ISSN 2347-3614

UNIQUE JOURNAL OF PHARMACEUTICAL AND BIOLOGICAL SCIENCES

Available online: www.ujconline.net

Research Article

# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND DEXAMETHASONE SODIUM PHOSPHATE

Rangaraju D[1], Patel Sujit[2], Sambrekar Amruta[2], Karigar Asif[3], Nagesh C[2*]

[1]Production Manager, Karnataka Antibiotics Pvt. Ltd. Bangalore
[2]Department of Pharmaceutics, Maratha Mandal College of Pharmacy, Belgaum, Karnataka, India
[3]Department of Pharmaceutical Analysis, Maratha Mandal College of Pharmacy, Belgaum, Karnataka, India

Received 05-08-2013; Revised 26-08-2013; Accepted 16-09-2013

*Corresponding Author: **Dr. Nagesh C**
Professor and Head Department of Pharmaceutics, Maratha Mandal College of Pharmacy, Belgaum, Karnataka, India, E-mail: nagesh_73@rediffmail.com

## ABSTRACT

A simple precise, accurate, rapid and economical spectrophotometric method has been developed for simultaneous estimation of Ofloxacin and Dexamethasone sodium phosphate in pure form. It is done by Simultaneous equation method by using nm 287 nm and 242 nm as absorbance maxima ($\lambda_{max}$) for Ofloxacin and Dexamethasone sodium phosphate respectively. A Phosphate buffer (pH 7.4) was used as Solvent. Linearity was observed in the concentration range of 5 - 25 µg/ml for Ofloxacin and 2 - 10 µg/ml for Dexamethasone sodium phosphate respectively. The method was validated statistically.

**Keywords:** Ofloxacin (OFX), Dexamethasone sodium phosphate (DEXP), simultaneous equation, UV spectroscopy method, phosphate buffer (pH 7.4).

## INTRODUCTION

Ofloxacin (OFX) chemically (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. Ofloxacin is active against a wide variety of gram-positive and gram-negative organism, use in the treatment of urinary tract infection, conjunctivitis, gonorrhoea and respiratory tract infection. Dexamethasone sodium phosphate (DEXP) chemically (9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione-21 (dihydrogenphosphate) disodium salt), a synthetic glucocorticoid with prominent anti-inflammatory, is official in the United States Pharmacopoeia.

OFX and DEXP are formulated together in the form of eye/ear drops (solution), which is used in the treatment of conjunctivitis, keratitis and post operative cases of cataract extraction and trabeculectomy.

## MATERIALS AND METHODS

**Instruments**

Double beam UV Spectrophotometer (Shimadzu Model 1800) was employed with automatic wavelength correction with a pair of 1 cm matched quartz cell.

**Chemicals and reagents**

OFX and DEXP pure drugs were received as gift samples from Lincoln Pharmaceutical Ltd. (Ahmedabad, India) and Indian Opthalmics (Surendranagar, India) respectively.

**Preparation of standard stock solution & calibration curve**

Standard stock solution of pure drug containing 50 µg/mL of OFX and 20 µg/mL of DEXP were prepared in phosphate buffer (pH 7.4) system. The working standard solutions of these drugs were obtained by dilution of the stock solution in the phosphate buffer (pH 7.4).Series of solutions with conc. 5 - 25 µg/mL and 2 – 10 µg/mL of OFX and DEXP respectively were used to prepare calibration curve. Solutions were scanned and proposed methods were applied. For determination of absorptivity values, calibration curves using standard serial dilutions of individual drugs were plotted.

**Simultaneous determination**

The Simultaneous Equation Method of analysis based on the absorption of the drugs OFX and DEXP at their $\lambda_{max}$. Two wavelengths selected for the development of Simultaneous Equation are 287 nm ($\lambda_1$) and 242 nm ($\lambda_2$). Absorptivities of both the drugs at both the wavelengths were determined. Equations obtained for the estimation of concentration were,

$$C_x = \frac{(A_1 * y_2) - (A_2 * y_1)}{x_1 y_2 - x_2 y_1}$$

$$C_y = \frac{(A_2*x_1) - (A_1*x_2)}{x_1y_2 - x_2y_1}$$

Where $A_1$ and $A_2$ are absorbance of sample solution at 287 nm and 242 nm respectively.
$x_1$ = Absorptivity of OFX at 287 nm
$x_2$ = Absorptivity of OFX at 242 nm
$y_1$ = Absorptivity of DEXP at 287 nm
$y_2$ = Absorptivity of DEXP at 242 nm
$C_x$ and $C_y$ are concentration of OFX and DEXP in sample solution

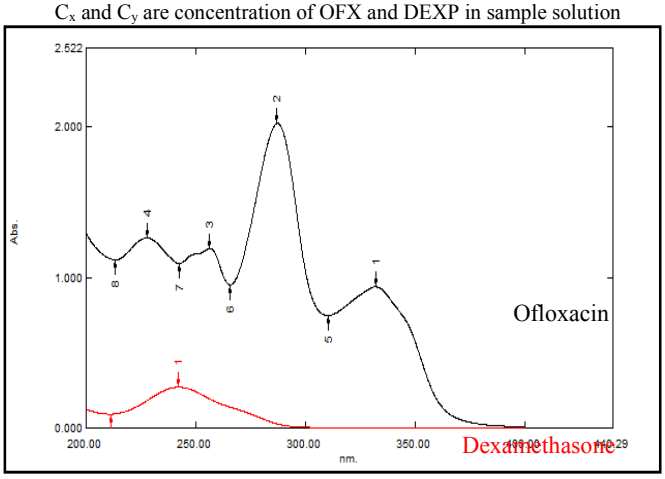


**Fig. 1: Overlay spectra of Ofloxacin and Dexamethasone**

## METHOD OF VALIDATION

### Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range is 5-25 µg/mL for Ofloxacin and 2-10 µg/mL for Dexamethasone.

### Robustness of the study

Small deliberate changes in the wavelength (±5 nm) were introduced and the effect on the results was examined.

### Precision of the method

Precision of the methods was determined by repeating assay si x times. To study intraday precision method was repeated 6 ti mes in a day and the average % RSD was calculated. Similarly the method was repeated on 3different days and average % R SD was calculated.

### Accuracy

Accuracy was confirmed by recovery study as per ICH guidelines Q2R1 at three different concentration levels 80 %, 100 %, 120 % by replicate analysis (n = 3). Here to a sample solution drug solutions were added and then percentage of drug content was calculated.

## RESULTS AND DISCUSSION

The calibration curves were found to be linear over the ranges 1200-3000 ng $spot^{-1}$ and 400- 1000 ng $spot^{-1}$ for OFX and DEXP respectively. Characteristic parameters for the regression equation and correlation coefficients are given in Table 1. The linearity of the calibration curves was validated by the high value of correlation coefficients of the regression.
Effect of small deliberate changes in the wavelength (±5 nm) was introduced and the results were reported in Table 2.
The results of the intraday and interday precision are shown in Table 3(a) and Table 3(b) respectively. The values of relative standard deviation (RSD) of the intraday and interday determinations show that the proposed method is precise.
The % recovery was found to be in the range of 89.7 to 95.3 for Ofloxacin and 85.3 to 94.2 for Dexamethasone. From the recovery study it is clear that the method is accurate for quantitative estimation of Ofloxacin and Dexamethasone, as the statistical parameters are within the acceptance range. The results of are shown in Table 4.

**Table 1: Spectral and Linearity Characteristic Data**

| Parameters | Ofloxacin | Dexamethasone |
|---|---|---|
| $\lambda_{max}$ (nm) | 287 | 242 |
| Linearity range (µg/ml) | 5-25 | 2-10 |
| Correlation coefficient ($R^2$) | 0.9997 | 0.9999 |
| Slope (m) | 0.0727 | 0.027 |
| Regression equation | y = 0.0727x | y = 0.027x |

**Table 2: Robustness study data**

| Drugs | Averages | S.D. | R.S.D. |
|---|---|---|---|
| OFX | 0.748 | 0.004647 | 0.6212 |
| DEXP | 0.271 | 0.001702 | 0.6280 |

**Table 3: (a). Intraday Precision (n=6)**

| Drugs | Averages | S.D. | R.S.D. |
|---|---|---|---|
| OFX | 0.760 | 0.003649 | 0.4798 |
| DEXP | 0.265 | 0.003472 | 1.3073 |

**Table 3: (b). Interday Precision (n=3)**

| Drugs | Averages | S.D. | R.S.D. |
|---|---|---|---|
| OFX | 0.760 | 0.002236 | 0.4801 |
| DEXP | 0.265 | 0.001224 | 1.3101 |

**Table 4: Result of Recovery Study**

| | % Recovery ± SD | |
|---|---|---|
| **Drug** | **Ofloxacin** | **Dexamethasone** |
| 80 % | 89.7 ± 0.24 | 85.3 ± 0.34 |
| 100 % | 91.4 ± 0.31 | 90.6 ± 0.11 |
| 120 % | 95.3 ± 0.19 | 94.2 ± 0.41 |

## CONCLUSION

A new simple, accurate, sensitive and economical UV spectrophotometric method was developed for the simultaneous analysis of Ofloxacin and Dexamethasone sodium phosphate. The developed methods were validated and from the statistical data, it was found that the methods were linear and precise and can be successfully applied for the analysis in laboratories and for quality control purposes.

## ACKNOWLEDGEMENTS

The authors are thankful to KAPL, Bangalore for providing gift samples of drugs. The authors are highly thankful to Maratha Mandal College of Pharmacy Belgaum, Karnataka, India for providing all the facilities to carry out the work.

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**Source of support: Nil, Conflict of interest: None Declared**