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Development and Validation of Spectrophotometric and Colorimetric Method for the determination of Nitazoxanide in its Bulk and Pharmaceutical Dosage Form (Tablets)

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Abstract: Two simple and sensitive spectroscopic methods in UV region and Visible region were developed for the estimation of Nitazoxanide in its pharmaceutical dosage forms.Method A is based on Nitazoxanide showing its absorption maxima at 238.3nm in acetonitrile and water(9:1).The method B is based on the reaction of Nitazoxanide with 1ml,1% ferric chloride and 2ml,0.1%MBTH to produce a greenish color ,characteristic light absorption at 732nm.These method obey Beer- Lambart law at a concentration range of 5-40mcg/ml and 50-250mcg/ml respectively. The % recoveries were found out to be 98.75 – 100.45.The results obtained with the proposed methods are in good agreement with the labeled amounts when tablet dosage forms were analysed.
Keywords: Nitazoxanide,MBTH,Spectroscopy,Method Development.

INTRODUCTION

Nitazoxanide is chemically N-(5-nitrothiazoly) salicilamide acetate which is a new antiprotozoal drug used in the treatment of c ryprtosporidiosis in immunecomprised patients including those with AIDS or HIV infectuion[1].It is used in helminth infections. The anti-protozoal activity of nitazoxanide is believed to be due to interference with the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme dependent electron transfer reaction which is essential to anaerobic energy metabolism.It has also been shown to have activity against influenza A virus. The mechanism appears to be by selectively blocking the maturation of the viral hemagglutinin at a stage preceding resistance to endoglycosidase H digestion. This impairs hemagglutinin intracellular trafficking and insertion of the protein into the host plasma membrane [2-4] Though not official in any pharmacopoeia, sufficient literature has not been found for its qualitative and quantitative estimation.A survey of literature reveals that very few method & solvents were available for the estimation of Nitazoxanide, but suffer from one disadvantage to the other, such as low sensitivity, lack of selectivity & simplicity.Hence it is proposed to improve the existing methods and to develop accurate, precise sensitive results for the assay of Nitazoxanide, in pharmaceutical dosage forms adopting different available analytical solvent and different techniques like UV-visible spectrophotometry.

MATERILS AND METHODS

Materials

Pure nitazoxanide, used, was gifted by Alembic Pharmaceutical, Borada, Gujurat, India. Solvents such as methanol, acetonitrile were of AR grade (Merck) used. Aqueous solutions of MBTH(0.1%)and ferric chloride(1%w/v) was freshly prepared. Tablets of Natezox were available in the local market. The spectrophotometric observation was made with JASCO V-630 spectrophotometer with 1cm matched quartzed cell.

Preparation of sample solution

Standard stock solution of nitazoxanide was prepared by dissolving 25 mg of drug in 25 ml of Acetonitrile : water (9 : 1) in volumetric flask to get a concentration of 1000µgm/ml for method A and for method B in methanol and water.

Preparation of calibration curve

The prepared stock solution was further diluted with Acetonitrile : water (9 : 1) to get a concentration of 100 µgm/ml. Different aliquots of drug solution were transferred separately in to a series of 10 ml volumetric flask and diluted to 10 ml with the solvent. Then the absorbances were measured at λmax 238.3nm against blank.

In method B **20**mg of drug (nitazoxanide) taken and dissolved in 6 ml of methanol and

2ml 4N HCl .0.24 gm of zinc dust was mixed slowly for reduction,kept for one hour for completion of reaction then filtered with whatmann filterpaper and finally the volume was made upto 20ml with methanol.

Different concentration of drug solution previously reagent mixed, i.e. 1ml ferric chloride (1%) and 2 ml MBTH (0.1%) were transferred separately in to a series of volumetric flasks. Then the absorbencies were measured at λmax 732nm against reagent blank. [5-6].The reaction is depicted in figure-1. The observations of Nitazoxanide concentrations and absorbances is shown in Table:1.

ASSAY RESULTS

For analysis of commercial formulation, 20 tablets containing nitazoxanide of each marketed formulation taken and powdered. The powder equivalent to 10 mg nitazoxanide was taken and dissolved in 10 ml of Acetonitrile : water (9 : 1), sonicated and filtered. Suitable aliquots from the filtrate were taken further diluted with the same solvent to get the concentrations within the linearity range of standard curve. The absorbances of the prepared solutions were measured at 238.3 nm against a blank method A and for method B the powder equivalent to 10 mg nitazoxanide taken and dissolve in methanol, sonicated and filtered. Suitable aliquots from the filtrate were taken further diluted with the water to get the concentrations within the linearity range of standard curve. Then to each dilution 1ml ferric chloride (1%) and 2 ml MBTH (0.1%) were mixed, so that greenish colour developed. The absorbances of the prepared solutions were measured at 732 nm against a blank.The results are given in Table:2.

Table: 1 PLOTTING OF CALIBRATION CURVE

METHOD A		**METHOD B**	
Concentration of nitazoxanide in µgm/ml	**Absorbance 238.3nm Wavelength**	**Concentration of nitazoxanide in µgm/ml**	**Absorbance at 238.3nm Wavelength**
5	.1458	50	.027
10	.3179	70	.074
12	.3731	90	.1058
15	.5061	100	.1194
20	.6262	150	.2144
25	.8025	200	.3135
30	.9415	250	.4044
35	1.1238		
40	1.2895		

Table:2 ASSAY RESULTS OF THE FORMULATION

Brand Name of the Tablet	Labeled amount of drug(mg)	Method A		Method B	
		Amount (mg) Found by the proposed method (n=3)	% Mean (±S.D.) Labeled amount (n=3)	Amount (mg) Found by the proposed method (n=3)	% Mean (±S.D.) Labeled amount (n=3)
Natezox	500	502.9	100.53 ± 0.19	498.58	99.71 ± 0.27
Nitazet	500	501.02	100.21±0.12	498.3	99.27±0.19

VALIDATION

Precision:-

To determine the precision 7 days measurement (intra-days and interday) were computed with coefficient of variation (C.V. %) for replicate samples (n = 5) using concentration 10, 20, 40µg/ml for method A and 100, 150, 200, 250µg/ml for method B.

Both the intra-day and interday samples were calibrated with standard curve concurrently prepared in the same day of analysis.The observations are given in Table:3.

Accuracy:-

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amount (80%, 100%. 120%) of bulk sample of nitazoxanide within the linearity range and added to the pre-analyzed formulation 10µgm/ml. from that percent recovery values were calculated. The observations are given in Table:4.

Table: 3 PRECISION OF THE PROPOSED FOR METHOD A and METHOD B

METHOD-A					METHOD-B				
Concentration of Nitazoxanide (µg/ml)	Observed concentration of Nitazoxanide(µg/ml)				Concentration of Nitazoxanide (µg/ml)	Observed concentration of Nitazoxanide(µg/ml)			
	Intra day		Inter day			Intra day		Inter day	
	Mean (n=3)	COV (%)	Mean (n=3)	COV (%)		Mean (n=3)	COV (%)	Mean (n=3)	COV (%)
10	10.08	1.13	10.07	0.41	100	98.63	1.45	97.89	1.80
20	20.36	0.47	20.45	0.105	150	148.36	1.4	148.04	0.43
40	40.71	0.101	40.94	0.38	200	198.05	0.87	198.05	0.74
					250	250.1	0.36	240.94	0.38

Table:4 RECOVERY STUDIES FOLLOWING METHOD A

Sample ID	Concentration (µgm/ml)		Theoretical Content (µgm/ml)	% recovery of pure drug		Statistical analysis		
	Pure drug	Formulation		Method A	Method B		Method A	Method B
S1 : 80%	8	10	9	99.16	98.75	Mean	99.33	99.60
S2 : 80%	8	10	9	98.33	99.60	SD	0.894	0.694
S3 : 80%	8	10	9	100.5	100.45	%RSD	0.90	0.696
S1 : 100%	10	10	10	99.8	99.5	Mean	99.53	99.77
S2 : 100%	10	10	10	99.35	99.79	SD	0.192	0.212
S3 : 100%	10	10	10	99.45	100.02	%RSD	0.19	0.213
S1 : 120%	12	10	11	99.63	100.14	Mean	100.07	99.85
S2 : 120%	12	10	11	100.13	99.84	SD	0.337	0.246
S3 : 120%	12	10	11	100.45	99.54	%RSD	0.336	0.245

Linearity

To establish the range of linearity between drug concentration and detector response and the drug concentrations of 5-40µg/mL for method A and 50, 70, 90, 100, 150, 200 & 250µg/mL for method B were used. Five replicates of analyte were measured.

Specificity and Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. The specificity of the method was evaluated with regard to interference due to presence of any other excipients.

Limit of detection and quantitation

The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where σ is the standard deviation of the blank and S is the slope of the calibration plot.

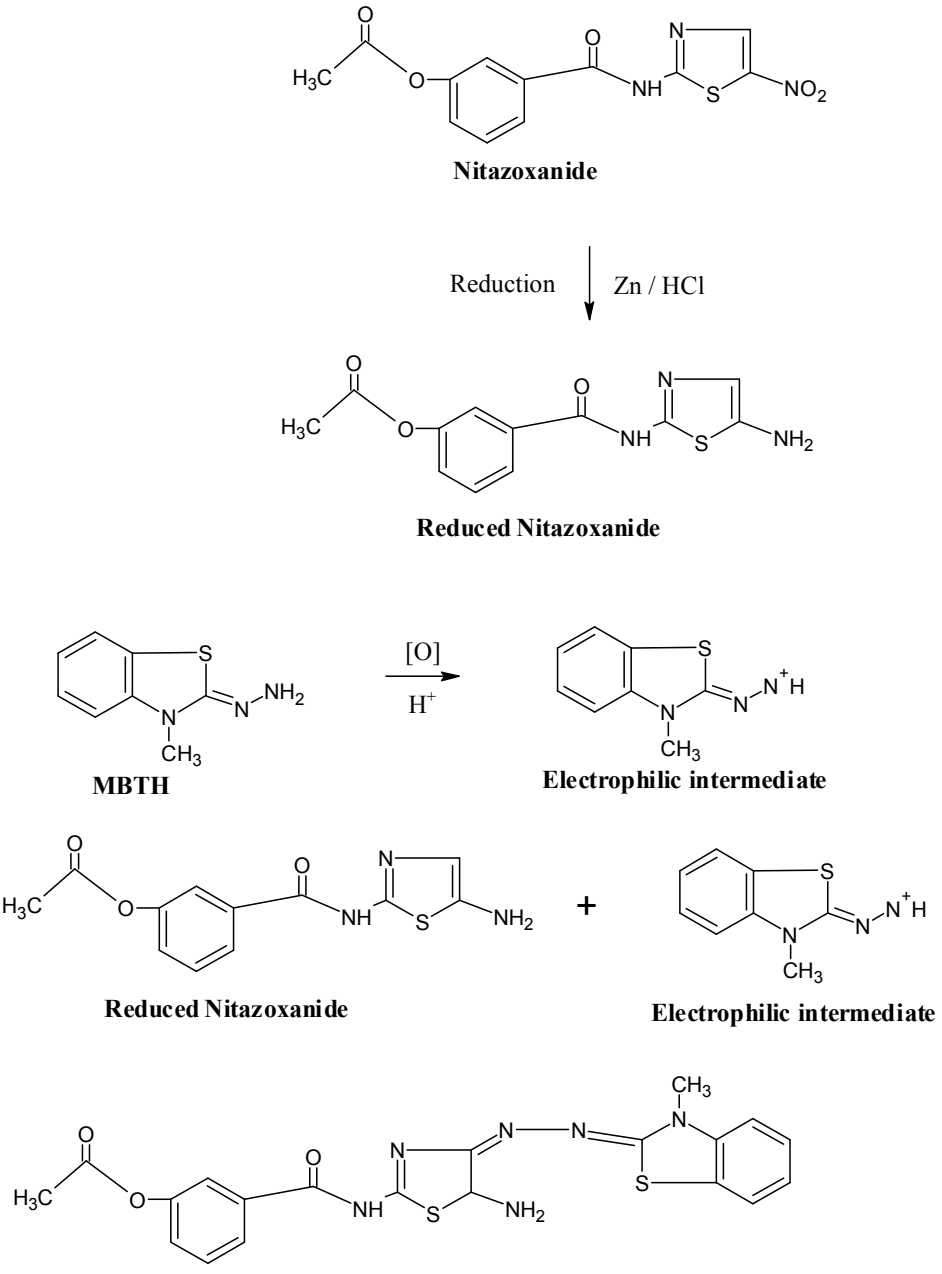

Figure-1:reaction of Nitazoxanide(reduction)with MBTH

Table: 5

Parameters	Results
λ max (nm)	732
Molar extinction coefficient	359.062×10^{3}
Beer's law (µg/mL)	50-250
Sandell's sensitivity (µg/cm^2/0.001)	87.03×10^{-2}
Limit of detection (LOD)	0.1147
Limit of quantitation (LOQ)	0.3824
Regression equation: $y = a + bx$	
Intercept (a)	0.0019
Slope (b)	0.0706
Correlation coefficient	0.9992
N	3

RESULT AND DISCUSSION

The method approached gives novelty to the development procedure. Presence of nitro group in the sample structure enables for reduction in the primary stage of reaction and then further reaction proceeds with MBTH. The optical characteristics show results which obeys Beer-Lambert limit and is summarized in Table:1.The precision value i.e, the interday and intraday standard deviation results were found to be within the limit and is summarized in Table: 3 .The % recovery of pure drug are found to be within the limit and standard deviation is 0.694 calculated from Statistical analysis which has been summarized in Table :4. The quantitative parameters for determination of Nitazoxanide in pharmaceutical dosage form are given in Table:5.

CONCLUSION

A new UV and a new colorimetric method has been developed for simultaneous analysis of Nitazoxanide and its tablet formulation. It has been shown above that the method is cost effective and less time consuming, accurate, reproducible, repeatable, linear, precise and selective, proving the reliability of the method. The scan results were very clear and obey Beer's law to a certain extend, which enables rapid quantitation of many samples in routine quality control. A minimal interference was observed from excipient. These results show the method could find practical application as a quality control tool for analysis of Nitazoxanide from their different pharmaceutical dosage forms in a quality control laboratory.

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