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Original Article

Comparison of Flurbiprofen Tablets Available In Pakistani Market and Their Absorption Studies

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Abstract

The aim of this present work was to compare different parameters of various brands of flurbiprofen tablets collected from different retail pharmacies in the local market of Pakistan. Four brands A, B, C and D were tested for weight variation, hardness, friability, disintegration dissolution, HPLC assay and in vitro absorption studies in rabbit skin, stomach and intestine by using the pre packed Column RT 250-4.6 Purospher® STAR RP-18 end capped (5 µm) and acetonitrile, phosphate buffer (pH 3.7) as mobile phase in the ratio of 1:1. Flurbiprofen was detected at 265 nm at the flow rate of 1 ml/min. Brand B was considered as referece. Similarity factor (f2) of brand B and C and brand B & D was found to be 61 and 51 and dissimilarity factors (f1) values were 5 and 9 respectively in same dissolution medium. Model dependent methods First order, Hixon Crowell and Weibull model were used. The method was found to be sensitive and linear in the range of 10 to 700 ppm with 0.999 coefficient of correlation. Everted sac absorption studies of selected formulation showed 50% of drug absorption from stomach in first 3 hours, 21% through intestine and very negligible through skin.

Keywords: Flurbiprofen, Dissolution studies, Everted Sac techniques, limit of detection (LOD)

Introduction:

Flurbiprofen is a propionic acid derivative which is mostly used as first line therapy for symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It showed 99% human serum albumin bounding at therapeutic concentrations with the short half-life of 3.9 hours. Figure 1 showed the chemical structure of Flurbiprofen ((*RS)*-2-(2-fluorobiphenyl-4-yl) propionic acid) as a weak acidic drug. Studies proved that flurbiprofen had an outstanding safety profile in the treatment of osteoarthritis, gout, spondylitis and rheumatoid arthritis, mild to moderate pain including migraine, postoperative analgesia, dysmenorrhea and sore throat due to its analgesic and anti-inflammatory action (2012).

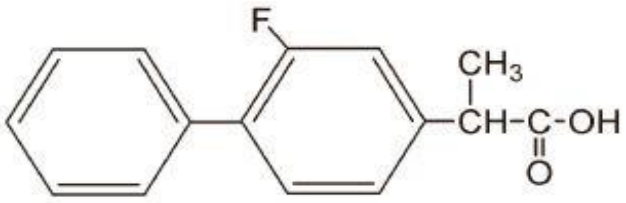

Figure 1. Chemical structure of flurbiprofen.

HPLC is a simple, accurate, rapid sensitive and applicable analytical method for determination of flurbiprofen in pharmaceutical preparation. Critical review of the literature showed a limited number of methods for the determine of

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flurbiprofen in pharmaceutical preparation by HPLC method (Aisha Qayyum et al., 2011). Everted sac technique was first established by Wilson and Wiseman in 1957 and explained by Karasov and Diamond in 1987 (Kershaw et al., 1960). Absorption of drugs through intestine is carried out by two types of transport channels present in the epithelium i.e., influx and efflux transport channels. Influx channels transports drug molecules from mucosal to serosal side of the intestine while efflux channels works in reverse direction (Mohd Aftab Alam et al., 2011). Two everted sac technique with and without use of cannula are recommended in previous literature (Mahmoud, 2004a, Christine Dixon and Mizen, 1977).

In this study, safest and most suitable brand among other available brands in Pakistan were selected. In vitro dissolution studies of four selected brands will be performed by using the different dissolution mediums of acidic and alkaline nature. Model independent approaches like similarity and dissimilarity tests was performed for selection of the best formulation. Simultaneous estimation of Flurbiprofen was performed by using HPLC. In vitro absorption studies was carried out on lab animals by using the everted sac techniques. Different routes like through skin, stomach and intestine was used for the complete absorption analysis.

MATERIALS AND METHODS;

Instruments and Chemicals

Analytical balance (Electronic Balance Model AED-300), High Performance Liquid Chromatographic system (SYKAM S5111), Prepacked Column RT 250-4.6 Purospher® STAR RP-18 endcapped (5µm), Rheodyne manual injector fitted with a 20 µl loop syringe (SGE Company, Australia), Monsanto hardness tester (locally made), friability apparatus (pharma PTZS D-63512 Hainsburg, Germany), Dissolution Apparatus Type II (Pharma PTZS D-63512 Hainsburg, Germany), UV-visible spectrophotometer (UV-3000 Germany), pH meter (370 pH meter, Jenway, Europe), Sonicator (Shenzhen Co. China) and Membrane Disc Filters Millipore, 0.45 µm pore size (Millipore Corporation, Billerica). A reference brand was a gift by Pfizer pharmaceuticals, and three more brands; Neo triad (Helicon Pharmaceutek), Flusaid (Alliance Pharmaceuticals), Dol (Wilshire Laboratories) were purchased from the local market. Potassium dihydrogen phosphate, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), and acetonitrile all were purchased from Merck Germany. All the chemicals purchased were of analytical grade. Double distilled water was used in the whole study.

Weight variation, Hardness and Friability Test

Six tablets of each brand were selected randomly, weighed and variation of weight was observed by calculating the standard deviation by using the Microsoft Excel 2007. Monsanto Hardness tester was used for testing hardness in kg of ten tablets of each brand. Similarly ten tablets of each brand were selected randomly for friability testing. Friabilator was operated at 25 rpm for four minutes. % Friability was calculated by using following formula

$$\text{Friability (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \qquad (1)$$

Where W_1 is the initial weight and W_2 is final weight after dusting

Disintegration and dissolution Test

Disintegration time of randomly selected six tablets was measured in 900 ml distilled water at 36.5±0.5°C while dissolution studies of formulations were performed by using USP-II dissolution apparatus with

temperature range of 35 ± 0.5 to 37 ± 0.5°C having 900ml phosphate buffer of pH 7.4 at 50 rpm. Five ml aliquot was withdrawn and replaced with fresh medium after different time intervals and observed at 247 nm.

Similarity and dissimilarity factor

Dissimilarity factor f_1 and Similarity factor f_2 were used to calculate the percentage difference among the brands at different time intervals.

$$f_1 = \left\{\frac{[\sum|Rt-Tt|]}{\sum Rt}\right\} \times 100 \quad (2)$$

$$f_2 = 50log\left\{\left[1+\frac{1}{n}\sum(Rt-Tt)^2\right]^{-0.5} \times 100\right\} (3)$$

Where n is the number of time points, R_t and Tt are the cumulative percentage dissolved at each time interval of Reference and test product, respectively. The values of f_1 should be less than 50(Leon et al., 2005).

Model Dependent Approaches

First order release kinetics can be expressed as

$$\frac{dC}{dt} = -Kc \quad (4)$$

Where K denotes the 1st order rate constant and have unit hour $^{-1}$. C is concentration of the above expression. In 1931 Hixson and Crowell found that the regular area of the particle has a directly proportional relation with the cube root of its volume. Mathematically expressed as

$$\sqrt[3]{A_o} - \sqrt[3]{A_t} = kt \quad (5)$$

A_o is amount of drug present initially in pharmaceutical dosage form, A_t is amount of drug at, time t and K is constant describing relation between particle's surface and volume. Weibull model is the descriptive model (not derived from a physical law) described by Langenbucher and used for the description of different dissolution profiles and expressed as

$$M = M_o\left[1 - e^{-\frac{(t-T)^b}{a}}\right] \quad (6)$$

M is the quantity of the drug which is dissolved per unit time t, M_o represents the total quantity of drug released, T represents the lag time. α parameter represents a scale that shows the time dependence and b denotes the shape of the curve (Sima sadray et al., 2010).

HPLC Chromatographic solution

Mobile phase were prepared by using the water, acetonitrile and glacial acetic acid in the ratio of 12: 7: 1 respectively. Stock solution 1 mg/ml was prepared by using the crushed powder of selected tablets and diluted in range 10-700 µg/ml. The isocratic flow rate was 1.0 ml/min. Detection was carried out using a wavelength of 247 nm. The developed method was validated according to International Conference on Harmonization (ICH) guidelines (Yamaoka et al., 1978).

Method Validation

To identify intervention of dosage form excipients being utilized in tablet as well as medium, specificity was assessed through the comparison of chromatograms of 6 replicate injections of placebo with the standard flurbiprofen. To calculate the linear relationship between the range of concentration of the API and the detector response linearity curve was used. Linearity curve was assessed by forming the dilution in 10, 250, 500, 700 µg/ml from stock solution 1 mg/ml.

System suitability was analyzed by means of preliminary mobile phase composition, pursued with ten insertions of similar standard. 10 consecutive injections were to

evaluate the system suitability on each day of method validation.

$$h = L/n \quad (7)$$

$$n = 16\left(\frac{t}{wb}\right)^2 \quad (8)$$

Where, h= theoretical plate values. W_b is the peak width of base, t is Retention time and L is the length of column. Similarly tailing factor is calculated by the following formula

$$T = w/2f \quad (9)$$

Where, w is the width at 5% of peak height and f is the distance between the maximum and leading edge of the peak. For quantification analysis the stability of solution is essential. Shelf life was estimated by placing sample at ambient temperature for twelve hours, as well as similar estimation was made at -15 to -20°C for seven days. All the experiments were repeated six times moreover consequences were within acceptance limit. Limit of detection (LOD) and lowest concentration of the analytical (LOQ) determined from 10 to 700 ppm as requirement of Food and Drug Administration (FDA) guidelines (Askholt and Nielsen-Kudsk, 1986). Accuracy is intimacy between supposed and results that were assessed via spiked placebo recovery method. Sample solutions with 10, 250, 500, and 700 ppm concentrations were prepared and spiked with placebo solution. Percentage recovery was estimated by comparing fraction of drug samples along with standard. Day to day precision was judged via 3 elected amounts for 3 successive days, while within a day precision was accomplished through investigation of control solution in triplicate all through linearity range initializing from beginning, middle as well as ending of day (Hanif et al., 2011).

Following equations were used to evaluate the theoretical plates of the reports

Analysis of Inter-day precision was carried out by selecting five concentrations for three consecutive days, whereas intraday precision was carried out by using four different concentrations i.e. 10, 250, 500 and 700 ppm.

Everted Sac Technique Flurbiprofen Absorption

An albino male rabbit having weight 800g was used for *in-vitro* absorption studies. Intestine and stomach was exposed, cleaned and placed in normal saline solution. Everted sac was used at room temperature 37°C,HCl solution of 1.2 pH for stomach and phosphate buffer of pH 7.2 was used for intestine (Zhang et al., 2006).

5ml aliquot of flurbiprofen was taken at different time intervals and absorbance was observed using UV/Visible spectrophotometer at 247nm and Percentage of flurbiprofen was calculated by equation

$$Concentration\ of\ drug = \frac{Abs_{Samp}}{Abs_{Std}}\ x100 \quad (10)$$

RESULTS AND DISCUSSIONS;

Physicochemical properties

All selected brands were evaluated by weight variation, hardness, friability and disintegration test and results were within limits as mentioned in standard pharmacopoeias. Similarly Shoaib et al reported the same thing (Shoaib et al., 2015).Mean weight of different brands ranges from 420.5 to 427.0 ± 5% and hardness varied from 7.89 to 10.1 Kg. Percentage friability of all brands were found to be less than 0.8% which was within Pharmacopeia limits. Disintegration time for different brands varied from 1.20 to 1.5 min. as shown in table 1.

Table:1 Physicochemical tests of different Flurbiprofen tablets

Brand	Weight (mg)	Hardness	Friability (%)	Disint (Min)
Limit	-	6-9	< 1	<15
A	424.5±4.5	9.80±0.78	0.48	1.30
B	427.0±5.2	10.1±0.71	0.52	1.20
C	420.5±5.3	7.97±0.57	0.73	1.34
D	421.7±4.8	7.89±0.56	0.75	1.50

Table: 2 Comparative study profiles by using similarity ***(f_1)***and dissimilarity***(f_2)*** factors

Brands	*(f_1)*	*(f_2)*
B and C	61	5
B and D	51	9

Investigations showed that all brands showed more than 60% release within first 5minutes, more than 75% and 85% release at 15 and 60 min respectively. The graphical presentation of data is shown in figure 3.

Comparison of different brands was performed successfully. Brand A showed more than 85% release within 15 minutes which was considered as biowaived and removed from comparative studies. Taking brand B as reference and compared it with C and D. In case of brand C, similarity factor was found to be 61 and dissimilarity factor was found to be 5. While in case of D, similarity factor was 51 and dissimilarity was found to be 9 as shown in table 3.

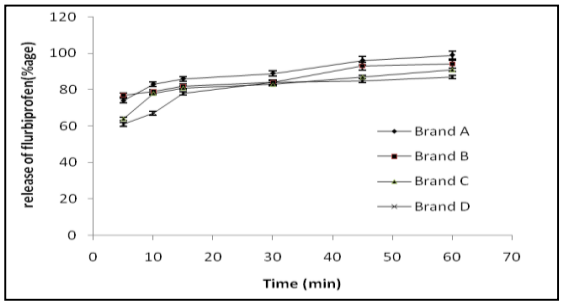

Figure 2: Percentage release of Flurbiprofen tablets of different brands

similarity was greater than 50% and dissimilarity was less than 15%, so both brands are similar with reference. Similarly Quality Control Studies on Gatifloxacin 200mg tablets available in the Pakistani market has been carried out (Hanif et al., 2011).

Regression of r^2 for first order, Hixson Crowell and Weibull ranges from 0.7266 to 0.9624, 0.8923 to 0.9897 and 0.9771 to 0.9874, respectively. Brand A was following Weibull model as the value of coefficient of correlation for this model was 0.9771.

Table: 3 Determination of coefficient of release profile of different brands of flurbiprofen tablets

	1st order		Hixson Crow		Weibull	
	$K(h^{-1})$	r^2	$K_H(h^{-1})$	r^2	*B*	r^2
A	0.218	0.878	0.026	0.967	0.491	0.977
B	0.212	0.726	0.025	0.892	1.605	0.974
C	0.158	0.962	0.024	0.955	0.172	0.987
D	0.124	0.962	0.023	0.989	0.285	0.978

Table.4. System suitability parameters of Flurbiprofen of Brand C

Consideration	Mean(n=10)	RSD(%)
Retention Time	2.457 min	0.006
Area	3131.87	0.174
Tailing Factor	321.548	0.986
Theoretical Plates	4156.6	1.02

Hixson Crowell model as shown in table 4.Observations showed that release of drug is according to Hixson Crowel model. Similar studies have been done by Hanif et al in 2011 after studying different brands of gatifloxacin available in Pakistani market (Khan et al., 2011). Brand B and C were also following Weibull model, but brand D was following

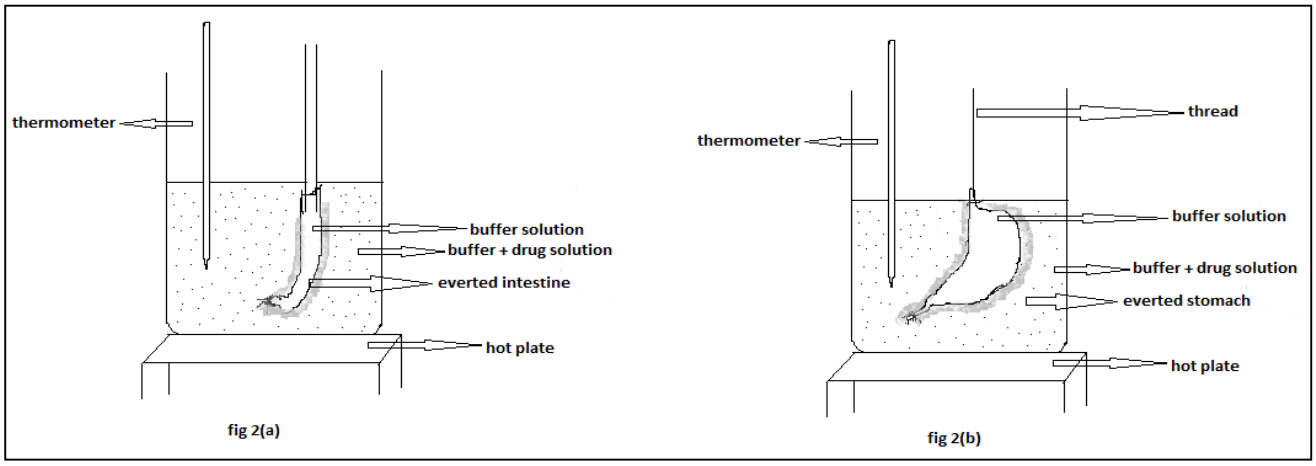

Figure 3(a). Everted intestine sac assembly. **Figure 2(b).** Everted stomach assembly

High Performance Liquid Chromatography

The method showed good sensitivity, selectivity, baseline resolved peaks and excellent resolution as required in method validation guidelines by the International Conference on the Harmonization of Pharmaceuticals for Human Use (ICH) Q2B. Accuracy, precision, linearity, specificity, solution stability, system suitability, LOD and LOQ were successfully calculated. Retention time for system suitability was 2.457min. Similarly percentage relative standard deviation (%RSD) of area was

Tailing Factor and theoretical plates were observed and showed in Table 4. All results were within limits of ICH guideline. Linearity curve of flurbiprofen had been plotted by taking concentration on horizontal axis and peak area on vertical axis.

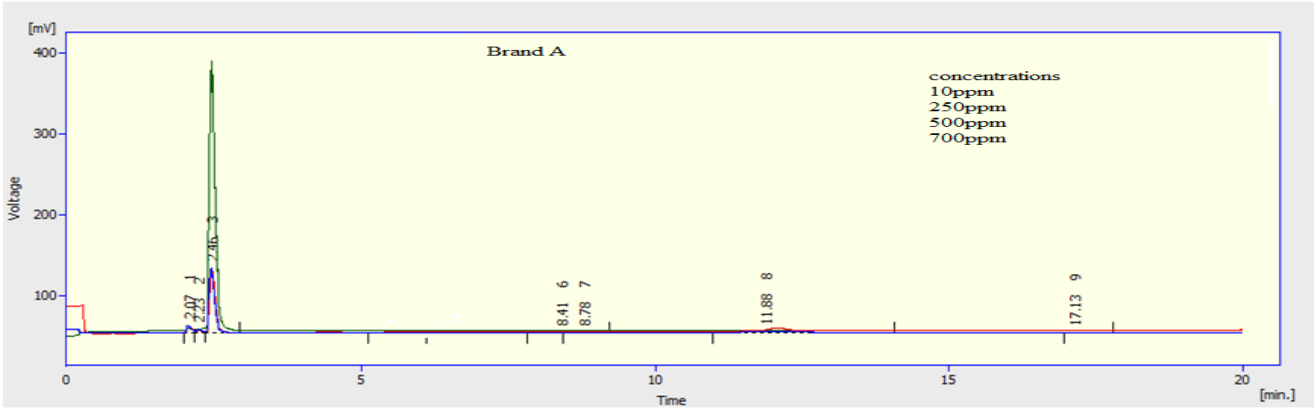

Figure 4: Specificity of the method at 1 ml/min flow rate of mobile phase, placebo and standard having total run time of 20/min of Brand C.

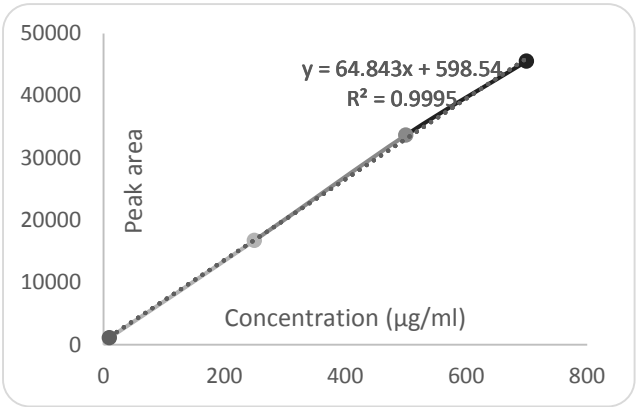

Figure 5: Linearity curve of flurbiprofen in different concentration band C.

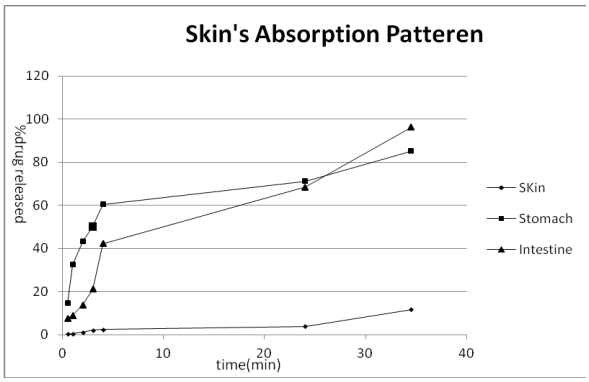

Figure 6: Absorption of flurbiprofen through skin, stomach and intestine Brand C.

0.175 Which was less than 2. %RSD values of tailing factor was 0.154 and was found within limit less than 2.For determination of linearity, four concentrations (Branch et al.,) with 10, 250, 500 and 700ppm were analyzed and Coefficient of Correlation was found to be 0.999 which showed linear regression. For the evaluation of resolution and reproducibility of the method, the results of the system suitability test are shown in Table 4. System suitability parameters include Relative Standard Deviation (RSD), Percentage of Retention Time, Peak area, the value of r^2 was 0.999 as shown in figure 5.

Conclusion

Comparative analysis of different Flurbiprofen brands available in Pakistani market was effectively accomplished. Dissolution profiles evaluated and Assay of Flurbiprofen was done via the use of HPLC method. HPLC method is specific, sensitive and reproducible. Validation of method was according to FDA rules and consequences were within acceptability criterion. *In vitro* absorption of Flurbiprofen was also assessed with the help of everted sac technique which revealed that absorption was maximum through stomach as compared to skin and intestine.

Table 5: Percentage recovery of flurbiprofen brand C.

S/N	Concentration (µg/ml)	Found (µg/ml)	Recovery (%)
1	10	9.6	96
2	250	245	98
3	500	480	96
4	700	686	98

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