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A Novel Stability Indicating Reverse Phase Ultra Performance Liquid Chromatography Method Development and Validation for Estimation of Related Compounds of Parecoxib Sodium in Parecoxib Sodium for Injection.

Dhananjay P. Dwivedi*, B. Ravi Kumar, Sridhar.Y, Prof.J. Sreeramlu1
**Research Scholar, Department of Chemistry, Rayalaseema University, Kurnool, A.P.*
[1]Department of Chemistry, SK University, Anantapur, A.P.
Corresponding Author: Dhananjay P. Dwivedi

Abstract*: A new and rapid stability-indicatingreversed-phase ultra performance liquid chromatography (UPLC) method was developed and validated for determination of Parecoxibimpurities in Injectionformulation. The chromatographic separation was carried out on an Acquity UPLC BEH C18, (100mmx2.1mm) 1.7µmusing a mobile phase consisting of Potassium dihydrogen phosphate buffer with pH 6.5, acetonitrile and methanol using gradient programme at a flow rate of 0.30 ml/min with an injection volume of 2µL andthe UV detection was performed at 240 nm. The method was validated for precision, accuracy, specificity, linearity, sensitivity and robustness. The proposed method can be applied for quality control, release and stability analysis of Parecoxib impurities in Injection formulation.*

Keywords*: Parecoxib, Stability-indicating, UPLC, Validation.*

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I. 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 cycloxygenase-2 (COX-2) inhibitor, has aroused great interests among researchers mainly because of its central role 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).

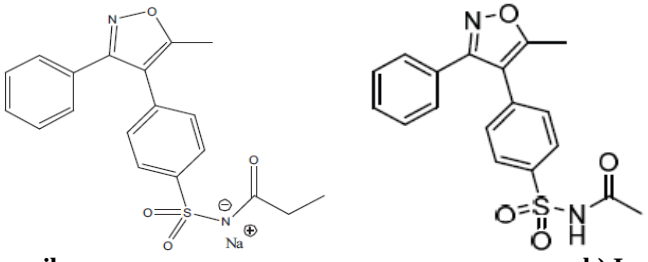
Parecoxibmolecular formula is C19H18N2O4S and molecular weight is370.423 g mol−1. The structural formula of Parecoxib and its impurities are depicted in Figure 1. Parecoxib is awhite to off-white, crystalline solid. The Parecoxib injection formulation is a sterile, preservative-free, clear, and colorless isotonic solution. Reconstitute the vial with 2 mL of sodium chloride 0.9% or glucose 5% and administer a single 40mg dose as an IV bolus over a few seconds or as a deep IM injection. An HPLC determination method was reported [6, 7] for Parecoxib and its impurities in drugsubstance. Studies on the estimation of Parecoxib and its impurities in an injection formulation were not available.

Parecoxib and its injection formulation are not official monographs in any of the pharmacopeia (USP, EP,BP, JP, and IP). Hence, no official methods have been reported for the estimation of Parecoxib and related substances in injection formulation. A literaturesurvey revealed several publications regarding the pharmacodynamics, pharmacokinetics, and Parecoxib estimation in plasma were reported [8, 9].

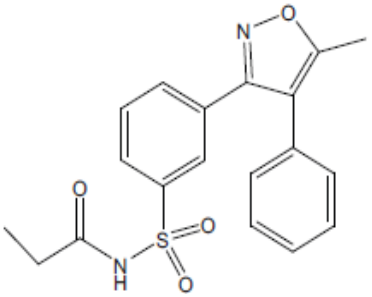
However, when it is required toincrease the throughput and reduce analysis cost,HPLC methods maynot be the first choice when comparedto UPLC methods.UPLC methods being simpler, faster and more economicmethods with decrease in run time and solventconsumptionthan HPLC,they represent an important alternative.

II. Chemical Structure Of Parecoxib And Its Impurities

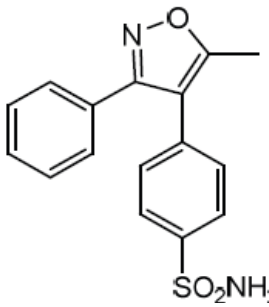
Fig.1.



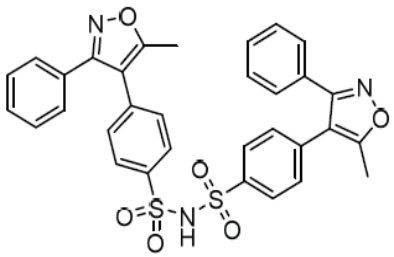
a) Parecoxib **b) Impurity-A**



c) Impurity-B



d)Impurity-C



e) Impurity-D

III. Materials And Methods

3.1 Chemicals And Drugs

Potassium dihydrogen phosphate (AR Grade) was used to prepare the buffer and obtained from Merck. Orthophosphoric acid (HPLC Grade) used to adjust the pH, was obtained from Qualigens. The organic solvents, Acetonitrile and Methanol (HPLC grade) were obtained from Rankem.The active pharmaceutical ingredient Parecoxib and its impurities were obtained from internally.

3.2 Instrument and Chromatographic condition:

The Integrated Acquity UPLC system used for the study was purchased from Waters Corporation, Milford, USA and equipped with Waters photodiode array detector (PDA). Data collection and analysis was performed using Empower software 2pro (Waters Corporation). The balance used for weighing the reference standards and samples was purchased from Metter Toledo. Separation was achieved on a Waters acquity UPLC BEH C18 column with dimensions of 100 mm x 2.1 mm I.D and a particle size of 1.7 µm. A simple mobile phase consisting of Potassium dihydrogen phosphate(0.01 M, pH 6.5) :Organic mixture (as Mobile phase A) and Buffer: Organic mixture (as Mobile phase B) was pumped into the UPLC chromatograph using a gradient program with varying compositions at a flow rate of 0.30 mL/ min, with a column temperature of 45°C throughout the run. Sample volume of 2µL was injected into the chromatograph and detected at 240 nm.
The final conditions summarized in the Table.No:1 and 2.

3.2.1 Preparation of standard and sample solution

A mixture of water and Acetonitrile in the ratio 60:40 was used as a diluent for preparing the solutions of standard and samples (diluent).

3.2.2 Standard stock solution

A standard stock solution was prepared by dissolving 40 mg of Parecoxib working standard in 100 mL of the diluent.

3.3.3 Preparation of standard solution for impurities determination (0.001 mg per mL)

The standard solution for the determination of impurities was prepared by diluting 3 mL of the standard stock solution into 50 mL with the diluent and further diluted 2 mL of the solution into 50 mL with the diluent to obtain a concentration of 0.001 mg per mL

3.3.4 Preparation of sample and placebo solution for impurities determination.

Sample vial was reconstituted with 2mL of 0.9% sodium chloride solution.Transferred the whole content of4 reconstituted vials into a 100 mL volumetric flask. Rinsed each vial with about 5 mL of diluent and transferred into the same volumetric flask. Diluted to volume with diluent and mixed well.

Pipetted 3 mL of above solution into 10mL volumetric flask, diluted to volume with diluent and mixedwellto obtain a concentration of 0.5 mg per mL.
Similarly the placebo solution was also prepared.

3.3.5 Preparation of spiked sample solution for impurities determination

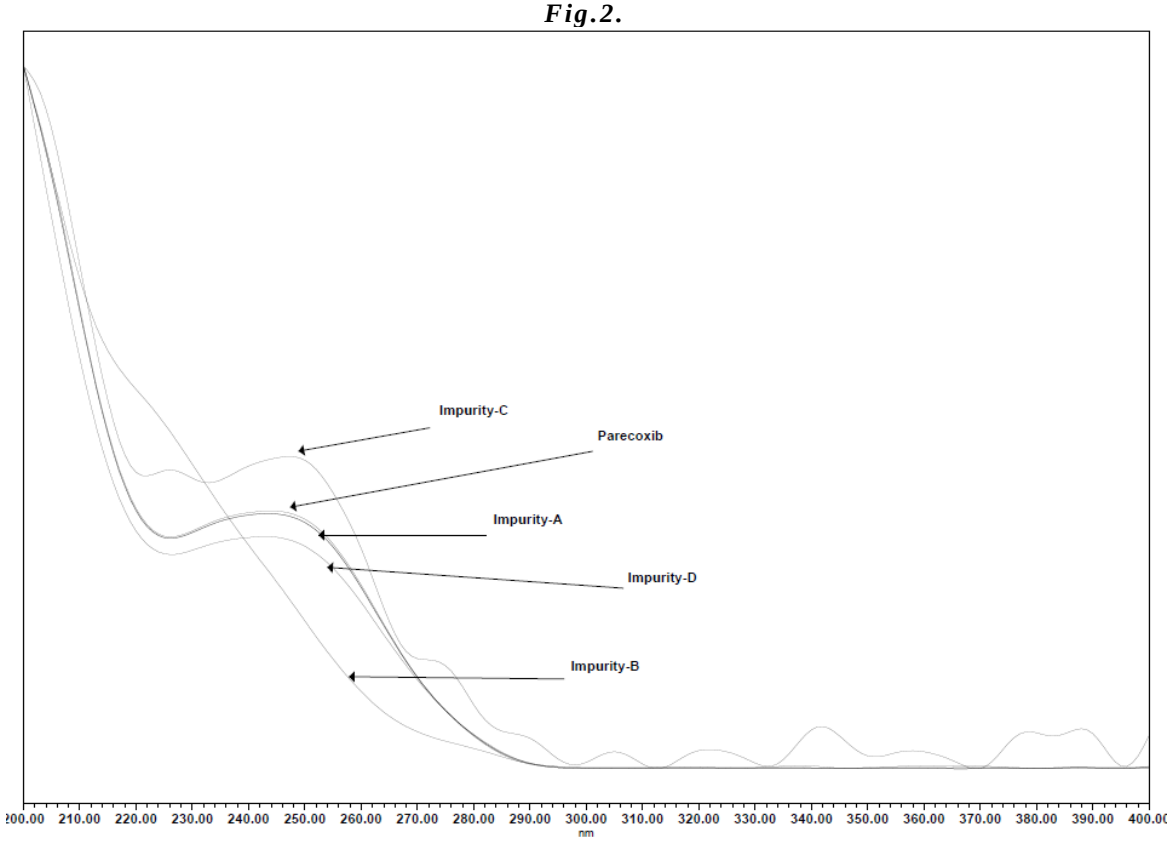
Stock solutions of all impurities were prepared by dissolving an appropriate quantity of each impurity in the diluent to obtain a concentration of 1 mg per mL. An appropriate volume of impurity stock solution was diluted with sample solution to get a final concentration of 0.5% for each impurity.

IV. Method Development And Optimization

4.1 Selection of detection wavelength

UV spectra of the drug and its impurities were recorded by scanning between 200 and 400 nm.
The spectra of drug and its impurities were overlaid and the wavelength 240 nm was selected where the main active analyte as well as its impurities have overlapped with sufficient response for detection and quantification. The overlay spectra of Parecoxib and its four impurities are shown in Figure 2.

Fig.2.



4.2 *Optimization of Chromatography.*

Parecoxib has ionizable amino group, and hence, the retention time of the drug and the resolution between parecoxib and impurity-B is dependent on the pH of the mobile phase. In the present study, the pH of the mobile phase was maintained at pH: 6.5 by the addition of Orthophosphoric acidsolution. Before initiating the development activity, information on type, condition in which impurities will arise and their acceptable limits were collected to define sample concentration and the Limit of quantification of the impurities. The chemical names and limits listed in the below table.no:1

The maximum daily dose of Parecoxib is 80mg/day. Based on the daily dose, the qualification threshold should not exceed 0.5%, and the identification threshold is 0.2%. Method development was targeted to cover a range of LOQ to 150% of qualification threshold for impurities. A systematic approach was adopted for the method development.

Table.No:1 Chemical names and Limits based on ICH for Impurities.

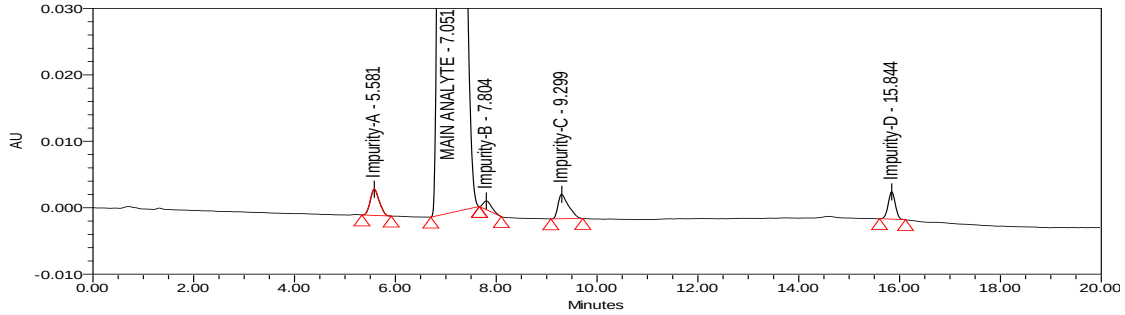
Nameof the Impurities	Chemical names	**ICH*Limits (QT**)**
Parecoxib	N - [4 -(5-Methyl-3-phenylisoxazol-4-yl) phenylsulfonyl]propionamide, sodium salt.	-
Impurity-A	N-{[4-(5-Methyl-3-phenylisoxazol-4-yl)phenyl]sulfonyl}acetamide. (Acetamide impurity)	0.5%
Impurity-B	N-{[3-(5-methyl-4-phenylisoxazol-3-yl)phenyl]sulfonyl}propanamide (Meta isomer impurity)	0.5%
Impurity-C	4-(5-Methyl-3-phenylisoxazol-4-yl)-benzenesulfonamide (Sulfonamide)	0.5%
Impurity-D	4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)-N-{[4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)phenyl]sulfonyl}benzenesulfonamide(Dimer impurity).	0.5%

*ICH: The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.

**QT: Qualification Threshold based on maximum daily dose.

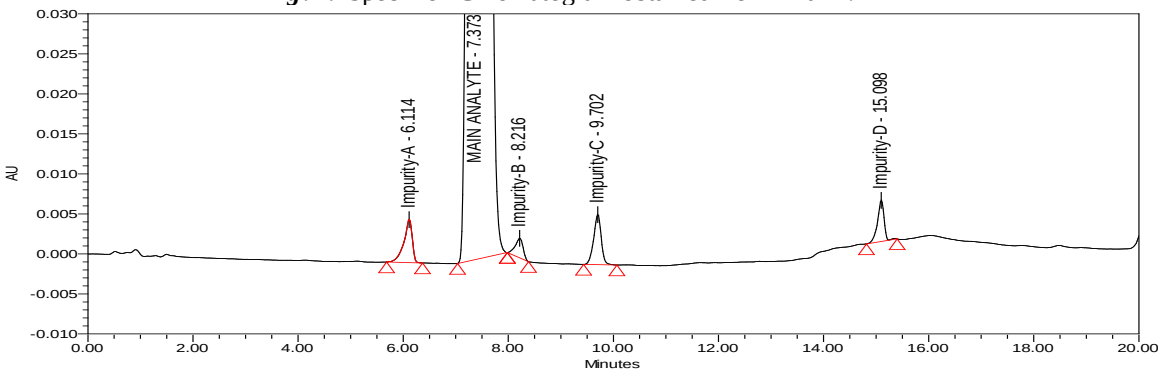
The mainaim of this method development is achieving a good separation between Parecoxib and Impurity-B (meta- isomer) peaks as they are very closely eluting. The resolution between these two peaks is influencing by the pH of the mobile phase, Column oven temperature and organic variation.Various mobile phase compositions were consecutively tested with buffer pH 6.0.

Fig.3. Specimen Chromatogram obtained from Trial-1:



Another attempt was made by changing the pH of the buffer to 6.5 and keeping other parameters unchanged.The resolution was improved between two peaks. The pattern obtained for trial 2 is depicted in Figure 4.

Fig.4. Specimen Chromatogram obtained from Trial-2:



The next trial was made by increasing the column oven temperature. The remaining chromatographic parameters were unchanged. All impurities were appropriately separated from each other. The resolution between Parecoxiband impurity B is further improved. The optimized chromatographic conditions are listed in Table:1 and 2. A specimen chromatogram obtained from the final method parameters is illustrated in Figure 5.

Fig.5. Specimen Chromatogram obtained from final method:

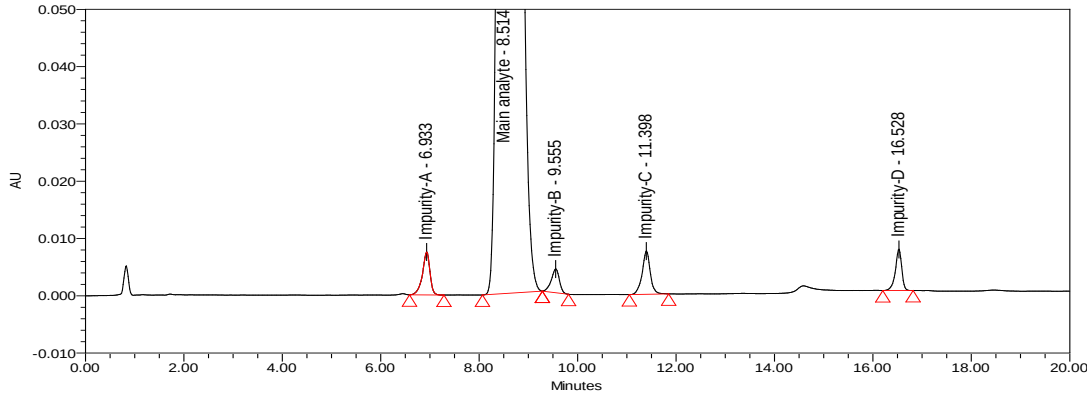


Table No.: 1. Optimized Chromatographic Conditions.

Chromatograph	WatersAcquity UPLC system
Mobile phase	**Mobile Phase A:**Potassium dihydrogen phosphate(0.01 M, pH 6.5) andOrganic mixture(Methanol:Acetonitrile,80:20) is 95:5v/v **Mobile Phase B:**Potassium dihydrogen phosphate(0.01 M, pH 6.5) and Organic mixture(Methanol:Acetonitrile,80:20) is 10:90v/v Mobile Phase B: Buffer : Acetonitrile (20:80v/v) (80:20%v/v)
Column	Acquity UPLC BEH C_{18}, (100mmx2.1mm)1.7µm size)
Detector	PDA
Flow rate	0.3 ml/min
Wavelength detection	240 nm
Injection volume	2µL
Column Oven Temperature	45°C
Run time	20 min
Diluent	Acetonitrile: Water (40:60 v/v)

Table No. : 2. Gradient programme.

Time	Flow(ml/min)	%Mobile Phase-A	%Mobile Phase-B
Initial	0.30	70	30
2.00	0.30	65	35
10.0	0.30	35	65
15.0	0.30	35	65
16.0	0.30	70	30
20.0	0.30	70	30

V. Method Validation

Results and Discussion:

The optimized method was fully validated for determination of impurities as per ICH guidelines, (Q2A (R1) validation of analytical procedures. The method was validated for specificity, precision, accuracy, linearity,Limit of Detection (LOD), Limit of Quantification (LOQ) and Robustness.

1. System Suitability.

System suitability parameters were measured to verify the system performance for the intended analysis. Hence, system precision was determined on six replicate injections of standard preparations and the relative standard deviation (% RSD) was evaluated. In addition to the % RSD, USP tailing, and USP plate count were also evaluated and found to be satisfactory, as per current USP requirements for a chromatographic peak (USP General Chapter<621>). The system suitability results obtained are presented in Table 3.

Table No. : 3. System suitability data.

System Suitability Parameters	**Results from Standard Solution**
Peak Tailing	1.1
Theoretical plates	15204
%RSD	0.5

2. Precision.

Precision of the test method was evaluated by injecting six individual samples spiked with all three impurities into the chromatograph. The % RSD values from the six individual test preparations were found to be below 2% for all three impurities. The ruggedness (intermediate precision) of the method was determined using another system and column for the analysis in a different day. The precision data are listed in Table 4. The data indicate that a low % RSD, concluding that the method is precise for impurities determination.

Table No. : 4. Precision data.

Impurity Name	**%RSD value**			
	Impurity-A	Impurity-B	Impurity-C	Impurity-D
Method Precision	0.5	0.6	0.5	0.8
Intermediate Precision	0.4	0.7	0.5	0.6

3. Accuracy.

Accuracy of the analytical procedure expresses the degree of closeness of the obtained results with true values. Samples for accuracy were prepared in triplicate by spiking impurities at different levels. The covered levels for impurities were LOQ, 50% , 100% and 150% of the qualification Threshold (0.5%) with respect to the sample concentration (0.5mg/ mL)• From the response of the analyte peaks, the amounts recovered (in %) and % RSD were reported. Accuracy results are summarized in Table 5.

The data indicate that the % recovery of impurities lies in the range of 91.5-108.5 with an RSD of between 0.3 and 3.6%. This indicates that the method is accurate and precise.

Table No. : 5. Recovery of Impurities data.

Impurity Name	**Accuracy Data**			
	LOQ %w/w (%RSD)	50% %w/w (%RSD)	100% %w/w (%RSD)	150% %w/w (%RSD)
Impurity-A	103.5(3.6)	105.3(1.2)	99.9(0.1)	107.4(0.3)
Impurity-B	103.2(2.8)	92.9(1.6)	91.5(0.6)	95.8(0.3)
Impurity-C	100.4(2.0)	103.9(1.6)	100.8(0.7)	108.5(0.6)
Impurity-D	107.6(3.2)	97.1(1.8)	98.9(0.8)	100.1(0.3)

4. Limit of Detection and Limit of Quantisation.

The LOD and LOQ values were established by Signal to Noise ratio. Results are shown in Table no.6

Table No. : 6. Impurities LOD and LOQ Concentrations.

Impurity name	**Data based on signal to noise ratio (S/N)**			
	LOQ Concentration (%w/w)	**S/N ratio**	**LOD Concentration (%w/w)**	**S/N ratio**
Parecoxib	0.0410	11.08	0.0123	3.30
Impurity A	0.0334	10.50	0.0100	2.80
Impurity B	0.0478	10.88	0.0143	3.80
Impurity C	0.0315	12.62	0.0095	3.55
Impurity D	0.0376	11.51	0.0113	2.49

5. Linearity.

The linearity of the analytical method was tested to check its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. Hence, different concentrations of individual impurities and standard working solution of Parecoxib were prepared and injected into the UPLC, and the chromatograms were recorded. The linearity of detector response was determined by plotting a graph of peak areas versus concentrations. Plots of linearity experiments are illustrated in Figure 6. The correlation coefficients, >0.996 for impurities and 0.999 for Parecoxib, indicate that the method satisfies a linear relationship between the concentration and peak response. The linearity range is between the limit of quantification (LOQ) and 150% of the sample concentration for all impurities. The linearity data for all four impurities and Parecoxib are listed in Table 7 and the linearity plots are depictedin the below Fig. No: 6

Fig.6.

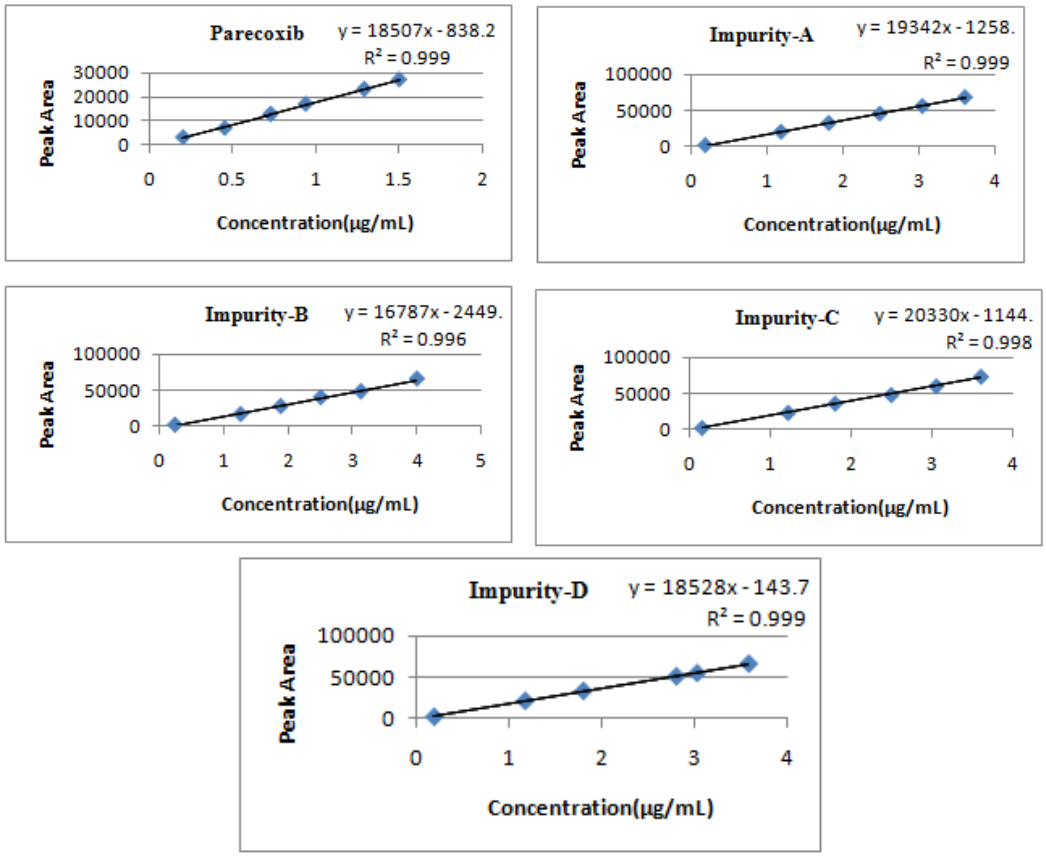


Table No. : 7. Linearity data.

S.No.	Impurity	Equation	R^2
1	Impurity-A	y=19342x-1258	0.999
2	Parecoxib	y=18507x-838.2	0.999
3	Impurity-B	y=16787x-2449	0.996
4	Impurity-C	y=20330x-1144	0.998
5	Impurity-D	Y=18528x-143.7	0.999

6. Specificity.

The specificity of the RP-HPLC method was checked by comparison of chromatogram obtained from standard, placebo and sample. The Placebo did not should not show any interference at retention of main analyte peak and at impurities.

6.1 *Forced degradation study.*

Forced degradation studies were conducted on samples and on the plain placebo to prove the specificity and stability-indicating power of the method. Specificity was determined by exposing test solution to oxidation by hydrogen peroxide, acid hydrolysis, base hydrolysis, heat and photolytic stress. A detailed procedure has been reported. The stressed samples were then further diluted with the diluent and chromatographed as per the proposed method. The peak purity of Parecoxib peak was evaluated using the PDA. The purity angle should be less than the purity threshold. The results are summarized in Table.No:8 and the degradation chromatograms are from Fig.no:7 to12

Table No. : 8. Degradation data.

Stress condition	%degradation	Peak purity		Purity flag
		Purity Angle	Purity Threshold	
Treated with 1N HCL solution for 24hrs on bench top.	0.20	0.231	0.420	NO
Treated with 1N NaOH solution for 24hrs on bench top.	0.86	0.168	0.400	NO
Treated with 30% H_2O_2 solution for 2hrs on bench top.	23.72	0.159	0.320	NO
Treated with heat at 80℃ for 5 days.	2.61	0.278	0.541	NO
Exposed to sunlight 1.2 million lux hrs for 6days.	0.27	0.187	0.500	NO
Exposed to UV-Light 200 watts/square meter for 6days.	0.20	0.279	0.484	NO

Fig. 7. Acid degradation Sample.

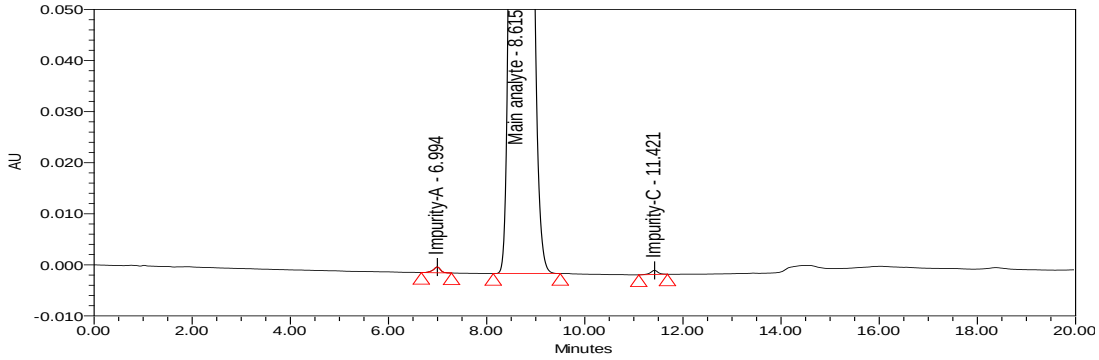


Fig. 8. BasedegradationSample.

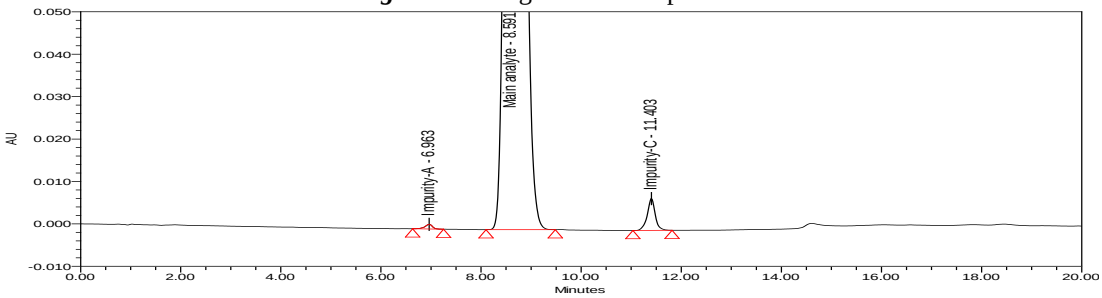


Fig. 9. Peroxidedegradation Sample.

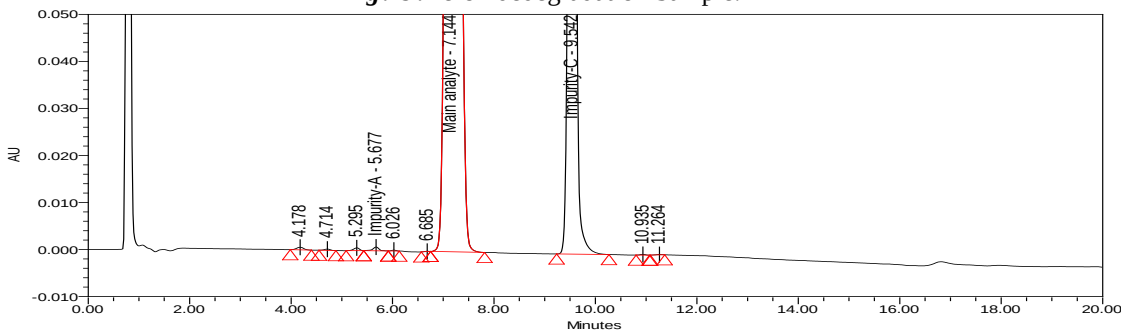


Fig. 10. ThermaldegradationSample.

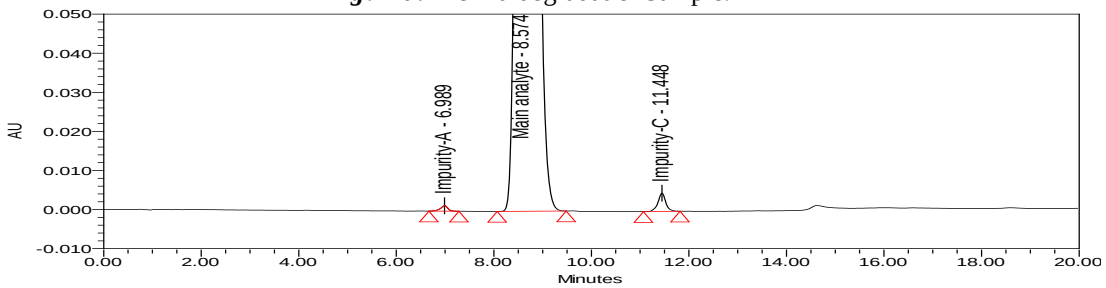


Fig. 11. UV exposed Sample.

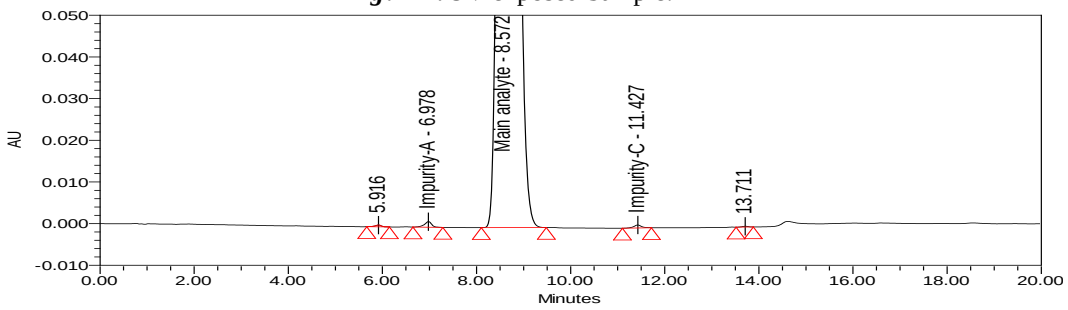
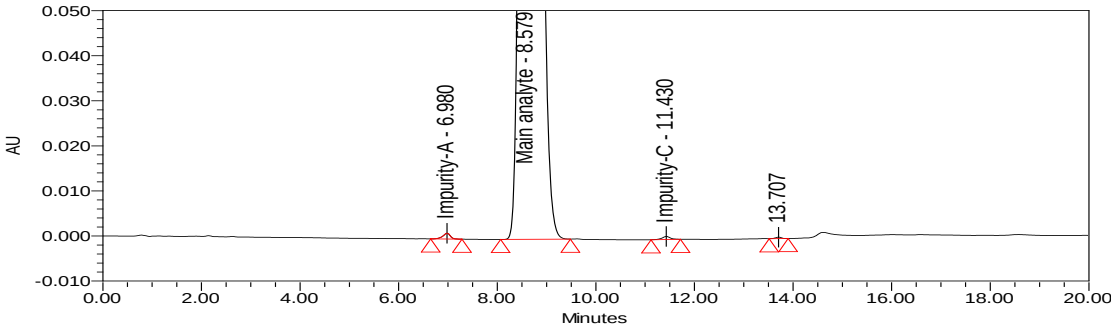


Fig. 12. Sunlight exposed Sample.



7. Robustness.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the method is evaluated by varying method parameters such as Flow rate, Buffer pH, Column oven temperature and mobile phase composition and determiningthe effect (if any) on the results of the method.

The system suitability was evaluated by injecting the Standard solutions. Sample solution spiked with known impurities at specification limit was injected to monitor the RRT of impurities.Result are shown in Table no.10, 11

Table No. : 9. Robustness conditions.

Parameters	**Change of conditions**	
Flowvariation (ml/min)	High(+)	0.33
	Actual	0.30
	Low(-)	0.27
Buffer pH variation	High(+)	6.70
	Actual	6.50
	Low(-)	6.30
Column oven temp. variation (°C)	High(+)	50
	Actual	45
	Low(-)	55
Organic variation in Mobile phase-A (10% Absolute)	High(+)	50
	Actual	45
	Low(-)	40
Buffer variation in mobile phase-B (10% Absolute)	High(+)	110
	Actual	100
	Low(-)	90

Table No. : 10. Robustness data.

System suitability parameters	**Robustness Parameters**										
	Control	**pH (+)**	**pH (-)**	**Flow (+)**	**Flow (-)**	**Temp (+)**	**Temp (-)**	**Organic (+)**	**Organic (-)**	**Buffer (+)**	**Buffer (-)**
USP tailing	0.95	0.94	0.95	0.96	0.94	0.97	0.95	0.94	0.95	0.93	0.96
USP plate count	25880	24651	27189	26157	2391	25170	28140	29163	26380	24328	27593
%RSD	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.3	0.2	0.2	0.2

Table No. : 11. Robustness data.

Impurity Names	**Robustness Parameters (RRT values)**										
	Control	**pH (+)**	**pH (-)**	**Flow (+)**	**Flow (-)**	**Temp (+)**	**Temp (-)**	**Organic (+)**	**Organic (-)**	**Buffer (+)**	**Buffer (-)**
Impurity-A	0.82	0.82	0.81	0.82	0.81	0.81	0.82	0.82	0.82	0.82	0.82
Impurity-B	1.11	1.11	1.12	1.10	1.10	1.11	1.11	1.09	1.11	1.11	1.10
Impurity-C	1.31	1.31	1.30	1.31	1.31	1.31	1.30	1.30	1.31	1.31	1.31
Impurity-D	1.81	1.81	1.81	1.80	1.83	1.79	1.82	1.79	1.82	1.81	1.80

VI. Conclusions

The rapid gradient reverse phase UPLC method, developed for the quantitative analysis of Parecoxib related substances in pharmaceutical dosage forms. Satisfactory results were obtained from validation of the method and shows that the method is precise, accurate, linear, specific, and robust.The method is stability-indicating and can be used for routine analysis of production samples, and checking the stability of samples of Parecoxib Injection.

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