ORIGINAL ARTICLE

Press Coated Pulsatile Release Flurbiprofen Tablets: A Preclinical Pharmacokinetic Investigation

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Abstract

Aim: The present study was designed to conduct a pharmacokinetic study for developed flurbiprofen press coated pulsatile release tablets (PRT), which are intended to treat early morning surge in rheumatoid arthritis. **Settings and Design:** A simple, accurate, specific and Linear reverse phase high-performance liquid chromatography method has been developed and validated for the estimation of Flurbiprofen in rabbit plasma. The formulated tablets were tested *in vivo* in New Zealand white male rabbits for various pharmacokinetic parameters. **Materials and Methods**: The method of estimation of flurbiprofen was developed and validated using Waters symmetry C18 (100*4.6 mm and 3.5 um) and 10 mm phosphate buffer: Acetonitrile (55:45) (pH 4.0) at 245 nm with 1.0 ml/min flow rate using Ibuprofen as an internal standard. The developed estimation method of flurbiprofen was used to conduct a pharmacokinetic study in twelve male New Zealand rabbits, which received optimized rapid release core tablets, and press coated PRT of flurbiprofen, respectively. Pharmacokinetic parameters were computed to compare the mean lag time required between administration of dosage and drug release in pulsatile formulation with that of immediate release dosage formulation. **Results:** The estimation method has shown repeatability and reproducibility. The extract action recoveries of Flurbiprofen were between 90% and 110%. The pharmacokinetic estimations proved the capability of press release coated tablets to achieve drug release after the desired lag time. The C_{max} of press-coated tablets was 45.10 ± 9.71 μg mL^{-1} at T_{max} of 8 h whereas it was 54.2 ± 2.18 μg/mL^{-1} at 2 h in case of immediate release tablets. The area under the curve for the immediate release and pulsatile release coated tablets was 356.82 μg mL^{-1} h^{-1} and 324.21 μg mL^{-1} h^{-1}. **Conclusion:** Pulsatile tablets of flurbiprofen were successfully evaluated, which provided a desirable lag time followed by desirable drug release.

Key words: Flurbiprofen, press-coated tablet, rheumatoid arthritis

INTRODUCTION

Oral formulations of flurbiprofen are indicated for the acute or long-term symptomatic treatment of rheumatoid arthritis (RA), osteoarthritis and ankylosing spondylitis. Flurbiprofen is a nonsteroidal anti-inflammatory agent, a propionic acid derivative with antipyretic and analgesic activity.[1] The anti-inflammatory effect of flurbiprofen occurs through reversible inhibition of cyclooxygenase, the enzyme responsible for the conversion of arachidonic acid to prostaglandin G2 (PGG2) and PGG2 to PGH2 in the prostaglandin synthesis pathway.[2] This effectively decreases the concentration of prostaglandins involved in inflammation, pain, and swelling.

RA is a long-term autoimmune disorder that primarily affects joints. RA typically manifests with signs of inflammation, with the affected joints being swollen, warm, painful and stiff, particularly early in the morning on waking or following prolonged inactivity.[3] Increased stiffness early in the morning is often a prominent feature of the disease and typically lasts for more than an hour.[4] RA requires time-dependent drug

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release for maximum therapeutic benefit.[5] Development of pulsatile tablets is one of the promising time specific systems that release the drug after a predetermined lag time for treating this disease condition occurring in the early hours.[6]

In the literature survey carried out, there were only few estimation procedures of flurbiprofen in rabbit plasma,[7-9] therefore, an attempt was made to develop and validate a simple, accurate, precise, selective reverse phase high performance liquid chromatography (RP-HPLC) method for determination of flurbiprofen in rabbit plasma so that it was used further in carrying out the pharmacokinetic study.

MATERIALS AND METHODS

Materials and instruments

Flurbiprofen, Explotab, Methocel LV Premium E50, and Ibuprofen were gift samples from Hetero drugs, Hyderabad, India. All other chemicals used were of analytical and HPLC grade procured from S.D. Fine chemicals (Make: Merck and Fisher Scientific). Shimadzu LC 2010CHT HPLC was used along with Lab solutions Software with column waters symmetry.

Analytical method for estimation of flurbiprofen

The analysis of flurbiprofen was done by Shimadzu LC 2010CHT HPLC which was used along with Lab solutions Software with column waters symmetry. The chromatographic separation was performed by phenomenex Gemini C18 (250.0 × 4.6 mm, 5 µ). The mobile phase consisted of 10 mm potassium dihydrogen phosphate (KH2PO4) buffer and acetonitrile buffer (pH 4.0 ± 0.2) with a volumetric ratio of 55: 45 at a flow rate of 1.0 ml/min. The UV detector was set at 245 nm, and injection volume was 20 µL. The calibration curve was generated for concentrations ranging from 0.5 to 75.0 µg/ml. Mobile phase gave good separation of main peak, diluent, plasma peak, and internal standard peak. Efficiency and asymmetry were also within the acceptance criteria. The resolution between main peak and internal standard was more than 2.0.

Preparation of mobile phase

1.36 g of potassium monobasic phosphate was accurately weighed and transferred into 1000 ml of water, to it 1 ml of triethylamine was added, and pH of the solution was adjusted to 4.0 ± 0.05 using diluted orthophosphoric acid, the solution was filtered through 0.45 um filter paper (PVDF membrane filter, make: Millipore). Further, 550 ml of buffer was mixed with 450 ml of acetonitrile and degassed by sonication to acquire a volumetric ratio of 55:45.

Preparation standard solution

Stock solution was prepared by weighing 50 mg of flurbiprofen in 100 ml of mobile phase. The internal standard stock solution was prepared by weighing 50 mg of ibuprofen in 100 ml of mobile phase. Each 5 ml of above solutions were pipetted out separately transferred into 50 ml volumetric flask than the volume was makeup to achieve 50 ml (50 µg/ml) using mobile phase.

Sample solution

The plasma sample was previously extracted using liquid - liquid extraction in a 10 ml volumetric flask to which 1 ml internal standard was added and mobile phase was used to make up the rest volume, after thorough mixing the solution was transferred into centrifuge tube and centrifuged at 3000 rpm for 5 min and the clear supernatant was collected and analyzed.

System suitability and precision

The system suitability is done by taking the area of standard preparation for five replicas to verify the working of the analytical system used. It should meet system suitability criteria.[10]

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample.

The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements. The system suitability parameter was carried out at 100% standard solution (50 µg/ml).

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range. To demonstrate linearity of the test method, five standard solutions with concentration ranging from 10% to 150% of test concentration were prepared by spiking standard stock solution.

Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interferences from blank and plasma. Plasma and diluent were injected into HPLC system, and interference was compared by direct comparison method.

Limit of quantification (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision, accuracy, respectively. It can be determined by S/N ratio or by linearity. LOQ is calculated by the standard deviation of response and the slope from 10% to 150% of linearity. The required dilutions were prepared, each in triplicate for calculating LOQ. LOQ = 10 σ/S; where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.[11]

Method precision

In method precision, a homogeneous sample of a single batch is analyzed for 6 times. This indicates whether a method gives consistent results or not for a single batch. The obtained results are tabulated.

Accuracy and recovery

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Recovery of flurbiprofen was estimated with various concentrations ranging from 50% to 150% of target concentration (0.025 mg/ml) by spiking the standard drug solution. Recovery was conducted in triplicate preparation at 50%, 100%, and 150% level. The samples were centrifuged at 3000 rpm for 10 min. The supernatant was separated and injected into HPLC system.

Robustness

The robustness is determined by varying the flow rate from 0.9 ml/min to 1.1 ml/min. The prepared standard solution of flurbiprofen was injected by varying the flow rate from 0.9 ml/min to 1.1 ml/min along with the optimized method.

Pharmacokinetic studies

In vivo pharmacokinetic studies were conducted in New Zealand white male rabbits weighing 2.5-3 kg, by following all ethical guidelines for investigations in laboratory animals, which was approved by the Institutional Animal Ethical Committee. (1505/PO/a/11 CPCSEA). The study was carried out to compare the pharmacokinetics of optimized pulsatile release tablets (PRT) and rapid release core tablet (RRT) of flurbiprofen. The precompression parameters of the powder blend of the tablets, the preparation methods, various physical evaluation tests and *in vitro* drug release studies including their lag time are completely discussed in Reddy *et al*.[12] (development and characterization of press coated tablet of flurbiprofen: A chronotherapeutic approach. Asian Journal of Pharmaceutics) from that study the optimized RRT and PRT were selected for the pharmacokinetic study. The overnight fasted male New Zealand white rabbits were divided into 2 groups (n =6) and treated orally with RRT's and PRTs, respectively. Tablets were administered orally by the help of a gastric intubation tube.[13] After oral administration of tablets 1 ml of blood sample was collected at each time point 30 min, 1 hr, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hrs from the marginal ear vein of the rabbit. Rabbits were held in rabbit restainers during blood sampling. The blood was collected into tubes containing ethylenediamine tetraacetic acid. The plasma was separated immediately at 10000 rpm for 10 min using cold centrifugation and stored at −20°C until future analysis. The above discussed method was opted for the preparation of HPLC plasma samples and injected into HPLC system and detected by UV detector at 245 nm for the content of flurbiprofen in the formulated tablets.

RESULTS AND DISCUSSION

The aim of this work was focused on to develop and validate a simple, precise RP-HPLC method for estimation of flurbiprofen in formulated dosage forms. Different mobile phases such as acetonitrile and sodium dihydrogen phosphate, acetonitrile and potassium dihydrogen orthophosphate were used in different compositions with different flow rates but peak resolution, tailing factor, retention time were not satisfactory. Finally, potassium dihydrogen phosphate (KH2PO4) buffer and acetonitrile (pH 4.0 ± 0.2) with a volumetric ratio of 55:45 at a flow rate of 1.0 ml/min was selected. The chromatographic separation was done using Phenomenex Gemini C18 column (250.0 × 4.6 mm, 5 µ) with a flow rate of 1 ml/min with a run time of 10 min, at 245 nm [Figure 1].

System suitability and linearity

The system suitability tests were conducted before performing the validation, and the parameters were within the acceptance criteria like % relative standard deviations (RSD) of peak areas of 6 injections were ≤2%, theoretical plate count was >2000, and peak tailing was <2 [Table 1]. Hence, the proposed system is precise and suitable for further analysis. The linearity range of flurbiprofen was in the range of 5-75 µg/ml with a linear regression equation y (flurbiprofen) = 9206x + 5939 (r^2 = 0.999) [Figure 2 and Table 2].

Table 1: System suitability of flurbiprofen

System suitability	Observed value	Acceptance criteria
% RSD for area from five replicates standards	1.04	NMT 2.0
Theoretical plates	8030	NLT 200
Asymmetry	1.08	NMT 2.0

RSD: Relative standard deviations

Table 2: Determination of linearity

Volume taken from standard solution (ml)	Volume make up to in ml with mobile phase	Concentrations (µg/mL)	Level (%)	Peak area
1	100	5	10	45957
2	100	10	20	95436
5	100	25	50	226916
5	50	50	100	456532
3	25	60	120	556516
3	20	75	150	690061

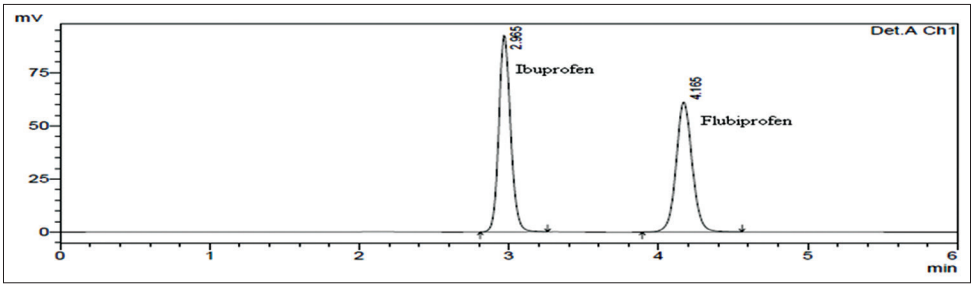

Figure 1: High-performance liquid chromatography of standard solution

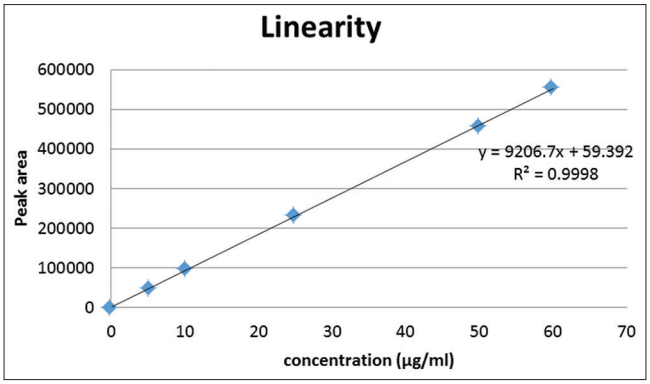

Figure 2: Linearity graph of flurbiprofen

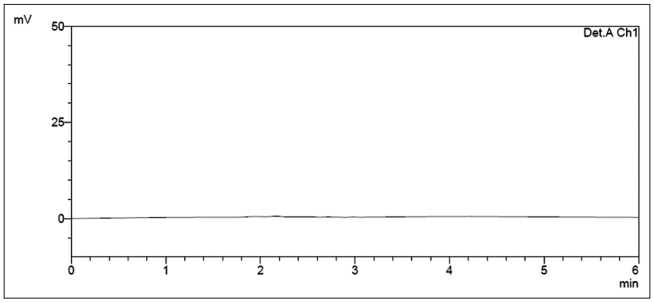

Figure 3: High-performance liquid chromatography chromatograph of plasma

Specificity, LOQ and precision

When plasma and diluent were injected into HPLC system, no interference was observed from the diluent and plasma peaks at the retention time of standard and internal standard peaks [Figure 3]. LOQ of flurbiprofen was calculated using slope and Y-intercept from concentrations ranging 0.5-75 µg/ml, and it was found to be 3 µg/mL from the linearity

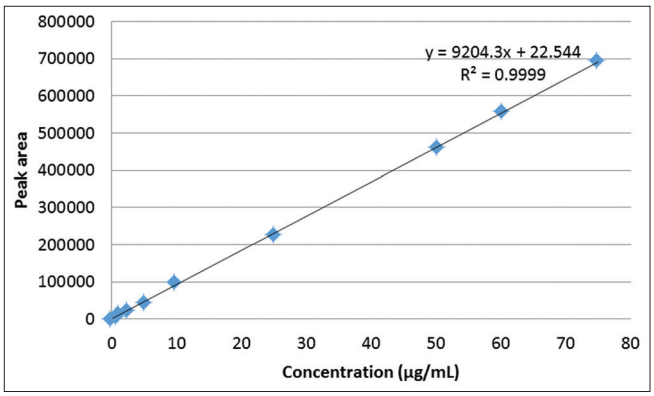

Figure 4: Linearity curve for limit of quantification

(calibration curve) [Table 3 and Figure 4]. Furthermore, the S/N ratio was satisfactory 15 from 1 µg/ml. Six replicates injections of same concentration were analyzed in the precision, and the % RSD of flurbiprofen were within acceptable limits. Hence, the method was precise [Table 4].

Recovery

The percentage recoveries of flurbiprofen from various samples analyzed were found to be between 97.7% and 100.4%. The results of the recovery studies undoubtedly demonstrate the accuracy of the proposed method [Table 5 and Figure 5].

Robustness

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample. It was

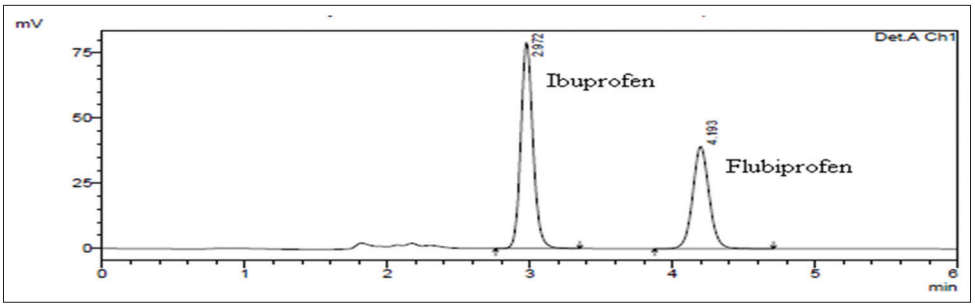

Figure 5: High-performance liquid chromatography chromatograph of flurbiprofen in rabbit blood plasma

Table 3: Determination of LOQ

Concentrations (µg/mL)	Area
0.5	4590
1	9343
2.5	22652
5	45957
10	95436
25	226916
50	456532
60	556516
75	690061

LOQ: Limit of quantification

Table 4: Determination of precision for flurbiprofen

Name of the sample	% Assay	Concentration in µg/mL
Method precision-1	93.52	46.76
Method precision-2	93.78	46.89
Method precision-3	95.3	47.65
Method precision-4	92.18	46.09
Method precision-5	89.34	44.67
Method Precision-6	97.52	48.76
Avg.	93.60667	46.80333
%RSD	2.6	2.96704

RSD: Relative standard deviations

confirmed that by the deliberate changes in the flow rate parameters there was no significant changes in % RSD, theoretical plates and tailing factor [Table 6].

Pharmacokinetic studies

The developed method was successfully applied for pharmacokinetic study in the New Zealand Rabbits and the results are as follows. In case of RRT flurbiprofen was detected within 30 min after its oral administration in rabbits whereas it was more than 5 h in PRT which clearly indicated that there was a predominant lag time before the drug release. The absorption of drug was rapid in case of RRT's, i.e., a C_{max} of 54.2 ± 2.18 µg/ml^{-1} was attained at a T_{max} of 2 h. In PRT's a C_{max} of 45.10±9.71 µg ml^{-1} was attained at a T_{max} of 8 h, no drug was detected till the end of 4th h in all the six rabbits, indicated same absorption pattern as PRT's but after a lag time. The other pharmacokinetic parameters determined are $AUC_{(0-\infty)}$ 352.86 µg ml^{-1} h^{-1}, elimination rate constant 0.052 h^{-1}and half-life of 13.32 h for RRT's. Similarly, for PRT's they were $AUC_{(0-\infty)}$ 324.21 µg ml^{-1} h^{-1}, elimination rate constant 0.047 h^{-1} and half-life of 14.74 h. From the above results, it was clear that there was no major difference in the pharmacokinetic parameters of PRT's and RRT's.

The results of the *in vivo* bioavailability test indicated that drug release from a pulsatile tablet is similar to that of RRT thereby providing prolonged and desired lag time [Figure 6].

Table 5: % recovery of drug sample in the presence of plasma and internal standard

Name of the sample (%)	Area obtained	µg/mL added	µg/ml obtained	% recovery	Mean
50 sample_1	226757	25.01	24.78	99.1	99.0
50 sample_2	225455	25.01	24.84	99.3	
50 sample_3	226555	25.01	24.65	98.6	
100 sample_1	456554	50.88	49.88	98.0	97.9
100 sample_2	456667	50.88	49.76	97.8	
100 sample_3	456565	50.88	49.72	97.7	
150 sample_1	690939	75.63	75.9	100.4	98.8
150 sample_2	690999	75.63	74.17	98.1	
150 sample_3	693383	75.63	74.09	98.0	

Table 6: Validation parameters of evaluated method

Validation parameter	Acceptance criteria	Results		
System precision	%RSD from five replicates of standard NMT 2.0			
Specificity	Plasma shall not show any peak at the retention time of standard and internal standard peaks	No interference was observed hence method is specific		
Method precision	%RSD of % assay results should be NMT 10.0	2.96		
	The % assay of flurbiprofen should not be<85.0 and not more than 115.0	93.0		
Linearity and range	Correlation coefficient (r) should be NLT 0.99	0.999		
LOQ	As per S/N ratio	1.0µg/mL		
Accuracy	The % recovery of flurbiprofen should not be<95.0 and not more than 105.0 for all levels	98.8		
	% RSD for recoveries of 9 determinations should be not more than 10.0	0.60		
Robustness (flow rate)		Lower	As per method	Higher
		0.9 ml/min	1.0 ml/min	1.1 ml/min
	Theoretical plates	6064	8030	6015
	% RSD from five replicate injections of standard NMT 2.0	1.09	1.04	1.11
	Tailing factor	1.01	1.08	1.23

RSD: Relative standard deviations

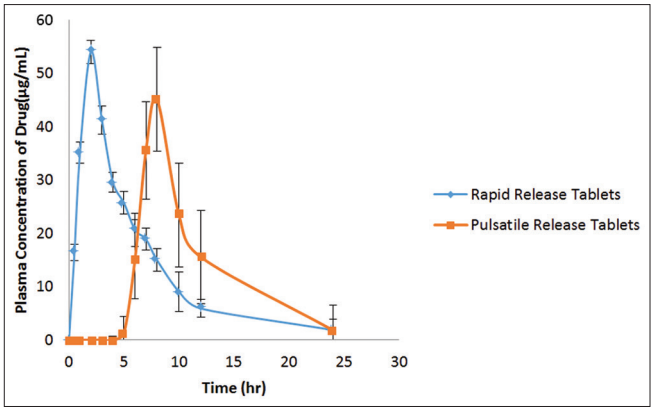

Figure 6: Plasma drug concentration-time curves for pharmacokinetic study in rabbits. All points are presented as mean ± SD, = 6

CONCLUSION

The proposed HPLC method was simple, specific, accurate, and precise. The method was suitable for its intended purpose of determining the content of flurbiprofen from the formulated tablets. The developed method was successfully applied for pharmacokinetic study which revealed that the pulsatile tablets released the drug after a sufficient and specified lag time. Thus the flurbiprofen PRT with desired lag time and time controlled release were developed and evaluated *in vivo* in rabbits, further the tablets should be evaluated in human subjects, which would meet the strategy for chronotherapy of arthritis.

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