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A validated stability indicating HPTLC method for determination of nitazoxanide

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A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for nitazoxanide in bulk drug and in formulations was developed and validated. Method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. Solvent system consisted of ethyl acetate-toluene- methanol (4:6:1, v/v/v). Nitazoxanide was subjected to hydrolysis, oxidation, photolysis and thermal decomposition to establish a validated stability-indicating HPTLC method. Extensive degradation occurred in alkaline medium and in oxidative stress condition and degradation product was well separated from pure drug. Densitometric analysis of nitazoxanide was carried out in absorbance mode at 350 nm. Linear regression analysis data for mean calibration plots showed good linear relationship with r^2 = 0.9997 in 400-1600 ng per spot. Method was validated with respect to linearity, precision, accuracy, specificity and robustness. The limits of detection and quantitation were 15 and 50 ng per spot, respectively. HPTLC method could effectively separate drug from its degradation product and can be employed as a stability-indicating method.

Keywords: HPTLC, ICH, Nitazoxanide, Stability-indicating method, Stress studies

Introduction

Nitazoxanide[1] [2-{(5-nitro-1, 3-thiazol-2-yl) carbamoyl} phenyl] ethanoate, mp, 202° is a yellowish crystalline powder that is slightly soluble in acetone and chloroform and very slightly soluble in methanol and water. It is a synthetic antiprotozoal agent for oral application[2]. It is indicated for the treatment of diarrhoea caused by *Cryptosporidium parvum* and *Giardia lamblia* parasites. Antiprotozoal activity of nitazoxanide is believed to be due to interference with the pyruvate: ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction, which is essential to anaerobic energy metabolism. Following oral administration in humans, nitazoxanide (Fig. 1a) is rapidly hydrolyzed to an active metabolite, tizoxanide (desacetyl-nitazoxanide) (Fig. 1b), which undergoes conjugation, primarily by glucuronidation[3,4]. International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2)[5] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures.

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Since no studies have described the determination of nitazoxanide in bulk drug and pharmaceutical dosage form, this study attempts to describe a selective, precise, accurate and stability indicating high-performance thin layer chromatographic (HPTLC) method.

Experimental Details

Materials

Nitazoxanide was received as a gift sample from Ind-swift Laboratories Ltd. (Punjab, India). All chemicals were of analytical reagent grade and purchased from Merck (Mumbai, India).

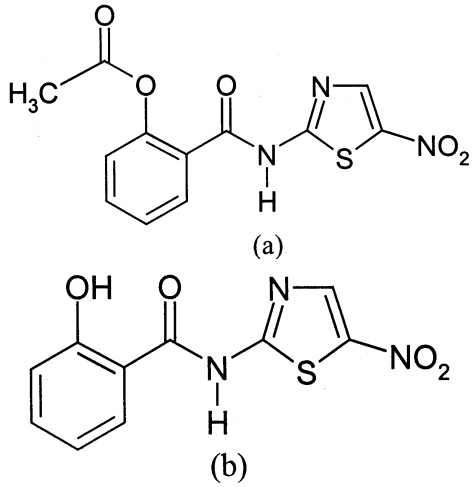

Fig. 1—Structure of: a) nitazoxanide; and b) tizoxanide

HPTLC Instrumentation

Samples were spotted as bands (width, 6 mm) with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm x 10 cm with 250 μm thickness, E. Merck, Germany) using a Camag Linomat IV (Switzerland). A constant application rate (0.1 μl/s) was employed and space between two bands was 6 mm. Slit dimension was kept at 5 mm x 0.45 mm, and 10mm/sec scanning speed was employed. Mobile phase consisted of ethyl acetate-toluene- methanol (4:6:1, v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. Optimized chamber saturation time for mobile phase was 15 min at room temperature. Length of densitogram run was 8 cm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 350 nm. Tungsten lamp was used as source of radiation.

Calibration Curve of Nitazoxanide

A stock solution of nitazoxanide (200 ng/μl) was prepared in acetonitrile. Different volumes of stock solution (2, 3, 4, 5, 6, 7 and 8 μl) were spotted on TLC plate to obtain concentrations of 400, 600, 800, 1000, 1200, 1400 and 1600 ng per spot of nitazoxanide, respectively. The data of peak area vs drug concentration were treated by linear least-square regression analysis.

HPTLC Validation

HPTLC method was validated with respect to precision, robustness, accuracy and specificity as per ICH guidelines[6,7]. For precision, repeatability of sample application and measurement of peak area were carried out by making three replicates of the lowest, middle and highest concentration in the above range (400, 1000 and 1600 ng per spot) on the same day. These studies were also repeated on different days with freshly prepared samples to determine intermediate precision and it showed very low %RSD of peak area of nitazoxanide. Blank acetonitrile was spotted six times following the HPTLC method. As per ICH guidelines, signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ. For robustness, mobile phases having different composition of ethyl acetate-toluene- methanol (3.9:6.1:1 and 4.1:5.9:1, v/v/v) were tried along with the original mobile phase at the concentration of 1000 ng per spot. For specificity, the peak purity of nitazoxanide was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot. The accuracy was assessed by conducting the recovery studies (80, 100, and 120 %) of pure drug from marketed formulation, by standard addition method.

Analysis of Marketed Formulation

To determine the content of nitazoxanide in conventional tablets (label claim, 500 mg nitazoxanide per tablet), 20 tablets were weighed, finely powdered and powder equivalent to 500 mg nitazoxanide was weighed. The drug from powder was extracted with acetonitrile. To ensure complete extraction of drug, it was sonicated for 30 min and volume made up to 100 ml with acetonitrile. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and diluted to get a concentration of 500 μg/ml. Filtered solution (2 μl) was applied (1000 ng per spot) on TLC plate followed by development and scanning as described under HPTLC instrumentation. Analysis was done in triplicates and the possibility of excipient interference was studied.

Stress Degradation Studies

All degradation studies were carried out at a drug concentration of 1 mg ml^{-1}. Neutral hydrolysis was carried out in water under refluxing for 2 h at 70 °C. Acid hydrolysis was done using 0.1 *N* HCl under refluxing for 30 min at 50 °C. Alkali hydrolysis was carried out using 0.1 *N* NaOH at room temperature for 30 min. Oxidative studies were conducted at room temperature in 3% H_2O_2 for 3 h. Thermal degradation studies[8, 9] were carried out by exposing the drug powder to dry heat at 80 °C for 12 h. Photo-degradation studies[10, 11] were performed by exposing the solutions to sunlight during the daytime (60,000-70,000 lux) for 2 days. Samples were withdrawn at appropriate time and subjected to HPTLC analysis at a concentration of 1000 ng per spot.

Results and Discussion

Optimization of Mobile Phase

HPTLC method was optimized with a view to develop a stability-indicating assay method. Degraded products and pure drug were spotted on TLC plates and run in different solvent systems. Toluene-ethyl acetate (6:4) showed a good peak shape for pure drug but degradation product formed does not show proper

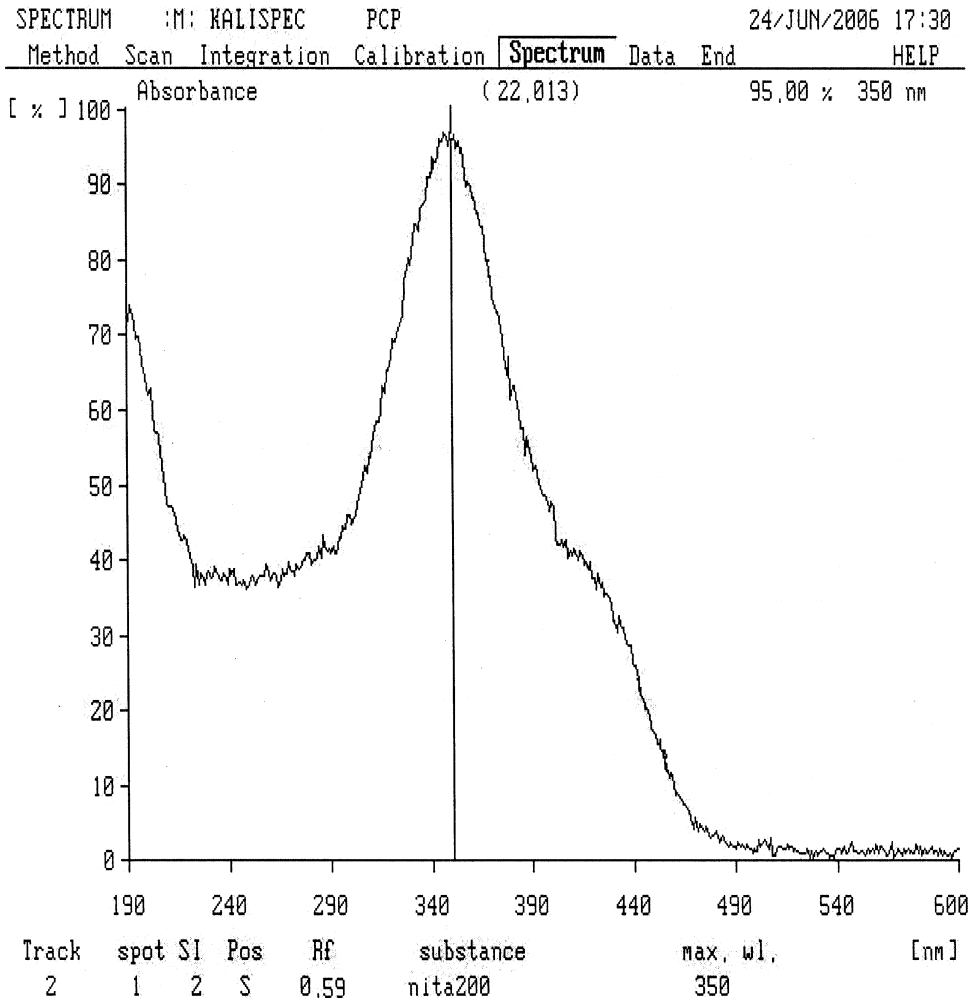

Fig. 2—Spectra of pure drug

peak shape. Slight addition of methanol to the mobile phase resulted in good peak shape for degradation product and pure drug. Finally, toluene-ethyl acetate-methanol (6:4:1, v/v/v) gave good separation along with sharp and symmetrical peak. The chamber was saturated for 20 min with the mobile phase to get good separation. Fig. 2 shows spectra of nitazoxanide and Fig. 3 A the densitogram of nitazoxanide showing pure drug (1000 ng per spot) at peak 1.

Calibration Curve

The linear regression data for the calibration curve (n=3) showed a good linear relationship over the concentration range 400-1600 ng per spot with r^2 0.9997.

Validation of Method

Precision

Triplicates of three different concentrations (400, 800, and 1600 ng/spot solutions) were used for repeatability studies in the same day. Average concentrations and % RSDs were found to be 403.93 ± 1.309, 804.91 ± 0.766 and 1600.84 ± 1.458 respectively. These studies were also repeated on different days with freshly prepared samples to determine intermediate precision and it showed very low %RSD of peak area of nitazoxanide. Average concentrations and % RSDs were found to be 402.89 ± 1.654, 801.98 ± 0.942 and 1601.52 ± 1.118 respectively.

Limit of Detection and Quantitation (LOD and LOQ)

Signal to noise ratios were LOD (3:1) and LOQ (10:1). LOD and LOQ were found to be 15 ng/spot and 50 ng/spot respectively for nitazoxanide.

Robustness

The low values of %RSD obtained after introducing small changes in mobile phase composition indicated robustness of the method.

Specificity

Specificity of method was by means of complete separation of pure drug from the degradation products. Peak purity of nitazoxanide and degradation product were assessed by comparing their respective spectra at peak start, apex and peak end positions of the spots. The r (S, M) = 0.9999 and r (M, E)=0.9998 for pure drug and r (S, M) = 0.9998 and r (M, E)=0.9995 for degradation products were obtained. Moreover the specificity of the HPTLC method was determined as per ICH guidelines by applying the method to a marketed formulation found any interference from any excipients.

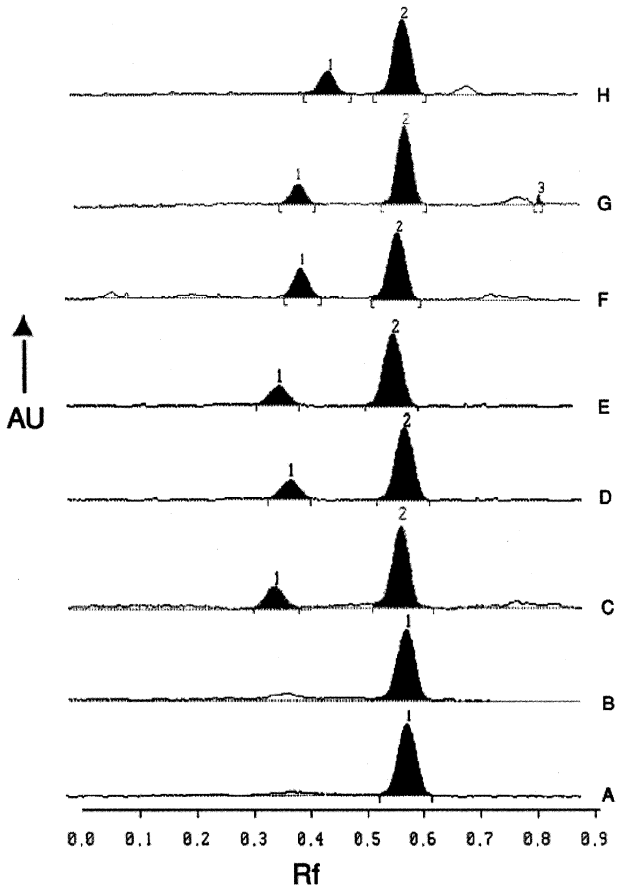

Fig. 3—A) Densitogram of nitazoxanide showing pure drug (1000 ng per spot) (peak 1); B) Densitogram of marketed formulation showing the peak of nitazoxanide (peak 1); C) Densitogram of acid degradation product (peak 1) at 50 ° C for 30 min; D) Densitogram of neutral hydrolysis product (peak 1) at 70 °C for 2 h; E) Densitogram of alkali hydrolysis product (peak 1) at room temperature for 30 min; F) Densitogram of oxidative hydrolysis product (peak 1) at room temperature for 3 h; G) Densitogram of photolysis product (peak 1) kept for 2 days; H) Densitogram of dry heat product (peak 1) at 80°C temperature for 12 h

Accuracy

Percentage recovery was calculated of pure drug from marketed formulation by standard addition of pure drug at three known concentrations (80%, 100% and 120%) and excellent recoveries were obtained at each levels. The respective % recovery and % RSDs for three levels (80%, 100% and 120%) were found to be 100.41 ± 1.306, 100.01 ± 1.361 and 100.34 ± 1.771 respectively.

Analysis of Marketed Formulation

There was no interference from the excipients commonly present in the tablets. The drug (yield, 101.17%) had low %RSD (1.05%) that indicated the suitability of this method for routine analysis of nitazoxanide in pharmaceutical dosage forms. Densitogram (Fig. 3B) of marketed formulation showed the peak of nitazoxanide (peak 1).

Stress Degradation Studies

Nitazoxanide under different ICH recommended stress conditions suggested the following degradation behaviour (Table 1).

Acidic Condition

Drug decreased (19.12 %) on refluxing at 50 °C in 0.1 *M* HCl for 30 min forming degradation product at R_f 0.39 (Fig. 3C). Degradation in acid was faster than in water and slower than in alkali.

Neutral (water) Condition

Drug decreased (16.34 %) on refluxing in water at 70 °C for 2 h at R_f 0.40 (Fig. 3D).

Table 1—Summary of degradation study

Conditions	Temperature	Degradation of pure drug		Degradation products	
		%	R_f	R_f	λ_{max}
Acid hydrolysis*	50 °C	19.12	0.59	0.39	340
Neutral hydrolysis*	70 °C	16.34	0.60	0.40	341
Alkali hydrolysis	Room temperature	11.96	0.59	0.39	338
Oxidation	Room temperature	27.67	0.59	0.40	340
Dry heat	80 °C	25.10	0.60	0.41	340
Photolysis	NA	13.65	0.60	0.38	339

*Refluxed

Alkali Degradation

Reaction, carried out initially with 0.1 *M* NaOH at 50 °C, was so fast that whole of the drug was degraded in 30 min. Subsequently, studies were performed with 0.1 *M* NaOH at room temperature for 30 min and degradation (11.96 %) was observed at R_f 0.39 (Fig. 3E).

Oxidative Condition

Drug was found to be highly labile to oxidation also. Reaction was carried out initially with 3% H_2O_2 at 50 °C for 30 min and the reaction was so fast that whole of the drug was degraded in 30 min. Subsequently, studies were performed with 3% H_2O_2 at room temperature for 3 h, and degradation (27.67 %) was formed at R_f 0.4 (Fig. 3F)

Photolytic Condition

Drug was comparatively stable after exposure to sunlight. Drug after exposure to daylight continuously for 2 days showed degradation (13.65 %) at peak at R_f 0.38 (Fig. 3G).

Dry Heat Study

Nitazoxanide exposed to intense dry heat at 80 °C for 12 h showed degradation (25.10 %) at peak at R_f 0.41 (Fig. 3H).

Conclusions

A new HPTLC densitometric method has been developed for the analysis of nitazoxanide in bulk drug and in pharmaceutical dosage form. Drug has been found more labile to hydrolysis. The degradation product formed in all the stress conditions were at the same R_f and moreover the spectra were also found to be same, indicating that they are of same chemical nature. Method is stability–indicating, which separates the degradation products formed under variety of conditions. Method proved to be simple, accurate, precise, specific and selective. Hence it is recommended for the routine analysis of the drug and degradation products in stability samples by the industry.

Acknowledgement

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References

1 *http://redpoll.pharmacy.ualberta.ca/drugbank/cgi-in/getCard.cgi?CARD=APRD00558.txt*, 2006 (accessed on 01.06.2006).

2 *http://www.centerwatch.com/patient/drugs/dru811.html* 2006 (accessed on 01.06.2006).

3 *http://www.healthsquare.com/newrx/ali1659.htm* 2006 (acces-sed on 01.06.2006).

4 Dubreuil L, Houcke I, Mounton Y & Rossignol J F, *In vitro* evaluation of activities of nitazoxanide and tizoxanide against anaerobes and aerobic organisms, *Antimicrob Agents Chemother,* **40** (1996) 2266-2270.

5 ICH, *Stability Testing of New Drug Substances and Products Q1A (R2)* (International Conference on Harmonization, IFPMA, Geneva) 2003, 1-18.

6 ICH, *Validation of Analytical Procedures: Methodology Q2 (R1)* (International Conference on Harmonization, IFPMA, Geneva) 1996, 1-13.

7 Carstenson J T & Rhodes C T, *Drug Stability Principles and Practices* (Marcel Dekker, Inc, New York) 2000, 209-260.

8 Singh S & Bakshi M, Guidance on conduct of stress test to determine inherent stability of drugs, *Pharm Tech On-line,* **24** (2000), 1-14.

9 Tipre D N & Vavia P R, Oxidative degradation study of Nitrendepine using stability indicating, HPLC, HPTLC and spectrometric method, *J Pharm Biomed Anal,* **24** (2001) 705-714.

10 Singh S, Singh B, Bahuguna R, Wadhwa L & Saxena R, Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay, *J Pharm Biomed Anal,* **41** (2006) 1037-1040.

11 ICH, *Stability Testing: Photo Stability Testing Of New Drug Substances and Products Q1B* (International Conference on Harmonization, IFPMA, Geneva) 1996, 1-8.