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In vitro bioequivalence profile of Penicillin V tablets marketed in Kisangani, Democratic Republic of the Congo: Assay and dissolution kinetics of generic 250 mg and originator 600 mg

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ABSTRACT

Introduction

Phenoxymethylpenicillin is commonly presented in various generic forms and rarely as proprietary brands. Given the prevalence of counterfeit products and the increased use of generic drugs in sub-Saharan Africa, ensuring drug quality is critical to meet legal requirements for bioequivalence and interchangeable dispensation.

Purpose

The purpose of this in vitro study was to meticulously compare the bioequivalence of 250 mg generic Penicillin V tablets with an originator brand marketed in Kisangani, focusing on physicochemical properties and dissolution kinetics based on European Medicines Agency (EMA) standards and USP Pharmacopoeia (USP 43-NF 38, 2013) guidelines.

Methods

Identification and assay were performed using a UV-visible spectrophotometer, and dissolution kinetics were conducted at pH levels of 1.2, 4.5, and 6.8 with appropriate dissolution equipment. The fit factor method was applied to compare dissolution profiles across these pH levels.

Results

The findings are significant, revealing that while the generic tablets weighed half as much (340 mg) as the originator tablets (770 mg), both showed acceptable mass uniformity and contained approximately 250 mg of active ingredient. Dissolution profiles were comparable, with difference factors (f1) < 15 and similarity factors (f2) > 50. In acidic media (pH 1.2 and 4.5), full release occurred in under 15 minutes, while in basic intestinal-type medium (pH 6.8), an 80% release rate was observed.

Conclusion

These results support the interchangeability of the generic and originator Penicillin V products, while potential in vivo variations cannot be ruled out.

INTRODUCTION

A pharmaceutical equivalent drug has the same active ingredient, dosage, concentration/strength, and route of delivery as a brand drug. Two drugs are considered bioequivalent if they are metabolized and processed by the body in the same way. This issue was addressed with the introduction of generic medications in the 1960s, which are growing in popularity (Abelli et al., 2001). Compared to generic formulations, brand-name drugs are often significantly more expensive. Thus, it is imperative that generic medications be widely prescribed to reduce the overall cost of care for patients (Abelli & Becart, 2009). In developing countries, it is recommended that less expensive, non-innovative medications be used to lower healthcare costs. However, concerns persist about the quality of generic medications, as the prevalence of counterfeit drugs is high. Worldwide, efforts are underway to establish quality control systems for generic medications that are safe, accessible, and reliable (Brossard, 2006).

In the Democratic Republic of the Congo (DRC), a developing nation where much of the population lives in poverty, there is a pressing need for lower-cost, high-quality generic drugs. Studies have shown the presence of substandard products, including antibiotics that are adulterated or under-dosed, despite clinical evidence that the efficacy of antibiotics depends on a direct correlation between their pharmacokinetics and pharmacodynamics (Naseem et al., 2018; Cardenas, 2016). The increasing prevalence of counterfeit drugs in low-resource settings such as the DRC necessitates rigorous evaluation of generic medications to ensure their safety and efficacy (Mahano et al., 2021). Phenoxymethylpenicillin, one of the most prescribed antibiotics for Gram-positive bacterial infections in the DRC, is frequently presented in various generic forms and rarely in proprietary brands. For such a widely used antibiotic, especially in underdeveloped regions, there is a high risk of adulteration (Moreno et al., 2007). Studies have been conducted to evaluate the bioequivalence of penicillin V; for example, Moreno et al. (2007) published a comparative study examining this parameter in two phenoxymethylpenicillin brands. Piovani et al. (2015) compared antibiotherapeutic outcomes between children treated with brand-name and generic medications, finding that 17.5% of children (57,346) received at least one recurrent prescription. The rate of regular prescriptions was slightly lower in children receiving a generic antibiotic (OR 0.96; 95% CI [0.93, 0.98]) compared with a brand-name antibiotic. The percentage of hospitalizations in children initially treated with brand (1.01%; 95% CI [0.98, 1.08]) or generic (1.03%; 95% CI [0.96, 1.06]) antibiotics did not significantly differ (p = .43). In children receiving amoxicillin-clavulanate, the hospitalization rate was slightly higher in the brand-name group (p = .002), while there was no difference for other active pharmaceutical ingredients.

According to the Biopharmaceutical Classification System (BCS) and its relevance to bioequivalence, in vivo bioequivalence studies might not be necessary for solid dosage forms of some medications, including penicillin V (BCS, 2001). Nevertheless, measuring pharmaceutical equivalence is crucial in predicting bioequivalence. This study aims to compare Penicillin V 250 mg generic tablets with the brand Ospen® 600 mg marketed in Kisangani, focusing on identification, quantitative assay, and dissolution kinetics, using standards outlined by the European Medicines Agency (EMA), United States Pharmacopoeia (USP), and British Pharmacopoeia (BP).

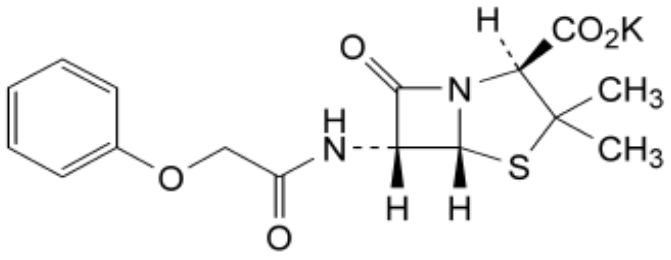
METHODS

Study Design and Site

This experimental study focused on comparing an originator drug with two generic Penicillin V brands available in various pharmacies in Kisangani City. The laboratory tests were conducted at the "Laboratoire National de Contrôle Qualité" (LNCQ-LAPHAKI) in Kinshasa, DR Congo.

Tested Drugs

Figure 1:
Penicillin V Chemical Structure



Penicillin V potassium (see **Figure 1**) is a beta-lactam antibiotic classified under BCS Class 3 due to its low

intestinal permeability and high solubility (Ulbach, 2017). The two generic tablet preparations were sourced from community pharmacies, while the branded Ospen® was obtained from Sandoz GmbH, Kundl, Austria. For identification purposes, a Penicillin V raw powder (Batch number: 200618047; Date of manufacture: 11/2018; Expiration date: 10/2022) was used. It appeared as a white microcrystalline powder, soluble in water but practically insoluble in methanol, with a pH of 7.24 and an absorbance of 0.2870.

Chemicals and Reagents

The reagents included an acid buffer at pH 1.2 (KCl and HCl), an acetate buffer at pH 4.5 (acetic acid and Na acetate), and a phosphate buffer at pH 6.8 (Na-dihydrogen phosphate and K-dihydrogen phosphate), all provided by CUPRO lab (Lemba-Kinshasa, DRC). Ultra-pure distilled water was produced in the lab using a Milli-Q ultrapure device, and Penicillin V raw material (R.M.) was used as the chemical reference substance (CRS) supplied by North China Pharmaceutical (Shandong).

Equipment

The equipment included an analytical balance (FA2004N), an ultrasonic bath (LPK/SA-05), a test dissolver (DISS-06, Hangzhou, China), a UV-visible spectrophotometer (ZUZI 4481/1), and standard laboratory materials (volumetric flasks, beakers, Erlenmeyer flasks, volumetric pipettes, syringes, watch glasses, petri dishes, spatulas, mortar and pestle, graduated cylinders, etc.).

Preparation of Solutions

1. **Buffer Solution at pH 1.2:** To prepare, add 50 ml of distilled water in a 200 ml volumetric flask, followed by 50 ml of 0.2N KCl (prepared by weighing 1.49 g in 100 ml) and 85 ml of 0.2N HCl (prepared by weighing 0.73 g in 100 ml), then adjust to the mark with distilled water.
2. **Buffer Solution at pH 4.5:** Mix 11.55 ml of concentrated 99.7% acetic acid in 1L of distilled water to make a 0.2M solution (S1). Weigh 16.4g of Na acetate in 1L of distilled water to make a 0.2M solution (S2). Then, mix 46.3 ml of S1 and 3.7 ml of S2 in a 100 ml volumetric flask, and adjust with distilled water.
3. **Buffer Solution at pH 6.8:** In a 1L volumetric flask, add 250 ml of distilled water, 28.80g Na dihydrogen phosphate, and 11.45g potassium dihydrogen phosphate. Use a Vortex mixer and magnetic stirrer for homogenization, then adjust to the mark with distilled water.
4. **Reference Solution (Raw Material):** On a dry watch glass, weigh 100 mg of Penicillin V, place it through a funnel into a 25 ml volumetric flask, and fill to the mark with distilled water, achieving a concentration of 80 µg/ml. Perform three independent tests.
5. **Originator Drug:** Crush 10 Penicillin V tablets, weigh 100 mg of OSPEN® on a watch glass, place it in a 100 ml flask, dissolve in distilled water, and ultrasonicate for 10 minutes. Transfer 2 ml of this solution to a 25 ml flask and fill with distilled water for an 80 µg/ml concentration. Perform three independent tests.
6. **Generic Sample Solutions:** Follow the same procedure as for the originator drug to prepare an 80 µg/ml concentration. Perform three independent tests.
7. **Solutions for Dissolution Tests:** Draw 10 ml of the dissolving solution at specified intervals (15, 30, 45, 60 minutes), place 4 ml in a 25 ml flask, and adjust with distilled water to reach 80 ppm Penicillin V. Perform three independent tests.

Identification

To identify Penicillin V, add 2 mg of powder, 0.05 ml of water, and 2 ml of sulfuric acid-formaldehyde to a test tube. Stir the mixture until it turns reddish-brown, then submerge the test tube in water for one minute, where a reddish-brown hue will appear.

Quantification and Comparability

To ensure accuracy per ISO 17025/2015 standards, we followed USP (43-NF 38, 2013) and B.P. (2013) procedures for sample assay and raw material assay, respectively. Solutions were analyzed using a UV-visible spectrophotometer at a 268 nm wavelength. Equation 2 was applied to calculate f1 and f2 values, confirming dissolution kinetic profiles with $f1 < 15$ and $f2 > 50$.

Equation 1:
Formula to calculate f1 and f2

$$f_1 = \frac{\sum_t^n (R_t - T_t)}{\sum_t^n R_t}$$

$$f_2 = 50 \times \log\left(\left[1 + \frac{1}{n}\sum_t^n (R_t - T_t)^2\right]^{-0.5} \times 100\right)$$

RESULTS

Identification

As shown in Table I, the identification test was positive for all samples, confirming the presence of the target compound in the analytical samples for both the originator drug and generics. All analyzed samples conformed to the standards of the American Pharmacopoeia.

Table 1:
Identification Test and Quantitative Assay for Raw Material and Samples

Products	ID test	Percentage	Specifications	Reference	Decision
Raw material	Positive	101.30%	98.00 – 102.00 %	(BP 2013)	Conform
Ospen®	Positive	109.70%	90.00 – 120.00 %	(USP 43-NF 38, 2013)	Conform
Generic 1	Positive	109.30%	90.00 – 120.00 %	(USP 43-NF 38, 2013)	Conform
Generic 2	Positive	115.50%	90.00 – 120.00 %	(USP 43-NF 38, 2013)	Conform

Mass Uniformity

Table 2 presents the mass uniformity test results for the originator drug and generics 1 and 2. None of the masses deviated from the specification by more than ±5%. Similar to Ospen®, both generic products passed the mass uniformity test.

Table 2:
Comparative Mass Uniformity of the Reference and Generics

	Ospen 600 mg			Generic 1 250 mg			Generic 2		
Tablet	*Weight (mg)*	*Mean %*	*SD (%)*	*Weight (mg)*	*Mean %*	*SD (%)*	*Weight*	*Mean %*	*SD (%)*
1	772.5	100.19	-0.19	343	98.5	1.5	356.1	101.48	-1.48
2	769.2	99.76	0.24	348.7	100.13	-0.13	348.8	99.4	0.6
3	767.6	99.56	0.44	350.1	100.53	-0.53	357.2	101.79	-1.79
4	774.5	100.45	-0.45	344.5	98.92	1.08	351.1	100.05	-0.05
5	776.4	100.7	-0.7	340.8	97.86	2.14	348.3	99.26	0.74
6	769	99.74	0.26	353.5	101.51	-1.51	350.8	99.97	0.03
7	763.7	99.05	0.95	353	101.36	-1.36	350.7	99.94	0.06
8	773.9	100.37	-0.37	341.5	98.06	1.94	349.6	99.63	0.37
9	771.3	100.03	-0.03	342.5	98.35	1.65	342.5	97.6	2.4
10	771.8	100.1	-0.1	344.8	99.01	0.99	353.4	100.71	-0.71
Mean	770.99	100	-0.95 +0.70	348.2	99.42	-1.92 +1.52	350.9	99.98	-2.39 +1.80

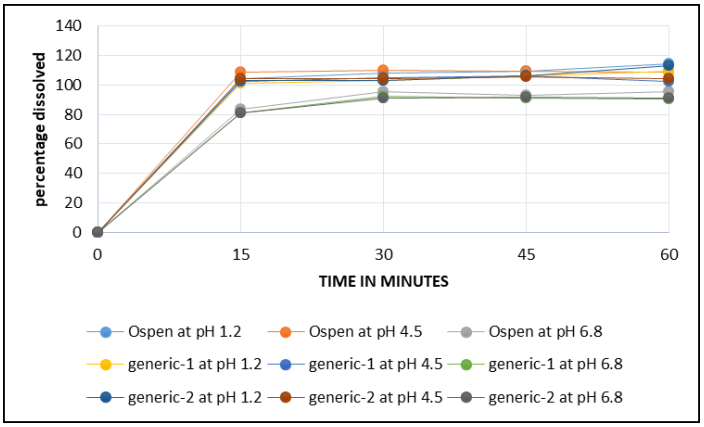
Dissolution Tests

Table 3 and Figure 2 illustrate the dissolution profiles of the originator and generic tablets at different pH levels. After 15 minutes, dissolution reached nearly 100% at pH 1.2 and 4.5, but was approximately 80% at pH 6.8.

Table 3:
Dissolution Kinetics: t-Test for Two-Sample Assuming Unequal Variance

		Time				*Statistical difference*	
pH	**Sample**	**15 min**	**30 min**	**45 min**	**60 min**	**MATCHING**	**Significance *t*- test**
1.2	Ospen	104.59	108.31	109.59	114.46		
	Generic 1	101.49	102.98	106.56	109.12	OS1.2/G11.2	0.008
	generic-2	102.87	102.83	106.45	113.15	OS1.2/G21.2	0.053
4.5	Ospen	108.65	109.74	109.48	108.64	OS1.2/OS4.5	0.961
	Generic-1	102.75	104.99	106.02	102.32	OS4.5/G1.4.5	0.004
	Generic-2	104.48	104.6	105.72	104.18	OS4.5/G2.4.5	0.326
6.8	Ospen	83.72	95.56	93.21	95.80	OS1.2/OS6.8	0.002
	Generic 1	80.77	92.30	91.27	90.36	OS6.8/G1.6.8	0.019
	Generic-2	80.94	91.31	91.83	91.29	OS6.8/G2.6.8	0.021

Figure 2:
Dissolution Kinetics



To validate our results and compare the profiles of each generic with the originator drug, we employed the "fit factor" method, calculating the difference factor (f1) and similarity factor (f2). This method typically requires 12 tablets; however, we conducted the test with 5 tablets for each generic. Two dissolution curves are considered similar if the f1 factor is less than 15 and the f2 factor is 50 or greater. Here, n represents the number of sampling points (n=4 in this study), Rt denotes the reference dissolution at time t, and Tt denotes the test form dissolution at time t. Using the t-test, we found a significant difference between Ospen® and the generics in a pH 6.8 medium, while there was no significant difference ($p = 0.537$) between the two generics.

DISCUSSION

Pharmaceutical products should not be dispensed in pharmacies without marketing authorization. For instance, in Europe, the European Medicines Agency (EMA) or

national agencies authorize pharmaceuticals under a centralized authorization system (Piovani et al., 2015; Grace et al., 2019; Even-Adin, 2002). The Regulatory Affairs Manager, responsible for launching a product on the market, must apply to the relevant authority in each nation to obtain marketing authorization. A marketing authorization dossier, prepared in compliance with European standards and protocols (Annex to Directive 2001/83/EC), must be submitted to registration authorities for review and approval (Naseem et al., 2018; Niwa et al., 2016; Sawaya, 2012; Rahim & Naqvi, 2024).

For two medications containing the same active pharmaceutical ingredient (API) to be considered similar, bioequivalence parameters must align with regulatory standards. Generics, as copies of the original medication, must meet the same purity, potency, and stability standards, as mandated by pharmacopoeia, unless exceptions apply (e.g., alternative synthesis routes or formulations that may alter impurity or degradation profiles). Bioequivalence studies evaluate the resemblance between the original drug and the generic, based on factors such as dissolution, solubility, and permeability (Rahim et al., 2024).

In the Democratic Republic of the Congo (DRC), there is limited evidence that all medications in pharmacies hold formal marketing authorization. However, sporadic checks, such as those conducted in this study, are useful in ensuring interchangeability between drugs from different sources. Our analysis of three randomly selected Penicillin V samples showed that the generics and Ospen® had similar characteristics based on reference pharmacopoeia standards. The dissolution kinetic profiles were also comparable, with difference factors (f1) less than 15 and similarity factors (f2) greater than 50. In acidic media (pH 1.2 and 4.5), the content was released within 15 minutes, while in a basic, intestinal-type medium (pH 6.8), 80% dissolution was achieved. The fit factor method, applied across three pH levels, confirmed the bioequivalence of these products.

Annke et al. (1998) evaluated three examples of immediate-release drugs containing phenoxymethylpenicillin potassium, glimepiride, and levofloxacin, each with distinct solubility characteristics. According to the biopharmaceutics classification system, phenoxymethylpenicillin potassium and levofloxacin showed high solubility, while glimepiride was less soluble. Based on in vitro and in vivo comparisons, as well as biopharmaceutical classification, Annke et al. recommended dissolution acceptance criteria: 80% in 30 minutes for phenoxymethylpenicillin potassium, 80% in 15 minutes for glimepiride, and 80% in 30 minutes for levofloxacin. Typically, a dissolution limit ensures active ingredient release within a specified time for immediate-release formulations. Phenoxymethylpenicillin potassium and levofloxacin, both classified as Class 1 drugs, do not necessitate an in vitro/in vivo correlation, as their absorption may be gastric emptying-dependent. In contrast, glimepiride is categorized as Class 2, where an in vivo dissolution profile correlation is not expected due to low pH solubility.

Priyanka et al. (2017) assessed the bioequivalence of generic and branded amoxicillin capsules in healthy volunteers. They analyzed log-transformed pharmacokinetic (PK) parameters (Cmax, AUC0–t, AUC0–∞) using a two one-sided test ANOVA in SAS, while Tmax and minimum inhibitory concentration (MIC) were assessed with the Wilcoxon rank-sum test in GraphPad Prism. The geometric mean ratio for Cmax met bioequivalence criteria, while the confidence limits for AUC0–t and AUC0–∞ fell below the criteria. The time above MIC for the generic was significantly shorter than for the branded version.

CONCLUSION

This research aimed to enhance quality and biopharmaceutical control for drugs marketed in Kisangani, focusing on Penicillin V tablets. The in vitro dissolution test revealed similar dissolution kinetics between the generics and originator drug at pH 1.2, 4.5, and 6.8, as similarity factors (f2) were all above 50. The study confirmed the interchangeability requirements for these Penicillin V products, while acknowledging potential in vivo variation. This research underscores the potential impact of such studies on drug policy and patient care in regions like Kisangani, where counterfeit and generic medications are widespread.

Ethics Approval: Nil required.

Conflicts of Interest: None declared.

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Borive, A. M.[1]: Nil identified
Uwizeyimana, F.[3]: Nil identified
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Kadima, N. J.[4]: Nil identified
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