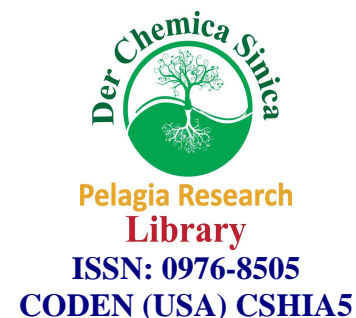




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A Validated RP-HPLC Method for Simultaneous Estimation of Nitazoxanide and Ofloxacin in Pharmaceutical Formulation

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ABSTRACT

A validated reverse phase high performance liquid chromatography method has been developed for the simultaneous determination of Nitazoxanide and Ofloxacin in combined dosage form. Chromatography was carried out on a Cosmosil 5 C₁₈ (4.6 mm × 250 mm, 5 μm) using Acetonitrile: 0.005 M triethylamine Buffer in the ratio of 55:45 (v/v) as the mobile phase at a flow rate of 1.0 mL/min and eluents were monitored at 240 nm. The calibration curves were linear over the range of 160-240 μg/mL for Nitazoxanide and 400-600 μg/mL for Ofloxacin. The average retention time of Dexrabeprazole and Domperidone was found to be 6.051 min and 2.106 min respectively. The results of the analysis have been validated statistically and by recovery studies.

Key words: Nitazoxanide, Ofloxacin, RP-HPLC, Validation.

INTRODUCTION

Nitazoxanide is chemically 2-(Acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide and ofloxacin is chemically 9-Fluoro-2, 3-dihydro-3 methyl-10 (4-methyl-1-piprazinyl)-7-oxo- 7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid (Fig.1). A combination of 200 mg of ofloxacin and 500mg of nitazoxanide is available commercially as tablets (Netazox-OF). An ofloxacin and nitazoxanide combination is indicated to antibacterial and antiprotozoal activity. The combination of nitazoxanide and ofloxacin is antiparasitic and antibacterial which is effective against a wide variety of protozoa, helminthes and gram-negative organisms. Oral bioavailability is good and well tolerated, with mild gastrointestinal side effects. Used in Giardia intestinalis induced diarrhea in patients [1]. This new combination was recently developed by pharmaceutical companies. In the process of development, fast and reliable analytical method is required for the simultaneous determination of both drugs in this compound formulation.

Literature survey reveals that, spectrophotometric [2] and RP-HPLC[3] method is available for estimation of NT in single dosage form. Ofloxacin (OF) is chemically 9-fluoro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-Oxo-7H-pyrido (1, 2, 3-di) - 1, 4-benzoxazine carboxylic acid. It is a fluoroquinolone derivative. It is used mainly as an antibacterial. It is official in USP[4] (United State Pharmacopoeia), BP[5] (British Pharmacopoeia) and EP[6] (European Pharmacopoeia). Literature survey reveals that spectrophotometric[7], HPLC[8], RP-HPLC[9], and HPTLC[10] methods are available for determination of Ofloxacin from pharmaceutical preparations and biological formulation. The aim of this study was to develop a precise, specific, accurate and sensitive method for simultaneous determination of Nitazoxanide and Ofloxacin combination by RP-HPLC method.

MATERIALS AND METHODS

Materials

Standard gift samples of Nitazoxanide and Ofloxacin were provided by Emcure Nitazoxanide and Ofloxacin was obtained as gift sample from Indswift laboratories, Pvt Ltd Samba Jammu. Combined dose capsule formulation the tablet dosage form, Nitazete-O (claim: 700mg Nitazoxanide and 200mg Ofloxacin, were purchased from local market. All chemicals and reagents used were of HPLC-AR grade of S.D. Fine chemicals were used for the study.

Instrumentation

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were per-formed using Empower- version 2 software.

Chromatographic conditions

Thermo Cosmosil 5c18 (4.6 mm i.d. × 250 mm) was used as stationary phase. Acetonitrile: 0.005 M triethylamine Buffer in the ratio of 45:55 % v/v was used as mobile phase and was filtered before use through 0.45 μ membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 240 nm. To ascertain the suitability of the proposed chromatographic conditions, system suitability tests were carried out and the results are shown in Table 1. Chromatogram of standard solution containing Nitazoxanide and Ofloxacin is shown in Fig. 1.

Table 1: System Suitability Parameters

System Suitability Parameter	Component	
	Nitazoxanide	Ofloxacin
Retention times (RT in min)	6.051	2.106
Theoretical plates (N)	17675.262	5492.729
Tailing factor (AS)	5492.729	1.319656
Resolution (RS)	25.64	25.64

Preparation of standard calibration curves (Linearity)

Standard stock solution of Nitazoxanide and Ofloxacin were prepared by transferring 200 mg of Nitazoxanide and 800 mg Ofloxacin in 100ml volumetric flask. Sufficient amount of mobile phase was added, sonicated and remaining volume was made up to the mark with mobile phase. Aliquots of standard stock solution were appropriately diluted with mobile phase to obtain concentration range of 400-600μg/ml for Nitazoxanide and 160-240μg/ml for Ofloxacin. The

diluted standard solutions with varying concentration were injected (in triplicate) into the HPLC system separately and chromatographed under above mentioned chromatographic conditions. Chromatographic peaks were recorded at 240 nm using UV detector. The calibration curves of mean peak area versus concentration were plotted.

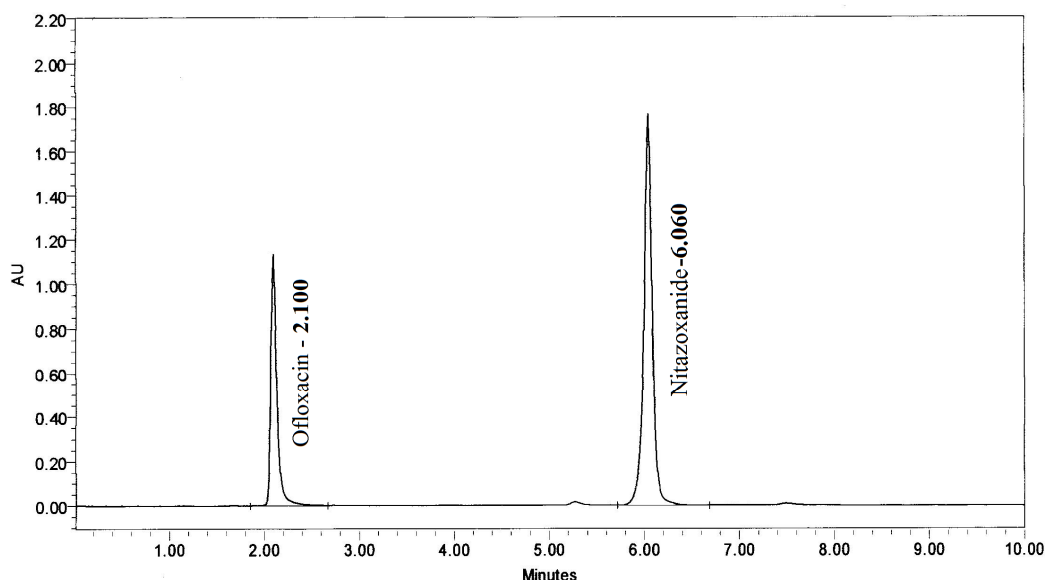


Figure 1: Typical Chromatogram of Nitazoxanide and Ofloxacin

Analysis of Capsule formulation

For the estimation of drugs in the commercial formulations, twenty capsules were uncapped weighed and average weight was calculated. The powder equivalent to 20 mg Nitazoxanide and 25 mg of Ofloxacin was transferred to 100 ml volumetric flask; 50 ml portion of mobile phase was added and sonicated for 20 min. and then volume was made up to the mark with mobile phase. The resulting solution was mixed and filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 8.4 $\mu\text{g/ml}$ of Nitazoxanide and 6 $\mu\text{g/ml}$ of Ofloxacin. The diluted solutions were filtered through 0.20 μ filter. From the filtrate, 20 μl was injected in to the column and chromatographed under above mentioned chromatographic conditions. Each sample solution was injected and chromatographed in triplicate. Six such samples were prepared and analyzed. Content of Nitazoxanide and Ofloxacin in capsule was calculated by comparing mean peak area of sample with that of the standard. Results of analysis of capsule formulation are shown in Table No. 2.

Table 2: Results of Analysis of Capsule Formulation

Component	Label claim * (mg/capsule)	Amount found * (mg/capsule)	Percent label claim*
Nitazoxanide	20	19.96	99.33
Ofloxacin	25	25.12	100.75

*Average of six determinations

Method Validation**Accuracy**

To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, adding known amount of each drug to the preanalysed capsule powder, at three levels 80 %, 100 % and 120 % of the label claim. Recovery studies were carried out in triplicate at each level. The results of recovery studies were expressed as percent recovery and are shown in Table No. 3

Table 3: Result of recovery studies

Spike level	Amt. Taken (mg)		Amt. of pure drug added (mg)		% recovery (mg)	
	Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin
80	19.929	8.082	9.782	7.968	99.81	101.43
100	24.84	9.988	15.2775	8.970	99.08	100.18
120	29.682	12.015	25.0595	11.973	99.11	100.65

Precision

Intra-day precision was determined by analyzing the capsule samples at three different time intervals on the same day and for inter-day precision capsule samples were analyzed on three different days. Standard deviation for intra-day and inter-day assay precision was calculated. Results of precision studies are shown in Table No. 4.

Table 4: Result of Precision studies

Parameters	Component	% Estimation
Intra-day	Nitazoxanide	100.18
	Ofloxacin	99.82
Inter-day	Nitazoxanide	100.02
	Ofloxacin	99.96

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

LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

Robustness

Robustness of the proposed method was ascertained by deliberately changing the chromatographic conditions such as change in flow rate of the mobile phase (± 0.1 mL/min), change in composition of the mobile phase (± 1 ml) and change in pH of the buffer solution used in mobile phase. Effect of change in chromatographic parameters on resolution and tailing factor of peak was studied.

RESULTS AND DISCUSSION

The proposed chromatographic system was found suitable for effective separation and quantitation of Nitazoxanide (RT-6.051min) and Ofloxacin (RT-2.106min) with good resolution, peak shapes and minimal tailing. The peak areas of the drugs were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.9996 and 0.9997 for Nitazoxanide and Ofloxacin, respectively. The sample recoveries from the formulation were in good agreement with their respective label claim indicating that there is no interference from the capsule excipients. The method exhibited good selectivity and sensitivity. Percent recoveries for Nitazoxanide and Ofloxacin were 99.38 % and 100.75%, respectively indicating accuracy of the proposed method. %RSD for capsule analysis, recovery studies and intra-day & inter-day precision studies is less than 2. LOD and LOQ were found to be 8.4µg/ml for Nitazoxanide and 6.27µg/ml for Ofloxacin, respectively. The results of robustness study also indicated that the method is robust and is unaffected by small deliberate variations in the method parameters.

CONCLUSION

The proposed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the estimation of Nitazoxanide and Ofloxacin in bulk and marketed formulation.

REFERENCES

- [1] Guerrant R L, Hughes J M, and Lima N I, *Clin Infect Dis.*, **2005**, 40, 1173-1180.
- [2] Kapse GK, Prabhakar G and Appala SR. *Indian J. Pharm. Sci.*, **2006**, 68, 403- 406.
- [3] Narayana LKV, Manohara YN and Appala RS, *Indian drugs.*, **2006**, 43, 503-506.
- [4] United State Pharmacopoeia. 29 National formulary, Rockville, USA, **2006**, 1574.
- [5] British Pharmacopoeia. British Pharmacopoeia Commission Office, London: U.K. **2002**, 1, 1247-48.
- [6] European Pharmacopoeia, Published in accordance with convention on the elaboration of European Pharmacopoeia. **2004**, 2, 2131.
- [7] Kasture VS, Bhagat AD, Puro NC, More PS. Bhandari NK. *Indian Drugs.*, **2004**, 41: 51-53.
- [8] Kraas E. and Hirle A. *Fresenius' J. Ana. Chem.*, **1986**, 324, 354-357.
- [9] Zeng S, Zhang L. and Liu ZQ. *Yao Xue Xue Bao.*, **1994**, 29, 223- 227.
- [10] Gandhimathi M, Ravi TK and Nilima S. *Indian J. Pharm. Sci.*, **2006**, 68, 838- 840.