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# Simultaneous spectrophotometric estimation of ofloxacin and ornidazole by first order derivative spectroscopy method in combined dosage form

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**ABSTRACT**

*The objective of the study was to develop a simple, accurate, precise and rapid UV first order derivative spectrophotometric method with subsequent validation by using ICH guidelines for the determination of ofloxacin and ornidazole by using 0.1N hydrochloric acid as the solvent in combined dosage form. The proposed first order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence 278 nm and 293.6 nm were selected for the estimation of ofloxacin and ornidazole respectively. The linearity of the proposed method was found in the concentration range of 0.5 – 10 µg /ml ($r^2$= 0.9995) for ofloxacin and 2.0 – 30 µg /ml ($r^2$= 0.9971) for ornidazole respectively. The percentage mean recovery was found to be 100.069 % for ofloxacin and 100.08 % for ornidazole respectively. The method was also statistically validated for its linearity, accuracy and precision. Both intra and inter day variation showed less percentage (%) RSD values indicating high grade of precision of this method.*

**Keywords:** UV spectrophotometric estimation, ofloxacin, ornidazole, validation.

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## INTRODUCTION

Ofloxacin is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin [1] is a fluorinated carboxy-quinolone. It is a racemate, (±)- 9-fluro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It is official in BP [2], USP [3], and EP [4]. The assay procedure mentioned in these pharmacopoeias uses non aqueous titration for estimation of ofloxacin. Literature survey reveals spectrophotometric methods [5, 6, 7, 8 ], atomic absorption spectrometry [5], spectro-flurometry [5], HPLC [9] and microbiological method [10] for its determination.

Ornidazole [1] is a 5-nitro-imidazole derivative used as anti-infective agent. It is not official in any Pharmacopoeia. Literature survey reveals that ornidazole is estimated by voltametry [11] and HPLC [12] methods for its determination in dosage forms and biological fluids. Ofloxacin and ornidazole in combined tablet dosage form is available in the market, has gained increasing acceptance in diarrhoea, bacterial and protozoal infections. Spectrophotometric [13, 14] and HPTLC [15] methods have been established for their simultaneous estimation in tablet dosage form. This proposed work presents simple, accurate and reproducible UV spectrophotometric methods for simultaneous determination of ofloxacin and ornidazole in tablet dosage form.

## MATERIALS AND METHODS

**Instrument and reagents**

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software.

Reference standards of ofloxacin and ornidazole were obtained from reputed firm with certificate of analysis.

Hydrochloric acid was used of AR grade.

**Preparation of standard drug solutions**

100 mg standard ofloxacin was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of 0.1N hydrochloric acid for 15 minutes. The volume was made up to the mark with 0.1N hydrochloric acid to give a stock solution of ofloxacin of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1N hydrochloric acid to give a working standard solution of concentration 100 µg/ml.

Similarly 100 mg standard ornidazole was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of 0.1N hydrochloric acid for 15 minutes. The volume was made up to the mark with 0.1N hydrochloric acid to give a stock solution of ornidazole of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1N hydrochloric acid to give a working standard solution of concentration 100 µg/ml.

**Estimation from tablets**

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 20 mg of ofloxacin and 50 mg of ornidazole was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

**Method: First order derivative method**

**(a) For ofloxacin**

For the selection of analytical wavelength, 10 µg/ml solution of ofloxacin was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 278 nm.

**(b) For ornidazole**

For the selection of analytical wavelength, 10 µg/ml solution of ornidazole was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 293.6 nm.

**Preparation of calibration curves**

Series of solutions containing 0.5 – 10 µg/ ml of ofloxacin and 2.0 -30 µg/ ml of ornidazole were used to determine linearity of the proposed method respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to first order derivative spectra [Fig. 1(a), 1(b)].

**Fig. 1(a): Overlay spectra of first order derivative of ofloxacin in the concentration range of 2 – 10 µg/ ml**

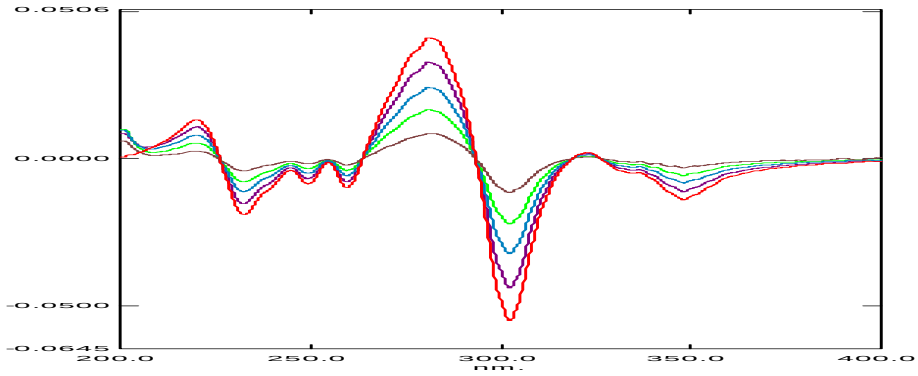
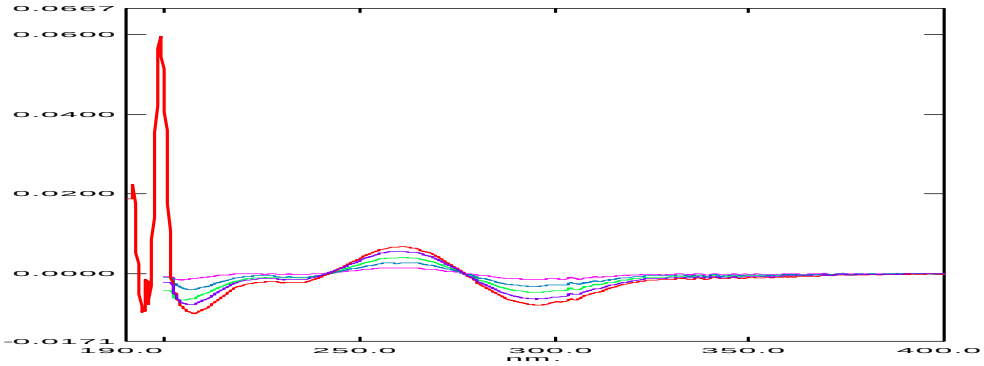



**Fig. 1(b): Overlay spectra of first order derivative of ornidazole in the concentration range of 2 – 10 µg/ ml**




After observing the overlain first order derivative spectra of ofloxacin and ornidazole, the zero crossing points of both drugs were selected for analysis of other drug. The first wave length selected was 278 nm, the zero crossing point of ornidazole where ofloxacin showed considerable absorbance. The second wavelength was 293.6 nm, the zero crossing point of ofloxacin, where ornidazole showed considerable absorbance. The calibration curves were plotted of dA/ dλ against concentrations [Fig. 2 (a), 2(b)].

**Fig.2 (a): Calibration curve of ofloxacin in the concentration range of 2-10 µg/ml**

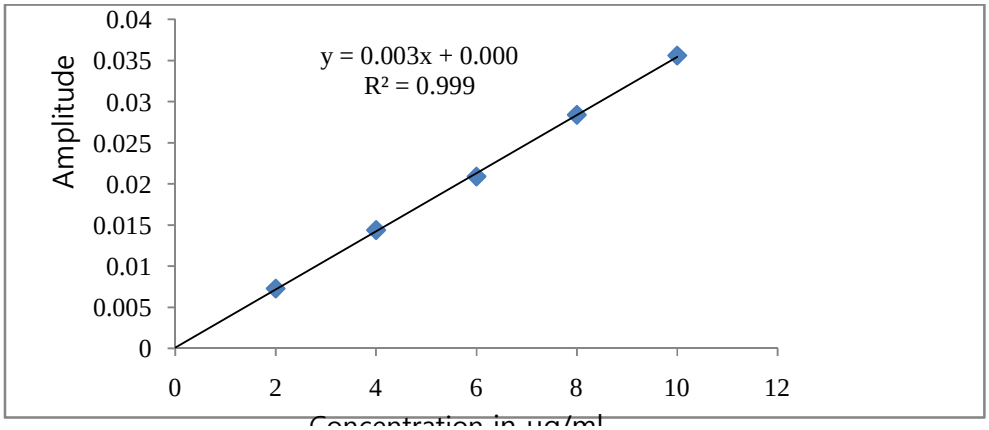
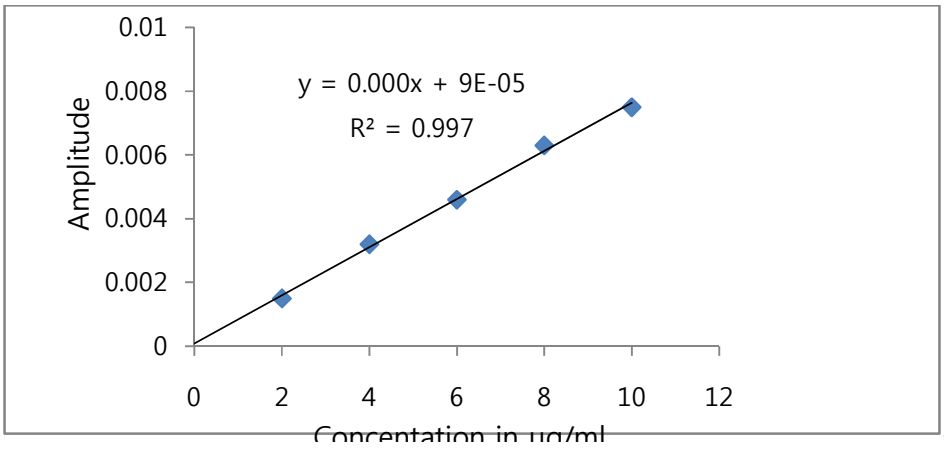


**Fig.2 (b): Calibration curve of ornidazole in the concentration range of 2-10 µg/ml**




Results of the analysis are given in table 1.

**Table 1: Values of results of optical and regression of drugs**

| Parameter | Ofloxacin | Ornidazole |
|---|---|---|
| Detection Wavelength (nm) | 278 | 293.6 |
| Beer Law Limits (µg/ml) | 0.5-10 | 2.0-30 |
| Correlation coefficient($r^2$) | 0.9995 | 0.9971 |
| Regression equation (y=b+ac) | | |
| Slope (a) | 0.0035 | 0.0008 |
| Intercept (b) | 0.0001 | 0.00009 |

**Estimation from tablets**

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 20 mg of ofloxacin and 50 mg of ornidazole was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N hydrochloric acid to give concentration as 100 µg /ml of each drug. Such solution was scanned in the range of 200-400 nm against 0.1 N hydrochloric acid as blank. The absorbance spectra were converted to first order derivative spectra. Calculations were done as per the equations. The concentrations of ofloxacin and ornidazole present in tablets were calculated by substituting the values of absorbance in linearity equations.

(a) For ofloxacin Y = 0.0035x + 0.0001
(b) For ornidazole Y = 0.0008x + 0.00009

**Method Validation**

These methods were validated according to ICH guidelines.

**Accuracy**

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for ofloxacin and ornidazole was found in the range of 98.05 % to 101.22 %. (Table 2).

**Table 2: Statistical evaluation of the data subjected to accuracy**

| Level of % recovery | Amount present in µg/ml | | Amount added in µg/ml | | Amount found in µg/ml | | % Recovery | | Mean % recovery | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OFL | ORN | OFL | ORN | OFL | ORN | OFL | ORN | OFL | ORN |
| 80% | 2.0 | 5.0 | 1.6 | 4.0 | 3.61 | 9.20 | 100.27 | 100.22 | 99.75 | 100.03 |
| | 2.0 | 5.0 | 1.6 | 4.0 | 3.53 | 9.11 | 98.05 | 101.22 | | |
| | 2.0 | 5.0 | 1.6 | 4.0 | 3.63 | 8.88 | 100.83 | 98.66 | | |
| 100% | 2.0 | 5.0 | 2.0 | 5.0 | 4.02 | 10.02 | 100.50 | 100.20 | 100.08 | 100.03 |
| | 2.0 | 5.0 | 2.0 | 5.0 | 4.03 | 9.98 | 100.75 | 99.80 | | |
| | 2.0 | 5.0 | 2.0 | 5.0 | 3.96 | 10.01 | 99.00 | 100.10 | | |
| 120% | 2.0 | 5.0 | 2.4 | 6.0 | 4.41 | 11.03 | 100.23 | 100.27 | 100.07 | 100.18 |
| | 2.0 | 5.0 | 2.4 | 6.0 | 4.45 | 11.05 | 101.13 | 100.45 | | |
| | 2.0 | 5.0 | 2.4 | 6.0 | 4.35 | 10.98 | 98.86 | 99.81 | | |
| **Confidence interval** | | | | | | | 99.96±0.69 | 100.08±0.44 | | |

*OFL = Ofloxacin, ORN = Ornidazole*

## Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ofloxacin and ornidazole. For both the drugs concentration range was found to be 0.5-10 µg/ml for ofloxacin and 2.0-30 µg/ml for ornidazole.

## Precision

The method precision was established by carrying out the analysis of tablets powder blend containing 200 mg of ofloxacin and 500 mg of ornidazole. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were well within limits 99.00 % and 100.75 % for ofloxacin and 99.80 % and 100.48 % for ornidazole respectively indicating the sample repeatability of the method. The results obtained are tabulated in table 3**.**

**Table 3: Statistical evaluation of the data subjected to method of precision**

| Sr. No. | Sample No. | % Assay | |
|---|---|---|---|
| | | OFL | ORN |
| 1 | 1 | 100.5 | 100.2 |
| 2 | 2 | 100.75 | 99.80 |
| 3 | 3 | 99.00 | 100.10 |
| 4 | 4 | 100.02 | 100.46 |
| 5 | 5 | 100.37 | 100.35 |
| 6 | 6 | 100.65 | 100.48 |
| **Mean % assay** | | 100.215 | 100.23 |
| **%R.S.D.** | | 0.6458 | 0.2572 |

Intra-day precision was estimated by assaying tablets powder blend containing 200 mg of ofloxacin and 500 mg of ornidazole. The assay was carried out for the drugs by using proposed analytical method in six replicates. The results were average for statistical evaluation.

Inter-day precision was estimated by assaying tablets powder blend containing 200 mg of ofloxacin and 500 mg of ornidazole for three consecutive days (i.e. 1$^{st}$, 3$^{rd}$ and 5$^{th}$ days). The statistical validation data for intra and inter day precision is summarized in table 4.

**Table 4: Summary of validation parameter for intra-day and inter-day**

| Sr. No. | Parameters | Ofloxacin | Ornidazole |
|---|---|---|---|
| 1 | Intra-day precision | 99.60% | 99.45% |
| | (N=3)amount found ± % R.S.D. | 0.24847 | 0.03446 |
| 2 | Inter-day precision | 98.484 | 98.762% |
| | (N=3)amount found ± % R.S.D. | 0.13607 | 0.00768 |

Both intra- day and inter-day precision variation found to be less in % RSD values. It indicates high degree of precision of the method.

## RESULTS AND DISCUSSION

The developed first order derivative spectrophotometric method for simultaneous determination of ofloxacin and ornidazole in tablet formulation was found to be simple and convenient for the routine analysis of two drugs. The method is used to eliminate the spectral interference from one of the two drugs while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug as the selected wavelength. Reason for not using simultaneous equation and absorbance ratio methods were not used as there is maximum spectral overlap and more difference in the absorbance. The proposed method is accurate, precise and reproducible. It is confirmed from validation data as given in tables 1 to 4. The % RSD was found to be less than 1, which indicates validity of method. Linearity was observed by linear regression equation method for ofloxacin and ornidazole in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity figure 2 (a) and 2 (b).

The assay results obtained by proposed method is shown in table 2 are in good agreement. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. Method is simple, accurate, precise, reliable, rapid, sensitive, reproducible and economical. It is validate as per ICH guidelines.

## CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of ofloxacin and ornidazole in combined dosage form. The method does not require any ratio of first order derivative as suggested in literature [8]. The amplitude of first order derivative can be directly used to assay of formulation. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adopted for routine quality control analysis.

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