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Estimation of Valdecoxib in Pharmaceutical Dosage Forms by Reverse Phase HPLC

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A rapid and sensitive reverse phase high performance liquid chromatographic method has been described for the estimation of valdecoxib in bulk samples and pharmaceutical dosage forms using RP C-18 column. The mobile phase consisted of 0.01 M ammonium acetate in water and acetonitrile in the ratio 50 : 50 (v/v) and was pumped at 1.5 mL/min at 40°C. The detection was carried out at 243 nm and the calibration curve was linear in the range of 5 to 150 μg/mL. The method was statically validated for its linearity, precision and accuracy. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The detection limit was found to be 5 μg/mL. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining valdecoxib in bulk drug samples or in pharmaceutical dosage forms.

Key Words: Reverse phase HPLC; Valdecoxib, Pharmaceutical formulations.

INTRODUCTION

Valdecoxib (I) is a new anti-inflammatory drug that is highly selective for inhibition of the inducible form of cyclooxygenase (COX-2)[1]. This drug has recently been approved by US FDA for treatments of rheumatoid arthritis, osteoarthritis and pain[2–6]. Cyclooxygenase is responsible for prostaglandin synthesis. The enzyme exists as two isoforms[7]: a constitutive form, COX-1 and an inducible form, COX-2. The constitutive COX-1 appears to be responsible for most of the physiological prostaglandin production associated with gastric lining cytoprotection. In contrast, the inducible COX-2 is involved in acute inflammatory response including joint inflammation. The selective inhibition of COX-2 while preserving COX-1 function provides an anti-inflammatory and analgesic effect without compromising the gastrointestinal tract[8–10]. Clinical studies have demonstrated that COX-2 inhibitors lead to a significant reduction in joint pain, joint tenderness/pain, and joint swelling with a statistically-significant lower incidence of gastric ulceration[11, 12].

Chemically, valdecoxib is a 4-(5-methyl-3-phenyl-4-isoxazolyl) benzene sulfonamide and is not official in any pharmacopoeia. So far no assay procedure

has been reported for the determination of this drug either in bulk drug samples or pharmaceutical formulations The aim of this study is to develop a simple, rapid, precise and accurate reversed phase HPLC method for the determination of valdecoxib in bulk drug samples and in pharmaceutical dosage forms.

EXPERIMENTAL

Valdecoxib (assigned purity 99.98%) was *gratis* sample from M/s Torrent Pharmaceuticals Ltd, Ahmedabad, India. Acetonitrile and water used were of HPLC grade (Qualigens). The commercially available valdecoxib tablets that claimed to contain 5 mg or 10 mg of drug were procured from the local market.

Quantative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC class VP series) with two LC-10AT pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), a disposable guard column LC-18 (Pelliguard)^{TM}, LC-18, 2 cm, Supelco, Inc., Bellefonte (PA) and RP C-18 column (150 mm × 4.6 mm I.D.; particle size 5 μm) was used. The HPLC system was equipped with the software "Class-VP series", version 6.01 (Shimadzu).

Chromatographic conditions: The contents of the mobile phase were 0.01 M ammonium acetate in water and acetonitrile in the ratio 50 : 50 v/v that were filtered before use through 0.45 μm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 mL/min which yielded a column back pressure of 130–148 kg/cm^2. The run time was set at 15 min and column temperature was maintained at 40°C. The volume of the injection loop was 20 μL. Prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluent was monitored at 243 nm and the data were acquired, stored and analyzed with the software "Class-VP series", version 6.01 (Shimadzu).

Procedure: About 50 mg of valdecoxib was accurately weighed and taken in a 50 mL volumetric flask in 30 mL of mobile phase, sonicated to dissolve and diluted up to the volume with mobile phase to obtain a 1 mg/mL solution. From this stock solution 5 mL was taken and made upto 100 mL with mobile phase, resulting in a 50 μg/mL solution of valdecoxib. The standard solution prepared as above was injected into the column at a flow rate of 1.5 mL/min six times successively. The mean of six readings was taken as the retention time The regression of the drug concentration over the drug peak area was calculated and it was used to estimate the amount of valdecoxib in tablet dosage forms.

Valdecoxib standard solutions containing 5 to 150 μg/mL were prepared and each concentration level was used to calculate the linearity of results obtained. A graph was plotted for concentration *vs.* area under curve (AUC).The correlation coefficient was also calculated. Valdecoxib solutions containing 5, 50 and 150 μg/mL were used to study reproducibility and stability. The solutions were analyzed for 8 h at an interval of 2 h while storing the sample solution at room temperature. The test solutions were also analyzed after 24 and 48 h while maintaining them at < 5°C in a refrigerator for finding out the intra- and inter-day

variations of proposed HPLC analysis. Recovery studies were carried out by adding known amount of valdecoxib to the preanalyzed samples and subjecting them to the proposed HPLC method.

Estimation of valdecoxib in tablet dosage forms: Ten tablets were weighed and crushed to fine powder. An accurately weighed portion of the powder, equivalent to 50 mg of valdecoxib, was transferred to a 100 mL volumetric flask containing about 30 mL of methanol The contents of the flask were sonicated to dissolve valdecoxib, made up to volume with methanol and the resulting solution was filtered through a 0.45 μm filter. 1 mL of this solution was taken in a 100 mL volumetric flask and made up to volume with mobile phase. This solution (20 μL) was injected in triplicate into the column. The mean values of peak area (six determinations) were calculated and the drug content in the tablet was quantified using the regression equation obtained with the standard drug solution. The same procedure was followed for the estimation of valdecoxib in two different brands of tablet dosage forms.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of valdecoxib in bulk samples and pharmaceutical preparations. The column pressure varied from 138 to 140 kgf/cm^2. The retention time of valdecoxib was 8.789 min. Each concentration of the samples was injected six times and the same retention time was observed. The mean of peak area at each concentration was also calculated. A good linear relationship ($r = 0.9998$) was observed between the concentration of valdecoxib and peak area (Table-1). Valdecoxib solutions containing 5, 50 and 150 μg/mL were analyzed by the proposed HPLC method for finding out intra- and inter-day variations (Table-2). When a known amount of the drug solution was added to the powdered sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method, there was a high recovery (Table-3) of valdecoxib (100.13 ± 0.082) indicating that the proposed procedure for the determination of valdecoxib in the tablet dosage form is highly accurate.

TABLE-1
CALIBRATION OF THE HPLC METHOD

Concentration of valdecoxib (μg/mL)	Mean peak area ratio (n = 6)	CV (%)
5	216118	0.8
25	1075591	0.7
50	2161487	0.6
75	3157093	0.8
100	4332071	0.6
150	6328866	0.8

TABLE-2
INTER- AND INTRA-DAY PRECISION FOR VALDECOXIB ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED METHOD

Concentration of valdecoxib (μg/mL)	Observed concentration of valdecoxib (μg/mL)			
	Intra day		Inter-day	
	Mean (n = 6)	CV (%)	Mean (n = 6)	CV (%)
5	5.02	0.97	5.04	1.12
50	50.04	0.19	49.95	0.43

TABLE-3
EXPERIMENTAL VALUES OBTAINED IN THE RECOVERY TEST FOR VALDECOXIB TABLETS BY PROPOSED HPLC

Amount of drug added (μg) to drug solution/ powdered tablet formulation	Recovery from drug solution		Recovery from powdered tablet formulation	
	Mean (± s.d.) amount (μg) found (n = 6)	Mean (± s.d.) % recovery (n = 6)	Mean (± s.d.) amount (μg) found (n = 6)	Mean (± s.d.) % recovery (n = 6)
5	5.02 ± 0.12	100.02 ± 0.17	5.07 ± 0.05	99.79 ± 0.59
50	50.03 ± 0.08	99.94 ± 0.32	50.08 ± 0.04	100.13 ± 0.082
150	150.01 ± 0.127	99.80 ± 0.49	150.08 ± 0.15	100.12 ± 0.10

TABLE-4
MEAN (± s.d.) AMOUNT OF VALDECOXIB IN TABLET DOSAGE FORM BY THE PROPOSED HPLC METHOD

Brand of tablet	Labelled amount of drug (mg)	Mean (± s.d.) amount (μg) found (n = 6) by the proposed method	Mean (± s.d.) % labelled amount (n = 6)
ABC	10	100.8 ± 0.01	100.08 ± 0.19
Abc	20	19.96 ± 0.15	99.8 ± 0.05
XYZ	10	10.02 ± 0.11	100.4 ± 0.07
xyz	20	20.11 ± 0.35	100.06 ± 0.35

The drug content in the tablets was quantified using the proposed analytical method. The mean content of valdecoxib in four different tablet dosage forms is shown in Table-4. The absence of additional peaks in test solution indicates no interference of the excipients used in the tablet dosage form. The tablets were found to contain 99.8% to 100.4%.

In conclusion, the proposed HPLC method was found to be simple, precise, highly accurate, specific and less time consuming. Hence, it can be used for the routine determination of valdecoxib in bulk samples and in its pharmaceutical preparations.

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