SPECTROPHOTOMETRIC DETERMINATION OF
METRONIDAZOLE IN PHARMACEUTICAL
PREPARATIONS

Saffaj T*, Charrouf M., Abourriche A., Aboud Y., Bennamara A., Maoufoud S.,
Berrada M.

Laboratoire de Chimie Organique Biomoléculaire, Faculté des Sciences Ben M’Sik,
Avenue Cdt D. El Harti BP 7955, Casablanca, Maroc.
E-mail: saffajt@yahoo.fr, Fax: 212 22 70 46 75

Abstract- A rapid and sensitive spectrophotometric method is proposed for determination of
Metronidazole. The method depends on the reduction of Metronidazole molecule with zinc
dust and hydrochloric acid flowed by diazotation and coupling with 8-quinolinol to give
colored chromogens easily measured spectrophotometrically which has $\lambda_{max}= 437$ nm.
The experimental conditions were optimized and bey’s law was obeyed over the applicable
concentration ranges. Both techniques were applied successfully to a wide variety of
pharmaceutical preparations.

Keywords: Metronidazole, Diazotation, 8-quinolinol, spectrophotometry.

1- INTRODUCTION

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is used as antiprotozoal,
antiamebic and antibacterial drugs. Excellent reviews have been published on the activity and
pharmacokinetics of this drug.

Several methods have been reported for determination of metronidazole which
includes volumetric, gravimetric, polarographic, CPG, TLC, HPLC, voltammetric, derivative
spectrophotometry, flow injection analysis, official methods and spectrophotometry. Most of
the spectrophotometric methods reported suffer from the disadvantage, like narrow range of
determination, requires heating or extraction, long time for the reaction to complete, use of
non-aqueous systems, stability of the coloured product formed, etc.

This paper describes sensitive and simple spectrophotometric method for the
determination of metronidazole in either pure form or in its pharmaceuticals formulations.
The method is based on the reduction of metronidazole molecule with zinc dust and
hydrochloric acid flowed by diazotation and coupling with 8-quinolinol.

The scientific novelty of the present work is that the reagents used in both the method
is easily available and the chemistry of these reagents is already well established. The
reactions involved with these reagents are simple, rapid and sensitive in their range of
determination compared with other established methods. As Metronidazole is important class
of imidazole compounds known for their antiamebic and antiprotozoal activity, this
determination in pharmaceutical is of great importance.
2- MATERIAL AND METHODS

2-1 Instrumentation

A Perkin-Elmer 551 UV-Visible spectrophotometer was used.

2-2 Reagents

All chemicals used were of analytical-reagent grade. 8-quinolinol was purchased from . sodium nitrite was purchased from . Metronidazole was obtained as gifts from Aventis Pharma. All other reagents and solvents were of analytical-reagent grade.

2-3 Solutions

Accurately weighed (100 mg) Metronidazole was transferred to a 100 ml beaker. Add 1g of zinc dust along with 20ml 1M hydrochloric acid. Stir well and wait for 1h at room temperature, filter and the filtrate was diluted with ethanol to 100ml in a volumetric flask. The working standard solution of the reduced Metronidazole containing 100µg ml\(^{-1}\) was prepared by further dilution. A 1% 8-quinolinol solution in 1M HCl and a 10% solution of hydroxide de sodium were kept in amber-glass volumetric flasks. A 1% sodium nitrite solution and a 5% ammonium sulfamate solution were prepared separately in distilled water.

2.4 Procedure

aliquots of the working standard solution of reduced Metronidazole were transferred into 25 ml calibrated flasks. 1ml of 1M HCl was added, cool in an ice bath and add 1ml of 1% NaNO\(_2\), stir the solution for 2 min. Add 2ml of 5% ammonium sulfamate, stir the solution for 3 min and add 1 ml of 1% of 8-quinolinol. After 2min add 2ml of 10% of NaOH and made up to the mark with ethanol.

2.5 Assay of pharmaceutical tablets

twelve tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the drug was reduced as mentioned in and the filtrate was made up to 100ml and an aliquot of this solution was treated as described above for pure sample in both the method.

3- RESULTS AND DISCUSSION

The spectrophotometric method for the determination of Metronidazole is based on the reduction of the nitro to an amino group with zinc dust and hydrochloric acid flowed by diazotation and coupling with 8-quinolinol to give colored product.

3-1 Spectral characteristics and reaction mechanism

the absorption spectra of the coloured product with \(\lambda_{\text{max}} = 437\) nm is shown in. The reagent blank has practically negligible absorption at this wavelength. The stochiometric equation derived was shown in scheme 1.
scheme 1: Reaction sequence for the formation of azo colored product

3.2 Optimization of reactions conditions

the factors affecting color development, reproducibility, sensitivity, and conformity with Beer’s law were investigated.

It was found that, 1-3 ml of 1M HCl, 1-4 ml of 1% NaNO$_2$ solution, 2-5 ml of 5% ammonium sulfamate, 1-3 ml of 1% 8-quinolinol and 1-3 ml of 10% NaOH solution were necessary to achieve maximum colour intensity.

The excess of nitrite sodium could be removed by the addition of 2ml of 5% ammonium sulfamate solution. An excess of ammonium sulfamate has no effect on the colour intensity of the product formed.

3.3 Quantification

Beer’s law is obeyed over the Metronidazole concentration range of 1-10 µg / ml. The proposed procedure is validated by determining various optical parametrs, which are listed in.
Table 1: parameters for the spectrophotometric determination of Metronidazole

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>437</td>
</tr>
<tr>
<td>Beer’s law range ($\mu$g ml$^{-1}$)</td>
<td>1-10</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$cm$^{-1}$)</td>
<td>$2.15 \times 10^4$</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.111</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.037</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.997</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>1.22</td>
</tr>
</tbody>
</table>

a. $y = ax + b$ where $x$ is the concentration of Metronidazole

3.4 analysis of pharmaceutical preparation.

Application of the proposed method to the determination of Metronidazole drug in its dosage forms was successfully made; the results are presented in table 2. The excellent recoveries obtained indicated the absence of any interference from the excipients.

Table 2: Analysis of Metronidazole in pharmaceutical preparation

<table>
<thead>
<tr>
<th>Commercial Formulations analyzed</th>
<th>Label claim in mg</th>
<th>Recovery$^a$, %($\pm$ RSD$^b$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagyl® 250</td>
<td>250/tablet</td>
<td>99.5 ($\pm$ 1.3)</td>
</tr>
<tr>
<td>Flagyl® 500</td>
<td>500/tablet</td>
<td>98.2($\pm$ 2.5)</td>
</tr>
<tr>
<td>Nidazol® 500</td>
<td>500/tablet</td>
<td>101.3($\pm$1.8)</td>
</tr>
</tbody>
</table>

a. Average of 5 determination. b. Relative standard deviation.

4- CONCLUSION

The method is found to be simple, economical, selective and more sensitive than most of the spectrophotometric methods reported. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing Metronidazole showed no interference from the common excipients. Hence, this approach could be considered for the determination of Metronidazole in the quality control laboratories.

5- REFERENCES