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Development of a HPLC Method for the Estimation of Celecoxib in Human Plasma

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Varying quantity of celecoxib (25 to 1500 ng) was added to drug-free human plasma (0.5 mL) and mixed with 0.5 mL of acetonitrile. The mixture was vortexed for 5 min and centrifuged at 3,000 rpm for 10 min and the supernatant liquid was filtered. Fifty microlitres of the resultant filtrate was injected into a reverse phase C-18 column using a mobile phase consisting of methanol and acetic acid (pH adjusted to 3.0 with dilute ammonia solution) in the ratio of 68 : 32% v/v at a flow rate of 1.5 mL/min. The eluents were monitored at 254 nm. The method was validated for its linearity, precision and accuracy. The present HPLC method was found to be simple, precise, specific, less time consuming and accurate for the estimation of celecoxib in human plasma.

Key words: Celecoxib; Human plasma, Estimation, HPLC.

INTRODUCTION

Two HPLC methods[1,2] and one LC-MS method[3] have been reported for the estimation of celecoxib in human plasma. Some of the reported HPLC methods[2, 3] required coulometric and electrochemical detectors and the process is considered tedious. However, the HPLC methods using the most commonly available columns are preferred. In the present study a sensitive, accurate and precise HPLC method (external standard) has been developed for the estimation of celecoxib in human plasma using RP C-18 column and simple UV detection.

EXPERIMENTAL

Celecoxib was a gift sample from M/s Torrent Laboratories Ltd., Ahmedabad, India. Glacial acetic acid and ammonia were of analytical grade and supplied by M/s Qualigens, Mumbai, India. Acetonitrile, methanol and water used were of HPLC grade (Qualigens).

Instrumentation: A gradient high pressure liquid chromatography (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 5 μm; YMC, Inc., Wilmington, NC

28403, U.S.A) were used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

HPLC conditions: The mobile phase components, methanol and acetic acid (pH adjusted to 3.0 with dilute ammonia solution), were filtered through 0.2 μm membrane filter before use and pumped from the solvent reservoir in the ratio of 68 : 32% v/v to the column at a flow rate of 1.5 mL/min which yielded column back-pressure of 82–110 kg/cm^2. The detector sensitivity was set at 0.0001 a.u.f.s. The volume of each injection was 50 μL.

Calibration solutions: Six sets of plasma samples with varying drug concentrations were prepared by spiking drug-free plasma with an appropriate volume (100 μL) of a known amount of celecoxib so as to give a concentration range of 25 to 1500 ng/0.5 mL of plasma.

An aliquot of plasma (0.5 mL) was accurately measured into a 10 mL glass tube with a teflon-lined cap, followed by addition of 0.5 mL of acetonitrile. The mixture was vortexed to ensure complete mixing of contents for 5 min and centrifuged for 10 min at 3,000 rpm. The supernatant liquid was filtered through 0.2 μm membrane filter and fifty microlitres of the filtrate was injected in to reverse phase C-18 column and the eluents were monitored at 254 nm. The peak area of celecoxib was recorded and the regression of plasma concentration of celecoxib over its peak area was calculated using the least squares method of analysis.

Precision: Aliquots of drug-free plasma (0.5 mL) were spiked with celecoxib solutions (100 μL) so as to obtain concentrations of 50, 100 and 250 ng/0.5 mL of plasma. Each plasma sample was treated, as described above, and the filtrate was injected into the HPLC column (n = 5). Each sample was prepared in triplicate on three consecutive days, and injected into the HPLC column (n = 5) to observe the precision of the method.

Accuracy: The preanalyzed plasma samples containing 50 ng/ 0.5 mL were added with known quantity of celecoxib (50, 100 or 250 ng/0.5 mL) and subjected to the proposed HPLC method, in triplicate. The difference in the measured concentration and that of the added quantity (50, 100 or 250 ng/0.5 mL) expressed as per cent recovery.

RESULTS AND DISCUSSION

The run time of the present HPLC method was set at 18 min. Celecoxib appeared on the chromatogram at about 8.8 min (Fig. 1). When the same filtrate was injected 5 times, the retention time of the drug was the same. Table-1 shows the mean peak area of celecoxib solutions for 6 such determinations. When the concentration of celecoxib and its respective peak area was subjected to regression analysis by least squares method, a high correlation coefficient was observed (r = 0.99989) in the range of 25 to 1500 ng/0.5 mL. The regression of celecoxib concentration over its peak area ratio was found to be $Y = -10041.66 + 324.89653X$ where 'Y' is the peak area ratio and 'X' is the concentration of celecoxib. This regression equation was used to estimate the amount of celecoxib in plasma or in validation study (precision and accuracy).

TABLE 1
CALIBRATION CURVE FOR THE ESTIMATION OF CELECOXIB IN HUMAN PLASMA BY HPLC METHOD

Amount of celecoxib added to 0.5 mL human plasma (ng)	Peak area *	C.V. (%)
0	0	0.00
25	1797	1.61
50	7464	1.54
100	22181	0.85
250	69305	1.42
500	148891	0.95
1000	312708	1.72
1500	480133	1.10

*Mean of six determinations
Regression equation: Y= –10041.66 + 324.89653 X (r = 0.99989)

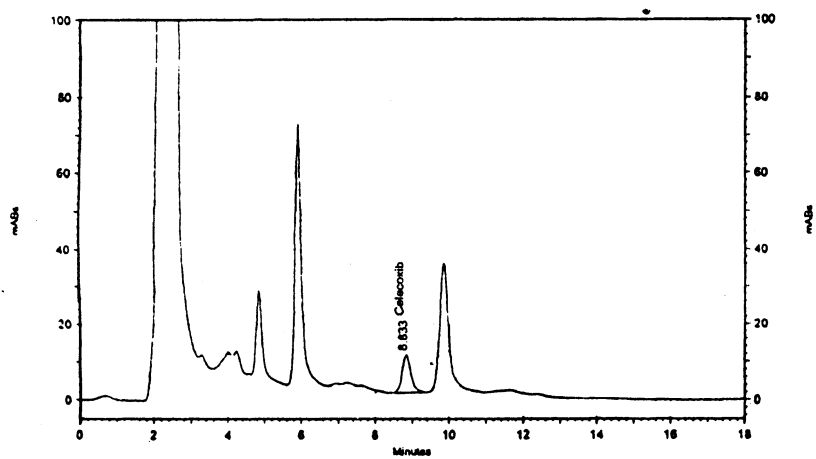

Fig. 1. Typical HPLC chromatogram of celecoxib in human plasma

The present HPLC method was also validated for intra- and inter-day variation. To assess the precision, drug-spiked plasma samples (0.5 mL) containing known quantity of the drug (50, 100 and 250 ng/mL) were subjected to the present HPLC method. The plasma samples were treated, as per the procedure described above, and the resultant filtrate was repeatedly injected on the same day and on three different days. The coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 1.9%. Also, the inter-day variation (3 days and five injections) was found to be less than 2%. Thus, the results show

that this HPLC method is highly reproducible. When a known amount of drug solution (50, 100 or 250 ng/mL) was added to preanalyzed plasma samples (100 ng/mL), there was a high recovery (97.67%) of celecoxib indicating that this HPLC method is highly accurate. The results of the study show that the present HPLC method is simple, precise and highly accurate.

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