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Der Pharma Chemica, 2012, 4 (3): 1140-1144
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

RP-HPLC Method for Estimation of Nitazoxanide in Oral Suspension Formulation

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ABSTRACT

A simple, precise, accurate, rapid and reproducible reverse phase high performance liquid chromatographic method was developed for estimation of Nitazoxanide in oral suspension formulation. The mixture of acetonitrile: ammonium dihydrogen phosphate buffer (0.075 M) in the ratio 45:55 (% v/v) adjusted to pH 3.0 with ortho-phosphoric acid was used as mobile phase. The detection of the Nitazoxanide in oral suspension formulation was carried out at 240 nm and flow rate was set to 1.5 ml/min. Linearity was obtained in the concentration range of 5 to 25 µg/mL of Nitazoxanide with correlation coefficients of 0.997. The results of the analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method.

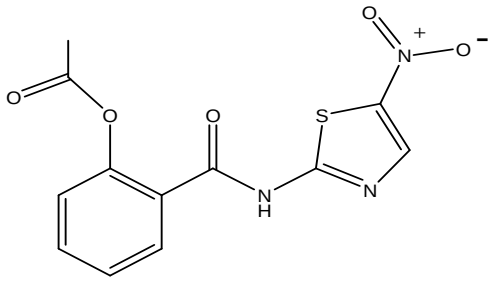
Keywords: RP-HPLC, Nitazoxanide, Oral Suspension

INTRODUCTION

Nitazoxanide (NTZ) chemically [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl] acetate [1], is an anti-amoebic and anthelmintic agent. It is indicated for amoebiasis, helminthiasis, giardiasis, fascioliasis, trichomoniasis and cryptosporidiosis, including those with AIDS or HIV infections [2, 3]. It is not yet official in any of the pharmacopoeia. Literature survey revealed RP-HPLC method for its determination in bulk drug and pharmaceutical formulation [4-10]. Therefore, it was thought worthwhile to develop simple, precise, accurate reverse phase HPLC method for simultaneous estimation of NTZ in oral suspension dosage form.

MATERIALS AND METHODS

Pharmaceutical Grade NTZ (Batch No. SNT 0606023) was supplied as a gift sample by Ind-Swift Laboratories Limited, Samba, (Jammu and Kashmir), India. The oral Suspension Dosage form (Netazox®, Ind-swift Limited, Parwanoo – 173 220. Himachal Pradesh, India; Batch No. NTS-08011; Label Claim: 100mg/5mL) was procured from the local market. All chemicals used were of HPLC grade.



Nitazoxanide (NTZ)

Chromatographic conditions

The HPLC system (Agilent Technologies, Agilent 1120 Compact LC system) equipped with isocratic pumping system, Rheodyne manual injector (injection capacity 20 µl) and a UV detector (VWD G4286A) was used for chromatographic separation. Pre-filtered samples (20 µL) were injected into a LCGC Qualisil BDS C_{18} (4.6 × 250mm, 5µ) revered phase column at ambient temperature. The mobile phase system consisted of acetonitrile-ammonium dihydrogen phosphate buffer (45:55, % v/v) adjusted to pH 3.0 with ortho-phosphoric acid and was run in isocratic mode at a flow rate was 1.5 mL/min through the column. The run time was 5 min per injection and elute was monitored at a wavelength of 240 nm. (Figure 1).

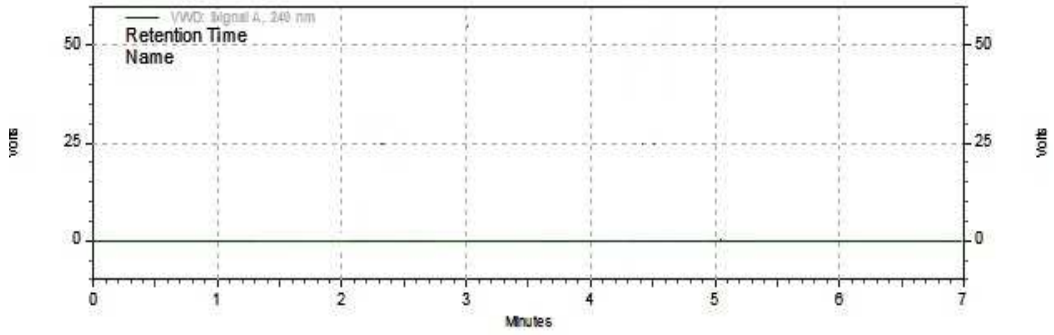


Fig. 1 Chromatograms of Blank mobile phase.

Preparation stock solution of NTZ

Accurately weighed NTZ (50 mg) was transferred to 50 ml volumetric flask, dissolved and diluted up to 50 ml with acetonitrile to get the stock solution of 1 mg/ml of NTZ.

Preparation of working standard solution of NTZ

Ten ml of stock solution was diluted to 100 ml with mobile phase to get the working standard solution of 100µg/ml of NTZ.

Calibration curve of NTZ

Aliquots of working standard solution were diluted with mobile phase to obtain the calibration standards of 5, 10, 15, 20 and 25 µg/ml of NTZ. All the calibration standard solutions were filtered through 0.45 µ nylon membrane filter before injection to the chromatographic system. A calibration curves was constructed by plotting the peak area versus the corresponding drug concentration. The least square linear regression analysis was performed on the calibration data. The slope and co-relation a coefficient (R^2) of the calibration curve were determined.

Quantitation of NTZ in oral suspension Dosage forms:

To determine the content of NTZ in oral suspension dosage form (Label claim: 100mg/5mL) the whole content of one bottle was removed and then mixed thoroughly. The volume equivalent to 30 mg of NTZ was taken and dissolved in 50 ml of acetonitrile. The resulting solution (0.5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with mobile phase. The final solution was filtered through 0.45µ membrane filter using

injection filter. A 20 µl of the filtrate was injected into the chromatographic system. The peak area of the NTZ was determined and concentration was found using linear regression equation obtained from calibration curve.

System suitability parameter

The chromatographic system used for analysis must pass the system suitability limits before sample analysis can commence. The capacity factor, injection repeatability (n=6), tailing factor and number of theoretical plate for the NTZ peak were the parameters tested on a solution containing 15 µg/mL of NTZ, in order to support analytical method validation parameters like accuracy and precision.

Validation of the proposed method

The proposed method was validated for parameters such as linearity, precision, accuracy, robustness, and specificity, limit of detection and limit of quantification. The linearity and range for the proposed method was established by running the calibration standards five times. The analytical recovery experiments were carried out, by standard addition method, to check the accuracy of the developed method and to study the interference of formulation additives. Recovery studies were performed at three concentration levels in triplicate. From the total amount of drug found, the percentage recovery was calculated. The precision of the method was determined in terms of inter-day and intra-day precision. The inter-day precision was assessed across the linearity range by analyzing the calibration standards on five different days where as the intra-day precision was determined by running calibration standards five times on same day. The robustness of the method was checked by deliberately changing the flow rate, mobile phase composition and pH of the mobile phase. The limit of detection (LOD) and limit of quantification (LOQ) was calculated based on signal to noise ratio.

RESULTS AND DISCUSSION

Chromatographic conditions

Optimization of the chromatographic conditions was performed based on system suitability parameters such as capacity factor, tailing factor, injection repeatability, and number of theoretical plates obtained for NTZ. The mobile phase of 0.075M Ammonium dihydrogen phosphate buffer: Acetonitrile (45:55) was found to be satisfactory and gave symmetric and well resolved peaks for NTZ in oral suspension dosage form. The retention time for NTZ in oral suspension dosage form was 4.46 min. (Figure 2).

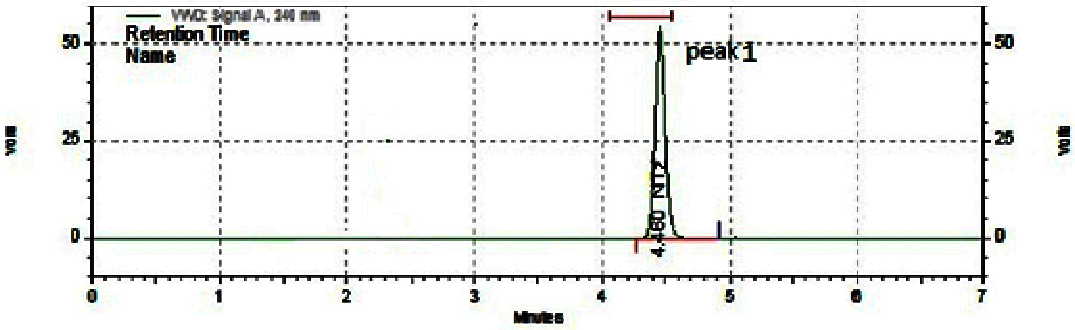


Fig. 2 Chromatograms of Standard solution of NTZ (15 µg/ml)

The wavelength 240 nm was selected for determination based on maximum absorption of drug and best detector response at this wavelength. The system suitability parameters for the proposed method were shown in Table 1.

Table 1 System suitability parameters for the proposed method

Parameter	NTZ
Retention time (R_t) in minutes	4.46 minutes
Tailing factor(T_f)	1.53 %
*Injection repeatability(% RSD)	0.0075
Number of theoretical plates (N)	5188

**RSD of peak areas of five consecutive injections of 15 µg/ml of NTZ*

Validation of method

The calibration curve for NTZ was obtained by plotting the peak area of NTZ versus their concentration over the range of 5-25 µg/ml and was found to be linear with r =0.9987 for NTZ (Figure 3). The recovery of NTZ was found to be in the range 99.06-100.58% in Table 2. The data for inter-day and intra-day precision are presented in Table 3. The validation parameters for the proposed method are summarized in Table 4.

Table 2 Results of recovery studies on NTZ oral suspension*

Drug	Initial Conc.(µg/ml)	Conc. Added (µg/ml)			%Recovery		
		A	B	C	A	B	C
NTZ	5	5	10	15	99.03	99.07	99.10
	10	5	10	15	100.58	100.50	100.49
	15	5	10	15	100.01	100.05	100.15
	20	5	10	15	99.59	99.69	99.00
	25	5	10	15	100.02	100.40	100.45

***Conc.:** Concentretion, Result are means five times, Recovery is more then 99%.*

Table 3 Precision data for proposed method*

Std. Conc. (µg/ml)	NTZ		
	Mean peck area*	SD	%RSD
5	2126421	478.5297	0.0225
10	4169560	560.447	0.013
15	6147297	465.2854	0.0075
20	81858730	5287.51	0.00645
25	10464000	478.5297	0.004573

***Std. Conc.:** Standard Concentration, **SD:** Standard deviation, **RSD:** relative standard deviation*Mean of five peck areas.*

Table 4 Summary of validation parameters*

Parameters (Units)	NTZ
Linearity Range (µg/ml)	5 - 25 µg/mL
Percentage Recovery (%)	99.06-100.58
LOD (µg/ml)	0.25
LOQ (µg/ml)	0.77
Slope (m)	412592.1886
Intercept (b)	15864.14
Co Relation coefficient	0.9997
Specificity/ Selectivity	No interference
Stability of sample solution	>24 hours

NTZ: *Nitazoxanide, SST: system suitability test, * calculated 5% per height,* ***LOD:*** *limit of detection,* ***LOQ:*** *limit of Quantitation*

Analysis of marketed formulation containing NTZ

The liquid chromatographic method was applied for estimation of NTZ in oral suspension dosage form. The results are shown in Table 5. The peak of NTZ was separated using proposed method without any interference from the inactive ingredients (Figure 3).

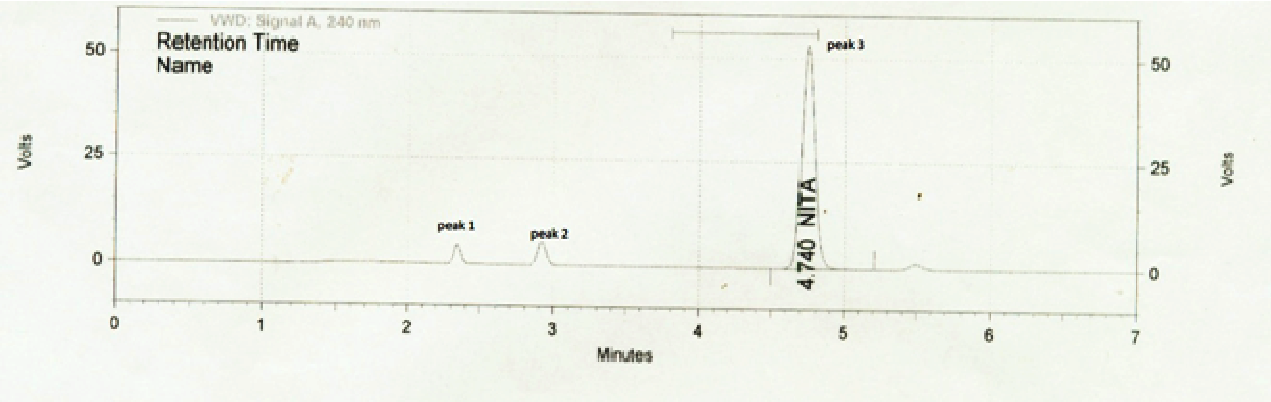


Fig. 3 Chromatograms of Marketed formulation containing NTZ. Peak 1 and Peak 2 Excipient peak chromatogram showing retention time, 4.74 min peak 3 for marketed Nitazoxanide (NTZ) oral suspension formulation.

Table 5 Results of analysis of marketed oral suspension formulation of NTZ*

Drug	Conc. Taken (µg/ml)	Conc. Found (µg/ml)	SD	%RSD
NTZ (Label Claim: 100mg/5mL)	5	5.0044	0.002074	0.041
	10	10.0048	0.001483	0.014
	15	15.026	0.011402	0.075
	20	20.032	0.014832	0.074
	25	25.022	0.013038	0.0521

***Conc.:** Concentration, **SD:** Slandered deviation, **RSD:** relative standard deviation, result are means five times.*

CONCLUSION

The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of NTZ in their oral suspension dosage form.

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