AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Betamethasone Sodium Phosphate and Ofloxacin in their Combined Dosage form

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ABSTRACT

A simple, sensitive, precise, accurate and economic Q-absorption ratio method for the quantitative determination of betamethasone sodium phosphate and ofloxacin in bulk and combined dosage form has been developed and validated. Q absorption ratio method uses the ratio of absorbances at two selected wavelengths, one which is iso-absorptive point and other being the λ-max of one of the two components. Betamethasone sodium phosphate and ofloxacin shows an iso-absorptive point at 247nm in methanol, the second wavelength used was 289nm, which is λ-max of ofloxacin. The linearity was obtained in the concentration range of 2-12 ug/ml for both the drugs in distilled water. The proposed method is recommended for routine analysis. The results of analysis have been validated statistically and by recovery studies.

Keywords: Betamethasone sodium phosphate, Ofloxacin, Q-absorption ratio method, Validation.

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Received 14 March 2014, Accepted 24 March 2014

Please cite this article in press as: Varshney P. *et al.*, Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Betamethasone Sodium Phosphate and Ofloxacin in their Combined Dosage form. American Journal of PharmTech Research 2014.

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. 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[1, 2.] It is official in IP, BP, and USP that describes spectrophotometric and HPLC method for its estimation [3-5]. Lack of its mineralo corticoid properties makes betamethasone particularly suitable for treating cerebral edema and congenital adrenal hyperplasia[4] and used for its anti inflammatory or immunosuppressive properties. It prevents and controls inflammation by controlling the rate of protein synthesis, depressing the migration of polymorph nuclear leukocytes and fibroblasts, and reversing capillary permeability and lysosomal stabilisation. The anti-inflammatory actions of corticosteroids are thought to involve lipocortins, phospholipase A_2 inhibitory proteins which, through inhibition arachidonic acid, control the biosynthesis of prostaglandins and leukotrienes.

Ofloxacin (OFLO) is chemically, 7-fluoro-2-methyl-6-(4-methylpiperazin-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[6-8] used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy.[9, 10] It is official in IP, BP and USP which describes potentiometric and titrimetric method for its estimation[11-13]. The chemical structure of betamethasone sodium phosphate and ofloxacin is shown in the (figreu 1 and 2) respectively.

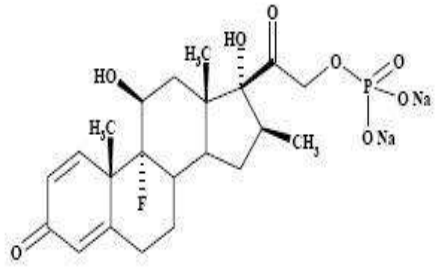

Figure 1: Betamethasone sodium phosphate

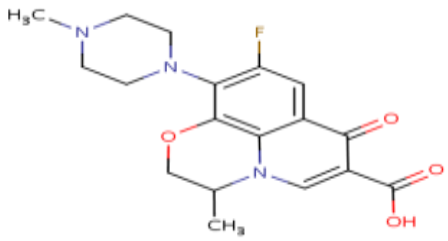

Figure 2: Ofloxacin

A detailed literature survey revealed spectrophotometric[14], HPLC[15], RP-HPLC[16, 17], HPTLC[18, 19], 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. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for estimation of both drugs in their combined dosage forms.

MATERIALS AND METHODS

Instruments

A Shimadzu model 1800 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 2.33).

Reagents and Chemicals

Betamethasone sodium phosphate and Ofloxacin were obtained as a gift sample from Sun Pharmaceuticals Industries Ltd, Vadodara. Distilled water was used in the study. The commercial fixed dose combination product eye drops (OMEFLOX-BM) was procured from the local market. All other chemicals and reagents used were of analytical grade.

Preparation of Standard Stock Solution

An accurately weighed quantity of Betamethasone sodium phosphate (10 mg) and Ofloxacin (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of betamethasone sodium phosphate (100 µg/ml) and ofloxacin (100 µg/ml). Working standard solutions of 10µg/ml for both BMS and OFLO was prepared separately in distilled water and scanned in the range of 200 nm-400 nm.

Preparation of Sample Solution

The sample solution was prepared using formulation to get the final concentration containing 3ug/ml (BMS) and 9ug/ml (OFLO). Absorbance of sample solution was simultaneously estimated at 289nm, and at its iso-absorptive point 247nm.

Development of Q-Absorption Ratio Method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that BMS and OFLO show an iso-absorptive point at

247 nm. The second wavelength used is 289 nm, which is the λ-max of ofloxacin. Six working standard solutions having concentration range of 2-12 ug/ml for both the drugs were prepared in distilled water and the absorbances at 247 nm (iso-absorptive point) and 289 nm (λ-max of ofloxacin) were measured and absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

$$Cx = \{(Q_M - Qy)/ (Qx - Qy)\} * (A/ax_1)$$

$$Cy = A/ ax_1 - Cx$$

Where, Q_M= Absorbance of sample at 289 nm/ Absorbance of sample at 247 nm

Q_X = Absorptivity of BMS at 289 nm/ Absorptivity of BMS at 247 nm

Q_Y = absorptivity of OFLO at 289 nm/ Absorptivity of OFLO at 247nm

A = Absorbance of sample at iso-absorptive point.

ax_1 = Absorptivity of ofloxacin at iso-absorptive point.

VALIDATION OF PROPOSED METHOD

The developed method was validated for the simultaneous assay determination of betamethasone sodium phosphate and ofloxacin as per ICH guidelines Q2 (R1) validation of analytical procedures: text and methodology[20].

Linearity (Calibration curve):

The calibration curves were plotted over a concentration range of 2-12 µg/ml for both betamethasone sodium phosphate and ofloxacin. Accurately measured standard stock solutions of each BMS (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) and OFLO (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with distilled water. The absorbances of solution were then measured at 240 nm and 289 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Precision (Repeatability):

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for BMS and OFLO (4 µg/ml for both drugs) 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 BMS and OFLO (4, 6 and 8 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

Accuracy was determined by calculating recovery of BMS and OFLO by the standard addition method. From working sample solution of test (100 µg/ml of both), 1 ml of solution were taken and increasing aliquots of combined working standard solution (0.08, 0.1 and 0.12 ml from 100 µg/ml of BMS and 0.24, 0.3, 0.36 ml of OFLO) were added and diluted to 10 ml with distilled water. These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for BMS and OFLO. The amount of betamethasone sodium phosphate & ofloxacin was calculated at each level by absorbance ratio method & % recoveries were computed.

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 × σ/S

LOQ = 10 × σ/S

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

Analysis of betamethasone sodium phosphate and ofloxacin in marketed formulation (Eye drop)

The sample solution was prepared using formulation to get the final concentration containing 3ug/ml (betamethasone sodium phosphate) and 9ug/ml (ofloxacin). Absorbance of sample solution was simultaneously estimated at 289nm, and at its iso-absorptive point 247nm.

RESULTS AND DISCUSSION

The optical and regression analysis data and validation parameters are shown in (Table 1). The calibration curve 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). Overlain spectra of BMS and OFLO are shown in (Figure 5 and 6). A Q-absorption spectrum showing iso-absorptive point is shown in (Figure 7). The spectra showing absorbance of both BMS and OFLO in marketed formulation is shown in (Figure 8).

Table 1: Optical and Regression Analysis Data and Validation Parameter of Q- Absorption Ratio Method

Parameters	BMS	OFLO
Concentration range (µg/ml)	2-12 µg/ml	2-12 µg/ml
Molar Absorptivity	2×10^5	2.9×10^5

($L\ mol^{-1}cm^{-1}$)			
Sandell's Sensitivity ($\mu g/cm^2$/0.001absorbance unit)		0.00258	0.00124
Slope		0.04	0.082
Intercept		0.011	0.008
Correlation coefficient		0.9988	0.9974
Accuracy (recovery, n = 3) ± S.D	80%	101.84 ± 0.014	100.72 ± 0.032
	100%	103.33 ± 0.038	99.11 ± 0.02
	120%	102.64 ± 0.011	99.83 ± 0.03
Repeatability % RSD (n=6)		0.5753	0.6518
Intraday (n = 3)		0.27-0.49	0.23-1.62
Interday (n = 3)		0.30-0.85	0.32-1.34
LOD (mcg/ml)		0.4763	0.4125
LOQ (mcg/ml)		1.44	1.25

Table 2. Statistical analysis for precision of proposed method

Drug	Conc. of drug (µg/ml)	%RSD (n=3) Intraday	Interday
Betamethasone sodium phosphate	4	0.27	0.41
	6	0.42	0.85
	8	0.49	0.30
Ofloxacin	4	0.38	0.76
	6	1.62	1.34
	8	0.23	0.32

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	80%	1	0.8	1.8	101.84	0.78
	100%		1	2	103.33	1.84
	120%		1.2	2.2	102.64	0.51
OFLO	80%	3	2.4	5.4	100.72	0.59
	100%		3	6	99.11	0.35
	120%		3.6	6.6	99.83	0.46

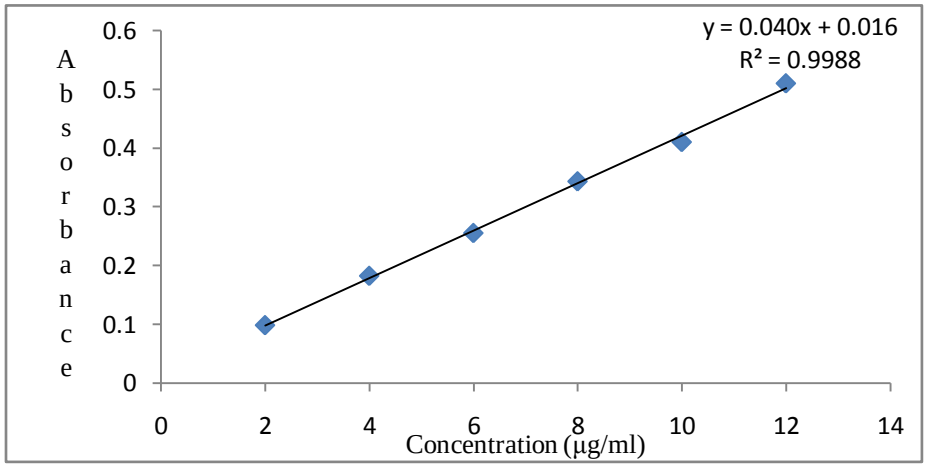

Figure 3: Calibration curve of betamethasone sodium phosphate

Table 4.Analysis of BMS and OFLO in marketed formulation

Formulation (eye drops)	Labeled amount (mg/ml)		Amount found (mg/ml)		% Label claim Assay ± S.D	
	BMS	OFLO	BMS	OFLO	BMS	OFLO
	1	3	0.99	2.97	99.16± 0.017	99.05±0.01

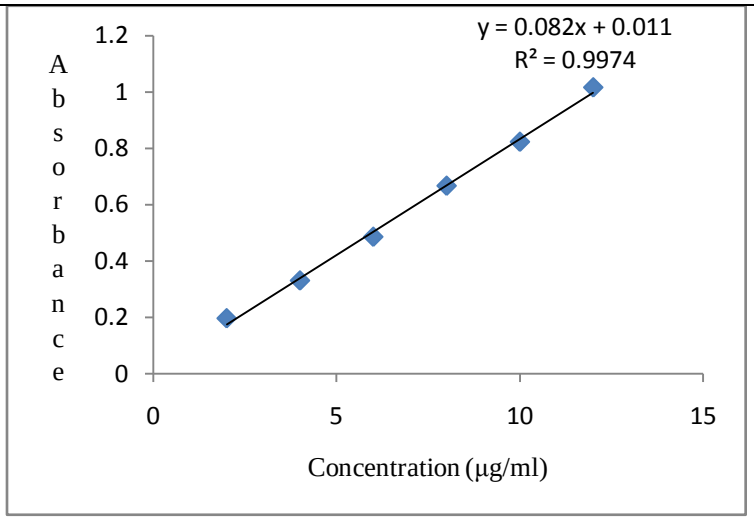

Figure 4: Calibration curve of ofloxacin

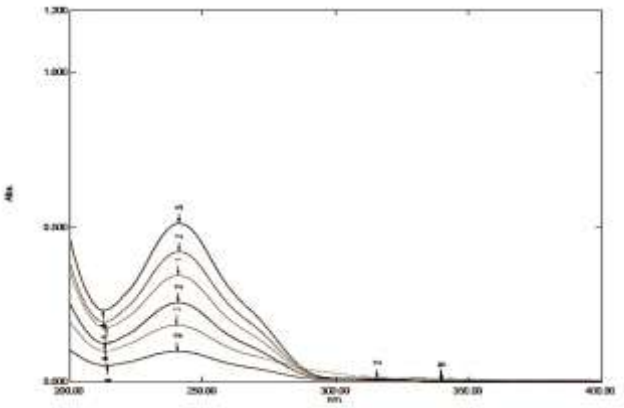

Figure 5: Overlain spectra of betamethasone sodium phosphate

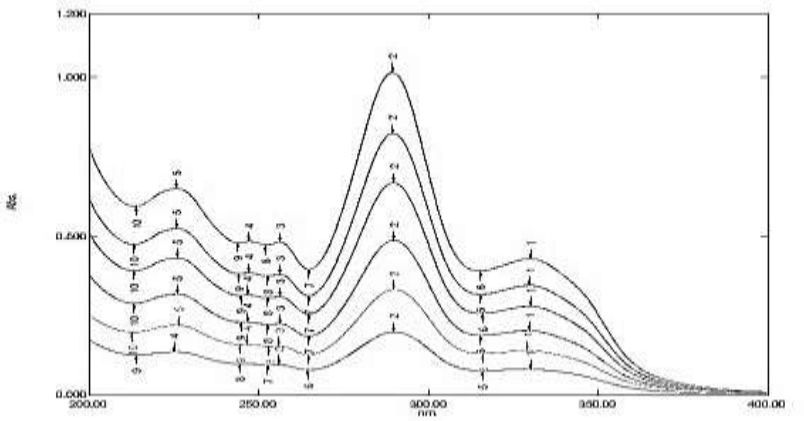

Figure 6: Overlain spectra of ofloxacin

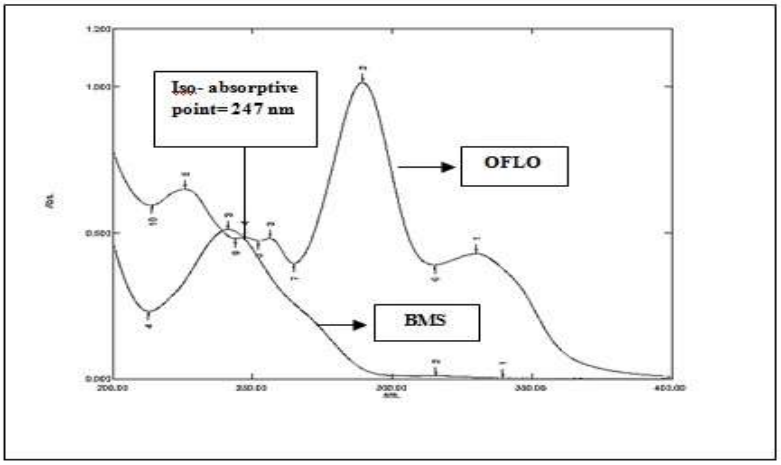

Figure 7: Overlain spectra showing iso-absorptive point 247 nm

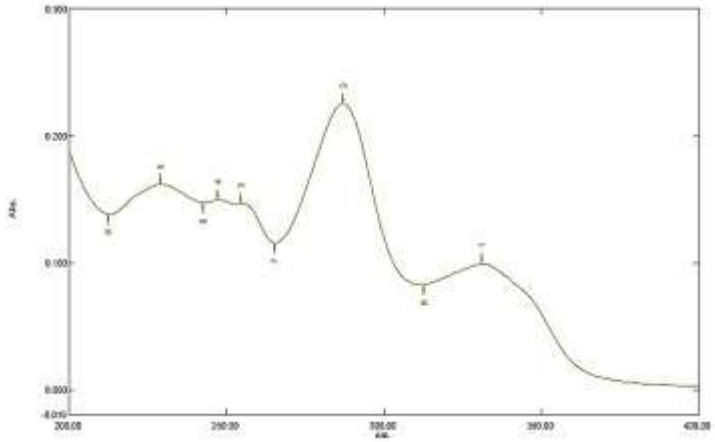

Figure 8: Spectra of BMS and OFLO in marketed formulation

CONCLUSION

The evaluation of obtained values suggests that the proposed method provide simple, sensitive, precise, accurate and economic quantitative analytical method for simultaneous determination of betamethasone sodium phosphate and ofloxacin in pure and combined dosage form. After validating proposed method as per ICH guidelines and correlating the obtained values with the standard values, satisfactory results were obtained. The sample recoveries in formulation were in good agreement with their respective label claims and they suggest non interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine estimation of betamethasone sodium phosphate and ofloxacin in combined dosage form.

ACKNOWLEDGMENTS

The authors are thankful to Sun Pharmaceuticals Ltd., Vadodara, Gujarat, India for providing gift sample of betamethasone sodium phosphate and ofloxacin for research. The authors are highly

thankful to Pioneer Pharmacy Degree College, Vadodara Gujarat, India for providing all the facilities to carry out the work.

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