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Simultaneous Estimation of Difluprednate and Moxifloxacin Hydrochloride In Ophthalmic Emulsion by UV- Visible Spectroscopy

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ABSTRACT

Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of difluprednate and moxifloxacin hydrochloride in combined dosage form have been developed. First method employs formation and solving of simultaneous equation using 241nm and 288 nm as two analytical wavelengths for both drugs in distilled water. The second method is a Q value analysis based on measurement of absorptivity at 241nm and 264nm (as an ISO-absorptive point). Difluprednate at their respective λmax 241 nm and 288 nm and at iso-absorptive point 264 nm shows linearity in a concentration range of 10-20 µg/mL and similarly moxifloxacin at their respective λmax 288 nm and 241 nm and at iso-absorptive point 264 nm shows linearity in a concentration range of 1-5 µg/mL. The results of the analysis have been validated statistically.

Keywords Difluprednate, Moxifloxacin, UV spectroscopy, Simultaneous equations, Q – analysis

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INTRODUCTION

Difluprednate is a topical corticosteroid indicated for the treatment of infammation and pain associated with ocular surgery. It is a butyrate ester of 6(α), 9(α)-difluoro prednisolone acetate. Difluprednate is abbreviated DFBA, or difluoroprednisolone butyrate acetate. It is indicated for treatment of endogenous anterior verity. [1] Moxifloxacin hydrochloride (MOXI) chemically is 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS, 7aS) octahydro 6H-pyrrolo [3, 4-b] pyridin-6-yl]-4-oxo-1, 4 dihydroquinoline- 3-carboxylic acid. It is a new-generation, 8- methoxyquinolone derivative of fluoroquinolone antibacterial agent. Moxifloxacin have an azabicyclo substitution at C-7, which is responsible for improved Gram-positive activity. Moxifloxacin is active against broad spectrum of pathogens, encompassing Gram- negative, Gram-positive bacteria including resistant strain Streptococcus pneumonia. It is available for oral and parenteral administration which is specially used to treat respiratory and skin infection. [1, 2]

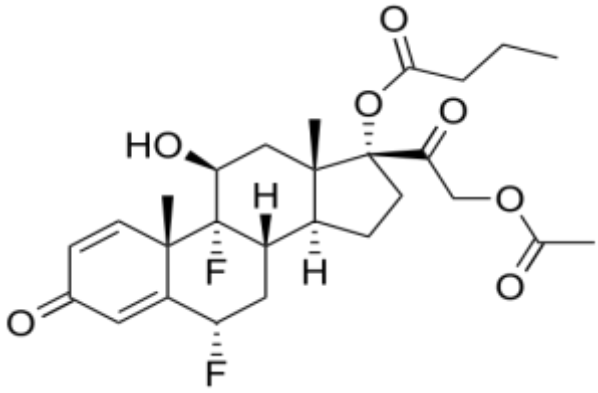

Figure 1. Chemical Structure of Difluprednate

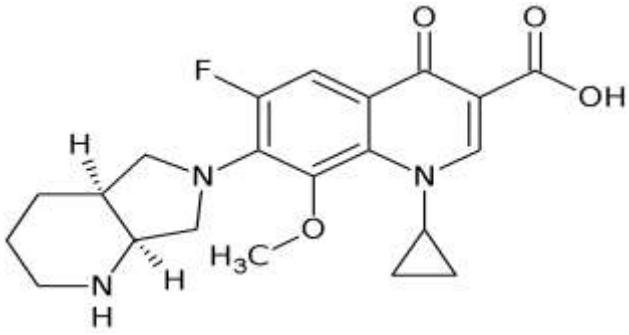

Figure 2. Chemical Structure of Moxifloxacin

The combination of difluprednate and moxifloxacin in ophthalmic solution form is use to treat the conjunctivitis. This combination is preferred because difluprednate is a corticosteroid used in inflammatory ocular conditions and along with inflammatory condition, the risk of bacterial infection exists. To prevent this infection moxifloxacin is given in combination.

Day to day number of drugs and their formulations either in single or combined dosage form are marketed. Combination of difluprednate and moxifloxacin is one of new drug combination. The combination of difluprednate and moxifloxacin is not official in any of the Pharmacopoeia. Hence no official method is available for estimation of these drugs in combined dosage form. So it is thought of interest to develop and validate UV spectroscopic methods in ophthalmic emulsion. This paper describes two simple, rapid, accurate, precise and economical methods for simultaneous determination of difluprednate and moxifloxacin in ophthalmic emulsion.

MATERIALS AND METHODS

Reagents & Instruments

A UV-VIS spectrophotometer Shimadzu UV-1800, single pan electronic balance, was used for the experimental purpose. Double distilled water was used throughout the study. Difluprednate and moxifloxacin were obtained as a gift sample from Pioneer Pharmacy Degree College, Vadodara. All the other reagents used were of analytical grade.

Determination of absorption maxima

Accurately weighed 10 mg of difluprednate was transferred to a 100 ml volumetric flask and volume was made up with the methanol to get a solution of concentration 100 µg/ml. Further aliquots were made with distilled water to get the concentration range of 10-20ug/ml. Solution of moxifloxacin was also prepared in a similar way to get a concentration of 1-5ug/ml µg/ml. The resulting solutions were scanned in the spectrum mode over the range of 200-400 nm. Difluprednate showed an absorbance peak at 241 nm, whereas moxifloxacin at 288 nm .

Sample Preparation Method

The sample solution was prepared using Miflox-DF formulation to get the final concentration containing 0.5 ug/ml (difluprednate) and 5ug/ml (moxifloxacin). Absorbance of sample solution was simultaneously estimated at 241nm, 288nm, and also at its iso-absorptive point 264nm.

Method I (simultaneous equation method)

Two wavelengths selected for the method are 241 nm and 288 nm that are absorption maxima's of difluprednate and moxifloxacin respectively. Standard stock solution(s) of 100 µg/ml each of difluprednate and moxifloxacin were prepared separately in methanol. The stock solutions of both the drugs were further diluted separately with distilled water to get a series of standard solutions of concentrations 10-20µg/ml for difluprednate and 1-5ug/ml for moxifloxacin.[3,4] The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations.

Concentrations in the sample were obtained by using the following equations:

$$Cx = \frac{A_2\ a_{y1} - A_2\ a_{y2}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

$$Cy = \frac{A_1\ a_{x2} - A_2\ a_{x1}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

Where, A1 and A2 are absorbances of mixture at 241 nm and 288 nm respectively, ax_1 and ax_2 are absorptivities of difluprednate at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of moxifloxacin at λ_1 and λ_2 respectively. C_x and C_y are concentrations of difluprednate and moxifloxacin respectively.

Method II (absorbance ratio or Q-analysis method)

From the overlay spectrum of difluprednate and moxifloxacin, two wavelengths were selected one at 288 nm, the Iso-absorptive point for both the drugs and the other at 241 nm, λ_{max} of moxifloxacin. [5, 6, 7] The absorbances of the standard and sample solutions prepared in a similar manner as in the previous method, were measured and the absorptivity values for both drugs at the selected wavelengths are presented in Table I. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula**,**

$$Cx = \{(QM-Qy)/ (Qx-Qy)\}* (A1/ax1)$$

$$Cy = \{(QM-Qx)/ (Qy-Qx)\}* (A1/ay1)$$

Where,

$$Q_M = \frac{\text{Absorbance of sample at 241nm}}{\text{Absorbance of sample at 264 nm}}$$

$$Q_X = \frac{\text{Absorptivity of Difluprednate at 241 nm}}{\text{Absorptivity of Difluprednate at 264 nm}}$$

$$Q_Y = \frac{\text{Absorptivity of Moxifloxacin at 241 nm}}{\text{Absorptivity of Moxifloxacin at 264nm}}$$

A1 = Absorbance of sample at iso-absorptive point

ay1 = Absorptivity of Moxifloxacin at iso-absorptive point.

ax1= Absorptivity of Difluprednate at iso-absorptive point.

METHOD VALIDATION

The described methods have been validated for the assay of both the major components of bulk

drug using following ICH parameters.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 10-20ug/ml for difluprednate and 1-5ug/ml for moxifloxacin and linear regression analysis was carried out. The regression coefficients are reported in Table I and II.

Precision

Precision was studied to find out intra and inter-day variations in the test method of difluprednate and moxifloxacin. Calibration curves prepared in medium were run in triplicate in the same day for three days. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are tabulated in Table III.

Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out using Milflox-DF formulation. Results of recovery studies were presented in Table IV.

RESULTS AND DISCUSSION

The overlay spectra of DFBA and MOXI exhibit λmax at 241 nm and 288 nm for DFBA and MOXI respectively which are quite separate from each other. Additionally an iso-absorptive point was observed at 264 nm. Standard calibration curves for difluprednate and moxifloxacin were linear with correlation coefficients (r^2) values in the range of 0.9816- 0.9939 at all the selected wavelengths. The method was repeated for the same day and % RSD was found to be <1.5% for DFBA and <2 for MOXI, similarly the method was repeated for different days and % RSD was found to be <2 for DFBA and MOXI. The accuracy of the method was confirmed by recovery studies from synthetic mixtures at three different levels of standard additions.

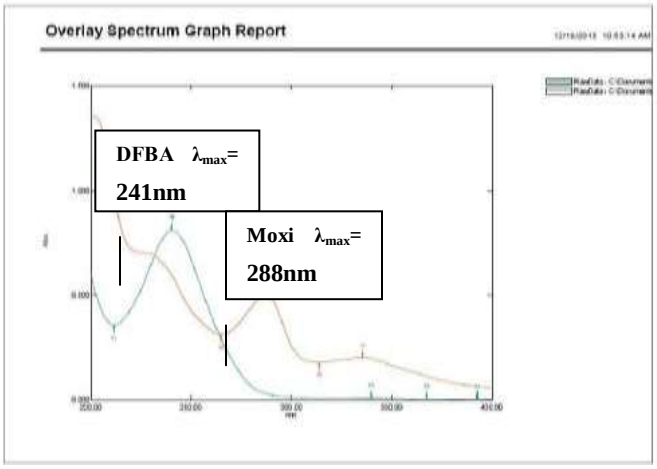

Figure 3.Spectra showing absorption maxima of difluprednate & moxifloxacin

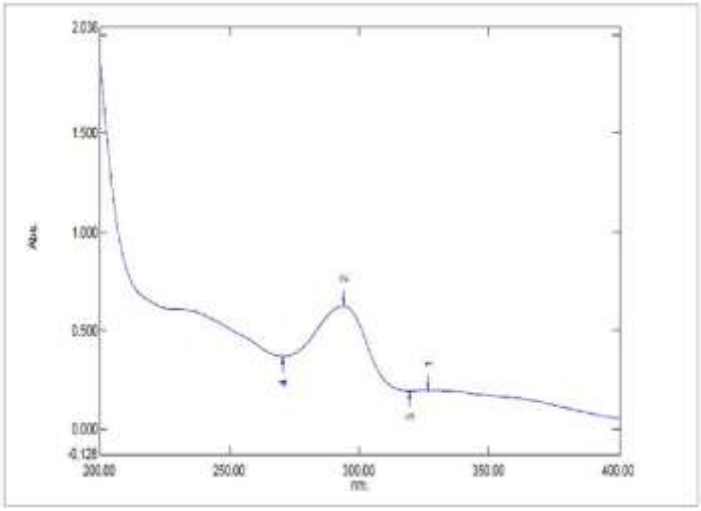

Figure 4.Spectra showing absorption of difluprednate & moxifloxacin in formulation (Milflox-DF)

Table 1.Linear regression analysis of calibration curves of difluprednate

Concentration (µg/ml)	Method 1 Difluprednate		Method 2 Difluprednate
	241 nm	288 nm	Iso-absorptive point 264nm
10	0.451	0.032	0.183
12	0.501	0.026	0.196
14	0.575	0.023	0.220
16	0.645	0.03	0.250
18	0.718	0.023	0.273
20	0.807	0.025	0.306
$A^{1\%}_{1cm}$	414.11	19.016	160.73

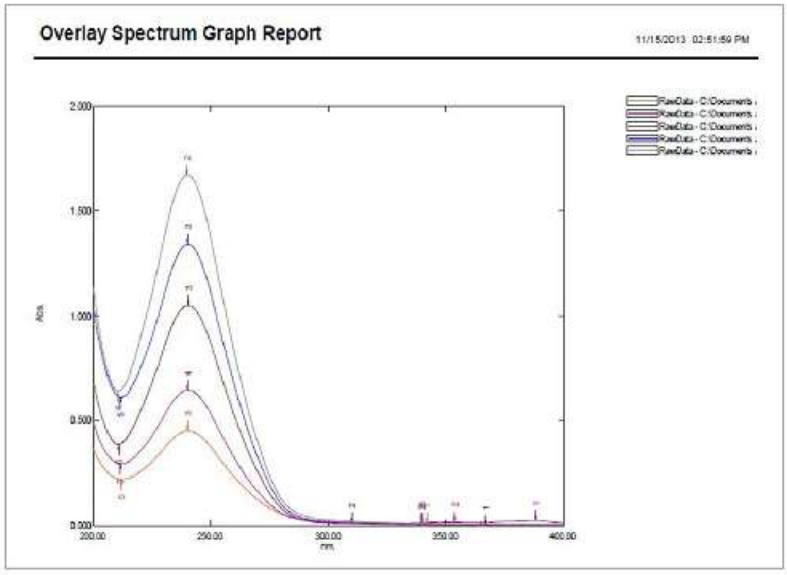

Figure 5: Linearity spectra of difluprednate at 241 nm

Table 2.Linear regression analysis of calibration curves of moxifloxacin

Concentration (µg/ml)	Method 1		Method 2
	Moxifloxacin		Moxifloxacin
	288 nm	241 nm	Iso-absorptive point 264nm
1	0.101	0.071	0.018
2	0.201	0.196	0.075
3	0.301	0.300	0.149
4	0.404	0.387	0.149
5	0.499	0.581	0.312
$A^{1\%}_{1cm}$	1005.26	963.9	409.632

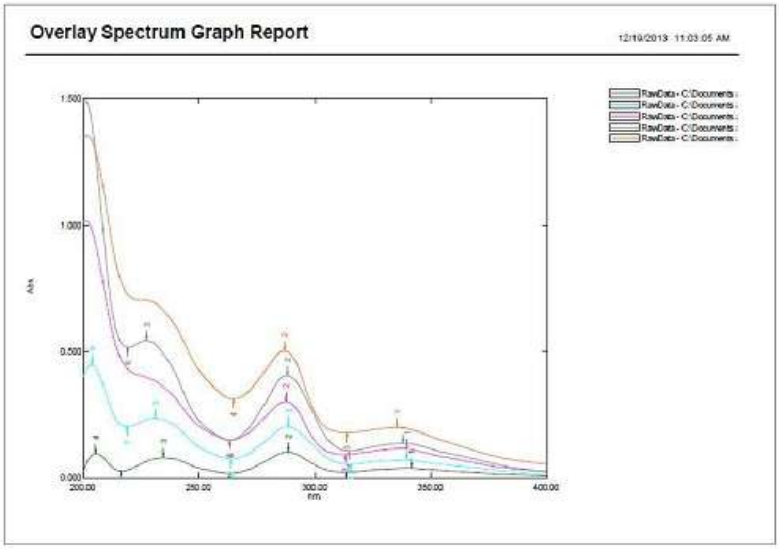

Figure 6: Linearity spectra of moxifloxacin at 288 nm

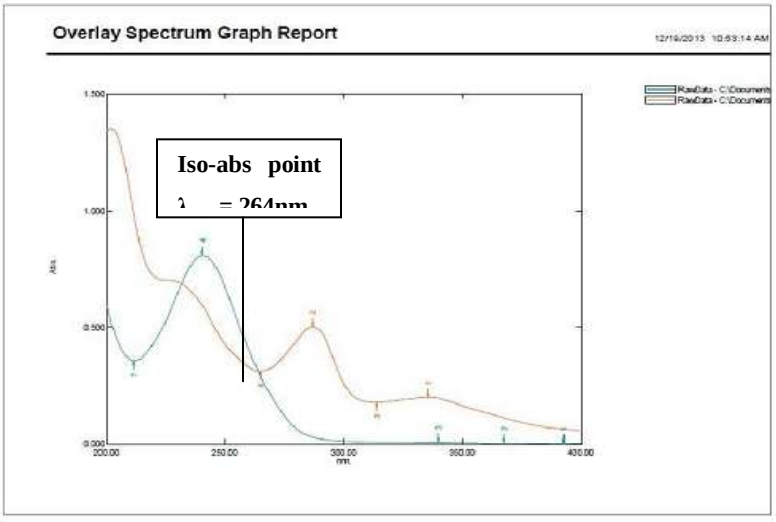

Figure 7.Q-Absorption spectra at iso absorptive point 264 nm

Table 3.Results of precision study (Intra-day and inter-day)

Methods	**Inter-Day Precision (n=3)% RSD**		**Intra-day Precision (n=3) % RSD**	
Simultaneous Equation				
	MOXI	**DFBA**	**DFBA**	**MOXI**
Method – I	0.10	1.79	1.67	0.18
Q- Absorption				
	Inter-day Precision (n=3)% RSD		Intra-day Precision (n=3)% RSD	
Method – II	0.23		0.32	

Table 4: Recovery study of difluprednate and moxifloxacin

% Amount added	**Actual Concentration taken(ug /ml)**		**Amount Recovered ug/ml**		**Average % Recovery**		**% RSD**	
	DFBA	**MOXI**	**DFBA**	**MOXI**	**DFBA**	**MOXI**	**DFBA**	**MOXI**
80	2.9	9	2.87	9.2	98.9	102.2	0.23	1.67
	2.9	9	2.87	8.9	98.9	98.9		
	2.9	9	2.88	9.1	99.3	101.1		
100	3.5	10	3.48	10.1	99.4	101.0	0.18	1.15
	3.5	10	3.49	9.9	99.71	99		
	3.5	10	3.48	10.1	99.4	101		
120	4.1	11	4.98	11.1	97.56	100.9	0.15	1.88
	4.1	11	3.99	11.2	97.31	101.81		
	4.1	11	4.00	10.8	97.56	98.18		

CONCLUSION

The proposed methods for simultaneous estimation of difluprednate and moxifloxacin in combined dosage forms were found to be simple, accurate, precise, economical and rapid. In both the methods percentage recovery was found be to 100% and % RSD found to be less than 2% for both the drugs. Hence, these methods can be employed for routine analysis in quality control laboratory.

REFERENCES

1. "Difluprednate", 15th December 2013,www.drugbanjk.ca/drugs/DB06781
2. "Moxifloxacin", 15th December 2013, www.drugbank.ca/DB00218
3. Government of India, "Ministry of Health and Family Welfare", Indian Pharmacopoeia, 2007, volume- II, pp. 540.
4. Yasueda S, Kimura M, Ohtori A, Kakehi K. Analysis of an anti-inflammatory steroidal drug, Difluprednate in aqueous humor by combination of semi-micro HPLC and column switching method. J Pharma Biomedical Analysis, 2003, 30(6): 1735-1742.

5. Misra M, Misra A.K. Simple and validated UV-Spectroscopic method for estimation of moxifloxacin HCL in bulk and formulation. J Global Pharmatechnology, 2010, 2(6): 21-27.
6. Vyas PJ, Dave JB, Patel CN. Simultaneous estimation of moxifloxacin HCL and bromfenac sodium in eye drops by spectrophotometric method. Int J Pharma Sci Res 2012, 3(7): 2137-2142.
7. Chaudhari BJ, Patel B. Development and validation of Q-absorption ratio method for simultaneous estimation of cefixime and moxifloxacin HCL in synthetic mixture. Am J Pharmtech Res 2012, 2(5): 823-832.