ACTA CHROMATOGRAPHICA, NO. 18, 2007

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR ANALYSIS OF CELECOXIB (CXB) IN BULK DRUG AND MICROEMULSION FORMULATIONS

S. Baboota[1,*], *S. Faiyaz*[1], *A. Ahuja*[1], *J. Ali*[1], *S. Shafiq*[1], and *S. Ahmad*[2]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India
[2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India

SUMMARY

A simple, economic, selective, precise, and stability-indicating HPLC method has been developed and validated for analysis of celecoxib (CXB), a selective COX-2 inhibitor, both in bulk drug and in microemulsions. Reversed-phase chromatography was performed on a C_{18} column with methanol–water, 75:25 (%, *v*/*v*), as mobile phase at a flow rate of 1.25 mL min^{-1}. Detection was performed at 250 nm and a sharp peak was obtained for CXB at a retention time of 4.8 ± 0.01 min. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 0.27–80 μg mL^{-1}; the regression coefficient was 0.996 and the linear regression equation was $y = 48415x + 54359$. The detection (LOD) and quantification (LOQ) limits were 0.086 and 0.2625 μg mL^{-1} respectively. The method was validated for accuracy, precision, reproducibility, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of CXB. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quantification of CXB with high precision and accuracy.

INTRODUCTION

Celecoxib (CXB) is a selective cyclooxygenase-2 (COX-2) inhibitor used for treatment of rheumatoid arthritis and osteoarthritis [1,2]. CXB has

analgesic, antipyretic, and anti-inflammatory activity as a result of selective inhibition of the enzyme COX-2 and does not inhibit platelet aggregation [3]. In contrast with other non-steroidal anti-inflammatory drugs (NSAIDs) it has neither acute nor chronic gastrointestinal toxicity [1–4]. CXB is also used for treatment of colon cancer [5], ultraviolet (UV) light-induced skin cancer [6], and breast cancer [7].

A thorough literature survey has revealed that a limited number of spectrophotometric, fluorimetric, voltammetric, electrophoretic, and chromatographic methods have been reported for analysis of CXB [8]. An ultraviolet (UV) spectrophotometric method based on absorption at 251 nm was used for assay of CXB in bulk drugs and capsules [9]. The method was unsuitable for assay of CXB in microemulsion formulations, because oil peaks interfered with the CXB peak. It was, therefore, thought worthwhile to develop a stability-indicating chromatographic method for assay of CXB in bulk drugs and pharmaceutical dosage forms. Chromatographic methods using acetonitrile and buffer as mobile phase have been reported for assay of CXB in biological fluids [10–16], bulk drugs [17–20], and pharmaceutical dosage forms [21] but only two methods are available for analysis of CXB in pharmaceutical dosage forms which use methanol–water (85:15) as mobile phase [22,23]. In one of these methods a mass spectrophotometer was used as detector [22].

The principal objective of this study was, therefore, to develop a new, simple, economical, selective, precise, reproducible, and stability-indicating high-performance liquid chromatographic (HPLC) method with a wide linear range and good sensitivity for assay of CXB in the bulk drug and in microemulsion formulations using UV detection. In the method proposed the mobile phase was used directly for dilution of the formulation after filtration, and then further used for analysis. Direct use of the mobile phase as diluent for formulations in quantitative analysis minimizes errors that occur during tedious extraction procedures. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines [24].

EXPERIMENTAL

Chemicals

CXB was obtained as a gift from Ranbaxy Research Laboratory, Gurgaon, India. HPLC-grade methanol was purchased from Merck, India.

High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR grade.

Chromatography

Chromatography was performed, under ambient conditions, with Shimadzu HPLC equipment comprising quaternary LC-10A VP pumps, a variable-wavelength programmable UV–visible detector, SPD-10AVP column oven, and a SCL 10AVP system controller. Samples (20 μL) were injected by means of a Rheodyne injector fitted with a 20-μL loop. The instrumentation was controlled by use of Class-VP 5.032 software. Compounds were separated on a 25 cm × 4.6 mm i.d., 5-μm particle, 516 C_{18} DB reversed-phase column (Supelco). The mobile phase was methanol–water, 75:25, at a flow rate of 1.25 mL min^{-1}. The eluate was monitored at 250 nm.

Method Development

A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of CXB in the bulk drug and in microemulsions. These included methanol–water, 75:25 (% *v*/*v*), acetonitrile–water, 75:25, methanol–water, 50:50, methanol–water, 95:5, methanol–phosphate buffer (pH 3.5–6.5), 80:20, and acetonitrile–phosphate buffer (pH 3.5–6.5), 80:20. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The same solvent mixture was used for extraction of the drug from the formulation containing excipients.

Method Validation

Linearity

A stock solution of CXB (100 μg mL^{-1}) was prepared by dissolving 50 mg drug in 100 mL mobile phase then transferring 2 mL of this solution to a 10-mL volumetric flask and diluting to volume. Solutions of different concentration (0.27–80 μg mL^{-1}) for construction of calibration plots were prepared from this stock solution. The mobile phase was filtered through a 0.45-μm membrane filter and delivered at 1.25 mL min^{-1} for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 250 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

Accuracy, as Recovery

Accuracy was determined by the standard addition method. Previously analyzed samples of CXB (8 μg mL^{-1}) were spiked with 0, 50, 100, and 150% extra CXB standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), *RSD* (%), bias (%), and standard error of mean (*SEM*) were calculated for each concentration.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of CXB at four different concentrations (4, 8, 12, and 16 μg mL^{-1}) were determined in triplicate.

Reproducibility

The reproducibility of the method was checked by determining precision on a different instrument, analysis being performed by another person in different laboratory. For both intra-day and inter-day variation, solutions of CXB at four different concentrations (4, 8, 12, and 16 μg mL^{-1}) were determined in triplicate.

Specificity

The specificity of the method was determined by exposing a solution (8 μg mL^{-1}) of the sample to acidic (0.1 M HCl), basic (0.1 M NaOH), and oxidising (3% H_2O_2) stress conditions. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak.

Detection (LOD) and Quantification (LOQ) Limits

LOD and LOQ were determined by the standard deviation ($S_{y/x}$) method. Blank samples were injected in triplicate and the peak area of this blank was calculated. LOD and LOQ were determined from the slope, S, of the calibration plot and the standard deviation of the response for the blank sample, $S_{y/x}$, by use of the formulae LOD = 3.3 × $S_{y/x}/S$ and LOQ = 10 × $S_{y/x}/S$.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of CXB. Robustness was determined by changing the mobile phase flow rate to 1 and 1.5 mL min^{-1} and the concentration of methanol in the mobile phase to 73 and 77%.

Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 48 h under laboratory bench conditions (32 ± 1°C) and under refrigeration (8 ± 0.5°C).

Analysis of CXB in Microemulsion

To determine the CXB content of the microemulsion (label claim 20 mg mL^{-1}), 1 mL microemulsion was suitably diluted with mobile phase to furnish 100 mL stock solution. This solution was sonicated for 10 min then analyzed for drug content. The analysis was repeated in triplicate. The possibility of interference of excipients with the analysis was studied.

RESULTS AND DISCUSSION

Method Development

The HPLC procedure was optimized with a view to developing a method for stability-indicating assay. No internal standard was used because no extraction or separation step was involved. Of several solvents and solvent mixtures investigated methanol–water 75:25 (%, *v*/*v*) was found to

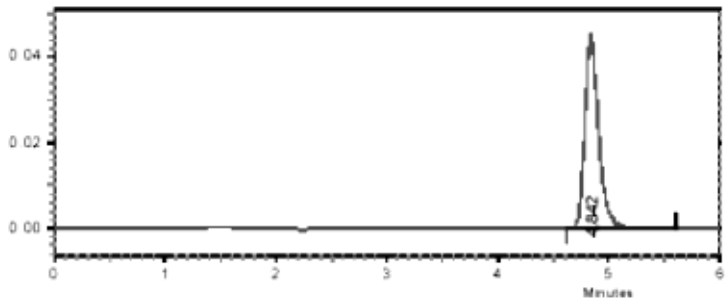

Fig. 1

HPLC chromatogram obtained from celecoxib in methanol–water 75:25 (% *v*/*v*), t_R 4.8 min

furnish sharp, well-defined peaks with very good symmetry (1.25) and low t_R (4.8 min) (Fig. 1). With acetonitrile–water 75:25 as mobile phase t_R was 5.9 min (Fig. 2) and peak shape and sensitivity were poor. Acetonitrile was also more expensive than methanol. Methanol–water 95:05 (Fig. 3) and methanol–water 50:50 (Fig. 4) did not furnish sharp, well-defined peaks and other mobile phases tried either resulted in much lower sensitivity or did not give well defined peaks in a short time, and so were not considered.

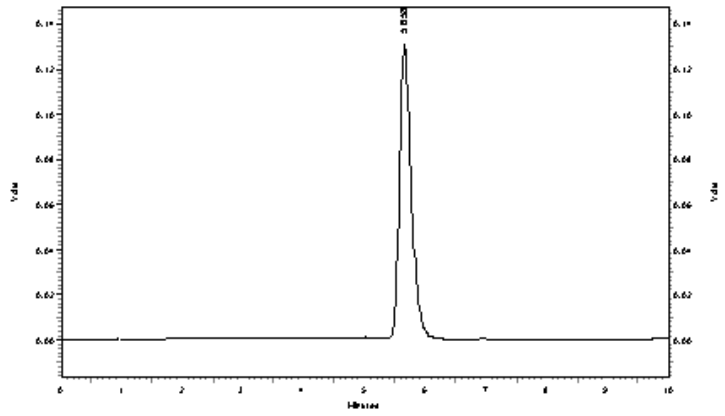

Fig. 2

HPLC chromatogram obtained from celecoxib in acetonitrile–water 75:25 (% *v*/*v*), t_R 5.9 min

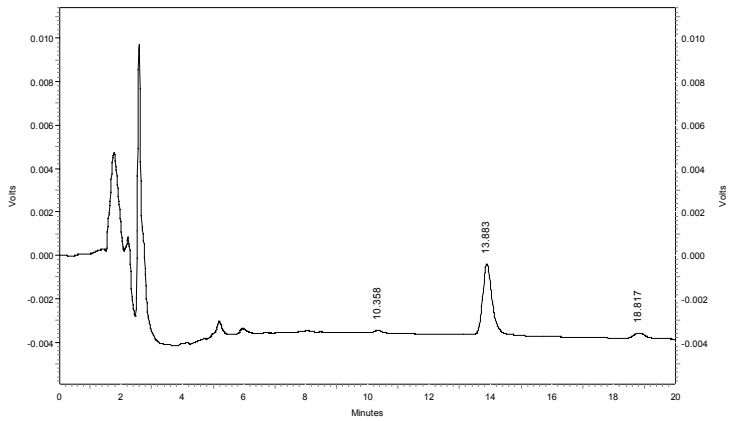

Fig. 3

HPLC chromatogram obtained from celecoxib in methanol–water 50:50 (% *v*/*v*)

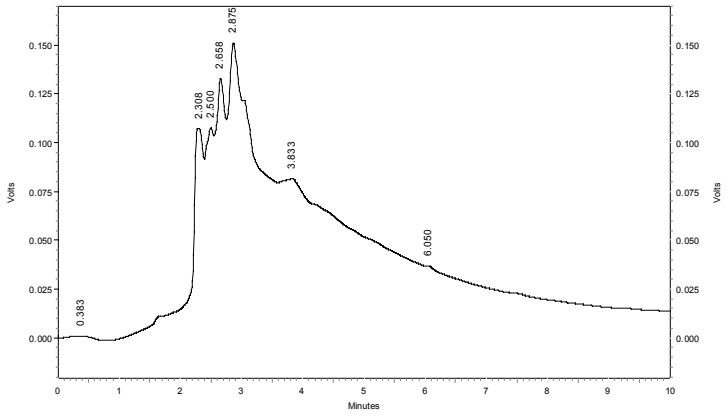

Fig. 4

HPLC chromatogram obtained from celecoxib in methanol–water 95:5 (% *v*/*v*)

The final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvent, and methanol–water 75:25 (%, *v*/*v*) was selected as the optimum mobile phase. Under these conditions the retention time and asymmetry factor were 4.8 ± 0.01 min and 1.25 ± 0.03, respectively.

Validation of the Method

Linearity

The calibration plot of peak area against concentration was linear in the range investigated (0.27–80 μg mL^{-1}). Calibration data, with their relative standard deviations, % *RSD*, standard error, and 95% confidence intervals are listed in Table I.

The low values of *RSD* and standard error show the method is precise. Statistical calculations were performed at the 5% level of significance. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range. The linear regression equation was $y = 48415x + 54359$ and the regression coefficient was 0.996. Other linear regression data are given in Table II. This performance was superior to that of other currently used methods [9,23]. The correlation coefficient was indicative of high significance. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not devia-

Table I

Calibration data for CXB with methanol–water 75:25 (%, *v*/*v*) as mobile phase

Concn (μg mL^{-1})	Mean area ± *SD* (*n* = 3)	*RSD* (%)	Standard error	95% Confidence interval	
				Lower value	Upper value
00.27	67431 ± 256.33	0.38	147.80	66795	68067
00.5	23664 ± 360.64	1.50	208.22	22511	24303
01.0	46310 ± 225.01	0.48	129.91	45674	46792
02.0	104611 ± 846.56	0.80	488.76	102412	106618
04.0	203388 ± 2193.62	1.07	1266.50	198980	209882
06.0	297949 ± 4080.26	1.36	2343.10	284353	304517
08.0	409408 ± 5668.98	1.38	3273.00	391417	419585
10	502372 ± 3518.43	0.70	2031.40	493416	510898
12	697107 ± 5686.56	0.82	3283.10	679718	707973
14	781115 ± 2813.18	0.36	1625.20	775147	789133
16	927884 ± 4915.03	0.52	2828.80	913446	937790
20	1156918 ± 3640.25	0.31	2101.70	1145048	1163135
40	2071982 ± 33014.52	1.59	19061.00	2000290	2164329
60	2930455 ± 51328.06	1.75	29634.00	2815834	3070866
80	3875772 ± 60963.03	1.57	35197.00	3688923	3991828

Table II

Linear regression data for the calibration plot (*n* = 3)

Linearity range (μg mL^{-1})	0.27–80
Regression equation	$y = 48415x + 54359$
Correlation coefficient	0.996
Slope ± *SD*	48415 ± 102.321
Intercept ± *SD*	54359 ± 81.231
Slope without intercept ± *SD*	49716 ± 120.974
Standard error of slope	59.076
Standard error of intercept	46.076
95% Confidence interval of slope	48669.204–48160.795
95% Confidence interval of intercept	54557.265–54160.735
Bias of intercept	−0.0261

te from linearity. There were no significant differences between the slopes of standard curves constructed on different days.

Accuracy, as Recovery

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 99.84–

101.01%. The values of recovery (%), *RSD* (%), percentage bias, and *SEM* listed in Table III indicate the method is accurate.

Table III

Accuracy of the method (n = 3)

Amount (%) of drug added to analyte	Theoretical content (μg mL^{-1})	Concn found (μg mL^{-1}) ± *SD*	Recovery (%)	*RSD* (%)	Bias (%)	*SEM*
0	8	8.02 ± 0.128	100.29	1.59	+0.299	0.074
50	12	12.08 ± 0.125	100.69	1.03	+0.695	0.072
100	16	16.16 ± 0.080	101.01	0.49	+1.010	0.046
150	20	19.97 ± 0.194	99.84	0.97	−0.162	0.112

Precision

Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation whereas intermediate precision was determined by measuring inter-day variation for triplicate determination of CXB at four different concentrations (4, 8, 12, and 16 μg mL^{-1}). Results from determination of repeatability and intermediate precision, expressed as *RSD* (%), are listed in Table IV. The low values of *RSD* indicate the repeatability of the method.

Table IV

Precision of the method

Concn (μg mL^{-1})	Repeatability (intra-day precision)			Intermediate precision (inter-day)		
	Mean area ± *SD* (n = 3)	*SEM*	*RSD* (%)	Mean area ± *SD* (n = 3)	*SEM*	*RSD* (%)
4	247244.7 ± 3850.75	2223.30	1.55	243890.0 ± 4602.40	2657.27	1.88
8	448173.0 ± 2254.59	1301.93	0.50	445154.7 ± 5017.66	2897.03	1.12
12	643443.7 ± 2159.26	1246.68	0.33	640151.7 ± 4513.91	2606.18	0.70
16	839226.3 ± 1104.47	637.68	0.13	841651.0 ± 4093.55	2363.48	0.48

Reproducibility

Reproducibility was checked by measuring the precision of the method in another laboratory on a different instrument with analysis performed by another person. Both intra-day and inter-day precision were de-

termined. There were no significant differences between *RSD* (%) values for intra-day and inter-day precision, which indicates the method is reproducible. The results from determination of reproducibility are listed in Table V.

Table V

Reproducibility of the method

Concn (μg mL^{-1})	Repeatability (intra-day precision)			Intermediate precision (inter-day)		
	Mean area ± *SD* (*n* = 3)	*SEM*	*RSD* (%)	Mean area ± *SD* (*n* = 3)	*SEM*	*RSD* (%)
4	246687.3 ± 4265.25	2462.61	1.72	247460.0 ± 4563.00	2634.57	1.84
8	445767.0 ± 5333.44	3079.35	1.19	450904.7 ± 4771.50	2754.91	1.05
12	645493.3 ± 7456.00	4304.88	1.15	644604.7 ± 10616.20	6129.45	1.64
16	834830.7 ± 9681.10	5589.55	1.16	843762.0 ± 11059.60	6384.29	1.31

Specificity

The specificity of the method was determined by exposing a solution of CXB to stress conditions, i.e. 0.1 M HCl, 0.1 M NaOH, and 3% H_2O_2. There was no degradation of CXB in the presence of 0.1 M HCl or 0.1 M NaOH and no significant change in peak area and retention time of CXB. In the presence of 3% H_2O_2 it was found there was a substantial change in the peak area of CXB, but not in the retention time. The results from these tests are listed in Table VI and a chromatogram obtained from

Table VI

Results from forced degradation of CXB solution. (The concentration of the solution analysed was 8 μg mL^{-1})

Stress conditions	Mean area ± *SD* (*n* = 3)	*SEM*	Number of degradation products (t_R)	CXB remaining (μg mL^{-1})	Amount recovered (%)
0.1 M HCl	444281.3 ± 3959.11	2285.86	–	8.05	100.67
0.1 M NaOH	440881.0 ± 1152.51	665.42	–	7.98	99.79
3% H_2O_2	356290.7 ± 4015.58	2318.46	1 (6.2 ± 0.03)	6.23	77.95

CXB after treatment with 3% H_2O_2 is shown in Fig. 5. A degradation product (peak 2 in Fig. 5) eluted with a retention time of 6.2 ± 0.03 min. The UV spectra of pure CXB (Fig. 6) and undegraded CXB (peak 1, t_R 4.8 min in Fig. 5) were compared and found to be similar with regard to

λ_{max} and appearance. This indicated the specificity of the method. The results from stress testing, including separation of the degradation product and quantification of CXB after exposure to stress conditions show the method is stability-indicating.

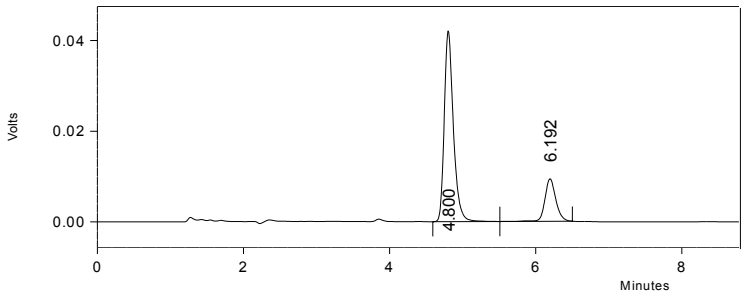

Fig. 5

HPLC chromatogram obtained from celecoxib in methanol–water 75:25 (% *v*/*v*) in the presence of 3% H_2O_2 (peak 1, t_R 4.8 min; peak 2, t_R 6.2 min)

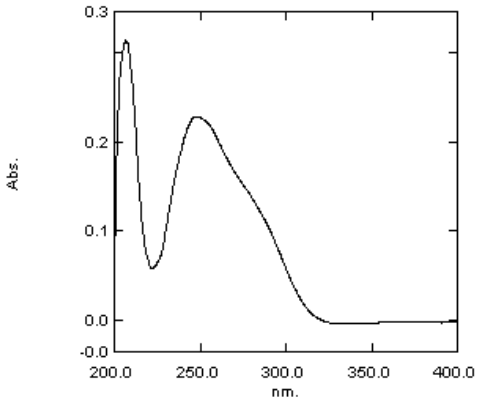

Fig. 6

UV spectrum of celecoxib in methanol–water 75:25 (% *v*/*v*)

Detection (LOD) and Quantification (LOQ) limits

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.086 and 0.263 μg mL^{-1}, respectively, which indicated the method can be used for detection and quantification of CXB over a very wide range of concentrations.

Robustness

There was no significant change in the retention time of CXB when the composition and flow rate of the mobile phase were changed. The low values of the *RSD*, shown in Tables VII and VIII, indicated the robustness of the method.

Table VII

Results from testing of the robustness of the method by changing the composition of the mobile phase. (The concentration of the solution analysed was 8 μg mL^{-1})

Mobile phase composition (methanol–water)			Mean area ± *SD* (n = 3)	*SEM*	Mean t_R ± *SD* (min)	% Bias in t_R	%*RSD*
Original	Used	Level					
75:25	73:27	−2	439032.0 ± 6838.54	3948.35	4.739 ± 0.03	−2.06	1.55
	75:25	0	445551.3 ± 2876.37	1660.72	4.810 ± 0.01	0	0.64
	77:23	+2	430350.3 ± 7057.6	4074.83	4.623 ± 0.04	−4.54	1.63

Table VIII

Results from testing of the robustness of the method by changing the mobile phase flow rate. (The concentration of the solution analysed was 8 μg mL^{-1})

Flow rate (mL min^{-1})			Mean area ± *SD* (n = 3)	*SEM*	Mean t_R ± *SD* (min)	% Bias in t_R	%*RSD*
Original	Used	Level					
1.25	1.0	−0.25	441758.3 ± 3607.45	2082.82	4.764 ± 0.03	−1.65	0.81
	1.25	–0	446513.3 ± 2077.46	1199.46	4.812 ± 0.01	0	0.46
	1.5	+0.25	433683.7 ± 2771.26	1600.03	4.721 ± 0.03	−2.47	0.63

Stability

The drug was stable when stored for 48 h at laboratory temperature (32 ± 1°C) and under refrigeration (8 ± 0.5°C) in methanol–water 75:25 (% *v*/*v*).

Analysis of CXB in a Microemulsion Formulation

A single peak was observed at the retention time of CXB when the solution of the microemulsion formulation was chromatographed. There was no interaction between CXB and excipients present in the microemulsion. The CXB content was found to be 99.46% and the *RSD* was 0.94%. It may, therefore, be inferred that degradation of CXB in the microemul-

sion formulation had not occurred. The low *RSD* indicated the suitability of this method for routine analysis of CXB in pharmaceutical dosage forms.

CONCLUSIONS

This HPLC method is accurate, precise, reproducible, specific, and stability-indicating. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of an economical and readily available mobile phase, UV detection, lack of extraction procedures, low t_R, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of CXB in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range. The method can be successfully used for routine analysis of CXB in bulk drug and in pharmaceutical dosage forms without interference. Study of the effects of exhaustive stress conditions, and separation and identification of the degradation products, is in progress in our laboratory.

ACKNOWLEDGEMENTS

The authors are thankful to Ranbaxy Research Laboratory, Gurgaon, India, for providing the sample of Celecoxib as a gift. The authors are also grateful to the University Grants Commission for providing a Research Award to Dr Sanjula Baboota.

REFERENCES

[1] J. Fort, Am. J. Orthop., **28**, 13 (1999)
[2] A. Gaurl, A.M. Martel, and J. Castner, Drugs Future, **22**, 711 (1997)
[3] G.S. Gies, Scand. J. Rheumatol., **109**, 31 (1999)
[4] M.M. Goldberg, Clin. Ther., **21**, 1497 (1999)
[5] T. Kawamori, C.V. Rao, K. Seibert, and B.S. Reddy, Cancer Res., **58**, 409 (1998)
[6] S.M. Fisher, H.H. Lo, G.B. Gordon, K. Seibert, G. Kellof, R.A. Lubet, and C.J. Conti, Mol. Carcinog., **25**, 231 (1999)

[7] R.E. Harris, G.A. Alshafie, H. Asbou-Issa, and K Seibert, Cancer Res., **60**, 2101 (2000)
[8] R.N. Rao, S. Meena, and A.R. Rao, J. Pharm. Biomed. Anal., **39**, 349 (2005)
[9] R.N. Saha, C. Sajeev, P.R. Jadhav, S.P. Patil, and N. Srinivasan, J. Pharm. Biomed. Anal., **28**, 741 (2002)
[10] O.I. Wong, N. Tsuzuki, M. Rhichardson, M.H. Rytting, W.R. Konishi, and T. Higuchi, Heterocycles, **26**, 315 (1987)
[11] M.N.E.I. Kousy, J. Pharm. Biomed. Anal., **20**, 185 (1999)
[12] R.W. Mathew and F. Jamali, J. Chromatogr., **616**, 59 (1993)
[13] C. Giachetti, A. Assandri, G. Zalano, and E. Brembilla, Biomed. Chromatogr., **8**, 180 (1994)
[14] H.S. Chow, N. Anavy, D. Salazar, D.H. Frank, and D.S. Albert, J. Pharm. Biomed. Anal., **34**, 167 (2004)
[15] E. Stormer, S. Bauer, J. Kirehhiener, J. Brockmoller, and I. Roots, J. Chromatogr B, **783**, 207 (2003)
[16] M. Zhang, G.A. Moore, S.J. Gardiner, and E.J. Begg, J. Chromatogr B, **830**, 245 (2006)
[17] G. Jayasagar, M.K. Kumar, K. Chandrasekar, P.S. Prasad, and Y.M. Rao, Pharmazie., **57**, 619 (2002)
[18] M.A. Hamid, L. Novotny, and H. Hamza, J. Chromatogr. B, **753**, 401 (2001)
[19] U. Werner, D. Werner, A. Phal, R. Mundkowski, M. Gllich, and K. Brune, Biomed. Chromatogr., **16**, 56 (2000)
[20] U. Satyanarayana, D. Rao, Y.K. Kumar, J.M. Babu, P.R. Kumar, and J.T. Reddy, J. Pharm. Biomed. Anal., **35**, 951 (2004)
[21] F. Schoenberger, G. Hienkele, T.E. Murdter, S. Brenner, U. Koltz, and U. Hoffman, J. Chromatogr. B, **768**, 255 (2002)
[22] H. Jalalizadeh, M. Amini, V. Ziace, S.A. Farsam, and A. Shafice, J. Pharm. Biomed. Anal., **35**, 665 (2004)
[23] P.M. Dhabu and K.G. Akamanchy, Drug Dev. Ind. Pharm., **28**, 815 (2002)
[24] International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonised Triplicate Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process on November 1996 by the ICH Steering Committee, IFPMA, Switzerland