



QUALITY AND *IN-VITRO* BIOEQUIVALENCE EVALUATION OF COMMERCIAL ASCORBIC ACID TABLETS IN ZARIA METROPOLIS, KADUNA STATE, NIGERIA

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ABSTRACT

Ascorbic acid (vitamin C) is an essential nutrient widely used as a dietary supplement. With the increasing availability of multiple generic brands in the market, assessing their quality and therapeutic equivalence is crucial to ensure patient safety and efficacy. This study aimed to assess the *in-vitro* bioequivalence of eight commercial brands of ascorbic acid tablets (coded VC01–VC08) available in Zaria, Nigeria. Physicochemical properties were analyzed using UV-Visible spectrophotometry, and dissolution studies were conducted in simulated physiological media (pH 1.2, 4.5, and 6.8) using USP Apparatus II (paddle method) at 100 rpm and 900 mL media volume, maintained at 37 ± 0.5 °C. The dissolution profiles were compared using model-independent approaches: difference factor (f_1) and similarity factor (f_2). Results showed that brands VC02 and VC07 met the bioequivalence criteria with $f_2 > 50$ and $f_1 < 15$ across all three media. Brands VC03, VC05, VC06, and VC08 failed to meet these criteria, while VC04 had variable results depending on pH. Dissolution efficiencies (D.E %) and assay results also supported the findings, with only VC02 and VC07 showing acceptable drug release and content within official limits. The study concludes that VC02 and VC07 are bioequivalent to the comparator brand VC01 and may be used interchangeably.

Keywords: Ascorbic acid, dissolution, fit factors, generic, *in-vitro* bioequivalence, innovator brand, physicochemical parameters

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INTRODUCTION

Ascorbic acid (vitamin C) is a water-soluble vitamin with diverse biological functions, including antioxidant activity, collagen synthesis, and enhancement of immune response [1, 2]. Owing to its role in health maintenance and disease prevention, it is among the most widely consumed over-the-counter supplements globally. This high demand has led to a proliferation of generic brands, particularly in developing countries like Nigeria, where quality assurance practices can vary significantly [3].

Generic substitution is acceptable only if products are pharmaceutically equivalent and bioequivalent to the innovator brand [4]. While regulatory authorities mandate compliance with pharmacopeial standards, reports of substandard and therapeutically inequivalent medicines in circulation remain a public health concern [5]. *In-vitro* bioequivalence testing, including dissolution profiling, serves as an important surrogate for *in-vivo* bioavailability studies, particularly for drugs like ascorbic acid that are highly soluble and permeable [6].

This study was therefore undertaken to assess the *in-vitro* bioequivalence of various brands of ascorbic acid tablets marketed within Zaria metropolis, Kaduna

State. By comparing their physicochemical properties and dissolution profiles against pharmacopeial specifications and a reference standard, the study aimed to determine whether marketed products are therapeutically interchangeable, thereby providing data relevant to consumer safety and regulatory oversight.

MATERIALS AND METHODS

Sample collection: Eight different brands of ascorbic acid (vitamin C) tablets, each labeled to contain 100 mg of ascorbic acid, were randomly purchased from community pharmacies within Zaria metropolis, Kaduna State, Nigeria. The brands were coded as VC01 – VC08 to ensure anonymity during analysis.

Reagents and equipment: Analytical grade reagents and distilled water were used throughout the study. Ascorbic acid reference standard powder (99.9% purity) was used as the standard. The equipment employed included an analytical balance (Mettler Toledo), UV-Visible spectrophotometer (ShimadzuUV-1800), Roche friabilator, melting point apparatus, and USP dissolution apparatus II (paddle method).

Method development and validation

Three UV–Spectrophotometry methods for the determination of ascorbic acid in simulated physiological media (pH 1.2, 4.5 and 6.8) were developed and validated according to ICH guideline 2006 as follows:

Preparation of the dissolution medium

All three test medium of pH 1.2, 4.5 and 6.8 were prepared as specified in the USP/BP.

pH 1.2 buffer: A quantity (8.33 mL) of 12 N Hydrochloric acid was measured and was transferred into 1000 mL volumetric flask containing distilled water and the volume was made up to the mark with distilled water. This was then labeled as 0.1 N HCl (pH 1.2 buffer).

pH 4.5 buffer: 2.99 g of sodium acetate was weighed and dissolved in 1000 mL volumetric flask containing 200 mL of distilled water, then 14 mL of 2 N acetic acid was added and volume was made up to the mark with distilled water. The pH was adjusted to 4.5 using 0.2 N NaOH or HCl as the case may be.

pH 6.8 buffer: 50 mL of 0.2M monobasic potassium phosphate (KH_2PO_4) solution was placed in a 200 mL volumetric flask, then 22.40 mL of 0.2 M of sodium hydroxide (NaOH) was added and volume was made up to the mark with distilled water. The pH was adjusted to 6.8 using 0.2 N NaOH or HCl as the case may be.

Preparation of ascorbic acid stock solution

Stock solutions (100 $\mu\text{g}/\text{mL}$) were prepared by dissolving 10 mg of ascorbic acid standard powder in 100 mL of each of the prepared simulated physiological media (pH 1.2, 4.5 and 6.8).

Determination of wavelength of maximum adsorption

The wavelengths of ascorbic acid maximum absorption in the various media were obtained by scanning the 16 $\mu\text{g}/\text{mL}$ solutions of the drug in the various media against their respective blanks.

Construction of calibration curve

A six-point ascorbic acid calibration curve in each medium was constructed by preparing solution of concentrations 2, 4, 8, 16, 32, 64 $\mu\text{g}/\text{mL}$ in the different simulated physiological media using serial dilution. The developed methods were validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and specificity as described below.

Linearity

Standard solutions of ascorbic acid were prepared at appropriate concentration ranges in each dissolution medium (pH 1.2, 4.5, and 6.8). The absorbance of each solution was measured at the predetermined maximum wavelength (λ_{max}). Calibration curves were constructed by plotting absorbance against concentration, and linear regression analysis was performed to determine the correlation coefficient (R^2).

Accuracy

Accuracy of the methods was evaluated using the recovery method at three concentration levels (80%, 100%, and 120%) of the nominal concentration. The percentage recovery of ascorbic acid was calculated for each level.

Precision

Precision was assessed in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was evaluated by analysing six replicate samples on the same day, while inter-day precision was determined over three consecutive days. Results were expressed as percentage relative standard deviation (%RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve using ICH-recommended equations.

Specificity

Specificity was assessed by evaluating the ability of the method to measure ascorbic acid in the presence of tablet excipients without interference at the analytical wavelength.

Dissolution studies

In-vitro dissolution testing was carried out using USP apparatus II (paddle), in 900 mL of 0.1 N HCl (pH 1.2), acetic buffer (pH 4.5) and phosphate buffer (pH 6.8) all at 37 ± 0.5 °C, paddle speed 100 rpm. Samples (5 mL) were withdrawn at 5, 10, 15, 30, 45 and 60 minutes, filtered (using Whatman filter paper No. 1), and analyzed spectrophotometrically at 242, 256 and 264 nm respectively. Dissolution studies were conducted in the three simulated physiological media and the data generated was used for bioequivalence evaluation.

Bioequivalence evaluation: Dissolution profiles were compared with the reference brand using difference factor (f_1) and similarity factor (f_2) in each of the three simulated physiological media. A generic product was considered bioequivalent to the reference (VC01) if it met the following in all three media: $f_1 \leq 15$, $f_2 \geq 50$, and D.E. within $\pm 10\%$ of the reference [8].

$$f_1 = \frac{\{[\sum t = 1 n |R_t - T_t|] / [\sum t = 1 n R_t]\} \cdot 100}{(1)}$$

$$f_2 = 50 \cdot \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^n n (R_t - T_t)^2 \right] \right\} \quad (2)$$

$$D.E = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \cdot x \cdot (t_2 - t_1)} \times 100 \quad (3)$$

Data analysis

The dissolution profiles of the generics and innovator brand were generated graphically and the fit factors

(difference factor (f_1) and similarity factor (f_2)) and dissolution efficiency (% D.E.) model were calculated using Microsoft Excel, 2016.

RESULTS AND DISCUSSION

All samples were locally manufactured in Nigeria within their expiry dates as at the time the study was conducted (table 1). The samples were uncoated, round in shape and red in colors (except VC04, which was white) and exhibited no physical defects such as cracks or discoloration. All the samples have manufacturing dates, expiry dates, NAFDAC registration numbers and batch numbers.

Table 1: Labelled information of the various ascorbic acid tablet samples

SN	Brand Code	Source	Batch No.	MFG Date	Exp. Date	NAFDAC Reg. No.
1	VC01	Nigeria	3447C	10/2023	10/2026	04-4053
2	VC02	Nigeria	T2409279	07/2024	06/2027	04-472
3	VC03	Nigeria	CC351	07/2024	12/2026	04-5000
4	VC04	Nigeria	04-5270	07/2023	07/2025	VCW23143
5	VC05	Nigeria	SC24211	06/2024	05/2027	A11-100422
6	VC06	Nigeria	055	03/2024	03/2027	A4-7699
7	VC07	Nigeria	IBBN-04161	04/2023	04/2026	B4-0280
8	VC08	Nigeria	10506924	06/2024	07/2027	04-2333

Figures 1-3 show the UV absorption spectrum for ascorbic acid in pH 1.2, 4.5 and 6.8 with maxima at 242, 256 and 264 nm respectively. The three methods developed for the quantification of ascorbic acid exhibited strong linearity in accordance with Beer-Lambert’s law. The calibration curves (Figures 4-6) showed a direct proportional relationship between absorbance and concentration, with correlation coefficients (R^2) close to unity, confirming the suitability of the methods.

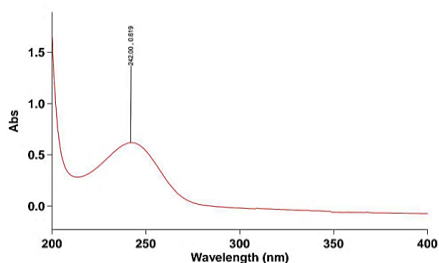


Figure 1: UV spectrum of ascorbic acid in buffer solution pH 1.2

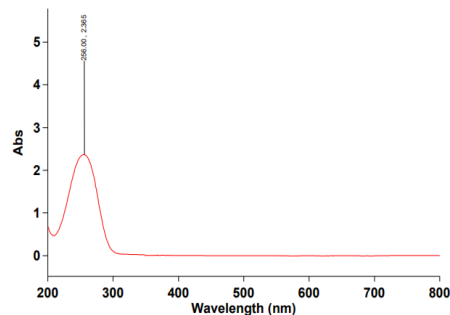


Figure 2: UV spectrum of ascorbic acid in buffer solution pH 4.5

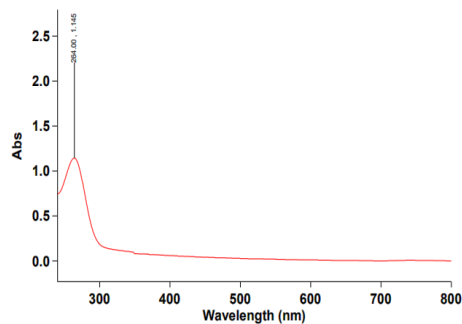


Figure 3: UV spectrum of ascorbic acid in buffer solution pH 6.8

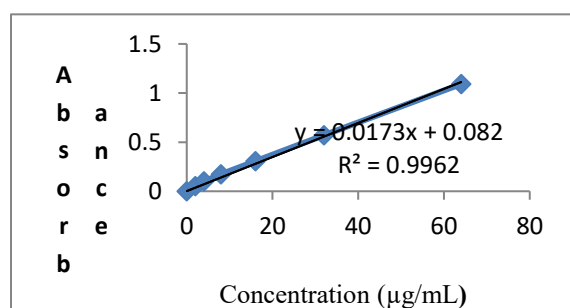


Figure 4: Calibration curve of ascorbic acid in pH 1.2

The therapeutic effectiveness of a dosage form is a function of the amount of drug released into the body fluids, followed by its absorption into the circulatory system [13], thus making the *in-vitro* dissolution (bioequivalence) studies of solid dosage form imperative and by extension determination of the dissolution rate of a dosage form in mimicking the physical action of ingested drugs.

Table 2: Accuracy and Percentage recovery of Method 1 (Ascorbic acid in 0.1 N HCl pH 1.2)

SN	Amount added	Amount found (µg/ml) n = 3	Mean Rec (%) (µg/ml) n = 3	% Er (µg/ml) n = 3
1	20	18.49	92.45	1.13
2	25	26.53	106.12	6.12
3	30	31.69	105.63	5.63

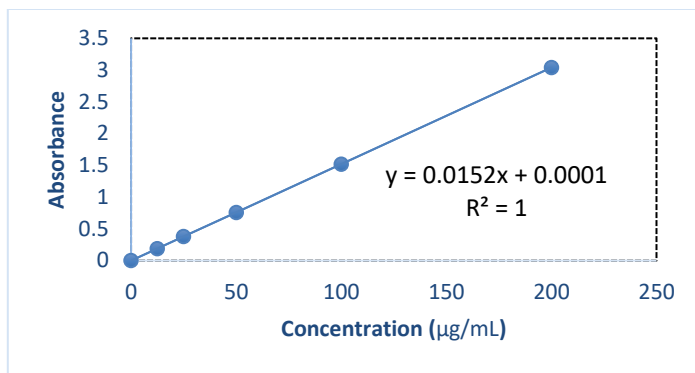


Figure 5: Calibration curve of ascorbic acid in pH 4.5

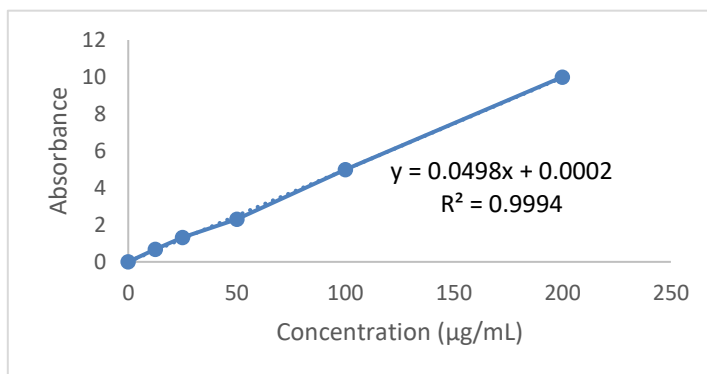


Figure 6: Calibration curve of ascorbic acid in pH 6.8

Table 3: Accuracy and percentage recovery of method 2 (Ascorbic acid in acetic buffer pH 4.5)

SN	Amount added	Amount found (µg/ml) n = 3	Mean (Rec) (µg/ml) n = 3	% Recovery (µg/ml) n = 3
1	20	19.32	96.6	3.40
2	25	24.68	98.72	1.28
3	30	28.06	93.53	6.47

Table 4: Accuracy and percentage recovery of method 3 (Ascorbic acid in phosphate buffer pH 6.8)

SN	Amount added	Amount found (µg/ml) n = 3	Mean (Rec) (µg/ml) n = 3	% Rec (µg/ml) n = 3
1	20	20.47	102.35	2.35
2	25	24.11	96.44	3.56
3	30	28.45	94.83	5.17

The methods were accurate due to large percentage recovery which range from 92.45 to 106.12 with a minimal error of 1.13 to 6.12 % in pH 1.2, also 93.53 to 98.72 with a minimal error of 1.28 to 6.47 % in pH 4.5 and 94.83 to 102.35 with a minimal error of 2.35 to 5.17 % in pH 6.8.

Intra and Inter- day precisions

The coefficient of variation (CV) was <15% in all the simulated physiological media; this is shown in Table 5.

Table 5: Precision of the developed methods

	Method 1 (pH 1.2)	Method 2 (pH 4.5)	Method 3 (pH 6.8)
Intra-day Absorbance (Mean±SD)	0.767±0.04 5	0.662±0.04 3	2.431±0.08 1
CV	5.907 %	6.51 %	3.321 %
Inter-day Absorbance (Mean±SD)	0.813±0.06 51	0.724±0.07 47	2.534±0.12 32
CV	8.0009 %	10.313 %	4.8602 %

The developed method was precise within day, due to low relative standard deviations in all the three media (5.907, 6.51 and 3.321 in pH 1.2, 4.5 and 6.8). It was also found to be precise between days, due to low relative standard deviations in all the three media (8.0009, 10.313 and 5.8602 in pH 1.2, 4.5 and 6.8).

Table 6: Limit of detection and limit of quantitation in the developed methods

SN	LOD(µg/mL)	LOQ(µg/mL)
Method 1	2.35	7.12
Method 2	2.67	8.10
Method 3	4.00	12.14

The developed methods were also found to be accurate as it can detect as low as 2.35, 2.67 and 4.00 µg/mL of the analyte in pH 1.2, 4.5 and 6.8 respectively and can quantify as low as 7.12, 8.10 and 12.14 µg/mL of the analyte.

For any brand to be considered *in-vitro* bioequivalent with the innovator, US FDA, WHO and EMEA require that the dissolution profile should be similar in at least three simulated physiological media (pH 1.2, 4.5, 6.8 and/or 7.4) [14]. In addition, generic brand is considered *in-vitro* equivalent with its branded counterpart when the similarity factor (f_2) is ≥ 50 and the difference factor (f_1) is ≤ 15 , and also dissolution efficiency of ± 10 %. Tables 7-9 show the fit factors (f_1 and f_2) and dissolution efficiency (% D.E) of ascorbic

acid tablets in the different dissolution medium (pH 1.2, 4.5 and 6.8).

Table 7: Fit factors (f_1 and f_2) and % D.E of ascorbic acid tablet samples in pH 1.2

SN	Brands	f_1	f_2	D.E
1	VC01	-	-	6.26
2	VC02	2.82	64.20	6.14
3	VC03	21.98	28.62	4.75
4	VC04	8.72	50.31	6.38
5	VC05	46.34	25.69	6.91
6	VC06	14.14	36.89	6.73
7	VC07	6.61	55.34	6.67
8	VC08	14.27	39.23	7.07

Table 8: Fit factors (f_1 and f_2) and % D.E of ascorbic acid tablet samples in pH 4.5

SN	Brands	f_1	f_2	D.E
1	VC01	-	-	6.7
2	VC02	13.73	64.88	6.2
3	VC03	18.53	34.41	5.74
4	VC04	23.49	31.18	5.21
5	VC05	25.79	27.62	5.05
6	VC06	21.67	34.39	5.7
7	VC07	11.29	73.58	5.33
8	VC08	25.33	30.20	5.19

Table 9: Fit factors (f_1 and f_2) and % D.E of ascorbic acid tablet samples in pH 6.8

SN	Brands	f_1	f_2	D.E
1	VC01	-	-	6.7
2	VC02	11.24	51.60	5.77
3	VC03	15.97	41.30	5.45
4	VC04	14.60	64.57	5.32
5	VC05	1.49	88.93	4.69
6	VC06	24.71	35.12	5.9
7	VC07	11.15	50.64	5.71
8	VC08	18.52	39.21	5.32

DISCUSSION

The fit factors (f_1 and f_2) for samples VC02 and VC07 were within the acceptable range limit across the three media (pH 1.2, 4.5 and 6.8), hence they are bioequivalent and therefore can be used interchangeably with comparator sample VC01 as shown in Tables 7 – 9. Sample VC04 was within the acceptable range in two media (pH 1.2 and 6.8) and the other brands (VC03, VC05, VC06 and VC08) were not bioequivalent in any of the simulated physiological media, and thus cannot be used interchangeably with the comparator brand

VC01. The systemic bioavailability of these generics will be low in addition to therapeutic efficacy.

The dissolution efficiency (% D.E) is a measure of the extent to which the active ingredients dissolves in a solvent/medium. If the % dissolution efficiencies are within the acceptable range limits of (± 10), it can be concluded that the reference and test dissolution profiles are similar [15]. However, based on the dissolution efficiency (% D.E) model, all tested samples demonstrated values within the acceptable limits across the three simulated physiological media (pH 1.2, 4.5, and 6.8) as presented in Tables 5 - 9. This showed that all the samples have similar extend of adsorption.

CONCLUSION

This study demonstrated that while all ascorbic acid tablet brands met pharmacopeial quality standards, only two were bioequivalent to the reference. Four brands failed dissolution similarity requirements, highlighting the need for strengthened regulatory oversight to ensure drug quality and protect public health, thus 25 % of the samples were bioequivalent to the innovator sample VC01, therefore, they may be used interchangeable with each other in clinical use.

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CONFLICT OF INTEREST

Authors declare there was no conflict of interest.

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