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RESEARCH ARTICLE

MJM BIOLABS

Evaluation of Post-Reconstituted Flucloxacillin Dry Powder for Suspension in Nakuru Town, Kenya

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ABSTRACT

Beta-lactam antibiotics are compounds which are commonly used to treat variety of bacterial infections. Flucloxacillin powder for suspension is among the most prescribed β- lactam antibiotics in Kenya.The β-lactam ring is readily hydrolyzed under varied pH and elevated temperature conditions. This degradation can result in subtherapeutic dosages and development of antimicrobial resistance. This study aimed to evaluate the effect of storage temperature on the stability of four brands of flucloxacillin dry powder for suspension. Reconstituted samples of flucloxacillin dry powder for suspension were stored in three temperature conditions: cold (4^0C), room (25^0C), and intermediate (30^0C). The content of flucloxacillin was determined by use of UV Spectroscopy at a wavelength of 219nm. An antimicrobial test was performed to determine the susceptibility of *Staphylococcus aureus* (a Gram-positive) and *Escherichia coli* (Gram-negative) to flucloxacillin using the Disk Diffusion method. The stability of the samples was evaluated every 2 days up to 10 days. The UV spectrum results showed a decreasing trend in flucloxacillin content over the 10 days of analysis. The microbial analysis results indicated that flucloxacillin retained its antimicrobial activity best under cold storage conditions. The zones of inhibition for *Staphylococcus aureus* for brand A at day 7 were 33.105mm, 31.055mm and 23.055mm at 4^0C, 25^0C and 30^0C respectively. A similar trend was observed across the other three brands, with zone diameters decreasing as the temperature increased. *Escherichia coli* demonstrated resistance to flucloxacillin, as inhibition zones remained below 17mm under all storage conditions. The decrease in content and antimicrobial activity of flucloxacillin samples stored at 25^0C and 30^0C temperatures indicates that non-compliance with prescribed storage conditions may lead to subtherapeutic level dosing and contribute to poor treatment outcomes.

Keywords: Beta-lactam,flucloxacillin, microbial, stability, temperature

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INTRODUCTION

Infectious diseases pose a major health challenge in Africa, contributing significantly to both illness and death across the region (World Health Organization, 2022). Conditions such as respiratory infections, skin infections, and sepsis are widespread, highlighting the essential role of effective antimicrobial treatments. Antibiotics, especially beta-lactam drugs are frequently used to manage these infections due to their proven effectiveness against a range of bacterial pathogens (O'Neill, 2016; Centers for Disease Control and Prevention, 2021).

Antimicrobial resistance (AMR) is becoming an increasingly serious public health concern, leading to challenges in treatment efficacy. This problem is often fueled by the misuse of antibiotics, presence of poor-quality medicines in the market, and may also result from improper storage or inadequate manufacturing practices (WHO, 2022). When antibiotics lose their effectiveness, infections last longer, treatment becomes more expensive, and the risk of complications or even death increases. To help prevent this, it's crucial to maintain high-quality standards through Good Manufacturing Practices (GMP), ensure proper storage of medicines, and enforce regulatory oversight. These efforts are key to preserving the effectiveness of antibiotics and protecting public health.

Antibiotics are primarily preventive and therapeutic agents for bacterial infections and represent the most commonly prescribed drugs (Leekha, Terrell, & Edson, 2011). According to a survey conducted by the Ministry of Medical Services and the Ministry of Public Health and Sanitation (2020), 60% of all prescriptions in Kenya were beta-lactam antibiotics, signifying their important role in health care. Beta-lactams (monobactams, penicillins, cephalosporins and carbapenems) contain a highly reactive beta-lactam ring. Bacterial cell wall synthesis is inhibited by their association with penicillin-binding proteins (Farhat et al., 2022). This inhibits the synthesis of the essential structural component peptidoglycan, resulting in bacterial cell lysis and subsequent cell death. A study conducted by Nakuru Provincial Hospital (Bosibori, 2017) showed that penicillin class of antibiotics, especially flucloxacillin, were the most prescribed antibiotics (11.2%) of all prescriptions.

Beta-lactams are recognized for their inherent instability due to the composition of the beta lactam ring, which is susceptible to acid/base hydrolysis, leading to the formation of penicilloic acid (an inactive compound) (Leekha, Terrell, & Edson, 2011). They are therefore prone to degradation under various environmental conditions, impacting their overall stability and efficacy (Mora-Ochomogo & Lohans, 2021). Beta-lactams are commonly formulated as capsules and powders for reconstitution. Drugs in powder form retain potency for a short time after reconstitution. The post-reconstitution stability of dry powder suspensions (DPS) presents multifaceted concerns related to physical, microbiological, chemical, therapeutic and toxicological stability (Pokharana, Vaishnav, Goyal, & Shrivast, 2018). Reconstituted drug products are predisposed to degradation, primarily due to presence of water, which accelerates the degradation process, potentially compromising the drug's quality, safety, and effectiveness (Obat, Kipsang and Maru, 2022). This accelerated degradation is often linked to inadequate storage conditions, affecting the stability of commonly prescribed DPS formulations (Obitte, Chukwu, Odimegwu, & Nwoke, 2009).

Pharmaceutical manufacturers assign a shelf life to drug products from studies performed under controlled environmental conditions. The label recommendation for the storage of flucloxacillin DPS states that once reconstituted, it should be stored in a refrigerator and used within 7 days. The World Health Organization (WHO) defines room and refrigerator temperatures as15°C to 25°C and 2°C to 8°C, respectively. Post-reconstituted DPS should be stored under the prescribed conditions to achieve the intended therapeutic effect. Degradation of antibiotics can lead to administration of subtherapeutic doses, which is a possible contributor to development of antimicrobial resistance. (LibreTexts Biology, 2016).

In this study, the stability of four brands of flucloxacillin dry powder for suspension were evaluated. The unique structure of flucloxacillin makes the reconstituted dry powder susceptible to hydrolysis, leading to potential degradation and reduced efficacy over time. Patients are required to comply with prescribed storage conditions for the drug substance to achieve optimal therapeutic effect.

METHODS

Study Design

The study adopted a laboratory-based experimental design and was conducted in Nakuru County, Kenya.

Materials, Chemicals and Reagents

Four brands of flucloxacillin sodium dry powder for oral suspension 125 mg/5 mL, including the innovator product were used in this study. All four brands were pharmaceutical equivalents, containing flucloxacillin sodium at a strength of 125 mg/5 mL, formulated as dry powder for oral suspension, intended for oral use.

Table 1:
Batch Numbers, Manufacturing Dates, and Expiry Dates of Study Samples

Sample	Manufacturing Date	Expiry Date
A	January, 2024	December, 2026
B	July, 2023	June, 2025
C	September, 2023	August, 2025
D	January, 2024	December, 2026

Bacterial strains (*Staphylococcus aureus* ATCC 25923 (test organism) and *Escherichia coli* ATCC 25922 (negative control)) obtained from Kenya Medical Research Institute (KEMRI), Mueller-Hinton Agar (MHA) plates, sterile antibiotic disks, sterile swabs, cotton-tipped sterile applicators, sterile forceps, sterile inoculating loop, incubator set at 37^0C, sterile saline solution (0.9% NaCl), sterile petri dishes 90 mm diameter, Vernier caliper, laminar airflow cabinet, sterile disposable syringes, alcohol wipes, conical flasks, autoclave.

Sample collection

Convenient non-random sampling was employed to collect flucloxacillin samples in their commercial packs from pharmacies in Nakuru Town, Kenya. The brands were obtained in bottles, properly labelled and within the indicated expiration dates.

UV-Vis Spectrophotometric Assay of Flucloxacillin

The flucloxacillin samples were reconstituted and stored at room (25^0C), refrigeration (4^0C), and intermediate (30^0C) temperatures for 10 days. For each brand, the active pharmaceutical ingredient (API) present in reconstituted samples was analyzed in triplicate on day 1, 3, 5, 7 and 10. UV-Vis spectrophotometry was employed at λmax 219nm to quantify the API content in each sample as follows (Dey, et al., 2010):

The reconstituted drug samples were shaken to ensure homogeneity. Two mL was pipetted from each sample and transferred into 50 mL volumetric flasks. This was made to volume with 0.1N NaOH solution with continuous shaking and filtered. One mL of the filtrate was transferred into 100 mL volumetric flasks, made to volume with 0.1N NaOH to achieve a concentration of 10 µg/mL and the UV absorbance reading taken at 219 nm. The UV reading of reconstituted samples stored under the three study conditions were recorded on day 1, 3, 5, 7 and 10.

A standard solution of flucloxacillin containing 10µg/mL was prepared by dissolving 10 mg of flucloxacillin sodium working standard in 10 milliliters of 0.1N NaOH and diluting 1 mL to 100 mL. The results of the assay were recorded and the concentrations of the different samples were determined using the equation:

$$\text{Assay (\%w/w)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard weight (mg)}}{\text{Standard dilution}} \times \frac{\text{Test dilution}}{\text{Test weight (mg)}} \times \text{Purity of the standard}$$

Microbial Disk Diffusion Test

Mueller-Hinton Agar was infected with *Staphylococcus aureus* and used for the disk diffusion test. The *Staphylococcus aureus* bacterial colonies were obtained by subculturing from a stock culture. A loopful of the stock culture was streaked onto fresh nutrient agar and incubated at 37°C for 24 hours. An autoclave set at 121°C and 15 psi for 15 minutes was used to sterilize the plates. The plates were dried and labeled.

The Mueller-Hinton agar was poured onto the sterile plates under a laminar flow cabinet and left to cool and harden. The bacterial colonies were blended and suspended in sterile normal saline. A sterile swab was used to spread the bacteria on the agar plates. The bacteria were cultured for 24 hours at 37°C (Bauer, Perry, & Kirby, 1959). The negative control used was *Escherichia coli* because it is known to be resistant to flucloxacillin (Bauer, Perry, & Kirby, 1959) (Bauer, Perry, & Kirby, 1959).

Using a pair of forceps, the disks were carefully put on top of the agar plate containing the bacteria and gently squeezed to achieve full contact with the agar surface. The plates were incubated for 24 hours at 37°C, and the outcomes were monitored. The zones of inhibition were measured using a Vernier caliper after incubation to the closest millimeter. Zones of inhibition were recorded on days 1, 3, 5, 7 and 10.

Samples kept at the three temperature conditions were subjected to the disk diffusion test. The samples kept under refrigeration served as the control for the analysis (Klu, Apepteng, Bright, Mintah, & Katsekpor, 2018).

Data analysis

Data were entered into a computerized database for analysis. Excel software and statistical product and service solution (SPSS) were utilized to record values and generate graphs to visualize the results. The data were summarized using descriptive statistics (standard deviation, frequency distributions and mean).

Ethical considerations

The Kabarak University Research Ethics Committee (KUREC), Kabarak University School of Pharmacy and National Commission for Science, Technology, and Innovation (NACOSTI) (Research license no. 722492) approved and authorized the research.

RESULTS

Chemical analysis

Table 2 presents the UV-Vis absorbance measurements of flucloxacillin samples stored at refrigerated (4°C), room temperature (25°C), and intermediate temperature (30°C) conditions. The results show a decrease in absorbance values over the 10-day period for brands A, B, C, and D stored at 4°C, 25°C, and 30°C, indicating a reduction in the concentration of the active pharmaceutical ingredient (API) with prolonged storage. Notably, the decrease was more pronounced at higher temperatures.

Table 3 displays the percentage concentration of flucloxacillin in samples stored at 4°C, 25°C, and 30°C over a 10-day period. The data reveals that the content of flucloxacillin in brands A, B, C and D declined progressively, with the steepest decline observed at 30°C.

Figure 1 presents the trend of the sample percentage concentration of active ingredient for intermediate temperature, room temperature and refrigerated temperature over the 10-day period. All four brands showed a general trend of decreasing stability as temperature increased. However, some samples stored at intermediate temperatures exhibited higher percentage concentrations than those stored at room or refrigerated conditions. This non-Arrhenius behaviour was construed as out of specification results based on the microbial results obtained for the same samples.

Microbial results

Table 4 summarizes the average zones of inhibition (ZOI) for *Staphylococcus aureus* under different storage conditions. Figure 2 presents the trend of the zones of inhibition of *Staphylococcus aureus* for intermediate, room and refrigerated temperatures over the 10-day period for the four brands. The antimicrobial activity, as indicated by ZOI and the decreasing trend in Table 4 and Figure 2 respectively, was best preserved in samples stored under refrigeration, while significant reductions were observed at intermediate and room temperatures. The reduction in the zones of inhibition, along with the decreasing trend over the 10-day period for all the brands assessed, indicates a decline in flucloxacillin's efficacy when stored at room and intermediate temperatures and preserved efficacy under refrigerated conditions. Table 5 outlines the average zones of inhibition for *Escherichia coli*,

which served as the negative control. Figure 3 presents the trend of the zones of inhibition of *Escherichia coli* for intermediate, room and refrigerated temperatures over the 10-day period. The results confirmed the specificity of flucloxacillin's antimicrobial activity, as no significant inhibition was observed, which is consistent with the known resistance of E. coli to flucloxacillin.

Figure 1:

Trend of Sample Concentration at 25^{0}C, 30^{0}C and 4^{0}C over 10 Days for Four Brands

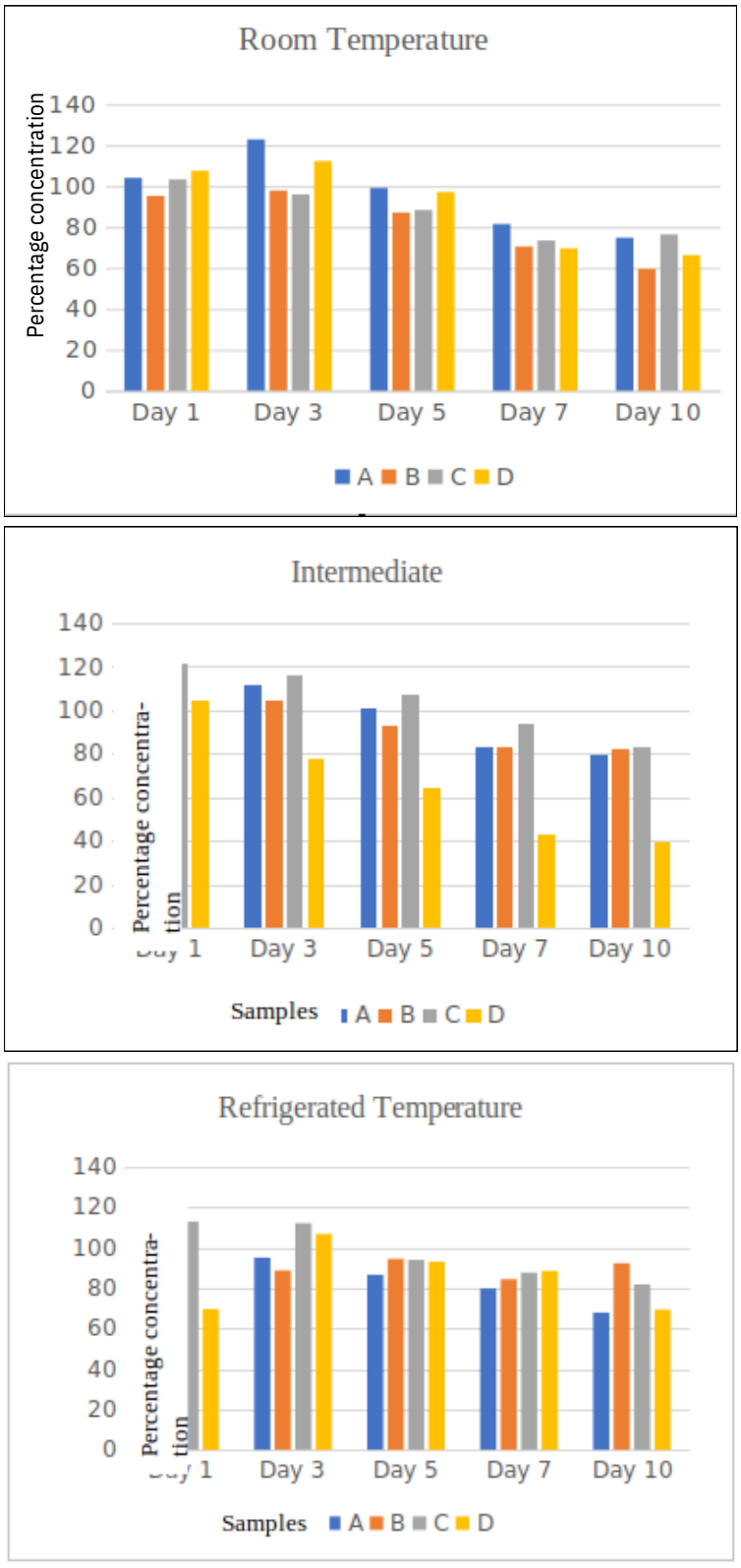


Table 2:

UV-Vis Absorbance Measurements for the Samples at 25^0C, 30^0C and 4^0C

	Room Temperature				Intermediate Temperature				Refrigeration				
Days	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**Std. Abs.**
Day 1	0.909	0.523	0.522	0.522	0.764	0.611	0.615	0.572	0.551	0.379	0.59	1.703	
	0.617	0.434	0.54	0.595	0.63	0.576	0.621	0.521	0.667	0.421	0.568	0.497	
	0.453	0.512	0.733	0.541	0.623	0.634	0.632	0.515	0.572	0.461	0.585	0.219	
Average	0.535	0.490	0.531	0.553	0.627	0.607	0.623	0.536	0.597	0.420	0.581	0.358	0.474
Day 3	0.621	0.464	0.493	0.517	0.544	0.541	0.604	0.26	0.498	0.368	0.567	0.556	
	0.591	0.423	0.496	0.545	0.558	0.525	0.582	0.396	0.482	0.537	0.609	0.58	
	0.687	0.619	0.487	0.666	0.614	0.545	0.595	0.404	0.485	0.461	0.551	0.509	
Average	0.630	0.502	0.492	0.576	0.572	0.537	0.594	0.400	0.488	0.455	0.576	0.548	0.507
Day 5	0.562	0.466	0.504	0.535	0.471	0.513	0.548	0.361	0.451	0.508	0.495	0.496	
	0.543	0.494	0.452	0.529	0.581	0.488	0.579	0.347	0.487	0.52	0.514	0.518	
	0.501	0.451	0.475	0.51	0.58	0.823	0.608	0.336	0.466	0.504	0.515	0.495	
Average	0.535	0.470	0.477	0.525	0.544	0.5005	0.578	0.348	0.468	0.511	0.508	0.503	0.498
Day 7	0.44	0.37	0.365	0.424	0.453	0.449	0.515	0.241	0.431	0.476	0.497	0.518	
	0.452	0.429	0.468	0.411	0.494	0.477	0.531	0.24	0.463	0.481	0.459	0.477	
	0.482	0.39	0.405	0.339	0.453	0.477	0.53	0.238	0.454	0.4468	0.523	0.498	
Average	0.458	0.396	0.413	0.391	0.467	0.468	0.525	0.240	0.449	0.475	0.493	0.498	0.529

Day 10	0.438	0.318	0.368	0.344	0.443	0.467	0.475	0.234	0.422	0.524	0.509	0.394	
	0.393	0.346	0.468	0.382	0.472	0.461	0.47	0.218	0.37	0.546	0.438	0.468	
	0.44	0.348	0.464	0.402	0.439	0.468	0.473	0.209	0.363	0.501	0.446	0.318	
Average	0.424	0.337	0.433	0.376	0.451	0.465	0.473	0.220	0.385	0.524	0.464	0.393	0.555

Table 3:

Percentage Concentration of Flucloxacillin in the Samples at 25^{0}C, 30^{0}C and 4^{0}C

	Room Temperature				Intermediate Temperature				Refrigerated Temperature			
Days	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Day 1	104.01	95.20	103.23	107.44	121.80	118.01	121.05	104.20	116.00	81.72	112.95	69.60
Day 3	122.77	97.83	95.88	112.25	111.47	104.65	115.69	77.95	95.16	88.73	112.18	106.85
Day 5	99.06	87.03	88.26	97.08	100.66	92.61	107.01	64.39	86.60	94.49	94.00	93.08
Day 7	81.43	70.46	73.37	69.57	82.97	83.15	93.40	42.61	79.89	84.45	87.65	88.48
Day 10	74.70	59.47	76.40	66.29	79.57	82.04	83.33	38.85	67.88	92.33	81.86	69.35

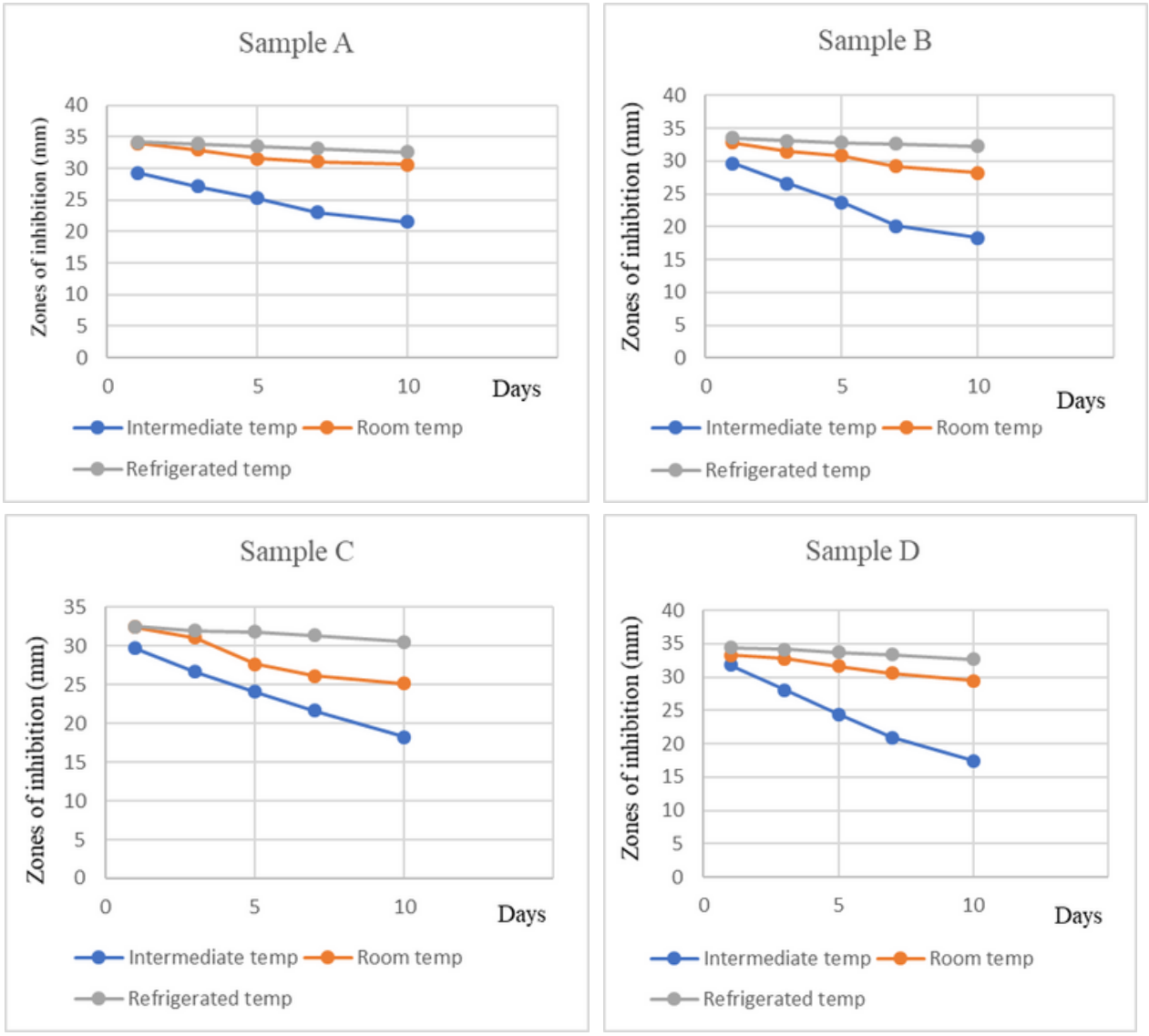
Table 4:

Average Zones of Inhibition of Staphylococcus aureus at 30^{0}C, 25^{0}C, and 4^{0}C

Staphylococcus aureus Zones of Inhibition (mm)												
	Intermediate Temperature				*Room Temperature*				*Refrigerated Temperature*			
Days	***A***	***B***	***C***	***D***	***A***	***B***	***C***	***D***	***A***	***B***	***C***	***D***
1	*29.33*	*29.64*	*29.73*	*31.77*	*33.98*	*32.76*	*32.45*	*33.26*	*34.16*	*33.51*	*32.53*	*34.43*
3	*27.11*	*26.58*	*26.65*	*28.075*	*32.96*	*31.435*	*31.095*	*32.735*	*33.86*	*33.1*	*32.005*	*34.175*
5	*25.285*	*23.695*	*24.065*	*24.39*	*31.52*	*30.81*	*27.67*	*31.605*	*33.49*	*32.77*	*31.875*	*33.7*
7	*23.055*	*20.07*	*21.66*	*20.9*	*31.06*	*29.22*	*26.175*	*30.605*	*33.105*	*32.65*	*31.41*	*33.4*
10	*21.495*	*18.315*	*18.265*	*17.45*	*30.58*	*28.2*	*25.19*	*29.42*	*32.59*	*32.27*	*30.52*	*32.65*

Figure 2:

Trend of the Zones of Inhibition of Staphylococcus aureus at 30^{0}C, 25^{0}C, and 4^{0}C



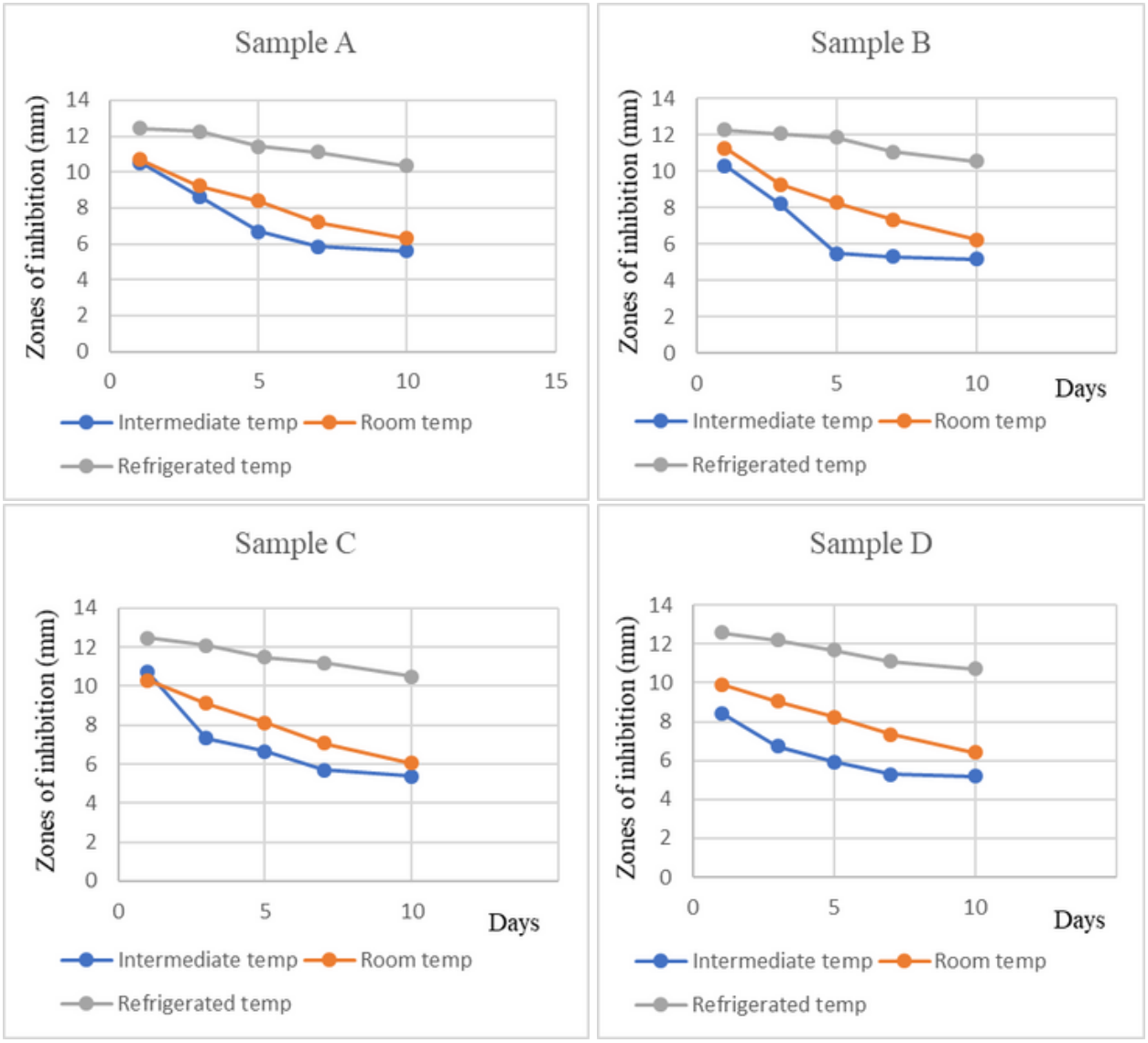
Escherichia coli Zones of Inhibition

Table 5:

Average Zones of Inhibition of Escherichia coli at 30^{0}C, 25^{0}C, and 4^{0}C

***Escherichia coli* Zones of Inhibition (mm)**												
	Intermediate Temperature				**Room Temperature**				**Refrigerated Temperature**			
Days	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	10.535	10.33	10.705	8.435	10.68	11.27	10.29	9.93	12.44	12.29	12.47	12.57
3	8.655	8.19	7.315	6.745	9.225	9.275	9.125	9.045	12.255	12.06	12.085	12.185
5	6.685	5.465	6.66	5.915	8.385	8.265	8.12	8.255	11.43	11.855	11.485	11.685
7	5.84	5.295	5.695	5.275	7.215	7.335	7.085	7.35	11.125	11.07	11.18	11.105
10	5.61	5.18	5.39	5.205	6.295	6.25	6.055	6.41	10.35	10.545	10.475	10.705

Figure 3:
Trend of Zones of Inhibition of Escherichia coli at 30^{0}C, 25^{0}C, and 4^{0}C



DISCUSSION

The British Pharmacopoeia 2019 states that content of flucloxacillin on storage should be 80 to 120% of the label claim. In this study, the UV-Vis spectrophotometric absorbance values of the reconstituted flucloxacillin dry powder suspension decreased with increase in temperature. According to the Arrhenius equation for stability, the reconstituted flucloxacillin samples stored at refrigeration temperature (4°C) should be most stable and show minimal or no degradation during the 10-day storage period as per the label claim. However, in this study, some samples stored at intermediate temperatures exhibited higher percentage concentrations than those stored at both room and refrigeration temperatures, which was contrary to the anticipated degradation patterns. In this research, these values were construed as out of specification (OOS) results, based on the microbial results obtained for the same samples.

An out of specification (OOS) root cause analysis was conducted to identify potential factors contributing to these results (U.S. Food and Drug Administration, 2006). For analysis of flucloxacillin in oral suspension, the USP prescribes high-performance liquid chromatography (HPLC) procedure which is known for its high sensitivity, specificity, and selectivity. The UV method used to determine the content of API in this study may have contributed a systematic error in regard to accuracy and reproducibility of absorbance measurements. Also, one of the samples was very viscous, hence the possibility of viscosity measurement errors for small volumes. Accurate volume measurements are critical in analysis as they minimize variability and enhance reproducibility. Furthermore, volume

measurement bias of the experimenters may have contributed to the OOS results. The OOS investigation highlighted areas that require improvement to ensure the accuracy of the chemical analysis results.

The microbial analysis results indicate the ability of flucloxacillin to inhibit the growth of *Staphylococcus aureus* over a period of time under various storage conditions. The zones of inhibition are measured in millimeters (mm). The Clinical and Laboratory Standards Institute (CLSI) provides breakpoints to interpret the zones of inhibition, which help to assess antimicrobial effectiveness. According to CLSI guidelines for flucloxacillin, the susceptibility threshold is as follows: resistance is 17 mm, intermediate resistance is 18-20 mm and susceptible microorganisms have a ZOI of 21 mm (Clinical and Laboratory Standards Institute, 2020). The trends that were observed indicate that antimicrobial activity was preserved at refrigerated temperature and significant reduction occurred at intermediate temperature:

- At refrigerated temperature (4°C), the zones of inhibition remained relatively consistent over the 10-day period despite the slight decrease. For example, the zones of inhibition for brand A against *Staphylococcus aureus* at day 1 and 10 were 34.16 and 32.59 respectively. This indicates that the antimicrobial activity of flucloxacillin is preserved when stored under refrigerated temperature. According to CLSI standards, zones of inhibition for flucloxacillin against *Staphylococcus aureus* should be ≥ 21 mm to be considered susceptible. The results consistently meet or exceed this threshold, indicating effective antimicrobial activity.
- At room temperature (25°C), a noticeable decrease in the zones of inhibition over the 10-day period was observed. The antimicrobial activity was initially comparable to that observed in refrigerated temperature, but a gradual decrease indicates that the efficacy of flucloxacillin diminishes over time at room temperature.
- At intermediate temperature (30°C), a reduction in the zones of inhibition is observed within the first 5 days, and the trend continued to decline sharply over the 10-day period. This indicates that flucloxacillin rapidly loses its antimicrobial effectiveness when stored at higher temperatures. The zones of inhibition quickly drop below the CLSI susceptibility threshold, indicating that the drug becomes ineffective.

The negative control helped in understanding the specificity of flucloxacillin against its intended target, *Staphylococcus aureus*, and ensured that the observed antimicrobial effects were not due to nonspecific inhibition. *Escherichia coli* was used as the negative control. The findings confirmed that flucloxacillin's antimicrobial effects were specific and did not inhibit *Escherichia coli.*

The consistent zones of inhibition observed at refrigerated conditions (4°C) highlight that flucloxacillin is most stable and effective when stored in a refrigerator. This stability ensures that the drug remains above the CLSI susceptibility threshold, preserving its therapeutic efficacy.

The gradual reduction in antimicrobial activity at room temperature conditions suggests that flucloxacillin undergoes some degree of degradation at 25°C, reducing its effectiveness. While it remains somewhat effective over the 10-day period, the declining trend and eventual drop below the CLSI threshold indicate a risk of insufficient dosage if not used within a shorter period.

The sharp decline in antimicrobial activity at 30°C is indicative of significant degradation of flucloxacillin. The rapid loss of effectiveness at this temperature makes it unsuitable for storage in warmer climates or conditions where refrigeration is not available, as the drug quickly falls below the CLSI susceptibility threshold.

The results demonstrate the importance of proper storage conditions to maintain the efficacy of flucloxacillin dry powder suspension. Inadequate storage, particularly at higher temperatures, can lead to a substantial loss of antimicrobial activity, resulting in therapeutic failure and contributing to antimicrobial resistance.

CONCLUSION

This study concludes that the decrease in content of the drug substance and the antimicrobial activity of flucloxacillin at room and intermediate temperatures suggests that poor storage is a risk factor that may lead to subtherapeutic dosing. The microbial analysis indicates that reconstituted flucloxacillin should be stored in a refrigerator (2-8°C) to maintain its antimicrobial efficacy over the 10-day period. If stored at room temperature (25°C), the suspension should be used within 7 days. Storage

at high temperatures (30°C) should be avoided to prevent rapid degradation and loss of antimicrobial activity.

RECOMMENDATIONS

Based on the OOS investigation results, a repeat experiment is recommended, taking into account the corrective actions to eliminate the possible causes of error. Extended studies are recommended to evaluate the stability of flucloxacillin suspensions beyond 10 days. This will help to establish comprehensive guidelines for storage duration and conditions.

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