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RESEARCH PAPER

STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF NSAIDS BASED FORMULATION

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A simple, precise, and accurate RP-HPLC method has been developed to estimate flurbiprofen in nanoparticles. The chromatographic separation is achieved using Phenomenex Luna C18 (250 x 4.60 mm, 5µm) analytical column and the mobile phase consisting of acetonitrile and pH 5.6 phosphate buffer 63:37% (v/v) pH adjusted to 5.6 using orthophosphoric acid at a flow rate of 1.0 ml min^{-1}. The UV detection is carried out at 248nm using a photodiode array detector. The retention time of flurbiprofen is found to be 9.6 min. The proposed method has been validated for specificity, linearity, accuracy, precision, ruggedness, and robustness. Linear calibration curves were obtained in the concentration of 5-25 µg ml^{-1} for flurbiprofen. The skin permeation profile of flurbiprofen nanoparticles shows that the transdermal delivery of anti-inflammatory drugs can give better treatment compared to conventional gels; hence, the developed method can be used for the routine analysis of flurbiprofen nanoparticles.

Key words: Flurbiprofen, RP-HPLC, NSAIDs, Nanoparticles, Retention time, Validation.

INTRODUCTION

Flurbiprofen, chemically [(±)-2-(2-fluorobiphenyl-4-yl) propionic acid], is a potent racemic nonsteroidal anti-inflammatory and analgesic drug used in treatment of gout, osteoarthritis, rheumatoid arthritis, and sunburns [1]. Flurbiprofen was one of the least permeable drugs across the skin among a series of lipophilic drugs. Aryl propionic NSAIDS, like flurbiprofen, can antagonize pupillary constriction during intraocular surgery by inhibiting the cyclooxygenase through polymorphonuclear leukocyte infiltration reduction, preventing myosis-induced surgical trauma during cataract extraction [2]. Although flurbiprofen has potent pharmacological activities after oral administration, it also has adverse effects secondary to prostaglandin inhibition, gastropathy, prolonged bleeding time, fluid retention, and acute renal insufficiency [3]. The half-life of flurbiprofen in plasma is about 6 hours and can be used as an in vivo probe for CYP2C9 activity. The major metabolic process is hydroxylation to form 4-hydroxy flurbiprofen [4-7].

Nanotechnology has been applied extensively in medicine, offering novel approaches to illness diagnosis and treatment. Nanoparticles, which are colloidal particles with a diameter of 10–1000 nm, have the potential to improve drug solubility and absorption, lessen the need for frequent dosages, and facilitate the enhanced bioavailability [8]. The capacity of nanomedicines to encapsulate medications or affix therapeutic chemicals to target tissues with controlled release is becoming more widely acknowledged [9]. Drug distribution, gastro-

intestinal harm prevention, and therapeutic practice are all facilitated by nanostructures. For effective medication distribution that improves the treatment of the disease, polymeric nanoparticles are perfect. To maximize performance, limit toxicity, and guarantee efficacy and safety in therapeutic applications, nanoparticle design includes regulating size and shape, surface functionalization, core stability, biocompatibility, release kinetics, targeting, and accumulation [10, 11]. Pharmaceutical drug development depends on the stability of drug nanoparticles, which necessitates the choice of suitable stabilizers. The stability of nano-formulations can be increased by combining surfactants, proteins, amino acids, dendrimers, polymers, plant extracts, and cryoprotectants. Nonetheless, researchers continue to concentrate on convenient, regulated, monitored, and targeted medication delivery methods [12]. Affordability, gender-sensitive medication delivery, symbiotic drug delivery, safety, and more environmentally friendly drug delivery methods are all crucial [13].

Literature survey reveals that few methodologies and techniques using different instruments and matrixes had been reported previously for the analysis of flurbiprofen as HPLC- UV assays for its determination in biological material [14, 15] HPTLC [16], primary metabolite determination using HPLC fluorescence system from serum [17], pre-column derivatization for the combined analysis of flurbiprofen, ketoprofen and etodolac in human plasma using RP- HPLC [18], HPLC analysis in rat plasma [19], LC-MS/MS determination in human plasma using hydrophilic polymer column and chiral phase liquid chromatographic stereospecific analysis of major metabolites [20]. However, this is the first time reporting the estimation of flurbiprofen in nanoparticles using RP-HPLC.

EXPERIMENTAL

Chemicals

Methanol (HPLC Grade) was purchased from SD Fine Chemicals Rade) were purchased from Qualigens Fine Chemicals, Mumbai. Milli Q water was used throughout Ltd, Mumbai, potassium orthophosphate (AR Grade), and potassium hydroxide (AR Grade).

Equipment

Analysis was performed on a chromatographic system of Shimadzu Prominence consisting of an LC 20 AD liquid pump. Chromatographic separation was achieved on Phenomenex Luna C18 (250 × 4.60 mm, 5 µm) analytical column. Data acquisition was made with LC solution v.1.24 Spinchrome-1 software. The peak purity was checked with the photodiode array detector.

Preparation of the standard

Flurbiprofen (25 µg/ml) USP standard stock solution was prepared by transferring 100.0 mg of flurbiprofen to a 100 ml volumetric flask containing 10 ml of methanol. It was then, sonicated for 15 min. The solution was diluted up to volume using Milli Q water; appropriate dilutions were made to prepare a 25 µg/ml solution using Milli Q water.

Preparation of flurbiprofen nanoparticles

Nanoparticles were prepared by the solvent evaporation method involving poly lactide co-glycolide polymer and Tween 20 surfactant using a magnetic stirrer with stirring for 12 h. The obtained particles were centrifuged at 12000 rpm using a cooling centrifuge.

In-vitro skin permeation studies

The permeability of flurbiprofen through goat skin (purchased at the local market) was measured *in vitro* with a Franz diffusion cell system after removing hair, subcutaneous fat, and connective tissue. The excised skins were washed with 0.9% saline solution and then used for permeation studies. Analysis was performed by using Franz diffusion cells with an effective diffusion area of 3.14 cm^{-2}. The excised skin samples were clamped between the donor and receiver compartments of the Franz diffusion cell; the stratum corneum side faced the donor compartment, and the dermal side faced the receiver compartment [21, 22]. The 1 ml of prepared nanoparticle was administered on the stratum corneum. The receptor chamber was filled with 34 phosphate buffer, pH 7.4. The diffusion cell was thermostated at 37 °C and stirred at 600 rpm using a magnetic stirrer throughout the experiment. Samples of 2 ml aliquots were drawn from the receiver compartment and replaced immediately with an equal volume of fresh phosphate buffer pH 7.4 at 30 mins and 1,2,3,4,5,6,7,8,12, and 24 h, analyzed by RP-HPLC.

Online HPLC analysis of permeate samples

The chromatographic separation was achieved on a Shimadzu Prominence consisting of LC 20

AD liquid pump, a Phenomenex C18 column (250 × 4.6 mm, 5 µm), and using acetonitrile-phosphate buffer (pH 5.6) 62:38% (*v/v*) as mobile phase. The pH of the buffer was adjusted with orthophosphoric acid to pH 5.5; the pH of the buffer was selected according to the pKa value of flurbiprofen. The eluate was monitored at 248 nm. The flow rate of 1.0 ml/min was kept to achieve an adequate retention time of 9.6 min.

Skin deposition study

To evaluate the loss of drug over dermal layers the skin deposition test at the end of the permeation study was determined by cutting the effective permeation areas of skins, which were then washed with 40 % aqueous methanol to remove surface adhered materials, wiped, and dried at 40 °C for 12 h, homogenized in 3 ml of methanol: Water (2:1 *v/v*), centrifuged at 5000 RPM for 10 mins, 0.5 ml of clear supernatant was processed and analyzed.

Encapsulation efficiency

To 0.2 ml of flurbiprofen nanoparticle containing (1.1 mg/ml), pipetted using a micropipette, was added 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath. The flurbiprofen-containing nanoparticles were separated from the untrapped drug by centrifugation at 5,000 rpm at 37 °C for 30 min. The supernatant was recovered and assayed by HPLC for flurbiprofen content.

The percentage of drug encapsulation (EP (%) was calculated by the following equation:

$$EP\ \% = [(C_t - C_r)/C_t] \times 100\ \%$$

where Ct is the theoretical amount of flurbiprofen added, and Cr is the amount of flurbiprofen detected only in the supernatant.

Optimization of the chromatographic conditions

The chromatographic conditions were optimized by using different buffers, like phosphate, acetate, and citrate, for mobile phase preparation. After a series of screening experiments, it was concluded that phosphate buffer gave better peak shapes than its acetate and citrate counterparts. The chromatogram obtained with acetonitrile is better than methanol, with a good resolution between the surfactant peak and flurbiprofen peak. Furthermore, the reported pKa value of flurbiprofen was 4.22, and solubility at pH 6.0 was 0.4 mg/ml, making it suitable to select the buffer pH of the mobile phase [23].

Method validation

The optimized chromatographic conditions were validated by evaluating linearity, method and system precision, robustness, intra-day, inter-day variability, and solution stability by ICH guideline Q2 (R1) as shown in **Table 1**. The robustness of the method was determined by purposely altering the final experimental conditions, like flow rate and pH of the mobile phase. The nominal concentrations of flurbiprofen were 5 and 25 µg/ml. The response function was determined by preparing a standard solution at five different concentration levels using different analysts on two different days. The precision of this method was determined by injecting the standard solution of the analytes five times [7]. The robustness of the method was calculated by a deliberate change in chromatographic conditions, i.e. change in flow rate by ±0.1 ml/min, and a change in pH of the buffer by ±0.1 unit [24, 25].

Short-term stability studies of the formulation

The physical stability of the flurbiprofen nanoparticles was evaluated after storage for 2 days under different temperature conditions. Exact volumes of each nanoparticle were stored in closed glass bottles and placed at room temperature or 4-8°C (refrigerator) away from direct light [26]. Aliquots were withdrawn at 2 days to determine their stability.

RESULT AND DISCUSSION

Linearity was determined in the range of 5 to 25 µg/ml [7]. The correlation coefficient 'r^2' values were found to be 0.9867, as shown in **Figure 1**. Typically, the regression equation for the calibration curve was found to be y = 95831x - 130387 (**Table 2**). The USP theoretical plate was found to be greater than 6000, and the tailing factor was found to be around 1.0. In peak purity analysis with a photodiode array detector, the purity angle was less than the purity threshold. A typical chromatogram showing flurbiprofen peak at the retention time of 9.6 is shown in **Figure 5**. The %RSD of peak area for the system, method precision, intra-inter-day variability, analyst-analyst variability, and robustness was found to be less than 3.0 % as shown in **Table 1**. By comparing the peak areas at different time intervals, the developed nanoparticles were observed to be stable in nature.

Table 1. Method validation parameters of flurbiprofen nanoparticles

S. No.	Precision and variability	% RSD	Robustness	Conditions changed	% RSD*	Solution stability (room temp)	Peak area
1	Method precision	1.17	Altered Flow (ml min^{-1})	0.9	0.38	0 h	4902549
2	Inter day	1.16		1.0	2.50		
3	Intra day	0.8		1.1	2.25	12 h	4671498
4	Analyst-1	4.32	Altered Buffer pH	pH 5.4	1.90		
5	Analyst-2	1.54		pH 5.5	2.50	24 h	4440862
				pH 5.6	2.40		

**% RSD- Relative standard deviation for peak area*

Table 2. System suitability and linearity parameters of flurbiprofen nanoparticles

S. No.	System suitability	Flurbiprofen peak	Linearity	Flurbiprofen peak
1	Tailing factor	0.98	Slope	95831
2	Theoretical plates	61629	Intercept	130387
3	Retention time	9.6	r^2	0.9867
4	Peak purity index	1.0		
5	Single point threshold	0.99		

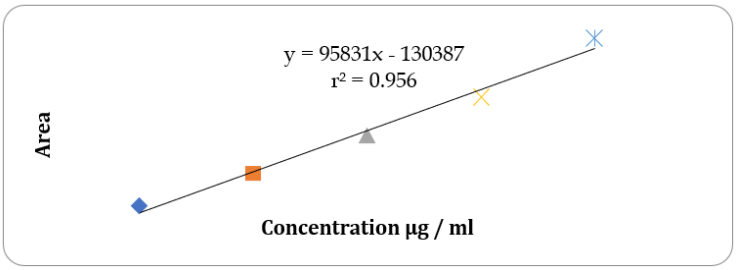

Fig. 1. Linearity of flurbiprofen

The results of skin permeation studies indicate that the F3 formulation showed a better permeation of 94.0 % permeation during 24 h compared to conventional formulation and formulations 1 and 2, respectively.

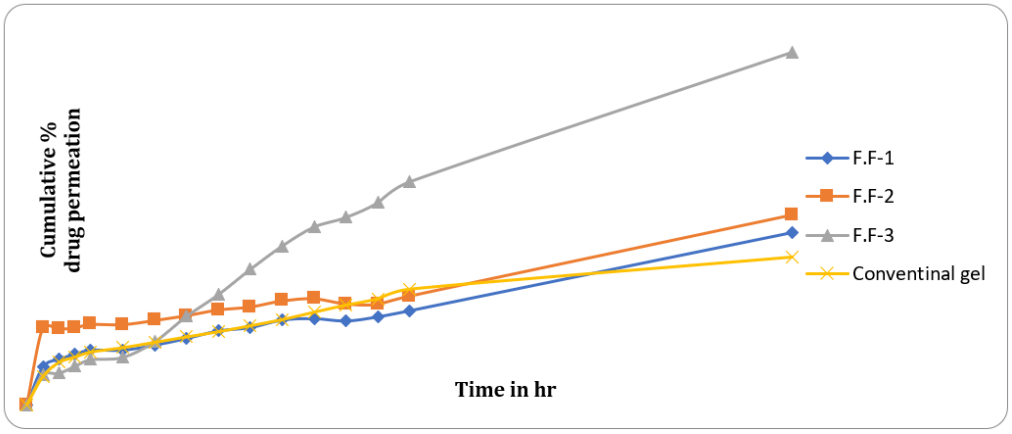

Fig. 2. Representative skin permeation of flurbiprofen nanoparticle

HPLC/other analysis for flurbiprofen

NSAIDS like flurbiprofen generally exhibit high protein binding. Protein leakage at the inflammatory site may be due to drug residence time at the diseased site; hence, this present experimental model will be useful to evaluate

flurbiprofen disposition in the skin [27]. The surfactant used in this formulation to lower the interfacial tension creates problems in UV analysis, giving a minimum absorbance in the blank formulation so blank correction is desirable at every time interval to avoid this nominal difficulty the method is shifted to HPLC, here tween 20 gives a sharp peak at the retention time of 4.9 (**Figure 3**) thus this peak was neglected for calculation only drug component peak at RT 9.6 (**Figures 3, 4**) taken into consideration producing an accurate result. The peak purity profile of flurbiprofen is illustrated in **Figure 5**.

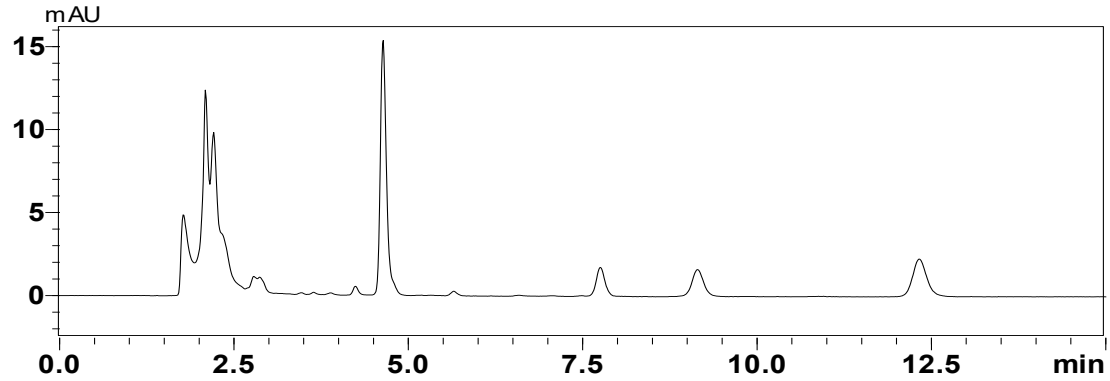

Fig. 3. A typical chromatogram of blank formulation

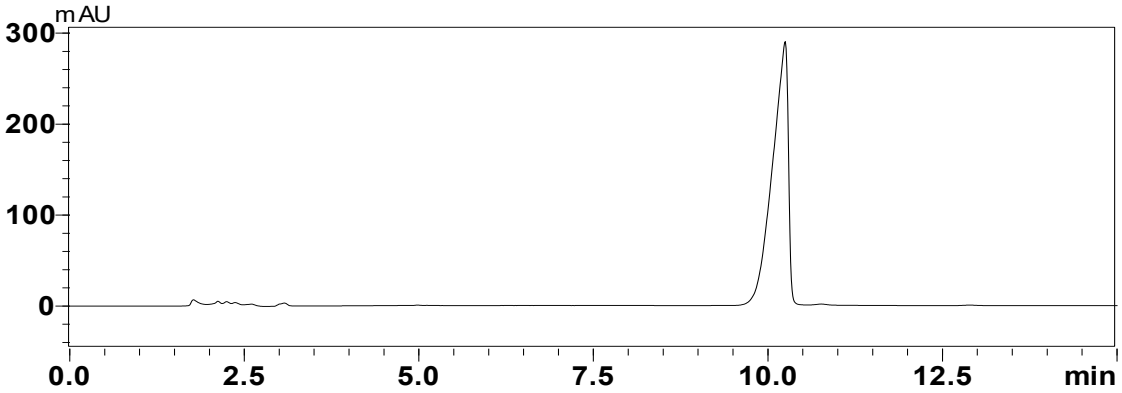

Fig. 4. A typical chromatogram of flurbiprofen standard solution (25 µg/ml)

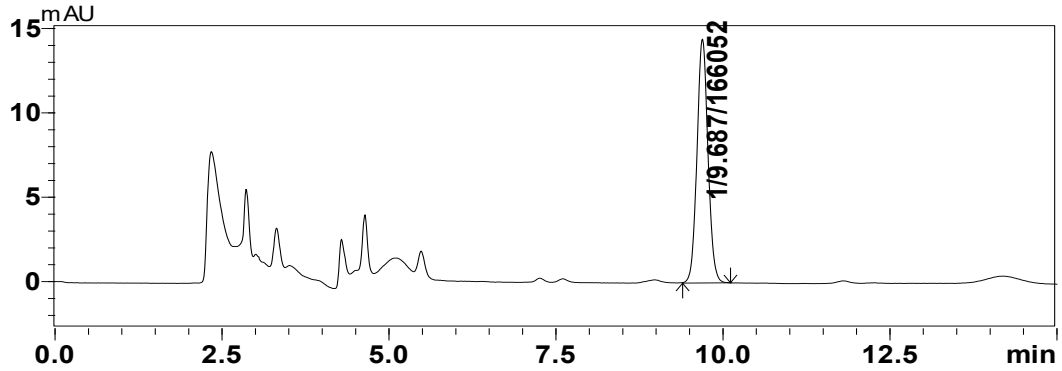

Fig. 5. A typical 12 h permeation chromatogram of flurbiprofen nanoparticle

The HPLC procedure was optimized for flurbiprofen-loaded Eudragit L100 nanoparticles using the Central Composite Design (CCD). The technique was created with a stationary phase and an auto-injector. The analytes were water, methanol, orthophosphoric acid, and aceto-nitrile, and the column measured 4.6 × 250 mm. With positive outcomes and a correlation coefficient of 0.994, the method was validated [28]. To identify flurbiprofen in human plasma, a high-performance liquid chromatography - HPLC approach was developed. Tested on an Ace C18

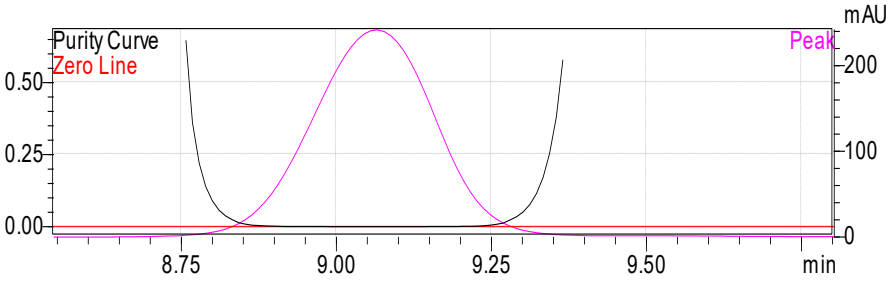

Fig. 6. Peak purity profile of flurbiprofen

column, the method's accuracy is better than 3.67% and its precision values are less than 4.47 [29]. The flurbiprofen calibration curve had a mean recovery of 97.07±0.008 to 103.66±0.013 and was linear and accurate (r ≥ 0.9996). Dissolution tests of flurbiprofen controlled release matrix tablets validated the method's practicality. It is appropriate for routine quality control analysis since it is simple, and has a short run-cycle time, high specificity, and accuracy [30]. To identify flurbiprofen in pharmaceutical dosage form, Rajani et al created and validated a reversed-phase high performance liquid chromatography (HPLC) approach. A Hypersil BDS column and a mobile phase consisting of acetonitrile and 0.01 M potassium dihydrogen phosphate buffer are used in the procedure. System appropriateness, linearity, accuracy, precision, specificity, limit of quantification (LOQ), limit of detection (LOD), stability of sample and standard stock solutions, and resilience are some of the validation factors. Flurbiprofen's calibration graph is linear from 12.5 to 75 µg/ml, and its retention duration is 3.1 mins. Flurbiprofen tablet quality can be regularly checked using this method at pharmaceutical companies and quality control labs [31].

CONCLUSION

A simple, reproducible, linear, precise, affordable, and accurate RP-HPLC method has been developed and validated to estimate flurbiprofen in nanoparticles. The method is very specific as the flurbiprofen peak is well separated from its interfering peak due to polymer and surfactant used in the formulation, with a short chromatographic time course of 13 mins. This analytical method enables quantification of flurbiprofen ranging from 2.5 to 25 µg/ml, with acceptable precision, linearity, inter-day and intra-day precision, which is considered to be a promising technique that has an obvious advantage as compared to the conventional analytical techniques for routine quality control analysis work.

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