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A Novel RP-HPLC method for the Quantification of Celecoxib in Formulations

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of celecoxib in tablet dosage form. Isocratic elution at a flow rate of 1.5ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: ACN: 60:40 (V/V). The UV detection wavelength was 220 nm and 20µl sample was injected. The retention time for celecoxib was 3.57 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of celecoxib in tablet dosage form and bulk drug.

Keywords: celecoxib, RP-HPLC, UV detection, recovery, precise, 220 nm

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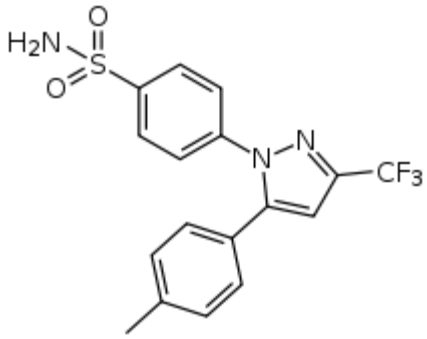
INTRODUCTION

Celecoxib is a sulfa non-steroidal anti-inflammatory drug (NSAID) and selective COX-2 inhibitor used in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis. It is marketed by Pfizer. It is known under the brand name Celebrex or Celebra for arthritis and Onsenal for polyps.



Celecoxib is licensed for use in osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, ankylosing spondylitis and to reduce the number of colon and rectal polyps in patients with familial adenomatous polyposis. It was originally intended to relieve pain while minimizing the gastrointestinal adverse effects usually seen with conventional NSAIDs. In practice, its primary indication is in patients who need regular and long term pain relief: there is probably no advantage to using celecoxib for short term or acute pain relief over conventional NSAIDs, except in the situation where non-selective NSAIDs or aspirin cause cutaneous reactions (urticaria or "hives"). In addition, the pain relief offered by celecoxib is similar to that offered by paracetamol (acetaminophen). Pfizer sells celecoxib under the brand name Celebrex, and is available as oral capsules containing 50 mg, 100 mg, 200 mg or 400 mg of celecoxib.

MATERIAL AND METHODS

Materials

Working standard of celecoxib was obtained from well reputed research laboratories. HPLC grade water, methanol, Acetonitrile was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), A manual Rheodyne injector with a 20 µl loop

was used for the injection of sample. PEAK LC software were used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of celecoxib were scanned in the range of 200 -400 nm against mobile phase as a blank. celecoxib showed maximum absorbance at 260 nm. So the wavelength selected for the determination of celecoxib was 220 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of a Methanol, Acetonitrile 60:40 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.5 ml/min. Detection was performed at 220 nm with 10 min runtime.

Standard and sample solutions

A 10 mg amount of celecoxib reference substance was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 ml volumetric flask to obtain 80 ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations. A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of, celecoxib was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 30 ml mobile phase were added and the solution was sonicate for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 10 µg/ml.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria

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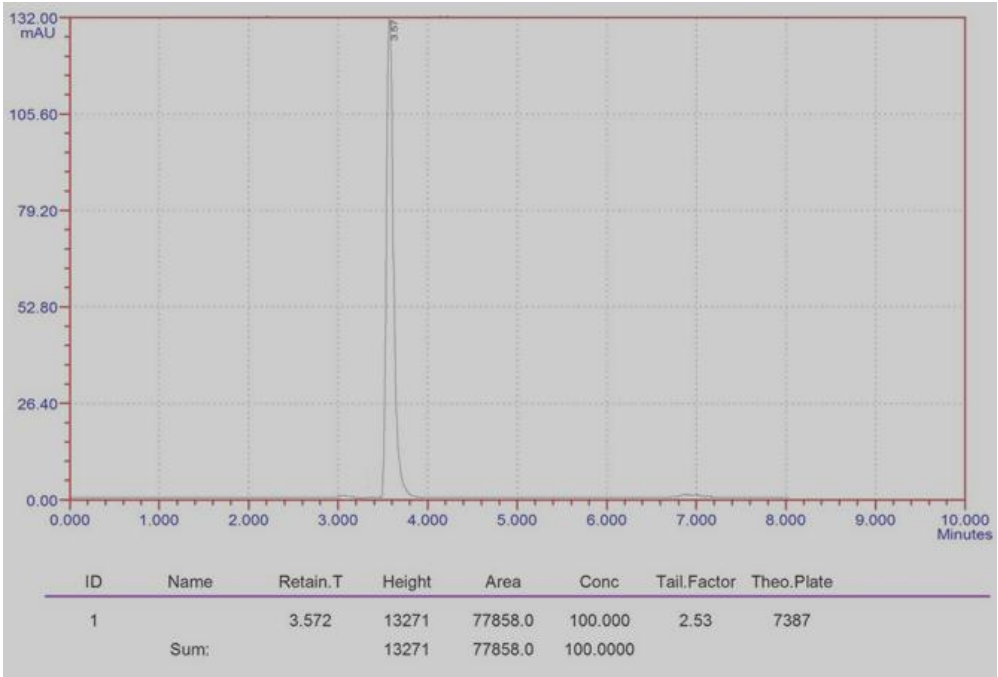
set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table 1: System suitability parameters

Mobile phase	Methanol : Acetonitrile 60:40 (v/v)
Pump mode	Isocratic
pH	5.2
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	220nm
Injection Volume	20 µl
Flow rate	1.5 ml/min
Run time	10 minutes
Retention Time	3.57 minutes

Figure 2: Typical chromatogram for Celecoxib



Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 20,30,40,50,60,70,80 µg/ml. for celecoxib. The linearity

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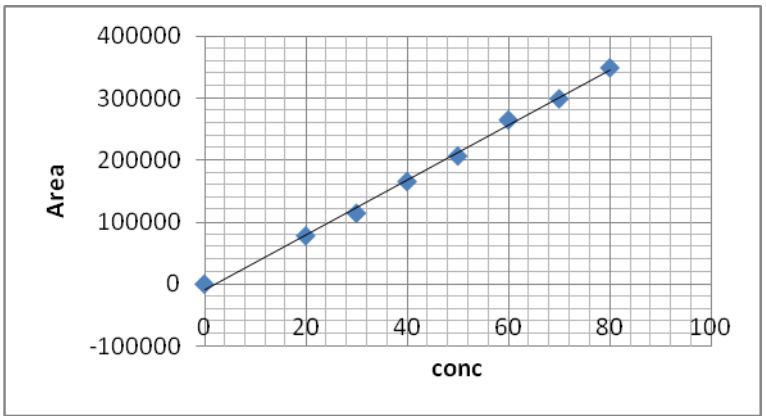
of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -17923 + 4577x (r= 0.998). Linearity values can shown in Table: 2

Level	Concentration of Lornoxicam In ppm	peak area
Level - 1	20	77858
Level - 2	30	114242
Level - 3	40	165434
Level - 4	50	206673
Level - 5	60	264737
Level - 6	70	298931
Level - 7	80	348893
Range:6ppm - 12ppm	Slope Intercept Correlation coefficient	4577 -17923 0.998

Table 1.



Graph 1.

Precision

To study precision, six replicate standard solutions of celecoxib(80 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be 1.23 which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3

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Precision Results for celecoxib:

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD (Acceptance criteria ≤ 2.0%)
celecoxib	80	1	368035	**1.23**
		2	366761	
		3	372462	
		4	369180	
		5	365588	
		6	358893	

Table 3.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After1 ppm dilution Peak was not clearly observed, based on which 1ppm is considered as Limit of Detection and Limit of Quantification is 3.3 ppm.

Parameter	Measured Value
Limit of Quantification	3.3 ppm
Limit of Detection	1.0 ppm

Table 4.

Accuracy:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. The standard addition method was performed at 50%, 100% and 150% level of 20 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table. Satisfactory recoveries ranging from to 98%, to 101% were obtained by the proposed method. This indicates that the proposed method was accurate.

Recovery Results

	Conc. ppm	Area	% of recovery	RSD
50%	30	114601	100.3	1.22
	30	115119	100.7	
	30	112498	98.4	
100%	40	166777	100.8	1.35
	40	162392	98.1	
	40	165066	99.7	

150%	50	205844	99.5	1.20
	50	203728	98.5	
	50	208709	100.9	
			Mean:99.65	Mean:1.25

CONCLUSION

The proposed method for the assay of celecoxib in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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