Development and validation of a method for simultaneous estimation of ofloxacin and ornidazole in different dissolution media

**Introduction:** Ofloxacin and ornidazole in a combined tablet dosage form is available in the market. This combination has gained increasing acceptance in diarrhea caused due to bacterial and protozoal infections. Ofloxacin and ornidazole are also combined in the capsule dosage form to modify its release pattern in different studies. Spectrophotometric and HPTLC methods have been reported for their simultaneous estimation in the tablet dosage form in specific solvents. This paper presents a simple, accurate, and reproducible spectrophotometric method for simultaneous estimation of ofloxacin and ornidazole in the tablet dosage form in different dissolution media. The reported method is helpful in determination of ofloxacin and ornidazole during a dissolution study. **Materials and Methods:** A simple, sensitive, accurate, and economical spectrophotometric method based on the simultaneous equation was developed for the estimation of ornidazole and ofloxacin simultaneously in the tablet or capsule dosage form in different dissolution media at different pH values. **Results:** Ofloxacin showed absorption maxima at 294 nm in 0.1 N HCl and at 287 nm in phosphate buffer pH 6.8 and phosphate buffer pH 7.4 while ornidazole showed absorption maxima at 277 nm in 0.1 N HCl and at 319 nm in two buffers, respectively. The linearity was obtained in the concentration range of 1–8 μg/ml for ofloxacin and 4–26 μg/ml for ornidazole. **Discussion:** The concentrations of the drugs were determined by the simultaneous equation method. The results of analysis have been validated statistically and by recovery studies. **Key words:** Dissolution media, ornidazole, ofloxacin, validation

**INTRODUCTION**

been reported for their simultaneous estimation in the tablet dosage form. This paper presents a simple, accurate, and reproducible spectrophotometric method for simultaneous determination of ofloxacin and ornidazole in a tablet dosage form in different dissolution media. The reported method is helpful in determination of ofloxacin and ornidazole during the dissolution study.

MATERIALS AND METHODS

Materials
Ofloxacin was received as a gift sample from Racspeed Pharma, Ahmedabad, India. Ornidazole was purchased from Yarrow Chem Product, Mumbai, India. The tablets (referred as T1 and T2) of the said combination were purchased from a local pharmacy (The label claim for both T1 and T2 was to contain 200 mg of ofloxacin and 500 mg of ornidazole). All the chemicals used were of either pharmaceutical or analytical grade.

Instrument
All the absorbance measurements were made on a double-beam UV visible spectrophotometer (Shimadzu, Kyoto, Japan, model UV – 1800) with matched quartz cuvettes.

Methods

Preparation of standard drug solution
The standard stock solutions of ofloxacin and ornidazole were separately prepared by dissolving 100 mg of ofloxacin or ornidazole in 100 ml of three different dissolution media. A stock solution of ofloxacin and ornidazole were further diluted with respective media to get a standard solution of concentration 100 μg/ml.

Study of Beer–Lambert’s law
The standard solution of ofloxacin (5 μg/ml) and ornidazole (12 μg/ml) in three different dissolution media namely 0.1 N HCl, phosphate buffer pH 6.8, and phosphate buffer pH 7.4 were prepared and scanned in the entire UV range to determine the λ_max of both the drugs. The λ_max of ofloxacin was found to be 294 nm in 0.1 N HCl and 287 nm in both the phosphate buffers. The λ_max of ornidazole was found to be 277 nm in 0.1 N HCl and 319 nm in both the phosphate buffers. A series of standard solution were prepared in different dissolution media in the concentration range of 1–40 μg/ml using a working standard solution. The absorbance of those standard solutions was taken at λ_max in respective media, and calibration curves were plotted at these wavelengths. The linearity observed was in the concentration range of 1–8 μg/ml and 4–25 μg/ml for ofloxacin and ornidazole, respectively [Figures 1 and 2].

Determination of E (1%, 1 cm) value at selected wavelength
The E (1%, 1 cm) value of ofloxacin and ornidazole was calculated at λ_max in the respective media. The simultaneous equations were formed using calculated absorptivity values for each media. The simultaneous equations for 0.1 N HCl (Eqs. 1 and 2), phosphate buffer pH 6.8 (Eqs. 3 and 4), and phosphate buffer pH 7.4 (Eqs. 5 and 6) are given below.

\[ C_X = 1.34 \times 10^{-3} A_1 - 9.2 \times 10^{-4} A_2 \]  
\[ C_Y = 5.53 \times 10^{-3} A_2 - 2.99 \times 10^{-3} A_1 \]  
\[ C_X = 3.28 \times 10^{-3} A_2 - 1.277 \times 10^{-3} A_1 \]  
\[ C_Y = 1.597 \times 10^{-3} A_1 - 9.32 \times 10^{-4} A_2 \]  
\[ C_X = 3.418 \times 10^{-3} A_2 - 1.585 \times 10^{-3} A_1 \]  

where \( A_1 \) and \( A_2 \) are the absorbance of samples at 294 and 277 nm in 0.1 N HCl, respectively. \( C_X \) and \( C_Y \) are the concentration of ofloxacin and ornidazole, respectively.

Assay of the standard laboratory mixture
The laboratory mixtures of different concentration of both the drugs were prepared from stock solution in their respective media. Their absorbance value at the two selected wavelength was recorded, and quantitative estimation of the drugs was carried out by solving simultaneous equations. The results of the recovery study of the physical mixtures are shown in Table 1.

Assay of tablet formulation
Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 50 mg of ofloxacin was taken in a 50 ml volumetric flask and dissolved in 25 ml of 0.1 N HCl; it was further diluted up to the mark with same solvent. The solution was filtered, and a filtrate was diluted to get 5 μg/ml concentration of ofloxacin. The solution was then read at two selected wavelengths. The concentration of the drug was determined by solving simultaneous equations. The results of the assay are shown in Table 2.
**In vitro dissolution studies**

The *in vitro* drug release rate method of a combined tablet is not official. It was carried out using USP dissolution test apparatus 2 (paddle method). The dissolution test was performed in 900 ml of 0.1 N HCl maintained at 37 ± 0.5 °C for 2 hour at a paddle speed of 50 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus hourly, and the samples were replaced with fresh dissolution medium. The samples were filtered through a 0.45 µm membrane filter and diluted to a suitable concentration with respective media. Absorbance of these solutions was measured at $\lambda_{max}$ of both the drugs in that media using a double-beam UV visible spectrophotometer [Figure 3]. The dissolution medium was replaced with phosphate buffer pH 6.8 after 2 h and the procedure was repeated for 3 h again and lastly dissolution was carried out in phosphate buffer pH 7.4 [Table 3].

**Validation of the analytical method**

To study the accuracy and precision of the proposed method, recovery studies were carried out by addition of a known amount of standard drug solutions of ofloxacin and ornidazole to pre-analyzed tablet solution. The resulting solutions were then analyzed by a proposed method. Results of recovery studies were found to be satisfactory.

**RESULTS AND DISCUSSION**

The proposed method was found to be simple, accurate, and reproducible for routine simultaneous estimation of ofloxacin and ornidazole in different dissolution media. As the $\lambda_{max}$ of these two drugs differ more than 20 nm, the simultaneous equation...
method was tried for their simultaneous estimation in formulation in different media. The standard deviation, percentage recovery indicates the precision and accuracy of the method. Since no any method is available for determining ornidazole and ofloxacin in a combined dosage form during a dissolution study, this method is very useful for those who want to study the release pattern of the combination of both the drugs.

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REFERENCES


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