

Original Article

Development and validation of RP-HPLC method for the assay of Celecoxib capsule

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ABSTRACT

Celecoxib is 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene-1-sulfonamide. A simple and accurate reversed phase liquid chromatography method (HPLC) method was developed for the quantitative estimation of celecoxib, a selective COX-2 inhibitor in capsule formulations. The drug was chromatographed on a reversed-phase C-18 column. Eluents were monitored at a wavelength of 220 nm using a mixture (600:400:1:1) of acetonitrile, Water, Triethylamine and Ortho-phosphoric acid. The retention time of Celecoxib was found to be 9.5 minutes. The flow rate of the mobile phase was 1.0 ml/min at room temperature. The percentage recovery lies in the range of 99.53%–99.75%. 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, selectivity and intermediate Precision. Due to its simplicity and accuracy, we believe that the method will be useful for routine quality control analysis. The method was validated as per ICH guidelines.

1. INTRODUCTION

Celecoxib is a sulfa non-steroidal anti-inflammatory drug (NSAID) (Fig.1) 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 [1,2]. It is marketed by Pfizer under the brand name *Celebrex* or *Celebra* for arthritis. Celecoxib is available by prescription in capsule form. Information collected from previous research has played an important role to develop for quantitative estimation of celecoxib from capsule dosage form [3,4]. It is used for relief and management of osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis, acute pain, primary dysmenorrhea and oral adjunct to usual care for patients with familial adenomatous polyposis [5,6]. The present study focused to develop and validate the RP-HPLC method for the assay of celecoxib capsule.

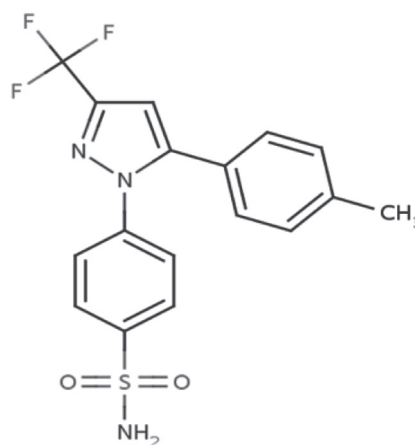


Fig. 1. Chemical Structure of Celecoxib

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

Ultra gradient grade Acetonitrile, water and Triethylamine and O-Phosphoric acid was prepared in the ratio of (600:400:1:1) was purchased from Merck Ltd. Acetonitrile of HPLC grade was obtained from Sigma-Aldrich Pvt. Ltd. Orthophosphoric acid (AR Grade) and Triethyl amine was supplied by Fisher Scientific Ltd (Mumbai, India) and Merck specialties Pvt. Ltd. respectively. All aqueous solutions and buffers were prepared using water that was purified using Millipore-Qs Gradient A10s (Millipore). Celecoxib Working standard and test sample were procured from Central Drug Laboratories and Akums Drugs & Pharmaceuticals Ltd. respectively.

2.2 HPLC Instrumentation and Analytical Conditions

The liquid chromatography separation was performed using a Shimadzu scientific instrument (Shimadzu Corporation; Kyoto, Japan) UFLC-Auto sampler and HPLC-1500 series (Water 1515) Isocratic Pump and (Water 2487) dual Lambda absorbance detector (WATERS) with Model No. Alliancee 2695 and FTIR (8400S). Liquid chromatographic separations were achieved using Lichro CART C-18, 250 mm × 4.6 mm, 5 μm (Merck Scientific, USA) and Inertsil ODS Column (150 × 4.6 mm), 5 μm OHS10077, consisting of C18 which is manufactured by G.L. Sciences, USA. Magnetic stirrer (Remi equipments Pvt. Ltd.), Electronic Balance (Mettler), KBR Press (Technosearch Ltd.), Ultra Sonicator (Spectral Lab), pH Meter (Eutech instruments Ltd.), Digital Weighing Balance (Sartorius) were used during the process of Validation. An injection volume of 20 μL was used for each analysis. Mobile phase consisted of Acetonitrile, water and Triethylamine and O-Phosphoric acid (600:400:1:1). The flow rate of the mobile phase was set at 1.0 mL/min.

2.3 Preparation of Mobile Phase

A mixture of Acetonitrile, water and Triethylamine and O-Phosphoric acid prepared in the ratio of (600:400:1:1). The solution was filtered through 0.45 μ nylon membrane filter and degas.

2.4 Preparation of Standard Solution and Test Solution

2.4.1 Preparation of Standard solution

50 mg. of Celecoxib working standard was weighed accurately and transferred into a 50 ml. volumetric flask and 10 ml of acetonitrile was added. Volumetric flask was sonicated to dissolve the contents and make up the volume 50 ml with mobile phase. Solution was filtered through 0.45 μ nylon membrane filter.

2.4.2 Preparation of Test Solution

20 capsules were selected from composite sample and open each capsule with out loosing any part of shell, remove the contents as complete as possible and crush the content finely. Finely crushed powder was accurately weighed and transferred equivalent to

about 50 mg of Celecoxib into a 50 ml. volumetric flask. 10 ml. of acetonitrile was added and shake well to dissolve and make up the volume 50 ml with mobile phase. Solution was filtered through 0.45 μ nylon membrane filter. [7]

2.5 Method Validation

A full method validation was performed according to guide- lines set by the USFDA & ICH Guidelines. [8-10] The validation of this procedure was performed in order to evaluate the method in terms of selectivity, sensitivity, range, the linearity of response, accuracy, precision and intermediate precision.

2.5.1 Specificity

Equal volume (about 20 μl.) of standard preparation and test preparation were separately injected into the chromatograph. Chromatograms were recorded and measured the responses for major peaks.

2.5.2 Linearity

Equal volume (about 20 μl.) of standard preparation and test preparation were separately injected at different concentration 14000 mcg to 26000 mcg into the chromatograph. Chromatograms were recorded and measured the responses for major peaks.

2.5.3 Precision

Equal volume (about 20 μl.) of standard preparation and test preparation were separately injected into the chromatograph. Chromatograms were recorded and measured the responses for major peaks.

2.5.4 Accuracy

It was obtained by Recovery studying using the standard addition method, Equal volume (about 20 μl.) of standard preparation and test preparation were separately injected into the chromatograph. Chromatograms were recorded and measured the responses for major peaks.

2.5.5 Intermediate Precision

Equal volume (about 20 μl.) of standard preparation and test preparation were separately injected into the chromatograph. Chromatograms were recorded and measured the responses for major peaks.

3. RESULTS

3.1 Optimization of the Chromatographic Condition

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of celecoxib in pharmaceutical formulations. Mobile phase tried for this purpose were Water: acetonitrile (90:10 V/V), acetonitrile: Water (70:30 V/V), acetonitrile: water (60:40 V/V), The condition that gave the best resolution and symmetry was selected. Same solvent system was used for the extraction of the drug from the formulation containing excipients which was used for quantification. (Table 1).

Table 1. Trial Taken For Optimization Condition

Sr. No.	Trails Taken	Observation	Remarks
1	Mobile Phase: Buffer : Solvent Mixture: (40:60) v/v Flow rate 1.0 ml/min Detector wavelength : 230 nm Injection volume :10 µL Column: Inertsil ODS-2, (150 × 4.6 mm), 5µm	Tailing and Retention time High	Not Satisfactory
2	Mobile phase : Buffer: Solvent Mixture: (40:60) v/v Flow rate:1.3 ml/min Column: Inertsil ODS-2, (150 x4.6mm),5µm	Retention time satisfactory but low theoretical plate	Not Satisfactory
3	Column: Inertsil ODS-2, (150 × 4.6 mm), 5µm Flow rate: 1.0 ml/minute Detector: UV Detector wavelength: 230 nm Injection volume: 10 µL Run time: 8 minute Diluent: Buffer: Acetonitrile (50:50) v/v Mobile phase: Buffer: Solvent Mixture: (50:50) v/v	Tailing low, Retention time good, USP Plate High, Area High	Not Satisfactory

3.2 Method Optimization

3.2.1 Selection of detection wavelength

Celecoxib showed absorbance at 220 nm. So the wavelength selected for the determination of Celecoxib was 220 nm.

3.2.2 Selection of proper column:

Lichro CART C-18, 250 mm × 4.6 mm, 5 µm

3.2.3 Selection of chromatographic conditions:

Optimized chromatographic conditions for estimation of celecoxib are finalized as shown in Table 2.

Table 2. Optimized chromatography conditions

Column	Lichro CART C-18, (250 × 4.6 mm), 5 µm 1.0 ml/minute
Flow rate	1.0 ml/minute
Detector	Water 2487 dual Lamda absorbance detector.
Detector wavelength	220 nm
Injection volume	20 µl
Run time	15 minutes
Mobile phase	Acetonitrile, water and TEA and O-Phosphoric acid (600:400:1:1)

3.3 Method Validation

3.3.1 Selectivity and Specificity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Selectivity was ascertained in different samples of celecoxib by comparing the chromatograms of celecoxib standard (Fig. 2). All the peaks were properly resolved from each other and peak purity of all the peaks in the spiked sample was passed. Besides, no peak of placebo was found at the RT of the compound which showed the specificity of the method. The specificity was found to be under limit. Specificity for celecoxib was 0.007.

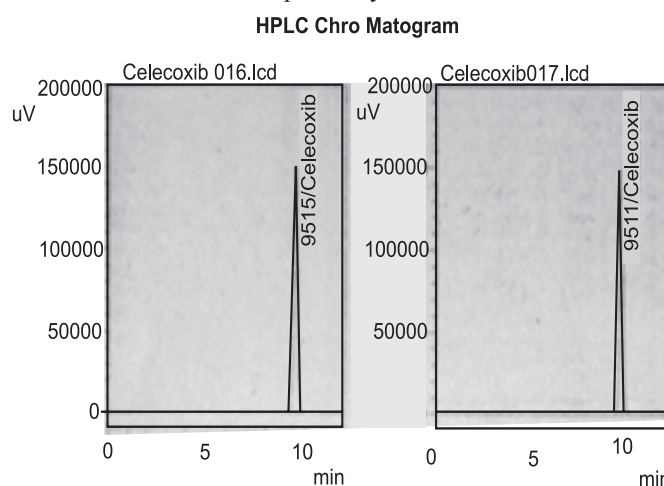


Fig. 2. Chromatogram of capsule formulation

3.3.2 Linearity and Sensitivity

The method was validated and calibration standard curve containing celecoxib was linear over the concentration range of 14000 mcg-26000 mcg with a correlation coefficient (r) of 0.999940 (Fig. 3). The intercept with the y-axis was not significantly different from zero. (Table 3)

Table 3. Observation for Linearity and Range

S.No.	Concentration (µg)	Area
1	14	1349782
2	16	1540515
3	18	1728374
4	20	1913866
5	22	2090892
6	24	2276545
7	26	2458736
Correlation coefficient		0.999940

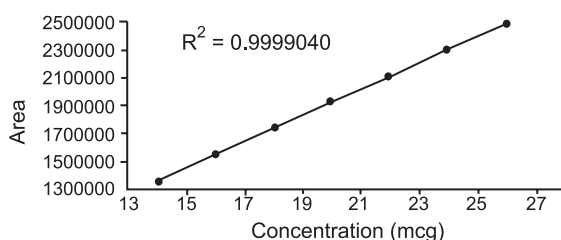


Fig. 3. Calibration curve of Celecoxib

3.3.3 System Precision

RSD of 0.6555% was obtained for Celecoxib from the six injections of system precision study, which was within the limit of 2%. Hence the system was found to be precise (Table 4).

Table 4. Observation for Precision

S. No.	Label claim (in mg)	Observation (in mg.)	% of Label Claim	Mean percentage	Relative standard deviation in %	Acceptance Criteria
1.	100.00	100.43	100.54	99.70	0.6555	RSD Not more than 2%
2.	100.00	99.24	99.95			
3.	100.00	100.88	100.10			
4.	100.00	100.61	99.72			
5.	100.00	98.96	98.80			
6.	100.00	99.91	99.07			

3.3.4 Accuracy

Accuracy of the method was determined by analyzing quality control samples at three concentrations within the calibration curve range to validate reproducibility. The accuracy limit is the percentage recovery should be the range of 98.0%-102%. The validation of the development method shows that the accuracy is well within the limit. The accuracy of the method was determined by performing recovery studies by a standard addition method in which pre-analyzed samples were taken and standard drug was added at 3 different levels. The % recovery lies in the range of 99.53% - 99.75%. The table summarizes accuracy values for quality control samples. (Table 5).

Table 5. Observation for Accuracy

S.No.	Known amount added in the placebo (in mg.) Individual value		Recovery in mg.		% of Recovery
			Average value	Mean percentage	
1.	80%	80.10	79.66	79.81	99.64
2.	80%	80.10	79.38		
3.	80%	80.10	80.40		
1.	100%	99.99	99.02	99.74	99.75
2.	100%	99.99	99.59		
3.	100%	99.99	100.60		
1.	120%	119.86	120.06	119.30	99.53
2.	120%	119.86	118.76		
3.	120%	119.86	119.08		

3.3.5 Intermediate Precision

Intermediate Precision of the method was determined by analyzing quality control samples at four concentrations within the calibration curve range to validate reproducibility. Intermediate precision was done at inter day analysis by two analysts by HPLC. The maximum variation against 1 is 1.893 (limit is less than 2%). These results above indicate that the present method has good accuracy, precision and reproducibility. (Table 6)

Table 6. Observation for Intermediate Precision

S. No.	Parameter validated	Analyst I	Analyst II	Maximum variation against Analyst 1 in %	Acceptance Criteria
1.	Assay	100.79	100.43	-0.358	NMT than 2%
2.	Assay	99.49	99.24	-0.252	
3.	Assay	98.97	100.88	1.893	
4.	Assay	100.37	100.61	0.239	
5.	Assay	99.84	98.96	-0.889	
6.	Assay	100.71	99.81	-0.902	

3.3.6 Robustness

Robustness of the method were investigated by varying the instrumental conditions such as the wavelength of detection (± 5 nm), column oven temperature ($+ 5$ °C), pH of buffer (± 0.2 pH unit), % organic (± 2 mL absolute). System suitability of the standard solution was checked at each variable condition and data was found to be within the acceptable range. (% RSD NMT2).

4. CONCLUSION

A validated RP-HPLC analytical method has been developed for the determination of celecoxib in capsule dosage form. The proposed method was simple, accurate, precise, specific and suitable to use for the routine analysis of celecoxib in capsule dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS and GC-MS. These methods are complicated, costly and rather time consuming than a simple HPLC-UV method. The assay was linear from 14000 mcg- 26000 mcg. In the accuracy % recovery is 98.70 and % RSD is 0.655 it meets criteria according to ICH Guideline. The result of this study showed the stability of celecoxib during storage, processing and throughout the validation. This method was used successfully for the quality assessment of celecoxib capsules with good precision and low cost.

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