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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN HYDROCHLORIDE AND KETOPROFEN IN BULK FORM

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ABSTRACT

A simple, specific and fast reverse phase liquid chromatographic method is established for determination of Moxifloxacin Hydrochloride and Ketoprofen in bulk drugs and pharmaceutical formulations. Chromatographic separations for separation of Moxifloxacin hydrochloride and Ketoprofen were achieved within 8 minutes by use of Inertsil ODS-3V C8 column (150 X 4.6 mm, 5µm) as stationary phase with mobile phase containing 10 mM potassium dihydrogen phosphate buffer with triethylamine (pH 3.5±0.05 adjusted with dilute phosphoric acid) acetonitrile and methanol (40:30:30 v/v/v) at a flow rate of 1.0 ml/ min-. Detection was performed at 306 nm using Prominence UV-Visible detector. The method was validated in accordance with ICH guidelines. Response was a linear function of concentrations over the range of 60-140 µg/ ml for Moxifloxacin hydrochloride and 48-112µg/ml for Ketoprofen. Limit of quantification was found to be 5.89, 5.29 and limit of detection 1.94, 1.75µg/ml for Moxifloxacin hydrochloride and Ketoprofen respectively. Accuracy and precision values of both within run and between-run obtained from six different sets of three quality control samples analyzed in separate occasions for both the analytes ranged from 98.39% to 99.59% respectively. The developed and validated method was successfully applied to quantitative determination of Moxifloxacin hydrochloride and Ketoprofen in pharmaceutical bulk formulation.

KEY WORDS

Acetonitrile, Chromatography, Accuracy, Moxifloxacin, Ketoprofen

INTRODUCTION:

Moxifloxacin hydrochloride (MOX) **(Figure.1)**, 1-Cyclopropyl-6-fluoro-1, 4-dihydro-8- methoxy-7 [(4aS, 7aS)-octahydro-6Hpyrrolo [3, 4-b] pyridin-6-yl]-4-oxo-3 quinoline carboxylic acid hydrochloride **(Figure 1)**, is a synthetic fourth generation broad-spectrum fluoroquinolone antibiotic. It acts by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV, which are involved in DNA replication and metabolism [1].

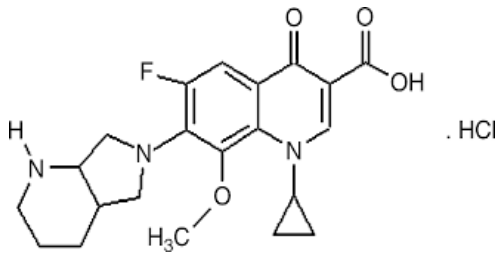

Figure.1: Structure of Moxifloxacin Hydrochloride

Ketoprofen, 2-(3-Benzoylphenyl) propionic acid **(Figure.2)**, ketoprofen is indicated for short term management of moderate to severe pain. It can be used instead of steroidal anti inflammatories in cases, where a raised intra ocular pressure (glaucoma) is to be avoided. The primary mechanism of action responsible for ketorolac's anti-inflammatory, anti-pyretic, and

analgesic effect is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX) ketoprofen is a non-selective cox inhibitor.

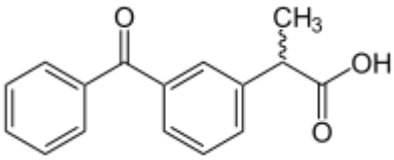

Figure.2: Structure of Ketoprofen

Various methods like UV spectrophotometry [1,2] estimation in biological fluids by HPLC [3,4] HPTLC [5] were reported for determination of MOX with other drugs in literature. Similarly, ketoprofen was determined using HPLC [6,7,8] methods. However, a few analytical methods were also reported for the simultaneous determination of moxifloxacin hydrochloride and ketoprofen in a mixture by UV spectrophotometry [9], liquid chromatography mass spectrometry [10], stability indicating RP-HPLC method [11,12,13] and HPTLC method [14]. An extensive review of the literature did not reveal any simple economical HPLC method for simultaneous determination of both the drugs. Therefore, attempts were made to develop and validate simple, precise, and sensitive, isocratic reverse phase high performance liquid chromatographic method for simultaneous determination of both drugs in bulk formulations.

MATERIALS AND METHODS:

Equipment:

The HPLC system consisted of Shimadzu LC-20A system equipped with model LC-20AT pump, SPD-20A prominence UV-visible detector (set at 306 nm) and a Rheodyne injection valve with a 20 µL loop. Peak areas were integrated using spinchrome CFR software program. The experimental conditions were optimized on an Inertsil ODS-3V C8 column (150X4.6 mm, 5µm) at room temperature.

Chemicals and reagents:

Moxifloxacin Hydrochloride, Ketoprofen were procured from Aurobindo pharma Ltd. Methanol and Acetonitrile used were of HPLC grade from EMerk and ortho-phosphoric acid, pure potassium dihydrogen phosphate, triethylamine-analytical grade from Merck, HPLC-grade water generated from a Milli-Q water purification system, was used throughout the analysis.

Determination of Detection Wavelength:

Accurately weighed and transferred about 100 mg each of moxifloxacin hydrochloride and Ketoprofen standard into a 100 ml volumetric flask separately, then added to it about few ml of methanol and sonicated for 10 minutes to dissolve and diluted up to mark with diluent. This produced standard stock solution (1000µg/ml). Further 1ml of above solution was transferred into 10 ml volumetric flask and volume was made up with diluent. Finally, 1 ml of above solution is diluted to 10 ml using diluent and mixed well. The concentration of the working standard solution thus produced is 10µg/ml. The working standard solutions of moxifloxacin hydrochloride and ketoprofen(10µg/ml) were scanned over the range of 190-400 nm. Both the drugs showed good response at 306 nm, therefore 306 nm was selected for further study. The UV absorption Spectrum of Moxifloxacin hydrochloride and Ketoprofen is shown in Figure.3.

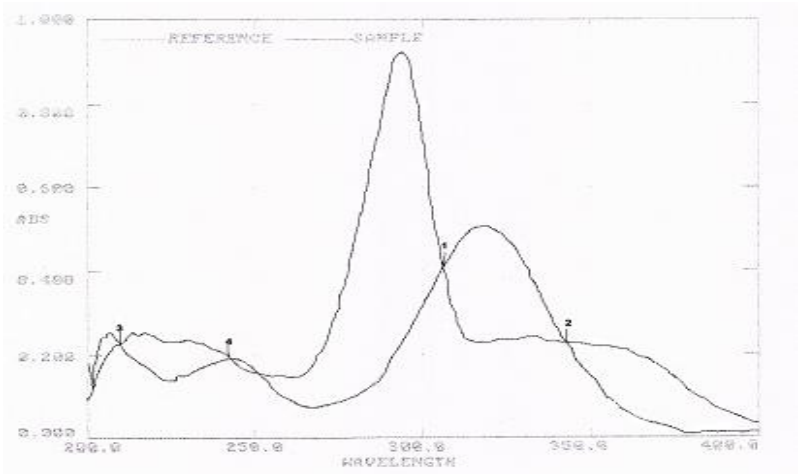

Figure 3: Overlaid UV absorption spectrum of Moxifloxacin hydrochloride and Ketoprofen

Chromatographic conditions

Mobile phase consisted of methanol, Acetonitrile and 10 mM potassium dihydrogen phosphate (pH 3.5±0.05) in the ratio of (30:30:40 v/v/v, respectively). Flow rate of the mobile phase was 1.0 ml/ min and all chromatographic experiments were performed at room temperature (25°C ± 2°C).

Preparation of Buffer Solution: 2.72 gm of potassium dihydrogen phosphate was dissolved in 1000mL MilliQ water, and then add 1ml of triethylamine (TEA) and pH of this solution was adjusted to 3.5±0.05 with ortho phosphoric acid. The solution was mixed well and then filtered through 0.45µ filter paper.

Preparation of Mobile phase: Mobile phase was prepared by mixing buffer solution of pH 3.5±0.05, Acetonitrile and methanol in the ratio 40:30:30, v/v/v. The mobile phase is then filtered through 0.45µ membrane and sonicated for 8 min.

Preparation of Working Standard Solution: Accurately weighed 100 mg of Moxifloxacin hydrochloride and Ketoprofen standard drug and transferred to 100 ml volumetric flasks and dissolved in 100 ml of mobile phase. From the above stock solution 1ml of MOX solution and 0.8ml of Ketoprofen solution was transferred to 10 ml of volumetric flask and was made up to with diluent. The working standard solution produced contains 100µg/ml of MOX and 80µg/ml of Ketoprofen.

Preparation of Sample Solution: The 5ml of combined solution (MOX-Ketoprofen) containing 0.5%w/v of moxifloxacin HCL and 0.4%w/v of Ketoprofen directly transferred into 25ml of volumetric flask. About 10 ml of mobile phase was added, sonicated to mix, diluted up to volume with mobile phase solvent and mixed (100µg/ml of MOX and 80µg/ml of Ketoprofen), which was assayed and quantified.

Optimized method:

Standard solutions ranging from 60-140µg/ml for moxifloxacin Hydrochloride (60, 80, 100, 120 and 140µg/ml) and 48-112µg/ml for Ketoprofen (48, 64, 80, 96 and 112µg/ml) were prepared. From Each solution 20µl was injected in to the optimized chromatographic system and chromatogram was recorded (**Figure 4**). Areas obtained from chromatograms were taken and a graph was plotted for both the drugs by taking areas on the y- axis and concentrations on the X-axis.

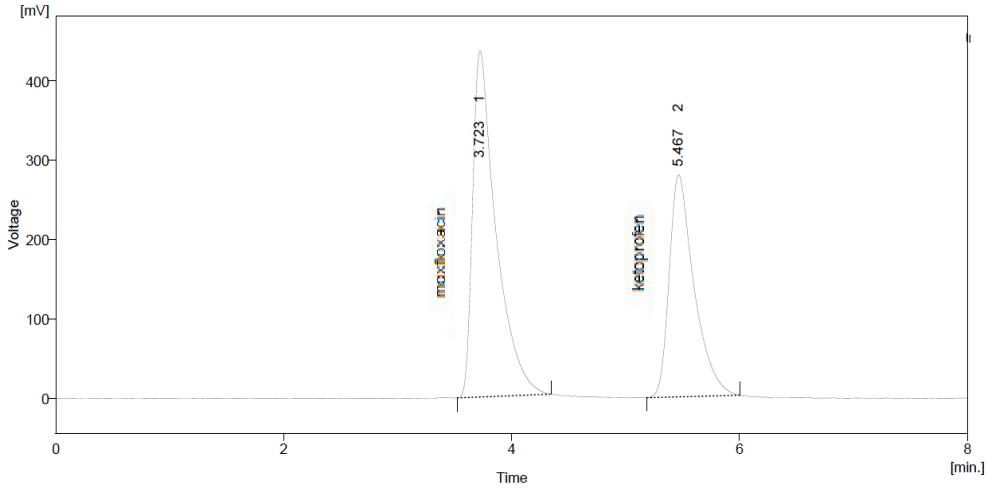

Figure.4: Typical chromatogram of Moxifloxacin hydrochloride and Ketoprofen

RESULTS:

Method Validation:

The proposed method was validated in compliance with the ICH guidelines and successfully applied for determination of Moxifloxacin hydrochloride and Ketoprofen in their bulk formulations.

System Suitability Studies:

The system suitability studies were done for parameters like theoretical plates, tailing factor, retention time, resolution were studied and presented in **Table-1.**

Table-1: Results of system suitability of the proposed method

Sr.No.	System suitability parameters	Moxifloxacin hydrochloride	Ketoprofen
1	Relative Standard Deviation (%RSD)	0.60	0.72
2	Tailing factor (T_f)	1.6	1.4
3	Resolution (Rs)	4.7	--
4	Retention time (Rt)	3.723	5.467
5	Theoretical plates (N)	2798	3222

Specificity:

The method was found to be selective as no significant interfering peak is observed at the retention times of MOX and Ketoprofen which were 3.770, and 5.487 min. respectively. Total chromatographic run time was 8 min and shows the representative chromatograms of blank and spiked with analytes **(Figure 5&6).**

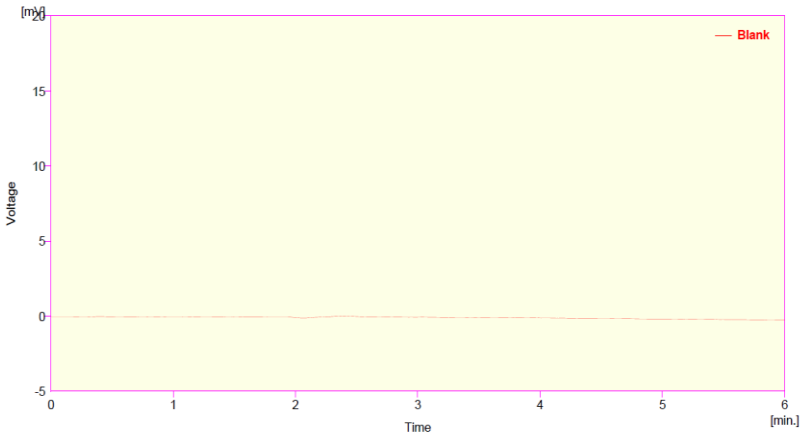

Figure.5: Blank chromatogram

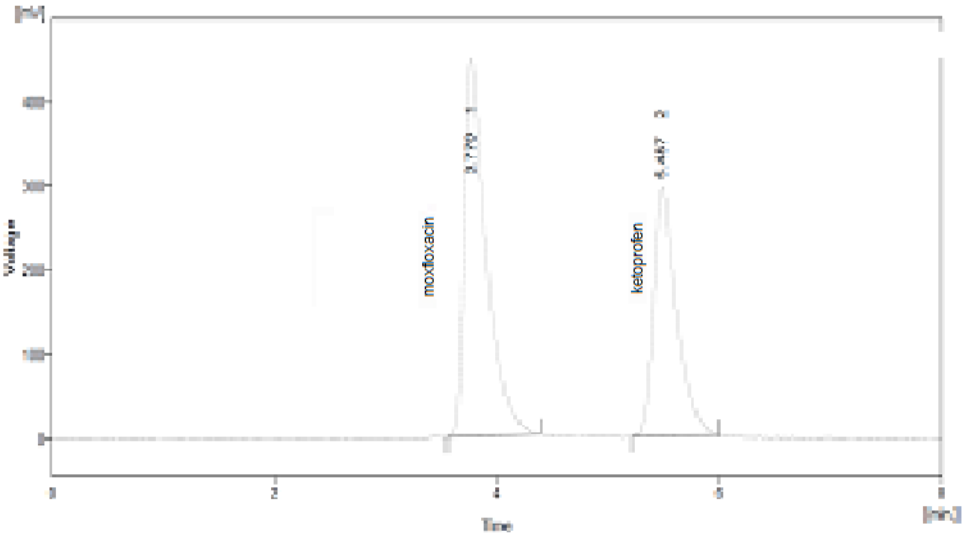

Figure.6: Standard chromatogram of Moxifloxacin hydrochloride and Ketoprofen

Linearity:

Linear calibration plots of the proposed method were obtained over concentration ranges of 60 140µg/ml for Moxifloxacin hydrochloride (60, 80, 100, 120 & 140µg/ml) and 48-112µg/ml for Ketoprofen (48, 64, 80, 96 & 112µg/ml). Each solution was prepared in triplicate. Regression coefficients were found to be 0.998 for MOX and Ketoprofen **(Figure.7 & 8).**

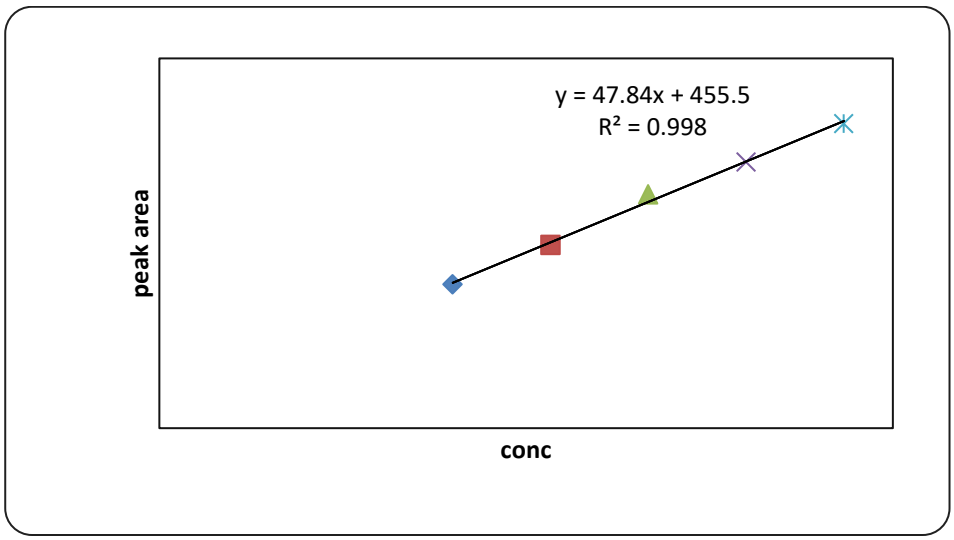

Figure.7: Linearity plot for Moxifloxacin Hydrochloride

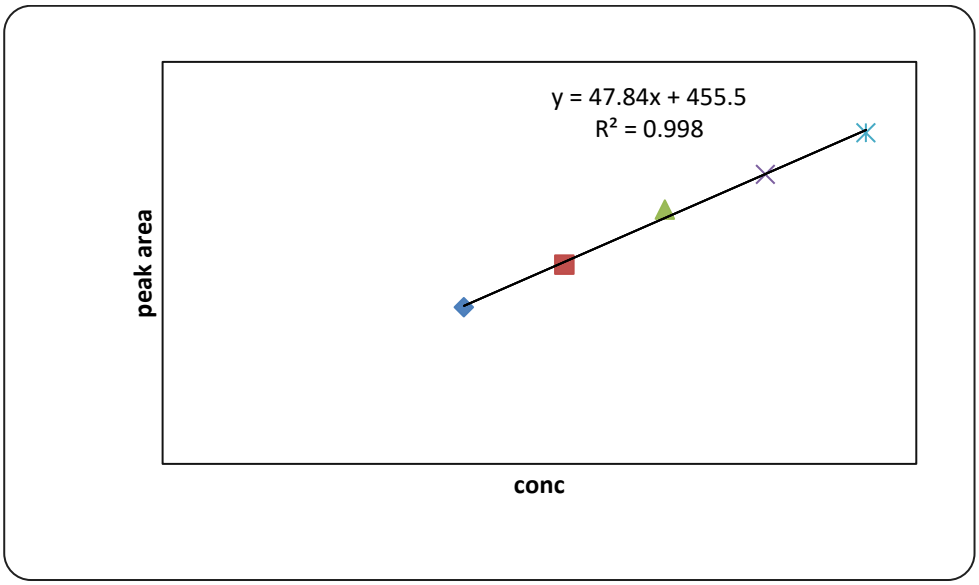

Figure.8: Linearity plot for Ketoprofen

Accuracy:

The standard addition method was used to demonstrate the accuracy of the proposed method. For this purpose, known quantities of Moxifloxacin HCL and Ketoprofen were supplemented to the previously analyzed sample solution and then experimental and true values compared. Three levels of solutions were made corresponding to 80, 100 and 120% of nominal analytical concentration (100µg mL^{-1} MOX, 80µg mL^{-1} for Ketoprofen). Standard preparation & Sample preparations was injected into the HPLC and % RSD for Moxifloxacin HCL and Ketoprofen peaks in Standard preparation was calculated.

Table -2: Accuracy data for the proposed method

	Area		SD		%RSD	
Accuracy	MOX	Ketoprofen	MOX	Ketoprofen	MOX	Ketoprofen
80%	6209.874	4262.569	23.32	27.89	0.37	0.65
100%	7605.479	5256.158	32.2	26.01	0.42	0.49
120%	8488.269	5866.268	42.032	41.03	0.49	0.69

Recovery: The Recovery of an analytical method should be established across its range. The study was performed by making three different standard concentrations 80%, 100% and 120% of known amounts of studied drugs. The Recovery was found for Moxifloxacin HCL 80%- 98.63%, 100%- 101.26% and 120%- 98.25% and for Ketoprofen 80%- 98.43%, 100%- 99.47% and 120%- 98.92%.

Precision:

System precision:

The system precision was carried out to ensure that the analytical system is working properly. Standard preparation was injected six times into the HPLC and %RSD for Moxifloxacin HCL and Ketoprofen peaks in Standard preparation was calculated. The retention time and area of six determinations was measured and % RSD 0.60 for MOX, 0.72 for Ketoprofen.

Robustness studies:

Robustness is done by changing deliberately the optimized parameters like flow rate and detection wavelength, Rt and area under changed conditions are compared under the optimum conditions.

Table -3: Robustness data for Moxifloxacin HCL &Ketoprofen

Condition	Variation	Retention time		Average area (n=3)		%RSD	
		MOX	Ketoprofen	MOX	Ketoprofen	MOX	Ketoprofen
Detection wave length 306nm	Detection wavelength 304nm	3.792	5.631	7216.712	4088.621	0.040	0.0231
	Detection wavelength 308nm	3.779	5.628	5545.69	4473.814	0.008	0.027
Flow rate 1 ml/min	Less flow 0.8ml/min	4.694	6.942	7674.9	5302.049	0.03	0.0
	More flow 1.2ml/min	3.212	4.735	5252.604	3580.76	0.085	0.67

Ruggedness:

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments and hence the proposed method was found to be rugged.

Table -4: Ruggedness data for Moxifloxacin HCL &Ketoprofen

Sr.No.	Moxifloxacin HCL		Ketoprofen	
	Analyst 1 (Area)	Analyst 2 (Area)	Analyst 1 (Area)	Analyst 2 (Area)
1	6262.783	6221.290	4267.555	4294.025
2	6263.781	6220.399	4267.552	4294.082
3	6261.788	6222.292	4267.544	4294.029
4	6262.668	6223.293	4267.551	4293.035
5	6261.783	6228.290	4267.054	4295.025
6	6263.781	6224.291	4267.002	4294.029
Average	6262.764	6223.309	4267.376	4294.037
Std. Deviation	0.815	20561	0.246	0.574
% RSD	0.013	0.041	0.0057	0.013

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection was established 1.94 and 1.75µg/ml for MOX and Ketoprofen respectively. Limit of quantification was established 5.89 and 5.29µg/ml for MOX and Ketoprofen respectively.

Assay:

20µL of Standard solution and sample solution were injected separately into the chromatography system and the peak areas responses for the analyte peaks were measured and substituted in the formula to calculate % recovery.

Table -5: Assay results of Moxifloxacin hydrochloride and Ketoprofen

product	ingredient	Label value (%w/v)	% Recovery
Moxifloxacin HCl + Ketoprofen bulk form	Moxifloxacin HCl	0.5	98.39
	ketoprofen	0.4	98.59

DISCUSSION:

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation with good peak symmetry was obtained with Inertsil ODS-3V C18 (150x 4.6mm, 5µm) column using mobile phase containing potassium dihyrogen phosphate (pH 3.5±0.05): Acetonitrile: methanol (40:30:30 v/v/v) at a flow rate of 1ml/min. Quantification was achieved with UV detection at 306nm based on peak area. The retention time for Moxifloxacin hydrochloride and Ketoprofen were found to be 3.7567min and 5.487min, respectively. The optimized method was validated as per ICH guidelines. The System suitability parameters observed by using this optimized condition were reported. A linearity range of 60-140µg/ml with correlation coefficient 0.998 was established for Moxifloxacin hydrochloride and 48-112µg/ml with correlation coefficient 0.998 was established for Ketoprofen. The precision of the proposed method was carried in terms of the repeatability and the %RSD values of moxifloxacin hydrochloride was found to be 0.57% and of ketoprofen was found to be 0.74% and reveal that the proposed method is precise. The % recovery for Moxifloxacin hydrochloride and Ketoprofen was calculated 99.38% and 98.99% respectively. The LOD and LOQ values for moxifloxacin hydrochloride were 1.94µg/ml, 5.89µg/ml respectively and for ketoprofen were found to be 1.75µg/ml, 5.29µg/ml. The study of robustness in the present method shows no significant changes either in the peak area or Rt. The results of analysis of commercial formulation indicated that there is no interference due to common formulation excipients with the developed method. Therefore, the proposed method can be used for routine analysis of these two drugs in their combined pharmaceutical bulk form.

CONCLUSION:

A simple, sensitive, and accurate method using reverse phase HPLC was described for simultaneous determination of Moxifloxacin hydrochloride and Ketoprofen in pharmaceutical bulk form. The proposed method was validated by testing its linearity, accuracy, precision, limit of detection, limit of quantitation and specificity. The developed and validated RP-HPLC method that has significant advantages over the previously published method as it provides simple mobile phase composition for chromatographic separation, shorter run time for analysis, simple sample preparation as well as improved sensitivity. Therefore, this new method leads to a simple, feasible, cost effective, rapid method with high degree of accuracy and specificity to quantify simultaneously Moxifloxacin hydrochloride and Ketoprofen in pharmaceutical formulations with HPLC. It will be extremely helpful for successfully analyzing the Moxifloxacin hydrochloride and Ketoprofen in ophthalmic bulk formulations.

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