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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN & BROMFENAC BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Moxifloxacin and Bromfenac in Tablet dosage form. Chromatogram was run through STD BDS 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1%OPA: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1%OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 275 nm. Retention time of Moxifloxacin and Bromfenac were found to be 2.102 min and 3.188. %RSD of the Moxifloxacin and Bromfenac were and found to be 1.3 and 0.4 respectively. %Recovery was obtained as 99.90% and 99.51% for Moxifloxacin and Bromfenac respectively. LOD, LOQ values obtained from regression equations of Moxifloxacin and Bromfenac were 0.09, 0.05 and 0.26, 0.16 respectively. Regression equation of Moxifloxacin is y = 14556x + 7263, y = 7758.x + 454.1 of Bromfenac. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Moxifloxacin, Bromfenac, RP-HPLC.

INTRODUCTION

Chemically Moxifloxacin (MXF) the bactericidal action of moxifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known to play a key role in the partitioning of the chromosomal DNA during bacterial cell division. Structure of the MXF was shown in figure 1 (A).[1]

Chemically Bromfenac (BRF) the mechanism of its action is thought to be due to its ability to block prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2. Prostaglandins have been shown in many animal models to be mediators of certain kinds of intraocular inflammation. In studies performed in animal eyes, prostaglandins have been shown to produce disruption of the blood-aqueous humor barrier, vasodilation, increased vascular permeability, leukocytosis, and increased intraocular pressure.Structure of the BRF was shown in figure 1 (B).[2]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of MXF and BRF, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

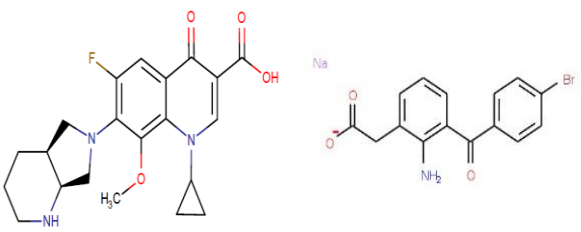

Figure 1: Structure of (A)Moxifloxacin (B) Bromofenac.

MATERIALS AND METHODS

Reagents and Chemicals: Moxifloxacin and Bromfenac pure drugs (API), Combination Moxifloxacin and Bromfenac Eye drops(M Bromica), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, BDS C18 (4.6 x 150mm, 5µm). As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The BDS C18 (4.6 x 150mm, 5µm) column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of OPA (0.1%) and Acetonitrile was taken in the ratio of (55:45%v/v) mobile phase for the investigation with a flow rate of a 1 ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 275nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Buffer: Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water

***Preparation of mixture Standard stock solution*:** Accurately Weighed and transferred 25mg & 4.5mg of Moxifloxacine and Bromofenac working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution. (2500µg/ml of Moxifloxacin and 450µg/ml Bromfenac)

Preparation of Sample (Tablet) stock solutions: A Test solution containing 250µg/ml of MOXI (45µg/ml of BROMO) was prepared by appropriate dilution of the sample stock solution (containing 0.5% of Moxifloxacin hydrochloride and 0.09% of Bromofenac sodium as per the label claim).

Optimized chromatographic conditions

Column Used : BDS C18 (4.6 x 150mm, 5µm)
Mobile phase : 55% OPA (0.1%): 45% Acetonitrile
Flow rate : 1ml/min
Wavelength : 275.0 nm
Temperature : 30◦C
Injection Volume : 10.0µl

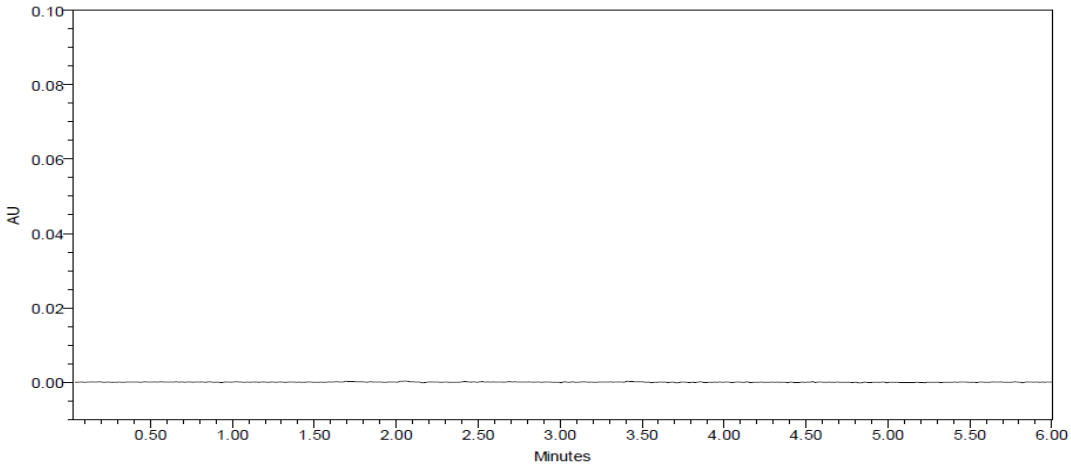

Figure 2: Blank chromatogram.

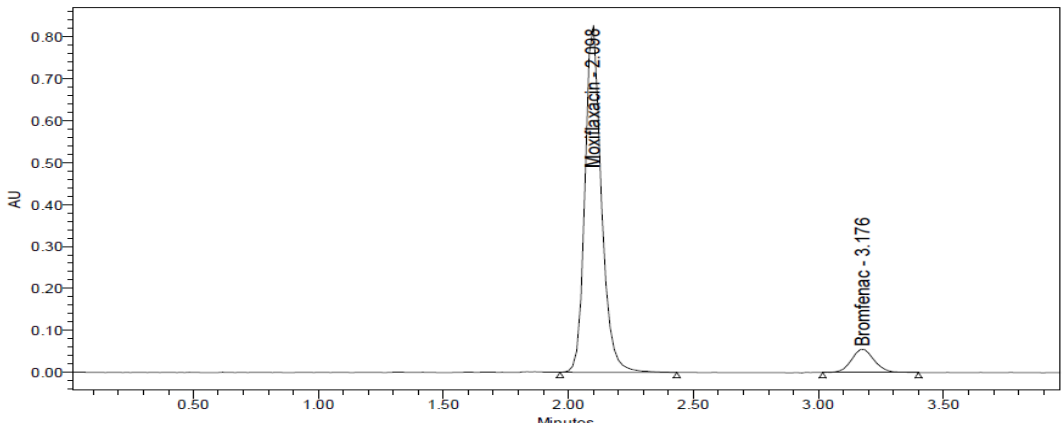

Figure 3: Chromatogram of standard mixture of MXF & BRF.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Moxifloxacin	2.098	3633078	1.20	6	5371
2	Bromfenac	3.176	344593	1.09	7.5	6022

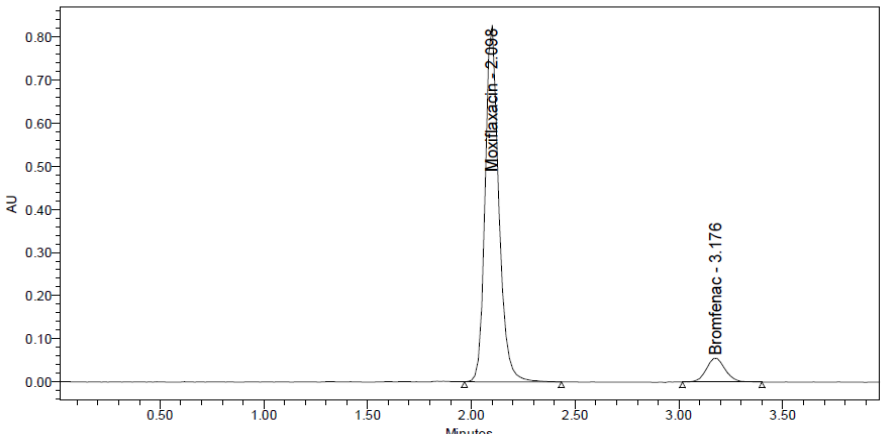

Figure 4: Chromatogram of sample mixture of MXF & BRF.

VALIDATION

The above optimized chromatographic method has been validated for the assay of MXF & BRF using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Moxifloxacin and Bromfenac (MXF & BRF)drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Moxifloxacin (25mg)+ Bromfenac (4.5mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Moxifloxacin (25mg)+ Bromfenac (4.5mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Moxifloxacin and Bromfenac. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for MXF was found to be y = 14556x + 7263 (slope, intercept and correlation coefficient were found to be 14556, 7263 and 0.999 respectively) and linear over beer's range of 62.5-375 µg/ml. The regression equation for BRF was found to be y = 7758x + 454.1 (slope, intercept and correlation coefficient were found to be 7758, 454.1 and 0.999 respectively) and linear over beer's range of 11.25-67.5µg/ml. Linearity graph of MXF & BRF were shown in Figure 5 & 6 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Moxifloxacin and Bromfenac were and found to be 1.3 and 0.4 respectively. %RSD of method precision for Moxifloxacin and Bromfenac were and found to be 0.8 and 0.5 respectively. % recovery was obtained as 100.23% and 100.21% for Moxifloxacin and Bromfenac respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug MXF & BRF and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.90% and 99.51% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for MXF & BRF was found to be 0.09µg/ml and 0.05µg/ml respectively. LOQ for MXF & BRF was found to be 0.26µg/ml and 0.16µg/ml respectively. Summary of all the validation parameter shown in table 3.

DEGRADATION

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Moxifloxacin and Bromfenac in Tablet dosage form was developed and the

proposed method as suitable for routine analysis of MXF & BRF.

Table 1: Linearity table for MXF & BRF.

Moxifloxacin		Bromfenac	
Conc (µg/mL)	Peak Area	Conc (µg/mL)	Peak area
0	0	0	0
62.5	905100	62.5	905100
125	1859127	125	1859127
187.5	2728211	187.5	2728211
250	3656959	250	3656959
312.5	4532964	312.5	4532964
375	5473079	375	5473079

Table 2: System precision table of MXF and BRF.

S. No	Area of Moxifloxacin	Area of Bromfenac
1.	3616686	343573
2.	3644357	346129
3.	3689188	342730
4.	3614120	347143
5.	3627632	344933
6.	3606486	343047
Mean	3633078	344593
S.D	30464.6	1782.1
%RSD	0.8	0.5

Table 3: summary of validation data of MXF & BRF.

Parameters		Moxifloxacin	Bromfenac	LIMIT
Linearity Range (µg/ml)		62.5-375 µg/ml	11.25-67.5µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		14556	7758	
Intercept(c)		7263	454.1	
Regression equation (Y=mx+c)		y = 14556x + 7263	y = 7758.x + 454.1	
Assay (% mean assay)		99.90%	99.51%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		1.3	0.4	NMT 2.0%
Method precision %RSD		0.8	0.5	NMT 2.0%
Accuracy %recovery		100.23%	100.21%	98-102%
LOD		0.09	0.05	NMT 3
LOQ		0.26	0.16	NMT 10
Robustness	**FM**	0.2	0.4	%RSD NMT 2.0
	FP	0.4	0.3	
	MM	1.0	0.8	
	MP	0.5	1.0	
	TM	0.6	1.7	
	TP	0.6	1.5	

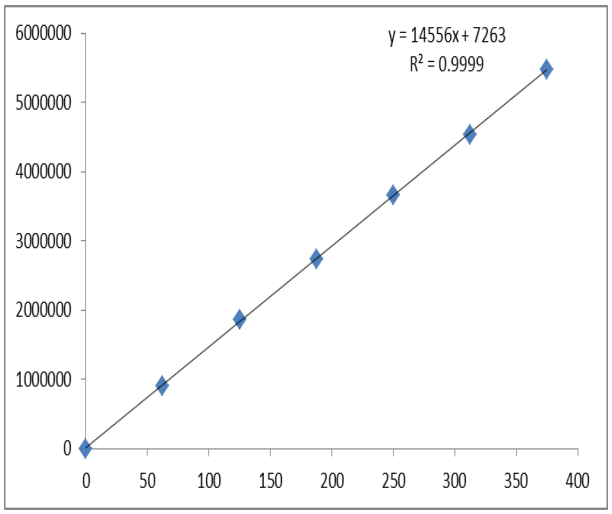

Fig No. 7: Linearity curve of Moxifloxacin.

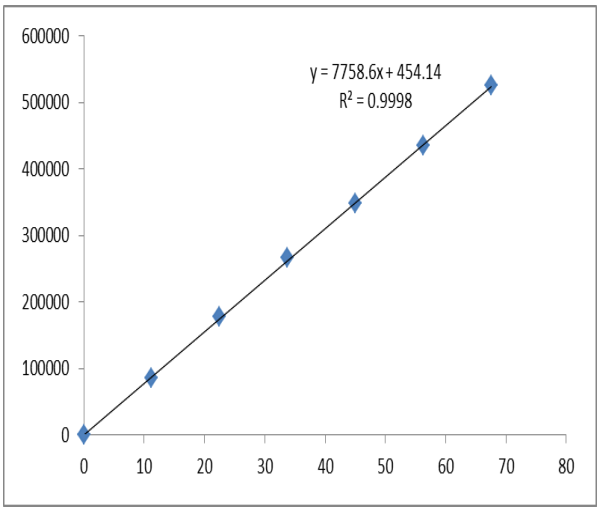

Fig No. 8: Linearity curve of Bromfenac.

Table no. 4: Degradation Data of Moxifloxacin.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.30	0.278	0.446
2	Alkali	4.86	0.340	0.530
3	Oxidation	3.34	0.240	0.405
4	Thermal	1.19	0.261	0.365
5	UV	2.63	0.250	0.372
6	Water	0.80	0.276	0.371

Table no.5: Degradation Data of Bromfenac

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.01	6.166	7.802
2	Alkali	3.43	9.865	11.905
3	Oxidation	4.86	4.166	4.736
4	Thermal	3.48	2.547	2.904
5	UV	3.74	2.225	3.128
6	Water	0.89	2.764	3.116

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