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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLURBIPROFEN IN TOPICAL FORMULATION BY LIQUID CHROMATOGRAPHY

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Abstract: A simple, rapid, sensitive, and specific high-performance liquid chromatography (HPLC) method was developed and validated for quantification of flurbiprofen in a topical gel developed and formulated in our laboratory. RP-HPLC analysis was performed using Jasco HPLC system, equipped with a UV-2077 PLUS detector. The procedures for the determination of flurbiprofen were carried out on Inertsil ODS 3V C18 (5µm; 150mm × 4.6 mm), column in an isocratic mode. The mobile phase consisting Potassium dihydrogen phosphate buffer of pH 6.8**:** ACN in the ratio of 68:32 at a flow rate of 1ml/min and effluents were monitored at 247 nm. The retention time of flurbiprofen was found to be 3.55 mins respectively. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) and robustness were studied as reported in the International Conference on Harmonization guidelines.Calibration curves was linear with a correlation coefficient of 0.999 over the concentration range of 5-30 µg/ml and precise (with % RSD < 2).It is suggested that the method should be used for the routine quality control analysis of flurbiprofen in topical dosage forms.

Keywords: Chromatography, flurbiprofen, RP-HPLC, quality control, validation

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INTRODUCTION

Flurbiprofen is chemically 2-(3- fluoro-4-phenylphenyl) propanoic acid and is a potent non steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic activity [(1)]. It has been effectively used in the treatment of rheumatoid arthritis, osteoarthritis, gout, ankylosing spondylitis, soft tissue trauma, acute tendonitis and bursitis, primary dysmenorrhea[(2)]. Flurbiprofen is official in BP[3] and USP[4]. Literature survey reveals the availability of several analytical methods being developed and validated for flurbiprofen alone or in combination with other drugs [(5-8)]. Various methods have been developed either for bulk or in oral dosage form. But no analytical method has been reported for estimation of flurbiprofen in topical gel. The present study reports a simple, rapid, specific, precise, and validated HPLC method and is applicable for quantification of flurbiprofen in a topical gel developed and formulated in our laboratory.

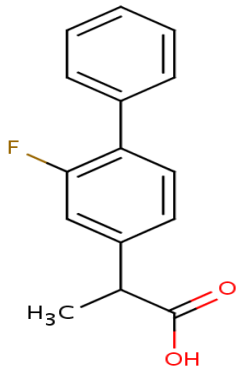

Figure 1: Structure of flurbiprofen.

MATERIALS AND METHODS:

Instrumentation:

Chromatography was performed on Jasco Binary system with, two PU2080 PLUS intelligent HPLC pumps, UV2075 PLUS intelligent UV detector, Solvent Mixing module MX-2080-31, Rheodyne® manual injector system, LCNet II / ADC system interface and Borwin® Chromatography Software, Jasco Corporation, Japan and 5µm Inertsil ODS 3V C18 column having dimensions of 150mm × 4.6 mm id was used.

Reagents and solvents:

The reference sample of Flurbiprofen was provided as gift sample from FDC Ltd. Mumbai. HPLC grade Acetonitrile, and all other chemicals purchased from S.D. Fine Chemicals (Mumbai, India). HPLC grade water obtained from Milli-Q water purification system was used throughout the

study. Whatman filter paper No. 42 was used for filtration. Topical gel developed and formulated in our laboratory is used for analysis.

Chromatographic parameters:

Equipment: HPLC equipped with UV detector.

Column: Inertsil ODS 3V C18 column (150 x 4.6 mm, 5 µ)

Flow rate: 1.0 ml/min

Mobile phase: Potassium dihydrogen phosphate buffer of pH 6.8**:** ACN (68**:**32)

Wavelength: 247 nm

Injection volume: 20 µl

Preparation of mobile phase:

Buffer Solution: 6.8gm of potassium dihydrogen orthophosphate was weighed and dissolved in 1000ml double distilled water. The pH 6.8 was adjusted by using 1M sodium hydroxide and orthophosphoric acid. Then this solution was passed through 0.45µ membrane filter. (Ultipore N –66R Nylon 66; Pall Corp.,).

The mobile phase was prepared by mixing buffer solution and acetonitrile in ratio of 68**:**32. It was filtered through 0.45 µm membrane filter and degassed by ultrasonicator for 15 min.

Preparation of analyte solution

1) Preparation of standard stock solution

Stock solution was prepared by dissolving 10 mg of flurbiprofen standard in 100ml of mobile phase separately to get a solution containing 100µg/mL for flurbiprofen. The working standard solution was prepared by diluting 1mL stock solution to 10mL with mobile phase to obtain a solution containing 10µg /mL flurbiprofen.

2) Preparation of sample solution

200 mg of gel was weighed and transferred to a 100 ml volumetric flask. Small quantity of mobile phase was added, and the solution was vortexed and sonicated for 15 minutes with intermittent swirling to get uniform dispersion and the volume was made up to 100ml with mobile phase to obtain sample stock solution of concentration 100 µg/mL. The solution was filtered through 0.45 µm PTFE filter. The working sample solution was prepared by diluting 1 mL

stock solution to 10mL with mobile phase to obtain a solution containing 10/µg mL flurbiprofen.

METHOD VALIDATION [9-12]

The method was validated for system suitability, linearity, accuracy, precision, robustness and limit of detection and quantification in accordance with ICH guidelines.

System suitability:

System suitability testing is performed to ensure system performance before and during analysis, which demonstrates that system is operating properly and is ready to deliver results with acceptable precision. The tests were carried out on freshly prepared standard solution of flurbiprofen using five replicate injections.

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. Specificity of the method was demonstrated by injecting the blank solution, standard solution and sample solution the responses were determined.

Linearity:

The linearity of the method was investigated by using concentrations of 5 to 30µg/mL. Analysis was performed in triplicates. These were injected in the HPLC system and chromatograms recorded. The peak area for flurbiprofen was calculated and respective calibration curves were plotted against concentration.

Limit of detection and quantification:

The detection limit (DL) and Quantification Limit (QL) were calculated from the linearity graph using the formula,

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = standard deviation of the response (peak area).

S = slope of calibration curve.

Precision:

Intermediate precision (inter-day precision) and repeatability (intra-day precision) of the method was verified by analyzing the six different samples at 100 % concentrations as per test method on different day. The peak area was recorded and relative standard deviation (RSD) was calculated for both series of analysis.

Accuracy:

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels viz. 80%, 100% and 120%. The percentage recoveries were calculated.

Robustness:

Here, the influence of small but deliberate variations of the analytical parameters was examined. The following three factors were selected for change:

i) Flow rate of the mobile phase (1.0 ± 0.1 ml/min),

ii) Change in composition of mobile phase (ACN**:** Buffer (±) 2%)

RESULTS AND DISSCUSSION:

Method development:

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of acetonitrile and water were used in which drug did not responded properly. The choice of buffer was done based on pKa value of drug i.e. 4**.**22 so buffer range from pH 4.8-7.3 selected to work on. The acetonitrile and various phosphate buffers are tried (i**.**e**.** pH 4**.**8, 6**.**8, 7**.**3) as mobile phases.

Our objective of the chromatographic method development was to achieve a peak tailing factor < 2, retention time in between 2 and 12 min, along with good resolution. This objective was obtained using mobile phase potassium dihydrogen phosphate buffer (pH 6.8)**:** ACN (68**:**32v/v) and with the flow rate 1mL/min. Inertsil ODS 3V C18, (150×4.6 mm, 5µm) was selected as the stationary phase on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time. Quantification was achieved with UV detection at 247 nm. The retention time was found to about 3**.**55 min. Representative chromatograms of standard flurbiprofen and flurbiprofen in topical gel are shown in **Figure 2** and **Figure 3** respectively.

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 1. The analytical method validation was carried out as per ICH method validation guidelines.

Linearity, Detection and Quantitation Limits: Calibration curve representing the relation between the concentrations of drugs versus the peak area was constructed. Results show linear relationship in the range of 5-30 µg /ml. The response for the drug was linear and the regression equation was found to be y=133514x - 13578 and correlation coefficient was found to be 0.9999 the results are given in Table 2 and Figure 4. The LOD was found to be 0.3µg/mL for flurbiprofen and LOQ was found to be 0.8µg/mL. These values indicate that the method is sensitive.

Precision: In the precision studies, RSD of mean assay values was found to be 0.722% for flurbiprofen. These %RSD values which are well below 2% indicate that the repeatability of this method is satisfactory. Thus there exists a closeness of agreement in repeated measurements of peak response. The intermediate precision study revealed that the method is rugged with %RSD values of 0.5214 for performing the analysis by performing the analysis on another day respectively. As evident the RSD values of the data obtained are well below 2% indicating that method can be repeated successfully on different day. (Table 3,4 & 5)

Accuracy: Accuracy studies indicated that the percent recoveries were obtained from the difference between the areas of spiked and unspiked samples. The mean recovery of the added standard drug was 99.21%. This means recovery value is well within the range of 98-100%, indicating the method is accurate. (Table 6)

Specificity: Specificity studies indicated good resolution was obtained between the drugs and excipients showing complete separation of flurbiprofen. No interference from excipients, impurities, or degradation products ensured that the peak response was due to flurbiprofen only. Good resolution was obtained between the drugs and excipients showing complete separation of flurbiprofen.

Robustness: Robustness studies signified that the results of the method remained unaffected by small, deliberate changes in the flow rate and mobile phase composition. The RSD of mean assay values was found to be 0.77 % with a flow rate of 0.9 mL/min. The RSD of mean assay values was found to be 0.50% with a flow rate of 1.1 mL/min. Also, RSD of mean assay values was found to be 1.01% at mobile phase composition of 66.64:32 v/v and 0.80% at mobile phase composition of 69.36:32 v/v. The RSD values of the data obtained are well below 2% indicating

that method is robust i.e. it is reliable during normal use. All the validation data are summarized in Table 7.

The assay results obtained by using the proposed method for the analysis of developed gel formulation containing flurbiprofen (5%w/w) were in good agreements with the labeled amounts of flurbiprofen. The average contents of flurbiprofen in developed formulation were 99.21%.

Table 1: System suitability parameters [flurbiprofen (10 µg/ml)].

Parameters	Results
Theoretical Plate counts±%RSD	2132.6±1.39
Peak asymmetry	1.27
Retention time (min) (n=5)	3.55
SD of retention time	0.03
% RSD of retention time	0.55
Average peak area (mAU) (n=5)	1382712
% RSD of peak area	0.38

Table 2: Linearity of flurbiprofen

Concentration (ppm)	Mean response (peak area)
5	648567.2
10	1336656
15	1986763
20	2646287
25	3318947
30	4000250

Correlation Coefficient	0.9999
Regression equation	y = 133514x - 13578
Slope	133514
Y-intercept	-13578

Table 3: Precision of flurbiprofen in topical formulation.

Drug	Sample 1	Sample 2	Sample3	Sample4	Sample5	Sample 6	Mean ±%RSD
Flurbiprofen (% Assay)	98.12	97.55	97.88	98.64	99.50	98.84	98.42 ± 0.72

Table 4: The intermediate precision (day change) of flurbiprofen in topical formulation.

Drug	Sample 1	Sample 2	Sample3	Sample4	Sample5	Sample 6	Mean ±%RSD
Flurbiprofen (% Assay)	99.28	98.21	98.64	98.12	98.66	97.83	98.46 ± 0.52

Table 5: Precision for flurbiprofen on Day 1 and Day 2.

Day	% Assay
1	98.42
2	98.46
Mean	98.44
SD	0.02
%RSD	0.02

Table 6: Recovery studies of flurbiprofen in topical formulation

Drug	Recovery Level(%) (n=3)	%Recovery ±%RSD (%)	Mean Recovery ±%RSD (%)
Sample	80	99.05±1.36	99.21 ± 0.33
	100	98.99±0.93	
	120	99.59±0.65	

Table 7: The robustness of flurbiprofen in topical formulation

Conditions	Plate number	Asymmetry	% Assay	%RSD of Assay
Flow rate(-10%)	2165	1.01	98.40%	0.77
Flow rate(+10%)	2048	1.00	97.91%	0.50
ACN: Buffer (- 2%)	2055	1.01	99.99%	1.01
ACN: Buffer (+2%)	2155	1.03	100.51%	0.80

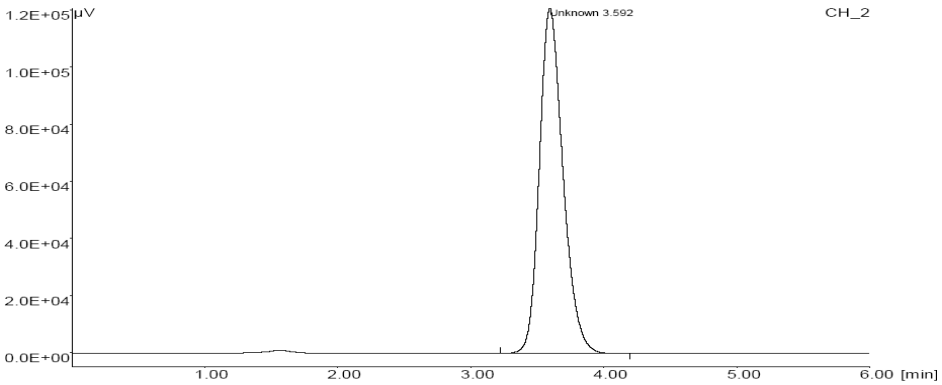

Figure 2: Chromatogram of standard flurbiprofen.

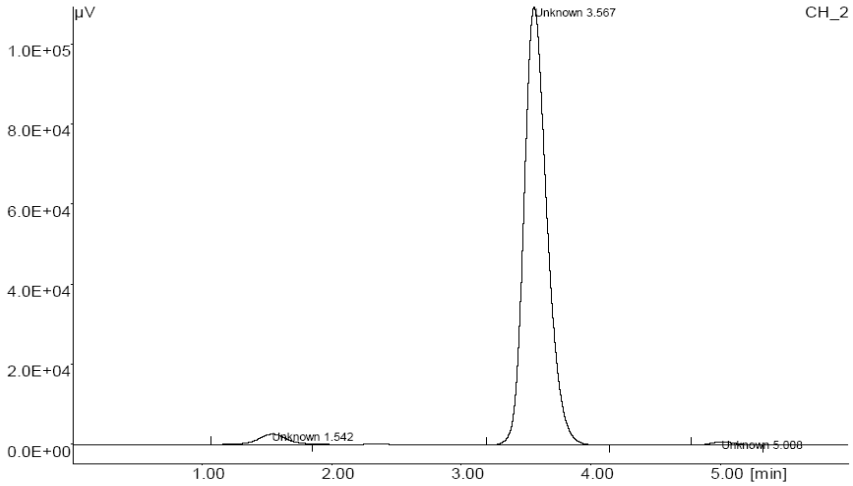

Figure 3: Chromatogram of flurbiprofen in Topical Formulation.

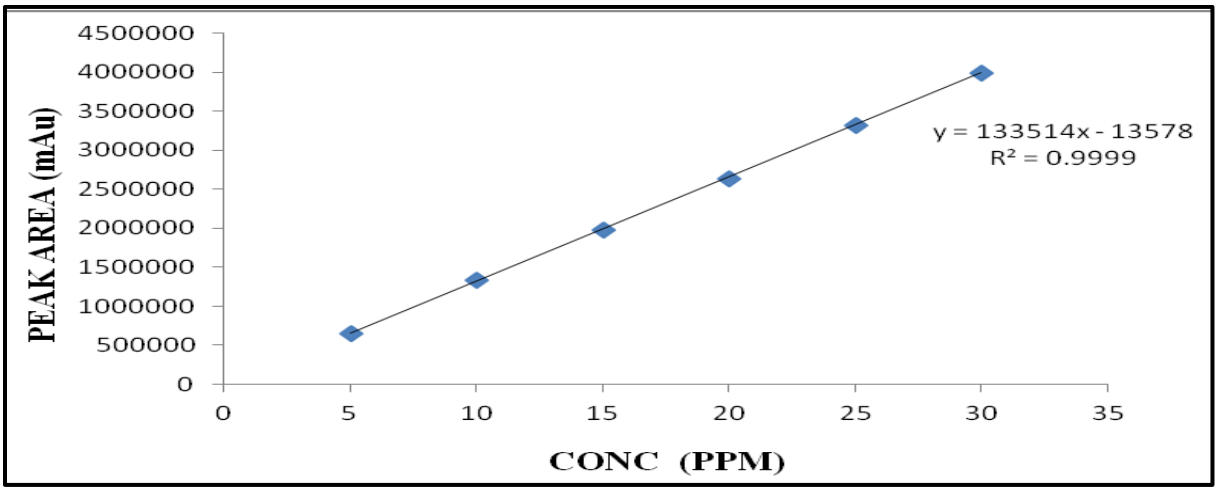

Figure 4: Calibration curve for Standard flurbiprofen.

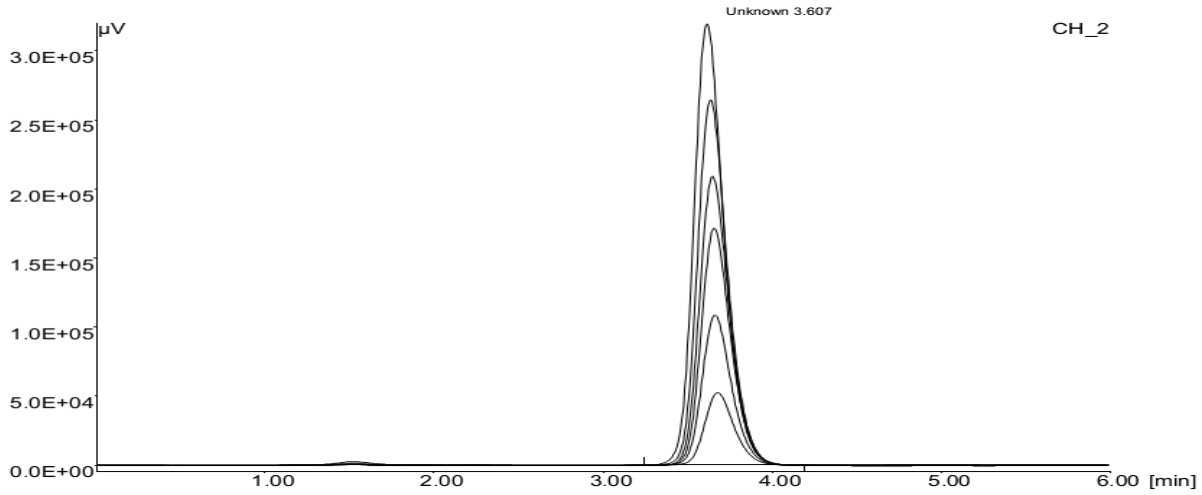

Figure 5: Overlay chromatogram for linearity of standard flurbiprofen.

CONCLUSION:

The proposed RP-HPLC method was simple, precise, economic, accurate, rapid and reproducible determination of flurbiprofen in topical gel developed and formulated in our laboratory.

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