



## RPHPLC DEVELOPMENT AND VALIDATION OF NITAZOXANIDE IN TABLET DOSAGE FORM

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## ABSTRACT

A simple, precise, accurate, rapid and reproducible RPHPLC procedure was developed for determination of Nitazoxanide (NTZ) in tablet dosage form at a single wavelength. The mobile phase used was a combination of methanol: water: acetonitrile (30:40:30% v/v/v). The detection of NTZ was carried out at 330nm & flow rate was set to 1ml/min. Linearity was obtained in the concentration range of 5-25µg/ml of NTZ with correlation coefficients of 0.9948. The results of the analysis were tested and validated statistically for various parameters according to ICH guidelines and recovery studies confirmed the accuracy of the proposed method.

**Keywords-** Nitazoxanide, RPHPLC, Development, Validation

## INTRODUCTION

Nitazoxanide (NTZ) is chemically, N-(5-nitro-2-thiozoly) salicylamide acetate is an anantiamoebic and anthelmintic agent. It is indicated for amoebiasis, helminthiasis, giardiasis, fasciasis, trichomoniasis and cryptosporidiosis, including those with AIDS or HIV infections<sup>1-3</sup>. NTZ is not official in any of the pharmacopoeia. It found mentioned in Martindale, The Complete Drug Reference<sup>4</sup>. Very few analytical methods like RPHPLC and spectrophotometric methods are reported in literature for the quantitative estimation of NTZ in bulk drug and pharmaceutical dosage forms<sup>5-8</sup>. Therefore, it was thought worthwhile to develop simple, precise, accurate RPHPLC method for determination of NTZ in tablet dosage form.

## EXPERIMENTAL

## Materials and Instruments

Pharmaceutical grade NTZ (Batch no. SNTX0107030) was supplied as a gift sample by Ind-Swift Laboratories Limited, Samba, (Jammu and Kashmir), India. The tablet dosage form (Nizonide, Batch no. 8MKT40, mfg dt. 09/2007 and Exp. dt. 08/2009) was procured from the local market (Label claim: 500mg NTZ) marketed by Ind-Swift Laboratories Limited, Samba, (Jammu and Kashmir), India. All chemicals used were of HPLC grade and were purchased from Loba chemicals, Mumbai, India.

System consist of Jasco PU 1580 HPLC used consist of pump with precision loop injector (Rheodyne 7725) of injection capacity 20 µl. Detector consists of photodiode array detector (Jasco UV 1575); the reversed phase column used was C<sub>8</sub> intersil (250×4.6 mm i.d. with particle size 10 µm) at ambient temperature.

## Mobile phase

A mixture of Methanol: Water: Acetonitrile (30:40:30 % v/v/v) was used as mobile phase which was filtered through a Whatman filter paper no.42.

## Preparation of standard stock solution

The standard stock solution of NTZ (5mg/ml) was prepared in methanol and diluted to get working concentration (5-25 µg/ml).

## Preparation of sample stock solution

20 tablets were taken, their average weight was determined and crushed to a fine powdered, equivalent to 500 mg of NTZ was weighed and dissolved in 40 ml of methanol and sonicated for about 10-15 min, finally volume was made to mark with methanol. The solution was filtered through Whatman filter paper no.42 to a 100 ml volumetric flask and volume was made up to mark with methanol to get sample stock solution which was further diluted with methanol to get required concentration in linearity range (5-25µg/ml). All solutions were stored at room temperature; these solutions were shown to be stable during the period of study.

## Method development

A volume of 20 µl of each standard was injected into column HPLC with Hamilton Syringe. All measurements were repeated five times for each concentration and respected calibration curves were constructed by plotting the peak area versus the corresponding drug concentration. The slope and co-relation coefficients were determined. (Table1)

Table 1: Linearity and range of NTZ

Sr. No.	Concentration of NTZ in µl	Area
1	5	2253
2	10	4392
3	15	6107
4	20	8418
5	25	10215

Table 2: Method precision for NTZ in tablet dosage form

Drug	Conc taken (µg/ml)	Conc found (µg/ml)	SD	% RSD	Found (Label claim)	
					%	Mg
NTZ (Label claim: 500 mg)	5	5.04	0.004	0.07	100.04	500.03
	10	10.02	0.001	0.03	100.06	500.06
	15	15.06	0.063	0.53	100.18	500.11
	20	20.01	0.108	1.05	100.12	500.04
	25	25.03	0.112	0.42	100.03	500.01

Conc: Concentration SD: Standard Deviation, RSD: Relative Standard Deviation,

Results are average of five replications.

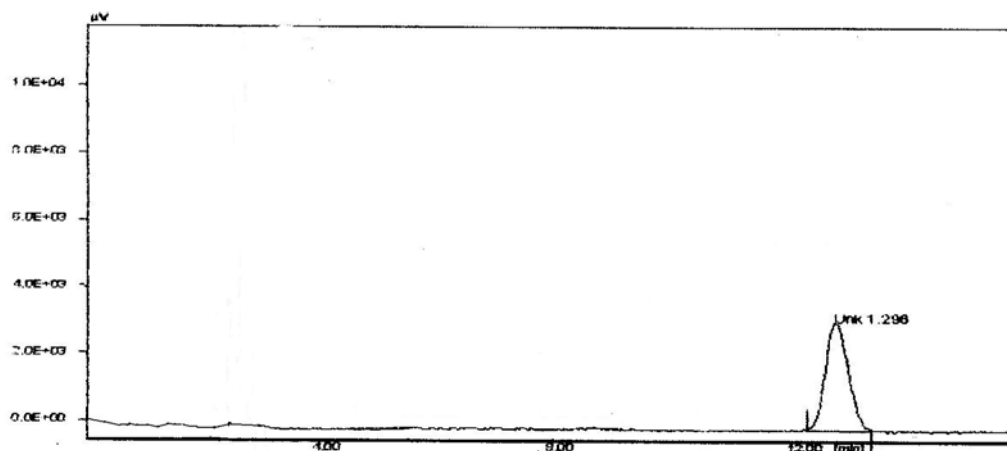


Fig. 1: Standard Chromatogram of NTZ (Rt 12.54) at 330nm.

To determine the content of NTZ in tablet dosage form (Label claim: 500 mg). A 20  $\mu$ l of above sample stock solutions diluted (5-25  $\mu$ g/ml) were injected one by one to the HPLC column with the help of Hamilton Syringe. The results are reported in Table 2 and the amount of drug was determined.

Optimization of mobile phase and flow rate were performed based on peak parameters such as height tailing factor, theoretical plates, capacity factor, run-time and resolution. The mobile phase Methanol: Water: Acetonitrile (30:40:30 % v/v/v) was found to be satisfactory and gave a symmetrical and well resolved peaks for NTZ. The wavelength 330 nm was selected for the determination based on maximum absorption of NTZ and best detector response at this wavelength.

#### Validation of the developed method

The developed method for the determination of NTZ was validated as per ICH guidelines (ICH 1996).

#### Linearity

Appropriate dilutions of standard stock solution (5-25  $\mu$ g/ml) were assayed as per the developed method for NTZ. To establish linearity of the proposed method, five separate series of solutions of NTZ were prepared from the stock solutions and analyzed.

#### Accuracy

To check the accuracy of developed method and to study the interference of formulation additives analytical recovery experiments were carried out by standard addition 80%, 100% and 120% of the label claim. (Table 3)

#### Precision (Interday and Intraday precision and Different analysts)

The interday and intraday precisions were determined by assay of the sample solution on the different day and same day at different time intervals and by different analysts respectively. (Table 4)

#### LOD and LOQ

The LOD and LOQ of NTZ by proposed methods were determined using calibration standards. LOD and LOQ were calculated as  $3.3 \sigma/s$  and  $10 \sigma/s$  respectively where  $s$  is slope of the calibration curve and  $\sigma$  is the standard deviation of  $y$ -intercept of regression equation.

### RESULTS AND DISCUSSION

#### Analytical validation

##### Linearity

Linearity range for NTZ estimation was found to be 5-25  $\mu$ g/ml at a selected wavelength.

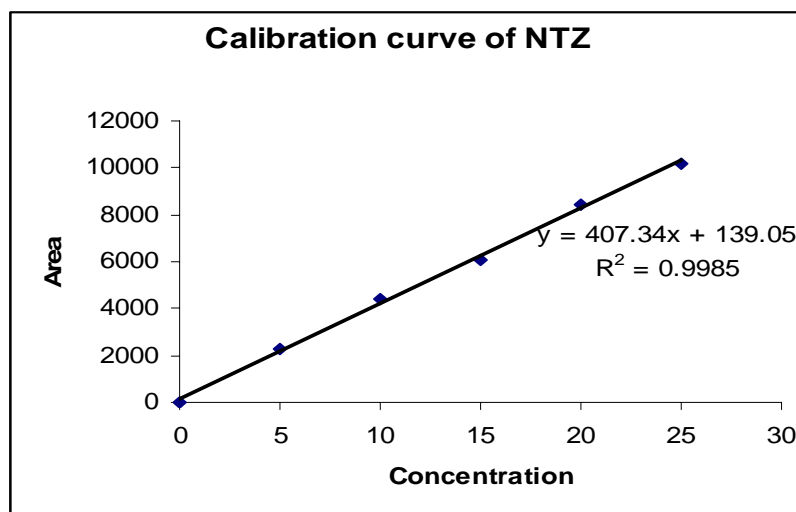


Fig. 2: Calibration curve of NTZ

**Accuracy**

The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The mean of %

recovery (% RSD) were found to be low values (<2.0) for the developed method. This result revealed that any small change in the drug concentration in the solution could be accurately determined by the developed analytical method.

**Table 3: Recovery study of NTZ using the proposed HPLC method**

Drug	Level of recovery	% Mean recovery	SD	%RSD
NTZ	80	500.12	0.008	0.56
	100	500.57	0.018	0.18
	120	500.43	0.012	0.19

Average of three determinations, SD: Standard Deviation, RSD: Relative Standard Deviation.

**Precision**

Precision was determined by studying the intermediate precision. Intermediate precision study expresses within laboratory variation in same day and different days. In intermediate precision study, % RSD values were not more than 2.0 % in developed method. RSD values for developed method for NTZ were found well within the acceptable range indicating that the developed method have excellent repeatability and intermediate precision.

**Table 4: Intraday, interday, different analysts, LOD and LOQ**

Parameter	NTZ
% RSD Intraday	0.191
% RSD Interday	0.231
% RSD Different Analysts	0.103
LOD	25 ng/ml
LOQ	75 ng/ml

RSD: Relative Standard Deviation LOD: Limit of Detection LOQ: Limit of Quantitation

**LOD and LOQ**

From data (standard deviation of y- intercept of regression equation and slope of calibration curve), it was possible to calculate the detection and quantitation limit. For developed method the LOD and LOQ value for NTZ was found to be 25ng/ml and 75ng/ml respectively.

**Table 5: System suitability parameters**

SST and other parameters	NTZ
Theoretical plates (N)	15878
Resolution (Rs)	6.13
Linearity range ( $\mu\text{g/ml}$ )	5-25
% recovery	99-101
Drug recovered	500.02
LOD ( $\mu\text{g/ml}$ )	25
LOQ ( $\mu\text{g/ml}$ )	75
Tailing factor	1:29
Retention time (min)	12.54
Slope (m) in tablet form	66218
Intercept (b) in tablet form	5821
Co-relation coefficient	0.9948
Specificity/Selectivity	No interference
Stability of sample solution	$\geq 24$ hrs

The proposed validated method describes a new RPHPLC method for the determination of NTZ in tablet dosage form. The method gives good resolution. The method was validated and found to be simple, sensitive, accurate and precise. Percent 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 tablet dosage form.

SST: System Suitability Test Calculated at 5% peak height, LOD: Limit of Detection, LOQ: Limit of Quantitation

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