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Development and Validation of Liquid Chromatographic Method for the Simultaneous Estimation of Ofloxacin and Ketorolac Tromethamine In Combined Dosage Form

V. Ramakrishna¹, P.V.V.Satyanarayana¹, B.Hari Babu^{1*}

1. Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, INDIA.

ABSTRACT

An isocratic, reversed phase-HPLC method was developed and validated for the quantitative determination of Ofloxacin and Ketorolac Tromethamine in combined-dosage form. A thermo hypersil BDS C18 (250mmx4.6mm, particle size 5 μ m) column with mobile phase containing water (p^H - 2.8 adjusted with ortho phosphoric acid) and methanol in the ratio of 60: 40, v/v was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 241 nm. The retention times of ofloxacin and ketorolac tromethamine were 4.671min and 5.751 min respectively. The correlation co-efficient values for both the ofloxacin and Ketorolac tromethamine were found to be 1. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of ofloxacin and Ketorolac tromethamine in formulations was found to be 100% confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision, the method can be successfully applied to the estimation of Ofloxacin and Ketorolac tromethamine in combined dosage form.

Keywords: RP-HPLC, Ofloxacin and Ketorolac Tromethamine.

*Corresponding Author Email: : dr.b.haribabu@gmail.com

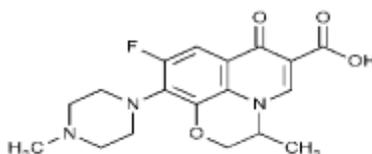
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INTRODUCTION

Ofloxacin :(\pm)

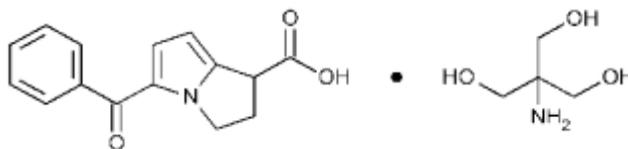
9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid is synthetic fluoroquinolone. It is broad-spectrum antimicrobial agent for oral administration which is active against both gram positive and gram negative bacteria. It functions by inhibiting DNA gyrase a type II topoisomerase and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.



Chemical structure Ofloxacin

Ketorolac tromethamine:

Ketorolac tromethamine (\pm)-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid with 2-amino-2-(hydroxymethyl)-1,3-propanediol is a non-selective cyclooxygenase (COX) inhibitor. Its anti-inflammatory, antipyretic, and analgesic effects are due to inhibition of prostaglandin synthesis by competitive blocking of the enzyme (COX). It is 800 times more potent than aspirin.



Chemical structure of Ketorolac Tromethamine

MATERIALS AND METHODS

Instrumentation:

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and thermo hypersil BDS column (250mmx4.6mm, particle size 5 μ m).

Chemicals and Reagents :

The working standards of Ofloxacin and Ketorolac Tromethamine were provided as gift samples from Dr. Reddy's Laboratories Ltd., Hyderabad. Marketed formulation of combination was purchased from local market. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions:

The mobile phase consisting of water (pH - 2.8 adjusted with orthophosphoric acid) and methanol (HPLC grade) were filtered through 0.45 μ m membrane filter, before use degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 241nm and the run time was 8min. The volume of injection loop was 10 μ l prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of the Standard Solution of Ofloxacin**Ofloxacin:**

Accurately weighed quantity, 15.00 mg of Ofloxacin was transferred into 50ml of volumetric flask and adds 30ml of water and sonicate for 15 min. Make up the volume with water. 5mls of the above solution was transferred into 50ml volumetric flask and diluted to the mark with water.

Preparation of Standard Solution of Ketorolac Tromethamine**Ketorolac Tromethamine:**

Accurately weighed quantity, 25mg of ketorolac was transferred into 50ml of volumetric flask and adds 30ml of water and sonicate for 15mins make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

Preparation of Sample Solution

Accurately measured 5mg of sample into 50ml of volumetric flask added 25ml of water and sonicated for 30mins and make up the volume with water and filtered through the 0.45 μ m membrane filter paper Transfer above solution 5ml into 50 ml volumetric flask and make up the volume with water.

RESULTS AND DISCUSSIONS**System Suitability Studies:**

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Specificity:

The specificity was established by preparing a Ofloxacin and Ketorolac tromethamine standard

at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

Table1: System Suitability Parameters

Parameters	Ofloxacin	Ketorolac Tromethamine
Correlation Coefficient	1	1
Regression Equation	$y = 9490x$	$y = 15632x$
LOD	0.106509	0.163043
LOQ	0.35503	0.543478
Theoretical plates	6770	9879
Tailing	1.559	1.394

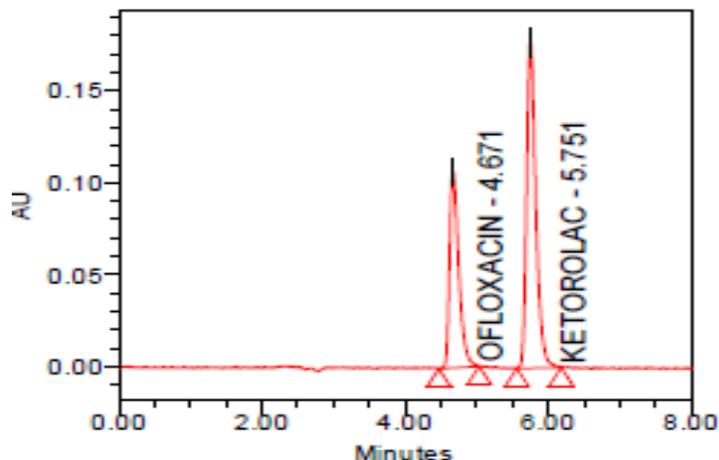


Figure. 1: Standard chromatogram for Ofloxacin and Ketorolac Tromethamine

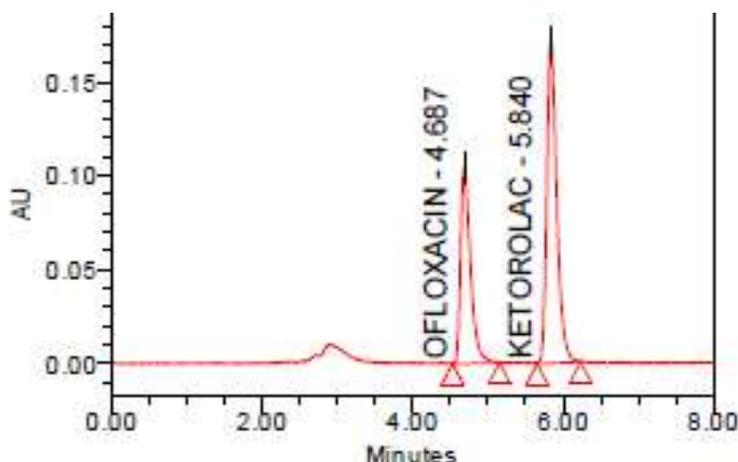


Figure. 2: Formulation chromatogram for Ofloxacin and Ketorolac Tromethamine

ACCURACY AND PRECISION

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three concentration levels. Each level was repeated six times. The percentage recovery and standard deviation of the percentage recovery were calculated. The mean recoveries of Ofloxacin and Ketorolac tromethamine were found to be 100% and 100% . The results

indicated good accuracy of the method for the determination of analysed drugs as revealed by mean recovery data (Table-3&4).

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies the assay on six test preparations were performed be injected into the chromatographic system as per the test method. The %assay of drug and percentage RSD were calculated. The inter-day variations of the method also determined by using six replicate injections of the same concentration and analyzed on three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Figure 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2a&2b).

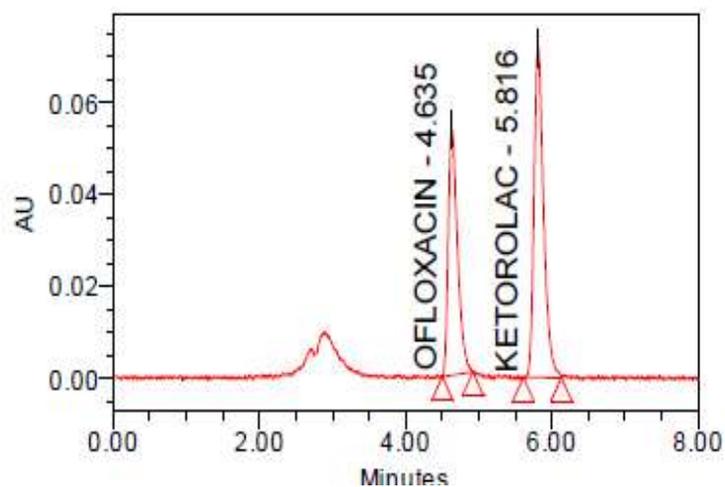


Figure. 3: Accuracy Chromatograms-50% of Ofloxacin and Ketorolac Tromethamine

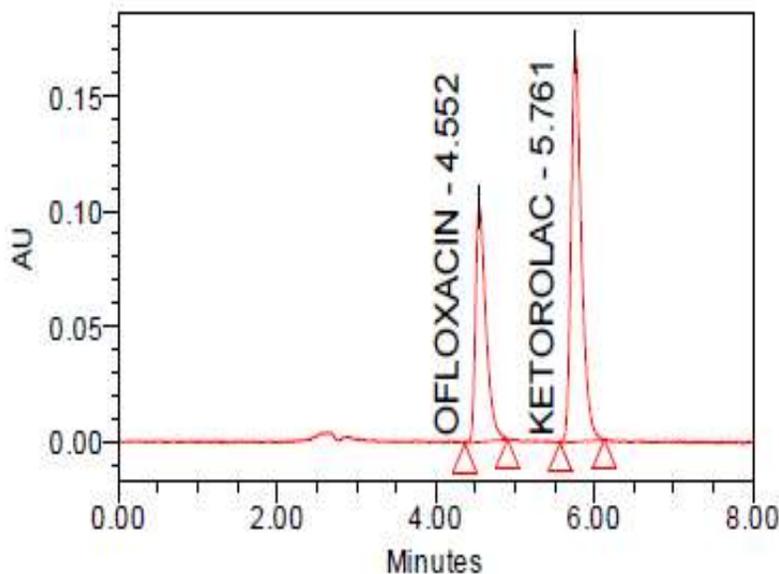


Figure. 4: Accuracy Chromatograms-100% of Ofloxacin and Ketorolac Tromethamine

Table 2a : Precision Studies(intra-day)

S.No	Sample Weight	Area (ofl)	Area (ket)	%Assay (ofl)	%Assay (ket)
1	5.00	950108	1565851	99	100
2	5.00	950502	1561607	99	100
3	5.00	950660	1561587	99	100
4	5.00	950113	1562781	99	100
5	5.00	950852	1560900	99	100
6	5.00	950992	1562500	99	100
Avg Assay:				99	100
STD				0.04	0.11
%RSD				0.04	0.11

Table 2b : Precision Studies(inter-day)

S.No	Sample Weight	Sample Area-1	Sample Area-2	% Assay	% Assay
1	5.00	954648	1546831	100	99
2	5.00	953294	1573648	99	100
3	5.00	953845	1557628	99	99
4	5.00	958735	1569264	100	100
5	5.00	956412	1562497	100	100
6	5.00	952769	1568974	99	100
Avg Assay:				100	100
STD				0.23	0.62
%RSD				0.24	0.63

Table 3: Accuracy for Ofloxacin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	2.50	475276	14.850	14.87	100	100
50%	2.50	475510	14.850	14.88	100	
50%	2.50	475664	14.850	14.88	100	
50%	2.50	475985	14.850	14.89	100	
50%	2.50	475909	14.850	14.89	100	
50%	2.50	475235	14.850	14.87	100	
100%	5.00	950756	29.700	29.75	100	100
100%	5.00	950506	29.700	29.74	100	
100%	5.00	950287	29.700	29.73	100	
150%	7.50	1428641	44.550	44.70	100	100
150%	7.50	1423783	44.550	44.55	100	
150%	7.50	1423372	44.550	44.54	100	
150%	7.50	1420167	44.550	44.44	100	
150%	7.50	1423037	44.550	44.53	100	
150%	7.50	1427953	44.550	44.68	100	

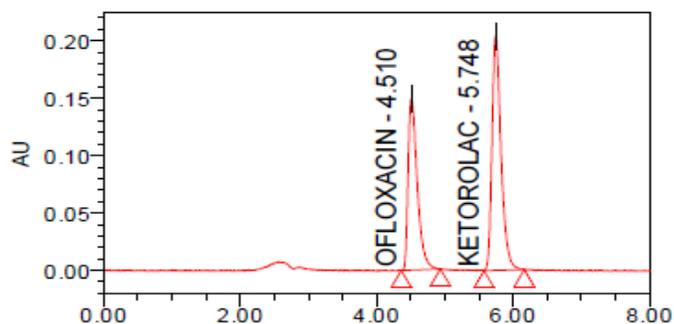


Figure. 5: Accuracy Chromatograms-150% of Ofloxacin and Ketorolac Tromethamine

Table 4: Accuracy for Ketorolac Tromethamine

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	2.50	782164	25.000	24.93	100	100
50%	2.50	782034	25.000	24.93	100	
50%	2.50	782478	25.000	24.94	100	
50%	2.50	782331	25.000	24.94	100	
50%	2.50	782483	25.000	24.94	100	
50%	2.50	783610	25.000	24.98	100	
100%	5.00	1568448	50.000	49.99	100	100
100%	5.00	1566277	50.000	49.92	100	
100%	5.00	1562528	50.000	49.80	100	
150%	7.50	2343758	75.000	74.70	100	100
150%	7.50	2346912	75.000	74.80	100	
150%	7.50	2344199	75.000	74.72	100	
150%	7.50	2340727	75.000	74.61	99	
150%	7.50	2347246	75.000	74.81	100	
150%	7.50	2344881	75.000	74.74	100	

LINEARITY AND RANGE

Chromatographic method was tested for linearity by plotting peak area against concentration of solutions. The plot of peak area versus the respective concentrations of Ofloxacin and Ketorolac tromethamine were found to be linear in the concentration range of 15-45µg/ml and 25-75µg/ml respectively. The regression equation for

Ofloxacin is $y = 9490x$ with a coefficient of correlation (R^2) of 1. The regression equation for Ketorolac tromethamine is $y = 15632x$ with a coefficient of correlation (R^2) of 1. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Ofloxacin and Ketorolac Tromethamine shows in Figure 9 and the results for calibration curves are given in Fig 7&8.

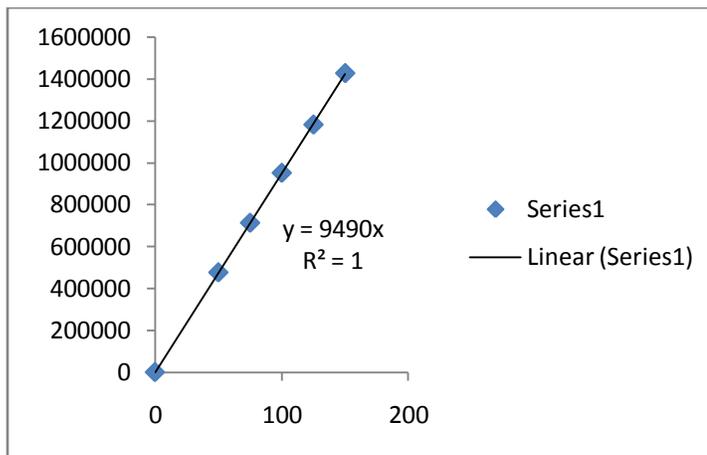


Figure. 7: Linearity Curve for Ofloxacin

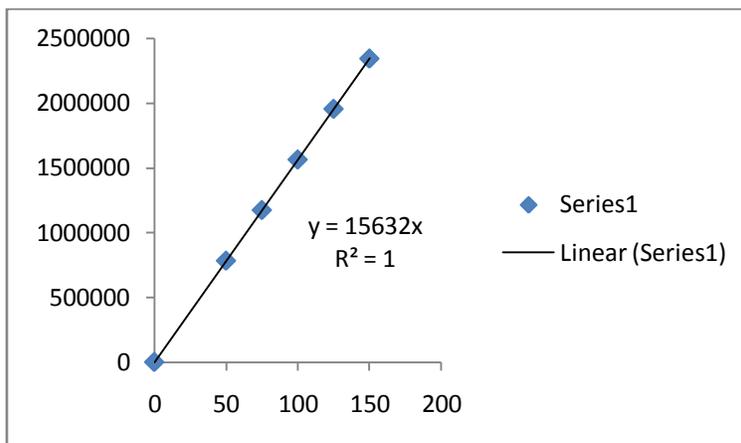


Figure. 8: Linearity Curve for Ketorolac Tromethamine

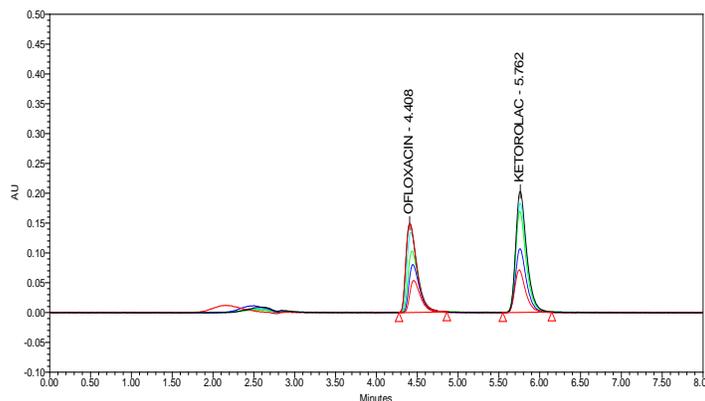


Figure.9: Overlay chromatograms of Linearity for Ofloxacin and Ketorolac Tromethamine
ROBUSTNESS

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, is rugged and robust. The results for robustness study is shown in (Table-5&6)

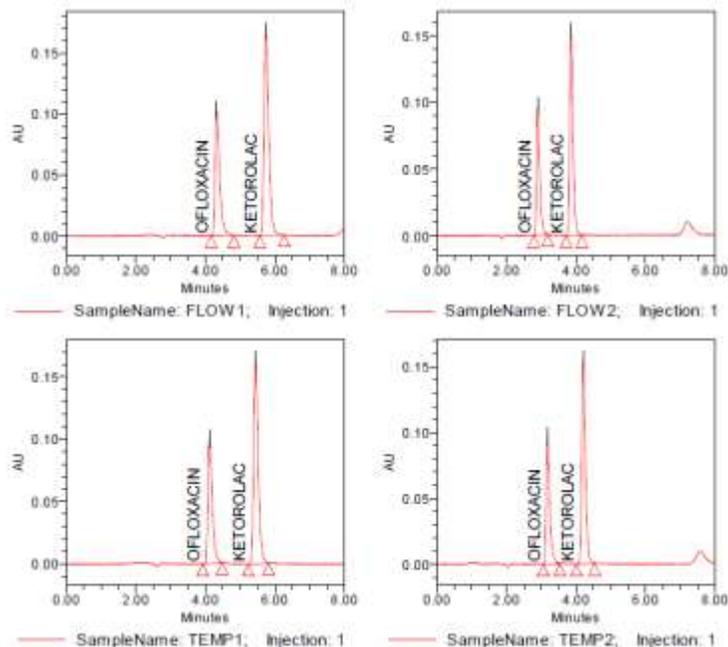


Table 5: Robustness for Ketorolac Tromethamine

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	s/n
1	TEMP2	1	KETOROLAC	4.208	1124007	5.439	1.408	8155	95.440
2	FLOW1	1	KETOROLAC	5.741	1569899	5.689	1.427	9153	181.438
3	FLOW2	1	KETOROLAC	3.854	1025809	5.463	1.422	8094	147.762
4	TEMP1	1	KETOROLAC	5.441	1474718	5.598	1.420	8903	164.811
Mean									
% RSD									

Table 6: Robustness for Ofloxacin

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	s/n
1	FLOW2	1	OFLOXACIN	2.894	615632		1.739	5134	93.505
2	TEMP2	1	OFLOXACIN	3.175	690032		1.792	4828	60.001
3	FLOW1	1	OFLOXACIN	4.322	960155		1.802	5466	111.519
4	TEMP1	1	OFLOXACIN	4.116	882252		1.731	5076	101.187
Mean									
% RSD									

System suitability results were given by Table 1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 (limit of %RSD is less than 2.0%) so we can say that system is suitable for the analysis. Method specificity was concluded by fig:1&2. In standard chromatogram and formulation chromatogram (figure: 1&2) there was no interference from placebo and excipients peaks with standard and analytic peaks. These results demonstrate the absence of interference from other materials. Hence it confirms the specificity of the proposed method. The result given in Table 2 says that the method precision passed for both Ofloxacin and Ketorolac Tromethamine studies. The

method accuracy was evaluated by recovery studies. Ofloxacin and Ketorolac Tromethamine recovery was founded 100% (as per ICH 97%- 103%) and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given above fig: 7&8. and plot the graph at three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be $y = 9490x$ for ofloxacin and $y = 15632x$ for ketorolac Tromethamine. The intra-day and inter-day variations was calculated in terms of %RSD and results was found to be intra-day and inter-day respectively. Method robustness results were given by table 5&6.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Ofloxacin and Ketorolac Tromethamine in pharmaceutical dosage forms. Hence, this method can be easily and conveniently adopted for routine quality control analysis of Ofloxacin and Ketorolac Tromethamine in pure and in its pharmaceutical dosage forms.

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