

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF NORFLOXACIN AND ORNIDAZOLE IN TABLET DOSAGE FORM

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ABSTRACT: Three simple, accurate and economical methods have been developed for the estimation of Norfloxacin and Ornidazole in tablet dosage form. First method is based on the simultaneous equations, wavelengths selected for analysis were 273.0 nm ($\lambda_{\max}$ of Norfloxacin) and 318.5 nm ($\lambda_{\max}$ of Ornidazole) respectively, in 0.1 N NaOH. Second method is Q-analysis method, based on absorbance ratio at two selected wavelengths 297.0 nm (iso-absorptive point) and 318.5 nm ($\lambda_{\max}$ of Ornidazole). Third method is first order derivative spectroscopy using 297.5 nm (zero cross for Norfloxacin) and 264.0 nm (zero cross for Ornidazole). The linearity was obtained in the concentration range of 4-20 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ for Norfloxacin and Ornidazole, respectively. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Norfloxacin, Ornidazole, Simultaneous equation, Q-analysis, Derivative spectroscopy.

INTRODUCTION

Norfloxacin (NF), chemically 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid¹, is a synthetic broad spectrum fluoroquinolone antibacterial agent used in the treatment of urinary and genital tract infection. Ornidazole (OZ), chemically 1-chloro-3-(2-methyl-5-nitro-imidazol-1-yl) propan-2-ol, is an antimicrobial agent used in treatment of susceptible protozoal infections and anaerobic bacterial infection². Norfloxacin is official in USP, BP and IP whereas Ornidazole is not official in any pharmacopoeia. Both the drugs are marketed as combined dose tablet formulation and the ratio of NF:OZ is 400:500 mg. Literature survey revealed that a number of methods have been reported for estimation of Norfloxacin³⁻⁶ and Ornidazole⁷⁻¹² individually or in combination with other drugs. However, there is no analytical method reported for the simultaneous estimation of Norfloxacin and Ornidazole in a combined dosage formulation. Present work describes three simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of NF and OZ in tablet formulation.

EXPERIMENTAL

Instrument A double-beam Shimadzu UV- Visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a

pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

Materials Standard gift sample of Norfloxacin was provided by Emcure Pharmaceuticals Ltd., Pune and Ornidazole by Aristo Pharmaceuticals Pvt. Ltd., Mumbai. Combined dose Norfloxacin and Ornidazole tablets (NORRIT-ORD, 400 mg Norfloxacin and 500 mg Ornidazole; manufacture by Ind-Swift Limited, Chandigarh), were purchased from local market.

Solvent 0.1 N sodium hydroxide was prepared from analytical reagent grade sodium hydroxide in double distilled water and used as a solvent.

Stock solution: Standard stock solutions of NF (100 $\mu\text{g/ml}$) and OZ (100 $\mu\text{g/ml}$) were prepared and used for the analysis.

Procedure

Method A: Simultaneous Equation Method

For the selection of analytical wavelength, solutions of NF and OZ (20 $\mu\text{g/ml}$, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the overlain spectra of both drugs (Fig.1), wavelengths 273.0 nm ($\lambda_{\max}$ of NF) and 318.5 nm ($\lambda_{\max}$ of OZ) were selected for the simultaneous equations. The calibration curves for NF and OZ were prepared in the concentration range of 4-20 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ at both the wavelengths respectively. The absorptivities values were

determined for both the drugs at both the wavelengths and following equations were used, $A_1 = 110.9C_{NF} + 15.3C_{OZ}$ (1) and $A_2 = 43.7C_{NF} + 28.4C_{OZ}$ (2). Where A_1 and A_2 are absorbances of the sample at 273 nm and 318.5 nm respectively, 110.9 and 43.7 are absorptivities of NF at 273.0 and 318.5 nm, respectively, 15.3 and 28.7 are the absorptivities values of OZ at 273.0 nm and 318.5 nm respectively. C_{NF} is the concentration of NF and C_{OZ} is the concentration of the OZ. The mixture concentration was determined by using the equation (1) and (2).

Method B: Absorption Ratio Method

In the absorption ratio method, from the overlain spectra of both drugs (Fig.1), wavelengths 297.0 nm (iso-absorptive point) and 318.5 nm (λ_{max} of OZ) were selected for the analysis. The calibration curves for NF and OZ were plotted in the concentration range of 4-20 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ at both the wavelengths respectively. The absorptivities values were determined for both the drugs at both the wavelengths. From the following set of equations the concentration of each component in sample can be calculated $C_x = Q_m - Q_y / Q_x - Q_y \cdot A_1 / a$ (1) and $C_y = Q_m - Q_x / Q_y - Q_x \cdot A_1 / a$ (2). Where C_x = concentration of NF, C_y = concentration of OZ, A_1 = Absorbance of sample at iso-absorptive wavelength 297.0 nm, a = Mean absorptivity of NF and OZ at iso-absorptive wavelength 297.0 nm, Q_m = ratio of absorbance of sample solution at 318.5 nm and at 297.0 nm, Q_x = ratio of absorptivities of NF at 318.5 nm and at 297.0 nm and Q_y = ratio of absorptivities of OZ at 318.5 nm and at 297.0 nm.

Method C: First Order Derivative Spectroscopy

In first order derivative spectroscopy, solutions of NF and OZ (20 $\mu\text{g/ml}$, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative spectra was selected for analysis of both drugs. The zero crossing wavelengths 297.5 nm (zero cross for NF) and 264.0 nm (zero cross for OZ) were selected for the analysis. The calibration curves for NF and OZ were plotted in the concentration range of 4-20 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ at both the wavelengths respectively. The concentration of the individual drug present in the mixture was determined against the calibration curve in quantitation mode.

Application of the proposed method for the determination of NF and OZ in tablets

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 80 mg of NF was transferred to 100.0 ml volumetric flask and ultrasonicated for 10 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 5.0 ml was transferred to a 100.0 ml volumetric flask and appropriately diluted with 0.1 N NaOH to obtain 8 $\mu\text{g/ml}$ of NF and 10 $\mu\text{g/ml}$ of OZ. The concentration of both NF and OZ were determined by measuring the absorbance of the sample at 273.0 nm, 318.5 nm (Method-A) and at 297.0 nm, 318.5 nm (method B) in the spectrum mode and values were substituted in the respective formulae to

obtain concentrations. For Method-C concentration of both NF and OZ were determined by measuring the absorbance of the sample at 297.5 nm and 264.0 nm in first order spectrum mode. The results of the tablet analysis were calculated against the calibration curve in quantitation mode.

Results of tablet analysis are shown in Table No. 1.

Validation

The methods were validated with respect to linearity, accuracy, precision and selectivity.

Accuracy To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels 80%, 100% & 120%. Percent recovery for ERD, by all three methods, was found in the range of 98.27 % to 101.07 %.

Linearity

The linearity was obtained in the concentration range of 4-20 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ for Norfloxacin and Ornidazole, respectively in all three methods.

Precision: The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intraday assay precision) and on three different days (Interday precision). Result of intraday and interday precision is expressed in % RSD. Percent RSD for Intraday assay precision was found to be 0.0863 (for NF) and 0.3373 (for OZ) in simultaneous equation method, 0.1994 (for NF) and 0.2121 (for OZ) in absorbance ratio method and 0.7229 (for NF) and 0.9274 (for OZ) in first order derivative spectroscopy. Interday assay precision was found to be 0.1389 (for NF) and 0.5223 (for OZ) in simultaneous equation method; 0.3202 (for NF) and 0.2751 (for OZ) in absorbance ratio method. 0.5495 (for NF) and 0.7901 (for OZ) in first order derivative spectroscopy

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of NF and OZ. In simultaneous equation method, wavelengths selected for analysis were 273.0 nm (λ_{max} of NF) and 318.5 nm (λ_{max} of OZ). In Q-analysis method, wavelengths selected were 297.0 nm (iso-absorptive point) and 318.5 nm (λ_{max} of OZ). In first order derivative spectroscopy zero crossing wavelengths 297.5 nm (zero cross for NF) and 264.0 nm (zero cross for OZ) were selected for analysis. Linearity for detector response was observed in the concentration range of 4-20 $\mu\text{g/ml}$ (for NF) and 5-25 $\mu\text{g/ml}$ (for OZ). Percent label claim for NF and OZ in tablet analysis, by all the methods, was found in the range of 98.15 % to 101.03 %. Standard deviation and coefficient of variance for six determinations of tablet sample, by both the methods, was found to be less than ± 2.0 indicating the precision of both the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for NF and OZ, by all three methods, was found in the range of 98.27 % to 101.07 %, values of standard deviation and coefficient of variation were satisfactorily

low indicating the accuracy of both the methods. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can

be employed for routine quality control of Norfloxacin and Ornidazole in combined dose tablet formulation.

Table1: Analysis of Tablet Formulation

Method	Component	Label Claim (mg/tab)	Amount Found (mg/tab)	Estimated Label Claim* (%)	S.D.
A	NF	400	398.89	99.73	±0.5932
	OZ	500	496.60	99.32	±0.8720
B	NF	400	396.51	99.13	±0.6855
	OZ	500	498.89	99.78	±0.7358
C	NF	400	400.10	100.03	±0.7842
	OZ	500	498.62	99.68	±0.9286

* denotes average of six determinations, S.D.-Standard Deviation.

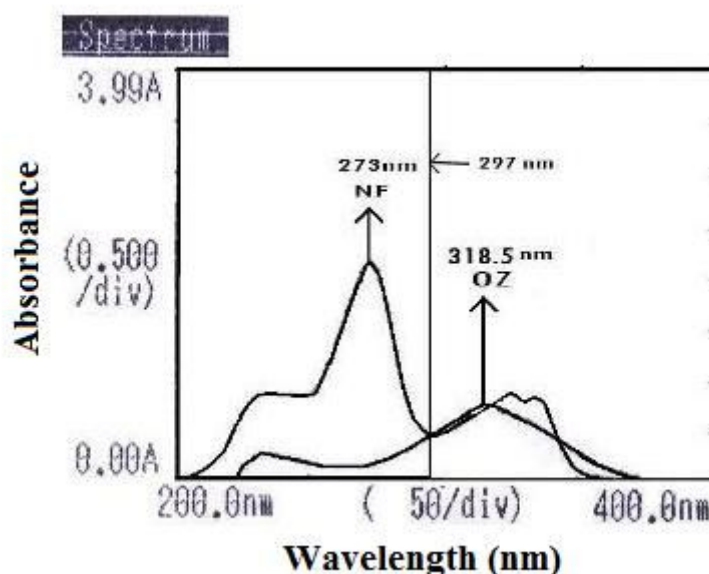


Fig.-1: Overlain Spectra of Norfloxacin (NF) and Ornidazole (OZ)

ACKNOWLEDGEMENTS

The authors are very thankful to Dr. Avinash D. Deshpande, Director of Pharmacy, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, Pimpri, Pune for providing necessary facilities. The authors also thankful to Emcure Pharmaceuticals Ltd., Pune and Aristo Pharmaceuticals Pvt. Ltd., Mumbai for providing gift samples of drugs NF and OZ.

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