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Comparative quality assessment of generic brands of ceftriaxone sodium injection marketed in Ibadan, South-West Nigeria

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Abstract

Ceftriaxone is a broad-spectrum bactericidal agent. Due to lower prices of generic brands of ceftriaxone sodium as compared to the innovator brand, the populace mostly uses them and hence, the need to comprehensively investigate and compare the pharmaceutical quality of innovator with generic brands marketed in Ibadan, South-West Nigeria. Standard physical and chemical tests for quality control of parenterals as stipulated by the British Pharmacopoeia 2013 were performed on 13 brands. The basic functional groups were identified by Infrared spectrophotometry. A Liquid Chromatography method was used for quantitative determination of ceftriaxone sodium. The bactericidal activity of ceftriaxone sodium and brands were investigated by using clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus species*. The FTIR spectra for the innovator and the generics were superimposable. For clarity test, almost all samples were observed to give a clear solution, but unclear solution was seen in samples B, E and I. The LC method was linear over a concentration range of 7.8125 – 250 µg/mL (r^2 = 0.9996). Microbiological efficacy using MIC determination of the generic products evaluated against several clinically significant organisms gave conclusive results with the generic products showing equivalent efficacy to the reference formulation except in few cases. The results obtained from this study conform to that of the British Pharmacopoeia specifications, giving an indication that all the sampled generics are pharmaceutically equivalent. There is need to improve on local production and to increase the post-marketing surveillance, as some products do not have NAFDAC registration numbers.

Keywords: Ceftriaxone; Physicochemical analysis; Pharmaceutical equivalence; Microbiological analysis

INTRODUCTION

Ceftriaxone sodium (Fig. 1) chemically (6R,7R)-7-[2-(2-Amino-4-tiazo lyl) glyoxylamido]-3-[[(2,5-dihydro-6-hydroxy-2-metyl-5-oxo-as-triazin-3-yl)tio] metyl]-8-oxo-5-tia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid 7²-(Z)-(O-metyl oxime) [1], disodium salt, hemihepterhydrate, chemical formula $C_{18}H_{16}N_8Na_2O_7S_3$•3.5$H_2O$ and a molecular weight of 661.59 g/mol is a broad-spectrum bactericidal agent which belongs to the third-generation cephalosporin. It was patented, manufactured, and marketed in 1982 as Rocephin® by Roche Pharmaceuticals, Basel, Switzerland and it is active *in vitro* against a wide range of Gram-positive and Gram-negative organisms, which include beta-lactamase producing strains [2].

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Since Rocephin® patent expired in Europe in the year 2000 and in North America in 2005; more products that are generic are now made available.

Ceftriaxone sodium antibacterial activity is produced through the inhibition of mucopeptide synthesis in the bacterial cell wall and by binding to one or more of the penicillin-binding proteins, which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thereby hindering bacterial cell wall synthesis. This leads to subsequent cell death by lysis because on-going activity of cell wall autolytic enzymes continues while cell-wall assembly is arrested [3]. Ceftriaxone sodium is intramuscularly absorbed and it possesses a complete bioavailability after both intramuscular and intravenous administration. The major route of elimination is urinary excretion. As a result of this, 33-67% of dose is excreted in urine as unchanged drug and the remaining fraction is eliminated in faeces through bile. Hence, biliary elimination is significant for ceftriaxone sodium [4].

A generic brand is a pharmaceutical product usually intended to be interchangeable with an innovator product that is manufactured without a license from the innovator company and marketed after the expiry date of the patent or other exclusive rights. Generic drugs are marketed under a non-proprietary or approved name rather than a proprietary or brand name [2]. Several studies have been conducted on the possibility of substituting generic products for the innovator brands. In many instances, generic substitutions were confirmed. However, in some other cases, the generic products were found to be inferior to the innovator brands. In a comprehensive and systematic review [5] of available literature between 2008 and 2012 on the possibility of generic substitution of brands of antibacterial agents, wide-ranging results were obtained. Of the 37 studies evaluated, 14 (37.8%) suggested that some generic products may be inferior to the innovator in terms of purity (n = 2), *in vitro* activity (n = 3), *in vivo* efficacy in experimental models (n = 4), clinical efficacy (n = 2), taste (n = 2), or compliance and acceptability in children (n = 1). The authors reviewed that majority of *in vitro* studies (78.6%) found no significant difference between generic products and the innovator. One limitation was that most (5/6) *in vivo* studies suggesting a difference between generic products and the innovator brands were performed in an animal model that is not validated for the evaluation of the efficacy of antibacterial agents. It is obvious that extrapolation of such results to human setting will be with a lot of caution. The authors concluded that the diversity of results obtained precludes any generalizations of research findings. This thus implies that regular and consistent pharmaceutical and therapeutic equivalence studies must be done in order to ensure that the goals of clinical treatments are accomplished.

Generic brands of ceftriaxone sodium cost several times less than the innovator branded product. Therefore, it is likely that hundreds of thousands of patients are exposed to generic ceftriaxone for the treatment of potentially life-threatening infections.

Some studies have compared original and generic versions of ceftriaxone and results have shown that many generics failed to meet the quality standards of the branded product [6-8], thus causing reduced efficacy, increased risk of clinical failure, and/or increased risk of emergence of resistant isolates for the generics [9]. However, from available literature, none of such studies has considered the generic brands circulating in South-west Nigeria. In a recent Nigerian study in the South-South region, seven brands were accessed and evaluated [10]. However, judging from the proximity of Ibadan to the Lagos port where imported products mostly come in into the country, we were able to

access thirteen generic brands of ceftriaxone powder for injection. This gives a comprehensive sampling allowing many brands to be included in the pharmaceutical equivalence study.

The aim of this research is to assess, quantify and compare the pharmaceutical quality of innovator brand of ceftriaxone sodium for injection with its generic brands that are marketed in Ibadan, south - west Nigeria in order to provide evidence of pharmaceutical and therapeutic equivalence that can guarantee and provide confidence for interchangeability or generic substitution. Analyses were carried out according to the British Pharmacopoeial requirements.

EXPERIMENTAL

Materials. Samples of the ceftriaxone sodium injection innovator and twelve (12) others readily accessible generic brands manufactured in Switzerland, China, India, Portugal, Korea and Nigeria were purchased in March, 2016 from various registered pharmacies in Ibadan, Oyo state, Nigeria. They were all within their expiration dates as at the time of analyses. These samples were stored in a cool place, prevented from having direct access to light and they were tested within their expiration dates. The sampled generics represent the largest proportion of ceftriaxone sodium for injection 1000 mg easily accessible. Table 1 provides the description of the ceftriaxone brands sampled.

Chemicals and reagents. Ceftriaxone reference sample, HPLC grade acetonitrile (Fisher Scientific, Leicestershire, UK) and methanol (BDH, Poole England) were used in the assay of content of ceftriaxone sodium injection samples. Potassium dihydrogen phosphate crystals (KH_2PO_4) were obtained from Merck (Darmstadt, Germany). Phosphoric acid (H_3PO_4) was obtained from BDH (Briare, France) and sodium hydroxide, also from BDH. A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to further purify the demineralised water

Equipment. The equipment used are; sterilizer (Health Quip Medical Products, England), incubator (GALLENHAMP), analytical weighing balance (Pioneer Ohaus, China), PHS - 3C pH meter (KERN AU 220-4, China), Fourier Transform Infrared (FTIR) Spectrometer (PerkinElmer Spectrum BXII) (Liantrisant UK), the LC system from CECIL Instruments comprising of CECIL CE 4100 ADEPT series dual piston pump, CECIL CE 4200 ADEPT series UV-VIS variable wavelength detector and column oven (CECIL CE 4601) ADEPT series was used for the content assay of the ceftriaxone brands. The LC system is equipped with a workstation, Powerstream® Chromatography System Manager (v. 4.2) ADEPT series CECIL CE 4900.

Methods. Analyses carried out on the generic brands are physical, physicochemical, chemical and microbiological analyses, using British Pharmacopoeia, 2013 method [11]. The assay was carried out using a newly developed reversed phase HPLC method reported by Adegoke [12].

Physical analysis. The primary and secondary packages of the various brand samples were visually examined carefully to check for compliance with requisite labeling and packaging information.

Physicochemical analysis (acidity or alkalinity determination). The acidity/ alkalinity analysis was carried out on all the 13 brands. The pH value was determined using pH meter.

Identification test using Fourier transform infrared spectrophotometer. Analysis was done using Fourier Transform Infrared Spectrophotometer (PerkinElmer Spectrum BXII) and spectrum was recorded at 350 – 4000 cm^{-1}. Solid-state samples of each of the brands were prepared in KBr and then

compressed into pellets for sample analysis. The various vibration brands produced by the innovator product and the generic brands were compared using principal vibrational bands.

Colour of vial content. Colour of vial content (powder) was assessed visually by examining a small quantity of powder placed on a flat white background. The British Pharmacopoeia (2013) [11] specification for colour of ceftriaxone sodium for injection is almost white or yellowish powder.

Clarity and colour of solution. The British Pharmacopoeia (2013) [11] specification for clarity and colour of solution was carried. A solution containing the equivalent of 1.20% w/v of ceftriaxone in carbon dioxide free water was prepared alongside the reference solution.

Assay of ceftriaxone brands using high performance liquid Chromatography. A high performance liquid chromatographic method was used for the assay of the 13 brands of ceftriaxone sodium injection. Final chromatographic separations were achieved on a RP 18 chromatographic column; 125 x 4 mm 5 µm Lichrospher (HICHROM, UK). Separation was accomplished with a mobile phase gradient mixture of mobile phase A (0.067 M KH_2PO_4, pH 7.5) and B (methanol) pumped at a flow rate of 1.0 mL/min. The injection volume was 20 µL and UV detection was performed at 220 nm. A 2-day calibration plot of reference ceftriaxone in the diluent was done using the peak area obtained in the optimization step as a guide to the minimum and maximum data points on the calibration range.

Sensitivity of organisms to standard antibiotic discs. The sensitivity test was determined on two selected organisms, which are *Staphylococcus aureus* and *Escherichia coli*, with the use of standard antibiotic discs, using Broth dilution and Agar diffusion methods. From the stock solution of the organisms, a loopful of the organism was taken into 5 mL of the nutrient broth using platinum wire giving a clear solution. The mixture was incubated for 24 hours. A turbid solution was observed after incubating. 0.1 mL of the overnight cultured organism was added to 9.9 mL of sterile distilled water, which gave a 10^{-2} fold dilution. 0.1 mL of the resultant dilution was added to 20 mL of nutrient agar, shaken and poured into sterile plate that has been pre-labeled. The mixture was placed down for 10 minutes for it to solidify. Antibiotic discs were placed on the solidified agar and allowed to diffuse for 10 minutes. The plate was incubated for 24 hours. Zones of inhibition were measured after incubation period.

Susceptibility testing. Four different organisms were used and their susceptibilities to ceftriaxone at three different concentrations (100, 50 and 25 µg/mL) for each sample were examined. The organisms used were isolated from patients. The organisms used are: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* species and *Pseudomonas aeruginosa*. Agar diffusion method was used for the susceptibility testing. The nutrient agar serves as the means for the organisms to grow. Sterile plates used were incubated for 24 hours, after which the plates were observed visually and the zones of inhibition produced were measured. Both positive (gentamicin) and negative (sterile water) controls were set up at the same time.

Statistical analysis. Statistical analysis was conducted using one-way analysis of variance (ANOVA) between the reference (innovator) product and generic brands to evaluate the equivalence of content of active ingredient. The results obtained for susceptibility tests were statistically evaluated using ANOVA and Dunnett multiple comparison test.

Determination of minimum Inhibitory concentration. The minimum inhibitory concentration (MIC) was determined on two

selected organisms, which are *Staphylococcus aureus* and *Escherichia coli*, using broth dilution method at 6 different concentrations (50, 25, 12.5, 6.25, 3.02 and 1.5 µg/mL). Negative control consisting of broth alone was set up simultaneously. Test tubes were incubated for 24 hours and MIC values were read independently by two observers in order to remove all forms of bias.

RESULTS AND DISCUSSION

The comparative analyses carried out among the 13 brands encompassed both chemical and microbiological works. Wide-ranging results were obtained from the comprehensive analyses carried out on the innovator ceftriaxone sodium injection sample and the twelve generic brands that were obtained for this study.

Acidity/Alkalinity test. The first physicochemical character analyzed is pH. The result obtained from this test is presented in Table 2. The pH is an integer, which represents conventionally the hydrogen ion concentration of an aqueous solution. British Pharmacopoeia [11] specification for pH of a solution containing the equivalent of 12.0% w/v of Ceftriaxone sodium is 6.0 to 8.0.

The pH of a pharmaceutical substance and the eventual product has a great influence on the rate of decomposition of most drugs, solubility, partition coefficient, stability and optimal microbial effectiveness. Weakly acidic and basic drugs show good solubility when they are ionized and they decompose faster when they are ionized. All samples tested satisfied the 2013 British Pharmacopoeia's requirement for pH.

Identification test. Regions of the functional groups found in both the reference product and the generics of ceftriaxone sodium were compared. Typical IR spectral results are presented in Fig. 2. The IR spectral interpretation shows that the spectra obtained from the formulation matches with original spectra of drug. The spectra revealed identical functional groups for all ceftriaxone brands. FTIR studies for ceftriaxone showed characteristic peaks at 3433.36 cm^{-1} (N-H_{str} mode of H-bonded amide group), 1746.27 cm^{-1} (C=O_{str}), 1607.66 cm^{-1}(oxime C=N_{str}) and 1399.82 cm^{-1} (Amines C-N_{str}) for the reference product A. The results are presented in Table 2. A critical overview of the results presented for the FTIR identification test in the results reveals very small variation in the important vibrational bands identified above. For N-H_{str}, the variation was within the range of ± (0.0004 – 0.0007); C=O gave ±(0.0001 – 0.0002); C=N_{str} ±(0.0001 – 0.0002) and C-N_{str} gave ±(0.001 – 0.002). The low variation in the values from the several brands connotes that the spectra are superimposable and hence confirms the identity of ceftriaxone in all the brands accessed for this study.

Colour of vial content. The results for the colour of the vial content are presented in Table 2. On inspection of the labels on the vials and the packs of different brands, all brands tested met the BP's [11] specification for labelling of ceftriaxone sodium for injection. The BP specifies that ceftriaxone sodium for injection should be almost white or yellowish powder. All the brands except brand E are off-white while Brand E was observed to be white. This can be attributed to the fact that all samples are still within their expiration dates. The test for colour of vial content is particularly important in a tropical climatic setting where extremes of humidity, sunlight and heat are prevalent. Any of the environmental conditions in exaggerated levels often catalyses decomposition of chemical compounds and in particular drugs and their formulations. Discolouration or development of a completely new colour is therefore indicative of decomposition. When a product is stored properly such physical qualities as colour, odour, amorphous or crystalline nature are maintained and in consequence, this will determine the integrity of the products in terms of their

biopharmaceutical, microbiological, physical and chemical properties.

Clarity and colour of solution. The colour of the sample solution is shown in Table 2. On visual inspection of the solutions of each drug sample, almost all samples were observed to give a clear solution, but unclear and slightly yellowish solution was observed in samples B, E and I. The BP [11] stipulates that solutions of ceftriaxone injection prepared as recommended should be clear and should not be more coloured than reference solution. This additional clarity test provides a further check on the physical state of the formulations. In particular, test for clarity serves the purpose of ensuring that undissolved particles are excluded. Presence of undissolved particles carries the deleterious consequences of occluding fine capillaries when injected.

Ceftriaxone sodium content. A 2-day calibration plot of ceftriaxone in the chromatographic diluent solution was done using the peak area obtained in the optimization step as a guide to the minimum and maximum data point on the calibration range. The calibration curve of ceftriaxone was linear over the concentration range of 7.8125 – 250 µg/mL and a calibration curve with slope of 29.4696, intercept of -60.2823, correlation coefficient of 0.9998 and coefficient of determination of 0.9996 was obtained (Fig. 3). From the value of the correlation coefficient, it shows that an excellent curve is obtained (when r is greater than 0.99 signifies an excellent curve). The concentration of ceftriaxone in each of the samples was obtained through extrapolation from the Beer-Lambert's plot. The results obtained for the assay of the 13 brands of Ceftriaxone injection powder are presented in Table 3. From the assay of the brands using the LC method, all samples met the BP specifications and also none has a relative standard deviation that is higher than 2.5% from the statistical analysis. Analysis of variance (ANOVA) gave a p >0.05 showing that the content of the active ingredients in the brands are not statistically significantly different.

Microbiological analysis

Antimicrobial activity: Sensitivity of organism to standard antibiotic disc. The organisms were sensitive to few of the antibiotics while others were resistant. For gentamicin, cotrimoxazole and chloramphenicol, *Staphylococcus aureus* was sensitive to the organism, while it was resistant to all other antibiotics tested such as Augmentin, amoxicillin, erythromycin, tetracycline and cloxacillin. For the other organism, *Escherichia coli*, all the antibiotics tested could not produce growth inhibition, which implies that double dose of the drug will be needed. The result obtained is defined by the status of the organism.

The main reason of carrying out this analysis is to know the resistance activity, sensitivity profile and nature of the organism used for the MIC. The two organisms used for the sensitivity test are those used for the minimum inhibitory concentration analysis. Organisms used in this analysis are clinical isolates obtained from University College Hospital, UCH, Ibadan, Oyo State. Most of the clinical isolates used are resistant strains and their sensitivity profile shows that many of them are multi-drug resistant. This means the sensitivity of the cephalosporins should be well appreciated on them. This also justifies why cephalosporins are currently preferred in the management of multi-drug resistant bacterial infections and thus necessitating the investigation into the generic substitution status for the antibiotics.

Table 1: Details of the Ceftriaxone sodium injection powders sampled

Sample	Branded	Country of Origin	Manufacturing Date	Expiry Date	NAFDAC Reg. No.
A	Yes	Switzerland	03/2015	03/2018	Yes
B	Yes	India	05/15	04/18	No
C	Yes	Portugal	09/2014	09/2017	Yes
D	Yes	Korea	28/02/2014	27/02/2017	Yes
E	Yes	Korea	05/11/2014	04/11/2017	Yes
F	Yes	Nigeria	11/2015	11/2018	Yes
G	Yes	China	07/2014	07/2017	Yes
H	Yes	China	09/2015	09/2018	No
I	Yes	China	09/2014	09/2017	Yes
J	Yes	India	10/2015	09/2018	Yes
K	Yes	India	08/2014	07/2017	Yes
L	Yes	China	08/2015	08/2018	No
M	Yes	China	06/2015	06/2018	Yes

Table 2: Physicochemical characteristics of generic ceftriaxone products tested

Sample	pH	IR bands of functional groups (cm^{-1})				Colour of the vial content	Clarity and colour of solution
		N-H	C=O	C=N	C-N		
A	6.860 ± 0	3433.36	1746.27	1607.66	1399.82	Off-white	Clear solution
B	6.550 ± 0.011	3429.50	1747.88	1606.79	1399.73	Off-white	Trace of yellow
C	6.727 ± 0.005	3428.57	1747.58	1607.52	1399.85	Off-white	Clear solution
D	6.720 ± 0.010	3439.77	1747.15	1608.01	1400.82	Off-white	Clear solution
E	6.937 ± 0.118	3439.77	1747.03	1607.38	1399.40	White	Trace of yellow
F	6.860 ± 0.026	3428.57	1747.73	1607.14	1399.50	Off-white	Clear solution
G	6.437 ± 0.005	3432.78	1745.40	1607.22	1375.05	Off-white	Clear solution
H	6.730 ± 0.026	3428.57	1747.61	1607.40	1399.69	Off-white	Clear solution
I	6.820 ± 0.017	3429.75	1745.73	1606.42	1376.02	Off-white	Trace of yellow
J	6.400 ± 0.010	3434.17	1747.21	1608.00	1399.51	Off-white	Clear solution
K	6.667 ± 0.005	3432.46	1745.57	1607.44	1376.35	Off-white	Clear solution
L	6.497 ± 0.023	3438.57	1746.95	1606.20	1370.12	Off-white	Clear solution
M	6.717 ± 0.015	3431.59	1746.45	1607.15	1399.00	Off-white	Clear solution

Table 3: Assay of Ceftriaxone Injection powder HPLC method

Drug formulation	Concentration found[a*]	Amount in g of ceftriaxone	Mean Recovery ± S.D.[b] (%)	RSD (%)
A	201.035	1.01	100.51 ± 0.98	0.98
B	204.025	1.02	102.01 ±1.20	1.18
C	203.309	1.02	101.65 ±3.00	2.95
D	203.2377	1.02	101.62 ±1.39	1.36
E	206.285	1.03	103.14±1.02	0.99
F	203.634	1.01	101.82±1.99	1.96
G	201.541	1.01	100.77±1.55	1.54
H	200.930	1.00	100.47±0.72	0.71
I	201.342	1.01	100.67±0.48	0.47
J	207.787	1.04	103.89±2.05	1.98
K	202.107	1.01	101.05±1.09	1.08
L	200.667	1.00	100.33±0.69	0.69
M	201.336	1.00	100.67±0.48	0.48

[a]*n=4;* ***Concentration expected = 200µg/mL;*** [b] ***BP specification = 92-108%*** *[11]*

Table 4: Zones of inhibition of generic brands of ceftriaxone sodium injection samples

Generic sample	Concentration of ceftriaxone (µg/mL)	Zones of inhibition (mm)			
		Bacillus species	*E. coli*	*Pseudomonas aeruginosa*	*Staphylococcus Aureus*
A	100	17	18	15	30
	50	12	12	12	20
	25	-	-	-	-
B	100	15	20	18	25
	50	12	15	-	20
	25	-	-	12	12
C	100	30	25	24	25
	50	25	20	-	18
	25	-	-	-	15
D	100	26	20	-	22
	50	20	12	-	18
	25	-	-	-	15
E	100	30	22	24	30
	50	22	15	15	25
	25	18	-	-	15
F	100	28	25	22	25
	50	22	18	15	24
	25	-	-	-	20
G	100	28	20	30	28
	50	25	-	20	25
	25	-	-	-	20
H	100	25	-	20	25
	50	20	-	12	20
	25	-	-	-	18
I	100	20	18	20	30
	50	15	12	-	22
	25	12	-	-	18
J	100	24	-	15	20
	50	18	-	-	18
	25	-	-	-	12
K	100	24	29	23	25
	50	18	21	-	-
	25	15	15	15	20
L	100	25	24	20	22
	50	20	-	15	15
	25	14	-	-	-
M	100	28	20	30	28
	50	18	18	15	25
	25	-	15	-	20
	Control	30	20	17	22

Table 5: Minimum inhibitory concentrations of innovator and generic brands of ceftriaxone injection against *Staphylococcus aureus*

Sample	1	2	3	4	5	6	Control (broth)
A	-	-	-	+	+	+	-
B	-	+	+	+	+	+	-
C	-	+	+	+	+	+	-
D	-	+	+	+	+	+	-
E	-	+	+	+	+	+	-
F	-	-	+	+	+	+	-
G	+	+	+	+	+	+	-
H	-	+	+	+	+	+	-
I	+	+	+	+	+	+	-
J	-	+	+	+	+	+	-
K	-	-	-	+	+	+	-
L	-	-	+	+	+	+	-
M	-	+	+	+	+	+	-

$^{+}$ Growth, $^{-}$ No growth; 1: 50 µg/mL, 2: 25 µg/mL, 3: 12.5 µg/mL, 4: 6.25 µg/mL, 5: 3.02 µg/mL, 6: 1.5 µg/mL

Table 6: Minimum inhibitory concentrations of innovator and generic brands of ceftriaxone injection against *Escherichia coli*

SAMPLE	1	2	3	4	5	6	Control (broth)
A	-	-	-	+	+	+	-
B	-	-	+	+	+	+	-
C	-	+	+	+	+	+	-
D	-	-	-	+	+	+	-
E	-	-	+	+	+	+	-
F	-	-	-	+	+	+	-
G	-	-	+	+	+	+	-
H	-	-	-	+	+	+	-
I	-	-	-	+	+	+	-
J	-	+	+	+	+	+	-
K	-	-	-	+	+	+	-
L	+	+	+	+	+	+	-
M	-	-	+	+	+	+	-

$^{+}$ Growth, $^{-}$ No growth; 1: 50 µg/mL, 2: 25 µg/mL, 3: 12.5 µg/mL, 4: 6.25 µg/mL, 5: 3.02 µg/mL, 6: 1.5 µg/mL

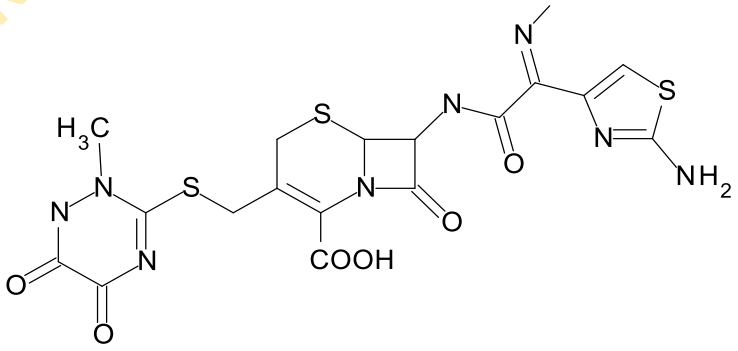

Figure 1: Chemical structure of Ceftriaxone (Richards *et al*, 1984)

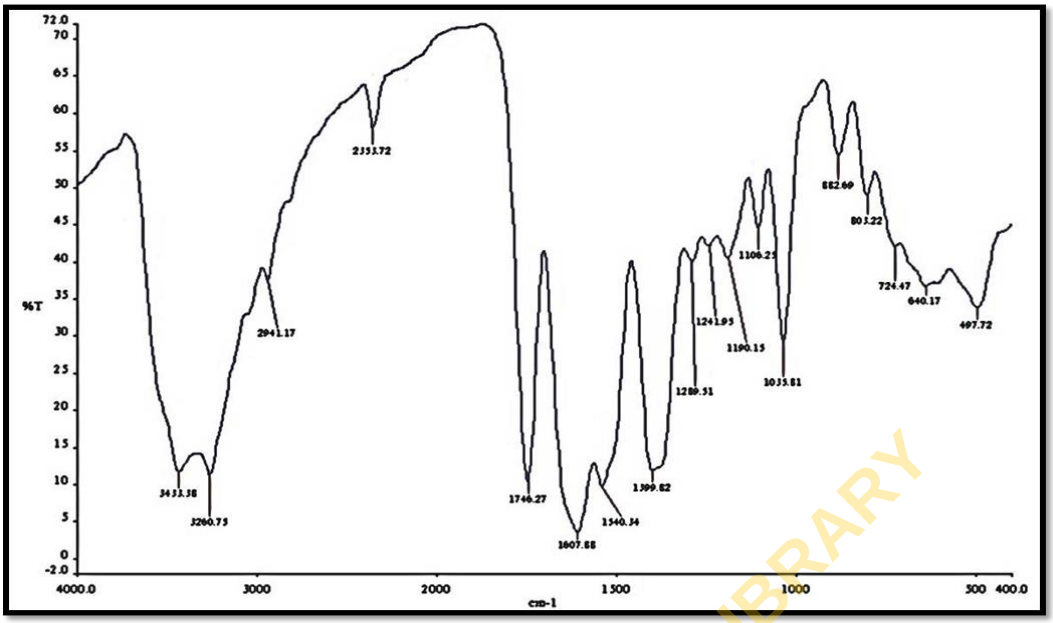

Figure 2: Typical IR spectra of a brand of ceftriaxone sodium injection

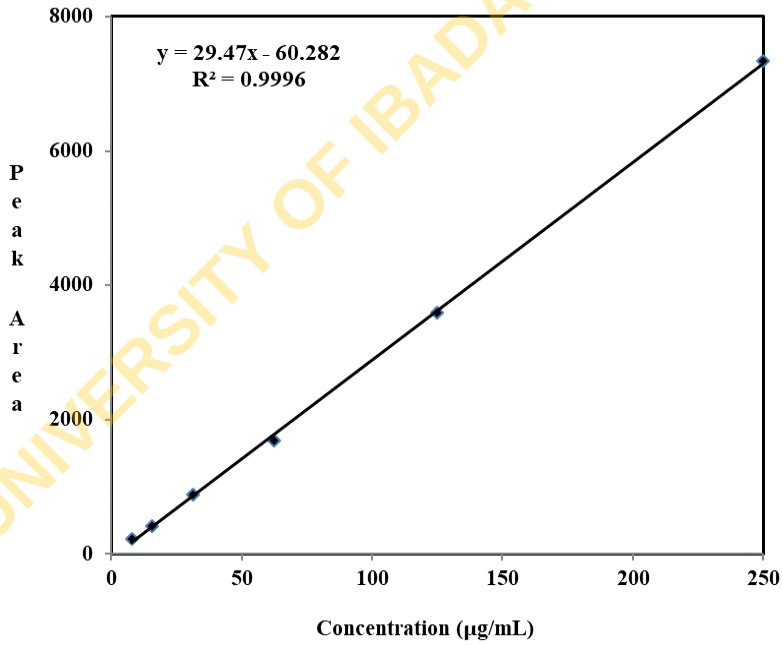

Fig. 3: Calibration Curve for determination of Ceftriaxone in injection dosage form

Antimicrobial activity: Susceptibility testing. From the results obtained (Table 4), sample B has the lowest and sample C and F have the highest zones of inhibition at 100 µg/mL. Sample B has the lowest and samples C and G have the highest zones of inhibition at 50 µg/mL. Sample I has the lowest and sample F has the highest zone of inhibition at 25 µg/mL concentration for *Bacillus* species. The samples of different brands of ceftriaxone sodium tested produced distinct and clearly defined zones of growth inhibition more on *Staphylococcus aureus*, compared to *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus* species inoculated agar. The size

of zones of inhibition produced with respect to the concentration of drug used for the test indicates that the microorganism is susceptible to the antibacterial activities of the different brands of ceftriaxone sodium tested, most especially at 100 µg/mL and 50 µg/mL. Little zones of inhibition were produced at concentration level of 25 µg/mL. However, differences were observed in the magnitude of zones of inhibition for each of the brands assessed. From the results, it shows that there was significant influence of brand type on the zones of growth inhibition produced on all the organisms tested especially *Staphylococcus aureus*. This variation can be attributed to formulation and manufacturing factors and thus account for why some of the brands may be more efficacious than some other brands. Also, excipients used could cause a decrease or increase on the zones of inhibition. The results produced for the sensitivity testing of the various brands of ceftriaxone injection are presented in Table 4. ANOVA and Dunnett multiple comparisons test as post-Hoc test were adopted. When compared to brand A which is the innovator product, all concentration levels for brand B produced very highly significant zones of inhibition ($p<0.001$) for all the organisms except against *Pseudomonas aeruginosa* at the lowest concentration of 25 µg/mL which happened to be an outlier. For sample C, there was no statistical significant difference at 100 and 50 µg/mL against *Bacillus* species ($p>0.05$). Brand F gave the best results relative to the innovator product against *Bacillus* species as there was no significant difference in the zones of inhibition at all concentration levels ($p>0.05$). Brand M was equally effectively as the innovator brand A at the highest concentrations of 100 µg/mL ($p>0.05$) against *Bacillus* species and *Pseudomonas aeruginosa*.

Antimicrobial activity: Determination of minimum inhibitory concentration. The MIC was determined at six different concentrations on two selected microorganism. Lower minimum inhibitory concentration indicates higher potency for the drug. The concentration that can inhibit the organism is low and therefore drug is active at low MIC. It shows that ceftriaxone is effective even at low concentrations. The results for the MIC determination are presented in Tables 5 and 6 respectively for *Staphylococcus aureus* and *Escherichia coli*. Only brand K gave similar MIC compared to innovator brand A against *Staphylococcus aureus* while brands D, F, I and K were equally effective as the innovator brand A against *E. coli*.

Conclusion

The pharmaceutical quality tests carried out on the innovator and twelve generics of ceftriaxone sodium for injection using standard quality control test, with the aim of evaluating the quality and quantity of the active pharmaceutical ingredient, antimicrobial activity and also whether they are pharmaceutically equivalent has been confirmed. The results obtained from this study conform to that of the British Pharmacopoeia, 2013 specifications. From the assay, it can be concluded that all generic brands are equivalent to the innovator as their content of active ingredients are within the specified range. Although, the samples passed majority of the tests, there is a need to improve on the indigenous manufacturing drive as 9 of the 13 brands (69.2%) were manufactured by foreign, companies for marketing in Nigeria while only one product (7.69%) is made locally in Nigeria. The disturbing observation that three of the products (representing 23.1% of the total sampled) do not possess NAFDAC registration numbers still calls for caution. If in spite of the aggressive drive of NAFDAC, some products as sensitive as injectables could still be found in the market without official registration then renewed efforts at post-marketing surveillance is needed as

much as national re-orientation on the part of importers and retailers.

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