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RESEARCH PAPER

A Stability-Indicating HPLC Method to Determine Celecoxib in Capsule Formulations

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ABSTRACT

A simple and accurate high-performance liquid chromatographic (HPLC) method was developed to determine Celecoxib in capsule formulations. The drug was chromatographed on a reversed-phase C-18 column. Eluents were monitored at a wavelength of 251 nm using a mixture (85:15) of methanol and water. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for linearity, accuracy, precision, and selectivity. Due to its simplicity and accuracy, we believe that the method will be useful for routine quality control analysis.

INTRODUCTION

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide) is a diaryl pyrazol class of non-steroidal anti-inflammatory drug (NSAID). It exhibits anti-inflammatory, analgesic, and antipyretic activities by selectively inhibiting cyclooxygenase-2 (COX-2) prostaglandin synthesis. It is indicated to relieve the signs and symptoms of rheumatoid arthritis and osteoarthritis.[1] Celecoxib received priority review rating and was approved by the U.S. FDA on 12th December, 1998. It is available as 100 mg and 200 mg capsule dosage forms. Its metabolism is primarily mediated via cytochrome P450 2C9. Three inactive metabolites, a primary alcohol, the corresponding

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carboxylic acid, and its glucuronide conjugate have been identified in human plasma.

A literature survey revealed some high-performance liquid chromatographic (HPLC) methods for Celecoxib determination from human plasma,[2–4] no method was found for Celecoxib determination in formulations.

This article describes the development and validation of a stability-indicating method for the assay of Celecoxib in capsules.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a dual piston reciprocating pump from Jasco Corp., Japan (model PU 1580), ultraviolet (UV)-visible detector from Jasco Corp., Japan (model UV 1575), and a rheodyne injector.

Materials, Reagents, and Chemicals

An authentic working standard for Celecoxib was gifted by Khandelwal Laboratories, Mumbai, India. Methanol (HPLC grade) was purchased from S.D. Fine-Chem Ltd., Mumbai, India. Water of HPLC grade was obtained by glass distillation of normal distilled water and passed through a 0.22-μm membrane filter.

Local commercial capsule formulations were used for analysis. They contained 100 mg of Celecoxib in a matrix of lactose monohydrate, crosscarmellose sodium, sodium lauryl sulfate, magnesium stearate, povidone sodium, and titanium dioxide.

Chromatographic Conditions

The experiment was performed on a 250 mm × 4.6 mm (ID), 5-μm particle HiQSil RP C-18 column.

The mobile phase consisted of 85% methanol and 15% water. The mobile phase was filtered through a nylon membrane (pore size 0.22 μm). The mobile phase was degassed before use. The chromatography was performed at room temperature using a flow rate of 0.8 mL/min. The run time was 10 min. Eluents were monitored at 251 nm. The volume of each injection was 20 μL.

Working Standard Solution

A standard solution of Celecoxib, 1 mg/mL, was prepared in methanol.

Sample Solution

For the preparation of the sample solution, powder from 10 capsules was weighed. Powder equivalent to 100 mg Celecoxib was placed in a 100-mL volumetric flask, 70 mL of methanol was added, and the flask was kept at room temperature for an hour. The mixture was then diluted to 100 mL with methanol, thoroughly mixed, and filtered.

Preparation of Solutions Used for Assay Validation

For the study of Celecoxib response linearity, different standard solutions were prepared in mobile phase at concentrations ranging from 1 to 100 μg/mL.

System precision was evaluated by performing five consecutive injections of Celecoxib standard solution. Method precision was evaluated by six repeated assays of the same lot of commercial formulation. Assay accuracy was assessed at 50%, 75%, 100%, 125%, and 150% Celecoxib by recovery experiments.

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the method. Intentional degradation was attempted using acid, base, and hydrogen peroxide. A degradation sample was prepared by taking 10 mg Celecoxib in a 100-mL volumetric flask followed by addition of 70 mL methanol. Celecoxib was dissolved properly by shaking the flask. The volume was adjusted to 100 mL with methanol. Degradation experiments were performed by taking 10 mL of the above solution in each of three different 50-mL round-bottomed flasks. To the first flask, 10 mL of 1 N HCl was added for acidic degradation. To the second flask, 10 mL of 1 N NaOH was added for basic degradation. To the third flask, 10 mL of 30% H_2O_2 was added for oxidative degradation. All the three flasks were refluxed for about 8 hr. After completing the degradation treatments, samples were allowed to cool to room temperature and treated as follows. First, two samples were neutralized by

adding 1 N Na_2CO_3 to the first flask and 1 N HCl to the second flask. Hydrogen peroxide from the third flask was destroyed by adding 1 N sodium bisulfite solution. Samples were injected and analyzed against a control sample (lacking degradation treatment).

Procedure

All solutions were prepared on a weight basis and solution concentrations were also measured on a weight basis to avoid the use of an internal standard. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Acceptable results for the number of theoretical plates, tailing factor, precision, and detector linearity criteria were required before sample analysis.[5] Quantification was accomplished using an external standard method. Each solution was injected in triplicate and the relative standard deviation (RSD) was required to remain below 2% on a Celecoxib peak area basis.

RESULTS AND DISCUSSION

System Suitability

A suitability test was applied to representative chromatograms to check various parameters such as column efficiency, peak tailing, RSD of peak area, and capacity factor. The results obtained were within acceptable limits (Table 1). Thus, the system meets suitability criteria.

Selectivity

Selectivity of the developed method was assessed by sample degradation studies. The percentage of Celecoxib recovered is shown in Table 2. Degradation peaks, when observed, were resolved from the Celecoxib peak (Fig. 1A–D).

Table 1

System Suitability Parameters

No.	Parameter	Value	Acceptance Limits
1	Theoretical plates	> 3500	More than 3000
2	Tailing factor	0.85	Less than 2
3	RSD of peak area	0.675	Less than 2%
4	Capacity factor	1.15	Not less than 1

Linearity

Five solutions containing Celecoxib at concentrations ranging from 2 to 50 μg/mL were analyzed (Table 3). The curve of the peak area vs. concentration proved linear.

The regression line equation was $y = 4E + 06x - 8338.4$, with a correlation coefficient of $r^2 = 0.9995$.

The plot of the peak area response vs. micrograms injected is shown in Fig. 2.

Assay

The Celecoxib contents found in capsules by the proposed method are listed in Table 4. Low RSD values (<1%) indicate that the method is precise and accurate.

Precision

Precision was considered at two levels of International Conference on Harmonization (ICH) suggestions:[6] repeatability and intermediate precision.

Repeatability was evaluated by analyzing five replicate injections of Celecoxib standard solution, giving an RSD of 0.675 and minimal variation in retention time (Table 5).

Intermediate precision was determined by carrying out two accuracy assays on two lots of commercial formulations one week apart by two different operators with the same equipment (Table 6). For each accuracy assay the mean values were 99.46% and 99.51%.

Table 2

Degradation of Celecoxib

Condition	Time (hr)	Recovery (%)	R_T of Degradation Products (min)
Acid 1 N HCl, reflux	8	48	3.077
Base 1 N NaOH, reflux	8	70	3.1
H_2O_2 30%, reflux	8	82	3.11, 6.082

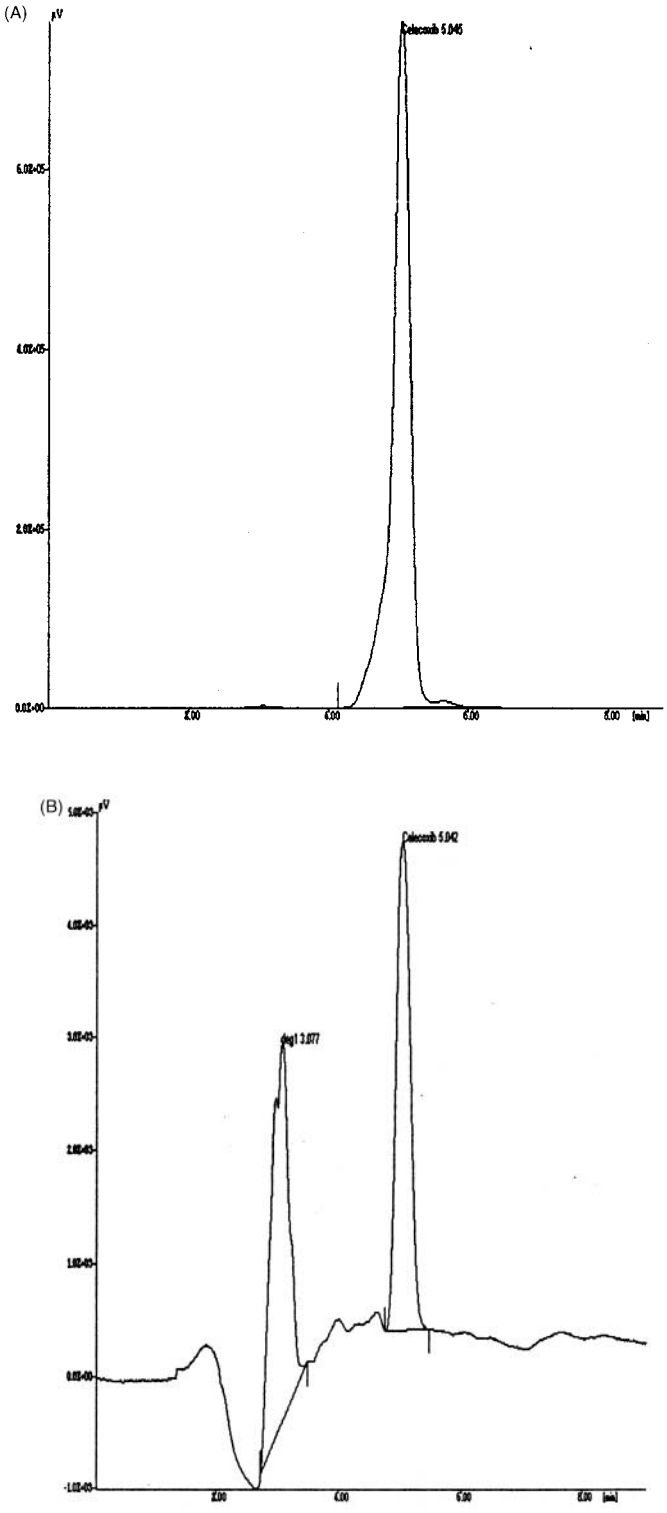
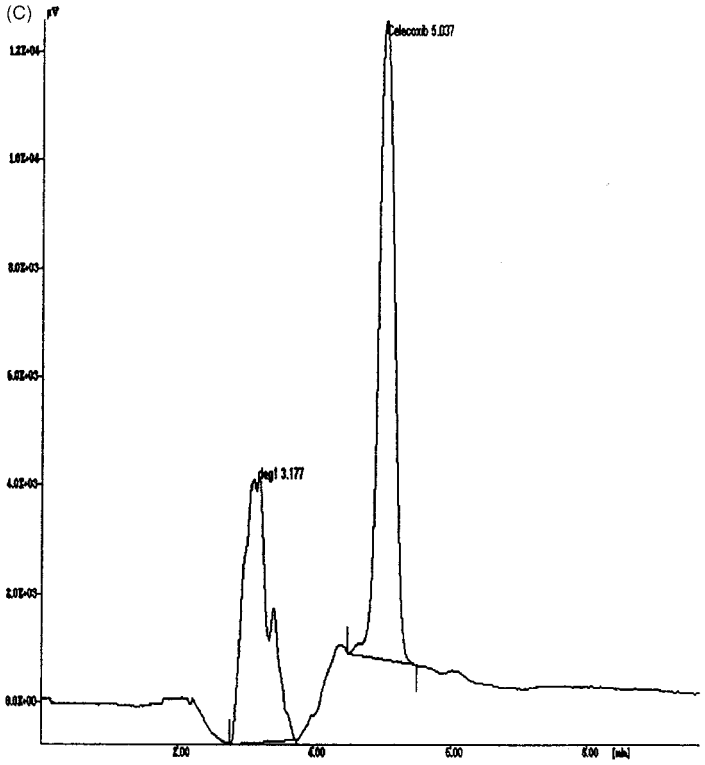


Figure 1. Chromatogram of (A) standard Celecoxib (control sample, lacking degradation); (B) acidic degradation (deg1 = degradation product); (C) basic degradation (deg1 = degradation product); (D) oxidative degradation (deg1, deg2 = degradation products).



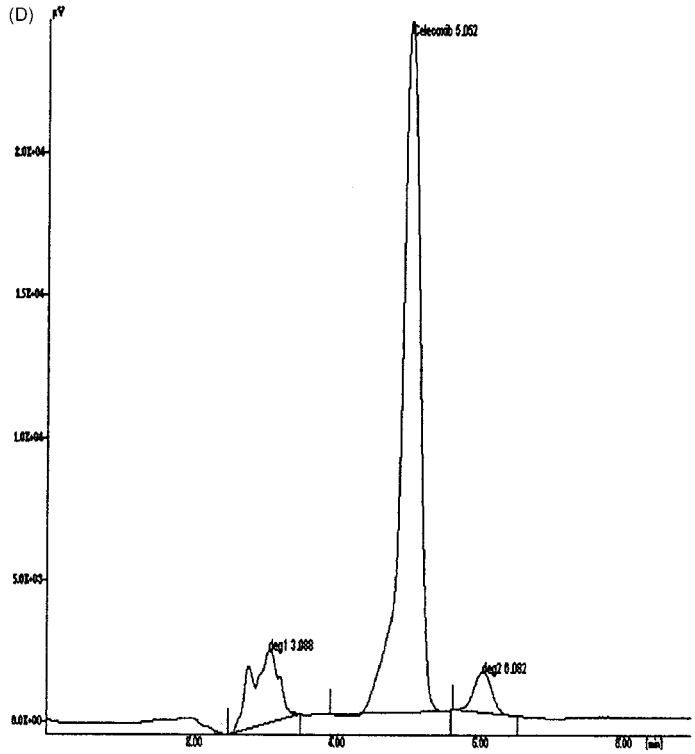


Figure 1. Continued.

Table 3

Linearity Data

Conc. (μg/mL)	Injected (μg)	Average Peak Area Response	RSD (%)
2	0.04	171,530	1.2
5	0.1	408,935	1.6
10	0.2	786,263	1.4
20	0.4	1,528,637	0.5
50	1.0	3,998,243	1.8

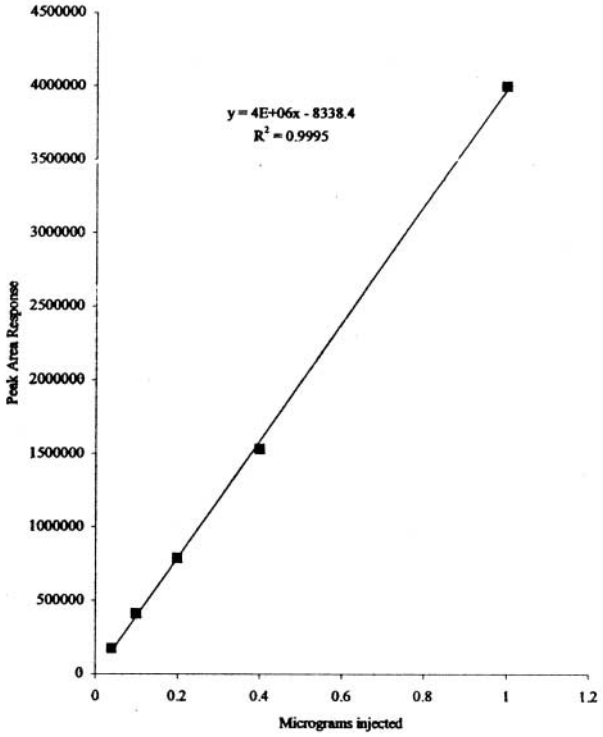


Figure 2. Linearity of peak area response vs. micrograms injected.

Accuracy

The percentage recovery of Celecoxib of lot 1 ranged from 96.89% to 102.21% with a mean value of 99.66%. Lot 2 data ranged from 97.09% to 102.62% with a mean value of 99.51% (Table 6).

The Celecoxib recovery achieved shows that there was no interference from excipients present in the capsule.

Table 4

Precision of the Assay Method

Sample No.	Celecoxib (%)	RSD (%)
1	102.3	0.75
2	104.2	0.85
3	102.1	0.62
4	105.3	0.98
5	103.7	0.83
6	101.8	0.97
Mean	103.23	1.35

Table 5

System Precision Data

No.	Peak Area	Retention Time (min)
1	395,066.7	4.963
2	39,272.8	4.965
3	400,058.2	4.962
4	396,452.1	4.973
5	396,991.3	4.965
%RSD	0.675	0.087

Stability of Sample Solution at Room Temperature

The sample solution injected after 8 hr failed to show any appreciable change (Table 7).

CONCLUSIONS

The investigation has developed a stability-indicating HPLC procedure for the determination of Celecoxib in dosage form. This method proved to be simple, since it does not contain a buffer system. Also, solution concentrations were measured on a weight basis to avoid the use of an internal standard. Calibration plots obtained using this method of analysis resulted in a coefficient of correlation of 0.9995. The precision of the Celecoxib chromatographic response was calculated from five replicate injections of the same solution prepared at the nominal analytical concentration, and showed an RSD of 0.675%.

Table 6

Recovery Analysis

%W/W	Amount Present (mg)	Amount Found (mg)	Recovered (%)	Average Recovered $n=3$
Lot 1				
	14.77	14.45	97.83	
50	15.65	15.90	101.60	100.24
	15.55	15.75	101.29	
	17.58	17.03	96.89	
75	18.00	17.99	99.94	98.42
	17.29	17.02	98.43	
	19.56	19.22	98.26	
100	20.51	20.97	102.21	99.95
	20.53	20.40	99.37	
	22.51	22.30	99.11	
125	23.40	23.31	99.61	100.00
	22.71	23.00	101.28	
	24.64	24.54	99.51	
150	24.90	24.56	98.39	98.70
	24.95	24.50	98.20	
Mean ($n=12$)				99.46
Lot 2				
	15.20	14.90	98.02	
50	15.30	15.35	100.32	99.11
	14.75	14.60	98.98	
	17.85	17.70	99.15	
75	17.45	17.60	100.85	99.03
	17.20	16.70	97.09	
	20.50	20.20	98.54	
100	20.25	20.50	101.24	100.09
	19.75	19.85	100.51	
	22.57	22.04	97.69	
125	22.46	23.05	102.62	100.40
	22.60	22.80	100.89	
	24.70	24.37	98.66	
150	24.40	24.10	98.77	98.93
	24.96	24.80	99.36	
Mean ($n=12$)				99.51

According to recovery studies performed at different percentage levels, the extraction of the active component was shown to be quantitative.

Selectivity was demonstrated, showing that the Celecoxib peak was free of any interference from degradation products.

Table 7

Stability of Sample Solution

Time	Celecoxib Peak Area	Mean Area	% Decrease in Area
Initial (0.0 hr)	395,066.5		
	392,720.8	395,948.5	
	400,058.1		
8.0 hr	393,491.0		0.8
	393,057.4	392,702.0	
	391,557.6		

The analytical method has been shown to be stability-indicating through the analysis of samples stressed under either acidic or basic conditions with applied heat, indicating that the proposed method can be used in a stability assay.

The proposed reversed-phase HPLC method is simple, precise, rapid, and selective for the determination of Celecoxib and may be employed for its assay in dosage formulations.

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