METHOD DEVELOPMENT AND VALIDATION OF CILNIDIPINE IN TABLET DOSAGE FORM BY USING ULTRA VIOLET SPECTROPHOTOMETRY

Abstract

A simple, quick, and accurate UV Spectroscopic method was developed to quantify celecoxib in API and tablet formulations. Celecoxib was used to treat rheumatoid arthritis and osteoarthritis. The proposed procedure was developed using acetonitrile and distilled water as the solvents in a 50:50 volume-to-volume ratio, and it was optimized using a Shimadzu UV-1800 ENG240V at a maximum wavelength of 240 nm with an absorbance of 0.558. The sensitivity, robustness, accuracy, and precision of the improved technique Degradation studies were also conducted and determined to be within the constraints. The regression coefficient was 0.996, and the linearity ranged from 2.5 to 12.5 µg/ml. LOD and LOQ values were 0.16 µg/ml and 0.052 µg/ml, respectively. According to ICH Q2R1, a straightforward, exact, and reliable approach was created.

Keywords: Celecoxib, acetonitrile, Spectrophotometer, ICH Guidelines.

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I. INTRODUCTION

4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-y) Celecoxib Benzenesul fonamide is a non-steroidal anti-inflammatory medication (NSAID) that is a diaryl pyrazol. It has anti-inflammatory, analgesic, and antipyretic effects by specifically blocking the synthesis of prostaglandins by the enzyme cyclooxygenase-2 (COX-2)[3,4]. It is prescribed to treat osteoarthritis and rheumatoid arthritis symptoms. The U.S. FDA gave celecoxib a priority review rating and approved it on December 12th, 1998. There are dose formulations for it in capsules of 100 mg and 200 mg. Cytochrome P450 2C9 is principally responsible for its metabolism. Human plasma has been found to contain three inactive metabolites, including a primary alcohol, the matching carboxylic acid, and its glucuronide conjugate [1, 2].

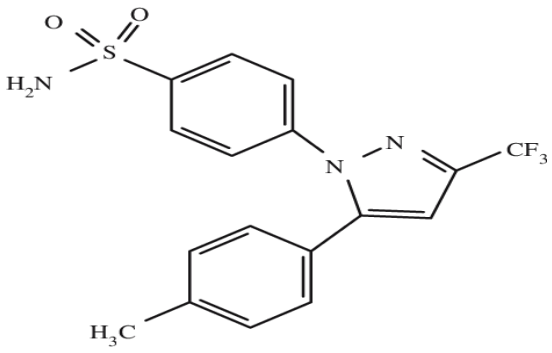

Figure 1: Chemical Structure of Celecoxib

II. MATERIAL AND METHODS

1. **Material:** Shimadzu UV-1800 ENG240V for UV Visible Spectroscopy, Shimadzu ATY224 for electronic balance, Sonica 2200MH for digital ultrasonic cleaner, Infra Digi ISO 9001-2015 for hot air oven, and Monoquartz for UV Cabinet are among the instruments used.

2. **Chemicals:** AcetoNitrile (HPLC grade Merck), Water for HPLC (Merck), and Celecoxib (obtained from Carbanio).

3. **Solubility Study:** Different tests were conducted using various solvents and concentrations based on the drug's solubility. Acetonitrile and distilled water were the only appropriate solvents left.

4. **Preparation of Standard Solution:** A 10 mL calibrated, clean, and dry volumetric flask was filled with 10 mL of solvent (acetonitrile: distilled water, 50:50% v/v) after being precisely weighed and transferred to the flask. Shake it vigorously and sonicate it (main stock solution, 1,000 g/mL) to improve solubility.Pipette 0.1 mL of the aforementioned standard solution into a 10 mL volumetric flask, then add solvent to get the volume to the required level. The solution's concentration was 100 µg/mL.

5. **Preparation of Sample Solution:** 20 tablets were precisely weighed, the average weight of each tablet was calculated, and the tablets were then ground into a powder using a

clean mortar and pestle. Put 117 mg of cilnidipine, which is the equivalent weight of the tablet powder, into a 10 mL volumetric flask. 10 mL of the solvent should be added, sonicated to completely dissolve it, filtered if necessary, and then added to the remaining volume. (1000µg/mL). Pipette 0.1 ml of the aforementioned sample solution into a 10 mL volumetric flask, then add more to the desired volume. The solution's concentration was 100 g/mL.

6. **Determination of Maximum Wavelength:** To get the spectrum of the maximum wavelength in UV-Visible spectroscopy, a 100 µg/mL concentration of cilnidipine solution was scanned between 200 nm and 400 nm. At 240 nm, cilnidipine's absorbance peaked [5,6].

III. VALIDATION

1. **System Suitability:** The suitability of the system has been assessed by taking six replicates of the prepared concentration, i.e., 7.5 g mL, placed in the Spectrophotometer setting the optimised conditions and measuring the absorbance. It was found to have a %RSD of 0.204.7-9].

2. **Linearity:** Prepared standard stock concentration (1000 µg / ml),pipette out 2.5ml,5 ml, 7.5 ml, 10 ml and 12.5 ml transferred in Individual 10 ml Marked with solvent. Calibration curve (range 2.5 – 12.5 µg/ ml) ,orrelation coefficient (0.996) absorbance plotted against drug solution concentration

3. **Precision:** Precision was performed for Intra-day & Inter-day. Precision Intra-day and Inter-day Precision were measured using 3 different time points within a day. Inter-day Precision was measured using 3 different days. %RSD for the approach was found to be within acceptable range.

4. **Accuracy:** A percent recovery study was conducted for the developed UV. The percent recovery study was performed at 50 %, 100 % and 150 %. A known amount of drug standard solution celecoxib was added to the pre-analyzed sample solution at three levels. [11-13].

5. **Robustness:** Small, incremental modifications (solvent composition and wavelength) of the developed method were evaluated for robustness.

6. **Sensitivity (LOD and LOQ):** The limit of Detection (LOD) and limit of Quantification (LOQ) for celecoxib was determined experimentally using LOD = (3 X SD/SLOPE) and LOQ = (10x SD/SLOPE).

IV. RESULTS AND DISCUSSION

Selecting a solvent system is one of the most important steps in the development of an ultraviolet spectrometric method. Therefore, numerous solvent systems have been tested in order to define the ultraviolet spectrometry method. Acetonitrile: Distilled water (50:50 v/v) was selected as solvent. Validation parameters were performed according to ICH guidelines [14]. The linearity of test results is directly proportional to the analyte concentration. The

linear concentration range is follow Beer's-lamberts Law and ranges from 2.5 to 12,5 µg/ ml. The linear regression coefficient of the calibration curve was found to be 0.996 (Table 1).

The intra-day and inter-day characteristics were used to determine the precision. Intra-day precision(% RSD) range from 1.01-1.2 and the inter-day precision(% RSD) range from 1.01-1.18. The approach is precise as evidenced by the% RSD being less than 2. The outcomes are recorded in Table 1. Three levels of accuracy testing were done: 50%, 100%, and 150%. By injecting sample solutions at six duplicates for 100% and six replicates for 50% and 150% solutions, the method's percent mean recovery was obtained. Celecoxib's recovery rate was discovered to be between 99.5-99.03 percent. The% recovery shows that the procedure developed was very accurate.LoD and LoQ values presented in table 1.

Table 1: Summary of Validation Parameters

S.No	Parameter	Result
1	Linearity range	2.5-12.5 µg/ml
2	Correlation coefficient	0.996
3	% Recovery	99.5-99.03
4	Precision (% RSD)	Intra-day →1.01-1.2 Inter-day→ 1.01-1.18
5	LOD	0.052 µg/ml
6	LOQ	0.16 µg/ml

The robustness results shown in table 2 and illustrate the robustness of the associate degree analytical technique in the face of minute but intentional changes in methodology parameters and provide an indication of its dependability under typical conditions.

Table 2: Summary of Robustness

Parameter	Condition	Mean ± SD	% RSD
Solvent composition	4.5:5.5 %v/v	0.807±0.004	0.49
	5:5 %v/v	0.563±0.002	0.44
	5.5:4.5 %v/v	0.965±0.004	0.46
Wave length	236 nm	0.547±0.0015	0.27
	240 nm	0.559±0.0015	0.27
	244 nm	0.569±0.003	0.52

The % assay for celecoxib in the marketed formulation was performed using the developed method. The result of the % assay was 100.51%. Based on the stress studies, the degradation of celecoxib was determined under the following stress conditions: Acid, Alkaline, Oxidized and Photolytic. The percentage degradation in all of these stress conditions was with limits. The developed method was able to quantitatively quantify celecoxib under the presence of degradation products, demonstrating its specificity. Table 4 shows the results.

Table 4: summary of stress Studies

S. No	Condition	% Degradation
1	Acid	9.1%
2	Base	10.01%
3	Peroxide	9.3%
4	Photo	9.8%
5	Thermal	9.9%

V. CONCLUSION

A method has been developed to estimate the amount of celecoxib present in API and the corresponding tablet dosage form by UV spectroscopy. This method has been validated according to ICHQ2R1 and ICHQ1A (R2) Guideline. Linearity ranged from 2.5-12.5µg/ml. %RSD in accuracy, precision and robustness was <2 indicating that parameters are within guidelines. LOD and LOQ were 0.0525µg/ml and 0.165µg/ml. stress studies were also conducted for the tablet dosage formulation. The solvent formulation is acetonitrile, and distilled water is relatively inexpensive compared to many of the solvents reported in literature. Therefore, this method can be considered sensitive, cost-effective, repeatable and significantly fast in the test of in celecoxib API as well as dosage form.

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REFERENCES

[1] Budavari S. The Merck index: An encyclopedia of chemicals, drugs and biologicals, Merck Research Laboratories, Division of Whitehouse Station, NJ: Merck and Co. Inc, 2001; pp 86.
[2] Hardman JG, Limbird LE, Gilman AG. Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw Hill, New York, 2001; pp 871.
[3] Clemett D, Goa KL, “Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain” Drugs 2000; 59(4):957-80.
[4] Mengle-Gaw L, Hubbard RC, Karim A. A study of the platelets effects of SC-58635, a novel selective cox-2 inhibitor. Arthiritis Rheum 1997; 40 Suppl: S93.
[5] Vimal Ravi, Kokilambigai K S, Kavitha J, Seetharaman R, Lakshmi K S, Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations. Journal of medical pharmaceutical and allied sciences,2022;11(2): 4672 – 4678.
[6] Sidhdhapara M, Patel B, Parmar A, Development and Validation of Spectrophotometric Method for Simultaneous Determination of Cilnidipine and Olmesartan medoximil in Tablet Dosage Form. Int. J. Pharm. Biol. Sci.2014; 4, 97–101.
[7] Haripriya M, Antony N, Jayasekhar P, Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Cilnidipine and Telmisartan in Tablet Dosage Form Utilizing Simultaneous Equation and Absorbance Ratio Method, Int. J. Pharm. Biol. Sci. 2013;3, 343–348.
[8] Safhi MM. Spectrophotometric method for the estimation of cilnidipine in bulk and pharmaceutical dosage forms. Oriental journal of chemistry. 2013;29(1):131.
[9] Chaudhari PP, Bhalerao AV. Method validation for spectrophotometric estimation of cilnidipine. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(5):96-8.

[10] Lingyun H, Gaoyun H, Yanbin Z, Jianhao L. RP-HPLC determination of cilnidipine and its related substances. West Pharmacy. 2011;19:70-1.
[11] Zhang X, Zhai S, Zhao R, Ouyang J, Li X, Baeyens WR. Determination of cilnidipine, a new calcium antagonist, in human plasma using high performance liquid chromatography with tandem mass spectrometric detection. Analytica chimica acta. 2007 Sep 26;600(1-2):142-6.
[12] 10. Lee HW, Seo JH, Lee HS, Jeong SY, Cho YW, Lee KT. Development of a liquid chromatography/negative-ion electrospray tandem mass spectrometry assay for the determination of cilnidipine in human plasma and its application to a bioequivalence study. Journal of chromatography B. 2008 Feb 1;862(1-2):246-51.
[13] Karmalkar HS, Vaidya VV, Gomes NA, Choukekar MP, Kekare MB. Determination of cilnidipine from pharmaceutical formulation by high performance thin layer chromatographic method. Analytical chemistry. 2008;7(8):573-6.
[14] International Conference on Harmonization (2005) ICH harmonised tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov 2005.

