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EFFECT OF SOLVENTS ON SPECTROPHOTOMETRIC ESTIMATION OF TINIDAZOLE IN BULK AND DOSAGE FORMS

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ABSTRACT

Tinidazole is a synthetic antiprotozoal agent. It demonstrates activity both *in vitro* and in clinical infections against the following protozoa: *Trichomonas vaginalis*, *Giardia duodenalis* (also termed *G. lamblia*), and *Entamoeba histolytica*. A simple, sensitive, accurate and reproducible UV/visible spectrophotometric method were developed for the determination of Tinidazole in bulk and pharmaceutical dosage forms. Drug obeyed beer's law in the concentration range of 2-20 µg/ml. The method was validated for several parameters such as Linearity, Accuracy, Precision and Robustness as per the ICH guidelines. The drug exhibited maximum wavelength (nm) in 0.1N Sodium hydroxide solutions, 375 nm when compared with 0.1N Hydrochloric acid, Ethanol, and Distilled water due to the effect of solvent and chromophoric group. It was conclude that the drug, Tinidazole showed absorption maximum (λ_{max}) in different manner in different solvents like 0.1N Hydrochloric acid, Ethanol, Distilled water and 0.1N Sodium hydroxide solution at 294 nm, 312 nm, 319 nm and 375 nm respectively. The percentage recovery values which is close to100 % indicates reproducibility of method and there is no interference from the excipients presents in the formulation (tablets) that developed method was found to be sensitive, accurate, and precise and the most reproducible result. The authors conclude that the proposed spectrophotometric method for the estimation of Tinidazole can be used for routine analysis of Tinidazole in bulk as well as in tablet dosage form.

KEYWORDS: Tinidazole, UV Spectrophotometer, Validation, ICH guideline.

INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. Analytical techniques play a very important and active role in various studies carried out in pharmaceutical analysis. The major areas are

- Assay of the drug from raw material, bulk drugs and formulations.
- Detection and quantification of probable impurities and metabolites.
- Accelerated stability studies.
- *In-vitro* dissolution studies.
- Quantitative estimation of drugs and/or its metabolites from biological fluids.

Ultraviolet spectroscopy is concerned with the study of absorption of UV radiation which range from 200 to 400 nm. But compounds which are colorless absorb radiation in the UV region.[1] In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, there by the molecule undergoes transition from ground state to excited state. This absorption is characteristic and depends on the mixture nature of electron present.[2] The intensity of absorption depends on the concentration and path length as given by beer-Lambert's law. According to the Beer's-Lambert's Law, absorbance is proportional to concentration, and Absorbance versus concentration plot is a straight line.[3]

The expression of Beer-Lambert law is- **$A = \log (I_0/I) = ECl$** Where, A = absorbance, I_0 = intensity of light incident upon sample cell I = intensity of light leaving sample cell, C = molar concentration of solute L = length of sample cell (cm.), E = molar absorptivity.

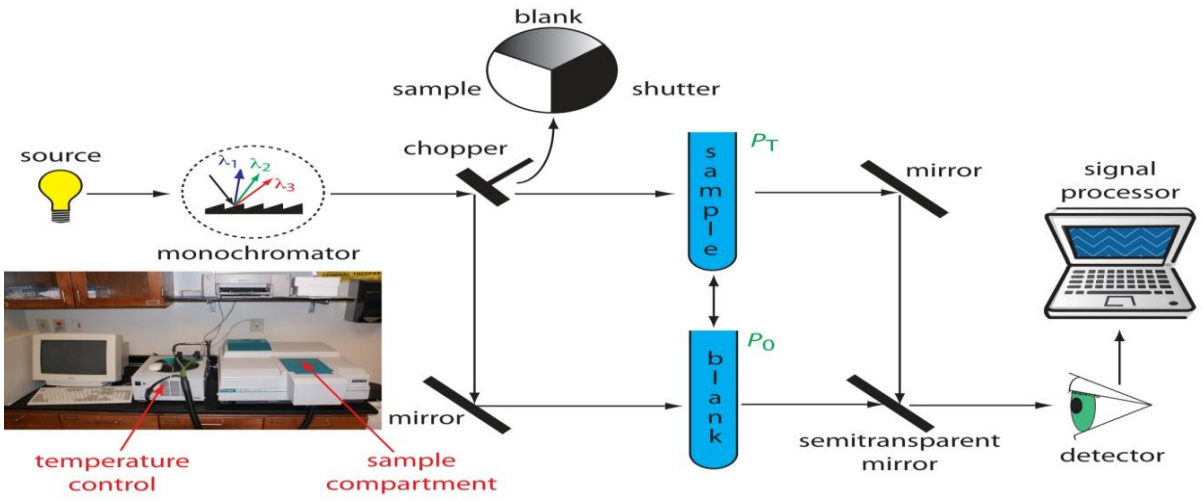

Fig. 1: Schematic diagram of a double beam scanning spectrophotometer.

The main objectives of the work was to develop new analytical method for the estimation of Tinidazole drug preferably by UV- VISIBLE Spectrophotometer in different solvents like 0.1N Hydrochloric acid, Ethanol, Distilled Water and 0.1N Sodium hydroxide solution.[4] The method also validated for various analytical parameters according to ICH guidelines. To applied the proposed method for the analysis of drug in their bulk and Pharmaceutical dosage form (marketed tablets) by Recovery studies. Tinidazole is a synthetic antiprotozoal agent containing nitroimidazole group, the nitro group of Tinidazole is reduced in *Trichomonas* by a ferredoxin-mediated electron transport system. Used for the treatment of trichomoniasis caused by *T. vaginalis* in both female and male patients.[8,9] Also for the treatment of giardiasis caused by *G. duodenalis* in both adults and pediatric patients older than three years of age and for the treatment of intestinal amebiasis and amebic liver abscess caused by *E. histolytica* in both adults and pediatric patients older than three years of age. As well as Bacterial Vaginosis, Nongonococcal urethritis, Sexually Transmitted Disease.[10,11,12]

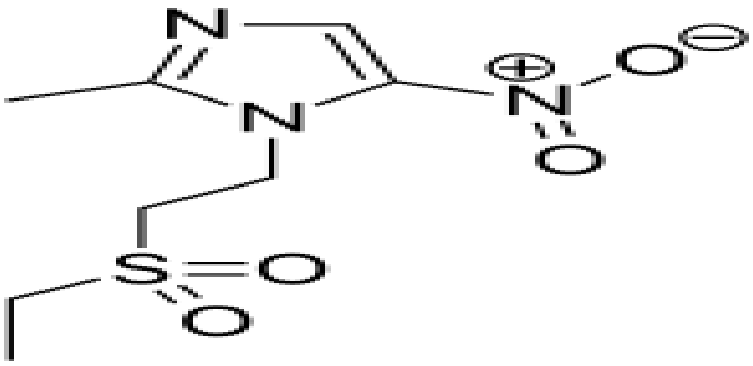

Fig. 2: Chemical structure of Tinidazole

MATERIALS AND METHODS

Instrument: Analytical Technologies double beam spectrophotometer with UV Probe software version 2 was used to develop the analytical method. The above instruments had automatic wavelength accuracy 0.1 nm and matched quartz cells with 1 cm cell path length, Ultra Sonicator and Weighing balance (Shimadzu, Japan) were used for this work.

Material: Tinidazole was gifted from Golden Streak Pvt Ltd Hyderabad, India. The commercially available marketed tablets tindamax 500 mg, mankind, India Ltd. were obtained from the market. Hydrochloric acid, Ethanol, and sodium hydroxide was used as solvents were obtained from Amul Scientific, India. The Distilled water was used obtained from Water purification unit.

Method development

Preparation of Standard solution: A spectrum of the working standards was obtained by scanning from 200-400 nm against the solvent as blank to fix absorption maximum using double beam UV-Visible spectrophotometer. Here using various solvents like 0.1N Hydrochloric acid, Ethanol, Distilled Water and 0.1N Sodium hydroxide solution for the estimation of Tinidazole. A Standard stock solution was prepared by accurately weighed 100 mg of Tinidazole in 100 ml of volumetric flask and dissolved in 0.1N HCl to obtain a concentration 1 mg/1ml or 1000 µg/ml (Standard Stock I). Pipette out 10 ml of stock solution - I and make up to the volume 100 ml with 0.1N HCl to get desired concentration of 100 µg/ml (Standard Stock II). From the stock –II solution prepared various concentrations; the same procedure repeated with other solvents like Ethanol, Distilled water and 0.1N Sodium hydroxide solutions.[7]

Selection of wavelength for analysis of Tinidazole: Accurately measured 1.0 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted to 10 ml to give concentration of 10 µg/ml and it was used for initial spectral scan in the UV range of 200-400 nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution by allegation method.[5]

Preparation of serial dilutions: The serial dilutions were prepared from the standard stock II solution to get a respective concentration of 2, 4, 6, and 8 up to 20 µg/ml.[6]

Method validation: The proposed method was validated for various parameters such as linearity and range, accuracy, precision, robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.[4]

Linearity and range: The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 2-20 µg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml)

versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software.[13]

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of Tinidazole was determined at each levels of the amount; three determinations were performed. The percentage recovery was calculated as mean ± standard deviation.[14]

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days, and the absorbance were recorded. All study was performed in triplicates. The result was indicated by calculating percentage RSD.[15]

Robustness: The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[16]

Ruggedness: The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.[17]

RESULTS AND DISCUSSION

Selection of wavelength: It was conclude that the drug, Tinidazole showed absorption maximum (λ_{max}) in different manner in different solvents like 0.1N Hydrochloric acid, Ethanol, Distilled water and 0.1N Sodium hydroxide solution at 294 nm, 312 nm, 319 nm and 375 nm respectively. Therefore the observed λ_{max} values were used for further work to analyze the test samples in bulk and pharmaceutical dosage form. Among the four solvents

0.1N Sodium hydroxide solution showed greater absorbance was 0.671 at the highest wavelength of 375 nm which is due to the effect of solvent like 0.1N Sodium hydroxide solution on Tinidazole when compared with other solvents like 0.1N Hydrochloric acid, Ethanol and distilled water. The lowest absorbance was 0.633 at the lowest wavelength of 294 nm which is due to the effect of solvent like 0.1N Hydrochloric acid on Tinidazole. Different wavelength and absorbance of Tinidazole in different solvents because of change of chromophoric groups; it is called as solvent effect of absorption maximum of Tinidazole. The greater wavelength of Tinidazole in 0.1N Sodium hydroxide solution is due to greater solubility of drug in alkali medium.

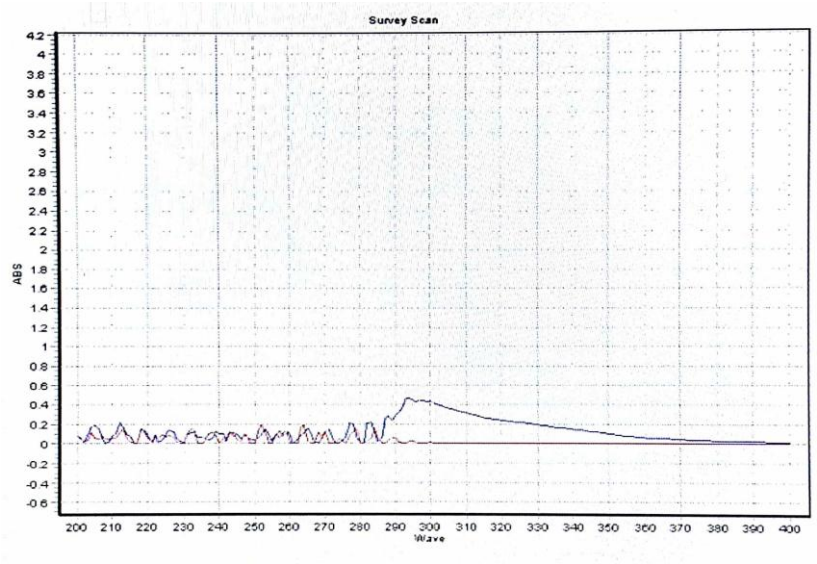

Fig. 3: Absorption maximum (λ_{max}) Tinidazole in 0.1N HCl (294 nm)

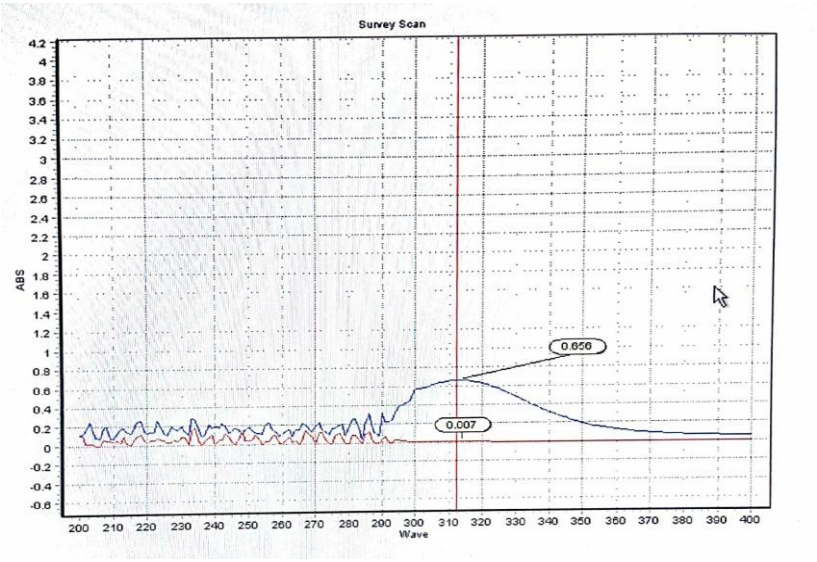

Fig. 4: Absorption maximum (λ_{max}) Tinidazole in Ethanol (312 nm)

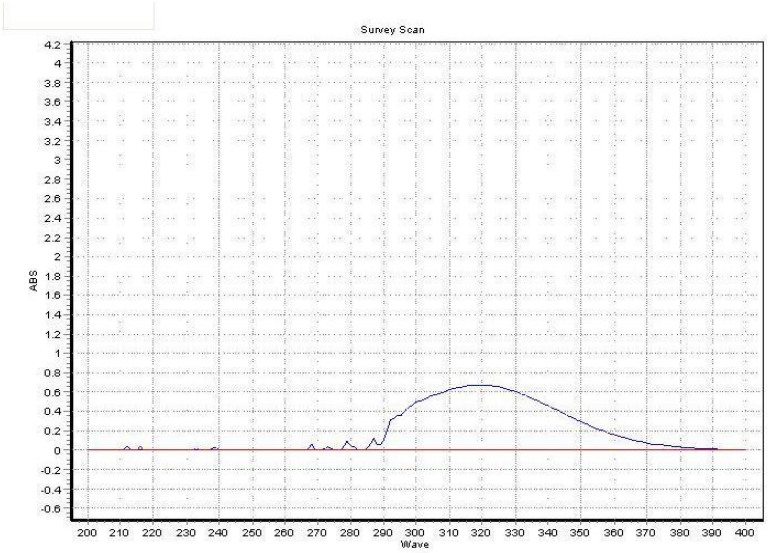

Fig. 5: Absorption maximum (λ_{max}) Tinidazole in Distilled Water (319 nm)

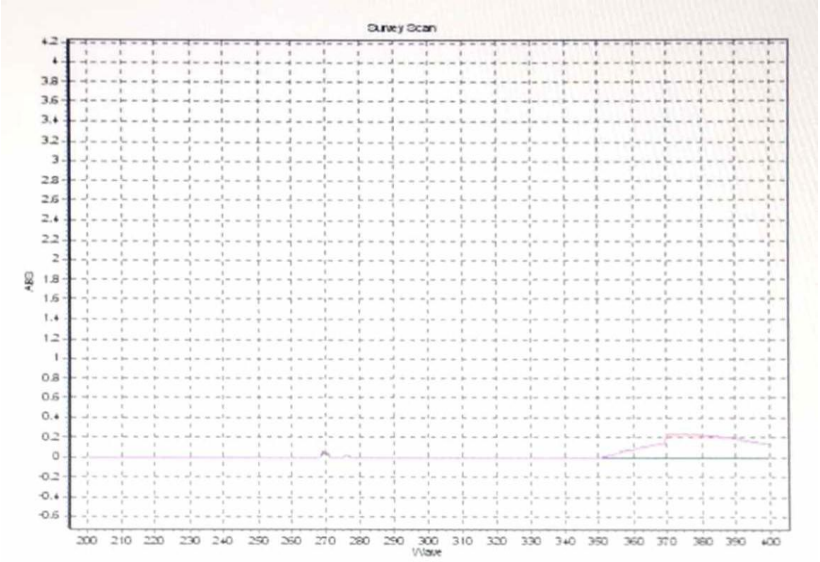

Fig. 6: Absorption maximum (λ_{max}) Tinidazole in 0.1N NaOH (375 nm)

Linearity and range

The linearity for the developed method was investigated by replicate analysis (n=3) at six concentration levels (2-20 µg/ml) of reference standard Tinidazole. The absorbance obtained at respective concentration was recorded and graph was plotted shows good linear correlation coefficient from the UV probe software. The linearity was shown in table 1, 2, 3 and 4 and fig. 7, 8, 9 and 10.

Table. 1: Standard calibration curve of Tinidazole in 0.1N HCl.

Flask No.	Volume of Stock-II (ml)	Volume made up to (ml)	Concentration (µg/ml)	Absorbance at 294 nm
1	0.2	10	2	0.067
2	0.4	10	4	0.120
3	0.6	10	6	0.183
4	0.8	10	8	0.238
5	1.0	10	10	0.297
6	1.2	10	12	0.375
7	1.4	10	14	0.429
8	1.6	10	16	0.488
9	1.8	10	18	0.574
10	2.0	10	20	0.633

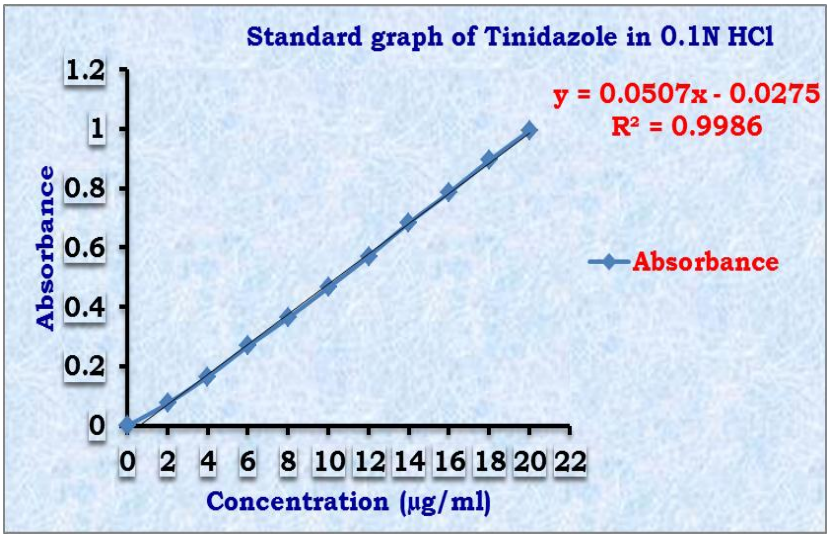

Fig. 7: Standard calibration curve of Tinidazole in 0.1 N HCl

Table. 2: Standard calibration curve of Tinidazole in ethanol at 312 nm.

Flask No	Volume of Stock-II (ml)	Volume made up to (ml)	Concentration (µg/ml)	Absorbance at 312 nm
1	0.2	10	2	0.078
2	0.4	10	4	0.134
3	0.6	10	6	0.225
4	0.8	10	8	0.284
5	1.0	10	10	0.363
6	1.2	10	12	0.431
7	1.4	10	14	0.487
8	1.6	10	16	0.545
9	1.8	10	18	0.618
10	2.0	10	20	0.689

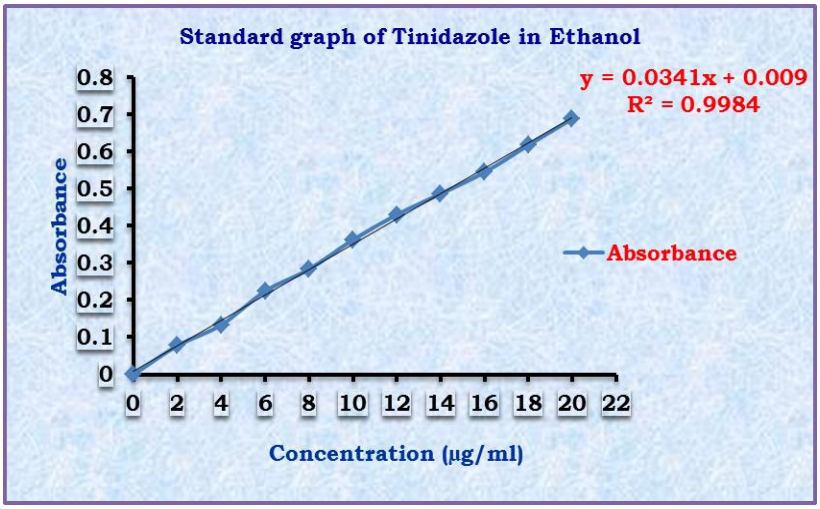

Fig. 8: Standard graph of Tinidazole in Ethanol

Table. 3: Data of Standard graph of Tinidazole in Distilled water at 319 nm.

Flask No.	Volume of Stock-II (ml)	Volume made up to (ml)	Concentration (µg/ml)	Absorbance at 319 nm
1	0.2	10	2	0.079
2	0.4	10	4	0.158
3	0.6	10	6	0.218
4	0.8	10	8	0.289
5	1.0	10	10	0.348
6	1.2	10	12	0.425
7	1.4	10	14	0.506
8	1.6	10	16	0.567
9	1.8	10	18	0.638
10	2.0	10	20	0.673

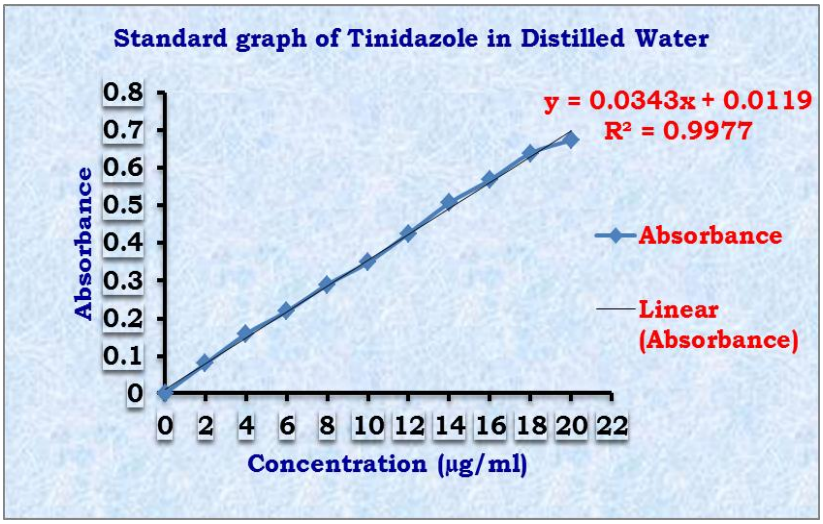

Fig. 9: Standard graph of Tinidazole in distilled water at 319 nm

Table. 4: Data of Standard graph of Tinidazole in 0.1N NaOH at 375 nm.

Flask No.	Volume of Stock-II (ml)	Volume made up to (ml)	Concentration (µg/ml)	Absorbance at 319 nm
1	0.2	10	2	0.107
2	0.4	10	4	0.165
3	0.6	10	6	0.245
4	0.8	10	8	0.304
5	1.0	10	10	0.363
6	1.2	10	12	0.438
7	1.4	10	14	0.499
8	1.6	10	16	0.547
9	1.8	10	18	0.61
10	2.0	10	20	0.671

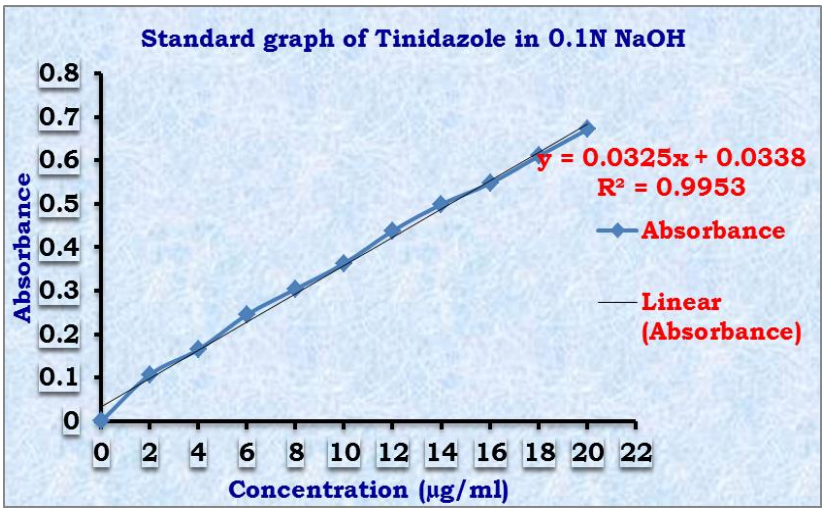

Fig. 10: Standard graph of Tinidazole in 0.1N NaOH at 375 nm

Accuracy: The accuracy was determined in triplicate by analysing Percentage recovery of Tinidazole by standard addition method. The percent recovery obtained indicates non-interference from the excipients used in the formulation. The results were shown in table 1,2,3,4.

Table. 5: Recovery studies: Analysis of marketed formulation.

Solvent	Label claim amount (mg)	Amount obtained (mg)	% RSD*	S.D*	Percentage Recovered	Standard error
0.1N HCl*	Tindamax Tablet - 500	499.61± 0.362	0.362	0.963	99.92± 0.94	0.08
Ethanol	500	498.82± 0.516	0.516	0.632	99.76± 0.16	0.24
Distilled Water	500	496.14± 0.721	0.721	0.794	99.22± 0.21	0.78
0.1NSodium hydroxide	500	499.73± 0.829	0.829	0.856	99.94± 0.35	0.06

***HCl-** Hydrochloric acid, **S.D**-Standard Deviation, **R.S.D**-Relative Standard Deviation, n=3

Method precision: The precision of proposed method was determined by Intra-day and Inter-day precision, and it was expressed in terms of percent relative standard deviation (% RSD). For Inter-day and Intra-day percentage RSD were found in the range of 0.048 at 4 µg/ml, 0.036 at 8 µg/ml and 0.070 at 4 µg/ml, 0.052 at 8 µg/ml respectively as shown in table 6.

Table. 6: Analysis of precision.

Amount taken (µg/ml)	Intraday precision		Inter day precision	
	Amount found	% RSD	Amount found	% RSD
4	3.99	0.070	3.95	0.048
4	3.96	0.070	3.92	0.048
4	3.97	0.070	3.93	0.048
8	7.98	0.052	7.96	0.036
8	7.96	0.052	7.94	0.036
8	7.98	0.052	7.95	0.036

Table. 7: Summary of analytical parameter.

Parameter	0.1N HCl	Ethanol	Distilled Water	0.1N NaOH
Absorption maximum (λ_{max}) nm	294	312	319	375
Beer's range (µg/ml)	2-20	2-20	2-20	2-20
Correlation co-efficient (r^2)	0.998	0.998	0.997	0.995
Regression	y = 0.050x ± 0.027	y = 0.034x ± 0.0059	y = 0.034x ± 0.011	y = 0.032x ± 0.033
Intercept	0.027	0.059	0.011	0.033
Slope (m)	0.050	0.034	0.034	0.032

Robustness: The robustness of the proposed method the solutions of 2µg/ml of standard Tinidazole solution was prepared and analysed by a change in wavelength. The wavelength was selected λ_{max} in 0.1N HCl, Ethanol, Distilled water and 0.1N NaOH i.e. 294 nm, 312 nm, 319 nm and 375 nm respectively for standard Tinidazole solution.

Ruggedness: The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days. To establish ruggedness of the proposed method, the solutions of 2 µg/ml of standard Tinidazole solution was prepared and analysed with the change in the different analyst.

CONCLUSION

The drug of Tinidazole exhibited different wavelength and absorbance in different solvents because of the change of chromophoric Group, it is called as the effect of solvent on absorption maximum of Tinidazole. Thus the conclusion was made that the proposed UV

Spectrophotometric method were found to be simple, rapid, accurate, precise, linear and more economical method has been developed for the quantitative estimation of Tinidazole in bulk and dosage form (tablets). The method is validated as per the ICH guidelines, and it is found that the developed method is robust and sensitive. Hence, this method can be successfully and suitably acquired for routine quality control analysis of Tinidazole in bulk and dosage form (tablets).

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