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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF BETAMETHASONE AND OFLOXACIN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:
A precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Betamethasone and Ofloxacin in pharmaceutical tablet dosage forms. The chromatographic analysis was performed using a Phenomenex C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase comprising 0.01 N potassium dihydrogen phosphate (KH_2PO_4) buffer at pH 4, acetonitrile, and methanol in the ratio of 60:30:10 (v/v/v). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 265 nm. Retention time of Betamethasone and Ofloxacin were found to be 2.1min and 4.1min. The method was validated according to ICH Q2(R1) guidelines for parameters including linearity, precision, accuracy, sensitivity, robustness, and system suitability. The developed method proved to be simple, economical, and suitable for routine quality control analysis in pharmaceutical industries.

INTRODUCTION

Betamethasone is a potent glucocorticoid corticosteroid widely used for its anti-inflammatory, immunosuppressive, and anti-allergic properties. It is employed in treating various conditions, including dermatological disorders, autoimmune diseases, respiratory disorders, and ophthalmic conditions. Due to its high potency, precise quantification of Betamethasone is critical to ensure therapeutic efficacy while avoiding potential side effects like adrenal suppression, osteoporosis, and immunosuppression.

Ofloxacin is a broad-spectrum fluoroquinolone antibiotic commonly used to treat bacterial infections such as respiratory tract infections, urinary tract infections, and ophthalmic and otic infections. It acts by inhibiting DNA gyrase and topoisomerase IV, enzymes essential for bacterial DNA replication and repair. Accurate quantification of Ofloxacin is necessary to maintain appropriate therapeutic levels, ensure bacterial eradication, and prevent the development of resistance.

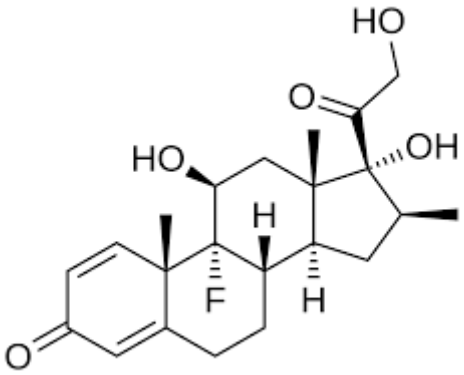

Fig. 1 Chemical structure of Betamethasone

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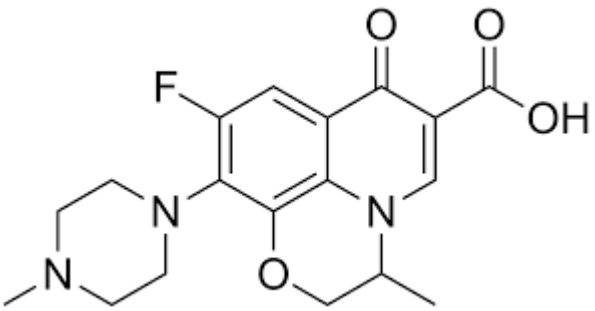

Fig.2 Chemical structure of Ofloxacin

Combination formulations containing Betamethasone and Ofloxacin, such as ophthalmic solutions, are frequently prescribed for the management of mixed infections and inflammatory conditions. Analytical methods for simultaneous estimation of these drugs must be sensitive, accurate, precise, and rapid, considering the challenges posed by their differing chemical structures and physicochemical properties.

Reversed Phase High Performance Liquid Chromatography (RP-HPLC) is a widely preferred analytical technique due to its high resolving power, reproducibility, and suitability for routine analysis. Method development and validation according to International Conference on Harmonisation (ICH) guidelines are imperative to ensure the method's reliability and regulatory compliance.

The current study focuses on developing and validating a simple, robust, and cost-effective RP-HPLC method for the simultaneous determination of Betamethasone and Ofloxacin in pharmaceutical dosage forms. The method is optimized for critical parameters such as retention time, resolution, linearity, accuracy, precision, detection and quantitation limits, and robustness. The validated method can be employed for quality control and routine analysis in pharmaceutical industries.

MATERIALS AND METHODS

Materials

Betamethasone and Ofloxacin, combination formulation (Oflatop D Eye/Ear Drops), distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, triethylamine, and ortho-phosphoric acid were used.

Instruments

The HPLC instrument used was the WATERS HPLC 2965 system equipped with an Auto Injector and PDA Detector, operated via Empower 2 software. A UV-VIS spectrophotometer (PG Instruments T60) with a bandwidth of 2 mm and 10 mm matched quartz was used for absorbance measurements of Betamethasone and Ofloxacin solutions.

Methods

Preparation of Buffer

A 0.01 N KH_2PO_4 buffer was prepared by accurately weighing 1.36 g of potassium dihydrogen orthophosphate and dissolving it in 900 ml of Milli-Q water in a 1000 ml volumetric flask. The solution was degassed by sonication and made up to volume with Milli-Q water. The pH was adjusted to 4 using diluted ortho-phosphoric acid.

Standard Preparation:

Accurately Weighed and transferred 10mg of Betamethasone and 30 mg of Ofloxacin working Standards into 100ml clean dry volumetric flask, add 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

Sample Preparation:

1 ml from the formulation was transferred to 10ml volumetric flask and made up to the mark with diluents. From the above solution 1 ml was diluted to 10ml.

Linearity: Linearity solutions are prepared such that 0.25 ml, 0.5ml, 0.75 ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Betamethasone and Ofloxacin are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 2.5µg/ml, 5µg/ml, 7.5µg/ml, 10µg/ml, 12.5µg/ml, 15µg/ml of Betamethasone and 7.5µg/ml, 15µg/ml, 22.5µg/ml 30µg/ml, 37.5µg/ml, 45µg/ml of Ofloxacin .

RESULTS AND DISCUSSION

Method development:

An optimized RP-HPLC method was developed utilizing a Phenomenex C18 column (150 mm × 4.6 mm, 5 µm) to achieve efficient separation. The mobile phase comprised a mixture of 0.01 N potassium dihydrogen phosphate (KH_2PO_4) buffer at pH 4, acetonitrile, and methanol in the ratio of 60:30:10 (v/v/v), delivering effective resolution and peak shape. The flow rate was maintained at 1.0 mL/min, and the detection was carried out at a wavelength of 265 nm. The column oven temperature was set at 30°C to ensure reproducibility and

stability of retention times. The diluent used for sample and standard preparation consisted of water and acetonitrile in a 50:50 ratio. A fixed injection volume of 10 µL was employed for all chromatographic runs, contributing to consistent and reliable quantification.

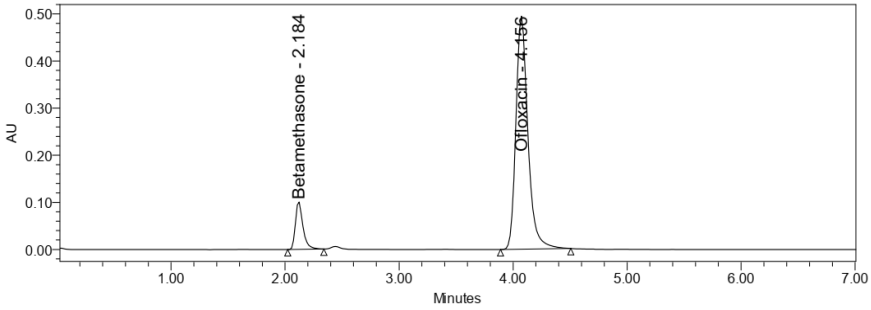

Fig 3. Optimized chromatogram of Betamethasone and Ofloxacin

1. System Suitability

The retention times for Betamethasone and Ofloxacin were 2.1 min and 4.1 min, respectively. Theoretical plates were 2014 for Betamethasone and 4875 for Ofloxacin. Tailing factors were 1.02 and 1.42, respectively. Resolution was found to be 4.2, meeting ICH requirements.

2. Linearity

Linearity was confirmed in the concentration ranges of 2.5–15 µg/ml for Betamethasone and 7.5–45 µg/ml for Ofloxacin. Regression equations were:

Betamethasone: $y = 45035x + 6902.7$ ($R^2 = 0.999$)

Ofloxacin: $y = 104186x + 28554$ ($R^2 = 0.9998$)

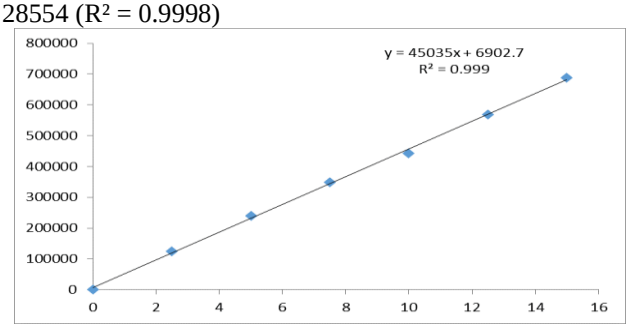

Fig: 4 Calibration curve of Betamethasone

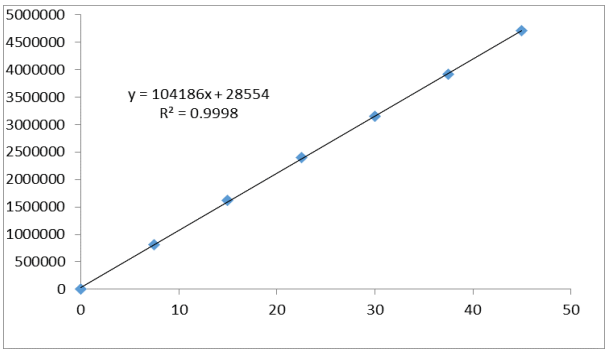

Fig: 5. Calibration curve of Ofloxacin

3. Precision

Intraday Precision

%RSD for Betamethasone and Ofloxacin were 0.9 and 0.8, respectively, indicating high precision.

Interday Precision

%RSD values after 24 hours were 1.6 for Betamethasone and 1.1 for Ofloxacin, confirming reproducibility.

4. Accuracy

Three different concentrations (50%, 100%, and 150%) were analyzed in triplicate. Average recovery was:

- Betamethasone: 98.4%
- Ofloxacin: 100.08%

Table: 1. Accuracy results of Betamethasone and Ofloxacin

Sample	Amount taken (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	AVG
Betamethasone	5	4.87	97.4	98.4
	10	9.91	99.1	
	15	14.81	98.73	
Ofloxacin	15	14.69	97.93	100.08
	30	29.86	99.53	
	45	46.25	102.77	

Limit of Detection (LOD)

- Betamethasone: 0.50 µg/ml
- Ofloxacin: 0.90 µg/ml

Limit of Quantification (LOQ)

- Betamethasone: 1.53 µg/ml
- Ofloxacin: 2.70 µg/ml

7. Robustness

Method robustness was confirmed under slight variations in flow rate, mobile phase composition, and temperature. All %RSD values remained within acceptable limits (<2%).

8. Assay

Assay of tablet formulation showed average content of:

- Betamethasone: 98.04%
- Ofloxacin: 100.52%

%RSD values were 1.63% and 1.1%, respectively, confirming method suitability for routine QC analysis.

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Betamethasone and Ofloxacin in Tablet dosage form. Retention time of Betamethasone and Ofloxacin were found to be 2.1min and 4.1min. %RSD of the Betamethasone and Ofloxacin were and found to be 0.9 and 0.8 respectively. %Recovery was Obtained as 98.4 and 100.08 for Betamethasone and Ofloxacin, respectively. LOD, LOQ values are obtained from regression equations of Betamethasone and Ofloxacin were 0.50 and 0.90 & 1.53 and 2.70, respectively. Regression equation of Betamethasone is $y = 45035x + 6902.7$, and of Ofloxacin is $y = 104186x + 28554$. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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