

## Research Article

# UV-Spectrophotometric Determination of Tinidazole in Bulk and Pharmaceutical Dosage Form Using Hydrotropic Solubilization Technique

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## ABSTRACT

Hydrotropic solvents may proper choice to preclude the use of organic solvents so that, a simple, accurate, novel, safe and precise method could developed for estimation of poorly water soluble drug, tinidazole. Solubility of tinidazole is increased by using 1M piperazine as a hydrotropic agent. tinidazole showed the maximum absorbance at 318 nm in method A, 314-322 nm in method B and 312 nm in method C. At these wavelengths, hydrotropic agent and other tablet excipients did not show any significant interference in the spectrophotometric assay. The developed methods were found to be linear in the range of 5-30 µg/ml with correlation coefficients (R) of 0.997, 0.998 and 0.992 respectively. The mean percent label claim of tablets of tinidazole in formulation estimated by the proposed methods was found to be 98.66. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be good accordance with the prescribe values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and dosage form in industries.

**Keywords:** Tinidazole; piperazine; AUC, Hydrotropic solubilization technique.

## INTRODUCTION

The term hydrotropic agent was first introduced by Neuberg (1916), to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. Hydrotropic agents consist generally of two essential parts, an anionic group and hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is prerequisite for a hydrotropic substance. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Hydrotropes commonly used includes sodium benzoate, sodium acetate, sodium salicylate, nicotinamide, urea, trisodium citrate, sodium ascorbate, piperazine, caffeine, potassium citrate etc. hydrotropic agents have been

observed to enhance the solubility of various substances in water.

Tinidazole (Figure 1) is a 5-nitroimidazole derivative used for the treatment of protozoal infections including giardiasis, amoebiasis and vaginal trichomoniasis. The IUPAC name for tinidazole is 1-(2 ethylsulfonyl ethyl)-2-methyl-5-nitroimidazole<sup>3</sup>. The British Pharmacopoeia (2009) specifies non-aqueous titration method using acetic perchloric acid for the assay of tinidazole<sup>7</sup>. Gas liquid chromatography<sup>7</sup>, high performance liquid chromatography<sup>8</sup>, voltammetry and spectrophotometry<sup>1-6</sup> are some of the methods found in the literature for the determination of tinidazole.

The objective of present investigation is to develop simple, precise, accurate and ecofriendly UV-spectrophotometric AUC and first order derivative methods for determination of tinidazole in bulk and in tablet dosage form using piperazine as a hydrotropic solubilizing agent. The developed methods were validated as per ICH guidelines.

## MATERIALS AND METHODS

### Chemicals and reagents

Tinidazole (99.4%) was obtained as gift sample from laboratories, Hyderabad, India. Pharmaceutical tablet formulation of TINVISTA tab 300 mg purchased from local market. Piperazine (A.R Grade;Qualigens) and distilled water used for the study.

### Instrumentation

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software ,version 2.21 was used for spectral measurements with 10mm matched quartz cells . Shimadzu balance (BL-220H) was used for weighing.

### Selection of solvent

1M piperazine solution was used as a solvent for developing spectral characteristics of a drug. The selection was made after assessing the solubility in different hydropropic solvents like sodium acetate, sodium benzoate, urea, sodium chloride, citric acid.

### Preparation of reagent solution

1M piperazine solution was prepared by 8.614 gm of piperazine pure chemical was weighed and dissolved in 10 ml distilled water and the volume was made upto the mark with distilled water in 100 ml volumetric flask.

### Preparation of standard stock solution

Working standard tinidazole 10 mg was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in 1 ml of 1M piperazine solution. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000 $\mu$ g/ml. It was further diluted with distilled water to get the concentration of 100 $\mu$ g/ml. from this solution a series of aliquots were prepared for further method development.

### Method A

#### Absorption maxima method

For the selection of analytical wavelength 10 $\mu$ g/ml solution of tinidazole was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum  $\lambda_{max}$  of tinidazole, 318 nm was selected for the analysis. The calibration curve was prepared in concentration range of 5-30  $\mu$ g/ml at 318 nm. The calibration curve for tinidazole was plotted in the concentration v/s absorbance and regression equation was calculated. (fig. 2&3).

### Method B

#### Area under curve method

For the selection of analytical wavelength 10 $\mu$ g/ml solution of tinidazole was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 314-322 nm. Area calculation processing item calculates area bound by curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression equation was calculated for the determination of amount of tinidazole in tablet formulation.(fig.4&5)

### Method C

#### First order derivative spectroscopy

It involves the conversion of normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often, these plots reveal spectral detail that is the lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily accurately using derivative methods. In this method, 10 $\mu$ g/ml solution of piroxicam was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivatised from zero to second order. First order derivative spectra of drug showed a sharp peak at 312 nm, which was selected for its quantification. The calibration curve for tinidazole was plotted in the concentration range of 2.5-17.5  $\mu$ g/ml at 312 nm. The concentration of drug present in the solution was determined against the calibration curve in quantization mode.(fig. 6&7)

#### Estimation of tinidazole in tablet formulation

For the estimation of tinidazole in the commercial formulation, 20 tablets each containing 300 mg of tinidazole were weighed and average weight calculated. Triturate the tablets, for the analysis of drug ,quantity of powder equivalent to 10 mg of tinidazole was

transferred to 10 ml volumetric flask and dissolved in 1M piperazine solution. It was filtered with Whatman filter paper no.41 and then volume made up to the mark with water to obtain a stock solution of 1000 µg/ml of piroxicam. Further dilutions of the stock solution were made in distilled water to get required concentration. In method A, the concentration of tinidazole was determined by measuring absorbances of sample solution at 318 nm. In method B, the concentration of tinidazole was determined by measuring absorbances of sample solution in wavelength range of 314-322 nm. In method C, first order derivative spectroscopy the concentration of tinidazole was determined by measuring amplitude difference at  $\lambda_{max}$  312 nm. Result of tablet analysis are shown in table no. the assay procedure was repeated 6 times (n=6) (table.1).

#### Method validation

The method was validated according to ICH guidelines to study accuracy, linearity and precision.

#### Linearity

In order to find out linearity range of proposed UV-spectrophotometric method, studies were carried out by plotting absorbances of analyte against concentrations of the analyte. A good linear relationship ( $r^2=0.992, 0.998$  &  $0.992$  for method A, B & C respectively) was observed between concentrations of tinidazole and the corresponding absorbance. The regression of tinidazole concentration over its absorbance was found to be  $y=0.028x+0.04, 0.279x-0.033$  &  $0.031x+0.019$  for method A, B & C (where y is the absorbance and x is the concentration of tinidazole). The slope, intercept and the correlation coefficient of the drug were shown in table.2

#### Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (2.5 µg, 5 µg, 7.5 µg) of bulk sample to the pre-analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed 6 times. The 105% recovery of the drug was calculated. The results were shown in the table.3

#### Precision

Precision is the degree of repeatability of an analytical method under normal operation

conditions, precision of the method was determined with standard and the test sample. Precision is of 3 types

1. System precision
2. Method precision
3. Intermediate precision
  - a. Intraday precision
  - b. Inter day precision

Method precision was achieved by repeating the same procedure of preparation solution six times and injecting.

System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this absorbance and % of drug of six determinations is measured and % RSD should be calculated.

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

#### LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

The standard deviation and response of the slope-

$$\text{LOD}=3.3 * \text{standard deviation } (\sigma) / s$$

#### LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy.

The standard deviation and response of the slope-

$$\text{LOQ}=10 * \text{standard deviation } (\sigma) / s$$

#### RESULTS AND DISCUSSION

For quantitative estimation of tinidazole in bulk and tablet dosage form three validated methods were proposed for method A, the absorbance maxima was found to be 318 nm, for method C  $\lambda_{max}$  at 312 nm was selected and for method B area under curve in the range of 314-322 nm were selected for the analysis. The % assay by the three methods was found to be 98.66% in method A, 99.62% in method B and 99.00% in method C. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be in the range of 106.2%,

102.01%, 102.19%. Hence, the proposed were validated in terms of linearity, precision, and accuracy. The present work provides an accurate and sensitive method for the analysis of tinidazole in bulk and tablet formulation.

### CONCLUSION

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and %RSD calculated for the methods are within the limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of tinidazole in bulk and formulation. The proposed methods were found to be simple, economical, eco-

friendly, rapid, precise and accurate for the determination of tinidazole in tablet dosage form. There is good scope for other poorly water soluble drugs which may be tried to get solubilised in 1M piperazine solution (as hydrotropic agent) to carry out their spectrophotometer analysis excluding the use of costlier and unsafe organic solvents. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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**Table 1: Results of marketed formulation analysis**

| Proposed methods | Label claim (mg) | Test conc (µg/ml) | Amount found (µg/ml) | %Assay | %RSD |
|------------------|------------------|-------------------|----------------------|--------|------|
| A                | 300mg            | 7.5               | 7.40                 | 98.66  | 0.92 |
| B                | 300mg            | 7.5               | 7.47                 | 99.62  | 0.85 |
| C                | 300mg            | 7.5               | 7.43                 | 99.00  | 0.92 |

**Table 2: Optical characteristics of the proposed methods**

| S.NO. | Parameter               | Method A        | Method B          | Method C           |
|-------|-------------------------|-----------------|-------------------|--------------------|
| 1     | Linearity(µg/ml)        | 5-30            | 2.5-17.5          | 2.5-17.5           |
| 2     | Linearity equation      | $y=0.028x+0.04$ | $Y=0.0279x-0.033$ | $Y=0.0307x+0.0311$ |
| 3     | Slope±SD                | 0.028±0.0056    | 0.0279±0.0065     | 0.0307±0.0058      |
| 4     | Intercept ±SD           | 0.04±0.0026     | 0.033±0.0049      | 0.0311±0.0036      |
| 5     | Correlation coefficient | 0.997           | 0.998             | 0.992              |

**Table 3: Recovery studies of proposed methods**

| Method   | Level of recovery | Pre analyzed conc(µg/ml) | Amount added(µg/ml) | Amount found(µg/ml) | %Recovery |
|----------|-------------------|--------------------------|---------------------|---------------------|-----------|
| Method A | 50                | 5                        | 2.5                 | 7.425               | 98.66     |
|          | 100               | 5                        | 5                   | 10.71               | 107.4     |
|          | 150               | 5                        | 7.5                 | 14.00               | 112       |
| Method B | 50                | 5                        | 2.5                 | 7.412               | 98.88     |
|          | 100               | 5                        | 5                   | 9.972               | 99.72     |
|          | 150               | 5                        | 7.5                 | 13.43               | 107.44    |
| Method C | 50                | 5                        | 2.5                 | 7.45                | 99.33     |
|          | 100               | 5                        | 5                   | 9.96                | 99.66     |
|          | 150               | 5                        | 7.5                 | 13.45               | 107.60    |

**Table 4: Precision studies of proposed methods**

| Method precision |                  |             |        |
|------------------|------------------|-------------|--------|
| Method           | Test conc(µg/ml) | Mean±SD     | %RSD   |
| A                | 10(µg/ml)        | 9.95±0.0010 | 0.2808 |
| B                | 10(µg/ml)        | 9.97±0.0021 | 0.2540 |
| C                | 10(µg/ml)        | 9.92±0.0041 | 0.3102 |

## System precision

| Method | Test conc( $\mu\text{g/ml}$ ) | Mean $\pm$ SD    | %RSD   |
|--------|-------------------------------|------------------|--------|
| A      | 10( $\mu\text{g/ml}$ )        | 9.96 $\pm$ 0.008 | 0.2266 |
| B      | 10( $\mu\text{g/ml}$ )        | 9.93 $\pm$ 0.005 | 0.2526 |
| C      | 10( $\mu\text{g/ml}$ )        | 9.91 $\pm$ 0.004 | 0.3102 |

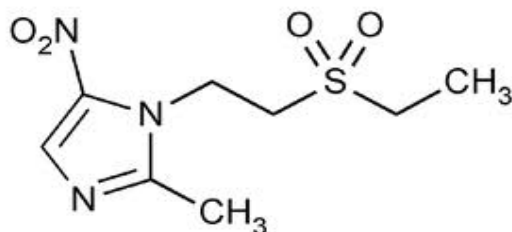
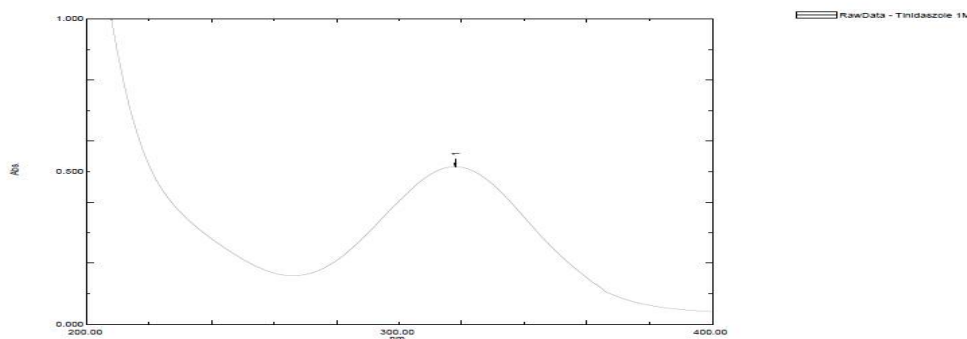


Fig. 1: structure of tinidazole

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Fig. 2: Absorption maxima spectrum of tinidazole

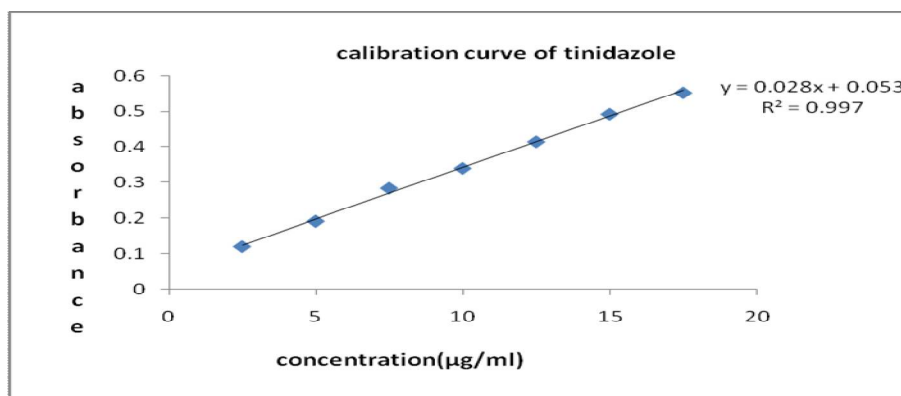
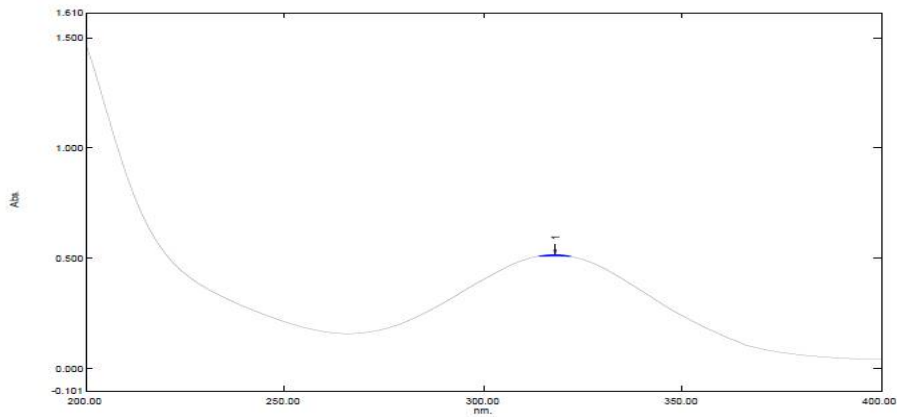


Fig. 3: calibration curve of tinidazole in method A

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Fig. 4: AUC spectrum of tinidazole

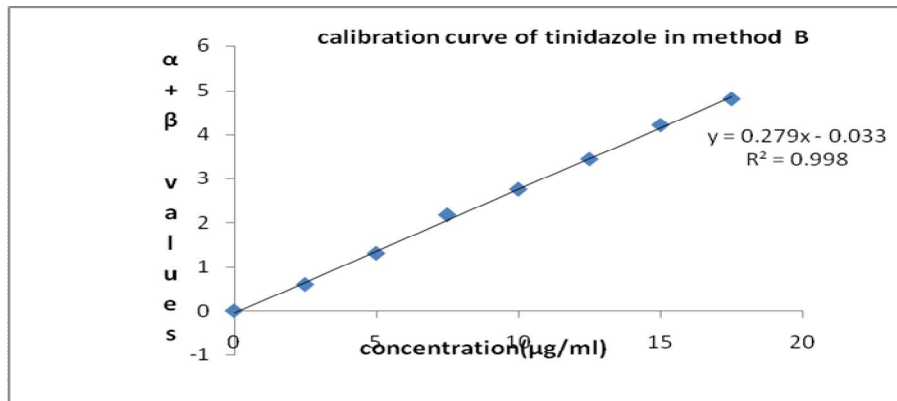
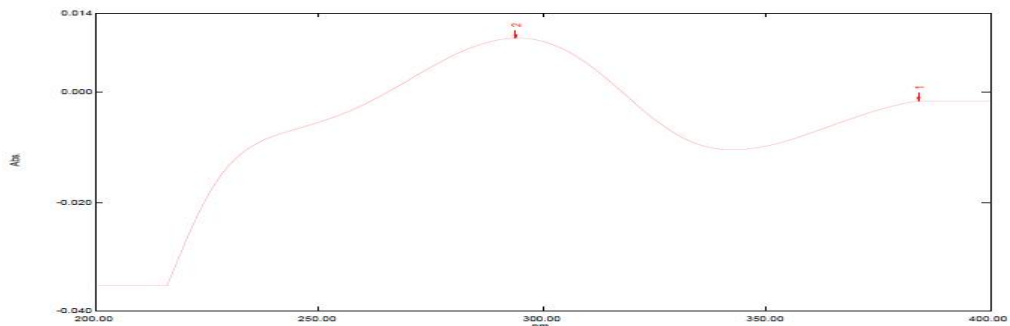


Fig. 5: calibration curve of tinidazole in method B

## Active Spectrum Graph Report

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Data Set: Dataset 022949 - Tinidazole 1M piperazine(16-12-14)



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Fig. 6: first order derivative spectrum of tinidazole

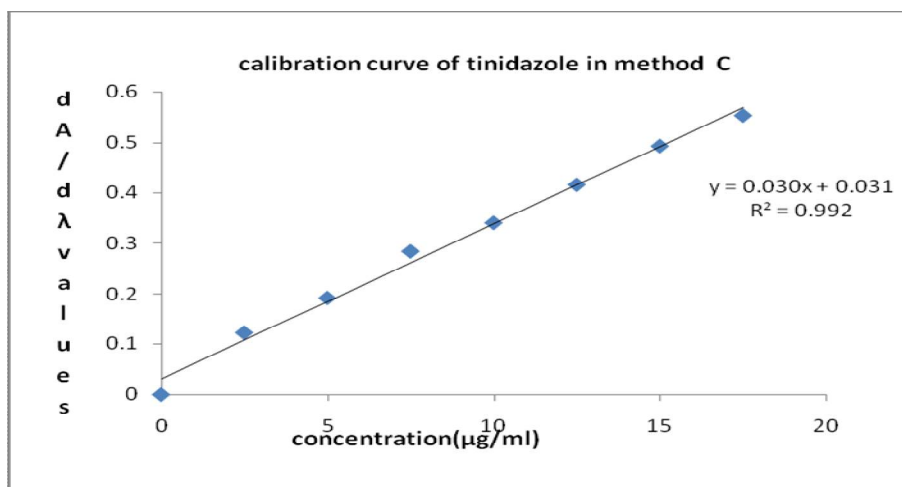


Fig. 7: Calibration curve of tinidazole in method C

#### REFERENCES

1. Luna-itol. Simple spectroscopic determination of tinidazole in formulation and serum, anal chim acta. 1997;340(1-3):241-244.
2. shingal DM-itol. Spectrocolorimetric estimation of tinidazole from pharmaceutical preparation. Indian drugs. 1997;24(7):363-364.
3. patel RB-itol. Colourimetric method for the estimation of tinidazole eastern pharmacist. 1985;28:137-138.
4. Bhatkar RG-itol. Spectrophotometric analysis of tinidazole Easter-pharmacist. 1982;25:117.
5. Devani MB-itpl. rapid method of analysis of metronadazole and tinidazole in tablets. Indian Journal of pharmaceutical sciences. 1981;43:15-152.
6. kamalapurkar OS-itol. Colourimetric estimation of tinidazole in pharmaceutical dosage form. Journal of pharmaceutical sciences. 1979;68:599-601.
7. Safwan ashour-itol. Capillary gas-liquid chromatographic determination of tinidazole in pharmaceutical dosage forms. Research gate. 2010;69.
8. Khaja pasha-itol. RP HPLC method for the analysis of tinidazole in pharmaceutical dosage form and bulk drug. International journal of pharmacy and pharmaceutical sciences. 2010;2: 46-47.
9. British Pharmacopoeia Vol. III, United Kingdom: The Stationery office on behalf of MHRA. 2009;2037.