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Stability studies of reconstituted oral amoxicillin suspension (125mg/5ml) under different temperature storage conditions

Authors
Rose Obat[(1)]; Nathan Kipsang[(2)]; Martha Maru[(3)]
Main author email: roseobat@kabarak.ac.ke

Abstract
The research objective was to determine the physical stability and concentration of amoxicillin reconstituted oral suspension (125mg/5ml) stored under room temperature of 25°C and refrigeration temperature of 2-8°C for day 1, 3, and 7. Three different brands of amoxicillin samples were used. Samples from the reconstituted amoxicillin suspension was assayed by HPLC to determine the concentration of the active and examined physically for taste, odour and colour change. Reconstituted antibiotic suspensions must be stored at room temperature or in a refrigerator to maintain potency throughout their use. However, a good number of patients do not adhere to storage specifications resulting in the degradation of the product and subsequent loss of potency. The drug concentration for all three brands during the days analysed ranged from 88.94-102.17(%) for refrigerated and 89.57-99.7(%) for reconstituted amoxicillin at room temperature. The odour for days 1, 3 and 7 is fruity. The colour was pale yellow for days 1 and 3, while on day 7, it was creamy yellow. The taste was sweet on days 1 and 3, while on day 7, it was sweet/low bitter. The three brands maintained their potency and physical stability as required in the British pharmacopoeia. The results for day one for all the drugs fall within the accepted range. Drug K has its percent concentration the lowest. Microbiological studies is recommended for the drug to ascertain its microbial stability.

Key terms: Stability, amoxicillin, room temperature, refrigerated temperature.

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1.0 INTRODUCTION

Amoxicillin is a semi-synthetic derivative of penicillin beta-lactam antibiotic. Amoxicillin works against bacterial growth by interfering with cell wall synthesis. Amoxicillin is active against different diseases originating from gram-negative or gram-positive bacteria, including bronchitis, pneumonia, otitis media, skin infections, syphilis, and gonorrhoea. Due to many hydroxyl groups present in its structure, solubility, absorption and subsequent distribution, amoxicillin is found to be extensive in the body fluids, making it a more potent agent and thus is the most commonly used penicillin. Amoxicillin is formulated as an amoxicillin trihydrate. It is marketed as a capsule, dispersible tablet or dry powder for reconstitution. Reconstituted dry powder poses a risk in terms of therapeutic, microbiological, physical, chemical and toxicological stability (Pokharana et al., 2018). In addition, after reconstitution, a drug should maintain maximum stability standards during the duration of use under different storage conditions such as humidity, direct light and temperature. However, if not stored correctly, then the product can degrade and lose its potency. Amoxicillin once reconstituted, it is required that the medicine is stored under low temperatures, preferably in a refrigerator/ cool, dry area/ room temperature. However, some limitations such as lack of refrigerator, unreliable power supply and caregivers' ignorance lead to failure to adhere to these important storage instructions hence degradation of the product and subsequent loss in quality, efficacy and safety, which endangers the user.

2.0 LITERATURE REVIEW

Penicillin, such as amoxicillin, is one of the most prescribed antibiotics due to its effectiveness in managing a wide range of common infections, cost-friendly and availability (Barker et al., 2017). Penicillin consists of the lactam ring, which is joined to a five-membered ring containing a thioether group as well as an amine group located in the positions first and fourth, respectively (Hoemann, 2012). 6-aminopenicillins acid (6-APA) is the penicillin nucleus. It is essential in all penicillin antibiotics, with the nucleus being the R1 position that can be substituted with different side chains to give products of different spectrums of activities, this manipulation of the R1 position has led to the development of broad-spectrum drugs, one being amoxicillin. (Tripathi, 2013).

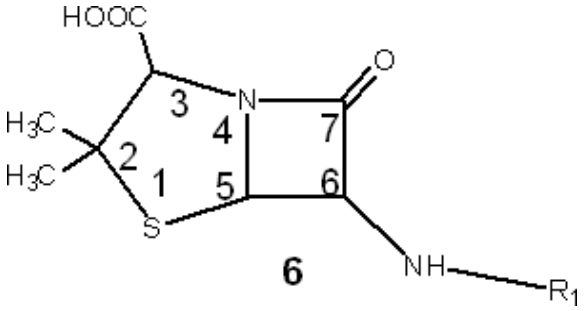

Figure 1: Amoxicillin structure

Amoxicillin is semi-synthetic Penicillin that has been substituted at position 6 with 2- amino 2 (4-hydroxyphenyl) acetamido group. The addition of a hydroxyl group on the phenyl ring makes it superior for oral use (Lima et al., 2020). Amoxicillin trihydrate inhibits bacteria's growth by hindering the conjoining of polymers, thereby stopping cell wall synthesis (Prescott, 2013). Its antibacterial spectrum matches that of penicillin-G, but amoxicillin is found to be superior against gram-negative bacilli shifting its category as an extended spectrum Penicillin. Amoxicillin is absorbed well in the body when taken orally, it is well distributed in the body fluids and excreted through the kidneys by active tubular secretion and glomerular filtration (Castagnola et al., 2021). Amoxicillin is available in various dosage forms, such as capsules, dry powder for suspensions, injections, and tablets. Various national and multinational pharmaceutical manufacturers manufacture the drug.

Amoxicillin was brought into the market in the 1970s (Ozhathil & Wolf, 2022). It is stable in acidic conditions and is presented as amoxicillin trihydrate with a yellowish-white or greyish-white colour and amoxicillin sodium, a white or slightly pink amorphous powder, which is highly hygroscopic with a little sulfurous odour upon reconstitution. The solubility of amoxicillin is sensitive to pH changes such that any slight increase in pH automatically increases its solubility. Amoxicillin pKa value ranges from 2.67, representing the carboxylic group in the solution, 7.11

representing the amine group and 9.5, representing the hydroxyl group at 37°C with moderate solubility at a pH range of 4 to 6 (Mat et al., 2004). Amoxicillin has antimicrobial activity on many diseases that originate from being infected with gram-positive bacteria, such as staphylococcus, and gram-negative bacteria, such as Escherichia coli, among others in human beings and animals (Rybak, 2004). Amoxicillin is useful in the treatment of infections such as otitis media, tonsillitis, laryngitis, pharyngitis, bronchitis, urinary tract infections, gonorrhoea and Pneumonia (Mombelli & Samaranayake, 2004).

Amoxicillin can easily degrade with an increase in temperatures whether the drug is kept in an open container or sealed. Amoxicillin undergoes first-order degradation under controlled humidity (Ford, 2004); hence, stability is very important. The ability of a drug substance or pharmaceutical ingredient to maintain the desired characteristics of identity, strength, composition and broadly quality within the shelf-life is referred to as stability. The stability of the drug is affected by extreme temperatures, humidity, light, and packaging material. Stability studies are done to determine the possible drug substance degradation mechanisms, pathways, and possible degradation products. It is expected that reconstituted drug substances will degrade faster due to the presence of water molecules that promote degradation leading to a drop in drug quality, safety and efficacy.

The major factors that lead to an increased rate of degradation of the reconstituted antibiotic for oral use include poor storage conditions such as high temperatures, high humidity and even exposure to direct sunlight. Most stability studies done on amoxicillin are on intervals between zero and seventh day or between zero and tenth day after reconstitution, looking at the effect of varying the temperature, humidity, type of water used to reconstitute and, in some cases, light.

The University of Sargodha, Sargodha, Pakistan, studied the stability of amoxicillin drug product in different packaging material; among the many factors that accelerate or initiates the degradation process of amoxicillin drugs includes heat, light and moisture. The study concluded after 6 months, and it clearly showed that the concentration of amoxicillin powder for reconstitution in the amidoxime generic brand had lost its potency to as low as 78 per cent on the shelf at the pharmacy at room temperatures while the Amoxil brand had up to 94.5 per cent active pharmaceutical ingredient.

A 5-day study carried out at the University of Port Harcourt, Nigeria, on the effects of storage conditions on oral antibiotics such as cefuroxime, amoxicillin/clavulanic acid, and azithromycin showed that temperature is a major factor of degradation of in-home use of antibiotics (Stanley & Igala, 2017). A different study was carried out by the clinical pharmacy and bio pharmacy department at the University of Lagos, Nigeria, on the stability of reconstituted amoxicillin /clavulanate potassium under simulated storage conditions at home, the study was carried out at room temperature of 20 ℃ and at 8 ℃ for 11 days to determine chemical stability of both drugs, amoxicillin was found to be stable up to 7 days in both temperatures (Akinleye et al., 2012).

3.0 METHODOLOGY

The experimental study was carried out in the Pharmaceutical chemistry laboratory after reconstituting three different brands of amoxicillin trihydrate 125mg/5mls dry powder. The drug concentration and physical stability were determined on days 1, 3 and 7. Materials and Equipment: three generic brands of amoxicillin oral powder for reconstitution (125mg/5ml), Distilled water, High-Performance liquid chromatography (HPLC) and refrigerator.

Table 1: Batches of 3 Brands of Amoxicillin 125mg/5ml Manufacturing Date and Expiry Date

NO	Name of brand 125MG/5ML	Batch no	Manufacturing date	Expiry date
1.	M	2201297	01/2022	12/2025
2.	K	81142	4/2022	3/2025
3.	E	1G98	07/2021	06/2024

Procedure
This study was done using British pharmacopoeia 2011.

Buffer preparation
2.7g/L of potassium dihydrogen phosphate was weighed, and the pH of the solution was adjusted to 5.0 using dilute KOH or dilute orthophosphoric acid.

Table 2: Buffer Preparation

Mobile phase:	Buffer and Acetonitrile in the ratio of 90:10, respectively.
Flow rate:	1.0ml/min
Detector wavelength:	254nm
Temperature:	30 degrees
Diluent:	Water
Column	ODS-C18(Lunar-250mm X 4.6mm)
HPLC model used	Shimadzu, LC-2030C plus

Standard preparation
In a 100ml volumetric flask, 143.75mg of amoxicillin trihydrate working standard was weighed. The standard was then dissolved with 30ml of the diluent and sonicated for approximately 20mins until all the standards were fully dissolved. It was then left to cool to room temperature, and the diluent was added to the mark. The standard solution was then filtered into a clean, dry vial using a 0.45-micron meter filter.

Sample preparation
The sample quantity equivalent to 125mg of amoxicillin was weighed and put into a 100ml volumetric flask. The sample was mixed with 30ml of the diluent to dissolve; it was then sonicated for approximately 20mins until all the samples had been fully dissolved. It was allowed to cool to room temperature, and top up was made to the mark using the diluent. The filtered sample was placed inside a clean, dry vial using a 0.45-micron meter filter. Injection volume: 20µL.

Standard injection
Five standard replicates weres injected, and the HPLC system suitability parameters were met.

Sample injection
Two samples of each brand from room and refrigerator temperature weres injected, and the assay was calculated.

4.0 RESULTS AND DISCUSSION

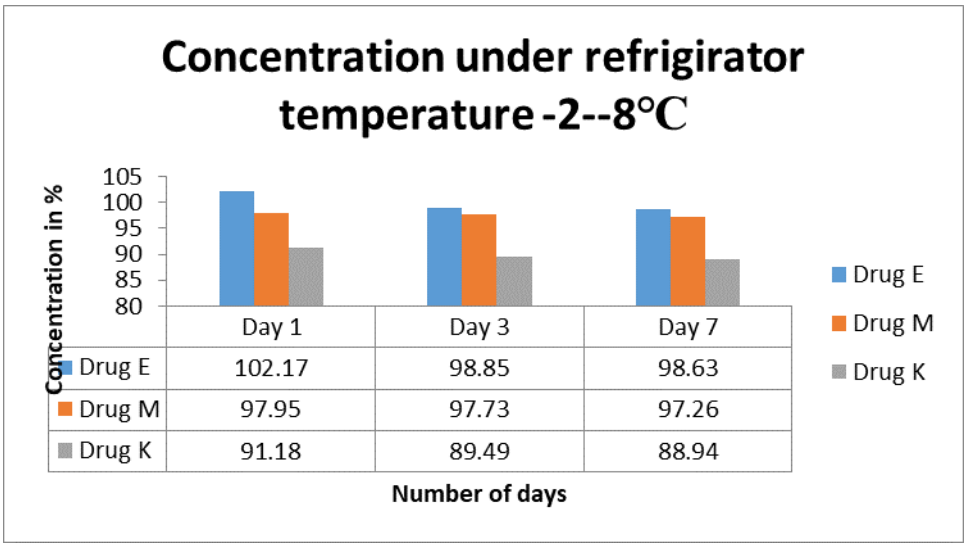

Figure 2: Concentration under Refrigerator Temperature -2 to -8 °C

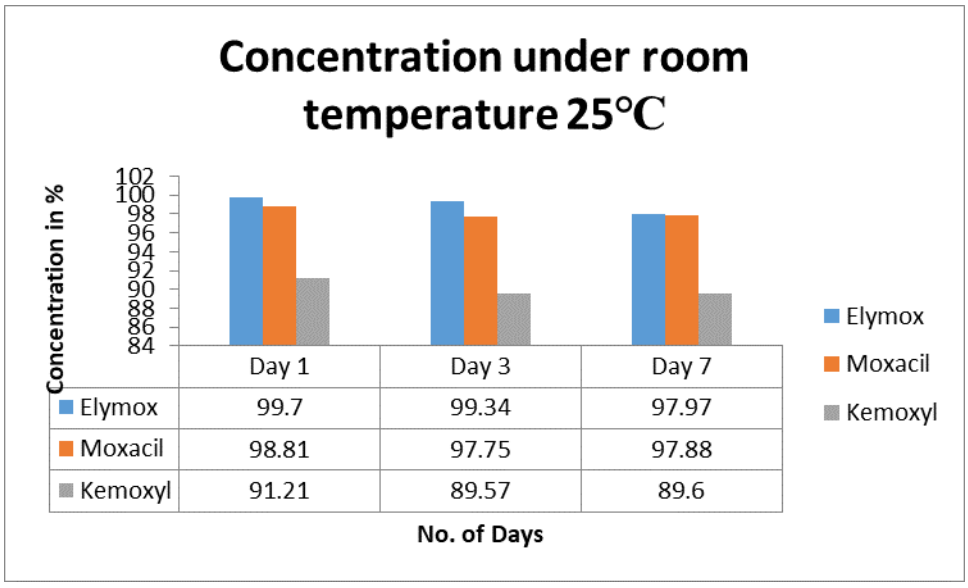

Figure 3: Concentration under Room Temperature 25°C

Table 3: The Physical Characteristics 3 Brands of Amoxicillin

		DRUG M	DRUG E	DRUG K
Day 1				
	odour	fruity	fruity	fruity
	colour	Pale yellow	Pale yellow	Pale yellow
	taste	sweet	sweet	sweet
Day 3				
	odour	fruity	fruity	fruity

	colour	Pale yellow	Pale yellow	Pale yellow
	taste	sweet	sweet	sweet
Day 7				
	odour	fruity	fruity	fruity
	colour	Cream yellow	Cream yellow	Cream yellow
	taste	Sweet/low bitter	Sweet/ low bitter	Sweet/ low bitter

For suspension to be themed potent and safe for use, British pharmacopoeia(BP) specify that its concentration should be 80-120 per cent of its active ingredient hence all the brands were found to be potent, safe and stable throughout the two storage conditions. Drug M lost about 1 per cent, drug E 5 per cent and drug K 2 per cent of the initial concentration in both storage conditions. The rate of degradation of drug M is very low, and the potency is because of late manufacturing and long expiry compared to the rest. The extent of degradation of all the drugs resembles other studies previously done by Peace et al. (2012) on the stability of co-amoxiclav suspension.

Factors that may lead to the drug losing its potency during storage include exposure of the drug to oxygen, direct sunlight, high temperatures, drug incompatibility with its excipients and moisture. The drugs M, E and K had specific taste and colours as stated in BP. all the suspensions had a sweet taste that changed to low bitter by the 7^{th} day, whereas the fruity smell was maintained throughout the study. The colour of the drugs M, E and K was milky white and tend to change by day 7 to distant yellow colour.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Reconstituted oral amoxicillin suspension is stable at temperatures between -2 and 29°C for a period of 7 days; this is seen in the concentrations of drug M, K, and drug E results obtained. In addition, the drugs' colour, taste and odour had no significant change.

Recommendations: The results for day one for all the drugs fall within the accepted range, drug K has its percent concentration the lowest, and microbiological studies should be done on this drug to ascertain microbial stability. In addition, studies should be done to determine the concentration of drug, microbial, physical and toxicological stability of reconstituted amoxicillin for a duration of 10 days of storage.

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