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NOVEL ISOCRATIC RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND BECLOMETHASONE DIPROPIONATE

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ABSTRACT: The present paper describes a novel, isocratic, simple, precise, accurate, and robust reversed-phase high-performance liquid chromatographic (RP-HPLC) method development and validation for simultaneous quantitative estimation of Ofloxacin and Beclomethasone dipropionate in an Ophthalmic/Otic formulation. The proposed chromatographic estimation was carried out isocratically using Protonsil C18 (250 × 4.6mm) SH 5.0µm column, the mixture of 0.02 M potassium dihydrogen phosphate buffer (pH adjusted to 3 using *ortho*-phosphoric acid): acetonitrile in a ratio of 30:70 v/v with a flow rate of 1 mL/min was used as mobile phase and column oven adjusted to 30°Cwith injection volume 10µL. The ultraviolet (UV) detection was carried out at 234 nm. The retention time of ofloxacin and beclomethasone dipropionate was found to be 2.67±0.2 min and 7.42±0.2 min, respectively. Calibration curves were linear over the tested concentration range of 4 to 20µg/mL. The present study reveals that this novel isocratic HPLC method is well-validated, reliable, and can be used for routine analysis of ofloxacin and beclomethasone dipropionate in an otic formulation containing these as one of the ingredients.

INTRODUCTION: Ofloxacin is a fluoro-quinolone derivative with excellent *in-vitro* activity against many Gram-positive and Gram-negative organisms. It has shown a large potency against many common bacterial pathogens and also a good activity against various mycobacteriacae, legionella species, rickettsiaceae and even multiple drug-resistant nosocomial isolates [1-4].

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It helps to reduce bacterial infection and to some extent, a fungal infection. Beclomethasone is a glucocorticosteroid that acts as an anti-inflammatory drug that prevents allergy symptoms such as stuff/runny nose, itchy nose/ eyes/ ear, and throat sneezing. It reduces inflammation in the outer parts of the ear (Otitis externa), and the infection does not go further to the eardrum [5-8].

The selected marketed formulation (Liotic Ear drops) relieves pain, congestion, and swelling caused by middle ear inflammation and reduces yeast or bacterial infection. This formulation has three constituents from the most important contents selected for quantification, namely ofloxacin and beclomethasone dipropionate.

Ofloxacin is a fluoroquinolone derivative which is act as antibacterial. A literature survey revealed that although several isocratic HPLC methods are reported for quantification of ofloxacin and beclomethasone dipropionate alone or in combination with other constituents 9-13, so so far, simultaneous HPLC, simultaneous of ofloxacin and beclomethasone dipropionate is not yet reported. Hence it was worth developing a novel HPLC method for the selected combination.

MATERIALS AND METHODS: HPLC grade beclomethasone dipropionate (purity 99%) was procured from Rusi Pharma Pvt. Ltd., Mumbai in Maharashtra. HPLC grade ofloxacin (purity 99%) was procured from Chemdyes Corporation, Rajkot, India. A multicomponent formulation named Liotic Ear drops (Luco Pharmaceuticals, Mumbai, India) used for analysis was procured from the local market. HPLC grade solvents were purchased from Thomas Baker Chemicals Pvt. Ltd. The commercially available Ofloxacin and BD calm ear drop with a label claim of ofloxacin 3% w/v and beclomethasone dipropionate 0.025% w/v.

Chromatographic Conditions: RP-HPLC Shimadzu LC Prominence-i 2030 model with Lab Solution software was employed in this method. The separation of ofloxacin and beclomethasone dipropionate was carried on Shimadzu Protonsil C-18 (250×4.6mm) SH 5.0µm column.

The mobile phase used was 0.02 M potassium dihydrogen phosphate of pH 3 and acetonitrile in the ratio (30:70 v/v) at a flow rate of 1mL/min, injection volume was 10 µL, column temperature was 30°C, and ofloxacin and beclomethasone diproponate were detected at 234 nm using UV-Visible spectrophotometer.

Selection of Wavelength: Standard solutions of ofloxacin and beclomethasone dipropionate were prepared and scanned separately by UV spectrophotometer in the range of 200–400nm.

The overlain UV spectrum of ofloxacin and BD is obtained in **Fig 1**. The 235nm wavelength was selected as the detection wavelength for detecting ofloxacin and beclomethasone dipropionate.

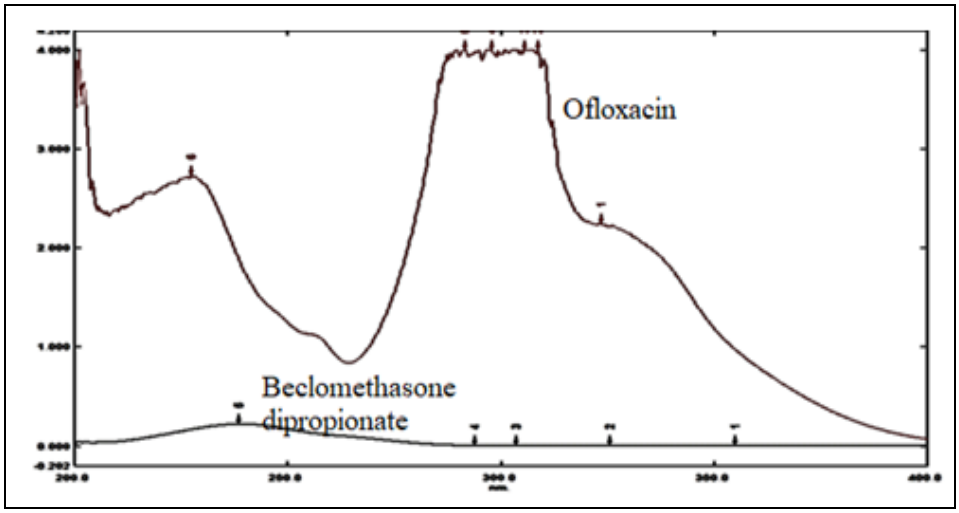

FIG. 1: UV SPECTRA OF OFLOXACIN AND BECLOMETHASONE DIPROPIONATE

Preparation of 0.02 M Phosphate Buffer (pH 3): 6.80g of potassium dihydrogen phosphate buffer was accurately weighed and dissolved in 950mL of water. The pH was adjusted to 3 with *ortho*-phosphoric acid, and the volume was made up to 1000mL in a volumetric flask. The solution was then filtered through 0.45 µm filter paper and sonicated before use.

Preparation of Standard Stock Solution: The standard stock solutions containing 100mg each of ofloxacin and beclomethasone dipropionate were prepared separately in a 100mL volumetric flask, and then the volume was made up with diluents (30:70 Phosphate buffer pH 3 and acetonitrile respectively) to obtain a stock solution of 1000µg/mL.

The stock solutions were further diluted in order to obtain working concentrations of 30 µg/mL (ofloxacin) and 2.5 µg/mL (beclomethasone dipropionate), respectively.

Preparation of Sample Solution: One ml of sample solution (marketed formulation) was taken in 10 ml of volumetric flask and diluted with the same diluent that was used in the preparation of the standard solution. Then sonication was done till constituents get dissolved into it. The final solution is filtered with a filter paper of 0.45 µm pore size. Then, 10 µL of the sample was injected into HPLC using an autosampler.

RESULTS AND DISCUSSION:

Method Development: Different trials are carried out using methanol/acetonitrile with varying ortho-phosphoric acid and potassium dihydrogen phosphate pH. When a mixture of *ortho*-phosphoric acid: methanol (40:60 v/v) was used, the peak of ofloxacin was broad and doam-shaped, but BD is not eluted. When an *ortho*- phosphoric acid: acetonitrile (40:60 v/v) was used as a mobile phase, both peaks resolved properly, but BD peak has an asymmetry of 2.1. After taking different trials by varying diluent and their concentration, a satisfactory result is achieved with mobile phase 0.02M phosphate buffer of pH 3: acetonitrile in a ratio of 30:70 v/v, respectively. The injection volume was kept 10µL. The flow rate and run time were set as 1.0 mL/min and 10 minutes, respectively. The column temperature was set at 30°C, and the detection was carried out at 235nm. The retention time of ofloxacin and BD obtained was 2.65±0.2 and 7.24±0.2 min, respectively. Chromatograms of standards and sample solution are shown in **Fig. 2 and 3.**

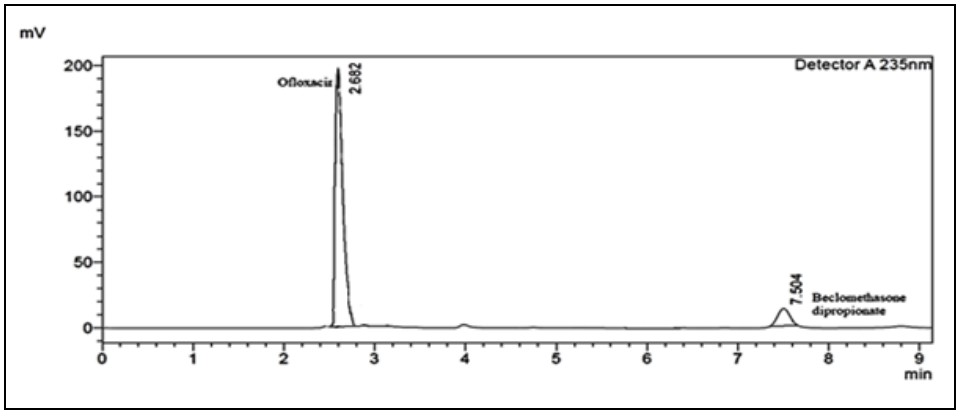

FIG. 2: CHROMATOGRAPH OF STANDARD SOLUTION

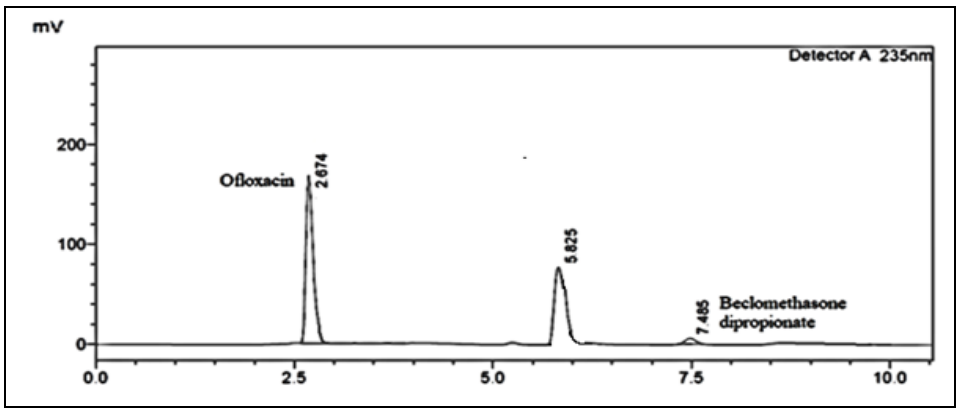

FIG. 3: CHROMATOGRAPH OF SAMPLE SOLUTION

System Suitability: Chromatograms of mixed standard and sample solution reveal that the peaks obtained are just because of the marker compound and the blank has no peak at the retention time of ofloxacin and beclomethasone dipropionate. Hence, the method is said to be specific.

TABLE 1: SYSTEM SUITABILITY RESULT

Parameters	Acceptance limit	Ofloxacin	Beclomethasone dipropionate
Retention time (min)	-	2.65	7.42
Number of theoretical plates	NLT 2000	8659	101211
Tailing Factor	NMT 2	1.21	1.23

Method Validation: The developed method was evaluated for specificity, linearity, precision, and accuracy robustness, so it was proved to be specific, accurate, and precise.

Hence, the method is useful for routine assay analysis. These acceptance criteria were taken into account concerning the ICH guidelines, and the developed HPLC method was validated according to it [14, 15].

Specificity: It is the ability to assess the analyte unequivocally in the presence of components that may be expected to be present. Chromatograms of mixed standard and sample solution reveal that the peaks obtained are just because of the marker compound and blank has no peak at the retention time of ofloxacin and beclomethasone dipropionate. Hence, the method is said to be specific.

Precision: The precision was assessed as 1 System precision 2 Method precision.

System Precision includes six replicate injections of the standard solutions individually or as a mixture of standard solutions at working concentrations. The % RSD was calculated concerning the individual areas of the peak of each marker should be less than 2% as per ICH guidelines which are shown in **Table 2**.

TABLE 2: SYSTEM PRECISION RESULT

S. no.	% Assay of Ofloxacin	% Assay of Beclomethasone dipropionate
1	99.43967703	99.45802851
2	100.1974029	100.1313304
3	99.87573274	100.9002458
4	99.14948826	100.7055837
5	100.0043622	101.2775468
6	100.8974773	98.92786051
Mean ± SD	0.618	0.902
% RSD	0.611	0.901

Method Precision: This includes six replicate injections of the sample solution at working concentrations to check the consistency of the developed method. The results are shown in **Table 3**.

TABLE 3: METHOD PRECISION RESULT

S. no.	% Assay of Ofloxacin	% Assay of Beclomethasone dipropionate
1	101.2247967	99.59028424
2	98.77499506	100.5882139
3	99.87568445	100.1805685
4	100.253461	101.1945291
5	100.650165	98.83052945
6	99.60992755	100.7365006
Mean ± SD	0.852	0.857
% RSD	0.851	0.855

Linearity: Linearity was evaluated by analyzing the plot area as a function of the concentration of the analyte. Calibration curves of ofloxacin and beclomethasone dipropionate were constructed individually as shown in **Fig. 4** and **Fig. 5**.

These curves obtained with different concentrations of standard solution of ofloxacin and beclo-methasone and that was found to be linear.

The correlation coefficients (R2) were > 0.99 for each marker. The results are calculated and tabulated in **Table 4.**

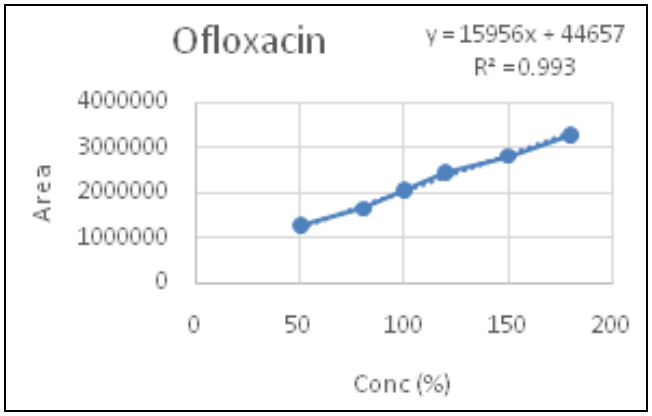

FIG. 4: CALIBRATION CURVE OF OFLOXACIN

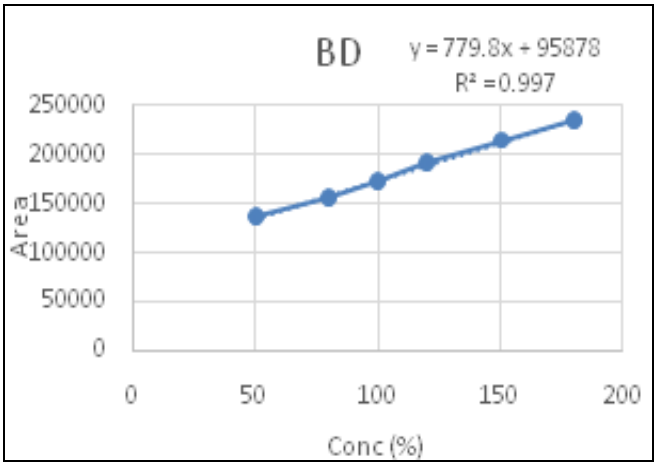

FIG. 5: CALIBRATION CURVE OF BECLOMETHASONE DIPROPIONATE

TABLE 4: LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Parameters	Ofloxacin	Beclomethasone dipropionate
Linearity(µg/mL)	15-54	1.25-4.5
Equation of regression line	y = 15956x + 446570	y = 779.89x + 95878
(R^2)	0.9937	0.9973
Intercept	446570	95878

Accuracy: Recovery of ofloxacin and beclomethasone dipropionate from formulation was checked by spiking a known quantity of standards at three concentration levels (*i.e.* 80%, 100% and 120% of the quantified amount) to the test samples in triplicate using HPLC shown in **Table 5.**

TABLE 5: % RECOVERY RESULTS

Compounds	Level	Sample (µg/mL)	Standard added (µg/mL)	Total amount	Amount recovered	% Recovery
Ofloxacin	80%	15	12	27	26.99	99.96
	100%	15	15	30	30.01	100.12
	120%	15	18	33	32.98	99.01
Beclomethasone dipropionate	80%	1.25	0.75	2	1.98	99.23
	100%	1.25	1.25	2.5	2.49	100.05
	120%	1.25	1.75	3	2.98	98.56

Robustness: The robustness of the analytical method was evaluated by making deliberate changes in the flow rate of the mobile phase (± 0.2 ml/ min) wavelength (± 2nm) and column temperature (± 2°C) and its results are shown in **Table 6**.

TABLE 6: ROBUSTNESS RESULTS

Parameters	Deviation	% RSD of assay	
		Ofloxacin	Beclomethasone dipropionate
Flow Rate	0.8mL/min	0.96	0.41
	1.2mL/min	0.76	0.57
Column Temperature	26^0C	0.47	0.85
	30^0C	0.53	0.63
Wavelength	233nm	0.67	1.08
	237nm	1.12	0.86

CONCLUSION: The present paper describes a novel rapid RP-HPLC method for the simultaneous estimation of ofloxacin and beclomethasone dipropionatein a pharmaceutical dosage form.

Those drugs have good resolution with a short analysis time below 10 minutes, making it an economic one. The developed isocratic HPLC method was found to be simple, linear, accurate, precise and robust, which makes it versatile and valuable for the simultaneous estimation of two drugs in the pharmaceutical dosage form.

This method obtained acceptable values of precision and accuracy at all levels as per guidelines for assay validation.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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