VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF SECNIDAZOLE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate, precise and sensitive HPLC-UV method was developed for the determination of secnidazole in pharmaceutical formulations. This method developed and validated in the present study. The mobile phase consists of mixed buffer, Methanol and Acetonitrile in the ratio 60: 30: 10 and adjusts the p^H to 6.8 with dilute sodium hydroxide solution. This was found to give a sharp peak of Secnidazole at a retention time of 2.705 min. HPLC analysis on column Ace, C8, 150 X 4.6, 5µ of Secnidazole was carried out at a wavelength of 254nm, with a flow rate of 1.0 ml min^{-1} linear regression analysis data for the Calibration curve showed a good linear relationship with regression coefficient 0.998 in the concentration range of 25 µg/ml^{-1} to 150 µg/ml^{-1}.The linear regression equation was Y=11361×-9156.The developed method was employed with a high degree of precision and accuracy for the analysis of Secnidazole. The method was validated for accuracy, precision, robustness, detection and quantification limits as for ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicate that this method was successfully applied to quantification of Secnidazole.

Keywords: Secnidazole; HPLC, Validation.

INTRODUCTION

Secnidazole which is an antifungal, antiprotozoal drug is used in treatment of amoebiasis, giardiasis, trichomoniasis, and bacterial vaginasis[1]. It is not official in any pharmacopoeia. Chemically it is (RS)-1-(2-methyl-5-nitroimidazole-1yl) propane-2-ol [2]. There is no information in literature on the stability behavior of this drug underhydrolytic, oxidative and thermal condition. As of now, information in literature is limited to the fate of the drug under photolytic conditions in organic solvents. The drug followed photochemical rearrangement and degradation pattern similar to metronidazole[3]. In another report, amethanolic solution of the drug was exposed to artificial sunlight for 24 h, and 2-methyl-5-nitroimidazole and 2-hydroxypropanol were found as the major degradation products[4].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[5-10].Differential pulse polarographic[11], supercritical fluid chromatographic[12], GLC[13] and HPLC[9, 14&15] methods. Adsorptive strippingvoltammetry[16] and HPLC[17&18] methods have been reported for its determination in biological fluids. For stability testing, four different analytical methods, viz. derivative spectroscopy, HPLC, TLC densitometry and colorimetry were developed by Moustafa and Bibawy.In this paper we describe a simple, rapid, sensitive and specific method for the determination of secnidazole in pharmaceutical formulations. HPLC with UV detection was employed. Compared with the published HPLCmethod described above, we have developed a sensitive procedure. The low of detection (LOD) and low limit of quantification (LOQ) were 2.228 and 6.752 µg/ml.

INSTRUMENTS, CHEMICALS AND METHOD

Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature. Waters (alliance) HPLC System is equipped with Empower software-2 software. Secnidazole sample was obtained from. Rantus Pharma Pvt. Ltd Hyderabad. Secnidazole tablet was purchased from local market. The solvent used Potassium dihydrogen orthophosphate and dipotasssium hydrogen phosphate (HPLC grade), Acetonitrile (AR grade), these chemicals were purchased from Merck Chemicals (Tirupati, (AP) India).

Selection of mobile phase

Chromatographic separation studies were carried out Waters HPLC 2 2695 series consisting 4 pump, C-18, column on the working standard solution of Secnidazole (10µg/ml). Initially, trials were carried out using Mixed Phosphate Buffer and Acetonitrile in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials, Mixed Phosphate Buffer: Acetonitrile (pH adjusted to 6.5 with Potassium dihydrogen orthophosphate and dipotasssium hydrogen phosphate) (60: 30: 10 v/v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatographic conditions

Column	:	Ace, C8, 150 X 4.6, 5µ.
Flow Rate	:	1.0 ml/min
Wave length	:	254 nm

Column temperature	:	30°C
Injection volume	:	20 µL
Diluent	:	Mobile Phase
Elution type	:	Isocratic
Needle wash solution	:	Water: Acetonitrile (90:10)
Needle wash solution	:	Water: Acetonitrile (90:10)

Preparation of Standard stock solution

20mg of Secnidazole reference standard was weighed accurately and transferred in 100ml volumetric flask. Drug was dissolve in Mixed Phosphate Buffer and Acetonitrile (60: 30: 10 v/v/v) and volume was made up to 100ml with same solvent. So as to get the concentration 100µg/ml. 1ml standard stock solution of Secnidazole was then diluted in 10ml Phosphate Buffer, methanol and acetonitrile (60: 30: 10 v/v/v) to get working standard solution 10µg/ml.

Preparation of mobile phase

Mobile phase was prepared by Mixture of Phosphate Buffer, methanol and acetonitrile (pH adjusted to 6.8 with Potassium dihydrogen orthophosphate) (60: 30: 10 v/v/v), filtered through 0.45µ membrane filter paper and then sonicated on ultra sonic water bath for 30min.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using Mixed Phosphate Buffer and Acetonitrile (60: 30: 10 v/v/v) and scanned over the range of 200 - 400nm and the spectra was obtained. It was observed that Secnidazole showed considerable absorbance at 254 nm.

Chromatogram of Secnidazole

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate).Standard solution of Secnidazole was injected to get the chromatogram. The retention time for Secnidazole was found to be 2.705 min. Chromatogram of Secnidazole is shown in (Figure- 3)

Validation of Analytical methods

The validation for HPLC method development was performed using parameters like Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness.

Linearity

The standard stock solution containing 100µg/ml of Secnidazole to prepare range of standard solutions containing six different concentrations of analyte. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 10-60µg/ml. The results obtained are shown in (table 1). The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure -2).

Precision

The precision of the method was demonstrated by intraday and inter-day variation studies. In the inter day studies, 3 different concentrations 20, 40 and 60µg/ml were injected in stabilized chromatographic conditions and were analyzed in triplicate. The percentage RSD was calculated. The result obtained for intraday variations are shown in (table- 3 & 4). In the inter day variation studies, 20, 40 and 60µg/ml were injected in stabilized chromatographic conditions and were analyzed. This procedure was repeated once a day for three consecutive days. The percentage RSD was calculated. The result obtained for inter-day variations are shown in (table-2& 3).

Accuracy

To check accuracy of the method, recovery studies were carried out by mixing standard drug solution to pre analyzed sample solution at three different levels 50%, 100% and 150%. Basic concentration of sample chosen was 20µg/ml of Secnidazole bulk drug solution to which 40and 60 µg/ml of Secnidazole tablet solution was added. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of Secnidazole were calculated by using linearity equation. The results obtained are shown in (table -4).

ASSAY

Standard préparation

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume With diluent

Sample Preparation

Transfer sample quantitatively equivalent to 40 mg of Secnidazole in to 100 mL volumetric flask add 100 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay the results are shown in table-8.

Assays result

Secnidazole = 99.73 %

Limit of Detection (LOD)

LOD is calculated from the formula:

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. In the range

S = slope of the calibration curve.

LOD = Secnidazole: **2.228 µg/ml**

Limit of Quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

LOQ = Secnidazole: **6.752µg /ml.**

RUGGEDNESS

The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days. % of RSD on Day-1& Day-2.The % of RSD of areas from six injections should not be more than 2.0%.The results shown in table -5&6.

ROBUSTNESS

Robustness was performed by injecting the Secnidazole standard solution in to the HPLC by altering the flow rate and column oven temperature from the normal chromatographic conditions. The results are tabulated in (table-9).Summary of validation parameters or Secnidazole

Validation Parameter Secnidazole	
Linearity Equation	Y=11361x -9156
(r2)	0.999
Range	10 – 60µg/ml
Precision (% RSD)	
Intraday	0.07%
Inter day	0.140%
Accuracy (% recovery)	100.75%, 99.96%, 100.77%
LOD	2.228µg/ml
LOQ	6.752µg/ml

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (98% to 102%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the %recoveries ranging from 98% to 102%. The LOD and LOQ were found to be 2.228µg/ml and 6.752µg/ml indicating the sensitivity of the method. The method was also found to be robust as indicated by the % RSD values which are less than 2%.

CONCLUSION

All the above factors lead to the conclusion that the proposed method as accurate, precise, simple, sensitive, robustsness and cost effective and can be applied successfully for the estimation of Secnidazole bulk and pharmaceutical formulation.

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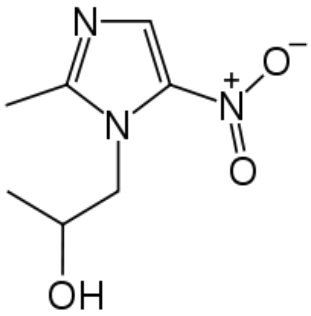


Figure: 1. Chemical Structure of Secnidazole

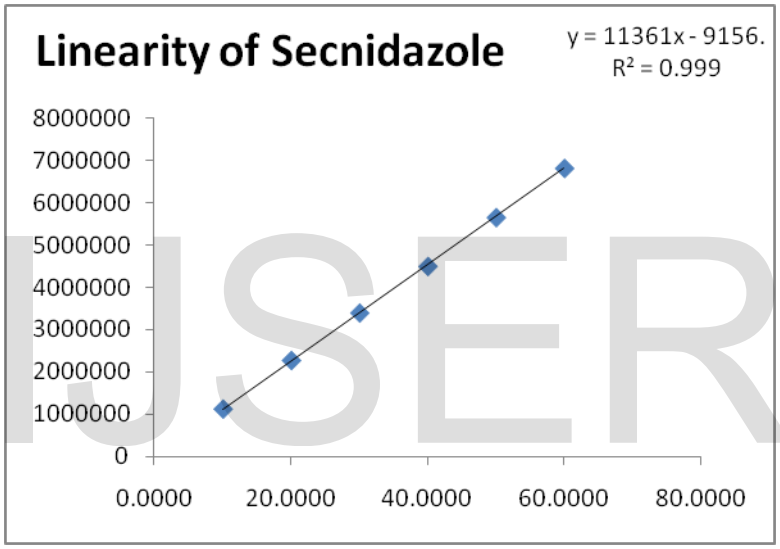
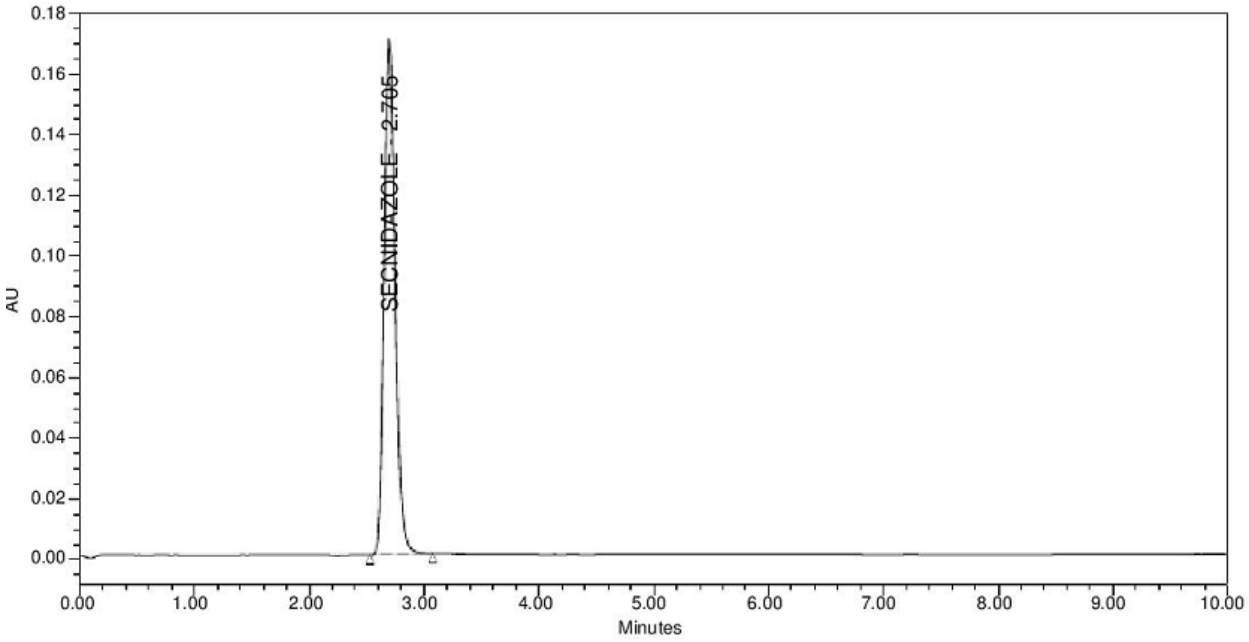


Figure: 2. Linearity of Secnidazole



	Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
1	SECNIDAZOLE	2.705	1127033	169757	100.00	1.15	3824

Figure: 3. Chromatogram of Secnidazole

Table: 1. Linearity of Secnidazole

%	Conc(mcg)	**Area**
25	10.0000	1127033
50	20.0000	2277865
75	30.0000	3402701
100	40.0000	4505264
125	50.0000	5664100
150	60.0000	6828120

Table: 2. System Precision of Secnidazole

S No	Name	RT	Area
1	Injection-1	2.723	4501316
2	Injection-2	2.721	4502666
3	Injection-3	2.721	4520125
4	Injection-4	2.725	4500984
5	Injection-5	2.727	4501893
6	Injection-6	2.72	4500323
Avg		2.723	4504551
Std Dev		0.003	7671.1
% RSD		0.100	0.17

Table: 3. Method Precision of Secnidazole

S No	Name	RT	Area
1	Solution-1	2.718	4499865
2	Solution-2	2.719	4499902
3	Solution-3	2.721	4508343
4	Solution-4	2.724	4504633
5	Solution-5	2.725	4501982
6	Solution-6	2.727	4503012
Avg		2.722	4502956
Std Dev		0.004	3214.5
% RSD		0.131	0.07

Table: 4. Accuracy of Secnidazole

Accuracy-50		Accuracy-100		Accuracy-150	
S No	Area	S No	Area	S No	Area
Injection-1	2271029	Injection-1	4500988	Injection-1	6809283
Injection-2	2269043	Injection-2	4501328	Injection-2	6808659
Injection-3	2265945	Injection-3	4502022	Injection-3	6805129
Avg	2268672	Avg	4501446	Avg	6807690.333
amt Recoverd	**50.37**	amt Recoverd	**99.96**	amt Recoverd	**151.16**
%Recovery	**100.75**	%Recovery	**99.96**	%Recovery	**100.77**

Table: 5. Results of Ruggedness -D1

S No	Name	RT	Area
1	Injection-1	2.718	4499865
2	Injection-2	2.719	4499902
3	Injection-3	2.721	4508343
4	Injection-4	2.724	4504633
5	Injection-5	2.725	4501982
6	Injection-6	2.727	4503012
Avg		2.722	4502956
Std Dev		0.004	3214.5
% RSD		0.131	0.07

Table: 6. Results of Ruggedness -D2

S No	Name	RT	Area
1	Injection-1	2.725	4522018
2	Injection-2	2.727	4525903
3	Injection-3	2.727	4525954
4	Injection-4	2.730	4529022
5	Injection-5	2.732	4532190
6	Injection-6	2.735	4540112
Avg		2.729	4529200
Std Dev		0.004	6340.8
% RSD		0.136	0.140

Table: 7. Inter-Intraday Precision Results of Secnidazole

S No	Name	RT	Area
1	Injection-1	2.718	4499865
2	Injection-2	2.719	4499902
3	Injection-3	2.721	4508343
4	Injection-4	2.724	4504633
5	Injection-5	2.725	4501982
6	Injection-6	2.727	4503012
7	Injection-7	2.725	4522018
8	Injection-8	2.727	4525903
9	Injectoion-9	2.727	4525954
10	Injection-10	2.730	4529022
11	Injection-11	2.732	4532190
12	Injection-12	2.735	4540112
	AVG	2.726	4516078.00
	STDEV	0.00504	14519.224
	%RSD	0.18	0.32

Table: 8. Results of Assay

4508437	20	10	50	100	99.6	1312	100.000	Result
4510313	50	100	26.3	10	100	1000		99.73%

Table: 9. Robustness of secnidazole

Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
Secnidazole(F)	3.484	5809348	623318	100.00	1.34	3209
	2.591	4258366	565242	100.00	1.23	2734
Secnidazole(T)	2.724	4539142	579997	100.00	1.26	2808
	2.735	4558575	581999	100.00	1.26	2765

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