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Quality Control and *In Vitro* Bioequivalence Studies on Four Brands of Ciprofloxacin Tablets Commonly Sold In Uyo Metropolis, Nigeria

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ABSTRACT

The quality control and in vitro bioequivalence of four brands of ciprofloxacin hydrochloride tablets commonly sold in Uyo, Nigeria, were assessed through the evaluation of the uniformity of weight, friability test, hardness, disintegration test, dissolution rate, and non-aqueous titration procedure with the use of crystal violet solution as indicator. All the brands complied with the official specification for uniformity of weight, hardness and disintegration time. However, for the friability test, one of the four brands (Cefroden), failed to meet the USP specification of maximum friability value of 1%. The dissolution rate profile revealed that one of the four brands (i.e. Cefroden) did not attain up to 70% dissolution throughout the period of the determination, while the other brands had above 70% release in less than 45min. The non-aqueous titrimetric procedure showed that three brands have values within the range specified for content uniformity in the USP (95-105%), while the remaining one brand (Cefroden) gave a lower value. Three of the four brands evaluated in this study could be regarded as being biopharmaceutically and chemically equivalent, while one brand is obviously a sub-standard product. The non-aqueous titrimetric procedure used in this study is simple, inexpensive, and easy to use and could be used in routine monitoring of the quality of ciprofloxacin HCl tablets, especially in the absence of high technology equipments that are not easily available in most developing countries. The analysis of variance showed that there is significant difference ($p < 0.001$) in the release profile of the four brands of ciprofloxacin tablets. Cipronol having the highest release profile while Cefroden had the least.

INTRODUCTION

The need to select one product from several generic drug products of the same active ingredients during the course of therapy is a cause of concern to a healthcare practitioner. The first stage in ascertaining the therapeutic equivalence of any drug product involves ascertaining the chemical and biopharmaceutical equivalency of such drug products [1].

Drug products that are chemically and biopharmaceutically equivalent must be identical in strength, quality, purity as well as content uniformity, disintegration and dissolution rate. The need to ensure that the generic and branded drug products are pharmaceutically and therapeutically equivalent cannot be over emphasized.

The safety and efficacy of drug products can be guaranteed when their quality is reliable and reproducible from batch to batch. To ensure the requisite quality, drug manufacturers are required to test their products during and after manufacturing and at various intervals during the shelf life of the product [2]. The quality of medicines is an integral part of access in light of ensuring that the pharmaceutical products are fit for their intended use, comply with the requirement of the marketing authorization and do not expose consumers to risks. To attain this objective there must be a system of quality assurance, which incorporates aspects including product development, manufacture, distribution, and storage.

The objective of this work was to assess the quality of these four brands of ciprofloxacin tablets commercially available in Uyo, Nigeria. The findings can serve as source of information to manufacturers and regulatory agencies like NAFDAC (National Agency for Food, Drug Administration and Control)

EXPERIMENTAL SECTION

Materials:
Cefroden, Cipox, Ciprocare and cipronol tablets were purchased from Amela Pharmaceuticals, Uyo in Nigeria. All other chemicals were of analytical grade.

Weight Variation Determination:
20 tablets from each generic were weighted individually using a weighing balance (Mettler 1180). The average weights of the tablet as well as their percentage deviation were calculated.

Tablet dimensions:
The dimensions of the tablets were determined using the micrometer screw gauge. The thickness and diameter of the tablets were determined. Five tablets were used for this determination.

Hardness test
The hardness of 10 tablets selected randomly from each of the batches after equilibrating at room temperature for 24 h was determined in an automatic hardness tester (Erweka, Model TBH - 28). The mean hardness was calculated.

Friability
The weight of 20 tablets selected from each batch at random was determined collectively as initial weight, WA. The tablets were placed in a friabilator (Erweka); set to rotate at 25 rpm for 4 min. At the end of the run, the tablets were de-dusted and weighed (WB). Friability was calculated from the equation.

$$F = (WA - WB)/WA \times 100$$

The test was repeated five times and the mean value determined.

Disintegration time determination
Erweka disintegration test apparatus (Model DT4) was used based on the British Pharmacopoeia, 2003 method [3]. The disintegration medium was 0.1 N HCI, maintained at 37 ± 0.5°C. Five tablets from each batch were used for the test. The disintegration time was taken as the mean time needed for the tablets to break into particles small enough to pass through the screen into the disintegration medium.

Content Uniformity Test:
Preparation of perchloric acid:
Perchloric acid was prepared by the reaction of nitric acid and ammonium perchlorate. Nitrous oxide was given off and the resulting perchloric acid was collected. Preparation of mercuric II acetate: 2g of metallic mercury was weighed out and dissolved in 50ml acetic acid to produce mercuric II acetate solution.

Determination :
The four different brands of ciprofloxacin HCl tablets were tested for uniformity of their drug content.Amounts of the crushed tablet material equivalent to 0.3g of pure ciprofloxacin hydrochloride in the tablet dosage form of the innovator brand were weighed. These were dissolved in 15ml glacial acetic acid, followed by the addition of 1.5ml of freshly prepared mercuric (II) acetate solution and 5ml 0f acetic anhydride . The solution was titrated against 0.1M aqueous perchloric acid using 0.5%w/v crystal violet solution as indicator until a bluish – green end point. Blank titrations were carried out using 15ml glacial acetic acid. Titre values were adjusted by deducting the blank determination from the assay. The procedure was carried out in triplicates.
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Dissolution profile studies:
Erweka dissolution apparatus was used, employing the British Pharmacopoeia 2003 method (3). One tablet was placed in the apparatus and rotated at 100 rpm. The dissolution medium was 1000 ml 0.1 N HCL, maintained at 37 ± 0.5°C. 5 ml portions of the dissolution medium were withdrawn using a pipette fitted with a non-adsorbent cotton wool at predetermined time intervals. Each 5 ml sample withdrawn was replaced by an equivalent fresh dissolution medium, maintained at 37 ± 0.5°C. The solution was analyzed after colour development using a Sp6-450 UV/VIS spectrophotometer at 270 nm.

Statistical Analysis:
The disintegration time, percentage friability, uniformity of weight and other physical properties was analyzed with simple statistics, while dissolution profiles were analyzed for significant differences by one- way analysis of variance (ANOVA) using a graph-pad instat 3 software.

RESULTS AND CONCLUSION

Tablet properties :
Tables 1-3 shows properties of four commercially available ciprofloxacin tablets. From the results obtained, the uniformity of weight determinations for all the brands gave values which

complies with the official books specification for weight uniformity, as none of the brands deviated by up to 5% from the mean value (Table 1). Also, the different brands of ciprofloxacin had good mechanical strength as all four brands had mean hardness values within the range 5.10 - 5.47kg/cm^2 (Table 1). The compendial specification for uniformity of weight states that for tablets weighing more than 324 mg, weights of not more than two tablets should deviate from the average weight by more than 5% [5]. Deviations for tablets were within the range. These values were within compendial standard [5].

Crushing strength test shows the ability of tablets to withstand pressure or stress during handling, packaging and transportation. It is the property of a tablet that is measured to assess its resistance to permanent deformation. Furthermore, the mechanical strength of a tablet determines the disintegration time and the rate of dissolution. For the mechanical strength of a tablet to be satisfactory, the minimum requirement is 4 kg [6]. All the tablets met the specifications implying that tablets have good mechanical strength.

Friability is another mechanical property of a tablet with compendial (specification not more than 1% [5]. While crushing strength test is a bulk deformation of the tablet, friability is a surface deformation which may be enhanced by the morphology of the tablet [7]. Virtually all the tablets met compendial specification for friability, except cefroden.

However, for friability test which is a measure of the ability of the tablets to withstand abrasion during handling, transportation etc, it was observed that Cipox, Ciprocare and Cipronol had appreciably low friability values .i.e. within the range of 0.1% to 0.2%. The hardness and friability values thus indicate that these three tablets can withstand the stress associated with transportation and dispensing processes. However, the brand Cefroden had friability value of about 20%. Cefroden thus failed the friability test as the USP and other reference / standard books gave an allowable friability value $\leq$ 1%. Most of the tablets in the generic brand Cefroden broke in halves along the middle line. Cefroden, having failed the friability test would thus not be able to withstand the stress associated with transportation and dispensing process. The USP specifies that the disintegration time for film-coated tablets should not exceed 30 min; all the four brands passed the disintegration test as they all disintegrated in less than 6minutes.

Table 1: Uniformity of weight, thickness and hardness determination of four brands of ciprofloxacin hydrochloride tablets.

Brand	Uniformity of weight (g)	Hardness(kg/cm^2)
Cipox	0.830 ± 0.009	5.47 ± 0.28
Ciprocare	1.096 ± 0.008	5.40 ± 0.21
Cefroden	0.738 ± 0.013	5.33 ± 0.18
Cipronol	0.766 ± 0.008	5.10 ± 0.35

Table 2: Mean tablet dimensions

Brand	Thickness (mm)	Width (mm)	Length(mm)
Ciprocare	7.18 ± 0.04	9.27 ± 0.01	19.39 ± 0.01
Cefroden	4.48 ± 0.02	9.04 ± 0.01	18.02 ± 0.01
Cipronol	5.26 ± 0.02	9.12 ± 0.02	19.31 ± 0.02
Cipox	6.11 ± 0.02	9.21 ± 0.02	16.27 ± 0.01

**Determination was carried out 5times.*

Table 3: Friability and disintegration time determinations for the four brands of ciprofloxacin tablets

Brand	Friability (%)	Disintegration time (min)
Cipox	0.12 ± 0.16	3.094 ± 0.09
Ciprocare	0.12 ± 0.16	5.982 ± 0.24
Cefroden	20.2 ± 3.70	3.296 ± 0.07
Cipronol	0.20 ± 0.01	5.162 ± 0.11

Content of active ingredient.

The results gotten from the assessment of the percentage content of active ingredient in the four brands of ciprofloxacin tablets, showed that three of the given four brands gave values within the ranges 95-99.8%, while one brand (Cefroden) had values below 95%. The USP specifies 95-105% drug content for ciprofloxacin tablets. Thus, the three brands, Cipox, Cipronol and, Ciprocare conformed to the pharmacopoieal standards for percentage content of active ingredient, but the brand Cefroden did not conform to the USP standards as it had percentage content less than the lower limit (95%).

Table 4: Percentage content of active drug

Brand	Cipox	Cefroden	Cipronol	Ciprocare
% content	96.3 ± 1.85	93.70 ± 1.35	97.83 ± 2.17	95.77 1.63

Dissolution profiles of Tablets:

The time taken for 50% and 70% of the drug to be released ($T_{50\%}$ and $T_{70\%}$) respectively and the maximum cumulative amount of drug release (C_{max}) were used to characterize the release profiles of ciprofloxacin tablets (Tables 5 and 6). During the *in vitro* drug release studies, all formulations were observed for physical integrity at different time intervals. After about two minutes, the tablets Cefroden had swelled and was the first to burst at the sides. However, with increasing time, there was no further change in the integrity of the Cefroden tablets. Cipox , Ciprocare and Cipronol attained T_{70} values in less than 45minutes. The brand Cefroden however, did not attain 70% release of its active drug all through the duration of the study. Also, Cmax values after the one- hour duration of the study were 97.5 and 95.0% for Cipox and Ciprocare respectively. The brand Cipronol only released 80% of its active drug at the end of the study. Cefroden however, was able to release only 40% of the active drug . This release profile of Cefroden raises some controversy. This is due to the fact that the Cefroden actually had a short disintegration time of about 3.30mins. One would have expected that Cefroden would have maximum release of the drug. This goes to certify that although disintegration is important for the dissolution of a drug, the mere fact that a drug passes the disintegration test does not necessarily mean that it would also pass the dissolution test and hence, have high bioavailability.

There was no significant difference between the release profile of Cipox and Ciprocare ($p > 0.05$) and between Ciprocare and Cipronol ($p > 0.05$).there however was significant difference between the brands Cefroden and Cipox ($p < 0.05$), between Cefroden and Ciprocare ($p < 0.05$), between Cefroden and Cipronol ($p < 0.05$) and between Cipox and Cipronol ($p < 0.05$), the drugs Ciprocare and Cipox and also Ciprocare and Cipronol can be said to be bioequivalent and can thus be substituted for each other.

Table 5: *In vitro* dissolution profile.

time(min)	Cefroden(%)	Cipox (%)	Ciprocare(%)	Cipronol (%)
5	3.0	7.2	29.5	40.0
10	7.0	18.0	45.0	62.0
15	10.0	24.0	54.0	67.5
20	12.5	38.0	60.0	75.0
25	18.0	41.7	67.5	82.0
30	22.4	50.0	74.0	87.0
35	26.0	59.5	80.0	92.5
40	27.7	64.8	83.5	95.0
45	30.0	71.0	88.0	95.0
50	32.0	75.0	91.0	98.0
55	36.4	80.0	92.0	98.0
60	40.0	80.0	95.0	99.5

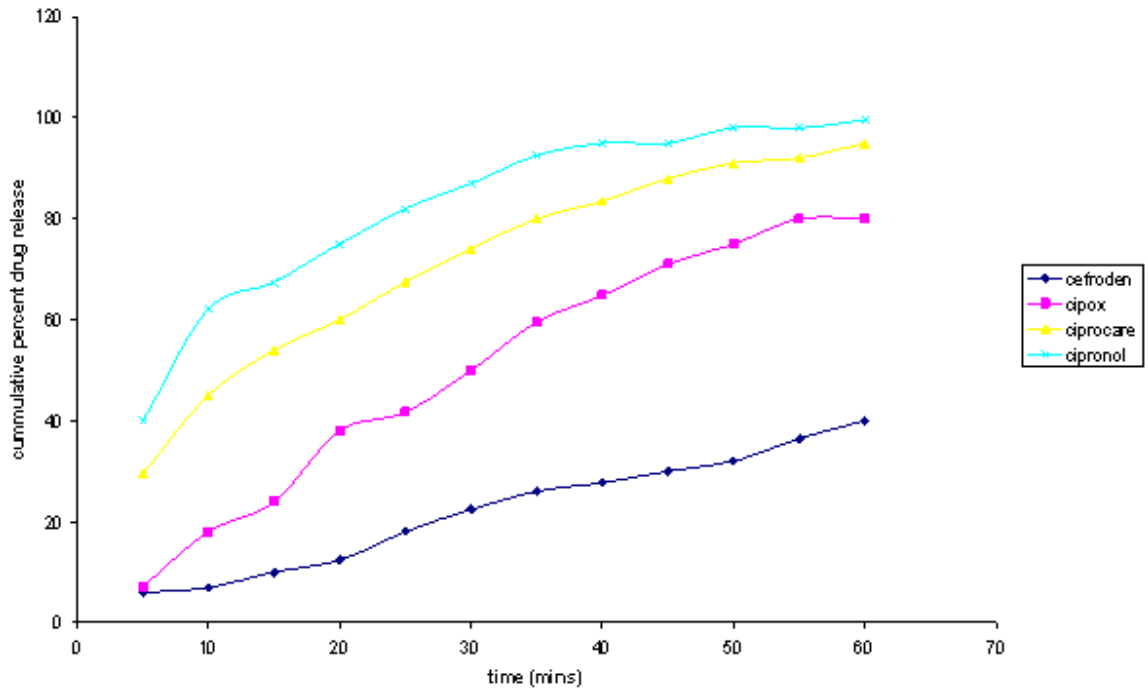

Fig. 1: Release profile of four brands of ciprofloxacin tablets

Table 6: *In vitro* dissolution test: percentage release

Sample	T_{50} (min)	T_{70} (min)	Cmax (%)
Cipox	30.0	45.0	99.5
Cipronol	7.0	17.0	80.0
Cefroden	-	-	40.0
Ciprocare	11.5	27.0	95.0

Drug Release Kinetics and Mechanism of Release:
In order to investigate the release kinetics and mechanism, the dissolution data were fitted into different kinetic models namely zero order, first order, Higuchi and Korsmeyer models [8-11].

Ideally, an immediate release tablet should release the required quantity a drug with predetermined kinetics in order to attain and maintain an effective drug plasma concentration (Merchant *et al.,* 2006). To achieve this, the tablet should be formulated so that it releases the drug in a predetermined and reproducible manner. Tables 5-7 show the release Kinetics.

From the results in table 4d ,the brand Cipox follows Higuchi kinetics with highest linearity(r^2 = 0.9913) via non- fickian or anomalous diffusion (n = 0.46). Higuchi kinetics describes the release of drugs from a drug as a square root of time dependent process.

Cipronol follows First order kinetics (r^2 = 0.9569) which the describes release from systems where drug release rate is concentration dependent. This release is via Fickian diffusion.

Ciprocare follows Korsmeyer model with highest linearity (r^2 = 0.9913) via super case II-transport. Korsmeyer.

The release of Cefroden also follows Korsmeyer model (r^2 =0.9934) via super case II-transport.

Table 7: Kinetics and mechanism of release for the four brands of ciprofloxacin

Sample	Zero order	First order	Higuchi	Korsmeyer	N
Cipronol	0.8417	0.9569	0.9333	0.9551	0.350
Ciprocare	0.9345	0.9896	0.9876	0.9913	0.960
Cipox	0.9711	0.9905	0.9913	0.9831	0.460
Cefroden	0.9897	0.9921	0.9823	0.9934	1.030

CONCLUSION

The increasing need for drugs and drug products to treat the various diseases that affects mankind, and the poverty level that exists in most developing countries , especially Nigeria, has led World Health Organisation to continually support the use of generic drugs . With this support comes the problem of fake, adulterated and substandard drugs. There thus arises the need for adequate quality assurance and control of drugs and also assessment of bioavailability of the different generic drugs in circulation to ascertain that the drugs being sold can actually be trusted to produce the desired effect similar to the standard drug.

All the brands complied with the official specification for uniformity of weight , hardness and disintegration. In general, the tablets showed good friability profiles, since most had friability values of less than 1.0% [4]. Only one of the four brands only one brand (Cefroden), failed to meet the USP specification of maximum friability value of 1%.

The determination of the percentage content of active drug and in vitro dissolution studies among other tests are important pointers to the quality of drugs.

This research showed that the four different brands are biopharmaceutically and chemically equivalent and thus cannot be used interchangeably. However ,there was no significant difference between the brands Cipox andCciprocare and also Ciprocare and Cipronol as shown by the analysis of variance ($p > 0.05$). These pairs can thus be said to be chemical and biopharmaceutical equivalents. The brand Cipronol had the highest percentage content of active ingredient (98.7%), it also had a good release profile releasing over 99% the drug in one hour. The brand Cefroden is obviously sub-standard.

As it lower content of the active than the lower boundary limit given by the USP (i.e. 93.7% while the USP specifies 95-105%). Also, Cefroden never attained 70% release of the active drug throughout the duration of the determination, it only attained 40% release (i.e. Cmax= 40%). Clearly this drug when taken would not be able to produce the desired therapeutic effect.

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