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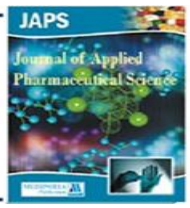

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Stability of reconstituted amoxicillin clavulanate potassium under simulated in-home storage conditions

Nwokoye Peace, Oyetunde Olubukola and Akinleye Moshood

Nwokoye Peace, Oyetunde Olubukola
Department of Clinical Pharmacy & Biopharmacy University of Lagos, Nigeria.

Akinleye Moshood
Department of Pharmaceutical Chemistry University of Lagos, Nigeria.

For Correspondence
Oyetunde Olubukola
Department of Clinical Pharmacy & Biopharmacy University of Lagos, Nigeria.
Tel:234-8028975338

ABSTRACT

Oral suspensions of antibiotics are mainly available as dry powders for reconstitution. Many reconstituted antibiotic suspension is to be kept refrigerated in order to get the optimal benefit from the drug. However, many patients do not keep to the specified storage conditions for different reasons like no refrigerator and irregular power supply that may result in various degrees of degradation of the product. Pharmacists are therefore challenged on how to counsel patients when there is no refrigeration or erratic power supply for refrigeration. This study investigated stability of amoxicillin-clavulanate potassium suspension in simulated in-home conditions of erratic power supply and no refrigeration. Amoxicilin clavulanate suspensions were reconstituted and stored in three different in-home storage conditions with temperature ranging between 5-29^{o}C over a period of 10 days. Samples from the suspension were assayed using a validated HPLC method. Percentage concentrations of amoxicillin-clavulanate potassium were over 90% up to fifth day, degradation was extensive by seventh day with amoxicillin concentration falling below 80% in two conditions while clavulanate had values less than 70% in all the three conditions. Reconstituted amoxicillin clavunate potassium stored at room temperature (27-29^{o}C) is stable for five days, use of reconstituted suspension that was not properly refrigerated after the fifth day should be discouraged.

Keywords: stability, amoxicillin-clavunate potassium, home – storage, suspension.

INTRODUCTION

Antibiotics for oral suspension are mainly available as dry powders for reconstitution. Many reconstituted antibiotic suspension is to be kept refrigerated in order to get the optimal benefit from the drug. However, many patients do not keep to the specified storage conditions for different reasons like no refrigerator and irregular power supply resulting in various degrees of degradation of the product. In Nigeria power outage is common. Power supply is intermittent daily and outages can last for several hours to days at a stretch. This is not unique to Nigeria, for example a work carried out in Basrah Iraq showed that there is extended power outage, an average of 14 hrs/day (Jassim, 2010). Antibiotic was chosen for this work because more often than not reconstituted antibiotic requires refrigeration, a condition that may be difficult to meet in many resource limited environments as ours and also studies in Basrah Iraq and Sudan have shown that antibiotic is the most commonly encountered drug stored and consumed by patients in their homes and of the antibiotics stored, the beta-lactam antibiotics of penicillin and cephalosporin derivatives constituted the highest percentage at 26.43% and 22% respectively (Jassim, 2010; Yousif, 2002).

Instructing patients on storage of reconstituted antibiotics at home is a challenge for pharmacists in this situation. This study is important to ascertain effect of in-home storage conditions on stability of reconstituted suspensions of amoxicillin/clavulanic and give insight on appropriate pharmacists' instructions when adequate refrigeration is unachievable.

Suspensions of amoxicillin/clavulanic acid are available for use in children and must be refrigerated (2-8^{o}C) to maintain effectiveness once reconstituted. Liquid formulations generally tend to have much shorter shelf-lives than solid formulations and once opened should be used within 2 weeks to avoid any microbial contamination or reduction in activity (Obitte *et al*., 2009; Kiying and Lauwo, 1993). The nature of syrup formulations in terms of added adjuncts such as sweetening, flavouring, suspending, stabilizing and preserving agents makes the liquid formula a complex one that is very prone to physical, chemical and microbiological instability (Obitte *et al.*, 2009). Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications, to maintain its identity, strength, quality, and purity throughout the retest or until expiry date period. Stability testing of an active substance or finished product provides information on how the quality of drug substance or drug product varies with time, influenced by a variety of environmental factors such as temperature, humidity and light**.** Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. Stability testing also provides information about the degradation mechanisms, potential degradation products, possible degradation pathways of drug as well as interaction between the drug and the excipients in drug product (Lalitha *et al.*, 2010; Uzunovic and Vranic, 2008). Climatic conditions can expose medications to dangerous temperatures that can potentially degrade the drug and often, unnoticed, example is Basrah where the summer heat can reach up to 50°C (Jassim, 2010), exceeding the U.S. Pharmacopeia's definition of room temperature (20-25 °C [68-77 °F]). High temperature and humidity accelerate deterioration, not only during transportation from overseas but also in warehouses and people's homes (Kiying and Lauwo, 1993). An appropriate storage condition for reconstituted antibiotic is defined as keeping the medicines under refrigeration (2-8^{o}C). Many homes in rural areas of developing nation may not have refrigerators or lack power supply, and even where there is refrigerator and power supply there may be erratic supply. Therefore medicines are stored at room temperature or kept in frigdes that has no power supply for several hours in a day thereby exposing these drugs to excessive temperatures far more than the room temperatures which ultimately may cause decomposition of both the excipients and active ingredient(s). Yousif, (2002**)**, in a Sudanese study reported that the rate of unsuitable storage conditions of drugs was 26.0%, compared to 31.8% in the Papua New Guinea study and that there was a higher rate of inappropriate storage in rural areas due to the lack of refrigeration. Drugs are chemicals that react to external stimuli such as, heat, humidity, light, microbial agents and dust. In many cases, such reactions can only lead to physical changes such as discoloration of the drug product. In many other cases, the reaction may affect the drug more seriously leading to the reduction or elimination of its effectiveness and/or potency. There are cases of drugs that, when affected, not only the failure of the drug to exert a therapeutic effect, but also cause adverse effects on the patient's health. Therefore sstorage conditions must not be taken lightly (Obitte *et al.*, 2009). Studies have shown different drug in-home storage practices, some store or keep their drugs on the dinning table, top of the refrigerator, first aid boxes, in their bags, in the car, closed cupboard or drawer, suit case, in the kitchen and even the bathroom and these practices may result to degradation (Obitte *et al.*, 2009; Naidoo, 2006; Kiyingi and Lauwo, 1993)**.** Instances of unsuitable storage often involve liquid preparations stored on open shelves, and reconstituted oral antibiotic powders stored for more than the recommended period after reconstitution or kept at freezing point (Kiyingi and Lauwo, 1993).

A study carried out to determine the chemical stability of amoxicillin and potassium clavulanate (250/62 Co-amoxiclav) oral suspension stored at room temperature 20°C and 8°C over a period of 11 days showed amoxicillin is stable for 7 days at both temperatures. Potassium clavulanate maintains at least 90% of its initial concentration for 7 days at 8°C but shows more than 40% degredation in the same time period at room temperature of 20°C. The time taken for the original concentration of potassium clavulanate to drop to 90% of its value at room temperature of 20°C is 2 days (Mehta *et al*., 2008**).** Another study by Tu *et al*. to determine the stability of amoxicillin trihydrate- clavulanate potassium in original containers and unit dose oral syringes showed that amoxicillin trihydrate is stable for at least 10 days in the original containers and all types of oral syringes at 5°C whereas clavulanate- potassium is stable for 11.1 days in original containers and less than 5 days in all types of oral syringes at the same temperature (Tu *et al.*, 1988). All these studies pointed at the importance of storage conditions in the stability of both solid and liquid drug formulations. The objective of this study is to determine the stability of reconstituted amoxicillin-clavulanic acid oral suspension under simulated in-home storage conditions where there is no refrigeration or where there is perennial power outage. This study assessed the impact of different home storage conditions on the stability of reconstituted oral suspensions. It also enhances our knowledge on appropriate patients' counsel on drug storage in such environments.

METHOD

Sample collection

A brand of amoxicillin-clavulanate potassium (228.5 mg/5 ml) from the same batch was used for this study. It is available as dry powder containing 200 mg amoxicillin (as amoxicillin trihydrate) and 28.5 mg clavulanic acid (as potassium clavulanate) per 5 ml for reconstitution in water for oral use. The samples were purchased from a reputable and registered pharmacy and were within the stated expiry date on pack.

Reference Standards

Amoxicillin 200mg and Potassium clavulanate reference standards were procured from United States Pharmacopoeia (USP) and Sigma-Aldrich respectively.

Reagents

All reagents such as sodium dihydrogen orthophosphate anhydrous, sodium hydroxide and ortho-phosphoric acid were of analytical grades while Methanol was of HPLC grade.

Instrumentation and chromatographic Conditions

Amoxicillin and clavulanic acid were assayed using a modified stability HPLC method developed by Tippa and Singh (2010). The analysis was carried out using HPLC system (Agilent 1100) series coupled with in-line degasser, quaternary pump, variable wavelength detector and data analysis was done using the chemstation® software. The analytical column was Zobax® C18, 150 x 4.6 mm, 5 µm particle size. The mobile phase was a mixture of methanol and potassium dihydro orthophosphate (10:90%v/v) adjusted to pH 5 with ortho-phosphoric acid. The flow rate was 1ml/min.The UV detection was set at 220 nm for simultaneous detection of amoxicillin and clavulanic acid. 20 µL of the sample was injected into the HPLC and the system was maintained at ambient temperature.

Preparation of stock and calibration solutions

Standard stock solution containing 1000 µg/ml of amoxicillin and 200 µg/ml of clavulanate was prepared by dissolving amoxicillin (10mg) and clavulanate potassium (2mg) reference standard in distilled water. Five different concentrations (50 - 400 µg/ml) of amoxicillin and (10 – 80 µg/ml) of clavulanate mixtures were prepared from stock solution for calibration and linearity study. The responses were measured as peak areas and plotted against gradient concentrations.

Sample preparation and estimation of amoxicillin and clavulanic acid

Eighteen samples of amoxicillin-clavulanate potassium (228.5mg/5ml) oral suspension were freshly reconstituted with table water. The reconstituted preparations were distributed into groups (n=6) and were subjected to three different simulated conditions that represent the different in-home storage conditions. Samples stored under condition A were refrigerated with fluctuating temperatures between 5-25°C due to power outages during the period. Samples in condition B were stored inside a cupboard with room temperatures of 27-29°C and samples stored under condition C were submerged in a bowl filled water at room temperatures of 27-29°C for a period of 10 days. A room thermometer was used to assess room temperature and temperature of fridge during power outages. 5ml of each sample was collected, diluted to a solution containing 100µg/ml of amoxicillin and 20µg/ml of clavulanic acid and filtered using 0.45µm syringe filter on each day of the analysis. The filtrate was injected into HPLC and the respective peak areas were plotted into calibration equation.The concentrations of amoxicillin and clavulanic acid remaining after storage on day 1, 5, 7 and10 were extrapolated from the line of regression. The percentage assay purities were then evaluated.

RESULTS AND DISCUSSION

The results of the study are summarized in tables 1 and 2 which show the concentrations of amoxicillin and clavulanate respectively as percentage assay.

Table. 1: Percentage Concentration of Amoxicillin ± SEM.

Storage Conditions	Refrigerated with fluctuating power (5-25°C)	Stored in cupboard at room temperature (27-29°C)	Submerged in water at room temperature (27-29°C)
Day	Concentration (%)	Concentration (%)	Concentration (%)
1	106.03 ± 6.95	101.96 ± 6.72	97.07 ± 2.26
5	96.03 ± 5.14	105.4 ± 5.34	104.44 ± 6.57
7	79.64 ± 6.62	78.79 ± 2.66	99.23 ± 4.28
10	83.2 ± 12.18	75.13 ± 11.43	85.36 ± 10.63

Table. 2: Percentage Concerntration of Clavulanate ± SEM.

Storage conditions	Refrigerated with fluctuating power (5-25°C)	Stored in cupboard at room temperature (27-29°C)	Submerged in water at room temperature (27-29°C)
Day	Concentration (%)	Concentration (%)	Concentration (%)
1	145.73 ± 11.10	140.39 ± 7.2	141.74 ± 9.25
5	116.64 ± 4.90	109.18 ± 9.87	113.26 ± 13.65
7	69.84 ± 2.99	55.24 ± 2.63	64.19 ± 3.36
10	80.95 ± 16.44	52.77 ± 1.67	53.67 ± 3.56

The suspensions were judged to be stable if the components maintained at least 90% of the label concentrations. During the test period, both components were found to be stable for 5 days under the three simulated home storage conditions (Table 1and 2). Amoxicillin component maintained at least 90% of its label concentration for 7 days in condition C submerged in water at room temperatures of 27-29°C), but showed more than 21% degredation in the same time period in conditions A (refrigerated between fluctuating temperatures of 5-25°C) and B (stored inside a cupboard with room temperatures of 27-29°C) (Figure 1). The percentage concentration of amoxicillin on day 5 for conditions B and C was found to be higher than that on day 1, this may be due to sampling or instrumental error. Potassium clavulanate showed more than 30% degradation as from day 7 in the three storage conditions. Also the concentrations of both components tend to be higher with the samples under condition C during the period of the study when compared to those in condition B (figure2). Both components were degraded extensively by day 10 under the three simulated home storage conditions.

The result of this study is similar to the work done by Naidoo (2006), which showed that only amoxicillin suspension stored between 2°C and 8°C for 7 days showed the lowest level of degradation.

The result is also in line with the study performed by Mehta *et al.* (2008) which showed that amoxicillin component of co-amoxiclav oral suspension is more stable than clavulanate-potassium when stored at the same temperature condition.

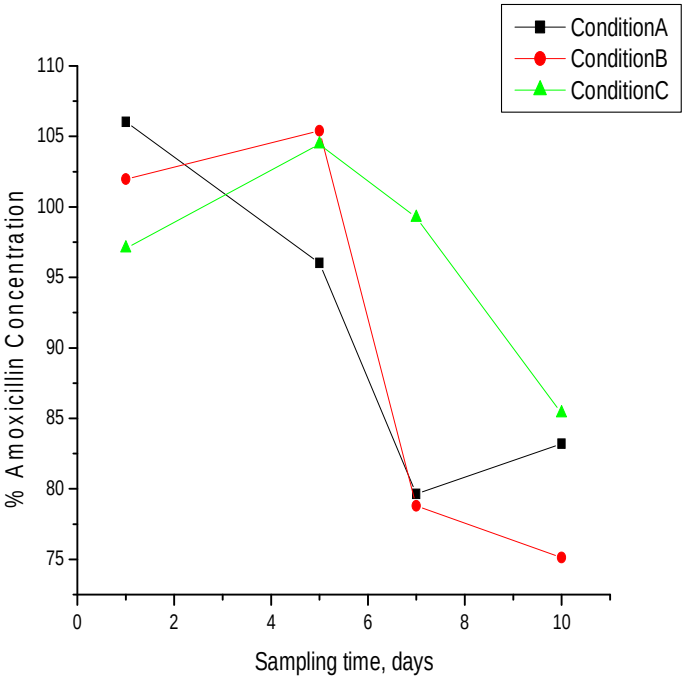

Fig.1: Degradation of amoxicillin versus time period under different in –home storage conditions.

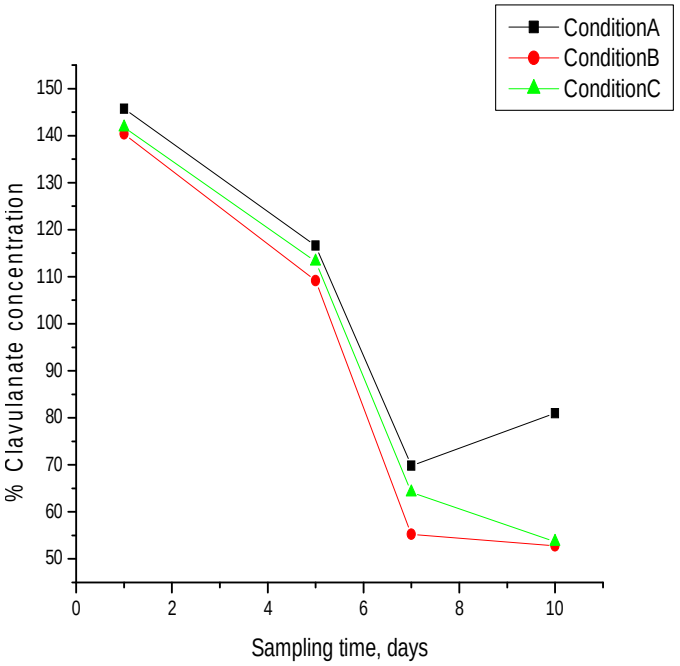

Fig. 2: Degradation of clavulanate versus time period under different in –home storage conditions.

CONCLUSION AND RECOMMENDATION

Reconstituted amoxicillin-clavulanic acid oral suspension is stable for at least 5 days when stored at 5-29^{o} C. When stored submerged in water at room temperature the amoxicillin constituent was stable for 7 days. Further work is required on home storage of reconstituted antibiotics in situation where there is no refrigeration or irregular power supply. Reconstituted amoxicillin clavulanate potassium stored at room temperature (27-29^{o}C) is stable for five days hence use of reconstituted suspension that was not properly refrigerated after the fifth day should be discouraged.

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