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Development and validation of a liquid chromatographic method for the simultaneous estimation of Moxifloxacin and Keterolac in opthalmic dosage form

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ABSTRACT

*A novel RP-HPLC method has been developed and validated for the estimation of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in opthalmic dosage form. The elution followed an isocratic mode in an INERTSIL ODS column (250*4.6mm), with mobile phase KH2PO4 buffer (pH 3.5): Methanol: Acetonitrile in the ratio of 40:40:20 at a flow rate of 1.0ml / min and the effluents were monitored at 306 nm. The retention times for Moxifloxacin and Keterolac Tromethamine were found to be 2.440 and 5.503 respectively. The proposed method was validated for system suitability, linearity, precision, accuracy specificity, robustness, LOD and LOQ as per ICH guidelines. The method was found linear within the range of 60-140 µg/ml for Moxifloxacin and 48-112 µg/ml for Keterolac Tromethamine with correlation coefficient 0.999 and 0.998 respectively. Percentage recoveries of Ketorolac and Moxifloxacin were found to be in the range of 99.83% - 99.91% and 99.74% - 99.65% respectively. The LOD and LOQ of Ketorolac and Moxifloxacin were 1.73 µg/ml & 5.25 µg/ml and 1.63 µg/ml, 4.96 µg/ml respectively Proposed method can be successfully applied for the quantitative determination of Ketorolac and Moxifloxacin in Bulk drug and Pharmaceutical dosage form.*

Key words: Moxifloxacin Hydrochloride, Ketorolac Tromethamine, RP-HPLC.

INTRODUCTION

Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent developed by Bayer AG (initially called BAY 12-8039). Its IUPAC name is 7-[(4aS,7aS)-octahydro-1H-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. It is soluble in water, ethanol and DMSO. It is marketed worldwide (as the hydrochloride) under the brand names Avelox, Avalox, and Avelon for oral treatment. In most countries, the drug is also available in parenteral form for intravenous infusion[1,2]. Moxifloxacin is also sold in an ophthalmic solution (eye drops) under the brand names Vigamox, and Moxeza for the treatment of conjunctivitis (pink eye). It differs from earlier antibacterials of the fluoroquinolone class such as levofloxacin and ciprofloxacin in having greater activity against Gram-(+) bacteria and anaerobes. Because of its potent activity against the common respiratory pathogen Streptococcus pneumoniae, it is considered a "respiratory quinolone." In 1999 Avelox was approved by the U.S. Food and Drug Administration (FDA) for use in the United States [3].

Ketorolac or ketorolac tromethamine is a non-steroidal anti-inflammatory drug (NSAID) from the family of heterocyclic acetic acid derivatives, and is also used as an analgesic. Ketorolac was developed in 1989 by Syntex Corp. It was approved by FDA on 30 November 1989 and introduced as*Toradol* by Syntex. The ophthalmic form was approved by FDA on 9 November 1992 and was introduced as *Acular*eye drops by Allergan under license from Syntex. Ketorolac in its oral and intramuscular preparations present as a racemic mixture of both (*S*)-(−)-ketorolac, the active isomer, and (*R*)-(+)-ketorolac [4,5].

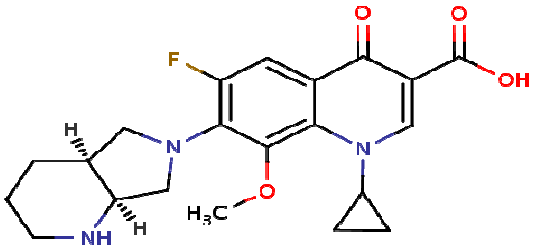

Fig no: 1- Structure of Moxifloxacin

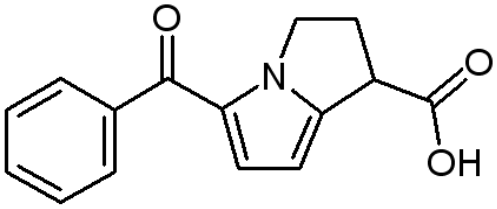

Fig no: 2- Structure of Keterolac

Review of literature revealed a few RP-HPLC , HPTLC and UV-Spectrophotometric methods have been reported for the determination of Moxifloxacin and Keterolac in bulk and pharmaceutical dosage forms individually and also in combination with other drugs[1-12]. But there a very few methods reported for simultaneous quantification of Moxifloaxacin and Keterolac in combined pharmaceutical dosage forms [13,14]. Hence the present work is focused on the development and validation of an analytical method using RP-HPLC for simultaneous estimation of Moxifloxacin and Keterolac that potentially creates an affordable method which can be adopted for routine quality control analysis.

MATERIALS AND METHODS

Instrumentation:
The present work was executed on Shimadzu HPLC system with absorbance detector using Phenomenex column (250mm, 4.6mm, 5µm) provided with a binary pump facility. The signal output was recorded and interpreted through LC-solutions software. The elution was carried out through an INERTSIL ODS column (250*4.6mm**).**

Chemicals and Reagents:
Moxifloxacin and Keterolac working standards were procured from Divi's labs as gift samples. HPLC grade water, Methanol (HPLC grade), Sodium Dihydrogen phosphate of AR grade were procured from MERCK (India) Ltd, Ortho phosphoric acid (HPLC grade) was procured from Emplura, Hydrochloric acid, Sodium Hydroxide were procured from SDFCL and Hydrogen peroxide was procured from Qualigens.

Determination of Detection Wavelength:
A solution of 100 µg/ml of Ketorolac Tromethamine and Moxifloxacin HCl were prepared in methanol. The resulting solutions were scanned individually in UV-Visible spectrophotometer. The optimal response for both of them was obtained at 306 nm. Hence 306 nm was selected as detection wavelength. The UV spectrums were summarized in **fig no, 4 & 5.**

Preparation of standard solution:
50 mg of Moxifloxacin HCl and 40mg of Ketorolac Tromethamine were accurately weighed and transferred into a 50 ml clean dry volumetric flask, about 10 ml of diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml of Moxifloxacin HCl and 800 µg/ml of Ketorolac Tromethamine (Stock solution).From this 1mL of the solution was transferred to 10 mL Volumetric flask and diluted to volume with diluents. (This solution contains a concentration of 100 µg/ml of Moxifloxacin HCl and 80 µg/ml of Ketorolac Tromethamine)

Preparation of sample solution:
5mL of the solution was transferred from the vial into a 50 ml clean dry volumetric flask, about 10 ml of diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent to give a concentration of 100 µg/ml of Moxifloxacin HCl and 40 µg/ml of Ketorolac Tromethamine.

Chromatographic conditions:
A reverse phase HPLC with INERTSIL ODS column (250*4.6mm**)** was used for elution at ambient temperature. The mobile phase was pumped through the column at a flow rate of 1ml/min. The sample injection volume was 20µl.The detector was set to wavelength of 306 nm and chromatographic run time was set to 10 minutes.

Method development
The method development was started with initial chromatographic conditions as stated above. The first trial was initiated with composition of KH2PO4 Buffer (pH 3.0): ACN (40: 60). In the above trial the peak symmetry of both drugs and efficiency was not good. Trial was repeated with change in mobile phase i.e. methanol: phosphate buffer (60:40), separation was good but base noise is more. Another trial was initiated with change in mobile phase i.e. Phosphate buffer pH 4.5: ACN (40: 60 v/v) but the same problem persists. In order to avoid these shortcomings the mobile phase composition was changed to KH2PO4 pH 3.5: MeOH: ACN (40:20:40 v/v/v), but this ended in poor resolution. Another trial was initiated with Phosphate Buffer: ACN: Methanol (40:30:30 v/v/v) where the system suitability parameters did not meet the acceptance criteria. Finally the method was optimized with KH2PO4: Methanol: ACN (40:40:20 v/v/v). The analytes were eluted with good resolution and the chromatogram has passed all the system suitability parameters. The retention times for Moxifloxacin & Keterolac were found to be 2.440 and 5.503 respectively. The chromatogram is shown in the **figure-3** and the chromatogram characteristics are given in **table-1.**

Method Validation:
The proposed method for the simultaneous estimation of Moxifloxacin and Keterolac Tromethamine in ophthalmic preparation was validated as per ICH guidelines by the following parameters.

System suitability:
Sample solutions of Moxifloxacin and Keterolac Tromethamine were injected in replicates as per the procedure. From the standard chromatogram system suitability parameters like retention times, tailing factor, theoretical plates and peak areas are evaluated through % RSD. The results are summarized in the table **-2.**

Linearity:
50 mg of Moxifloxacin HCl and 40mg of Ketorolac Tromethamine were accurately weighed and transferred into a 50 ml clean dry volumetric flask, about 10 ml of diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent to give a concentration of 500 µg/ml of Moxifloxacin HCl and 400 µg/ml of Ketorolac Tromethamine. From this various concentrations equivalent to 60-140 µg/ml for Moxifloxacin and 48-112 µg/ml for Keterolac Tromethamine were prepared and injected in replicates. A graph of peak area vs. concentration was plotted and the correlation coefficient was calculated and was represented in **fig-4&5**. The results were summarized in the **tables -3.**

Precision:
Precision was evaluated by injecting the sample solutions at 100 µg/ml of Moxifloxacin and 80 µg/ml of Keterolac Tromethamine. The results were summarized in the **tables-4.**

Accuracy:
The accuracy of the proposed method was evaluated in triplicates by recovery studies at various concentrations of Moxifloxacin and Keterolac Tromethamine equivalent to 80,100 &120%. The percentage recovery values were calculated and reported in the **table-5.**

Specificity:
It is ability of a method to measure the analyte of interest specifically in presence of matrix and other components. Solutions of blank, sample and standard were injected as per the test procedure. The chromatograms were represented as **figures-6,7 & 8.**

Limit of Detection and Limit of Quantification:
The detection limit of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated.

Limit of Quantification is the lowest concentration of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy.

LOD = 3.3 σ / S , LOQ = 10 σ / S

σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves

The results were summarized in **table-6**.

Robustness:
The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition , detection wavelength etc .The parameter was determined by differing physical flow rate and detection wavelength .

Effect of variation of flow rate:
The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 100 ppm of Moxifloxacin HCl &80 ppm of Ketorolac Tromethamine was prepared and analysed using the varied flow rates along with method flow rate.The results are summarized

Effect of variation of organic phase composition:
Standard solution 100 ppm of Moxifloxacin HCl &80 ppm of Ketorolac Tromethamine was prepared and analysed using the varied wavelength at 304 nm and 308 nm.

The results were summarized in **table-7.**

RESULTS AND DISCUSSION

The proposed method for the simultaneous estimation of Moxifloxacin and Keterolac was optimized by a series of trials. At each trial the mobile phase composition was changed to improve the fineness of the chromatogram. Finally the method was optimized with the mobile phase Phosphate Buffer: ACN: Methanol (40:30:30 v/v/v). The retention times of Moxifloxacin and Keterolac were found to be 2.440 and 5.503 respectively. The chromatogram has fulfilled the system suitability parameters.

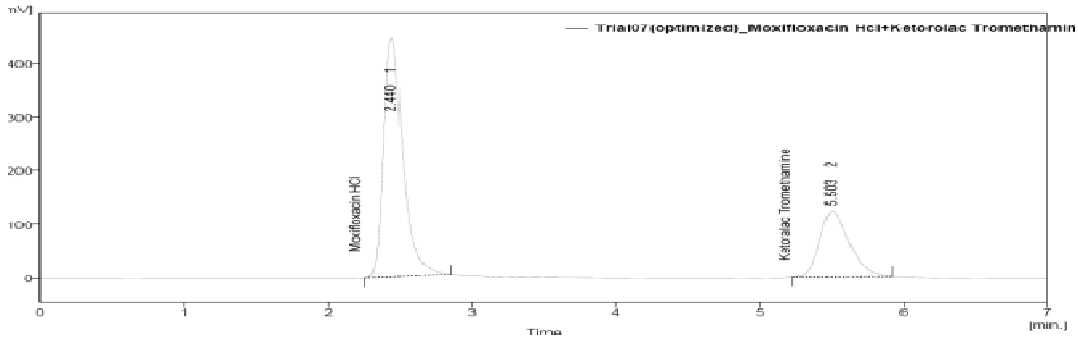

Fig no:3 - Chromatogram of Optimized trial

Table no: 1- Chromatogram characteristics of Moxifloxacin and Ketorolac Tromethamine

S. No.	Name	Rt (min)	Peak Area	Asymmetry	Efficiency	Resolution
1	Moxifloxacin	2.440	4112666	1.700	2683	-
2	Keterolac	5.503	1721694	1.324	3467	10.014

System suitability:
For five replicate injections system suitability parameters like number of theoretical plates, USP Tailing and % RSD were found to be within specified limits

Table no: 2- System Suitability data of Moxifloxacin and Ketorolac Tromethamine

	Moxifloxacin				Keterolac Tromethamine			
Injection	**RT**	**Area**	**USP Plate Count**	**USP Tailing**	**RT**	**Area**	**USP Plate Count**	**USP Tailing**
1	3.793	6287002	2061	1.829	5.597	4301232	3813	1.957
2	3.777	6253429	2114	1.722	5.583	4300460	3795	1.957
3	3.767	6293425	2901	1.806	5.547	4284740	3417	2.000
4	3.793	6287002	2061	1.829	5.597	4301232	3813	1.957
5	3.803	6283523	2938	1.806	5.607	4318300	3492	1.958
Mean	3.7866	6280876	2415	1.7984	5.5862	4301193	3666	1.965
S.D		15.75498				11.87319		
%RSD		0.25084				0.276044		

Linearity:
The linearity of the method was determined by five replicate injections at 60-140% & 48 -112% concentration levels of Moxifloxacin and Keterolac respectively. Linearity of detector response was established by plotting graph between concentrations versus average area counts of the analytes.The results are as follows:

Table no: 3- Linearity data of Moxifloxacin and Keterolac

	Keterolac Tromethamine		Moxifloxacin	
S. No.	**Concentration**	**Area**	**Concentration**	**Area**
1	48	2722955	60	3918028
2	64	3565002	80	5198951
3	80	4326582	100	6467322
4	96	510644	120	7797049
5	112	5798935	140	8993952
Correlation Coefficient		0.9989		0.9998

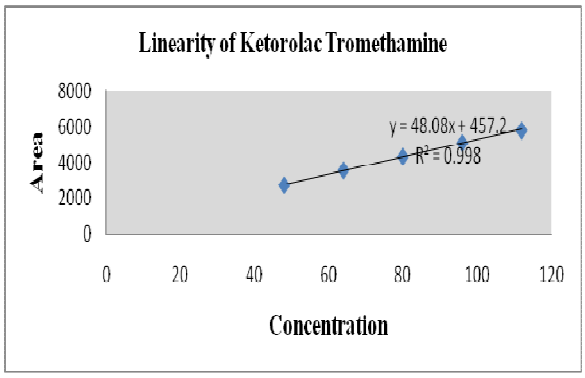

Fig no: 4-Calibration curve of Keterolac

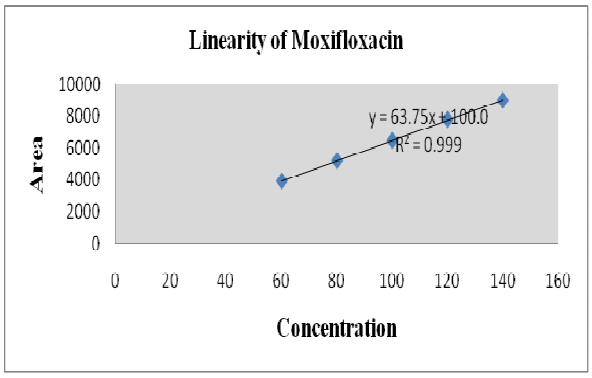

Fig no: 5-Calibration curve of Moxifloxacin

Table no: 4-Precision data of Moxifloxacin and Keterolac

Ketorolac Tromethamine			Moxifloxacin	
Injection	**Rt**	**Area**	**Rt**	**Area**
1	5.467	4246118	3.723	6213.644
2	5.517	4233389	3.777	6167.919
3	5.527	4248341	3.777	6199.594
4	5.477	4302932	3.760	6233.710
5	5.473	4226850	3.763	6177.762
6	5.413	4215588	3.740	6128.529
Avg	5.479	4245536	3.7567	6186.860
S D		30.646		37.198
%RSD		**0.72**		**0.60**

Precision:
The standard solution was injected for six times and the area for all six injections was measured in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:
Accuracy was performed by recovery studies in triplicate for various concentrations of Ketorolac Tromethamine and Moxifloxacin HCl equivalent to 80, 100, and 120 %. The recovery at each level was determined and reported.

Table no: 5-Accuracy data of Moxifloxacin and Keterolac Tromethamine

Level	Amount Added	Amount recovered	% recovery	mean recovery	Amount Added	Amount recovered	% recovery	mean recovery
80	64+16	79.80	99.75	99.82	80+20	99.08	99.08	99.74
	64+16	80.01	100.01		80+20	100.18	100.18	
	64+16	79.78	99.72		80+20	99.96	99.96	
100	80+16	95.86	99.85	99.94	100+20	120.06	100.05	99.83
	80+16	96.01	100.01		100+20	119.90	99.91	
	80+16	95.98	99.97		100+20	119.45	99.54	
120	96+16	111.69	99.72	99.91	120+20	138.98	99.27	99.65
	96+16	112.01	100.00		120+20	139.76	99.82	
	96+16	112.04	100.02		120+20	139.84	99.88	

Specificity:
Solutions of blank Standard and Sample were prepared as per test procedure and injected into the HPLC system and the Chromatograms were recorded.

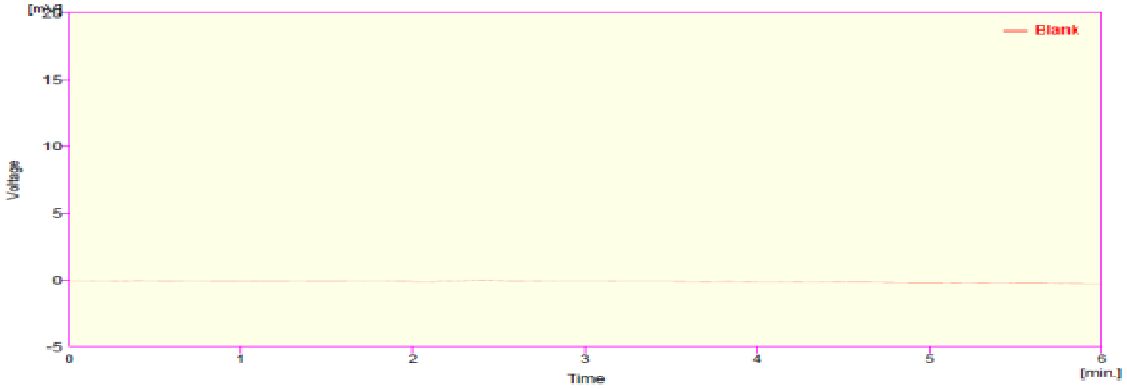

Fig no: 6- Chromatogram of Blank

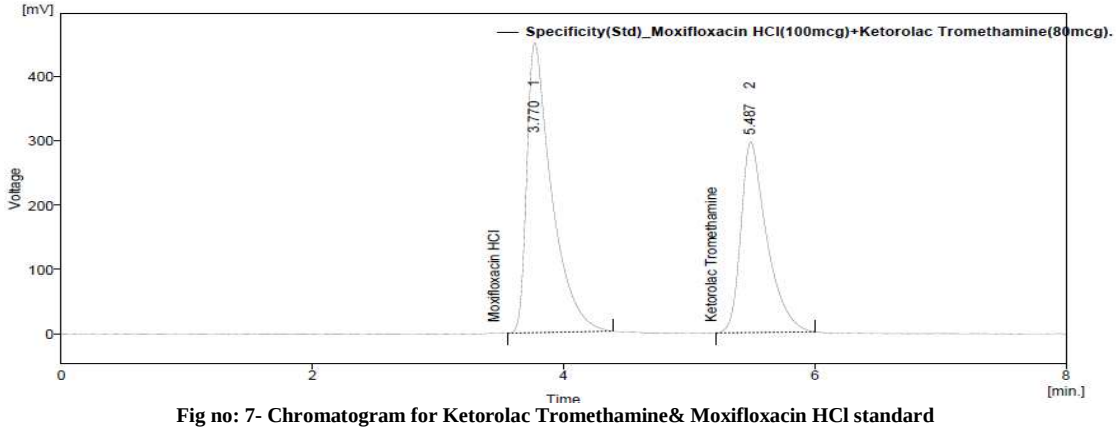

Fig no: 7- Chromatogram for Ketorolac Tromethamine& Moxifloxacin HCl standard

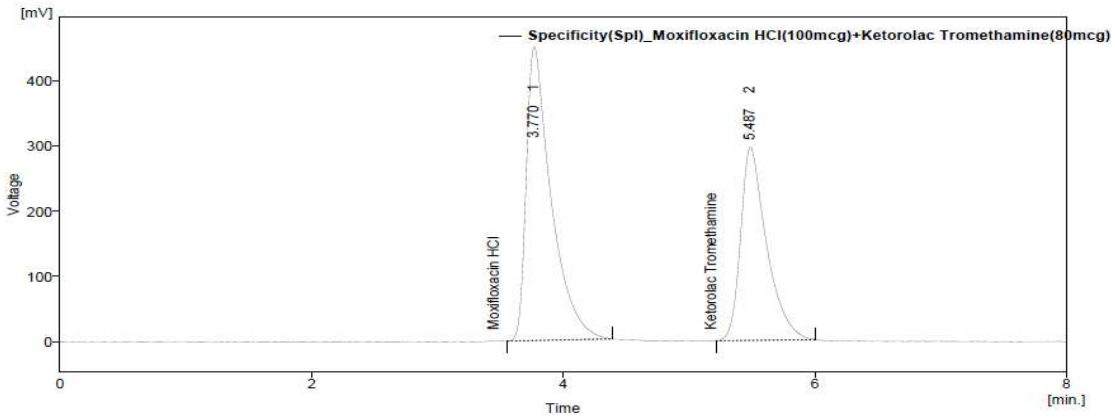

Fig no: 8- Chromatogram for Ketorolac Tromethamine& Moxifloxacin HCl sample

Limit of Detection and Limit of Quantification:

The Limit of Quantification was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

Table no: 6- LOD & LOQ data of Moxifloxacin and Keterolac

S . no:	Parametre	Moxifloxacin	Keterolac
1	**LOD**	1.63 µg	1.73 µg
2	**LOQ**	4.96 µg	5.26µg

Robustness :

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and detection wavelength.

Table no: 7- Robustness data of Moxifloxacin and Keterolac

Parameters		USP Tailing		USP Plate count	
		Moxifloxacin	Keterolac	Moxifloxacin	Keterolac
Flow Rate (ml/min)	0.8	1.146	1.107	2167	3716
	1.0	1.605	1.840	2134	3766
	1.2	1.286	1.652	2981	3433
Wavelength (nm)	304	1.649	1.840	2761	3823
	306	1.829	1.957	2989	3479
	308	1.645	1.820	2476	3636

CONCLUSION

An attempt was made to develop a simple, accurate, economical and precise method for the routine analysis of Moxifloxacin & Keterolac Tromethamine in Occular Dosage form. Finally the method was optimized by using KH_2PO_4 buffer (pH 3.5): Methanol: Acetonitrile in the ratio of 40:40:20 at a flow rate of 1.0ml / min. The proposed method was validated for system suitability, linearity, precision, accuracy, specificity, robustness, LOD and LOQ. From the validation results it has been evident that the method was linear, precise, accurate, sensitive and robust. Therefore the proposed method could be a good approach for obtaining reliable results and suitable for the routine analysis of Moxifloxacin and Keterolac in Bulk drug and ophthalmic Pharmaceutical dosage form.

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