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ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES OF SECNIDAZOLE IN API & PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC TECHNIQUE

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Abstract:
The project was aimed at developing an analytical method to quantify Secnidazole drug either alone or in tablet formulation, including stability studies with the aid of RP-HPLC technique. An analytical method was finalised and inspected consistency of the method by performing the different validation parameters like system suitability, specificity, linearity, accuracy, precision, LOD, LOQ, Robustness and assay. The degradation studies were performed under different physical and chemical conditions by following the ICH guidelines. The column used was Inertsil ODS Column C_{18} (4.6×250mm)5µm of Shimadzu.

The HPLC was Shimadzu make with UV PDA detector and model 20AD. In the stream lining the analytical method, we have settled on to use the mobile phase with the combination of Methanol: 0.1% OPA (90:10 v/v). The drug was detected at 314 nm on UV-Visible spectrophotometer. The retention time was at 2.953 min with the run time of 10 min. The linearity range of Secnidazole was from 2 µg/ml to 10µg/ml and the Regression coefficient calculated to be (R^2) 0.999. The corresponding recognition limits (LOD and LOQ) of the Secnidazole was 0.3µg/ml and 0.9µg/ml respectively. Precision studies were carried out and the RSD values were found to be less than two. The degradation studies were successfully conducted. The significant advantages were reduction of retention time at 1ml/min and the mobile phase used was quite cheaper than the reported methods. The other part was that, the usability of the method to quantify even though the drug was degraded nearly to 10 % in presence of the unknown degradants. The method is also sensitive, reproducible, quick and economical.

Keywords: Secnidazole, Methanol, OPA, HPLC, ICH Guidelines, Secnil.

1. Introduction

The main objective of this project is to develop and validate a simple, precise and accurate method by using RP-HPLC method. Secnidazole is chemically as (2-Methyl-5 nitro1H imidazol-yl)propan-2-ol. The empirical formula $C_{17}H_{11}N_3O_3$ is and molecular weight is 185.18 g/mol. The drug was soluble in water, soluble in organic solvents such as ethanol, chloroform, DMSO and diethyl formamide (DMF).

Senidazole enters micro-organisms by passive diffusion and activated by reduction of 5- nitro group. The intra cellular reduction occurs via the pyruvate-ferredoxin oxidoreductase complex and results in a concentration gradient across the cell membrane which, improve transport of the parent drug into the cell. Because the electron affinity of the 5-nitroimidazole is greter than that of the reduced ferredoxin, the drug disturbing the normal electron flow, which, in turn, enhances transport of the active form of drug into the cell. Because the electron affinity of the 5-nitroimidazole is much more than that of reduced ferrrdoxin, the drug disturbing the normal electron flows. DNA is the intracellular target of the 5-nitroimidazoles. Drug induced DNA damage results in the strand breakage, loss of the helical structure and impaired template function. Furthermore, cytotoxicity may be greatest in micro-organisms with DNA containing a high percentage of adenine and thymine[1].

Nasiruddin Alumad Farooqui, et al., developed and validated a RP-HPLC method for the estimation of secnidazole in pure and pharmaceutical dosage form at Wavelength of 228nm; mobile phase composition of 0.01M Potassium di hydrogen phosphate and Acetonitrile (85:15); retention time being 11.81 min; Linearity concentration was obtained between 30-70 µg/ml[2]. Tanzina Sharmin et al., analyzed the drug at wave length of 310nm in mobile phase of methanol and water(60:40) at flow rates of 1.0 ml/min. The retention time was at 4.147 min[3]. Ali Gamal Ahmed Al-Kaf et al., performed separation on C_{18} column (25cm x 0.46cm.5µm) using mobile phase consisting of water, methanol, acetonitrile in ratio (73: 17: 10). A flow rate was set at 1mL/min; the detection wavelength was set at 228nm[4]. Sunitha rani et al., selected mobile phase of Phosphate buffer and acetonitrile(85:15) at flow rate of 1.0ml/min, the drug eluted at retention time of 7.15min[5]. Rajan V. Rele and Sandip P. Patil made use of the column C_{18} (50mm x 4.6mm, 3µm) with ambient temperature. The mobile phase consisted of buffer: methanol in proportion 80:20 % (v/v). The detection was carried out at wavelength 310nm, set the flow rate at 1 ml/min and the peak appeared with a retention time of 3.94min. The linear ranges were 50-150 µg/ml for secnidazole[6]. Neeharika Yamsani et al., have used mobile phase of 50% buffer and 50% acetonitrile at flow rate of 1.5ml/min; retention time being 2.235 min[7]. Akash shelke, Someshwar mankar et al., disclosed method for estimation of secnidazole API and pharmaceutical dosage at wave length of 310nm with flow rate 1.0ml/min considering the mobile phase- buffer and methanol(80:20)[8].

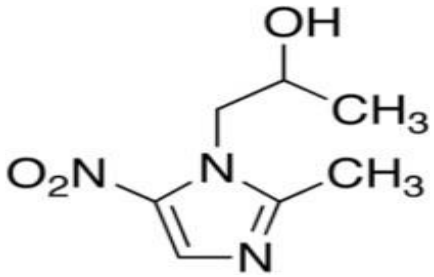


Figure 1: Chemical structure of Secnidazole

The objective of the present work was to develop a chromatographic method for determination of Secnidazole and to validate the method by using various parameters.

2. Methodology

2.1 Materials

HPLC (Shimadzu LC-20AD), UV Visible Spectroscopy (Shimadzu UV-1800 ENG240V), Electronic balance (Shimadzu ATY224), Digital ultrasonic cleaner (SONICA2200MH), hot air oven (INFRA DIGI ISO 9001-2015), UV Cabinet (MONOQUARTZ).

2.1.2 Chemicals
Secnidazole (Carbanio), Methanol (HPLC grade, Merck), Water for HPLC(Merck), Ortho Phosphoric acid (HPLC grade, Merck).

2.1.3 Preparation of standard stock solution:
Standard stock solution was prepared by dissolving, accurately weighed 10mg of Secnidazole Active Pharmaceutical Ingredient(API) in Methanol: 0.1% OPA(90:10) and the mobile was slowly added to reach the mark on the 10 ml volumetric flask (1^0 stock solution 1000µg/ml).

2.1.4 Preparation of secondary standard stock solution:
Secondary standard stock solution was prepared by pipetting 1ml of stock solution and volume of mobile phase added to make up to the mark on the 10ml volumetric flask(2^0 stock solution 100µg/ml).

2.1.5 Chromatographic condition in RP-HPLC
HPLC analysis was performed using Shimadzu Corporation, equipped with reservoir tray, column oven, detector(PDA). Reverse phase column of C_{18} packed with particle size of 5µm with internal diameter 4.6 mm, 250 mm length of the column. The mobile phase and the drug solution were filtered using micropore filter of 0.45µm pore size.

The various dilutions of Secnidazole in the concentration of 2-10µg/ml was prepared and the solutions were injected using a 20 µl fixed loop into the chromatographic system at the flow rate of 1ml/min. The output from the column was monitored at 314 nm and chromatograms were analyzed. The peak was eluted at 2.953 min. The method was extended for determination of Secnodazole in pharmaceutical dosage form. Initially, 10 tablets of Secnidazole were taken, each tablet weighted individually and the average weight of 10 tablets was calculated. i.e.,12.3g.

The equivalent weight to 10mg was calculated from the average weight of 10tablets and label claim and equivalent weight was found to be 12.30mg. Next 12.3mg of secnidazole powder weighted accurately, transferred into 10ml volumetric flask made up to the mark of volumetric flask using mobile phase, mixed well, sonicated and finally the solution was filtered through 0.45µm filter. Six optimized concentration were prepared from the above solution. The concentration was injected into HPLC and peak response was clearly noted. Further dilutions were made with mobile phase to obtain working standard of 10µg/ml.

The solution was filtered using micropore filter paper of 0.45µ pore size. The concentration of the drug in tablet sample solution was calculated by comparing with peak area of standard. The parameters such as system suitability, linearity, LOD and LOQ are done by API whereas precision, accuracy, robustness, and degradation studies are done by the tablet powder. The proposed method was validated as per the ICH guidelines[9-10].

3. Results and Discussion
3.1 System suitability:
Standard solution of 10µg/ml was prepared and six injections are injected into the chromatographic system. From the system suitability studies, it was observed that all the parameters were within limits viz Retention time (2.953min), tailing factor(1.193), peak area (436099). Table 1 contains the values for the system suitability parameter and Figure 2 showing the chromatogram for optimized conditions.

Figure-2 showing the λ_{max} for Secnidazole

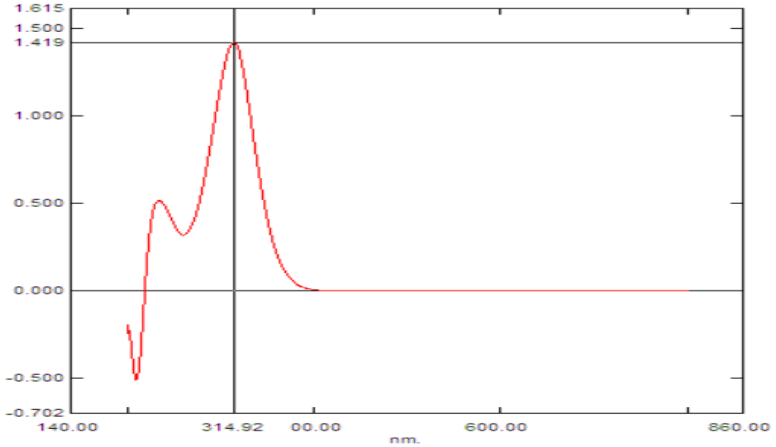


Figure 3: Chromatogram of optimised conditions Secnidazole in the mobile phase of Methanol: 0.1% OPA; 90:10.

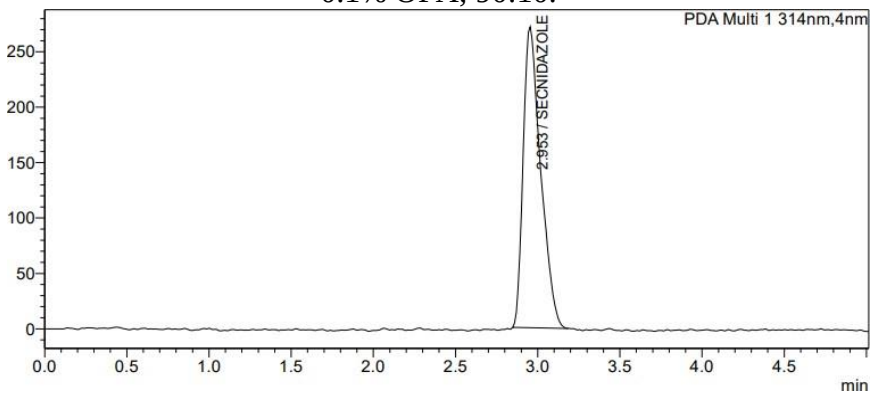
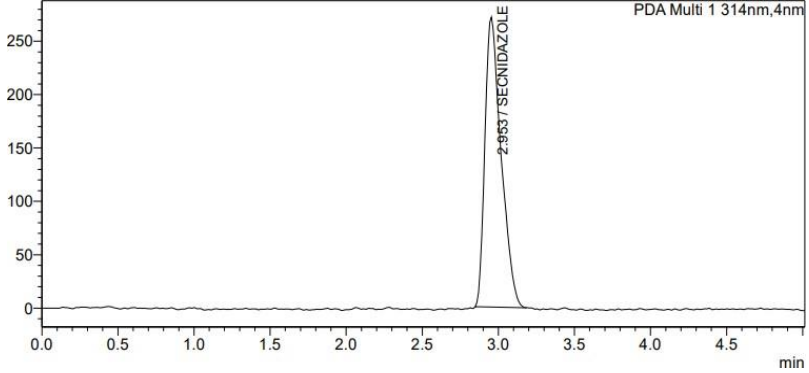


Table 1: System suitability parameters for proposed HPLC method

S. No	Peak Area	Ret Time	Plate Count	Peak Height	Tailing factor
1	436099	2.953	3259	689513	1.193
2	439742	2.96	3192	689513	1.204
3	423695	2.95	3210	674542	1.184
4	421527	2.958	3175	689547	1.153
5	439822	2.954	3243	695614	1.197
6	425791	2.89	3328	679874	1.198
Average	431112.67	2.94	3234.50	683270.67	1.26
STDEV	8371.48	0.03	55.43	9804.76	0.02
% RSD	1.94	0.91	1.71	1.43	1.59
Limits	_	_	>2000	_	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0	<2.0

Figure 4: Representative chromatogram of System suitability.



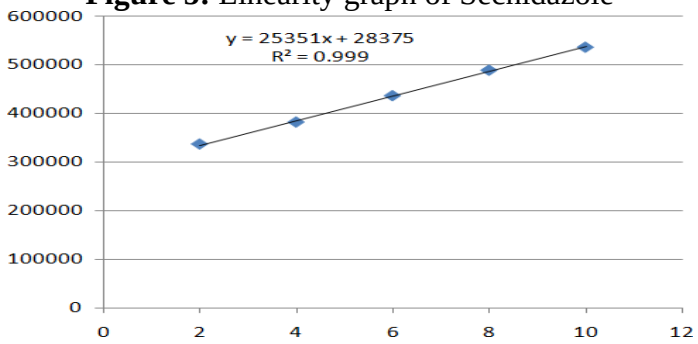
3.2 Linearity:

Linearity range was found to be 2 µg/ml to 10µg/ml for Secnidazole. The correlation coefficient was found to be 0.999. A calibration curve was prepared by plotting peak area as a function of concentration of drug solution. The values for calibration curve are presented in table 2 and in the Figure 4 graph is accomplished for the same.

Table 2: Linearity for proposed HPLC method

S. No	Linearity level	Conc. µg/ml)	Peak area
1	50%	2	336342
2	75%	4	381744
3	100%	6	436099
4	125%	8	488776
5	150%	10	536336

Figure 5: Linearity graph of Secnidazole



3.3 Precision:

Concentration of 100% was prepared and six injections were injected into the chromatographic system and %RSD was found to be within the limits(limit <2). The values being presented in table 3, table 4 for inter day precision and intraday precision respectively. The chromatograms for the same are presented in figure no 6 and 7.

Table 3: Inter day precision peak areas for the Day 1, Day 2 and Day 3

S. No	Day 1	Day 2	Day 3
1	442791	426328	425107
2	449270	436697	419057
3	442503	420394	424742
4	443155	435587	426473
5	441598	423512	431781
6	441415	428410	417658
Average	443455.33	428488.00	424136.33
STDEV	2927.85	6522.71	5157.10
% RSD	0.66	1.52	1.22
Limits	**% RSD: <2.0**	**% RSD: <2.0**	**% RSD: <2.0**

Table 4: Intraday precision peak areas within the day

S. No	9:00 AM	1:00 PM	5:00 PM
1	442791	442046	442353
2	449270	448383	443556
3	442503	431194	438434
4	443155	449882	434799
5	441598	442405	443081
6	441415	442183	425691
Average	443455.33	442682.17	437985.67
STDEV	2927.85	6588.56	6893.18
% RSD	0.35	0.45	0.61
Limits	**% RSD: <2.0**	**% RSD:<2.0**	**% RSD: <2.0**

Figure 6: Representative chromatogram of Inter day Precision

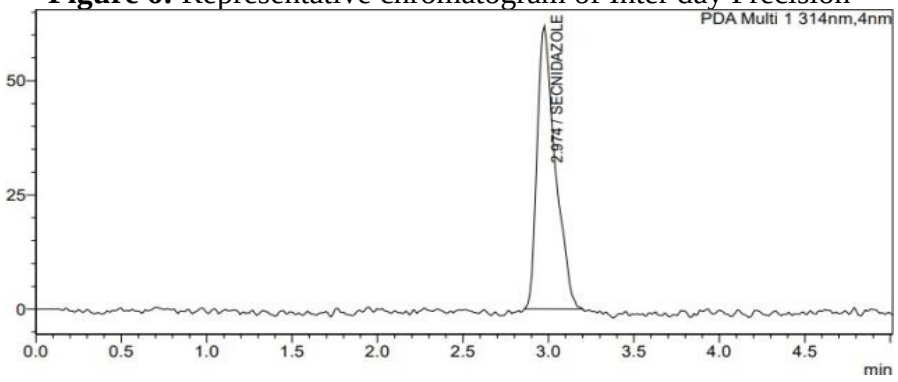
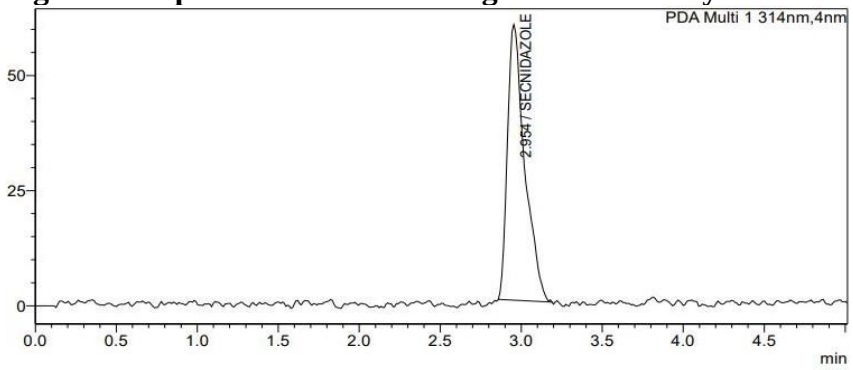


Figure 7: Representative chromatogram of Intraday Precision



3.4 Accuracy:

Series of 50%, 100% and 150% solutions were prepared by taking the tablet powder equivalent to Secnidazole drug. Resulting chromatograms for the above concentrations were analyzed and percentage recovery was found to be within limits i.e., 98-102%. The values being presented in figure in table 5 and the resulted chromatograms are presented in figure 8.

Figure 8: Representative chromatogram of Accuracy : (100%)

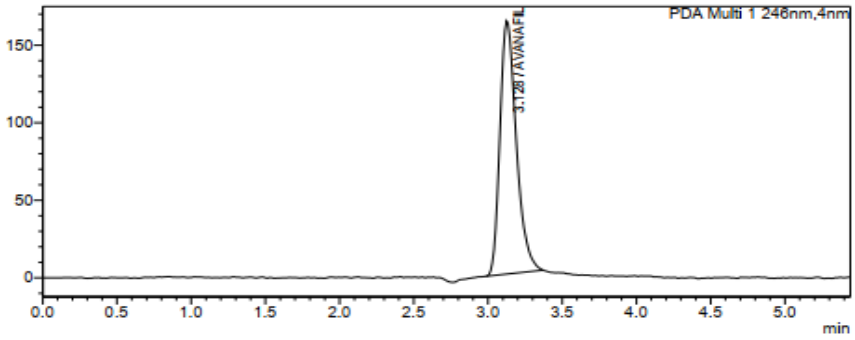


Table 5: Values of Accuracy at 50%, 100 % and 150 %

Accuracy level	Peak area	Conc Taken(µg/ml)	Conc Added(µg/ml)	Total conc Found (µg/ml)	Recovery concentration(µg/ml)	Mean % Recovery
50%	513692	6	3	9.0703	3.0703	101.16
	514939	6	3	9.1194	3.1194	
	509752	6	3	809148	2.9148	
100%	586336	6	6	11.9358	5.9358	100.75
	591431	6	6	12.1368	6.1368	
	589562	6	6	12.0630	6.0630	
150%	656325	6	9	14.6966	8.6966	99.33
	669841	6	9	15.2297	9.2297	
	661325	6	9	14.8938	8.8938	

3.5 LOD & LOQ:

The limit of detection (LOD) was found to be 0.3 µg/mL and the limit of quantification (LOQ) was found to be 0.9 µg/mL, these parameters are summarized in Table 6 and the chromatograms are summarized in figure 9 (a) and 9(b).

Table 6: values for LOD and LOQ for Secnidazole

Parameters	**Slope from Linearity**	**SD of peak from system suitability**
	25351	2298.5
LOD = 3 x SD/Slope	0.3 µg/ml	
LOQ = 10 x SD/Slope	0.9µg/ml	

Representative Chromatograms for LOD and LOQ

Figure 9(a) LOD

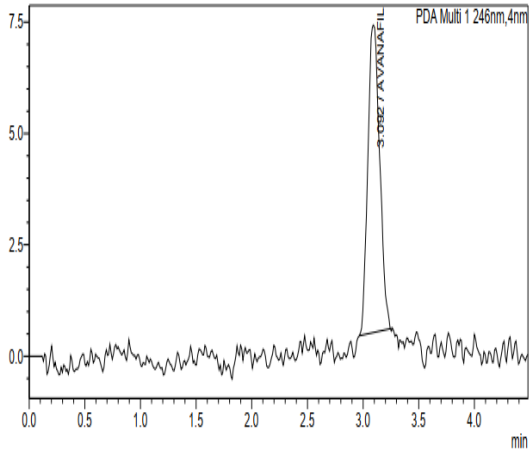
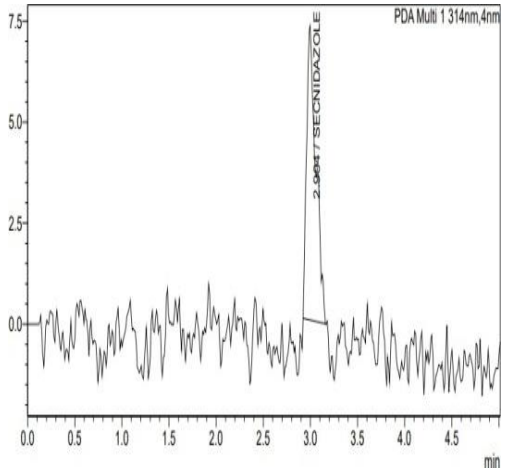


Figure 9(b) LOQ



3.6 Robustness:

A sample solution of 100% concentration was prepared and injected into the chromatographic system by following the below chromatographic conditions.]

The observed values are within the acceptance limits (Limits: %RSD<2.0) and are shown in table 7.

Table 7: Robustness

Parameter	**Condition**	**Condition**	**RT**	**Peak area**	**Theoretical plates**	**% Assay**
Flow (ml/min) min±0.2 ml	-0.2ml/min	0.8ml/min	3.692	536681	3748	102.22
	+0.2ml/min	1.2ml/min	2.489	416827	3075	98.43
Temp (°C) min±5°C	-5^{0}C	25°C	2.98	477112	3261	100.07
	+5^{0}C	35°C	2.966	499515	3056	100.37
Wave length (nm) min±5 nm	-2nm	244 nm	2.985	511760	3224	101.37
	+2nm	248 nm	2.987	508929	3227	101.01

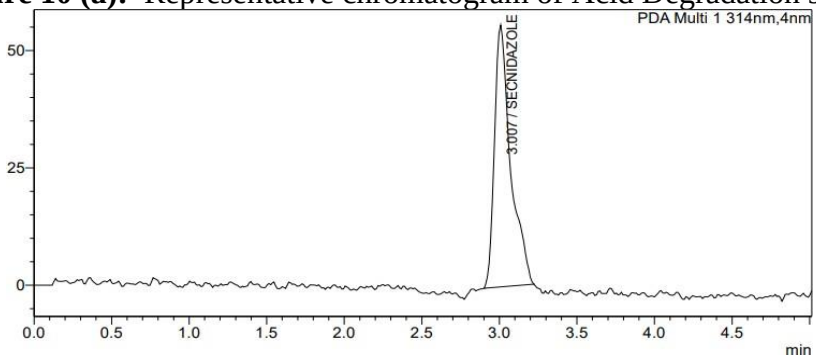
DEGRADATION STUDIES

1. Acid Degradation:

1ml of dosage form (10µg/ml) was taken and 1ml of 0.1 N HCl was added in a 10 ml volumetric flask and warmed at 60°C for 10min.

The solution was cooled and 1ml of 0.1N Sodium hydroxide was added and make up to the mark using mobile phase and the solution was injected into the HPLC system and the peak responses were recorded and the chromatogram is shown in figure 10 (a) and in table 8 s.no 1.

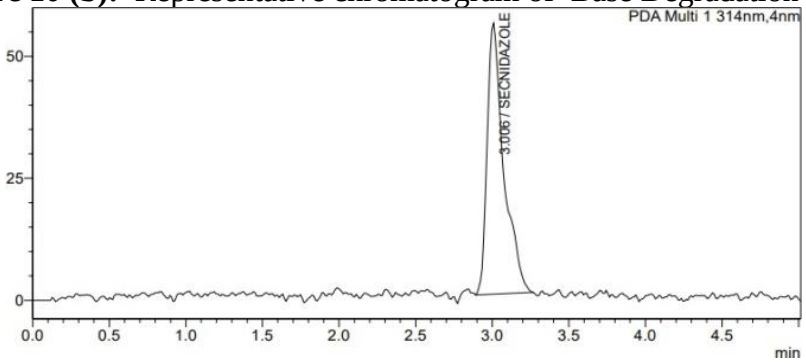
Figure 10 (a): Representative chromatogram of Acid Degradation study



2. Base Degradation:

1ml of dosage form (10µg/ml) and 1ml of 0.1N sodium hydroxide was added in a 10 ml volumetric flask and warmed to 60°C for 10min. The solution was cooled and 1ml of 0.1N HCl was added and make up to the mark using mobile phase and the solution was injected into HPLC system and the peak responses were recorded and the chromatogram is shown in figure 10 (b) and in table 8 s.no 2.

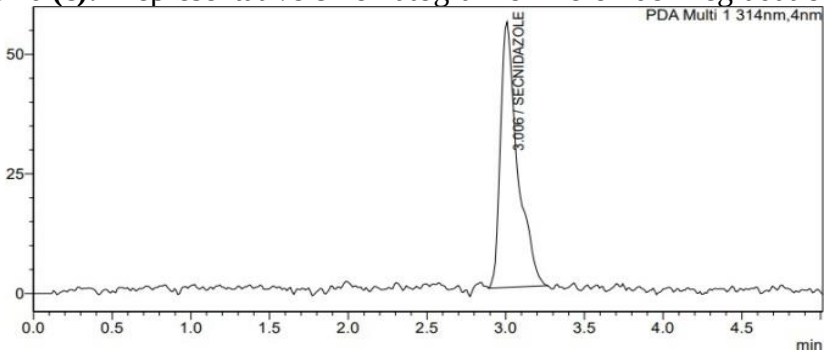
Figure 10 (b): Representative chromatogram of Base Degradation study



3. Peroxide Degradation:

1ml of dosage form (10µg/ml) and 1ml of 3% H_2O_2 was added in a 10ml volumetric flask and this volumetric flask was warmed to 60°C and make up to the mark using mobile phase. The solution was injected into HPLC system and the peak responses were recorded and the chromatogram is shown in figure 9 (c) and in table 8 s.no 3.

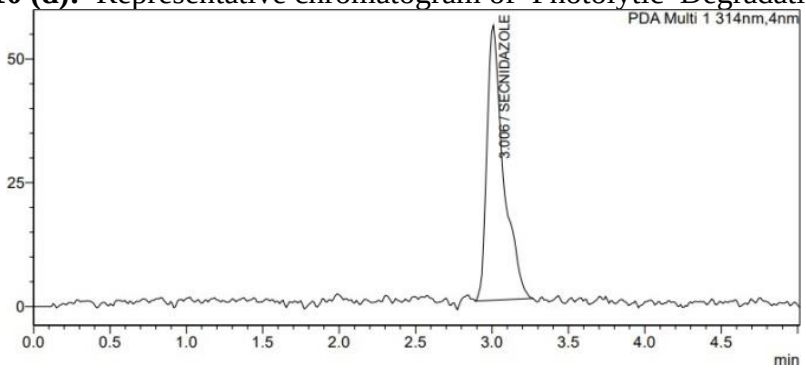
Figure 10 (c): Representative chromatogram of Peroxide Degradation study



4. Photolytic Degradation:

1ml of dosage form (10µg/ml) was transferred into 10 ml volumetric flask and was placed in hot air oven for 15min and make up to the mark using mobile phase. The solution was injected into HPLC system and the peak responses were recorded and the chromatogram is shown in figure 10 (d) and in table 8 s.no 4.

Figure 10 (d): Representative chromatogram of Photolytic Degradation study



5. UV Degradation:

1ml of dosage form (10µg/ml) was transferred into 10ml volumetric flask and was placed in UV Cabinet for 1hour and make up to the mark using mobile phase. The solution was injected into HPLC system and the peak responses were recorded and the chromatogram is shown in figure 10 (e) and table no 8 S.No 5.

Figure 10 (e): Representative chromatogram of UV Degradation study

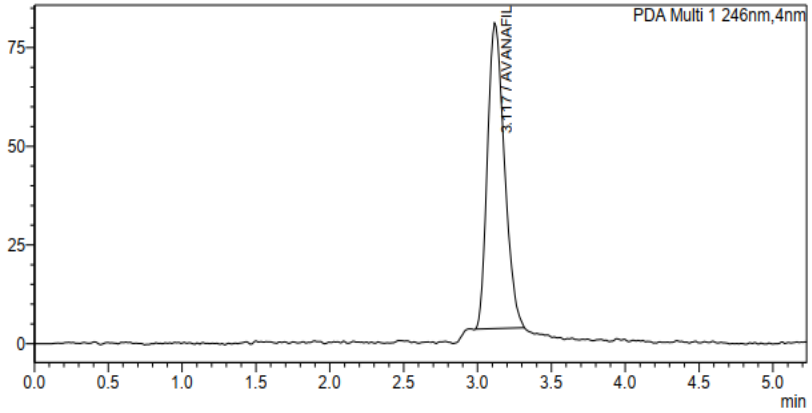


Table 8: Degradation studies of Secnidazole

S.No	Condition	Peak Area	% Assay	% Degradation
1	Acid Degradation	446395	91.836	8.164
2	Base Degradation	418531	91.086	8.914
3	Peroxide Degradation	412559	94.372	5.628
4	Photolytic Degradation	407258	91.637	8.363
5	UV Degradation	396003	90.537	9.463

Summary table of the parameters for the method development, validation and degradation studies

Table no.9

S. No	Parameters	HPLC Results
1	Mobile phase	Methanol : OPA (90:10v/v)
2	Wave length detection	314 nm
3	Calibration range (µg/ml)	2-10 µg/ml
4	Regression equation	y = 25351x + 283752
5	Correlation coefficient (r^2)	0.999
6	Retention time	2.953 min
7	Systems Suitability	1.94 %
8	LOD (µg/ml)	0.301 µg/ml
9	LOQ (µg/ml)	0.911 µg/ml
10	Accuracy	98-102 %

11	Inter-day Precision(%RSD)	0.66-1.57%
12	Intraday Precision(%RSD)	0.66-1.57%
13	Robustness Flow rate	98.43-102.85%
14	Robustness Temperature	99.21-100.37%
15	Robustness Wave length	99.21-101.56%
16	Acid degradation	8.164%
17	Base degradation	8.914%
18	H_2O_2 degradation	5.628%
19	Photolytic degradation	8.363%
20	UV Degradation	9.463 %

4. Conclusion:

A method for the estimation of Secnidazole in API and it's tablet dosage form was developed using RP-HPLC. The method was successfully validated following ICH Q2R1 guidelines. The linearity was in the range of 2µg/ml to 10µg/ml and the theoretical plates were found well beyond 2000. The %RSD for accuracy, precision, and robustness were all found <2 which indicates that the parameters are within the limits of the guidelines. The LOD & LOQ were found to be 0.3µg/ml and 0.9µg/ml respectively. The degradation studies were also performed to the tablet dosage form. The %assay was found to be within the limits 98%- 102%.

The mobile phase used is methanol and OPA which are comparatively cheaper than many solvents used in the literature. Thus, we can consider that this method as sensitive, economical, reproducible and considerably rapid in the assay of Secnidazole in API and dosage form.

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