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Method Of Simultaneous Estimation of Nitazoxanide and Ofloxacin from Bulk and Tablet Dosage form Using RP-HPLC

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ABSTRACT:
For the simultaneous determination of Nitazoxanide and Ofloxacin in tablet dosage form, a simple reverse phase liquid chromatographic method was developed and validated. A mobile phase containing acetonitrile, methanol, and 0.4 M citric acid (60:30:10, v/v/v) was used for the separation. Thermo C18 (4.6mm*250 mm, 5) column was used, with a flow rate of 1 mL/min and UV detection at 300 nm. Nitazoxanide and ofloxacin had retention times of 3.071 and 12.47 minutes, respectively. The described method was linear over a concentration range of 5-30 g/mL for Nitazoxanide (r2>0.999) and 2-12 g/mL for Ofloxacin (r2>0.998). The mean percentage recovery for Nitazoxanide was 99.96% and 101.05% for Ofloxacin. Nitazoxanide and Ofloxacin had limits of detection (LOD) of 0.3788 and 0.0929 g/mL, respectively. The limit of quantification (LOQ) for Nitazoxanide and Ofloxacin, respectively, was 1.1479 g/mL and 0.2816 g/mL. The study's findings revealed that the proposed RP-HPLC method is simple, fast, precise, accurate, and cost effective, making it suitable for routine determination of Nitazoxanide and Ofloxacin in bulk and tablet dosage forms.

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

I. INTRODUCTION:

Chemically, nitazoxanide [NTZ] is N-(5-nitro-2-thiazolyl) salicylamide acetate [Fig.1(a)]. is a nitrothiazole benzamide derivative synthesized. It is an antiprotozoal with a broad spectrum. It is used to treat amoebiasis, helminthiasis, giardiasis, and other parasitic infections.[1]

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

Few methods, such as UV spectrometry, have been reported for quantitative determination of NTZ and OFX in single and combined samples[3-5] and RP- HPLC[6-9]. Although HPLC methods have been reported, the linear range

reported is higher, with high values for LOD and LOQ. As a result, reported methods are less sensitive. As a result, the development of a simple, precise, accurate, and sensitive reverse phase HPLC method for simultaneous estimation of NTZ and OFX in tablet dosage form was thought worthwhile.

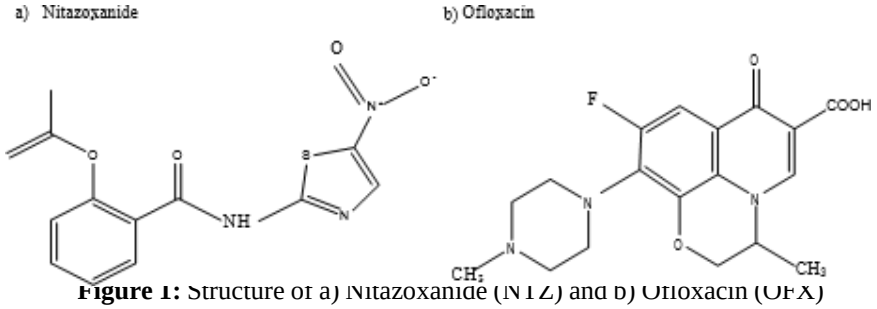

Figure 1: Structure of a) Nitazoxanide (NTZ) and b) Ofloxacin (OFX)

II. MATERIAL AND METHODS

Instrumentation

RP-HPLC was performed using a JASCO HPLC system outfitted with a PU 2080 Plus pump and a PU 2010 Plus UV detector. Rheodyne sample injection port (50 l) was used to inject samples. The column HiQSil C18 (250 x 4.5mm, i.d. 5 m) was used. Borwin software was used for data acquisition and integration (version 1.5). Furthermore, an electronic weighing balance (Schimadzu AY120) and an ultrasonicator (PRAMA SM15VS) were used.

Material and Reagent

Pure drug samples (API) of nitazoxanide and ofloxacin were obtained as gift samples from the Aurangabad Research Centre. Without further purification, the drug samples were used. The ELGA LAB WATER purification system provided HPLC grade water (PURELAB UHQ-11, United Kingdom). The methanol used in HPLC was HPLC grade (LOBA Chemie,). NIZONIDE-O tablets containing Nitazoxanide 500 mg and Ofloxacin IP 200 mg, manufactured by Lupin Pvt. Ltd., were obtained from a 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.

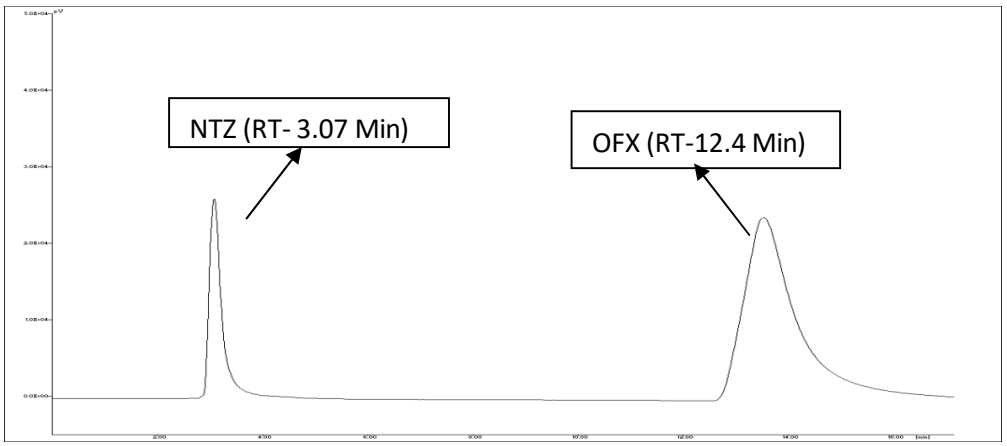

Fig 2: Chromatogram of standard Mixture NTZ & OFX (10 µg/ml of each)

Standard stock solutions

NTZ and OFX stock solutions were made by dissolving accurately weighed 10 mg of drug samples in 10 ml of HPLC grade methanol (1000 g/ml) separately. 5 ml of the above solution was pipetted and diluted to 50 ml to produce 100 g/ml of NTZ and OFX, respectively. Working solutions

Working standard solutions were made from a standard stock solution of 100 g/ml by dilution with mobile phase to achieve a final concentration of 5.0-30.0 g/ml of NTZ and 2.0-12.0 g/ml of OFZ for HPLC.

Calibration curves for the HPLC method

By diluting the standard stock solutions with mobile phase, standard solutions with concentrations ranging from 5.0-30.0 g/ml for NTZ and 2.0-12.0 g/ml for OFX were prepared. Chromatograms were taken after injecting 50 l of each standard solution. NTZ and OFX had retention times of 3.071 and 12.47 minutes, respectively. Calibration curves were created by plotting average peak areas versus concentrations. The calibration curves for both drugs are depicted in Figs. 3 and 4, respectively.

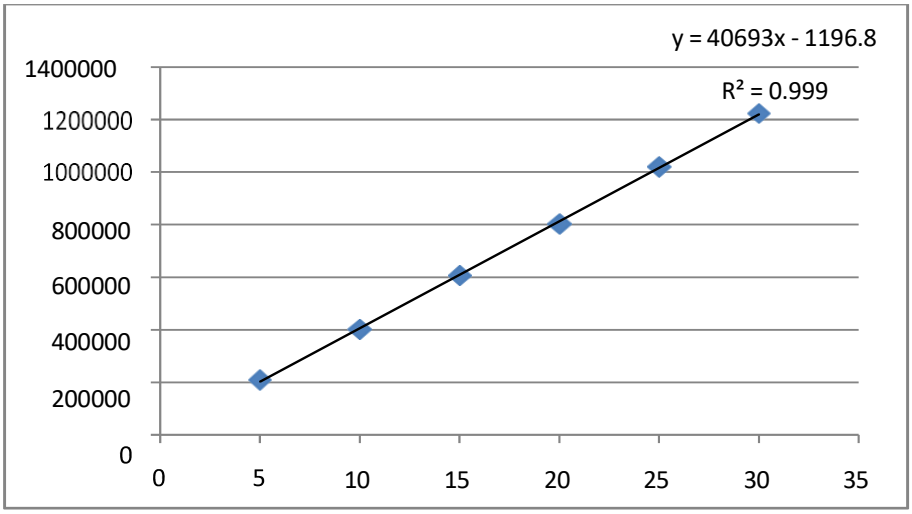

Fig.3: Calibration Curve of NTZ

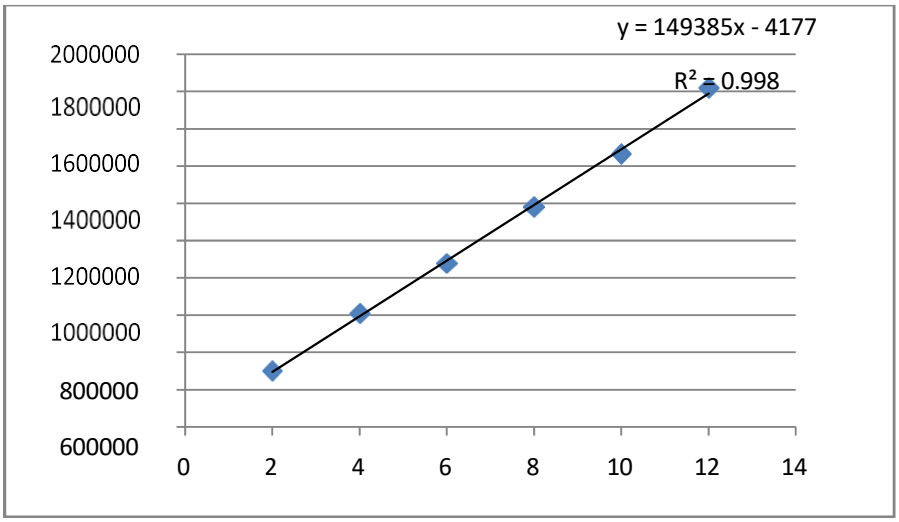

Fig.4: Calibration Curve of OFX

Analysis of drugs in marketed formulation

Twenty tablets containing 200 mg of Ofloxacin and 500 mg of Nitazoxanide were weighed and finely ground. A powder equivalent to 50 mg Nitazoxanide (20 mg Ofloxacin) was weighed and transferred to a 50 ml volumetric flask. To this, HPLC grade methanol was added and sonicated for 10 minutes; the volume was increased to 50 ml with HPLC grade methanol to obtain a 1000 g/ml solution. Whatmann filter paper was used to filter the solution. The filtrate was diluted with mobile phase to obtain a final assay solution with a concentration of 10 g/ml of NTZ (4 g/ml of OFX). The procedure was repeated six times, and the percentage was calculated using a linear equation.

VALIDATION OF PROPOSED HPLCMETHOD[10]

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

Specificity

Peak purity profile studies were used to determine the method's specificity. The peak purity values were found to be greater than 0.996, indicating that no other peak of degradation product, impurity, or matrix interfered.

Linearity

A series of standard solutions containing six different concentrations of each compound was analyzed to determine the linearity of the HPLC detector response for the determination of NTZ and OFX. Calibration curves for NTZ and OFX were linear over concentration ranges of 5.0-30.0 g/ml and 2.0-12.0 g/ml, respectively. Regression analysis was performed on NTZ and OFX, with coefficients of determination (r2) of 0.999 and 0.998, respectively.

Precision

The method's precision was demonstrated through intra-day and inter-day variation studies. In the intra-day studies, three replicates of three different concentrations were tested in a single day, and the percentage RSD was calculated. Three different concentrations were analyzed on three consecutive days for the interday variation studies, and the percentage RSD was calculated. Tables 1 and 2 show the results obtained for intra-day and inter-day variations, respectively.

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

The LOD and LOQ were calculated from the linearity data using the formulas LOD = 3.3/S and LOQ = 10/S, where = standard deviation of intercept of linearity equations and S = slope of the analyte calibration curve. LOD was 0.3788 g/ml and 0.0929 g/ml for NTZ and OFX, respectively, and LOQ was 1.1479 g/ml and 0.2816 g/ml for 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 – 1196.8r^2 = 0.999	y = 149385x - 4177r^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 performed as described in the section on drug analysis in marketed formulation. The procedure was repeated six times. The area was measured after the injection of the sample solution. The linear equation was used to calculate concentration and percentage recovery. Table 3 displays the obtained results.

Accuracy

To validate the method, recovery studies were performed by spiking the standard drug into the NIZONIDE-O tablet sample solution at three different concentrations of 50, 100, and 150%. The sample solution's basic concentration was set at 10 g/ml. The linearity equation was used to calculate the percentage of recovery. Table 4 displays the obtained results.

Robustness

The method's robustness was determined by running the analysis under conditions in which the mobile phase composition, detection wavelength, and flow rate were changed and the effect on the area was noted.

III. RESULT AND DISCUSSION

In the concentration ranges of 5.0 to 30.0 g/ml for Nitazoxanide and 2.0 to 12 g/ml for Ofloxacin, the proposed method was found to be simple and linear. Recovery studies and %RSD less than 1.5 indicated that the method was accurate and precise. Furthermore, the LOD and LOQ Nitazoxanide were 0.3788 g/ml and 1.1479 g/ml, respectively, and Ofloxacin were 0.0929 g/ml and 0.2816 g/ml, respectively. As a result, the method is sensitive. Table 5 displays the results summaries.

IV. CONCLUSION

The proposed RP-HPLC method for estimating Nitazoxanide and Ofloxacin simultaneously in combined dosage forms was found to be sensitive, accurate, precise, simple, and fast. As a result, the current RP-HPLC method can be used for routine analysis of raw materials and formulations.

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