Research Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Betamethasone sodium phosphate and Ofloxacin in Eye Drops

Dhara Patel[1*], Varshney Priya[1], Dhananjay Meshram[1] and Sharav Desai[2]

[1]Department of quality assurance, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India.

[2]Department of Pharmaceutical microbiology and biotechnology, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India.

ABSTRACT

A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for the simultaneous analysis of betamethasone sodium phosphate and ofloxacin in eye drops. The separation was carried out using a mobile phase consisting of water and acetonitrile with pH 2.0 adjusted with orthophosphoric acid in the ratio of 30: 70 % v/v. The column used was Shim pack XR ODS II (150 mm x 3 mm id, 5 µm) with flow rate of 1 ml / min using UV detection at 247 nm. The described method was linear over a concentration range of 1-6 µg/ml and 3-18 µg/ml for the assay of betamethasone sodium phosphate and ofloxacin respectively. The retention times of betamethasone sodium phosphate and ofloxacin were found to be 7.06 ± 0.038 and 2.64 ± 0.029 min respectively. Method was validated statistically and recovery studies were carried out. The proposed method has been applied successfully to the analysis of cited drugs either in pure form or in pharmaceutical formulations with good accuracy and precision. The method here in described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.

Keywords: Betamethasone sodium phosphate, Ofloxacin, RP-HPLC, Validation.

INTRODUCTION

Chemically, betamethasone sodium phosphate (BMS) is disodium; [2-[(8S,9R,10S,11S ,13S,14S,16S,17R)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo 6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] phosphate[1]. Betamethasone sodium phosphate is a synthetic glucocorticoid given orally, parenterally by local injection, inhalation or applied topically in the management of various disorders in which corticosteroids are indicated. It is official in IP[1], BP[2], and USP[3] that describes spectrophotometric and HPLC method for its estimation. Ofloxacin (OFLO) is chemically, 7-fluoro-2-methyl-6-(4-methyl piperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0{5,13}] trideca-5(13),6,8,11-tetraene-11-carboxylic acid, is a fluoroquinolone antibacterial agent used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy.[4,5]. It is official in IP[6], BP[7] and USP[8] which describes potentiometric and titrimetric method for its estimation.The chemical structure of betamethasone sodium phosphate and ofloxacin is shown in the (Fig 1 and 2) respectively. A detailed literature survey revealed spectrophotometric[9-11], HPLC[12-16], HPTLC[17, 18], and stability indicating RP-HPLC[19-21] method for both betamethasone sodium phosphate and ofloxacin as individual and with other drug combination. The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of betamethasone sodium phosphate and ofloxacin in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of betamethasone sodium phosphate and ofloxacin in combined dosage forms.

MATERIALS AND METHODS

Betamethasone sodium phosphate and Ofloxacin were obtained as a gift sample from Sun Pharmaceuticals Industries Ltd, Vadodara.Methanol, Water, Acetonitrile and distilled water was used in the study.The

commercial fixed dose combination product containing 0.1% w/v betamethasone sodium phosphate and 0.3% w/v ofloxacin was procured from the local market. All other chemicals and reagents used were of HPLC grade. RP-HPLC instrument (Shimadzu, LC-20 AD) equipped with a UV-Visible detector, aShim pack XR ODS II 150 mm x 3 mm id, 5 µmcolumn was used. Chromatograms were automatically obtained by LC-solution system software. Chromatographic separations were obtained by gradient elution mode which was performed using a mobile phase containing Water and Acetonitrile (pH adjusted to 2.0 using orthophosphoric acid) in the ratio of 30:70 %(v/v) at a flow rate of 1ml/min through Shim pack XR ODS II (150 mm x 3 mm id, 5 µm). The selective detection of the column effluent was monitored at a wavelength of 247nm. Injection volume was 20µl.

Preparation of standard stock solutions

A stock solution of BMS and OFLO (100 µg/ml) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-ml volumetric flasks and dissolving in methanol and diluted to 100 ml with same solvent upto the mark.

Preparation of sample solution

Test solution was done in triplicate, by adding 1ml of unfiltered sample stock solution (containing 1000µg/ml of BMS and 3000 µg/ml of OFLO) in a test tube. The contents of test tube were then shaken for some time and cautiously filtered through whatmann filter paper and the filtrate was collected in to 100ml volumetric flask. In order to collect the remnants of the solution, the test tube and filter paper were washed with small quantities of diluent, and the washings were used to make up the solution upto the mark. The resultant solution was filtered through 0.45µ membrane filter. 3 ml of this solution (100ml) was pipetted out and was transferred to a 10ml volumetric flask and finally make up this solution upto the mark with the methanol. Now this solution contains 3µg/ml of BMS and 9µg/ml of OFLO. The obtained mean peak areas were substituted in the regression equation of linearity curve and the amount was calculated.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[22].

Calibration curve (Linearity)

Calibration curves were constructed by plotting Peak areas vs. Concentrations of BMS and OFLO, and the regression equations were calculated. The calibration curves were plotted over the concentration range 1-6 µg/ml for BMS and 3-18 µg/ml for OFLO. Accurately measured standard working solutions of BMS (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml) and OFLO (0.3, 0.6, 0.9, 1.2, 1.5, 1.8 ml) were transferred to a series of 10ml of volumetric flasks and diluted to the mark with methanol. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions described above.

Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for betamethasone sodium phosphate and ofloxacin (3 µg/ml for BMS and 9 µg/ml for OFLO) without changing the parameter of the proposed spectrophotometry method.

Intermediate 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 over a period of 1 week for 3 different concentrations of standard solutions of betamethasone sodium phosphate (3, 4, and 5µg/ml) and ofloxacin (9, 12 and 15 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

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, i.e., 3.3 for LOD and 10 for LOQ) 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.

RESULTS AND DISCUSSION

The regression analysis data and validation parameters for the methods are shown in Table 1. The calibration curve for BMS and OFLO is shown in Figure 3 and 4. The method was found to be precise and accurate which was evident from its low %RSD values Table 2 and 3. The results of the assay are shown in the Table 4. Results of robustness study are shown in Table 5. The results for system suitability are shown in Table 6. Chromatogram of baseline, standard BMS and OFLO individual and in mixture are shown in the Figure 5, 6,7 and 8.

In this proposed method, the linearity is observed in the concentration range of 1-6 µg/ml for BMS and 3-18 µg/ml for OFLO with co-efficient of correlation, (R^2) = 0.9973 and (R^2) = 0.9962 for BMS and OFLO, respectively at 247 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the BMS and OFLO in combined dosage form without any interference of excipients.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Parameters		RP-HPLC method	
		BMS	OFLO
Concentration range (µg/ml)		1-3	3-18
Slope		49992	28963
Intercept		6371.6	18178
Correlation coefficient		0.9973	0.9962
LOD (µg/ml)		0.113	0.422
LOQ (µg/ml)		0.343	1.279
Accuracy ± S.D (n=3)	50%	100.55 ± 0.0503	100.36 ± 0.100
	100%	100.16 ± 0.0416	102.49 ± 0.1571
	150%	99.80 ± 0.0435	101.95 ± 0.1800
Repeatability (% RSD, n=6)		1.17	1.31
Intraday precision (n=3)		0.89-1.62	0.32-1.38
Interday precision (n=3)		0.59-1.38	0.37-0.88
Assay ± S.D		99.66 ± 0.055	99.88 ± 0.137

Table 2: Statistical analysis for precision of proposed method

Drug	Conc. of drug (µg/ml)	%RSD (n=3)	
		Intraday	Interday
Betamethasone sodium phosphate	3	1.62	1.38
	4	1.19	0.59
	5	0.89	1.07
Ofloxacin	9	0.32	0.48
	12	1.38	0.37
	15	1.05	0.88

Table 3: Statistical analysis for accuracy of proposed method

DRUGS	Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Total amount of drug (µg/ml)	%Recovery (n = 3)	%RSD
BMS	50%	2	1	3	100.55	1.66
	100%		2	4	100.16	1.03
	150%		3	5	99.80	0.87
OFLO	50%	6	3	9	100.36	1.10
	100%		6	12	102.49	1.27
	150%		9	15	101.95	1.17

Table 4: Analysis of BMS and OFLO in marketed formulation

Formulation (eye drops)	Labeled amount (mg/ml)		Amount found (mg/ml)		% Label claim ± S.D Assay	
	BMS	OFLO	BMS	OFLO	BMS	OFLO
	1	3	0.99	2.99	99.66 ± 0.055	99.88 ± 0.137

Table 5: Results of Robustness study

Sr. No.	Parameters	Variation	Assay % of BMS	Assay % of OFLO
1	Flow rate (±0.2 ml/min)	a) 0.8 ml/min	101.86	101.16
		b) 1.0 ml/min	99.66	99.88
		c) 1.2 ml/min	101.70	101.26
2	Wavelength (±2 nm)	a) 245 nm	101.40	101.62
		b) 247 nm	99.90	100.1
		c) 249 nm	100.85	100.36
3	Mobile phase composition %v/v (± 2)	a) 68:32	101.40	101.16
		b) 70:30	100.50	100.28
		c) 72:28	100.85	101.43

Table 6: Results of System suitability parameters

Parameters	Data obtained	
	BMS	OFLO
Retention Time	7.06 ± 0.03834	2.64 ± 0.0296
Theoretical plates per column	3579.20 ± 72.0915	8904.082± 33.5664
Symmetry factor/Tailing factor	1.69 ± 0.0494	1.4± 0.0563
Capacity factor	2.05	0.115
Separation factor	17.9	
Resolution	15.115	

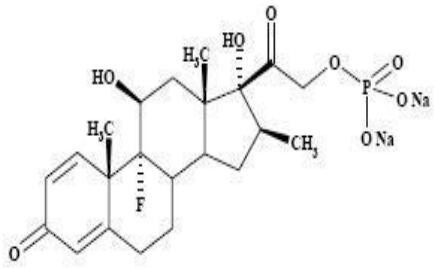

Fig. 1: Chemical structure of Betamethasone sodium phosphate

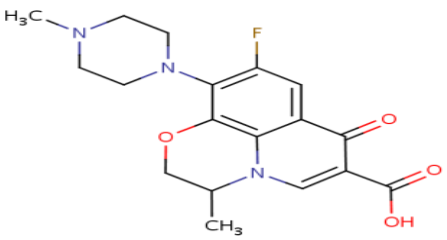

Fig. 2: Chemical structure of Ofloxacin

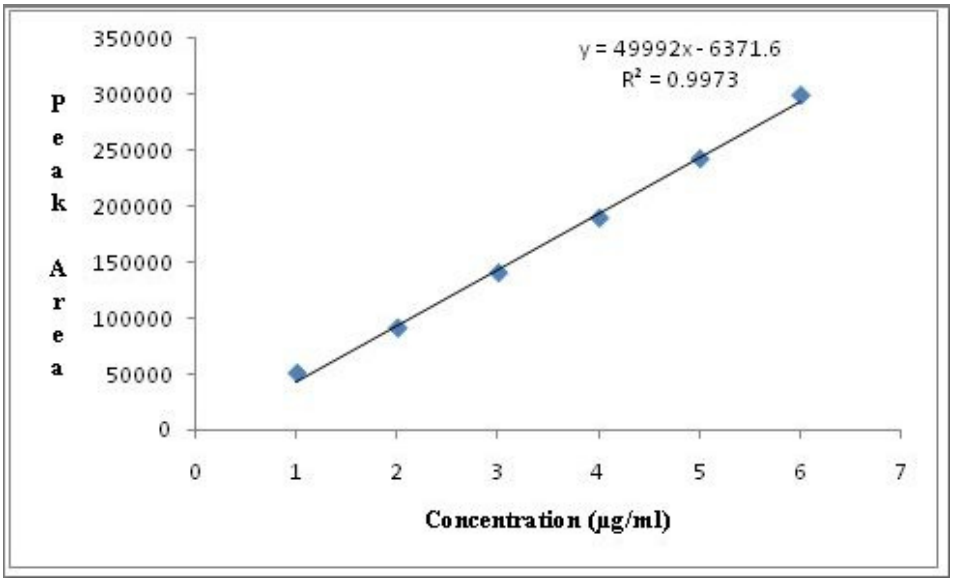

Fig. 3: Calibration curve of BMS

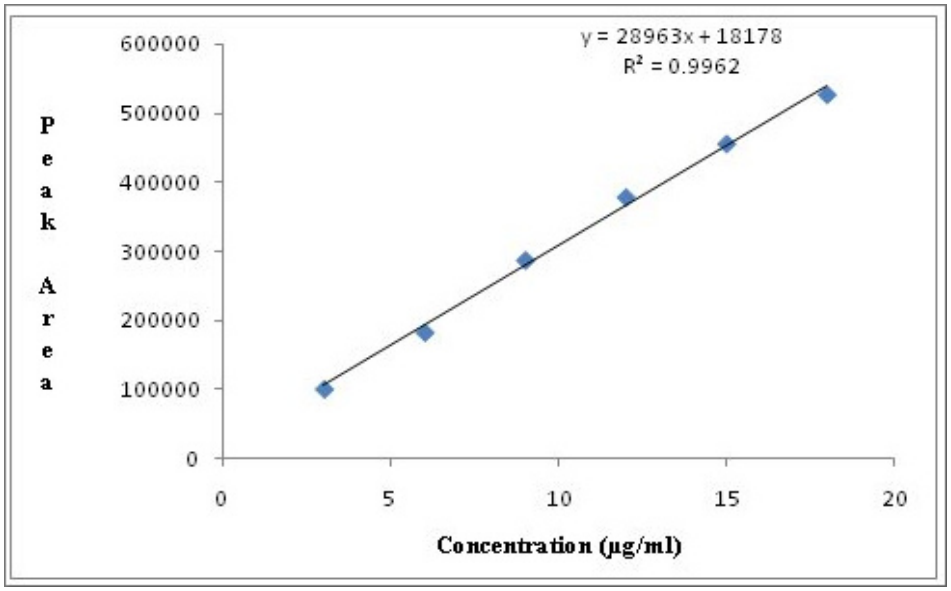

Fig. 4: Calibration curve of OFLO

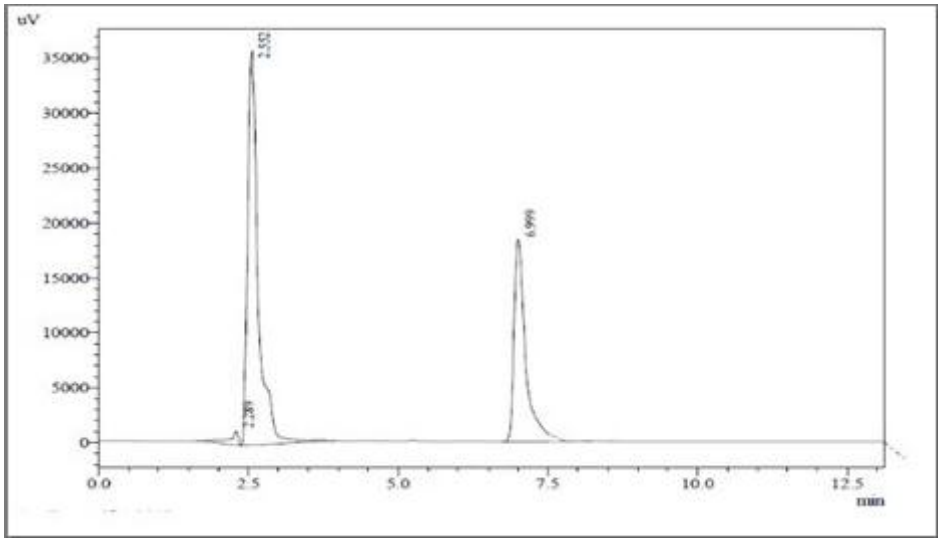

Fig. 5: Chromatogram of BMS and OFLO

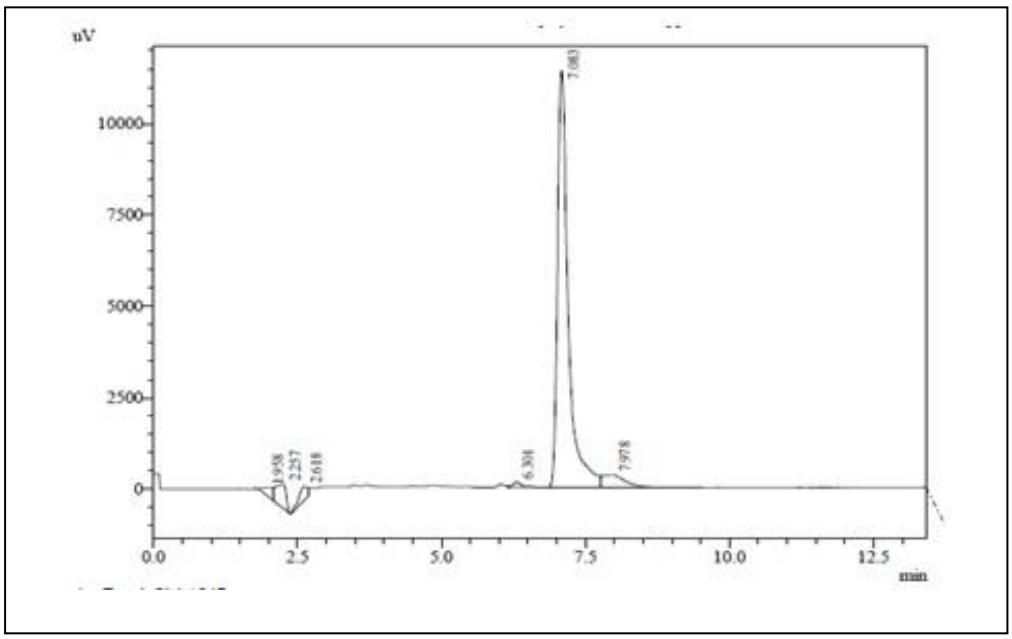

Fig. 6: Standard chromatogram of BMS

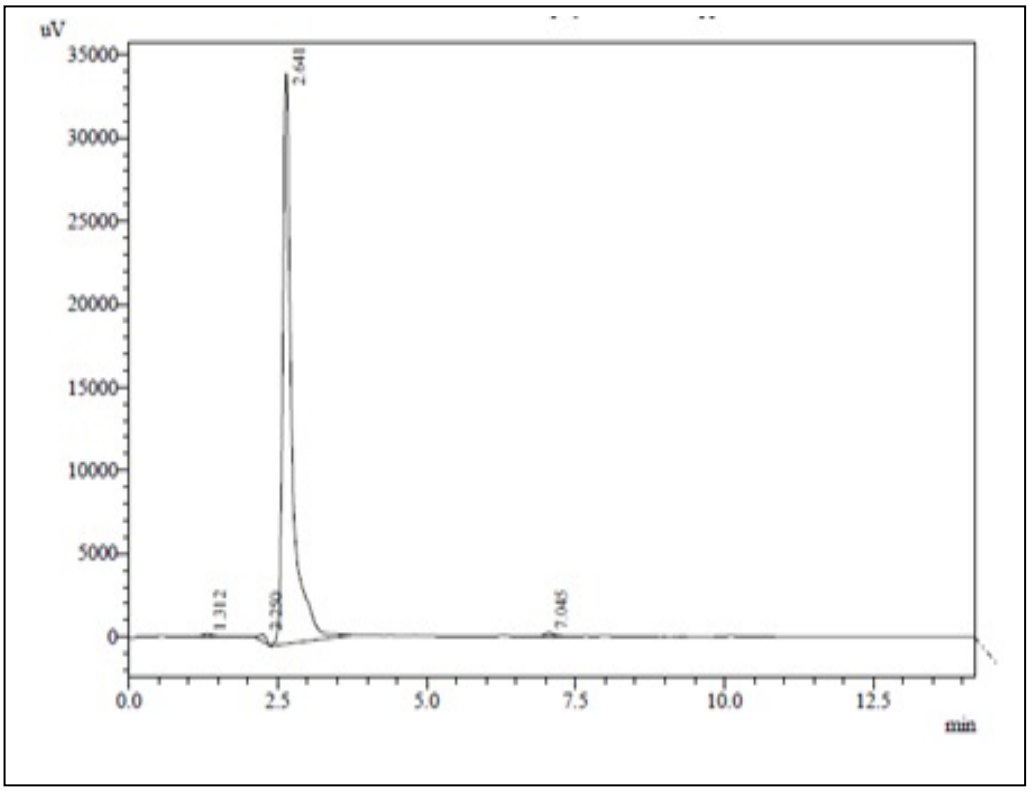

Fig. 7: Standard chromatogram of OFLO

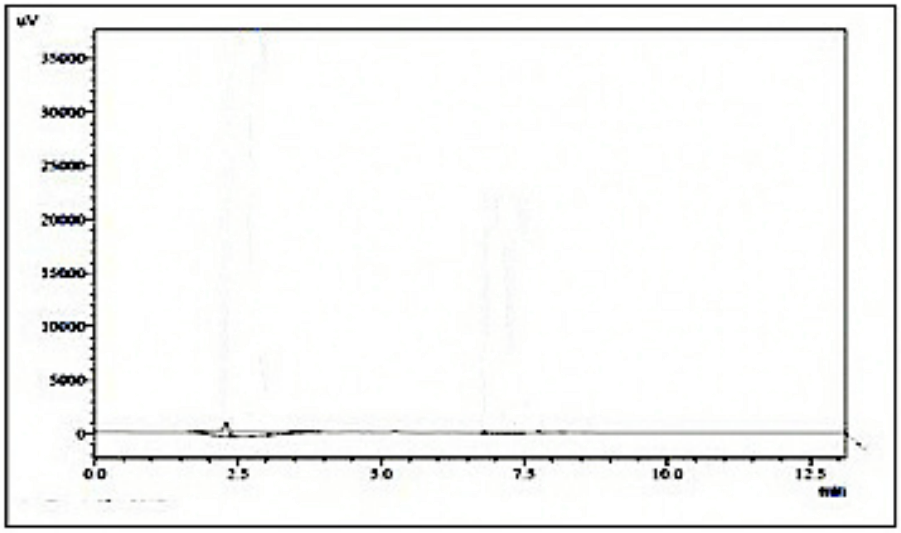

Fig. 8: Chromatogram of baseline

REFERENCES

1. Indian Pharmacopoiea. Vol. 2. New Delhi: Controller of Publication, Govt. of India, Ministry of Health and Family Welfare. 2007: 181-182.
2. British Pharmacopoiea, Vol. 3. London: HMSO Publication. 2009: 8112-8113.
3. United State of Pharmacopoeia 31 National Formulary 26, Vol. 1. The standard of quality. United State Pharmacopoeial Convention Inc. 2009: 1515.
4. EF. James Reynolds. Martindale: The Extra Pharmacopoeia: 30th Ed, London: The Pharmaceutical Press; 1993.
5. SC. Sweetman, Martindale: The Complete Drug Reference. 35th ed. London: The Pharmaceutical Press; 2007: 207.
6. Indian Pharmacopoeia. Vol. 3. New Delhi: Controller of Publication, Govt. of India, Ministry of Health and Family Welfare. 2007: 854-855.
7. British Pharmacopoeia, Vol. 2. London: HMSO Publication; 2010: 1546.
8. United State of Pharmacopoeia 31 National Formulary 26, Vol. 2. The standard of quality. United State Pharmacopoeial Convention Inc. 2009: 2847.
9. Awen BZ, Hwisa NT, Rao C, Nama S and Katakam P. Development and validation of a new spectrophotometric method for the determination of Betamethasone in bulk and pharmaceutical dosage forms. Int J PharmBiomed Res. 2010; 1: 87-89.
10. Wankhede SB, Prakash A and Chitlange SS. Simultaneous spectrophotometric estimation of ofloxacin and satranidazole in tablet dosage form. Asian J Res Chem. 2008; 1: 9-11.
11. Kumar S, Sahni V, Chawla P and Saraf SA. Development and validation of analytical method for simultaneous estimation of diclofenac sodium and ofloxacin in bulk and ophthalmic formulations using UV-visible spectrophotometry. Int J Pharm Sci and Nanotech. 2011; 4: 1399-1402.
12. Ankam R, Mukkanti K and Khan M. Simultaneous HPLC determination of butenafine hydrochloride and betamethasone in a cream formulation. Ind J Pharm Sci. 2009; 71: 547–551.
13. Simon, Alice. Cabral, Lúcio Mendes and Sousa, Valeria Pereira de. Development and validation of an analytical method by HPLC for simultaneous quantification of betamethasone dipropionate and betamethasone sodium phosphate in injectable suspension. Química nova. 2012; 35: 593-600.
14. Rege PV and Mapari R. Simultaneous quantification of ofloxacin and ornidazole from combined pharmaceutical drug formulation by HPLC. Int JPharma Bio Sci. 2011; 2: 51-58.
15. Dharuman J, Vasudevan M, Somasekaran KN, Dhandapania B,

Ghodea PD,Thiagarajana M. RP-HPLC method development and validation for the simultaneous estimation of ofloxacin and tinidazole in tablets. Int JPharmtechRes. 2009; 1: 121-124.
16. Ghosh SJ, Darbar S, Chowdhury PP, Chattopadhyay SP and Chakraborty MR. Simultaneous determination and validation of ofloxacin and ornidazole in combined dosage pharmaceutical formulation. Int J Pharmtech Res. 2010; 2: 367-374.
17. Lestyo W, Gunawan I. HPTLC determination of betamethasone in tablets and its validation. J LiquChromatogr&Relat Technol. 2003; 26: 2709-17.
18. Rao J, Sethy K and Yadav S. Validated HPTLC method for simultaneous quantitation of cefixime and ofloxacin in bulk drug and in pharmaceutical formulation. Int J ComprehenPharma. 2011; 4: 1-4.
19. Johnston SE, Gill NL, Wei YC, Markovich R and Rustum AM. Development and validation of a stability-indicating RP-HPLC method for simultaneous assay of betamethasone dipropionate, chlorocresol, and for the estimation of betamethasone dipropionate related compounds in a pharmaceutical cream and ointment. J Chromatogra Sci. 2010; 48: 733-41.
20. Natarajan S and Bhanu R. Development and validation of a stability indicating HPLC method for simultaneous estimation of ofloxacin and ornidazole. The Indian Pharmacist. 2005; 4: 79-84.
21. Razzaq SN, Muhammad A, KhanI U and Irfana M. Stability indicating HPLC method for the simultaneous determination of Ofloxacin and Ketorolac tromethamine in pharmaceutical formulations. Analytic Meth. 2012; 4: 2121-2126.
22. ICH, Q2 (R1). Validation of Analytical Procedures, Text and Methodology. Proceedings of the International Conference on Harmonization, Geneva. 2005.