ABSTRACT

Stability testing confirms the safety and quality of an active pharmaceutical ingredient or product. The shelf life of *Picralima nitida* (herbal drug) and glibenclamide were evaluated using the bio-based dose-response relationship method by the use of animal model based on their pharmacological activity. Glibenclamide was used as the comparative drug for the assessment of the specifications for *Picralima nitida*. Their shelf life was estimated by means of accelerated stability testing on the basis of the first-order kinetics of degradation and the time required to degrade 10% of a drug at 27°C (*t*_{10%}). The influence of storage time (1, 2, 3 and 4 weeks) and temperature (45, 60, and 70 °C) on the stability of the drug samples were studied. Their half-life (*t*_{1/2}) and the toxicity level (LD_{50}) were also estimated. The concentrations of the drugs were found to decline with an increase in storage time and temperature. The shelf life of glibenclamide and *Picralima nitida* were found to be 10.54 and 3.15 weeks, respectively; the half-life of glibenclamide
and *Picralima nitida* were found to be 70 and 20.94 weeks, respectively. Their pharmacological activity varied due to the pharmacokinetic profile of the animal models. Also, *Picralima nitida* extract was found to be practically nontoxic on the tested animals (LD<sub>50</sub> = 14.97 g/kg). From the study, it was observed that glibenclamide (used as a comparative drug) aided in the estimation of the capacity of *Picralima nitida* to retain its specification (quality and safety) for treatment under the influence of environmental conditions.

**Keywords:** Accelerated stability study; Degradation kinetics; Glibenclamide; *Picralima nitida*; Shelf life estimation; bio-based dose-response relationship method; Pharmacological activity; Hypoglycemic effect.

### 1. INTRODUCTION

The purpose of stability testing is to provide the safety and quality of an active pharmaceutical ingredient (API) or finished product [1]. It also provides information on (i.) the variation of a drug under the influence of environmental factors including temperature, humidity, and light, (ii.) its capability to retain its physical, chemical, microbiological, toxicological, protective and informational specifications [1]. Stability studies also guarantee the identity, potency, and purity of the active ingredients, as well as the formulated products for regulatory approval [2,3].

Stability testing involves complex processes due to varieties of influencing factors such as the interaction between the active ingredients and excipients, manufacturing process, type of dosage form, container/closure system used for packaging, environmental conditions encountered during shipment, storage and handling. Chemical degradation reactions like oxidation, reduction, hydrolysis or racemization play a vital role in the stability of a pharmaceutical product. Chemical degradation depends on the concentration of the reactants, pH of the drug substance, rate of radiation, type of catalysts, type of raw materials used and the length of time between manufacture and consumption of the product [4]. A pharmaceutical product may undergo physical changes (appearance, consistency, uniformity, clarity of the solution, moisture content, particle size, shape, and package integrity) due to the impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing, and shearing. Microbiological changes such as the growth of microorganisms in non-sterile products and changes in the efficacy of the preservative used can also affect the stability of a pharmaceutical product [5].

Herbal medications generally contain many constituents with low concentration of the active constituents. Stability testing of a herbal drug is very challenging due to (i.) the entire herb is regarded as the active material, irrespective of the defined therapeutic activity of the constituents [6], (ii.) the climate condition, time of harvesting and biological variance. The consumption of natural drugs is important owing to their economic significance and ease of access with regards to the declining efficacy of synthetic drugs including the cost, convenience and increasing contradiction [7,8].

*Picralima nitida* extracts have been stated to be effective for the treatment of several ailments [2]. Glibenclamide {5-chloro-N-[4-(cyclohexyl carbamoyl) sulfamoyl]-2-methoxybenzamide}, also known as glyburide is an antidiabetic drug [2]. Glibenclamide is amongst the only two oral antidiabetics in the World Health Organization model list of essential medicines [9,10]. Besides, glibenclamide has been indicated to improve the outcome in animal stroke models by inhibiting brain swelling [11].

In our previous study [2], shelf-life of *Picralima nitida*, glibenclamide, ciprofloxacin, and pefloxacin were determined using the UV spectrometry physicochemical standard technique. The shelf life of ciprofloxacin, pefloxacin, and glibenclamide were obtained as 535.18, 298.17 and 134.31 weeks, respectively. Their half-life were also obtained as 3553.85, 1980 and 891.89 weeks, respectively. The shelf life and half-life of *Picralima nitida* could not be evaluated using the UV technique because of the existence of complex metabolites in herbal drugs, which can lead to the irregular rise in absorbance value and instability. This led to the development of a novel method that can be applied to determine the shelf-life and half-life of *Picralima nitida* (the herbal drug). The objective of this study is to evaluate the shelf life of *Picralima nitida* (herbal drug) using the bio-based dose response technique via animal model, which was also compared to that of an orthodox...
material and temperature on their degradation rate was investigated. The acute toxicity (LD$_{50}$) of Picralima nitida was also determined.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of the Drug Samples

Picralima nitida (Apocynaceae) seeds were collected from Ihembo, Anambra state, Nigeria. The pods were washed with clean water and cut to expose the interior part. The seeds were detached from the pulp. The seeds were dried at room temperature for 7 days and the testae were detached manually. The seeds were dried further and crushed. The resulting powder was passed through a 0.25 μm sieve and stored in an airtight container. 200 g of the stored powder was defatted using n-hexane and dried. Methanol of 1 L was added to cover two-thirds of the volume of the container containing the dried defatted seed powder and allowed to stand for 48 h with occasional shaking. The mixture was filtered and the filtrate was allowed to dry at room temperature. The resinous extract obtained was stored in a refrigerator until further usage to avoid further reaction.

Glibenclamide (hypoglycemic drug) tablets BP 5 mg (diatab®), which was produced in 2009 by Mayer and Baker, Nigeria PLC, Ikeja, Lagos State, Nigeria was acquired from a pharmaceutical store in Awka, Anambra State, Nigeria on February 19, 2012.

2.2 Animal

The animals used in this study consists of 5 weeks old Swiss albino mice (18 – 25 g) and Swiss albino rats (120 – 150 g). The animals were housed and acclimatized to the environment of the animal house of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu campus, Nigeria. Prior to the experiments, the animals were abstained from food overnight before measuring their fasting blood glucose level because rodents are nocturnal animals.

2.3 Acute Toxicity Study of Picralima nitida

The study was performed using the Lorke method [12] with a total of 16 Swiss albino mice of weight: 18 - 25 g. This was done in two phases but due to the lack of visible signs of acute toxicity and mortality of Picralima nitida seed extract, the study was then extended to 3 phases. In the first phase, 3 groups of 3 mice each were orally administered 10, 100 and 1000 mg/kg of the seed extract and signs or symptoms of toxicity and mortality were observed for a period of 24 h and weighed daily for 14 days. For the second phase, 3 mice were distributed into 3 groups of an animal each which were orally administered 1600, 2900 and 5000 mg/kg of the seed extract, observed for another 24 h and weighed daily for 14 days. Finally, for the third phase, 4 mice were distributed into 4 groups of an animal each given extremely high doses of 10000, 14000, 16000 and 20000 mg/kg of the seed extract followed by close observation over the period of 24 h and weighed daily for 14 days.

2.4 Bioassay with Animal Model

Standard plots of percentage glycemic change against Log dose for glibenclamide and Picralima nitida were achieved by dividing the rats into groups of stock concentration of the drugs (glibenclamide 2.5 mg/mL in water of doses: 1, 2, 3, 4 mg/kg and Picralima nitida extract 2.5 mg/mL in water of doses: 25, 50, 75, 100, 125, 250 mg/kg). Each group contains 5 rats which were dosed individually according to their calculated weights. Blood samples were withdrawn from their tails and their normal glucose level was measured with the aid of a glucometer (mL/dL) before and after 2 h of drug administration. The mean glycemic change and percentage glycemic change of each group was calculated using Eq. 1:

\[
\% \text{ Percentage glycemic change} = \left( \frac{\text{Blood glucose level after 2 h of oral admin. (mean)} - \text{Normal blood glucose level (mean)}}{\text{Normal blood glucose level (mean)}} \right) \times 100
\] (1)

The stock solutions of the drugs were then subjected to different temperatures (45, 60, and 70 °C) and the samples were collected at one-week interval for one month to obtain the percentage glycemic change in which the Log doses were extrapolated from the regression equations of their standard plots. The corresponding final concentrations were estimated using Eq. 2:
\[
\frac{C_0}{D_0} = \frac{C_1}{D_1}
\]

(2)

Where \( C_0 \) = Initial concentration (mg/mL), \( C_1 \) = final concentration (mg/mL), \( D_0 \) = initial dose (mg/kg), and \( D_1 \) = final dose (mg/kg).

All experiments were carried out in the year 2012.

3. RESULTS AND DISCUSSION

3.1 Estimation of Picralima nitida Lethal Dose for 50% (LD_{50})

No visible sign of toxicity and weight loss was observed after post administration but mortality occurred at Picralima nitida doses of 16000 and 20000 mg/kg. The presence of alkaloids, saponins and their derivatives in Picralima nitida may contribute to the mortality, which was documented to have a hepatotoxic effect [13–15]. A study by Ilodigwe et al. [16] on sub-chronic toxicological studies on Picralima nitida leaf extract dictated hepatocellular injury that leads to intracellular enzymes permeability into the bloodstream, which is accompanied by elevated serum Aspartate aminotransferase (AST) and Alanine aminotransaminase (ALT).

Also, cholestasis was dictated due to biliary obstruction or hepatic infiltration accompanied by elevated serum Alkaline phosphatase (ALP).

The LD_{50} was calculated as follows [12]:

\[
LD_{50} = \sqrt{(D_0 \times D_{100})}
\]

(3)

Where \( D_0 \) is the highest dose that gave no mortality (14000 mg/kg), and \( D_{100} \) is the lowest dose that produced mortality (16000 mg/kg).

The lethal dose (LD_{50}) for the Picralima nitida extract was estimated to be 14967 mg/kg \( \approx 14.97 \) g/kg. This implies that the extract is practically nontoxic on the test animals [17].

3.2 Bioassay Standard Plots for Glibenclamide and Picralima nitida

The percentage glycemic change against Log dose (standard plots) of glibenclamide and Picralima nitida are presented in Figs. 1-2. The regression equations obtained were used to calculate the final drug concentrations after the accelerated stability studies at different storage conditions (times and temperatures).

![Fig. 1. Bioassay standard plot for glibenclamide via animal model](image1)

![Fig. 2. Bioassay standard plot for Picralima nitida via animal model](image2)
3.3 Effect of Storage Time and Temperature on Drug Concentration

The accelerated stability experiment was performed by stressfully conditioning the drug at high temperatures of 45, 60, and 70°C for a period of 0, 1, 2, 3 and 4 weeks. An initial drug concentration of 2.5 mg/mL glibenclamide and 100 mg/mL Picralima nitida was used for the experiment. The residual concentrations of glibenclamide and Picralima nitida at the different storage times and temperatures were determined as shown in Figs. 3-4 (The plot of concentration of the drug substances remaining against time). It can be observed that the concentrations of glibenclamide and Picralima nitida decreased with increasing storage time. Increase in the storage temperature also improved the decomposition of glibenclamide, and Picralima nitida owing to the fact that molecules are likely to move more rapidly with intensified kinetic energy [18]. This entails that the storage temperature and time have a great impact on the drugs’ concentrations.

3.4 Degradation Kinetics

The accelerated degradation kinetics experiment was performed at elevated temperatures of 45, 60, and 70°C. The degradation kinetic constant (k) was calculated using the first-order rate equation stated as follows [2]:

\[ \ln C = \ln C_0 - k_1 t \]  

Where C is the residual drug concentration at time, t; C₀ is the initial drug concentration at time (t = 0); k₁ is the degradation rate constant.

The degradation rate constant, k₁ were calculated (Table 1) from the slopes of the linear plot of Ln conc. and time (Figs. 5-6). The average k₁ can be calculated each week at temperatures of 45, 60 and 70°C. The correlation coefficients (Figs. 5-6) indicates that the degradation data of glibenclamide and Picralima nitida fit or didn’t fit into the first-order model at all studied temperatures.

![Fig. 3. Effect of storage time and temperature on glibenclamide concentration using animal model](image)

![Fig. 4. Effect of storage time and temperature on Picralima nitida concentration using animal model](image)
The theory of accelerated stability analysis is centered on the Arrhenius equation [2]. The influence of temperature on the degradation kinetics was also studied using the Arrhenius equation. The Arrhenius equation gives a correlation between temperature and rate of reaction [2]. The values of the degradation rate constant, \( k \) at 27°C (\( k_{27} \)) were deduced from the Arrhenius plots (Figs. 7-8) (Ln \( k \) against 1/T) using Eq. 4 [19,20]:

\[
\ln k = \ln A - \frac{E_a}{RT}
\]  

Where \( A \) is the frequency of molecular collisions occurring between the molecules or Arrhenius factor, \( E_a \) is the energy of activation (kJ/mol.K), \( T \) is the absolute temperature (K) and \( R \) is the ideal gas constant (8.314 J/mol.K).

The rate of degradation can also be determined by the energy of activation of a chemical reaction [2,21]. The activation energies were calculated from the slopes of the Arrhenius plots (Figs. 7-8) using Eq. 5:

\[
\text{Slope} = \frac{E_a}{R} \tag{5}
\]

The activation energy, \( E_a \) for the degradation of glibenclamide (Table 1) was found to be high (since \( E_a > 50 \text{ kJ/mol.K} \)) but 49.11 kJ/mol.K was obtained for Picralima nitida. The higher the activation energies, \( E_a \), the lesser the influence of temperature on the degradation reaction. Based on the result, temperature contributed to the drugs’ degradation [22] and the pharmacokinetic profile of the animal model also contributed to its degradation.

The shelf-life of glibenclamide and Picralima nitida were estimated using Eq. 6 [2]:

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**Fig. 5. Degradation kinetics of glibenclamide using animal model**

**Fig. 6. Degradation kinetics of Picralima nitida using animal model**
between two reaction rate constants. The another parameter of importance to drug stability are found to be 10.54 and 3.15 weeks, respectively (Table 1).

Another parameter of importance to drug stability is the half-life ($t_{1/2}$), which is the period of time required for the concentration of a drug to be reduced by one-half of its original concentration. The half-life of the substances were estimated at 27°C (Table 1) using Eq. 7:

$$t_{1/2} = \frac{\ln 2}{k_{27}} = \frac{0.693}{k_{27}}$$

(7)

The half-life of glibenclamide and *Picralima nitida* were found to be 70 and 20.94 weeks, respectively.

3.6 $Q_{10}$ Method for Shelf-Life Estimation

Simonelli and Dresback’s $Q_{10}$ (the factor by which rate constant increases for a 10°C rise in temperature) method for shelf-life determination [23] was also applied in this study. It is the ratio between two reaction rate constants. The commonly used $Q_{10}$ values of 2, 3 and 4 relate to the activation energy of reaction at room temperature.

$$Q_{10} = \frac{K_{(T+10)}}{K_T}$$

(8)

$$Q_{10} = \exp \left[\frac{-E_a}{R} \left(\frac{1}{T+10} - \frac{1}{T}\right)\right]$$

(9)

$Q_{10} = 4$, provides the higher estimate for the increase in rate with increasing temperature and estimate the maximum likely decrease in shelf life with increasing temperature; $Q_{10} = 2$, provides the lower estimate for the decrease in rate with decreasing temperature and provide the most conservative estimate of the increase in shelf life with decreasing temperature; $Q_{10} = 3$ gives our most likely estimate.

$Q_{10}$ is applied in the prediction and estimation of the expiration date or shelf-life of drug materials at varying storage temperatures (Eq. 10 and Table 2) and is independent of the reaction order.

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{(T_2-T_1)/10}}$$

(10)

Where $t_{90}(T_2)$ is the estimated shelf life, $t_{90}(T_1)$ is the shelf-life at a given temperature. $T_1$ and $T_2$ are the varying temperatures.

Fig. 7. Arrhenius plot for glibenclamide using animal model

Fig. 8. Arrhenius plot for *Picralima nitida* using animal model
Table 1. The degradation rate constants calculated at different temperatures, shelf life and half-life of glibenclamide and \textit{Picralima nitida}

<table>
<thead>
<tr>
<th>Drug substance</th>
<th>Temp. (°C)</th>
<th>$K_1$ (week$^{-1}$)</th>
<th>Absolute Temp. (K)</th>
<th>$1/T$</th>
<th>Ln K</th>
<th>Arrhenius factor (A)</th>
<th>Activation energy, $E_a$ (kJ/mol.K)</th>
<th>Shelf-life at 27°C (weeks)</th>
<th>Half-life at 27°C (weeks)</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>27</td>
<td>0.0099</td>
<td>300</td>
<td>0.0033</td>
<td>-4.61389</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.0394</td>
<td>318</td>
<td>0.00315</td>
<td>-3.23399</td>
<td>5.73×10$^6$</td>
<td>67.62</td>
<td>10.54</td>
<td>70</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.185</td>
<td>333</td>
<td>0.003</td>
<td>-1.6874</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.2376</td>
<td>343</td>
<td>0.00292</td>
<td>-1.43717</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picralima nitida</td>
<td>27</td>
<td>0.0331</td>
<td>300</td>
<td>0.00333</td>
<td>-3.408</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.0869</td>
<td>318</td>
<td>0.00315</td>
<td>-2.443</td>
<td>1.15×10$^6$</td>
<td>49.11</td>
<td>3.15</td>
<td>20.94</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.308</td>
<td>333</td>
<td>0.003</td>
<td>-1.17766</td>
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<td></td>
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<tr>
<td></td>
<td>70</td>
<td>0.3103</td>
<td>343</td>
<td>0.00292</td>
<td>-1.17022</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. The estimated shelf-life using the Q_{10} method at room temperature

<table>
<thead>
<tr>
<th>Drug substance</th>
<th>Q_{10}</th>
<th>Shelf-life at 27°C (weeks)</th>
<th>Shelf-life at 4°C (weeks)</th>
<th>Shelf-life at 37°C (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>2.33</td>
<td>10.54</td>
<td>73.75</td>
<td>4.52</td>
</tr>
<tr>
<td>Picralima nitida</td>
<td>1.85</td>
<td>3.15</td>
<td>12.97</td>
<td>1.7</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The shelf life of *Picralima nitida* (herbal drug) and glibenclamide (orthodox drug) have been investigated. The stability/shelf life study was done using the bio-based dose respond relationship technique. The shelf life, t_{10%} (the time required to degrade 10% of a drug at 27°C) was determined via the accelerated stability studies. The stability studies were performed at temperatures of 45, 60, and 70°C and time of 1, 2, 3 and 4 weeks. The first-order degradation kinetics constant (k), half-life (t_{1/2}), and toxicity level (LD_{50}) were also evaluated. Storage temperature and time were found to have a great influence on the concentration of the drugs. The shelf life of glibenclamide and *Picralima nitida* were obtained as 10.54 and 3.15 weeks, respectively. The use of glibenclamide (orthodox drug) as a comparative drug assisted in the estimation of *Picralima nitida* (herbal drug) capacity to retain its specification (quality and safety) for treatment under the influence of environmental conditions. From the study, the shelf life of glibenclamide and *Picralima nitida* can be evaluated based on their pharmacological activity using the bio-based dose respond relationship technique but varied due to their pharmacokinetic profile of the animal models.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been reviewed and approved by the appropriate ethics committee of COOUTH Amaku, P.M.B., 5022 Awka, Anambra State, Nigeria with reference number COOUTH/AA/VOL.I.031.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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