Stability and In Vitro Dissolution Studies of Metronidazole Tablets and Infusions

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ABSTRACT
The aim of this study was to compare metronidazole tablets (500 mg) and infusions (500 mg/100 mL) obtained from Saudi and Egyptian suppliers. Evaluation of the tablets included weight variation, hardness, friability, drug content, disintegration time, and dissolution profiles. Stability of the tablets in their original packages after 3-month storage at various temperatures (75% relative humidity) was evaluated, and degradation kinetics was determined. The infusion solutions underwent accelerated stability testing. The Q10 method was used to estimate the shelf life of metronidazole infusions at room temperatures (75% relative humidity) of various climates. Results revealed that all tablets complied with USP specifications, and degradation was slowest at room temperature (20 °C). The mechanism of drug release for all tablets at all temperatures conformed to the Korsmeyer–Peppas model. Metronidazole intravenous infusion solutions stored at 40 °C or 50 °C for 90 days exhibited good stability. The studied Saudi infusion brand is more stable than Egyptian one. The metronidazole tablets and infusion solutions complied with USP specifications and showed similar results in quality control testing. Standard quality control measures should be maintained to ensure safety and efficacy of drug products, especially in climates with extreme temperatures.

KEYWORDS: Metronidazole; Riazole; Amrizole; stability study; expiry date; infusion; quality control; dissolution.

INTRODUCTION
Metronidazole is classified as an antiamoebic, anti-amoebicidal, antiprotozoal, and antibacterial drug in the World Health Organization’s Essential Medicines List (1). Metronidazole is commonly used in combination with other antibiotics and either bismuth compounds or proton pump inhibitors to treat peptic ulcer disease caused by Helicobacter pylori. Metronidazole is also used to treat periodontal disease caused by Gardnerella vaginalis and infections caused by anaerobic bacteria including intra-abdominal infections, skin and skin structure infections, gynecological infections, bacterial septicemia, bone and joint infections, central nervous system infections, lower respiratory tract infections, and endocarditis (2–4). Metronidazole is rapidly absorbed, with a bioavailability approaching 100% (5, 6). The solubility of metronidazole in water at room temperature is 64.8 mg/mL at pH 1.2 and approximately 10 mg/mL at pH 2.5–8.0 (7).

The stability of a drug is a primary concern in climates with extreme temperatures. Previous studies (8, 9) have reported the stability and other quality control parameters of various brands of metronidazole tablets. In a study testing metronidazole tablets marketed in Zaria, Nigeria, only 60% of the tested metronidazole drugs passed the quality control test (10, 11). Another study testing the bioavailability of 10 brands of metronidazole tablets from different manufacturers reported wide variations in hardness, weight uniformity, friability, disintegration time, absolute drug content, and dissolution efficiency. Only some of the brands showed acceptable tablet characteristics. These results are clinically important because drugs are expected to conform to label claims and exhibit satisfactory bioavailability (12–14).

In this study, we evaluated the effect of temperature on the stability of two brands of metronidazole tablets and intravenous infusion solutions manufactured in Saudi Arabia and Egypt.

METHODS AND MATERIALS
Materials
Metronidazole tablets (Riazole 500 mg and Amrizole 500 mg) were kindly provided by Riyadh Pharma (Riyadh, Saudi Arabia) and Amriya Pharmaceutical Industries (Alexandria, Egypt), respectively. Metronidazole intravenous infusion solutions (500 mg/100 mL; sterile, nonpyrogenic) were kindly provided by Pharmaceutical...
Solution Industry (Jeddah, Saudi Arabia) and Amriya Pharmaceutical Industries (Alexandria, Egypt). Distilled water was used to reconstitute metronidazole.

Storage of Metronidazole Tablets at Different Temperatures
All of the tested samples were within their shelf life and expiry dates. The tablets were stored at different temperatures (4, 20, and 40 °C) at 75% relative humidity (RH) for 3 months and then tested as follows.

Dissolution Test
Dissolution testing was conducted according to USP 32 (15). Dissolution rates of metronidazole tablets were determined in 900 mL of HCl (0.1 M) at 37 ± 0.5 °C using an Erweka dissolution apparatus (Automated Dissolution Testing Apparatus Type I, Germany) at a rotational speed of 100 rpm. Triplicate samples (5 mL) were removed at 5-min intervals over 60 min and replaced immediately with an equal volume of 0.1 M HCl to maintain sink conditions. Three experimental runs were performed, and the results were averaged. The amount of metronidazole that dissolved over 60 min was determined by measuring absorbance at 277 nm using a UV–vis spectrophotometer (Spectro UV–VIS Auto Scanning UV–2602).

Hardness Test (Crushing Strength)
Tablet hardness was evaluated using an Erweka hardness tester. The average force required to break 10 randomly selected tablets of each metronidazole brand was calculated. USP 32 states that a crushing strength of 4–8 kg is acceptable for uncoated, non-sustained-release tablets.

Disintegration Test
Six tablets from each metronidazole batch were placed in the six tubes of the apparatus and immersed in distilled water at 37 ± 0.5 °C. The time required for all six tablets to break into particles and pass into the disintegration medium was recorded. As specified by USP 32, if one tablet failed to disintegrate within 30 min, the disintegration test was repeated on 12 additional tablets. Sixteen of the 18 tablets tested disintegrated completely within 30 min.

Drug Content Test
All samples examined were within their shelf life and expiry dates. After storage at 4 °C (refrigerator), 20 °C (room temperature), or 40 °C (oven) at 75% RH for 1 week, 1 month, 2 months, or 3 months, the tablets were subjected to a drug content test (n = 3 for each condition). One tablet was transferred to a 250-mL volumetric flask with 100 mL diluted hydrochloric acid (1:100) and shaken for 30 min, according to USP 32 specifications. The filtrate (100 mL) was transferred to a 100-mL volumetric flask, diluted to volume with 0.1 M HCl pH 1.2 (1:100), and mixed to obtain a solution that was approximately 0.2 mg/mL. The absorbance of this test solution and a standard solution of USP Metronidazole RS (approx. 20 µg/mL) in a 1-cm matched cell was determined concomitantly at 277 nm using a UV–vis spectrophotometer. Diluted hydrochloric acid (1:100) was used as a blank, and the mean of at least three absorbance readings was calculated. The quantity of metronidazole (mg) in the tablets was calculated using the following equation:

\[
\frac{(TC/D)(A_u/A_s)}{A} = \frac{1}{k} - \left(A - \frac{E_a}{2.303 RT}ight) \left(\log A - \frac{E_a}{2.303 RT}\right)
\]

where \( T \) is the label quantity (mg), \( C \) is the concentration (µg/mL) of USP Metronidazole RS in the standard solution, \( D \) is the concentration (µg/mL) of the test solution, \( A_u \) is the absorbance of the test solution, and \( A_s \) is the absorbance of the standard solution. Acceptable values are 90–110% of the labeled amount.

Stability of Metronidazole Intravenous Infusion Solutions
The stability of the metronidazole intravenous infusion solutions was evaluated under accelerated storage conditions of 40 °C and 50 °C (75% RH) for 7, 30, 60, and 90 days. The shelf life under recommended storage conditions may be estimated by storing the product under extreme conditions (e.g., temperature, humidity, light, and radiation). Temperature is the most common acceleration factor for chemical, pharmaceutical, and biological products because its relationship with the degradation rate is characterized by the Arrhenius equation (eq 2).

Shelf life was estimated using the \( Q_{10} \) method, where \( Q_{10} \) is the factor by which the rate constant increases for a 10 °C rise in temperature. The absorbance of the samples in 0.1 N HCl was determined at 277 nm using a UV–vis spectrophotometer. Equation 17 describes the relationship between temperature and degradation rate, where \( A \) is absorbance, \( R \) is the rate, and \( T \) is temperature (°C).

\[
k = A e^{\left(-\frac{E_a}{RT}\right)}
\]

where \( A \) is the pre-exponential factor, \( E_a \) is activation energy, \( R \) is the universal gas constant, and \( T \) is temperature in degrees Kelvin.

\[
\log k = \log A - \frac{E_a}{2.303 RT}
\]
For $k_1$ and $k_2$, we can rearrange in the following manner:

$$\log k_2/k_1 = -\mathit{Ea}/2.303 \mathit{R} \left( 1/T_2 - 1/T_1 \right) \quad (4)$$

or

$$\log k_2/k_1 = \mathit{Ea} \left( T_2 - T_1 \right) / 2.303 \mathit{R} T_2 T_1 \quad (5)$$

$$Q_{10} = e^{[-\mathit{Ea}/(\mathit{R} (1/10 - 1/T))] \quad (6)$$

or

$$Q_{10} = e^{[\mathit{Ea} \cdot 1/10 \mathit{R} (1/10 + 1/T)] \quad (7)$$

For an arbitrary temperature change $\Delta T$,

$$Q_{\Delta T} = e^{[\mathit{Ea} \cdot \Delta T/R (T + \Delta T) \ T]} \quad (8)$$

Multiplying the exponential term by $10(T+10)/10(T+10)$, gives the following:

$$Q_{\Delta T} = e^{[\mathit{Ea} \cdot \Delta T/R (T + 10) \ T]} \quad (9)$$

or

$$Q_{\Delta T} = e^{[\mathit{Ea} \cdot \Delta T/R (T + 10) \ T \times \Delta T / (T + 10) \ T]} \quad (10)$$

or

$$Q_{\Delta T} = e^{[\mathit{Ea} \cdot \Delta T/R (T + 10) \ T \ \Delta T / (T + 10) \ T]} \quad (11)$$

or

$$Q_{\Delta T} = Q_{10} \left( T + 10 / (T + \Delta T) \right] \quad (12)$$

$$T = 300 \mathit{K}, (T + 10) \ T \equiv 1$$

(For a 10–20 °C interval, it is almost equal to 1.)

Therefore,

$$Q_{\Delta T} = k_T \Delta T \ / k_T = Q_{10} \Delta T/10 \quad (13)$$

In this way, for a 10–20 °C interval,

$$Q_{\Delta T} = Q_{10} \Delta T/10$$

To evaluate the effect of temperature on shelf life, we can correlate the $Q_{\Delta T}$ value with shelf life. Degradation reactions generally follow zero-order, first-order, or pseudo-first-order kinetics. The shelf life in these cases is:

$$t_{90} = 0.1 \left[ D_0 \right] / k_0 \quad (14)$$

where the degradation process is zero-order, and $D_0$ is the initial concentration,

$$t_{90} = 0.105/k_1 \quad (15)$$

where the reaction is a first-order process and $0.1$, $D_0$, and $0.105$ are the constants.

Therefore, shelf life can be written as $t_{90} = a / kT$.

For $T_1$ and $T_2$,

$$t_{90} (T_1) = a / kT_1 \text{ and } t_{90} (T_2) = a / kT_2 \quad (16)$$

Since, $T_2 = T_1 + \Delta T$,

$$t_{90} (T_2) = a / k (T_1 + \Delta T) \quad (17)$$

Using $k \left( T + \Delta T \right) / k_T = Q_{10} \Delta T/10$ of eq 16 in the above equation, we get

$$t_{90} (T_2) = a / \left( kT_1, Q_{10} \Delta T/10 \right)$$

Because $t_{90} (T_1) = a / kT_1$:

$$t_{90} (T_2) = t_{90} (T_1)/Q_{10} \Delta T/10 \quad (18)$$

The $Q_{10}$ equation (eq 17) is independent of the order of reaction. $Q_{10}$ has low, average, and high estimate values (2, 3, and 4, respectively). A zero- or first-order kinetics reaction occurs at elevated temperatures and at the recommended storage temperature. The same model was used to fit the degradation patterns at each temperature.

Kinetics Study

The in vitro release mechanisms of the drug in tablets and infusions were evaluated using four kinetic models (Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Model name</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zero order</td>
<td>$Q_t = Q_0 + k_0 t$</td>
</tr>
<tr>
<td>2</td>
<td>First order</td>
<td>$\log Q_t = \log Q_0 + k_1 t /2.303$</td>
</tr>
<tr>
<td>3</td>
<td>Higuchi</td>
<td>$Q = k_T t^{1/2}$</td>
</tr>
<tr>
<td>4</td>
<td>Korsmeyer–Peppas</td>
<td>$M_t / M_\infty = k^n$</td>
</tr>
</tbody>
</table>

Model parameters were obtained by linear regression. $Q$ and $M$ denote fraction of drug released up to time $t$. $K$ is the rate constant, and $n$ is the release exponent.

Statistical Analysis

Stability of the intravenous infusion solutions over time was analyzed using regression analysis.

RESULTS AND DISCUSSION

Characterization of Metronidazole Tablets

Figure 1 shows dissolution profiles of the 500-mg metronidazole tablets, demonstrating that dissolution was within USP limits (i.e., not less than 85% of the labeled amount of metronidazole dissolved in 60 min). However, dissolution fluctuated in tablets that were stored at 40 °C, because the dissolution medium pH was 1.5.

Table 2 shows the remaining drug (log percent) over time in tablets stored at 4 °C, 20 °C, or 40 °C (75% RH) for 3 months. The highest concentration of metronidazole in the tested tablets was 482 mg per tablet after storage at 20 °C for 7 days, and the lowest concentration was 339 mg per tablet after storage at 40 °C for 90 days. The drug content was highest when the tablets were stored...
at room temperature. As shown in Table 3, the hardness values of the tablets exceeded 4–8 kg, meeting USP 32 specifications. Tablets stored at 4 °C had the highest hardness values (>14 kg). Disintegration times were longest for tablets stored at 4 °C (4.6 and 3.7 min for Amrizole and Riazole 500 mg, respectively) and shortest for tablets stored at 40 °C (2.5 and 2.7 min for Amrizole and Riazole 500 mg, respectively).

Kinetic Modeling of Metronidazole Release from Tablets

We tested zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetics models of metronidazole release from tablets using dissolution data and release data. The best-fit model with the highest correlation coefficient ($R^2$) was the Korsmeyer–Peppas model. The $n$ values for all metronidazole tablets (stored at 4 °C, 20 °C, or 40 °C for...
3 months) were less than 0.45, which indicates that the drug release mechanism was Fickian diffusion (i.e., net flux from regions of high concentration to regions of low concentration).

In the present study, dissolution tests of Riazole and Amrizole tablets met USP requirements at all storage temperatures (4 °C, 20 °C, and 40 °C, 75% RH) with few differences, indicating that metronidazole tablets are stable over a wide temperature range. However, the ideal storage temperature is 20 °C, and the tablets should not be stored in the refrigerator. These results are in accordance with the results of previous studies (9, 16).

Degradation Kinetics of Metronidazole Infusion Solution
In addition, the stability of Egyptian and Saudi metronidazole infusions (500 mg/100 mL) was studied at 37 and 50 °C (75% RH) over 7, 30, 60, and 90 days. The linear relationship between the logarithm of percent drug remaining and time indicates that degradation of metronidazole infusion solution follows pseudo-first-order kinetics (Figure 2).

Effect of Temperature on Shelf Life of Metronidazole Intravenous Infusion Solution
The effect of higher temperatures on the degradation of the two metronidazole intravenous infusion solutions was evaluated; degradation was more rapid at 50 °C than at 40 °C (Table 4). The shelf life at 40 °C was estimated at 1.9 years based on the rate constant at this temperature from eq 14, and the shelf life at 25 °C was calculated using eq 17. There are three estimates for shelf life at 25 °C because Q10 has three values to represent low, average, and high estimates. However, the activation energy indicated a lower estimate value. Using the Q10 value of 2 in eq 17, expiry dates for the Saudi brand (usually 6 months shorter than shelf life) were estimated at 4.15 years at 25 °C, 5.8 years at 20 °C, and 2.4 years at 33 °C, while the Egyptian brand showed different expiry dates that were 3.4 years at 25 °C, 4.8 years at 20 °C, and 1.9 years at 33 °C, where these represent room temperatures in the Asian subcontinent, winter zone, and tropical zone, respectively. Metronidazole intravenous infusion solutions stored at 40 °C or 50 °C over 90 days exhibited good stability. The rate constant values (Table 4) were used in modified Arrhenius equations to identify activation energy ($E_a$), which was 8.7 kcal/mole for this preparation throughout the accelerated stability studies. The infusion solution was clear, and the pH was within the USP range at 40 °C (6.11, 6.10, 6.11, 6.12, and 6.13) and 50 °C (6.11, 6.10, 6.08, 6.09, and 6.1) for 0, 7, 30, 60, and 90 days, respectively.

Metronidazole intravenous infusion solution (500 mg/100 mL) exhibited good stability when stored at 40 °C or 50 °C (75% RH) for up to 90 days. Degradation followed pseudo-first-order kinetics. A previous study (11) reported degradation rate constants of $14.1604 \times 10^{-5}$ and $30.1 \times 10^{-5}$ day$^{-1}$ for 40 °C and 50 °C, respectively.

CONCLUSION
The four commercially available metronidazole products manufactured in the Middle East were physically and chemically equivalent with few exceptions. The quality control methods used in this study are useful for monitoring batch-to-batch consistency of drug release. The therapeutic equivalence of the drugs must also be investigated by comparing their dissolution profiles.

ACKNOWLEDGMENTS
The authors thank Riyadh Pharma for their generous gift of metronidazole powder and tablets. The authors are grateful to the Research Centre of Science and Medical Studies Departments at King Saud University for their financial support.

CONFLICT OF INTEREST
The authors report no conflict of interest.
Table 4. Results of Accelerated Stability Tests and Calculated Half-Life of Metronidazole Intravenous Infusion Solution (100 mL) Stored at 40 °C and 50 °C

<table>
<thead>
<tr>
<th>Time</th>
<th>Drug % mean</th>
<th>Conc. Mean</th>
<th>Abs. Mean</th>
<th>Drug % mean</th>
<th>Conc. mean</th>
<th>Abs. Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 °C</td>
<td></td>
<td></td>
<td>40 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>S</td>
<td>0.674</td>
<td>500</td>
<td>100</td>
<td>0.674</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.674</td>
<td>500</td>
<td>100</td>
<td>0.674</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>S</td>
<td>0.629</td>
<td>466.864</td>
<td>93.372</td>
<td>0.6193</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>0.642</td>
<td>476.261</td>
<td>95.252</td>
<td>0.628</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>S</td>
<td>0.620</td>
<td>460.435</td>
<td>92.087</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>0.639</td>
<td>474.035</td>
<td>94.807</td>
<td>0.621</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>S</td>
<td>0.612</td>
<td>454.500</td>
<td>90.901</td>
<td>0.588</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>0.631</td>
<td>468.101</td>
<td>93.620</td>
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<tr>
<td></td>
<td>90 days</td>
<td>S</td>
<td>0.606</td>
<td>449.555</td>
<td>89.911</td>
<td>0.580</td>
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<tr>
<td></td>
<td></td>
<td>E</td>
<td>0.624</td>
<td>462.908</td>
<td>92.581</td>
<td>0.602</td>
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<tr>
<td></td>
<td>50 °C</td>
<td>K</td>
<td>19.5 × 10⁻⁵ days⁻¹</td>
<td>34.5 × 10⁻⁵ days⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>28.2 × 10⁻⁶ days⁻¹</td>
<td>41.23 × 10⁻⁶ days⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 °C</td>
<td></td>
<td>1.0203 y</td>
<td>0.833 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.47365 y</td>
<td>0.697 y</td>
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<td></td>
</tr>
</tbody>
</table>

S = Riazole 500-mg tablet (manufactured in Saudi Arabia).
E = Amrizole 500-mg tablet (manufactured in Egypt).

REFERENCES