A comparative study on the dissolution profiles and Equivalent assessment of some the generics of Aceclofenac tablets available in Lagos Nigeria

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ABSTRACT

The dissolution behavior of a reference standard brand and four generic equivalent brands of Aceclofenac tablets purchased from the Lagos metropolis was compared in this study. A variation of excipients is contained in the different formulations, influences of these on the release characteristics of the varying aceclofenac brands was evaluated in gastric pH (1.2), and intestinal pH (4.5 and 6.8). Physicochemical parameters such as hardness, disintegration, friability and content uniformity, were assessed in comparison with the reference standard brand coded RS-1. The studied formulations reflected a dissolution profile exhibiting variations in the release characteristics among the different brands. Only one of the generic brands had a > 80% of aceclofenac released within 60 minutes in SIF complying with the United States pharmacopeia *in vitro* dissolution specifications for drug release. The study showed that only one of the generic brands was similar to the reference standard brand *f*2 being 66 in SIF dissolution media, and can thus be used interchangeably with the reference standard brand, the other two brands had dissimilar dissolution patterns. There is the need for constant market vigilance to ensure that products marketed are similar to innovator products to facilitate ease of interchangeability by health care providers.

Key words: Aceclofenac tablets, Dissolution, Market vigilance

INTRODUCTION

By blocking cyclo-oxygenase enzymes (COX-1 and COX-2) required to produce prostaglandins thus relieving pain and inflammation, non-steroidal anti-inflammatory drugs (NSAIDs) exert their pharmacological action (Yamazaki *et al.*, 1997, The Merck Manual, 2011). NSAIDs are very effective in the management of ankylosing spondylitis, rheumatoid and osteoarthritis, however because these conditions require the use of these drugs over a prolonged period of time complications such gastric erosion, obstruction and or perforation may occur representing the major adverse reactions to the use of this class of drugs.

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Aceclofenac (Figure 1), exhibits analgesic and anti-inflammatory properties being a potent inhibitor of the enzyme cyclo-oxygenase directly blocking prostaglandin synthesis is a newer derivative of the diclofenac group of NSAIDs. (The Merck Manual, 2011). *In vitro* data have shown that COX-1 and COX-2 are inhibited by aceclofenac in whole blood assays, however increased selectivity for COX-2 was elucidated. It is this selectivity for COX-2 that gives the unique reduced gastro intestinal ulceration compared to diclofenac and thus makes it a better choice in prolonged pain management (Brogden and Wiseman 2006; Martindale 2005).

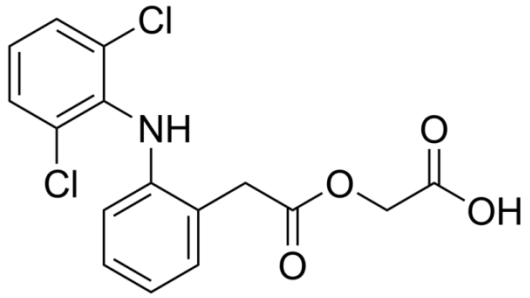

Figure 1 Aceclofenac structure

In vitro test outlined in the biopharmaceutical classification system (BCS) has been used to reduce *in vivo* bioequivalence requirements relying on the specific gastrointestinal solubility and permeability of active pharmaceutical ingredient, thus ensuring that reduced timelines and resources management during generic drug development is obtainable. The BCS is being used to ensure regulatory standards are adhered to all over the world. It is also indispensible in research and development during *in vivo* drug design (CDER 2000; WHO, 2006). Aceclofenac a BCS class II drugs, has low water solubility but high permeability. The bioavailability of Aceclofenac is limited by its solvation rate; this influences its absorption from tablets after oral administration (O'Hara *et al.*, 1997, Ilomuanya *et al.*, 2012). The release of the aceclofenac from the dosage form will depend on its dissolution and the permeability across the GIT under physiological conditions. A correlation between the *in vivo* bioavailability and the *in vitro* solvation can thus be extrapolated (Polli *et al.*, 1997).

Comparative evaluation of the physicochemical properties of one reference standard brand and four generics of Aceclofenac brands available in Lagos, Nigeria was carried out in this study, as well as evaluation of the equivalence of these brands of Aceclofenac using their dissolution data to establish similarity in profiles among the varying generic brands.

MATERIALS AND METHOD

MATERIALS

Aceclofenac (ACE) pure standard, one reference standard brand, Zerodol® (RS-1) and four generic brands of Aceclofenac tablets Table 1 sourced via random sampling from community pharmacies in Lagos Nigeria were used. Potassium dihydrogen phosphate (Thomas Baker® UK), sodium bicarbonate (Sigma Aldrich® USA), NaOH (Thomas Baker® UK), Methanol (Sigma Aldrich® USA) and distilled water were used throughout the study. All solvents and reagents used were of analytical grade and the dissolution media used was always freshly prepared.

METHOD

Uniformity of weight

Twenty tablets of each of the Aceclofenac brands were weighed individually using a Mettler Toledo analytical balance. The mean and standard deviation were calculated and the percentage deviation was determined.

Table 1 Brands of Aceclofenac used each containing 100mg of active constituent.

Brand code	Brand Name	Batch Number	Manufacturing date	Expiry Date	Country of origin	NAFDAC* REG. No.
RS -1	Zerodol	10:2021	10/2012	09/2016	INDIA	A4-2257
ACE-2	Acefen	FIO68	06/2011	05/2016	INDIA	A4-5599
ACE-3	Oetco	BOTT-010	06/2011	05/2016	INDIA	A4-1781
ACE-4	Acycor	12485	06/2012	05/2016	INDIA	A4-4595
ACE-5	Medinac	1002	03/2012	02/2016	INDIA	A4-4192

Hardness

Twenty tablets of each brand were utilized for this test. The tablet hardness was determined by Monsanto hardness tester (Labtech, India). The reading was noted. The reading indicates the hardness of the tablet in kg/cm^2.

Friability

Twenty tablets of each brand was weighed and then introduced into an automated Erweka® TA friabilator. After 100 revolutions, the tablets were re-weighed, recorded and the friability calculated.

Disintegration test

Twenty randomly selected tablets was taken from each of the brands and placed on the mesh in the disintegration tester operated in vertical oscillatory mode utilizing 0.1N HCL and Simulated intestinal fluid pH 6.8 as media for disintegration. The time required for absence of tablet residue from the surface of the mesh to occur was recorded as the disintegration time (Bertocchi *et al.*, 2005).

Drug content

Twenty tablets of each sample were finely powered and 100 mg was weighed out using an analytical balance, transferred to 100 ml volumetric flasks containing 50ml of phosphate buffer (pH6.8) and analyzed for the content of aceclofenac sodium using UV-visible spectrophotometer at 275 nm. The drug content of each sample was estimated from their standard curve (Figure 2) and percentage purity calculated.

***In vitro* Dissolution Studies**

Four generic formulations coded as ACE 2, ACE-3, ACE-4 and ACE-5 and the standard reference brand coded RS-1 were studied. Dissolution was carried out on 12 units of each formulation according to FDA/USP specification to enable statistical analysis, using an Intech® dissolution tester Series 2600 USA, USP apparatus-II (Paddle) at 37 ± 0.5°C at a speed of 50 rpm in 900 ml of dissolution media. Three dissolution media were utilized 0.1N HCL pH 1.2, simulated intestinal fluid phosphate buffer pH 4.5 and 6.8. From each dissolution vessel 5mls of sample was withdrawn and filtered using a millipore filter 0.45µm at specific time intervals, after which dissolution medium replacement constituting the exact volume withdrawn took place. Samples were analyzed utilizing UV spectrophotometry at 275 nm after the calibration curve has been established.

STATISTICAL EVALUATION

One-way ANOVA applied using Microsoft Excel 2007.

Similarity factor (f_2) and Difference factor (f_1) analysis

Comparison and evaluation of dissolution profile data was was statistically analyzed using similarity factor f_2 (equation 1) and difference factor (equation 2)

$$f_2 = 50 \log \{[1 + (^1/m) \sum_{j=1}^{m} Wj(Rj - Tj)(Rj - Tj)\ ]^{-0.5} \times 100\} \ldots\ldots\ldots \text{Equation 1}$$

f_1 = {[[Rj – Tj] / $\sum_{j=1}^{m}$ Rj] ×100}……..Equation 2

Where m reflects the number of time points utilized, Rj ,the cumulative percent of the reference product dissolved at specifically selected time points, Tj, the cumulative percent of the generic brand dissolved at specifically selected time points (Ilomuanya *et al.,* 2012, Shah *et al.*, 1998; Ocana *et al.*, 2009; Duan *et al.,* 2011.)

RESULTS AND DISCUSSION

The brands of aceclofenac utilized in this study were of the 100 mg strength and were utilized within their shelf life.

Compendial standards for weight variation, drug content and disintegration time were used as a basis of comparison between the reference standard brand RS-1 and the four generics evaluated in this study. The average weight of the varying brands differed considerably with RS-1 having a mean weight of 185.2 mg and ACE-4 having a mean weight of 407.7 mg. However there was no significant variation in the weight of the tablets within each brand. The difference in the mean tablet weights is indicative of the use of varying excipients and manufacturing procedures in formulation of these varying brands. These differences in composition of excipients are relevant as disparities arising from varying physicochemical characteristics exhibited by these brands may be directly

Table 2 Physicochemical properties of the Aceclofenac tablets n=20

Brand code	Mean Tablet weight (mg)	Hardness (kg/cm^2)	Disintegration time in SIF (seconds)	Friability (%)	% Drug Content
RS -1	185.2 ± 0.014	2.32 ± 0.114	30.5 ± 0.10	0.05 ± 0.0321	100.5 ± 0.021
ACE-2	300.3 ± 0.037	9.46 ± 0.102	45.8 ± 0.10	0.03 ± 0.0125	98.9 ± 0.328
ACE-3	325.5 ± 0.021	5.44 ± 0.203	72.4 ± 0.08	0.07 ± 0.0201	101.9 ± 0.109
ACE-4	407.7 ± 0.023	5.22 ± 0.110	39.9 ± 0.09	0.10 ± 0.0331	94. 9 ± 0.217
ACE-5	198.7 ± 0.004	5.02 ± 0.100	90.9 ± 0. 15	0.05 ± 0.0231	109.0 ± 0.124

All the values are expressed as mean ± standard deviation

Table 3 Dissolution data in three different media at 60 minutes

	Aceclofenac in 0.1N HCL			**Aceclofenac in Phosphate buffer pH 4.5**			**Aceclofenac in simulated intestinal fluid pH 6.8**		
	% Dissolved mean ± SD	f_1	f_2	% Dissolved mean ± SD	f_1	f_2	% Dissolved mean ± SD	f_1	f_2
RS -1	2.77 ± 0.23	-	-	34.78 ± 0.23	-	-	98.67 ± 0.05	-	-
ACE-2	2.8 ± 0.03	15	98	25.76 ± 0.04	47	44	92.8 ± 0.38	6	66
ACE-3	2.5 ± 0.12	12	99	8.96 ± 0.03	82	32	74.81 ± 0.01	39	26
ACE-4	1.2 ± 0.09	64	87	7.86 ±0.33	87	30	59.7 ± 0.04	37	27
ACE-5	1.29 ± 0.37	76	86	22.89 ± 0.1	47	44	63.77 ± 0.22	27	34

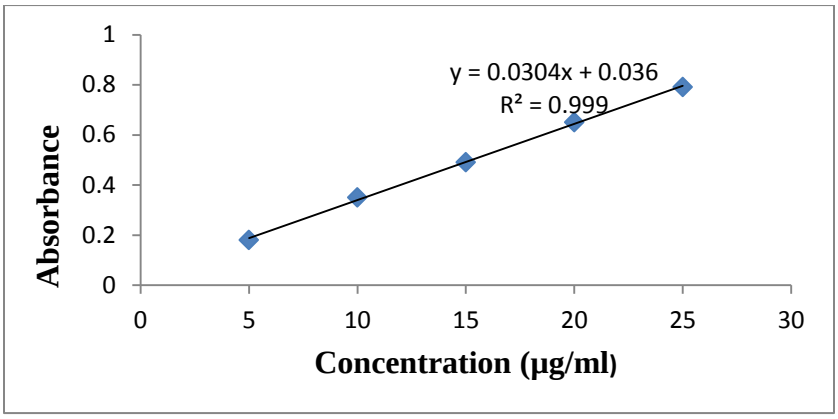

Figure 2 Calibration curve of Aceclofenac

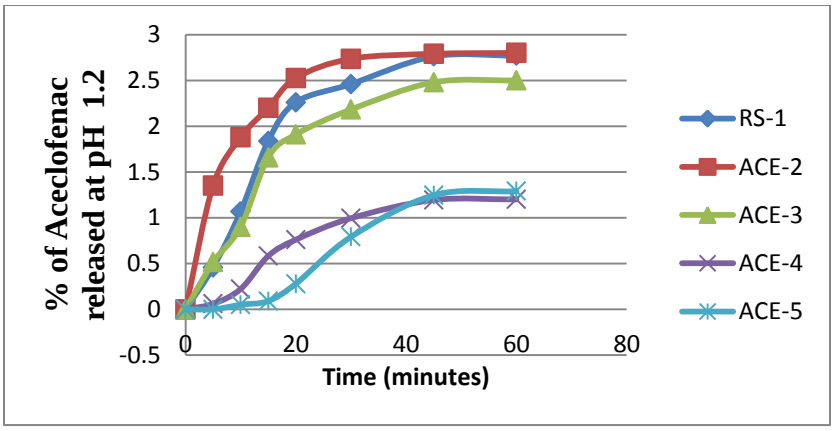

Figure 4: Dissolution profile of the reference standard brand and four generics of Aceclofenac tablets in pH 1.2

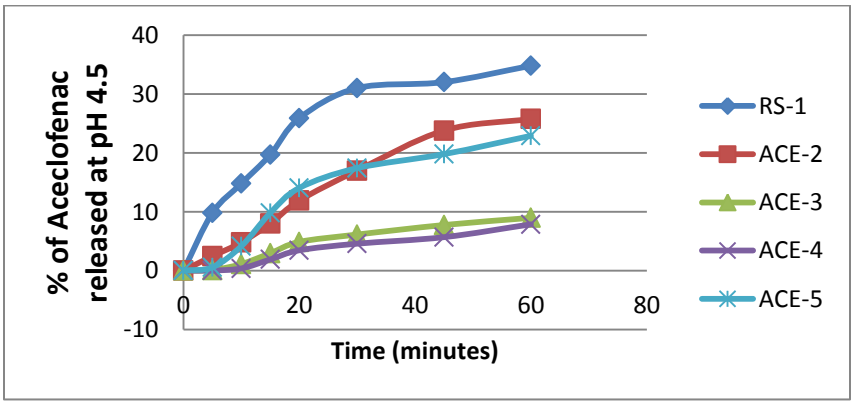

Figure 5 Dissolution profile of the reference standard brand and four generics of Aceclofenac tablets in pH 4.5

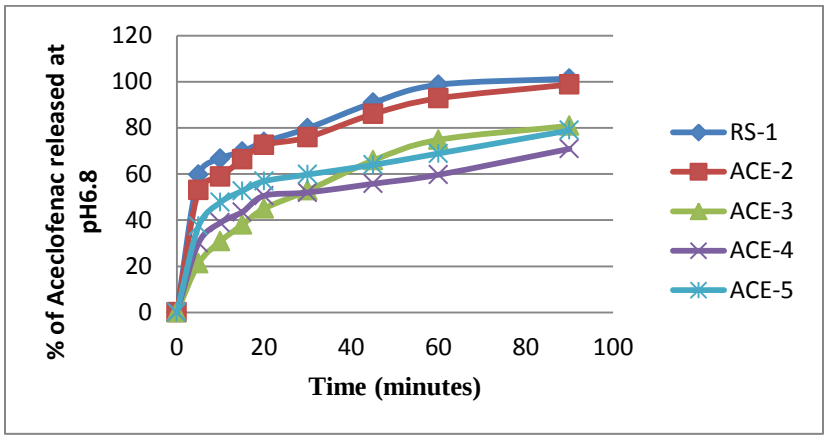

Figure 6: Dissolution profile of the reference standard brand and four generics of Aceclofenac tablets in pH 6.8

linked to it. The potency/ drug content of the tablets, an indication of how much active ingredient is present in the formulation was assessed. BP specification states that drug content should not be less than 95% and not more than 105%. Drug content, a reflection of the potency of the tablets was found to fall within compendial range for RS-1, ACE-2, ACE-3 and ACE-4 having aceclofenac content between 94.9% - 101.9%. ACE-5 having drug content constituting 109% did not meet compendial specification. Variation in the % drug content between the brands and the RS-1 reflected a statistically significant difference ($P < 0.05$).

The tablets' ability to withstand mechanical stress which may result in chipping cracking or breakages during the varying stages of handling prior to getting to the final consumer was assessed as essential criteria for tablet quality. Tablet hardness was obtained as ranges between 2.32 kg/cm^2 – 9.46 kg/cm^2 (as shown in Table 2). RS-1 required the least amount of force to effect fracture compared to ACE-2 which had the highest value for tablet hardness. At 95% confidence interval, ANOVA reflected significant differences between the reference standard brand RS-1 and all the generics with $P < 0.05$. Due to the fact that the British Pharmacopoeia (BP) has no specifications for tablet hardness and hardness itself can't be classified as an absolute indicator of tablet strength, friability of the tablets were assessed (British Pharmacopoeia 2012). All the tablets RS-1 and ACE-2 through to ACE-5 complied with BP specification for friability (≤ 1%) (British Pharmacopoeia 2012), having friability of 0.03 – 0.1%. This reflected an indication of good formulation practices culminating in sufficient mechanical strength of the tablets assessed. Disintegration studies reflected that RS-1 had the fastest disintegration time of 30.5 seconds compared with the slowest disintegration time of ACE-5, 90.9 seconds. The reference standard having the lowest crushing strength also was seen to have the fastest disintegration time compared with other generics, having higher crushing strength/ hardness had much longer disintegrations time. These values are indicative of the rate determining step in drug release and absorption. Dissolution studies were carried out for the varying brands in three different dissolution media reflecting three different pH environments in a pH range of 1.2 to 6.8 characterizing the

pH of the gastro intestinal tract from stomach to the small bowel/intestine where absorption of the drug takes place. Due to the fact that aceclofenac is a BCS class II, its bioavailability limited by its solvation rate. The release of aceclofenac from the tablets thus depends on the dissolution of the drug i.e. aceclofenac in the appropriate media. In acidic media, pH 1.2, over a 60 minute period aceclofenac was not released in appreciable concentrations into this media. At 60 minutes less than 3% of aceclofenac was released into this media as shown in figure 4. The similarity factor *f*2 for all the generic brands were similar to the reference standard RS-1, with *f*2 values > 50 (i.e. 87-99). In phosphate buffer pH 4.5 (Figure 5) all the generics showed a dissimilar profile with the reference standard brand used with *f*2 values < 50 (i.e. 30 – 44 as in Table 3), less than 40 % of aceclofenac was obtained in this medium after 60 minutes. Graphical interpretation of the release profile of the reference standard brand RS-1 in simulated intestinal fluid pH 6.8 over a 60 minute period showed very good release in this media, with almost 80% of the aceclofenac released into the dissolution medium by 30 minutes and over 90% released by 45minutes (Figure 6). This result is similar to that obtained by other workers Soni and Chotai in 2010 where aceclofenac brands available commercially in India were also assessed. Aceclofenac is largely absorbed through the small intestine to reduced gastro intestinal irritation, thus making SIF the ideal dissolution media. These values were similar to the release curve exhibited by RS-1 and ACE-2, which had close to 90% of aceclofenac released into the media at 45 minutes. *f*2 values showed that only ACE-2 showed strong similarity with the reference standard brand *f*2 = 66 and very low dissimilarity profile *f*1 = 6. ACE-3, 4 and 5 had dissimilar dissolution profiles compared with RS-1. FDA/CDER in its publication detailing the industry waiver of *in vitro* bioavailability and bioequivalence study guidance for BCS class II drugs states that *f*1 and *f*2 must have values corresponding to and (0- 15) and (50 -100) respectively, to ensure that there exist equivalence between two dissolution profiles and by extension two pharmaceutical products with respect to drug release (FDA/CDER 2000). The USP specifies for both APIs that the amount of drug released in a dissolution experiment after 60 min should not be less than 80% of the labeled amount in pH 6.8 phosphate buffer i.e. simulated intestinal fluid (USP 30-NF 25), dissolution data profile shows that utilizing *f*1 and *f*2 as the criteria for comparison ACE-2 was from the point of difference and similarity directly comparable to the reference brand RS-1. ACE3, 4 and 5 had variable dissolution profiles in SIF, ANOVA showed significantly varied release profiles when compared to RS-1, with % of aceclofenac released ranging from 59.7 – 74.81% at 60 minutes. These values together with their corresponding similarity and dissimilarity factor values obtained via model independent approach (Table 3) showed that the dissolution profiles were not similar to that of the reference standard brand. At 90 minutes, dissolution of ACE-3 and ACE-5 averaged 80% of aceclofenac released into the dissolution medium. Only ACE-4 however did not achieve an 80% aceclofenac release throughout the duration of the dissolution study.

Different approaches elucidated in FDA guidance based on BCS were used to compare the profiles obtained via dissolution carried out in this study. The model independent approach utilized via *f*1 and *f*2 as the criteria for comparison of dissolution profiles was applicable and useful. Due to the fact that aceclofenac a

non-selective COX inhibitor is used to manage chronic pain it is appropriate that drug regulatory agencies in developing countries such as Nigeria adopt these *in vitro* dissolution comparison approaches. This will serve as drug evaluation and licensing standards leading to proliferation of generics brands that meet compendial specification for drug release and by extension are as effective as the standard reference brand or innovator product for which they may be interchanged with in community or hospital pharmacies at the point of sales to the patient.

CONCLUSION

The four generics brands and the reference standard brand of aceclofenac evaluated in this study were within compendial specified limits for hardness, friability, drug content and disintegration. Only one of the brands ACE-2 was assessed to be pharmaceutically equivalent to the reference standard brand RS-1 at the time of this study. A high variation in the dissolution drug release profiles of the varying brands of aceclofenac tablet assessed has been recorded. Initial physicochemical and pre chemical tests carried out prior to dissolution testing however showed that all the studied brands contained the labeled amount of aceclofenac within official limits. There is thus need for market vigilance to ensure that products marketed are similar to the innovator or reference standard products by the regulatory agencies to facilitate ease of interchangeability by physicians and pharmacists.

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