DEVELOPMENT AND VALIDATION OF SIMULTANEOUS
SPECTROPHOTOMETRIC ESTIMATION OF DOXYCYCLINE
AND TINIDAZOLE INTO TABLET DOSAGE FORMS

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ABSTRACT
A simple, sensitive, rapid and precise spectrophotometric method has been developed and
validated for Simultaneous estimation of Doxycycline and Tinidazole from bulk and its
tablet dosage forms. Doxycycline and Tinidazole shows absorption maxima at 273 and
317 nm in Double distilled Water. The first method developed employs formation and
solving simultaneous equations using 273 and 317 nm as two wavelengths for the
formation of equations. Second method is absorbance ratio method (Q-analysis) in which
wavelengths selected were 341 nm as iso-absorptive point and 317 nm as $\lambda_{\text{max}}$ of
Tinidazole. Linearity were observed in the concentration range of 5-25 $\mu$g/ml for both the
drugs. The recovery studies established the accuracy of the proposed method and the
results were validated as per ICH guidelines. The procedures were successfully applied
for the simultaneous determination of both drugs in pure form and in commercial tablet
dosage formulations.

Key words: Doxycycline, Tinidazole, Simultaneous equation method, Absorbance ratio
method.

INTRODUCTION
Doxycycline, 2-(amino-hydroxy-methylidene)-4-dimethylamino-5,10,11,12a-
tetrahydroxy-6-methyl- 4a, 5a, 6-tetrahydro-4H-tetracene-1, 3,12-trione is a member of
the tetracycline antibiotics group and is commonly used to treat variety of infections like
chronic sinusitis, syphilis. Tinidazole, 1-(3-chloro-2-hydroxypropyl)-2-methyl-
5nitroimidazole is an antiamoebic drug [1-3]. Both the drugs are formulated in a binary solid
dosage form for the treatment of gastro-intestinal infectious diseases. Doxycycline and
Tinidazole was determined by HPLC\cite{4}. No simultaneous spectrophotometric method has been reported for both the components in a combined dosage forms. The aim of this paper was to develop the Vierodt’s method and absorbance ratio method for estimating Doxycycline and Tinidazole simultaneously in their mixture form.

**EXPERIMENTAL METHODS**

**Instrument, reagents and chemicals**

A dual-beam Shimadzu UV-visible spectrophotometer 1700 and doubled distilled water were used. Gift samples of Doxycycline and Tinidazole were procured from Cipra Labs, Hyderabad.

**Preparation of pure stock solution**

100 mg of each drugs were accurately weighed and transferred to two separate 100 ml volumetric flasks, dissolved in double distilled water to obtain first stock solution and from resulting stock solutions 10ml of sample transferred to two separate 100ml volumetric flask and make up to 100ml with double distilled water to get concentration 100 µg/ml as a second stock.

**Preparation sample solution**

Commercially available tablets were selected. Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 30 mg of Doxycycline and 180 mg of Tinidazole was transferred to 100 ml volumetric flask and contents were sonicated for 10 min. The volume was made up to the mark with double distilled water. The resulting solution was filtered through Whatmann filter paper No. 41. Aliquot were appropriately diluted with double distilled water to get final concentration of 3µg/ml and 18µg/ml of each drug respectively. The absorbances of standard and sample solutions were measured at 273, 317 and 341 nm (iso-absorptive point) using solvent blank. The results were calculated by the formula.

**Selection of λ\text{max}**

From second stock solutions, working standard solutions were prepared by appropriate dilution of aliquot portions with double distilled water to get final concentration of 25 µg/ml each and were scanned in the wavelength range of 200-400 nm to determine the λ\text{max}. Doxycycline shows λ\text{max} at 273 nm and Tinidazole at 317 nm, respectively (Figure a).
Figure a
Concentration of sample is found out by solving this equation.

\[
\begin{align*}
A & = \frac{A_1 a_1 - A_2 a_2}{a_2 a_1 - a_1 a_2}, \\
B & = \frac{A_1 a_2 - A_2 a_1}{a_2 a_1 - a_1 a_2}
\end{align*}
\]

Where,

\( A_1 \) and \( A_2 \) → Absorbance of diluted mixture at 317 and 273 nm respectively.

\( c_x \) and \( c_y \) → Concentration of Tinidazole and Doxycycline respectively (g/L).

\( a_{x1} \) and \( a_{x2} \) → Absorptivities of Tinidazole at 317 and 273 nm respectively.

\( a_{y1} \) and \( a_{y2} \) → Absorptivities of Doxycycline at 317 and 273 nm respectively.

**Absorption ratio method (Q-analysis)**

In quantitative assay of two components by Q-analysis method, absorbances were measured at the iso-absorptive wavelength and maximum absorption of one of the two components. From overlain spectra of Doxycycline and Tinidazole shown in figure (Fig.a), absorbances were measured at the selected wavelengths i.e. 341 nm (iso-absorptive wavelength) and 317 nm (wavelength of maximum absorption of Tinidazole). From the following sets of equations, the concentration of each component in sample can be calculated.

Concentration of Tinidazole \( (C_T) = (Q_0 - Q_D) / (Q_T - Q_D) \times A_3 / a_{T3} \)

Concentration of Doxycycline = \( A_3 / a_{D3} - (C_T) \)

Where,

\( A_1 \) and \( A_3 \) = Absorbance of sample solution at 317 nm and 341 nm (iso-absorptive wavelength).

\( Q_0 = A_1 / A_3, \ Q_T = a_{T1} / a_{T3} \ Q_D = a_{D1} / a_{D3} \).
RESULTS AND DISCUSSION

An attempt was made to develop a simple, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of Doxycycline and Tinidazole in their combined dosage forms. The molar absorptivity for both the drugs were calculated at three wavelengths 273 nm ($\lambda_{\text{max}}$ of Doxycycline), 317 nm ($\lambda_{\text{max}}$ of Tinidazole) and 341 nm (isoabsorptive wavelength). Both the drugs obey Beer Lambert's law in the range of 5-25 μg/ml. Percentable claimed for Doxycycline and Tinidazole in tablet analysis, by both methods, was found in the range of 100.02% to 100.12%. Standard deviation and coefficient of variance for six determination of tablet sample, by both methods, was found to be less than ±1.452 indicating the precision of the methods. Linearity of the calibration graphs was validated by the high value of Correlation coefficient of the regression (Table 1). Standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of the methods. Based on the results obtained, it is found that the proposed methods are accurate, precise and reproducible. It can be employed for routine quality control analysis of Doxycycline and Tinidazole in combined tablet dosage forms.

TABLE 1: OPTICAL CHARACTERISTICS AND OTHER PARAMETER

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>273 nm</th>
<th>317 nm</th>
<th>341 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law range µg/ml</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5-25</td>
<td>5-25</td>
<td>5-25</td>
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<tr>
<td>Molar absorptivity L/mol/cm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.3866X10^4</td>
<td>2.077X10^4</td>
<td>8.177X10^3</td>
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<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4.11X10^-4</td>
<td>5.34X10^-4</td>
<td>5.14X10^-4</td>
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<tr>
<td>Relative Standard Deviation</td>
<td></td>
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<tr>
<td></td>
<td>0.0891</td>
<td>0.4192</td>
<td>0.089</td>
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<tr>
<td>Correlation Coefficient (r)</td>
<td></td>
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<tr>
<td></td>
<td>0.9996</td>
<td>0.9986</td>
<td>0.9654</td>
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<tr>
<td>Slope (b)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.0306</td>
<td>0.0089</td>
<td>0.0908</td>
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<td>Intercept (a)</td>
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<tr>
<td></td>
<td>0.004</td>
<td>-0.0021</td>
<td>0.0198</td>
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<tr>
<td>Limit Of Quantification (LOQ)</td>
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<tr>
<td></td>
<td>0.1343</td>
<td>0.6</td>
<td>0.15</td>
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<tr>
<td>Limit Of Detection (LOD)</td>
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<td></td>
<td>0.0443</td>
<td>0.198</td>
<td>0.0498</td>
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<tr>
<td>% Range of Error With 0.05 level</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4.3469X10^-4</td>
<td>5.64X10^-4</td>
<td>3.16X10^-4</td>
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<tr>
<td>With 0.01 level</td>
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<tr>
<td></td>
<td>1.43X10^-4</td>
<td>8.35X10^-4</td>
<td>4.67X10^-4</td>
</tr>
</tbody>
</table>

Y=bC+a where C is the concentration of Doxycycline and Tinidazole in μg/ml and Y is absorbance unit
TABLE 2: RESULTS OF TABLET ASSAY FOR CONTENT OF DOXYCYCLINE AND TINIDAZOLE BY USING PROPOSED METHODS

<table>
<thead>
<tr>
<th></th>
<th>Simultaneous equation method</th>
<th>Graphical absorbance ratio method</th>
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<tbody>
<tr>
<td></td>
<td>Recovery(%)± S.D.(n=6)</td>
<td>Recovery(%)± S.D.(n=6)</td>
</tr>
<tr>
<td>Doxycycline Tablet</td>
<td>100.15 ±1.452</td>
<td>101.12±0. 236</td>
</tr>
<tr>
<td>SD(%)</td>
<td>1.45</td>
<td>0.23</td>
</tr>
<tr>
<td>Tinidazole Tablet</td>
<td>101.12±0.752</td>
<td>101.02±0.143</td>
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<tr>
<td></td>
<td>0.74</td>
<td>0.14</td>
</tr>
</tbody>
</table>

CONCLUSION

The simultaneous estimation methods described are applicable to the estimation of Doxycycline and Tinidazole in the pure as well as combined dosage forms.

REFERENCES


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