CODEN [USA]: IAJPBB **ISSN: 2349-7750**

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3346999

Available online at: http://www.iajps.com ***Research Article***

SIMULTANEOUS ESTIMATION OF KETOROLACTROMETHAMINE AND OFLOXACIN IN OPHTHALMIC PREPARATIONS BY RP-HPLC METHOD

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Article Received: May 2019 **Accepted:** June 2019 **Published:** July 2019

Abstract:
A reversed- phase High Performance Liquid Chromatography (RP-HPLC) method for the rapid and accurate quantification of Ketorolactromethamine and Ofloxacin. The detection was carried out by Shimadzu 250×4.6mm, 5µm column using mixture of buffer KH_2PO_4 (Potassium dihydrogen phosphate) Ph adjusted to (±)4.0 and Methanol (60:40) with a flow rate of 1.5ml/min. The eluents were detected at 298nm and the Retention time for Ketorolactromethamine and Ofloxacin was found to be 2.395 and 9.779min. Linear responses for Ketorolactromethamine and Ofloxacin were 5-25 µg/ml, 5-25 µg/ml with correlation factor of 0.998 and 0.998 respectively. The method was validated as per ICH guidelines for specificity, linearity, accuracy and robustness. The validated method was successfully employed for the simultaneous determination Ketorolactromethamine and Ofloxacin.

Key Words: *Ketorolactromethamine , Ofloxacin, RP-HPLC, NSAID.*

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Please cite this article in press Jahnavi Kutty K et al., ***Simultaneous Estimation Of Ketorolactromethamine And Ofloxacin In Ophthalmic Preparations By RP-HPLC Method.,*** *Indo Am. J. P. Sci, 2019; 06[07].*

INTRODUCTION:

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for cost effective estimation of Ketorolactromethamine and Ofloxacin in combination.

Ofloxacin is an anti-microbial drug and chemically it is 9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-10-(-4-methyl-1-piperizinyl)-7-oxo-7H-pyridol[1,2,3-de]-1,4-benzoxaine-6-carboxylic acid.

Ketorolactromethamine has analgesic property.chemically it is 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid,2-(hydroxyl-methyl)-1,3-propanediol.officially in USP.

Ofloxacin and ketorolactromethamine combination available in market as ophthalmic preparations only. The main objective is to develop analytical method Selecting the HPLC separation mode.Selecting/ optimizing the mobile phase.Selecting column for analysis. Selecting the appropriate detector system.Selecting appropriate gradient/ isocratic medium.Selecting appropriate flow rate, temperature.

MATERIALS AND METHODS:

Experimental: Liquid chromatographic system was manufactured by shimadzu and equipped with PDA detector. Rheodyne injector with20 µl loop volume and HPLC column- Agilent RP-C_{18} ODS (250 X 4.6mm), 5µm. Weighing was done on an Electronics Balance of Single pan balance of model AX-200-shimadzu. p^H of buffer was maintained by p^H analyzer, digital electronics model-7007.

Ofloxacin and Ketorolactromethamine was obtained from Dr.Reddy's laboratories Hplc grade water afrom fischer scientific,methanol wasobtainedfrommerk,potassium dihydrogen phosphate was obtained from ARgrades.

Mobile phase: Methanol:0.05 Mpotassium dihydrogen phosphate buffer(60:40v/v) pH was adjusted to 4.0 with orthophosphoric acid Potassium dihydrogen sulfate (6.8045g) was dissolved in hplc grade water (500mL) was added to get 0.05Msolution and ph was adjusted to (±)4.0and filtered through 0.45µmembrane filters.

Chromatographic conditions: The ideal condtions of mobile phase containing Methanol:0.05M potassium dihydrogen phosphate buffer(60:40v/v).pH was adjusted to(±)4.0with orthophosphoric acid as it resolves peaks of ketorolactromethamine and ofloxacin.the flow rate was set to 1.5ml/min uv detection of wave length at 298 nm was carried out at ambient column temperature.

PREPARATION OF STANDARD STOCK SOLUTION:

Standard stock solution: Standard stock solution was prepared by dissolving separately 10 mg of ketorolactromethamine and ofloxacin in 10 ml volumetric flask. Dissolved and diluted with methanol up to the mark to get concentration of 100 µg/ml each of ketorolactromethamine and ofloxacin.

PREPARATION OF WORKINGSTANDARD SOLUTION:

Working standard solution: Working standard solution were prepared by taking 0.5, 1.0, 1.5, 2.0, 2.5 into 10 ml volumetric flask and diluted up to the mark with the mobile phase to get 5-25µg/ml each of ketorolactromethamine and 5-25µg/ml for ofloxacin.

Chromatographic conditions: The ideal condtions of mobile phase containing Methanol:0.05M potassium dihydrogen phosphate buffer(60:40v/v). pH was adjusted to(±)4.0 with orthophosphoric acid as it resolves peaks of ketorolactromethamine and ofloxacin.the flow rate was set to 1.5ml/min uv detection was carried out at ambient column temperature

Method optimization: The chromatographic separation was performed using Agilent C_{18} (250 x 4.6 mm, 5µm) column. For selection of mobile phase, various mobile phase compositions were observed for efficient elution and good resolution. The mobile phase consisting of methanol and phosphate buffer (Potassium dihydrogen phosphate, 0.05 N) in the ratio of 60:40 (v/v) was found to be the optimum composition for efficient elution of analyte. The mobile phase was injected to the column at a flow rate 1.5ml/min for 15 min. Column temperature was maintained at 30°C. Analyte was monitored at 298 nm using UV-detector. The retention time of ketorolactromethamine and ofloxacin was eluted at 2.0min and 9.78min respectively with good resolution. Mobile phase was used as diluents during the preparation of standards and test samples.

METHOD VALIDATION:

System suitability: System suitability test should be carried out to verify the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected into the system. The system suitability parameters was evaluated from resolution, retention

times (RT) and theoretical plates (N) was evaluated for six injections of standard solution at 100 µg/ml of ketorolactromethamine and ofloxacin. The results are tabulated in the table no 1 and chromatogram was shown in figures no 3.

Linearity: The linearity of an analytical method was carried out to check its ability to elute test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of concentration ketorolactromethamine 5-25µg/ml & ofloxacin 5-25µg/ml. From the linearity studies calibration curve was plotted and concentrations were subjected to the least square regression analysis to calculated regression equation. The regression coefficient was found to be 0.995 for ketorolactromethamine and ofloxacin showing good linearity for two drugs. The results are tabulated in the table no2 and chromatograms was shown in figures no 4, 5.

Precision: The precision of an analytical method is a measure of the random error and are defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements. The results are tabulated in the table no 3 and chromatograms were shown in figures no 6.

Intra-day: Repeatability expresses the precision under the same operating condition over a short interval of time. Repeatability is also termed as Intra-assay precision. The results are tabulated in the table no 4 and chromatograms were shown in figures no 7.

Intermediate precision (Day_ Day Precision): Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all five replicate sample preparations ware determined and mean % assay value, standard deviation & % RSD was calculated. The results are tabulated in the table no 5 and chromatograms were shown in figures no 8.

Accuracy: The accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value finds. Recovery of the method was determined by spiking an amount of the pure drug (50%, 100% and 150%) at the three different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug. The results are tabulated in the table no 6,7 and chromatograms were shown in figures no 9,10 and 11.

Robustness: The robustness of an analytical method 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.Small deliberatechanges in method like Flow rate, mobile phase ratio, and wavelength are made but there were no recognized change in the result and are within range as per ICH Guide lines.Robustness conditions like Flow minus (1.2ml/min), Flow plus (1.8ml/min), mobile phase minus (55:45), mobile phase plus (60:45), Wavelength minus (290) and Wavelength plus (305) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed and all are within the limit. The results are tabulated in the table no 8,9 and chromatograms were shown in figures no 12-15.

SUMMARY:

A simple, sensitive, precise and specific Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for estimation of accurate quantification of ketorolactromethamine and related substances in liquid dosage form. The separation was performed on Shimadzu C_{18} (250×4.6mm, 5µm) chromatographic column. The mobile phase was mixture of Water and Methanol (60:40). The flow rate was 1.5mL/ min and detection was performed at 298 nm. According to guidelines, system suitability parameters constitute integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The developed method was validated according to ICH guidelines. The linear response was observed in the range of 5-25µg /ml for ketorolactromethamine and ofloxacin 5-25µg/ml ketorolactromethamine and ofloxacin related substances respectively. The proposed method had adequate specificity for estimation of ketorolactromethamine and ofloxacin and related substances in opthalmic dosage form. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98 – 102 %. System precision, method precision and intermediate precision were found to be within limits and method was found to be robust. The results of assay showed good agreement with label claim.. The method was validated statistically and was applied successfully for estimation of ketorolactromethamine and ofloxacin.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of ketorolactromethamine and ofloxacin was done by Reverse Phase HPLC. The mobile phase used consists of methanol and Potassium di hydrogen phosphate buffer in the ratio of 60:40. An SHIMADZU 250 x 4.6 mm, 5µm was used as the stationary phase. The detection was carried out using UV detector set at 298nm. The solutions are chromatographic at a constant flow rate of 1.5 ml/ min. Retention time of ketorolactromethamine and ofloxacin was found to be 2.480 min and 4.890 min. The method was developed by using RP HPLC. The results obtained are subjected to the statistical validation. The values of RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries vary with was 99.98% and 100.06% for of ketorolactromethamine and ofloxacin. The results obtained on the validation parameters met the ICH requirements. It is inferred that the method was found to be simple, specific, precise and linear. The method was found to have suitable applications in routine laboratory analysis with high degree of accuracy and precision.

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Table 1 System suitability parameters for Ketorolactromethamine and Ofloxacin.

S.no	KETOROLACTROMETHAMINE			OFLOXACIN		
Inj	RT(min)	TP	Asymmetry	RT(min)	TP	Asymmetry
1	2.400	7654	1.3	9.779	33190	1.2
2	2.395	7165	1.6	9.750	34873	1.3
3	2.393	7713	1.5	9.766	32457	1.4
4	2.396	7174	1.2	9.797	41617	1.2
5	2.397	7568	1.3	9.762	33219	1.3
6	2.395	7432	1.4	9.771	34219	1.6

Table 2 Linearity table for Ketorolactromethamine and Ofloxacin

S.no	Ketorolactromethamine		Ofloxacin	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	5	232205	5	73588
2	10	459282	10	136430
3	15	651457	15	207739
4	20	895208	20	271807
5	25	1092821	25	351568
	R^2= 0.998		R^2= 0.998	

Table 3 System precision table of Ketorolactromethamine and Ofloxacin

S.no	Ketorolactromethamine	Ofloxacin
1	457265	139766
2	448784	138207
3	445212	137888
4	456762	139232
5	442872	138125
6	452686	138289
Mean	450596.8	138584.5
S.D	5979.869	740.4428
%RSD	1.3271	0.53429

Table 4 Intra-day table of Ketorolactromethamine, Ofloxacin

S.no	Ketorolactromethamine	Ofloxacin B
1	449123	138026
2	455698	139726
3	445286	139745
4	454376	137872
5	445698	137897
6	457329	139873
Mean	451251.7	138856.5
S.D	5242.689	1015.711
%RSD	1.16181	0.731483

Table 5 Intermediate precision table of Ketorolactromethamine, Ofloxacin

S.no	Ketorolactromethamine	Ofloxacin
1	443284	135372
2	453769	139672
3	455267	139877
4	443698	137683
5	458281	136586
6	456782	138787
Mean	451846.8	137996.2
S.D	6646.386	1785.736
%RSD	1.470938	1.294047

Table 6 Accuracy table of Ketorolactromethamine

%Concentration (at specification Level)	Area			Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	559282	555523	755403.23	5.00	4.78	95.6	98.96
	546431						
	560856						
100 %	746242	744631.7		10.0	10.23	102.3	
	739214						
	748439						
150 %	961434	966055		15.0	14.86	99.0	
	970245						
	966486						

Table 7.7 Accuracy table of Ofloxacin

%Concentration (at specification Level)	Area			Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	Sample Area	Average	Standard Area				
50 %	176430	178231	1243281.3	5.0	5.025	100.5	98.43
	178240						
	180023						
100 %	251642	250509		10.0	9.56	95.6	
	249621						
	250264						
150 %	3312067	3301104		15.0	14.89	99.2	
	3299621						
	3291624						

Table 7 Robustness data for ketorolactromethamine

S.No	Parameter	Ketorolactromethamine		
		RT (min)	Theoretical plate count	Resolution
1	Standard	2.403	6448	0.00000
2	Change in organic phase ratio(-)55:45	2.400	6444	0.00000
3	Change in organic phase ratio(+)60:45	2.403	6448	0.00000
4	Change in flow rate(-) 1.2ml/ min	2.402	6753	0.00000
5	Change in flow rate(+)1.8ml/ min	2.404	6929	0.00000
6	Change in wavelength (-)290	2.404	6824	0.00000
7	Change in wavelength (+)305	2.396	6664	0.00000

Table 8 Robustness data for Ofloxacin

S.No	Parameter	Ofloxacin		
		RT (min)	Theoretical plate count	Resolution
1	Standard	10.232	17444	15.068
2	Change in organic phase ratio(-)55:45	9.779	33190	17.616
3	Change in organic phase ratio(+)60:45	10.015	17444	13.938
4	Change in flow rate(-) 1.2ml/ min	10.012	19393	14.572
5	Change in flow rate(+)1.8ml/ min	10.015	19760	14.721
6	Change in wavelength(-)290	9.980	19830	14.706
7	Change in wavelength (+)305	9.797	18353	14.352

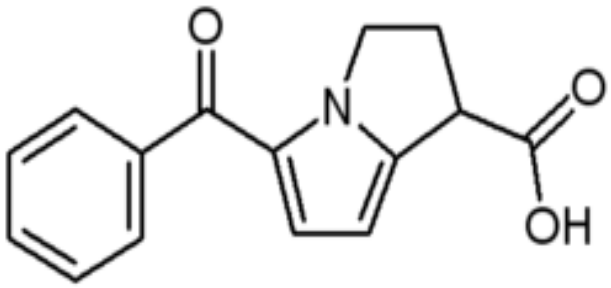


Fig 1 structure of Ketorolactromethamine

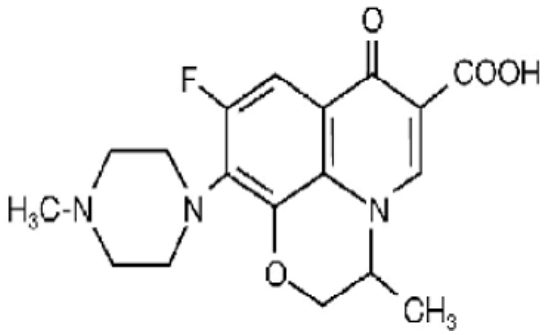


Fig 2 structure of Ofloxacin

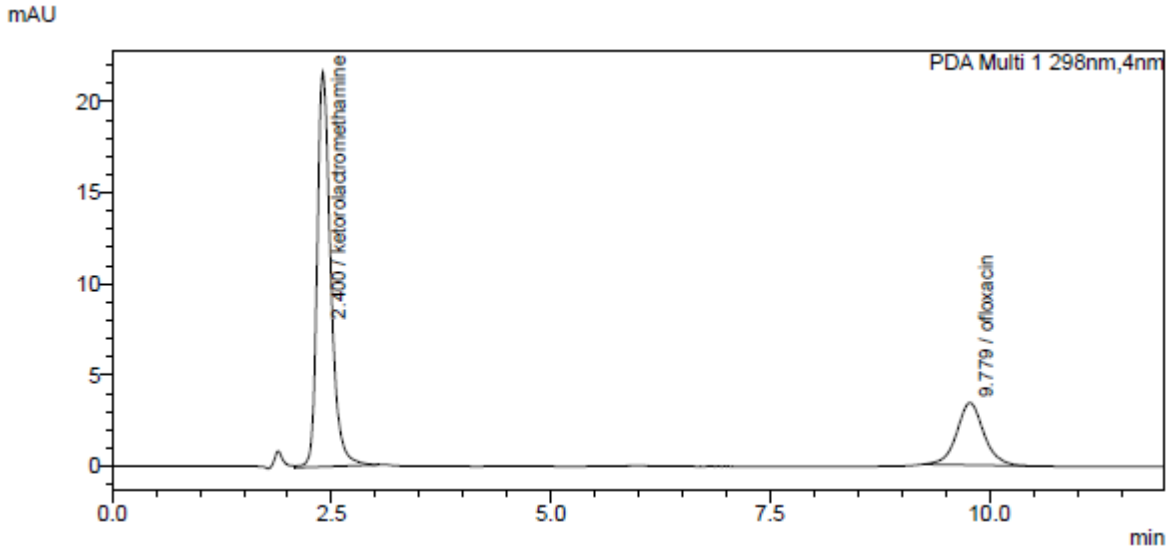


Fig 3 System Suitability Chromatogram

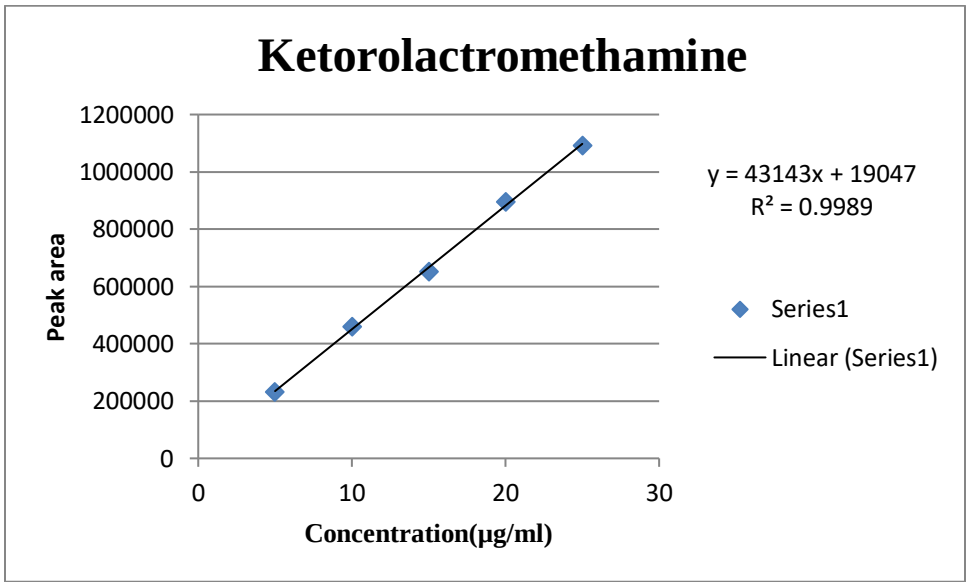


Fig 4Calibration curve of Ketorolactromethamine

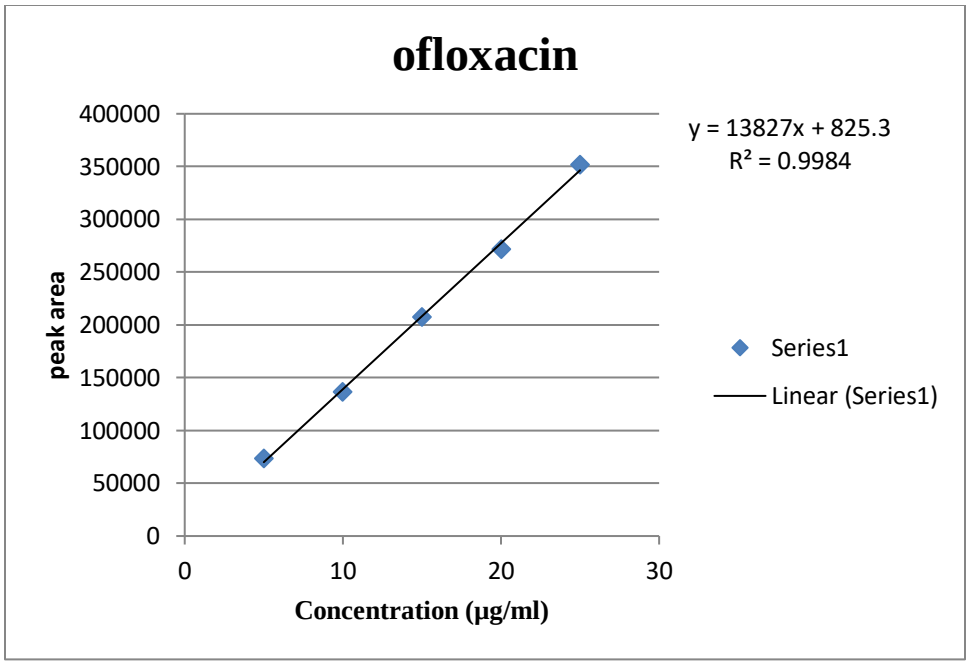


Fig 5 Calibration curve of Ofloxacin

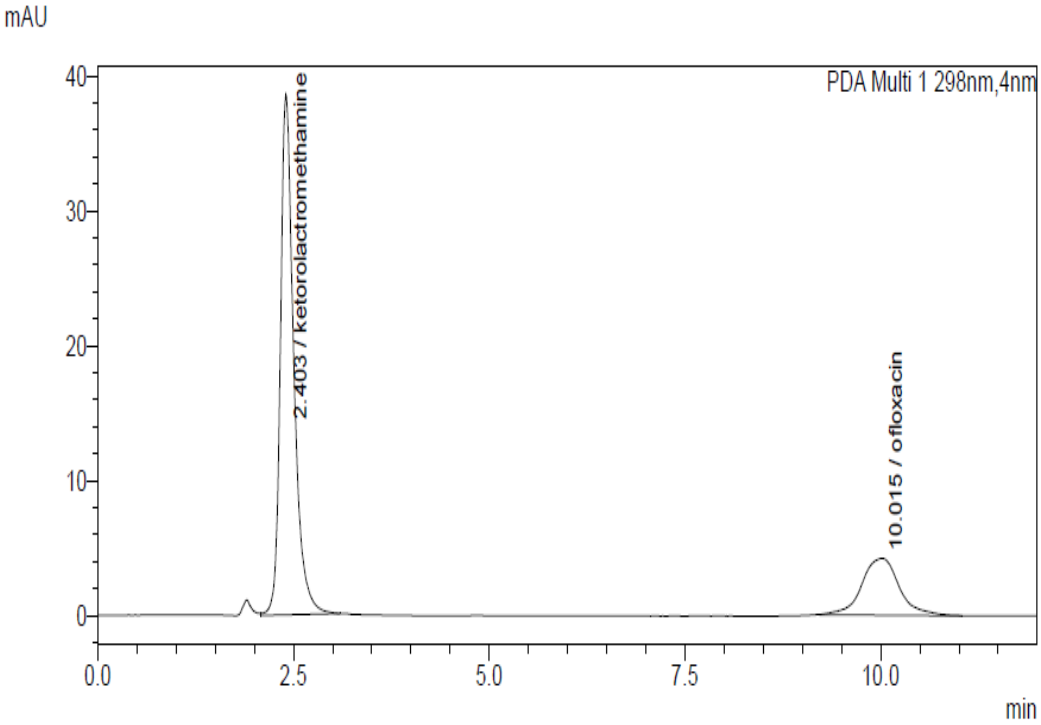


Fig.6 System precision chromatogram injection

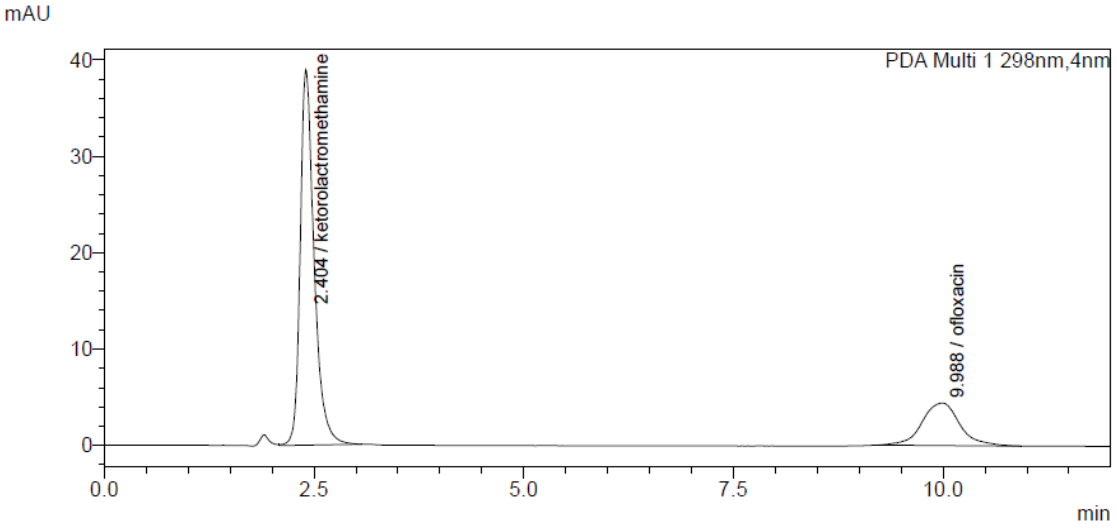


Fig.6 Intra-day chromatogram injection

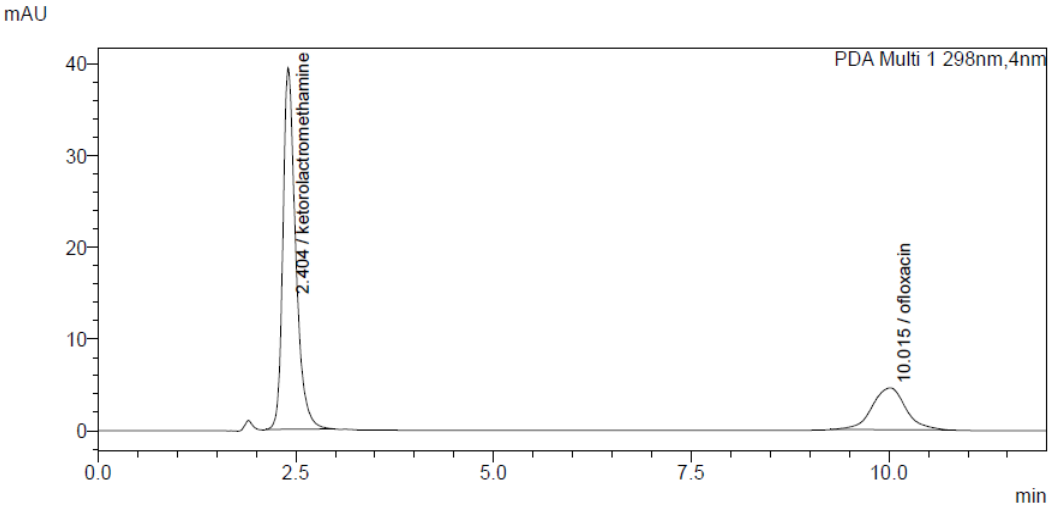


Fig.7Intermediate precision chromatogram injection

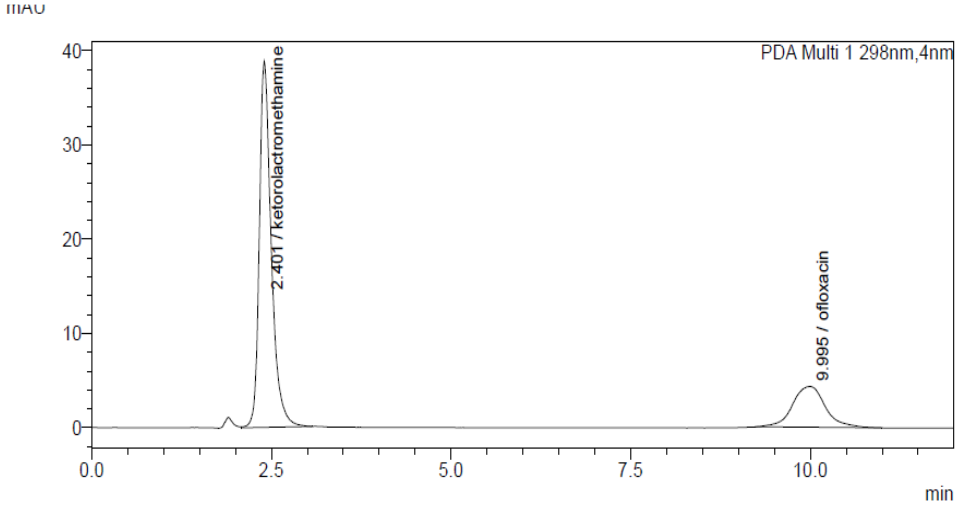
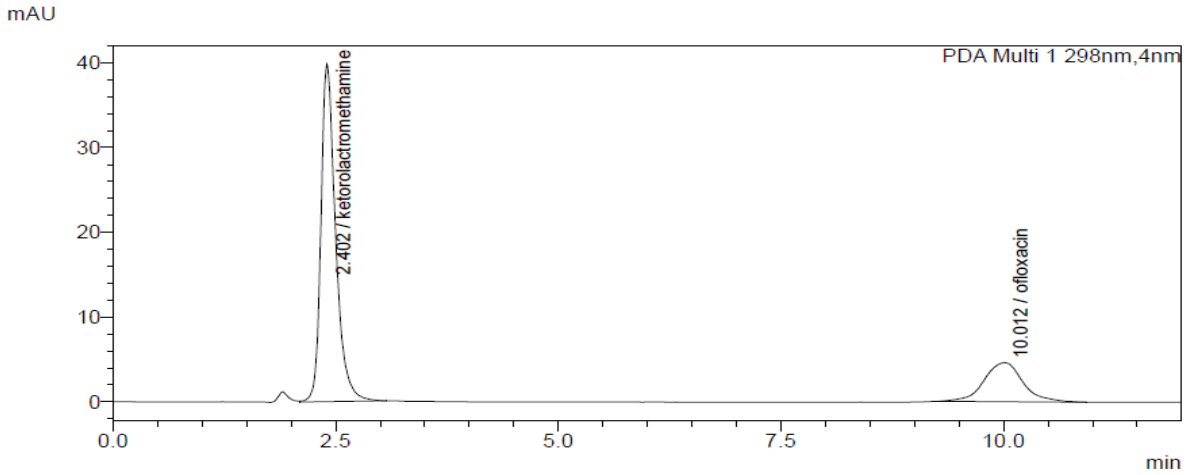


Fig 8 Accuracy 50% chromatogram



<Peak Table>

Fig 9Accuracy 100% chromatogram

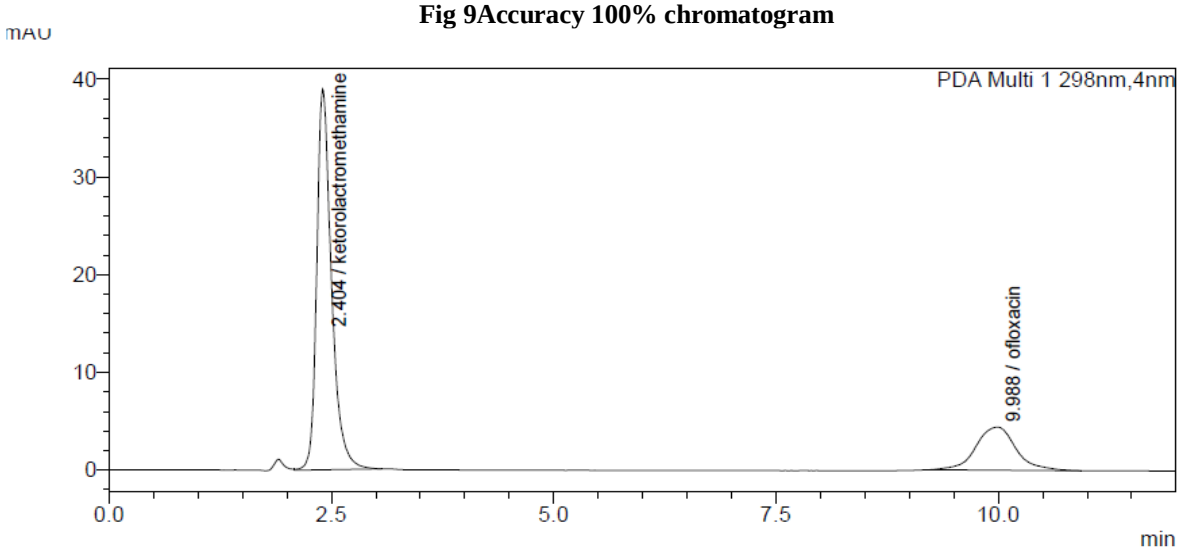


Fig 10 Accuracy 150% chromatogram

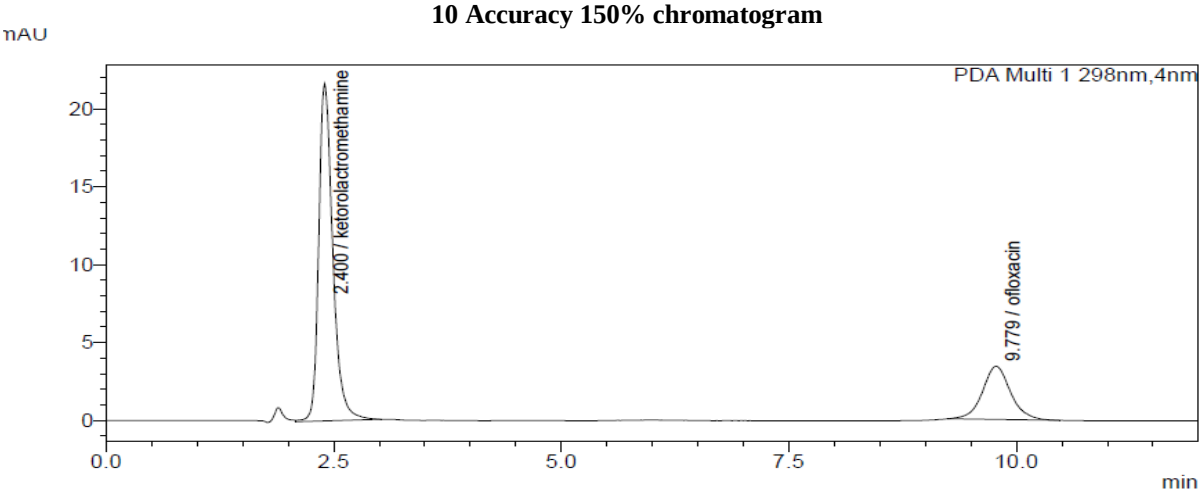


Fig 11Mobile phase minus chromatogram injection

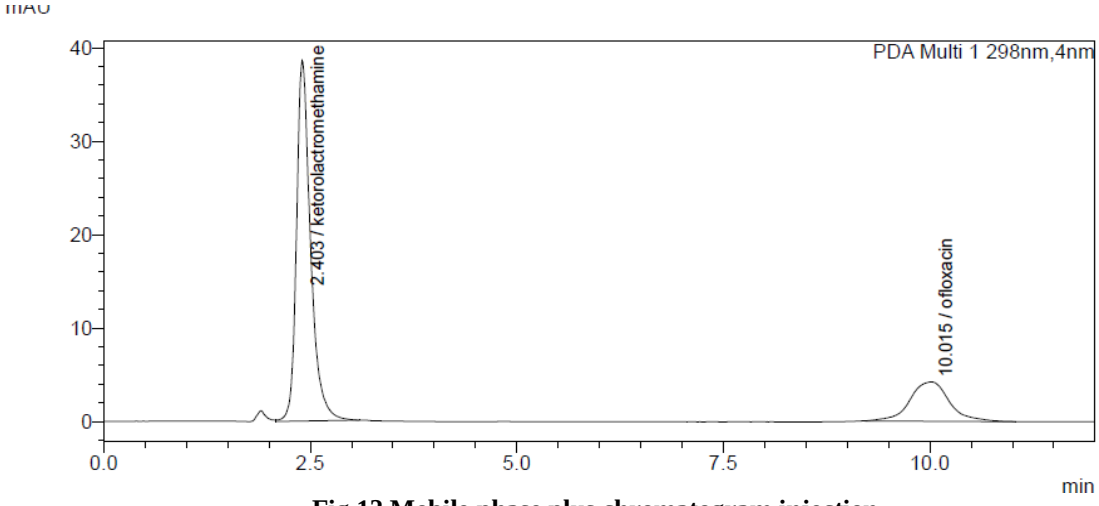


Fig 12 Mobile phase plus chromatogram injection

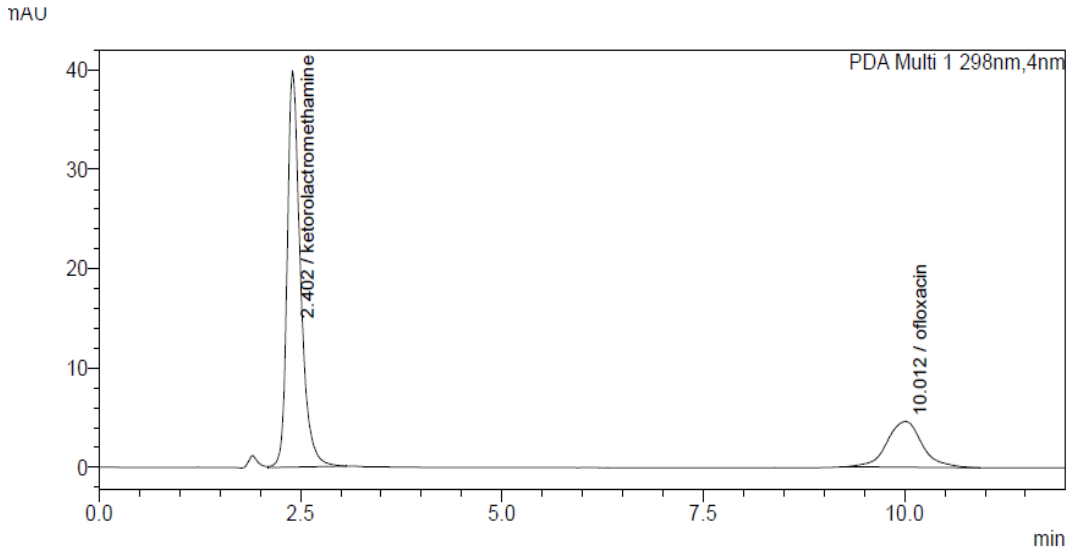


Fig 13 Flow minus chromatogram injection

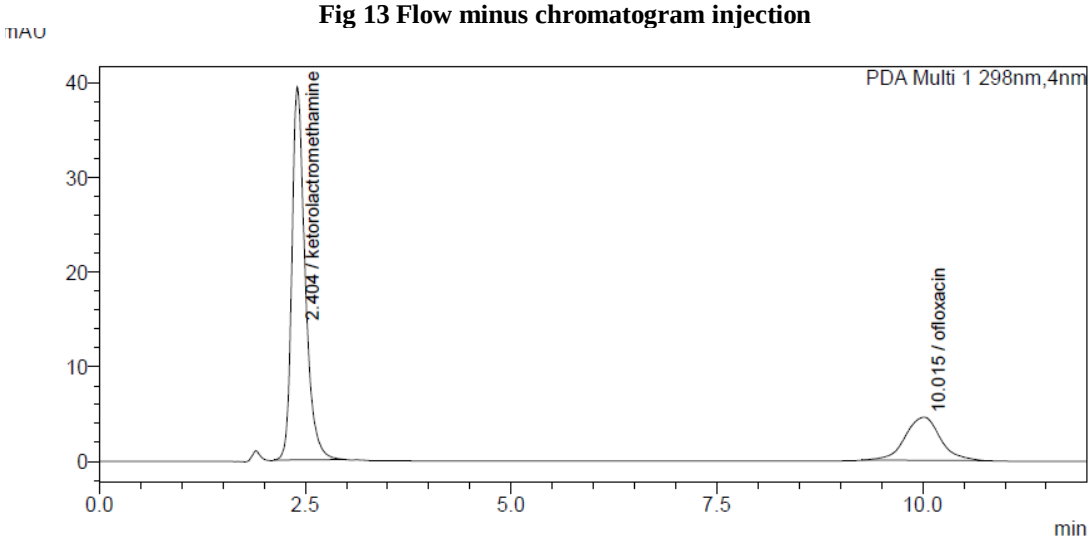


Fig 14Flow plus chromatogram injection

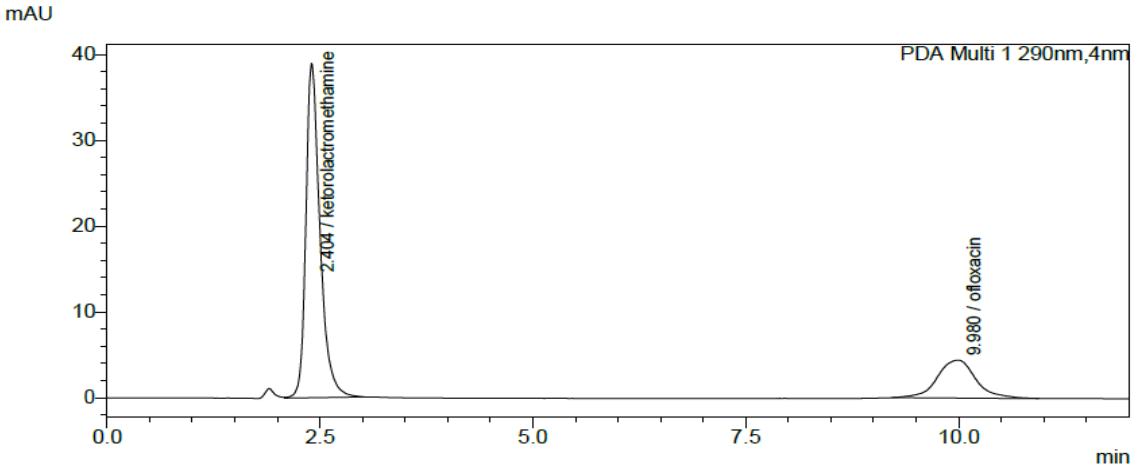


Fig 7.23Wavelength minus chromatogram injection

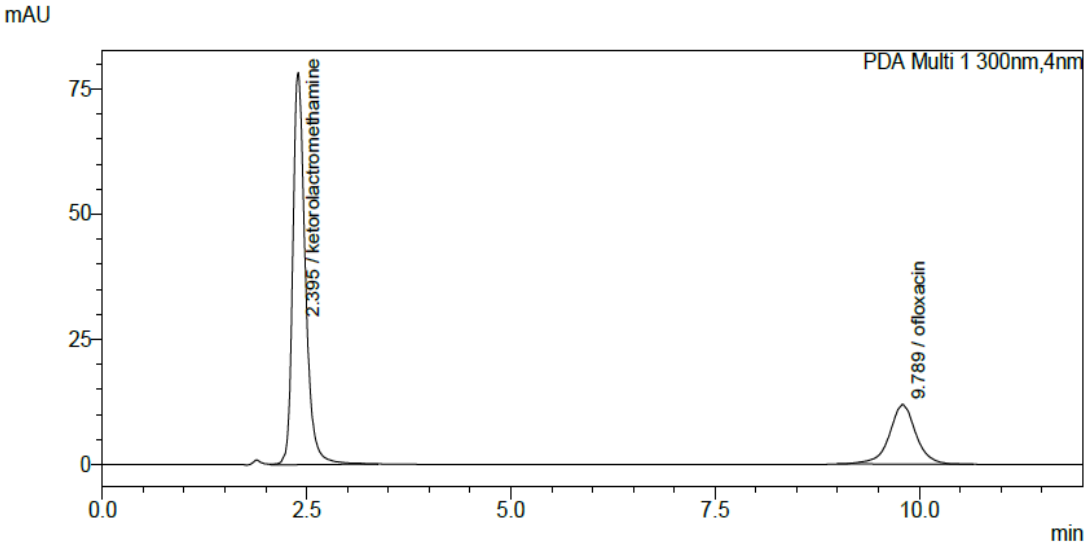


Fig 15 Wavelength plus chromatogram injection