Research Article

In Vitro Bio-Equivalence Studies on Commercial Formulations Containing Paracetamol and Ibuprofen

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ABSTRACT

The most widely used product categories in the pharmaceutical sectors are solid dosage forms, particularly tablets. To determine the in-vitro bioequivalence of tablets containing paracetamol and ibuprofen, this study was done. One of the most often prescribed antipyretic and analgesic medications in the world is paracetamol (acetaminophen). It is the recommended prescription for people who cannot use non-steroidal anti-inflammatory drugs, including those with bronchial asthma, haemophilia, salicylate hypersensitivity, peptic ulcers, pregnancy, or nursing. The conditions dysmenorrhoea, headaches, including migraines, postoperative pain, dental pain, and musculoskeletal and joint disorders such ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis are among those for which ibuprofen is most frequently used today. It also brings down fever. Different formulations of paracetamol and ibuprofen are available in the market with various brand names hence in order to enhance solubility and permeability different techniques and different excipients materials are used thus the in-vitro dissolution rate may vary from one formulation to another formulation. So, there is a need to conduct in-vitro bioequivalence studies for these formulations in order to identify the differences (if any) among the formulations and to select better formulation.

Keywords: Paracetamol, Ibuprofen, in-vitro dissolution studies, excipients, bioequivalence studies.

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INTRODUCTION

According to BCS classification the paracetamol and ibuprofen belongs to the class III and class II respectively.

Figure 1: BCS Classification

The bioavailability of BCS class II and class III medications is therefore improved by several methods.

Approaches to enhance the bioavailability

Class II – high permeability, low solubility

- Micronization and surfactant addition.
- Emulsion and micro-emulsion formulation, as well as the usage of complexing agents like cyclodextrin's.

Class III –low permeability, high solubility

- Demand the development of technologies that overcome the fundamental, absolute or regional permeability constraints.
- Class III materials include peptides and proteins, and the technology used to handle these substances are advancing today.

The word "bioequivalence" refers to the drug material reaching systemic circulation at the same pace and to the same relative extent in two or more similar dosage forms. They will have identical plasma concentration-time profiles, i.e., there won't be any appreciable statistical differences. The FDA mandates that bioequivalence studies be conducted in order to demonstrate average bioequivalence in drug absorption in terms of pharmacokinetic (PK) parameters like the area under the blood and/or plasma concentration-time curve (AUC) and peak concentration (C_{max}) before small molecule generic drug products can be approved. In reality, if the 90% confidence interval for the ratio of geometric means of the key PK parameter is entirely within the bioequivalence limits of (80%, 125%), we may assert that a test drug product is bioequivalent to an inventive (reference) drug

product. Based on log-transformed data, the confidence interval for the ratio of geometric means of the major Pk parameter is determined. The largest dose of a medication provided as an instant release formulation must be soluble in 250 ml or less of aqueous medium with a pH range of 1.2 to 6.8 at 37 °C in order for it to be deemed highly soluble. The substance is allowed to pass through biological membranes as part of the dynamic and intricate process known as permeability. When the percentage of the dose that is absorbed or the absolute bioavailability is equal to or higher than 90%, a medication is said to be extremely permeable.

MATERIALS AND METHODS

Materials:

Chemicals used: potassium di hydrogen phosphate,

Sodium hydroxide pellets,

Paracetamol crude drug,

Ibuprofen crude drug,

Hydrochloric acid (0.1N)

Tablet brands used:

Table 1: Tablets brands used

S.No	Tablet Name & brand name	Strength	Mfg. date	Exp. date	Batch.no
1	Combiflam (SANOFI)	Ibuprofen-400mg Paracetamol-325mg	01-04-2022	12-04-2023	2NG-420
2	Flexon (ARISTO)	Ibuprofen-400mg Paracetamol-325mg	04-03-2021	10-02-2024	DPC210676
3	Ibugesic plus (CIPLA)	Ibuprofen-400mg Paracetamol-325mg	05-09-2020	15-08-2023	AF20F57

Construction of Calibration Curve for Paracetamol and Ibuprofen: [23]

Preparation of stock solution: The calibration curve was created in phosphate buffer at pH 7.2. A stock solution of 1 mg/ml was produced by properly weighing 25 mg of paracetamol and 25 mg of ibuprofen, then transferring them into a 25 ml volumetric flask and adding 10 ml of 7.2 phosphate buffer to dissolve them.

Working standards: Further dilutions were made with 7.2 phosphate buffer to obtain 2 to 10µg/ml concentrations of paracetamol and ibuprofen and the absorbance was measured at 244&228nm respectively.

Evaluation of marketed Tablets using quality control tests:[24, 25]

Parameters:

- Weight variation
- Friability test
- Hardness test
- Tablet disintegration
- % Drug content
- Tablet dissolution

Weight variation: [26]

Weight variation was done to make sure that each tablet had the right amount of medication. Using an analytical scale, 20 tablets were individually weighed for the test. The average weight was then calculated, and the individual tablet weights were compared to the average. The following formula is used to determine the percentage of weight fluctuation.

Formula: -

$$\%\ deviation = \frac{individual\ weight\ of\ tablet - average\ weight\ of\ tablet}{average\ weight\ of\ tablet} \times 100$$

Friability test: [27]

Cleanly dust the tablets, then weighed 10 of them. Put the tablets in the drum, then spin it 100 times. The tablets should been taken out, cleaned of any loose dust, and correctly weighed. The test was only performed once unless the results were unclear or the weight loss exceeds the desired amount, in which case the test was repeated twice and the average of the three tests was calculated. A weight decrease of no more than 1. 0% (from the mean of the three tests or from a single test) was acceptable.

Formula:

$$\%\ friability = \frac{weight\ before\ friability - weight\ after\ friability}{weight\ before\ friability} \times 100$$

Hardness test: [28]

The hardness of tablets determines how resistant they were to capping, abrasion, or breakage during storage, transportation, and handling before to use. The forced necessary to broke or crushed a tablet when it was put on its edge was referred to as the tablet hardness. It was occasionally referred to as pill crushing strength. The Monsanto typed (make: singhla) hardness tester was used to conduct the hardness test. As an anvil strikes a tablet with forced, an instrument measures how much forced was needed to broke the tablet. The tablet was sandwiched

between two anvils, and the forced needed to broke the tablet was measured by applying pressure to the anvils. Four tablets from each formulation were subjected to the crushing strength test

Tablet Disintegration: [28]

The tool was a glass or plastic tube with an opened end and a rust-resistant no 10 mesh sieve attached to the other end. The tube was kept floating in a bath of watered or other suitable liquid that had a thermostat set at 370c. Unless otherwise specified in the specific monograph, the tube may travel up and down at a steady rate of 30 times per minute over a distance of 75 mm. Put one tablet and one disc in each of the six tubes of the basket, then operate the equipment with watered kept at 37oc as the immersion liquid for the duration of the period recommended in each individual monograph. The basket from the liquid by raising it.

Drug Content:

Twenty tablets, each containing 100 mg of paracetamol, were weighed, and 81.25 mg of ibuprofen were collected and dissolved in 7.2 phosphate buffers. After 10 minutes of sonication, the solution was transferred to a 100 ml volumetric flask. The volume was made up using the same buffer as the solution. A membrane filter was used to filter 20 ml of fluid. The 10 ml of filtrate was added to a 100 ml volumetric flask and diluted to the appropriate level. Using P^H7.2 buffer, 5 ml of the solution were collected and increased to 25 ml. Analysing the sample at 228 and 244 nm, respectively, allowed researchers to determine how much ibuprofen and paracetamol were present.

Dissolution: [27]

$_PH$	: 7.2
Volume	: 900ml
R P M	: 75
Time of samples with drawn	: 5mins
Total number of samples with drawn	: 6
Total time	: 30min

In-vitro dissolution parameters

The USP Type 2 (paddle type) dissolving apparatus was used to conduct the in-vitro dissolution research of tablets. At 75 rpm and 37 0.5°C, the test was conducted in 900 cc of 7.2 phosphate buffers. After a period of 30 minutes, the specified volume (5 ml) of samples was taken out at predetermined intervals (5, 10, 15, 20, 25, and 30 min.) and replaced with the same volume of the dissolution medium. The samples were appropriately diluted after being filtered via a 0.2-m membrane filter. The obtained samples were analysed for paracetamol and ibuprofen estimate at max 244nm and 228nm, respectively. The amount of drug dissolved from the tablets was calculated from the observed absorbance value by using absorption maximum co-efficient method. The relevant mathematical equations are furnished below.

$$C_x = \frac{A_2 a y_{1-} A_1 a y_2}{a x_2 a y_{1-} a x_1 a y_2}$$

$$C_y = \frac{A_1 a x_{2-} A_2 a x_1}{a x_2 a y_{1-} a x_1 a y_2}$$

C_x = Concentration of "x" (Paracetamol)

C_y = Concentration of "y" (Ibuprofen)

A_1 = Absorbance of Paracetamol

A_2 = Absorbance of Ibuprofen

ax_1 = Absorptivity of "x" at λ_1

ay_1 = Absorptivity of "y" at λ_1

ax_2 = Absorptivity of "x" at λ_2

ay_2 = Absorptivity of "y" at λ_2

Determination of *In-vitro* dissolution parameters: -

The dissolution data is fitted into different models such as first order, zero order and Hixson-Crowell cube root law by using disso 2.3.2.0. The dissolution rate constant and the time required for dissolution of 50% and 90% of the drug from the tablet dosage forms was calculated separately for each drug. From the data the extent of dissolution was also investigated by calculating dissolution efficiency. The dissolution efficiency was calculated by trapezoid rule.

Statistical treatment of *in-vitro* dissolution parameters: -

The dissolution parameters observed for each drug from each formulation were treated statistically with one way ANOVA followed by Dunnett's t test by using Minitab 16 statistical software, product version 16.2.3.0.

K=-slope ×2.203

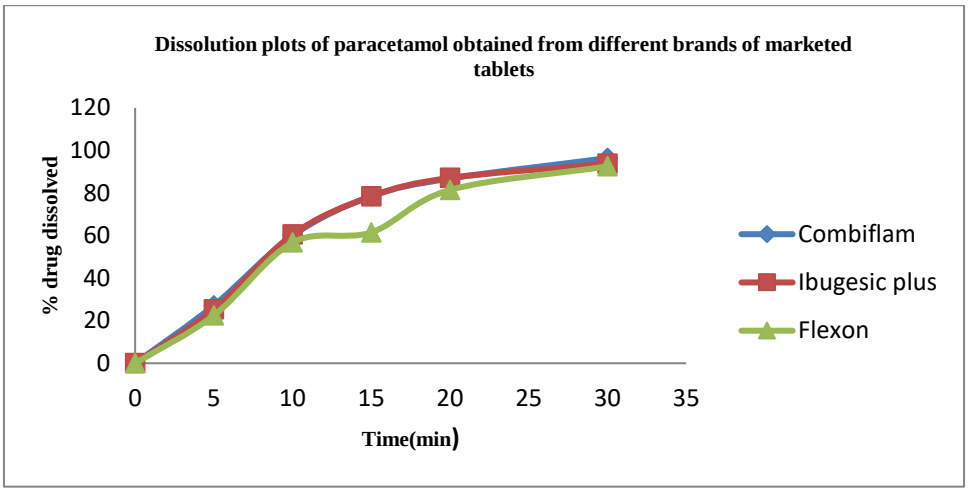
$$DE = \frac{1/2[(C1 + C2)(T2 - T1) + (C2 + C3)(T3 - T2) + \cdots .]}{25}$$

RESULTS AND DISCUSSION

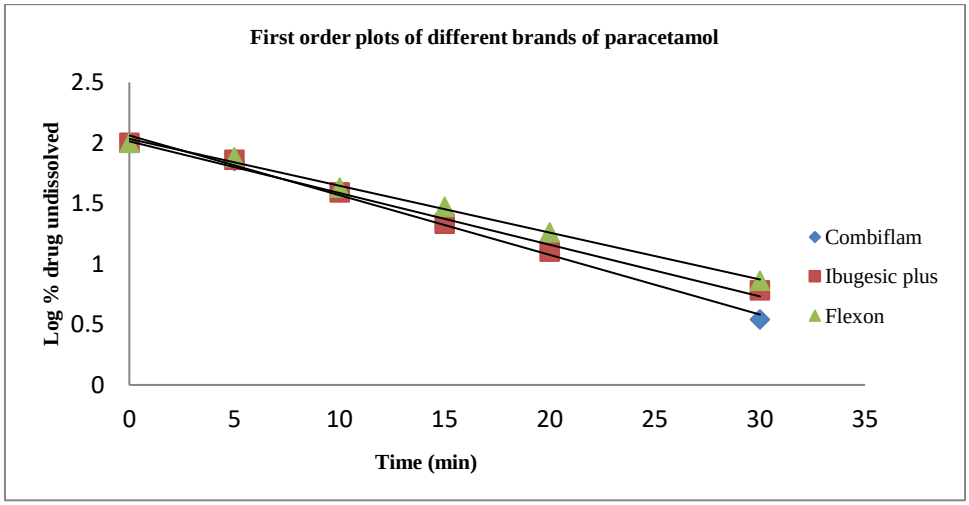
Table 2: Physical characteristics

Tablet name	Avg. wt. Variation (mg)	HardnessTest (kg/cm²)	Friability (%)	Disintegration (min)	% Drug Content (Assay)	
					Paracetamol	Ibuprofen
Combiflam	915.94±4.45	4.5±7.0	0.435±0.02	3.68±0.04	99	99.07
Ibugesic plus	835.28 ±4.80	4.0±0.40	0.647±0.06	1.33±0.06	99.75	99.69
Flexon	926.24±4.25	3.3±0.25	0.521±0.03	4.3±0.07	101	100.5

Paracetamol



Graph 1: Dissolution plots of paracetamol obtained from different brands of marketed tablets

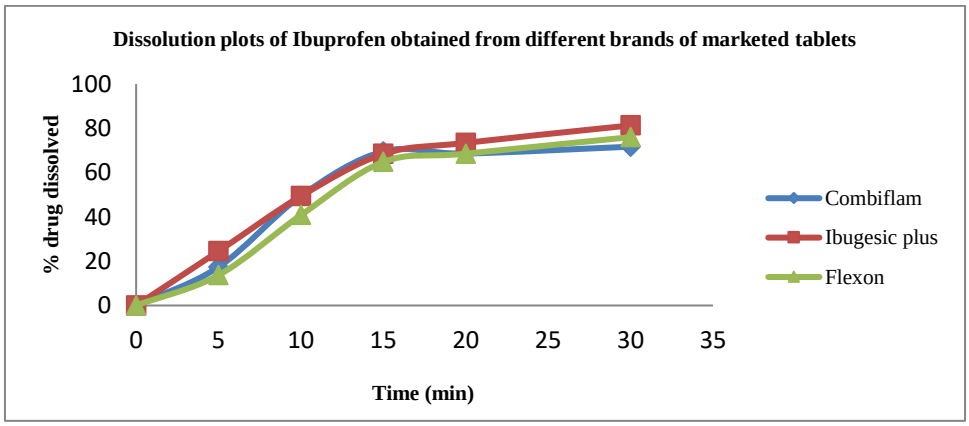


Graph 2: First order plots of different brands of paracetamol

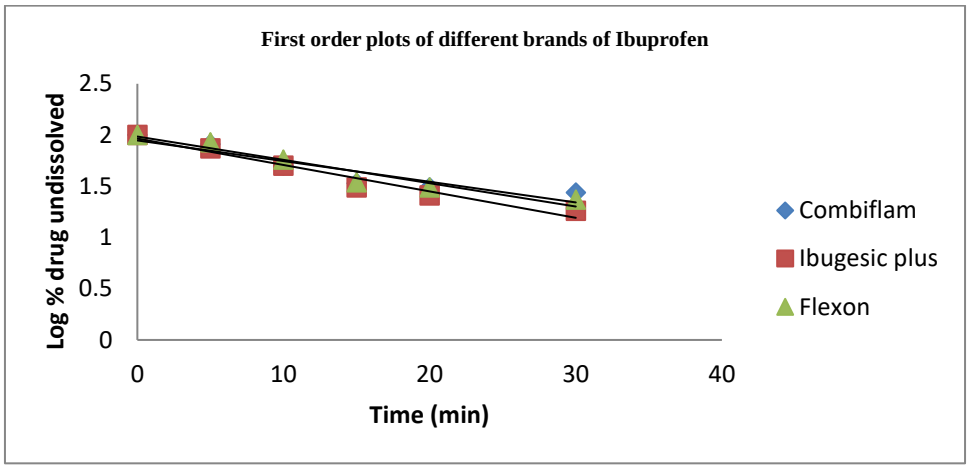
Table 3: *In-vitro* dissolution kinetics of paracetamol observed from different brands of marketed tablets.

Parameters	Combiflam	Ibugesic plus	Flexon
Slope	-0.055	-0.04	-0.0436
K(min^{-1})	0.127	0.112	0.100
$t_{50\%}$ (min)	5.463	6.171	6.89
$t_{90\%}$(min)	18.13	20.50	22.90

Ibuprofen



Graph 3: Dissolution plots of Ibuprofen obtained from different brands of marketed tablets



Graph 4: First order plots of different brands of Ibuprofen

Table 4: *In-vitro* dissolution kinetics of Ibuprofen obtained from different brands of marketed tablets.

Parameters	Combiflam	Ibugesic plus	Flexon
Slope	-0.023	-0.029	-0.026
K(min^{-1})	0.054	0.067	0.06
$t_{50\%}$ (min)	12.84	10.2	11.56
$t_{90\%}$(min)	42.65	34.21	38.42

Table 5: *In-vitro* dissolution kinetics "DE" values of Paracetamol and Ibuprofen

Paracetamol						Ibuprofen					
Source	**DF**	**SS**	**MS**	**F**	**P**	**Source**	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	42.584	21.292	47.58	0.000	Factor	2	57.0648	28.824	44.57	0.000
Error	6	2.685	0.448			Error	6	3.881	0.647		
Total	8	45.269				Total	8	61.529			

DISCUSSION

Most of the drugs are suffering with poor oral Bioavailability due to either low aqueous solubility or not having adequate permeability through the biological membranes. To overcome these challenges, the manufacturers prefer to alter the physico-chemical properties of the drug or the formulation or composition of the formulation may be changed as mentioned in table-2. The strategies used by one manufacturer may differ from the strategies used by other manufacturers. Due to these variations, the formulations may be pharmaceutically equivalent but they may not bio-equivalent. So, the studies were undertaken to compare the marketed formulations based on *in-vitro* dissolution rate. The tablets were subjected to various compendial and non-compendial Quality Control tests. The tablet satisfied Weight Variation test IP. The observed % deviation wasfound to be less than permeated deviation (5%). The hardness was found to be within the range (3.5-4.5 kg/cm^2). The result indicating that the tablets are having good mechanical strength. Further the tablets were also tested for drug content, disintegration and friability and the observed results are presented in table 2. The drug content was found to be 99-101%for Paracetamol and 99.07-109.5% for Ibuprofen. The Assay requirements for paracetamol and ibuprofen as per IP are 95 to 105% and 98.5 to 101%. All the tablets passed Assay requirements for Paracetamol tablets IP and Ibuprofen tablets IP. The disintegration time was found to be less than 15mins for marketed tablets. All the tablets passed the disintegration time requirement for paracetamol IP and Ibuprofen IP. The friability was found to be less than 1%. All the tablets passed the friability limit requirements IP. All three brands procured from the local market were subjected to in-vitro dissolution studies by using apparatus-2. The dissolution data observed from commercial formulation was presented. To ascertain the kinetics of drug release, the graph was plotted between time and log % drug undissolved. The resulting first order graphs were showed in graph-2,4. Various in vitro dissolution parameters such as K and DE values were calculated and the results are placed in table- 5. These parameters were further treated statistically with one way ANOVA followed by Dunnett's t test. Significant differences in dissolution parameters were

detected from these formulations. Based on dissolution rate the formulations ranked as:

Combiflam>Ibugesic plus>Flexon

Based on DE values the formulations are ranked as:

Ibugesic plus>Flexon>Combiflam

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Flexon>Ibugesic plus>Combiflam

Based on DE values the formulations are ranked as

Ibugesic plus>Flexon>Combiflam

CONCLUSION

- All the marketed formulations (combination tablets of paracetamol & ibuprofen) satisfy the compendial requirements i.e., the dissolution requirements of paracetamol & ibuprofen as per the individual tablet monographs.
- The marketed formulation satisfied the dissolution requirements of paracetamol. As per IP the % paracetamol to be dissolved from the tablets should be 80% and the observed % drug dissolved is found to be greater than the specified limit
- The marketed formulation satisfied the dissolution requirements of Ibuprofen. As per IP the % Ibuprofen to be dissolved from the tablets should be 50% and the observed % drug dissolved is found to be greater than the specified limit.

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