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### RP-UPLC method development and validation for the simultaneous estimation of Moxifloxacin and Bromfenac in bulk and pharmaceutical dosage form

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#### ABSTRACT

The objective of the present study is to develop Accurate, precise, simple and reliable RP-UPLC method for the simultaneous estimation of the Moxifloxacin and Bromfenac in pharmaceutical dosage form. Mobile phase with 0.1% Ortho phosphoric: Acetonitrile in the ratio of 55:45 was pumped through SB C8 100 x 3 mm, 1.8 $\mu$  column at a rate of 0.3ml/min at a temperature of 30°C and optimized wavelength was 275nm. Retention time of 1.221min and 1.901 min and %RSD of 0.8% and 0.5% and %Recovery of 100.23% and 100.21% were obtained for Moxifloxacin and Bromfenac, respectively. The Limit of Detection (LOD) and Limit of quantification (LOQ) values obtained from regression equations of Moxifloxacin and Bromfenac were 0.09, 0.26 and 0.05, 0.16, respectively. Regression equation of Moxifloxacin is  $y = 14556x + 7263$ , and  $y = 7758x + 454.1$  of Bromfenac. Retention times and run time were decreased and the Recovery percentage was high which indicates Accuracy of Method, so the method was precise and reliable which is also economical that can be apt to be adopted in regular Quality control test in Industries.

**Keywords:** Moxifloxacin, Bromfenac, UPLC

#### INTRODUCTION

Bromfenac is a non-steroidal anti-inflammatory drug (NSAID) for ophthalmic use. Ophthalmic NSAIDs are becoming a cornerstone for the management of ocular pain and inflammation [1-2]. Their well-characterized anti-inflammatory activity, analgesic property, and established safety record have also made NSAIDs an important tool to optimize surgical outcomes. Bromfenac ophthalmic solution 0.09% (Xibrom, Senju Pharmaceuticals, Japan) is a sterile topical NSAID for ophthalmic

use. Each milliliter of Xibrom contains 1.035 mg bromfenac sodium (equivalent to 0.9 mg bromfenac free acid). Bromfenac sodium is designated chemically as sodium 2-amino-3-(4-bromobenzoyl) phenylacetate sesquihydrate, with an empirical formula of  $C_{15}H_{11}BrNNaO_3$  and molecular weight of 383.17. The osmolality of Xibrom ophthalmic solution is approximately 300 mOsmol/kg. The commercially available formulation is buffered to pH 8.3 and contains polysorbate 80 as solubilizer and benzalkonium chloride (0.005%) as preservative. It shows good

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ocular penetration, and significant amounts are also absorbed systemically after topical administration. Moxifloxacin, sold under the brandname Avelox among others, is an antibiotic used to treat a number of bacterial infections.[3] This

includes pneumonia, conjunctivitis, endocarditis, tuberculosis, and sinusitis. [4-5] It is used by mouth, by injection into a vein, or as an eye drop. Common side effects include diarrhea, dizziness, and headache. Severe side effects may include spontaneous tendon ruptures, nerve damage, and worsening of myasthenia gravis. Safety of use in pregnancy or breastfeeding is unclear.<sup>[4]</sup> Moxifloxacin is in the fluoroquinolone family of medications. It usually results in bacterial death through blocking their ability to duplicate DNA. Moxifloxacin was approved for use in the United States in 1999. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.<sup>1</sup> The wholesale cost in the developing world is 0.45 USD to 2.70 USD per day, as of 2015. In the United States, as of 2017, the wholesale cost is about 4.00 USD per day. The literature survey was carried out for the simultaneous estimation of Moxifloxacin and Bromfenac, few analytical methods are methods available for Montelukast and Ebastine individually and in combination with other drugs [6-12]. According to literature survey there is no official method for the estimation of Moxifloxacin and Bromfenac by ultra performance liquid chromatography (UPLC) in pharmaceutical dosage forms. Hence, an attempt has been made to develop new method for the simultaneous estimation and validation of Moxifloxacin and Bromfenac in pharmaceutical formulation in accordance with the ICH guidelines.

## MATERIALS AND METHODS

### Chemicals and Reagents

Moxifloxacin and Bromfenac pure drugs (API) were obtained from spectrum Pharma research solutions and DUOBROM Eye drops was purchased from local Pharmacy store. All the chemicals and solvents like Distilled water, Acetonitrile, Phosphate buffer, Methanol,

Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid are from RANKEM-Mumbai.

### Instruments and Chromatographic Conditions

Electronics Balance-Denver, P<sup>H</sup> meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS UPLC Acquity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Moxifloxacin and Bromfenac solutions. The mobile phase used was 0.1% Ortho phosphoric: Acetonitrile in the ratio of 55:45 run through SB C8 100 x 3 mm, 1.8 $\mu$ .column at a rate of 0.3ml/min. for 3 min at Temperature 30°C and Optimized wavelength was 275nm at the injection volume of 3 $\mu$ L.

### Preparation of Solvents and Solutions

#### Diluent

Diluent was selected Based on the solubility of the drugs, Water: Acetonitrile (50:50) were taken as diluent.

#### Preparation of 0.1% Ortho phosphoric acid Buffer

0.3ml of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

#### Preparation of Mobile Phase

Mobile phase was prepared by mixing 0.1% Ortho phosphoric: Acetonitrile in the ratio of 55:45 and sonicated using ultrasonic bath to degas and subjected to vacuum filtration with 0.45 $\mu$  Millipore Nylon filter.

### Preparation of Standard stock solutions

Accurately Weighed and transferred 25mg of Moxifloxacin and 4.5mg of Bromfenac working Standards into a 10 ml&10ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

### Preparation of Standard working solutions (100% solution)

Accurately about 0.3ml of above each stock solution was pipetted out and transferred into a 10ml volumetric flask and the final volume was made up with diluent. (250 µg/ml of Moxifloxacin and 45 µg/ml of Bromfenac).

### Preparation of Sample stock solutions

An Accurately measured volume of ophthalmic solution equivalent to (DUOBROM (Eye) (5 ml)) equivalent to 25 mg and 4.5mg of Moxifloxacin and Bromfenac, respectively transferred into a 25ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters (1000 µg/ml of Moxifloxacin and 180 µg/ml of Bromfenac). 2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (250 µg/ml of Moxifloxacin and 45 µg/ml of Bromfenac).

### Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

### Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

### Linearity

**25% Standard solution:** 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (62.5 µg/ml of Moxifloxacin and 11.25 µg/ml of Bromfenac)

**50% Standard solution:** 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (125 µg/ml of Moxifloxacin and 22.5 µg/ml of Bromfenac)

**75% Standard solution:** 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (187.5 µg/ml of Moxifloxacin and 33.8 µg/ml of Bromfenac)

**100% Standard solution:** 1.0ml each from two standard stock solutions was pipetted out and made up

to 10ml. (250 µg/ml of Moxifloxacin and 45 µg/ml of Bromfenac)

**125% Standard solution:** 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (312.5 µg/ml of Moxifloxacin and 56.25 µg/ml of Bromfenac)

**150% Standard solution:** 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (375 µg/ml of Moxifloxacin and 67.5 µg/ml of Bromfenac)

### Accuracy

**Preparation of Standard stock solutions:** Accurately Weighed and transferred 25mg of Moxifloxacin and 4.5mg of Bromfenac working Standards into a 10 ml & 10ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

**Preparation of 50% Spiked Solution:** 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 100% Spiked Solution:** 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 150% Spiked Solution:** 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

### Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (60:40) mobile phase plus (50:50) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

### LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.03ml each of Moxifloxacin and Bromfenac solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

### LOQ sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Moxifloxacin and Bromfenac solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent. Samples were injected in duplicates.

### System Suitability

By preparing standard solutions of Moxifloxacin (250ppm) and Bromfenac (45ppm) the system suitability parameters were determined the solutions were injected six times and the parameters like peak tailing, resolution and the USP theoretical plate count were assessed to check whether the results complies with Recommended limits.

### Assay of Moxifloxacin and Bromfenac

Sample solutions were injected in to the UPLC system and scanned at 275 nm from which the % of drug was estimated.

## RESULTS & DISCUSSIONS

### Optimization of Chromatographic Conditions

To develop and establish a suitable RP-UPLC method for simultaneous estimation of Moxifloxacin and Bromfenac in bulk and Pharmaceutical dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1. The final analysis was performed by using 55% Ortho phosphoric acid:45% Acetonitrile at a flow rate of 1.0 ml/min. samples were analyzed at 275 nm detector wave length and at an injection volume of 3  $\mu$ L using

discovery SB C8 100 x 3 mm, 1.8m. column with run time of 3 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Moxifloxacin and Bromfenac, the optimized chromatogram was obtained as shown in (Figure-3).

### Chromatographic conditions

<b>Flow rate</b>	: 0.3ml/min
<b>Column</b>	: SB C8 100 x 3 mm, 1.8 $\mu$ .
<b>Detector wave length</b>	: 275nm
<b>Column temperature</b>	: 30°C
<b>Injection volume</b>	: 3 $\mu$ L
<b>Run time</b>	: 3min
<b>Diluent</b>	: Water:Acetonitrile (50:50)

### Validation

Linearity was established for Moxifloxacin (62.5-375 $\mu$ g/ml) and Bromfenac (11.25-67.5 $\mu$ g/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as  $y = 14556x + 7263$  for Moxifloxacin and  $y = 7758x + 454.1$  for Bromfenac, Correlation coefficient ( $R^2$ ) was determined as 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4&5) for Moxifloxacin and Bromfenac respectively. Retention times of Moxifloxacin and Bromfenac were 1.221 min and 1.901 min respectively. Where no interfering peaks in blank and placebo at retention times of these drugs were not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and Triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 100.23% and 100.21% for Moxifloxacin and Bromfenac respectively were shown in (Table-2). % RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Moxifloxacin and Bromfenac the repeatability was obtained as 0.8% and 0.5% respectively for Moxifloxacin and Bromfenac and the % RSD for intermediate Precision was obtained as 1.5%, 0.6% for Moxifloxacin and Bromfenac, Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard

deviation of response and slope of the calibration curve Moxifloxacin and Bromfenac. The detection limit values were obtained as 0.09 and 0.26 and Quantitation limit were found to be 0.05 and 0.16 for Moxifloxacin and Bromfenac respectively as given in (Table-4).

Robustness of the method to study the effect of Robustness conditions like Flow minus 0.27ml/min, Flow plus 0.33ml/min, mobile phase minus 60B:40A, mobile phase plus 50B:50A, temperature minus 25°C and temperature plus 35°C was maintained and samples were injected in duplicates. %RSD was within the limit as shown in (Table-5). The system suitability parameters like Retention time, Resolution, USP plate count and peak asymmetry or Tailing evaluated to check whether the results comply the prescribed limits and shown in (Table-6). The assay of the marketed

product An Accurately measured volume of ophthalmic solution equivalent to 25 mg and 4.5mg of Moxifloxacin and Bromfenac respectively was used to perform assay and the Average % of drug was found to be 99.90 and 99.51% for Moxifloxacin and Bromfenac respectively the results were shown in (Table-7) and the chromatograms for Moxifloxacin and Bromfenac standard drugs and ophthalmic solution dosage forms were shown in (Figure-6, 7) respectively.

### Degradation Studies

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 (Table 8&9).

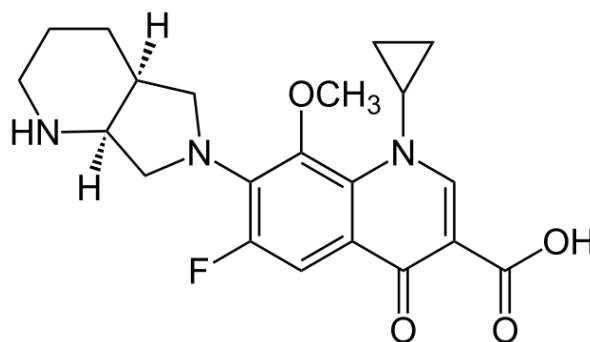


Figure-1: Chemical Structure of Moxifloxacin

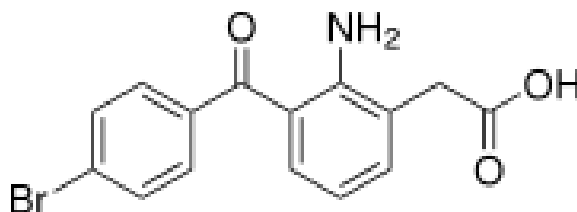


Figure-2: Chemical Structure of Bromfenac

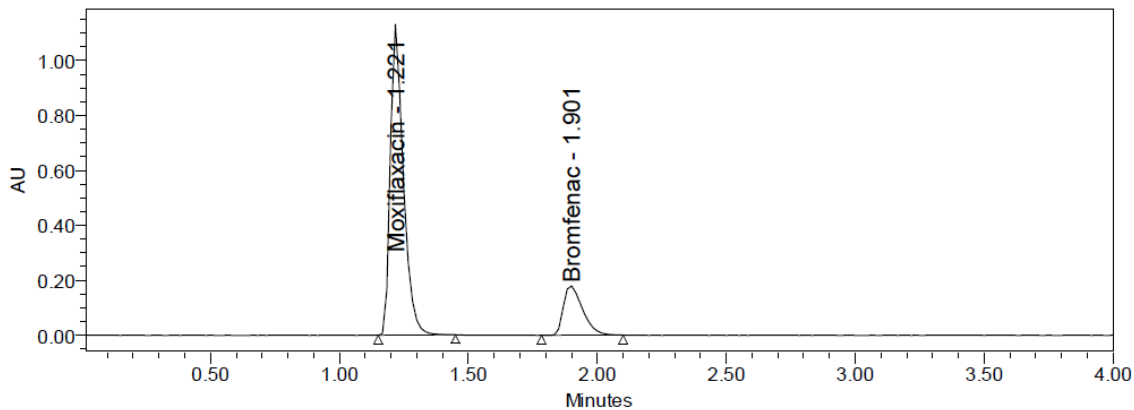


Figure-3: Optimized Chromatogram of Moxifloxacin and Bromfenac

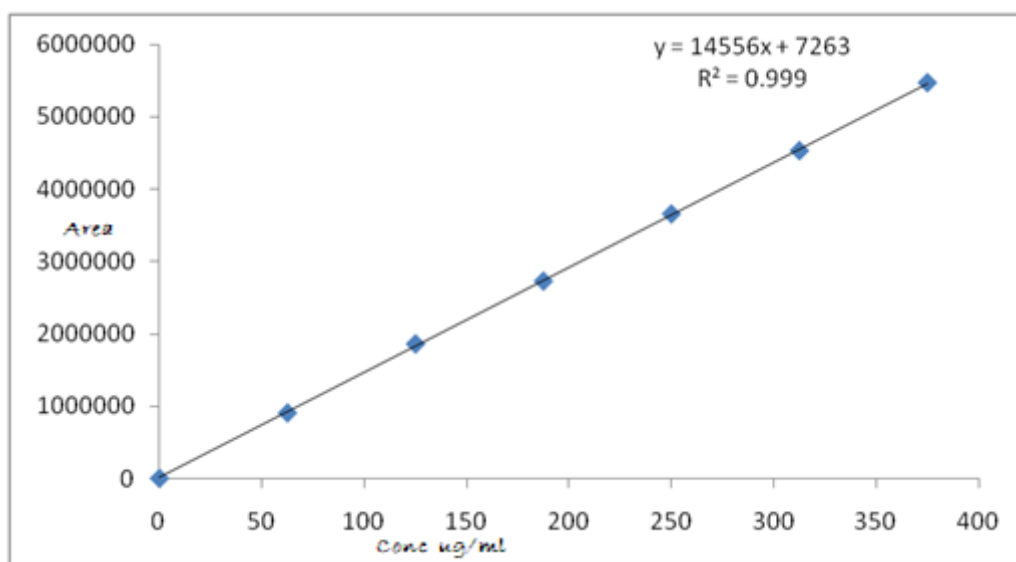


Figure-4: Linearity Curve of Moxifloxacin

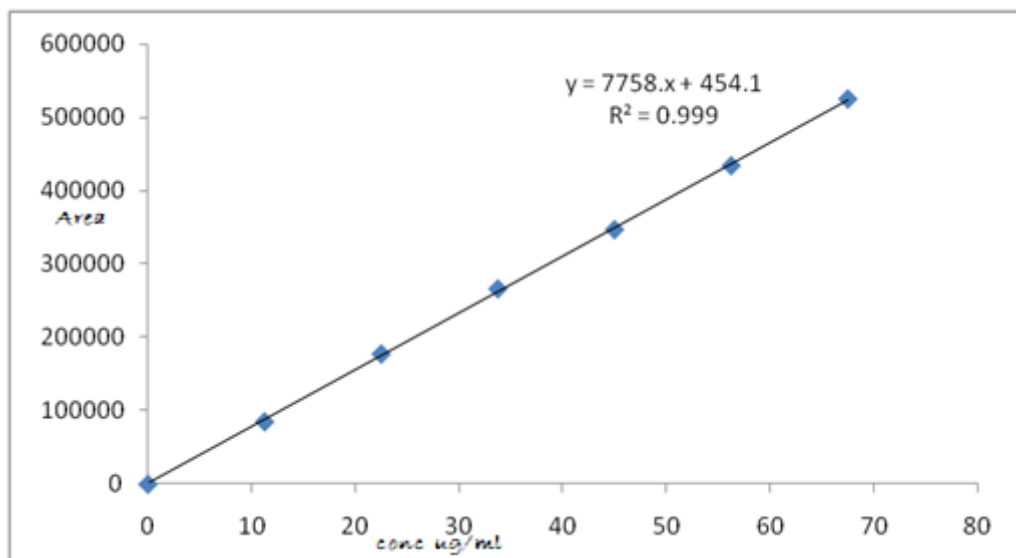


Figure-5: Calibration Curve of Bromfenac

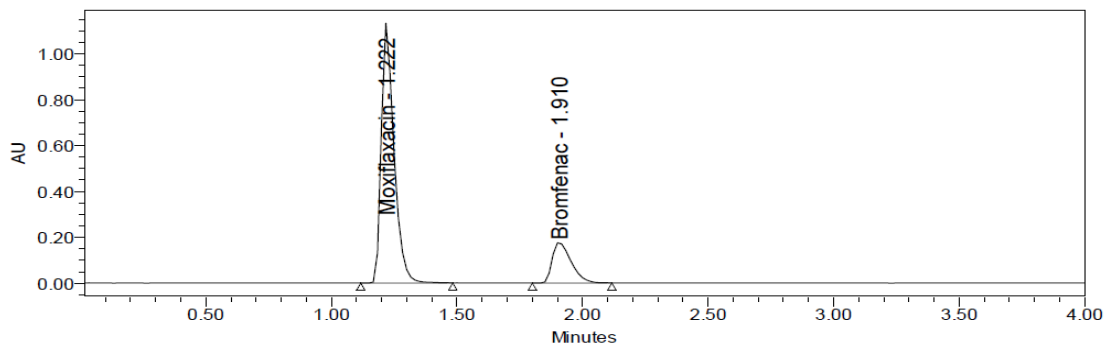


Figure-6: Standard Chromatogram of Moxifloxacin and Bromfenac

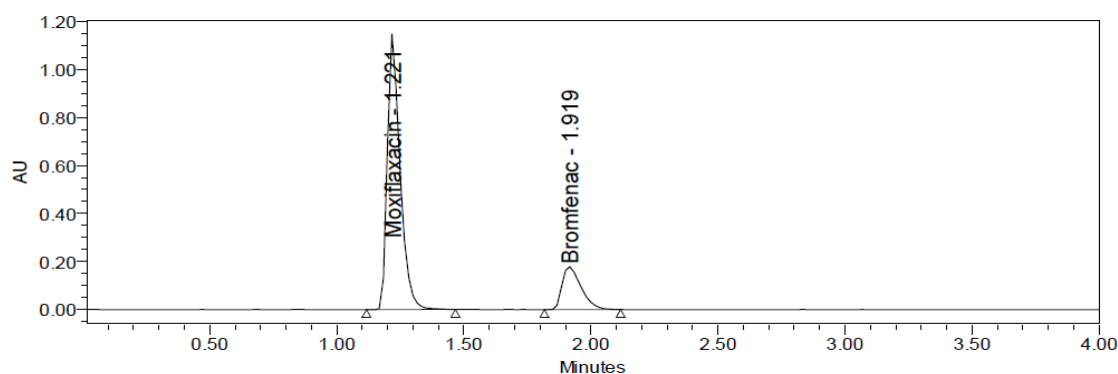


Figure-7: A Sample Chromatogram of Moxifloxacin and Bromfenac Pharmaceutical Dosage Form

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-UPLC	WATERS UPLC SYSTEM equipped with quaternary pumps with TUV detector
Mobile phase	Buffer and ACN: taken in the ratio 55:45.
Flow rate	0.3ml/min
Column	SB C8 100 x 3 mm, 1.8 $\mu$ .
Detector wave leng	275nm
Column temperatu	30°C
Injection volume	3 $\mu$ L
Run time	3 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Moxifloxacin 1.221 min and Bromfenac 1.901 m
Theoretical Plates	Moxifloxacin 2617 and Bromfenac 2856

Table-2: Accuracy results of Moxifloxacin and Bromfenac

Conc.	Moxifloxacin			Bromfenac		
	Amount added ( $\mu$ g/ml)	Amount recovered ( $\mu$ g/ml)	% Recovery	Amount added ( $\mu$ g/ml)	Amount recovered ( $\mu$ g/ml)	% Recovery
50%	125	126.05	100.84	22.5	22.29	99.06
	125	125.00	100.00	22.5	22.72	100.97
	125	124.55	99.64	22.5	22.50	99.99

	250	248.76	99.50	45	45.56	101.25
100%	250	247.92	99.17	45	45.00	100.00
	250	249.49	99.80	45	45.26	100.58
	375	377.77	100.74	67.5	67.64	100.21
150%	375	378.64	100.97	67.5	67.88	100.56
	375	380.43	101.45	67.5	67.02	99.28
<b>Mean % Recovery</b>			<b>100.23%</b>	<b>Mean % Recovery</b>		<b>100.21%</b>

**Table-3: Precision Results of Moxifloxacin and Bromfenac**

S.No	Repeatability		Intermediate precision	
	Area of Moxifloxacin	Area of Bromfenac	Area of Moxifloxacin	Area of Bromfenac
1.	3616686	343573	3553380	340676
2.	3644357	346129	3555806	341627
3.	3689188	342730	3650588	337395
4.	3614120	347143	3531203	343987
5.	3627632	344933	3530178	340976
6.	3606486	343047	3637075	339774
<b>Mean</b>	3633078	344593	3576372	340739
<b>S.D</b>	30464.6	1782.1	53513.7	2168.5
%RSD	0.8	0.5	1.5	0.6

**Table-4: LOD and LOQ values of Moxifloxacin and Bromfenac**

Molecule	LO	LOQ
Moxifloxacin	0.0	0.2
Bromfenac	0.0	0.1

**Table-5 Robustness Data of Moxifloxacin and Bromfenac**

S.no.	Condition	%RSD of Moxifloxacin	%RSD of Bromfenac
1	Flow rate (-) 0.9ml/min	0.6	0.9
2	Flow rate (+) 1.0.3ml/min	0.8	0.9
3	Mobile phase (-) 35B:65A	0.7	0.7
4	Mobile phase (+) 45B:55A	0.8	1.1
5	Temperature (-) 25°C	0.3	0.5
6	Temperature (+) 35°C	1.1	1.1



**Table-6: System Suitability Parameters Results of Moxifloxacin and Bromfenac**

S no	Moxifloxacin			Bromfenac				
	Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	1.217	2617		1.31	1.901	2856	1.41	5.8
2	1.220	2629		1.31	1.902	2950	1.41	5.8
3	1.221	2635		1.31	1.907	3182	1.51	5.8
4	1.222	2477		1.32	1.910	3015	1.50	5.7
5	1.233	2659		1.32	1.925	3083	1.53	5.8
6	1.234	2655		1.31	1.931	3001	1.43	5.9

**Figure-7: Assay Results of Moxifloxacin and Bromfenac**

S.No	Moxifloxacin			Bromfenac		
	Standard Area	Sample area	% of Drug	Standard Area	Sample area	% of Drug
1.	3594213	3616686	99.45	347112	343573	99.22
2.	3697170	3644357	100.21	347288	346129	99.95
3.	3607220	3689188	101.45	344762	342730	98.97
4.	3602142	3614120	99.38	345094	347143	100.25
5.	3597103	3627632	99.75	343661	344933	99.61
6.	3678079	3606486	99.17	345650	343047	99.06
<b>Mean</b>	3629321	3633078	99.90	345595	344593	99.51
<b>S.D</b>	45778.9	30464.6	0.84	1404.0	1782.1	0.5
<b>%RSD</b>	1.3	0.8	0.84	0.4	0.5	0.5

**Table 6.12 Degradation Data of Moxifloxacin**

S.NO	Degradation Condition	% Drug Degraded
1	Acid	5.30
2	Alkali	4.86
3	Oxidation	3.34
4	Thermal	1.19
5	UV	2.63
6	Water	0.80

**Table 6.13 Degradation Data of Bromfenac**

S.NO	Degradation Condition	% Drug Degraded
1	Acid	6.01
2	Alkali	3.43
3	Oxidation	4.86
4	Thermal	3.48
5	UV	3.74
6	Water	0.89

## CONCLUSION

A new Accurate, Precise, Simple and reliable method for the simultaneous estimation of the Moxifloxacin and Bromfenac in Pharmaceutical Dosage Form has been developed. The method developed was validated and was found to be sensitive, accurate, precise and reliable for the analysis of Moxifloxacin and Bromfenac in Bulk and Pharmaceutical dosage forms. The Results obtained were within the prescribed limits of ICH

Guidelines and shown accuracy and preciseness of the method developed. As the Retention times were decreased and that run time was less the method can be effectively adopted in regular quality control testing in industries which is also economical too. Finally it can be concluded from the results that the method developed was simple and accurate with robust and reliability as added values to the method.

## REFERENCES

- [1]. Jones J, Francis P. Ophthalmic utility of topical bromfenac, a twice-daily nonsteroidal anti-inflammatory agent. *Expert Opin Pharmacother.* 10(14), 2009, 2379-85.
- [2]. Hoy SM. Bromfenac Ophthalmic Solution 0.07%: A Review of Its Use After Cataract Surgery. *Clin Drug Investig.* 35(8), 2015, 525-9.
- [3]. <https://en.wikipedia.org/wiki/Moxifloxacin>
- [4]. <http://www.rxlist.com/jardiance-drug/overdosage-contraindications.html>
- [5]. <http://www.news-medical.net/health/Moxifloxacin-Mechanism.aspx>
- [6]. Lakshmi Sushma B, Madhusudhan G, Jayashree A, Srinivasulu P, Koti Reddy Y. Development and validation of stability indicating UPLC method for the quantitative determination of related substances in moxifloxacin hydrochloride. *Journal of Pharmacy Research.* 9(4), 2015, 211-216.
- [7]. Koppala S, Reddy VR, Anireddy JS. Development and Validation of a New Stability-Indicating RP-UPLC Method for the Quantitative Determination of Bromfenac Sodium and Its Impurities in an Ophthalmic Dosage Form. *J Chromatogr Sci.* 54(9), 2016, 1514-1521
- [8]. Desai SP, Kalaveni KJ, Malairajan P. Development and Validation for the Simultaneous Estimation of Moxifloxacin Hydrochloride and Bromfenac Sodium by RP- HPLC. *Journal of Pharmacy Research.* 9(2), 2015, 129-133.
- [9]. Shirisha V, Sunil Kumar Chaitanya P, Rohini Reddy G and Deepthi Reddy K et al., Development and validation of an analytical method for the simultaneous quantification of Moxifloxacin and Bromofenac ophthalmic solution by using RP-HPLC. *Der Pharmacia Lettre.* 7(7), 2015, 29-41.
- [10]. Shine Sudev, Vishnumanikandan N, Sapna Shrikumar, Ajmal Shareef K, Najuma salim. Simultaneous HPLC method development and validation of moxifloxacin hydrochloride and bromofenac sodium in pharmaceutical formulation. *Int. J. of Pharmacy and Analytical Research.* 4(1), 2015, 75-2
- [11]. Shuchi Desai, Hitesh Dalwadi, Kesha Desai et al. Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Moxifloxacin Hydrochloride and Bromfenac Sodium in Combined Dosage Form. *J Pharm Sci Bioscientific Res.* 5(6), 2015, 572-578.
- [12]. Haritha Reddy N, Samidha T, Sushma E, Sreekanth G. Simple RP-HPLC method development and validation for simultaneous estimation of moxifloxacin hydrochloride and bromofenac sodium in eye drops. *Intl J Pharma Pharmaceu Sci.* 5, 2013, 689-698.