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Determination of in-vitro Equivalence of Paracetamol Tablets

Athri D. Rathnayake[1], Uthpali Mannapperuma[2], Dhanusha Thambawita[2], Kamal P. B. Herath[3], Priyadarshani Galappatthy[2], Ravindra L. Jayakody[2]

[1]Department of Chemistry, University of Colombo, Sri Lanka

[2]Department of Pharmacology, University of Colombo, Sri Lanka

[3]*M. S. J. Industries (Ceylon) (Private) Ltd, Sri Lanka*

ABSTRACT

Bioequivalence studies are the usually accepted method to determine the therapeutic equivalence of two drug products. Because in-vivo bioequivalence studies are time consuming and expensive to conduct, major regulatory authorities have introduced biowaivers for some selected medicines belonging to BCS class 1 and III drugs. Comparative dissolution tests are used in biowaiver procedure to waiver the bioequivalence requirement. We performed this study to see whether two brands of paracetamol tablets are bioequivalent using the in-vitro methodology. In the first stage of this research study, British Pharmacopeia 2012 quality tests were performed on the two selected paracetamol tablet products to determine whether they are pharmaceutically equivalent. In the second stage in-vitro equivalence of the two products was determined using the biowaiver testing procedure given by the World Health Organization. Dissolution profiles were generated at pH values, 1.2, 4.5 and 6.8. Results were compared through two model independent methods, difference factor (f_1) and similarity factor (f_2). The two paracetamol tablet products tested, complied with all the quality requirements of the British Pharmacopeia 2012. For the two products, the difference factor (f_1) was below the 15 and similarity factor (f_2) was above the 50 in all dissolution test conditions. These results confirm that the two products are pharmaceutically equivalent. The test product is also bioequivalent to the reference product in-vitro, and therefore they can be interchangeable during clinical use. This study shows that in-vivo bioequivalence testing can be waived using the in-vitro method, for some pharmaceutical products such as paracetamol tablets.

KEYWORDS: *Paracetamol tablets, Biowaivers, Dissolution profiles*

corresponding author: Athri D. Rathnayake, eMail: athridewmina@gmail.com

1. INTRODUCTION

Paracetamol is an over the counter medication used widely as an analgesic agent for symptomatic management of mild to moderate pain. Also, it has excellent antipyretic properties and is used to reduce the body temperature in patients with fever (Kalantzi *et al.*, 2006; Ellis *et al.*, 2002). In Sri Lanka, as well as in other countries many brands and generics of paracetamol (500 mg) tablets are available. These are formulated to be pharmaceutically equivalent to each other. Pharmaceutically equivalent drug products contain same molar amount of the same active pharmaceutical ingredient in the same dosage form if they meet comparable standards and administered by the same route. Pharmaceutical equivalence does not necessarily imply similarity of therapeutic effect. Therefore, there are concerns when it comes to substituting one brand of a pharmaceutically equivalent drug product to another in clinical practice (WHO, 2006; USP, 2012).

Pharmaceutically equivalent drug products should be bioequivalent (or therapeutically equivalent) to each other to be given interchangeably during the clinical practice. Two drug products are bioequivalent if they are pharmaceutically equivalent and their effects can be expected to be essentially the same after administration of the same molar dose under the same conditions. Bioequivalence (BE) testing is a comparative test, which uses specified criteria for comparison and have predetermined BE limits for such criteria (HHS/FDA, 2003; WHO, 2006a).

There are several *in-vivo* and *in-vitro* approaches to determine the equivalence of two pharmaceutically equivalent drug products. These approaches include pharmacokinetic studies, pharmacodynamic studies, comparative clinical trial studies and *in-vitro* dissolution studies (HHS/FDA, 2003; WHO, 2006) Because *in-vivo* BE studies are time consuming and expensive to conduct, major regulatory authorities such as United States Food and Drug Administration (US-FDA) and World Health Organization (WHO) have introduced biowaivers. The biowaiver approach, waives *in-vivo* BE studies by the means of comparative *in-vitro* dissolution test and it's based on the Biopharmaceutical Classification System (BCS). There are only several pharmaceutical formulations which are currently being considered for biowaivers. According to the WHO, paracetamol is a BCS class I active pharmaceutical ingredient (highly soluble and highly permeable) thus making it eligible to be considered for biowaivers (HHS/FDA, 2003; WHO, 2006).

In biowaiver studies, comparison of dissolution profiles is done for two pharmaceutically equivalent products under identical conditions. In order to compare the dissolution profiles, similarity factor (f_2) and difference factor (f_1) are used. The f_1 calculates the relative error based on the percent difference between the two dissolution profiles.

$$f_1 = \{[\sum_{t=1}^{n} |R_t - T_t|] / \sum_{t=1}^{n} R_t]\}.100.$$

The f_2 calculates the percent similarity between the two dissolution profiles.

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

In above equations, *n* is the number of sampling time points used to draw a drug dissolution profile, R_t is dissolution value of the

reference batch at time t and T_t is dissolution value of the test batch at time t.

Generally a f_1 value up to 15 (0-15) and a f_2 value greater than 50 (50-100) indicates the equivalence of the two dissolution profiles between two pharmaceutical products. A f_2 of 50 represents an average 10 % difference of drug release at all sampling time points, while a f_2 of 100 represents zero average difference of drug release at all sampling time points (HHS/FDA, 1997).

This study was carried out to investigate the *in-vitro* equivalence of selected two paracetamol 500 mg tablet products, taking the market leader product as the reference product and the brand currently utilized in the public sector as the test product. In order to declare the pharmaceutical equivalency of these two paracetamol tablet products quality testing methods of British Pharmacopeia 2012 (BP 2012) paracetamol tablet monograph were used.

2 MATERIALS AND METHODS

2.1 Materials

Reference paracetamol tablets (Panadol) was obtained as a donation for the purpose of the research study from GlaxoSmithKline, Sri Lanka. Test paracetamol tablets (SPMC Paracetamol) were obtained from the Lady Ridgeway Hospital for Children via request from the Medical Supplies Division, Ministry of Health, Sri Lanka. Paracetamol working standard, 4-aminophenol, and tetrabutylammonium hydroxide were obtained as a donation from Astron Limited, Sri Lanka for the paracetamol related substance test. All the chemicals used for the research study were analytical reagent grade. For the related substance test HPLC graded chemicals were used.

2.2 Quality Testing for Two Products of Paracetamol 500 mg Tablets According to the BP 2012

Uniformity of mass: Uniformity of mass test was performed on CAS CAY 120 electronic balance. Three trials were conducted for the 2 products separately (BP, 2012).

Assay: Analysis of paracetamol was done according to the BP 2012 Paracetamol tablets monograph assay using CAS CAY 120 electronic balance and JASCO V-560 UV/VIS spectrophotometer. Three trials were performed for each product (BP, 2012).

Friability of paracetamol tablets: The friability of randomly selected eleven tablets were investigated using EP 420A-FR Electronic balance and ERWEKA Friability Tester. Three trials were performed for each product (BP, 2012).

Resistance to crushing of paracetamol tablets: Hardness of randomly selected 10 tablets was investigated using ERWEKA hardness tester. Three trials were performed for each tablet product [8].

Dissolution: A volume of 900 ml (± 1 %) of phosphate buffer pH 5.8 (Prepared according to the BP 2012) was placed in the each vessel of the PHARMA TEST dissolution apparatus. Dissolution medium was equilibrated to 37 ± 0.5 ^{0}C. Six tablets were randomly selected for the dissolution test. Prior to commencement of the test, care was taken to exclude air bubbles from the surface of the tablets. Paddles were rotated at 50 revolutions per minute, for 45

minutes. Sample medium was collected at 5, 10, 15, 20, 30, 45 minutes from all 6 vessels using the auto sampler system. One milliliter of the collected samples was diluted to 10.00 ml with 0.1M sodium hydroxide. One milliliter of the resulting solutions were diluted up to 10.00 ml with 0.1M sodium hydroxide. This dilution procedure was carried out for all 36 samples and absorbance was measured using VARIAN CARY-50 Bio UV/VIS spectrophotometer at 257 nm using 0.1M sodium hydroxide in the reference cell. Percentage release of the drug from paracetamol tablets was determined. Dissolution profile was plotted for each tablets. The same procedure was followed for the two tablet products separately.

Related substance test: Analysis of 4-aminophenol content in the paracetamol tablets was done using high performance liquid chromatography (SHIMADZU HPLC machine and C_8 stainless steel column with dimension of 25 cm X 4.6 mm) according to the BP 2012 paracetamol tablets related substance test (BP, 2012).

Following solutions were prepared immediately before the test was started and covered with aluminum foil in order to protect those from light.

Solution - 1: Powdered tablet from each product containing 0.2 g of paracetamol was dispersed in 8.00 ml of the mobile phase using sonicator. Sufficient volume of mobile phase was added to produce 10.00 ml of the solution. Resulting solution was mixed well and filtered. Separate solutions have been made for the two paracetamol products using above procedure.

Solution – 2: Volume of 1.00 ml of solution 1 has been diluted to 20.00 ml with mobile phase. Volume of 1.00 ml of this solution has been diluted up to 20.00 ml with mobile phase.

Solution – 3: Volume of 100.00 ml containing 0.002 % w/v 4-aminophenol in the mobile phase and volume of 100.00 ml containing 0.002 % w/v paracetamol reference standard in the mobile phase were mixed together to produce 200 ml of the solution.

Preparation of mobile phase and adjustment of mobile phase parameters were done as follows.

Volume of 1000.0 ml of HPLC graded methanol containing 4.60 g of a 40 % v/v solution of tetrabutylammonium hydroxide was made and filtered using pore size of 0.45 micrometer cellulose acetate filter paper. Volume of 1000.0 ml of 0.05 M disodium hydrogen orthophosphate (analytical reagent grade of commerce) and 1000.0 ml of 0.05 M sodium dihydrogen orthophosphate (analytical reagent grade of commerce) were mixed together and filtered using pore size of 0.45 micrometer cellulose acetate filter paper. In order to make the 1000 ml of mobile phase, 250 ml of methanol containing 1.15 g of 40 %v/v solution of tetrabutylammonium hydroxide were mixed with 750 ml of the mixture of 0.05M sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate. Mobile phase flow rate was adjusted to 1 ml per minute at a temperature of 35 ^{0}C. Volume ratio has been adjusted as 1 volume of methanol contains 40 % v/v solution of tetrabutylammonium hydroxide to 3 volume of phosphate buffer. Detection wavelength was adjusted to 245 nm.

Injection of prepared solutions and results interpretation of obtained chromatograms were done according to BP 2012.

2.3 Determination of *in-vitro* Equivalence of Paracetamol 500 mg Tablets

For each product at each pH (pH 1.2 HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer), a volume of 900 ml (±1 %) of the dissolution media was placed in each and every vessel of the PHARMA TEST dissolution apparatus. Dissolution medium was equilibrated to 37 ± 0.5 ^{0}C. Twelve tablets were randomly selected for the dissolution test. Prior to the commencement of the test care was taken to exclude air bubbles from the surface of the tablets. Paddles were rotated at 75 revolutions per minute, for 45 minutes. Samples of dissolution medium were collected at 5, 10, 15, 20, 30, 45 minutes from all 6 vessels using auto sampler system. The dilution procedure described in the BP 2012 was carried out to dilute all the 72 samples and their absorbance was measured using VARIAN CARY-50 Bio UV/VIS spectrophotometer according to the procedure describe under the section 2.2 dissolution test. Average percentage release of the drug from paracetamol tablets in each time point was determined in order to apply those values for the calculation of f_2 and f_1.

3 RESULTS AND DISCUSSION

3.1 Quality Testing for Two Products of Paracetamol 500 mg Tablets According to the BP 2012

Uniformity of mass: Results of uniformity of mass test for both products are illustrated in Table 1. According to the BP uniformity of mass test, for tablets weighing more than 250 mg, permissible percentage deviation of the mass is 5%. In order to comply for the requirement of uniformity of mass test, more than 2 of the individual masses should not deviate from the average mass by more than the percentage deviation, and none should deviate more than twice the percentage (BP, 2012). Thus both products complied with the requirements for BP uniformity of mass test.

Assay: Results achieved from analysis of active ingredient (paracetamol) in both products are given in Table 1. As per BP 2012 specifications, the content should not be less than 95% and not more than 105% of labeled amount of the tablets (BP, 2012). Results indicate the reference product assay value is in the acceptable limits. Despite the second assay trial failing by 0.6%, the average content of the labeled amount of the test product is in the accepted BP range.

Friability of paracetamol tablets: Friability test is to ensure that tablets possess a suitable mechanical strength to avoid abrasion, crumbling or breaking on handling or subsequent processing. Results achieved from the friability test in both products are given in Table 1. A BP 2012 specification for friability is a maximum loss of mass not greater than 1.0 % (BP, 2012). Thus both products comply with the specifications of the BP

Resistance to crushing of tablets: The hardness or crushing strength of tablets assesses the ability of tablets to withstand handling without fracturing or chipping. The results were interpreted as average hardness of 10 tablets, minimum hardness value, and maximum hardness value according to the BP (BP, 2012). Results achieved from hardness test for both products are given in Table 1.

Dissolution: Dissolution profiles for the two products are shown in Figure 1 and Figure 2. According to the harmonized dissolution

criteria, no unit must release the drug product less than 80 % of the labeled amount in 45 minutes. Hence both products comply with the BP dissolution test.

Related Substance Test: The major impurity of paracetamol raw material is 4-aminophenol. The quantity of 4-aminophenol present in a paracetamol tablet must be strictly controlled as 4-aminophenol has nephrotoxic and teratogenic effects (Ca˘linescu *et al.*, 2012). Because of the resolution factor between the two principal peaks of the chromatogram obtained with the reference solution is 5.587 which is greater than 4.0, the HPLC test was accepted as a valid test according to BP 2012.

The chromatogram obtained with solution - 1 for reference product, the area of the peak correspond to 4-aminophenol is not greater than area of corresponding peak of 4-aminophenol in the chromatogram obtained with standard solution - 3. The chromatogram obtained with solution - 1 for test product, the area of the peak corresponds to 4-aminophenol is not greater than area of corresponding peak of 4-aminophenol in the chromatogram obtained with standard solution - 3. No other impurity (peaks correspond to impurities in solution - 1 of both products) is greater than the area of the principal peak obtained with solution - 2 of both products. Thus the levels of 4-aminophenol in both the paracetamol tablet products were below the level specified in the BP.

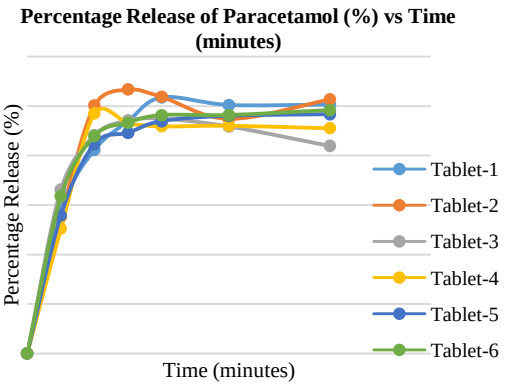

Figure 1. Dissolution profiles of six tablets of the reference product at pH 5.8 phosphate buffer

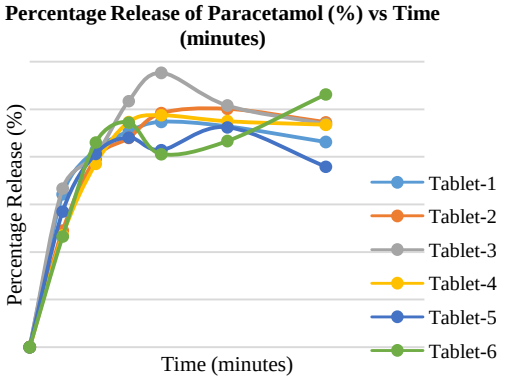

Figure 2. Dissolution profiles of six tablets of the test product at pH 5.8 phosphate buffer

TABLE 1. Results of the Uniformity of Mass, Assay, Friability Test and Hardness Test (Test for Resistant to Crushing of Tablets).

Test	Reference Product			Test Product		
	Trial 1	***Trial 2***	***Trial 3***	***Trial 1***	***Trial 2***	***Trial 3***
Uniformity of mass						
Accepted mass deviation range (g)	0.5647-0.6241	0.5612-0.6203	0.5637-0.6230	0.5435-0.6007	0.5969-0.5401	0.5436-0.6008
Number of tablets outside the deviation range	None	None	None	None	2	None
Assay						
Content of paracetamol as a percentage of labeled amount (%)	103.7	99.9	101.1	97.2	94.4	96.6
Friability test						
Percentage loss of mass (%)	0.4539	0.0736	0.0369	0.1964	0.0862	0.5255
Hardness test						
Average hardness (N)	151	148	146	130	135	131
Minimum hardness (N)	136	136	136	099	099	097
Maximum hardness (N)	156	156	154	148	148	148

3.2 Determination of *in-vitro* Equivalence of Paracetamol 500 mg Tablets

Dissolution tests (Table 2) were carried out in order to determine the *in-vitro* equivalence of two paracetamol tablet products adopting the biowaiver testing procedure proposed by the WHO. Dissolution profile comparison (Figure 3, Figure 4 and Figure 5) of the two paracetamol tablets (in all the three dissolution media) was done according to the recommendations given by US-FDA. Calculated values of f_1 and f_2 are given in Table 2.

Because the f_2 for all three dissolution media is within the range of 50-100 and the f_1 for all three dissolution media is within the range of 0-15 there is a similarity of dissolution profile of both products in all the three dissolution media.

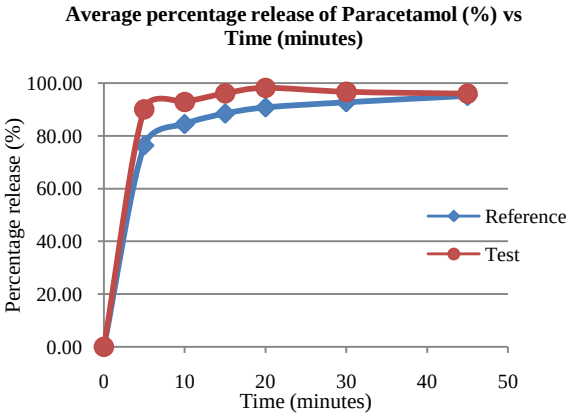

Figure 3. Dissolution profiles of paracetamol tablets in pH 1.2 HCl media

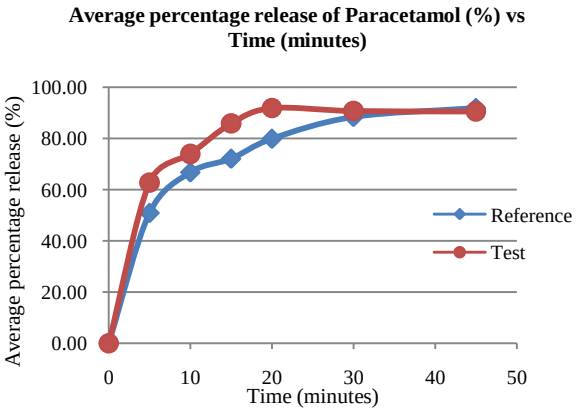

Figure 4. Dissolution profiles of paracetamol tablets in pH 4.5 acetate buffer

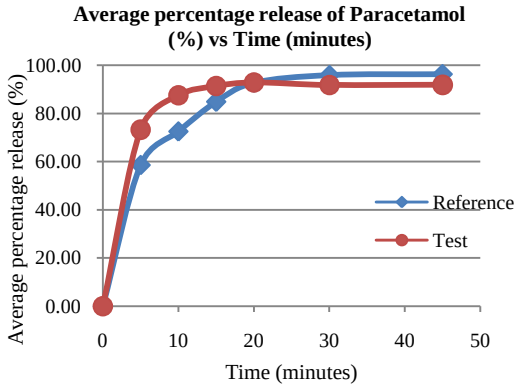

Figure 5. Dissolution profiles of paracetamol tablets in pH 6.8 phosphate buffer

TABLE 2. Dissolution Tests Carried Out In Order to Determine the *in-vitro* Equivalence of Paracetamol Tablets and Calculated Similarity Factor (f_2) and Difference Factor (f_1) .

Test	Products	Number of tablets	Dissolution media	f_1	f_2
1	Reference	12	pH 1.2 HCl Solution	7.971	56.18
	Test	12	pH 1.2 HCl Solution		
2	Reference	12	pH 4.5 Acetate Buffer	10.18	51.17
	Test	12	pH 4.5 Acetate Buffer		
3	Reference	12	pH 6.8 Phosphate Buffer	5.554	51.44
	Test	12	pH 6.8 Phosphate Buffer		

4. CONCLUSION

Reference paracetamol tablets comply with all the BP 2012 quality specifications such as the uniformity of mass, assay, dissolution, related substances test, hardness test, and friability test. Test paracetamol tablets also comply with all the BP quality specifications. It can be concluded that the test product is pharmaceutically equivalent to the reference product.

In-vitro equivalence test was carried out as the second stage of the research study. Similarity factor was calculated for all the three dissolution media. Similarity factor is 56.18 for the pH 1.2 HCl solution, 51.17 for the pH 4.5 acetate buffer and 51.44 for the pH 6.8 phosphate buffer. Since the similarity factor is within the accepted range (50 - 100) for profile similarity in all three media, the test paracetamol tablet brand demonstrates equivalent dissolution to the reference paracetamol tablet brand in-vitro. Thus it can be concluded that the two products can be substituted with each other during the clinical practice. Results of this study indicates the possibility of post marketing evaluation of pharmaceutically equivalent products in-vitro as a substitute for in-vivo bioequivalence testing.

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