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STABILITY- INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF OXAPROZIN

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ABSTRACT

A simple, sensitive, precise, and validated RP-HPLC method has been developed for the analysis of Oxaprozin API and for its stability-indicating assay. The solvent system and wavelength were optimized in order to maximize the sensitivity of the proposed method; Oxaprozin shows the maximum absorbance at 220 nm by using a PDA detector. A Waters HPLC system, with Hemochrom C18 column 150 mm X4.6mm column is employed for the analysis using Acetonitrile and 0.1% Formic acid in the ratio of 60:40 respectively as mobile phase. The method was validated for accuracy, precision, linearity, specificity, stressed studies, robustness, etc. Linearity was observed in the concentration range of 1-5% and gave a mean correlation coefficient of 0.999. The developed RP-HPLC method was found to be accurate, precise, and stability indicating, and has application in API pharmaceutical manufacturing.

KEYWORDS: Oxaprozin, RP-HPLC, Development, Validation, Forced degradation.

1. INTRODUCTION

Oxaprozin (4, 5-diphenyl-2-oxazole propionic acid) is a non-narcotic, non-steroidal anti-inflammatory drug (NSAID), used to relieve the inflammation, swelling, stiffness, and joint pain associated with osteoarthritis and rheumatoid arthritis.[1]

The anti-inflammatory effects of Oxaprozin are believed to be due to the inhibition of cyclooxygenase in platelets which leads to the blockage of prostaglandin synthesis.[1]

Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Oxaprozin is a non-selective NSAID, with a cell assay system showing lower COX-2 selectivity implying higher COX-1 selectivity.[1]

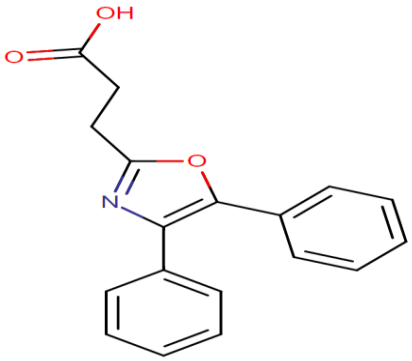

Fig No 1: Chemical structure of the drug.[1]

The literature survey reveals that few RP-HPLC methods were reported for estimating Oxaprozin. However, the Methods were found to be incompatible with LC-MS, Expensive, and complicated, and no stability-indicating methods are reported.

Hence, the objective of this work is to develop and validate a new, linear, simple, rapid, Accurate, efficient, Reproducible, and MS- compatible RP-HPLC method for the analysis of Oxaprozin and which could also be applied to its stability studies.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagent: Oxaprozin was purchased from Dhamtec Pharma. HPLC-grade water and other reagents such as Acetonitrile, Methanol, and IPA were procured from Ultra-Pure Pvt Ltd, Goregaon, Mumbai, India.

2.2 Instrumentation: The estimation of oxaprozin was carried out using a WATERS HPLC system with PDA Detector using Empower software as an integrator, Analytical Balance (Mettler Toledo), pH meter (Lab India) and a Sonicator (Spectralab) The column used for separation of Oxaprozin is Hemochrom C18 column 150 mm X4.6mm.

1.3 Preparation of Solution

1.3.1 Preparation of Standard Stock Solution – In a 10 ml volumetric flask, 10 mg of Oxaprozin was accurately weighed and transferred Sample was dissolved and diluted

with diluent (50% ACN+ 50% Water) up to the mark Solution A (1000 µg/ml).1ml of Solution A was taken in 10 ml volumetric flask and was diluted with diluent to the mark Solution B (100 µg/ml). Further 1 ml of solution B was diluted to 10 ml with diluent (10 µg/ml) (Solution C) and was used as a working standard.

1.3.2 Preparation of Mobile Phase - A mixture of 40% acetonitrile and 60% of 0.1 Formic acid. (Prepared by adding 1 ml of Formic Acid in 999ml of water).

1.3.3 Preparation of Diluent – Diluent was prepared by mixing of 50ml of water and 50ml of Acetonitrile.

2.3 Initial Method Development

2.3.1 Choice of Wavelength: In a 10 ml volumetric flask, 10 mg of Oxaprozin was accurately weighed and transferred Sample was dissolved and diluted with diluent (50% ACN+ 50% Water) up to the mark Solution A (1000 µg/ml).1ml of Solution A was taken in 10 ml volumetric flask and was diluted with diluent to the mark Solution B (100 µg/ml). Further 1 ml of solution B was diluted to 10 ml with diluent (10 µg/ml). UV spectrum of a solution having a concentration of 10µg/ml was recorded using diluent as blank. It showed absorbance at a wavelength of 220 nm, so it was selected λ max for Oxaprozin.

2.3.2 Choice of Column: The selection of the column is given in Table No 1.

Table No 1: Experimental trials for choice of columns.

Column	Observation	Inference
C8	Poor retention of the analyte	Broad and poor peak shape
C18	Improved retention of analyte	Improved peak shape

2.3.3 Choice of Mobile phase: The selection of mobile phase is given in Table No 2.

Table No 2: Experimental trials for selection of mobile phase.

Combination	Observation	Inference
MeOH: Water	High baseline noise	ACN required to reduce baseline interference
Acetonitrile:0.1% Formic Acid	Broad peak shape and inconsistent retention time	Change in mobile phase ratio required
Acetonitrile:0.1 FA (40:60)	Symmetric peak shape and precise retention	Mobile phase finalised

3.0 METHOD VELIDATION[2] - The developed method was validated on the parameters such as system suitability, linearity, precision, accuracy, the limit of detection (LOD) and limit of quantification (LOQ), Ruggedness, and Forced degradation studies.

A) System Suitability- System suitability testing is an integral part of any analytical procedure. System suitability testing was carried out by injecting 6 replicates of 3µg/ml Standard Oxaprozin solution. In this test, system suitability parameters like %RSD of Peak area, retention time, and number of theoretical plates (NTP) were evaluated.

B) Specificity The specificity of the method was determined by recording the chromatogram of the Blank and the standard solution. Specificity signifies the identification of analyte, interference from other peaks, and peak purity.

Peak purity data reveals that the Oxaprozin peak was homogeneous and there were no co-eluting peaks at the retention time of the Oxaprozin peak.

C) Linearity and Range- The method's linearity was evaluated in the range of 1.0µg/mL–5.0µg/mL for Oxaprozin. Drug levels of these concentrations were prepared and each linearity level was injected into HPLC, chromatograms and peak area were recorded for all the peaks. The calibration curve was plotted as the mean peak area of the analyte against the concentration of the drug in µg/mL.

D) Precision - The instrument precision was evaluated by determining the absorbance of the standard solution six times repeatedly. The results are reported in terms of relative standard deviation for the same. The intra-and inter-day variation for the determination was carried out in triplicate for the standard solution.

E) Accuracy- The % recovery study was performed by using a minimum of three concentration levels, each in triplicate determinations.

It was carried out by of Higher, middle and lower concentration of range in triplicates, and the mean %RSD was calculated.

F) Limit of Detection and Limit of Quantification - The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the Standard solution of Oxaprozin using the developed HPLC method. This was done until a signal-to-noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ, respectively.

4.0 FORCED DEGRADATION STUDIES

A forced degradation study was carried out by treating the sample under the following conditions

a) **Acid Degradation** - 4ml of the solution(1mg/ml) solution taken in 10ml volumetric flask and volume was made up to 10 ml with 0.1N HCl and the solution kept for 24,48,72 hrs respectively and at mentioned hrs taken 1ml of solution and then neutralized with the same molar concentrations of sodium hydroxide solution and the volumes were made up with the diluent to give a solution of 40µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

b) **Base Hydrolysis** - 4ml of the solution(1mg/ml) solution taken in 10ml volumetric flask and volume was made up to 10 ml with 0.1N Sodium hydroxide and the solution kept for 24,48,72 hrs and at mentioned hrs taken 1ml of solution respectively and then neutralized with the same molar concentrations of acid solution and the volumes were made up with the diluent to give a solution of 40µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

c) **Oxidative Degradation** - 4ml of the solution (1000µg/ml) was pipetted out in a 10ml volumetric flask and the volume was made up to 10ml with 6% H2O2. And the solution kept at such at room temperature for 6,12,24,48 hours. and at mentioned hrs taken 1ml of solution respectively and then the volume was made up with the diluent to give a solution of 40µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

d) **Thermal Degradation** - 4ml of the solution 1 (1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was kept in water bath at 60℃ for 6,12,24,48,72 hours. The volume was made up with the diluent to give a solution of 40µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

e) **Photolytic Degradation** - 4ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was kept in sunlight for 6,12,24,48,72 hrs. The volume was made up with the diluent to give a solution of 40µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

5.0 RESULT AND DISCUSSSION

The summery of Optimized Chromatographic Conditions is stated in Table 3.

Table 3: Optimized Chromatographic Conditions.

Analytical technique	Optimized conditions	Oxaprozin
RP-HPLC	Mobile Phase	Acetonitrile:0.1% Formic Acid in water (40:60)
	Column	Hemochrom C18 (150 mm*4.6mm*5um)
	Flow rate	1.0ml/min
	Injection Volume	10 µl
	Column Oven Temperature	28º c
	Detector	Water 2996 PDA
	Mode	Isocratic
	Wavelength	220 nm

5.1 **System Suitability**- The 3ug/ml standard of oxaprozin were injected in Six replicate and the mean of system suitability parameters were obtained and mentioned in table 4.

Table 4: System suitability data for Oxaprozin.

Sr. No.	System suitability parameters	Observations	Acceptance criteria
1	Oxaprozin Standard Solution	3.00µg/mL	--
2	Area % RSD	1.003%	NMT 2%
3	Retention Time	5.447	--
4	NTP	5562.56	NTP > 2000
5	Symmetry Factor	0.9345	0.8-2.0

5.2 **Specificity** - The method was quite selective for Oxaprozin as there was no other interfering peak seen around the retention time of Oxaprozin. Also, the baseline did not show any significant peak. Thus, the method was found to be highly specific for Oxaprozin. Representative chromatogram for a blank in Figure and Oxaprozin standard is in Figure 2.

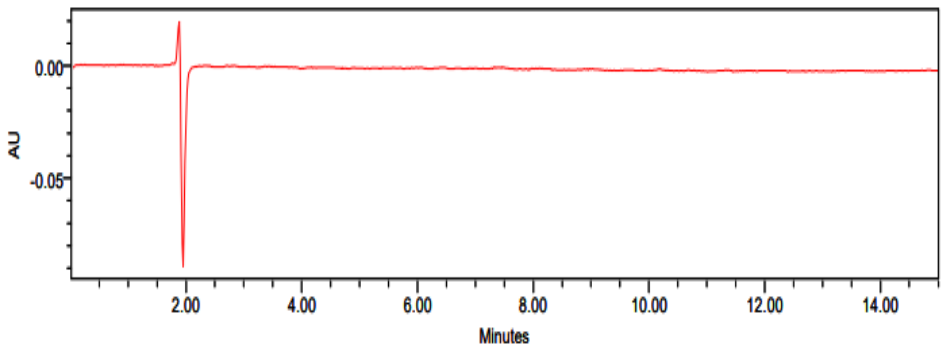

Fig. 2: Chromatogram of blank.

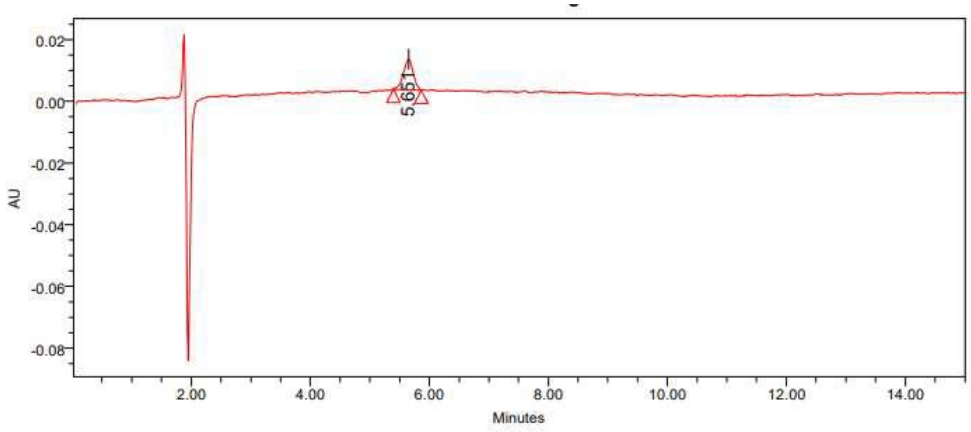

Fig. 3: Chromatogram for Oxaprozin standard solution (3µg/ml).

5.3 Linearity – The linearity was confirmed within the range 1.00µg/mL - 5.00µg/mL. The Correlation Coefficient (R^2) was found to be 0.9991 and the equation of the line was found to be **y = 43812x - 22804** as evident from the calibration curve. Thus, the data showed that the response to be linear. This clearly indicates that an excellent correlation existed between the peak area and concentration of the analyte. The calibration curve is shown in Figure 4.

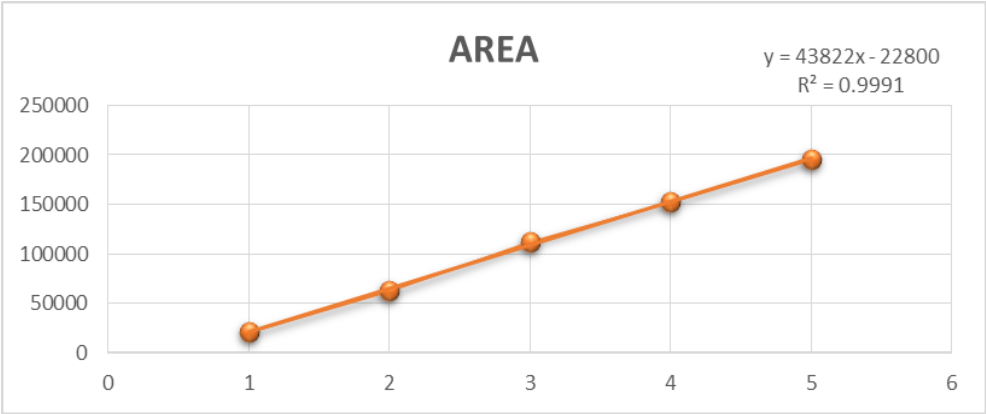

Fig 4. Calibration curve for Oxaprozin.

Table 5: Linearity Data for Oxaprozin.

Concentration (µg/ml)	Peak Area								
	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6	Mean	SD	%RSD
1	21100	21278	21217	21342	21234	21287	21243	75.46	0.35
2	62456	62886	61826	62354	62790	62124	62406	364.91	0.58
3	112464	112353	110470	111435	112178	111876	111796	682.81	0.61
4	152907	152172	152483	152565	152780	153056	152660	291.75	0.19
5	194943	195768	194978	195234	194897	195546	195227	328.13	0.16

5.4 Precision - The precision studies were carried out for the developed analytical method by injecting six replicate injections of the concentration 1.0µg/ml, 2.0µg/ml, and 3.0µg/ml (50%,

100% and 150% of the working level). Intra and Inter-day precision studies were carried out by estimating the corresponding responses for the solutions of above three concentrations levels on the same day and on a different day respectively. The % RSD values of the intra-day precision & Interday precision study were found to be 0.92% & 0.73% for oxaprozin. This is confirmed that the method developed to be Precise.

5.5 Accuracy: % Recovery study was performed using a minimum of 3 concentration levels, each in triplicate determinations. It was carried out by spiking blank concentrations of 50%, 100% and 150% of working level (1.0µg/ml, 2.0µg/ml, and 3.0µg/ml) and obtaining the percent recovery by putting the values of the areas of the peak obtained in the calibration curve to obtain the values of the concentration injected.

Results for % Recovery summarized in Table 6.

Table 6: Accuracy data of Oxaprozin.

Sr. No.	Concentration (µg/ml)	% Recovery
1	1	100.88
2	2	101.60
3	3	99.60

5.6 The Limit of Detection (LOD) and Limit of Quantification (LOQ): The Limit of Detection (LOD) and Limit of Quantification (LOQ) for the developed method were determined by progressively injecting low concentrations of the Standard solution of Oxaprozin using the developed HPLC method. This was carried out until a signal to noise ratio of NLT 3:1 and NLT 10:1 is obtained for LOD and LOQ respectively.

Stability Indicating Assay Method of Oxaprozin by HPLC

Forced degradation was performed to prove that the method is stability-indicating. Oxaprozin was degraded by subjecting to different stress conditions including. acid hydrolysis, base hydrolysis, oxidative degradation, thermal degradation and photolytic degradation to suggest forced degradation behaviour. Chromatogram of forced degradation studies are shown below.

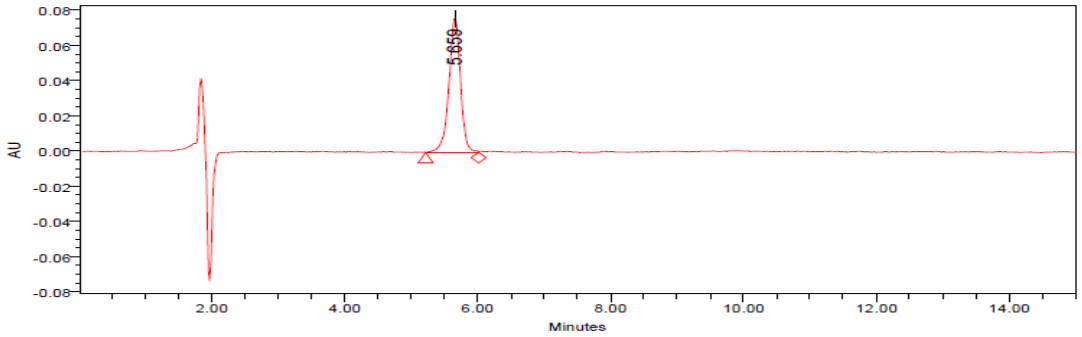

Fig. 5: Chromatogram of standard stock solution (40 µg/ml).

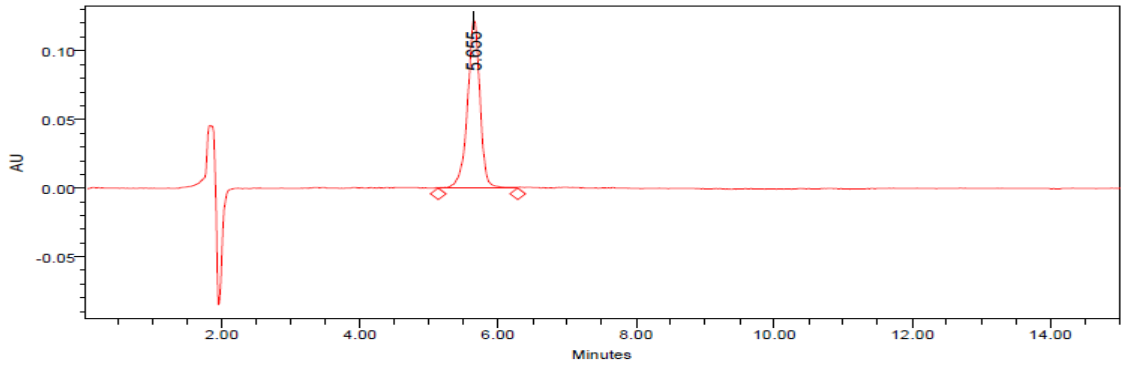

Fig 6: Chromatogram of Acid Hydrolysis after 72 hrs.

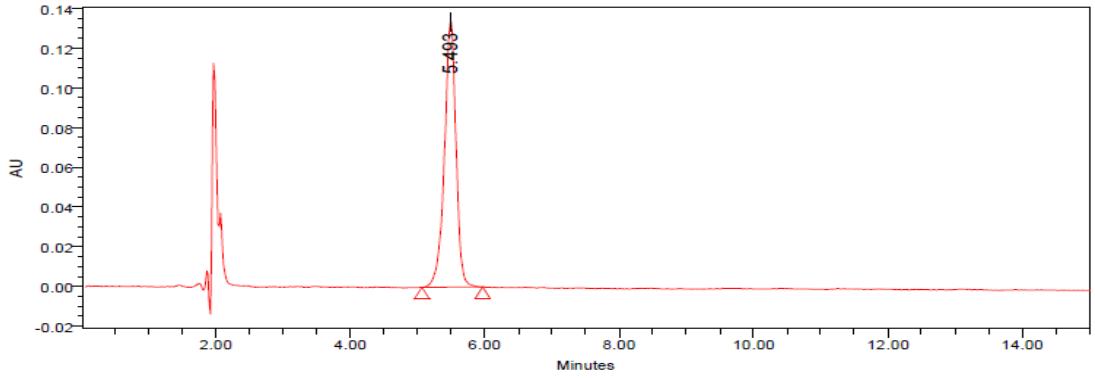

Fig 7: Chromatogram of Base Hydrolysis after 72 hrs.

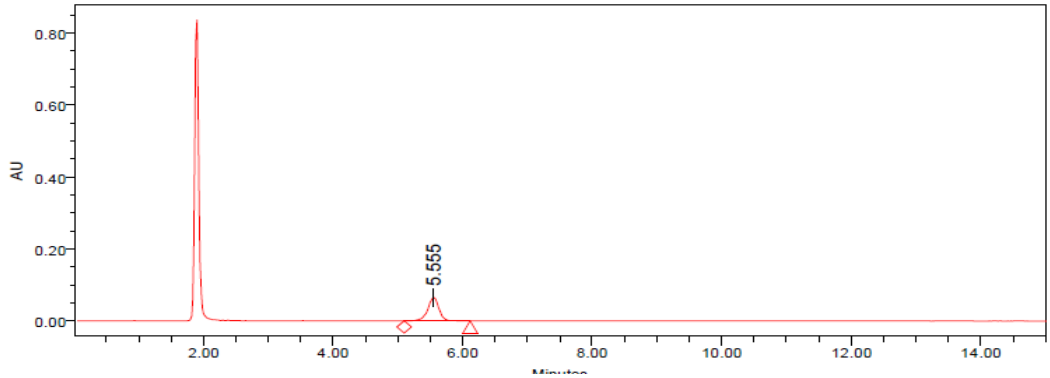

Fig 8: Chromatogram of Peroxide Degradation after 72 hrs.

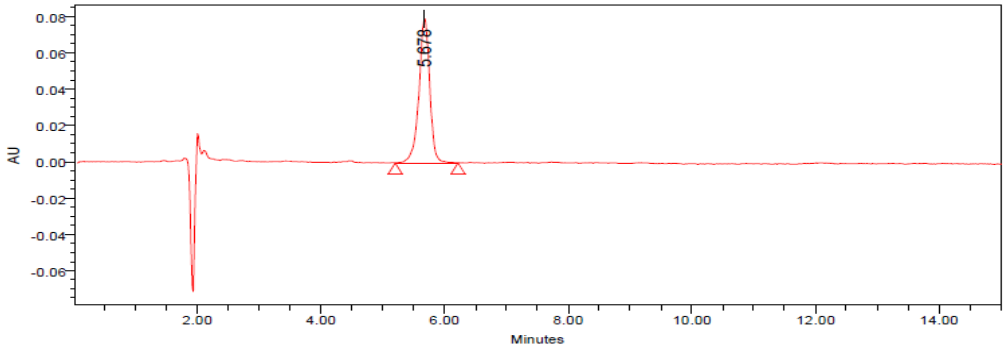

Fig 9: Chromatogram of Thermal Degradation after 72 hrs.

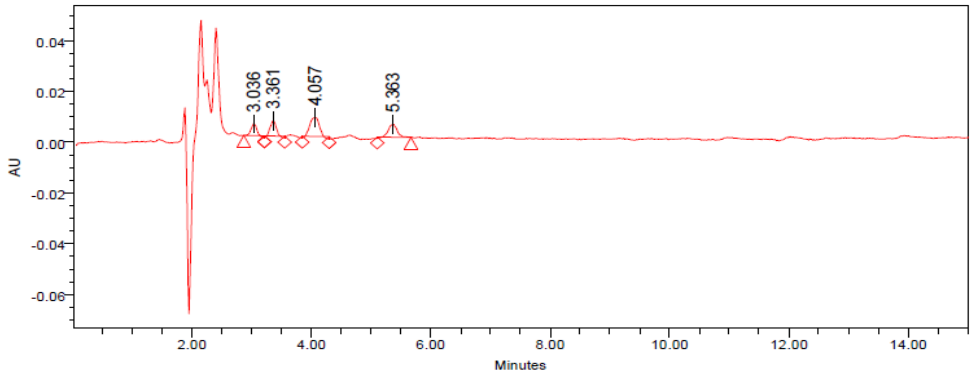

Fig 10: Chromatogram of Photolytic Degradation after 24 hrs.

Table No 7: Forced Degradation data for Oxaprozin.

Sr.No.	Degradationcondition	Retention time of degradationproducts (min)	% degradation	Concentration
1	Acid Hydrolysis	-	-	40µg/ml
2	Base Hydrolysis	-	-	40µg/ml
3	Peroxide Degradation	-	-	40µg/ml
4	Thermal Degradation	-	-	40µg/ml
5	Photolytic Degradation	3.03, 3.36, 4.07	78.12%	40µg/ml
	Total Degradation		78.12%	

CONCLUSION

The RP-HPLC assay method developed for determination of Oxaprozin is linear, accurate, precise, rapid and specific as evident from the validation results thus can be used to provide a convenient and reproducible approach for determination of Oxaprozin. The Developed method for the drug is also stability indicating and can be conveniently used for quality control to determine the assay in regular product development, production and stability samples for routine analysis of the samples.

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