

A simple high-performance liquid chromatographic practical approach for determination of flurbiprofen

Muhammad Akhlaq^{1,2},
Gul Majid Khan¹, Abdul Wahab¹,
Arshad Khan¹, Abid Hussain¹,
Asif Nawaz¹, Hamdy Abdelkader^{2,3}

¹Faculty of Pharmacy, Gomal,
University, D.I. Khan, KPK, Pakistan,
²School of Pharmacy, University of
Auckland, Auckland, New Zealand,
³Department of Pharmaceutics,
Faculty of Pharmacy,
Minia University, Minia, Egypt

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ABSTRACT

A simple, rapid, sensitive, and specific high-performance liquid chromatography (HPLC) assay for flurbiprofen has been developed and validated practically. The chromatography was conducted using Gemini C18 column (5 μ m; 4.6 mm \times 250 mm, Phenomenex, California, USA). The mobile phase containing disodium hydrogen phosphate solution (30 mM) pH 7.0 and acetonitrile (50:50); and the isocratic flow rate of 1.0 ml/min were used in the current study. Detection was made at 247 nm. The calibration curve was linear ($r \geq 0.9996$) over the concentration range of 5-50 μ m/ml. Mean percentage (%) recovery \pm % relative standard deviation (RSD) ranged from 97.07 ± 0.008 to 103.66 ± 0.013 . Within-day and between-day precision were also in acceptable range of 98.83 ± 0.004 to 104.56 ± 0.009 . In order to confirm the practical applicability of the method developed, flurbiprofen controlled release matrix tablets were subjected to the dissolution studies and the release rate was analyzed. The reported HPLC for flurbiprofen provides several advantages of simplicity, high specificity, accuracy, and very short run-cycle time. It is suggested that the method should be used for the routine quality control analysis of flurbiprofen pure drug and its dosage forms.

Key words: Chromatography, flurbiprofen, isocratic, quality control, validation

INTRODUCTION

Flurbiprofen (FLB) (propionic acid derivative) is an important non steroidal anti-inflammatory analgesic drug and is usually recommended to the patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gout.^[1] It is also recommended for the treatment of vernal keratoconjunctivitis,^[2] ocular gingivitis,^[3] and postoperative ocular inflammation,^[4] radio-protection, protection of post-irradiation myelo-suppression, and inhibition of colon tumor.^[5] It has been investigated that

flurbiprofen had an excellent safety profile and showed dose-dependent central nervous system penetration when its safety, tolerability, and pharmacokinetics were studied in healthy elderly individuals.^[6] With its ever increasing use in the clinical efficacy and pharmaceutical industry, there is a need to develop a sensitive, accurate, simple, rapid, reproducible, and more practically applicable analytical method for the determination of flurbiprofen. United States Pharmacopoeia^[7] and British Pharmacopoeia^[8] have recommended the methods for the analysis of flurbiprofen active drug and in tablet, using a stainless steel column (15 \times 3.9 cm), with a mobile phase of water: acetonitrile: glacial acetic acid (60: 35: 05) at a flow rate of 1 ml/min. Literature showed only limited number of methods for the determination of pure flurbiprofen. Sajeev *et al.* reported a liquid chromatographic method for the determination of flurbiprofen in pure form and in formulations RP C18 column with mobile phase composition of methanol-acetonitrile-phosphate buffer (pH 5.6, 40:20:40). The retention time was found to be 4.3 min.^[3] Foda *et al.* reported a method for the determination of flurbiprofen in pharmaceutical dosage forms. UV detector was used and the retention times were found to be 8.94 and 6.27 min both for flurbiprofen and internal standard.^[9] Different high-performance liquid chromatography (HPLC) methods for the determination

Address for correspondence:

Dr. Muhammad Akhlaq,
Office No. 301-2.78, Level-2, Science Building,
23 Symonds Street, University of Auckland, New Zealand.
E-mail: akhlaqhayat@hotmail.com

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of flurbiprofen in various biological fluids were also found. Szpunar *et al.* investigated the pharmacokinetics of flurbiprofen in man regarding area/dose relationship using HPLC technique.^[10] Geisslinger *et al.* reported an enantioselective HPLC assay for the quantitation of the enantiomers of flurbiprofen in human plasma. Acid glycoprotein (AGP) column (100 mm × 4.0 mm, I.D., 5 µm, Grom, Herrenberg, Germany.), the mobile phase 5% 2-propanol and 1 mM diethyloctylamine in 20 mM phosphate buffer (pH 6.5) were used and the detection was made at 246 nm.^[11] Hence, it could be concluded that no simple and rapid chromatographic methods have been published till date for the quantitation of DCL-Na except for various pharmacokinetic studies. The present study reports a simple, rapid, specific, precise, and validated HPLC method for the quantitative estimation of flurbiprofen.

MATERIALS AND METHODS

Materials

Disodium phosphate, acetonitrile and flurbiprofen were received from Sigma-Aldrich, New Zealand. Ethyl cellulose standard Premium and hydroxypropylmethylcellulose (Dow chemical co., Midland USA). All other chemicals used were of HPLC and analytical grades.

Methods

High-performance liquid chromatography method development

In brief, an HPLC system (Agilent 1200, Agilent Corporation, Germany) comprising a quaternary pump, an automatic sampler and a photodiode array (PDA) detector was used with data acquisition by ChemStation® software (Agilent Corporation, Germany). The chromatographic separation was achieved using a Gemini C18 column (5 µm; 4.6 mm × 250 mm, Phenomenex, California, USA) maintained at 25°C. The mobile phase was prepared and premixed containing fifty parts (by volume) of disodium hydrogen phosphate solution (30 mM) pH 7.0 fifty parts (by volume) of acetonitrile (50:50 v/v). The isocratic flow rate was 1.0 ml/min. An acetonitrile: water (50:50 v/v) mixture was used as a rinse solution for the injector, and the injection volume was fixed at 5 µl. Detection was carried out using a wavelength of 247 nm. The peak purity for FLB was also determined.

The developed method was validated according to International Conference on Harmonization (ICH) guidelines.^[12]

FLB 10 mg pure drug was accurately weighed, transferred into a 10 ml volumetric flask and dissolved in triple-distilled water which was obtained in-house by reverse osmosis and commercially known as Milli-Q water (MilliQ, Millipore,

USA) to yield a stock solution of 1 mg/ml. From the stock solution, a serial dilution was performed using the acetonitrile: water (50:50 v/v) mixture to yield a standard calibration curve with a concentration range from 5 to 50 µg/ml.

Linearity

The linearity of the calibration curve was checked over the range of 5–50 µg/ml.

Limit of detection and limit of quantitation

Limit of detection (LOD) is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value, calculated using equation 1.

$$LOD = \frac{3\sigma}{S} \quad (1)$$

Where σ is standard deviation of the intercept and S is slope of the calibration curve.

Limit of quantitation (LOQ) is the lowest amount of analyte in a sample, which can be quantitatively determined in suitable precision and accuracy calculated using equation 2.

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

Precision and accuracy

Intra-day and inter-day variabilities were determined by repeated injections of quality control (QC) samples. The QC samples were prepared at 8, 25, and 45 µg/ml representing low, middle, and high controls, respectively. Accuracy was assessed by comparing the predicted concentrations of the QC samples with the nominal 8, 25, and 45 µg/ml concentrations.

The HPLC-PDA detector with the Agilent ChemStation software allowed on-line data acquisition of UV-spectra between 190 and 400 nm during peak elution. The PDA detector provides more information on sample composition than single-wavelength detection.^[13,14] UV spectra were obtained at five different points across the FLB peak; two points before the peak apex (leading front), one point at the apex and two points after the apex (tailing front). The peak purity was assessed by examining the similarity of the UV spectra obtained at the five points. If an impurity or degradation product co-elutes with the FLB peak, the five UV spectra obtained across the peak are different. The peak purity analysis was carried out for all FLB samples throughout the study.

Practical Applicability

Two selected FLB controlled release matrix tablets were subjected to the release studies and different samples were obtained at predetermined time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hrs. The samples were analyzed by

HPLC to confirm the practical applicability of the method developed.

($R^2 > 0.9996$). Percentage (%) recovery \pm % RSD ranged from 97.07 ± 0.008 to 103.66 ± 0.013 [Table 1].

RESULTS AND DISCUSSION

High-performance Liquid Chromatography Method Development

The system suitability parameters were found to be within acceptable limits. An analytical run time of 15 min was optimized for each sample. The retention time of FLB under the experimental conditions was 3.2 min and well resolved from the formulation used [Figure 1]. The following validation parameters were estimated and evaluated according to the ICH guidelines:^[12,15]

Linearity

The calibration curves were linear in the range of 5-50 $\mu\text{g/ml}$

Limit of Detection and Limit of Quantitation

LOD and LOQ were estimated to be 0.173 $\mu\text{g/ml}$ and 0.578 $\mu\text{g/ml}$, respectively.

Precision and accuracy

The precision and accuracy of the method were reported as the %RSD and % recovery of the QC samples [Table 2]. The inter-day and intra-day precision for the three samples was found to be between 0.001 to 0.027%, and 0.004 to 0.030%, respectively. These values comply with the acceptance criteria of the ICH guidelines.^[12]

The purity of FLB peaks was checked all over the study using a PDA detector and Agilent ChemStation software

Table 1: Reverse predicted concentrations, % recovery and regression coefficient (R^2)

Set number	Nominal concentration ($\mu\text{g/ml}$)						R^2	Slope	Intercept
	5	10	15	20	30	50			
1	5.196	10.00	14.45	19.91	30.07	50.00	0.9998	0.051	1.000
2	5.169	9.827	14.51	19.56	30.47	50.06	0.9996	0.138	1.004
3	5.180	9.817	14.51	19.41	30.62	50.14	0.9995	0.168	1.006
4	5.200	9.841	14.76	19.71	30.54	49.93	0.9997	0.036	1.001
5	5.170	9.873	14.56	19.46	30.40	50.03	0.9989	0.120	1.002
Mean	5.183	9.872	14.56	19.61	30.42	50.03	0.9996	13.375	6.4988
% RSD	0.013	0.005	0.008	0.001	0.001	0.014			
% Recovery	103.6	98.72	97.07	98.07	101.4	100.0			

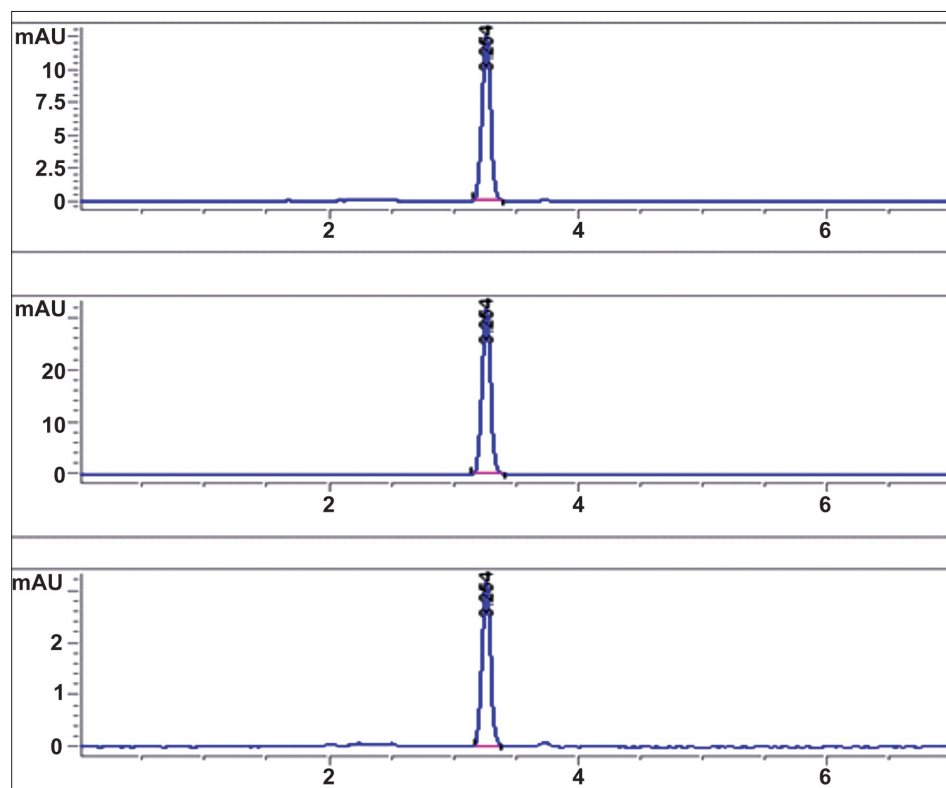


Figure 1: Representative HPLC chromatograms for FLB at three different concentrations in mobile phase

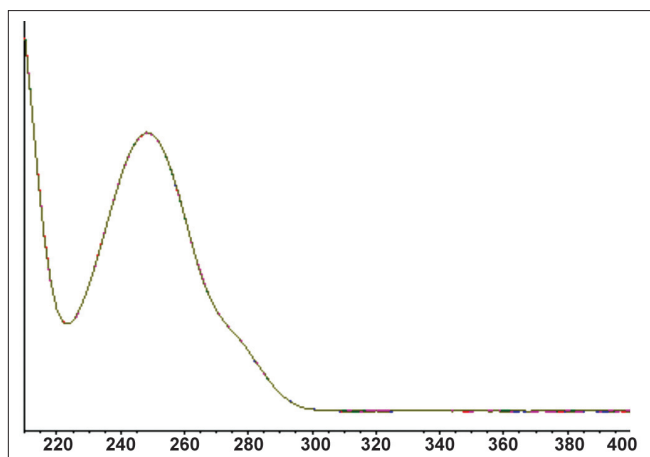


Figure 2: PDA-absorption spectra of the FLB peak from a standard solution

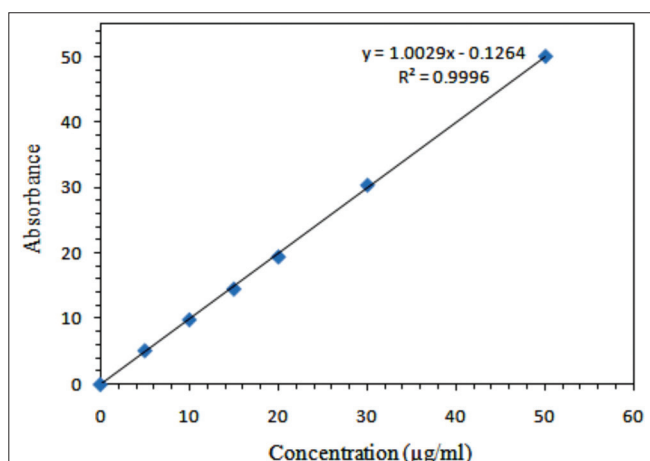


Figure 3: Mean standard HPLC calibration curve for FLB (n = 5)

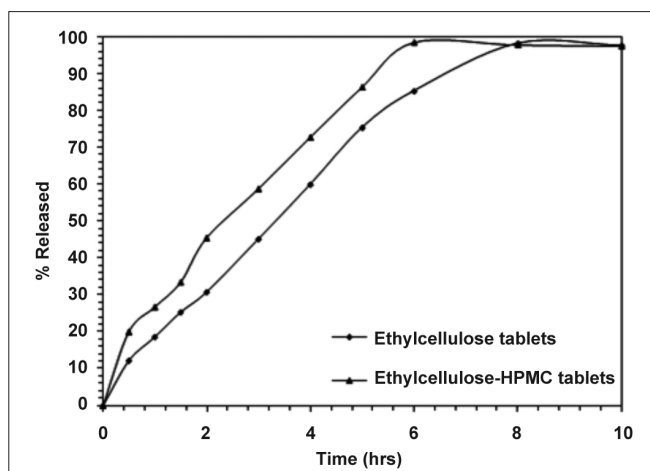


Figure 4: FLB release data from two selected controlled release matrix tablets

through examining the UV spectra at four predetermined points on the eluted drug peak from beginning to end.^[13,16] Figure 2 is a representative example showing FLB spectra acquired during elution. The examined points were 2

Table 2: Precision and accuracy data of the QC samples (Results were expressed as mean values, n = 5)

	Nominal concentration (µg/ml)	
	Intra-day 8 (µg/ml)	Inter-day 25 (µg/ml)
Mean	8.365	25.344
% RSD	0.009	0.027
% recovery	104.56	101.37

upslope points, a base point and a down-slope point for purity determination, indicating a single component and a pure peak. The calibration curve [Figure 3] showed the best linearity of $R^2 = 0.9996$.

Practical Applicability

The release data is shown in the Figure 4, indicating about 98% release of FLB from the matrix tablets formulated with ethylcellulose and ethylcellulose-hydroxypropymethylcellulose after 8:00 and 6:00 hrs, respectively. It could be observed that the release studies confirmed the applicability of the HPLC method developed for FLB pure drug and dosage form.

CONCLUSION

It could be concluded that a simple, rapid, and a specific high-performance liquid chromatographic assay for flurbiprofen has been developed and validated practically. The isocratic flow rate of 1 ml gave the retention time of FLB 3.2 min, which was well resolved from the formulation used under the experimental conditions to confirm its practical applicability.

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