Asian Journal of Chemistry Vol. 19, No. 4 (2007), 2527-2530

Visible Spectrophotometric Determination of Nitazoxanide in Bulk and Pharmaceutical Dosage Forms

K.V. LAKSHMI NARAYANA and Y.N. MANOHARA*
Department of Pharmaceutical Analysis, National College of Pharmacy
Shimoga-577 201, India
E-mail: manohara_yn@yahoo.com

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of nitazoxanide, in bulk drug and pharmaceutical dosages forms. Methods were based on the formation of light yellow and bluish green coloured chromogens of drug with *p*-dimethylaminocinnamaldehyde and folin-ciocalteu reagent, which were measured at 390 and 765 nm and linearity in the concentration range of 6-30 and 2-10 µg/mL of the drug, respectively. The developed methods were found to be precise and accurate from the statistical validation of the analysis.

Key Words: Spectrophotometric, Nitazoxanide.

INTRODUCTION

Nitazoxanide[1,2] is chemically, N-(5-nitro-2-thiazoly)salicylamide acetate and it is a new antiprotozoal drug used in the treatment of cryptosporidiosis in immunocompromised patients, including those with AIDS or HIV infection. It has been used in helminth infections[3-7]. It is not official in any pharmacopoeia and spectrophotometric analytical reports are not found in literature for its quantitative estimation in bulk drug and pharmaceutical dosage forms.

Two simple and sensitive visible spectrophotometric method have been developed for the quantitative estimation of nitazoxanide after converting it to its reduced from, N-(5-nitro-2-thiazoly)salicylamide acetate, by using zinc dust and 4N hydrochloric acid in methanol at room temperature.

Method **A** is based on the reaction of reduced nitazoxanide with *p*-dimethylaminocinnamaldehyde (PDACA) to form light yellow coloured chromogen with an absorption maximum (λ_{max}) at 390 nm and Beer's law is obeyed in the concentration range of 6-30 µg/mL.

Method **B** is based on the reaction of reduced nitazoxanide with folin-ciocalteu reagent in presence of sodium carbonate to form bluish green coloured chromogen exhibiting absorption maximum at 765 nm and obeyed Beer's law in the concentration range of 2-10 µg/mL. These methods have

been successfully extended to the pharmaceutical dosage forms (tablets) containing nitazoxanide.

EXPERIMENTAL

A Shimadzu UV-Visible double beam spectrophotometer (model 1601) with 1 cm matched quartz cells were used for spectral measurements.

All chemicals used are of A.R. grade from S.D. Fine Chem., Mumbai (i) ethanolic solution of *p*-dimethylaminocinnamaldehyde was prepared by adding 30 mL of 95 % ethanol, 180 mL of 1-butanol and 30 mL of conc. HCl to 1 g of *p*-dimethylaminocinnamaldehyde, (ii) folin-ciocalteu reagent was prepared and used after dilution with water (1:2), (iii) zinc dust, (iv) 4N hydrochloric acid, (v) methanol and (vi) sodium carbonate (12 % w/v in water)

Working standard of drug and sample solutions: About 100 mg of nitazoxanide (pure or formulation) was accurately weighed and dissolved in 30 mL of methanol. The methanolic solution of nitazoxanide was treated with 10 mL of 4N hydrochloric acid and 1.2 g of zinc dust was added in portions while shaking. After standing for 1 h at room temperature, the solution was filtered through cotton wool. The residue was washed with 10 mL portions of methanol three times and the total volumes of the filtrate were made up to 100 mL with methanol (1 mg/mL). The final concentration of reduced nitazoxanide was brought to 100 µg/mL with methanol. In case of formulation, one brand of commercially available tablets were analyzed by the proposed methods. 10 Tablets of nitazoxanide each containing 500 mg were accurately weighed and powereded. Tablet powder equivalent to 100 mg of nitazoxanide was taken.

Assay

Method A: Aliquots of reduced nitazoxanide ranging from 0.6-3.0 mL (1 mL = 100 µg/mL) was transferred into a series of volumetric flasks. To each of the above 1 mL of ethanolic PDACA was added. After 5 min, the volume was brought to the mark with methanol. The absorbance of light yellow coloured chromogen was measured at 390 nm against reagent blank. The coloured species was stable for more than 5 h. The amount of nitazoxanide present in the sample was computed from calibration curve.

Method B: Aliquots of reduced nitazoxanide ranging from 0.2-1.0 mL (1 mL = 100 µg/mL) was transferred into a series of 10 mL volumetric flasks. To each of the above add 2.5 mL of sodium carbonate solution followed by 2 mL of folin-ciocalteu reagent and a reaction time of 15 min is allowed and the solution is made upto 10 mL with distilled water. The absorbance of the bluish green coloured chromogen was measured at 765 nm against reagent blank. The coloured species was stable for more than 6 h. The amount of nitazoxanide present in the sample was computed from calibration curve.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the other fixed by observing the effect produced on the absorbance of the coloured species. Various parameters involved in the coloure development, like the concentration of the differenet reagents and time involved for maximum colour development, were optimized. The optical characteristics and precision of the methods are given in Table-1, together with regression equations (obtained by linear least square treatment) for the calibration plots. The precision and accuracy were found by analyzing eight replicate samples of the known amount of the drug and the results are summarized in Table-1. Two commercially available dosage forms of nitazoxanide are analyzed by both the methods and the results obtained are given in Table-2. As an additional check on the accuracy of methods, recovery experiments were performed by adding known amounts of pure drug to preanalyzed dosage form and percent recovery values obtained are listed in Table-2.

Recovery experiments indicate the absence of interference from the commonly encountered pharmaceutical additives and excipients. The proposed methods can be employed for the routine determination of nitazoxanide in bulk sample and pharmaceutical formulations.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

	Method **A**	Method **B**
λ_{max} (nm)	390	765
Beer's law limits (µg/mL)	6-30	2-10
Molar absorptivity (L mol^{-1} cm^{-1})	9.1165×10^3	2.0346×10^4
Sandell's sensitivity (µg/mL 0.001-absorbance unit)	0.0340	0.0152
Regression equation (Y*)		
Slope (b)	0.0300	0.0670
Intercept (a)	0.0030	0.0072
Correlation coefficient (r)	0.9998	0.9980
RSD (%)	0.34427	0.6763
Range of errors**		
Confidence limits with 0.05 level	0.0020	0.0030
Confidence limits with 0.01 level	0.0030	0.0044

*Y = a + bC where C is the concentration of nitazoxanide in µg/mL and Y is the absorbance at the respective λ_{max}, **for eight measurements.

TABLE-2
EVALUATION OF NITAZOXANIDE IN PHARMACEUTICAL DOSAGE FORMS

Sample (tablets)	Labelled amount (mg)	Amount obtained (mg)*			Recovery (%)**	
		Proposed methods		Reference Method UV	Proposed methods	
		A	B		A	B
1	500	497.2 ± 0.002	498.9 ± 0.004	499.6 ± 0.002	99.52 ± 0.05	99.86 ± 0.03

*Mean and standard deviation of 8 determinations.

**Mean and standard deviation of 8 determinations.

(100 mg of nitazoxanide was added and recovered)

ACKNOWLEDGEMENTS

The authors are thankful to Ind-Swift Limited, Parwanoo (H.P.) for gift sample of nitazoxanide for research and management, National Education Society, Shimoga for providing all facilities to carry out the present work.

REFERENCES

1. S.C. Sweetman, in ed.: Martindale, The Complete Drug Reference, Pharmaceutical Press, London (UK), edn. 33, p. 598 (2002).
2. M.J. O'Neil, The Merck Index, An Encyclopeadia of Chemicals, Drugs and Biologicals, Merck & Co., Inc., NJ, edn. 13, p. 6596 (2001).
3. R. Cravier, *Eur. J. Med. Chem. Chim. Ther.*, **13**, 539 (1978).
4. L. Debreuil, *Antimicrob. Agent Chemother.*, **40**, 2266 (1996).
5. J.R. Murphy and J.C. Friedmann, *J. Appl. Toxicol.*, **5**, 49 (1985).
6. A. Stockis, *Int. J. Clin. Pharmacol. Ther.*, **34**, 349 (1996).
7. J.F. Rossignol and H. Maisonneuve, *Am. J. Trop. Med. Hyg.*, **33**, 511 (1984).

(*Received*: 19 November 2005; *Accepted*: 27 November 2006) AJC-5281