



## Original Article

## Accelerated Stability Study of Metronidazole Infusion 100 ml

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## Abstract

Metronidazole infusion had been prepared and studied under acceleration by heat at 37 and 50 °C temperatures. Physical and chemical evaluation had been carried out on 4<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> day, respectively. Chemical assay had been performed by UV light absorption spectroscopy. After 3 months stability data were analyzed by statistical regression analysis and plotted against time. It followed pseudo first order degradation kinetics. Rate constants were found out from this plot and set up in Arrhenius equation to find out the energy of activation. The rate constants were  $14.1604 \times 10^{-5}$  and  $30.1 \times 10^{-5} \text{ days}^{-1}$  for 37 and 50 °C, respectively. According to  $Q_{10}$  method this value was used to determine the exact  $Q_{10}$  factor, which indicated its lower estimate value. Taking this lower value the expire dates were predicted for 5.3 yrs, 3.8 yrs, and 2.2 yrs, at 20 °C, 25 °C and 33 °C, respectively. These temperatures were the room temperatures of winter zone, Asian subcontinent and tropical zone, respectively.

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## Introduction

In the past before a product is marketed it has been the practice to assess the stability by placing it on storage test. This test is time consuming and expensive. Hence accelerated stability technique was devised during the product development stage that will enable rapid prediction of the long term stability of a product within short period of time<sup>1</sup>.

This prediction of shelf life may be made by acceleration the decomposition process by temperature, humidity, light and radiation. In most cases acceleration of chemical decomposition can be achieved by raising the temperature of the preparation. Using  $Q_{10}$  method, shelf life might be almost predicted. It is an approximated method to predict the shelf life of a drug and it needs a few stability data from the acceleration at, at least one or two temperatures. Only in case of first one, the introduction of energy of activation value can

provide the prediction of almost accurate shelf life. The term  $Q_{10}$  is the factor by which the rate constant increases for a 10 °C rise in temperature<sup>2</sup>.

$$Q_{10} = k_{(T+10)}/k_T \quad \dots\dots (1)$$

The  $Q_{10}$  factor can also be calculated from the exponential form of Arrhenius equation derivatives:

$$k = A \cdot \exp(-Ea/RT) \quad \dots\dots\dots(2)$$

Where k is the reaction rate constant of any order, A and Ea are the constants and T is the absolute temperature. R is the gas constant (1.987 cal/mol/°). In simplified form it can be written as:

$$\text{Log } k = \text{log } A - Ea/2.303 RT \quad \dots\dots\dots (3)$$

For  $k_1$  and  $k_2$  respectively, we can rearrange in the following way,

$$\text{Log } k_2/k_1 = -Ea/2.303 R (1/T_2 - 1/T_1) \quad \dots\dots\dots (4)$$

Or

$$\text{Log } k_2/k_1 = Ea (T_2 - T_1) / 2.303 RT_2 T_1 \quad \dots\dots\dots (5)$$

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Now,  $Q_{10} = \exp [-Ea/R (1/T_{+10} - 1/T)] \dots\dots (6)$

Or

$$Q_{10} = \exp [Ea. 10/ R(T+10)T ] \dots\dots\dots (7)$$

For an arbitrary temperature change  $\Delta T$ ,

$$Q_{\Delta T} = \exp [Ea. \Delta T/ R(T+\Delta T)T ] \dots\dots\dots (8)$$

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

$$Q_{\Delta T} = \exp [Ea/R\{ \Delta T. 10(T+10)/ (T+ \Delta T)T. 10(T+10)\}]$$

Or

$$Q_{\Delta T} = \exp [Ea/R \{ 10/(T+10)T \times \Delta T(T+10)/ (T+ \Delta T)10\}]$$

Or

$$Q_{\Delta T} = \exp\{Ea/R \{ 10/(T+10)T\}^{[\Delta T(T+10)/(T+\Delta T)10]}$$

Or

$$Q_{\Delta T} = Q_{10}^{[(\Delta T/10)(T+10)/(T+\Delta T)]}$$

Since  $T \equiv 300K$ ,  $(T+10)/ (T+\Delta T) \equiv 1$  (For 10 to 20 °C interval it is almost equal to 1)

$$\text{So, } Q_{\Delta T} = k_{T+\Delta T} / k_T = Q_{10}^{\Delta T/10} \dots (9)$$

In this way, for 10 to 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 the shelf life. Most of the degradation reactions usually follow either zero order or first order or pseudo first order. The shelf lives in these cases:

$$t_{90} = 0.1 [D_0]/k_0 \dots\dots\dots (10)$$

Where, the degradation process is zero order and  $D_0$  is the initial concentration.

$$t_{90} = 0.105/k_1 \dots\dots\dots (11)$$

Where the reaction is a first order process, and 0.1,  $D_0$ , 0.105 all are respective constants.

So the shelf lives can be written in a general correlating order of reactions,

$$t_{90} = a / k_T$$

For  $T_1$  and  $T_2$ ,

$$t_{90(T1)} = a / k_{T1} \text{ and } t_{90(T2)} = a / k_{T2}$$

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

$$t_{90(T2)} = a / k_{(T1+\Delta T)} \dots\dots (12)$$

Using  $k_{(T+\Delta T)} / k_T = Q_{10}^{\Delta T/10}$  of equation (9) in above equation, we get:

$$t_{90(T2)} = a / (k_{T1} \cdot Q_{10}^{\Delta T/10})$$

Since,  $t_{90(T1)} = a / k_{T1}$  So we can write,

$$t_{90(T2)} = t_{90(T1)} / Q_{10}^{\Delta T/10} \dots\dots(13)$$

This equation (13) is known as  $Q_{10}$  equation and independent of order of reaction.  $Q_{10}$  has got three values: 2, 3 and 4 known as low, average and high estimate values respectively<sup>2</sup>.

Metronidazole (2-methyl-5 nitroimidazole-1 ethanol) is a synthetic antibacterial agent that is used primarily in the treatment of various anaerobic infections such as intra abdominal infections, skin and skin structure infections, gynecologic infections, central nervous system infections, bacterial septicemia, bone and joint infections, lower respiratory tract infection and endocarditis<sup>3</sup>. Metronidazole was reported to undergo hydrolysis in aqueous media due to the presence of photolytically generated hydroxyl radicals<sup>4</sup>. Light irradiation has more effect on the degradation of metronidazole in solution than that of with sonic energy<sup>5</sup>. The Arrhenius plot showing the temperature dependence of metronidazole degradation indicated the estimation of the activation energy of 15.3 Kcal/mole and a half life of 963 h at room temperature in 0.1 M acetate buffer, pH 3.1 with ionic strength 0.5<sup>6</sup>. In line to the connection of this study it seems to be important for further research to find out the degradation kinetics and shelf life of this drug at various room temperatures. Here we utilized  $Q_{10}$  method for the evaluation of shelf life of metronidazole in infusion for different room temperatures (20, 25 and 33 °C), where 20, 25 and 30 °C are the room temperatures of winter zone, Asia subcontinent and tropical zone, respectively.

## Materials and Methods

Metronidazole, citric acid, sodium phosphate dehydrate, sodium chloride, water for injection and other apparatus available in sterile section; 0.1 N NaCl and spectrophotometer. All the raw materials were of commercial grade.

Raw materials including metronidazole were checked and weighed according to manufacturing formula (Table-1). 2/3 portion of WFI (water for injection) of total volume was taken in a master vat. Metronidazole was added in it. Stirring has to be continued until clear solution was obtained. Sodium chloride was dissolved in another vat and added to the master vat. Citric acid and sodium phosphates were dissolved in a beaker and added to the master vat with stirring. Finally, the volume was adjusted upto the mark. pH of the adjusted finished product was checked by a pH meter. The change in pH of the product throughout the stability studies was within USP range, 4.5 to 7 (Table-III). After chemical analysis, filtration, filling the solution in 100 ml pre-sterilized vial and sealing were performed perfectly. Then the sealed vials were allowed to sterilize by autoclaving at 15 lbs. Pressure and 121 °C. Solution was analyzed by UV light absorption spectrophotometer (shimadzu 160) at 277 nm, using 0.1 N HCl solution on 4<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> days of the storage.

## Results

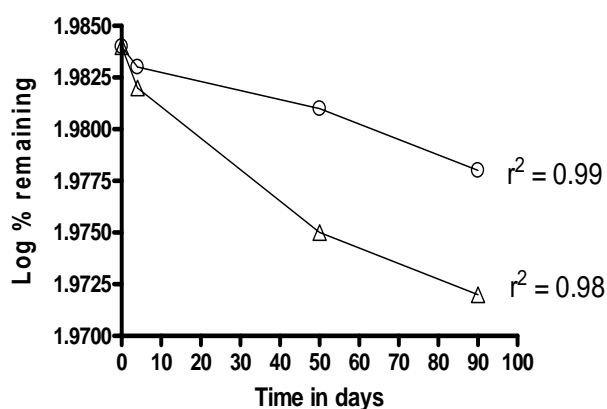
Representative sets of stability profiles for metronidazole infusion at different storage temperature (37 and 50 °C) were showed in Table II. Mean values were taken for % remaining with a coefficient of variation range, 0.001-0.003.

In figure 1, there was a linear relationship when log percent remaining was plotted against storage time. The degradation rate constants were

determined from the slope values of the plots by statistical regression analysis<sup>7</sup>. These were  $14.1604 \times 10^{-5} \text{ days}^{-1}$  and  $30.1 \times 10^{-5} \text{ days}^{-1}$  for 37 and 50 °C, respectively. The correlation coefficient values of the regression lines were -0.989 and -0.979, respectively.

**Table I.** Manufacturing formula of metronidazole infusion

| Composition  | Qnt/100 ml<br>in mg | Qnt/Batch<br>in gm |
|--|---------------------|--------------------|
| Metronidazole BP<br>(pyrogen free)                 | 500.00              | 25.226             |
| Sodium Phosphate<br>dehydrate BP<br>(Pyrogen free) | 150.00              | 7.5                |
| Citric acid BP<br>(Pyrogen free)                   | 42                  | 2.1                |
| Sodium Chloride BP<br>(Pyrogen free)               | 750                 | 37.5               |
| WFI BP   | qs to 100 ml        | qs to 5 L          |



**Fig. 1:** Pseudo first order degradation kinetics of metronidazole in infusion

**Table II.** Statistical data of accelerated stability test of metronidazole infusion 100 ml

| Time<br>(days) | Data for 37 °C storage |             | Data for 50 °C storage |            |
|----------------|------------------------|-------------|------------------------|------------|
|                | % Remaining            | Mean ± SD   | % Remaining            | Mean ± SD  |
| 0              | 96.5                   | 96.5        | 96.5                   | 96.5       |
| 4              | 96.38, 95.84, 96.12    | 96.11±0.27  | 96.12, 95.84, 96.08    | 96.01±0.15 |
| 50             | 95.84, 95.84, 95.84    | 95.84       | 94.75, 94.48, 95.02    | 94.75±0.27 |
| 90             | 95, 95.29, 94.76       | 95.01± 0.26 | 93.64, 93.37, 93.64    | 93.55±0.15 |

The temperature dependence of metronidazole degradation at 37 °C and 50°C in infusion was studied. Both the rate constant values were set up in modified Arrhenious equation (5) to find out the

**Table III.** Data of physical parameters throughout the study

| Time (days) | 37 °C storage                      |      | 50 °C storage                      |      |
|-------------|------------------------------------|------|------------------------------------|------|
|             | Physical Appearance (Transparency) | pH   | Physical Appearance (Transparency) | pH   |
| 0           | +++                                | 6.11 | +++                                | 6.11 |
| 4           | +++                                | 6.08 | +++                                | 6.12 |
| 50          | +++                                | 6.08 | +++                                | 6.06 |
| 90          | +++                                | 6.14 | +++                                | 6.10 |

## Discussion

### *Degradation Kinetics*

The linear relationship between logarithmic drug percent remaining and time indicates the degradation process is a pseudo first order kinetic. The slope value should provide the rate constant value as well as the half life of the drug at the respective temperatures.

### *Thermal effect and shelf life studies*

Due to increased temperature thermal effect markedly influenced the energy of activation to a lower value than that of room temperature<sup>5</sup>, which triggers the degradation process. Accordingly the shelf life at 37 °C was 1.9 yrs taking the rate constant of this temperature in equation (11). Using the  $Q_{10}$  equation in equation (13) the shelf life for 25 °C was calculated using the shelf life 1.9 yrs. Since  $Q_{10}$  has got three values as lower, average and higher estimate values, so at 25 °C there are three values for the shelf life. But the activation energy indicated only to lower estimate value<sup>8</sup> for all these three room temperatures.

Therefore taking  $Q_{10}$  value 2 in equation (13), the expiry dates (usually 6 months less than shelf life) were predicted for 3.8 yrs at 25°C, 5.3 yrs at 20 °C and 2.2 yrs at 33 °C, where these room temperatures represent the room temperatures of Asian subcontinent, winter zone and tropical zone respectively. Throughout the accelerated stability studies the physical appearance of the infusion was clear and pH was within the USP range.

## Conclusion

In order to find out the shelf lives of metronidazole infusion at various room temperatures, accelerated stability studies was performed at 37 and 50°C for a period of 90 days. The degradation kinetics was a pseudo first order kinetics and taking the rate

$E_a$  (Energy of activation) value. It was 11.63 kcal /mole for this preparation.

The pH range was 4.5 to 7 throughout the accelerated stability studies (Table III).

constant value  $Q_{10}$  method was explored to find out the shelf lives of the respective room temperatures. Using  $Q_{10}$  equation, the expiry dates of the metronidazole infusion were predicted 3.8 yrs, 5.3 yrs and 2.2 yrs for 25, 20 and 33 °C room temperatures.  $Q_{10}$  method has been a milestone in predicting the shelf life of the drug substances in solution, where the degradation process is a temperature sensitive one.

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