Development and validation of analytical methods for the simultaneous estimation of Nimorazole and Ofloxacin in tablet dosage form

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A b s t r a c t

Two simple, rapid, accurate and precise spectrophotometric methods have been developed for simultaneous estimation of Nimorazole and Ofloxacin from tablet dosage form. Method I involves formation of 'simultaneous equations' at 304 nm (λ max of Nimorazole) and 287.5 nm (λ max of Ofloxacin); while Method II involves, formation of 'Absorbance ratio equation' at 301 (isoabsorptive point) and 287.5 nm (λ max of Ofloxacin) using distilled water as a solvent. The linearity was observed in the concentration range of 5- 25 µg/ml for Nimorazole and 2-10 µg/ml for Ofloxacin. The results of analysis have been validated statistically and by recovery studies and were found satisfactory.

Kewords: Ofloxacin, Nimorazole, Simultaneous equation method, Absorbance ratio method.

Introduction

Ofloxacin (OFX) is a fluoroquinolone derivative with potent activity against a broad spectrum of bacteria. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid[1] (Figure 1). It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases [1].
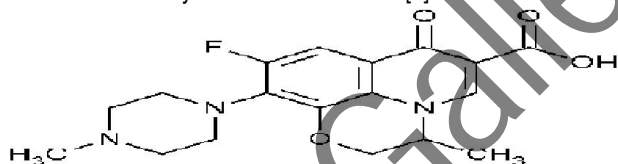

Figure.1 Structure of OFX

Nimorazole (NIM) is a 5-nitroimidazole, which is closely related to Metronidazole in structure and activity. Nimorazole is used as a hypoxic sensitizer concomitantly with radiotherapy for head and neck cancers and could from the similarities with Metronidazole theoretically lead to increased effect of anticoagulant therapy. Nimorazole chemically known as 4-[2-(5-nitro-1*H*-imidazole-1-yl)ethyl] morpholine 2 (Figure. 2) [2].

Figure 2 structure of NIM

Literature survey assured that very few analytical methods such as HPLC [3-6], UV [7-12] were reported for the estimation of OFX and NIM either individually or combined with other drugs. only two methods are reported for estimation of Ofloxacin and Nimorazole in combination by RP-HPLC [13] and Vierordt's [14] method So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous estimation of Ofloxacin and Nimorazole from tablet dosage forms using Vierordt's method and Absorbance ratio method.

Material and Methods

Instrumentation

Shimadzu 1601 UV-VIS double beam spectrophotometer with UV probe software was used. Absorbance Absorbance of measurements were recorded with a pair of 1cm matched quartz cells.

Chemicals and Reagents

Working standards of NIM (99.68 %) and OFX (99.35%) were kindly supplied by Lupin Pvt. Ltd. (Jammu and Kashmir) and Zim Laboratory Ltd. (Nagpur). distilled water was used throughout the experiment. The pharmaceutical formulation used in this study was NIMORAZ O tablets (Lupin Ltd, Mumbai, India) procured from the local market and labelled to contain 200mg OFX and 500mg NIM per tablet.

Preparation of standard stock solution

10 mg of OFX and 25 mg NIM were weighed accurately and transferred in to a separate 50 volumetric flasks and sufficient water was added to dissolve the drug and then sonicated for 10

minutes. Finally the volume was adjusted up to the mark with water.5ml was pip petted out from each stock solution in to a 50ml volumetric flask and the volume was adjusted to 50mL with water, and again pipetted out 5ml from OFX stock solution and 2ml from NIM stock solution in 10 volumetric flasks so as to get the final concentration of 10 µg/mL of OFX and 10 µg/mL for NIM.

Methods

Method I: Simultaneous Equation Method

In simultaneous equation method,   when no region can be found free from overlapping spectra of two chromophore
it is still possible to device a method based on measurements at two wavelengths.
Two dissimilar chromophores must necessary have different powers of light absorption at some point or in linear absorption spectra.
If samples contain two absorbing drugs (X and Y), each of which absorbs at the λmax of the other, it may be possible to determine both drugs by the technique of simultaneous equations
From overlain spectra (Figure.1) 287.5nm λmax for Ofloxacin and 301 nm λmax for Nimorazole were selected for formation of simultaneous equation of two drugs.

$$Cx = \frac{A2\,ay1 - A1\,ay2}{ax2\,ay1 - ax1\,ay2}$$

$$Cy = \frac{A1\,ax2 - A2\,ax1}{ax2\,ay1 - ax1\,ay2}$$

Where, A1 and A2 were absorbance of sample at 287.5 nm and 301 nm respectively, ax1 and ax2 are absorptivity of Ofloxacin at 287.5 nm and 301nm, ay1 and ay2 are absorptivity of Nimorazole at 287.5 nm and 30 nm.
Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components. Results of which are reported in Table.1.

Method II: Absorbance Ratio Method

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths is a constant value independent of concentration or Path length.
In the quantitative assay of two components in admixture by the absorbance by the absorbance ratio method, absorbance's are measured at two wavelengths one being the λmax of one of the components (λ2) and the other being a wavelength of equal

absorptivity of the two components (λ1), that is , an isoabsorptive point.
From overlain spectra (Figure 1.) 301 nm (Isobestic point) and 287.5 nm λmax for Ofloxacin were selected for formation of Absorbance ratio equation of two drugs.
The absorbance at 301 nm and 287.5 nm for NIM and   OFL were measured. The absorptivity values of each drug at both wavelengths were determined. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$Cx = \frac{Qm - Qy}{Qx - Qy} * \frac{A1}{ax1} \quad \text{——— III}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} * \frac{A1}{ay1} \quad \text{——— IV}$$

QM, QX, and QY were obtained as below:
QM = A2/A1, QX = ax2/ax1, QY = ay2/ay1
Where, A1 and A2 were absorbance of sample at 301 nm and 287.5 nm respectively, ax1 and ax2 are absorptivity of Ofloxacin at 301 nm and 287.5 nm, ay1 and ay2 are absorptivity of Nimorazole at 301 nm and 287.5 nm.
Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components. Results of which are reported in Table.1

Assay of tablet formulation

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed sample equivalent to 200 mg of OFX and 500mg of NIM was taken in a stoppered volumetric flask (100.0ml); 40ml of water was added and sonicated for 20 min. The solution was filtered through Whatmann filter paper (No 41) and the volume was made up to the mark with the same solvent. The aliquot portions of above solutions were further diluted with solvent to get final concentration of about 200 mcg/ml of OFX and 500 mcg/ml of NIM, respectively and absorbances were measured at 287.5 nm, 304 nm and 301.0 nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2 and equation 3 and 4**.** The results are reported in the Table 2.

Recovery studies

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100%, and 120%) within range of linearity for both the drugs. Results are reported in table 3.

Results and Conclusion
Method I: Simultaneous Equation Method

UV-spectrophotometric method using simultaneous equation was developed. OFX showed absorbance maxima at 287.5 nm and NIM at 304.0 nm. Linearity was observed in the concentration rage of 2 - 10 µg/ml for OFX and 5 -25 µg/ml for NIM correlation coefficient was found to be 0.9996 and 0.9998 at 287.5 nm and 304 nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for OFX and NIM was found to be 99.50 and 101.50, respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis.

Method II: Absorbance Ratio Method

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 301 nm (isoabsorptive point) and 287.5 nm (λ max of Ofloxacin) Linearity was observed in the concentration range of 2 - 10 µg/ml and 5 - 25 µg/ml correlation coefficient was found to be 0.999 and 0.998 respectively The proposed method was applied for pharmaceutical formulation; % label claim for OFX and NIM was found to be 99.26 and 101.27, respectively. The low C.V. value indicates method is accurate and precise.

Conflict of Interest

The authors declare no conflict of interest.

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