Development and Validation of Spectrophotometric Methods for the Estimation of Nitazoxanide in Tablet Dosage Forms

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Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of nitazoxanide, in bulk drug and pharmaceutical dosage forms. Methods were based on the formation of reddish yellow coloured and green coloured chromogens, which were measured at 544 nm and 715 nm, respectively. The results obtained with the proposed methods are in good agreement with the labelled amounts when tablet dosage forms were analysed.

Nitazoxanide[1,2] (I) is chemically, N-(5-nitro-2-thiazoly) salicylamide acetate, which is a new antiprotozoal drug used in the treatment of cryptosporidiosis in immune-compromised 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 tablet dosage forms.

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

Method A is based on the oxidation of the reduced nitazoxanide with ferric chloride to form reddish yellow coloured chromogen with the absorption maximum at 544 nm and obeyed Beer's law in the concentration range of 100-500 µg/ml. Method B is based on the oxidation followed by complex formation reaction of reduced nitazoxanide with potassium ferricyanide in presence of ferric chloride to form green coloured chromogen with absorption maximum at 715 nm and Beer's law is obeyed in the concentration range of 20-100 µg/ml. Spectrophotometric parameters are established for the standardization of the methods including statistical analysis of data. These methods have been successfully extended to tablets containing nitazoxanide.

A Shimadzu UV/Vis double beam spectrophotometer (model 1601) with 1 cm matched quartz cells were used for all the spectral measurements. All the chemicals used were of A.R. grade.

About 100 mg of nitazoxanide (pure or equivalent formulations) was accurately weighed and dissolved in 30 ml of methanol. The methanol solution of nitazoxanide was treated with 10 ml of 4 N 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 volume of the filtrate was made up to 100 ml with methanol (1 mg/ml). The final concentration of the reduced nitazoxanide was brought to 100 µg/ml with methanol.

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In case of formulation, commercially available tablets (Nitacure® 500 mg, Alembic, Kanjari) was analysed by the proposed methods. Ten tablets of nitazoxanide, each containing 500 mg of the drug, were accurately weighed and powdered. Tablet powder equivalent to 100 mg of nitazoxanide, was transferred to a 100 ml volumetric flask. The contents were dissolved in methanol and made volume made to 100 ml with methanol. The resulting solution was filtered through the grade-I filter paper and volume made to 100 ml with methanol. Further reduction and dilution was carried out in the same manner as described for standard solution.

In method A, fresh aliquots of the reduced nitazoxanide, ranging from 1-5 ml (1 mg/ml) were transferred into a series of 10 ml volumetric flasks. To each flask, 1 ml of ferric chloride (0.5% w/v) was added and kept for 5 min, for complete colour development. The volumes were made up to the mark with methanol. The absorbance of the reddish yellow coloured chromogen was measured at 544 nm against the reagent blank. The coloured species was stable for more than 7 h. The amount of nitazoxanide present in the sample was computed from the calibration curve.

In method B, fresh aliquots of reduced nitazoxanide ranging from 0.2-1 ml (1 mg/ml) were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of ferric chloride (0.5% w/v) and 1ml of potassium ferricyanide (0.2% w/v) were added and made up to the mark with water. The absorbance of the green coloured chromogen was measured at 715 nm against reagent blank. The colour was stable for more than 8 h. The amount of nitazoxanide present in the sample was computed from calibration curve

The results of the above methods were compared with the results obtained with UV spectrophotometric method. Solution of reduced nitazoxanide in methanol either pure or formulation (1 mg/ml) was prepared. Aliquots of reduced nitazoxanide ranging from 0.2-1.0 ml were transferred into a series of 10 ml volumetric flasks. The volumes were made up to the mark with methanol and the absorbance of the solutions was measured at 302 nm against the solvent blank. The amount of nitazoxanide present in the sample was computed from the calibration curve.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1. The regression analysis using method of least squares was performed for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table 1. The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) calculated from the eight measurements ¾ of the upper Beer's law limits of nitazoxanide are given in Table 1. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for the dosage forms (Table 2) confirms the suitability of the methods for pharmaceutical dosage forms when compared to UV method. The proposed methods are reaction specific and eliminate interference from impurities.

The optimum conditions for colour development for methods A and B were established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of the coloured species and incorporated

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION

Parameters	Method A	Method B
λ_{max} (nm)	544	715
Beer's law limits (μg /ml)	100-500	20-100
Molar absorptivity (l/mol.cm)	3.1960×10^2	2.1475×10^3
Sandell's sensitivity (μg/ml/ cm^2/0.001 absorbance unit)	0.0950	0.0145
Regression equation (Y*)		
Slope (b)	0.0010	0.0069
Intercept (a)	0.0033	0.0024
Correlation coefficient (r)	0.9995	0.9990
% RSD	0.5442	0.9110
Range of errors**		
Confidence limits with 0.05 level	0.001889	0.004255
Confidence limits with 0.01 level	0.002793	0.006296

*Y= bC+a, 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 FORM

Sample (tablets)	Label claim mg/tablet	Amount obtained (mg)*			% Recovery**	
		Proposed methods		Reference method UV	Proposed methods	
		A	B		A	B
1	500	497.5±0.002	495.4±0.004	499.60.002	99.57±0.05	99.16± 0.03

*Mean±SD of eight determinations. **Mean±SD of eight determinations. (100 mg of nitazoxanide was added and recovered)

in the procedures. To evaluate the validity and reproducibility of the methods, known amounts of the pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed methods. The percent recoveries are given in Table 2. Interference studies revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium trisilicate, that are usually present in the tablet dosage forms did not interfere at their regularly added levels.

The proposed methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of nitazoxanide in bulk drug and its pharmaceutical dosage forms (tablets) in a routine manner.

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