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Research Article

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RP-HPLC method for simultaneous estimation of Ofloxacin and Nitazoxanide in tablet formulation

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ABSTRACT

A simple, precise, accurate, rapid and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for simultaneous estimation of Ofloxacin and Nitazoxanide (NTZ) on RP C-18 column (Shimadzu liquid chromatograph LC-10ATVP) using Tetrabutyl ammonium hydrogen sulphate: methanol: acetonitrile (20:20:60) as mobile phase at a flow rate of 0.5ml/min and the detection wave length was 280nm. The retention time for OFX and NTZ was found to be 4.67 and 7.79 min respectively. The recovery was greater than 98% with RSD less than 1.00. The proposed method was validated for precision, accuracy, linearity, range, robustness and ruggedness.

Keywords: Ofloxacin, Nitazoxanide, Reverse phase High Performance liquid chromatography, Method validation

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INTRODUCTION

Ofloxacin is chemically (±) 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. It is a synthetic fluoroquinolone derivative. They inhibit the enzyme bacterial DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. In gram positive bacteria the major target of fluoroquinolone action is a similar enzyme topoisomerase IV which nicks and separates daughter DNA strands after DNA replication [1]. Nitazoxanide (NTX) is chemically [2-[(5-nitro-1, 3-thiazol-2-yl) carbamoyl] phenyl] ethanoate. It is a prodrug which on absorption is converted to the active form tizoxanide, an inhibitor of PFOR enzyme. It is used in the treatment of giardiasis, cryptosporidiosis, and amoebic dysentery as luminal amoebicide. It is a salicylamide congener of the anthelmintic niclosamide used for the treatment of giardiasis active against E. Histoltica, T. Vaginalis, cryptosporidium and some other protozoa and helminthes [1]. Literature survey reveals that spectrophotometric[2-13], potentiometry[14] ,Liquid chromatography[15], phosphorometry[16], capillary electrophoresis[17], HPLC[19-23] and HPTLC[24] methods are available for determination of Ofloxacin and Nitazoxanide from pharmaceutical preparations. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Ofloxacin and Nitazoxanide in tablet dosage form. This work is concerned with application of simple, precise, accurate and reproducible method for the simultaneous estimation of Ofloxacin and Nitazoxanide from combined dosage form.

EXPERIMENTAL SECTION

2.1 Chemicals and Reagents

OFX and NTZ were obtained as gift samples from Discovery mankind pharmaceuticals, New Delhi. Tetrabutyl ammonium hydrogen sulphate, methanol, acetonitrile were of reagent grade. The pharmaceutical preparation of combination of Ofloxacin and Nitazoxanide is Zenflox tablet (Discovery mankind pharmaceuticals) commercial formulation of OFX and NTZ is available in ratio of 200mg and 500mg as tablet.

2.2 Instrumentation

Shimadzu liquid chromatograph LC-10 ATVP and RP-C18 column was used. A Rheodyne injector with a 20ml loop was used for the injection of sample.

2.3 Chromatographic condition

The mobile phase containing Tetrabutyl ammonium hydrogen sulphate: methanol: Acetonitrile (20:20:60) was found to resolve OFX and NTZ. The mobile phase was filtered on a 0.45 micron membrane filter. The flow rate was set to 0.5ml/min, both drugs showed good absorbance at 280nm which was selected as wavelength for further analysis.

2.4 Calibration curve

Calibration curves were prepared by taking appropriate aliquots of standard OFX and NTZ stock solutions in different 50ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentration of 5,10,15,20,25,30 µg/ml of OFX and 5,10,15,20,25,30 µg/ml of NTZ. Standard solutions were injected through 20µl loop system and chromatograms were obtained using 0.5ml/min flow rate. The effluent was monitored at 280nm calibration curve was constructed by plotting average peak area against concentration.

2.5 Validation of the method

The developed method was validated in terms of specificity, linearity, limit of quantifications, limit of detection, accuracy, precision, Robustness, Ruggedness and system suitability studies.

2.6 Sample preparation

A total of 20 tablets were accurately weighed and triturated with glass motar and pestle. An amount equivalent to one tablet (containing 200mg OFX and 500mg NTZ) was transferred to a 100ml dried volumetric flask (Table 1).

Table 1. Result of OFX and NTZ in marketed formulation

MARKETED FORMULATION	DRUG	% AMOUNT FOUND	% RSD
Zenflox (Discovery mankind)	OFX	100.20 ± 0.33	0.33
	NTZ	99.94 ± 0.70	0.74

RSD:Relative standard deviation

The compounds were first dissolved in 20ml of mobile phase and it was sonicated for 10min then the volume was adjusted to 100ml with mobile phase from the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get a concentration of 200µg/ml of Ofloxacin and 500µg/ml of Nitazoxanide.

The volume was made upto mark and the solution was filtered through 0.2 micron nylon membrane filter 20µl of the resulting solution was injected (Figure 3).

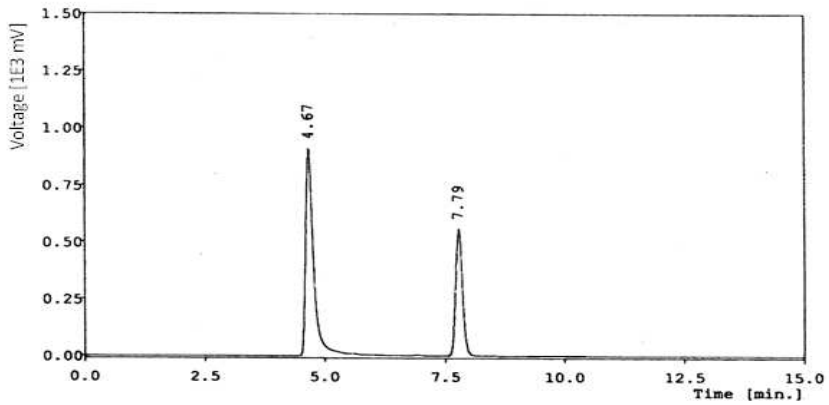

Fig. 3. Cromatogram of Ofloxacin and Nitazoxanide by HPLC

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable RP-HPLC method for the simultaneous estimation of OFX and NTZ different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination.

3.1 Method validation

The proposed HPLC method was validated as per ICH guidelines[25].

3.2 Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix. The peak purity of OFX and NTZ were assessed by comparing the retention time (RT) of standard OFX and NTZ. Good correlation was obtained between the retention time of standard and sample of OFX and NTZ.

3.3 Linearity

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity range of OFX and NTZ were found to be 160.8-242.5µg/ml and 400-600µg/ml respectively. The coefficient of correlation (r) is 0.9981 and 0.9990 respectively (Figure 1 and 2).

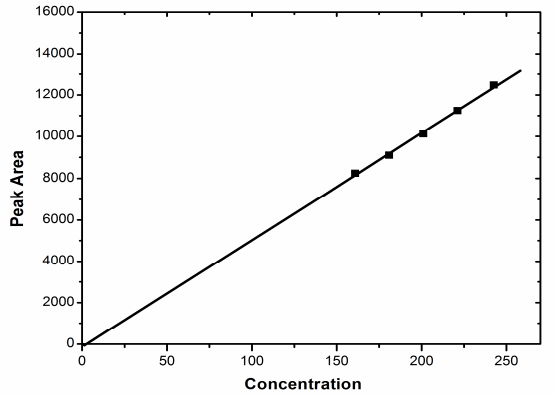

Fig. 1. Linearity of Range of Ofloxacin

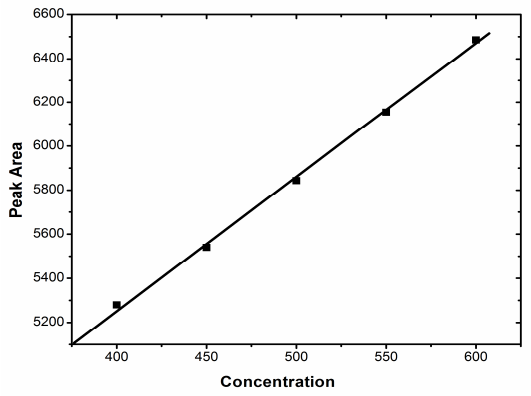

Fig. 2. Linearity of Range of Nitazoxanide

3.4 Accuracy (Recovery studies)

The accuracy of an analytical method is the closeness of the results obtained by that method to the results obtained by that method to the true value. Accuracy of the method, (recovery studies were performed in triplicate by standard

addition method at 80%, 100% and 120%. The percentage recovery should be within 98-102%. Results of recovery studies are shown in Table 2.

Table 2. System suitability parameters

PARAMETER		OFX	NTZ
Linearity range(µg/ml)		160.8-242.5	400-600
Correlation coefficient		0.9981	0.999
Slope		52.068	6053.52
Retention time(min)		4.67	7.79
Resolution factor		6.2	6.2
Tailing factor		1	1
L.O.D(µg/ml)		2.04	0.000431
L.O.Q(µg/ml)		6.183	0.0001306
Accuracy	80%	99.88±0.45	99.74±0.68
	100%	100.55±0.37	100.06±0.41
	120%	100.94±0.27	99.71±0.75

3.5 Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sample of a homogenous sample. Percentage relative standard deviation (%RSD) was found to be less than 2%.

3.6 Limit of detection (LOD)

It is the lowest amount of analyte in sample that can be detected but not necessarily quantities as an exact value under the sated, experimental conditions. The detection limits is usually expressed as the concentration of analyte. It is given by

$$LOD = \frac{3.3X\sigma}{m}$$

σ – standard derivation of the response

m- slope of the calibration curve

3.7 Limit of Quantitation (LOQ)

The quantitation limit of an analytical procedure is the lowest amount of analyte which can be quantitatively determined with suitable precision and accuracy.

$$LOQ = \frac{10X\sigma}{m}$$

σ – Standard derivation of the response

m- Slope of the calibration curve

3.8 Ruggedness

The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition such as different laboratories different analyst, different instruments, different lots of reagents, different elapsed assay time different assay temperature, different days. The standard and sample solutions were prepared by different analysts on different days and the resulting solution were injected and chromatograms are recorded.

3.9 Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by analysis of aliquots from homogeneous lots by differing physical parameters that may differ but were still within the

specified parameter of the assay for example change in physical parameters like flow rate and lambda max. The results are shown in Table.3.

Table 3. Ruggedness and Robustness

PARAMETERS		OFX	NTZ
Intraday		100.14	99.48
Inter day		99.06	98.92
Flow rate	0.4	101.53%	100.74%
	0.6	101.62%	99.05%
Lambda max	278nm	101.79	101.15
	282nm	101.68	99.04

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of OFX and NTZ in bulk as well as in pharmaceutical preparations statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than one. The developed method can be used for routine quantitative simultaneous estimation of pharmaceutical preparation.

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