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Simultaneous spectroscopic determination of tinidazole and clotrimazole from combined dosage forms from pharmaceutical formulations

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ABSTRACT

A new spectroscopic method for the simultaneous and separate estimation of Tinidazole and Clotrimazole in the binary tablet formulations has been described. The method is based on the estimation of the one drug in presence of another drug by absorbance difference method. The Tinidazole and Clotrimazole solutions were scanned over the range 200 to 315 nm. In this method, two wavelengths 240 nm and 285 nm were selected for Tinidazole. At these wavelengths the absorbance is almost zero and in case of Clotrimazole there should be considerable absorbance difference. Similarly, the two wavelengths 200 nm and 235 nm were selected for Clotrimazole; at these two-wavelengths difference is almost zero and there should be considerable absorbance difference in case of Tinidazole. In the Tinidazole and Clotrimazole solution the absorbance values of four wavelengths 240,285,200 and 235 nm were measured. The amount of Clotrimazole is directly proportional to the absorbance difference 200 and 235 nm. Similarly, the amount of Tinidazole is directly proportional to the absorbance difference between 240 and 285 nm. © 2011 Trade Science Inc. - INDIA

KEYWORDS

Spectrophotometry;
Tinidazole;
Clotrimazole;
Pharmaceutical formulations.

INTRODUCTION

The combination formulations of Tinidazole and Clotrimazole have been in the market for their using anaerobic infections. Literature describes various methods for the analysis of Tinidazole^[1-3] and Clotrimazole^[4-11] as individual drug products. Only one spectrophotometric method for simultaneous analysis for Tinidazole and Clotrimazole has been cited. No method for simultaneous analysis of Tinidazole and Clotrimazole in binary tablet formulation has been reported by absorbance difference method. The aim of the present work is to develop simple, rapid, precise, and reproducible and economic method for simultaneous analysis of the binary

drug formulations by using absorbance difference method with out any interference from the other.

EXPERIMENTAL

A Spectronics-1001 plus spectrophotometer with 10mm quartz cells was used for absorbance values of the drug solution. All the chemicals used were of analytical grade. AR grade methanol was used as solvent.

Preparation of standard solutions

Pure Tinidazole (50mg) was dissolved in the 50mL methanol. Further, one mL of the stock solution was further diluted to 50 mL with methanol to get working

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concentration of 20 $\mu\text{g/mL}$.

Pure Clotrimazole (50mg) was dissolved in the 50mL methanol. Further, One mL of the stock solution was further diluted to 50 mL with methanol to get working concentration of 20 $\mu\text{g/mL}$.

Preparation of mixed solution

Two solutions, first contained 20 $\mu\text{g/mL}$ of Tinidazole and the second contain 20 $\mu\text{g/mL}$ Clotrimazole were used as mixed solution. Four mixed standard solutions were made by taking 4, 3, 2 and 1 mL of Tinidazole solution in a series of test tubes and Clotrimazole stock solution was also added t series of test tubes to keep total volume 5 mL (TABLE 1).

TABLE 1 : Concentration of the two components in the four mixed standards

Standard No	Volume of TZ (mL)	Amount of TZ ($\mu\text{g/mL}$)	Volume of CZ (mL)	Amount of CZ ($\mu\text{g/mL}$)
1	4	80	1	20
2	3	60	2	40
3	2	40	3	60
4	1	20	4	80

TZ= Tinidazole CZ= Clotrimazole

Calibration curves of tinidazole and clotrimazole

Various aliquots (5, 6, 7 and 8 mL) of Tinidazole solution were transferred into series of 10mL standard flasks and the volume in each flask is adjusted to 10 mL with methanol. The absorbance of these solutions was

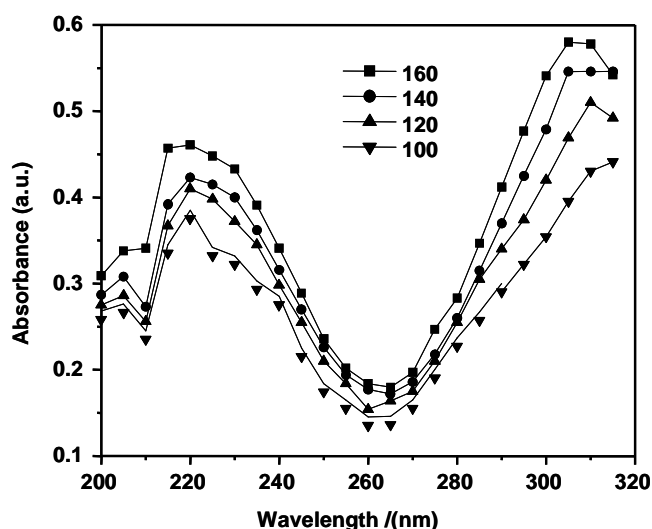


Figure 1 : Overlain spectrum of tinidazole. Absorbance spectra obtained form different concentrations ($\mu\text{g/mL}$) of tinidazole solutions.

scanned over the range 200 to 315 nm. Overlain spectrum of Tinidazole was showed in the Figure 1.

Again various aliquots (5, 6, 7 and 8mL) of Clotrimazole solution were transferred into series of 10 mL volumetric flask and the volume in each flask was adjusted to 10 mL with methanol. These solutions were scanned over the range 200 to 315 nm. Overlain spectrum of Clotrimazole is shown in the Figure 2.

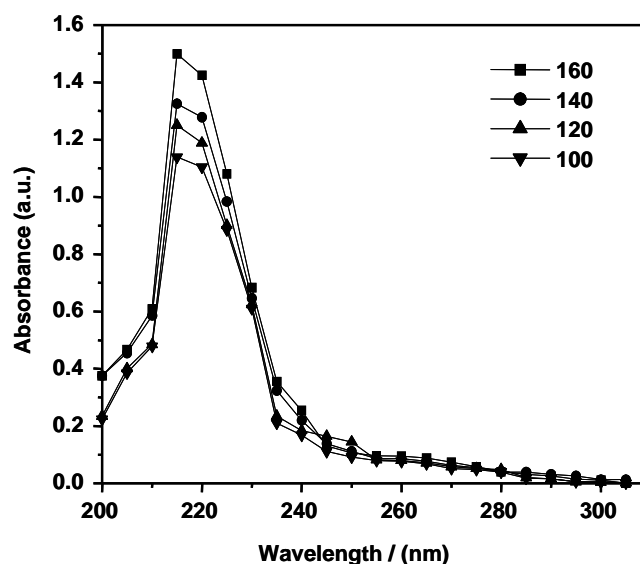


Figure 2 : Overlain spectrum of clotrimazole. Absorbance spectra obtained form different concentrations ($\mu\text{g/mL}$) of clotrimazole solutions.

Two wavelengths 240 and 285 nm were selected for Tinidazole: At these two wavelengths the absorbance values are almost zero and in case of Clotrimazole at the same wavelength 240 and 285 nm have maximum absorbance difference. A calibration curve was drawn between the absorbance difference values of Clotrimazole and the amount of Clotrimazole in $\mu\text{g/mL}$. The amount of Clotrimazole present in the sample was estimated from the calibration curve. Similarly, two wavelengths 200 and 235 nm was selected for Clotrimazole; at these two wavelengths the absorbance difference was almost zero and in case of Tinidazole, maximum absorbance difference values were at same wavelength 200 and 235 nm. A calibration curve was drawn between the absorbance difference values of Tinidazole and the amount of Tinidazole in $\mu\text{g/mL}$. The amount Tinidazole present in the sample was estimated from the calibration curve.

Various aliquots of mixture of Tinidazole and Clotrimazole solution in different proportion were trans-

ferred into a series of test tubes and the volume in each test tube was made 5 mL with methanol. The absorbance values were measured at two wavelengths 200 nm and 235 nm for estimation of Tinidazole and two wavelength 240 nm and 285 nm for estimation of Clotrimazole. A calibration graph was drawn between the absorbance difference values of Clotrimazole and amount of Clotrimazole present in $\mu\text{g/mL}$. A linear curve in each case was obtained. The linear curve obtained indicates that it obeys beers law and suitability of this method for the simultaneous determination of the two drugs in admixture.

Estimation of tinidazole and clotrimazole in pharmaceutical formulation

Tablets were weighed and powdered. 50mg of the tablets containing the two drugs Tinidazole and Clotrimazole were taken in 50 mL standard flask and dissolved in 30 mL methanol by vigorously shaking and the volume was made up to the mark. The solution was then filtered through whatmann filter paper no 41 and the solution was diluted to get a final concentration of $20\mu\text{g/mL}$ of Tinidazole and $20\mu\text{g/mL}$ of Clotrimazole. The sample solution were measured at 200 and 235 nm for Tinidazole and 240 and 285 nm for Clotrimazole in a spectronics-1001 plus spectrophotometer. The results are represented in the TABLE 2.

Validation of the method

The method was validated in terms of linearity, accuracy, precision, specificity and reproducibility of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Tinidazole ($20\mu\text{g/mL}$) and Clotrimazole ($20\mu\text{g/mL}$) and measured the absorbance values at 200 and 235 nm for Tinidazole and at 240 and 285 nm for Clotrimazole in a spectronics-1001 plus spectrophotometer. Calibration curves were constructed by plotting the absorbance difference values against the amount of drug in $\mu\text{g/mL}$.

Statistical analysis

A statistical analysis was performed in the statically significant variables using the statistical software. The following parameters were determined: coefficient of variation, standard deviation and student t-test.

Recovery experiment

To ensure the accuracy and reproducibility of the results obtained recovery experiments were performed by adding a known amount of standard drug to previously analyzed pharmaceutical preparation. The results are recorded in TABLE 2.

TABLE 2 : Estimation of tinidazole and clotrimazole in pharmaceutical preparations

Sample	Label Claim (mg/tab)		Amount found by Proposed method (mg)**		%Recovery by Proposed method*	
	TZ	CZ	TZ	CZ	TZ	CZ
Tablet 1	250	8	249.98	8.04	99.99	100.50
Tablet 2	250	8	249.86	8.02	99.94	100.25

TZ= Tinidazole CZ= Clotrimazole

*Average five determinations

** Determination of tinidazole and Clotrimazole in combined dosage form pharmaceutical preparations by proposed method.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, rapid, sensitive, precise, reproducible and accurate spectrophotometric method for the estimation of simultaneous determination of Tinidazole and Clotrimazole in pharmaceutical dosage forms. The proposed absorbance difference method was simple, less time consuming, low cost and found to be one of the best versatile analytical technique employed for routine analysis purposes like assay and pharmaceutical formulation. No method for the simultaneous analysis of Tinidazole and Clotrimazole in binary tablet formulations has been reported in literature by absorbance difference method. The content of Tinidazole and Clotrimazole in two different tablet dosage forms is shown in TABLE 2. The absorbance of various aliquots of the mixture of Tinidazole and Clotrimazole solutions was measured at two wavelengths 200 and 235 nm for Tinidazole and 240 and 285 nm for Clotrimazole. A calibration curve was drawn between the absorbance difference of Tinidazole and the amount of Tinidazole in $\mu\text{g/mL}$. The amount of Tinidazole present in the sample was computed from the calibration curve. Similarly, for the estimation of Clotrimazole, a calibration curve was plotted between the absorbance difference values of Clotrimazole against the amount of Clotrimazole in $\mu\text{g/}$

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mL. The amount of Clotrimazole in the sample was estimated from the calibration curve.

The results obtained by the proposed method are in good agreements with the label claim of the tablets. The additive and excipients usually present in the tablets do not interfere. As a check on accuracy of the method, recovery experiments were performed and percent recovery values also tabulated (TABLE 2). The statistical analysis was studied by the proposed method. The values of standard deviation and coefficient of variation were satisfactory low, indicating the accuracy and the reproducibility of the method. Student t-test shows that the calculated 't' values are less than the theoretical value 2.78 with 2 degree of freedom at 5% level of significance, including that there is no significant difference between the proposed and official values (TABLE 3).

TABLE 3 : Statical analysis of estimation of clortimazole and tinidazole

Sample	S.D.		C.V		^a t _{cal}	
	TZ	CZ	TZ	CZ	TZ	CZ
Tablet 1	0.3847	0.2236	0.076	0.118	0.172	1.000
Tablet 2	0.3420	0.2408	0.068	0.120	0.523	1.301

S.D=Standard deviation. C.V=Coefficient of variation.

TZ= Tinidazole. CZ=Clotrimazole

Calculated 't' value by proposed method. Theoretical values at 95% confidence limit's' 2.78.

CONCLUSION

In conclusion, the results indicate that the proposed absorbance difference method was found to be new, simple, environment-friendly, cost-effective, rapid, precise and accurate, and it can be used for the routine analysis of simultaneous determination of Tinidazole and Clotrimazole in pharmaceutical formulations.

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