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Validated HPLC method for the determination of celecoxib in human serum and its application in a clinical pharmacokinetic study

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A simple high performance liquid chromatographic method using UV detection for the determination of celecoxib, a specific COX 2 inhibitor, in serum was developed. Serum samples containing the internal standard, tolbutamide, are eluted through a C18, Wakosil column. After extracting with dichloromethane, the eluent is monitored at 250 nm. The mobile phase comprised of 10 mM potassium dihydrogen ortho phosphate (pH 3.2) and acetonitrile (50 : 50 v/v) with a flow rate of 1 ml/min. Retention times of celecoxib and tolbutamide were 9.6 and 3.5 min, respectively. The mean absolute recovery value was about 70–80%, while the intra day and inter day coefficient of variation and percent error values of the assay method were less than 10%. The calibration curve was linear over a concentration range of 10–1000 ng/ml.

1. Introduction

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID), and the first specific inhibitor of cyclo-oxygenase-2 (COX-2) to be approved by the US FDA. The selective inhibition of COX-2 is thought to lead to a reduction in the unwanted effects of NSAIDs like upper gastrointestinal complications [1]. Celecoxib is indicated for the relief of signs and symptoms of osteoarthritis and rheumatoid arthritis.

The rate of absorption of celecoxib is moderate when given orally. Celecoxib is extensively protein bound, and has an apparent volume of distribution of 455 ± 166 l/kg in humans. It is eliminated following biotransformation to carboxylic acid and glucuronide metabolites that are excreted in urine and faeces. Celecoxib is metabolized primarily by the cytochrome P450 (CYP) 2C9 isozyme and has elimination half life of about 11 h in healthy individuals [2].

For pharmacokinetic studies, a method that allows an accurate measurement of low concentrations of celecoxib in serum is needed. Various analytical methods including HPLC [3–7], have been developed for the determination of celecoxib in biological samples.

This paper describes a simple HPLC method which enables the determination of celecoxib with great accuracy, even in low concentrations in serum. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Investigations, results and discussion

2.1. Chromatography

Typical chromatograms corresponding to individual blank serum and celecoxib spiked control serum are shown in Figs. 1 and 2. The chromatogram of the serum sample of one of the volunteers obtained 2 h after oral administration of celecoxib is shown in Fig. 3. No endogenous interfering peaks were visible in the individual blank serum at the retention times of celecoxib and tolbutamide, thereby confirming the specificity of the analytical method. Both the analyte and the internal standard (I.S.) were well separated with retention times of 9.6 and 3.5 min, respectively. Sys-

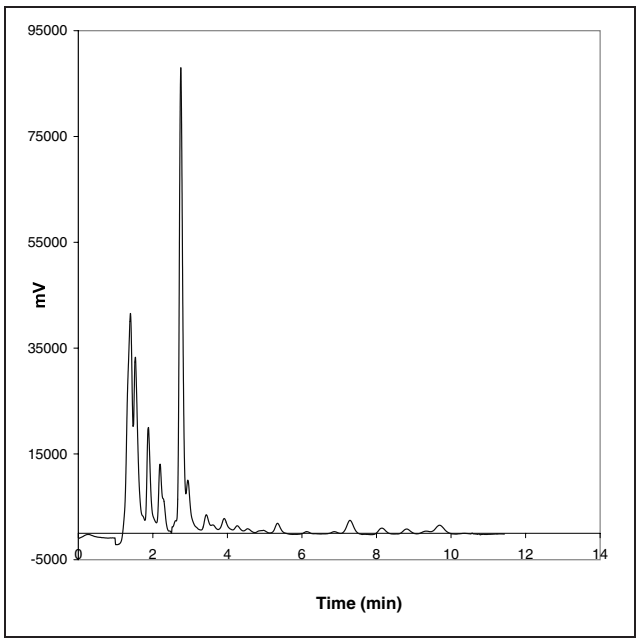

Fig. 1: Typical HPLC chromatogram for analysis of celecoxib: blank serum

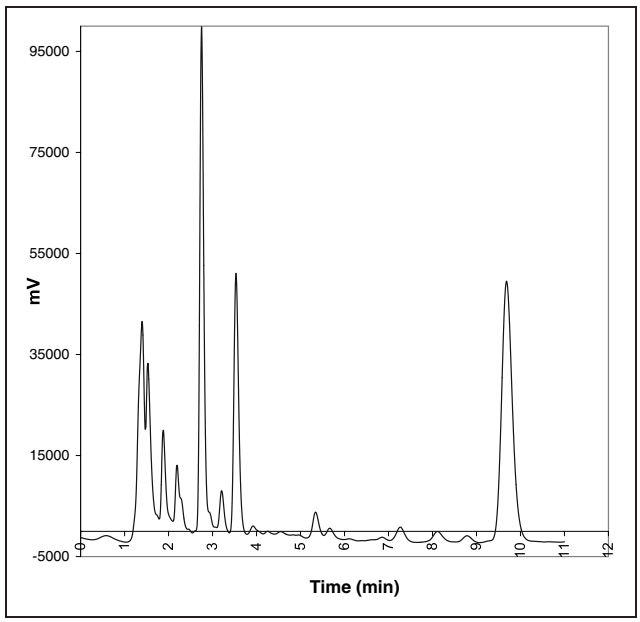

Fig. 2: Typical HPLC Chromatogram for analysis of celecoxib: blank serum spiked with 500 ng/ml of celecoxib

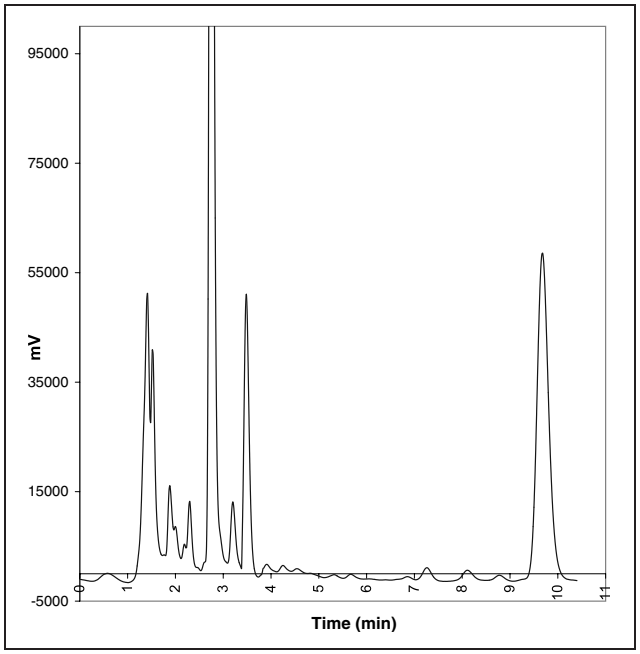

Fig. 3: Typical HPLC chromatogram for the analysis of celecoxib: serum sample collected 2 h after dosing. The respective concentration was 615 ng/ml of celecoxib

tem suitability parameters for the method were as follows: theoretical plates for celecoxib and I.S. were 2528 and 1627 respectively, tailing factor was less than 1.5 for both celecoxib and I.S. and resolution between celecoxib and I.S. was 10.98.

2.2. Quantification

The ratio of peak area of celecoxib to that of I.S. was used for the quantification of celecoxib in serum samples. The calibration curves were linear in the concentration range 10–1000 ng/ml. The calibration/regression equation is $y = mx + c$, where y represents the peak area ratio of celecoxib to I.S., x represent the concentrations of celecoxib, m is slope of the curve and c is the intercept. The equation of the calibration curve obtained from 8 points was $y = 0.0153x + 0.166$; ($r^2 = 0.9995$).

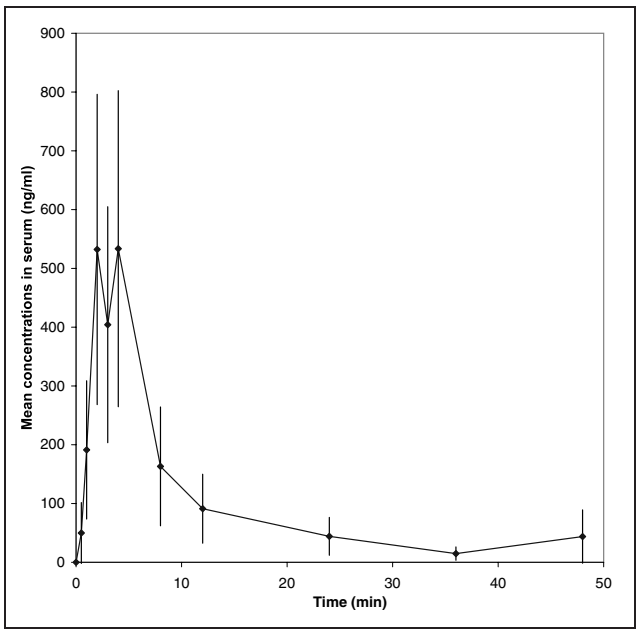

Fig. 4: Serum concentrations verses time profile of celecoxib after 200 mg oral administration. The data points are mean ± SD of 12 observations

Table 1: Intra and inter-day precision of determination of celecoxib in serum

Concentration (ng/ml)	Day	Mean concentrations (ng/ml)		
		Mean	S.D.	R.S.D
Intra day variation (n = 3)				
10	0	9.74	0.41	4.23
	1	10.54	0.34	3.22
	5	10.14	0.25	2.52
	10	9.88	0.48	4.85
100	0	98.88	6.32	6.39
	1	99.48	4.48	4.50
	5	100.56	2.58	2.56
	10	99.12	4.39	4.42
500	0	498.21	12.6	2.52
	1	489.14	22.35	4.56
	5	496.24	30.91	6.22
	10	496.74	16.04	3.22
1000	0	1000.65	33.92	3.38
	1	995.24	36.52	3.66
	5	984.24	44.38	4.50
	10	979.98	40.66	4.14
Inter day variation (n = 12)				
10		10.2	0.42	4.17
100		98.5	4.04	4.10
500		494	16.54	3.34
1000		992.24	27.88	2.80

The limit of quantification (LOQ), established by determining the concentrations of four spiked calibration standards having a reproducibility with a relative standard deviation (RSD) less than 20% and an accuracy of 80 to 120%, was found to be 10 ng/ml. Using this method, it is possible to further, increase the sensitivity by increasing the serum/injection volume.

The intra-day precision of the assay was determined by analyzing three spiked serum samples at each concentration on the same day. For the determination of inter-day precision, the samples were analyzed on four different days. The intra-day RSD ranged from 2.52–4.85, 2.56–6.39, 2.52–6.22 and 3.38–4.50 for 10, 100, 500 and 1000 ng/ml, respectively (Table 1). These values were within the limits (<15%) specified for inter and intra-day precision [8, 9].

The recovery of celecoxib from serum was estimated at 10, 100, 500 and 1000 ng/ml concentrations. Serum samples (in triplicates) containing celecoxib and I.S. were extracted (0.5 ml serum with 6 ml of dichloromethane) and analyzed. Four samples containing similar concentrations

Table 2: Recovery and accuracy of the method proposed

Concentration (ng/ml)	Absolute recovery (%)		Accuracy (%)	
	Mean ± S.D. (n = 3)	Range (min–max)	Mean ± S.D. (n = 3)	Range (min–max)
10	72.6 ± 1.34	71.5–74.1	95.9 ± 2.13	95.9–97.8
100	73.56 ± 2.41	71–75.8	96.2 ± 2.06	94.5–98.5
500	73.26 ± 3.78	69.1–76.5	98.5 ± 2.2	96.4–100.8
1000	78.56 ± 2.43	76.4–81.2	99.9 ± 1.54	98.2–101.2

of celecoxib in methanol were directly injected and peak areas were measured. Absolute recovery was calculated by comparing the peak areas for direct injection of pure celecoxib with that obtained from extracted serum samples spiked with the same amount of celecoxib and processed similarly. The absolute recoveries ranged from 70–80% (Table 2). The accuracy of the method was verified by comparing the concentrations of celecoxib measured in extracted serum with the actual concentrations added.

The mean serum concentration Vs time profile of celecoxib in 12 human volunteers following oral administration of 200 mg celecoxib is shown in Fig. 4. A peak concentration of 544.89 ± 73.91 ng/ml (C_{max}, mean $\pm$ SD) for celecoxib was reached at 4 ± 0.48 h (t_{max}, mean $\pm$ SD). The half-life was found to be 9.3 ± 1.5 h. Systemic exposure, area under the serum concentration ($AUC_{(0-\infty)}$) was found to be 4632.42 ± 742.75 ng · h/ml. These parameters were comparable with those reported earlier [10–12].

2.3. Conclusion

These experiments confirm that the presented method for determination of celecoxib in human serum is simple, sensitive, specific, precise and accurate and requires relatively small volumes of serum (500 µl). The calibration curve was linear in the concentration range between 10 and 1000 ng/ml and hence the method is suitable for conducting pharmacokinetic studies.

3. Experimental

3.1. Materials

Celecoxib and tolbutamide pure samples were gifted by Panacea Biotech, Chandigarh, India and Cadila Health Care, Ahmedabad, India, respectively. Acetonitrile (HPLC grade) was obtained from Qualigens Chemicals, Mumbai, India. Potassium dihydrogen ortho-phosphate (AR grade) and Dichloromethane (AR grade) were purchased from E. Merck (India) Limited, Mumbai, India. Celecoxib 200 mg capsules (Revebra® 200) were obtained from Dr. Reddy's Laboratories, Hyderabad, India. Double distilled water was used during the entire HPLC procedure.

3.2. Standard solutions

Primary stock solutions of 1 mg/ml of celecoxib and tolbutamide were prepared in methanol and stored at 4 °C. Appropriate dilutions of celecoxib were made in methanol to produce working stock solutions of 100, 10, 1 µg/ml and 100, 10 ng/ml. These dilutions were used to spike serum in the preparation of calibration curves. The I.S. working stock solution (200 µg/ml) was made from the primary stock solution using methanol for dilution. Calibration samples were prepared by spiking 500 µl of individual blank serum with the appropriate amount of drug on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control human serum in bulk of appropriate concentrations (10,100, 500 and 1000 ng/ml) and stored at −20 °C.

3.3. Extraction procedure

To 500 µl of serum, 20 µl of tolbutamide solution (200 µg/ml) were added and mixed well. To this, 6 ml of dichloromethane were added and vortexed for 5 min followed by centrifugation at 3500 rpm for 15 min. The organic phase was separated and evaporated under nitrogen gas. The residue was reconstituted in 100 µl of mobile phase and 20 µl of this solution was spiked on to the HPLC column.

3.4. Chromatographic conditions

The HPLC system (Shimadzu, Japan) consisted of a LC-10AT solvent delivery module, SPD-10A, and an UV-Visible spectrophotometric detector with LC10 software. The column used was SGE C18 (stainless steel column of length 15 cm and internal diameter of 4.6 mm packed with porous silica spheres of 5 µ diameter). The mobile phase consisting of potassium dihydrogen orthophosphate (10 mM, pH 3.2) and acetonitrile mixture (50 : 50) was used at a flow rate of 1.0 ml/min. The eluate was monitored at 250 nm. The sensitivity was set at 0.01 AUFS.

3.5. Linearity and limit of quantification

The calibration samples were prepared by spiking 500 µl of control human serum with the appropriate amount of celecoxib and I.S. on the day of analysis. The LOQ was defined as the lowest concentration at which the RSD and deviation from the nominal concentration were less than 20%.

3.6. Precision

Samples for the determination of precision were prepared by appropriately spiking control human serum in bulk, to get concentrations of 10, 100, 200 and 1000 ng/ml. At each concentration, 500 µl aliquots were distributed into screw-capped tubes and stored at −20 °C. Three replicates at each concentration were processed as described in the sample preparation on day 0, 1, 5, and 10 to determine intra day and inter day reproducibility. The precision of the method at each concentration was calculated as the RSD.

3.7. Recovery and accuracy

The recovery from serum samples was determined by comparing the amount of celecoxib from serum samples with that of recovery standards, which were processed similarly without serum matrix. The accuracy of the procedure was determined by expressing the mean calculated concentration as a percentage of the spiked/nominal concentration.

3.8. Application to a clinical pharmacokinetic study

The assay method was used to determine celecoxib concentrations in serum following oral administration of a celecoxib 200 mg tablet to 12 healthy male human volunteers after an overnight fast. Blood samples (5 ml) were withdrawn from the ante cubital vein at the intervals of 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 36 and 48 h following drug administration. The samples were allowed to clot and centrifuged at 3500 rpm for 15 min. The serum was separated and stored at −20 °C until the commencement of analysis.

Pharmacokinetic parameters like peak serum concentrations (C_{max}), time to reach peak concentrations (T_{max}), area under the curve (AUC), elimination half-life ($t_{1/2}$), volume of distribution (Vd/f) and total clearance (CL/f) for celecoxib were obtained for each subject using a computer program RAMKIN (Krishna, unpublished work) intended for calculation of model independent parameters.

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