JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

The Simultaneous Estimation of Moxifloxacin Hydrochloride and Bromfenac Sodium in Eye Drops by UV

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ABSTRACT:

A simple, rapid, accurate, precise and economical UV spectrophotometric method for the simultaneous determination of moxifloxacin hydrochloride and bromfenac sodium in combined eye drops using simultaneous equation method has been developed. The method is based on the simultaneous equations for analysis of both the drugs using distilled water as solvent. Moxifloxacin hydrochloride has absorbance maxima at 289 nm and bromfenac sodium has absorbance maxima at 268 nm in distilled water. The linearity was obtained in the concentration range of 2-10 µg/ml and 4-20 µg/ml for moxifloxacin hydrochloride and bromfenac sodium respectively. The concentrations of the drugs were determined by using simultaneous equations method. The mean recovery was 99.64±0.53 and 99.62± 0.04 for moxifloxacin hydrochloride and bromfenac sodium respectively. The method was found to be simple, accurate and precise and was applicable for the simultaneous determination of moxifloxacin hydrochloride and bromfenac sodium in eye drops. In conclusion, spectrophotometric methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation and results of analysis were validated statistically and by recovery studies.

KEY WORDS: Moxifloxacin hydrochloride, bromfenac sodium, simultaneous equation method, uv spectrophotometer

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INTRODUCTION:

Moxifloxacin HCl is a fourth generation fluroquinolone, the antimicrobial activity of which depends upon inhibition of DNA gyrase (bacterial topoisomerase II), an enzyme necessary for DNA replication, transcription, repair and recombination.

Moxifloxacin has *in-vitro* and *in-vivo* activities against wide range of gram+ve and gram-ve bacteria[1].Moxifloxacin hydrochloride is official in british pharmacopoeia[2].Various methods like UV spectrophotometry[3,4,5],RP-HPLC[6],Extractive spectroscopy[7],spectrophotometric method for simultaneous determination of MOXI with other drug are reported in literature for estimation of MOXI in pharmaceutical dosage forms[8]. Bromfenac is a nonsteroidal antiinflammatory drug (NSAID) that has anti-inflammatory activity. The mechanism of its action is thought to be due to its ability to block prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2[9].Bromfenac sodium is not official in any pharmacopoeia. RP-HPLC method was reported in plasma[10].MoxifloxacinHCl (MOXI) is 1- Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*) octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4- oxo-1,4 dihydroquinoline-3- carboxylic acid hydrochloride. Bromfenac sodium is 2-[2-amino-3-(4-bromobenzoyl) phenyl] acetic acid [11]. For routine analysis, a simple and cost effective analytical method is preferred. The objective of the present study was to develop a simple, precise,

accurate and economic analytical method with better detection range, for the estimation of moxifloxacin Hcl & bromfenac sodium in pharmaceutical formulation. In the analytical method developed, water was used as analytical media, as both drug was found to be stable in water & also water is economic as compare to other media so this method is simple, precise, accurate and economic. The developed method was validated as per ICH guidelines and suitable statistical tests were performed on validation data.

MATERIALS AND METHODS

Chemicals and Reagents

Moxifloxacin HCl was obtained as a gift sample from Selvok pharmaceuticals Co, Bilimora, Gujarat, India and Bromfenac sodium was obtained as a gift sample from Enaltec Labs Private ltd, Mumbai, India. Marketed eye drop contains 5mg/ml moxifloxacin HCl and Bromfenac sodium 0.9mg/ml of drug. All other chemicals used were of analytical grade. Distilled water and caliberated glasswares were used throughout the work.

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. A Reptech electronic weighing analytical balance based on EMFC technology was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of MOXI (50 mg) and BROM (50 mg) were transferred to a separate 50 ml volumetric flask and dissolved and diluted to the mark with distilled water to obtain standard solution having concentration of MOXI (1000 µg/ml) and BROM (1000 µg/ml). Accurately measured 10 ml of both the solutions were transferred to 100ml of volumetric flask and diluted to the mark with distilled water to obtain solution having concentration 100 µg/ml of MOXI and BROM.

Method

The standard solutions of MOXI (10 ug/ml) and BROM (10 µg/ml) were scanned separately in the UV range of 200-400 nm to determine the λmax of both the drugs. The λmax of MOXI and BROM were found to be 289 nm and 268 nm respectively. Five standard solutions having concentration 2, 4, 6, 8 and 10 µg/ml for MOXI and 4, 8, 12, 16 and 20 µg/ml for BROM were prepared in distilled water using the solutions having concentration 100 µg/ml. The absorbance of resulting solutions was measured at 289 nm and 268 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of MOXI and BROM in sample solution was determined by solving the respective simultaneous equations generated by using

absorptivity coefficients and absorbance values of MOXI and BROM at these wavelengths. Wavelengths of MOXI 289nm, wavelength of BROM 268 nm were selected for formulation of the simultaneous equation method. The absorptivities (A1%, 1 cm) of both the drugs at the wavelengths were determined. The absorbance and absorptivities values at the particular wavelength were substituted in the following to obtain the concentration [12].

$$C_x = \frac{A2a1y - A1a2y}{a2xa1y - a1xa2y} \tag{1}$$

$$C_y = \frac{a2xA1 - a1xa2}{a2xa1y - a1xa2y} \tag{2}$$

Where,

A^1, A^2 — are absorbance of the mixture,

a^1_x, a^2_x – are absorbance of the x,

a^1_y, a^2_y — denote absorptivities of Y at 289, 268nm respectively,

C_X = concentration of MOXI.

C_Y = concentration of BROM

Validation of the proposed method:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [13].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-10 µg/ml and 4-20 µg/ml for M0XI and BROM respectively. Accurately measured standard solutions of MOXI (2, 4, 6, 8, and 10 ml) and BROM (4, 8, 12, 16 and 20 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water. The absorbances of the solutions were measured at 289 and 268 nm against distilled water as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of MOXI and BROM.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of MOXI and BROM by the standard addition method. Known amounts of standard solutions of MOXI and BROM were added at 80, 100 and 120 % level to prequantified sample solutions of MOXI and BROM (5000 µg/ml for MOXI and 900 µg/ml for BROM). The amounts of MOXI and BROM were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of MOXI and BROM in combined eye drops

One ml of drop contain 5000ug/ml MOXI and 900 ug/ml BROM.5 ml of 1000 ug/ml of std BROM was added to it as standard addition for making concentration of BROM within the calibration range. It is made up to 100 ml with distilled water so concentration of MOXI and BROM becomes 50 and 59 ug/ml of MOXI and BROM respectively. Eye drops was soluble in water so no extractions was necessary. The aliquot of 10 ml was transferred to 100 ml volumetric flask and volume was adjusted to the mark with distilled water so concentration becomes 5 ug/ml and 5.9 ug/ml for MOXI and BROM respectively. The absorbances of the eye drop solution i.e. A1 and A2 were recorded at 289 nm and 268 nm and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MOXI and BROM at these wavelengths.

RESULTS AND DISCUSSION

In this method linearity was observed in the concentration range of 2-10 μg/ml for MOXI and 4-20 μg/ml for BROM. Validation parameters are presented in Table-1.Marketed brand of eye drop was analyzed and amount of MOXI and BROM determined by proposed methods was 99.6% and 103.33% Table-2. The proposed methods were validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery at 80,100 and 120 % level. The % recovery ranges from 99.2 to 100.23 for MOXI and BROM respectively and are presented in Table-3.Precision was calculated as inter and intraday variations for both drugs.

The proposed methods were found to be simple, accurate and rapid for the routine determination of MOXI and BROM in eye drops. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision. This method can be successfully used for simultaneous estimation of MOXI and BROM in eye drops.
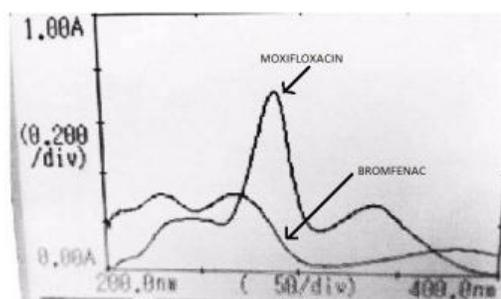
Figure: Overlay spectra of MOXI and BROM

TABLE-1 REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER OF THE CALIBERATION CURVES

Parameters	MOXI		BROM	
Wavelength (nm)	289	268	289	268
Beer's law limit (µg /ml)	2-10	2-10	4-20	4-20
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.1767x + 0.0167 0.1767 0.0167	y = 0.0619x + 0.0032 0.0619 0.0032	y = 0.08x + 0.0035 0.08 0.0035	y = 0.1663x − 0.0083 0.1663 0.0083
Correlation coefficient (r^2)	0.9998	0.9982	0.9999	0.9999
LOD (µg/ml)	0.11	0.30	0.19	0.06
LOQ (µg /ml)	0.34	0.93	0.59	0.20
Precision(% RSD,n=3) Interday Intraday	1.9-2.0 6.0-7.0	1.6-1.8 6.0-8.0	0.7-1.9 4.0-5.0	1.9-2.0 3.0-4.0

TABLE-2 RESULTS OF ANALYSIS OF EYE DROPS

Drugs	Simultaneous equation method % ± SD(n=5)
MOXI	99.72 ± 0.58
BROM	99.598 ± 4.58

n = number of replicates

TABLE-3 RESULTS OF THE RECOVERY STUDIES

Level of recovery %	Amount of pure drug added (ml)		Simultaneous equation method % recovery	
	MOXI(1000ug/ml)	BROM(100ug/ml)	M0XI	BROM
80	4	4.6	99.5	99.62
100	5	6.0	99.2	99.66
120	6	7.2	100.23	99.58
Mean % recovery			99.64	99.62
SD*			0.529	0.04
CV**			0.53	0.04

*SD = Standard deviation ** CV = coefficient of variance

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