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IN-VITRO BIOEQUIVALENCE STUDY AND EFFECT OF FOOD BEVERAGES ON VARIOUS BRANDS OF ACECLOFENAC AVAILABLE IN INDIAN MARKET

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ABSTRACT

In this study nine different brands of instant release formulations of Aceclofenac were selected for bioequivalence study and two brands of sustained release formulations of Aceclofenac were selected to study the effect of food beverages on the drug release profile. General quality parameters of like thickness, diameter, hardness, weight variation and disintegration time of tablets were evaluated. Disintegration time of different brands in ten different media was determined. All the brands complied with the official specification for uniformity of weight, disintegration time and hardness. In phosphate buffer pH 6.8 higher drug release was found and the drug release profile was similar for all brands in phosphate buffer pH 6.8. When compared to other beverages delayed drug release was observed in milk. Even though there were some minor differences in hardness and disintegration time, the dissolution profiles appeared to be similar and not significantly different for the products of various manufactures.

KEY WORDS: Bioequivalence, NSAID, BCS class, Alcohol.

1. INTRODUTION

Drug's therapeutic efficacy mainly depends on its availability in the systemic circulation. The rate-limiting step in the absorption process of a drug from the gastro intestinal tract is the drug's dissolution rate (Chiba, 1991). Most of the poorly soluble drugs suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability. So, constant surveillance on these drugs are carried out by the Government, manufacturers and other respective authorities to make sure that high quality drugs are made available in the market (Tanjinatus Shams Oishi, 2011). Generic drugs must qualify the standards of innovator product.

The extent of drug absorption in the body fluids depends on the release and dissolution of active ingredients from a dosage form. *In-vitro* dissolution study can be used to assess *in-vivo* performance of a drug. Dissolution test can be used to differentiate between acceptable and unacceptable products (Ngwuluka, 2009) and the quality of the drug product can also be determined with the help of this test. It also aids in the developing process of new drug products.

Interaction between food and drugs play an important role on drug treatment and the potential side effects that may arise. The clinical significance of drug-food interactions can be variable and can lead to a loss of therapeutic efficacy or toxic effects of drug therapy. In general the effect a food on drugs leads to reduction in the drug's bioavailability. There are some advantages and disadvantages of the effect of food on drugs. In some cases it may lead to side effects, toxicity or unsuccessful drug therapy. On the other hand they may also increase the efficiency of drug or prevent the occurrence of undesirable effects. So it is essential to know about the interaction of drug with the food, to assess the effect of consuming any food or beverage while undertaking certain medications.

Aceclofenac belongs to a class of Non-Steroidal Anti-Inflammatory Drug (NSAID) which contains 99 to 101 percent of 2-[[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid. Aceclofenac is practically insoluble in water and falls under bio pharmaceutics classification system (BCS) class II that is low solubility, high permeability (Ashraful, 2011). Despite being unofficial in BP (British Pharmacopeia, 2010) or USP (US Pharmacopeia, 2009), Aceclofenac tablets are being marketed. No pharmacopoeial tests available.

The aim of this study was to obtain the dissolution profiles of nine brands of Instant release Aceclofenac tablets available in the Indian pharmacies and study the effect of various food beverages on sustained release formulation of Aceclofenac. The rate of dissolution of instant release (IR) tablet was compared in three different dissolution media such as phosphate buffer (pH 6.8), 0.1 N HCl (pH 1.2) and distilled water, in order to mimic *in-vivo* conditions as close as possible to get the necessary data for establishing similarity in profiles among the different brands. And the dissolution rate of Aceclofenac sustained release tablets was done on eight different

food beverages including three different percentages of ethanol to mimic the alcoholic drugs available for human consumption.

2. MATERIALS AND MATERIALS

Different brands of Aceclofenac were purchased from the retailed pharmacies in Tamil Nadu, India. The details of the nine brands of IR and two brands of SR tablets were given in the table 1. The details of different brands of food beverages used for disintegration and dissolution study were shown in table 2.

Uniformity of Thickness and Diameter: Thickness of tablet varies because of difference in the density of the granulation, the pressure applied to the tablets and also the speed of tablet compression. The variation in the thickness or diameter of the tablet need not change its weight. The thickness variation limits allowed are ± 5% of the size of the tablet (Gennaro, 2000). British pharmacopeia introduced a standard for tablet diameter and the stated diameter can deviate by ± 5% up to 12.5 mm and by ± 3% above 15mm. Three tablets from each brand of were randomly chosen and their diameter and thickness was measured using Vernier Callipers. The average diameter and thickness of the tablets with their standard deviations were calculated and tabulated.

Weight Variation: Tablet is designed to contain a specific amount of drug. To check whether tablet contain a proper amount of drug, uniformity of dosage form should be routinely measured. Uniformity of dosage form can be demonstrated by two methods, content uniformity and weight variation. If uncoated or film coated tablets contain 25 mg or more drug substance that comprise 25 % of each tablet weight, weight variation is applicable for the test of uniformity of dosage form. Therefore considering the amount of active ingredient (100 mg) in each dosage form in the present study, weight variation method was performed. Weight variation was calculated by taking ten tablets from each brand. An analytical weighing balance (BL-220H, Shimadzu Corporation, Japan) was used for weighing the tablets. From that the average weights for each brand and percentage deviation were calculated and tabulated. The percentage deviation allowed by USP for weight variation is ±10% for tablets weighing 130mg or less, ±7.5% for tablet weighing more than 130mg to 324mg and ± 5%for tablet weighing more than 324mg (Gennaro, 2000).

Hardness: The hardness of the tablet determines its resistance towards chipping, abrasion or breakage under condition of storage, transportation and handling (Gennaro, 2000). Weight of the material used, space between the upper and lower punches and pressure applied during compression are some of the factors on which the hardness of the tablets depends on. The hardness also depends on the nature and quantity of excipients used during formulation (Ashok, 1993). Here hardness test was performed using Monsanto hardness tester. Three tablets from each brand were chosen randomly. The pressure at which the tablets break or crush was recorded.

Disintegration test: Three tablets from each brand were dropped into the basket tube of the USP disintegration apparatus (DT 1000, Labindia, Mumbai, India) and loaded into a one-litre glass beaker filled with 900 ml of the media and then lowered into the thermostat water bath. Ten different media were taken and disintegration time of the each tablet was noted. The temperature of the bath was maintained at 38.0 ± 0.5 °C throughout the test period, so that temperature of the media can be maintained as 37.0 ± 0.5 °C. The time at which the tablet was completely disintegrated was noted (Monica, 2010).

Dissolution test: Under *in-vitro* conditions, the drug release from the solid dosage form was studied by dissolution testing and used to find the factors that affect the bioavailability of a drug. In the dissolution test the drug release is studied as a function of time. This test thus describes the overall rate of release of the drug. The dissolution tests (DS 8000, Labindia, Mumbai, India) for bioequivalence study of nine brands of instant release tablets of Aceclofenac was carried out using the paddle type apparatus operated at 75 rpm in three different media phosphate buffer of pH 6.8, 0.1N HCl (pH 1.2)and distilled water. Three tablets were chosen randomly from each brand and were put into the dissolution medium of 900ml. 10 ml sample was withdrawn at intervals for one hour and equal amount of fresh medium was introduced into the system in order to maintain the sink condition,. Temperature was maintained at 37 ± 0.5^0C throughout the study. The absorbance reading was determined at 275 nm using UV-Vis spectrophotometer against the blank. The concentration of released drug was determined from the calibration curve of pure Aceclofenac.

To understand the effect of the food beverages on the drug release profile of two different brands of sustained release formulation of Aceclofenac, the tablets were subjected for dissolution in five different food beverages. The food beverages available in the market such as coca-cola, milk and based on the alcohol content

available in the alcoholic beverages, alcohol (ethanol) content is mixed in water and used as media(approximate alcohol content in : brandy=40%, beer=16%, wine=6%). This test was carried out in rotating basket type apparatus operated at 75 rpm. The designated volume of the beverages was included in the medium for 1 hr to stimulate *in-vivo* conditions (Anagha Joshi, 2010). After 1 hr the medium was replaced with the fresh 6.8 pH phosphate buffer at 37±0.5°C. Samples were withdrawn at every one hour up to 6 hrs.

3. RESULTS AND DISCUSSION

Nine different brands of Aceclofenac were studied for their thickness, diameter, weight variation, hardness, disintegration and dissolution. The nine brands of Aceclofenac are named accordingly as alphabetic order from A-I and two brands of sustained release tablets, Acemol and Hifenac were named as J and K. The summary of results of diameter, thickness, weight variation, hardness for both IR and SR tablets were shown in table 3. The diameter of the tablets varied from 8mm to 12mm where as thickness of the tablets varied from 3.3mm to 5.13mm. Brand B had the highest diameter of 11.15 and brand D had smallest diameter of 8.05mm. The diameter and thickness of SR tablets showed slightest variation, Acimol was 10.1mm in diameter and 5.68 mm in thickness and Hifenac had diameter of 10.51 mm and 4.4 mm thickness.

Uniformity of weight serves as a monitor to Good Manufacturing Practices (GMP) as well as the amount of Active Pharmaceutical Ingredient (API). The average weight and standard deviation were calculated. From the values it was found that the weight of the brands lies between 0.176g to 0.392g.

Hardness is usually referred as non – compendial test. Among the nine brands, brand E and G showed crushing strength with more than 5 kg/cm² where as other brands show less than 5 kg/cm². Brand E requires highest pressure strength of about 11.166Kg/cm²to break where as brand I break at 1.833 Kg/cm². Acimol SR needs a pressure of 8.33 Kg/cm² to break while the pressure required by Hifenac was 3Kg/cm².

Disintegration test is important as it relates with the dissolution study. The churning motion of a disintegrating device more closely resembles gastric contractions. Disintegration time of all the nine brands of IR tablets was within limit. According to BP specifications the uncoated tablets should disintegrate within 15 min and film coated tablets in 30 min, while the USP specifies that both uncoated and film coated tablets should disintegrate within 30 min. Aceclofenac tablets were film coated and maximum time for disintegration was found 7 min 39 sec in case of brand Movon in milk. However the disintegration time varies with media. For sustained release tablets, Hifenac disintegrated fastly in phosphate buffer pH 6.8 within 15 minutes while Acimol disintegrated in 0.1N HCl in 61 minutes.

Disintegration time of a drug can be affected by hardness, compression factor, moisture in granules, hydrophilic and hydrophobic nature of the drug as well as the disintegration media used. The colloidal nature of the milk may alter the rate of disintegration of the drug. Hence in milk slower disintegration time was observed when compare with phosphate buffer pH 6.8, Due to the presence of preservatives, flavours, carbon dioxide and other substances, the disintegration time of Aceclofenac in coca-cola was not the same as in phosphate buffer.

From the results it was clear that for both sustained release and instant release formulations, the disintegration time was high in milk. The disintegration time of the tablets increases with increase in the % content of alcohol. Although patients are often counselled to take medications, especially pain relief medications, with milk to decrease the potential for gastric irritation, this study shows that milk delays disintegration of both SR and IR tablets.

The results of dissolution test for instant release tablets were graphically represented in fig 1,2&3. Three media (pH 6.8 Phosphate buffer, 0.1 N HCl and Distilled water) were considered for the study in order to mimic the *in-vivo* conditions. According to pharmacopeia, no mathematical evaluations are needed if >80% of the drug release was observed within first 15 min. In phosphate buffer, the solubility of Aceclofenac was 1538.7 ± 1.215 µg/ml. Drug release from nine brands was almost uniform in phosphate buffer pH6.8. In our study, around 80% drug release was observed in first 15 min and almost 100% drug was released at the end of 60 min from all the brands when phosphate buffer pH 6.8 was used as dissolution medium. Drug release of Aceclofenac in distilled water was much slower as it is poorly water soluble. However the brand Zerodol showed 80% of drug release within the first 15 min while Zix and Dolokind showed around 50% drug release at the end of 60 min. During the first 15 minutes, 93% of drug release was observed in Zerodol, 76% in Topnac, 69% in Hifenac while the other

brands showed less than 50% drug release. The percentage drug release of Aceclofenac in 0.1N HCl was very low. Less than 10% drug release was observed in all the nine brands. This is because of the pKa value of drug.

Table.1.Details of the different brands of Aceclofenac instant release (IR) and sustained release (SR)tablets

Code	Brand	Company	Mfg date	Exp date	Price (per 10 tablets)
A	Aceclo (100mg)	Aristo Pharmaceuticals pvt. Ltd	Jun-12	Jun-14	26.93
B	Dolokind (100mg)	Discovery mankind	Apr-12	Mar-14	14
C	Dolowin (100mg)	Microlabs Limited	Jul-12	Dec-14	28.5
D	Hifenac (100mg)	Intas pharmaceuticals	Sep-12	Aug-15	27.02
E	Movon (100mg)	IPCA Lab Ltd	Nov-12	Oct-14	30
F	Topnac (100mg)	Systopic Laboratories	Oct-11	Sep-13	14.5
G	Synofen (100mg)	Zeta Laboratories pvt Ltd	Jun-12	May-15	18
H	Zerodol (100mg)	IPCA Lab Ltd	Nov-12	Oct-14	30
I	Zix (100mg)	Jenburkt	Aug-10	Jul-13	27.79
J	Acimol SR (200mg)	Leeford healthcare	Dec-11	Nov-13	45
K	Hifenac SR (200mg)	Intas pharmaceuticals	Dec-12	Nov-15	41.84

Table.2.Different Media used for disintegration and Bioequivalence study

S.No	Media
1	Phosphate buffer(pH 6.8)
2	0.1N HCl (pH 1.2)
3	Water
4	6% Ethanol
5	16% Ethanol
6	40% Ethanol
7	Milk
8	Coca cola
9	Mountain Dew
10	Bovonto

Table.3. Results of physical parameter tests

Brand	Shape	Diameter (mm)	Thickness (mm)	Average Weight (g)	Hardness (Kg/Cm²)
A	Spherical	9.133±0.018	3.88±0.04	0.245±0.003	4
B	Spherical	11.153±0.037	4.31±0.009	0.392±0.001	3.666±0.235
C	Spherical	10.82	4.66±0.05	0.267±0.001	4.5±1
D	Spherical	8.05±0.009	3.69±0.065	0.213±0.001	4.166±0.235
E	Spherical	8.16	3.33±0.009	0.186±0.001	11.166±0.235
F	Flat,oval	12.72	5.133±0.04	0.263±0.004	2.166±0.288
G	Spherical	10.95±0.009	4.84±0.016	0.327±0.002	8.666±0.235
H	Spherical	8.1	3.4	0.176±0.007	3.666±0.288
I	Spherical	9.366±0.009	4.04	0.278±0.002	1.833±0.288
J	Spherical	10.1	5.68±0.043	0.392±0.006	8.33±0.235
K	Spherical	10.51±0.019	4.4±0.009	0.376±0.001	3

n= 3 ± standard deviation

Table.4.Comparative disintegration profile of IR tablets of Aceclofenac

Media	A	B	C	D	E	F	G	H	I
PB (pH6.8)	11″	14″	4′ 45″± 7″	1′ 13″± 2″	35″±7″	5 ″	11″	1′31″ ± 22″	10 ″±1″
0.1N HCl	9″ ± 2″	52″±3″	2′ 26″± 8″	4″	30″±1″	3 ″	3″	17″±3″	5″
Water	5 ″±1″	6″±1″	1′ 22″± 6″	41″±1″	29″±2″	28″±1″	9″	43″±2″	15″±2″
Milk	2′ 1″	27″±1″	4′38″±14″	2′35″± 1″	7′39″±11″	1′24″±2″	2′18″±13″	7′11″±13″	1′52 ″±2″
Coke	14″±1″	55″±7″	2′20″±10″	2′40″±16″	43″±1″	49 ″	40 ″±6″	38 ″	13 ″±1″
Mountin dew	16″	10″±2″	1 ′32″± 3″	1′20″±16″	45 ″±3″	27″± 3″	30 ″	38 ″	20 ″±2″
Bovonto	2′29″±5″	32″±1″	1′ 53″ ±2″	4′ 52″± 8″	55″±14″	1′06″±5″	51 ″±6″	1′37 ″	29 ″±2″
6% Ethanol	1′18″±10″	12″±2″	1′ 27″ ±1″	1′58″±15″	1′01″±11″	31″±1″	46″±3″	1′13 ″± 2″	13 ″±4″
16% Ethanol	1′2″± 4″	11″±1″	50″	2′ 3″± 10″	53 ″	1′04″±2″	52″	25 ″	22 ″±2″
40% Ethanol	2′9″± 4″	36″±4″	6 ′40″ ± 22	8′ 16″± 9″	1′24 ″±1″	2′06″±3″	2′17 ″± 7″	1′50 ″	13 ″±4″

Table.5.Comparative disintegration profile of SR tablets of Aceclofenac

Media	Acimol SR®	Hifenac SR®
PB(pH 6.8)	77′18″±12′	15′
0.1N HCl (pH 1.2)	61′12″±8′	52′30″±2′
Water	90′30″±10′	70′16″±5′
Milk	160′52″±15′	135′48″±8′
Coke®	98′40″±7′	105′20″±13′
Mountain dew®	92′35″±3′	60′52″±3′
Bovonto®	35′36″±1′	66′18″±4′
6%Ethanol	96′16″±4′	70′15″±3′
16%Ethanol	95′25″±9′	79′22″±12′
40%Ethanol	145′20″±3′	97′20″±2′

Figure.1.Comparative dissolution profile of different brands of Aceclofenac in phosphate buffer (pH 6.8)

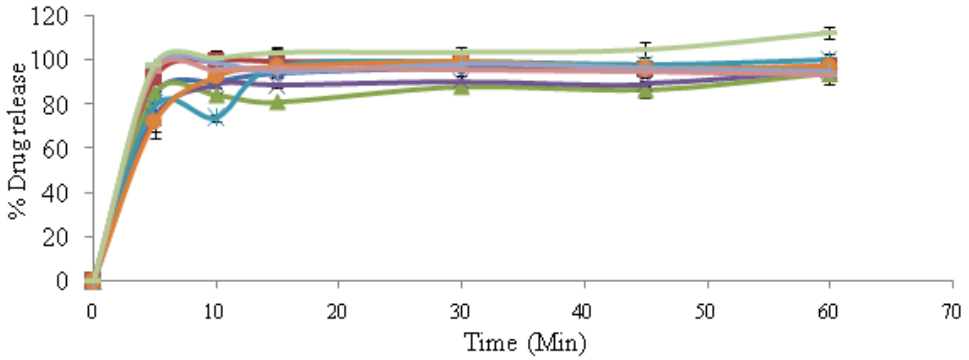


Figure.2.Comparative dissolution profile of different brands of Aceclofenac in distilled water

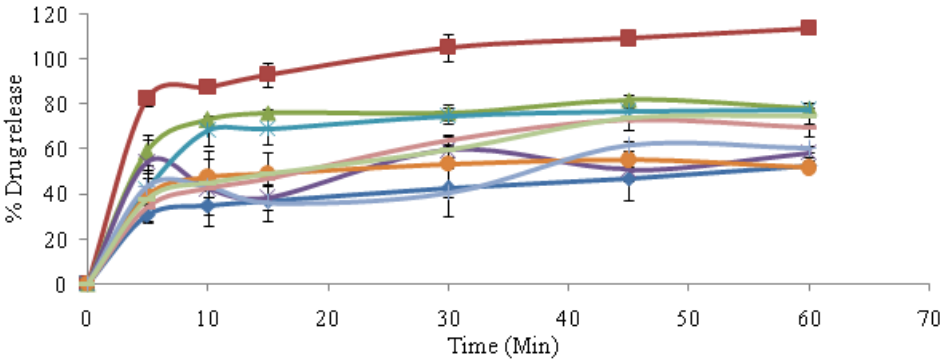


Figure.3.Comparative dissolution profile of different brands of Aceclofenac in 0.1N HCl pH 1.2

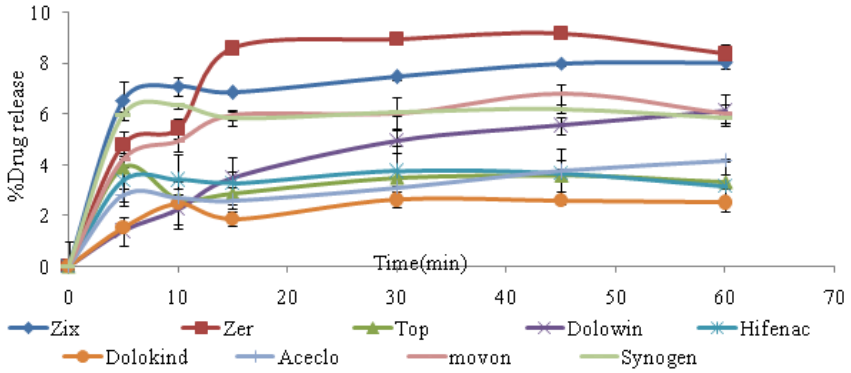
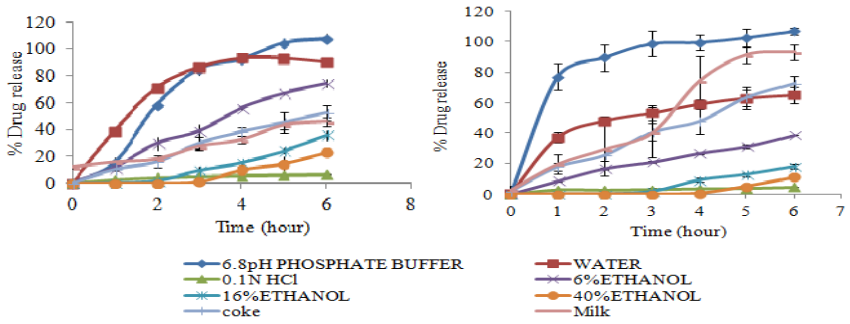


Figure.4.Comparative dissolution profile of (a) Acimol SR and (b) Hifenac SR (200mg) in different media

Fig.4a **Fig.4b**



4. CONCLUSION

Our results indicate that all the nine brands of Aceclofenac seem to have high dissolution rate and also good bioavailability. Both the brands of Aceclofenac sustained release tablets showed similar dissolution profile when different media was used. When compared with phosphate buffer the drug release was faster in milk than Coca cola and slow in 6 % ethanol, slower in 16% ethanol and slowest in 40% ethanol. This indicated that the drug release was affected by the strength of alcohol used in the food beverages. Our results suggest that the drug release is influenced by food beverages which have ability to disrupt the sustained release mechanism. The changes in the dissolution profile observed in sustained release formulations depend on the drug solubility and the food beverages. In this case, both earlier and delayed drug release was observed when different food beverages were used as the dissolution media. Based on the finding from this study it was concluded that despite some differences in physical parameters, the dissolution characteristics of all brands of Aceclofenac appeared to be similar, which proved the bioequivalence of the products.

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