

Rapid and Sensitive Spectrofluorimetric Method for the Estimation of Celecoxib and Flurbiprofen

S. CHANDRAN, P. R. JADHAV, P. B. KHARWADE AND R. N. SAHA*

Pharmacy Group, Birla Institute of Technology and Science, Pilani-333 031, India.

In this study new, rapid and sensitive spectrofluorimetric methods for the quantitative estimation of celecoxib and flurbiprofen in pure form and in their pharmaceutical dosage forms were developed. The solvent systems, wavelengths of detection (excitation and emission) were optimized in order to maximize the sensitivity and minimize the cost of analysis for both the drugs. No extraction procedure was employed for analysis of these compounds in their formulation matrix, which reduced the time of sample preparation. The excitation and emission wavelengths were found to be 256 nm and 403 nm respectively for celecoxib in water and 250 nm and 314 nm respectively for flurbiprofen in 1:1 mixture of methanol and 0.1N sulphuric acid. The linear regression equations obtained by least square regression method for fluorescence intensity (FI) and concentration in ng/ml (conc) were $FI=1.2874 \times conc + 22.647$, for celecoxib; and $FI=27.7970 \times conc + 46.049$, for flurbiprofen. The limit of detection as per the error propagation theory was found to be 4.97 ng/ml and 0.99 ng/ml for celecoxib and flurbiprofen respectively. The developed methods were successfully employed with high degree of precision and accuracy for the estimation of total drug content in two commercial capsule formulations of celecoxib and two ophthalmic drops of flurbiprofen. The results of analysis were treated statistically, as per International Conference on Harmonization guidelines for validation of analytical procedures, and by recovery studies. It was concluded that developed methods are simple, accurate, sensitive, precise and reproducible and could be applied directly and easily to the pharmaceutical preparations of celecoxib and flurbiprofen.

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-H-pyrazol-1-yl] benzene sulfonamide), a selective cyclooxygenase-2 (COX-2) inhibitor, is a newer nonsteroidal anti-inflammatory drug (NSAID) indicated to relieve the signs and symptoms of rheumatoid arthritis and osteoarthritis with efficacy comparable to other NSAIDs (e.g., naproxen and diclofenac) in such pathophysiological states¹. As celecoxib specifically inhibits the COX-2 pathway, it has a lesser chance to cause gastropathy and GI bleeding^{1,2}. Celecoxib has also been reported to have chemopreventive activity in case of colon carcinogenesis³, UV light-induced skin cancer⁴ and breast cancer⁵. Flurbiprofen, ((±)-2-(2-fluoro-4-biphenyl) propionic acid), is also an important nonsteroidal anti-inflammatory drug with efficacy comparable to other NSAIDs in the treatment of rheumatoid arthritis⁶⁻¹⁰. As both the drugs are being widely used, necessity of a rapid and sensitive method with low detection range was felt for routine and repetitive analysis.

A survey of literature has not revealed any simple spectrofluorimetric method for estimation of celecoxib or flurbiprofen in pure form and in pharmaceutical dosage form. Few liquid chromatographic methods have been reported for estimation of celecoxib in bulk drugs and formulations¹¹⁻¹⁴ using UV detection. Several liquid chromatographic methods have been reported for estimation of flurbiprofen in biological fluids employing fluorescence detection¹⁵⁻¹⁸. Various liquid chromatographic methods developed using UV detection for analysis of flurbiprofen in pure form, pharmaceutical formulations and biological samples have been reported and reviewed by this group¹⁹. Fluorescence detection has been preferred in these methods due to the interference evident in the chromatograms from UV detection.

In the present study, simple, accurate and reproducible spectrofluorimetric analytical methods, with better detection/quantitation level and devoid of complications and cost of LC method, were developed for estimation of celecoxib and flurbiprofen in pure form and in their pharmaceutical dosage forms. In both the methods, no

*For correspondence

E-mail: msaha@bits-pilani.ac.in

extraction step is utilized, thus reducing the time and error involved in the estimation. The developed methods were used to estimate the total drug content in two commercially available capsules of celecoxib and two commercially available ophthalmic drops of flurbiprofen. The results of the analysis were validated by statistical methods and as per USP²⁰, ICH guidelines²¹. The results of analysis were further validated by recovery studies.

MATERIALS AND METHODS

Pure celecoxib and flurbiprofen were obtained as gift samples from Cheminor Drugs Limited, Hyderabad; and Ophtho Remedies, Allahabad, respectively. HPLC grade acetonitrile, methanol and concentrated sulphuric acid were purchased from Merck, Mumbai. High quality pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model SA 67120). Two commercially available capsules of celecoxib (Celact, Sun Pharmaceuticals Ltd., Vadodara; and Colcibra, Crosslands, Mumbai) were selected from the local market on random basis. These capsules contained 200 mg celecoxib and common additives like diluents (lactose, aerosil), glidants and lubricants (talc, magnesium stearate). Two commercially available ophthalmic drops of flurbiprofen (Flur, Nicholas Piramal India Ltd., Dhar; and Ocuflur, FDC Ltd., Aurangabad) were selected from the local market on random basis. These ocular drops contained flurbiprofen sodium USP- 0.03% w/v and excipients like phenyl mercuric nitrate, hydroxypropylmethylcellulose and aqueous buffered vehicle or water for injection IP. A scanning spectrofluorimeter (Jasco model FP-777, Tokyo, Japan) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220-750 nm, and 10 mm quartz cells was used for fluorescence intensity measurement.

Method development:

Different solvent systems were used to develop a rugged, quick and suitable spectrofluorimetric method for the quantitative determination of celecoxib and flurbiprofen in pure form and in their respective pharmaceutical formulations. The final decision on the suitability of a solvent system for method development of the two drugs was based on cost, sensitivity, solvent noise (fluorescence), quenching effect of the solvent, sample preparation time and steps involved, adaptability of the method for estimation of the drugs in their pharmaceutical

dosage form and minimization of interference from commonly employed excipients in pharmaceutical formulations.

Calibration curve:

Separate stock solutions of both the drugs were prepared by dissolving 10 mg of drug in 100 ml (final volume) of 25% v/v acetonitrile in water to get a final concentration of 100 µg/ml. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) of celecoxib in the above media were determined by scanning a suitable dilution of the stock in high pure water using the scanning spectrofluorimeter. From the stock solution, various dilutions were made using high pure water to obtain solutions of 50, 100, 200, 400, 600, 800 and 1000 ng/ml, and the fluorescence intensity was measured for each dilution. For the analysis of flurbiprofen, the solvent system used for preparing standard dilutions was 1:1 mixture of methanol and 0.1N sulphuric acid. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) of flurbiprofen in the above media were determined by scanning a suitable dilution of the stock using the scanning spectrofluorimeter. From the stock solution, various dilutions were made using the above solvent system to obtain solutions of 10, 25, 50, 100, 200, 250 and 300 ng/ml, and fluorescence intensity was measured for each dilution. The PMT gain mode was kept at medium for all determinations.

The calibration curve values for the two drugs by the proposed methods are listed in Table 1. A regression analysis was performed on the calibration curve values, and the results of one-way ANOVA test for linearity²² are presented in Table 2.

Method validation:

Following procedures were employed to determine various validation parameters of the two developed methods^{20,21}. Accuracy and precision were determined by five replicate analyses per concentration at three different standard concentrations (high, medium and low) within the range of the standard curve. The analysis was carried out as per the previous section. For establishing linearity, five separate series (in duplicate) of solutions of the drug, 50-1000 ng/ml for the celecoxib and 10-300 ng/ml for flurbiprofen, were prepared from the stock solution and analyzed. Series of five solutions of celecoxib (400 ng/ml) and flurbiprofen (100 ng/ml) were prepared from the stock solution meant for method validation and analyzed to determine specificity.

TABLE 1: CALIBRATION CURVE VALUES OF THE PROPOSED METHODS IN ESTIMATION OF STANDARD SOLUTION OF CELECOXIB AND FLURBIPROFEN BY SPECTROFLUORIMETRY

Celecoxib (in water)				Flurbiprofen (in methanol: 0.1N H ₂ SO ₄ ; 1:1)			
Conc. (ng/ml)	Fluorescence Intensity ^a	CV (%)	Std. Error	Conc. (ng/ml)	Fluorescence Intensity ^a	CV (%)	Std. Error
50	85±4	4.2	1.61	10	292±8	2.83	2.62
100	169±7	4.0	3.04	25	734±29	3.88	9.01
200	273±10	3.5	4.23	50	1484±43	2.91	13.66
400	532±11	2.1	5.08	100	2791±119	4.25	37.46
600	777±20	2.5	8.82	200	5525±277	5.01	87.59
800	1068±36	3.3	15.96	250	7155±235	3.28	74.16
1000	1310±23	1.8	10.40	300	8248±379	4.59	119.72

^aAverage of ten determinations with standard deviation

TABLE 2: ONE-WAY ANOVA TEST FOR LINEARITY OF PURE CELECOXIB AND FLURBIPROFEN SOLUTION BY THE PROPOSED METHODS

Source of variation	Degree of freedom (DF)	Sum of squares (SS)	Mean sum of squares (MS)	F-value	
				F _{Calc}	F _{Crit} ^a
Celecoxib					
Between Group	4	1.5434×10 ³	3.8585×10 ⁴	0.0018	2.6896
Within Group	30	6.6007×10 ⁶	2.2002×10 ⁵		
Total	34	6.6022×10 ⁶			
Flurbiprofen					
Between Groups	4	4.1249×10 ⁵	1.0312×10 ⁵	0.0097	2.6896
Within Group	30	3.1946×10 ⁸	1.0649×10 ⁸		
Total	34	3.2199×10 ⁸			

^aTheoretical value of F(4, 30) based on one-way ANOVA test at P=0.05 level of significance.

Limit of detection (LOQ) and quantitation (LOD) were calculated on the basis of response and slope of the regression equation of the two methods. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the two methods. Ruggedness was determined for both the developed methods by varying the analyst for analyzing standard and test solution of the two drugs (100 and 600 ng/ml for celecoxib and 25 and 250 ng/ml for flurbiprofen) in triplicate. Robustness of the proposed method for celecoxib was determined by varying the quality of water (single, double or triple distilled) and studying their effect on fluorescence intensity of blank and drug. For determining the robustness of developed method for flurbiprofen, relative proportion of methanol in the solvent system was varied (48, 50, 52%) and the effect on sensitivity of the method studied.

Analysis of commercial formulations by the proposed methods:

Two commercially available capsule formulations of celecoxib from the Indian market were taken randomly for estimation of total drug content per capsule by the proposed method. For each brand, 20 capsules were weighed, contents were thoroughly mixed and an accurately weighed aliquot amount (equivalent to 5 mg of

celecoxib) was transferred to a series of 25 ml volumetric flasks (five in each case) and volume was made using 25% v/v acetonitrile in water. The resulting solutions were filtered through Whatman filter paper no. 1 and suitably diluted with water to get final concentration within the limits of linearity for the proposed method for celecoxib. From the fluorescence intensity value, the drug content per capsule was calculated on an average weight basis.

Two commercially available ophthalmic drops of flurbiprofen from the Indian market were selected randomly for estimation of total drug content per ml of the ophthalmic drops by the proposed method. For each brand, contents of ten containers were mixed and an aliquot volume (equivalent to 1 mg of flurbiprofen) was transferred to a series of 25 ml volumetric flasks (five in each case) and volume was made using 25% v/v acetonitrile in water. The resulting solutions were filtered through Whatman filter paper no.1 and suitably diluted using 1:1 mixture of methanol and 0.1N sulphuric acid to get final concentration within the limits of linearity for the proposed method for flurbiprofen. From the fluorescence intensity, the drug content per ml of different brands of ophthalmic drops was calculated on an average concentration basis.

Recovery studies:

Recovery studies were performed to keep an additional check on the accuracy of the developed assay methods. Known amount of pure drug was added to pre-analyzed samples of commercial dosage forms. The percent analytical recovery was calculated by comparing concentration obtained from the spiked samples with actual added concentration.

RESULTS AND DISCUSSION

During development of the proposed method for both the drugs, solubility of the drug was a major problem. In pure aqueous solvents, the drugs are difficult to be solubilised. For this reason, 25% v/v acetonitrile in water was used for preparing the primary stock solution for both the drugs. For the analysis of celecoxib, various solvent systems (either alone or in combination with water, 25 to 75% v/v) investigated were high pure water, water saturated with ether, methanol, acetonitrile, dioxane, diethyl ether, 0.1N sulphuric acid, 0.1N sodium hydroxide, 0.1N hydrochloric acid, 0.3% glacial acetic acid, phosphate buffers of various pH (5.2-8.0). Similarly for the assay method for flurbiprofen, various solvents (either alone or in combination, 25 to 75% v/v, with water) studied were high pure water, methanol, ethanol, acetonitrile, dioxane and diethyl ether, dimethyl formamide, 0.1N sulphuric acid, 0.1N sodium hydroxide, and 0.1N hydrochloric acid. The above solvents were also used in combinations like methanol: 0.1N sulphuric acid (25 to 75%) and acetonitrile: methanol (40 to 70%). The final decision of using water for celecoxib and methanol: 0.1N sulphuric acid (1:1) for flurbiprofen as the solvent for analysis was based on sensitivity, interference, ease of preparation, suitability for drug content estimation and stability studies, time and cost—in that order. No interference was observed from various formulation additives on the fluorescence pattern and intensity of the two drugs in the proposed solvent systems.

The excitation and emission wavelength (λ_{ex} and λ_{em} respectively) of celecoxib in water was found to be 256 nm and 403 nm, respectively. The drug concentration in water showed a linear relationship with the fluorescence intensity in the range 50-1000 ng/ml. The λ_{ex} and λ_{em} of flurbiprofen in methanol: 0.1N sulphuric acid (1:1) was found to be 250 nm and 314 nm, respectively. The drug concentration in the selected solvent system showed a linear relationship with the fluorescence intensity in the range 10-300 ng/ml.

The statistical analysis of data obtained for the estimation of celecoxib and flurbiprofen in pure solution indicated high level of precision for the proposed method as evidenced by the low standard deviation values and standard error (Table 1). The low values of coefficient of variation (Table 1) further established the precision of the proposed methods.

The linear regression equation was obtained as $FI=1.2874 \times conc+22.647$ for celecoxib and as $FI=27.7970 \times conc+46.049$ for flurbiprofen, where, FI is the measured fluorescence intensity, and the concentration of pure drug solution is expressed in ng/ml. Linearity of the regression equation and negligible scatter of points were demonstrated from the correlation coefficient values of 0.9996 and 0.9997 for the analysis of celecoxib and flurbiprofen respectively. The reported slope values without intercept (1.2965 for celecoxib analysis and 28.1684 for flurbiprofen analysis) on the ordinate suggested that the calibration lines of both the drug solutions in their respective solvent systems did not deviate from the origin as the above obtained value was within the 95% confidence limits of the slope (1.2480 to 1.3267 for celecoxib and 27.0410 to 28.5554 for flurbiprofen). Thus the linearity characteristics of the proposed methods for celecoxib and flurbiprofen could be practically considered as 0-1000 ng/ml and 0-300 ng/ml respectively. The precision of the fit for both the drugs was further confirmed from the low standard error values of the intercept, slope and the estimate.

A one-way ANOVA test²² was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5% significance levels in both the methods (Table 2).

The developed methods were validated as per standard procedures^{20,21}. The LOD was obtained as 4.97 ng/ml for celecoxib and 0.99 ng/ml for flurbiprofen. LOQ was obtained as 16.58 ng/ml and 3.32 ng/ml for celecoxib and flurbiprofen, respectively. The accuracy for the two methods, reported in terms of percentage relative error, was found to be 99.76 ± 0.52 and 100.13 ± 0.45 for celecoxib and flurbiprofen, respectively. The precision in terms of relative standard deviation (RSD) was determined to be 0.52% and 0.45%, respectively for celecoxib and flurbiprofen. The low values of these parameters reflect excellent measurement accuracy and precision of the

proposed methods of estimation of celecoxib and flurbiprofen. The developed methods were found to be highly rugged with the accuracy (%) of analysis of various standard and test solution by different analysts (100 and 600 ng/ml for celecoxib and 25 and 250 ng/ml for flurbiprofen in triplicate) varying from 99.83 to 99.95% for celecoxib and 99.46 to 100.10% for flurbiprofen. The % RSD for intra- and inter- day variations in the analysis was below 2.0% and 3.0% respectively in the estimation of celecoxib and 1.5% and 2.3% respectively in case of flurbiprofen. Fluorescence intensity of the test solution decreased when the quality of water was changed (inferior quality like single distilled or undistilled) in case of celecoxib analysis, and when the relative proportion of methanol and 0.1N sulphuric acid was changed by $\pm 2.0\%$ in case of flurbiprofen analysis.

Drug content from the capsules of celecoxib and ophthalmic drops of flurbiprofen was determined using the respective developed methods using pure drug solution as reference standard. The results of the studies are presented in Table 3. The estimated drug content with low values of standard deviation and coefficient of variation established the precision of the proposed methods. The accuracy of the results of estimation was further tested by recovery study. The analytical recoveries (%) varied between 99.76 to 102.13% in case of celecoxib and 99.45 to 101.65% in case of flurbiprofen.

Recovery experiments using the developed assay procedures indicated the absence of commonly encountered interference from pharmaceutical excipients used. The reported F-value of a two-way ANOVA test²², without replication, suggested that there was no significant difference in the mean recoveries of the samples (Table 4) in both the cases.

The proposed spectrofluorimetric methods of estimation of celecoxib and flurbiprofen were found to be accurate, precise, and easier compared to other reported methods. They can be easily adapted for routine analysis in quality control laboratories and formulation design and development laboratories, for estimation of flurbiprofen and celecoxib in pure form and in its formulations. There are no extractions or complicated sample preparation steps involved, thus decreasing the error and time involved in drug content estimation. The sample recoveries in all formulations were in good agreement with their respective label claims and thus suggested non-interference of formulation excipients in the estimation, which presents an added advantage over earlier reported methods. The LOQ and LOD of the proposed methods at nanogram level were lower than the earlier reported spectroscopic methods for the two drugs, making it a viable alternative for routine assay procedures for drugs at very low level on par with existing chromatographic techniques. Also, the proposed methods can be adopted

TABLE 3: RESULTS OF THE ASSAY OF PURE CELECOXIB AND FLURBIPROFEN AND THEIR COMMERCIAL FORMULATIONS BY THE PROPOSED METHODS

Sample	Label claim	Recovery			
		Mean ^a	C.V. (%)	S.E.	A.R. (%)
Analysis of celecoxib formulation					
Pure drug solution ^b		100.2 \pm 0.5	0.49	0.29	100.6 \pm 0.5
CELACT	200 mg/cap	201.2 \pm 1.7	0.84	0.49	100.6 \pm 0.8
COLCIBRA	200 mg/cap	203.8 \pm 0.4	0.22	0.13	101.9 \pm 0.2
Analysis of flurbiprofen formulation					
Pure drug solution ^c		50.4 \pm 0.4	0.80	0.4677	100.8 \pm 0.8
FLUR	300 μ g/ml	307.7 \pm 0.3	1.08	0.6373	102.6 \pm 1.1
OCUFLUR	300 μ g/ml	304.7 \pm 0.7	0.23	0.1325	101.6 \pm 0.2

^aMean and S.D. for triplicate determinations, ^b100 μ g/ml, ^c50 μ g/ml, C.V.- Coefficient of variation, S.E.- Standard error and A.R.- Analytical recovery

TABLE 4: TWO-WAY ANOVA TEST (WITHOUT REPLICATION) FOR LINEARITY IN ESTIMATION OF CELECOXIB AND FLURBIPROFEN IN COMMERCIAL FORMULATIONS

Source of variation	Celecoxib					Flurbiprofen				
	SS ^a	DF ^a	MS ^a	F _{Calc}	F _{Crit}	SS ^a	DF ^a	MS ^a	F _{Calc}	F _{Crit}
Within the brand	0.5961	2	0.2981	0.6466	19.0000 ^b	1.5058	2	0.7529	1.4594	19.0000 ^b
Between the brands	2.5350	1	2.5350	5.4995	18.5128 ^c	1.4336	1	1.4336	2.7789	18.5128 ^c
Error	0.9219	2	0.4610			1.0318	2	0.5159		
Total	4.053	5				3.9711	5			

^aSS- Sum of squares; DF-Degree of freedom; MS-Mean sum of squares, ^bTheoretical value of F (2,2) based on two-way ANOVA test at $P = 0.05$ level of significance, ^cTheoretical value of F (1,2) based on two-way ANOVA test at $P = 0.05$ level of significance.

for cleaning analysis in pharmaceutical dosage form manufacturing units. They can also be used for dissolution or similar studies.

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