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## Development and Validation of a RP-HPLC Method for the Quantitation and Dissolution Studies of Parecoxib.

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### ABSTRACT

An isocratic high-performance liquid chromatography (HPLC) procedure was developed for the dissolution rate studies and quantitative determination of parecoxib in solid dosage forms and in active pharmaceutical ingredient. HPLC separation was carried out by reversed phase chromatography on a Synergi® fusion C18 column (150 mm x 4.6 mm i.d.; 4 µm particle size), held at 30 °C. The mobile phase consisted of water, pH 7.0/acetone nitrile (52:48,v/v), run at a flow rate of 1.0 mL/min and with UV detection at 210 nm. Method validation investigated parameters such as the linearity ( $r^2=0.9999$ ), range, precision, accuracy, robustness and specificity. The method yielded good results with a quantitation limit of 50 ng/ml and a detection limit of 10 ng/mL. The dissolution test conditions and the dissolution medium was chosen as 0.5% of sodium lauryl sulfate in water at a stirring rate of 75 rpm. The described method can be successfully applied for the analysis of tablets, active pharmaceutical ingredient and drug dissolution studies.

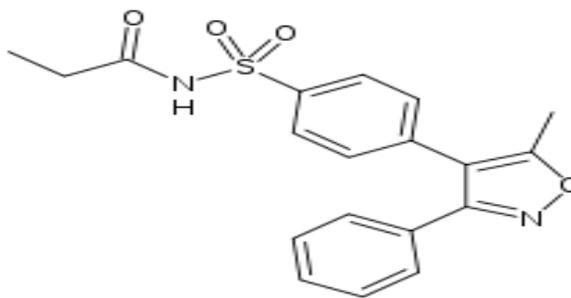
**Keywords:** Parecoxib, Dissolution, RP-HPLC, Validation.

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## INTRODUCTION

Parecoxib is a prodrug of valdecoxib which is chemically described as *N*-{[4-(5-methyl-3-phenylisoxazol-4-yl)phenyl]sulfonyl} propanamide, a novel highly selective cyclooxygenase-2 (COX-2) inhibitor investigated for the treatment of many inflammatory diseases such as rheumatoid arthritis, osteoarthritis and pain, causing fewer gastrointestinal complications than conventional non-steroidal anti-inflammatory drugs (NSAIDs) [1]. Dissolution test has emerged in the pharmaceutical field as a very important tool based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be solubilised. Therefore the dissolution studies are used not only to assess batch-to batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for screening and proper assessment of different formulations.



## STRUCTURE OF PARECOXIB

Moreover, the *in vitro* dissolution profiles obtained from dissolution rate studies have also been used for the successful characterization of the *in vivo* behavior of drugs [2-4]. A liquid chromatography-tandem mass spectrometry assay was developed and validated after solid phase extraction for estimation of parecoxib and its hydroxylated metabolite in human plasma [5]. The RP-HPLC method with UV detection was also described for the pharmacokinetic study and determination of parecoxib in human plasma [6,7]. The recent findings related to the adverse cardiovascular events or serious skin reactions, showed that additional pharmaceutical and clinical studies and the safety monitoring will be important to understand the risk and evaluate the safe dosage level for this class of drugs [8,9].

The aim of the present work was to develop and validate a sensitive RP-HPLC method to be applied to the analysis and dissolution rate studies of parecoxib in tablets, contributing therefore for the quality control and safety of this type of pharmaceutical preparation.

## MATERIALS AND METHODS

### Chemicals and reagents:

The parecoxib reference standard was generously supplied by IICT (Hyderabad, India). Parecoxib tablets containing 10, 20 and 40 mg of active substance were obtained from

commercial sources and used within their shelf life period. The HPLC-grade acetonitrile was purchased from Spectrochem (Pune, India). Analytical grade hydrochloric acid, sodium hydroxide and potassium dihydrogen phosphate monobasic were obtained from Magnum Labs (Hyderabad, India). All chemicals used were of pharmaceutical or special analytical grade. For all analyses, double-distilled water filtered through a 0.45  $\mu\text{m}$  membrane filter was used.

#### **Apparatus and Chromatographic Conditions:**

A Vankel VK7010 (VanKel Technology Group, Cary, USA), a paddle-stirrer type of apparatus, was used integrated with a VK 8000 dissolution sampling station, VK type bidirectional peristaltic pump. A Shimadzu HPLC system (Shimadzu, Kyoto, Japan) was used, equipped with an SCL-10AVP system controller, LC-10 ADVP pump, DGU-14A degasser, SIL-10ADVP autosampler and an SPD-M10AVP photodiode array (PDA) detector. The detector was set at 210 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP® V 6.12 software program. The experiments were carried out on a reversed phase Phenomenex® (Torrance, USA) Synergi fusion C18 column (150 mm x 4.6 mm I.D., with a particle size of 4  $\mu\text{m}$  and pore size of 80 Å). A security guard holder (4.0 mm x 3.0 mm I.D.) was used to protect the analytical column. The HPLC system was operated isocratically and the column was operated at 30 °C using a mobile phase of water, pH 7.0 adjusted with NaOH 0.1M/acetonitrile (52:48, v/v). Before use the mobile phase was filtered through a 0.45  $\mu\text{m}$  membrane filter (Millipore®) and run at a flow rate of 1.0 mL/ min. The injection volume was 10  $\mu\text{l}$  for both standard and samples and all determinations were carried out in five replicates. For the robustness test a reversed phase Shimadzu (Kyoto, Japan) C18 Shim-Pack CLC-ODS column (150 mm x 4.6 mm i.d.; 5  $\mu\text{m}$  particle size and pore size of 100 Å ) and an Ace (Aberdeen, Scotland) C18 column (150 mm x 4.6 mm i.d.; 5  $\mu\text{m}$  particle size and pore size of 80 Å), were also used.

#### **Parecoxib reference standard solutions:**

The stock solution of parecoxib was prepared by weighing 10 mg of the reference standard which was transferred into a 10 mL volumetric flask and diluting to volume with methanol. The concentration of this solution was 1 mg/mL and was stable for at least two months stored in refrigerator at 4 °C. Working standard solutions of parecoxib were prepared daily by diluting the stock solution to an appropriate concentration in water/methanol (60:40, v/v).

#### **Parecoxib sample solutions:**

Twenty tablets of each sample containing 10, 20 and 40 mg of parecoxib were separated, accurately weighed and crushed to a fine uniform particle size powder. An appropriate amount of each concentration was transferred into an individual 50 mL volumetric flask, diluted to volume with methanol and sonicated for 10 minutes, obtaining concentrations of 1 mg/mL (stock solution). Working sample solutions of parecoxib were prepared daily by diluting the stock solution to an appropriate concentration in water/ methanol (60:40, v/v).

**Validation of the chromatographic method:**

Once the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters: linearity, range, precision, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ), specificity and system suitability tests, following the ICH guidelines [10].

**Linearity and range:**

The range of linearity was determined by constructing three calibration curves. For the construction of each calibration curve seven standard solutions of parecoxib at different concentrations in the range of 0.05-150 µg/mL were prepared. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Each measurement was carried out in five replicates of 10 µl injections for the standard solution to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of parecoxib to obtain the calibration curve. The seven concentrations of the standard solution were subjected to regression analysis to calculate calibration equation and correlation coefficients.

**Precision:**

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing twelve repeated analysis of the same working solution of parecoxib, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter-day) and also by another analyst performing the analysis in the same laboratory (between-analysts).

**Accuracy:**

To confirm the accuracy of the proposed method, a total of 15 determinations were performed using 3 concentration levels covering the specified range.

**Robustness:**

The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.8 to 1.2 mL/min, the mobile phase composition with water pH 7.0/acetonitrile (55:45; 50:50; 48:52; 45:55), the wavelength in the range of 200 to 370 nm, the temperature of the column between 25 °C to 40 °C and using different columns.

**Limit of detection and limit of quantitation**

Limit of detection (LOD) and limit of quantitation (LOQ), were calculated based on the ICH guidelines [10].

**Specificity:**

Specificity of the method towards the drug was established through determination of purity peak of parecoxib in the working standard solution using a PDA detector.

**System suitability:**

To ensure the validity of the analytical procedure, a system suitability test was established. Data from five injections of 10  $\mu\text{L}$  of the working standard solution containing 100  $\mu\text{g/mL}$  were used for the evaluation of the system suitability parameters like asymmetry, number of theoretical plates, retention time and area, by the CLASS-VP® V 6.12 software.

**Analysis of tablet dosage forms:**

The analysis of the pharmaceutical tablet dosage forms of parecoxib, containing 10, 20 and 40 mg, was carried out by the proposed RPHPLC method against the reference standard.

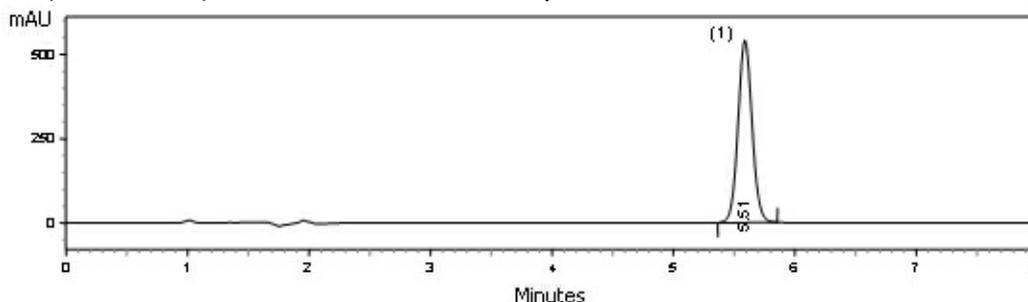
***In vitro* drug dissolution rate studies for tablets:**

The dissolution rate studies of parecoxib from tablets were performed on a paddle-stirrer type of apparatus by a semi-automated system. Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products 11,12 in 900 mL of different media of HCl 0.1 M (50 and 75 rpm), 0.25% and 0.5% of sodium lauryl sulphate in water (50 and 75 rpm), and distilled water (50 and 75 rpm), for 120 min. The temperature of the cell was maintained at  $37 \pm 0.5$  °C by using a thermostatic bath. At each sample time interval, an exact volume of the sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium to maintain a dissolution sink condition. A correction factor was included in the calculations to account for the drug lost in the sampling. At predetermined time intervals (0, 5, 10, 15, 30, 60, 90 and 120 min) for the development of the methodology and (0, 3, 5, 7, 10, 15, 20, 30, 45 and 60 min) for the dissolution studies, the concentrations of parecoxib in the dissolution medium were determined by the proposed RP-HPLC method. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to calculate the *in vitro* dissolution data (n=12) by the linear regression equation [11].

**RESULTS AND DISCUSSION**

A reversed-phase HPLC method was proposed as a suitable method for the determination of parecoxib in drug dissolution studies and tablet dosage form. The best chromatographic conditions were adequately selected. Figure 1 shows a typical chromatogram of parecoxib standard solution with a symmetrical peak, well separated from the solvent front. The retention time observed (5.51 min) allows a rapid determination of the drug. The calibration curves for parecoxib were constructed by plotting the area of the peaks versus concentration. Linearity was observed in a concentration range from 0.05 to 150  $\mu\text{g/mL}$ . This

concentration range corresponds to 0.05 to 150% of the target concentration of 100 µg/mL. A linear regression by the least squares method was then applied. The value of the determination coefficient ( $r^2 = 0.9999$ ) showed excellent linearity of the calibration curve for the method.



**FIGURE 01:** RP- HPLC chromatogram of parecoxib reference standard 100 µg/ml Peak 1: parecoxib.

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample.

Repeatability was studied by calculating the relative standard deviation (RSD) for twelve determinations of the concentration of 100 µg/mL, performed on the same day and under the same experimental conditions. The results of parecoxib determinations in the working standard solution with the relative standard deviation calculated as 0.06% are shown in Table 1. Intermediate precision includes the estimation of variations in analysis when a method is used within laboratories, on different days, by different analysts, and on different equipments.

**TABLE 01:** Parecoxib concentrations obtained in the analysis of twelve determinations by RP-HPLC to show method repeatability. a Mean of twelve determinations.

Sample	Theoretical Amount (µg/mL)	Experimental Amount (µg/mL)	Purity a (%)	RSD (%)
1	100.0	100.08	100.01	0.06
2	100.0	99.97		
3	100.0	100.08		
4	100.0	99.91		
5	100.0	99.95		
6	100.0	100.01		
7	100.0	99.98		
8	100.0	99.99		
9	100.0	100.04		
10	100.0	99.98		
11	100.0	100.13		
12	100.0	100.02		

**TABLE 02:** Between-day precision data of RP-HPLC for parecoxib. a Mean of five replicates; b Mean of three days.

Sample	Day	Purity a (%)	Mean b	RSD%
1	1	100.03	100.04	0.22
	2	100.27		
	3	99.84		
2	1	99.70	99.92	0.19
	2	100.04		
	3	100.03		

**TABLE 03:** Between-analysts precision data of RP-HPLC for parecoxib. a Mean of five replicates; b Mean of two analysts.

Sample	Analyst	Purity a (%)	Mean b	RSD (%)
1	A	100.07	99.92	0.21
	B	99.77		
2	A	99.89	100.12	0.33
	B	100.35		
3	A	99.80	99.99	0.27
	B	100.18		

**TABLE 04:** Recovery of parecoxib standard solution added to sample solution analyzed by RP-HPLC for parecoxib. a Mean of five replicates analyses; b Accuracy: obtained concentration expressed as % of the nominal concentration.

Theoretical Amount (µg/mL)	Experimental Amount (µg/mL)	Accuracy (%)	RSD (%)
80	79.92	100.03	0.12
100	100.02		
120	120.18		

The intermediate precision was assessed by analyzing two working standard solutions on three different days (inter-day, Table 2); the RSD values obtained were 0.22 and 0.19%, respectively. Between-analysts precision was determined by calculating the RSD for the analysis of three

working standard solutions by two analysts; the values were found to be 0.21, 0.33 and 0.27%, respectively (Table 3).

**TABLE 05:** Chromatographic conditions and range investigated during robustness testing. a Mean of five replicates

Variable	Range Investigated	Parecoxib a (%)	Optimized Value
Flow rate (mL/min)	0.8	100.91	1.0
	1.0	100.07	
	1.2	100.33	
Column Temperature (°C)	25	100.44	30
	30	100.06	
	35	99.83	
	40	99.70	
Injection Volume (µL)	5	99.62	10
	10	100.04	
	20	99.45	
Percent Acetonitrile	45	99.75	48
	48	100.19	
	50	101.09	
	55	99.35	
Wavelength (nm)	200-370	–	210

**TABLE 06:** Results of the system suitability test

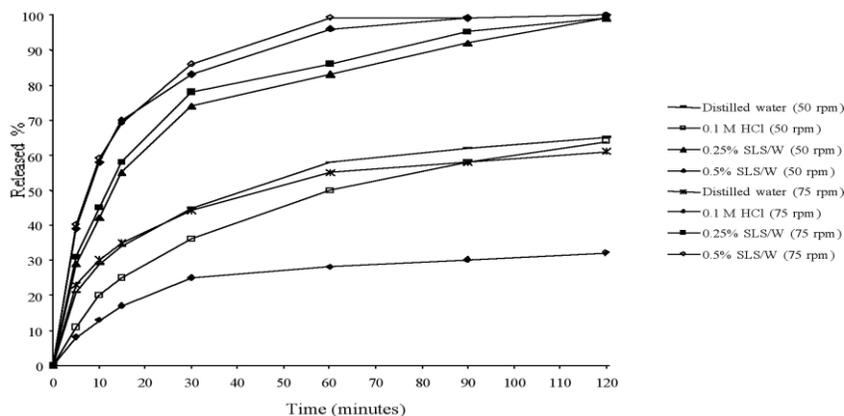
Parameter	Minimum	Maximum	RSD (%)	Status
Asymmetry	1.19	1.20	0.376	Passed
Theoretical Plates	9493	9828	1.207	Passed
Retention Time	5.510	5.529	0.159	Passed
Area	4192705	4221212	0.315	Passed

The accuracy of an analytical method is the closeness of the test results obtained using the proposed method and the true value. The accuracy was assessed from five replicates determinations of three different solutions containing 80, 100 and 120 µg/mL. The absolute means obtained were 99.90, 100.02 and 100.15% respectively, with a mean value of 100.03% and RSD of 0.12% as shown in Table 4. It is evident that the method is accurate within the desired range. The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered were: variations in the flow rate, percentage of acetonitrile in the mobile phase, column temperature and wavelength. The results and the experimental range of the selected variables are given in Table 5, together with the optimized values. There

were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing thus that the method is robust. The equivalence of the columns evaluated by the system suitability tests demonstrated also non significant column-to-column variability. The LOD and LOQ were obtained from the slope and the standard deviation of the intercept from three calibration curves determined by a linear regression line as defined by ICH. The LOD and LOQ were found to be 10 and 50 ng/mL, respectively.

These values were also used in an experimental assay confirming the calculation. The studies with the PDA detector showed that the parecoxib peak was free from any co eluting peak, demonstrating thus that the proposed method is specific for the analysis of parecoxib. The system suitability tests were also carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed. Results of system suitability tests are given in Table 6 showing that the parameters are within the suitable range. The RP-HPLC method validated in this paper was used to evaluate the potency of parecoxib in the same batches of tablet forms used for the dissolution studies, with the labeled claim of 10, 20 and 40 mg. The results obtained in the analysis were expressed as percentage of the drug and were: 100.45%, 99.81% and 100.75%, respectively. The *in vitro* studies were performed using eight different dissolution conditions.

As parecoxib is a poorly water-soluble drug with relatively low bioavailability, a surfactant agent sodium lauryl sulfate (SLS), was added in the media to improve the solubility of the drug. As shown in Figure 2 the best dissolution rate profile was achieved with water as the medium containing 0.5% of sodium lauryl sulfate with a paddle rotating at 75 rpm, that was chosen to carry out the dissolution tests of the batches of parecoxib containing 10, 20 and 40 mg. The concentrations of parecoxib in the dissolution medium were evaluated using the proposed RPHPLC method and the coefficients of variation at the earlier time points, up to 15 minutes, were within 7.99-12.92% (acceptable < 20%) and the other time points within 0.99-3.8% (acceptable < 10%) (n=12) [12].



**FIGURE 02:** Comparison of dissolution profiles of parecoxib tablets in different dissolution media with paddles (Apparatus 2) using the proposed RP-HPLC.

## CONCLUSION

The data validation shows that the RP-HPLC method is accurate, robust and possesses excellent linearity and precision characteristics. This method can be successfully used for the quantitation of parecoxib as active substance, in dissolution studies and in tablet dosage forms. The dissolution data are reliable and precise. This approach is valid not only for the parecoxib, but also for other solid dosage forms, therefore, contributing for the establishment of procedures to assure the quality, safety and efficacy of the developed drug product.

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