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Simultaneous HPLC method development and validation of moxifloxacin hydrochloride and bromofenac sodium in pharmaceutical formulation

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ABSTRACT

This paper deals with the simultaneous reversed – phase HPLC method development for the for the simultaneous determination of Moxifloxacin Hydrochloride and Bromofenac Sodium in Pharmaceutical preparation. The chromatographic separation was carried out using a STD Hyber C18 column, 150× 4.6mm i.d, 5µ particle size in isocratic mode with flow rate of 1mLmin^{-1} and the detection was carried out by Photo diode array detector at 280nm. The mobile phases consist of 0.01N Sodium Dihydrogen Ortho Phosphate buffer (pH 4 ±0.5) and Acetonitrile in the ratio of 65:35 (v/v). The total chromatographic analysis per sample was approximately 6 mints. Retention times for Moxifloxacin Hydrochloride and Bromofenac Sodium were found to be 2.26 and 5.72 min respectively. A linear response curve was observed over the concentration range of 25-150µgmL^{-1} and 4.5-27µgmL^{-1} for Moxifloxacin Hydrochloride and Bromofenac Sodium. The method was statistically validated as per ICH guidelines and % RSD was found to be less than 2 indicating high degree of accuracy and precision of the proposed HPLC method. Hence the proposed method can be applied for the quantitative analysis of Moxema and Bromday eye drops.

Keywords: HPLC, Moxifloxacin Hydrochloride, Bromofenac Sodium, Method Development & Validation.

INTRODUCTION

Moxifloxacin Hydrochloride (MOX) (Fig.1) 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octa-hydropyrrolo [3, 4-*b*] pyridin-6-yl]-4-oxo-1, 4-dihydro quinoline-3-carboxylic acid hydrochloride is a fourth generation fluoro quinolone antibiotic used to treat respiratory infections, including acute sinusitis, acute exacerbations of chronic bronchitis, and community-acquired pneumonia, as well as dermatological infections[1,2].

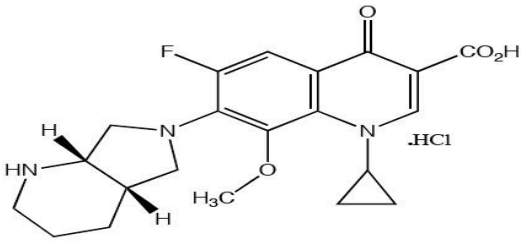

Fig.1 The chemical structure of Moxifloxacin Hydrochloride

Bromofenac Sodium (BRO) (Fig.2) is 2-[2-amino-3-(4-bromobenzoyl) phenyl] acetic acid. It is a non-steroidal anti-inflammatory drug (NSAID) that has anti-inflammatory action. It is indicated for the treatment of ocular inflammation and pain after cataract surgery[1, 3].
Literature review reveals different methods for the analysis of Moxifloxacin and Bromofenac in pharmaceuticals by using spectroscopic method[4-6], Fluorimetric method[7], Colorimetric method[8,9] and HPLC method[10-16.] In the present study, a new HPLC method was developed and validated for the simultaneous estimation of MOX and BRO.

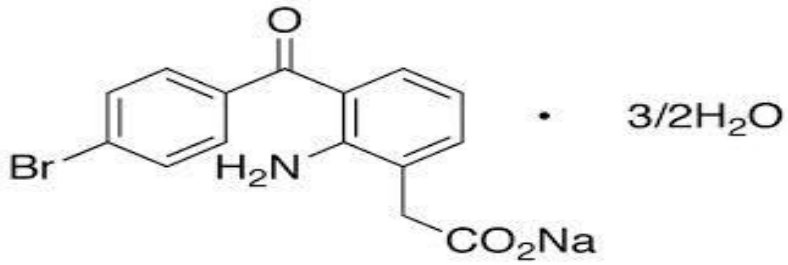

Fig.2 The chemical structure of Bromofenac Sodium

EXPERIMENTAL

MATERIALS AND METHODS

Apparatus and software

Chromatography measurements were made on Waters Alliance equipped with a Waters 2695 separations module, and Waters 2996 PDA detector. The system was controlled through a system controller. Data acquisition was performed by the Empower- 2 software. The mobile phase was degassed using power sonic sonicator (Hwashin technology, Seoul, Korea). Absorbance spectra were recorded using an UV-Visible spectrophotometer (Model UV-1800, Japan) employing quartz cell of 1.00 cm of path length (Fig.3). The rest of the calculations for the analysis were performed by use of Micro soft Excel 2007 software (Microsoft, USA).

Chemicals and Reagents

Working standards of MOX and BRO were donated by Pharma Analytical Lab, Puducherry. Ophthalmic dosage form was procured from local market with the brand name Moxeza by ALCON Laboratories containing 0.5% w/v of Moxifloxacin and 0.09% w/v of Bromofenac with the brand name Bromday by ISTA Pharmaceuticals. HPLC Grade Water and Acetonitrile were of HPLC grade supplied by Merck, Sodium di-hydrogen phosphate and Ortho-phosphoric acid of analytical grade supplied by Rankem, were used during the study.

Buffer Preparation

Accurately weighed 1.41gm of sodium di-hydrogen Ortho phosphate in a 1000 mL^{-1} of Volumetric flask, add about 900 mL^{-1} of HPLC Grade Water and sonicate for few minute to degas and finally make up the volume with HPLC water and then the pH was adjusted to 4.0 with dil. Ortho Phosphoric acid .

Standard Preparation

Accurately Weighed and transferred 10mg & 4.5mg of Moxifloxacin and Bromofenac working Standards into 10 mL^{-1} and 25 mL^{-1} clean dry volumetric flasks separately, add 3/4th amount of diluents, sonicate for 30 minutes and make up to the final volume with diluents. 1 mL^{-1} was pipette out from the stock solution and transferred in to a 10 mL^{-1} volumetric flask and then make up to the final volume with diluents.

Sample Preparation

Locally available marketed formulation contains 0.5 %w/v of Moxifloxacin hydrochloride and 0.09% w/v of Bromofenac sodium was taken. From the Above formulation 2 mL^{-1} was taken and diluted to 10 mL^{-1} with diluents. Further 1 mL^{-1} was taken from the sample stock solution into a 10 mL^{-1} volumetric flask and made up with diluents.

Chromatographic Procedure

Chromatographic separations were achieved using STD Hyber C18 analytical Column (150 x 4.6mm, 5µ). The mobile phase consists of Phosphate buffer (pH 4 ±0.5) and Acetonitrile in the ratio 65:35. The injection volume of sample was 10 µL. The column temperature was maintained at 30ºC. The mobile phase was degassed by ultra sonication before pumping into HPLC system. The flow rate was set at 1mLmin^{-1} and the wave length 280 nm was selected for detection. Table No.1 shows the best optimum chromatographic separation condition.

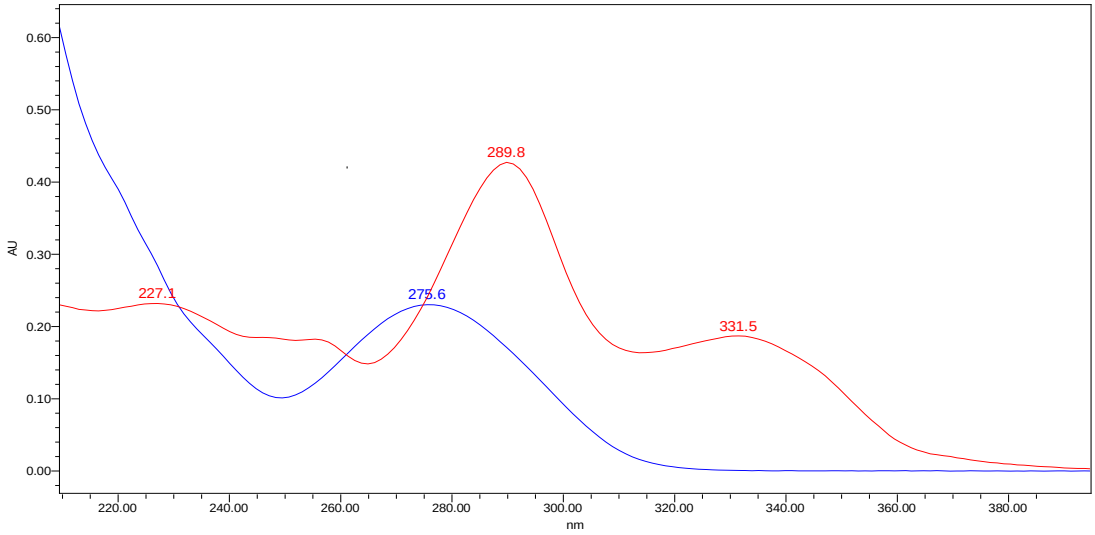

Fig.3 UV- Overlay Spectrum of Moxifoxacin and Bromofenac

METHOD DEVELOPMENT

Several trials were performed for the method development and the best peak separation with least fronting factor was found to be the Sixth trail with RT of 5.72 for Bromofenac sodium and 2.26 for Moxifloxacin. Best peak optimal separation condition and the corresponding chromatograms shown in Fig 4.

Method Validation

Validation studies were conducted using the final assay conditions based on the principles of validation described in the ICH guidelines "Text on Validation of Analytical Procedures"[17] and "Q2B, Validation of Analytical Procedures: Methodology"[18]. Key analytical parameters, including, specificity, accuracy, precision, linearity, detection limit and quantitation limit were evaluated.

Table.1 Best chromatographic conditions

Sl. No.	Chromatographic conditions	
1	Mode of separation	Isocratic elution
2	Mobile phase	Buffer (pH 4.0 ±0.5) and Acetonitrile(65:35)
3	Column	STD HYBER C18, (150 mm x 4.6 ,5 µ)
4	Flow rate	1.0 ml/ min
5	Detection wavelength	280 nm
6	Injection volume	10 µl
7	Column oven temperature	30 °C
8	Run time	6.0 min

System suitability

System suitability tests are an integral part of liquid chromatographic method. These tests are used to verify that the chromatographic system is adequate for the intended analysis. The system suitability test was performed for theoretical plates (not less than 2000) and tailing factor (less than 2), the results were within the acceptable limit are summarized in Table 2.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity experiments were performed for both the components and the response were found to be linear in the range of 25-150 ppm for Moxifloxacin and 4.5-27 ppm for Bromofenac. The regression equation of calibration curves were y = 14953.3x + 722.1 for Moxifloxacin hydrochloride and y= 62666x+5976.9 for Bromofenac sodium.

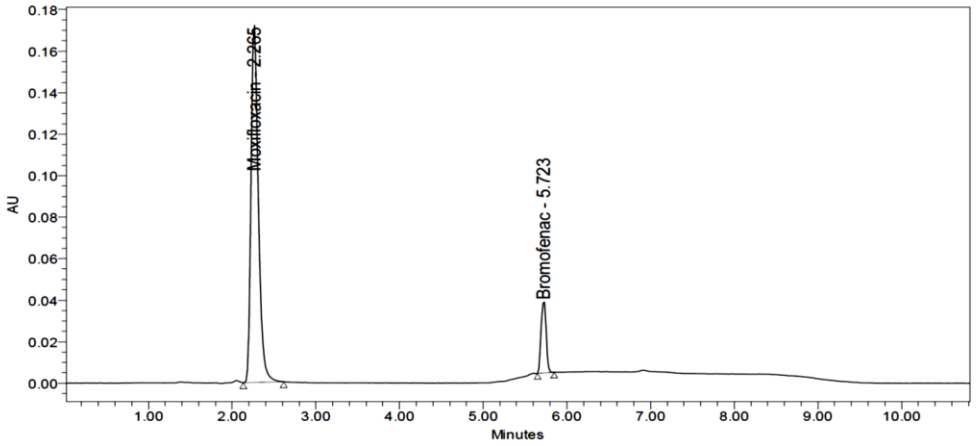

Fig No.4 Chromatogram of best separation condition for MOX and BRO

Sl. No.	Parameters	Moxifloxacin Hydrochloride	Bromofenac Sodium	Acceptable criteria
1	Tailing factor	1.32	1.04	Less than 2
2	Theoretical plates	3334	46820	Not less than 2000
3	Retention time	2.26	5.77	Less than 10
4	Mean Area	1823789	326777	-
Std. Dev. (Mean Area)		9401.8	1268.8	-
% RSD		0.5	0.4	Less than 2 %

Table 2 System suitability parameter of Moxifloxacin and Bromofenac

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. In this study, the chromatograms of standard and sample are identical with nearly same retention time. No interference due to placebo, mobile phase and sample at the retention time of analyte which shows that the method was specific.

Accuracy

The accuracy of the method was determined by recovery experiment. Accuracy was determined by performing the recovery experiment at 50%, 100% and 150% of the target analyte concentration in the commercial formulation. The % recovery of analytes at each concentration (n = 3) and mean % recovery (n = 9) for each analyte were determined. In this respect, the mean recovery (n = 3) at each concentration should be within the acceptance criteria of bias, ±2%.

Limit of detection

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. The LOD for MOX and BRO standard solutions were found to be 0.58µg.mL^{-1} and 0.52µg.mL^{-1} respectively.

Limit of Quantification

Limit of Quantification (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level. The LOQ for Moxifloxacin and Bromofenac standard solutions were found to be 2.78µg.mL^{-1} and 1.98µg.mL^{-1} respectively.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation, and is normally expressed as the percent coefficient of variation (%CV). Precision may be performed at two different levels: intra-day and inter-day precisions. Precision data representing the % CV values for both intra-day and inter-days were less than 2.0%, this indicates that the proposed method is precise.

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.

In the present work Robustness of the method was determined by making slight changes in the composition of mobile phase ± 2 %, flow rate by ±0.2 mL^{-1} and temperature by ± 2ºC. It was observed that were no marked changes in the retention time and area of the chromatograms and the % RSD was less than 2 %, which demonstrated that the RP-HPLC method developed was robust.

RESULTS AND DISCUSSIONS

The HPLC method was developed by using, Std Hyber C18 column (150 mm X 4.6 mm, 5µ) with mobile phase consist of Sodium di-hydrogen phosphate buffer (adjusted pH to 4.0 using Ortho- phosphoric acid) and Acetonitrile (65:35). Flow rate was set at 1.0 mLmin-1 with UV detection at 280 nm and the injection volume was 10 µl, with the run time of 6.0 minutes.

The last step of the present study was to check method's validation for specificity, linearity, accuracy, intra/inter-day precision, and robustness. All the validation parameters were found to be well within the acceptance criteria, Table 3. The system suitability parameters also reveals that the values within the specified limit for the proposed method. Theoretical plates and tailing factor for Moxifloxacin hydrochloride and Bromofenac sodium were found to be NLT 2000 and NMT 2.0 respectively, Table 2. The developed HPLC method was specific in relation to the placebo. All placebo chromatograms showed no interference peaks. An excellent linearity was established at six levels in the range of 25-150 µg/ml for MOX & 4.5-27 µg/mL for BRO with R^2 of more than 0.999 for both the analytes. The LOD and LOQ were estimated as 0.58 and 2.78 µg/ml for MOX, 0.52 and 1.98 µg/ml for BRO respectively. Accuracy (n = 9), assessed by spike recovery, were found to be 100.08 and 100.00 % for MOX and BRO respectively,

which were within acceptable ranges of 100 ± 2%. The intra and inter-assay precision (n = 6) was confirmed since, the %C.V. were well within the acceptable limit of ≤ 2 and ≤ 3, respectively. Robustness study reveals that small changes did not alter the retention times, retention factor, and resolution more than 2% and therefore it would be concluded that the method conditions are robust.

From the above results, it was concluded that, the method was accurate, reproducible, repeatable, linear, precise and selective, proving reliability of method. The run time is relatively short i.e., 6.0 min which enables rapid quantitation of many samples in routine quality control analysis. These results show the method could find practical application as a quality control tool for estimation of the MOX and BRO in quality control laboratories.

Application of the method As a final step, commercial products Moxeza (0.5% w/v) (MOX) and Bromday (0.09% w/v) (BRO) with the brand name were assayed by the proposed HPLC method. 50µg/ml of MOX and 9µg/ml of BRO was prepared from suitable dilution and filtered through 0.45micron membrane filter. The solution was injected through 10µl loop system and chromatograms were obtained at flow rate of 1.0 $mLmin^{-1}$. The concentrations of both solutions.

Sl. No	Parameter		Requirement	Results MOX	BRO	Acceptance criteria
1	Linearity		Correlation coefficient	0.999	0.999	NLT-0.999
2	Specificity		Interference	specific	specific	No interference
3	Accuracy &		50 %	100.03%	99.98%	98-102%
	% Recovery		100 %	100.29%	99.77%	
			150 %	99.94%	100.27%	
4	LOD		μgmL^{-1}	0.58	0.52	-
5	LOQ		μgmL^{-1}	2.78	1.98	-
6	precision	Intra-day	% RSD	0.41	0.6	NMT-2%
		Inter-day	% RSD	0.75	0.64	
7	Robustness	Mobile Phase	63:37	0.43	0.61	NMT-2%
			67:33	0.48	0.56	
		Temp.	(28°C)	0.5	1.4	
			(32°C)	0.4	0.5	
		Flow rate	0.9 $mLmin^{-1}$	1.1	0.0	
			1.1 $mLmin^{-1}$	0.2	0.1	

Table 3 VALIDATION PARAMETERS FOR MOX AND BRO

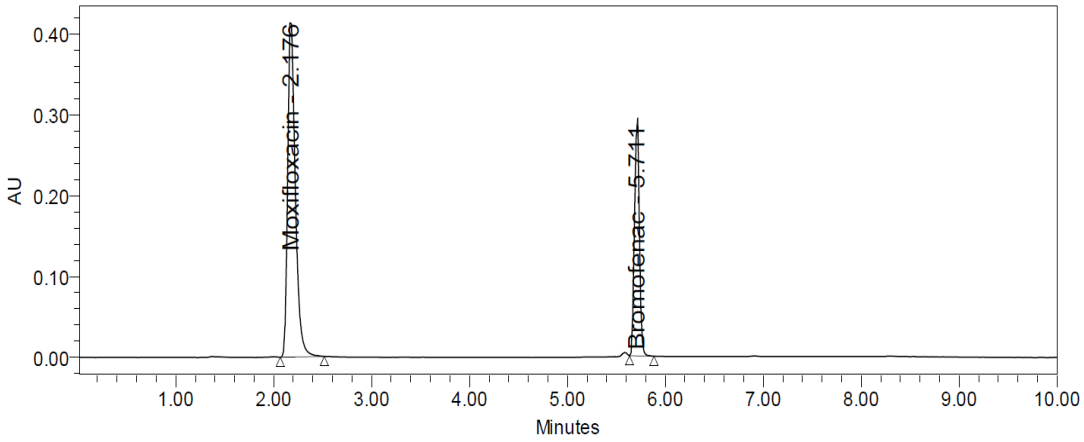

Fig.5 Chromatogram showing assay of Moxifoxacin and Bromofenac

were measured at 280 nm and representative chromatograms are presented in (Fig.5). The percentage of drug content was calculated for MOX and BRO, which showed a Good agreement between the assay results and the label claim of the product. The % C.V. for both formulations was < 2, indicating the precision of the analytical methodology.

CONCLUSION

A simple, fast and cost effective HPLC method was successfully developed for simultaneous estimation of Moxifloxacin hydrochloride and Bromofenac sodium eye drops formulation. The proposed method was validated for the various experimental parameters according to ICH guidelines. Influence of the mobile phase, column oven temperature and flow rate was evaluated for the proposed method. The MOX and BRO analytes were well resolved and separated within 6 min. The developed HPLC method provides high through put for simultaneous determination of MOX and BRO with excellent accuracy, precision, specificity, and robustness. Hence this method can be applied for the simultaneous quantification of both MOX and BRO in Ophthalmic solution for the routine analysis.

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