DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF TINIDAZOLE BY USING DERIVATIVE SPECTROSCOPY

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ABSTRACT

Tinidazole is used in the treatment of protozoal infections. Two simple, sensitive, accurate and economical spectrophotometric methods have been developed for the estimation of Tinidazole in bulk drug and pharmaceutical formulation. Methods are based on measurements of first order and second order derivative spectroscopy adopted to eliminate spectral interference, in which derivative amplitude was measured at 295nm and 213nm respectively. The drug obeys Beer’s law in the concentrations range of 4 - 40μg/ml. The results of analysis were validated for accuracy, precision, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ). These results were found to be satisfactory. The proposed methods are simple, sensitive, rapid, economical and suitable for routine quality control applications in pharmaceutical formulation. This method is found to be useful in the method development of tinidazole.

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INTRODUCTION

Tinidazole is a 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole. Molecular formula: C₈H₁₃N₃O₄S. Tinidazole, a synthetic imidazole derivative, is widely used in the oral treatment of several protozoal infections - trichomoniasis, giardiasis and amoebiasis. Among the protozoal organisms inhibited by Tinidazole are Trichomonas vaginalis, Trichomonas foetus, and Entamoeba histolytica. Tinidazole is the most preferred choice of drug for intestinal amoebiasis.

This drug is to be delivered to the colon for its effective action against Entamoeba histolytica where in the trophozoites reside in the lumen of the caecum and large intestine and also adhere to the colonic mucus and epithelial layers. But the pharmacokinetic profile of Tinidazole indicates that the drug is completely and promptly absorbed after oral administration.[1]

![Figure No.1 Structure of Tinidazole.](image)

A literature revealed that few methods are available like LC method for the estimation of Tinidazole in human plasma [2] and other methods like UV [3-7], RP-HPLC[8-9], Colorimetry [10], Differential Pulse Polarography [11] are used for the estimation of Tinidazole in single or combination with other drug. No derivative spectrophotometric method is reported for Tinidazole until now. The derivative spectroscopic method used for eliminate the spectral interference and to locate the hidden peaks. It is used for quantification of active pharmaceutical ingredient.[12-17]. Derivative methods has been not developed for the determination of Tinidazole. So we developed method for estimation of Tinidazole by derivative spectroscopy. The aim of this study is to develop a simple, rapid, precise and accurate Derivative spectrophotometric methods for the determination of Tinidazole in bulk drug samples or in pharmaceutical dosage form.

MATERIALS & METHODS

Tinidizole, pure substance was obtained from Aarti Drug Limited, Mumbai and it used as such without further purification. The solution was prepared in distilled water and its maximum absorbance was found at wavelength of 317 nm. All measurements were taken on UV-Visible spectrophotometer (Shimadzu-1800). The normal spectrum was transform into first order and second order derivative and wavelengths were found to be 295 nm and 213 nm respectively.

Preparation of stock solution

Accurately 10 mg of Tinidazole was weighed into to 100 ml of clean and dry volumetric flask and dissolved in 70 ml double distilled water and the volume was made up to 100 ml to get a stock solution of 100μg/ml. This stock solution was used for making dilutions for calibration curve. All solutions were freshly prepared prior to analysis.

Method A: UV Spectroscopy

Dilutions of stock solution (100μg/ml) were prepared in the range of 4-40μg/ml and scanned in the spectrum mode from 200nm-400nm. From the spectra of drug (Figure No. 2), λ max of Tinidazole was found to be 317 nm. The calibration curve (Figure No. 3) was prepared in the range 4-40μg/ml. The amount of drug present in the sample solution was computed from its calibration curve.

![Figure No. 2. Zero Order Spectra.](image)
Method B: UV Derivative spectroscopy

In this method, 4-40μg/ml solutions of Tinidazole were prepared from stock solution (100μg/ml) and scanned in the spectrum mode from 200 nm to 400 nm. First order derivative spectra were selected for analysis of drug. First order derivative spectra of drug showed a sharp peak at 295 nm (Figure No. 4) which was selected for its quantification. Similarly second order derivative spectroscopy adopted to eliminate spectral interference, in which derivative amplitude were measured at 213nm (Figure No. 5). The overlay spectra’s of first and second order derivative spectroscopy are shown in (Figure No. 6 and 7). The calibration curves for Tinidazole of first order (Figure No. 8), second order (Figure No. 9) were plotted in the concentration range of 4-40μg/ml. The amount of drug present in the sample solution was computed from its calibration curve.
Figure No. 5. Second Order Derivative Spectra

Figure No. 6. Overlay Spectra of First Order Derivative Spectroscopy

Figure No. 7. Overlay Spectra of Second Order Derivative Spectroscopy.
METHOD OF VALIDATION

Linearity

A calibration curves were constructed at optimum experimental conditions using zero, first and second derivative absorbance’s versus concentration in the range of 4-40μg/ml. From calibration curve data, high value of the correlation coefficient was found and the value shows very good linearity of the calibration graph and adherence of the method to Beer’s law.

Accuracy

The accuracy of the method was assessed, based on recovery study. The technique of standard addition method was used to assess accuracy of the method. For this purpose a concentration of 80%, 90% 100%, 110% and 120% was selected. In this the absorbance of the sample after standard addition were measured. The results are reported in terms of % recovery in Table No. 1.

<table>
<thead>
<tr>
<th>Method</th>
<th>% Recovery</th>
<th>±S.D.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>100.10</td>
<td>0.4331</td>
<td>0.4327</td>
</tr>
<tr>
<td>First order derivative spectroscopy</td>
<td>102.36</td>
<td>0.4505</td>
<td>0.4401</td>
</tr>
<tr>
<td>Second order derivative spectroscopy</td>
<td>99.66</td>
<td>1.080</td>
<td>1.083</td>
</tr>
</tbody>
</table>

Precision

For Intraday and Interday precisions of the method, solutions of Tinidazole were prepared at three concentration levels (low, mid and high) each in triplicate. These solutions were analyzed respectively three times within one day and three consecutive days.

Limit of Detection and Limit of Quantification

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines were used to calculate the LOD and LOQ.

Assay Determination

Ten tablets were weighed and triturate to obtained powder. An accurately weighed 100 mg powder of Tinidazole and transferred to clean and dry 100 ml volumetric flask and dissolved in 70 ml of distilled water. The volume was made up to the mark
using distilled water to get concentration of 100μg/ml. From above solution pipette out 10 ml in 100 ml volumetric flask and volume was made up to the mark using distilled water get concentration of 100μg/ml. The resulting solution was filtered through Whatman filter paper no.41. From the above prepared solution (filtrate), further dilution was prepared to get the concentration of 20μg/ml. The absorbance was measured at the selected wavelength and concentrations were determined. The analysis was done in triplicate.

RESULTS AND DISCUSSION
First order, second order derivative spectroscopy was adopted in estimation of Tinidazole to eliminate spectral interference, in which derivative amplitude was measured at 295 nm, 213nm respectively. UV spectrophotometric measurements in which Tinidazole was dissolved in distilled water and exhibited absorption maximum at 317 nm have been used for drug to obey Beer’s law in the concentration range of 4 - 40μg/ml. The method was validated for various parameters like linearity, accuracy, precision, recovery, limit of detection and quantitation and ruggedness shown in Table No. 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>UV spectroscopy</th>
<th>Derivative spectroscopy</th>
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<tbody>
<tr>
<td></td>
<td>First Order</td>
<td>Second Order</td>
</tr>
<tr>
<td>λ max</td>
<td>317 nm</td>
<td>295 nm</td>
</tr>
<tr>
<td>Linearity (μg/ml)</td>
<td>4 - 40 μg/ml</td>
<td>4 - 40 μg/ml</td>
</tr>
<tr>
<td>Regression equation (Y = mx + c)</td>
<td>0.0417x + 0.0525</td>
<td>0.0087x + 0.0050</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0525</td>
<td>0.005</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0417</td>
<td>0.0087</td>
</tr>
<tr>
<td>Correlation Coefficient (r)</td>
<td>0.9983</td>
<td>0.9991</td>
</tr>
<tr>
<td>Accuracy</td>
<td>101.31 ± 0.5054</td>
<td>102.36 ± 0.4505</td>
</tr>
<tr>
<td>Precision (S.D.)</td>
<td>101.25 ± 0.2645</td>
<td>100.33 ± 0.8621</td>
</tr>
<tr>
<td>LOD</td>
<td>0.2284 μg/ml</td>
<td>1.631 μg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.6923 μg/ml</td>
<td>3.942 μg/ml</td>
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<tr>
<td>Ruggedness</td>
<td>101.30 ± 0.2828</td>
<td>100.55 ± 0.7778</td>
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</table>

CONCLUSION
The developed UV spectroscopic, derivative spectroscopic method gives sensitive, accurate, precise and economical results for determination of Tinidazole in marketed formulation (tablet) and easily applied for routine analysis. The most striking feature of these methods is its simplicity and rapidity. The developed method was validated for various parameters like linearity, accuracy, precision, LOD, LOQ and ruggedness. The developed methods were successfully applied for determination of the drug in commercial formulation. The most important effect of derivative spectroscopy is that broad bands are suppressed relative to sharp bands and this suppression increases with increasing derivative order. A common, unwanted effect in spectroscopy is baseline shift. This may arise either from instrument (lamp or detector instabilities) or sample handling (cuvette repositioning) effects. Derivative spectra always eliminates such baseline shifts and improves the accuracy of quantification.

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REFERENCES