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Development of Visible Spectrophotometric Methods for the Estimation of Nitazoxanide in Bulk and Pharmaceutical Formulation Using Ferric Chloride

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Abstract: Simple, accurate, precise and economical procedure for UV, simultaneous estimation of first and second derivative of Nitazoxanide single component tablet dosage form has been developed utilizing concept of standard addition. The method is based upon determination of Nitazoxanide at 218.5 nm absolute ethanol. Nitazoxanide at their respective λ max 274 nm and 297 nm shows linearity in the concentration range of 5-30 µg/ml. For the second method, first derivative Spectrophotometry, the response (dA/dλ) of standard solutions was measured at 277 nm. Calibration curve was constructed by plotting dA/dλ values against concentrations, 5-40 μgml^{-1} of Nitazoxanide standards in ethanol. For second derivative Spectrophotometry, the response ($d_2A/d\lambda_2$) of standard solutions was measured at 314 nm. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.

Key words: Nitazoxanide · Simultaneous equation method · First derivative · Second derivative spectroscopy · Ferric chloride

INTRODUCTION

Nitazoxanide is chemically 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide and it is used as antiprotozoal drug. The molecular formula is $C_{12}H_9N_3O_5S$. Nitazoxanide is used for treatment of diarrhea caused by *Giardia lamblia/intestinalis* or *Cryptosporidium parvum* [1]. This novel agent has a broad spectrum of activity against many other gastrointestinal pathogens, including bacteria, roundworms, flatworms and flukes [2]. Nitazoxanide is used in many areas of the world, especially in Central and South America, as a broad-spectrum parasiticidal agent in adults and children.The main work has been to establish simple, accurate, rapid and sensitive UV method, useful for routine and quality control of nitazoxanide in pharmaceutical dosage form [3]. Literature survey reveals that few methods like UV-visible spectroscopic, FT-IR and HPLC for determination of newer drugs in pharmaceutical dosage forms [4]. Extensive literature survey revealed that three colorimetric methods have been reported for determination of Nitazoxanide as single component [1]. Also single UV spectrophotometric method is available for determination of Nitazoxanide in dosage form [4]. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [5-19]. The method was validated according to ICH guidelines [21].

Experimental: UV Visible spectrophotometer was employed with spectral bandwidth of 1 cm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). All chemicals and reagents used were of AR/HPLC grade, Chloroform, ammonia (SD'S) and methanol (A.R. Ranbaxy Ltd. New Delhi) were used for mobile phase preparation and as solvent. All chemicals used in this study were of analytical grade and used without further purification. Nitarid® and Nizonide® marketed samples were from Cipla Ltd. Mumbai and Lupin Ltd. Mumbai respectively were procured from local market.

Determination of λ Max:

Preparation of Stock Solution: An accurately weighed quantity of 100mg of pure nitazoxanide was dissolved in a minimum quantity of ferric chloride. Absorbance was measured and derivative spectra were recorded over

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the wavelength range 200-400 nm in two matched quartz cells with a 1 cm light path using a double beam 1700 UV-Visible spectrophotometer.

Preparation of Working Solution: From the above stock solution 25 ml was transferred into 100 ml volumetric flask and volume was made up to the mark with ferric chloride to make 100µg/ml. Then the sample was scanned with UV Spectrophotometer in the range 200-400nm against absolute ethanol as blank and the wavelength corresponding to maximum absorbance was noted which is its λmax i.e. at 218.5 nm.

Preparation of Sample Solutions: Twenty tablets of Nitazoxanide were weighed and powdered in glass mortar. Powder equivalent to 10 mg of the drug was weighed accurately and transferred to 100 ml volumetric flask, dissolved in about 20ml of phosphate buffer pH 5.2 with frequent shaking and made up the volume to the mark with phosphate buffer pH 5.2 to obtain the concentration of 100 µg/ml. The solution was filtered through Whatmann filter paper No.41.The filtrate was diluted suitably with phosphate buffer pH 5.2 to get the concentration of 5µg/ml. The absorbance of sample solution was measured at 254 nm and the amount of Nitazoxanide present in tablet formulation was determined by extrapolating from the calibration curve.

Simultaneous Equation Methods [20]: Simultaneous equation method of analysis is based on the absorption of drug (Nitazoxanide) at the wavelength maximum of the each other. Wavelengths were selected for the developments of the simultaneous equations was218.5 nm, λmax of Nitazoxanide.The absorptivity values E (1%, 1cm) determined for Nitazoxanide at 274 nm and 297 nm. These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of drug.

$$CNIT = \frac{(A_2 \times 247\text{-}A_1 \times 274)}{34215} \quad (1)$$

$$CNIT = \frac{(A_1 \times 214\text{-}A_2 \times 297)}{34215} \quad (2)$$

Where C_{NIT} are concentrations of Nitazoxanide in g /10 mL. A_1 and A_2 are the absorbance of the mixture at 274 nm and 297 nm, respectively.

First-Derivative Spectrophotometry [20]: The spectrums obtained in method was derivatized to get first order derivative spectra and the response (dA/dλ) of the spectra were measured at 277 nm and then calibration curve was constructed by plotting concentration (5-40 µgml-1) versus response (dA/dλ) at 300 nm.

Second-Derivative Spectrophotometry: The term derivative spectroscopy refers to a technique in which the rate of change of spectral intensity with wavelength is the slope of the spectrum is measured. It represents an elegant way of resolving overlapping spectra and has been successfully used for the determination of drugs alone or in mixture. The spectrums obtained in method was derivatized to get second order derivative spectra and the response (d2A/dλ2) of the spectra were measured at 260 nm and then calibration curve was constructed by plotting concentration (10-50 μgml^{-1}) versus response (d2A/dλ2) at 314 nm.

Preparation of Calibration Curve: Aliquots of 0.1 to 1 ml portions of the standard solution were transferred to a series of calibrated 10 ml volumetric flasks and volume was adjusted with phosphate buffer pH 5.2. Solutions were scanned in the range of 200-400 nm against blank (phosphate buffer pH 5.2). The absorption a maximum of solutions was found to be at 254 nm the absorbance of solutions was measured at 254 nm against blank.

Analysis of Commercial Formulation: Content of powder equivalent to 100 mg of Nitazoxanide was taken and added in 1000 ml of solvent system sonicated for 10 min after sonication volume was made up to 100 ml. 1ml of this stock solution was diluted to 10 ml to get concentration equal to 10 µg/ml of Nitazoxanide. This solution is scanned in range 200-400 nm taking solvent system as blank. The spectra obtained were converted Simultaneous equation method concentrations were determined from regression equations generated from calibration graph.

Method Development: The newly developed method was validated according to the ICH guidelines [21] with respect to specificity, linearity, accuracy, precision and robustness. System suitability was established by injecting standard solution and results are given in Table 1.

Table 1: Optical Characteristics Data.

	Nitazoxanide					
	Method I		Method II		Method III	
Parameters / Working λ	274 nm	297 nm	277 nm	300 nm	260 nm	314 nm
Beer's law limit (µg/ml)	5-30	5-30	5-40	5-40	10-50	10-50
Absorptive E (1%,1cm)*	274	274	280	294	287	305
Molar absorptivity (l/mol.cm)*	1548	3064	6590	3553	4532	6730
Correlation coefficient*	0.9986	0.9990	0.9983	0.9989	0.9993	0.9996
Intercept*	0.0154	0.0261	0.0325	0.0465	0.0230	0.0674
Slope*	0.143	0.231	0.331	0.494	0.574	0.653

*Average of six estimation

Linearity: For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for Nitazoxanide in the range of 5 -35 µg mL^{-1}. The correlation coefficient ('r') values were >0.999 (n = 6). The regression equations for the calibration curve was found to be y = 0.3215x+0.0654.

Specificity: The specificity of the UV method, where complete separation of Nitazoxanide was noticed in presence of tablet placebo. In addition there was no any interference at the retention time of Nitazoxanide of tablet solution. In peak purity analysis with detector; purity angle was always less than purity for all the analytes.

Precision: The precision of repeatability was studied by replicate (n=5) analysis of tablet solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The result revealed the precision with % RSD (0.457 %) and (0.864%), respectively for intraday and inter day precision.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ values were found to be 0.038 µg mL^{-1} and 0.067 µg mL^{-1}, Nitazoxanide.

Ruggedness and Robustness: Ruggedness of the method was estimated by preparing six dilutions of the Nitazoxanide as per the proposed method and each dilution injected in duplicate using different analyst on different days.

RESULTS AND DISCUSSION

In order to ascertain the suitability and reproducibility of the proposed method, recovery studies were carried out by adding known quantities of standard Nitazoxanide (80,100,120%) to the tablet and the mixtures were analyzed by the proposed method. This method utilizes the active analogue principle that lies at the spectroscopic method [5-19]. Three samples were prepared for each recovery level. The percentage recovery of Nitazoxanide was found to be 100.32 ± 0.211 % indicating that there is no interference by the excipients in the method. Intra-day precision was evaluated by analyzing six test samples of Nitazoxanide. The intermediate precision (inter-day precision) of the method was determined by evaluating the samples of Nitazoxanide on different days and by two different analysts in the same laboratory. The assay and relative standard deviation (RSD) values are 99.32%, 0.137 and 100.32%, 0.326 respectively. Nitazoxanide exhibits its maximum absorption at 218.5 nm and obeyed Beer's law in the range of 5-30 µg/ml. Linear regression of absorbance Vs concentration yielded equation y=0.3215x+0.0654 with a correlation coefficient of 0.9998. Short-term stability can be evaluated by analyzing either working solutions or matrix-based samples added to working solutions and kept at room temperature before the extraction step. The working solutions were left at room temperature for at least 1h. The matrix-based samples added to working solutions were left at room temperature for 24hrs. The recoveries of Nitazoxanide from the standard mixture solution were found to be 100.11%. Derivative Spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery Studies

Method	Drug	Lab. Claim (mg/tab)	Amt.found* mg/tab.	%	S.D.*	% COV	S.E*	Amt. Added At (%)	mg/ml	% Rec.#
I	NIT	500	499.97	99.98	0.267	0.653	0.165	80	499.6	101.09
								100	500.24	99.97
								120	500.15	100.06
II	NIT	500	500.07	100.03	0.540	0.384	0.432	80	500.04	100.02
								100	501.43	101.65
								120	500.53	100.50
III	NIT	500	500.32	100.32	0.438	0.174	0.774	80	501.31	101.04
								100	500.10	100.17
								120	499.96	99.96

NIT- Nitazoxanide, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery.

Table 3:Validation Parameters

Method	Drug	LOD*µg/ml	LOQ*µg/ml	Precision (% COV) Intraday n=6	Interday* I st day	II nd day	III rd day
I	NIT	0.7653	0.8796	0.6512	0.56340	0.3265	1.2317
II	NIT	1.2374	1.1134	0.9564	0.74584	0.4631	1.0932
III	NIT	1.6543	1.2467	1.0321	0.8942	0.6504	1.2104

NIT- Nitazoxanide, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation

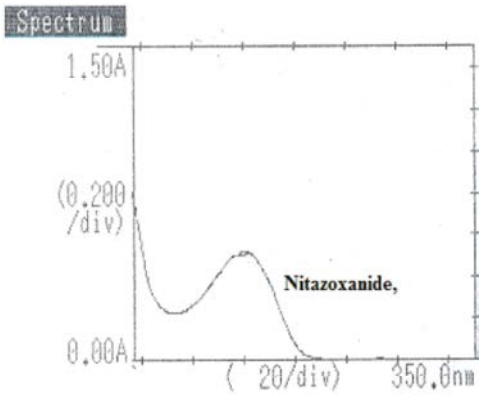

Fig. 1: Overlain Spectra Nitazoxanide,

qualitative and quantitative analysis of various compounds including pharmaceuticals. Hence methods II and III were carried out for Nitazoxanide. For Method II, 277 nm is selected because at 263 nm and 289 nm peaks are distorted and maximum wavelength of the peaks as well as zero crossing point is not remaining constant. Zero crossing point and maximum wavelength are not remaining constant for each concentration. The recoveries of Nitazoxanide which was evaluated by the percent recovery studies at concentration levels of 80, 100 and 120% were found to be in the acceptable range. Excipients used in the formulation did not interfere with response of the drug at its analytical wavelengths. Also, no significant change in response of Nitazoxanide was observed by changing parameters such as wavelength range and slit width. The intra-day and inter-day precision values (%RSD) were calculated and lying in the acceptable range for Nitazoxanide. Ruggedness of proposed methods were determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range.

CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise, reproducible and can be used for dissolution studies as well as routine quality control analysis of Nitazoxanide in bulk and tablet formulation. The developed Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non inference of formulation excipients in the estimation.

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