

SEPARATION AND DETERMINATION OF PROCESS-RELATED IMPURITIES OF CELECOXIB IN BULK DRUGS USING REVERSED PHASE LIQUID CHROMATOGRAPHY

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Abstract

The present paper describes the development of a new isocratic reverse phase liquid chromatographic method for the separation and determination of celecoxib in the presence of its process-related impurities. The method was found to be simple, sensitive, precise, robust and accurate. Successful separation of the drug from the process impurities was achieved on an Inertsil ODS C18 column (250 mm X 4.6 mm, 5 μ) using phosphate buffer (pH adjusted to 3.5): acetonitrile (45-55v/v) as eluent, at a flow rate of 1.0 mL/min and the detection wavelength was set at 250 nm. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis, humidity and thermal degradation. The degradation of celecoxib was observed under oxidative environment. The method was developed and validated with respect to specificity, system suitability, linearity, accuracy, precision, LOD (limit of detection), LOQ (limit of quantification) and robustness as per ICH (International Conference on Harmonisation) guidelines.

Rezumat

Lucrarea prezintă o nouă metodă isocratică HPLC cu faze inversate pentru separarea și dozarea celecoxibului în prezența impurităților sale. Metoda dezvoltată este simplă, sensibilă, precisă, robustă și prezintă o înaltă acuratețe. Metoda a fost validată conform normelor ICH (International Conference on Harmonisation) în vigoare.

Keywords: Celecoxib, Process-related impurities, Validation.

Introduction

Celecoxib (Figure 1) is a non steroidal anti-inflammatory drug (NSAID) indicated to relieve the symptoms of rheumatoid arthritis and osteo-arthritis [1]. Celecoxib exhibits anti-inflammatory, analgesic and anti-pyretic activities by selective inhibition of cyclooxygenase -2 (COX-2), the inducible isoform of cyclooxygenase, involved in the prostaglandin synthesis, and does not inhibit platelet aggregation. In contrast, most of the

NSAIDs inhibit both isoforms of cyclooxygenases (COX-1 and COX-2) and inhibit platelet aggregation [2]. It is chemically designated as (4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzenesulfonamide) and its empirical formula is $C_{17}H_{14}N_3O_2S$, having a molecular weight of 381.373g/mol [3].

As per literature survey few analytical methods were reported for the analysis of celecoxib in pharmaceutical formulations and in human plasma by HPLC/UV detection [4-9] or fluorescence detection [10] and MEKC [11]. So far to our knowledge, no analytical method for determination of process-related impurities in celecoxib API (active pharmaceutical ingredient) is reported in literature. So it is essential to develop a HPLC method which will serve as a rapid and reliable method for the determination of process related impurities in celecoxib. The method has been thoroughly validated as per the ICH guidelines [12].

Experimental

Materials

Samples of celecoxib and its process-related impurities: impurity-B, impurity-C and impurity-D were obtained from Pharmazell R&D Centre, Andhra Pradesh, India. HPLC grade acetonitrile, analytical grade dipotassium hydrogen phosphate and orthophosphoric acid were obtained from Merck. High purity water was prepared by using Milli - Q academic purification system (Millipore). 4-methyl acetophenone (impurity-A) was purchased from SL Drugs & Pharmaceuticals, Andhra Pradesh, India. The chemical structures and nomenclature of impurities were reported in Table I.

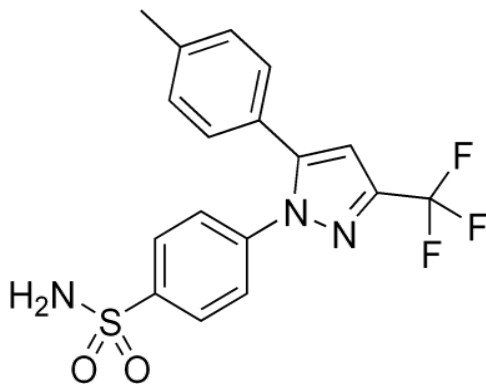
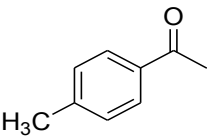
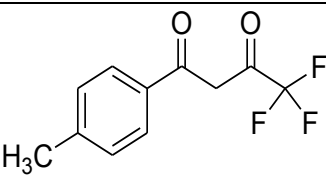
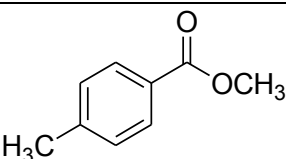
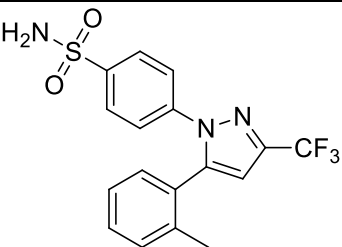


Figure 1
Chemical structure of celecoxib

Table I

Chemical structures of process related impurities of celecoxib

	Name of the Impurity	Structure	Impurity Code
1	4-methyl acetophenone		Impurity-A
2	trifluoroacetyl 4-methyl acetophenone		Impurity-B
3	methyl-4-methyl benzoate		Impurity-C
4	4-[5-(2-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzene sulphonamide		Impurity-D

Equipment

The LC system used for method development, forced degradation studies and method validation consisted of a Waters Alliance system with 2695 model Quaternary gradient system auto sampler and photodiode array detector. The output signal was monitored and processed using empower software. Photo stability studies were carried out in a photo stability chamber. Thermal stability studies were carried out in a dry air oven.

Solutions

Mobile phase

- A. Buffer: 10 mM aqueous dipotassium hydrogen phosphate was prepared and the pH was adjusted to 3.5 ± 0.05 with orthophosphoric acid. It was filtered through a $0.45 \mu\text{m}$ nylon membrane filter prior to use and degassed in an ultrasonic bath.
- B. Acetonitrile

Dilution: A mixture of phosphate buffer and acetonitrile (45:55 v/v) mobile phase was used as diluent.

Impurity mixture

About 37.5 mg of celecoxib and its process-related impurities (impurity A, B, C and D) were weighed accurately and transferred into a 100 mL volumetric flask, and the volume was made up to the mark with diluent. 5 mL of this solution was made up to 50 mL with diluent to get a concentration of 37.5 µg/mL. This impurity stock solution was adequately diluted to study the accuracy, precision, linearity, limit of detection and limit of quantitation.

Celecoxib standard solution

Accurately weighed 50 mg of celecoxib working standard were transferred into a 50 mL volumetric flask containing 30 mL of diluent. The contents of the flask were sonicated for 15 min to dissolve the drug completely and the volume was made up to 50 mL with diluent (1 mg/mL) and injected into the system.

Sample solution

Accurately weighed 50 mg of sample were transferred into a 50 mL volumetric flask. To this solution, 30 mL of diluent were added, sonicated for 15 min to dissolve the drug completely and the volume was made up to 50 mL with diluent and injected into the system.

Forced degradation samples for the specificity study

The stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation and UV degradation. Celecoxib was heated with aqueous 0.1 N HCL at 80° C for 24 hrs and separately with aqueous 0.1 N NaOH at 80° C for 24 hrs to study formation of degradation products under acidic and basic conditions.

Celecoxib sample was heated with 5 % potassium permanganate solution at 80° C for 3 hrs to study the formation of degradation products under oxidative condition. Celecoxib sample was exposed to UV light (254 nm) for 24 hrs and another sample was kept at 105° C temperature for 24 hrs respectively.

Chromatographic conditions

An Inertsil ODS C18 analytical column (250 mm X 4.6 mm, 5 µ) was used for the analysis at 25° C. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20 µL. The UV detector was set to a wavelength of 250 nm for the detection.

Results and Discussion*Method development*

The main objective of the chromatographic method was to separate celecoxib and its process-related impurities, by using different columns and different buffer systems with different pH ranges and organic modifiers.

Based on literature, the method development started with a C18 column and mobile phase composed of phosphate buffer and methanol in the ratio 50:50v/v. In this mobile phase, the separation was not good and peaks splitted. Many experiments were conducted by using different columns, different buffers and different organic modifier concentrations were tried. Even so, no improvement was observed and hence methanol was replaced with acetonitrile. Some improvement was observed, but the impurity peaks were merging, so further trials were carried out by verifying the pH and concentration of buffer and acetonitrile. Finally, the separation was assessed with C18 (250 mm X 4.6 mm, 5 μ) column using 0.01 M phosphate buffer with pH 3.5 adjusted with ortho phosphoric acid and acetonitrile in the ratio (45:55 % v/v) at a flow rate of 1.0 mL/min, which gave sharp peaks with minimum tailing and good resolution for both drug and impurities. These impurities in the drug substance were identified based on the retention time and relative retention time.

Method validation

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its process-related impurities. The specificity of the developed HPLC method was performed by injecting blank solution and standard solution spiked with process-related impurities separately. The chromatograms are shown in Fig.2 and 3.

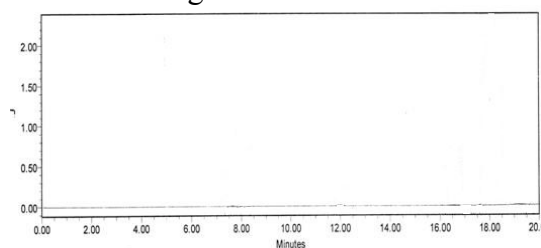


Figure 2

Chromatogram of blank solution

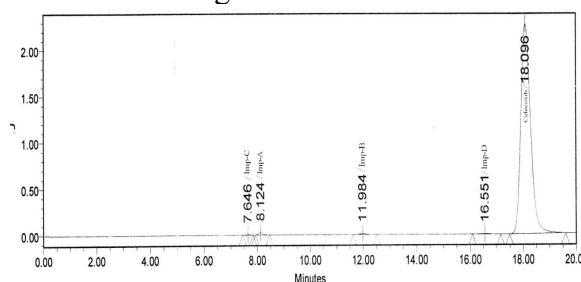


Figure 3

Chromatogram of celecoxib spiked with impurities

System Suitability

It is an integral part of the development of a chromatographic method to verify that the resolution and reproducibility of the system are adequate enough for the analysis to be performed. It is based on the concept that the equipment, electronics, analytical operations and samples constituting an integral system could be evaluated as a whole. Parameters such as plate number (N), asymmetry or tailing factors (A_s), relative retention time (RRT), resolution (R_s), reproducibility (% R.S.D) and retention time (RT) were determined and the results of the method are presented in Table II

Table II
Results of the system suitability study

Name	RT	RRT	Resolution (R_s)	Theoretical plate(N)	Peak asymmetry(A_s)
Impurity-A	8.07	0.45	-	11424	1.11
Impurity-B	11.89	0.66	10.5	13534	1.07
Impurity-C	7.59	0.42	1.57	10069	1.07
Impurity-D	16.32	0.91	8.86	12702	1.05
Celecoxib	17.87	1	2.53	13017	1.04

Linearity

Calibration curves were performed for celecoxib and its impurities in the concentration ranges of 0.373 to 2.268 $\mu\text{g/mL}$. The linearity plot was drawn taking the concentration on X-axis and the mean peak area on Y-axis. The data were subjected to statistical analysis using a linear-regression model and the parameters are shown in Table III.

Table III
Parameters of linearity curves of celecoxib and its impurities

	Slope	Intercept	Correlation coefficient
Celecoxib	78888	387.53	0.9998
Impurity A	135273	8297.1	0.9972
Impurity B	69623	3854.1	0.9953
Impurity C	29365	212.93	0.9954
Impurity D	52505	-557.2	0.9997

Accuracy/Recovery

The accuracy of the test method was determined by analyzing celecoxib drug substance spiked with impurities at three different concentration levels of 50 %, 100 %, and 150 % of each in triplicate at the specified limit. The mean recoveries of all the impurities were calculated and the values are reported in Table IV.

Table IV
Recovery studies for the impurities of celecoxib

Impurity name	Spike level (%)	Concentration spiked ($\mu\text{g/mL}$)	Concentration recovered ($\mu\text{g/mL}$)	% Recovery ^a
	50	0.76	0.73	96.92
Impurity-A	100	1.52	1.51	99.34
	150	2.28	2.18	95.76
	50	0.74	0.72	97.74
Impurity-B	100	1.48	1.44	97.52
	150	2.26	2.23	98.82
	50	0.66	0.63	95.38
Impurity-C	100	1.33	1.28	96.16
	150	1.99	1.93	97.07
	50	0.72	0.71	97.75
Impurity-D	100	1.45	1.41	97.29
	150	2.18	2.12	97.22

a; average of three determinations

Precision

The precision of the method for the determination of impurities related to celecoxib was studied for repeatability and intermediate precision at 100 % level. Repeatability was demonstrated by analyzing the standard solution spiked with impurities for six times. The % R.S.D for the peak area of each impurity was calculated. The intermediate precision was demonstrated by analyzing the same sample of celecoxib by two different analysts on two different days (Inter-day). Intra-day variations of impurities related to celecoxib were expressed in terms of % R.S.D and results are given in Table V.

Table V
Precision studies for celecoxib and its impurities

Name	Method precision % R.S.D(n=6)	Intermediate precision	
		Intra day % RSD(n=6)	Inter day % RSD(n=6)
Celecoxib	0.32	0.46 $\pm$ 0.08	0.42 $\pm$ 0.08
Impurity-A	0.98	0.92 $\pm$ 0.03	0.89 $\pm$ 0.07
Impurity-B	0.48	0.51 $\pm$ 0.06	0.49 $\pm$ 0.03
Impurity-C	0.74	0.72 $\pm$ 0.04	0.69 $\pm$ 0.06
Impurity-D	0.82	0.76 $\pm$ 0.02	0.89 $\pm$ 0.04

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. They were determined by measuring the magnitude of the analytical background by blank samples and calculating the signal-to-noise ratio for each compound by injecting a series of solutions. The values were shown in Tables VI and VII, and the chromatograms in Fig. 4 and 5.

Tables VI

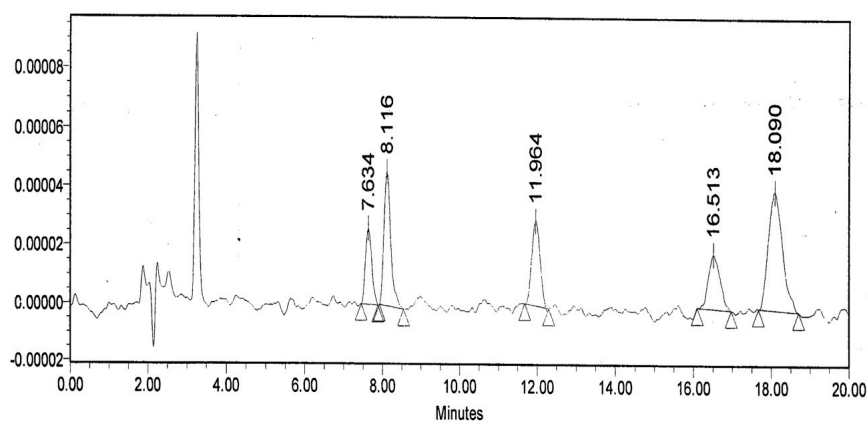
Results for limit of quantitation

S. No	Name	Concentration (µg/mL)	Observed signal to noise ratio
1	Impurity-A	0.378	10.4
2	Impurity-B	0.373	9.9
3	Impurity-C	0.373	9.8
4	Impurity-D	0.377	9.6
5	Celecoxib	0.374	10.2

Tables VII

Results for limit of detection

S. No	Name	Concentration (µg/mL)	Observed signal to noise ratio
1	Impurity-A	0.126	3.4
2	Impurity-B	0.124	3.2
3	Impurity-C	0.124	2.9
4	Impurity-D	0.125	3.1
5	Celecoxib	0.124	3.1

**Figure 4**

Typical chromatogram for LOQ

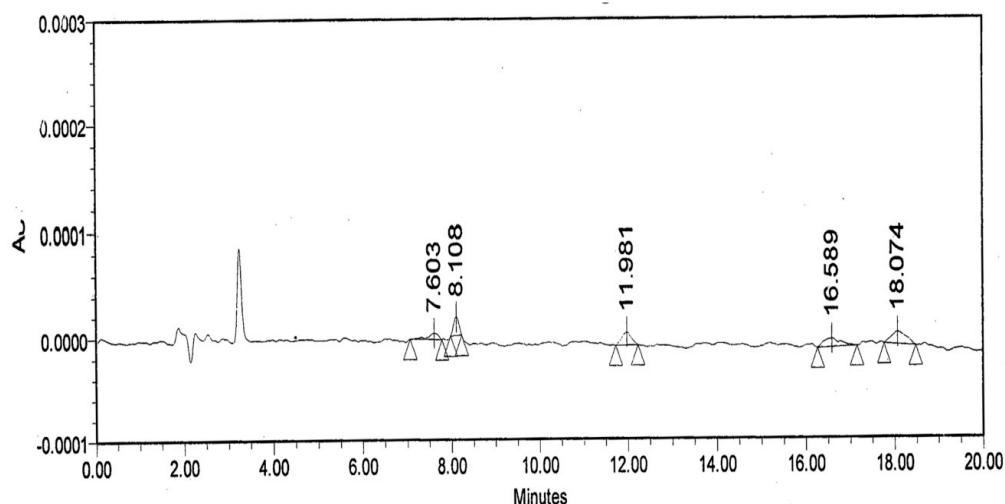


Figure 5
Typical chromatogram for LOD

Robustness

To determine the robustness of the developed method, chromatographic conditions were deliberately altered. The parameters selected were changed regarding the flow rate (± 0.2 mL/min), the pH of the buffer (± 0.2), in the ratio of mobile phase (± 4 %) and the column temperature (± 5 °C), the rest of the chromatographic conditions for each alteration study being kept constant.

Results of forced degradation studies

Stability testing of an active substance or finished product provide evidence on how the quality of a drug substance or drug product varies in time, influenced by a variety of environmental conditions like temperature, humidity, light etc.

The performed degradation study showed that celecoxib undergone significant degradation only in the presence of potassium permanganate. The oxidation degradation peaks were separated well from the main peak. Peak separation, peak purity results showed that the method is specific and capable of picking up all the degradation peaks. Hence it was concluded that the method was very selective and stability indicative and suitable for the determination of impurities in the pure drug.

The summary of the forced degradation study is given in Table VIII and the chromatograms are shown in Fig. 6.

Table VIII
Results of forced degradation studies

Stress condition	Purity angle	Purity threshold	Assay (%)	Degradation
Acid degradation	0.490	11.523	98.7	No degradation peak was observed
Alkali degradation	0.470	12.062	98.4	No degradation peak was observed
Thermal degradation	0.186	0.663	99.4	No degradation peak was observed
Humidity degradation	0.105	0.552	99.7	No degradation peak was observed
Photolytic degradation	0.140	0.636	99.5	No degradation peak was observed
Oxidative degradation	0.196	9.541	82.3	Observed significant degradation of about 18%. The major degradation peaks are at 6.2 min. The degradation peaks are well resolved from main peak and also from known impurities peaks. These degradation peaks are not matching with any of the known peaks

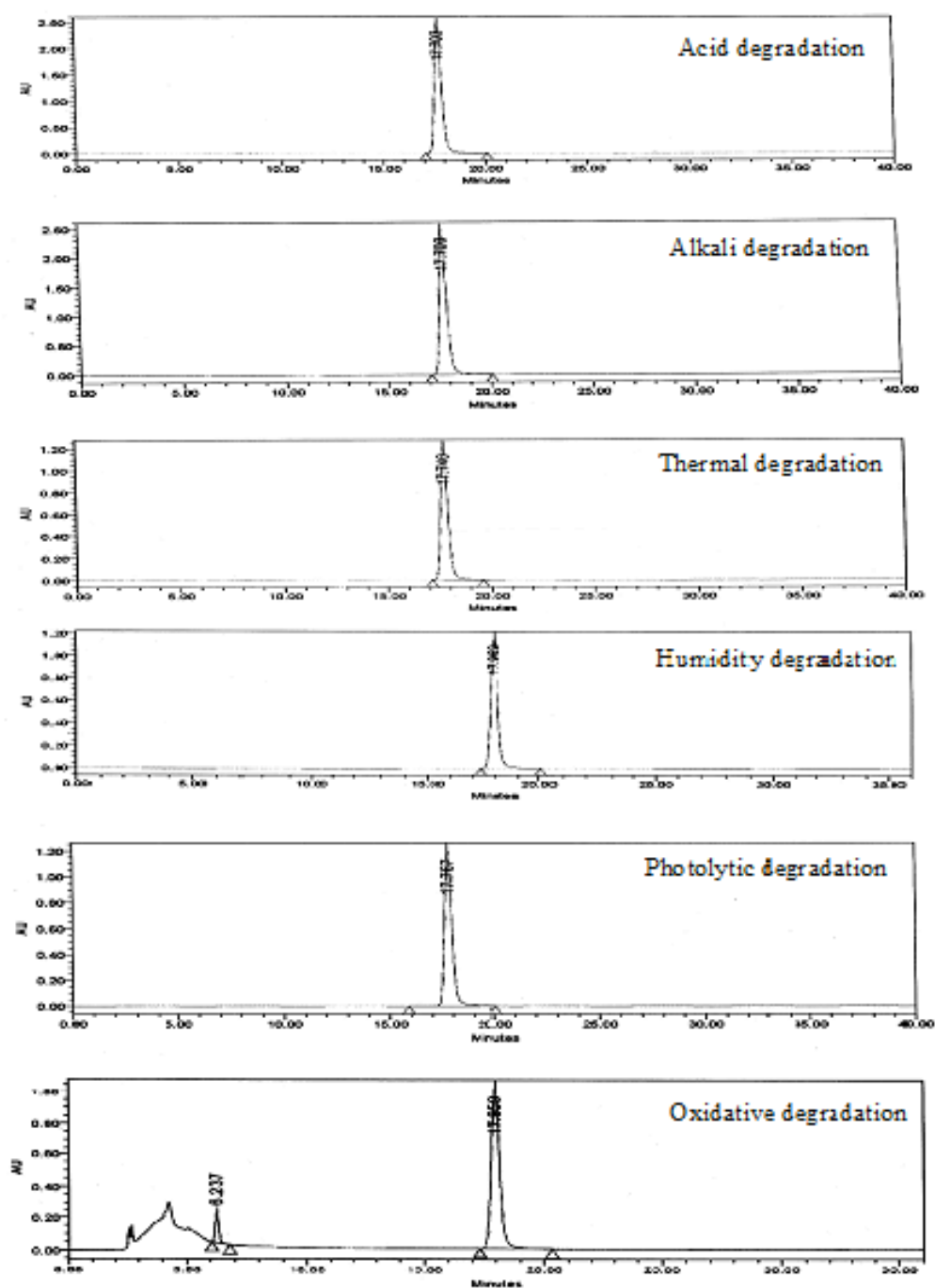


Figure 6
Chromatograms of forced degradation studies

Conclusions

A new isocratic RP-HPLC method was developed for the separation and determination of process-related impurities in celecoxib and validated as per ICH guidelines. The method was found to be simple, sensitive, precise, robust and accurate. Therefore, this method can be used for routine testing as well as stability analysis of celecoxib drug substance. All statistical results (Mean, % RSD and % recovery) were within the acceptance criteria.

Acknowledgements

The authors are thankful to Pharmazell R&D Centre, Andhra Pradesh, India for providing the celecoxib and its impurities samples.

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