HPLC Method for the Determination of Celecoxib in Human Plasma and its Applications in Pharmacokinetics and Bioequivalence Studies

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(Received 27th July 2007, revised 9th October 2007)

Summary: A sensitive, precise and fast reverse phase HPLC method was developed and validated for quantitative analysis of celecoxib in human plasma in order to carry out bioequivalence studies. Celecoxib was extracted using liquid-liquid extraction method and analyzed on BDS Hypersil C8 (150 mm × 4.6 mm × 5 μm) column, with mobile phase of composition acetonitrile: water: triethyleamine (50: 50: 0.05), pH 5 was adjusted with acetic acid, delivered at a rate of 1.5 ml/ min, using UV-detector at 254 nm. The standard curve was linear (r^2 = 0.9911) in the range of 0.032–2 μg/ ml. Intra day and inter days expressed by relative standard deviation (R.S.D.) was less than 3 % and inaccuracy was less than 4 %. The extraction recovery of Celecoxib from human plasma was greater than 89 %. The assay has been used to analyze samples collected for the purpose of bioequivalence studies of two brands of celecoxib in healthy human volunteers. Furthermore, this method can be used to analyze samples of celecoxib collected in real clinical situations.

Introduction

Celecoxib (CEL) 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazole-1-yl] benzene-sulfonamide [1] (Fig. 1) is a non-steroidal anti inflammatory drug (NSAID) approved for the treatment of rheumatic pain and osteoarthritis. It selectively inhibits cyclooxygenase-2 (COX-2) [2], the inducible isoform of cyclooxygenases, involved in prostaglandin synthesis [3]. Celecoxib is deficient in a carboxylic group present in most NSAIDs and therefore, are able to orient into the COX-2 enzyme in a selective manner that differs from that of other NSAIDs [4].

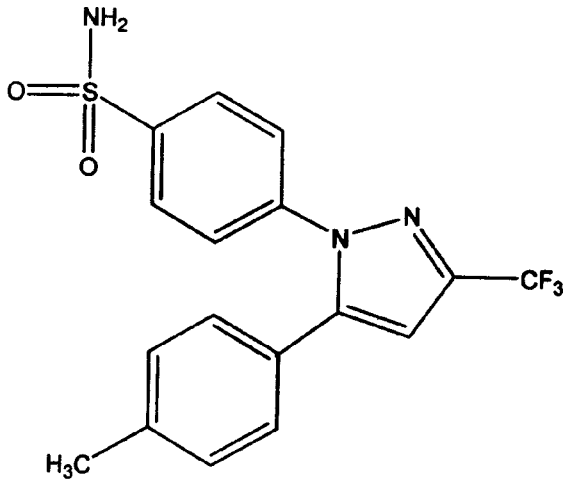

Fig. 1: Chemical structure of celecoxib.

Different analytical methods have been described in literature for the analysis of celecoxib in human plasma and pharmaceutical formulations. These methods include different extraction procedures, sample processing with different detection techniques employed such as thin layer chromatography (TLC) [5], first derivative Spectrophotometry [5], adsorptive stripping voltammetry [6], micellar electrokinetic chromatography (MEKC) [7], liquid chromatography (LC) [8], liquid chromatography-mass spectrometry (LC-MS) [9], high performance liquid chromatography with solid-phase extraction (HPLC-SPE) [10], high performance liquid chromatography with UV detector [2, 11] and high performance liquid chromatography with fluorimetric detection [12, 13]. Although different bio-analytical methods are available in literature but the present method shows greater sensitivity, precision and accuracy. The fastness, cost effectiveness and simple extraction procedure make it ideal for quantitative analysis of plasma drug concentrations.

Results and Discussion

Standard curve of celecoxib in plasma shows the concentration *vs* response (peak area)

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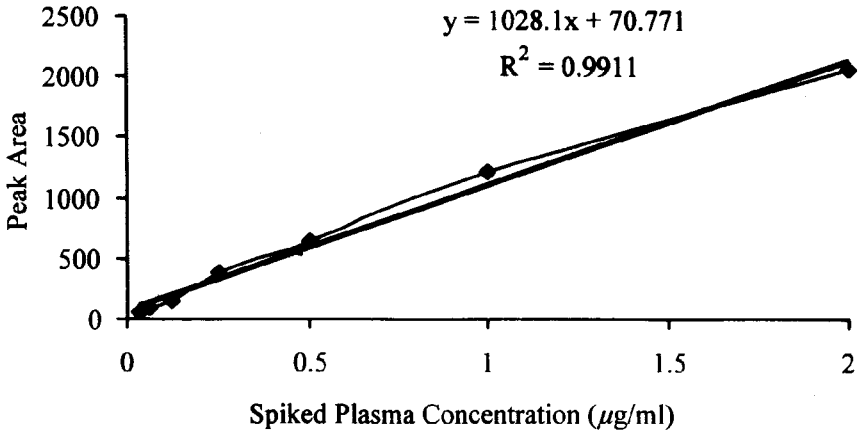

Fig. 2: Standard curve of Celecoxib in plasma.

that was linear in the range of 0.032–2.0 μg/ ml (Fig. 2). The mean value of correlation coefficient (r^2) was 0.9911. The average slope and intercept of the regression equation were 1028.1 ± 0.2 and 70.771 ± 0.05, respectively (Table-1). The intra day and inter days variations of the assay were determined at celecoxib concentrations of 0.032, 0.250, 0.500 and 2.000 μg/ ml and values of precision and accuracy were calculated (Table-2). Percent recovery for each dilution was calculated by dividing extracted plasma samples with plasma free dilutions. The average recovery for all samples was 89.36 ± 1.18 % (Table-3). The bioavailability parameters of celecoxib including C_{max}, T_{max} and AUC for both the reference and test products were calculated (Table-4). A typical chromatogram showing the separation of celecoxib plasma samples on C8 stationary phase (Fig. 3).

The use of suitable-solvents makes it possible to extract Celecoxib which is a weak acid drug from human plasma samples. The present method is a modification and simplification of all above methods making the extraction easy and fast with comparable extraction efficiency. This method is based on liquid liquid extraction using suitable-solvent system for withdrawl of drug from plasma samples as well as separation and quantification of drug using UV detection. The discrepancies of the previous methods were removed. In a reported method, the retention time of celecoxib was more than 20 minutes [14] which is time consuming where as in our method the retention time is 5.8 minutes which shows that the method is fast. The reported extraction efficiency of 70 % [14] was increased to greater than 89 % by using extraction solvent chloroform instead of isooctane-isopropanol with complete resolution. This shows that our method is more efficient than the

Table-1: Mean ± Standard deviation of slope, intercept and correlation coefficient of calibration curve equation (n = 3).

Parameters	Mean ± S.D.
Slope	1028.1 ± 0.2
Intercept	70.771 ± 0.05
Correlation coefficient	0.9911 ± 0.0009

Table-2: Intra day and inter days variability of assay.

Concentration Added (μg/ ml)	Mean Concentration Found (μg/ ml) (Mean ± S.D.)	Accuracy (% difference)	Precision (% R.S.D.)
Intra-day (n = 5)			
0.032	0.032 ± 0.0008	0	2.5
0.250	0.251 ± 0.0027	0.4	1.0
0.500	0.508 ± 0.0053	1.6	1.0
2.000	2.046 ± 0.0464	2.3	2.2
Inter-days; over three different days (n = 15)			
0.032	0.031 ± 0.0009	-3.1	2.9
0.250	0.250 ± 0.0018	0	0.7
0.500	0.503 ± 0.0027	0.6	0.5
2.000	2.024 ± 0.0329	1.2	1.6

Table-3: Percentage recovery of celecoxib from human plasma at various concentrations (n=3).

Concentration (μg/ ml)	Percentage Recovery ± S.D. (μg/ ml)
0.032	89.56 ± 0.97
0.250	89.05 ± 1.23
0.500	89.54 ± 1.32
2.00	89.29 ± 1.19
	Mean ± S. D. = 89.36 ± 1.18

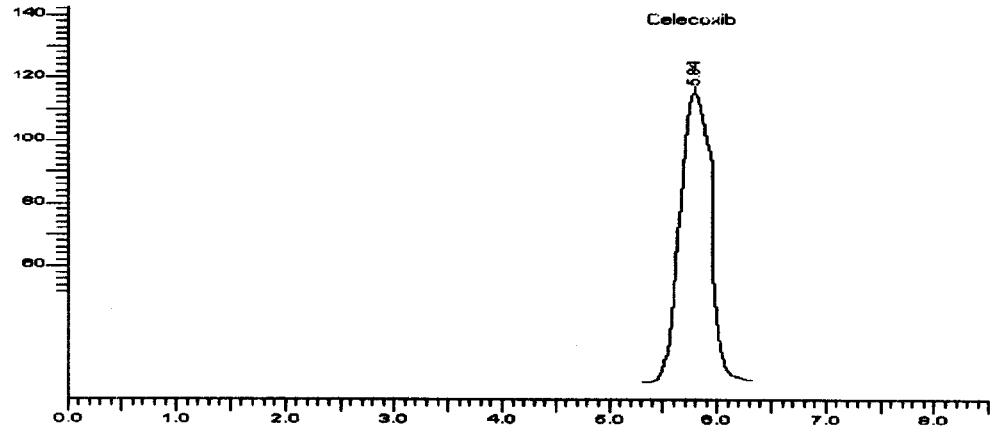

Fig. 3: Chromatogram showing separation of celecoxib on C8 Column.

Table-4: Comparison of bioavailability parameters of two brands of celecoxib capsules in the oral dose of 200 mg in healthy human subjects.

Parameters	Reference (Celbexx) (mean ± S. D.)	Test (Rheuoxib) (mean ± S.D.)
C_{max} (μg/ ml)	1.064 ± 0.030	1.009 ± 0.061
T_{max} (h)	3.250 ± 0.886	3.500 ± 0.535
AUC (μg h/ ml)	29.315 ± 2.799	25.931 ± 3.926

previously used methods for the extraction of celecoxib from human plasma. However, chloroform has also been used as an extraction solvent by Schonberger *et al.*, [12], using 8 ml chloroform, 0.5 ml plasma, 0.5 ml saturated sodium chloride solution and 1 ml acetonitrile with a final volume of 10 ml. Practically this large volume causes difficulties during the process of extraction. Additionally, in certain cases, creation of emulsion prevented the separation of aqueous and organic phase. By using smaller volumes of extraction solvent, the present method is easy to perform and cost effective as compared to the method used by Schonberger, *et al.*, (2002) [12]. Shafiee *et al.*, (2004) used 40 % acetonitrile in mobile phase gave tailing below flow rate of 0.9 ml/ min. In our method, the concentration of acetonitrile was increased to 50 % with a flow rate of 1.5 ml/ min producing sharp peaks. Chow *et al.*, (2004) used high performance liquid chromatography and solid-phase extraction method showing extraction recovery greater than 88 %. In the present method the extraction recovery was greater than 89 % with a simple liquid liquid extraction procedure as compared to complex solid phase extraction method. It shows that present method is more efficient, easy to perform and rapid. The method is also checked for sensitivity, LOQ was determined to be 32 ng/ ml compared to LOQ determined by Chow *et al.*, (2004) as 40 ng/ ml. This shows that the present method is more sensitive.

Intra day and inter - day precision expressed by relative standard deviation (R.S.D) is less than 3 % and inaccuracy is less than 4 % while R.S.D. for intraday and inter days variability of method presented by Chow *et al.*, (2004) was 5 % and 12 %, respectively. These results show that the present method is more sensitive, efficient, precise, accurate and rapid compared to the methods published previously.

Experimental

Chemicals and Reagents

Celecoxib was a kind gift by Getz Pharma Pvt. Ltd., HPLC grade acetonitrile; methanol and analytical grade chloroform were purchased from Merck-Germany. All other chemicals and solvents were of analytical grade and used without any further purification.

Experimental Design

The study consisted of a single oral dose of two treatments, two-way crossover design. The study was conducted at the Bioequivalence Studies Centre, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur.

The study employed the subjects to receive single oral dose of celecoxib 200 mg on an empty stomach. The subjects were instructed to fast over night prior to treatment. The standardized breakfast and lunch was given to subjects according to the study protocols. One week wash out period was given for second treatment.

Sample Collection

A 20 gauge venous cannula was interleaved into forearm for collection of blood samples. A blood sample of 3 ml was collected before drug was given (zero time) and then at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 hours after dosing of Celecoxib capsule. Blood samples were centrifuged at 3500 rpm for 10 minutes and plasma was collected. The plasma samples were then frozen at -20 °C until assay [4, 5].

Preparation of Mobile Phase

The mobile phase was filtered through a 0.45 μm membrane filter before use. The mobile phase consisted of acetonitrile: water: triethyleamine (50: 50: 0.05). The pH of mobile phase was adjusted with glacial acetic acid (100 %) to 5. The mobile phase was then degassed by passing Nitrogen gas for about 2-3 minutes until complete degassing of mobile phase was ensured.

Preparation of Stock Solution

Stock solution of celecoxib was prepared by dissolving drug in acetonitrile to give a final concentration of 2 mg/ ml. Standard solutions were obtained by diluting this solution with acetonitrile to give concentrations over the range of 0.032-2 μg/ ml.

Extraction Procedure

Extraction procedure was based on liquid-liquid extraction method. In extraction procedure 0.5 ml of drug solution was spiked with 0.5 ml of blank plasma in 5 ml glass centrifuge tube. Protein precipitation was carried out by adding 20μl of perchloric acid and vortex mixed for 1 min. 2 ml of chloroform was then added in centrifuge tube and vortex mixed for further 1 min and centrifuged at 3500 rpm for 10 minutes. The upper layer was discarded and the clear organic layer was taken in glass tube. The organic layer was evaporated under the gentle stream of nitrogen at 50°C using sample concentrator. The residue was reconstituted with 20 μl of mobile phase and was injected through 20 μl injection loop into HPLC system for assay.

Preparation of Standard Curve

Standard curve was constructed to encompass anticipated range of plasma Celecoxib concentration found in healthy volunteers. Blank plasma was spiked with celecoxib drug solutions to give concentration of 0.032, 0.064, 0.125, 0.25, 0.5, 1 and 2 μg/ ml, extraction procedure was same as described above. Injections of 20 μl were injected and spectra were taken of each concentration. The peak areas were noted for each concentration. Mean recovery of celecoxib was greater than 89 %. The intra day (within run) and inter days (between run) accuracy and precision of method were determined on three separate days.

High Performance Liquid Chromatography

Analysis was performed by using Perkin Elmer Liquid Chromatography, with a pump series 200, Perkin Elmer U.V. detector series 200 set at 254 nm. A reverse phase system was used consisting of BDS Hypersil C8 column (150 mm x 4.6 mm I.D x 5 μm particle size).

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