



RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF POLMACOXIB IN SOLID DOSAGE FORM AND APPLICATION TO DEGRADATION STUDY

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ABSTRACT

Stability indicating assay method is reported for determination of polmacoxib from its capsule formulation. The polmacoxib is relatively a new molecule and very few analytical methods have been reported for its determination from biological fluid. Hence, a new HPLC method has been developed and validated with high accuracy and simplicity. The method was applied for stress degradation study of the drug. The separation of analyte and degradation products were obtained on Inertsil ODS 3V (250mm × 4.6mm), 5µm column, using the gradient elution of ammonium acetate buffer (Mobile Phase - A) and acetonitrile (Mobile Phase - B), with a flow rate of 1.5 mL/min. The eluent was monitored at 240 nm and the total run time was 28 min. Retention time of polmacoxib was found to be 10.8 min. Force degradation of polmacoxib was carried out under acidic, basic, oxidative, thermal, photo and humidity conditions. Polmacoxib has been found to degrade in selected oxidative degradation conditions. The method was validated as per ICH guidelines. The linearity of the method was in the concentration range of 50 – 150 µg/mL with the linearity equation $Y = 19.658x + 3.434$ ($r^2 = 0.999$). The method was identified as sufficiently specific to separate degradation products from the main analyte. The method had proven its potential for its application in routine estimation and stability study of polmacoxib.

Keywords: Forced degradation, HPLC, Method validation, Polmacoxib

INTRODUCTION

Polmacoxib was developed as Acelex, CG100649 and approved in South Korea for its use in treatment of osteoarthritis in the February 2015. Chemically Polmacoxib is, 4-(3-(3-Fluorophenyl)-5, 5-

dimethyle-4-oxo-4, 5-dihydrofuran-2-yl)-benzenesulfonamide (Figure 1). Polmacoxib is a non-steroidal anti-inflammatory drug and a dual inhibitor of COX-2 and carbonic anhydrase enzyme [1].

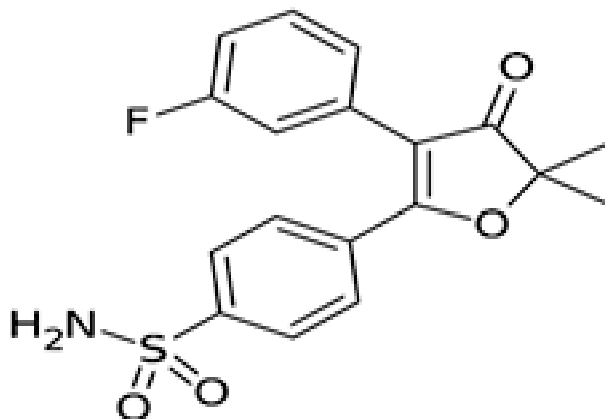


Figure 1: Chemical structure of Polmacoxib

Literature studies show few analytical methods reported for estimation of Polmacoxib from various matrices. Kim et al developed HPLC-MS/MS method for pharmacokinetic and pharmacodynamics study of CG10049 in healthy volunteers [2]. Choi et al developed HPLC-MS/MS method to study the coadministration of ketoconazole with CG10049 [1]. Hirankarn et al developed HPLC-MS/MS method for determination of polmacoxib metabolism in healthy volunteers and use this information to define the clinical development strategy [3]. Kyeon et al carried out solid state studies for polymorphism, solubility and dissolution of CG100649 using various techniques like UV spectroscopy, differential scanning

calorimetry and powder X-ray diffraction method [4]. Lee et al reported safety and efficacy study of polmacoxib in comparison with celecoxib [5].

From, the available literature it can be concluded that, no analytical method has been available in the public domain for estimation of polmacoxib and its stability study. Hence, there is a need to develop a simple HPLC method for estimation of the drug which can be used in routine for quality control of the API and formulation of polmacoxib.

The objective of this study was to formulate and validate a stability-indicating assay method for determining polmacoxib in its capsule dosage form

using HPLC. The established analytical procedure underwent validation through assessments of system suitability, linearity, precision, accuracy, specificity, robustness, ruggedness, and solution stability, adhering to the guidelines set forth by the International Conference on Harmonization (ICH).

EXPERIMENTAL

Chemicals and reagents

Polmacoxib API (purity-98.9%) was obtained from Zydus Healthcare Ltd. (Ahmedabad, India). Pharmaceutical capsule dosage form (label claim - 2 mg) and placebo were obtained from Zydus Healthcare Ltd. (Ahmedabad, India).

HPLC grade acetonitrile and ammonium acetate were procured from Merck (Mumbai, India). HCl, NaOH and H₂O₂ were of AR grades and procured from Qualigens (Mumbai, India), HPLC grade water (Milli Q or equivalent) was used throughout the experimental work.

Instrumentation

HPLC experiment was carried out on Agilent 1260 series LC system (manufactured by Agilent Technologies, USA) with UV detector and also a PDA detector. The output signal was observed and converted using chromelion software. The analytical column used for the separation was Inertsil ODS 3V (250mm X 4.6mm, 5 μ) column. The other equipments used were ultra sonicator, analytical balance and pH meter.

Chromatographic conditions

Chromatographic separation was achieved at 30°C temperature on a reversed phase column with 25°C sample cooler temperature. Solvent A comprised a 0.02 M ammonium acetate buffer, while Solvent B contained HPLC-grade acetonitrile. The mobile phase was maintained at a flow rate of 1.5 mL/min. The HPLC gradient was kept as Time / % B : 0/40, 12/50, 15/80, 20/80, 20.5/40, 28/40. The analyte was detected at a wavelength of 240 nm, with an injection volume of 10 μ L. Acetonitrile was utilized as the diluent.

Preparation of standard solution

A precise quantity of 50 mg of polmacoxib API was weighed and placed into a 50 mL volumetric flask. Subsequently, 25 mL of acetonitrile was added to the flask and sonicated at a temperature below 25°C until the powder was fully dissolved. The volume was made up to the mark with acetonitrile to have stock solution of polmacoxib having concentration 1000 μ g/mL. A 5 mL aliquot was taken from this solution and transferred into a 50 mL volumetric flask. The volume was then adjusted to the mark using acetonitrile to achieve a final concentration of 100 μ g/mL of polmacoxib.

Preparation of test solution

20 capsules were taken and accurately weighed on the analytical balance. The whole content of the capsules was collected and cleaned empty shells were

weighed again to calculate the average net content of the capsule.

Ten intact capsules were collected, and their contents, equivalent to approximately 20 mg of polmacoxib, were transferred into a 200 mL volumetric flask. About 100 mL of diluent was added, and the mixture was sonicated at a temperature below 25°C for 35 minutes, with intermittent shaking for 30 seconds every 5 minutes. The volume was adjusted to the mark using diluent and thoroughly mixed. The solution was then filtered through a 0.45 µm MDI-manufactured PVDF filter, discarding the initial 3 mL of the filtrate. The final expected concentration of the test solution was 100 µg/mL polmacoxib.

Method validation

The analytical method validation was carried out as per ICH method validation guidelines Q2 (R1) [6]. The optimized chromatographic conditions were validated by evaluating system suitability, linearity, precision, accuracy, specificity, robustness, ruggedness and solution stability.

System suitability

The system suitability test was carried out by injecting the diluted working standard solution of polmacoxib API (100 µg/mL) into the HPLC system. Five replicates of the solution were injected and system suitability parameters like number of theoretical plates, tailing factor and peak area were determined. The relative standard deviation of the responses were calculated.

Linearity

The linearity of the developed method was evaluated across a concentration range spanning 50% to 150% of the target concentration (100 µg/mL). The concentrations 50, 80, 100, 120, 150 µg/mL of polmacoxib were prepared by withdrawing suitable aliquots from the stock solution of polmacoxib (1000 µg/mL). The solutions were injected into the HPLC system and area under the curve was measured for each concentration. The plot of concentration v/s peak area was constructed to find out the equation of line.

Precision (Repeatability)

Method precision was demonstrated by injecting six test samples of polmacoxib (100 µg/mL) into the HPLC system and analyzed. The areas of all 6 injections were recorded and standard deviation, % relative standard deviation and % assay were calculated.

Accuracy

The accuracy of the developed method was assessed by preparing recovery samples at three distinct levels: 50%, 100%, and 150%. These samples were generated by spiking a known quantity of API into a placebo and were prepared in triplicate for each level. The percentage recovery of the added amount was then calculated. The precision of the recovery at each level was evaluated by determining the percentage relative standard deviation of the triplicate recovery results.

Specificity

To evaluate the interference from placebo the specificity of the test method was demonstrated by studying the peak purity and interference from placebo, mobile phase and diluent.

Robustness

The robustness of the method was validated by performing system suitability tests under both standard conditions and various modified conditions. Working standard solution of polmacoxib (100 µg/mL) was taken and five replicates of this solution were injected in HPLC system with changes in optimized chromatographic conditions like organic phase ratio of mobile phase was changed by $\pm 2\%$, flow rate of mobile phase was changed by $\pm 10\%$ and column temperature was changed by $\pm 5^\circ\text{C}$ to that of the optimized chromatographic conditions. Parameters like % relative standard deviation, number of theoretical plates, tailing factor were evaluated for each of altered conditions.

Ruggedness

It is also known as intermediate precision. A test sample solution (100 µg/mL) was prepared, and a precision study was conducted by injecting six replicates of a single batch sample. The study was performed by two different analysts, on separate days, using two distinct columns and two different instruments. The % relative standard deviation of six samples

and absolute difference between two sets of data was calculated.

Solution stability

Stability of analytical solution and mobile phase were determined by preparing API and sample solution (100 µg/mL) and stored at 25°C . Solutions of API and sample were chromatographed into HPLC system at regular intervals for minimum 36 hr using the same mobile phase. The area of the analyte peak for both API and sample solutions were recorded. For stability of analytical solution the % deviation of analyte peak area from initial peak area of API and sample solutions were calculated and for stability of mobile phase the system suitability parameters like % relative standard deviation, number of theoretical plates, tailing factor for standard solution were evaluated.

Forced degradation study

Forced degradation study was conducted on Polmacoxib API and capsule formulation [7] [8].

Acid degradation

For the acid degradation study, ten capsules were opened, and their contents (equivalent to approximately 20 mg of Polmacoxib) were transferred along with the empty shells into a 200 mL volumetric flask. 10 mL of acetonitrile was added to the flask, and the mixture was sonicated for 10 minutes. Subsequently, 5 mL of 5N HCl was added and flask was maintained

in a water bath at 80°C for 2 hours. The acid was then neutralized using 5 mL of 5N NaOH solution. Following this, 100 mL of acetonitrile was added, and the solution was sonicated for 30 minutes. The final volume was adjusted to the mark with acetonitrile and thoroughly mixed. The prepared solution was then filtered through a 0.45 µ filter before being injected into the HPLC system. The similar procedure was carried out by taking 20 mg of polmacoxib API in place of capsule formulation.

Alkali degradation

The alkali degradation was carried out in the similar manner as that of the acid degradation condition. For alkali degradation, initially 5 mL of 5 N NaOH was taken as the stressor and 5 N HCl was added later as the neutralizing solution. The similar procedure was carried out by taking 20 mg of polmacoxib API in place of capsule formulation.

Oxidative degradation

For the oxidative degradation study, ten capsules were opened, and their contents (equivalent to approximately 20 mg of Polmacoxib), along with the empty shells, were transferred into a 200 mL volumetric flask. 10 mL of acetonitrile was added, and the mixture was sonicated for 10 minutes. Subsequently, 5 mL of 10% H₂O₂ was added and the flask was maintained in a water bath at 80°C for 2 hours. Following this, 100 mL of acetonitrile was added, and the solution was sonicated for 30 minutes.

The final volume was adjusted to the mark with acetonitrile and thoroughly mixed. The prepared solution was then filtered through a 0.45 µ filter before being injected into the HPLC system. The similar procedure was carried out by taking 20 mg of polmacoxib API in place of capsule formulation.

Photolytic degradation

Solid drug product and capsule formulations were kept in different petridishes and exposed in photo stability chamber under UV light (1.2 million lux hr.) for 96 hr. For capsule formulation, 10 intact capsules were opened and the whole content of capsules was transferred along with the empty capsule shells (equivalent to about 20 mg of polmacoxib) in to a 200 mL volumetric flask. 10 mL of acetonitrile was added into the flask and sonicated for 10 min. The volume was made up to the mark with acetonitrile. The similar procedure was carried out for polmacoxib API by taking 20 mg of API powder.

Thermal degradation

Solid drug product and capsule formulations were kept in different petridishes and kept in hot air oven at 105°C for 24 hr. For capsule formulation, 10 intact capsules were opened and the whole content of capsules was transferred along with the empty capsule shells (equivalent to about 20 mg of polmacoxib) in to a 200 mL volumetric flask. 10 mL of acetonitrile was added into the flask and sonicated for 10 min. The volume was

made up to the mark with acetonitrile. The similar procedure was carried out for polmacoxib API by taking 20 mg of API powder.

Humidity degradation

Solid drug product and capsule formulations were kept in different petridishes and kept in humidity chamber at 40°C/75% relative humidity for 4 days. For capsule formulation, 10 intact capsules were opened and the whole content of capsules was transferred along with the empty capsule shells (equivalent to about 20 mg of polmacoxib) in to a 200 mL volumetric flask. 10 mL of acetonitrile was added into the flask and sonicated for 10 min. The volume was made up to the mark with acetonitrile. The similar procedure was carried out for polmacoxib API by taking 20 mg of API powder.

RESULTS AND DISCUSSION

Optimization of chromatographic condition

Selection of solvent

The solvent was selected on bases of solubility study of polmacoxib in acetonitrile (100%), water (100%) and acetonitrile : water (50:50) [9] [10]. The polmacoxib showed very good solubility of 30 mg/mL in acetonitrile (100%) hence, it was selected as the solvent for polmacoxib.

Selection of wavelength

A 10 µg/mL solution of polmacoxib was prepared using acetonitrile as the solvent and analyzed using a UV-Visible spectrophotometer within the wavelength range of 200–400 nm to identify the drug's absorption maxima. From the spectra it was observed that at 240 nm polmacoxib had maximum absorbance. Hence, 240 nm was selected as the wavelength for determination of polmacoxib.

Selection of chromatographic condition

Mobile Phase: Various combinations of mobile phase were tried in isocratic mode to get good peak shape of polmacoxib. But, isocratic mobile phase was not giving the desired results hence, the mobile phase was changed from isocratic (Buffer:ACN/40:60) to gradient programme. In isocratic mode poor resolution and greater width of analyte peak was observed. In gradient programme peak shape was better as compared to isocratic.

Diluent: It was changed from ACN:Water (50:50) to Acetonitrile (100%). Drug was not sufficiently soluble in mixture of acetonitrile and water; so, 100% acetonitrile was used as a diluent.

Flow rate: It was changed from 1.0 mL/min to 1.5 mL/min. At flow rate of 1.5 mL/min the retention time of analyte was decreased.

Method validation

System suitability

The results of the system suitability parameter showed that, the peak of polmacoxib was very sharp and symmetrical. The average theoretical plates were found to be 17318 and tailing factor was found to be 1.0 with % relative standard deviation of peak area about 0.3%.

Linearity

The linearity of the method was performed in the range of 50 – 150 µg/mL of polmacoxib. The method showed excellent linearity with correlation coefficient 0.999 and equation of the straight line $Y = 19.658X + 3.434$. The linearity curve for the polmacoxib is shown in the Figure 2. The overlay chromatograms for the linearity is shown in Figure 3.

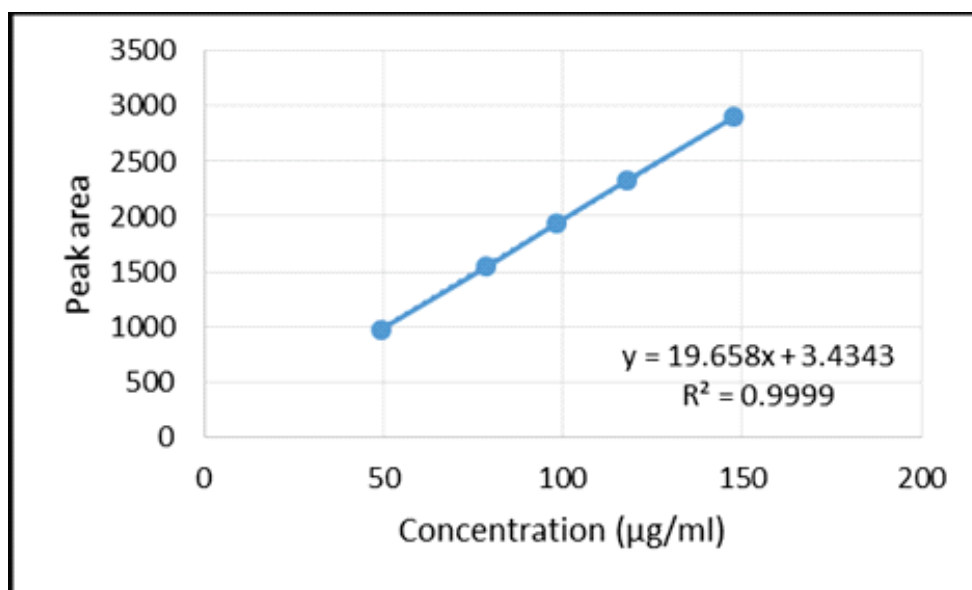


Figure 2: Linearity curve for polmacoxib (50 – 150 µg/mL)

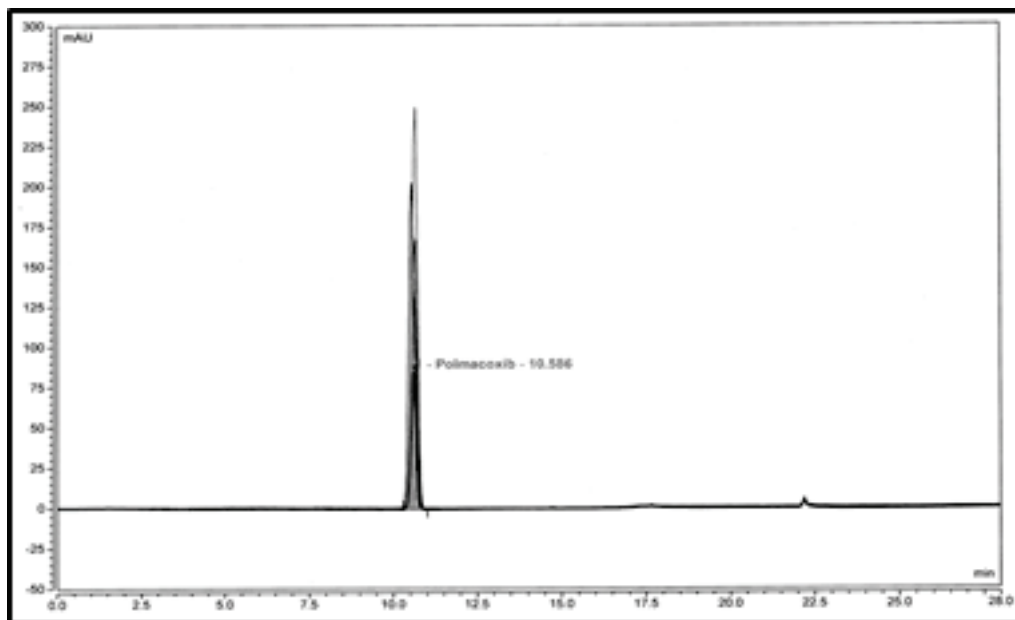


Figure 3: Overlay chromatograms for linearity of polmacoxib (50 – 150 µg/mL)

Precision

The method precision was carried out by injecting replicate sample injections. The average % assay of polmacoxib was found to be 98.7 % with relative standard deviation of 0.6%. The lower value of % relative standard deviation indicates the method is precise.

Accuracy

Accuracy of proposed method was determined by preparing recovery samples at three different level of 50%, 100%, and 150%. The mean % recovery for the amount added was found to be between 99.4 - 101.2 % of polmacoxib. The relative standard deviation for the three concentration level was found to be between 0.2 – 0.5 %. The results of the accuracy study is shown in Table 1.

Table 1. Accuracy study results for polmacoxib

Recovery level	Set No.	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean	%RSD
50%	1	9.919	10.028	101.1	101.2	0.5
	2	9.919	9.992	100.7		
	3	9.919	10.085	101.7		
100%	1	19.839	19.829	100.0	100.4	0.5
	2	19.839	20.030	101.0		
	3	19.839	19.908	100.3		
150%	1	29.759	29.518	99.2	99.4	0.2
	2	29.759	29.649	99.6		
	3	29.759	29.598	99.5		

Specificity

For specificity study, the peak purity spectra of polmacoxib peak and peak area was determined. Further the chromatograms of blank, polmacoxib API

and sample solution of polmacoxib capsules were compared to find out any additional interfering peak from the excipients. The peak purity spectra of polmacoxib is shown in Figure 4.

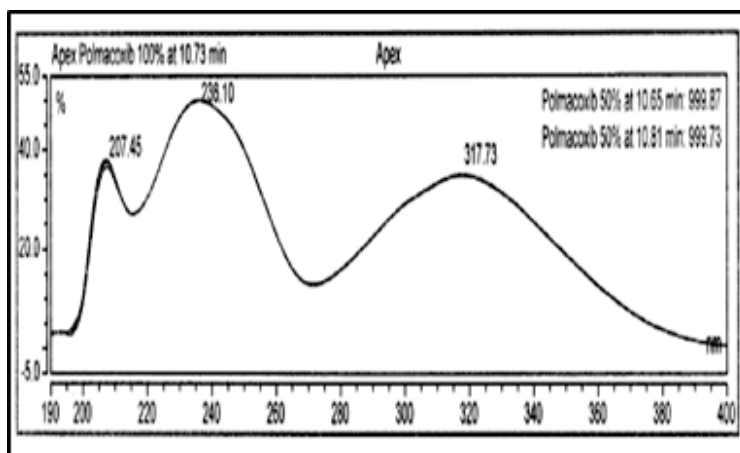


Figure 4: Peak purity spectra of polmacoxib

Robustness

The robustness of the methods was checked by carrying deliberate small changes in the optimized chromatographic conditions. The results of the robustness is

shown in Table 2. The results indicates that, the when there is decrease in organic ratio of the mobile phase, the number of theoretical plates increase alongwith the increase in the retention time of polmacoxib.

Table 2: Robustness study results for polmacoxib

Condition	% RSD*	Retention Time*	Theoretical plates*	Tailing factor*
Normal condition	0.3	10.6	17318	1
Mobile phase organic ratio was changed to -2%	0.2	11.3	23476	1
Mobile phase organic ratio was changed to +2%	0.1	9.5	16568	1
Column temperature was changed to 5°C	0.4	10.9	19440	1
Column temperature was changed to -+5°C	0.1	10.1	18702	1
Flow rate was changed to -10%	0.1	11.4	20658	1
Flow rate was changed to +10%	0.2	9.7	17794	1

*n=5

Ruggedness

The ruggedness study was performed by 2 different analysts using 2 different system and the same experimental conditions. The results of the ruggedness study are shown

in Table 3. The results indicates that there is not much deviation in the results obtained by 2 different analysts for the % assay of polmacoxib (98.7 % and 98.4 % for analyst 1 & 2 respectively).

Table 3. Ruggedness study results for polmacoxib

Sample	% Assay of polmacoxib	
	Analyst 1 / Instrument 1	Analyst 2 / Instrument 2
1	98.4	98.8
2	98.7	98.8
3	98.8	99.0
4	98.2	98.4
5	98.2	97.2
6	99.7	97.9
Average	98.7	98.4
% RSD	0.6	0.7

Solution stability

The polmacoxib API and sample solutions showed excellent stability in the solvent and mobile phase. There is no much difference in the peak area was observed. The % relative standard deviation of the peak area was found to be less than 2% during the 36 hr storage period.

Assay from capsule formulation

The developed method was successfully applied to carry out assay of polmacoxib

from the capsule formulation. The sample solution (100 µg/mL) was prepared from the capsule formulation and injected in HPLC system under optimized experimental conditions.

The retention time of polmacoxib peak was 10.90 min with 1932.405 peak area and 22963 theoretical plates. Observed tailing factor of the peak was 1. Calculation of % Polmacoxib per capsule as carried out using following formula and % Polmacoxib was found to be 98.8 % present in capsule.

$$\% \text{Polmacoxib} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{10} \times \frac{P}{100} \times \frac{1}{L.C} \times 100$$

Where, AT = peak area of polmacoxib in sample preparation

AS = peak area of polmacoxib in standard preparation

WS = weight of working standard taken in mg

DS = dilution of standard solution

DT = dilution of sample solution

P = percentage potency of working standard

L.C = label claim

Forced Degradation Study

Acid degradation

The polmacoxib API solution does not show any degradation peak in acidic degradation condition. However, the sample solution of polmacoxib showed a

very minute unknown degradation peak retention time 5.52 min. The % assay was found to be 102.8 % and 105.1 % for polmacoxib API and sample solutions respectively. The chromatogram for blank, and acid degradation product is shown in Figure 5A and 5B.

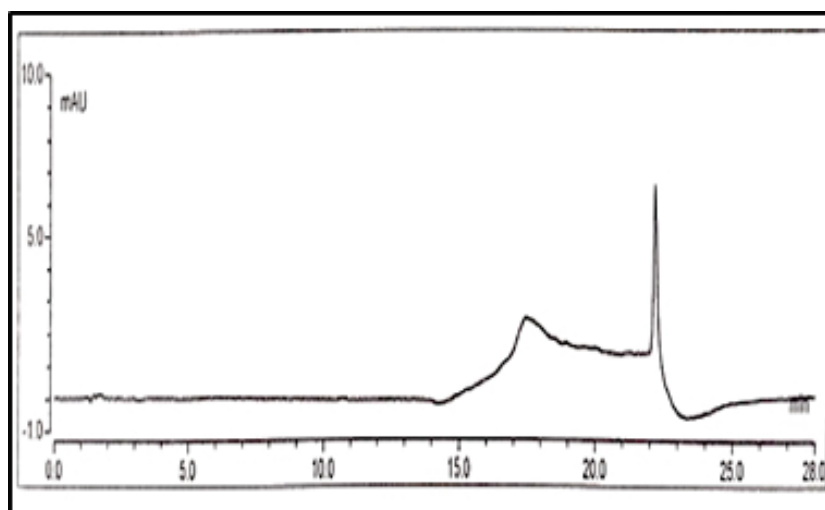


Figure 5A: Chromatogram for blank (Acid degradation)

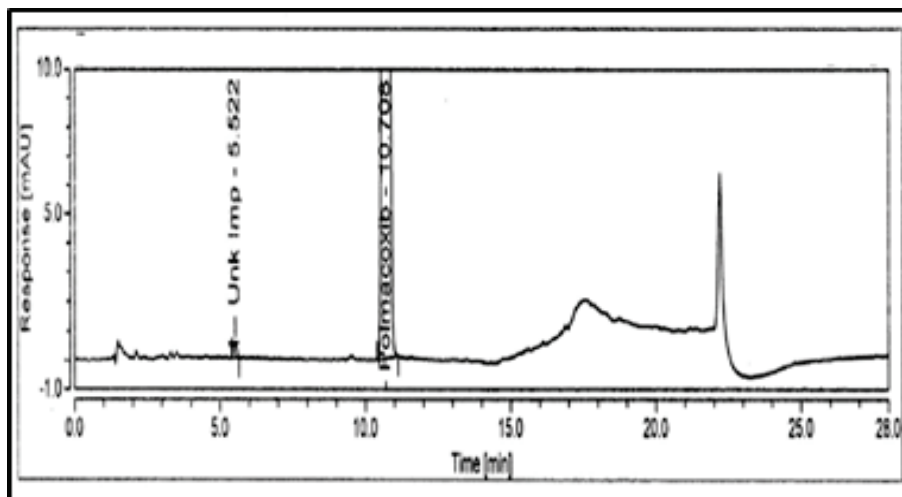


Figure 5B: Magnified chromatogram for polmacoxib sample (100 µg/mL, 5mL 5N HCl Acid degradation)

Alkali degradation

In alkali degradation study, the polmacoxib API solution showed one unknown peak at retention time 7.644 while, the polmacoxib sample solution was found to be stable in alkali degradation conditions. The % assay of polmacoxib was found to be 92.49%

and total degradation was found to be 7.5% in polmacoxib API solution. While, in polmacoxib sample solution, the % assay was found to be 99.5%. The chromatogram for blank and alkali degradation of polmacoxib API solution is shown in Figure 6A and 6B respectively.

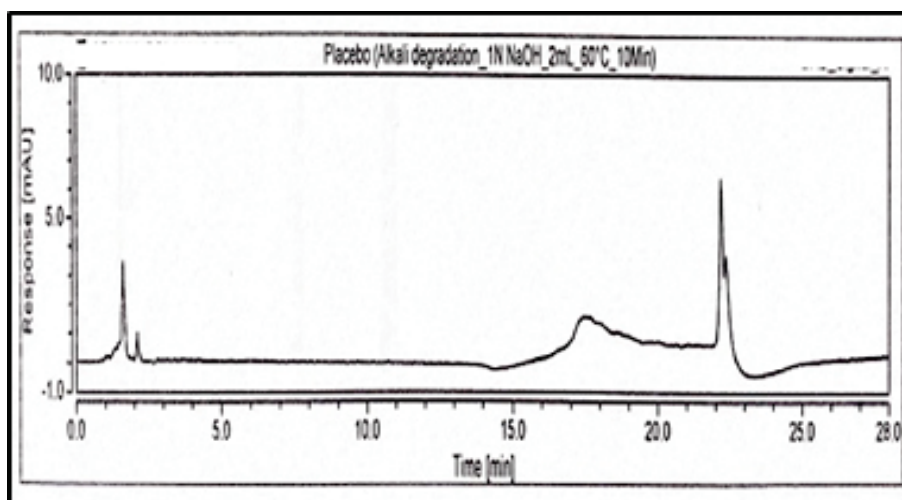


Figure 6A: Chromatogram for blank (Alkali degradation)