e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(10); 388-395

Original Research Article

In-Vitro Bioequivalence Studies of Commercialized Vildagliptin Tablets in India

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Received: 23-08-2022 / Revised: 22-09-2022 / Accepted: 08-10-2022

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Conflict of interest: Nil

Abstract

The objective of this work is to evaluate the *in-vitro* behavior of marketed vildagliptin tablets. Six marketed Vildagliptin tablets and the innovator were evaluated. Physicochemical parameters such as percentage weight deviation, hardness, friability, drug content, disintegration, dissolution, similarity factor and difference factors were analyzed as per IP. Dissolution profiles were evaluated using USP apparatus type 2 at 50 rpm and 900 ml of phosphate buffer in pH 6.8. All the marketed products under study were bioequivalent to the innovator. B-2 & B4 showed least similarity values and highest difference factor values but lies within the acceptable range. B-6 showed the highest similarity value and least difference factor value with that of the innovator product. The results indicate that at pH 6.8 all marketed vildagliptin tablets included in this study seem to have good overall quality with good dissolution rate when compared to that of innovator product.

Keywords: Bioequivalence, Vildagliptin, difference factor, similarity factor.

Introduction

Bioequivalence is the study of various brands of an unchanging drug and allure portion of drug or other consumable forms. Two various formulations of similar drugs are bioequivalent when their rate of destruction and incorporation is similar. As skilled is an increase in result and use of general drugs, the need for bioequivalence study is climbing as well [1].

Nowadays drug's cost increases on account of the high-priced original drug. This cost may be decreased by substituting low general brands. For this, common brand concede possibility be therapeutically equivalent to the original drug. In order to find this, bioequivalent studies are transported [2].

According to the US Food and Drug Administration (FDA) bioequivalence defined as ''the property wherein two drugs with identical active ingredients or two different dosage forms of the same drug possess similar bioavailability and produce the same effect at the site of physiological activity'' [3].

Two common ways to conduct a bioequivalence study are in-vitro and in-vivo. In-vivo bioequivalence studies are generally performed in humans and animals by measuring the rate and the extent of drug absorption into the bloodstream after drug administration. The in-vivo study gives a lot reliable information but there are many parameters that cannot be controlled. So we have to do a series of tests and the costs are also

important. In-vitro bioequivalence studies are performed in a dissolution device. All biological necessities conditions are provided and samples are taken and analyzed regularly. It also makes it possible to mimic biological conditions. In-vitro studies reduce the cost and number of attempts. It also offers advantages in terms of ethical considerations and drug performance [4].

Biowaiver is an exemption granted by the US FDA to conduct in-vivo bioequivalence studies. This implies that it is not necessary for generics to conduct in-vivo studies for product approval. Instead, a resolution test can be resumed. Biowaiver can only be recommended for solid oral immediate release products (85% release in 30 min) with highly soluble drugs in a pH range of 1-7.5 [5].

Vildagliptin

Vildagliptin is the second member of the DPP-IV inhibitor class of drugs licensed for the treatment of type 2 diabetes mellitus (T2DM). Vildagliptin belongs to a group of BCS Class III and cyan pyrrolidine inhibitors, where its glycyl Xaa complex is replaced. The nitrile group on the pyrrolidine ring of vildagliptin has been vital both for its high potency and facilitating oral administration [6].

Mechanism of action of Vildagliptin [4]

Glucagon-1-like peptide (GLP-1) and glucose- dependent insulin otropic peptide (GIP) are incretin hormones that regulate blood sugar levels and maintain glucose homeostasis. GLP-1 and GIP activity are estimated to contribute more than 70% of the insulin response to oral glucose challenge. They stimulate insulin secretion in a glucose-dependent manner through G-protein-coupled GIP and GLP-1 receptor signaling. In addition to its effects on insulin secretion, GLP-1 is involved in stimulating island neoplasia and in differentiating and attenuating apoptosis of pancreatic beta cells. Incretin hormones also have effects outside the pancreas, such as adipogenesis and myocardial function. In type 2 diabetes, GLP-1 secretion is reduced and the insulin-secreting effect of GIP is greatly reduced.

Vildagliptin exerts a hypoglycemic effect by selectively inhibiting dipeptidyl peptidase-4 (DPP-4), an enzyme that rapidly cleaves and inactivates GLP-1 and G IP upon release from intestinal cells. DPP-4 cleaves the oligopeptide after the second amino acid at the N-terminus. Inhibition of DPP-4 significantly prolongs the half-life of GLP-1 and G IP and increases the levels of active circulating incretin hormones. The duration of DPP-4 inhibition with vildagliptin is dose-dependent. Vildagliptin is administered on an empty stomach. It reduces postprandial blood sugar and HbA1c level. It increases the sensitivity of alpha and beta cells to glucose and increases glucose-dependent insulin secretion. Fasting and postprandial glucose levels are reduced and postprandial lipid and lipoprotein metabolism is improved.

Half life [4]

The mean elimination half-life after intravenous administration is approximately 2 hours. After oral administration, the elimination half-life is approximately 3 hours.

Structure of Vildagliptin [7]

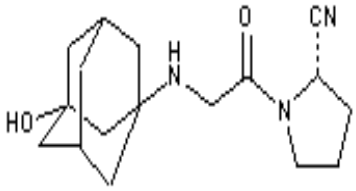

Figure 1: Structure of Vildagliptin

Materials

Pure Vildagliptin was obtained as gift sample from Chandra Labs, Hyderabad. Six marketed generic brands of Vildagliptin tablets as well as the innovator brand were purchased from the different pharmacies. Furthermore, the reagents including KH2PO4, K2HPO4, 0.1N HCl and all other chemicals used were of analytical grade.

The innovator brand was selected and labeled as I (Vilgrip-50, Medilords). Also, the different other marketed brands were selected and labeled as B-1 (Torglip-50, Torrent Pharma), B-2 (Viltus – 50, Aretaeus Pharmaceutical Pvt Ltd), B-3 (Vildus-50, Lividus Pharmaceutical Pvt Ltd), B-4 (Vildaglar-50, Larion Life Sciences Pvt Ltd), B-5 (Vinglyn-50, Zydus Candiva) and B-6 (Vildanat-50, Natco Pharma Limited).

Methodology

Standard curve of Vildagliptin [1]

Vildagliptin can be estimated spectrophotometrically at 210 nm. 100 mg of Vildagliptin was taken in 100 ml volumetric flask and dissolved in 2 ml of ethanol and then volume was made up to 100 ml with 0.1N HCl of pH 1.2 to prepare the primary stock solution of 1mg / ml. From this stock solution 2 ml was made up to 100 ml thus giving a concentration of 20 □g /ml giving secondary stock solution. Aliquot of standard drug solution ranging from 2 to 20 ml were transferred into 10 ml volumetric flask and were diluted up to the mark with 0.1N HCl with pH 1.2. Thus, the final concentration ranges from 2 – 20 g/ml. Absorbance of each solution was measured at 210 nm against 0.1N HCl as a blank. A plot of concentrations of drug versus absorbance was plotted. The same procedure is repeated with buffer solution for obtaining standard curve at pH 6.8.

Weight variation [8]

Factors that affect the weight of the tablet include the press machine, head pressure, machine speed, and powder flow characteristics. Weight variation is calculated by taking 20 tablets from each brand. Analytical balances are commonly used to weigh tablets. Weighted averages and percentage deviations from the mean were calculated for each tablet. According to the pharmacopoeia, no more than two individual weights should deviate from the average weight.

Friability [8]

Friability is a phenomenon in which the surface of the tablet is damaged or damaged by mechanical impact. This test is done to ensure that the edges of the tablet are not broken. The appliance used is a Roche Friabilator. Calculate the initial weight (W1) of 10 randomly selected tablets. After the tablets are sent through the friabilator at 25 rpm for 4 minutes, the final weight (W2) is calculated. The percentage loss is determined by the formula:

% Friability = [(W1-W2)/W1] *100

Hardness [9]

Hardness testing is important because it determines the resistance of the tablets to breakage, abrasion or breakage during storage, transport and handling before use. The hardness of the tablet is affected by the space between the upper and lower perforations during compression, the weight of the material used and the pressure applied during compression. The different types of devices used to measure hardness are Monsanto or Stokes hardness testers, Pfizer hardness testers etc. The crushing strength was determined with a tablet hardness tester (Monsanto, UK). Tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Uniformity of content [9]

Weighed and powdered 20 tablets of each generic product and the innovator brand product. The powder equivalent to 100 mg of vildagliptin was taken and transferred to 100 ml of volumetric flask. Then, the

volume made up to 100 ml with 0.1 N HCl. Vigorous shaking was done to dissolve the powdered material. After proper dilution, absorbance values were measured at the maximum wavelength (λ max) of these concentrations was measured using a UV-VIS spectrophotometer a blank. Maximum wavelength was obtained by scanning all samples from 200 to 400 nm and this was 210 nm.

Disintegration [10]

There are different types of disintegration devices depending on the drug, but the principle and composition are the same. The unit consists of a basket of 6 tubes of the same diameter. Each tube has a wire mesh attached. A piston motor is used to move the basket. The entire assembly is immersed in a container containing the test medium. 6 tablets from each brand were employed for the test in a freshly prepared medium, 0.1N HCl at 37°C. The disintegration time was taken to be the time no particle remained on the basket of the system.

Dissolution [11,12]

Before executing dissolution test, to calculate the concentration, series of diluted solutions of pure Vildagliptin were prepared and standard curve was drawn. The obtained curve was linear with a correlation coefficient of 0.9989.

The dissolution test was executed USP apparatus II with the rate of 50 rpm at 37°C on tablets of each brand. The dissolution medium was 900 ml of phosphate buffer (pH=6.8).

Dissolution profile was drawn by withdrawing 5 ml of dissolution samples at different time intervals up to 60 min and replaced with the same volume of dissolution medium. Subsequently, samples were assayed by ultraviolet spectrophotometer at an absorbance wavelength of 210 nm. The concentration of each sample was determined from a calibration curve. The main purpose of performing dissolution study for test and reference product was to compare product's dissolution profiles.

The dissolution profiles were compared using two model independent parameters: the difference factor (F1) and the similarity factor (F2) derived from the dissolution profiles. The F1 factor measures the percentage difference between two concentration curves and the F2 factor shows similarity between them over all time points. F1 is zero and F2 is 100 when the test and reference drug profiles are identical. The F1 increases and F2 decreases proportionally as the dissimilarity between them increases. Two dissolution profiles are avowed similar if F1 is between 0 and 15 and if F2 is between 50 and 100. F1and F2 can be calculated from following equations

$$F1 = \{[\Sigma t = 1\ n \mid Rt-Tt \mid] / [\Sigma t = 1\ nRt]\} \times 100.$$

$$F2=50 + \log\{[1+ (1/n)\sum t=1^{*}\ n\ (Rt-Tt)\ 2]-0.5\times100\}$$ Where,

Rt and Tt are the cumulative percentage of dissolved drug for the reference and test formulation at time t, respectively. n is the number of time points.

Table 1: Samples of marketed Vildagliptin tablets

S.No	Brand Number	Brand Name	Mfg Date	Manufacturing Company	Country	Dosage form
1	I	Vilgrip -50	11/2021	Medilords	India	Tablet
2	B-1	Torglip-50	12/2021	Torrent Pharma	India	Tablet
3	B-2	Viltus-50	07/2021	Aretaeus Pharmaceutical Pvt Ltd	India	Tablet
4	B-3	Vildus-50	01/2022	Lividus	India	Tablet

				Pharmaceutical Pvt.Ltd		
5	B-4	Vildaglar-50	12/2021	Larion Life Sciences (p)Ltd	India	Tablet
6	B-5	Vinglyn-50	11/2021	Zydus Candiva	India	Tablet
7	B-6	Vildanat-50	09/2021	Natco Pharma Limited	India	Tablet

Results and Discussion

Table 2: Standard curve of Vildagliptin

S.No	Conc (µg/ml)	Absorbance (nm)	
		pH 1.2	pH 6.8
1	2	0.026	0.034
2	4	0.049	0.065
3	6	0.073	0.095
4	8	0.095	0.122
5	10	0.12	0.152
6	12	0.141	0.179
7	14	0.164	0.201
8	16	0.19	0.234
9	18	0.216	0.263
10	20	0.238	0.289

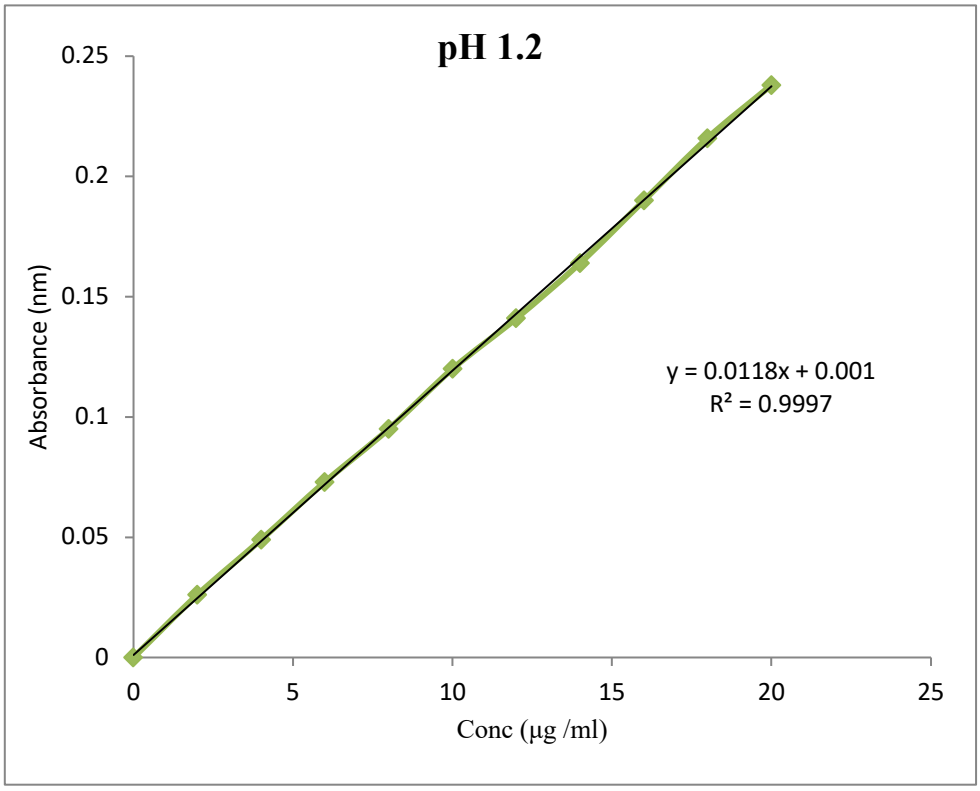

Figure 2: Standard Curve of Vildagliptin at pH 1.2

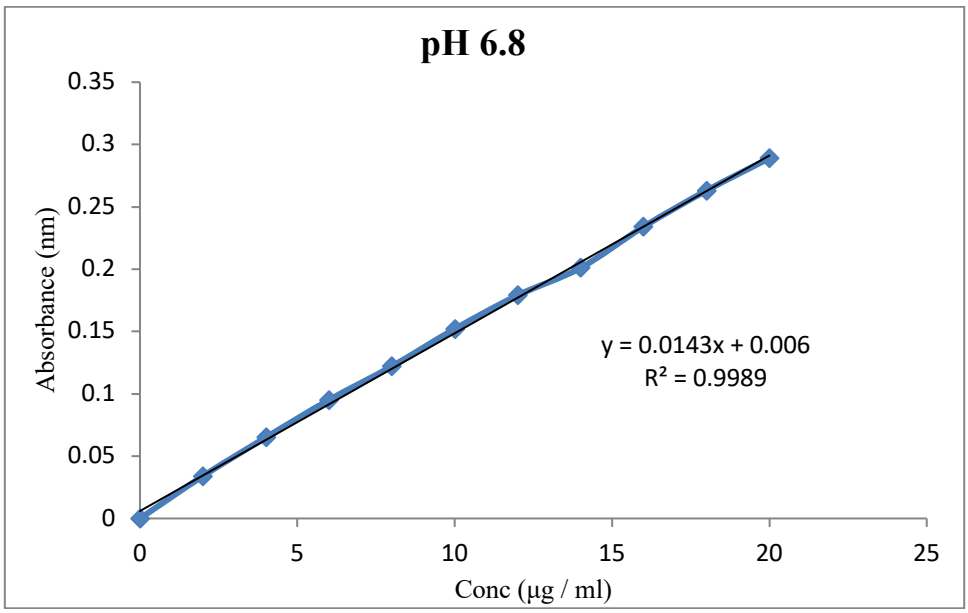

Figure 3: Standard Curve of Vildagliptin at pH 6.8

Table 3: Physicochemical properties of various brands of Vildagliptin tablets

Brand Code	Weight of the tablet (mg)	% Weight deviation	Hardness (kg/cm^2)	Friability (%)	Drug content (%)	Disintegration Time (sec)
B-1	200	0.34	5.2	0.213	98.44	182
B-2	200	0.12	5.1	0.304	99.38	171
B-3	220	0.26	5.3	0.352	99.12	189
B-4	200	0.51	5.2	0.228	98.65	164
B-5	190	0.17	5.1	0.402	99.57	195
B-6	170	0.23	5.2	0.363	98.89	178
I	200	0.47	5.3	0.282	99.93	153

All the commercialized tablet brands of Vildagliptin were analyzed for weight variation test. The information of weight variations are given in Table 3. All the tablets were found to be within the weight variation limits when compared with the USP guidelines. Consistency in weight of tablets guarantees consistency of dose units. Variation in weight of tablets should be decreased to least for uniform dosage to be given to the patients.

All the commercialized vildagliptin tablet brands have been analyzed for friability testing using Roche friabilator to check the resistance of abrasion during conveyance and packing. Percentage friability was calculated as per USP. The result was diverged from 0.213- 0.402% for all brands as shown in Table 3. Thus, the friability test was transcended by all brands.

Tablets hardness test outcomes of all the commercialized vildagliptin tablet brands have been shown in Table 3. The value of hardness was found to be in the orbit of 5.1 – 5.3 kg /cm^2. So, all the brands transcended the hardness test.

Data of disintegration studies are shown in Table 3. The average disintegration time of the all tablets was found to be less than 4 minutes and thus undoubtedly transcended the disintegration test. The innovator product showed a maximum of 153 sec for complete disintegration.

The outcome of percentage purity of all the brands is shown in Table 3. The drug content was appraised by comparing with the calibration curve.

Table 4: Dissolution, Difference factor & Similarity factor of various brands of Vildagliptin tablets

Brand Code	Dissolution % at 30 mins	Dissolution % at 60 mins	Difference Factor F1	Similarity Factor F2
B-1	73.34	98.37	6.34	66.29
B-2	71.46	99.13	12.11	51.72
B-3	75.42	98.75	5.56	66.64
B-4	69.81	99.26	12.4	50.98
B-5	77.52	98.29	7.03	59.94
B-6	80.63	99.02	2.72	79.82
I	79.13	99.68	-	-

B-6 showed highest dissolution value of 80.63% at 30 min and B-4 showed the least dissolution value of 69.81% at 30 mins. However, all the marketed tablets showed maximum dissolution at the end of 60 mins. For two dissolution profiles to be considered similar and bioequivalent, F1 should be between 0 and 15 while F2 should be between 50 and 100.

The calculated F1 and F2 values are shown in Table 4. B-2 & B4 showed least similarity values of 51.72 & 50.98 and highest difference factor values of 12.11 & 12.4 but lies within the acceptable range. B-6 shows the highest similarity value of 79.82 and least difference factor value of 2.72 with that of the innovator product. Significant differences were not observed in both parameters and this confirmed similarity between all brands formulations compared with innovator product. Taking all results in account it was understood that the brands may be used interchangeably.

Conclusion

Post-market observing is extremely vital for powerful clinical outcome and this study has underscored that chemical identicalness doesn't demonstrate bioequivalence. Our *in vitro* results show that at pH 6.8 all commercialized vildagliptin tablets intromitted for this study appear to have good overall quality with good dissolution rate. Each one of them can be considered bioequivalent with the picked innovator brand. From this review, it was perceived that cost may not be guaranteed to show the genuineness and viability of a medication product.

Acknowledgement

All the authors would like to thank management authorities and Principal in Excel College of Pharmacy, Komarapalayam, Tamilnadu for supplying analytical lab facilities for this study. This paper is based on a part of a B.Pharm "Practice School" report submitted to The Tamilnadu Dr MGR Medical University, Chennai.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication.

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