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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLURBIPROFEN IN TABLET DOSAGE FORM BY LIQUID CHROMATOGRAPHY

B. Rajani[1,*], G. Bangar Raju[2] and K. Mukkanti[1]

[1]Center for Chemical Sciences and Technologies, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-500085
[2]Department of Chemistry, SRKR Engineering College, Bhimavaram, AndhraPradesh-534202

***Corresponding author e-mail:** rajanibonthu6@gmail.com

ABSTRACT

A simple reversed-phase high performance liquid chromatography (HPLC) method for the determination of Flurbiprofen in pharmaceutical dosage form was developed and validated. The chromatographic separation was achieved on Hypersil BDS (100 x 4.6 mm, 5µ) column. The mobile phase, 0.01 M potassium dihydrogen phosphate buffer and acetonitrile (52:48) were delivered at a flow rate of 1.0 ml/min. The eluent was monitored using PDA detection at 246 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention time for Flurbiprofen was 3.1 min. The calibration graph for Flurbiprofen was linear from 12.5 to 75 µg/mL. The interday and intraday precisions (relative standard deviation) were less than 1.0. The method can be employed for routine quality control of Flurbiprofen tablets in quality control laboratories and pharmaceutical industries.

Key words: Flurbiprofen, HPLC, Validation

INTRODUCTION

Flurbiprofen is a potent non-steroidal anti-inflammatory drug (Figure 1). It has been effectively used in the treatment of rheumatoid arthritis, osteoarthritis, gout, sunburn, ankylosing spondylitis, soft tissue trauma, acute tendonitis and bursitis, primary dysmenorrhea, and bone pain in cancer patients[1-5]. Because of its clinical advantages, there is an increase in the number of Flurbiprofen formulations in the market for a variety of indications. Therefore, there is a need for a sensitive and reliable analytical method for the estimation of Flurbiprofen in pharmaceutical formulations. A single method that can be used for the estimation of a variety of formulations will give an additional advantage. UV spectroscopic and high-performance liquid chromatography methods (with electrospray tandem mass spectrometry, UV and fluorescence detectors) were reported for the estimation of Flurbiprofen in biological fluids, such as plasma, serum, and urine[5-10]. The present study was aimed at developing a sensitive reverse-phase HPLC method suitable for routine and selective analysis of Flurbiprofen in a variety of commercially available pharmaceutical preparations as per ICH guidelines[11].

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Alliance waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and convenient for LC with class Empower-2 software.

Reagents and chemicals: The reference sample of Flurbiprofen was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was

used throughout the study. Commercial tablets (Arflur-50, FDC Limited) were purchased from the local pharmacy.

Chromatographic condition: The mobile phase consisted of phosphate buffer and acetonitrile was taken in ratio of 52:48 at a flow rate of 1.0 mL/min. Hypersil BDS, (100 x 4.6 mm, 5m) was used as the stationary phase. 246 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution:
Accurately Weighed and transferred 10 mg of Flurbiprofen working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents.

***Preparation of Working Standard Solutions*:** Aliquot of 0.125, 0.25, 0.375, 0.5, 0.625 & 0.75 mL were pipette out from stock solution into 10 mL volumetric flask and volume was made up to 10 mL with diluent. This gives the solutions of 12.5, 25, 37.5, 50, 62.5 and 75µg/mL for Flurbiprofen.

***Preparation of phosphate Buffer*:**
Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degass to sonicate and finally make up the volume with water, then pH adjusted to 4.8 with dil. Ortho phosphoric acid solution.

Sample preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 30mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation: Parameters such as systems suitability, Linearity, accuracy, specificity, LOD & LOQ and robustness were performed according to the ICH guidelines.

RESULTS AND DISCUSSION

Method development:
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which the drug did not responded properly. The organic content of mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, phosphate buffer: acetonitrile were taken in isocratic ratio: 52: 48 and with flow rate of 1.0 mL/min was employed. Hypersil BDS, (100 x 4.6 mm, 5m) was selected as the stationary phase to reduce the tailing of the peak. 246 nm was selected as the detection wavelength for PDA detector. The retention time was found to about 3.1 min and the results were shown in Table 1 and Figure 2.

Method Validation:
System suitability: A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 3. The analytical method validation was carried out as per ICH method validation guidelines.

Linearity: The linearity range was found in the range of 12.5-75 µg/mL. The response for the drug was linear and the regression equation was found to be Y=51556X+18942 and correlation coefficient was found to be 0.9999 and the results are given in Table 2 and Figure 3.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intra-day precision and inter-day precision.

Intra-day precision: To study the intra-day precision, six replicate standard solutions of Flurbiprofen were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.86 which are well within the acceptable criteria of not more than 2.0.

Inter-day precision: To study the inter-day precision, six replicate standard solutions of Flurbiprofen were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.2 which are well within the acceptable criteria of not more than 2.0.

Specificity: The effect of wide range of excipients and other additives usually present in the formulation of Flurbiprofen in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Flurbiprofen.

Limit of detection and limit of quantification: A calibration curve was prepared using concentrations in the linearity range (expected detection limit range).

The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Flurbiprofen were 0.17 and 0.52 µg/mL, respectively (Table 3).

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD was found to be 0.45. Satisfactory recoveries ranging from 99% to 101% were obtained by the proposed method. This indicates that the proposed method was accurate.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Tablet Analysis*:* The Content of Flurbiprofen in the tablets was found by the proposed method. RSD values for Flurbiprofen are found to be 0.301 and results were shown in table.4.

CONCLUSION

A new precise accurate and simple HPLC method was developed and validated for the estimation of Flurbiprofen in tablet dosage form. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of Flurbiprofen tablets in QC laboratories and industries.

Figure 1: Structure of Flurbiprofen

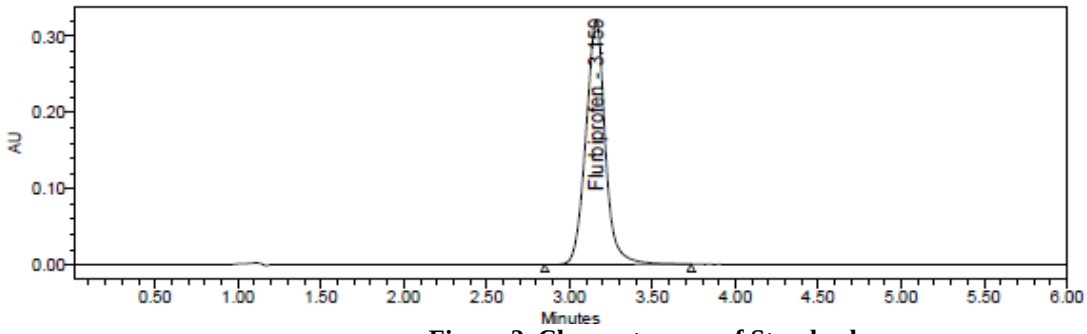

Figure 2. Chromatogram of Standard

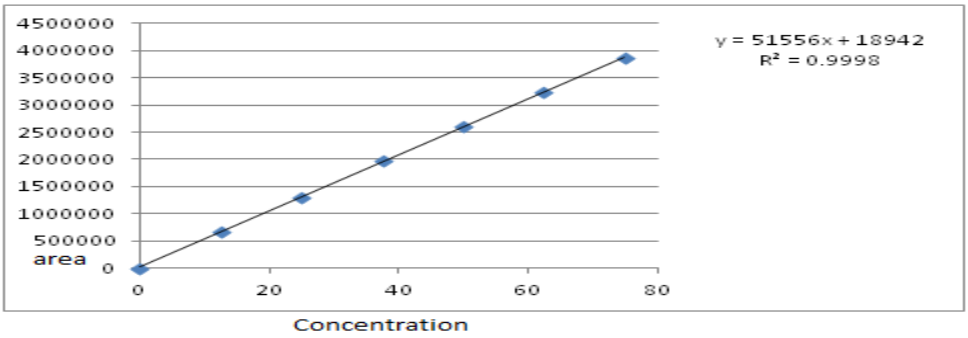

Figure 3. Linearity curve

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Buffer:Acetonitrile (52:48)
2	pH	4.8(+/-0.5)
3	Diluent	Methano:Water(50:50)
4	Column, make	Hypersil BDS, Thermo Electron Corporation
5	Column temperature	30^0C
6	Wave length	246nm
7	Injection volume	10ul
8	Flow rate	1.0ml/min
9	Run time	6mins
10	Retention time	3.07mins

Table 2: Linearity results

S. No.	Concentration in µg/mL	Area
1	12.5	667472
2	25	1304815
3	37.5	1974285
4	50	2615352
5	62.5	3243424
6	75	3860738

Table 3: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (µg/mL)	12.5-75 µg/mL
2	Correlation coefficient	0.999
3	Theoretical plates (N)	4822
4	Tailing factor	1.05
5	LOD (µg/mL)	0.17 µg/mL
6	LOQ (µg/mL)	0.52 µg/mL
7	Regression Equation	Y=51556+18942

Table 4: Assay results

S. No.	Formulation	Label claim	Amount found	%Assay
1	**Flurbiprofen**	50 mg	49.94mg	100.1%

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