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Analytical method development and validation of Secnidazole in the tablet dosage form by RP-HPLC method

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ABSTRACT
Objective of the present work is to develop and validate a simple, cost effective, sensitive and fast HPLC method for the analysis of Secnidazole. A Shimadzu HPLC system with Luna 5µm C_{18} column is employed for the analysis using Methanol:H_2O (60:40, v/v) as mobile phase. Signal from Secnidazole is detected at 310nm by UV Spectrophotometer. The proposed method is fully validated and found to be linear over a workable drug concentration, accurate, precise and robust. This fast and inexpensive method is suitable for research laboratories as well as for quality control analysis in pharmaceutical industries.

***Key Words*:** Secnidazole, HPLC, Nitroimidazole, pharmaceutical, Shimadzu, validation.

INTRODUCTION

Secnidazole is an antiprotozoal drug. It is a derivative of 5-nitromidazole which is closely related to Metronidazole (figure 1). It is a category of 6 Anti-infective drugs, Anti-protozoal drugs, Antiamebic and antigiardiasis drugs. It is of Synthetic origin and belongs to Nitroimidazole. Secnidazole belongs to Amebicides pharmacological group.

Secnidazole is used to treat intestinal ameobiasis, fiardiasis, trichomoniasis and bacterial vaginosis. These disease symptoms can be treated with a single once only dose of Secnidazole. Secnidazole does not differ from Metronidazole, being a nitroimidazole derivative. However, prolonged plasma half-life of Secnidazole makes it the first choice for treatment of many diseases (Gillis and Wiseman, 1996). Various method validations for Secnidazole has been developed using different analytical methods for the determination of Secnidazole in pharmaceutical dosage formulations (Misiuk, 2010, Farooqui *et al*., 2010, Bansode *et al*., 2013, Jain *et al*., 2014, Baraka *et al*., 2014). These methods are used either alone or in combination with other drugs. HPLC method is more suitable than others because operational pressures are significantly higher (50-350 bar) and requires very small sample amount to be separated (Shabir, 2003).

Among the existing methods for determination of Secnidazole in pharmaceutical dosage forms, most are time consuming, expensive, complex in nature and damage the susceptibly of the column (Alhalabi *et al*., 2012, Farooqui *et al*., 2010, Yanamandra *et al*., 2011). The aim of the present work was to attempt the development of new HPLC methods for Secnidazole which are superior to prior developed methods with respect to cost of analysis, analysis time, sensitivity and simplification. When the method is developed it must be validated to check that it meets performance characteristics. Typical analytical characteristics used in method validation are accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range and robustness. Further, the proposed method was validated as per ICH guideline (ICH, 2005).

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade of Secnidazole was supplied as a generous gift from Incepta Pharmaceuticals Limited, Saver, Bangladesh, Secnidazole 1000mg (Secnid DS by Square Pharmaceuticals Ltd, Bangladesh) was procured from local market (Noakhali, Bangladesh). Methanol HPLC grade from Active Fine Chemicals Ltd, Bangladesh, Methanol Reagent grade from Active Fine Chemicals Ltd, Bangladesh, HPLC grade water and Distilled-water were used.

Instrument used and Chromatographic conditions

HPLC system compromised of Column Oven (Model: CT-10ASvp Made by Shimadzu Corporation Japan), Prominence Liquid Chromatographs, (Model: LC20AT, Japan), Prominence Degassing unit (Model: DGU-20A3R, Japan) and Prominence UV/ VIS Detector (Model: SPD-20A,

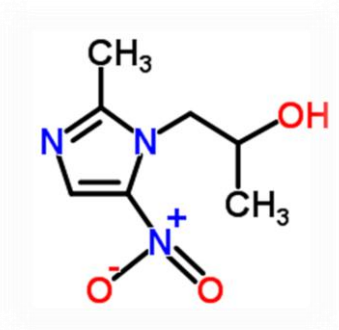

Figure 1: Chemical structure of Secnidazole.

Tanzina Sharmin and Mariyam Akter contributed equally to this work and should be considered as joint first authors

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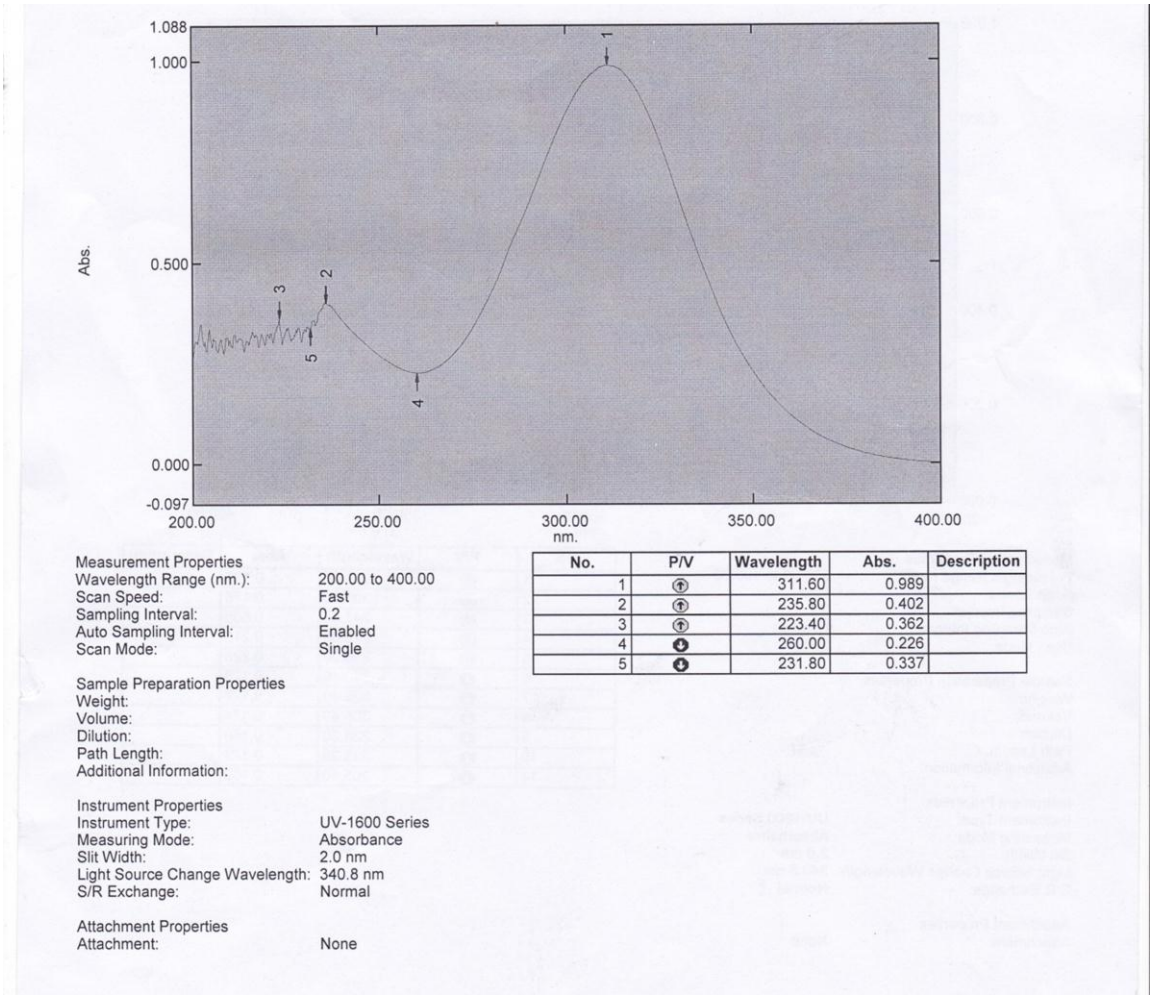

No.	P/V	Wavelength	Abs.	Description
1	↑	311.60	0.989	
2	↑	235.80	0.402	
3	↑	223.40	0.362	
4	↓	260.00	0.226	
5	↓	231.80	0.337	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Fast
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Single

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Instrument Properties
Instrument Type: UV-1600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Light Source Change Wavelength: 340.8 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Figure 2: UV spectrum of Secnidazole.

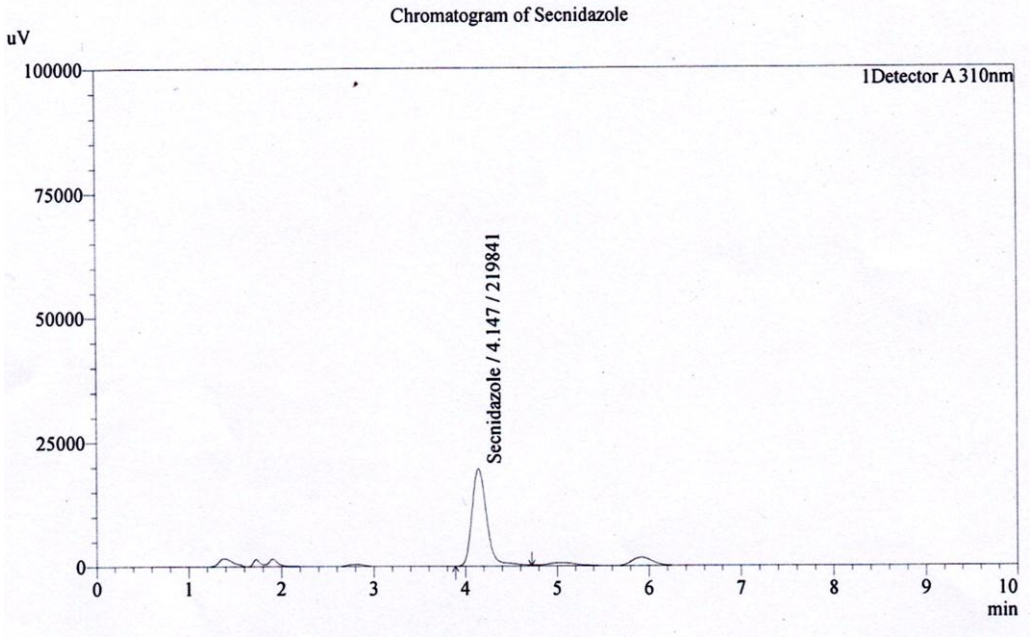

Figure 3: Typical Chromatogram of Secnidazole.

Japan). The chromatographic separations were performed using Luna 5µm C_{18} 100A (Size 150×4.60mm) as column, Methanol: H_2O (60:40, v/v) as mobile phase, flow rate 1ml/min with isocratic elution and retention time of 4.147min etc. The wavelength 310nm was detected by UV Spectrometer (Model: UV-1800, Made by Shimadzu Corporation) (figure 2).

Standard and sample preparation

An accurately weighed quantity of 25mg of Secnidazole was placed into a 100ml volumetric flask and dissolved in reagent grade methanol up to 100ml. Then, 2ml were withdrawn in a 25ml volumetric flask. The volume was made with mobile phase to made final concentration of 20µg/ml. The solutions were passed through a 0.45µm nylon membrane filter before injection. To prepare sample solution, same procedure was followed to make the concentration of standard solution and sample solution same. Briefly, the average weight of 20 tablets was taken. Then these 20 tablets were grinded and an equivalent weight of 25mg of Secnidazole standard was taken from grinded powder. Accurately weighted sample was poured in a 100ml volumetric flask and about 60ml of methanol was added. Sample solution then was sonicated for 10 minutes. After 10 minutes volume of sample solution was made up to 100ml and then sample solution was filtered using Whatman filter paper. The dilution from this stock solution was same as standard solution.

Method development and optimization

For the simultaneous determination of Secnidazole with an intention to develop a precise, accurate, simple, rapid, sensitive and specific way, isocratic RP-HPLC method was optimized. Secnidazole was very soluble in the methanol, ethanol, chloroform, acetic acid and water. But methanol and water are easily available and low cost. So, different mobile phases were tried with different fractions of methanol and water. A satisfactory resolution was achieved using a mixture of methanol and water in the ratio of 60:40 (v/v). The optimum wavelength for detection of Secnidazole was 310nm. The flow rate of Secnidazole was 1ml/min at ambient temperature for column oven (25°C). The injection volume was 20µL and run time was 10 min. The retention time of Secnidazole was observed at 4.147min (figure 3).

System Suitability and Method validation

All the system suitability parameters were assessed as per ICH guidelines (ICH, 2005).

RESULTS AND DISCUSSION

Linearity and range

The linear regression data for the calibration curves showed good linear relationship over the concentration range of 20-60µg/ml for Secnidazole. Typically, the regression equations for the calibration curve was found to be y = 95882x-2E + 06 (r2 = 0.990) for Secnidazole. The calibration curve was plotted by considering the peak areas(y) versus corresponding concentration(x) (figure 4, table 1).

Limit of detection (LOD) and limit of quantitation (LOQ)

For this study, three replicates of analyte at lowest concentrations were measured and quantified. The equations are LOD=3.3×σ/S and LOQ=10×σ/S where, 'σ' is standard deviation of lowest three concentrations and 'S' is slope of linearity equation. The LOD and LOQ were 0.33µg/ml and 0.99µg/ml respectively (table 2).

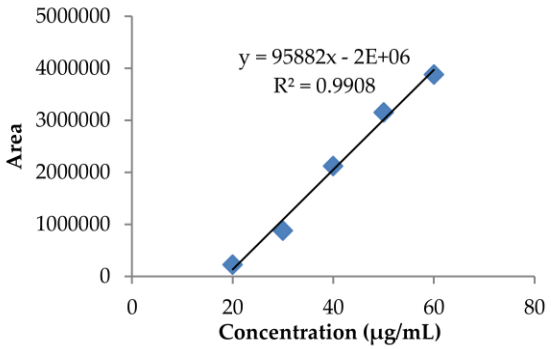

Figure 4: Linearity curve of Secnidazole.

Table 1: Linearity study of Secnidazole.

Sr. no.	Concentration [µg/ml]	Peak Area	Slope	Intercept	R^2 Value
1	20	219841			
2	30	879906			
3	40	2117130	95882	06	0.990
4	50	3150969			
5	60	3878387			

Table 2: LOD and LOQ data.

Sr. No.	Conc. (µg/ml)	Area	σ	S	LOD (µg/ml)	LOQ (µg/ml)
1	4	68942				
2	6	78654	9497.811	95882	0.33	0.99
3	8	87936				

Table 3: Precision data.

Sr. no.	Sample preparation	Sample wt. (mg)	Area of sample	Assay (mg)	Average value	SD	% RSD
1	Sample 1	29.00	217969	985.22			
2	Sample 2	29.03	217572	983.43			
3	Sample 3	29.20	216965	974.17	980.77	3.98	0.41
4	Sample 4	29.09	217492	980.89			
5	Sample 5	29.10	218340	982.53			
6	Sample 6	29.16	217892	978.35			

Table 4: Robustness of Secnidazole.

Sr. no	Sample preparation	Sample wt. (mg)	Area of sample	Assay (mg)	Average value	SD	% RSD
1	Sample 1	29.16	225931	980.54			
2	Sample 2	29.18	226889	984.70			
3	Sample 3	29.09	226524	985.38	982.44	4.09	0.42
4	Sample 4	29.15	224857	975.88			
5	Sample 5	29.20	226583	981.10			
6	Sample 6	29.12	226910	987.06			

Table 5: Accuracy of Secnidazole.

Sample	Area of sample	Theoretical value (mg)	Experimental value (mg)	% of recovery	Mean
80% S -1	219277	831.96	831.15	99.90	
80% S -2	219577	831.96	831.52	99.94	99.93
80% S -3	219960	831.96	831.67	99.96	
100% S -1	223244	1039.94	1039.59	99.96	
100% S -2	226533	1039.94	1039.32	99.94	99.95
100% S -3	229456	1039.94	1039.42	99.95	
120% S -1	226889	1247.93	1247.46	99.96	
120% S -2	227658	1247.93	1247.19	99.94	99.94
120% S-3	227387	1247.93	1247.11	99.93	

Precision

The precision of the developed HPLC method was expressed in terms of percent relative standard deviation (% RSD). At first, standard sample (20µg/ml) was run for six times. After confirmation of system suitability parameters (number of theoretical plates, tailing factors etc.), six samples were injected. Then % RSD was calculated as 0.406 (table 3). % RSD values less than 2, revealed high precision of the method.

Robustness

There are different ways to check the robustness of methods such as variations of pH in a mobile phase, variations in mobile phase composition, different columns (different lots and/or suppliers), flow rate, detection wavelength, temperature etc. In this study, standard deviation of peak areas was calculated by changing two parameters. Temperature was increased to 30ºC and flow rate was changed to 0.8ml/min. % RSD was found to be 0.416 (< 2) (table 4). Being less than 2, the values of % RSD indicated the robustness of the method. It was observed that there were no marked changes in chromatograms.

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100, and 120% of the standard concentration of Secnidazole (20µg/ml). Recovery was found in the range from 99.93-99.95%. The recovery of Secnidazole by proposed method is satisfactory as percent of relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0% (table 5).

CONCLUSION

The developed HPLC method for the qualification of Secnidazole was performed in mobile phase methanol: water (60:40, v/v). The modalities adopted in experiment were successfully validated as per ICH guidelines. It is thus inferred that this newly developed method was found to be accurate, simple, precise, and reproducible. The current method can be conveniently applied for quality control analysis in industry. The short run time of this method will significantly reduce the analysis time and cost.

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