

Selective RP-HPLC Determination of Celecoxib in Capsules

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A simple and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of celecoxib in capsules. The quantification was carried out using a Partisil C-18 octa decyl silane column 250 × 4.6 mm i.d., 5 µm particle size in isocratic mode, with mobile phase comprising acetonitrile and water in the ratio 60 : 40 (v/v). The flow rate was 1.0 mL/min and the detection was carried out at 254 nm. The retention time of the drug was 6.35 min and the method produced linear response in the concentration range of 1–40 µg/mL. The proposed method was statistically evaluated and can be applied for routine quality control analysis of celecoxib in capsules.

Key Words: RP-HPLC, Celecoxib, Capsules.

INTRODUCTION

Celecoxib¹ is a non-steroidal anti-inflammatory drug. Chemically it is (4-(4-methyl phenyl)-3-(trifluoro methyl)-1H-pyrazole-1-yl)-benzene sulfonamide). It acts by the inhibition of prostaglandin synthesis through the selective inhibition of COX-2 isoenzyme, which is upregulated during inflammatory process. It is indicated during acute or chronic pain associated with inflammation, dental pain, dysmenorrhoea, osteo-arthritis and rheumatoid arthritis. Literature survey reveals that few HPLC^{2,3} methods for estimation of celecoxib were reported. The proposed method was simple, fast, accurate and precise for the estimation of celecoxib in capsules.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Shimadzu HPLC class VP series) with two LC-AT VP pumps, variable wavelength programmable UV-Visible detector SPD-10A VP, CT0-10 AS VP column oven, SCL-10A VP system controller (Shimadzu) and RP C-18 column (250 × 4.6 mm i.d.; particle size 5 µm) was used.

Pure sample of celecoxib was received from Dr. Reddy's Laboratories; Hyderabad. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai and triple distilled water was used for preparing the mobile phase.

Chromatographic conditions: The chromatographic column used was a 250 × 4.6 mm Partisil C-18 with 5 µm particles. Both acetonitrile and triple distilled water were filtered through 0.4 µm membrane filter and sonicated before use. The

flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 60 : 40 (acetonitrile : water). The column was maintained at 30°C and the detection was carried out by UV detector at 254 nm. The injection volume was 20 μ L.

Procedure: About 50 mg of pure sample of celecoxib was weighed accurately and transferred to a 50 mL volumetric flask and dissolved in 25 mL of the mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 1–40 μ g/mL were made in 10 mL volumetric flasks. The solutions prepared as above were filtered through 0.4 μ m membrane filter and then 20 μ L of filtrate was injected each time into the column at a flow rate of 1 mL/min. Evaluation of the drug was performed with UV-Visible detector at 254 nm. Peak area was recorded for all the peaks. The plot of peak area vs. the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed. This regression equation was used to estimate the amount of celecoxib in capsules.

Estimation of Celecoxib in capsule dosage forms: Capsule powder equivalent to 100 mg was taken in 100 mL volumetric flask and 50 mL mobile phase was added. The solution was sonicated for complete solubility of the drug, made up to the mark with the mobile phase and filtered through a 0.4 μ m membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to volume with the mobile phase and mixed well. Each of these solutions (20 μ L) was then injected five times into the column. From the peak areas, the drug content in the capsules was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise HPLC method for the analysis of celecoxib in capsule dosage forms. A typical chromatogram was shown in Fig. 1. The retention time for celecoxib was 6.35 min.

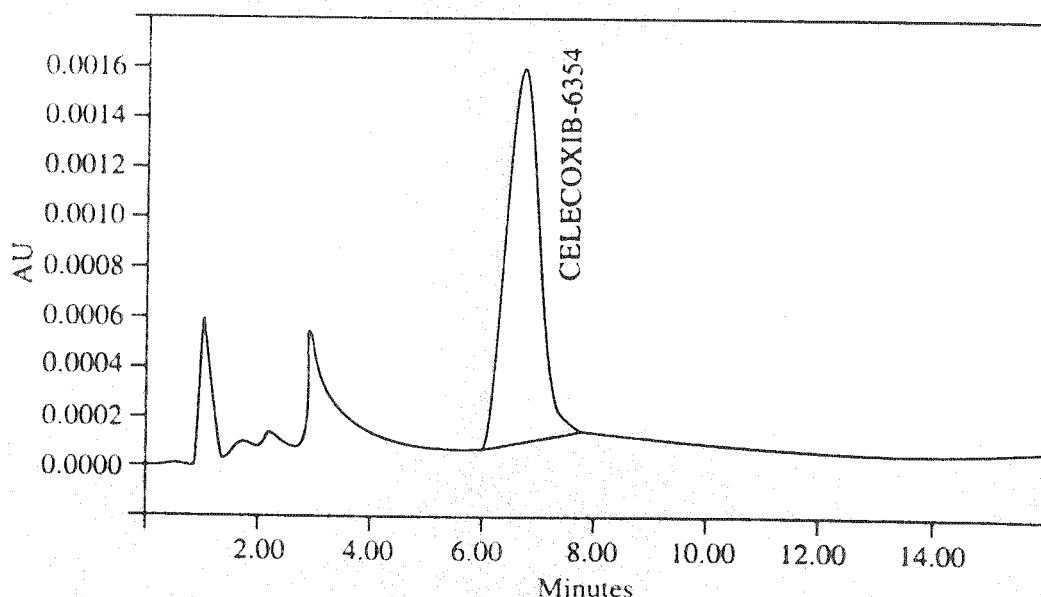


Fig. 1. Model chromatogram for celecoxib

The peak areas from such different concentrations set up as above were calculated and are shown in Table-1. A good linear relationship ($r = 0.9999$) was observed between the concentration of celecoxib and the respective peak area. The regression curve was constructed by linear regression fitting and its mathematical expression was $y = 5030.06x + 46.09$ (where y is the peak area and x is the concentration of celecoxib). The intra-day and inter-day variations of the method were determined using three replicate injections of four different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise.

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration ($\mu\text{g/mL}$)	Peak area
1	5031
2	10042
3	16214
4	20121
5	24210
10	50321
20	100710
40	201275

Regression Equation from 1–40 $\mu\text{g/mL}$
 $Y = 5030.06X + 46.09$ ($r = 0.9999$)

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of celecoxib ($\mu\text{g/mL}$)	Observed concentration of celecoxib ($\mu\text{g/mL}$)			
	Intra-day		Inter-day	
	Mean ($n = 3$)	Coefficient of variance (%)	Mean ($n = 3$)	Coefficient of variance (%)
5	4.91	0.81	4.83	0.99
10	9.83	0.34	9.78	0.57
15	14.73	0.31	14.67	0.51
20	19.51	0.26	19.35	0.59

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values are shown in Table-3. About 99.8% of celecoxib could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

TABLE-3
RESULTS OF RECOVERY STUDY

Amount of drug added (μg)	Recovery from drug solution		Recovery from capsule formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
10	10.02	100.2	10.03	100.3
20	19.96	99.8	19.99	99.95
30	30.05	100.16	30.01	100.03

The drug content in the capsules was quantified using the proposed analytical method. The mean amount of celecoxib in three different brands of capsule dosage forms is shown in Table-4. The capsules were found to contain 99.94–100.07% of the drug. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of celecoxib in capsule dosage forms within a short analysis time. The method was duly validated by evaluation of the required parameters.

TABLE-4
ASSAY OF CELECOXIB IN CAPSULE DOSAGE FORMS

Brand	Labelled amount of drug (mg)	Mean (\pm s.d.) amount (mg) found by the proposed method (n = 5)	Mean (\pm s.d.) % labelled amount (n = 5)
I	200	199.97 \pm 0.03	99.96 \pm 0.05
II	200	199.88 \pm 0.23	99.94 \pm 0.12
III	200	200.03 \pm 0.01	100.07 \pm 0.02

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