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Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Moxifloxacin Hydrochoride and Bromfenac Sodium in Combined Dosage Form

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ABSTRACT:

A newer, simple, accurate and sensitive Absorbance ratio method is developed for the simulataneous estimstion of Moxofloxacin Hydrochloride (MOX) and Bromfenac sodium (BROM) in combined dosage form. In Absorbance ratio method MOX and BROM both obeyed Beer's law in the concentration range of 1 - 16 µg/ml. Absorbance ratio method was developed using two wavelenghts which are 275 nm (isobestic point) and 291 nm (λmax of MOX). Methanol:water (10:90 v/v) was used as a solvent. The results of the analysis were analyzed and validated statistically and recovery studies were carried out as per ICH guidelines. It can be used for routine analysis of both drugs in bulk as well as in pharmaceutical formulations.

KEY WORDS: Moxifloxacin Hydrochloride (MOX), Bromfenac Sodium (BROM), Absorbance Ratio Method

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INTRODUCTION:

Moxifloxacin is a 4th generation synthetic fluoroquinolone antibacterial Agent. It is chemically 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline-3-carboxylic acid. Bromfenac Sodium is Non steroidal anti-inflammatory agent. Chemically it is Sodium [2-amino-3-(4-bromobenzoyl) phenyl].Clinically a combination is being used in the treatment of post operative inflammation and pain. The combination of Moxifloxacin and Bromfenac is not official in any official pharmacopoeia. A literature survey revealed that only a few methods based on HPLC[1-3], Spectrometry[4-11] and HPTLC[12] were reported for the determination of Moxifloxacin and HPLC was reported for the determination of Bromfenac but no single method is reported for the simultaneous estimation of Moxifloxacin and Bromfenac in pharmaceutical dosage form.

Hence in the present study a physical mixture of Moxifloxacin and Bromfenac was being taken for simultaneous estimation by UV method. This present investigation describes a rapid, accurate and precise UV method of Moxifloxacin and Bromfenac in combination using Methanol:water (10:90% v/v) as a solvent. In which two wavelengths are used 275 nm (isobestic point) and 291 nm (λ_{max} of MOX).

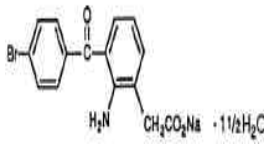

Figure:1: Structure of Moxifloxacin Hydrochloride

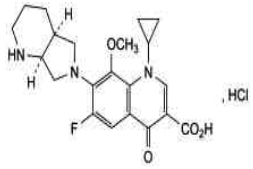

Figure:2: Structure of Bromfenac Sodium

MATERIALS AND METHODS:

Apparatus:

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched quartz cells. All weighing was done on Mettler Toledo electronic analytical balance.

Reagents and Chemicals:

Moxifloxacin Hydrochloride was gifted by Vital Healthcare, Vapi and Bromfenac sodium was gifted by Enaltec Labs, Mumbai.

Methanol and water were used as solvents and calibrated glasswares were used throughout the work.

Marketed Formulation:
Combined eyedrop formulation was purchased from

Preparation of Standard solution:

Moxifloxacin (MOX) standard stock solution: (1000 µg/ml)

100 mg of MOX standard was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved by adding 10 ml of methanol and volume was made up to the mark with water to give a solution containing 1000 µg/ml MOX. From this solution 5 ml was transfer to 100 ml volumetric flask. The volume was adjusted to the mark with the methanol:water (10:90) to give a solution containing 50 µg/ml MOX.

Bromfenac (BROM) standard stock solution: (1000 µg/ml)

100 mg of BROM standard was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved by adding 10 ml of methanol and volume was made up to the mark with water to give a solution containing 1000 µg/ml BROM. From this solution 5 ml was transfer to 100 ml volumetric flask. The volume was adjusted to the mark with the methanol:water (10:90) to give a solution containing 50 µg/ml BROM.

Selection of Analytical Wavelength

1 - 16 µg/ml solutions of MOX were prepared in methanol:water(10:90) and spectrum was recorded between 200-400 nm. Spectrums for above concentration were obtained with n=3. Similarly 1 -16 µg/ml solutions of BROM were prepared in Methanol:water(10:90) and spectrum was recorded between 200-400nm. MOX showed λ_{max} at wavelength 291 nm and BROM showed λ_{max} at wavelength at 267 nm.

The overlain spectrums of MOX and BROM at different concentration were recorded. The Wavelength, for simultaneously detection of both drugs by Absorbance ratio method was 275 nm.

Method:

Calibration curve for the MOX (1 – 16 µg/ml)

Appropriate volume of aliquot from standard MOX stock solution was transferred to different volumetric flasks of 50 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 1, 4, 7, 10, 13 and 16µg/ml. The curve of each solution against the Methanol:water(10:90) was recorded. Absorbance at 291 nm and 275 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Calibration curve for the BROM (1 - 16 µg/ml)

Appropriate volume of aliquot from standard BROM stock solution was transferred to different volumetric flasks of 50 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 1, 4, 7, 10, 13 and 17µg/ml. The curve of each solution against the Methanol:water (10:90) was recorded. Absorbance at 291 nm and 275 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined.

Preparation of Sample solution:

From the Ophthalmic formulation , (0.5 % w/v MOX & 0.09 % w/v BROM), 1.1 mL taken in 100 mL volumetric flask and the volume was adjusted to mark with Methanol:water (10:90). This was working sample solution having strength 550 µg/mL of MOX & 1 µg/mL of BROM. From this solution, 10 ml was taken in 100 ml volumetric flask. This was working sample solution having strength 5.5 µg/mL of MOX and 1 µg/mL of BROM.

Validation of spectrophotometric method:

(1) Accuracy

Accuracy was determined by calculating recovery of MOX and BROM by the standard addition method. Known amounts of standard solutions of MOX and BROM were added to a pre-quantified test solutions of MOX (5.5 µg/mL) and BROM (1 µg/mL). Each solution was measured in triplicate, and the recovery was calculated by measuring absorbance.

(2) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

(3) Repeatability

Standard solutions of MOX were prepared of linearity range and spectrums were recorded. Absorbance was measured at 291 nm and 275 nm. The absorbance of the same concentration solution was measured six times and RSD was calculated.

In the similar manner solutions of BROM were prepared and spectrums were recorded. Absorbance was measured at 291 nm and 275 nm . The procedure was repeated for six times and RSD was calculated.

(4)Intra and inter day precision

Variation of results within the same day (intra day), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing MOX and BROM individually for three times in the same day at 291 nm and 275 nm.

Inter day precision was determined by analyzing both the drugs individually daily for three days at 291 nm and 275 nm.

(5) Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

RESULT AND DISCUSSION:

From overlain spectra of MOX and BROM it is clear that MOX exhibited λ_{max} at 291 nm and BROM exhibits λ_{max} at 267 nm. The overlain spectra of MOX and BROM reveals that the both the drug exhibits distinct λ_{max} and also isobestic point was found at 275 λ_{max}. For estimation of MOX and BROM using Q Ratio Absorption method was decided to be used. In this method two wavelengths are required. One wavelength is selected at which MOX shows maximum absorbance, while second wavelength is selected as isobestic point.

Calibration data at 291 and 275 nm for MOX and 291 and 275 nm BROM are shown in Table. Calibration curves for MOX and BROM were plotted between absorbance and concentration. The following equations for straight line were obtained for MOX and BROM.

Linear equation for MOX at 291 nm, **Y = 0.0816x + 0.0460**

Linear equation for MOX at 275 nm, **Y = 0.0399x + 0.0096**

Linear equation for BROM at 291 nm, **Y = 0.0141x + 0.0539**

Linear equation for BROM at 275 nm, **Y = 0.0399x + 0.0096**

The developed Q Absorption Ratio method was validated. The linear range, correlation coefficient, detection limit and standard deviation for MOX and BROM by Spectrophotometry method are shown in Table. Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 98.18- 101.21 % for MOX and 98.00-101.00% for BROM respectively at 275nm and 98.36-100.24% for MOX and 98.00-102.00% for BROM at 291nm. Precision

was calculated as repeatability and intraday and interday variation for both the drugs. The LOD and LOQ for MOX and BROM was found to be 0.202µg/mL and 0.614 µg/mL at 275 nm respectively and 0.065 and 0.437 µg/mL LOD for MOX and 0.197 and 1.31 µg/mL LOQ for BROM at 291 nm respectively. Summary of validation parameters are shown.

Marketed formulation was analyzed by the proposed method and assay result of marketed formulation is shown.

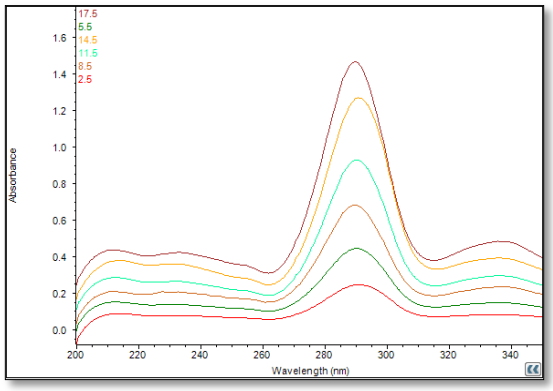

Figure:2: Overlain spectra of MOX at 291nm

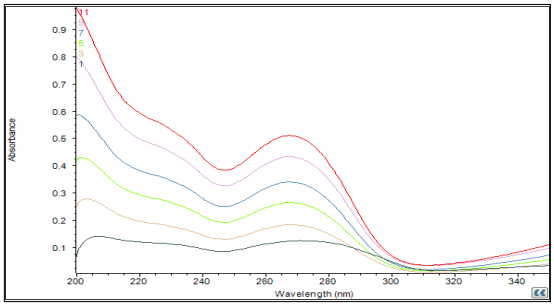

Figure:3: Overlain spectra for BROM at 265nm

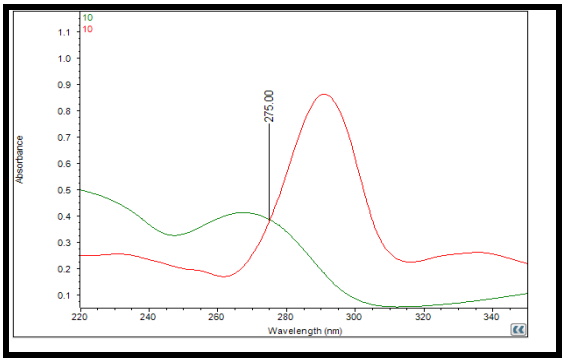

Figure:4: Overlay of MOX and BROM (10 µg/ml) at 275 nm

Table:1: Result of calibration readings at 275 nm for MOX and BROM in Methanol:water (10:90)

Concentrations (µg/ml)	Absorbance at 275 nm Mean ± S.D. (n=3)
1	0.050±0.00177
4	0.170±0.00276
7	0.294±0.00264
10	0.399±0.00260
13	0.526±0.00244
16	0.653±0.0025

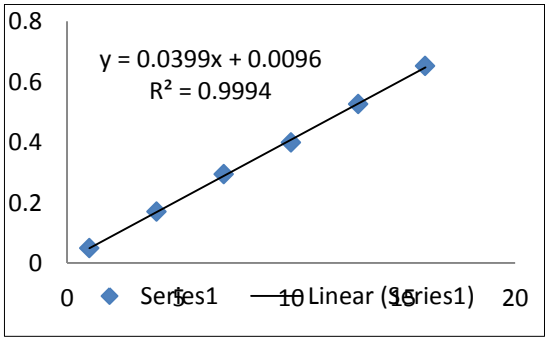

Figure:5: Calibration curve of MOX and BROM at 275nm

Concentrations (µg/ml)	Absorbance at 291 nm Mean ± S.D. (n=3)
1	0.132±0.00173
4	0.356±0.00234
7	0.623±0.00203
10	0.879±0.000911
13	1.098±0.00151
16	1.349±0.00114

Table:2: Result of calibration readings at 291 nm for MOX in Methanol:water (10:90)

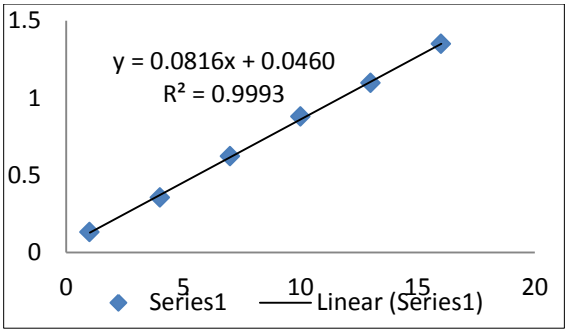

Figure:6:Calibration curve of MOX at 291 nm

Concentrations (µg/ml)	Absorbance at 291 nm Mean ± S.D. (n=3)
1	**0.068±0.00191**
4	**0.108±0.00207**
7	**0.154±0.00151**
10	**0.198±0.000911**
13	**0.235±0.00234**
16	**0.279±0.00250**

Table:3: Result of calibration readings at 291 nm for BROM in Methanol:water (10:90)

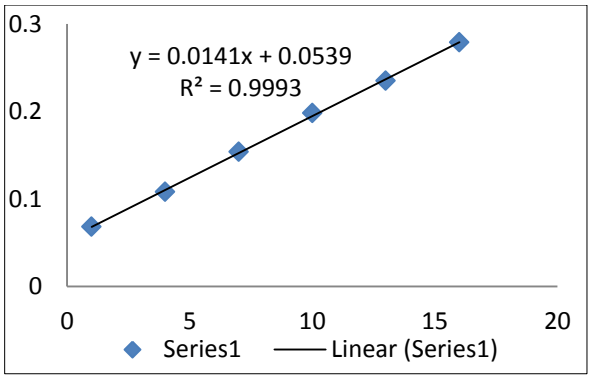

Figure:7: Calibration curve of BROM at 291 nm

Table:4: Statistical data for MOX and BROM (at 275 nm) by Q Absorption Ratio method

Parameter	MOX(at 275 nm)	BROM (at 275nm)
Linear Range (µg/ml)	1-16	**1-16**
Slope	0.0399	**0.0399**
Intercept	0.0096	**0.0096**
Limit of Detection (µg/ml)	0.202	**0.202**
Limit of Quantitation (µg/ml)	**0.614**	**0.614**

Table:5: Statistical data for MOX and BROM (at 291 nm) by Q Absorption Ratio method

Parameter	MOX (at 291nm)	BROM (at 291nm)
Linear Range (µg/ml)	1-16	**1-16**
Slope	0.0816	**0.0141**
Intercept	0.0460	**0.0539**
Limit of Detection (µg/ml)	0.065	**0.437**
Limit of Quantitation (µg/ml)	**0.197**	**1.32**

Table:6: Determination of Accuracy of MOX and BROM (at 275nm)

Amt of sample		Amt. of drug added		Amt. recovered		% Recovery	
MOX (µg/ml)	**BROM (µg/ml)**	**MOX (µg/ml)**	**BROM (µg/ml)**	**MOX (µg/ml)**	**BROM (µg/ml)**	**MOX %**	**BROM %**
5.5	1	0	0	5.30	2.41		
5.5	1	2.75	0.5	2.7	0.49	98.18	**98.00**
5.5	1	5.5	1	5.49	1.01	99.81	**101.00**
5.5	**1**	**8.25**	**1.5**	**8.35**	**1.48**	**101.21**	**98.66**

Table:7: Determination of Accuracy of MOX and BROM (at 291 nm)

Amt of sample		Amt. of drug added		Amt. recovered		% Recovery	
MOX (µg/ml)	BROM (µg/ml)	MOX (µg/ml)	BROM (µg/ml)	MOX (µg/ml)	BROM (µg/ml)	MOX %	BROM %
5.5	1	0	0	5.38	0.987		
5.5	1	2.75	0.5	2.74	0.49	99.63	**98.00**
5.5	1	5.5	1	5.41	1.02	98.36	**102.00**
5.5	**1**	**8.25**	**1.5**	**8.27**	**1.48**	**100.24**	**98.66**

Table:8: Precision data for MOX and BROM at 275nm

MOX at 275nm			BROM at 275nm		
Conc. (µg/ml)	Intraday (n =3)	Interday (n =3)	Conc. (µg/ml)	Intraday (n =3)	Interday (n =3)
7	0.18	0.89	7	0.18	0.88
10	0.32	0.78	10	0.34	0.75
13	0.28	0.55	13	0.25	0.58

Table:9: Precision data for MOX and BROM at 291nm

MOX at 291nm			BROM at 291nm		
Conc. (µg/ml)	Intraday (n =3)	Interday (n =3)	Conc. (µg/ml)	Intraday (n =3)	Interday (n =3)
7	0.24	0.37	7	0.80	1.94
10	0.20	0.29	10	0.77	1.51
13	0.13	0.18	13	0.55	1.75

Table:10: Summary of validation Parameters of absorabance ratio method

Parameters	MOX (275 nm)	BROM (275 nm)	MOX (291 nm)	BROM (291nm)
Linearity range	2.5-17.5	1-11	2.5-17.5	1-11
Recovery%	98.18-101.21	98-101.00	98.36-100.24	98-102
Repeatability (RSD, n=6)	0.18	0.15	0.06	0.21
Precision(RSD)			0.13-0.24	0.55-0.80
Intra-day (n=3)	0.18-0.32	0.18-0.34	0.18-0.37	1.51-1.94
Inter-day (n=3)	0.55-0.89	0.58-0.88		

Table:11: Assay Results of Marketed Formulation (absorbance ratio)

Formulation	Actual concentration µg/ml		Amount obtained µg/ml (n=3)		% MOX	% BROM
	MOX	BROM	MOX	BROM		
Eyedrops	5.5	1	5.29±0.0174	0.996±0.00158	96.18	99.6

CONCLUSION:

The low value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage forms without any interference due to the other components present in the formulations. Hence this study presents simple,

accurate, precise and rapid spectroscopic analytical method for the simultaneous estimation of these two drugs in combined dosage form.

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