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SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN HCl AND BROMFENAC SODIUM IN EYE DROPS BY SPECTROPHOTOMETRIC METHODS

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Keywords:
Moxifloxacin Hydrochloride,
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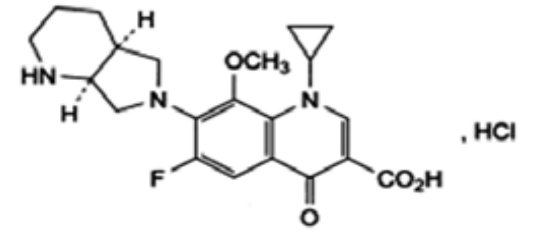
ABSTRACT

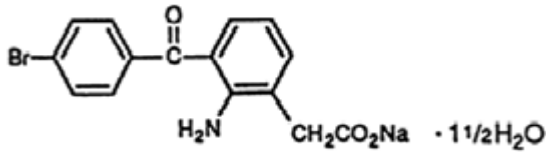
Moxifloxacin Hydrochloride is a fluorinated quinolone antibacterial and Bromfenac Sodium is a non-steroidal anti-inflammatory. The combination formulation is used for the treatment of the reduction of post operative inflammatory conditions of the eye. Three new, simple, accurate and precise UV spectrophotometric methods have been developed and validated for the simultaneous determination of Moxifloxacin Hydrochloride (MOX) and Bromfenac Sodium (BROM) in their combined dosage forms. Method - I is based on simultaneous equation method using two wavelengths, 294 nm (λmax of MOX) and 265 nm (λmax of BROM). Method - II Q-absorption Ratio method using two wavelengths, 294 nm (λmax of MOX) and 276.66 nm (Isoabsorptive point). Method - III involves the use of First order derivative technique. Here 301.71 nm, the zero crossing point of Bromfenac Sodium, was selected for the determination of Moxifloxacin Hydrochloride and 293.22 nm, the zero crossing point of Moxifloxacin Hydrochloride, was selected for the determination of Bromfenac Sodium. Methanolic HCL (0.1 M) was the solvent used in all three methods. Moxifloxacin showed linearity in the range of 1-14 µg/ml and Bromfenac showed linearity in the range of 1-14 µg/ml in all the methods. All methods were validated statistically and recovery studies were carried out. All methods were found to be accurate, precise and reproducible. These methods were applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 102.0% of the labelled value for both Moxifloxacin and Bromfenac. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

INTRODUCTION: Moxifloxacin Hydrochloride is 1-Cyclopropyl- 6- fluoro- 8- methoxy- 7- [(4a*S*, 7a*S*)-octa hydro- 6*H*- pyrrolo[3, 4*b*] pyridin- 6- yl]- 4- oxo-1, 4-dihydroquinoline-3-carboxylic acid hydrochloride [1]. It is a prescription drug indicated for the treatment of bacterial infections. Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication [2, 3, 4]. Bromfenac Sodium is Sodium [2-amino-3-(4-bromobenzoyl) phenyl] [5]. It acts thought to block prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2. Prostaglandins have been shown mediators of certain kinds of intraocular inflammation.

Prostaglandins have been shown to produce disruption of the blood-aqueous humor barrier, vasodilation, increased vascular permeability, leukocytosis, and increased intraocular pressure [5, 6]. The chemical structures of MOX and BROM are shown in **Fig. 1(A) (B).**



A



B

FIG. 1: CHEMICAL STRUCTURE OF (A) MOXIFLOXACIN HYDROCHLORIDE; (B) BROMFENAC SODIUM [1, 5]

A detailed survey of analytical literature for MOX revealed several methods based on varied techniques, viz, HPLC [7, 8, 9, 10], Spectrophotometry [11, 12], High-Performance Thin- Layer Chromatography (HPTLC) [13, 14]. Similarly, a survey of the analytical literature for BROM revealed methods based on HPLC for determination in pharmaceuticals [15, 16].

According to detailed survey of analytical literature none of the reported analytical procedures describes a simple and satisfactory UV spectrophotometric method for simultaneous determination of MOX and BROM in their combined dosage forms. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combination drug products containing MOX and BROM.

MATERIALS AND METHODS:

Instrumentation: 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. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31).

An Electronic analytical balance (Acculab) and an ultrasonic bath were used in the study.

Materials and reagents: MOX and BROM bulk powder was gifted by Zydus Cadila Health Care Pvt. Ltd., Ahmadabad, india and Enaltec lab Pvt. Ltd., Mumbai, india respectively. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade and Hydrochloric acid AR Grade were procured from S.D.Fine Chemicals Ltd., Mumbai, India.

Standard and Test Solutions:

Preparation of standard solution: An accurately weighed quantity of MOX (10 mg) and BROM (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with Methanolic HCL to obtain standard solution having concentration of MOX (100 µg/ml) and BROM (100 µg/ml).

Preparation of test solution: From the Opthalmic formulation ,4- Quin brom (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 methanolic HCl. This was working sample solution having strength 55 µg/ml of MOX & 10 µg/ml of BROM.

Methods:

Simultaneous Equation Method: In this method, seven working standard solutions having concentration 2-14 µg/ml for MOX and 1-13 µg/ml for BROM were prepared in Methanolic HCL and the absorbance at 294 nm (λ-max of MOX) and 265 nm (λ-max of BROM) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad \text{..... (1)}$$

$$Cy = \frac{A1ax2 - A2\ ax1}{ax2ay1 - ax1ay2} \quad \text{..... (2)}$$

Where, A_1, A_2 are absorbance of mixture at 294 nm (λ1) and 265 nm (λ2) respectively, ax1 and ax2 are absorptivities of MOX at λ1 and λ2 respectively, ay1

and ay2 are absorptivities of BROM at λ1 and λ2 respectively, Cx and Cy are concentrations of MOX and BROM respectively.

Q-Absorption Ratio Method: This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths are a constant value, independent of concentration or path length [17, 18, 19, 20]. Two wavelengths, 276.66 nm (Isoabsorptive point) and 294 nm (λ_{max} of MOX) were selected for the formation of Q-absorbance equation. The absorptivity co-efficient of each drug at both the wavelengths were determined.

The concentration of individual components, MOX and BROM may be calculated using the following equations

$C_{MOX}= (Qm–Q_{BROM}/Q_{MOX}–Q_{BROM})*A1/ax1$ ..... (1)

$C_{BROM}= (Qm–Q_{MOX}/Q_{MOX}–Q_{BROM})*A1/ay1$ ..... (2)

Where, Qm= A2 / A1, Q_{MOX} = ax2 / ax1 & Q_{BROM} = ay2/ay1; A1 and A2 are absorbance of sample solution at Isoabsorptive point (276.66 nm) and λ_{max} of MOX (294 nm) respectively; ax1 and ax2 are the absorptivities of MOX at 276.66 and 294 nm respectively and ay1 and ay2 are the absorptivities of BROM at the two wavelengths respectively.

First Derivative Spectroscopy Method: First derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to them zero-crossing wavelength of the derivative spectrum of another Component [4, 5, 6, 7]. In this method, 301.71 nm was selected for the determination of MOX, which is the zero crossing point of BROM and 293.22 nm, the zero crossing point of MOX, was selected for the determination of BROM.

Method Validation: All the methods were validated as per ICH guidelines for parameters like linearity, accuracy, precision, limit of detection, limit of quantitation [22].

RESULTS AND DISCUSSION: In the present work, three methods, namely, Simultaneous equation method, Q-absorption ratio method and first derivative spectroscopy method were developed for the simultaneous spectroscopic estimation of MOX and BROM in commercially available opthalmic dosage forms. Methanolic HCL was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of the opthalmic formulation were observed.

Simultaneous Equation Method: Estimation of drugs by Simultaneous Equation equation method was carried out at 294 nm (λ_{max} of MOX) and 265 nm (λ_{max} of BROM). The standard solutions of MOX and BROM were prepared to determine the absorptivity values of the subject analyte at the two selected wavelengths. The method showed good linearity in the range of 2-14 µg/mL for MOX and 1-13 µg/mL for BROM. Overlain spectra of both drugs shown in **figure 2**.

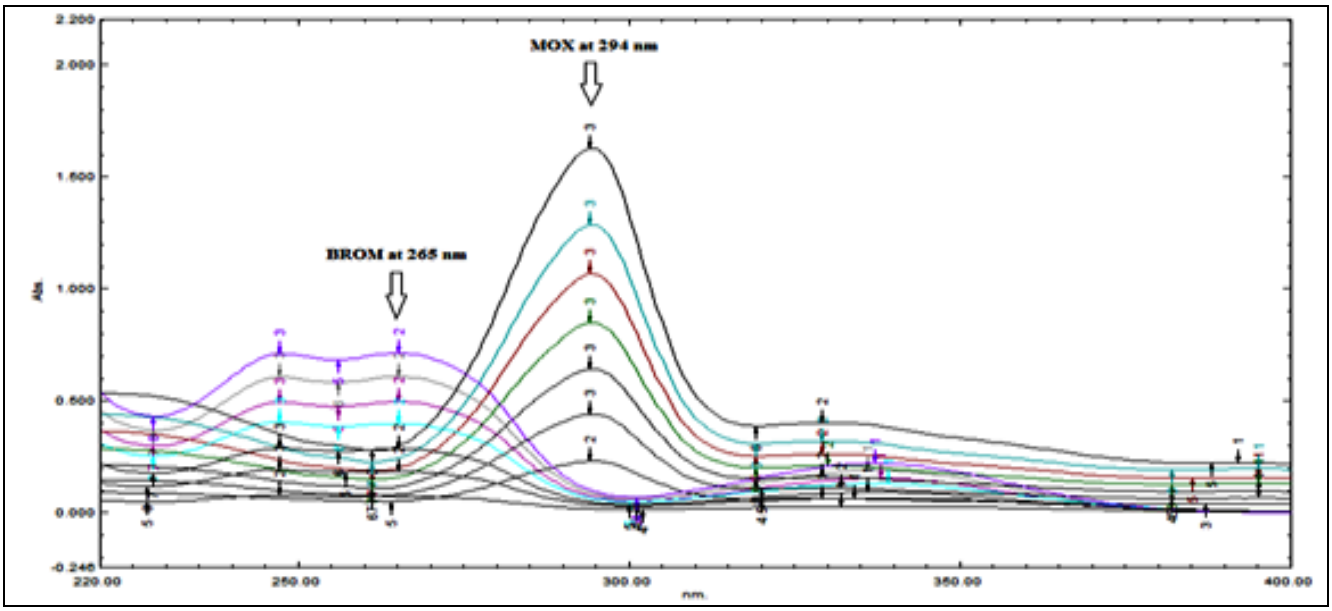


FIG. 2: OVERLAIN ZERO ORDER SPECTRA OF MOX AND BROM (SIMULTANEOUS EQUATION METHOD)

Q-Absorption Ratio Method: As shown in **Figure 3**, the overlain spectra of both drugs show a Reproducible Iso-absorptive point at 276.66 nm. Thus estimation of drugs by Q-absorbance ratio equation method was carried out at 276.66 nm (Isoabsorptive point) and 294 nm (λ_{max} of MOX).

The standard solutions of MOX and BROM were prepared to determine the absorptivity values of the subject analyte at the two selected wavelengths. The method showed good linearity in the range of 1-13 µg/mL for MOX and 1-13 µg/mL for BROM.

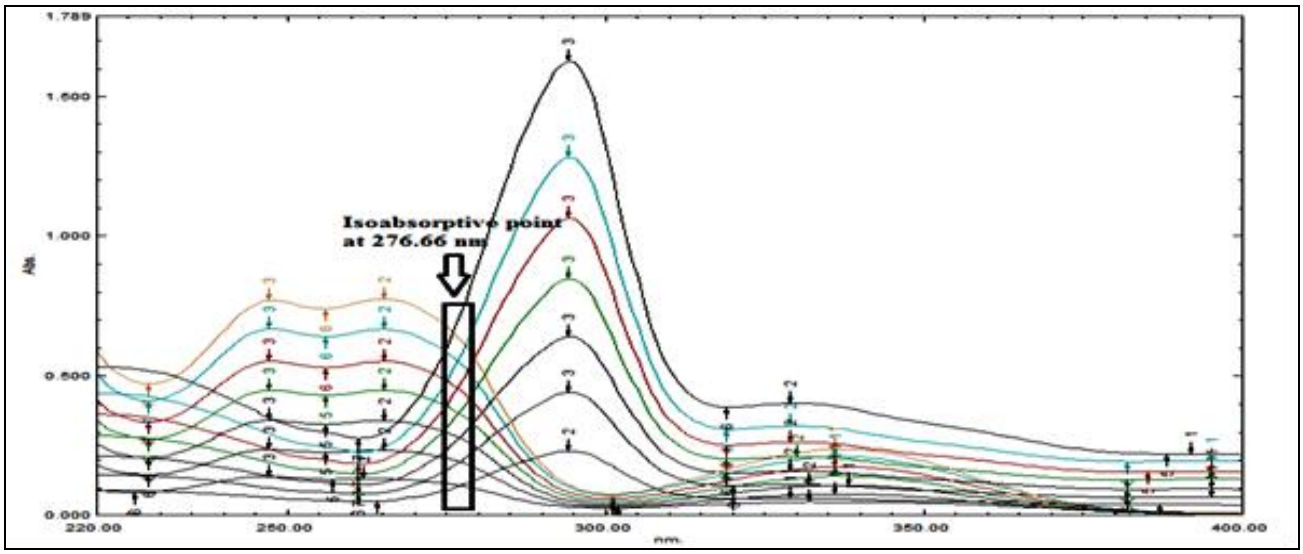


FIG. 3: OVERLAIN ZERO ORDER SPECTRA OF MOX AND BROM (Q-ABSORPTION RATIO METHOD)

First Derivative Spectroscopy Method: In this method, the absorption spectra of standard solutions of MOX and BROM were recorded in the range of 200 nm to 400 nm as shown in Figure 2. The 1st derivative spectra, obtained were traced with smoothing at Δλ=2 nm for determining zero cross points for both the drugs as shown in **Figure 4**. It was found that the 1st derivative spectrum of MOX crosses zero point at 293.22 nm and that of BROM crosses zero point at 301.71 nm. The amplitudes at 301.71 nm were plotted against the respective concentrations of MOX and the amplitudes at 293.22 nm were plotted against the respective concentrations of BROM. The method showed good linearity in the range of 2-14 µg/mL for MOX and 1-13 µg/mL for BROM.

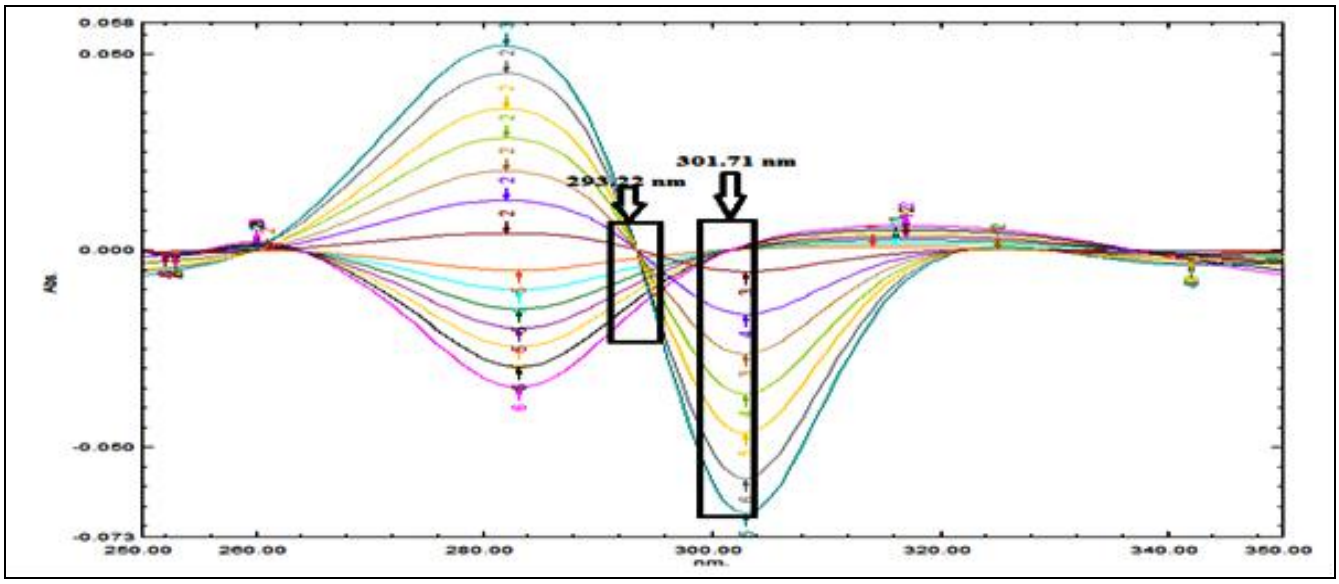


FIG. 4: FIRST DERIVATIVE OVERLAIN SPECTRA OF MOX AND BROM

Method Validation: The developed methods were validated for parameters like linearity, precision, accuracy, LOD, LOQ. the data for which are presented in the following **Tables 1-5**. The low value of R.S.D. value indicates that all the methods are precise and accurate.

TABLE 1: DATA SHOWING LINEARITY OF THE DEVELOPED METHODS

Methods	Simultaneous Equation Method		Q-Absorption ratio method		First derivative spectroscopy method	
Parameters	**MOX**	**BROM**	**MOX**	**BROM**	**MOX**	**BROM**
Linearity Range	2-14 µg/ml	1-13 µg/ml	1-13µg/ml	1-13µg/ml	2-14µg/ml	1-13µg/ml
Slope	0.105	0.051	0.104	0.0045	-0.005	-0.001
Intercept	0.014	0.034	0.025	0.016	0.0003	0.0001
Correlation co-efficient	0.999	0.999	0.999	0.999	0.999	0.999

TABLE 2: DATA SHOWING ACCURACY OF THE DEVELOPED METHODS

DRUG	Amt. taken (µg/ml)	Amt. added (µg/ml)	Amt. added %	% mean recovery(±s.d.) n=3		
				Simultaneous Equation Method	Q-Absorption ratio method	First derivative spectroscopy method
MOX	5.5	1.38	25 %	98.23 ± 0.25	98.85 ± 1.03	98.02 ± 0.86
	5.5	2.75	50 %	98.56 ± 0.89	101.23 ± 0.58	99.58 ± 0.42
	5.5	4.18	75 %	99.87 ± 0.78	100.56 ± 0.84	101.89 ± 1.10
BROM	1	0.25	25 %	99.13 ± 0.85	98.25 ± 0.85	98.82 ± 0.53
	1	0.50	50 %	99.56 ± 0.47	100.13 ± 1.05	98.58 ± 1.22
	1	0.75	75 %	100.47 ± 0.40	101.59 ± 0.74	101.49 ± 0.48

(n = number of repetition)

TABLE 3: DATA SHOWING PRECISION OF THE DEVELOPED METHODS

Methods		Simultaneous Equation Method (%RSD) (n=3)		Q-Absorption ratio method (%RSD) (n=3)		First derivative Spectroscopy method (%RSD)(n=3)	
		MOX	**BROM**	**MOX**	**BROM**	**MOX**	**BROM**
System precision	**Intraday**	0.25	0.59	0.42	0.55	0.58	0.22
	Interday	0.53	0.50	0.27	1.13	0.44	1.03
Method precision	**Intraday**	0.37	0.46	0.35	0.67	0.36	0.65
	Interday	0.77	0.38	0.76	0.31	0.24	0.38

(n = number of repetition)

TABLE 4: DATA SHOWING LOD AND LOQ OF THE DEVELOPED METHODS

Methods	Simultaneous Equation Method		Q-Absorption ratio method		First derivative spectroscopy method	
	MOX	**BROM**	**MOX**	**BROM**	**MOX**	**BROM**
LOD(µg/ml)	0.062	0.097	0.072	0.115	0.132	0.495
LOQ(µg/ml)	0.190	0.294	0.253	0.332	0.400	0.894

TABLE 5: RESULT OF ANALYSIS OF FORMULATION

Methods	Simultaneous Equation Method		Q-Absorption ratio method		First derivative spectroscopy method	
	MOX	**BROM**	**MOX**	**BROM**	**MOX**	**BROM**
%Assay	98.6	99.5	98.34	99.2	98.86	99.18
S.D. (n=3)	0.55	0.39	0.44	0.28	0.38	0.56

(n = number of repetition)

CONCLUSION: The developed spectroscopic methods are found to be simple, sensitive, accurate and precise and can be used for routine analysis of MOX and BROM. The developed methods were validated as par ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of MOX and BROM in combination as a single drug in bulk as well as in pharmaceutical formulations.

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REFERENCES:

1. British Pharmacopoeia, London, United Kingdom: Stationary office, on behalf of medicine and health care products regulatory agency, 2009, vol-1, 4051-54, 3349-52.
2. Rang, H.P., Dale, M.M., Ritter, J.M. and Flower, "Pharmacology", *Elsevier publication house*, 2001, 6th Edition, pp. 647-648.
3. Joel, G.H., "Goodman and Gilman's the Pharmacological basis of therapeutics", *McGraw hill publishers*, *medical publishing division*, 2001, 9th edition, pp. 1637-38.
4. Elsea SH, Osheroff N, Nitiss JL,"Cytotoxicity of quinolones toward eukaryotic cells, Identification of topoisomerase II as the primary cellular target for the quinolone CP-115, 953 in yeast", *J Biol Chem*, 1992, *267 (19)*, 13150–3. http://en.wikipedia.org/Quinolone
5. Walsh, David A, Moran H, "Anti-inflammatory agents-Synthesis and pharmacological evaluation of 2-amino-3-benzoylphenylacetic acid and analogs",*Journal of Medicinal Chemistry* , 1984 , *27(11)*, 1379–88. http://en.wikipedia.org/Bromfenac
6. D. A. Walsh *et al, "*Prostaglandin synthetase inhibitor; analog of amfenac"*, Journal of Medicinal* chemistry, 1984, *27*,.1379.http://www.drugfuture.com/chemdata/bromfenac
7. Sultana N, Arayne MS, Akhtar M, Shamim S, Gula S, and Mehboob M., "High-Performance Liquid Chromatography Assay for Moxifloxacin in Bulk,Pharmaceutical formulations and Serum", *J. Chin. Chem. Soc,* 2010*, 57(4A),* 1-10.
8. Subbaiah PR, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM, "Method Development and Validation for estimation of Moxifloxacin HCl in tablet dosage form by RP-HPLC method", *Pharm Anal Acta,* 2010, *1*,109.
9. Djurdjevic P, Ciric A, Djurdjevic A, Stankovc MS, "Optimization of separation and determination of moxifloxacin and its related substances by RP-HPLC", *JPBA of Elsevier*, 2009, *50*, 117–126.
10. Ulu. ST. *et al,* "High-performance liquid chromatography assay for moxifloxacin Pharmacokinetics in human plasma", *Journal of Pharmaceutical and Biomedical Analysis*, 2007, *43(1),* 320-324.
11. Misra M, Misra AK, Zope P, Panpalia GM, Dorle AK, "Simple and validated Uv- spectroscopy method for estimation of Moxifloxacin in bulk and formulation", *Journal of Global Pharma Technology,* 2010, *2(6)*, 21-27.
12. Dhumal DM, Shirkhedkar AA, and Surana SJ, "Quantitative determination of Moxifloxacin.HCl in bulk and ophthalmic solution by UV-spectrophotometry and first order derivative using area under curve*", Der Pharmacia Lettre*, 2011, *3(3),* 453-456.
13. Vandana, Chaudhary AK, "A Validated HPTLC Method for Estimation of Moxifloxacin Hydrochloride in Tablets", *African Journal of Pharmaceutical Sciences and Pharmacy.* 2010*, 1(1),* 74-84.
14. Motwani ST, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar S, "Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on Moxifloxacin", *Analytica Chimica Acta*, 2007, *582*, 75–82.
15. KAN J, Biao WU, Hong-xiao CUI, "RP-HPLC determination of content and related substances of bromfenac sodium", *Chinese Journal of Pharmaceutical Analysis,* 2009, *04,* 051.
16. Osman MA, Dunning LK, Cheng LK, Wright GJ, "Determination of bromfenac in plasma by high-performance liquid chromatography", *journal of chromatography biomediacal application,* 1989, *81 (489)*, 452-458.
17. Skoog DA., Holler FJ. And Nieman TA. In Principle of Instrumentation Analysis; 5th Edn; Thomas Asia Pvt Ltd., Singapore, 2005, 580.
18. Sharma BK. In Instrumental Method of Chemical Analysis; 25th Edn; Krishna Prakashan Media Ltd, Meerut, 2006, 183-186.
19. Beckett AH. And Stenlake JB. In Practical Pharmaceutical Chemistry, 4th Edn, CBC Publication and Distributors, New Delhi, 1977, 275-300.
20. Ewing GW. Instrumental methods of chemical analysis. Singapore: McGraw-Hil; 1985. 51.

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