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Development and Validation of a Simple and Rapid UV Spectrophotometric Method for Assay of Nitazoxanide in Pharmaceutical Dosage Forms

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Abstract

This paper reports the development and validation of a simple UV spectrophotometric method for the assay of nitazoxanide in tablets and powder for oral suspension. The method was linear in the range of 5 and 50 µg/ml presenting a good correlation coefficient (r = 0.9996, n = 7). Precision and accuracy analyses showed low relative standard deviation (< 2.50%) and good recoveries (94.01-113.9%). The procedure was found to be accurate, precise, linear, robust, simple and cost effective. It did not require any polluting reagents and can be applied to assay of nitazoxanide in pharmaceutical dosage forms notably tablets and powder for oral suspensions.

Key words: Nitazoxanide, tablets, suspensions, UV spectrophotometry.

Introduction

Nitazoxanide (NTZ) is a new antiparasitic and antiprotozoal agent having broad-spectrum of activity. It is a nitrothiazole derivative and its chemical name is 2-acetyloxyl-N-(5-nitro-2-thiazolyl) benzamide (Fig. 1) (Rossignol *et al.*, 1976). NTZ was first described in 1975 by Jean Francois Rossignol and was initially developed as a veterinary anthelminthic with activity against intestinal nematodes, cestodes and trematodes. NTZ was approved by the US Food and Drug Administration (FDA) in 2002 for use in human beings (Fox *et al.*, 2005). It is used for treating both intestinal protozoal infections and helminthiasis (Raether *et al.*, 2003). It is also used for treating diarrhea caused by *Giardia lamblia* as well as for cryptosporidiosis in immune-compromised patients, including those with AIDS or HIV infection (Cravier *et al.*, 1978; O'Neil *et al.*, 2001; Murphy *et al.*, 1985; Stockis *et al.*, 1996; Rossignol *et al.*, 1984).

NTZ is a light yellow/pink crystalline powder that is poorly soluble in ethanol, practically insoluble in water with a molecular mass of 307.283 gm/mole and molecular formula of $C_{12}H_9N_3O_5S$. After ingestion, it is converted to the active metabolites tizoxanide and tizoxanide glucuronide. In plasma, more than 99% of NTZ is bound to proteins. It is available in the market as tablets and oral suspensions.

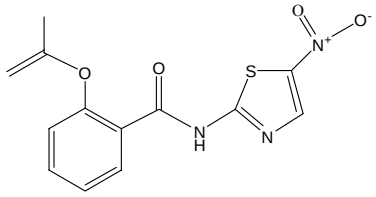

Figure 1. Chemical structure of NTZ

Although no official method for the determination of this drug in oral formulation has been described yet, NTZ in pharmaceutical formulations (tablets and powder for oral suspension) can be estimated by liquid chromatography (LC) (Gopu *et al.*, 2007; Jadhav *et al.*, 2007; Rane *et al.*, 2008; Malesuik *et al.*, 2008), LC-mass spectrometry (LC-MS) (Stockis *et al.*, 1996; Zhao *et al.*, 2008), high performance-thin layer chromatography (HP-TLC), RP-HPLC and UPLC (Kalta *et al.*, 2008). Although various analytical methods have been reported for determination of NTZ in bulk as well as in pharmaceutical formulations, the reported chromatographic methods necessitate sample pretreatment and time-consuming extraction steps prior to analysis of the drug (Sakamoto *et al.*, 2008). Several of these methods require the use of hazardous and expensive chemicals, which make the process not only a challenge for the environment but also complex. Moreover, these methods require expensive equipments and considerably skilled personnel.

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In this paper, we describe a simple UV- spectophotometric method for the determination of NTZ in tablet and powder for suspension formulations. The method has been optimized and validated as per the ICH guidelines (Jadhav *et al.*, 2005).

Materials and Methods

Apparatus: A Shimadzu UV-Visible spectrophotometer (UVmini-1700, Shimadzu Corporation, Kyoto, Japan) with matching quartz cells was used for all absorbance measurements.

Materials and reagents: All chemicals and reagents were of analytical or pharmaceutical grade. NTZ was kindly supplied by Incepta Pharmaceuticals Ltd. (Bangladesh) and was used as the reference standard. A standard solution of NTZ was prepared by dissolving 50 mg of NTZ in 50 ml of ethanol, transferred to a 100 ml volumetric flask and the volume was adjusted to the mark with ethanol to obtain a stock solution of 500 µg/ml. Five different tablets and five powder samples for suspension of NTZ coded as NTZ-ta to NTZ-te and NTZ-pa to NTZ-pe were purchased from local market.

Determination of λ_{max}: An ultra violet spectrophotometric scanning (190-380 nm) was carried out with the reference solution (5.0µg/ml) to select the λ_{max} for detection of nitazoxanide.

Recommended procedure: Aliquots of 500 µg/ml from the standard stock solution of NTZ were pipetted into a six series of 50.0 ml volumetric flasks. Then the mixture was serially diluted with ethanol to get NTZ solutions of 5.0, 10.0, 15.0, 20.0, 25.0 and 50.0 µg/ml.. The contents of each flask were mixed well and immediately transferred to the spectrophotometric cell. The absorbance was recorded at its λ_{max} 344 nm.

Procedure for the determination of NTZ in pharmaceutical formulations: The test sample solutions containing NTZ at a concentration of 20.0 µg/ml were prepared. For this 20 tablets from each sample were weighed and the average weight was calculated. The contents of 20 tablets were obtained by gently peeling of the hard shells and then crushed to a fine powder and an amount equivalent to 100 mg of NTZ was transferred to each of the 100.0 ml volumetric flask and 70.0 ml of ethanol was added and left for 10.0 minutes for complete dispersion of the drug. Then the flasks were shaken ultrasonically for 20.0 minutes and the solution was filtered through Whatman no.1 filter paper (Whatmann International Limited, Kent, UK). The residue was washed well with ethanol for complete recovery of the drug. After filtration an aliquot of 1.0 ml of this solution was transferred into a 50.0 ml volumetric flask and the volume was adjusted upto the mark with ethanol. In case of powder for oral suspension, at first suspension was made by adding purified water (according to direction in the label). From this suspension, 5.0 ml aliquot was taken in 100.0 ml volumetric flask and about 70.0 ml of ethanol was added, sonicated for 20 minutes and diluted up to the mark (100.0 ml) with ethanol to get a concentration of 1.0 mg/ml. The solution was filtered through Whatman no.1 filter paper and 1.0 ml of 1.0 mg/ml NTZ was further diluted to 50 ml in a volumetric flask with ethanol to produce a final concentration of 20 µg/ml. The assay was completed following the same proposed method used for the determination of NTZ.

Method validation

The developed analytical method has been validated for specificity, linearity, limit of detection, precision, accuracy and robustness as mentioned below.

Specificity: The reference standard and quality control samples of NTZ were subjected to stress conditions (i.e. light, heat etc). Each stressed sample was measured to determine the content of NTZ and the results were compared with those for an unstressed time zero reference solution. The reference assay value for each unstressed product was evaluated and the contents of degradation in the stressed and control samples were estimated relative to the assay value. The system response was examined for the interference or overlaps if any, with NTZ responses at 344 nm.

Linearity: The linearity was evaluated with six standard solutions: 5.0, 10.0, 15.0, 20.0, 25.0 and 50.0 µg/ml. The determination was repeated five times at each concentration level. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Limits of detection (LOD) and limit of quantification (LOQ): Limits of detection (LOD) and quantitation (LOQ) for the assay were calculated using the following equations (Mustafa *et al*., 2000).

LOD = 3.3 ×S_o /*b* and LOQ = 10 × S_o/*b,* where S_o and *b* are the standard deviation and the slope of the calibration line.

Precision and accuracy: Intra-day precision and accuracy of the proposed method were determined by replicate analysis (n= 4) of calibration standards at three concentration levels (5.0, 20.0 and 50.0 µg/ml). Inter-day precision and accuracy were determined by assaying the calibration standard at the same concentration levels for four consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and coefficient of variance (CV,%) of the observed concentration as compared to the theoretical one, respectively. For the method to be precise the %CV determined at each concentration level, should not exceed 15% (Bansal *et al*., 2007).

Robustness: Robustness of the proposed method was determined by changing the pH of the medium by ± 0.2 units and by maintaining the solutions at room temperature (25 ± 2 ºC) for 3 h to test the stability of NTZ in the working diluent (ethanol at pH 4.5).

Potency test: The potency of the tested tablet and powder for suspension formulations were determined by the proposed validated method.

Results and Discussion

A simple and rapid UV spectrophotometric assay method has been developed and validated for analysis of nitazoxanide (NTZ) in tablets and powder for suspension.

In order to verify the absence of interference of excipients on the analysis of NTZ tablets and powder for suspension, a sample was prepared with all the excipients present in the tablets but without the drug (placebo). Absorption spectra did not show any potential interference of the tablet excipients at λ_{max} of 344 nm.

A good linear relationship was evident between the absorbance and concentration in the range of 5.0 to 50.0 µg/ml (Fig. 2). The correlation coefficient was 0.9996 (Table 2) indicating good linearity. The representative linear equation was Y=0.0469x + 0.0235, calculated by the least squares method. The limit of quantification (LOQ) was found as 0.907 µg/ml while the limit of detection (LOD) was 0.299 µg/ml.

The intra-day and inter-day precision and accuracy were determined and listed in Tables 3 and 4 respectively. Since all the values of accuracy and % CV are well within the acceptable range of 15%, the results indicated that the method is reliable, reproducible and accurate.

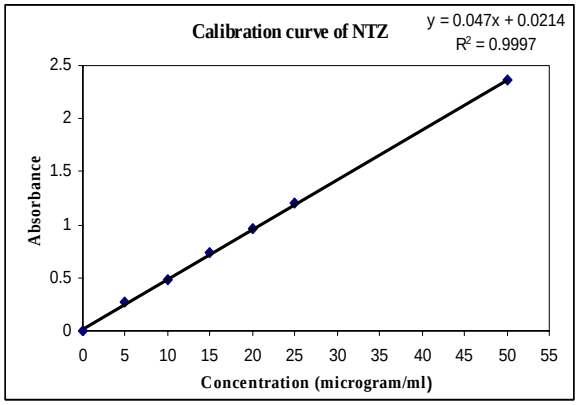

Figure 2. Calibration curve for NTZ

Table 1. Absorbance of NTZ solutions of varying concentrations at 344 nm

No.	Nitazoxanide (NTZ)	
	Conc. (µg ml^{-1})	Absorbance
1	0	0.00
2	5	0.27
3	10	0.48
4	15	0.74
5	20	0.97
6	25	1.20
7	50	2.36

Table 2. Regression data for calibration of NTZ

Traits	Slope	Intercept	R^2
Mean	0.0458	0.0284	0.9996
Standard deviation	0.0013	0.0042	0.0020
CV (%)	2.7490	14.6670	0.2050
SEM	0.0006	0.0019	0.0009

Table 3. Intra-day precision and accuracy study of standard NTZ

Intra-day precision and accuracy (n = 4 replicates)							
	NTZ concentration (µg/ml)					Accuracy (%)	CV (%)
Declared Conc. (µg/ml)	Calculated concentration (µg/ml)				Mean ± SD		
	1	2	3	4			
05.0	05.2	05.3	05.3	05.2	05.3 ± 0.03	105.22	0.61
20.0	19.4	19.2	21.5	19.6	19.9±1.06	99.47	5.31
50.0	52.1	52.1	49.6	51.3	51.3±1.17	102.54	2.27

Table 4. Inter-day precision and accuracy study of NTZ

Intra-day precision and accuracy (n = 4 consecutive days)							
	NTZ concentration (µg/ml)					Accuracy (%)	CV (%)
Declared Conc. (µg/ml)	Calculated concentration (µg/ml)				Mean ± SD		
	1	2	3	4			
05.0	05.2	05.6	05.4	05.5	5.5 ± 0.013	108.96	2.45
20.0	20.4	21.5	22.7	21.7	21.6 ± 0.80	108.00	3.72
50.0	54.4	50.9	50.0	52.9	51.0 ± 2.79	102.19	5.46
05.0	05.2	05.6	05.4	05.5	5.5 ± 0.013	108.96	2.45

Table 5. Extinction coefficient of NTZ

Conc. (µg/ml)	Conc. (M)	Absorbance (λ_{max}= 344 nm)	Extinction co-efficient, € (M^{-1}/cm)
5.0	1.627×10^{-5}	0.273	16779.35
10.0	3.254 × 10-5	0.482	14812.54
15.0	4.881 × 10-5	0.743	15222.29
20.0	6.508 × 10-5	0.971	14920.10
25.0	8.135 × 10-5	1.202	14775.66
50.0	16.270 ×10-5	2.360	14505.22
	Mean		15169.20

In addition, the reliability of the proposed method was also evaluated by means of the determination of the extinction coefficient of NTZ using Beer-Lambert's Law (Table 5).

After the validation of the newly developed UV spectrophotometric method, the potency of marketed formulations was determined by the proposed validated method and the results are shown in Table 6. Among the different marketed brands used, the potency of all the brands was found to be within the limit of 94.012 – 113.9837%. According to USP 2011, the potency range of standard nitazoxanide is 98.0-102.0%.

Table 6. Potency determination of the NTZ formulations

Dosage form	SL. No.	Sample code	Amount claimed (mg)	Amount found (mg) (Mean ± SD)	Potency (%)
Tablet	1	NTZ-ta	500.0	470.0 ± 5.365	94.01
	2	NTZ-tb	500.0	569.9 ± 3.427	113.98
	3	NTZ-tc	500.0	536.5 ± 0.533	107.30
	4	NTZ-td	500.0	526.7 ± 0.307	105.35
	5	NTZ-te	500.0	517.1 ± 3.123	103.42
Powder for suspension	6	NTZ-pa	100.0	105.0 ± 2.370	105.01
	7	NTZ-pb	100.0	102.5 ± 0.268	102.46
	8	NTZ-pc	100.0	104.3 ± 0.274	104.32
	9	NTZ-pd	100.0	103.8 ± 0.967	103.74
	10	NTZ-pe	100.0	096.1 ± 0.906	96.07

For the recovery analysis, the concentration present and the percentage recovery were calculated by average of four replicate analyses of each formulation. The studies were carried out at different level of concentrations by spiking different amount of standard drug to the pre-analyzed sample and the contents were re-analyzed by the proposed method and the results are summarized in Table 7.

Table 7. Recovery studies of NTZ

Amount claimed (µg)	Amount of pure drug added (µg)	Amount of drug recovered (µg)	Potency (%)
45.0	5	49.2	98.4
50.0	10	60.336	100.56
55.0	15	69.776	99.68
60.0	20	80.088	100.11

Conclusion

The results obtained and the statistical parameters for determination of NTZ in pharmaceutical dosage forms demonstrated that the proposed UV spectrophotometry method is simple, accurate, fast and precise. The method showed high sensitivity, acceptable linearity and accuracy. Therefore it could be easily used for the analysis of pure drug. Moreover, the method uses simple reagents with minimum steps and time for sample preparation, which allow it to be useful for routine analyses and quality-control assays of NTZ in tablets and powder for suspension dosage form.

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