Section: Research Paper

Development and Validation of TLC-Densitometric Method for Simultaneous Estimation of Moxifloxacin HCL and Ketorolac Tromethamine in Bulk Drug and Marketed Formulation

Ujwala Wasnik[1], Rushikesh Nikalje[2], Khandoba Birajdar[3], Sameer Lakade[4] ,

Sidheshwar Jadhav[2], Padmaja Kore[2], Minal Harde[2]*

[1]Taywade College of Pharmacy, Koradi, Nagpur, Maharashtra, India.
[2]PES's Modern college of pharmacy, Nigdi, Pune, 411044, Maharashtra, India.
[3]Bharati Vidyapeeth College of Pharmacy, Kolhapur-416013, MS India
[4]RMD Institute of Pharmaceutical Education & Research, Pune, Maharashtra, India.
* Corresponding Author
Dr. Minal T Harde
Modern College of Pharmacy,
Yamunanagar, Nigdi, Pune -411044
Email id : minalresearch@gmail.com
ORCID id : 0000-0002-4963-4165

ABSTRACT

Objective: A simple novel, accurate, precise, and reproducible TLC-densitometric method for simultaneous estimation of Moxifloxacin HCl (MXF) and Ketorolac Tromethamine (KTA) has been developed for routine estimation.

Methods: The proposed method was validated using ICH validation parameters like linearity, accuracy, precision, LOD and LOQ, recovery studies and repeatability studies. The chromatographic separation of the drugs was achieved on Merck precoated silica gel aluminium plates 60 F_{254} as the stationary phase and the solvent system consisting of mobile phase chloroform: methanol: Formic acid (8.5: 1.5: 0.05 v/v/v) was utilized. Densitometric evaluation was performed at 305 nm, which is the iso-absorptive point for the simultaneous estimation of both drugs.

Results: Both MXF and KTA were satisfactorily resolved with obtained R_f values 0.71±0.02 and 0.18±0.05 respectively. The accuracy and reliability of the method was assessed by evaluation of linearity for MXF and KTA, which obtained as 100-350 ng/spot for both drugs with the regression coefficient of 0.9981 and 0.9995, respectively. Precision study found to be intra-day % RSD (0.72–1.01) and inter-day % RSD (1.15 –1.10) for MXF and intra-day % RSD (0.89–

1.09) and inter-day % RSD (0.85 –1.12) for KTA**.** The detection and quantification limits were found to be 8.17 and 24.76 ng/spot for MXF, 17.63 and 53.42 ng/spot for KTA, respectively.

Conclusion: The proposed method was validated in terms of Linearity, Range, Accuracy, Precision, Specificity and Robustness in accordance with ICH guidelines. The method was successfully applied to the estimation of MXF and KTA in marketed formulation.

KEYWORDS: HPTLC, ICH guidelines, Method development, Quantification, Validation.

INTRODUCTION

Moxifloxacin HCl is chemically 8-methoxy fluoroquinolone derivative [1-cyclopropyl -6-fluro-1,4-dihydro-8-methoxy-7-{(4aS,7aS-octa-hydro-6H-pyrrolol(3,4b) pyridine-6-yl)}-4-oxo-3-quinoline carboxylic acid, monohydrochloride] (Figure 1)[1]. It is yellow crystalline solid having molecular formula $C_{21}H_{24}FN_3O_4$. MXF is used along with first line anti-tuberculosis drugs to shorten the duration of treatment of tuberculosis[2]. MXFis a fourth -generation fluroquinolone antibiotic, used to treat bacterial infection of the respiratory tract such as pneumonia, bronchitis and sinusitis. MXF readily absorbed from gastrointestinal tract and penetrates easily into target tissues and fluids[3]. It inhibits function of topoisomerase II and IV[4,5]. It has a narrow absorption window and absorbed primarily in the proximal portions of gut[6]. Moxifloxacin is commonly used to treat acute and subacute conjunctivitis, bacterial keratitis, bacterial endophthalmitis and keratoconjunctivitis[7].

Ketorolac Tromethamine is chemically 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid,2-(hydroxymethyl)-1,3propanediol and official only in USP[8](Figure 2). It has a molecular formula $C_{15}H_{13}NO_3$.C4H11NO3 with molecular weight 255.27 g/mol[9]. It has analgesic and anti-inflammatory activity [10,11]. It is administered intravenously, intramuscularly and orally as the water soluble tromethamine salt to treat moderate pain or, together with reduced opioid doses, for severe pain[12]. Ketorolac ophthalmic may be administered in conjunction with other ophthalmic medications, such as antibiotics, beta-adrenergic blocking agents, carbonic anhydrase inhibitors, cycloplegics, and mydriatics[13]. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins[14].

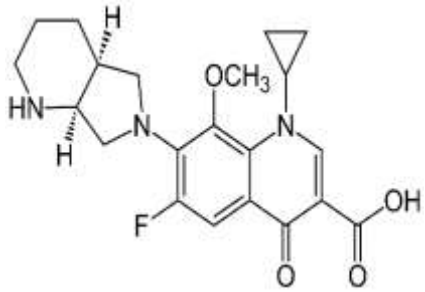

Figure 1: Structure of MXF

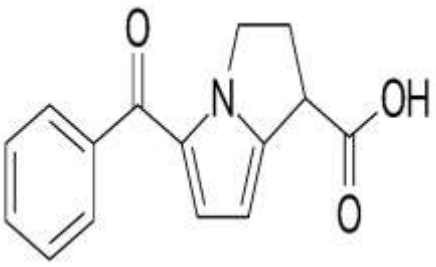

Figure 2: Structure of KTA

Combination of MXF and KTA is used for the dealing of postoperative inflammation and infection following cataract surgery[15]. A bacterial infection sign may consist of red eyes, pain, swelling of eyes, itching and blurry vision. A combination of MOXI and KETO is used as anti-infective and antiseptic in the form of eye drops[16]. MXF and KTA are official in British Pharmacopoeia (BP) and United State Pharmacopoeia but combination is not yet official in any pharmacopoeia.

Few methods such as spectroscopy[17], HPLC[18,19] HPTLC[20] and spectroscopy[21], HPLC[22], HPTLC[23] have been reported for individual drugs MXF and KTA respectively. Some of the simultaneous estimation methods of MXF and KTA includes spectroscopy[24,25], HPLC[26,27,28], LC-MS method.

Today HPTLC is fast becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major merits of HPTLC are several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus decreasing the analysis time and cost per analysis. It facilitates automated application and scanning in situ. A simple, correct, consistent and precise high performance thin layer chromatography method can be developed for simultaneous determination of MXF and KTA.

To the best of our knowledge, there is no simultaneous quantitation method available for quantitation of MXF and KTA using TLC- densitometry for ophthalmic formulation. Thus, present study was a successful effort, to develop a scientific, HPTLC method for quantitative estimation of MXF and KTA in marketed formulation as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents:

The pharmaceutical grade working standards of MXF and KTA were obtained from Micro labs, Bangalore (Karnataka, India). Fixed dose combination of ophthalmic formulation (Brand Name: APDROPS-KT^{TM}) each containing 5 mg w/v of MXF and 5 mg w/v of KTA were purchased from local pharmacy shop. Chloroform, methanol, formic acid and all other chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation:

The samples were spotted in the form of bands of width 6 mm with a Camag 100µL sample syringe (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium plate G 60 $_{F254}$ (20×10) with 250 µm thickness; (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland) as a sample applicator. A constant application rate of 0.1 µg/ spot was employed and space between two bands was 5mm. The plates were prewashed with

methanol and activated at 110^{o} C for 5 min prior to chromatography. The mobile phase consisted of chloroform: methanol: Formic acid (8.5: 1.5: 0.05 v/v/v). Linear ascending development was carried out in a 12 cm × 4.7 cm × 12.5 cm twin through glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 2) at relative humidity of 60 % ± 5. The length of chromatogram run was 8cm and approximately 10 min saturation time was kept for each chromatographic run. Following the development, the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance absorbance mode at 305 nm and operated by Win CATS software (V 1.3.0). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts**.**

Preparation of Standard Stock Solutions

10 mg of MXF and 10 mg KTA was dissolved in 10 ml methanol to obtain 1000 µg/mL as a standard stock solution. From the standard stock solutions, diluted mixed standard solutions were prepared containing 100 µg/mL for MXF and 100 µg/mL for KTA respectively, by diluting 1 ml of standard stock solution in 10 ml methanol. The stock solution was stored properly.

Chromatographic condition

The HPTLC procedure was optimized with a view to develop a simultaneous assay method for MXF and KTA dosage form respectively. The mixed standard stock solution (100 µg/mL of MXF and 100 µg/mL of KTA) was taken and sample was spotted on to HPTLC plates and run-on different solvent systems. Finally mobile phase composed of Chloroform: methanol: formic acid (8.5: 1.5: 0.05 v/v/v) was found optimum.

In order to decrease the neckless effect, HPTLC chamber was saturated for 15 min using saturation pads. The mobile phase was run which takes around 10 min for complete development of the TLC plate.

Assay of marketed formulation

To determine the content of MXF and KTA in ophthalmic drop preparation (containing 5 mg/ml of each drug). 1 ml of marketed ophthalmic formulation was pipette out and transfer to 10 ml volumetric flask and make up the volume up to mark with methanol to get concentration of 1000 µg/ml. The solution was further diluted to get concentration (100 ng/µL of each drug) was spotted for assay of MXF and KTA. Analysis was carried out at λ max 305 nm. The peak area of standard and the sample bands were compared to obtain the

concentration of ophthalmic formulation and % estimation of drug, standard deviation and % relative standard deviation was calculated.

Validation of the method

Validation of the optimized HPTLC method was carried out with respect to the evaluating parameters linearity, precision, Limit of detection, limit of quantification, recovery studies in accordance to ICH guidelines[29].

Linearity and range

From the mixed standard stock solution, 100 µg/mL of MXF and 100 µg/mL of KTA was taken, solution was spotted on TLC plate to obtain final concentration 100- 350 ng/spot for MXF and KTA. Linearity of the method was studied by applying six concentrations of the drug, each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. Calibration coefficients, slopes, and intercept were analysed from coefficient equation.

Precision

The precision of this method was verified by repeatability and intermediate precision studies. Repeatability studies were carried out by analysis of three different concentrations (100 ng/spot, 200 ng/spot and 300 ng/spot for both the drugs MXF and KTA respectively) six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for MXF and KTA by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. To determine the LOD and LOQ, sequential dilutions of mixed standard solution of MXF and KTA were prepared from the standard stock solution. The samples were applied to HPTLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank.

Recovery studies

Accuracy of the method was carried out by applying the method to drug sample (MXF and KTA combination tablet Ophthalmic drop) to which known amount of MXF and KTA standard powder corresponding to 80, 100 and 120 % of label claim had been added (standard addition method), mixed and the powder was extracted and analysed by running chromatogram in optimized mobile phase.

RESULTS AND DISCUSSION

Chromatographic method optimization for the densitometric measurements

During optimization several mobile phase compositions were tried using mixture of various polar and relatively non-polar solvents. Among several compositions of mobile phase, chloroform: methanol: Formic acid (8.5: 1.5: 0.05 v/v/v) gave better resolution and peak shape with the acceptable Rf values of 0.71±0.02 and 0.18±0.05 respectively for MXF and KTA is represented in Figure 3.

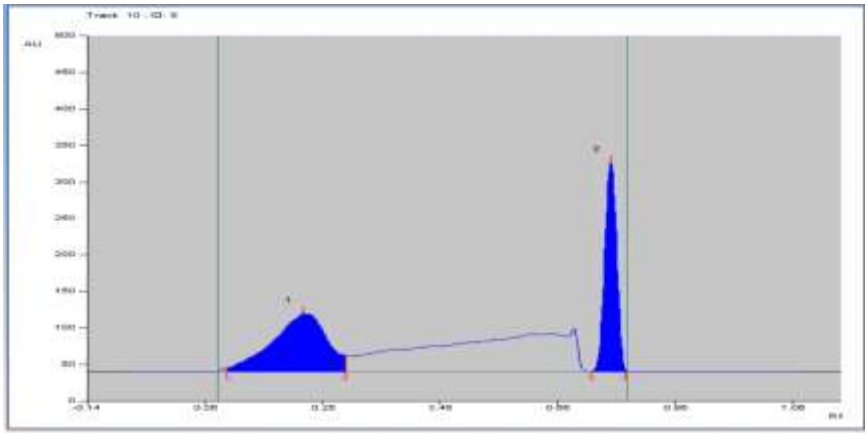

Figure 3: HPTLC Chromatogram of Marketed Formulation

Linearity

The drug response was found to be linear (regression coefficient were 0.9981 for MXF and 0.9995 for KTA) over the concentration range between 100-350 ng/spot for both MXF and KTA. The regression equations show slopes (m) and intercept(c) values 12.526 and 743.59 for MXF, 11.233 and 637.09 for KTA respectively (Figure 4 and 5). Where y indicates the peak area and x indicates concentration in ng/ml.

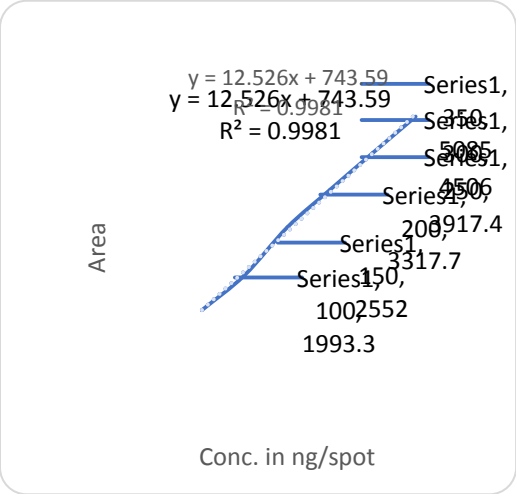

Figure 4: Calibration Graph for MXF

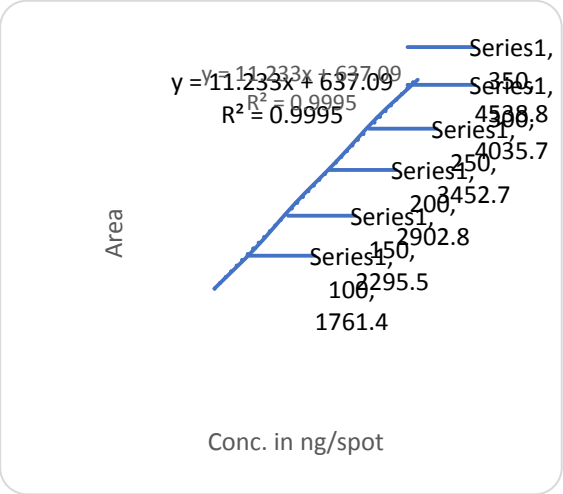

Figure 5: Calibration Graph for KTA

Precision

Precision was determined by studying the intermediate precision and repeatability over the concentration range of 100 ng/spot to 300 ng/spot for both drugs MXF and KTA. The results of the repeatability and intermediate precision experiments are shown in **Table I**. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, as recommended by ICH guidelines.

Table I: Precision Study

Intraday study			**Inter Day study**		
Drug	Concentration in ng/spot	% RSD	Drug	Concentration in ng/spot	% RSD
MXF	100	0.72	MXF	100	1.15
	200	0.60		200	0.95
	300	1.01		300	1.10
KTA	100	0.89	KTA	100	0.85
	200	1.02		200	0.95
	300	1.09		300	1.12

LOD and LOQ

The LOD and LOQ were found to be 8.17 ug/ml and 24.76 ug/ml for MXF, 17.63 ug/ml and 53.42 ug/ml for KTA, respectively. Signal-to-noise ratio for LOD and LOQ found to be 3:1 and 10:1 respectively.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2. The obtained values of % RSD, as shown in **Table-II,** indicated the robustness of the method.

Recovery studies

As shown from the data in **Table II** good recoveries of the MXF and KTA in the range from 99.63 % w/w to 100.11 % w/w were obtained at various added concentrations. The study showed that the results within acceptable limit of above 99 % and below 101% and lower values of RSD indicate the proposed method is accurate. The percentage recovery shows the method is free from interference of the excipients used in the formulations.

Table II: Recovery studies

Level of Recovery	Drug	Amount of drug applied ng/spot	% Recovery	S.D.	% RSD
80 %	MXF	80	100.11	± 0.94	0.93
	KTA	80	99.88	± 0.65	0.65
100%	MXF	100	99.63	± 0.58	0.58
	KTA	100	99.80	± 0.45	0.45
120%	MXF	120	99.70	± 0.96	0.96
	KTA	120	99.77	± 0.98	0.98

Repeatability studies

Repeatability was assessed by spotting MXF (2 ul) and KTA (2 ul) concentration of drug solution 5 times on a TLC plate followed by development of plate and recording peaks area for 5 spots **(Table- III).**

Table III: Repeatability Studies

Drug	Applied amount(ul)	No. of scan	% RSD of sample application	% RSD of measurements
MXF	2	1	0.78	-
KTA	2	1	0.62	-
MXF	2	5	-	0.92
KTA	2	5	-	0.81

Assay of marketed formulation

The commercial eye drop formulation, (APDROPS-KT^{TM}) was quantitatively determined using the developed HPTLC method. The analysis of marketed formulation was based on comparing the mean peak area of standard band with that of sample peak area. The results obtained after formulation analysis were in good agreement with those of label claim. The result and statistical analysis is presented in **Table IV.**

Table IV: Assay of commercial ophthalmic preparation

Drug	Label Claim mg/ml	Amount of drug estimated	% Label claim	Standard Deviation	% RSD
MXF	5	4.96	99.2	±0.85	0.82
KTA	5	5.02	100.4	±0.67	0.45

CONCLUSION

A simple, sensitive and selective validated HPTLC method has been developed as per ICH guidelines for estimation of MXF and KTA in bulk drug and pharmaceutical formulation. The validation study proved that the developed method is precise, specific and accurate. Specificity study proved that the method is suitable for the analysis of MXF and KTA as bulk drug and in pharmaceutical formulation without any interference from the excipients. 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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