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Method Development and Validation of Secnidazole in Solid Dosage Form by RP-HPLC

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Abstract: *A simple, rapid and precise method is developed and validated for the estimation of Secnidazole in solid dosage form by RP-HPLC. The proposed method produced results in accordance with the ICH guidelines. The assay value for Secnidazole is found to be 100.26%. The linearity of Secnidazole was found to be linear with a correlation coefficient of 0.999. The method developed and validated is precise with an RSD value of 0.17. Hence the proposed method is useful in routine analysis and the method is capable of producing good sensitivity.*

Keywords*: Secnidazole, linearity, correlation coefficient, assay and ICH.*

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I. Introduction

High Pressure Liquid Chromatography (HPLC) sometimes called High Performance Liquid Chromatography is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The techniques of HPLC are so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation. In reverse phase technique, a non-polar stationary phase is used and the mobile phase is polar in nature. Hence polar components get eluted first and non-polar compounds are retained for a longer time. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster, columns used in the mode of chromatogram are ODS (Octadecyl silane) or C_{18}, C_8, C_4, etc. [1, 2].

Method development and optimization in liquid chromatography is still an attractive field for theoreticians and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution. Alternate analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intended to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory [3, 4].

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength and quality, for the quantification of the drug substances and drug products. Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method [5, 6].

II. Experimental

2.1: List of Instruments:

S. No.	INSTRUMENT	MODEL
1	HPLC	WATERS, software: Empower, UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

2.2: List of Chemicals:

S. No.	CHEMICAL	SUPPLIER
1	Secnidazole	Supplied by Pharmatrain
2	KH_2PO_4	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

2.3: Method development:

2.3.1: Optimized Chromatographic Conditions:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and UV detector

Temperature : Ambient
Column : Waters C18 (4.6 x 150mm, 5.0µm)
Buffer : Phosphate buffer pH 6.0
Mobile phase : 50% buffer: 50% Acetonitrile
Flow rate : 1.0 ml per min
Wavelength : 232 nm
Injection volume : 20 µl
Run time : 8min.

2.3.2: Preparation of buffers and mobile phase:

2.3.2.1: Preparation of pH 6 phosphate buffer: Dissolve 6.8gms of potassium dihydrogen orthophosphate in 1000ml of water and sonicate for 2 minutes and adjust the pH for 6.0 and then sonicate for 2 min.

2.3.2.2: Preparation of mobile phase: Mix a mixture of above buffer 600ml (60%) and 400ml methanol HPLC (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

2.4: Method Validation:

2.4.1: Assay:

2.4.1.1: Preparation of standard solution: Accurately weigh and transfer 100mg of Secnidazole working standard into a 10ml clean dry volumetric flask, add diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 1ml of Secnidazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 3ml of Secnidazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

2.4.1.2: Preparation of sample solution: Accurately weigh and transfer 100mg of Secnidazole equivalent weight of the sample into a 10ml clean dry volumetric flask, add about 7ml of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent (stock solution). Further pipette 1ml of Secnidazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 3ml of Secnidazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

2.4.1.3: Procedure: Inject 20µL of the standard, sample into the chromatographic system and measure the areas for the Secnidazole peaks and calculate the assay.

2.4.2: Linearity:

2.4.2.1: Preparation of stock solution: Accurately weigh and transfer 100mg of Secnidazole working standard into a 10ml clean dry volumetric flask, add about 7ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 1ml of Secnidazole the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

2.4.2.2: Preparation of Level-I solution (100ppm of Secnidazole): 1ml of stock solution is taken in 10ml of volumetric flask, dilute up to the mark with diluents.

2.4.2.3: Preparation of Level-II solution (200ppm of Secnidazole): 2ml of stock solution is taken in 10ml of volumetric flask, dilute up to the mark with diluents.
2.4.2.4: Preparation of Level-III solution (300ppm of Secnidazole): 3ml of stock solution is taken in 10ml of volumetric flask, dilute up to the mark with diluents.
2.4.2.5: Preparation of Level-IV solution (400ppm of Secnidazole): 4ml of stock solution is taken in 10ml of volumetric flask, dilute up to the mark with diluents.
2.4.2.6: Preparation of Level-V solution (500ppm of Secnidazole): 5ml of stock solution is taken in 10ml of volumetric flask, dilute up to the mark with diluents.
2.4.2.7: Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.
2.4.3: Precision:
2.4.3.1: Preparation of stock solution: Accurately weigh and transfer 100mg of Secnidazole working standard into a 10ml clean, dry volumetric flask, add about 7ml of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Secnidazole from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 3ml of Secnidazole from the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.
2.4.3.2: Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC.

III. Results And Discussions

3.1: Optimized chromatographic conditions:

Column	:	Waters C18 (4.6 x 150mm, 5.0µm)
Mobile phase	:	50% Phosphate buffer pH6: 50% Acetonitrile
Flow rate	:	1.0 ml per min
Wavelength	:	232 nm
Injection volume	:	20 µl
Run time	:	10min.

Figure 1: Optimized Chromatogram of Secnidazole

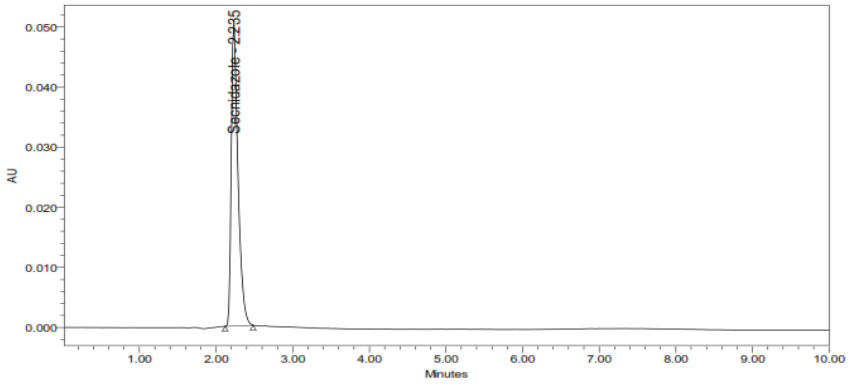

Table 1: System suitability studies of Secnidazole

S. No.	Name	Rt (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Secnidazole	2.235	1085263	58148	1.65	2524.84

The separation of analytical peak was good. The plate count also above 2000 and tailing factor below 2. The condition is taken as optimized method.
3.2: Assay:

Figure 2: Chromatogram of Standard

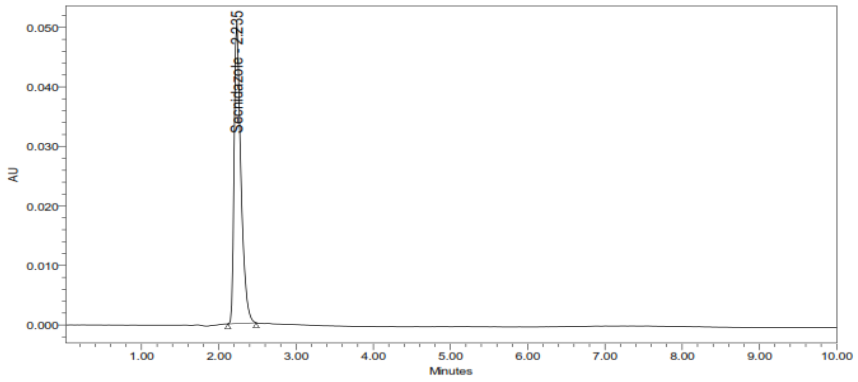

Figure 3: Chromatogram of Sample

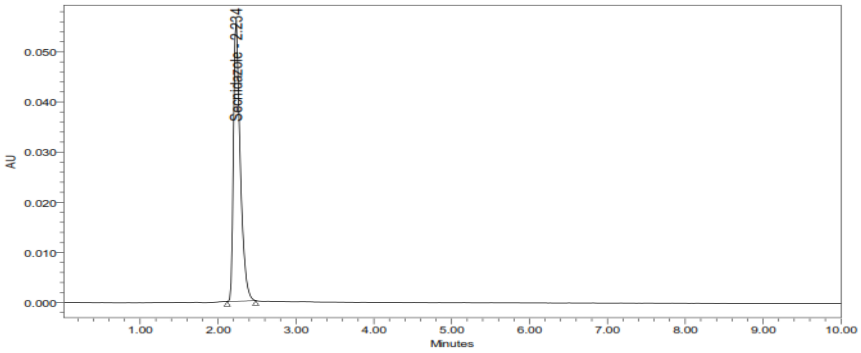

Table 2 : Assay of Secnidazole

	Label Claim (mg)	**% Assay**
Secnidazole	2000	100.26

3.3: Linearity:

Figure 4: Chromatogram for Linearity I (100ppm Secnidazole)

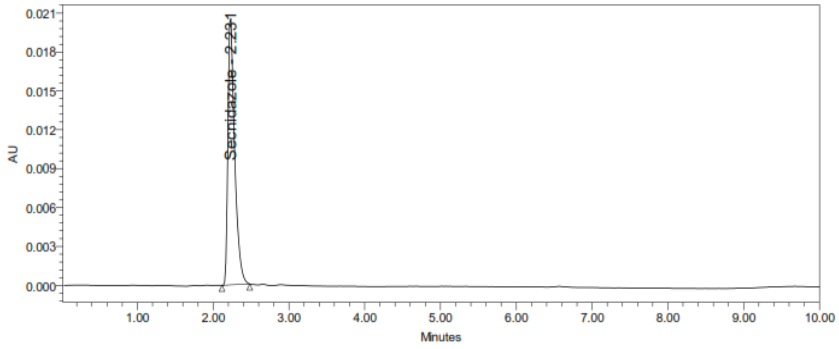

Figure 5: Chromatogram for Linearity II (200ppm Secnidazole)

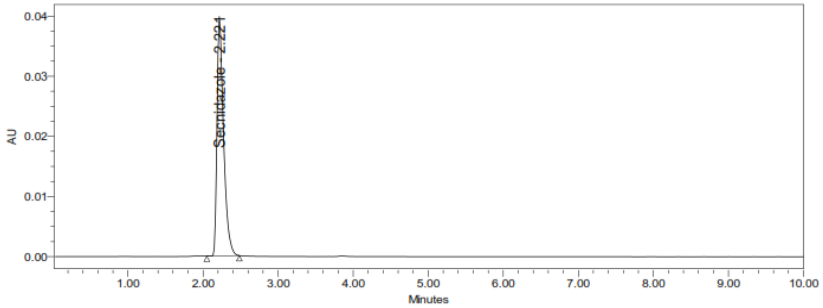

Figure 6: Chromatogram for Linearity III (300ppm Secnidazole)

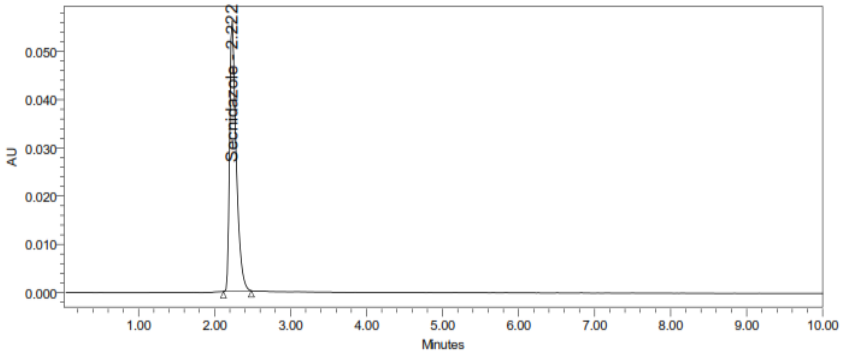

Figure 7: Chromatogram for Linearity IV (400ppm Secnidazole)

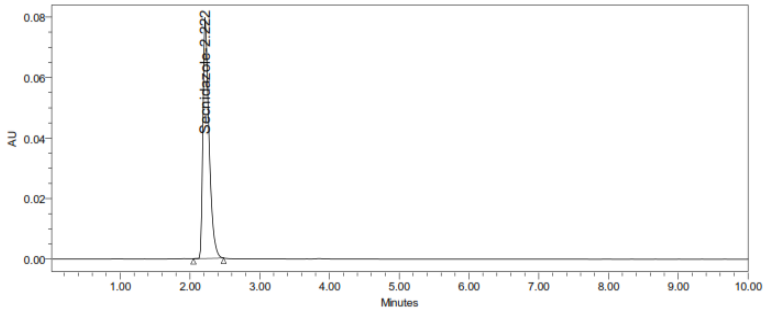

Figure 8: Chromatogram for Linearity V (500ppm Secnidazole)

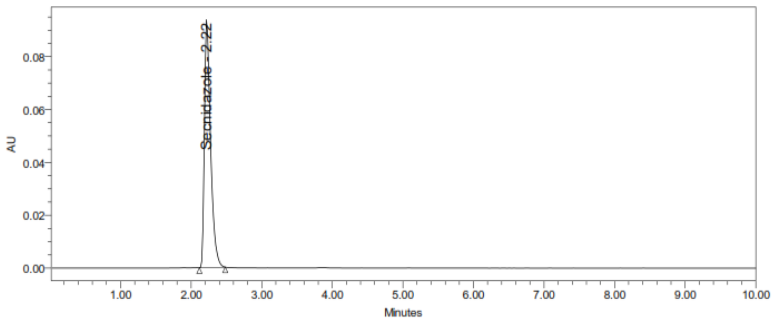

Table 3: Area of different concentrations of Secnidazole

S. No.	Secnidazole	
	Concentration (µg/ml)	Area
1	100	388060
2	200	744106
3	300	1061095
4	400	1413273
5	500	1725673

Figure 9: Calibration graph of Secnidazole

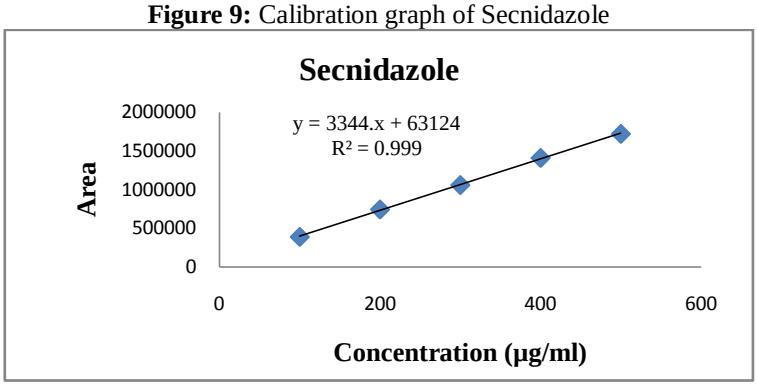

Table 4: Analytical performance parameters of Secnidazole

Parameters	Secnidazole
Slope (m)	3344.4
Intercept (c)	63124
Correlation coefficient (R^2)	0.999

Acceptance criteria: Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999.

3.4: Precision:

Figure 10: Chromatogram for Precision 1

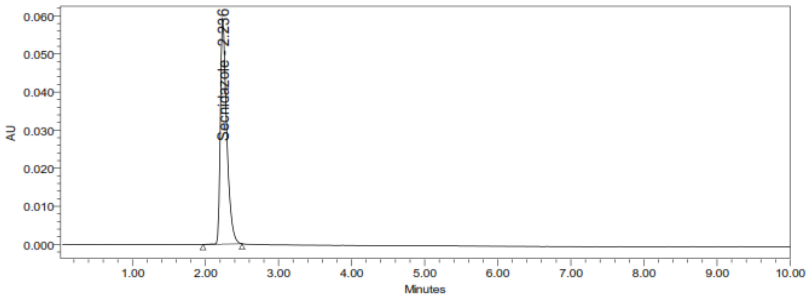

Figure 11: Chromatogram for Precision 2

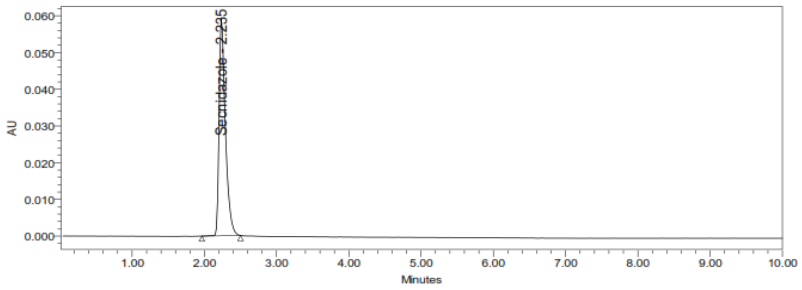

Figure 12: Chromatogram for Precision 3

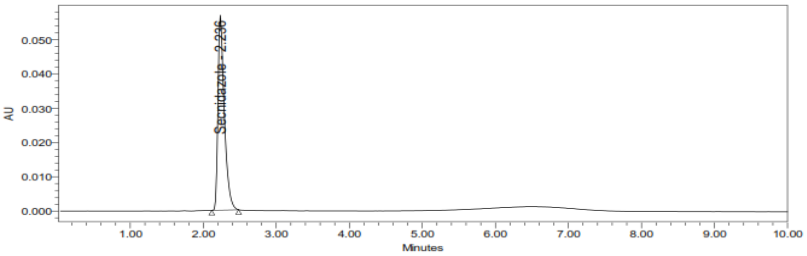

Figure 13: Chromatogram for Precision 4

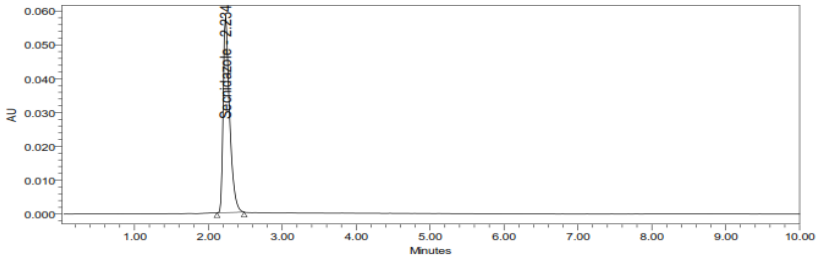

Figure 14: Chromatogram for Precision 5

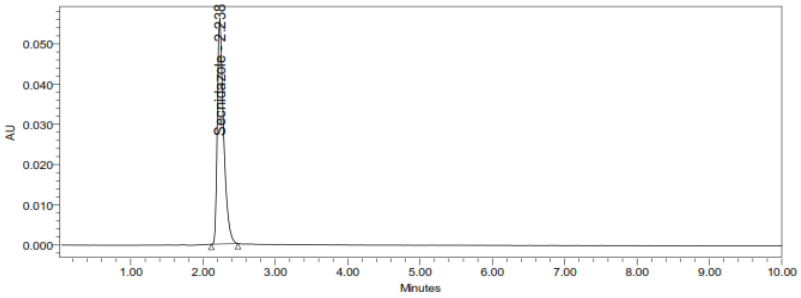

Figure 15: Chromatogram for Precision 6

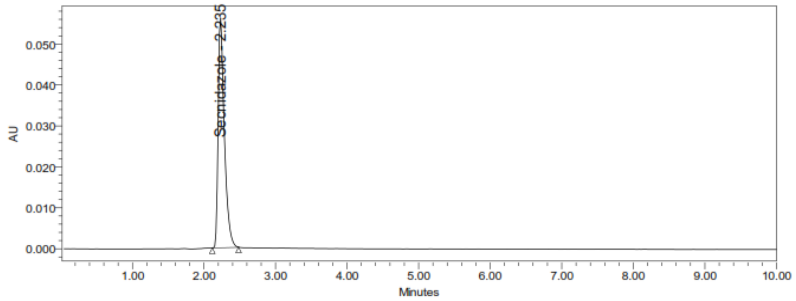

Table 5: Precision results of Secnidazole

Injection	Area
Injection-1	1062317
Injection-2	1062789
Injection-3	1066303
Injection-4	1063099
Injection-5	1067011
Injection-6	1064738
Average	1064376.17
Standard Deviation	1787.75
%RSD	0.17

IV. Conclusion

The estimation of Secnidazole was done by RP-HPLC. The assay of Secnidazole was performed with tablets and the assay was found to be 100.26, which shows that the method is useful for routine analysis. The linearity of Secnidazole was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD, which should not be more than 2.0% and the method shows a precision of 0.17 for Secnidazole which shows that the method is precise.

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