Section: Research Paper

Development and Validation of RP-HPLC Methods for Simultaneous Determination of Moxifloxacin HCl and Ketorolac Tromethamine in Bulk and Marketed Formulation

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ABSTRACT:

Objective

A rapid, accurate, selective, and sensitive RP-HPLC method has been developed and validated for estimation of moxifloxacin hydrochloride (MXF) and ketorolac tromethamine (KTA) in the bulk and ophthalmic formulation.

Method: The chromatographic separation was achieved on ODS Phenomenex C18 column (250mm × 4.6mm, 5 µm). The compound was separated using mobile phase 0.025M Potassium dihydrogen phosphate solution: acetonitrile: methanol (30:35:35v/v/v). The pH of mobile phase was adjusted to 3.0 with ortho-phosphoric acid, flow rate was 1.0 ml/min and 25°C column temperature and detection wavelength was set at 300 nm for overall analysis.

Results: The retention time of MXF and KTA was obtained at 2.67 and 5.39 min, respectively. The linearity study was performed in the concentration range of 2 to 20 µg/ml for MXF and KTA with correlation coefficient was found to be 0.999 for both drugs. The percentage purity of MXF and KTA was obtained in the range 98-102%. The limit of detection was 0.148 µg/ml and 0.272µg/ml and limit of quantification was 0.449 µg/ml and 0.824 µg/ml, respectively for MXF and KTA.

Conclusion: The method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, limit of detection, limit of quantification and robustness. Thus, proposed

method can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs without any interference of excipients.

KEYWORDS: Chromatography, ICH guidelines, Method Validation, NSAID, Simultaneous estimation.

INTRODUCTION:

Moxifloxacin hydrochloride (MXF) is belonging to fluoroquinolone family of drugs which is used to treat variety of bacterial diseases such as sinusitis, endocarditis, conjunctivitis, pneumonia and TB. By preventing the bacteria from duplicating DNA, it typically kills them. MXF is a broad spectrum antibiotic which is effective against gram-positive and gram-negative bacteria. It is broad-spectrum antibiotic that prevents cell replication by impending DNA gyrase, a type II topoisomerase and topoisomerase IV enzymes required to split bacterial DNA.[1,2] Chemically, moxifloxacin hydrochloride is known as 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-[(4aS,7aS)-octahydro-6-H-pyrrolo[3,4-b]pyridine-6-yl]Hydroxy-4-oxo-3-quinolinecarboxylic acid.[3] (Figure 1)

Ketorolac tromethamine is a non-steroidal anti-inflammatory medicine (NSAID) that is 800 times more effective than acetylsalicylic acid in terms of analgesic efficacy (aspirin).[4] It has both analgesic and anti-inflammatory activity. KTA is primarily used to treat post-operative ocular inflammation.[5] When administered systemically, the drug does not cause pupil constriction because its mode of action is to suppress prostaglandin manufacture. KTA is chemically known as (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Figure 2).

In the form of eye drops, a combination of MXF and KTA is employed as an antiseptic and anti-infective.[5] The official monographs describe the procedure for individual assay of MXF and KTA using RP-HPLC and potentiometry, respectively. MXF and KTA was analyzed by various techniques either alone or combination with other drugs. The analytical method exists for MXF alone includes determination by spectrophotometry[6,7] and high performance liquid chromatography (HPLC)[8,9] in pharmaceutical dosage form. For the determination of MXF in biological fluids, methods such as voltammetry[10] and capillary electrophoresis with laser induced fluorescence [11] has been reported. In human plasma, MXF and other fluoroquinolones estimated through HPLC.[12] Several analytical techniques has been published for the quantification of KTA alone using HPLC method [13], the detection of KTA and its impurities by capillary electrochromatography[14], gas chromatography-mass spectrometry [15] and the assay of KTA in pharmaceutical matrices using differential pulse polarography [16].

The main objective of presented study was to design accurate, sensitive, simple and rapid reverse phase HPLC techniques for the simultaneous estimation of both KTA and MXF in bulk and pharmaceutical dosage form. The combination of MXF and KTA has not been included in any authoritative pharmacopoeias. [17]

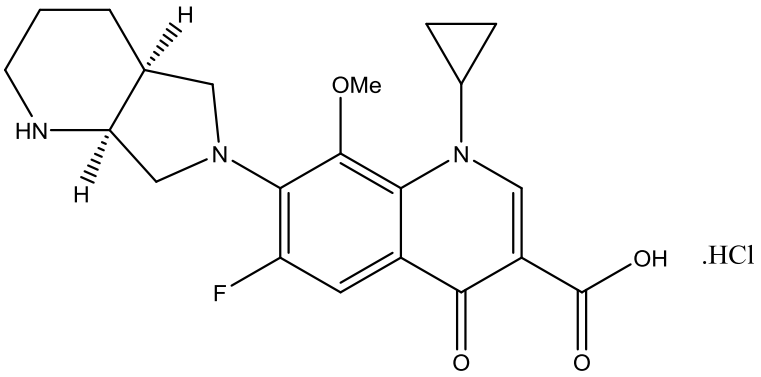

Figure 1: Structure of Moxifloxacin Hydrochloride

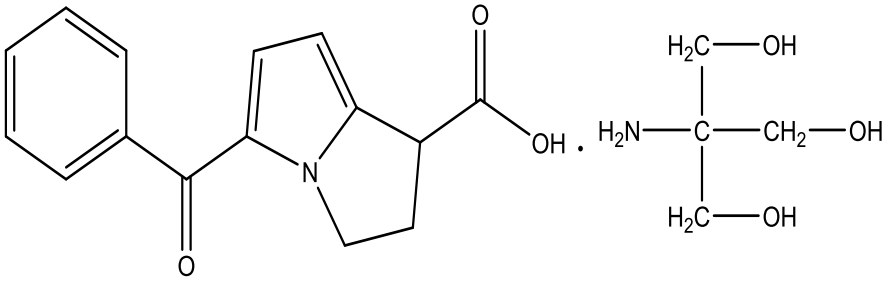

Figure 2: Structure of Ketorolac tromethamine

EXPERIMENTAL PROCEDURES:

Materials:

Moxifloxacin HCL and Ketorolac Tromethamine were obtained from Micro labs, Bangalore. Methanol, potassium dihydrogen phosphate and acetonitrile purchased from Merck (Darmstadt Germany) of analytical reagent grade. APDROPS-KT^{TM} and Miliflox Plus ^{TM} eye drop formulation were purchased from local pharmacy. This formulation has label claim of 5mg w/v of MXF and KTA each. It also may contain isotonicity modifier sodium chloride, buffering agents like boric acid or monobasic sodium phosphate and preservatives like benzalkonium chloride and chlorobutanol.

Instrumentation:

A Cyber lab HPLC instrument-LC- 100B equipped with Rheodyne 773Si injection valve with a 20 µl loop volume and Binary gradient pump was used. This system also includes UV-VIS (UV 100) detector operated at wavelength 300nm. Data were acquired and processed by using DS-100 control data system software. Chromatographic separation was performed using ODS

Phenomenex C18 (250×4.6mm, 5µm). Gradient mixer GM-100, pump LC100 reciprocating HPLC pump.

Preparation of standard stock solution:

About 10 mg of MXF and 10 mg of KTA were accurately weighed and transferred into 100 mL volumetric flask. The content of the volumetric flask was dissolved in mobile phase to get 100µg/ml of MXF and KTA. Working standard solution was freshly obtained by diluting the standard stock solution with mobile phase during analysis time. The stock solution and working standard solution were protected from light during analysis.

Chromatographic condition:

Chromatographic separations were carried out on a ODS Phenomenex C_{18} column (250×4.6mm, 5µm). A mixture of 0.025M potassium dihydrogen phosphate solution, acetonitrile and methanol (30:35:35v/v/v) was used as the mobile phase. The pH buffer was adjusted at 3 with ortho-phosphoric acid. The injected volume of sample was 20µl with flow rate 1.0ml/min. Wavelength of 300nm was used as detection at which both drugs gave good response.

Analysis of eye drop formulation:

To determine the content of MXF and KTA in ophthalmic formulation (containing 5mg/ml of each drug), accurately about 2 ml of sample was pipette out and transfer into 10 ml volumetric flask. Further dilutions were made from resultant solution to make a volume up to the mark with methanol to get concentration (100ng/µL of each drug). Resultant solution was spotted for assay of MXF and KTA. The unknown concentration of formulation was determined by regression equation.

METHOD VALIDATION:

Linearity –A feature of the analytical process is the ability to obtain witness results (within a certain range), which directly proportional to the analytical intensity (quantity) of the sample. Linearity was assessed by observing the visual meaning of the plot in the form of material or analytical function.[18]

Specificity- Specificity is the ability of the analytical method to distinguish between the analytes and the other components in the sample matrix. Specificity is the study of the chromatographic method is performed by the separation of the analyte form the other potential components such as impurities, degradants or excipients.[18]

Accuracy- It is analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be assessed using minimum of nine determinations over of three concentration levels covering the specified range. The acceptance criterion for accuracy is the relative standard deviation (%RSD) for all recovery values should not be more than 2%. [18]

Precision **–** The accuracy of analysis procedure for detecting proximity of agreement (distribution rate) between a string of measurement taken from multiple samples from the same homogenous sample below specified conditions. Three levels were considered: repetition, average and reproducibility.[19]

Limit of Detection (LOD) - The limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value.

$LOD = 3.3\ \sigma/S$

σ – the standard deviation of X- intercept

S- the slope of the calibration curve [20]

Limit of Quantification (LOQ) – The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable.

$LOQ = 10\sigma/S$

Where, σ- the standard deviation of the response

S- the slope of the calibration curve[21]

Robustness:

The robustness of an analytical procedure 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. Six samples' solutions were prepared and analyzed under the established conditions and the various analytical parameters like flow rate of mobile phase, mobile phase composition and pH.[21]

RESULT AND DISCUSSION

Results of the validated method proved, performance and characteristics to meet the requirement of standard analytical application.

Optimization of chromatographic condition:

Spectroscopic analysis of compound showed that MXF and KTA have maximum absorbance (λ_{max}) at 295nm and 320 nm respectively. The chromatographic detection was performed at 300 nm using a UV-Visible detector. In a trial of mobile phase, it was observed that when a combination of drugs was injected, MXF and KTA together give a single peak. Chromatographic conditions were optimized by changing the mobile phase composition with ratio of solvents. The optimized mobile phase was determined as a mixture of 0.025M Potassium dihydrogen phosphate solution: acetonitrile: methanol (30:35:35v/v/v) at flow rate 1.0 ml/min. Under these condition, MXF and KTA were eluted at 2.67 and 5.39 minutes, respectively. The obtained chromatogram is depicted as Figure 3.

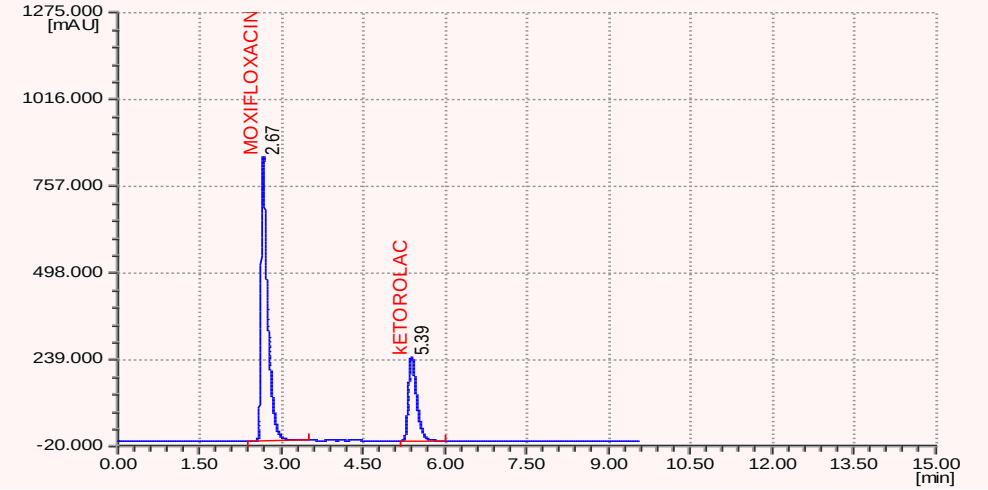

Figure 3: Chromatogram of MXF and KTA

System suitability:

System suitability study was performed to evaluate the parameters like tailing factor, theoretical plates, resolution and % RSD for replicate injections. The result are within limits and given in Table I.

Table I: Validation and system suitability parameter

Parameters	MXF	KTA
Linearity range (µg/ml)	2-20	2-20
Correlation coefficient	0.9999	0.9999
Limit of detection (µg/ml)	0.1482	0.2721
Limit of quantification (µg/ml)	0.4492	0.8247
Retention time (min)	2.67±0.10	5.39±0.10
Tailing factor	1.81	1.55
Theoretical plates	3790	3099
Resolution factor	5.453	

Linearity:

The linearity of the measurement was evaluated by analyzing different concentrations 2-20 µgm. /ml of the standard solution of MXF and KTA. The calibration curve was plotted, concentration against mean peak area and the regression equation was computed. The coefficient of correlation (R^2) for MXF and KTA were found to be 0.999 and 0.999 respectively. The calibration curve of MXF and KTA is presented as figure 4.

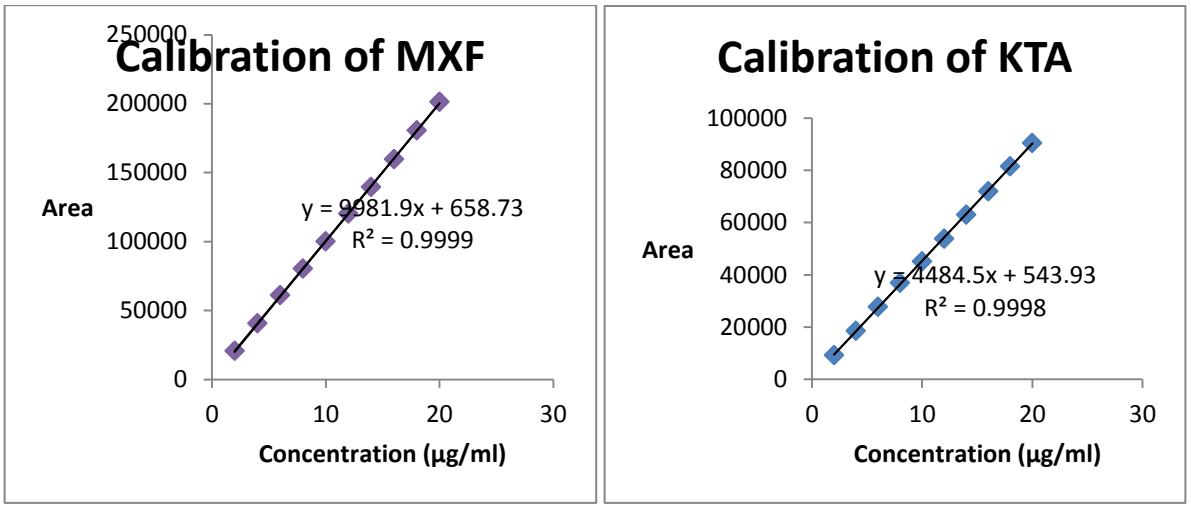

Figure 4: Calibration curve of MXF and KTA

Accuracy:

The results of accuracy study are expressed in terms of percent recovery. The percent recovery at three levels (80 %, 100 % and 120 %) was found to be in the range of 98-102 %. Statistical analysis of recovery studies are shown in Table II.

Table II: Recovery study of MXF and KTA

Level of % recovery	% Mean recovery		Standard deviation*		% RSD	
	MXF	KTA	MXF	KTA	MXF	KTA
80	98.66	99.81	± 0.3350	± 0.3915	0.3395	0.3922
100	99.73	99.86	± 0.6027	± 0.7371	0.6043	0.7381
120	99.99	99.99	± 0.6357	± 0.7903	0.6357	0.7903

mean of three determinations

Precision:

Repeatability and reproducibility of the proposed method was determined by intra-day and interday precision studies. The formulation was assayed three times on the same day (intra-day) and on three consecutive days (inter-day). The results of precision studies were expressed in terms of relative standard deviation (RSD) less than 2 of the percent label claim determined by developed method shown in Table III.

Table III: Intraday and interday precision study of MXF and KTA

Parameter	Drug	Mean*	Standard deviation	% RSD
Intra day Precision	MXF	99.91	± 0.8645	0.8652
	KTA	99.89	± 0.5463	0.5469

Inter day Precision	MXF	98.85	± 1.0526	1.0648
	KTA	98.98	± 0.9864	0.9965

mean of six determinations

Specificity:

The chromatogram of formulation showed only two peaks at retention time of 2.67 min and 5.39 min respectively for MXF and KTA (Figure 3), indicating that there is no any interference of any of the excipients of the formulation.

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

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) linear were separately determined based on the standard deviation (σ) of the response and the slope (S) of the calibration curve, using the formula LOD=3.3 σ /S and LOQ=10 σ /S. The LOD and LOQ for MXF and KTA were estimated.

The limit of detection for was 0.1482µg/ml and 0.2721 µg/ml for MXF and KTA and limit of quantification was 0.4492µg/ml and 0.8247µg/ml for MXF and KTA respectively as shown in Table IV.

Table IV: LOD and LOQ for MXF and KTA

Parameter	**MXF (µg/ml)**	**KTA (µg/ml)**
Limit of Detection (LOD)	0.1482	0.2721
Limit of Quantification (LOQ)	0.4492	0.8247

Robustness:

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done.The robustness of the method was evaluated for mobile phase flow rate, solvent composition, pH and the influence of these changes on specific peak characteristics like retention time and tailing factor were evaluated.

Table V: Robustness study of MXF and KTA

Parameters	**Level**	**Retention Time**		**Tailing Factor**	
		MXF	KTA	MXF	KTA
Flow rate (ml/min)	0.9	2.66	5.38	1.98	1.70
	1	2.67	5.39	1.81	1.55
	1.1	2.68	5.40	1.92	1.70
	Mean	2.67	5.39	1.90	1.65
	S.D	± 0.01	± 0.01	± 0.086	± 0.086
Mobile phase Composition	68:32	2.89	5.88	2.02	1.75
	70:30	2.65	5.40	1.81	1.55

(v/v)	72:28	2.51	5.21	1.98	1.79
	Mean	2.68	5.49	1.93	1.69
	S.D	± 0.192	± 0.345	± 0.111	± 0.128
pH	2.9	2.68	5.42	1.89	1.52
	3	2.65	5.40	1.81	1.55
	3.1	2.61	5.39	1.78	1.56
	Mean	2.64	5.40	1.82	1.54
	S.D	± 0.035	± 0.015	± 0.056	± 0.020

Application of method: analysis of real sample-

The validated method has been successfully applied to determine MXF and KTA concentration in ophthalmic formulation. Average content of 99.2% and 100.4% of the label claim was obtained respectively, which was in good agreement with label claim for the formulation

Table VI: Assay of marketed formulation

Drug	**Label claim (mg/ml)**	**Amount of drug estimated (mg/ml)***	**% Label claim**	**S.D ***	**%RSD**
MXF	5	4.96	99.2	± 1.21	0.82
KTA	5	5.02	100.4	± 1.41	0.45

mean of six determinations

CONCLUSION:

A simple, rapid, accurate and precise HPLC analytical method has been developed and validated for the routine analysis of MXF and KTA in API and pharmaceutical dosage forms. The validation results of developed RP-HPLC method was in accordance to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective. Statistical analysis proved that the method is repeatable, reproducible, accurate and specific for the analysis of MXF and KTA. The suggested method was found to be less time consuming and cost effective and may be more advantageous for routine analysis of drug in marketed formulation. It may be extended to study the degradation kinetics of MXF and KTA also for its estimation in plasma and other biological fluids.

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