



## New Precise And Accurate Method For The Determination Of Calibration Curves Of Some Selective Advanced Medicinal Compounds

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**Abstract-** The main objective of this technique is to determine the calibration curves of Nitazoxanide and Roxithromycin. By using solvent Methanol and phosphate buffer of pH 7.4 for Nitazoxanide where as solvent Deionised Water and phosphate buffer of pH 7.4 for Roxithromycin the Beer's law is obeyed in the concentration range of 4-20 µg/ mL and the graph of the drug shows a straight line with correlation coefficient of 0.9920 for Nitazoxanide. For Roxithromycin the Beer's law is obeyed in the concentration range of 20-70 µg/ mL and the graph of the drug shows a straight line with correlation coefficient of 0.9840. The assay method of both drugs are validated by accuracy and precision of the proposed method. The results are validated as per the Q2B directions of International conference on Harmonization.

**Keywords** – Nitazoxanide, Roxithromycin. Methanol, Deionised Water and phosphate buffer of pH 7.4

### I. INTRODUCTION

Nitazoxanide is a synthetic derivative of Nitrothiazolyl-salicylamide, Hermitic and Anti Protozoal agent and used to treat Fasciasis, Amoebiasis, Trichomoniasis[2] and Cryptosporidiosis[3]. Chemically this drug is 2-acetyloxy-N-(5-nitro-2-thiazolyl)benzamide[1]. The IUPAC name of NTZ is [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl] acetate. The chemical formula of this compound is  $C_{12}H_9N_3O_5S$ , It has a calculated molecular weight of 307.28 g/mol. It is a crystalline light yellow powder and insoluble in water, partially soluble in ethanol and freely soluble in DMSO and Methanol. It is not official in any of the pharmacopoeia and It found mentioned in Martindale, The Complete Drug Reference[4].

The molecular structure of the drug is shown in the fig:1. Literature survey of Nitazoxanide reveals that Different spectrophotometric methods have been reported for the determination of Nitazoxanide which includes using Dimethyl Formamide & Phosphate Buffer by Deshpandey M.M et al[5], only Dimethyl Formamide by Shailendra Pandey et al[6], Methanol by Madhuri. D. Game[7], Singh H.P et al[9], and Sunitha Sharma et al [11], Ethanol by Senthil Raja.M[8] and Ferric Chloride & Ethanol by Sharma .S. et al[10]. Therefore, an attempt was made to develop a low cost precise, accurate spectrophotometric method to determine calibration curve for the estimation of Nitazoxanide in bulk and tablet dosage form.

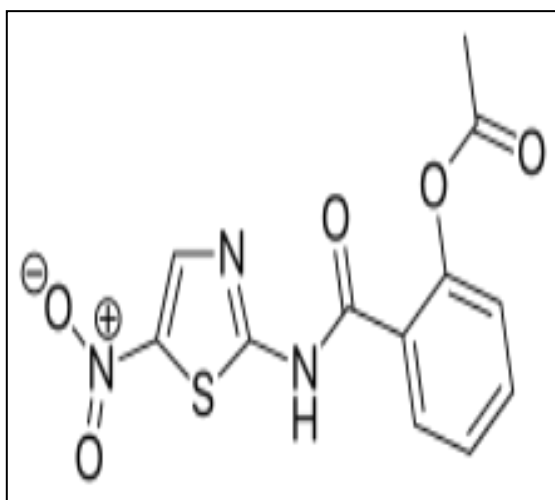


FIG 1: Structure of Nitazoxanide

Roxithromycin [15] is a New generation Erythromycin, semi synthetic macrolide antibiotic, nullifies growth of bacteria and their synthesis of proteins. The molecular formula of the drug is  $C_{41}H_{76}N_2O_{15}$  and molecular mass of 837.047 g/mol. It is a Semi synthetic White solid and freely soluble in DMSO, Methanol, Ethanol, DMF and Deionised Water. The molecular structure of the drug is shown in the fig:2. Literature survey of Roxithromycin reveals that Different spectrophotometric methods have been reported [12-19]. Therefore, an attempt was made to develop a low cost precise and accurate spectrophotometric method for the estimation of Roxithromycin in bulk and tablet dosage form.

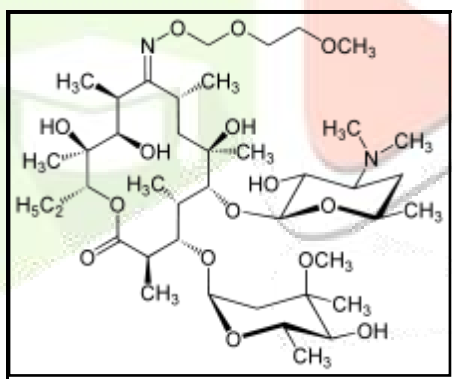


FIG : 2 structure of Roxithromycin

## MATERIALS AND METHODS FOR NITAZOXANIDE :

**Instruments and Apparatus :** The absorbance of the drug were carried out by using shimadzu company model 1700 UV-visible double beam spectrophotometer with 1 cm matched quartz cell, spectral band width is 1 nm, supported by UV win 5.0 software .

**Reagents and Chemicals:** All chemicals are AR grade. Methanol and phosphate buffer of pH 7.4 is used throughout the analysis. Pharmaceutical formulation of Nitazoxanide was supplied by Mankind pharmaceuticals, Hyderabad. Methanol and phosphate buffer 7.4 purchased from Merck India Ltd, Mumbai. Commercially available tablets namely Zindax and Paramix procured from Medwin pharmacy, Hyderabad

### Selection of Solvent:

Methanol, and phosphate buffer of pH 7.4.[13] are used throughout the analysis.

### Selection of Method and Wave Length:

UV scan range of 300 nm to 400 nm was selected for the proposed method of Nitazoxanide . The wavelength corresponding to maximum absorbance was found at 340 nm and calibration curve was taken at 340 nm shown in fig:3. The intercept of calibration line of the drug was determined by Linear regression Analysis.

### Preparation of Standard Solutions of Nitazoxanide :

The 100 mg of standard (pure) drug of Nitazoxanide is weighed accurately and dissolved in 100 ml methanol solvent then transferred into 100 ml volumetric flasks to prepare 1000 µg/ mL[12] stock solution of Nitazoxanide .Then to get different aliquots of Nitazoxanide was prepared by pipetting out 0.4,0.6,0.8,10,12,14,16,18 and 20 mL were taken in nine 10 ml volumetric flasks.To each flask 2mL of phosphate buffer of pH 7.4 solution is added, then all stock solutions of the drug were scanned in the UV scan range of lambda max ( $\lambda_{max}$ ) 300 nm to 400 nm to determine maximum absorbance of this method .The calibration curve was plotted in the concentration range of 4-20 µg/ mL. The wavelength corresponding to maximum absorbance of Nitazoxanide measured at 340nm against methanol as blank shown in fig:3.

### Preparation of Sample Solutions of Nitazoxanide :

For the analysis of Nitazoxanide two commercial brands namely Zindax (50mg) and Paramix (50mg),tablets were purchased from Medwin pharmacy ,Hyderabad .Twenty tablets of the drug was weighed accurately and powdered , then 100 mg of the drug in powdered form dissolved in 100 ml of methanol and sonicated for few minutes and filtered by using whatmann filter paper No.42. The filtrate of 10µg/mL concentration is taken in a nine 10 ml volumetric flasks. To each 10 ml flask 2mL of phosphate buffer of pH 7.4 solution is added. Then absorbance of Nitazoxanide measured at 340nm against Methanol as blank.

### Determination of $\lambda_{Max}$ :

UV scan range of 300 nm to 400 nm was selected to determine maximum absorbance by using 10 µg/ml solution of the drug , the wave length corresponding to maximum absorbance was found at 340 nm for this drug. The spectrophotometric spectrum Nitazoxanide is shown in fig.3

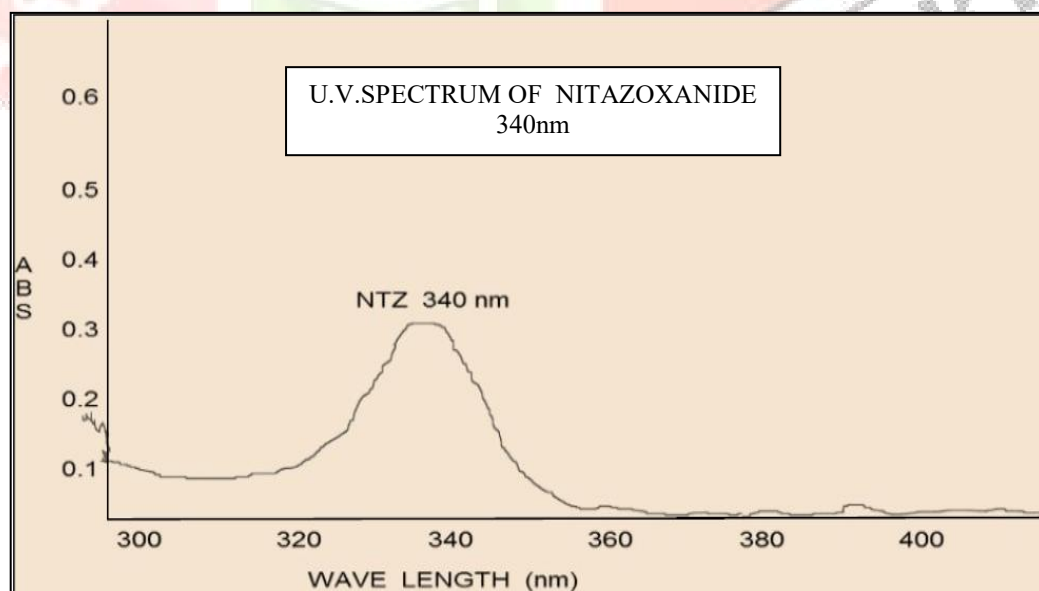


FIG :3 U. V. SPECTRUM OF NITAZOXANIDE

TABLE : 1 Optical Parameters of Nitazoxanide

S.no	Parameter	Nitazoxanide
1	$\lambda_{\text{Max}}$ (nm)	340 nm
2	Beer's Law Limit ( $\mu\text{g}/\text{mL}$ )	4-20
3	Correlation Coefficient( $r^2$ )	0.9920
4	Regression Equation ( $Y= a+bc$ )	0.047X-0.0580
5	Intercept (a)	0.0580
6	Slope (c)	0.047
7	SD	5.4772
8	Mean	12
9	Variance	30
10	LOD (%)	0.384
11	LOQ (%)	1.165

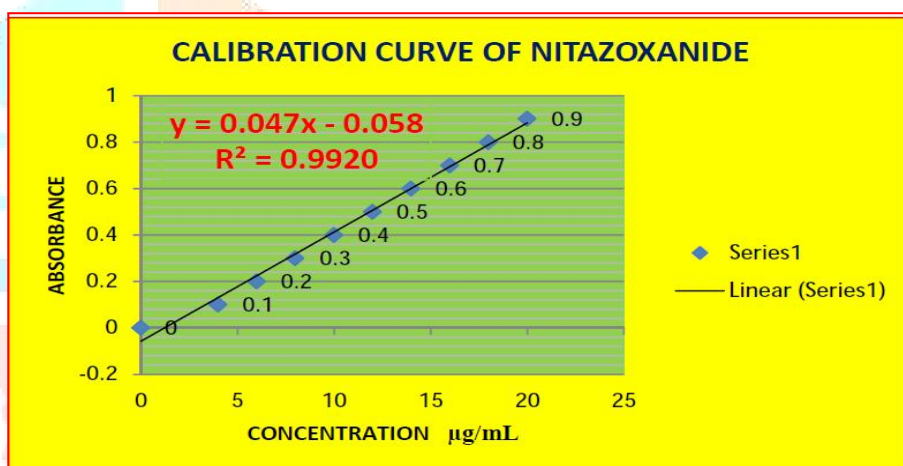


Fig 4 : Calibration Curve of Nitazoxanide

**Preparation of Calibration Curve :**

On the basis of experimental results, calibration curve is plotted and shown in fig: 4 in the concentration range of 4-20  $\mu\text{g}/\text{mL}$  of nine standard solutions of Nitazoxanide in methanol as blank. UV scan range of 300 nm to 400 nm was selected to determine maximum absorbance of the drug. In this method the wavelength corresponding to maximum absorbance was found at 340 nm.

**Validation of Method[14]**

The spectrophotometric estimation of Nitazoxanide is validated as per the directions of International conference on Harmonization to determine linearity, precision, accuracy, LOD and LOQ of the proposed method.

**Linearity and Range :**

Standard stock solution of Nitazoxanide in appropriate dilution were assayed as per the proposed method According to Beer's – Lambert's law the concentration range of Nitazoxanide was found to be 4-20  $\mu\text{g}/\text{mL}$ , So that the calibration curve in the figure : 4 is linear in the given concentration range.

**Precision :**

The precision of the proposed method of Nitazoxanide was estimated by using drug concentration of Nitazoxanide were analyzed six times in a day (intra-day precision) and six continuous days (inter-day precision). Data is given in the table-2

**Accuracy :**

The Accuracy of the proposed method of Nitazoxanide was estimated by using standard addition method. This process is carried out by adding different amounts namely 80% ,100% and 120% of the pure sample of the drug to the pre-analysed formulation. Accuracy data of the drug is shown in the table-2

S.NO	NAME OF THE SAMPLE	LAELED AMOUNT (mg/capsule)	AMOUNT FOUND* (mg)	PRECISION	
				INTER DAY	INTRADAY
1	ZINDAX	50	49.2	0.0076	0.0072
2	PARAMIX	50	48.05	0.0083	0.0069

(\*average of 6 determinations)

**Table : 2 Accuracy and Precision of Nitazoxanide**

**LOD and LOQ:**

LOD is Limit of Detection and LOQ is Limit of Quantitation[15]. The LOD and LOQ of Nitazoxanide were determined (Table : 1) by using standard deviation of the response and slope approach as per the directions of International Conference on Harmonization (ICH) guidelines. The limits of detection (LOD) is calculated by using the equation  $LOD = \frac{3s}{k}$  Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) The limits of quantitation (LOQ), is calculated by using the equation  $LOQ = \frac{10S}{K}$  Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean).

**Recovery Studies of Nitazoxanide :**

Recovery of Nitazoxanide were performed to know the accuracy of the proposed method. This process is done by adding a known quantity of pure drug to a pre-analysed sample. The result of analysis of the drug is notified in the table: 3

**Table : 3 Recovery studies of Marketed Formulations of Nitazoxanide**

S.NO	NAME OF THE SAMPLE	LAELED AMOUNT (mg/capsule)	%LEVEL	AMOUNT FOUND* (mg)	% RECOVERY
1	ZINDAX	50	120	49.2	98.4
2	PARAMIX	50	80	48.05	96.1



**MATERIALS AND METHODS FOR ROXYTHROMYCIN :****Instruments and materials:**

A Shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1 cm matched quartz cell. All the chemicals used were of analytical grade and procured from Merck India Ltd, Hyderabad. Pharmaceutical formulation of Roxithromycin was procured from Cipla Pharmaceuticals Ltd, Hyderabad. Commercially available tablets namely Rotip(75mg) and Roxyfin(75mg) procured from Medwin pharmacy, Hyderabad,

**TABLE : 4 Optical Parameters of ROXYTHROMYCIN**

S.NO	PARAMETER	ROXYTHROMYCIN
1	$\lambda$ Max (nm)	420 nm
2	Beer's Law Limit ( $\mu$ g/mL)	20-70
3	Correlation Coefficient( $r^2$ )	0.9840
4	Regression Equation ( $Y= a+bc$ )	0.017X-0.0860
5	Intercept (a)	0.0860
6	Slope (c)	0.017
7	SD	18.7082
8	Mean	45
9	Variance	350
10	LOD (%)	1.313
11	LOQ (%)	3.980

**Selection of Solvent:**

Deionised Water and phosphate buffer of pH 7.4[21] are used throughout the analysis.

**Selection of Method and Wave Length:**

UV scan range of 380 nm to 500 nm was selected for the proposed method of Roxithromycin. The wavelength corresponding to maximum absorbance was found at 420 nm and calibration curve was taken at 420 nm. The intercept of calibration line of the drug was determined by Linear regression Analysis shown in fig:4.

**Preparation of standard stock solution and calibration curve:**

The 10 mg of standard (pure) drug of Roxithromycin is weighed accurately and dissolved in 100 ml Deionised water then transferred into 100 ml volumetric flasks to prepare 1000  $\mu$ g/mL[20] stock solution of Roxithromycin. Then to get different aliquots of 2 to 7 ml of standard stock solution were transferred into series of 10 ml volumetric flasks and made upto mark by adding Deionised water to get the given concentration range. To each flask 2mL of phosphate buffer of pH 7.4 solution is added, then all stock solutions of the drug were scanned in the UV scan range of  $\lambda$ max ( $\lambda$ max) 380 nm to 500 nm to determine maximum absorbance for this method. The calibration curve was plotted in the concentration range of 20-70  $\mu$ g/mL shown in fig:5. The wavelength corresponding to maximum absorbance of Roxithromycin measured at 420nm against Deionised water as blank shown in fig:4.

**Preparation of sample solution**

For the analysis of Roxithromycin two commercial brands namely Rotip (75mg) and Roxyfin (75mg) tablets were purchased from Medwin pharmacy, Hyderabad. Twenty tablets of the drug was weighed accurately and powdered, then 10 mg of the drug in powdered form dissolved in 100 ml of Deionised water and sonicated for few minutes and filtered by using whatmann filter paper No.42. The filtrate of 10 $\mu$ g/mL concentration is taken in a six 10 ml volumetric flasks. To each 10 ml flask 2mL of phosphate buffer of pH 7.4 solution is added. Then absorbance of Roxithromycin measured at 420nm against Deionised water as blank

**Validation of Method[22]:**

The spectrophotometric estimation of Roxithromycin is validated as per the directions of International conference on Harmonization to determine linearity, precision, accuracy, LOD and LOQ of the proposed method .

**Linearity and Range :**

Standard stock solution of Roxithromycin in appropriate dilution were assayed as per the proposed method According to Beer's – Lambert's law the concentration range of Roxithromycin was found to be 20-70 µg/ mL ,So that the calibration curve in the figure : 5 is linear in the given concentration range.

**Precision :**

The precision of the proposed method of Roxithromycin was estimated by using drug concentration of Roxithromycin were analyzed six times in a day (intra-day precision) and six continuous days (inter-day precision). Data is given in the table-5

**Accuracy :**

The Accuracy of the proposed method of Roxithromycin was estimated by using standard addition method .This process is carried out by adding different amounts namely 80% ,100% and 120% of the pure sample of the drug to the pre-analysed formulation. Accuracy data of the drug is shown in the table-5

**LOD and LOQ:**

LOD is Limit of Detection and LOQ is Limit of Quantitation . The LOD and LOQ of Roxithromycin were determined (Table : 4) by using standard deviation of the response and slope approach as per the directions of International Conference on Harmonization (ICH) guidelines[22]. The limits of detection (LOD) is calculated by using the equation  $LOD = \frac{3s}{k}$  Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) The limits of quantitation (LOQ), is calculated by using the equation  $LOQ = \frac{10S}{K}$  Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean).

**Recovery Studies of Roxithromycin :**

Recovery of Roxithromycin were performed to know the accuracy of the proposed method . This process is done by adding a known quantity of pure drug to a pre-analysed sample. The result of analysis of the drug is notified in the table: 6

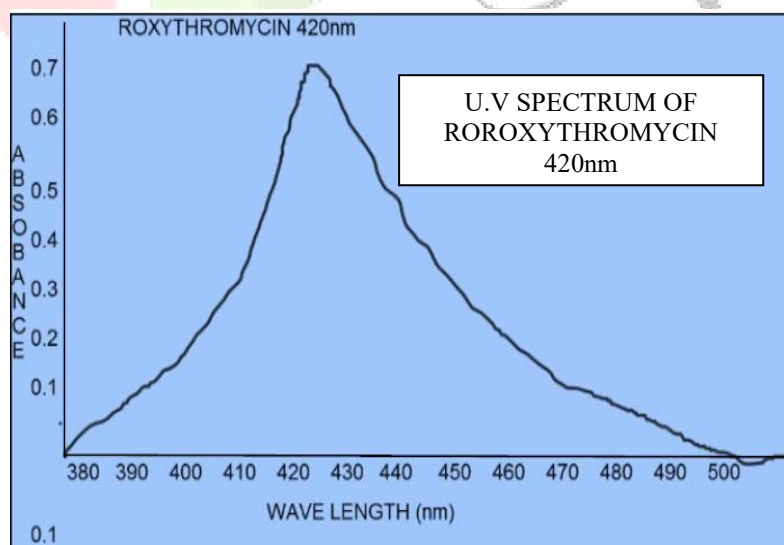
**FIG :4 U. V. SPECTRUM OF ROXYTHROMYCIN**

FIG : 5 Calibration Curve of Roxithromycin

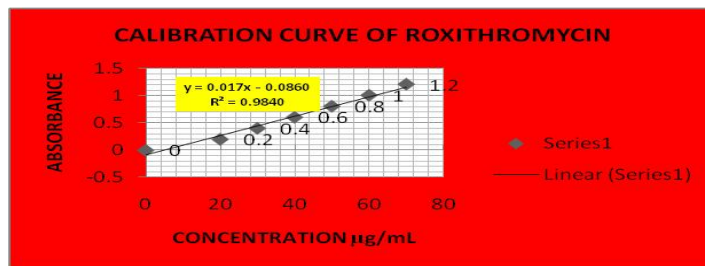


Table no.5 Precision &amp; Accuracy of Roxithromycin

S.no	Name of the sample	Labeled Amount (mg/capsule)	Amount Found* (mg)	Precision	
				Inter day	Intra day
1	Rotip	75	74.23	0.0061	0.0068
2	Roxyfin	75	74.01	0.0075	0.0082

(\*average of 6 determinations)

Table no.6 Recovery studies of Marketed Formulations of Roxithromycin

S.no	Name of the sample	Labeled Amount (mg/capsule)	%Level	Amount Found* (mg)	% Recovery
1	Rotip	75	120	74.23	98.9733
2	Roxyfin	75	80	74.01	98.68

## RESULTS AND DISCUSSION

The U.V Spectrum of standard stock solutions of Nitazoxanide and Roxithromycin shows absorption maximum at 340 nm and 420nm respectively, then the calibration curve is obtained by plotting a graph of absorbance versus concentration, the Beer – lamberts' law was verified from the data of calibration curve of the drug under investigation. The calibration curve of the Nitazoxanide and Roxithromycin is shown in the figures 4 and 5 respectively. The linearity was observed between 4-20 µg/ mL for Nitazoxanide and 20-70µg/ mL for Roxithromycin. The graph of this drug shows a straight line with correlation coefficient of 0.9920 for Nitazoxanide and 0.9840 for Roxithromycin. The assay method of the both drugs was validated by the accuracy and precision of the proposed method shown in tables of 2 and 5. The % recovery of 98.4-98.6 shows accuracy of the proposed method. The validated optical, statistical parameters, LOD and LOQ data of Nitazoxanide is given in table: 1 where as for Roxithromycin is given in table :4

## CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise, economic and can be used for routine quality control analysis of Nitazoxanide and Roxithromycin in bulk as well as in pharmaceutical dosage form.

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