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RP-HPLC Method Development and Validation of Moxifloxacin Hydrochloride & Ketorolac Tromethamine in Bulk and its Pharmaceutical Formulation

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Abstract: ***A simple, rapid, precise, and accurate, reversed phase high performance liquid chromatographic method was developed and validated for simultaneous determination of Moxifloxacin Hydrochloride (MOX) & Ketorolac Tromethamine (KET). The chromatographic separation was achieved on Grace 4.6 (id) x 250 mm column at detector wavelength of 319 nm, using an mobile phase consisting of methanol and buffer in a ratio of 50:50 pH 4.4 at a flow rate 1ml/min. The retention time for Moxifloxacin Hydrochloride (MOX) & Ketorolac Tromethamine (KET). were found to be 5.218 and 7.149 respectively. The method showed adequate precision with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentration used. The HPLC method developed in this study showed specificity and selectivity with linearity in the working range and good precision and accuracy, making it very suitable for quantification of Moxifloxacin Hydrochloride & Ketorolac Tromethamine in ophthalmic solution. The analytical procedure is reliable and offers not only advantages in terms of speed but also low cost of reagents.***

Keywords: Moxifloxacin Hydrochloride & Ketorolac Tromethamine, HPLC etc

1. Introduction [1,2,3,4]

Analytical chemistry may be defined as the science and art of determining the composition of material in terms of elements or compounds contained in it. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative method is the information about the identity of atomic or molecular species or functional groups in sample. A quantitative method provides numerical information as to the relative amount of one or more of these components[1-4]. Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial- and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.

Moxifloxacin.HCl (Fig. 1) is a fourth generation fluroquinolone, the antimicrobial activity of which depends upon inhibition of DNA gyrase (bacterial topoisomerase II), an enzyme necessary for DNA replication, transcription, repair and recombination. Moxifloxacin has in-vitro and in-vivo activities against wide range of gram+ve and gram-ve bacteria. Ketorolac tromethamine is a potent nonnarcotic analgesic compound with cyclooxygenase inhibitory activity which has been developed for oral and parenteral use. Moxifloxacin HCl (MOX) is 1- Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS) octahydro-6H- pyrrolo[3,4-b] pyridin-6-yl]-4- oxo-1,4 dihydroquinoline-3- carboxylic acid hydrochloride. Ketorolac tromethamine (KET) is 1H-Pyrrolizine-1- carboxylic acid, 5benzoyl-2, 3dihydro, with 2 amino-2- (hydroxymethyl)-1, 3- propanediol For routine analysis, a simple and cost effective analytical method is preferred. The objective of the present study was to develop a simple precise, accurate and economic analytical method with better detection range, for the estimation of Moxifloxacin HCl & ketorolac tromethamine in bulk and pharmaceutical formulation.

2. Experimental

Chemical, Reagents and Solution

MOX and KET gratis sample from pharmaceutical industry India, the formulation of this drug is buy from local market i.e. Moxicip KT cipla Ltd. eye drop. HPLC grade Methanol, Acetonitrile and Water from Loba chemical India

The stock standard solutions of MOX and KET were freshly prepared by dissolving 10 mg of each drug in 10 ml Methanol. The stock standard solutions were further diluted with Methanol to obtain a concentration of 50µg/mL of both MOX and KET. The λmax was determined on Shimadzu UV-Visible spectrophotometer (Model UV-1800) in the range 200-400 nm using Methanol as blank. The solution of mixture exhibited maxima at about 319 nm.

HPLC Instrumentation and Chromatographic condition:

After determination of λmax of mixture 319 nm wavelength selected for evaluation of chromatographic parameter. The chromatographic separation was achieved Grace 4.6 (id) x 250 mm column with UV detector. The standard solution containing mixture of MOX and KET was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each

mobile phase was filtered through Whatman filter paper No. 42., well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions.

From various mobile phases tried, mobile phase containing. Methanol: Phosphate buffer (50:50), pH 4.4, flow 1 ml/ min was selected, since it gives sharp reproducible retention time for MOX and KET.(Figure 3,4,5)

System suitability test:
System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline Was obtained. A 20 µL std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded as shown in Table No.1

Analysis of standard laboratory mixture and marketed formulation to see feasibility of the proposed methods.

Preparation of laboratory mixture (standard and sample):
The standard solution of MOX & KET were prepare and mixed properly, also sample solution were prepare to obtain laboratory mixtures containing a concentration in a ratio of marketed formulation.

The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration. The laboratory mixture and formulation Result were recorded and compare as shown in Table No. 2

Method Validation
The analytical method was validated for various parameter according to ICH guideline [3]

Linearity
The linearity of the method was determined at five concentration level ranging from 80-120%. The graph plotted as the concentration of the drug Vs peak area depicted in (Figure. .6 and 7)

Precision
Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method. Table no 3

Accuracy
It was ascertained on the basis of recovery studies performed by standard addition method. Table no.4

Robustness
To evaluate the robustness of the method, the chromatographic condition were deliberately altered and the resolution between MOX and KET was evaluated. Table no. 3
The chromatographic condition selected on various flow rate, different pH were tried

Ruggedness:
The studies of ruggedness were carried out under two different conditions-
1) Days (Interday & Intraday)
2) Different Analyst.

The summary of result shows in table no 3

Specificity:
Specificity was measured as ability of the proposed method to obtain well separated peak for MOX and KET without any interference from component of matrix.
Mean retention time for –
MOX– .218
KET – 7.149

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

3. Result and Discussion

A simple and reproducible RP-HPLC procedure was developed and validated as per ICH guidelines for simultaneous determination of Moxifloxacin HCl and Ketorolac tromethamine. After development of the method it was validated for specificity, accuracy, linearity, precision, and robustness. The chromatographic separation was achieved on Grace 4.6 (id) x 250 mm column at detector wavelength of 319 nm, using an mobile phase consisting of methanol and buffer in a ratio of 50:50 pH 4.4 at a flow rate 1ml/min. The retention time for Moxifloxacin HCl and Ketorolac tromethamine were found to be 5.2 and 7.1 respectively. The linearity studies were performed and found good result. It was evaluated by the visual inspection of the plot Absorbance vs. concentration (r^2 = 0.999 for MOX & r^2 = 0.9993 for KET). The precision was checked and found to be within limits (C.V. - 0.528 for MOX & 0.5039 for KET), hence the method is precise. Accuracy also show good result (C.V. - 0.522 for MOX & 0.471 for KET). The Specificity study shows no any interference.

4. Future Scope

The Method can use for estimation of drugs in Biological fluid.

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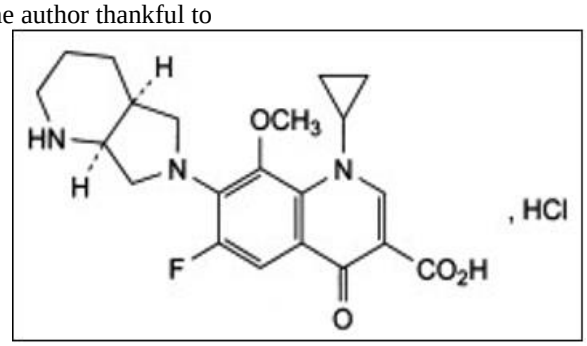

Figure 1: Moxifloxacin HCl

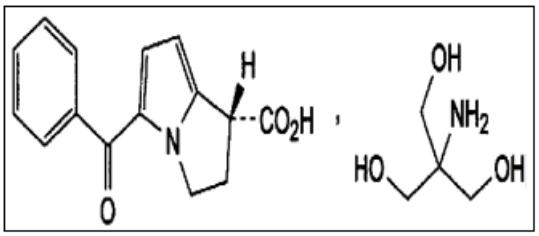

Figure 2: Ketorolac tromethamine

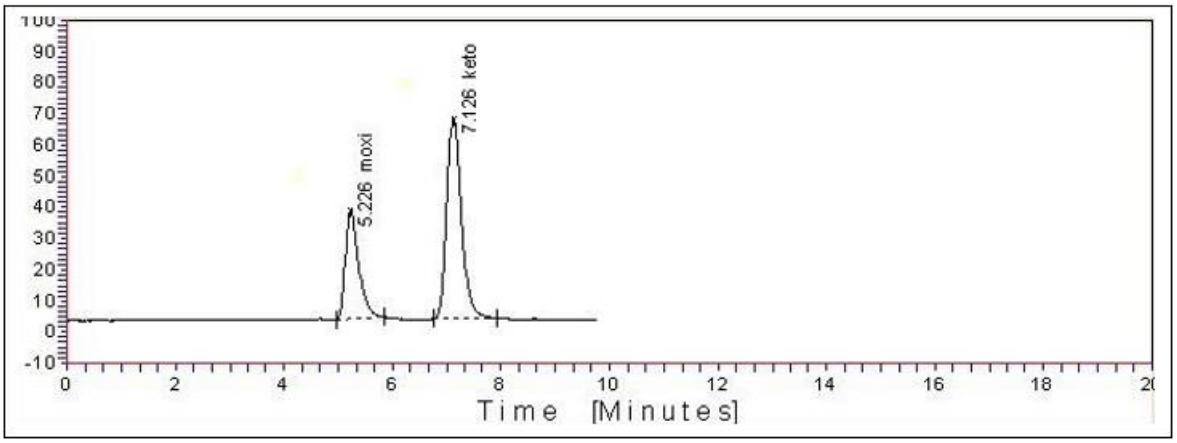

Figure 3: Chromatogram obtained by using MEOH: KH_2PO_4 Buffer (10mM):(50:50 PH 4.4)as mobile phase

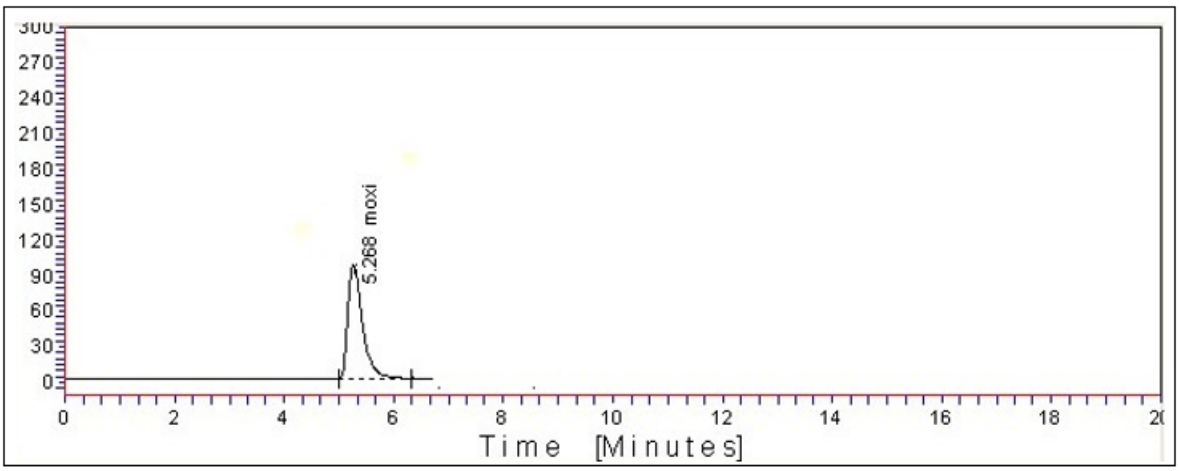

Figure 4: Chromatogram obtained by using MEOH: KH_2PO_4 Buffer (10mM):(50:50 PH 4.4)as mobile phase of MOX.

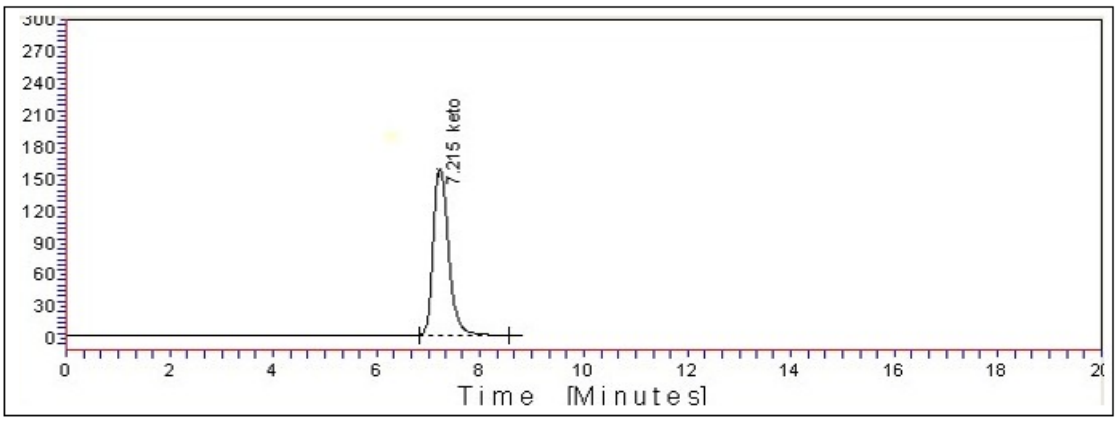

Figure 5: Chromatogram obtained by using MEOH: KH_2PO_4 Buffer (10mM):(50:50 PH 4.4)as mobile phase of KET.

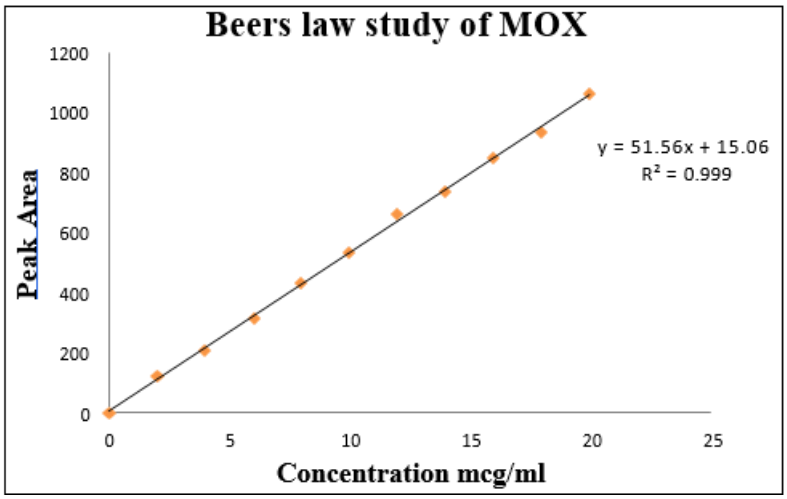

Figure 6: Plot of linearity and range study for MOX

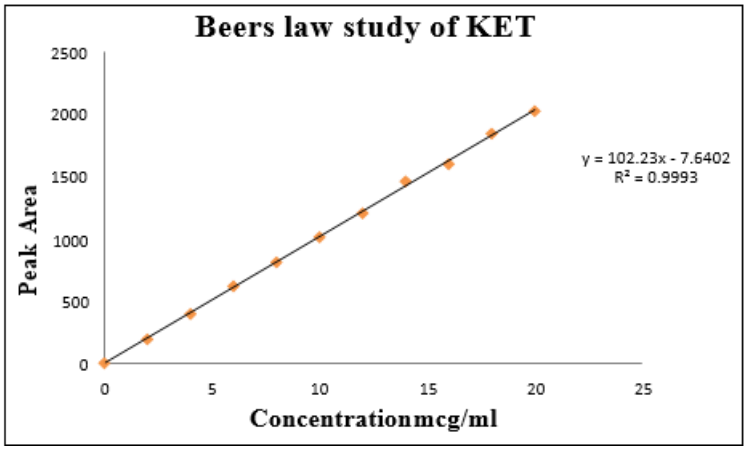

Figure 7: Plot of linearity and range study for TOBRA

Table 1: Result of System Suitability Study

S. No	Peak area		Retention Time		Asymmetry		Efficiency	
	MOX	KET	MOX	KET	MOX	KET	MOX	KET
1	3038.588	6067.448	5.219	7.118	1.773	1.502	60351.612	112275.688
2	3032.567	6062.343	5.208	7.1	1.775	1.518	60358.617	112287.876
3	3032.577	6062.876	5.213	7.089	1.784	1.511	60338.771	112288.798
4	3038.567	6060.998	5.211	7.123	1.78	1.518	60450.654	112377.354
5	3038.582	6067.442	5.209	7.118	1.779	1.51	60365.771	112245.765
Mean	3036.176	6064.221	5.212	7.1096	1.7782	1.5118	60373.09	112295.1
± S.D	3.2901	3.0212	0.0043	0.0144	0.0043	0.0066	44.492	49.163
C.V	0.1083	0.0498	0.0825	0.2025	0.2418	0.4365	0.0736	0.0437

Table 2: Summary of Laboratory Mixture & Marketed Formulation Analysis By RP- HPLC Method

Sr. No.	Sample	Statistical data	% Estimation		% Recovery	
			MOX	KET	MOX	KET
1.	Standard Laboratory mixture	Mean	101.17	99.78	-	-
		S.D.	0.3104	0.593	-	-
		C.V.	0.3068	0.594	-	-
2.	Moxicip- KT (marketed formulation)	Mean	100.06	100.26	99.56	99.39
		S.D.	1.36	0.41	0.5198	0.469
		C.V.	1.35	0.40	0.522	0.471

Table 3: Summary of validation parameter

S. No.	Parameter	Value (CV)	
		MOX	KET
1	Specificity	No interference	
2	Precision	0.528	0.5039
3	Accuracy	0.6426	0.5654
4	Intraday	1.025	1.053
5	Interday	1.36	0.41
6	Different Analyst	0.36625	0.299049

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