METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES OF TINIDAZOLE IN ITS API AND FORMULATION BY USING UV-SPECTROSCOPIC AND RP-HPLC METHODS

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Abstract:
The main objective of this project is to develop and validate a simple, precise and accurate method by using UV-Spectroscopic and RP-HPLC methods. Tinidazole is an antibiotic used for bacterial infections and parasitic infections, which is caused by Trichomonas vaginalis, Entamoeba histolytica. It is soluble in organic solvents such as ethanol, DMSO, dimethyl formamide, sparingly soluble in aqueous buffers. Its pka value is 4.70. From the literature review, the solvents used are methanol: buffer; acetonitrile: water: methanol; methanol were the mobile phases. Column C18 (4.6×250mm); 5µm. The λ_{max} was found at 315nm for the drug whose mobile phase was finalised as acetonitrile: 0.1% IPA-90:10 after performing different trials. System suitability parameters that included retention time (2.689 min), area (430651), number of theoretical plates (4355), tailing factor (1.012) found to be within limits. Linearity range was from 10µg/ml to 50µg/ml and the regression value was 0.9919. Accuracy and precision were within the limits. Robustness studies were performed. LOD and LOQ values were 1.4µg/ml and 4.8µg/ml respectively. Degradation studies were also performed and was found to be within the limits. It is a simple and less economical method as the Retention time was 2.6min which is less compared to other methods. A simple, precise, accurate method was developed and validated according to ICH Q2R1 guidelines.

Keywords: Tinidazole, Acetonitrile, IPA, UV Spectroscopy, HPLC, ICH Guidelines and Tiniwell.

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INTRODUCTION

Tinidazole is a nitroimidazole antitrichomonal agent for both adults and pediatric patients older than three years of age, effective against Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia infections. Tinidazole is designated chemically as a 1-[2-(ethyl sulphonyl) ethyl]-2-methyl 5-nitroimidazole. It is slightly soluble in acetone and dichloromethane. Sparingly soluble in methyl alcohol and practically insoluble in water. (P. Deepika et al.2014). The free nitro radical generated as a result of this reduction is responsible for the antiprotozoal activity. (Randa Bakheet Ahmed et al.2019). The empirical formula is $C_8H_{13}N_3O_4S$ and molecular weight is 247.273 g/mol. It is a synthetic nitroimidazole derivatives used as an antiprotozoal, antibacterial agent, chemically reduced tinidazole was shown to release nitrites and cause damage to purified bacterial DNA in vitro. Oral absorption of tinidazole is found to be 100%. (P.Jyothi et al.2018).

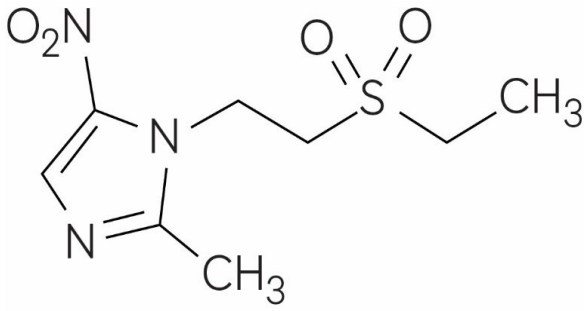

Figure 1: Chemical structure of tinidazole

Tinidazole crosses the placental barrier and is secreted in breast milk. It is excreted by the liver and the kidneys. It is excreted in the urine mainly as unchanged drug (approximately 20-25% of the administered dose). Approximately 12% of the drug is excreted in the feces. (M.A. QADEER et al.2020).

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

MATERIAL AND METHODS

Material

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).

Chemicals

Tinidazole(Carbanio), Acetonitrile (HPLC grade, Merck), Methanol (HPLC grade Merck), Water for HPLC (Merck), Isopropyl alcohol (HPLC grade Merck).

Preparation of standard stock solution for UV method:

Standard stock solution was prepared by dissolving, accurately measured 10mg of Tinidazole in ACN: 0.1%IPA and the volume was made up to 10 mg in 10 ml volumetric flask (1^0 stock solution 1000µg/ml).

Preparation of secondary standard stock solution for UV method:

Secondary standard stock solution was prepared by pipetting 1ml of stock solution and the volume was made up to 10 ml in 10ml volumetric flask (2^0 stock solution 100µg/ml).

Chromatographic condition in RP-HPLC

HPLC analysis was performed using Shimadzu Corporation equipped with reservoir tray, column oven, detector (PDA). Reverse phase column of C18 packed 5 µm with 4.6×250mm internal diameter particles with the mobile phase consisting of Acetinitrile:0.1% Isopropyl alcohol (90:10, v/v). The standard solution of Tinidazole was prepared by dissolving 10mg of Tinidazole in 10ml of diluent and subsequent dilutions were made with mobile phase to obtain working standard of 10µg/ml. The mobile phase and the drug solution were filtered using micropore filter of 0.45µ pore size. The various dilutions of Tinidazole in the concentration of 10-50µg/ml was prepared. The solutions were injected using a 20 µl fixed loop into the chromatographic system at the flow rate of 1ml/min and the effluents were monitored at 315 nm, chromatograms were recorded.

The peak was eluted at 2.6 min. The method was extended for determination of Tinidazole in pharmaceutical dosage form. The pharmaceutical dosage form containing 500mg strength was taken. 20 tablets of Tinidazole (containing 500 mg) were weighed and powdered in glass mortar and the powder equivalent to 100mg of Tinidazole was transferred into 100ml volumetric flask and diluents(mobile phase) was used to make up the volume to 100ml. 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.

RESULTS AND DISCUSSION

Calibration curve of Tinidazole

Table 1: Linearity for UV method

Concentration µg/ml	Absorbance at 315nm
10	0.155
20	0.347
30	0.515
40	0.691
50	0.846

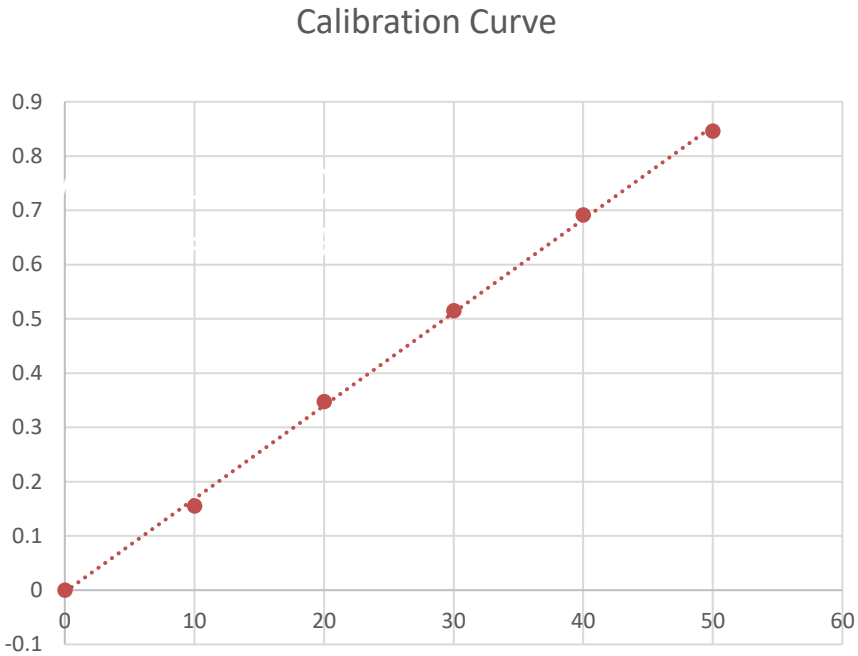

Figure 2: Linearity graph of Tinidazole by UV method

Precision

Intraday Precision of Tinidazole for UV method

Table 2: Intraday precision for UV method

S. No.	Conc.(µg/ml)	Conc. Found(µg/ml)	Percentage %	Mean %	*SD	% RSD
1	30	29.9	99.6	100.0	0.61	0.61
2		30.3	100			
3		30.4	101.3			
4		30.1	100.3			
5		29.9	99.6			
6		29.9	99.6			

Interday Precision of Tinidazole for UV method

Table 3: Interday precision by UV method

S. No.	Conc.(µg/ml)	Conc. Found(µg/ml)	Percentage %	Mean %	*SD	% RSD
1	30	29.75	99.16	99.9	0.58	0.58
2		29.9	99.66			
3		30.3	101			
4		30.0	100.0			
5		30.1	100.3			
6		29.9	99.6			

Recovery studies

Table 4: Recovery studies for UV method

S. No.	% added	Conc. (µg/ml)	Conc. Added (µg/ml)	Conc. found	Conc. recovered	% Recovery (×)	Mean ±SD	% RSD
1	50%	29.9	15	44.9	15	99.9	0.65	0.65
2			15	45	15.1			
3			15	44.8	14.9			
4	100%		30	59.8	29.9	99.3	0.3	0.30
5			30	59.7	29.8			
6			30	59.6	29.7			
7	150%		45	75.2	45.3	100.5	0.12	0.11
8			45	75.2	45.3			
9			45	75.1	45.2			

Limit of Detection & Limit of Quantification

Table 5: LOD & LOQ for UV method

S. No.	Parameters	Tinidazole
1	LOD(µg/ml)	0.194
2	LOQ (µg/ml)	0.588

System suitability:

Standard solutions were prepared and injected into the chromatographic system. From the system suitability studies, it was observed that all the parameters were within limits viz rt(2.69min), theoretical plates(4330), tailing factor(1.14), peak area(2448779), HETP(31.754).Table 6 is showing the system suitability parameters and Figure 4(a-f) showing the system suitability chromatograms.

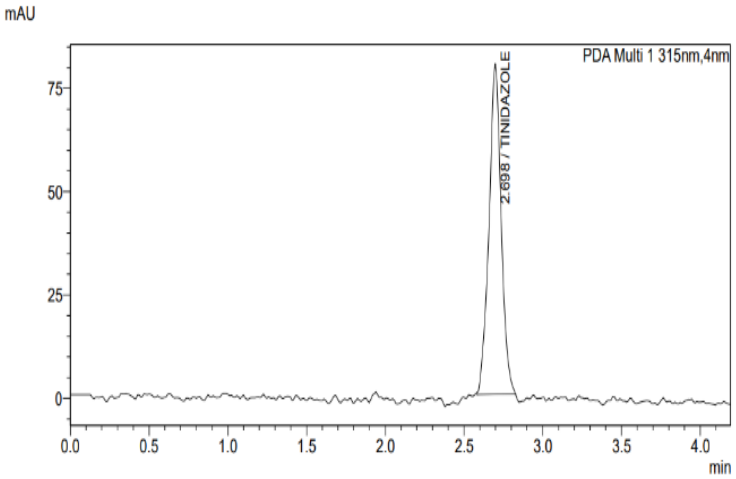

Figure 3: Chromatogram of Tinidazole in the mobile of acetonitrile:0.1% IPA; 90:10.

Table 6: System suitability parameters for proposed HPLC method

S. No	Peak Area	Ret Time	Plate Count	Peak Height	Tailing
1	2345657	2.694	4724	442790	1.137
2	2415802	2.692	4248	437603	1.151
3	2480380	2.691	4240	452181	1.148
4	2500242	2.691	4202	451279	1.151
5	2493769	2.692	4256	452196	1.145
6	2456825	2.691	4315	451045	1.152
Average	2448779.16	2.69	4330.83	447849	1.14
STDEV	24122.97	0.00094	80.02	2518.04	0.00401
% RSD	0.98	0.03	1.84	0.56	0.35
Limits	_	_	>2000	_	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0	<2.0

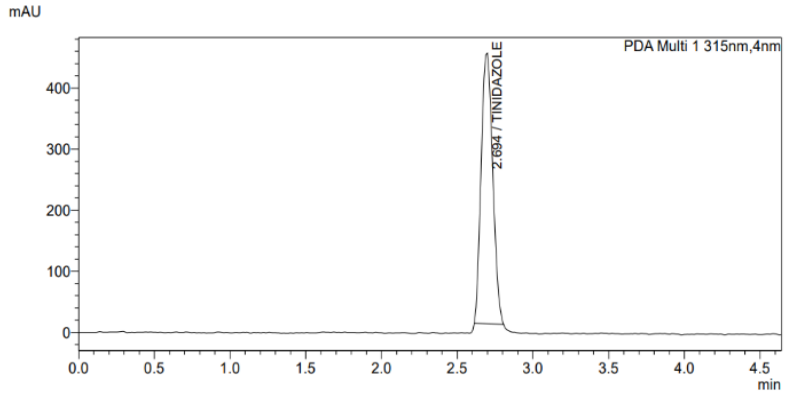

Figure 4: System suitability chromatogram

Linearity:
Linearity range was found to be 10-50µg/ml for Tinidazole. The correlation coefficient was found to be 0.991. A calibration curve was prepared by plotting peak area as a function of concentration of drug solution. The calibration curve is shown in Figure 5(a-e) and values of the same are presented in Table 7.

Table 7: Linearity for proposed HPLC method

S. No	Conc. (µg/ml)	Peak area
1	10	1794382
2	20	2306823
3	30	2615300
4	40	3147701
5	50	3447902

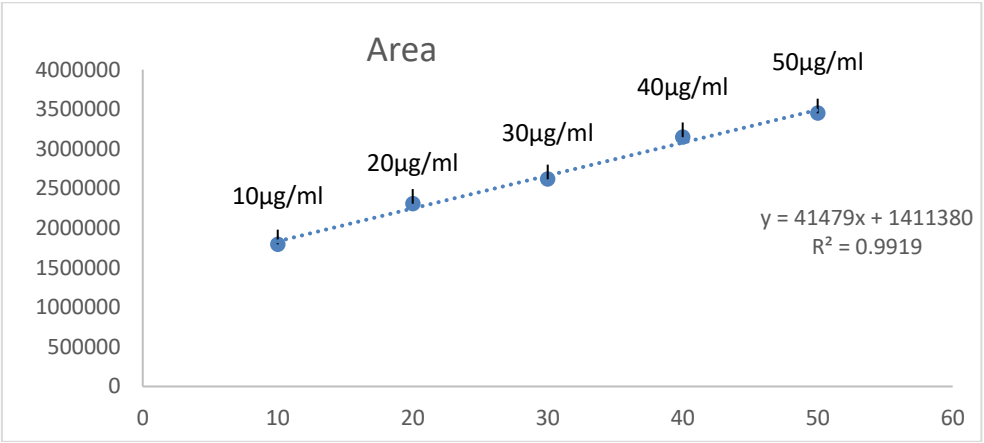

Figure 5 Linearity chromatograms of Tinidazole 10-50µg/ml

Precision:
Concentration of 100% was injected into the chromatographic system and it was found to be within the limits. The %RSD was less than 2. The values being presented in table 8 and the chromatograms are presented in figure 6.

Table 8: Precision for proposed HPLC method

S. No	Day 1	Day 2	Day 3
1	2345657	2693740	2814575
2	2415802	2703333	2835830
3	2480380	2629508	2802219
4	2500242	2757964	2857411
5	2493769	2731607	2963097
6	2456825	2642970	2764759
Average	2448779.16	2693187	2839648.5
STDEV	24122.97	20291.34	27813.48
% RSD	0.98	0.75	0.97
Limits	**% RSD: <2.0**	**% RSD: <2.0**	**% RSD: <2.0**

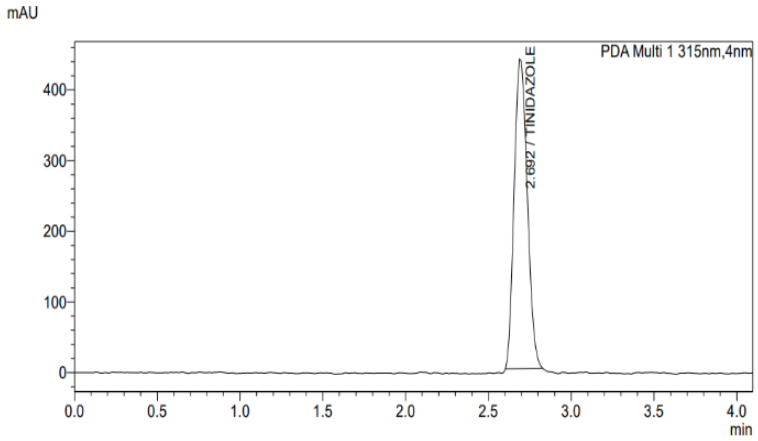

Figure 6 Precision chromatograms

Accuracy:

Series of 50%, 100% and 150% solutions were prepared by taking the tablet powder equivalent to Tinidazole 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 9 and the resulted chromatograms are presented in figure 7.

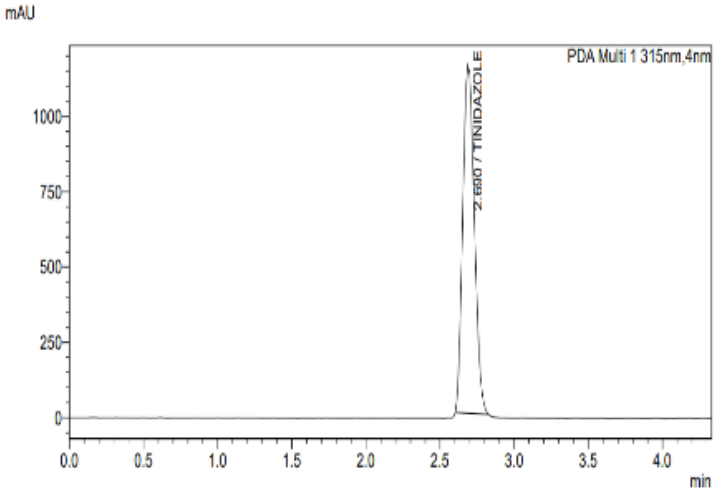

Figure 7: Accuracy chromatograms: (100%)

Table 9: Accuracy for proposed HPLC method

Accuracy level	Peak area	Amount taken	Amount added	Amount found	% Recovery	Mean % Recovery
50%	4709795	30	15	14.8	99.2	100.6
	4765620	30	15	15.4	102.8	
	4718858	30	15	14.9	99.8	
100%	6216860	30	30	29.2	97.5	99.1
	6292083	30	30	29.9	99.8	
	6296745	30	30	30	100	
150%	7746741	30	45	46.1	102	101.6
	7711674	30	45	46.5	103	
	7870356	30	45	44.9	99.9	
50%	4709795	30	15	14.8	99.2	100.6
	4765620	30	15	15.4	102.8	
	4718858	30	15	14.9	99.8	
100%	6216860	30	30	29.2	97.5	99.1
	6292083	30	30	29.9	99.8	
	6296745	30	30	30	100	
150%	7746741	30	45	46.1	102	101.6
	7711674	30	45	46.5	103	
	7870356	30	45	44.9	99.9	

LOD & LOQ:

The limit of detection (LOD) was found to be 1.4 µg/mL and the limit of quantification (LOQ) was found to be 4.8µg/mL, these parameters are summarized in Table 10 and the chromatograms are summarized in figure 8.

Table 10: LOD and LOQ for HPLC method

Parameters	Slope from Linearity	SD of peak from system suitability
	41479	20260.37
LOD = 3 x SD/Slope	1.4µg/ml	
LOQ = 10 x SD/Slope	4.8µg/ml	

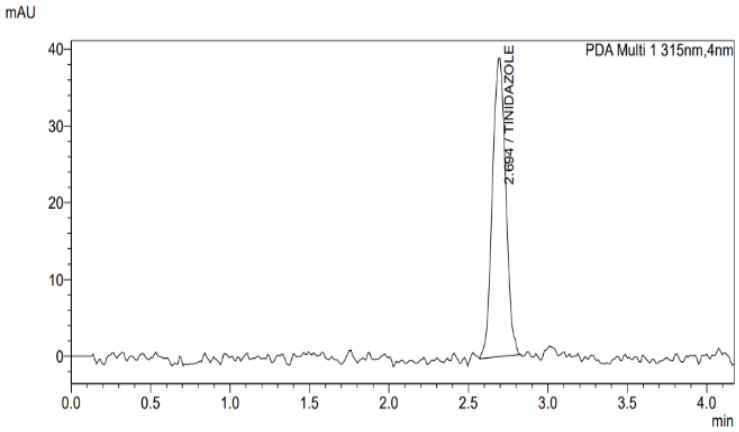

Figure 8(a): Chromatograms for LOD

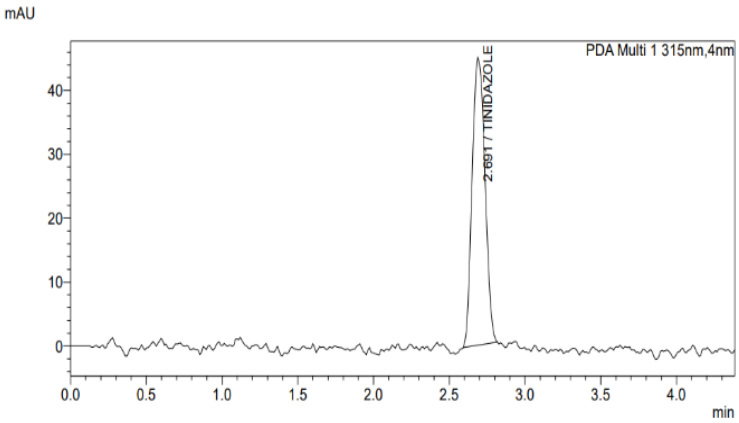

Figure 8(b): Chromatograms for LOQ

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.

Table 11: Robustness for HPLC method

Parameter	Condition	Condition	RT	Peak area	Theoretical plates	% Assay
Flow (ml/min) min±0.2 ml	-0.2ml/min	0.8ml/min	3.355	3148996	4725	98%
	+0.2ml/min	1.2ml/min	2.250	3278684	3969	101%
Temp (°C) min±5°C	-5^{0}C	25°C	2.695	3154222	4403	98%
	+5^{0}C	35°C	2.680	3206907	4151	99.8%
Wave length (nm) min±5 nm	-5nm	310 nm	2.689	3129427	4157	97%
	+5nm	320 nm	2.691	3370189	4180	102.2%

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).

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).

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 10(c).

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).

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).

Table 12: Degradation studies

S. No	Condition	Peak Area	% Assay	% Degradation
1	Acid Degradation	1614774	92.4	7.6
2	Base Degradation	1561721	90	10
3	Peroxide Degradation	1550038	90	10
4	Photolytic Degradation	1597440	92.4	7.6
5	UV Degradation	1558789	90	10

Chromatograms of Degradation studies

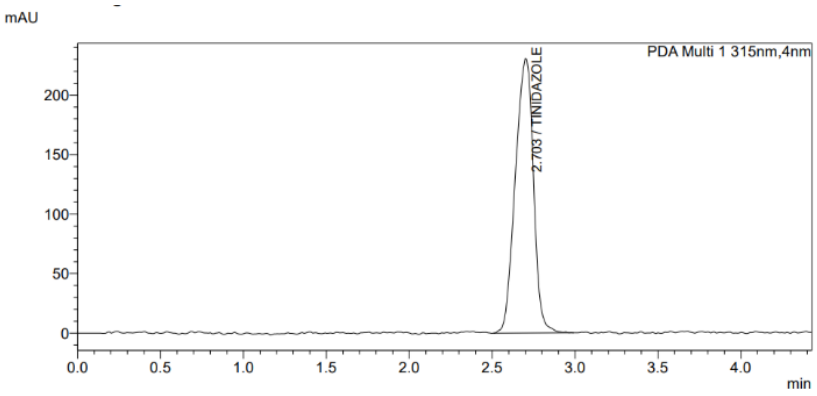

Figure 9 (a): Acid Degradation

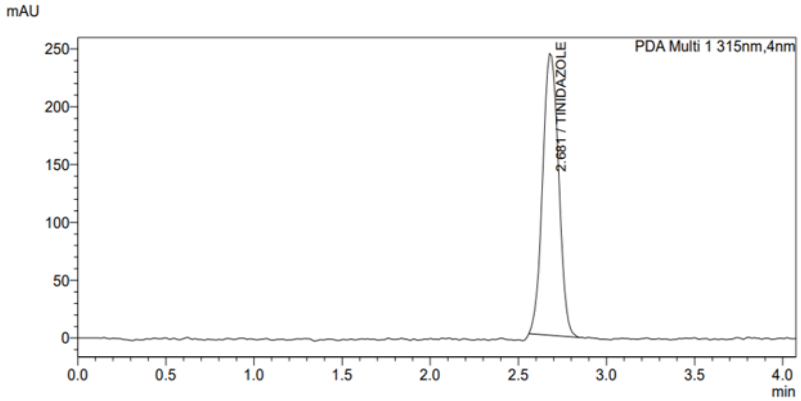

Figure 9(b): Base Degradation

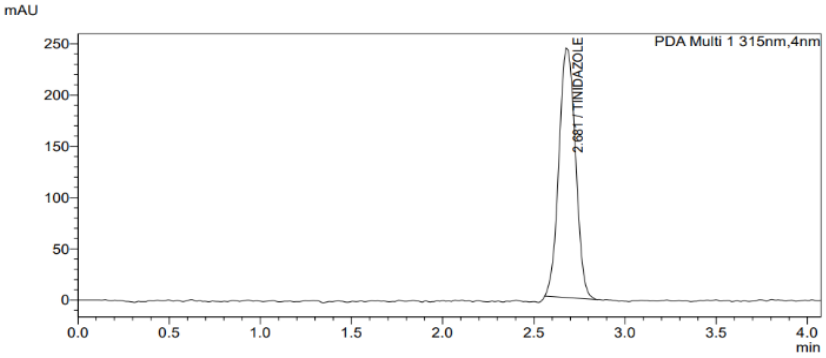

Figure 9(c): Peroxide Degradation

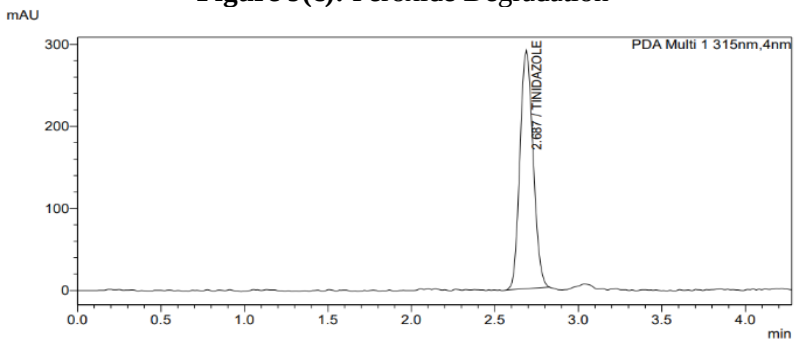

Figure 9(d): Photolytic Degradation

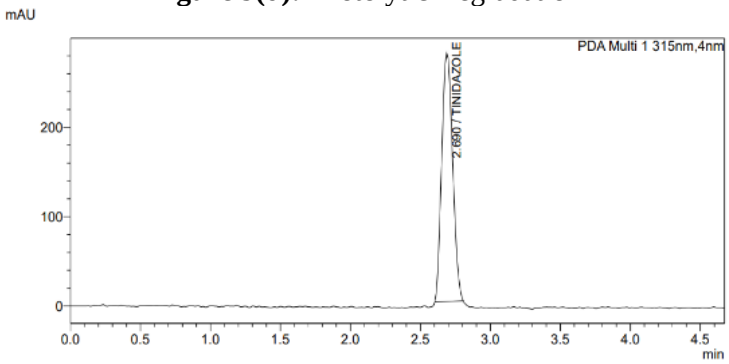

Figure 9(e): UV Degradation

Table no.7 Summary

S. No	Parameters	HPLC Results	UV Results
1	Mobile phase	Acetonitrile: IPA (90:10v/v)	Acetonitrile: IPA (90:10v/v)
2	Wave length detection	315 nm	315nm
3	Calibration range (µg/ml)	10-50µg/ml	10-50µg/ml
4	Regression equation	y = 41479x+1411380	y = 0.017x-0.003
7	Correlation coefficient (r^2)	0.991	0.999
8	Retention time	2.69 min	-
9	Systems Suitability	0.35	-
10	LOD (µg/ml)	1.2µg/ml	0.194µg/ml
11	LOQ (µg/ml)	4.8µg/ml	0.588µg/ml
12	Accuracy	99-101%	99.3-100.5%
13	Inter-day Precision(%RSD)	0.75-0.98	0.98
14	Intraday Precision(%RSD)	0.86-1.12	0.61
15	Robustness Flow rate	98-101%	-
16	Robustness Temperature	98-99.8%	-
17	Robustness Wave length	97-102.2%	-
18	Acid degradation	92.4%	91.4%
19	Base degradation	90%	92%
21	H_2O_2 degradation	90%	88%
22	Photolytic degradation	92.4%	90.4%
23	UV Degradation	90%	90.8%

Conclusion:

The estimation of Tinidazole in API and its tablet dosage form was developed using UV spectroscopy and RP-HPLC techniques. The methods were successfully validated following ICH Q2R1and Q1A guidelines. The linearity was in the range of 10µg/ml to 50µg/ml and the %RSD for accuracy, precision, and robustness were all within the limits of <2 %.

The mobile phase/solvent used was acetonitrile and 0.1% IPA(90:10) which are comparatively cheaper than many solvents used in the literature. Thus, we can consider these methods as sensitive, economical, reproducible and considerably rapid in the assay of Tinidazole in API and dosage form.

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