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Research Article

Spectrophotometric Methods for the Determination of Nitaoxanide in Bulk Drug and its Pharmaceutical Formulation

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ABSTRACT

Objectives: A simple spectrophotometric method was developed and validated for the determination of Nitaoxanide in pharmaceutical dosage forms. Two visible spectrophotometric methods have been described for the assay of Nitaoxanide bulk form or dosage forms.
Methods: Method A is based on the formation of Schiff's base and it was condensed with 4 hydroxybenzaldehyde. Method B is based on diazotization and coupling method with phluroglucinol. The methods are done in UV Visible spectrophotometric method having maximum absorbance at 460 nm.
Results: Regression analysis of Beers law plots showed good concentration range of 10-50µg/ml for method A and B and gives reproducible results.
Conclusion: Due to its simplicity of the method it may be used for determining Nitaoxanide in bulk and dosage forms.

Keywords: Spectrophotometry, Nitaoxanide, 4 hydroxybenzaldehyde, phluroglucinol

INTRODUCTION

Nitaoxanide is a synthetic nitro thiazolyl-salicylamide derivative approved for the treatment of infectious diarrhea [1] caused by Cryptosporidium parvum and Giardia lamblia. This novel agent has a broad spectrum of activity against many other gastrointestinal pathogens, including bacteria, round worms, flat worms and flukes. Nitaoxanide is used in many areas of the world, especially in Central and South America, as broad-spectrum parasiticidal agents in adults and children. In oral administration it is rapidly hydrolyzed to its active metabolite, Nitaoxanide, which is observed 1-4 hours after administration. It is excreted in the urine, bile and faeces. Chemicaly known as 2-[(5-nitro-1, 3-thiazol-2-yl) carbamoyl] phenyl acetate. A number of methods such as spectrophotometric [2-8], colorimetric [9.10] HPLC [11-13], HPTLC [14] , RP HPLC [15-18] for the estimation of Nitaoxanide. The present communication describes 3 UV spectroscopic methods in bulk form and dosage form by using different reagent 4-hydroxy benzaldyhyde and phluroglucinol having maximum absorbance at 460 and 450 nm.

EXPERIMENTAL

INSTRUMENTS

A Perkin Elmer EZ 301 UV Visible double beam spectrophotometer with 1cm matched quartz cell was used for the spectral and absorbance measurements. The analytical balance shimadzu is 0.1 mg. The electronic balance AY220.

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REAGENTS

All the chemicals and reagents were of analytical grade and the solutions were prepared in distilled water. Aqueous solution of 4 hydroxybenzaldehyde and Conc. HCl were prepared for method A. Aqueous solutions of sodium nitrite and Ammonium sulphate and phluroglucinol were prepared for method B for diazotization reaction and coupling method.

ANALYTICAL PROCEDURES

METHOD A[19]

Preparation of 4-hydroxy benzaldyhyde (2%)

It was prepared by dissolving 2g of 4-hydroxy benzaldyhyde in 100ml methanol.

Preparation of standard stock solution

25mg of Nitaoxanide was dissolved in 5ml of methanol and was treated with 2.5ml 5N HCl and 200mg Zn powder with continuous stirring for 20mts at room temperature. It was then filtered and the residue was washed with methanol. Then the volume was made upto25ml with methanol (Stock solution I, 1000µg/ml)

Absorption spectra of coloured species

5ml of standard stock solution I was pipetted into 50ml volumetric flask and it was diluted to 50ml with methanol (Stock solution II, 100µg/ml). From this 3ml was pipetted out into 10ml volumetric flask and 1ml of 2% 4-hydroxy benzaldyhyde, 1ml of Conc. HCl were added and kept aside for 5mts. The volume was made up to 10ml with methanol. The final volume concentration of the solution was 30µg/ml. The absorbance was measured between 340-480nm against reagent blank. Readings were shown in Table no:1 and plotted in Graph no:1

METHOD B

Spectrophotometric determination of Nitaoxanide by diazotization coupling method using phluroglucinol[20]

Preparation of 4N HCl

It was prepared by dissolving 34ml of conc. HCl in 100ml distilled water.

Preparation of 0.1%w/v sodium nitrite

It was prepared by dissolving 0.1g of sodium nitrite in 100ml distilled water.

Preparation of 0.5%w/v of ammonium sulphate

It was prepared by dissolving 0.5g of ammonium sulphate in 100ml distilled water.

Preparation of 0.5%w/v phluroglucinol

It was prepared by dissolving 0.5g of phluroglucinol in 100ml distilled water.

Preparation of standard stock solution

10mg of Nitaoxanide was accurately weighed and dissolved in 5ml methanol. The methanolic solution of Nitaoxanide was treated with 200mg of Zn powder and 2.5ml of 4N HCl and kept aside for 1hour at room temperature. The solution was filtered and the volume was made up to 10ml with methanol. (1000 µg/ml).

Absorption spectra of coloured species

5ml of standard stock solution I was pipette into 50ml volumetric flask and made up with methanol. From this 3ml was pipetted out into 10ml volumetric flask followed by the addition of 1ml conc. HCl, 1ml of 0.1%sodium nitrite, 1ml of 0.5%w/v ammonium sulphate and 1ml of 0.5%w/v phluroglucinol were added. Finally the volume was made up to 10ml with methanol. The final concentration of the solution was 30µg/ml. The absorbance was scanned between 400-500nm against reagent blank. Readings were shown in Table no 2 and plotted in Graph no 2.

BEERS'S LAW PLOT

Beer's law states that the fraction of the monochromatic radiant energy absorbed on passing through a solution is directly proportional to concentration of the absorbent.

$Log_{10} I_0/I_t = K.C$

where,

K-Proportionality constant

C-Concentration

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I_0-I intensity of incident light
I_t-Intensity of transmitted light

Having fixed other parameter, Beer's law plot was constructed by measuring the absorbance of various concentration of drug solution against reagent blank.
5ml of stock solution-1 was pipetted in 50ml volumetric flask and made up to 50ml with methanol. The concentration of solution was 100µg/ml (Stock solution-II). From stock solution II, various aliquots of 1ml, 2ml, 3ml, 4ml and 5ml were pipetted out into a 10ml volumetric flask followed by addition of 1ml, 2% 4-hydroxybenzaldehyde and 1ml conc. HCl and kept aside for 5mts.The volume was made up to 10ml with methanol to produce concentration in the range of 10-50µg/ml. Absorbance of each solution was observed at 460nm against reagent blank. The readings were recorded in Table no 3and graphically plotted in Graph no 3.

INTERFERENCE STUDIES

The interference studies of additives used in the formation of tablet were done by distributing them individually in distilled water and set aside for 10mts before filtering. The filtrate was diluted and preceded as per tablet assay and the absorbance was measured at 460nm against reagent blanks. This procedure was repeated 5 times of each additive and the average value for each additive was given the following Table no 4.

RECOVERY STUDIES

In order to justify the reliability and suitability of the proposed method, recovery studies were carried out. The recovery experiment was performed on Nitaoxanide tablet. The powder equivalent to 25mg was accurately weighed and dissolved in 5ml methanol and was treated with 200mg Zn powder and 2.5ml 4N HCl with continuous stirring for 1hr at room temperature. It was filtered and washed with methanol. An aliquot of 5ml of standard solution (1mg/ml) of pure sample of Nitaoxanide was added to the flask. It was shaken well and the volume was made upto 25ml with methanol and the procedure for the assay of Nitaoxanide was followed. The experiment was repeated 5 times. The results were recorded in Table no 5. The percentage recovery was calculated by using the formula:-

$$\% \text{ of Recovery} = \frac{\text{Avg content from recovery} - \text{Avg content from assay}}{\text{Amount of std drug added}} \times 100$$

PRECISION [21]

The precision of an analytical method is the degree of reproducibility among the individual test results when the procedure was applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as standard deviation or percentage RSD of a series of measurements. The precision study was done based on the data obtained from Table no: 7 and the results were shown in Table no 6.

ACCURACY [22]

Accuracy is the closeness of the test results obtained by the procedure to the true value. Accuracy of the proposed method was evaluated by comparing the average value obtained by the proposed method with that of reported method (standard method) and results were given Table no 7.

RESULTS AND DISCUSSION

METHOD I

SPECTROPHOTOMETRIC DETERMINATION – I

Under the experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. The drug obeys Beer's law in the concentration range of 10-50µg/ml with coefficient of variation of 0.2735 at λ_{max} 460nm. The percentage recovery value was

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104.93% and the results of interference studies shows that the excipients have no effect in the absorption of drug in this method. Results of this method were compared with standard method and the value obtained in the proposed method was closely agreed with the standard method.

The proposed method was practicable for routine analytical purpose of Nitaoxanide in its formulations.

METHOD II

SPECTROPHOTOMETRIC DETERMINATION – I

Absorption spectral analysis showed the maximum wavelength at 450nm. The drug obeys Beer's law in the concentration range of 10-50µg/ml. The percentage recovery value was 101.812% and the results of interference study shows that the excipients have no effect in the absorption of drug in this method. The results obtained from the tablet assay showed low range of coefficient of variation in percentage about 0.1891. The value of standard deviation and %RSD were low indicating that the proposed method is précised. The proposed method was simple, precise and reproducible for the routine estimation of Nitaoxanide in bulk drug as well as in its pharmaceutical formulation.

Absorption spectra for the drug Nitaoxanide by using 4-hydroxybenzaldehyde

TABLE NO: 1

WAVELENGTH(nm)	ABSORBANCE
380	0.087
390	0.094
400	0.112
410	0.139
420	0.173
430	0.214
440	0.250
450	0.285
460*	0.291
470	0.268
480	0.233
490	0.158
500	0.090

The maximum absorbance was measured at 460nm.

Calibration curve for nitazoxanide using 4-hydroxy benzaldehyde

GRAPH NO: 1

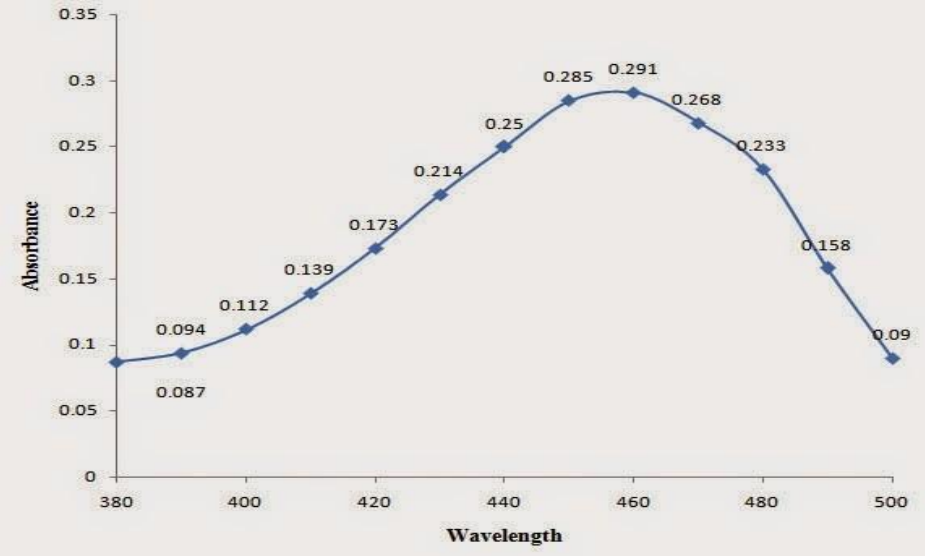

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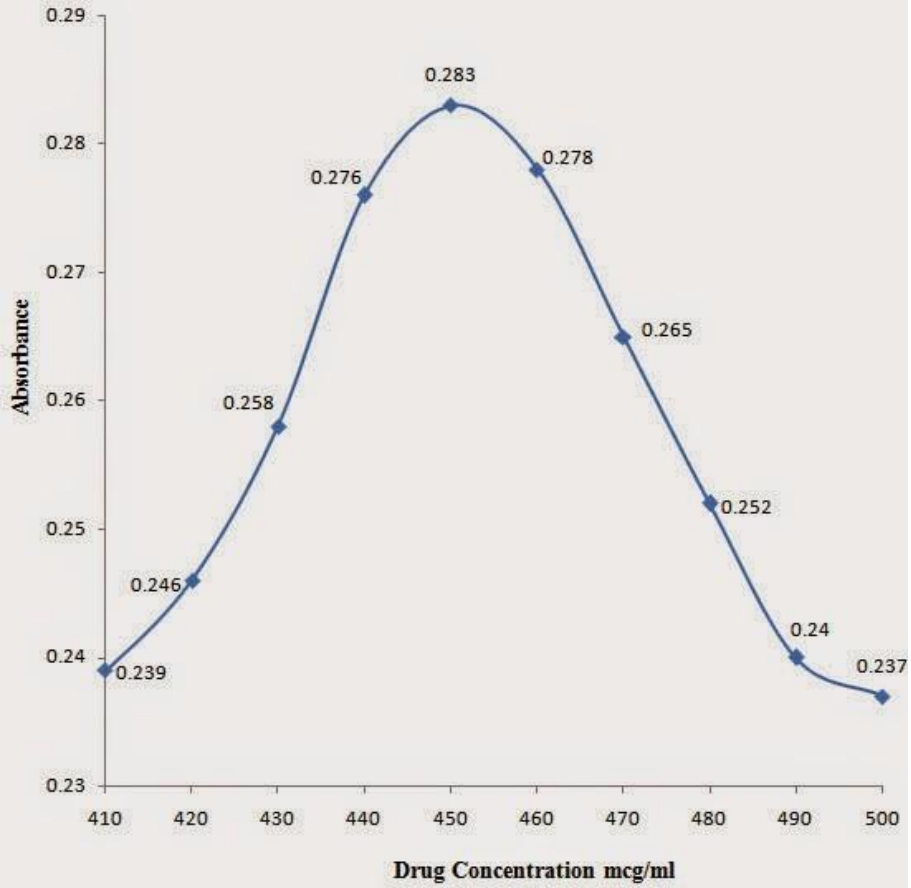
TABLE NO 2

WAVELENGTH	ABSORBANCE
410	0.239
420	0.246
430	0.258
440	0.276
450*	0.283
460	0.278
470	0.265
480	0.252
490	0.240
500	0.237

The maximum absorbance was measured at 450nm.

Calibration curve for nitazoxanide using phluroglucinol

GRAPH NO 2



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BEER'S LAW PLOT

TABLE NO 3

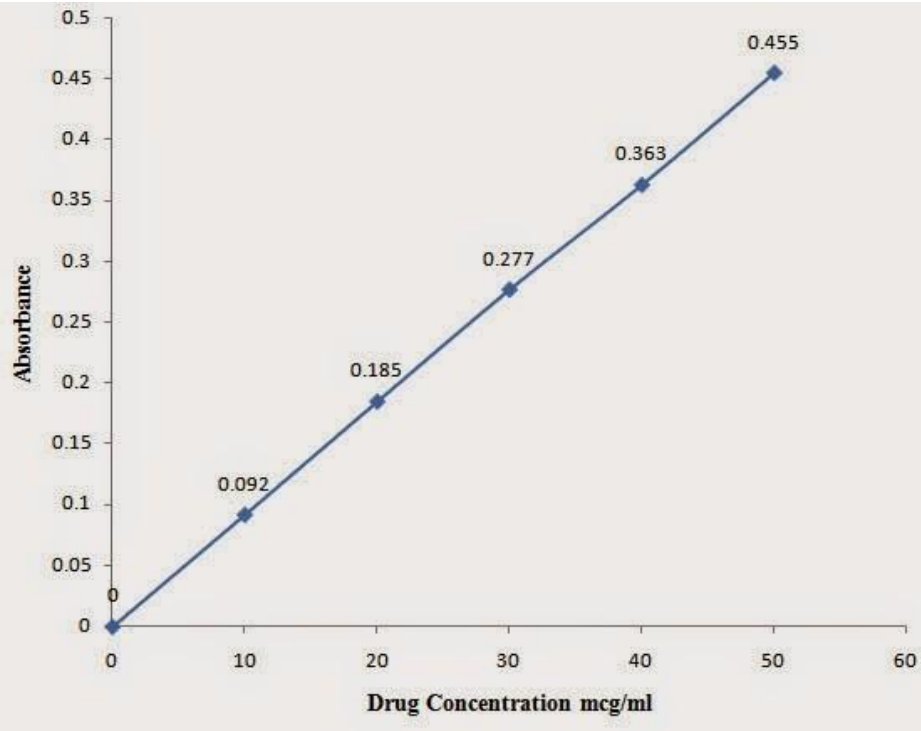
Drug concentration (µg/ml)	Absorbance
10	0.092
20	0.185
30	0.277
40	0.363
50	0.455

Beer's law was obeyed in the concentration range of 10-50µg/ml

Beers Law plot

Calibration curve for nitazoxanide using 4-hydroxy benzaldehyde

GRAPH NO 3



INTERFERENCE STUDIES

TABLE NO 4

SI no:	Name of the excipients	Absorbance at 460nm
1	Talc	0.001
2	Lactose	0.003
3	Starch	0.002
4	Magnesium state	0.002

The results show that the effect of interference studies in this spectroscopic method was found to be negligible.

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TABLE NO 5

Sl no	Brand name	Avg wt of tablet (mg)	Wt. of std drug (mg)	Std absorbance	Wt. of tablet powder (mg)	Pure drug added	Abs of recovered sample	%recovery
1	NIZONIDE - 500mg	1261.9	25.7	0.30	63.3	5	0.298	104.93%
2					63	5	0.296	
3					64	5	0.300	
4					64.1	5	0.301	
5					63.6	5	0.299	

TABLE NO 6

Sl no:	Name of tablet	Standard Deviation (SD)	Coefficient of variation(%RSD)
1	NIZONIDE	1.3623	0.2735

TABLE NO 7

Sl no:	Amount obtained by standard method	Amount obtained by proposed
1	499.05mg	498.73mg

CONCLUSION

A new spectrophotometric method has been developed for simultaneous analysis of Nitaoxanide and its tablet formulation. The proposed methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of Nitaoxanide in bulk drug and its pharmaceutical dosage forms in a routine manner. Analysis of authentic samples containing Nitaoxanide showed no interference from the common additives and excipients. The results were very clear and obey Beer's law, which gives rapid quantitation of many samples in quality control. The method used as a quality control tool for analysis of Nitaoxanide in a quality control.

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