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SPECTROPHOTOMETRIC ESTIMATION FOR SIMULTANEOUS ESTIMATION OF TINIDAZOLE AND DILOXANIDE FUROATE USING HYDROTROPIC AGENTS

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Abstract

A simple, accurate, and precise spectrophotometric method was developed for the simultaneous estimation of Tinidazole (TDZ) and Diloxanide Furoate (DXF) using hydrotropic agents. The poor aqueous solubility of both drugs was enhanced using a combination of 2M Sodium citrate and 8M Urea (1:1), which provided stability, solubility, and minimal spectral interference. TDZ and DXF showed λmax at 291.0 nm and 257.0 nm, respectively, with distinct absorption spectra allowing simultaneous analysis. Linearity was observed in the concentration range of 5–25 µg/mL with regression equations Y = 0.056X – 0.0067 (R^2 = 0.9997) for TDZ and Y = 0.0486X + 0.0045 (R^2 = 0.999) for DXF. Recovery studies at 80%, 100%, and 120% levels yielded mean recoveries of 98.66–98.89% for TDZ and 98.88–98.99% for DXF with %RSD < 1, indicating high accuracy. Validation parameters, including repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility, confirmed precision and robustness. Application of the method to marketed tablet formulations demonstrated label claim compliance with low %RSD values. The proposed method is rapid, cost-effective, and suitable for routine quality control of TDZ and DXF in combination formulations.

Introduction

Tinidazole (TDZ) is a nitroimidazole derivative widely used as an antiprotozoal and antibacterial agent. It is effective against anaerobic bacteria and protozoal infections, including amoebiasis and giardiasis (Sweetman, 2011). Diloxanide furoate (DXF), a derivative of dichloroacetamide, is primarily used in the treatment of intestinal amoebiasis due to its luminal amoebicidal activity (Martindale, 2020). Both drugs are often formulated together to achieve a synergistic effect in amoebic infections.

Quantitative estimation of drugs in combination formulations is essential for quality control and therapeutic efficacy. Spectrophotometry is a simple, rapid, and cost-effective analytical technique widely used for drug estimation. However, many drugs, including TDZ and DXF, have poor aqueous solubility, which can limit their

accurate spectrophotometric analysis (Aher et al., 2015).

Hydrotropic agents have emerged as an effective strategy to enhance the solubility of poorly soluble drugs. Hydrotropy involves the addition of large amounts of a solute (hydrotrope) to an aqueous solution, leading to increased solubility of the target compound without altering its chemical structure (Patel et al., 2013). Hydrotropic solubilization has been successfully applied to various drugs for spectrophotometric, chromatographic, and formulation purposes.

Several studies have demonstrated that the combination of hydrotropic agents can provide synergistic solubility enhancement for poorly soluble drugs, enabling accurate and reliable spectrophotometric estimation (Aher et al., 2015; Patel et al., 2013). In this study, TDZ and DXF were simultaneously estimated using a mixture of 2M Sodium citrate and 8M Urea, which provided maximum solubility, stability, and minimal interference at their respective λmax. The development of such a method is valuable for routine analysis of fixed-dose combination formulations in quality control laboratories.

Material and Methods

Material

Tinidazole (TDZ) and Diloxanide Furoate (DXF) were procured as pure drug samples, and marketed tablet formulations were used for analysis. Analytical grade chemicals and hydrotropic agents, including 2M Sodium citrate and 8M Urea, were employed to enhance solubility. Distilled water was used throughout the study. UV-Visible spectrophotometer with quartz cells, volumetric flasks, pipettes, and standard laboratory glassware were utilized for preparation of solutions, scanning, and analysis. All materials and instruments used were of analytical grade and suitable for spectrophotometric investigations.

Methods

Solubility

Solubility of TDZ and DXF was determined at 25±1°C. Accurately weighed 10mg TDZ and DXF was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed visually.

Selection of solvent system

TDZ and DXF were scanned in various hydrotropic agents in the spectrum mode over the UV range of 200–400 nm to identify a suitable medium for their

simultaneous estimation. Among the different hydrotropic systems tested, the combination of 2M Sodium citrate and 8M Urea in a 1:1 ratio was found to be the most appropriate. This system effectively solubilized both drugs, ensuring their complete dissolution, and provided chemical stability over the analysis period. Additionally, both TDZ and DXF exhibited clear and well-defined spectral characteristics in this medium, with no interference at their respective λmax values, confirming the suitability of this hydrotropic system for UV analysis (Aher et al., 2015). Furthermore, the solubility of TDZ and DXF was significantly enhanced, with more than 8-fold increase for TDZ and more than 7-fold increase for DXF, making the 2M Sodium citrate: 8M Urea (1:1) system ideal for accurate and reliable spectrophotometric estimation.

Establishment of stability profile

Stability of both drugs was observed by dissolving TDZ and DXF in 2M Sodium citrate: 8M Urea (1:1) solution used as solvent. Solution of TDZ and DXF was prepared in the conc. of 10µg/ml and 10µg/ml respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution (Mary and Babuan, 2015).

Linearity range and calibration graph

Preparation of Standard and Working Solutions for TDZ and DXF

Preparation of Standard Stock Solution (Stock-A):

Standard stock solutions were prepared by dissolving 10 mg of each drug separately in 8 mL of a mixed hydrotropic solution containing 2M Sodium citrate: 8M Urea (1:1) in a 1:1 ratio. The solutions were sonicated for 10 minutes to ensure complete solubilization of the drugs, and the volume was then adjusted to 10 mL with the same mixed hydrotropic solution to obtain a concentration of 1000 µg/mL (Stock-A) for both TDZ and DXF.

Preparation of Sub-Stock Solution (Stock-B):

From Stock-A, 1 mL aliquots of TDZ and DXF were withdrawn using a pipette and transferred into separate 10 mL volumetric flasks. Each was diluted up to 10mL with distilled water, resulting in a concentration of 100 µg/mL (Stock-B) for both drugs.

Preparation of Working Standard Solutions:

For TDZ: Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were withdrawn from Stock-B and transferred into 10 mL volumetric flasks. Each flask was then diluted up to 10 mL with RO water to obtain working solutions of 5, 10, 15, 20, and 25µg/mL, respectively.

For DXF: Similarly, 0.5, 1.0, 1.5, 2.0, and 2.5 mL aliquots were withdrawn from Stock-B, transferred into 10 mL volumetric flasks, and diluted up to 10mL with RO water to achieve concentrations of 5, 10, 15, 20, and 25 µg/mL, respectively (Rane *et al.,* 2015).

Selection of Wavelength for Linearity:

Solutions of 10µg/mL of TDZ and DXF were prepared separately and scanned in spectrum mode over the range of 200–400 nm. The maximum absorbance was observed at 278 nm for TDZ and 257 nm for DXF. Both drugs demonstrated linearity in the concentration range of 5–25 µg/mL at their respective λ_{max} values. Calibration curves were plotted with absorbance versus concentration to confirm linearity (Maheshwari *et al.,* 2010).

The selected wavelengths for further linearity and analytical studies were 278 nm for TDZ and 257 nm for DXF.

Selection of wavelength for linearity

Solutions of 10µg/ml of TDZ and 10µg/ml DXF were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of TDZ and DXF was observed at 278.0 nm and 257.0 nm, respectively.

TDZ and DXF showed linearity in the concentration range of 5-25µg/ml and 5-25µg/ml at their respective maxima (Maheshwari *et al.,* 2010). Calibration curve was plotted, absorbance versus concentration.

Method (Simultaneous equation method)

Study of overlay spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of TDZ and 10µg/ml of DXF were scanned in the spectrum mode over the range of 200-400 nm against water as blank and the overlain spectra of the two were recorded. TDZ showed an absorbance peak at 278.0 nm, whereas DXF at 257.0 nm. The overlain spectra also showed isoabsorptive points at 260.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Khan, 2015).

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two

wavelengths selected for the method are 278.0 nm and 257.0 nm that are λ_{max} of TDZ and DXF respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$CTDZ = \frac{A1ay2 - A2ay2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(1)$$

$$C\,DXF = \frac{A1ax2 - A2ax2}{ax1ay2 - ax2ay1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(2)$$

Where, A_1 and A_2 are absorbances of mixture at 278.0 nm and 257.0 nm respectively, ax_1 and ax_2 are absorptivities of TDZ at λ_1 (278.0 i.e. λ_{max} of TDZ) and λ_2 (257.0 i.e. λ_{max} of DXF) respectively and ay_1 and ay_2 are absorptivities of DXF at λ_1 and λ_2 respectively. C_{DXF} and C_{TDZ} are concentrations of TDZ and DXF respectively. The overlain spectra of both the drugs in 1:1 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the TDZ and DXF.

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio (Chauhan *et al.,* 2015).

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of TDZ and DXF to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels (Ravisankar *et al.,* 2014).

Analysis of tablet sample

Twenty marketed tablets of TDZ and DXF were weighed and ground to a fine powder; amount equal to 10mg of TDZ was taken in 10 ml volumetric flask. The DXF present in this amount of tablet powder was 10mg. Then 8 ml of 2M Sodium citrate: 8M Urea

(1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times (Devi *et al.,* 2013; Daksh *et al.,* 2015).

Results and Discussion

The UV-spectroscopic analysis of Tinidazole (TDZ) and Diloxanide Furoate (DXF) was performed to develop a reliable method for their simultaneous estimation using hydrotropic agents. The λmax of TDZ and DXF were determined to be 291.0 nm and 257.0 nm, respectively (Figures 1 and 2). The overlain spectra of both drugs (Figure 3) indicated distinct absorption maxima with minimal spectral overlap, confirming the suitability of the selected wavelengths for simultaneous estimation.

Linearity studies were conducted in the concentration range of 5–25 µg/mL for both TDZ and DXF. The regression equations obtained were $Y = 0.056X - 0.0067$ ($R^2 = 0.9997$) for TDZ and $Y = 0.0486X + 0.0045$ ($R^2 = 0.999$) for DXF (Table 7.1), demonstrating excellent correlation between absorbance and concentration. This indicates that the method is highly linear within the specified range and suitable for quantitative analysis.

Recovery studies were performed at 80%, 100%, and 120% levels to evaluate the accuracy of the method (Table 7.2). TDZ showed mean % recovery ranging from 98.66% to 98.89%, while DXF ranged from 98.88% to 98.99%. The low standard deviation (SD) and relative standard deviation (% RSD) values (<1%) indicate high precision and reliability of the developed method.

Further validation parameters, including repeatability, day-to-day variation, analyst-to-analyst variation, and reproducibility, were evaluated (Table 7.3). For TDZ, the mean values ranged from 97.04% to 98.65% with % RSD between 0.066 and 0.121, while DXF showed mean values from 97.98% to 98.39% with % RSD between 0.104 and 0.128. These results confirm the method's robustness, precision, and consistency across different analysts and time intervals.

Analysis of the marketed tablet formulations of TDZ and DXF revealed that the amount found was 295.45 mg for TDZ (98.48% of label claim) and 249.88 mg for DXF (99.95% of label claim) with low SD and % RSD values (Table 7.4). This demonstrates that the method is accurate and suitable for routine quality control of combination formulations.

The developed spectrophotometric method using hydrotropic agents provides a simple, rapid, and precise approach for the simultaneous estimation of TDZ and DXF in bulk and marketed formulations. The method's linearity, accuracy, precision, and reproducibility make it appropriate for analytical and quality control applications.

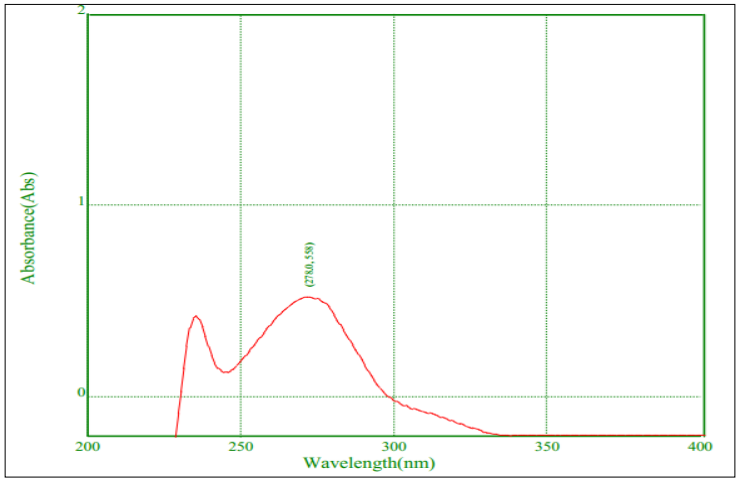


Figure 1: Determination of λ_{max} of TDZ

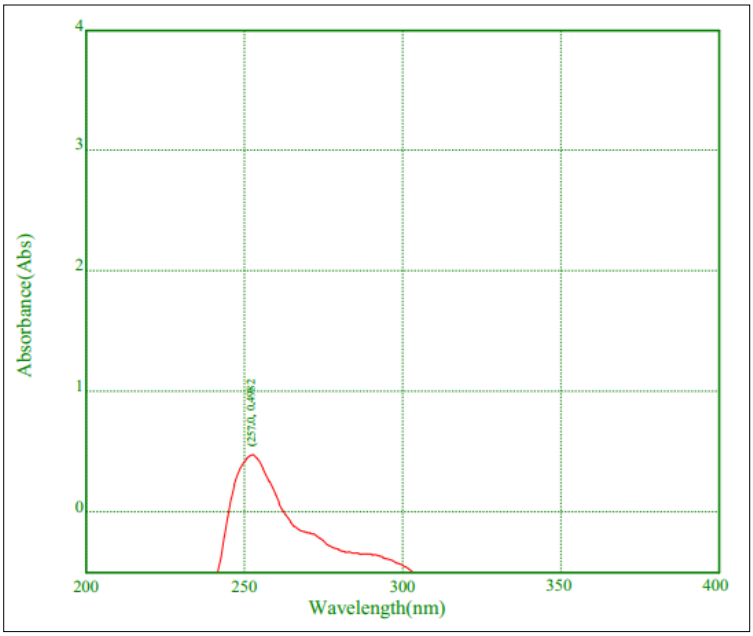


Figure 2: Determination of λ_{max} of DXF

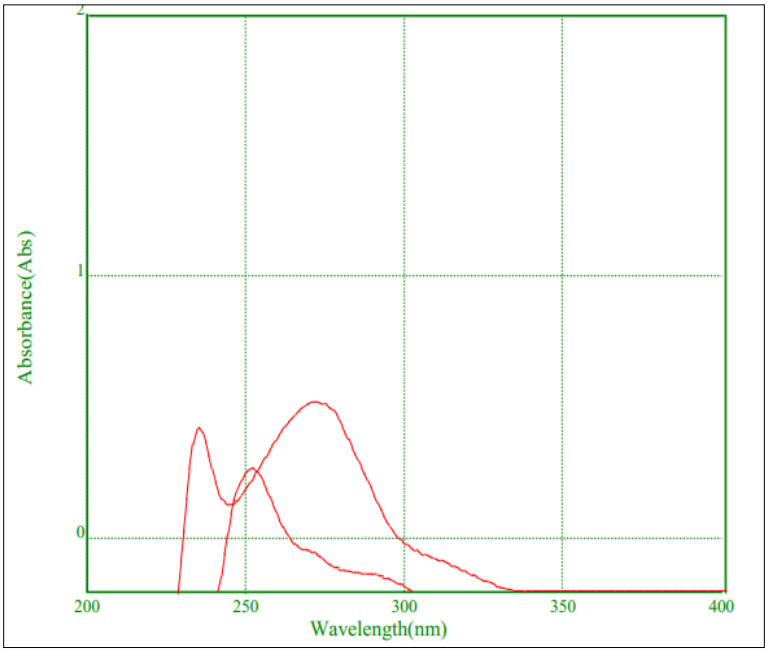


Figure 3: Overlain Spectra of both drugs

Table 1: Statistical data for linearity

S. No.	Drug	λmax (nm)	Concentration Range (µg/mL)	Regression Equation (Y = mx + c)	Correlation Coefficient (R^2)
1	Tinidazole (TDZ)	291.0	5 – 25	Y = 0.056X – 0.0067	0.9997
2	Diloxanide furoate (DXF)	257.0	5 – 25	Y = 0.0486X + 0.0045	0.999

Table 2: Results of Recovery Study

Drug	Recovery Level	Mean % Found	SD	% RSD
Tinidazole (TDZ)	80 %	98.89	0.422	0.427
	100 %	98.66	0.192	0.194
	120 %	98.86	0.559	0.565
Diloxanide Furoate (DXF)	80 %	98.96	0.671	0.678
	100 %	98.99	0.759	0.767

	120 %	98.88	0.567	0.573

Table 3: Results of Validation Parameters

Parameter	Drug	Mean (%)	SD	%RSD
Repeatability	Tinidazole (TDZ)	97.04	0.117	0.121
	Diloxanide Furoate (DXF)	98.39	0.117	0.119
Day-to-Day Variation	Tinidazole (TDZ)	98.21	0.100	0.103
	Diloxanide Furoate (DXF)	97.98	0.124	0.126
Analyst-to-Analyst Variation	Tinidazole (TDZ)	98.65	0.065	0.066
	Diloxanide Furoate (DXF)	98.21	0.126	0.128
Reproducibility	Tinidazole (TDZ)	98.35	0.112	0.114
	Diloxanide Furoate (DXF)	98.22	0.103	0.104

Table 4: Analysis of tablet formulation of TDZ and DXF

Drugs	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
TDZ	300	295.45	98.48	0.225	0.246
DXF	250	249.88	99.95	0.245	0.257

Conclusion

A simple, rapid, and reliable spectrophotometric method was successfully developed for the simultaneous estimation of Tinidazole (TDZ) and Diloxanide Furoate (DXF) using hydrotropic agents. The combination of 2M Sodium citrate and 8M Urea (1:1) significantly enhanced the solubility and stability of both drugs, allowing accurate and reproducible measurements. The method demonstrated excellent linearity, precision, accuracy, and robustness, as confirmed by recovery studies and validation parameters including repeatability, day-to-day, analyst-to-analyst variations, and reproducibility. Analysis of marketed tablet formulations showed results in close agreement with label claims, with low %RSD values, highlighting the method's suitability for routine quality control. This spectrophotometric approach is cost-effective, environmentally friendly, and

practical for simultaneous estimation of TDZ and DXF in bulk and pharmaceutical dosage forms.

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