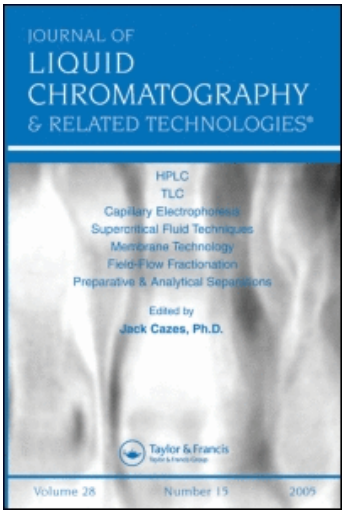

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Development and Validation of a Stability-Indicating HPTLC-Densitometric Method for Satranidazole

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Development and Validation of a Stability-Indicating HPTLC-Densitometric Method for Satranidazole

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Abstract: A sensitive, selective, precise, and stability-indicating high performance thin layer chromatography (HPTLC) method for analysis of satranidazole both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene/acetonitrile (60:40, v/v). Densitometric analysis of satranidazole was carried out in the absorbance mode at 314 nm. This system was found to give compact spots for satranidazole (R_f value of 0.53 ± 0.02, for six replicates). Satranidazole was subjected to acid and alkaline hydrolysis, oxidation, and photo degradation. The drug undergoes degradation under acidic and basic conditions, oxidation, and photo degradation. Also, the degraded products were well resolved from the pure drug with significantly different R_f values. The method was validated for linearity, precision, limit of detection (LOD), limit of quantitation (LOQ), and accuracy. Linearity was found to be in the range of 100–500 ng/spot with a significantly high value of correlation coefficient $r^2 = 0.9979 \pm 0.66$. The LOD and LOQ were 50 and 85 ng/spot, respectively. Statistical analysis proved that the method is repeatable and specific for the estimation of the said drug. As the method could

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effectively separate the drug from its degradation products, it can be employed as a stability-indicating one. Moreover, the proposed HPTLC method was utilized to investigate the kinetics of the alkali degradation process.

Keywords: Satranidazole, HPTLC, Stability-indicating, Degradation

INTRODUCTION

Satranidazole, 3-(1-Methyl-5-nitroimidazol-2-yl)-1-(methylsulfonyl) imidazolidin-2-one (Figure 1) is used in the treatment of intestinal and hepatic amoebiasis, giardiasis, trichomoniasis, and anaerobic infections.[1–4] Satranidazole is also available in combination with ofloxacin. Satranidazole is not official in any pharmacopoeia. Few reports for analysis of satranidazole are available in literature; various spectrophotometric methods have been reported for satranidazole in bulk drug and pharmaceutical formulation.[5]

To our knowledge, no article related to the stability-indicating HPTLC determination of satranidazole has ever been mentioned in literature. The International Conference on Harmonization (ICH) guideline entitled *Stability Testing of New Drug Substances and Products* requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.[6] Susceptibility to hydrolysis is one of the required tests. Also, oxidative and photolytic stability studies are required. 5-Nitroimidazoles are sensitive to alkali and photodegradation.[7–10] Therefore, a reliable and rapid determination method needs to be developed, which could also be used to obtain the optimum separation of the degradation components from the parent compound. An ideal stability-indicating method is that which resolves the standard drug as well as its degradation products. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering the analysis time and cost per analysis. The primary goal of the present work is to develop an accurate, specific, repeatable, and stability-indicating HPTLC method for the determination of satranidazole in the presence of its degradation products and related impurities, for assessment of purity of bulk drug and stability of its bulk dosage forms. The proposed method was validated as per ICH guidelines.[11,12] Alkali induced degradation kinetics was investigated by quantitation of the drug by a validated stability-indicating HPTLC method.

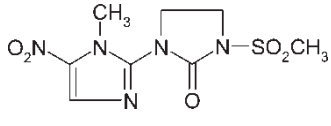

Figure 1. Chemical structure of satranidazole.

EXPERIMENTAL

Materials

Pharmaceutical grade satranidazole was kindly supplied as a gift sample by Alkem laboratories Ltd., India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. Tablets were procured from local markets.

HPTLC Instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 F–254 (10 × 10 cm with 250 μm thickness, E. Merck), using a Camag Linomat V. The plates were prewashed with methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 4 × 0.45 mm and 10 mm/s scanning speed was employed. The mobile phase consisted of toluene/acetonitrile (6:4, v/v) and 15 mL of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C ± 2). The length of the chromatogram run was approximately 8 cm. Subsequent to development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner III and was operated by WINCATS software.

Calibration Curves of Satranidazole

A stock solution of satranidazole (200 μg/mL) in methanol was prepared. Standard curves were constructed over a concentration range of 100 to 500 ng following the procedure described earlier. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

Method Validation

Precision

Precision of the method was determined with the satranidazole. An amount of the satranidazole powder equivalent to 100% of the label claim of the drug was accurately weighed and assayed. System intra-day repeatability was determined by six replicate determinations and six replication measurements of

each sample solution at the analytical concentration of 400 ng/spot. The repeatability of sample application and measurement of peak area for the active compound were expressed in terms of percentage relative standard deviation (%RSD). The %RSD was found to be less than 2%. Intermediate precision was assessed by the assay of two sets of six samples on different days (inter-day precision). The intra- and inter-day variation for determination of satranidazole was carried out at three different concentration levels: 200, 300, and 500 ng/spot, respectively.

Limit of Detection and Limit of Quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained above. The signal-to-noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1.

Accuracy

The analyzed samples were spiked with extra 50%, 100%, and 150% of the standard satranidazole, and the mixtures were reanalyzed by the proposed method. The experiments were conducted six times. This was done to check for the recovery of the drug at different levels in the formulations. The within day and day-to-day variation were studied and the percent recovery was computed using the following formula:

$$\%\text{recovery} = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \times 100$$

where X is the amount of standard drug added, Y is the amount of drug found by the proposed method, and N is the total number of observations.

Analysis of the Marketed Formulation

Twenty tablets of a marketed tablet formulation were weighed and powdered. Accurately weighed amounts of the tablet triturate corresponding to the label claim of the drug was dissolved in methanol and subjected to sonication for 15 min. The volume was made up to 100 mL and filtered. The filtrate, after suitable dilution, was spotted onto the plate followed by development and scanning as discussed above. The analysis was repeated six times.

Forced Degradation of Satranidazole

A stock solution containing 20 mg satranidazole in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability-indicating property and specificity of the proposed method. In all degradation studies, the average peak area of satranidazole after application (400 ng/spot) of six replicates was obtained.

Preparation of Acid- and Base-Induced Degradation Product

To 1 mL of the methanolic stock solution, 10 mL each of 0.1 N HCl and 0.1 N NaOH were added separately. These mixtures were placed at 40°C. The forced degradation in acidic and basic media was performed in the dark, in order to exclude the possible degradative effect of light. The resultant solutions of 20 μL (400 ng/spot) were applied on TLC plates and the chromatograms were run as described previously.

Preparation of Hydrogen Peroxide-Induced Degradation Product

Subsequently, the drug was exposed to 6% H_2O_2 at room temperature for a period of 3 h. The resultant solution of 20 μL (400 ng/spot) were applied on TLC plates and the chromatograms were run as described previously.

Photochemical Degradation Product

The photochemical stability of the drug was also studied by exposing the stock solution (200 μg/mL) to direct sunlight for 4 h. The diluted solution (20 μL) (400 ng/spot) were applied on TLC plates and chromatograms were run as described in the above.

Study of Alkali-Induced Degradation Kinetics

Accurately weighed 20 mg of drug was dissolved in 100 mL methanol. Of this standard solution, 1 mL was transferred into a 10 mL conical and diluted to the mark with 0.1 N NaOH. At the specified temperature (34°C) and specified time intervals the contents of the flask (1 mL) were transferred to the flask. Then 20 μL was spotted to get the final concentration of 400 ng/spot. The experiment was carried out in six replicates. The concentration of the remaining drug was calculated for each temperature and time interval. The data were further analyzed and degradation kinetics constants were calculated.

RESULTS AND DISCUSSION

Development of the Optimum Mobile Phase

The TLC procedure was optimized with a view to develop a stability-indicating assay method. Both the pure drug and the degraded drug solutions were spotted on the TLC plates and run in different solvent systems. Toluene alone gave a spot that exhibited tailing phenomena. The mobile phase toluene:acetonitrile (6:4, v/v) gave good resolution with R_f value of 0.53 for satranidazole. The peak was sharp and symmetrical (Fig. 2). Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Validation of the Method

Linearity

Figure 3 shows the densitogram of the standard satranidazole to be used for calibration. It was obtained after accurate optimization of the operative conditions chiefly affected by the slit dimensions and scanning speed. The calibration points were obtained in triplicate at five levels over a range of 100–500 ng of the analyte, by applying 5, 7, 10, 15, 20, and 25 μL of the standard solution.

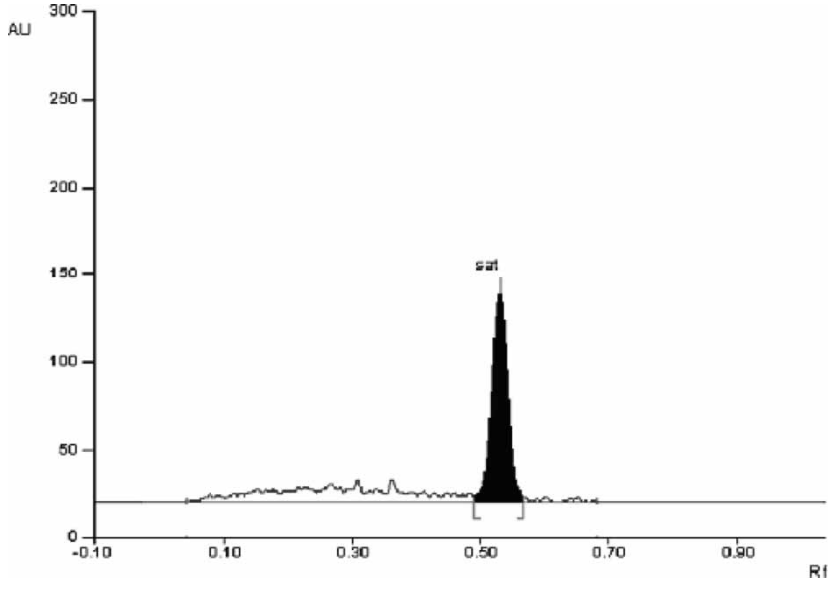

Figure 2. Chromatogram of standard satranidazole (400 ng/spot); peak 1: *R*f: 0.53 ± 0.02, mobile phase toluene: acetonitrile (6:4, v/v).

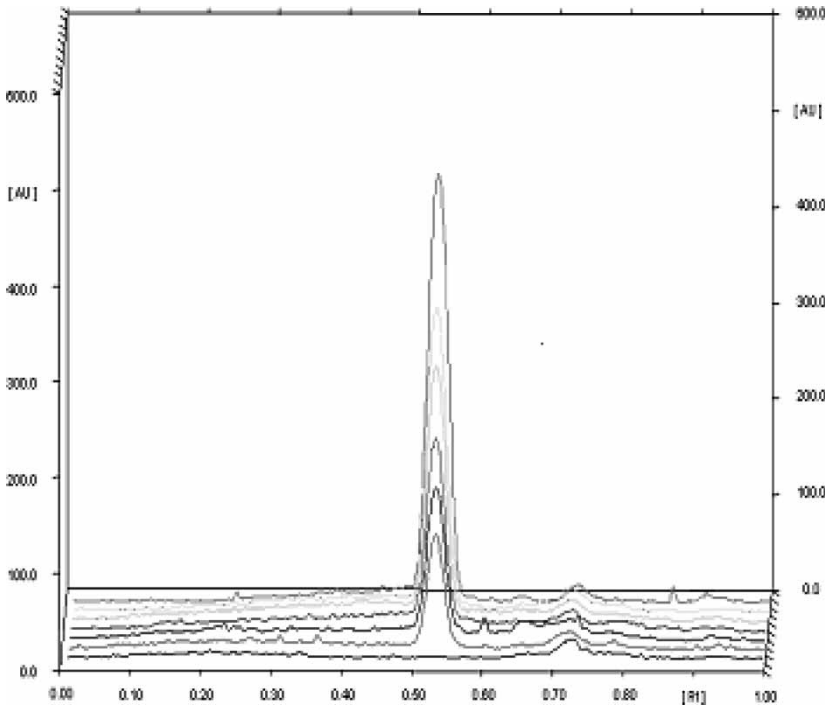

Figure 3. Scanning profile of TLC chromatogram of 0.1, 0.15, 0.2, 0.3, 0.4, and 0.5 μg/spot Satranidazole.

The equation for the curve $y = 22.976x + 487.33$ ($n = 6$) was calculated by linear regression analysis, assuring method linearity over the mass range studied with correlation coefficient $R^2 = 0.9979$.

Precision

The repeatability of sample application and measurement of peak area expressed in terms of %RSD was found to be 0.98, for six replicate determinations. The %RSD for intra- and inter-day variation of the satranidazole peak area at three different concentration levels 200, 300, and 500 ng/spot are 1.70, 0.30, and 0.98.

LOD and LOQ

The signal-to-noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 50 and 85 ng/spot, respectively.

Accuracy

The proposed method, when used for extraction and subsequent estimation of satranidazole from the pharmaceutical dosage form (after spiking with 50%, 100% and 150% of additional drug), afforded recovery of 99–101%, as listed in Table 1.

Analysis of the Marketed Formulation

A single spot at R_f 0.53 was observed in the chromatogram of the drug samples extracted from tablet. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.28 ± 0.42% with a %RSD of 0.83 for six replicate determinations. It may, therefore, be inferred that degradation of satranidazole had not occurred in the marketed formulations that were analyzed by this method. The good performance of the method indicated the suitability of this method for routine analysis of satranidazole in pharmaceutical dosage form (Table 2).

Table 1. Accuracy[a]

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	RSD (%)
0	100	99.28	0.88
50	150	99.83	0.70
100	200	101.23	0.82
150	250	100.94	0.58

[a]n = 6.

Table 2. Summary of validation parameters

Parameter	Data
Linearity range	100–500 ng/spot
Correlation coefficient	0.9979 ± 0.66
Limit of detection	50 ng/spot
Limit of quantitation	85 ng/spot
Accuracy (n = 6)	100.12 ± 0.59
Precision (%RSD)	
Repeatability of application (n = 6)	0.98
Inter-day (n = 6)	1.16
Intra-day (n = 6)	0.997

Stability-Indicating Property

Acid- and Base Induced Degradation Product

The chromatograms of the acid degraded samples for satranidazole showed two additional peaks at R_f values of 0.40 and 0.72 (Fig. 4). Degradation studies performed using a base showed additional peaks at R_f of 0.12, 0.43, as shown in (Fig. 5a-c). Satranidazole first degraded to the unstable impurity of R_f. These unstable impurities again decomposed, and disappeared completely after 90 min. The concentration of the drug was found to be changing from the initial concentration, indicating that satranidazole undergoes degradation under acidic and basic conditions.

Hydrogen Peroxide Induced Degradation Product

The sample degraded with 6% v/v hydrogen peroxide (Fig. 6) showed additional peaks at R_f values of 0.17 and 0.74. The spots of the degraded products were well resolved from the drug spots.

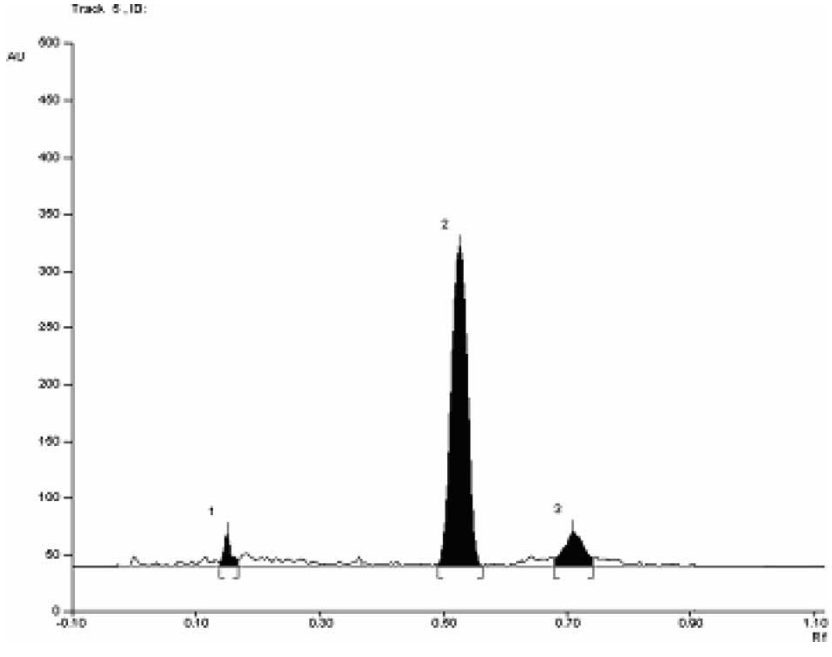

Figure 4. Chromatogram of acid (0.1 N HCl, 1 h, room temperature) treated satranidazole; peak 2: satranidazole, *R*f: 0.53; peak 1: degraded, *R*f: 0.14 and peak 3: degraded, Rf: 0.71.

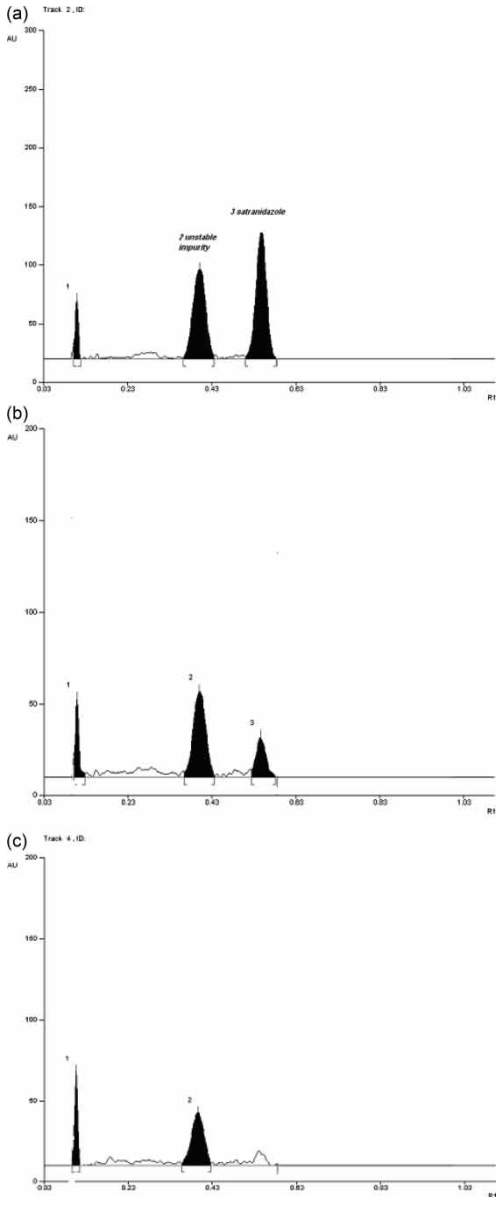

Figure 5. (a) Chromatogram of base (0.1 N NaOH, 30 min., room temperature) treated satranidazole; peak 1: degraded, *R*f: 0.12, peak 2: unstable product Rf: 0.43, peak 3: satranidazole, *R*f: 0.54. (b) Chromatogram of base (0.1 N NaOH, 60 min., room temperature) treated satranidazole; peak 1: degraded, *R*f: 0.12, peak 2: unstable product Rf: 0.43, peak 3: satranidazole, *R*f: 0.54. (c) Chromatogram of base (0.1 N NaOH, 90 min., room temperature) treated satranidazole; peak 1: degraded, *R*f: 0.12, peak 2: unstable product Rf: 0.43.

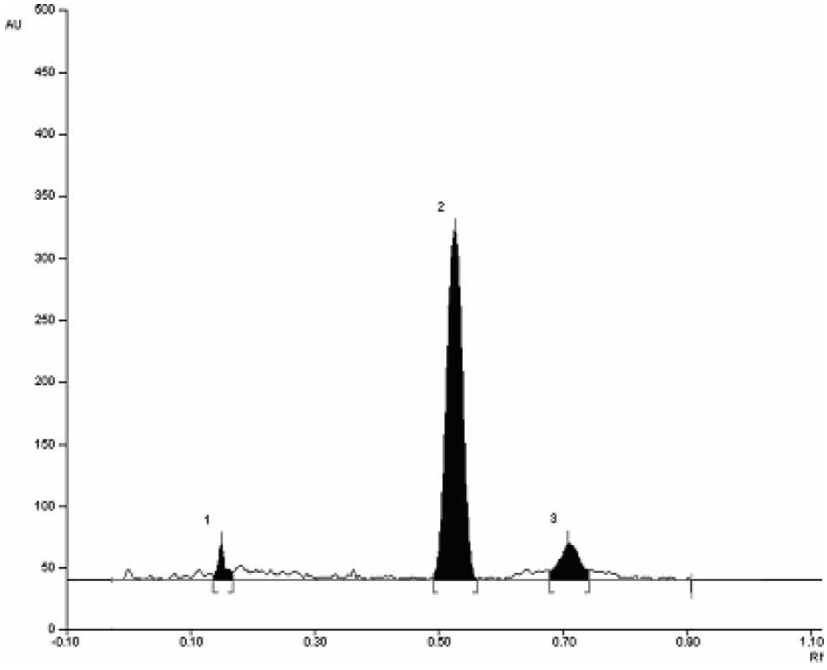

Figure 6. Chromatogram of hydrogen peroxide (6% w/v, reflux for 30 min, room temperature) treated satranidazole; peak 1and 3: degraded, *R*f: 0.17 and 0.74, peak: 2 satranidazole, *R*f: 0.53.

Photochemical Degradation Product

The photo degraded sample showed five additional peaks at R_f of 0.16, 0.32, 0.63, 0.75, and 0.89, when the drug solution was left in the day light for 4 h (Fig. 7).

The stability studies indicate that the drug is susceptible to acid base hydrolysis, as well as oxidative and photo degradation. The lower R_f values of base degraded product and oxidized products indicated that they were more polar than the analyte itself. The higher *R*f values of acid degraded, and photo degraded components indicated that they were less polar than the analyte itself.

Degradation Kinetics

In the basic medium, a decrease in the concentration of drug with increasing time was observed (see Fig. 8). The influence of temperatures on the degradation process in basic medium is shown in Fig. 8. At the tested temperatures (34°C), the degradation process followed pseudo-first order kinetics.

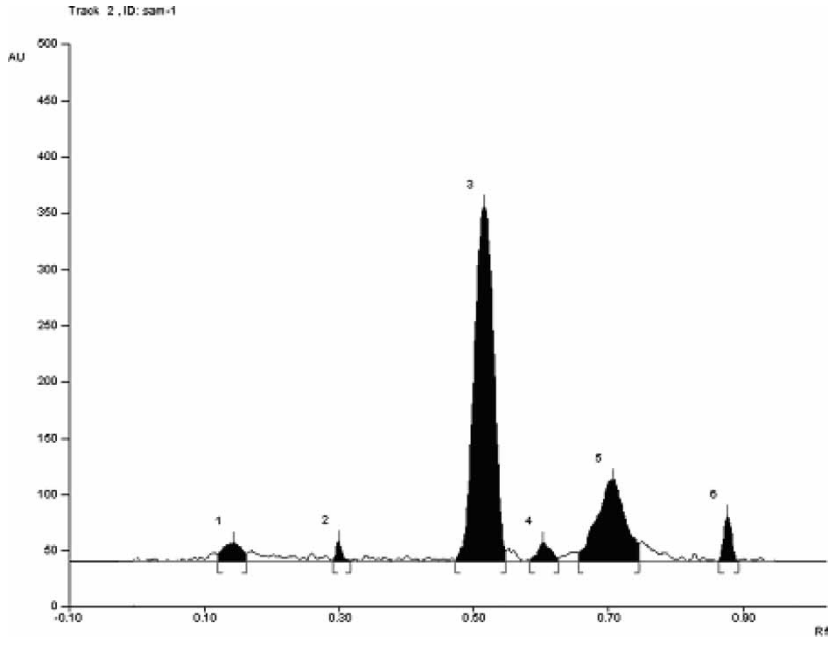

Figure 7. Chromatogram of photo degraded satranidazole; peak 1, 2, 4, 5, 6 degraded products:peak 3 satranidazole, *R*f: 0.55.

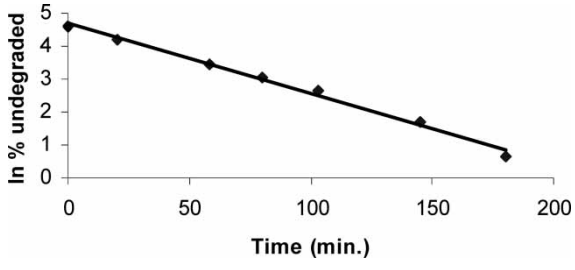

Figure 8. Pseudo first-order plots for the degradation of Satranidazole with 0.1 N NaOH at 34°C.

CONCLUSION

The developed HPTLC technique is precise, specific, accurate, and stability-indicating. Statistical analysis proves that the method is suitable for the analysis of satranidazole in bulk drug and in pharmaceutical formulations without any interference from the excipients. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. Furthermore, it can be concluded that the impurities

present in the drug could be due to hydrolysis or oxidation during processing and storage of the drug. The above results showed the suitability of the proposed method for the base induced degradation kinetic study of satranidazole. The degradation rate constant and $t_{1/2}$ of satranidazole can be predicted.

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