effect. The mean result of the in vivo disintegration time was 62.33 s. Which is acceptable according to European Pharmacopeia.

**Table 4.** *In vivo* taste masking and disintegration time of the optimized formula

| Volunteer No. | Disintegration time (s) | Taste masking (0-3) |
|---|---|---|
| V1 | 65 | 0 |
| V2 | 60 | 1 |
| V3 | 62 | 1 |
| V4 | 63 | 0 |
| V5 | 59 | 0 |
| V6 | 65 | 0 |
| Mean | 62.33±2.503 | |

#### *3.8.2. Pharmacokinetic parameters evaluation*

The VRD plasma concentration time profiles from the optimized formulation of VRD-ODT and the marketed Levitra tablet (Bayer AG, Leverkusen, Germany) are represented in Figure 6. The values of Cmax, tmax and AUCo-24, t1/2, Kel, MRT, and volume of distribution (Vd), for VRD from these formulations, are summarized in Table 5. The results indicated that optimized VRD-ODTs bioavailability (F) compared with the marketed tablet was 125.429 %. This data indicated that ODT's improved the bioavailability of VRD over the marketed tablets. The oral absorption of VRD from ODTs was obviously higher when compared with the marketed tablets which was obvious from the value of Cmax that increased significantly from 24.58 ng/ml for the marketed tablet to 36.38 ng/ml (for the optimized VRD-ODTs.

In addition, the tmax of optimized VRD-ODTs shortened to 1 h when compared with tmax of 2 h for the marketed tablets which indicated that the onset of action of VRD from optimized ODTs was accelerated in comparison with the marketed tablets. The analysis of variance showed that there were significant differences among the samples ($P< 0.05$) taken at 0.5, 0.75, 1, and 1.25 h from the two groups of volunteers indicating the significant improvement achieved by the ODTs. Complexation of VRD with β-CD followed by its formulation as taste masked ODTs enhance both the solubility and dissolution rate that reflects on the availability of VRD ready for absorption [48]. Accordingly, optimized VRD-ODTs is a promising approach for improved VRD bioavailability.

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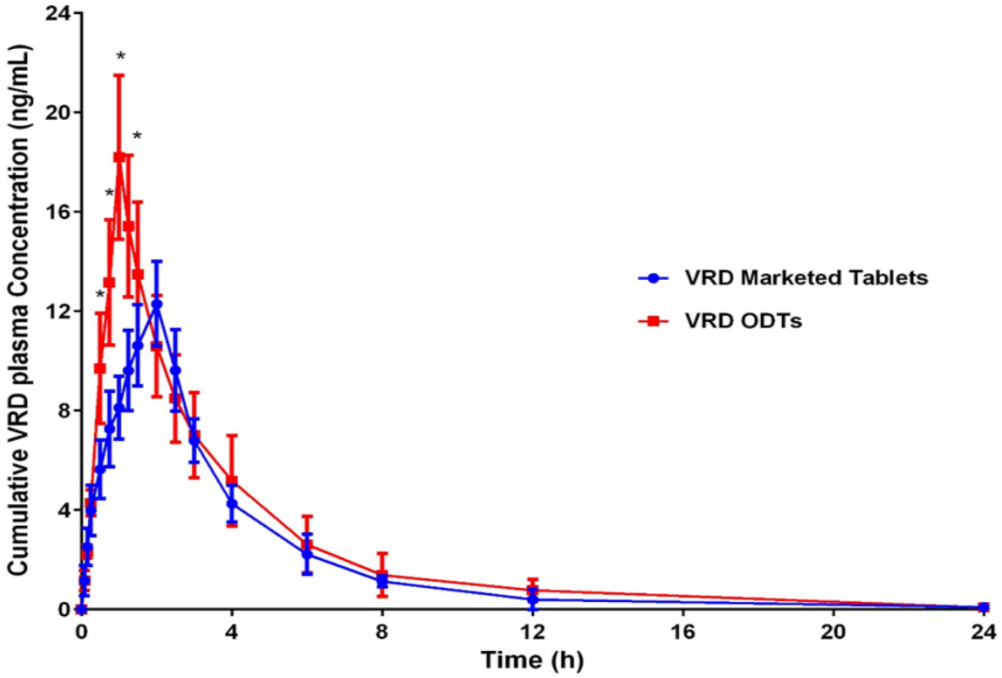


**Figure 6.** The mean plasma concentration-time profiles of VRD after oral administration of a single oral dose (10 mg) of the marketed Levitra tablet and optimized VRD-ODTs

**Table 5.** Pharmacokinetic parameters ± SD of VRD following the administration of a single oral dose (10 mg) of either the VRD marketed tablet, or the optimized VRD-ODTs

| Pharmacokinetic parameter | VRD marketed tablet | Optimized VRD-ODTs |
|---|---|---|
| $C_{max}$ (ng/ mL) | 24.58767 ± 5.11 | 36.38219 ± 3.91* |
| $t_{max}$ (h) | 2.0 ± 0.13 | 1.0 ± 0.21* |
| $AUC_{(0-24)}$ (ng.h/ mL) | 93.376 ± 9.12 | 117.6253 ± 10.32* |
| $AUC_{(24-\infty)}$ (ng.h/ mL) | 0.92 ± 0.72 | 0.650553 ± 0.15 |
| $AUC_{(0-\infty)}$ (ng.h/ mL) | 94.297 ± 7.18 | 118.2758 ± 34.34 |
| AUMC (24-end) ng.hr$^2$/mL | 369.1667 | 478.1427 |
| AUMC (24-end) ng.hr$^2$/mL | 22.09666 | 15.61327 |
| AUMC (0-end) ng.hr$^2$/mL | 391.2633 | 493.756 |
| $K_{el}$ ($h^{-1}$) | 0.209 ± 0.01 | 0.230573 |
| $t_{1/2}$ (h) | 3.317 ± 0.63 | 3.005555 ± 0.53 |
| MRT (h) | 4.149 ± 0.93 | 4.174615 ± 1.33 |
| CL (mL/h) | 1.924577 | 1.455188 |
| Relative bioavailability (%) | -- | 125.429 |

**Notes:** *significant difference at $P < 0.05$ (unpaired t test).

**Abbreviations:** VRD, Vardenafil; ODTs, oral disintegrating tablets; AUC, area under the time–concentration curve; $C_{max}$, maximum plasma concentration; $K_{el}$, elimination rate constant; MRT, mean residence time; $t_{max}$, time to reach $C_{max}$.

## 4. Conclusions

The present study proves that the inclusion complex of VRD with ß-CD increased its aqueous solubility in 1:2 molar ratio and masked its bitter taste. The optimized formulation compromises between the hardness and the disintegration time. The studied tablet's excipients showed the varied impact to fulfill all the required characteristics of ODTs. However, incorporation of an optimized concentrations, via using the design of the experiment, of these excipients achieved the best balance and therefore produced VRD-ODTs with superior characteristics. The developed tablet offers a taste-masked, satisfactory hardness and short disintegration time for the rapid release of the drug. The pharmacokinetic data point out to the improved bioavailability of VRD over the marketed tablets. In addition, in vivo data found that the oral absorption of VRD from ODTs was obviously higher than that from the marketed tablets. Moreover, the tmax was shortened to 1 h in comparison with two h for the marketed tablets which indicated the rapidity of onset of action of VRD and hence improved patient efficacy and satisfaction.

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