ORIGINAL RESEARCH

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NITAZOXANIDE AND OFLOXACIN FROM BULK AND TABLET DOSAGE FORM.

Santosh V. Gandhi*[1], Parag L. Khairnar[1], Atul P. Chaudhari[2]

[1] AISSMS College of Pharmacy, Kennedy Road, Near R. T. O., Pune - 411001, Maharashtra, India

[2] Smt. S. S. Patil Institute of Technology (Pharmacy), Chopda- 425107, Dist. Jalgaon, Maharashtra, India

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ABSTRACT:

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Nitazoxanide and Ofloxacin in tablet dosage form. The separation was carried out using a mobile phase containing acetonitrile, methanol and 0.4 M citric acid, (60:30:10, v/v/v). The column used was Thermo C_{18} (4.6mm*250 mm, 5 µ) with flow rate of 1 mL / min using UV detection at 300 nm. The retention time of Nitazoxanide and ofloxacin was 3.071 min and 12.47 min, respectively. The described method was linear over a concentration range of 5-30 µg/mL (r^2>0.999) for Nitazoxanide and 2-12 µg/mL (r^2>0.998) for Ofloxacin. The mean % recovery was found to be 99.96 % for Nitazoxanide and 101.05 % for Ofloxacin. The limit of detection (LOD) for Nitazoxanide and Ofloxacin were found to be 0.3788 and 0.0929 µg/mL, respectively. Whereas the limit of quantification (LOQ) for Nitazoxanide and Ofloxacin was 1.1479 µg/mL and 0.2816 µg/mL, respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of Nitazoxanide and Ofloxacin bulk drug and in its tablet dosage form.

KEYWORDS: Nitazoxanide; Ofloxacin; RP-HPLC; Method Validation.

Corresponding Author: Santosh V. Gandhi
E-mail: santoshvgandhi@rediffmail.com
Phone: +91-9689932181

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INTRODUCTION:

Nitazoxanide [NTZ] chemically N-(5-nitro-2-thiazolyl) salicylamide acetate [Fig.1(a)] is a synthetic nitrothiazole benzamide derivative. It is a broad spectrum antiprotozoal. It is indicated for amoebiasis, helminthiasis, giardiasis etc.[1]

Ofloxacin [OFX] is 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7 H-pyrido [1,2,3-de] [1,4] benzoxazine-6-carboxylic acid [Fig.1(b)]. It is a synthetic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone. It is used to treat certain infections including bronchitis, pneumonia and infections of the skin, bladder, urinary tract, reproductive organs, and prostate[2].

Few methods are reported for quantitative determination of NTZ and OFX in single and in combination such as UV Spectrometry[3-5] and RP-HPLC[6-9]. Although there are reported HPLC methods; the linear range reported are higher with high value for LOD and LOQ. Thus reported methods are less sensitive. Therefore, it was thought worthwhile to develop simple, precise, accurate and sensitive reverse phase HPLC method for simultaneous estimation of NTZ and OFX in tablet dosage form.

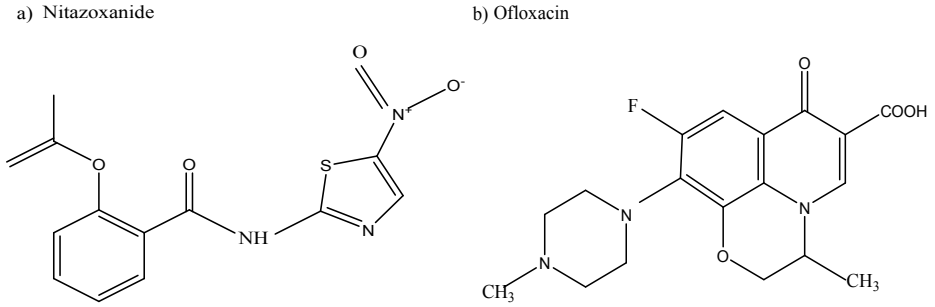

Figure 1: Structure of a) Nitazoxanide (NTZ) and b) Ofloxacin (OFX)

MATERIAL AND METHODS

Instrumentation

The RP-HPLC was carried on JASCO HPLC system equipped with PU 2080 Plus pump and PU 2010 Plus UV detector. Samples were injected through Rheodyne sample injection port (50 µl). HiQSil C18 Column (250 x 4.5mm, i.d. 5 µm) was used. Data acquisition and integration was performed using Borwin software (version 1.5). In addition, electronic weighing balance (Schimadzu AY120), Ultrasonicator (PRAMA SM15VS) was used.

Material and Reagent

Pure drug samples (API) of Nitazoxanide and Ofloxacin were obtained from Wockhardt Research Centre, Aurangabad as gift samples. The drug samples were used without further purification. HPLC grade water was obtained from ELGA LAB WATER purification system (PURELAB UHQ-11, United Kingdom). Methanol used for HPLC was of HPLC grade (LOBA Chemie, Mumbai, MH, India). NIZONIDE-O tablets manufactured by Lupin Pvt. Ltd. containing Nitazoxanide 500 mg and Ofloxacin IP 200 mg were procured from local pharmacy shop.

Chromatographic Conditions

The mobile phase was prepared by mixing acetonitrile, methanol and 0.4 M citric acid in ratio (60:30:10 % v/v/v) and filtered through 0.45 µm membrane filter and sonicated for 10 min for degassing. The flow rate was 1 ml/min. Quantitation based on peak area was achieved using UV detector at 300 nm. All determinations were performed at ambient temperature. The representative chromatogram is shown in Fig.2.

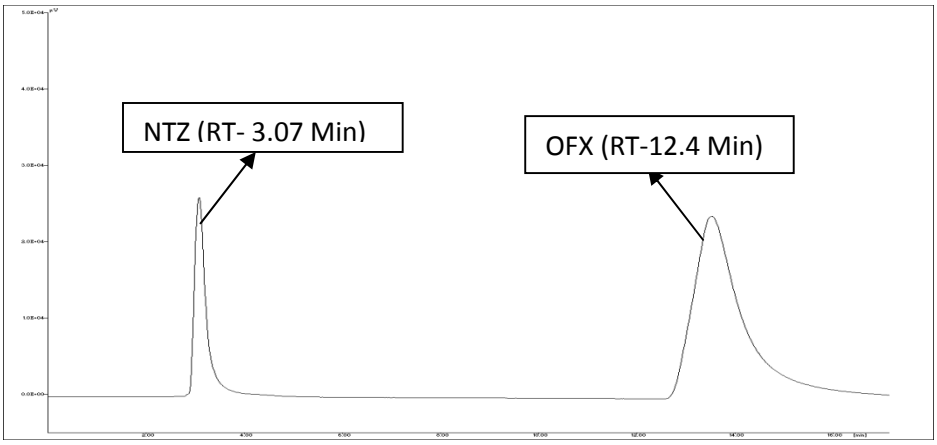

Fig 2: Chromatogram of standard Mixture NTZ & OFX (10 µg/ml of each)

Standard stock solutions

Stock solution of NTZ and OFX were prepared by dissolving accurately weighed 10 mg of drug samples in 10 ml of HPLC grade methanol, separately (1000 µg/ml). From above solution further 5 ml was pipetted and diluted to 50 ml to produce 100 µg/ml of NTZ and OFX, each.

Working solutions

Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with mobile phase, to obtain final concentration of 5.0-30.0 µg/ml of NTZ and 2.0-12.0 µg/ml of OFZ for HPLC.

Calibration curves for the HPLC method

From the standard stock solutions, standard solutions of different concentration ranging from 5.0-30.0 µg/ml and 2.0-12.0 µg/ml for NTZ and OFX, respectively were prepared by diluting with mobile phase. 50 µl of each standard solution was injected and chromatograms were recorded. The retention time of NTZ and OFX were 3.071 min and 12.47 min respectively. Calibration Curves were constructed by plotting average peak areas against respective concentrations. Calibration curve for both drugs are shown in Fig. 3 and Fig 4, respectively.

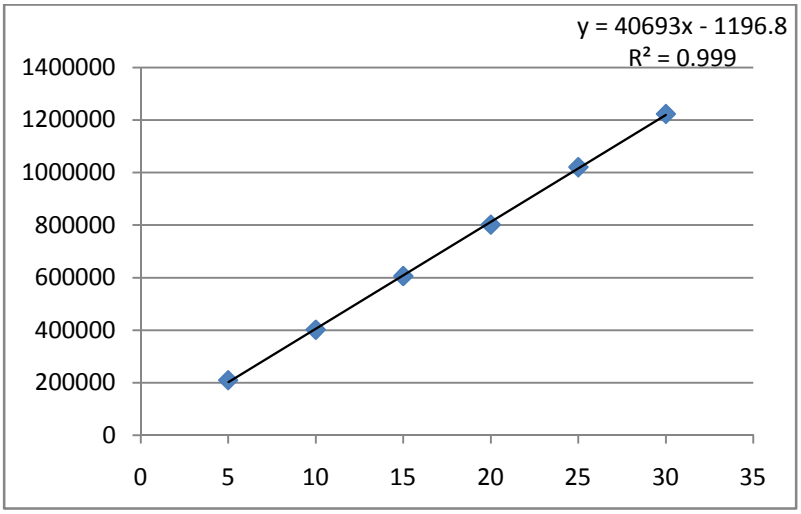

Fig.3: Calibration Curve of NTZ

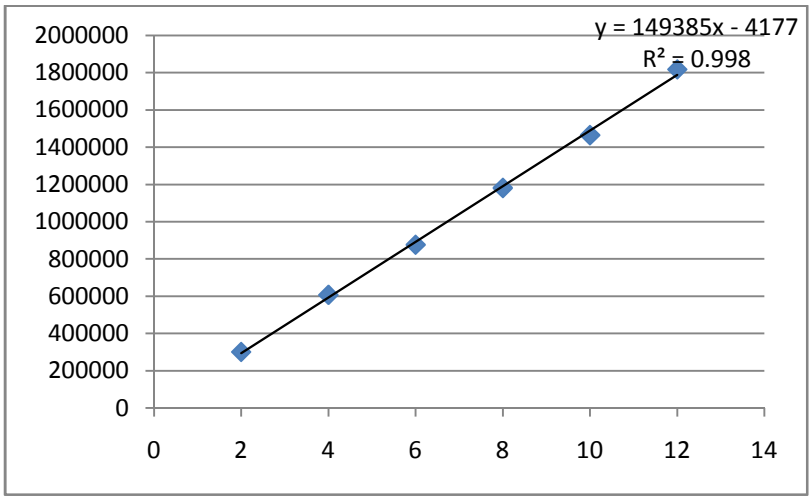

Fig.4: Calibration Curve of OFX

Analysis of drugs in marketed formulation

Twenty Tablets, each containing 200 mg of Ofloxacin and 500 mg Nitazoxanide were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Nitazoxanide (20 mg of Ofloxacin) was weighed and transferred to 50 ml volumetric flask. To this, HPLC grade methanol was added and sonicated for 10 min; the volume was made up to 50 ml with HPLC grade methanol to get solution of 1000 µg/ml. The solution was filtered using whatmann filter paper. From the filtrate appropriate dilutions were made with mobile phase to obtain final assay solution having concentration 10 µg/ml of NTZ (4 µg/ml of OFX). The procedure was repeated for six times and percentage was determined from linear equation.

VALIDATION OF PROPOSED HPLC METHOD[10]

For validation of the developed method, the ICH Q2 (R1) guidelines were followed. The requirement for drug assay follows these topics: linearity, precision, accuracy, specificity, robustness, LOD and LOQ.

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.996, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

Linearity study of HPLC detector response for determination of NTZ and OFX was evaluated by analyzing a series of standard solutions of six different concentrations of each compound. Calibration curves constructed were linear over the concentration range of 5.0-30.0 µg/ml and 2.0-12.0 µg/ml for NTZ and OFX respectively. Regression analysis has been carried out with coefficient of determination (r^2) 0.999 and 0.998 for NTZ and OFX, respectively.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1 and Table 2, respectively.

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y

intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD was found to be 0.3788 µg/ml and 0.0929 µg/ml of NTZ and OFX respectively, LOQ was found to be 1.1479 µg/ml and 0.2816 µg/ml of NTZ and OFX respectively.

Table 1: Intra-day Precision of Nitazoxanide and Ofloxacin

Intra-day Precision					
Nitazoxanide			**Ofloxacin**		
Conc.(µg/ml)	Avg. area	% RSD[a]	Conc.(µg/ml)	Avg. area	% RSD[a]
10	398469.4	0.773	4	604148.9	1.596
15	604895.3	0.637	6	872597.8	0.927
20	797256.9	1.151	8	1178385	1.118

[a]RSD: Relative Standard Deviation

Table 2: Inter-day Precision of Nitazoxanide and Ofloxacin

Inter-day Precision					
Nitazoxanide			**Ofloxacin**		
Conc.(µg/ml)	Avg. area	% RSD[a]	Conc.(µg/ml)	Avg. area	% RSD[a]
10	405755.8	1.212	4	610480.2	0.547
15	607658.4	1.518	6	881051.8	1.389
20	807449.7	0.699	8	1184643	0.613

[a]RSD: Relative Standard Deviation

Table 3: Assay of Marketed Formulation

Sl. No.	**Amount Present (µg/ml)**		**Amount Found (µg/ml)**		**% Assay**	
	NTZ	OFX	NTZ	OFX	NTZ	OFX
1	10	4	9.941	4.053	99.410	101.321
2	10	4	10.012	4.059	100.120	101.473
3	10	4	10.123	3.991	101.230	99.780
4	10	4	9.892	4.056	98.922	101.401
5	10	4	10.128	4.037	101.281	100.932
6	10	4	9.879	4.057	98.790	101.421
Mean			9.996	4.042	9.996	4.042
SD[b]			0.110	0.026	0.110	0.026
% RSD[a]			1.106	0.648	1.106	0.648

[a]RSD: Relative Standard Deviation, [b]SD: Standard Deviation

Table 4: Accuracy of NTZ and OFX

Level of % Recovery	Mean (% Recovery)		± SD[a]		% RSD[a]	
	NTZ	OFX	NTZ	OFX	NTZ	OFX
50	102.471	99.902	1.490	0.832	1.454	0.832
100	101.552	100.564	0.109	1.071	0.106	1.065
150	101.410	100.394	1.003	0.631	0.970	0.628

[a]RSD: Relative Standard Deviation, [b]SD: Standard Deviation

Table 5: Summary of Validation Parameters

Sl. No.	Validation Parameter	NTZ	OFX
1.	Regression Equation	y = 40693x – 1196.8 r^2 = 0.999	y = 149385x - 4177 r^2 = 0.998
2.	Range	5.0 - 30.0 µg/ml	2.0 – 12.0 µg/ml
3.	Intra-day precision (% RSD[a])	0.854	1.214
4.	Interday precision (% RSD[a])	1.143	0.850
5.	Limit of Detection (µg/ml)	0.378	0.092
6.	Limit of Quantitation (µg/ml)	1.147	0.281
7.	Assay (Mean ± %RSD[a])	99.961 ± 0.142	101.050 ± 0.647
8.	Accuracy (Mean % recovery)	101.811	100.280
9.	Robustness (% RSD[a])	Robust	Robust
10.	Specificity	Specific	Specific

[a]RSD: Relative Standard Deviation

Assay

NIZONIDE-O tablet formulation analysis was carried out as mentioned under section analysis of drugs in marketed formulation. Procedure was repeated for six times. Sample solution was injected and area was recorded. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 3.

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the NIZONIDE-O tablet sample solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate were altered and the effect on the area were noted.

RESULT AND DISCUSSION

The proposed method was found to be simple and linear in the concentration range of 5.0 to 30.0 µg/ml for Nitazoxanide and 2.0 to 12 µg/ml for Ofloxacin, respectively. The method was found to be accurate and precise as indicated by recovery studies and %RSD not more than 1.5. Moreover LOD and LOQ Nitazoxanide were found to be 0.3788 µg/ml and 1.1479 µg/ml, respectively and for Ofloxacin were 0.0929 µg/ml and 0.2816 µg/ml, respectively. Thus the method is sensitive. The summaries of results are shown in Table 5.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Nitazoxanide and Ofloxacin in combined dosage forms was found to be sensitive, accurate, precise, simple and rapid. Hence the present RP –HPLC method may be used for routine analysis of the raw materials and formulations.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED