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# Determination of celecoxib in pharmaceutical formulations using UV spectrophotometry and liquid chromatography

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**Abstract**

A new UV spectrophotometric method (UV method) and a reversed phase liquid chromatographic method (LC method) for the quantitative estimation of celecoxib, a selective COX-2 inhibitor, in pure form and in solid dosage form were developed in the present study. The linear regression equations obtained by least square regression method, were $Abs = 4.949 \times 10^{-2} \cdot Conc.$ (in μg/ml) $+ 1.110 \times 10^{-2}$ for the UV method and Area under the curve $= 5.340 \times 10^{1} \cdot Conc.$ (in ng/ml) $+ 3.144 \times 10^{2}$ for the LC method, respectively. The detection limit, as per the error propagation theory, was found to be 0.26 μg/ml and 25 ng/ml, respectively, for the UV and LC methods. The developed methods were employed with a high degree of precision and accuracy for the estimation of total drug content in three commercial capsule formulations of celecoxib. The results of analysis were treated statistically, as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures, and by recovery studies. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods. 

*Keywords:* Celecoxib; UV spectrophotometry; Liquid chromatography; Pharmaceutical formulations

## 1. Introduction

Celecoxib is a new nonsteroidal anti-inflammatory drug (NSAID) indicated to relieve the signs and symptoms of rheumatoid arthritis and osteoarthritis. Celecoxib demonstrates comparable or better efficacy to other NSAIDs (e.g. naproxen and diclofenac) in these pathophysiological states [1]. Celecoxib exhibits anti-inflammatory, analgesic, and antipyretic activities by selective inhibition of cyclooxygenase-2 (COX-2), the inducible isoform of cyclooxygenase, involved in the prostaglandin synthesis, and does not inhibit platelet aggregation. In contrast, most of the NSAIDs inhibit both isoforms of cyclooxygenases (COX-1 and COX-2) and inhibit platelet aggregation [2]. Due to celecoxib's specificity for the COX-2 inhibition, it has the potential to cause less gastropathy and risk of GI bleeding [1,3]. Celecoxib has also been indicated for its chemopreventive activity in case of colon carcinogenesis [4], UV light induced skin cancer [5] and breast

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cancer [6]. As the use of celecoxib is increasing rapidly, it is very much essential to develop simple and suitable analytical methods for its estimation in bulk and in formulations. Such methods should provide better sensitivity and selectivity and could be easily adapted for routine quality control analysis, dissolution or similar studies. One such method has been reported [7] recently by this group for estimation of nimesulide in bulk and in formulations.

A survey of literature has not revealed any UV-visible spectrophotometric method, however two liquid chromatographic methods [8,9] have been reported. The first one is normal phase HPLC method with complicated sample preparation and column switching procedure with UV detection for determination of celecoxib in human plasma [8]. The other one is reverse phase HPLC method [9], which utilized phosphate buffer (pH 4.8; 0.01 M):acetonitrile as mobile phase system and the percentage recovery obtained for celecoxib from its solid dosage form was found to be less (90–94%). The first one is not simple and is only suitable for estimation of celecoxib in human plasma. In the latter the preparation of mobile phase is not simple as it contains a buffered media, the drug solution was prepared in solvent different from the mobile phase and also internal standard was employed. Moreover, limit of detection and quantitation are reported only for celecoxib impurities, namely, 4-hydrazino benzene sulfonamide and 1-(4-methyl phenyl)-4,4,4-trifluro butan-1,3-dione.

In the present study, two simple, economical, accurate and reproducible analytical methods with better detection ranges for estimation of celecoxib in pure form and in its solid dosage forms were developed. This paper describes a UV spectrophotometric method (UV method) for estimation of celecoxib in 50% v/v acetonitrile in phosphate buffer (pH 5.6) at 251 nm and a reverse phase high performance liquid chromatographic method (LC method) using RP-C8 column in 65:35 mixture of acetonitrile:water with UV detection at 230 nm. The UV method was aimed at developing an easy and rapid assay method for celecoxib without any time consuming sample preparation steps for routine analysis, to be adopted in quality control and drug testing laboratories, and at the same time ensure satisfactory recovery during drug estimation from pharmaceutical formulations. Liquid chromatography was attempted to demonstrate the utility of UV detection for the estimation of celecoxib coupled with simple and economical mobile phase and reasonable analysis time with high precision. The methods were also assessed for their suitability as stability indicating assay. In both the proposed methods there is no need to extract the drug from the formulation excipient matrix thereby decreasing the error in quantitation. Formulation sample can be directly used after dissolving and filtration. The developed methods were used to estimate the total drug content in three commercially available capsules of celecoxib. The results of the analysis were validated by statistical methods [10,11] and recovery studies.

## 2. Experimental

### 2.1. Chemicals

Celecoxib was obtained as a gift sample from Cheminor Drugs Limited, Hyderabad, India. HPLC grade acetonitrile was purchased from Merck, India. Sodium dihydrogen phosphate A.R. and disodium hydrogen phosphate A.R. were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. High pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model Elix SA 67120). Three commercially available capsules of celecoxib were selected from the local market on a random basis. These capsules normally contain common additives like diluents (lactose, aerosil, etc.), glidants and lubricants (magnesium stearate, etc.).

### 2.2. Equipment

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, and 10 mm matched quartz cells with spectra manager software was used for all absorbance measurements. For LC estimations, a Jasco model HPLC

equipped with two-pump system (PU-1580), Rheodyne injector (7725i) fitted with a 20 μl loop, UV detector (UV-1575) and BORWIN-I software was used.

### 2.3. Chromatographic conditions

The chromatographic column used was a reverse phase $4.6 \times 250$ mm Inertsil® C8 HPLC column (GL Sciences Inc.) with 5 μm particles. The column and the HPLC system were kept in ambient conditions. The mobile phase was acetonitrile–water (65:35) delivered at a flow rate of 1.25 ml/min. The injection volume was 20 μl. The eluate was analyzed at a wavelength of 230 nm.

### 2.4. Method development

To develop a rugged and suitable UV spectrophotometric and LC method for the analysis of celecoxib in formulations, different solvents systems were used. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipient matrix for estimation of the drug content.

### 2.5. Preparation of standard curve for UV method

A stock solution of celecoxib was prepared by dissolving 10 mg of drug in 100 ml of 50% v/v acetonitrile–sodium phosphate buffer (pH 5.6) mixture to get a final concentration of 100 μg/ml. The composition of the buffer was 0.1 M $NaH_2PO_4 \cdot H_2O$ (94.8 parts) and 0.1 M $Na_2HPO_4 \cdot 7H_2O$ (5.2 parts). The $\lambda_{max}$ of celecoxib in the above media was determined by scanning a suitable dilution of the stock. From the stock solution, various dilutions were made to obtain solutions of 1, 5, 10, 15 and 20 μg/ml, and absorbance was measured for each dilution. The results are listed in Table 1. The stability of the drug in the solvent system and during actual analysis was also investigated.

### 2.6. Preparation of standard curve for LC method

A stock solution (100μg/ml) of pure drug was prepared by dissolving 5 mg celecoxib in 50 ml of 65:35 acetonitrile:water mixture. 1 ml of this solution was transferred to 10 ml volumetric flask and the volume was made to obtain a solution of 10 μg/ml. From this solution, concentrations of 100, 200, 400, 600, 800 and 1000 ng/ml were made in series in 10 ml volumetric flasks for the purpose of calibration curve.

Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase acetonitrile:water (65:35) was passed through the same. The mobile phase filtered through 0.22 μm membrane filter using Millipore HPLC solvent filtration assembly, was delivered at 1.25 ml/min for column stabilization. During this period, the baseline was continuously monitored. The wavelength of detection was selected at 230 nm. The prepared dilutions were injected serially. The obtained peaks were integrated and the area under the peak was calculated. The stability of the solution of celecoxib during analysis was determined by repeated analysis of samples during the course of the experiment on the same day and also on different days after storing at laboratory bench conditions and in the refrigerator. The results are listed in Table 1. Chromatogram parameters, retention time and asymmetry factor, were standardized.

### 2.7. Method validation

(a) Accuracy and precision: Five separate solutions of celecoxib (10 μg/ml for UV method and 500 ng/ml for LC method) standard and test solution were prepared in duplicate from freshly prepared stock solution and analyzed as per the procedure given in Sections 2.5 and 2.6 for UV and LC methods, respectively.

(b) Linearity: Five separate series of solutions of the drug, 1–20 μg/ml for UV method and 100–1000 ng/ml for the LC method were prepared from the stock solution and analyzed.

(c) Specificity: Series of five solutions of the drug in 10 μg/ml for UV method and 500 ng/ml of LC method were prepared from the stock

Table 1
Calibration curve points of the proposed methods for estimation of celecoxib

| UV method (in 50:50 acetonitrile–sodium phosphate buffer, pH 5.6) | | | | LC method (in 65:35 acetonitrile–water) | | | |
|---|---|---|---|---|---|---|---|
| Concentration of the solution (μg/ml) | Mean absorbance value[a] | Coefficient of variation (%) | Standard error | Concentration of the solution (ng/ml) | Mean area value[a] | Coefficient of variation (%) | Standard error |
| 1 | 0.0557 ± 0.0007 | 1.21 | 0.0003 | 100 | 5375.06 ± 132.47 | 2.46 | 54.0786 |
| 5 | 0.2658 ± 0.0038 | 1.42 | 0.0015 | 200 | 11507.24 ± 361.89 | 3.14 | 147.7414 |
| 10 | 0.5061 ± 0.0025 | 0.49 | 0.0010 | 400 | 21382.44 ± 405.16 | 1.89 | 165.4066 |
| 15 | 0.7503 ± 0.0063 | 0.84 | 0.0026 | 600 | 32366.01 ± 889.28 | 2.75 | 363.0467 |
| 20 | 1.0017 ± 0.0072 | 0.72 | 0.0029 | 800 | 43104.31 ± 612.45 | 1.42 | 250.0313 |
| | | | | 1000 | 53701.47 ± 182.72 | 0.34 | 74.5929 |

[a] Average of ten determinations with standard deviation.

solution meant for method validation and analyzed.

(d) Limit of detection (LOQ) and quantitation (LOD): LOQ and LOD were calculated on the basis of response and slope of the regression equation for UV method and signal-to-noise ratio in case of LC method. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the two methods.

(e) Ruggedness: It was determined for both the methods by varying the analyst, instrument (Jasco UV spectrophotometer model 7800 for UV method and Waters HPLC equipped with two-pump system (model 501), Rheodyne injector (7725i) fitted with a 20 μL loop, UV detector (Lambda max model 481) and Aimil chromatography data station with WIN-ACDS software was used for LC method), and different columns of same make for LC method.

(f) Robustness: Robustness of the method was determined by varying the pH of phosphate buffer between 5.4 and 5.8 and performing the analysis at 48, 50, and 52% acetonitrile in UV method. For determining the robustness of LC method the % of acetonitrile was varied (62, 65, 68%) and the effect on retention time and peak parameters studied.

### *2.8. Estimation of celecoxib from three commercial capsule formulations by the proposed methods*

Three commercially available capsules of celecoxib (Brand A, B and C) were taken randomly from the Indian market for estimation of total drug content per capsule by the proposed methods. 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 50% v/v acetonitrile–sodium phosphate buffer (pH 5.6 mixture) in case of UV method and with 65:35 acetonitrile:water in case of LC method, respectively. The resulting solutions in both the cases were filtered through Whatman filter paper no. 1 and suitably diluted to get final concentration within the limits of linearity for the respective proposed methods (as given in Table 4). From the absorbance value (UV method) and the area under the curve (LC method), the drug content per capsule (on an average weight basis) was calculated. The results are tabulated in Table 5.

### *2.9. Recovery studies*

To keep an additional check on the accuracy of these developed assay methods, recovery experiments were performed by adding the known amount of pure drug to pre-analyzed samples of commercial dosage forms. The percent analytical recovery calculated by comparing the concentration obtained from spiked samples with actual added concentration and the values are listed in Table 5. The effect of formulation excipients on UV absorbency of the drug was studied by adding common excipients to the known concentration of the pure drug sample and drug concentration estimated.

## 3. Results and discussion

### *3.1. Method development*

For UV method, various solvent systems investigated were high pure water, methanol, acetonitrile, 0.1 N NaOH, 0.1 N HCl, phosphate buffers of various pH (5.2–8.0), acetate buffer (pH 3.6–5.6) and citrate buffer (pH 3.0–7.0). All the buffers were prepared by the method reported by Gomori [14] and analyzed for absorbency for same concentration of the drug. The above solvents were also used in combinations viz., methanol:water (40–70%), acetonitrile:water (35–70%), methanol:acetonitrile (40–60%). Acetonitrile with different buffers (organic phase varied from 30 to 70%) was also employed to improve the sensitivity. In such combinations, pH of the buffer selected was one in which the drug gave maximum stability and absorbency. The final decision for using 50% acetonitrile in phosphate buffer pH 5.6 as the solvent was based on sensitivity, ease of preparation, suitability for drug

content estimation and stability studies, time and cost in that order.

Again in case of LC method mobile phase investigated were methanol:water (20–80%), acetonitrile:water (20–80%), methanol:phosphate buffer (pH 5.0–6.2) (20–80%) and acetonitrile: phosphate buffer (pH 5.0–6.2) (20–80%). Mobile phase and flow rate selection were based on peak parameters (height, asymmetry, tailing), baseline drift, run time, ease of preparation of the mobile phase, need for pH adjustment and cost (in that order). Internal standard was not used, as there was no extraction or separation step involved. Glacial acetic acid at 0.5% level in the above investigated mobile phase improved the peak parameters, but its use was given up in favor of long term stability of the column.

### 3.2. UV method

The spectra of celecoxib in 50:50 acetonitrile:sodium phosphate buffer (pH 5.6) is shown in Fig. 1. The $\lambda_{max}$ was found to be 251 nm. The statistical analysis of data obtained for the estimation of celecoxib in pure solution indicated a high level of precision for the proposed method as evidenced by low standard deviation values (Table 1). The low values of standard error and coefficient of variation (Table 1) further established the precision of the proposed method. The drug solutions were stable for a period of 48 h in the solvent system used, indicating that the proposed method can be used as a stability indicating method. As there was no microbial growth in the used media, use of sodium azide (1%) was not required.

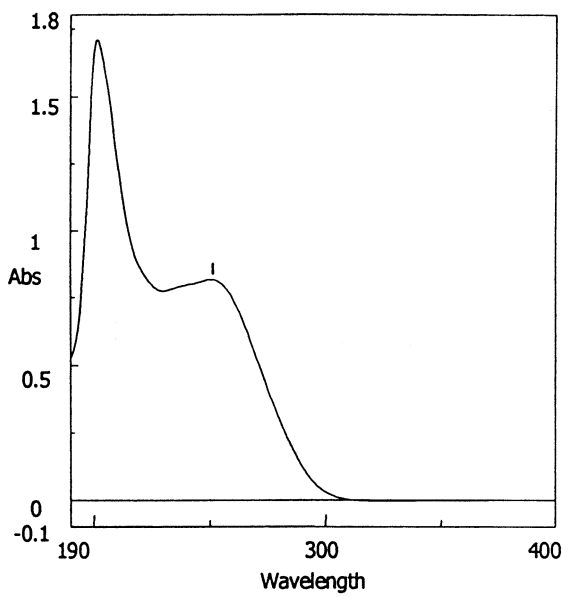


Fig. 1. UV spectrum of celecoxib in 50% v/v acetonitrile in sodium phosphate buffer (pH 5.6).

Table 2
Results of least square regression analysis of data for the estimation of celecoxib by the proposed methods

| Statistical parameters | UV method | LC method |
|---|---|---|
| Regression equation[a] | $Y = 4.949 \times 10^{-2} \cdot X + 1.110 \times 10^{-2}$ | $Y = 5.340 \times 10^{1} \cdot X + 3.144 \times 10^{2}$ |
| Correlation coefficient ($r$) | 0.9999 | 0.9999 |
| Standard error of slope | $3.560 \times 10^{-4}$ | $4.208 \times 10^{1}$ |
| Standard error of intercept on ordinate | $4.360 \times 10^{-3}$ | $2.554 \times 10^{2}$ |
| Standard error of the estimate | $5.400 \times 10^{-3}$ | $3.282 \times 10^{2}$ |
| 95% Confidence interval of slope | $4.836 \times 10^{-2}$, $5.062 \times 10^{-2}$ | $5.224 \times 10^{1}$, $5.457 \times 10^{1}$ |
| 95% Confidence interval of intercept | $-2.770 \times 10^{-3}$, $2.497 \times 10^{-2}$ | $-3.947 \times 10^{2}$, $1.024 \times 10^{3}$ |
| Slope without intercept | $4.979 \times 10^{-2}$ | $5.357 \times 10^{1}$ |

[a] Based on five calibration values.
$Y$ is the absorbance (UV method) and area under the peak (LC method). $X$ is the concentration of the drug in μg/ml (UV method) and in ng/ml (LC method).

The linear regression equation obtained was $Y = 4.949 \times 10^{-2} \cdot X + 1.110 \times 10^{-2}$ ($r = 0.9999$), where $Y$ is the absorbance and $X$ is the concentration (in μg/ml) of pure celecoxib solution. The correlation coefficient values obtained were highly significant for the method (Table 2). The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of celecoxib solutions in 50:50 acetonitrile:sodium phosphate buffer (pH 5.6) did not

deviate from the origin as the above obtained values were within the confidence limits (Table 2). The precision of the fit was further confirmed from the standard error values of the intercept, slope and the estimate.

A one-way ANOVA test [12,13] 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 this method (Table 3).

### 3.3. LC method

A typical chromatogram for celecoxib using C8 RP-HPLC column with mobile phase, composed of acetonitrile and water (65:35), at 1.25 ml/min flow rate is shown in Fig. 2. The $\lambda$ of detection was fixed at 230 nm so that there was less interference from mobile phase with highest sensitivity according to UV analysis. The statistical analysis [10–13] of data obtained for the estimation of celecoxib in pure solution indicated a high level of precision of the proposed method.

The calibration curve area (μv.s) versus concentration (ng/ml) was found to be linear. Values obtained for the calibration curve points and their standard deviation, coefficient of variance and standard error are presented in Table 1. Statistical calculations were done at 5% level of significance. The low values of standard deviation, standard error and coefficient of variation (Table 1) established the precision of the proposed method. The drug was stable during analysis and for a period of 48 h stored at room temperature and under refrigerated conditions in acetonitrile:water (65:35) mixture.

The linear regression equation obtained for the proposed LC method was $Y = 5.340 \times 10^1 \cdot X + 3.144 \times 10^2$, ($r = 0.9999$) where $Y$ is the area under the peak in μv.s and $X$ is the concentration in ng/ml. The correlation coefficient value was highly significant (Table 2). The reported slope value without intercept on the ordinate, at 95% confidence limits, suggested that the calibration line of celecoxib solution did not deviate from the origin as the above values were within the confidence limits (Table 2). The retention time and asymmetry factor were found to be $8.047 \pm 0.006$ min and $1.186 \pm 0.041$, respectively.

As done for the UV method, a one-way ANOVA test [12,13] was performed for the LC method 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 this method as well (Table 3).

### 3.4. Validation of the developed methods

The developed methods were validated according to the standard procedures [10,11] and the

Table 3
One-way ANOVA test for linearity of pure celecoxib 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] |
| UV method | | | | | |
| Between group | 5 | $7.84 \times 10^{-5}$ | $1.57 \times 10^{-5}$ | 0.0001 | 2.6207 |
| Within group | 24 | 3.3927 | $1.41 \times 10^{-1}$ | | |
| Total | 29 | 3.3928 | | | |
| LC method | | | | | |
| Between groups | 5 | $6.44 \times 10^5$ | $1.61 \times 10^5$ | 0.0005 | 2.6207 |
| Within group | 24 | $8.71 \times 10^9$ | $3.49 \times 10^8$ | | |
| Total | 29 | $8.72 \times 10^9$ | | | |

[a] Theoretical value of $F(5, 24)$ based on one-way ANOVA test at $P = 0.05$ level of significance.

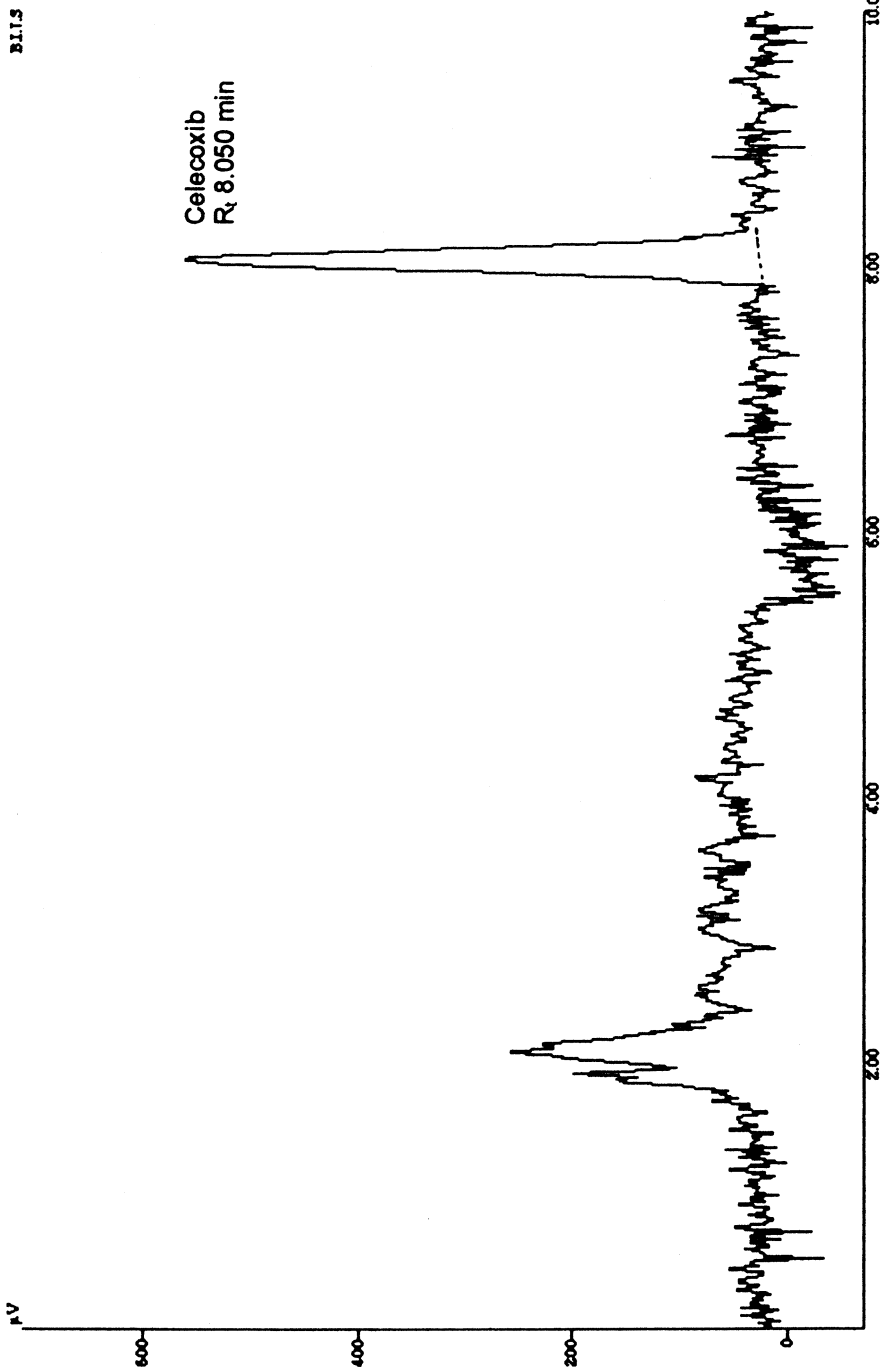


Fig. 2. A typical chromatogram of celecoxib at LOQ (75 ng/ml) [mobile phase, acetonitrile:water (65:35); flow rate, 1.25 ml/min; column, RP C8; and $\lambda$ of detection, 230 nm].

Table 4
Validation report for the determination of celecoxib in standard solutions by UV method and LC method

| Analytical parameter | Results | |
|---|---|---|
| | UV method | LC method |
| Accuracy (%) | 100.82 ± 0.17 | 99.83 ± 0.15 |
| Precision (%) | 99.83 | 98.76 |
| | 99.75 | 99.00 |
| | 99.87 | 98.89 |
| | 99.98 | 98.88 |
| | 100.03 | 99.92 |
| | RSD[a] = 0.10 | RSD[a] = 0.43 |
| Linearity | 1–20 μg/ml | 100–1000 ng/ml |
| Specificity | A 10 μg/ml solution of celecoxib will show an absorbance of 0.5061 ± 0.0025 at 251 nm | A 500 ng/ml solution of celecoxib will give an area of 26513.14 ± 0.0004 at 230 nm using RP-C8 column in, acetonitrile–water (65:35) mobile phase at a flow rate of 1.25 ml/min |
| Limit of detection[b] | 0.26 μg/ml | 25 ng/ml |
| Limit of quantitation[b] | 0.88 μg/ml | 75 ng/ml |
| Ruggedness (%) | 99.87 ± 0.80 | 99.83 ± 0.15 |

[a] Relative standard deviation.
[b] Based on standard deviation of the response and the slope of the regression curve in case of UV method and signal-to-noise ratio in case of LC method.

results obtained are tabulated in Table 5. The linearity range of celecoxib solution in case of UV method was found to be 1–20 μg/ml at a $\lambda_{max}$ of 251 nm and for LC method was obtained as 100–1000 ng/ml. Since the reported slope values without intercept fell within 95% confidence limits for both the methods, the linearity characteristics of the proposed UV and LC methods could be practically considered as 0–20 μg/ml and 0–1000 ng/ml, respectively. The limit of detection (LOD) (0.26 μg/ml and 25 ng/ml for UV and LC methods, respectively) and limit of quantitation (LOQ) (0.88 μg/ml and 75 ng/ml, respectively) are given in Table 4. However, in the case of the LC method, the lowest quantity detected was 12.5 ng/ml, and the lowest quantity accurately quantified was 50 ng/ml (but it was omitted from the calibration curve due to constraint of signal-to-noise ratio requirement) [10]. Both the proposed methods were found to be rugged when analyst or equipment or column (in the case of LC method) were varied. The accuracy of these estimations varied between 99.07–100.67% and 99.68–99.97% for UV and LC methods, respectively.

For the developed UV method, varying the pH of the phosphate buffer from 5.4 to 5.8 did not significantly affect the sensitivity of the method. The method employed [14] for the preparation of the buffer (pH 5.6) was such that the variation in pH was less than or equal to ± 0.05. Although varying the percentage of acetonitrile did not affect the linearity range of the UV method, a maximum ± 5% change was observed in the absorbency at different concentrations of the calibration curve. For LC method, changing the acetonitrile percentage in the mobile phase changed the retention time by only ± 0.3 min and the peak parameters were most optimized at 65% level. The validation parameters of the proposed methods are presented in Table 4. The intra- and inter-day variations calculated on the basis of percentage relative standard deviation on replicate set of calibration samples ($n = 5$ at each concen-

tration for each method) was less than 2% for UV method and less than 5% for HPLC method.

In Table 4, the accuracy is reported in terms of % relative error and precision in terms of % RSD. The low values of these parameters reflect excellent measurement accuracy and precision of the proposed methods of estimation of celecoxib.

### 3.5. Recovery studies

The two methods were evaluated by estimation of celecoxib in pharmaceutical formulations by the proposed methods and analysis of pure drug solution as reference. The results are presented in Table 5. The percentage recovery from these formulations by the proposed methods varied from 95.60 to 99.82% for UV method and 97.44 to 99.86% for LC method. The estimated drug content with low values of standard deviation established the precision of the proposed methods. The accuracy of the results of estimation was further tested by recovery experiments by adding known amount of pure drug to pre-analyzed samples of the formulation. The average accuracy was 99.05% for UV and 99.1% for LC method. Common formulation excipients in the concentration normally used did not affect the UV absorbency of the drug. Recovery experiments using the developed assay procedures further indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulations. The reported $F$-value of a two-way ANOVA test, without replication [11], suggested that there was no significant difference in the mean recoveries of the samples (Table 6) in both the methods. This was another reason internal standard was not used in the LC method.

## 4. Conclusions

The proposed methods of estimation of celecoxib were found to be accurate, precise, and easy. As the LOQ of the proposed UV method is very low (0.88 μg/ml), the method can be adopted for routine quality testing and dissolution studies. The LOQ and LOD of the proposed LC method are lower than the earlier reported works. The LC method is found to be superior to earlier reported methods, as the mobile phase is simple to prepare and economical. No extraction procedure is involved and there is no need to use internal standard. Also, the medium for dissolving the drug and the mobile phase (LC) or solvent for analysis (UV) is the same. The percentage recoveries of the proposed LC method (97.44–99.86%) are much higher than the earlier reported method [9] where the recovery was only 90.7–93.8%. 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. These methods were better when compared to other reported methods and thus can be used effectively, without separation

Table 5
Results of the assay of pure celecoxib and commercial formulations by the proposed methods

| Sample | Label claim (mg/capsule) | Percentage recovery | | | | | |
|---|---|---|---|---|---|---|---|
| | | UV method | | | LC method | | |
| | | Mean[a] | CV% | AR (%) | Mean[a] | CV% | AR (%) |
| Pure drug solution[b] | | 100.82 ± 0.17 | 0.17 | 99.8 | 99.20 ± 2.54 | 2.56 | 99.4 |
| Brand A | 100 | 95.60 ± 1.48 | 1.55 | 98.4 | 98.01 ± 1.55 | 1.58 | 98.5 |
| Brand B | 100 | 99.82 ± 2.54 | 2.54 | 99.8 | 99.86 ± 1.15 | 1.15 | 99.6 |
| Brand C | 100 | 96.67 ± 2.73 | 2.74 | 98.2 | 97.44 ± 2.21 | 2.27 | 98.9 |

CV, coefficient of variation; AR, analytical recovery.
[a] Mean and standard deviation for five triplicate determinations.
[b] 100 mg in 1000 ml.

Table 6
Two-way ANOVA test (without replication) for linearity in estimation of celecoxib in various commercial formulations by the proposed methods

| Source of variation | UV method | | | | | LC method | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS[a] | DF[a] | MS[a] | $F_{Calc}$ | $F_{Crit}$[b] | SS[a] | DF[a] | MS[a] | $F_{Calc}$ | $F_{Crit}$[b] |
| Between the brands | 28.8406 | 2 | 14.4203 | 4.8772 | 6.9443 | 9.5669 | 2 | 4.7834 | 1.2715 | 6.9443 |
| Within the brand | 20.2548 | 2 | 10.1274 | 3.4253 | 6.9443 | 2.2115 | 2 | 1.1057 | 0.2939 | 6.9443 |
| Error | 11.8267 | 4 | 2.9567 | | | 15.0487 | 4 | 3.7623 | | |
| Total | 60.9221 | 8 | | | | 26.8270 | 8 | | | |

[a] SS, sum of squares; DF, degree of freedom; MS, mean sum of squares.
[b] Theoretical value of $F(2, 4)$ based on two-way ANOVA test at $P = 0.05$ level of significance.

and interference, for routine analysis of celecoxib in pure form and its formulations and can also be used for dissolution or similar studies.

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