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Simultaenous Quantification Of Parecoxib And Its Potential Impurities In Injection Formulation By Reversed Phase Chromatography

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Abstract
This study explores the reversed phaseliquidchromatography (HPLC) for the simultaneous quantification of Parecoxib and its four potential impurities in an injection formulation. The separation of impurities from Parecoxib was achieved on a Zorbax XDB C18 150 x 4.6, 3.5 µm, using a simple inexpensive buffer (potassium phosphate, 0.02 M), a mixture of acetonitrile and methanol as the mobile phase. The mobile phase was pumped at a flow rate of 1.0 mL min-1 using a simple linear gradient elution mode. The sample components were detected by UV-VIS detector at 245 nm. The method is capable of resolving close eluting analytes with a minimum resolution of 3.0. Forced degradation studies were conducted to establish the stability indicating power of the method, product sensitivity towards acid hydrolysis, base hydrolysis, oxidation, thermal and light. The stability indicating power of the method. A linear correlation of 0.999 was achieved between the analytes concentration and detector response between LOQ to 150% of the target specifications. The method is linear, accurate and precise between LOQ and 150% of the target concentration.

Keywords: *HPLC, Parecoxib,Injection formulation, Potential impurities, Method Development and Validation.*

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I. Introduction

Parecoxib is a novel second generation cycloxygenase-2 (COX-2) inhibitor. It is soluble in water and is a pro-drug. It is chemically ([4-(5-methyl-3-phenyl-1,2-oxazol-4-yl) phenyl]sulfonylpropanoylazanide, with formula $C_{19}H_{18}N_2O_4S$; molecular weight 370.422 g mol^{-1} [1-2].Example of other COX-2 inhibitors include Celecoxib, Roficoxib, Valdecoxib, Etoricoxib etc. Parecoxib sodium is used in treatment of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, fewer gastrointestinal complications [3]. The USFDA UNII (Unique Ingredient Identifier) code for Parecoxib sodium is EB87433V6F.UNII contains complete scientific information on Parecoxib sodium. The description of Parecoxib API is "white to off-white, crystalline solid". It is formulated as sterile for injection. Each vial/powder contains 40 mg parecoxib (as 42.36 mg parecoxib sodium). After reconstitution, the concentration of parecoxib is 20 mg/ml. It is marketed under the brand name of Dynastat [4]. The chemical structure of Parecoxib is shown in Figure 1 and list of its potential impurities with their nature are tabulated in table 1. Impurity A (Acetamide impurity), Impurity B (Meta Isomer), Impurity C and Impurity D (Dimer) are the four potential impurities of Parecoxib.

A thorough literature studies shows no pharmacopoeia methods on either Parecoxib sodium or its injection formulation. Fewliteratures are available on the pharmacodynamics, pharmacokinetics, and plasma studies [5-9]. A very few analytical literature are available on Parecoxib sodium injection.An RP-HPLC method was published by shaikh et. al for the determination of Parecoxib in human plasma and pharmaceutical formulations [10]. The method uses acetonitrile-water (92:8 v/v) as the mobile phase and the separation was achieved on CLC C18 (5 µ, 25 cm×4.6 mm i.d.) column. The quantification is based on an internal standard (Ibuprofen). The UV detection was carried at 200nm. G. Saccomanni et. al reported a LC-MS method on the determination of parecoxib and valdecoxib simultaneously in canine plasma. A reversed phase column ODS 2 was used. The mobile phase used was a mixture of acetonitrile and ammonium acetate (10 mM; pH 5.0) in the ratio 55:45, *v/v, pumped at a flow rate of* 0.4 mL min−1[11]. One UPLC-MS/MS method is available on the determination of Parecoxib and its metabolite in rat plasma. The separation was carried out on a Kinetex C18 column (2.1 mm × 50 mm, 2.6 µm) with a gradient elution using methanol (A) and a 2 mM ammonium acetate aqueous solution (B) [12]. A review article on the applications of different analytical techniques for coxibs is

reported StarekM et. Al [13]. An RP-HPLC method was published by Lakshmi et. al for the quantitation and dissolution of Parecoxib [14]. One HPLC-DAD method is available on the determination of impurities of Parecoxib [15]. One UPLC method is available on the determination of impurities of Parecoxib [16]. Few more literatures are available on the quantification of Parecoxib in biological samples by using UPLC/HPLC with mass detector [17-18].

So far to our knowledge there is no single HPLC method available for the simultaneous quantification of Parecoxib and its four potential impurities in injection formulation.

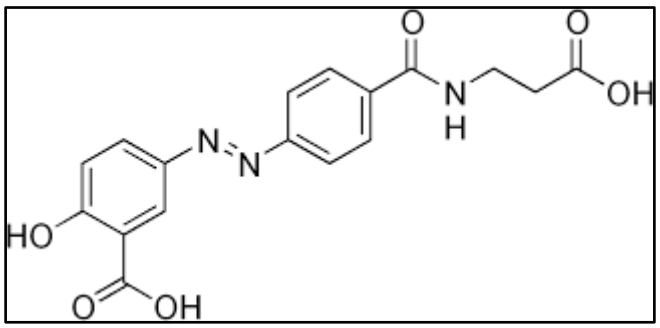
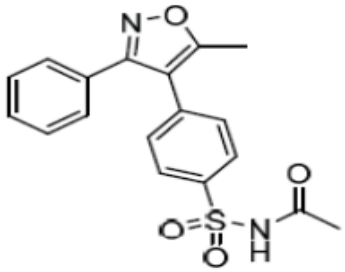
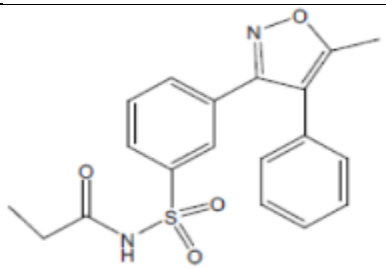
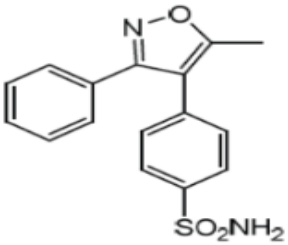
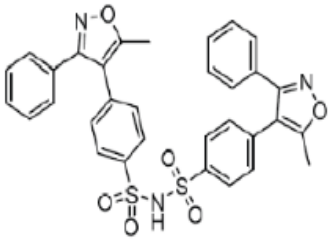


Figure 1. Chemical Structure of Parecoxib

Table 1. Names, Chemical Structures, and Nature of Parecoxib Impurities

Name	Chemical structure	Nature
Impurity A		Process Impurity
Impurity B		Process Impurity
Impurity C		Degradation Impurity
Impurity D		Process Impurity

II. Materials and Methods

2.1 Chemicals, Reagents, Standards, and Instruments

The HPLC grade acetonitrile, and methanol were procured from J.T.Baker. The buffer salt Potassium Phosphate (KH_2PO_4 A.R. grade) used in the preparation of mobile phase were obtained from Merck, India. HPLC grade ortho phosphoric acid used to adjust the pH of the mobile phase buffer, was obtained from Fischer Scientific Ltd. The samples used in the study were market samples. The reference standards and impurity standards were procured from custom manufacturer.

An integrated HPLC system is from Waters Corporation, Milford, USA, and equipped with a Waters photodiode array detector (PDA). Data collection and analysis were performed using the Empower software 2pro (Waters Corporation). Balances used for weighing the reference standards and samples were from Mettler Toledo. The columns Zorbax XDB C18 150 x 4.6, 3.5 µm, was procured from Agilent Technologies. Class A glassware used in conducting the experiments and validations was from Duran and Borosil, India.

2.2. Chromatographic Conditions

The separation of principal peak (Parecoxib) and its potential impurities was achieved on a Zorbax XDB C18 column having dimensions of 150 mm x 4.6 mm I.D and a particle size of 3.5 µm. The mobile phase consists of a buffer solution (KH_2PO_4, 0.02 M, pH 6.2) and acetonitrile pumped into the chromatographic system using a linear gradient program (Gradient Table 1) at a flow rate of 1.0 mL/ min. The chromatographic column was maintained at a temperature of 40°C throughout the run. The sample size was 10 µL. The detection wavelength chosen as 245 nm based on lambda maxima depicted by active compound and individual impurities.

Gradient Programme-1

Time (min)	Mobile phase A (Buffer: Organic) (90:10)	Mobile phase B (Buffer: Organic) (80:20)
0	75	25
25	75	25
40	40	60
50	40	60
55	75	25
60	75	25

2.2 Standard and Sample Preparations

2.2.1 Diluent Preparation

A mixture of HPLC grade acetonitrile and Milli Q waterin the ratio of 50:50 v/v was used as the diluent, for the preparation of standards, system suitability solutions, and sample solution.

2.2.2 *Preparation of Parecoxib working standard stock solution:*

Accurately weighed and transferred 50 mg of Parecoxibworking standard into 100 mL volumetric flask. Added 25 mL of the diluent and sonicated for 5 minutes to dissolve the contents. Made up to the mark with the diluent ($500 \mu g mL^{-1}$).

2.2.3 Preparation of impurity mixed stock solution:

Accurately weighed and transferred 2.5 mg of each impurity of Parecoxibinto50 mL volumetric flask. Added 10 mL of the diluent and sonicated for 5 minutes to dissolve the contents. Made up to the mark with the diluent.

2.2.4 Preparation of Standard Solution for assay determination of Parecoxib

Pipette out 5 mL of the working standard stock solution into 10 mL volumetric flask. Add 2 mL of the diluent, swirl to mix and make up to the mark with the diluent to obtain a concentration of 250 $\mu g mL^{-1}$.

2.2.5 Preparation of Standard Solution for impurities determination of Parecoxib

Pipette out 2 mL of the working standard stock solution into 100 mL volumetric flask. Add 20 mL of the diluent, swirl to mix and make up to the mark. Further dilute 2 mL of the above solution into 20 mL with the diluent to obtain a concentration of $1 \mu g mL^{-1}$.

2.2.6 Preparation of Sample Solution (Parecoxib for Injection 40 mg/vial)

Taken five vials of Parecoxibfor injection sample, reconstituted each vial as recommended in the PIL. Pooled the contents of all five vials. Pipetted 1.5 mL of the sample into 50 mL volumetric flask. Added20 of the diluent and sonicate for 5minutes to mix the contents. Make up to the mark with the diluent to get the final concentration of $600 \mu g mL^{-1}$.

2.2.7 Preparation of sample solution spiked with impurity mixture

Pipette1.5 mL of the reconstituted sample into 50 mL volumetric flask. Added 15mL of the diluent and sonicated for 5 minutes to mix the contents. Add 1.5 mL of the impurity mixed stock solution, and diluted up to the mark with the diluent. Mixed well for get a final concentration of 0.2% of target sample concentration.

2.2.8 Preparation of Placebo Solution:

Transferred placebo equivalent to 60 mg of Parecoxib into 50 mL volumetric flask. Added 20 mL of the diluent and sonicated for 5minutes to mix the contents. Make up to the mark with the diluent.

III. Results And Discussion

3.1 Method development and optimization

The detection wavelength was determined by injecting a detectable concentration of Parecoxib and individual impurity standard into the chromatographic system having photodiode array(PDA) detector. Parecoxib sodiumand its impurities exhibitsmaximum absorbance at 245 nm. Hence the detection wavelength was selected as 245 nm.

3.1.1 Selection of Chromatographic Conditions

Based on the structure of Parecoxib/impurities and functional groups present, the method development was initiated using the principles of reversed phase chromatography(RPC). RPC is the first choice for neutral, acid, and basic molecules having molecular weight less than 2000 Da [19].Based on the reported pKa (pKa=6.7) of the molecule Parecoxib sodium [1], a simple phosphate buffer (KH_2PO_4, 0.02M) with pH 6.5 was chosen and initial trials were conducted using acetonitrile as the isocratic mobile phase (50:50) v/v.The stationary for separating the impurities and principal peak was a reversed phase octadesylsilyl column 150 mm x 4.6 mm, 5µm, at 30 ºC, and The injection volume was chosen as 10 µL to keep minimum possible load so as to achieve repeatable Gaussian peak. The detection wavelength was 245 nm.

Method Development and Optimisation

With above chromatographic conditions, impurity mixed solution was injected into the chromatographic system. The inference was acetamide impurity, impurity C along with Parecoxib were eluted within 25 minutes of run time. Other two impurities appears to be non-polar and hence were not observed with these chromatographic conditions. In the next trial the run time was increased to 50 minutes and injected the impurity mixed solution. No impact was observed. Further different attempts were made with other buffer solutions like Na_2HPO_4 (0.02M, pH 7.0), sodium perchlorate (0.1 M, pH 5.6), and acetonitrile was replaced with methanol. The impurities were not separated from each other and the baseline not proper. Now using KH_2PO_4 (0.02 M, pH 6.5) and acetonitrile in gradient (T/%B 0/10, 20/45, 35/55, 45/55, 50/10, 60/10) elution mode. Dimer impurity eluted at 46 minutes. Meta isomer impurity was merged with the principal peak. The column replaced with Zorbax XDB C18 150 x 4.6 mm, 3.5 µm. Different trials were conducted using different gradient programmes, pH (6.0 to 6.8) and column oven temperature (30 ºC- 38 ºC).

All peaks were separated from each other with minimum resolution of 1.0 at pH 6.3, and column oven temperature 38 ºC. Dimer impurity was eluted at the retention time of 34 minutes.Meta-isomer is the closest eluting impurity to Parecoxib with a resolution of 1.0. Further the resolution between the two peaks was improved (Resolution 3.45)byoptimization of the column oven temperature to 40 ºC and pH to 6.2.The retention time of Parecoxib is about 15 minutes and the run time is 55 minutes. The final optimized method parameters are described in the section 2.2. The optimized chromatogram obtained is shown in figure 2.

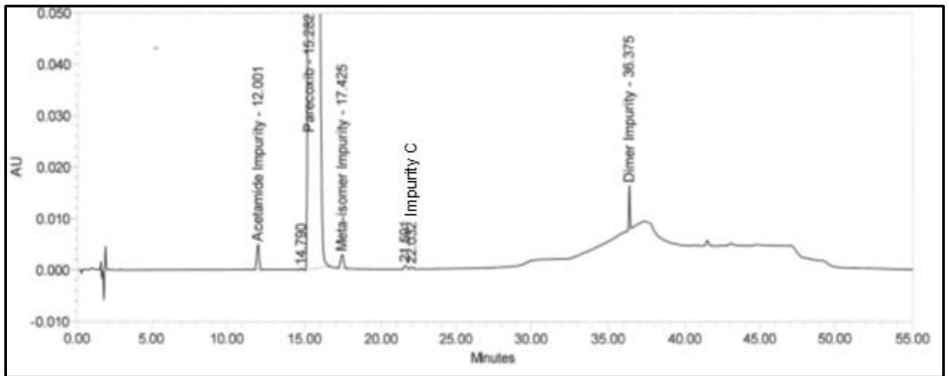


Figure 2. Chromatogram obtained from Optimized parameters

IV. Method Validation

The developed and optimized HPLC method was taken up for validation. The method validation was carried out in accordance with the validation guidelines (Q2R1) recommended by ICH [20]. The developed HPLC method was validated with respect to system suitability, specificity, accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. Each validation parameter is explained in detailed in the sections from 4.1 to 4.9.

4.1 System Suitability and System Precision

The system suitability and system precision for the intended analysis were evaluated from five and six replicate injections of standard solution respectively. The system suitability parameters like USP plate counts, USP tailing factor, USP resolution and %RSD for Parecoxib peak were determined and evaluated (Table2). The data shows Parecoxib and meta-isomer impurity were well resolved, and the area of the Parecoxib peak was precise with and RSD 0.5%.

Table 2. Results of System Suitability and System Precision

Peak Name	Retention Time(min)	USP Plate Counts	USP Tailing	USP Resolution[$]	%RSD *	System Precision %RSD **
Parecoxib	15	39271	1.0	3.45	0.5	0.4

[$] *Resolution between Parecoxib peak and Meta isomer impurity*
[*] *Data from five replicate injections*
[**] *Data from six replicate injections*

4.2 Specificity

4.2.1 Diluent interference

The diluent was injected as such into the chromatograph to assess its interference (if any) at the retention time of Parecoxib and its potential impurities. No interference is found at the retention time of Parecoxib and its impurities (Figure3).

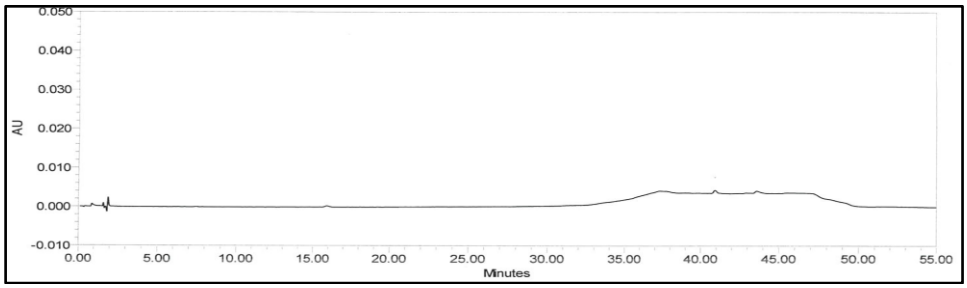


Figure 3. Typical Chromatogram of Diluent

4.2.2 Placebo interference

The placebo solution was injected as such into the chromatograph to assess its interference (if any) at the retention time of Parecoxib and its potential impurities. No interference is found at the retention time of Parecoxib and its impurities (Figure4).

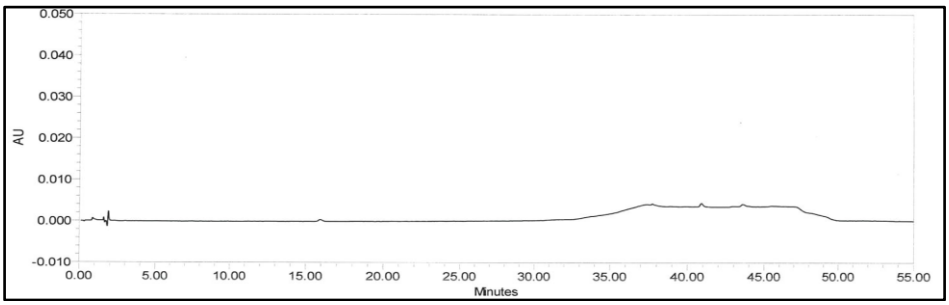


Figure4. Typical Chromatogram of Placebo

4.2.3 Interference due to specified impurities of Parecoxib

To assess the interference due the specified impurities of Parecoxib, a sample solution spiked with small portion (1%) of listed impurities (as mentioned in the Table1) was injected into the chromatographic system. The Parecoxib peak was evaluated for the homogeneity by measuring the purity angle and purity threshold using the empower software. Assay of Parecoxib Sodium in presence of the specified impurity mixture was calculated and compared with that of the unspikedsample (Table 3).Assay of the sample spiked with impurities was found 98.4% and that of pure sample was 98.7%. The absolute difference (0.3) is very small and indicates that the assay of Parecoxib was unaffected in presence of its impurities. The purity data indicate that purity angle is less than the purity threshold for all three samples, which concludes that Parecoxib peak is homogenous, and the new method is analyte specific.

Table3. Comparison of Assay Results and Peak Purity Results of Sample Spiked with Impurity and Unspiked Sample

Sample Details	% Assay	Purity Angle	Purity Threshold	Purity Flag*
Unspiked sample-1	99.1	0.123	0.365	No
Unspiked sample-2	97.9	0.342	0.784	
Unspiked sample-3	99.0	0.165	0.272	
%Average	98.7	NA		
Spiked sample-1	98.0	0.243	0.674	
Spiked sample-2	98.7	0.246	0.547	
Spiked sample-3	98.5	0.336	0.376	
%Average assay	98.4	NA		
Difference between average assays of unspiked and spiked sample				
0.3				
**Purity flag 'No' indicates, peak is homogenous (Empower software)* *Peak is homogeneous if purity angle is less than purity threshold*				

4.2.4 Interference from degradation products

To assess the interference due to the degradation products, Parecoxib Sodium for injection and placebo solutions were exposed to various stress conditions. The stressed and neutralized samples were then diluted with the diluent to obtain a concentration of about 600 µg mL^{-1}. The obtained solutions were chromatographed as per the optimized methodology. All chromatograms were processed by using the Empower 2pro software. The homogeneity of Parecoxibpeakand specified impurities from the stressed samples was evaluated from the purity angle and peak threshold data using the PDA detector. The stress parameters, stress conditions, %degradation in each stress parameter are listed in Table4.The data of stress study indicate that degradation was observed in base hydrolysis (2.8%), oxidation (30.5%), acid hydrolysis (0.81%) and thermal degradations (6.0%). The purity angle was less than the purity threshold in all the stress conditions which indicates that Parecoxib peakand observed specified impurity are homogeneous in presence of its degradation impurities and the method is stability indicating. The chromatograms of forced degradation studies are shown in figure 5-10.

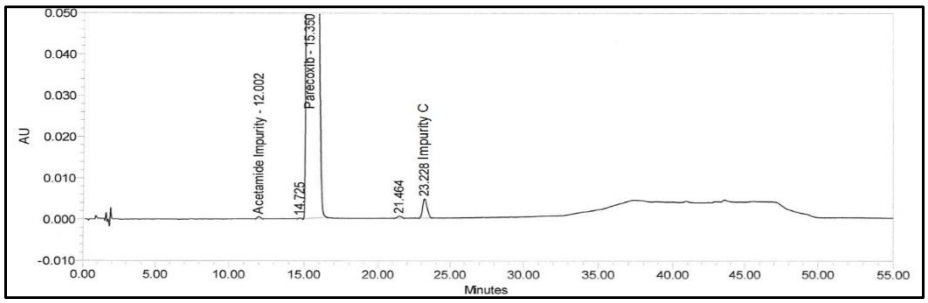


Figure5.Acid Stressed Sample Chromatogram

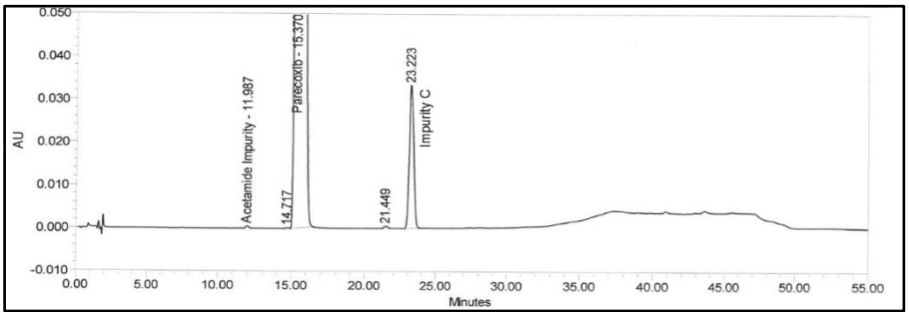


Figure 6. BaseStressed Sample Chromatogram

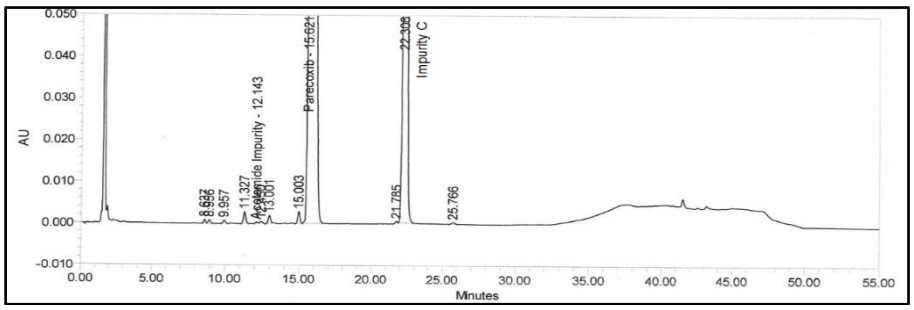


Figure 7. Peroxide StressedSample Chromatogram

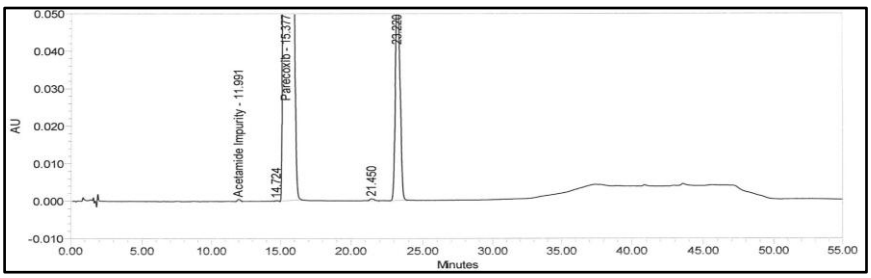


Figure 8. Thermal StressedSample Chromatogram

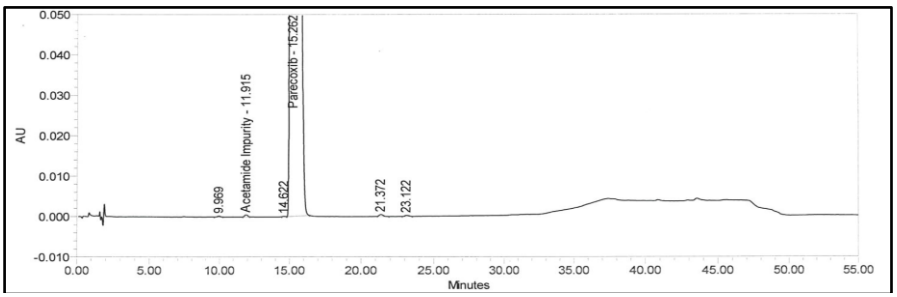


Figure 9. Sample Chromatogram from UV Degradation

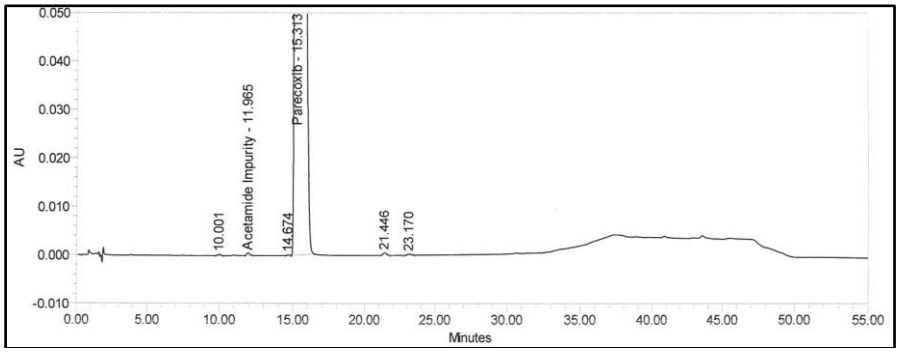


Figure 10.Sample Chromatogram from Light Degradation

Table 4. Results of Stress Study and Peak Purity Data

Parameter	Stress Conditions	%Assay of Degraded Sample A	%Degradation w.r.t. Control B *	Purity Angle	Purity Threshold
Control sample	No exposure	99.4	NA	0.334	0.544
Acid hydrolysis	0.5 ml of 1N HCl for 24 hours at room temperature	98.6	0.81	0.253	0.524
Base hydrolysis	1ml of 1N NaOH for 24 hours at room temperature	96.6	2.8	0.315	0.565
Oxidation	1ml of 30% H_2O_2 for 4 hours at room temperature	69.1	30.5	0.432	0.634
Thermal degradation	105ºC for 15 hours	93.4	6.0	0.444	0.754
Photolytic degradation (UV)	200-Watt hours / m^2	99.2	0.18	0.456	0.661
Photolytic degradation (light)	1.2 Million lux hours	99.2	0.18	0.434	0.773

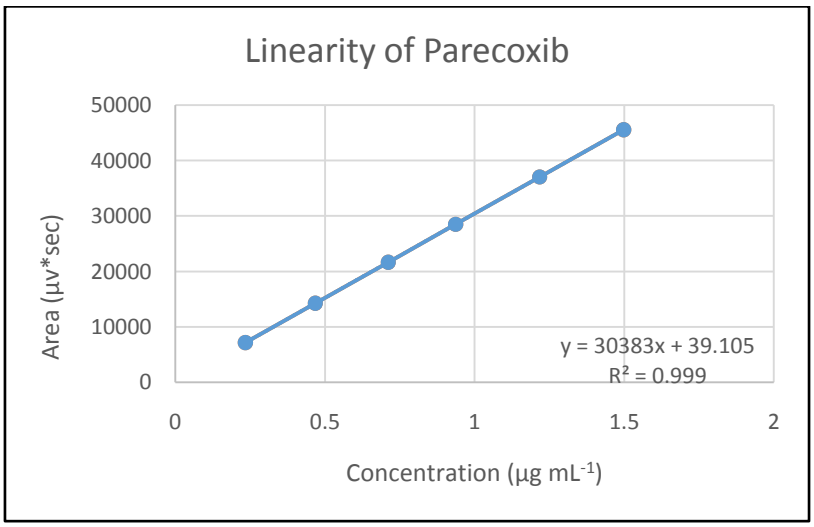
**B= (99.4- A)/99.4*100*

4.3 Linearity

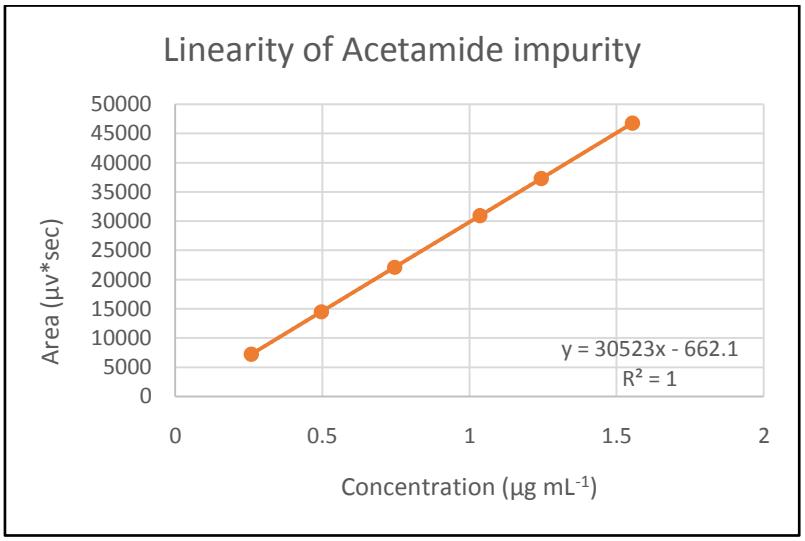
Linearity of detector response was demonstrated from LOQ to 150% of target specifications of the impurity.Using minimum six calibration levels (LOQ, 50%, 75%, 100%, 125% and 150%) with respect to sample concentration (600 µg mL^{-1}) for impurities determination. The linearity solutions were prepared from a standard stock solution by appropriate dilutions. Each solution was chromatographed, and area response was recorded. The data was evaluated by using linear regression method. The correlation coefficient (R^2) was found greater than 0.999 for all specified impurities and for Parecoxib peak (Table 5). A linearity graph is plotted between the concentration and areas (Figure 11-This indicates an excellent linear relationship between the concentrations and obtained peak areas by the proposed method.

Table 5. Summary of Regression Parameters

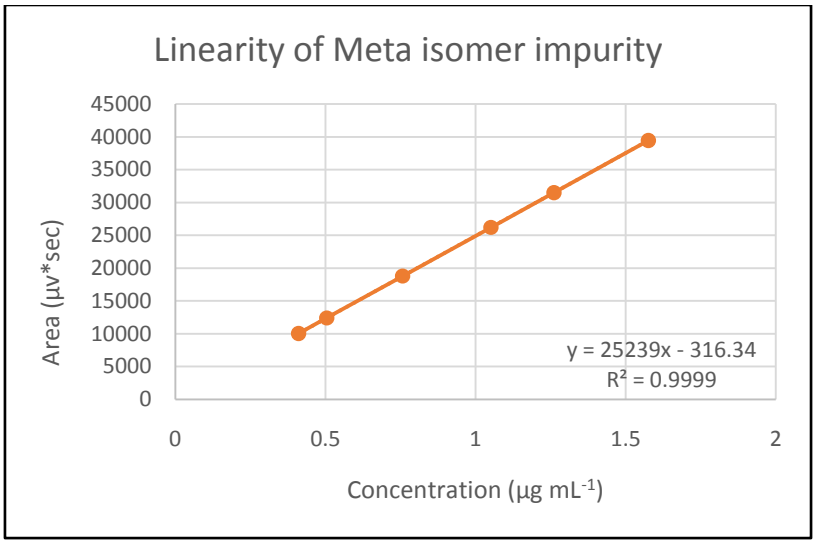
S. No.	Parameter	Obtained Values				
		Parecoxib	Imp A	Imp B	Imp C	Imp D
1	Correlation coefficient(R^2)	0.9999	1.000	0.9999	0.9995	1.000
2	Slope	30383	30523	25239	30673	30157
3	Y-Intercept	39.105	- 662.18	- 316.34	-1708.4	-396.77
4	Residual sum of squares	2000875.248	114705.237	259023.866	156943.453	435688.796



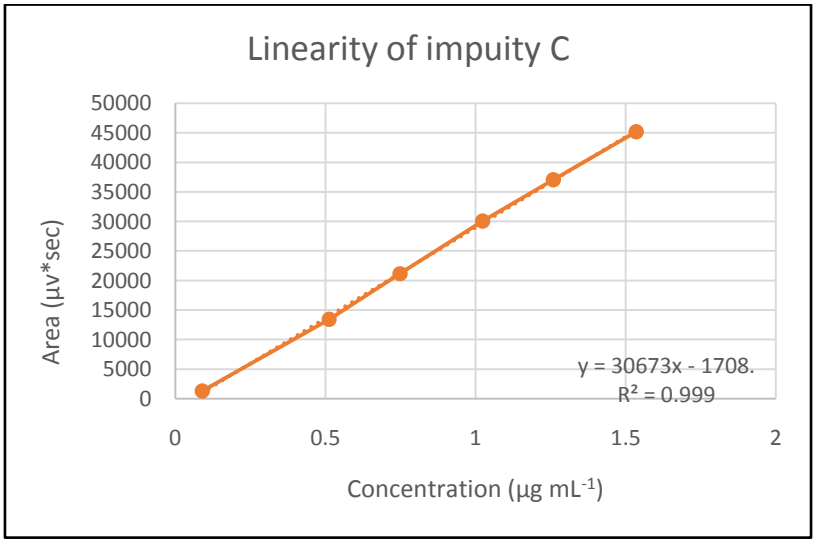
(a)


Linearity of Acetamide impurity
Area (µv*sec)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
0.5
1
1.5
2
Concentration (µg mL-1)
y = 30523x - 662.1
R² = 1

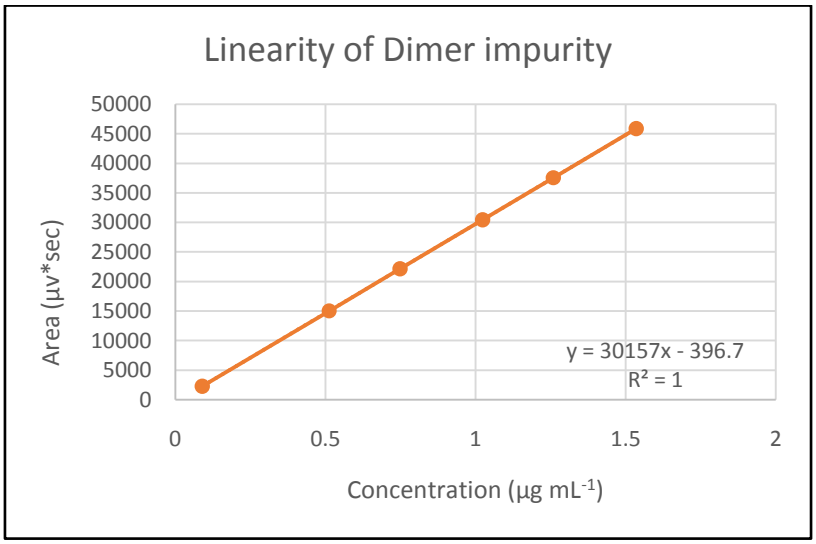
(b)


Linearity of Meta isomer impurity
Area (µv*sec)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
0.5
1
1.5
2
Concentration (µg mL-1)
y = 25239x - 316.34
R² = 0.9999

(c)


Linearity of impuity C
Area (µv*sec)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
0.5
1
1.5
2
Concentration (µg mL-1)
y = 30673x - 1708.
R² = 0.999

(d)



(e)

Figure 11. Calibration plot for (a) Impurity 1 (b) Impurity 2 (c) Impurity 3 and (d) Impurity 4

4.4 Accuracy

The accuracy of the analytical procedure expresses the degree of the closeness of the obtained results to the true values. A study of accuracy (recovery) was performed on known amount of placebo by spiking active pharmaceutical ingredient and impurities at specification level. Samples were prepared as per the proposed method at different levels i.e., LOQ, 50%, 100%, and 150% of target analyte concentration in triplicate (n=3) for impurities. For assay, the amount of Parecoxib was spiked at levels, 50%, 100%, and 150% into the placebo. Each preparation was injected in duplicate (n=2) into the chromatographic system. From the average of the two areas obtained, calculated the amount of Parecoxib and each individual impurity recovered from the placebo for each recovery levels (Table 6). Mean and relative standard deviation of all three recovery levels were calculated and assessed for accuracy of the method. The minimum and maximum recovery of Parecoxib was 98.9 and 99.2 with % RSD ranging from 0.4 to 0.9 which is well within the acceptance criteria. The minimum and maximum recovery of impurities lied between 96.2 % and 101.1 % which also well within in the acceptance criteria for impurities. These results show that method is capable of extract the impurities and active compound from the placebo precisely.

Table 6. Results of accuracy experiment

Name of analyte Peak	% Mean Recovery and %RSD							
	LOQ		50%		100%		150%	
Parecoxib Sodium	99.2	0.7	98.9	0.9	98.7	0.7	99.2	0.4
Impurity-A	100.1	1.1	98.1	1.2	96.2	1.8	96.9	1.3
Impurity-B	96.7	0.9	98.8	1.4	97.8	1.5	98.2	0.9
Impurity-C	100.1	0.4	99.4	0.7	97.2	0.7	99.9	0.5
Impurity-D	101.1	1.2	98.6	1.6	97.8	1.0	97.9	1.0

4.5 Precision

Method Precision and Intermediate Precision (Ruggedness)

Precision or repeatability of the test method was evaluated by analyzing six samples of the same batch as per the proposed method. Intermediate precision or ruggedness of the method was performed by analyzing six samples of the same batch as per the proposed method on another day, using another column and system. A comparison of system suitability parameters is made between the method precision and intermediate precision and are tabulated in Table 7-8. The average assay obtained in M.P and I.P are 99.9 and 99.7 respectively. The %RSD of 12 precision results is 0.63 which is well below the acceptance criteria for Precision. This shows that the method is precise.

Table 7. Comparison of System Suitability Parameters

System suitability Parameters	Results	
	Method Precision	Intermediate Precision
USP tailing	1.0	1.1
USP plate count	39871	40339

USP resolution	3.33	3.20
% RSD of five standard injections	0.32	0.46
Retention Times		
Parecoxib	15.123	15.730
Acetamide	12.031	12.112
Meta isomer impurity	17.876	17.110
Impurity C	22.342	22.118
Dimer Impurity	36.948	35.998

Table 8. Comparison of Method Precision and Intermediate Precision

Sample No	Method Precision					Intermediate Precision				
	I	II	III	IV	V	I	II	III	IV	V
1	99.4	0.48	0.54	0.51	0.45	98.6	0.49	0.52	0.54	0.47
2	98.7	0.48	0.55	0.52	0.45	98.9	0.48	0.52	0.53	0.46
3	99.4	0.49	0.53	0.53	0.44	99.3	0.49	0.53	0.55	0.46
4	99.0	0.48	0.53	0.53	0.44	98.0	0.48	0.54	0.54	0.48
5	98.8	0.49	0.54	0.53	0.43	98.5	0.47	0.52	0.54	0.46
6	98.8	0.48	0.55	0.53	0.45	98.3	0.48	0.53	0.54	0.46
Mean (n=6)	99.0	0.48	0.54	0.53	0.44	98.6	0.48	0.53	0.54	0.47
% RSD (n=12)	**0.32**	1.07	1.66	1.59	1.84	**0.46**	1.56	1.55	1.17	1.80

I-Parecoxib, II- Acetamide, III- Meta isomer, IV- impurity C, V- Dimer impurity

4.6 Range

The range of the analytical method falls between 50 to 150% of Parecoxib concentration in the sample for assay and LOQ to 150% of specification levels for impurities determination, in which it has been demonstrated to have a suitable level of precision, Accuracy, and Linearity.

4.7 Limit of Detection and Quantification

This limit was defined as the lowest concentration level that provided a peak area with signal to noise ration higher than 3:1 for detection and 10:1 for quantification. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal to noise ratio (Table 7.19).

Table 9. Results of LOD and LOQ

Name of analyte Peak	%w/w			
	LOD	LOQ	S/N-LOD	S/N-LOQ
Impurity-A	0.003	0.02	4	11
Impurity-B	0.009	0.04	4	12
Impurity-C	0.01	0.04	3	9
Impurity-D	0.005	0.01	4	10

4.8 Robustness

Robustness study was performed on the chromatographic parameters which are susceptible to change during the preparation of solution, handling, and operation of the instrument. The parameters studied are mobile phase flow rate, column oven temperature, mobile phase pH. The mobile phase flow rate was studied at ±10% from the optimized flow rate. The column temperature on was studied at ±5 °C. The variation in mobile pH was studied at ±0.2 units. The system suitability parameters like USP resolution, USP Tailing and relative standard deviation were evaluated (Table 10).

Table 10. Results of Robustness/Ruggedness Experiment

S. No.	Condition	Actual Condition	Altered Condition	USP Resolution	USP Plates	%RSD
1	**Control**	**--**	**--**	**3.45**	**1.0**	**0.5**
2	Flow (in mL min^{-1})	1.0	0.90	3.44	1.1	0.66
			1.10	1.99	1.1	0.59
3	Column oven temperature (°C)	40	35	2.05	1.1	0.47
			45	3.50	1.1	0.66
4	Mobile pH	6.20	6.00	2.3	1.1	0.60
			6.40	2.7	1.1	0.49

The above data indicates that USP resolution, is very critical and depends on the flow rate and column oven temperature. USP tailing factor and %RSD are not impacted due to the deliberate changes made to the chromatographic conditions.

V. Conclusions

A rapid, simple, sensitive, accurate and reliable RP-HPLC method was developed and validated for the determination of Parecoxib inaninjection formulations.In this method Parecoxib Sodium and its impurities were quantified simultaneously in single chromatographic run with high degree of accuracy and precision. The stability indicating power of the method was established through stress studies. All the degradationproducts formed during stress studies were wellseparated from the analyte peak which is evident from the peak purity data. The method discusses the nature of the molecule under stress conditions where it was shown that the compound sensitive towards oxidation (30.5%), followed by thermal degradations (6.0%). The validation data meets the acceptance criteria for the validation parameters as per the current ICH guidelines.

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