*Drug Development and Industrial Pharmacy*, 31:779–783, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500216378

Taylor & Francis
Taylor & Francis Group

# A New Stability—Indicating RP-HPLC Method to Determine Assay and Known Impurity of Celecoxib API

**A. S. Jadhav and M. S. Shingare**
Department of Chemistry, Dr. B. A. M. University, Aurangabad, India

**ABSTRACT** A simple, rapid and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed to determine assay and known impurity of Celecoxib API. The chromatographic separation was performed on reversed-phase C-18 column. Eluents were monitored on photo-diode array detector at a wavelength of 254 nm using a mixture (40:60) of buffer and acetonitrile. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for forced-degradation study, linearity and range, accuracy, precision, stability of analytical solutions, and selectivity. Due to its simplicity, rapidity, and accuracy, we believe that the method will be useful to determine assay and known impurity of Celecoxib.

**KEYWORDS** RP-HPLC, Celecoxib, COX-2, Validation, Active pharmaceutical ingredients

## INTRODUCTION

Celecoxib (4-[5-(4-methyl phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulfonamide) is a diaryl pyrazol that belongs to the general class of diaryl heterocyclic inhibitors and non-steroidal anti-inflammatory drugs (NSAID). Its unique structure differs from salicylates, pyrazolones, fenamic acid, acetic and propionic acid, and oxicam-based NSAIDs (Compound No. 1). 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 osteoarthrit's. Celecoxib received priority review rating and was approved by the U.S.FDA on December 12, 1998. Its metabolism is primarily mediated via cytochrome P450 2C9. Three inactive metabolites, a primary alcohol, the corresponding carboxylic acid, and its glucuronide conjugate have been identified in human plasma (Hassan et al., 2004; Pramod & Krisnacharya, 2002).

Address correspondence to M. S. Shingare, Department of Chemistry, Dr. B. A. M. University, Aurangabad 431004, India; E-mail: profmsshingare@rediffmail.com

**TABLE 1** System Suitability Parameter

| Sr. no | Parameter | Results | Acceptance limits |
|---|---|---|---|
| 1 | Theoretical plates | 15285 | More than 3000 |
| 2 | Asymmetry factor | 0.75 | Less than 2 |
| 3 | Capacity factor | 1.79 | More than 1 |
| 4 | Resolution | 2.5 | More than 2 |
| 5 | RSD of peak area | 0.501 | Less than 2% |

A literature survey revealed some high-performance liquid chromatographic (HPLC) methods for Celecoxib determination from formulations and human plasma (Pramod & Krisnacharya, 2002; Rajesh, 2004), no method was found for determination of Celecoxib and Cox-2 having resolution 2.5.

This article describes the development and validation of a stability-indicating method for the assay and known impurity (Compound No.2) in Celecoxib API.

## EXPERIMENTAL

### Instrumentation

Quantitative HPLC analysis was performed on a high-performance liquid chromatography which consisted of a dual piston reciprocating two LC-10AT VP pumps from Shimadzu Corp., Japan (model HPLC class 10AT), photo-diode array detector from Shimadzu Corp., Japan (model SPD-10M VP), and auto sampler of SCL-10A series. The HPLC system was equipped with data acquisition and processing software "Class-LC-10 series" Shimadzu Corp., Japan.

### Materials, Reagents, and Chemicals

An authentic working standard for Celecoxib and known impurity COX-2 (Regiosomer of Celecoxib) was gifted by Wockhardt Pharmaceuticals Ltd., Aurangabad, India. Potassium dihydrogen orthophosphate, ortho phosphoric acid, and water of HPLC grade were purchased from Qualigen India Ltd., Mumbai, India. The test sample was gifted from Vorian Laboratories Ltd., Secunderabad, India.

### Chromatographic Condition

The experiment was performed on a 250mm $\times$ 4.6mm (ID) $\times$ 5 μm particle size YMC ODS-A C-18 stainless steel column. The mobile phase consisted of 40% buffer (0.067 M potassium dihydrogen orthophosphate) and 60% acetonitrile. The pH of buffer was adjusted to four with orthophosphoric acid. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) and degassed with a helium spurge for 10 min. The chromatography was performed at room temperature using a flow rate of 1 ml/min. The run time was set to 15 min. Eluents were monitored on photo-diode array detector at a wavelength of 254 nm. The volume of each injection was 20 μl.

### Standard and Sample Solution

Standard and sample solution of Celecoxib 100 μg/ml each was prepared in mobile phase separately and a mixture of standard solution of Celecoxib and known impurity (COX-2) was prepared 100 μg/ml and 5 μg/ml respectively.

### Preparation of Solutions Used for Assay Validation

For the study of Celecoxib response linearity, different solutions were prepared at concentrations ranging from 0.5 to 75 μg/ml.

System precision was evaluated by performing five consecutive injections of Celecoxib standard solution. Method precision was evaluated by six repeated assays

**TABLE 2** Degradation of Celecoxib

| Sr. no | Condition | Degradation time (hr) | Fall in assay (%) |
|---|---|---|---|
| 1 | Acid 1N HCl, reflux | 8 | 18 |
| 2 | Base 1N NaOH, reflux | 8 | 13 |
| 3 | $H_2O_2$ 30%, reflux | 8 | 07 |

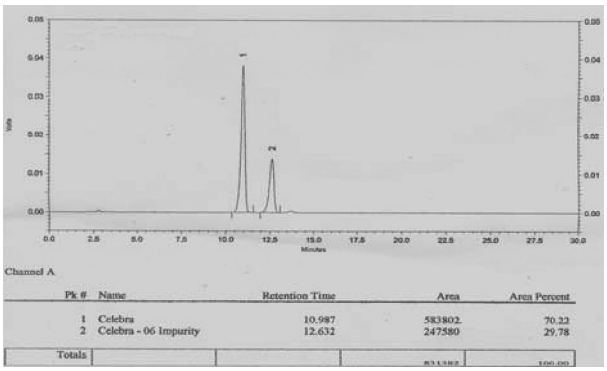



**FIGURE 1** **HPLC Chromatogram of Standard Celecoxib and Known Impurity COX-2 (Lacking of Degradation).**

of Celecoxib sample solution. Assay accuracy was assessed at 100%, 110%, and 120% 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 100 mg Celecoxib in a 100 ml volumetric flask followed by 70 ml acetonitrile. Celecoxib was dissolved properly by shaking and sonicating the flask. The volume was adjusted to 100 ml with acetonitrile taking 10 ml of the above solution in three different 50 ml round bottomed flasks to perform degradation experiments. To the first flask, 10 ml of 1N HCl was added for acidic degradation. To the second flask, 10 ml of 1N NaOH was added for basic degradation. To the third flask, 10 ml of 30% H2O2 was added for oxidative degradation. All three flasks were refluxed for about eight hours. After completing the degradation treatments, samples were allowed to cool to room temperature and treated as follows. The pH of the first flask was adjusted to six (neutralized) with dilute sodium hydroxide. The pH of the second flask was adjusted to six (neutralized) with dilute hydrochloric acid. To the third flask, adding 1N sodium bisulfite solution destroyed hydrogen peroxide. The volume of all three flasks was adjusted to 50 ml with acetonitrile. Samples were injected and analyzed against control samples (lacking of 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 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 (USP 27/NF 22, 2004). Quantification was accomplished using an external standard method. Each solution was injected in triplicate and relative standard deviation (RSD) was required to remain below 2% on a Celecoxib peak area basis. Data were acquired, stored, and analyzed with the software "Class-LC-10 series version 3.1" (Shimadzu).

# 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, resolution, and capacity factor. The results met acceptance criteria (Table 1).

## Selectivity

Selectivity of the developed method was assessed by sample degradation studies and peak purity evaluation. The retention time for Celecoxib peak was 10.9 min and for known impurity (Compound

**TABLE 3** **Linearity Data**

| Sr. no | Concentration injected (μg/ml) | Average peak area | RSD (%) |
|---|---|---|---|
| 1 | 1 | 62144 | 0.700 |
| 2 | 50 | 3006614 | 0.840 |
| 3 | 75 | 4475767 | 0.310 |
| 4 | 100 | 6006380 | 0.442 |
| 5 | 125 | 7512506 | 0.563 |
| 6 | 150 | 8967503 | 0.930 |

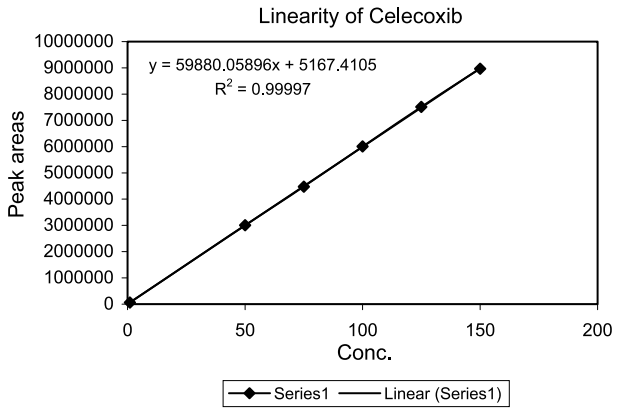



**FIGURE 2** **Linearity Graph of Peak Areas Response Against Concentration Injected.**

No. 2) was 12.6 min. The fall in assay of Celecoxib is shown in Table 2. Peak purity curve of Celecoxib peak evaluation by photo-diode array detector shows up 0.9983 and down 0.9856. Degradation study of Celecoxib is shown in Fig. 1.

## Linearity and Range

Six solutions of Celecoxib at concentration ranging from 1 to 150 μg/ml were analyzed (Table 3). The graph of the peak area against concentration proved linear. The correlation coefficient of $R^2 = 0.9999$. The plot of the peak area response against concentration in μg/ml injected is shown in Fig. 2. Range of the analytical method will be for the interval between upper and lower levels of an analyze including these levels which show precision and accuracy of the method.

## Method Precision

The assays of Celecoxib found in test sample by the proposed method are shown in Table 4.

**TABLE 4** **Method Precision**

| Sr. No | Assay of Celecoxib (%) |
|---|---|
| 1 | 99.13 |
| 2 | 98.95 |
| 3 | 99.04 |
| 4 | 99.12 |
| 5 | 98.92 |
| 6 | 98.87 |
| Mean | 98.98 |
| SD | ±0.158 |
| RSD | 0.16 |

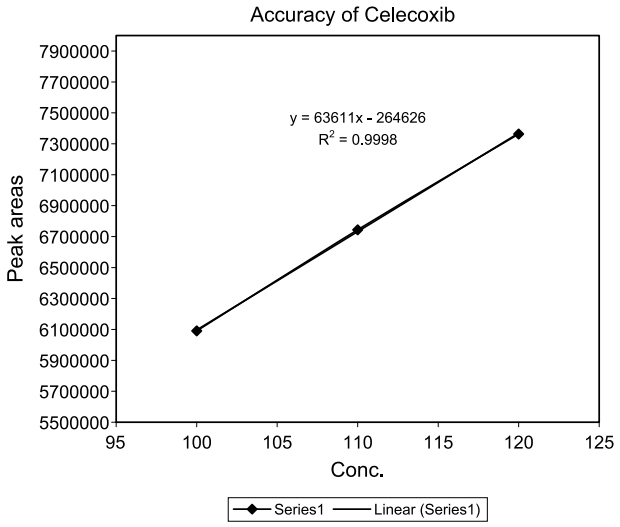



**FIGURE 3** **Accuracy Graph of Peak Areas Response Against Micrograms Injected.**

## Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the true value. Accuracy of the Celecoxib assay method is to be inferred from specificity, linearity, and precision data. Accuracy experiment of Celecoxib was carried out at 100%, 110%, and 120%. Accuracy of the Celecoxib is shown in Fig. 3.

## Stability of Analytical Solution

Stability of the standard solution was studied by injecting the prepared solution at periodic intervals into the chromatographic system up to about 48 hours. The cumulative RSD calculated for the area count of Celecoxib peak should not be more than 2%. Data is shown in Table 5.

**TABLE 5** **Stability of Analytical Solution**

| Sr. no | Times (hrs.) | Average peak area |
|---|---|---|
| 1 | Initial | 3054786 |
| 2 | 4 | 3100113 |
| 3 | 12 | 3023457 |
| 4 | 24 | 3113190 |
| 5 | 48 | 2998563 |
| | Cumulative RSD | 1.188% |

## CONCLUSION

The new RP-HPLC stability-indicating method was developed to determine assay of Celecoxib API. In this method, known impurity (Compound No. 2) was separated from Celecoxib peak. This method proved simple and accurate. All solution concentrations were measured on a weight basis to avoid the use of an internal standard. Calibration curve of peak area to drug concentration obtained using this method of analysis resulted in coefficient of correlation of 0.9999. The method precision of the Celecoxib chromatographic response was calculated from six repeated samplings of homogenous sample showed RSD of 0.16%. Recovery studies were performed at 100%, 110%, and 120% levels and resulted in a coefficient of correlation ($r^2$) 0.9998. Peak purity curve of Celecoxib peak evaluation by photo-diode array detector shows up 0.9983 and down 0.9856, and degradation studies in under acidic, basic, and oxidative showing that proposed analytical method was selectivity and stability indicating. The proposed RP-HPLC analytical method is simple, precise, and selective for the determination of Celecoxib and its known impurity (Compound No. 2).

## ACKNOWLEDGMENTS

We would like to thank Prof. T. K. Chordekar, Head, Department of Chemistry, Dr. B. A. M University, Aurangabad for encouragement, and Wockhardt Pharmaceuticals Ltd, Aurangabad, and Vorian Laboratories Ltd., Secunderabad, India for donation of drugs.

## REFERENCES

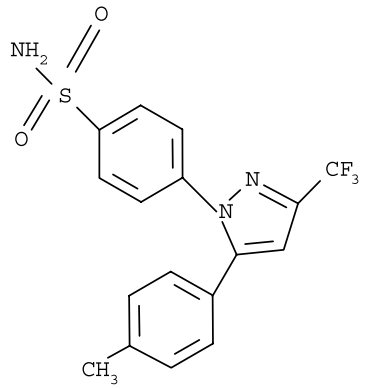
Hassan, J., Mobsen, A., Vahid, Z., Ali, S., Hassan, F., & Abbas, S. (2004). Determination of celecoxib in human plasma by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, *35*(3), 665–670.

Pramod, M. D., & Krisnacharya, G. A. (2002). A stability-indicating HPLC method to determine Celecoxib in capsule formulations. *Drug Development and Industry Pharmacy*, *28*(7), 815–821.

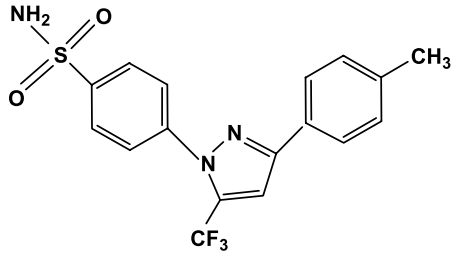
Rajesh, K. (2004). *Applications of pharmacokinetic principles in drug development* (pp. 405–407). New York: Plenumus.

USP 27/NF 22 (2004). *The United States Pharmacopeia* (pp. 2622–2625). Rockville, MD: The National Formulary.

*Q2A and Q2B International Conference on Harmonization*. www.ich.org.



Compound No.1



Compound No.2