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Research Article

Development and Validation of Stability-Indicating HPTLC Method for Estimation of Secnidazole in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

A simple, selective, precise and stability-indicating high-performance thin layer chromatography (HPTLC) method for the analysis of Secnidazole both in bulk drug and pharmaceutical formulation has been developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of Toluene: methanol: triethylamine (4: 1: 0.4 *v/v/v*). The system was found to give compact spot for Secnidazole (R_f value of 0.50 ± 0.03). Densitometric analysis of Secnidazole was carried out in the absorbance mode at 313 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.999 ± 0.0017 with respect to peak area in the concentration range 200–1200 ng per spot. The mean values ± SD of slope and intercept were 10.081 ± 1.56 and 3607.5 ± 1.48, respectively, with respect to peak area. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 6.85 and 18.96 ng per spot, respectively. Secnidazole was subjected to acid and alkali hydrolysis, oxidation, light and thermal degradation. The drug not showed any degradation under acidic, basic, light, oxidation and thermal exposure conditions. This indicates that the drug is stable to acid, base, light (Photo degradation), oxidation and thermal exposure conditions. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of investigated drug. The proposed developed HPTLC method can be applied for the identification and quantitative determination of Secnidazole in bulk drug and pharmaceutical formulation.

Keywords: Secnidazole, HPTLC, validation, Stability and Degradation

1. Introduction

Secnidazole is nitroimidazole drug and chemically it is (*RS*)-1-(2-methyl-5-nitroimidazole-1yl) propane-2-ol [1]. Secnidazole is an orally active antifungal, antiprotozoal drug is used in treatment of amoebiasis, giardiasis, trichomoniasis, and bacterial vaginasis [2]. In an infection, the infecting organism seeks to utilize the host's resources to multiply. The injecting organism, or pathogen, interferes with the normal functioning of the host and can lead to chronic wounds, gangrene, loss of an infected limb and even death. The nitro group of Secnidazole (nitroimidazole) is chemically reduced by ferrodoxin which is Ferrodoxin- Linked Metabolic process. After entering into the microorganism by diffusion, its nitro group is reduced to intermediate compound which cause cytotoxicity, by damaging DNA. Its selectivity high activity against anaerobic organism has suggested interference with electron transport from NADPH or other reduced substrate. Secnidazole is completely absorbed after oral administration, Secnidazole is having abdominal pain, dizziness, Neurological disturbance and headache as adverse effects [3].

A detailed literature survey for Secnidazole revealed that several analytical techniques have been described for Secnidazole determination. For example, UV-Spectrophotometric method for estimation of Secnidazole [4-5]. Several RP-HPLC [6-7], UPLC [8], Colorimetric estimation [9], Adsorptive Stripping Voltammetry [10], LC-MS [11-12] and GC-MS [13] were developed for estimation of Secnidazole in meat, egg, animal feed and Pharmaceutical dosage forms.

However only few methods have been developed and validated for pharmaceutical dosage forms. Hence, our study reports a simple, precise and economic, stability-indicating HPTLC method for determination of Secnidazole in Tablet formulation. The method was validated according to ICH guidelines [14].

2. Experimental

2.1 Material and reagents

Secnidazole bulk drug and tablet Secnidazole (1000 mg) were obtained from Cadila Pharmaceuticals (Mumbai, India). Hydrochloric acid and sodium hydroxide pellets were obtained from Merck Laboratories Ltd., India. Methanol, Hydrogen peroxide, toluene and triethylamine are also obtained from Merck Specialties Private Ltd., India. All chemicals used are of HPLC grade.

2.2 Instrumentation and Chromatographic Conditions

The samples were spotted in the form of bands of 6 mm width with a Camag microliter syringe on precoated silica gel aluminium plates 60 F_{254} (20 × 20 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60 ^{0}C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of toluene: methanol: triethylamine (4: 1: 0.4 *v/v/v*), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 20 × 20 cm twin trough glass chamber (Camag,

Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature. 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 3 and was operated by Win-CATS software. Finally, the mobile phase consisting of toluene: methanol: triethylamine (4: 1.5: 0.4 *v/v/v*) gave a sharp and well-defined peak at R_f value of 0.50 **(Figure 1)**. Finalized chromatographic conditions were expressed in **Table 1**.

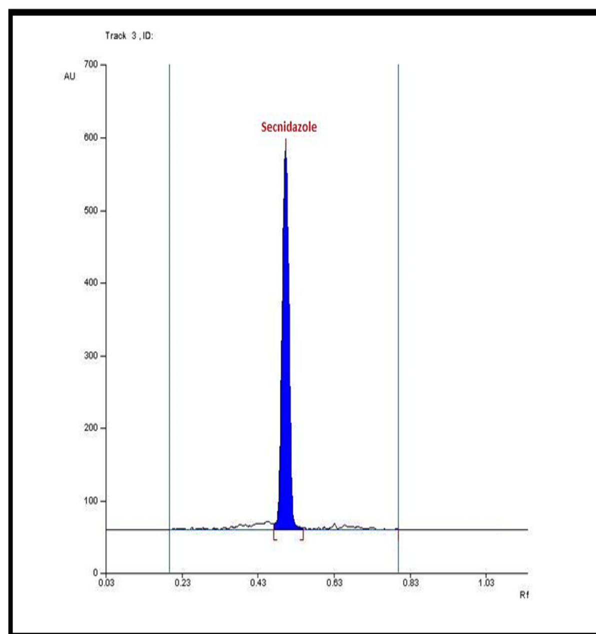

Figure 1: Densitogram of standard Secnidazole (R_f 0.50 ± 0.03), measured at 313 nm, mobile phase toluene: methanol: triethylamine (4: 1: 0.4 *v/v/v*).

Table 1: Finalized chromatographic conditions

Parameters	Specifications
Stationary phase	Aluminum backed silica gel 60 F-254 TLC plates, (10 cm × 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) prewashed with methanol
Mobile phase	toluene: methanol: triethylamine (4: 1: 0.4 *v/v/v*)
Chamber saturation	20 minutes
Migration distance	80 mm
Activation of prewashed plate	10 min
Band width	6 mm
Slit dimensions	6.00 x 0.45 mm
Radiation source	Deuterium lamp
Scanning wavelength	313 nm
Distance between bands	15.0 mm

2.3 Preparation of standard stock solutions

An accurately weighed quantity of 10 mg Secnidazole was transferred to 10 mL volumetric flask and dissolved in methanol, and volume was made up to mark with the same solvent to obtain concentration of 1000 ng/µL. Aliquots of standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µL of Secnidazole was applied on TLC plates with the help of

microliter syringe, using Linomat 5 sample applicator to obtain the concentration of 200, 400, 600, 800, 1000 and 1200 ng per spot.

3. Method Validation

3.1 Linearity and range:

Aliquots of standard stock solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µL of Secnidazole was applied on TLC plates with the help of microliter syringe, using Linomat 5 sample applicator to obtain the concentration of 200, 400, 600, 800, 1000 and 1200 ng per spot. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated 6 times. The calibration curve was plotted by considering the peak areas versus corresponding concentration.

3.2 Sensitivity:

In present study the sensitivity parameter was evaluated by determining the LOD and LOQ of the drug. The LOD and LOQ parameter was evaluated by using the slope of line and standard deviation obtained from calibration curve studies. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using equations LOD = $3.3 \times N/B$ and LOQ = $10 \times N/B$, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

3.3 Specificity:

Method specificity was evaluated for interference of closely related impurities and excipients in the analysis of drug solution. The specificity of the method was ascertained by analyzing the standard drug and sample. The spot for Secnidazole in sample was confirmed by comparing the R_f values and spectra of the spot with that of the standard. The peak purity of Secnidazole was assessed by comparing the spectra at three different levels, i.e. peak start (S), peak apex (M) and peak end (E) positions of the spot.

3.4 Accuracy:

To check the accuracy of the method, recovery studies were carried out at three different levels 80, 100 and 120 %. Base level concentrations of analytes in tablet formulation used were 600 ng/spot for Secnidazole.

3.5 Precision:

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng per spot of Secnidazole). The intra and inter-day variation for the determination of Secnidazole was carried out at three

different concentration levels of 400, 600 and 800 ng per spot.

3.6 Robustness:

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions of toluene: methanol: triethylamine (4: 1.2: 0.4 and 3.8: 0.7: 0.4 *v/v/v*) were tried, and chromatograms were run. The volume of mobile phase and saturation time was varied in the range of ± 5%. Time from spotting to chromatography and from chromatography to scanning was varied from 5, 20 and 40 min.

3.7 Ruggedness:

Ruggedness of the method was performed by spotting 600 ng of Secnidazole by two different analysts, keeping same experimental and environmental conditions.

3.8 Application of proposed method for estimation of drug in Tablet formulation:

Twenty tablets were weighed and finely powdered. Amount of tablet powder equivalent to 10 mg of Secnidazole was weighed accurately, transferred to 100 ml volumetric flask and shaken with 50 ml methanol for 15 min. Volume was made up to 100 mL with methanol and ultrasonicated for 15 min. Solution was then filtered through Whatmann filter paper No. 41. The solution was suitably diluted with methanol to get concentration 100 ng/µL of Secnidazole. 6 µL of each drug was applied in the form of bands on the TLC plate in order to get 600 ng/spot concentration. The plate was developed using mobile phase, containing toluene: methanol: triethylamine (4: 1: 0.4 *v/v/v*).

4. Forced degradation studies

The drugs were subjected to stress conditions of acid hydrolysis, alkali hydrolysis, oxidation, thermal degradation and light degradation. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and intrinsic stability of the molecule. Specificity is the ability of the method to measure the analyte response in presence of its potential impurities.

4.1 Acid- and base-induced degradation

Ten milligrams of Secnidazole was separately dissolved in 10 mL of methanolic solution of 5 N HCl and 5 N NaOH. These solutions were reflux for 24 hrs. at 60^0C temperature respectively in the dark in order to exclude the possible degradative effect of light. One millilitre from the above solutions was taken and neutralized and then diluted up to 10 mL with methanol. The resultant solutions were applied on TLC plate in triplicate (6 µL each, i.e. 600 ng per spot).

4.2 Hydrogen peroxide-induced degradation

Ten milligrams of Secnidazole was separately dissolved in 10 mL of methanolic solution of hydrogen peroxide (10.0%, *v/v*). The solution was kept for 48 hrs. at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on TLC plate in triplicate (0.6 µL each, i.e. 600 ng per spot).

4.3 Photochemical degradation

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 48 hrs. The resultant solution (0.6 µL, i.e. 600 ng per spot) was applied on a TLC plate.

4.4 Dry heat degradation

The powdered drug stored at 60°C for 48 hrs. under dry heat condition showed no significant degradation. In all degradation studies, the average peak areas of Secnidazole after application (600 ng per spot) of three replicates were obtained.

5. Results and Discussions

5.1 Validation of the Method

5.1.1 Linearity and Range

The linear regression data for the calibration curves showed good linear relationship over the concentration range 0.2 - 1.2 ng/µL for Secnidazole (n = 6). Typically, the regression equations for the calibration curve was found to be y = 10.081x + 3607.5 (r^2 = 0.999) for Secnidazole. The calibration curve was plotted by considering the peak areas versus corresponding concentration (Figure 2). The results were expressed in Table 2 and 3D linearity spectra of Secnidazole shown in Figure 3.

Table 2: Linearity Study of Secnidazole

Sr. No	Concentration in [ng/spot]	Peak area mean ± S.D.	% R.S.
1	200	5477.02 ± 45.69	0.83
2	400	7754.80 ± 41.49	0.53
3	600	9678.87 ± 39.98	0.41
4	800	11787.09± 38.78	0.32
5	1000	13662.43± 58.11	0.42
6	1200	15623.71± 56.33	0.36

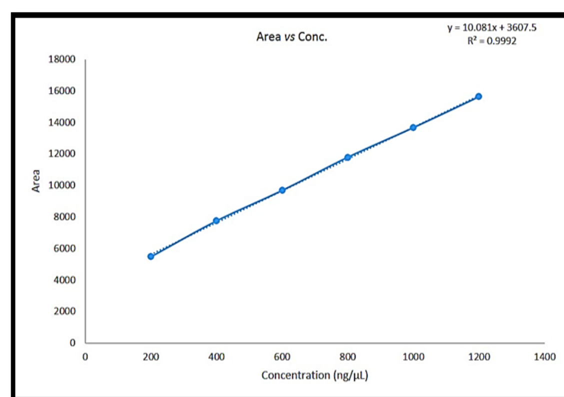

Figure 2: Calibration Curve of Secnidazole; Y = 10.081 X + 3607.5; Whère, Corrélation coefficient = 0.999, Slope = 10.081, Intercept = 3607.5

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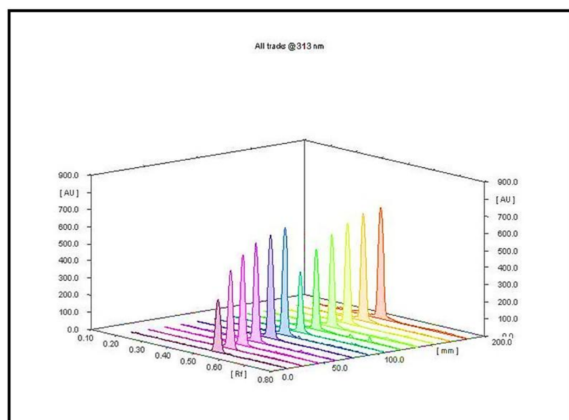

Figure 3: The 3D Linearity spectra of Secnidazole standard drug solution.

5.1.2 Sensitivity:

The linearity equation was found to be Y = 14.147 X + 2366. The Limit of Quantification and Limit of Detection for Secnidazole were found to be 18.96 ng and 6.85 ng, respectively. [Where, N = 26.83, B = 14.147]. This indicates the adequate sensitivity of the method.

5.1.3 Specificity

The peak purity of Secnidazole was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e. r^2 (S, M) = 0.99993 and r^2 (M, E) = 0.995. Good correlation (r^2 = 0.999) was also obtained between standard and sample spectra of Secnidazole (Figure 4).

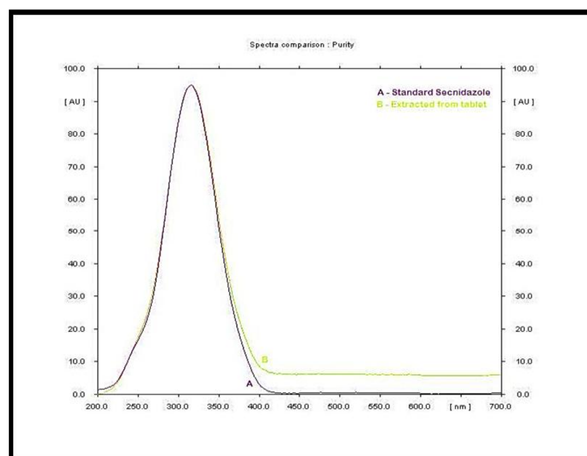

Figure 4: Peak purity spectra of standard Secnidazole (A), sample (B) extracted from Secnidazole tablet, scanned at the peak-start, peak-apex, and peak-end

5.1.4 Accuracy

The proposed method when used for subsequent estimation of Secnidazole from the pharmaceutical dosage formed after over spotting with 80%, 100% and 120% of additional drug afforded good recovery of Secnidazole. The amounts of drug added and determined and the percentage recovery are listed in Table 3, which showed that the % amount found was between 99.30 % and 100.02 % with % R.S.D. >2.

Table 3: Recovery studies

Drug	Initial Amount [ng/spot]	Amount added (%)	Amount recovered ± S.D.[ng/band] [n=3]	% Recovered	% RSD
Secnidazole	400	80	714.99± 4.94	99.30	0.69
	400	100	800.22± 1.62	100.02	0.20
	400	120	875.82± 4.68	99.52	0.53

5.1.5 Precision

The precision of the developed HPTLC method was expressed in terms of percent relative standard deviation (% RSD). The results, presented in Table 4 and results of repeatability expressed in Table 4.1. % R.S.D. values found to be less than 2, revealed high precision of the method.

Table 4: Precision Studies (Intra- day and Inter- day)

Drug	Conc.[ng/spot]	Intra day %Amount found [ng]		Inter day %Amount found [ng]	
		Mean ± SD [n= 3]	% RSD	Mean ± SD [n= 3]	% RSD
Secnidazole	400	100.31 ± 0.97	0.96	101.67 ± 6.85	0.67
	600	98.57 ± 1.12	1.13	98.24 ± 5.13	0.52
	800	99.64 ± 0.76	0.76	100.51 ± 4.52	0.44

Table 4.1: Results of Repeatability Studies

Drug	Amount Taken (ng/spot)	Amount Found (ng)	Amount Found %
Secnidazole	600	558.27	98.04
	600	590.04	98.34
	600	591.86	98.64
	600	594.01	99.00
	600	591.16	98.52
	600	596.66	99.44
	Mean ± SD	592.00± 2.97	98.66 ± 0.49
	%RSD	0.5023	0.5028

5.1.6 Robustness

The standard deviation of peak areas was calculated for each parameter, and % RSD was found to be < 2. The low values of % RSD values, shown in Table 5, indicated the robustness of the method.

Table 5: Results of Robustness Studies

Parameters	± SD of peak area	% RSD
Mobile phase composition		
toluene : methanol: triethylamine (4: 1.2: 0.4 *v/v/v*)	10.39	0.10
toluene : methanol: triethylamine (3.8: 0.7: 0.4 *v/v/v*)	15.64	0.16
Mobile Phase volume		
5 mL	9.87	0.10
10 mL	20.87	0.21
Saturation Time		
15 Min.	11.19	0.11
25 Min.	10.61	0.11
Time from spotting to chromatography	16.16	0.32
Time from chromatography to scanning	13.34	0.29

5.1.7 Ruggedness

Peak area was measured for same concentration solutions, six times by two analyst. The results are given in Table 6, showed that the % R.S.D. was less than 2 and % amount found was between 99.97 % and 100.01 %.

Table 6: Results of Ruggedness

Analyst	%Amount found ± SD	%RSD [n=3]
I	99.97 ± 0.064	0.64
II	100.01 ± 0.089	0.89

5.1.8 Analysis of the Marketed Formulation

A single spot at R_f 0.40 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablet. The low % RSD value indicated the suitability of this method for the routine analysis of Secnidazole (Figure 5) in pharmaceutical dosage forms. The results expressed in Table 7.

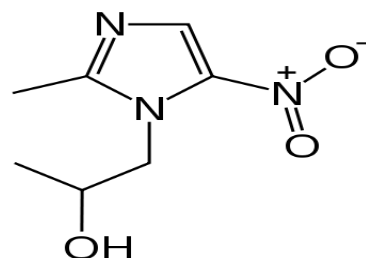

Figure 5: Structure of Secnidazole

Table 7: Analysis of Marketed Tablet Formulation

Brand Name: SECNIL FORTE **Mfg. By:** Abbott Healthcare Pvt. Ltd.

Batch No.: SFA1006 **Average weight:** 1236.30 mg

Drug	Amount Taken (ng/spot)	Amount Found (ng)	Amount Found %
Secnidazole	600	587.87	97.97
	600	588.81	98.13
	600	593.16	98.86
	600	587.75	97.95
	600	592.98	98.83
	600	591.98	98.86
	Mean ± SD	590.42± 2.55	98.04 ± 0.43
	%RSD	0.4332	0.4369

5.2 Force Degradation

Samples applied on TLC plates not showed any additional peak after scanning, it only showed spot of pure drug, which confirmed by spectral scanning. The spot of Secnidazole was well resolved from each samples applied on TLC plates. In each cases, the concentration of the drug was not changing from the initial concentration, indicating that Secnidazole not showed degradation under acidic, basic, light, oxidation and thermal exposure conditions. This indicates that the drug was not susceptible to acid–base hydrolysis, light,

oxidation and thermal degradation and there was no change seen in R_f value of drug. The results are listed in **Table 8**

Table 8: Forced degradation studies

Agent	Exposure time	Condition	Degradants peak	R_f	% Recovery
5 N HCl	24 hrs. (reflux)	60^0 C	Not Found	-	98.25
5 N NaOH	24 hrs. (reflux)	60^0 C	Not Found	-	99.02
10% H_2O_2	48 hrs.	60^0 C	Not Found	-	97.12
Dry Heat	48 hrs.	60^0 C	Not Found	-	98.28
Light	48 hrs.	Sunlight	Not Found	-	97.54

6. Conclusions

The modalities adopted in experiment were successfully validated as per ICH guidelines. The developed HPTLC method was found to be accurate, simple, precise, specific and stability-indicating and can be conveniently applied for quality control analysis in industry and is having short run time which significantly reduces the analysis time and cost. Statistical analysis proves that the method is repeatable and selective for the analysis of Secnidazole as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. Method concluded that the drug was stable in all stress conditions, it can be employed as a stability-indicating one.

Acknowledgments

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