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UV- VISIBLE PHOTOMETRIC ESTIMATION OF COX 2 BLOCKER IN BULK AND ITS DOSAGE FORM

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ABSTRACT

A new simple, precise, sensitive, highly specific and economical ultraviolet spectrophotometric method for the determination of Celecoxib in bulk and its pharmaceutical Formulation (dispersible tablets and capsules) has been developed. The λ_{max} values of Celecoxib in methanol and 0.001N sodium hydroxide in the ratio 2:1 v/v were 253.2 nm. Beer's law is obeyed over concentration range of 8 to 22 µg/ml with correlation coefficient r > 0.9998. The result of analysis for the method has been validated stastically and by recovery studies.

KEYWORDS: Cox 2 Blocker; UV-VIS Spectrophotometric Estimation.

1. INTRODUCTION

Celcoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonami- de, a non-steroidal anti inflammatory drug (NSAID), is a highly selective COX-2 inhibitor and primarily inhibits this isoform of cyclooxygenase. Celecoxib is approximately 7.6 times more selective for COX-2 inhibition over COX-1 with minimal gastrointestinal adverse drug reactions. Pharmacological studies have shown that celecoxib is effective in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms.[1] Recent clinical trials performed have proved its efficacy in the treatment of cervical dysplasia[2], chemoprevention of colorectal cancer[3] and prostate cancer.[4]

Several analytical methods, such as UV spectrophotometric[5-7] high performance liquid chromatography [HPLC][8-13], spectrofluorimetry[14] and micellar electrokinetic chromatography (MEKC)[15] of celecoxib in bulk and pharmaceutical formulation have been reported. Various solvents were employed for different methods however; these solvents are suffering from one or other disadvantages like accuracy, stability, sensitivity and specificity.

Hence it is thought worthwhile to develop a simple and precise spectrophotometric method using methanol and 0.001N sodium hydroxide in the ratio 2:1 v/v as a solvent for the estimation of celecoxib in bulk and its dosage form.

2. EXPERIMENTAL

2.1. Instruments and reagents

Spectrophotometric analysis was carried out on a Systronic 2210 double beam spectrophotometer with a fixed slit width (2 cm) using a pair of 1 cm matched quartz cells. All weighing were performed on an electronic single pan balance (Citizen). Calibrated borosilicate glass wares were used in the study. Pure celecoxib sample was kindly provided by Zydus Cadila Laboatories (Ahmedabad, India). Celecoxib tablets, Celact (Formulation I, Sun Pharmaceutical Industries Ltd., Mumbai) and capsules, Zycel (Formulation II, Zydus Cadilla, Mumbai) were procured from local drug stores. Other chemicals and solvents were of analytical grade.

2.2. Selection of Solvent

Different solvents were tested to establish absorption maxima of celecoxib at 10 µg/ml concentration. The absorbance intensities followed the order methanol and 0.001N sodium hydroxide solution (2:1 v/v) methanol and 0.001N sodium hydroxide solution (1:1 v/v) > methanol (corrected by the corresponding blank). Accordingly, methanol and 0.001N sodium hydroxide solution (2:1 v/v) (λ_{max} 253.2 and absorbance intensity 0.575) was selected the best solvent. Results are presented in Table 1.

Table 1: Selection of solvent.

Concentration (µg/ml)	Solvent	λ_{max}	Absorbance
10	Methanol	248	0.354
10	Methanol and 0.1N sodium hydroxide solution	251.1	0.456
10	Methanoland0.001N sodium hydroxide solution	253.2	0.575

Among the three solvents, methanol and 0.001N sodium hydroxide (2:1 v/v) showed greater absorbance 0.575 nm at λ_{max} 253.2 and selected as a solvent for the estimation of celecoxib.

2.3. Preparation of Standard Stock Solutions

Standard stock solution was prepared by dissolving 50 mg of celecoxib in a mixture of methanol and 0.001N sodium hydroxide (2:1 v/v) to get a concentration of 1000 µg/ml.

Working solution was prepared by further diluting this stock solution to obtain a concentration of 100 µg/ml.

2.4. Preparation of calibration curve

To prepare calibration standards, 0.8 to 2.2 ml of working standard solutions were diluted to obtain final drug concentration of 8 to 22 µg/ml and linearity was studied. Linearity relationship was observed in the range 8 to 22 µg/ml (Fig. 1) against a reagent blank as reference at 253.2 nm (Table 2).

Table 2: Linearity table of Celecoxib.

Sl.No.	Concentration (µg/ml)	Absorbance
1	8	0.462
2	10	0.575
3	12	0.685
4	14	0.792
5	16	0.896
6	18	1.012
7	20	1.127
8	22	1.238

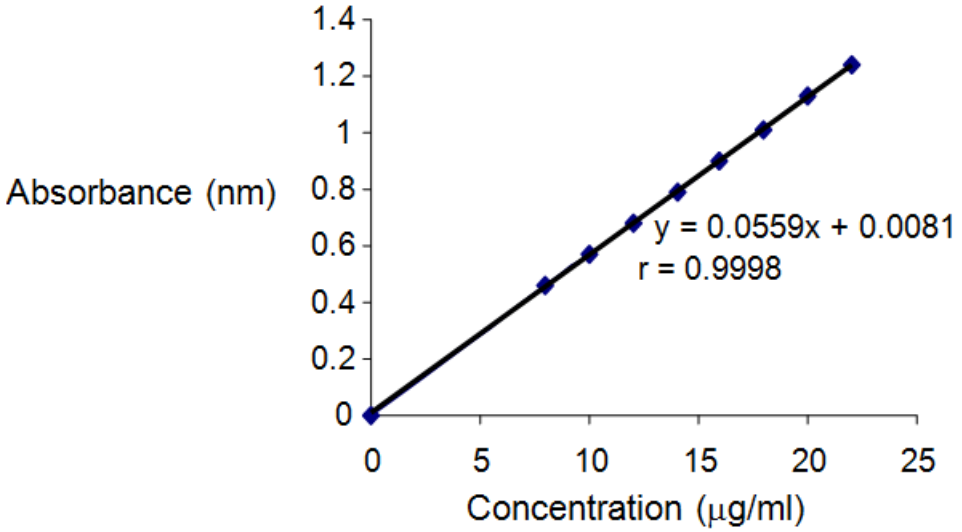

Fig.1. Calibration curve of celecoxib

2.5. Estimation of Celecoxib in tablets and capsules

20 tablets and capsules were weighed separately and powdered (content of capsules) and a portion of powder equivalent to 200 mg of celecoxib was then separately transferred to beaker and extracted with 100 ml of mixture of methanol and 0.001N sodium hydroxide (2:1 v/v) for 35 min. The suspension was filtered; the filtrate was diluted to 200 ml with the same

solvent. A 5 ml portion of this solution was diluted to 50 ml and then further diluted to obtain a concentration of 12 µg/ml, 14 µg/ml and 16 µg/ml of celecoxib. The absorbances of these solutions were determined at 253.2 nm against a reagent blank. The amount of celecoxib determined in both the sample is presented in Table 3.

Table 3: Analysis of formulations I and II by proposed method.

Formulation	Lablled amount of celecoxib(mg)	*Amount obtained (mg)	% Of drug present	% RSD
Celact (tablet)	200	197.74±0.631	99.11	0.531
Zycel (capsule)	200	195.91±0.720	98.30	0.605

*Each value is average of three determinations ± standard deviation

2.6. Validation criteria

2.6.1. Repeatibility

The repeatability of the method was established by carrying out (n = 8) analysis of the analyte (16 µg/ml) using the proposed method. The low value of % relative standard deviation (0.161) showed that the method is precise. The results obtained are shown in Table 4.

Table 4: Repeatability of the proposed method.

Concentration of celecoxib in (µg/ml)	Absorbances	Statistical analysis
16	0.895	Mean: 0.895 S.D: 0.00143 % R.S.D:0.161
16	0.893	
16	0.896	
16	0.896	
16	0.895	
16	0.894	
16	0.897	
16	0.897	

2.6.2. Accuracy

The accuracy of the method was evaluated by calculating recovery of celecoxib by standard addition method at concentration of 80%, 100%, and 120% of the target level in tablets and capsules. The results are presented in Table 5. The percent recovery was found to be 99.82% for tablets and 100.04% for capsules.

Table 5: Recovery data of tablets by the proposed method.

Sample ID	Concentration of pure drug (µg/ml)	Concentration of tablet formulation (µg/ml)	% Recovery of pure drug	Statistical analysis
S_1 : 80 %	8	10	99.33	Mean 99.82
S_2 : 80 %	8	10	99.78	S.D 0.5031
S_3 : 80 %	8	10	100.55	% RSD 0.5033
S_4 : 100%	10	10	99.98	Mean 99.23
S_5 : 100%	10	10	99.08	S.D 0.5900
S_6 : 100%	10	10	98.55	% RSD 0.5958
S_7 : 120%	12	10	100.64	Mean 100.21
S_8 : 120%	12	10	100.36	S.D 0.1765
S_9 : 120%	12	10	100.18	% RSD 0.1772

Table 6: Recovery data of capsules by the proposed method.

Sample ID	Concentration of pure drug (µg/ml)	Concentration of capsule formulation (µg/ml)	% Recovery of pure drug	Statistical analysis
S_1 : 80 %	8	10	100.66	Mean 100.6
S_2 : 80 %	8	10	100.62	S.D 0.2021
S_3 : 80 %	8	10	100.58	% RSD 0.2003
S_4 : 100 %	10	10	99.91	Mean 100.08
S_5 : 100 %	10	10	100.89	S.D. 0.5523
S_6 : 100 %	10	10	99.41	% RSD 0.501
S_7 : 120 %	12	10	99.37	Mean 99.49
S_8 : 120 %	12	10	99.59	S.D. 0.1712
S_9 : 120 %	12	10	99.54	% RSD. 0.185

2.6.3. Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies using three different concentrations of drug. In intra-day studies drug was analyzed on the same day while inter-day precision was determined by analyzing drug for three days over a period of one week and results are presented in Table 7.

Table 7: Results of precision studies.

	Inter-day		Intra-day	
Amount taken (µg/ml)	**amount found (µg/ml)**	**% RSD**	**amount found (µg/ml)**	**% RSD**
14	13.96	0.162	13.93	0.148
14	13.97		13.91	
14	13.94		13.92	
16	15.86	0.134	15.98	0.211
16	15.89		15.92	
16	15.86		15.86	
18	17.93	0.253	17.92	0.158
18	17.84		17.80	
18	17.97		17.96	

2.6.4. Ruggedness

Ruggedness of the proposed method was determined by carrying out the experiment on different instruments by different analyst under similar environmental condition and results are presented in Table 8.

Table 8: Results of ruggedness studies.

	Analyst 1		Analyst 2	
Amount taken (µg/ml)	**Amount found (µg/ml)**	**%RSD**	**Amount found (µg/ml)**	**%RSD**
16	15.87	0.138	15.92	0.130
16	15.93		15.93	
16	15.86		15.97	

Table 9: Validation parameters.

Parameters	Results
Absorption maximum(nm)	253.2
Beer's law limit (µg/ml)	8-22
Absorptivity	
Sandell's sensitivity (µg/cm^2/0.001)	567.2
% Relative standard deviation	
% Range of error	0.01812
0.05 confidence limit	0.161
0.01 confidence limit	
Limit of detection (µg/ml)	0.101
Limit of quantitation (µg/ml)	0.014
Correlation coefficient	0.082
Slope	
Intercept	0.2611
	0.9998
	0.055
	0.0081

3. RESULTS AND DISCUSSION

The present study was carried out to develop a simple, accurate and sensitive UV-visible photometric method for the determination of celecoxib in tablets and capsules. In the present investigation methanol and 0.001N sodium hydroxide in the ratio 2:1 v/v was found to be a better solvent. Linearity was observed in the range 8-22 µg/ml. The results showed that an excellent correlation (r > 0.9998) exists between response factor and concentration of drug within the concentration range indicated above. Limit of detection (LOD) and limit of confidence (LOQ) were determined. The values of LOD and LOQ were 0.082 and 0.261 µg/ml, respectively (Table 9). The experiment was repeated three times in a day for intra-day and on three different days over a period of one week for inter-day precision. The method was found to be precise as % RSD for intra-day and inter-day precision were 0.181 and 0.186, respectively (Table 7). Accuracy of the method was calculated by % mean recovery (n = 3). The recovery studies were carried out by the addition of standard analyte to the preanalyzed sample. The concentrations of standard spiked to both the sample were 8 to 12 µg/ml of celecoxib. The mean % recovery was found to be 99.84 for tablets (Celact) and 100.04 for capsules (Zycel). For ruggedness, study was carried out for two different parameters i.e., instrument and analyst. Low values of % RSD demonstrated the ruggedness of the proposed method. The assay values for the marketed formulations I and II were found to be within the limit as listed in Table 3. The low RSD indicated the suitability of the proposed method for routine analysis of celecoxib in pharmaceutical dosage forms.

4. CONCLUSIONS

The proposed method was found to be simple, precise, accurate and sensitive. High percentage recovery showed that the method was free from interference of excipients used in the formulation. Values of LOD and LOQ showed that the proposed method was sensitive enough to analyze the drug in bulk as well as in its pharmaceutical formulation. Hence the proposed method renders suitable for routine analysis in quality-control laboratories.

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