WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 1278-1287. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN AND PREDNISOLONE ACETATE IN PHARMACEUTICAL PREPARATION

Pinal B. Patel* and Satish A. Patel

Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana, Gujarat, India.

Article Received on
31 Jan. 2017,

Revised on 20 Feb. 2017,
Accepted on 13 March 2017

DOI: 10.20959/wjpr20174-8201

***Corresponding Author**
Pinal B. Patel
Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana, Gujarat, India.

ABSTRACT

A simple and sensitive first order derivative spectrophotometric method was developed for the simultaneous estimation of Moxifloxacin and Prednisolone Acetate in pharmaceutical dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 258 nm (ZCP of Moxifloxacin) for Prednisolone Acetate and 303 nm (ZCP of Prednisolone Acetate) for Moxifloxacin. The two drugs comply with beer's-lambert's law over the linearity range of 2-40 µg/ml for Moxifloxacin and 5-80 µg/ml for Prednisolone Acetate. The method was validated as per ICH guidelines in terms of linearity, accuracy (recovery study), precision (repeatability, intraday, interday precision), limit of detection and limit of quantification. All the validation parameters were found to be within acceptable limits. The method was found to be simple, sensitive, rapid, cost effective, accurate and precise for the routine analysis of both the drugs in pharmaceutical dosage form.

KEYWORDS: Moxifloxacin, Prednisolone Acetate**,** First order derivative spectrophotometric method, Zero crossing point, Pharmaceutical dosage form, Validation.

INTRODUCTION

Moxifloxacin (MOX) (Figure 1) is chemically 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3. It is a broad spectrum antibacterial drug that is used for the treatment of bacterial infections.[1] Its antibacterial spectrum includes enteric gram (−) rods, atypical bacteria and streptococcus pneumoniae and anaerobic bacteria. It differs from earlier antibacterials of the fluoroquinolone class such as levofloxacin and ciprofloxacin in having greater activity against gram-positive bacteria and anaerobes. Because of its potent activity against the common respiratory pathogen streptococcus pneumoniae, it is considered a "respiratory quinolone."[2] It is official in Indian Pharmacopoeia (IP)[3], United State Pharmacopoeia (USP)[4], British Pharmacopoeia (BP)[5] and European Pharmacopoeia (EP).[6] IP, USP, BP and EP describe LC method for its determination. Literature review reveals RP-HPLC[7], HPTLC[8] and spectrophotometry[9] methods for determination of MOX in alone. Prednisolone Acetate (PRD) (Figure 2) is chemically Pregna-1, 4-diene-3, 20-dione,21-(acetyloxy)-11,17-dihydroxy-,(11β). It is a topical anti-inflammatory agent for ophthalmic use.[1] Prednisolone is a corticosteroid drug with predominant glucocorticoid and low mineralocorticoid activity, making it useful for the treatment of a wide range of inflammatory and autoimmune conditions such as asthma, uveitis, pyoderma gangrenosum, rheumatoid arthritis.[2] This drug is official in Indian Pharmacopoeia (IP)[10], United State Pharmacopoeia (USP)[11], British Pharmacopoeia (BP)[12], European Pharmacopoeia (EP)[13] and Japanese Pharmacopoeia (JP).[14] IP, USP, EP, BP and JP describe LC method for its estimation. Literature survey reveals HPLC[15] and spectrophotometry[16-18] methods for determination of PRD alone. The present invention relates to a fixed dose combination comprising one or more antibiotics and one or more steroidal anti-inflammatory agents for the treatment of ocular infections. The combination is not official in any pharmacopoeia; hence no official method is available for simultaneous estimation of these two drugs. Literature survey reveals RP-HPLC[19], Stability indicating HPLC[20], HPTLC[21] and spectrophotometry (simultaneous equations method)[22] methods for estimation of MOX and PRD in combined dosage form. Literature survey reveals only single spectrophotometric method based on simultaneous equation for estimation of this two drugs in mixture; hence, it is thought of interest to developed and validate alternative spectrophotometric method for simultaneous estimation of MOX and PRD in combined dosage form. The present manuscript describe new simple, accurate, precise and sensitive UV spectrophotometric method based on absorbance correction for simultaneous estimation of MOX and PRD in combined ophthalmic formulation.

MATERIALS AND METHODS

Materials

Pure sample of MOX was obtained from Taj Pharmacutical Ltd, Ahmedabad, Gujarat. PRD was provided as a gift sample from Maharshi Pharma Chem Private Ltd, Ahmedabad, Gujarat. Methanol (S. D. Fine Chemicals Ltd, Mumbai) was used in the study as solvent. All the chemicals used were of analytical grade. 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. A sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) were used in the study.

Methods

Preparation of Standard Stock and Working Standard Solutions

Standard stock solution of MOX and PRD was prepared by accurately weighing 10 mg of pure drug powder to separate 100 ml calibrated volumetric flask, dissolved and diluted up to mark with methanol to obtain 100 µg/ ml of each drugs. Aliquots of MOX and PRD and were suitably diluted with methanol to obtain the final concentration in the range of 2 to 40 µg/ml and 5 to 80 µg/ml for MOX and PRD, respectively.

Methodology

The standard solution of MOX (20 µg/ml) and PRD (20 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. The two spectra were overlain and it appeared that MOX showed zero crossing at 258 nm, while PRD showed zero crossing at 303 nm. At the zero crossing point (ZCP) of MOX (258 nm), PRD showed a first-derivative absorbance, whereas at the ZCP of PRD (303 nm), MOX showed a first-derivative absorbance. Hence 303 and 258 nm was selected as analytical wavelengths for determination of MOX and PRD, respectively. These two wavelengths can be employed for the determination of MOX and PRD without any interference from the other additives in their combined dosage formulation.

VALIDATION OF THE DEVELOPED METHOD

The method was validated as per the International Conference on Harmonization (ICH) guidelines.[23]

Linearity (Calibration curve)

The calibration curves were plotted a concentration range of 2 - 40 µg/ml for MOX and 5 – 80 µg/ml for PRD. Accurately measured standard solutions of MOX (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml) and PRD (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-derivative absorbance was measured at 303 nm for MOX and 258 nm for PRD. The calibration curves were constructed by plotting derivative absorbances versus concentrations and the regression equations were calculated.

Method precision

Repeatability

The standard solutions of MOX and PRD (10 µg/ml and 20 µg/ml) was prepared. The absorbance was measured at selected wavelength six times on same day without changing the parameters of the developed method and % RSD was calculated.

Intraday and Interday precision

The intraday variation (% RSD) was determined by analysis of three standard solutions of MOX and PRD (10, 20 and 30 µg/ml and 10, 30 and 50 µg/ml) three times on the same day. Interday variation (% RSD) was determine by analysis of three standard solutions of MOX and PRD (10, 20 and 30 µg/ml and 10, 30 and 50 µg/ml) three times on the three different days for period of one week and % RSD was calculated.

Accuracy (recovery study)

The accuracy of an analytical procedure is the closeness of agreement between the value which is accepted as true value and the value found. The recovery experiment were carried out by adding known amount of standard solution of MOX and PRD at 50%, 100% and 150% level to prequantified sample solution of MOX (5 µg/ml) and PRD (10 µg/ml). The amount of MOX and PRD were analyzed by proposed method.

Limit of detection and limit of quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio by the use of standard deviation of the response and the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

LOD = 3.3 X σ/S

LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

Determination of MOX and PRD in their combined ophthalmic formulation

The eye-drop (1.0 ml) containing 5 mg of MOX and 10 mg of PRD was transferred to 25 ml volumetric flask. Methanol (10 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with methanol to get a final concentration of 5 µg/ml of MOX and 10 µg/ml of PRD. The resulting solution was analyzed by proposed methods.

RESULT AND DISCUSSION

In first derivative spectrophotometric method, the foremost and prime need is that the drugs should comply with the beer's law at selected wavelengths. Linear correlation was obtained between absorbance and concentration of MOX and PRD in the concentration ranges of 2-40 µg/ml and 5-80 µg/ml, respectively. The standard solutions of MOX and PRD were scanned separately in the UV range and zero-order spectra (Figure 3) thus obtained was then processed to obtain first-derivative spectra. The two derivative spectra showed maximum absorbance at 258 nm (ZCP of MOX) for PRD and 303 nm (ZCP of PRD) for MOX. First-derivative absorbances (D1) were recorded 303 nm for MOX and 258 nm for PRD (Figure 4). First derivative spectra give good quantitative determination of both the drugs at their respective wavelengths without any interference from the excipients in their combined dosage formulation.

Table 1: Recovery Data of MOX and PRD by first order derivative spectrophotometric Method

% Level (n=3)	Amount of drug taken (µg/ml)		Amount standard added (µg/ml)		% Recovery ± S.D	
	MOX	PRD	MOX	PRD	% MOX ± S.D	%PRD ± S.D
50%	5	10	2.5	5	101.1 ± 0.80	100.8 ± 1.03
100%	5	10	5	10	101.6 ± 1.03	99.53 ± 0.64
150%	5	10	7.5	15	99.33 ± 0.93	100.2 ± 0.76

S.D. is standard deviation and n is number of replicate.

Table 2: Analysis of MOX and PRD in ophthalmic formulation by developed method

Formulation	Label claim (mg)		Amount found (mg)		% Label claim ± S.D (n=3)	
EYE DROPS	MOX	PRD	MOX	PRD	MOX	PRD
	5	10	4.96	10.1	99.20 ± 0.50	101.0 ± 1.10

S.D is standard deviation and n is number of replicate.

Table 3: Regression analysis data and summary of validation parameters by proposed first order derivative spectrophotometric method

Parameter	First derivative spectrophotometric Method	
	MOX	PRD
Wavelength	303 nm	258 nm
Beer's Law Linearity Range (µg/ml)	2-40	5-80
Slope	0.0059	0.0009
Intercept	0.0024	0.0006
Correlation Coefficient (r^2)	0.9993	0.9997
Accuracy ± S.D. (%Recovery, n= 3)	100.7 ± 1.19	100.2 ± 0.63
Repeatability (% RSD , n= 6)	0.87	0.61
Intraday Precision %RSD (n = 3)	0.53 - 0.91	0.56 - 1.10
Interday Precision %RSD (n = 3)	0.79 - 0.94	0.71 - 1.29
LOD (µg/ml)	0.19	1.29
LOQ (µg/ml)	0.59	3.90

LOD = Limit of detection, LOQ = Limit of quantification, RSD = Relative standard deviation, S. D. = Standard deviation, n = number of replicates.

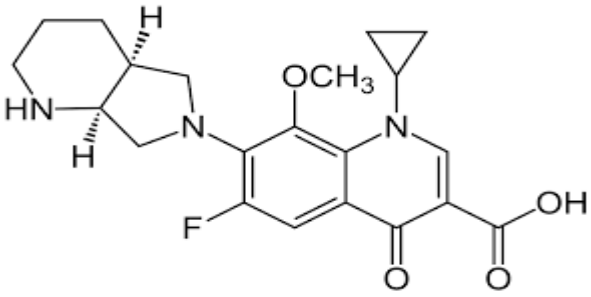

Figure 1: Structure of Moxifloxacin

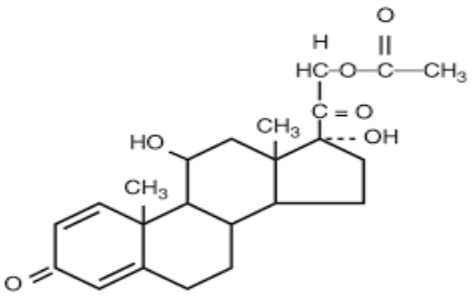

Figure 2: Structure of Prednisolone Acetate

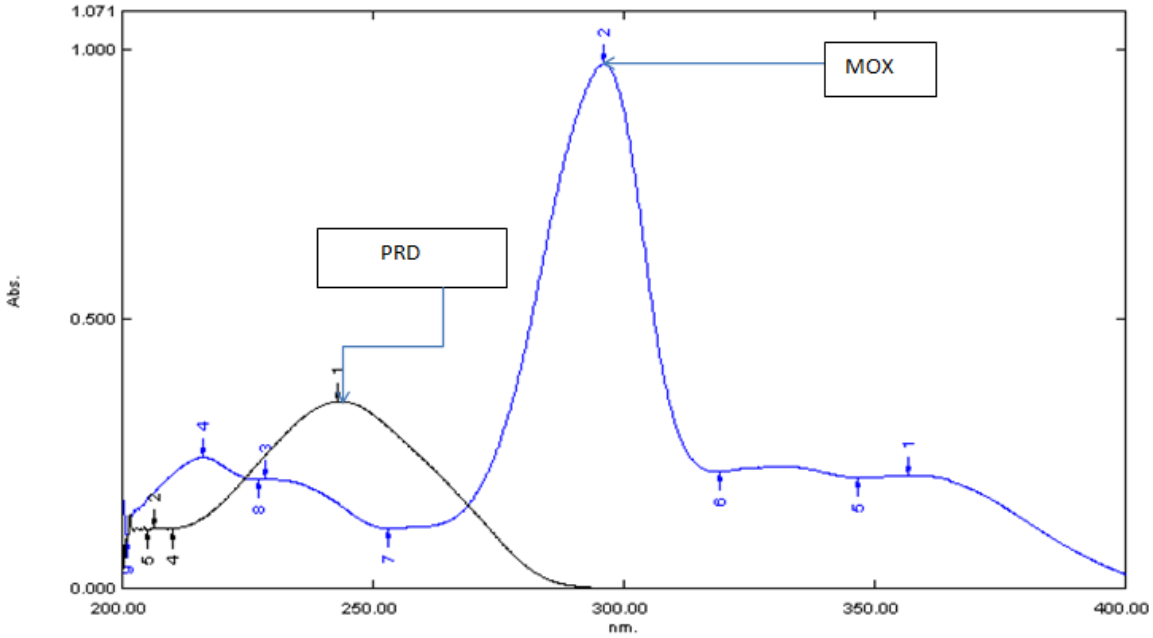

Figure 3: Overlain zero order spectra of MOX (20 µg/ml) and PRD (20 µg/ml)

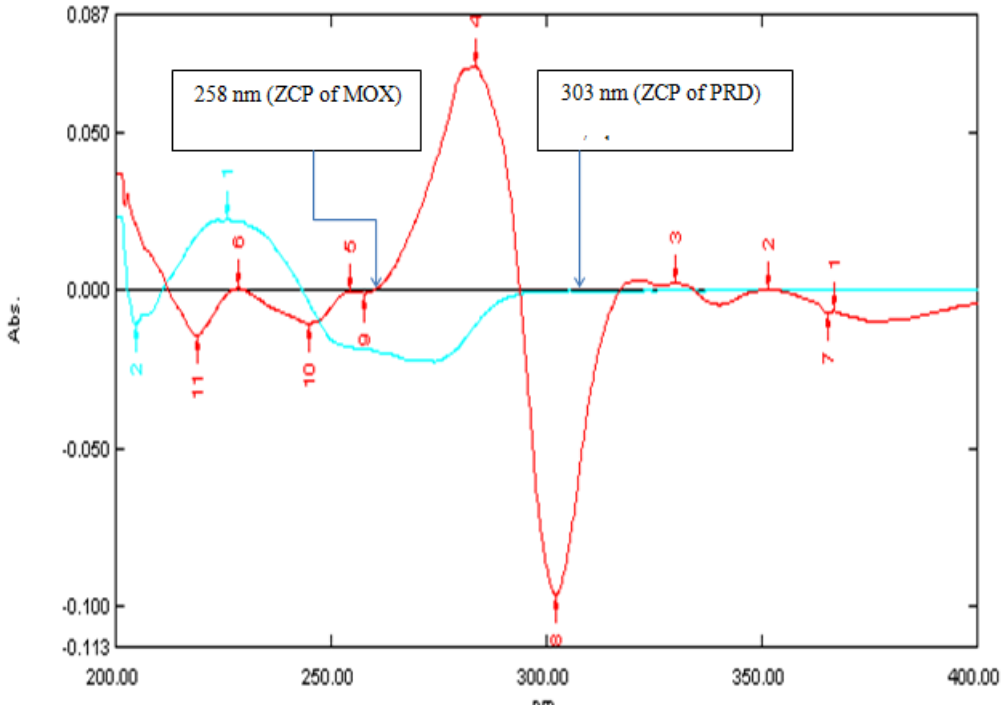

Figure 4: Overlain first-order derivative spectra of MOX (20 µg/ml) and PRD (20 µg/ml)

The validation parameters were studied at all the selected wavelengths for the developed method. All the validation parameters were found to be within acceptable limits. The % recoveries were found to be in the range of 99.33 – 101.60% for MOX and 99.53 – 100.80% for PRD (Table 1). The precision of method was determination by repeatability, intraday, interday precision and was expressed as the % RSD which indicates good method precision

(Table 3), The LOD and LOQ for MOX at 303 nm were found to be 0.19 µg/ml and 0.59 µg/ml, respectively. The LOD and LOQ for PRD at 258 nm were found to be 1.29 µg/ml and 3.90 µg/ml, respectively. All the regression and validation parameters are summarized in Table 3. The proposed spectrophotometric method was successfully applied to determine MOX and PRD in pharmaceutical dosage form. MOX and PRD content in marketed eye drops were found to be 99.20% and 101.0%, respectively indicates non-interference from excipients (Table 2).

CONCLUSION

The first order derivative spectrophotometric method was developed for simultaneous determination of MOX and PRD in binary mixture. Method was found to be precise and accurate as can be reflected from validation parameters data. Developed method was efficiently applied for determination of MOX and PRD in pharmaceutical formulation and there for method can be extended for the routine QC analysis of both drugs in formulation.

ACKNOWLEDGEMENT

The authors are thankful to was obtained for providing gift sample of Taj Pharmacutical Ltd, Ahmedabad, Gujarat. and Maharshi Pharma Chem Private Ltd, Ahmedabad, Gujarat. MOX and PRD for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES

1. O'Neil MJ. The Merck Index (2006): An Encyclopedia of Chemicals, drugs and Biological. 14th edition New Jersey; Published by Merck Research Laboratories, Division of Merck and co., Inc., Whitehouse Station, Prednisolone acetate and Moxifloxacin HCl 2006; 59: 115–39.
2. Tripathi, K. D. (6). (2006). Essentials of Medicinal Pharmacology. Jaypee Brothers Medicinal Publication (P) Ltd., New Delhi. 688-693/203.
3. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare Ghaziabad, Indian Pharmacopeia Commission, 7th edition., 2014; 3: 2254-2256.
4. USP 36 NF 31 United State Pharmacopoeia, U.S. Pharmacopeia Convention. Twinbrook Parkway, Rockville, MD, 2013; 3: 4412 - 4415.
5. British Pharmacopoeia, The Department of Health, Social Services and Public Safety, London Her Majesty's. Stationary office, 6th edition., 2010; 2: 1459-1460.

6. Europian Pharmacopoeia, volume 2,6th edition EDQM European Directorate for the Quality of Medicines & Healthcare, Council of Europe Strasbourg, 6th edition, 2007; 2: 2451-2452.
7. Subbaiah P. Rama, Kumudhavalli M .V, Saravanan C, and Chandira R. Margret. Method Development and Validation for Estimation of Moxifloxacin HCl in Tablet dosage form by RP-HPLC method. Pharmaceutica Analytical Acta, 2010; 1(1): 2153-2435.
8. Guerra FL, Paim CS, Steppe M, Schapoval EE. A Validation HPTLC Method for Estimation of Moxifloxacin Hydrochloride in Tablets, Pharmaceutical Methods. 2010; 1(1): 1086-92.
9. Tarkase Kailash N., Admane Swati S., Sonkhede Neha G. and Shejwal Seema R. Development and Validation of UV-Spectrophotometric Methods for Determination of Moxifloxacin HCL in Bulk and Pharmaceutical Formulations. Der Pharma Chemica, 2012; 4(3): 1180-1185.
10. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare Ghaziabad, Indian Pharmacopeia Commission, 7th edition., 2014; 3: 2541-2545.
11. USP 36 NF 31 United State Pharmacopoeia, U.S. Pharmacopeia Convention. Twinbrook Parkway, Rockville, MD, 2013; 3: 4878-4887.
12. British Pharmacopoeia, The Department of Health, Social Services and Public Safety, London Her Majesty's. Stationary office, 6th edition., 2010; 2: 3025-3029.
13. Europian pharmacopoeia, volume 2,6th edition EDQM European Directorate for the Quality of medicines & Healthcare, Council of Europe Strasbourg, 6th edition, 2007; 2: 2741-2746.
14. Japanese pharmacopoeia. Society of Japanese Pharmacopoeia, Shibuya Tokyo, Japan 15thedition, 2006; 1021-1026.
15. Maria Nella Gai, Elizabeth Pinilla, Claudio Paulos, Jorge Chávez, Verónica Puelles and Aquiles Arancibia. Determination of Prednisolone and Prednisone in Plasma, Whole Blood, Urine and Bound-to-Plasma Proteins by High-Performance Liquid chromatography. J. Chrom. Sci., 2005; 43(5): 201-206.
16. R. Ashok, P.P. Prakash and Tamil R. Selvan. Development and Validation of Analytical method for Estimation of Prednisolone in Bulk and Tablets using UV-visible spectroscopy, Int. J. Pharm. Pharmac. Sci., 2011; 3(4): 184-186.
17. Bhusnure O. G., Bawage M. S. and Gholve S. B. Eco-friendly and cost-effective UV spectroscopy Method for the estimation of Prednisolone sodium Phosphate in bulk and Pharmaceutical dosage form, Int. J. of Pharmac. Sci. and Res., 2015; 6(1): 327-332.

18. Raval Kashyap E., V. S. Subrahmanyam, A. R. Sharbaraya. Development and Validation UV Spectroscopy method for the estimation of Prednisolone in bulk and dosage form. J. Chem. and Pharmac. Res., 2012; 4(2): 1090-1096.
19. Reddy Haritha N, Samidha T, Mangangkhomba Mangang K. H, Sudheer kumar.D, Sreekanth G. Simple RP-HPLC Method for Simultaneous Estimation of Moxifloxacin hydrochloride and Prednisolone acetate in eye drops, Indo. Am. J. of Pharmac. Res. 2013; 3(10): 8008-8018.
20. Razzaq Syed Naeem, Khan Islam Ullah, Irfana Mariam and Syed Saleem Razzaq. Stability indicating HPLC method for the simultaneous Determination of Moxifloxacin and Prednisolone in Pharmaceutical Formulations. Chem Cent Journal. 2012; 6(1): 94.
21. Raut Ganesh S., Shirkhedkar Atul A. Simultaneous Determination of Prednisolone acetate and Moxifloxacin hydrochloride in bulk and in eye drop using RP-HPTLC. J. of Liq. Chrom. & Rel. Tech. 2013; 37(4): 528-537.
22. Patel Rajesh, Sayaendra K. Shrivastava, Bhandari Priya and Patidar Arun, Simultaneous estimation of Moxifloxacin HCl and Prednisolone acetate from eye drop formulation, Int. J. of Phar. & Lifesciences, 2012; 3(11): 2111-2114.
23. International Conference on Harmonization. (2005). Q2R1: Validation of Analytical Procedure: Text and Methodology. The Third International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland.