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Original Article

# SIMULTANEOUS DETERMINATION OF MOXIFLOXACIN HYDROCHLORIDE AND DIFLUPREDNATE BY RATIO DERIVATIVE SPECTROPHOTOMETRY

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**ABSTRACT**

**Objective:** Ratio derivative spectrophotometric method has been developed for the simultaneous determination of Moxifloxacin HCl (MH) and Difluprednate (DIFLU) in Phosphate Buffer pH 7.4.

**Methods:** In this method, the overlapping spectra of MH and DIFLU were well resolved by making use of the first-derivative of the ratios of their direct absorption spectra. The derivative ratio absorbances of MH and DIFLU were measured at λmax 325.8 and λmax 238.0 nm, respectively for their quantification. MH and DIFLU were determined in the concentration range of 2-10 µg/ml and 4-20 µg/ml respectively.

**Results:** The method was validated as per the ICH guideline and accuracy, precision are found to be within the acceptable limit. The limits of detection and quantitation were found to be 0.1144 and 0.3466 µg/ml, respectively for MH and 0.0311 and 0.094 µg/ml, respectively for DIFLU.

**Conclusion:** The proposed ratio first derivative spectrophotometric method is novel, rapid, simple, sensitive, accurate, precise and does not require separation of MH and DIFLU hence successfully applied for simultaneous estimation of MH and DIFLU in marketed eye drops (liquid dosage form).

**Keywords:** Simultaneous determination, Moxifloxacin HCl, Difluprednate, Ratio derivative spectrophotometric method.

## INTRODUCTION

### Moxifloxacin Hydrochloride [MH]

Moxifloxacin HCl is (4aS - cis) - 1 - Cyclopropyl - 6 - fluoro - 1, 4 - dihydro - 8 - methoxy - 7 - (octahydro - 6H - pyrrolol [3, 4 - b] pyridin - 6 - yl) - 4 - oxo - 3 - quinoline carboxylic acid monohydrochloride (Fig.1). Moxifloxacin hydrochloride (HCl) is a fourth-generation fluoroquinolone with a new 8-methoxy derivate of fluoroquinolones with enhanced activity *in vitro* against gram positive bacteria and maintenance of activity against gram negative bacteria. It is an anti-infective agent useful in the treatment of eye infection such as bacterial conjunctivitis, keratitis and keratoconjunctivitis. It is currently available as eye drops (0.5%). It is administered at dosing interval of 1 drop in the affected eye 3 times a day for 7 days [1-5].

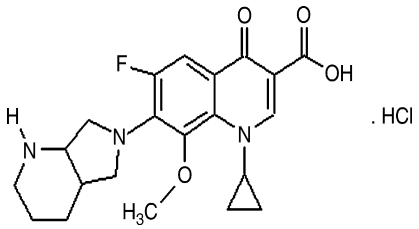



**Fig. 1: Structure of Moxifloxacin HCl**

### Difluprednate (DIFLU)

Difluprednate (difluoroprednisolone butyrate acetate or DFBA) is a synthetic difluorinated prednisolone derivative. The chemical name is 6α, 9difluoro-11β, 17, 21-trihydroxypregna-1, 4-diene-3, 20-dione 21-acetate 17-butyrate (Fig. 2) (CAS number 23674-86-4). Difluprednate 0.05% is indicated for the treatment of inflammation and pain associated with ocular surgery. [1-3, 6].

Nowadays, MH has been marketed in combination with DIFLU as eye drops (liquid dosage forms), DIFLUMOX (Moxifloxacin Hydro chloride 0.5 % and 0.05 % Difluprednate) is useful for anti-inflammatory, anti-allergy effect mainly in eye diseases, such as conjunctivitis, pain after ocular surgery, inflammation, keratitis.

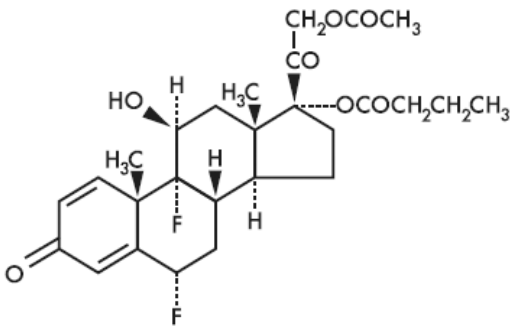



**Fig. 2: Structure of Difluprednate**

Scientific literature reports that there are many methods reported for the determination of MH individually and in combination with other drugs like ketorolac, bromfenac sodium, prednisolone, dexamethasone sodium phosphate, cefixime trihydrate, loteprednol etabonate etc. based on reversed-phase HPLC method, UV-Spectroscopic, Q-Absorbance Ratio Method. [7-13].

For the determination of DIFLU, there was no combination with other drugs have been found. Several analytical methods were reported includes semi-micro HPLC and column switching method, HPLC method [14, 15]. MH is official in IP, BP, and USP. DIFLU is unofficial in IP, BP, USP. [16, 17]

To the best of our knowledge, no spectro photometric method has been reported for the estimation of mentioned drugs in formulation. Therefore, the goal of present work is to develop a simple procedure that could be applied in quality control laboratories for the simultaneous determination of both drugs. This work aims to present simple, accurate and precise ratio-derivative spectro

photometric method for the simultaneous determination of MH and DIFLU in eye drops dosage form.

## MATERIALS AND METHODS

### Instrumentation

An UV-Visible Spectrophotometer (Simadzu - 1800, Japan) with 10 mm matched quartz cells was used for Spectrophotometric method. All weighing were done on electronic balance (Model Shimadzu AUW – 220 D). Ultrasonicator (Model 5.5 150 H) was used for sample solution preparation.

### Reagents and chemicals

Analytical pure samples of MH was obtained as a gift samples from Marck Bioscience Ltd., Kheda, Gujarat, India. and DIFLU was obtained as a gift samples from Ajanta Pharmaceuticals, Mumbai. These samples were used without further purification. Eye drop formulation "DIFLUMOX" manufactured by Ajanta Pharmaceuticals, Mumbai, Maharashtra, India. was purchased from the local market containing MH (25 mg) and DIFLU (2.5 mg) per sample (5 ml). Disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride were purchased from Astron Chemicals, Ahmedabad. Gujarat, India.

### Preparation of Standard Solutions and Calibration Curve

Standard stock solutions each containing 1000 µg/ml of MH and DIFLU were prepared separately in the phosphate buffer pH 7.4. The working standard solutions (100 µg/ml) of mentioned drugs were obtained by dilution of the respective stock solution in phosphate buffer pH 7.4. For verification of Beer's law, a series of dilutions in the concentration range of 2-10 µg/ml for MH and 4-20 µg/ml for DIFLU were prepared separately to establish calibration curve.

### Ratio first derivative Spectrophotometric method

The method involves dividing the spectrum of formulation by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using appropriate dilutions of standard stock solution, the standard solutions of MH (6 µg/ml) and DIFLU (4 µg/ml) were prepared and their zero order spectra recorded over the range 200-400 nm using phosphate buffer pH 7.4 as blank. The ratio spectra of different MH standards at increasing concentrations were obtained by dividing each with the stored zero order spectrum of standard solution of DIFLU (4 µg/ml) and the first derivative of these spectra traced with the interval of Δλ= 8 nm, illustrated in Fig.4. Similarly, the ratio derivative spectra of the solutions of DIFLU at different concentrations were obtained by dividing each with the stored zero order spectrum of standard solution of MH (6 µg/ml) and the first derivative of these spectra traced with the interval of Δλ= 8 nm, illustrated in Fig.5. From Fig. 4 and 5, 325.80 nm and 238.0 nm as wavelength maxima (λmax) was selected for the simultaneous determination of MH and DIFLU in marketed eye drop formulation, respectively.

### Method Validation

The method was validated as per ICH Q2 (R1) guideline [18]. Intraday and inter day precision was studied by analyzing three replicates of standard solutions at three concentrations level. The accuracy studies were carried out at different concentrations by spiking (50, 100 and 150 %) a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by the developed method. The limit of detection (LOD = 3.3 σ/s, where σ is the standard deviation of response and s is slope) and limit of quantitation (LOQ=10σ/s) of MH and DIFLU was calculated.

### Analysis of marketed Eye Drops

For the analysis of marketed eye drops, 5 ml was measured accurately and a quantity equivalent to 50 mg of MH and 5 mg of DIFLU was weighed and dissolved in 50 ml phosphate buffer pH 7.4 with the aid of ultrasonicator for 15 min and solution was filtered through Pre-filter + PVDF (0.45 µm) into a 100 ml volumetric flask and volume was made up to mark with phosphate buffer pH 7.4 as a diluent. The solution was suitably diluted with phosphate buffer pH 7.4 to get a concentration of 50 µg/ml of MH and 5 µg/ml of DIFLU. The prepared solution were analysed in triplicate and the amount of MH and DIFLU in formulation was calculated as per following. MH = Derivative amplitude at λmax 325.80, DIFLU = Derivative amplitude at λmax 238.0

## RESULTS AND DISCUSSION

### Ratio first derivative spectrophotometric method

The ratio spectra of different MH standards at increasing concentrations in phosphate buffer pH 7.4 obtained by dividing each with the stored zero order spectrum of standard solution of DIFLU are shown in Fig.4 (a) and the first derivative of these spectra traced with the interval of Δλ= 8 nm are illustrated in Fig.4 (b). Similarly, the ratio derivative spectra of the solutions of DIFLU in different concentrations in phosphate buffer pH 7.4 traced with the interval of Δλ= 8 nm by using the zero order spectra of MH as divisor by computer aid is demonstrated in Fig. 5. Here, the standard spectra of 6.0 µg/ml of MH and 4.0 µg/ml of DIFLU were considered as suitable for the determination of DIFLU and MH respectively, as divisor. The Δλ found as optimum for the first derivative of their ratio spectra was 8 nm. From the Fig. 4 (b) and Fig. 5 (b), wavelength maxima 325.80 nm and 238.0 nm were selected for the determination of the MH and DIFLU respectively in the assay of pharmaceutical preparation, eye drops dosage form, due to its lower R.S.D. value and more suitable mean recovery.

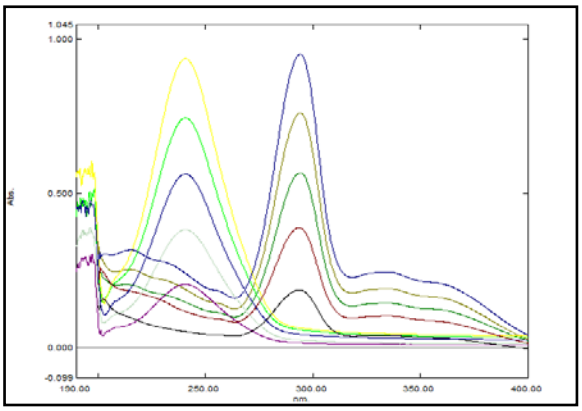
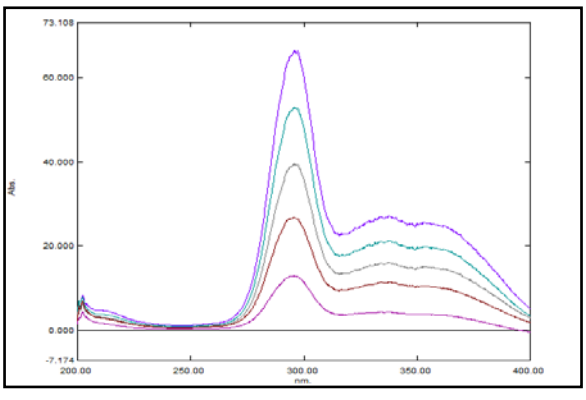



**Fig. 3: Zero order Overlay spectra of Moxifloxacin HCl and Difluprednate.**




**(A)**

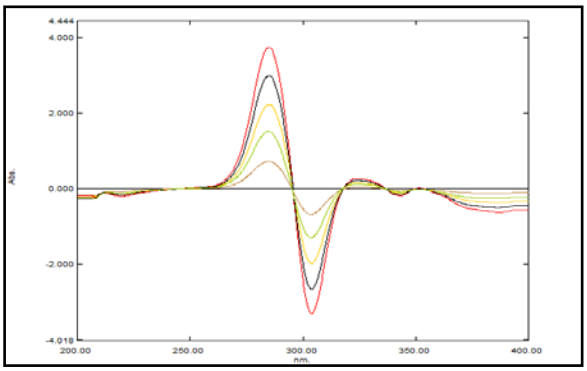
### Method validation

Validation of the methods has been performed according to ICH recommendations.

### Linearity

The calibration range for MH and DIFLU was established through considerations of the practical range necessary according to Beer–

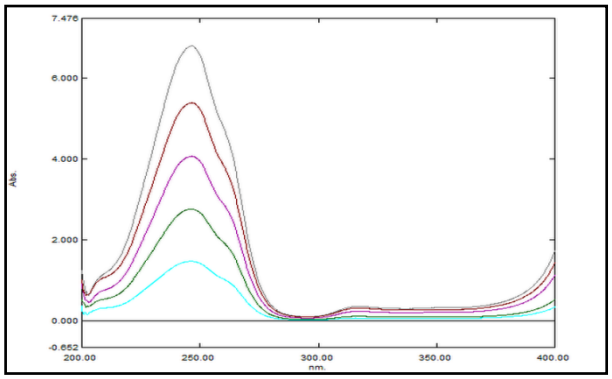
Lambert's law. The linearity response was determined by analyzing 5 independent levels of concentrations in the range of 2-10 µg/ml and 4-20 µg/ml at 325.80 nm for MH and at 238.0 nm for DIFLU respectively. The values of correlation coefficients of MH and DIFLU were close to unity indicating good linearity, the characteristic parameters for the constructed equations are summarized in Table 1.



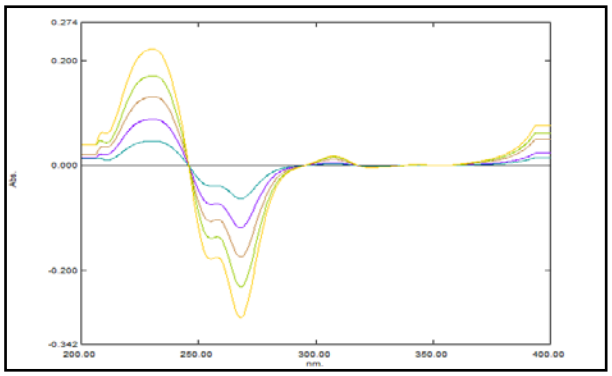
**(B)**

**Fig. 4: (A) Ratio derivative spectra of Moxifloxacin HCL, 4 µg/ml Difluprednate as divisor**

**(B) Ratio first order derivative spectra of Moxifloxacin HCl (λmax 325.80, Δλ = 8)**



**(A)**



**(B)**

**Fig. 5: (A) Ratio derivative spectra of Difluprednate, 6 µg/ml Moxifloxacin HCl as divisor**

**(B) Ratio first order derivative spectra of Difluprednate (λmax 238.0, Δλ = 8)**

**Table 1: Analytical parameters of proposed method**

| Parameters | MH | DIFLU |
|---|---|---|
| Wavelength (nm) | 325.80 | 238 |
| Linearity range (µg/ml) | 2-10 | 4-20 |
| Regression equation | y = 0.075x - 0.771 | y = 0.010x + 0.001 |
| Correlation coefficient | 0.999 | 0.997 |
| SD of intercept | 0.002608 | 0.0002 |
| SD of slope | 0.001517 | 0.000548 |
| CI of intercept | 0.8254-0.7153 | 0.0106-0.0140 |
| CI of slope | 0.0737-0.0763 | 0.0099-0.0118 |
| LOD (µg/ml) | 0.1144 | 0.0311 |
| LOQ (µg/ml) | 0.3466 | 0.094 |

n=5 replicates, CI means confidence interval; SD means standard deviation

**Precision**

The intraday precision was carried out through three replicate analysis of 4, 6 and 8 µg/mL of MH and 4, 8 and 12 µg/ml of DIFLU. The interday precision was also evaluated through three replicate analysis of the pure drug samples for three consecutive days at above mentioned concentration levels. The developed method is found to be precise as the % RSD values for intraday and interday precision were less than 2% (Table 2).

**Table 2: Precision studies**

| Amount of drug (µg/ml) | Intraday precision | | Interday precision | |
|---|---|---|---|---|
| | Amount of drug found ± SD (µg/ml) | %RSD | Amount of drug found ± SD (µg/ml) | %RSD |
| **MH** | | | | |
| 4 | 3.954784 | 1.218446 | 3.923239 | 0.966377 |
| 6 | 5.949176 | 0.539984 | 5.938661 | 0.715597 |
| 8 | 7.915528 | 0.731644 | 7.890992 | 0.887264 |
| **DIFLU** | | | | |
| 4 | 3.923333 | 1.790256 | 3.91 | 1.423981 |
| 8 | 7.93 | 0.702114 | 7.930 | 0.630517 |
| 12 | 11.76667 | 1.29818 | 11.7 | 1.709402 |

n=3 replicate; SD means standard deviation; %RSD means relative standard deviation.

**Accuracy**

Accuracy of methods was assured by applying the standard addition technique where good percentage recoveries were obtained and the accuracy of the proposed methods were confirmed (Table 3).

The recovery studies were carried out by adding known amount of standard to samples at 50, 100 and 150 % level and analyzed by the proposed method, in triplicate.

**Sensitivity**

The limit of detection and limit of quantitation were determined based on the standard deviation of response (y-intercept) and slope of the calibration curve according to ICH guideline [21].

The limits of detection and quantitation were found to be 0.1144 and 0.3466 µg/ml, respectively for MH and 0.0311 and 0.094 µg/ml, respectively for DIFLU.

**Analysis of marketed eye drops**

The proposed method was applied for the simultaneous determination of MH and DIFLU in commercial eye drops formulation and amount of MH and DIFLU were found to be 95%and 101.4 % respectively as shown in Table 4. The percent recoveries of the amount of MH and DIFLU in marketed eye drops, expressed as a percentage assay were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients that normally present in solution.

**CONFLICT OF INTERESTS**

Declared None

**Table 3: Recovery studies for determination of MH and DIFLU in semi-solid dosage form**

| Drugs | Taken (µg/ml) | % Level | Amount of std added (µg/ml) | Total amount of drug Found (µg/ml) | % Recovery ± SD | % RSD |
|---|---|---|---|---|---|---|
| MH | 4 | 50% | 2 | 5.917 | 98.62±0.21 | 0.2205 |
| | | 100% | 4 | 7.954 | 99.42±0.57 | 0.5820 |
| | | 150% | 6 | 9.953 | 99.53±0.43 | 0.4404 |
| DIFLU | 8 | 50% | 4 | 11.939 | 96.13±0.70 | 0.7372 |
| | | 100% | 8 | 15.943 | 98.68±0.85 | 0.8706 |
| | | 150% | 12 | 19.936 | 98.66±1.06 | 1.0753 |

n= 3 replicates; SD means standard deviation; %RSD means relative standard deviation

**Table 4: Determination of MH and DIFLU in marketed eye drops**

| Formulation | Drug | Label Claim | % Assay ± SD | % RSD |
|---|---|---|---|---|
| DIFLUMOX | MH | 50 mg | 95% ±0.687 | 0.697 |
| | DIFLU | 5 mg | 101.4%±0.223 | 0.234 |

n=3 replicates; SD means standard deviation; %RSD means relative standard deviation.

**CONCLUSION**

The proposed ratio first derivative spectrophotometric method for simultaneous determination of MH and DIFLU was found to be novel, rapid, simple, sensitive, accurate, precise and easy to be understood and applied. Distinct advantages of the proposed method include the simplicity and rapidity of sample preparation, good sensitivity and a cost effective methodology. Hence, the proposed method could be regarded as useful alternative to the chromatographic techniques in the routine quality control of title drugs either alone or in combination with a relatively inexpensive instrumentation for simultaneous estimation of MH and DIFLU in their binary mixtures.

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