Biowaiver assessment of some commercially available brands of valsartan tablets using *in vitro* methods

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ABSTRACT

Background: Generic medicines must be therapeutic equivalents with the innovator brand before substitution is appropriate.

Objective: This study was carried out to evaluate the *in vitro* equivalence of valsartan (a BCS Class III drug) tablets under Biowaiver conditions.

Methods: Physicochemical parameters were assessed in accordance with BP and USP specifications. Therapeutic equivalence of the innovator and commonest generic brands were investigated using *in vitro* methods.

Results: The tested valsartan brands investigated complied with Compendia specifications for tablets. Valsartan tablets were not very rapidly dissolving as per WHO Biowaiver testing specifications. Both test and reference products were poorly soluble in acidic medium (pH 1.2), while 85% of the drug was released at 15 minutes in pH 6.8. Dissolution profiles of the test and innovator brands were similar at pH 1.2 and 6.8 (f_2: 65 and 69 respectively), and dissimilar at pH 4.5 (f_2: 30). The generic valsartan tablets evaluated in this study showed pharmaceutical equivalence with the innovator brand. The test and reference products were not however very rapidly dissolving as required for BCS Class III drugs.

Conclusion: The valsartan tablet brands tested did not meet WHO BCS-based biowaiver conditions. I*n vivo* bioequivalence studies are recommended to ascertain therapeutic equivalence and interchangeability.

Key words: Generic, Valsartan, Biowaver, Interchangeability, Innovator

Evaluation de bio-dérogation de certaines marques de comprimés valsartan disponibles dans le commerce utilisant *in vitro*

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RESUME

Contexte: Les médicaments génériques doivent être des équivalents thérapeutiques de la marque novatrice avant qu'une substitution soit appropriée.

Objectif: Cette étude fut réalisée pour évaluer l'équivalence *in vitro* des comprimés valsartan (un médicament BCS Classe III) sous les conditions de bio-dérogation.

Méthodes: Les paramètres physico-chimiques furent évalués en conformité avec les normes BP et USP. L'équivalent thérapeutique des marques novatrices et les plus couramment génériques ont été examinées à l'aide de méthodes *in vitro*.

Résultats: L'examen des marques de valsartan testées s'accorde avec les normes Compendia pour les médicaments. Les comprimés Valsartan ne se dissolvaient pas très vite par rapport aux normes de test de bio-dérogation de l'OMS. Les produits de test et les produits de référence avaient une faible solubilité dans le médium acide (pH 1,2), alors que 85% du médicament était libéré à partir de 15 minutes en pH 6,8. Les profils de dissolution du test et les marques novatrices étaient semblables à pH 1,2 et 6,8 (f_2: 65 et 69 respectivement), et dissemblables à pH 4,5 (f_2: 30). Les comprimés génériques valsartan examinés dans cette étude ont montré une équivalence pharmaceutique avec la marque novatrice. Les produits de test et de référence ne dissolvaient cependant pas très rapidement comme requis pour les médicaments BCS Classe III.

Conclusion: Les marques de médicament valsartan testées n'ont pas satisfait les conditions de bio-dérogation basées sur le BCS de l'OMS. Les études de bioéquivalence *in vivo* sont recommandées pour établir l'équivalence thérapeutique et l'interchangeabilité.

Mots-clés: Générique, Valsartan, Bio-dérogation, Interchangeabilité, novatrice

INTRODUCTION

A generic drug is a pharmaceutical product, usually intended to be interchangeable with an innovator product, which is manufactured without a license from the innovator company and marketed after the expiry date of the patent or other exclusive right. In other words, generic medicines should be comparable to the innovator product in dosage form, strength, route of administration, quality and performance characteristics and intended use. They are thus, advocated and promoted as a measure of limiting healthcare costs and improving accessibility to medicines. Nevertheless, while the economic need of cost containment is unquestionable, it is very pertinent to ensure that patients' health is not compromised as well, since generic medicines can only be interchangeable with their innovator counterparts when they are pharmaceutically and therapeutically equivalent.

Drug release, disintegration and dissolution are usually the main focus of bioequivalence studies (BE); which could involve *in vivo* or *in vitro* studies. With the introduction of the Biopharmaceutics Classification System (BCS) however, *in vivo* bioequivalence studies could be waived for immediate release solid oral dosage forms for BCS Classes I (High Solubility, High Permeability)[1], and III (High Solubility, Low Permeability) drugs.[2–5] Consequently, only *in vitro* testing may be used to determine bioequivalence for Classes I and III drugs. *In vitro* dissolution tests based on BCS are acceptable surrogates for establishing the bioequivalence of generics with innovator products.

Drug absorption is determined by release of Active Pharmaceutical Ingredient (API) from drug product, the dissolution of the drug under physiological conditions and the permeability across the gastrointestinal tract. Based on this, *in vitro* dissolution testing is vital in predicting *in vivo* performance of a drug product. Dissolution testing also serves as a tool to distinguish between acceptable and unacceptable drug products.

Valsartan is a potent, orally active non peptide tetrazole derivative chemically known as 3-methyll-2-[pentanoyl-[[4-[2-(2H-tetrazoyl-5yl) phenyl] phenyl] methyl]amino]-butanoic acid with empirical formula $C_24H_29N_5O_3$ and molecular weight of 435.519g/mol (Fig. 1). Valsartan is a white coloured powder that is freely soluble in ethanol, methanol, acetonitrile and sparingly soluble in water. The drug is listed officially in USP monograph along with its three impurities (R)-N-valeryl-N-{[2'- (1H-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine, (S)-N-butyryl-N- {[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine and (S)-Nvaleryl-N-{[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]-methyl}valine benzyl ester.

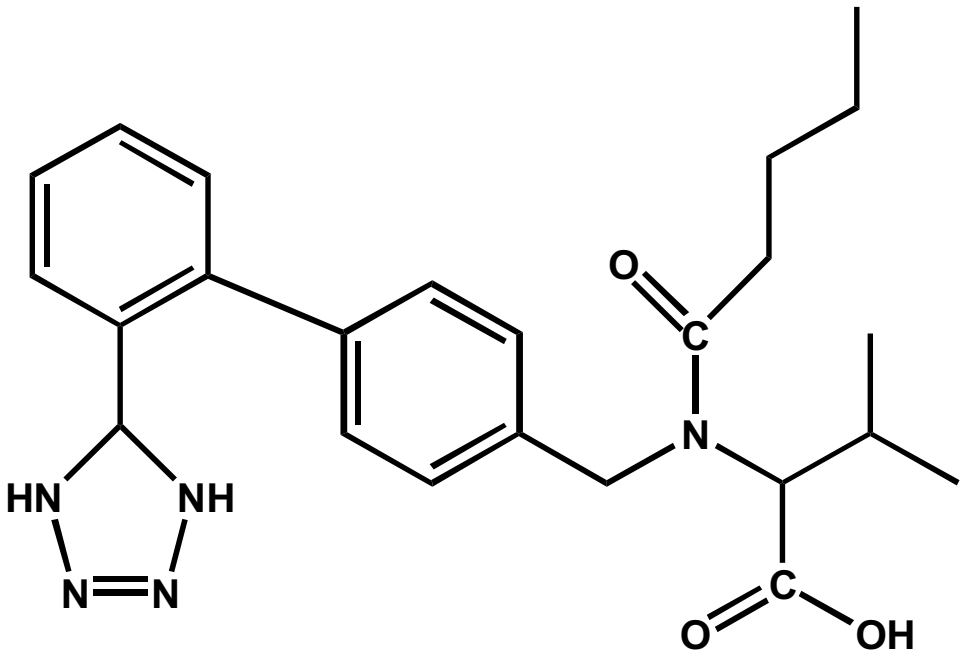

Figure 1: Chemical structure of valsartan

Valsartan appears in the melting range of 105-110˚C and the specific rotation [α]D/20 in methanol being 68˚. The partition coefficient of Valsartan is 0.033 (log P=1.499), suggesting that the compound is hydrophilic at physiological pH. The compound is stable under storage in dry conditions.[14] Valsartan is a tetrazole derivative that contains acid (pKa=4.73) and carboxylic (pKa=3.9) groups making the compound soluble in the neutral pH range.[6]

It is an antihypertensive and used in the treatment of congestive heart failure, post-myocardial infarction. Valsartan acts by blocking the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selective binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland.[7] It is a BCS Class III drug with low permeability, poor metabolism and high solubility.[8, 9] Valsartan has only limited distribution outside the plasma compartment and is extensively bound to the plasma proteins (94- 97%) and hence is only limited distributed outside plasma compartment. Because of the presence of carboxylic groups Valsartan is soluble in neutral pH range and is mainly present in the ionized form at physiological pH. The volume of distribution at steady state is about 17 L.[6]

Upon expiration of the patency of valsartan, various generics of the drug are being imported into the country. It is thus imperative to ensure that all brands circulating in the country at any point in time conform to compendia requirements and most importantly, are therapeutically equivalent to the innovator product. Six brands of the drug are currently available in the Nigerian market namely: Diovan (Novartis®–innovator), Carvals® (Ranbaxy), Joltan® (Joswe), Valsartan (Actavis®), Valsoten® (Alpha), and Valsartan (Teva®).

We report for the first time the biowaiver assessment some commercially available brands of valsartan using *in vitro* methods to determine the appropriateness of their interchangeability with the innovator brand. *In vitro* dissolution profile was carried out to ascertain their release in three different dissolution media employed while HPLC-UV was used to assay the dissolution samples at different predetermined sampling time interval.

METHODS

Materials

Valsartan pure reference standard was obtained from USP® with LOT# LOL 195. The innovator product for valsartan 80 mg tablets; Diovan®, coded as DVN, and three generics coded CVL, ATV and JTN respectively were purchased from registered wholesale pharmaceutical outlets in Lagos state, Nigeria. All chemicals and reagents used were of analytical grade: Potassium dihydrogen orthophosphate (SureChem®), Sodium hydroxide (Riedel-de-Haën®), Glacial Acetic Acid (Sigma-Aldrich®), Ammonium acetate (Riedel-de-Haën®), concentrated Hydrochloric acid (BDH®), Acetonitrile (HPLC grade) (Sigma-Aldrich®).

Physicochemical studies

The following physicochemical studies were carried out: uniformity of weight, hardness test, friability test, disintegration test, and chemical assay.

Uniformity of weight

This was carried out by randomly selecting twenty tablets from each of the 4 brands; these were weighed individually with an analytical weighing balance (Ohaus® Adventure USA). The average weights for each brand and percentage deviation from the mean value were determined.

Hardness test

Ten tablets were randomly selected from each brand, this were individually placed between the platens of an integrated hardness, thickness and diameter tester (Campbell®, Model DHT 250). The pressure at which each tablet got crushed was recorded.

Friability test

Ten tablets of each brand were weighed and subjected to abrasion using a Roche friabilator (Erweka® Gmbh, Germany) at 25 rev/min for 4 min. The tablets were then weighed and compared with their initial weights and percentage friability was obtained.

Disintegration test

Six tablets were placed in a tablet disintegration tester (Campbell® Model TD-400) filled with distilled water and maintained at 37±0.5^{0}C. The tablets were considered completely disintegrated when all the particles passed through the wire mesh and time was recorded.

Chromatographic conditions

The chemical assay was carried out using high performance liquid chromatography coupled with ultraviolet spectrometer (HPLC-UV) method as reported in literature with slight modification.[7] Chromatographic conditions were achieved by using Agilent® HPLC-UV consisting of reverse phase Eclipse

Apparatus II (paddle method) at 75 revolution per minutes. A single–point dissolution test was carried out for the tone brands of valsartan tablets. This was done as a comparable requirement for establishing the duality of tablets. Three dosage units of each product were evaluated in 900 ml of phosphate buffer. Sample aliquot of 2 ml was withdrawn at 30 min, filtered, and analyzed.

The innovator and the most frequently used generic brand were then subjected to dissolution studies in three different media. Twelve dosage units of each product were evaluated in 900 ml of each of the following media: 1.0 N HCl (pH 1.2), acetate buffer (pH 4.2), and phosphate buffer (pH 6.8). Dissolution samples (2 ml) were simultaneously withdrawn at predetermined sampling time interval range (2 – 60) minutes and replaced with fresh 2 ml of appropriate medium in order to maintain sink condition. The withdrawn samples were filtered using 0.45 hm millipore filter, diluted appropriately with the mobile phase, and analyzed using an Agilent® HPLC-UV machine.

The actual concentrations of valsartan in the respective brands at the different sampling times were determined using valsartan reference standard calibration curve.

Statistical analysis

The data obtained from the experiments were statistically analyzed using Microsoft Excel worksheet 2010 (Microsoft®). Results are expressed as mean ± SD. Analytical data obtained from the experiments were analyzed with simple statistics. Dissolution profiles were analyzed using similarity factor (f_2):

$$f_2 = 50 \log\{[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100$$

where, R_t and T_t are the cumulative percentage dissolved at time point t for reference and test products, respectively, and n is the number of pool points.

RESULTS

All the brands of valsartan tablets assessed showed acceptable uniformity of weight, hardness and friability with comparable specifications. ^{01} Disintegration time, dissolution and assay results of all the brands were all within USP permissible limits (Table 1). The results of the in vitro release studies of the innovator and generic brand studied in three different media (pH 1.2, 4.2 and 6.8) are shown in Table 2. Figs. 2 -4 show dissolution

In vitro dissolution studies

The dissolution studies were carried out using USP Apparatus C-II (100mm X 4.6mm, 3.2hm) column, dual-gradient pump with mobile flow rate observed at 1.0 ml\min and UV detector wavelength set at 248 nm coupled with auto sampler injector set at 20 hL. The mobile phase consists of water, acetonitrile and glacial acetic acid (250:420:1).

Preparation of valsartan standard stock and working solution

The standard stock solution was prepared by accurately weighing 20 mg of valsartan pure reference standard into a 20 ml volumetric flask, 20 ml of methanol was added and sonicated for 10 minutes after which the solution was subsequently made up to 20 ml volume with methanol to obtain a 1.0 mg\ml valsartan standard stock solution. The working solution was prepared by meticulously measuring 3.1252 ml from the freshly prepared stock solution into 25 ml volumetric flask and made up to 25 ml volume with the HPLC mobile phase mixture to obtain 20 hg\ml working solution.

Gradient calibration concentration range of 20 – 100 hg\ml was used to obtain the calibration curve used to obtain the calibration curve which was used for the assay of drug content while 1 – 10 hg\ml was used for dramatification of the dissolution samples.

Assay of drug content

Twenty tablets were randomly selected, weighed and pulverized. An accurately weighed portion of the tablet powder equivalent to 100 mg of valsartan was transferred into a 100 ml volumetric flask. Methanol (20 ml) was added and the solution was sonicated for 30 minutes to prepare achieve 1mg\ml stock solution. The solution was cooled to room temperature, made up to volume with methanol, and filtered through a 0.45hm Millipore filter. Working solution of 20 hg\ml of valsartan samples were prepared by measuring 2 ml of the stock solution into a 100 ml volumetric flask and made up to volume with the HPLC mobile phase solution (Water: Acetonitrile: Glacial Acetic Acid). The same protocol was used for the tone brands of the drug respectively.

The contents of valsartan in the tablets of the respective brands were determined using the linear regression equation (y = 44.20x – 7.63, R^2 = 0.999) obtained from the valsartan reference standard calibration curve.

profile curve of the respective brands in different dissolution media. Both the reference and test products were not very rapidly dissolving ($\geqslant$ 85% of labeled amount not released within 15 min) in the three media. The dissolution profiles of the two brands are also not superimposable in all three media.

Table 1: Physicochemical Data of valsartan tablets

Brands	Mean wt. (mg) n= 20	Hardness (KgF) n=10	Friability (%) n= 10	Disintegration time (mins) n=6	Assay (%) n= 20	Dissolution (%) pH=6 .8, t=30 min, n= 3
DVN	160.55±0.73	9.11±0.59	0.25±0.02	1.00±0.21	104.2±0.53	86.5±0.12
CVL	255.01±0.66	8.09±0.51	0.34±0.01	1.25±0.31	102.1±1.74	103.4±1.45
JTN	208.45±0.88	9.50±0.60	0.17±0.15	1.16±0.02	103.4±1.45	88.1±0.55
ATV	188.1±0.21	9.18±0.51	0.19±0.03	1.28±0.14	99.6±2.65	87.7±0.59

Results are expressed as mean ± SD. Limits for hardness, friability, disintegration time and assay are 4-7kgF, <1%,<15% and 95-105% respectively

Table 2: *In vitro* dissolution data of valsartan reference and test products with their f_2

		DVN (Reference)	ATV (Test)	f_2
Media	Time (min)	% released (Mean ± SD)		
pH 1.2	5	0.14±0.76	0.00±0.00	
	15	0.32±0.02	1.65±0.23	
	30	4.22±0.70	9.89±1.64	65
	45	10.28±1.65	15.29±1.15	
	60	12.14±1.83	19.76±1.19	
pH 4.5	5	19.44±1.01	28.25±1.09	
	15	71.63±1.19	60.25±0.26	
	30	74.61±1.67	117.78±0.28	
	45	115.11±1.20	126.17±1.14	30
	60	123.39±1.85	132.83±1.01	
pH 6.8	5	76.24±1.16	81.76±0.55	
	15	85.11±1.06	85.56±1.18	
	30	86.18±1.26	88.91±1.57	69
	45	88.18±1.35	84.33±0.67	
	60	89.17±1.80	83.77±0.28	

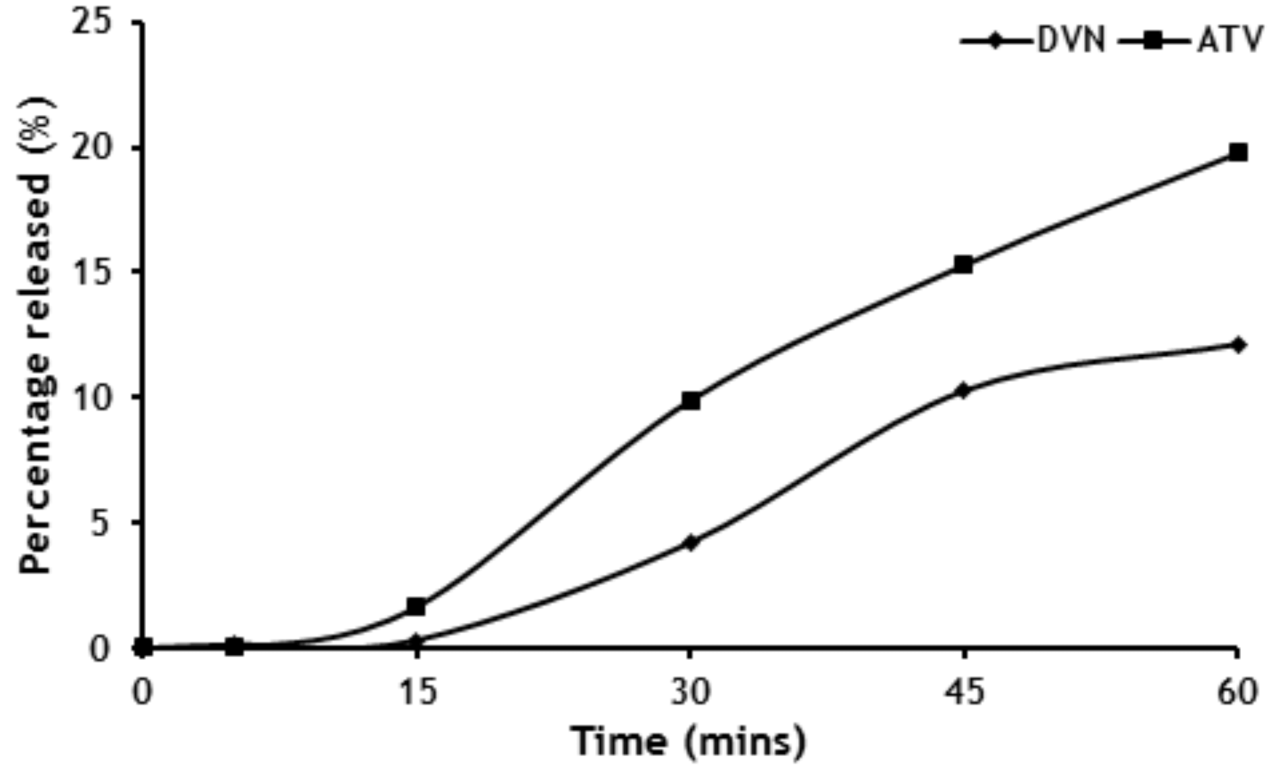

Fig. 2: Dissolution rate profiles of DVN and ATV in pH 1.2

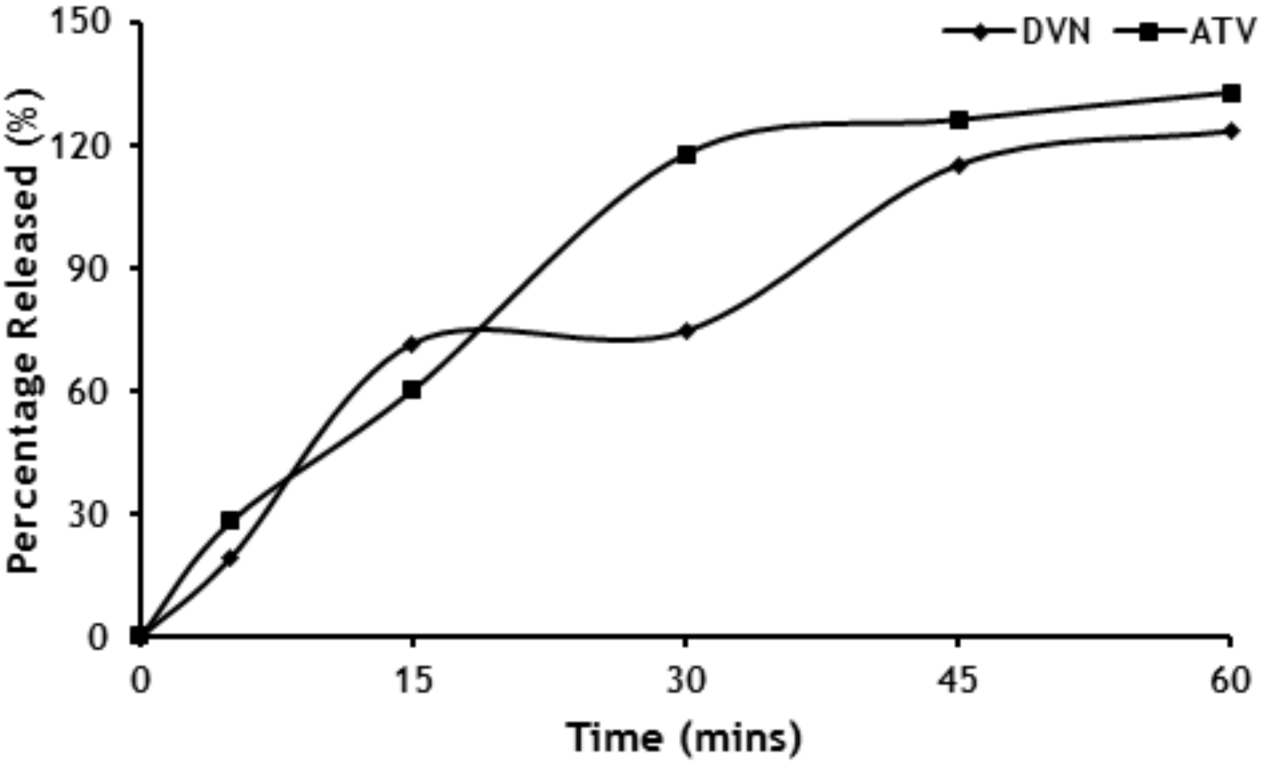

Fig. 3: Dissolution rate profiles of DVN and ATV in pH 4.5

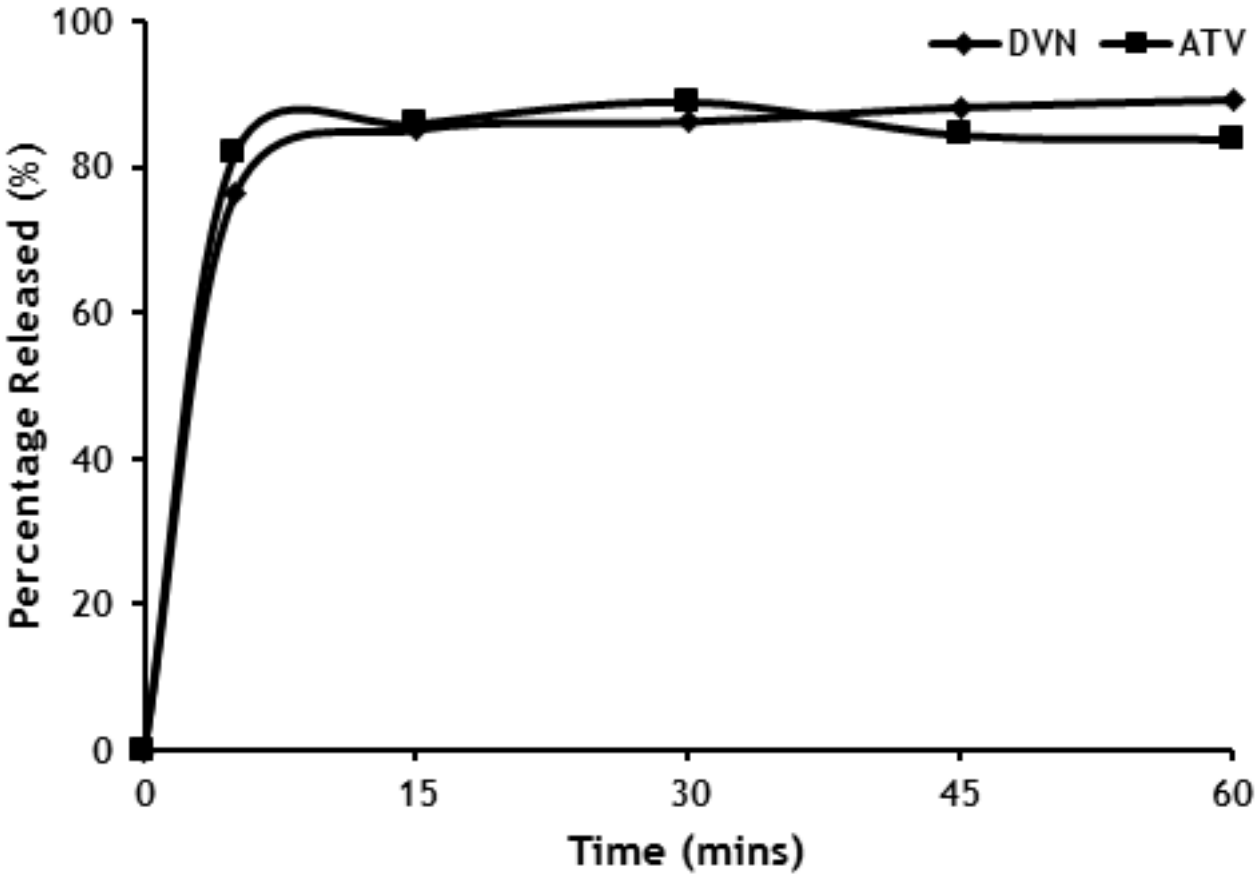

Fig. 4: Dissolution rate profiles of DVN and ATV in pH 6.8

DISCUSSION

The physicochemical characteristics of all the valsartan tablets brands tested were within the USP specified limits for immediate release oral dosage forms (Table 1). These assure drug product quality and pharmaceutical equivalence of the generics with the innovator brand. Therapeutic equivalence is often assessed using *in vivo* bioequivalence (BE) studies; however, they are often costly[11], and involve invasive procedures. The Biopharmaceutics Classification System (BCS) has evolved over the years, into a tool that can be used to reduce *in vivo* BE requirements using *in vitro* dissolution tests.[12]

Immediate release drug products should exhibit rapid or very rapid *in vitro* dissolution characteristics for exemption from *in vivo* pharmacokinetic study. BCS Class III compounds which are characterized by high solubility and low permeability should be very rapidly dissolving to qualify for a BCS-based Biowaiver. For immediate-release products of this class, it is assumed that if their dissolution is very rapid ($\geqslant$ 85% release of labeled amount within 15 min) under all physiological pH conditions, they are expected to behave like oral solutions *in vivo*, since the rate-limiting step in the absorption process is intestinal permeability. These drugs exhibit a high variation in the rate and extent of drug absorption. Since they have rapid dissolution, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage form factors.[13]

In the present study, both the test and reference products of valsartan tablets were not very rapidly dissolving, and did not achieve 85% release of the labeled API within 15 min in all the three media. In acidic medium, very poor solubility was observed (< 20% dissolved at 60 min); higher amount of valsartan was released in acetate medium from both products, though not up to 85% at 15 min. However, at pH 6.8, both products had 85% release of API at 15 min (Table 2, Figs. 2 - 4). The poor solubility in acidic medium can be attributed to the pH dependent solubility of valsartan. Valsartan contains two acidic functional moieties with pKa values of 3.9 and 4.7 and one asymmetric center and (co)exists in solution at physiological pH values as the un-dissociated acid, the mono-anion and the di-anion. A rise from pH 4 to pH 6 increases the solubility of

valsartan by a factor of about 1000, but favours the anionic form and decreases lipophilicity. *In vitro* dissolution is rapid and complete at pH 5.0 and above and is solubility-limited at pH 3.0 and below. This may explain the observed low solubility in acidic medium. Since the solubility of valsartan increases in the pH range 4-8 and lipophilicity decreases in the same range, the rate of absorption of valsartan may be influenced by intestinal pH profile along the gastrointestinal (GI) tract. This has been demonstrated using an *in vitro* model of intestinal absorption (Caco-2 cells), where the absorption rate was observed to decrease as pH increased in the range 6-7.5.[14]

The absorption of a Class III drug is likely limited by its permeability and less dependent upon its formulation. Therefore, if the *in vitro* dissolution of a Class III drug product is rapid under all physiological pH conditions (i.e. not less than 85% release of API within 30 mins), and the amount and nature of excipients is not expected to affect bioavailability, its *in vivo* behaviour will be similar to oral solution.[2] For instance, Jantratid *et al*.[15] reported that the *in vivo* absorption performance of ten rapidly dissolving IR products containing Cimetidine (a BCS Class III compound) were similar despite considerable differences in their *in vitro* dissolution profiles. Likewise, simulations have shown that formulations of Metformin (a BCS Class III drug) that released 100% drug *in vitro*, in a time period equal to or less than two hours, are indicated to be bioequivalent.[16] Similarly, a bioequivalence study between a generic product of metformin and the innovator product, with similar dissolution profiles in three media covering the physiological pH range, which were rapidly dissolving (both brands only releasing 89% within 30 min), showed *in vitro-in vivo* correlation. In a study by Cheng *et al*.[17], the primary bioequivalence parameters C_{max}, T_{max}, AUC_{0-t}, and AUC_{0-}^{∞} for the test products were similar to those of the reference product using log-transformed data. They concluded that a biowaiver by *in vitro* dissolution profiles was justified by the bioequivalence data for metformin. Homsek *et al.*[18] also justified Metformin hydrochloride biowaiver criteria based on bioequivalence study. Their results indicated that differences in drug dissolution observed *in vitro* were not reflected in *in vivo* results. In their conclusion, such data support the existing evidence that Class III drugs are eligible biowaiver candidates, even if a very rapid dissolution criterion is not met.

Ideally, when both the test and the reference products dissolve 85% or more of the label amount of the API within 15 min in all three dissolution media, then a profile comparison is unnecessary. In this study however, this condition was not met, hence the need for dissolution profile comparison. Similarity factor (f_2) was thus calculated to test dissolution similarity in order to ascertain equivalence. Dissolution profiles of the reference and test products were similar at pHs 1.2 and 6.8 (f_2 = 65 and 69 respectively), and dissimilar at pH 4.5 (f_2 = 30). The BCS guidance specifies that the test and reference dissolution profiles are considered similar if both products have at least 85% dissolution in 15 min or if comparison of profiles by the f_2 test results in an f_2 value of at least 50.[19] Both test and reference products in this study did not meet this requirement.

For BCS Class III cases, possible differences in content of excipients are considered critical, especially in cases where the absorption of the drug is very low (i.e. below 50%) and in cases of absorption windows, i.e. absorption in the area of the proximal part of the gastrointestinal tract. Therefore, the generics should match the comparator with regard to the excipients (qualitatively and quantitatively) as much as possible, to lower the risk of an inappropriate decision on equivalence. The possible effect of excipients on the dissolution of valsartan tablets was not evaluated because the excipients used in the formulations were not listed on the packaging.

There have also been advocacies for biowaivers for Class III drugs that are rapidly dissolving (85% dissolved in 30 min).[20, 21] This advocacy was because, bioavailability of this class is independent of drug dissolution; therefore, generic drugs with differing *in vitro* dissolution will not necessarily exhibit different *in vivo* performance.

The significance of the observed *in vitro* difference between the tested brands may be further evaluated and confirmed by *in vivo* BE studies, in order to establish therapeutic equivalence and generic substitution.

CONCLUSION

All the brands of valsartan tablets evaluated in this study showed pharmaceutical equivalence. The test and reference products did not however meet WHO biowaiver requirements for BCS Class III drugs. Apparently, *in vivo* studies will be needed to establish therapeutic equivalence and interchangeability of the valsartan tablet brands. These results further emphasize the need for continuous monitoring of marketed drug products to ensure their safety, consistency, conformity, therapeutic equivalence to innovator brands, and enable generic substitution by

healthcare providers.

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