Simultaneous Spectrophotometric Estimation of Norfloxacin and Ornidazole in Tablet Dosage Form

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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 0.1N 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 µg/ml and 5-25 µg/ml for norfloxacin and ornidazole, respectively. The results of the analysis have been validated statistically and by recovery studies.

Key words: Norfloxacin, ornidazole, simultaneous equation, Q-analysis, derivative spectroscopy

Norfloxacin (NF), chemically 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid[1], is a synthetic broad spectrum fluoroquinolone antibacterial agent used in the treatment of urinary and genital tract infection[2,3]. 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 infection[4,5]. NF is official in USP[1], BP[6] and IP[7] whereas OZ is not official in any pharmacopoeia. Both the drugs are marketed as combined dose tablet formulation in the ratio of NF:OZ 400:500 mg. Literature survey revealed that a number of methods have been reported for estimation of NF[8-11] and OZ[12-17] 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.

A double-beam Shimadzu UV/Vis spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy of ±0.5 nm and a pair of 1-cm matched quartz cells, was used to measure absorbance of the resulting solution. Standard gift sample of norfloxacin was provided by Emcure Pharmaceuticals Ltd., Pune and ornidazole by Aristo Pharmaceuticals Pvt. Ltd., Mumbai. Combined dose NF and OZ tablets (Norrit-Ord, 400 mg norfloxacin and 500 mg ornidazole; Ind-Swift Ltd., Chandigarh), were purchased from the local pharmacy. Sodium hydroxide, 0.1N, was prepared from analytical reagent grade sodium hydroxide in double distilled water and used as a solvent. Standard stock solutions of NF (100 µg/ml) and OZ (100 µg/ml) were prepared and used for the analysis.

For the selection of analytical wavelength for the simultaneous equation method (Method-A), solutions of NF and OZ (20 µg/ml, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 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 µg/ml and 5-25 µg/ml at both the wavelengths respectively. The absorbivity values were determined for both the drugs at both the wavelengths and following Eqs 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 of OZ at 273.0 nm and 318.5 nm, respectively. C<sub>NF</sub> is the concentration of NF and C<sub>OZ</sub> is the concentration of the OZ. The mixture concentration was determined by using the Eqns. 1 and 2.

In the absorption ratio method (Method-B), from the overlain spectra of both drugs (fig.1), wavelengths 297.0 nm (iso-absorptive point) and 318.5 nm (λ<sub>max</sub> of OZ) were selected for the analysis. The calibration curves for NF and OZ were plotted in the concentration range of 4-20 µg/ml and 5-25 µg/ml at both the wavelengths respectively. The absorptivity values were determined for both the drugs at both the wavelengths. From the following set of Eqns the concentration of each component in the sample can be calculated, C<sub>x</sub> = Q<sub>m</sub>–Q<sub>y</sub>/Q<sub>x</sub>–Q<sub>y</sub>×A<sub>a</sub>/a (1) and C<sub>y</sub> = Q<sub>m</sub>–Q<sub>x</sub>/Q<sub>y</sub>–Q<sub>x</sub>×A<sub>a</sub>/a (2), where C<sub>x</sub> is the concentration of NF, C<sub>y</sub> is the concentration of OZ, A<sub>a</sub> is the absorbance of sample at iso-absorptive wavelength 297.0 nm, a is the mean absorptivity of NF and OZ at iso-absorptive wavelength 297.0 nm, Q<sub>m</sub> is the ratio of absorbance of sample solution at 318.5 nm and at 297.0 nm, Q<sub>x</sub> is the ratio of absorptivities of NF at 318.5 nm and at 297.0 nm and Q<sub>y</sub> is the ratio of absorptivities of OZ at 318.5 nm and at 297.0 nm.

In first order derivative spectroscopy (Method-C) solutions of NF and OZ (20 µg/ml, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative spectrum 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 µg/ml and 5-25 µg/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.

For the estimation of drugs in the commercial formulations, 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 containing 40 ml of 0.1N NaOH and ultrasonicated for 10 min and diluted to the mark with 0.1N NaOH. The solution was then filtered through a Whatmann filter paper No. 41. From the filtrate 5.0 ml was transferred to a 50.0 ml volumetric flask and diluted to the mark with 0.1N NaOH to obtain 8 µg/ml of NF and 10 µg/ml of OZ. The concentration of both NF and OZ was determined by measuring the absorbance of the sample at 273.0 nm and 318.5 nm (Method-A) and at 297.0 nm and 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 was 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.

Recovery studies were carried out by standard addition method at three different levels 80%, 100% and 120%. The % recovery of NF and OZ in the sample mixture was determined. The results of tablet analysis and recovery studies obtained by proposed method were validated by statistical evaluation and are recorded in Tables 1 and 2.

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of NF and OZ. Percent label claim for NF and OZ in tablet, 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 in the range of ±0.1578 to ±0.7216 and 0.1603 to 0.7250, respectively indicating the accuracy of proposed methods. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible and economical and can be employed for routine quality control of norfloxacin and ornidazole in combined dose tablet formulation.

ACKNOWLEDGEMENTS

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

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The naturally occurring xanthone glycoside mangiferin has been isolated by column chromatography from the ethanol extract of stem bark of *Mangifera indica*. Mangiferin was further converted to 5-(N-phenylaminomethyleno) mangiferin, 5-(N-p-chlorophenylaminomethyleno) mangiferin, 5-(N-2-methylphenylaminomethyleno) mangiferin, 5-(N-p-methoxyphenylaminomethyleno) mangiferin, 5-(N,N-diphenylaminomethyleno) mangiferin, 5-(N-α-napthylaminomethyleno) mangiferin and 5-(N-4-methylphenylaminomethyleno) mangiferin. Mangiferin and its analogues were characterized by melting point and Rf value determination and through spectral technique like UV, IR, and NMR spectral analysis. The synthesized compounds were screened for antimicrobial activity.

**Key words:** Antifungal, antimicrobial, *Mangifera indica*, mangiferin