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Research Article

A Validated Stability – Indicating Liquid Chromatographic Method for Secnidazole

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Abstract

The research work describes development of stability – indicating reverse phase – HPLC method for Secnidazole in the presence of its degradation products, generated from forced degradation studies. Secnidazole was subjected to forced degradation under acidic, basic, photolytic and oxidative conditions. Successful separation of drug from degradation products formed under forced degradation was achieved on a C 18 column (250 mm × 4.6 mm, 5 mcm) using methanol : water (60 : 40 v/v) as a mobile phase at a flow rate of 1mL/min. The detection was carried out at 310 nm. The method was validated for linearity, range, accuracy and precision.The developed method can be used to evaluate quality of regular production samples and also to test stability samples of secnidazole tablets. The intra and inter day results at each level were subjected to one way ANOVA and F values at each level were obtained as a ratio of between mean square and within mean square. The F values were found to be within limits. And the t-test value was obtained as 1.9725.

Key words: Secnidazole**,** Secnidazole tablets, HPLC, forced degradation studies, method validation.

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1. Introduction

Secnidazole is chemically 1-(2-hydroxypropyl)-2- methyl-5-nitroimidazole (Figure 1). It is a relatively new antiprotozoal agent used in the treatment of amoebiasis and has also been tried in trichomoniasis. [1] It is not official in any pharmacopoeia. Secnidazole is completely absorbed after oral administration. The adverse effects of

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secnidazole are abdominal pain, dizziness, Neurological disturbance and headache.

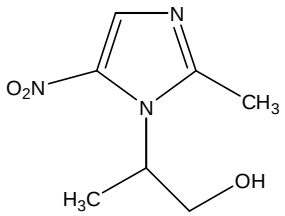


Figure 1: Structure of Secnidazole

Several analytical procedures have been reported for the determination of secnidazole in pharmaceutical formulations, either alone or in combination with other drugs. These include spectrophotometric, [6-11] differential pulse polarographic, [12] supercritical fluid chromatographic, [13] GLC [14] and HPLC methods. [2,10] For stability testing, four different analytical methods, viz. derivative spectroscopy, HPLC, TLC densitometry and colorimetry were developed. But only few stability-indicating HPLC methods for drug analysis has been reported.

The aim of present work is to develop an accurate, specific, and repeatable stability – indicating HPLC method for the estimation of secnidazole from tablets in presence of its possible degradation products. Further, the proposed method was validated as per ICH guideline Q2 (R1).

2. Experimental

Chemicals and reagents

Pharmaceutical grade secnidazole was supplied as a gift sample by Meditab Speacialities Pvt. Ltd., Daman. Methanol used was of HPLC grade and other chemicals and solvents used in the studies were of AR grade and purchased from S.D. Fine Chemicals, Mumbai, India. Double distilled water was used throughout the experiment. The 0.45 µ Nylon filter papers were purchased from Pall India Pvt. Ltd, Mumbai (India).

HPLC Instrumentation and Chromatographic Conditions

HPLC system used consisted of pump PU – 2080 plus (JASCO, Tokyo, Japan with Rheodyne loop injector (7725 *i*) of capacity 25 mcL and detection was carried out by UV – 2077 detector. The data acquisition was done using Borwin chromatograohy software version 1.50. The chromatographic seperations were carried out on a HiQsil C18 column (250 mm × 4.6 mm, 5 mcm, Kya Technologies Corporation, JAPAN).

Mobile phase consisted of methanol : water (60 : 40 % v/v). The mixture was filtered through 0.45 micron membrane filter, ultrasonically degassed and used. Flow rate of 1mL/min with iscocratic elution and the column eluent being monitored at 310 nm at ambient temperatures. The injection volume was 20 mcL.

Degradation studies

Standard secnidazole with a concentration of 1 mg/ml was used in all the degradation studies. The bulk drug was subjected to hydrolysis by refluxing the sample solution in hydrochloric acid (1N, 0.5N, and 0.1N), sodium hydroxide (1N, 0.5N, 0.1N, 0.01N) at temperature of 100^0 C. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase. For the oxidative studies, the standard secnidazole solution was subjected to stress conditions in 30 % and 3 % hydrogen peroxide at room temperature for different time intervals and then heated in water bath for 10 min to remove excess of hydrogen peroxide. Photo degradation studies were

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performed by exposing the drug powder to direct sunlight for different time interval. At the end of exposure, the drug samples were quenched, diluted with mobile phase so as to achieve a concentration of 10 mcg/mL with respect to the initial concentration of the drug and subjected to chromatographic analysis. In each case, suitable blanks and controls were used to preclude errors. Appearance of secondary peak and reduction in area of the drug peak was taken as an indication of degradation. [1]

Estimation of secnidazole from tablets

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 10 mg of secnidazole was transferred to a 100 ml volumetric flask and shaken with 70 ml of methanol for 10 min. The volume was made up to 100 ml with methanol. The solution was filtered. From the filtrate appropriate dilutions were made mobile phase. The tablet sample solution was injected and chromatogram was obtained. The peak area of the secnidazole was calculated. Using the regression equations and peak areas of the sample, the amount of secnidazole in the sample was calculated. The amount of secnidazole per tablet was thus found. [2]

Method Validation

Analytical method validation was carried out as per ICH method validation guideline Q2 (R1).

Linearity and range

Standard stock solution of secnidazole was prepared in methanol and mixed to obtain final concentration of about 1 mg/mL.

From the above stock solution, dilutions were made with mobile phase to get seven calibration standards with concentrations of 2, 4, 6, 8, 10, 12, 14 mcg/mL. The calibration standards were analyzed in three replicates and the peak areas were plotted against corresponding drug concentrations and were subjected to least square linear regression to get an equation for line of best fit. The slope and intercept of the least square line were noted. [15]

Accuracy and Precision

The accuracy and precision were evaluated by fortifying a powder mixture of blank tablets with amounts of drug corresponding to 80, 100, 120% of label claimed and analyzing the resulting mixtures in three replicates over three days. The % recovery of added drug and % relative standards deviation (RSD) were taken as measures of accuracy and precision, respectively. Also, the results obtained were subjected to one way ANOVA and between – day mean square compared to the within – day mean square by F-test . [15]

Specificity

The specificity of the proposed method was established by the complete separation of secnidazole in presence of its degradation products. Blank tablets of secnidazole were chromatographed before and after subjected to stress conditions. Absence of peaks in the blank runs at the retention time of drug and degradation products was taken as indication of specificity. [15]

3. Results and Discussion

Development and optimization of the stability-indicating HPLC method

Different mobile phases were tried and a best resolution was achieved with an initial run of a mixture of methanol and water in the ratio of 60:40 (v/v) to separate secnidazole from its degradation

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products. The optimum wavelength for detection was 310 nm at which better detector response was obtained. The retention time for secnidazole was observed to be 4.32 min.

Analysis of stressed sample

Secnidazole was found to degrade under acidic, alkaline, oxidative, and photolytic conditions, while it was stable under dry heat and wet heat conditions. Table 1 summarizes the degradation behavior of secnidazole and the retention times of degradation products obtained under tested conditions. Figure 2 gives the representative chromatograms of stressed samples of secnidazole showing well resolved peaks of degradation products under various stress conditions.

Validation of the method

Linearity and range

In the calibration studies it was found that the calibration line was linear in the range of 2-14 mcg/ml (Figure 3). The response for the drug was linear and the calibration equation was y = 48871x + 54281 with R^2 = 0.998. The drug content was found to be 100% ±1.32 of the added amount indicating that the method can be used for analysis of secnidazole from tablet formulation. The t-test value was obtained as 1.9725.

Accuracy and precision

The data obtained from precision and accuracy experiments are summarized in Table 2. Mean values of amount added and intraday % RSD values were very low indicating acceptable accuracy and precision of the method. The intra and inter day results at each level were subjected to one way ANOVA and F values at each level were obtained as a ratio of between mean square and within mean square. The F values were found to be found to be less than the tabulated F (2,6) at α=0.05 (Tabulated F=5.14). This indicated that there was no significant difference between intra and inters –day variability, suggesting good intermediate precision of the method.

Specificity

The chromatograms of blank and placebo solutions showed no interfering peak at the retention time of the drug indicating specificity of the developed method.

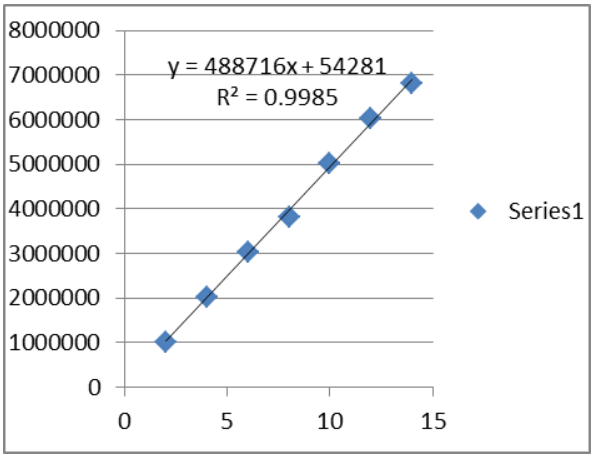


Figure 3 : Linearity of Secnidazole

4. Conclusion

This study presents a simple and validated stability-indicating HPLC method for the estimation of secnidazole in the presence of its degradation products. The developed method is specific, accurate, precise and robust. All the peaks of the degradation products formed during forced degradation studies were well separated from the analyte peak. This demonstrates that the developed method was specific and stability-indicating**.** The developed method can be used for routine analysis of secnidazole tablets or for assay of secnidazole tablets from stability batches.

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Table 1: Percent degradation of secnidazole and retention time of degradation products

Sr. No	Condition	Drug peak area at zero time (mcV.sec)	Drug peak area of stressed samples (mcV.sec)	Retention time(s) of degradation products (min)	% Degradation
1	Refluxed in 0.1N HCL for 45 mins	5188614.595	4646562.364	2.417	10.45 %
2	Refluxed in 0.01N NaOH for 15 mins	5188614.595	4555432.75	1.808	2.21%
3	Kept in 3% H_2O_2 for 30 mins	3996951.486	2649963.500	2.083 , 2.742, 3.767	33.71%
4	Exposed to direct sunlight for 14 hr	3996951.486	2916549.500	2.967	15.07%
5	Refluxed in H_2O for 15 h	-	-	No Degradation Product	No Degradation Product
6	Kept in oven at 70˚C for 23 h	-	-	No Degradation product	No Degradation Product

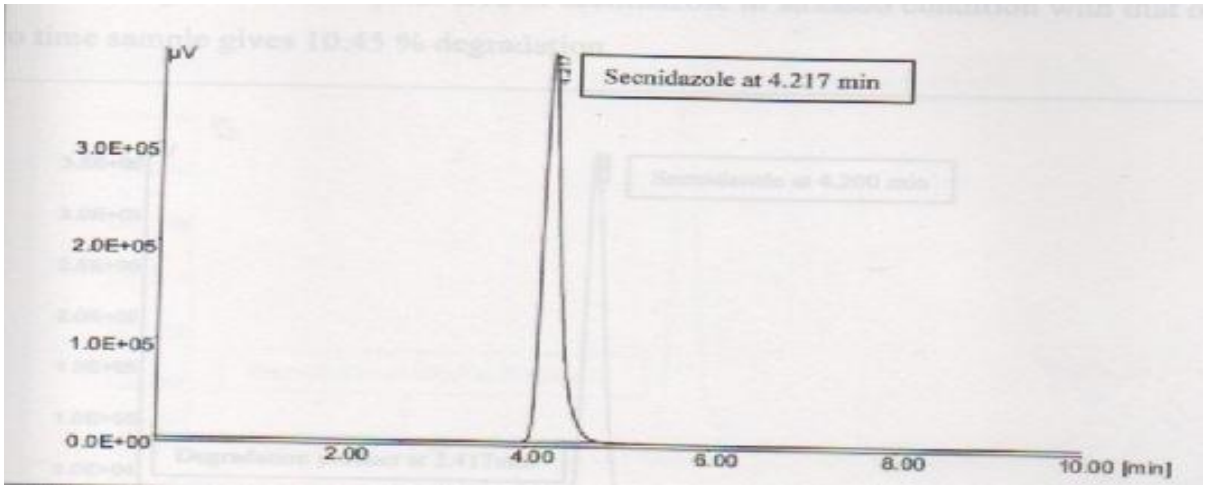


Figure 2: Representative Chromatogram of Secnidazole

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Table 2: Accuracy and Precision table

Amount added (mg)	Amount found (mg)			Within mean square	Between mean square	F
	Days					
	1	2	3			
800mg	807.5	807.3	799.8			
	804.5	807.5	805.9	11.567	3.017	0.2608
	807.0	800.5	807.4			
Mean	806.3	805.1	804.3			
% RSD	0.20	0.49	0.50			
1000mg	1003.6	1002.6	1007.5			
	1002	991.4	989.4	48.729	11.454	0.2351
	1004.0	1006.1	1002.0			
Mean	1003.2	1000.0	999.6			
% RSD	0.10	0.76	0.92			
1200mg	1195.5	1184.3	1197.4			
	1199.4	1197.7	1198.8	43.284	17.160	0.3965
	1197.5	1197.8	1184.1			
Mean	1197.4	1193.2	1193.4			
%RSD	0.16	0.65	0.67			

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Table 3: Results from regression analysis and system suitability of secnidazole

Sr. No.	Description	Result
1.	Retention time (min)	4.325
2	Linearity range (µg/ml)	2-14
3	Slope (m)	48871
4	Correlation coefficient (r)	0.998
5	Tailing factor	1.21
6	Theoretical plates	9595.90

Table 4: Determinations of active ingredients in formulation

Sr. No.	Sample	Label Claimed (mg/ml)	Amount Found (mg/ml)	%Labeled Claim
1	secnidazole	1000	1015.7	101.5
2	secnidazole	1000	990.7	99.0
3	secnidazole	1000	995.3	99.5

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