International Journal of PharmATA

2022; 2(3): 72-81

Research Articles

Development and Validation of HPLC Method for the Determination of Flurbiprofen in Pharmaceutical Preparations

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ABSTRACT:
In this study, a new and rapid high-performance liquid chromatography (HPLC) method was developed for the determination of flurbiprofen in pure and pharmaceutical preparations. The method was developed on the Ace C_{18} column using a mobile phase of acetonitrile-0.05 M potassium dihydrogen phosphate solution (60:40, v/v) adjusted to pH 3.5 with phosphoric acid. The eluent was monitored by UV detection at 254 nm. The analysis was performed in less than 6 min with a flow rate of 1.0 mL min^{-1}. Calibration curve was linear over the concentration range of 0.10-5.0 µg mL^{-1}. Intra- and inter-day precision values for flurbiprofen were less than 4.56, and accuracy (relative error) was better than 4.00%. The mean recovery of flurbiprofen was 99.8% for pharmaceutical preparations. The limits of detection (LOD) and quantification (LOQ) were 0.03 and 0.10 µg mL^{-1}, respectively. Also, the method was applied for the quality control of two commercial flurbiprofen dosage forms to quantify the drug and to check the formulation content uniformity.

Keywords: Flurbiprofen, HPLC, pharmaceutical preparation, validation

Received	Accepted	Published
23.06.2022	24.06.2022	06.07.2022

To cite this article:
Yılmaz B, Bayrak B, Kadıoğlu Y. Development and Validation of HPLC Method for the Determination of Flurbiprofen in Pharmaceutical Preparations. International Journal of PharmATA. 2022; 2(3); 72-81.

1.INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed agents worldwide to treat a variety of pain-related conditions, including arthritis and other rheumatic diseases. In addition, epidemiological studies have shown that long-term use of NSAIDs reduces the risk of developing Alzheimer's disease and delays its onset [1-3]. Flurbiprofen (Figure 1) is used for the treatment of rheumatoid arthritis, degenerative joint disease, osteoarthritis, ankylosing spondylitis, acute musculoskeletal disorders, low back pain and allied conditions [4-7]. It contains a fluorine atom in its molecular structure, producing better effects at a lower therapeutic dose and with fewer adverse effects compared with similar drugs.

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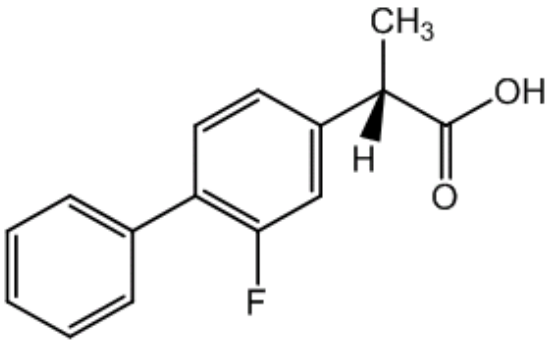

Figure 1. Chemical structure of flurbiprofen.

Several methods have been reported for the determination of flurbiprofen including high performance liquid chromatography (HPLC) [8-19] and liquid chromatography-mass spectrometry (LC-MS) [20]. Over the last 20 years, several HPLC methods using UV or fluorescence detection have been reported for the estimation of flurbiprofen either alone or together with their metabolites in plasma/serum [8-13], in urine [14-18] and in ocular fluids [19].To date, no method is reported till date for the determination of flurbiprofen by HPLC in pharmaceutical preparations. Therefore, we report an HPLC with UV method for the determination of flurbiprofen in pharmaceutical preparations. The developed method was validated by using linearity, stability, precision, accuracy and sensitivity parameters according to International Conference on Harmonization (ICH) guidelines. The method uses a simple mobile phase composition and a rapid run time of 6 min. Hence, this method can be used for the analysis of a large number of samples.

2.MATERIAL AND METHODS

2.1.Chemicals and reagents

Flurbiprofen was obtained from Sigma (St. Louis, MO, USA). Methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Majezik and Frolix tablets containing flurbiprofen were obtained from Pharmacy (Erzurum, Turkey). HPLC-grade organic solvents were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Distilled water was prepared as required by using aquaMAX™ ultra, Young instrument (Korea) ultra water purification system.

Instrumentation

An Agilent 1260 Elmer series 200 HPLC system equipped with programmable UV/Vis detector and OpenLAB ChemStation software was used. The HPLC mobile phase was composed of acetonitrile-0.05 M potassium dihydrogen phosphate solution (60:40, v/v) adjusted to pH 3.5 with phosphoric acid. Separation was achieved using an Ace C_{18} column (5 µm, 4.6×250 mm i.d.) with a flow rate of 1.0 mL/min. The eluent was monitored by UV detection at 254 nm.

2.2.Preparation of the standard and quality control solutions

The stock standard solution of flurbiprofen was prepared with methanol to a concentration of 50 μg mL^{-1} and stored at 4 ^{0}C under refrigeration. The six standard solutions from 0.1 to 5.0 μg mL^{-1} (0.1, 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 μg mL^{-1}) in methanol were made by a serial dilution. Three quality control (QC) samples at the concentrations of 0.75, 3.0 and 5.0 μg mL^{-1} were prepared from the stock standard solution.

2.3.Procedure for pharmaceutical preparations

The average tablet mass was calculated from the mass of Majezik and Frolix tablets. They were then finely ground, homogenized and a portion of the powder was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 15 min to aid dissolution and then filtered through a Whatman No 42 paper. An appropriate volume of the filtrate was diluted further with methanol so that the concentration of flurbiprofen in the final solution was within the working range and then analyzed by HPLC.

2.4.Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

3.RESULTS AND DISCUSSION

3.1.Method development and optimization

The development of the RP-HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in the routine quality control analysis. An RP-HPLC method was proposed as a suitable method for the estimation of flurbiprofen in the pharmaceutical dosage form. A good separation was achieved using an Ace C_{18} column (5 μm, 4.6×250 mm i.d.). The chromatographic conditions were adjusted to provide a good performance of the assay. The method involved a mobile phase consisting of acetonitrile-0.05 M potassium dihydrogen phosphate solution (60:40, v/v) adjusted to pH 3.5 with phosphoric acid accomplished at 254 nm. The retention time was 5.4 min at a flow-rate of 1 mL min^{-1} and the injection volume was 10 μl. The total run time for an assay was approximately 10 min. The integrator attenuation was 10 and the chart speed was 0.3 cm min^{-1}. The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figure 2 shows a typical chromatogram obtained from the analysis of a standard flurbiprofen using the proposed method. As shown in Figure 2, flurbiprofen was eluted forming a

symmetrical peak and well separated from the solvent front. The observed retention time allowed a rapid determination of the drug.

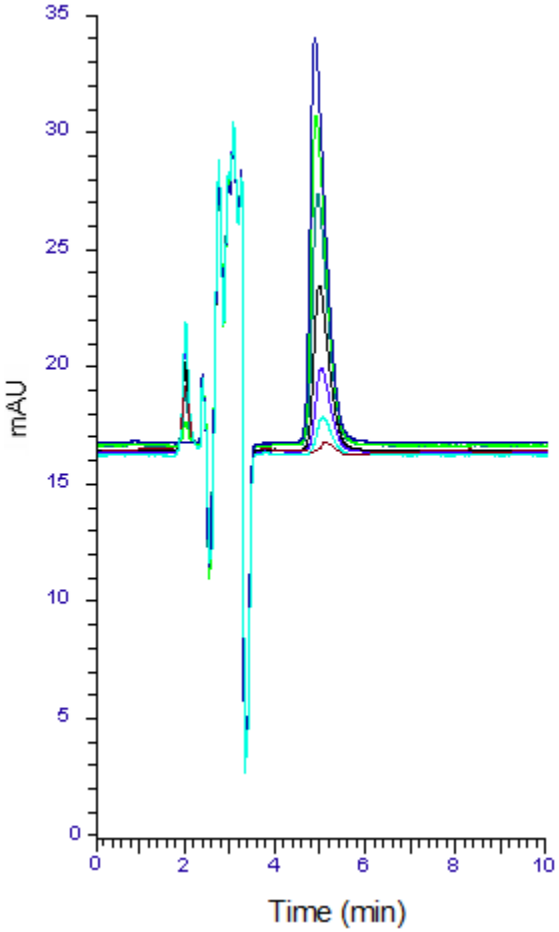

Figure 2. HPLC-UV chromatograms of flurbiprofen (0.1, 0.25, 0,5, 1.0, 2.0, 4.0 and 5.0 µg mL^{-1}).

3.2.Validation of the method

3.2.1.System suitability

A system suitability test of the chromatography system was performed before each validation run. Five replicates injections of a system suitability/calibration standard and one injection of a check standard were made. Area relative standard deviation, tailing factor and efficiency for the five suitability injections were determined. The check standard was quantified against the average of the five suitability injections. For all sample analyses, the tailing factor was ≤ 1.04, efficiency ≥ 2318 and %RSD ≤1.26%.

3.2.2.Linearity

The calibration curve was constructed for the flurbiprofen standard by plotting the concentration of compound versus peak area response. Standard solutions containing 0.10, 0.25, 0.5, 1.0, 2.0, 4.0, and 5.0 μg mL^{-1} of flurbiprofen were prepared and 10 μL was injected into the HPLC column. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The regression equations were calculated from the calibration graphs, along with the standard deviations of the slope (Sb) and intercept (Sa) on the ordinate (Table 1).

Table 1. Linearity of flurbiprofen

Method	Range (μg mL^{-1})	LR[a]	Sa	Sb	R	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
HPLC	0.10-5.0	y=536.4x+76.13	10.24	11.28	0.9996	0.03	0.10

[a]Based on three calibration curves, LR:Linear regression, Sa: Standard deviation of intercept of the regression line, Sb:Standard deviation of the slope of regression line, R: Coefficient of correlation, x: flurbiprofen concentration, LOD: Limit of detection, LOQ: Limit of quantification y: peak area

3.2.3.Accuracy and precision

Accuracy of the assay method was determined for both intra-day and inter-day variations using the six time analysis of the QC samples. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days). Accuracy and precision calculated for the QC samples during the intra- and inter-day run are given in Table 2.

Table 2. Precision and accuracy of flurbiprofen

Method	Added (μg mL^{-1})	Intra-day			Inter-day		
		Found ± SD	Accuracy	Precision RSD%[a]	Found± SD	Accuracy	Precision RSD%[a]
HPLC	0.75	0.72 ± 0.025	-4.00	3.47	0.74 ± 0.021	-1.33	2.83
	3.0	3.07 ± 0.136	2.33	4.43	2.97 ± 0.071	-1.00	2.39
	5.0	5.08 ± 0.214	1.60	4.21	5.09 ± 0.232	1.80	4.56

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

The accuracy ranged from 1.33% to 2.67% and precision from 2.39% to 4.56%. All the values were within the acceptance criteria of 5.0 %.

3.2.4.Sensitivity

Limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10, with precision (% RSD) and accuracy (% bias) within ±10%. LOD and LOQ values of the HPLC method were determined to be 0.03 and 0.10 μg mL^{-1}, respectively.

3.2.5. Stability

Stability studies indicated that the samples were stable when kept at room temperature, 4 and -20 ^{0}C refrigeration temperature for 8 h (short-term) and refrigerated at 4 and -20 ^{0}C for 72 h (long-term). The results of stability studies were given in Table 3 and no significant degradation was observed.

Table 3. Stability of flurbiprofen in solution

Stability (%)		Room temperature stability (Recovery % ± RSD)		Refrigeratory stability, +4 °C (Recovery % ± RSD)		Frozen stability, - 20 °C (Recovery % ± RSD)	
Method	Added (μg mL^{-1})	8 h	24 h	24 h	72 h	24 h	72 h
HPLC	0.50	99.7 ± 3.17	101.2 ± 3.84	99.1 ± 3.47	101.2 ± 4.26	97.4 ± 4.25	101.2 ± 3.92
	2.5	101.4 ± 3.64	102.3 ± 4.68	101.4 ± 5.06	98.2 ± 4.27	101.3 ± 4.78	99.4 ± 3.74
	4.5	98.3 ± 3.24	98.6 ± 4.09	101.3 ± 3.62	99.6 ± 5.84	99.2 ± 3.49	98.2 ± 4.98

RSD: Relative standard deviation of six replicate determinations

3.2.6.Recovery

Recovery studies by spiking different concentrations of pure drug in the pre-analyzed tablet samples within the analytical concentration range of the proposed method. The added quantities of the individual drugs were estimated by the above method. The results of recovery studies were found to be satisfactory and the results are presented in Table 4.

Table 4. Recovery of flurbiprofen in pharmaceutical preparations by HPLC method

Pharmaceutical preparation	Added (μg mL^{-1})	Found ± SD	Recovery (%)	RSD [a] (%)
Majezik	0.5	0.51 ± 0.018	102.0	3.53
tablet	2.5	2.52 ± 0.102	100.8	4.04
(1.0 μg mL^{-1})	3.5	3.51 ± 0.109	100.3	3.10
Frolix	0.5	0.50 ± 0.020	100.0	4.00
tablet	2.5	2.49 ± 0.129	99.6	5.18
(1.0 μg mL^{-1})	3.5	3.48 ± 0.112	99.4	3.20

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

3.2.7.Comparison of the methods

Flurbiprofen is a non-steroidal anti-inflammatory agent, one of the propionic acid group, which has significant anti-inflammatory, analgesic and antipyretic properties. In this study, a fast and simple HPLC method is employed in the analysis of commercial preparations in the drug industry. The proposed method is used so much because it is a method easy to apply. Also, Pharmacopoeias [21-23] have reported titrimetric and liquid chromatographic methods for the analysis of flurbiprofen in pure form and pharmaceutical formulations. Titrimetric method involves dissolving about 0.5 g of accurately weighed flurbiprofen in 100 mL of alcohol (previously neutralized with 0.1 M sodium hydroxide versus to the phenolphthalein endpoint) and then, titrating the same (after adding phenolphthalein) with 0.1 M sodium hydroxide versus till the first appearance of faint pink colour that persists for not less than 30 seconds. Each ml of 0.1 M sodium hydroxide is equivalent to 24.43 mg of flurbiprofen. Another method has recommended the HPLC method for analysis of related substances in pure flurbiprofen and assay of flurbiprofen in pharmaceutical dosage form (tablet and ophthalmic drop). The methods recommended using a mobile phase of water-acetonitrile-glacial acetic acid (60:35:5, *v/v*) at a flow rate of 1 ml min^{-1}, using UV detection (254 nm) on a stainless steel column (4 μm, 3.9×15 cm i.d.).

The present work describes the validation parameters stated either by USP 26 [21] or by the ICH guideline [24] to achieve HPLC method for the determination of flurbiprofen. The proposed method is very effective for the assay of flurbiprofen in two different tablets. The validity of the proposed method was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of

drug was estimated by the proposed method. Each level was repeated six times. The results were reproducible with low SD and RSD. No interference from the common excipients was observed. The RSD for intra- and inter-day variation was less than 4.56% for the HPLC method, which falls well below the acceptance criteria described by Shah et al. [25].

In comparison with earlier reported and official methods for estimation of flurbiprofen in pharmaceutical formulations the proposed HPLC method gave a lower LOD and LOQ at 30 and 100 ng ml^{-1} when compared to 100 ng ml^{-1} and 1 mg ml^{-1} of the earlier two proposed methods [26,27]. The proposed methods also gave a comparable or in most cases lower range of the calibration plot. Unlike reported methods, the proposed method does not utilize a special extraction step for recovering the drug from the formulation excipients matrices thereby decreasing the degree of error and time in estimation. The proposed methods of estimation of flurbiprofen is, therefore, more accurate and precise, rugged, reproducible and easier compared to other reported methods. Also, the sample recoveries in all formulations were in good agreement with their respective label claims and thus suggested the validity of the methods and non-interference of formulation excipients. The results show the high reliability and reproducibility of the method.

4.CONCLUSIONS

A rapid and simple isocratic HPLC method for the determination of flurbiprofen has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, recovery and precision. The chromatographic run time of 6 min allows for the analysis of a large number of samples in a short period. Therefore, the method is also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and raw materials.

Conflict of Interest

Author has no personal financial or non-financial interests.

REFERENCES

1. Townsend KP, Pratico D,Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs.FASEB J. 2005; 19(12): 1592-1601.

2. Vega E, Egea MA, Garduno-Ramírez ML, García ML, Sánchez E, Espina M et al,Flurbiprofen PLGA-PEG nanospheres: Role of hydroxy-β-cyclodextrinon ex vivo human skin permeation and in vivo topicalanti-inflammatory efficacy. Colloids Surf. B. 2013; 110: 339-346.

3.Tamborini L, Romano D, Pinto A, Bertolani A, Molinari F, Conti P, An efficient method for the lipase-catalysed resolution and in-line purification of racemic flurbiprofen in a continuous-flow reactor. J. Mol. Catal., B Enzym. 2012; 84: 78-82.
4.Babu GMM, Prasad CD, Himasankar K, Gourishankar V, Kumar NK, Murthy KR, Development of new controlled release formulation of flurbiprofen: in vitro-in vivo correlation. Indian J Pharm Sci. 2002; 64(1): 37-43.
5. Kagkadis KA, Rekkas DM, Dallas PP, ChoulisNH,A freeze-dried injectable form of flurbiprofen: development and optimisation using response surface methodology.Int. J. Pharm.1998; 161(1): 87-94.
6. Muraoka A, Tokumura T, Machida, Evaluation of the bioavailability of flurbiprofen and its β-cyclodextrin inclusion complex in four different doses upon oral administration to rats. Eur J Pharm Biopharm. 2004; 58(3): 667-671.
7. Poul J, West J, Buchanan N, Grahame R,Local action transcutaneous flurbiprofen in the treatment of soft tissue rheumatism.Br. J. Pharmacol. 1993; 32(11): 1000-1003.
8. Guo CC, Tang YH, Hu HH, Yu LS, Jiang HD, Zeng S, Analysis of chiral non-steroidal anti-inflammatory drugs flurbiprofen, ketoprofen and etodolac binding with HSA. J Pharm Anal. 2011; 1(3): 184-190.
9. Askholt J, Nielsen-Kudsk F, Rapid HPLC-determination of ibuprofen and flurbiprofen in plasma for therapeutic drug control and pharmacokinetic applications. Acta Pharmacol. Sin.1986; 59(5): 382-386.
10. Chi SC, Kim H, Lee SC,High performance liquid chromatographic analysis of flurbiprofen in rat plasma. Anal. Lett. 1994; 27(2): 377-389.
11. Johnson VA, Wilson JT,Flurbiprofen analysis in plasma and breast milk by high-performance liquid chromatography.J. Chromatogr. A. 1986; 382: 897-901.
12. Adams WJ, Bothwell BE, Bothwell WM, VanGiessen GJ, Kaiser DG. Simultaneous determination of flurbiprofen and its major metabolite in physiological fluids using liquid chromatography with fluorescence detection. Anal. Chem.1987; 59(11): 1504-1509.
13. Hutzler JM, Fyre RF, Tracy TS, Sensitive and specific high-performance liquid chromatographic assay for 4'-hydroxyflurbiprofen and flurbiprofen in human urine and plasma. J. Chromatogr. B. 2000; 749(1): 119-125.
14. Kang JH, Oh DH, Oh YK, Yong CS, Choi HG, Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS). Eur J Pharm Biopharm. 2012; 80(2): 289-297.
15. Pe'hourcq F, Jarry C, Bannwarth B,Chiral resolution of flurbiprofen and ketoprofen enantiomers by HPLC on a glycopeptide-type column chiral stationary phase. Biomed. Chromatogr. 2001; 15(3): 217-222.

16. Geisslinger G, Menzel-Soglowek S, Schuster O, Brune K, Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma. J. Chromatogr. B. 1992; 573(1): 163-167.
17. Knadler MP, Hall SD, High performance liquid chromatography analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine. J. Chromatogr. B. 1989; 494: 173-182.
18. Hirai T, Matsumoto S, Kishi I, Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. J. Chromatogr. B. 1997; 692(2): 375-388.
19. Riegel M, Ellis PP, High-performance liquid chromatographic assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids. J. Chromatogr. B. 1994; 654(1): 140-145.
20. Mano N, Narui T, Nikaido A, Goto J,Separation and determination of diastereomeric flurbiprofen acyl glucuronides in human urine by LC/ESI-MS with a simple column-switching technique. Drug Metab. Pharmacokinet.2002; 17(2): 142-149.
21. Rockville MD. United States Pharmacopoeia. 24th ed. United States Pharmacopoeial Convention; 2000: 748-750.
22. British Pharmacopoeia. British Pharmacopoeial Commission. London; 1993: 292-293.
23. The Pharmacopoeia of India. Indian Pharmacopoeial Commission. New Delhi; 1996: 328-329.
24. International Conference on Harmonisation (ICH) of Technical requirements for Registration of Pharmaceuticals for Human Use: Harmonised Tripartite Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process on November by the ICH Steering Committee, Published by IFPMA, Switzerland; 1996.
25. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A et al, Analytical method validation: bioavailability, bioequivalence, and pharmacokinetics studies. J Pharm Sci. 1992; 81(3): 309-312.
26. Beaulieu N, Cyr TD, Lovering EG,Validation of methods for the assay of flurbiprofen and flurbiprofen sodium, related compounds and volatile impurities in raw materials and tablets. Drug Dev Ind Pharm. 1991; 17(13): 1843-1855.
27. Mathew M, Gupta VD, Bethea C,Quantitation of flurbiprofen in tablets using high performance liquid chromatography. Drug Dev Ind Pharm. 1993; 19(4): 493-498.