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Research article

A validated HPTLC method for estimation of Nitazoxanide in bulk and formulation

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Abstract

A simple, specific, accurate, reproducible and precise high performance thin layer chromatographic method was developed for estimation of Nitazoxanide (NTZ) in pharmaceutical formulation. This method involved Camag high performance thin layer chromatography (HPTLC) system comprising of Linomat IV sample applicator with Merck precoated aluminium sheets Silica Gel 60 F254 of 10 x 10 cm size and 200 mm thickness. The mobile phase consisting of ethyl acetate : iso-octane (5 : 5 v/v). The detection was carried out densitometrically using UV detector in absorbance mode at 360 nm. The Rf value was 0.44, proving good resolution. The method is sensitive to detect Nitazoxanide (NTZ). The correlation coefficient was >0.99. The method was validated for accuracy, precision, specificity, linearity, range, ruggedness and robustness. The proposed method was successfully used to determine the drug content of marketed formulation.

Keywords: Nitazoxanide, HPTLC, validation.

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Introduction

Nitazoxanide (NTZ), synthetic antimicrobial agent having broad spectrum of activity against intracellular and extracellular protozoa, helminthes, aerobic and anaerobic bacteria [1-9]. It is a drug of choice against cryptosporidial diarrhoea in AIDS [6,9]. Chemically it is 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide as shown by its structure [10-12]. Nitazoxanide is rapidly metabolized by ester hydrolysis to an active metabolite tizoxanide (desacetyl-nitazoxanide)[4,10-12].Pharmacokinetic studies using HPLC method for the estimation of its active metabolite tizoxanide (desacetyl nitazoxanide) in blood plasma have been reported [12]. This paper presents simple, rapid and cost effective HPTLC method for the estimation of nitazoxanide.

Materials and Methods

Nitazoxanide (NTZ) working standard was procured as a gift sample from M/s. Ind–Swift pharmaceuticals, Parwanoo (H. P.). Commercial tablet formulations were procured from local market namely Zonaxid (N_1) and Netazox (N_2) containing 500mg Nitazoxanide per tablet. All

chemicals used were of HPLC or AR grades. Distilled water and Whatmann filter paper Grade-I were used throughout the experimental work.

HPTLC studies

Preparation of Samples: Standard solution A

An accurately weighed quantity of NTZ (~25 mg) was taken in 50.0 mL volumetric flask and dissolved in about 35.0 mL acetonitrile and the volume was made up to the mark (Conc. 0.5 mg/mL) with acetonitrile. 1.0 mL of this solution was transferred to 10.0 mL volumetric flask containing 1.0 mL acetonitrile and the volume made up to the mark with pH 3.5 acid phthalate buffer (Conc. 50 µg/mL) [13].

Development of HPTLC technique

5 µL bands of standard solution A (6mm band width) were applied on Merck pre-coated Silica Gel 60 F_{254} TLC Plate of 10 x 10 cm size and 200 µm thickness with application speed 10 sec/µL using CAMAG LINOMAT IV sample applicator. The optimized mobile phase system used was ethyl acetate : iso-octane (5 : 5 v/v). Linear ascending development was carried out in a twin through glass chamber, saturated with mobile phase. The optimized saturation time was 10min at room temperature (20 ± 5 ^{0}C). The plates were developed till 70mm. The plates were dried at room temperature. The bands on the developed TLC plate were scanned over the wavelength range 200-500 nm. The wavelength selected for densitometric determination was 360 nm. The selection of wavelength was based on its high absorptivity at 360.0 nm for better sensitivity of determination.

Standard calibration curve

Standard solution A was applied on TLC plate by micro litre syringe with the help of LINOMAT IV sample applicator in the range of 1-8 µL (50 to 400 ng NTZ). After development, the plate was scanned at 360 nm with the help of CAMAG TLC SCANNER III, which was attached to a computer controlled by CAT's software version 4. Peak height and peak area were recorded for each concentration of drug. Concentration v/s response curves were constructed and they are depicted in Plate No. 1. The results of the study are also given in tabular form in the Table No 1.

Sample solution

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 25 mg of NTZ was taken into 50.0 mL volumetric flask and about 35.0 mL of acetonitrile was added to it. The flask was shaken for 15 minutes and the volume was adjusted to 50.0 mL with acetonitrile. Then the solution was centrifuged for 5 min at 2000 rpm and 1.0 mL portion of the clear supernatant liquid was transferred to 10.0 mL volumetric flask containing 1.0 mL of acetonitrile and the volume was made up to the mark with pH 3.5 acid-phthalate buffer.

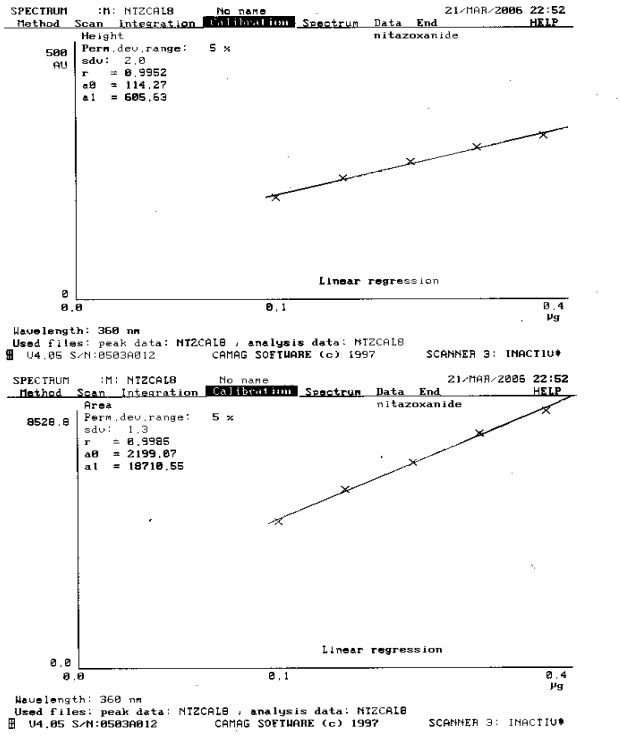

Plate 1. Concentration-response curve for NTZ (a) by height (b) by area

Two bands of standard solution and six bands of sample solution of equal volume (5 µL) were applied on TLC plate as 6 mm band and the plates were developed and scanned as per the optimized chromatographic conditions. A typical densitogram obtained for standard and sample solution is shown in Plate No. 2. Results of estimation of NTZ in tablet are shown in Table No. 2

Table 1. Standard calibration curve / Linearity range

Sr. No.	Linearity range (µg)		Coefficient of correlation		Y- intercept		Slope	
	By height	By area	By height	By area	By height	By area	By height	By area
N_1	0.147-0.343	0.147-0.343	0.9952	0.9985	114.27	2199.07	605.63	18710.55

Table 2. Result of estimation of NTZ in tablet

Sr. No.	Sample code	Avg. weight of tablet (mg)	Labelled claim (mg/tablet) NTZ	% labelled claim (Mean ±S.D)*	
				Height	Area
1	N_1	919.88	500	100.59±0.7224	101.01±1.2102
% RSD				0.7182	1.2148
2	N_2	1280.38	500	100.22±0.5797	100.56±1.1861
% RSD				0.5784	1.1795

* mean of five observations

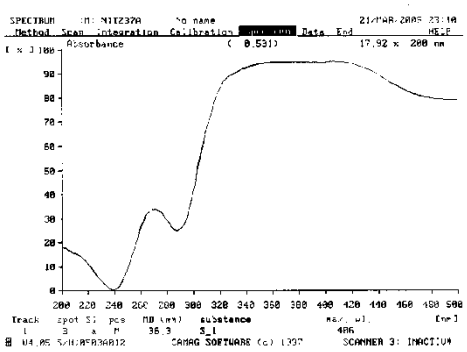

Plate 2. Densitogram of NTZ (a) tablet and (b) standard

Method validation

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method at different levels of labelled claim (i.e. 60 to 120 % of labelled claim). Results of accuracy (recovery) studies are shown in Table No. 3.

Precision

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of estimation of NTZ by proposed method was ascertained by replicate analysis of homogeneous samples of tablet powder. Results are as shown in Table No. 2.

Specificity

Accurately weighed seven quantities of finely powdered tablets each equivalent to about 25 mg NTZ were treated for 24 hrs under following different conditions.

1. Normal (untreated)
2. At 40^0C after addition of 1.0 mL of 0.1N NaOH (alkali)
3. At 40^0C after addition of 1.0 mL of 0.1N HCL (acid)
4. At 40^0C after addition of 1.0mL of 3% H_2O_2 (oxide)

5. At 50^{0}C (Heat)
6. In UV chamber
7. At 75% RH

After 24 hours, plates were developed and scanned as described earlier and results were calculated by comparing height and peak area response of sample with standard. Results are summarised in Table No. 3. The densitogram obtained under different storage conditions are depicted in Plate Nos. 3,4,5,6,7,8,9.

Table 3. Validation results

Sr. No.	Parameter	Conditions	% labelled claim (Mean ±S.D)	
			By height	By area
1	Specificity	Normal	100.90±0.658	100.60±0.889
		Acid	96.75±0.235	97.49±0.311
		Alkali	-	-
		Peroxide	48.68±0.788	46.50±0.131
		UV	101.13±0.221	102.35±0.439
		Heat	99.08±0.556	99.93±0.358
		75 %RH	100.90±0.122	102.20±0.882
2	Ruggedness	Different Analyst	100.20 ±0.632	99.76 ± 0505
		Different days	100.4 ±0.472	100.54±0.463
3	Robustness	Change in wavelength	99.08±0.101	100.79±0.448
			% Recovery (Mean ±S.D)	
4	Accuracy	-	100.12 ±1.527	100.34±0.738

Linearity and range

The study was performed by applying different volumes of standard solution A and developing the plates. The graph plotted as the amount of drug applied v/s response depicted in Plate No. 1 was found to be straight line. The result are summarised in Table No. 1.

Ruggedness

The samples were analyzed by using proposed method for tablet sample N_1 by three different analysts and on different days. Results are as shown in Table No. 3.

Robustness

The samples were analyzed by using proposed method for tablet sample N_1 by deliberate changes in scanning wavelength by ±5 nm. Results are as shown in Table No. 3

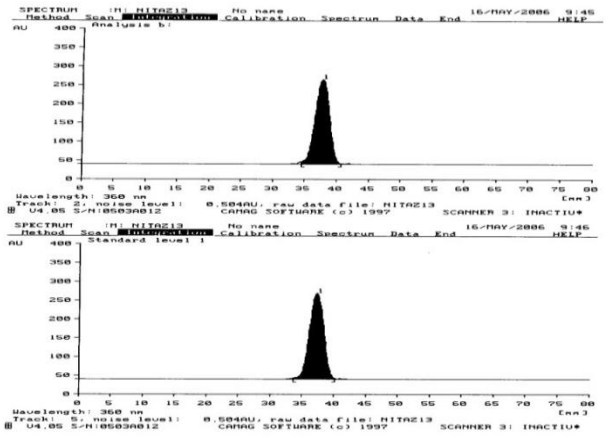

Plate 3. Specificity of untreated sample

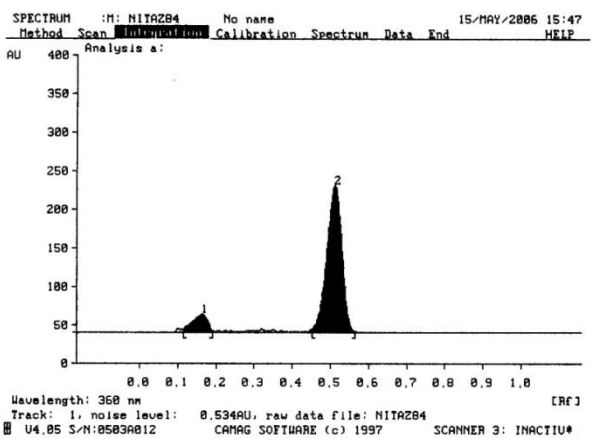

Plate 4. Specificity of Acid treated sample

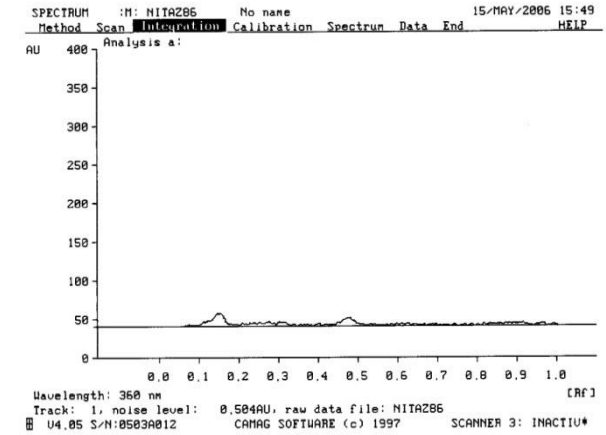

Plate 5. Specificity of base treated sample

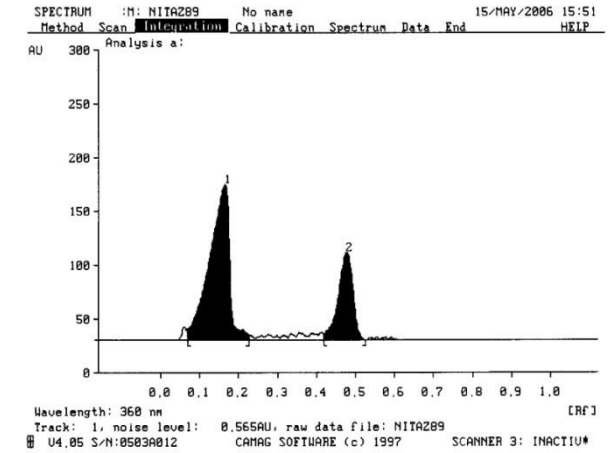

Plate 6. Specificity of peroxide treated sample

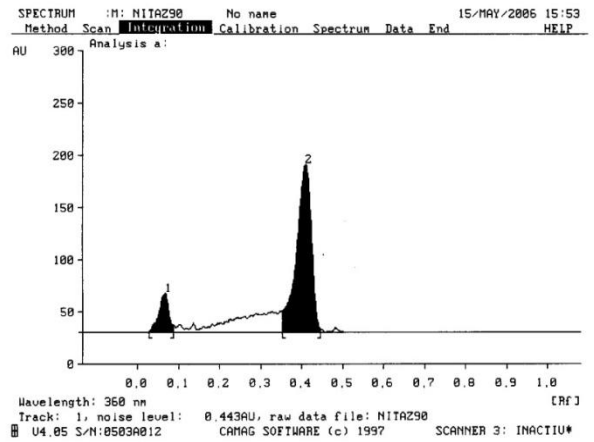

Plate 7. Specificity of UV treated sample

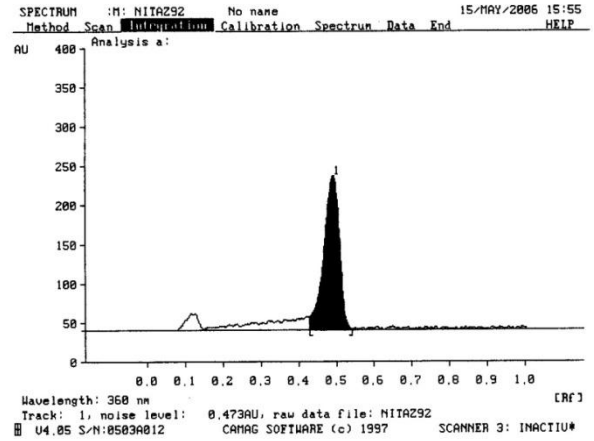

Plate 8. Specificity of 75 % RH treated sample

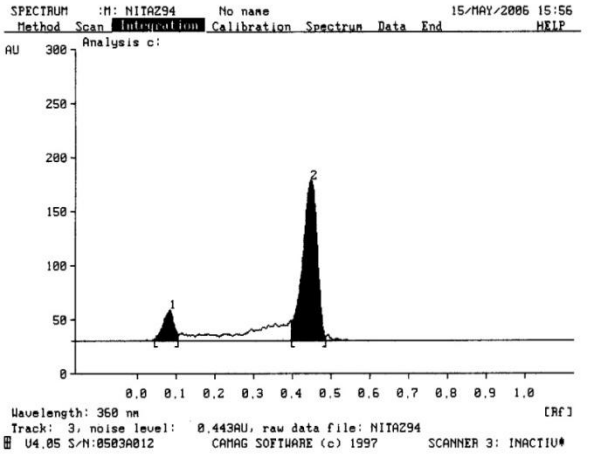

Plate 9. Specificity of 50^{0}C treated sample

Result and Discussion

A single well resolved peak with a considerable Rf value (0.44) was obtained, with ethyl acetate : iso-octane (5:5 v/v) as mobile phase. The concentration response plots (as per peak height and peak area) of the drug shows linearity over the concentration range of 0.147-0.343 μg with correlation coefficient values well above 0.99. Recoveries of the drug were observed to be very close to 100 %, representing the accuracy of the method and also non interference of excipients. Replicate estimations of NTZ in tablet by proposed method yielded quite concurrent results, shows repeatability of the method.

In case of method specificity estimation of NTZ, the results of sample treated with 3% H_2O_2, were much lower (~47%) with an additional peak (Plate No. 6), also the results were also low (~97%) for samples treated with 0.1 N HCl (Plate No. 4) with additional peak observed, indicating that it had undergone degradation and method is capable of estimating NTZ specifically in presence of its peroxide, and acid degradation products. No peak was observed in case of alkali treated samples, which reveals that the drug has undergone degradation and this degradation product is either beyond the detection limits of scanning wavelength (360.0 nm) or it might not have traveled along with the mobile phase or may have moved with mobile phase (Plate No. 5).

Estimation of NTZ in 75% RH, UV and heat exposed samples (Plate Nos. 7,8,9) have shown no significant difference in the results and were close to normal samples, which indicate that there is either no degradation or otherwise the proposed method is incapable of detecting it. Above study shows that method is able to resolve the impurity and degradation products from the drug of interest.

Operating of proposed method by different analysts and on three different days observes reproducible results with maximum % RSD of the order of 0.63 and 0.47 respectively indicates the ruggedness of the method in the hands of different expert analyst.

The results obtained by proposed HPTLC method are quite concurrent and accurate for the drug by peak area as well as by peak height consideration. The statistical analysis proves that the method is simple, rapid, reproducible, reasonably specific and rugged. Hence, it may be employed for routine quality control of NTZ in pharmaceutical formulation.

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