China Petroleum Processing and Petrochemical Technology

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2269-2278

ACIDIC, ALKALINE, OXIDATIVE, PHOTOLYTIC AND DRY HEAT STRESS TESTING OF CELECOXIB

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Abstract:
The Celecoxib formulation and bulk drug were studied to degradation conditions and degradation was well alienated and determined from the drug and excipients on SunFire C18 column using a blend of Acetonitrile: Water (70: 30 v/v) pH 7.0 in isocratic mode at a flow rate of 0.8mL/min at an ambient temperature of 28°C with the detection wavelength at 251nm. The retention time of Celecoxib was found 4.277min. The linearity was performed in the concentration range of 30-110 ppm with a correlation factor of 0.999 for Celecoxib. The percentage purity of the Celecoxib tablet was found 99.98%. The drug was found to degrade under acid, and alkali conditions but found stable under photolytic conditions.

Keywords: Celecoxib, RP-HPLC, Method Development, Method Validation, Stability, Tablet (CELACT-MD).

1. INTRODUCTION:

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Analytical method development and validation play significant roles in the discovery, development, and manufacture of drugs. Analytical methods are necessary to validate for providing reliable and scientific data for regulatory submissions. These methods are needed for passing quality control tests, checking the stability of the drug and formulation, and for documentation purposes. In addition, drug control is a pharmaceutical discipline of principal importance. It is so necessary to ensure the quality, safety, and efficacy of drugs, which fulfills one of the significant tasks of pharmaceutical studies[1].

Celecoxib is a selective noncompetitive inhibitor of cyclooxygenase-2 (COX-2) enzyme and a non-steroidal anti-inflammatory drug. The inhibition of this enzyme reduces the synthesis of metabolites that include (PGE2), prostacyclin PGI2, TXA2, PGD2, and PGF2. Resultant inhibition of these mediators leads to the lessening of pain and swelling[2,3] Literature survey on the analytical methods for Celecoxib revealed that a few of the analytical methods were published for Celecoxib by using Spectrophotometry, HPLC, and HPTLC[4-9]. The present study aimed to develop a cost-effective method that will be used for the usual analysis of the drug Celecoxib and its formulations.

2. MATERIAL AND METHODS[10-14]:

Materials

Pharmaceutical grade Celecoxib was kindly supplied as a gift sample from Mylan Laboratories Ltd. All the chemicals were purchased from S.D. Fine Chemicals, Mumbai (India). Acetonitrile, water, and methanol (all HPLC Grade), Concentrated Hydrochloric Acid, Sodium Hydroxide, and Hydrogen Peroxide (AR Grade) were used for the experiment. A membrane Filter was used of 0.45µ purchased from Pall India Ltd. Mumbai (India).

Methods

Celecoxib was evaluated with different parameters like solubility, melting point, UV spectra, IR spectra, and chromatography. A Jasco V-630 spectrophotometer with matched pair cells of 10mm Quartz was used for the measurement of UV Absorbance in the range of 400-200nm. The IR spectrum of the drug sample was recorded as potassium bromide (KBr) pellets at a resolution of 4 cm^{-1} over a range of 4000-400 cm^{-1}. The peaks in the spectrum of Celecoxib were compared with the principal peak of the IR spectrum reported in the literature.

Celecoxib was subjected to HPLC chromatographic analysis with C18 column (250×4.6 mm, 5 µm) using a mobile phase of different strength, flow rate as 1 ml/min at the wavelength of 251 nm. Celecoxib was studied for a variety of stress conditions to affect the degradation of about 5-20% like acid, alkali, wet heat, effect by oxidation, light and dry heat. The stressed samples were then used for chromatographic separation. Analytical method validation was carried out as per ICH method validation guideline Q2 (R1).

Results

A. Evaluation

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Melting point of the procured reference standard of Celecoxib was determined and found in the range of temperature of 156 - 158°C. The drug was found to be freely soluble in Acetonitrile, Acetone, and Methanol. The UV Absorption spectrum of Celecoxib showed a λmax at 251nm in methanol when scanned in the range of 400-200nm.

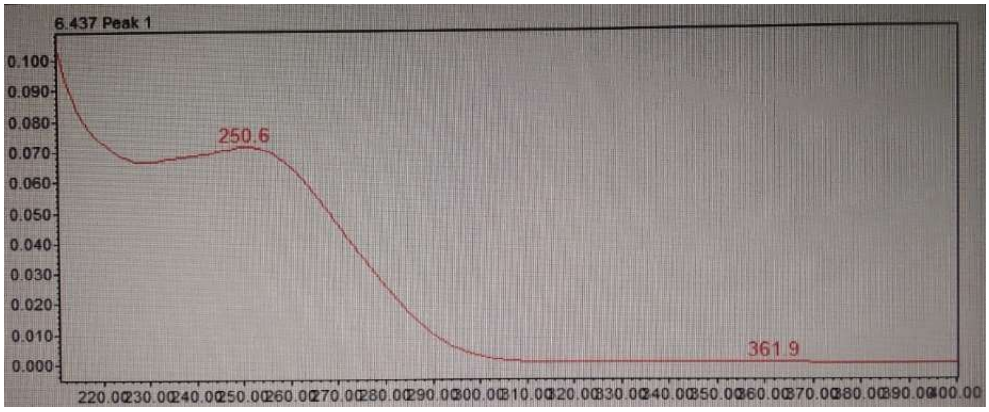

Figure No:01 UV Spectrum of Celecoxib

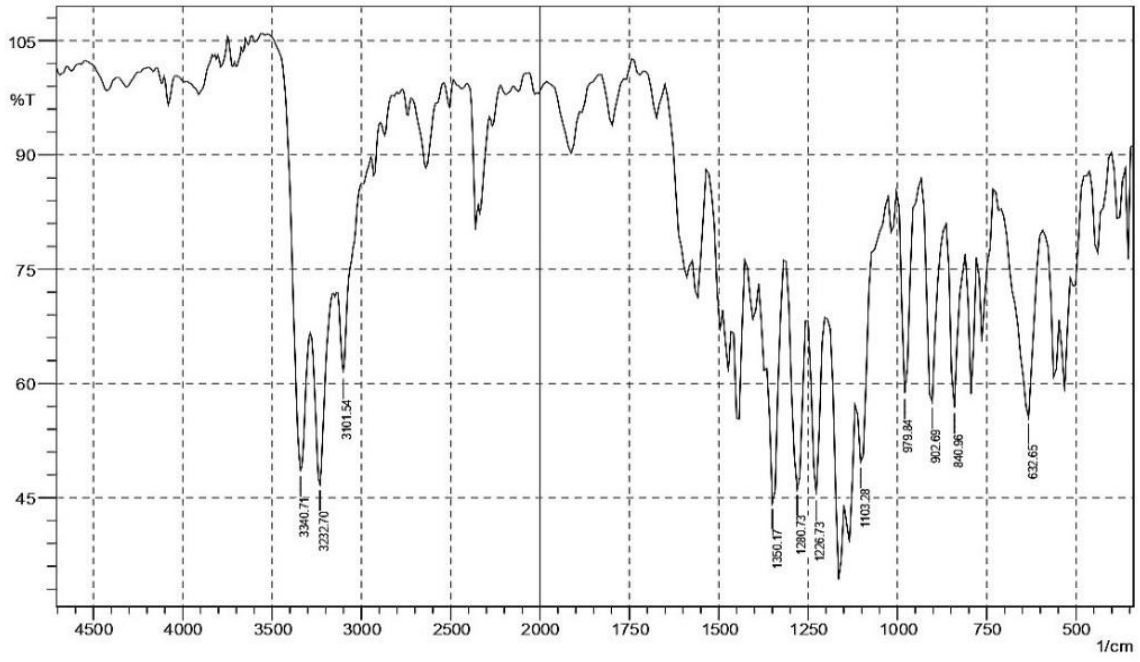

Figure No:02 Infrared Spectrum of Celecoxib

Table No:01 Interpretation of Infrared Spectrum of Celecoxib

Wave Number cm^{-1}	**Assignment**
3232-3340	NH2 Stretching
1226-1350	S O Stretching (Sulphonamide group)
1100-1200	NH bending

800-1100	CH bending
1500-1600	NH Stretching

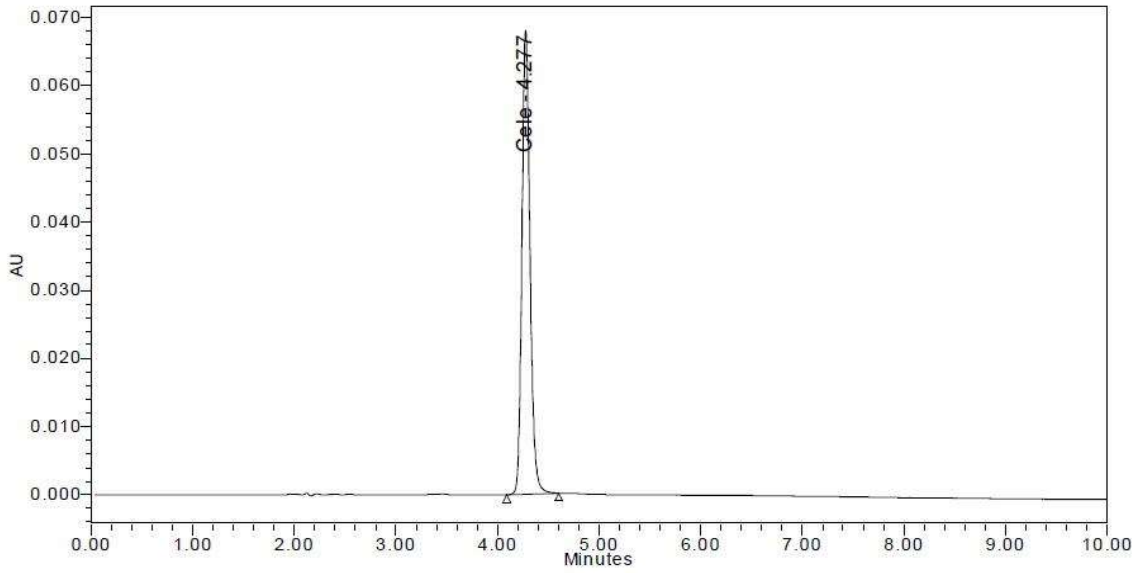

Figure No 03: Chromatogram of Celecoxib in Acetonitrile: Water (70: 30 v/v) pH 7.0, 1mL/min

B. Analytical Method Validation:

1. System Suitability

Table No.02: System Suitability Parameters

Celecoxib (20PPM)	**Retention Time (min)**	**Area**	**Tailing Factor**
Standard 1	4.277	310075	1.1621
Standard 2	4.275	310289	1.1550
Standard 3	4.276	310164	1.1648
Standard 4	4.277	310265	1.1706
Standard 5	4.278	310284	1.1662
	Mean	**310215.4**	
	SD	**93.4574**	
	%RSD	**0.03012**	

2. Experiment Calibration

A calibration standard in the range of 30-110µg/mL was analyzed and regression analysis was carried out from plot of peak area v/s concentration.

Table No.3: Calibration Curve of Celecoxib

Sr. No	**Concentration**	**Area**
1	30	310075

2	50	620150
3	70	930225
4	90	1230300
5	110	1550375
	Slope	**15467**
	Intercept	**1000**
	Correlation	

3. Specificity

The Specificity of the proposed method was established by the complete separation of Celecoxib in presence of excipients and degradation Product. The method was declared specific because there were no interfering peaks at retention time of Celecoxib and peak was well resolved from the peaks of all potential degradation products.

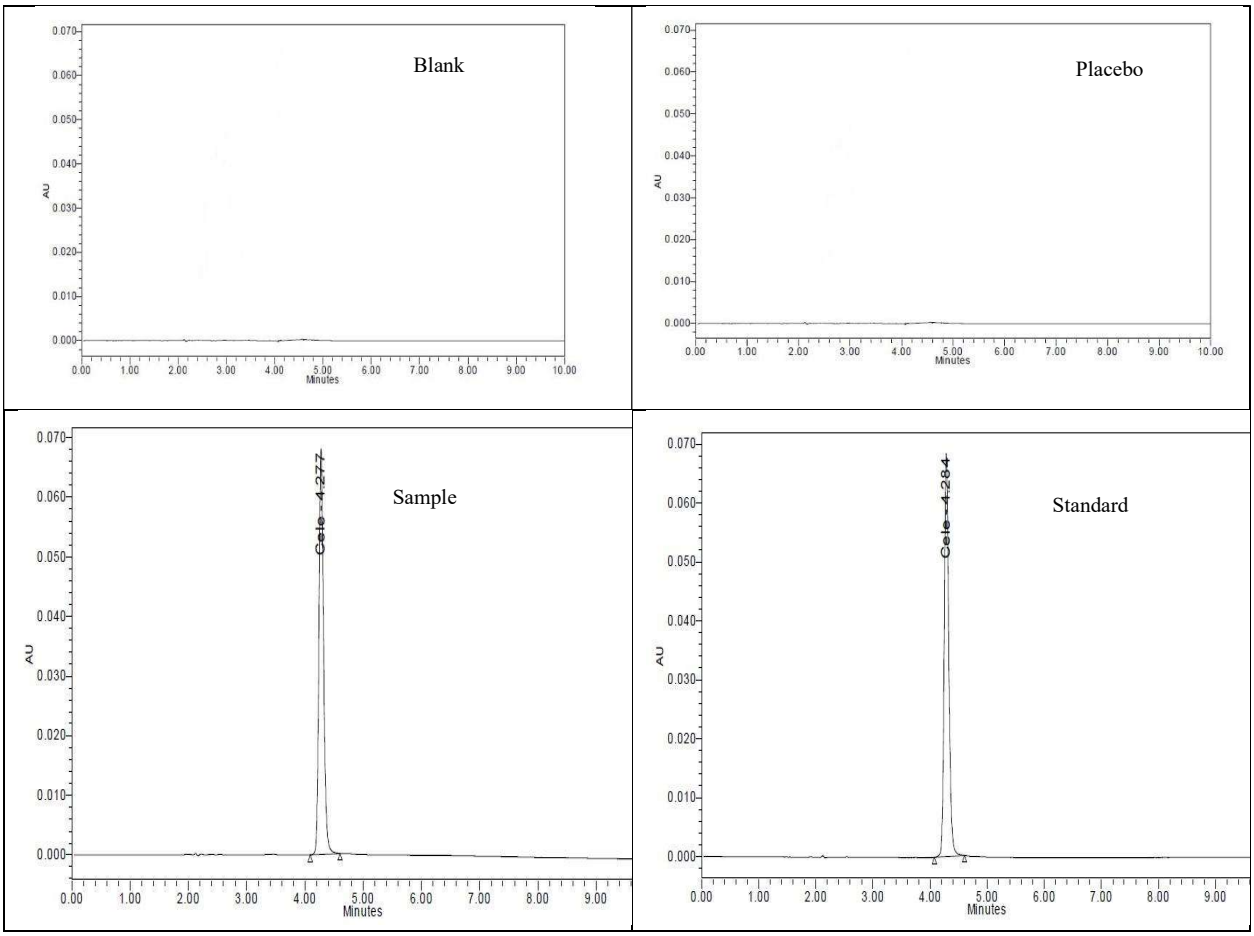

Figure No. 04: Chromatograms for Specificity

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4. Accuracy

The accuracy was evaluated by fortifying a powder mixture of blank tablet formulation with amount of drug corresponding to 80%, 100% and 120% of label claimed and analysing the resulting mixture in three replicates. The % recovery of added drug and % Relative Standard Deviation (RSD) were taken as measures of accuracy. The data obtained from precision and accuracy experiments are summarized in Table No:04 mean value of amount found were very close to the amount added and % RSD values were very low indicating acceptable accuracy of method.

Table No. 04: Accuracy Results

S.N.	Conc Level	Conc. (µg/mL) Sample Solution Taken	Conc. (µg/mL) Std. Stock Solution Added	Area	Avg Peak Area	Conc. Found (µg/mL)	% Recovery	SD	% RSD
1	80%	20	16	556089	**556188**	**35.89**	**99.69**	**99.50**	**0.0178**
		20	16	556288					
		20	16	556188					
2	100%	20	20	617986	**617485**	**39.85**	**99.62**	**501**	**0.0811**
		20	20	616984					
		20	20	617485					
3	120%	20	24	679250	**679539**	**43.87**	**99.70**	**289**	**0.0425**
		20	24	679828					
		20	24	679539					

5. Intermediate Precision:

Intermediate precision expresses within-laboratories variations on different days, with different analysts, and different equipment. The inter-day precision expresses the same concentration level determined on consecutive days. The inter-day variations calculated for 3 days and 3 different analysts respectively are expressed in terms of % RSD values. At each day and each analyst, the RSD values were below 1.0%, indicating a good inter-day precision.

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Table No.05: Intermediate Precision

Sr. No	Conc. in µg/mL	Conc. in %	Peak Area at Analyst 1st	Peak Area at Analyst 2nd	Peak Area at Analyst 3rd
1	20	100%	310052	310521	310531
2	20	100%	310265	309817	309807
3	20	100%	310145	310043	308409
4	20	100%	308612	308509	310343
5	20	100%	310419	309954	310255
6	20	100%	310021	310155	309964
Avg. Peak Area			**309991**	**30933**	**309884**
Standard Deviation			**656.8308**	**691.2309**	**768.7490**
% RSD			**0.2119**	**0.2230**	**0.2480**

6. Reproducibility

Table No.06: Reproducibility Results

Sr. No	Conc. in µg/mL	Conc. in %	Peak Area at Day 1st	Peak Area at Day 2nd	Peak Area at Day 3rd
1	20	100%	308910	309611	309478
2	20	100%	310024	309825	309692
3	20	100%	309899	309700	309567
4	20	100%	309711	309612	309479
5	20	100%	310123	309924	309791
6	20	100%	310249	310050	309917
Avg Peak Area			**309969.33**	**309734.4**	**309654**
Standard Deviation			**200.9404**	**178.0741**	**178.0741**
% RSD			**0.0648**	**0.0574**	**0.0575**

7. Calculation Of LOD

The LOD of the method was found to be 0.910µg/mL

8. Calculation Of LOQ

The LOQ of the method was found to be 2.76µg/mL

C. Stress Study of Celecoxib

Photolytic Degradation

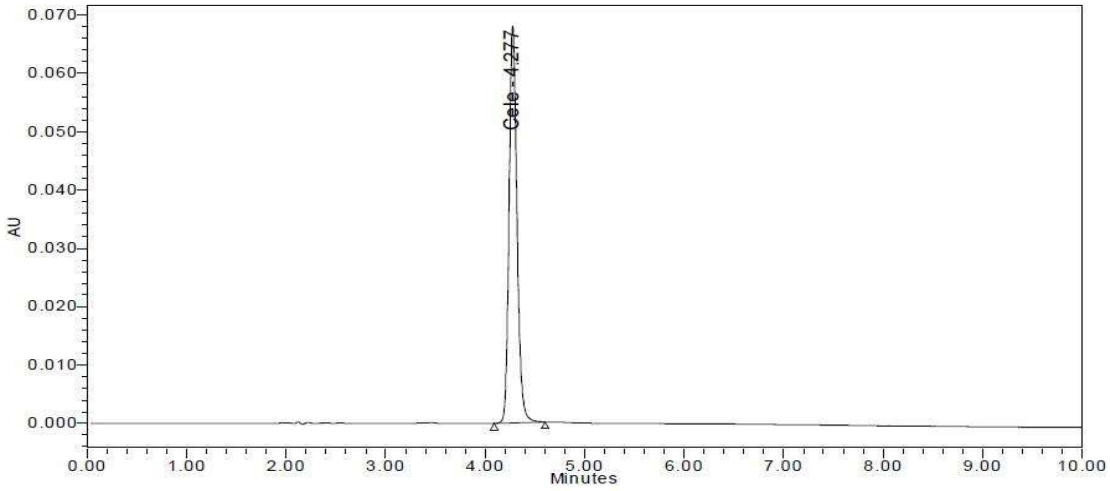

Figure No. 05: Photolytic Degradation chromatogram of Celecoxib no degradants Peak, 'Celecoxib RT: 4.2

The drug was exposed to direct sunlight for 1 month. When samples were analyzed, no additional peak was observed.

Table No. 07: Forced Degradation Results

Sr. No	Stress Condition	Drug peak area at zero-time sample	The drug peak area of the stressed sample	Retention time of degradation product (min)	% Degradation
1	Acid Hydrolysis (1 N HCl of 1Hr)	312264	276376	4.2574	11.42%
2	Alkali Hydrolysis (1 N NaOH of 24 Hrs)	311164	286558	4.253	7.82%
3	Oxidative (3% v/v H_2O_2) in direct room temperature	-	-	-	-
4	Photolytic (exposed to direct sunlight for 1 Month)	311368	302294	No Degradation Peak	2.91%
5	Dry Heat 80 °C (Kept in oven for 8 Hrs)	-	-	-	-

5. SUMMARY AND CONCLUSION

Stress testing of Celecoxib was carried out under Acidic, alkaline, oxidative, photolytic, and dry heat conditions.

The HPLC analysis of Celecoxib was carried out using SunFire C18 Column during the stress studies Celecoxib was found to degrade under acidic, alkaline, and oxidative conditions but stability was shown to dry heat as well as photolytic conditions. The degradation products and tablet excipients were well resolved from the drug by the mobile phase of Acetonitrile: Water (70:30 v/v). The developed method was validated as per ICH Guidelines. The method was established to be accurate, precise, robust as well and linear in the range of 30-110µg/mL.

The LOD and LOQ were found to be 0.910µg/mL and 2.76µg/mL respectively. It can be established that the HPLC method developed for Celecoxib can discriminate among the drugs and then degradation products.

ACKNOWLEDEMENT

The authors would like to thank MET's Institute of Pharmacy for their support for performing all laboratory works.

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