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Comparative *in vitro* equivalence evaluation of some Aceclofenac generic tablets marketed in Bangladesh

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Abstract
Availability of copious generic brands in local drug market makes the health professionals confused to select the desired quality product. This study was designed to assess the bioequivalence of six generic Aceclofenac tablets from different manufacturers using *in vitro* dissolution study in order to minimize health risk factors. Other general quality assessments of these tablets like diameter, thickness, hardness, friability, weight variation, disintegration time were also evaluated according to the established protocols. Using a validated UV spectrophotometric method, active ingredients were assayed. All brands complied with the official specification for weight variation and disintegration time but only two brands complied in case of friability. Assay value was recorded within 92.68% to 100.51%. The dissolution profiles showed intra brand and inter brand variability. Only three brands achieved 80% dissolution within 60 minutes. Test results were subjected to statistical analysis to compare the dissolution profile. Model independent approaches of difference factor (f_1) and similarity factor (f_2) were employed and the data revealed that only two brands may be used interchangeably. Such study serves as a good cursor for assessment of *in vitro* parameters of commercially available products.

Keywords: Bangladesh, Aceclofenac, In vitro equivalence, Dissolution test, Generic tablets, Similarity factor (f_2)

Introduction
To evaluate the quality, therapeutic efficacy and safety of commercially available medicine, post market monitoring serves as a confidential tool [1]. Information obtained from such monitoring can accelerate the improvement of existing regulations and product development [2]. In this research physical parameters of commercially available aceclofenac tablets were evaluated.

Aceclofenac (ACF) is a non steroidal anti inflammatory cytokine inhibitor which is broadly used for the symptomatic treatment of pain and inflammation specifically in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis with the recommended dose of 100 mg twice daily [3,4]. The drug works by inhibiting the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever [3, 5-7]. The incidence of gastric ulcerogenicity of ACF has been reported to be significantly lower than that of the other frequently prescribed NSAIDs, for instance, 2-folds lesser than naproxen, 4-folds lesser than diclofenac, and 7-folds lesser than indomethacin [8].

Aceclofenac ($C_{16}H_{13}Cl_2NO_4$), chemically [(2-{2, 6-dichlorophenyl) amino} phenylacetooxyacetic acid], is a crystalline powder with a molecular weight of 354.19 [9-11]. It is practically insoluble in water with good permeability [10, 11]. It is metabolized in human hepatocytes and human microsomes to form [2-(2',6'-dichloro-4'-hydroxy- phenylamino) phenyl] acetoxyacetic acid as the major metabolite, which is then further conjugated [12].

According to the Biopharmaceutical Classification System (BCS) drug substances are classified to four classes upon their solubility and permeability [13-16]. Aceclofenac falls under the BCS Class II, poorly soluble and highly permeable drugs [10]. So, dissolution rate limited absorption is shown by ACF that gives rise to difficulties in pharmaceutical formulations for oral delivery, which may lead to under medication or overmedication as the steady state concentration values fall or rise beyond the therapeutic range [10, 17]. Therefore constant surveillance on marketed aceclofenac tablets by the government, manufactures and independent research groups is essential to ensure availability of quality medicines.

Since no such recent information of the local market is available on widely used BCS Class-II NSAID, aceclofenac, an initiative was taken in this study to evaluate the quality of some

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commercially available generic aceclofenac tablets in the Bangladeshi market with special emphasis on disintegration and dissolution study due to their massive significance in predicting bioavailability and product quality. Six units from each brand were tested for dissolution. Other general quality parameters of these tablets like diameter, thickness, hardness, friability, weight variation, disintegration time were also determined according to the established protocols. Test results were subjected to statistical analysis to compare the dissolution profile. Model independent approaches of difference factor (f_1) and similarity factor (f_2) were also employed.

2. Materials and Methods
2.1. Materials
2.1.1. Drug
Standard of aceclofenac was a kind gift from Aristo Pharma Ltd, Bangladesh.

2.1.2. Dosage form
Aceclofenac tablets (100 mg) from six different brands were purchased from local drug store of Dhanmondi, Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, production and expiry dates. They were randomly coded as A, B, C, D, E, F and stored properly.

2.1.3. Solvents and reagents
Potassium dihydrogen phosphate (Lot No: P21010D, Daejung Chemicals & Metals Co. Ltd.) and sodium hydroxide (Batch No: PA344CB01, Qualikems Fine Chem Pvt. Ltd.) were of analytical-reagent grade and obtained from South Korea and India respectively. Distilled water was used during the study.

2.2. Methods
2.2.1. Determination of diameter and thickness
20 tablets from 6 brands were taken and both the diameter and thickness of the tablets was measured with an electronic digital caliper (MEGA Digital Clipper) in order to determine the average diameter and thickness.

2.2.2. Hardness test
The crushing strength (KgF) was determined with an Automatic Tablet Hardness Tester (8M, Dr Schleuniger, Switzerland). The force applied to the edge of the tablet was gradually increased by moving the screw knob forward until the tablet was broken. Ten tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

2.2.3. Friability test
Ten tablets from each brand were weighed and subjected to abrasion by employing a Veego friabilator (VFT-2, India) which was operated at 25 RPM for 4 minutes. After 100 revolutions the tablets were again weighed. The loss in weight indicated the friability.

2.2.4. Determination of uniformity of weight
20 tablets from each of the 6 brands were weighed individually with an analytical weighing balance (AY-200, Shimadzu, Japan). The average weight for each brand was determined as well as the percentage deviation from the mean value were calculated using the formula given by Banker and Anderson [18].

2.2.5. Disintegration test
Six tablets from each brand were employed for the test in distilled water at 37±0.5 °C using a Tablet Disintegration Tester (Model: VDT-2, Veego, India). As stated by Alderborn [19], the disintegration time (DT) was taken as the time when no particle remained on the basket of the system.

2.2.6. Dissolution test
The dissolution test was undertaken using Tablet Dissolution Tester (TDT-08L, Electrolab, India) in 6 replicates for each brand involving USP apparatus-II (paddle) at 50 RPM. The dissolution medium was 900 ml of phosphate buffer (P^H 6.8) which was maintained at 37±0.5 °C. In all the experiments, 10 ml of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50 and 60 min and replaced with equal volume to maintain an ideal sink condition. Samples were filtered and then assayed by UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 273 nm. To determine the concentration of sample, help from the standard curve of pure API (Figure 1) was taken. Using the $Y = mX + C$ equation, sample concentration was calculated.

2.2.7. Assay
Twenty tablets from each brand were weighed and finely powdered. The powder equivalent to 100 mg of aceclofenac was taken and dissolved in phosphate buffer (P^H 6.8). Flasks were subjected to sonication to dissolve the powdered material. Then the solution was filtered. The filtrate was suitably diluted. Absorbance values were then measured at the maximum wavelength (λ_{max}) of these concentrations using a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan). Maximum wavelength (λ_{max}) was obtained by scanning samples from 200 to 400 nm and it was found 273 nm.
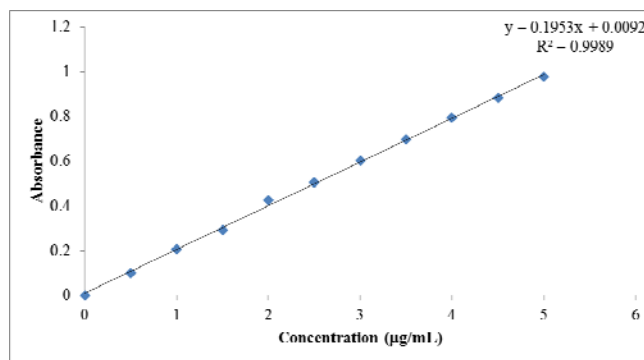

Fig 1: Standard Curve of Aceclofenac (R^2 indicates Correlation Coefficient)

2.2.8. Data analysis
The uniformity of weight was analyzed with simple statistics while the dissolution profiles were analyzed by difference factor (f_1) and similarity factor (f_2).

3. Results and Discussions
3.1. Diameter test
By monitoring the diameter and thickness of the tablets at regular intervals, potential problems relating to tablet weight and hence content uniformity can be detected at an early stage [20]. From the data mentioned in Table 1, it has been found that among six brands brand-C had highest average diameter (11.26 mm) where as brand-B had lowest average diameter (7.22 mm).

3.2. Thickness test
With increasing thickness, there is a decrease in hardness due to compression force, on the other hand with decreasing thickness there is an increase in hardness. So tablets of the same batch having lower thickness show greater hardness. The average thickness of Brand A, B, C, D, E, F were found 3.98 mm, 4.75 mm, 4.10 mm, 4.48 mm, 3.58 mm and 3.61 mm respectively as shown in Table 1. In consideration of average thickness, the variation of thickness was satisfactory for all brands.

3.3. Hardness test
Hardness has impact on disintegration. If the tablet is hard then it cannot disintegrate within the specified time and if the tablet is soft then it becomes hard to withstand the handling during coating or packaging. Therefore, adequate tablet hardness and resistance to powdering and friability are necessary requisites for quality products [21]. Oral tablets normally have a hardness of 4 to 8 or 10 kg. In general, if the tablet hardness is too high, disintegration test is performed before rejecting the batch. And if the disintegration is within limit, the batch is usually accepted [20]. According to Table 1, brand-C had maximum hardness of 5.42 kgF where as brand-E had the lowest hardness of 0.28 kgF among the six brands. Here, only one brand was within the range but since the hardness test is an unofficial test [22] and later their disintegration time (DT) was found satisfactory, the batches were considered as of good quality.

Table 1: A summary of the quality control tests undertaken on different brands of ACF tablets.

Brand code	Diameter (mm)*	Thickness (mm)*	Hardness (KgF)*	Friability (%)	Weight deviation (gm)	DT (min)*	% Drug content
A	9.22±0.03	3.98±0.05	1.79±0.90	1.52	0.35±3.98	5.78±0.27	96.18
B	7.22±0.03	4.75±0.07	0.38±0.21	3.13	0.23±3.78	0.60±0.28	94.95
C	11.26±0.02	4.10±0.02	5.42±1.60	1.28	0.22±4.08	3.52±1.47	97.73
D	8.50±0.02	4.48±0.04	0.50±0.43	0.22	0.19±5.31	0.58±0.27	92.68
E	9.21±0.02	3.58±0.05	0.28±0.26	0.04	0.27±3.69	3.11±1.03	96.08
F	8.21±0.02	3.61±0.04	0.32±0.36	1.05	0.16±7.54	0.84±0.35	100.51

*Values are expressed as mean±SD

Table 2: Dissolution profile of six brands of ACF tablets (values are expressed as mean±SD).

Time (min)	% Drug release					
	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
0	0±0	0±0	0±0	0±0	0±0	0±0
10	29.57±0.98	30.03±1.12	44.94±0.93	29.78±1.70	34.08±0.66	45.85±0.28
20	40.12±1.36	37.61±0.56	55.48±1.09	37.83±0.62	37.80±0.84	57.64±1.04
30	50.81±1.80	49.55±1.40	67.36±1.65	46.30±0.80	50.97±0.47	68.18±0.73
40	60.80±2.35	60.12±2.80	72.99±0.94	59.44±3.32	60.25±0.75	74.76±0.88
50	70.11±1.73	70.24±5.26	81.22±2.32	68.17±4.16	69.12±3.14	83.46±0.80
60	77.97±3.23	81.49±5.01	87.19±2.87	78.22±3.11	79.92±1.17	88.58±1.25

3.4. Friability test
Friability assessment reveals good mechanical strength of the tablets [23]. The compendial specification for friability is not more than 1% [24]. Usually harder the tablets less will be the percentage friability and vice versa [21]. As shown in Table 1, two brands (D and E) had percent friability below 1% which indicates tablets from other four brands (A, B, C and F) may face difficulty during storage or transportation. Among six brands, brand-B showed maximum friability (3.13%) where as brand-E showed minimum friability (0.04%).

3.5. Test of uniformity of weight
Weight variation does serve as a pointer to good manufacturing practices (GMP) maintained by the manufacturers as well as amount of active pharmaceutical ingredient (API) contained in the formulation [24]. The limit of deviation is ±10% for tablets weighing 130 mg or less, ±7.5% for tablet weighing more than 130 mg to 324 mg and±5% for tablet weighing more than 324 mg. According to USP not more than two tablets should cross the single limit and none of them should cross the double of the limit. The weight variation for all the tablets used in this study showed compliance with the offi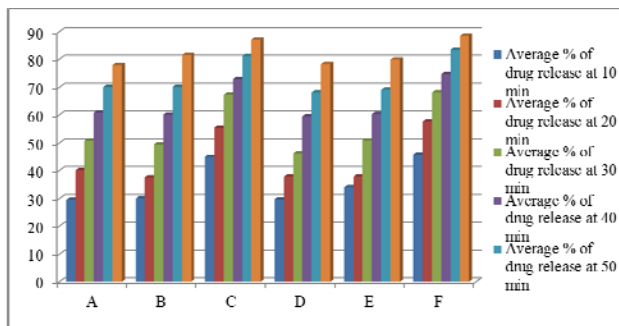cial specifications of USP. Here, as depicted in Table 1, brand F showed the highest deviation, one tablet crossed the limit but it did not cross the double limit of 15%. And brand E showed least deviation among all the six brands.
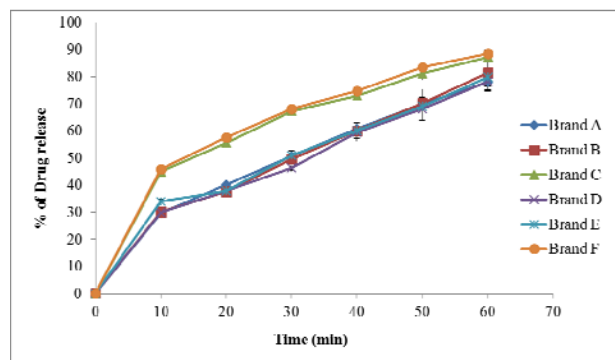
3.6. Disintegration test
Disintegration plays an important role in a tablet's dissolution. Therefore type, concentration, and efficiency of disintegrates to a large extent affects the dissolution [25]. BP specifies that uncoated tablets should disintegrate within 15 minute which is 30 minute in case of USP [22]. Table 1 shows all the brands met the official criteria. Here, brand A took maximum time of 5.78 minute and brand D took the minimum time of 0.58 minute to disintegrate.

3.7. Dissolution test
In vitro release profile (Table 2) shows only three brands achieved 80% dissolution within 60 minutes. Intra-brand (within a brand) dissolution profile in Figure 2 and inter-brand (brand to brand) dissolution profile in Figure 3 reveals that brand F showed maximum % of drug release (88.58%) where as brand A showed minimum % of drug release (77.97%) in 60 minutes.

Fig 2: Intra brand Dissolution Profile of Six Brands of Aceclofenac Tablets

Fig 3: Inter brand Dissolution Profile of Six Brands of Aceclofenac Tablets. Vertical bars represent mean±SD

The evaluation showed that release pattern of drugs were different among the six brands and brand A, D and E fail to comply the official specification (not less than 80% within 60 minutes).

3.8. Comparison of dissolution data

Difference factor (f_1) and similarity factor (f_2) were calculated to compare the dissolution profile. Difference factor (f_1) is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. The following equations were used to calculate difference factor (f_1) and similarity factor (f_2).

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100$$

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^{n} (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

Where n is the number of time points, R_t is the dissolution value of reference product at time t and T_t is the dissolution value for the test product at time t. Similarity factor (f_2) has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products by the Committee for Proprietary Medicinal Products (CPMP) to compare dissolution profile. Two dissolution profiles are considered similar and bioequivalent, if f_1 is between 0 and 15 and f_2 is between 50 and 100 [10, 22, 26].

Table 3: Calculated difference factor (f_1) and similarity factor (f_2) of six generic ACF tablets.

Brand	Difference Factor (f_1)	Similarity Factor (f_2)
A	19.50	43.33
B	19.59	42.62
D	21.86	40.61
E	18.83	43.75
F	2.27	85.77

Table 3 shows the f_1 and f_2 values of different brands in respect of brand C as a reference brand. It reveals only for brand F, f_2 value were more than 50 and f_1 were less than 15. So, brand F and brand C and can be used interchangeably.

3.9. Assay

Analysis of drug potency in tablets indicates the presence of drug in dosage form and their stability [27]. Here, Table 1 depicts that the active content of all the brands were in between 92.68% (brand-D) and 100.51% (brand-F). The result indicates there was no significant variation in content of active moiety in their dosage form among the six companies and all are within the USP specification of 100±10%.

4. Conclusions

In the current industrial practice, to compare with the multi brand generic molecules and to provide enough therapeutic activity of the dosage form, *in-vitro* tests play a significant role. The presented data exhibits that only two brands can be used interchangeably. This study states the need for constant surveillance on the marketed drugs by the regulatory bodies with the view to ascertain quality medicines although *in vivo* testing is required for final comments regarding the quality of marketed brands of aceclofenac.

5. Acknowledgement

6. References

1. Hailu GS, Gutema GB, Hishe HZ, Ali YS, Asfaw AA. Comparative In Vitro Bioequivalence Evaluation of Different Brands of Amoxicillin Capsules Marketed in Tigray, Ethiopia. Int. J Pharm Sci Nanotech. 2013; 6(1):1966-1971.
2. Chandrasekaran AR, Yi HC, Yang CAC, Cheang LW, LS Ping. Post-marketing in vitro equivalency evaluation of paracetamol tablets in Kedah, Malaysia. Int. J Pharm Sci Nanotech. 2011; 4(2):1403-1407.
3. Ahmed T, Ferdous A, Biswas SK, Sharif F. Assesments of Dissolution Profile of Aceclofenac Tablets Available in Bangladesh. S. J Pharm Sci. 2010; 3(1):1-3.
4. Sivakumar R, Venkataraman PE, Natarajan R, Ganeasn V, Ali KAA. Comparative *in vitro* Evaluation of Commercial Aceclofenac Tablets. Int. J Chem Sci. 2009; 7(3):1591-1594.
5. Raza K, Kumar M, Kumar P, Malik R, Sharma G, Kaur M *et al.* Topical Delivery of Aceclofenac: Challenges and Promises of Novel Drug Delivery Systems. Biomed Res. Int. 2014; 2014:1-11.
6. Yamazaki R, Kawai S, Matsuzaki T, Kaneda N, Hashimoto S, Yokokura T *et al.* Aceclofenac blocks prostaglandin E2 production following its intracellular conversion into cyclooxygenase inhibitors. Eur. J

Pharmacol. 1997; 329(2-3):181-187.

1. Trevor AJ, Katzung BG, Masters SB. Katzung & Trevor's Pharmacology: Examination & Board Review. Edn 6, Lange Medical Books/McGraw-Hill Medical Publishing Division, 2002, 175.

7. Brogden RN, Wiseman LR. Aceclofenac: a review of its pharmacodynamic properties and therapeutic potential in the treatment of rheumatic disorders and in pain management. Drugs. 1996; 52(1):113-124.

8. Aceclofenac. [Weblink: http://www.chemspider.com/Chemical-Structure.64809.html]. [Visited on 20 January, 2016].

9. Islam SMA, Islam S, Shahriar M, Dewan I. Comparative in vitro dissolution study of Aceclofenac Marketed Tablets in Two Different Dissolution Media by Validated Analytical Method. J Appl Pharm Sci. 2011; 1(9):87-92.

10. Drug Profiles: Aceclofenac. Institute of Pharmaceutical Sciences and Research Center, Bhagwant University. [Weblink:http://shodhganga.inflibnet.ac.in/bitstream/1060 3/28916/8/08_chapter%203.pdf]. [Visited on 20 January, 2016].

11. Bort R, Ponsoda X, Carrasco E, Gomez-Lechon MJ, Castell JV. Metabolism of aceclofenac in humans. Drug Metab. Disos. 1996; 24(8):834-841.

12. Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP *et al*. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. Pharm. Res. 2002; 19(7):921-925.

13. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H *et al*. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian J. Pharm. Sci. 2014; 9(6):304-316.

14. Dahan A, Miller JM, Amidon GL. Prediction of Solubility and Permeability Class Membership: Provisional BCS Classification of the World's Top Oral Drugs. AAPS. J 2009; 11(4):740-746.

15. Yasir M, Asif M, Kumar A, Aggarval A. Biopharmaceutical Classification System: An Account. Int. J Pharm Tech Res. 2010; 2(3):1681-1690.

16. Harsh S, Patel K, Padhyay UMU. Formulation and evaluation of controlled release colon targeted micro sponge of Aceclofenac. Pharma Innovation J 2014; 3(10):81-87.

17. Banker GS, Anderson NR. The Theory and Practice of Industrial Pharmacy. Edn 3, Lea & Febiger Verghese publishing house, Philadelphia, 1986, 293-303.

18. Alderborn G. The Design and Manufacture of Medicines. Edn 1, Churchill livingstone: Elsevier, Hungary, 1988, 441-481.

19. Bano R, Gauhar S, Naqvi SBS, Mahmood S. Pharmaceutical evaluation of different brands of Levofloxacin tablets (250 mg) available in local market of Karachi (Pakistan). Int. J Curr Pharm Res. 2011; 3(1):15-22.

20. Saleem M, Shahin M, Srinivas B, Begum A. Evaluation of tablets by friability apparatus. Int. J Res Pharm Chem. 2014; 4(4):837-840.

21. Oishi TS, Nimmi I, Islam SMA. Comparative *in vitro* Bioequivalence Analysis of Some Generic Tablets of Atorvastatin, a BCS Class II Compound. Bangladesh Pharm J. 2011; 14(1):61-66.

22. Kumar P, Ganure AL, Subudhi BB, Shukla S, Upadhyay P. Design and comparative *in-vitro* and *in-vivo* evaluation of starch–acrylate graft copolymer based salbutamol sulphate sustained release tablets. Asian J Pharm Sci 2015; 10(3):239-246.

23. Nayak AK, Pal D. Comparative in vitro Bioequivalence Analysis of Some Ciprofloxacin HCl Generic Tablets. Int. J Pharm Sci Res. 2010; 1(8):51-57.

24. Mangal M, Thakral S, Goswami M, Thakur N. Comparative study between various reported disintegration methods for fast dissolving tablets. African J Basic & Appl Sci. 2012; 4(4):106-109.

25. Popy FA, Dewan I, Parvin MN, Islam SMA. Evaluation of In Vitro Equivalence for Tablets Containing the Poorly Water-Soluble Compound Atrovastatin. Dissolut. Technol. 2012; 19(4):30-33.

26. Abbirami V, Sainithya P, Shobana A, Devi DR, Hari BNV. Review on In-vitro Bioeqivalence Studies and its Methodologies. Int. J Chem Tech Res. 2013; 5(5):2295-2302.